O1

Following the Proton: A Journey Through Bioenergetics

Joachim Heberle[a]

[a]*Freie Universität Berlin, Exp. Molecular Biophysics, Arnimallee 14, 14195 Berlin, Germany*

E-mail address: joachim.heberle@fu-berlin.de

A central mechanism in bioenergetics in any living cell is chemiosmosis. As devised by Peter Mitchel, protons are actively translocated across a biological membrane (e.g., the inner mitochondrial membrane), creating the proton motive force, which stores free energy in a the form of an electrochemical gradient. Here, protons (H^+ ions) are not merely participants but the core mediators of energy transduction: they store energy as a gradient, transfer it across membranes, and release it in a controlled manner to drive essential biological processes like ATP synthesis.

Proton transfer is achieved through spatiotemporal variations in hydrogen-bonded networks, i.e. the movement of protons at different timescales and distances – from femtoseconds to seconds and from less than 1 Å to 10 nm. Protonation dynamics can tune and control the redox potentials and pKa values of individual residues or catalytically active groups, and they can drive and respond to the conformational changes of proteins and the water clusters associated with them [1].

As a personal reflection on my journey to explore bioenergetic principles, I will showcase examples of membrane proteins - particularly microbial rhodopsins [2,3] and cytochrome c oxidase [4] - as model platforms to dissect proton transfer with high spatial and temporal resolution. A central aspect of my work has been the development and application of time-resolved [5] and surface-enhanced infrared spectroscopy [6] to monitor protein structural dynamics in real time. These studies have enabled us to establish a detailed mechanistic understanding of how light absorption induces coordinated conformational changes that drive proton pumping across membranes, thereby generating electrochemical gradients essential for cellular energy storage.

In addition to proton pumps, my group has extended these approaches to channelrhodopsins [7] and other optogenetic tools, elucidating how light-gated ion channels achieve ion selectivity and gating on ultrafast timescales. This work connects fundamental bioenergetics with applications in neuroscience.

Overall, my research aims to uncover the physicochemical principles governing energy transduction in biological systems. By integrating spectroscopy, structural biology, and theoretical approaches [8, 9], we seek to establish a comprehensive framework that links protein dynamics to function, with implications for bioenergetics, photobiology, and the design of light-driven biomolecular systems.

[1] V.A. Lorenz-Fonfria, C. Bamann, T. Resler, R. Schlesinger, E. Bamberg, J. Heberle, Temporal evolution of helix hydration in a light-gated ion channel correlates with ion conductance, Proc Natl Acad Sci USA 112(43) (2015) E5796-804.

[2] J. Heberle, J. Riesle, G. Thiedemann, D. Oesterhelt, N.A. Dencher, Proton migration along the membrane surface and retarded surface to bulk transfer, Nature 370(6488) (1994) 379-382. [3] J. Heberle, Proton transfer reactions across bacteriorhodopsin and along the membrane, Biochim Biophys Acta 1458(1) (2000) 135-147.

[4] F. Baserga, P. Langner, L. Schubert, J.P. Storm, R. Schlesinger, J. Heberle, Proton-Coupled Electron Transfer in Cytochrome c Oxidase: Heme a Controls the Protonation Dynamics of E286, ChemPhysChem 26(24) (2025) e202500539.

[5] T. Kottke, V.A. Lorenz-Fonfria, J. Heberle, The Grateful Infrared: Sequential Protein Structural Changes Resolved by Infrared Difference Spectroscopy, J Phys Chem B 121(2) (2017) 335-350. [6] J. Kozuch, K. Ataka, J. Heberle, Surface-enhanced infrared absorption spectroscopy, Nat Rev Method Prime 3(1) (2023) 70.

[7] V.A. Lorenz-Fonfria, J. Heberle, Channelrhodopsin unchained: structure and mechanism of a light-gated cation channel, Biochim Biophys Acta 1837(5) (2014) 626-642.

[8] P. Skopintsev, D. Ehrenberg, T. Weinert, …, Nogly, I. Schapiro, C. Milne, J. Heberle, J. Standfuss, Femtosecond-to-millisecond structural changes in a light-driven sodium pump, Nature 583(7815) (2020) 314-318.

[9] S. Mous, G. Gotthard, D. Ehrenberg, …, J. Standfuss, I. Schapiro, J. Heberle, P. Nogly,
Dynamics and mechanism of a light-driven chloride pump, Science 375(6583) (2022) 845-851.

O2

Structures of the intermediates of the catalytic cycle of the bacterial cytochrome *c* oxidase

Hartmut Michel[a], Daniel Hatlem[a], Di Wu[a], Schara Safarian[a], Daniela S. Barreiro[a], Muhammad T. Hafeez[b], Raaif Siddeeque[b], Frédéric Melin[b], Petra Hellwig[b]

aMax Planck Institute of Biophysics, Frankfurt am Main, Germany [b]Laboratoire de bioélectrochimie et spectroscopie, Université de Strasbourg, France

E-mail address: Hartmut.Michel@biophys.mpg.de

Presenting author: Hartmut Michel

We have recently determined the structures of the intermediate states of the catalytic cycle of the cytochrome c oxidase from *Paracoccus denitrificans* using a recombinant form of the enzyme by electron cryomicroscopy.[1] We have no repeated this work using an ATCC wild type of this enzyme achieving resolutions of up to 1.6 Å. The results agree well with those of our previous work. The oxidized form (Ostate) contains a peroxide in the binuclear active site bridging the heme a_3 iron and Cu_B agreeing well with the results of resonance Raman spectroscopy. We do not find any oxoferryl groups in the P- and the Fstates. We provide evidence that the P-state contains a neutral dioxygen species and the F-state a superoxide molecule which would be converted to a peroxide upon reduction by another electron leading back to the O-state. The classical cycle has to be rewritten.

[1] F. Kolbe, Cryo-EM structures of intermediates suggest an alternative catalytic reaction cycle for cytochrome *c* oxidase, Nature Communications 12(6903) (2021), https://doi.org/10.1038/s41467-021-27174-y

O3

Structural mechanism of proton pumping by ba_3-type cytochrome c oxidase

Jonatan Johannesson, Doris Zorić, Adams Vallejos, Arpitha Kabbinale, Emil Sandelin, Carl Johan Wallentin, Richard Neutze and Gisela Brändén

Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

Cytochrome c oxidase (CcO) utilizes the energy released as oxygen is reduced to water to pump protons across a biological membrane[1]. Despite being heavily scrutinized, it is not understood how the chemical reaction drives proton pumping against the electrochemical gradient. Here we apply time-resolved serial femtosecond X-ray crystallography[2] to observe structural changes in the ba_3type CcO from Thermus thermophilus[3] upon exposure to oxygen. The reaction is triggered upon illumination of a caged oxygen compound by a UV laser flash. After 3.3 ms, water molecules are observed to order and disorder within the active-site, creating and disrupting water mediated pathways for proton transport through the enzyme. Based on these results and the principle of electroneutrality[4], we propose a step-by-step atomistic model for proton pumping by ba_3-type CcOs.

---

[1] Kaila, V. R., Verkhovsky, M. I. & Wikstrom, M. Proton-coupled electron transfer in cytochrome oxidase. Chem Rev 110, 7062-7081 (2010).

[2] Branden, G. & Neutze, R. Advances and challenges in time-resolved macromolecular crystallography. Science 373, eaba0954 (2021).

[3] Soulimane, T. et al. Structure and mechanism of the aberrant ba(3)-cytochrome c oxidase from thermus thermophilus. EMBO J 19, 1766-1776 (2000).

[4] Mitchell, R. & Rich, P. R. Proton uptake by cytochrome c oxidase on reduction and on ligand binding. Biochim Biophys Acta 1186, 19-26 (1994).

O4

Dissecting the conformational complexity and mechanism of cytochrome *bd* biogenesis

Haoran Fu[1,4], Elena Olkova[2], Ahmad R. Mehdipour[3], Bowen Dong[1], Wenjing Gao[3], Shengzhuo Ma[1], Huaina Jin[3], Yiwen Zhang[5],

Jiaqi Fu[6], Dirk Bald[7], Mark Shepherd[8], Quanshun Li[1,5], Di Wu[4,5,9], Hartmut Michel[4,5], Schara Safarian[4,5,9]

Affiliations

[1] *Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, School of Life Sciences, Jilin University, Changchun, Jilin, China*

[2]*Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt/Main, Germany*

[3]*Center for Molecular Modeling (CMM), Ghent University, Zwijnaarde, Belgium*

[4] *Department and Emeritus Group of Molecular Membrane Biology, Max Planck Institute of Biophysics, Frankfurt, Germany*

[5] *China-Singapore Joint Laboratory on Liver Disease Research, The First Hospital of Jilin University, Changchun, Jilin, China*

[6] *Center of Pathogen Biology and Infectious Diseases, The First Hospital of Jilin University; Changchun, Jilin, China*

[7]*Amsterdam Institute for Life and Environment, AIMMS, Faculty of Science, Vrije University of Amsterdam, Amsterdam, Netherlands*

[8]*School of Biosciences, RAPID Group, University of Kent, Canterbury, UK*

[9] *Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Frankfurt, Frankfurt, Germany*

Abstract:

Iron-bound cyclic tetrapyrroles (hemes) serve as essential redox-active cofactors in bioenergetic enzymes, yet the molecular mechanisms governing heme transport and insertion into respiratory chain complexes remain poorly understood. To address this gap, we employed an integrative approach combining cellular, biochemical, structural, and computational methods to characterize the structure and function of CydDC, a heterodimeric bacterial ABC transporter.

We present multi-level evidence establishing CydDC as a dedicated heme transporter essential for the functional maturation of cytochrome *bd* — a terminal oxidase of significant pharmaceutical relevance and an emerging drug target against *Mycobacterium tuberculosis* and other pathogens. Using a systematic single-particle cryogenic electron microscopy (cryo-EM) workflow coupled with atomistic molecular dynamics (MD) simulations, we mapped the conformational landscape of CydDC across discrete stages of substrate binding and occlusion with high resolution.

Our MD simulations reveal a previously uncharacterized substrate entry mechanism: heme accesses the transporter laterally from the membrane-water interface, inserting into the transmembrane region of CydDC. This lateral entry is enabled by a pronounced asymmetric inward-facing conformation of the transporter — an architectural feature critical for substrate accommodation. During the binding trajectory, the heme propionate groups engage in sequential electrostatic interactions, first with positively charged surface residues at the membrane interface, and subsequently with complementary residues deep within the substrate-binding pocket. Strikingly, this progressive engagement induces a 180° reorientation of the heme macrocycle, suggesting that proper positioning of the cofactor is actively coordinated by the transporter architecture rather than occurring stochastically.

Building on these findings, we subsequently focused on elucidating the biogenesis of the cytochrome *bd* CydA subunit, with particular emphasis on the heme insertion cascade facilitated by CydDC. Cytochrome *bd* coordinates three heme cofactor - b_{558}, b_{595}, and *d* - whose precise and sequential incorporation is a prerequisite for assembly of a catalytically competent complex. Our recent structural, computational, and experimental investigations reveal key molecular determinants governing the direct interaction interface between CydA and CydDC. We identify specific conformational adaptations in both proteins that enable a coordinated, stepwise heme insertion mechanism during cytochrome *bd* maturation.

Together, these findings establish a comprehensive mechanistic framework for heme trafficking and cofactor insertion in a medically important respiratory oxidase, with broad implications for understanding ABC transporter function and the development of novel antimicrobial strategies targeting the bacterial electron transport chain.

O5

Mitochondrial bioenergetics through the lens of evolution and disease Vamsi K. Mootha[a,b]

[a]*Howard Hughes Medical Institute*
[b]*Department of Systems Biology and Medicine, Harvard Medical School*

E-mail address: vamsi_mootha@hms.harvard.edu
Presenting author: Vamsi K. Mootha

In this talk, I will present our lab's latest efforts to utilize a systems-approach to investigate mitochondrial bioenergetics both in health and in disease. First, I will present our efforts to systematically characterize mitochondrial proteomes and their evolutionary diversity. Second, I will present our lab's efforts aimed at understanding the genetic underpinnings of human mitochondrial disease and how they are alleviated by low ambient oxygen.

O6

The effect of depleting respirasome formation in mice expressing COX7A2L

Nils-Göran Larsson

Department of Medical Biochemistry and Biophysics,
Karolinska Institutet, Stockholm, Sweden

E-mail address: nils-goran.larsson@ki.se
Presenting author: Nils-Göran Larsson

The respiratory chain enzyme complexes interact to form supercomplexes but the role of these structures has been much debated. There is accumulating evidence that that they do not facilitate electron shuttling and catalysis. We recently reported the creation and extensive characterization of C57BL/6N mice homozygous for the *Uqcrc1*^{DelEED} knock-in mutation that drastically decreases the formation of respirasomes by disrupting the interaction between Complex I and III (Milenkovic et al, Cell Metabolism 2023). Unexpectedly, these mice had normal respiratory chain function and normal physiology. We have now extended these studies by generating homozygous *Uqcrc1*^{DelEED} mice that also express COX7A2L (a.k.a. SCAF1) on the 129 genetic background. We have performed extensive bioenergetic and phenotypic characterization of these mice and will report the outcome of these experiments with regard to respiratory chain function, organ histology and physiological assessment.

O7

Folate and NAD cofactors at crossroads of metabolic regulation and disease

Saara Forsström, Liliya Euro, Anu Suomalainen

Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland.

Discoveries of mitochondrial disease mechanisms have increased the understanding of tissue-specific stress responses and systemic regulation of metabolism. Our data from mitochondrial disease model systems have uncovered important regulatory roles of mitochondria in tissue-specific cellular metabolic responses, especially those involving folate-driven one-carbon cycle and NADcofactors. Folate, vitamin B9, carries glucose-derived one-carbon units and drives cellular nucleotide, lipid and amino acid synthesis to support cellular growth and repair pathways. NAD-forms deriving from vitamin B3, regulate the direction of many of these growth pathways, their ratios having important roles in activation of fasting response. Here, we report our findings of these metabolic cofactors as contributors to metabolic stress responses to mitochondrial diseases and as targets of intervention.

O8

In mitochondrial disease respiratory complex III_2 assembles complex I via toxic intermediate

Maria G. Ayala-Hernandez[a], Anetzy Bermudez Torales[a], Hannah Camille Tan[a], Claire B. Montgomery[b], Abhilash Padavannil[a], Dominic T. Kurian[c], Andrew Maclean[d], Gino Cortopassi[b] and James A. Letts[a,e]

[a]Department of Molecular and Cellular Biology, University of California, Davis, United States

[b]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, United States [c]Institute for Immunology and Infection Research, University of Edinburgh, United Kingdom [d]Roslin Institute, University of Edinburgh, United Kingdom

[e]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia

E-mail address: james.letts@monash.edu (jaletts@ucdavis.edu)
Presenting author: James Letts

Mutations in mitochondrial complex I can cause severe metabolic disease [1–3] . Although no treatments are available for complex I deficiencies, chronic hypoxia improves lifespan and function in a mouse model of the severe mitochondrial disease Leigh syndrome caused by mutation of complex I subunit NDUFS4 [4–6]. To understand the molecular mechanism of NDUFS4 mutant pathophysiology and hypoxia rescue, we investigated the structure of complex I in respiratory supercomplexes isolated from NDUFS4 mutant mice. We identified complex I assembly intermediates bound to complex III_2, supporting the cooperative assembly model [7–9]. Further, an accumulated complex I intermediate is structurally consistent with pathological oxygen-dependent reverse electron transfer, revealing unanticipated pathophysiology and hypoxia rescue mechanisms. Thus, the build-up of toxic intermediates and not simply decreases in complex I levels underlie mitochondrial disease.

[1] F. Distelmaier, W.J.H. Koopman, L.P. van den Heuvel, R.J. Rodenburg, E. Mayatepek, P.H.G.M. Willems, J.A.M. Smeitink, Mitochondrial complex I deficiency: from organelle dysfunction to clinical disease, Brain, 132 (2008) 833–842.

[2] D.M. Kirby, M. Crawford, M.A. Cleary, H.H. Dahl, X. Dennett, D.R. Thorburn, Respiratory chain complex I deficiency: an underdiagnosed energy generation disorder, Neurology, 52 (1999) 1255–1264.

[3] Y.-Y. Ma, X.-Y. Li, Z.-Q. Li, J.-Q. Song, J. Hou, J.-H. Li, L. Sun, J. Jiang, Y.-L. Yang, Clinical, biochemical, and genetic analysis of the mitochondrial respiratory chain complex I deficiency, Medicine, 97 (2018) e11606.

[4] J.D. Meisel, M. Miranda, O.S. Skinner, P.P. Wiesenthal, S.M. Wellner, A.A. Jourdain, G. Ruvkun, V.K. Mootha, Hypoxia and intra-complex genetic suppressors rescue complex I mutants by a shared mechanism, Cell, 187 (2024) 659-675.e18.

[5] I.H. Jain, L. Zazzeron, R. Goli, K. Alexa, S. Schatzman-Bone, H. Dhillon, O. Goldberger, J. Peng, O. Shalem, N.E. Sanjana, F. Zhang, W. Goessling, W.M. Zapol, V.K. Mootha, Hypoxia as a therapy for mitochondrial disease, Science, 352 (2016) 54–61.

[6] M. Ferrari, I.H. Jain, O. Goldberger, E. Rezoagli, R. Thoonen, K.-H. Cheng, D.E. Sosnovik, M. ScherrerCrosbie, V.K. Mootha, W.M. Zapol, Hypoxia treatment reverses neurodegenerative disease in a mouse model of Leigh syndrome, Proc. Natl. Acad. Sci., 114 (2017) E4241–E4250.

[7] M. Protasoni, R. Pérez-Pérez, T. Lobo-Jarne, M.E. Harbour, S. Ding, A. Peñas, F. Diaz, C.T. Moraes, I.M. Fearnley, M. Zeviani, C. Ugalde, E. Fernández-Vizarra, Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV, The EMBO Journal, 265 (2020) 11409–19.

[8] M.A. Calvaruso, P. Willems, M. van den Brand, F. Valsecchi, S. Kruse, R. Palmiter, J. Smeitink, L. Nijtmans, Mitochondrial complex III stabilizes complex I in the absence of NDUFS4 to provide partial activity, Hum. Mol. Genet., 21 (2012) 115–120.

[9] E. Fernández-Vizarra, C. Ugalde, Cooperative assembly of the mitochondrial respiratory chain, Trends Biochem. Sci., 47 (2022) 999–1008.

O9

Exploring mitochondrial molecular architecture across eukaryotes *in situ* Mariia Melnik[a], Benjamin D. Engel[a], Florent Waltz[a]

a University of Basel, Biozentrum, Basel, Switzerland

E-mail address: florent.waltz@unibas.ch
Presenting author: Florent Waltz

In mitochondria, five membrane-bound complexes, collectively known as the respiratory chain, regenerate ATP from ADP by coordinating electron transfer with proton pumping. The native organization of these complexes into higher-order structures remains a subject of ongoing debate. Using focused ion beam (FIB) milling combined with cryo-electron tomography (cryo-ET), we investigate the native architecture and organization of mitochondrial complexes *in situ*. This approach provides unprecedented, high-resolution snapshots of the mitochondrial landscape in its near-native state.
We previously revealed the detailed spatial arrangement of respiratory complexes in the green alga *Chlamydomonas reinhardtii*, where ATP synthases and respiratory complexes segregate into curved and flat crista membrane domains, respectively. In this organism, respiratory complexes I, III, and IV assemble into a single type of supercomplex[1].
Now, we explore how these complexes are organized across diverse organisms and, using a mutant-based approach, investigate the potential roles of these supercomplexes in maintaining efficient mitochondrial function.

[1] Florent Waltz, Ricardo D. Righetto, Lorenz Lamm, Thalia Salinas-Giegé, Ron Kelley, Xianjun Zhang, Martin Obr, Sagar Khavnekar, Abhay Kotecha, Benjamin D. Engel. In-cell architecture of the mitochondrial respiratory chain. Science. 387 (6740), 1296-1301 (2025)

Topic list:
S2. Bioenergetic complexes & supercomplexes

Contribution type:
Talk – T

O10

Structural basis for coupling methyl-transfer to sodium pumping in the Mtr complex

Tristan Reif-Trauttmansdorff[1], Anuj Kumar[1], Tomas Pascoa[1], Eva Herdering[2], Stefan Bohn[3], Ruth A. Schmitz[2], Georg K. A. Hochberg[4,5], Jan M. Schuller[5*]

1 – Philipps-University Marburg, Department of Chemistry and Center for Synthetic Microbiology (SYNMIKRO), Karl-von-Frisch-Strasse 14, Marburg, Germany

2 – Institute for General Microbiology, Christian Albrechts University, Am Botanischen Garten 1-9, Kiel, Germany.

3 – Helmholtz Munich Cryo-Electron Microscopy Platform, Helmholtz Munich, Neuherberg, Germany.

4 – Philipps-University Marburg, Department of Biology and Center for Synthetic Microbiology

(SYNMIKRO), Karl-von-Frisch-Strasse 14, Marburg, Germany

5 - Microbes-for-Climate (M4C) Cluster of Excellence, Marburg, Germany.

* Corresponding author.

E-mail address: jan.schuller@synmikro.uni-marburg.de
Presenting author: Jan M. Schuller

Methanogenic archaea use a fundamentally unusual strategy for biological energy conservation. In these organisms, the membrane-bound N5-methyl-H4MPT:coenzyme M methyltransferase (Mtr) couples the transfer of a methyl group directly to Na+ pumping across the membrane. This reaction constitutes the sole energy-conserving step in most methanogenic pathways and represents one of the most distinctive mechanisms of chemiosmotic energy conversion in biology. In contrast to canonical bioenergetic systems such as Complex I and Rnf/Nqr, Mtr is driven by group-transfer chemistry rather than redox chemistry. Yet despite decades of biochemical work, the structural basis by which methyl-transfer chemistry is converted into a sodium motive force has remained unresolved.

Here, we present cryo-electron microscopy structures of the methanogenic Mtr complex captured under strictly anaerobic conditions in catalytically relevant states. These structures define the catalytic core of the enzyme and reveal how engagement of the corrinoid-containing MtrA subunit is coupled to coordinated rearrangements within the membrane-embedded MtrCDE trimer. Focused classification resolves distinct positional states of MtrA that likely correspond to functionally relevant intermediates along the catalytic cycle, thereby providing a structural framework for substrate delivery, methyl transfer and product release. Comparison of these states shows that catalysis is coupled to opening of a gated, hydrated ion-conduction pathway across the membrane. Together, these observations define how substrate engagement and methyl transfer are linked to activation of the ion-translocation pathway and provide a mechanistic framework for sodium pumping by the Mtr complex.

Our results place Mtr alongside Complex I and Rnf as a primary ion-translocating bioenergetic machine, but they also highlight a crucial difference. In Complex I and Rnf, local redox reactions at flavin, quinone or iron–sulfur cofactors are coupled to long-range conformational changes that drive ion transport. In Mtr, by contrast, the driving chemistry is not electron transfer but group transfer. Thus, Mtr reveals a fundamentally different solution to biological energy conservation: the direct conversion of methyl-transfer chemistry into a transmembrane sodium gradient. These findings establish a structural basis for methyl-transfer-driven sodium pumping and show that group-transfer reactions, no less than redox chemistry, can serve as primary drivers of chemiosmotic energy conversion.

O11

Rebuilding Power: Structural Strategies to Rescue OXPHOS Dysfunction

Cristina Ugalde[a,b,c]

[a] *Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid 28041, Spain* [b] *Centro de Investigaciones Biológicas 'Margarita Salas' (CIB-CSIC), Madrid 28040, Spain* [c] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), U723, Madrid, Spain.*

E-mail address: cristina.ugalde@cib.csic.es

Presenting author: Cristina Ugalde

Abstract text.

Mitochondrial oxidative phosphorylation (OXPHOS) depends not only on the assembly of individual respiratory complexes but also on their dynamic organization into higher-order structures that adapt energy conversion to metabolic changes.[1,2] How defects in complex assembly propagate to global metabolic and structural dysfunction, and whether these can be reversed, remains incompletely understood. Here, we identify a late-stage assembly checkpoint in mitochondrial complex I (CI) that links enzyme maturation to the formation of respiratory supercomplexes. We show that catalytically competent CI intermediates can accumulate prior to full assembly, indicating that acquisition of the N- module and enzymatic activity precedes completion of the complex. Instead, progression to a mature, supercomplex-competent state requires completion of a distal membrane-arm module that stabilizes key structural interfaces within the respiratory chain.

Disruption of this late assembly step leads to a coordinated remodeling of the respiratory chain, characterized by respirasome disassembly and redistribution of complexes III and IV into alternative configurations, accompanied by broader metabolic adaptation. Strikingly, we demonstrate that targeted structural modulation of complex IV is sufficient to overcome this defect, restoring CI maturation, re-establishing supercomplex organization, and rewiring mitochondrial function toward a more efficient energetic state.

Together, our findings uncover a fundamental principle of mitochondrial biology whereby late-stage CIV assembly governs both structural organization and metabolic output. This work highlights the intrinsic plasticity of the OXPHOS system and establishes structural reprogramming of the respiratory chain as a potential strategy to rescue mitochondrial dysfunction.

[1] Fernández-Vizarra, E., López-Calcerrada, S., Sierra-Magro, A., Pérez-Pérez, R., Formosa, L. E., Hock, D. H., Illescas, M., Peñas, A., Brischigliaro, M., Ding, S., Fearnley, I. M., Tzoulis, C., Pitceathly, R. D. S., Arenas, J., Martín, M. A., Stroud, D. A., Zeviani, M., Ryan, M. T., & Ugalde, C.
(2022). Two independent respiratory chains adapt OXPHOS performance to glycolytic switch. *Cell Metabolism*, 34(11), 1792-1808.e6. https://doi.org/10.1016/j.cmet.2022.09.005

[2] Liang C, Padavannil A, Zhang S, Beh S, Robinson DRL, Meisterknecht J, Cabrera-Orefice A, Koves TR, Watanabe C, Watanabe M, Illescas M, Lim R, Johnson JM, Ren S, Wu YJ, Kappei D, Ghelli AM, Funai K, Osaka H, Muoio D, Ugalde C, Wittig I, Stroud DA., Letts JA, Ho L. 2025.
Formation of I2+III2 supercomplex rescues respiratory chain defects. Cell Metabolism 37 - 2, pp.
441 - 459. 2025. https://doi.org/10.1016/j.cmet.2024.11.011

O12

Diversification of gear ratio of ATP synthase Hiroyuki Noji[a]

[a]*Graduate School of Engineering, University of Tokyo*

E-mail address: hnoji@g.ecc.u-tokyo.ac.jp
Presenting author: H. Noji

ATP synthase is a central enzyme in bioenergetics that synthesizes ATP by harnessing proton flow driven by the proton-motive force across biological membranes. The protonto-ATP ratio (H+/ATP) of ATP synthase—referred to here as the “gear ratio”—is one of the key parameters governing the stoichiometry of energetic coupling between redox potential (via the proton-motive force) and ATP synthesis.

We previously demonstrated that this ratio is tunable by a rational engineering approach that was not observed in nature at the time [1]. By increasing the number of peripheral stalks and the ion-conducting subunit ‘*a*’, we raised the H+/ATP ratio by approximately two- to threefold.

Notably, soon after our report, the Xu group identified a naturally occurring ATP synthase with a double-stalk architecture in an anoxygenic phototrophic bacterium [2], demonstrating that multi-stalk configurations also exist in natural systems. Motivated by this finding, we revisited sequence databases of ATP synthases with the aim of systematically identifying signatures of multi-stalk architectures.

In this presentation, we introduce newly identified multi-stalk ATP synthases and describe their structural basis and functional properties. Based on these findings, we further discuss the physiological roles of these systems, as well as possible evolutionary scenarios underlying the diversification of gear ratios in ATP synthase.

[1] H. Ueno, K. Yasuda, N. Hamaguchi-Suzuki, R. Marui, N. Adachi, T. Senda, T. Murata, H. Noji, Engineering of ATP synthase for enhancement of proton-to-ATP ratio. Nat. Commun. 16, (2025), 5410. https://doi.org/10.1038/s41467-025-61227-w

[2] X. Zhang, J. Wu, Z. Min, J. Wang, X. Hong, X. Pei, Z. Rao, X. Xu, Structure of ATP synthase from an early photosynthetic bacterium *Chloroflexus aurantiacus*. Proc. Natl. Acad. Sci. U.S.A. 122(13), (2025), e2425824122. https://doi.org/10.1073/pnas.2425824122

with the topic marking (see list below)

Topic list:
S6. ATP synthase and ATPases

Contribution type:
Poster – P and Talk – T (invited talk)

O13

How mitochondria assemble human ATP synthase

John E. Walker[a]

[a]*MRC Mitochondrial Biology Unit, University of Cambridge, UK*

E-mail address: jew30@cam.ac.uk
Presenting author: John E. Walker

Human ATP synthase is an assembly of 28 subunits of 17 kinds [1]. Mitochondrially encoded ATP6 and ATP8 are synthesized inside the organelle[2], whereas the 15 nuclear encoded subunits are imported. The lecture will describe how the ATP synthase is built from these 17 components, emanating from two distinct cellular sources, three of them assembled in multiple copies. We have established the following features. First, assembly factors are required to construct the oligomeric $\alpha_3\beta_3$- and the c_8-rings, whereas the introduction of single copy subunits does not [3, 4]. Most likely, the $\alpha_3\beta_3$-ring is built, around the γ-subunit built into a pre-assembled central stalk. Second, the F_1-domain and the rotor, consisting of the central stalk and the c_8-ring, can be assembled independently [3]. Third, subunit ATP-6, which provides the two proton half channels, and ATP8, are introduced into an almost complete enzyme complex [5]. Fourth, in the absence of subunit c, an F_1-peripheral stalk complex (subunits OSCP, F_6, b and d) plus membrane subunits e, f and g is built [6]. The two membrane α-helices of subunit b together with subunits e, f and g form a wedge that encapsulates specific phospholipids [7]. A striking feature of this assembly process is that it avoids building incomplete and uncoupled intermediate assemblies that could wastefully either hydrolyze ATP or dissipate the proton-motive force. Thus, all the characterized intermediates with an intact F_1-module and capable of ATP hydrolysis, but lacking proton half-channels,and incapable of ATP synthesis, are prevented from hydrolyzing ATP by IF_1, the unidirectional protein inhibitor of ATPase hydrolysis [8]. Thus, IF_1 is another assembly factor. Equally important is that the two proton half-channels are created at the end of the assembly process by inserting ATP6 (accompanied by ATP8) into the almost complete complex [5]. The addition of subunit j apparently locks ATP6 in place. A final step is the addition of the k-subunit involved in linking dimeric complexes, possibly with subunit g, into the rows of dimeric ATP synthases on the tips of the cristae. The assembly of human ATP synthase via the formation of the intermediate F_1-module, the c_8-ring and the rotor, and the PS-module, adds further support to the proposal that the ATP synthase arose by the independent evolution of its F_1 and F_o modules [8].

[1] J.E. Walker, The ATP synthase: the understood, the uncertain and the unknown. Biochem. Soc. Trans. 41, (2013),1-16. https://doi.org/10.1042/BST20110773

[2] I.M. Fearnley, J.E. Walker, Two overlapping genes in bovine mitochondrial DNA encode membrane components of ATP synthase EMBO J. 5, (1986) 2003-2008. https://doi.org/10.1002/j.14602075.1986.tb04456.x

[3] J. He, J. Carroll, S. Ding, I.M. Fearnley, J.E. Walker, Assembly of the catalytic module and the rotor of human ATP synthase EMBO J. (2026) submitted

[4] J. Carroll, J. He, S. Ding, J.E. Walker, TMEM70 and TMEM2542 help to assemble the rotor ring of human ATP synthase and interact with assembly factors for complex I Proc Natl Acad Sci 118, (2021) e2100558118 https://doi.org/10.1073/pnas.2100558118

[5] J. He, H.C. Ford, J. Carroll, S. Ding, I.M. Fearnley, J.E. Walker, Persistence of the permeability transition in the absence of subunit c of human ATP synthase Proc Natl Acad Sci 114, (2017) 3409-3414 https://doi.org/10.1073/pnas.1702357114

[6] J. He, H.C. Ford, J. Carroll, C. Douglas, E. Gonzales, S. Ding, I.M. Fearnley, J.E. Walker, Assembly of the membrane domain of ATP synthase in human mitochondria Proc Natl Acad Sci 115, (2018) 2988-2993 https://doi.org/10.1073/pnas.1722086115

[7] T.E. Spikes. M.G. Montgomery, J.E. Walker Structure of the dimeric ATP synthase from bovine mitochondria Proc Natl Acad Sci 115, (2020) 23519-23526 https://doi.org/10.1073/pnas.2013998117

[8] J.E. Walker, A. Cozens Evolution of ATP synthase Chemica Scripta 28B, (1986) 263-272

O14

High-resolution cryoEM of mitochondrial ATP synthase and complex I

Werner Kühlbrandt
Department of Structural Biology
Max Planck Institute of Biophysics
Frankfurt, Germany

Biological energy conversion in mitochondria is carried out by the large membrane protein complexes of the inner mitochondrial membrane. For several years, cryoEM has been the main tool for investigating the structure of mitochondrial ATP synthase and complex I at high resolution, as a prerequisite for understanding how they work. Single-particle cryoEM of the *Polytomella* ATP synthase dimer from the unicellular eukaryote *Polytomella sp* has yielded maps of the membranespanning rotor/stator complex at 2 to 2.2 Å resolution. 3D classification reveals three different rotary states, each with well-resolved water molecules forming wires for proton transfer from the matrix lumen to the protonation site at the interface of the *c*-ring rotor and stator. Ion pairs and hydrogen bonds between rotor and stator define three 9° to 14° substeps in the ten 36° steps of ring rotation that drives rotary catalysis. To complement our high-resolution single-particle studies, we use cryoET of FIB lamellae to determine the *in situ* structure of ATP synthase dimers and complex I in *Polytomella* mitochondria within intact cells. The current sub-tomogram average maps of ATP synthase and complex I *in situ* stand at 4.2 Å and 5.3 Å resolution. The maps indicate unexpected, functionally important differences between the complexes purified in detergent solution and in their native membrane environment.

O15

Complementary approaches for understanding water oxidation reaction in Photosystem II with serial X-ray crystalloraphy and X-ray spectroscopy

Asmit Bhowmick[a], Miao Zhang[a], Philipp Simon[a], Hiroki Makita[a], Erzsi Szilagyi[a], Natalie Minnetian[a], Aaron Brewster[a], Nicolas Sauter[a], Johannes Messinger[b], Vittal Yachandra[a], Jan Kern[a], Junko Yano[a]

[a] *Lawrence Berkeley National Laboratory, USA* [b] *Umea University, Sweden*

E-mail address: JYano@lbl.gov
Presenting author: Junko Yano

In metalloenzymes, metal centers are responsible for the rearrangement of electrons, protons, and atoms to carry out electron transfer and catalytic reactions. These reactions occur under ambient conditions in a highly controlled manner by utilizing the flexibility of the protein and water network. Enzymatic reactions and their kinetics are typically studied using spectroscopic methods in solution, while structural determination is achieved through X-ray crystallography, often employing cryo-cooled crystals. However, correlating structural information with spectroscopic data independently obtained from solution samples remains challenging. The advent of X-ray free-electron lasers (XFELs) and serial crystallography has enabled in situ determination of intermediate states, providing a tool for characterizing enzymatic reactions.[1] By combining structural studies with simultaneous on-line in situ spectroscopy, one can gain deeper understanding of enzyme catalysis and protein dynamics. Our goal is to understand the interplay between metal catalytic centers and their environment by following the dynamics of catalytic reactions. We combine X-ray crystallography and X-ray emission spectroscopy (XES) with in situ activation to follow sequential reaction steps under functional conditions, and have also used shot-by-shot X-ray absorption spectroscopy (XAS) for dilute metalloenzyme samples.[2]

Using this approach, we are investigating light-driven water oxidation in Photosystem II (PS II).[1,2] PS II carries out this reaction by coupling four-electron oxidation of water at the oxygen-evolving complex (OEC, the Mn_4CaO_5 cluster and surrounding water and amino acid residues) with one-electron photochemistry at the reaction center. It cycles through five intermediate S-states (S_0 to S_4), corresponding to the abstraction of four successive electrons. During this multielectron catalysis, spatially and temporally controlled transport of electrons, protons, and water substrate to and from the OEC is essential for maintaining catalytic efficiency and turnover. In our recent study, we improved crystallographic resolution to confirm stable intermediate structures. Anomalous diffraction data was used to accurately model Mn positions, providing a solid foundation for determining oxygen positions—particularly important for the S_3 state, the last stable intermediate before O–O bond formation. We confirmed the additional ligand ligated to the Mn1 position. Combining structural information and Mn XES data with kinetic modeling, we hypothesize the intermediate steps, associated time constants, and oxidation states for the S-state transitions, with particular emphasis on the $S_3 \rightarrow [S_4] \rightarrow S_0$ step. A two-electron reduced ($Mn(III)Mn(IV)_3$) peroxo-like intermediate state satisfactorily describes the transition from S_3 to S_0.

[1] J. F. Kern *et al*., Structures of the intermediates of Kok's photosynthetic water oxidation clock, Nature 563 (2018) 421–425, https://doi.org/10.1038/s41586-018-0681-2

[2] I. Bogacz *et al*., X-ray Absorption Spectroscopy of Dilute Metalloenzymes at X-ray Free-Electron Lasers in a Shot-by-Shot Mode, J. Phys. Chem. Lett. 16 (2025) 3778-3787, https://doi.org/10.1021/acs.jpclett.5c00399

[3] A. Bhowmick *et al*., Structural evidence for intermediates during O_2 formation in Photosystem II, Nature 617 (2023) 629–636, https://doi.org/10.1038/s41586-023-06038-z

O16

Mechanism of binding of quinone to the catalytic sites of cytochrome bc_1

Rafał Pietras[a], Anna Wójcik-Augustyn[a], Bohun Mielecki[a], Marcin Sarewicz[a], Marcin Jaciuk[b,c], Łukasz Koziej[b], Sebastian Glatt[b,d], Artur Osyczka[a]

[a] *Faculty of Biochemistry, Biophysics and Biotechnology, Department of Molecular Biophysics, Jagiellonian University, Kraków, Poland*

[b] *Małopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland*

[c] *National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland*

[d] *Department for Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria*

E-mail address: artur.osyczka@uj.edu.pl
Presenting author: Artur Osyczka

Cytochrome bc_1 ($cytbc_1$) is one of the key components of electron transport chains. The catalytic cycle of $cytbc_1$ involves joint operation of the quinone binding sites: the Q_o site (Q_o) which oxidizes ubiquinol and the Q_i site (Q_i) which reduces ubiquinone. Q_o, unlike Q_i, undergoes active-inactive transition associated with macroscopic motion of the domain containing Rieske cluster (ISP-HD) towards-away from cytochrome b subunit (cytb). Molecular details of quinone-protein interactions associated with this transition remain elusive. Here, we reflect on new insights provided by high-resolution CryoEM structures of bacterial $cytbc_1$ and quantum mechanical (QM) calculations[1]. We overview the structure of wild-type enzyme for which subclasses with different positions of ISP-HD were identified and the structure of the ISP-HD motion mutant in which the ISP-HD homogenously occupies position close to cytb (bposition). These structures disclosed different positions of quinone at Q_o depending on whether ISP-HD is at b-position (active Q_o state) or has moved away from that position (inactive Q_o site). The head group of quinone bound in active Q_o occupies position considered to be catalytically-relevant. The position of quinone when Q_o is inactive appears to reflect a prebound state. The structures also revealed specific conformational changes of quinone-binding pocket associated with the active-inactive Q_o transition. We discuss mechanistic consequences of these findings and the new quinone binding mode proposed for Q_o. We also describe details of the position of quinone at Q_i and positions of several water molecules in this region. The way quinone interacts with cytb at Q_i was found to differ from the way it interacts with the region of Q_o which implicated different binding modes for quinone for either of the sites. We discuss these two distinct quinone binding modes as specific strategies for efficient operation of sites differing in architecture, dynamics and catalytic mechanism.

[1] Rafał Pietras, Anna Wójcik-Augustyn, Bohun Mielecki, Marcin Sarewicz, Marcin Jaciuk, Łukasz Koziej, Sebastian Glatt, Artur Osyczka, Distinct ubiquinone binding at oxidation and reduction sites of cytochrome bc_1, *submitted.*

O16

Far-Red Photosystem II: Less Energy but New Light

Ho Fong Leong[a], Giovanni Consoli[a], Geoffry A. Davis[a], Ben Hancox-Lachman[a], Kenta Renard[a], Fiazall Tufail[a], Lauren E. Lee[a], Lucas Gautier[a], James W. Murray[a], Andrea Fantuzzi[a], A. William Rutherford[a]

a
Department of Life Sciences, Imperial College; London, SW7 2AZ, United Kingdom.

E-mail address: a.rutherford@imperial.ac.uk,
Presenting author: A. William Rutherford

Far-red light photoacclimation enables some cyanobacteria to survive in shaded environments by using far red/near-infrared light[1,2]. This represents an unexpected case where the energy demanding reactions of oxygenic photosynthesis are done but using less energy. In recent years our work has focused on how this has been achieved[2-5]. In far-red Photosystem II, modified protein subunits replace the usual ones, allowing the incorporation of four chlorophyll *f* molecules and one chlorophyll *d.* These pigments have Qy absorptions that are red-shifted and from under the spectrum of the chlorophyll *a* manifold and are also distinguishable from each other [2]. A comparative study of far-red Photosystem II from *Chroococcidiopsis thermalis* PCC 7203 and *Calothrix* sp. NIES-3974 using Cryo-EM, sequence comparisons and spectroscopy provided the following results. i) In *C. thermalis*, the structure includes a previously undetected, far-red-exclusive subunit, PsbH2', which forms part of a chlorophyll *f* binding site and could interact with the far-red allophycocyanin. ii) Chlorophyll d is present in the Chl_{D1} site and is assigned as the primary electron donor, and four chlorophyll *f* molecules are identified, one of which differs from previous reports[6]. iii) In *Calothrix, psbH2'* is absent, and only two of the chlorophyll f sites are present. iv) Comparative phylogenetic, structural, and spectroscopic analyses allow the assignment of specific wavelengths to all the red-shifted chlorophylls, an advance not possible in chlorophyll-a-only photosystems due to spectral overlap. These assignments provide an unprecedented basis to model and test excitation energy transfer in Photosystem II.

[1] F. Gan, S. Zhang, N.C. Rockwell, S.S. Martin, J.C. Lagarias, D.A. Bryant, Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. Science 345, 1312–1317 (2014). doi:
10.1126/science.1256963

[2] D.J. Nürnberg, J. Morton, S. Santabarbara, A. Telfer, P. Joliot, L. A. Antonaru, A.V Ruban, T. Cardona, E. Krausz, A. Boussac, A. Fantuzzi, A.W. Rutherford, Photochemistry beyond the red limit in chlorophyll f–containing photosystems. Science 360, 1210–1213 (2018).
doi:10.1126/science.aar8313

[3] G. Consoli, F. Tufail, H.F. Leong, S. Viola, G.A. Davis, N. Rew, D. Medranda, M. Hofer, P.
Simpson, M. Sandrin, B. Chachuat, J. Nelson, T. Renger, J.W. Murray, A. Fantuzzi, A.W.
Rutherford Locating the missing chlorophylls f in far-red photosystem I. Science. https://doi.org/10.1126/science.ado6830 (2025)

[4] G. Consoli, H.F. Leong, G.A. Davis, T. Richardson, A. McInnes, J.W. Murray, A. Fantuzzi & A.W. Rutherford Structure of a stripped-down and tuned-up far-red phycobilisome. Commun. Biol.
8, 907 (2025). https://doi.org/10.1038/s42003-025-08326-y

[5] S. Viola, W. Roseby, S. Santabarbara, D. Nürnberg, R. Assunção, H. Dau, J. Sellés, A. Boussac, A. Fantuzzi, A.W. Rutherford, Impact of energy limitations on function and resilience in long-wavelength Photosystem II. Elife 11, e79890 (2022). doi: 10.7554/eLife.79890

[6] C. J. Gisriel, G. Shen, D. A. Flesher, V. Kurashov, J. H. Golbeck, G.W. Brudvig, M. Amin, D.A. Bryant, Structure of a dimeric photosystem II complex from a cyanobacterium acclimated to far-red light. Journal of Biological Chemistry 299, 102815 (2023). doi:10.1016/j.jbc.2022.102815

O17

Far-Red Photosystem II: Less Energy but New Light

Ho Fong Leonga, Giovanni Consolia, Geoffry A. Davisa, Ben Hancox-Lachmana, Kenta Renarda , Fiazall Tufailaa, Lauren E. Leea, Lucas Gautiera, James W. Murraya, Andrea Fantuzzia, A. William Rutherforda aDepartment of Life Sciences, Imperial College; London, SW7 2AZ, United Kingdom.

E-mail address: a.rutherford@imperial.ac.uk,

Presenting author: A. William Rutherford

Far-red light photoacclimation enables some cyanobacteria to survive in shaded environments by using far red/near-infrared light1,2 . This represents an unexpected case where the energy demanding reactions of oxygenic photosynthesis are done but using less energy. In recent years our work has focused on how this has been achieved2-5 . In far-red Photosystem II, modified protein subunits replace the usual ones, allowing the incorporation of four chlorophyll f molecules and one chlorophyll d. These pigments have Qy absorptions that are red-shifted and from under the spectrum of the chlorophyll a manifold and are also distinguishable from each other 2 . A comparative study of far-red Photosystem II from Chroococcidiopsis thermalis PCC 7203 and Calothrix sp. NIES-3974 using Cryo-EM, sequence comparisons and spectroscopy provided the following results. i) In C. thermalis, the structure includes a previously undetected, far-red-exclusive subunit, PsbH2', which forms part of a chlorophyll f binding site and could interact with the far-red allophycocyanin. ii) Chlorophyll d is present in the ChlD1 site and is assigned as the primary electron donor, and four chlorophyll f molecules are identified, one of which differs from previous reports6 . iii) In Calothrix, psbH2' is absent, and only two of the chlorophyll f sites are present. iv) Comparative phylogenetic, structural, and spectroscopic analyses allow the assignment of specific wavelengths to all the red-shifted chlorophylls, an advance not possible in chlorophyll-a-only photosystems due to spectral overlap. These assignments provide an unprecedented basis to model and test excitation energy transfer in Photosystem II.

[1] F. Gan, S. Zhang, N.C. Rockwell, S.S. Martin, J.C. Lagarias, D.A. Bryant, Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. Science 345, 1312–1317 (2014). doi: 10.1126/science.1256963

[2] D.J. Nürnberg, J. Morton, S. Santabarbara, A. Telfer, P. Joliot, L. A. Antonaru, A.V Ruban, T. Cardona, E. Krausz, A. Boussac, A. Fantuzzi, A.W. Rutherford, Photochemistry beyond the red limit in chlorophyll f–containing photosystems. Science 360, 1210–1213 (2018). doi:10.1126/science.aar8313

[3] G. Consoli, F. Tufail, H.F. Leong, S. Viola, G.A. Davis, N. Rew, D. Medranda, M. Hofer, P. Simpson, M. Sandrin, B. Chachuat, J. Nelson, T. Renger, J.W. Murray, A. Fantuzzi, A.W. Rutherford Locating the missing chlorophylls f in far-red photosystem I. Science. https://doi.org/10.1126/science.ado6830 (2025)

[4] G. Consoli, H.F. Leong, G.A. Davis, T. Richardson, A. McInnes, J.W. Murray, A. Fantuzzi & A.W. Rutherford Structure of a stripped-down and tuned-up far-red phycobilisome. Commun. Biol. 8, 907 (2025). https://doi.org/10.1038/s42003-025-08326-y

[5] S. Viola, W. Roseby, S. Santabarbara, D. Nürnberg, R. Assunção, H. Dau, J. Sellés, A. Boussac, A. Fantuzzi, A.W. Rutherford, Impact of energy limitations on function and resilience in long-wavelength Photosystem II. Elife 11, e79890 (2022). doi: 10.7554/eLife.79890

[6] C. J. Gisriel, G. Shen, D. A. Flesher, V. Kurashov, J. H. Golbeck, G.W. Brudvig, M. Amin, D.A. Bryant, Structure of a dimeric photosystem II complex from a cyanobacterium acclimated to far-red light. Journal of Biological Chemistry 299, 102815 (2023). doi:10.1016/j.jbc.2022.102815

O18

Describing the Coupling of Charge Transfer and Chemical Reactions in Proteins: A Microstate Model Large Enzyme Complexes

G. Matthias Ullmann[a] [a]*Computational Biochemistry, University of Bayreuth, 95440 Bayreuth, Germany*

E-mail address: ullmann@uni-bayreuth.de
Presenting author: G. Matthias Ullmann

Charge transfer within biological macromolecules plays a crucial role in many fundamental biological processes, including photosynthesis and respiration. In these systems, protons or electrons are exchanged between titratable residues or redox-active cofactors, and these transfers are often strongly coupled. Computational approaches based on continuum electrostatics have become standard tools in theoretical biochemistry for studying the function of even highly complex biomolecular systems. Such methods make it possible to account for environmental factors like pH, redox potential, and membrane potentials. When combined with statistical thermodynamics, they enable the calculation of equilibrium properties, for example, the probabilities of specific protonation or oxidation states.[1] Beyond equilibrium thermodynamics, these electrostatic calculations can also inform reaction kinetics. Because the charge transfer rate between two sites depends on the instantaneous charge configuration of surrounding sites, it is essential to model the system at the level of discrete microstates. In this microstate-based framework, each transition between microstates is assigned a unique rate constant, and interactions between all sites involved in charge transfer arise naturally from the formalism. This methodology is applied to study electron transfer kinetics in the tetraheme subunit and the special pair of the reaction center of *Blastochloris viridis* and the F_{420}-Reducing [NiFe]-Hydrogenase Complex, which contains several iron-sulfur clustes. The results demonstrate that continuum electrostatic calculations, in combination with an established rate law, can be used to derive electron transfer rate constants. Using the microstate approach, the relaxation kinetics following photo-oxidation of the special pair are simulated and shown to agree well with experimental observations. A flux analysis further reveals the individual steps of the electron transfer pathway. Overall, this microstate-based kinetic simulation represents an important step toward bridging structural biology and systems biology by enabling the analysis of complex biomolecular kinetics directly from structural data.

[1] Ullmann, G.M. and Roy, R. R. (2025). The Multisite Microstate Model: A Theoretical Approach for Analyzing Charge Transfer in Proteins. In Iron-Sulfur Clusters (eds S. Leimkühler, G. Schwarz, O. Lenz and O. Einsle). DOI: 10.1002/9783527843596.ch18

O19

Photosynthesis in the far-red: mechanisms and challenges Roberta Croce[a]

[a]*Department of Physics and Astronomy, Faculty of Science, VU University Amsterdam, the Netherlands*

E-mail address: r.croce@vu.nl
Presenting author: Roberta Croce

Photosynthesis in oxygenic organisms typically operates within the photosynthetically active radiation (PAR) range of 400–700 nm, where chlorophyll a absorbs most efficiently. However, some cyanobacteria have evolved the ability to perform photosynthesis beyond this range, using far-red light. Studying these systems provides valuable insight into how the photosynthetic machinery can adapt to capture and utilize lower-energy photons. The main challenges in far-red photosynthesis lie in both the efficient harvesting of long-wavelength photons and their effective use in photochemical reactions, given their reduced energy. To overcome these limitations, photosynthetic organisms employ a variety of strategies: they introduce new far-red–absorbing pigments and tune the absorption properties of existing pigments through their protein environment. These adjustments are accompanied by structural adaptations in antenna complexes and photosystems that help maintain high photochemical efficiency.
In this presentation, I will discuss the natural strategies that support far-red photosynthesis and how these solutions may guide efforts to engineer similar capabilities into plants. This work opens promising avenues for expanding the spectral range of photosynthesis and improving light-use efficiency in future crop systems.

O20

ATP Synthase Redox Regulation Couples Dark Bioenergetics to Senescence in Arabidopsis

Lando Lebok[a], Jürgen Eirich[b], Ana Carolina Vilchez[c], Iris Finkemeier[b], Till Ischebeck[c], Felix Buchert[a]

a
Plant Biochemistry and Biotechnology, Institute of Plant Biology and Biotechnology, University of Münster, Schlossplatz 8, 48143 Münster, Germany.
b Plant Physiology, Institute of Plant Biology and Biotechnology, University of Münster,
Schlossplatz 7, 48149 Münster, Germany
c
Green Biotechnology, Institute of Plant Biology and Biotechnology, University of Münster, Schlossplatz 8, 48143 Münster, Germany

E-mail address: f.buchert@uni-muenster.de
Presenting author: Felix Buchert

The chloroplast ATP synthase (CF_OF_1) balances the free energy stored in the proton motive force (pmf) with the phosphorylation potential [ATP]/([ADP][Pi]). In vascular plants, this balance is adjusted via thiol modulation of the γ-subunit Cys199/Cys205 pair, lowering the pmf threshold to drive ATP synthesis/hydrolysis in the reduced (dithiol) state [1]. Oxidation to a disulfide in the dark suppresses ATP hydrolysis and thereby conserves ATP at low pmf [2]. However, the physiological relevance of this switch remains unclear, as Cys199/Cys205 mutants in Arabidopsis thaliana are viable [3]. Here, we combine optical spectroscopy – covering chlorophyll fluorescence kinetics, ECS-based pmf quantification, and cytochrome b_6f redox measurements – with targeted mutants of the leaf-type γ-subunit in A. thaliana to revisit the role of CF_OF_1 thiol modulation in terrestrial photosynthesis. We confirm that thiol-based regulation is dispensable for plant survival and demonstrate that introduction of an algal γ-hairpin motif [4] partially overrides disulfide-dependent inactivation of CF_OF_1. By quantitatively linking the dark-induced senescence readout F_v/F_m to the dark pmf amplitude, we identify the γ-Cys199Ser mutant as a key line for integrated physiological and multi-omics analyses. Under extended darkness, wild-type plants exhibit impaired photosystem II activity and reduced electron transfer upon reillumination, whereas γ-Cys199Ser remains functionally active. This phenotype correlates with attenuated dark-induced senescence responses, including increased abundance of proteins associated with photosynthesis and metabolites linked to suppression of cell death. We propose that failure to downregulate ATP hydrolysis in the dark alters chloroplast energetic status, thereby interfering with the coordination of senescence and nutrient remobilization programs. Sustained dark ATPase activity preserves photosynthetic competence upon return to light and is associated with broader metabolic reprogramming across cellular compartments. Consistent with findings from root-type γ-subunit mutants [5], these results establish γ-subunit thiol modulation of CF_OF_1 as a central regulator linking chloroplast bioenergetics with senescence and stress-responsive pathways.

[1] U. Junesch, P. Gräber, Influence of the redox state and the activation of the chloroplast ATP synthase on proton-transport-coupled ATP synthesis hydrolysis, Biochim Biophys Acta, 893 (1987) 275-288. https://doi.org/10.1016/0005-2728(87)90049-1

[2] D.R. Ort, K. Oxborough, In situ regulation of chloroplast coupling factor activity, Annu Rev Plant Physiol Plant Mol Biol, 43 (1992) 269-291. https://doi.org/10.1146/annurev.pp.43.060192.001413

[3] G. Wu, D.R. Ort, Mutation in the cysteine bridge domain of the g subunit affects light regulation of the ATP synthase but not photosynthesis or growth in Arabidopsis, Photosynthesis Research, 97 (2008) 185-193. https://doi.org/10.1007/s11120-008-9315-0 [4] L. Lebok, F. Buchert, The chloroplast ATP synthase redox domain in Chlamydomonas reinhardtii eludes activity regulation for heterotrophic dark metabolism, Proc Natl Acad Sci U S A, 121 (2024) e2412589121. https://doi.org/10.1073/pnas.2412589121

[5] K. Kohzuma, J.E. Froehlich, G.A. Davis, J.A. Temple, D. Minhas, A. Dhingra, J.A. Cruz, D.M. Kramer, The Role of Light-Dark Regulation of the Chloroplast ATP Synthase, Frontiers in Plant Science, 8 (2017) 1248. https://doi.org/10.3389/fpls.2017.01248

O21

Title

Environmental signal integration in Chlamydomonas reinhardtii

Authors

1Dimitris Petroutsos

Affiliations

Department of Organismal Biology, Uppsala University, Uppsala, Sweden

Keywords

Photoprotection, light perception, signal transduction, photoreceptors, transcription factors

Abstract

In photosynthetic organisms, light functions both as an energy source driving the conversion of CO_2 into carbohydrates used for growth and storage, and as an environmental signal regulating development and cellular physiology. Excessive light exposure, however, can generate oxidative stress and ultimately lead to cell death. To counteract these harmful effects, photosynthetic organisms employ energy-dependent quenching (qE), a photoprotective mechanism that dissipates excess excitation energy as heat. In Chlamydomonas reinhardtii, qE mainly depends on the effector protein LHCSR3 and, to a lesser extent, LHCSR1. Central to the regulation of both qE and carbohydrate metabolism is the plasma membraneassociated blue-light photoreceptor PHOTOTROPIN (PHOT), which controls the accumulation of LHCSR transcripts as well as starch, the major storage carbohydrate in Chlamydomonas. By combining phosphoproteomics, co-immunoprecipitation, genome-wide RNA sequencing (RNA-seq) followed by gene regulatory network (GRN) analysis, we identified proteins interacting with PHOT as well as with transcription factors that collectively regulate qE and starch metabolism in Chlamydomonas. In this talk, I will present results on two kinases, PHOTOTROPIN-MEDIATED SIGNALING KINASE 1 and 2 (PMSK1 and PMSK2), which mediate the regulation of qE and starch accumulation, as well as a PP2C phosphatase and a leucine-rich repeat protein that appear to be components of this signaling network. I will further discuss transcription factors involved in these processes and integrate these findings into the current understanding of photoreceptor-mediated signaling and metabolic regulation in microalgae.

O22

Deciphering thermogenic functionality of UCP1 by evolutionary reconstruction Martin Jastroch[a], Paul G. Crichton[b], Susanne Keipert[a], Michael J. Gaudry[a]

[a]*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden*

[b]*Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, UK*

E-mail address: martin.jastroch@su.se
Presenting author: Martin Jastroch

Non-shivering thermogenesis (NST) is one of the most fascinating evolutionary achievements, enabling high body temperatures and successful radiation of endothermic vertebrates into cold environments[1]. In many mammals the mechanism of adaptive NST relies on the high abundance of mitochondria and its uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) [2]. UCP1 dissipates the proton motive force and uncouples mitochondrial respiration from ATP homeostasis, thereby accelerating oxidative processes to increase heat output. UCP1 is activated by free fatty acids and potently inhibited by cytosolic nucleotides. Yet, the regulatory and molecular mechanisms of UCP1 function have not been fully resolved[3]. In humans, dissipating excessive nutrient energy via mitochondrial recruitment and UCP1 activation in adipose tissue represents an attractive route to reduce obesity and its comorbidities. To unravel pathways governing UCP1 function that could be instrumental in therapeutically targeting human adipose tissue, we use unique animal models to receive evolutionary clues on how UCP1-dependent thermogenesis was wired into adipose tissue. Functional comparison of UCP1 variants, which have been tuned during the course of vertebrate evolution, enable us to identify the origin of UCP1-dependent thermogenesis, its physiological significance, and opens a new window into the understanding of structure-function relationships. Specifically, our view on BAT evolution has been transformed recently, highlighting that UCP1-dependent thermogenesis emerged late during mammalian evolution in the stem placental ancestor [4]. The adaptation of UCP1 function during species diversification of placentals allowed to scrutinize previous claims on important functional motifs, such as the histidine pair motif [5]. The repeated loss of UCP1 functionality in various placental species uncovers thermoregulatory selection pressures[6,7,8] and uncovers new structure-function relationships. Reconstructing the evolution of UCP1 expands our tool box to unravel unknown elements of UCP1 regulation and will help to pinpoint the molecular mechanism of UCP1 proton transport.

[1] R. Oelkrug, E.T. Polymeropoulos, M. Jastroch, Brown adipose tissue: physiological function and evolutionary significance. J. Comp.Physiol. B 185, (2015), 587-606. doi: 10.1007/ s00360-015-0907-7.

[2] D.G. Nicholls, Fifty years on: How we uncovered the unique bioenergetics of brown adipose tissue. Acta Physiol (Oxf). 237, (2023), e13938. doi: 10.1111/apha.13938.

[3] R. Cavalieri, M.A.S. Dela Rosa, C.A. Cotrim, D. Copeman, M. Aris, C. Eke, H. Staggs-Sandy, P.G. Crichton, A structural context for the mechanisms of Uncoupling Protein 1 in brown fat thermogenesis. Acta Physiol (Oxf). In press.

[4] S. Keipert, M.J. Gaudry, M. Kutschke, M. Keuper, M.A.S. Dela Rosa, Y. Cheng, J.M. Monroy
Kuhn, R. Laterveer, C.A. Cotrim, P. Giere, F. Perocchi, R. Feederle, P.G. Crichton, D. Lutter, M. Jastroch, Two-stage evolution of mammalian adipose tissue thermogenesis. Science 384, (2024), 1111-1117. doi: 10.1126/science.adg1947.

[5] M.J. Gaudry, A. Bundgaard, M. Kutschke, K. Ostatek, M.A.S. Dela Rosa, P.G. Crichton, J.
Reznick, M. Jastroch, Natural Mutation in Naked Mole-Rat UCP1 Refutes Importance of the Histidine Pair Motif for Proton Conductance and Thermogenesis. Acta Physiol (Oxf) 241:e70109, (2025). doi: 10.1111/apha.70109.

[6] M.J. Gaudry, M. Jastroch, J.R. Treberg, M. Hofreiter, J.L.A. Paijmans, J. Starrett, N. Wales, A.V. Signore, M.S. Springer, K.L. Campbell, Inactivation of thermogenic UCP1 as a historical contingency in multiple placental mammal clades. Sci Adv. 3(7):e1602878, (2017). doi: 10.1126/ sciadv.1602878.

[7] M.J. Gaudry, T.J. Fyda, M. Jastroch, Evolution of pinniped UCP1 is not linked to aquatic life but to neonatal thermogenesis and body size. Proc Natl Acad Sci U S A. 119:e2118431119, (2022). doi: 10.1073/pnas.2118431119.

[8] M.J. Gaudry, J. Khudyakov, L. Pirard, C. Debier, D. Crocker, P.G. Crichton, M. Jastroch, Terrestrial Birth and Body Size Tune UCP1 Functionality in Seals. Mol Biol Evol. 41:msae075, (2024). doi: 10.1093/molbev/msae075.
Pia Ädelroth, Terezia Kovalova, Mateusz Janczak, Ana Gamiz-Hernandez, Daniel Lundin, Soni Sharma, Parul Singh, Jóhanna Vilhjálmsdóttir, Dan Sjöstrand, Ville Kaila, Martin Högbom.
Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.

O23

E-mail address: piaa@dbb.su.se Presenting author: Pia Ädelroth

The two cytochrome *bd*s in the respiratory chain of *Mycobacterium smegmatis*

Mycobacterium smegmatis is a gram-positive Actinobacterium and a close relative of the important pathogen *M. tuberculosis*. The *M. smegmatis* respiratory chain is branched at the level of the menaquinol pool, with the final oxygen reduction step being carried out either by the *bcc-aa*$_3$ supercomplex [1, 2] or the cyt. *bd* quinol oxidase [3]. The *bd*-type oxidases are strictly prokaryotic proteins with high O_2 affinity, and the protein expressed from the *M. smegmatis* (*Ms*) *cydAB* genes (coding for the *bd*-I) is highly homologous to the *M. tubercolosis* cyt *bd*. Interestingly, the *M. smegmatis* genome contains an additional (*appBC*) gene cluster, coding for a putative second *bd* protein (*bd*-II). We have expressed and purified this *bd*-II from its native host and determined its structure in LMNG by cryoEM to 2.8 Å resolution [4]. The *M. smegmatis bd*-II belongs to a previously uncharacterized evolutionary group (qOR-2 [5]), and has low sequence identity to the *bd*-I. We show that the *Ms bd*-II contains the same heme components as the *bd*-I; hemes b^{558}, b^{595} and a heme *d,* but that unlike the *Ms bd-I,* the heme *d* active site is ligated by a Glu in the oxidised state. Using multiscale simulations we propose that coordination changes of this Glu and a Phe nearby regulate substrate access into the active site. The *Ms bd*-II is more promiscous in its substrate preferences and ligand binding properties than the *Ms bd*-I. The possible advantages of these differences for the bacterium, and the evolutionary differences in the *bd* superfamily will be discussed.

[1] B. Wiseman, R. G. Nitharwal *et al.*, Structure of a functional obligate complex III2IV2 respiratory supercomplex from Mycobacterium smegmatis. Nat. Struct. Mol. Biol. 25, (2018), 1128-1136. http://doi.org/10.1038/s41594-018-0160-3

[2] H. Gong, J. Li *et al.*, An electron transfer path connects subunits of a mycobacterial respiratory supercomplex. Science 362, (2018). http://doi.org/10.1126/science.aat8923

[3] W. Wang, Y. Gao *et al.*, Cryo-EM structure of mycobacterial cytochrome bd reveals two oxygen access channels. Nat. Commun. 12, (2021), 4621. http://doi.org/10.1038/s41467-021-24924-w

[4] T. Kovalova, M. Janczak *et al.*, The Mycobacterium smegmatis bd-II terminal oxidase employs a carboxylate shift mechanism. Proc. Natl. Acad. Sci. USA. 123, (2026), e2515348123. http://doi.org/doi:10.1073/pnas.2515348123

[5] R. Murali, R. B. Gennis, J. Hemp, Evolution of the cytochrome bd oxygen reductase superfamily and the function of CydAA' in Archaea. The ISME journal 15, (2021), 3534-3548. http://doi.org/10.1038/s41396021-01019-4

O24

Allosteric activation of DNA polymerase gamma as a therapeutic strategy in POLG-related mitochondrial disease

Maria Falkenberg[a]

[a]University of Gothenburg, Institute of Biomedicine E-mail address: maria.falkenberg@medkem.gu.se Presenting author: Maria Falkenberg

Mitochondrial DNA (mtDNA) replication is carried out by DNA polymerase gamma (POLγ), a heterotrimer consisting of the catalytic POLγA subunit and two accessory POLγB subunits. Mutations in POLG, encoding POLγA, are among the most common causes of inherited mitochondrial disease and currently lack effective treatment options. Restoring POLγ function therefore represents an attractive therapeutic strategy.

In collaboration with Pretzel Therapeutics, we have identified PZL-A, a small-molecule activator that restores enzymatic activity in four common disease-associated POLG variants. We now extend this work by analysing approximately 50 additional pathogenic POLG mutations to assess the broader therapeutic potential of allosteric activation. We also present structural and biochemical data that define the mechanism underlying POLγ activation by PZL-A. Using single-particle cryo-EM in combination with enzymatic assays, we map the binding site of the compound to the POLγA– POLγB interface and show that this interaction stabilizes an active conformation of the holoenzyme. The binding site is located at a structurally conserved region of the holoenzyme, providing a mechanistic basis for the broad mutational rescue observed.

Together, these findings strengthen the rationale for therapeutic activation of POLγ and broaden the range of genotypes that may benefit from treatment with PZL-A.

O25

"High-energy" metastable (O_H) and "as isolated" (O) states of the oxidized bovine cytochrome *c* oxidase

Daniel Jancura[a], Adriana Tomkova[a], Tereza Sztachova[a], Vladimir Berka[b], Jonatan Johannesson[c], Marian Fabian[d]

[a] *Department of Biophysics, Faculty of Science, University of P. J. Safarik, Kosice, Slovakia* [b] *Department of Internal Medicine, University of Texas Health Science Center, Houston, Texas, USA*
[c]*Faculty of Science, University of P. J. Safarik, Kosice, Slovakia* [d]*Center for Interdisciplinary Biosciences, Technology and Innovation Park, University of P.J. Safarik, Kosice, Slovakia*

E-mail address: daniel.jancura@upjs.sk
Presenting author: Daniel Jancura

Mitochondrial cytochrome c oxidase (CcO) catalyzes the electron transfer (ET) from ferrocytochrome *c* to O_2 leading to water formation and contributes to the formation of electrochemical proton gradient on inner mitochondrial membrane. The current models of the proton pumping in CcO rely on the existence of a "high-energy" metastable O_H state, which is formed immediately after oxidation of the reduced CcO with oxygen. A part of the energy released in the redox reactions of the CcO catalytic cycle is stored in this O_H state. The O_H is supposed to relax to the stable non-pumping "as isolated" oxidized state (O). It was proposed that the catalytic heme a_3-Cu_B center of these two forms should differ in protonation and ligation states and the transition of O_H-to-O was suggested to be associated with a proton transfer into this center. We have investigated spectroscopic and thermodynamic properties of both O and O_H states as well kinetics of ET to the catalytic sites of these states. It was found that there are no observable differences between O and O_H states in the UV-Vis absorption and EPR spectra, and the rates of the heme a_3 and Cu_B reduction are very similar in both states [1]. It was shown that there is no uptake of a proton from external medium during the supposed relaxation of O_H. Moreover, the interactions of O and O_H with H_2O_2, resulting in the formation of the P and F states, were similar in the kinetics and the amounts of the formed ferryl states [2]. These results suggest that there is no difference in the protonation and ligation states of the catalytic sites of O and O_H. Finally, we have, using ITC measurements, determined that the energy released by ET from cytochrome *a* to the catalytic center during F-to-O transition is ~1 eV [3]. If the F-to-O_H transition is associated with the translocation of two charges across the membrane, against a membrane potential of 200 mV, the residual energy accumulated in the O_H state would be 0.6 eV. If we accept the fact that the redox potentials of heme a_3 and Cu_B in O and O_H forms are similar, the energy for proton pumping might be stored in a non-equilibrium structure of oxidized cytochrome *a* produced immediately after ET to the catalytic center of the F state. This assignment is also implicated by the substantial entropic change that accompanies the F-to-O transition.

[1] D. Jancura, V. Berka, M. Antalik, J. Bagelova, R.B. Gennis, G. Palmer, M. Fabian, Spectral and kinetic equivalence of oxidized cytochrome c oxidase as isolated and „activated" by reoxidation. J. Biol. Chem. 281, (2006), 30319-30325, https://doi.org/10.1074/jbc.M605955200

[2] D. Jancura, A. Tomkova, T. Sztachova, V. Berka, M. Fabian, Examination of „high-energy" metastable state of the oxidized (O_H) bovine cytochrome c oxidase: proton uptake and reaction with H_2O_2. Archives Biochem. Biophys. 747 (2023), art. numb. 109758, https://doi.org/ 10.1016/j.abb.2023.109758

[3] A. Tomkova, T. Sztachova, J. Johannesson, D. Jancura, M. Fabian, Thermodynamics of the transition from the ferryl (F) state to the oxidized form of the solubilized cytochrome c oxidase: implication for the proton pumping. Archives Biochem. Biophys. 774 (2025), art. numb. 110638, https://doi.org/10.1016/j.abb.2025.110638

O26

A crucial role for liver mitochondria in thermogenesis in complex III-deficient mice

Rishi Banerjee[a,b], Divya Upadhyay[a,b], Tomáš Zarybnický[b], Satu Kuure[b,c], Vilma Wanne[a,b], Vineta Fellman[a,b,d,e], Janne Purhonen[a,b,f], Christa Kietz[a,b], and Jukka Kallijärvi[a,b]

a Folkhälsan Research Center, Helsinki, Finland
b Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Finland
c GM-unit, Laboratory Animal Center, Helsinki Institute of Life Science, University of Helsinki, Finland
d Children's Hospital, University of Helsinki, Finland
e Department of Clinical Sciences, Lund, Pediatrics, Lund University, Sweden
f Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden

E-mail address: jukka.kallijarvi@helsinki.fi
Presenting author: Jukka Kallijärvi

Both basal and adaptive thermogenesis in homeothermic animals rely heavily on mitochondria. Small animals, such as mice, have high metabolic rates and surface area-to-volume ratios, and require intense thermogenesis to maintain euthermia as compared to humans. Hypothermia has been reported in some mouse models of mitochondrial disease, but the underlying physiological mechanisms remain largely unexplored. *Bcs1l*$^{p.S78G}$ knock-in mice, modelling human neonatal complex III (CIII)-deficiency, develop chronic hypothermia after weaning and provide an excellent experimental model for such studies. We restored complex III function in juvenile *Bcs1l*$^{p.S78G}$ mice using recombinant adeno-associated viral vectors (rAAVs) expressing wild-type *Bcs1l* under a broad, liver- or muscle-specific promoter. We assessed how CIII deficiency modulates basal and adaptive thermogenesis in the targeted tissues and in the brown adipose tissue (BAT). The *Bcs1l*$^{p.S78G}$ mice developed hypothermia after P25, correlating with progressing decline in CIII activity, and the main adaptive thermogenic organs skeletal muscle and BAT failed to compensate for it. Disrupted foot pad innervation suggested sensory neuropathy and impaired thermosensation as a contributor to the thermoregulatory failure. The mutant BAT showed inflammation, macrophage infiltration, repressed fatty acid oxidation, and failure to induce Uncoupling protein 1 (UCP1) and adrenergic signaling basally and upon acute cold challenge. Surprisingly, hepatocyte-specific restoration of CIII allowed maintenance of near-normal body temperature at normal housing temperature (23ºC), whereas skeletal muscle targeting was required for acute cold tolerance. Transgenic alternative oxidase (AOX), which dissipates respiratory energy directly as heat, prevented hypothermia both at 23ºC and in acute cold (10ºC, 7 h). Housing the mutant mice at as high as 35ºC was required to force normal body temperature, and this relieved their hepatic metabolic stress and senescence. We conclude that mitochondrial respiration in hepatocytes is essential for thermal homeostasis in juvenile mice. Our findings highlight hypothermia as a consequence of mitochondrial dysfunction, particularly in small animal models, and the underappreciated role of the liver in thermoregulation.

O27

Heme redox potentials and cooperative interactions in the obligate $CIII_2CIV_2$ supercomplex from Mycobacterium smegmatis

Frederic Melin[a], Rowan Walters[b], Ben Hartmann[b], Jamie Blaza[b] and Petra Hellwig[a]

[a]Laboratoire de Bioélectrochimie et Spectroscopie, UMR 7140, Chimie de la Matière Complexe, Université de Strasbourg-CNRS 4, Rue Blaise Pascal, 67081 Strasbourg, France [b]York Structural Biology Lab, Department of Chemistry, University of York, United Kingdom.

E-mail address: fmelin@unistra.fr
Presenting author: Frederic Melin

Respiratory complexes do not only exist as discrete proteins inside the membrane, but tend to self-assemble into supramolecular architectures known as supercomplexes or respirasomes.[1] Their existence was already proposed 40 years ago, with the "solid model" of closely packed respiratory membrane proteins complexes, but isolation of stable and functional associations of respiratory complexes was achieved mainly in the last two decades, thanks to improved solubilization procedures with mild detergents. Organization into supercomplexes is now believed to be a common feature of membrane proteins of both the respiration and photosynthetic chains. These supramolecular structures seem to play a role in the stabilization of the individual enzymes, but their functional role is less clear.[2] They may allow a direct and more efficient transfer of reactive intermediates and substrates between protein active sites. There is evidence in particular of quinol (QH_2) channeling between complexes I and III and cytochrome *c* between complexes III and IV in supercomplexes.[3] It also seems that the release of reactive oxygen species (ROS) is minimized in supercomplexes.[2]
Here we focus on the cytochrome *bcc:aa*$_3$ supercomplex from *Mycobacterium smegmatis*, a close but non-pathogenic relative to *M. tuberculosis*. This supercomplex has menaquinol:O_2 oxidoreductase activity without exogenous cytochrome *c* and it contains a central cytochrome *bcc* dimer ($CIII_2$) flanked by individual cytochrome *aa*$_3$ oxidases (CIV) on both sides.[4,5] To get insight into the thermodynamics of the electron transfer processes involved in the supercomplex, we have determined the redox potentials of all the hemes by UV/Visible potentiometric titrations in a thin layer electrochemical cell. The values obtained closely match those previously reported for the *Corynebacterium glutamicum* supercomplex.[6] We also discuss the degree of anti-cooperativity between the heme centers using different models of interaction.

[1] R. Vartak, C.A.-M. Porras, Y. Bai, Respiratory supercomplexes: structure, function and assembly. Protein Cell 4 (2021) 582-590. https://doi.org/10.1007/s13238-013-3032-y

[2] P. Brzezinski, A. Moe, P. Ädelroth, Structure and Mechanism of Respiratory III–IV Supercomplexes in Bioenergetic Membranes. Chem. Rev. 121 (2021) 9644-9673. https://doi.org/10.1021/acs.chemrev.1c00140

[3] E. Lapuente-Brun, R. Moreno-Loshuertos, R. Acín-Pérez, A. Latorre-Pellicer, C. Colás, E. Balsa, E. Perales-Clemente, P.M. Quirós, E. Calvo, M.A. Rodríguez-Hernández, P. Navas, R. Cruz, Á. Carracedo, C. López-Otín, A. Pérez-Martos, P. Fernández-Silva, E. Fernández-Vizarra, J.A. Enríquez, Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. Science 340 (2013) 1567-1570. https://doi.org/10.1126/science.1230381

[4] H. Gong, J. Li, A. Xu, Y. Tang, W. Ji, R. Gao, S. Wang, L. Yu, C. Tian, J. Li, H.-Y. Yen, S. Man Lam, G. Shui, X. Yang, Y. Sun, X. Li, M. Jia, C. Yang, B. Jiang, Z. Lou, C.V. Robinson, L.-L. Wong, L.W. Guddat, F. Sun, Q. Wang, Z. Rao, An electron transfer path connects subunits of a mycobacterial respiratory supercomplex, Science (2018) 362, eaat8923. https://doi.org/10.1126/science.aat8923

[5] D. Riepl, A. P. Gamiz-Hernandez, T. Kovalova, S. M. Krol, S. L. Mader, D. Sjöstrand, M. Högbom, P. Brzezinski, M. Högbom, V.R.I. Kaila, Long-range charge transfer mechanism of the III_2IV_2 mycobacterial supercomplex. Nat. Commun. 15 (2024) 5276. https://doi.org/10.1038/s41467-024-49628-9

[6] W.-C. Kao, T. Kleinschroth, W. Nitschke, F. Baymann, Y. Neehaul, P. Hellwig, S. Richers, J. Vonck, M. Bott, C. Hunte, The obligate respiratory supercomplex from Actinobacteria. Biochim. Biophys. Acta Bioenerg. 1857 (2016) 1705-1714. https://doi.org/10.1016/j.bbabio.2016.07.009

O28

Subcellularly targeted AMPfret nanosensors reveal spatiotemporal propagation of energy stress and discriminate physiological from toxic responses

Roland Abi Nahed[a], Ali Hussein[a], Cécile Cottet-Rousselle[a], Francesco Aulicino[b], Alaa Al Assi[a], Eric Fontaine[a], Marc Billaud[c], Martine Cordier-Bussat[c], Marie Carrière[d], Aurélie Dupont[e], Imre Berger[b], Uwe Schlattner[f]

a
Univ. Grenoble Alpes, Inserm U1055, LBFA, Grenoble, France
b Univ. of Bristol, Bristol Synthetic Biology Centre and School of Biochemistry, Bristol, UK
c
Univ. Claude Bernard Lyon1, Inserm U1052, CNRS UMR5286, CRCL, Lyon, France.
d
Univ. Grenoble Alpes, CEA, CNRS, IRIG-SyMMES, CIBEST, Grenoble, France
e
Univ. Grenoble Alpes, CNRS, LIPhy, Grenoble, F-38000, France.
f
Univ. Grenoble Alpes, LBFA, Grenoble, and Institut Universitaire de France, Paris, France

E-mail address: uwe.schlattner@univ-grenoble-alpes.fr
Presenting author: Uwe Schlattner

Real-time, non-invasive quantification of intracellular energy dynamics in living mammalian cells remains a major challenge, particularly at subcellular and single-cell resolution. To address this, we developed AMPfret[1,2], a genetically encoded FRET-based nanosensor derived from AMP-activated protein kinase (AMPK), a central regulator of cellular energy homeostasis that senses physiologically relevant ATP:AMP and ATP:ADP ratios.

We generated HEK293T clonal cell lines with stable expression of AMPfret targeted to distinct subcellular compartments, including the cytosol, plasma membrane, and mitochondrial surface or intermembrane space. This approach enabled simultaneous monitoring of energy status across cellular compartments and revealed pronounced heterogeneity in baseline energy levels within clonal populations. Upon exposure to oxidative and mitochondrial stressors, including the ROS generator tert-butyl hydroperoxide (Luperox) and the uncoupler FCCP, AMPfret imaging uncovered distinct spatiotemporal response patterns. Energy stress responses varied in both amplitude and kinetics between compartments, indicating that intracellular energy imbalance is not uniform but instead propagates in a coordinated, wave-like manner across the cell.

The early temporal dynamics of the AMPfret signal consistently distinguished between physiological (adaptive and reversible) and toxic (persistent and deleterious) stress. Mild or transient increases in FRET corresponded to effective metabolic compensation, whereas delayed, sustained, and high-amplitude responses were associated with toxic stress and loss of energy homeostasis. Single-cell analyses further revealed substantial variability in stress responses within genetically identical populations, emphasizing the importance of resolving cellular heterogeneity when studying bioenergetics and toxicity.

Together, these results establish AMPfret as a powerful tool for mapping the spatiotemporal dynamics of cellular energy states with high resolution. By linking subcellular energy sensing to functional outcomes, this approach provides new insights into how energy stress signals propagate within cells and offers a versatile platform for energy-based toxicity screening, drug evaluation, and systems-level studies of cellular metabolism.

[1] M. Pelosse, C. Cottet-Rousselle, C. M Bidan, A. Dupont, K. G, I. Berger, U. Schlattner, Synthetic energy sensor AMPfret deciphers adenylate-dependent AMPK activation mechanism. Nat. Commun. 10(1), (2019), 1038. https://doi.org/10.1038/s41467-019-08938-z

[2] R. Abi Nahed, M. Pelosse, F. Aulicino, F. Cottaz, I. Berger, U. Schlattner, FRET-based sensor for measuring adenine nucleotide binding to AMPK. Methods Mol. Biol. 2882, (2025),15-45. https://doi.org/10.1007/978-1-0716-4284-9_2.

O29

Stepwise ATP translocation into the endoplasmic reticulum by human SLC35B1

Ashutosh Gulati[1], Do-Hwan Ahn[1], Albert Suades[1], Yurie Hult[1], Gernot Wolf[2], So Iwata[3], Giulio SupertiFurga[2], Norimichi Nomura[2] & David Drew[1]

[1]Department of Biochemistry and Biophysics, Science for Life Laboratory, Stockholm University, Stockholm, Sweden

[2] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria [3] Department of Cell Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

E-mail address:ddrew@dbb.su.se

Presenting author: ddrew@dbb.su.se

Abstract[1]

ATP generated in the mitochondria is exported by an ADP/ATP carrier of the SLC25 family. The endoplasmic reticulum (ER) cannot synthesize ATP but must import cytoplasmic ATP to energize protein folding, quality control and trafficking. It was recently proposed that a member of the nucleotide sugar transporter family, termed SLC35B1 (also known as AXER), is not a nucleotide sugar transporter but a long-sought-after ER importer of ATP. Here I will present that human SLC35B1 does not bind nucleotide sugars but indeed executes strict ATP/ADP exchange with uptake kinetics consistent with the import of ATP into crude ER microsomes. A CRISPR–Cas9 cell-line knockout demonstrated that SLC35B1 clusters with the most essential SLC transporters for cell growth, consistent with its proposed physiological function. We have further determined seven cryogenic electron microscopy structures of human SLC35B1 in complex with an Fv fragment and either bound to an ATP analogue or ADP in all major conformations of the transport cycle. We observed that nucleotides were vertically repositioned up to approximately 6.5 Å during translocation while retaining key interactions with a flexible substrate-binding site. We conclude that SLC35B1 operates by a stepwise ATP translocation mechanism, which is a previously undescribed model for substrate translocation by an SLC transporter.

[1] Gulati A, Ahn D, Suades A, Hult Y, Wolf G, Iwata S, Superti-Furga G, Nomura N, Drew D. Step-wise translocation of ATP into the ER by human SLC35B1. Nature (2025) 643:855–864.

Topic list:
S9. Transporters and channels

Contribution type:
Talk – T

O30

Structure of giant kelp Photosystem I-FCP uncovers drivers of antenna evolution across the red lineage

Jenevieve Weissman[1,3], Pablo Maturana[1,3], Hui-Ming Oung[1], Reece Riddle[1], Gabrielle Wyatt[1], Viktoria Dubinin[1], Philipp Zerbe[1], María Maldonado[1,2]

[1]*Department of Plant Biology, University of California-Davis, Davis, USA.*
[2]*Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia.*
[3]*Equal contribution.*

E-mail address: maria.maldonado@monash.edu
Presenting author: María Maldonado

Brown algae and other red-algae-derived organisms are major contributors to global CO_2 fixation via photosynthesis. To understand the photosynthetic function of brown algae, we obtained the structure of giant kelp *Macrocystis pyrifera* photosystem I (PSI) with a fucoxanthin-chlorophyllprotein (FCP) antenna and compared it to known structures from the red-algal lineage. We identified differences in *M. pyrifera*'s antenna composition, architecture, and chlorophyll networks, as well as a pronounced variation in transmembrane hydrophobic thickness across the PSI-FCP supercomplex, with implications for photochemical function. Our work lays the foundation to understand kelp's high photosynthetic productivity and reveals drivers of antenna conservation and diversification for the red lineage.

O31

Molecular basis of substrate recognition and transport by the human mitochondrial citrate carrier SLC25A1

Jianhui Li[a], Denis A. Lacabanne[a], Martin S. King[a], Jonathan J. Ruprecht[a], Alice P. Sowton[a], Stephany Jaiquel Baron[a], Shane M. Palmer[a], Els Pardon[b,c], Jan Steyaert[b,c], Vanessa Leone[d], Edmund R. S. Kunji[a]

[a]*MRC Mitochondrial Biology Unit, University of Cambridge, The Keith Peters Building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, United Kingdom.* [b]*VIB-VUB Center for Structural Biology, VIB, Pleinlaan 2, B-1050 Brussels, Belgium.*
[c]*Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.* [d]*Department of Biophysics and Data Science Institute, Medical College of Wisconsin, Milwaukee, WI 53226-3548, USA.*

E-mail address: ersk2@cam.ac.uk
Presenting author: Edmund Kunji

The mitochondrial citrate carrier (SLC25A1) [1,2] is a nuclear-encoded member of the SLC25 mitochondrial carrier family in the inner membrane [2,3]. The citrate carrier has a broad substrate specificity, including dicarboxylates, tricarboxylates and phosphoenolpyruvate, and uses the proton motive force to drive their import [1]. This transporter links mitochondrial energy production with cytosolic biosynthetic pathways, thereby coordinating metabolic flux in response to cellular demands. Its main function is to export citrate (or isocitrate) from the mitochondrial matrix into the cytosol in exchange for malate. In the cytosol, citrate is converted into acetyl-CoA, which is a starting block for the synthesis of fatty acids, cholesterol, and other compounds of the cell [4]. Concurrently, the import of malate supports mitochondrial redox balance and sustains the tricarboxylic acid cycle. The citrate carrier is monomer and has an alternating access mechanism [5]. Dysregulation of this carrier has been implicated in a range of pathological conditions, including cancer, metabolic disorders, and rare genetic diseases [4,6]. Loss-of-function mutations in SLC25A1 are associated with combined D-2- and L-2-hydroxyglutaric aciduria, a neurometabolic disorder characterised by developmental delay and mitochondrial dysfunction [4,7]. Here we present our recent advances in understanding the complex transport mechanism of this carrier.

[1] Chappell JB, Robinson BH. Penetration of the mitochondrial membrane by tricarboxylic acid anions. Biochem Soc Symp. 1968;27:123-133

[2] Kunji ERS, Mavridou V, King MS, Cimadamore-Werthein C, Baron SJ, Jones SA, et al. The peculiar properties of mitochondrial carriers of the SLC25 family. Biochem J. 2025;482(15):1011-1042, https://doi.org/10.1042/Bcj20253171

[3] Kunji ERS, King MS, Ruprecht JJ, Thangaratnarajah C. The SLC25 Carrier Family: Important Transport Proteins in Mitochondrial Physiology and Pathology. Physiology (Bethesda). 2020;35(5):302-327, https://doi.org/10.1152/physiol.00009.2020

[4] Majd H, King MS, Smith AC, Kunji ERS. Pathogenic mutations of the human mitochondrial citrate carrier SLC25A1 lead to impaired citrate export required for lipid, dolichol, ubiquinone and sterol synthesis. Biochim Biophys Acta. 2018;1859(1):1-7, https://doi.org/10.1016/j.bbabio.2017.10.002

[5] Cimadamore-Werthein C, King MS, Lacabanne D, Pyrihova E, Jaiquel Baron S, Kunji ER. Human mitochondrial carriers of the SLC25 family function as monomers exchanging substrates with a ping-pong kinetic mechanism. EMBO J. 2024;43(16):3450-3465, https://doi.org/10.1038/s44318-024-00150-0

[6] Mosaoa R, Kasprzyk-Pawelec A, Fernandez HR, Avantaggiati ML. The Mitochondrial Citrate Carrier SLC25A1/CIC and the Fundamental Role of Citrate in Cancer, Inflammation and Beyond. Biomolecules. 2021;11(2), https://doi.org/10.3390/biom11020141

[7] Nota B, Struys EA, Pop A, Jansen EE, Fernandez Ojeda MR, Kanhai WA, et al. Deficiency in SLC25A1, encoding the mitochondrial citrate carrier, causes combined D-2- and L-2-hydroxyglutaric aciduria. Am J Hum Genet. 2013;92(4):627-631, https://doi.org/10.1016/j.ajhg.2013.03.009

O32

Directionality of electron and proton transfers in Type II reaction centers

M.R. Gunner[a,b], Rongmei Judy Wei[b], Jose Ortiz-Soto[a]
First N. N. Last[a], First N. N. Last[b] (Example: Jane G. Doe[a])

[a]*Department of Physics City College of New York CUNY;* [b]*Department of Chemistry CUNY Graduate Center*

E-mail address: mgunner@ccny.cuny.edu
Presenting author: Marilyn Gunner

All photosynthetic reaction centers have two symmetrical paths for electrons to go from the primary donor (bacterio)chlorophyll to the terminal acceptor. In type II proteins, Photosystem II (PSII) and purple non-sulfur bacterial reaction centers (bRCs), the electron transfers along one path, while in type I system (PSI) electrons both paths. Type II systems add to the proton gradient by coupling reduction of the quinone terminal electron acceptor to proton uptake on the negative side of the protein. In PSII protons are released to the positive side as water is oxidized. MultiConformation Continuum Electrostatics (MCCE) calculations determined the free energy of electron transfer down the active and inactive branches of wild-type bRCs and mutated protein that supports 'wrong-way' electron transfer. The electron transfers down the path at more positive electrostatic potential, showing protein electrostatics helps control electron transfer reactions. MCCE also examined the proton transfer paths in bRCs and PSII. Complex networks leading to the secondary quinone in bRCs provides a robust way to deliver protons. In PSII, the complexity of the network may provide an entropy barrier for water oxidation.

O33

Mechanism of loosely coupled transport by EmrE and its functional impact *in vitro* and in bacteria Ashley Hiett[a], Ryan Klevens[a], Juliet Cheng[a], Merissa Brousseau[a,b], Peyton Spreacker[a,c], Katherine Henzler-Wildman[a]

[a]*Department of Biochemistry, University of Wisconsin - Madison*
[b]*Present address: School of Medicine and Public Health, University of Wisconsin - Madison* [c]*Present address: Labcorp, Madison, Wisconsin*

E-mail address: henzlerwildm@wisc.edu
Presenting author: Katherine Henzler-Wildman

The Small Multidrug Resistance (SMR) transporters are among the smallest known active transporters. They were named for their ability to perform proton-coupled antiport, harnessing the proton motive force to drive efflux of toxic compounds in bacteria. However, the quaternary ammonium compound (QAC) subfamily of SMR transporters, exemplified by EmrE, are only loosely coupled. They can perform antiport with variable proton/small molecule stoichiometry and can even perform drug-gated proton uniport. EmrE is promiscuous, with transport stoichiometry changing with substrate concentration and pH. In addition, the identity of the small molecule determines whether coupled or uncoupled transport is the dominant function. Here we investigate the mechanism of loosely coupled transport and how switching transport activity impacts bacterial bioenergy metabolism. We have found that the C-terminal tail acts as a secondary gate regulating access to the transport pore[1]. Our new structure EmrE shows key interactions between the tail and adjacent loops that stabilize the fully closed conformation of this secondary gate to fully occlude the transport pore. Substrates initially interact with the surface of EmrE, disrupting the C-terminal gate and allowing transport to proceed[1,2]. Properties of the small molecule, including substrate charge, determine whether the substrate progresses to the primary binding site in the cores of EmrE and thus influence partitioning between coupled and uncoupled transport modes. In the native *E. coli*, uncoupled proton leak through EmrE can be significant enough to disrupt bacterial energy metabolism and growth, leading to enhanced susceptibility rather than resistance[2,3]. Thus, EmrE transport activity can be either beneficial or detrimental to the native bacteria, depending on the identity of the small molecule substrate.

[1] M. Brousseau, D. Teng, N.E. Thomas, G.A. Voth, K.A. Henzler-Wildman. The C-terminus of the multi-drug efflux pump EmrE prevents proton leak by gating transport. eLife 14, (2025), RP105525. https://elifesciences.org/articles/105525.

[2] P.J. Spreacker, N.E. Thomas, W.F. Beeninga, M. Brousseau, C.J. Porter, K.M. Hibbs, K.A. Henzler-Wildman. Activating alternative transport modes in a multidrug resistance efflux pump to confer chemical susceptibility. Nat. Commun. 13(1), (2022), 7655. https://www.nature.com/articles/s41467-022-35410-2.

[3] P.J. Spreacker, A.K. Wegrzynowicz, C.J. Porter, W.F. Beeninga, S. Demas, E.N. Powers, K.A. Henzler-Wildman. Functional promiscuity of small multidrug resistance transporters from Staphylococcus aureus, Pseudomonas aeruginosa, and Francisella tularensis. Mol. Microbiol.
121, (2024), 798-813. https://onlinelibrary.wiley.com/doi/10.1111/mmi.15231.

O34

Thylakoid-wide loss of protein segregation upon state transitions in Chlamydomonas reinhardtii

Wojciech J. Nawrockia, Wojciech Wietrzynskib, Sofie van Dorstb, Eduard Eliasc, Kajwal Patraa , Olli Virtanenc, Doran I.G.B. Raccahd, Roberta Crocec, Benjamin D. Engelb

aUMR7141, CNRS-Sorbonne Université, Institut de Biologie Physico-Chimique, Paris, France

bBiozentrum, University of Basel, Switzerland

cBiophysics of Photosynthesis, Department of Physics and Astronomy, Faculty of Science, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

dUTex Austin, Texas, USA

E-mail address: wojciech.nawrocki@cnrs.fr
Presenting author: W.J.N.

Bioenergetic membranes evolved to host a dense matrix of energy-transducing enzymes. In oxygenic photosynthesis, the architecture of thylakoids faces an implicit trade-off: since Photosystems II (PSII) and I (PSI) electron-transfer in series, they should be in close proximity to minimize diffusion of soluble carriers between them. However, as both photosystems use membrane-embedded antennae (LHCII) with nearly isoenergetic pigments, their proximity could lead to competition for excitation energy (EE), a competition that would strongly favor PSI due to its higher excitation energy transfer (EET) efficiency and quenching rate. The green lineage of photosynthetic eukaryotes resolved this conflict by laterally segregating PSII and PSI complexes into appressed grana regions and thylakoid lamellae, respectively. In either of those regions, virtually absolute separation of the two Photosystems was observed [1]. Here, we demonstrate—using in situ structural and functional approaches—that during state transitions (a regulatory process aimed at redistributing EE between the two photosystems [2]), this lateral heterogeneity is largely lost. We show that, despite minor mesoscale changes, appressed regions containing PSI exhibit de facto local destacking. Finally, we employ membrane-scale modeling to reveal molecular details of membrane organization during state transitions. This work provides snapshots of large-scale bioenergetic membrane reorganization in response to physiological stimuli. [1] Wietrzynski, Wojciech, et al. "Charting the native architecture of Chlamydomonas thylakoid membranes with single-molecule precision." Elife 9 (2020): e53740. [2] Wollman, Francis-André. "State transitions reveal the dynamics and flexibility of the photosynthetic apparatus." The EMBO journal 20.14 (2001): 3623-36

O35

Role of the MmpS5L5 Efflux Pump in Potentiating the Evolution of Untreatable Multi-Drug-Resistant Strains of *Mycobacterium tuberculosis*

Gregory M Cook, Matthew B McNeil

Department of Microbiology and Immunology
University of Otago
PO Box 56, Dunedin
New Zealand

E-mail address: greg.cook@otago.ac.nz
Presenting author: Greg Cook

Abstract

The MmpS5–MmpL5 efflux system in *Mycobacterium tuberculosis* plays a critical role in exporting the ironchelating siderophores mycobactin and carboxymycobactin, thereby enabling iron acquisition from host tissues. This process is essential for mycobacterial survival, metabolism, and virulence. In addition to siderophores, the MmpS5–MmpL5 efflux pump recognizes and exports a broad spectrum of anti-tubercular compounds, including bedaquiline (BDQ) and several preclinical candidates. However, the molecular mechanisms underlying substrate recognition and transport by this system have remained poorly understood.

Here, we report several key advances in elucidating the role of the MmpS5–MmpL5 efflux system in drug resistance. Using *M. tuberculosis*, we demonstrate that both genetic disruption and chemical inhibition— via *verapamil*—of MmpS5–MmpL5 significantly enhance the efficacy of BDQ and other efflux substrates. Furthermore, genetic inhibition of MmpS5–MmpL5 expression restores drug susceptibility in previously resistant strains. We also present the first atomic-resolution structure of the fully assembled MmpS5– MmpL5 efflux complex, providing critical insights into its mechanism of action. Finally, we show that overexpression of MmpL5 potentiates drug resistance in *M. tuberculosis*.

Collectively, these findings establish MmpS5–MmpL5 as a central mediator of drug resistance and a promising target for therapeutic intervention in tuberculosis.

S9_T_G_Cook

O36

TBA

O37

Roles of reactive oxygen species in bacterial bioenergetics

Christoph von Ballmoos

University of Bern, Department of Chemistry, Biochemistry and Pharmaceutical Sciences

E-mail address: Christoph.vonballmoos@unibe.ch
Presenting author: Christoph von Ballmoos

Electron transfer reactions are central to bacterial metabolism, allowing cells to convert external energy sources into key metabolites such as NADH and ATP. Many of these processes are carried out by membrane proteins that couple redox reactions to the generation of a proton-motive force, which is then used by the F_1F_0 ATP synthase to produce ATP or to support other cellular functions. Among the available electron acceptors, oxygen stands out due to the large amount of free energy released upon its complete reduction. However, its chemistry is not trivial: full reduction requires four electrons, while partial reduction leads to reactive oxygen species (ROS), which are considered harmful and associated with oxidative stress. At the same time, ROS can serve signaling roles and are actively produced by enzymes such as NADPH oxidase during host defense(1).

At host–microbe interfaces such as the gut, this creates a particular situation: while the gut lumen is largely anaerobic and electron acceptors are limiting, host cells have access to oxygen and can locally generate superoxide or hydrogen peroxide as part of the immune response. It has been shown that this host response affects microbial communities by providing a growth advantage to facultative anaerobes, including pathogens such as *Salmonella enterica* or *Escherichia coli*(2,3).

In our lab, we use bottom-up reconstitution approaches to better understand the function of bioenergetic proteins in isolation. In this talk, I will discuss our experiments addressing the hypothesis whether in facultative anaerobes, oxygen generated in the cytoplasm by catalase from host-derived hydrogen peroxide can act as a terminal electron acceptor for quinol oxidases(4,5).

