Opening lecture

Dr. Elise Pepermans

Affiliation:
Immunespec

Title:
High-sensitive immunopeptidomics for better, safer biotherapeutics

Abstract:
Accurate characterization of MHC-presented peptides is essential to understand adaptive immune responses in both therapeutic safety and cancer immunotherapy development. We apply high-sensitivity immunopeptidomics/MHC-associated peptide proteomics (MAPPs) to define the repertoire of naturally presented peptides with high depth and confidence.
For biotherapeutics, our optimized MAPPs workflow enables detailed mapping of MHC associated peptides derived from therapeutic molecules, supporting data-driven immunogenicity risk assessment. We demonstrate how MAPPs-derived peptide profiles correlate with clinical immunogenicity, highlighting their predictive potential in preclinical evaluation.
In the context of cancer, we will touch upon how integration of next-generation sequencing within our NeoEpitopipe enables identification of MHC-presented targets for immunotherapy, including cancer antigens, neoantigens, and dark antigens.

Plenary lecture:

Dr. Thibaux Van der Stede

Affiliation:
VIB-UGent center for inflammation Research
Core Move lab
UGent department of Movement and Sport sciences

Title:
A muscle physiologist's attempt at applying proteomics technologies

Abstract:

Skeletal muscle is a heterogeneous tissue composed of multinucleated myofibers and diverse mononuclear cell populations. It exhibits exceptional plasticity, enabling robust structural and metabolic adaptations to external stimuli. Exercise training is one of the most potent drivers of this plasticity, promoting extensive remodeling of muscle and contributing to broad metabolic and cardiovascular health benefits. Advances in mass-spectrometry-based proteomics and phosphoproteomics now allow us to characterize these adaptations with unprecedented depth. In this talk, I will highlight our recent work integrating transcriptomics and proteomics to dissect muscle fiber heterogeneity and identify molecular signatures that distinguish fiber types. I will also present how exercise training reshape the skeletal muscle proteome. Finally, I will discuss (phospho)proteomic data from clinically relevant contexts such as insulin resistance, illustrating the central role of skeletal muscle in the metabolic dysfunction underlying type 2 diabetes. Together, these findings show how proteomics can advance our understanding of muscle biology, exercise adaptation, and metabolic disease.

Selected abstracts flash talks

Flash talk 1

Presenter
Laura Van Moortel

Affiliation:
Host-pathogen interactions lab
VIB Center for Medical Biotechnology

Title:
Immunopeptidomics-based design of mRNA vaccine formulations against Mycobacterium tuberculosis

Abstract:

Every year, more than 1 million people die from infections with Mycobacterium tuberculosis, to date still the most deadly infectious disease worldwide. Antibiotics (over)use is causing increased levels of antimicrobial resistance (AMR), which is why M. tuberculosis is listed as one of the most critical pathogens on the WHO AMR watchlist. Vaccination is a very effective strategy to mitigate AMR and reduce mortality, but to date, the only marketed vaccine against M. tuberculosis offers very poor protection in adults. Furthermore, the development of novel, more effective vaccines for bacteria such as M. tuberculosis is held back by a lack of known antigens.
Advances in the field of mass spectrometry-based proteomics have revolutionized the field of immunopeptidomics, making the latter a very potent approach to select candidate antigens via the identification of bacterial epitopes presented on major histocompatibility complexes (MHCs). Following a proof-of-concept study where we designed mRNA vaccine formulations against the intracellular bacterial model pathogen Listeria monocytogenes, we have further optimized all aspects of our immunopeptidomics workflow to increase its sensitivity and sample throughput. This optimized pipeline is being used throughout the EU-funded BAXERNA project (www.baxerna.eu) for the development of mRNA vaccines against three clinically problematic bacteria, including M. tuberculosis. For the antigen discovery, THP-1 and U937 macrophage cell lines were infected with M. tuberculosis for 24h or 72h and processed with our immunopeptidomics pipeline. We were able to identify more than 200 MHC class I-presented M. tuberculosis peptides from 175 proteins, of which 15 proteins were selected for further evaluation as vaccine candidates. mRNA-liquid nanoparticle formulations combining two or more of these candidate antigens were evaluated for their protective efficacy following prime-boost vaccination in mice. We found that a multi-valent mRNA vaccine formulation encoding the five most protective antigens provides protection comparable to BCG in the lungs in a murine model of M. tuberculosis infection. Furthermore, in the lungs, our 5-valent mRNA vaccine candidate surpasses the protection of BCG alone when administered as a booster for BCG. Together, this work might lead to a much-needed improved vaccine against M. tuberculosis.

Flash talk 2

Presenter
Emma De Jonge

Affiliation:s
AMCB
UCLouvain – LIBST

Title:
Proximity labeling identification of HOXA1 partners that could account for its exceptional longevity

Abstract:

HOXA1 is a transcription factor (TF) that plays a critical role in embryogenesis, particularly in the development of the heart, and its loss of function in mice can lead to several defects including bicuspid aortic valve (BAV). Interestingly, Hoxa1 is not expressed in the developing heart itself, but transiently in migrating progenitors that will reach it.
However, recent discoveries made in our laboratory have shown that the HOXA1 protein is still present in these migrating cells when reaching the heart, and this, in the absence of gene expression for at least 48 hours. Considering that TF are viewed as having a fairly rapid turnover allowing a dynamic control of their target genes, this raises the question of why does HOXA1 interactants that could contribute to its exceptionally long lifespan as well as what regulates HOXA1 longevity, both questions amenable by characterizing the HOXA1 interactome.
We initiated HOXA1 interactomic screenings based on proximity labelling of interactors with biotin. Proximity biotinylation techniques, such as TurboID, are proving ideal for capturing transient and unstable protein-protein interactions including the ones involved in protein stability like ubiquitination or deubiquitination.
Our TurboID experiments were performed in pluripotent murine cell line P19 upon retinoic acid induced differentiation. Cells were transfected with constructs encoding N- or C-terminal Turbo protein fusion to HOXA1 and biotinylated interactors have been harvested on streptavidin beads to proceed with mass spectrometry identification.
A 459-protein HOXA1 proxisome was identified, including proteins involved in protein stability, such as ubiquitin ligases. A series of proteins involved in cardiac development and BAV were also found which might be relevant to the cardiogenic activity of migratory cells colonizing the heart and harboring long-life HOXA1.
Interaction between HOXA1 and its proxisome proteins will be tested by orthogonal assays and, if confirmed, and found relevant in heart development, gain and loss-of-function experiments will be performed to establish their impact on the stability, transcriptional activity and potential cardiogenic function of HOXA1.

