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The evolution of a Na^+-sensitive *Vibrio cholerae* mutant unmasks the moonlighting aminopeptidase PepA as a regulator of *nhaB* Na^+/H^+ antiporter gene expression

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The pathogenic bacterium *Vibrio cholerae* is native to seawater and can therefore be cultivated in nutrient media with increased salt concentration. To maintain osmotic balance, *V. cholerae* uses Na^+/H^+ antiporters and an Na^+-translocating NADH:quinone oxidoreductase (Na^+-NQR).

The exact contribution of the various Na^+/H^+ antiporters to maintain Na^+ and H^+ homeostasis in *V. cholerae* is unclear. However, genetic studies indicate the major Na^+/H^+ antiporter NhaA and the Na^+-NQR are required by the bacteria for Na^+ resistance at alkaline conditions. Here we show that the growth defect of a *V. cholerae nhaA nqr* mutant at increased Na^+ concentrations and alkaline pH is relieved by the rapid acquisition of suppressor mutations. The suppressor mutants could be assigned to two classes. We identified (i) mutations in the promoter of the *nhaB* gene encoding a Na^+/H^+ antiporter and (ii) mutations that either affect the expression level of the *pepA* gene or DNA binding activity of the encoded multifunctional aminopeptidase PepA. The characterization of the P_{nhaB} and P_{pepA} promoters of the suppressor mutants, membrane potential measurements and comparative proteome analyses identified PepA as a novel factor controlling Na^+ homeostasis in *V. cholerae.*

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Spin-Dependent Electronic Reconfiguration and the Stability of the Semiquinone Intermediate at the Q_o site of Cytochrome bc_1

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Cytochrome bc_1 is a homodimeric enzyme in biological energy-conserving systems that catalyzes the oxidation of ubiquinol (QH_2) at the Q_o site and proton transfer across the membrane. The central catalytic reaction involves electron bifurcation (EB) into two separate cofactor chains. Despite decades of intensive study, the molecular mechanism of electron bifurcation (EB) remains elusive. The canonical mechanism of EB assumes a sequence of reactions governed by the relationship between the equilibrium midpoint redox potentials of the cofactors, primarily the 2Fe2S cluster and heme b_L, and the proposed potentials of the ubiquinone (Q)/semiquinone (SQ)/QH_2 triad[1]. However, several spectroscopic observations reveal the formation of species that are difficult to reconcile with this framework. To explain the origin of these species, we recently developed a new EB mechanism — Emergent Electron Transfer (EMET) — derived from quantum mechanical (QM) calculations[2]. EMET predicts the existence of these species and proposes distinct microstates and lowest-energy electronic configurations during SQ formation.

Here, we quantitatively test these predictions using continuous-wave and pulse EPR spectroscopy on isolated bacterial cytochrome bc_1 from *Rhodobacter capsulatus* in the presence of antimycin[3]. We detect SQ spin-coupled to the reduced 2Fe2S cluster (SQ-2Fe2S^{red}). Quantitative analysis reveals that the population of SQ-2Fe2S^{red} exceeds that of reduced heme b_L, demonstrating that SQ forms predominantly in monomers containing oxidized heme b_L. Furthermore, pulse EPR distance measurements place SQ at the Q_o-site position corresponding to the stigmatellin-binding location, confirming its formation directly within the catalytic pocket.

Among the electronic configurations considered, QM calculations indicate that the state containing reduced heme b_H, oxidized heme b_L, and SQ-2Fe2S^{red} possesses the lowest energy, explaining its stability under steady-state turnover. Additionally, we observed a 1:1 ratio between SQ-2Fe2S^{red} and uncoupled 2Fe2S^{red}, which provides strong support for the EMET mechanism. This ratio is directly predicted by EMET and arises from stochastic spin selection of the electron transferred from heme b_L during the formation of ferro- or antiferromagnetic coupling between SQ and the cluster. Because antiferromagnetic coupling is unstable and results in complete reversion of the reaction (reduction of SQ to QH_2), the observable SQ-2Fe2S^{red} occupancy is expected to equal that of uncoupled 2Fe2S^{red}, precisely as observed experimentally.

Our results provide experimental support for the EMET mechanism, in which electron bifurcation proceeds through previously unconsidered microstates of the enzyme. These findings shed new light on the molecular mechanisms of EB.

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The EMET (Emergent Electron Transfer) Mechanism of Electron Bifurcation in cytochrome bc_1: A Combined Spectroscopic and Theoretical Study

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Quinone-based Electron Bifurcation (QBEB) is a unique redox reaction catalyzed by cytochrome bc_1. In this process, electron transfer (ET) pathways from ubiquinol (QH_2) diverge into two acceptor branches, which is necessary for efficient energy conversion by the enzyme. Canonical explanations of QBEB are based on redox midpoint potentials (E_m values) of the cofactors accepting electrons from QH_2: the 2Fe2S cluster ($E_m \approx +300$ mV) and heme b_L ($E_m \approx -120$ mV). These E_m values suggest that the first electron is transferred from QH_2 to the 2Fe2S cluster, and from the resulting semiquinone (SQ) the second ET to the heme b_L occurs, producing quinone (Q). However, the canonical QBEB model faces unresolved mechanistic problems including short-circuit reactions (internal energy-wasting side reactions) that have not been satisfactorily addressed[1].

To uncover the fundamental principle underlying EB, we applied density functional theory (DFT) method using large cluster models, each containing both electron acceptors (2Fe2S and heme b_L) to identify stationary points defining the QBEB mechanism[2]. The potential energy surface and the location of frontier molecular orbitals, surprisingly, revealed a scenario that differs from canonical QBEB. Unexpectedly, the architecture of QH_2-binding site imposes spin and charge polarization on the benzoquinone ring, which induces proton-coupled electron transfer: a proton from QH_2 is accepted by a conserved glutamate residue on the cytochrome *b* subunit (E295 in *R. capsulatus*), while ET occurs from QH_2 to heme b_L. Completion of QH_2 oxidation to the product requires ET from heme b_L to heme b_H. However, if this ET is blocked, our theoretical results predict the formation of SQ ferromagnetically coupled to reduced 2Fe2S, which indeed is detected by electron paramagnetic resonance spectroscopy in the antimycin-inhibited enzyme[3]. The theoretical study revealed that ET from QH_2 to heme b_L is feasible only in the presence of oxidized 2Fe2S at the Q_o site, despite the apparent lack of direct involvement of the latter in this process. These results indicate that heme b_L, 2Fe2S and QH_2 should be treated as an emergent system that possesses its specific properties which cannot be inferred when the system is divided into separate components.