Further, I will discuss our findings on the mechanism of two ROS producing, membraneintegral heme-containing proteins that mediate electron transfer across membranes , NADPH oxidase from *Streptococcus pneumoniae* and superoxide oxidase from *E. coli*, including potential partner proteins and small-molecule modulators (6–8).

[1] Vermot A, Petit-Härtlein I, Smith SME, Fieschi F. NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology. Antioxidants (Basel). 2021 Jun 1;10(6):890. doi:10.3390/antiox10060890

[2] Rivera-Chavez F, Lopez CA, Bäumler AJ. Oxygen as a driver of gut dysbiosis. Free Radic Biol Med. 2017 Apr;105:93–101. doi:10.1016/j.freeradbiomed.2016.09.022

[3] Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, et al. Gut inflammation provides a respiratory electron acceptor for Salmonella. Nature. 2010 Sep 1;467(7314):426–9. doi:10.1038/nature09415

[4] Chanin RB, Winter MG, Spiga L, Hughes ER, Zhu W, Taylor SJ, et al. Epithelial-Derived Reactive Oxygen Species Enable AppBCX-Mediated Aerobic Respiration of Escherichia coli during Intestinal Inflammation. Cell Host Microbe. 2020 Dec 9;28(6):780-788 e5. Located at: 33053375. doi:10.1016/j.chom.2020.09.005

[5] Crowley SM, Vallance BA. Microbial Respiration in the Colon: Using H_2O_2 to Catch Your Breath. Cell Host Microbe. 2020 Dec 9;28(6):771–3. Located at: 33301714. doi:10.1016/j.chom.2020.11.006

[6] Dubach VRA, San Segundo-Acosta P, Murphy BJ. Structural and mechanistic insights into Streptococcus pneumoniae NADPH oxidase. Nat Struct Mol Biol. 2024 Jul 22;1–9. doi:10.1038/s41594-024-01348-w

[7] Abou-Hamdan A, Mahler R, Grossenbacher P, Biner O, Sjöstrand D, Lochner M, et al. Functional design of bacterial superoxide:quinone oxidoreductase. Biochim Biophys Acta Bioenerg. 2022 Oct 1;1863(7):148583. doi:10.1016/j.bbabio.2022.148583

[8] Lundgren CAK, Sjöstrand D, Biner O, Bennett M, Rudling A, Johansson AL, et al. Scavenging of superoxide by a membrane-bound superoxide oxidase. Nat Chem Biol. 2018 Aug;14(8):788–93. Located at: 29915379. doi:10.1038/s41589-018-0072-x

O38

Understanding the powerhouse of Gram-positive and -negative pathogens to identify and design new targets and leads

Gerhard Grüber[a]

a
Nanyang Technological University, School of Biological Sciences, Singapore 637551, Singapore

E-mail address: GGrueber@ntu.edu.sg
Presenting author: Gerhard Grüber

Infectious diseases remain one of the leading causes of death worldwide, with substantial implications for public health and the global economy. Recent studies estimated that nearly 5 million deaths in 2021 were associated with bacterial antimicrobial resistance, and over 1 million were directly attributable to it[1].

Gram-positive nontuberculous mycobacteria (NTM) and the Gram-negative bacterium *Acinetobacter baumannii* are representatives of multi- and pan-drug resistant pathogens. NTM infections are increasing in their global prevalence, morbidity and mortality and frequently surpass the global incidence of tuberculosis infections in developed countries. *A. baumannii* is described as one of the top ten pathogens world-wide and is regarded as one of the most antibiotic-resistant bacterium[1]. It causes infections in the hospital and community, most commonly presenting as pneumonia. Community-acquired infections are frequently observed, leading to mortality rates above 60%.

NTM and *A. baumannii* are non-fermenting and strictly aerobic pathogens, depending on the oxidative phosphorylation (OXPHOS) pathway. The presentation will highlight new fundamental insights, revealing the differences of the OXPHOS pathways among both pathogens and most importantly to the human mitochondrial cousin, paving the way to identify new targets, hit- and lead molecules.

[1] WHO Bacterial Priority Pathogens List. Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance. (2024). Available online at: https://www.who.int/publications/i/item/9789240093461 (Accessed March 8, 2025).

O39

Tubular respiratory architectures redefine quinone interaction in prokaryotic bioenergetics M. Broc[a], M. V. Cherrier[b], P. S. Garcia[a], R. Osman[b], F. Seduk[a], S. Grimaldi[c], C. Y. Botté[d], F. Pierrel[e], G. Schoen[f], Y. Nicolet[b], A. Walburger[a], A. Magalon[a]

[a]*Aix Marseille Univ, CNRS, LCB, IMM, IM2B, Marseille, France*
[b]*Univ. Grenoble-Alpes, CEA, CNRS, IBS, Metalloproteins Unit, Grenoble, France* [c]*Aix Marseille Univ, CNRS, BIP, IMM, IM2B, Marseille, France.*
d
Apicolipid Team & Gemeli Platform, Institut pour l'Avancée des Biosciences, CNRS UMR5309, INSERM U1209, Université Grenoble Alpes
[e]*Univ. Grenoble-Alpes, CNRS, Grenoble INP, TIMC, Grenoble, France*
[f]*Univ Grenoble-Alpes, CNRS, CEA, IBS, Electron Microscopy and Methods Unit, Grenoble, France*

E-mail address: magalon@imm.cnrs.fr
Presenting author: Axel Magalon

Quinones are central components of prokaryotic bioenergetics, coupling a broad range of oxidoreductases to respiratory electron-transfer chains. In the classical view, access to the quinone pool relies on two main structural solutions: either quinone-reacting enzymes contain integral membrane subunits, or they interact directly with the membrane surface through amphipathic regions in monotopic configurations. Recent work now points to an additional organizational principle based on proteinaceous tubular assemblies terminated by a conserved Helical Membrane Plug-in (HMP) motif [1].

This architecture first emerged from the study of the ForCE system, where a tubular scaffold appears to provide a dedicated interface between a soluble redox enzyme, namely a formate dehydrogenase, and the membrane quinone pool. More broadly, comparative analyses indicate that this HMP-based organization is not restricted to a single respiratory module, but is associated with at least four distinct classes of quinone-reacting enzymes [1]. Within this context, type II NADH:quinone oxidoreductase (NDH-II) deserves to be revisited. NDH-II is widely distributed and classically described as a monotopic enzyme that associates peripherally with the membrane through a C-terminal amphipathic region. However, recent structural observations indicate that, in *Bacillus subtilis*, NDH-II can instead operate in association with an HMP-containing tubular partner. Strikingly, the C-terminal region that in canonical NDH-II enzymes mediates membrane interaction appears here to be repurposed for docking onto the HMP tube. Rather than an isolated variation, this organization points to an unexpected structural plasticity in how respiratory enzymes access membrane quinones.

Taken together, these findings broaden current views of prokaryotic respiratory chains. They support the idea that quinone exchange may be organized not only by direct membrane contact, but also through dedicated protein assemblies that shape access and routing of hydrophobic electron carriers. Recognizing this additional modality opens new perspectives on the evolution of respiratory systems, on the spatial organization of energy-converting pathways, and on the interpretation of the many uncharacterized bioenergetic modules emerging from microbial genomics.

[1] Broc M, Cherrier MV, Uzel A, Arias-Cartin R, Arnoux P, Brasseur G, Seduk F, Guigliarelli B, Legrand P, Pierrel F, Schoehn G, Maté MJ, Martin L, Grimaldi S, Nicolet Y, Magalon A, Walburger A. A scaffold for quinone channeling between membrane and soluble bacterial oxidoreductases. Nat Struct Mol Biol. 32(11), (2025), 2196-2202. https://10.1038/s41594-025-01607-4

O40

UCP3 couples branched-chain amino acid metabolism to fatty acid oxidation and glucose sparing

Amer Ahmed[a], Ruggiero Gorgoglione[b], Daria Di Molfetta[a], Giorgia Natalia Iaconisi[a], Antonello Caponio[a], Luigi Palmieri[a], Vincenza Dolce[c], Giuseppe Fiermonte[a]

[a]*Department of Biosciences, Biotechnologies and Environment, University of Bari, 70125 Bari, Italy*
[b]*Department of Medicine and Surgery, LUM University, Casamassima, 70010, Italy*
[c]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende, Italy*

E-mail address: giuseppe.fiermonte@uniba.it
Presenting author: Giuseppe Fiermonte

Mitochondrial uncoupling protein 3 (UCP3) has been genetically and functionally linked to metabolic disorders, including obesity, insulin resistance, and type 2 diabetes (T2D)[1,2]. In animal models, reduced UCP3 expression compromises fatty acid oxidation (FAO) and contributes to metabolic dysfunction; however, its precise molecular role remains incompletely understood[3]. UCP3 expression is modulated by nutrient availability, particularly by branched-chain amino acids (BCAAs) and fatty acids, both of which are frequently altered in T2D.

In this study, we provide evidence that UCP3 functions as a mitochondrial metabolite transporter. In our experimental systems, UCP3 mediates an electroneutral exchange between mitochondrial aspartate and cytosolic malate, coupled to proton translocation. In differentiated human myotubes, this activity promotes a metabolic shift toward increased reliance on fatty acid oxidation, reducing glucose utilization and recapitulating features of a fasting-like metabolic state. Additionally, UCP3 activity supports the generation of glutamine from fatty acid–derived carbon, a pathway that appears compromised in certain diabetic conditions.

At the mechanistic level, UCP3 contributes to ammonia handling during BCAA catabolism and helps maintain mitochondrial oxaloacetate levels, thereby sustaining efficient FAO. Reduced UCP3 function leads to compensatory induction of UCP2, which facilitates net aspartate export from mitochondria and may further limit oxaloacetate availability, exacerbating defects in fatty acid metabolism.

Overall, our findings identify UCP3 as a regulator of mitochondrial metabolite exchange that influences substrate preference and metabolic flexibility in muscle cells, providing new insights into its role in metabolic homeostasis and its potential contribution to T2D-associated dysfunction.

[1] Schrauwen, P. et al. Uncoupling protein 3 content is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 50, 2870–2873 (2001). https://doi.org/10.2337/diabetes.50.12.2870

[2] Bhatti, A. A., Rana, S., Khalid, M., Nawaz, H. & Irfan, M. UCP3 gene variants and obesity in a Pakistani sample population. Sci Rep 15, 33135 (2025). https://doi.org/10.1038/s41598-025-18434-8

[3] Bezaire, V., Seifert, E. L. & Harper, M. E. Uncoupling protein-3: clues in an ongoing mitochondrial mystery. FASEB J 21, 312–324 (2007). https://doi.org/10.1096/fj.06-

O41

Role of respiratory oxidases in photosynthetic electron transport in *Synechococcus elongatus* PCC 7942

Fabrizio Iacono[a], Eve Giovannetti[a], Samuele Perin[b], Yagut Allahverdiyeva-Rinne[b], Stefania Viola[a]

[a] *Photosynthesis & Environment, BIAM, UMR7265, CEA/CNRS/AMU, Saint-Paul-Lez-Durance, France*
[b]*Molecular Plant Biology, Department of Life Technologies, University of Turku, Finland*

E-mail address: stefania.viola@cea.fr
Presenting author: Stefania Viola

In cyanobacteria, respiratory enzymes are present in the thylakoid membranes, where they are connected with the photosynthetic electron transport chain (ETC) [1]. Respiratory complexes can thus participate in electron transport during illumination, contributing to the regulation of ATP and NADPH production for CO_2 fixation. The obligate photoautotrophic cyanobacterium *Synechococcus elongatus* PCC 7942 possess two haem-copper cytochrome *c* oxidases (Cox and Cbb) and a cytochrome *bd* quinol oxidase (Cyd) that could share electron carrier pools with Photosystem II and Photosystem I, and participate in the generation of trans-thylakoid *pmf*. However, the effects of the activity of on the photosynthetic efficiency of *S. elongatus*, as well as their precise localization and molecular context, remain poorly understood.

We investigated the participation of the three respiratory oxidases in regulating the photosynthetic efficiency of *S. elongatus*, using a combination of genetics, biochemistry and non-invasive biophysical methods. Our *in vivo* spectroscopy measurements [2] show that Cox and Cyd play the main bioenergetic role, maintaining a trans-thylakoid *pmf* in the dark and acting as electron sinks during dark-to-light transition. While Cox and Cyd contribute to *pmf* generation and fine-tune the redox poise of the intersystem chain at the light onset, their absence doesn't affect photosynthetic electron transport and CO_2 assimilation under continuous illumination, where O_2 reduction by the Flavodiiron proteins (FLVs) predominates [3]. However, mutants lacking both Cox and Cyd have lower rates of CO_2 assimilation at the light onset, and in continuous low light when FLVs are also absent, suggesting that they help energizing the cellular carbon concentrating mechanism. We could not detect an activity for Cbb, that we show accumulates to much lower levels than Cox and, especially, Cyd. Our biochemical analyses show that the three oxidases form independent supercomplexes, whose composition and subcellular localization we are currently investigating to gain a deeper understanding of their molecular function and interactions. Altogether, our results provide new insights on the consequences of the connection with respiration on the photosynthetic activity of *S. elongatus*, revealing the role of Cox and Cyd both in dark respiration, and in priming electron transport upon illumination and the acquisition of inorganic carbon.

[1] D. J. Lea-Smith et al., Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria, BBA Bioenergetics 1857(3), (2016), 247-255.
https://doi.org/10.1016/j.bbabio.2015.10.007

[2] S. Viola et al., Probing the electric field across thylakoid membranes in cyanobacteria, PNAS
116(43), (2019), 21900-21906. https://doi.org/10.1073/pnas.1913099116

[3] Y. Allahverdiyeva et al., Interplay between Flavodiiron proteins and photorespiration in *Synechocystis* sp. PCC 6803, JBC 286(27), (2011), 24007-24014.
https://doi.org/10.1074/jbc.M111.223289

O42

VDAC structure in mitochondrial connectivity and modulation

Vito De Pinto, G. Battiato, S. Conti-Nibali, Salvatore A. M. Cubisino, Francesca Guarino, Simona Reina

Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy

E-mail address: vito.depinto@unict.it
Presenting author: Vito De Pinto

For decades, the Voltage-Dependent Anion Channel (VDAC), formerly known as the mitochondrial porin, was considered a simple pore enabling nearly free permeability across the outer mitochondrial membrane. This simplified view has been progressively dismantled through the discovery of three mammalian isoforms (VDAC1, VDAC2, and VDAC3) with the gradual and often serendipitous attribution of diverse cellular roles beyond passive metabolite exchange. VDACs are now considered an important hub on the surface of the outer mitochondrial membrane, allowing cross-talk between the organelle and cytosol, and playing a role in apoptosis and other cell pathways[1] due to their interaction with over 154 proteins including metabolic enzymes, cytoskeletal proteins, apoptogenic factors, and intra-organelle receptors[2]. Recent advances in cryo-electron microscopy have catalyzed a breakthrough in VDAC research; in particular, the definition of the oligomeric structure of its assembly is reshaping our understanding of its role in mitochondrial physiology[3].
In light of such background, in this report we summarize the main research lines and the results produced in our laboratory:

(a) Identification of the specific functions of each VDAC isoform. Since human VDAC isoforms—VDAC1, VDAC2, and VDAC3—display 75% sequence similarity, we focused on their different composition of cysteines. We recently revealed several disulfide bonds, and we show that replacing all cysteine residues with alanine in human VDAC2 increases both the conductance and voltage-gating in response to positive potentials. Moreover, we suggest that cysteine residues are necessary to maintain VDAC2 protein stability. In human VDAC3, removal of cysteine residues completely reshapes the biophysical characteristics by turning small-sized and ungated channels into canonical high-conducting pores and, concurrently, confirms these amino acids are indispensable for VDAC3 to counteract ROS-induced oxidative stress.

(b) structural determination of VDAC interactions with small-molecule modulators and functional consequences. Thanks to an *AI-based* screening we selected a group of molecules built around a three-ring architecture that bind VDAC1 in the NADH-anchoring pocket. Electrophysiological, metabolic and organoid-based experiments show that they are highly effective in inducing cancer cell death[4]. Moreover, the metabolomic analysis reveals the bioenergetic consequences of VDAC inhibition.

(c) Herein, we aimed to decipher unambiguously the HK1-VDAC1 complex topology using crosslinking mass spectrometry. Accordingly, in vitro experiments were conducted using epitope-tagged recombinant proteins expressed in bacteria and purified through a combination of affinity and sizeexclusion chromatography. The challenging results will be shown.

[1] V. De Pinto, Renaissance of VDAC: new insights on a protein family at the interface between mitochondria and cytosol, Biomolecules 11 (2021) 107, https://doi.org/10.3390/biom11010107 [2] V. Shoshan-Barmatz, A. Shteinfer-Kuzmine, A. Verma, VDAC1 at the Intersection of
Cell Metabolism, Apoptosis, and Diseases, Biomolecules 10 (2020) 1485, https://doi.org/10.3390/ biom10111485

[3] H. Takeda et. Al., Oligomer-based functions of mitochondrial porin, Nat Commun. 16 (2025) 6854, https:// doi.org/10.1038/s41467-025-62021-4

[4] S. Conti-Nibali et al., VDAC1-interacting molecules promote cell death in cancer organoids through mitochondrial-dependent metabolic interference, iScience 27 (2024) 109853 https:// doi.org/ 10.1016/j.isci.2024.109853

O43

Atomic resolution structure of spinach rubisco reveals protons and dynamics

Nicholas Croy[a], Guillaume Gaullier[a], Patricia Saura[b], Simonas Masiulis[c], Asmit Bhowmick[d], Jan F. Kern[d], Oliver Raschdorf[e], Inger Andersson[e,f], Ville R. I. Kaila[b], Cecilia Blikstad[a], Johannes Messinger[a,g]

[a]*Department of Chemistry – Ångström, Uppsala University, Uppsala, Sweden.*
[b]*Department of Biochemistry and Biophysics, Stockholm University, Arrhenius Laboratories for Natural Sciences, Stockholm, Sweden.*
[c]*Materials and Structural Analysis Division, Thermo Fisher Scientific, Eindhoven, the Netherlands.*
[d]*Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA.*
[e]*Laboratory of Molecular Biophysics, Department of Cellular and Molecular Biology, Uppsala University, Uppsala, Sweden.*
[f]*The European Extreme Light Infrastructure, Dolni Brezany, Czech Republic.* [g]*Department of Plant Physiology, Umeå University, Umeå, Sweden.*

E-mail address: johannes.messinger@umu.se
Presenting author: Johannes Messinger

Photosynthetic organisms sustain life on Earth by storing solar energy in biomass. Central to this process is rubisco, the enzyme that catalyzes the fixation of CO_2 to ribulose-1,5-bisphosphate, providing the primary gateway for inorganic carbon into the biosphere. Rubisco's catalytic efficiency is a major determinant of crop productivity and global carbon flux, making it a longstanding target for protein engineering. Yet, attempts to enhance its performance through rational design have met limited success due to an incomplete understanding of rubisco's catalytic mechanism. Here, we report an atomic resolution (1.25 Å) cryo-EM structure of spinach rubisco in complex with the transition-state analogue 2-carboxyarabinitol-1,5-bisphosphate. Supported by large-scale quantum/classical (QM/MM) calculations, our structural analysis reveals protonation equilibria within the active site and unexpected structural flexibility across large protein regions despite the exceptionally tight ligand binding. Our findings provide new insight into the complex interplay of protonation equilibria and conformational sampling, suggesting a novel basis for rubisco's rational redesign utilizing strategies that rely on a combination of dynamic and electrostatic control.[1]

[1] N. Croy et al., Atomic resolution structure of spinach rubisco reveals protons and dynamics, bioRxiv (2025). https://doi.org/10.64898/2025.12.22.696010

O44

A novel mechanism of regulation of UCP1-mediated respiratory uncoupling

F. Gellé[1], R. Moussa[1], S. Masscheleyn[1], S. Gutche[2], A. Belicar[1], D. Castro[1], J. Hénin[2] and B. Miroux[1]

1. Biochimie des protéines membranaires, Université Paris Cité, CNRS, Institut de Biologie PhysicoChimique, Paris, France;
2. Laboratoire de Biochimie Théorique, Université Paris Cité, CNRS, Institut de Biologie PhysicoChimique, Paris, France;

Bruno.miroux@ibpc.fr
Presenting Author: Bruno Miroux
Topic: S9

Uncoupling Protein 1 (UCP1) dissipates energy as heat in brown adipose tissue, positioning it as a promising therapeutic target for metabolic disorders. In this study, we investigate the mechanism by which purine nucleotides inhibit UCP1-mediated respiration uncoupling. We previously identified two hydrophobic amino acids, I187 and W281, through molecular dynamics simulations and high-resolution respiration experiments. These residues, mutated in alanine, suppress the inhibitory effects of nucleotides on UCP1-dependent uncoupling in yeast mitochondria.

The UCP1(I187A, W281A) mutant protein was purified from yeast mitochondria and reconstituted into proteoliposomes. Proton transport assays revealed that the basal activity of the mutant matches the fatty acid-activated activity of the wild-type protein.

Using the cryo-EM structure of human UCP1, we compared the dynamics of ATP within the binding pocket of wild-type UCP1 and the UCP1(I187A, W281A) mutant. Our results demonstrate that ATP acts as a molecular switch, adopting two distinct poses: one that inhibits proton transport and another that permits it, even in the absence of fatty acids. In the UCP1(I187A, W281A) mutant, ATP predominantly adopts the non-inhibiting conformation, whereas in the wild-type protein, ATP favors the inhibiting conformation.

This model elucidates the basal uncoupling activity of UCP1 and proposes a novel mechanism for the competitive interaction between nucleotides and fatty acids.

1. Gagelin, A., Largeau, C., Masscheleyn, S., Piel, M.S., Calderón-Mora, D., Bouillaud, F., Hénin, J., and Miroux, B. (2023). Molecular determinants of inhibition of UCP1-mediated respiratory uncoupling. Nat. Commun. *14*, 2594. https://doi.org/10.1038/s41467-023-38219-9.

2. Moussa, R., Gellé, F., Masscheleyn, S., Pozza, A., Le Bon, C., Moncoq, K., Bonneté, F., and Miroux, B. (2025). Fine-tuning the yeast GAL10 promoter and growth conditions for efficient recombinant membrane protein production and purification. Protein Sci. Publ. Protein Soc. *34*, e70125. https://doi.org/10.1002/pro.70125.

O45

Deciphering far-red pigment absorption in chlorophyll-f-containing Photosystem II

Ho Fong Leong[a], Giovanni Consoli[a], Geoffry A. Davis[a], Ben Hancox-Lachman[a], Kenta Renard[a], Fiazall Tufail[a], Lauren E. Lee[a], Lucas Gautier[a], James W. Murray[a], Andrea Fantuzzi[a], A. William Rutherford[a] [a]*Department of Life Sciences, Imperial College London, London, SW7 2AZ, United Kingdom*

E-mail address: ho.leong18@imperial.ac.uk
Presenting author: Ho Fong Leong

Oxygenic photosynthesis relies mainly on chlorophyll *a* for both light-harvesting and photochemistry. However, other types of chlorophyll exist to broaden the absorbance and photochemical capabilities of the cell. In particular, chlorophylls *d* and *f* are found in a diverse set of cyanobacteria that is capable of acclimating to far-red light (700 - 800 nm) via a process known as far-red light photoacclimation.[1,2] During this process, certain subunits of Photosystems are replaced with far-red homologues that bind these chemically red-shifted chlorophylls in specific positions. For far-red Photosystem II, subunits D1, D2, CP43, CP47, and sometimes PsbH are replaced, allowing the incorporation of some chlorophyll f molecules and one chlorophyll *d*. When incorporated into Photosystem II, these pigments are spectrally distinct from the manifold of chlorophyll *a* and from each other.[2] Here, we present a comparative study of far-red Photosystem II from *Chroococcidiopsis thermalis* PCC 7203 and *Calothrix* sp. NIES-3974 using cryo-electron microscopy and low-temperature spectroscopy. Based on sequence comparisons and electrostatic potential analyses, four chlorophyll f positions are assigned in *C. thermalis*, with one differing from previous reports in the literature.[3,4] An additional, previously undetected far-red subunit is also identified. Conversely, in *Calothrix* sp., the same analyses show that two of the long wavelength chlorophyll *f* sites, including the newly assigned chlorophyll *f* in *C. thermalis,* are absent. By comparing these two variants of far-red Photosystem II, absorption wavelengths are specifically assigned to all the far-red chlorophylls. Due to spectral overlap, this was previously not possible in "canonical" chlorophyll-*a*-only photosystems. This work highlights and makes use of the genetic diversity and the conserved features of far-red light photoacclimation to deconvolve pigment absorption in far-red Photosystem II, and lays the basis for modelling excitation energy transfer in the system.

[1] F. Gan, S. Zhang, N.C. Rockwell, S.S. Martin, J.C. Lagarias, D.A. Bryant, Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. Science, 345(6202), (2014), 1312–1317. https://doi.org/10.1126/science.1256963

[2] D.J. Nürnberg, J. Morton, S. Santabarbara, A. Telfer, P. Joliot, L.A. Antonaru, A.V. Ruban, T. Cardona, E. Krausz, A. Boussac, A. Fantuzzi, A.W. Rutherford, Photochemistry beyond the red limit in chlorophyll f-containing photosystems. Science, 360(6394), (2018), 1210–1213. https://doi.org/10.1126/science.aar8313

[3] C.J. Gisriel, G. Shen, M.Y. Ho, V. Kurashov, D.A. Flesher, J. Wang, W.H. Armstrong, J.H. Golbeck, M.R. Gunner, D.J. Vinyard, R.J. Debus, G.W. Brudvig, D.A. Bryant, Structure of a monomeric photosystem II core complex from a cyanobacterium acclimated to far-red light reveals the functions of chlorophylls d and f. The Journal of biological chemistry, 298(1), (2022), 101424. https://doi.org/10.1016/j.jbc.2021.101424

[4] C.J. Gisriel, G. Shen, D.A. Flesher, V. Kurashov, J.H. Golbeck, G.W. Brudvig, M. Amin, D.A. Bryant, Structure of a dimeric photosystem II complex from a cyanobacterium acclimated to far-red light. The Journal of biological chemistry, 299(1), (2023), 102815. https://doi.org/10.1016/j.jbc.2022.102815

O46

Acyl-CoA binds directly to UCP1 to interplay with nucleotide and fatty acid regulation of its uncoupling activity

Scott A. Jones*[b], Hannah R. Staggs-Sandy*[a], Danielle M. Copeman[a], Eric Y.C. Mao[b], Martin S. King[b], Mehmethan Aris[a], Callum Eke[a], Edmund R.S. Kunji[b], and Paul G. Crichton[a]

[a]*Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, United Kingdom*

[b]*MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge Biomedical Campus, Cambridge, United Kingdom*

*These authors contributed equally

E-mail address: ersk2@cam.ac.uk, P.Crichton@uea.ac.uk
Presenting author: Paul G. Crichton

Uncoupling Protein 1 (UCP1) catalyses proton leak across the mitochondrial inner membrane of brown adipose tissue to facilitate heat production for thermoregulation in mammals[1]. Encouraging UCP1-dependent energy expenditure is a potential avenue to help combat metabolic disease. During the stimulation of brown adipocytes, fatty acids are generated from triglyceride stores to both fuel thermogenesis and directly activate UCP1, overcoming its inhibition by cytosolic nucleotides. The free fatty acids are converted to acyl-CoA species ahead of shuttling into the mitochondrial matrix to be broken down by beta-oxidation. Historically, there has been some evidence that acyl-CoA species may influence UCP1 activity, but outcomes have been inconsistent and a clear role in UCP1 regulation has not been established (e.g. refs [1,2,3]). How ligands interplay to regulate UCP1 and potentially involve metabolites additional to fatty acids and nucleotides is not clear.
Here, we reveal a potent interaction of acyl-CoA with UCP in molecular detail. Ligand interaction and thermostability analysis shows that key species among variants interact strongly with isolated UCP1, impacting underlying bonding and nucleotide binding. Proton leak measurements detail the effects of acyl-CoA on UCP1 activity, clarifying an interplay with established ligand regulators to modulate proton conductance. We present the structure of UCP1 with bound palmitoyl-CoA, determined by cryogenic electron microscopy, revealing the positions of the CoA moiety as well the acyl chain, both bound in the central cavity. The latter provides the first clue how fatty acids may bind to the water-filled cavity. Hence, we propose that acyl-CoA species may represent a distinct regulator of UCP1 in the acute control of brown fat thermogenic energy expenditure.

[1] D.G. Nicholls, Mitochondrial proton leaks and uncoupling proteins, Biochim Biophys Acta Bioenerg 1862(7) (2021) 148428, https://doi.org/10.1016/j.bbabio.2021.148428

[2] B. Cannon, U. Sundin, L. Romert, Palmitoyl coenzyme A: a possible physiological regulator of nucleotide binding to brown adipose tissue mitochondria. FEBS Lett. 74 (1977) 43–6, https:// doi.org/10.1016/0014-5793(77)80748-5

[3] P. Ježek, J. Houstek, Z. Drahota, Alkaline pH, membrane potential, and magnesium cations are negative modulators of purine nucleotide inhibition of H^+ and Cl^- transport through the uncoupling protein of brown adipose tissue mitochondria, J. Bioenerg. Biomembr. 20 (1988) 603–622, https:// doi.org/10.1007/BF00768922

O47

Uncovering the role of Flv3 in nitrogen metabolism in *Synechocystis spp*. PCC 6803 Samuele Perin[a], Tuomas Huokko[a], Yagut Allahverdiyeva[a]

[a] *PhotoMicrobes group, Molecular Plant Biology, University of Turku, Finland*

E-mail address: samuele.perin@utu.fi
Presenting author: Samuele Perin

Abstract

Flavodiiron proteins (FDPs; also referred to as flavoproteins, Flvs) are modular enzymes widely distributed in Bacteria and Archaea. The evolution of cyanobacteria and oxygenic photosynthesis was accompanied by the functional diversification of canonical bacterial FDPs. In cyanobacteria, FDPs typically consist of three domains: a β-lactamase-like domain, a flavodoxin-like domain, and a flavin reductase–like domain. In the model cyanobacterium *Synechocystis spp*. PCC 6803 (*Synechocystis*), four FDPs (Flv1–4) form hetero-oligomeric complexes (Flv1/3 and Flv2/4) that catalyse the four-electron reduction of O_2 to H_2O downstream of PSI, using reduced Fed as the electron donor (Helman et al., 2003, Allahverdiyeva et al., 2013, Santana-Sanchez et al., 2019, Nikkanen et al. 2024). This reaction functions as a safety valve, preventing over-reduction of the photosynthetic electron transport chain and limiting the formation of reactive oxygen species. Importantly, Flv3 homo-oligomers do not reduce O_2, but they still contribute to photoprotection by facilitating the recovery of CO_2 fixation and P700 oxidation under fluctuating light conditions (Mustila et al., 2016). This observation suggests that FDPs participate in additional, as yet uncharacterized, electron transfer pathways beyond O_2 photoreduction. Our recent BiFC assays, revealing an interaction between Flv3 and the large isoform of FNR_L (Nikkanen et al., 2024), open up the possibility of a NADPH-dependent uncharacterized pathway.

We uncover a previously unrecognized role of Flv3 in nitrogen metabolism. We show that loss of Flv3 abolishes growth when arginine is supplied as the sole nitrogen source, a phenotype not observed in wild-type or other FDP-deficient strains. Using advanced biophysical and biochemical approaches available through the Fin-BioFoundry infrastructure in our laboratory, we performed a comprehensive characterization of the Δ*flv3* mutant. Despite its severe growth defect, Δ*flv3* cells remain viable and maintain oxygen evolution rates comparable to the wild type. However, although they do not fully reproduce nitrogen deprivation responses, they exhibit features of nitrogen starvation, including chlorosis and phycobilisome degradation. Targeted metabolomic analyses further reveal disruptions in nitrogen assimilation pathways, suggesting the presence of a metabolic bottleneck underlying the observed phenotype.

Together, our findings establish a previously unrecognized link between flavodiiron proteins and arginine catabolism and may also play a signaling role in nitrogen assimilation. These insights expand our understanding of the integration between photosynthetic electron transport and nitrogen metabolism in cyanobacteria and may inform future strategies to enhance productivity in crops and microalgal systems.

Reference List

Allahverdiyeva, Y., Mustila, H., Ermakova, M., Bersanini, L., Richaud, P., Ajlani, G., Battchikova, N., Cournac, L., & Aro, E.-M. (2013). Flavodiiron proteins Flv1 and Flv3 enable cyanobacterial growth and photosynthesis under fluctuating light. *Proceedings of the National Academy of Sciences*, *110*(10), 4111–4116. https://doi.org/10.1073/pnas.1221194110

Helman, Y., Tchernov, D., Reinhold, L., Shibata, M., Ogawa, T., Schwarz, R., Ohad, I., & Kaplan, A. (2003). Genes Encoding A-Type Flavoproteins Are Essential for Photoreduction of O2 in Cyanobacteria. *Current Biology*, *13*(3), 230–235. https://doi.org/10.1016/S0960-9822(03)00046-0

Mustila, H., Paananen, P., Battchikova, N., Santana-Sánchez, A., Muth-Pawlak, D.,

Hagemann, M., Aro, E.-M., & Allahverdiyeva, Y. (2016). The Flavodiiron Protein Flv3 Functions as a Homo-Oligomer During Stress Acclimation and is Distinct from the Flv1/Flv3 Hetero-Oligomer Specific to the O2 Photoreduction Pathway. *Plant and Cell Physiology*, *57*(7), 1468–1483.

https://doi.org/10.1093/pcp/pcw047

Nikkanen, L., Vakal, S., Hubáček, M., Santana-Sánchez, A., Konert, G., Wang, Y., Boehm, M., Gutekunst, K., Salminen, T.A. and Allahverdiyeva, Y. (2025), Flavodiiron proteins associate pH-dependently with the thylakoid membrane for ferredoxin-1-powered O2 photoreduction. New

Phytol, 246: 2084-2101. https://doi.org/10.1111/nph.70114

Santana-Sanchez, A., Solymosi, D., Mustila, H., Bersanini, L., Aro, E.-M., & Allahverdiyeva, Y. (2019). Flavodiiron proteins 1–to-4 function in versatile combinations in O2 photoreduction in cyanobacteria. *eLife*, *8*, e45766. https://doi.org/10.7554/eLife.45766

O48

Redox-Driven CO_2 Hydration by NDH-1: Long-Range Coupling in a Divergent Complex I System

Zhifen Zhang, Minquan Zhang, Swarnali Mukherjee, Robert L Burnap
Department of Microbiology & Molecular Genetics, Oklahoma State University, Stillwater OK, USA

E-mail address: rob.burnap@okstate.edu
Presenting author: Rob Burnap

Complex I–type enzymes represent an ancient and modular family of bioenergetic machines that couple long-range electron transfer to proton translocation across membranes. While their canonical role in respiration is well established, divergent members of this family have evolved specialized coupling functions that extend beyond proton motive force generation. Cyanobacterial NDH-1 complexes provide a compelling example, where variant isoforms (NDH-1_3/1_4) incorporate CO_2 uptake proteins (Cup) proteins[1] that enable redox-driven CO_2 hydration within the CO_2 concentrating mechanism, that is essential for high-efficiency photosynthesis [2].

To probe this coupling, we engineered a *Synechococcus elongatus* PCC7942 strain expressing exclusively the NDH-1_4 complex. Inhibition of the CupB active site using ethoxzolamide (EZ) not only reduced CO_2 hydration activity, but also slowed cyclic electron flow, as measured by delayed Photosystem 1 re-reduction, and abolished proton pumping monitored by acridine orange fluorescence[3]. These results provide direct evidence that perturbation of CO_2 hydration at a distal Zn site propagates through the complex to influence electron transfer and proton translocation.

These observations support a model in which redox energy released during plastoquinone reduction is transmitted over ~150 Å through proton-coupled pathways embedded within the membrane domain. Two mechanistic interpretations are considered. In a “product removal” framework, proton transfer through reconfigured pathways favors vectorial CO_2 hydration by removing protons from the catalytic site. Alternatively, a “trapping” mechanism posits transient stabilization of protons within proton-loading sites, without obligatory transmembrane release. Both models invoke adaptations of the ancestral proton-pumping architecture, yet differ in how proton flux is partitioned. Evidence for a mechanism involving the trapping of the bicarbonate product and its energetic release involving modified proton pumping will be presented and discussed in terms of energetic analogies to ATP binding and release by ATP synthase.

The findings highlight general principles of long-range proton-coupled electron transfer and illustrate how modular elements of Complex I can be repurposed to support new bioenergetic functions.

[1] M. Shibata, H. Ohkawa, T. Kaneko, H. Fukuzawa, S. Tabata, A. Kaplan, T. Ogawa, Distinct constitutive and low-CO_2-induced CO_2 uptake systems in cyanobacteria: genes involved and their phylogenetic relationship with homologous genes in other organisms, Proceedings of the National Academy of Sciences of the United States of America, 98 (2001) 11789-11794.

[2] R.L. Burnap, M. Hagemann, A. Kaplan, Regulation of CO_2 concentrating mechanism in cyanobacteria, Life (Basel), 5 (2015) 348-371. https://www.mdpi.com/2075-1729/5/1/348

[3] Z. Zhang, M. Zhang, R.L. Burnap, Action at a distance: The remarkable coupling of CO2 uptake to electron transfer in specialized cyanobacterial NDH-1 complexes, Proceedings of the National Academy of Sciences, 122 (2025) e2511786122. https://doi.org/10.1073/pnas.2511786122

Talk – T

O49

Aralar couples metabolic state to mitochondrial cristae organization through Opa1

Luca Scorrano[a,b]

[a] *Department of Biology, University of Padova, Via Ugo Bassi 58/B, 35121, Padova, Italy*
[b] *Veneto Institute of Molecular Medicine (VIMM), Via Orus 2, 35129, Padova, Italy*
E-mail address: luca.scorrano@unipd.it
Presenting author: Luca Scorrano

Mitochondria adapt to cellular cues through continuous cycles of fusion and fission that define their architecture. Yet, the mechanisms linking metabolic state to mitochondrial dynamics remain poorly understood. Through MS-based complexomic screening, we identified the Ca^{2+}-sensitive aspartate–glutamate carrier Agc1 as an interactor of Opa1, a key regulator of mitochondrial fusion and cristae organization. Agc1, a component of the malate–aspartate shuttle, dimerizes to efficiently transfer glycolysis-derived NADH into mitochondria. We find that Agc1 dimerization is driven by high glucose, coupling elevated glycolytic NADH to mitochondrial function. Loss of Agc1 reduces mitochondrial NADH accumulation, dissipates membrane potential under starvation, and impairs respiration. Reconstitution with dimerization-deficient or Ca^{2+}-insensitive Agc1 mutants uncovers the structural requirements necessary to sustain mitochondrial activity and cell viability. Notably, Agc1 ablation leads to rapid cristae disruption upon starvation, accompanied by reduced Opa1 oligomerization. Together, these findings identify Agc1 as a metabolic sensor that links fuel availability to mitochondrial cristae remodeling through its dimerization and interaction with Opa1.

O50

Evolutionary incremental complexity in the formation of the cytochrome c oxidase catalytic center

Michele Brischigliaro[a,b], Suleva Povea-Cabello[a,b], Kristýna Čunátová[a,b,c], Alfredo CabreraOrefice[d,e], Jimin Pei[f], Cinzia Franchin[a], Marco Roverso[a], Sara Bogialli[a], Giorgio Arrigoni[a],
Susanne Arnold[g], Qian Cong[f], Massimo Zeviani[a,b,h], Carlo Viscomi[a,b,i], Erika FernándezVizarra[a,b,j]

[a]University of Padova, Padua, Italy. [b]Veneto Institute of Molecular Medicine, Padua, Italy. [c]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic. [d]Goethe University Frankfurt, Frankfurt am Main, Germany. [e]Justus-Liebig-Universität Gießen, Giessen, Germany. [f]University of Texas Southwestern Medical Center, Dallas, TX, USA.
[g]University of Cologne, Cologne, Germany. [h]IRCCS Women and Children's Hospital "Burlo-Garofolo", Trieste, Italy. [i]University of Milano, Milan, Italy. [j]University of Zaragoza, Huesca, Spain.

E-mail address: erikafv@unizar.es
Presenting author: Erika Fernández-Vizarra

Cytochrome c oxidases (CcO) are the terminal oxidases in electron transfer systems performing respiration, thus, catalysing the oxidation of cytochrome c and the reduction of molecular oxygen to water. The mitochondrial enzyme, also known as complex IV (cIV), is located in the inner membrane and is a copper-heme oxygen reductase containing cytochromes a and a3, evolutionarily related to the CcO of aerobic bacteria. During evolution there has been an increase in the complexity of CcO, having augmented subunit number from three in the bacterial enzymes, to fourteen in human cIV. In addition, eukaryotes have developed assembly factors to coordinate metal center insertion and to chaperone multi-subunit assembly intermediates. Heme A synthesis is a requirement for CcO activity both in bacteria and in mitochondria, where the first step, i.e., the transformation of heme B to heme O is catalysed by conserved heme O synthase enzymes, the activity of which is performed in mitochondria by a protein denominated COX10 [1, 2]. By combining proteomics, bioinformatics and functional approaches we have found that in human mitochondria, COX10 interacts specifically with COA8, a protein only present in metazoan organisms whose loss-of-function causes mitochondrial cIV deficiency and leukoencephalopathy [3, 4]. The exact molecular role of COA8 remained enigmatic as well as the molecular basis of its redox-mediated regulation [4]. In this work we have determined that, in human mitochondria, the functional interaction of COX10 with COA8 is necessary to sustain appropriate heme A levels and, consequently, for proper cIV assembly and function. Therefore, cIV biogenesis in higher eukaryotes appears to involve enhanced regulatory complexity, enabling dynamic adaptation of respiratory activity to meet escalating energy requirements or respond to metabolic stress.

[1] Tzagoloff, A., et al., On the functions of the yeast COX10 and COX11 gene products. Biochem Mol Biol Int, 1993. 31(3): p. 593-8.

[2] Valnot, I., et al., A mutation in the human heme A:farnesyltransferase gene (COX10) causes cytochrome c oxidase deficiency. Hum Mol Genet, 2000. 9(8): p. 1245-9.

[3] Melchionda, L., et al., Mutations in APOPT1, Encoding a Mitochondrial Protein, Cause Cavitating Leukoencephalopathy with Cytochrome c Oxidase Deficiency. Am J Hum Genet, 2014. 95(3): p. 315-25.

[4] Signes, A., et al., APOPT1/COA8 assists COX assembly and is oppositely regulated by UPS and ROS. EMBO Mol Med, 2019. 11(1).

O50

Computer simulations of molecular gates and switches in the membrane domain of respiratory complex I

Vivek Sharma[a,b], Amiel Abettan[a], Mahdi Torabi[a], Jasmine E. Aaltonen[a], Erik Endres[a]

[a] *Department of Physics, University of Helsinki, Helsinki, Finland*
[b] *HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

E-mail address: vivek.sharma@helsinki.fi
Presenting author: Vivek Sharma

Respiratory complex I is a large redox-driven proton pump. Despite numerous cryo-EM, mutagenesis and computer simulation studies, several fundamental questions on its mechanism remain unanswered.[1] Among these, one key unresolved issue is how complex I transfers protons in its ~150 Å long membrane domain and what kind of gates and switches are needed to ensure the directionality of proton transfer. Two such putative gating elements that span its membrane domain are; a histidine switch[2,3] and a tyrosine gate[4]. Using enhanced sampling MD simulations, hybrid QM/MM and free energy calculations on high-resolution cryo-EM structures, we show that these molecular switches can play an important role in proton directionality. The gating and switching functions are found to be driven by tautomerism, charge-change based hydrogen bond restructuring and long-range electrostatic effects. The simulation-based findings advance our mechanistic understanding of respiratory complex I.

[1] A. Djurabekova, J. Lasham, O. Zdorevskyi, V. Zickermann, V. Sharma, Long-range electron proton coupling in respiratory complex I — insights from molecular simulations of the quinone chamber and antiporter-like subunits. Biochemical Journal 481(7) (2024) 499-514, https://doi.org/10.1042/BCJ20240009.

[2] E., Endres, M. Torabi, M. Jousmäki, K. V. Huynh, C. Pecorilla, O. Zdorevskyi, V. Zickermann, V. Sharma, Molecular and Energetic Basis of Histidine Switch Dynamics in Respiratory Complex I.
bioRxiv 2026-01 (2026), https://doi.org/10.64898/2026.01.12.698557.

[3] C. Pecorilla, A. Altmeyer, O. Haapanen, Y. Lee, V. Zickermann, V. Sharma, Conformational dynamics of a histidine molecular switch in a cation/proton antiporter. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1866(4) (2025) 149563, https://doi.org/10.1016/j.bbabio.2025.149563.

[4] L. Simsive, O. Zdorevskyi, V. Sharma, Proton Transfer through a Charged Conduit in Respiratory Complex I: Long-Range Effects and Conformational Gating. Journal of Chemical
Information and Modeling 65(19) (2025) 10600-10612, https://doi.org/10.1021/acs.jcim.5c01365.

O52

From Damage to Recovery: Stress-Dependent Maintenance Turnover of Mitochondrial OXPHOS Complexes

Paulina Castañeda-Tamez[a], Ilka Wittig[a,b]

a Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Germany
b Goethe University, Center for Functional Proteomics, Germany

E-mail address: wittig@med.uni-frankfurt.de
Presenting author: Ilka Wittig

Maintenance of mitochondrial oxidative phosphorylation (OXPHOS) complexes is crucial for sustaining bioenergetic function, especially in differentiated and post-mitotic cells. While de novo assembly pathways have been extensively studied, growing evidence suggests that OXPHOS complexes are continuously maintained through selective turnover and repair processes. Defects in these quality control pathways are associated with human disease, emphasising the importance of coordinated chaperone- and protease-driven maintenance by mitochondrial service factors. However, the physiological triggers that initiate these processes are still largely unknown. Here, we combined complexome profiling with pulse-SILAC labelling to investigate how defined mitochondrial stress conditions shape the transition from damage to recovery in OXPHOS complex maintenance turnover. Preliminary data from a human cell line (HEK) were obtained by applying mitochondrial stressors, including transient inhibition of mitochondrial translation, redox stress, and combined OXPHOS inhibition, followed by pulse labelling to detect recovery-related incorporation of newly synthesised proteins within native protein complexes. Different stress conditions produced markedly distinct turnover signatures. Transient inhibition of mitochondrial translation caused a moderate but consistent increase in the incorporation of newly synthesised proteins across multiple OXPHOS complexes, consistent with compensatory recovery-phase replacement. Conversely, combined OXPHOS inhibition resulted in a pronounced loss of complex abundance together with strongly reduced incorporation of newly synthesised subunits, indicating impaired recovery and suppressed replacement capacity. Under the tested conditions, redox stress caused only minor effects on assembled OXPHOS complexes. Overall, these findings suggest that mitochondrial maintenance is not constant but depends on the type of stress stimulus, defining whether OXPHOS complexes undergo effective recovery via maintenance turnover or fail to be replaced. Ongoing research in differentiated cells (e.g. C2C12 myotubes) and mouse tissues will investigate how these mechanisms function in post-mitotic systems and support long-term mitochondrial health.

Protonation states defining quinone binding and catalysis in respiratory complex I Guilherme M. Arantes[a,b], Carolina S. Pereira[a], Injae Chung[c], John J. Wright[c], Judy Hirst[c]

[a]Department of Biochemistry, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil [b]Department of Biology, Massachusetts Institute of Technology, Cambridge, USA [c]MRC Mitochondrial Biology Unit, University of Cambridge, The Keith Peters Building, Cambridge Biomedical Campus, Cambridge, UK

E-mail address: garantes@iq.usp.br

O53

Presenting author: Guilherme M. Arantes

Respiratory complex I couples NADH oxidation to quinone (Q) reduction and drives proton translocation across the mitochondrial inner membrane. Despite the availability of high-resolution structural data, the molecular details underlying quinone reactivity and its coupling to proton transfer remain unresolved, largely due to the dynamic nature of protonation equilibria and substrate binding. Here, we combine enhanced-sampling simulations with constant-pH molecular dynamics to determine the protonation states of key catalytic residues in representative cryo-EM structures of mammalian complex I along its catalytic cycle. Our results indicate that residues at the Q-headgroup binding site and near the N2 FeS cluster adopt well-defined protonation patterns: Tyr, His, and Asp are in the states (TyrH, His, AspH) prior to reaction with bound ubiquinone[1], and transition to (Tyr , His, Asp) upon quinone reduction, consistent with a net two-proton transfer and^{-} $^{-}$ formation of ubiquinol. By integrating these predictions with structural data capturing Q in multiple binding poses, we propose a detailed sequence of binding intermediates traversed by quinone during reduction[2]. Extending this analysis, we characterize the protonation states of numerous residues within the Q-binding cavity and the membrane domain. This enables the identification of control points that may regulate proton flux and couple quinone chemistry to proton pumping.

[1] Chung I, Wright JJ, Bridges HR, Ivanov BS, Biner O, Pereira CS, Arantes GM, Hirst J, Cryo-EM structures define ubiquinone-10 binding to mitochondrial complex I and conformational transitions accompanying Q-site occupancy, Nat. Comm. 13, (2022), 2758, https://doi.org/10.1038/s41467022-30506-1

[2] Chung I, Pereira CS, Wright JJ, Arantes GM, Hirst J, Post-catalysis structures of mitochondrial complex I with ubiquinol-10 bound in the active site, Nat. Comm. 17, (2026), https://doi.org/10.1038/s41467-026-70030-0

O54

The Dynamics of Proton Transport Processes on the Surface of Biological Membranes Nadav Amdursky[a]

aChemistry, School of Mathematical and Physical Sciences, The University of Sheffield, UK

E-mail address: n.amdursky@sheffield.ac.uk

It is well established that the surface of biological membranes plays a fundamental role in the proton circuitry of bioenergetic systems, bridging proton producers to proton consumers, thereby expending Mitchell's chemiosmotic theory. In this talk, I will detail the latest developments from my group concerning the use of light-triggered fluorescent probes that can be tethered into the biological membrane and can either deprotonate or protonate following light excitation. These molecules are usually referred to as photoacids and photobases. Using the newly synthesised molecular probes, we investigate the role of the membrane as either a proton acceptor to the photoacid probe or a proton donor for the photobase probe. We primarily use ultrafast fluorescence to investigate the ultrafast proton transfer reaction between the photoacid/photobase and its membrane environment, as well as slower processes that can be correlated with proton diffusion around the probe. We find that the membrane composition, fluidity, and phase have a large impact on its ability to serve as a proton source/sink. We further demonstrate that the activity of reconstituted ATP synthase highly influences proton transfer reactions on the surface of the membrane, whereas the proton sink capability of an active ATP synthase induces an ultrafast proton transfer from the photoacid to the membrane surface.

[1] N. Amdursky, Y. Lin, N. Aho, and G. Groenhof, Exploring fast proton transfer events associated with lateral proton diffusion on the surface of membranes, Proc. Natl. Acad. Sci. USA 116, 2443-2451 (2019).

[2] A. Ramanthrikkovil Variyam, M. Rzycki, A.A. Stuchebrukhov, D. Drabik, and N. Amdursky, Proton diffusion on the surface of mixed lipid membranes highlights the role of membrane composition, Biophys. J., 123, 4200-4210 (2024).

[3] A. Ramanthrikkovil Variyam, M. Rzycki, R. Nandi, A.A. Stuchebrukhov, D. Drabik, and N. Amdursky, The role of lipid phase and temperature in proton barrier and proton migration on biological membranes, Chem. Sci., 16, 5194 - 5204 (2025).

[4] H. Flegel, A. Ramanthrikkovil Variyam, N. Amdursky, and C. Steinem, ATP Synthase Activity Boosts Membrane Proton Acceptance and Lateral Diffusion, Proc. Natl. Acad. Sci. USA, 123, e2510444123 (2026).

Contribution type:
T

O55

Cryo-EM reveals the gating mechanism of ATP synthase leak channel and its role in mitochondrial permeability transition

Amrendra Kumar[1], Juliana da Fonseca Rezende e Mello[1], Yangyu Wu[2], Louis Betz[1],

Chris Palles[1], Nelli Mnatsakanyan[1, 3*]

[1]Department of Cell and Biological Systems, Penn State College of Medicine, Hershey, PA, USA

[2]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT, USA

[3]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT,

USA

E-mail address: nvm5654@psu.edu

Presenting author: Nelli Mnatsakanyan, PhD

The mitochondrial F_1F_o ATP synthase is a unique enzyme that uses rotation of its own subunits to synthesize ATP. ATP synthase plays a vital role in cell survival and death by tightly regulating its catalytic and leak-channel activities. The catalytic mechanisms of ATP synthesis and hydrolysis have been extensively studied over the past few decades. However, the gating and regulatory mechanisms of the ATP synthase c-subunit leak channel (ACLC) and its role in the mitochondrial permeability transition (mPT) remain unclear. In this work, we investigated the effects of various compounds of therapeutic significance on the ACLC activity and mPT-induced cell death. Single-particle cryoelectron microscopy (cryo-EM) and planar lipid bilayer electrophysiological recordings were employed to investigate the gating mechanism of ACLC using purified porcine heart ATP synthase. The obtained cryo-EM structures of ATP synthase bound to different compounds suggest a generalizable activation mechanism for ACLC that involves structural distortions of the c-ring and deviations from its symmetry. These findings will enable the development of structure-based drug design targeting ATP synthase to treat mPT-induced diseases.

Topic list: S6. ATP synthase and ATPases

Contribution type: Talk – T

O56

ComplexomA: a user-friendly R/Shiny platform for comprehensive analysis and visualization of complexome profiling data

Jan Dreute[a], Alfredo Cabrera-Orefice[a]

[a] *Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University, Giessen, Germany*

E-mail address: alfredo.cabrera@biochemie.med.uni-giessen.de
Presenting author: Alfredo Cabrera-Orefice

Complexome profiling has emerged as a powerful mass spectrometry-based approach to investigate the composition, assembly and dynamics of protein complexes, particularly providing valuable insights into mitochondrial biology [1]. Despite its potential, downstream data analysis remains a major bottleneck due to heterogeneous outputs from different proteomics workflows and the lack of complete, user-friendly computational solutions.

To address these limitations, we developed “ComplexomA”, an R-based software platform that delivers a complete, user-friendly, one-stop solution for the analysis of standard complexome datasets. Implemented as an interactive Shiny application, ComplexomA integrates data processing, visualization, and interpretation within a single environment, effectively lowering the barrier for comprehensive complexome analysis. The tool supports direct import and post-processing of output files from widely used proteomics pipelines, including MaxQuant, FragPipe and DIA-NN, and generates post-processed outputs compatible with spreadsheet-based visualization (e.g., Excel).

ComplexomA offers extensive functionality for data curation, normalization and near-complete flexibility in profile editing, enabling refined protein migration patterns and improved data quality. Multiple approaches are implemented to identify co-migrating proteins, alongside diverse visualization methods such as heatmaps, line plots, t-SNEs and UMAP representations. Notably, our tool enables the rapid generation of publication-quality figures, significantly accelerating the path from data acquisition to dissemination.

Importantly, ComplexomA integrates curated protein complex resources, including MitoCarta 3.0, CORUM and Complex Portal, enabling automated mapping to known complexes. It further retrieves functional annotations, including subcellular localization and Gene Ontology terms, directly from UniProt, enhancing biological interpretation. The platform supports identification of curated complexes, prediction of novel assemblies and comparative analyses across experimental conditions.

ComplexomA is designed as a continuously evolving platform, with planned support for quantitative proteomics strategies such as SILAC and TMT. The tool will be openly released via GitHub and will actively incorporate user feedback and community-driven feature development, with the goal of establishing a comprehensive and widely adopted standard for complexome data analysis.

[1] A. Cabrera-Orefice, A. Potter, F. Evers, J.F. Hevler, S. Guerrero-Castillo, Complexome Profiling—Exploring Mitochondrial Protein Complexes in Health and Disease. Front. Cell Dev. Biol. 9 (2022) 796128. https://doi.org/10.3389/fcell.2021.796128

O57

Analysis of proton transport in *E. coli* and *Pseudomonas aeruginosa* ATP synthases P. Ryan Steed[a], Amy E. Defnet[a], William J. Fairley VI[a], Juan A. Gaspar-Rivera[a], Sam J. Shepard[a], Ariel D. Stewart[a] [a]*University of North Carolina Asheville, Asheville, North Carolina, USA*

E-mail address: psteed@unca.edu
Presenting author: P. Ryan Steed

Bacterial bioenergetics is an emerging target for novel antibiotics against multidrug resistant infections. *Pseudomonas aeruginosa* (PA), a Gram-negative bacterium identified as a serious threat by world health agencies, requires a functional ATP synthase to catalyze the transduction of the H^+ gradient to the chemical energy of ATP even under anaerobic growth conditions. Previous work has demonstrated that inhibitors of PA ATP synthase prevent growth of multidrug resistant strains of PA, and the recently determined cryo-EM structure of PA ATP synthase revealed distinct features of the enzyme that may be targeted by future classes of inhibitors. One such feature is a novel metal coordination site in the N-side H^+ half channel of the membrane-embedded F_o sector, which appears to plug the channel.[1] To further understand the H^+ transport mechanism and how it differs from the better-characterized *E. coli* ATP synthase, we are exploring the unique H^+ translocation pathway by assaying *in vitro* gradient-driven ATP synthesis, ATP-driven H^+ pumping, and passive H^+ permeability activities of subunit *a* mutants. We have identified residues in the Nside channels of PA and *E. coli* F_o where mutations asymmetrically alter H^+ pumping and ATP synthesis. Furthermore, mutations of the metal binding site in PA altered H^+ pumping activity consistent with the hypothesis that the bound metal ion in PA F_o is regulating the direction of H^+

movement. These results further define the roles of N-side channel residues in H^+ translocation and further define a potential antibiotic target within bacterial ATP synthases.

[1] M. Sobti, A.P. Gunn, S.H.J. Brown, L. Zavan, V.F. Fraunfelter, A.L. Wolfe, C.A. McDevitt, P.R. Steed, A.G. Stewart. Distinct structural features of Pseudomonas aeruginosa ATP synthase revealed by cryo-electron microscopy. Nat. Commun. 17,(2026) 406. https://doi.org/10.1038/ s41467-025-67100-0

Ultrastructural mapping of an anaerobic microbial eukaryote lacking a canonical FeS biogenesis system

O58

Princess R. Cabotaje[a,b], Anastasiia Borodina[b], Yumiko Masukagami[b], Michelle Leger[b], Nikolaj Brask[a], Roxanne A. Beinart[c], Hidetaka Nomaki[d], Filip Husnik[b], Courtney Stairs[a]

[a]*Department of Cell and Molecular Biology (ICM), Molecular Evolution, Uppsala University, 751 24, Uppsala, Sweden* [b]*Evolution, Cell Biology, and Symbiosis Unit, Okinawa Institute of Science and Technology, 904-0495, Okinawa, Japan*

[c]*Graduate School of Oceanography, University of Rhode Island, Narragansett, 02882, Rhode Island, USA*

[d]*Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Kanagawa, 237-0061, Japan*

E-mail address: princess.cabotaje@icm.uu.se
Presenting author: Princess R. Cabotaje

Protists represent approximately 70% of all eukaryotic species, yet arguably remain the leaststudied fraction of the entire tree of life.[1] Some depend on mitochondrion-related organelles (MROs) and prokaryotic symbionts as adaptations to low oxygen conditions, with electrons typically exchanged via H_2 (*i.e.*, syntrophy).[2,3] H_2-mediated syntrophy fuels the metabolism of prokaryotic partners, which produce key molecules of major geochemical cycles, and is hypothesized to be important in driving transition to anaerobic lifestyle.[3,4] However, the (ultra-)structural and bioenergetic aspects of such interactions remain insufficiently understood. Here, we examine H_2-producing protist *Pygsuia biforma* and its sulfate-reducing bacterial (SRB) symbiont by tracking sulfur metabolism at cellular and organellar scales. *P. biforma* is the only protist known to replace the mitochondrial iron-sulfur cluster (ISC) assembly system with an archaeal-type Sulfur Utilization Factor-like Minimal System (SMS) protein complex.[5] It remains unclear whether the protist acquires sulfur for FeS biogenesis directly from the H_2S generated by its syntrophic SRB partner. To differentiate contact-dependent versus gas-mediated sulfur exchange, we physically separated anaerobically ^{34}S-labeled SRB from *P. biforma* using a 10-kDa dialysis membrane that permits gas and metabolite exchange. High-resolution volume electron microscopy (EM) will enable 3D reconstructions of MROs, cytoplasm, and symbiosis-dependent organelle morphologies, paired with correlative transmission EM-Nanoscale Secondary Ion Mass Spectrometry (TEM-NanoSIMS) to map sulfur isotopes at subcellular resolution. We anticipate ^{34}S enrichment in *P. biforma* to demonstrate metabolic exchange, with ultrastructures revealing syntrophic interfaces, thereby deepening insights into MRO evolution and eukaryotic anaerobiosis.

[1] F. Husnik, et al., Bacterial and archaeal symbioses with protists, Curr. Biol., 31(13), (2021), R862–R877. https://doi.org/10.1016/j.cub.2021.05.049

[2] F. Burki, Mitochondrial Evolution: Going, Going, Gone. Curr. Biol., 26, (2016), R410–R412. https://doi.org/10.1016/j.cub.2016.04.032

[3] B. E. L. Morris, et al., Microbial syntrophy: interaction for the common good, FEMS Microbiol. Rev., 37, (2013), 384–406. https://doi.org/10.1111/1574-6976.12019

[4] R. M. R. Gawryluk, C. W. Stairs, Diversity of electron transport chains in anaerobic protists, Biochim. Biophys. Acta, Bioenerg. 1862(11), (2021), 148334. https://doi.org/10.1016/ j.bbabio.2020.148334

[5] C. W. Stairs, et al., A SUF Fe-S cluster biogenesis system in the mitochondrion-related organelles of the anaerobic protist Pygsuia. Curr. Biol., 24, (2014), 1176–1186. https://doi.org/ 10.1016/j.cub.2014.04.033

O59

Monitoring subunit rotation in a single F_oF_1-ATP synthase by quantum sensing

Michael Börsch[a, b, c], Iván Pérez[a,c], Lukas Spantzel[a], Fahimeh Rashidi[a], Thomas Heitkamp[a]

[a] *Jena University Hospital, Jena, Germany*
[b] *Abbe Center of Photonics, Friedrich Schiller University, Jena, Germany*
[c] *Carl-Zeiss-Stiftung Center QPhoton, Jena-Stuttgart-Ulm, Germany*

E-mail address: michael.boersch@uni-jena.de
Presenting author: Michael Börsch

For 25 years we have recorded subunit rotation of the *E. coli* enzyme F_oF_1-ATP synthase in solution [1]. We introduced single-molecule FRET (smFRET) measurements to study subunit rotation and regulatory conformational changes in individual F_oF_1-ATP synthases in liposomes. However, observation times of single, freely diffusing proteoliposomes are limited to tens of milliseconds by Brownian motion using a confocal microscope. To counteract diffusive motion actively in real time, we have built a fast anti-Brownian electrokinetic trap (ABEL trap, see [2]) with a laser focus pattern and electrode feedback. Increased observation times for about one second in the ABEL trap were achieved. Fast subunit rotation in F_oF_1-ATP synthases was recorded at different ATP concentrations revealing broad distributions of ATP hydrolysis rates from enzyme to enzyme, and changing speeds in time traces of a single enzyme [3]. ABEL-smFRET was used to unravel the mechanism of ADP inhibition of single F_oF_1-ATP synthase [4].

However, the ABEL trap observation times are still limited by photobleaching of the FRET fluorophores. How can we overcome this limit? Nitrogen-vacancy (NV) centers in nanodiamonds (10 to 100 nm diameter) can be applied as single fluorescent quantum sensors. The extraordinary photo-physical properties such as very high photo-stability and non-blinking behavior allow for optical detection of magnetic resonance due to the NV^- triplet spin states as well as nanoscale distance measurements, and the appropriate surface chemistry allows for specific attachment to membrane proteins [5]. To develop a nanodiamond-based alternative for smFRET, we determined the different molecular brightness, spectral ratio, diffusion coefficient, surface charge and multiexponential fluorescence lifetimes for nanodiamonds one by one in solution. We will report on our progress in monitoring the fluorescence lifetime changes of the NV^- center as a novel approach to record subunit rotation of a single diffusing F_oF_1-ATP synthase for tens to hundreds of seconds.

References

[1] M. Börsch et al., Stepwise rotation of the □-subunit of EF_oF_1-ATP synthase observed by intramolecular single-molecule fluorescence resonance energy transfer, FEBS lett. 527 (2002) 147-152, https://doi.org/10.1016/S0014-5793(02)03198-8

[2] A. E. Cohen, W. E. Moerner, Suppressing Brownian motion of individual biomolecules in solution, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 4362–4365, https://doi.org/10.1073/pnas.0509976103

[3] T. Heitkamp et al., Fast ATP-Dependent Subunit Rotation in Reconstituted FoF1-ATP Synthase Trapped in Solution, J. Phys. Chem. B 125 (2021) 7638−7650, https://doi.org/10.1021/acs.jpcb.1c02739

[4] I. Pérez et al., Mechanism of ADP-Inhibited ATP Hydrolysis in Single Proton-Pumping FoF1-ATP Synthase Trapped in Solution, Intl. J. Mol. Sci. 24 (2023) 8442, https://doi.org/10.3390/ijms24098442

[5] A. Sadžak et al., Impact of Surface Functionalization on NV Quantum Properties: Implications for Biosensing with Fluorescent Nanodiamonds, Adv. Funct. Mat. 36 (2026) e21387, https://doi.org/10.1002/adfm.202521387

Contribution type: T

O60

Toward Mechanistic Resolution of Nonequilibrium Transport in Coupled Bioenergetic Complexes

Hannah Weckel-Dahman,[a] Ryan Carlsen,[a] Jessica M.J. Swanson[a] [a] *Department of Chemistry, University of Utah, Salt Lake City, UT*
E-mail address: j.swanson@utah.edu
Presenting author: Jessica Swanson

Bioenergetic transformations commonly involve ion channels, transporters, and redox-active enzymes responding to electrochemical gradients in a dynamic and coupled manner. Despite substantial progress resolving molecular mechanisms for specific systems under equilibrium conditions, extending mechanistic insight to biologically-relevant nonequilibrium conditions— particularly across multiple coupled complexes—remains an outstanding challenge.

To this end, we are developing multiscale responsive kinetic modeling (MsRKM),[1] an experimentally guided theoretical framework that integrates molecular-level insights from simulations with macroscopic observables to extract nonequilibrium transport mechanisms. By explicitly incorporating electrochemically responsive rates, MsRKM captures how transport processes evolve dynamically with voltage, ion gradients, and local environments—enabling mechanistic interpretation of current–voltage and current–concentration relationships and their dependence on molecular-scale features.

Our results demonstrate that chemical and electrical gradients—even when equal in Nernstian magnitude—drive fundamentally different transport behaviors. For example, ClC-ec1 exhibits gradient-dependent mechanisms while the Shaker K^+ channel redistributes flux into essential offpathway intermediates in response to varying gradients.[2] MsRKM further resolves the physical origins of voltage- and concentration-dependent current profiles (I–V vs. I–μ) and explains rectification by linking ion binding site locations to voltage sensitivity.[3] It has revealed how rate and flux limiting steps can be directly interpreted from electrophysiology assays.[4]

Building on this foundation, we are developing a generalizable extension of MsRKM to systems of coupled bioenergetic complexes with the integration physics-informed AI. These approaches combine mechanistic constraints from statistical mechanics with data-driven inference to identify nonlinear, non-Markovian, and cross-complex coupling effects. In particular, we aim to uncover how protonation equilibria, polarization responses, and kinetic pathways become coordinated across interacting complexes—including shared electrochemical gradients and intermediate exchange—while maintaining physical interpretability and consistency with experimental data.

These ongoing efforts aim to contribute a quantitative framework for capturing the nonequilbrium responses of channel, transporter and redox enzymes and to help resolve how interacting bioenergetic complexes coordinate flux and energy transduction across scales.

[1] H Weckel-Dahman, R Carlsen, JMJ Swanson, Multiscale Responsive Kinetic Modeling: Quantifying Biomolecular Reaction Flux under Varying Electrochemical Conditions, J. Chem. Theory Comput. 21 (1) (2025) 374-389. https://doi.org/10.1021/acs.jctc.4c00872

[2] H Weckel-Dahman, R Carlsen, A Daum, M He, TG Southam, JMJ Swanson, Ion Channel Reaction Networks: Dielectric Screening and the Importance of Off-Pathway Flux, J. Chem. Theory Comput. Special Issue on Markov State Modeling 21 (2025) 3700-3711. https://doi.org/10.1021/acs.jctc.4c01569

[3] R Carlsen, H Weckel-Dahman, JMJ Swanson, Nernst Equilibrium, Rectification, and Saturation: Insights into Ion Channel Behavior, Biophys. J. 123 (2024) 1-12. https://doi.org/10.1016/j.bpj.2024.10.016

[4] From Flux to Function: Extracting Mechanistic Insights from Ion Channels via I-V and I-μ Analyses, H Weckel-Dahman, R Carlsen, A Daum, JMJ Swanson J. Phys. Chem. B (2026) 130 2733-2742. https://doi.org/10.1021/acs.jpcb.5c06019 https://pubs.acs.org/toc/jpcbfk/130/10

O61

Ca^{2+}-Induced Conformational Changes in Mammalian ATP Synthase

Anton Kavaleuski and Leonid Sazanov
Institute of Science and Technology Austria

Elevated Ca^{2+} concentrations in the mitochondrial matrix induce the opening of a non-specific channel in the inner mitochondrial membrane known as the permeability transition pore (PTP). Although the molecular entity of the PTP remains formally unresolved, accumulating evidence supports ATP synthase underlying the pore formation.

In this work, we used cryo-EM to compare the conformations of native murine ATP synthase with those of the enzyme incubated with Ca^{2+} and the PTP inducers Bz-423 and PhAsO, to determine structural changes associated with PTP opening.

From cryo-EM maps at up to 2.3 Å resolution, we identify distinct structural features associated with Ca^{2+} alone or in combination with the PTP inducers. These include displacement of the β-barrel in a catalytic subunit, conformational changes in the OSCP subunit, and a pronounced shift of the peripheral stalk. These rearrangements are consistent with a mechanism in which structural signals propagate from Ca^{2+} binding at the catalytic site toward the membrane region of ATP synthase through the peripheral stalk.

Another key finding is the identification of a binding dwell (ATP-waiting) conformation that has not previously been structurally characterized in mammalian ATP synthase. This conformation is also associated with Ca^{2+} treatment; however, its relevance to PTP formation remains unclear.

Importantly, we find that these structural changes affect the three rotary states of ATP synthase unequally, highlighting a potential link between the enzyme's threefold pseudosymmetry and PTP formation.

We propose that the observed rearrangements represent early structural events leading to PTP opening. Furthermore, we characterize the effects of several wellknown PTP inducers on these conformational changes. These findings provide a mechanistic explanation for the contribution of ATP synthase to PTP opening.

O62

Nuclear isoforms and higher-order organisation of cytochrome *c* oxidase

A. Maréchal[a]

a
Department of Structural and Molecular Biology, University College London, London WC1E 6BT, UK

E-mail address: a.marechal@ucl.ac.uk
Presenting author: Amandine Maréchal

Cytochrome *c* oxidase, or complex IV (CIV), is the terminal enzyme of our respiratory chains and, as such, is often referred to as the electron transport chain pacemaker. It is embedded in the inner membrane of mitochondria, where it catalyzes the reduction of molecular oxygen to water, conserving the released redox energy through coupled transmembrane proton transfers. While the molecular details of this coupling mechanism remain a matter of debate, it has proven even more elusive to understand how CIV activity is regulated.

Mammalian CIV is composed of up to 14 protein subunits of dual genetic origin. Three are encoded by the mitochondrial DNA and form the catalytic core, while the remaining eleven are encoded by the nuclear genome. It has been proposed that some of these nuclear-encoded subunits evolved to regulate core CIV activity. Of particular interest is a small subset that can exist as distinct isoforms depending on tissue type, developmental stage, or oxygen availability, with evidence for roles in allosteric regulation and, more recently, in respiratory supercomplex formation. The role of these supercomplexes, and the physiological advantage they may confer compared to the individual enzymes, remain incompletely understood and are the focus of intense research efforts.

Combining structural data obtained by single-particle cryo-EM and in situ cryo-electron tomography, generated by us and others, I will review the current understanding of how CIV isoforms influence membrane organisation through higher-order assembly of the respiratory chain.

O63

Molecular Determinants of Oxygen Reactivity in Bacterial Terminal Oxidases: Functional Roles of Acidic Residues in Cytochrome *bd*

Raaif Siddeeque[a], Lucia Heger[b], Thorsten Friedrich[b], Frédéric Melin[a] and Petra Hellwig[a*]
a Laboratoire de Bioélectrochimie et Spectroscopie, UMR 7140, Chimie de la Matière Complexe, Université de Strasbourg – CNRS 4, rue Blaise Pascal, 67081 Strasbourg, France
b Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany;

E-mail address: *hellwig@unistra.fr*
Presenting author: Petra Hellwig

Terminal oxidases are essential components of aerobic respiratory chains in both prokaryotes and eukaryotes, catalyzing the final step of the electron transport chain. These membrane proteins mediate the transfer of electrons from reduced electron carriers (such as cytochrome *c* or quinols) to the terminal electron acceptor, molecular oxygen, thereby reducing it to water. They play a central role in aerobic respiration and energy metabolism, adapting to diverse environmental and physiological conditions across different organisms. First, an overview of the electrochemical properties of terminal oxidases from various organisms will be presented, highlighting their remarkable adaptability, with redox potentials spanning more than 500 mV. [1,2] Particular emphasis will be placed on bacterial oxidases, especially cytochrome *bd*. Members of the cytochrome *bd* superfamily are found exclusively in prokaryotes and play key roles in protection against oxidative stress, virulence, adaptability, and antibiotic resistance. [3]

Recent structural, electrochemical, and spectroscopic studies have provided new insights into oxygen reduction, coupled protonation processes, and interactions with small signaling molecules such as NO. [4, 5] Notably, several conserved acidic residues are located in the vicinity of the three hemes and along the proton transfer pathways to the active site. The roles of residues E445, D105, D58, and E99 in electron transfer and proton uptake are investigated.

FTIR difference spectroscopy allows the protonation states of these residues to be probed. In addition, electrocatalytic responses obtained by protein film voltammetry will be presented, providing insight into the complex and finely tuned electron and proton transfer processes catalyzed by these enzymes.

.

[1] F. Melin, P. Hellwig, Redox Properties of the Membrane Proteins from the Respiratory Chain, Chem. Rev. 120 (2020) 10244–10297.

[2] P. Hellwig, The electrochemical properties of the highly diverse terminal oxidases from different organisms, Bioelectrochemistry 165 (2025) 108946.

[3] V.B. Borisov, S.A. Siletsky, A. Paiardini, D. Hoogewijs, E. Forte, A. Giuffrè, R.K. Poole, Bacterial Oxidases of the Cytochrome bd Family: Redox Enzymes of Unique Structure, Function, and Utility As Drug Targets, Antioxid. Redox Signal. 34(16) (2020) 1280–1318.

[4] S. Safarian, A. Hahn, D.J. Mills, M. Radloff, M.L. Eisinger, A. Nikolaev, J. Meier-Credo, F.
Melin, H. Miyoshi, R.B. Gennis, J. Sakamoto, J.D. Langer, P. Hellwig, W. Kühlbrandt, H. Michel, Active site rearrangement and structural divergence in prokaryotic respiratory oxidases, Science 366 (2019) 100–104.

[5] J. Kägi, I. Makarchuk, D. Wohlwend, F. Melin, T. Friedrich, P. Hellwig, E. coli Cytochrome bd I Requires Asp58 in the CydB Subunit for Catalytic Activity, FEBS Lett. 596 (2022) 2418–2424.

O64

UV-Raman evidence of Y and W radicals in the cytochrome c oxidase -Y280H, -W272H and -Y167F/O_2/H_2O_2 reactions

Constantinos Varotsis[a], Takehiro Ohta[b], Kerstin Budiman[c], Oliver-Matthias H. Richter[d], Teizo Kitagawa[b], Bernd Ludwig[d] and Eftychia Pinakoulaki[e]

a
Department of Chemical Engineering, Cyprus University of Technology, 3603 Limassol, Cyprus
b Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences 5-1 Higashiyama, Myodaiji, Okazaki, Aichi 444-8787 Japan
c
Max-Planck Institut für Biophysik, D-60439 Frankfurt am Main, Germany
d Institute of Biochemistry, Biozentrum, Johann Wolfgang Goethe-Universität, Marie-Curie-Str. 9, D-60439 Frankfurt/M., Germany
e
Department of Chemistry,University of Cyprus, 2109 Aglantzia, Cyprus

E-mail address: c.varotsis@cut.ac.cy
Presenting author: Constantinos Varotsis

In cell respiration, tyrosyl radicals play a crucial role in the formation of ferry intermediates which are stabilized for efficient energy conservation and proton translocation. The properties of the P and F ferryl intermediates in the reactions of oxidized cytochrome c oxidase (CcO) from P. denitrificans with H_2O_2 were investigated by monitoring the 244-nm resonance Raman spectra of tyrosine-280, tyrosine-167 and tryptophan-267 mutants. At early times in the reaction, the RR spectra of the reactions of Tyrosine-280 and wild type are nearly identical and showed the formation of bands characteristic of tyrosine/tyrosinate and tryptophan residues. The decay of these transient species to the oxidized form of the enzyme is completed within 15 minutes after mixing with H_2O_2. The reaction of MV CcO Y280 with O_2 was also investigated by Uv-Raman spectroscopy. The time evolution of the transient species formed will be presented.

[1] E. Pinakoulaki, V. Daskalakis, T. Ohta, O-H Richter, K. Budiman, T. Kitagawa, B. Ludwig, C. Varotsis, The protein effect in the structure of two ferryl-oxo intermediates at the same oxidation level in the heme copper binuclear center of cytochrome c oxidase, J. Biol. Chem. 288 (2013) 20261-20266, https://doi.org/10.1074/jbc.m113.468488.

[2] V. Daskalakis, S.C. Farantos, C. Varotsis, Assigning vibrational spectra of ferryl oxo intermediates of cytochrome c oxidase by periodic orbits and molecular dynamics, J. Am. Chem.
Soc. 130 (2008) 12385 12393, https://doi.org/10.1021/ja801840y.

[3] E. Pinakoulaki, V. Daskalakis, C. Varotsis The origin of the FeIV=O intermediates in cytochrome aa_3 oxidase, Biochimica et Biophysica Acta (BBA)-Bioenergetics 1817 (2012) 552-557, https://doi.org/10.1016/j.bbabio.2011.07.009.

O65

Geochemical origin of bioenergetics: Aqueous phosphorylation with substrates, catalysts and conditions of serpentinizing hydrothermal vents

Manon Schlikker, Nadja Hoffman, William F. Martin
Institut for Molecular Evolution
University of Düsseldorf, Germany

Life is a chemical reaction—an energy releasing chemical reaction, the individual steps of which are catalyzed by enzymes. How, and where, the first prokaryotic cells arose before there were enzymes is of interest. At the core of the life process is metabolism, a network of
~400 chemical reactions that generate the building blocks of life. The reactions of metabolism entail a number of substrate conversions that are, by themselves, not energetically favourable, requiring energetic coupling to ATP hydrolysis in order to make the overall (coupled) reaction exergonic. Today, ATP in growing cells is continuously supplied by (i) chemiosmiosis via ATP synthases, which harness ion gradients generated at the plasma membrane by the coupling of redox reactions to ion pumping, in addition to (ii) substrate level phosphorylations (SLP), which couple redox reactions of carbon substrates to the formation of organophosphates (acyl phosphates or enolphosphates) with sufficient group transfer potential to phosphorylate ADP. But chemiosmotic ATP synthesis and SLP are enzymatic processes. Before there were enzymes, how could phosphate have become the universal energy currency of metabolism? In recent work we and our collaborators have found that native metals (Ni^0, Fe^0, Co^0, Ni_3Fe) that naturally occur in serpentinizing hydrothermal vents replace, in pure alkaline water, ~150 enzymes of microbial metabolism via the acetyl-CoA pathway by converting H_2 and CO_2 to pyruvate and citric acid cycle intermediates and reductively aminating the 2-oxoacids to amino acids. Following Bernhard Schink's work on bacteria that obtain phosphate and electrons from phosphite, HPO_3^{2-}, we explored phosphite as a primordial phosphorylating agent. Phosphite naturally occurs in serpentinizing hydrothermal vents, as do platinum group elements in their native state (Pd^0, Rh^0, Ru^0, Ir^0, Pt^0). We find that in pure water at pH 9, 25–100 °C over 2–72 h, solid state Pd^0 catalyzes the phosphorylation (i) of glycerol, ribose, glucose, serine and cytidine, forming the biologically relevant phosphoester bonds, (ii) of phosphate residues, forming phosphoanhydride bonds in pyrophosphate, polyphosphates and ADP, and (iii) of acetate forming acetyl phosphate. These reactions go forward because phosphite oxidation to phosphate and H_2 ($E_0¢ = –690$ mV) is exergonic with $DG_0¢ = –46$ kJ·mol$^{–1}$. The simplicity and broad substrate specificity of these aqueous reactions suggest that the origin of phosphate dependent bioenergetics could reside in phosphite dependent phosphorylation. At the origin of cells, enzymes and phosphate replaced Pd^0 and HPO_3^{2-} as the mechanism of phosphorylation.

O66

Structural phylogenetics to unravel the evolutionary history of bioenergetics.

Sofia Appelgren, Karim H. Kholeif, Mustafa R. Törer, Kaija Walter, Jediael Ng, Lennart Randau, Georg Hochberg

[a]*Affiliation 2* [b]*Affiliation 2*

E-mail address: hochberg@staff.uni-marburg.de
Presenting author: Georg Hochberg

The origin and diversification of bioenergetic molecular machines is an important unsolved problem in evolutionary biochemistry. The enzymes function in today's diverse respiration chains have deep evolutionary origins that are often obscured by extensive horizontal transfer. Phylogenetic trees can be difficult to infer reliably because of extensive sequence divergence and even if they can be inferred, these trees are usually unrooted and leave the direction of evolution unknown. The order in which particular kinds of respiration chains evolved relative to one another therefore remains unsolved. We have used a new technique that infers phylogenies from structures, not sequences to overcome this problem. We make use of structural symmetry within proteins to root trees that are otherwise unrootable. This for example allowed to us to resolve the origin of the heme copper oxidases, a family of enzymes crucial for both aerobic and nitrate respiration. The same technique even allows us to resolve events in time that predated the last common ancestor of all life on earth. Structural phylogenogetics thus represents a valuable new tool to peer deeper into the biological past than was possible before.

O67

Reconstructing Early Bioenergetics: Evolution of Heme and Quinone Biosyntheses Val Karavaeva[a,b], Anwar Hiralal[a] , Filipa L Sousa[a] [a] *Functional and Evolutionary Ecology, University of Vienna, Austria b Vienna Doctoral School of Ecology and Evolution, Austria*

E-mail address:filipa.sousa@univie.ac.at
Presenting author: Filipa L Sousa

Understanding how early life harnessed energy is central to reconstructing the coevolution of biology and Earth's geochemical environment[1,2]. Microbial diversity and it's evolution cannot be fully captured by organismal phylogenies alone, but must instead be grounded in the evolution of bioenergetic systems and their components. In this context, cofactors such as hemes and quinones are of particular interest, as they form the core of electron transport chains and underpin both respiration and photosynthesis.

Here, we combine comparative genomic and phylogenomic analyses with biogeochemical constraints to investigate the evolutionary history of heme and quinone biosynthesis pathways across Archaea and Bacteria. Building on the framework that metabolic evolution is modular, mosaic, and strongly shaped by lateral gene transfer [1,2], we reconstructed the distribution, diversification, and inferred ancestry of key biosynthetic genes related with heme or quinone biosynthesis. Our analyses reveal distinct trajectories for these pathways and allowed us to build a scenario for the origin and diversification of proteins depending on those chemical compounds.

By integrating pathway evolution with the changing availability of electron donors and acceptors on early Earth, we propose a stepwise model in which the emergence of cofactor biosynthesis expanded the accessible redox landscape for early microbes, revising the current framework for quinone[3] and heme evolution [4,5]. This approach provides a bridge between genomic data and bioenergetics history, illustrating how the evolution of a limited set of bioenergetic "building blocks" contributed to the vast metabolic diversity observed today. Moreover, our findings underscore the importance of cofactor evolution as a key driver of bioenergetic innovation and offer new constraints on the timing and sequence of early metabolic transitions.

[1] Padalko A, *et al*, Bioenergetics evolution: the link between Earth's and Life's history, Philos Trans R Soc Lond B Biol Sci 380 (2025), 20240102, https://doi.org/10.1098/rstb.2024.0102 [2] Baymann F, *et al* The redox protein construction kit: pre-last universal common ancestor evolution of energy-conserving enzymes. Philos Trans R Soc Lond B Biol Sci. 358 (2003), 267-74, https://doi.org/10.1098/rstb.2002.1184.
[3] - Schoepp-Cothenet B, *et al,* On the universal core of bioenergetics. Biochim Biophys Acta. 1827 (2013), 79-93,
[4] - Karavaeva V, *et al,* Heme biosynthesis evolution: early and late innovations, submitted
[5] - Hiralal A, *et al*, Quinone diversity and evolution: what do genomes tell us?, in preparation

O68

Unraveling the molecular mystery of the catalytic specificities of Mo-*bis* PGD enzymes, the workhorse of bioenergetics: mission impossible?

Ines Bouchoul[a], Gwendoline Degré[a], Bastien Marchis-Mouren[a], Audrey Tempier[a], Julie Szyttenholm[a], Simon Duval[a], Barbara Schoepp-Cothenet[a]

[a]*Aix-Marseille Université, CNRS, BIP-UMR 7281, IM2B, Marseille, France*

E-mail address: schoepp@imm.cnrs.fr
Presenting author: Barbara Schoepp-Cothenet

Abstract

For over ten years, enzymes containing molybdenum (Mo) or tungsten (W) have been recognised for their fascinating diversity[1,2]. Found in both prokaryotes and eukaryotes, these enzymes are the true workhorses of bioenergetics. They carry out redox reactions in all the major geochemical cycles — the carbon, sulfur and nitrogen cycles — and are even involved in converting numerous toxic substances, including xenobiotics. The Mo/W-bis-PGD sub-family has been identified as the most diverse and oldest of the Mo/W-enzymes[3,4]. While the phylogeny of this group clearly highlights evolutionary links between different enzymes, research has struggled to decipher the molecular basis of the evolution of this superfamily and the specificity of each enzyme identified to date[5,6]. The data presented here forms part of our group's ongoing efforts to identify the molecular basis of enzyme specificity in arsenic redox reactions, with a particular focus on the evolutionary links between these enzymes and their phylogenetic neighbours[7,8].

[1] Hille, R. Molybdenum and tungsten in biology. Trends in biochemical sciences 27, 360–7 (2002), https://doi.org/10.1016/s0968-0004(02)02107-2

[2] Grimaldi, S., Schoepp-Cothenet, B., Ceccaldi, P., Guigliarelli, B. & Magalon, A. The prokaryotic Mo/W-bisPGD enzymes family: a catalytic workhorse in bioenergetic. Biochimica et biophysica acta 1827, 1048–1085 (2013), https://doi.org/10.1016/j.bbabio.2013.01.011

[3] Schoepp-Cothenet, B. et al. The ineluctable requirement for the trans-iron elements molybdenum and/or tungsten in the origin of life. Scientific reports 2, 263 (2012), https://doi.org/10.1038/srep00263

[4] Wells, M., Kim, M., Akob, D. M., Basu, P. & Stolz, J. F. Impact of the Dimethyl Sulfoxide Reductase Superfamily on the Evolution of Biogeochemical Cycles. Microbiol Spectr 11, e0414522 (2023), https://doi.org/10.1128/spectrum.04145-22

[5] Magalon, A., Ceccaldi, P. & Schoepp-Cothenet, B. The prokaryotic Mo/W-bisPGD enzymes family. in Molybdenum and Tungsten Enzymes: Biochemistry (ed. Hille, R.; S.) 143–191 (RSC Publishing, Cambridge/UK, 2016), https://doi.org/10.1039/9781782623915-00143

[6] Arias-Cartin, R. et al. Redox cofactors insertion in prokaryotic molybdoenzymes occurs via a conserved folding mechanism. Scientific reports 6, 37743 (2016), https://doi.org/ 10.1038/srep37743

[7] Szyttenholm, J. et al. The controversy on the ancestral arsenite oxidizing enzyme; deducing evolutionary histories with phylogeny and thermodynamics. Biochimica et biophysica
acta. Bioenergetics 1861, 148252 (2020), https://doi.org/10.1016/j.bbabio.2020.148252

[8] Degré, G. et al. Demonstration of the Role of Both a Ttr and a Psr Homologue Enzymes in the Respiration of Tetrathionate by an Environmental Bacterium Shewanella sp. ANA-3. Environ Microbiol 28, e70258 (2026), https://doi.org/10.1111/1462-2920.70258

with the topic marking S13

Contribution type: P (T)

O69

Rescuing Ischemic Brain Injury by Rewiring Mitochondrial Electron Flow

Alexander Galkin[a], Belem Yoval-Sánchez[a], Ivan Guerrero[a], Qiuying Chen[b], Marten Szibor[c], Giovanni Manfredi[a], Ilka Wittig[d,e]

a Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA
b Department of Pharmacology, Weill Cornell Medicine, New York, USA
c Department of Cardiothoracic Surgery, Center for Sepsis Control and Care, Jena University Hospital, Jena, Germany
d Institute for Cardiovascular Physiology, Goethe University, Frankfurt am Main, Germany
e Functional Proteomics Center, Goethe University, Frankfurt am Main, Germany

E-mail address: alg2057@med.cornell.edu
Presenting author: Alexander Galkin

Mitochondrial dysfunction is a central driver of ischemia-reperfusion (IR) brain injury, yet the mechanistic link between metabolic imbalance and mitochondrial impairment remains unresolved. Reverse electron transfer (RET), driven by a highly reduced quinone pool, has been implicated as a major source of pathological reactive oxygen species (ROS) in mitochondria.[1]

We employed the neonatal brain hypoxia-ischemia model combined with spatial MALDI imaging and LC-MS metabolomics to define metabolic flux alterations in vivo. Mitochondrial function, ROS production, and flavin mononucleotide (FMN) content were assessed in isolated intact brain mitochondria and tissue samples.

IR induced a distinct metabolic signature characterized by rapid accumulation of RETsupporting substrates, such as glycerol 3-phosphate and succinate, together with degradation of adenylate pool. Upon reperfusion, oxidation of these substrates drove RET, resulting in the highest rates of ROS production and progressive inactivation of complex I. Mechanistically, RET promoted dissociation of the complex I FMN cofactor without evidence of covalent protein modification, linking enzyme over-reduction to catalytic impairment.

To mitigate RET, we used transgenic mice expressing alternative oxidase (AOX), which bypasses complexes III and IV by oxidizing the quinone pool by oxygen. AOX expression did not alter respiratory chain composition, supercomplexes assembly, or basal respiration, but selectively limited RET flux. As a result, AOX presence markedly reduced mitochondrial ROS generation, preserved FMN binding to complex I, and maintained enzymatic activity. *In vivo*, AOX expression significantly reduced infarct size, prevented FMN dissociation from complex I and improved neurofunctional outcomes, demonstrating robust and sustained neuroprotection.

These findings identify RET-driven FMN dissociation from complex I as a central mechanism of mitochondrial dysfunction in IR brain injury. Modulation of quinone pool redox state via AOX effectively rewires mitochondrial electron flux, preventing RET and preserving complex I integrity. Our findings reveal a novel strategy for rewiring mitochondrial electron flux to reduce IR brain injury, highlighting modulation of the electron flow in the respiratory chain as a potential therapeutic approach.

[1] V. Ten and A. Galkin, Mechanism of mitochondrial complex I damage in brain ischemiareperfusion injury. A hypothesis. Mol Cell Neurosci. 100, (2019) 103408, https://doi.org/10.1016/j.mcn.2019.103408

O70

Nicotinamide nucleotide transhydrogenase is part of a supercomplex and plays opposite roles during mitochondrial oxidative and energy stress conditions

Weiwei Li[a,b,c], Inge van der Stelt[a], Els M.A. van de Westerlo[c,d], Merel J.W. Adjobo-Hermans[c,d], Jaap Keijer[a] and Werner J.H. Koopman[a,b,c]

a
Human and Animal Physiology, Wageningen University and Research, Wageningen, The Netherlands.
b
Department of Pediatrics, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands.
c
Radboud Center for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.
d
Department of Medical BioSciences, Radboud Center for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.

E-mail address: Werner.Koopman@radboudumc.nl
Presenting author: Werner J.H. Koopman

Nicotinamide nucleotide transhydrogenase (NNT) is a mitochondrial inner membrane (MIM) protein [1]. In forward mode, NNT mediates proton (H^+) influx into the mitochondrial matrix to drive the conversion of ($NADP^+$ + NADH) into (NADPH and NAD^+). In this way, NNT function connects to mitochondrial hydrogen peroxide (H_2O_2) removal (via NADPH) and electron transport chain (ETC) function (via H^+ influx and NADH). Under pathological conditions, NNT operates in reverse, thereby consuming NADPH, producing NADH, and mediating matrix H^+ efflux. It is still incompletely understood how forward- and reverse-mode NNT action is regulated and how this action dynamically integrates with the spatial homeostasis of H_2O_2, NADH and NADPH in the mitochondrial matrix and cytosol of intact living cells. Here, we addressed this question by analyzing the effects of NNT knockdown (NNT-KD) in HEK293 cells during resting conditions, exogenous H_2O_2 addition and mitochondrial uncoupling. Quantified parameters included mitochondrial oxygen consumption rate (OCR), mitochondrial membrane potential (□□), and NAD(P)H autofluorescence. In addition, we generated cell lines stably expressing proteinaceous sensors (HyPer, SypHer, Inap1, Inap3) for analysis of mitochondrial and cytosolic H_2O_2, H^+ and NADPH levels. Our results provide evidence that: (1) NNT-KD primarily alters mitochondrial parameters, (2) H_2O_2 stimulates NNT forward-mode activity, (3) a minor reduction in trans-MIM pH gradient (□pH) does not activate NNT, (4) a moderate □pH reduction stimulates NNT reverse-mode activity, and (5) a large sustained □pH reduction inhibits NNT activity. We propose that NNT activation is beneficial during oxidative stress conditions but detrimental upon partial □pH dissipation. This suggests that NNT plays an important role during mitochondrial dysfunction in (cell and animal models of) human disease.

[1] Z. Gan, I. van der Stelt I, W. Li, L. Hu, J. Song, S. Grefte, E. van de Westerlo, D. Zhang, E.M. van Schothorst, H.L. Claahsen-van der Grinten, K.J. Teerds, M.J.W. Adjobo-Hermans, J. Keijer, W.J.H. Koopman, Mitochondrial Nicotinamide Nucleotide Transhydrogenase: Role in Energy Metabolism, Redox Homeostasis, and Cancer. Antioxid. Redox. Signal. 41, (2024), 927956. doi: 10.1089/ars.2024.0694.

O71

ATP Synthase and IMM Rewiring in Senescent Cardiomyocytes

Silke Morris[a], Nico Marx[a], Gonzalo Barrientos[b], Isidora Molina-Riquelme[b], Frank Schmelter[a], Hugo E. Verdejo[c], Stefan Peischard[d], Guiscard Seebohm[d] , Verónica Eisner[b], Karin B. Busch[a]

[a]Institute of Integrative Cell Biology and Physiology, Faculty of Biology, University of Muenster, Muenster, Germany [b]School of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago de Chile, Chile [c]Departamento de Enfermedades Cardiovasculares, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago de Chile, Chile [d]Department of Cardiovascular Medicine, Institute for Genetics of Heart Diseases (IfGH), University Hospital Münster, Münster, Germany

E-mail address: buschkar@uni-muenster.de Presenting author: Karin. B. Busch

Aging is a natural process that affects heart function. Heart failure is one of the leading causes of death worldwide. Often, heart failure is caused by an energy crisis in cardiomyocytes, which primarily rely on oxidative phosphorylation (OXPHOS) to produce ATP. Our goal was to identify the mechanisms that lead to energy crises and to decipher the involvement of ATP synthase. Since human heart tissue is difficult to obtain, we studied the function, assembly, and spatiotemporal behavior of ATP synthase in a human senescent cell model. We differentiated human induced pluripotent stem cells (hiPSCs) into cardiomyocytes and used rat neonatal ventricular cardiomyocytes (CM) for comparison. Senescence was induced by mild doxorubicin treatment and thoroughly characterized [1]. We investigated the relationship between the spatiotemporal organization and function of ATP synthase in control and senescent CM, and its impact on energy metabolism, Ca^{2+} oscillations, and contractility. We observed decreased mitochondrial ATP levels, alongside a change in ATP synthase stoichiometry, including a loss of F_O subunit e and an increase in F_O subunit c relative to F_1 subunit β, indicating a loss of dimers. Consistently, we found increased ATP synthase mobility in the inner mitochondrial membrane [2]. ATP synthase reorganization was linked to altered mitochondrial ultrastructure and increased fenestrations [3]. Overall, the disintegration of ATP synthase increased ATPase capacity and the opening of the mitochondrial permeability transition pore (mPTP), both of which are functions linked to ATP synthase.

In the cytosol, delayed Ca^{2+} clearance resulted in irregular contractile activity. We suggest that this was at least partially linked to decreased ATP availability, which is needed for several Ca^{2+} pumps. Together, our findings imply that compromised ATP synthase organization and altered cristae architecture create unfavorable conditions for optimal ATP synthesis. Combined with more frequent mpTP opening, this reduces the ATP pool supply, which may create a vicious cycle of impaired energy homeostasis and declining cardiac function.

[1] S. Morris, I. Molina-Riquelme, G. Barrientos, F. Bravo, G. Aedo, W. Gomez, D. Lagos, H. Verdejo, S. Peischard, G. Seebohm, O.E. Psathaki, V. Eisner, K.B. Busch, Inner mitochondrial membrane structure and fusion dynamics are altered in senescent human iPSC-derived and primary rat cardiomyocytes, Biochim Biophys Acta Bioenerg 1864(2) (2023) 148949. https://doi.org/10.1016/j.bbabio.2022.148949

[2] S. Morris, N. Marx, G. Barrientos, I. Molina-Riquelme, F. Schmelter, H.E. Verdejo, S. Peischard, G. Seebohm, V. Eisner, K.B. Busch, Disruption of ATP Synthase Spatiotemporal Organization, Ca2+ Dynamics, and Contractile Function in Senescent Cardiomyocytes, Aging Cell 25(2) (2026). https://doi.org/10.1111/acel.70388

[3] I. Molina-Riquelme, G. Barrientos, L. Breitsprecher, W. Gomez, F. Diaz-Castro, S. Morris, G. Almarza, A. Del Campo, L. Garrido-Olivares, H.E. Verdejo, O.E. Psathaki, K.B. Busch, V. Eisner, Multiscale mitochondrial cristae remodeling links Opa1 downregulation to reduced OXPHOS capacity in aged hearts, Proc Natl Acad Sci U S A 123(1) (2026) e2508911123. https://doi.org/10.1073/pnas.2508911123

O72

Title

Benjamin D. Engel[a], Mariia Melnik[a], Forent Waltz[a], Manon Demulder[a], Philippe Van der Stappen[a], Lorenz Lamm[b], Fanny Le Blanc[a], Davide Tamborrini[a], Sofie van Dorst[a], Wojciech Wietrzynski[a]

[a]*Biozentrum, University of Basel, Switzerland* [b]*Helmholtz AI, Helmholtz Munich, Germany*

E-mail address: ben.engel@unibas.ch
Presenting author: Benjamin Engel

In this talk, I will show how our group uses cryo-electron tomography (cryo-ET) to explore bioenergetic organelles, which extract energy from the environment and thereby sustain life on our planet. We are investigating how these globally important organelles are built, repaired, and adapt to environmental stress. The talk will explore the molecular organization of crista membranes in mitochondria[1] and thylakoid membranes in chloroplasts[2], the latter of which regenerates biological energy from just sunlight and water. I will showcase some of our recent efforts to visualize how thylakoid architecture adapts to changing environmental conditions. We will also take a look pyrenoids, microcompartments in chloroplasts that enable Rubisco to efficiently fix atmospheric CO_2[3,4]. Finally, I will share our lab's preliminary work "taking cryo-ET into the field" to visualize environmental samples at molecular resolution[5].