Flash talk 3

Presenter
Laura De Clerck

Affiliation:s
ProGenTomics,UGent

Title:
Potential of Proteomics in Forensic Phenotyping: A Focus on Biological Sex Determination
Abstract:

Forensic DNA analysis is well established for phenotyping, providing valuable investigative leads. Recently, proteomics, the large-scale study of proteins, is increasingly recognized as a complementary tool to DNA analysis, particularly for enhancing the evidential value of traces and especially in cases involving degraded samples or challenging matrices. This study aims to extract phenotypic traits directly from whole blood proteomes, using biological sex determination as a starting point. Using LC–MS/MS, proteomes from 100 whole blood samples of known sex were used to train a biological sex classifier. Cross-validation of the model demonstrated the potential of proteomics for accurate sex classification. Key peptides, such as from pregnancy zone protein and ceruloplasmin, were identified as highly important features. To further evaluate the model, mock case samples were generated to simulate real-world case scenarios. However, a large portion of these mock samples were incorrectly classified, which was caused by batch effects. Based on our findings, transitioning from an untargeted assay to a maximally performant analytical targeted assay is the next crucial step needed for implementation into routine forensic application. Overall, this study advocates for the inclusion of proteomics as part of the forensic phenotyping toolkit, while addressing the challenges, opportunities, and recommendations in its implementation.

Flash talk 4

Presenter
Christopher Kune

Affiliation:s
Mass Spectrometry Laboratory,Unviersity of Liege

Title:
Smarter Libraries for Faster DIA: A Sequential Digestion Strategy to Enhance Proteome Coverage in DDA-based Spectral Libraries

Abstract:

Large-cohort studies are increasingly required in multifactorial proteomics, demanding high-throughput and scalable workflows. Sample preparation automation and fast LC-MS instrumentation, including short-gradient chromatography combined with Data Independent Acquisition (DIA), have enabled rapid data generation. However, DIA data processing needs high-quality spectral libraries, which is a current bottleneck. While AI-generated libraries offer comprehensive coverage, their large size leads to time-consuming data searches. In contrast, DDA-based libraries reduce search space but limit peptide identifications to those observed in DDA runs. Here, we propose a novel strategy combining sequential protease digestion, peptide fractionation and DDA acquisitions on the timsTOF SCP. In this approach, three replicate samples are separately digested with trypsin, Arg-C, or Lys-C, followed by peptide fractionation and a final digestion with trypsin. This yields tryptic peptides distributed differently across fractions based on the initial protease, enhancing orthogonality between fractionation and LC separation.
Compared to conventional post-trypsin fractionation, our method identifies nearly 50% more peptides. Applied to 10-minute DIA runs, libraries from sequential digestion yield 10% more protein IDs without notably increasing processing time. This strategy enhances peptide coverage and search efficiency, making it ideal for large-scale DIA proteomics requiring both speed and depth.

Flash talk 5

Presenter
Fatemeh Mirzadeh Sarcheshmeh

Affiliation:s
Mass Spectrometry Laboratory,Unviersity of Liege
Adrem Data Lab
Computer science, University of Antwerp

Title:
AI-Driven Metadata Annotation for Reproducible Mass Spectrometry-Based Omics Research

Abstract:

Mass spectrometry-based proteomics generates vast amounts of data, yet the effective reuse and discovery of these datasets remain challenging due to incomplete and inconsistent metadata. Metadata which provides critical contextual information such as experimental conditions, sample characteristics, and data processing details is essential for making data FAIR (findable, accessible, interoperable and reusable). To address these challenges, our working group aims to develop intelligent, automated workflows for comprehensive metadata extraction, harmonization, and integration into PRIDE, the largest public proteomics data repository.
Our working group has established a systematic approach to automated metadata extraction from scientific publications. We curated a corpus of open-access publications linked to PRIDE datasets and manually annotated 27 publications to create a gold standard benchmark. We then evaluated multiple natural language processing approaches including GPT-4, BERT-based models, and DocETL—a LLM-powered framework for complex document processing pipelines.
We implemented DocETL to automatically extract and standardize proteomics metadata across multiple dimensions. The pipeline employs specialized extraction modules targeting: (1) biological information including organism, tissue, cell lines, disease characteristics, and clinical parameters; (2) experimental design encompassing biological and technical replicates, sample treatments, and genetic modifications; (3) sample preparation details including reduction, alkylation, digestion enzymes, post-translational modifications, and enrichment strategies; (4) chromatographic separation parameters such as fractionation methods, gradient times, and flow rates; (5) mass spectrometry instrumentation including instrument models, fragmentation methods, acquisition modes, and mass tolerance settings; and (6) data files and experiment types. The framework incorporates automated quality control through multi-round gleaning for iterative validation, entity resolution modules to harmonize terminology variations (e.g., standardizing organism names, cell lines, and instrument models), and aggregation operations to generate organism-level and instrument-level statistics across the literature.
Our evaluation against manually annotated publications demonstrates that DocETL effectively extracts comprehensive metadata with high accuracy and consistency, significantly outperforming traditional NLP approaches.

Flash talk 6

Presenter
Michel Dierckx

Affiliation:s
Mathematics and Computer Science Department, University of Antwerp
Computer science, University of Antwerp

Title:
Scalable mass-spectrometry-based molecular phylogeny with TreeMS2

Abstract:

Traditional molecular phylogenetics relies on DNA and RNA sequences, which provide a detailed record of evolutionary history but reflect only an organism's genetic blueprint. In contrast, proteomes and metabolomes represent the realized molecular phenotype. Therefore, mass spectrometry (MS)-based molecular phylogenetics has the potential to uncover biochemical convergence and divergence patterns that sequence-based analyses fail to capture.

Even though vast public repositories of proteomic and metabolomic data are available, these resources remain largely untapped. The main challenge is the lack of scalable methods for reconstructing phylogeny directly from MS data. Existing methods rely on exhaustive spectral comparisons and cannot scale efficiently to the millions of spectra needed for large-scale MS-based phylogenetics.