This became the foundation of a new mechanism, termed EMET (Emergent Electron Transfer), which provides a relatively flat energy landscape that naturally aligns with existing experimental evidence[2]. The EMET framework successfully explains the high efficiency and intrinsic shortcircuit suppression of electron bifurcation without requiring *ad hoc* structural constraints, establishing emergence as a vital concept in bioenergetics.

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P210

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

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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.

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P211

Does the δ Subunit of *Polytomella parva* ATP Synthase Controls its Hydrolytic Activity?

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Mitochondrial ATP synthase catalyzes the formation of ATP from ADP and Pi in most eukaryotic organisms. In the colorless alga *Polytomella parva*, this enzyme exhibits an atypical composition, particularly in those subunits forming the peripheral arm and those involved in dimerization.[1] In addition to ten lineage-specific subunits (Asa1–10) apparently absent in other ATP synthases, the catalytic α and β subunits possess amino acid extensions in their N- and C-terminal regions, respectively.[2] To date, the ATP synthase of *Polytomella parva* appears to lack the peptide IF_1, the natural inhibitor of ATP hydrolysis, which has been found bound to the enzyme in all the "atypical" ATP synthases from poorly studied eukaryotic lineages studied to date. [3–5] In addition, the δ subunit—homologous to the bacterial ε subunit and responsible for linking the hydrophilic and hydrophobic regions of the enzyme— also exhibits an atypical N-terminal extension. Due to the possible proximity of the N-terminal extension of the δ subunit to the conserved DELSEED motif of the β subunit belonging to the enzyme's catalytic core, we hypothesized that the δ subunit could regulate the hydrolytic activity of the algal ATP synthase and evaluated this possibility experimentally. However, the biochemical results, complexomic profiling analysis, and 3D structural data suggest the absence of an IF_1 peptide and clearly indicate that the ATPase of this alga may be regulated solely by ADP-Mg levels.

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Enterococcus faecalis extracellular electron transport

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Extracellular electron transport (EET) is the process where electrons, generated in metabolism in the cytoplasm, are transported out from the cell to an electron acceptor, which can be a soluble redox compound, an electrode, or another cell. Electrons can also pass in the opposite direction, for example, from an electrode to a cell to drive biosynthesis. By EET, electrons from the oxidation of NADH in the cytoplasm can be transported directly or indirectly to an electrode and thereby generate electric current. This is the key feature of microbial fuel cells and therefore of biotechnological interest. The overall impact of EET in nature and molecular details behind the process are far from understood [1].
The gram-positive lactic acid bacterium *Enterococcus faecalis*, found in the intestine of animals, is an opportunistic human pathogen and shows EET [2]. In the gut microbiota, EET might be of considerable importance because it can support syntrophic metabolism and growth of various kinds of cells. The poster presents the current level of understanding of EET in *E. faecalis* and, for example, that cytochrome *bd* activity attenuates EET in this bacterium.

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P213

VDAC1 as a target of Amyloid-β Oligomers: linking channel dysfunction to mitochondrial bioenergetics in Alzheimer's disease

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Voltage-Dependent Anion-selective Channel 1 (VDAC1), the main pore forming protein of the outer mitochondrial membrane (OMM), mediates the exchange of ions and small metabolites, including ADP and ATP, between mitochondria and cytosol, thereby playing a central role in mitochondrial bioenergetics. In Alzheimer's disease, VDAC1 represents the principal mitochondrial binding site for amyloid-β (Aβ) peptide, whose accumulation at the OMM has been associated with mitochondrial dysfunction and oxidative stress. To investigate the molecular effects of Aβ on VDAC1 function, we analyzed the electrophysiological properties of the channel in the presence of $A\beta_{1\text{-}42}$ oligomers (oAβ) using the planar lipid bilayer technique. Under physiological conditions, VDAC1 displays a conductance of approximately 3.5nS in 1M KCl, voltage-dependent gating between open and closed states and a preference for anions. Exposure to oAβ increased VDAC1 conductance at positive potentials (e.g. +10mV) and altered its voltage dependency. Most notably, oAβ induced a marked shift in ion selectivity from anionic to cationic, suggesting a structural perturbation of the β-barrel architecture of the channel. To evaluate the functional consequences of these alterations, mitochondrial respiration was assessed in permeabilized SH-SY5Y neuroblastoma cells exposed to Aβ oligomers. High-resolution respirometry revealed a significant reduction in basal oxygen consumption, maximal respiratory capacity and ADP-linked respiration, indicating impaired oxidative phosphorylation efficiency [1]. Overall, our findings provide further evidence that the molecular interplay between Aβ oligomers and VDAC1 represents a critical event in the development of mitochondrial dysfunction. By linking alterations in channel electrophysiology to deficits in mitochondrial respiration, this study deepens our understanding of the VDAC1-Aβ interaction and provides a rationale for the development of VDAC1-targeting molecules capable of counteracting Aβinduced mitochondrial dysfunction.

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P214

Investigating metabolic changes in a citrin-deficient cell model

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Citrin, encoded by the *SLC25A13* gene, a member of the SLC25 solute carrier family, is located in the inner mitochondrial membrane (IMM), and exchanges mitochondrial aspartate with cytosolic glutamate and a proton. Citrin deficiency (CD) is a debilitating and sometimes fatal condition, which arises from >120 different mutations found in *SLC25A13* [1]. It is a heterogenous condition with three recognised stages: an infant stage, neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), followed by a period termed failure to thrive and dyslipidemia (FTTDCD), which may deteriorate into adolescent/adult-onset citrullinaemia type II (CTLN2) or now called adolescent and adult citrin deficiency (AACD). Citrin is primarily expressed in the liver, and CD mutations predominantly affect the liver in patients. A mammalian isoform called aralar (*SLC25A12*) is also found in the IMM, especially in excitable tissues. Defective citrin affects multiple metabolic pathways via dysfunction of the malate-aspartate shuttle, causing impaired energy metabolism, urea cycle dysfunction, and toxic accumulation of ammonia [2]. However, the metabolic characteristics of CD remain incompletely understood. The generation of suitable models of CD is a key strategic objective to further the understanding of the underlying biology of the disease and potentially allow the development and testing of novel therapies of the human condition. To this end, single allele and double allele citrin knock-out clonal models were generated by CRISPR-Cas9 gene editing in the established hepatoblastoma cell line, HepG2. Deletion of citrin from HepG2 cells resulted in a severe growth defect, and by high resolution respirometry and transcriptome and proteome analyses we showed a significant derailment of bioenergetic capacity and alterations to transcript and protein abundances. These data, and additional investigations of metabolite levels, will be used for identifying the specific metabolic pathways disrupted in these knock-out cells and determine how the level of citrin expression influences the specific phenotype observed. Future work will investigate whether established nutritional supplements or alternative therapeutic interventions may rescue the phenotypes observed in these cell models.