[1] Waltz F, Righetto RD, Lamm L, Salinas-Giegé T, Kelley R, Zhang X, Obr M, Khavnekar S, Kotecha A, Engel BD (2025). In-cell architecture of the mitochondrial respiratory chain. *Science*. 387: 1296-1301.

[2] Wietrzynski W, Lamm L, Wood WHJ, Loukeri M-J, Malone L, Peng T, Johnson MP, Engel BD (2025). Molecular architecture of thylakoid membranes within intact spinach chloroplasts. *eLife*.14: RP105496.

[3] Shimakawa G , Demulder M, Flori S, Kawamoto A, Tsuji Y, Nawaly H, Tanaka A, Tohda R, Ota T, Matsui H, Morishima N, Okubo R, Wietrzynski W, Lamm L, Righetto RD, Uwizeye C, Gallet B, Jouneau PH, Gerle C, Kurisu G, Finazzi G, Engel BD*, Matsuda Y* (2024). Diatom pyrenoids are encased in a protein shell that enables efficient CO2 fixation. *Cell*. 187: 5919-5934.e19.

[4] Kumar G, Barrett J, Van der Stappen P, Payne-Dwyer A, Nam O, Shepherd J, Frame L, Howard J, Malis V, Degut C, Hodgkinson M, Richter R, Schaefer C, Plevin M, Hondele M, Leake M, Engel BD, Mackinder L (2026). Sticker number modulates pyrenoid condensate assembly to support algal fitness. *bioRxiv*. 2026.01.27.701992.

[5] Leisch N, Baars S , Beavis T, Bertucci P, Bhickta C , Bonadonna M, Brannon CM, BurguésPalau L, Cherek P, Chevalier F, Decelle J, Demulder M, Dey G, Dudin O, Duke E, Engel BD, Flaum E, Flori S, Gallet B, Guichard P, Halavatyi A, Hamel V, Jacobovitz M, Juery C, Laporte M, Mattei S, Mikus F, Mocaer K, Moog K, Olivetta M, Pavie M, Pepperkok R, PerezBoerema A, Planat L, Prakash M, Pyle EW, Rhodes CR, RomeroBrey I, Ronchi P, Rosa H, Rubio Ramos A, SaintDonat C, Schwab Y, Shah H, Steyer AM, Svetlove A, Toullec G, Vincent F, Wiegand T, Wietrzynski W, Yee D, Zwahlen S (2026). An Advanced Mobile Laboratory to enable field-based microbial ecology and cell biology across scales. *bioRxiv*. 2026.02.23.707475.

O73

Mechanistic and bioenergetic aspects of the core sulfate reduction pathway

Max Yin[a], Raquel Bernardino[b], Américo G. Duarte[b], Gonçalo Manteigas[b], Jose A. Brito[b], Inês Cardoso Pereira[b], Bonnie J. Murphy[a]

[a]*Max Planck Institute of Biophysics, Frankfurt am Main, Germany*
[b]*Instituto de Technologia Química e Biológica António Xavier, Univesidade Nova de Lisboa, Oeiras, Portugal*

E-mail address: bonnie.murphy@biophys.mpg.de
Presenting author: Bonnie Murphy

Dissimilatory sulfate reduction (Dsr) plays an important role in global sulfur and carbon cycles, with particular relevance to global methane emissions. Dsr metabolism is also important in human health, as sulfide production in the gut is associated with inflammation and disease. Although the soluble enzymes catalyzing steps of the Dsr pathway are well characterised, the membrane-bound machineries linking sulfate (and sulfite) reduction to bioenergetics are poorly understood.

In this talk I will discuss cryo-EM structures of membrane-bound complexes of Dsr metabolism and complementary biochemical results that allow us to better understand the bioenergetics of Dsr. Together, these results allow us to understand how sulfur reactivity is controlled within the cell, how the sulfide release step is catalysed, and how these reactions are linked to electron and proton movement at the membrane.

O74

A novel oxidative phosphorylation supercomplex shapes the mitochondrial cirstae Yiqi Hu[a], Vivek Sharma[b], Yue Liu[a], Alexey Amunts[c], Long Zhou[a]

[a]*School of Basic Medicine, Zhejiang University, Hangzhou 310058, China* [b]*Department of Physics, University of Helsinki, Helsinki 00014, Finland*
[c]*Institute of Bio-Architecture and Bio-Interactions, Shenzhen Medical Academy of Research and Translation, Shenzhen, Guangdong, China*

E-mail address: longzhou@zju.edu.cn
Presenting author: Long Zhou

Mitochondrial oxidative phosphorylation (OXPHOS) is often viewed as two spatially segregated domains on the cristae membrane. ATP synthase dimers and their higher-order rows accumulate at regions of high curvature, most prominently along crista edges where they contribute directly to membrane bending, whereas respiratory chain complexes I-IV and their supercomplexes tend to populate flatter membrane regions and are often viewed as discrete, laterally mobile assemblies[1]. This canonical picture provides an intuitive physical complement to Mitchell's chemiosmotic concept, in which boundary conditions and membrane geometry help shape and confine the proton circuit. But it also leaves fundamental gaps, such as how are the sources and sinks of the proton circuit coordinated at the membrane scale and whether respiratory chain complexes can also directly participate in long-range crista architecture? Recent advances in in situ structural biology suggested stacked respiratory assemblies in species with tubular cristae[2]. In parallel, transient co-localization of all OXPHOS complexes was also reported in mammalian mitochondria[3]. However, a definitive structural description is still missing.

Here, we address these gaps using model parasites belonging to the eukaryotic supergroup of Discoba. We use single particle cryo-electron microscopy to resolve the structure of a novel supercomplex involving the oxidative and phosphorylative components in its highly specialized mitochondria. This assembly is enabled by a non-canonical subunit module that repurposes the canonical ATP synthase dimerization interface. Genetic perturbation and bioenergetic characterizations reveal that the primary consequence of disrupting this supercomplex is the remodeling of discoidal cristae, which further induces a drop in the maximal efficiency of chemiosmotic coupling. We further validate the existence of this novel supercomplex in situ via cryo-electron tomography and sub-tomogram averaging and reveal periodic organization of such co-assembly over long distances, tiling the native crista membrane with defined registers. In conclusion, these findings demonstrate that the sophisticated higher-order OXPHOS array is tuned to safeguard mitochondria both morphologically and functionally.

[1] K.M. Davies, M. Strauss, B. Daum, J.H. Kief, H.D. Osiewacz, A. Rycovska, V. Zickermann, W. Kühlbrandt, Macromolecular organization of ATP synthase and complex I in whole mitochondria,
Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 14121–14126. https://doi.org/10.1073/pnas.1103621108 [2] W. Zheng, P. Chai, J. Zhu, K. Zhang, High-resolution in situ structures of mammalian respiratory supercomplexes, Nature 631 (2024) 232–239. https://doi.org/10.1038/ s41586-024-07488-9
[3] S. Nesterov, Y. Chesnokov, R. Kamyshinsky, A. Panteleeva, K. Lyamzaev, R. Vasilov, L.
Yaguzhinsky, Ordered Clusters of the Complete Oxidative Phosphorylation System in Cardiac
Mitochondria, Int. J. Mol. Sci. 22 (2021) 1–10. https://doi.org/10.3390/ijms22031462

O75

Structure of a photosynthesome supercomplex from purple phototrophic bacteria

Sebastian Pintscher[a], Pavol Bárdy[b], Pu Qian[c], Elizabeth C. Martin[c], Michał Rawski[d], Andrew Hitchcock[c], James N. Blaza[b], C. Neil Hunter[a]

a
Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
b Department of Chemistry, University of York, York, United Kingdom
c
School of Biosciences, University of Sheffield, Sheffield, United Kingdom
d National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland

E-mail address: sebastian.pintscher@uj.edu.pl
Presenting author: Sebastian Pintscher

The photosynthetic apparatus of purple phototrophic bacteria (PPB) is localized within specialized membrane vesicles known as chromatophores and comprises the reaction center light-harvesting complex I (RC-LH1) core complex, the peripheral light-harvesting complex 2 (LH2) antenna complex, and the cytochrome bc_1 complex. While structures of the individual complexes have been reported, how they associate with one another in native membranes has only been partially revealed using atomic force microscopy.

Here, we use cryogenic electron microscopy (cryo-EM) to reveal the higher order structure of a photosynthetic supercomplex from the model PPB Rhodobacter sphaeroides. The supercomplex, which we refer to as a 'photosynthesome', is a symmetrical dimer consisting of a central cytochrome bc_1 dimer, two RC-LH1 dimers and two LH2 complexes. The configuration of the photosynthesome is determined by specific interactions between subunit IV of the cytochrome bc_1 complex and the RC-LH1 complex, and additional interactions between the LH1 and LH2 complexes. The geometry of the photosynthesome allows for a short distance between cytochrome bc_1 and RC-LH1, required for a rapid exchange of electrons via cytochrome c_2. Moreover, they are arranged so the central cavity of cytochrome bc_1 directly faces the opening of the RC-LH1 dimer, creating an enclosed quinone pool that allows for efficient exchange of quinones and quinols between these two complexes.

Our structure answers longstanding questions about previously observed kinetic effects and interactions between elements of the photosynthetic chain of PPB, and the requirement of subunit IV of the cytochrome bc_1 complex for optimal phototrophic growth.

We further demonstrate the power of this in situ cryo-EM approach by determining high-resolution structures of PPB membrane protein complexes in their natural lipid environment.

O76

Insights into the biogenesis of complex I and the molecular mechanism of tafazzin

Jonathan Schiller[a], José G. Rosas Jiménez[b], Monalisa Sarkar[a], Janet Vonck[c], Gerhard Hummer[b,d], Volker Zickermann[a,d]

[a] *Institute of Biochemistry II, University Hospital, Goethe University, 60438 Frankfurt am Main, Germany.* [b] *Department of Theoretical Biophysics, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany.* [c] *Department of Structural Biology, Max Planck Institute of Biophysics, 60438 Frankfurt am Main, Germany.* [d] *Cluster of Excellence SubCellular Architecture of Life (SCALE)*

E-mail address: Zickermann@med.uni-frankfurt.de Presenting author: Volker Zickermann

The structure of respiratory complex I has been determined at high-resolution, but much less is known about the structural basis of its assembly.[1,2] To investigate the biogenesis of the proximal proton pump module (P_P module) of complex I, we purified assembly intermediates associated with complex I assembly factor 1 (NDUFAF1) and determined their structures using cryoEM.[3] Recently, we resolved the structure of a detergentsensitive intermediate associated with the yet uncharacterized assembly factors TPR50 and CMC1L at 2.2 Å resolution. We now understand in molecular detail how assembly factors define a structural pathway and control the timing of the P_P module assembly process. Interestingly, the transacylase tafazzin, which plays a key role in the remodeling of cardiolipin (CL)[4], is also an integral part of P_P module assembly intermediates in the aerobic yeast *Yarrowia lipolytica*.[5] Until now, the tafazzin reaction has been thought to be independent of coenzyme A (CoA). However, our high-resolution structure clearly shows an acyl-CoA molecule bound to tafazzin. By combining cryo-EM with structure predictions and molecular dynamics simulations, we were able to gain detailed insights into a CoAmediated transacylation mechanism. These findings explain how pathogenic mutations cause tafazzin dysfunction that is associated with Barth syndrome, a severe multisystem disorder.

[1] E. Laube, J. Schiller, V. Zickermann, J. Vonck, Using cryo-EM to understand the assembly pathway of respiratory complex I, Acta Crystallogr D Struct Biol 80(Pt 3) (2024) 159–173, https://doi.org/10.1107/S205979832400086X

[2] I. Vercellino, L.A. Sazanov, The assembly, regulation and function of the mitochondrial respiratory chain, Nat Rev Mol Cell Biol 23(2) (2022) 141–161, https://doi.org/10.1038/s41580-021-00415-0

[3] J. Schiller, E. Laube, I. Wittig, W. Kühlbrandt, J. Vonck, V. Zickermann, Insights into complex I assembly: Function of NDUFAF1 and a link with cardiolipin remodeling, Sci Adv 8(46) (2022) eadd3855, DOI:10.1126/sciadv.add3855

[4] M. Schlame, Y. Xu, The Function of Tafazzin, a Mitochondrial PhospholipidLysophospholipid Acyltransferase, J Mol Biol 432(18) (2020) 5043–5051, https://doi.org/10.1016/j.jmb.2020.03.026

[5] J.G. Rosas Jiménez, J. Schiller, J. Vonck, G. Hummer, V. Zickermann, Coenzyme A is bound to tafazzin – a paradigm change for transacylation, bioRxiv (2026) 2026.02.05.703992.

O77

A novel and widespread mechanism of membrane-coupling drives microbial respiration

Ashleigh Kropp[1,2,3], Kazem Asadollahi[1#], Jamie A. Stapleton[4#], Luka Simsive[5#], Pok Man Leung[3#], Rachel L. Darnell[6#], Christopher K. Barlow[7], Benjamin G. Hartmann[9], Nicholas D. J. Yates[4], Daniel R. Fox[1,2,3], Oleksii Zdorevskyi[5], Vivek Sharma[5,8], James N. Blaza[9], Alison Parkin[4], Chris Greening[3], Rhys Grinter[1,2,3*]

[1] Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia

[2] Centre for Electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Parkville, Victoria, Australia

[3] Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

[4] Department of Chemistry, University of York, York, YO10 5DD, UK

[5] Department of Physics, University of Helsinki, Helsinki, Finland

[6] Centre for Bacterial Cell Biology, Newcastle University Biosciences Institute, Newcastle University, Newcastle-upon-Tyne, UK

[7] Monash Proteomics & Metabolomics Platform, Department of Biochemistry, Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

[8] HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki, Finland

[9] York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5DD, UK

Email address: Ashleigh.kropp@unimelb.edu.au
Presenting author: Ashleigh Kropp

Microbes occupy diverse and often extreme environments that encounter constant challenges, including fluctuating resource availability. As microbes frequently obtain nutrients from the extracellular environment, their survival is often reliant on metabolic flexibility. Consequently, many microbes have unique enzymes that drive respiration and energy generation.

Like all organisms, microbes rely on adenosine triphosphate (ATP) to power cellular processes. The key site of ATP generation for aerobic microbes is the membraneassociated respiratory chain. Here, membrane-localised respiratory enzymes couple the oxidation of fuel compounds to the reduction of terminal electron acceptors, such as oxygen, via specific membrane-localised respiratory enzymes. The transfer of electrons in this process generates energy that produces an electrochemical gradient that powers ATP synthesis via ATP synthase. Integral to this are membrane-localised respiratory quinones that carry electrons between these respiratory enzymes.

As the microbial membrane is a site of numerous cellular processes, area on the membrane is highly sought-after. Unlike mitochondria and other eukaryotic membranebound organelles, microbes often have limited membrane capacity for respiration. Here, I present a novel strategy microbes employ to overcome membrane congestion and promote metabolic flexibility. The strategy, called long-range quinone transport, uses a specialised family of proteins we termed Respiratory Chain Coupling Proteins (RCCPs).

Employing genomic surveys and phylogenetic analysis, we found that RCCPs are widespread in bacteria and archaea and are associated to varied respiratory enzymes. Through high-resolution cryo-EM structures and structural modelling we show that RCCPs form a tetrameric protein tube that have an internal hydrophobic chamber and externally scabold respiratory enzymes. Further, through molecular dynamics simulations, biochemical and physiological experiments we show that the RCCP tube interacts transiently with the membrane to extract quinones into the hydrophobic chamber. The quinones are then delivered to the associated enzymes for electron transfer, allowing for respiration in the cytoplasm. Our findings demonstrate that longrange quinone transport is an essential driver of microbial respiration, promoting metabolic flexibility and microbial survival.

O78

The structure-based assembly mechanism of the mitochondrial respiratory chain

Ahad Ali Kazmi[1,2], Irene Vercellino[1,2]

[1]Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons (ER-C), Structural Biology (ER-C-3), Forschungszentrum Jülich GmbH, 52428 Jülich, Germany
[2]Heinrich Heine University Düsseldorf, Department of Chemistry, 40225 Düsseldorf, Germany

i.vercellino@fz-juelich.de

The mitochondrial respiratory chain is a set of enzymes involved in the conversion of energy from food to ATP, a molecule that can be used to fuel cellular processes. Since the respiratory chain enzymes are large protein complexes, formed by over 100 proteins overall, an important open question in Bioenergetics is how these respiratory chain complexes are assembled. The importance of this process is highlighted by the myriad of neurodegenerative and cardiological diseases that occur when proper assembly is perturbed.

The baker's yeast, *Saccharomyces cerevisiae*, is an established model organism for mitochondrial research due its ability to utilize respiration or fermentation for growth. This enables the genetic manipulation of the respiratory chain complexes for structural purposes, which results in the disruption of mitochondrial respiration, without causing cell death. By using single particle cryo-electron microscopy (cryo-EM), we were able to visualize different assembly intermediates of the yeast respiratory chain complexes. We were also able to visualize how these intermediates assemble into higher-order structures called supercomplexes, known to be the physiological state in which the respiratory chain enzymes exist in the mitochondrial membrane. These assembly mechanisms revealed in *S.cerevisiae* are a promising starting point for understanding general strategies of respiratory chain assembly in other respiring eukaryotes.

O79

Substrate binding by respiratory complex I

Thorsten Friedrich

Albert-Ludwigs-Universität Freiburg, Institut für Biochemie, Albertstr. 21, 79104 Freiburg, Germany

E-mail address: Friedrich@bio.chemie.uni-freiburg.de

Energy-converting NADH:ubiquinone oxidoreductase, respiratory complex I, is central to cellular energy metabolism by coupling electron transfer from NADH to quinone with proton translocation across the membrane [1]. Electrons are transferred from the primary acceptor flavin mononucleotide (FMN) *via* a chain of iron-sulfur (Fe/S) clusters to quinone. The conserved Fe/S cluster N1a is located in electron transfer distance to the FMN and is not part of the main electron transfer chain. It has either a low or a high redox potential. We showed by x-ray crystallography that reduction of high potential N1a increases the binding affinity towards NAD^+, preventing an untimely leakage of the nucleotide [2]. The conformational changes underlying this process have also been determined by cryo-electron microscopy (cryo-EM) [3]. From N1a electrons might travel *via* the FMN to the quinone binding site. It is widely accepted that the chemistry of quinone reduction drives proton translocation. The quinone sequentially takes two electrons from the distal Fe/S cluster N2 forming the fully reduced quinone species. This species is protonated by conserved tyrosine and histidine residues. We mutated these residues and a nearby conserved aspartate residue individually and in combinations. Mutation of the tyrosine residue led to the (partial) loss of the EPR signals of cluster N2. However, cryo-EM showed the presence of the cluster in full occupancy. Furthermore, these mutations significantly impeded electron transfer, with the tyrosine replacement exerting the most pronounced effect. Importantly, only the histidine mutation led to a loss of redox-driven proton translocation. Cryo-EM revealed a compensatory structural rearrangement that allowed another conserved histidine in a double variant to restore the original function. We propose that protonation of the reduced quinone leads to histidine deprotonation, disrupting its salt bridge with aspartate and triggering further conformational changes that drive proton translocation. Thus, histidine deprotonation enables energy conversion in complex I.

[1] Kaila, V.R.I. and Wikström, M. Architecture of bacterial respiratory chains. Nat. Rev. Microbiol. 19, (2021), 319−330. https://doi.org/10.1038/s41579-020-00486-4

[2] Schulte, M., Frick, K., Gnandt, E., Jurkovic, S., Burschel, S., Labatzke, R., Aierstock, K., Fiegen, D., Wohlwend, D., Gerhardt, S., Einsle, O., and Friedrich, T. A mechanism to prevent production of reactive oxygen species by *Escherichia coli* respiratory complex I. Nat. Commun. 10 (2019), 2551. https://doi.org/10.1038/s41467-019-10429.

[3] Wohlwend, D., Seifermann, T. Gnandt, E., Vranas, M., Gerhardt. S. and Friedrich, T. Structural changes shifting the redox potential of the outlying cluster N1a in respiratory complex I. Structure 34 (2026), 175183. https://doi.org/10.1016/j.str.202510.016

O80

Leonid Sazanov

Institute of Science and Technology Austria, Am Campus 1, Klosterneuburg 3400, Austria
sazanov@ist.ac.at

Complex I mechanism

Complex I couples NADH:ubiquinone oxidoreduction to the translocation of protons across the membrane by a mechanism which is currently debated. Previously, we have solved cryo-EM structures of ovine and *E. coli* complex I in different redox conditions, including catalytic turnover [1, 2]. Unexpectedly, only one antiporter-like subunit, the distal ND5, appears to be capable of ejecting protons into intermembrane space (IMS). We proposed a mechanism in which large conformational changes around the quinone (Q) binding cavity couple the redox reaction to proton translocation during "open" to "closed" state transitions of the enzyme. In the "open" state Q cavity is widely open, allowing quinone to come in/out. In the "closed" state the cavity is tightly enclosed around bound quinone but is connected by a newly formed water wire to the central hydrophilic axis of the membrane domain. Therefore, the protons needed to complete quinone reduction have to come from the central axis. This initiates a "domino effect"-like cascade of electrostatic interactions within the antiporter-like subunits, ultimately resulting in the ejection of four protons per catalytic cycle from subunit ND5 into IMS. The water wire is controlled by a pi-gate in ND6 subunit, which likely prevents futile leak of protons from the central axis into the open Q cavity. In a new work we show the presence of His-gate in subunit ND5, at the other end of the membrane arm. This gate likely redirects protons entering from the matrix side either towards the ND5 IMS exit or towards the rest of the central axis, preventing shortcuts. We also show that the features of open-to-closed state transitions during turnover are reproduced in the further species of mitochondrial and bacterial complex I. Additionally, a defined open-ready state is now observed in several species and it likely represents a "waiting" state before quinone approaches and enters the Q cavity. Thus, the overall catalytic cycle is likely to comprise three, rather than two, different conformations of complex I.

References

[1] V. Kravchuk, O. Petrova, D. Kampjut, A. Wojciechowska-Bason, Z. Breese, L. Sazanov, A universal coupling mechanism of respiratory complex I, Nature 609(7928) (2022) 808-814. [2] D. Kampjut, L.A. Sazanov, The coupling mechanism of mammalian respiratory complex I, Science 370(6516) (2020).

O81

Towards the Molecular Mechanism Underlying Long-Range Energy Transduction in Complex I

Ville R. I. Kaila
Department of Biochemistry and Biophysics, Arrhenius Laboratories for Natural Sciences, Stockholm University, Stockholm, Sweden.
E-mail address: ville.kaila@dbb.su.se
Presenting author: Ville R. I. Kaila

Complex I is a gigantic redox-driven proton pump that powers cellular respiration by a remarkable long-range proton-coupled electron transfer (PCET) process, extending more than 200 Å away from the active site. Despite major advances over the last decade, its energy transduction mechanism remains poorly understood and much debated. Here, I describe our recent breakthroughs in understanding the challenging long-range proton-electron coupling process in Complex I. By developing an integrative multi-layered approach, we guide biochemical, spectroscopic, and high-resolution cryo-EM experiments with theory- and datadriven predictions, and identify how molecular locks and gates control key protonation reactions in the membrane domain of Complex I. We show how both mutations of identified coupling site, as well as substitutions linked to mitochondrial diseases, result in drastic perturbations of the long-range charge transfer activity. Moreover, by employing rational bottom-up and top-down protein engineering approaches, we show that the process is controlled by intrinsic electric field effects that result in conformational and hydration changes, mediated by allosteric effects. Our findings suggest that Complex I employs an electrical wave-propagation mechanism to efficiently transduce energy across large molecular distances, while on a general level, several bioenergetic machines operate via similar intrinsic electric field effects to power the generation of a proton motive force.

O82

How does complex I pump protons? Defining the proton-wiring diagram and identifying proton-transfer gates and control points in the membrane domain

Judy Hirst

MRC Mitochondrial Biology Unit, University of Cambridge, UK jh480@cam.ac.uk

Respiratory complex I, a cornerstone of mitochondrial metabolism and a locus of mitochondrial dysfunction and disease, captures the free energy from ubiquinone reduction by NADH to pump protons across an energy-transducing membrane, contributing to the proton-motive force that drives ATP production by oxidative phosphorylation. In recent years, remarkable developments in cryo-EM have enabled numerous high-resolution structures of complex I to be solved in multiple states from mammalian species, as well as from model bacterial and fungal systems. These structures have defined the architecture and framework of the catalytic machinery, including hydrated networks of residues in the membrane domain, in great detail – but using static structures to construct the catalytic mechanism of energy transduction is challenging, and the apparent absence of large-scale conformational changes continues to obscure how energy is transferred to coordinate and control proton pumping. This talk will present new experimental data, from integrated structural, genetic and functional studies by the Hirst group, to determine the proton-wiring diagram for proton translocation across the membrane domain in complex I, and to identify the proton gates that control the connections between proton uptake and output channels to impose directionality on proton-transfer mechanisms and eliminate uncoupling short circuits.

O83

Alternative oxidases: mechanisms, functions and therapeutic opportunities

Anthony L Moore, Andrew McGowan and Luke Young.

Sussex Drug Discovery Centre, School of Life Sciences, University of Sussex,

Brighton BN1 9QG, U.K

The alternative oxidase (AOX) plays an important bioenergetic role in OXPHOS chains of many organisms, where it reduces levels of reactive oxygen species (ROS) by controlling the redox state of the quinol pool, protecting it against oxidative cell damage. In the pathogenic parasite of tsetse-flies, *Trypanosoma brucei*, the causative agent of African trypanosomiasis (sleeping sickness), AOX operates as the sole terminal oxidoreductase when the parasite resides in the bloodstream of its mammalian host since the parasite down-regulates all of its respiratory chain complexes apart from NDH1 and AOX. Interestingly in the human parasite microsporidia, which contain highly reduced forms of mitochondria (mitosomes), AOX plays a pivotal electron transport role during the parasites life cycle progression. In addition AOX also functions as a terminal oxidoreductase in several phytopathogenic fungi which not only threaten the world's food supply but also human health. Since AOX is not present in mammalian respiratory chains, the design of inhibitors is thus of significant biomedical and therapeutic interest. In this talk we will describe how knowledge of the composition of the catalytic site and hydrophobic channel has enabled the design of novel phytopathogenic and antiparasitic inhibitors and provided a molecular insight for the development of engineered AOX for the treatment of human mitochondrial dysfunctions.

O84

Title

Andrew E MacLean[a,b], Monika Singh[a], Shikha Shikha[a], Mariana Ferreira Silva[a], Alexander Mühleip[a,c] and Lilach Sheiner[a]

[a] School of Infection and Immunity, Glasgow Centre for Parasitology, University of Glasgow, Glasgow, UK

[b] Institute for Immunology and Infection Research, University of Edinburgh, Edinburgh, United Kingdom [c] Institute of Biotechnology, Helsinki Institute of Life Science HiLIFE, University of Helsinki, Helsinki, Finland

E-mail address: lilach.sheiner@glasgow.ac.uk
Presenting author: Lilach Sheiner

Abstract

Mitochondrial respiration is a bioenergetic pathway that enables key cellular metabolism and therefore, essential to life itself. Yet, for decades the understanding of how this critical pathway works has largely been informed by studies performed in a small group of organisms which are evolutionarily closely related to one another (primarily mammalians and yeast) and therefore manifest broadly siminlar biology. The field is thus underinformed about the various mechanisms that execte and control respiration in different biological settings.

Over the past decade, new technologies including cryoEM and cryoET structural biology alongside advanced proteomics methods, allowed us to extend the study of principles and mechanisms of mitochondrial respiration to various microbial eukaryotes. An understanding of respiration in divergent organisms is emerging revealing new mechanisms that were not easily detectable or do not exist in commonly studied models. Furthermore, the comparison between remotely related systems is helping resolve some of the fields' open questions.

Our team is interested in the organisms from the Myzozoan group, which include some of the most impactful parasitic pathogens of human and animals. We work with three such parasites: Perkinsus, *Toxoplasma* and *Plasmodium*. Our recent studies discovered the surprisingly different protein composition[1,2], structure and functional mechanism of bioenergetic enzymes in these parasites [2,3,4]. For example, clade-specific components mediate the formation of respiratory chain supercomplexes. Despite being mediated by proteins not found in yeast and mammals, and while assuming different orientation and angle between the complexes, Toxoplasma gondii III_2IV supercomplex shares functional features with its yeast and mammal parallels, indictaing a convergent evolution pathway for III_2IV formation [4], which is in line with importance for fitness.

In another example, supercomplex strcutural analysis and complexome studies in *Perkinsus* revealed a putative novel natural inhibitor of complex III [3, 5], and this is further supported by genetic work characterising its homolog in the related *Toxoplasma.*

Our work defines global functions of respiratory complexs across eukaryotes, reveals novel respiration control mechanims and raises exciting questions about the function of clade specific components in responding to species or growth condition needs.

[1] Maclean AE, Bridges HR, Silva MF, Ding S, Ovciarikova J, Hirst J, Sheiner L. PLoS Pathog. 202

[2] Maclean AE, Iorillo O, Faktorová D, Singh M, McGill S, Lukeš J and Sheiner L; doi:https://doi.org/10.64898/2025.12.18.695115

[3] Wú F, Mühleip A, Gruhl T, Sheiner L, Maréchal A and Amunts A; doi:https://doi.org/10.1101/2024.05.25.595893

[4] MacLean AE, Shikha S, Ferreira Silva M, Gramelspacher MJ, Nilsen A, Liebman KM, Pou S, Winter RW, Meir A, Riscoe MK, Doggett JS, Sheiner L and Mühleip A. Nat Struct Mol Biol. 2025

Pathogen induced formation of a nascent organelle derived from mitochondria

Xianhe Li[1,1], José M. Delgado[2], Yubai Sun[2], and Lena Pernas[1,2,2]

Affiliations:

[1]Metabolism of Infection Group, Max Planck Institute for Biology of Ageing, Cologne, Germany

[1] Dept. Microbiology, Immunology & Molecular Genetics, University of California Los Angeles, USA

[2] Howard Hughes Medical Institute, Chevy Chase, MD USA

O85

Pathogen induced formation of a nascent organelle derived from mitochondria

Xianhe Li[1,2], José M. Delgado[2], Yubai Sun[2], and Lena Pernas[1,2,3]

Affiliations:

[1]Metabolism of Infection Group, Max Planck Institute for Biology of Ageing, Cologne, Germany

[2] Dept. Microbiology, Immunology & Molecular Genetics, University of California Los Angeles, USA

[3]Howard Hughes Medical Institute, Chevy Chase, MD USA

Abstract:

Intracellular pathogens extensively remodel host cells to create environments that support their replication and nutrient acquisition, yet how they generate new host compartments remain poorly understood. Defining the mechanisms underlying host cell remodeling is critical for understanding disease pathogenesis. Here we show that the human parasite *Toxoplasma gondii* induces the biogenesis of acidified compartments derived from host outer mitochondrial membrane (OMM). Following infection, host mitochondria shed large OMM-derived structures termed SPOTs. SPOTs matured into multivesicular compartments that engulfed cytosolic proteins and active lysosomes. Lysosome acquisition by SPOTs required host ESCRT machinery and the parasite effector TgGRA7, and drove the acidification of the SPOT lumen. Disrupting SPOT acidification impaired parasite proliferation. These findings show that an intracellular pathogen coopts host machinery to generate an acidified compartment derived from mitochondria that promotes its replication, and uncover organelle biogenesis as a pathogen strategy to remodel host cells.

O86

The F1FO ATP synthase Mechanism

Wayne D. Frasch

Arizona State University, Tempe AZ 85287-4501

Single-molecule studies of the F1-ATPase show that oscillations of subunit-g during the catalytic dwell progress through three successive stages. Oscillations are initially centered at 0° (Stage-1), which decays with a rate constant consistent with ATP hydrolysis. In Stage-2, 80% and 20% of the oscillations are centered at 14° and 33°, respectively, where the latter is at about when ATP-binding is most common. In Stage-3, subunit-g returns to 0° to end the dwell, and retain the subunit-g position required for consecutive rotational events. Årrhenius analysis of power stroke velocity profiles had negative activation energies during the first 60° indicating elastic energy powered this rotation. At limiting [ATP], ATP-binding dwells were observed during the entire first 60° of the power-stroke where their occurrence fit to a parabola with a maximum at ~36°, comparable to the position at which activation energy reached a minimum. The power stroke velocity profile depended on the position of the ATP-binding dwell. Mutations of b- subunit catch loop residues and their electrostatic partners in subunit-g, which are known to substantially decrease ATPase and ATP synthases activity, dramatically changed oscillations during the catalytic dwell, and the power stroke velocity profile indicating that twisting the subunit-g coiled-coil provides elastic energy for both the dwell oscillations and power stroke. ATP binding induces the subunit-g to switch to the next catch-loop, which rewinds the coiled-coil for the next catalytic dwell and power stroke. The results are consistent with the subunit-g position determining catalytic site conformations. However, nucleotide occupancy can vary in each conformation, and rotation can proceed only when all three sites have the correct occupancy. At saturating ATP, c10-ring rotation of F1FO in lipid bilayer nanodiscs had transient dwells spaced every 36° indicating that FO stopped rotation as each c-subunit came into contact with subunit-a. The occurrence of these dwells increased inversely with pH that fit to a curve where the subunit-a input (pKa 5.6) and output (pKa 7.5) channels had to be protonated and unprotonated, respectively, indicating their dependence on concurrent H+ transfer to and from the c-ring. Mutations of amino acids in either the input or output changed both pKa's, indicating both channels comprise a single Grotthuss channel linked by c-ring rotation. Most transient dwells rotated 11° in the ATP synthase direction against the force of F1-ATPase rotation. These results support an ATP synthase mechanism where in Step-1, H+ transfer to and from the c-ring rotates the c-ring 11° to form a negatively charged cD61- carboxyl, which in Step-2 uses the electrostatic attraction to aR210+ to rotate the c-ring 25° and advance by one c-subunit.

These conclusions are supported by F1 and F1FO structures with comparable nucleotide occupancies and rotary positions of subunit-g as well as the c- ring relative to subunit-a, of which many structures did not previously fit with canonical ATPase and ATP synthase mechanisms. T aken together, the mechanisms of F1 and FO provide new insight how the rotational ATP synthase mechanism accommodates known variations in the number of c-ring subunits across the Tree of Life.

P1

The Na^+-pumping mechanism driven by redox reactions in the NADH-quinone oxidoreductase from *Vibrio cholerae* relies on dynamic conformational changes

Moe Ishikawa-Fukuda[1,2], Takehito Seki[3,4], Jun-ichi Kishikawa[5,6], Takahiro Masuya[1], Kei-ichi Okazaki[3,4], Takayuki Kato[6], Blanca Barquera[2], Hideto Miyoshi[1], and Masatoshi Murai[1]

[1]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Kyoto 606-8502, Japan, [2] Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, United States, [3]Research Center for Computational Science, Institute for Molecular Science, National Institutes of Natural Sciences, Okazaki, Aichi 444-8585, Japan, [4]Graduate Institute for Advanced Studies, SOKENDAI, Okazaki, Aichi 444-8585, Japan, [5]Faculty of Applied Biology, Kyoto Institute of Technology, Kyoto, Kyoto 606-8585, Japan, [6] Institute for Protein Research, The University of Osaka, Suita, Osaka5650871, Japan.

E-mail address: fukudm@rpi.edu
Presenting author: Moe Ishikawa-Fukuda

The Na^+-pumping NADH-quinone oxidoreductase (Na^+-NQR) is a central respiratory enzyme in many marine and pathogenic bacteria, adapting to the sodium-rich environment. Na^+-NQR consists of 6 subunits and couples electron transfer from NADH to ubiquinone via 6 cofactors with Na^+-pumping across the inner membrane, which forms the sodium motive force energizing crucial activities such as ATP synthase and flagella motility[1]. Previous X-ray crystallographic and cryo-electron microscopy structures of Na^+-NQR from *Vibrio cholerae*[2,3] suggested that the conformational changes during catalytic turnover drive electron transfer between cofactors. However, there is no firm evidence for these proposals. Here, we have revealed at least five distinct conformational states of Na^+-NQR by using deletion mutants of specific cofactors, inhibitors, or low-sodium conditions. Molecular dynamics simulations based on our structural data indicate that 2Fe-2S reduction in NqrD/E triggers Na^+ translocation by driving structural rearrangements in the membrane-embedded NqrD/E subunits, which subsequently influence NqrC and NqrF positioning. This study provides the first structural insights into the mechanism of Na^+ translocation coupled to electron transfer in Na^+-NQR.[4]

[1] Dibrov, P., Dibrov, E., and Pierce, G. N. Na^+-NQR (Na^+-translocating NADH:uniquinone oxidoreductase) as a novel target for antibiotics. *FEMS Microbiol*. Rev. 41, 653–671(2017).

[2] Hau, J-L., Kaltwasser, S., Muras, V., Casutt, M. S., Vohl, G., Claußen, B., Steffen, W., Leitner, A., Bill, E., Cutsail III, G. E., DeBeer, S., Vonck, J., Steuber, J., and Fritz, G. Conformational coupling of redox-driven Na^+-translocation in Vibrio cholerae NADH:quinone oxidoreductase. *Nat Struct Mol Biol*, 30, 1685-1694 (2023).

[3] Kishikawa, J., Ishikawa, M., Masuya, T., Murai, M., Kitazumi, Y., Butler, N. L., Kato, T., Barquera, B., and Miyoshi, H. Cryo-EM structures of Na^+-pumping NADH-ubiquinone oxidoreductase from *Vibrio cholerae*. *Nat. Commun.* 13, 4082 (2022).

[4] Ishikawa-Fukuda, M., Seki, T., Kishikawa, J., Masuya, T., Okazaki, K., Kato, T., Barquera, B., Miyoshi, H., and Murai, M. (2025) The redox driven Na^+-pumping mechanism in *Vibrio cholerae* NADH-quinone oxidoreductase relies on dynamic conformational changes. *Nat. Commun.* 17, 1394 (2026).

P2

High Resolution structure of *P. denitrificans* complex I in detergent micelle Serhat Dönmez[a], Leonid Sazanov[a] [a]*Institute of Science and Technology Austria, Klosterneuburg, Austria*

E-mail address: Serhat.Doenmez@ist.ac.at
Presenting author: Serhat Dönmez

Respiratory complex I (NADH:ubiquinone oxidoreductase) is a key energy-transducing enzyme that couples electron transfer from NADH to ubiquinone with proton translocation across biological membranes, thereby contributing to the proton-motive force required for ATP synthesis. Despite extensive structural characterization, the molecular mechanism underlying this coupling process remains hotly debated. Bacterial systems provide powerful models for structure–function studies because of their genetic accessibility; however, widely used models such as *Thermus thermophilus* and *Escherichia coli* present limitations, including thermophilic adaptations, non-physiological quinone usage, and the absence of a complete respiratory chain. *Paracoccus denitrificans*, close relative to the mitochondrion, is an alternative and more physiologically relevant bacterial model. Its enzyme displays high structural and functional similarity to the mammalian counterpart and uniquely contains eukaryotic-like accessory subunits absent in other bacterial systems. We purified highly homogeneous and stable wild-type complex I from *P. denitrificans* without the use of affinity tags. The enzyme purified in dodecyl-maltoside detergent retained high activity and was analysed by cryo-EM, yielding a structure at 2.3 Å resolution. The structure of detergent-solubilized enzyme reveals the presence of three eukaryotic-type supernumerary subunits, as well as an additional L-isoaspartyl-O-methyltransferase associated with the core complex, as reported recently for the nanodisk-embedded enzyme [1]. A native quinone molecule is resolved in the shallow binding site of the quinone-binding cavity. The enzyme adopts a single, well-defined closed conformation, which is also a unique feature of *P. denitrificans* complex. The conformational changes during catalytic turnover will be investigated to provide insights into the coupling mechanism of complex I.

[1] Ivanov, B.S., Bridges, H.R., Jarman, O.D. et al. Structure of the turnover-ready state of an ancestral respiratory complex I. Nat Commun 15, 9340 (2024).
https://doi.org/10.1038/s41467-024-53679

P3

Capturing the respiratory chain in action with time-resolved cryo-EM Kaitlyn A. Kiernan[a],
Leonid Sazanov[a a] Institute of Science and Technology Austria, Klosterneuburg, Austria
E-mail address: kaitlyn.kiernan@ist.ac.at
Presenting author: Kaitlyn Kiernan

The respiratory chain plays a crucial role in generating metabolic energy for cellular life. It is powered by four large membrane protein complexes that harness redox chemistry to power proton translocation across the mitochondrial membrane and drive ATP synthesis. Respiratory enzymes undergo many conformational transitions to tightly coordinate electron transfer to proton pumping. Decades of research have uncovered the basic principles of this process, but most structural insights have come from static snapshots that fail to represent the dynamic nature of these molecular machines. To address this, we have implemented time-resolved cryo-EM to visualize transient structural states of the largest enzyme of the respiratory chain, respiratory complex I (NADH:ubiquinone oxidoreductase). We have constructed a device enabling controlled substrate delivery and rapid freezing of cryo-EM grids on the millisecond timescale. This approach allows us to trap short-lived conformational states during turnover and resolve precisely how complex I
couples electron transfer from NADH to quinone with proton pumping. Ultimately, this work will advance our understanding of mitochondrial bioenergetics and establish a broadly applicable approach for probing the structural dynamics of large, membrane-bound molecular assemblies in action.

P4

Diversity and in situ organization of algal mitochondrial respiratory supercomplexes Mariia Melnik[a], Ben Engel[a], Florent Waltz[a]

aBiozentrum, University of Basel, Switzerland

E-mail address: mariia.melnik@unibas.ch
Presenting author: Mariia Melnik

Mitochondrial respiratory complexes can assemble into supercomplexes, or respirasomes, whose function remains unclear. To visualize their organization within the native membrane context, we use cryogenic electron tomography (cryo-ET) to capture 3D native cellular volumes where molecular details can be resolved. With this approach, we investigate the diversity of respiratory supercomplexes and aim to understanding their function, including a potential role in shaping mitochondrial architecture.

In the green alga *Chlamydomonas reinhardtii*, we reported the first description of respirasomes in native cells, identifying a single respirasome type, $CI_2CIII_4CIV_6$, localized on the flat regions of lamellar cristae.[1] Respirasomes in this organism are spatially segregated from rows of ATP synthase dimers, which localize on the curved cristae rims. We are now investigating the phenotype of mutants lacking complete complexes and/or subunits involved in supercomplexes stabilization and observe changes in overall cristae architecture and supercomplexes organization.

We also investigate mitochondrial architecture in the unicellular marine diatom alga *Phaeodactylum tricornutum*. There, we observe characteristic tubular cristae and multiple respirasome populations of different stoichiometries and localizations. Notably, some respirasomes are not strictly segregated from ATP synthase. Some assemblies stably associate with ATP synthase, forming a CI-CV "OXPHOSome" that integrates the entire respiratory chain.

Our results reveal a pronounced correlation between respirasome organization and mitochondrial membrane morphology. Characterizing this relationship will advance understanding of how molecular organization shapes higher-order organelle architecture.

[1] F. Waltz, R.D. Righetto, L. Lamm, T. Salinas Giegé, R. Kelley, X. Zhang, M. Obr, S. Khavnekar, A. Kotecha, B.D. Engel, In cell architecture of the mitochondrial respiratory chain. *Science* 387, (2025), 1296– 1301. https://doi.org/10.1126/science.ads8738

P5

Interdomain bridges and their roles in complex I regulation and catalysis

Daniel N. Grba[a], Chris Seunggyu Lee[a,b], John J. Wright[a], Bozhidar S. Ivanov[a], Judy Hirst[a].

[a]*MRC Mitochondrial Biology Unit, University of Cambridge*
[b]*Centre for Medicines Discovery, Nuffield Department of Health, University of Oxford*

E-mail address: dng26@cam.ac.uk
Presenting author: Daniel N. Grba

Complex I (CI; NADH ubiquinone oxidoreductase) is central to energy generation and metabolic homeostasis. However, in mammalian cells it can contribute to adverse outcome pathways under challenging conditions; for example, during ischemia, mammalian CI transitions from a turnoverready, structurally "closed" state toward a dormant "open" state, where this transition prevents CI from functioning in reverse during reperfusion where it produces reactive oxygen species [1]. Generally speaking, the closed state includes ordered substrate-binding loops at the interface of the two major arm domains of CI; the open state contains disordering of these loops and subsequent relaxation at this junction. Unfortunately, simpler, genetically tractable CI models do not recapitulate the same behavior, compromising mechanistic studies into this form of regulation. We recently showed that structure of isolated CI from the yeast *Pichia pastoris* (*Pp*-CI) adopts distinct closed and open states that resemble those of mammalian CI, and that there was a direct correlation of these states with a unique subunit, NUQM, that bridges the two major arms of the complex. This interdomain bridging, is integral to defining the energy landscape of the larger conformational changes of CI, including those associated with the active–deactive transition. We look at analogous interdomain bridges, and their absences, across multiple species and use these to consider their roles in stability, regulation, as well as proposed turnover mechanisms .

P6

Protonation-linked conformational gating coordinates vectorial proton transfer in respiratory complex I

John J. Wright[a], William Fisher[a], Man Nok Choy[a], Guilherme M. Arantes[b] Robert A. Waddell[a], Daniel N. Grba[a] , Judy Hirst[a]

[a] *The Medical Research Council Mitochondrial Biology Unit, University of Cambridge* [b]*Department of Biochemistry, Instituto de Química, Universidade de São Paulo*

E-mail address: jjw86@cam.ac.uk
Presenting author: John J. Wright

Respiratory complex I (NADH:ubiquinone oxidoreductase) is a central enzyme in cellular energy metabolism, converting the free energy of NADH oxidation and ubiquinone reduction into a transmembrane proton gradient that drives ATP synthesis during oxidative phosphorylation. Despite extensive structural characterisation across multiple species, the mechanism underlying energy coupling and proton pumping remains unresolved. The apparent absence of large-scale conformational changes within the membrane domain of this intricate redox-driven proton pump has obscured our understanding of how proton transfer is gated and coordinated. High-resolution structures have identified conserved hydrated networks within the membrane domain comprising chains of ionisable residues proposed to mediate proton transfer, however their connectivity, regulation, and response to physiological variables such as pH are not well understood. Here, we investigate how pH modulates the structural and functional properties of mammalian complex I by determining high-resolution (2.2 Å) cryo-EM structures of complex I from *Bos taurus* captured under acidic (pH 6.0) and alkaline (pH 9.2) conditions. These structures enable detailed visualisation of hydrogen-bonding networks within the membrane domain and reveal histidine gating mechanisms in the antiporter-like subunits that govern proton-transfer directionality. By integrating structural analysis with constant pH molecular dynamics (CpHMD), we identify control points that coordinate these proton transfer processes. Together, these results clarify principles underlying proton-coupled energy transduction in respiratory complex I.

P7

Mechanistic insights into the DsrMKJOP complex, a central respiratory complex in dissimilatory sulfite reduction

Raquel M. Bernardino[a], Ana C. C. Barbosa[a], Andreia I. Pimenta[a], José A. Brito[a], Max Dongsheng Yin[b], Bonnie J. Murphy[b], Inês A. C. Pereira[a]

a Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVA de Lisboa, Oeiras, Portugal, b Redox and Metalloproteins Research Group, Max Planck Institute of Biophysics, Frankfurt am Main, Germany

E-mail: rm.bernardino@itqb.unl.pt

Presenting author: Raquel M. Bernardino

Microbial reduction of sulfur compounds plays a key role in driving the global sulfur and carbon cycles [1]. Dissimilatory sulfite reduction (DSR) is a central pathway in microbial sulfur metabolism and is believed to be an ancient respiratory process sustaining life on early Earth. Furthermore, its relevance to human health has become increasingly recognized, as gut microbial sulfide production has been associated with chronic inflammatory disorders and cancer [2].

Over the last years, our group has made significant advances in elucidating the biochemical mechanisms underlying sulfite reduction and its coupling to energy conservation. The core of the DSR pathway comprises the cytoplasmic sulfite reductase DsrAB, its essential partner DsrC, and the membrane-bound DsrMKJOP complex. In this pathway, DsrAB, together with its cosubstrate DsrC, reduces sulfite to a zero-valent sulfur (S^0), forming a trisulfide in DsrC [3]. The DsrC-trisulfide is subsequently reduced to sulfide by the transmembrane DsrMKJOP complex via menaquinol oxidation, a process likely coupled to the generation of a proton motive force [4].

Despite the essential role of this membrane complex in linking sulfite reduction to chemiosmotic energy conservation, the molecular mechanism of DsrMKJOP remain poorly understood. Here, functional and structural insights into this respiratory complex will be presented, providing new perspectives on how energy is conserved during dissimilatory sulfite reduction.

References:

1. Rabus, R. et al. Adv Microb Physiol (2015) doi:10.1016/bs.ampbs.2015.05.002
2. Pimenta, A. I. et al. Adv Microb Physiol (2024) doi:10.1016/BS.AMPBS.2024.04.003.
3. Santos, A. A. et al. Science (2015) doi:10.1126/science.aad3558
4. Barbosa, A. C. C. et al. PNAS (2024) doi:10.1073/PNAS.2313650121

P8

In situ characterisation of the effect of SCAF1 on mitochondrial respiratory supercomplexes, mitochondrial network and morphology in human embryonic kidney cells

Ines Zouhair[a,b], Emma Buzzard[b], Valentina Loconte[c], Cristina Ugalde[d,e], Karen Davies[b] and Amandine Maréchal[a,f]

[a] *Department of Structural and Molecular Biology, University College London, London WC1E 6BT, UK*

[b] *eBIC, Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK* [c] *B24 beamline, Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK*

[d] *Centro de Investigaciones Biologicas Margarita Salas (CIB-CSIC), Madrid 28040; Spain* [e] *Instituto de Investigacion Hospital 12 de Octubre, Madrid 28041, Spain*

[f] *Institute of Structural and Molecular Biology, Birkbeck College, London WC1E 7HX, UK*

E-mail address: ines.zouhair.19@ucl.ac.uk
Presenting author: Ines Zouhair

Mitochondrial respiratory supercomplexes (SCs) vary in composition and stoichiometry across species and mammalian tissue types, suggesting that their evolution has adapted to different cellular metabolic requirements. In addition, changes in SC populations have been observed in response to metabolic shifts and in health versus disease. However, the regulation and biological significance of SC formation in relation to energy production remains uncertain. In mammals, complex IV forms different SCs via its COX7A subunit. COX7A can exist as one of three isoforms—COX7A1, COX7A2 or COX7A2L (SCAF1)— which are differentially expressed depending on tissue type. Mutant human cell lines selectively expressing only one COX7A isoform showed differences in mitochondrial respiratory chain (MRC) organisation and cellular bioenergetic phenotypes, suggesting a direct link between COX7A isoforms, SC populations and metabolism (Fernández-Vizarra et al., 2022, Cell Metabolism). In recent years, efforts have been made to characterise SCs in absence of detergent, in their native environment, using cryogenic electron tomography (cryo-ET) on mitochondrial membranes or even whole cells. Several structures of the MRC promoted by COX7A2 (C-MRC) have been solved and provided novel information on the different COX7A2-led SC populations' occurrence *in situ* as well as the existence of higherorder assemblies. Here, we have been targeting the MRC promoted by SCAF1 (S-MRC). Using cryo-ET on isolated mitochondrial membranes from HEK293T COX7A2^{KO} cell lines exclusively expressing SCAF1, we aimed to characterise the different SCAF1-led SC populations occurring in the native membrane environment and will be reporting preliminary *in situ* structures of S-MRC SCs observed to contain complexes I and III. Furthermore, we used cryo-soft X-ray tomography (cryo-SXT) to investigate the impact of the C- and S-MRC at the cellular level. Indeed, Fernández-Vizarra et al. showed that HEK293T WT and COX7A2^{KO} cells displayed different responses when grown under glycolytic or OXPHOSinducing conditions, with COX7A2^{KO} cells exhibiting a longer doubling time under OXPHOSinducing conditions, highlighting the distinct roles of the C-MRC and S-MRC in meeting different bioenergetic demands. Therefore, we collected cryo-SXT data on both cell lines grown in glucose or galactose medium to study the effect of SCAF1 and associated S-MRC on mitochondrial ultrastructure and network. Overall, our *in situ* and multi-scale approach to characterising WT and SCAF1 expressing cell lines, integrating molecular, ultrastructural and metabolic changes, provides a novel insight into how different MRC organisations can impact a cell's response to different energetic needs.

P9

Redox-driven proton-pumping in respiratory alternative complex III, ACIII, from *Rhodothermus marinus*

Catarina Guerreiro-Oliveira[a], Filipa Calisto[a], Janet Vonck[b], Manuela M. Pereira[a]

a
University of Lisbon, Faculty of Sciences, Department of Chemistry and Biochemistry and BioISI - Biosystems & Integrative Sciences Institute, Campo Grande, C8, 1749-016 Lisboa, Portugal.
b
Department of Structural Biology, Max Planck Institute of Biophysics, Max-von-Laue Str. 3, 60438, Frankfurt am Main, Germany.

E-mail address: fc65196@alunos.fc.ul.pt Presenting author: Catarina Guerreiro-Oliveira

Rhodothermus marinus is an obligately aerobic, thermohalophilic, and Gram-negative bacterium, with optimum growth at 65 °C and 1−2 % sodium concentration.[1,2] Its respiratory chain contains an respiratory Alternative Complex III (ACIII), a quinol:cytochrome *c* oxidoreductase complex functionally analogous but structurally distinct from canonical bc_1 complexes.[3] ACIII from *R. marinus* was the first complex in this defined class of oxidoreductases to be isolated and characterized.[4,5] It is composed of eight subunits, including ActA, which accommodates five *c*-type hemes, and ActE, a monoheme *c* subunit.[6,7] Subunits ActC and ActF have previously been shown, by single-particle cryo-electron microscopy (cryo-EM) structure, to contain putative proton pathways, suggesting the existence of two proton-pumping sites in ACIII and framing it as a redox-driven proton pump.[7] Here, we present a new cryo-EM structure of ACIII from *R. marinus* at 2.14 Å resolution, providing unprecedented detail of the complex in its complete eight-subunit assembly. This high-resolution structure reveals ordered water molecules within the previously identified proton channels, supporting their role as functional proton transfer pathways. We will further discuss how these structural features relate to ACIII's redox-driven proton-pumping mechanism and address its proton/electron stoichiometry. Functional studies are in progress using liposome-reconstituted ACIII. Fluorescence spectroscopy assays with SNARF-1 and DiSC3(5) were performed to monitor pH and membrane potential variations. The obtained results will be discussed providing new insights into ACIII's redox-driven protonpumping stoichiometry.

[1] G.A. Alfredsson, J.K. Kristjansson, S. Hjorleifsdottir, K.O. Stetter, Rhodothermus marinus, gen. nov., sp. nov., a thermophibic, halophilic bacterium from submarine hot springs in Iceland. J. Gen. Microbiol., 134(2) (1988), 299−306. https://doi.org/10.1099/00221287-134-2-299

[2] O.C. Nunes, M.M. Donato, M.S.Da Costa, Isolation and Characterization of Rhodothermus Strains from S. Miguel, Azores. Syst. Appl. Microbiol., 15(1) (1992), 92−97. https://doi.org/10.1016/S07232020(11)80144-X

[3] M.M. Pereira, J.N. Carita, M. Teixeira, Membrane-bound electron transfer chain of the thermohalophilic bacterium Rhodothermus marinus: a novel multihemic cytochrome bc, a new complex III. Biochemistry, 38(4) (1999), 1268−1275. https://doi.org/10.1021/bi9818063

[4] M.F. Yanyushin, M.C. del Rosario, D.C. Brune, R.E. Blankenship, New Class of Bacterial Membrane Oxidoreductases. Biochemistry, 44(30) (2005), 10037−10045. https://doi.org/10.1021/bi047267l

[5] M.M. Pereira, P.N. Refojo, G.O. Hreggvidsson, S. Hjorleifsdottir, M. Teixeira, The alternative complex III from *Rhodothermus marinus* — a prototype of a new family of quinol:electron acceptor oxidoreductases. FEBS Lett., 581(25) (2007), 4831−4835. https://doi.org/10.1016/j.febslet.2007.09.008

[6] P.N. Refojo, M. Teixeira, M.M. Pereira, The alternative complex III of *Rhodothermus marinus* and its structural and functional association with caa3 oxygen reductase. Biochim. Biophys. ActaBioenerg., 1797(8) (2010), 1477−1482, https://doi.org/10.1016/j.bbabio.2010.02.029

[7] J.S. Sousa, F. Calisto, J.D. Langer, D.J. Mills, P.N. Refojo, M. Teixeira, W. Kühlbrandt, J. Vonck, M.M. Pereira, Structural basis for energy transduction by respiratory alternative complex III. Nat. Commun., 9 (2018), 1728. https://doi.org/10.1038/s41467-018-04141-8

P10

Deciphering the Deactive to Active transition in bovine Complex I

Ketul Saharan[a], John J. Wright[a], Daniel Grba[a], and Judy Hirst[a]

[a]Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom

Email address: ks2166@cam.ac.uk
Presenting Author: Ketul Saharan

Respiratory complex I couples NADH oxidation and ubiquinone reduction to pump protons across the mitochondrial membrane. While its mechanism is fundamental to bioenergetics, transitions between the active (closed) and deactive (open) states [1] of the mammalian enzyme, and their relevance to catalysis, are not fully understood [2]. While some models propose that a distribution of open and closed states is maintained during catalysis, viewing the open state as an on-pathway intermediate [3], other views argue it represents an off-cycle resting form [4]. Here, we use a combination of biochemical and structural methods to study the activation of bovine complex I in Q_{10}-containing proteoliposomes upon the addition of substrates. Starting with a fully deactive (100% open) population, we monitor the transition from open to closed by correlating biochemical labelling and single-particle cryo-electron microscopy. We compare the results from different biochemical systems, including mitochondrial membranes and submitochondrial particles, under a range of experimental conditions, and observe that, in all cases, the enzyme shifts substantially towards the closed state. However, we have not yet succeeded in observing a 100% closed population. We discuss technical and biological reasons for the differences between the systems and conditions, and the implications for understanding the mechanisms of catalysis and regulation.

[1] E. Maklashina, A.B. Kotlyar, G. Cecchini, Active/de-active transition of respiratory complex I in bacteria, fungi, and animals, Biochim Biophys Acta, 1606 (2003) 95-103

[2] I. Chung, D. Grba, J.J. Wright, J. Hirst, Making the leap from structure to mechanism: are the open states of mammalian complex I identified by cryoEM resting states or catalytic intermediates?, Curr Opin Struct Biol, 77 (2022) 102447.

[3] D. Kampjut, L.A. Sazanov, Structure of respiratory complex I – An emerging blueprint for the mechanism, Curr Opin Struct Biol, 74 (2022) 102350.

[4] D. N. Grba, J. J Wright, Z. Yin, W. Fisher, J. Hirst, Molecular mechanism of the ischemiainduced regulatory switch in mammalian complex I, Science 384 (2024), 1247-1253.

P11 The structure and function of dimeric cytochrome *c* dependent Nitric Oxide Reductase from *Paracoccus denitrificans*.

Finja König[a], Katarzyna Jankowska[a], Maximilian Kahle[a], Sofia Appelgren[a,b] and Pia Ädelroth[a]. [a] Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden [b] Department of Biology, Philipps University Marburg, Marburg, Germany
Presenting author: Finja König, finja.konig@dbb.su.se

The enzyme cytochrome *c* dependent Nitric Oxide Reductase (*c*NOR) is part of the branched respiratory chain of the bacterium *Paracoccus denitrificans (Pd)* enabling the organism's anaerobic respiration of nitrogen compounds, where it catalyzes the reduction of nitric oxide to nitrous oxide. *c*NOR is a homologue to complex IV of the respiratory chain. The protein consists of two subunits, NorC and NorB. NorC is so named for its *c*-heme which accepts electrons from soluble cytochrome *c*, these are passed to the NorB subunit with contains two *b*-hemes of which one *b*-heme and a nonheme iron make up the active site for NO-reduction. The presence of the non-heme iron in the active site is dependent on two chaperone proteins NorQ and NorD [1]. Expression of the protein without these chaperones results in an inactive *c*NOR lacking the non-heme iron.

Using Cryo-EM we have solved the structure of *Pd c*NOR. In contrast to the only previously solved *c*NOR structure, a crystal structure of the enzyme from *P. aeruginosa* [2], our structure shows a dimer of two *c*NOR proteins ($NorB_2C_2$). A dimeric structure has been reported for the related quinol dependent Nitric Oxide Reductase (*q*NOR) [3] for which the dimeric form is more active than the monomer. In our structure the dimer interface is formed by NorB transmembrane helix 9 and 11, this is different from the interface reported for *q*NOR.

To investigate the importance of the *c*NOR dimer we have introduced mutations to the surface of NorB at the interface between the two *c*NOR proteins. Blue Native PAGE results indicate that some of these mutations affect dimer formation. We are investigating dimer formation further using Mass Photometry, and also look at potential effects on nitric oxide reduction activity and non-heme iron content. We hope to elucidate whether the dimer is biologically relevant for the activity of *c*NOR and/or for iron-insertion by the chaperones.

(1) Kahle, M.; Ter Beek, J.; Hosler, J. P.; Ädelroth, P. The Insertion of the Non-Heme FeB Cofactor into Nitric Oxide Reductase from P. Denitrificans Depends on NorQ and NorD Accessory Proteins. *Biochim. Biophys. Acta BBA - Bioenerg.* 2018, *1859* (10), 1051–1058. https://doi.org/10.1016/j.bbabio.2018.05.020.

(2) Hino, T.; Matsumoto, Y.; Nagano, S.; Sugimoto, H.; Fukumori, Y.; Murata, T.; Iwata, S.; Shiro, Y. Structural Basis of Biological N_2 O Generation by Bacterial Nitric Oxide Reductase. *Science* 2010, *330* (6011), 1666–1670. https://doi.org/10.1126/science.1195591.

(3) Jamali, M. A. M.; Gopalasingam, C. C.; Johnson, R. M.; Tosha, T.; Muramoto, K.; Muench, S. P.; Antonyuk, S. V.; Shiro, Y.; Hasnain, S. S. The Active Form of Quinol-Dependent Nitric Oxide Reductase from *Neisseria Meningitidis* Is a Dimer. *IUCrJ* 2020, *7* (3), 404–415. https://doi.org/10.1107/S2052252520003656.

P12

Analysing Respiratory Chain Complexes from *Corynebacterium glutamicum*

Nick Hiatt,[1,2,3], Wei-Chun Kao[1], Carola Hunte[1,4]

[1] *Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine, University of Freiburg, Freiburg, Germany*

[2] *Faculty of Biology, University of Freiburg, Freiburg, Germany*

[3] *SGBM -Spemann Graduate School of Biology and Medicine, University of Freiburg, Freiburg, Germany* [4] *CIBSS, Centre for Integrative Biological Signalling Studies, Freiburg, Germany nick.hiatt@biochemie.uni-freiburg.de,* wei-chun.kao@biochemie.uni-freiburg.de, *carola.hunte@biochemie.uni-freiburg.de*

Corynebacterium glutamicum is an aerobic Gram-positive bacterium of major industrial relevance for amino acid production and of biomedical interest due to its close phylogenetic relationship to pathogens such as *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis*. A hallmark of *C. glutamicum* is its branched electron transport chain, making it an attractive model for studying bacterial respiration and the organization and assembly of respiratory protein machineries. Cellular respiration is essential for aerobic life and relies on oxidative phosphorylation, in which the proton motive force generated by respiratory chain complexes drives ATP synthesis via ATP synthase. In *C. glutamicum*, the terminal oxidase (complex IV) is organized as *bcc-aa*$_3$ supercomplex, featuring a di-heme cytochrome *c* subunit within the *bcc* complex. How such an intricate respiratory machinery assembles and is maintained remains poorly understood.

We investigate the assembly and structural organization of respiratory chain complexes in *C. glutamicum*. To enable rapid assessment of complex integrity, identification of higher-order assemblies and assembly intermediates, we optimized membrane protein solubilization using a range of detergents and established native gel analysis suitable for complexome profiling and comparative analysis of assembly defects in future mutagenesis studies. In parallel, we developed purification and sample preparation strategies for cryo-electron microscopy, yielding several high-resolution structures of respiratory protein machineries. We present here the cryo-EM structure of respiratory complex II (succinate:menaquinone oxidoreductase), which catalyzes electron transfer from succinate to menaquinone. The complex comprises three subunits: the flavoprotein SdhA, the iron–sulfur protein SdhB, and the membraneanchoring subunit SdhC containing two hemes. The structure was resolved in its trimeric state at 2.4 Å resolution. Functional characterization of the complex complements the structural analysis. Together, these data provide a framework for understanding the organization and assembly of respiratory chain machineries in *C. glutamicum*.

P13

Integrative mechanistic explorations of the Q_o active site of the mycobacterial III_2IV_2 supercomplex

Terezia Kovalova, Ana P. Gamiz-Hernandez, Daniel Riepl, Sylwia Król, Dan Sjöstrand, Peter Brzezinski, Martin Högbom, Ville R. I. Kaila

Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, 106 91 Stockholm, Sweden
E-mail address: terezia.kovalova@dbb.su.se; ville.kaila@dbb.su.se Presenting author: Terezia Kovalova

Mycobacterium tuberculosis is a pathogenetic bacteria, with emerging multi-resistance strains resulting in severe global health threats. Development of novel drugs against is thus of great biomedical importance. One of the most promising drug targets is the $CIII_2CIV_2$ respiratory supercomplex of *Actinobacteria,* as this protein assembly is structurally distinct from its mammalian counterpart. To derive a detailed understanding of the mechanistic principles linked to its menaquinol oxidoreductase activity, we probe here the function of key residues of the Q_o site in the *M. smegmatis* $CIII_2CIV_2$ supercomplex by combining site-directed mutagenesis experiments and molecular simulations with biochemical activity assays and cryo-electron microscopic studies. Mutation of the identified results in drastic perturbation of the activity (0-50%), while our highresolution (2.3-3.0 Å) structures of the variants resolve distinct conformational changes linked to the function of the supercomplex. Our combined findings guide functional studies as well as the design of new drugs against mycobacteria.

P14

Time-resolved studies of nitric oxide turnover in cytochrome *c* oxidase

Johan Glerup[a], Elin Hellquist[a], Gabrielle Wehlander[a] and Gisela Brändén[a].
[a]*University of Gothenburg*

E-mail address: johan.glerup@gu.se
Presenting author: Johan Glerup

Cytochrome *c* oxidase (C*c*O) is a key enzyme in oxidative phosphorylation, where it couples the reduction of oxygen to water with proton pumping. Some C*c*Os can also reduce nitric oxide (NO) to nitrous oxide (N_2O). We study this reaction in the ba_3-type C*c*O from *Thermus thermophilus* using photolabile and pHdependent NO donors to deliver NO directly into the active site. We follow changes after NO-release by serial crystallography and UV-Vis spectroscopy. I will present the first crystallographic models of NO bound ba_3-type C*c*O and discuss the reaction intermediates. In accordance with previous studies, we observe that NO turnover proceeds far slower than oxygen turnover [1], presenting both advantages and challenges for time-resolved crystallography. The work highlights current technical limitations when working under anaerobic conditions and triggering reactions with photocages for experiments at synchrotron and XFEL facilities.

[1] Giuffrè, A., Stubauer, G., Sarti, P., Brunori, M., Zumft, W.G., Buse, G., Soulimane, T., 1999. The hemecopper oxidases of Thermus thermophilus catalyze the reduction of nitric oxide: Evolutionary implications. Proc. Natl. Acad. Sci. U.S.A. 96, 14718–14723. https://doi.org/10.1073/pnas.96.26.14718

P15

Rotatory activity of ATP Synthase tunes membrane elasticity through lateral packing modulation: evidence and implications Iván López-Montero[a,b,c]

[a]*Departamento de Química Física, Universidad Complutense de Madrid, Av. Complutense s/n 28040 Madrid, Spain*

[b] *Instituto Pluridisciplinar, Universidad Complutense de Madrid, Paseo Juan XXIII 1, 28040 Madrid, Spain*

[c] *Instituto de Investigación Sanitaria Hospital Doce de Octubre (imas12), Av. Córdoba s/n, 28041 Madrid, Spain*

E-mail address: ivanlopez@quim.ucm.es
Presenting author: Iván López-Montero

Membrane elasticity and lateral lipid packing are central determinants of cellular function, yet the extent to which active proteins can directly remodel these properties remains poorly understood. Our work aims to elucidate how the rotatory activity of ATP synthase impacts membrane mechanics, lipid self-organization and mesoscale structural dynamics in model membrane systems. ATP synthase was reconstituted into lipid bilayers of controlled composition, including vesicular and tubular architectures, and its rotational activity was modulated under physiologically relevant biochemical conditions. Membrane structural and mechanical responses were characterized using time-resolved fluorescence microscopy, flickering spectroscopy, and complementary techniques sensitive to mechanical response. The primary observable is a rotation-dependent reduction of lateral lipid packing [1], which acts as a central driver of multiple downstream effects. Decreased packing facilitates curvature-dependent redistribution and sorting of ATP synthase into lipid nanofilaments [2], enhances membrane fluctuations [3], and allows active control over the size of lipid domains [4]. These results demonstrate that ATP synthase activity imposes nonequilibrium stresses on the bilayer, which directly modulate both elasticity and mesoscale organization in a feedback loop between protein dynamics and membrane structure. In conclusion, ATP synthase functions as an active modulator of membrane mechanics, demonstrating that protein rotational dynamics can directly tune lateral lipid packing and drive self-assembly in lipid membranes. This bidirectional coupling between enzymatic activity and membrane properties provides mechanistic insight into the regulation of mitochondrial membrane architecture and dynamics.

.

[1] D. Valdivieso González, M.A. Sacristán, P. Natale, B. Orgaz, M.P. Lillo, V. Almendro-Vedia, I. López-Montero, Elastic remodeling of model and cell membranes by rotating ATP synthase, Cell Rep. Phys. Sci. 6 (2025) 102567. https://doi.org/10.1016/j.xcrp.2025.102567

[2] D. Valdivieso González, M. Makowski, M.P. Lillo, F.J. Cao-García, M.N. Melo, V.G. Almendro-Vedia, I. López-Montero, Rotation of the c-Ring promotes the curvature sorting of monomeric ATP synthases, Adv. Sci. 10 (2023) e2301606.
https://doi.org/10.1002/advs.202301606

[3] V.G. Almendro-Vedia, P. Natale, M.N. Melo, S. Bonneau, F. Monroy, F. Joubert, I. López-Montero, Nonequilibrium fluctuations of lipid membranes by the rotating motor protein F_1F_o-ATP synthase, Proc. Natl. Acad. Sci. U.S.A. 114 (2017) 11291–11296.
https://doi.org/10.1073/pnas.1701207114

[4] N. Carrillo-Godoy, D. Valdivieso González, P. Natale, H. Ritacco, F.J. Cao-García, V.G. Almendro-Vedia, I. López-Montero, Protein nanorotors control the size of lipid domains in phase-separated monolayers, J. Colloid Interface Sci. 698 (2025) 138061.
https://doi.org/10.1016/j.jcis.2025.138061

P16

Role of dynamic lipid-protein scaffold in quinone transport by respiratory chain coupling proteins

L.Simsive[a], A. Kropp[b], R. Grinter[b], V. Sharma[a,c], O.Zdorevskyi[a]
[a]Department of Physics, University of Helsinki, Helsinki, Finland
[b]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Australia
[c]HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki, Finland

E-mail address: oleksii.zdorevskyi@helsinki.fi
Presenting author: Oleksii Zdorevskyi

Throughout evolution, bacteria have developed a range of sophisticated survival strategies to endure diverse environmental conditions.[1,2] A key aspect of this adaptability lies in the inner bacterial membrane, which is densely populated with proteins involved in various metabolic pathways, including aerobic and anaerobic respiration, fermentation, and carbon fixation. To accommodate the high density of proteins within the membrane, nature has evolved specialized structures named respiratory chain coupling proteins (RCCPs).[3] These tube-like elongated membrane-anchored conduits facilitate electron transfer between the solvent-exposed dehydrogenases and membrane-associated quinone pool.

While recent advances have led to high-resolution RCCP structures,[3,4] their functional mechanisms remain largely unexplored. In this study, we employ large-scale atomistic molecular dynamics simulations to investigate the dynamics of lipids and quinones with RCCP-associated Type-2 NADH Dehydrogenase (NDH2) from Bacillus subtilis.[4] The simulation data reveal that phospholipid head groups effectively occupy the surface region of RCCP-NDH2 interface keeping the tube interior dehydrated. The long-time scale simulation data also show that the stabilization of membrane-associated RCCP can be achieved by tilting of the entire RCCP-NDH2 complex relative to the lipid bilayer, which might facilitate the uptake of membrane-bound quinones. The high mobility of lipid tails and quinones inside the RCCP conduit create favorable conditions for efficient long-range quinone transport. These findings[5] provide novel insights into the functional roles of RCCPs in bacterial respiration, with potential implications for protein engineering and the development of biocatalytic systems.

P17

Investigating *Mycobacterium abscessus* cytochrome *bcc*:aa_3 oxidase QcrB subunit reveals key insights that paves the way to the development of new targeting agents Emilia Tan Xin Yi[1], Vikneswaran Mathiyazakan[1], and Gerhard Grüber[1]

1 School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Republic of Singapore

E-mail address: EM0001YI@E.NTU.EDU.SG
Presenting author: Emilia Tan Xin Yi

Abstract

The threat of non-tuberculous mycobacteria (NTM) infection globally has become increasingly apparent [(1,2)]. Among these NTMs, *Mycobacterium abscessus* (*Mab*) has been notoriously known to be difficult to treat due to its resistance to numerous classes of antibiotics including standard first line antituberculosis drugs [(3–5)]. Being a non-fermentative obligate aerobe, *Mab* is highly dependent on the oxidative phosphorylation (OXPHOS) pathway to meet its energetic demands. The cytochrome *bcc*:aa_3 supercomplex is a terminal oxidase in the OXPHOS pathway responsible for the translocation of protons to maintain redox homeostasis and the reduction of oxygen to water. Q203 (Telacebec) a cytochrome *bcc*:aa_3 supercomplex inhibitor bacteriostatic in *Mycobacterium tuberculosis* (*Mtb*) H37Rv was found to be ineffective against *Mab*. Here we report that D311 and L314 at the Q_o site of QcrB subunit are the key amino acids identified responsible for conferring resistance in *Mab* to Q203. This was confirmed through mutational studies investigating growth inhibition, intracellular ATP and oxygen consumption rate (OCR) of the mutants in the presence of Q203. The experiments revealed that D311E/L314A double mutant display growth inhibition, decrease in ATP level and an increase in OCR when incubated with Q203 compared to wild type. To achieve a better grasp of the evolutionary aspect of the Q_o site of QcrB of *Mtb*, *Mycobacterium smegmatis* and *Mab*, D311E/L314I double mutant was generated. Q203 tested against this mutant exhibited an intermediate effect between *Mtb* and *Mab*. Homology modeling studies on the QcrB Q_o site showed a large cavity present in *Mtb* but this cavity gradually shrank in *M. smegmatis* and furthermore in *Mab* [(6)]. This finding supports the identified D311 and L314 polymorphisms that led to the resistance to Q203. Overall,
these key findings are important, and they help build the foundation needed for medicinal chemist to develop compounds capable of inhibiting the *Mab* cytochrome *bcc*:aa_3 supercomplex.

1. Sorayah R, Manimekalai MSS, Shin SJ, Koh WJ, Grüber G, Pethe K. Naturally-Occurring Polymorphisms in QcrB Are Responsible for Resistance to Telacebec in *Mycobacterium abscessus*. ACS Infect Dis. 2019 Dec 13;5(12):2055–60. doi:10.1021/acsinfecdis.9b00322

2. Ratnatunga CN, Lutzky VP, Kupz A, Doolan DL, Reid DW, Field M, et al. The Rise of Non-Tuberculosis Mycobacterial Lung Disease. Front Immunol. 2020 Mar 3;11:303. doi:10.3389/fimmu.2020.00303

3. Chopra S, Matsuyama K, Hutson C, Madrid P. Identification of antimicrobial activity among FDA-approved drugs for combating Mycobacterium abscessus and Mycobacterium chelonae. J Antimicrob Chemother. 2011 Jul 1;66(7):1533–6. doi:10.1093/jac/dkr154

4. Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus. Nat Rev Microbiol. 2020 Jul;18(7):392–407. doi:10.1038/s41579-020-0331-1

5. Dartois V, Dick T. Toward better cures for *Mycobacterium abscessus* lung disease. Fang FC, editor. Clin Microbiol Rev. 2024 Dec 10;37(4):e00080-23. doi:10.1128/cmr.00080-23

6. Sorayah R, Manimekalai MSS, Shin SJ, Koh WJ, Grüber G, Pethe K. Naturally-Occurring Polymorphisms in QcrB Are Responsible for Resistance to Telacebec in *Mycobacterium abscessus*. ACS Infect Dis. 2019 Dec 13;5(12):2055–60. doi:10.1021/acsinfecdis.9b00322

P18

Mammalian respiratory supercomplexes: Uncovering their role in mitochondrial bioenergetics

Andy Dominguez[a], Jelena Misic[b], Nils-Göran Larsson[b], Leonid Sazanov[a] [a] *Institute of Science and Technology Austria, Klosterneuburg, Austria* [b] *Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden*

E-mail address: andy.dominguezrodriguez@ist.ac.at

Presenting author: Andy Dominguez

The physiological role of mammalian respiratory-chain supercomplexes remains unresolved, particularly under conditions in which canonical respirasome architecture is perturbed but oxidative phosphorylation is preserved. Here, we investigated supercomplex remodeling in mitochondria from UQCRC1delEED mice[1], in which the canonical complex I–complex III_2 interface is destabilized. High-molecular-weight assemblies were purified from hepatic mitochondria after digitonin solubilization using optimized MonoQ anion-exchange chromatography and micro-size-exclusion chromatography, thereby improving the recovery and stability of fragile assemblies. Biochemical analysis revealed a marked increase in the content of the free complex I together with the appearance of a novel high-molecular-weight complex, not present in WT mitochondria. We have solved the preliminary cryo-EM structure of the newly formed supercomplex, providing structural insight into how respiratory-chain architecture is changing in response to perturbation of established interaction interfaces.

[1] Milenkovic, D. *et al.* Preserved respiratory chain capacity and physiology in mice with profoundly reduced levels of mitochondrial respirasomes. *Cell Metab.* 35, 1799-1813.e7 (2023)

P19

Structures of *Tetrahymena thermophila* mitochondrial megacomplex integrating the entire respiratory chain

Fangzhu Han[a], Mengchen Wu[a], Long Zhou[a]

[a]School of Basic Medicine, Zhejiang University, Hangzhou 310058, China

E-mail address: mengchenwu@zju.edu.cn

Presenting author: Mengchen Wu

Mitochondrial respiration chain is responsible for the electron transport step of oxidative phosphorylation and is composed of four trans-membrane complexes I-IV (CI-CIV). Supercomplexes are assembled from these four complexes with defined structures, including $CICIII_2$, $CICIII_2CIV$ or the respirasome, $CIII_2CIV_2$ and $CI_2CIII_2CIV_2$ or the megacomplex in the mammalian mitochondria. On the other hand, complex II or the succinate dehydrogenase, providing an alternative entry point into the respiratory chain, has not been discovered as part of any supercomplex until recently. Moreover, accumulating evidences show that the higher-order assemblies of respiratory chain complexes contribute to the shaping of the diverse crista shapes across the Eukaryota Domain. Thereby, the respiratory chain constitutes an additional driving force of crista morphogenesis apart from the classic factors such as the ATP synthase multimers, the mitochondrial contact site and cristae organizing system (MICOS) and the optic atrophy 1 (OPA1).

Here, we investigate the structural organization of respiratory chain supercomplexes/megacomplexes in *Tetrahymena thermophila*, a model ciliate with characteristic tubular mitochondrial cristae. Using cryo-electron microscopy (cryoEM), we resolve the high resolution structure of a respiratory megacomplex in the composition of $CIV_2(CICIII_2)_2CII_2$ and a medium resolution structure of
$(CIV_2CICIII_2CII_2)_2$. Both megacomplexes most likely represent different ways of fragmentation of the original spiral-shaped, long-range respiratory chain array on the tubular cristae. The megacomplex $CIV_2(CICIII_2)_2CII_2$ forms a half ring-shaped architecture, in which the obligatory dimeric CIV2 acts as a central linker connecting two copies of supercomplex $CICIII_2$ and two copies of CII. This arrangement conforms to the curvature of the ciliate tubular cristae with a diameter of about 40 nm. Additionally, the larger $(CIV_2CICIII_2CII_2)_2$ is C2 symmetric and the relative position of its two asymmetric units, $CIV_2CICIII_2CII_2$, implicates the spatial register between adjacent $CIV_2(CICIII_2)_2CII_2$ copies in its long-range arrangement.

Mechanism wise, both our results[1] and the similar work published at about the same time[2] demonstrate close proximity between cytochrome *c* binding sites of $CIII_2$ and CIV_2, essential for efficient electron transfer. Functional symmetry of $CIII_2$, as demonstrated by the conformational flexibilities of the Rieske domains, is disrupted in the smaller supercomplex $CICIII_2$ but restored in the megacomplex $CIV_2CICIII_2CII_2$, suggesting the integration of an additional electron source of CII activates the originally inactive CIII protomer copy. This further hints ubiquinone channelling inside the full scale respiratory chain assembly of *Tetrahymena thermophila*. Overall, the work unravels the next-level complexity of respiratory chain organization beyond the classical landscape described for mammalian mitochondria.

[1] F. Han, Y. Hu, M. Wu, Z. He, H. Tian, L. Zhou, Structures of Tetrahymena thermophila respiratory megacomplexes on the tubular mitochondrial cristae. Nat. Commun. 14, (2023), 2542. https://doi.org/10.1038/s41467-023-38158-5

[2] A. Mühleip, R.K. Flygaard, R. Baradaran, O. Haapanen, T. Gruhl, V. Tobiasson, A. Maréchal, V. Sharma, A. Amunts, Structural basis of mitochondrial membrane bending by the I–II–III2–IV2 supercomplex. Nature 615, (2023), 934–938. https://doi.org/10.1038/s41586-023-05817-y

P20

Role of COX6A1 in the structural and functional organization of the mitochondrial respiratory chain

Alba Cantalejo-Rodrigo[a] and Cristina Ugalde[a,b]

[a] *Centro de Investigaciones Biológicas Margarita Salas (CIB), 28040, Madrid, Spain*
[b]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), U723, Madrid, Spain*

E-mail address: alba.cantalejo@cib.csic.es
Presenting author: Alba Cantalejo Rodrigo

The mitochondrial respiratory chain (MRC) generates the proton motive force used by the ATP synthase to drive ATP production during oxidative phosphorylation (OXPHOS). Beyond their catalytic roles, respiratory complexes are structurally and functionally interconnected, assembling into supramolecular structures known as supercomplexes (SCs). In mammals, the most abundant SC is the respirasome ($I_1III_2IV_1$), whose formation is closely linked to CI maturation and stability. In particular, association of fully or partially assembled complex IV (CIV) stabilizes complex I (CI) within respirasomes through still unclear mechanisms.[1] CIV biogenesis follows a modular assembly pathway in which distinct structural modules are incorporated sequentially. Among these, the COX3 module represents a late-stage assembly unit that includes accessory subunits such as COX7A and COX6A. Notably, isoforms of COX7A within this module define two predominant respirasome species, the C- and S-respirasomes, which differ in how the distal membrane arm of CI (P_D module) interacts with CIV.[2] Recent studies have further identified COX6A as an alternative mediator of the interaction between CIV and CI during SC formation.[3] Despite these findings, the molecular mechanisms regulating the specific interactions between CI and CIV that determine the formation of different SC configurations remain unclear, and how COX6A regulates CIV assembly and activity is still unknown. Here, we investigate the role of the COX3 module—focusing on the COX6A1 isoform—in CIV biogenesis and mitochondrial SC stability.

To investigate the role of COX6A1, we generated COX6A1 knockout ($COX6A1^{KO}$) lines in a HEK293T cellular background using CRISPR-Cas9 technology. Genomic indel sequencing together with subsequent SDS-PAGE and immunoblotting analyses confirmed the loss of COX6A1 expression. Blue-Native PAGE and *in-gel activity* (IGA) assays revealed that COX6A1 depletion disrupts COX3 module assembly, leading to reduced CIV enzymatic activity and a reorganization of supercomplex distribution. This study provides insights into the regulatory role of the COX3 module in mitochondrial CIV biogenesis and the structural organization of mammalian MRC.

[1] Lobo-Jarne T, Pérez-Pérez R, Fontanesi F, Timón-Gómez A, Wittig I, Peñas A, SerranoLorenzo P, García-Consuegra I, Arenas J, Martín MA, Barrientos A, Ugalde C. (2020). Multiple pathways coordinate assembly of human mitochondrial complex IV and stabilization of respiratory supercomplexes. EMBO J 39(14):e103912. https://doi.org/10.15252/embj.2019103912.

[2] Fernández-Vizarra, E., López-Calcerrada, S., Sierra-Magro, A., Pérez-Pérez, R., Formosa, L. E., Hock, D. H., Illescas, M., Peñas, A., Brischigliaro, M., Ding, S., Fearnley, I. M., Tzoulis, C., Pitceathly, R. D. S., Arenas, J., Martín, M. A., Stroud, D. A., Zeviani, M., Ryan, M. T., & Ugalde, C. (2022). Two independent respiratory chains adapt OXPHOS performance to glycolytic switch. Cell Metabolism, 34(11), 1792-1808.e6. https://doi.org/10.1016/j.cmet.2022.09.005

[3] Vercellino I, Sazanov LA. (2025). SCAF1 drives the compositional diversity of mammalian respirasomes. Nat Struct Mol Biol, 32(11):2371. https://doi.org/10.1038/s41594-025-01721-3.

Monalisa Sarkar[1,2], Jonathan Schiller[1], Neele Manik[1], Katharina Friedreich[1], Alfredo Cabrera-Orefice[3,4], Janet Vonck[2], Volker Zickermann[1]

[1]*Structural Bioenergetics Group, Institute of Biochemistry II, Goethe University, Frankfurt am Main, Germany*
[2]*Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany*
[3]*Center for Functional Proteomics, Goethe University, Frankfurt am Main, Germany*
[4]*Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University, Giessen, Germany.*

janet.vonck@biophys.mpg.de, zickermann@med.uni-frankfurt.de

P21

I_1III_2 supercomplex formation on deletion of a complex IV assembly factor COA1

Complex I is the first protein complex of the mitochondrial respiratory chain. Its structure consists of 14 conserved central subunits along with accessory subunits arranged in an Lshaped structure comprising of the membrane arm and the matrix arm [1]. With 45 subunits and at least 20 different assembly factors in human complex I, the assembly of this machinery is a very intricate and complicated process. While the pathways of this assembly processes are known [2], its structural basis is mostly unknown. More insights into the assembly process have been provided by our efforts focusing on the assembly factor NDUFAF1 [3] and we are now investigating the role of subsequent assembly factors. Additionally, complex I is observed to form supercomplexes with the other respiratory complexes at various stoichiometric ratios. Supercomplex formation is more commonly observed in higher organisms, although the function of such assemblies is still up for debate.

COA1 is a particularly interesting assembly factor because it appears to function in two biogenesis pathways. Despite being a recognized cytochrome oxidase assembly factor, it has also been shown to be involved in human complex I assembly. Recently, we were successful in deletion of the COA1 gene in the fungus *Yarrowia lipolytica* and observed the assembly process of mitochondrial complex IV to be significantly hindered. Surprisingly, we also observed complex I and complex III dimers forming supercomplexes in this strain. The I_1III_2 supercomplex has been identified in a number of plant and animal species, unlike *Y. lipolytica*, which normally has relatively little supercomplex abundance [4].

[1] E. Laube, J. Schiller, V. Zickermann, and J. Vonck, Using cryo-EM to understand the assembly pathway of respiratory complex I, *Acta Crystallogr D Struct Biol*, vol. 80, no. Pt 3, pp. Feb. 2024, 159–173.

[2] S. Guerrero-Castillo, F. Baertling, D. Kownatzki, H.J. Wessels , S. Arnold, U. Brandt , L. Nijtmans, The assembly pathway of mitochondrial respiratory chain complex I. Cell Metab., 25(1) (2017) 128-139.

[3] J. Schiller, E. Laube, I. Wittig, W. Kühlbrandt, J. Vonck, V. Zickermann, Insights into complex I assembly: Function of NDUFAF1 and a link with cardiolipin remodeling, Science Advances, 8 (2022) eadd3855.

[4] E. Nübel, I. Wittig, S. Kerscher, U. Brandt, H. Schägger, Two-dimensional native electrophoretic analysis of respiratory supercomplexes from Yarrowia lipolytica. Proteomics, 9, (2009) 2408-2418.

P22

Structure-function studies of the respiratory supercomplexes of the thermoacidophilic archaeon, *Sulfolobus acidocaldarius*

Paulina Borkowska[a], Joanna Florek[a], Hanna Pryputniewicz[a], Katarzyna Lorencik[b], Paulina Indyka[c], Michał Rawski[c], Arkadiusz Borek[a], Sebastian Pintscher[a]

[a]*Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland* [b]*School of Biosciences, University of Sheffield, Sheffield, United Kingdom* [c]*National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland*

E-mail address: paulina.borkowska@uj.edu.pl; sebastian.pintscher@uj.edu.pl Presenting author: Paulina Borkowska

Sulfolobus acidocaldarius is a thermoacidophilic archaeon that utilizes an unusual set of respiratory complexes, characterized by a surprising redundancy in the type and function of these enzymes. Within the *S. acidocaldarius* genome, three alternative terminal oxidases (SoxB, SoxM, and DoxB) and three cytochrome *bc*-like complexes ($SoxCL_2$, SoxNL, and SoxGF) have been identified[1,2]. It was proposed that SoxB and SoxM form obligatory supercomplexes with $SoxCL_2$ and SoxGF, respectively, while DoxB and SoxNL are standalone enzymes. Thus far, the redundancy of the *S. acidocaldarius* respiratory system remains unexplained. Moreover, no structures of these complexes were determined.

Herein, using the native organism as a source, we performed the extraction and partial purification of SoxM- and SoxB-comprising complexes. Using cryo-EM, we determined their structures, and then we used novel computational tools, AlphaFold and ModelAngelo, to identify previously unknown subunits present in these assemblies. We show that the $SoxABCDL_2$ supercomplex comprises at least two additional cytochromes *a*, both with an unusual heme A. Moreover, we found that the blue copper protein, SoxE, is a core subunit of SoxEFGHIM and may function as a mobile electrontransfer protein in a way similar to the Rieske protein in canonical *bc*-type cytochromes. Based on the unusual SoxM assembly, we hypothesize that the SoxEFGHIM may form a multimeric megacomplex in native membranes.

To gain insight into the function of these enzymes, we designed a simple kinetic assay of the terminal oxidase complexes, harnessing a soluble version of SoxE and isolated native caldariellaquinone-6. We developed a system for heterologous production of soluble SoxE in *E. coli*, which we are currently optimizing. We hope that our contribution will help to fill the existing gaps in knowledge on the long-neglected archaeal respiratory enzymes.

[1] G. Schäfer, M. Engelhard, V. Müller, Bioenergetics of the Archaea, Microbiology and Molecular Biology Reviews 63 (1999) 570–620. https://doi.org/10.1128/mmbr.63.3.570-620.1999.

[2] L.F. Bischof, et al., Early response of *Sulfolobus acidocaldarius* to nutrient limitation, Frontiers in Microbiology 10 (2019) 1–17. https://doi.org/10.3389/fmicb.2018.03201.

P23

Probing the supramolecular architecture of the *Drosop* respiratory supercomplexes

Mayla G. Zanchetta[a], Murilo F. Othonicar[b]; Marcos T. Oliveira[c]

[1]Department of Biochemistry and Immunology, Ribeirão Preto Medical School (FMRP-USP), University of São Paulo, Ribeirão Preto, Brazil

E-mail address: maylagabriela@usp.br
Presenting author: Mayla G. Zanchetta

Respiratory supercomplexes (SCs) are supramolecular assemblies of mitochondrial electron transport chain complexes that contribute to the stabilization of individual complexes, optimization of electron transfer kinetics, and organization of the inner mitochondrial membrane[1–3]. Although their functional significance remains under debate, accumulating evidence supports a predominantly structural role in shaping cristae architecture and modulating respiratory efficiency[4]. Recent cryo-electron microscopy studies have resolved the architecture of mammalian respirasomes at near-atomic resolution, revealing conserved interaction interfaces among complexes I, III_2, and IV, stabilized by both protein–protein and protein–lipid interactions[5–7]. In this context, structural elements of complex IV, including the subunits COX7A1 and COX7A2, together with supercomplex assembly factor 1 (SCAF1/COX7A2L), have been implicated in stabilizing $CIII_2$–CIV interactions within CIV-containing SCs[8]. *Drosophila melanogaster* has a single COX7A subunit, which is a homologue to both mammalian COX7A2 and SCAF1; however, its functional contribution to SC organization remains unresolved. Here, we investigated the roles of COX7A in SC organization in *D. melanogaster* mitochondria by integrating biochemical and *in silico* structural approaches. SC organization and stability were assessed by blue native polyacrylamide gel electrophoresis, following RNAi-mediated depletion of COX7A, while comparative structural modeling between human COX7A2 and SCAF1, and *Drosophila* COX7A was performed to evaluate structural conservation and infer potential interaction interfaces. Structural predictions revealed divergence in regions putatively involved in intermolecular contacts, suggesting altered interaction surfaces despite overall sequence and structural similarities. Consistently, COX7A depletion did not abolish SC formation under the conditions tested, indicating that COX7A is not required for SC maintenance in fruitflies. Collectively, these findings demonstrate that COX7A-like proteins are not universally required for SC stability, and support a model in which $CIII_2$–CIV and CI-CIV associations are mediated by a plethora of determinants, highlighting the evolutionary diversification of mechanisms governing SC organization in animal species.

[1] Schägger H, Pfeiffer K. Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J.* 2000;19(8):1777–1783. (https://doi.org/10.1093/emboj/19.8.1777)

[2] Enríquez JA. Supramolecular organization of respiratory complexes. *Annu Rev Physiol.* 2016;78:533–561. (https://doi.org/10.1146/annurev-physiol-021115-105031)

[3] Cogliati S, Enríquez JA. Mitochondrial respiratory chain supercomplexes: structure, function and regulation. *Biochim Biophys Acta Bioenerg.* 2021;1862(1):148408. (https://doi.org/10.1016/j.bbabio.2020.148408)

[4] Brischigliaro M, et al. Structural rather than catalytic role for mitochondrial respiratory chain supercomplexes. *eLife.* 2023;12:e88084. (https://doi.org/10.7554/eLife.88084.3)

[5] Guo W, et al. Structure of the mammalian respirasome. *Nature.* 2017;548:80–85. (https://doi.org/10.1038/nature20157)

[6] Letts JA, Fiedorczuk K, Sazanov LA. The architecture of respiratory supercomplexes. *Sci Adv.* 2020;6(16):eaba7509. (https://doi.org/10.1126/sciadv.aba7509)

[7] Yang Y, et al. Structure of the human mitochondrial respiratory megacomplex $I_2III_2IV_2$. *Nature.* 2024;628:934–941. (https://doi.org/10.1038/s41586-024-07487-w)

[8] Zhou Y, et al. Structural basis for SCAF1-mediated assembly of respiratory supercomplexes. *Nat Struct Mol Biol.* 2024;31.
(https://doi.org/10.1038/s41594-024-01255-0)

P24

Structural and Functional Insights into QrcABCD Mechanism for Energy Conservation

Américo G. Duartea,§ , David C. Medinaa , Gonçalo Manteigasa , Max. D. Yinb , Teresa Catarinoa , José A. Britoa , João B. Vicentea , Bonnie. J. Murphyb , Inês A. C. Pereiraa

aInstituto deTecnologia Química e Biológica António Xavier (ITQB NOVA), Universidade NOVAde Lisboa, Av. da República, 2780 -157 Oeiras, Portugal

bRedox and Metalloproteins Research Group, Max Planck Institute of Biophysics; Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany

E-mail address: americoduarte@itqb.unl.pt

Presenting author: Américo G. Duarte

The QrcABCD quinone reductase complex is an electrogenic complex present in sulfate-reducing bacteria of the phylum Desulfobacterota. It functions as a cytochrome c_3:menaquinone (MK) oxidoreductase that transfers electrons from periplasmic hydrogen or formate oxidation to the MK pool. Within this complex, the QrcC and QrcD subunits form a redox dimer (QrcCD) that catalyzes MK reduction coupled to proton uptake from the cytoplasm. Homologues of QrcCD occur in many bacterial redox complexes operating in diverse bioenergetic contexts.

In this work, a homologous overexpression system for QrcABCD was established in Nitratidesulfovibrio vulgaris Hildenborough and used to produce variants carrying substitutions in key amino acid residues proposed to participate in energy conservation. Growth analyses of the mutant strains, combined with activity assays of the corresponding Qrc variants reconstituted in proteoliposomes, revealed the essential role of residues located in the MK-binding site on the P-side of the membrane and in a putative proton uptake pathway from the cytoplasm to this site.

The results support a mechanism in which QrcABCD conserves energy by generating a proton motive force through the uptake of electrons and protons from opposite sides of the membrane, without active proton pumping, validated by the recently determined cryo-EM structure of the Qrc complex solved with high resolution. In this structure we observe the arrangement of the four subunits and their redox cofactors, additional transmembrane helices, and a quinone molecule bound in a pocket of QrcD facing the P-side of the membrane. Together, these data provide new insights into the molecular mechanism of QrcABCD and expand our understanding of the structural diversity and functional adaptations of related quinone oxidoreductases in bacterial energy metabolism.

P25

NDUFB7 Dysfunction: Impact on Respirasome Assembly and Mitochondrial Bioenergetics

Paula San Román Ruiz[a,b], Alfonso Gómez del Val[c], Erika Férnández-Vizarra[d], Miguel A. Martín Casanueva[a,b], Cristina Ugalde[a,b,c]

[a]*Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid 28041, Spain* [b]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), U723, Madrid, Spain*
[c]*Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), 28040 Madrid, Spain*
[d]*Facultad de Ciencias de la Salud y del Deporte, Universidad de Zaragoza, Zaragoza 50009, Spain*

E-mail address: paula.roman.imas12@h12o.es
Presenting author: Paula San Román Ruiz

Mochondrial complex I (CI) deficiency is the most common cause of oxidative phosphorylation (OXPHOS) disorders and is frequently associated with severe mitochondrial encephalomyopathies. In mammals, CI assembly and stability depends on its association with complexes III and IV into supramolecular structures termed supercomplexes (SCs), particularly the respirasome (SC $I_1III_2IV_1$), where structural interactions between CI and CIV are largely mediated by NDUFB7, a structural subunit of the CI distal membrane arm (PD-ND5 module). Defining how NDUFB7 contributes to respirasome assembly is therefore essential for understanding the structural organization of the mitochondrial respiratory chain (MRC) and the mechanisms underlying mitochondrial disease.