Here we introduce TreeMS2, an open-source tool for large-scale molecular phylogenetic analysis from MS-based proteomic and metabolomic data. TreeMS2 represents each spectrum as a binned vector, applies sparse random projections for dimensionality reduction, and uses approximate nearest-neighbour search to rapidly identify similar spectra across samples. This information is then used to infer a distance matrix that quantifies dissimilarity between samples without relying on sequence information or protein databases.

TreeMS2 was applied to a bacterial proteomics dataset comprising 303 proteomes from 119 genera and five phyla, totaling over 13 million MS/MS spectra. The analysis completed in approximately 3 hours on two 14-core CPUs, demonstrating its scalability. The resulting phylogenetic tree showed strong agreement with established bacterial taxonomy, down to the genus level. Moreover, unexpected placements could be matched to known mislabeling errors, illustrating TreeMS2's usability for unsupervised quality control. In addition, TreeMS2 was applied for species identification. A nearest-neighbor classifier trained on TreeMS2 distances correctly assigned 46 of 46 genera and 31 of 32 species in an independent isolate dataset.

Beyond bacterial phylogenetics, TreeMS2 was successfully applied to metabolomics and single-cell proteomics datasets, demonstrating its flexibility. TreeMS2 is available as open-source software at https://github.com/MichelDierckx/TreeMS2, establishing a practical foundation for integrating molecular and evolutionary landscapes.

Flash talk 7

Presenter
Ina Devos

Affiliation:
Centre for Ethics, Department of Philosophy

Title:
Ethics of Proteomics: A Scoping Review

Abstract:

Proteomics research and applications are becoming increasingly relevant for biomedical and clinical contexts, which raises the ethical stakes. However, while bioethical scholarship has substantially engaged with human genomics, human proteomics has been largely ignored as a topic of interest. Current bioethical engagement is sparse and largely comes from life science researchers themselves. In our scoping review, we investigated their publications to map the ethical topics and common narratives in discussions of ethics in human proteomics. We employed a two-stage analysis of included publications. First, we identified the topics discussed as being ethically relevant. Second, we identified common narratives around these topics using thematic analysis. This approach provides an overview of the content, structure, and tensions of scholarly discussions on the ethics of human proteomics.
In the 37 included publications, we identified 22 ethical topics of discussion. Most of the topics, such as public benefits, privacy, and incidental findings, are familiar topics from the more general ethics of biotechnologies. Additionally, our thematic analysis highlights 6 common narratives: (1) ethics of proteomics requires multidisciplinary attention, (2) aspirational benefits of (open) proteomics, (3) protection of individuals, (4) balancing the benefits/individual protections trade-off, (5) resources and equity in proteomics, and (6) dimensions beyond the proteomics experiment. Investigating these highlights some unaddressed assumptions and tensions in the ethics discussions. For example, we identified a perceived link between aspired societal benefits and openly available proteomic data, on the one hand, and between the protections afforded to individual participants or data subjects and the limitations of proteomic data availability, on the other.
We suggest that these assumptions inform an unhelpful trade-off narrative between realizing aspired societal benefits and protecting individual research subjects. It strongly limits the possibility for nuanced, complex and creative approaches in which to interests of those seeking protection are aligned, rather than oppositional to those of research and the 'public good'. Therefore, we argue for interdisciplinary proteomic-bioethical research that critically examines these commonly discussed issues, concepts, and narratives, while closely engaging with the specificities and realities of proteomics research practices.

Posters

Poster 1

Presenter
Amine Mohamed,BERKAL

Affiliation:
MolSys Mass Spectrometry Lab, Univ. of Liège

Title:
Sweat Proteomics: A Non-Invasive Approach for Biomarker Discovery with Optimized and Fully Automated Sample Preparation

Abstract:

Author(s):
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Sweat is a promising biofluid for biomarker discovery due to its non-invasive collection and rich molecular content. Its proteome reflects key physiological processes and offers opportunities for disease monitoring via mass spectrometry-based proteomics.

We developed a fully automated workflow combining NanoOrange™ protein quantification with a streamlined One-Pot digestion protocol, enabling direct analysis of raw sweat without pretreatment steps that can cause protein loss. The study included 411 sweat samples and 6 pooled quality controls, covering several diseases—cystic fibrosis, asthma, bronchiectasis, respiratory infections/cough, and growth or digestive disorders—alongside healthy individuals. A total of 2,413 proteins were identified; after stringent quality filtering, 1192 proteins with 92% data completeness were retained.

Identified proteins participate in different important biological processes, including innate immunity, oxidative stress, and metabolic pathways. After adjusting for age, sex, and Body Mass Index (BMI), differential expression analyses revealed several candidate biomarkers distinguishing cystic fibrosis, along with additional disease-associated biomarkers across the cohort.

These results demonstrate the strong potential of sweat as a non-invasive source for disease characterisation and health monitoring. Ongoing work aims to validate the identified biomarkers and further establish sweat-based proteomics as a reliable tool for clinical applications.

Poster 2

Presenter
Amélie,De Maesschalck

Affiliation:
ProGenTomics, Ugent
Laboratory for pharmaceutical biotechnology, Ghent University

Title:
Differentiation therapy in Acute Myeloid Leukemia: How does the interplay between histone epigenetics and metabolism define stemness?

Abstract:

Acute Myeloid Leukemia (AML) is a heterogeneous hematologic malignancy characterized by increased numbers of immature white blood cells called myeloblasts. In essence, AML is a disease of blocked differentiation. Cells fail to mature properly and instead continue to proliferate.
Recent advancements in differentiation therapy have led to the approval of Revumenib by the FDA in 2024. Revumenib is an anti-menin inhibitor, which triggers a massive differentiation event in AML cells. This is reflected in a brief spike in cell counts, after which the leukemic cells die, ultimately leading to remission. Mechanistically, it breaks the interaction of MEN1 with methyltransferase 2A (KMT2A), which methylates lysine 4 of histone H3 (H3K4me). This interaction is a dependence in acute leukemia caused by either rearrangement of KMT2A or mutation of the nucleophosmin 1 gene (NPM1). KMT2A rearrangements occur in up to 10% of acute leukemias and have an adverse prognosis, whereas NPM1 mutations occur in up to 30%, forming the most common genetic alteration in AML.
ProGenTomics has recently developed a multi-omics mass spectrometry (MS)-based approach that integrates three biomolecular fractions all extracted from a single cell pellet: the histone epigenome, the metabolome and the proteome. Together, these fractions capture both the regulatory layer (the epigenome) and the functional output (the metabolome and proteome) of a cell, thereby reflecting the phenotype. To understand the impact of (driver) mutations on these three biomolecular fractions in AML, this workflow has been successfully applied to a cohort of 18 genetically different AML cell lines (6 replicates each) in collaboration with Prof. Tim Lammens (Lab for pediatric hematology-oncology, UZ Gent). This created an extremely coherent and low-noise data matrix since all omics fractions were extracted from the same cell pellet. Importantly, these cell lines differ only in their genomic background and cellular history, providing just a snapshot in time, which makes it unable to reveal causal relationships between the different layers. The next crucial step is capturing the temporal dynamics of these molecular changes upon therapeutic challenge, allowing us to establish causal relationships and identify key interactions that drive AML differentiation. By applying our newly established multi-omics workflow to a timelapse experimental design, we can study the underlying dynamics following anti-Menin therapy.

