Cell type-resolved proteome of mitochondria dysfunction

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Mitochondrial diseases present with diverse clinical manifestations that can affect multiple organs across all ages. What makes these diseases particularly challenging is that patients carrying the very same mutation can show completely different trajectories (e.g., some facing organ failure and others presenting only mild symptoms). This variability reflects an unresolved aspect of these disorders: certain cell types adapt and remain resilient to impaired mitochondrial metabolism, while others fail and drive pathology. To examine cell-type-specificity in mitochondrial dysfunction, we are building a spatial and single-cell proteomics pipeline designed to deliver cell-type-resolved protein data across multiple tissues simultaneously. Using a validated mouse model carrying asymmetric heteroplasmy in mt-tRNAAla, Deep Visual Proteomics (DVP) will be applied across 20 tissues and 30 cell types. Tissue microarrays (TMA) are used to guarantee simultaneous analysis of multiple tissues on a single slide, and multiplexed imaging allows precise cell-type identification. Mass spectrometry analysis was performed using an Orbitrap Astral coupled with an Evosep ENO system. Our preliminary results show that TMA scales up the original DVP workflow, supporting simultaneous proteome profiling at single-cell resolution. Across different tissues, the pipeline identified over 3,000 proteins per cell type. Together, TMA and DVP enable body-wide proteome mapping in a single workflow. Given its spatial resolution and proteome coverage, this approach represents a valuable tool for understanding why mitochondrial dysfunction affects some cell types and spares others.

Integrating MALDI-MSI and spatial transcriptomics reveals peptide-specific plaque microenvironments in Alzheimer's disease

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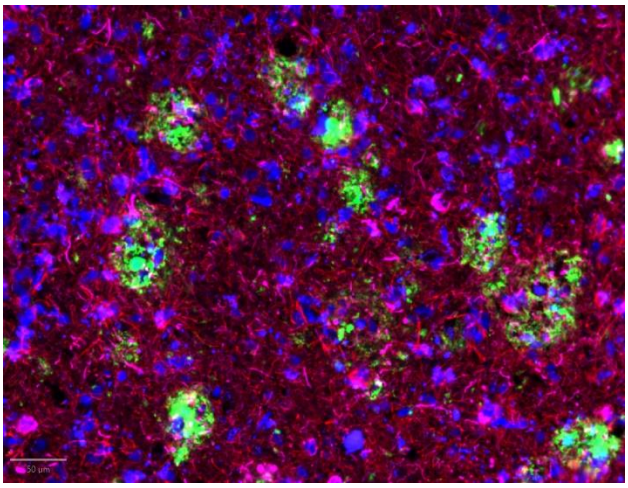
Alzheimer's disease is characterized by heterogeneous amyloid-β (Aβ) plaque morphologies and peptide compositions, but whether distinct plaque chemistries are associated with specific local transcriptional programs in human brain tissue remains unknown. Here, we integrated MALDI mass spectrometry imaging with GeoMx spatial transcriptomics to link plaque-associated Aβ peptide burden with spatial gene expression programs in Alzheimer's disease cortical tissue.

Fresh-frozen human cortical sections were analyzed by MALDI mass spectrometry imaging to map Aβ peptide species directly in tissue. MALDI-MSI enabled label-free detection of multiple plaque-associated peptides, including full-length, N-terminally truncated, and pyroglutamate-modified Aβ species. Peptide ion images were used to assess Aβ distributions across cored plaques, coarse-grained plaques, and cerebral amyloid angiopathy. Adjacent sections were immunostained for amyloid pathology and cell-state markers, followed by whole-transcriptome spatial profiling of plaque-associated regions, enabling comparison of local peptide composition with transcriptional programs in corresponding microenvironments.

