20th Central and Eastern European Proteomic Conference

June 14 – 17, 2026 | Zagreb, Croatia

BOOK OF ABSTRACTS

Welcome to the 20thCEEPC Zagreb 14-17 June 2026

Dear Participants,

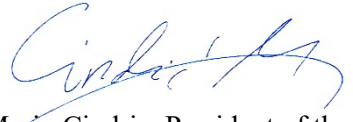
It is our great pleasure to welcome you to this year's CEEPC, held for the first time in Zagreb, Croatia. This event is organised to foster connectivity, knowledge and student exchange, and protein science across borders. Over the past three years, conferences have taken place in Vilnius, Vienna, and Budapest, and now in Zagreb, connecting East and West geographically, and the whole world scientifically.

Croatia hosts two main mass spectrometry events each year: the Workshop on Mass Spectrometry in Life Sciences (Zagreb) and Mass Spectrometry in Biotechnology and Medicine (Dubrovnik). This year, the 20th CEEPC will contribute significantly to the mass spectrometry community.

We hope this conference will foster new partnerships and future discoveries that will shape the future of proteomics, biomedical, and biotech research in Croatia, Europe, and beyond.

Thank you for joining us. We look forward to making your stay at the conference pleasant and fruitful.

Warm regards,



Mario Cindric, President of the Organizing Committee and the Organizing team

Marijana Erk	Rudjer Boskovic Institute
Renata Biba	Croatian Mass Spectrometry Society
Amela Hozić	Proconventa d.o.o.
Lucija Vujević	Suresh Jivan Gadher and CEEPC
Conscius d.o.o.	country representatives

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE | June 14 – 17, 2026 Zagreb, Croatia

PRELIMINARY PROGRAM

SUNDAY | JUNE 14	
17.00-18.00	Registration and coffee
18.00-18.10	20thCEEPC Welcome - Prof. Mario Cindrić
18.10-19.00	**Keynote Lecture** Prof. David Goodlett A BRIEF HISTORY OF PROTEOMICS: FROM PROTEIN CHEMISTRY TO SPATIAL PROTEOMICS
19.00	Get together – wine and snacks

MONDAY | JUNE 15	
Morning Session | Cancer Proteomics | Chair: Dr. Suresh Jivan Gadher	
09.00-10.00	Morning coffee
10:00–10:10	Dr. Suresh Jivan Gadher: Celebrating 20 years of CEEPC milestone
10.10-11.00	**Keynote Lecture** Prof. Ted Hupp DEVELOPMENT OF NOVEL ANTIBODY-BASED DETECTION PLATFORM FOR USE IN OESOPHAGEAL ADENOCARCINOMA
11.00-11.35	Prof. Javier Alfaro A PHYSICAL-EVIDENCE-BASED ATLAS REVEALS HOW MHC CLASS I PEPTIDE PROMISCUITY SHAPES CANCER ANTIGEN LANDSCAPES (20min lecture + Q&A)
11.35-12.10	Dr. Irena Đapić NOVEL MOLECULAR TECHNOLOGIES FOR INVESTIGATION OF TISSUES AND BIOFLUIDS (20min lecture + Q&A)
12.10-12.45	Dr. Katarina Davalieva FROM TISSUE PROTEOMICS TO URINE BIOMARKERS IN UROLOGICAL CANCERS: AN INTEGRATIVE PIPELINE FOR DISCOVERY AND VALIDATION (20min lecture + Q&A)
12.45-13.00	An open forum and round-table discussions
13.00-14.00	Lunch break

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE | June 14 – 17, 2026 Zagreb, Croatia

PRELIMINARY PROGRAM

Afternoon Session MultiOmics & Proteomics | Chair: Dr. Manghes Bhide	
14.00-15.00	**Keynote Lecture** Prof Laura Bindila MULTI-OMICS STRATEGIES FOR NEURO-IMMUNO METABOLISM PHENOTYPING IN HEALTH AND DISEASES
15.00-15.35	Dr. Kathirvel Alagesan REBEL, REBEL: BREAKING NORMS WITH PROTEASE MULTIPLEXING FOR GLYCOPROTEOMICS (20min lecture + Q&A)
15.35-16.10	Dr. Anita Horvatić DECODING MOLECULAR INTERPLAY IN CANINE INTERVERTEBRAL DISC HERNIATION: A MULTI-OMICS PERSPECTIVE (20min lecture + Q&A)
16.10-16.45	Prof. Dr. Frank Schmidt MULTI-PROTEOMICS REVEALS SIGNIFICANT PROTEIN AND INFECTION-RELATED ANTIBODY ALTERATIONS IN TYPE 2 DIABETES (20min lecture + Q&A)
16.45-17.15	Cofee Break
Top-down Bottom-up proteomics | Dr. Katarina Davalieva	
17.15-17.50	Dr. Renata Biba DIRECTED PEPTIDE FRAGMENTATION ENABLES DE NOVO SEQUENCING FOR BIOTYPIZATION OF COMPLEX PROTEOMES (20min lecture + Q&A)
17.50-18.25	Dr. David Kilgour EXORCISING THE GHOSTS IN YOUR DATA: A MULTILAYER APPROACH TO FILTERING OUT SUSPECT TOP-DOWN ASSIGNMENTS (20min lecture + Q&A)
19.00	Wine and snacks | Poster session with designated committee

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE | June 14 – 17, 2026 Zagreb, Croatia

PRELIMINARY PROGRAM

TUESDAY | JUNE 16	
Morning Session | Emerging New Technologies, Single Cell & Cell Proteomics | Chair: Dr. David Goodlett	
9.00-10.00	Morning coffee | Poster session, Q&A
10.00-11.00	**Keynote Lecture** Prof. Dr. Ljiljana Paša-Tolić FROM SINGLE CELLS TO TISSUES: ADVANCED SAMPLING AND MASS SPECTROMETRY FOR ULTRASENSITIVE SINGLE-CELL AND SPATIAL PROTEOMICS AND METABOLOMICS
11.00-11.35	Dr. Peter Verhaert SPATIAL ATMOSPHERIC PRESSURE IONIZATION HIGH-RESOLUTION MS ANALYSIS OF ENDOGENOUS (NEURO)PEPTIDES AND METABOLITES IN PATHOLOGISTS' FFPE SAMPLES (20min lecture + Q&A)
11.35-12.10	Ass. Prof. Gabriel Mazzucchelli FROM SINGLE CELLS TO LARGE COHORTS: BOOSTING PROTEOMICS THROUGH ENHANCED PEPTIDE RECOVERY AND ROBUST LC-MS PERFORMANCE (20min lecture + Q&A)
12.10-12.45	Dr. Ondrej Lacina (Altium international) DATA QUALITY AS THE FOUNDATION: AUTOMATED SAMPLE PREPARATION AND PROTEIN CHARACTERIZATION IN LIFE SCIENCE RESEARCH (20min lecture + Q&A)
12.45-13.10	Dr. Karla Košpić (Biocentar Zagreb) PROTEOMICS HELPDESK: A CRO PERSPECTIVE ON FEASIBILITY, CONSTRAINTS, AND ANALYTICAL STRATEGY SELECTION (20min lecture + Q&A)
13.00-14.00	Lunch break
Afternoon Session | Antimicrobial Proteomics | Dr. Laura Bindila	
14.00-15.00	Dr. Manges Bhide TARGETED DELIVERY SYSTEM TO TRANSPORT ANTIMICROBIAL PEPTIDES ACROSS BLOOD BRAIN BARRIER (20min lecture + Q&A)
15.35-16.10	Assist Prof Marko Močibob PROTEOMIC PROFILING OF BACILLUS THURINGIENSIS RESPONSES TO NATURAL INHIBITORS TARGETING AMINOACYL-TRNA SYNTHETASES (20min lecture + Q&A)
16.10-16.45	Prof Biljana Balen SURFACE COATING-DEPENDENT PHYTOTOXICITY OF SILVER NANOPARTICLES: ANTIOXIDANT AND PROTEOMIC RESPONSES IN PLANTS AND GREEN ALGAE (20min lecture + Q&A)
16.45-17.15	Coffee Break

Afternoon Session | Foodomics | Chair: Dr. Biljana Balen	
17.15-17.50	Dr. Ramesh Katam ROOTH PROTEOMICS INSIGHT INTO SALINITY-INDUCED METABOLOMIC SWITCHING IN PISTACHIO ROOTSTOCKS (20min lecture + Q&A)
17.50-18.25	Dr. Josipa Kuleš DON'T CRY OVER SPILLED MILK: TRANSLATING MILK PROTEOMICS INTO RAPID DIAGNOSTIC TOOLS FOR SUSTAINABLE DAIRY PRODUCTION (20min lecture + Q&A)
18.25-19.00	Dr Danijela Bakarić TEMPERATURE-INDUCED DENATURATION OF BOVINE SERUM ALBUMIN: RECONCILING THE RESPONSES OBTAINED BY DIFFERENT EXPERIMENTAL TECHNIQUES (20min lecture + Q&A)
20.00	Conference Dinner

WEDNESDAY | JUNE 17	
Morning Session | Biomedical Proteomics | Chair: Dr. Mario Cindrić	
9.00-10.00	Morning coffee
10.00-11.00	**Keynote Lecture** Dr. Suresh Jivan Gadher SIMULTANEOUS MONITORING OF GENE AND PROTEIN EXPRESSION IN HIV PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY AND AT INCREASED RISK OF AIDS-DEFINING CANCERS
11.00-11.30	Prof. Lovorka Grgurević PROTEOMICS IN BIOMEDICAL RESEARCH WITH EMPHASIS MUSCULOSKELETAL DISEASES AND TUMOR BIOMARKERS (20min lecture + Q&A)
11.30-12.00	Prof. Jyotsna Batra IDENTIFICATION OF A MICROPEPTIDE LINKED TO CANCER STEM CELL REGULATION AND CHEMORESISTANCE (20min lecture + Q&A)
12.00-12.15	Dr. Mario Cindrić: Best Poster Awards and Concluding remarks
12.15-12.30	21thCEEPC

Welcome to the 20thCEEPC Zagreb 14-17 June 2026

Dear Participants,

It is our great pleasure to welcome you to this year's CEEPC, held for the first time in Zagreb, Croatia. This event is organised to foster connectivity, knowledge and student exchange, and protein science across borders. Over the past three years, conferences have taken place in Vilnius, Vienna, and Budapest, and now in Zagreb, connecting East and West geographically, and the whole world scientifically.

Croatia hosts two main mass spectrometry events each year: the Workshop on Mass Spectrometry in Life Sciences (Zagreb) and Mass Spectrometry in Biotechnology and Medicine (Dubrovnik). This year, the 20th CEEPC will contribute significantly to the mass spectrometry community.

We hope this conference will foster new partnerships and future discoveries that will shape the future of proteomics, biomedical, and biotech research in Croatia, Europe, and beyond.

Thank you for joining us. We look forward to making your stay at the conference pleasant and fruitful.

Warm regards,

Mario Cindric, President of the Organizing Committee and the Organizing team

Marijana Erk
Renata Biba
Amela Hozić
Lucija Vujević
Conscius d.o.o.
Rudjer Boskovic Institute
Croatian Mass Spectrometry Society
Proconventa d.o.o.
Suresh Jivan Gadher and CEEPC country representatives

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE

June 14 – 17, 2026
Zagreb, Croatia

Keynote Lectures

A BRIEF HISTORY OF PROTEOMICS: FROM PROTEIN CHEIMSTRY TO SPATIAL PROTEOMICS

D. R. Goodlett[1]

1) University of Victoria, Department of Molecular Life Sciences, Victoria, BC, CANADA

We will review the technical developments leading to what we now refer to as shotgun proteomics or bottom-up proteomics as most often practiced for qualitiative description of the proteins in a sample (1). This history covers the time span from the late 1970s, when the triple quadrupole mass spectrometer was developed, to the late 1990s, when data dependent acquisition came in to play, demonstrating how the basics of nanoLC-ESI-MS were ready and waiting from work in protein chemistry and immunopeptidomics when the term proteomics was coined in 1994. It will highlight how being clever is critical to success in science and often more important than simpling being 'smart'. Futhermore, the development of data independent methods that came out of work in the early 2000s will also be reviewed (2). Finally, we will finish with how the revolution in single cell proteomics has empowered direct from specimen analysis (3). Such analysis of proteomes directly from the environment in which they were produced *in vivo,* as opposed to proteomes defined after *ex vivo* expansion, will be discussed as the next wave in discovery science, aka hypothesis generation (4). We will finish with discussion of the intersection of mass spectromety imaging and bottom-up protemics, so-called spatial proteomics.

References

1. Aebersold, R and Goodlett, DR (2001) Mass spectrometry in proteomics. *Chem Rev*. Feb;101(2):269-95. PMID: 11712248.
2. Chapman JD, Goodlett DR, Masselon CD. (2014) *Mass Spectrom Rev*. Nov-Dec;33(6):452-70. PMID: 24281846
3. Eshghi A, Xie X, Hardie D, Chen MX, Izaguirre F, Newman R, Zhu Y, Kelly RT, Goodlett DR. (2023) Sample Preparation Methods for Targeted Single-Cell Proteomics. *J Proteome Res*. 2023 Jun 2;22(6):1589-1602. PMID: 37093777
4. Nartey LK, Weke K, Yuen V, Kibsey P, Chen MX, Goodlett DR. (2026) Proteomic Adaptations of *Escherichia coli* in Urinary Tract Infection Patients. *J Proteome Res*. 2026 Feb 16. PMID: 41698857

DEVELOPMENT OF NOVEL ANTIBODY-BASED DETECTION PLATFORM FOR USE IN OESOPHAGEAL ADENOCARINCOMA

Jack Brydon[1],Tomas Henek[2],Lenka Hernychova[2],Radovan Krejci[2],Borivoj Vojtesek[2,3],JR O'Neill[4,5],Ted Hupp[1,]

1 Institute of Genetics and Cancer (IGC), University of Edinburgh, Edinburgh, Scotland, UK
2 Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic.
3 Laboratory of Growth Regulators, Institute of Experimental Botany, The Czech Academy of Sciences, Olomouc, Czech Republic
4 Cambridge Oesophagogastric Centre, Addenbrooke's Hospital, Hills Rd, Cambridge CB2 0QQ
5 Department of Surgery, University of Cambridge, Cambridge, United Kingdom

Abstract

Oesophageal adenocarcinoma (OAC) is a GI cancer of high unmet clinical need. Identifying highly penetrant thersanostic targets is required to improve patient outcomes. Using cancer tissue and two normal adjacent tissues (gastric and squamous epithelium), mass spectrometry methodologies were applied in individual patient tissue sets to identify patterns of tumour specific proteins. One proteome pattern identified GPA33[1,2] to be a superior tumour-specific target to an emerging theranostic target (Claudin18.2)[3]. An OAC tumour microarray including normal adjacent squamous, normal gastric tissue, and involved lymph nodes, was used to compare the expression of GPA33 and with other theranostic targets including Claudin18.2, HER2, and PD-L1. GPA33 and Claudin18.2 generally exhibit mutually exclusive expession in cancer tissue indicating they represent two distinct diagnostic groups[4]. A scFv-phage display library[5] was screened against GPA33 to isolate novel antibodies that were evaluated for their utility as a theranostics.