Poster 3

Presenter
Charlotte,Adams

Affiliation:
Department of Informatics, University of Antwerp
Adrem data lab

Title:
Investigating the Indistinguishability of Positional Isomers

Abstract:

Mass spectrometry is an essential technique for the confident identification of complex peptide mixtures. However, a key limitation persists: certain peptides are analytically indistinguishable, displaying nearly identical retention times and fragmentation patterns. This indistinguishability can lead to ambiguous or incorrect peptide annotations, which may have severe consequences when misidentifying isoforms results in misleading conclusions.

To examine this limitation, recent studies have employed peptide property prediction tools to simulate mass spectrometry experiments in silico. By predicting fragment ion intensities and retention times, these models enable a theoretical assessment of peptide indistinguishability across entire proteomes. In this project, we focused specifically on the indistinguishability of positional isomers, peptides that differ only in the position of specific amino acids. We investigated how the identity of the swapped amino acids, the location of the swap, and the surrounding sequence context influence spectral similarity.

Our findings largely confirmed known effects. For example, interchanging leucine and isoleucine produced minimal spectral changes (median spectral similarity score: 0.94), whereas substitutions involving proline resulted in more pronounced differences (median spectral similarity score: 0.51), compared to swaps not involving proline (median spectral similarity score: 0.65). A better understanding of these effects can improve the interpretation of spectrum annotations and contribute to more reliable peptide identification in complex biological samples.

Poster 4

Presenter
Matthieu Hodeige

Affiliation:s
CBIO and MSLab
de Duve Institute
MolSys

Title:
Linking spatial to functional proteomics on heterogeneous samples: The pixel-by-pixelshotgun proteomic approach.

Abstract:

M.Hodeige[1,2], C.Kune[2], V.Bertrand[2], M.Fleron[3], D.Baiwir[3], M.Herfs[4], G.Eppe[2], L.Gatto[1], G.Mazzucchelli[2,3]
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[3]GIGA Proteomics Facility, University of Liège, Liège, Belgium
[4]Laboratory of Tumor and Development Biology, GIGA-Cancer, University of Liège, Liège, Belgium

Proteins are crucial for assessing cell health and activity, yet proteomics of biological tissues faces challenges due to sample heterogeneity. Mass spectrometry imaging offers spatial information with limited functional insights, while laser capture microdissection (LCM) shotgun proteomics provides detailed proteome data but sacrifices spatial resolution. We propose a technique, called pixel-by-pixel shotgun proteomics (Pixel2 Proteomics, P2P), combining laser microdissection with proteomics. This approach was tested on mouse squamo-columnar junctions that connect a stratified squamous epithelium to a glandular epithelium. The interest of this tissue lies in the well-established emergence of precancerous lesions in these junctions. Tissues were initially preserved in formalin-fixed paraffin-embedded (FFPE) form and sectioned into 5 µm slices. The 5 µm tissues were microdissected into areas of 2,000 µm² (10–20 cells), following a systematic grid pattern. To develop this method, it was important to identify a laser microdissection system that could combine maximal recovery rate with high throughput. To achieve this goal, we compared two systems: the LMD7 laser microdissector from Leica and the CellCut system from MMI. We highlighted several critical parameters, including recovery affected by static electricity, laser precision, biological tissue degradation, and the implementation of sample preparation after dissection, thereby revealing the advantages and limitations of both approaches. On average, for all pixels that were successfully recovered and analyzed with either system on the studied biological tissue, between 500 and 1500 proteins could be identified per 2000 µm² pixel.

Poster 5

Presenter
Clémence Balty

Affiliation:
MassProt,UCLouvain

Title:
Enrichment-Free Detection of Tau Proteoforms in CSF and Plasma Using a SCOPE-Inspired MS Workflow

Abstract:

Tauopathies are neurodegenerative diseases defined by the accumulation of misfolded tau aggregates. Tau exists as six isoforms generated through alternative splicing, producing either 3R or 4R isoforms. Tauopathy diagnosis relies mostly on post-mortem brain analysis, where each disease displays characteristic patterns of isoform aggregation. For Alzheimer's disease, the tauopathy with the highest prevalence, 3R and 4R isoforms are found in equal quantities in tau aggregates. In contrast, only 4R tau aggregates are found in Cortico-Basal Degeneration and some Frontotemporal Lobar Degeneration cases, while only 3R tau aggregates are found in Pick's disease and other FTLD cases.

Our recent absolute MS quantification of tau in brain tissue demonstrated that isoform abundance differs only in the aggregated fraction and not in the soluble fraction, suggesting that tau aggregation is not driven by changes in isoform expression but by isoform-specific post-translational modifications (PTMs). Tau displays an extensive PTM landscape, including phosphorylation, ubiquitination, and acetylation, which can modulate its structural state and aggregation propensity. In previous work on brain tissues, we identified tauopathy-specific PTMs and PTM combinations with potential pro- or anti-aggregation effects.

Developing ante-mortem biomarkers for tauopathies requires unbiased characterization of tau PTMs in biofluids, where tau is present at very low concentration (~50 fmol/mL) and is fragmented. In literature, immunoprecipitation-based enrichment remains the dominant strategy to study tau in biofluids but introduces strong epitope-dependent biases. To overcome these limitations, we adapted the SCOPE single-cell proteomics workflow to enable enrichment-free, MS-based detection of tau and its proteoforms in CSF and plasma. Our approach uses a carrier composed of soluble and insoluble tau from brain tissues (containing the entire tau sequence and previously mapped PTMs), thus sensitively triggering tau peptide identification and quantification in biofluid samples.