Differences in peptide-associated transcriptional signatures were detected across plaque morphologies. In cored plaques, Aβ1–42-associated signatures involved synaptic markers including SYT1, CPLX1, DLGAP3, and SCG2, alongside axonal cytoskeleton and stress-response genes such as NEFL, NEFM, MAP2, HSPA1A/B, and HSP90AA1/AB1. Within the same plaque class, Aβ3pE–42 captured a more restricted program involving HAPLN4 and SPARC. In coarse-grained plaques, Aβ1–40-associated signatures pointed toward barrier/glial and metabolic programs, represented by AQP4, CLDN5, S100B, APOC1, C1QA, and DLAT. In cerebral amyloid angiopathy, Aβ11pE–40-associated signatures reflected vascular remodeling, proteostasis/RNA-processing stress, and neuronal–glial perturbation.

Together, these results link amyloid peptide chemistry to divergent neuronal, glial, stress-response, and vascular plaque microenvironments in human Alzheimer's disease.

Figure 1. Example of spatial transcriptomics design



Representative immunofluorescence image of Alzheimer's disease cortical tissue used for spatial transcriptomic profiling. Tissue sections were stained for nuclei/SYTO13, amyloid-β plaques, phosphorylated tau at pTau217, and GFAP-positive astrocytes. This multiplex staining enabled identification of plaque-associated microenvironments and selection of regions of interest for downstream GeoMx whole-transcriptome profiling. The image illustrates the spatial relationship between amyloid deposits, tau pathology, astroglial response, and surrounding cellular architecture used to guide plaque-focused transcriptomic analysis.