Conclusions

Mass spectrometry was applied to identify patterns of proteins correlating with OAC tumour tissue. Claudin18.2 and GPA33 usually exhibit mutually exclusive expression indicative of two different OAC progression pathways. These features indicate that GPA33 forms a compelling clinically relevant target in OAC. The antibody discovery pipeline we present includes scFv library screening against a native folded membrane receptor domain which yielded a monoclonal antibody with an unusual redox-regulated epitope. This provides a novel diagnostic and therapeutic tool for use in OAC.

References

1 O'Neill, J. R. et al. Quantitative Shotgun Proteomics Unveils Candidate Novel Esophageal Adenocarcinoma (EAC)-specific Proteins. Mol Cell Proteomics 16, 1138-1150, (2017).

2 O'Neill, J. R. et al. Multi-Omic Analysis of Esophageal Adenocarcinoma Uncovers Candidate Therapeutic Targets and Cancer-Selective Posttranscriptional Regulation. Mol Cell Proteomics 23, 100764, (2024).

3 Carstens, E. J. et al. Modeling and addressing on-target/off-tumor toxicity of claudin 18.2 targeted immunotherapies. Nat Commun 16, 9651, (2025).

4 Brydon, J. e. a. Targetting a Redox Regulated Epitope in GPA33 Enables a Novel Antibody-based Detection Platform for Oesophageal Adenocarincoma Cellular and Molecular Biology Letters accepted for publication (2026).

5 Lisowska, M. et al. The development of a canine single-chain phage antibody library to isolate recombinant antibodies for use in translational cancer research. Cell Rep Methods 5, 101008, (2025).

MULTI-OMICS STRATEGIES FOR NEURO-IMMUNO METABOLISM PHENOTYPING IN HEALTH AND DISEASES

Laura Bindila

Clinical Lipidomics Unit, Institute of Physiological Chemistry, University Medical Center of JGU Mainz, Germany

Many of the pressing chronic diseases, including neurodegenerative, cardiovascular, immune diseases, and cancer, affect multi-organ function across brain-periphery axis leading to compounded consequences of multiple organ failures and challenges in disease management. Therefore, modern medical research is increasingly shifting the paradigm from organ-centric to multi-organ, systemic investigation, and disease management.

Multi-omics strategies of clinical samples are essential in advancing systemic medicine investigation of diseases, enabling maximum output of molecular fingerprints and integrated pathways. Analytical pipelines enabling efficient co-extraction of multiple molecular classes: proteins and genes, proteins and metabolites, metabolites and genes from multiple sample types: cells, tissues, retrospective tissues, biological fluids, etc are in development and in focus for system medicine and systems biology investigation.

Integrated lipidomics and transcriptomic approaches and pipelines tailored to immune organs and central nervous system as well as cell models of inflammatory diseases will be here presented and discussed. Applications of such pipelines in Parkinson, cardiovascular diseases and cancer will be discussed for their potential to phenotype the neuro-immuno metabolism in patient subgroups and determine bioactive molecular fingerprints for disease and therapeutic targets.

Modern, 4D-lipidomic approaches combined with RNA sequencing or targeted RNA anaylsis enable a higher resolution view of the metabolic switch with diseases than classical clinical lipid markers.

References

1. Baker D, Gonzalez Escamilla G, Janitschke D, Devaux Y, Schröter N, Groppa S, Bindila L. (2025) prm-PASEF-Based Quantification and Isomeric Model for Extended Coverage of Human Plasma Lipidome in Parkinson's Disease. *Anal Chem*. 97(44):24295-24305. PMID: 41143626.

2. Giang VH, Gonzalez-Escamilla G, Mirzac D, Rotaru L, Herz D, Groppa, and Bindila L (2025). Extended coverage of human serum glycosphingolipidome by 4D-RP-LC TIMS-PASEF unravels association with Parkinson's disease. Nat. Commun. 16(1):4567. PMID: 40379659.

3. Del Barrio Calvo C, Bindila L. (2024). Integrated cellular 4D-TIMS lipidomics and transcriptomics for characterization of anti-inflammatory and anti-atherosclerotic phenotype of MyD88-KO macrophages. Front Cell Dev Biol. 12:1450971. eCollection.PMID: 39247623

4. Post JM, Lerner R, Schwitter C, Lutz B, Lomazzo E, Bindila L. (2022) Lipidomics and Transcriptomics in Neurological Diseases. J Vis Exp. 18;(181). doi: 10.3791/59423.

FROM SINGLE CELLS TO TISSUES: ADVANCED SAMPLING AND MASS SPECTROMETRY FOR ULTRASENSITIVE SINGLE-CELL AND SPATIAL PROTEOMICS AND METABOLOMICS

L. Paša-Tolić[1]

1) 1Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA

This presentation highlights recent advances that boost the sensitivity, throughput, and spatial resolution of mass spectrometry-based omics, from single cells to intact tissues, with an emphasis on proteomics workflows. We introduce a high throughput single-cell proteomics platform combining nanodroplet sample preparation (nanoPOTS), dual-column LC, TMTpro 32 plex labeling, and real-time spectral-library searching. This system quantifies thousands of proteins across more than 1,000 primary immune cells, resolving major immune cell classes and revealing new protein-level markers. We then highlight spatial proteomics and metabolomics approaches that pair advanced tissue-sampling strategies with high-resolution MS imaging (MSI) to map proteins, metabolites, and lipids directly in tissues capturing molecular variation in situ. We demonstrate an integrated spatial multiomics workflow in a plant-pathogen model of sorghum anthracnose (*Colletotrichum sublineola*). Using laser capture microdissection, nanoPOTS LC-MS proteomics, and MALDI-MSI, we quantify ~7,000 plant proteins across diverse cell types within their native tissue context, and ~3,000 fungal proteins in infection zones, while spatial metabolomics reveals genotype-specific metabolic gradients. These datasets capture pathway activation and biomolecular signatures across disease progression and biocontrol conditions. Together, these developments define a unified framework for probing cellular heterogeneity, tissue organization, and host-pathogen interactions omics across diverse biological systems.

SIMULTANEOUS MONITORING OF GENE AND PROTEIN EXPRESSION IN HIV PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY AND AT INCREASED RISK OF AIDS-DEFINING CANCERS

D. Vangelov[1], R. Emilova[1], Y. Todorova[1], S.J. Gadher[2], M. Nikolova[1]

1) National Centre of Infectious and Parasitic Diseases, 1504 Sofia, Bulgaria.

2) Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008 USA.

Keywords: HIV, ART, immune monitoring, viral reactivation, chronic inflammation

The ability to simultaneously measure multiple secreted proteins and the corresponding gene expression levels of HIV patients receiving continuous antiretroviral therapy (cART) is prerequisite to ensuring patient benefits and long-term effectiveness of ART treatment. Interrogation of such patient samples for molecular signature of the disease together with clinical parameters such as mitochondrial mass, incomplete immune reconstitution, and CD4+ T-cell counts in people living with HIV (PLWH), may help better understand disease progression and efficacy of treatment.

Here, we demonstrate messenger RNA (mRNA) expression using QuantiGene™ assay on a multiplexing platform. Human peripheral blood mononuclear cells (hPBMCs) stimulated with phytohemagglutinin (PHA) were harvested at 24 hours and assayed for specific mRNA targets on a QunatiGene Human 8plex panel. Quantification of CCL2, CLDN5, CSF2, CXCL8, IL17F, IL25, IL6 and OCLN genes were performed on a multiplexing system.

Additionally, whole blood samples from PLWH on continuous ART (A, n=15), with CD4AC >500 cells/µl and CD4/CD8 ratio > 0.9, or untreated (B, n=10) were collected during routine immune monitoring. Mitochondrial mass (MM), mitochondrial membrane potential (MMP) and mitochondrial superoxide levels (MSL) in CD4 and CD8 T cells were determined using flow cytometry

Conclusion

Increased MSL in T lymphocytes of cART- PLWH suggested elevated oxidative stress and possible mitochondrial damage. Additionally, higher MM found in cART- suggested mitochondrial health disruption as well as on-going biogenesis. Complementing whole blood assays with hPBMCs based Quantigene mRNA assay, provided a more holistic view of the antiretroviral therapy for HIV by helping to screen for molecular signature of the disease at gene level. Such a combined approach can elevate patient blood sample screening to a 'multi-omics' level, providing a high-level interrogation of clinical parameters concurrently with gene expression networks of the disease.

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE

June 14 – 17, 2026
Zagreb, Croatia

Talks

A PHYSICAL-EVIDENCE-BASED ATLAS REVEALS HOW MHC CLASS I PEPTIDE PROMISCUITY SHAPES CANCER ANTIGEN LANDSCAPES

Michal Mateusz Waleron[1,2,3,†], Aleksander Palkowski[1,2,3,†], Ashwin Adrian Kallor[1,†], Emilia Daghir-Wojtkowiak[1,†], Piyush Borole[4], Mikolaj Kocikowski[1], Karol Polom[5,6], Fabio Marino[7], Beatriz Monterde[8], Michele Mastromattei[9], Davide Venditti[9], Mark Stares[10], The KATY Consortium, Ashita Singh[10], Theodore Hupp[10], Christophe Battail[11], Catia Pesquita[12], Luis Zapata[8], Stefan N. Symeonides[10], Ajitha Rajan[4], Alexander Laird[10,13,14,‡,], Fabio Massimo Zanzotto[9,‡,*], Javier Antonio Alfaro[1,2,3,4,‡,*]*

1) *International Centre for Cancer Vaccine Science, University of Gdansk, Poland*
2) *The Riddell Centre for Cancer Immunotherapy, University of Calgary, Canada*
3) *Department of Biochemistry and Molecular Biology, University of Calgary, Canada*
4) *School of Informatics, University of Edinburgh, Informatics Forum, United Kingdom*
5) *Academy of Applied Medical and Social Sciences, Poland*
6) *Gastrointestinal Surgical Oncology Department, Greater Poland Cancer Centre, Poland*
7) *Faculty of Science, Biomolecular Mass Spectrometry and Proteomics, Utrecht, The Netherlands*
8) *The Institute of Cancer Research, United Kingdom*
9) *Department of Enterprise Engineering, University of Rome Tor Vergata, Italy*
10) *Cancer Research UK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, UK*
11) *Université Paris-Saclay, CEA, France*
12) *LASIGE, Faculdade de Ciências da Universidade de Lisboa, Portugal*
13) *Institute of Genetics and Cancer, The University of Edinburgh. United Kingdom*
14) *Department of Urology. Western General Hospital., University of Edinburgh, United Kingdom*

† *These authors are joint-first equal-contributing authors.* ‡ *These authors jointly-directed this work*

MHC ligands can be strikingly promiscuous: some peptides bind across multiple MHC alleles, and some alleles present unusually broad repertoires, although many peptide–allele pairings remain restricted and neither trend is universal. Understanding how neoantigens are shared across diverse human leukocyte antigen backgrounds can guide selection of immunotherapy targets with wider population coverage and improve patient stratification across modalities. Here we define antigen-presentation promiscuity at five levels: peptides, MHC alleles, individuals, populations, and genomic regions. We operationalize these levels in the CARMEN release, harmonizing 2,323 samples from 72 papers spanning tissues, with an emphasis on cancer. From this dataset, we learn a low-dimensional representation and derive a quantitative promiscuity metric based on physically detected presentation. We then map promiscuity and immunological versatility across all five levels. Gene and mutation analyses highlight recurrent cancer mutations in highly promiscuous regions, highly mutated genes that appear to evade presentation, and genomic features associated with immunotherapy response in clinical cohorts.

NOVEL MOLECULAR TECHNOLOGIES FOR INVESTIGATION OF TISSUES AND BIOFLUIDS

Irena Đapić[1], Renata Biba[2], Lucija Vujević[2], Amela Hozić[2], Ana Turčić[3], Željka Vogrinc[3] and Mario Cindrić[2]

1) Laboratory for Synthetic methodologies in Organic Chemistry, Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute
2) Laboratory for Bioanalytics, Department of Molecular Medicine, Ruđer Bošković Institute
3) Department of Laboratory Diagnostics, University Hospital Centre Zagreb

Proteome profiling of biological material with the advance of novel technologies has progressed in its ability to give detailed molecular insights from human tissues and biofluids. Proteomics allows us direct examination of proteins, revealing small molecular changes often missed by other techniques. Even tough mass spectrometry is essential tool for proteomic analysis in tissues and biofluids majority of the challenges are represented by the size and heterogeneity of the tumours as well as their location (i.e. within the brain). Thus it is essential to develop sensitive and robust methods for understanding tumor biology and to develop targeted therapies.

Here we focused on development of platforms for detecting proteins in different sample types such as small biopsy-sized tissue samples in glioblastoma (GBM) and cerebrospinal fluid from multiple sclerosis patients. In GBM we investigated how tissue type affects protein detection and the overall sensitivity of our methods by comparing various protein extraction and analytical techniques. Additionally, we are examining how MDM2 inhibition influences protein and peptide presentation in GBM cells, utilizing novel fluorophore-peptide probes to explore MDM2's role in regulating MHC peptide presentation. To this end we synthesized several peptide-fluorophore conjugates and studied their interactions with MDM2.

By applying multidimensional chromatography we analyzed cerebrospinal fluid samples from multiple sclerosis patients. To achieve this we employed various stationary phases such as QMA, SCX, and HLB to explore the specific properties of peptides after sample fractionation. Developing this comprehensive platform enabled us to detect a greater number of disease-related proteins which might give deeper insights into the molecular mechanisms underlying multiple sclerosis.