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P215

Electron transfer in Streptococcus pneumoniae NADPH Oxidase (SpNOX)

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Reactive oxygen species (ROS) arise from the incomplete reduction of O_2 to water and include molecules such as superoxide, hydrogen peroxide, and hydroxyl radicals. Although elevated ROS levels damage cellular macromolecules and lipids, they also play key roles in signaling and, in eukaryotes, function as a first line of microbial defense.[1] In eukaryotic cells, ROS originate predominantly from electron leakage within the electron transport chain, but are also produced deliberately by NADPH oxidases (NOXs).[2] Recently, bacterial homologues of eukaryotic NADPH oxidases have been identified, among which the *Streptococcus pneumoniae* NOX (SpNOX) was particularly interesting as it revealed a conserved architecture consisting of an FAD-containing cytosolic domain connected to a membrane-embedded di-heme module, but lacking the regulatory domains characteristic of eukaryotic NOXs.[3]

Here, we characterize purified SpNOX using multiwavelength steady-state spectroscopic measurements, allowing simultaneous monitoring of NADPH oxidation and the redox states of FAD and the hemes. We find that the hemes remain largely reduced during turnover and that the reaction with oxygen is slow, making electron transfer to oxygen the likely rate-limiting step.

We find that bovine cytochrome c is a significantly more efficient electron acceptor than oxygen, accelerating the reaction rate of the protein upon its addition. In particular, cytochrome c also serves as a good acceptor when SpNOX is reconstituted into liposomes and electrons are transported across the membrane, suggesting that O_2 may not represent the physiological electron acceptor of SpNOX.

Finally, we observed greatly accelerated NADPH oxidation and oxygen consumption when shortchain mena- and ubiquinone analogues were supplied in catalytic quantities. Our data suggest that quinones intercept electron transfer from FAD to the hemes, initiating quinone-mediated redox cycling and superoxide formation without participation of the hemes. This unexpected observation suggests a potential interaction between bacterial NADPH oxidases and environmental redox-active quinones.

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P216

High-resolution in situ structures of plant photosystem supercomplexes

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Abstract

Photosynthesis converts light into chemical energy through the coordinated action of Photosystem I (PSI) and Photosystem II (PSII) within thylakoid membranes. Although structures of isolated photosystems are available, their native organization in chloroplasts remains unknown. Here, using in situ cryo-electron microscopy, we directly imaged rice chloroplasts and determined atomic-resolution structures of photosystem supercomplexes in their native membrane environment. We resolved a $C_2S_2M_2L_4$-type PSII–LHCII supercomplex, including four LHCII antenna trimers not retained in purified preparations. Excitation-energy transfer calculations closely reproduce in vivo measurements, confirming its physiological relevance. We also resolved asymmetric PSII-LHCII dimers and higher-order assemblies, and propose that PSII forms a trans-lumenal and trans-stromal "skeleton" that shapes thylakoid morphology and supports grana stacking. We also obtained structures of PSI–LHCI–LHCII and PSI–LHCI supercomplexes. Together, these structures reveal extensive networks of lipids, pigments, and cofactors, providing the first molecular framework for understanding how the native architecture of plant photosystems supports their exceptional photon-to-electron efficiency.

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Glutamine Transport Regulates Lysine Catabolism and mTOR Signaling in Cancer Cells

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Cancer cells frequently exhibit increased glutamine dependence to sustain anabolic metabolism and growth signaling [1]. The glutamine transporters ASCT2 (SLC1A5) and SNAT1 (SLC38A1) have been implicated in cancer metabolism; however, their functional relationship and contribution to bioenergetic status remains poorly characterized [1,2].
In this study, we investigated the expression, glycosylation status, and functional interplay of glutamine transporters across 12 human cancer cell lines. Western blot analysis identified two ASCT2-immunoreactive bands at approximately 80 and 50 kDa. PNGase-F treatment shifted the upper band to the lower molecular weight species, confirming that the 80 kDa band corresponds to glycosylated ASCT2, whereas the 50 kDa band represents the unglycosylated form.
Comparative analysis across multiple cancer cell lines revealed substantial variability in the proportion of glycosylated ASCT2, suggesting differences in transporter maturation, processing, and membrane trafficking.
To evaluate the bioenergetic profile of the cancer cells, level of lysine catabolism and mTOR signaling in the cells were measured through Western Blot analysis. Unexpectedly, the level of lysine catabolic enzyme, AASS, significantly and negatively correlates with expression of ASCT2; while there was no correlation between ASCT2 level and mTOR signaling output.
To further assess the contribution of glutamine transporters to nutrient-sensing pathways, siRNA-mediated knockdown of ASCT2 and SNAT1 was performed in the ovarian-cancer cell line SKOV3. ASCT2 silencing reduced ASCT2 protein abundance by *circa* 80% and decreased phosphorylation of ribosomal protein S6 (pS6) by approximately 30%, indicating suppression of mTOR signaling. SNAT1 knockdown also reduced ASCT2 protein levels and produced a comparable reduction in pS6, supporting a functional connection between these transporters. However, expression of AASS remains unchanged following ASCT2 or SNAT1 silencing, indicating that the observed signaling effects are unlikely to be mediated through changes in lysine metabolism [3].
Collectively, these findings support a model in which SNAT1 and ASCT2 operate as a coordinated glutamine transport network that contributes to mTOR pathway activation in cancer cells. Whether this is mediated through bioenergetic change or direct regulation of the mTOR pathway remains unelucidated. These results highlight transporter-mediated nutrient sensing as a potentially targetable therapeutic mechanism in cancer.