To investigate the cellular pathophysiological consequences of NDUFB7 loss, we generated two genome-edited human cell models: a knockdown ($NDUFB7^{KD}$) expressing 10% of normal NDUFB7 levels and a full knockout ($NDUFB7^{KO}$). Using an integrative approach combining blue-native electrophoresis, complexome profiling, and bioenergetic assays, we show that the absence of NDUFB7 disrupts the PD-ND5 module and impaired the supramolecular architecture of the MRC. Specifically, NDUFB7 deficiency compromised the association of CI with complexes III and IV, resulting in a marked reduction in respirasome levels and global CI destabilization. Notably, $NDUFB7^{KD}$ cells accumulated two catalytically-active late CI assembly intermediates with NADH dehydrogenase activity, accompanied by compensatory remodelling of the MRC, including increased levels of SC III_2IV_1 and free complexes III and CIV. Functionally, these structural alterations compromised mitochondrial respiration, driven by a specific decrease in CI activity due to defects in its assembly and stability. Consistently, high-resolution respirometry revealed a significant decrease in the CI-dependent P/O ratio, indicating impaired OXPHOS efficiency. Notably, NDUFB7-deficient cells recapitulate the bioenergetic defects observed in patients carrying *NDUFB7* mutations, providing a robust and physiologically relevant platform to study CI-related mitochondrial disorders. Overall, this work advances our understanding of CI structural organization and the mechanisms governing respirasome assembly. Our results demonstrate the existence of catalytically active CI assembly intermediates, suggesting that partial functionality can persist despite structural disruption. By elucidating how NDUFB7 loss impairs respirasome formation and MRC organization, our findings shed light on the molecular basis of mitochondrial and neurodegenerative diseases and point to potential therapeutic strategies aimed at stabilizing MRC supramolecular architecture.

P26

Mitochondrial–cytosolic redox crosstalk via the alternative oxidase and lactate dehydrogenase: growth coordination in *Drosophila*

Geovana S. Garcia[a], Marcos T. Oliveira[a]

[a]*Department of Biochemistry and Immunology - Ribeirão Preto School of Medicine (FMRP), University of São Paulo (USP), Brazil*

E-mail address: garcia_gs@usp.br, marcos.t.oliveira@usp.br
Presenting author: Geovana S. Garcia

Drosophila melanogaster is a powerful model for investigating disease-relevant metabolic adaptations, as larval growth depends on aerobic glycolysis, a metabolic strategy resembling tumor metabolism[1–3]. The mitochondrial alternative oxidase (AOX), absent in vertebrates and most insects, has emerged as a promising therapeutic candidate for mitochondrial disorders[4,5] . AOX enables electron transport by bypassing complexes III and IV (CIII and CIV), reducing oxygen to water without contributing to the proton gradient[6]. Although traditionally considered active only under conditions of impaired CIII/CIV function, our study demonstrates that xenotopic expression of AOX, specifically from the tunicate *Ciona intestinalis*, profoundly affects larval development, growth, and responses to environmental cues, even in the absence of overt defects in the electron transport system[7,8]. Notably, AOX-expressing larvae consistently exhibit increased lactate dehydrogenase (LDH) expression, suggesting a functional link between mitochondrial electron transport, lactate metabolism, and growth regulation. Here, we aim at investigating how coordinated modulation of AOX and LDH reshapes larval metabolism and redox balance using double-transgenic flies as model. Developmental and growth parameters were assessed through indirect metabolic readouts, including viability, body mass, and size, alongside analyses of mitochondrial physiology using oxygen consumption measurements and microscopy. We found that *Ldh* knockdown is lethal during the larval stage. However, AOX expression partially rescues this phenotype, albeit with a marked reduction in larval biomass. Conversely, AOX induces pupal lethality in the context of *Ldh* overexpression, further exacerbating the reduction in biomass observed with AOX alone. Metabolomic analyses indicate that AOX promotes lipid metabolism remodeling in both larvae and pupae. Consistently, tissue-specific analyses in the fat body reveal alterations in lipid droplet morphology and size. Furthermore, AOX expression increases cysteine and γ-glutamyl-cysteine levels, metabolites associated with antioxidant defense via the Xc^- system and protection against lipid peroxidation. These findings suggest a potential link between AOX activity and redox homeostasis, which remains to be validated in combined AOX–LDH modulation backgrounds. In addition, respirometry data point to a noncanonical lactate oxidation pathway linked to the coenzyme Q pool in *Drosophila* larvae, highlighting a previously unrecognized metabolic interaction between LDH and the electron transport system. Together, our results reveal that AOX is not merely a bypass for impaired respiration but actively rewires metabolic and redox networks, uncovering a complex interplay between mitochondrial function and lactate metabolism during development.

[1] D. Drummond-Barbosa, J.M. Tennessen, Reclaiming Warburg: Using developmental biology to gain insight into human metabolic diseases, Dev. 147 (2020). https://doi.org/10.1242/dev.189340.

[2] J.M. Tennessen, C.S. Thummel, Coordinating Growth and Review Maturation — Insights from Drosophila, Curr. Biol. 21 (2011) R750. https://doi.org/10.1016/J.CUB.2011.06.033.

[3] J.M. Tennessen, K.D. Baker, G. Lam, J. Evans, C.S. Thummel, The Drosophila estrogen-related receptor directs a metabolic switch that supports developmental growth, Cell Metab. 13 (2011) 139–148. https://doi.org/10.1016/J.CMET.2011.01.005.

[4] M. Szibor, C. Schenkl, M.R.O. Barsottini, L. Young, A.L. Moore, Targeting the alternative oxidase (AOX) for human health and food security, a pharmaceutical and agrochemical target or a rescue mechanism?, Biochem. J. 479 (2022) 1337. https://doi.org/10.1042/BCJ20180192.

[5] S. Saari, G.S. Garcia, K. Bremer, M.M. Chioda, A. Andjelković, P. V. Debes, M. Nikinmaa, M. Szibor, E. Dufour, P. Rustin, M.T. Oliveira, H.T. Jacobs, Alternative respiratory chain enzymes: Therapeutic potential and possible pitfalls, Biochim. Biophys. Acta - Mol. Basis Dis. 1865 (2019) 854–866. https://doi.org/10.1016/j.bbadis.2018.10.012.

[6] A.E. McDonald, D. V. Gospodaryov, Alternative NAD(P)H dehydrogenase and alternative oxidase: Proposed physiological roles in animals, Mitochondrion 45 (2019) 7–17.

https://doi.org/10.1016/j.mito.2018.01.009.

[7] G.S. Garcia, M.F. Othonicar, A.T.P. Campos, E.A. Kilbourn, K.C. Bícego, J. Lerchner, J.M. Tennessen, M.T. Oliveira, The alternative oxidase reconfigures the larval mitochondrial electron transport system to accelerate growth and development in Drosophila melanogaster, Elife 14 (2025). https://doi.org/10.7554/ELIFE.106202.1.

[8] C.A. Couto-Lima, S. Saari, G.S. Garcia, G.H. Rocha, J. ten Hoeve, E. Dufour, M.T. Oliveira, Impairment of Muscle Function Causes Pupal Lethality in Flies Expressing the Mitochondrial Alternative Oxidase, Biomolecules 15 (2025) 570.
https://doi.org/10.3390/BIOM15040570/S1.

P27

Reverse redox loop-driven coupling of fatty acid β-oxidation to CO_2 reduction during syntrophic methane production

Dennis Kosian[a], Lin Zhang[b,c], Lena Appel[a], Lorenz Heidinger[b], Oliver Einsle[b], Matthias Boll[a]

[a] Faculty of Biology – Microbiology, University of Freiburg, Freiburg, Germany [b] Institute of Biochemistry, University of Freiburg, Freiburg, Germany [c] Shenzhen University of Advanced Technology, Guangdong, China

E-mail address: dennis.kosian@biologie.uni-freiburg.de
Presenting author: Dennis Kosian

The microbial conversion of organic matter to methane is a key process in the global carbon cycle and a major contributor to annual biogas production on Earth[1]. To oxidize primary fermentation products such as short-chain fatty acids and alcohols to methane, methanogenic archaea depend on syntrophic associations with secondary fermenting bacteria. The syntrophic model bacterium *Syntrophus aciditrophicus* is able to oxidize the C_4 fatty acid butyrate to acetate and formate, two typical substrates for methanogenesis. In this pathway, CO_2 serves as an intermediate electron acceptor for electrons derived from β-oxidation. A longstanding question has been how the energetically unfavourable electron transfer from acyl-coenzyme A (acyl-CoA; $E^{0'} \approx -10$ mV) via electron-transferring flavoprotein (ETF) to CO_2 ($E' \approx -290$ mV under syntrophic conditions) is achieved[2]. We recently identified a membrane-integral ETF:(methyl)menaquinone oxidoreductase (EMO) as the key component of this process. Overall, CO_2 reduction is proposed to occur via a proton-motive force-driven reverse redox loop involving EMO and a membrane-bound formate dehydrogenase, with methylmenaquinone (MMK, $E^{0'} = -156$ mV) acting as an interprotein electron carrier[3]. The 79-kDa EMO—distinct from canonical mitochondrial and bacterial ETF:ubiquinone oxidoreductases— contains heme *b* cofactors within its transmembrane domain and iron-sulfur (FeS) clusters in the cytoplasmic, ETF-interacting region. Cryo-electron microscopy structures of EMO from *S. aciditrophicus* in complex with ETF reveal unprecedented cofactor arrangements, including a stacked heme *b* and noncubane FeS clusters. These clusters are characteristic of B-subunits of soluble heterodisulfide reductases (HDRs), where they form the catalytic site for disulfide reduction[4]. Phylogenetic analyses suggest an evolutionary relationship between EMO, HDRs and other prokaryotic proteins harbouring non-cubane FeS cluster binding motifs. Notably, the EMO-ETF complex exhibits pronounced conformational flexibility in the FAD domain of ETF, reminiscent of the dynamics observed between mitochondrial ETF and medium-chain acyl-CoA dehydrogenase[5]. Together, these findings provide mechanistic insights into the electron transfer between ETF and the (M)MK pool in prokaryotes, a fundamental process not only in syntrophic but also in pathogenic organisms such as *Mycobacterium tuberculosis*[6].

[1] P.N. Evans, J.A. Boyd, A.O. Leu, B.J. Woodcroft, D.H. Parks, P. Hugenholtz, G.W. Tyson, An evolving view of methane metabolism in the Archaea, *Nat. Rev. Microbiol.* 17, (2019), 219-232. https://doi.org/10.1038/s41579-018-0136-7

[2] J.R. Sieber, M.J. McInerney, R.P. Gunsalus, Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation, *Annu. Rev. Microbiol*. 66, (2012), 429-452. https://doi.org/10.1146/annurev-micro-090110-102844

[3] M. Agne, S. Estelmann, C.S. Seelmann, J. Kung, D. Wilkens, H. Koch, C. van der Does, S.V. Albers, C. von Ballmoos, J. Simon, M. Boll, The missing enzymatic link in syntrophic methane formation from fatty acids, *Proc. Natl. Acad. Sci. U.S.A.* 118(40), (2021), e2111682118. https://doi.org/10.1073/pnas.2111682118

[4] T. Wagner, J. Koch, U. Ermler, S. Shima, Methanogenic heterodisulfide reductase (HdrABC-MvhAGD) uses two noncubane [4Fe-4S] clusters for reduction, *Science* 357(6352), (2017), 699-703. https://doi.org/10.1126/science.aan0425

[5] H.S. Toogood, A. van Thiel, J. Basran, M.J. Sutcliffe, N.S. Scrutton, D. Leys, Extensive Domain Motion and Electron Transfer in the Human Electron Transferring Flavoprotein·Medium Chain Acyl-CoA Dehydrogenase Complex, *J. Biol. Chem*. 279(31), (2004), 32904-32912. https://doi.org/10.1074/jbc.M404884200

[6] T. Beites, R.S. Jansen, R. Wang, A. Jinich, K.Y. Rhee, D. Schnappinger, S. Ehrt, Multiple acyl-CoA dehydrogenase deficiency kills *Mycobacterium tuberculosis* in vitro and during infection, *Nat. Commun*. 12(6593), (2021). https://doi.org/10.1038/s41467-021-26941-1

P28

Native state structure determination of respiratory supercomplexes in mammalian mitochondrial inner membrane.

Takahiro Masuya[1,2#], Atsuki Nakano[3#], Shinsuke Akisada[3,4#], Moe Ishikawa-Fukuda[2], Kaoru Mitsuoka[4], Hideto Miyoshi[2] Masatoshi Murai[2], and Ken Yokoyama[3]

[1]*Affiliation: Department of Applied Biological Chemistry, Osaka Metropolitan University*
[2]*Affiliation: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University* [3]*Affiliation: Department of Molecular Biosciences, Kyoto Sangyo University* [4]*Affiliation: Research and Development, Sumitomo Pharma. Co., Ltd.*
[5]*Affiliation: Research Center for Ultra-High Voltage Electron Microscopy, Osaka University*

E-mail address: masuya.takahiro.5e@kyoto-u.ac.jp
Presenting author: Takahiro Masuya

Understanding the functional mechanisms of membrane protein complexes requires structural analysis within their native membrane environment. Here, we applied cryo-electron microscopy to determine the native state tructures of respiratory supercomplexes (SCs) on submitochondrial particles (SMPs) isolated from bovine heart mitochondria. In addition to the previously reported SC compositions $CI_1CIII_2CIV_1$ and $CI_1CIII_2CIV_2$, we identified a new assembly with the composition $CI_1CIII_2CIV_3$ as well as a $CI_2CIII_4CIV_6$ megacomplex.

Focused classification of CI within the SC ($CI_1CIII_2CIV_3$) revealed two distinct subclasses corresponding to closed and open conformations. In the closed conformation, a narrow ubiquinone-access tunnel was occupied by a density likely corresponding to ubiquinone. Within this SC, CIII forms a two-fold symmetric dimer ($CIII_2$). Two subclasses of $CIII_2$ were observed that were nearly identical except for the conformation of the iron–sulfur protein, a catalytic subunit containing a 2Fe–2S cluster involved in electron transfer. The three CIV units within the SC ($CI_1CIII_2CIV_3$) occupy distinct positions relative to CI and $CIII_2$. One CIV is located adjacent to the distal end of CI through interactions with the ND5 subunit, another resides in the space between CI and $CIII_2$, and the third is positioned adjacent to $CIII_2$.

Structural determination using SMPs was achieved without solubilizing the mitochondrial inner membrane, enabling the identification of previously unreported SC assemblies, including $CI_1CIII_2CIV_3$ and $CI_2CIII_4CIV_6$. Compared with analyses using intact mitochondria[1], the SMPbased approach offers practical advantages. SMPs are straightforward to prepare and, owing to their smaller vesicle size, allow reliable and reproducible cryo-grid preparation, which is technically challenging with intact mitochondria. This approach therefore provides a robust platform for native state structural characterization of mitochondrial inner membrane protein assemblies[2].

[1] Zheng, W. et al. High-resolution *in situ* structures of mammalian respiratory supercomplexes. *Nature* 631, 232-239 (2024)

[2] A. Nakano, T. Masuya, S. Akisada et al. *In situ* structure determination of ATP synthase oligomers and Respiratory Supercomplexes in mammalian mitochondrial inner membrane. *Nat. Commun.* In press.

P29

Forward or Reverse? Superoxide Oxidase as a Versatile Player in Bacterial Electron Transport

Yannick Bärtschi[1], Abbas Abou Hamdan[1], Thomas Nauser[2], Christoph von Ballmoos[1]

[1]*Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Switzerland*
[2]*Department of Chemistry and Applied Biosciences, ETH Zürich, Switzerland*

E-mail address: yannick.baertschi@unibe.ch
Presenting author: Yannick Bärtschi

Reactive oxygen species (ROS) are generated both deliberately by enzymatic systems and inadvertently through single-electron transfer to molecular oxygen, for example in the respiratory chain. As elevated ROS levels cause oxidative stress and cellular damage, cells employ detoxification enzymes such as superoxide dismutase and catalase to convert superoxide and hydrogen peroxide into water and oxygen.[1] In contrast, membrane-embedded cytochrome b_{561} (CybB) has been proposed to shift excess electrons from superoxide to the quinone pool, contributing to the proton motive force (pmf) via transmembrane electron transport while generating reduced quinol and molecular oxygen. Accordingly, CybB was classified as a superoxide:ubiquinone oxidoreductase.[2] This reaction was found to occur in the diffusion-limited range. We used pulse radiolysis to generate defined amounts of superoxide on sub-microsecond timescales, which provided insights into kinetic rates and key residues involved in the reaction. Consistently, CybB-mediated quinol formation was confirmed using a constant enzymatic superoxide supply combined with solvent extraction and HPLC analysis.

Notably, CybB can also operate in the reverse direction and has been proposed to act as a quinol-dependent superoxide producer, indicating the dual functionality of the enzyme.[3] While the formation of superoxide is clearly enzyme-mediated, intrinsic rates of the purified enzyme were slow but could be strongly accelerated by superoxide-consuming molecules, indicating that the superoxide-bound form is kinetically trapped. In this context, we identified the periplasmic soluble protein cytochrome b_{562} (CybC) as a potential physiological electron acceptor for CybB. Interestingly, electron transfer from CybB to CybC occurs independently of oxygen, and mutational analysis reveals that electrostatic interactions between the two proteins are critical for the reaction.

1. Johnson, L. A. & Hug, L. A. Distribution of reactive oxygen species defense mechanisms across domain bacteria. *Free Radic. Biol. Med.* 140, 93–102 (2019).

2. Lundgren, C. A. K. *et al.* Scavenging of superoxide by a membrane-bound superoxide oxidase.

Nat. Chem. Biol. 14, 788–793 (2018).

3. Abou-Hamdan, A. *et al.* Functional design of bacterial superoxide:quinone oxidoreductase.

Biochim. Biophys. Acta - Bioenerg. 1863, (2022).

P30

FADS2–LSD1 interaction: structural determinants and potential role in Alzheimer's disease

Alessia Nisco[a], Martina Tripicchio[a], Angela Sposato[a], Michele Galluccio[b], Lara Console[b], Katia Zanier[c], Cesare Indiveri[b], Maria Barile[a]

[a] *Department of Biosciences, Biotechnologies, and Environment, University of Bari Aldo Moro, Italy*

[b] *Department of DiBEST (Biologia, Ecologia e Scienze della Terra), University of Calabria, Arcavacata di Rende, Italy* [c] *Biotechnology and Cell Signaling (CNRS/Université de Strasbourg, UMR 7242), Ecole Superieure de Biotechnologie de Strasbourg, Illkirch, France*

E-mail address: alessia.nisco@uniba.it Presenting author: Alessia Nisco

Flavin adenine dinucleotide (FAD) is an essential redox cofactor involved in multiple metabolic and regulatory processes. Its biosynthesis is catalyzed by flavin adenine dinucleotide synthase (FADS), which converts flavin mononucleotide (FMN) into FAD in a tightly regulated reaction required for maintaining intracellular flavin homeostasis. Dysregulation of flavin metabolism has been increasingly associated with human diseases due to the widespread dependence of cellular pathways on flavin-dependent enzymes [1]. Among these, lysine-specific demethylase 1 (LSD1) is a FAD-dependent histone demethylase that regulates chromatin remodeling and gene expression by removing mono- and di-methyl groups from histone H3 and H4. LSD1 also targets non-histone substrates, further expanding its regulatory functions. Moreover, LSD1dependent pathways, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, regulate mitochondrial function and oxidative stress responses that are critically altered in Alzheimer's disease (AD) [2,3]. Interestingly, the FAD precursor vitamin B2 has been reported to exert a protective effect against ROS-induced brain damage in AD, likely through activation of the Nrf2 signaling pathway [4].

We aimed to investigate whether FADS plays a role in AD through LSD1-dependent pathways. A protein– protein interaction between FADS isoform 2 (FADS2) and LSD1 has been previously demonstrated *in cellulo* [5]; however, the molecular determinants underlying this interaction remain to be defined.

Structural modeling was used to predict amino acids likely to participate in the FADS2–LSD1 interface, which were then targeted by site-directed mutagenesis. Mutant constructs were generated, verified by Sanger sequencing, and cloned into Gateway® vectors for *Gaussia princeps* complementation assay (GPCA)-based interaction studies in HEK293T cells. Protein–protein interactions were quantified by measuring normalized luminescence ratios in triplicate experiments.

Mutations targeting interface residues resulted in approximately a 50% reduction in interaction compared to wild-type FADS2, whereas a control mutation outside the predicted interface showed no significant effect, confirming the specificity of the interaction surface. These findings support the existence of a defined FADS2–LSD1 interaction interface that may be relevant for regulating LSD1 function in AD. In this context, we are currently introducing circulating cells from AD patients as a novel model to investigate potential alterations in the FADS2–LSD1 axis.

[1] M. Tolomeo, A. Nisco, P. Leone, M. Barile, Development of Novel Experimental Models to Study Flavoproteome Alterations in Human Neuromuscular Diseases: The Effect of Rf Therapy, Int J Mol Sci . 2020 Jul 26;21(15):5310. doi: 10.3390/ijms21155310.

[2] Y. Li, Y. Zhao, X. Li, L. Zhai, H. Zheng, Y. Yan, Q. Fu, J. Ma, H. Fu, Z. Zhang, Z. Li, Biological and therapeutic role of LSD1 in Alzheimer's diseases. Front Pharmacol. 2022 Oct 25:13:1020556. doi: 10.3389/fphar.2022.1020556.

[3] M. C. B. D'Alessandro, S. Kanaan, M. Geller, D. Praticò, J. P. L. Daher, Mitochondrial dysfunction in Alzheimer's disease. Ageing Res Rev. 2025 May:107:102713. doi: 10.1016/j.arr.2025.102713.

[4] R. Zhao, H. Wang, C. Qiao, K. Zhao, Vitamin B2 blocks development of Alzheimer's disease in APP/PS1 transgenic mice via anti-oxidative mechanism. Trop. J. Pharm. Res. 2018 June; 17 (6): 10491054 https://doi.org/10.4314/tjpr.v17i6.10.

[5] A. Nisco, A. Sposato, M. Ardone, P. Leone, R. A. Cardone, L. Console, C. Indiveri, K. Zanier, M. Barile, Supplying LSD1 with FAD in pancreatic cancer: A matter of protein-protein interaction? Arch Biochem Biophys 2025 Feb:764:110291. doi: 10.1016/j.abb.2025.110291.

P31

A putative Nfn soluble transhydrogenase in the bacterial chemolithoautotroph *Aquifex aeolicus*

Pascale Infossi[a], Amine Ali-Chaouche[a], Arlette Kpebe[a], Carole Baffert[a], Myriam Brugna[a], Marianne Guiral[a]

[a]*Bioenergetics and Protein Engineering Laboratory (BIP-UMR 7281), CNRS-Aix-Marseille Université, Marseille, FRANCE*

E-mail address: guiral@imm.cnrs.fr
Presenting author: Guiral Marianne

Aquifex aeolicus is a chemolithoautotrophic bacterium that oxidizes hydrogen or inorganic sulfur compounds as energy sources, reduces O_2 and assimilates CO_2 via the reverse tricarboxylic acid

(TCA) cycle[1]. We have characterized two low-redox potential [4Fe-4S] ferredoxins (Fd6 and Fd7, with E_0 values of -440 and -460 mV, respectively) from the soluble content of *A. aeolicus*[2] and demonstrated that they are likely the physiological electron donors of the pyruvate:ferredoxin oxidoreductase (PFOR) and 2-oxoglurarate:ferredoxin oxidoreductase (OGOR) enzymes involved in the carbon fixation pathway[2]. One long-standing question is the origin of the reducing equivalents and the nature of the enzyme(s) used for low-potential ferredoxin reduction. *A. aeolicus*, and most of the chemolithoautotrophic bacteria that use the reverse TCA cycle for CO_2 assimilation, grow using energy sources with redox potentials more positive than those of ferredoxins. Therefore, Fd reduction must be energy-driven.

In order to identify the enzymes that reduce Fd6 and Fd7, pulldown experiments were undertaken. Numerous proteins, captured by ferredoxins, were identified, including PFOR and OGOR. Two proteins with homologies to the two subunits of the electron-bifurcating NADH-dependent ferredoxin:NADP oxidoreductase (NfnAB) [3] were identified as partners of both Fd6 and Fd7 and could form such a complex. This putative enzyme could catalyze the endergonic reduction of Fd with NADPH, driven by the exergonic reduction of NAD^+ by NADPH. Both proteins were produced recombinantly to investigate their ability to form a complex and to characterize them.

[1] M. Guiral, M.-T. Giudici-Orticoni, Microbe Profile: *Aquifex aeolicus*: an extreme heat-loving bacterium that feeds on gases and inorganic chemicals, Microbiology 167(1) (2021), doi:10.1099/ mic.0.001010

[2] L. Prioretti, G. D'Ermo, P. Infossi, A. Kpebe, R. Lebrun, M. Bauzan, E. Lojou, B. Guigliarelli, M.T. Giudici-Orticoni, M. Guiral, Carbon Fixation in the Chemolithoautotrophic Bacterium *Aquifex aeolicus* Involves Two Low-Potential Ferredoxins as Partners of the PFOR and OGOR Enzymes,
Life (Basel) 13(3) (2023) 627, doi: 10.3390/life13030627

[3] J.K. Demmer, H. Huang, S. Wang, U. Demmer, R.K. Thauer, U. Ermler, Insights into Flavinbased Electron Bifurcation via the NADH-dependent Reduced Ferredoxin:NADP Oxidoreductase
Structure, J. Biol. Chem. 290(36) (2015) 21985-21995, doi:10.1074/jbc.M115.656520

P32

Deciphering the photosynthetic pathway of *Halorhodospira halophila*

Emmanuella Serwaa[a,b], Jean Alric[b], Barbara Schoepp-Cothenet[a]

[a]: Bioénergétique et Ingénierie des Protéines (BIP), UMR 7281, CNRS, 13009, Marseille, France.
[b] : Institut de Biosciences & biotechnologies d'Aix-Marseille (BIAM), CNRS, 13115, Cadarache, France

Email address: eserwaa@imm.cnrs.fr

Presenting author: Emmanuella Serwaa

Halorhodospira halophila is a strict anaerobe and obligate phototroph, belonging to purple sulphur bacteria. It utilizes sulphur compounds (but not exclusively) as electron donors to supply its anoxygenic photosynthesis. Currently, little information is known about its photosynthetic pathway. Previous studies propose a metabolic network with a high degree of "redundancy", with multiple soluble electron carriers, including four high potential iron-sulphur proteins (HiPIPs) and four cytochromes, as well as several soluble and membranous enzymes, all funneling electrons to the central photosynthetic reaction centre (RC). The soluble carriers do not directly reduce the special pair of the RC, but a tetraheme subunit, which in turn reduces P. One of the questions is the respective role of each of the eight electron carriers. To address this, we performed heterologous production, purification, and biochemical characterization of the eight electron carriers and determined their ability to donate electrons to the RC using UV-Vis and EPR spectroscopy. Some studies also report that a HiPIP is tightly bound to the RC. This RC-bound HiPIP may detach to accept electrons from a Rieske/cyt*b* complex to re-reduce the RC or act as an intermediary between the soluble carriers and the RC, probably for some but not all. This raises the question of which soluble carriers depend on this bound HiPIP. Furthermore, the cofactors in this photosynthetic network are reported to exhibit unusually low redox potentials, which is atypical for purple sulphur bacteria. To confirm this, we performed redox titration of the special pair and the tetraheme subunit as well as the soluble electron carriers, providing more information about the functioning of this photosynthetic network.

1. G. D'Ermo, S. Audebert, L. Camoin *et al*., Environ Microbiol. (2024) 26(6):e16655. doi: 10.1111/14622920.16655.
2. C. Lieutaud, J. Alric, M, Bauzan, W. Nitschke & B. Schoepp-Cothenet. P*roc Natl Acad Sci U S A*. (2005) 102(9):3260-3265. doi:10.1073/pnas.0407768102.
3. B. Schoepp-Cothenet, C. Lieutaud, F. Baymann *et al.*, *Proc Natl Acad Sci U S A*. (2009) 106(21):8549-8554. doi:10.1073/pnas.0813173106.
4. K. Tani, R. Kanno, K.V.P. Nagashima, et al. A Native LH1–RC–HiPIP Supercomplex from an Extremophilic Phototroph. Commun Biol 8, 42 (2025). https://doi.org/10.1038/s42003-024-07421-w

P33

Electron bifurcation – a tale of interdependant proton and electron transfer reactions

Wolfgang Nitschke[a], Simon Duval[a], Barbara Schoepp-Cothenet[a], Frauke Baymann[a]

a
BIP/IMM/CNRS, Marseille, France

E-mail address: baymann@imm.cnrs.fr
Presenting author: Frauke Baymann

The process of electron bifurcation is a crucial element of bioenergetic mechanisms: a pair of electrons from an electron donor is split and the two electrons are directed towards spatially and thermodynamically dissimilar acceptors, one of higher and the other one of lower redox midpoint potential than the initial donor, thus increasing the local electrochemical disequilibrium. This mechanism was first observed in the Qo site of Rieske/cyt*b* complexes and more recently discovered to operate in many flavoenzymes. Based on the empirical observation that strongly inverted redox potentials are correlated to the occurance of electron bifurcation [1,2,3] and on the fact that such inverted redox potentials can theoretically result from modifications of the pK-values of the protonatable oxygen atoms of quinones or the protonatable nitrogen atoms of flavins, we wrote a routine to calculate the theoretical, experimentally inaccesible, redox midpoint potentials of the individual one-electron transitions as a function of pH. Taking together available experimental results on the redox midpoint potential of the respective 2-electron donors in aqueous solution and in aprotic medium, the pH-dependance of the 2-electron transition of the cofactor in its binding site, structural details of the binding pocket influencing the pK-values of the protonatable groups of the cofactors and nearby amino acids, we addressed the question of how the protein environment sets the redox properties of the cofactor. For the structurally diverse flavoproteins it turned out that completely unrelated protein folds can result in the same redox behavior of the flavin if the pattern of H-bondings of the nitrogen atoms N1, N3 and N5 is comparable. For Rieske/cyt*b* complexes, detailed information gathered over decades on the redox properties of the electron acceptors – the Rieske iron sulfur cluster and the b_L-heme - allows us to propose a more detailed reaction mechanism.

[1] A.R. Crofts, S. Hong, C. Wilson, R. Burton, D. Victoria, C. Harrison, K. Schulten, The mechanism of ubihydroquinone oxidation at the Qo -site of the cytochrome bc1 complex. Biochim. Biophys. Acta 1827 (2013), 1362–1377. http://dx.doi.org/10.1016/j.bbabio.2013.01.009

[2] L. Bergdoll, F. Ten Brink, W. Nitschke, D. Picot, F. Baymann, From low-to high-potential bioenergetic chains: Thermodynamic constraints of Q-cycle function. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1857 (2016), 1569-1579. http://dx.doi.org/10.1016/j.bbabio.2016.06.006 [3] W. Nitschke, MJ Russell, Redox bifurcations: mechanisms and importance to life now, and at its origin. BioEssays 34 (2012) 106-109. https://doi.org/10.1002/bies.201100134

P34

Traceless cofactor regeneration from formate for CYP450 enzyme cascades

Ping Xiao[a], Jibira Yakubu[b], Amit V Pandey[b], Christoph von Ballmoos[a]

[a]*University of Bern*, Department *of Chemistry, Biochemistry and Pharmacy (DCBP),* Freiestrasse 3, 3012 Bern, *Switzerland*
[b]*University of Bern*, Department *for BioMedical Research (DBMR),* Murtenstrasse 28, CH-3008 Bern, *Switzerland* Email address: ping.xiao@unibe.ch

Ping Xiao

Cytochrome P450 enzymes are a versatile superfamily of heme-containing monooxygenases that play a central role in the metabolism of both endogenous compounds and xenobiotics. Their broad substrate specificity makes them essential for the metabolism of clinically relevant drugs and for physiological processes such as hormone biosynthesis. In addition, their remarkable catalytic versatility has made them highly valuable in applications ranging from personalized medicine and drug development to biosensing and synthetic biology [1]. Numerous in vitro platforms, such as liver microsomes, whole-cell systems, and purified enzyme preparations, have been developed to investigate CYP450 catalysis [2]. A common feature of these systems is their dependence on a continuous supply of NADPH as the primary electron donor. However, this is a major limitation because external NADPH is costly and unstable, and NADPH regeneration systems can produce byproducts that may affect CYP450 activity and complicate product purification. Here, using formate dehydrogenase (FDH) and soluble transhydrogenase (SthA), we present a two-enzyme system that continuously supplies NADPH from low-cost formate to drive CYP450 catalysis, while producing CO_2 as the only byproduct that readily leaves the solution as a gas. We first expressed and purified FDH and SthA, and then systematically evaluated their individual activities in different buffer systems, as well as the effects of temperature, pH, and metal ions on their catalytic activity. We next examined their coupling efficiency, reaction duration, and substrate conversion efficiency. Finally, the NADPH regeneration system was applied to support CYP450 protein cascades in both bactosome-based and purified enzyme systems. In conclusion, we successfully developed an FDH- and SthA-based two-enzyme NADPH regeneration system that enables continuous CYP450 catalysis using low-cost formate. Its efficiency and compatibility with different CYP450 platforms highlight its potential for future applications in biocatalysis, synthetic biology, and pharmaceutical development.

Reference

[1] M. Zanger, Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol. Ther. 138(1), (2013), 103-141. https://doi.org/10.1016/j.pharmthera.2012.12.007.

[2] L. Marques, A Review on New Frontiers in Drug-Drug Interaction Predictions and Safety Evaluations with In Vitro Cellular Models. Pharmaceutics. 17(6), (2025), 747. https://doi.org/10.3390/pharmaceutics17060747.

P35

Superoxide- and Proton-Coupled Electron Transport Between Redox Proteins of the Respiratory and Photosynthetic Chains

Pau Gorostiza[a,b,c]

[a]*Catalan Institution for Research and Advanced Studies (ICREA), Barcelona 08010* [b]*Institute for Bioengineering of Catalonia (IBEC), Barcelona 08028*
[c]*Networking Biomedical Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN)*

E-mail address: pau@icrea.cat
Presenting author: Pau Gorostiza

Interprotein electron transport is an essential process in cell respiration and photosynthesis. It takes place between redox proteins and complexes, and it displays an outstanding efficiency and environmental adaptability. Although the biochemistry of intra-protein electron transport processes is well characterized, the dynamics of inter-protein electron transport require nanoscale experimental methods to be understood understand.

Electrochemical scanning tunneling microscopy (ECSTM) is a unique tool to study electronic materials and redox molecules including proteins. It offers single molecule resolution and allows working in aqueous solution, in nearly physiological conditions in the case of proteins, and under full electrochemical control[1]. ECSTM also allows performing conductance measurements by currentpotential and current-distance tunneling spectroscopy[2], notably between cognate redox partner proteins.

Recent and ongoing ECSTM conductance results of the laboratory in the respiratory[3-5] and photosynthetic chains[6-9] will be discussed. They include the mechanism of long-distance electron transport through the aqueous solution between redox partner proteins cytochrome *c* and respiratory complex III.[5] The current flowing between individual protein pairs extends beyond tunneling distances and is electrochemically gated. It is mediated by reactive oxygen species like superoxide anions and by protons with a high kinetic isotope effect that suggests strong coupling between proton and electron reactions.

[1] M. López-Martínez *et al., Small* 2017, 13, 36. DOI: https://doi.org/10.1002/smll.201700958

[2] J. M. Artés *et al., J Am Chem Soc* 2012, 134, 20218. DOI: https://doi.org/10.1021/ja3080242

[3] A. Lagunas *et al., Nat Commun* 2018, 9, 5157. DOI: https://doi.org/10.1038/s41467-018-07499-x

[4] A. M. J. Gomila *et al., Nat Commun* 2022, 13, 7100. DOI: https://doi.org/10.1038/s41467-02234809-1

[5] A. Lagunas *et al., Small* 2025, 21, e01286. DOI: https://doi.org/10.1002/smll.202501286

[6] M. López-Martínez *et al., Angew Chem Int Ed Engl* 2019, 58, 13280. DOI: https://doi.org/10.1002/anie.201904374

[7] R. A. Zamora *et al., ACS Nano* 2022, 16, 15155.DOI: https://doi.org/10.1002/smll.202104366

[8] M. López-Ortiz *et al., Small* 2022, 18, e2104366. DOI: https://doi.org/10.1002/smll.202104366

[9] M. López-Ortiz *et al., ACS Nano* 2023, 17, 20334. DOI: https://doi.org/10.1021/acsnano.3c06390

P36

Why Ferredoxins and HiPIPs Favor Dierent Redox Transions in [4Fe-4S] Clusters: An Electrostac Analysis

Maxim Janzen[a], Rajeev R. Roy[a], G. Matthias Ullmann[a] [a]*Computational Biochemistry, University of Bayreuth, 95440 Bayreuth, Germany*

E-mail address: maxim.janzen@uni-bayreuth.de, Rajeev-Ranjan.Roy@uni-bayreuth.de , ullmann@unibayreuth.de
Presenting author: Maxim Janzen

Iron-sulfur clusters are among the most ancient and versatile redox cofactors in biology, mediating electron transfer in photosynthesis, respiration, and numerous metabolic pathways. Two major classes of [4Fe-4S]-Cys_4 electron carrier proteins illustrate the remarkable functional range of this cofactor: ferredoxins (Fds) operate via the low-potential transition (LPT: $[4Fe\text{-}4S]^{1+}/[4Fe\text{-}4S]^{2+}$) at −300 to −600 mV, whereas high-potential iron-sulfur proteins (HiPIPs) employ the high-potential transition (HPT: $[4Fe\text{-}4S]^{2+}/[4Fe\text{-}4S]^{3+}$) at +100 to +350 mV. Strikingly, although both protein classes bind an identical inorganic core assembled by the same biosynthetic machineries, no protein is known to utilize both transitions for its biological function. What prevents a ferredoxin from accessing the HPT, or a HiPIP from using the LPT? Here, we apply continuum electrostatic calculations based on model redox potentials that we recently derived using a virtual model compound approach to a set of high-resolution crystal structures of Fds, HiPIPs, and nitrogenase Fe proteins. This computational analysis allows us to calculate the redox potentials for both the native and the hypothetical non-native transition in each protein and to decompose the individual energy contributions to the redox potentials. We find that transferring the cluster from aqueous solution into the low-dielectric protein interior shifts both transitions to lower redox potentials, but the LPT is affected more strongly than the HPT. As a result, the separation between the two transitions increases from about 700 mV in solution to approximately 1200 mV in the protein, matching values observed for model clusters in organic solvents. This asymmetry follows from the Born solvation energy, which scales with the square of the ionic charge: the higher-charged redox states involved in the LPT incur a larger desolvation penalty. Protein background charges, including protein dipoles and hydrogen bonds, then shift the redox potentials of both transitions by nearly the same amount, tuning the native transition into the physiological redox window while pushing the non-native transition outside of the biologically accessible range. This shift is substantially larger in Fds than in HiPIPs, consistent with the more extensive hydrogen-bonding network around the cluster in ferredoxins. Other contributions like the interaction with titratable groups and entropic effects further fine tune the potentials. Together, these results show that the exclusive use of a single redox transition in each protein class is an inherent consequence of fundamental electrostatic principles: desolvation inflates the gap between the two transitions, and protein charges provide a linear shift that can position only one of them at a physiologically
relevant potential.[1,2]

[1] Rajeev Ranjan Roy, Maxim Janzen, and G. Matthias Ullmann, Fundamental Electrostatics Explains Why Ferredoxin- and HiPIP-Type Proteins Favor Different Redox Transitions in [4Fe-4S]-Cys4 Clusters, J. Phys. Chem. B, Article ASAP (2026), https://doi.org/10.1021/acs.jpcb.6c00593

[2] Rajeev Ranjan Roy, Maxim Janzen, and G. Matthias Ullmann, Protein-Based Determination of Model Redox Potentials for the High-Potential and Low-Potential Redox Transition of [4Fe-4S]-Cys4 Clusters Using Continuum Electrostatics. *J. Chem. Theory Comput.* (2026), *22*, 738– 749, https://doi.org/10.1021/acs.jctc.5c01761

P37

CybB and its paralogs contribute to compensation of redox backpressure in *E. coli*

Abbas Abou-Hamdan, Yannick Bärtschi, Roman Mahler, Ana-Marija Stanic, Marcia Rohrer and Christoph von Ballmoos

[1] *Department of Chemistry, Biochemistry and pharmaceutical Sciences, University of Bern, 3012 Bern, Switzerland*

Email address: Abbas.abouhamdan@unibe.ch Presenting author: Abbas ABOU-HAMDAN

Reactive oxygen species (ROS) are highly reactive oxygen-derived molecules generated as natural by-products of aerobic metabolism. At controlled levels, they serve important signaling functions; however, when their accumulation exceeds the cell's detoxification capacity, they induce oxidative damage to DNA, proteins, and lipids(*1*). To mitigate ROS toxicity, bacteria have developed a range of enzymatic defense mechanisms. Superoxide dismutases (SODs) convert superoxide radicals into hydrogen peroxide (H_2O_2), which is subsequently detoxified by catalases and peroxidases. In anaerobic organisms, superoxide reductases (SORs) provide an alternative pathway by reducing superoxide to H_2O_2 using electron donors such as rubredoxin. Beyond these soluble systems, *Escherichia coli* encodes CybB, a membranebound diheme cytochrome b_{561} that has been characterized in vitro as a superoxide:quinone oxidoreductase(*2*). CybB catalyzes reversible redox reactions involving superoxide and quinones via transmembrane electron transfer between its heme groups(*3, 4*). Despite this established biochemical activity, its physiological function remains unclear. Interestingly, *cybB* expression is elevated during exponential growth and appears independent of oxygen availability, suggesting a role beyond classical oxidative stress responses(*2*). Homologs of CybB are widespread across bacteria and are often present as multiple paralogs, such as YodB and YceJ in *E. coli*, whose biological roles are still poorly understood.

In this study, we combined phylogenetic, biochemical, and genetic approaches to investigate CybB and its paralogs. Analysis using the EggNOG database revealed their broad distribution across species. Biochemical characterization of YodB and YceJ, together with targeted gene deletions, allowed us to assess functional overlap with CybB. Thermodynamic analyses suggest that under aerobic conditions, CybB preferentially operates in the superoxideproducing direction. Functionally, CybB becomes critical under membrane hyperpolarization conditions induced by ATP synthase inactivation. Athough a ΔcybB strain grows normally under standard conditions, it exhibits severe growth defects when respiratory imbalance is induced, a phenotype restored by complementation. Together, these findings identify CybB as a conditional redox regulator that alleviates electrochemical stress during respiration. This work highlights the complexity of superoxide metabolism and reveals functional diversification within the CybB family, with implications for bacterial stress adaptation and pathogenicity. 1. K. Sigler, J. Chaloupka, J. Brozmanová, N. Stadler, M. Höfer, Oxidative stress in microorganisms—I. *Folia Microbiologica* 44, 587-624 (1999).

2. C. A. K. Lundgren *et al.*, Scavenging of superoxide by a membrane-bound superoxide oxidase. *Nature Chemical Biology* 14, 788-793 (2018).

3. A. Abou-Hamdan *et al.*, Functional design of bacterial superoxide:quinone oxidoreductase. *Biochimica et biophysica acta. Bioenergetics* 1863, 148583 (2022). 4. D. Riepl *et al.*, Molecular Principles of Proton-Coupled Quinone Reduction in the Membrane-Bound Superoxide Oxidase. *Journal of the American Chemical Society* 147, 6866-6879 (2025).

The priority of the external NADH dehydrogenase for electron transfer in the respiratory chain of *Saccharomyces Cerevisiae* is substrate and electron flux dependent

Corinne Pellon[a], Mélanie Martins Pinto[a], Stéphane Arbault[b], Michel Rigoulet[a], Patrick Paumard[a] and Anne Devin[a]

[a] *IBGC, UMR 5095, CNRS/Université de Bordeaux, 33077 Bordeaux, France*

[b] *CBMN, UMR 5248, CNRS/Bordeaux INP, 33600 Pessac, France*

E-mail address: corinne.pellon@ibgc.cnrs.fr
Presenting author: Corinne Pellon

Cell homeostasis and proliferation require chemical energy produced in the form of ATP by different pathways of the cellular energy metabolism. Redox reactions of those pathways also lead to the reduction of NAD+ into NADH. The cell therefore needs to maintain ATP/ADP and NADH/NAD+ ratios within a sustainable range. Mitochondria are essential in this regard as they ensure most of the ATP synthesis and NADH reoxidation in aerobic cells through the oxidative phosphorylation (OXPHOS) system within the mitochondrial inner membrane. Though, cytosolic NADH is not the only substrate of the OXPHOS system as it includes various external and internal dehydrogenases with different substrates. There are competition processes between those dehydrogenases [1,2] for the electron transfer towards quinones, which are the central electron carriers of the system. Understanding how the cellular NADH/NAD+ ratio is maintained at an adequate level then proves to be a major but complex issue of energy metabolism. To address this issue, we studied the electron competition processes on isolated mitochondria from *S. cerevisiae*. We first used an electrochemical method previously used in our team for mitochondrial metabolism study [3] to simultaneously measure mitochondrial respiration and the quinones redox state in realtime. We studied those parameters at different energetic states in presence of one or two substrates of the mitochondrial dehydrogenases: the external (Ndep) and internal (Ndip) NADH dehydrogenases, the glycerol-3-phosphate dehydrogenase (Gut2p), and the succinate dehydrogenase (SDH). With a single substrate, the bioenergetic profile obtained from the electrochemical method proved to be specific of the substrate introduced and the quinone redox state strong correlation with the respiration rate added to its relevance in our conditions. Our study with multiple substrates showed an apparent priority of Ndep over the other dehydrogenases, and consequently priority of reoxidation of cytosolic NADH within the cell, but it actually depends on the experimental conditions. Indeed, we found out that if Ndep kinetic properties are largely in favor of its priority for electron transfer, the competition processes are however not that straightforward as our results showed that they are substrate and energetic state dependent. We were notably able to demonstrate that the SDH always manages to oxidize its substrate despite the activity of Ndep and that the priority of Ndep over Ndip, and so of cytosolic over mitochondrial NADH oxidation, is weakened when the respiration rate increases.

[1] I.L. Påhlman, C. Larsson, N. Averét, O. Bunoust, S. Boukebeur, L. Gustafsson and M. Rigoulet, Kinetic Regulation of the Mitochondrial Glycerol-3-phosphate Dehydrogenase by the External NADH Dehydrogenase in Saccharomyces cerevisiae, J Biol Chem. 277(31) (2002), 27991-27995, https://doi.org/10.1074/jbc.M204079200

[2] O. Bunoust, A. Devin, N. Avéret, N. Camougrand, M. Rigoulet, Competition of Electrons to Enter the Respiratory Chain : A New Regulatory Mechanism of Oxidative Metabolism in *Saccharomyces Cerevisiae*, J Biol Chem. (2005) 280(5) (2005), 3407-3413, https://doi.org/10.1074/jbc.M407746200

[3] M. Martins Pinto, S. Ransac, J.P. Mazat, L. Schwartz, M. Rigoulet, S. Arbault, P. Paumard et A. Devin. Biochim Biophys Acta Bioenerg, Mitochondrial quinone redox states as a marker of mitochondrial metabolism, 1865(2) (2024), 149033, https://doi.org/10.1016/j.bbabio.2024.149033

P39

Multiscale Computational Insights into Long-Range Electron Transfer in Hydrogendependent CO_2 Reductase

Jonathan Lasham[a], Oleksii Zdorevskyi[a] [a]*Department of Physics, University of Helsinki, Finland*
E-mail address: jonathan.lasham@helsinki.fi
Presenting author: Jonathan Lasham

Acetogenic bacteria have evolved specialized strategies to survive in extreme environments, including hydrogen metabolism, carbon fixation, and aldehyde oxidation. A common feature of these processes is their reliance on hydrogenases, which utilize hydrogen to supply electrons for carbon dioxide conversion across a wide range of metabolic pathways.[1–3] Hybrid hydrogenases contain extended chains of redox cofactors which often assemble into higher-order filaments, enabling efficient electron transfer over distances of hundreds of nanometers.[1–4] Among them, hydrogen-dependent CO_2 reductase (HDCR) is one of the most efficient known enzymes for CO_2 reduction, yet the functional role of its filamentous organization remains unclear.[4,5] Here, we investigate how filament formation modulates electron transfer in HDCR from *Thermoanaerobacter kivui*.[4] Our classical molecular dynamics simulations reveal that deletion of the C-terminal helices destabilizes the stalk–periphery interface, disrupting the stability of iron–sulfur clusters required for efficient electron transfer. This explains the reduced activity of mutants observed in recent biochemical studies.[4] To further probe electron transfer within a multiscale framework, we combine classical simulations with electronic structure methods to estimate the key Marcus parameters: electron coupling coefficients, reorganization energies, and thermodynamic driving force. Our analysis identifies key protein regions that modulate electron transfer energetics and highlights potential pathways for electron transfer. These findings provide molecular-level insight into the function of HDCR and its filament-dependent activity.

[1] J.-Z. Zhang et al., Engineered acetogenic bacteria as microbial cell factory for diversified biochemicals, Front. Bioeng. Biotechnol. 12 (2024) 1395540. https://doi.org/10.3389/ fbioe.2024.1395540

[2] X. Feng et al., An electron-bifurcating “plug” to a protein nanowire in tungsten-dependent aldehyde detoxification, Proc. Natl. Acad. Sci. U.S.A. 122 (2025) e2501900122. https://doi.org/ 10.1073/pnas.2501900122

[3] A. Winiarska et al., A bacterial tungsten-containing aldehyde oxidoreductase forms an enzymatic decorated protein nanowire, Sci. Adv. 9 (2023) eadg6689. https://doi.org/10.1126/ sciadv.adg6689

[4] H.M. Dietrich et al., Membrane-anchored HDCR nanowires drive hydrogen-powered CO_2 fixation, Nature 607(7920) (2022) 823–830. https://doi.org/10.1038/s41586-022-04971-z

[5] H.M. Dietrich, V. Müller, Ferredoxin as a physiological electron donor for carbon dioxide fixation to formate in a bacterial carbon dioxide reductase, ACS Catal. 13(18) (2023) 12374–12382. https:// doi.org/10.1021/acscatal.3c02753

P40

Contrasting binding pockets of the two flavins of Bifurcating Electron Transferring Flavoprotein observed through FTIR spectroelectrochemistry

María González-Viegas[a], Federico Baserga[a], Anne-Frances Miller[b], Maria-Andrea Mroginski[c] and Joachim Heberle[a]

[a] *Department of Physics, Freie Universität Berlin, Berlin, Germany* [b]*Department of Chemistry, University of Kentucky, Lexington KY, U.S.A.* [c]*Department of Chemistry, Technische Universität - Berlin, Berlin, Germany*

Email-address: maria.gonzalez.viegas@fu-berlin.de
Presenting author: María González-Viegas

Abstract text:

Flavin-based electron bifurcation couples an unfavorable (endergonic) reaction to a favorable (exergonic) reaction, producing highly energetic reducing agents. Bifurcating electron-transferring flavoproteins (Bf-ETFs) catalyze this reaction through the activities of two chemically identical flavin cofactors that exhibit contrasting physicochemical properties [1]. However, the mechanisms by which the protein environment tunes their reactivities remain poorly understood. To address this question, we applied FTIR difference spectroscopy in combination with potentiometric titrations [2] of the Bf-ETF from *Sulfolobus acidocaldarius*. By jumping across the determined midpoint potential, we recorded potentiostatic difference IR spectra, thereby resolving experimentally the spectral responses of particular flavins at specific redox states. Our results show distinct IR signatures for the two flavins of Bf-ETF, demonstrating that IR spectroscopy can discriminate the two FAD binding sites. H/D exchange measurements and comparison to previous literature allowed assignment of cofactor normal modes to some of the observed bands and identification of hydrogen bonds between the cofactors and the protein environment. In addition, the IR spectra indicate changes in protein secondary structure linked to the flavin redox states. Overall, this study highlights IR spectroscopy as a powerful technique to obtain informative structural information about Bf-ETFs, and establishes a framework for applying the method to other complex redox enzymes.

[1] W. Buckel, R. K. Thauer, Flavin-based electron bifurcation, a new mechanism of biological energy coupling. Chemical reviews, 118(7), (2018) 3862-3886. https://doi.org/10.1021/acs.chemrev.7b00707

[2] F. Baserga, et al., Quantification of local electric field changes at the active site of cytochrome c oxidase by fourier transform infrared spectroelectrochemical titrations. Frontiers in Chemistry 9 (2021): 669452. https://doi.org/10.3389/fchem.2021.669452

P41

Systematic disruption of Tyr/Trp pathways amplifies the ROS formation 25-fold in multicopper oxidase: theoretical predictions of ET pathways and RRDE electrochemical studies

Carla Scharbach[a,b], Frank H. Pilz[a,b], Patrycja Kielb[a,b]

[1]*Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Wegelerstr. 12 53115 Bonn, Germany*
2
Transdisciplinary Research Area 'Building Blocks of Matter and Fundamental Interactions', University of Bonn, Bonn, Germany

E-mail address: kielb@uni-bonn.de
Presenting author: Patrycja Kielb

Multicopper oxidases catalyze the four-electron reduction of O_2 to water in a structurally constrained protein matrix. Efficient catalysis requires efficient electron delivery to the buried trinuclear cluster while protecting against partially reduced oxygen species. The protein scaffold imposes spatial confinement on both electron transfer and reactive intermediates, raising the question of how internal redox architecture support catalysis. Aromatic residues such as tyrosine and tryptophan have been proposed to form hole-hopping redox networks that buffer oxidizing equivalents. Such pathways have been identified in *Streptomyces coelicolor* laccase (SLAC) and experimentally shown to form tyrosyl radical during single oxygen turnover[1], yet their quantitative role in suppressing reactive oxygen species (ROS) formation has remained unclear. In our recent work[2], we combine kinetic modeling of hole-transfer pathways with electrochemical measurements to directly probe the protective function of these networks in SLAC. Calculated hole-transfer kinetics identify a network of ~40 Tyr/Trp pathways with residence times faster than enzymatic turnover. Systematic disruption of these pathways by site-directed mutagenesis sequentially reduces network connectivity without directly perturbing the active site. By immobilizing SLAC variants on MWCNT-coated glassy carbon electrodes and employing rotating ring–disk electrochemistry (RRDE), we simultaneously quantified electrocatalytic activity and H_2O_2 production. As such, we observed that blocking aromatic redox pathways dramatically increased incomplete O_2 reduction. A single mutation that abolishes all relevant pathways results in a >25-fold increase in ROS formation. These results provide direct quantitative evidence that extended aromatic redox networks act as intrinsic protective circuits.

[1] P. Kielb, C. Teutloff, R. Bittl, H.B. Gray, J.R. Winkler Does Tyrosine Protect S. coelicolor Laccase from Oxidative Degradation or Act as an Extended Catalytic Site? J. Phys. Chem. B 126(40) (2022) 7943-7949.

[2] Scharbach C., Pilz F.H., Ciaccafava A., Kielb P., Systematic disruption of Tyr/Trp pathways amplifies the ROS formation 25-fold in multicopper oxidase, preprint on ChemRxiv 2025

P42

Disentangling the biotic flow of electrons in the continental subsurface

Guillermo Climent Gargallo[a], Benoit de Pins[a], Marco Moracci[a], Katriona Edlmann[b]

[a]Department of Biology, University of Naples Federico II, Naples, Italy [b]School of Geosciences, The University of Edinburgh, Edinburgh, UK

E-mail address: guillermo.climent.gargallo@gmail.com
Presenting author: Guillermo Climent Gargallo

The carbon economy we are highly dependent on has accelerated the rate of global climate change, with important and harmful consequences for the ecosystem. In order to mitigate and favour a transition to a greener and low-carbon energy grid, Underground Hydrogen Storage (UHS) could allow the storage of excess renewable energy in suitable deep subsurface porous rocks and salt caverns in the form of hydrogen gas to supply demands in times of need. One of the major risks these operations face is the extant microbial populations living in these rock formations, which can consume the stored hydrogen and produce undesired byproducts, such as methane or hydrogen sulfide. Oligotrophic in nature and confined within faults, pores, crevices, and many other formations, these environments push bioenergetics to its limits, with several reported cases of syntrophy and reliance on hydrogen as the main electron donor. The subsurface is in great part enriched in electron donors with respect to the surface, mainly due to the in-situ presence of hydrogen, methane and other gases that are generated within the planetary interior as well as the lack of widespread acceptors, such as oxygen, nitrate, nitrite, ferric iron, manganese (IV), sulfate and carbon dioxide. Other limitations that potentially shape the composition and constraint the rapid growth of these communities comprise organic carbon, phosphorous and trace element sources, pH, high temperatures and pressures as well as the mobilization of nutrients by percolating water, which is conditioned by rock porosity. Additionally, when compared to other ubiquitous environments, these subsurface species lack a comprehensive biogeochemical characterization and several aspects of their metabolism remain unknown, such as their usage of redox species and their surrounding geochemistry. Knowing the extent to which these microorganisms can mobilize electrons from donors to acceptors is critical to further advance our understanding of these extreme, underexplored environments and, ultimately, to assess the risk of UHS operations. In this work, we explore the presence of different oxidoreductases together with the flow of electrons they enable, employing public datasets related to the deep subsurface as a case study, with the objective of understanding their potential usage of hydrogen and other redox species with relevance to UHS.

P43

High Resolution Structures of Cytochrome *c* Oxidase Catalytic Intermediates Daniel Hatlem[a], Daniela S. Barreiro[a], Di Wu[a], Schara Safarian[a], Hartmut Michel[a]

[a]*Molecular Membrane Biology Emeritus Group, Max Planck Institute of Biophysics, Frankfurt am Main, Germany*

E-mail address: Daniela.Barreiro@biophys.mpg.de
Presenting author: Daniela S. Barreiro

Cytochrome *c* oxidases are membrane proteins that receive electrons from *c*-type cytochromes to catalyze the four-electron reduction of molecular oxygen to water. Simultaneously, these proteins facilitate the translocation of four protons across the inner membrane, enabling energy conservation in the form of an electrochemical gradient. Although their atomic structures have been known for over 25 years, the exact catalytic mechanism of these membrane proteins remains a subject of debate. Our recent Cryo-EM high resolution structures of different catalytic intermediates suggest that the oxidized form of the enzyme (O-state) features a peroxide bridge between the Fe and Cu_B atoms of the catalytic center, while the peroxide state (P-state) contains a neutral dioxygen molecule and the ferryl state (F-state) binds superoxide.[1] These findings suggest that the commonly accepted catalytic model for cytochrome *c* oxidases may need to be reconsidered and turned by 180 degrees. [1] To further complement the structural data, Raman and EPR spectroscopy will be used to confirm the nature of each distinct catalytic intermediate.

[1] F. Kolbe, Cryo-EM structures of intermediates suggest an alternative catalytic reaction cycle for cytochrome *c* oxidase, Nature Communications 12(6903) (2021), https://doi.org/10.1038/s41467021-27174-y

P44

Deciphering the critical mechanistic and regulative elements of *Mycobacterium abscessus* cytochrome *bd* oxidase

Subha Suvetha Kathalingam[a], Vikneswaran Mathiyazakan[a], Kevin Pethe[a-d], and Gerhard Grüber[a,e]

[a] Nanyang Technological University, School of Biological Sciences, Singapore 637551, Singapore [b] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 636921, Singapore [c] National Centre for Infectious Diseases (NCID), Singapore 308442, Singapore [d] Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Singapore 639798, Singapore [e] Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR), Singapore 138671, Singapore

E-mail address: subhasuv001@e.ntu.edu.sg
Presenting author: Subha Suvetha Kathalingam

The increasing global incidence of pulmonary non-tuberculous mycobacteria (NTM) infections has become a serious public health concern [1-2]. *Mycobacterium abscessus* (*Mab*) has emerged as one of the most commonly isolated and clinically challenging pathogens [3]. The intrinsic mechanisms present in *Mab* limit the efficacy of classical anti-tuberculous drugs and majority of antimicrobials, severely constraining treatment options [3,4]. The clinical barrier imposed by *Mab* could be circumvented by targeting novel target spaces within the bacterium. The non-fermentative *Mab* relies on the oxidative phosphorylation (OXPHOS) pathway to meet its energy requirements and the successful clinical validation of the pathway in *Mycobacterium tuberculosis* provides confidence for adopting the strategy in *Mab*. The branched respiratory pathway, within the OXPHOS pathway, comprises of an energetically efficient cytochrome *bcc*:*aa*$_3$ oxidase and an alternate terminal oxidase cytochrome *bd* (cyt-*bd*) which possesses higher oxygen affinity compared to its counterpart [5]. The redirection of electron flux via cyt-*bd*, in the event of inhibition of the cytochrome *bcc*:*aa*$_3$ or F-ATP synthase [6], highlights the importance of cyt-*bd* as a potential virulence factor and the need to characterize the oxidase in *Mab*. To perform a structure-guided investigation, we generated a homology model of the *Mab* cyt-*bd* focusing on the key structural and catalytic elements in the oxidase: Alternate Menaquinol-Binding Site (AMS), Oxygen channel (OC) and Redox Modulation Site (RMS). An alanine scan was performed to understand the physiological relevance of the key residues using a heterologous system, which was employed to monitor ATP synthesis and oxygen consumption on the whole cell level. The findings revealed that the mutations of W9 (AMS), E98 (OC) and F103 (OC) resulted in abolishment of oxidase function while the C285 (RMS) mutation led to partial loss of function. In summary, the biochemical characterization of the *Mab* cyt-*bd* oxidase paved way to the identification of critical mechanistic and regulative elements that are crucial for the enzyme function. These insights serve as blueprints to guide future Structure-Based Drug Discovery campaigns for anti-NTM therapies, addressing unmet needs in treating multidrug-resistant *Mab* infections.

[1] Prevots, D. R., Shaw, P. A., Strickland, D., Jackson, L. A., Raebel, M. A., Blosky, M. A., Montes de Oca, R., Shea, Y. R., Seitz, A. E., Holland, S. M., and Olivier, K. N. (2010) Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *American journal of respiratory and critical care medicine* 182, 970-976.

[2] Ratnatunga CN, Lutzky VP, Kupz A, Doolan DL, Reid DW, Field M, Bell SC, Thomson RM, Miles JJ. The Rise of Non-Tuberculosis Mycobacterial Lung Disease. Front Immunol. 2020 Mar 3;11:303. doi: 10.3389/fimmu.2020.00303. PMID: 32194556; PMCID: PMC7062685.

[3] Zweijpfenning, S. M. H., van Ingen, J., and Hoefsloot, W. (2018). Geographic Distribution of Nontuberculous Mycobacteria Isolated From Clinical Specimens: A Systematic Review. Semin. Respir. Crit. Care Med. 39 (3), 336–342. doi: 10.1055/s-0038-1660864.

[4] Nessar, R., Cambau, E., Reyrat, J. M., Murray, A., and Gicquel, B. (2012) *Mycobacterium abscessus*: a new antibiotic nightmare. *The Journal of antimicrobial chemotherapy* 67, 810-818.

[5] Cook GM, Hards K, Dunn E, Heikal A, Nakatani Y, Greening C, Crick DC, Fontes FL, Pethe K, Hasenoehrl E, Berney M. Oxidative Phosphorylation as a Target Space for Tuberculosis: Success, Caution, and Future Directions. Microbiol Spectr. 2017 Jun;5(3):10.1128/microbiolspec.tbtb2-0014-2016. doi: 10.1128/microbiolspec.TBTB2-0014-2016. PMID: 28597820; PMCID: PMC5480969.

[6] Harrison, S.H., Walters, R.C., Cheung, CY. *et al.* Remission spectroscopy resolves the mechanism of action of bedaquiline within living mycobacteria. *Nat Commun* 16, 11018 (2025). https://doi.org/10.1038/s41467-025-65928-0.

P45

Escherichia coli cytochrome *bd*-II displays significant quinol peroxidase activity

Lucia Heger[a], Pia Gescher[a], Jonas Hentschel[a], Jan Kägi[a], Hannes Kucher[b], Bernhard Breit[b] and Thorsten Friedrich[a] [a] *Institute of biochemistry, Albert-Ludwigs-Universität Freiburg, Germany* [b] *Institut of organic chemistry, Albert-Ludwigs-Universität Freiburg, Germany.*

E-mail address: Lucia.heger@bio.chemie.uni-freiburg.de
Presenting author: Lucia Heger

Cytochrome *bd* enzymes are bacterial terminal oxidases only present in bacteria and some archaea, making them attractive targets for novel antimicrobials [1]. They contribute to the protonmotive force, by coupling the reduction of oxygen to water with the oxidation of quinols [2]. Moreover, they are expressed under oxygen-limited conditions and contribute to the detoxification of reactive oxygen or nitrogen species, supporting bacterial survival and stress adaptation [3]. *Escherichia coli* contains two cytochrome *bd*s (*bd*-I and *bd*-II) that share a similar structure. Therefore, *bd*-I and *bd*-II are often considered to perform the same reaction [2,4]. This would be atypical for *E. coli*, suggesting that each enzyme is adapted to distinct physiological functions. It is already known that cytochrome *bd*-I displays quinol peroxidase activity [5]. Here, we measured the cytochrome *bd*-II quinol peroxidase activity as Q_1H_2 oxidation at 260 nm, *via* UV-vis spectrometry in an anaerobic tent. Our results indicate that cytochrome *bd*-II has a higher catalytic efficiency as a quinol peroxidase than as a quinol oxidase. We could not detect catalase activity of the preparation. Based on its structure, the peroxidase reaction is directly linked to transmembrane charge separation and cytochrome *bd*-II would define a new class of enzymes, namely energyconverting quinol peroxidases [2,5].

[1] J. Kägi, W. Sloan, J. Schimpf *et al.*, Exploring ND-011992, a quinazoline-type inhibitor targeting quinone reductases and quinol oxidases. Sci Rep 13, 12226 (2023). https://doi.org/10.1038/ s41598-023-39430-w

[2] A. Theßeling, T. Rasmussen, S. Burschel, D. Wohlwend, J. Kägi, T. Friedrich, Homologous *bd* oxidases share the same architecture but differ in mechanism, Nat. Commun., 10 (2019) 1-7. https://doi.org/10.1038/s41467-019-13122-4

[3] V. B. Borisov, E. Forte, Bioenergetics and Reactive Nitrogen Species in Bacteria, Int. J. Mol. Sci., 23 (2022) 7321. https://doi.org/10.3390/ijms23137321

[4] A. Grauel, J. Kägi, T. Rasmussen *et al.*, Structure of *Escherichia coli* cytochrome *bd*-II type oxidase with bound aurachin D. Nat. Commun., 12 (2021) 1-11. https://doi.org/10.1038/ s41467-021-26835-2

[5] S. Al-Attar, Y. Yu., M. Pinkse *et al.*, Cytochrome *bd* Displays Significant Quinol Peroxidase Activity. Sci Rep 6, 27631 (2016). https://doi.org/10.1038/srep27631

P46

Spectroscopic Investigation of the O2 Reduction Intermediates by Cytochrome c Oxidase from *Paracoccus denitrifican*

Muhammad Tahir Hafeeza, Raaif Siddeequea, Daniel Hatlemb, Frederic Melina, Petra Hellwiga ,Hartmut Michelb
aFaculty of Chemistry, University of Strasbourg, Laboratoire de Bioelectrochimie et Spectroscopie, 4 Rue Blaise pascal UMR 7140, 67081 Strasbourg, France
bMax Planck Institute of Biophysics, Frankfurt am Main, Germany
E-mail address: Muhammad-tahir.hafeez@etu.unistra.fr

Presenting author: Muhammad Tahir Hafeez

Understanding oxygen reduction in cytochrome c oxidase (CcO) has long been central to bioenergetics research. Since the early mechanistic investigations of the late 1970s [1,2], our knowledge of this enzyme has expanded considerably. Spectroscopic, kinetic, and structural studies have provided major insights into how the enzyme reduces O_2 to water while coupling the reaction to proton pumping. The precise nature, structure, and interconversion of key intermediates are still debated, and different mechanistic models [3-5] have been proposed to explain experimental observations. As a result, a complete and unified description of the oxygen reduction mechanism at the binuclear centre is still an active area of debate, especially after the recent Cryo-EM studies of intermediate states [4].

Our work explores the nature of intermediates (P-state, F-state, O-state and E-state) [4,6] of the catalytic cycle of Oxygen reduction at the binuclear centre of cytochrome c oxidase from Paracoccus denitrificans. By using UV-visible and Resonance Raman (RR) spectroscopic approaches, we aim to get structural insights into those intermediates. Catalytic intermediates were generated by controlled chemical manipulation of Isolated CcO, which is well defined in the literature [7]. Then, UV–visible absorption spectroscopy was systematically performed to monitor and verify successful state formation.

To minimize perturbations associated with drying or immobilization and to maintain the structural and functional integrity of the enzyme during spectroscopic analysis, we performed all measurements in capillary tubes. Because the catalytic intermediates involve oxygen- derived species bound at the heme a_3–CuB binuclear centre, isotopic labelling experiments were carried out to enable unambiguous vibrational assignments. The P-state RR spectra reveal oxygen-isotope–sensitive bands attributable to Fe–oxygen moieties (Fe[II]–O_2 or Fe[III]–O_2-). In the F-state, distinct isotope–sensitive vibrational features indicate changes in oxygen coordination within the binuclear center, potentially involving CuB-associated oxygen species. The O-state spectra display isotope–sensitive vibrational bands compatible with a peroxide-derived, Fe–O–O–Cu bridging structure at the active site. E-state spectrum showed the presence of Fe–Hydroxy and Oxoferryl species (Fe[IV]=O2-) but Isotopic labelling is yet to be done to confirm these assignments.

P47

Mechanistic and Functional characterization of Cytochrome *bd* Oxidases in *Mycobacterium smegmatis*

Parul Singh[1], Mateusz Janczak[1] and Pia Ädelroth[1]

[1] Department of Biochemistry and Biophysics, Stockholm University, Sweden

Email: parul.singh@dbb.su.se

Presenting Author: Parul Singh

Cellular respiration relies on membrane-bound electron transfer chains that generate a proton motive force. In bacteria, oxygen reduction is catalyzed by heme-copper oxidases (HCuOs) and cytochrome *bd* oxidases. In contrast to HCuOs, which couple electron transfer to proton pumping, cytochrome *bd* oxidases are non-proton pumping enzymes unique to prokaryotes, characterized by high O_2 affinity and quinol oxidation, enabling function under microaerobic and stress conditions. This study aims to resolve the mechanisms of electron and proton transfer in cytochrome *bd* oxidases and to assess their variability across the family. Using biochemical and biophysical approaches, including activity assays and spectroscopic characterization, we investigate the two terminal *bd* oxidases in *Mycobacterium smegmatis*, both of which have structures defined to atomic resolution [1, 2]. Emphasis is placed on defining their different kinetic properties, redox behavior, and structure-function relationships. Our data suggests that the *Ms bd*-II is much more promiscuous in quinol utilization than the *bd*-I and that the role of the heme b^{595} is also more versatile in *Ms bd*-II. These results will provide mechanistic insight into cytochrome *bd* function and clarify the distinct physiological roles of the two oxidases, contributing to our understanding of mycobacterial respiration and its potential as a therapeutic target.

References:

[1]. Wang W, Gao Y, Tang Y, Zhou X, Lai Y, Zhou S, Zhang Y, Yang X, Liu F, Guddat LW, Wang Q, Gong H, Rao Z, Cryo-EM structure of mycobacterial cytochrome bd reveals two oxygen access channels. Nat Commun. 12, (2021), 4621, https://doi.org/10.1038/s41467-02124924-w

[2]. Terezia Kovalova, Mateusz Janczak, Ana P. Gamiz-Hernandez, Daniel Lundin, Soni Sharma, Johanna Vilhjálmsdóttir, Dan Sjöstrand, Ville R.I. Kaila, Martin Högbom, Pia Ädelroth, The *Mycobacterium smegmatis bd*-II terminal oxidase employs a carboxylate shift mechanism. Proc Natl Acad Sci USA. 123(11), (2025), e2515348123, https://doi.org/10.1073/pnas.2515348123

P48

Electron distribution in the one-electron reduced E-state of bovine cytochrome c oxidase

Jonatan Johannessona , Marian Fabianb , Daniel Jancurac

aFaculty of Science, University of P. J. Safarik, Jesenna 5, 041 54, Kosice, Slovak Republic
bCenter for Interdisciplinary Biosciences, Technology and Innovation Park, University of P. J. Safarik, Jesenna 5, 041 54, Kosice, Slovak Republic
cDepartment of Biophysics, Faculty of Science, University of P. J. Safarik, Jesenna 5, 041 54, Kosice, Slovak Republic

E-mail address: jonatan.goran.johannesson@upjs.sk
Presenting author: Jonatan Johannesson

Mitochondrial cytochrome c oxidase (CcO) couples the exergonic reduction of oxygen to water to the formation of an electrochemical gradient via the sequential four electron reduction and protonation of the intermediates of the catalytic sites of CcO. Moreover, for each electron delivered to the CcO catalytic site, a proton is translocated across the membrane.

The proton pumping mechanism in CcO is still a matter of intensive debates [1] . The proton pumping in the oxidative phase of the catalytic cycle (PM-to-F and F-to-OH transitions) is relatively well described. However, the mechanism of this process in the reductive phase is less understood. It was suggested that energy release during the oxidative phase is stored in a “high-energy” metastable state (OH) of the oxidized CcO [2] . Subsequently, the reduction of this OH form by two electrons, sequentially generating the one-electron reduced E form (O→E) and two-electron reduced R state (E→R), should be linked to the proton pumping [3] . However, the properties of the E-state are still not very well known and studied. In this work, we characterize the electron distribution in the one-electron reduced E-state of bovine CcO. This state was prepared by two-electron reduction of the ferryl F form with CO. This reaction generates a relatively stable (tens of minutes) E-state. According to the UV-Vis absorption spectra of the E-form, the electron is localized almost exclusively on heme a at pH 9.5. However, at the acidic pH 6.5, only a few percent of reduced heme a is observed and the electron supposedly resides on the spectrally invisible CuB. The presence of electron on CuB is confirmed by the appearance of EPR g=6 signal in the E state at low pH. The electron distribution is fully reversible which is demonstrated by the appearance of electron on heme a after pH change of the E sample prepared at pH 6.5 to pH 9.5. This observation indicates the pH-dependent electron distribution between heme a and CuB: Fea 2+ CuB 2+ ↔ Fea 3+ CuB + . The pH dependence of the electron distribution in the E state in the pH region 6.5 – 9.5 shows redox dependency -56 mV/pH for CuB center and -20mV/pH for heme a. The localization of the electron on CuA and/or heme a3 was not detected in the studied pH range. The consequences of the present findings for elucidation of the proton pumping mechanism in CcO are discussed.

[1] M. Wikström, R. B. Gennis, P. R. Rich, Structures of the intermediates in the catalytic cycle of mitochondrial cytochrome c oxidase, Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1864 (2), (2023), 148933, ISSN 0005-2728, https://doi.org/10.1016/j.bbabio.2022.148933
[2] M.I. Verkhovsky, A. Jasaitis, M.L. Verkhovskaya, J.E. Morgan, J. E., M. Wikstrom,Proton translocation by cytochrome c oxidase, Nature 400, (1999), 480-483, https://doi.org/10.1038/22813
[3] V .R. Kaila, M.I. Verkhovsky, M. Wikstrom, Proton-coupled electron transfer in cytochrome oxidase, Chem Rev 110(12), (2010), 7062-81, https://doi.org/ 10.1021/cr1002003

P49

Cytochrome *bd* oxidase from *Solidesulfovibrio fructosivorans* and why its cooler than *E. coli*'s

Mary de La Sayette [a], Arlette Kpebe [a], Frédéric Melin [b], Petra Hellwig [b], Yonghua Li-Beisson [c], Bertrand Legeret [c], Stéphane Grimaldi [a], Myriam Brugna [a], James Sturgis[d], Eric Pilet [a]

[a]Laboratoire de Bioénergétique et Ingénierie des Protéines, UMR 7281, BIP, 13009, Marseille, France

[b]Laboratoire de Bioelectrochimie et Spectroscopie, UMR 7140, Université de Strasbourg, CNRS, 67081 Strasbourg, France

[c]Heliobiotech Facility, BIAM-Cadarache CEA/CNRS/Aix-Marseille Université (UMR 7265) St Paul-lez-Durance

[d]Laboratoire d'Ingénierie des Systèmes Macromoléculaires UMR 7255 LISM, 13009, Marseille, France

E-mail address: msayette@imm.crns.fr and epilet@imm.crns.fr
Presenting author: Mary de La Sayette and Eric Pilet

The cytochrome *bd* oxidase (cyt*bd*) is an O_2 reductase in procaryote respiratory chains involved in both oxygen reduction and resistance to redox stress [(1)]. The cyt*bd* has been well characterized in *Escherichia coli (Ec)*(cytbd_{Ec}) and other human pathogenic bacteria. From cyt*bd* diversity, raises

questions about the protein activity: the importance of lipids [(2)], the redox potential of the hemes, and the localization of quinol binding site(s). Our preliminary results on cyt*bd* from *Solidesulfovibrio fructosivorans (Sf)* shows this enzyme shares common features with both mycobacterial (Mt) enzymes (small Q-loop, absence of additional small subunit in the operon) and with *E. coli* enzyme (absence of SS bridge in Q-loop). Moreover, the lipid content in *Sf* is different compared to *Mt* or *Ec*. Cytbd_{Sf}, homologously produced with the operon under the control of its native promoter, is purified by affinity after solubilization by 1% UMNG. The O_2 reduction capability of the enzyme, measured on a Clark electrode, allows enzymatic characterization focusing on two aspects: the nature of the substrates (Quinol, O_2) and an inhibitor study (CN, CO, NO and H_2S). Our results show that Cyt*bdSf* KI_{CN} in mM range, similar to cytbd_{Ec} confirming cyt*bd* as a CN insensitive enzyme. Analyses by both TLC and mass spectrometry reveal an unusual lipid content: absence of cardiolipins, an odd number fatty acids, and the presence of ceramides and diamino fatty acids. Further analyses in the characterization study include catalytic flexibility assay, spectroscopic characterization, redox potential, UV/Visible spectroscopy, EPR and FTIR. Hemes redox potential, substrate specificity, and inhibitors effect on catalysis have been explored by means of Protein

Film Voltametry, potentiometric titrations monitored by UV/Visible spectroscopy[(3)] and oxygraphy. The integration of our results and those from *Mt* and *Ec* will allow for a have a better understanding of the diversity of cyd*bd* and lead to a characterization of the mechanism of this important family of enzymes.

[1] T. Friedrich *et al.*, Recent Advances in Structural Studies of Cytochrome *bd* and Its Potential Application as a Drug Target. *International Journal of Molecular Sciences* 23,(2022).

[2] A. H. Asseri *et al.*, Cardiolipin enhances the enzymatic activity of cytochrome *bd* and cytochrome *bo(3)* solubilized in dodecyl-maltoside. Scientific Reports 11, (2021).

[3] I. Makarchuk *et al.*, Mutating the environment of heme b_{595} of *E. coli* cytochrome *bd-I* oxidase shifts its redox potential by 200 mV without inactivating the enzyme. *Bioelectrochemistry* 151, (2023)

P50

P51

Confirmation and functional characterization of Ni-NTA binding motif in cytochrome bo_3 quinol oxidase from *Escherichia coli*

Y. Gao[a], R.M.A. Heeren[a]

[a] The Maastricht MultiModal Molecular Imaging institute, M4i, Maastricht University, Maastricht, Netherlands

E-mail address: y.gao@maastrichtuniversity.nl
Presenting author: Y. Gao

Abstract text: Cytochrome bo_3 oxidase (cyt bo_3) from *E. coli* is frequently reported to bind to Ni–NTA resin and to co-purify as a contaminant during the isolation of unrelated His-tagged membrane proteins. As *E. coli* remains one of the most widely used expression systems, affinity purification using His-tag fusion proteins and Ni–NTA chromatography represents a standard strategy. The unintended retention of cyt bo_3 on Ni–NTA resin therefore poses a recurring challenge. Identification of the Ni–NTA binding motif within cyt bo_3 is essential for the rational design of an engineered *E. coli* expression strain that minimizes this contamination. This would improve the homogeneity of purified membrane proteins and facilitate downstream structural and functional studies. In this work, we applied cryo-EM Single Particle Analysis (SPA) to determine the structure of cyt bo_3 from *E. coli* and identified a potential Ni–NTA binding motif. A truncation variant lacking this motif was then generated and purified using the Ni–NTA column. The integrity and composition of the truncated complex were evaluated by SDS–PAGE, anti-His Western blotting, and peptide identification by bottom-up proteomics following trypsin/Lys-C digestion. The truncation variant has been further analyzed by cryo-EM and native mass spectrometry (MS) to explore the functionality of the potential binding motif. A cryo-EM structure of cyt bo_3 assembled in peptidisc was resolved at 2.8 Å resolution and the N-terminal region of subunit III could be examined and is proposed to be the binding motif with Ni-NTA [1]. Following truncation of this proposed binding motif, a substantially larger proportion of cyt bo_3 was recovered in the wash fraction from the Ni–NTA column, indicating reduced affinity for the resin. Mass spectrometry validated the complex composition. Protein structure prediction by AlphaFold supported similar structural frame of the truncated variant compared to the native complex. Additional structural and functional characterization including activity assays, cryo-EM SPA and native MS are in progress. The current preliminary results confirmed that the proposed N-terminal region of subunit III is indeed responsible for Ni–NTA binding, providing a basis for minimizing cyt bo_3 contamination during purification of His-tagged membrane proteins expressed in *E. coli*. Further functional characterization of the binding motif is necessary to determine whether it can be truncated from the *E. coli* genome without impairing bacterial growth, thereby evaluating the feasibility of generating such an expression strain for recombinant protein expression.

[1] Y. Gao, Y. Zhang, S. Hakke, R. Mohren, L. Sijbers, P.J. Peters, R.B.G. Ravelli, Cryo-EM structure of cytochrome bo(3) quinol oxidase assembled in peptidiscs reveals an "open" conformation for potential ubiquinone-8 release, Biochim Biophys Acta Bioenerg 1865(3) (2024) 149045.

with the topic marking
S4. Terminal oxidases

Contribution type:
Talk – T

P52

Inhibition of mitochondrial transcription activates fatty acid oxidation via transcript-level sensing prior to decrease of OXPHOS capacity

Taolin Yuan[1], Polyxeni Papadea[1], Florian Rosenberger[1,2], Stefan Siira[3], Aleksandra Filipovska[3], Patric Giavalisco[4], Jelena Misic[1], Nils-Göran Larsson[1,*]

[1]Department of Medical Biophysics and Biochemistry, Karolinska Institutet, Sweden
[2]Department of Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Martinsried, Germany
[3]Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University, Bentley, WA 6102, Australia
[4]Metabolomics Core Facility, Max Planck Institute for Biology of Ageing, Cologne, Germany
Taolin.yuan@ki.se Presenter:
Taolin Yuan

Mitochondria are critical for cellular metabolism through their roles in bioenergetics, biosynthesis and intracellular signaling. Mitochondrial function requires both nuclear and mitochondrial gene expression. Our previous study reported that paradoxical metabolic benefits were induced by inhibition of mtDNA transcription in a mouse model with dietinduced obesity. *Per oral* treatment of mice with inhibitors mitochondrial transcription (IMTs) for four weeks markedly decreased mitochondrial transcript levels and reduced OXPHOS capacity in the liver. Surprisingly, IMT treatment normalized pre-diabetes, normalized the body weight, and reversed hepatosteatosis in mice fed a high-fat diet (HFD). To better understand possible mechanisms underlying these remarkable metabolic benefits, we investigated whether isolated OXPHOS complex deficiencies contribute to the metabolic phenotypes. Several liver-specific knockout mouse models with isolated complex I (CI), complex IV (CIV), or combined CI and CIV deficiencies were used. These knockout mice showed impaired adaptation to HFD and gained less body weight on HFD in comparison with controls. However, the fatty acid oxidation rate of liver mitochondria was impaired in comparison with controls. Body composition analyses revealed a decrease of both fat and lean mass in IMT-treated HFD-fed knockout mice. Furthermore, double knockout of CI and CIV resulted in liver pathology and a fourfold increase in the liver to body weight ratio. Together, these data demonstrate that liver-specific OXPHOS deficiency interferes with fatty acid oxidation, results in energy crisis and liver pathology. This study thus shows that OXPHOS deficiency do not recapitulate the metabolic benefits observed in the IMT-treated obese mice. In agreement with this, transcriptomics analysis showed that the fatty acid oxidation pathway was activated as an early response to IMT treatment. This response occurred when mitochondrial transcript levels were reduced by half, while no significant decrease in OXPHOS protein levels was yet observed. Liver mitochondrial respiration profiling showed an increased fatty acid oxidation and preserved CI- and CII-driven respiration. Collectively, our data suggests that sensing the mitochondrial transcript levels rather than OXPHOS protein levels drives the activation of fatty acid oxidation in liver.

red0;Proton exit routes in complex I explored with MD simulations and free energy calculations

Amiel Abettan[a], Jonathan Lasham[a], Erik Endres[a], Vivek Sharma[a,b]

[a] Department of Physics, University of Helsinki, Helsinki 00014, Finland [b] HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki 00014, Finland

E-mail address: amiel.abettan@helsinki.fi
Presenting author: Amiel Abettan

Energy (ATP) production is central to cellular metabolism. In human cells, most of the ATP is generated by large enzymatic complexes embedded in the mitochondrial inner membrane. High-resolution cryo-EM structures have revealed the detailed architecture of these complexes, yet their molecular mechanisms remain unclear and actively debated.[1] In particular, the pathways enabling proton translocation across the membrane through Complex I (CI) remain difficult to characterize. Here, we investigate the structural dynamics of antiporter-like subunits, and the putative proton exit routes towards the P side of the membrane using atomistic molecular dynamics simulations. We identify scenarios when the water mediated proton network connecting key residues open and close and their triggering mechanisms. AWH-based free energy methods provide quantitative insights on the energetics of open/closed transitions. These findings provide mechanistic insights into the P-side proton transfer routes and refine the current models of energy conversion in complex I.[2]

[1] M. Wikström, C. Pecorilla, V. Sharma, The mitochondrial respiratory chain, Enzymes 54 (2023) 15–36, https://doi.org/10.1016/bs.enz.2023.05.001

[2] Djurabekova A, Lasham J, Zdorevskyi O, Zickermann V, Sharma V. Long-range electron proton coupling in respiratory complex I - insights from molecular simulations of the quinone chamber and antiporter-like subunits. Biochem J. 2024 Apr 10;481(7):499-514. doi: 10.1042/BCJ20240009.

P53

Inhibition of mitochondrial transcription activates fatty acid oxidation via transcript-level sensing prior to decrease of OXPHOS capacity

Taolin Yuan1, Polyxeni Papadea1 , Florian Rosenberger1,2 , Stefan Siira3 , Aleksandra Filipovska3 , Patric Giavalisco4 , Jelena Misic1 , Nils-Göran Larsson1,*

1Department of Medical Biophysics and Biochemistry, Karolinska Institutet, Sweden
2Department of Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Martinsried, Germany
3Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University, Bentley, WA 6102, Australia
4Metabolomics Core Facility, Max Planck Institute for Biology of Ageing, Cologne, Germany
Taolin.yuan@ki.se
Presenter: Taolin Yuan

Mitochondria are critical for cellular metabolism through their roles in bioenergetics, biosynthesis and intracellular signaling. Mitochondrial function requires both nuclear and mitochondrial gene expression. Our previous study reported that paradoxical metabolic benefits were induced by inhibition of mtDNA transcription in a mouse model with diet- induced obesity. Per oral treatment of mice with inhibitors mitochondrial transcription (IMTs) for four weeks markedly decreased mitochondrial transcript levels and reduced OXPHOS capacity in the liver. Surprisingly, IMT treatment normalized pre-diabetes, normalized the body weight, and reversed hepatosteatosis in mice fed a high-fat diet (HFD). To better understand possible mechanisms underlying these remarkable metabolic benefits, we investigated whether isolated OXPHOS complex deficiencies contribute to the metabolic phenotypes. Several liver-specific knockout mouse models with isolated complex I (CI), complex IV (CIV), or combined CI and CIV deficiencies were used. These knockout mice showed impaired adaptation to HFD and gained less body weight on HFD in comparison with controls. However, the fatty acid oxidation rate of liver mitochondria was impaired in comparison with controls. Body composition analyses revealed a decrease of both fat and lean mass in IMT-treated HFD-fed knockout mice. Furthermore, double knockout of CI and CIV resulted in liver pathology and a fourfold increase in the liver to body weight ratio. Together, these data demonstrate that liver-specific OXPHOS deficiency interferes with fatty acid oxidation, results in energy crisis and liver pathology. This study thus shows that OXPHOS deficiency do not recapitulate the metabolic benefits observed in the IMT-treated obese mice. In agreement with this, transcriptomics analysis showed that the fatty acid oxidation pathway was activated as an early response to IMT treatment. This response occurred when mitochondrial transcript levels were reduced by half, while no significant decrease in OXPHOS protein levels was yet observed. Liver mitochondrial respiration profiling showed an increased fatty acid oxidation and preserved CI- and CII-driven respiration. Collectively, our data suggests that sensing the mitochondrial transcript levels rather than OXPHOS protein levels drives the activation of fatty acid oxidation in liver.

P54

Conformational dynamics in the terminal Complex I membrane subunit Nqo12

Jobst Liebau[a], Patricia Saura[a], Sofia Badolato[a], Ana P. Gamiz-Hernandez[a], Adel Beghiah[a], Ville R. I. Kaila[a]

[a] *Department of Biochemistry and Biophysics, Stockholm University, Svante Arrhenius väg 16C, 106 91 Stockholm, Sweden*

E-mail address: jobst.liebau@dbb.su.se

Presenting author: Jobst Liebau

To transduce energy, the membrane domain of Complex I actively pumps protons across the membrane, against an external proton gradient. However, the mechanism of this proton conduction and its regulation remain poorly understood. Here, we investigate the isolated, terminal antiporterlike subunit Nqo12 of Complex I from *T. thermophilus*. Employing ^{19}F-NMR, we observe that Nqo12 adopts distinct conformations, which show pH-dependent population changes. Consistently, the pH-dependence of the conformational ensemble is decoupled when a central conserved titratable residue is mutated into a non-titratable residue. Employing NMR dynamics experiments, we demonstrate that the antiporter module undergoes conformational exchange between the pHindependent and the pH-dependent conformations. We further observe that mutation of a functionally crucial ion-pair residue that results in gate opening, gives rise to a shift in the conformational ensemble of the antiporter module, and an increased overall apparent pK_a. Our findings are consistent with constant pH-MD simulations, indicating that electric field effects control the conformational ensemble, and corroborate the central role of buried ion-pairs in controlling the proton pumping in Complex I. Based on the combined NMR data and MD simulations, we suggest a mechanism, in which the antiporter module samples various conformations that control proton conduction via electric field effects, modulating the pK_a of titratable residues along the proton pathways.

P55

Cryo-EM structure of a novel type of complex I homologue (NDH) from *Sulfolobus acidocaldarius*

Hanna Pryputniewicz[a], Joanna Florek[a], Paulina Borkowska[a], Paulina Indyka[b], Michał Rawski[b], Beatrycze Nowicka[c], Sebastian Pintscher[a]

[a]Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland [b]National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland [b]Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

E-mail address: sebastian.pintscher@uj.edu.pl
Presenting author: Hanna Pryputniewicz

In mitochondrial and bacterial respiratory electron transport systems, complex I is a key component that delivers electrons to the quinone pool by coupling NADH oxidation to quinone reduction. However, in the case of the oxygen-respiring Archaea, the presence of a functional complex I counterpart in their membranes remains uncertain[1].

Herein, we show a 2.9 Å cryo-EM structure of the NDH complex isolated from native membranes of the thermoacidophilic archaeon, *Sulfolobus acidocaldarius*. Our structure reveals which parts of the NDH are evolutionarily conserved, as well as novel structural motifs specific to Archaea and adaptations unique to *Sulfolobus* that can be crucial for its survival in a harsh environment. Importantly, the gentle protocol used to isolate the complex resulted in the retention of supernumerary subunits and unique bound ligands. The extra subunits, not present in the core *ndh* operon, were identified as indole-3-glycerol phosphate synthase and a dimeric 2-oxoacid:ferredoxin oxidoreductase (OFOR). We proposed that OFOR and NDH can be functionally coupled via a small redox carrier. In the search for a plausible candidate, we identified Zn-containing ferredoxin (Zn-Fdx) as the most abundant small redox protein in *S. acidocaldarius* cells. This allowed us to propose the putative mechanism of action of this newly discovered NDH homologue.

If our hypothesis on the NDH-OFOR complex mechanism is correct, it will mean that *S. acidocaldarius* has a rewired electron transport system, in which the Krebs cycle is directly coupled to a complex I homologue instead of succinate dehydrogenase. Moreover, our results provide us with a new insight into the early evolution of bioenergetic systems.

[1] G. Schäfer, M. Engelhard, V. Müller, Bioenergetics of the Archaea, Microbiology and Molecular Biology Reviews 63 (1999) 570–620. https://doi.org/10.1128/mmbr.63.3.570-620.1999.

P56

Investigations into proton transfer pathways in respiratory complex I from *Paracoccus denitrificans* by mutagenesis.

Robert A. Waddell[a], Harry Messent[a], John J. Wright[a], Spencer Regan-Smith[a], Emily Smith[a], Judy Hirst[a]

[a]*Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom*

E-mail address: raw84@cam.ac.uk
Presenting author: Robert A. Waddell

Respiratory complex I is situated at a nexus of human metabolism, coupling the reduction of NADH and oxidation of quinone to the pumping of four protons across the inner mitochondrial membrane. Studying the coupling mechanism remains challenging due the diLiculty in resolving proton movements across such a large protein. Structures of complex I have provided some insight, resolving three likely candidates for proton pumping pathways in the antiporter-like subunits ND2, ND4 and ND5. This has created a mismatch between the biochemical-derived four proton stoichiometry and the three proton translocating antiporter-like subunits. Further structural and simulation data have been used to propose a number of conflicting mechanisms to resolve the mismatch that diLer in their number of proposed proton uptake and output channels [1,2,3]. We therefore used unmarked genomic mutagenesis in *Paracoccus denitrificans*, a genetically tractable close relative of the proto-mitochondrion to investigate the proton pathways [4]. Mutagenesis allows the substitution of amino acids to alter the local chemistry and probe the role of individual side chains. By applying this to potential proton channels in complex I, molecular details can be built up and used to diLerentiate proposed mechanisms. In this work, we focus on residues within the conserved architecture of the antiporter-like subunits to question extant complex I mechanisms and to restrict the opportunity space for future complex I mechanistic proposals.

[1] V. R. I. Kaila. Resolving Chemical Dynamics in Biological Energy Conversion: Long-Range Proton-Coupled Electron Transfer in Respiratory Complex I. Accounts Chem. Res. 54(24), (2021), 4462–4473. https://doi.org/10.1021/acs.accounts.1c00524

[2] V. Kravchuk, O. Petrova, D. Kampjut, A. Wojciechowska-Bason, Z. Breese & L. Sazanov, (A universal coupling mechanism of respiratory complex I. Nat. 609, (2022), 808–814. https://doi.org/10.1038/s41586-022-05199-7

[3] K. Parey, J. Lasham, D. J. Mills, A. Djurabekova, O. Haapanen, E. G. Yoga, H. Xie, W. Kühlbrandt, V. Sharma, J. Vonck, & V. Zickermann, High-resolution structure and dynamics of mitochondrial complex I-Insights into the proton pumping mechanism. Sci. Adv., 7(46), (2021), 3221. https://doi.org/10.1126/sciadv.abj3221

[4] O. D. Jarman & J. Hirst, Membrane-domain mutations in respiratory complex I impede catalysis but do not uncouple proton pumping from ubiquinone reduction. PNAS Nexus, 1, (2022), 1–12. https://doi.org/10.1093/pnasnexus/pgac276

P57

Structural elucidation of the mechanism for inhibitor resistance in the Na^+-translocating NADH-ubiquinone oxidoreductase from *Vibrio cholerae*

Moe Ishikawa-Fukuda[a,b], Jun-ichi Kishikawa[c,d], Takahiro Masuya[a], Takeshi Ito[b], Nicole L. Butler[b], Danielle McFee[b], Takayuki Kato[c], Blanca Barquera[b]. Hideto Miyoshi[a], Masatoshi Murai[a]

[a] *Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

[b] *Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, United States,*

[c] *Institute for Protein Research, Osaka University, Suita 565-0871, Japan,* [d] *Faculty of Applied Biology, Kyoto Institute of Technology, Kyoto 606-8585, Japan.*

E-mail address:murai.masatoshi.5s@kyoto-u.ac.jp
Presenting author: Masatoshi Murai

Na^+-translocating NADH-ubiquinone oxidoreductase (Na^+-NQR) is a unique redox-driven Na^+pump. This enzyme is exclusively present in prokaryotes, including the human pathogens *Vibrio cholerae* and *Neisseria gonorrhoeae*, making it a promising target for highly selective antibiotics. Korormicin A, a natural product, and a specific and potent inhibitor of *V. cholerae* Na^+-NQR, may become a lead compound for the relevant drug design. Previous studies demonstrated that the G141A mutation in the NqrB subunit (NqrB-G141A) confers moderate resistance to korormicin A (about 100-fold). However, the efficiency of photoaffinity labeling of the mutant enzyme by a photoreactive korormicin derivative was the same as in the wild-type enzyme. Because of these apparently conflicting results, the molecular mechanism underlying the korormicin A-resistance remains elusive. In this study, we determined the cryo-EM structure of the *V. cholerae* NqrB-G141A mutant in the presence of bound korormicin A, and compared it to the corresponding structure from the wild-type enzyme. The toxophoric moiety of korormicin A binds to the mutant enzyme similarly to how it binds to the wild type. However, the added bulk of the alanine-141 excludes the alkyl side chain from the binding cavity, resulting in a decrease in the binding affinity. In fact, isothermal titration calorimetry revealed that the binding affinity of korormicin to the NqrB-G141A mutant is significantly weaker compared to the wild-type. These findings indicate that the inhibitory potency of korormicin A is reduced in the NqrB-G141A mutant due to decreased binding affinity within the altered binding cavity [1].

[1] M. Ishikawa-Fukuda, et al., Structural elucidation of the mechanism for inhibitor resistance in the Na^+-translocating NADH-ubiquinone oxidoreductase from *Vibrio cholerae*, Biochemistry 64 (2025) 1963-1972. https://doi.org/10.1021/acs.biochem.5c00069

P58

Dynamics and energetics of a tyrosine gate in respiratory complex I Jasmine E. Aaltonen[a], Luka Simsive[a] , Vivek Sharma[a,b]

[a]Department of Physics, University of Helsinki, Helsinki, Finland [b]HiLIFE Institute of Biotechnology, University of Helsinki, Helsinki, Finland

E-mail address: jasmine.aaltonen@helsinki.fi
Presenting author: Jasmine E. Aaltonen

While the role of respiratory complex I is well established, the mechanistic details around how the four protons are pumped across the membrane remain poorly understood. Among the many proposed pathways, the E channel is believed to be involved in proton pumping. Within this E channel are located several acidic residues, including a highly conserved tyrosine. In a previous study[1], we found that the tyrosine sidechain undergoes a large conformational change, which depended on the protonation states of the surrounding acidic residues. The rotation of tyrosine sidechain between gauche (+) and gauche (-) dihedrals was found to be critical for proton transfer in this region, suggesting the conserved tyrosine to have a gating function. In this study, we investigate the effect of local environment on the dynamics of tyrosine rotamerisation via free energy simulations. By systematically examining the energetic landscape of tyrosine dynamics within the narrow E channel, we aim to elucidate how the local environment modulates its conformational transitions. These findings will provide new insights into the coupling between conformational dynamics and proton pumping in the E channel region of complex I.

[1] Simsive, L.; Zdorevskyi, O.; Sharma, V. Proton Transfer through a Charged Conduit in Respiratory Complex I: Long-Range Effects and Conformational Gating. *J. Chem. Inf. Model.* 2025, *65* (19), 10600–10612. https://doi.org/10.1021/acs.jcim.5c01365.