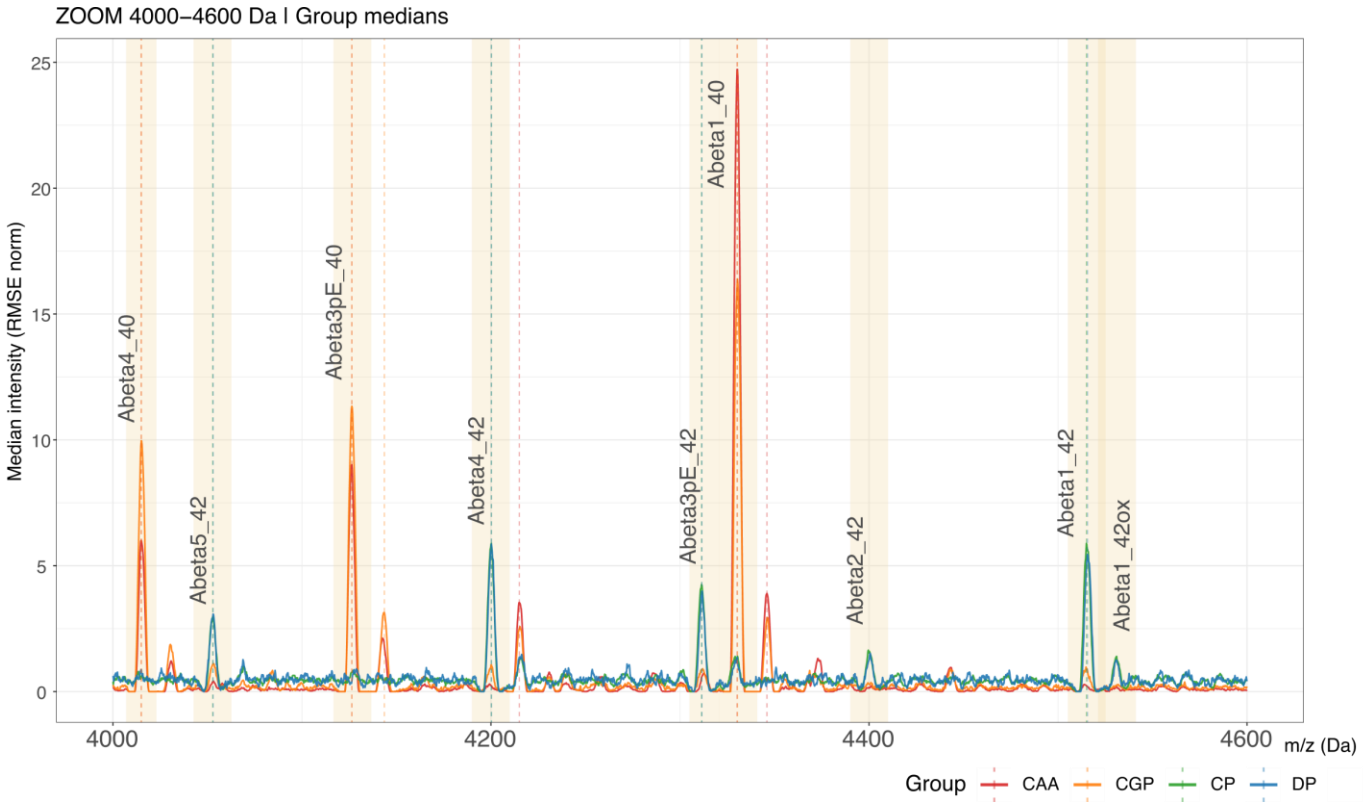


Figure 2. MALDI-MSI-based Aβ peptide profiling

Representative MALDI-MSI Aβ peptide profiles showing differences in peptide abundance across plaque classes in Alzheimer's disease cortical tissue. Annotated peaks correspond to multiple Aβ species, including full-length, truncated, and pyroglutamate-modified peptides such as Aβ1–40, Aβ1–42, Aβ3pE–40, Aβ3pE–42, Aβ4–40, Aβ5–42, and Aβ11–40. Differences in peak intensities between plaque classes indicate plaque-type-specific variation in Aβ peptide composition, providing a molecular basis for linking plaque chemistry to spatial transcriptomic signatures in corresponding plaque microenvironments.

Functional proteomics in representative species of the human gut microbiome

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Changes in the gut microbiome community composition are linked to different diseases. Yet, the poor annotation of many of proteins of gut microbiome species makes it difficult to uncover the molecular mechanisms driving disease or promoting health. In this project, we employed a high-throughput proteomics approach using a panel of 96 drugs to perturb the proteome of 30 representative gut bacterial species. Regardless of their class, drugs previously reported to induce broad-spectrum growth inhibition responses also induced the largest proteomic changes. Specifically, we observed higher abundance of proteins of central carbon metabolism in Phocaeicola vulgatus treated with the antipsychotic loxapine. In parallel, levels of glycolysis metabolites were decreased in the same condition. Different evolution experiments conducted by prolonged exposure to loxapine resulted in the isolation of resistant bacteria, and we confirmed a base deletion in all mutants by whole genome sequencing. Notably, protein and metabolite abundance changes showed high correlation between loxapine and the antihistamine promethazine, suggesting a similar mechanism of action of both drugs in P. vulgatus. Our approach provides a resource for future investigation of drugs from diverse classes reported to influence gut bacterial species but with unknown targets. Altogether, this information is key to uncover the function of the proteome of these largely uncharacterized species and shift microbiome studies towards a mechanistic understanding of disease.

Phylum-wide structural diversity of Bacteroidota O-glycans

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The unique and species-specific protein O-glycosylation in the Bacteroidota phylum remains poorly characterized, and this study investigates the diversity in glycan structures and biosynthetic pathways underlying this modification across the whole phylum.

Bottom-up global proteomics without glycopeptide enrichment was performed on over 100 Bacteroidota strains to determine their monosaccharide composition using nanoflow liquid chromatography coupled to a Thermo Fisher Orbitrap Exploris 480 mass spectrometer with a stepped HCD method. The data was analysed using open search strategies and the diagnostic ion mapping feature of MSFragger. In addition, glycans were purified and their stereochemistry and linkages were determined by NMR. To identify the biosynthetic pathways of these glycans, sequence- and structure-based protein alignment tools were used to identify loci encoding glycosylation-related genes (LFGs), followed by deletion of putative glycosyltransferase genes and determination of glycan structure by proteomics analysis.

Glycan monosaccharide compositions revealed significant structural diversity across the phylum. For selected species, complete glycan structures including stereochemistry and linkages were resolved. LFGs were identified in the analysed Bacteroides and Phocaeicola species, and integration of the monosaccharide composition data allowed the assignment of putative functions to LFG genes. The predicted functions of several LFG glycosyltransferases were confirmed by deletion of the respective genes.