We applied this method to cerebrospinal fluid and plasma and obtained ~80% sequence coverage and detected ~30 PTMs. Ongoing work will analyze CSF samples paired with brain tissue from the previous study to assess whether disease-specific PTM signatures detected in the brain are observed in biofluids, paving the way for isoform- and PTM-based biomarkers for 3R/4R tauopathies.

Poster 6

Presenter
Lee Meunier

Affiliation:
MASSPROT,De Duve Institute

Title:
Implementing Thermal Proteome Profiling as a tool to identify off-targets of compounds of interest: from sample preparation to data processing.

Abstract:

Lee Meunier [1], Didier Vertommen [1], Clémence Balty [1], Gaëtan Herinckx [1], Brecht Permentier [2], Julien Olivet [2], Mark H. Rider [1], and Manuel Johanns [1].
[1] UCLouvain, De Duve Institute, MASSPROT platform
[2] KU Leuven, Rega Institute

Thermal Proteome Profiling (TPP) is a liquid chromatography-coupled mass spectrometry (LC-MS)-based workflow for performing proteome-wide analyses of protein-ligand interactions. This is achieved through monitoring protein thermal stability changes upon ligand binding, after subjecting samples to heat treatments in presence or absence of compounds.
TPP is a relevant method of identification of drug targets. It can be applied to various compounds to identify either unknown targets responsible for beneficial effects, or unintended targets responsible for harmful effects. The latter are termed off-targets.
Our laboratory has been working on implementing and refining a standard TPP workflow, from sample preparation to data processing.
A tailored sample preparation protocol was first established. It was validated by immunoblotting and LC-MS on lysates of mouse embryonic fibroblasts (MEF) using trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor known to cause thermal stabilisation of HDAC1. Relative quantification by LC-MS was achieved using Tandem Mass Tags (TMT) labelling.
LC-MS data was then processed using the open-source TPPR package for proteome-wide analysis of melting curves and melting temperature shifts. Evaluation of the MS dataset obtained from MEF lysates treated with TSA highlighted a series of identified target proteins, among which HDAC1 was confirmed as the most statistically significant hit. Other analysis pipelines, such as the two R packages NPARCand MSstatsTMT, are based on temperature-per-temperature thermal stability variations rather than melting temperature shifts. They are currently being evaluated using the same dataset, to enable comparison of proteins identified as hits by each pipeline.
We are currently applying this TPP workflow to identify (off-)targets of compounds of interest, including modulators of the AMP-activated protein kinase (AMPK).
Ultimately, this approach will be applied to protein-targeting drugs which are already in clinical use, e.g. SGLT2 inhibitors and GLP1 receptor agonists, in kidney and heart cells and/or lysates. This could contribute to refining their characterisation and, hopefully, elucidate the origin of some of some of their side effects.

Poster 7

Presenter
Maxine,Vergucht

Affiliation:
Impens lab, VIB - UGent Center for Medical Biotechnology

Title:
Dissecting the substrate repertoire of ISG15 E3 ligases during infection.

Abstract:

Maxine Vergucht[1,2], Denzel Eggermont[1,2], Katie Boucher[1,2], Fabien Thery[1,2], Francis Impens[1,2]
[1]VIB-UGent Center for Medical Biotechnology, VIB, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium
[2]UGent Department of Biomolecular Medicine, Ghent University, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium

During infection, the production of interferon stimulated gene 15 (ISG15) is a paramount host response. ISG15 is a ubiquitin-like protein which conjugates to target proteins via an enzymatic cascade similar to ubiquitination involving an E1 activating enzyme, E2 conjugating enzyme and E3 ligase, a process referred to as ISGylation. During viral infection, co-translational ISGylation of both viral and host proteins by the E3 ligase HERC5 lead to a general antiviral state, mainly through loss-of-function of modified proteins. In contrast, certain host ISGs were recently shown to be activated by ISGylation, often catalyzed by the E3 ligases ARIH1 and TRIM25. This suggests that HERC5-independent ISGylation can also lead to gain-of-function effects, which encouraged us to map the substrate repertoire of ISG15 E3 ligases by mass spectrometry.
To distinguish between HERC5-dependent and HERC5-independent ISGylomes, we performed a proteome-wide analysis of ISG15 sites affected by HERC5 knockdown. We made use of anti-K-ε-GG antibodies to enrich for ISG15-modified peptides and included ISG15 KO cells in our experiments to distinguish bona fide ISGylation sites in HeLa cells treated with interferon-α, with or without knockdown of HERC5. Samples were analyzed on both timsTOF SCP and Q Exactive HF instruments leading to 1,011 and 1,380 ISG15 sites on 759 and 908 proteins, respectively, in a background of in total 8,030 and 9,836 ubiquitin sites. Hierarchical clustering separated HERC5-dependent from HERC5-independent ISG15 sites. In total, we identified 533 proteins uniquely modified by HERC5, 524 proteins uniquely modified independent of HERC5, and 87 proteins targeted by both HERC5 and other E3 ligase(s). Interestingly, the HERC5-independent ISGylated targets were significantly enriched in proteins linked to innate immunity, while HERC5-modified substrates were enriched in proteins linked to metabolism and translation. Biochemical and functional validation of selected targets is ongoing, in order to confirm HERC5 (in)dependent modification and potential antimicrobial activity.