P59

Zn^{2+} disrupts proton-translocation pathways in respiratory complex I

George-Valentin Datcu[a], Daniel N. Grba[a], Bozhidar S. Ivanov[a], Marcelo Mesa-Costa-Lima[a], Judy Hirst[a]

[a]Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom

E-mail address: gvd23@cam.ac.uk
Presenting author: George-Valentin Datcu

Respiratory complex I (CI) of bacterial and mitochondrial energy-transducing membranes exploits the exergonic reduction of quinone by NADH to drive the movement of four protons against the electrochemical gradient. Extensive hydrated networks of hydrogen-bonded polar residues intercalated with ordered water molecules define the proton pumping elements in the antiporter-like subunits and mediate the long-range energy transfer from the quinone-binding site.[1] Divalent transition metal cations have been suggested to inhibit CI by obstructing key residues in these proton-translocation pathways [2,3]. Here, we characterise the inhibition of isolated *Paracoccus denitrificans* CI by Zn^{2+} and determine a 2.2Å cryo-EM structure of the Zn^{2+}-bound state in the presence of NADH. In the membrane arm, we identify a Zn^{2+} ion inserted within a putative proton channel and further validate its contribution to disrupting catalysis through site-directed mutagenesis.

[1] Grba DN, Hirst J. Mitochondrial complex I structure reveals ordered water molecules for catalysis and proton translocation. Nat Struct Mol Biol. 2020 Oct;27(10):892-900. doi: 10.1038/s41594-020-0473-x

[2] Sharpley MS, Hirst J. The inhibition of mitochondrial complex I (NADH:ubiquinone oxidoreductase) by Zn2+. J Biol Chem. 2006 Nov 17;281(46):34803-9. doi: 10.1074/jbc.M607389200

[3] Schulte M, Mattay D, Kriegel S, Hellwig P, Friedrich T. Inhibition of Escherichia coli respiratory complex I by Zn(2+). Biochemistry. 2014 Oct 14;53(40):6332-9. doi: 10.1021/bi5009276

P60

Structural basis of complex I biogenesis

Neele Manik[a], Monalisa Sarkar[a,b], Janet Vonck[b], Volker Zickermann[a]

[a] *Structural Bioenergetics Group, Institute of Biochemistry II, Goethe University, Frankfurt am Main, Germany*
[b]*Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt am Main, Germany*

E-mail address: manik@med.uni-frankfurt.de
Presenting author: Neele Manik

The proton-pumping NADH:ubiquinone oxidoreductase (complex I) is a central component of the mitochondrial respiratory chain and plays an essential role in oxidative phosphorylation (OXPHOS). Its assembly is a highly coordinated, multi-step process involving more than 40 structural subunits and more than 20 assembly factors. Although many of these factors have been identified, their structures and molecular functions in complex I biogenesis are poorly understood.[1, 2]

In previous work, we focused on the assembly factor NDUFAF1 and its associated assembly intermediates to obtain structural insight into early stages of complex I biogenesis. Structural characterization of P_P-module intermediates showed that the assembly factors NDUFAF1 and CIA84, coordinate the early stages of P_P -module assembly by organizing central subunits into a core unit that serves as a starting point for the P_P-module assembly.[3]

Building on these findings, we now extend our investigation to additional assembly factors involved in later stages of the assembly pathway. We focus on factors associated with the P_P-module as well as those involved in the integration of the Q-module. By characterizing the resulting assembly intermediates and determining their structures using cryo-electron microscopy (cryo-EM), we aim to further elucidate the structural organization of these modules and the mechanisms that coordinate their stepwise integration during complex I biogenesis.

[1] Guerrero-Castillo S, Baertling F, Kownatzki D, et al. The Assembly Pathway of Mitochondrial Respiratory Chain Complex I. *Cell Metab*. 2017;25(1):128-139. doi:10.1016/j.cmet.2016.09.002

[2] J. Schiller, E. Laube, I. Wittig, W. Kühlbrandt, J. Vonck, and V. Zickermann, “Insights into complex I assembly: Function of NDUFAF1 and a link with cardiolipin remodeling,” 2022. doi:10.1126/sciadv.add3855

[3] Laube E, Schiller J, Zickermann V, Vonck J. Using cryo-EM to understand the assembly pathway of respiratory complex I. *Acta Crystallogr Sect Struct Biol*. 2024;80(3):159-173.
doi:10.1107/S205979832400086X

P61

Cryo-EM structure of a complex I assembly intermediate reveals the function of assembly factors

Jonathan Schiller[a], Alfredo Cabrera-Orefice[b], José G. Rosas Jiménez[c], Monalisa Sarkar[a,d], Katharina Friedrich[a], Gerhard Hummer[c], Volker Zickermann[a], Janet Vonck[d,e]

[a] *Institute of Biochemistry II, University Hospital, Goethe University, Frankfurt, Germany* [b] *Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University, Giessen, Germany* [c] *Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt, Germany* [d] *Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt, Germany* [e] *Central Electron Microscopy Facility, Max Planck Institute of Biophysics, Frankfurt, Germany*

E-mail address: janet.vonck@biophys.mpg.de
Presenting author: Janet Vonck

Complex I or NADH:ubiquinone oxidoreductase is the first protein complex of the mitochondrial respiratory chain with more than 44 subunits. Its assembly is a very intricate process involving at least 20 assembly factors. The structural basis of this process is mostly unknown[1].

We study complex I assembly in the yeast *Yarrowia lipolytica*[2,3]. We have determined a high-resolution cryo-EM structure of an assembly intermediate of the proton pumping module that contain two assembly factors in the intermembrane space, TPR50 and CMC1L, both essential for complex I biogenesis in *Y. lipolytica*. TPR50 appears to function as a chaperone for transmembrane helices of several subunits and for the C-terminus of subunit NDUFA5. CMC1L acts as a placeholder for subunit NDUFB5, preventing premature P_D module insertion. The assembly intermediate also contains the cardiolipin remodeling enzyme tafazzin, dysfunction of which in humans causes Barth syndrome, a severe multisystem disorder[4]. We identified a previously unknown acyl-CoA in the high-resolution structure, and based on structure predictions and MD simulations we propose a model for the transacylation mechanism of tafazzin[5].

[1] E. Laube, J. Schiller, V. Zickermann, J. Vonck, Using cryo-EM to understand the assembly pathway of respiratory complex I, Acta Crystallogr D Struct Biol 80 (2024) 159–173, https://doi.org/10.1107/S205979832400086X

[2] K. Parey, O. Haapanen, V. Sharma, H. Köfeler, T. Züllig, S. Prinz, K. Siegmund, I. Wittig, D. J. Mills, J. Vonck, W. Kühlbrandt, V. Zickermann, High-resolution cryo-EM structure of respiratory complex I: mechanism, assembly, and disease. Sci Adv 5 (2019) eaax9484, https://doi.org/10.1126/sciadv.aax9484.

[3] J. Schiller, E. Laube, I. Wittig, W. Kühlbrandt, J. Vonck, V. Zickermann, Insights into complex I assembly: Function of NDUFAF1 and a link with cardiolipin remodeling, Sci Adv 8 (2022) eadd3855, https://doi.org/10.1126/sciadv.add3855

[4] M. Schlame, Y. Xu, The function of tafazzin, a mitochondrial phospholipid- lysophospholipid acyltransferase, J Mol Biol 432 (2020) 5043–5051, https://doi.org/10.1016/j.jmb.2020.03.026

[5] J.G. Rosas Jiménez, J. Schiller, J. Vonck, G. Hummer, V. Zickermann, Coenzyme A is bound to tafazzin – a paradigm change for transacylation, bioRxiv (2026), https://doi.org/10.64898/2026.02.05.703992

P62

Hydration of proton pathways in *E. coli* complex I hampers energy conversion

Maximilian F. Weiß[a], Niklas Hoja[b], Caroline Harter[a], Ville R. I. Kaila[b], Thorsten Friedrich[a] [a]*Institute of Biochemistry, University of Freiburg, 79104 Freiburg, Germany* [b]*Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

E-mail address: maximilian.weiss@biochemie.uni-freiburg.de
Presenting author: Maximilian Weiß

NADH:ubiquinone oxidoreductase, respiratory complex I, is a key enzyme in cellular respiration, coupling electron transfer from NADH to ubiquinone (Q) with proton translocation across the membrane. In *Escherichia coli*, the enzyme is composed of 13 subunits, designated NuoA – NuoN[1]. On the basis of simulations and experiments, a proton-pumping mechanism [2] has been proposed in which an electric wave propagates along the membrane arm, thereby triggering proton uptake from the N-side and proton release to the P-side. In this framework, proton translocation proceeds through each antiporter-like subunit (NuoM,

NuoN and NuoL), as well as through the E-channel formed by NuoA, NuoH, NuoJ and NuoK.[3] Complementary structural analyses have given rise to the "NuoL-only" model, which postulates distinct "open" and "closed" conformational states driven by the quinone chemistry, with proton uptake from the Nside mediated by NuoL and NuoM and proton release to the P-side occurring exclusively *via* NuoL, owing to the absence of hydrated proton-conducting pathways in NuoM and NuoN.[4] Here, we introduced the mutations F408D in NuoM and F396D in NuoN which are homologous to the key residue D400 on NuoL to enhance the hydration of the proposed proton-conducting pathways. The catalytic activity of the variant in cytoplasmatic membranes as well as isolated show diminished electron transfer and proton translocation activity, while MD simulations demonstrate a rapid hydration of the proton outlet pathways. Together with ongoing structural analysis, our data suggest that a tightly regulated proton transport across NuoM and NuoN is central for establishing a coupled proton pumping machinery in Complex I.

[1] Hoeser, F. et al., Respiratory complex I with charge symmetry in the membrane arm pumps protons, Proc. Natl. Acad. Sci. 119 (2022).

[2] Kaila, V.R.I., Long-range proton-coupled electron transfer in biological energy conversion: towards mechanistic understanding of respiratory complex I, J. Roy. Soc. Interfaces 141 (2018)

[3] Kim, H. et al., Quinone Catalysis Modulates Proton Transfer Reactions in the Membrane Domain of Respiratory Complex I, J. Am. Chem. Soc. 145 (2023).

[4] Kravchuk, V. et al., A universal coupling mechanism of respiratory complex I, Nature 609 (2022).

P63

Thilo Seifermann[1], Jonas Hentschel[1], Anton Altmeyer[2,3], Zoé Kupek[1], Volker Zickermann[2,3], Thorsten Friedrich[1]

[1]Institute of Biochemistry, Albert-Ludwigs-Universität Freiburg, Germany

[2]Institute of Biochemistry II, University Hospital, Goethe Universität Frankfurt am Main, Germany.

[3]Centre for Biomolecular Magnetic Resonance, Institute of Biophysical Chemistry, Goethe Universität Frankfurt am Main, Germany. thilo.seifermann@bio.chemie.uni-freiburg.de

Mutation of highly conserved residues in MrpA-like subunit NuoL of *E. coli* respiratory complex I

The NADH:ubiquinone oxidoreductase (complex I) is the first enzyme of respiratory chains that oxidizes NADH and reduces quinone coupling this reaction with the translocation of protons across the membrane. The enzyme is L-shaped consisting of a membrane and peripheral arm. NADH is oxidized by FMN transferring two electrons to ubiquinone via several iron-sulfur clusters. Proton translocation takes place in the membrane arm [1]. The proton pumping subunit NuoL in the membrane arm is structurally similar to the multiple resistance and pH adaption (Mrp) cation/proton antiporter subunit MrpA [2]. A highly conserved histidine is positioned at the junction of the two half channels for proton translocation and the horizontal hydrophilic axis. This histidine shows protonation-dependent conformational changes in structural analysis and MD simulations [3,4]. In this work the histidine and nearby residues were mutated and the impact of the mutations on proton pumping and Q-reduction measured.

[1] Gnandt E, Dörner K, Strampraad MFJ, de Vries S, Friedrich T. The multitude of iron-sulfur clusters in respiratory complex I. Biochim Biophys Acta. 2016 Aug; 1857(8):1068-1072. doi: 10.1016/j.bbabio.2016.02.018. Epub 2016 Mar 2. PMID: 26944855.

[2] Yu H, Schut GJ, Haja DK, Adams MWW, Li H. Evolution of complex I-like respiratory complexes. J Biol Chem. 2021 Jan-Jun; 296:100740. doi: 10.1016/j.jbc.2021.100740. Epub 2021 May 3. PMID: 33957129; PMCID: PMC8165549.

[3] Djurabekova A, Haapanen O, Sharma V. Proton motive function of the terminal antiporterlike subunit in respiratory complex I. Biochim Biophys Acta Bioenerg. 2020 Jul
l;1861(7):148185. doi: 10.1016/j.bbabio.2020.148185. Epub 2020 Mar 19. PMID: 32171794.

[4] Lee Y, Haapanen O, Altmeyer A, Kühlbrandt W, Sharma V, Zickermann V. Ion transfer mechanisms in Mrp-type antiporters from high resolution cryoEM and molecular dynamics simulations. Nat Commun. 2022 Oct 14;13(1):6091. doi: 10.1038/s41467-022-33640-y. PMID:
36241630; PMCID: PMC9568556.

P64

Redox chemistry of the FMN-cofactor in respiratory complex I

Lars Vollmer, Daniel Wohlwend, Thorsten Friedrich
Institute of Biochemistry, University of Freiburg, 79104 Freiburg, Germany

E-mail address: lars.vollmer@bio.chemie.uni-freiburg.de
Presenting author: Lars Vollmer

NADH:ubiquinone oxidoreductase, respiratory complex I, is a key component in energy metabolism coupling NADH oxidation to proton translocation. Its dysfunction is associated with neurodegenerative diseases such as Parkinson's and Alzheimer's disease, most likely through an enhanced production of reactive oxygen species (ROS). The mechanism of ROS formation is not yet fully understood, but it is generally accepted that they are formed by the redox chemistry at the flavin mononucleotide (FMN) cofactor. It was proposed that FMN can reversibly dissociate from complex I, halting the transfer of electrons and thus decreasing ROS production. However, this is not supported by X-ray data, showing that the FMN is bound with full occupancy and low B-factors in both, the oxidized and reduced state of NuoEF, the electron input module of complex I, and experiences no change in binding by the oxidation state.[1] A preliminary cryo-electron microscopy structure of the dithionite reduced enzyme at a resolution of 2.2 Å supports the findings of the Xray data. First trials of a dataset with added NADH have yet not been successful for both X-ray and cryo-EM. Additionally, the redox potential of FMN in the presence of NADH was determined in complex I and NuoEF by electrochemical titration. Free FMN has a redox potential of -207 mV that is shifted to -330 mV in NuoEF of *Aquifex aeolicus* and to -350 mV in complex I of *Escherichia coli*.

[2] In the presence of NADH, the FMN redox potential is shifted to more positive values, leading to thermodynamically stronger binding of FMN than previously assumed. Furthermore, the role of Fe/ S cluster N1a was investigated. Mitochondrial complex I produces predominantly superoxide, while *E. coli* complex I forms H_2O_2. It was speculated that this difference arises from the more negative redox potential of the nearby Fe/S cluster N1a. Altering the redox potential of N1a by site-directed mutagenesis did not alter the produced ROS species.[3,4] However, it is shown that the redox potential of N1a influences that of FMN.

[1] M. Schulte *et al*., A mechanism to prevent production of reactive oxygen species by *Escherichia coli* respiratory complex I, Nat. Commun. 10 (2019) 2551.

[2] S.G. Mayhew, The effects of pH and semiquinone formation on the oxidation-reduction potentials of flavin mononucleotide. A reappraisal, Eur. J. Biochem. 265 (1999) 698-702.

[3] J.A. Birrell *et al*., Investigating the function of 2Fe-2S cluster N1a, the off-pathway cluster in complex I, by manipulating its reduction potential, Biochem. J. 456 (2013) 139–146.

[4] D. Wohlwend *et al.*, Structural changes shifting the redox potential of the outlying cluster N1a in respiratory complex I, Structure 34 (2026) 175-183.

P65

Deprotonation of a conserved histidine residue enables energy conversion in respiratory complex I

Jonas Hentschel[a], Tim Schlosser[a,b], Caroline Harter[a], Maximilian Weiß[a], Niklas Hoja[a,c], Thilo Seifermann[a], Lorenz Heidinger[a], Daniel Wohlwend[a], Thorsten Friedrich[a]

a Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Germany.

b Present address: Institut für Pharmazeutische Wissenschaften, Albert-Ludwigs-Universität Freiburg, Germany.

c Present address: Department of Biochemistry and Biophysics, Stockholm University, Sweden.

E-mail address: Jonas.hentschel@bio.chemie.uni-freiburg.de
Presenting author: Jonas Hentschel

Respiratory complex I is the largest and most intricate enzyme of the respiratory chains and serves as the primary entry point for electrons into cellular energy metabolism. The L-shaped complex consists of a peripheral arm, where electron transfer occurs, and a membrane arm responsible for proton translocation [1]. Complex I catalyzes the transfer of electrons from NADH via FMN and a series of iron-sulfur (Fe/S) clusters to ubiquinone (Q) in the peripheral arm. Electron transfer is linked to proton translocation by the membrane arm. The mechanism of long-range proton-coupled electron transfer (PCET) is still under debate [2,3]. To investigate the role of conserved residues involved in quinone chemistry, we analyzed variants of *Escherichia coli* complex I, in which key residues near the distal Fe/S cluster N2 in subunit NuoCD were replaced by non-protonatable residues. In particular, the conserved tyrosine $Y277^{CD}$ and histidine $H228^{CD}$ were mutated, together with a nearby conserved aspartate residue $D329^{CD}$. Functional consequences were assessed by enzymatic activity measurements and protontranslocation assays in reconstituted proteoliposomes. The structure of the variants was determined by cryo-electron microscopy, and the Fe/S clusters were analyzed by EPR spectroscopy. Notably, only mutation of $H228^{CD}$ abolished redox-driven proton translocation, whereas the $Y277^{CD}$ variants retained proton-pumping activity, albeit with reduced activity. Our results indicate that the conserved histidine $H228^{CD}$ plays an essential role in coupling quinone reduction to proton translocation. We propose that protonation of the reduced quinone by this histidine weakens its salt bridge with the conserved aspartate residue. This event likely initiates conformational changes in the membrane arm that propagate along the complex, consistent with the “electrical wave” model of proton translocation in respiratory complex I [4].

[1] Friedrich, T. On the mechanism of respiratory complex I. *J. Bioenerg. Biomembr.* 46, 255-268 (2014). doi: 10.1007/s10863-014-9566-8

[2] Kaila, V. R. I. Long-range proton-coupled electron transfer in biological energy conversion: towards mechanistic understanding of respiratory complex I. *J. R. Soc. Interface* 15, 20170916. doi: 10.1098/rsif.2017.0916

[3] Kravchuk, V., Petrova, O., Kampjut, D., Wojciechowska-Bason, A., Breese, Z. & Sazanov, L. A universal coupling mechanism of respiratory complex I. *Nature* 609, 808-814 (2022). doi: 10.1038/ s41586-022-05199-7

[4] Kim, H., Saura, P., Pöverlein, M. C., Gamiz-Hernandez, A. P. & Kaila, V. R. I. Quinone catalysis Modulates Proton Transfer Reactions in the Membrane Domain of Respiratory Complex I. *J. Am. Chem. Soc*. 145, 17075-17086 (2023). doi: 10.1021/jacs.3c03086

P66

Characterization of the Antiporter-Like Subunit Nqo14 of Respiratory Complex I

Sofia Bilancioni[1], Sofia Badolato[1], Ville R.I. Kaila[1]

[1]Department of Biochemistry and Biophysics, Stockholm University, 106 91, Stockholm, Sweden

Presenting author: sofia.bilancioni@dbb.su.se

Corresponding author: ville.kaila@dbb.su.se

The respiratory Complex I initiates the electron transport chain and couples it to proton pumping across the inner mitochondrial membrane, generating the proton motive force used to produce ATP. While the electron transfer mechanism is well-known, the exact molecular principles by which Complex I powers proton pumping across its membrane domain remains a topic of debate. We have proposed a long-range proton translocation mechanism, in which conformational and electrostatic changes drive proton pumping across antiporter-like subunits in the membrane domain [1]. In support of this model, the isolated Nqo13 and Nqo12 have been successfully characterized, confirming their role in proton translocation[2].Here in this project, we isolate and dissect Nqo14 (NuoN/ND2) subunit from the intact bacterial Complex I, and develop an optimized workflow for the characterization of its proton transport activity in proteoliposomes. We identify key conserved residues that modulate the proton transfer, and study their effects in both Nqo14 as well as the full Complex I. Our findings broaden our mechanistic understanding of the antiporter-like subunit of Complex I, their individual roles, as well as their collective function in the proposed long-range proton pumping mechanism.

(1) Ville R. I. Kaila; *Long-range proton-coupled electron transfer in biological energy conversion: towards mechanistic understanding of respiratory complex I. J R Soc Interface* 1 April 2018; 15 (141): 20170916. https://doi.org/10.1098/rsif.2017.0916

(2) Beghiah, A., Saura, P., Badolato, S., Kim, H., Zipf, J., Auman, D., Gamiz-Hernandez, A. P., Berg, J., Kemp, G. & Kaila, V. R. I. *Dissected antiporter modules establish minimal proton-conduction elements of the respiratory complex I. Nat. Commun.* 15, 9098 (2024).

P67

Characterizing redox-driven proton pumping of the bacterial Complex I in minirespiratory chains

Eric Mattiuz[a], Adel Beghiah[a], Ville R. I. Kaila[a]

[a] Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden

email address: eric.mattiuz@dbb.su.se, ville.kaila@dbb.su.se Presenting author: Eric Mattiuz

Complex I is the primary entry point of oxidative phosphorylation (OXPHOS) in mitochondria and in the plasma membrane of bacteria. It oxidizes NADH and reduces quinone in its hydrophilic domain, which drives proton-pumping via an elusive long-range coupling mechanism[1 2]. Despite great advances in both structural, biophysical, and computational-based analyses of Complex I, its overall coupling mechanism remains poorly understood. Over the years, different biophysical assays were developed to determine proton-pumping stoichiometries[3]. However, these methods often rely on proteins purified in the presence of detergents such as DDM, which can compromise the functional integrity of Complex I[4]. Here, we demonstrate how an LMNG-purified alternative oxidase (AOX) from *Trypanosoma brucei* can be used to accurately characterize proton pumping. Using established absorbance or fluorescent probes such as oxonol VI and ACMA, we assess the proton-coupled electron transfer activity of Complex I in the presence of AOX. Taken together, the established proteoliposome assays allow us to study Complex I and its mutants at high turnover conditions, to characterize the mechanism perturbation of precise point mutation.

1. Kaila, V. R. I. Long-range proton-coupled electron transfer in biological energy conversion: towards mechanistic understanding of respiratory complex I. *J R Soc Interface* 15, 20170916 (2018).
2. Beghiah, A. *et al.* Dissected antiporter modules establish minimal protonconduction elements of the respiratory complex I. *Nat Commun* 15, 9098 (2024).
3. Biner, O., Fedor, J. G., Yin, Z. & Hirst, J. Bottom-Up Construction of a Minimal System for Cellular Respiration and Energy Regeneration. *ACS Synth. Biol.* 9, 1450–1459 (2020).
4. Bridges, H. R. *et al.* Structural Basis of Mammalian Respiratory Complex I Inhibition by Medicinal Biguanides. *Science* 379, 351–357 (2023).

P68

Investigation of a kinetic gate controlling the proton-coupled electron transfer in Complex I

Mariona Martínez[a], Adel Beghiah[a], Niklas Hoja[a], Ville R. I. Kaila[a]

[a]Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden.

Email address: mariona.martinez@dbb.su.se, ville.kaila@dbb.su.se

Presenting author: Mariona Martínez

Complex I is the first enzyme of the respiratory chain that transduces energy by transferring electrons from NADH to ubiquinone, which triggers the pumping of four protons across the membrane domain of Complex I [1,2]. Yet, despite extensive studies, the coupling mechanism of Complex I is still unknown. Here, we combine highresolution cryo-EM structure determination, site-directed mutagenesis, biochemical and biophysical assays with multiscale simulations to gain insights into the role of functional sites that triggers proton pumping in Complex I. Based on our combined data, we identify residues that affect the π/α-transition of a conserved trans-membrane helix in NuoJ, and show how these residues control the conformational changes initiating the proton pumping process in Complex I [3]. Taken together, our integrative approach, reveal molecular principles underlying the π/α-transition as well as molecular details underlying the resting/activated states of Complex I during the initiation of the proton pumping process.

References

[1] Kaila, V. R. I. & Wikström, M. Architecture of bacterial respiratory chains. Nat Rev Microbiol 19, 319–330 (2021).

[2] Kaila, V. R. I. Long-range proton-coupled electron transfer in biological energy conversion: towards mechanistic understanding of respiratory complex I. J R Soc Interface 15, 20170916 (2018).

[3] Beghiah A. (in preparation).

P69

Mechanistic Insights into Conformational Switching Network of Complex I

Pauline van Beers[a], Niklas Hoja[a], Adel Beghiah[a], Ville R.I. Kaila[a]

[a]Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden.

Email address: paulinevanbeers@gmail.com, ville.kaila@dbb.su.se

Presenting Pauline van Beers

Complex I, or NADH:ubiquinone oxidoreductase, is the first complex in the respiratory chain where it participates in the generation of the proton motive force (PMF) across the membrane [1–4]. Complex I oxidizes NADH and reduces quinone in its hydrophilic domain, which couples to proton translocation across its membrane domain, up to 200 Å away from the site of quinone reduction. Quinol formation in the active site is believed to induce conformational changes within a switching network that propagate a signal towards the membrane domain to initiate proton pumping [5, 6]. Previous studies suggest that the TM5-6 loop of NuoH and TM3 of NuoJ play an important role in these conformational changes, although the precise mechanism coupling oxidoreductase activity to proton translocation remains unknown. Here, we combine site-directed mutagenesis and activity measurements with molecular dynamics simulations to investigate the coupling mechanism. We identify a unique ion pair network in the TM5–6 loop of NuoH that mediates the crosstalk between quinol-induced conformational changes, with mutations of the sites resulting in drastic perturbation of the redox-coupled pumping activity. Overall, our findings provide new insights into how the oxidoreductase activity triggers the proton pumping in Complex I.

[1] Kaila, V. R. I.; Wikström, M. Architecture of Bacterial Respiratory Chains. *Nat Rev Microbiol* 2021, *19* (5), 319–330.

[2] Mitchell, P. Coupling of Phosphorylation to Electron and Hydrogen Transfer by a ChemiOsmotic Type of Mechanism. *Nature* 1961, *191* (4784), 144–148.

[3] Wirth, C.; Brandt, U.; Hunte, C.; Zickermann, V. Structure and Function of Mitochondrial Complex I. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 2016, *1857* (7), 902–914.

[4] Kaila, V. R. I. Long-Range Proton-Coupled Electron Transfer in Biological Energy Conversion: Towards Mechanistic Understanding of Respiratory Complex I. *J. R. Soc. Interface.* 2018, *15* (141), 20170916.

[5] Fedor, J. G.; Jones, A. J. Y.; Di Luca, A.; Kaila, V. R. I.; Hirst, J. Correlating Kinetic and Structural Data on Ubiquinone Binding and Reduction by Respiratory Complex I. *Proc. Natl. Acad. Sci. U.S.A.* 2017, *114* (48), 12737–12742.

[6] Kim, H.; Saura, P.; Pöverlein, M. C.; Gamiz-Hernandez, A. P.; Kaila, V. R. I. Quinone Catalysis Modulates Proton Transfer Reactions in the Membrane Domain of Respiratory Complex I. *J. Am. Chem. Soc.* 2023, *145* (31), 17075–17086.

P70

Electron transfer energetics in yeast respiratory complex I

Ido Dan[a], Eleanor Clifford[a], Josh Lian[a], Ignacio Gispert Contamina[a], James W. Hindley[a,b], Oscar Ces[a], Maxie M. Roessler[a]

a *Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, White City Campus, London W12 0BZ, UK*

b *Department of Chemistry, King's College London, Britannia House, 7 Trinity Street, London SE1 1DB, UK*

E-mail address: i.dan24@imperial.ac.uk
Presenting author: Ido Dan

Respiratory complex I (R-CI, NADH:ubiquinone oxidoreductase), the first protein complex in the electron transport chain, catalyses two-electron transfer from NADH, through a chain of seven FeS clusters, to the electron carrier coenzyme Q_{10} in the inner mitochondrial membrane. Investigation of the electron transfer energetic profile is a key step towards understanding the redox catalysis reaction, and the coupling mechanism to proton pumping across the membrane.[1] In this work, the reduction potentials of four Fe-S clusters in a yeast R-CI were investigated using an established CW-EPR potentiometric titrations.[2] Our observed reduction potentials are positively shifted compared to reported values of other complex I species. This surprising result raises questions about how conserved the clusters' energy profile is across species and its effect on the catalytic activity, while also providing a step toward establishing a robust eukaryotic model for studying respiratory complex I.

[1] J. Hirst, M.M. Roessler, Energy conversion, redox catalysis and generation of reactive oxygen species by respiratory complex I, Biochimica et Biophysica Acta (BBA) - Bioenergetics 1857 (2016) 872–883. https://doi.org/https://doi.org/10.1016/j.bbabio.2015.12.009. [2] J.J. Wright, E. Salvadori, H.R. Bridges, J. Hirst, M.M. Roessler, Small-volume potentiometric titrations: EPR investigations of Fe-S cluster N2 in mitochondrial complex I, J. Inorg. Biochem. 162 (2016) 201–206. https://doi.org/https://doi.org/10.1016/j.jinorgbio.2016.04.025.

P71

Reconstitution of mammalian complex I into proteoliposomes for structural and functional studies in a native-like membrane environment

A. A. Kulikova[a], M. E. L. Catarinelli [b], L. A. Sazanov[a]

[a]Institute of Science and Technology Austria, Sazanov Group, Klosterneuburg, Austria [b]Division for Biomolecular and Cellular Medicine, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

E-mail address: akulikov@ist.ac.at
Presenting author: A. A. Kulikova

Respiratory complex I is a key enzyme of oxidative phosphorylation whose activity and conformational dynamics are strongly influenced by the membrane environment. However, studying the enzyme under physiologically relevant conditions remains challenging.

Here, we establish a workflow for reconstitution of ovine complex I into proteoliposomes, combining controlled lipid composition, biochemical characterization, and cryoEM sample preparation. The reconstituted enzyme retains full functional activity, remains well coupled, and is capable of proton pumping, making it suitable for structural analysis in a membrane setting.

Our preliminary cryo-EM analysis confirms the feasibility of visualizing complex I embedded in such liposomes under turnover conditions, providing a foundation for future high-resolution studies and investigation of its mechanism under near-native conditions and proton motive force.

P72

Toward Highly Efficient Artificial Respiratory Chains: Integrating Tunable Membranes and Complex I

Stefan T. Täuber[a], Christoph von Ballmoos[a]

[a]*University of Berne, Department of Chemistry, Biochemistry and Pharmaceutical Sciences*

E-mail address: stefan.taeuber@unibe.ch
Presenting author: Stefan T. Täuber

Artificial minimal respiratory chains are simplified, membrane-based systems that replicate key steps of biological electron transport. Built from selected redox enzymes and carriers and reconstituted in liposomes, they generate proton gradients that can power ATP synthesis, enabling controlled studies of energy transduction.

Membrane protein complexes are typically incorporated into vesicles via detergent-mediated reconstitution, a method that can lead to heterogeneous orientation of the inserted proteins, which in turn has a direct effect on functionality. Therefore, reconstitution conditions must be carefully tuned at the system level to control orientation. However, adjustments that favor one component can negatively affect others, which is why optimizing insertion conditions in a holistic manner pose reoccurring challenges.[1]

A conundrum which we faced revolved around the incorporation of the peripheral NADH:quinone oxidoreductase NDH-2 into our proteoliposomal systems, which requires negatively charged lipid environments for its activity. However, these same lipid conditions impair the correct orientation of the membrane-integrated bo_3 oxidase, which inserts into positively charged membranes in the favorable direction. We were able to resolve this conflict by implementing the strategy of incorporating ionizable lipids into our vesicles, which enables dynamic control over membrane surface charge through pH changes, allowing sequential optimization of enzyme orientation and activity within the same system.[2]

Our future efforts focus on systematically refining reconstitution profiles to balance enzyme orientation, activity, and compatibility across increasingly complex assemblies. A key objective of ours is to replace NDH-2 with respiratory Complex I. Unlike NDH-2, Complex I is an integral membrane enzyme that not only catalyzes NADH oxidation but also actively contributes to proton translocation, pumping four protons per NADH oxidized. Its incorporation would eliminate reliance on a peripheral enzyme while significantly enhancing coupling efficiency between electron transfer and proton motive force generation.

In parallel, we would like to explore how diverse lipid compositions, from charged and zwitterionic lipids to the already established tunable ionizable system, will be critical for optimizing both protein insertion and functional performance of our systems. We would also like to integrate alternative terminal oxidases with more predictable orientation dynamics, which may further improve system robustness and adaptability. Ultimately, the overarching goal of ours is to maximize ATP yield per NADH consumed by fine-tuning all components of the artificial respiratory chain. Achieving this will provide not only a highly efficient minimal bioenergetic platform but also deeper insight into the design principles governing biological energy conversion.

[1] Rigaud JL, Lévy D. Reconstitution of membrane proteins into liposomes. Methods Enzymol. 2003;372:65-86. doi: 10.1016/S0076-6879(03)72004-7. PMID: 14610807.

[2] Sabina Deutschmann, *et al.*, Modulating Liposome Surface Charge for Maximized ATP Regeneration in Synthetic Nanovesicles
ACS Synthetic Biology 2024 13 (12), 4061-4073
DOI: 10.1021/acssynbio.4c00487

P74

Paracoccus denitrificans complex I: A compete and fully integrated model platform for respiratory complex I

Bozhidar S. Ivanov, Robert A. Waddell, George-Valentin Datcu, Marcel Wittenberg, Harry Messent, Daniel N. Grba, John J. Wright & Judy Hirst

Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, UK

Email: bi226@cam.ac.uk

Respiratory complex I converts the free energy of NADH oxidation and ubiquinone reduction into vectorial proton translocation to support oxidative phosphorylation, yet the mechanism by which redox chemistry in the peripheral arm is coupled to long-range proton pumping across the membrane domain remains unresolved. This is because few model systems permit high-resolution structural analysis, quantitative functional measurements and targeted mutagenesis across the membrane arm, all within the same system. Here we present *Paracoccus denitrificans* complex I as an integrated experimental platform and describe how recent methodological advances have converged to make this system accessible for combined biochemical, biophysical and structural interrogation. These advances include streamlined purification and structural characterisation of intact and catalytically active enzyme [1], development of a fully genetically tractable strain [2], targeted membrane-domain mutagenesis [3], reconstitution into liposomes that retain proton-pumping activity, and reconstitution into nanodiscs compatible with both cryo-EM and steady-state functional analysis [1,4]. Mutagenesis in the antiporterlike subunits enabled quantitative analysis of catalytic activity, reversibility, and proton-pumping stoichiometry, showing that functionally impaired variants remain tightly coupled and providing mechanistic insight into conserved charged residues and proposed proton-uptake features [3]. In parallel, reconstitution of the enzyme into large nanodiscs yielded a high-resolution cryo-EM structure and demonstrated a membrane-mimetic system that accommodates Q_{10} and auxiliary redox partners for steady-state catalysis, thereby linking functional and structural analyses [4]. Together, these advances show that the *Paracoccus* model has evolved from a useful bacterial proxy into a fully integrated platform in which genetics, kinetics, proton-pumping assays, near-native membrane reconstitution and cryo-EM can be deployed on a mitochondrial-like enzyme that uses Q_{10} and retains key architectural and functional similarities to its eukaryotic counterpart [1-4]. This framework now enables integrated testing of mechanism from residue-level perturbation to turnover-compatible structural analysis and provides a robust foundation for defining how redox chemistry is coupled to long-range proton translocation.

[1] B.S. Ivanov, H.R. Bridges, O.D. Jarman, J. Hirst, Structure of the turnover-ready state of an ancestral respiratory complex I. Nat. Commun. 15, (2024), 9340. https://doi.org/10.1038/s41467-024-49340-0 [2] O.D. Jarman, O. Biner, J.J. Wright, J. Hirst, Paracoccus denitrificans: a genetically tractable model system for studying respiratory complex I. Sci. Rep. 11, (2021), 10143. https://doi.org/10.1038/s41598021-89641-4

[3] O.D. Jarman, J. Hirst, Membrane-domain mutations in respiratory complex I impede catalysis but do not uncouple proton pumping from ubiquinone reduction. PNAS Nexus 1(5), (2022), pgac276. https://doi.org/10.1093/pnasnexus/pgac276

[4] B.S. Ivanov, J. Hirst, Oversized nanodiscs for combined structural and functional investigation of multicomponent membrane protein systems. Sci. Rep. 15, (2025), 29070. https://doi.org/10.1038/s41598-025-29070-0

P75

Molecular Principles Underlying Quinone Binding and Proton-Coupled Electron Transfer in Complex I

Ana P. Gamiz-Hernandez[a], Ville R. I. Kaila[a]

[a]*Department of Biochemistry and Biophysics, Stockholm University, SE-10691 Stockholm, Sweden*

E-mail address:ana.gamiz-hernandez@dbb.su.se
Presenting author: Ana P. Gamiz-Hernandez

Quinone binding and its subsequent reduction to quinol establish the initial steps that activate the proton pumping in Complex I. However, the exact nature and protonation states of the residues participating in the catalysis remain debated. By combining free energy simulations and constant pH-molecular dynamics (MD) simulations of the primary quinone binding site from various organisms, we probe here conserved protonation patterns relevant for the catalysis. We identify how conserved ion-pairs undergo conformational changes upon quinone/quinol diffusion through the Q-tunnel that influence the proton affinity of nearby residues. We find that the Q binding is favored by protonation of conserved aspartate and histidine residues, whilst reduction of the quinone favors their deprotonation reaction. Our classical MD as well as hybrid quantum/classical (QM/MM) simulations further suggest that the formation of a hydrogen-bonded semiquinone intermediate stabilizes the negative charge and favors proton-coupled electron transfer (PCET) of a subsequent electron transfer from the FeS chain. QM/MM free energy sampling as well in silico mutagenesis of the identified residues allow us to understand the energetics of the $QH2/QH^-$ formation. Taken together, our findings provide thermodynamic and kinetic boundaries for understanding the redox-driven proton pumping in Complex I.

P76

Disease mutation perturbs long-range charge transfer reactions in the respiratory Complex I

Patricia Saura[a], Adel Beghiah[a], Terezia Kovalova[a], Franziska Höser[b], Thorsten Friedrich[b] and Ville R. I. Kaila[a]

[a]Department of Biochemistry and Biophysics, Stockholm University, 10691 Stockholm, Sweden [b] Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Germany

E-mail address: patricia.saura@dbb.su.se
Presenting author: Patricia Saura

Complex I is the most intricate and least understood protein complex of the respiratory chain. It initiates the mitochondrial electron transport chain by oxidizing NADH and reducing quinone, which couples to the translocation of protons across its membrane domain, more than 200 Å away from its catalytic site. Importantly, around half of all human mitochondrial disorders are related to mutations in Complex I, with several mutations occurring near functional sites. Despite decades of research, the molecular principles of its long-range redox-driven proton-transfer coupling mechanism remain poorly understood and highly debated. In this work, we use an integrative approach combining multiscale molecular simulations, biochemical and biophysical experiments, with structural studies to investigate the molecular basis of the charge transfer across the central E-channel region of Complex I. We find that mutation of a conserved central carboxylate, linked to Leigh's syndrome, results in a drastic reduction of oxidoreductase and proton translocation activities, consistent with a drastic increase in the proton transfer free energy barrier based on our quantum/classical (QM/MM) simulations, but without perturbing the structure of the region, as revealed by our high resolution cryo-EM structures. Our combined findings suggest that the identified carboxylate constitutes a switch point in the coupling region, and show how disease mutations perturb the long-range charge transfer mechanism.

[1] Beghiah A., Saura P., Kovalova T., Höser F., Friedrich T., Kaila V. R. I., A Carboxylate Switch Point Controls Long-Range Energy Transduction in Respiratory Complex I (in review). bioRxiv 2026.01.27.702182; doi: https://doi.org/10.64898/2026.01.27.702182

P77

An ancient carbon monoxide–driven respiratory system composed of a CODH nanowire and a basal complex-I

Anuj Kumar[a†], Florian P. Rosenbaum[b†], Sofia Appelgren[c], Georg Hochberg[c], Volker Müller[b*], Jan M. Schuller[a*]

[a]*Department of Chemistry, Philipps-University of Marburg, Marburg, Germany*

[b]*Department of Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University, Frankfurt am Main, Germany* [c]*Department of Biology, Philipps-University of Marburg, Marburg, Germany*

E-mail address: kumaran@staff.uni-marburg.de, vmueller@bio.uni-frankfurt.de, jan.schuller@synmikro.uni-marburg.de
Presenting author: Anuj Kumar

Carbon monoxide (CO) is a highly toxic atmospheric pollutant that disrupts aerobic life and anaerobic processes. Despite its toxicity, CO uniquely couples redox metabolism to carbon assimilation by functioning simultaneously as an electron donor and a carbon intermediate [1,2]. Carboxydotrophic acetogens oxidize CO through carbon monoxide dehydrogenase (CODH), generating CO_2 while reducing low-potential electron carriers (ferredoxins) that fuel cellular energy metabolism [3]. However, the respiratory systems that channel electrons from CO oxidation into membrane-bound energy conservation remain poorly understood. Here, we purified both components of a CO-driven electron transfer system from the acetogenic bacterium Moorella carbonis, revealing that monofunctional CODH co-purifies with a membrane-bound respiratory complex. Using anaerobic cryo-EM single-particle analysis, we found that CODH assembles into an extended filament. A 2.5 Å structure revealed that each repeating unit consists of a dimeric CODH associated with a poly-ferredoxin HycB subunit, whose C-terminal helices oligomerize to form a continuous iron–sulfur cluster backbone that organizes the catalytic units into an enzymatically decorated nanowire. In parallel, we determined the structure of the associated membrane-bound Complex I–type respiratory enzyme at 2.0 Å resolution, revealing a dimeric assembly. This enzyme displays a primitive architecture consisting of a minimal catalytic head that binds menaquinone-7 and connects to the membrane arm. The complex lacks accessory modules for NADH or F420 cofactor binding and instead couples ferredoxin oxidation to reduction of the menaquinone-7. In vitro assays confirmed that the CODH nanowire catalyzes CO oxidation and efficiently reduces bacterial ferredoxin. In situ cryo-electron tomography of intact cells revealed long CODH filaments positioned near the cytoplasmic membrane, appearing either membraneassociated or distributed within the cytoplasm, suggesting a spatial organization that facilitates electron transfer to membrane-bound complexes. Structural phylogenetic analysis of HycB proteins revealed that CODH-decorated nanowires are among the earliest enzyme-decorated nanowires to evolve. Likewise, evolutionary analysis suggests that the acetogenic respiratory complex represents an ancestral form out of which photosynthetic complex I oxidoreductases evolved. Together, these findings reveal a bioenergetic system in which an enzymatic nanowire links CO oxidation to energy conservation, pointing to an ancient mode of CO-driven respiration.

[1] O. Meyer, H. G. Schlegel, Biology of aerobic carbon monoxide-oxidizing bacteria. *Annu. Rev. Microbiol.* 37, 277–310 (1983). https://doi.org/10.1146/annurev.mi.37.100183.001425 [2] M. Diender, A. J. Stams, D. Z. Sousa, Pathways and bioenergetics of anaerobic carbon monoxide fermentation. *Front. Microbiol.* 6, 1275 (2015). https://doi.org/10.3389/fmicb.2015.01275 [3] A. Galani, D. Sipkema, D. Z. Sousa, Hot prospects: harnessing thermophilic microbes for syngas fermentation, *Trends Biotechnol.* 2025;0. doi: https://doi.org/10.1016/j.tibtech.2025.04.017

P78

Beyond assembly: ACAD9 stabilizes a novel extended conformation of mitochondrial complex I

Christian Buratto[a], Alessandro Grinzato[b], Marco Bortolus[a], Roberto Battistutta[a], Gabriele Giachin[a]

[a]Department of Chemical Sciences, University of Padova, Padova, Italy [b]Department of Biomedical Sciences, University of Padova, Padova, Italy

E-mail address: christian.buratto@phd.unipd.it
Presenting author: Christian Buratto

Complex I (CI) is the largest enzyme of the mitochondrial respiratory chain, coupling NADH oxidation to proton pumping across the inner mitochondrial membrane. The Acyl-Coenzyme A dehydrogenase family member 9 (ACAD9) is a dual-function mitochondrial protein involved in both long chain fatty acids β-oxidation (FAO) and CI assembly.[1,2] Although ACAD9 is essential for mitochondrial function and is implicated in CI deficiency, its structural and functional interplay with assembled CI remains poorly understood. Here we report a biophysical and structural characterization of the interaction between ACAD9 and the fully assembled bovine CI. SEC-SAXS, SEC-MALS and microscale thermophoresis demonstrated a stable low-micromolar interaction in solution consistent with a 1:1 stoichiometry between one ACAD9 dimer and CI. Cryo-EM analysis showed that bovine CI alone adopts the previously reported deactive conformation[3], whereas the structure obtained on the CI-ACAD9 co-eluted SEC fraction revealed a previously unreported extended state at 2.3 Å resolution. Upon ACAD9 binding, fully assembled CI undergoes membrane-arm elongation and expanded inter-subunit separations that propagate into the catalytic core, while retaining all canonical subunits and cofactors in the map; notably, the EPR signals of the redox cofactors remain unaltered. Crosslinking mass spectrometry identified ACAD9 contacts with matrix-exposed CI subunits in the membrane arm, supporting a defined interaction interface. These findings identify ACAD9 as a structural modulator of Complex I, revealing a regulatory role beyond complex biogenesis. By shifting fully assembled Complex I into a novel extended state, ACAD9 emerges as a potential determinant of Complex I conformational plasticity.

[1] Fabbian, S., Masciovecchio, B., Schievano, E. & Giachin, G. Hidden β-γ Dehydrogenation Products in Long-Chain Fatty Acid Oxidation Unveiled by NMR: Implications on Lipid Metabolism. ACS Bio Med Chem Au 5, 262–267 (2025).

[2] Nouws, J., Te Brinke, H., Nijtmans, L. G. & Houten, S. M. ACAD9, a complex I assembly factor with a moonlighting function in fatty acid oxidation deficiencies. Hum. Mol. Genet. 23, 1311–1319 (2014).

[3] Chung, I. et al. Cryo-EM structures define ubiquinone-10 binding to mitochondrial complex I and conformational transitions accompanying Q-site occupancy. Nat. Commun. 13, 2758 (2022).

P79

Biophysical characterization of engineered modules of respiratory Complex I Sofia Badolato[1], Hyunho Kim[1], Vladana Sušić[1], Tobias Fritz[1], Tiana Dusil[1], Niklas Hoja[1], Ville R.I. Kaila[1]

[1]Department of Biochemistry and Biophysics, Stockholm University, 106 91, Stockholm, Sweden

Presenting author: sofia.badolato@dbb.su.se
Corresponding author: ville.kaila@dbb.su.se

The respiratory Complex I (NADH:quinone oxidoreductase) is the first enzyme of the respiratory chain that plays a crucial role for energy transduction in the cell. Complex I transfers electrons from NADH to quinone, which is coupled to the pumping of four protons across the inner mitochondrial membrane, contributing to the generation of a proton motive force that powers the synthesis of ATP. Despite its fundamental role and its association to several mitochondrial diseases, the molecular mechanism of Complex I is still highly debated. To investigate the stoichiometry and the mechanism of the long-range proton pumping, we have used a bottom-up approach, by dissecting and decoupling the full complex into minimal proton conducting modules of the membrane domain. Using data-driven approaches combined with molecular simulations, we engineer a proton conducting antiporter-like dimer and probed the stoichiometry of a dissected complex. By combining biochemical activity assays and proton pumping measurements in proteoliposomes with cryo-electron microscopy and molecular dynamics simulations, we elucidate key mechanistic principles underlying the proton translocation and coupling efficiency of Complex I.

P80

From Simulations to Experiments: Relating Redox Chemistry to Proton Transfer in Complex I

Niklas L. Hoja[1], Jonas Hentschel[2,a], Hyunho Kim[1,a], Thilo Seifermann[2], Adel Beghiah[1], Tim Schlosser[3], Patricia Saura[1], Thorsten Friedrich[2,*], Ville R. I. Kaila[1,*]

[1] Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden.

[2] Institut für Biochemie, Albert-Ludwigs-Universität Freiburg, Germany

[3] Institut für Pharmazeutische Wissenschaften, Albert-Ludwigs-Universität Freiburg, Germany. [a] These authors contributed equally.

[*]Corresponding authors: Thorsten Friedrich, E-mail: thorsten.friedrich@bio.chemie.uni-freiburg.de and Ville R. I. Kaila, E-mail: ville.kaila@dbb.su.se

The generation of a proton motive force (PMF) from redox energy is a universal principle of respiratory energy conversion. Respiratory Complex I catalyzes the PMFgeneration through a remarkable long-range coupling mechanism that connects electron transfer with proton pumping across distances more than 200 Å. Yet, the molecular basis by which local quinone chemistry drives proton translocation at distant sites has remained elusive. Here, we addressed this question through a closely integrated combination of multiscale QM/MM simulations, site-directed mutagenesis, activity measurements in proteoliposomes, and high-resolution cryo-EM. These approaches complement one another: While simulations predict the pathways of proton transfer and the elements of conformational change, mutagenesis and functional assays allow for the verification of their mechanistic relevance, and cryo-EM experiments elucidate the structural context of the observed effectsOur combined analysis identifies Tyr156 in subunit NuoH as a key mechanical switch, involved in coupling conformational communication to proton pumping. However, mutations at this site disrupt conformational signaling, while largely preserving the proton transfer reaction itself. Together, our findings uncover critical coupling sites in the redoxdriven proton transport of Complex I and demonstrate the power of an interdisciplinary approach for resolving long-range energy transduction mechanisms in large membrane protein assemblies.

P81

Integrative investigations of the quinone-triggered proton translocation mechanism of *E. coli* Complex I

Adel Beghiah[a], Niklas Hoja[a], Mariona Martínez[a], Patricia Saura[a], Hyunho Kim[a], Ville R. I. Kaila[a]

[a] Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden

email address: adel.beghiah@dbb.su.se, ville.kaila@dbb.su.se Presenting author: Adel Beghiah

Complex I is the first enzyme involved in energy transduction of the respiratory chains, generating a proton motive force (pmf) that drives the synthesis of ATP1,2. Complex I catalyzes a NADH-driven reduction of quinone (Q), which triggers the translocation of four protons across the membrane through the antiporter-like subunits3, up to 200 Å away from the Q reduction site. Despite decades of work, the proton-coupled electron transfer (PCET) of Complex I is still poorly understood and highly debated4,5. Here, we combine site- directed mutagenesis, biochemical, and biophysical characterisation with high-resolution cryo-EM structure determination and multi-scale simulations to decipher key steps triggering the redox-driven proton pumping in Complex I. We identify a specific conserved switch point, which communicates redox signals from the Q site to the antiporter-like subunits.6,7 More broadly, our findings reveal mechanistic insights into the long-range PCET reaction of respiratory Complex I.Kaila, V. R. I.; Wikström, M. Architecture of bacterial respiratory chains. Nat. Rev. Microbiol. (2021), 19, 319−330.

1. Mitchell, P. Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of Mechanism. Nature (1961), 191, 144−148.
2. Beghiah, A., Saura, P., Badolato, S. *et al.* Dissected antiporter modules establish minimal protonconduction elements of the respiratory complex I. *Nat. Commun.* 15, 9098 (2024).
3. Kaila V. R. I. Accounts of Chemical Research (2021) 54 (24), 4462-4473.
4. Kravchuk, V. *et al*. "A universal coupling mechanism of respiratory complex I." *Nature* vol. 609,7928 (2022): 808-814.
5. Beghiah, A. *et al*. A Carboxylate Switch Point Controls Long-Range Energy Transduction in Respiratory Complex I. https://doi.org/10.64898/2026.01.27.702182 7. Beghiah, A. *et al*. In preparation.

P82

Modulation of F_1F_O-ATPase kinetics and mitochondrial respiration by the redox-active antioxidants N-acetylcysteine and sodium ascorbate

Cristina Algieri[a], Antonia Cugliari[b], Patrycja A. Glogowski[b], Fabiana Trombetti[b], Silvia Buscaroli[c], Micaela Fabbri[b], Ettore Federici[c], Salvatore Nesci[b]

aDepartment of Life Sciences, Health and Health Professions, Link University, Rome, Italy bDepartment of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy cMitochon srl, San Lazzaro di Savena (BO), Italy

E-mail address: salvatore.nesci@unibo.it Presenting author: Salvatore Nesci

Abstract.

Mitochondrial F_1F_O-ATPase is a key regulator of cardiac energy metabolism and redox balance, and its activity can be modulated by the mitochondrial redox environment [1]. The present study aimed to investigate the effects of two high antioxidant capacity biological molecules, N-acetylcysteine (NAC) and sodium ascorbate (vitamin C), on mitochondrial F_1F_O-ATPase activity and mitochondrial respiration in isolated swine heart mitochondria. The hydrolytic activity of the F_1F_O-ATPase was assessed in the presence of NAC and sodium ascorbate, either individually or in combination, with a specific focus on the incompetive activation induced by sodium ascorbate. Mitochondrial respiratory activity was also evaluated by measuring oxygen consumption supported by substrates feeding electrons into complex I and complex II of the electron transport chain. In parallel, the concentration of free thiol groups was measured as an indicator of mitochondrial redox status and to assess the ability of NAC and sodium ascorbate to preserve cysteine residues of mitochondrial proteins in a reduced state. Both antioxidants modulated F_1F_O-ATPase hydrolytic activity, with sodium ascorbate showing a distinct kinetic profile consistent with a progressive activation of the enzyme. NAC and sodium ascorbate also influenced mitochondrial respiration, with measurable effects of activatio or inhibition of complex I– or complex II–supported respiration, respectively. Furthermore, treatment with either compound increased the availability of free thiol groups, indicating an improved mitochondrial redox environment, while their combined administration exerted an enhanced protective effect compared to single treatments. In conclusion, these findings demonstrate that NAC and sodium ascorbate modulate F_1F_O-ATPase activity and respiratory function through redox-dependent mechanisms, supporting their potential role in preserving mitochondrial function in cardiac tissue under conditions associated with oxidative stress

[2].

[1] S.-B. Wang, C.I. Murray, H.S. Chung, J.E. Van Eyk, Redox Regulation of Mitochondrial ATP Synthase, Trends in Cardiovascular Medicine 23 (2013) 14–18. https://doi.org/10.1016/j.tcm.2012.08.005.

[2] C. Algieri, F. Oppedisano, F. Trombetti, M. Fabbri, E. Palma, S. Nesci, Selenite ameliorates the ATP hydrolysis of mitochondrial F1FO-ATPase by changing the redox state of thiol groups and impairs the ADP phosphorylation, Free Radic Biol Med 210 (2024) 333–343. https://doi.org/10.1016/j.freeradbiomed.2023.11.041.

P83

Structural analysis of ATP synthase embedded in lipid bilayer vesicles

Ken Yokoyama
Faculty of Life Sciences, Kyoto Sangyo University

E-mail address: yokoken@cc.kyoto-su.ac.jp
Presenting author: Ken Yokoyama

Most membrane proteins function by exploiting membrane potentials and ion concentration gradients generated across lipid bilayers. Therefore, to obtain structures that faithfully represent functional states, membrane proteins should be analyzed while embedded in native membranes or membrane-mimetic lipid bilayer vesicles (liposomes).
Here, we reconstituted a V/A-type ATP synthase together with bacteriorhodopsin into liposomes and captured the ATP-synthesizing state driven by a light-induced proton motive force (pmf) using cryo-electron microscopy single-particle analysis. Under pmf-loaded conditions, we confirmed the presence of synthesized ATP at the catalytic sites of the V1 domain. In addition, a torsional distortion was observed between the rotor and stator components. This observation suggests that the rotational torque generated by proton translocation is transiently stored as elastic twisting within the complex before being utilized for ATP synthesis.
We also determined the structure of mitochondrial ATP synthase directly within the inner mitochondrial membrane without detergent solubilization. The structures revealed ATP synthase dimers connected by the inhibitory factor IF1, as well as tetrameric assemblies formed by the faceto-face association of two dimers. The curvature of these assemblies closely matched the curvature observed at the tips of cristae membranes. Furthermore, we constructed a full-length main-chain model of the Fo subunit e. Notably, no previously reported density was observed within the c-ring, indicating that the c-ring is largely hollow and does not contain immobilized lipid molecules.
Together, these results demonstrate that structural analysis of ATP synthase in lipid membrane environments provides critical insights into functional conformations and supramolecular assemblies that cannot be obtained from analyses of solubilized protein complexes.

Contribution type:P

P84

pH determination at mitochondrial ATP synthase subunit e via local pH sensors Greta Bussmann[a], Changjiang You[b], Abigail Robson[c], Nicholas Tomkinson[c], Karin Busch[a]

[a]*Institute of Integrative Cell Biology and Physiology, Department of Biology, University of Münster, Münster, Germany*
[b]*Division of Biophysics, Department of Biology/Chemistry, University of Osnabrück, Osnabrück, Germany*
[c] *Group of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom*

E-mail address: g_buss03@uni-muenster.de
Presenting author: Greta Bussmann

Mitochondrial adenosine triphosphate (ATP) synthesis relies on a finely tuned interplay between the electron transport chain, the proton motive force (pmf) and the F_1F_O-ATP synthase. During oxidative phosphorylation, ATP synthesis is promoted by the pmf generated across the inner mitochondrial membrane. The local availability of protons at the p-side of the inner mitochondrial membrane in the intracristal space at the cristae rims, where most of the ATP synthase is located, shapes the activity of the enzyme [1]. Previously, our group has demonstrated – using the pH-sensitive fluorescent protein sEcGFP - that proton distribution across and within cristae is heterogeneous, challenging the classical view of homogeneous bulk phases in the matrix and intermembrane space [2].

Here, we tested the feasibility of two red-emitting pH-sensors, pH-mScarlet ($\lambda_{Excitation}$: 514 nm and 561 nm; $\lambda_{Emission}$: 610 nm) and SNARF1 ($\lambda_{Excitation}$: 534 nm; $\lambda_{Emission}$: 580 nm and 640 nm), for mitochondrial pH-measurements. The emission spectra of these sensors are distinct from green fluorescent sEcGFP and would therefore allow simultaneous pH-measurements across the inner mitochondrial membrane. To deploy pH-mScarlet and SNARF1 for local pH-determination, we attached them to the ATP synthase subunit e in a mammalian cancer cell line (HeLa). SNARF1 was conjugated to the HaloTag-Ligand (SNARF-1-HTL), which is a substrate for HaloTag. This was genetically fused to subunit e.

Using confocal microscopy, plate reader assays, *in situ* calibration, metabolic substrate switching and inhibitors of the oxidative phosphorylation, the sensitivity and dynamic range of the pH sensors was assessed under various conditions. The (ratio-metric) calibration using different pH values enabled us to convert fluorescence intensity ratios into pH values. Next, we recorded pH changes induced by metabolic modulation and inhibition of the OXPHOS system. We found that dye concentration and the time dependency of metabolic adaption are critical parameters for reliable pH determination. If these parameters are appropriately addressed, SNARF-1-HTL should be a suitable sensor for quantitative mitochondrial pH measurements.

In contrast, pH-mScarlet was unreliable for pH-quantification, due to its poor dynamic response in the alkaline range and limited sensitivity to subtle pH changes. Consequently, pH-mScarlet is better suited for exploratory, relative assessments than for quantitative pH measurements.

[1] Strauss, M., Hofhaus, G., Schröder R.R., Kühlbrandt W.. Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. EMBO J. 2008 Apr 9; 27(7): 1154-60. 2008. doi: 10.1038/emboj.2008.35

[2] Rieger, B., Junge W., Busch K.B. Lateral pH gradient between OXPHOS complex IV and F_1F_O ATP-synthase in folded mitochondrial membranes. Nature Communication 5, 3103, 2014. doi: 10.1038/ncomms4103 P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, Nature 191(4784) (1961) 144–148, https://doi.org/10.1038/191144a0

P85

The *Mycobacterium abscessus* F-ATP synthase and its druggability

Tuck Choy. Fong[a], Wuan-Geok. Saw[b], Vikneswaran. Mathiyazakan[a], Chui Fann. Wong[a], Gerhard. Grüber[a]

[a]*School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore*
[b]*NTU Institute of Structural Biology, Nanyang Technological University, Singapore 636921, Singapore*

E-mail address: tuckchoy001@e.ntu.edu.sg
Presenting author: Tuck Choy. Fong

Increasing global incidence rate of nontuberculous mycobacteria (NTM) pulmonary infections is an emerging public health crisis, with *Mycobacterium abscessus* (*Mab*) being one of the most virulent and treatment-refractory of these pathogens. *Mab* exhibits extensive intrinsic and acquired drug resistance mechanisms that neutralize most antimicrobials targeting it, causing a clinical conundrum. Dependence on the oxidation phosphorylation (OXPHOS) pathway for growth and survival in this strictly aerobic opportunistic pathogen, establishes an attractive target for treatment. The F-ATP synthase that forms the core of the OXPHOS pathway to generate the ubiquitous adenosine triphosphate (ATP), is essential for virtually all biochemical processes in the cell. We performed genomic tag knock-in to enable selective purification and subsequently, single particle analysis via cryo-EM to construct the model of the F_1 and F_O-domains. Our high-resolution structures provided a blueprint for *in-silico* studies, mapping out essential epitopes in regulating ATP biosynthesis and its respective binding pockets. Notably, the atypical *Mab* subunit δ-extension was structurally observed to potentially play a role in conducting torsional stress, maintaining the structural integrity of the hexamer head of F_1-domain[1]. Finally, the structural insights enable medicinal chemistry efforts to develop advanced anti-NTM lead compounds.

[1] Fong, T. C., Saw, W.-G., Mathiyazakan, V., Wong, C. F., & Grüber, G. (2026). The Mycobacterium abscessus F-ATP synthase structure reveals mechanistic elements enabling rational drug design to combat NTM lung disease. *Structure*, *34*(3), 508-520.e3.
https://doi.org/10.1016/j.str.2025.12.005

P86

The C-terminus of subunit e is essential to switch the ATP synthase into the mitochondrial permeability transition pore

Federica Boscolo Nata[a], Paolo Bernardi[a,b] and Michela Carraro[a]

[a]Department of Biomedical Sciences, University of Padova, Padova, Italy [b]Department of Molecular Pathobiology, New York University, New York, USA

E-mail address: federica.boscolonata@phd.unipd.it
Presenting author: Federica Boscolo Nata

The mitochondrial permeability transition (PT) refers to a sudden increase in the inner mitochondrial membrane permeability caused by the opening of the PT pore (PTP), a Ca^{2+}-activated, high-conductance channel. Identifying the molecular identity of the PTP has remained a central goal in mitochondrial biology due to its roles in Ca^{2+} homeostasis and cell death. Nowadays, the mitochondrial ATP synthase is considered the *bona fide* PTP. However, in absence of the ATP synthase subunits e and g, that were shown to be crucial for channel formation, an alternative permeability pathway mediated by the adenine nucleotide translocator (ANT) might emerge [1].

Despite the growing understanding of the PT, the mechanism of channel formation by the ATP synthase remains elusive. A recent hypothesis suggests that a channel forms within the c-ring of the ATP synthase after the removal of the lipid plug filling the rotor. This theory, called the "death finger" model, is supported by the high-resolution structures of the ATP synthase which revealed a physical connection between subunit e and lysolipids filling the c-ring [2]. According to this model, Ca^{2+} binding to the F_1 domain would induce a conformational change that is transmitted along the peripheral stalk to subunit e which leads to pore formation by pulling out lipids from the rotor.

To test this hypothesis, we combined site-directed mutagenesis with functional analyses of mitochondrial properties in HeLa cells to disentangle ATP synthase catalysis from its channel activity. Using this genetic framework, we show that truncation of the C-terminus of ATP synthase subunit e or the point mutation of its terminal lysin (eK69A), preserves enzyme function while profoundly altering PTP behavior. Our results identify this region as a critical determinant of Ca^{2+}-induced pore opening and reveal a previously masked contribution of the ANT, highlighting the molecular complexity underlying PTP formation.

[1] A. Carrer, L. Tommasin, J. Šileikytė, F. Ciscato, R. Filadi, A. Urbani, M. Forte, A. Rasola, I. Szabò, M. Carraro, P. Bernardi, Defining the molecular mechanisms of the mitochondrial permeability transition through genetic manipulation of F-ATP synthase, Nature Communications. 12 (2021) 4835–4835. [2] G. Pinke, L. Zhou, L.A. Sazanov, Cryo-EM structure of the entire mammalian F-type ATP synthase, Nature Structural and Molecular Biology. 27 (2020) 1077–1085.

P87

Cryo-EM structure of the chloroplast ATP synthase from *Chlamydomonas reinhardtii*

Katarzyna Lorencik[1], Sebastian Pintscher[2], Katherine H. Richardson[1], Matthew S. Proctor[1], Philip J. Jackson[1], Michał Rawski[3], James N. Blaza[4], Andrew Hitchcock[1], C. Neil Hunter[1] and Matthew P. Johnson[1]

[1]*Plants, Photosynthesis & Soil, School of Biosciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom.*
[2]*Faculty of Biochemistry, Biophysics and Biotechnology, Department of Plant Biotechnology, Jagiellonian University, Kraków, Poland*
[3]*National Synchrotron Radiation Centre SOLARIS, Jagiellonian University, Kraków, Poland*
[4]*York Structural Biology Laboratory, Department of Chemistry, University of York, United Kingdom*

E-mail address: k.lorencik@sheffield.ac.uk
Presenting author: Katarzyna Lorencik

The chloroplast ATP synthase (CF_1F_o) is a multisubunit molecular machine that catalyses ATP synthesis coupled to transmembrane proton transport. Within the chloroplast, this rotary enzyme is uniquely adapted to synchronize ATP production with light availability.[1, 2, 3] The green alga *Chlamydomonas reinhardtii* offers a simplified yet representative system to study the assembly and regulation of the photosynthetic apparatus. However, our structural understanding of the algal ATP synthase has lagged behind that of land plants.[4] Here we report the 2.2 Å resolution cryo-EM structure of the CF_1F_o complex from the model green alga *C. reinhardtii*, revealing the 13-subunit c-ring and providing a complete structural framework for its unique redox-regulated energy transduction. Interfaces between subunits a and c form two half-channels for proton translocation, supporting a Grotthuss-like mechanism. Resolved rotational states, each with distinct nucleotide occupancies, reveal how nucleotide binding and release are coordinated with rotary catalysis, offering mechanistic insight into molecular energy conversion. These findings advance our understanding of light-driven energy conversion in photosynthetic eukaryotes while providing a framework for engineering these processes in algae and plants.

[1] H. Konno, T. Nakane, M. Yoshida, H. Ueoka-Nakanishi, S. Hara, T. Hisabori, Thiol Modulation of the Chloroplast ATP Synthase is Dependent on the Energization of Thylakoid Membranes, *Plant Cell Physiol*. 53(4), (2012), 626–634. https://doi.org/10.1093/pcp/pcs018.

[2] L. Lebok, F. Buchert, The chloroplast ATP synthase redox domain in *Chlamydomonas reinhardtii* eludes activity regulation for heterotrophic dark metabolism, *PNAS* 121 (46) e2412589121, (2024), https://doi.org/10.1073/pnas.2412589121.

[3] K. Akiyama, SI. Ozawa, Y. Takahashi, K. Yoshida , T. Suzuki, K. Kondo, K-i. Wakabayashi, T. Hisabor, Two specific domains of the γ subunit of chloroplast FoF1 provide redox regulation of the ATP synthesis through conformational changes, *PNAS* 120 (6) e2218187120, (2023), https://doi.org/10.1073/pnas.2218187120.

[4] A. Hahn, J. Vonck, DJ. Mills, T. Meier, W. Kühlbrandt, Structure, mechanism, and regulation of the chloroplast ATP synthase, *Science* 360, eaat4318, (2018), http://dx.doi.org/10.1126/science.aat4318.

P88

Rewiring of Mitochondrial ATP Synthesis Upon Synaptic Stimulation

Othman Abu Shelbayeh[a], Nico Marx[a], Bledi Petriti[b], Elizabeth Jonas[b†], Karin Busch[a†]

[a]*Institute of Integrative Cell Biology and Physiology, Department of Biology, University of Münster, Münster, Germany.*
[b]*Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, USA.*
[†]*These authors contributed equally as senior authors.*

E-mail address: othman.abushelbayeh@uni-muenster.de
Presenting author: Othman Abu Shelbayeh

Long-term potentiation (LTP) involves stimulation of neurons and enlargement of specific synapses, which is a fundamental process underlying early memory formation. The underlying process of protein biosynthesis is known to be highly energy-consuming, underscoring the critical importance of optimized energy conversion. The proton motive force (PMF) across the inner membrane (IM) serves as the driving force for ATP synthesis. The PMF is generated by the primary proton pumps of the electron transport chain (ETC), also known as respiratory chain. The IM does not exhibit complete tightness for ions, but rather demonstrates permeability including a large ion channel within the ATP synthase c-ring [1]. While the proton pumps of the ETC are located on the cristae sheets, the ATP synthase is located on the cristae tips along the rims. The cristae ultrastructure and the enzymatic complexes located within it, are by no means rigid entities. Rather, they exhibit an optimum of altered mobility and dynamics depending on the energetic state of the cell [2]. We hypothesized that an increase in metabolic efficiency, which supplies ATP for LTP, is based on PMF optimization. We hereby propose two mechanisms: (a) a decrease of the leakiness of the IM for ions, e.g. through closure of the ATP synthase linked ion channel, and (b) structural cristae changes that optimize OXPHOS coupling. To test this hypothesis, we used advanced techniques, including STED microscopy for cristae imaging, tracking and localization microscopy (TALM) for the spatiotemporal localization of the ATP synthase, and fluorescence lifetime imaging microscopy (FLIM) to determine the inner membrane tension after LTP stimulation in neurons. We found changes in the cristae ultrastructure, ATP synthase mobility, and inner membrane compression in the dendritic mitochondria following LTP stimulation. We conclude that these alterations are crucial for meeting the increased energy demands of the stimulated neurons.

[1] Mnatsakanyan, N., Park, HA., Wu, J. et al. Mitochondrial ATP synthase c-subunit leak channel triggers cell death upon loss of its F1 subcomplex. Cell Death Differ 29, 1874–1887 (2022). https://doi.org/10.1038/s41418-022-00972-7

[2] Rieger, B., Arroum, T., Borowski, MT. et al. Mitochondrial F1FO ATP synthase determines the local proton motive force at cristae rims. EMBO Rep 22, EMBR202152727 (2021). https://doi.org/10.15252/embr.202152727

P89

Small Subunit, Big Impact: DAPIT's Role in ATP Synthase

Marie-Theres Borowski, Pia-Marie Ott, Karin Busch

Institute of Integrative Cell Biology and Physiology, Faculty of Biology, University of Muenster, 48149 Muenster, North-Rhine-Westphalia, Germany

E-mail address: m_boro05@uni-muenster.de
Presenting author: Marie-Theres Borowski

Mitochondrial ATP synthase uses the proton motive force to synthesize ATP during oxidative phosphorylation. The reaction is reversible, allowing ATP hydrolysis and proton pumping across the inner mitochondrial membrane (IMM). In mammals, ATP synthase dimers are localized at cristae edges, forming arrays that shape cristae membrane architecture. [1]. Several small peptides influence the supramolecular structure of ATP synthase. For example, inhibitory factor 1 (IF1) controls the activity and organization of ATP synthase by blocking reverse rotation and preventing ATP hydrolysis. IF1 overexpression alters IMM ultrastructure and affects the spatiotemporal organization of ATP synthase, suggesting a link between enzyme activity and membrane architecture [2]. While IF1 modulates enzyme activity and oligomerization [3], the DAPIT subunit (diabetes-associated protein in insulin-sensitive tissues) likely only stabilizes dimerdimer interactions [4]. However, the role of DAPIT subunit and its regulatory mechanisms remain poorly understood. Here, we investigated the effects of DAPIT on ATP synthase organization, function and cellular physiology. We found that DAPIT overexpression stabilized ATP synthase levels and promoted ATP synthesis under glycolytic conditions despite reduced mitochondrial mass. The increased amount of ATP synthase resulted from delayed ATP synthase turnover and led to indirect cellular effects like increased cell proliferation and altered morphology. In contrast, DAPIT knockout reduced ATP synthase oligomerization status, limiting mitochondrial respiration and cell proliferation. DAPIT is therefore essential for maintaining ATP synthase oligomers and optimizing energy metabolism.

[1] T. B. Blum, A. Hahn, T. Meier, K. M. Davies, W. Kuhlbrandt, Dimers of mitochondrial ATP synthase induce membrane curvature and self-assemble into rows, PNAS, 116 (2019) 4250-4255. [2] B. Rieger, T. Arroum, M. T. Borowski, J. Villalta, K. B. Busch, Mitochondrial F1 FO ATP synthase determines the local proton motive force at cristae rims, EMBO Rep, 22 (2021) e52727. [3] J. Gu, L. Zhang, S. Zong, R. Guo, T. Liu, J. Yi, P. Wang, W. Zhuo, M. Yang, Cryo-EM structure of the mammalian ATP synthase tetramer bound with inhibitory protein IF1, Science, 364 (2019) 1068-1075.
[4] J. He, H. C. Ford, J. Carroll, C. Douglas, E. Gonzales, S. Ding, I. M. Fearnley, J. E. Walker, Assembly of the membrane domain of ATP synthase in human mitochondria, PNAS, 115 (2018) 2988-2993.

P90

Functional analysis of novel wing domain subunits in the Toxoplasma gondii ATP synthase complex

Monika Singh[a], Mariana F. Silva[a], Alexander Mühleip[b] and Lilach Sheiner[a]

[a] Institute of Infection, Immunity and Inflammation, University of Glasgow, UK [b] University of Helsinki, Finland

E-mail address: Monika.Singh@glasgow.ac.uk

Presenting author: Monika Singh

ATP synthase, a membrane bound multi-subunit enzyme is an integral part of the eukaryotic mitochondrial oxidative phosphorylation [1]. The general architecture and subunit composition of ATP synthase is well studied in common model organisms, and the catalytic mechanism is considered conserved across eukaryotes [1], but little is known about this critical complex in *Apicomplexa*. Using cryo-electron microscopy, we have characterized the *Toxoplasma gondii* ATP synthase complex, which reveals its new structural features [2]. *T. gondii* ATP synthase integrates 17 new subunits not seen in other systems and conserved across Myzozoa [2]. Some are involved in forming a unique ATP synthase hexamer, which defines the apicomplexan bulbous cristae [2], but the role of others is largely unknown. Curiously, three new subunits, ATPTG1, ATPTG4 and ATPTG14, form a distinct peripheral matrix-exposed "wing" region. We applied conditional gene knockdown approach to investigate their function. Our results show that these subunits are essential for tachyzoite parasite survival even in culture, yet the different mutants exhibit distinct phenotypic outcomes: ATPTG1 and ATPTG14 depletion results in immediate arrest in the lytic cycle, while ATPTG4 depletion allows some replication before parasite fails to grow. Additionally, depletion of these ATPTGs disturbs ATP synthase complex stability, suggesting that these subunits have similar roles together with the joint formation of the wing. In conclusion, our study aims to provide new insights into unique features of *T. gondii* ATP synthase, enhancing our understanding of its role in parasite's mitochondrial biology and probable targets for therapeutic intervention.

[1] Kühlbrandt, W. Structure and mechanisms of F-Type ATP synthases. *Annual Review of Biochemistry*, *88*(1), (2019), 515–549. https://doi.org/10.1146/annurev-biochem-013118-110903

[2] A. Mühleip, R. K. Flygaard, J. Ovciarikova, A. Lacombe, P. Fernandes, L. Sheiner, & A. Amunts, ATP synthase hexamer assemblies shape cristae of Toxoplasma mitochondria. *Nature Communications*. *12*(1), (2021), 120. https://doi.org/10.1038/s41467-020-20381-z

P91

The structural insights for the rotary mechanism of prokaryotic ATP synthase driven by proton motive force.

Jun-ichi Kishikawa[a,b], Atsuki Nakano[b,c], Ken Yokoyama[b]

[a]*Fac. Appl. Biol., Kyoto Inst. Tech.* [b]*Dept. Adv. Life. Sci., Kyoto Sangyo Univ.*
[c]*Dept. Appl. Chem., Univ. Tokyo*

E-mail address: kishijun@kit.ac.jp
Presenting author: Jun-ichi Kishikawa

ATP synthases are rotary molecular machines essential for energy production, utilizing the proton motive force (pmf) across the membrane to drive the rotation of a membrane-embedded rotor ring relative to a stator, which is coupled to ATP synthesis in the hydrophilic catalytic domain. Despite their importance, the precise structural mechanisms by which the pmf is converted into mechanical rotation and how that rotation coordinates with catalytic sites have remained elusive. To address this, Cryo-electron microscopy (cryo-EM) was used to analyze the V/A-ATPase from *Thermus thermophilus* in both isolated and lipid-reconstituted environments under active conditions. The studies provided two primary sets of results regarding the membrane and catalytic domains. First, a 2.8 Å resolution structure of the V_O domain revealed the precise orientations of glutamate (Glu) residues within the c_{12}-ring [1]. Three Glu residues face a water channel, with one forming a critical salt bridge with a conserved arginine in the stator. Molecular dynamics simulations demonstrated that the protonation of specific Glu residues triggers unidirectional Brownian motion of the c_{12}-ring; conversely, when a key Glu remains unprotonated, the salt bridge persists and acts as a physical block to rotation. Second, structural analysis of the enzyme reconstituted into liposomes under an applied pmf captured the complex in an active state, with ATP molecules bound at two of the three catalytic sites [2]. This revealed an induced-fit mechanism where the catalytic site closes upon binding ADP and P*i*. By capturing multiple snapshots of the rotor ring at different positions, the study identified that a 31° rotation of the ring induces significant torsion in both the central rotor and the peripheral stator. In conclusion, these findings demonstrate that the rotary catalysis of ATP synthesis is driven by an asymmetric protonation of c_{12}-ring Glu residues that biases Brownian motion, while the resulting mechanical energy is managed through structural torsion and inducedfit conformational changes within the catalytic subunits.

[1] J. Kishikawa, Y. Nishida, A. Nakano, T. Kato, K. Mitsuoka, K. Okazaki, K. Yokoyama, Rotary mechanism of the prokaryotic Vo motor driven by proton motive force. Nat. Commun. 15, 9883 (2024) https://doi.org/10.1038/s41467-024-53504-x

[2] A. Nakano, J. Kishikawa, Y. Nishida, K. Sugawara, Y. Kan, C. Gerle, H. Shigematsu, K. Mitsuoka, K. Yokoyama, Structures of rotary ATP synthase from Thermus thermophilus during proton powered ATP synthesis. Sci. Adv. 11(42), eadx8771 (2025) https://doi.org/10.1126/sciadv.adx8771

P92

Running in Reverse: Does ATP Synthase lose its Oligomeric Organization?

Nico Marx[a], Rebeca Acín-Pérez[b], Marko Kostic[b], Alex Napior[b], Orian Shirihai[b], Karin B. Busch[a] [a]*Institute of Integrative Cell Biology and Physiology, Faculty of Biology, University of Muenster, Schlossplatz 5, 48149 Muenster, North-Rhine-Westphalia, Germany*

[b]*Division of Endocrinology, Department of Medicine, David Geffen School of Medicine, UCLA, 10833 Le Conte Ave, Los Angeles, CA 90095*

E-mail address: nico.marx@uni-muenster.de
Presenting author: Nico Marx

Mitochondrial ATP synthase or Complex V (CV) uses the proton motive force (PMF) generated by the electron transport chain to synthesize ATP during oxidative phosphorylation. The reaction is reversible: when respiratory function is impaired, CV hydrolyzes ATP and pumps protons across the inner mitochondrial membrane (IMM) into the intra-cristae space. In mammals, ATP synthase predominantly forms higher-order oligomers along cristae tips [1,2]. However, the interplay between the activity mode of CV and its oligomerization state is still not fully understood. Previous studies indicate that dissipation of the PMF is associated with increased ATP hydrolysis activity of CV and correlates with destabilization of dimers into monomers [3].
Here we use intact cells as well as isolated coupled mitochondria and induce a loss of PMF to increase ATP hydrolysis by CV. These treatments led to monomerization of the Complex V. We show that inhibition of the reverse mode of Complex V by the selective inhibitor (+)-Epicatechin [4] can protect against CV monomerization. Previous work using single molecule Tracking and Localization microscopy (TALM) of single CV complexes demonstrated that CV dimers show a lower mobility compared to the average enzyme population [5]. Consistent with this, we found that loss of PMF, accompanied by CV monomerization, led to increased mobility of ATP synthase within the IMM. Furthermore, this effect can be reversed by selective inhibition of ATP hydrolysis. Together, our findings establish a functional link between the catalytic direction of ATP synthase and its supramolecular organization, suggesting that ATP hydrolysis activity promotes dimer dissociation and increased enzyme mobility, with potential implications for cristae structure and mitochondrial function.

[1] T.E. Spikes, M.G. Montgomery, & J.E. Walker, Structure of the dimeric ATP synthase from bovine mitochondria, Proc. Natl. Acad. Sci. (2020). U.S.A. 117 (38) 23519-23526, https://doi.org/10.1073/pnas.2013998117

[2] K.M. Davies, M. Strauss, B. Daum, J.H. Kief, H.D. Osiewacz, A. Rycovska, V. Zickermann, & W. Kühlbrandt, Macromolecular organization of ATP synthase and complex I in whole mitochondria, Proc. Natl. Acad. Sci. (2011). U.S.A. 108 (34) 14121-14126, https://doi.org/10.1073/pnas.1103621108

[3] B. Daum, A. Walter, A. Horst, H.D. Osiewacz, & W. Kühlbrandt, Age-dependent dissociation of ATP synthase dimers and loss of inner-membrane cristae in mitochondria, Proc. Natl. Acad. Sci. (2013). U.S.A. 110 (38) 15301-15306, https://doi.org/10.1073/pnas.1305462110.

[4] R. Acin-Perez, C. Benincá, L. Fernandez del Rio et al. Inhibition of ATP synthase reverse activity restores energy homeostasis in mitochondrial pathologies. EMBO J 42 (2023). https://doi.org/10.15252/embj.2022111699

[5] K. Salewskij, B. Rieger, F. Hager, T. Arroum, P. Duwe, J. Villalta, S. Colgiati, C. P. Richter, O. E. Psathaki, J. A. Enriquez, T. Dellmann, K. B. Busch, The spatio-temporal organization of mitochondrial F1FO ATP synthase in cristae depends on its activity mode. Biochimica et Biophysica Acta (BBA) – Bioenergetics Volume 1861, Issue 1, (2020). https://doi.org/10.1016/j.bbabio.2019.148091.

P93

Electric field effects drive protonation dynamics and c-ring rotation of ATP synthase

Sarah C. Rossamann[a], Patricia Saura[a], Maximilian Allgwer[a], Ana P. Gamiz-Hernandez[a], John E. Walker[b], Ville R. I. Kaila[a]

[a] Department of Biochemistry and Biophysics, Stockholm University, 10691, Stockholm, Sweden. [b] Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, UK.

E-mail address: sarah.rossmann@dbb.su.se
Presenting author: Sarah Christina Rossmann

ATP synthase employs the proton motive force (PMF) generated by the respiratory chain to synthesize ATP. While its overall rotatory mechanism is well-known, the molecular basis of how the PMF-driven protonation of the c-ring drives its rotation, remain debated. Here, we investigate the molecular mechanism of the PMF-driven c-ring rotation and the effect of disease-related mutations by combining large-scale atomistic molecular dynamics (MD) simulations, constant pH-MD simulations, and hybrid quantum mechanics/molecular mechanics (QM/MM) free energy simulations. We find that a Grotthuss-mediated protonation of c-ring carboxylates is modulated by electric field effects that drive the rotatory motion of the c-ring, and result in p*K* shifts of both the inlet and outlet proton channels. We also find that a
the protonation states of the inlet and outlet proton pathways show strong coupling effects, while disease-related mutations perturb both the hydration state of the proton pathways and result in structural alterations. Our combined findings suggest a general mechanism underlying the PMF-driven proton transfer reactions that drive c-ring rotation, and how the process is guided by electric fields.

P94

Cryo-EM structure of the *Rhodobacter sphaeroides* ATP synthase

Hannah Takahashi[a]*, Katarzyna Lorencik[a]*, Katherine H. Richardson[a], Pu Qian[a], Philip J. Jackson[a], Sebastian Pintscher[b], Matthew P. Johnson[a], James N. Blaza[c], C. Neil Hunter[a] and Andrew Hitchcock[a]

a
Plants, Photosynthesis & Soil, School of Biosciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom.
b
Faculty of Biochemistry, Biophysics and Biotechnology, Department of Plant Biotechnology, Jagiellonian University, Kraków, Poland
c
York Structural Biology Laboratory, Department of Chemistry, University of York, United Kingdom Plants, Photosynthesis & Soil, School of Biosciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom.
**These authors contributed equally.*

E-mail address: htakahashi1@sheffield.ac.uk
Presenting author: Hannah Takahashi

Purple phototrophic bacteria (PPB) are model organisms for studying light harvesting and electron transport in anoxygenic photosynthesis. PPB operate a cyclic photosynthetic electron transport chain involving the RC-LH1 core complex, cytochrome bc_1 and cytochrome c_2, generating a proton motive force to drive ATP synthesis. While the structure of RC-LH1 [1] and cytochrome bc_1 [2] are known, structural studies of the ATP synthase from PPB have not yet been reported. Here, we present the 2.9 Å resolution cryo-EM structure of the *Rhodobacter* (*Rba.*) *sphaeroides* ATP synthase, revealing an additional subunit bound to F_1 (ζ) and 11 c-ring subunits. The ζ subunit is bound in a similar way to ATPase Inhibitory Factor 1 (IF1) in the mitochondrial enzyme and has been suggested to inhibit ATP hydrolysis in other proteobacteria [3]. We are making use of the genetically tractable *Rba. sphaeroides* system to determine the function of ζ and its role in ATP synthase regulation. Mass spectrometry data of our ATP synthase preparations indicates the presence of multiple isomers of the complex encoded by up to three distinct operons found throughout the *Rhodobacter* genome. The function, regulation and relative abundance of these isomers is not currently understood, but our data confirms that genes belonging to all three operons are expressed during photoheterotrophic growth, suggesting a complex regulatory and assembly process. In summary, our work completes the set of structures of the purple bacterial photosynthetic energy generating machinery, revealing *Rba. sphaeroides* ATP synthase possesses unique and unexplored genetic and structural features.

[1]Swainsbury, David J. K., Pu Qian, Andrew Hitchcock, and C. Neil Hunter. 'The Structure and Assembly of Reaction Centre-Light-Harvesting 1 Complexes in Photosynthetic Bacteria'.
Bioscience Reports 43, no. 5 (2023): BSR20220089. https://doi.org/10.1042/BSR20220089.

[2]Swainsbury, David J. K., Frederick R. Hawkings, Elizabeth C. Martin, et al. 'Cryo-EM Structure of the Four-Subunit Rhodobacter Sphaeroides Cytochrome Bc1 Complex in Styrene Maleic Acid Nanodiscs'. *Proceedings of the National Academy of Sciences* 120, no. 12 (2023): e2217922120. https://doi.org/10.1073/pnas.2217922120.

[3] Varghese, Febin, James N. Blaza, Andrew J. Y. Jones, Owen D. Jarman, and Judy Hirst.
'Deleting the IF1-like ζ Subunit from *Paracoccus denitrificans* ATP Synthase Is Not Sufficient to Activate ATP Hydrolysis'. *Open Biology* 8(1) (2018): 170206. https://doi.org/10.1098/rsob.170206.

P95

ATP-synthase subunits e,g do not shape rims of narrow cristae but determine cristae inflation and MICOS-SAM connections in INS-1E cells

Petr Ježek[a], Jitka Špačková[a], Hana Engstová[a], Jan Tauber[a], Nicole Brychová[a], Katarína Smolková[a], Andrea Dlasková[a]

[a]*Department of Mitochondrial Physiology, No.75, Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 142 20, Czech Republic*

E-mail address: jezek@biomed.cas.cz

Presenting author: Petr Ježek[a]

Attempting to verify/reject hypothesis that narrow cristae are maintained by ordered/stabilized ATP-synthase dimeric arrays[1], we studied INS-1E cells having CRISPR/Cas9-deleted ATPsynthase F_O-membrane subunits[2] e plus g. Subunits f, 6.8PL, b, d, and F6 were also missing. Resulting Δeg cells exhibited fragmented mt-network with frequent OMM rings containing circular thin giant cristae lamellae. Cristae junctions were highly reduced in mt-tubular sections of Δeg cells, orienting cristae in parallel with the longitudinal axis of mt-network tubules. This correlated with MICOS detachment from OMM SAM, absence of ATP-synthase tetramers and highly reduced dimers in BN-PAGE of Δeg mt-samples. Controls (scrambled sgRNA) inflated cristae at 3 mM glucose, narrowing them at 11- and 20-mM glucose. In contrast, Δeg cells locked always narrow morphology with a few proteome compensations, exhibited lower ATP-elevation responses on glucose without glutamine and ~20% insulin secretion rate. The persistence of thin cristae despite incomplete/destabilized Δeg ATP-synthases suggests that cristae form spontaneously double membrane lamellae. Subunits e plus g are paradoxically required for cristae inflation hypothetically forming a channel ensuring osmotic mechanism, when water penetrates the intracristal space (cristal lumen; ICS) by osmolarity disbalance caused by cation plus anion influx. While cations could be translocated by the mitochondrial ATP-sensitive K^+ channel, as judged by glibenclamide sensitivity[3]; anions can be transported by a putative anion channel, which may be speculatively regarded to be identical with the phenomenon of permeability transition pore (PTP) [2] [4]. This identity is suggested by considering the subunits e as a "Death Finger"[2]. Also, subunits e plus g are required for MICOS-SAM connections. We conclude that stabilized arrays of complete ATPsynthase dimers do not determine the thin cristae and that physiological role of subunits e may include cristae inflation.

[1] P. Ježek, M. Jabůrek, B. Holendová, H. Engstová, A. Dlasková, Mitochondrial Cristae Morphology Reflecting Metabolism, Superoxide Formation, Redox Homeostasis, and Pathology, Antioxid. Redox. Signal. 39 (2023), 635−683. https://doi.org/10.1089/ars.2022.0173.

[2] G. Pinke, L. Zhou, L.A. Sazanov, Cryo-EM structure of the entire mammalian F-type ATP synthase. Nat. Struct. Mol. Biol. 27 (2020), 1077–1085. https://doi.org/10.1038/s41594-020-05038.

[3] L. Plecitá-Hlavatá, H. Engstová, L. Alán, T. Špaček, A. Dlasková, K. Smolková, J. Špačková, J. Tauber, V. Strádalová, J. Malínský, M. Lessard, J. Bewersdorf, P. Ježek, Hypoxic HepG2 cell adaptation decreases ATP synthase dimers and ATP production in inflated cristae by mitofilin down-regulation concomitant to MICOS clustering, FASEB J. 30 (2016) 1941−1957 https://doi.org/ 10.1096/fj.201500176.

[4] P. Bernardi, C. Gerle, A.P. Halestrap, E.A. Jonas, J. Karch, N. Mnatsakanyan, E. Pavlov, S.S. Sheu, A.A. Soukas, Identity, structure, and function of the mitochondrial permeability transition pore: controversies, consensus, recent advances, and future directions. Cell Death Differ. 30 (2023), 1869-1885. https://doi.org/10.1038/s41418-023-01187-0.

P97

Uncovering the link between ATP synthase and the TCA cycle by crosslinking mass spectrometry

Jelena Misic[1], Laura Pérez Pañeda[2], Tereza Kadavá[2], Albert J.R. Heck[2], Nils-Göran Larsson[1]

[1]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden

[2]Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and
Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Padualaan 8, Utrecht 3584 CH, the Netherlands and Netherlands Proteomics Center, Padualaan 8, Utrecht 3584 CH, the Netherlands

E-mail address: jelena.misic@ki.se
Presenting author: Jelena Misic

ATP production in mitochondria requires tight coordination between the oxidative phosphorylation (OXPHOS) system, comprising complexes I–V, and the tricarboxylic acid (TCA) cycle. Although complex II is considered the only direct structural link between these pathways, it remains unclear whether other OXPHOS components contribute to this connection. Using in-solution crosslinking mass spectrometry (XL-MS), quantitative proteomics, blue native PAGE (BN-PAGE), and complexome profiling, we investigated the organization and interactome of ATP synthase (complex V) in murine heart mitochondria under physiological and pathological conditions. We found that, in wild-type hearts, the F_1 catalytic head of ATP synthase formed extensive contacts with TCA cycle enzymes, establishing a previously unrecognized link between OXPHOS and central carbon metabolism. Loss of the mitochondrial RNA-stabilizing protein LRPPRC, which impairs mtDNA gene expression, destabilized ATP synthase and strengthened these interactions. In *Lrpprc* knockout hearts, ATP synthase dysfunction was accompanied by enhanced binding of ATPase inhibitory factor 1 (ATIF1) to the F1 head through its N-terminal inhibitory region, consistent with inhibition of ATP hydrolysis and a shift toward an energy-conserving state. Together, these findings demonstrate that ATP synthase is integrated into a broader mitochondrial metabolic network and that its interactome is remodeled in response to stress.

P98

Title: Structural studies of the human V-ATPase containing the a2-isoform

Caitlin Bricault[a], Andrew Hillowe[a], Stuti Sharma[a]

[a]*Stony Brook University, Stony Brook, NY-11794, USA.*

E-mail address: stuti.sharma@stonybrook.edu
Presenting author: Stuti Sharma

The V-ATPase is an ATP-dependent proton pump, found on the intracellular membranes of eukaryotic cells. This multisubunit, rotary ATPase acidifies intracellular organelles to create an optimal environment for fundamental processes such as pH and ion homeostasis, endocytosis, vesicular traGicking, degradation of macromolecules and the secretion of hormones and neurotransmitters. The V-ATPase can be divided into two functional subcomplexes - the V1-ATPase and the V0 proton channel, whose activity is structurally and functionally coupled in the holo-enzyme. Unneeded V-ATPase activity is switched oG by the reversible disassembly of V1 and V0, a phenomenon which is conserved from yeast to humans. Additionally, in mammals, V-ATPase subunits are expressed as tissue and/or organelle specific isoforms. For instance, the a-subunit is expressed as four isoforms, which often localize the V-ATPase to specific cellular compartments. The a2-isoform localizes the V-ATPase to endosomes and the Golgi, and mutations in this gene cause a congenital glycosylation defect called autosomal recessive cutis laxa type 2A (ARCL-2A) or the wrinkly skin syndrome. In this study, we have purified the a2-isoform containing human V-ATPase and determined its cryo-EM structures. The structures allow us to map missense mutations reported to cause ARCL-2A and identify features in this isoform, that are unique compared to other a-subunit isoforms. Additionally, we have observed that a2-containing V-ATPase is more labile compared to the a4-isoform containing enzyme, such that the V1 and Vo sub complexes are mostly disassembled in the purified complex. These observations highlight potential interplay between reversible disassembly of V-ATPase and its isoform composition.

P99

Analysis of Kinetic Experiments Suggest a Localized Model of Energy Coupling in ATP Synthase

Sunil Nath, Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, INDIA. E-mail: sunath@iitd.ac.in; sunil_nath_iit@yahoo.com

Cellular ATP is synthesized by the rotary motor F_1F_O-ATP synthase located in bacterial membranes, the cristae membranes in mitochondria, and the thylakoid membranes in chloroplasts. The results of kinetic experiments have generally been interpreted in terms of Mitchell's chemiosmotic theory, which is a delocalized theory, with its protonmotive driving force (pmf) arising solely from H^+ ion translocation. Historically however, experimental evidence for the theory was not water-tight, and acceptance of the theory was not universal. Alternative models of energy coupling have been developed [1–6] because several new experiments offer a different view. Proteoliposomes reconstituted with F_1F_O have been energized by acid-base H^+ transition along with valinomycin-induced diffusion potential of K^+ ions at various ATP, ADP and Pi concentrations and the initial rates of ATP synthesis/hydrolysis have been measured. In the experiments, the pH_{in} varied between 6.3 – 7.6, the ΔpH between 0.4 – 1.7, the ΔpK from 0.3 – 1.6, the Pi concentration from 0.1 – 10 mM, and the [ATP]/([ADP][Pi]) ratio from 0.1 – 50. It is shown here by calculation over the aforementioned *broad* range of experimental variables that the results of such kinetic biochemical experiments are quantitatively consistent with a localized model of energy coupling in which energy for synthesis of ATP is contributed by two ions, in this case K^+ and H^+. The biological implications of these findings for models of energy coupling and ATP synthesis in the F_1F_O-ATP synthase and in Complex I are discussed. Connections to nonequilibrium thermodynamics of the coupling process are made. In particular, the need for a *local* coupling—as opposed to the delocalized coupling postulated by chemiosmosis—in order to explain the cumulative experimental information is highlighted.

References

1. Brändén, M., Sandén, T., Brzezinski, P., Widengren J., 2006. Localized proton microcircuits at the biological membrane–water interface. *Proceedings of the National Academy of Science USA* 103, 19766–19770.

2. Nath, S., 2010. Beyond the Chemiosmotic Theory: Analysis of Key Fundamental Aspects of Energy Coupling in Oxidative Phosphorylation in the Light of a Torsional
Mechanism of Energy Transduction and ATP Synthesis – Invited Review Part 2. *Journal of Bioenergetics and Biomembranes* 42, 301–309.

3. Rieger, B., Junge, W., Busch, K., 2014. Lateral pH gradient between OXPHOS Complex IV and F_OF_1-ATP synthase in folded mitochondrial membranes. *Nature Communications* 5, 3103.

4. Toth, A., Meyrat, A., Ott, M., et al., 2020. Kinetic coupling of the respiratory chain with ATP synthase, but not proton gradients, drives ATP production in cristae membranes. *Proceedings of the National Academy of Science USA* 117, 2412– 2421.

5. Nath, S., 2021. Energy landscapes and dynamics of ion translocation through membrane transporters: A meeting ground for physics, chemistry, and biology. *Journal of Biological Physics* 47, 401–433.

6. Nath, S., 2026. Rethinking the classical chemiosmotic theory. *Biological Theory*
21. ONLINE FIRST: https://doi.org/10.1007/s13752-025-00499-3.

P100

V-ATPase Regulation by TLDc proteins: from Yeast to Neurodegenerative Disease

Rebecca A. Oot & Stephan Wilkens

SUNY Upstate Medical University, Department of Biochemistry and Molecular Biology, Syracuse, NY, USA

The vacuolar ATPase (V-ATPase) is a rotary molecular motor enzyme and dedicated proton pump. V-ATPase generates the acidic internal pH of subcellular compartments and organelles, such as endosomes, lysosomes and synaptic vesicles (SVs). As such, V-ATPase function is linked to diverse and fundamental cellular processes including endocytosis, protein traffic, degradation and neurotransmitter loading. V-ATPase activity is regulated by "reversible disassembly" wherein the catalytic ATPase subcomplex (V_1) is released from its membrane integral proton channel subcomplex (V_o). Upon dissociation, both subcomplexes are functionally silenced but the enzyme is reactivated upon V_1-V_o reassembly. A number of cellular signals converge on enzyme assembly state to drive this process (eg. nutrient levels, pH, viral infection) but the molecular details have remained unclear. Recently, we identified TLDc protein

Oxr1p to be a V-ATPase disassembly factor in yeast. The human genome encodes at least five TLDc proteins; Ncoa7, Oxr1, mEAK7, TBC1D24 and TLDC2. Using purified human TLDc proteins and lipid nanodisc reconstituted human V-ATPase, we previously found that Ncoa7,

Oxr1, TLDC2 and TBC1D24 inhibit V-ATPase by promoting its disassembly *in vitro*. Interestingly, mutations in TBC1D24 or V-ATPase subunit B are causative of neurological disease such as DOORS (deafness, onchodystrophy, osteodystrophy, intellectual disability and seizures) syndrome. Using our previous cryo-EM structure of the yeast Oxr1p:V-ATPase complex to examine the binding interface, revealed that i.) the interface is highly conserved, ii.) much of the interaction is contributed by subunit B and iii.) several disease mutations line the interface. Thus, we have made disease-causing mutations in human TLDc proteins, characterized their stability, and examined their effects on purified human V-ATPase function. We find that while wild type TBC1D24 inhibits human V-ATPase via disassembly, a DOORS causing missense mutant has no impact on V-ATPase function. We hypothesize that failure to negatively regulate V-ATPase will lead to hyperactivity, in turn resulting in disruption of pH dependent cellular processes. Notably, hyperacidification of lysosomes has been shown to reduce their degradative capacity and has been proposed to be a driver of neurodegenerative disease.