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P218

Targeting Parkinson-linked complex I deficiency: A small molecule compound enhances mitochondrial respiratory chain function in SH-SY5Y cells

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Parkinson's disease (PD) is currently the fastest-growing neurological disorder worldwide [1, 2], yet no disease-modifying therapies are currently available that can delay, halt, or reverse the disease progression [1, 3]. Previous research has highlighted mitochondrial dysfunction, specifically, quantitative and functional deficiencies of mitochondrial complex I (CI), as a significant factor in the brains and possibly other tissues of individuals with PD [4]. Moreover, recent evidence supports the existence of a possible subtype of idiopathic PD, which accounts for approximately 25% of cases, characterised by severe and widespread neuronal CI deficiency [5], highlighting mitochondrial CI as a relevant therapeutic target. Inspired by these findings, we developed a cell-based screening platform to identify a small molecule that enhances mitochondrial respiratory chain function. Using this approach, we identified Hit1, a small-molecule compound that upregulates mitochondrial complex I and other mitochondrial respiratory chain complexes in SHSY5Y cells. In addition, the Hit1 treatment improves mitochondrial respiration, rescues mitochondrial morphology from rotenone toxicity and triggers mitochondrial biogenesis via SIRT1-mediated activation of PGC-1α. These results suggest that Hit1 improves mitochondrial respiratory chain function and adaptive mitochondrial remodelling in SHSY5Y cells. Although further validation in disease-relevant models is required, Hit1 presents a promising candidate for modulating mitochondrial dysfunction associated with PD-linked CI deficiency.

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P219

Photosynthetic hydrogen production in cyanobacteria

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The cyanobacterium *Synechocystis* sp. PCC 6803 possesses a bidirectional NiFe hydrogenase HoxEFUYH (H_2ase). Fermentative hydrogen (H_2) is produced at the expense of carbohydrate oxidation under anoxic conditions in darkness, whereas photoH_2 production is connected to the photosynthetic electron transport chain and takes place in a transition state when dark adapted cells are illuminated. The bidirectional H_2ase subsequently consumes photoH_2 and feeds electrons back into the photosynthetic electron transport chain. We fused HoxYH and HoxUYH to different photosystem I (PSI) subunits with the aim to enhance photoH_2 production and to prevent its subsequent consumption [1-4]. PSI-H_2ase fusions were characterized *in vivo* and *in vitro* [2, 5]. Electron transfer between PSI and the H_2ase in PSI-H_2ase fusion mutants and and the physiology behind photoH_2 production in PSI-H_2ase fusion mutants and the wildtype will be discussed.

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P220

Characterization of GLUT8 as a Key Lysosomal Hexose Transporter Yunlong Qiu, Albert Suades, David Drew

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As the central degradative hub of the cell, the lysosome breaks down complex glycans into simple sugars. Despite the importance of nutrient recycling, the transporters responsible for exporting these products to the cytoplasm are not fully characterized. Here, we investigate GLUT8, a member of the Solute Carrier 2 (SLC2) family localized to the lysosomal membrane, which mediates the facilitative diffusion of hexoses. A defining feature of GLUT8 is its ability to recognize and transport multiple substrate types despite the structural constraints of the Major Facilitator Superfamily (MFS) fold. Our research aims to explore the molecular mechanism behind this multi-substrate recognition. We propose that GLUT8 utilizes a specialized conformational gating mechanism to achieve its substrate selectivity. Intriguingly, this mechanism is similar to the model observed in the plasmodium falciparum hexose transporter PfHT1, suggesting a conserved structural logic for multi-substrate recognition across different species

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P221

Membrane curvature modulates voltage-sensing domain coupling in the sperm-specific Na^+/H^+ exchanger SLC9C1

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The sperm-specific Na^+/H^+ exchanger SLC9C1 is an unusual voltage-regulated transporter that links membrane hyperpolarization to intracellular alkalinization, cAMP signalling and CatSper-dependent Ca^{2+} entry during sperm motility. Our previous cryo-EM studies of SLC9C1[1] in detergent and nanodiscs revealed a distinctive architecture in which voltage-sensing domains flank the homodimeric transporter core and are connected to cytoplasmic cyclic-nucleotide-binding domains through an unusually long S4 segment. However, how this electromechanical coupling operates in a real membrane environment remains poorly understood.

Here, we reconstituted SLC9C1 into artificial lipid membranes and developed a dedicated cryo-EM dataprocessing workflow to overcome the challenges associated with proteoliposome-embedded membrane proteins. This approach enabled near-atomic-resolution structural determination of SLC9C1 in a membrane environment. Our structures reveal that the voltage-sensing domains are highly sensitive to membrane curvature, undergoing positional and conformational rearrangements relative to the transmembrane dimer. These curvature-dependent changes are propagated to the downstream cyclic-nucleotide-binding domains, which adopt distinct conformations, including states in which the cytoplasmic domains become detached from the transmembrane domains or dissociated from one another. These rearrangements suggest a mechanism by which membrane geometry may relieve the inhibitory constraints imposed on the iontransporting domains and thereby prime SLC9C1 for activation.

Together, our work establishes an artificial membrane-based structural framework for understanding SLC9C1 regulation beyond detergent or flat nanodisc environments. In future experiments, we will impose defined membrane potentials across the artificial membrane system to visualize hyperpolarizationdependent conformational transitions directly. This strategy will allow us to dissect how physiological voltage changes regulate Na^+/H^+ exchange and downstream signaling through coordinated motions of the voltagesensing domains, cyclic-nucleotide-binding domains and transporter core.

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P222

Complex I: An Electrostatic Coulomb Pump Model Similar to Cytochrome c Oxidase

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I will present an extension of complex I multi-barrel electrostatic proton pumping model[1], which is similar in its physical principles to that of cytochrome c oxidase[2]. The model is based on the following:

1. The pump is driven by protonation of QH-; that is, a single protonation (delivering all redox energy of 800mV) drives four proton pumping[1].
2. The proton is coming from the E-channel, transmitting a *negative* charge to the central PT axis.