Poster 8

Presenter
Adillah Gul

Affiliation:
Impens Lab,VIB-UGent Center for Medical Biotechnology

Title:
Towards ultrasensitive immunopeptidomics for bacterial antigen discovery

Abstract:

Mass spectrometry-based immunopeptidomics is a powerful tool to identify antigenic peptides presented on major histocompatibility complexes (MHCs) and serves as an excellent strategy for antigen discovery in the context of vaccine development, among others, against infectious diseases and cancers. In our proof-of-concept study on the intracellular bacterial model pathogen Listeria monocytogenes, 500 million infected cells per condition were needed, drastically limiting its throughput and applicability. In my PhD project, I further optimized all aspects of sample preparation to reduce the required input material and to improve sensitivity and throughput. Sample preparation is now semi-automated in a 96-well format and requires only a few million cells or less. We combined sample preparation with LC-MS/MS on the ultra-sensitive TimsTOF SCP mass spectrometer. Data analysis uses a streamlined pipeline that combines four search engines (MSFragger, Sage, Comet, and PEAKS), followed by peptide rescoring with MS2Rescore (v.3), which includes predictions on retention time, ion mobility and peptide fragmentation. This approach effectively identifies bacterial epitopes and antigens with very high confidence. Using this optimized pipeline, we determined the working ranges for several cell lines in our lab. For instance, using only 25% of the eluate from 16 million JY cells, we identified over 13,500 MHC class I and 6,000 MHC class II peptides through DDA-PASEF acquisition. We demonstrate the ultra-sensitivity of our platform by identifying up to 1,000 peptides from as few as 20,000 JY cells. To validate the platform's utility, we conducted successful proof-of-concept studies to identify Listeria monocytogenes and Bovine Calmette-Guérin (BCG) antigens from infected U937 cells, using minimal input material. This optimized pipeline is currently being used throughout the EU-funded BAXERNA project (www.baxerna.eu) to discover candidate antigens against bacteria Mycobacterium tuberculosis, Mycobacterium ulcerans, and Acinetobacter baumannii.

Poster 9

Presenter
Moira Breëns

Affiliation:s
ADReM Data Lab,University of Antwerp

Title:
Exploring non-human species in the human immunopeptidome

Abstract:

Peptides bound to major histocompatibility complex (MHC)-proteins are presented on the cell surface to the adaptive immune system, which can recognise these peptides -- commonly referred to as epitopes -- and eliminate infected and malignant cell through an immune response. Mass spectrometry can be used to analyse these epitopes, however current research is limited by the use of the database-driven approach to the data analysis aspect. An alternative approach, one not limited by the database content, is based on de novo sequencing tools such as Casanovo and subsequently identifying these peptides and their origin.
We aim to investigate the presence of non-human epitopes in the healthy human immunopeptidome by utilising a BLAST search against a specifically compiled list of viruses, bacteria and archaebacteria that are known to infect humans, be part of the microbiome or otherwise associated with humans. A preliminary analysis was performed on the HLA Ligand Atlas data, BLASTing the de novo Casanovo output against the entirety of Swissprot, which already demonstrated the presence of viral, bacterial and archaea epitopes. Several established epitopes, amongst which from Epstein-Barr virus and Cytomegalovirus, were additionally found in the data.
These discoveries will contribute to the insight into the adaptive immune system and could aid in the development of immunotherapies and vaccines, as well as provide a pipeline for investigating the immunopeptidome in other contexts.

Poster 10

Presenter
Marina Pominova

Affiliation:
Bittremieux Lab,University of Antwerp

Title:
Extending peptide spectrum modeling to neutral loss fragments for improved fragmentation representation

Abstract:

Mass spectrometry-based proteomics employs spectrum prediction models to enhance peptide identification through rescoring and to generate high-quality in-silico spectral libraries. On standard HCD data of unmodified tryptic peptides and canonical b/y ions, state-of-the-art models such as Prosit achieve spectral angles close to experimental reproducibility, suggesting that performance is near a practical ceiling in this setting.
However, in many biological applications, fragmentation patterns deviate from the canonical b/y-ion model. Peptides with particular post-translational modifications (PTMs), such as phosphorylation or citrullination, can produce spectra dominated by neutral loss fragment ions rather than canonical backbone fragments. Neutral loss ions are also informative in complex settings with extremely large search spaces, such as immunopeptidomics or metaproteomics. Incorporating neutral losses into spectrum prediction would thus yield more realistic spectral profiles, enhance rescoring and PTM-site validation, and expand the applicability of predicted spectral libraries.
Yet, predicting such peaks remains challenging: the number of possible ion types grows combinatorially with the inclusion of multiple losses, relevant examples are underrepresented in most training datasets, and mass coincidences between ions complicate unambiguous annotation.
To address these limitations, we developed a transformer-based model that extends Prosit's concept to jointly predict canonical fragment ions and peaks with neutral losses. Instead of regressing intensities for a predefined set of ion types, the model outputs a set of the 50 most intense peaks in each spectrum, predicting both their ion types and intensities. Fragment ions are represented through a combination of labels describing backbone type, fragment length, charge, and presence of neutral losses such as H_2O or NH_3, allowing flexible extension to additional ion types. The model is trained on one million synthetic spectra from ProteomeTools, re-annotated with expert rule-based inclusion of neutral-loss fragments to minimize false positive assignments. It achieves a mean spectral angle of 0.901 on validation data and 0.711 on peaks containing neutral losses, demonstrating the feasibility of learning neutral-loss fragmentation patterns directly from data.

Poster 11

Presenter
Guillaume Deflandre

Affiliation:
CBIO,UCLouvain

Title:
PSMatch: a Bioconductor Package for Handling Peptide-Spectrum Matches Data

Abstract:
Authors: Guillaume Deflandre, Sebastian Gibbs, Laurent Gatto
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Loading, exploring and analysing the resulting Peptide-Spectrum Matches (PSMs) from a database search in Mass Spectrometry (MS)-based proteomics can be time-consuming. PSMatch is an R/Bioconductor package designed to handle this process by offering functionalities to streamline exploration and visualisation of PSM data. It provides functions to load PSM data from mzId or tabular files, generate theoretical fragment ions, model peptide-protein relations and facilitate visualisations.

Recent developments in PSMatch have focused on extending these functionalities to support post-translational modifications, enabling more accurate characterisation of modified peptides. Effort in identifying modified peptides is needed as it is these peptides that are expected to constitute a significant proportion of unidentified spectra. In fields such as single-cell proteomics or metaproteomics, where the identification rates pale by comparison with bulk approaches, this becomes even more prominent. Enabling users to benefit from a powerful and flexible R ecosystem to further explore these unidentified spectra is therefore paramount.

PSMatch is part of the R for Mass Spectrometry initiative, that develops an open and collaborative ecosystem of MS-based proteomics and metabolomics, offering efficient, scalable, and stable infrastructure for MS-based proteomics.