P101

Elucidation of a Mechanism for Photosynthetic CO_2 Capture in *Synechococcus elongatus*

Eve Giovannetti[a], Stefania Viola[a]

[a]*Photosynthesis & Environment, BIAM, UMR7265, CEA/CNRS/AMU, Saint-Paul-Lez-Durance, France*

E-mail address: eve.giovannetti@cea.fr
Presenting author: Eve Giovannetti

Cyanobacteria are prokaryotic microalgae capable of performing oxygenic photosynthesis. In these organisms, photosynthetic and respiratory complexes coexist within the thylakoid membrane. This unique organization entails the sharing of several components of the electron transport chain, notably certain carriers and the plastoquinone pool. Consequently, photosynthetic and respiratory pathways may compete for electrons.

The NADPH dehydrogenase-1 complex (NDH-1) plays a central role in this metabolic dynamic. It participates both in cyclic electron flow (CEF) around photosystem I (contributing to proton gradient generation and thus ATP synthesis) and in respiratory processes. Four forms of NDH-1 in cyanobacteria have been identified[1]. All are involved in CEF and play a major role in maintaining cellular bioenergetic balance.

Among these forms, the NDH-1_3 and NDH-1_4 complexes contain additional subunits : the carbonic anhydrases CupA and CupB. These enzymes catalyze the conversion of CO_2 into bicarbonate, a key step in the carbon-concentrating mechanism (CCM). The CCM increases the intracellular availability of inorganic carbon and limits CO_2 diffusion out of the cell. The unique coupling between ATP production and the CCM in the NDH-1_3 and NDH-1_4 complexes contributes to the optimization of carbon fixation[2].

However, the mechanisms linking electron transport, membrane polarization, and CCM efficiency remain poorly understood. In particular, how the different NDH-1 forms contribute to the regulation of electron fluxes and inorganic carbon dynamics, remains to be clarified.

To better understand these relationships, we characterize photosynthetic activity and CO_2 fixation in *Synechococcus elongatus* strains carrying targeted mutations in several NDH-1 subunits. We employ non-invasive biophysical methods to precisely analyze electron fluxes, electrochemical gradient dynamics, and gas exchange, associated with CO_2 fixation. This work aims to improve our understanding of the coupling between bioenergetics and carbon assimilation in cyanobacteria.

[1] Peltier, G., Aro, E. M., & Shikanai, T. (2016). NDH-1 and NDH-2 Plastoquinone Reductases in Oxygenic Photosynthesis. *Annual review of plant biology*, *67*, 55–80. https://doi.org/10.1146/annurev-arplant-043014-114752

[2] Zhang, Z., Zhang, M., & Burnap, R. L. (2025). Action at a distance: The remarkable coupling of CO_2 uptake to electron transfer in specialized cyanobacterial NDH-1 complexes. *Proceedings of the National Academy of Sciences of the United States of America*, *122*(36), e2511786122.
https://doi.org/10.1073/pnas.2511786122

P102

QM/MM refinement of cryo-EM data structural reveal protonation states of bioenergetics protein complexes

Hao Jiang[a], Sarah Rossmann[a], Patricia Saura[a], Johannes Messinger[b,c], Ville R. I. Kaila[a]

[a]*Department of Biochemistry and Biophysics, Stockholm University, Arrhenius Laboratories for Natural Sciences, Stockholm, Sweden.*

[b]*Department of Plant Physiology, Umeå University, Linnaeus väg 6 (KBC huset), Umeå, Sweden*

[c]*Molecular Biomimetics, Department of Chemistry – Ångström Laboratory, Uppsala University, Uppsala, Sweden*

E-mail address: hao.jiang@dbb.su.se
Presenting author: Hao Jiang

Recent high-resolution cryo-electron microscopy (cryo-EM) studies of Photosystem II (PSII) have revealed unprecedented structural details, including new water sites and hydrogen positions within the oxygen-evolving Mn_4CaO5 center.[1] While these developments provide a transformative basis for investigating the water-splitting mechanism, experimental cryo-EM maps often represent mixtures of catalytic intermediates, particularly a mixture of different S-states of the photocycle. To address this, we employ here a multi-scale computational framework building upon the methodology recently developed by our group.[2] Based on QM/MM calculations and highresolution cryo-EM data, we construct atomic models of different S states, considering distinct conformational-, protonation-, and spin states. We suggest that our optimized structures together with computed multi-state population refinement of electrostatic potential and difference maps, provide a direct comparison with the experimental cryo-EM maps. Our method allows us to assess the relative populations of S states, and to identify structural models that are most consistent with the observed features. Our framework provides a basis for interpreting mixed-state cryo-EM data and to enhance our understanding of the biological water-oxidation mechanism.

[1] R. Hussein, A. Graça, J. Forsman, A.O. Aydin, M. Hall, J. Gaetcke, P. Chernev, P. Wendler, H. Dobbek, J. Messinger, A. Zouni, W.P. Schröder, Cryo–electron microscopy reveals hydrogen positions and water networks in photosystem II, Science 384 (2024) 1349–1355, https:// doi.org/10.1126/science.adn6541

[2] N. Croy, G. Gaullier, P. Saura, S. Masiulis, A. Bhowmick, J. Kern, O. Raschdorf, I. Andersson, V R.I. Kaila, C. Blikstad, J. Messinger, Atomic resolution structure of spinach rubisco reveals protons and dynamics, bioRxiv (2025), https://doi.org/10.64898/2025.12.22.696010

P103

Where Do the Electrons Go? A Synechocystis Bioenergetics Model

Edwin Ortega-Arzola[1], Thomas Höfer[1,2], Jenny Zhang[1]

1 Yusuf Hamied Department of Chemistry, University of Cambridge

2 Technische Universität München

E-mail address: ejo34@cam.ac.uk

Presenting author: Edwin Ortega-Arzola

Cyanobacteria offer a robust platform for sustainable energy, oxygen, and biomass production. Previous studies have laid the groundwork for biophotoelectrochemical systems using cyanobacteria, demonstrating that their current electrical output and maintenance costs are far from their theoretical limits and shedding light on the potential for optimisation and use in biotechnological applications. Model-driven optimisation could boost outputs by accounting for energetic constraints and metabolite dynamics. We present a modelling framework that aims to standardise basal metabolic energy requirements and electron allocation while shedding light on key bottlenecks associated with reactive oxygen species and pH changes. The core of this approach is a detailed kinetic model of the main cyanobacterial metabolism and energy conversion, implemented in COMSOL Multiphysics [1,2].

[1] McCormick, Alistair J. et al., “Biophotovoltaics: oxygenic photosynthetic organisms in the world of bioelectrochemical systems”, Energy & Environmental Science, 2015.

[2] Tanaka, Kenya et al., Dark accumulation of downstream glycolytic intermediates confers robust initiation of photosynthesis in cyanobacteria, 2022.

P104

Local Thylakoid Unstacking Underlies State Transitions

S. VAN DORST[1], W. WIETRZYNSKI[1], K.K. PATRA[2], W.J. NAWROCKI[2], D.I.G.B. RACCAH[3], R. CROCE[4], B.D. ENGEL[1]

[1]*Biozentrum - University of Basel, Basel, Switzerland*

[2] *Laboratoire de Biologie du Chloroplaste et Perception de la Lumière chez les Microalgues, Sorbonne Université, Institut de Biologie Physico-Chimique, Paris, France.*

[3] *Department of Chemistry, University of Texas at Austin, Austin*

[4] *Department of Physics and Astronomy, Faculty of Science, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands*

Photosynthesis occurs in thylakoids, a membranous compartment containing the protein complexes of the electron transport chain and ATP synthase, densely packed to make up ~80% of the membrane volume. Green algae, like *Chlamydomonas*, inhabit dynamic niches such as the layered canopies and mixing bodies of water, where light fluctuates strongly. They adapt rapidly to changes in light intensity by tuning the energy delivered to photosystems through small, membrane-embedded antennae (LHCs). These state transitions equilibrate excitation energy within minutes, but their underlying mechanism remains contested. The prevailing model proposes that under reducing conditions, LHCIIs in stacked thylakoid regions, where Photosystem II is found, become phosphorylated and migrate to Photosystem I in unstacked membranes to balance electron transport.

Using a combination of in vivo spectroscopy, spectral cryo- light microscopy, and cryoelectron tomography, we instead find that state transitions involve phosphorylationdependent local thylakoid unstacking, which enables intermixing of photosynthetic complexes and redistribution of excitation energy.

P105

Time-Resolved Infrared Investigation of the Chloride-Ion Pump *Nm*HR Influenced by Solvent Salt and Detergent Concentration

Celina Wittenzellner[a], Mariafrancesca La Greca[b], Karoline-Luisa Lê Công[a], Aoife Redlich[a], Pit Langner[a], Sabine Oldemeyer[a], Ramona Schlesinger[b], Jacek Kozuch[c], Joachim Heberle[a]

[a]*Experimental Molecular Biophysics, Department of Physics, Freie Universität Berlin* [b]*Genetic Biophysics, Department of Physics, Freie Universität Berlin*
[c]*Institut of Physical und Theoretical Chemistry, Braunschweig Integrated Centre of Systems Biology (BRICS), Technische Universität Braunschweig*

E-mail address: wittenzec00@zedat.fu-berlin.de
Presenting author: Celina Wittenzellner

Microbial Rhodopsins are a family of light activated photoreceptors, which are of high interest for optogenetic applications, such as treatments for human disorders like Parkinson's or blindness. Therefore, it is important to fully understand the pumping mechanism of these nano-machines in detail. A new, promising candidate is the light activated chloride-ion pump *Nm*HR (*Nonlabens marinus* halorhodopsin).[1] Steady-state UV/Vis spectroscopy has shown that the *Nm*HR retinal absorbance band is highly sensitive to NaCl concentration changes, suggesting relevant electrostatic and/or structural changes at the chromophore when the chloride-binding site is occupied.[2] To obtain a more fundamental understanding of the chloride-ion pumping mechanism during the light activated photocycle of *Nm*HR, Fourier transform infrared (FTIR) difference spectroscopy and transient time-resolved infrared spectroscopy are used in combination. For the latter, a new home-built referenced quantum cascade laser (rQCL)-setup is used, which makes use of QCLs as a powerful IR-Laser source, with the aim to observe changes in the *Nm*HR photocycle at single amino acids and the chromophore at different salt concentrations. Additionally, previous time-resolved XFEL-data are correlated with the observed transient timeresolved infrared results for a better understanding of the chloride-ion pumping mechanism.[3]

[1] S. Yoshizawa, Y. Kumagai, H. Kim, Y. Ogura, T. Hayashi, W. Iwasaki, E.F. DeLong, & K. Kogure, Functional characterization of flavobacteria rhodopsins reveals a unique class of lightdriven chloride pump in bacteria. Proc. Natl. Acad. Sci. U.S.A. 111(18), (2014), 6732-6737, https://doi.org/10.1073/pnas.1403051111

[2] T. Tsukamoto, S. Yoshizawa, T. Kikukawa, M. Demura, and Y. Sudo, Implications for the Light-Driven Chloride Ion Transport Mechanism of *Nonlabens marinus* Rhodopsin 3 by Its Photochemical Characteristics. The Journal of Physical Chemistry B, 121 (9), (2017), 2027-2038, DOI:10.1021/acs.jpcb.6b11101

[3] Sandra Mous *et al.*, Dynamics and mechanism of a light-driven chloride pump. Science 375, (2022), 845-851, DOI:10.1126/science.abj6663

P106

Structural Basis of Redox-Coupled CO_2 hydration by the Cyanobacterial NDH-1MS' complex

Yat Kei Lo1,2, Florian Ruoß3, Jacqueline Thiemann3, Marc Nowaczyk3, Jan M. Schuller1,4

1 Philipps-University Marburg, Department of Chemistry and SYNMIKRO Research Center, Marburg, Germany
2 Department of Chemistry for Life Sciences, Uppsala University, Uppsala, Sweden
3 Plant Biochemistry, Faculty of Biology and Biotechnology, Ruhr-University Bochum, Bochum, Germany
4 Microbes-for-Climate (M4C) Cluster of Excellence, Marburg, Germany

E-mail address: yatkei.lo@kemi.uu.se
Presenting author: Yat Kei Lo

Microbial CO2 fixation accounts for approximately half of global primary production and forms the basis of food chains in diverse ecosystems [1]. However, this essential process is often limited by the scarcity of dissolved CO2 and the inefficiency of the key CO2-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [2]. To overcome these challenges, cyanobacteria evolved a specialized variant of the photosynthetic NAD(P)H dehydrogenase-like complex, NDH-1MS' which actively scavenges CO2 and convert it to bicarbonate against its chemical gradient. This energetically uphill process is driven by cyclic electron flow through electron transfer from ferredoxin to plastoquinone [3]. The molecular mechanism of energy-coupled CO2 hydration by NDH-1MS' so far remains largely unclear. Here, we present the cryo- EM structural analysis of NDH-1MS' isolated from the cyanobacterium Thermosynechococcus vestitus. The complex structurally resembles respiratory Complex I, while the transmembrane arm distal subunit, NdhF4 (corresponds to ND5/NuoL/Nqo12 of Complex I) forms a unique CO2-concentrating module together with the cytoplasmic subunit CupB. Our structure further reveals a putative ligand-bound active site buried within CupB, connecting to the proton transport pathway of NdhF4. This configuration differs from the active site previously proposed for the close homologue, NDH-1MS [4]. We hypothesize that NDH-1MS' functions as a metal-independent vectorial carbonic anhydrase which utilizes energy from proton-coupled electron transfer to catalyze unidirectional CO2 hydration.

[1] Hügler M, Sievert SM. Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. Ann Rev Mar Sci. 2011;3:261-89. doi: 10.1146/annurev-marine-120709-142712.
[2] Raven JA, Beardall J. The ins and outs of CO2. J Exp Bot. 2016; 67(1):1-13. doi: 10.1093/jxb/erv451. Epub 2015 Oct 14.
[3] Price GD. Inorganic carbon transporters of the cyanobacterial CO2 concentrating mechanism. Photosynth Res. 2011 Sep;109(1-3):47-57. doi: 10.1007/s11120-010-9608-y.
[4] Schuller JM, Saura P, Thiemann J, et al. Redox-coupled proton pumping drives carbon concentration in the photosynthetic complex I. Nat Commun. 2020 24;11(1):494. doi: 10.1038/s41467-020-14347-

P107

Functional Characterization of GFY Proteins in Acetate Metabolism in *Chlamydomonas reinhardtii*

Keqing Liu[a], Achille Marchand[a], Anaïs Scholivet[a], Alix Boulouis[a], Frédéric Carriere[a], Yonghua LiBeisson[b], Hélène Launay[a]

[a] *Aix Marseille University, CNRS, BIP, UMR 7281, IMM, FR 3479, 31 Chemin J. Aiguier, 13402 Marseille Cedex 20, France*

[b] *Aix- Marseille University, CEA, CNRS, Institute of Biosciences and Biotechnologies of AixMarseille, UMR 7265, CEA Cadarache, Saint-Paul-lez Durance, France*

E-mail address: kliu@imm.cnrs,fr

Presenting author: Keqing Liu

Chlamydomonas reinhardtii is a widely used model organism for studying photosynthesis and cellular bioenergetics, partly thanks to its ability to grow on acetate, enabling both mixotrophic and heterotrophic lifestyles. In algal cells, acetate is converted into acetyl-CoA, which can enter either the mitochondrial tricarboxylic acid (TCA) cycle or the peroxisomal glyoxylate cycle. Despite the importance of acetate metabolism for heterotrophic and mixotrophic growth, the intracellular trafficking of acetate and its metabolic partitioning between these pathways remain poorly understood. A previous study identified five GFY proteins in Chlamydomonas belonging to the GPR1/FUN34/YaaH protein family, whose members function as acetate transporters in bacteria and fungi. However, their role in acetate transport in Chlamydomonas has not yet been demonstrated. Interestingly, localization studies suggest that GFY proteins are associated with the membranes of specific microbodies distinct from those involved in fatty acid β-oxidation. Here, we aim to investigate the function of GFY proteins in acetate metabolism by generating single and double mutants using CRISPR–Cas9 genome editing and analyzing their effects on acetate utilization and metabolic flux under contrasting nutrient conditions.[1,2]

[1] Durante L, Hübner W, Lauersen KJ, Remacle C (2019) Characterization of the GPR1/FUN34/YaaH protein family in the green microalga Chlamydomonas suggests their role as intracellular membrane acetate channels. Plant Direct 3: e00148. https://doi.org/10.1002/pld3.148 [2] Lauersen KJ, Willamme R, Coosemans N, Joris M, Kruse O, Remacle C (2016) Peroxisomal microbodies are at the crossroads of acetate assimilation in the green microalga Chlamydomonas reinhardtii. Algal Research 16: 266–274. http://dx.doi.org/10.1016/j.algal.2016.03.026

P108

ATP-dependent potassium channel of the native mitochondrial inner membrane: electrophysiological and pharmacological characterization

Yevheniia Kravenska[a], Diego De Stefani[b], Vanessa Checchetto[a], Andrea Mattarei[c], Ildiko Szabo[a]

[a]*Department of Biology, University of Padova* [b]*Department of Biomedical Sciences, University of Padova*
[c]*Department of Pharmaceutical and Pharmacological Sciences, University of Padova*

E-mail address: yevheniia.kravenska@unipd.it
Presenting author: Yevheniia Kravenska

The mitochondrial ATP-dependent K^+ channel (mitoK-ATP channel, MITOK/MITOSUR complex) plays a key role in regulating cellular potassium and energy homeostasis and is one of the key molecular factors of cardioprotection. However, knowledge on its function and structure is extremely limited since the activity of the channel in intact membranes remains elusive, and even the molecular identity is debated: CCDC51(MITOK)+mitoSUR were identified in our previous work[1] while another study proposes ROMK channel isoform as a possible candidate[2]. Our study was devoted to a comprehensive electrophysiological and pharmacological analysis of the mitoK-ATP channel of cardiomyocytes using the sophisticated patch clamp technique in the mitochondria-attached configuration. The wildtype and MITOK-knockout mice were used for our experiments. MitoK-ATP channel modulation was tested with Mg/ATP and the mitochondriotropic derivative of diazoxide. Tertiapin-Q was used to block possible ROMK activity. For the first time, we demonstrate and describe mitoK-ATP currents in native cardiomyocytes. Channel conductances in both wildtype and MITOK-knockout mitochondria were heterogeneous. Meanwhile, the efficiency of mitoK-ATP modulators in wild-type cardiomyocytes was 2-4 times higher than in MITOKknockout ones. Tertiapin-Q has shown no action on the both types of currents. Based on the observed pharmacological profile and electrophysiological characteristics, we suggests the presence of more than one type of mitoK-ATP channels in the native membranes, one of which is not active in knockout mitochondria. We also shown the operation of mitoK-ATP in different substates and excluded the identification of ATP-sensitive activity due to ROMK.

[1] A. Paggio, V. Checchetto, A. Campo, R. Menabò, G. Di Marco, F. Di Lisa, I. Szabo, R. Rizzuto, D. De Stefani, Identification of an ATP-sensitive potassium channel in mitochondria, Nature 572(7771) (2019) 609-613. https://doi.org/10.1038/s41586-019-1498-3

[2] M. Laskowski, B. Augustynek, P. Bednarczyk, M. Żochowska, J. Kalisz, B. O'Rourke, A. Szewczyk, B. Kulawiak, Single-channel properties of the ROMK-pore-forming subunit of the mitochondrial ATP-sensitive potassium channel, Int. J. Mol. Sci. 20(21) (2019), 5323. https://doi.org/10.3390/ijms20215323

P109

Using Cryo-EM to Reveal How Calcium Controls the Mitochondrial ATPMg/Phosphate Carrier

Anna Ceesay[a], Denis Lacabanne[a], Jonathan J. Ruprecht[a], Steven P.D. Harborne[a],
Martin S. King[a], Fiona Fitzpatrick[a], Els Pardon[b], Jan Steyaert[b], Edmund R.S. Kunji[a]

[a]*Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, CB2 0XY, United Kingdom*
[b]*VIB-VUB Centre for Structural Biology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium.*

E-mail address: ersk2@cam.ac.uk
Presenting author: Anna Ceesay

The mitochondrial ATP-Mg/Phosphate carrier (APC), a member of the SLC25 family, exchanges adenine nucleotides and phosphate across the mitochondrial inner membrane to regulate the matrix adenine nucleotide pool in response to cellular energy demands. Unlike most of SLC25 members, APC consists of three distinct domains: an N-terminal regulatory domain containing four calcium-binding EF-hands, a loop domain harbouring an amphipathic α-helix, and a mitochondrial carrier domain [1-3]. This unique architecture allows transport activity to be dynamically regulated by cytosolic calcium levels, directly coupling mitochondrial metabolic output to cellular calcium signalling. The precise regulation of this transport activity is fundamental to mitochondrial function, and its dysregulation has been implicated in a broad range of human pathologies including metabolic disorders, neurodegeneration, and cancer [4].

Despite decades of biochemical investigation, the structural basis by which calcium controls transport activity in the intact protein remains one of the central outstanding questions in mitochondrial biology [1]. While previous studies have provided partial insight into the organisation of individual domains in isolation [2], a complete mechanistic picture of how the carrier transitions between active and inactive states has been lacking.

Here we present new structural and functional insights into the regulatory mechanism of APC. Using an integrated approach combining cryo-electron microscopy with biochemical and biophysical analyses, we provide a molecular framework for understanding how calcium signalling controls transport activity. Our findings reveal key structural elements that govern the inhibited state of the carrier. Furthermore, by extending our analysis across closely related APC family members, we demonstrate that calcium sensitivity is differentially tuned across paralogues, providing insight into how mitochondrial adenine nucleotide transport may be tailored to distinct physiological contexts.

[1] Harborne, S.P.D., et al., *Calcium regulation of the human mitochondrial ATP-Mg/Pi carrier SLC25A24 uses a locking pin mechanism.* Sci Rep, 2017. 7: p. 45383.

[2] Harborne, S.P.D., J.J. Ruprecht, and E.R.S. Kunji, *Calcium-induced conformational changes in the regulatory domain of the human mitochondrial ATP-Mg/Pi carrier.* Biochim Biophys Acta, 2015. 1847(10): p. 1245-1253.

[3] Harborne, S.P.D. and E.R.S. Kunji, *Calcium-regulated mitochondrial ATPMg/Pi carriers evolved from a fusion of an EF-hand regulatory domain with a mitochondrial ADP/ATP carrier-like domain.* IUBMB Life, 2018. 70(12): p. 1222-1232.

[4] Kunji, E.R.S., et al., *The SLC25 carrier family: Important transport proteins in mitochondrial physiology and pathology.* Physiology, 2020. 35(5): p. 302-327.

P110

Title Structure and mechanism of substrate discrimination of the human mitochondrial citrate carrier SLC25A1

Jianhui Li[a], Denis A. Lacabanne[a], Martin S. King[a], Jonathan J. Ruprecht[a], Alice P. Sowton[a], Stephany Jaiquel Baron[a], Shane M. Palmer[a], Els Pardon[b,c], Jan Steyaert[b,c], Vanessa Leone[d], Edmund R. S. Kunji[a]

[a]MRC Mitochondrial Biology Unit, University of Cambridge, The Keith Peters Building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, United Kingdom. [b]VIB-VUB Center for Structural Biology, VIB, Pleinlaan 2, B-1050 Brussels, Belgium. [c]Structural Biology Brussels, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium. [d]Department of Biophysics and Data Science Institute, Medical College of Wisconsin, Milwaukee, WI 53226-3548, USA.

E-mail address: ersk2@cam.ac.uk
Presenting author: Jianhui Li

The human mitochondrial citrate carrier (SLC25A1, CIC) mediates the exchange of tricarboxylate, dicarboxylates and phosphoenolpyruvate across the mitochondrial inner membrane, thereby linking mitochondrial and cytosolic metabolism[1,2]. The export of citrate is central to the generation of cytosolic acetyl-CoA, which underpins lipid biosynthesis and diverse anabolic pathways[3]. Consistent with this key metabolic role, CIC dysfunction is associated with severe metabolic disorders and cancer, highlighting its importance in both physiology and disease.

As a member of the mitochondrial carrier family, CIC is thought to operate through an alternating access mechanism, in which a central substrate binding site is exposed to either side of the membrane [4-6]. This transport process is driven by coordinated conformational changes that involve multiple structural elements and transient intermediate states. However, despite extensive biochemical and structural work on related carriers, the molecular basis of substrate recognition and the determinants of substrate activity in CIC remain poorly understood. In particular, how this carrier can recognise and bind a whole set of chemically diverse substrates, and how substrate transport may be coupled to proton translocation, remain unresolved questions.

Here, we combine structural and biophysical approaches to investigate the conformational landscape and substrate interactions of CIC. Our findings shed light on the complexity of substrate recognition in this carrier and provide a framework for understanding transport mechanisms across the SLC25 mitochondrial carrier family.

[1] Kunji, E.R.S., et al., The SLC25 carrier family: important transport proteins in mitochondrial physiology and pathology. Physiology (Bethesda), 2020. 35(5): p. 302-327.

[2] Majd, H., et al., Pathogenic mutations of the human mitochondrial citrate carrier SLC25A1 lead to impaired citrate export required for lipid, dolichol, ubiquinone and sterol synthesis. Biochim Biophys Acta, 2017. 1859(1): p. 1-7.

[3] Grunler, J., J. Ericsson, and G. Dallner, Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. Biochimica Et Biophysica Acta-Lipids and Lipid Metabolism, 1994. 1212(3): p. 259-277.

[4] Cimadamore-Werthein, C., et al., Human mitochondrial carriers of the SLC25 family function as monomers exchanging substrates with a ping-pong kinetic mechanism. EMBO J, 2024: p.
3450-3465.

[5] Ruprecht, J.J., et al., Structures of yeast mitochondrial ADP/ATP carriers support a domainbased alternating-access transport mechanism. Proc Natl Acad Sci U S A, 2014. 111(4): p. E426E434.

[6] Ruprecht, J.J., et al., The molecular mechanism of transport by the mitochondrial ADP/ATP carrier. Cell, 2019. 176(3): p. 435-447.

P111

Dynamical Insights on the Ion-capturing Function of the S-layer Protein

Erik Endres[1], David Dallemer[2,3], Guylaine Nuijten[4], Arne Moeller[2,3], Laura van Niftrik[4], Kristian Parey[2,3], Vivek Sharma[1,5]

[1]*Department of Physics, University of Helsinki, Helsinki, Finland*

[2]*Structural Biology section, Department of Biology/Chemistry, Osnabrueck University, 49076 Osnabrueck, Germany*
[3]*Center of Cellular Nanoanalytic Osnabrück (CellNanOs), Osnabrueck University, 49076 Osnabrueck, Germany*
[4]*Department of Microbiology, Radboud Institute for Biological and Environmental Sciences, Faculty of Science, Radboud University, Nijmegen, The Netherlands*
[5]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland*

E-mail address: erik.endres@helsinki.fi
Presenting author: Erik Endres

Anaerobic ammonium oxidizing (anammox) bacteria play a crucial role in the global nitrogen cycle, estimated to release 50% of all nitrogen gas into the atmosphere.[1] Their chemolithoautotrophic lifestyle requires them to absorb nitrogen containing molecular ions like ammonium and nitrite that only occur in low concentrations in the environment for the energygenerating anammox reaction. In this study,[2] we focused on the hexameric S-layer glycoprotein enveloping the anammox bacterium, presumed to be critical in the substrate filtering process. Based on new structural data obtained from cryo-electron microscopy combined with subtomogram averaging, we performed atomistic molecular dynamics simulations and identified hot spots for ion aggregation, giving further support to the hypothesis of the S-layer acting as a molecular sieve based on electrostatic interactions.[2,3] Our simulations show significant flexibility within and between each monomer of the structure, allowing for variable pore sizes.

[1] Kartal, B. *et al.* How to make a living from anaerobic ammonium oxidation. *FEMS Microbiol Rev* 37, 428–461 (2013). https://doi.org/10.1111/1574-6976.12014

[2] Dallemer, D. *et al.* Structure and Dynamics of Annamox Bacterial S-layer (Manuscript in Preparation)

[3] von Kügelgen, A. *et al.* Membraneless channels sieve cations in ammonia-oxidizing marine archaea. *Nature* 630, 230-236 (2024). https://doi.org/10.1038/s41586-024-07462-5

PI12

A novel mechanism of regulation of UCP1-mediated respiratory uncoupling

F. Gellé[1], R. Moussa[1], S. Masscheleyn[1], S. Gutche[2], A. Belicar[1], D. Castro[1], J. Hénin[2] and B. Miroux[1]

1. Biochimie des protéines membranaires, Université Paris Cité, CNRS, Institut de Biologie PhysicoChimique, Paris, France;
2. Laboratoire de Biochimie Théorique, Université Paris Cité, CNRS, Institut de Biologie PhysicoChimique, Paris, France;

gelle@ibpc.fr
Presenting Author: François Gellé
Topic: S9

Uncoupling Protein 1 (UCP1) dissipates energy as heat in brown adipose tissue, positioning it as a promising therapeutic target for metabolic disorders. In this study, we investigate the mechanism by which purine nucleotides inhibit UCP1-mediated respiration uncoupling. We previously identified two hydrophobic amino acids, I187 and W281, through molecular dynamics simulations and high-resolution respiration experiments. These residues, mutated in alanine, suppress the inhibitory effects of nucleotides on UCP1-dependent uncoupling in yeast mitochondria.

The UCP1(I187A, W281A) mutant protein was purified from yeast mitochondria and reconstituted into proteoliposomes. Proton transport assays revealed that the basal activity of the mutant matches the fatty acid-activated activity of the wild-type protein.

Using the cryo-EM structure of human UCP1, we compared the dynamics of ATP within the binding pocket of wild-type UCP1 and the UCP1(I187A, W281A) mutant. Our results demonstrate that ATP acts as a molecular switch, adopting two distinct poses: one that inhibits proton transport and another that permits it, even in the absence of fatty acids. In the UCP1(I187A, W281A) mutant, ATP predominantly adopts the non-inhibiting conformation, whereas in the wild-type protein, ATP favors the inhibiting conformation.

This model elucidates the basal uncoupling activity of UCP1 and proposes a novel mechanism for the competitive interaction between nucleotides and fatty acids.

1. Gagelin, A., Largeau, C., Masscheleyn, S., Piel, M.S., Calderón-Mora, D., Bouillaud, F., Hénin, J., and Miroux, B. (2023). Molecular determinants of inhibition of UCP1-mediated respiratory uncoupling. Nat. Commun. *14*, 2594. https://doi.org/10.1038/s41467-023-38219-9.

2. Moussa, R., Gellé, F., Masscheleyn, S., Pozza, A., Le Bon, C., Moncoq, K., Bonneté, F., and Miroux, B. (2025). Fine-tuning the yeast GAL10 promoter and growth conditions for efficient recombinant membrane protein production and purification. Protein Sci. Publ. Protein Soc. *34*, e70125. https://doi.org/10.1002/pro.70125.

PI13

Structural and functional analyses of plasma membrane sodium-proton transporters

Lukas M. Kmetsch[a,b,c], Niko Groß[a,b], Evgeny Mymrikov[a,b], Christophe Wirth[a,b], Wei-Chun Kao[a,b], Carola Hunte[a,b,c] *a Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine**

*b Faculty of Biology**

c CIBSS - Centre For Integrative Biological Signalling Studies, * University of Freiburg, Germany*

Email address: carola.hunte@biochemie.uni-freiburg.de

Presenting author: Lukas M. Kmetsch

In mammalian cells, the plasma membrane Na^+/H^+ exchanger 1 (NHE1) is a key regulator of cytosolic pH, particularly under conditions of metabolic challenge. NHE1 activity is governed by a cytosolic C-terminal domain that integrates regulatory inputs and modulates transporter conformation and activity [1].

To ensure correct cell surface localization and activity of NHE1, it is essential for the transporter to bind to one of the three calcineurin-like EF hand proteins. In addition, lipid-mediated regulation, including posttranslational lipidation and binding of specific phosphoinositides, is thought to play a central role in stabilizing distinct conformational states and adjusting transport activity. These observations underscore the importance of studying NHE1 within a native-like lipid environment to capture its physiologically relevant regulatory mechanisms, especially as current insights to this remain limited [2].

Here, we pursue an approach aimed at preserving the native membrane context during extraction and analysis of sodium-proton transporters. To establish transferable methodologies, we used the homologous prokaryotic transporter NhaA from *Salmonella* Typhimurium [3] and screened a series of 30 amphiphilic polymers of which seven were able to solubilize the homologous transporter from its membrane. We assessed the properties of these nanodiscs for size, by dynamic light scattering, and thermal stability, by nano differential scanning fluorimetry. From this we identified the best hits with currently ongoing cryogenic electron microscopy studies. This framework is complemented by functional assays designed to probe transport activity in controlled membrane environments. This is realized by using a pH sensitive fluorescent dye and reconstituting the isolated transporter into preformed liposomes.

Together, this work aims to bridge the gap between structural organization and functional regulation of sodium-proton exchangers, providing mechanistic insight into how protein-lipid and protein-protein interactions coordinate transporter activity in biological membranes.

[1] S. F. Pedersen und L. Counillon, „The SLC9A-C Mammalian Na+/H+ Exchanger Family: Molecules, Mechanisms, and Physiology - PubMed“. Zugegriffen: 27. August 2025. [Online]. Verfügbar unter: https://pubmed.ncbi.nlm.nih.gov/31507243/

[2] F. Becker *u. a.*, „Conformational regulation and target-myristoyl switch of calcineurin B homologous protein 3“, *eLife*, Bd. 12, S. e83868, Juli 2023, doi: 10.7554/eLife.83868.

[3] C. J. Lentes, S. H. Mir, M. Boehm, C. Ganea, K. Fendler, und C. Hunte, „Molecular characterization of the Na+/H+-antiporter NhaA from Salmonella Typhimurium“, *PloS One*, Bd. 9, Nr. 7, S. e101575, 2014, doi: 10.1371/journal.pone.0101575.

P114

Kinetics of UCP1 Inhibition: Guanine vs. Adenine Nucleotides in Functional Brown-Fat Mitochondria

Celso P. B. Sousa-Filhoa, Irina G. Shabalinaa , Barbara Cannona and Jan Nedergaarda

aDepartment of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, SE-106 91 Sweden

E-mail address: celso.sousa-filho@su.se
Presenting author: Celso P. B. Sousa-Filho

Uncoupling Protein 1 (UCP1) enables non-shivering thermogenesis in brown adipose tissue by dissipating the mitochondrial proton electrochemical gradient. Although UCP1 is classically inhibited by purine nucleotides, a systematic comparison across nucleotide types and mechanistic conditions has been lacking. Therefore, we comprehensively characterize the inhibitory effects of a broad panel of purine and pyrimidine nucleotides on both innate and fatty acid (oleate)-induced UCP1 activity in isolated mouse brown-fat mitochondria. Mitochondria were isolated from NMRI mice by differential centrifugation, oxygen consumption was measured polarographically utilizing an Oroboros O2k-FluoRespirometer. The incubation media contained bovine serum albumin to deplete endogenous free fatty acids, establishing innate UCP1 activity prior to rigorous titration-based inhibition analysis. Kinetic analyses were performed to determine IC_{50} values and Hill coefficients for various nucleotides, while fatty acid titration experiments assessed the resistance of nucleotide inhibition to reactivation. Kinetic analysis revealed that guanine nucleotides (GDP, GTP) are the most potent inhibitors of both innate and oleate-induced uncoupling, exhibiting significantly lower IC_{50} values and stronger cooperative inhibition (higher Hill coefficients) than adenine nucleotides (ATP, ADP). GMP displayed moderate inhibition, whereas AMP and pyrimidine nucleotides (CTP, UTP, CDP) had weak or negligible effects. A strong correlation between inhibition IC_{50} and oleate competition (EC_{50}) for GDP, GTP, and ATP - exclusively when calculated using free concentrations - supports a shared regulatory site on UCP1. Notably, ADP exhibited persistent cooperativity (Hill coefficient >1) even at free levels, suggesting a non-classical mechanism involving secondary interactions. FCCP-driven assessments confirmed that nucleotide-induced inhibition does not impair maximal oxidative capacity. Furthermore, these mouse inhibitory profiles correlate highly with classical data from hamster mitochondria. These results support a unified model in which GDP, GTP, and ATP regulate UCP1 through specific binding interactions at a primary site, with physiological potency shaped strictly by nucleotide identity, free concentration, Mg^{2+} availability, and activation context. Together, our findings refine the mechanistic understanding of UCP1 inhibition and highlight the distinct regulatory profiles of guanide and adenine nucleotides in

Topic list:

S9. Transporters and channels

Contribution type:

Poster – P

PI15

Title Mechanism and Structure of human Mitochondrial Phosphate Carrier (SLC25A3) Jiayue. Su[a], Martin S. King[a], Shane M. Palmer[a], Edmund R. S. Kunji[a]

[a]MRC Mitochondrial Biology Unit, University of Cambridge, The Keith Peters Building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, United Kingdom.

E-mail address: ersk2@cam.ac.uk
Presenting author: Jiayue Su

The mitochondrial phosphate carrier (SLC25A3, PIC), located in the mitochondrial inner membrane, imports inorganic phosphate into the matrix, thereby providing an essential substrate for ATP synthesis by the F_1F_0-ATP synthase as part of oxidative phosphorylation[1,2]. Consistent with this central bioenergetic role, impaired SLC25A3 function leads to severe mitochondrial dysfunction and is associated with autosomal-recessive mitochondrial phosphate carrier deficiency[3,4]. Thus, the carrier has important roles in both normal physiology and disease.

As a member of the SLC25 family, PIC is thought to follow the alternating access transport mechanism[5]. The transport process depends on conformational changes of PIC. Although some studies have addressed aspects of its function, the lack of a PIC structure and limited biochemical data make it difficult to explain how conformational changes occur and how transport is achieved[6].

To address these questions, we use structural and biophysical methods to show how PIC interacts with its substrate and how substrate transport is coupled to proton translocation. This study provides important insights that advance our understanding of the transport mechanism of PIC and other members of the SLC25 mitochondrial carrier family.

[1] Kunji, E.R.S., et al., The SLC25 carrier family: important transport proteins in mitochondrial physiology and pathology. Physiology (Bethesda), 2020. 35(5): p. 302-327.

[2] Kunji, E.R.S., et al., The peculiar properties of mitochondrial carriers of the SLC25 family. Biochem J. 2025 Jul 23;482(15):1047-78.

[3] Selamioglu A., et al., Expanding the Clinical Spectrum of Mitochondrial Phosphate Carrier Deficiency: A Case Report With Literature Review. Am J Med Genet A. 2026 Feb;200(2):438-445.

[4] Bhoj, E. J., et al., Pathologic Variants of the Mitochondrial Phosphate Carrier SLC25A3: Two New Patients and Expansion of the Cardiomyopathy/Skeletal Myopathy Phenotype With and Without Lactic Acidosis. JIMD Reports, 2015 19: 59–66.

[5] Ruprecht JJ, Kunji ERS. The SLC25 Mitochondrial Carrier Family: Structure and Mechanism. Trends Biochem Sci. 2020 Mar;45(3):244-258.

[6] Boulet A., et al., The mammalian phosphate carrier SLC25A3 is a mitochondrial copper transporter required for cytochrome c oxidase biogenesis.J Biol Chem. 2018 Feb 9;293(6):1887-1896.

P116

Characterisation of proposed pathogenic mutations in *SLC25A20* associated with carnitine-acylcarnitine translocase deficiency

Titus L. Mashilo, Martin S. King, Edmund R. S. Kunji
E-mail: tm813@cam.ac.uk
Presenting author: Titus Mashilo

Mitochondrial carnitine acyl-carnitine carrier (CAC) is a nuclear-encoded protein located in the mitochondrial inner membrane, which catalyses the import of acyl-carnitines from the cytosol to the mitochondrial matrix in exchange for free carnitine1 . Similar to other mammalian SLC25 carriers, the CAC has three homologous amino acid sequence repeats with the PX[DE]XX[RK] signature motif. The structural fold consists of six transmembrane helices and three short hydrophilic helices on the matrix side2 .

Carnitine acyl-carnitine translocase deficiency is a rare and life-threatening autosomal recessive disorder of fatty acid β–oxidation caused by mutations in the SLC25A20 gene. These mutations predominantly affect long-chain fatty acid metabolism, and symptoms usually manifest in early infancy with severe hypoketotic hypoglycaemia, hyperammonaemia, cardiomyopathy, and skeletal muscle weakness3,4 . This study reports the structural and biophysical characterisation of fourteen known pathogenic CAC mutations in order to understand their molecular and functional consequences better.

1. Indiveri C, Tonazzi A, Palmieri F. Identification and purification of the carnitine carrier from rat liver mitochondria. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 1990;1020(1):81-6.

2. Kunji E. 8.9 Structural and Mechanistic Aspects of Mitochondrial Transport Proteins. 2012.

3. Wendel U, Huizing M, Iacobazzi V, Ruitembeek W, Veenhuizen P, Ijlst L, et al. Carnitine. Acylcarnitine carrier deficiency: Identification of the molecular defect in a patient. JOURNAL OF INHERITED METABOLIC DISEASE. 1997;20:45.

4. Iacobazzi V, Invernizzi F, Baratta S, Pons R, Chung W, Garavaglia B, et al. Molecular and functional analysis of SLC25A20 mutations causing carnitine-acylcarnitine translocase deficiency. Human mutation. 2004;24(4):312-20.

PI17

Structural Changes during Turnover of the Human Mitochondrial Transporter ABCB10 Revealed by FTIR Difference Spectroscopy

Corinna Strothenke[a], Henry Bockhorn[a], Tilman Kottke[a]

[a]*Biophysical Chemistry and Diagnostics, Bielefeld University, Germany*

E-mail address: corinna.strothenke@uni-bielefeld.de
Presenting author: Corinna Strothenke

ATP binding cassette (ABC) transporters use the hydrolysis of ATP for the active transport of substrates across membranes. The human transporter ABCB10 is located in the inner mitochondrial membrane and protects cells from oxidative stress by exporting biliverdin. Unlike other ABC transporters, ABCB10 does not readily form the catalytically active outward-facing conformation, so structural information about its active state is missing. Here, we employed FTIR difference spectroscopy to access the outward-facing conformation under turnover conditions. By applying attenuated total reflection (ATR) FTIR spectroscopy, we non-invasively investigated full-length human ABCB10 under near-native conditions and simultaneously resolved ATP conversion, protein rearrangement and lipid response. We immobilized ABCB10 in membrane fragments on the internal reflection element and exchanged the buffer by perfusion. Activation of ABCB10 by ATP addition resulted in a reduction of α-helical signals and gain in β-sheet content as well as responses of lipid tails. The β-sheet response was assigned to dimerization of the nucleotide binding domains (NBDs) from comparison to signals observed by photolyzing caged ATP in ABCB10-NBD. Measurements under turnover conditions on full-length ABCB10 revealed the response of ABCB10 and lipids to biliverdin addition including additional changes in secondary structure and an accelerated release of the hydrolysis products. These results define the changes in secondary structure of ABCB10 that differ between futile and transport cycles, whereas lipid responses were similar in the presence and absence of substrate.

P118

The SLC7A10 amino acid transporter: new molecular insights into trafficking to plasma membrane and regulation by cholesterol.

Cesare Indiveri[a,b], Tiziano Mazza[a], Michele Galluccio[a], Lara Console[a], Martina Scarpelli[a], Raffaella Scanga[a], Mariafrancesca Scalise[a]

[a]*Department DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria, Via P. Bucci 4C, 87036 Arcavacata di Rende, Italy.*
[b]*CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnology, Via Amendola 165/A, 70126, Bari, Italy*

E-mail address: cesare.indiveri@unical.it
Presenting author: Cesare Indiveri

The relationships of membrane transporters with lipids is a very important object of investigation due the profound influence that lipids could exert on modulating transporter function and shaping their structures. This matter was recently substantiated by the discovery of lipids or lipid analogues strictly associated with several 3D structures of SLCs. The SLC7A10, also known as the Alanine Serine Cysteine 1 (ASC1) transporter, has a peculiar heterodimeric structure similarly to other few members of the SLC7 family. It forms a heterodimer with the glycoprotein CD98 (SLC3A2) stabilized by a disulfide bond, and by hydrophobic interactions. ASC1 mediates a sodiumindependent transport of L- and D-serine, L-cysteine, glycine, and is abundantly expressed in the central nervous system (CNS) and in adipose tissue. ASC1 dysfunctions correlate with neurological and metabolic diseases. Although the 3D structure of the heterodimer has recently been solved also highlighting some spots of plausible lipid nature, the role of the ancillary protein (CD98) and of the putative lipids are unknown. We employed *ex vivo*, *in vitro* and *in silico* approaches to explore these features. Using confocal microscopy and site-directed mutagenesis we demonstrated that N-glycosylated CD98 is required for routing ASC1 to the plasma membrane. We then investigated its influence on the stability of ASC1 and CD98 in HEK293 overexpressing both proteins. Depletion of cholesterol impaired the thermal stability of the heterodimer. To address the role of cholesterol on ASC1 functionality, we extracted the transporter from cells, following cholesterol depletion, and assayed its function in proteoliposomes. Cholesterol depletion strongly impaired ASC1 function. To define the effect on kinetics, recombinant cholesterol-free ASC1 was exploited. Cholesterol concentration dependent Vmax increase was observed without Km changes, indicating that the lipid facilitates the conformational transitions during the transport cycle. Cholesterol oxidation abolished the effects on transport indicating that ASC1 regulation relies on structurally specific cholesterol-protein interactions. The functional data were corroborated by molecular docking analyses identifying sterol-binding pockets, predicting a direct cholesterolprotein interaction(s). Site-directed mutagenesis of the residues predicted by the computational analysis, allowed us to identify the major ASC1 site for cholesterol interaction. The results on structure/function relationships of ASC1 with cholesterol also have potential relevance to understanding the mechanisms of human diseases associated to altered membrane composition typical of cholesterol-rich tissues, such as the nervous and adipose tissue.

P119

Bacterial production of human SLC transporters in a soluble functional state by chaperone co-expression

Michele Galluccio[a], Tiziano Mazza[a], Martina Scarpelli[a], Lorena Pochini[a,b], Cesare Indiveri[a,b]

[a]*Department DiBEST (Biologia, Ecologia, Scienze Della Terra) Laboratory, Biochemistry, Molecular Biotechnology and Molecular Biology, University of Calabria, Via P. Bucci 4C, Arcavacata di Rende, 87036, Italy*
[b]*CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnology, Via Amendola 165/A, 70126, Bari, Italy*

E-mail address: michele.galluccio@unical.it
Presenting author: Michele Galluccio

The SoLute Carrier superfamily (SLC) includes about 450 transport proteins that are key elements in maintaining cell homeostasis of amino acids, sugars, ions, vitamins, neurotransmitters, and metals [1]. For this reason, SLCs are associated with several human diseases, including many inborn errors of metabolism and neurological disorders. These aspects make transporters central to biomedical research. Although membrane proteins have been expressed using human cell lines, bacterial over-expression is a powerful tool for studying proteins since it is a low-cost and highyield system [2]. Indeed, *E. coli* has been successfully used to produce many SLC transporters even though in an insoluble form (inclusion bodies) from which the proteins need to be refolded by procedures generally based on previous urea treatment followed by on-column urea dilution and detergent exchange [2, 3]. To bypass this denaturation/refolding cycle that could be in some instances deleterious we have exploited the co-expression with DnaK, DnaJ and GrpE chaperones cloned in the pKJE7 plasmid under the control of the arabinose promoter. The selected expression host was NiCo21(DE3) a BL21-derived strain optimized for production and purification of Histagged recombinant proteins. The proposed strategy was tested on three SLC transporters from two different families (SLC22A16, SLC22A4, and SLC7A10). Interestingly, despite the high hydrophobicity of the target proteins, the co-expression of the chaperones allowed the production of the three SLC transporters in a soluble form in the absence of detergents. Several critical parameters were optimized including medium composition, IPTG and concentration, and growth temperature, each tailored for the specific transporter. The soluble fractions of induced cell lysates were purified by IMAC under native conditions during which the chaperons were released in the presence of 5 mM ATP magnesium. For SLC22A16 and SLC7A10 proteins, the simultaneous addition of 0.1% C12E8 in the presence of 0.02% cholesteryl hemisuccinate (CHEMS) was necessary to preserve the solubility, while SLC22A4 remained soluble in the absence of detergents. The functional state of the protein was confirmed after reconstitution in proteoliposomes where the transport of radiolabeled substrates was measured in the absence of interferences by other transporters. To our knowledge, this is the first example of bacterial expression of human SLC transporters in a soluble form, suitable for transport assays. The strategy may pave the way for application to other members of the SLC superfamily.

[1] E. Ferrada, G. Superti-Furga, A structure and evolutionary-based classification of solute carriers, iScience 25 (2022), 105096. https://doi.org/10.1016/j.isci.2022.105096.

[2] M. Galluccio, L. Console, L. Pochini, M. Scalise, N. Giangregorio, C. Indiveri, Strategies for Successful Over-Expression of Human Membrane Transport Systems Using Bacterial Hosts: Future Perspectives, Int J Mol Sci 23 (2022). https://doi.org/10.3390/ijms23073823.

[3] M. Galluccio, T. Mazza, M. Scalise, M. Tripicchio, M. Scarpelli, M. Tolomeo, L. Pochini, C. Indiveri, Over-Production of the Human SLC7A10 in E. coli and Functional Assay in Proteoliposomes, Int J Mol Sci 25 (2023). https://doi.org/10.3390/ijms25010536.

P120

Oxygen-dependent regulation of ion fluxes and intracellular pH in *Saccharomyces cerevisiae* exposed to acetic acid

Anahit Shirvanyan[a-c], Gohar Azbekyan[a-c], Nicoletta Guaragnella[d], Rodrigo LedesmaAmaro[e], Karen Trchounian[a-c]

[a]*Scientific-Research Institute of Biology, Faculty of Biology, Yerevan State University, 1 Alex Manoogian, 0025 Yerevan, RA*

[b]*Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 1 Alex Manoogian, 0025 Yerevan, RA*

[c]*Microbial Biotechnologies and Biofuel Innovation Center, Yerevan State University, 1 Alex Manoogian, 0025 Yerevan, RA*

[d]*Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Piazza Umberto 1, Bari, Italy*

[e]*Imperial College Centre for Engineering Biology, Bezos Centre for Sustainable Protein, Microbial Food Hub, Department of Bioengineering, Imperial College London, UK*

E-mail address: anahitshirvanyan@ysu.am
Presenting author: Anahit Shirvanyan

The study of acetic acid (AA) stress in yeast is crucial for understanding the mechanisms of acid tolerance and enhancing production efficiency. The mechanism of ion flux balance and intracellular pH regulation depending on oxygen availability remains poorly understood. Here, we investigated the combined effects of AA, acidic pH, and oxygen availability on plasma membrane Na^+, K^+, and H^+ fluxes and intracellular pH (pH_{in}) in two *S. cerevisiae* strains (ATCC 9804 and ATCC 13007) with distinct AA tolerance. The ion flux rates (J_i^+) were studied by potentiometric method using ion-selective electrodes, and the pH_{in} was determined by using spectrofluorometer via decrease in fluorescence of 9-aminoacridine upon its uptake by cells. Results showed that DCCD-sensitive J_{H^+} increased by □5.0-fold in ATCC 9804 and by □8.0-fold in ATCC 13007 under 50 mM AA stress. Total J_{Na^+} increases by 60-110% during 20-50 mM AA exposure of ATCC 9804 and ATCC 13007. DCCD-sensitive J_{K^+} was directly proportional to AA concentration up to 20 mM. Upon AA treatment, the DCCD-sensitive Na^+/H^+ ratio increased progressively, reaching up to □14.8 at 50 mM AA in both strains. The K^+/H^+ ratio was higher in control cells (□132.0 in ATCC 9804 and □180.0 ATCC 13007) under aerobic conditions, consistent with substantial K^+ influx coupled to H^+ efflux. Increasing AA concentration markedly reduced the K^+/H^+ ratio reaching up to □30.0 at 50 mM AA in both strains. ATCC 9804 maintained a pH_{in} of □6.3 in control and in the presence of 10 mM AA under aerobic conditions.
20 mM and 50 mM AA exposure decreased pH_{in} to □6.0 and to □5.9, respectively. A decrease in pH_{in} by approximately 0.4 units in ATCC 13007 was already observed at 10 mM AA. Under oxygen-limited conditions, 10 mM AA already results in 1.6- and 2.0-fold increase of $[H^+]_{in}$ in *S. cerevisiae* ATCC 9804 and ATCC 13007 strains, respectively. Thus, AA exposure increased DCCD-sensitive proton (J_{H^+}) and sodium (J_{Na^+}) fluxes while progressively impairing potassium uptake (J_{K^+}), resulting in nonlinear, strain-specific shifts in Na^+/H^+ and K^+/H^+ exchange ratios and a substantial increase in ion exchange imbalance. These effects were amplified under oxygen-limited conditions and correlated with reduced growth. Notably, the more tolerant strain maintained lower intracellular proton accumulation and more efficient ion flux coordination, particularly during aerobic growth. These insights have direct implications for strain selection and process optimization in industrial fermentations exposed to weak organic acid inhibition.

P121

Analyses of gene structure and protein sequences shed light on the evolution of the mitochondrial carrier family

Magnus Monné[a,b,*], Daniela Valeria Miniero[a,c], Luigi Palmieri[a,d] and Ferdinando Palmieri[a,d]

Affiliations

[a]Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Bari, Italy. [b]Department of Health Sciences, University of Basilicata, Potenza, Italy.

[c]Department of Medicine and Surgery, LUM University Giuseppe Degennaro, Casamassima, Italy. [d]CNR Institute of Biomembranes, Bioenergetics and Molecular

Biotechnologies (IBIOM), Bari, Italy. *Presenting author: Magnus Monné

(magnus.monne@unibas.it)

Mitochondrial carriers (MCs) belong to a eukaryotic protein superfamily of transporters, also called the solute carrier family 25 (SLC25), most of which are localized to the inner membrane of mitochondria. The protein sequences of MCs contain characteristic triplicated repeats of two transmembrane segments separated by a conserved signature motif. The three repeats make up a three-fold symmetric helical bundle with a centrallylocated substrate-binding site in the structure of the MC transporter domain. MCs transport a wide range of substrates, such as protons, inorganic ions, dicaboxylates, amino acids, nucleotides and cofactors, and may be divided into subfamilies based on their substrate specificity. We have mapped the exons in MC protein sequences of highly diversified organisms and analyzed the positions of the introns. The results demonstrate that several introns are found at identical positions within the MC transporter domain and that many of these positions are related by the three-fold symmetry. Many of these frequently occurring intron positions are particularly common among members of specific MC subfamilies and in groups of subfamilies transporting similar substrates. These findings suggest that the gene architectures of the present day MCs have been partially conserved from ancestral MCs. Based on this conclusion, a phylogenetic tree of the MC family was reconstructed taking into account the frequent and conserved intron positions as evidence for evolutionary links even between distantly-related MC homologs with low sequence similarities. Moreover, the locations of the intron positions in the MC structure imply that exon shuffling and recombination could have contributed to the diversification of substrate specificities in the evolution of the MC family. Work in progress based on analyses of sequence similarities in MC segments gives further support to these hypotheses.

P122

Human and mouse UCP1: bioenergetic similarities and discrepancies

Irina G. Shabalina[1], Luise Jacobsen[2], Glauber Rudá Feitoza Braz[1], Qimuge Naren[1], Björn Eriksson[3], Uzair Ali[1], Jiaping Li[1], Annika Ericsson[4], Barbara Cannon[1], Himanshu Khandelia[2] and Jan Nedergaard[1]

[1]*The Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden;* [2]*Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark*; [3]*Lipigon Pharmaceuticals AB, Umeå, Sweden*; [4]*CombiGene AB, Stockholm, Sweden*

E-mail address: jan.nedergaard@su.se

Presenting author: Jan Nedergaard

The structure of UCP1 has been known for several years now, but many basic functional features of this fascinating protein remain unsolved. These features include whether the high proton conductivity (that is the background for the thermogenic function of UCP1 and thus for the thermogenic function of brown adipose tissue) is an innate property of UCP1 – or whether it is induced by (membrane) fatty acids. Particularly, in reconstituted systems (e.g. black lipid membranes) UCP1 is not associated with a high proton conductivity, and the high conductivity observed in brown-fat mitochondria could therefore be a function of the particular mitochondrial membranes (and their fatty acids) in brown adipose tissue. It has also been suggested that humanUCP1 is divergent from e.g. mouse-UCP1 in that the high innate proton conductivity may not be seen in human-UCP1. There have also been suggestions that the inhibitory function of the purine nucleotide GDP is divergent between human- and mouse-UCP1 – and, in general, the way in which e.g. GDP induces inhibition of conductivity is not understood.

To approach several of these outstanding questions, we developed a system for ectopic expression of human- and mouse-UCP1 in mouse liver mitochondria through adeno-associated virus (AAV) delivery. We established through this that a high innate proton conductivity was introduced not only by mouse- but also by human-UCP1, demonstrating that the special environment of the brown-fat mitochondria (including their fatty acid profile) not is necessary for UCP1 function – and that present reconstituted systems thus are limited in reproducing innate UCP1 properties. We found that ectopic mouse-UCP1 is sensitive to GDP, as expected. Noteworthy, through molecular dynamics, we revealed a hitherto unobserved GDP-induced structural shift in UCP1, making GDP invisible from the outer compartment. This structure could be the background for the classical "(un)masking" phenomenon, as well as for other known structural changes, and it may indeed represent a "closed" configuration of UCP1. Human-UCP1 was indeed much less sensitive to GDP than was mouse-UCP1 – but ADP/ATP could fulfil the role as endogenous inhibitor of human-UCP1 activity. Through molecular dynamics we identified the amino acid mutation that was mainly responsible for this alteration in nucleotide sensitivity; the evolutionary significance of this mutation is unknown. Although we present important advances in understanding the inhibitory control of UCP1, main issues remain: is the physiological activator of UCP1 really fatty acids, moving through suggested pathways that are remarkable similar between the different UCPs and closely related proteins (UCP1/2/3, AAC) – or has the uniquely thermogenic UCP1 developed a unique stimulatory mechanism?

P123

Structural Insights into the Twin-Arginine Translocase (Tat) Receptor and Its Substrate Recognition Mechanism

Ziyu Zhao, Leonid Sazanov

Institute of Science and Technology Austria, 3400 Klosterneuburg, Austria.

E-mail: Ziyu.Zhao@ist.ac.at, sazanov@ist.ac.at
Presenting author: Ziyu Zhao

The twin-arginine translocase system uniquely transports fully folded proteins across biological membranes without compromising membrane integrity, yet its mechanism has remained elusive. The Tat system recognises substrates via a conserved twin-arginine signal peptide and operates across bacteria and plant organelles, powered by the proton motive force. Here, we present a cryogenic electron microscopy (cryo-EM) structure of the *Escherichia coli* trimeric $TatB_3C_3$ complex bound to its substrate SufI, assembled *in vivo*. The complex adopts an unexpected wide-open, bowl-shaped architecture, with potential membrane-thinning features and a polar inner cavity. Strikingly, the substrate engages the complex through a dual-contact mode: the signal peptide binds within one TatBC unit, while the folded domain interacts with an adjacent unit. This arrangement suggests a potential proofreading mechanism for substrate quality control. Our findings provide a structural framework for understanding substrate recognition and engagement by the Tat system, and support a model in which the entire Tat complex directly participates in substrate translocation.

P124

Measurement of basal proton leak through Uncoupling Protein 1

Luise Jacobsen, Peter Reinholdt and Himanshu Khandelia

Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, Odense 5230 M, Denmark

E-mail address: hkhandel@sdu.dk
Presenting author: Himanshu Khandelia

Uncoupling Protein 1 (UCP1) dissipates the mitochondrial proton gradient to generate heat, thereby short-circuiting ATP synthesis, and consuming glucose from the bloodstream. Thus, UCP1 is an important anti-obesity target. However, the molecular basis of proton leak remains debated. One of the key questions in the area is whether or not UCP1 has a ”basal” conductance, without the need for activation by fatty acids. We test the idea if transient water wires can form across UCP1 and provide a direct pathway for proton translocation [1].

We performed an aggregate of 20 µs all-atom molecular dynamics simulations of cryo-EM structures of UCP1 in di!erent functional states (apo, ATP-bound, and fatty-acid-bound) embedded in a realistic inner mitochondrial lipid bilayer [2]. We launched large ensembles of short QM/MM simulations to directly assess proton transport competence starting from snapshots containing pre-formed water wires. In these simulations, we placed an excess proton in the intermembrane space bulk water and monitoring proton migration.

We find three symmetrically positioned water grooves (WG) in UCP1, one of which is blocked by a Phe residue (Phe32). In WG3, across multiple states, we find a deeply buried hydration site in the central substrate-binding cavity (water site 1, WS1) stabilised by residues on TM5 and TM6 (notably S231 and the K269 backbone), with residence times reaching 100s of nanoseconds. The site coincides with an unassigned density in the apo cryo-EM map [3, 4]. Importantly, the binding of fatty-acid enhances the probability of forming continuous water wires. QM/MM simulations show that these transient wires can support proton leakage events across the protein consistent with a Grotthuss-like mechanism. The wire-prone grooves overlap with known cardiolipin interaction motifs, suggesting a link between lipid binding and hydration pathways. Finally, phenylalanine substitutions at key stabilising positions (S231F and Q132F/S231F) reduce hydration/wire formation.

Our data support a model in which there is significant basal water leak through UCP1, without need for fatty acids.

References

[1] Jonathan H Borowsky and Michael Grabe. Interactions of arachidonic acid with AAC1 and UCP1. Int. J. Mol. Sci., 26(21):10504, October 2025.

[2] Luise Jacobsen, Sneha Menon, Michael James Gaudry, Ali Asghar Hakami Zanjani, PeterReinholdt, Martin Jastroch, and Himanshu Khandelia. A novel model for proton transport mediated by uncoupling protein 1. Protein Sci., 34(11):e70357, November 2025.

[3] Yunlu Kang and Lei Chen. Structural basis for the binding of DNP and purine nucleotidesonto UCP1. Nature, 620(7972):226–231, June 2023.

[4] Scott A. Jones, Prerana Gogoi, Jonathan J. Ruprecht, Martin S. King, Yang Lee, ThomasZ¨ogg, Els Pardon, Deepak Chand, Stefan Steimle, Danielle M. Copeman, Camila A.
Cotrim, Jan Steyaert, Paul G. Crichton, Vera Moiseenkova-Bell, and Edmund R. S.
Kunji. Structural basis of purine nucleotide inhibition of human uncoupling protein 1.
Science Advances, 9(22):eadh4251, June 2023.

P125

Hnd, an electron-bifurcating hydrogenase, is involved in bacterial motility in *Solidesulfovibrio fructosivorans*

Arlette Kpebe[a], Olivia di Valentin[a,b], Natalie Payne[a], Chloé Guendon[a], Carole Baffert[a], Régine Lebrun[c], Sylvain Davidson[d], Yannick Combet-Blanc[d], Emmanuel Talla[b], Myriam Brugna[a]

[a] *Laboratoire de Bioénergétique et Ingénierie des Protéines, UMR7281, CNRS/AMU, 13402, Marseille, France* [b] *Laboratoire de Chimie Bactérienne, UMR7283, CNRS/AMU, 13402, Marseille, France* [c] *Plateforme Protéomique de l'IMM, FR3479, CNRS/AMU, 13402, Marseille, France*

[b] *Institut Méditerranéen d'Océanologie, UMR7294, CNRS/AMU//Univ. Toulon/IRD, 13288, Marseille, France*

E-mail address: mbrugna@imm.cnrs.fr
Presenting author: Myriam Brugna

Hydrogen metabolism is central to sulfate-reducing bacteria and relies on hydrogenases, which reversibly catalyze the oxidation of H_2 and the reduction of protons. *Solidesulfovibrio fructosivorans*, our model organism, possesses a complex hydrogenase system comprising six distinct enzymes located in different cellular compartments[1]. While this multiplicity and diversity of hydrogenases enable the bacterium to rapidly adapt its metabolism in response to environmental changes, they also complicate efforts to elucidate the physiological functions of these enzymes. Indeed, the roles of most hydrogenases in the energy metabolism of *S. fructosivorans* remain unclear.

In recent years, our research has focused on one hydrogenase in particular: the tetrameric cytoplasmic FeFe hydrogenase Hnd. We previously characterized this enzyme as a flavin-based electron-bifurcating hydrogenase[2], coupling the exergonic reduction of NAD^+ to the endergonic reduction of ferredoxin using electrons derived from H_2. Regarding its physiological role, we demonstrated that Hnd is involved in ethanol metabolism[3, 4]. Hnd is essential for *S. fructosivorans* growth on ethanol, and we proposed that it produces H_2 from NADH and reduced ferredoxin generated by an alcohol dehydrogenase and an aldehyde ferredoxin oxidoreductase during the conversion of ethanol to acetate. Hnd functions as a reversible hydrogenase: it consumes H_2 via an electron-bifurcating mechanism during pyruvate fermentation and produces H_2 through an electron-confurcating mechanism when ethanol serves as the electron donor.

To further clarify the physiological role of Hnd, we employed an omics approach. Here, we present the first results from quantitative proteomics, comparing the wild-type strain to an *hnd* deletion mutant. These findings confirm the involvement of Hnd in ethanol metabolism and, intriguingly, suggest a new function for Hnd in bacterial motility.

[1] C. Baffert, A. Kpebe, L. Avilan, M. Brugna, Hydrogenases and H_2 metabolism in sulfatereducing bacteria of the *Desulfovibrio* genus, Adv. Microb. Physiol. 74 (2019) 143-189. https:// doi.org/10.1016/bs.ampbs.2019.03.001

[2] A. Kpebe, M. Benvenuti, C. Guendon, A. Rebai *et al*., A new mechanistic model for an O_2protected electron-bifurcating hydrogenase, Hnd from *Desulfovibrio fructosovorans* BBA-Bioenergetics 1859 (2018) 1302-13012. https://doi.org/10.1016/j.bbabio.2018.09.364

[3] N. Payne, A. Kpebe, C. Guendon, C. Baffert *et al*., The electron-bifurcating FeFe-hydrogenase Hnd is involved in ethanol metabolism in *Desulfovibrio fructosovorans* grown on pyruvate, Mol. Microbiol. 117 (4) (2022) 907-920. DOI: 10.1111/mmi.14881

[4] A. Kpebe, C. Guendon, N. Payne, J. Ros *et al*. An essential role of the reversible electronbifurcating hydrogenase Hnd for ethanol oxidation in *Solidesulfovibrio fructosivorans*, Front. Microbiol 14 (2023) 1139276. https://doi.org/10.3389/fmicb.2023.1139276

P126

Role of FocA and FocB formate channels in proton flux of Escherichia coli during mixedcarbon fermentation at pH 5.5

Anush Babayan[1,2,3], Anait Vassilian[2], Anna Poladyan[1,2], Karen Trchounian[1,2,3]

[1]Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 0025 Yerevan, Armenia
[2]Scientific-Research Institute of Biology, Faculty of Biology, Yerevan State University, 0025 Yerevan, Armenia
[3]Microbial Biotechnologies and Biofuel Innovation Center, Yerevan State University, 0025 Yerevan, Armenia

E-mail address: a.babayan@ysu.am Presenting author:
Anush Babayan

Escherichia coli translocates formate/formic acid bidirectionally across the cytoplasmic membrane via the FocA and FocB formate channels during fermentation, which play an important role in pH regulation and membrane bioenergetics [1]. Depending on external pH and whether formate is supplied exogenously or generated intracellularly, the mechanisms of translocation differ.

This study aimed to characterize total proton flux (ΔJH_+) and its FOF_1-ATPase–dependent component in Escherichia coli BW25113 (wild-type) and ΔfocA, ΔfocB, and ΔfocAfocB mutants during anaerobic fermentation at pH 5.5. Cells were grown in peptone medium supplemented with glucose (11.1 mM), glycerol (137 mM), or formate (10 mM). Proton translocation was measured using a selective pH electrode, and ATPase involvement was assessed with 0.2 mM N,N′dicyclohexylcarbodiimide (DCCD).

When during experiments glucose was supplemented as a carbon fermentation, wild-type cells exhibited a total JH_+ of 0.55 mM min^{-1} per 10^9 cells. JH+ decreased by approximately 15% in the focA mutant, whereas the focB mutant showed an approximately 22% increase compared with the wild type, indicating differential contributions of the formate channels under acidic conditions. DCCD treatment revealed a significant ATPase-dependent component of proton flux. In wild-type cells, the DCCDsensitive part accounted for approximately 40% of total JH_+, whereas higher ATPase-dependent contributions were observed in the focA ~72% and focAfocB ~71% mutants, suggesting compensatory activation of FOF_1-ATPase when formate channel function is impaired.

With glycerol, proton fluxes were markedly lower (0.03 - 0.08 mM min^{-1} per 10^9 cells), emphasizing the importance of glucose for proton translocation under acidic conditions.

Exogenous formate induced net inward proton flux in all strains. Wild-type cells displayed a total JH_+ of -0.07 mM min^{-1}, whereas focB and focAfocB mutants showed pronounced inward fluxes (0.27 and - 0.34 mM min^{-1}), corresponding to approximately four- and five-fold increases relative to the wild type. The DCCD-sensitive component under formate conditions was negligible in most strains, indicating that proton influx was largely independent of FOF_1-ATPase and likely associated with formic acid transport through membrane channels.

Overall, during glucose fermentation, formic acid neutralization likely occurs not only through FocA and FocB channels but also via hydrogenase activity and the membrane-associated FOF_1-ATPase. In contrast, when exogenous formate is present, the mechanisms of formic acid neutralization appear to differ.

[1] L. Grigoryan, A. Babayan, A. Vassilian, A. Poladyan, G. Sawers, K. Trchounian, Escherichia coli FocA/B-dependent H^+ and K^+ fluxes: Influence of exogenous versus endogenous formate, Biophysical Reports 5 (2025) 100225.https://doi.org/10.1016/j.bpr.2025.100225

P127

Structural and Functional Characterization of MdaB from *Helicobacter pylori*

Arkadiusz Borek[a] and Piotr Wilk[b]

[a]Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

[b]Małopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland E-mail address: arkadiusz.borek@uj.edu.pl (A. Borek)

MdaB from *Helicobacter pylori* is an enzyme classified within the family of quinone reductases, specifically as an NAD(P)H-dependent oxidoreductase, and is implicated in the protection of bacterial cells against oxidative stress. It contributes to bacterial survival by helping cells withstand reactive oxygen species generated by the host immune system, maintaining redox balance, and enhancing virulence and colonization capacity. MdaB has also been investigated as a potential therapeutic target, as inhibition of this protein could compromise bacterial fitness.

Previous studies suggest quinone reductase activity of MdaB based on coupled assays; however, direct evidence of quinone reduction and product formation remains limited. To gain a deeper understanding of MdaB, we performed a full characterization of the protein. Its tertiary structure was determined for the first time using X-ray crystallography. In addition, comprehensive analyses of its enzymatic activity revealed previously unreported reactions that the protein may catalyze.

P128

Interplay Between FocA-Mediated Formate Transport and F_OF_1-ATPase in Regulating Proton Flux in *Escherichia coli*

Lilit G. Grigoryan [a,b,c], Liana M. Vanyan [a,b,c], Gary R. Sawers[d], Karen A. Trchounian [a,b,c]

[a]Department of Biochemistry, Microbiology, and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan, Armenia, [b]Research Institute of Biology, Faculty of Biology, YSU, Yerevan, Armenia, [c]Microbial Biotechnologies and Biofuel Innovation Center, YSU, Yerevan, Armenia [d]Institute for Biology/ Microbiology, Martin-Luther University Halle-Wittenberg, Kurt-Mothes-Str. 3, 06120, Halle (Saale), Germany

E-mail address: lilithgyan@gmail.com, lilitgrigoryan@ysu.am
Presenting author: Lilit G. Grigoryan

FocA is a bidirectional channel responsible for transport of formate/formic acid across bacterial membranes during anaerobic growth [1]. This study examined the role of FocA in proton flux (J_{H+}) in stationary-phase *Escherichia coli* cells grown in peptone medium supplemented with 2 g L^{-1} glucose. During the assay either 2 g L^{-1} (low) or 8 g L^{-1} (high) glucose was added. *N,N'*-dicyclohexylcarbodiimide (DCCD) was used to determine the contribution of F_OF_1-ATPase in total J_{H+}. Flux was measured using pH electrode (Hanna HI 1131, Portugal). The following strains were analyzed: DH4100 (parental strain), DH701 (*ΔfocA*), DH4200
($focA_{H209N}$), DH4300 ($focA_{T91A}$), DH4400 ($focA_{H209C}$), DH5000 (*ΔfhlA*) [1, 2].

In DH4100, J_{H+} was 1.32 mM min^{-1} per 10^9 cells with ~25% contribution from F_OF_1ATPase. The addition of high glucose did not significantly alter flux. In DH701 F_OF_1-ATPase contribution increased by ~40% under low glucose conditions. However, upon addition of high glucose J_{H+} decreased by ~40%, and F_OF_1-ATPase did not contribute to flux. DH4200 ($FocA_{H209N}$) displayed ~40% reduced J_{H+} during addition of both glucose concentrations, showing decreases in both DCCD-insensitive and F_OF_1-ATPase dependent components. In DH4300, F_OF_1-ATPase contribution increased by ~20% compared to DH4100 under low glucose, and high glucose reduced both total J_{H+} and F_OF_1-ATPase contribution by ~20%. In DH4400 F_OF_1-ATPase contribution was reduced by ~90% and ~85% compared to DH4100 under low and high glucose conditions respectively. In DH5000 (*ΔfhlA*), J_{H+} was ~30% lower compared to DH4100. The DCCD-insensitive component decreased by ~30% at both glucose concentrations, while F_OF_1-ATPase input decreased by ~20% under low glucose, and ~60% under high glucose.

Taken together, these results indicate that in strains defective in FocA-mediated formate efflux (DH701, DH4300) [3], F_OF_1-ATPase contribution to proton flux increases under low glucose conditions, possibly functioning as a compensatory mechanism to regulate intracellular pH, consistent with previously reported lower intracellular pH in these strains [1]. Notably, FoF_1ATPase contribution decreases upon the addition of high glucose. One possible explanation for the reduced F_OF_1-ATPase contribution under high glucose conditions is activation of alternative proton-balancing pathways. In contrast, strains restricted to formate export, without reimport capacity (DH4200, DH4400) [1, 2] show reduced F_OF_1-ATPase contribution compared to parental strain. The inability to reimport formate may alter proton flux dynamics without significantly affecting intracellular pH [1], thereby lowering the requirement for F_OF_1-ATPase dependent proton efflux. These findings support a functional link between FocA-mediated formate transport, F_OF_1-ATPase -dependent proton export, and glucose concentration in the media.

[1] Vanyan Liana, Kammel Michelle, Sawers Gary, and Trchounian Karen, "Evidence for bidirectional formic acid translocation in vivo via the Escherichia coli formate channel FocA," *Arch. Biochem. Biophys.*, vol. 752, Feb. 2024.

[2] M. Kammel, O. Trebbin, R. G. Sawers, and G. Sawers, "A FocA variant incapable of formate import but retaining formic acid efflux highlights the distinct mechanisms governing bidirectional formate translocation Title: FocA switches translocation mechanism depending on import or efflux direction Corresponding Author."

[3] M. Kammel and R. Gary Sawers, "The FocA channel functions to maintain intracellular formate homeostasis during Escherichia coli fermentation," *Microbiology (United Kingdom)*, vol. 168, no. 4, 2022, doi: 10.1099/mic.0.001168.

P129

Glucose availability and CRP regulation shape redox balance and proton flux in fermenting *Escherichia coli*

Lilit G. Grigoryan [a,b,c], Liana M. Vanyan [a,b,c], Karen A. Trchounian [a,b,c]

[a]Department of Biochemistry, Microbiology, and Biotechnology, Faculty of Biology, Yereva n State University, Yerevan, Armenia,
[b]Research Institute of Biology, Faculty of Biology, YSU, Yerevan, Armenia, [c]Microbial Biotechnologies and Biofuel Innovation Center, YSU, Yerevan, Armenia

E-mail address: k.trchounian@ysu.am
Presenting author: Karen A. Trchounian

During anaerobic fermentation different reducing equivalents and organic acids are generated[1]. CRP-dependent regulation of these pathways[2] can influence intracellular redox balance and proton load, potentially linking carbon availability to systems involved in proton motive force (*pmf*) maintenance and ion transport. In this study, we investigated NAD^+/NADH concentrations and proton flux rate (J_{H^+}) in *Escherichia coli* BW25113 parental strain and *Δcrp* mutant during fermentation of different glucose concentrations.

Total NAD concentrations in cells grown without glucose or with 2 g L^{-1} glucose did not differ between parental strain and *Δcrp*. However, growth at 8 g L^{-1} glucose significantly increased total NAD by 65% and 50% in BW25113 and *Δcrp*, respectively. Larger NAD(H) pool size is connected with high glycolytic flux occurring at high glucose concentrations.

Increasing glucose concentration decreased NAD^+/NADH ratio, indicating a shift toward more reduced intracellular state. Intracellular NADH levels in the *Δcrp* mutant were increased compared to the parental strain, being ~50% higher in glucose-free medium and ~25% higher when 2 g L^{-1} glucose was present. In contrast, when cells were grown with 8 g L^{-1} glucose, NADH levels were similar between two strains.

In the parental strain, total J_{H^+} remained relatively stable (1.3-1.6 mmol min^{-1} per 10^9 cells) under different growth conditions. In *Δcrp* mutant grown without glucose, addition of 2 g L^{-1} glucose resulted in the absence of F_OF_1-ATPase contribution to J_{H^+}. In the parental strain, proton recycling occurs through hydrogenase-dependent H_2 cycle where H_2 oxidation contributes to NAD^+ reduction, followed by NADH oxidation coupled with proton translocation through F_OF_1-ATPase. Decreased NAD^+/NADH ratio and absence of F_OF_1-ATPase mediated H^+ flux in the *Δcrp* mutant suggests that this mechanism operates less efficiently in the absence of CRP regulation. When 8 g L^{-1} glucose was added the flux through F_OF_1-ATPase in mutant did not differ from the parental strain. Possibly, addition of high glucose resulted in acidification of intracellular space, and F_OF_1-ATPase acted as a compensatory mechanism.

Taken together these results indicate that increasing glucose availability shifts the intracellular redox state toward more reduced conditions in *E. coli* and alters the mechanisms maintaining proton flux. In the absence of CRP regulation, cells maintain more reduced intracellular environment under carbon-limited conditions and display altered contributions of F_OF_1-ATPase to H^+ flux, suggesting that CRP contributes to finetuning of cellular redox balance and proton homeostasis during fermentation.

[1] T.J. Hackmann, The vast landscape of carbohydrate fermentation in prokaryotes, FEMS Microbiology Reviews 48(4) (2024), https://doi.org/10.1093/femsre/fuae016

[2] K. Kochanowski, H. Okano, V. Patsalo, J. Williamson, U. Sauer, T. Hwa, Global coordination of metabolic pathways in Escherichia coli by active and passive regulation, Mol Syst Biol 17(4) (2021), https://doi.org/10.15252/msb.202010064

P130

Anion and cation fluxes in *Escherichia coli* during glucose fermentation and implications for bioenergetic coupling

Liana Vanyan[a,b,c], Liza Jorkntsyan[a,b,c], Sunil Nath[d], Karen Trchounian[a,b,c]

[a]Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia [b]Scientific-Researach Institute of Biology, Yerevan State University, Yerevan, Armenia [c]Microbial Biotechnologies and Biofuel Innovation Center, Yerevan State University, Yerevan, Armenia
[d]Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, India

E-mail address: liana.vanyan@ysu.am

Presenting author: Liana Vanyan

Glucose fermentation in *Escherichia coli* produces organic acids such as formate, acetate, lactate, and succinate that must be transported across the cytoplasmic membrane. Under fermentative conditions, the F_OF_1-ATPase operates in ATP hydrolysis mode and contributes to proton translocation, yet the relationship between proton flux and transport of cations and anions remains insufficiently understood. In this study, cation and anion fluxes in whole *E. coli* cells were analyzed during glucose fermentation at pH 7.5.

Proton and potassium flux rates (J_{H^+} and J_{K^+}) were monitored using ion-selective electrodes, while samples for HPLC analysis were simultaneously collected from the same cell suspension during ion flux measurements to quantify the efflux of organic acids (formate, acetate, lactate, and succinate). Cells grown at different glucose concentrations low (2 g L^{-1}) and high (8 g L^{-1}) were harvested during stationary phase and during assays low glucose was supplemeted to monitor the kinetics. The contribution of F_OF_1-ATPase was estimated using inhibitor N,N′dicyclohexylcarbodiimide (DCCD).

When cells were grown with 2 g L^{-1} glucose, the total J_{H^+} was 5.82×10^{-3} mmol min^{-1} per 10^9 cells. Addition of DCCD reduced the proton flux to 3.80×10^{-3} mmol min^{-1}, corresponding to a DCCD-sensitive component of approximately 2.02×10^{-3} mmol min^{-1}. Under the same conditions, K^+ uptake occurred at rates of $0.95–1.03 \times 10^{-3}$ mmol min^{-1}. Initial efflux rates of fermentation-derived organic anions were 3.62×10^{-3} mmol min^{-1} for formate, 2.50×10^{-3} for acetate, 3.67×10^{-3} for lactate, and 1.19×10^{-3} for succinate. In the presence of DCCD, anion efflux rates decreased markedly to 0.42×10^{-3}, 0.18×10^{-3}, 0.44×10^{-3}, and 0.12×10^{-3} mmol min^{-1} for formate, acetate, lactate, and succinate, respectively.

At higher glucose concentrations, similar relationships between proton and ion fluxes were observed. DCCD-sensitive J_{H^+} increased to approximately 3.20×10^{-3} mmol min^{-1}. Under these conditions, the combined flux of succinate efflux and K^+ influx was $2.69 \pm 0.27 \times 10^{-3}$ mmol min^{-1}, again showing close quantitative agreement with the proton efflux rate. These results indicate that the coupling between F_OF_1-ATPase-mediated proton translocation and coordinated succinate/K^+ transport is maintained across different glucose concentrations.

These results demonstrate that the DCCD-sensitive proton efflux mediated by F_OF_1-ATPase is closely balanced by coordinated succinate efflux and K^+ influx, suggesting a coupled ion-transport mechanism that contributes to charge balance and energy transduction during fermentative metabolism in *E. coli*.

P131

Regulation of growth and O_2-tolerant [NiFe]-hydrogenase activity by the proton motive force and FoF_1-ATP synthase in *Cupriavidus necator* H16

Anna Poladyan[a,b], Ruzanna Arshakyan[a], Meri Iskandaryan[a,b]

[a] Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan, Armenia

[b] Research Institute of Biology, YSU, Yerevan, Armenia

E-mail address: apoladyan@ysu.am
Presenting author: Anna Poladyan

The chemolithoautotrophic *Cupriavidus necator* H16 plays an important role in the global hydrogen (H_2) cycle through its oxidation by O_2-tolerant [NiFe]-hydrogenases (Hyds). H_2 metabolism contributes significantly to cellular bioenergetics. Therefore, Hyds represent key components of bacterial homeostasis, enabling cells to maintain metabolic balance under different environmental and cultivation conditions. In this study, role of proton motive force and F_0F_1-ATP- synthase on growth and activity of Hyds of *C. necator* H16 were evaluated under different growth conditions.

Bacteria were cultivated micro-aerobically in glycerol-fructose-nitrogen (GFN) medium in the presence and absence of 1.5mM sodium azide (NaN_3), 50μM carbonyl cyanide-mchlorophenyl-hydrazone (CCCP) and N,N′-dicyclohexylcarbodiimide (DCCD). Whole-cell extracts, cytoplasmic and membrane fractions were treated with inhibitors at final concentrations of 4-10 mM. Heterotrophic growth of bacteria was monitored for 168h, 30°C, 150rpm. H_2-oxidizing Hyds activity in whole-cell extracts and membrane fractions (MBH) was determined by monitoring methylene blue reduction at 570nm. Activity in the cytoplasmic fraction (SH) was measured by NAD^+ reduction at 365nm at 30°C (Cary60 UV–Vis spectrophotometer).

The addition of inhibitors to the cultivation medium resulted in strong growth inhibition (50-90%) during the 1st days of cultivation. However, by the 7thday the inhibitory effect decreased to 18-27%. A slight decrease in oxidation-reduction potential (ORP) was observed from the 3rd day of cultivation, corresponding to growth stimulation and the transition to the stationary phase. In control and DCCD-containing cultures, a slight decrease in pH was observed from the 1stday of growth, whereas in CCCP- and NaN_3-treated cultures pH reduction was detected only from the 3rd and 4thdays, respectively. Analysis of H_2-oxidizing activity of whole-cell extracts revealed a 1677% decrease in Hyds activity depending on the inhibitor used and the bacterial growth phase. On the 7th day, MBH activity in the control GFN culture reached 14.2U (min, mg protein)$^{-1}$, whereas SH activity was 4.7U (min, mg protein)$^{-1}$. In inhibitor-treated samples, MBH activity decreased by 37-44%, while SH activity showed stronger inhibition ranging from 61-91%.

Treatment of whole-cell extracts showed concentration-dependent inhibition ranging from 23-100%, with complete inhibition observed only after DCCD treatment on days 3-4 of growth. In contrast, no detectable inhibition was observed in isolated cytoplasmic or membrane fractions. The results demonstrate that the activity of O_2-tolerant-Hyds in *C. necator* H16 is tightly regulated by the proton motive force, with FoF_1-ATP-synthase playing a central role in maintaining this electrochemical gradient, thereby indirectly modulating Hyd function and cellular energy balance.

.

P132

The role of Gad acid resistance system in the proton/potassium fluxes in *Escherichia coli* during fermentation

Heghine Gevorgyan[a,b], Stella Hakobyan[b], Meri Hayrapetyan[b], Sophie Brameyer[c], Kirsten Jung[c], Karen Trchounian[a,b]

[a] *Research Institute of Biology, Faculty of Biology, Yerevan State University, Yerevan 0025, Armenia;*

[b] *Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, Yerevan 0025, Armenia;*

[c] *Faculty of Biology: Microbiology, Ludwig-Maximilians-Universität München, Martinsried 82152, Germany*

E-mail address: heghine.gevorgyan@ysu.am

Presenting author: Heghine Gevorgyan

In *Escherichia coli*, the glutamate decarboxylase (Gad) system is a primary mechanism for maintaining intracellular pH during severe acid stress by consuming cytoplasmic protons during the conversion of glutamate to γ-aminobutyric acid (GABA), which is then exported via the GadC antiporter [1]. Moreover, proton efflux is coupled to potassium uptake during fermentation, a process mediated by the F_OF_1-ATPase [2]. In this study, the role of Gad system in the proton (J_{H^+}) and potassium flux rate (J_{K^+}) was determined at early stationary phase (3.5 h) of fermentative growth across a pH gradient representing various stress levels: pH 7.6 (no acid stress), 6.5 (mild), 5.8 (moderate), and 5.4 (strong). *Escherichia coli* K12 MG1655 wild type (WT) and MG1655 *ΔgadE* mutant were used. To understand the role of proton F_OF_1-ATPase in the flux rate, *N,N′*dicyclohexylcarbodiimide (DCCD) was used. It was shown that overall J_{H^+} was 3.68 mmol min^{-1} per 10^9 cells in WT under no acid stress condition, DCCD-sensitive flux was ~25%. Overall J_{H^+} was reduced with increasing acid stress level. The contribution of DDCD-sensitive J_{H^+} was also increased, reaching ~50%, ~80% and ~85% under mild, moderate and strong acid stress conditions, accordingly. Proton efflux was coupled to potassium uptake. Overall J_{K^+} was ~0.09 mmol min^{-1} per 10^9 cells in WT under no acid stress condition. It was decreased with rising acid stress level. DCCD treatment reversed the direction of K^+ flux in all conditions, reinforcing the model that K^+ uptake is coupled with H^+ efflux via the F_OF_1 [2]. Significant variation was determined in the *ΔgadE* mutant. While baseline J_{H^+} in the mutant remained similar to the WT, J_{K^+} was reduced by ~90% under no acid stress condition. Overall J_{H^+} was decreased in the *ΔgadE* under mild acid stress by ~30%, compared to WT, however, it was increased by ~50% and ~25% under moderate and strong acid stress conditions, accordingly. Moreover, the contribution of F_OF_1 was not significantly changed under moderate and strong acid stress. *ΔgadE* exhibited potassium uptake during acid stres conditions. It was decreased under mild (by ~75%) and strong acid stress (by ~40%) condition, compared to WT. The results demonstrate that the Gad system and F_OF_1ATPase work coordinately to regulate ion flux. Furthermore, the significant alterations in potassium and proton exchange within the *ΔgadE* mutant highlight GadE as a critical regulator for maintaining ion homeostasis and coupling during the bacterial acid stress response during fermentation.

[1] J. Schwarz, K. Schumacher, S. Brameyer, K. Jung, Bacterial battle against acidity. FEMS Microbiol. Rev. 46 (6), (2022), fuac037, https://doi.org/10.1093/femsre/fuac037

[2] A. Trchounian, K. Trchounian, Fermentation revisited: how do microorganisms survive under energy-limited conditions? Trends Biochem. Sci. 44 (2019) 391-400. https://doi.org/10.1016/j.tibs.2018.12.009

P133

Biochemistry and physiology of ubiquinone biosynthesis under anaerobic conditions

Katayoun Kazemzadeh[a], Sophie-Carole Chobert[a], Sophie Abby[a], Emmanuelle Bouveret[b], Murielle Lombard[c], Marc Fontecave[c], Frédéric Barras[b], Ludovic Pelosi[a] and Fabien Pierrel[a]

[a] *TIMC-TrEE, CNRS-Université Grenoble Alpes, 38000 Grenoble, France* [b] *SAMe Unit, Département de Microbiologie, Institut Pasteur, Paris, France* [c] *LCPB, Collège de France, Paris, France*

E-mail address: fabien.pierrel@univ-grenoble-alpes.fr
Presenting author: Fabien Pierrel

Bacteria synthesise several types of isoprenoid quinones, which play a key role in electron transfer chains. Menaquinone, which is widely distributed in bacteria, is typically associated with anaerobic metabolism, whereas ubiquinone (UQ) is generally considered an aerobic quinone. In addition to the classical UQ biosynthetic pathway, which requires molecular oxygen (O_2), we discovered an O_2-independent pathway that depends on three genes: *ubiT*, *ubiU* and *ubiV* [1]. In *Pseudomonas aeruginosa*, these genes are essential for denitrification under anaerobic conditions, positioning them as promising targets for antimicrobial strategies against this opportunistic pathogen [2]. Our work further demonstrates that *ubiT*, *ubiU* and *ubiV* are controlled by the O_2-sensing transcription factor FNR, and that they contribute to the development of *Escherichia coli* in the mouse intestine [3]. At the molecular level, we characterised UbiU and UbiV as founding members of a novel class of O_2-independent hydroxylases, revealing their ability to utilise an organic molecule as an oxygen donor for hydroxylation reactions [4]. Large-scale genomic analysis across the *Pseudomonadota* phylum (formerly *Proteobacteria*) highlights the widespread distribution of this O_2-independent pathway, alongside a remarkable quinone profile diversity [5]. The prevalence of this pathway suggests broad physiological significance, enabling bacteria to thrive in oxygen-limited environments, ranging from host-associated niches to anaerobic biotechnological processes. Our findings not only redefine the boundaries of quinone biosynthesis but also deepen our understanding of bacterial respiration in diverse ecological contexts.

[1] L. Pelosi, C.D. Vo, S.S. Abby, L. Loiseau, B. Rascalou, M. Hajj Chehade, B. Faivre, M. Gousse, C. Chenal, N. Touati, L. Binet, D. Cornu, C.D. Fyfe, M. Fontecave, F. Barras, M. Lombard, F. Pierrel, Ubiquinone Biosynthesis over the Entire O2 Range: Characterization of a Conserved O2-Independent Pathway, mBio 10 (2019) e01319-19. https://doi.org/10.1128/mBio.01319-19.

[2] C.-D.-T. Vo, J. Michaud, S. Elsen, B. Faivre, E. Bouveret, F. Barras, M. Fontecave, F. Pierrel, M. Lombard, L. Pelosi, The O2-independent pathway of ubiquinone biosynthesis is essential for denitrification in Pseudomonas aeruginosa, J Biol Chem 295 (2020) 9021–9032. https://doi.org/10.1074/jbc.RA120.013748.

[3] R. Arias-Cartin, K. Kazemzadeh Ferizhendi, E. Séchet, L. Pelosi, C. Loeuillet, F. Pierrel, F. Barras, E. Bouveret, Role of the Escherichia coli ubiquinone-synthesizing UbiUVT pathway in adaptation to changing respiratory conditions, mBio 14 (2023) e0329822. https://doi.org/10.1128/mbio.03298-22.

[4] K.K. Ferizhendi, P. Simon, L. Pelosi, E. Séchet, R. Arulanandam, M.H. Chehade, M. Rey, D. Onal, L. Flandrin, R. Chreim, B. Faivre, S.C.-D.-T. Vo, R. Arias-Cartin, F. Barras, M. Fontecave, E. Bouveret, M. Lombard, F. Pierrel, An organic O donor for biological hydroxylation reactions, Proc Natl Acad Sci U S A 121 (2024) e2321242121. https://doi.org/10.1073/pnas.2321242121.

[5] S.-C. Chobert, M. Roger-Margueritat, L. Flandrin, S. Berraies, C.T. Lefèvre, L. Pelosi, I. Junier, N. Varoquaux, F. Pierrel, S.S. Abby, Dynamic quinone repertoire accompanied the diversification of energy metabolism in Pseudomonadota, ISME J 19 (2025) wrae253. https://doi.org/10.1093/ismejo/wrae253.

P134

Hydrothermal origin of metabolic phosphorylation

Manon L. Schlikker[a], Nadja K. Hoffmann[a], William F. Martin[a]

[a]*Institute of Molecular Evolution*
Faculty of Mathematics and Natural Sciences
Heinrich Heine University Düsseldorf
40225 Düsseldorf, Germany

E-mail address: manon.schlikker@hhu.de
Presenting author: Manon L. Schlikker

Phosphate is central to modern bioenergetics and to all theories for the origin of life. How and where phosphate entered metabolism are unknown, but microbial physiology and geochemical environments can provide important clues. Some bacteria obtain electrons and energy from phosphite (HPO_3^{2-}), a reduced form of phosphate (HPO_4^{2-}), that naturally occurs in serpentinizing (H_2-producing) hydrothermal systems. Here we show that native palladium, which also occurs in serpentinizing hydrothermal systems, catalyzes the oxidation of phosphite to phosphate and H_2 in water at 25–100 °C in a highly exergonic reaction. Phosphite oxidation over Pd in water generates a reactive but yet unknown chemical intermediate, possibly metaphosphate, $[PO_3]^-$, that phosphorylates hydroxyl moieties in a wide spectrum of biologically relevant compounds including glycerol, ribose, glucose, serine and cytidine, forming phosphoester bonds at 25–100 °C in 2–72 h. The same conditions also phosphorylate (i) phosphate residues, forming phosphoanhydride bonds in pyrophosphate, polyphosphates and ADP, (ii) creatine, forming the phosphoramidate bond in phosphocreatine, and (iii) acetate, forming acetyl phosphate. The reactions proceed in the absence of sulfur, excluding thioester or metal sulfide intermediates. Phosphite-dependent phosphorylations under conditions of serpentinization identify a natural, geochemical source of prebiotic phosphorylation and furthermore identify a source of continuous, aqueous metabolic energy that overcomes thermodynamic barriers at origins. This novel route of phosphorylation explains the centrality of phosphate in heredity and energetics as a chemical inheritance from the site where metabolism and life arose.

[1] Schlikker, M. L., Hoffmann, N. K., & Martin, W. F. (2025). Hydrothermal origin of metabolic phosphorylation. *bioRxiv*, 2025-12.

P135

Comparative Analysis of Quinone-Reducing Enzymes from *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Patrícia.Pires[a], Manuela M. Pereira[a]

[a] BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, 1749-016 Lisboa, Portugal pmpires@ciencias.ulisboa.pt

In this work we aim at investigating the molecular and cellular roles of enzymes involved in the energy metabolism of *Staphylococcus aureus* (*S.aureus*) and *Pseudomonas aeruginosa* (*P.aeruginosa*), two prominent human opportunistic pathogens, which area major public health threat due to the increased incidence of their drug resistance. Both bacteria show a great capacity to adapt to diverse environmental conditions, especially during host colonization. The adaptability of these pathogens comes from their metabolic versatility, which in part is due to their ability to use several substrates by a vast array of quinone reductases that connect different metabolic pathways to the respiratory chain. [2,3] Importantly the two bacteria produce quinones with different structures and it is known that *P. aeruginosa* also produces 2-Heptyl-4-hydroxyquinoline-N-oxide (HQNO), a quinone analogue that works as an inhibitor of most quinone interacting proteins and which confers this bacterium a competitive advantage when colonizing the same niches with other bacteria, as the case of *S. aureus*. [4] In this way, the quinone reductases from *S. aureus* and *P. aeruginosa* were selected as studying systems. These included NADH dehydrogenases type II (Ndh-2s), Dihydroorotate: quinone oxidoreductases (DHOQOs), and Glycerol-3phosphate: quinone oxidoreductases (G3PQOs), which allow comparative studies and identification of structural/functional determinants. All these enzymes are flavin-containing monotopic enzymes, meaning they are attached to a single side of the lipid membrane, that catalyze the oxidation of various substrates and concomitant reduction of a quinone to quinol. [4-7] Some of our preliminary results show that HQNO has a different effect on homologous proteins from these two pathogens. HQNO has an inhibitor effect on proteins from *S.aureus* but seems to not alter the activity of the enzymes from *P.aeruginosa.*

[1]: Calisto, F., Sousa, F. M., Sena, F. V., Refojo, P. N. & Pereira, M. M. Mechanisms of Energy Transduction by Charge Translocating Membrane Proteins. Chemical Reviews (2021);

[2]: Patrícia N. Refojo , Filipa V. Sena, Filipa Calisto, F. M. S. and M. M. P. The plethora of membrane respiratory chains in the phyla of life. Advances in Microbial Physiology vol. 74 Elsevier Ltd, (2019);

[3]: Pires, P. M., Santos, D., Calisto, F., & Pereira, M. The monotopic quinone reductases from Staphylococcus aureus. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1865(4), 149488. 8 (2024);

[4]: Sousa, F. M., Refojo, P. N. & Pereira, M. M. Investigating the amino acid sequences of membrane bound dihydroorotate:quinone oxidoreductases (DHOQOs): Structural and functional implications. Biochim. Biophys. Acta - Bioenerg. 1862, 148321 (2021);

[5]: Sousa, F. M. et al. Unveiling the membrane bound dihydroorotate: Quinone oxidoreductase from Staphylococcus aureus. Biochim. Biophys. Acta - Bioenerg. 1864, (2023);

[6]: Sena FV, Sousa FM, Pereira AR, Catarino T, Cabrita EJ, Pinho MG, Pinto FR, Pereira MM. The two alternative NADH:quinone oxidoreductases from Staphylococcus aureus: two players with different molecular and cellular roles. Microbiol Spectr. (2024);

[7]: Yeh, J. I., Chinte, U. & Du, S. Structure of glycerol-3-phosphate dehydrogenase, an essential monotopic membrane enzyme involved in respiration and metabolism. Proc. Natl. Acad. Sci. U. S. A. 105, 3280–3285 (2008).