3. The central PT axis chain is not continuous (at room temperature), but consists of blocks separated by Coulomb gaps[4]. So is the E-channel separated from the central axis by a gap, to prevent immediate transfer of protons to Q-cavity (otherwise redox energy is lost without pumping, shortcircuiting). The negative charge at the “end” of E-channel pulls the protons of the central axis to their (four) proton loading sites, PLS’s. This creates a high-energy state (HES); its relaxation results in proton pumping, due to (assumed) connectivity of PLS’s to the P-site of the membrane.
4. The total energy of 800mV is equally divided in HES between the four pumping blocks due to electrostatic correlation of protons in the central axis.
5. Possible conformational changes originating upon ubiquinone binding - due to a tight bottleneck at the entrance of catalytic cycle, the correlated movement of TM helices in ND1 required to allow entrance of ubiquinone[3], and possible water expulsion – will be discussed. The conformational changes can be transmitted along the membrane arm, presumably to control PT gates; however, the ND5-single-exit model is deemed unlikely, due to the need of at least three PT gates, and complicated mechanics to operate the gates from a distant Q-site.
6. The proposed multi-barrel model is based on kinetic gating, as opposed to mechanical gating, similar to Cco[2].

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P223

A mouse model of ATPβ overexpression causes cell differentiation, lipid biogenesis, and behavioral performance changes

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ATP synthase β subunit (ATPβ) is part of the F1 (enzymatic or soluble complex) of the mitochondrial ATP synthase. F1 functions as an inactivation gate of the ATP synthase c-subunit leak channel. Recent studies, including our own, have shown that ATPβ expression is significantly reduced in Parkinson's disease models (DJ-1 KO mice, fibroblasts from patients carrying DJ-1 mutations), and ATPβ is known to decrease during aging, obesity, and other neurodegenerative conditions[1].

To investigate the physiological role of ATPβ, we generated a conditional LSL-ATPβtdTomato mouse line and crossed it with a β-actin-Cre mice to achieve ubiquitous ATPβ overexpression (ATPβ OE) in the organism. Mitochondrial ATPβ protein levels increased by more than 2-fold in ATPβ OE mice. MEFs (mouse embryonic fibroblasts) derived from ATPβ OE exhibited enhanced mitochondrial function (increased $\Delta\Psi_m$ and cellular ATP production). Comprehensive proteomic and puromycin-based nascent proteome analyses of MEFs revealed significant enrichment of pathways associated with cell differentiation, including skeletal muscle, osteoclast, adipocytes, and Th1 cells, and upregulation of pathways involved in lipid biosynthesis, remodeling, and vesicle transport. Cell cycle progression was downregulated. Consistent with the proteomic findings, Perilipin-2 (PLIN2), a lipid droplet (LD)-associated protein [2], and BODIPY staining were markedly increased (BODIPY in MEFs and PLIN2 immunohistochemistry in the striatum of 6-month-old male mice). This demonstrated that ATPβ increased both the number and size of LDs, promoting neutral lipid accumulation in the brain and MEFs.Whole-body MRI revealed that ATPβ OE mice have reduced body weight compared with WT controls. This reduction was primarily due to decreased lean mass rather than reduced adiposity. Moreover, ATPβ OE mice displayed impaired motor performance, including reduced treadmill endurance and poorer rotarod performance (slower running speeds, shorter running distances). These findings suggest ATPβ OE may alter skeletal muscle development, potentially affecting the balance between oxidative and glycolytic muscle fibers.

Collectively, these findings identify ATPβ as a critical metabolic regulator whose expression must be maintained within an optimal physiological range. Although ATPβ OE enhances mitochondrial bioenergetics at the cellular level, it produced long-term systemic consequences, potentially through stoichiometric imbalance and changes in ATP synthesis efficiency. ATPβ-induced LD accumulation may be a marker for cell differentiation which may have benefits in developmental and degenerative disease. However, excessive, or dysregulated muscle differentiation may impair plasticity and repair during normal muscle use. These results suggest that precise temporal and tissue-specific modulation of ATPβ may represent a more effective therapeutic strategy for metabolic and neurodegenerative disorders.

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P224

Unfolding Without Inactivation: Probing the Link Between Global Stability and Redox Cofactor Integrity in CpI

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CpI, one of three [FeFe]-hydrogenases from the bacterium Clostridium pasteurianum, is among the most catalytically active and well-studied hydrogenases [1]. The enzyme contains several redox-active cofactors, including iron-sulfur (FeS) clusters that mediate electron transfer and a unique CO- and CN^--ligated diiron "H-cluster", where H_2 interconversion takes place. These cofactors, however, render the enzyme highly oxygen-sensitive and cause the protein to degrade readily under non-native conditions.

Here, we apply a semi-rational protein design approach to engineer a more thermostable CpI by rigidifying surface-exposed secondary structure elements. The resulting variants, containing up to 26 mutations, were evaluated for changes in thermostability and H_2-evolution activity. Despite an apparent thermostability (T_m-value by SYPRO differential scanning fluorimetry [2]) reduced by more than 10°C relative to wildtype CpI ($T_{m,WT}$ = 55°C), the "standard" H_2-evolution activity at 37°C remained largely unaffected after extended heat exposure of all variants to temperatures up to 65°C. Activity was at least partially recovered upon return to 37°C, consistent with a reversible — rather than purely degradative — unfolding of the enzyme.

The direct effect of temperature on proton-reduction activity itself could not yet be unambiguously resolved, owing to chemical side reactions from the assay beyond standard conditions. Nonetheless, control experiments indicate that the integrity of the metal-cofactor environment, specifically cysteine-residues involved in FeS-cluster ligation, responsible for catalysis is largely unaffected by partial unfolding of the surrounding protein scaffold.

Together, these results suggest that global structural unfolding, as detected by SYPRO-DSF, and catalytic inactivation are not necessarily the same event: a substantially destabilized protein scaffold can still harbor a functionally intact redox-cofactor system. This decoupling has practical implications for thermostability engineering of metalloenzymes such as [FeFe]-hydrogenases, as T_m-value alone may underestimate the operational robustness of a variant under process-relevant conditions.

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P225

ADP-inhibition of ATP synthase as a membrane-potential stabilizer?

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F_OF_1-ATP synthase is usually viewed as a reversible molecular machine that converts the proton-motive force into ATP, or, under de-energized conditions, hydrolyses ATP to pump protons. The latter activity is prone to non-competitive inhibition by ADP[1]. This ADP-inhibited state is commonly interpreted as a protective mechanism that prevents wasteful ATP hydrolysis when the membrane is de-energized. Here I propose a different, hypothetical interpretation: ADP inhibition may allow ATP synthase to function as a voltage-dependent buffer of the membrane electric potential difference (□□).