Poster 12

Presenter
CederDens

Affiliation:
Adrem Data Lab
University of Antwerp, Department of Computer Science

Title:
Improvements to Casanovo, a deep learning de novo peptide sequencer

Abstract:

De novo peptide sequencing from tandem mass spectrometry (MS/MS) is a powerful strategy for characterizing peptides that are absent from reference databases, such as non-canonical, antibody-derived, or microbiome peptides. This capability makes de novo methods especially valuable in fields like immunopeptidomics and metaproteomics, where conventional database-driven approaches are limited. Accurately inferring peptide sequences directly from spectra is thus a crucial enabler for discovery in these contexts.
We have previously introduced the deep-learning-based Casanovo model to address this challenge. Casanovo maps MS/MS spectra to peptide sequences using a sequence-to-sequence transformer architecture, achieving state-of-the-art performance in de novo peptide identification.
Here, we report several advancements to Casanovo, aimed at (i) improving the interpretability and calibration of the scores assigned to predicted peptides, (ii) generalizing the software to function in database-search as well as pure de-novo contexts, (iii) reducing training and runtime costs, and (iv) providing robust workflows, visualization and user-interfaces to support adoption. The redesigned scoring system provides better-calibrated confidence estimates, enabling more effective thresholding and integration with downstream analyses. Additionally, we achieve faster training and inference while preserving the predictive performance of the original model, lowering the barrier to routine use.
Finally, Casanovo's database-search mode substantially increases the number of peptide-spectrum matches compared to traditional database search engines, owing to Casanovo's powerful learned score function, which provides well-calibrated, discriminative ranking of candidate peptide matches.
With these updates, Casanovo has become a more user-friendly and versatile tool for peptide sequencing. Its improved calibration, expanded functionality, including the database-search mode, and enhanced workflows broaden its applicability. Ongoing development continues to strengthen performance, usability, and integration with downstream proteomics pipelines.

Poster 13

Presenter
Thijs Lefever

Affiliation:
Lab of Pharmaceutical Biotechnology ,UGent

Title:
HS-Trap: a novel multi-omics-compatible sample preparation workflow for histone-analysis

Abstract:

Histone proteins are highly basic cellular components that wrap DNA to form nucleosomes, thereby compacting long strands of genomic DNA into the nucleus while also organizing chromatin structure. Beyond this packaging function, histones play a central role in regulating gene expression and thus biological function. How DNA is packaged, how tightly the nucleosomes are assembled, and how histone variants are incorporated all affect whether specific gene regions are accessible to transcription factors and other regulators. This regulatory capacity is the result of a rich repertoire of post-translational modifications (PTMs) and histone variants, which dynamically alter in response to environmental and cellular cues. For example, acetylation of lysine residues often disrupts histone-DNA contacts and favors transcriptional activation, while specific lysine methylations are linked to repressed chromatin states. With these mechanisms, histones occupy a central position in epigenetic regulation, which is increasingly recognized for its pivotal role in disease mechanisms and as a promising target for therapeutic intervention.

We aimed to develop a novel, automated sample preparation workflow for histone analysis that is compatible with multi-omics analysis. The method, termed HS-Trap, combines suspension trapping (S-Trap) technology with a promising derivatization reagent, trimethylacetic acid (TMA). When comparing TMA to the current derivatization with propionic acid (PA), it introduces a larger chemical group, which considerably enhances chromatographic separation and improves the identification of several histone isoforms. In addition, it reduces chemical artifacts while maintaining high data quality and reproducibility. By integrating a suspension filter system and 96-well plates, the workflow supports automation with a pipetting robot, which minimizes chemical and technical variability and substantially increases throughput. This is of high need as epigenetic dynamics are increasingly being investigated with large-scale studies and time-resolved experimental designs. Furthermore, we optimized histone extraction by replacing strong acids with acidified organic solvent mixtures, which improved the simultaneous extraction of the proteome, metabolome, and histone fractions from a single cell pellet.

In conclusion, this workflow paves the way for exploring cross-omics correlations and identifying epigenome-associated disease markers.

Poster 14

Presenter
Manel Berkemal

Affiliation:
Team Gofflot, LIBST, UCLouvain

Title:
Integrative multi-omics profiling of HOXA5 : new insights in neuronal cells.

Abstract:

HOXA5, a member of the HOX family of transcription factors, is a master regulator of morphogenesis and cell differentiation in a broad spectrum of tissues and organs. HOXA5 also plays important roles in successive steps of central nervous system (CNS) formation during embryonic and fetal development but less is known about its regulation and function at later stages. Our team provided evidence that HOXA5 is functional in the hindbrain during the first postnatal weeks, where it could participate in maturation of neuronal circuits after birth (Lizen et al., 2017a; Lizen et al., 2017b). To fully apprehend HOXA5 importance in late processes of CNS late development, it is essential to first characterize its transcriptional activity by identifying its direct genomic targets in neuronal cells and further to explore how the protein's regulation, stability and molecular interactions contribute to its overall function.To address these questions, we selected an in vitro model, the pluripotent murine P19 cell line, which can be efficiently differentiated into neurons thanks to a well-established protocol, combining retinoic acid treatment and embryoid body (EB) formation. This model provides a controlled system to study both neural progenitors (EB stage) and post-mitotic neurons at immature and mature stages. We first mapped HOXA5's genome-wide binding sites using Chromatin Immunoprecipitation followed by massively parallel Sequencing (ChIP-seq) at the EB stage. This identified consensus binding motifs, novel binding sites, and a set of direct target genes. Complementary to this, we generated an HOXA5 knockout (KO) P19 cell line using CRISPR-Cas9 and performed comparative proteomic analysis*, between KO and wild-type cells, revealing 1741 differentially expressed proteins upon HOXA5 loss. By overlapping these datasets, we were able to discriminate direct and indirect impact of HOXA5 loss. Indeed, for 114 out of the 1174 downregulated proteins, corresponding genes were identified in ChiP-Seq data suggesting direct targets (9.7% overlap). Similar observations were made for 68 out of the 567 upregulated proteins (12.0% overlap). Gene Ontology (GO) analysis of these direct targets revealed a significant enrichment for core neurodevelopmental processes, including Neural tube development, Axon development, or Neuron projection morphogenesis.