A simple mass spectrometry-based glycoproteomics method enables determination of O-glycan composition across the Bacteroidota phylum without glycoprotein enrichment, revealing diverse species-specific glycan structures. Integration of monosaccharide data with bioinformatic LFG analysis allows functional assignment of biosynthesis genes responsible for this modification.

Proteomic profiling of bone-metastatic prostate cancer uncovers potential drug targets for patients with especially poor outcomes

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Prostate cancer (PC) is a common, multifocal and unpredictable disease and a major cause of cancer-related mortality worldwide. The most prevalent site of distant metastasis in PC is bone. Bone metastases (bmPC) can be classified into molecular subgroups (MetA/MetB/MetC) based on transcriptomic profiles discovered in our lab. The subgroups differ in androgen receptor (AR) activity, cell cycle activity, and stromal response. MetB, with low AR activity and high proliferation, has the poorest prognosis following androgen deprivation therapy (ADT). New treatment strategies are needed for bmPC in general, and for MetB in particular. To further understand the molecular background of bmPC, we examined proteomic profiles using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a data-independent acquisition (DIA) approach. In total, the profiles of 7,735 proteins from 153 samples obtained from 120 unique patients (hormone-naïve (n=18), short-term castrated (n=11) and CRPC (n=91)) were analyzed in relation to MetA-C. The protein profiles were in consent with the transcriptomic profiles, showing AR and cell cycle activity heterogeneity between the subgroups. We further identified potential therapeutic target proteins for MetB, the relevance of which will be evaluated in PC cell lines and/or patient-derived organoids.

Deep spatial proteomic profiling of the hippocampal tangle proteome in Alzheimer's disease

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Introduction:

Neurofibrillary tangles composed of hyperphosphorylated tau are a central pathological hallmark of Alzheimer's disease (AD). However, the precise protein composition of individual tangles and the molecular alterations within tangle-bearing neurons remain incompletely understood. We applied spatially resolved mass spectrometry-based proteomics to comprehensively characterize the proteome of isolated tau tangles from human AD hippocampus.

Methods:

We performed MS-based proteomics on laser-capture microdissected tangles from frozen hippocampal sections of AD patients (n=10). Tau tangles were visualized using AT8 immunostaining, and 50 tangles per patient were isolated and pooled together with area-matched adjacent non-tangle tissue (three replicates per patient). Tryptic peptides were analyzed by LC-MS/MS on a high-resolution Orbitrap Eclipse mass spectrometer. Protein abundance differences between tangles and adjacent tissue were assessed using linear models.

Main results:

We reproducibly quantified ~1,500 proteins per 50 tangles (median CV=25%). Tau was strongly enriched in tangles compared with adjacent tissue (FC=4.5, padj<0.0001). Phosphorylated tau peptides at T181, T217, S262, and S404 showed 3-10-fold higher abundance in tangles (padj<0.0001), confirming marked tau hyperphosphorylation. In addition, proteins involved in protein degradation, including p62 and ubiquitin, as well as proteins linked to mRNA processing, were significantly enriched in tangles. Proteins reduced in tangle-bearing neurons were predominantly associated with extracellular matrix organization (e.g., LAMC1). Immunohistochemical validation is ongoing.

This spatial proteomics study provides an in-depth characterization of the AD tangle proteome from minute sample amounts, highlighting quantitative enrichment of specific phospho-tau species and implicating protein degradation, RNA processing, and extracellular matrix alterations as key processes in tangle-bearing neurons.

Mitochondrial ATP5IF1 Depletion Define the Proteomic Landscape of Acral Lentiginous Melanoma

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[2] UNAM, [3] Lund University, [4] National Autonomous University of México, [5] 2- Department of Translational Medicine, Lund University, Sweden

Acral lentiginous melanoma remains biologically undercharacterized despite its distinct clinical behavior and its frequent association with diagnostic delay and outcome disparities. We integrated AI-driven digital pathology with deep proteomics of routine FFPE melanoma sections to resolve subtype-specific proteomic states within their histologic context.