P136

ATP synthase architecture controls cristae formation and bioenergetic remodeling in *T. brucei*

Michaela Husová[a,b], Brian Panicucci[a], Martin Zoltner[c], Richard Wheeler[e], Bill Wickstead[d], Catarina Gadelha[d], Alena Zíková[a,b]

[a] *Institute of Parasitology, Biology Centre, ASCR, Czechia* [b] *Faculty of Science, University of South Bohemia, Czechia* [c] *BIOCEV, Department of Parasitology, Charles University, Czechia;* [d] *School of Life Science, University of Nottingham, UK*

[e] *Institute of Immunology and Infection Research, University of Edinburgh, UK*

E-mail address: azikova@paru.cas.cz

Presenting author: Alena Zíková

Mitochondrial cristae architecture is a key determinant of cellular bioenergetics, yet how it is established during cellular differentiation coupled with metabolic remodeling remains incompletely understood. *Trypanosoma brucei* undergoes a remarkable transition from the glucose-dependent mammalian bloodstream form to the amino acid–utilizing insect form, accompanied by extensive mitochondrial remodeling. During this process, the mitochondrion transforms from a simple tubular organelle with sparse cristae into a highly branched network enriched in densely packed cristae. Using transmission electron microscopy, proteomics, functional assays, and an RNAi screen, we systematically map this transition and show that structural maturation of the mitochondrion is accompanied by upregulation of the mitochondrial proteome, rewiring of electron flow from alternative oxidase to cytochrome-dependent complexes III and IV, and a functional switch of ATP synthase from hydrolysis to synthesis, enabling full engagement of oxidative phosphorylation (OXPHOS). Furthermore, we identify key regulatory players and demonstrate that cristae biogenesis precedes the complete assembly and activation of OXPHOS complexes. Strikingly, disruption of ATP synthase dimerization leads to severe defects in cristae architecture, resulting in a disorganized inner mitochondrial membrane. Despite normal assembly of respiratory complexes III and IV, mutant cells display markedly reduced respiration, fail to produce ATP via OXPHOS, and instead rely on substrate-level phosphorylation. These defects are associated with reduced mitochondrial mass, impaired network branching, and increased sensitivity to glucose, indicating compromised metabolic flexibility. Notably, while ATP synthase dimer-deficient parasites remain viable and can differentiate under in vitro conditions, they fail to complete differentiation when derived from infected mammalian hosts. This reveals a critical requirement for ATP synthase–dependent cristae architecture under physiological conditions. Restoration of dimerization fully rescues mitochondrial structure and function. Together, our findings identify ATP synthase dimerization as a central driver of proper cristae biogenesis and bioenergetic remodeling, establishing a direct link between mitochondrial membrane architecture and metabolic adaptation during the parasite life cycle progression.

P137

Factors affecting the allotopic expression of subunit II (Cox2) of yeast cytochrome c oxidase

Miriam Vázquez-Acevedo[a], Patrice P. Hamel[b], and D. González-Halphen[a].

[a]*Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico*
[b]*Department of Molecular Genetics, Ohio State University, Columbus, Ohio, USA*

E-mail address: dhalphen@ifc.unam.mx
Presenting author:D. González Halphen

Experimentally relocating mitochondrial genes to the nucleus (allotopic expression) holds promise for the development of treatments of mitochondrial diseases [1]. A mutation in the mitochondrial genome, yielding a defective protein, could be overcome in principle by the import of a nucleus-encoded, functional version of the protein. The high hydrophobicity of mitochondria-encoded proteins seems to be one of the main factors preventing this allotopic expression. Here, we focused on subunit II of cytochrome *c* oxidase (Cox2) to study which modifications may enable or improve its allotopic expression in yeast. Cox2 exhibits two transmembrane stretches (TMS1 and TMS2) embedded in the inner mitochondrial membrane (IMM) and an N_{out}-C_{out} topology, with both termini exposed to the intermembrane space (IMS). Cox2 can be imported from the cytosol into mitochondria in the presence of the W56R substitution [2], which decreases the protein hydrophobicity of TMS1 and allows partial respiratory rescue of a *cox2*-null strain. We explored other reported substitutions that decrease hydrophobicity of TMS2 [3], the role of the leader peptide, and the levels of the allotopically-produced Cox2. Based on our findings, we propose a mechanistic model for the import and assembly of the allotopically produced Cox2 subunit within mitochondria. We believe our findings are relevant for the design of new allotopic expression strategies.

[1] F. Nieto-Panqueva, D. Rubalcava-Gracia, P.P. Hamel, D. González-Halphen, The constraints of allotopic expression. Mitochondrion. 2023 73, (2023), 30-50. https://doi.org/10.1016/j.mito.2023.09.004.

[2] L. Supekova, F. Supek, J.E. Greer, P.G. Schultz, A single mutation in the first transmembrane domain of yeast COX2 enables its allotopic expression. Proc. Natl. Acad. Sci. USA. 107, (2010), 5047-5052. https://doi.org/10.1073/pnas.1000735107.

[3] K. Gombeau, S.A. Hoffmann, Y. Cai, A new set of mutations in the second transmembrane helix of the Cox2p-W56R substantially improves its allotopic expression in *Saccharomyces cerevisiae*. Genetics. 229, (2025), iyaf037.
https://doi.org/10.1093/genetics/iyaf037.

P138

Insights into the assembly of yeast mitochondrial respiratory complex III

Jorge Jimenez-Niebla[a,b,c], Christophe Wirth[a] , Roshan Jha[a], Jon Busto[a], Antoine Feignier[a,b,c], Neils Wiedemann[a], Wei-Chun Kao[a], Carola Hunte[a,d]

*[a]Institute of Biochemistry and Molecular Biology, ZBMZ, Faculty of Medicine * [b]Faculty of Biology * [c]SGBM -Spemann Graduate School of Biology and Medicine * [d]CIBSS - Centre for Integrative Biological Signalling Studies * *University of Freiburg, Germany*

E-mail address: jorge.jimenez@biochemie.uni-freiburg.de, wei-chun.kao@biochemie.unifreiburg.de, carola.hunte@biochemie.uni-freiburg.de
Presenting author: Jorge Jimenez-Niebla

Cellular respiration is essential for aerobic life and relies in eukaryotes on the efficient function of the mitochondrial respiratory chain. Mitochondrial complex III (cytochrome bc_1 complex) is a central proton-translocating component of the respiratory chain that generates proton motive force driving ATP synthesis and participates in the formation of respiratory supercomplexes. Proper function of complex III depends on its accurate assembly, and defects in this process are linked to human disease. The biogenesis of mitochondrial respiratory complex III is a tightly regulated, multistep pathway involving several assembly factors and chaperones, proceeding through defined intermediates as individual subunits are incorporated. However, key steps in this process remain poorly understood, largely due to limited structural information.
Here, we combine biochemical and structural approaches to elucidate the molecular mechanism of complex III assembly. Using cryogenic electron microscopy, we provide structural insights into assembly intermediates in *Saccharomyces cerevisiae*, with a particular focus on previously unresolved steps in the assembly pathway.

P139

Repair and Modular Turnover Dynamics of Mitochondrial Complex I: Differential Responses to Loss of the High-Turnover Subunits NDUFA6 and NDUFA7

Paulina Castañeda-Tamez[a], Matthias S. Leisegang[a,] Frederike Lam[a], Ralf P. Brandes[a,] Ilka Wittig[ab]

[a]Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Germany [b]Goethe University, Center for Functional Proteomics, Frankfurt, Germany

E-mail address: Castaneda-Tamez@med.uni-frankfurt.de
Presenting author: Paulina Castañeda-Tamez

Mitochondrial respiratory complexes maintain functionality through selective turnover and replacement of damaged subunits. The mechanisms coordinating damage recognition, targeted extraction, and subunit replacement remain poorly understood. In complex I, turnover preferentially targets peripheral N and Q modules via ClpXP-mediated removal and replacement of subunits. High-turnover components, including NDUFA6 and NDUFA7, are late-incorporating and weakly integrated, with the co-chaperone DNAJC30 facilitating their exchange.

Here, we investigated the role of the high-turnover subunits NDUFA6 and NDUFA7 in complex I stability and repair by generating cellular knockouts designed to locally destabilize the N module while preserving partial enzymatic activity. Complex I–dependent respiration was reduced in both models (38–65%), confirming functional impairment despite residual activity. Quantitative complexome profiling revealed distinct effects on complex I organization. Loss of NDUFA6 caused pronounced destabilization of the N module accompanied by a global reduction of other modules, consistent with structural collapse of the complex. In contrast, NDUFA7 deficiency resulted in a more homogeneous decrease in abundance across all modules (~25%). Both mutations triggered upregulation of mitochondrial quality control and service factors, including DNAJC30 and the chaperone DNAJA3, indicating activation of repair pathways. These findings suggest distinct cellular responses: a “demolition-and-rebuild” strategy following structural collapse in NDUFA6 KO cells, and a more controlled maintenance response in NDUFA7 KO cells. Ongoing pulse SILAC experiments combined with mitochondrial stress aim to resolve subunit-specific turnover kinetics and further dissect the balance between damage accumulation and repair. Together, our results provide evidence for distinct, subunit-dependent repair strategies in complex I and highlight modular turnover as a key principle of mitochondrial proteostasis.

P140

Reshaping mitochondria: AOX drives cristae ultrastructural remodeling in *Drosophila melanogaster*

Murilo F. Othonicar[a], Mike Gerards[b], Marcos T. Oliveira[a]

[a]Ribeirão Preto Medical School - University of São Paulo, Ribeirão Preto, São Paulo, Brazil [b]Maastricht Centre for Systems Biology - Maastricht University, Maastricht, Netherlands.

E-mail address: murilo_othonicar@usp.br

Presenting author: Murilo F. Othonicar

Respiratory supercomplexes (SCs) are supramolecular assemblies formed by mitochondrial electron transport chain complexes I, III, and IV. They have been proposed to enhance the efficiency of oxidative phosphorylation and are known to contribute to mitochondrial cristae architecture in conjunction with ATP synthase oligomers[1]. In some organisms, such as tunicates, SCs appear to be absent, likely due to the loss of key subunits. Notably, these species harbor an alternative oxidase (AOX), which enables electron flow through the respiratory chain independently of complexes III and IV [2]. Given the growing interest in the use of AOX in a therapeutic strategy for mitochondrial diseases, it is important to understand how its expression influences mitochondrial systems that typically depend on SC organization. In this study, we investigated the impact of AOX expression on the electron transport chain organization and mitochondrial ultrastructure in *Drosophila melanogaster* larvae. We combined blue native polyacrylamide gel electrophoresis (BN-PAGE) to assess SCs, cryo-electron tomography (cryo-ET) to examine cristae morphology, and quantitative PCR to evaluate mitochondrial DNA copy number and the expression of genes associated with mitochondrial dynamics and cristae regulation, such as *Opa1*, *Marf* (*MFN1/2* orthologue) and *Drp1*. Our results demonstrate that AOX-expressing larvae show an increase in the abundance of SCs and ATP synthase dimers. These molecular changes correlate with a higher density of cristae observed in cryo-ET reconstructions, without significant alterations in overall mitochondrial volume. Furthermore, AOX expression is associated with elevated transcript levels of *Opa1* and *Marf*, and no changes in mitochondrial DNA copy number, suggesting cristae remodeling by enhanced mitochondrial fusion, without a detectable role for mitochondrial biogenesis. Together, these findings indicate that AOX expression influences both the structural and functional organization of mitochondria, reflecting adaptive changes in mitochondrial metabolism. This work provides important insights into how AOX interacts with native respiratory chain organizations and highlights considerations for its application in therapeutic contexts, as well as for understanding the mechanisms governing mitochondrial architecture and bioenergetics.

[1] Waltz, F., Righetto, *et al*. (2025). In-cell architecture of the mitochondrial respiratory chain. Science, 387(6740), 1296-1301.

[2] Othonicar, M. F., Garcia, G. S., & Oliveira, M. T. (2024). The alternative enzymes-bearing tunicates lack multiple widely distributed genes coding for peripheral OXPHOS subunits. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1865(3), 149046.

P141

Effects of Cyclophilin D N-terminal cleavage: a dynamical perspective

Gabriele Coluccino[a]; Valentina Pia Muraca[a]; Paolo Bernardi[b]; Giovanna Lippe[a]; Alessandra Corazza[a]

[a]Department of Medicine (DMED), University of Udine, Udine, Italy; [b]Department of Biomedical Sciences, University of Padova, Padova, Italy;

E-mail address: gabriele.coluccino@uniud.it
Presenting author: Gabriele Coluccino

Protein function is tightly regulated by structure and dynamics. Cyclophilin D (CyPD) is a key modulator of the mitochondrial permeability transition pore (mPTP), an unselective channel involved in rapid Ca^{2+} release from mitochondria (transient openings) and in bioenergetic failure leading to cell death (prolonged openings).

We have previously shown that CyPD undergoes proteolytic cleavage in mammalian mitochondria. In *H. sapiens* the cleavage, putatively mediated by calpain 1, removes residues 1-13 of the N-terminal tail (NTT), generating a truncated form (ΔN-CyPD). Structural studies by Nuclear Magnetic Resonance (NMR) spectroscopy showed that the full-length (FL-CyPD) and ΔN-CyPD are indistinguishable in their shared regions.

However, biochemical and functional assays revealed that compared to FL-CyPD, ΔNCyPD (i) interacts more avidly with the OSCP subunit of F-ATP Synthase under nearphysiological ionic strength conditions and (ii) rapidly triggers F-ATP Synthase-mediated mPTP opening upon calcium stimulation.

Given the high structural similarity between the two forms, we investigated their protein dynamics. By adopting an integrated molecular dynamics and NMR-based approach, we gained insights into the conformational dynamics of CyPD. Molecular dynamics simulations (1 μs) and NMR relaxation experiments were performed by comparing the two proteins in buffer with or without 150 mM KCl, enabling the characterization of both FLCyPD and ΔN-CyPD in a controlled and biologically relevant setting. Our data show how the NTT modulates CyPD dynamics and sensitivity to KCl, offering a possible explanation for how its cleavage modulates CyPD-mediated regulation of mPTP opening.

P142

ATPIF1 preserves mitochondrial ultrastructure and β-cell integrity under stress conditions Andrea Dlasková[a], Bazila Bazila[a, b], Petr Ježek[a]

[a] Laboratory of Mitochondrial Physiology, Institute of Physiology, CAS, Prague, Czechia [b] First Faculty of Medicine, Charles University, Prague, Czechia

E-mail address: andrea.dlaskova@fgu.cas.cz
Presenting author: Andrea Dlasková

Mitochondria integrate metabolic and stress stimuli to regulate pancreatic β-cell function. ATP is a key signaling molecule in β-cells, driving plasma membrane depolarization and insulin secretion, and is primarily generated by mitochondrial ATP synthase. Inhibitory factor 1 (ATPIF1), an endogenous inhibitor of ATP synthase, has been shown to modulate mitochondrial function in β-cells [1,2]; however, its role in β-cell survival under stress remains poorly understood. This study aimed to investigate the role of ATPIF1 in β-cell stress responses and viability.

To this end, we generated CRISPR/Cas9-mediated ATPIF1 knockout (KO) INS-1E cells and isolated pancreatic islets from ATPIF1 KO mice. Cells and islets were exposed to metabolic overload, mitochondrial inhibitors, oxidative stress, and pro-inflammatory cytokines. Cell death was assessed by flow cytometry in INS-1E cells and by spinning-disk confocal microscopy in pancreatic islets. Mitochondrial ultrastructure was analyzed using transmission electron microscopy.

Loss of ATPIF1 significantly increased β-cell susceptibility to electron transport chain inhibition, metabolic and oxidative stress, and pro-inflammatory cytokines. This heightened vulnerability resulted in a marked increase in β-cell death under stress conditions and was mechanistically associated with enhanced cytochrome c release and induction of the ER stress marker BiP. These molecular changes were accompanied by pronounced structural remodeling of the endoplasmic reticulum and mitochondrial cristae.

We conclude that ATPIF1 plays a protective role in pancreatic β-cells by maintaining mitochondrial integrity and promoting cellular resilience under stress. These findings identify ATPIF1 as a key modulator of mitochondrial stress responses and highlight its importance in preserving β-cell function under conditions relevant to diabetes.

Supported by the Czech Science Foundation (Grant No. 24-10325S).

[1] A. Kahancová, F. Sklenář, P. Ježek, A. Dlasková, Overexpression of native IF1 downregulates glucose-stimulated insulin secretion by pancreatic INS-1E cells, Sci. Rep. 10 (2020). https://doi.org/10.1038/s41598-020-58411-x.

[2] A. Kahancová, F. Sklenář, P. Ježek, A. Dlasková, Regulation of glucose-stimulated insulin secretion by ATPase Inhibitory Factor 1 (IF1), 2018. https://doi.org/10.1002/1873-3468.12991.

P143

Complexome profiling reveals novel links between mitochondrial genome maintenance and OXPHOS assembly

Esperanza López-Merino[a], Alfredo Cabrera-Orefice[b] and Cristina Ugalde[a,c,d]

[a]*Laboratory of Mitochondrial Biomedicine, Center for Biological Research Margarita Salas (CIBCSIC), Madrid 28040, Spain*
[b]*Institute of Biochemistry, Faculty of Medicine, Justus-Liebig-University, Giessen 35392, Germany* [c]*Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid 28041, Spain*
[d]*Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), U723, Madrid, Spain*

E-mail address: esperanza.lopez@cib.csic.es
Presenting author: Esperanza López-Merino

Mitochondrial oxidative phosphorylation (OXPHOS) is the primary source of cellular ATP and relies on the coordinated assembly and maintenance of the mitochondrial respiratory chain (complexes I–IV) and ATP synthase (complex V). The biogenesis of these large multiprotein complexes requires tight coordination between nuclear and mitochondrial gene expression as well as faithful replication and maintenance of mitochondrial DNA (mtDNA). Defects affecting either respiratory chain assembly or mtDNA replication lead to severe mitochondrial diseases, highlighting the importance of better understanding how these processes are functionally interconnected.

In this work, we investigated the relationship between mtDNA maintenance and respiratory chain biogenesis using complexome profiling, a proteomic strategy that combines native separation of protein complexes with quantitative mass spectrometry. For this purpose, we used human SHSY5Y and HEK293T cell lines carrying mutations in OXPHOS subunits and mtDNA replication factors. By applying blue native PAGE (BN-PAGE) and high-resolution clear native PAGE (hrCNPAGE), we systematically compared the ability of these electrophoretic approaches to resolve respiratory chain complexes, supercomplexes and nucleoprotein assemblies associated with the mitochondrial replisome.

Our analyses indicate that perturbations in mitochondrial genome maintenance influence the organization of the mitochondrial complexome. This approach enables the identification of changes in respiratory chain assembly intermediates and alterations in supercomplex distribution and abundance. Thus, our data suggest that defects in mtDNA replication are accompanied by broader remodeling of the mitochondrial complexome, affecting the assembly landscape of OXPHOS complexes. These observations support the concept that mitochondrial genome maintenance and bioenergetic function are closely coordinated processes.

Together, this work highlights complexome profiling as a powerful tool to investigate the structural organization of the mitochondrial bioenergetic machinery and its perturbation in models of mitochondrial disease. By integrating the analysis of both OXPHOS complexes and mtDNA replication factors, this study provides new insights into the molecular links between mitochondrial genome stability and respiratory chain biogenesis.

P144

Intermediate stages in the origin of metabolism at a phosphorylating hydrothermal vent

Natalia Mrnjavac[1,†], Nadja K. Hoffmann[1,†], Manon L. Schlikker[1,†], Maximilian Burmeister[1], Loraine Schwander[1], Carolina García García[1], Max Brabender[1], Mike Steel[2], Daniel H. Huson[3], Sabine Metzger[1], Quentin Dherbassy[4], Bernhard Schink[5], Mirko Basen[6], Joseph Moran[4,7], Harun Tüysüz[8,9], Martina Preiner[10], William F. Martin[1]

[1] Institute of Molecular Evolution, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

[2] Biomathematics Research Centre, University of Canterbury, Christchurch, New Zealand

[3] Institute for Bioinformatics and Medical Informatics, University of Tübingen, Tübingen, Germany

[4] University of Strasbourg, CNRS, ISIS, Strasbourg, France

[5] Department of Biology, University of Konstanz, Konstanz, Germany

[6] Department of Microbiology, Institute of Biological Sciences, University of Rostock, Rostock, Germany

[7] Department of Chemistry and Biomolecular Sciences, University of Ottawa, Canada

[8] Catalysis and Energy Materials Group, IMDEA Materials Institute, Madrid, Spain

[9] Department of Heterogeneous Catalysis, Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany

[10] Microcosm Earth Center, Max Planck Institute for Terrestrial Microbiology and Philipps University Marburg, Marburg, Germany

† These authors contributed equally to this work.

E-mail address: nadja.hoffmann@hhu.de
Presenting author: Nadja K. Hoffmann

Abstract

The origin of life required the emergence of metabolism, an autocatalytic network of enzymatic reactions that synthesize amino acids, nucleotides and cofactors. At the origin of metabolism there were no enzymes—how did it start? Empirical studies addressing early metabolic evolution are lacking. Harnessing protein structures for metabolic enzymes, we identify intermediate states in primordial metabolic assembly. We show that enzymatic metabolism in the universal common ancestor was incomplete, undergoing final assembly independently in the lineages leading to Bacteria and Archaea. Native transition metals—Fe^0, Co^0, Ni^0, Pd^0— served as the catalytic forerunners of both enzymes and cofactors at metabolic origin while phosphite supplied energy, as it phosphorylates AMP to ADP and serine to phosphoserine using native metal catalysts in water. Phosphite and native metals occur in serpentinizing hydrothermal systems, identifying an energy-supplying, catalytic site of metabolic origin. Cofactors liberated nascent metabolism from native metal catalysts, engendering its autocatalytic state.

P145

Origin of Heterodisulfide Reductases Mustafa R. Törer[a], Georg Hochberg[a] [a] Department of Biology, Philipps-Universität Marburg; Marburg, 35043, Germany
E-mail address: toerer@staff.uni-marburg.de
Presenting author: Mustafa R. Törer

Heterodisulfide reductases break down the disulfide bond in the CoB-S-S-CoM (Hds) that is released by MCR and reduce the ferredoxin of FMD, connecting the initial and final step of methanogenesis. HdrABC complex has several homologs functioning in different metabolisms. HdrA homologs are oxidoreductases involved in metabolisms such as energy (e.g. Nfn), amino acid (e.g. GltD), sulfur metabolism (e.g. DsrL, sHdrA, QmoAB) and aromatic compound degradation (e.g. BamE). Similarly, HdrB scaffold is also widespread, involved in different parts of sulfur (DsrK, sHdrB, HdrD), carbon (e.g. LutA, GlcF, TfrB), and energy metabolism (e.g. EMO). This diversity of metabolisms represented in these two protein families presents a unique opportunity to order the emergence of these metabolisms in time. Methanogenesis has long been suspected to be the ancestral metabolism amongst these based on the fact that it is an autotrophic process. But proving this theory would require resolved and rooted phylogenies of these proteins. This has not been possible so far, because of extensive sequence divergence in both families and a lack of suitable outgroups. Here we overcome this problem by utilizing structural phylogenetics to infer highly resolved trees of these families using structural characters alongside amino acids for tree inference. We utilize the internal symmetry of these proteins to root their phylogenies without an outgroup. Using ancestral sequence reconstruction and our rooted trees, we infer a deep history of heterodisulfide utilization that may have preceded methanogenesis as we know it today.

P146

Investigating the diversity and origins of ubiquinone biosynthesis across eukaryotes Emma Bouvet[a], Fabien Pierrel[a], Romain Leroy[b], Laura Eme[b], Ludovic Pelosi[a], Sophie Abby[a] [a] Laboratoire TIMC, UMR 5525, CNRS, Université Grenoble Alpes, 38000, Grenoble, France
[B] Laboratoire Ecologie Société Evolution, CNRS, Université Paris-Saclay, AgroParisTech,
Gif-sur-Yvette, France
E-mail address: Emma.Bouvet@univ-grenoble-alpes.fr
Presenting author: Emma Bouvet[a]

Ubiquinone ("UQ"), also called coenzyme Q in eukaryotes, serves as a central electron carrier in bacterial and mitochondrial respiratory chains. Because mitochondria originated from an alphaproteobacterial ancestor, UQ metabolism represents a key link between bacterial and eukaryotic bioenergetics. Although the biosynthetic reactions leading to UQ are generally considered conserved, recent studies have revealed substantial diversity in the enzymes catalyzing key hydroxylation and decarboxylation steps in bacteria, challenging the concept of a canonical pathway.

To investigate the distribution and evolutionary origins of UQ biosynthesis enzymes, we combined large-scale annotation of bacterial and eukaryotic genomes with phylogenetic reconstruction and comparative genomics, complemented by experimental characterization of selected enzymes.

In bacteria, we have shown that gene duplications, transfers, and losses have extensively reshaped the UQ biosynthetic pathway within *Pseudomonadota*. The recent discovery of a novel decarboxylating/hydroxylating enzyme further highlights the evolutionary flexibility of this pathway. In contrast, UQ biosynthesis in eukaryotes has only been thoroughly characterized in three model organisms (yeast, human and plant), and the distribution of the "Coq" enzymes remains largely unexplored across eukaryotic diversity. The identification of a novel enzyme, CoqF, in the green lineage (*Archaeplastida*) suggests that alternative enzymatic solutions may exist and raises questions about the evolutionary origin of Coq enzymes and their relationship with bacterial homologs.

Our preliminary analyses reveal that most eukaryotic lineages encode a conserved core set of Coq enzymes, while others display lineage-specific absences or alternative homologs, resulting in a mosaic distribution of the pathway. Notably, several Coq "core" enzymes lack clear homologs in *Alphaproteobacteria*, the closest relatives of the mitochondrial ancestor. Ongoing phylogenetic analyses will clarify how the eukaryotic pathway emerged and whether it reflects multiple bacterial contributions rather than simple vertical inheritance from the mitochondrial ancestor.

P146

Coevolution in action: how the endosymbiont regulates the energy metabolism of *Angomonas deanei*

Azuil Barrinha[a], Ana C. Loyola-Machado[a,§], Marlon D. M. Dos Santos[b], Paulo C. Carvalho[b], Wanderley de Souza[a,c], Ana P. Valente[c,d], Antonio Galina[e] and Maria C. M. Motta[a,c] (Example: Jane G. Doe[a])

a

Laboratório de Ultraestrutura Celular Hertha Meyer, Centro de Pesquisa em Medicina de Precisão (CPMP), Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro-UFRJ, 21491-590, Rio de Janeiro, Rio de Janeiro, Brazil.

b

Laboratory for Structural and Computational Proteomics, Carlos Chagas Institute, Fiocruz, Paraná, Brazil. ᶜA Instituto Nacional de Ciência e Tecnologia em Biologia Estrutural e Bioimagens – Rio de Janeiro, Brazil. d Centro Nacional de RMN, Departamento de Biologia Estrutural, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. e Laboratório de Bioenergética e Fisiologia Mitocondrial, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. §

Present address:Department of Internal Medicine, Section of Digestive Diseases, Yale University School of Medicine, 333 Cedar Street, New Haven, CT, 06510, USA.

E-mail address: azuil@biof.ufrj.br
Presenting author: Azuil Barrinha

Endosymbiosis in trypanosomatids involves a mutualistic association between a symbiotic bacterium and a host protozoan, and it is an excellent model for studying metabolic coevolution and the origin of organelles[1]. This work investigated the influence of the symbionts on the metabolism of *Angomonas deanei* by comparing wild-type and aposymbiotic strains under different nutritional conditions. The presence of the symbiont enhanced cell proliferation in medium containing a single carbon source and increased O_2 consumption. Wild-type cells utilized oxidative phosphorylation to produce ATP, whereas aposymbiotic cells relied on substrate-level glycolysis, resulting in the excretion of greater amounts of fermentative products, such as acetate, succinate, and ethanol. Proteomic analysis revealed increased expression of glycolytic and fermentative enzymes by the aposymbiotic strain and oxidative phosphorylation enzymes by symbiontharboring cells. These findings highlight the role of the symbiotic bacterium in optimizing host metabolism and provide insights into the evolution of parasitism in trypanosomatids when *A. deanei* is compared with pathogenic species[2].

[1] A.C. Loyola-Machado, A.C. Azevedo-Martins, C.M.C. Catta-Preta, W. de Souza, A. Galina, M.C.M. Motta, The Symbiotic Bacterium Fuels the Energy Metabolism of the Host Trypanosomatid Strigomonas culicis, Protist 168(2) (2017) 253–269, https://doi.org/10.1016/j.protis.2017.02.001

[2] A. Barrinha, A.C. Loyola-Machado, M.D.M. dos Santos, P.C. Carvalho, W. de Souza, A.P. Valente, A. Galina, M.C..M Motta, Endosymbiosis in trypanosomatids: the bacterium regulates the intermediate and oxidative metabolism of the host cell. mSphere 10(11) (2025) e0045725, https://doi.org/10.1128/msphere.00457-25

P148

Dynamic evolution of quinone pathways and quinone repertoires in Bacteria Sophie-Carole Chobert[a,b], Morgane Roger-Margueritat[a], Felix Elling[c], Fabien Pierrel[a], Sophie S. Abby[a]

[a]Univ. Grenoble Alpes, CNRS, UMR 5525, VetAgro Sup, Grenoble INP, TIMC, Grenoble, France [b]Univ. Grenoble Alpes, CNRS, INRAE, IRD, Grenoble INP, IGE, 38000 Grenoble, France [c]Leibniz-Laboratory for Radiometric Dating and Isotope Research, Christian-Albrecht University of Kiel, Kiel 24118, Germany

E-mail address: sophie.abby@univ-grenoble-alpes.fr
Presenting author: Sophie Abby

Quinones serve as essential electron carriers in respiratory and photosynthetic electron transport chains, playing a central role in cellular energy metabolism. Their diversity – spanning low-potential menaquinone (MK) to high-potential ubiquinone (UQ), plastoquinone (PQ), and the recently discovered methyl-plastoquinone (mPQ) [1] – reflects the evolution and diversification of bacterial energy metabolism. However, the evolution of their biosynthetic pathways and dynamics underlying their distribution remain poorly understood.

To investigate this, we developed bioinformatics tools to annotate quinone biosynthetic pathways in genomes, and combined large-scale genomic annotation, phylogenetic analyses, and experimental characterization.

We here provide an overview of quinone pathway distribution across bacteria and underlying evolutionary processes. Our findings reveal a dynamic quinone repertoire in Proteobacteria (*Pseudomonadota*), marked by the ancestral innovation of UQ, differential losses of the oxygen-independent UQ pathway in specific lineages, and repeated gains of the low-potential MK pathway, likely through lateral gene transfer. These processes appear correlated with adaptations to varying oxygen levels [2]. While MK remains dominant across the tree of bacteria, an ancient UQ pathway was identified in *Desulfobacterota*, suggesting that UQ biosynthesis is not restricted to *Pseudomonadota* [3]. This finding points to ancient origins of the oxygen-independent UQ pathway relative to the classical UQ pathway. Additionally, frequent switches between the two MK biosynthetic pathways (Men and futalosine) were observed within the *Desulfobacterota* phylum, raising questions about the selective advantage of one pathway over the other. Recent work further characterized a missing hydroxylase in the PQ pathway, likely acquired via lateral gene transfer from the UQ pathway in *Pseudomonadota*, indicating that the contemporary PQ pathway may have evolved after the UQ pathway [4]. Finally, we identified mPQ, a novel high-redox-potential quinone exclusive to *Nitrospirota*, whose biosynthetic pathway suggests a shared evolutionary origin with PQ and UQ predating the Great Oxygenation Event (GOE) [1]. This study also highlights that, while high-potential quinones have recently been discovered in novel lineages, several lineages remain devoid of quinones – suggesting that further quinones may remain to be discovered.

Overall, our study offers a perspective on quinone evolution, from the diversification of their biosynthetic pathways and enzymes, to the adaptation of quinone repertoires at the phylum level. By integrating genomics, phylogenetics, and biochemistry, we contribute to understanding the co-evolution of quinones and energy metabolism, and their role in bacterial adaptation to changing dioxygen levels over geological time.

[1] F.J. Elling, F. Pierrel, S. Chobert, S.S. Abby, T.W. Evans, A. Reveillard, L. Pelosi, J. Schnoebelen, J.D. Hemingway, A. Boumendjel, K.W. Becker, P. Blom, J. Cordes, V. Nathan, F. Baymann, S. Lücker, E. Spieck, J.R. Leadbetter, K. Hinrichs, R.E. Summons, A. Pearson. A novel quinone biosynthetic pathway illuminates the evolution of aerobic metabolism. Proc. Natl. Acad. Sci. U.S.A., (2025). https://doi.org/10.1073/pnas.2421994122

[2] S. Chobert, M. Roger-Margueritat, L. Flandrin, S. Berraies, C.T. Lefèvre, L. Pelosi, I. Junier, N. Varoquaux, F. Pierrel, S.S. Abby, Dynamic quinone repertoire accompanied the diversification of energy metabolism in *Pseudomonadota.* The ISME Journal (2025), https://doi.org/10.1093/ismejo/wrae253

[3] S. Chobert, S. Kanwar, O. Lerouxel, N. Varoquaux, J. Michaud, L. Pelosi, F. Pierrel, S.S. Abby, Global distribution of isoprenoid quinones across Bacteria. BiorXiv preprint, (2025). https://doi.org/10.1101/2025.09.17.676790

[4] M. Roger-Margueritat, M.Beltran, J. Schnoebelen, L. Flandrin, W. Rezali, E. Michel, S.S. Abby, F. Pierrel, Heterologous plastoquinone production using a newly identified O2-dependent cyanobacterial. The FEBS Journal (2025). https://doi.org/10.1111/febs.70272

P149

The bioenergetic origins and evolutionary persistence of cytoplasmic potassium dominance

Nikolay Korolev

School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

E-mail address: sbs-nikolay.k@ntu.edu.sg
Presenting author: Nikolay Korolev

Potassium (K^+) is the primary intracellular cation across all domains of life, maintained against an extracellular environment typically dominated by sodium (Na^+). However, the origins and evolutionary conservation of the cytosolic K^+ dominance remain enigmatic.[1] This study provides a physicochemical explanation for the foundation of the asymmetric K^+/Na^+ distribution, identifying it as a requirement for bioenergetic stability in early chemiosmotic organisms.

The universal chemiosmotic mechanism involves high-intensity proton circuits. In rapidly dividing bacteria such as *E. coli*, the proton efflux rate reaches ~25 million H^+/sec. Therefore, even a 1% mismatch between proton efflux and influx generates membrane potential spikes exceeding 100 V/sec. Without a regulation mechanism, the lipid bilayer would reach its dielectric breakdown threshold (200 mV; 40 MV/m) within 40 milliseconds. Consequently, ion-conducting channels are a physical necessity for acting as voltage regulators and must be included as an integral part of the chemiosmotic mechanism.[2] The ion conductivity of most cation channels (e.g., KcsA, 100 million ions/sec) allows a single such channel to provide reliable bacterial membrane protection.

To maintain the proton motive force, these channels must be impermeable to protons. To avoid leakage (via a "proton hopping" mechanism), the channel pore must be anhydrous, and peptide backbone carbonyls are a natural choice to provide substituent coordination for dehydrated cations. Experimental data indicate that these carbonyl groups possess an intrinsic thermodynamic preference for K^+ over Na^+ with a free energy gain δg^{K}_{Na} = □100 □ □250 cal/mol.[3] This minor selectivity bias is exponentially amplified through 36 coordination events (32 within the selectivity filter and 4 at the entrance), resulting in a discrimination factor, K^{K}_{Na}, exceeding 1,000. Driven by metabolic current imbalances, the cytoplasm rapidly becomes enriched in K^+ shortly after membrane sealing (an *E. coli* cell contains ~100 million monovalent cations).

This initial K^+ enrichment, once established as a "side effect" of the channels' operation, led to an evolutionary lock-in. Fundamental biological processes were optimised for a K^+-rich environment.[1] Particularly, K^+ serves as a lubricant ("grease") for the Central Dogma, facilitating the transfer of histones during chromatin disassembly, whereas Na^+ acts as a competitive inhibitor ("sand") of acidic protein domains.[2, 3] Darwinian selection for reproductive velocity ensured that any shift toward Na^+ utilisation resulted in immediate competitive exclusion, fixing potassium as the foundational biological cation.

[1] A. Danchin, P.I. Nikel, Why nature chose potassium. J. Mol. Evol. 87 (2019) 271-288, https://doi.org/10.1007/s00239-019-09915-2

[2] N. Korolev, How potassium came to be the dominant biological cation: Of metabolism, chemiosmosis and cation selectivity since the beginnings of life. BioEssays 43 (2021) e2000108, https://doi.org/10.1002/bies.202000108

[3] N. Korolev, L. Nordenskiöld, Influence of alkali cation nature on structural transitions and reactions of biopolyelectrolytes. Biomacromolecules 1 (2000) 648-655, https://doi.org/10.1021/bm000042f

P150

Decreased proton cost for mitochondrial ATP production emerged in early metazoans Malte Marquardt, Thomas Visser, Marcel Vieira-Lara

[a]*Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ, Delft, The Netherlands*

E-mail address: m.l.marquardt@tudelft.nl
Presenting author: Malte Marquardt

The bioenergetic efficiency of oxidative phosphorylation depends on the proton-to-ATP (H^+/ATP)-ratio of its electron transport chain. The homooligomeric c-ring subunit of the F_oF_1-ATP-synthase mediates proton translocation and the number of c-subunit monomers forming the c-ring (stoichiometry) determines the H^+/ATP-ratio. For decades, c-ring stoichiometries were believed to be identical (10) in all eukaryotes. However, the discovery that some metazoan (animal) mitochondria contain an 8-subunit c-ring implies that ATP synthesis can be more proton-efficient. To date, nine mitochondrial c-ring stoichiometries have been determined experimentally. Yet, the evolution of c-ring stoichiometries in eukaryotes remains unknown. In this study, we optimized an AlphaFold-based c-ring prediction tool[1] to determine the mitochondrial H^+/ATP-ratio of over 3,000 eukaryote species. Through analysis of phylogeny and mapping of mitochondrial targeting sequences, we tracked the evolution towards lower H^+/ATP-ratios in metazoa, and predicted greater diversity among fungi than previously assumed. A decrease of c-ring stoichiometry to 8 coincided with (i) the appearance of the first metazoa and (ii) migration of its coding gene from the mitochondrial genome to the nucleus. Regulation of nuclear expression of ATP9 enables tighter control over ATP-Synthase assembly and has been demonstrated to drive tissue-specific variations in mitochondrial ATP-Synthase levels overall. Additionally, we identified key mutations likely responsible for the low H^+/ATP-ratio in metazoa such as I17V and A19V (reference: OLI1, *S. cerevisiae*) and reconstructed ancestral sequences to predict the c-ring stoichiometries of extinct sequence intermediates. Nuclear and mitochondrial genome engineering is currently being performed in the yeast *Saccharomyces cerevisiae* to test a selection of heterologous and reconstructed c-rings *in vivo*. This may provide unique insight into the coupling of stoichiometry and proton motive force in mitochondria as well as the bioenergetic impact of altered H^+/ATP-ratios on the cellular level.
In summary, we found that reduced c-ring stoichiometries coincide with gene transfer to the nucleus and are first and exclusively predicted in metazoans. This suggests that a more proton-efficient mitochondrial ATP production may have been an enabling factor for the emergence of multicellularity.

[1] Osipov, S. D., Zinovev, E. v., Anuchina, A. A., Kuzmin, A. S., Minaeva, A. v., Ryzhykau, Y. L., Vlasov, A. v., & Gushchin, I. Y. (2025). High-Throughput Evaluation of Natural Diversity of F-Type ATP Synthase Rotor Ring Stoichiometries. Proteins: Structure, Function, and Bioinformatics, 0, 1–13. https://doi.org/10.1002/PROT.26790

P151

The many survival strategies of the poly-extremotolerant yeast *Rhodotorula mucilaginosa*. Control of its mitochondrial metabolism.

S. Uribe-Carvajal[a], E. Hernández Torres[a], N. Chiquete Félix[a], P. Acosta-Valdelamar[a], O. Mendez-Romero[a], and C. Ricardez-García[a].

Universidad Nacional Autónoma de México, Instituto de Fisiología Celular. México DF, México.

E-mail address: suribe@ifc.unam.mx Salvador Uribe Carvajal.

All over the World, *Rhodotorula mucilaginosa* may be found in many diTerent extreme environments. It has been found in Anctartic ice, in heavily polluted sewage and in animal epithelia. *R. mucilaginosa* expresses several survival strategies, among which the aomponents of a highly branched respiratory chain (RC) are diTerentially expressed, leading to physiological uncoupling of oxidative phosphorylation as needed[1]. Accessory RC-subunits include two alternative NADH dehydrogenases, (NDH2), an alternative oxidase (AOX), a glycerol-phosphate dehydrogenase (GPDH) and a lactate/Cytochrome c oxido-reductase. In addition, a cytoplasmic fumarate dehydrogenase binds to mitochondria during the logarithmic phase. However, given the choice, and even at high oxygen concentrations, it expresses a mostly
fermentative metabolism where it produces a low amount of reactive oxygen species (ROS). Furthermore, when *R. mucilaginosa* is forced to grow aerobically on a respiratory substrate such as lactate, it increases its carotenoid production, which are, mostly b-carotene, torulene and thorularhodin: these carotenoids inactivate ROS. It was observed, however, that upon exposure to high amounts of ROS, these antioxidant molecules may be damaged, becoming prooxidants themselves[2]. In regard to survival in poor environments, *R. mucilaginosa* grows better than other yeast species on a single carbon source such as glutamine[3].

[1] Castañeda-Tamez et al., *BBA - Bioenergetics 1865 (2024) 149035. doi.org/10.1016/j.bbabio.2024.149035*

[2] Mosqueda-Martínez et al., *Front. Fungal Biol. (2024) 5:1378590. doi: 10.3389/Gunb.2024.1378590*

[3]Acosta-Valdelamar et al., (2026) Fermentation 2026, 12 36. *doi.org/10.3390/fermentation12010036*

P152

Using structural phylogenetics to map and root the evolutionary history of respiration Sofia Appelgren[a], Georg Hochberg[a] [a] Department of Biology, Philipps-Universität Marburg; Marburg, 35043, Germany
E-mail address: appelgre@staff.uni-marburg.de
Presenting author: Sofia Appelgren

Bioenergetic enzymes are ancient molecules, many of which are suggested to predate the last universal common ancestor. Prokaryotic respiration is very diverse, and prokaryotes use a wide range of electron donors and acceptors to create electrochemical gradients over their membranes. Despite this diversity, several of the metabolic pathways and enzymes consist of homologous subunits and thus share a common evolutionary history. However, due to enormous timescales and high evolutionary rates, the amino acid sequences of these homologs are very divergent. This causes problems during the construction of phylogenetic trees, thus hindering our ability to study how bioenergetic processes relate to each other phylogenetically. To overcome this issue, we have utilized the phenomenon that protein structures evolve much slower than amino acid sequences and aligned bioenergetic proteins based on their structure rather than on their amino acids. This have allowed us to construct phylogenetic trees of highly divergent but homologous enzymes and map evolutionary relationships that previously were hidden behind low sequence similarities. Rooting of very old protein families has until now been an issue due to the lack of obvious outgroups and high occurrence of horizontal gene transfer. By combining structural phylogenetics and symmetry rooting, utilizing the internal pseudosymmetry present in many bioenergetic enzymes, we can unambiguously root protein families which previously were considered unrootable. Using this method, we now present the roots for the phylogenetic trees of the superfamily of heme-copper oxidases, NrfD-like subunits including the cytochrome *bd* oxidases and the Mrp antiporter-like subunits found in Complex I-like enzymes. Thus, we provide polarity to the deep evolutionary history of respiration and shed light on how these protein families might have started out and subsequently evolved in accordance with the ever-changing environment around them. Using Ancestral Sequence Reconstruction, we have resurrected some of the very early bioenergetic enzymes and characterized them biochemically.

P153

Activity and quantity of Complex I together with low ROS efflux during anoxia-reoxygenation suggest depressed reverse electron transport (RET) in mitochondrial isolated from an invertebrate extremophile

Ravi C. Baker[a], Ethan A. Goldsmith[a], Masen Mannina[a], Amanda Le[a], Alexis Ducoing[a], Jacob T. Nguyen[a], Daniel A. Arabie[b], Steven C. Hand[a]

[a]Department of Biological Sciences, Louisiana State University, Baton Rouge, USA [b]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, USA

shand@lsu.edu

Presenting author: Steven C. Hand

Embryos of the invertebrate extremophile *Artemia franciscana* (brine shrimp) exhibit depressed metabolic states during anoxia and diapause that are survived for months to years with minimal oxidative damage during recovery [1,2]. To quantify Complex I in these embryos, mitochondria were isolated and permeabilized with alamethicin. Both the Complex I NADH-ubiquinone oxidoreductase activity and the quantity of Complex I flavin site [NADH:$APAD^+$ (3-acetylpyridine adenine dinucleotide) oxidoreduction][3] were two-fold higher during diapause compared to post-diapause. Artificial termination of diapause with H_2O_2 treatment returned the activity and flavin quantity back toward post-diapause values after 6 h of development. Virtually identical patterns for catalytic activity and the flavin site quantity suggest the upregulation of Complex I is due to increased Complex I content. The physiological role for this novel increase in Complex I during diapause is likely to support metabolic reactivation; certainly arrest of activity during diapause to avoid ROS production upon reactivation is not in play. Recently we have reported that isolated mitochondria from *A. franciscana* embryos display low H_2O_2 efflux (quantified with Amplex UltraRed) after anoxia/reoxygenation (A/R) when respiring on the Complex II substrate succinate[1]. Here we evaluated H_2O_2 efflux when mitochondria were supplied with NADH-linked substrates pyruvate+malate and with substrates promoting convergent entry of electrons into the Q-pool (succinate+malate+pyruvate). Forward electron transport through Complex I with pyruvate+malate promoted less H_2O_2 efflux in normoxic controls and after A/R when compared to succinate. Likewise, mitochondria respiring on succinate+pyruvate+malate exhibited lower ROS efflux than that observed with succinate alone, and this substrate combination also eliminated any ROS contribution by RET through Complex I as assessed with rotenone (an effective blocker of RET[3]); apparently, NADH-linked substrates fostered forward electron transfer that mitigated ROS production by RET through Complex I. As anticipated, all substrate combinations and oxygenation regimes exhibited several-fold increases in ROS efflux from mitochondria with addition of auranofin+dinitrochlorobenzene (inhibitors of endogenous ROS scavenging; peroxiredoxin 3 and glutathione peroxidase, respectively). Very importantly, even with succinate alone, the ROS generation by RET was minimal in *A. franciscana* mitochondria when compared to the large burst of ROS generated under identical conditions by mitochondria isolated from rat heart[4]. Our results suggest that Complex I from *A. franciscana* possesses intrinsic properties that may limit ROS release via RET and may provide translational applications for mammalian systems where tissue damage during ischemia-reperfusion is a concern. [supported in part by NSF grant IOS-1457061/IOS-1456809].

[1] D. Arabie, O. Moncrief, S. Shirmer, S. Hand, Low oxidative stress during mitochondrial recovery from anoxia in *Artemia franciscana*, an invertebrate extremophile. J. Comp. Physiol. B 195 (2025) 507-520. https://doi.org/10.1007/s00360-025-01631-2

[2] D. Arabie, D.A. and S.C. Hand, Defense capacities against oxidative stress in diapause and postdiapause embryos of *Artemia franciscana*. J. Exp. Zool. A 345 (2026) 196-208. https://doi.org/10.1002/jez.70051

[3] Z. Yin, N. Burger, D. Kula-Alwar, D. Aksentijević, H. Bridges, H. Prag, D. Grba, C. Viscomi, A. James, A. Mottahedin, T. Krieg, M. Murphy, J. Hirst, Structural basis for a complex I mutation that blocks pathological ROS production. Nat. Commun. 12 (2021) 707. https://doi.org/10.1038/s41467-021-20942-w [4] E. Chouchani, C. Methner, S. Nadtochiy, A. Logan, V. Pell, S. Ding, A. James, H. Cochemé, J. Reinhold, K. Lilley, L. Partridge, I. Fearnley, A. Robinson, R. Hartley, R. Smith, T. Krieg, P. Brookes, M. Murphy, Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. Nat. Med. 19 (2013) 753-759. https://doi.org/10.1038/nm.3212P.

P154

Mitochondrial NME4 deletion rewires kinase signaling and identifies FLT1/VEGFR1 as a link between mitochondrial dysfunction and cancer progression

Małgorzata Tokarska-Schlattner[a], Laurence Kay[a], Stéphane Attia[a], Cindy Tellier[a], Marie-Lise Lacombe[b], Mathieu Boissan[b], Uwe Schlattner[c]

[a]*Univ. Grenoble Alpes, Inserm U1055, LBFA, Grenoble, France*
[b]*Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine (CRSA), Paris, France; AP-HP, Hôpitaux Universitaires Pitié-Salpêtrière-Charles Foix, Paris, France*
[c]*Univ. Grenoble Alpes, LBFA, Grenoble, and Institut Universitaire de France, Paris, France*

E-mail address: malgorzata.tokarska-schlattner@univ-grenoble-alpes.fr Presenting author: Małgorzata Tokarska-Schlattner

The NME protein family comprises several nucleoside diphosphate kinases with distinct subcellular localizations and functions. Among them, NME4 is a mitochondrial isoform associated with the inner membrane, where it contributes to local GTP regeneration, cardiolipin transfer and metastasis suppression[1,2]. Although NME4 has been identified as a metastasis suppressor in cancer cell models, its physiological role in vivo and its mechanistic links to cancer progression remain incompletely understood.

To address this, we employed a germline NME4 knockout (KO) mouse model and performed unbiased, kinome-wide activity profiling of liver tissue. Loss of NME4 resulted in widespread alterations in protein kinase signaling networks compared with wild-type controls, revealing a global rewiring of signaling pathways consistent with dysregulation observed in NME4-deficient cancer cells. Kinase activity analysis identified a marked increase in the predicted activity of FLT1 (VEGFR1), a receptor for vascular endothelial growth factor (VEGF) and a key regulator of pro-survival and proliferative pathways, including Akt and ERK signaling. Enhanced activation of these pathways in NME4-KO liver was confirmed by immunoblotting, supporting a functional link between mitochondrial NME4 loss and pro-oncogenic signaling cascades.

FLT1 is a well-established mediator of angiogenesis and tumor progression, with elevated expression correlating with poor clinical outcomes. Conditioned media derived from breast tumor cells overexpressing NME4 decreased endothelial cell tube formation, reflecting inhibition of angiogenic activity. In line with this, analysis of human cancer datasets revealed a strong negative correlation between NME4 and FLT1 mRNA levels, reinforcing the relevance of this axis in malignancy.

Together, our findings uncover a mechanistic connection between mitochondrial dysfunction and altered kinase signaling, positioning FLT1 as a key downstream effector of NME4 loss. This work provides new insights into the signaling basis of the metastasis suppressor function of NME4 and highlights potential targets for therapeutic intervention in cancer.

[1] U. Schlattner, The complex functions of the NME family – a matter of location and biological activity? Int. J. Mol. Sci. 22, (2021) 13083. https://doi.org/10.3390/ijms222313083

[2] M.L. Lacombe, F. Lamarche, O. De Wever, T. Padilla-Benavides, A. Carlson, I. Khan, A. Huna, S. Vacher, C. Calmel, C. Desbourdes, C. Cottet, I. Hininger-Favier, S. Attia, B. Nawrocki-Raby, .
Raingeaud, V. Machon, J. Guitton, M. Le Gall, G. Clary, C. Broussard, P. Chafey, P. Thérond, D. Bernard, E. Fontaine, M. Tokarska-Schlattner, P. Steeg, I. Bièche, U. Schlattner,*, M. Boissan, The mitochondrially-localized nucleoside diphosphate kinase D (NME4) is a novel metastasis suppressor. BMC Biology 19, (2021), 228. https://doi.org/10.1186/s12915-021-01155-5.

P155

Mitochondrial redox signaling links branched-chain α-ketoacid and fatty acid metabolism to β-cell insulin secretion

Martin Jabůrek[a], Oleksandra Mozheitová[a], Jan Tauber[a], Eduardo Klöppel[a], Petr Ježek[a] *[a]Department of Mitochondrial Physiology, Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, 14220, Czech Republic*

E-mail address: martin.jaburek@fgu.cas.cz Presenting author:
Martin Jabůrek

Mitochondrial redox homeostasis critically regulates cellular signaling and pancreatic β-cell function. Our prior work shows that mitochondria-to-plasma membrane redox signaling drives fatty acid (FA) β-oxidation-dependent insulin secretion, with redox-activated mitochondrial phospholipase A2γ (iPLA2γ) mediating FA-stimulated, GPR40-dependent insulin release [1]. Preliminary data further indicate that redox signaling from branched-chain α-ketoacid (BCKA) metabolism stimulates insulin secretion in β-cells [2]. To elucidate shared metabolic and redox mechanisms, we performed dose-response assays with BCKAs in islets from wild-type (WT) and iPLA2γ-knockout (KO) mice under non-stimulatory glucose (0 or 5.5 mM). Insulin secretion and

H_2O_2 release were measured with selective inhibitors of iPLA2γ or GPR40. Additionally, siRNAmediated silencing of the BCKA dehydrogenase (BCKDH) complex inhibited FA-stimulated insulin secretion and H_2O_2 release in INS-1E cells. These findings demonstrate that BCKAs trigger iPLA2γ- and GPR40-dependent insulin secretion, while BCKDH silencing impairs FA-induced insulin secretion and H_2O_2 release. Together, they reveal bidirectional redox control between BCKA and FA metabolism in β-cell insulin secretion.

[1] M. Jabůrek, E. Klöppel, P. Průchová, et al., Mitochondria to plasma membrane redox signaling is essential for fatty acid β-oxidation-driven insulin secretion, Redox Biol. 75 (2024) 103283, https://doi.org/10.1016/j.redox.2024.103283

[2] E. Klöppel, E. P. Průchová, J. Tauber, et al., Redox signaling upon ß-like oxidation of branched-chain keto acids essentially stimulates insulin secretion in pancreatic ß-cells, Biochim. Et Biophys. Acta – Bioenergetics 1865 (2024) 108, https://doi.org/10.1016/j.bbabio.2024.149392

P156

Role of NDUFA4 and its isoforms in regulation of cytochrome *c* oxidase

Michal Knězů[ab], Kristýna Čunátová[b], Josef Houštěk[b], Tomáš Mráček[b], Petr Pecina[b]

a Department of Cell Biology Faculty of Science, Charles University, Prague, Czech Republic.
b
Laboratory of Bioenergetics, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic.

E-mail address: mich.knezu@gmail.com
Presenting author: Michal Knězů

Cytochrome *c* oxidase (COX, complex IV) function can be modulated by exchange of nDNA-encoded subunits' isoforms. These can be expressed in specific tissues to finetune the function of complex IV to best suit the cell type and situation. Isoforms of NDUFA4, considered the latest assembling subunit of COX, are studied in multiple contexts, ranging from immunology and virology to cellular biology, bioenergetics and cancer research. The two non-canonical isoforms NDUFA4L2 and NDUFA4L3, also known as COXFA4L2 or MISTRH and COXFA4L3, NMES1, MOCCI or MISTRAV, have been assigned roles in oxygen sensing in carotids or development of clear cell renal carcinoma and sperm development, autophagy and immune response respectively. Here we present a NDUFA4KO cell line, which we aim to use as a uniform platform to express and characterize the three individual gene isoforms. In the knockout of NDUFA4 in HEK293 cell line, all other subunits of complex IV are present, confirming that NDUFA4 is indeed the last assembled subunit of COX holoenzyme. While the amount of assembled complex IV is not decreased, we detected redistribution of its higher structures, particularly of the III_2IV supercomplexes. While no significant shift from oxidative to glycolytic metabolism was observed in intact cells, the KO model presents with a moderate OXPHOS dysfunction, with approximately 40% reduced respiratory capacity both in coupled and uncoupled state, as well as of COX measured as an isolated step in permeabilized cells. Furthermore, we observed changes in the relative contribution of respiratory dehydrogenases with enhanced utilization of glycerol-3-phosphate. Interestingly, complex IV without NDUFA4 possesses marginal increase in affinity for oxygen, indicated by lower p_{50} values. This suggests potential structural confines imposed on the oxygen binding by NDUFA4. Taken together, we suggest that NDUFA4 does not affect assembly of the complex, but functions as a regulatory subunit.

P157

Title: Regulation of mitochondrial proteostasis by the ribosome-associated quality controln mammals: molecular bases and physiopathological relevance.

Claude Lalou1,2, Victor Gidenspeger1,2, Agata Ars1,2, Clément Farout1,2, Alice Bibeyran2,3, Valérie Prouzet Mauleon2,3, Béatrice Turcq2,3 and Giovanni Bénard1,2, *

1 Laboratoire Maladies Rares: Génétique et Métabolisme, INSERM U1211, 33076 Bordeaux, France
3Plateforme Oncoprot, TBM-Core US 005, 33000 Bordeaux, France
* corresponding : giovanni.benard@inserm.fr

Abstract.

In mammals, over 99% of mitochondrial proteins are synthesized in the cytosol as precursors and subsequently imported into the organelle. Ensuring the quality of these newly synthesized proteins is essential to prevent the accumulation of defective proteins within mitochondria, which lead to mitochondrial dysfunctions. Our findings reveal that cytosolic translation actively contributes to the quality control of nascent mitochondrial proteins prior to their import in the mitochondria of mammalian cells. This quality control is exerted through the activation of a conserved mechanism known as the ribosome-associated quality control (RQC) machinery. Previously, we have identified that the E3 ubiquitin ligase, FBXL6 participates this process ensuring the quality of ribosomal proteins including mitochondrial ribosomal proteins1. Deletion of FBXL6 hampers the degradation of defective mitochondrial ribosomal proteins and results in mitochondrial dysfunction in mammalian cells. Our recent findings demonstrate that the RQC protein, ABCE1, accumulates to the mitochondrial surface in absence of FBXL6. This accumulation leads to mitochondrial degradation by mitophagy as previously reported 2. Notably, we demonstrated FBXL6- knockout mice display accumulation of ABCE1 on mitochondrial outer membrane in liver. Accordingly, mitochondrial content are decreased in this tissue leading to mitochondrial metabolic dysfunction and overall metabolic disturbances. Finally, we demonstrated that decreasing expression of ABCE1 through the iron-sulfur (Fe-S) cluster biogenesis pathway is sufficient to mitigate the deleterious effects of FBXL6 deletion in both cell and tissue.

Reference:

1. Lavie, J. et al. Article The E3 ubiquitin ligase FBXL6 controls the quality of newly synthesized mitochondrial ribosomal proteins ll ll The E3 ubiquitin ligase FBXL6 controls the quality of newly synthesized mitochondrial ribosomal proteins. (2023) doi:10.1016/j.celrep.2023.112579.

2. Wu, Z. et al. Ubiquitination of ABCE1 by NOT4 in Response to Mitochondrial Damage Links Co- translational Quality Control to PINK1-Directed Mitophagy. Cell Metab. 28, 130-144.e7 (2018)

P158

No UCP1 in the kidney*

Celso Pereira Batista Sousa-Filho[1], Natasa Petrovic[1]

[1]Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Sweden

email: natasa.petrovic@su.se

Objective

Interesting studies have been published that imply the presence of UCP1 protein in the kidney parenchyma, potentially contravening both the paradigm that UCP1 is only found in brown and beige adipocytes and broadening the (patho)physiological significance of UCP1. The kidney localization has been the direct outcome of immunohistochemical investigations, as well as the implied outcome with several lines of reporter mice. These studies thus require confirmation and physiological expansion.

Methods

With immunohistochemistry we investigated transversal sections through the kidney hilum, consistently including both perirenal bona fide brown fat and adjacent kidney tissue.

Results

In addition to observing UCP1 in the perirenal adipose tissue, we observed – using our in-house UCP1 antibody "C10" – well-defined immunopositive structures in the kidney parenchyma, in apparent agreement with the earlier descriptions. To corroborate this, we examined the interaction of the C10 UCP1 antibody with kidney sections from UCP1-ablated mice. We found that these completely UCP1-negative tissues reacted equally well to the C10 antibody. We then performed a similar test using the widely used antibody ab10983, earlier used in the kidney studies. The positive reaction in the kidney parenchyma remained in the UCP1-ablated mice, clearly invalidating the earlier results. In UCP1 qPRC studies, we were similarly unable to detect UCP1 mRNA above background. We also examined two highly specific antibodies (E9Z2V and ab209483) and found that these antibodies accurately reported UCP1 in the perirenal adipose tissue but fully failed to show a positive signal in the kidney parenchyma.

Conclusions

All data presented failed to provide any evidence that UCP1 is found in the kidney. Evidently, this conclusion also implies that the outcome with UCP1 reporter mice specifically concerning kidney expression of the UCP1 gene – but probably extrapolatable to other tissues – would need reconfirmation before being taken as evidence for the presence of UCP1 in non-adipose tissues.

* Sousa-Filho CPB, Petrovic N. No UCP1 in the kidney. Molecular Metabolism, 95: 102127, 2025.

Contribution type:
Poster – P

P159

Title: A new conception of the existence of the Closed 9-Stepped Cycle of Proton Conductance

First name: Ambaga Miyegombo, Tumen-Ulzii Ambaga, Buyantushig Tumen-Ulzii

Affiliation: "New Medicine" Medical University, Ulaanbaatar, Mongolia

E-mail address: ambaga@ncm.edu.mn

Presenting author: Ambaga Miyegombo

Abstract text.

Classical bioenergetics explains mitochondrial ATP synthesis through chemiosmotic coupling and proton motive force; however, these models remain functionally open with respect to systemic proton, carbon dioxide, and oxygen continuity across cellular, blood, and pulmonary compartments.

Here we propose the Closed 9-Stepped Cycle of Proton Conductance (C9SCPC) as an integrated bioenergetic framework that unifies mitochondrial metabolism, erythrocyte buffering, and respiratory gas exchange into a loss-free, cyclic system.

The methodology by which the Closed 9-Stepped Cycle of Proton Conductance was first established consists of several stages.

First, the incomplete equation

C6H12O6 + 6O2 = Energy + 6H2O + 6 CO2 was corrected and reformulated into a new, error-free versions:

Donor (CHO) + Membrane Redoxy potential Three state line system +ADP + Pi + O2 +Protons in the intermembrane space = ATP + heat + carbon dioxide + metabolic water + matrix protons

Second, the principal parameters of this newly established equation were arranged into nine sequential, mutually interconnected stages (1–9).

The presentation detailed the sequence:

Stage 1-CHO food molecules → $NADH/FADH_2$ formation

Stage 2-Hydrogen atom separation into proton + electrons

Stage 3-Electron transport chain initiation

Stage 4-Proton gradient establishment across mitochondrial membrane

Stage 5-ATP synthesis, heat generation, metabolic water formation

Stage 6-Exit of $CO_2 + H_2O \rightarrow H_2CO_3$ formation in serum

Stage 7-Hamburger shift: $HCO_3^- \leftrightarrow$ RBC exchange; Bohr–Haldane balance

Stage -8-Oxygen uptake via electrophilic substitution

Stage 9-Oxygen release via nucleophilic substitution → entry into mitochondria

This closed architecture introduces a system-level conservation principle for protons, resolving conceptual gaps between mitochondrial bioenergetics and whole-body gas transport. The model predicts measurable coupling between mitochondrial

redox state, bicarbonate flux, hemoglobin proton buffering, and oxygen delivery efficiency. gas analysis, and membrane redox measurements.

Within the Closed 9-Stepped Cycle of Proton Conductance, the 9th stage of each cycle gives rise to and conditions the 1st stage of the next cycle, forming a continuous, life-long process that persists without interruption until the end of an individual's life."

P160

P161

Hypomagnetic fields modulate lifespan, physical ability and mitochondrial metabolism in a *Pink1* model of neurodegeneration.

Jacob Reed[1], Mark Fromhold[4], Nicoleta Moisoi[3], Melissa Mather[2], Lisa Chakrabarti[1]

1 School of Veterinary Medicine and Science, University of Nottingham, UK.

2 Optics and Photonics Group, Faculty of Engineering, University of Nottingham, UK.

3 School of Pharmacy, De Montfort University, UK.

4 School of Physics and Astronomy, University of Nottingham, UK.

Email: Lisa.chakrabarti@nottingham.ac.uk

Magnetic fields (MFs) influence biological systems in a variety of contexts, including interplanetary space travel, artificial electromagnetic environments created by medical equipment, and magnetoreception in migratory animals. One area of growing interest is the biological impact of reducing the geomagnetic field (GMF). Hypomagnetic fields (HMFs; <0.004 μT) can be generated experimentally by shielding organisms from the Earth's magnetic field. While previous studies have examined the effects of HMF exposure in healthy model organisms, the potential therapeutic implications of reduced magnetic fields in disease models remain largely unexplored. Mitochondrial dysfunction is a key feature of ageing and many age-associated diseases, including Parkinson's disease. Although most cases of Parkinson's disease are idiopathic, mutations in PTEN-induced kinase 1 (PINK1) cause a rare autosomal recessive form of early-onset disease. Loss-of-function Pink1 models in Drosophila melanogaster reproduce several hallmarks of Parkinson's disease, including impaired mitochondrial function, locomotor deficits, degeneration of dopaminergic neurons, and reduced lifespan.

To investigate whether modulation of magnetic field strength influences mitochondrial physiology in a neurodegenerative disease model, we exposed wild-type (w^{1118}) and Pink1/B9 ($Pink1^{-}$) Drosophila melanogaster to hypomagnetic conditions. A MuMagnetic GA4 benchtop shielding apparatus (Magnetic Shields Ltd., UK) was used to generate a uniform internal magnetic field of approximately 5 nT, effectively eliminating the Earth's GMF (normally 25–60 μT). The effects of HMF exposure were assessed using several physiological and behavioural readouts, including lifespan analysis, locomotor performance measured by a climbing assay, mitochondrial respiration, and superoxide production. HMF exposure increased the lifespan of $Pink1^{-}$ mutant flies by approximately 20%, although their climbing ability was reduced. In contrast, wild-type flies exhibited decreased lifespan under HMF conditions but showed improved locomotor performance. Reactive oxygen species were captured using the spin probe TEMPOL, and changes in superoxide levels were quantified using nitrogen-vacancy (NV) centre quantum diamond sensors. High-resolution mitochondrial respirometry revealed increased mitochondrial complex II activity in flies maintained under HMF conditions. These findings demonstrate that hypomagnetic field exposure alters mitochondrial physiology and reactive oxygen species production in Drosophila melanogaster. Our results suggest that biological pathways responding to the absence of the geomagnetic field may represent novel, non-invasive targets for modulating mitochondrial dysfunction in neurodegenerative disease.

Topic list: S14. Mitochondrial physiology

Contribution type: Poster – P and
Talk – T

P162

Protonmotive force drives circuit-like signaling networks between mitochondria under metabolic stress

Nada Ahmed Selim[a], Shon A. Koren[b], Peter Girardi[b], Joel Rodwell[b], Andrew P. Wojtovich[a,b]

[a]Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY, USA [b]Department of Anesthesiology and Perioperative Medicine, University of Rochester Medical Center, Rochester, NY, USA

E-mail address: nada_ahmedselim@urmc.rochester.edu
Presenting author: Nada Ahmed Selim

Mitochondria must coordinate their activity to mount effective stress responses. While several mechanisms have been proposed for inter-mitochondrial communication, including ROS, physical contacts, and metabolite exchange, how mitochondria propagate signals in real time remains unclear. Using optogenetic control of ROS production, we found that mitochondria act as ROS sinks rather than sources, prompting us to look beyond ROS for the propagating signal [1]. We hypothesized that mitochondria communicate via protonmotive force (PMF), functioning similarly to voltage-based signaling in excitable cells [2]. To test this, we developed mtOFF, an optogenetic tool that harnesses light to precisely depolarize a single mitochondrion. Paired with an automated single-organelle tracking pipeline, mtOFF allowed us to map PMF signal propagation within living HeLa cells and primary neurons. PMF perturbations propagated up to 15 micrometers from the stimulated mitochondrion, dependent on ER-mitochondria calcium exchange that serves as a relay shaping signal reach. Functional connectivity between mitochondria emerged under metabolic stress, with stimulated mitochondria forming dense directed networks featuring hub organelles that controlled signal directionality, while unstimulated controls showed no network formation. In neurons, physiological activity drove compartment-specific PMF transients in the soma, dendrites, and axons, suggesting these same networks may relay local energetic demand across the mitochondrial population. These findings establish mitochondria as circuit-like signaling networks where PMF coordinates stress responses through structured, directional communication, redefining how we understand metabolic coordination at the organelle level.

[1] S.A. Koren, N. Ahmed Selim, L. De la Rosa, J. Horn, M.A. Farooqi, A.Y. Wei, A. Müller-Eigner, J. Emerson, G.V.W. Johnson, A.P. Wojtovich, All-optical spatiotemporal mapping of ROS dynamics across mitochondrial microdomains in situ, Nat. Commun. 14(1) (2023) 8230, https://doi.org/10.1038/s41467-023-44146-6

[2] N.A. Selim, A.P. Wojtovich, Mitochondrial membrane potential and compartmentalized signaling: Calcium, ROS, and beyond, Redox Biol. 86 (2025) 103859, https://doi.org/10.1016/j.redox.2025.103859

P163

The role of mitochondria in antipsychotic-induced metabolic syndrome.

Katherine R.H Mortimer[1], Mohammad Zia Ul Haq Katshu[2,3], Adam Dowle[4], Reinhard Stöger[5], Lisa Chakrabarti[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham, United Kingdom

[2]Institute of Mental Health, School of Medicine, University of Nottingham, Nottingham, United Kingdom

[3]Nottinghamshire Healthcare NHS Foundation Trust, Nottingham, United Kingdom

[4]Department of Biology, Bioscience Technology Facility, University of York, United Kingdom

[5]School of Biosciences, University of Nottingham, Nottingham, United Kingdom

Email: Lisa.chakrabarti@nottingham.ac.uk

Second-generation antipsychotics (SGAs) are widely prescribed for the treatment of severe psychiatric disorders such as Schizophrenia and Bipolar disorder. Although these drugs are effective in managing psychotic symptoms, their clinical use is frequently limited by metabolic side effects, including weight gain, dyslipidaemia, insulin resistance, and the development of metabolic syndrome. Among SGAs, olanzapine and clozapine carry the greatest metabolic risk. Clozapine is the most effective treatment for treatment-resistant schizophrenia, and olanzapine is widely used due to its strong efficacy. Both are consistently associated with obesity and hypercholesterolaemia, yet the underlying cellular mechanisms remain incompletely understood.Given the central role of mitochondria in cellular metabolism, we hypothesised that SGAs disrupt mitochondrial physiology, thereby contributing to metabolic disturbances. Using high-resolution respirometry in HepG2 hepatocyte cells, we examined the effects of olanzapine and clozapine on mitochondrial function. Both drugs significantly impaired mitochondrial respiration, characterised by reduced fatty acid oxidation-linked respiration and diminished oxidative phosphorylation capacity through both complex I- and complex II-linked pathways. These findings indicate substantial disruption of mitochondrial energy metabolism. Proteomic analysis further revealed that olanzapine markedly upregulates several enzymes within the mevalonate pathway, resulting in increased cholesterol biosynthesis. This metabolic reprogramming may exacerbate mitochondrial dysfunction and contribute to the development of hypercholesterolaemia associated with SGA-induced metabolic syndrome. We also explored pharmacological strategies to counteract these mitochondrial effects. Treatment with Metformin partially mitigated mitochondrial impairments, while the GLP-1 receptor agonist Liraglutide demonstrated more pronounced protective effects on mitochondrial function.

Together, these findings provide mechanistic insight into the mitochondrial and metabolic alterations induced by SGAs and suggest potential therapeutic strategies to reduce the metabolic burden associated with antipsychotic treatment.

Topic list: S15. Bioenergetics in health & disease
Contribution type: Poster – P and Talk – T

P164

A Strategy for Precise Detection of High-Risk Chronic Liver Disease using Nano-Diamond Quantum Sensing.

Chloe Sargent[1], Jacob Reed[1], Aloysius A. Aravinthan[2], Mark Fromhold[3], Melissa Mather[4], Lisa Chakrabarti[1]

1 School of Veterinary Medicine and Science, University of Nottingham, UK.

2 School of Medicine, University of Nottingham, UK

3 School of Physics and Astronomy, University of Nottingham, UK.

4 Optics and Photonics Group, Faculty of Engineering, University of Nottingham, UK.

Email: lisa.chakrabarti@nottingham.ac.uk

Chronic liver disease (CLD) is a growing cause of morbidity and mortality worldwide. Disease progression is highly variable: while many patients remain clinically stable for prolonged periods, a subset develops cirrhosis, liver failure, or liver cancer. Identifying which patients will progress remains a major clinical challenge. Current diagnostic tools can estimate fibrosis but do not reliably identify those at highest risk of complications. As a result, large numbers of patients undergo repeated monitoring while some high-risk individuals are not detected early enough for targeted surveillance or therapeutic intervention. Mitochondrial dysfunction is increasingly recognised as a key driver of CLD progression. In hepatocytes, impaired mitochondrial bioenergetics alters oxidative phosphorylation, disrupts cellular energy balance, and promotes the accumulation of reactive oxygen species (ROS). These highly reactive molecules contribute to oxidative damage, cellular senescence, and fibrogenic signaling pathways. Importantly, mitochondrial ROS production reflects underlying bioenergetic stress and may therefore serve as a mechanistic indicator of disease activity and progression.

In this study, we are applying diamond-based quantum sensing to directly detect ROS associated with mitochondrial dysfunction in liver tissue. The approach uses nanodiamonds containing nitrogen–vacancy (NV) centres, atomic-scale defects that act as highly sensitive quantum sensors. Through optically detected magnetic resonance (ODMR), NV centres can detect the magnetic signatures generated by individual free radicals with nanometre-scale sensitivity. This enables direct measurement of ROS in biological samples, overcoming the limitations of conventional assays that measure indirect or bulk markers of oxidative stress. Quantum ROS measurements will be integrated with detailed assessment of mitochondrial bioenergetic function, including analysis of respiratory activity and mitochondrial integrity in liver tissue samples. By combining quantum sensing with bioenergetic profiling and histological evaluation, we aim to identify cellular signatures linking mitochondrial dysfunction to disease severity.

We will present the first application of diamond quantum sensing to quantify reactive oxygen species in human liver biopsy samples and examine their relationship with mitochondrial bioenergetic dysfunction and clinical indicators of chronic liver disease severity. Preliminary findings demonstrate the feasibility of detecting ROS signals associated with mitochondrial stress using NV-centre sensors. These results highlight how quantum sensing technologies can provide direct insight into mitochondrial-driven pathology and may enable the development of mechanistic biomarkers for risk stratification and precision monitoring in chronic liver disease. Topic list: S15. Bioenergetics in health & disease

Contribution type: Poster – P and Talk – T

P165

A redox cycler- based theraphy againstin mitochondrial diseases

Davide Fontana[a], Daniele Bonesso[a], Michael Donadon[a], Andrea Mattarei[b], Lucia Biasutto[c,] Carlo Viscomi[d], Massimo Zeviani[d], Ildik Szab [a]

[a]Department of Biology, University of Padova, Padova, Italy.

[b]Department of Pharmacological and Pharmaceutical Sciences, University of Padova, Padova, Italy [c]CNR Institute of Neurosciences [d] Venetian Institute of Molecular Medicine

E-mail address: davide.fontana.5@studenti.unipd.it
Presenting author: Davide Fontana

Mitochondrial diseases represent the most common class of genetic inborn diseases related to metabolism, which are characterized by the presence of dysfunctional mitochondria. Furthermore, due to mitochondria being almost ubiquitarian cellular organelles, they are characterized by an highly heterogenous clinical presentation. Gene therapy can be envisioned as the only definitive cure. However, due to the difficulty in targeting the mitochondrial genome which resides inside the mitochondrial matrix, over the last decades several pharmacological approaches have been studied. In this context, we have previously identified a class of small molecules, the so-called redox cyclers, able to undergo cyclic reactions of reduction and oxidation by accepting electrons from donors with lower redox potential and then donating them to acceptors with higher redox potential (Peruzzo et al, 2021). In this context, pyocyanin was highlighted as the most promising candidate thanks to its redox potential which is between NAD^+/NADH, $CoQ/CoQH_2$ and cyt c (oxidized)/cyt c (reduced.). In vitro, pyocyanin demonstrated, when used in the sub-µM range, to be able to increase mitochondrial ATP production and mitochondrial oxygen consumption in both MAFs Ndufs4 KO cells, a model of complex I (CI) deficiency, and MEFs Ttc19 KO cells, a model of complex III deficiency, suggesting its ability in bypassing CI and CIII and alleviate the reducing equivalents overload caused by the dysfunction of such complexes. Furthermore, pyocyanin proved to be able to bypass such complexes regardless of the underlying mutation and to have beneficial effects on mitochondrial membrane potential. Following, pyocyanin and other redox cyclers were tested in vivo in disease models where they proved useful in recovering muscle and neurological functions without inducing any toxicity over long-period treatments.

Exploiting pyocyanin to treat mitochondrial disease due to respiratory complex III dysfunction. Peruzzo R, Corrà S, Costa R, Brischigliaro M, Varanita T, Biasutto L, Rampazzo C, Ghezzi D, Leanza L, Zoratti M, Zeviani M, De Pittà C, Viscomi C, Costa R, Szab I. Nat Commun. 2021 Apr 8;12(1):2103.

P167

Differential Modulation of Forward and Reverse Electron Flow at Complex I by Metformin Enables Selective Inhibition of Reverse Electron Transport and Protects Against Ischemia–Reperfusion Injury

Livia P. Ferreira[a], Leticia M. C. Nunes[a], Heberty T. Facundo[a] [a]*Faculdade de Medicina, Universidade Federal do Cariri, Ceará, Brazil*

E-mail address: heberty.facundo@ufca.edu.br
Presenting author: Heberty Tarso Facundo

Metformin has been reported to inhibit mitochondrial complex I at high concentrations; however, its potential to selectively modulate reverse electron transport (RET) without impairing forward electron flow remains unclear. Because RET-driven reactive oxygen species (ROS) production during ischemia–reperfusion is a major contributor to tissue injury, we investigated whether metformin could function as a pharmacological inhibitor of RET at concentrations that preserve forward respiration. Isolated mitochondria were exposed to metformin (0–25 mM) and oxygen consumption was measured in states 2, 3, and 4 using malate/glutamate as complex I substrates. Hydrogen peroxide (H_2O_2) production was quantified by the Amplex Red/horseradish peroxidase system. RET was induced by energizing mitochondria with succinate or with malate/glutamate in the presence of oligomycin, and ROS formation was assessed under these conditions. In parallel, a chemical RET system was established in lysed mitochondria using NAD^+ and decylubiquinol in the presence of antimycin A, cyanide, and malonate, allowing direct evaluation of RET flow through complex I by monitoring NADH formation. Complex I enzymatic activity was also assessed in lysed mitochondria. Metformin concentrations (3.12-12.5 mM) did not significantly alter oxygen consumption in any respiratory state with malate/glutamate. In contrast, 25 mM inhibited respiration. Forward electron transport supported by complex I substrates showed increased ROS production at 25–50 mM metformin, whereas lower concentrations did not significantly affect H_2O_2 generation. Strikingly, the same intermediate concentrations (3.12–12.5 mM) that preserved forward respiration and ROS production significantly suppressed RET-driven ROS formation during succinate-supported respiration. At 6.25-12.5 mM or above, metformin inhibited RET-derived ROS to levels comparable to rotenone. Consistent results were obtained in the chemical RET system, in which metformin prevented NADH accumulation driven by reverse electron flow from decylubiquinol to complex I. Notably, metformin did not significantly alter complex I enzymatic activity in lysed mitochondria. In isolated hearts subjected to ischemia–reperfusion in a Langendorff perfusion system, administration of metformin (3 mM) at reperfusion improved ratepressure product and left ventricular end developed pressure at 15 and 30 minutes of reperfusion compared to controls (vehicle-treated). These results identify a concentration window in which metformin selectively suppresses RET–driven ROS generation while preserving forward respiration and basal ROS production. This bioenergetically permissive inhibition of RET supports the feasibility of a proof-of-concept therapeutic strategy aimed at limiting ischemia–reperfusion injury without compromising mitochondrial function.

P168

The creatine/creatine kinase system in rat adipose tissue

Orane Lerouley[a], Valeria Cortés Garduño[a], Violette Duval[a], Stéphane Attia[a], Cécile Cottet-Rousselle[a], Amel Achouri[a], Cindy Tellier[a], Theo Wallimann[b], Malgorzata Tokarska-Schlattner[a], Uwe Schlattner[c], Laurence Kay[a]

[a]*Univ. Grenoble Alpes, Inserm U1055, LBFA, Grenoble, France* [b]*Department of Biology, ETH Zurich, Switzerland*
[c]*Univ. Grenoble Alpes, LBFA, Grenoble, and Institut Universitaire de France, Paris, France*

E-mail address: orane.lerouley@univ-grenoble-alpes.fr
Presenting author: Orane Lerouley

The creatine (Cr)/creatine kinase (CK) system is a well-established cellular energy buffer and shuttle, particularly in tissues with high or fluctuating energy demands. Beyond this classical role, it has been proposed to support UCP1-independent but ATP-dependent thermogenesis in beige and brown adipocytes through a putative mitochondria-localized, Cr-driven futile cycle involving phosphocreatine (PCr) turnover by brain-type CK (BCK) and TNAP acting as a mitochondrial phosphatase. Here, we investigated the basal properties of the Cr/CK system in adipose tissues of rats exposed to thermoneutral (28 °C), ambient (22 °C), or cold (5 °C) conditions. Using a combination of protein expression analysis, imaging, metabolite assessment, and mitochondrial respiration measurements, we characterized CK isoform distribution and functional activity in brown (BAT), beige, and white adipose tissue (WAT).

We found that, in total tissue lysates, cytosolic CK isoforms predominate, with muscle-type CK (MCK) enriched in BAT and BCK in WAT and beige fat. In contrast, mitochondrial CK isoforms were present at very low levels, with minor contributions from sarcomeric mitochondrial CK (smtCK) in BAT and ubiquitous mitochondrial CK (umtCK) in beige fat and WAT. Notably, adipose tissue heterogeneity (including CK-expressing vascular and neuronal components) must be considered when interpreting these data. Cold exposure, which may more directly reflect adipocyte-specific responses, modestly increased MCK and smtCK levels in BAT, while changes in beige fat and WAT were variable. In contrast, glycolytic markers and uncoupling protein UCP1 were robustly upregulated in both beige fat and BAT, with the latter confirming activation of classical thermogenic pathways. Neither BCK, TNAP, nor mitochondrial respiratory complex markers showed consistent cold-induced increases.

Respiration of isolated mitochondria from BAT revealed strong UCP1-dependent uncoupling further enhanced by cold, whereas WAT mitochondria lacked UCP1-mediated respiration. Mitochondria from both tissues displayed oligomycin-sensitive, ADP- and ATP-stimulated respiration, with ATP-stimulated respiration being particularly evident in WAT, suggesting the presence of ATPase/phosphatase activities. However, in both cases, this respiration was not affected by the TNAP inhibitor Sbi-425. Importantly, Cr supplementation only marginally increased respiration in mitochondria from cold-exposed WAT, while having no significant effect at ambient temperature, consistent with very low mitochondrial CK activity in mitochondrial lysates.

Together, these results support a basal role of the Cr/CK system in adipose tissue energetics, particularly under cold conditions, but so far provide no evidence for a significant contribution of a creatine-driven futile cycle to thermogenesis in rat adipocytes under these conditions.

P169

Elucidating the role of mitochondrial protein NDUFA4L2 in pulmonary arterial smooth muscle cells exposed to chronic hypoxia and pulmonary hypertension

Sandipan Jash[1], Arjang Adeli[1], Vidya Lakshmi[1], Fenja Knoepp[1], Oleg Pak[1], Werner Seeger[1,2], Norbert Weissmann[1], Luca Giordano[1], Natascha Sommer[1]

[1]Excellence Cluster Cardio-Pulmonary Institute (CPI), Universities of Giessen and Marburg Lung-Center (UGMLC), Member of the German Center for Lung Research (DZL), Justus-Liebig University Giessen, Germany [2]Institute for Lung Health (ILH), Giessen, Germany

Introduction: Pulmonary hypertension (PH) is caused by vascular remodelling of the pulmonary arteries (PA) induced by resistance to apoptosis and hyperproliferation of pulmonary arterial smooth muscle cells (PASMC). This phenotype is sustained by decreased mitochondrial respiration (MR) and increased anaerobic glycolysis, leading to a metabolic adaptation (MA). The mitochondrial NADH dehydrogenase 1a subcomplex subunit 4-like 2 (NDUFA4L2) is upregulated in several cell types exposed to chronic hypoxia (CH); however, its role in the MA of the PASMC exposed to CH and PH remains unclear. Materials and methods: RNAseq, western blot (WB) and immunostaining of the pulmonary arteries (PA) were used to quantify NDUFA4L2 in mice exposed to CH (10% O_2) and in lung biopsies of patients with idiopathic PH (IPAH). qPCR and WB were used to detect NDUFA4L2 expression in isolated mouse and human PASMC exposed to CH (1% O_2), and high-resolution respirometry to examine MR. The functional role of NDUFA4L2 in PASMC during CH was tested by siRNA-mediated downregulation, followed by cell proliferation (CelPro) and scratch assays, and WB to explore proliferative (pAKT, pERK), apoptotic (BAX, cleaved PARP, cleaved caspase-3) and metabolic (PDHK1, LDHA, PC, OXPHOS) markers. Results: NDUFA4L2 protein was upregulated in the lung homogenate and PA of mice exposed to CH, and in the lung biopsies from IPAH patients. *In vitro*, NDUFA4L2 mRNA and protein were upregulated in mouse and human PASMC, along with decreased MR. Silencing NDUFA4L2 in PASMC exposed to CH decreased cell survival and wound healing, increased protein levels of apoptotic markers and restored mitochondrial complex I and III subunits, and pyruvate carboxylase. Conclusion: Our results support a direct role of NDUFA4L2 in the pathogenesis of CH-induced PH. It controls mitochondrial function, apoptosis of the PASMC during CH. Future investigations using NDUFA4L2 KO mice will clarify its role in the pathophysiology of PH.

P170

Quercetin ameliorates the damage induced by atomoxetine in SH-SY5Y cells by enhancing mitochondrial function and reducing ROS production

Juan Carlos Corona, Sonia Carreón-Trujillo, Marcela González-Ordoñez and Alejandra Esparza-Sánchez

Laboratory of Neurosciences, Hospital Infantil de México Federico Gómez. Dr. Márquez 162, Colonia Doctores, Alcaldía Cuauhtémoc, C.P. 06720, Mexico City, Mexico.

E-mail address: jccc93@gmail.com
Presenting author: Juan Carlos Corona

Abstract

Introduction: Although the pathophysiology of attention-deficit/hyperactivity disorder (ADHD) remains elusive, increasing evidence suggests that oxidative stress and mitochondrial dysfunction in ADHD are associated with the pathophysiology [1-2]. ATX is a non-psychostimulant drug prescribed for the treatment of ADHD, and several studies in vivo and in vitro have demonstrated that ATX use induces cell damage, neuroinflammation, oxidative stress, and mitochondrial dysfunction [3-4]. Quercetin is a flavonoid compound present in a wide variety of vegetables and fruits; quercetin represents the highest percentage of total flavonoid intake. Thus, quercetin has been demonstrated to exert neuroprotective effects in several neurodegenerative and neurodevelopmental disorders as well as by its antioxidant properties [5], but the mechanism of protection is not clear. Aims: We investigated whether quercetin prevents cell death, oxidative stress, and mitochondrial dysfunction in SH-SY5Y cells exposed to ATX, and explored the interaction of pathways involved in neuroprotection. Methods: Human neuroblastoma SH-SY5Y cells were used, treated for 24 hours with different doses of ATX and quercetin; in addition, cells were pre-treated for 4 hours with quercetin and then with ATX for 24 hours. Cell viability, cytosolic and mitochondrial reactive oxygen species (ROS) production with DHE and MitoSOX, mitochondrial membrane potential ($\Delta\psi m$) with MitoTracker and Ca^{2+} levels with Fluo-4 were assessed. Results: Our data show that ATX treatment in SH-SY5Y cells decreases $\Delta\psi m$ and Ca^{2+} levels, while increasing cell death and the production of cytosolic and mitochondrial ROS. All these changes were reversed by pre-treatment of the cells with quercetin, which normalised the $\Delta\psi m$ and Ca^{2+} levels, decreasing cell death and ROS production. Conclusion: Our data indicate that quercetin is potentially neuroprotective, acting directly on oxidative stress and mitochondrial function in cells, and might provide a possible co-adjuvant therapy for the treatment of neurodevelopmental disorders such as ADHD.

[1] Giannoulis SV, Müller D, Kennedy JL, Gonçalves V, Systematic review of mitochondrial genetic variation in attention-deficit/hyperactivity disorder, Eur Child Adolesc Psychiatry. (2024) Jun;33(6):1675-1685. doi: 10.1007/s00787-022-02030-6.

[2] Corona JC, Role of Oxidative Stress and Neuroinflammation in Attention-Deficit/Hyperactivity Disorder, Antioxidants. (2020) Oct 23;9(11):1039. doi: 10.3390/antiox9111039.

[3] Corona JC, Carreón-Trujillo S, González-Pérez R, Gómez-Bautista D, Vázquez-González D, SalazarGarcía M, Atomoxetine produces oxidative stress and alters mitochondrial function in human neuron-like cells, Sci Rep. (2019) Sep 10;9(1):13011. doi: 10.1038/s41598-019-49609-9.

[4] Gür F, Cengiz M, Gür B, Alternations in nuclear factor kappa beta activity (NF-kB) in the rat brain due to long-term use of atomoxetine for treating ADHD: In vivo and in silico studies, Biochem Biophys Res Commun. (2021) Jan 1;534:927-932. doi: 10.1016/j.bbrc.2020.10.072. Epub 2020 Nov 2.

[5] Alvarez-Arellano L, Salazar-García M, Corona JC, Neuroprotective Effects of Quercetin in Pediatric Neurological Diseases, Molecules. (2020) Nov 28;25(23):5597. doi: 10.3390/molecules25235597.

Contribution type: Poster – P

P171

Mitochondrial Nicotinamide Nucleotide Transhydrogenase (NNT) is part of a supercomplex Weiwei Li[a,b,c], Maria Nino Quiroga[b,c], Inge van der Stelt[a], Els M.A. van de Westerlo[c,d], Joost C.M. Holthuis[e], Sebastian Bieker[e], Martijn A. Huynen[c,d], Jan C. van der Meijden[b,c], Merel J.W. Adjobo-Hermans[c,d], Jaap Keijer[a], Johannes N. Spelbrink[b,c] and Werner J.H. Koopman[a,b,c]

[a]*Human and Animal Physiology, Wageningen University and Research, Wageningen, The Netherlands.*
[b] *Department of Pediatrics, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands.*
[c]*Radboud Center for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.*
[d]*Department of Medical BioSciences, Radboud Center for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands.*
[e]*Department of Biology/Chemistry, Osnabrück University, Osnabrück, Germany.*

E-mail address: Weiwei.Li@radboudumc.nl
Presenting author: Weiwei Li

Nicotinamide Nucleotide Transhydrogenase (NNT) is embedded in the mitochondrial inner membrane (MIM) [1]. In forward mode, NNT mediates proton (H^+) influx into the mitochondrial matrix to drive the conversion of ($NADP^+$ and NADH) into (NADPH and NAD^+). In this way, NNT connects hydrogen peroxide (H_2O_2) removal in the mitochondrial matrix (via NADPH) to electron transport chain (ETC) function (via H^+ influx and NADH). Under pathological conditions, NNT operates in reverse, thereby consuming NADPH, producing NADH, and mediating matrix H^+ efflux. It still remains poorly understood how forward- and reverse-mode NNT action are regulated and how this action integrates with cellular and mitochondrial redox and energy homeostasis. According to all current experimental evidence, NNT is active as a homodimer [1,2,3]. However, in our current studies we provide evidence that NNT is part of a supercomplex (NNT-SC). Here, we will present our characterization of this NNT-SC using a variety of techniques.

[1] Z. Gan, I. van der Stelt, W. Li, L. Hu, J. Song, S. Grefte, E. van de Westerlo, D. Zhang, E.M. van Schothorst, H.L. Claahsen-van der Grinten, K.J. Teerds, M.J.W. Adjobo-Hermans, J. Keijer, W.J.H. Koopman, Mitochondrial Nicotinamide Nucleotide Transhydrogenase: Role in Energy Metabolism, Redox Homeostasis, and Cancer, Antioxid. Redox Signal. 41 (2024) 927–956, https://doi.org/10.1089/ars.2024.0694

[2] Kampjut D, Sazanov LA. Structure and Mechanism of Mitochondrial Proton-translocating Transhydrogenase. Nature. 2019 Sep;573(7773):291-295. doi: 10.1038/s41586-019-1519-2. Epub 2019 Aug 28. PMID: 31462775.

[3] Nesci S, Trombetti F, Pagliarani A. Nicotinamide Nucleotide Transhydrogenase as a Sensor of Mitochondrial Biology. Trends Cell Biol. 2020 Jan;30(1):1-3. doi: 10.1016/j.tcb.2019.11.001. Epub 2019 Nov 18. PMID: 31753532.

P172

Bilirubin Phototherapy Triggers Carbon Monoxide Release, Superoxide Production, and Mitochondrial Respiratory Decline In Vitro

Ales Dvorak[a] , Daniela Semlerova[a,c], Lucie Muchova[a], Jakub Vavra[a], David Krepelka[a], Libor Vitek[a,b]

Faculty of Medicine, Charles University, 12108
[b]4th Department of Internal Medicine, General University Hospital in Prague, 1st Faculty of Medicine, Charles University, 12108
[c] Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, 166 28, all Prague, Czech Republic

E-mail address: ales.dvorak@lf1.cuni.cz
Presenting author: Ales Dvorak

Introduction: Recent studies in human newborns have suggested the possible adverse effects of phototherapy (PT, 420–500 nm) used to treat neonatal jaundice, including increased mortality in premature infants and a higher incidence of allergies or even cancers. During PT, bilirubin (BR) is converted into photoproducts that are more readily excreted out of the human body. This study aimed to evaluate the biological effects of PT using in vitro models of immune cells and hepatocytes.

Materials & Methods: Jurkat cells (human immortalized T lymphocytes) and HepG2 (human hepatoblastoma) cells were treated with BR (0, 10, 20, and 50 μM) for 24 h and then exposed to blue light (BL; 460 nm, 2 h) in vitro or kept in the dark. Intracellular carbon monoxide (CO) and superoxide were measured by gas chromatography with a reduced gas detector (GC-RGD) and flow cytometry (MitoSOX dye ~488/510 nm), respectively. Cell viability was assessed by flow cytometry (forward/side scatter parameters) and MTT assay. Cellular respiration was analyzed by high-resolution respirometry (O2k, Oroboros; SUIT-003). The oxygen concentration in irradiated media (±BR) was measured using O2k equipped with BL. BR concentrations in irradiated samples were determined by LC-MS (Poroshell 120 EC-C18 column, gradient elution with 1 mM NH_4F/MeOH, positive SRM mode).

Results: Cell viability was unchanged except in cells treated with 50 μM BR. In HepG2 cells, PT significantly increased intracellular CO concentrations (30 vs. 50–90 pmol/1×10⁶ cells; $p < 0.05$ at 20 μM BR) as well as superoxide production, reaching a three-fold increase at 20 μM BR after irradiation. In Jurkat cells, BR (10 and 50 μM) increased superoxide production two times and six times, respectively ($p < 0.05$). CO concentrations were higher in irradiated cells (control vs. 10 μM BR: 25 vs. 100 pmol/1×10⁶ cells; $p < 0.05$). Routine and maximal respiration was significantly reduced after irradiation. PT decreased the BR concentration in the medium by ~80% and oxygen concentrations by ~90%.

Conclusion: PT increases intracellular CO and superoxide production in both cell types, which may contribute to the observed reduction in cellular respiration of Jurkat cells. Moreover, BR photoconversion is associated with substantial oxygen consumption in the medium. These findings suggest that PT-induced oxidative stress and oxygen depletion warrant further investigation as potential mechanisms underlying adverse clinical effects. Supported by Foundation of the Czech Society of Hepatology and by the Inter-Action project LUAUS26259 given by the Czech Ministry of Education.

P173

Mutant Spartin causes defects in mitochondrial protein import process and cellular bioenergetics

Nicola Rizzardi[a], Chiara Diquigiovanni[b], Luca Pincigher[a], Martina Salmi[a], Veronica Casalini[a], Francesca Valenti[a], Elena Bonora[b,c], Arianna Orsini[b], Erica Cataldi Stagetti[b], Luca Soliani[c], Duccio Maria Cordelli[b,c], Marilena D'Aurelio[d], Romana Fato[a], Christian Bergamini[a]

[a]Department of Pharmacy and Biotechnology, University of Bologna, Bologna 40126, Italy [b]Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy [c]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, 40128, Italy [d]Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA

nicola.rizzardi2@unibo.it:
Presenting author: Nicola Rizzardi

Hereditary spastic paraplegia (HSP) encompasses a large group of rare genetic neurological diseases characterized by degeneration of the upper motor neurons, spasticity and weakness of the lower limbs [1]. Troyer syndrome (TS) is an autosomal recessive form of HSP characterized by additional features such as short stature, cognitive impairment, distal amyotrophy and degeneration of corticospinal tract axons [2]. TS is caused by loss-of-function mutations in SPART, also known as SPG20, which encodes for Spartin, a multifunctional protein that consists of a N-terminal microtubule interacting and trafficking (MIT) domain and a C-terminal senescence domain.

To identify the overall altered biological pathways due to mutant Spartin, we performed RNA-sequencing in *i)* a genome-edited neuronal cell line with a homozygous SPART frameshift mutation vs.isogenic controls and *ii)* skin-derived fibroblasts from a patient carrying biallelic SPART variants. Then, functional studies to evaluate the mitochondrial bioenergetics were carried out. Differential expression and gene ontology analysis identified transcriptional and translational processes, including the oxidative phosphorylation, as top significantly downregulated biological pathways. Functional studies in mutant neuronal cells and fibroblasts demonstrated an altered mitochondrial network morphology, reduced oxygen consumption rate, and decreased expression of mitochondrial complex I and II enzymes. In both cell models, Spartin loss-of-function affected the mitochondria-associated membranes (MAMs) stability, leading to a decrease in mitochondrial and endoplasmic reticulum Ca2+ levels. We further observed a lower expression of the mitochondrial import of nuclear-encoded proteins, associated with a consequent decrease in CoenzymeQ10 (CoQ10) level. Wild-type (WT) Spartin re-expression restored mitochondrial function, ATP/ADP ratio, calcium homeostasis and protein import efficiency. Noteworthy, WT Spartin re-expression fully rescued the expression levels of several OXPHOS genes, including MT-ND3, MT-CO3, and MT-ATP6. CoQ10 supplementation recovered the ATP levels in both Spartin mutant cells. Remarkably, in two patients with homozygous SPART frameshift mutations, six months of Ubidecarenone treatment reduced fatigability and improved the 6-minute walk and the 10-meter run tests, suggesting enhanced aerobic capacity and endurance.

Our findings highlight the critical role of Spartin, as its loss-of-function leads to dysregulation of energy metabolism, subsequently affecting the transcription of genes involved in mitochondrial function. Moreover, our data support CoQ10 as a potential therapeutic approach for patients with TS.