The hypothesis is based on two established features of F_OF_1-regulation. First, ADP inhibition occurs at low proton-motive force and is relieved by energization of the membrane. Second, reactivation of the ADP-inhibited enzyme requires a proton-motive force higher than that required thermodynamically for ATP synthesis. This creates a threshold-like behavior. When primary proton-motive force generators, such as respiratory or photosynthetic electron-transfer chains, are weakly active, most ATP synthases remain inhibited and do not consume □□. When generator activity increases and □□ rises above the activation threshold, a fraction of ATP synthases becomes active, starts ATP synthesis, and imposes an additional proton conductance on the membrane. This should limit further growth of the potential and convert changes in generator activity into changes in the active fraction of ATP synthase rather than into large voltage fluctuations.

A minimal kinetic model considering only the electrical component of the proton-motive force confirms that ADP-inhibition can indeed stabilize □□ upon significant changes in the activity of the primary proton-motive force generators. The model is not intended as proof of an in vivo mechanism, but as a test of physical plausibility and a source of experimentally testable predictions. I suggest that ADP-inhibition and other regulatory mechanisms that inhibit the ATPase activity upon de-energization may represent a general regulatory principle contributing to membrane potential stabilization in bacteria, chloroplasts, and mitochondria.

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P226

Mechanistic insights into UCP1 inhibition by nucleotides

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The mitochondrial uncoupling protein 1 (UCP1) plays an important role in thermogenesis in brown adipose tissue. It does so by mediating proton leakage across the inner mitochondrial membrane, thereby dissipating the proton gradient generated by aerobic respiration as heat rather than for ATP production. Although structural and experimental studies have established that nucleotides inhibit UCP1-mediated proton transport, the detailed molecular basis of this inhibition remains poorly understood.

Here, we use atomistic molecular dynamics simulations to investigate the mechanism of nucleotide inhibition in UCP1. We identify local structural changes in a transmembrane helix that may be relevant to inhibition. Furthermore, by analyzing protein–nucleotide interactions and the intrinsic chemical differences between adenine and guanine nucleotides, we suggest a plausible molecular explanation for the differential responses of human and rodent UCP1 to these nucleotides, as recently identified in our laboratory.

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Oxidative stress-induced upregulation of riboflavin transporter 1 (*SLC52A1*) in human SH-SY5Y neuronal cell model: putative role of ATF4 and NRF2 in its transcriptional regulation

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Introduction/aims:
Mitochondrial bioenergetics critically depends on the availability of flavin cofactors FMN and FAD, derived from riboflavin (Rf, vitamin B2). In human cells, Rf is imported by three plasma membrane transporters — RFVT1, RFVT2, and RFVT3, encoded by *SLC52A1, SLC52A2* and *SLC52A3*, respectively. Then Rf is sequentially converted to FMN and FAD by riboflavin kinase (RFK, EC 2.7.1.26) and FAD synthase (FADS EC 2.7.7.2). FAD is the essential redox cofactor for mitochondrial flavoenzymes and some antioxidant enzymes.
Given the high energetic demand of neurons, flavin cofactor availability is particularly critical for neuronal function and survival. Consistently, a rare neurodegenerative disorder characterized by mitochondrial dysfunction, oxidative stress, and UPR activation is linked to pathogenic variants of *SLC52A2* and *SLC52A3 [1, 2]*. Oxidative stress and proteostasis dysregulation are also established hallmarks of common neurodegenerative diseases, including Alzheimer's disease (AD) [3].
We postulated here that alteration in Rf transporter expression may occur more broadly in neurodegeneration, and we investigated the transcriptional regulation of the Rf uptake machinery in human SH-SY5Y neuroblastoma cells under oxidative stress, as a neuronal model relevant to AD.

Methods:
Human neuroblastoma cell line (SH-SY5Y) was treated with increasing concentrations of H_2O_2 as an acute oxidative stress model. mRNA (RT-qPCR) and protein (Western blot) levels of Rf pathway components and stress-responsive transcription factors were analyzed.

Results:
H_2O_2 treatment induced a significant upregulation of *SLC52A1* (RFVT1) mRNA, alongside no significant changes in *SLC52A2* (RFVT2). Concomitantly, *ATF4* and *NFE2L2* mRNA levels doubled in response to the treatment. Consistent with a direct transcriptional link, *in silico* promoter analysis using JASPAR2026 identified the presence of putative CARE and ARE sequences within a region 2 kb in the upstream region of *SLC52A1* but not *SLC52A2*.

Conclusions:
Our data demonstrate that oxidative stress induces a differential transcriptional response of Rf transporters in human neuronal cells, possibly orchestrated by mediators of the integrated stress response. These findings suggest that dysregulation of Rf transporter expression under oxidative stress may represent a novel contributor to metabolic impairment in Alzheimer's disease and other neurodegenerative conditions, characterized by chronic oxidative stress and bioenergetic failure, warranting further investigation.

Fundings:
PNRR M6C2 – AGAINST AD2 - AddressinG brAIN choleSterol meTabolism in Alzheimer's Disease to identify novel disease biomarkers”

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P228

Towards a computational characterization of proton coupling in XylE C. Alleva[a], M. Bonaccorsit[a], L. Delemotte[b] and D. Drew[a] [a]*Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.* [b]*Department of Applied Physics, SciLifeLab, KTH Royal Institute of Technology, Stockholm, Sweden.*

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Sugar transporters have the fundamental role to take up energy into cells in the form of glucose and other hexoses; they belong to the major facilitator superfamily (MFS) and follow a rocker-switch alternating access model of transport. XylE, an E. coli homolog, has been used as a model to study functional characteristics of GLUTs. Yet, in contrast to most of the GLUTs, XylE is a proton-coupled sugar transporter. Previous studies [1,2] have suggested that substitution of one aspartate residue (D27) with an asparagine might be responsible for the difference in coupling. However, the presence of the same aspartate in GLUT2, despite it not being coupled to proton uptake, suggests that the coupling could involve more than simply this residue. Intriguingly, this aspartate belongs to a network of three protonatable residues, one aspartate, one glutamate and one arginine. We speculate that the proton coupling is achieved through an interplay of these residues which could all participate in the proton coupling process.
Our unbiased MD simulations suggest that the stability of different states along the translocation pathway is controlled by the protonation state of this central triad.
Here, we use constant pH simulations [3] in order to characterize the interplay between different residues, allowing for their protonation state to change dynamically.
Unexpectedly, the most stable protonation state differs from the one suggested in resolved structures[1]; this suggests that we do not yet grasp the tight coupling between protonation events and protein dynamics.
We then use enhanced sampling techniques [4,5] to characterize the protein dynamics in different conformational states.
Combining the two approaches, we strive to study the interplay between the state of all protonatable residues and progression along the transport cycle.Finally, with this combined approach we suggest a mechanism for the glucose inhibitory activity on XylE [6] that takes into account protein dynamics.