From Tissue Proteomics to Urine Biomarkers in Urological Cancers: An Integrative Pipeline for Discovery and Validation

Katarina Davalieva

Research Centre for Genetic Engineering and Biotechnology "Georgi D Efremov", Macedonian Academy of Sciences and Arts, Krste Misirkov 2, 1000 Skopje, North Macedonia

Urological malignancies such as prostate cancer (PCa) and bladder cancer (BCa) present significant clinical challenges due to limitations in early detection, risk stratification, and disease monitoring. Although proteomics has substantially advanced our understanding of these cancers, the translation of candidate biomarkers into clinically applicable, non-invasive tests remains limited. Here, we present an integrative proteomics strategy for biomarker discovery and validation in urological cancers.

Our work in PCa encompasses multiple tissue and body fluid proteomics studies employing 2D-DIGE and LC-MS/MS platforms, enabling deep proteome coverage and identification of proteins associated with disease initiation and progression. Correlation with clinical parameters revealed a subset of proteins associated with Gleason score and tumor stage, supporting their relevance for disease progression. Integration of these datasets with published tissue proteomics studies allowed prioritization of robust candidate biomarkers consistently identified across independent cohorts. To evaluate the translational potential of tissue-derived candidates, selected biomarkers were assessed in urine as a non-invasive matrix. Finally, seven prioritized PCa candidates (AZGP1, MDH2, FABP5, ENO1, GSTP1, GSTM2, EZR) were quantified in urine samples from 85 patients using ELISA[1]. Individual markers showed moderate performance, while biomarker panels improved diagnostic and prognostic accuracy, supporting the concept that multi-marker approaches outperform single analytes. The applicability of this strategy was further demonstrated in BCa. Label-free LC-MS/MS analysis of BCa tissues across disease stages identified proteins with consistent stage-associated regulation, among which NNMT and GALK1 showed strong diagnostic performance in urine and effectively discriminated non-invasive from invasive disease, with improved accuracy when combined.[2].

Overall, this work demonstrates that integrative analysis of tissue proteomics data, combined with targeted validation in body fluids, represents an effective strategy for identifying clinically relevant biomarkers. The proposed pipeline advances proteomics discoveries toward translation into non-invasive tools for urological cancer diagnosis and progression monitoring.

References

[1]Vujicic I, Rusevski A, Stankov O, Popov Z, Dimovski A, Davalieva K (2022) Potential Role of Seven Proteomics Tissue Biomarkers for Diagnosis and Prognosis of Prostate Cancer in Urine. Diagnostics (Basel). Dec 16;12(12):3184. doi: 10.3390/diagnostics12123184.

[2]Davalieva K, Kiprijanovska S, Ivanovski O, Trifunovski A, Saidi S, Dimovski A, Popov Z (2023) Proteomics Profiling of Bladder Cancer Tissues from Early to Advanced Stages Reveals NNMT and GALK1 as Biomarkers for Early Detection and Prognosis of BCa. Int J Mol Sci. Oct 6;24(19):14938. doi: 10.3390/ijms241914938.

REBEL, REBEL: BREAKING NORMS WITH PROTEASE MULTIPLEXING FOR GLYCOPROTEOMICS

Kathirvel Alagesan[1], Florian Kondrot[1], Emmanuelle Charpentier[1,2]

1 - Max Planck Unit for the Science of Pathogens, Charitéplatz 1, Berlin 10117, Germany
2 - Institut für Biologie - Molecular Microbiology, Humboldt-Universität zu Berlin, Berlin 10115, Germany

Multiple proteases improve protein sequence coverage but remain underutilized in routine data-dependent acquisition (DDA) proteomic workflows due to the increased LC–MS analysis time required. Here, we present a streamlined DDA multiplexing approach that combines three proteolytic digests into a single sample for glycoproteomics analysis. We first optimized data acquisition conditions for each protease using canonical fragmentation modes (higher-energy collisional dissociation (HCD), stepping HCD (sHCD), and electron transfer/higher-energy collisional dissociation (EThcD)) to identify the best consensus fragmentation settings across the proteases. Next, we demonstrate that applying these conditions to a protease-multiplexed sample derived from human peptides yields protein identifications and quantitative performances comparable to those of a single-protease analysis, while significantly increasing unique glycopeptide identification. Our protease-multiplexing approach reduces the LC-MS instrument time by approximately 66%, improves protein sequence coverage (close to 100%), and increases the number of unique N- and O-glycosides identified. Together, these results establish protease multiplexing as an efficient strategy for increasing glycoproteome depth within standard DDA workflows.

DECODING MOLECULAR INTERPLAY IN CANINE INTERVERTEBRAL DISC HERNIATION: A MULTI-OMICS PERSPECTIVE

A. Horvatić[1] *and M. Lipar*[2]

1 University of Zagreb Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

2 University of Zagreb Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia

Canine intervertebral disc herniation (IVDH) is a complex musculoskeletal disorder with both systemic and localised molecular effects. It is a critical condition, as it can cause severe pain, loss of bladder control, and permanent paralysis if not diagnosed and treated promptly. Due to its multifactorial nature and the lack of effective therapies to prevent disc degeneration and the potentially severe consequences of disease progression, a systems approach is needed for the diagnosis, monitoring of progression, and treatment of IVDH. By combining serum and CSF molecular profiles, our integrated model bridges the gap between localised spinal injury and systemic metabolic effects.

Integrated high-resolution LC-MS-based untargeted metabolomics and TMT-based proteomics were used to characterise the multi-omics landscape of both serum and CSF in dogs affected by IVDH. Additionally, serum zinc levels were measured. Locally, the CSF profile revealed an integromics signature dominated by key proteins FSTL1, SCG5, NUCB1, CRSP2, along with the metabolites N-acetyl-D-glucosamine and adenine, indicating active neuroinflammation, neuropathic pain signalling, and disc matrix degradation. Systemically, serum analysis identified significant dysregulation of the branched-chain amino acids, suggesting disrupted nitrogen and energy metabolism. Furthermore, elevated serum levels of ZAG and VTN, coupled with increased zinc concentrations, point toward antioxidant and immune-modulatory response to spinal trauma. Conversely, lowered levels of KLKB1 and SERPINA5 suggest altered blood coagulation.

By bridging localized neurodegenerative markers with systemic metabolic shifts, this integrated approach identifies a molecular fingerprint for IVDH which offers a robust framework for developing novel diagnostic tools and personalized therapeutic strategies. Our findings facilitate the monitoring of disease progression before clinical symptoms become irreversible, ultimately improving the standard of care for patients with degenerative spinal disorders.

References

1. Horvatić A, Kuleš J, Gelemanović A, Smolec O, Pirkić B, Pećin M, Rubić I, Mrljak V, Samardžija M, Lipar M. (2025) Combining Metabolomics and Proteomics to Reveal Key Serum Compounds Related to Canine Intervertebral Disc Herniation. Metabolites 15(6):396

2. Horvatić A, Gelemanović A, Pirkić B, Smolec O, Beer Ljubić B, Rubić I, Eckersall PD, Mrljak V, McLaughlin M, Samardžija M, Lipar M. (2021) Multi-Omics Approach to Elucidate Cerebrospinal Fluid Changes in Dogs with Intervertebral Disc Herniation. Int J Mol Sci. 22(21):11678

Multi-Proteomics Reveals Significant Protein and Infection-related Antibody Alterations in Type 2 Diabetes

F. Schmidt[1], M.-Al-Noubi[1], H. Sawarth[1], P. Vemulapalli[1], S. Choi[1], S. Chennakkandathil[1], S. Yousef[1], R. Dalloul[1], A. Halama[2], N. Stephan[2], K. Suhre[2]

1) Weill Cornell Medicine Qatar, Proteomics Core, Doha, Qatar
2) Weill Cornell Medicine Qatar, Bioinformatics Core, Doha, Qatar

Type 2 diabetes (T2D) is increasingly recognized as a chronic inflammatory disorder in which microbial exposure and immune modulation play critical roles. To dissect pathogen-specific immune signatures, we established a multi-stage proteomics and immuno-proteomics workflow combining multiplex antibody profiling, recombinant protein arrays, cellular assays, and translational mouse models. In the discovery stage, 67 low-risk (BSL-2) bacterial and fungal supernatants were screened on FLEXMAP 3D using plasma from 100 Central BioHub participants. Distinct antibody patterns differentiated T2D from controls, with the strongest responses against Staphylococcus aureus and Streptococcus pyogenes. In the verification stage, recombinant correctly folded virulence proteins (KREX™, 10 pathogens × 10 proteins) were profiled in 344 QMDiab samples. S. aureus antigens—including IsdB, SSL3, and Protein A showed particularly high IgG titers in T2D, while parallel Fc-sialylation analysis (SNA lectin) revealed reduced sialylated IgG fractions, consistent with a pro-inflammatory antibody phenotype. The KREX data were further correlated with 19 other omics datasets from QMDiab, including plasma proteomics, metabolomics, lipidomics, and clinical traits [1, 2, 3]. Network integration demonstrated that S. aureus-specific antibody intensities exhibited the highest connectivity to multi-omics modules related to inflammation, lipid metabolism, and glycemic control—indicating a central immunometabolic link between bacterial exposure and diabetic state. To validate mechanisms, human cell lines were exposed to 50 S. aureus toxins and secreted proteins, revealing congruent activation of inflammatory and metabolic-stress pathways. In a diabetic mouse wound-infection model, 50 normalized IgM S. aureus-specific antibody levels were measured, and fast restored anti-inflammatory proteomic profiles were detected after treatment with plasma pen. Distinct pattern were observed compared to human response. Together, these data connect pathogen-resolved antibody patterns and IgG Fc-glycosylation with systemic metabolic inflammation in T2D. S. aureus emerges as a dominant microbial correlate of immune dysregulation. The data were further compared to a sepsis-specific cohort. Pathogen-specific immunoprofiling combined with omics integration provides a powerful platform for biomarker discovery and therapeutic monitoring in metabolic disease.

References

1. HalamaA,ZaghloolS,TharejaG,KaderS,AlMuftahW,Mook-KanamoriM,SarwathH,Mohamoud YA, Stephan N, Ameling S, Pucic Baković M, Krumsiek J, Prehn C, Adamski J, Schwenk JM, Friedrich N, Völker U, Wuhrer M, Lauc G, Najafi-Shoushtari SH, Malek JA, Graumann J, Mook- Kanamori D, Schmidt F, Suhre K. A roadmap to the molecular human linking multiomics with population traits and diabetes subtypes. Nature Communications. 2024 Aug;15(1):7111.
2. Suhre K, Venkataraman GR, Guturu H, Halama A, Stephan N, Thareja G, Sarwath H, Motamedchaboki K, Donovan MKR, Siddiqui A, Batzoglou S, Schmidt F. Nanoparticle enrichment mass-spectrometry proteomics identifies protein-altering variants for precise pQTL mapping. Nature Communications. 2024 Feb;15(1):989.
3. Suhre K, Chen Q, Halama A, Mendez K, Dahlin A, Stephan N, Thareja G, Sarwath H, Guturu H, Dwaraka VB, Batzoglou S, Schmidt F, Lasky-Su JA. A genome-wide association study of mass spectrometry proteomics using a nanoparticle enrichment platform. Nat Genetics. 2025, 57. 2987–2996.

DIRECTED PEPTIDE FRAGMENTATION ENABLES *DE NOVO* SEQUENCING FOR BIOTYPIZATION OF COMPLEX PROTEOMES

R. Biba[1], Mihaela Pravica[1], L. Vujević[1], K. Košpić[2], A. Hozić[1], M. Cindrić[1]

1) Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
2) BICRO BIOCentre Ltd, Central Lab, Borongajska cesta 83 H, 10000 Zagreb, Croatia

De novo peptide sequencing is a powerful method for protein identification that does not rely on sequence databases, allowing the analysis of novel proteins, sequence variations, and organisms not represented in existing proteome databases. However, its wider use is constrained by incomplete and ambiguous fragment ion series produced during tandem mass spectrometry, which reduces sequence readout and confidence in peptide assignment. These limitations are especially significant for biotypization tasks, such as species identification in mixed or processed samples. To overcome this, we developed a peptide derivatization strategy based on *N*-terminal modification with 4-formylbenzene-1,3-disulfonic acid (4-FBDA), enabling chemically activated fragmentation and improving performance in both positive and negative electrospray ionization modes. The derivatization protocol was optimized on a set of 13 proteins covering a broad molecular weight range and structural complexity, and evaluated using data-independent acquisition (DIA) mass spectrometry. This approach enhanced the formation of complementary b- and y-ion series, significantly increased fragment ion yield, and improved spectral clarity and sequence interpretability in derivatized peptides compared to non-derivatized counterparts, resulting in a marked increase in merged sequence coverage (positive and negative ion modes) from 86.6% to 96.4% on average. To assess its applicability to complex systems, the method was applied at the proteome level across multiple species, enabling the generation of high-quality tandem mass spectra suitable for *de novo* sequencing. Finally, the approach was extended to the analysis of mixed proteomes in processed meat samples. Enhanced fragmentation and extended peptide sequence readouts support the differentiation of species-specific peptide signatures, enabling biotypization in complex food matrices and providing a robust method for protein identification in complex biological systems.

EXORCISING THE GHOSTS IN YOUR DATA: A MULTILAYER APPROACH TO FILTERING OUT SUSPECT TOP-DOWN ASSIGNMENTS

David P A Kilgour

1) Vibrat-Ion Ltd, Aberystwyth, UK

Top-down proteomics offers unparalleled insights into proteoform diversity, yet the reliability of fragment assignment remains a significant bottleneck. A decade ago, we identified a primary source of spectral "ghosts": the practice of performing charge state deconvolution prior to sequence assignment. We demonstrate that performing deconvolution on raw data – which often suffers from suboptimal mass accuracy before internal recalibration – propagates noise and necessitates dangerously wide mass-tolerance windows and prevents you from using some of the underlying statistics.