Primary melanomas including acral lentiginous, superficial spreading, nodular, and lentigo maligna subtypes were analyzed using an integrated pipeline combining AI-assisted digital pathology and high-throughput acoustic FFPE proteomics. Proteomic sections were bracketed by H&E slides and annotated in QuPath to generate composition-resolved maps of tumor, stromal and immune infiltration. Samples were processed using an optimized 96-well acoustic workflow and analyzed by data-independent acquisition mass spectrometry.

Integrated quantitative proteomics and computational digital pathology revealed that acral lentiginous melanoma defines a coherent and biologically distinct molecular state.

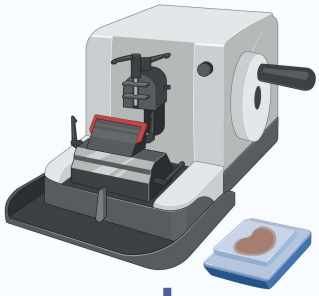
Acral tumors showed coordinated enrichment of translation and ribosome-associated pathways together with extracellular matrix and adhesion programs, while lipid metabolism, fatty acid biosynthesis, and peroxisomal pathways were consistently depleted, indicating systematic metabolic reprogramming.

Most strikingly, ATP5IF1 emerged as a selectively and consistently depleted factor in this subtype and remained significant after normalization for overall mitochondrial content and Complex V abundance, excluding global mitochondrial loss as an explanation. ATP5IF1 depletion coincided with a relative shift toward glycolytic protein representation, indicating a precise and subtype-specific stoichiometric remodeling of ATP synthase regulation.

Our novel data establishes acral lentiginous melanoma as a proteomically distinct entity characterized by translational activation, matrix remodeling, and mitochondria-centered metabolic rewiring.

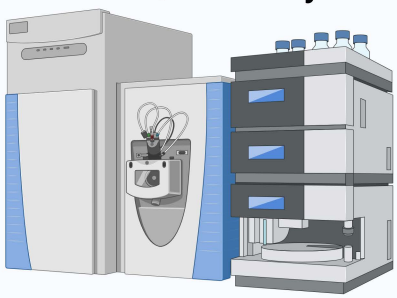
Melanoma Sample processing

2 slides FFPE
4 µm

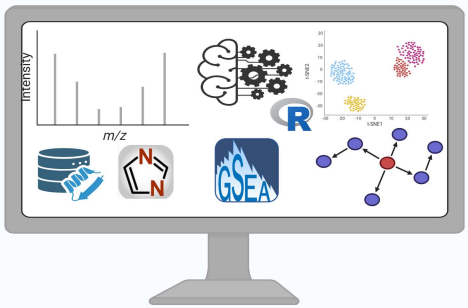


1- 4

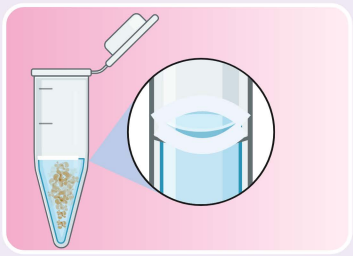
LC-MS/MS Analysis



Data Analysis

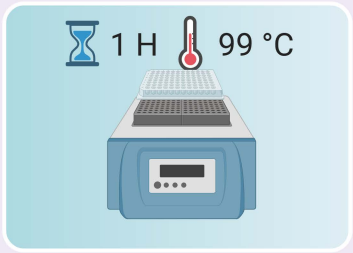


Process optimization



1

Deparaffinization

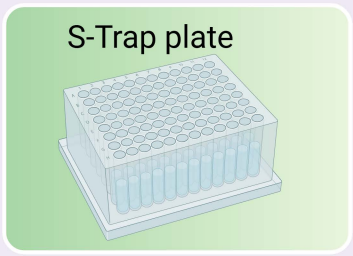


2

Decrosslinking

3

Homogenization



4

Trypsin digestion

cftr modulator therapy reshapes airway inflammation in cystic fibrosis: insights from proteomics and machine learning

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Background

For patients with cystic fibrosis (pwCF), CFTR modulators, including Elexacaftor/Tezacaftor/Ivacaftor (ETI), have remarkably improved lung function and quality of life. However, the molecular signature of airway inflammation in CF remains incompletely understood. This study aimed to characterise longitudinal changes in airway inflammation and sputum proteomes following ETI treatment.