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P229

Dynamics of Cytosolic and Mitochondrial ATP Pools in baker's yeast

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Adenosine 5′-triphosphate (ATP) serves as the universal energy currency in all living cells. In the budding yeast *Saccharomyces cerevisiae*, ATP is generated either in the cytosol through glycolysis or in the mitochondrial matrix via oxidative phosphorylation. Although the pathways of ATP biosynthesis have been extensively characterized and its central role in cellular energetics is well established, the dynamics of ATP concentrations in the cytosol and, in particular, within the mitochondrial matrix remain poorly understood.

In this study, ATP dynamics were monitored in living *S. cerevisiae* cells using genetically encoded FRETbased fluorescent ATP sensors (yATeam)[1] targeted either to the cytosol or to the mitochondrial matrix. We investigated how cytosolic and mitochondrial ATP pools respond to the addition of substrates and inhibitors of glycolysis and oxidative phosphorylation, and protonophores.

We found that, in wild-type yeast cells, glucose addition did not alter cytosolic ATP pool but markedly increased ATP level in mitochondrial matrix. Unexpectedly, subsequent addition of protonophore resulted in a further increase in mitochondrial ATP concentration. In contrast, cells lacking the mitochondrial ATP/ADP translocator exhibited the opposite response: glucose addition increased cytosolic ATP levels, while ATP concentration in the mitochondrial matrix remained unchanged, including after the addition of uncouplers. These findings demonstrate the remarkable stability of the cytosolic ATP pool and indicate that ATP/ADP exchange between the cytosol and the mitochondrial matrix plays a critical role in maintaining this homeostasis. In *rho*^{0} cells lacking mitochondrial DNA, both cytosolic and mitochondrial ATP pools exhibited more pronounced responses to the tested compounds, further supporting the conclusion that functional mitochondria are essential for stabilizing cytosolic ATP levels.

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

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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.

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Hypoxia and Reoxygenation Induce Higher-Order Respiratory Supercomplex Assembly in Cardiac Mitochondria

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The myocardium relies heavily on mitochondrial stress responses to sustain continuous contractile function and adapt to increases in cardiac output during both mild and severe stress1 . Prolonged exercise confers beneficial mitochondrial adaptations that improve cardiac resilience to more substantial stressors2. Recently, exercise training and genetic manipulation of CIII demonstrated formation of a megacomplex consisting of CI_2+$CIII_2$ (SC-XL) that conferred metabolic advantages associated with endurance training and recovery of cardiovascular function following ischemia/reperfusion (IR)3. Further, hypobaric hypoxia induced supercomplexes (SCs) in the heart acutely, suggesting hypoxia-driven SC assembly reflects immediate metabolic demand of the myocardium4. It remains unknown whether the acute absence and reintroduction of oxygen directly influence ETC protein-protein interactions in isolated cardiac mitochondria, and whether such exposure confers resilience to calcium overload.

Mitochondria isolated from C57BL/6 mouse hearts were stimulated to consume O2 with ADP and Pi in a sealed chamber, reducing pO2 to hypoxic levels. Samples aspirated following either 20’ or 5’ hypoxia (20’H, 5’H) and reoxygenation (15’R, 30’R) were immediately pelleted. Blue Native Gel Electrophoresis determined ETC arrangements. Ca2+-induced swelling was measured by absorbance at 540 nm.

A single cycle of either 20’H or 5’H, followed by 30’R, induced acute rearrangements of ETC complexes in isolated cardiac mitochondria. Increased band density in the HMW region of BN-PAGE gels, consistent with SC-XL, was observed during both hypoxic intervals and continued to increase throughout 15’R and 30’R. These increases occurred relative to total protein, total SCs, and respirasomes (CI+CIII2+CIVn). Reoxygenation following either interval induced significant elevations in free CI relative to overall SC expression. Dimers of CV (CV2) decreased following 20’H/30’R, while 5’H/30’R did not induce rearrangements of CV relative to baseline. Calcium- induced swelling was prevented in all 5’H/30’R mitochondria, while 20’H/30’R did not confer the same protection.

We demonstrate that hypoxia and reoxygenation drive rapid rearrangements of ETC protein-protein interactions in isolated cardiac mitochondria independently of extramitochondrial signaling. Strikingly, SC-XL induction appears to represent an initial, conserved mitochondrial stress response to low-oxygen, regardless of hypoxic duration. These rearrangements occur independently of functional outcome, evidenced by divergent calcium-handling responses between groups. Reduction of CV2 following 20’H may indicate a propensity for irreversible permeability when subjected to Ca2+ overload5–7. Conversely, preserved CV2 during 5’H/30’R may suggest transient permeability associated with ischemic preconditioning8,9. Future studies will examine whether hypoxia-driven SC remodeling is similarly inducible in skeletal muscle or represents a myocardial-specific adaptation to acute stress.