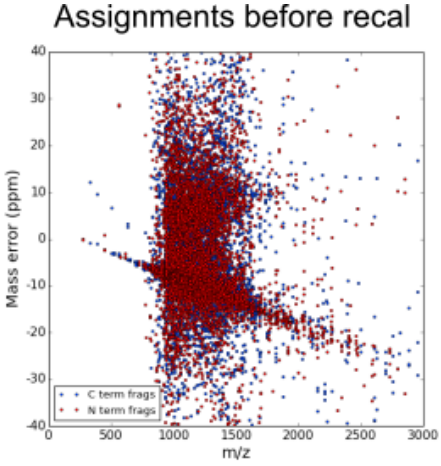
In this presentation, we revisit the "Assignment-First" paradigm we have been using for over 10 years. By assigning fragments directly to raw m/z spectra, we leverage theoretical masses to perform high-precision internal recalibration "on the fly." This approach, without deconvolution, significantly tightens the search space and permits more confident assignments and more rapid data interpretation. And, it permits us to move beyond simple "best-fit" assignments, we introduce a multilayered filtering strategy designed to exorcise false positives:

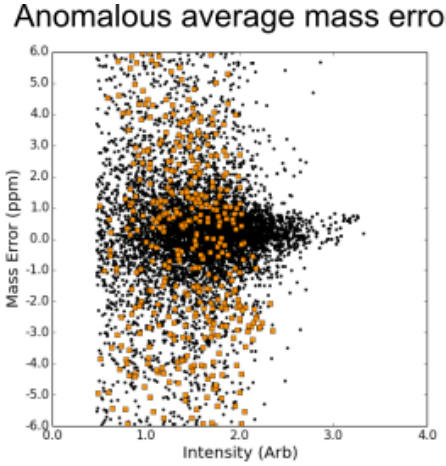
- Monte Carlo Precision Filter: Quantifying the mathematical "wobble" of a centroid by modeling the relationship between signal-to-noise (S/N) ratios and peak position uncertainty – and the effect of different peak geometries.

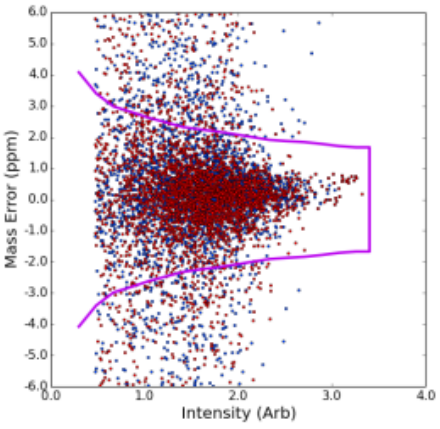
- Isotopic Combined Error Distribution: A consistency check that flags fragment clusters with average mass errors that are statistically anomalous relative to the global distribution of all assigned peaks.

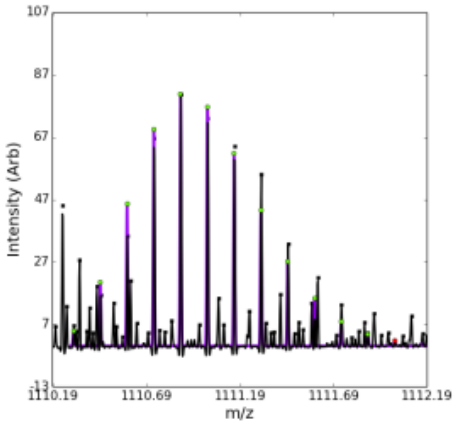
- Spectral Cosine Similarity: A common vector-based comparison ensuring the physical "shape" of the isotopic envelope matches the theoretical identity.

We have always followed the paradigm that we would rather output a smaller list of justifiable assignments (with objective confidence) than maximize a massive list of tentative assignments. Can we move away from the core tendency to compete only for who can get the most assignments in any data set?


Assignments before recal
Mass error (ppm)
m/z
C term frags
N term frags


Anomalous average mass error
Mass Error (ppm)
Intensity (Arb)


Monte Carlo S/N boundary
Mass Error (ppm)
Intensity (Arb)


Isotope peak intensities
Intensity (Arb)
m/z

SPATIAL ATMOSPHERIC PRESSURE IONIZATION HIGH-RESOLUTION MS ANALYSIS OF ENDOGENOUS (NEURO)PEPTIDES AND METABOLITES IN PATHOLOGISTS' FFPE SAMPLES

P.D.E.M. Verhaert[1,2], M. Giampà[3], G. Frache[4], M.M.H.A. Verhaert[2,5], R. Sciot[1], L. Zhang[6], L. Shan[6], D. Swaab[6] and B. Ghesquière[3]

1) Imaging and Pathology, Faculty of Medicine, Leuven University, Leuven, Flanders, Belgium
2) ProteoFormiX BV, Vorselaar, Flanders, Belgium
3) VIB Technologies, Metabolomics Core Facility, Leuven, Flanders, Belgium
4) Luxembourg Institute for Science and Technology, Belvaux, Luxembourg
5) Translational Oncology Research Centre, University of Brussels, Brussels, Belgium
6) Netherlands Institute for Neuroscience, Amsterdam, Netherlands

To maximally align mass spectrometry imaging (MSI) workflows with the realities of clinical pathology, we develop applications that make formalin-fixed paraffin-embedded (FFPE) tissues accessible for cutting-edge high-resolution (HR-)MSI. As such we help to lower the threshold for the adaptation of MSI in the field of pathology. We previously showed that spatial (top-down) MS histochemistry (MSHC) of neuropeptides and metabolites can be done directly of clinical FFPE samples[1] and that (nano)ESI tandem MS and ion mobility can be employed to validate neuropeptide annotations after LESA (liquid extraction surface analysis)[2].

Human FFPE tissue blocks from university hospital pathology and research institute biobanks are sectioned at 5 µm thickness (microtome) on standard glass microscope slides. Prior to HR-MSI, tissue sections are deparaffinized employing short xylene and ethanol dips[3]. For MALDI analyses, samples are matrix coated (generally DHB) using an automated heated nebulizer (SunCollect MALDI Sprayer, Sunchrom Friedrichsdorf, Germany or M5 Sprayer, HTX Technologies, Chapel Hill NC USA). An AP/MALDI UHR (MassTech, Columbia MD USA) source is mounted to different HR-MS instruments, including various orbitrap (ThermoFisher Scientific) systems. In addition, deparaffinized histological sections are directly analyzed in positive and negative ionization mode by DESI multi-reflecting TOF (MRT, Waters, Wilmslow, UK).

A variety of physiologically relevant biomolecules are imaged from FFPE tissue sections. MSHC mirrors the workflow of immunohistochemistry (IHC), improves molecular specificity, offers cellular spatial resolution (see Fig. 1) and can be applied directly to the millions of FFPE tissue blocks archived in biobanks worldwide. Recent developments allow AP/MALDI MSI and simultaneous MS/MS and ion mobility to be performed directly form a tissue section in one imaging experiment. Each extra dimension added to the MSHC multi-omics analysis and integrated with other pathology imaging modalities

currently in practice on (adjacent) FFPE sections, including IHC and H&E, helps stratifying patients, staging diseases, important for correct personalized diagnosis/prognosis.

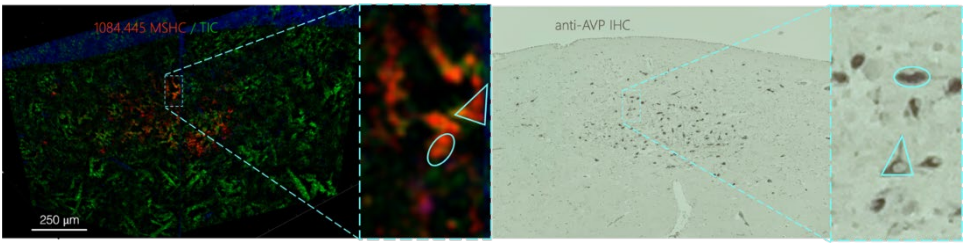


Figure 1. Selected ion (AP/MALDI orbitrap) MSHC (*m/z* 1084.445, 7x7 µm^2 pixel size; left) and anti-arginine vasopressin (AVP) IHC (right) of adjacent human FFPE hypothalamus sections (nucleus paraventricularis) showing different neuropeptide synthesizing cell bodies.

References

1. Frache G, Muddiman DC, Ospitalieri S, O'Connor CR, Thal DR, Verhaert PDEM (2025) Spatial atmospheric pressure MALDI HRMS analysis of histological sections of FFPE samples reveals a unique subset of the metabolome. Methods Mol Biol 2925:145-160
2. Cintron-Diaz YL, Gomez-Hernandez ME, Verhaert MMHA, Verhaert PDEM, Fernandez-Lima F (2022) Spatially resolved neuropeptide characterization from neuropathological formalin-fixed, paraffin-embedded tissue sections by a combination of imaging MALDI FT-ICR mass spectrometry histochemistry and liquid extraction surface analysis-trapped ion mobility spectrometry-tandem mass spectrometry. J Am Soc Mass Spectrom 33(4):681-687
3. Paine MRL, Ellis SR, Maloney D, Heeren RMA, Verhaert PDEM (2018) Digestion-free analysis of peptides from 30-year-old formalin-fixed, paraffin-embedded tissue by mass spectrometry imaging. Anal Chem 90(15):9272-9280

FROM SINGLE CELLS TO LARGE COHORTS: BOOSTING PROTEOMICS THROUGH ENHANCED PEPTIDE RECOVERY AND ROBUST LC-MS PERFORMANCE

C. Kune.[1], A. Gholizadeh[2], S. Tielen[3], M. Fléron[4], D. Baiwir[4], A. Mottais[5], A Bruffaert[5], D. Vandormael[6], L. Nguyen[3], Sophie Gohy[5] G. Eppe[1], T. Gilet[2], G. Mazzucchelli[1,4]

(1) University of Liege, MS Lab - Molsys, Liège, Liège, Belgium.
(2) University of Liege, Microfluidic Lab, Liège, Liège, Belgium.
(3) University of Liege, GIGA Stem Cells - Molecular Regulation of Neurogenesis, Liège, Liège
(4) University of Liège, GIGA Proteomics Facility, Liege, Liege, Belgium.
(5) Université Catholique de Louvain, Department of Pneumology, ENT and Dermatology, Institute of Experimental & Clinical Research
(6) Sirris, Liège, Liège, Belgium.

Advances in mass spectrometry have enabled single-cell proteomics, yet peptide loss due to surface adsorption remains a major bottleneck, particularly at low concentrations. Using HeLa tryptic digests, we demonstrated that adsorption follows a Langmuir model, leading to significant signal loss in dilute samples[1]. To mitigate this, we optimized a one-pot sample preparation workflow integrating polymer-based materials and nonionic detergents. The use of PMMA and PET vials, combined with PEO- or DDM-containing buffers, improved peptide recovery by up to 16-fold.

Building on this, we developed the WellOmics plate, a microfluidic chip in a 384-well format operated solely by centrifugation[2,3,4,5]. This platform should be able to automate the workflow from single-cell isolation to LC-MS injection, minimizes handling, reduces volumes to the sub-microliter scale, and enables high-throughput processing.

To enhance robustness, we introduce the Trap-Refocusing-Elute (TRE) nanoLC mode, combining online desalting with high-resolution chromatography. TRE supports flexible injection volumes (1–10 µL), ensures efficient transfer of low-abundance samples, and maintains sharp peak profiles comparable to direct injection. The method enables 25-minute analysis cycles on standard UPLC systems.

Importantly, this workflow was validated in large-cohort proteomics. Using a streamlined One-Pot digestion strategy with TRE nanoLC-MS, we analyzed several hundred low-input human sweat samples, identifying over 2,000 proteins with high data completeness and retaining ~1,200 after filtering. This demonstrates the ability to bridge ultra-low input analysis and population-scale studies for biomarker discovery.

Finally, to leverage short DIA runs, we developed a spectral library generation strategy based on sequential digestion and peptide fractionation, yielding 282,008 peptides and 9,588 proteins from HeLa tryptic digests.

Together, these innovations enhance peptide recovery, analytical robustness, and proteome depth, establishing a unified platform for both single-cell and large-scale proteomics with clinical potential.

References

1. Kune C, Tielens S, Baiwir D, et al. SIGNIFICANT IMPACT OF CONSUMABLE MATERIAL AND BUFFER COMPOSITION FOR LOW-CELL NUMBER PROTEOMIC SAMPLE PREPARATION. Anal Chem. 2025;97(7):3836-3845. doi:10.1021/acs.analchem.4c03709
2. Patent WO2025176316 (A1): FLUIDIC DEVICE FOR METERING AND DISPENSING A PLURALITY OF SAMPLES
3. Patent EP4669460 : (A1)FLUIDIC UNIT FOR DISCRETE ELEMENT TRAPPING
4. Gholizadeh A, Mazzucchelli G, Amoroso A, Gilet T. Modular Centrifugal Microfluidics for Sample Preparation. Anal Chem. 2025;97(23):12070-12079. doi:10.1021/acs.analchem.5c00076
5. Gholizadeh A, Mazzucchelli G, Amoroso A, Gilet T. Modular Centrifugal Microfluidics for Sample Preparation. Anal Chem. 2025;97(23):12070-12079. doi:10.1021/acs.analchem.5c00076

DATA QUALITY AS THE FOUNDATION: AUTOMATED SAMPLE PREPARATION AND PROTEIN CHARACTERIZATION IN LIFE SCIENCE RESEARCH

O. Lacina and S. Stipaničić

1) Altium International s.r.o., Na Jetelce 69/2, 190 00, Prague 9, Czech Republic
2) Altium International d.o.o., Karlovačka cesta 24, 10 000 Zagreb, Croatia

Discovery proteomics and biopharmaceutical analysis represent fundamentally different analytical challenges. Where proteomics deals with thousands of unknown proteins at trace concentrations, biopharma focuses on thorough characterization of a single well-defined molecule — its sequence, modifications, proteoforms, and impurities. Yet the two fields are deeply connected: proteins and peptides identified through biological research result in a better understanding of disease mechanisms and can ultimately lead to the development of modern biotherapeutics that must be produced, characterized, and controlled.

In both worlds, sample preparation is a well-recognized source of variability — one that can easily mask the biological signal or compromise drug batch approval. Variability introduced at this stage cannot be corrected downstream; it can only be measured more precisely. This presentation introduces the Agilent Bravo platforms as a common foundation for reproducible sample preparation across life science disciplines, from phosphopeptide enrichment and automated digestion to plasma extraction for metabolomic and lipidomic studies.