[1] J. Fink, Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms, Acta neuropathological 126(3) (2013) 307-28, doi: 10.1007/s00401-013-1115-8.

[2] H. Tawamie, Recurrent null mutation in SPG20 leads to Troyer syndrome, Molecular and Cellular Probes, 29(5) (2015), 315-8, doi: 10.1016/j.mcp.2015.05.006

P174

Bioenergetic Impairment and CoQ10 Deficiency in Phenylketonuria: Metabolic Insights and Therapeutic strategies

Martina Salmi[a], Nicola Rizzardi[a], Beniamino Benedetto Cattuti[a], Luca Pincigher[a], Francesca Valenti[a], Davide Gibellini[a], Dorina Mita[b], Michele Stecchi[c], Valeria Guarneri[b], Matteo Beretta[b], Lucia Brodosi[c], Christian Bergamini[a] and Romana Fato[a]

[a] Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy [b] Department of Medical and Surgical Sciences, University of Bologna, Via Zamboni 33, 40126, Bologna, Italy [c] Clinical Nutrition and Metabolism Unit, IRCCS AOUBO, Via Albertoni 15, 40138, Bologna, Italy.

E-mail address: martina.salmi2@unibo.it
Presenting author: Martina Salmi

Phenylketonuria (PKU, OMIM 261600) is an inherited metabolic disorder caused by mutations in the gene encoding phenylalanine hydroxylase (PAH), the enzyme that catalyses the conversion of phenylalanine (Phe) to tyrosine. PAH deficiency results in systemic accumulation of Phe, leading to severe cognitive impairments resulting from a neurotoxic effect of hyperphenylalaninemia [1].
The primary treatment for PKU is represented by a lifelong low-phenylalanine diet combined with amino acid supplement. Pharmacological therapies include Sapropterin dihydrochloride, a synthetic analogue of the natural PAH cofactor, tetrahydrobiopterin (BH4) and Pegvaliase administration as enzyme replacement therapy in adults [2]. Studies have shown that PKU is associated with reduced plasma levels of Coenzyme Q10 (CoQ10) and mitochondrial dysfunction, suggesting that the pathology may be considered as a secondary CoQ deficiency disorder [3].
We established an in vitro model of PKU by pharmacological inhibition of PAH using 4-chlorophenylalanine (4-CPA) in a hepatocellular carcinoma cell line (HepG2). The bioenergetic profile of this model showed that PAH inhibition reduces cell viability and impairs mitochondrial function, as evidenced by decreased ATP production, reduced mitochondrial membrane potential and altered oxygen consumption resulting in lower spare respiratory capacity.
Notably, 4-CPA-treated cells exhibited a significant reduction in intracellular CoQ10 levels (−54.6%), consistent with the decreased plasma CoQ10 levels observed in PKU patients, along with increased lipid peroxidation, measured using the fluorescent probe BODIPY C11. The supplementation of PAH-treated HepG2 cells with CoQ10 or with its biosynthetic precursor, 4hydroxybenzoic acid (4-HBA) restored intracellular CoQ10 content (56,6 pmol/mg of protein in 4CPA vs 130,4 pmol/mg of protein in 4-CPA treated with 4-HBA) , improved ATP levels (8 nmol/mg of protein in 4-CPA vs 16 nmol/mg of protein in 4-CPA treated with 4-HBA), and reduced lipid peroxidation. These findings support the notion that reduced CoQ10 levels are a hallmark of PKU and correlate to impaired oxidative phosphorylation, reduced ATP production and oxidative stress. The supplementation with both CoQ10 and its precursor 4-HBA increased intracellular CoQ10 levels and improved bioenergetic function, with 4-HBA demonstrating greater efficacy in enhancing cellular ATP production.
In conclusion, our results suggest that strategies aimed at increasing endogenous CoQ10, in addition to current therapeutic approaches, may offer additional clinical benefit for PKU patients.

[1] Nulmans I, Lequeue S, Desmet L, Neuckermans J, De Kock J. Current state of the treatment landscape of phenylketonuria. Orphanet J Rare Dis. 2025 Jun 5;20(1):281. doi: 10.1186/s13023-025-03840-y. [2] van Wegberg AMJ, MacDonald A, Ahring K, et al. European guidelines on diagnosis and treatment of phenylketonuria: First revision. *Mol Genet Metab*. 2025;145(2):109125. doi:10.1016/j.ymgme.2025.109125
[3] Pallotti F, Bergamini C, Lamperti C, Fato R. The Roles of Coenzyme Q in Disease: Direct and Indirect Involvement in Cellular Functions. Int J Mol Sci. 2021 Dec 23;23(1):128. doi: 10.3390/ijms23010128.

P175

In diet-induced fatty liver, ectopically expressed human UCP1 induces regulated mitochondrial uncoupling and lipid-droplet–associated remodeling

Irina G. Shabalina[a], Glauber R. F. Braz[a], Ingela Bergqvist[b], Naren Qimuge[a], Björn Eriksson[b], Annika Ericsson[c], Barbara Cannon[a], Jan Nedergaard[a]

[a] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden [b] Lipigon Pharmaceuticals AB, Umeå, Sweden [C] CombiGene AB, Stockholm, Sweden

E-mail address: irina.shabalina@su.se
Presenting author: Irina G. Shabalina

Excessive lipid buildup in the liver is a key feature of metabolic dysfunction-associated steatotic liver disease and signals an imbalance among lipid intake, storage, and breakdown. Strategies that boost hepatic fatty acid oxidation and energy release could therefore be a treatment approach to reduce liver fat. Uncoupling protein 1 (UCP1) is a mitochondrial proton transporter normally found in brown fat tissue, where it dissipates the proton gradient and encourages fatty-acid oxidation. Here, we examined whether delivering human UCP1 (hUCP1) via adeno-associated virus (AAV) to the mouse liver causes controlled mitochondrial uncoupling and modifies liver fat metabolism in obesity. Male C57BL/6J mice were fed a regular or high-fat diet and injected with liver-specific AAV vectors encoding hUCP1 through the bloodstream. We assessed hepatic expression, mitochondrial respiration, membrane potential, fatty acid oxidation, and liver triglyceride levels using immunoblotting, high-resolution respirometry, and biochemical assays. Liver ultrastructure was observed with transmission electron microscopy, followed by quantifying lipid droplet quantity, size, and mitochondria-lipid droplet contact points. AAV delivery resulted in stable, liver-restricted hUCP1 expression in mitochondria and caused dose-dependent mitochondrial uncoupling, as evidenced by increased baseline respiration and heightened fatty-acid-stimulated proton conductance, while maintaining oxidative phosphorylation and mitochondrial structure. In high-fat-fed mice, hepatic hUCP1 expression slightly reduced overall fat accumulation and significantly lowered liver triglycerides, whereas chow-fed mice showed minimal systemic effect despite detectable mitochondrial uncoupling. This difference between mitochondrial uncoupling capacity and overall health effects suggests that hepatic UCP1's influence depends on metabolic context and may involve diet-related regulation of UCP1 activity and/or structural changes in liver lipid pathways. Supporting this, ultrastructural studies using transmission electron microscopy showed fewer lipid droplets, more mitochondria-lipid droplet contacts, and increased peroxisomes in the livers of mice expressing hUCP1, indicating coordinated structural changes that facilitate fatty acid mobilization and oxidation. These results demonstrate that hepatic hUCP1 expression causes genuine mitochondrial uncoupling, increases fatty acid breakdown, and establishes structural links between lipid droplets and mitochondria, thereby improving fat management during dietary fat overload.

P176

Combined pyruvate, coenzyme Q10, and vitamin B3 supplementation supports mitochondrial function and neuroprotection under metabolic stress

Luca Pincigher[a]*, Nicola Rizzardi[a]*, Francesca Valenti[a], Martina Salmi[a], Luca Masin[b], Luca Agnifili[c], Matteo Sacchi[d], Alessio Martucci[e], Romana Fato[a], Christian Bergamini[a].

[a]*Department of Pharmacy and Biotechnology, FaBiT, University of Bologna, Via Irnerio 48, 40126 Bologna, Italy.*

[b]*KU Leuven, Leuven Brain Institute, Department of Biology, Animal Physiology and Neurobiology Section, Belgium.*
[c]*Ophthalmology Clinic, Department of Medicine and Aging Science, University G. d'Annunzio of Chieti-Pescara, Via dei Vestini s.n.c., Chieti, Italy.*

[d]*Ophthalmology Unit, Department of Medicine, Surgery, Pharmacology, University of Sassari, Viale San Pietro 43, Sassari,*
e
Ophthalmology Unit, Department of Experimental Medicine, University of Rome "Tor Vergata", Via Montpellier 1, Rome, Italy

*These authors share first co-authorship.

E-mail address: luca.pincigher2@unibo.it
Presenting author: Luca Pincigher

Mitochondrial dysfunction is increasingly recognized as a contributing factor in several ophthalmic diseases.[1] In glaucoma, progressive vision loss occurs despite intraocular pressure-lowering treatments suggesting additional pathogenic mechanisms. The optic nerve exhibits among the highest energy demands and oxygen consumption rates (OCR), making retinal ganglion cells (RGCs) vulnerable to metabolic stress and positioning mitochondria as therapeutic target.[2] For this work we used two cell models: Human CCF-STTG1 astrocytoma cells and primary murine RGCs. CCF-STTG1 cells were cultured either under standard or metabolic stress conditions (MSC; 25 mM galactose) and supplemented with a combination of pyruvate, coenzyme Q10 and vitamin B3 (Mix). Cellular growth, ATP levels, NAD(H) pool, OCR, mitochondrial morphology and membrane potential, inflammatory signaling, and apoptosis were assessed. In parallel, RGCs were cultured in microfluidic devices and subjected to axotomy to evaluate axonal regeneration. The intra-axonal ATP levels were measured using ATeam genetically encoded biosensor.

In astrocytoma cells cultured under MSC, Mix supplementation significantly increased ATP-linked and maximal respiratory capacity, together with cellular ATP levels and NAD(H), suggesting that improved oxidative metabolism facilitates NAD(H) biosynthesis. Mix treatment reduced oxidative stress and restored mitochondrial network morphology to control level. Moreover, Mix attenuated inflammatory responses, reducing NF-κB activation and IL-6 production, and decreased caspase3/7 activation following apoptotic stimulation.

In RGCs, Mix treatment significantly reduced axonal degeneration and promoted regeneration after injury. This neuroprotective effect was associated with preserved intra-axonal ATP levels, despite no detectable changes in mitochondrial size or density, suggesting enhanced mitochondrial efficiency rather than structural remodeling.

In conclusion, combined supplementation with pyruvate, CoQ10, and vitamin B3 produces synergistic effects on mitochondrial bioenergetics, redox balance, and NAD^+ metabolism, particularly under metabolic stress. This combination may represent a promising mitochondrialtargeted therapeutic strategy for diseases characterized by impaired energy metabolism and NAD(H) depletion, such as optic neuropathies. These data may provide the biochemical basis for future studies aimed at mitigating RGCs degeneration and slowing glaucoma progression.

Luca Pincigher was financed by EU—NextGenerationEU through Italian MUR under PNRR— Mission 4 Component 2, Investment 3.3 "(DM 117/2023) and by Indena S.p.A."

[1] N. Gueven, *et al*. Targeting mitochondrial function to treat optic neuropathy. Mitochondrion. 2017 Sep;36:7-14. doi: 10.1016/j.mito.2016.07.013.
[2] J.M. Harder, *et al*. Disturbed glucose and pyruvate metabolism in glaucoma with neuroprotection by pyruvate or rapamycin, Proc. Natl. Acad. Sci. 2020 Dec; 33619-33627. doi.org/10.1073/pnas.2014213117.

P177

Statin-Induced Metabolic Remodeling in Astrocytes: Impact on Coenzyme Q Homeostasis and the Bioenergetic Effects of CoQ10 Supplementation

Wieslawa Jarmuszkiewicz[a], Krzysztof Wojcicki[a], Wojciech Pijanowski[a], Lukasz Galganski[a] [a]*Laboratory of Mitochondrial Biochemistry, Faculty of Biology, Adam Mickiewicz University*

E-mail address: wiesiaj@amu.edu.pl

Presenting author: Wieslawa Jarmuszkiewicz

Although statins are extensively prescribed for cholesterol management, their impact on astrocyte oxidative metabolism remains insufficiently characterized. In this study, rat CTX TNA2 astrocytes were treated with 200 nM atorvastatin or simvastatin for six days to investigate alterations in coenzyme Q (CoQ) balance, cellular energetics, and mitochondrial dynamics. Exposure to either statin produced a comparable reduction in total cellular CoQ9 and CoQ10 (~35%), with a more pronounced depletion of their reduced antioxidant forms (60–75%). This decrease was associated with lower intracellular and mitochondrial reactive oxygen species (ROS), alongside activation of NRF2-mediated antioxidant defenses, including increased expression of superoxide dismutase 1 and glutathione reductase. Markers of metabolic stress, such as HIF1α and BDNF, were also elevated. Statin treatment induced a shift toward glycolytic metabolism, promoted mitochondrial fission and biogenesis, and disrupted oxidative phosphorylation, as indicated by diminished ATPlinked respiration, enhanced proton leak, and reduced ATP content. These findings suggest that astrocytes undergo adaptive metabolic remodeling favoring redox stability over efficient ATP production. Supplementation with CoQ10 replenished both oxidized and reduced CoQ pools and normalized ATP levels, without further lowering ROS, implying that its primary effect is the restoration of bioenergetic function rather than additional antioxidant activity. Collectively, the results highlight CoQ depletion as a key consequence of statin exposure in astrocytes and demonstrate a coordinated cytoprotective response that maintains redox homeostasis at the expense of mitochondrial energy generation. CoQ10 supplementation may therefore provide metabolic support under statin-induced stress conditions.

P178

Extracellular vesicles from Klebsiella pneumoniae facilitate bacterial pneumonia in intubated patients via metabolic reprogramming of alveolar macrophages

Mohammad Estiri[1, 2, 3], Julian Better[1, 2, 3], Lucas Kimmig[4], Victoria Ogungbemi-Alt[1,2,3], Siavash Mansouri[8], Marie Wiegand[5], Nurseda Yilmaz Demirel[6], Saveria ressin[5], Katharina Höfer[6], Bernd Schmeck[5], Moritz Fritzenwanker[7], Can Imirzalioglu[7], Torsten Hain[7], Anna-Lena Jung*[5], Susanne Herold*[1, 2, 3], Ulrich Matt*[1, 2, 3]

[1]Department of Medicine V, Internal Medicine, Infectious Diseases and Infection Control, Universities of Giessen and Marburg Lung Center (UGMLC), member of the German Center for Lung Research (DZL), Giessen, Germany, [2]CardioPulmonary Institute (CPI), Giessen, Germany, [3] Institute for Lung Health (ILH), Giessen, Germany, [4] University of Chicago, Pulmonary Disease and Critical Care Medicine, Chicago, United States, [5] Institute for Lung Research, Universities of Giessen and Marburg Lung Center, Philipps University Marburg, Hesse, Germany, [6] SYNMIKRO, Center for Synthetic Microbiology, Marburg, Germany, [7] Institute of Medical Microbiology, Universities of Giessen and Marburg Lung Center (UGMLC), Giessen, Germany, [8]Max Planck Institute for Heart and Lung Research Member of the German Center for Lung Research (DZL), Bad Nauheim, Germany
*Senior authors

E-mail address: Mohammad.Estiri@innere.med.uni-giessen.de
Presenting author: Mohammad Estiri

Introduction

Nosocomial pneumonia, defined as pneumonia developing at least 48 hours after hospital admission, remains a major clinical challenge, particularly in mechanically ventilated patients. Colonization of the respiratory tract with *Klebsiella pneumoniae (K. pneumoniae)* is a wellrecognized risk factor for infection, yet the mechanisms that facilitate progression from colonization to pneumonia are incompletely understood. This study investigates the role of bacterial extracellular vesicles (bEVs) released by *K. pneumoniae* in modulating host immune responses, with a focus on alveolar macrophages (AMs), which are key effector cells in pulmonary host defense. Methods bEVs were isolated from *K. pneumoniae* cultured in vitro. Murine and human AMs were obtained by bronchoalveolar lavage and stimulated with bEVs prior to infection with viable bacteria. Reactive oxygen species (ROS) were quantified by flow cytometry. Cytokines were measured using a multiplex bead-based assay. Oxygen consumption rate (OCR) and glycolysis were measured using an extracellular flux analyzer. Proteomics methods were done for identifying and quantifying proteins within the bEVs cargo. Mitochondrial dynamics were analyzed using Leica confocal microscopy.

Results

Preincubation with bEVs significantly impaired the bactericidal capacity of AMs, which was confirmed in vivo by enhanced bacterial outgrowth following intratracheal bEV administration. Mechanistically, bEVs did not affect cytosolic ROS but markedly reduced mitochondrial ROS (mtROS) production and cellular respiration during bacterial challenges. Similar effects were observed in human AMs, highlighting translational relevance. bEVs induced mitochondrial fusion in AMs, in contrast to the mitochondrial fission typically seen during bacterial responses, and this shift was associated with reduced mtROS and cytokine release. Notably, bEVs from Stenotrophomonas maltophilia did not produce these effects, indicating pathogen specificity. Further analysis showed that bEV-associated proteins, rather than nucleic acids, mediated these immunomodulatory effects. Proteomics identified outer membrane protein A (OmpA) as a key component responsible for impaired mtROS production and sustained mitochondrial fusion. Inactivation of bEV proteins restored macrophage bactericidal function. Importantly, bEVs from *K. pneumoniae*-colonized patients reproduced these effects, and AMs from these patients failed to undergo mitochondrial fission upon bacterial exposure, underscoring clinical relevance.

Conclusion

Overall, this study demonstrates that *K. pneumoniae*-derived bEVs compromise macrophage antimicrobial function by altering mitochondrial dynamics and metabolism. These findings provide mechanistic insight into how bacterial colonization may predispose to infection and highlight bEVs as potential therapeutic targets to prevent nosocomial pneumonia.

P179

Mitochondrial dysfunction rewires proton homeostasis and redox regulation under variable pH and glucose conditions in *Saccharomyces cerevisiae*

Liana Anikyan[a, b], Anahit Shirvanyan[a, b], Nicoletta Guaragnella[c], Karen Trchounian[a, b]

[a]Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia
[b]Scientific-Research Institute of Biology, Yerevan State University, 1 A. Manoogian Str., 0025 Yerevan, Armenia
[c]Department of Biosciences, Biotechnology and Environment, University of Bari Aldo Moro, Piazza Umberto 1, Bari, Italy

E-mail address: l.anikyan@ysu.am
Presenting author: Liana Anikyan

Mitochondria integrate ATP synthesis, proton transport, and redox balance; however, quantitative adaptation to combined glucose and extracellular pH stress under mitochondrial deficiencies remain insufficiently studied. This study investigates the role of mitochondrial activity in metabolic regulation and sensing mechanisms under varying glucose concentrations (5 and 20 g L^{-1}) and extracellular pH (3.0, 5.0, and 6.5). To dissect the role of mitochondrial activity, we used the □*hap4* mutant with impaired regulation of respiration and mitochondrial electron transport chain, and ρ^0 cells, lacking mitochondrial DNA and, consequently, functional mitochondria. Growth kinetics, proton flux (J_{H^+}); *N, N′*- dicyclohexylcarbodiimide (DCCD)-sensitive ATPase activity in whole cells and mitochondrial fractions, and alcohol dehydrogenase (ADH) activity were determined. Under 5 g L^{-1} glucose at pH 3.0, *Δhap4* and ρ^0 strains exhibited up to 2.5-fold and 4.0-fold reductions in growth, respectively, compared with wild type. In contrast, at 20 g L^{-1} glucose abolished pH dependence and increased ρ^0 growth 2.0-fold. While *S. cerevisiae* W303-1B maintained pH-independent growth, mitochondrial deficiency selectively impaired growth rate of strains at pH 3.0 and 5 g L^{-1} glucose conditions. Mitochondria regulated proton homeostasis in yeast under glucose limitation, while plasma membrane H^+ATPase controls intracellular ATP and H^+ levels during mitochondrial dysfunctions, with ρ^0 cells showing maximal reliance on non-mitochondrial ATPases. DCCD-sensitive proton flux scaled inversely with glucose availability, reflecting increased energetic demand under nutrient limitation and acid stress. ρ^0 cells exhibited the highest ADH activity (□46.8 U mg^{-1} protein), supporting fermentative NAD^+ regeneration. In wild-type cells, F_oF_1-ATPase contributed □75% of total DCCD-sensitive H^+ATPase activity at 5 g L^{-1} glucose and □60% during 24h growth in 20 g L^{-1} glucose containing media. In contrast, *Δhap4* showed an □2.0-fold reduction in F_oF_1-ATPase contribution under both glucose conditions. The highest ATPase activity (~250 nmol P_{in} min^{-1} μg^{-1} protein) was observed in ρ^0 cells at pH 6.5 and 5 g L^{-1} glucose, indicating compensatory upregulation of plasma membrane ATPases to maintain plasma membrane potential and intracellular pH. Thus, this study provides a systematic, quantitative analysis of how mitochondrial deficiencies in *S. cerevisiae* rewire proton flux, ATPase activity, and ADH-mediated redox regulation in response to combined pH and glucose levels, revealing graded compensatory mechanisms between partial (*Δhap4*) and complete (ρ^0) loss of mitochondrial functionality that have not been comprehensively described before. These findings provide a mechanistic framework for optimizing yeast performance under acidic and glucose-rich conditions, with direct relevance to industrial fermentation process design.

P180

VDAC1-Targeted NHK1 Peptide Recovers Mitochondrial Dysfunction in ALS primary fibroblasts

Iolanda R. Infantino[a], Stefano Conti-Nibali[a], Andrea Leotta[a], Andrea Magrì[b], Angela Messina[b]

[a]University of Catania, Department of Biomedical and Biotechnological Sciences [b]University of Catania, Department of Biological, Geological and Environmental Sciences

E-mail address: mess@unict.it

Presenting author: Angela Messina

Amyotrophic lateral sclerosis (ALS) is an uncurable neurodegenerative disorder caused by selective degeneration of spinal motoneurons. Mitochondrial dysfunction represents an early event and a common hallmark in both sporadic (sALS) and familial (fALS) forms.

Accumulation of misfolded mutant proteins (i.e. SOD1, TDP43, FUS) on mitochondria, in fALS as in sALS, correlates with drastic reduction of mitochondrial respiration and inhibition of metabolites exchanges, including ADP/ATP and NAD^+/NADH. Voltage-Dependent Anion-selective Channel 1 (VDAC1), the most abundant protein of the outer mitochondrial membrane, is responsible for these metabolites flux and represents the physiological receptor of the hexokinase I.

In ALS mitochondria, mutant SOD1 competes with hexokinase for binding to VDAC1 [1]. At this site, the synthetic NHK1 peptide specifically interacts with VDAC1 inhibiting the binding of SOD1-G93A to mitochondria and thus restoring mitochondrial function and the viability of SOD1-G93A NSC34 cells [2].

In this study, we investigated the effect of the NHK1 peptide on ALS primary fibroblasts. Human fibroblast cultures (from skin biopsies of patients with sALS and fALS, carrying mutations in TDP-43, FUS or C9orf72 gene) and mouse fibroblast cultures (derived from ear pinnae of SOD1-G93A mice) were studied by Seahorse/Oroboros high-resolution respirometry, cell viability and mitochondrial function analyses. Our results show that:

- Fibroblasts from ALS patients exhibit altered respiratory parameters compared to healthy controls, with differences between genetic subtypes. sALS fibroblasts show a pronounced deficit, whilst milder alterations characterize TDP-43 and FUS cases; C9orf72 fibroblasts show preserved basal and coupled respiration but reduced maximum respiratory capacity. All ALS fibroblasts showed a marked decrease in respiratory reserve.
- In fibroblasts from SOD1-G93A mice, the respiratory profile is more severe: mitochondria are strongly decoupled and all respiratory fluxes are drastically impaired.
- Treatment with the NHK1 peptide significantly improves mitochondrial function in SOD1-G93A mice fibroblasts, increasing all respiratory fluxes and cell viability. Similarly, NHK1 produces beneficial effects in both SOD1-G93A NSC34 cells and ALS patient fibroblasts, restoring both cell viability and mitochondrial respiration.

Therefore, the NHK1 peptide is a powerful positive modulator of mitochondrial function, able to restore respiratory capacity and energy reserves in ALS primary fibroblasts.

[1] A. Israelson et al. Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS, Neuron 2010 Aug 26;67(4):575-87. DOI: 10.1016/j.neuron.2010.07.019 [2] A. Magrì et al. Small hexokinase 1 peptide against toxic SOD1 G93A mitochondrial accumulation in ALS rescues the ATP-related respiration, Biomedicines 2021, 9, 948. doi: 10.3390/biomedicines9080948

P181

$NAD(P)^+$ Transhydrogenase Is Essential for Maintaining Mitochondrial Redox Homeostasis in Cardiac and Skeletal Muscle During Aging

Roger F. Castilho[a], Ana P. Dalla Costa[a], Claudia D. C. Navarro[b], Edilene S. Siqueira-Santos[a], Ericka F. D. Costa[a], Tiago R. Figueira[c], Anibal E. Vercesi[a], Annelise Francisco[d]

a
Department of Pathology, School of Medical Sciences, University of Campinas (UNICAMP), 13083 887 Campinas, SP, Brazil
b Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark
c
School of Physical Education and Sport of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil d Department of Experimental Medical Science, Faculty of Medicine, Lund University, Lund, Sweden

E-mail address: rogerc@unicamp.br
Presenting author: Roger F. Castilho

$NAD(P)^+$ transhydrogenase (NNT) is a mitochondrial enzyme that generates NADPH, thereby supporting redox balance in different tissues. However, its role in age-related functional decline in the heart and skeletal muscle remains incompletely understood. We evaluated multiple parameters in isolated mitochondria from NNT-deficient ($Nnt^{-/-}$) and wild-type ($Nnt^{+/+}$) mice at young, middleaged, and old ages. NNT deficiency was associated with increased mitochondrial H_2O_2 release when specific respiratory substrates were provided, in both cardiac and skeletal muscle mitochondria. In cardiac mitochondria, mitochondrial respiration and calcium retention capacity remained largely preserved during aging. In contrast, skeletal muscle from aged $Nnt^{-/-}$ mice exhibited reduced mitochondrial oxygen consumption in oxidative fibers, along with histological remodeling, particularly in the soleus muscle, which expresses the highest levels of NNT. The selective impact of NNT deficiency on skeletal muscle respiration, but not cardiac respiration, may reflect the metabolic characteristics of skeletal muscle, which typically remains in a resting state and therefore relies more heavily on NNT-derived NADPH to maintain redox homeostasis. In conclusion, these findings demonstrate that NNT is critical for preserving mitochondrial redox balance and function in both cardiac and skeletal muscle during aging.

P182

CagriSema-induced weight loss in obese rats depends on preservation of mitochondrial proton leak in skeletal muscle

Ida Blom[a,b], Julie M. Jacobsen[a], Anna T. Hölker[b], Barbara D. Eriksfoss[b], Kristine K. Lange[b], Jesper Havelund[c], Kirsten Raun[a], Martin Hey-Mogensen[a], Nils J. Færgeman[c], Anna Secher[a], Rune E. Kuhre[a], Steen Larsen[b], Jens F. Halling[a]

aResearch and Development, Novo Nordisk A/S, Måløv, Denmark

bDepartment of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

c Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

E-mail address: IDBM@novonordsik.com

Presenting author: Ida Blom

Weight loss triggers counter-regulatory mechanisms that reduce resting energy expenditure beyond what is explained by the weight loss per se - a phenomenon termed metabolic adaptation. CagriSema, a combination of semaglutide (a GLP-1 analogue) and cagrilintide (an amylin analogue), counteracts metabolic adaptation in diet-induced obese (DIO) rats. In this study, we aimed to investigate the underlying molecular mechanisms.

DIO male rats (n = 45) were fed a 45% high-fat diet and received daily subcutaneous injections of either vehicle or CagriSema (2 nmol/kg cagrilintide + 2 nmol/kg semaglutide) for 20 days. To distinguish CagriSema effects from effects solely attributable to the weight loss, a vehicle-treated, calorie-restricted, weight matched group (WM) was included. Food intake and body weight were recorded daily, and calorie supply to the WM rats was adjusted daily to match the weight loss in the CagriSema group. *Ex vivo* analyses comprised high-resolution respirometry to quantify mitochondrial function with a specific focus on proton leak in isolated mitochondria from skeletal muscle (soleus) and liver. Additionally, multi-omics profiling (metabolomics, lipidomics, and proteomics) of skeletal muscle (soleus) and liver was conducted.

CagriSema produced a ~15% vehicle-adjusted weight loss ($p < 0.0001$). To achieve the same weight reduction, WM rats required 22% fewer calories than the rats receiving CagriSema ($p < 0.005$). This difference is consistent with prior studies showing reduced energy expenditure in WM rats compared with those receiving CagriSema. Mitochondrial leak respiration in skeletal muscle was comparable between vehicle and CagriSema rats but was ~20% lower in the WM group relative to the CagriSema group (leak respiration at 0.2 mM succinate: $WM = 47.1 \pm 3.0$, $CagriSema = 57.8 \pm 2.4$ $pmol \cdot s^{-1} \cdot mL^{-1}$, $p < 0.02$). Liver mitochondrial respiration did not differ between groups. Multi-omics profiling of both skeletal muscle and liver revealed markedly fewer changes relative to vehicle in the CagriSema group than in the WM group.

Collectively, our data indicate that CagriSema counteracts metabolic adaptation at least in part by preserving mitochondrial proton leak in skeletal muscle. Moreover, based on the omics profiles, CagriSema-induced weight loss appears to bypass normal molecular responses to diet-induced weight loss in both liver and skeletal muscle.

P183

Energetic cost adjusted defense strategies enables stratified oxidative stress tolerance

Anjali V. Patil[1], Jiao Zhao[2], Snehal V. Khairnar[1], Laurence Yang[2], Amitesh Anand[1]

1. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India.

2. Department of Chemical Engineering, Queen's University, Kingston, Ontario, Canada.

Email address: amitesh.anand@tifr.res.in

Presenting author: Amitesh Anand

Abstract

Stress mitigation is central to cellular survival, particularly for bacteria that are continuously exposed to fluctuating microenvironments [1]. To cope with challenges such as heat, oxidative stress, pH shifts, and nutrient limitation, bacteria have evolved a broad repertoire of defense mechanisms that preserve cellular homeostasis [2,3]. While many individual stress-response pathways are well characterized, how bacteria tune their adaptive strategies to the magnitude of a given stressor remains largely unresolved. This question is especially relevant for oxidative stress, whose intensity varies widely across physiological niches and imposes distinct bioenergetic and selective constraints. Here, we investigate how bacteria modulate oxidative stress tolerance as a function of stress severity. Using four independent adaptive laboratory evolution experiments in *Escherichia coli*, spanning multiple paraquat concentrations and genetic backgrounds, we dissect adaptive trajectories across a gradient of superoxide stress.

By integrating multi-omic profiling with tailored genome-scale metabolic modeling, we uncover two fundamentally distinct tolerance strategies that differ in flux control and cellular resource allocation. Under low superoxide stress, *E. coli* adopts a low-cost strategy in which tolerance is achieved primarily by blocking a polyamine transporter that is co-opted for paraquat entry, thereby limiting intracellular radical generation and maintaining redox balance. In contrast, higher stress levels elicit a qualitatively different response characterized by substantial energetic investment. This high-stress program involves enhanced detoxification of reactive oxygen species, activation of active efflux systems, and metabolic rewiring that enables maintenance of a more oxidative aerotype despite elevated stress.

We further demonstrate that these stress-dependent flux regulation strategies interface with metabolic repair pathways, yielding additional fitness gains under sustained oxidative challenge. Together, our results reveal that *E. coli* deploys modular and energetically stratified stress-response programs that are selected based on stress magnitude and operational energy costs. This work provides a bioenergetic framework for understanding how bacteria dynamically balance stress tolerance, metabolic efficiency, and fitness across heterogeneous environments, offering generalizable principles for adaptive evolution under redox stress.

[1] Stress responses of bacteria, Current Opinion in Structural Biology 17 (2007) 755–760.

[2] J. Dawan, J. Ahn, Bacterial Stress Responses as Potential Targets in Overcoming Antibiotic Resistance, Microorganisms 10 (2022). https://doi.org/10.3390/microorganisms10071385.

[3] The classification of bacterial survival strategies in the presence of antimicrobials, Microbial Pathogenesis 155 (2021) 104901.

P184

Interrogating the role of c-MYC in mitochondrial complex III deficiency-induced liver disease

Oliver Ros[a,b], Nasrin Sultana[a,b], Rishi Banerjee[a,b,c], Janne Purhonen[a,b,d], Christa Kietz[a,b],

Vineta Fellman[a,b,e,f] and Jukka Kallijärvi[a,b,*]

[a]Folkhälsan Research Center; Haartmaninkatu 8, 00290 Helsinki, Finland;

[b]Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki; P.O.Box 63, 00014 University of Helsinki, Helsinki, Finland [c]Finnish Doctoral Programme in Oral Sciences (FINDOS)
[d]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden
[e]Department of Clinical Sciences, Lund, Pediatrics, Lund University, P.O.Box 117, 221 00 Lund, Sweden
[f]Children's Hospital, Helsinki University Hospital, P.O. Box 22, 00014 Helsinki, Finland

[*]Corresponding author (jukka.kallijarvi@helsinki.fi)

E-mail address: oliver.ros@helsinki.fi

Presenting author: oliver.ros@helsinki.fi

The *Bcs1l*$^{p.S78G}$ mouse model of mitochondrial complex III (CIII) deficiency based on the Finnish GRACILE founder mutation exhibits impaired CIII assembly and activity, leading to severe OXPHOS deficiency and multi-organ disease. Recently, we reported that the mice show striking juvenile-onset progeria with widespread DNA damage response, cell cycle arrest, and cellular senescence in tissues that regenerate via cell cycle re-entry of parenchymal cells, most significantly the liver. We showed that transcription factor c-MYC (MYC) is remarkably (1040-fold) induced in the juvenile mutant liver, possibly driving illicit cell cycle entry, DNA damage and senescence. Indeed, viral expression of Omomyc, a dominant negative inhibitor of MYC, attenuated the DNA damage. Here, we utilized a hypomorphic *Myc*$^{\square 2\text{-}540}$ allele, containing a large 540-kb deletion of an upstream super-enhancer of MYC, shown to restrict tissue-specific tumorigenesis, to modulate *Myc* expression in the *Bcs1l*$^{p.S78G}$ mice. The *Myc*$^{\square 2540}$ allele had a clear effect on mouse growth and on the MYC expression in the colon. Surprisingly, the *Myc*$^{\square 2\text{-}540}$ allele did not attenuate MYC expression in the liver or kidney, or the histopathology in these tissues. This indicates that the OXPHOS-deficiency induced MYC upregulation is independent of the large 5' regulatory region required for colon carcinogenesis. We have developed novel Omomyc-and 3'-miRNA-shRNA-based MYC blunting tools, which have shown high efficacy in transfected cells. We are next delivering these in vivo using rAAVs, aiming to reveal the role of MYC in the CIII deficiency-induced liver disease and hepatocyte senescence.

P185

Fibrinaloid microclot complexes, Long COVID, and ischaemic stroke

Douglas B. Kell[a,b] & Etheresia Pretorius[a,b]

[1] *Department of Biochemistry, Cell & Systems Biology, University of Liverpool, UK.* [2] *Departmentt of Physiological Sciences, University of Stellenbosch, South Africa*

E-mail address: dbk@liv.ac.uk

Presenting author: Douglas Kell

deposits', and later discovered that these are actually amyloid in nature, and can be induced by minuscule (highly substoichiometric) amounts of bacterial cell wall material [1-3] or the SARS-CoV-2 spike protein [4, 5]. Many diseases [6], including acute [7] and Long COVID [8] and Myalgic Encephalopathy/ Chronic Fatigue Syndrome (ME/CFS) [9] display large numbers of these 'fibrinaloid' microclots, that can also be produced *in vitro* [1, 10]. Along with the presence of anti-fibrinolytic proteins, this provides a ready explanation for a great many features of Long COVID, summarised at http://dbkgroup.org/longcovid/. By blocking up microcapillaries (the microcirculation) and hence O_2 transport to mitochondria, fibrinaloid microclots provide a ready mechanistic explanation for the multifarious symptoms of such diseases [11-15]. They are also highly predictive of mortality from sepsis (another disorder of the microcirculation), with an Odds Ratio exceeding 5 [16].

Amyloidogenic cross-seeding is common [17], and is highly predictive of observed amyloid(ogenicity) [18, 19]. Traditional Chinese Medicine recognises all of this under the term Blood Stasis [20]. Similarly amyloid structures are also found in the macrothrombi removed from patients following ischaemic stroke [21, 22], and likely have a similar origin.

1 Pretorius, E., Mbotwe, S., Bester, J., Robinson, C. J. and Kell, D. B. (2016) Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *J R Soc Interface*. 123, 20160539. https://doi.org/10.1098/rsif.2016.0539

2 Kell, D. B. and Pretorius, E. (2017) Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. *Progr Biophys Mol Biol*. 123, 16-41. https://doi.org/10.1016/j.pbiomolbio.2016.08.006

3 Pretorius, E., Page, M. J., Hendricks, L., Nkosi, N. B., Benson, S. R. and Kell, D. B. (2018) Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains. *J R Soc Interface*. 15, 20170941. https://doi.org/10.1098/rsif.2017.0941

4 Grobbelaar, L. M., Venter, C., Vlok, M., Ngoepe, M., Laubscher, G. J., Lourens, P. J., Steenkamp, J., Kell, D. B. and Pretorius, E. (2021) SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19. *Biosci Rep*. 41, BSR20210611. https://doi.org/10.1042/BSR20210611

5 Grobbelaar, L. M., Kruger, A., Venter, C., Burger, E. M., Laubscher, G. J., Maponga, T. G., Kotze, M. J., Kwaan, H. C., Miller, J. B., Fulkerson, D., Huff, W., Chang, E., Wiarda, G., Bunch, C. M., Walsh, M. M., Raza, S., Zamlut, M., Moore, H. B., Moore, E. E., Neal, M. D., Kell, D. B. and Pretorius, E. (2022) Relative hypercoagulopathy of the SARS-CoV-2 Beta and Delta variants when compared to the less severe Omicron variants is related to TEG parameters, the extent of fibrin amyloid microclots, and the severity of clinical illness. *Semin Thromb Haemost*. 48, 858-868. https://doi.org/10.1055/s-0042-1756306

6 Pretorius, E. and Kell, D. B. (2024) A Perspective on How Fibrinaloid Microclots and Platelet Pathology May be Applied in Clinical Investigations. *Semin Thromb Hemost*. 50, 537-551. https://doi.org/10.1055/s-0043-1774796

7 Pretorius, E., Venter, C., Laubscher, G. J., Lourens, P. J., Steenkamp, J. and Kell, D. B. (2020) Prevalence of readily detected amyloid blood clots in 'unclotted' Type 2 Diabetes Mellitus and COVID-19 plasma: A preliminary report. *Cardiovasc Diabetol*. 19, 193. https://doi.org/10.1186/s12933-020-01165-7

8 Pretorius, E., Vlok, M., Venter, C., Bezuidenhout, J. A., Laubscher, G. J., Steenkamp, J. and Kell, D. B. (2021) Persistent clotting protein pathology in Long COVID/ Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin. *Cardiovasc Diabetol*. 20, 172. https://doi.org/10.1186/s12933-021-01359-7

9 Nunes, J. M., Kruger, A., Proal, A., Kell, D. B. and Pretorius, E. (2022) The occurrence of hyperactivated platelets and fibrinaloid microclots in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). *Pharmaceuticals (Basel)*. 15, 931. https://doi.org/10.3390/ph15080931

10 Grixti, J. M., Theron, C. W., Salcedo-Sora, J. E., Pretorius, E. and Kell, D. B. (2024) Automated microscopic measurement of fibrinaloid microclots and their degradation by nattokinase, the main natto protease *J Exp Clin Appl Chin Med*. 5, 30-55. https://doi.org/10.62767/jecacm504.6557.

11 Kell, D. B., Laubscher, G. J. and Pretorius, E. (2022) A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications. *Biochem J*. 479, 537-559. https://doi.org/10.1042/BCJ20220016

12 Kell, D. B. and Pretorius, E. (2022) The potential role of ischaemia-reperfusion injury in chronic, relapsing diseases such as rheumatoid arthritis, long COVID and ME/CFS: evidence, mechanisms, and therapeutic implications. *Biochem J*. 479, 1653-1708. https://doi.org/10.1042/BCJ20220154

13 Kell, D. B. and Pretorius, E. (2023) Are fibrinaloid microclots a cause of autoimmunity in Long Covid and other post-infection diseases? *Biochem J*. 480, 1217-1240. https://doi.org/10.1042/BCJ20230241

14 Kell, D. B., Lip, G. Y. H. and Pretorius, E. (2024) Fibrinaloid Microclots and Atrial Fibrillation. *Biomedicines*. 12https://doi.org/10.3390/biomedicines12040891

15 Kell, D. B., Khan, M. A., Kane, B., Lip, G. Y. H. and Pretorius, E. (2024) Possible Role of Fibrinaloid Microclots in Postural Orthostatic Tachycardia Syndrome (POTS): Focus on Long COVID. *J Pers Med*. 14https://doi.org/10.3390/jpm14020170

16 Schofield, J., Abrams, S. T., Jenkins, R., Lane, S., Wang, G. and Toh, C. H. (2024) Microclots, as defined by amyloid-fibrinogen aggregates, predict risks of disseminated intravascular coagulation and mortality. *Blood Adv*. 8, 2499-2508. https://doi.org/10.1182/bloodadvances.2023012473

17 Kell, D. B. and Pretorius, E. (2024) Proteomic Evidence for Amyloidogenic Cross-Seeding in Fibrinaloid Microclots. *Int J Mol Sci*. 25, 10809. https://doi.org/10.3390/ijms251910809

18 Kell, D. B., Doyle, K. M., Salcedo-Sora, J. E., Sekhar, A., Walker, M. and Pretorius, E. (2025) AmyloGram reveals amyloidogenic potential in stroke thrombus proteomes. *Biochem J*. 482, 1689-1706. https://doi.org/10.1042/BCJ20253317

19 Kell, D. B. and Pretorius, E. (2025) The Proteome Content of Blood Clots Observed Under Different Conditions: Successful Role in Predicting Clot Amyloid(ogenicity). *Molecules*. 30, 668. https://doi.org/10.3390/molecules30030668

20 Kell, D. B., Pretorius, E. and Zhao, H. (2025) A Direct Relationship Between 'Blood Stasis' and Fibrinaloid Microclots in Chronic, Inflammatory, and Vascular Diseases, and Some Traditional Natural Products Approaches to Treatment. *Pharmaceuticals (Basel)*. 18, 712. https://doi.org/10.3390/ph18050712

21 Grixti, J. M., Chandran, A., Pretorius, J.-H., Walker, M., Sekhar, A., Pretorius, E. and Kell, D. B. (2024) The clots removed from ischaemic stroke patients by mechanical thrombectomy are amyloid in nature. *medRxiv*, 2024.2011.2001.24316555. https://doi.org/10.1101/2024.11.01.24316555

22 Grixti, J. M., Chandran, A., Pretorius, J. H., Walker, M., Sekhar, A., Pretorius, E. and Kell, D. B. (2025) Amyloid Presence in Acute Ischemic Stroke Thrombi: Observational Evidence for Fibrinolytic Resistance. *Stroke*. 56, e165-e167. https://doi.org/10.1161/STROKEAHA.124.050033

Contribution type:
Poster – P and Talk – T

P188

A PDK1–PDHA1–SCAF1 axis regulates mitochondrial respiratory chain organization during metabolic rewiring in cancer cells

Sandra López-Calcerrada[a] and Cristina Ugalde[a,b,c]

[a] *Instituto de Investigación Hospital 12 de Octubre (imas12), Madrid 28041, Spain* [b] *Centro de Investigaciones Biológicas 'Margarita Salas' (CIB-CSIC), Madrid 28040, Spain* [c] *Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), U723, Madrid, Spain.*

E-mail address: sandra.lopez.imas12@h12o.es

Presenting author: Sandra López-Calcerrada

Abstract text.

The mammalian mitochondrial respiratory chain (MRC) complexes I, III_2 and IV can assemble into supercomplexes (SCs) and respirasomes; however, the mechanisms regulating their biogenesis and functional relevance remain incompletely understood. Previous studies from our laboratory demonstrated the coexistence of two distinct MRC organizations in human cells and postmitotic tissues, defined by the preferential expression of complex IV COX7A subunit isoforms: i) the canonical C-MRC, characterized by COX7A1/COX7A2-containing respirasomes and free $CIII_2$ and CIV; and ii) the bioenergetically less-efficient COX7A2L/SCAF1-dependent SMRC, defined by SCAF1-containing respirasomes and SC III_2IV_1 [1,2]. The prevalence of each MRC organization is reversibly regulated by the activation state of the pyruvate dehydrogenase complex (PDC), a key regulator of the metabolic switch between glycolysis and oxidative phosphorylation (OXPHOS). PDC activation promotes the C-MRC organization to support efficient respiration under oxidative conditions, whereas PDC inactivation favors the S-MRC, sustaining OXPHOS upon metabolic rewiring toward glycolysis. Notably, this glycolytic switch is a hallmark of cancer, and increasing evidence suggests a role for SCAF1 in MRC functional adaptations in tumour cells. To gain deeper insight into the mechanisms underlying this interplay, we investigated PDC-mediated MRC reorganization in glioblastoma U-87 MG (U87) and pancreatic cancer MIA PaCa-2 (MP2) cell lines using dichloroacetate (DCA) treatment and biochemical analyses. Sustained PDC activation induced by DCA triggered a remodelling response characterized by reduced SCAF1 levels and disruption of the S-MRC, with cell-typespecific outcomes: U87 cells also lost the C-MRC organization, whereas MP2 cells favoured it through stabilization of SC I_1III_2 and the C-respirasome. To further assess the structural impact of PDC loss on the S-MRC, we analysed MP2 cells deficient in the PDHA1 subunit ($PDHA1^{KO}$ cells). Unexpectedly, PDHA1 loss resulted in decreased SCAF1 levels and disruption of the SMRC, both of which were restored upon re-expression of wild-type PDHA1. Similarly, expression of a non-phosphorylatable PDHA1 variant ($PDHA1^{AAA}$) -which renders PDC constitutively active- maintained low SCAF1 levels, destabilized the S-MRC, and promoted the C-MRC configuration. We found that PDHA1 levels positively correlated with both SCAF1 and PDK1 abundance, and that PDK1 overexpression alone was sufficient to induce SCAF1 expression. Overall, these findings suggest that induction of SCAF1 and the S-MRC requires both the presence of PDHA1 and its phosphorylation-mediated inactivation, revealing a regulatory PDHA1- PDK1-SCAF1 axis that may contribute to metabolic adaptation in cancer.

[1] Fernández-Vizarra, E., López-Calcerrada, S., Formosa, LE., Pérez-Pérez, R., Ding, S., Fearnley, IM., Arenas, J., Martín, MA., Zeviani, M., Ryan, MT., & Ugalde, C. (2021). SILAC-based complexome profiling dissects the structural organization of the human respiratory supercomplexes in $SCAFI^{KO}$ cells. *Biochimica et Biophyslca Acta - Bioenergetics*, 1862(7), 148414. https://doi.org/10.1016/j.bbabio.2021.148414.

[2] Fernández-Vizarra, E., López-Calcerrada, S., Sierra-Magro, A., Pérez-Pérez, R., Formosa, L. E., Hock, D. H., Illescas, M., Peñas, A., Brischigliaro, M., Ding, S., Fearnley, I. M., Tzoulis, C.,
Pitceathly, R. D. S., Arenas, J., Martín, M. A., Stroud, D. A., Zeviani, M., Ryan, M. T., & Ugalde, C. (2022). Two independent respiratory chains adapt OXPHOS performance to glycolytic switch. *Cell Metabolism*, 34(11), 1792-1808.e6. https://doi.org/10.1016/j.cmet.2022.09.005.

P189

Loss of CHCHD2 and CHCHD10 causes tissue-specific bioenergetic failure and severe myopathy driven by impaired postnatal myogenesis

Jule Gerlach [a], Juan Diego Hernandez Camacho [b], Hendrik Nolte [c], Frank Chenfei Ning [a], Joana a Rodrigues [a,e], David Alsina [c], Dusanka Milenkovic [d], Jelena Misic [a], Annelie Falkevall [d], Thomas c Langer [b], Timothy Wai [a#], Roberta Filograna [a#].

[a] Division of Molecular Metabolism, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden
[b] Institut Pasteur, CNRS UMR 3691, Mitochondrial Biology Unit, Université Paris Cité, Paris, France.
[c] Max-Planck-Institute for Biology of Ageing, Department of Mitochondrial Proteostasis, Cologne, Germany
[d] Division of Vascular Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.
[e] Centre for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm, Sweden.
corresponding author

Mutations in the mitochondrial proteins CHCHD2 and CHCHD10 cause severe neurodegenerative and muscular diseases, including Parkinson's disease and myopathy, yet their core physiological roles remain undefined.

CHCHD2 and CHCHD10 are highly conserved small proteins that share substantial sequence identity and localize to the mitochondrial intermembrane space. We previously demonstrated that whole-body *Chchd2* knockout (KO) mice exhibit a mild motor phenotype associated with reduced striatal dopamine levels and brain lipid dysregulation, despite no overt dopaminergic neuron loss or global OXPHOS impairment. *In vivo*, CHCHD2 and CHCHD10 assemble into a large molecular complex, and in the absence of CHCHD2, CHCHD10 can still form this complex, suggesting partial functional compensation. To address this redundancy, we generated *Chchd10* KO and *Chchd2/ Chchd10* double KO mouse models. While *Chchd10* KO mice showed no phenotype, double KO animals were smaller, with a marked reduction in lean mass and progressive muscle weakness. These mice exhibited pronounced OXPHOS dysfunction, primarily compromising complex I, in both heart and skeletal muscle. Strikingly, we identified tissue-specific metabolic and mitochondrial alterations: the heart undergoes early metabolic rewiring that preserves myocardial contractility into adulthood, whereas skeletal muscle displays severe lipid dysregulation. Morphologically, we found an accumulation of megamitochondria in the heart and enlarged mitochondria together with tubular aggregates in skeletal muscle. In addition, skeletal muscle showed a strong impairment of postnatal myogenesis caused by defective satellite cell function, likely driving the loss of muscle mass. Interestingly, in a mouse model of mitochondrial myopathy, the abundance and size of CHCHD2–CHCHD10 oligomers increase with the severity of mitochondrial dysfunction, supporting their role as a stress-responsive protein complex.

Together, our findings identify the CHCHD2–CHCHD10 complex as a key regulator of mitochondrial and tissue homeostasis and provide new insight into how its dysfunction drives tissue-specific pathology.

P190

From Childhood Trauma to immunocellular health: Unveiling the link between Childhood Maltreatment and mitochondrial health

Authors: Margareta Scherr[1], Karin de Punder[1], Alexander Karabatsiakis[1], Felix Scholkmann[2,3], Lea Toscano[4], Lisa Holper[4], Emmanuella Guenova[5,6]

[1] Department of Psychology, Clinical Psychology II, University of Innsbruck, Innsbruck, Austria

[2]Biomedical Optics Research Laboratory, Department of Neonatology, University Hospital Zurich, University of Zurich, Zurich, Switzerland

[3]Scholkmann Data Analysis Services, Scientific Consulting and Physical Engineering, Zurich, Switzerland

[4]University Hospital of Psychiatry, University of Zurich, Zurich, Switzerland

[5]Department of Dermatology, University Hospital and University of Lausanne, Lausanne, Switzerland [6] Department of Translationale Immundermatology, Johannes Kepler University Linz, Linz, Austria

The term childhood maltreatment (CM) refers to experiences of abuse, mistreatment, and neglect during childhood. While the negative impact of CM on mental health in adulthood is well documented from an epidemiological perspective, its effects at the cellular level remain unclear. This research investigates the relationship between CM and mitochondrial bioenergetics in immune cells of an adult Swiss cohort. Sixty-six participants aged between 18 and 64 years were recruited as part of the CCO-NIRS project “Brain mitochondrial cytochromec-oxidase assessed by fNIRS as a potential biomarker in depressive episodes”, conducted in collaboration between the Psychiatric University Hospital Zurich and the University of Innsbruck since October 2017. Peripheral blood mononuclear cells (PBMCs) were collected and cryopreserved from the participants without current or past psychiatric diagnoses. Mitochondrial respiration was measured using high-resolution respirometry (HRR). CM was retrospectively assessed via self-report using the Childhood Trauma Questionnaire (CTQ). The participants were categorized in three groups: none, mild or severe, based on the assessed trauma score and separated between women (n = 32) and men (n = 34). Linear regression analyses revealed a significant ($p < .05$) dose–response relationship between CM severity and routine respiration, uncoupled respiration and SRC in women after adjustment for age and BMI. This pattern may reflect a compensatory hyperactivation of mitochondria, potentially interpreted as a biological resilience mechanism. Conversely, in men, trauma load was not associated with mitochondrial function, indicating the absence of a dose–response relationship. The differences between men and women highlight the importance of examining biological stress responses in a sex - and gender sensitive manner rather than making generalized statements about “risk groups.” This raises the question of whether men possess other (examined or yet unidentified) protective factors, or whether their adaptive mechanisms operate on different hormonal levels.

P191

Beyond Pairwise Attractions: Density-Dependent Dry Coarse-Grained Models for Biomolecular Organization

Alexander Jussupow[a]

[a] *School of Chemistry, University College Dublin, Ireland*

E-mail address: alexander.jussupow@ucd.ie Presenting author: Alexander Jussupow

Bioenergetic processes occur in highly crowded and heterogeneous cellular environments, where weak multivalent interactions can drive large-scale organization, such as phase separation and organelle formation. Simulating these complex environments requires models that can access large length and time scales.

Dry coarse-grained force fields reduce computational cost by rescaling interaction terms to eliminate the need for explicit solvent molecules. However, many existing approaches rely predominantly on attractive pairwise interactions, which can lead to attraction-driven collapse, resulting in unrealistically dense local environments.

Here, we explore strategies for incorporating many-body dissipative particle dynamics (MDPD) into dry coarse-grained force fields. In contrast to force fields with conventional pairwise interactions, MDPD uses density-dependent interaction terms, providing a mechanism to mitigate attraction-driven collapse.

We demonstrate the effectiveness of this approach with a sequence-dependent model for intrinsically disordered proteins calibrated against experimental data. Furthermore, we propose a more transferable dry Martini-based MDPD model aimed at capturing the coupled organization of proteins and membranes, potentially providing a scalable route to simulate biomolecular assembly in bioenergetic systems under realistic crowded conditions.

P192

Impact of caloric restriction on ΔQCR6-dependent mitochondrial function.

Arias Gordillo Dylan, Ricardez García Carolina, Uribe Carvajal Salvador, Mendez Romero Ofelia Alejandra and Chiquete Félix Natalia.

Department of Genetics and Molecular Biology, Instituto de Fisiología Celular, UNAM, Mexico City, Mexico

E-mail address: dylanarias@ifc.unam.mx, cricardez@ifc.unam.mx, suribe@ifc.unam.mx.

Presenting author: Dylan Arias Gordillo

Abstract

Complex III, or cytochrome bc1, is an essential component of the mitochondrial respiratory chain responsible for electron transfer and for contributing to the proton-motive gradient. In *Saccharomyces cerevisiae*, this complex is composed of catalytic and structural subunits encoded in the nucleus, including Δ*QCR6*. This protein is assembled in the inner mitochondrial membrane as part of the BC1 complex and is necessary for its proper stability and function; its absence compromises respiratory activity [1].

Caloric restriction (CR), defined as the reduction of glucose in the culture medium (<1%), promotes respiratory metabolism and alters mitochondrial function [2]. It is known that CR modifies gene expression [3], including that of Δ*QCR6.* However, it is not known how CR affects the function of cytochrome bc1 or how mitochondrial function is modulated. That is why we decided to evaluate a Δ*QCR6* mutant of *S. cerevisiae* under CR.

In this project, we analyzed the regulation of caloric restriction in *S. cerevisiae* and the effects of the Δ*QCR6* subunit deletion on mitochondrial function by conducting experiments with whole cells and isolated mitochondria. Among the experiments performed, we measured the concentration of reactive oxygen species, ATP concentration, a yeast growth curve, and a glucose consumption curve. With isolated mitochondria, native polyacrylamide gels were prepared to analyze the activity of the respiratory complexes.

The results obtained were as expected: the mutant with the Δ*QCR6* deletion exhibits reduced respiratory activity and increased ROS production.

[1] Schmitt, M. E., et. al. (1990). *Subunit 6 of the yeast cytochrome bc1 complex is required for the assembly and regulation of the complex.* Journal of Biological Chemistry. https://doi.org/10.1016/S0021-9258(17)42253-8

[2] Tahara, E. B., et. al. (2013). *Calorie restriction hysteretically primes aging Saccharomyces cerevisiae toward more effective oxidative metabolism*. PLoS ONE, 8(5), e56388. https://doi.org/10.1371/journal.pone.0056388

[3] Arlia-Ciommo, A., et al. (2018). *Caloric restriction delays yeast chronological aging by remodeling carbohydrate and lipid metabolism, altering peroxisomal and mitochondrial functionalities, and postponing the onsets of apoptotic and liponecrotic modes of regulated cell death.* Oncotarget, 9(22), 16163–16184. https://doi.org/10.18632/oncotarget.24604

P193

Mitochondrial Function During Aging in *Saccharomyces cerevisiae*: Role of Caloric Restriction and Sirtuin2

Carolina Ricardez-García[a] and Salvador Uribe-Carvajal
[a] *Department of Genetics and Molecular Biology, Instituto de Fisiología Celular, UNAM, Mexico City, Mexico*

E-mail address: cricardez@ifc.unam.mx, suribe@ifc.unam.mx.
Presenting author: Carolina Ricardez-García

Abstract

Aging is a complex biological process strongly influenced by metabolic status and mitochondrial function. Caloric restriction (CR) is one of the interventions known to delay aging and extend lifespan across species, yet the underlying bioenergetic mechanisms remain incompletely understood. How glucose metabolism interfaces with mitochondrial regulation during aging represents a central unresolved question. Increasing evidence highlights sirtuins as key metabolic sensors that couple nutrient availability to mitochondrial function and longevity [1].

In this study, we investigate the impact of caloric restriction on mitochondrial bioenergetics during chronological aging in the yeast *Saccharomyces cerevisiae*, focusing on the role of the NAD^+dependent deacetylase SIR2. Chronological aging in yeast provides a powerful and well-established model to study conserved mechanisms of cellular aging under controlled metabolic conditions. Using wild-type and SIR2-related mutant strains, we analyze mitochondrial function under standard and caloric restriction conditions.

Mitochondrial and metabolic states were evaluated through measurements of oxygen consumption, glucose metabolism, fermentation, growth and viability, plus levels of ATP and reactive oxygen species. In addition native gel activity analyses of respiratory complexes and trehalose quantification. Our results indicate that caloric restriction induces remodeling of mitochondrial bioenergetics during aging. Importantly, these adaptations are strongly dependent on SIR2 activity, suggesting that this sirtuin acts as a critical mediator linking glucose availability to mitochondrial function.

These findings support the emerging central role of mitochondrial signaling in aging and health span [2], this work provides mechanistic insight into how metabolic interventions modulate mitochondrial function at the molecular level.

[1] Z. Ji, G.-H. Liu, J. Qu, et al., *Mitochondrial sirtuins, key regulators of aging*, Life Medicine 4(4) (2025) lnaf019, https://doi.org/10.1093/lifemedi/lnaf019

[2] D. Steffan, C. Pezzini, M. Esposito, A. Franco-Romero, *Mitochondrial aging in the CNS: Unravelling implications for neurological health and disease*, Biomolecules 15(9) (2025) 1252, https://doi.org/10.3390/biom15091252

P194

Streamlining the biolistic method for faster mitochondrial genome editing in yeast Malte Marquardt[a], Marcel Vieira-Lara[a]

[a]*Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ, Delft, The Netherlands*

E-mail address: m.l.marquardt@tudelft.nl
Presenting author: Malte Marquardt

The composition of the mitochondrial genome (mtDNA) greatly impacts the bioenergetic landscape of the cell as it encodes several proteins directly involved in oxidative phosphorylation. Investigations on the effect of mitochondrial variants on energy metabolism suffer from the limited efficacy of editing tools available for this genome. As the organelle does not naturally import DNA or RNA, biolistic bombardment is the only available technique for targeted sequence insertion or substitution in mitochondria to date[1]. Predominantly used in *S. cerevisiae,* the method delivers a gene of interest to mitochondria via bombardment of cells with DNA-covered carrier particles. From plasmid construction to obtaining a mutant, this process takes months. In this work, we removed bottlenecks in the standard workflow of biolistic bombardment and developed an updated version that significantly shortens the time from design to final mutant. This enables higher throughput in the generation of *S. cerevisiae* strains with engineered mtDNA.

In this method *S. cerevisiae* strains lacking mtDNA (ρ^0) are bombarded with plasmids carrying a gene variant of interest, using a commercially available high-velocity particle delivery system. We developed plasmids that contain a minimal backbone and are compatible with Golden Gate cloning. Out of many transformants that are obtained, a very small fraction also harbors the bombarded plasmids in their mitochondria. To identify this subset of mitochondrial transformants, all obtained transformants are mated to a non-respiring tester strain with defective mtDNA. During this process, the mitochondria fuse and the mtDNA recombines. The heterologous gene variant in the mitochondrial transformants replaces the dysfunctional allele in the tester strain mtDNA. The recombined mtDNA in the offspring can rescue oxidative phosphorylation and in turn respiration, which can be selected for on non-fermentable medium.
To facilitate the transfer of recombinant mtDNA to strains with any nuclear background of choice we developed a one-step mating protocol accommodating donor and recipient strains of either mating type. This eliminates the need for the laborious recovery of haploid parental strains of respiring offspring, determination of mating type, and a further time-sensitive mating procedure, shortening the time from design to target strain by several weeks. To achieve simultaneous construction of up to five strains per bombardment, we are currently trialing multiplexed bombardment steps.
The already realized improvements in combination with the envisioned multiplex bombardment strategy hold the potential of more than halving the time required for mitochondrial genome editing, enabling its use for screening panels of gene variants in parallel.

[1] Bonnefoy N, Remacle C, Fox TD (2007) Genetic transformation of *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii* mitochondria. Methods Cell Biol 80:525–548. https:// doi.org/10.1016/S0091-679X(06)80026-9

P195

Real-time mapping of metabolofunction in human neuronal networks

Julian van Reede[a,b,c,d], Jaap Keijer[d], Nael Nadif Kasri[c], Merel J.W. Adjobo-Hermans[e], Werner J.H. Koopman[a,b,d]

a

Department of Pediatrics, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands. [b]Radboud Center for Mitochondrial Medicine, Radboud University Medical Center, Nijmegen, The Netherlands. [c]Department of Human Genetics, Radboud University Medical Center, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands. [d]Human and Animal Physiology, Wageningen University, Wageningen, The Netherlands. [e]Department of Medical BioSciences, Radboud University Medical Center, Nijmegen, The Netherlands.

E-mail address: Julian.vanreede@radboudumc.nl

Presenting author: Julian van Reede

To function properly, neurons are organized in networks that require spatiotemporal coupling between electrical activity, energy metabolism and redox homeostasis[1]. During the last decade, the relevance of energy/redox metabolism in neuronal network function and connectivity has been clearly established in human brain disorders. Among the tools used, micro-electrode arrays (MEAs) have been particularly valuable as a non-invasive method to characterize neuronal activity and physiology, as well as disease-specific genotypephenotype correlations *in vitro*[2]. However, the absence of concurrent bioenergetic monitoring creates a significant interpretive gap. In this study we will design, validate and apply a novel on-chip platform ("NeurO$_2$ChipH") for synchronized analysis of neuronal energy metabolism and function ("metabolofunction"). By integrating MEA technology with high-resolution quantitative fluorescence microscopy, we set the first step towards such a platform for analysis of iPSC-derived excitatory cortical neurons. Our approach enables the simultaneous 2D mapping of network-wide electrical activity together with dynamic live-cell measurements of cellular and mitochondrial biosensors. First experiments revealed the degree of synchronicity between 2D electrical network bursting, localized cytosolic Ca^{2+} transients and fluctuations in mitochondrial membrane potential and morphology. We conclude that this multiplexed analysis holds great promise for studying mitochondrial (dys)function in neuronal networks, as well as other electrically active cell models.

[1] Š. Danačíková, P. Pecina, A. Pecinová, J. Svoboda, D. Vondrášek, D.A. Basello, T. Čajka, D. Hadraba, T. Mráček, V. Kořínek, J. Otáhal, Development of cellular energy metabolism during differentiation of human iPSCs into cortical neurons, Mol. Neurobiol. 63
(2025) 37. https://doi.org/10.1007/s12035-025-05284-8

[2] B. Mossink, A.H.A. Verboven, E.J.H. van Hugte, T.M.K. Gunnewiek, G. Parodi, K. Linda, C. Schoenmaker, T. Kleefstra, T. Kozicz, H. van Bokhoven, D. Schubert, N.N. Kasri, M. Frega, Human neuronal networks on micro-electrode arrays are a highly robust tool to study disease-specific genotype-phenotype correlations in vitro, Stem Cell Rep. 16 (2021) 2182–
2196. https://doi.org/10.1016/j.stemcr.2021.07.001

P196

Bioenergetic and functional characterization of fresh and cryopreserved mitochondria for cardiac transplantation

Elisa Zamprogno[1], Giada Carpi[1], Francesca D'Este[1], Marina Comelli[1], Antonio P. Beltrami[1,2], Giovanna Lippe[1], Sandro Sponga[3]

[1]Department of Medicine, University of Udine, Italy;
[2]Academic Hospital of Udine, ASUFC, Udine, Italy;
[3]Division of Cardiac Surgery, Cardiothoracic Department, University Hospital of Udine, Udine, Italy

Zamprogno.elisa@spes.uniud.it
Giovanna.lippe@uniud.it

Mitochondrial transplantation is an emerging strategy of myocardial protection against ischemic injury. This approach involves isolating healthy, respiration-competent mitochondria from a patient and delivering them into the damaged cardiac tissue. The ultimate goal is to exploit a personalized therapy that preserves myocardial function while patients wait for cardiac transplantation.

Given that isolated mitochondria often fail to survive calcium overload due to the long-lasting openings of the permeability transition pore (PTP), a condition likely in the bloodstream or in tissues, a first objective of our study was to test the efficacy of transplanting mitochondria derived from Cyclophilin (CyP) D knock-out HEK293 cells (kindly provided by Dr. M.Carraro, University of Padova), which exhibit a lower propensity to Ca-induced PTP opening, compared to HEK293 WT cells.

Moreover, to evaluate the feasibility of using frozen mitochondria aliquots in clinical settings, a second objective of our study was to assesses the impact of freezing/thawing cycles on the bioenergetic performance and transplantation efficacy of isolated mitochondria compared to fresh counterpart.

Mitochondria isolated from CyPD-KO and WT HEK293 cells resulted functionally active, both when freshly isolated and after cryopreservation, although freezing/thawing caused a partial reduction in the respiratory rate. CyPD-KO-derived mitochondria also showed a much higher Calcium Retention Capacity (CRC) and a higher Respiratory Control Ratio and coupling efficiency compared to WT. Despite these findings, the efficacy of transplantation, evaluated via MTT assay and cell counting, showed similar improvements in viability of healthy donor-derived pericytes following 24 h of co-incubation with either WT or CyPD KO mitochondria, suggesting that PTP desensitization plays a minor role in this process. While cryopreserved mitochondria did not produce the same statistical significance as fresh ones, both genotypes exhibited similar trends of increased pericytes viability and number after treatment. Using confocal microscopy, we confirmed that exogenous mitochondria, labelled with mitochondria-targeted GFP, were successfully internalized by pericytes following co-incubation.

In conclusion, we demonstrated that the isolation method allows to obtain respiration-competent mitochondria capable of internalization and enhancing recipient cell viability, although with a reduced activity post-cryopreservation, thus providing a foundation for the clinical translation of mitochondrial transplantation.

P197

Microfluidic Encapsulation of ATP-Producing Liposomes in GUVs: Toward Synthetic Mitochondria

Larissa Henke[1], Chao Song[2], Stavros Stavrakis[2], Christoph von Ballmoos[1]
[1]*Department of Biochemistry, University of Bern, Bern (Switzerland)*

[2]*Department of Chemistry and Applied Biosciences, ETH Zürich*

E-mail address: larissa.henke@unibe.ch
Presenting author: Larissa Henke

Energy production is fundamental to both natural and synthetic systems, yet quantifying ATP dynamics in reconstituted bioenergetic networks remains challenging. iATPSnFR2, a ratiometric fluorescent ATP sensor, offers a powerful solution by enabling real-time, quantitative monitoring of ATP synthesis (1). This sensor, based on a circularly permuted superfolder GFP within the εsubunit of bacterial F_0F_1 ATPase, provides affinity variants spanning 4 μM to 500 μM, enabling precise measurements across diverse ATP concentrations.

Here, we employed iATPSnFR2 to track ATP synthesis from synthetic proteoliposomes with bacterial ATP synthase and either proteorhodopsin or *bo*$_3$ oxidase embedded. Upon activation with light (proteorhodopsin) or the addition of ubiquinone and dithiothreitol (DTT) as a reducing agent (*bo*$_3$ oxidase), iATPSnFR2 confirmed ATP production. Using *bo*$_3$ oxidase, we achieved 40–50% ADP-to-ATP conversion and ATP synthesis rates of 2–3 μM/min. These findings highlight the efficiency of different energy-coupling strategies for ATP generation in synthetic systems. By enabling precise, real-time quantification of ATP levels, iATPSnFR2 is a powerful tool for investigating energy metabolism in artificial bioenergetic networks.

To integrate ATP-generating modules into cell-sized compartments, we developed a microfluidic platform to encapsulate these synthetic liposomes inside giant unilamellar vesicles (GUVs). GUVs were produced via double-emulsion solvent extraction, where droplets formed rapidly but required a slower solvent removal step (~1 h) to eliminate residual oil. Fluorescence microscopy, combined with automated image segmentation using Cellpose, enabled quantitative identification of ATTO647N-labelled liposomes encapsulated within Lissamine-Rhodamine-labelled GUVs. These ATP-producing liposomes function as modular energy units for synthetic cells, enabling sustained ATP supply to drive biochemical reactions. In future work, we aim to couple ATP production to cell-free lipase synthesis inside GUVs to achieve ATP-powered membrane degradation and programmable cargo release for synthetic biology and drug delivery applications.

References:

1. Marvin JS, Kokotos AC, Kumar M, Pulido C, Tkachuk AN, Yao JS, et al. iATPSnFR2: A high-dynamic-range fluorescent sensor for monitoring intracellular ATP. Proceedings of the National Academy of Sciences. 2024 May 21;121(21):e2314604121.

P198

Loss of dGTP in postmitotic tissues due to respiratory complex I instability

Marco Spinazzi[a], Xiaoshan Zhou[c], Janne Purhonen[c]

[a] *Neuromuscular Reference Center, Department of Neurology, CHU d'ANGERS/ INSERM U1083, Angers,France*

[b] *Neurobiology and Neuropathology Unit, Department of Pathology, CHU d'Angers, Angers, France* [c] *Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Sweden*

E-mail address: janne.purhonen@ki.se

Presenting author: Janne Purhonen

Recent biochemical investigations and high-resolution cryo-EM structures have revealed that the NDUFA10 subunit of respiratory complex I (CI) contains a tightly bound deoxyguanosine triphosphate (dGTP)[1,2]. The structural, functional, and physiological significance of this finding remains unclear. Here, we utilized mice lacking the accessory CI subunit NDUFS4, which is critical for the proper assembly and integrity of the complex, to assess how CI instability and deficiency alter nucleotide pools in the liver, kidney, heart, skeletal muscle, and brain (see refs.[3,4] for method details). Guanosine triphosphate (GTP) levels were preserved in the brain, skeletal muscle, and heart of *Ndufs4* knockout mice. In contrast, all five assessed tissues showed ~40% decrease in dGTP content. In the brain, renal cortex, and heart, this decrease was identical to that of *Dguok* knockout mice (a primary mitochondrial dGTP depletion). Differential metabolite extraction with increasing harshness showed that the difference arose exclusively from the tightly protein-bound dGTP pool. Labile dGTP pool was identical to that of control mice. Estimation of the stoichiometry between dGTP and CI abundances in mouse tissues, based on our quantifications and an absolute protein quantification data set[5], implied that both are present at near-equimolar amounts. Unlike DGUOK-deficient mice, *Ndufs4* knockout mice did not show mtDNA depletion, suggesting that CI-bound dGTP is not critical for mtDNA maintenance. We propose that 50-75% of dGTP in postmitotic cells may be bound to CI.

[1] D. Molina-Granada, E. González-Vioque, M.G. Dibley, R. Cabrera-Pérez, A. Vallbona-Garcia, J. Torres-Torronteras, L.A. Sazanov, M.T. Ryan, Y. Cámara, R. Martí, Most mitochondrial dGTP is tightly bound to respiratory complex I through the NDUFA10 subunit, Commun. Biol. 5 (2022) 1–16. https://doi.org/10.1038/s42003-022-03568-6.

[2] D.N. Grba, I. Chung, H.R. Bridges, A.-N.A. Agip, J. Hirst, Investigation of hydrated channels and proton pathways in a high-resolution cryo-EM structure of mammalian complex I, Sci. Adv. 9 (2023) eadi1359. https://doi.org/10.1126/sciadv.adi1359.

[3] J. Purhonen, R. Banerjee, A.E. McDonald, V. Fellman, J. Kallijärvi, A sensitive assay for dNTPs based on long synthetic oligonucleotides, EvaGreen dye and inhibitor-resistant high-fidelity DNA polymerase, Nucleic Acids Ress 48 (2020) e87. https://doi.org/10.1093/nar/gkaa516. [4] J. Purhonen, A. Hofer, J. Kallijärvi, Quantification of all 12 canonical ribonucleotides by real-time fluorogenic in vitro transcription, Nucleic Acids Res 52 (2023) e6. https://doi.org/10.1093/nar/gkad1091.

[5] J.R. Wiśniewski, H. Koepsell, A. Gizak, D. Rakus, Absolute protein quantification allows differentiation of cell-specific metabolic routes and functions, Proteomics 15 (2015) 1316–1325. https://doi.org/10.1002/pmic.201400456.

P199

The molecular mechanism of mitochondrial complex I inhibition by the hydrophilic biguanide metformin

Zhaoxiang He[a], Yiqi Hu[a], James A. Letts[b], Long Zhou[a]

[a]School of Basic Medicine, Zhejiang University, Hangzhou 310058, China [b]Department of Molecular and Cellular Biology, University of California, Davis, United States

E-mail address: yiqihu@zju.edu.cn

Presenting author: Yiqi Hu

Metformin is the only anti-type II diabetes biguanide with proven safety. Its mechanism of action depends on modulation of the ATP/AMP ratio, activation of the AMP-activated protein kinase (AMPK) and suppression of hepatic gluconeogenesis. The direct target of metformin however, is still much debated, with proposed candidates including mitochondrial respiratory complexes I and IV (CI and CIV), mitochondrial glycerol-3-phosphate dehydrogenase and PEN2-lysosomal V-type ATPase. It is well established that the reversible inhibition of the mitochondrial CI by metformin contributes to its blood glucose lowering efficacy. However, no structural information has been reported for metformin bound with CI or any other clinically relevant target before our study reported here[1].

We solve the cryo-EM structures of porcine respirasome in complex with metformin or the hydrophobic biguanide proguanil, a classic antimalarial agent. The structures show that metformin selectively binds CI in its globally closed conformational state, occuping the bottom of CI's ubiquinone (Q) channel and coexisting with the natural substrate Q. In this way, the quinone headgroup is displaced from the catalytically important H98/Y147/ $D199^{NDUFS2}$ triad which ligates and protonates Q during electron transfer from the iron-sulfur cluster N2. On the other hand, proguanil occupies both the entrance and the bottom sites of Q channel in both open and closed CI states. Molecular dynamics simulations validate that the structurally observed biguanide binding sites are indeed local minimums in respective binding energy profiles along the Q channel. In line with the observed structural features, we propose two CI state-dependent kinetic models, the competitive model and the ordered-binding inhibitor trapping model, for CI inhibition by proguanil and metformin, respectively. Using respirasome-reconsitituted proteoliposomes, the measured IC_{50} vs [Q], $K_{Q.APP}$ vs [biguanide] and $V_{MAX,APP}$ vs [biguanide] curves are well fitted by the model predicted equations. Therefore, distinct mechanisms are employed by the hydrophilic metformin and the hydrophobic proguanil to inhibit complex I. Metformin, probably entering CI via the matrix side crevice exclusively in the open/deactivated state, becomes trapped upon CI closure by incoming CoQ_{10} and can leave CI only when the complex opens up/deactivates again. On the other hand, the hydrophobic proguanil diffuses through the membrane-embedded Q channel entrance and functions via the classic 'cork-inbottle' mechanism blocking Q channel access by the natural substrate Q.

[1] Z. He, F. Teng, Y. Yang, R. Guo, M. Wu, F. Han, H. Tian, J. Wang, Y. Hu, Y. Jiang, L. Zhang, C. Xu, F. Yang, J. Zhou, S. Zhang, J.A. Letts, R. Zhou, L. Zhou, Hydrophilic metformin and hydrophobic biguanides inhibit mitochondrial complex I by distinct mechanisms. Nat. Struct. Mol. Biol. 33 (1) (2026), 100-111, https://doi.org/ 10.1038/s41594-025-01710-6

P200

Structural investigation on cytochrome c maturation system II

Céline Moog[1], Julia Seifermann[1], Lorena Ilcu[1], Anton Brausemann[1], Lin Zhang[1,2], Oliver Einsle[1]

[1]*Institute of Biochemistry, Albert-Ludwigs-Universität Freiburg, Albertstraße 21, 79104 Freiburg, Germany.*

[2]*Faculty of Synthetic Biology, Shenzhen University of Advanced Technology, Shenzhen, 518107, China.*

Email: celine.moog@biochemie.uni-freiburg.de

Presenting author: Céline Moog

Cytochromes *c* play a preponderant role in electron transport, sensing and redox catalysis, most prominently to O_2 yielding H_2O in aerobic respiration. The cofactor-deplete precursors of cytochromes are translocated via the Sec translocon to the periplasmic space, where heme cofactors are covalently attached to the protein chain by a multi-component maturation machinery. To this end, a processive heme lyase scans the apocytochrome peptide for the occurrence of distinct binding motifs, such as CX_nCH (mostly n =2), CXXCK, etc.. Thereafter the protein attains its final structure in the periplasmic space. Different organisms employ one of several known types of the heme maturation machinery. Among these, the most versatile and most thoroughly researched variants are the systems I (Ccm system) and II (Ccs). Ccm-type cytochrome c maturation systems are composed of up to nine individual protein components that are organized in two functional modules, a heme translocase (CcmABCD) and a cytochrome c heme lyase (CcmFGH(I)). Between the two complexes, the cofactor is transported via the heme chaperone CcmE. In the alternative system II, also called cytochrome *c* synthase, these functions are largely unified into the CcsBA complex, which itself doubles as a heme transporter.

The focus of my research lies on the cytochrome *c* maturation systems II, mostly occurring in β-, δ-, and ε-proteobacteria, Gram-positive bacteria, etc.. Our key methods are structural biology, which are crucial for understanding the mechanistic aspects of the transport of the porphyrin ring within the maturation system and the subsequent introduction of heme into the cytochrome *c* protein.

References

1. Sutherland, M. C. *et al.* Structure-Function Analysis of the Bifunctional CcsBA Heme Exporter and Cytochrome c Synthetase. *mBio* 9; 10.1128/mbio.02134-18 (2018).

2. Kranz, R., Lill, R., Goldman, B., Bonnard, G. & Merchant, S. Molecular mechanisms of cytochrome c biogenesis: three distinct systems. *Molecular microbiology* 29, 383–396; 10.1046/ j.1365-2958.1998.00869.x (1998).

3. Frawley, E. R. & Kranz, R. G. CcsBA is a cytochrome c synthetase that also functions in heme transport. *Proceedings of the National Academy of Sciences of the United States of America* 106, 10201–10206; 10.1073/pnas.0903132106 (2009).

4. Simon, J. & Hederstedt, L. Composition and function of cytochrome c biogenesis System II. *The FEBS journal* 278, 4179–4188; 10.1111/j.1742-4658.2011.08374.x (2011).

5. Ow, Y.-L. P., Green, D. R., Hao, Z. & Mak, T. W. Cytochrome c: functions beyond respiration. *Nat Rev Mol Cell Biol* 9, 532–542; 10.1038/nrm2434 (2008).

6. Ilcu, L., Denkhaus, L., Brausemann, A., Zhang, L., & Einsle, O. Architecture of the Heme-translocating CcmABCD/E complex required for Cytochrome c maturation. *Nature Communications*, *14*(1), 5190 (2023).

7 Brausemann, A., Zhang, L., Ilcu, L., & Einsle, O. Architecture of the membrane-bound cytochrome c heme lyase CcmF. *Nature chemical biology*, *17*(7), 800-805 (2021).

P201

Production of intact UCP2 in higher eukaryotic expression systems

Mehmethan Aris[a], Margeoux Dela Rosa[b], Danielle Copeman[a], Callum Eke[a], Hannah StaggSandy[a], Riccardo Cavalieri[b], Steven Harborne[b], Paul Crichton[a]

[a]*Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, United Kingdom*

[b]*Sygnature Discovery Ltd., Tytherington Business Park, Macclesfield, United Kingdom*

E-mail address: m.aris@uea.ac.uk, p.crichton@uea.ac.uk
Presenting author: Mehmethan Aris

Uncoupling protein 2 (UCP2) is a mitochondrial carrier and homologue of UCP1, the protein that facilitates thermogenesis in brown adipose tissue. Unlike UCP1, which is specific to brown fat, UCP2 occurs at lower abundance in a variety of different tissues and has been linked to metabolic and oncogenic disorders. Whether or not UCP2 catalyses mitochondrial proton leak like UCP1 is not clear, and there is now evidence that it potentially exchanges 4-carbon metabolites in a conventional carrier transport function. Hence, its biochemical function is unclear. The protein is unstable, and existing methods to produce UCP2 for purification using microbial systems generate too little protein or rely on protein 'refolding' from E.coli-generated inclusion bodies, which produce incorrectly folded carrier. Hence, new methods to generate sufficient intact UCP2 are needed to help clarify its function, role in pathologies and potential to be targeted. [1,2]

Here, we have utilised higher eukaryotic protein expression systems to generate intact UCP2 for subsequent purification and functional assessment. Our studies have focused on the use of both insect and mammalian cell systems, with initial construct and expression screening carried out to assess the suitability of various purification affinity tags and conditions. We found that all cell types were able to express recombinant UCP2, though only insect cells produced UCP2 in a form almost fully extractable in mild detergent, indicative of an appropriately folded protein. In further tests, we found that the particular insect cell line used was important for optimal expression levels, whereas other parameters were less critical (e.g. multiplicity of infection or glutamine addition). We observed that UCP2 exhibited a loss of solubility in mild detergent and temperature challenge tests, confirming limited stability, which we utilised to assess for potential stabilising agents (e.g. putative metabolite transport substrates). Our purification trials showed that UCP2 could be enriched with an appropriate affinity resin, which we are further optimising to allow subsequent functional characterisation of the protein.

[1] J. Nedergaard, B. Cannon, The 'novel' 'uncoupling' proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions, Exp. Physiol. 88(1) (2003) 65–84, https://doi.org/ 10.1113/eph8802502

[2] A. Vozza, G. Parisi, F. De Leonardis, F. M. Lasorsa, A. Castegna, D. Amorese, R. Marmo, V. M. Calcagnile, L. Palmieri, D. Ricquier, E. Paradies, P. Scarcia, F. Palmieri, F. Bouillaud, G. Fiermonte, UCP2 transports C4 metabolites out of mitochondria, regulating glucose and glutamine oxidation, Proc. Natl. Acad. Sci. U.S.A. 111(3) (2014) 960–965, https://doi.org/10.1073/pnas.1317400111

P202

Functional diversification of respiratory complex I in anaerobic bacteria through exchangeable electron input modules

Mirjam Odermatt[a], Lin Zhang[b,c], Kaija Walter[d], Leonie Stahmer[a], Dennis Kosian[a], Georg Hochberg[d], Oliver Einsle[b] & Matthias Boll[a] [a]Faculty of Biology – Microbiology, University of Freiburg, 79104 Freiburg, Germany. [b]Institute of Biochemistry, University of Freiburg, 79104 Freiburg, Germany. [c]Shenzhen University of Advanced Technology, Guangdong, China. [d]Max-Planck-Institute for Terrestrial Microbiology, 35043 Marburg, Germany.

E-mail address: mirjam.odermatt@biologie.uni-freiburg.de
Presenting author: Mirjam Odermatt

Respiratory complex I (NADH:quinone oxidoreductase) is typically described as a conserved entry point for catabolically-gained electrons via NADH into respiratory chains, with NADH commonly reduced during the citric acid cycle. In strictly anaerobic bacteria such as the model organism *Geobacter metallireducens*, central metabolism deviates from this canonical scheme: an $NADP^+$-dependent isocitrate dehydrogenase and an α-ketoglutarate:ferredoxin oxidoreductase operate alongside a primarily NAD^+-dependent malate dehydrogenase, resulting in the simultaneous generation of NADH, NADPH, and reduced ferredoxin. Due to the absence of annotated transhydrogenase systems, this raises the questions of how these distinct reducing equivalents are reoxidized during respiration, whether the electrons enter the menaquinone pool and how this affects respiratory complex I.

To address these questions, we opted for native enrichment of complex I from *G. metallireducens* and characterized the hydrophilic domain of the protein biochemically as well as structurally by single-particle cryo–electron microscopy. We found that *G. metallireducens* co-expresses two functionally distinct variants of respiratory complex I. Structural analysis revealed that both variants share a conserved core architecture but differ in their electron input modules. Biochemical analyses confirmed that the two assemblies indeed specifically oxidize different electron donors, consistent with homologous reducing equivalent-consuming protein subunits found in different cellular context.

Together, these findings show that respiratory complex I from *G metallireducens* acts as a modular platform that can incorporate two alternative electron input modules to channel electrons from multiple redox carriers into the respiratory chain.

P203

High-Turnover Redox-Coupled Proton Pumping of the Bacterial Complex I in Proteoliposomes

Vladana Sušić[a], Adel Beghiah[a], Terezia Kovalova[a], Ville R. I. Kaila[a,*]

[a]*Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, SE-106 91, Stockholm, Sweden*

[*]Corresponding Author E-mail address: ville.kaila@dbb.su.se Presenting author: Vladana Sušić

Functional characterization of proton translocation by energy-converting membrane protein complexes remains methodologically challenging. Here, using a novel reconstitution method, we incorporate *E. coli* Complex I (*Ec*CI) into liposomes, together with terminal oxidases that reoxidize the quinone pool and enable high catalytic turnover conditions. The resulting proteoliposomes are stable and well-ordered, as confirmed by our cryo-electron microscopic analysis, and can sustain large proton gradients without leaking, as monitored spectroscopically with pH-sensitive dyes, resulting in proton-pumping stoichiometry of around 1.8 H^+/e^-. The *Ec*CI is also active with menaquinone, but shows a reduced pumping stoichiometry. Taken together, our established setup provides an improved platform to probe redox-driven proton pumping of Complex I and related membrane proteins under high-turnover conditions.

[1] Sušić V, Beghiah A, Kovalova T, Kaila VRI (in preparation).

P204

Dynamic modulation of complex IV excess capacity by a redox-dependent uncoupling mechanism in a metabolically active bean beetle line

Clément J. L. Chevret[a], Pierre U. Blier[b], Mirko Đorđevid[c], Uroš Savkovid[c], Hélène Lemieux[a,d,e]

a Department of Medicine, University of Alberta, Edmonton, Alberta, Canada b Département de Biologie, Université du Québec à Rimouski, Rimouski, Québec, Canada

c
Department of Evolutionary Biology, Institute for Biological Research "Siniša Stankovid" - National

Institute of the Republic of Serbia, University of Belgrade, Serbia d Faculty Saint-Jean,
University of Alberta, Edmonton, Alberta, Canada

e Women and Children's Health Research Institute, University of Alberta, Edmonton, Alberta, Canada

E-mail address: chevret@ualberta.ca
Presenting author: C.J.L. Chevret

Despite growing evidence positioning mitochondria as central regulators of ageing, the contribution of individual components of the electron transport system (ETS) to longevity remains poorly defined. Across a wide range of animal species, Complex IV (CIV) displays a substantial excess capacity relative to the electron transfer capacity of the ETS. Classical bioenergetic theory predicts that such excess enzymatic capacity should exert little control over pathway flux. Whether this apparent surplus serves a specific physiological function, however, remains unresolved. Here, we investigated two lines of the bean beetle (*Acanthoscelides obtectus*) selected for contrasting life-history strategies: an early-reproduction line (E), characterized by a short lifespan (~7 days), and a late-reproduction line (L), with nearly doubled longevity (~13 days). This model is particularly relevant because larvae develop within a single bean and adults do not feed, imposing strict energetic constraints that drive the E line to maximize early-life metabolic output, whereas the L line prioritizes energy allocation toward somatic maintenance and lifespan extension. Measurements were conducted in 1-day-old individuals from both lines, in which CIV excess capacity had previously been shown to differ. By coupling high-resolution respirometry with fluorescence, we simultaneously monitored oxygen consumption and hydrogen peroxide (H_2O_2) production. CIV inhibition was used to determine CIV excess capacity under different pathway conditions: (i) NADH + succinate (NS pathway), (ii) NADH + succinate + proline (NSPro pathway), and (iii) NADH + succinate + proline + glycerophosphate (NSProGp pathway). In both lines and under each pathway condition, reactive oxygen species (ROS) production increased exponentially when CIV inhibition reached ~15% beyond the point at which excess capacity was exhausted. This conserved response supports the hypothesis that CIV excess capacity contributes to limiting ROS production and reveals the existence of a buffering range that persists beyond the point of excess-capacity exhaustion. This buffering property was preserved in both lines despite their different longevity. In the long-lived L line, as expected, CIV excess declined proportionally with increasing respiration when moving from the NS- to NSPro- toNSProGp-pathways. In contrast, in the E line, apparent CIV excess capacity increased at high flux (from NSPro to NSProGp) while ROS/O_2flux ratio decreased, despite a 30% stimulation of respiration by glycerophosphate. This result challenges theoretical expectations based on fixed enzyme control within the ETS.

Collectively, these findings indicate that CIV excess capacity is dynamically regulated rather than static. We propose that this regulation may involve a ROS-dependent uncoupling mechanism that enhances CIV catalytic performance while constraining ROS production under conditions of elevated electron flux

Contribution type: Talk

P205

Uncovering a VDAC1-Associated Mitochondrial Vulnerability in Pediatric B-Cell Acute Lymphoblastic Leukemia

Haripriya Santosh[a], Souvik Roy[a], Sameer Bakhshi[b], Jayanth Kumar Palanichamy[a], Anita Chopra[c], Archna Singh[a]

[a]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India [b]Medical Oncology, BRAIRCH, All India Institute of Medical Sciences, New Delhi, India [c]Lab Oncology, BRAIRCH, All India Institute of Medical Sciences, New Delhi, India
E-mail address: hari_priya@aiims.edu

Presenting author: Haripriya Santosh

Despite survival rates approaching 90%, relapse remains the leading cause of mortality in pediatric B-cell acute lymphoblastic leukemia (B-ALL), with chemoresistance and metabolic adaptation contributing to adverse outcomes.[1] Given the central role of mitochondrial metabolism in leukemic cell survival and therapy response, the voltage-dependent anion channel (VDAC), the major transit pore for metabolites and metal ions across the outer mitochondrial membrane, has emerged as a key regulator of mitochondrial function.[2] VDAC1 also regulates apoptosis through mitochondrial outer membrane permeabilization (MOMP), where stress-induced VDAC1 oligomerization facilitates the release of pro-apoptotic factors and activation of the apoptotic cascade.[3] To identify clinically relevant mitochondrial vulnerabilities associated with aggressive disease, RNA sequencing data from 50 pediatric BALL patients were stratified by median VDAC expression and subjected to Kaplan–Meier survival analysis, univariate Cox regression, and gene set enrichment analysis (GSEA). Although all VDAC isoforms were expressed in pediatric B-ALL samples, only elevated

VDAC1 expression was associated with inferior event-free survival (HR = 2.94, 95% CI: 1.25– 6.9, p = 0.0135). High VDAC1 was associated with enrichment of oxidative phosphorylation (OXPHOS) pathways (NES=1.84, FDR=0.032). Leading-edge genes from enriched pathways, including NDUFS3 (Complex I; HR≈3.4), ATP5F1A (Complex V; HR≈2.6), CYC1 (Complex III; HR≈2.4), and NNT (mitochondrial redox regulation; HR≈2.3), demonstrated adverse prognostic significance and positive correlation with VDAC1 expression, indicating coordinated mitochondrial respiratory remodeling in high-risk disease. Given that HK2 is a major mitochondrial binding partner of VDAC1 that couples glycolysis to mitochondrial ATP production and apoptosis regulation, we next investigated whether the VDAC1–HK2 axis contributes to the bioenergetic phenotype observed in VDAC1-high leukemias through mechanistic studies in NALM6 and REH cells. Subcellular fractionation and western blotting demonstrated HK2 localization in both mitochondrial and cytosolic compartments. Furthermore, peptide-mediated disruption of HK2–VDAC interaction impaired oxygen consumption, suggesting reduced mitochondrial bioenergetic capacity. Ongoing studies assessing HK2–VDAC co-localization and VDAC1 oligomerization aim to further define mechanisms underlying mitochondrial adaptation. Collectively, these findings identify a clinically adverse VDAC1-high/OXPHOS-enriched transcriptional state in pediatric B-ALL and provide preliminary evidence that HK2–VDAC interaction contributes to maintenance of mitochondrial bioenergetics, implying the HK2–VDAC axis as a potential metabolic vulnerability in aggressive B-ALL.

[1] C.B. Lill, S. Fitter, A.C.W. Zannettino, K. Vandyke, J.E. Noll, Molecular and cellular mechanisms of chemoresistance in paediatric pre-B cell acute lymphoblastic leukaemia, Cancer Metastasis Rev. 43(4) (2024) 1385–1399, https://doi.org/10.1007/s10555-024-102039

[2] V. Shoshan-Barmatz, E.N. Maldonado, Y. Krelin, VDAC1 at the crossroads of cell metabolism, apoptosis and cell stress, Cell Stress 1(1) (2017) 11–36, https://doi.org/10.15698/cst2017.10.104

[3] N. Keinan, D. Tyomkin, V. Shoshan-Barmatz, Oligomerization of the mitochondrial protein voltage-dependent anion channel is coupled to the induction of apoptosis, Mol. Cell. Biol.
30(24) (2010) 5698–5709, https://doi.org/10.1128/MCB.00165-10.

P206

Beyond the c-state: are dynamic state changes required for UCP1 proton leak?

Callum Eke, Mehmethan Aris, Danielle Copeman, Hannah Staggs-Sandy, and Paul G. Crichton*

Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, United Kingdom

E-mail address: wnr19kcu@uea.ac.uk; P.Crichton@uea.ac.uk

Presenting author: Callum Eke

Uncoupling Protein 1 (UCP1) is central to non-shivering thermogenesis in brown adipose tissue, where it catalyses proton leak across the mitochondrial inner membrane to generate heat. The protein is activated by free fatty acids and inhibited by purine nucleotides, which directly interact to regulate activity. Despite decades of research, the mechanism of UCP1 proton conductance remains unresolved. The protein is a member of the mitochondrial carrier family of solute transporters, which transition between cytoplasmic-open (c-state) and matrix-open (m-state) conformations to facilitate substrate transport. Though to what extent UCP1 uses dynamic movements and specific state changes for proton leak is not clear. Protein thermostability studies have suggested that activator binding is associated with significant molecular changes in the protein [1].

Recently, cryo-EM structures have been achieved using engineered nanobody or sybody binding partners to reveal human UCP1 in a proton impermeable c-state conformation, providing valuable molecular details on nucleotide binding and inhibition [2,3]. However, all available structures, including a ligand-free and a purported 'activator' dinitrophenol-bound structure, retain a similar c-state arrangement, lacking significant molecular changes. As such, the c-state has been proposed to be the only relevant state for activity, accompanying claims that sybody-bound UCP1 is not impeded and retains full activity [3].

Here, we have assessed the impact of both cytoplasmic-side and matrix-side binding nanobodies on the function of recombinant human and native ovine UCP1. Like all binding partners used to date, these species were raised against UCP1 in a nucleotide-inhibited c-state, where binding may restrict changes to other unresolved conformations [4]. Hence, an assessment of nanobody impact on activity has potential to help determine if dynamic movement and states other than the c-state are required in the UCP1 mechanism. Using protein thermostability analysis, we have found that both nanobody types successfully bind to purified UCP1 in our assay in a concentration-dependent manner, inducing thermal stabilisation of both ligand-free and GDP-bound forms. Our subsequent characterisation of these binding partners in UCP1 proteoliposome studies has provided valuable outcomes of mechanistic insight, allowing us to discern between proposed claims on the underlying dynamics of UCP1 that facilitate proton leak [1,3]. These findings are complemented by mutagenesis work to clarify the core principles involved.

[1] R. Cavalieri et al., Activating ligands of Uncoupling protein 1 identified by rapid membrane protein thermostability shift analysis, Mol. Metab. 62 (2022) 101526, https://doi.org/10.1016/j.molmet.2022.101526

[2] S.A. Jones et al., Structural basis of purine nucleotide inhibition of human uncoupling protein 1, Sci. Adv. 9 (2023) eadh4251, https://doi.org/10.1126/sciadv.adh4251

[3] Y. Kang, L. Chen, Structural basis for the binding of DNP and purine nucleotides onto UCP1, Nature 620 (2023) 226–231, https://doi.org/10.1038/s41586-023-06332-w

[4] S.A. Jones, J.J. Ruprecht, P.G. Crichton, E.R.S. Kunji, Structural mechanisms of mitochondrial uncoupling protein 1 regulation in thermogenesis, Trends Biochem. Sci. 49 (2024) 506–519, https://doi.org/10.1016/j.tibs.2024.03.005

P227

A novel Fus mutation causes protein mislocalization and late onset motor dysfunction in zebrafish

Christina Toumpa, Nils Dennhag and Eva Hedlund

Department of Biochemistry and Biophysics, Stockholm University.

Amyotrophic lateral sclerosis is a lethal neurodegenerative disease that causes progressive neuronal loss, muscle atrophy and paralysis. One of the proteins most commonly associated with the disease is FUS, a DNA-RNA binding protein with mainly nuclear localization under physiological conditions and a wide variety of cellular processes in which it participates. Mutations in the FUS gene primarily occur in the region responsible for the localization of FUS to the nucleus, and cause its mislocalization to the cytoplasm, where it acquires toxic gain-of-function properties. The correlation of mutations in FUS with the appearance of ALS pathology makes the protein an ideal target for disease modeling studies. Zebrafish is a relatively new promising model organism for research in ALS and offers many advantages including the high efficiency of CRISPR/Cas9-mediated mutagenesis, optical transparency and fast generation time. With this genome editing tool, we have created a line carrying a novel mutation in the zebrafish fus gene. The mutation caused partial mislocalization of Fus protein within motor neurons to the cytoplasm already within two days post fertilization. Nonetheless, no behavioral phenotypes were seen until the animals were six months old. At this time the zebrafish showed a swim test phenotype indicative of reduced fast motor units. This indicates that we have generated a late onset zebrafish model of ALS. We are now investigating if the level of mislocalized Fus protein has increased over time, or if it is the length of time a cell is exposed to a subthreshold level of mislocalized mutated Fus that triggers cellular stress and a disease phenotype. We are also further investigating neuropathological alterations causative of the swim phenotype.