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Compartmentalized Oxygen Generation from Hydrogen Peroxide Enables Anaerobic Oxidative Phosphorylation

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In the healthy mammalian gut, the small amount of oxygen diffusing from the blood into the colon is rapidly consumed by colonocytes during fatty acid oxidation, maintaining a strictly anaerobic lumen. Disruption of the gut microbiome by antibiotics or immune responses can increase oxygen availability, promoting the expansion of facultative anaerobes such as pathogenic Salmonella and E. coli, which contributes to dysbiosis and inflammatory bowel disease. Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) produced by many eukaryotic organisms as part of the immune defense against bacterial invasion. Recent findings show that facultative anaerobes can use host derived ROS as a source of oxygen, enabling oxidative phosphorylation even under anoxic conditions.[1] E. coli in particular is suggested to derive oxygen from H_2O_2 by catalase, which is then used by the bd-II oxidase as terminal electron acceptor.[2] Here, we developed a minimal bottom-up system to investigate how H_2O_2 dependent respiration of E. coli supports ATP synthesis under anaerobic conditions. Purified E. coli bd-I, bd-II, and bo_3 quinol oxidases were co-reconstituted with F1FO ATP synthase into small unilamellar vesicles (SUVs), while catalase was encapsulated within the vesicle lumen. Using a Clark-type oxygen electrode, we demonstrate that catalase-generated oxygen inside the liposomes is rapidly consumed by terminal oxidases fueled by ubiquinol oxidation, while maintaining anaerobic bulk conditions. The resulting proton motive force efficiently drives ATP synthesis even at low H_2O_2 concentrations. Importantly, ATP production required catalase encapsulation within the proteoliposomes, highlighting the necessity of localized oxygen generation for the coupling to respiration. Replacement of short-chain quinols with membrane embedded ubiquinone Q10 and glycerol-3-phosphate dehydrogenase (GlpD) confirmed that the system also functions with more native respiratory substrates, albeit with lower efficiency. Together, these results demonstrate that compartmentalized peroxide to oxygen conversion coupled to respiratory oxygen consumption constitutes an efficient strategy by which facultative anaerobes may exploit host derived peroxide under oxygen limited conditions and the developed proteoliposome platform further expands the synthetic biology toolbox for controlled ATP generation in minimal systems.

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Longitudinal quantification of ATP-related metabolic fluxes in single Saccharomyces cerevisiae cells

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Living systems harvest free energy from the environment and convert it into forms that power essential cellular processes. Although the biochemical network underlying energy metabolism has been extensively characterized, metabolic activity at the single-cell level remains poorly understood[1]. In particular, it is unclear to what extent metabolic fluxes can be measured longitudinally in individual cells. Metabolic activity can be defined as the sum of the product of reaction energy and reaction flux. Focusing on ATP-producing pathways, reaction energies are determined by the ATP/ADP ratio (phosphorylation potential), and key fluxes include glycolysis, the citric acid cycle, and oxidative phosphorylation. Longitudinal measurements are especially important in systems where genetically identical cells follow distinct trajectories, e.g. during development or in isogenic microbial populations[2,3]. Using Saccharomyces cerevisiae as a model organism, we investigate whether metabolic fluxes can be measured non-destructively at the single-cell level and which fluxes are experimentally accessible under these constraints.

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Testing the Heat Pump Hypothesis: An Experimental Strategy to Resolve the Hot Mitochondria Paradox

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Temperature is a fundamental parameter influencing virtually all biological processes, including gene expression, enzymatic activity, and membrane properties[1,2,3]. In endotherms, heat primarily arises as a by-product of metabolic reactions and is essential for maintaining constant body temperature [4,5]. While heat distribution and temperature gradients at the tissue level are well studied, these principles remain largely unexplored at the single-cell or subcellular scale. From a thermodynamic perspective, heat should be evenly distributed within the small volume of a cell. However, several studies have reported temperature gradients of up to several degrees, with mitochondria—the core metabolic organelles—as the proposed cellular "hot spots"[6,7] . Such findings, if correct, would fundamentally challenge our understanding of metabolic function and subcellular heat distribution, yet they remain highly controversial[8] .

We propose a novel thermodynamic model—the heat pump hypothesis—which posits that membrane proteins, such as respiratory chain complexes, actively transport heat across mitochondrial membranes, thereby generating substantial intracellular gradients. To test this, we developed a minimal artificial cellular system of liposomes with long-term, temperature-independent stability. Using encapsulation and in-liposome gene expression, we characterise the sensitivity of organic and protein-based sensors to temperature and other confounding factors.

Once nanothermometry is established in liposomes, we will create proteoliposomes, incorporating functional mitochondrial membrane proteins to directly assess their role in heat-gradient generation. In parallel, explorative studies using primary immortalized brown adipocytes have been initiated as an adaptable in vitro platform to validate findings from liposome studies.

In sum, our dual liposome–cell model approach provides the first comprehensive framework to rigorously test intracellular heat transport, with the potential to redefine the thermodynamic principles underlying cellular metabolism.

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Title: Bioluminescence as a possible light source for marine photosynthesis during the polar night

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The polar night poses a unique challenge to marine primary producers as prolonged darkness minimises photosynthetic activity. Recent data from the MOSAiC expedition and the mesopelagic ocean however suggest that phytoplankton can thrive at light levels close to the theoretical minimum of photosynthesis. Those light levels might already be reached through bioluminescence, naturally occurring not only in the mesopelagic but also during winter in polar oceans. Thus, our study addresses the question as to whether bioluminescence can act as a light source to help sustain biomass of phytoplankton during the polar winter. To test this, we developed an LED system to simulate bioluminescence using in-situ data. Our results show that under low, flashing light (< 1-10 µmol photons m−2 s−1), phytoplankton exhibit higher cell numbers and increasing photochemical efficiency compared with constant light, providing first insights into the role of bioluminescence in supporting photosynthetic marine organisms during the polar night.

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Vibrational spectroscopy of fluorous systems

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Abstract:

Per- and polyfluoroalkyl substances (PFAS) are widespread environmental pollutants that are highly persistent and resistant to degradation. Among them, long-chain perfluorocarboxylic acids, such as perfluorooctanoic acid (PFOA), are of particular concern because of their tendency to bioaccumulate and their adverse effects on human health. These properties are believed to originate from the unique omniphobic nature of perfluoroalkyl chains, which repel both water and lipids. In this work, we investigate the interactions of fluorinated molecules with their surroundings by combining Fourier-transform infrared (FT-IR) spectroscopy with density functional theory (DFT) and molecular dynamics (MD) simulations. First, we assess the suitability of C–F stretching vibrations as probes of local electric fields. We then study the structural organization of fluorinated molecules in self-assembled monolayers and the arrangement of interfacial water at fluorous surfaces using surface-enhanced infrared absorption spectroscopy (SEIRAS). Our findings reveal that perfluoroalkyl chains containing more than seven CF_2 units exhibit enhanced molecular ordering and that hydrophobic fluorous interfaces promote a distinct orientation of dangling water molecules.