The second part focuses on biopharmaceutical workflows for protein and peptide characterization using the Agilent 6545XT AdvanceBio LC/Q-TOF with ExD cell for electron capture dissociation — covering intact protein analysis, top-down fragmentation, labile PTM preservation, and amino acid isomer identification, where data quality requirements extend beyond reproducibility into regulatory compliance.

PROTEOMICS HELPDESK: A CRO PERSPECTIVE ON FEASIBILITY, CONSTRAINTS, AND ANALYTICAL STRATEGY SELECTION

K. Košpić, V. Kelava, Ž. Stanečić, R. Bertoša, and M. Lovrić

BICRO BIOCentre Ltd., Borongajska cesta 83H, 10000 Zagreb

A total of over 70,000 publications in the past 15 years[1] reflects the rapid expansion of proteomics and its increasing application across diverse scientific fields, where translating complex questions into analytical strategies remains a key challenge. In this lecture, an analytical perspective from an R&D Contract Research Organization (CRO) proteomics laboratory will be presented, operating at the interface between hypothesis-driven scientific inquiry and practical analytical execution. From initial project input to final data reporting, key aspects of experimental design and analytical workflow selection will be addressed, including rapid feasibility evaluation, selection of appropriate sample preparation steps, deciding on tailored analytical approach, and realistic budgeting considerations. The overarching goal is to ensure technical feasibility within instrumental capabilities and time and budget constraints, while remaining aligned with the underlying biological or chemical objective.

Although proteomics relies heavily on mass spectrometry (MS), practical examples will illustrate key decision-making in selecting the most appropriate analytical technique and instrument, including cases where High-Performance Liquid Chromatography (HPLC)–UV is chosen over LC–MS for protein quantification due to time and cost efficiency, while taking into account sequence homology between standards and target proteins. Furthermore, a case study on protein conjugate characterization will be presented, along with workflows for assessing therapeutic protein stability, highlighting challenges in reliably capturing degradation-related changes throughout the product lifecycle. In addition, a targeted proteomics workflow will be demonstrated through the development of multiple reaction monitoring (MRM) method based on a purified protein standard rather than synthetic peptides, incorporating *in silico* digestion, systematic screening of candidate transitions, and selection of robust transitions for reliable quantification of the target protein.

By illustrating practical decision-making across diverse analytical scenarios, this perspective demonstrates how proteomics projects can be translated from conceptual questions into robust and fast analytical outcomes within an applied bioanalytical setting.

References

1. Nice E.C (2022 The status of proteomics as we enter the 2020s: Towards personalised/precision medicine. Anal. Biochem. 644:113840-113850.

TARGETED DELIVERY SYSTEM TO TRANSPORT ANTIMICROBIAL PEPTIDES ACROSS BLOOD BRAIN BARRIER

M. Bhide[1,2]

[1]Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia
[2]Institute of Neuroimmunology, Slovak Academy of Sciences v. v. i., Dúbravská cesta 9, 845 10 Bratislava, Slovakia
bhidemangesh@gmail.com

Major obstacle for curing brain diseases is the blood-brain barrier (BBB), which impedes therapeutic agents from reaching the brain and targeting the related pathogens. The aim was to develop a drug delivery system derived from antimicrobial peptide and the CNS homing peptide. We developed antiviral and anti-borrelial peptides using combinatorial phage display and tested their activity and cell toxicity. The best peptides were further fused with CNS-homing peptides (such as Angiopep-2 or TGN) to increase their biodistribution in the brain parenchyma. In parallel, we also developed the novel-CNS homing peptide derived from OspA protein of the neuroinvasive *Borrelia* and fused with the antimicrobial peptide. In this way, we successfully produced fusion peptides against Tick-borne encephalitis virus, SARS-CoV-2 and neuroinvasive *Borrelia.* Development of all fusion peptides, their antimicrobial activity, and crossing across the BBB will be presented. We expect to generate clinically useful pilot results for the best-performing candidates for future translation and, at the same time, research data of general scientific interest useful to the broad scientific community. *Research was funded by grants VEGA 1/0381/23 and APVV-22-0084.*

PROTEOMIC PROFILING OF *BACILLUS THURINGIENSIS* RESPONSES TO NATURAL INHIBITORS TARGETING AMINOACYL-TRNA SYNTHETASES

M. Mocibob[1], *I. Gruic-Sovulj*[1]

1) University of Zagreb, Faculty of Science, Department of Chemistry, Horvatovac 102a, 10000 Zagreb, Croatia

Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes that catalyze the binding of a specific amino acid to its corresponding tRNA, a process known as "charging" or aminoacylation. Aminaocylated tRNA are utilized as the substrates for protein biosynthesis by the ribosome and play a crucial role in decoding of genetic information during ribosomal translation.

Although a single aaRS should suffice for incorporation of each amino acid into growing polypeptides, bacterial genomes often contain duplications of aaRS genes. A remarkable example of aaRS gene duplications is *Bacillus thuringiensis*, with 8 duplicated and one triplicated aaRS gene. Proteomic analysis revealed that a single aaRS paralogue is abundantly expressed under normal growth conditions, and acts as constitutive, housekeeping aaRS. In order to explore functional redundancy and possible role of aaRS paralogues in antibiotic resistance, we have treated the bacteria with mupirocin, indolmycin, halofuginone, and borrelidin, the natural products inhibiting isoleucyl-, tryptophanyl-, prolyl-, and threonyl-tRNA synthetases (IleRS, TrpRS, ProRS and ThrRS), respectively. In all four cases activation of cryptic, divergent aaRS genes in *B. thuringiensis* was observed (34-, 8-, 5-, and 40-fold for IleRS, TrpRS, ProRS, and ThrRS, respectively) upon antibiotic treatment. However, it was unequivocally confirmed only for the additional divergent IleRS that it confers mupirocin resistance to its host. Interestingly, proteomic profiling revealed that Ile, Trp, Pro and Thr biosynthesis genes were greatly upregulated in *B. thuringiensis* (up to 100-, 200-, 19- and 260-fold, respectively), and bioinformatic analysis has shown that the genes are organized in operons under control of the T-boxes, the transcription regulatory elements responsive to tRNA aminoacylation status. Comparison to closely related *Bacillus subtilis* has shown that these gene operons are unique to *B. thuringiensis* and revealed unexpected differences in amino acid biosynthesis and regulation in closely related species.

SURFACE COATING-DEPENDENT PHYTOTOXICITY OF SILVER NANOPARTICLES: ANTIOXIDANT AND PROTEOMIC RESPONSES IN PLANTS AND GREEN ALGAE

B. Balen[1], P. Peharec Štefanić[1], B. Komazec[1], D. Loknar[1], M. Tkalec[1], P. Cvjetko[1], D. Pavoković[1], R. Biba[2], K. Košpić[3] and M. Cindrić[2]

1) Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
2) Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia
3) Croatia BICRO BIOCentre Ltd, Central Lab, Borongajska cesta 83 H, 10000 Zagreb, Croatia

Silver nanoparticles (AgNPs) are widely used in consumer and industrial applications, resulting in their broad release and persistence in the environment. Because AgNPs tend to agglomerate and release Ag^+ ions, surface coatings such as citrate, polyvinylpyrrolidone (PVP), and cetyltrimethylammonium bromide (CTAB) are applied to enhance stability and modify interactions with biological systems. A primary mechanism of AgNP toxicity is the induction of oxidative stress, which triggers antioxidant defense responses in exposed organisms. As primary producers, plants and green algae play a crucial role in transferring AgNPs into terrestrial and aquatic food chains, raising concerns about potential risks to human health.

This study examined the coating-dependent phytotoxic effects of AgNPs using several model organisms, including green algae (*Chlorella vulgaris*), tobacco (*Nicotiana tabacum*), onion (*Allium cepa*), and duckweed (*Lemna minor*). In algae, both AgNP-citrate and AgNP-CTAB significantly increased peroxidase (PPX) and ascorbate peroxidase (APX) activities, while superoxide dismutase (SOD) activity increased significantly only with AgNP-CTAB. Proteomic analysis showed that most affected proteins were related to photosynthesis, with stress-response proteins displaying coating-specific expression patterns. In tobacco seedlings and adult plants, both AgNP-PVP and AgNP-CTAB induced oxidative stress, but CTAB-coated nanoparticles caused stronger activation of antioxidant enzymes, particularly catalase (CAT) and APX, along with more extensive proteomic changes. Root tissues showed more pronounced antioxidant responses than leaves, and coating type influenced specific pathways, including proline accumulation and glutathione metabolism. In onion roots, antioxidant enzyme responses varied, with increased SOD, APX, and PPX activities, but decreased CAT activity. Duckweed also showed altered antioxidant enzyme activities after exposure to both nanoparticle types.

Overall, these results demonstrate that AgNP surface coating plays a critical role in determining phytotoxicity, influencing both antioxidant responses and proteomic changes across species.

References

1. Biba R., Košpić K, Komazec B, Markulin D, Cvjetko P, Pavoković D, Peharec Štefanić P, Tkalec M, Balen B (2022) Surface coating-modulated phytotoxic responses of silver nanoparticles in plants and freshwater green algae. Nanomaterials 12:24

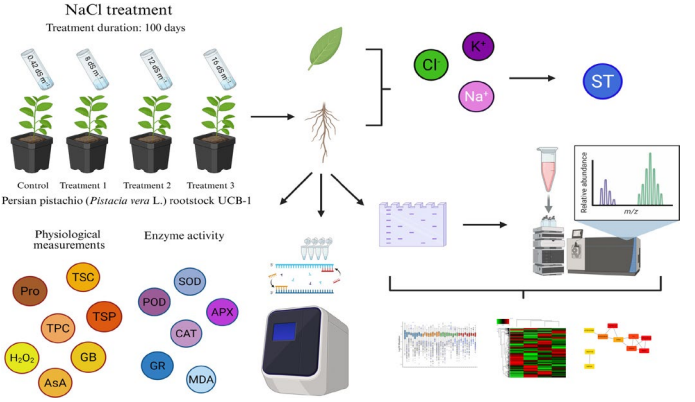
2. Biba R, Cvjetko P, Tkalec M, Košpić K, Peharec Štefanić P, Šikić S, Domijan A-M, Balen B (2022) Effects of silver nanoparticles on physiological and proteomic responses of tobacco (*Nicotiana tabacum*) seedlings are coating-dependent. Int J Mol Sci 23:15923
3. Komazec B, Cvjetko P, Balen B, Letofsky-Papst I, Lyons DM, Peharec Štefanić P (2023) The occurrence of oxidative stress induced by silver nanoparticles in *Chlorella vulgaris* depends on the surface-stabilizing agent. Nanomaterials 13:1967
4. Komazec B, Vitko S, Balen B, Cindrić M, Biba R, Peharec Štefanić P (2025) Multi-parameter analysis of photosynthetic and molecular responses in *Chlorella vulgaris* exposed to silver nanoparticles and ions. Toxics 13:627

ROOT PROTEOMIC INSIGHTS INTO SALINITY-INDUCED METABOLIC SWITCHING IN PISTACHIO ROOTSTOCKS

Ramesh Katam, Mohammad Akbari and Chase Weaver-Johnson

Department of Biological Sciences, Florida A&M University, Tallahassee FL 32307 USA

Soil salinization is an escalating threat to pistachio production in arid and semi-arid regions, demanding deeper molecular insight into root-mediated stress tolerance. As the primary interface for salt perception and ion regulation, roots orchestrate complex adaptive responses that remain largely unexplored in woody crops. Here, we present a quantitative proteomic dissection of salinity-induced remodeling in the UCB-1 pistachio rootstock. One-year-old plants were exposed to four sodium chloride regimes over 100 days to capture progressive stress adaptation. Root proteome profiling identified more than 1,600 proteins, of which 245 increased and 190 decreased significantly across salinity levels. Salinity triggered coordinated reprogramming of metabolic and regulatory networks. Proteins involved in folding, post-translational modification, and heat shock protection were strongly enriched, highlighting robust proteostasis mechanisms. Enhanced secondary metabolism and redox-related proteins supported detoxification and oxidative balance. Increasing stress intensified vesicle trafficking and transporter-associated proteins, reinforcing ion sequestration and homeostasis. Notably, signaling components predominated under moderate stress, whereas structural and membrane-stabilizing proteins became critical under severe conditions, revealing stage-specific adaptive strategies. Network analysis mapped to orthologs in Arabidopsis thaliana uncovered tightly connected clusters in carbohydrate and amino acid metabolism. This study delivers a systems-level proteomic framework for salinity tolerance in pistachio roots and identifies molecular targets for breeding resilient cultivars.



Effect of NaCl treatment on root proteome

DON'T CRY OVER SPILLED MILK: TRANSLATING MILK PROTEOMICS INTO RAPID DIAGNOSTIC TOOLS FOR SUSTAINABLE DAIRY PRODUCTION

J. Kuleš[1], D. Prišćan[2], V. Eraghi[2], R. Barić Rafaj[1], G. Bačić[3], N. Mačešić[3], M. Benić[4], V. Mrljak[2]

1) Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
2) Internal Diseases Clinic, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
3) Reproduction and Obstetrics Clinic, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
4) Department for Bacteriology and Parasitology, Croatian Veterinary Institute, Savska cesta 143, Zagreb, Croatia

Mastitis, i.e. inflammation of the mammary gland, is the most common infectious disease in cows. Although clinical mastitis is readily recognized, subclinical mastitis accounts for approximately 90–95% of all mastitis cases and may persist for prolonged periods without visible alterations in the udder or milk. Thus, subclinical mastitis represents one of the greatest challenges in modern dairy production due to its high prevalence, difficult detection, and significant economic impact on herd health and milk quality.

Early and reliable diagnosis of subclinical mastitis is therefore essential for improving treatment outcomes, reducing the duration and intensity of therapy, minimizing antimicrobial usage, and enhancing animal welfare and productivity. However, current diagnostic approaches lack sufficient sensitivity and specificity for early-stage detection, creating an urgent need for novel biomarkers suitable for rapid and practical on-farm application.

Ongoing research focused on the detection, characterization, and quantification of milk proteomic profiles associated with subclinical mastitis in dairy cows will be presented. Particular emphasis will be placed on the application of advanced quantitative bottom-up proteomics combined with bioinformatic analyses to identify and quantify protein biomarkers associated with early inflammatory processes in the mammary gland, providing new insights into host–pathogen interactions and disease pathophysiology.