Patients and methods

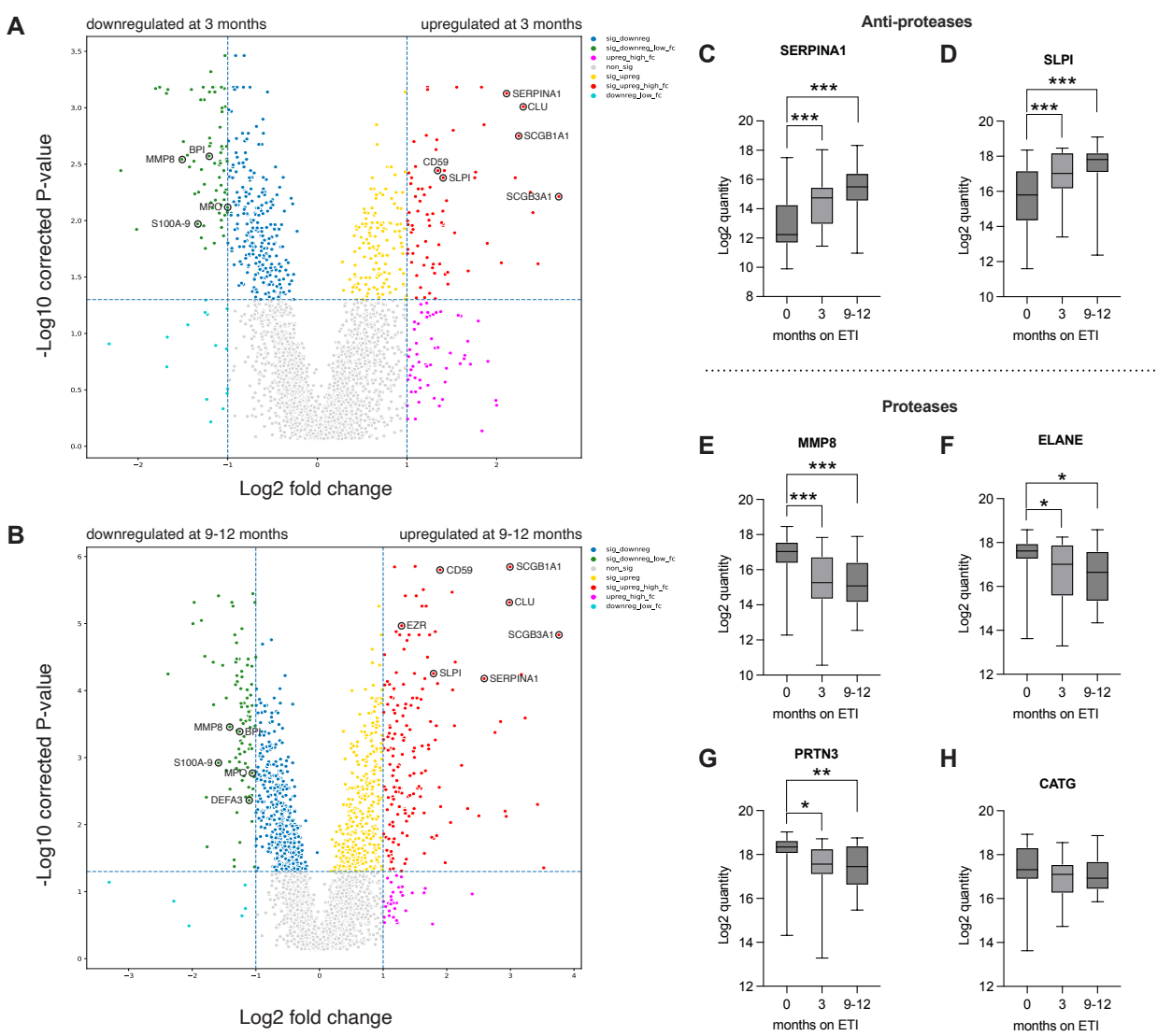
Sputum from pwCF (n=30) was collected before ETI initiation and after 3 and 9-12 months of treatment. Sputum from healthy controls (n=7) were included for comparison. Proteomes were analysed using data-independent acquisition liquid chromatography tandem mass spectrometry (DIA LC-MS/MS), and cytokines using Mesoscale assays. Differential expression analysis, k-means clustering, and correlations between airway proteomes and inflammatory cytokines were performed. Machine learning (XGBoost with bootstrapping approach) was applied to identify proteins predictive of ETI response.