Poster 15

Presenter
Denzel Eggermont

Affiliation:s
Francis Impens Lab,VIB-UGent Center for Medical Biotechnology

Title:
Multi-Omics Characterization Reveals ISGylation as a Key Regulator of GAPDH in Glycolytic Control

Abstract:

Ubiquitin-like protein ISG15 (interferon-stimulated gene 15) has been implicated in the regulation of central carbon metabolism, but conflicting findings across experimental systems have limited mechanistic insight. Here, we applied a multi-omics approach in cells ectopically expressing the ISGylation machinery independent of immune stimuli, to generate a systematic view of ISGylation in metabolic control. We found that ISGylation predominantly modifies metabolic proteins, including key glycolytic enzymes, and is associated with suppression of the energy-producing phase of glycolysis. Tracer metabolomics indicated a bottleneck at the steps catalyzed by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase 1 (PGK1), marked by accumulation of substrates and depletion of downstream metabolites. Functional assays demonstrated that ISGylation directly impairs GAPDH activity, and structural analysis revealed that ISG15 modifications cluster near structural elements important for catalysis. These findings identify GAPDH as a central metabolic checkpoint regulated by ISGylation and uncover a direct post-translational mechanism by which ISG15 controls energy metabolism.

Poster 16

Presenter
Bradley Ward
Affiliation:s

Integrated Pharmacometrics, Pharmacogenomics and Pharmacokinetics - **PMGK**
Louvain Centre for Toxicology and Applied Pharmacology **- LTAP**
Institut de recherche expérimentale et clinique - **IREC**

Title:
Longitudinal multi-omics network analysis reveals complement–coagulation rewiring in Long COVID

Abstract:

Post-acute sequelae of COVID-19 (PASC) is heterogeneous and mechanistically unresolved. We profiled matched plasma proteomics, metabolomics and whole-blood transcriptomics at acute infection and convalescence (~3-month later) in a Belgian cohort. Late-stage integrative multi-omic analysis via linear mixed models and multi-omic GSEA revealed sustained enrichment of complement cascade pathways from acute illness to follow-up in PASC compared with recovered patients.

Correlation networks integrated with prior-knowledge databases were explored using a novel differential-correlation rewiring approach to highlight dysregulated components. Among the top rewired nodes, coagulation regulators heparin cofactor II (SERPIND1) and alpha-1 antitrypsin (SERPINA1) and complement regulator complement factor H-related 5 (CFHR5) appear of particular interest due to their high network dysregulation and their overall importance to the regulation of the complement and coagulation pathways. Additionally, multiple minor hints appear to implicate prothrombin/thrombin (F2) with PASC, indicating altered thrombin context rather than large abundance changes. It is noteworthy that these insights only became obvious through an integrative network analysis as single-omic tests (limited by heterogeneity and small effects) yielded few FDR-significant hits.

These findings provide a systems framework for mechanistic validation and for exploring targeted interventions at the complement–coagulation–platelet interface in Long COVID.

Poster 17

Presenters :
Caroline Jachmann
Robbe Devreese
Robbin Bouwmeester

Affiliations :
Compomics, VIB-UGent Center for Medical Biotechnology

Title:
Proteobench

Abstract:

Introduction
Many instruments and data acquisition strategies are available for MS-based proteomics, and the data they acquire differs substantially and thus require tailored analysis algorithms. Dedicated bioinformatics workflows are in constant evolution and are often compared in published benchmark papers that are rarely comparable to each other, stand on their own, and can quickly become outdated. ProteoBench (proteobench.readthedocs.io) is a standalone platform that brings together software developers and users to provide an ever-evolving comparison of state-of-the-art proteomics data processing tools, workflows or analysis steps.
Methods
This open resource is structured in independent benchmark modules proposed and openly discussed by the community. Each module is designed to benchmark a specific aspect of proteomics data analysis. ProteoBench now hosts three active modules, three more are in development, and five are in discussion. Anyone can contribute by making workflow results publicly available, proposing new modules and/or contributing to the development of the platform.
Results
We will present the comparison of results available in currently active ProteoBench modules dedicated to DDA and DIA data quantification of precursor ions, from thirteen different software tools. These modules use a three-species benchmark dataset to evaluate the quantification depth and accuracy of data analysis workflows. Results show significant differences between tools in terms of both the number of quantified precursors and the quantification error. We will demonstrate how the ProteoBench API can be used to mine public ProteoBench data to explain differences between workflows, explore the impact of specific parameters, and highlight the advantages of having a constantly evolving benchmark.
Conclusions
This community endeavor allows the continuous benchmarking of workflow outputs, helps determining the impact of specific parameters, and provides a frame of reference for developers to benchmark their newly developed tools.

Poster 18

Presenter
Ina Devos
Affiliation:s
Centre for Ethics,Department of Philosophy
Title:
Ethics of Proteomics: A Scoping Review

Abstract:

Proteomics research and applications are becoming increasingly relevant for biomedical and clinical contexts, which raises the ethical stakes. However, while bioethical scholarship has substantially engaged with human genomics, human proteomics has been largely ignored as a topic of interest. Current bioethical engagement is sparse and largely comes from life science researchers themselves. In our scoping review, we investigated their publications to map the ethical topics and common narratives in discussions of ethics in human proteomics. We employed a two-stage analysis of included publications. First, we identified the topics discussed as being ethically relevant. Second, we identified common narratives around these topics using thematic analysis. This approach provides an overview of the content, structure, and tensions of scholarly discussions on the ethics of human proteomics.
In the 37 included publications, we identified 22 ethical topics of discussion. Most of the topics, such as public benefits, privacy, and incidental findings, are familiar topics from the more general ethics of biotechnologies. Additionally, our thematic analysis highlights 6 common narratives: (1) ethics of proteomics requires multidisciplinary attention, (2) aspirational benefits of (open) proteomics, (3) protection of individuals, (4) balancing the benefits/individual protections trade-off, (5) resources and equity in proteomics, and (6) dimensions beyond the proteomics experiment. Investigating these highlights some unaddressed assumptions and tensions in the ethics discussions. For example, we identified a perceived link between aspired societal benefits and openly available proteomic data, on the one hand, and between the protections afforded to individual participants or data subjects and the limitations of proteomic data availability, on the other.
We suggest that these assumptions inform an unhelpful trade-off narrative between realizing aspired societal benefits and protecting individual research subjects. It strongly limits the possibility for nuanced, complex and creative approaches in which to interests of those seeking protection are aligned, rather than oppositional to those of research and the ‘public good’. Therefore, we argue for interdisciplinary proteomic-bioethical research that critically examines these commonly discussed issues, concepts, and narratives, while closely engaging with the specificities and realities of proteomics research practices.