In addition, the translation of proteomic discoveries into practical diagnostic tools will be discussed, with special focus on the development of immunochemical and lateral flow assay platforms for rapid on-farm detection. Strategies including biological validation of candidate biomarkers, antibody production, and optimization of lateral flow tests will be presented as key steps toward the development of sensitive, user-friendly, and cost-effective point-of-care diagnostics for dairy herd management.

Overall, the presented research demonstrates the potential of integrating state-of-the-art proteomics with lateral flow technologies to create next-generation diagnostic solutions for earlier detection and improved control of subclinical mastitis, ultimately supporting more sustainable and precision-based dairy production systems.

TEMPERATURE-INDUCED DENATURATION OF BOVINE SERUM ALBUMIN: RECONCILING THE RESPONSES OBTAINED BY DIFFERENT EXPERIMENTAL TECHNIQUES

D. Bakarić[1], R. Petko[1], and S. Vojvodić[2]

1) Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička 54, 10000 Zagreb, Croatia (E-mail: danijela.bakaric@irb.hr)

2) Institute of Physiology, Pathophysiology and Biophysics, Department of Biomedical Sciences, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

Bovine serum albumin (BSA) is a water-soluble globular protein found in the blood of vertebrates, valued for its stability, availability, and low cost. It is widely studied as a model protein due to its non-toxic and non-antigenic properties, which prevent immune responses[1]. BSA maintains its native structure under normal conditions, but temperature-induced denaturation can enhance protein coatings for surface passivation[2]. Current research focuses on characterizing this phenomenon and how changes in secondary structures within specific temperature ranges impact macroscopic properties. Differential scanning calorimetry (DSC) has identified a temperature range in which significant thermotropic events occur (Fig. 1a). Fourier transform infrared (FTIR) and circular dichroism (CD; Fig. 1b) spectroscopies have related certain molecular-level changes to BSA denaturation3, although with some discrepancies. Ultimately, temperature-dependent refractometry provided insights into the system's macroscopic properties, helping reconcile findings from different spectroscopic techniques.

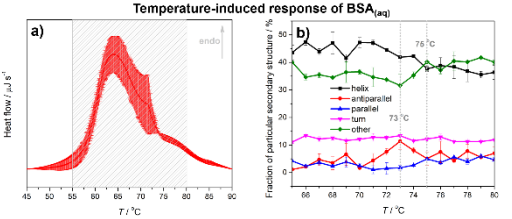


Fig. 1. a) DSC curve of BSA; b) temperature-dependent change in distribution of BSA secondary structures[4]

References

1. Nnyigide OS, Hyun K (2020) The protection of bovine serum albumin against thermal denaturation and gelation by sodium dodecyl sulfate studied by rheology and molecular dynamics simulation. Food Food Hydrocoll 103:105656, 11

2. Park JH, Jackman JA, Ferhan AR, Ma GJ, Yoon BK, Cho N-J (2018) Temperature-Induced Denaturation of BSA Protein Molecules for Improved Surface Passivation Coatings. ACS Appl. Mater. Interfaces 10(38):32047-32057.

3. Murayama K, Tomida M (2004) Heat-Induced Secondary Structure and Conformation Change of Bovine Serum Albumin Investigated by Fourier Transform Infrared Spectroscopy. Biochemistry 43:11526-1153

4. BeStSel - Protein Circular Dichroism Spectra Analysis, https://bestsel.elte.hu/index.php (07.05.2026)

PROTEOMICS IN BIOMEDICAL RESEARCH WITH EMPHASIS ON MUSCULOSKELETAL DISEASES AND TUMOR BIOMARKERS

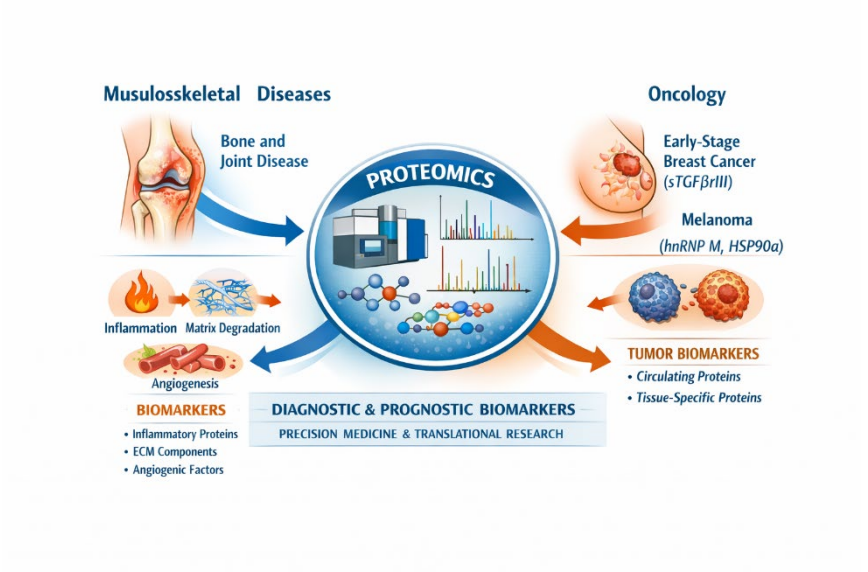
L. Grgurevic[1,2,3], R. Novak[1,2]

[1] *BIMIS – Biomedical Research Centre Šalata, University of Zagreb School of Medicine, Zagreb, Croatia*

[2] *Center for Translational and Clinical Research, Department of Proteomics, School of Medicine, University of Zagreb, Zagreb, Croatia*

[3] *Department of Anatomy, "Drago Perović", University of Zagreb, School of Medicine, Zagreb, Croatia*

Proteomics has become a central tool in modern biomedical research because it enables direct insight into protein composition, abundance and post-translational modifications, thereby capturing functional molecular interactions within biological systems (1). Recent advances in mass-spectrometry–based proteomics, particularly high-resolution LC-MS technologies and data-independent acquisition strategies, have significantly improved the ability to analyze complex biological samples such as plasma, synovial fluid, cartilage and bone. Musculoskeletal diseases represent a major clinical burden, and early diagnosis as well as monitoring of disease progression remain important challenges. Proteomic profiling provides valuable opportunities for identifying circulating or tissue-specific biomarkers that reflect ongoing pathological processes.A representative example is haemophilic arthropathy, a debilitating joint disease caused by recurrent intra-articular bleeding in patients with haemophilia (2,3). Our recent plasma proteomic analysis identified several differentially expressed proteins associated with inflammatory signaling, extracellular matrix turnover and angiogenesis, highlighting potential biomarkers linked to disease severity and joint degeneration (4). Beyond musculoskeletal diseases, proteomic technologies have also contributed significantly to biomarker discovery in oncology. Proteomic studies have identified circulating and tissue-specific proteins associated with tumor progression and patient prognosis. For example, soluble transforming growth factor-β receptor III (sTGFβrIII) has been proposed as a potential biomarker for early-stage breast cancer (7), while tissue proteomic profiling in melanoma has identified proteins such as heterogeneous nuclear ribonucleoprotein M (hnRNP M) and heat-shock protein 90α (HSP90α) as potential predictors of disease-specific mortality (8). These findings highlight the translational value of proteomics in identifying clinically relevant tumor biomarkers. Overall, proteomics provides a powerful framework for understanding disease mechanisms and discovering novel diagnostic and prognostic biomarkers. By bridging basic molecular research with clinical applications, proteomic approaches contribute to the development of more precise and personalized strategies in both musculoskeletal medicine and oncology, although broader clinical implementation still faces technological and cost-related challenges.



References

1. Aebersold R, Mann M. (2016). Mass-spectrometric exploration of proteome structure and function. Nature 15;537(7620):347-55.
2. Gualtierotti R, Solimeno LP, Peyvandi F. (2021) Hemophilic arthropathy: Current knowledge and future perspectives. J Thromb Haemost.19(9):2112-2121
3. Pulles AE, Mastbergen SC, Schutgens RE, Lafeber FP, van Vulpen LF.(2017). Pathophysiology of hemophilic arthropathy and potential targets for therapy. Pharmacol Res. 115:192-199
4. Kalebota N, Novak R, Hrkač S, Perić P, Salai G, Močibob M, Pranjić M, Zdráhal Z, Pustka V, Žerjavić NL, Milošević M, Vodanović M, Šalek SZ, Grgurević L.(2024). Proteomic exploration of potential blood biomarkers in haemophilic arthropathy. Health Sci Rep. 25;7(9):e70046.
5. van Bergen EDP, van Vulpen LFD, Schutgens REG, Mastbergen SC, Lafeber FPJG.(2021). Biochemical marker research in hemophilic arthropathy: A systematic review. Blood Rev.47:100781.
6. Martín-Márquez BT, Sandoval-García F, Corona-Meraz FI, Martínez-García EA, Sánchez-Hernández PE, Salazar-Páramo M, Fletes-Rayas AL, González-Inostroz D, Vazquez-Del Mercado M.(2023). Osteopontin: A Bone-Derived Protein Involved in Rheumatoid Arthritis and Osteoarthritis Immunopathology. Biomolecules. 9;13(3):502.
7. Grgurevic L, Novak R, Trkulja V, Hrkac S, Salai G, Bilandzic J, Hamzic LF, Milas I, Vucemilo T, Balja MP, Bilic K.(2020). Plasma levels and tissue expression of soluble TGFβrIII receptor in women with early-stage breast cancer and in healthy women: a prospective observational study. J Transl Med. 11;18(1):478
8. Košec A, Novak R, Konjevoda P, Trkulja V, Bedeković V, Grgurević L.(2019). Tumor tissue hnRNP M and HSP 90α as potential predictors of disease-specific mortality in patients with early-stage cutaneous head and neck melanoma: A proteomics-based study. Oncotarget. 19;10(62):6713-6722.

IDENTIFICATION OF A MICROPEPTIDE LINKED TO CANCER STEM CELL REGULATION AND CHEMORESISTANCE

Jyotsna Batra[1,2], Chamikara Liyanage[2], Achala Fernando[1,2]

[1]School of Biomedical Science Faculty of Health Sciences and Medicine, Bond University, Gold Coast, QLD 4226, Australia

[2]Translational Research Institute, The Centre for Genomics and Personalised Health, Queensland University of Technology, Brisbane, QLD 4102, Australia

Short open reading frames encoding micropeptides (miPEPs) less than 100 amino acids in length have recently emerged as important regulators of diverse biological functions. However, the functional role of cancer-specific miPEPs in cancer progression and therapeutic response remains largely unexplored. Genome-wide association studies have identified an association of Iroquois (IRX) clusters with multiple cancer risk. In this study, we identified 17 miPEPs generated from IRX clusters in prostate, breast, endometrial, and ovarian cancers using SWATH-MS/MS-based proteomic analysis. We found that an IRX4-derived miPEP, IRX4_PEP1, promotes prostate cancer (PCa) cell proliferation, migration, and invasion by interacting with heterogeneous nuclear ribonucleoprotein K (hnRNPK). Overexpression of IRX4_PEP1 leads to dysregulation of stem cell pathways by co-interaction with catenin beta-1 (CTNB1) and upregulation of prominent PCa stem markers, resulting in docetaxel resistance. IRX4_PEP1 expression is significantly upregulated in prostate tumour tissues compared with normal and is positively correlated with disease aggressiveness.

Our findings highlight IRX4_PEP1 as a regulator of PCa stemness and chemotherapy resistance, supporting its potential as a therapeutic target and as a diagnostic/prognostic biomarker.

References

1. Fernando A, Liyanage C, Srinivasan S, Janaththani P, Batra J (2023) Identification of a Micropeptide Linked to Cancer Stem Cell Regulation and Chemoresistance. bioRxiv. https://doi.org/10.1101/2023.03.14.532696

20th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE

June 14 – 17, 2026
Zagreb, Croatia

Posters

PERIPHERAL BLOOD CD14+ MONOCYTES IN LUMINAL BREAST CARCINOMA SUBTYPES: IN PRELIMINARY RESEARCH OF PROTEOMIC SIGNATURE CANDIDATES

Michal Alexovič, [1] Peter Bober[1], Miroslav Marcin[1], Jozef Parnica[1], Michal Marcin[2], Marek Lenárt[3], Dávid Tóth[4], Jozef Radoňak[3], Peter Urdzík[4], and Ján Sabo[1]

1) Department of Medical and Clinical Biophysics, Faculty of Medicine, University of Pavol Jozef Šafárik in Košice, 04011 Košice, Slovakia
2) Center of Clinical and Preclinical Research MEDIPARK, Faculty of Medicine, University of Pavol Jozef Šafárik in Košice, 04011 Košice, Slovakia
3) 1st Department of Surgery, University of Pavol Jozef Šafárik in Košice & Louis Pasteur University Hospital in Košice, 04011 Košice, Slovakia
4) Department of Gynaecology and Obstetrics, University of Pavol Jozef Šafárik in Košice & Louis Pasteur University Hospital in Košice, 04011 Košice, Slovakia

Peripheral blood CD14+ monocyte proteomes from LumA, LumB-HER2−, LumB-HER2+ breast carcinoma (BC) and benign cases were compared with those from healthy controls (HCs). Among the DAPs, *SRSF1, CSTB, KRT2, KRT5, HEL-S-11, APOB, APOE*, and *ITGA2B* were found significantly changed in all Lum BC vs. healthy controls (HCs), whereas in the benign vs. HCs comparisons, only *KRT2* and *KRT5* were DAPs found across all disease groups. *ENO1*, *KRT1*, and *AIDB* were among the top 10 hits in LumA, LumB-HER2− and LumB-HER2+, respectively, and can also likely be linked to Lum BC. GSEA showed that KEGG Gonadotropin Release Hormone, KEGG Leukocyte Trans-Endothelial Migration, and KEGG Calcium Signalling pathways were all downregulated in LumA. In LumB-HER2-, KEGG Ribosome was upregulated and KEGG Lysosome downregulated, while in LumB-HER2+, the HALLMARK MYC-Targets V2 pathway was downregulated. Such proteins may reflect BC-related immune responses and help to subcategorise disease cohorts via comparative proteomics.