Results

ETI induced general proteomic shifts, particularly related to decreased neutrophil degranulation and increased anti-proteases. Machine learning predicted proteins involved in RNA splicing, ER stress and lipid transport as contributors to treatment response. IL-1b, IL-8, and TNFa decreased with treatment, correlating with neutrophil-related proteins. In contrast, IL-6 levels increased and correlated with mucin O-glycosylation pathways. Despite these improvements, proteomic and cytokine profiles remained distinct from healthy controls.

Conclusion

ETI therapy reduces neutrophilic inflammation and restores protease/antiprotease balance but does not fully normalise airway biology comparable to healthy controls. Machine learning provides complementary insights into molecular determinants of ETI response, suggesting a role for RNA splicing, ER stress and lipid metabolism.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downregulated at 3 months
upregulated at 3 months
-Log10 corrected P-value
Log2 fold change
sig_downreg
sig_downreg_low_fc
upreg_high_fc
non_sig
sig_upreg
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downreg_low_fc
SERPINA1
CLU
SCGB1A1
CD59
SLPI
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BPI
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Log2 fold change
CD59
SCGB1A1
CLU
EZR
SCGB3A1
SLPI
SERPINA1
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Snapshot of Open Science indicators in publications of the Glycoproteomics and MS Proteomics Infrastructure Unit of SciLifeLab

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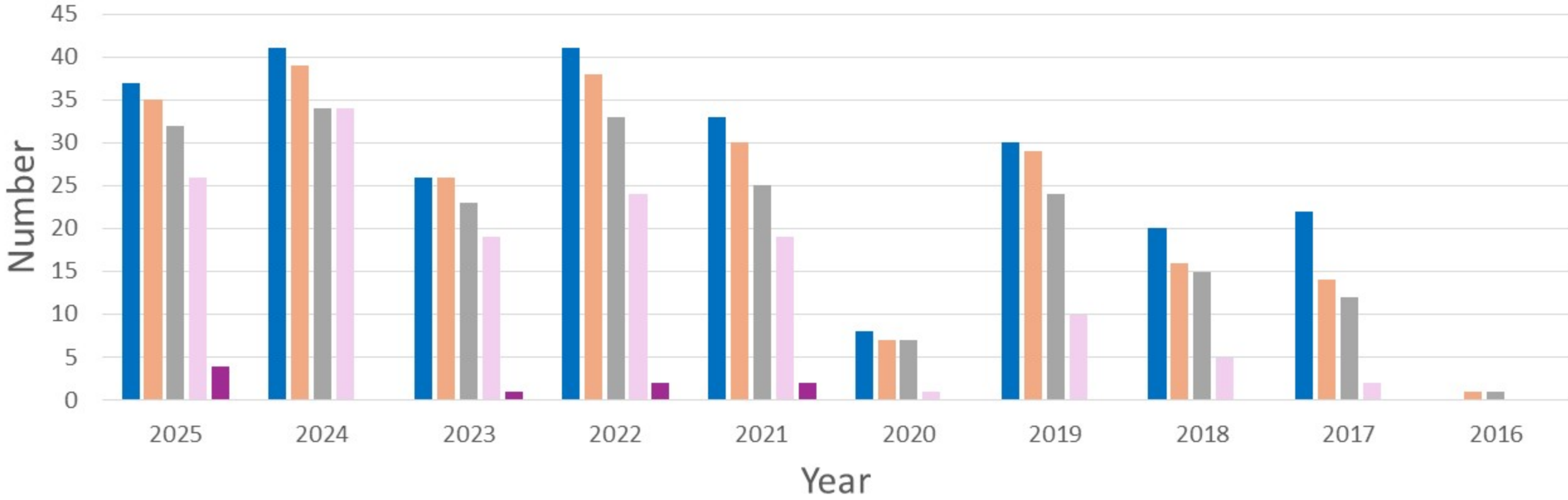
Open Science aims to modernize publicly funded scholarly research to make its research practices more transparent and efficient. Two building blocks of Open Science are Open Access to scholarly publications and access to research data that provide the basis for these publications. Research data serves a dual purpose. On the one hand, access to research data that corroborates scientific findings ensures transparency of scholarly research. On the other hand, reuse of research data saves costs and makes scholarly research more efficient. The FAIR-principles provide guidance on how researchers can describe and give access to their research data to comply with Open Science policies and to prepare them for reuse in future research. In this study, we analyze Open Science indicators in the publications of the Glycomics and MS Proteomics Infrastructure Unit of SciLifeLab to learn how researchers in this field practice Open Science. Our preliminary results indicate an increasing trend to submit data from MS-based proteomics to repositories of the ProteomeXchange Consortium like EBI-Pride and MassIVE. That applies also to glycoproteomics where GlycoPOST serves as a repository for data sharing. However, researchers also share large amounts of research data in supplementary documents that in large parts do not meet the recommendations of the FAIR-principles. This is a point that indicates a need to rethink the current forms of scholarly communication in life science. We discuss our findings in the context of Swedish recommendations for Open Science.

Publications of the SciLifeLab infrastructure for MS proteomics and glycoproteomics include many submissions to EBI-Pride, MassIVE and GlycoPOST, but the supplementary materials contain much proteomics an other data that is not FAIR.



Doping detection - from 2D-DIGE to semi-tryptic MS

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Autologous blood transfusion (ABT) using cryopreserved red blood cells (cryo-RBCs) enhances endurance performance but remains undetectable by any implemented anti-doping test. Over the past decade, our research has focused on identifying irreversible cryo-induced cytosolic RBC storage lesions as definitive biomarkers of ABT through three successive methodological phases.

The initial discovery phase employed two-dimensional difference gel electrophoresis (2D-DIGE), demonstrating that cryopreservation induces irreversible structural alterations and protein fragmentation within the RBC cytosol. Although this approach identified altered low-molecular-weight (<12 kDa) protein fragments, limited reproducibility, random proteolysis, and labor-intensive workflows restricted its suitability for routine anti-doping analysis.

To improve analytical performance, the platform evolved to shotgun proteomics using nano-UPLC-MS/MS following tryptic digestion. This enabled highly predictive machine-learning models that distinguished doped from clean samples irrespective of sex, altitude exposure, or elite endurance training. However, external validation revealed reduced diagnostic performance, suggesting that tryptic digestion fragmented endogenous peptides and eliminated cryopreservation-specific peptide signatures, contributing to model overfitting.

The current strategy employs semi-tryptic mass spectrometry, endogenous peptidomics, AI-assisted erythrocyte sorting, and extracellular vesicle proteomics to preserve naturally occurring peptide sequences and improve biomarker specificity and robustness. Because anti-doping tests must achieve exceptional analytical performance to withstand legal scrutiny, the ultimate goal is to develop a biomarker panel exceeding 95% sensitivity and specificity across independent validation cohorts. Beyond anti-doping, this erythrocyte-based proteogenomic platform has broad translational potential for liquid-biopsy diagnostics, particularly in cancer, where it may enable the discovery of highly specific peptide biomarkers for earlier detection and precision medicine.