FUNDING

Financial support was provided by the Slovak Research and Development Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic (VV-MVP-24-0135)

PRMT1 INHIBITION ENHANCES THERAPEUTIC EFFICACY OF CISPLATIN IN RESISTANT TESTICULAR GERM CELL TUMOR CELLS

L. Borszekova Pulzova[1], A. Holickova[1], K. Valoskova[1], M. Chovanec [1]

1) Cancer Research Institute, Biomedical Research Center of SAS, Dubravska cesta 9, Bratislava, Slovakia

Cisplatin (CDDP) is one of the front-line chemotherapeutic agents, while drug resistance is a great obstacle to its efficiency. Protein arginine methylation acts as a molecular switch and regulates essential cellular processes that dictate the life and death of the cell (1). While protein arginine methyltransferases (PRMTs) inhibition has recently been attracting attention as a potential therapeutic tool in cancer treatment and overcoming chemotherapy resistance, to our knowledge, no study has addressed the role of PRMTs in testicular germ cell tumors (TGCTs). To comprehensively characterize PRMTs in TGCT, we analyzed the expressions of all the PRMT family members across the patient cohort from the TCGA database and our previously sequenced TGCT cell lines. Among PRMTs, PRMT1 is the highest expressed gene in TGCT cell lines and patient cells. To better understand the role of PRMT1 in TGCTs and in resistance development, we inhibited the type I PRMTs using MS023 in cell line 2102EP and its resistant derivative, and employed MTT assay, EdU proliferation assay, clonogenic assays, live-cell imaging, in-cell western blotting, qRT-PCR, comet assay, and immunoflurescence. Our results showed that in both models, MS023 monotherapy had a low cytotoxic effect in cells/spheroids. Mechanistically, PRMT1 inhibition led to DNA damage induction and downregulation of DNA damage response factors expression. MS023-CDDP co-treatment did not show synergic effect. Conversely, we observed a strong synergy when cells were pre-treated with MS023 and then co-treated with CDDP. An increase in γH2AX foci observed following priming indicates accumulation of unresolved DNA double-strand breaks and decreased DNA damage repair efficiency.

Here, we show that pharmacologic inhibition of PRMT1 creates therapeutically exploitable vulnerability in TGCTs. Our research revealed that MS023 pre-treatment primed cells for subsequent chemotherapy and enhanced CDDP chemosensitivity. However, the specific relationship between PRMT1-mediated arginine methylation and CDDP warrants further investigation.

Acknowledgement: This work was supported by the EU's Next Generation EU through the Recovery and Resilience Plan for Slovakia under the project No. [09I03-03-V04-00430] and VEGA2/0100/25.

References

1. Xu J, Richard S (202) Cellular pathways influenced by protein arginine methylation: Implications for cancer. Molecular Cell, 2021; 81, 4357-4368

PRE-SURGICAL IMMUNE-PROTEOMIC PROFILES IN SERUM IDENTIFY INFLAMED OR SUPPRESSED IMMUNE STATES ASSOCIATED WITH PROSTATE CANCER PROGNOSIS

Indri Desiati[1], Selma Bozorgpana[1], Håkon Ramberg[2], Viktor Berge[3,4], Kristin A. Tasken[2,4], Guro F. Giskeødegård[5], Tone F. Bathen[1] , May-Britt Tessem[1,6]

1) Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), Trondheim, Norway.
2) Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway.
3) Department of Urology, Oslo University Hospital, Oslo, Norway.
4) Institute of Clinical Medicine, University of Oslo, Oslo, Norway
5) Department of Public Health and Nursing, Norwegian University of Science and technology (NTNU), Trondheim, Norway
6) Clinic of Surgery, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Abstract

Background: Prostate cancer progression is shaped by tumor-immune interactions and inflammatory processes, but non-invasive blood-based biomarkers associated with prognosis after radical prostatectomy remain limited. We aimed to characterize pre-surgical serum immune-proteomic profiles associated with biochemical recurrence (BCR; prostate specific antigen [PSA] > 0.2 ng/mL) and persistent PSA (PPSA; PSA > 0.1 ng/mL) after surgery, targeting proteins within immuno-oncology and inflammation.

Methods: Pre-surgical serum from two independent prostate cancer cohorts (Trondheim, n=136; Oslo, n=87) were analyzed using the Olink Inflammation and Immuno-Oncology panels, respectively. Group comparisons used one-way ANOVA with false discovery rate adjustment and multivariate modeling used Partial Least Squares Discriminant Analysis with fivefold cross-validation and recursive feature elimination. Prognostics associations were evaluated using Cox proportional hazards models.

Results: IL18 was consistently lower in patients with BCR and PPSA compared to non-recurrent patients, suggesting reduced antitumor immune activity present before surgery. PPSA was further characterized by lower TNFSF12, CCL19, and CD244 compared to non-recurrent patients. We observed a broader trend toward reduced protein levels across the panel in PPSA patients in both cohorts, consistent with a more immune-deserted profile. The protein-based model discriminated BCR and PPSA more accurately than the clinical-parameter model, which included age, PSA before surgery, biopsy Gleason Group, clinical T stage , and Prostate Imaging-Reporting and Data System (PI-RADS) score (Trondheim: BCR AUC 0.73 vs 0.64; PPSA AUC 0.83 vs 0.81; Oslo: BCR AUC 0.77 vs 0.54; PPSA AUC 0.89 vs 0.63). Combining proteins and clinical parameters further improved discrimination (Trondheim BCR AUC 0.75; PPSA AUC 0.91; Oslo

BCR AUC 0.75; PPSA AUC 0.88). Cox analyses identified proteins significantly associated with patients with BCR and PPSA compared to non-recurrent patients ($p<0.05$).

Conclusions: Serum protein signatures improved discrimination beyond presurgical clinical parameters, supporting their potential value for presurgical risk stratification with further validation in larger independent cohorts.

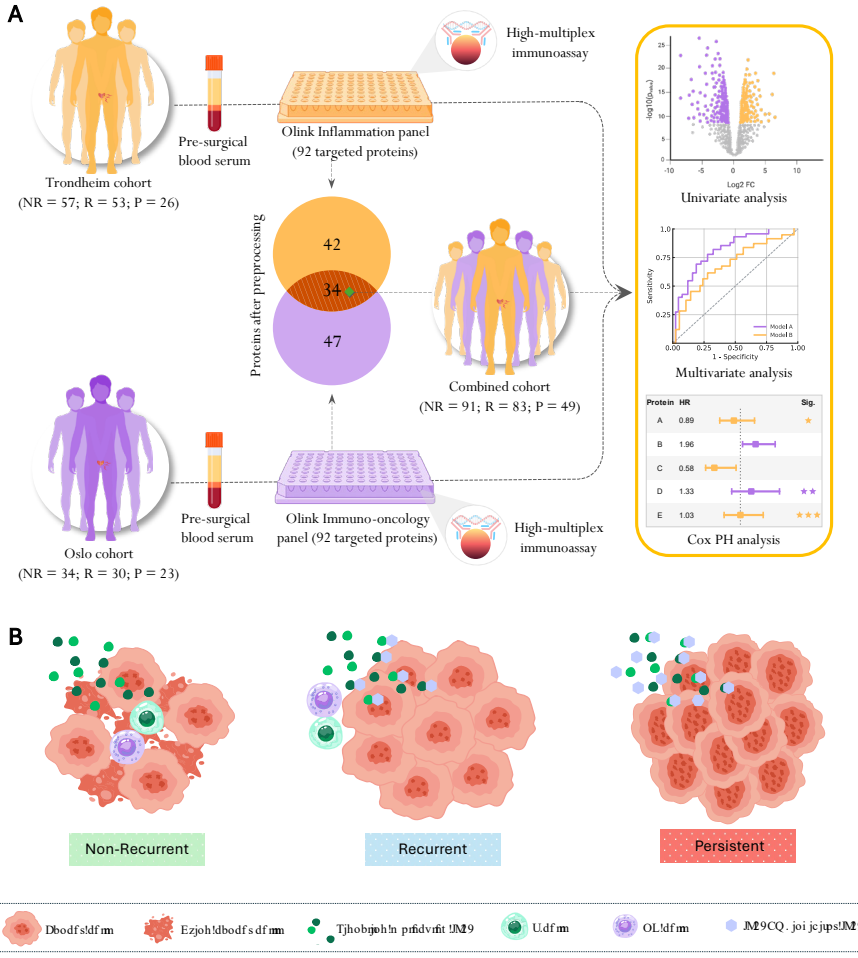


Figure 1. Study design and proposed IL18 signaling across outcome groups.
(A) Pre-surgical serum from the Trondheim and Oslo cohorts was profiled using Olink panels and integrated using overlapping proteins for prognostic biomarker analysis. **(B)** Higher IL18 in non-recurrent prostate cancer may support immune surveillance, whereas reduced IL18 in recurrent disease and profound IL18 loss with increased IL18BP in persistent disease may suppress antitumor immunity.

References

1. Kotb, A.F. and A.A. Elabbady, *Prognostic factors for the development of biochemical recurrence after radical prostatectomy.* Prostate Cancer, 2011. **2011**: p. 485189.
2. Hao, S., et al., *Analysis of risk factors for persistent PSA after radical prostatectomy: results from a high-volume center in Southeast China.* BMC Urol, 2024. **24**(1): p. 185.
3. Rebello, R.J., et al., *Prostate cancer.* Nat Rev Dis Primers, 2021. **7**(1): p. 9.
4. Sharma, U., et al., *The heat of the battle: inflammation's role in prostate cancer development and inflammation-targeted therapies.* Discov Oncol, 2025. **16**(1): p. 108.
5. Gong, D., et al., *Extensive serum cytokine analysis in patients with prostate cancer.* Cytokine, 2020. **125**: p. 154810.
6. Luo, J.L., et al., *Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin.* Nature, 2007. **446**(7136): p. 690–4.
7. Sanz, A.B., et al., *Inflammatory cytokines and survival factors from serum modulate tweak-induced apoptosis in PC-3 prostate cancer cells.* PLoS One, 2012. **7**(10): p. e47440.
8. Culig, Z., *Proinflammatory cytokine interleukin-6 in prostate carcinogenesis.* Am J Clin Exp Urol, 2014. **2**(3): p. 231–8.
9. Landskron, G., et al., *Chronic inflammation and cytokines in the tumor microenvironment.* J Immunol Res, 2014. **2014**: p. 149185.
10. Torrealba, N., et al., *Expression of several cytokines in prostate cancer: Correlation with clinical variables of patients. Relationship with biochemical progression of the malignance.* Cytokine, 2017. **89**: p. 105–115.
11. Ozga, A.J., M.T. Chow, and A.D. Luster, *Chemokines and the immune response to cancer.* Immunity, 2021. **54**(5): p. 859–874.
12. Novysedlak, R., et al., *The Immune Microenvironment in Prostate Cancer: A Comprehensive Review.* Oncology, 2025. **103**(6): p. 521–545.
13. Krossa, S., et al., *Spatial multi-omics identifies aggressive prostate cancer signatures highlighting pro-inflammatory chemokine activity in the tumor microenvironment.* Nat Commun, 2025. **16**(1): p. 10160.
14. Rani, A., P. Dasgupta, and J.J. Murphy, *Prostate Cancer: The Role of Inflammation and Chemokines.* Am J Pathol, 2019. **189**(11): p. 2119–2137.
15. Li, W. and J. Wang, *The current state of inflammation-related research in prostate cancer: a bibliometric analysis and systematic review.* Front Oncol, 2024. **14**: p. 1432857.
16. Chen, R., et al., *Evaluation of the clinical application value of cytokine expression profiles in the differential diagnosis of prostate cancer.* Cancer Immunol Immunother, 2024. **73**(8): p. 139.
17. Assarsson, E., et al., *Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability.* PLoS One, 2014. **9**(4): p. e95192.
18. Qin, W., et al., *MicroRNA-124 regulates TGF-alpha-induced epithelial-mesenchymal transition in human prostate cancer cells.* Int J Oncol, 2014. **45**(3): p. 1225–31.
19. Yin, J., et al., *AR-regulated TWEAK-FN14 pathway promotes prostate cancer bone metastasis.* Cancer Res, 2014. **74**(16): p. 4306–17.
20. Damodaran, S., et al., *Dysregulation of Sirtuin 2 (SIRT2) and histone H3K18 acetylation pathways associates with adverse prostate cancer outcomes.* BMC Cancer, 2017. **17**(1): p. 874.
21. Alvarez-Diaz, S., et al., *Cystatin D is a candidate tumor suppressor gene induced by vitamin D in human colon cancer cells.* J Clin Invest, 2009. **119**(8): p. 2343–58.
22. Halder, S., et al., *The Pleiotropic role, functions and targeted therapies of LIF/LIFR axis in cancer: Old spectacles with new insights.* Biochim Biophys Acta Rev Cancer, 2022. **1877**(4): p. 188737.
23. Ray, M., et al., *Inhibition of Granzyme B by PI-9 protects prostate cancer cells from apoptosis.* The Prostate, 2011. **72**(8): p. 846–855.
24. Hou, Q., et al., *Granzyme H induces apoptosis of target tumor cells characterized by DNA fragmentation and Bid-dependent mitochondrial damage.* Mol Immunol, 2008. **45**(4): p. 1044–55.
25. Bullock, T.N.J., *CD40 stimulation as a molecular adjuvant for cancer vaccines and other immunotherapies.* Cell Mol Immunol, 2022. **19**(1): p. 14–22.
26. Liu, Y., et al., *The Infiltration of IL-18-Related Immune Cells and Their Bidirectional Regulatory Roles in the Pathogenesis of Prostate Cancer.* The Prostate. **n/a**(n/a).

27. Li, Y., et al., *CXCL11 Correlates with Immune Infiltration and Impacts Patient Immunotherapy Efficacy: A Pan-Cancer Analysis.* Front Immunol, 2022. **13**: p. 951247.
28. Liu, Z.L., et al., *Angiogenic signaling pathways and anti-angiogenic therapy for cancer.* Signal Transduct Target Ther, 2023. **8**(1): p. 198.
29. Grizzi, F., et al., *Prostate Cancer Microvascular Routes: Exploration and Measurement Strategies.* Life (Basel), 2023. **13**(10).
30. Wu, R., et al., *Intratumoral PDGFB gene predominantly expressed in endothelial cells is associated with angiogenesis and lymphangiogenesis, but not with metastasis in breast cancer.* Breast Cancer Res Treat, 2022. **195**(1): p. 17–31.
31. Nakanishi, K., *Unique Action of Interleukin-18 on T Cells and Other Immune Cells.* Front Immunol, 2018. **9**: p. 763.
32. Dinarello, C.A., *IL-18: A TH1 -inducing, proinflammatory cytokine and new member of the IL-1 family.* Journal of Allergy and Clinical Immunology, 1999. **103**(1): p. 11–24.
33. Fujita, K., et al., *Immunomodulatory IL-18 binding protein is produced by prostate cancer cells and its levels in urine and serum correlate with tumor status.* Int J Cancer, 2011. **129**(2): p. 424–32.
34. Hong, W., et al., *New Insights of CCR7 Signaling in Dendritic Cell Migration and Inflammatory Diseases.* Front Pharmacol, 2022. **13**: p. 841687.
35. Buller, C.W., P.A. Mathew, and S.O. Mathew, *Roles of NK Cell Receptors 2B4 (CD244), CS1 (CD319), and LLT1 (CLEC2D) in Cancer.* Cancers (Basel), 2020. **12**(7).
36. Zheng, S., et al., *Tumor battlefield within inflamed, excluded or desert immune phenotypes: the mechanisms and strategies.* Exp Hematol Oncol, 2024. **13**(1): p. 80.
37. Hirz, T., et al., *Dissecting the immune suppressive human prostate tumor microenvironment via integrated single-cell and spatial transcriptomic analyses.* Nat Commun, 2023. **14**(1): p. 663.
38. van Loo, G. and M.J.M. Bertrand, *Death by TNF: a road to inflammation.* Nat Rev Immunol, 2023. **23**(5): p. 289–303.
39. Hassouneh, Z., et al., *The Laws of Attraction: Chemokines as Critical Mediators in Cancer Progression and Immunotherapy Response in Bladder Cancer.* Cancers (Basel), 2024. **16**(19).
40. Graff, J.R., et al., *eIF4E activation is commonly elevated in advanced human prostate cancers and significantly related to reduced patient survival.* Cancer Res, 2009. **69**(9): p. 3866–73.
41. Zhu, F., et al., *Eotaxin-1 promotes prostate cancer cell invasion via activation of the CCR3-ERK pathway and upregulation of MMP-3 expression.* Oncol Rep, 2014. **31**(5): p. 2049–54.
42. Rohena-Rivera, K., et al., *CCL-4 enhances prostate cancer migration and invasion by modulating integrin expression.* International Journal of Clinical and Experimental Medicine, 2016. **9**: p. 5426–5438.
43. Hay, Z.L.Z. and J.E. Slansky, *Granzymes: The Molecular Executors of Immune-Mediated Cytotoxicity.* Int J Mol Sci, 2022. **23**(3).
44. Kapiainen, E., et al., *The Amino-Terminal Oligomerization Domain of Angiopoietin-2 Affects Vascular Remodeling, Mammary Gland Tumor Growth, and Lung Metastasis in Mice.* Cancer Research, 2021. **81**(1): p. 129–143.
45. Gupta, A., et al., *Predictive value of plasma hepatocyte growth factor/scatter factor levels in patients with clinically localized prostate cancer.* Clin Cancer Res, 2008. **14**(22): p. 7385–90.
46. Escamilla, J., et al., *CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy.* Cancer Res, 2015. **75**(6): p. 950–62.

IDENTIFICATION AND STRUCTURAL MODELING OF THE NOVEL TTC33-ASSOCIATED NETWORK CORE (TANC) COMPLEX INVOLVED IN DNA DAMAGE RESPONSE

M. Drabko[1], R. Tomecki[1,5], M. Siek[1], A. Jurkiewicz[2], M. Ludwinek[1], K. Kobyłecki[1], D. Cysewski[3,6], A. Malinowska[3], M. Bakun[3], K. Goryca[4], Ł. S. Borowski[2,5], R. J. Szczęsny[2], R. Płoski[7], A. Tudek[1]

1) Laboratory of RNA Processing and Decay, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5A, Warsaw, Poland;
2) Laboratory of RNA Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5A, Warsaw, Poland;
3) Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5A, Warsaw, Poland;
4) Genomic Core Facility, Centre of New Technologies, University of Warsaw, Warsaw, Poland;
5) Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, A. Pawińskiego 5A, Warsaw, Poland;
6) present address: Medical University of Białystok, Clinical Research Centre, J. Kilińskiego 1, Białystok, Poland;
7) Department of Medical Genetics, Medical University of Warsaw, A. Pawińskiego 3c, Warsaw, Poland;
*Correspondence: atudek@ibb.waw.pl

Approximately 9% of human protein-coding genes lack defined functions, presenting a substantial gap in understanding cellular processes. Among these uncharacterized proteins is TTC33, a 30 kDa protein composed of tetratricopeptide (TPR) repeats and conserved exclusively in bony vertebrates. This study aimed to elucidate TTC33 function by characterizing its interaction partners, structural features, and cellular roles using the "function by proximity" approach.

We employed comparative label-free mass spectrometry (cLF-MS) to map the TTC33-associated network (TAN), identifying key interactors including WDR61, CCDC97, UNG, PP2A-B55α, PHF5A, and the SF3B subcomplex of U2. Large-scale purification and size-exclusion chromatography revealed a stable trimeric core complex (TANC) composed of TTC33, WDR61, and PHF5A, which co-purified in stoichiometric amounts.

Structural modeling using AlphaFold 3 and molecular dynamics simulations provided insights into TANC architecture. The model demonstrated that the negatively charged TPR2-3-4 concave of TTC33 recruits the positively charged PHF5A C-terminus via multiple electrostatic interactions. Deletion of the C-terminal 9 amino acids of PHF5A disrupted its binding to TTC33. Adjacent to the charged PHF5A patch is a putative RNA cross-linking site, and consistently, SF3B4 co-purified more efficiently with TTC33 in the absence of RNA.

Functional analysis through transcriptomic profiling revealed 421 differentially expressed transcripts in TTC33-depleted cells, including up-regulation of CGA, UNC13A, TMEM145,

and MMP24, and down-regulation of SFRP1. Immunofluorescence assays showed that TTC33 depletion led to redistribution of p53-S15P, a marker of DNA damage. Changes in TTC33 abundance resulted in reduced cellular proliferation and decreased resistance to hydrogen peroxide, highlighting its importance in cell viability and oxidative stress response.

This work identifies TANC as a novel complex involved in DNA damage response and provides structural insights into its assembly and function.

References

1. Fagerberg, Linn et al. “Analysis of the human tissue-specificexpression by genome-wide integration of transcriptomics andantibody-based proteomics.” Molecular & cellular proteomics : MCPvol. 13,2 (2014): 397-406. doi:10.1074/mcp.M113.035600
2. Abraham, M. et al. GROMACS 2025.2 Manual. 2025.2, Zenodo, 12maj 2025, doi:10.5281/zenodo.15387070.
3. Abramson, Josh et al. “Accurate structure prediction ofbiomolecular interactions with AlphaFold 3.” Nature vol. 630,8016(2024): 493-500. doi:10.1038/s41586-024-07487-w
4. Cretu, Constantin et al. “Molecular Architecture of SF3b andStructural Consequences of Its Cancer-Related Mutations.”Molecular cell vol. 64,2 (2016): 307-319.doi:10.1016/j.molcel.2016.08.036REFERENCESstate

NEW ADVANCES IN AI-EMPOWERED TISSUE CYTOMETRY AND PRECISION MEDICINE

R. C. Ecker[1,2,3] F. Mungenast[1] and J. Batra[2,3]

1) TissueGnostics GmbH, Taborstrasse 10/2, 1020 Vienna, Austria
2) Bond University, Faculty of Health Sciences & Medicine, Australia
3) 3School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia.

INTRODUCTION: The advent of single cell genomic technologies provides a wealth of valuable transcriptomic data, however lack alignment with cellular phenotypes in situ.
METHODS: Our research teams at TissueGnostics, Bond University and Queensland University of Technology have joined forces to combine TissueGnostics' existing tissue cytometry technology platform and established knowhow with innovative AI solutions to establish state-of-the-art solutions for precision medicine. An automated platform for tissue image cytometry has been established that allows to understand cellular interactions and quantify immune responses where they happen – in the tissue. This includes in-depth analysis of single cell-transcriptomic data and linking of RNA signatures with cellular expression profiles.
RESULTS: Tissue Image Cytometry permits to determine the in-situ phenotype of individual cells as well as histological entities, like glands, vessels or tumor foci. Applications include but are not limited to the exploration of immune responses in situ and the tumor microenvironment and/or the spatial organization of cellular subpopulations. To better understand the function of inflammatory cells in tumor development, type and number of inflammatory cells and their proximity to glandular/tumor structures have to be analyzed in-situ and correlated with disease state. Using Tissue Image Cytometry the time-consuming and error-prone human evaluation of stained histological sections can be approached with an observer-independent and reproducible technology platform, offering a high degree of automation, paired with user interaction at relevant points of the analytical workflow.
DISCUSSION & CONCLUSIONS: The Tissue Image Cytometry platform provides scanning in brighfield, widefield fluorescence, confocal, multispectral as well as multi-plexing and incorporates Machine & Deep Learning algorithms. It also promotes tissue cytometry to a new level of quality, where complex cellular interactions, intracellular expression profiles and signal transduction cascades can be addressed on the single-cell level but still in histological context, empowering precision diagnostics.

TARGETING OXIDATIVE STRESS IN CEREBROSPINAL FLUID OF DOGS WITH INTERVERTEBRAL DISC HERNIATION

A. Horvatić[1], O. Smolec[2], B. Pirkić[2] and M. Lipar[2]

1 University of Zagreb Faculty of Food Technology and Biotechnology, Pierottijeva 6, 10000 Zagreb, Croatia

2 University of Zagreb Faculty of Veterinary Medicine, Heinzelova 55, 10000 Zagreb, Croatia

Oxidative stress is an imbalance between excessive reactive oxygen species (ROS) and insufficient antioxidant defences in body, resulting in cellular, lipid, and DNA damage. It is implicated in many chronic diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. Recent findings in humans also highlights its role in intervertebral disc degeneration (IVD) and related conditions. In canine intervertebral disc herniation (IVDH), oxidative stress is reflected in microenvironment in the cerebrospinal fluid (CSF), providing a valuable source for identifying key molecular changes associated with disease pathogenesis.

In this study, CSF samples from dogs with IVDH and healthy controls were analysed using LC-MS-based proteomics and metabolomics. Among the significantly deregulated proteins (227 in total) and metabolites (73 in total), several oxidative stress–related proteins and metabolites were identified in CSF in relation to canine IVDH. The antioxidant enzyme paraoxonase 1 (PON1) was elevated, indicating a protective response aimed at maintaining redox balance and preventing lipid oxidation. This increase was confirmed by enzymatic activity measurements (189.6 ± 77.79 mU/L; $p = 0.0375$). Superoxide dismutase 1 (SOD1) was also elevated, reflecting activation of enzymatic defences that neutralise ROS. At the metabolite level, elevated pyroglutamic acid indicated disruption of glutathione metabolism, a key antioxidant pathway responsible for ROS detoxification. Increased glutamate indicates excitotoxicity, in which excessive receptor stimulation causes mitochondrial dysfunction and oxidative damage. Additionally, arginine metabolism contributes to oxidative stress through nitric oxide (NO) production, generating reactive nitrogen species (RNS) that promote neuronal injury and myelin degradation.

Overall, oxidative stress in IVDH is very complex and significant, driven by interconnected mechanisms including ROS accumulation, impaired glutathione cycling, nitric oxide–mediated damage, and excitotoxicity. Antioxidant proteins such as PON1 and SOD1 appear to counteract these effects, highlighting their potential role in disease modulation and as targets for future therapeutic strategies**.**

References

1. Horvatić A, Gelemanović A, Pirkić B, Smolec O, Beer Ljubić B, Rubić I, Eckersall PD, Mrljak V, McLaughlin M, Samardžija M, Lipar M. (2021) Multi-Omics Approach to Elucidate Cerebrospinal Fluid Changes in Dogs with Intervertebral Disc Herniation. Int J Mol Sci. 22(21):11678
2. Baka, R.D.; Ginoudis, A.; Botia, M.; Garcia-Martinez, J.D.; Savvas, I.; Giota, D.; Polizopoulou, Z. Assessment of Oxidative Stress-Related Markers and Inflammatory Proteins in Serum and CSF Samples of Dogs with Different Types of Epilepsy. Antioxidants **2026**, *15*, 282.

PROTEOMIC ANALYSIS OF TEARS AND MEIBUM OF PATIENTS WITH MEIBOMIAN GLAND DYSFUNCTION

G. Kecskeméti[1], Zs. Orosz[2], E. Tóth-Molnár[2], T. Janáky[1] and Z. Szabó[1]

1) Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary
2) Department of Ophtalmology, Albert Szent-Györgyi Health Centre, University of Szeged, Korányi Fasor 10-11, H-6720 Szeged, Hungary

Meibomian gland dysfunction (MGD) is a major cause of evaporative dry eye, arising from impaired lipid secretion, ductal obstruction, and progressive acinar atrophy. The recent TFOS DEWS III reports emphasize a shift toward mechanism-based, personalized management of dry eye, highlighting the need to identify molecular drivers of glandular dysfunction and tear-film instability. Proteomic profiling of meibum and tear fluid offers a powerful approach to uncover these mechanisms and supports the development of targeted, patient-specific therapeutic strategies [1].

This study aimed to comprehensively characterize the proteomic composition of tear and meibum samples, to determine proteins most likely originating from the Meibomian glands and to identify proteomic alterations associated with MGD. The findings are expected to contribute to the elucidation of the disease's molecular mechanisms, facilitate the discovery of biomarkers, and serve as a basis for the development of targeted, mechanism-based therapeutic strategies.

Meibum and tear samples were collected using Schirmer strips from healthy and MGD-affected individuals. The extracted proteins were digested with trypsin, and the resulting peptides were analyzed using a nanoLC-MS/MS system. Data were acquired in Data-Independent Acquisition (DIA) mode and subsequently processed with the DIA-NN 2.5.0 software.

Through correlation analysis of quantified proteins between different tear samples, combined with detection-frequency filtering across sample types, we identified more than 500 putative contaminant proteins, the majority of which were of cellular origin. These proteins were excluded from all subsequent analyses. After this filtering, statistical comparisons between control and patient groups were performed using more than 1,500 confidently quantified proteins in both the tear and meibum datasets. Proteins with significant differences in expression between the control group and the patient group were successfully identified in both meibum and tear samples.

References

[1] L. Jones *et al.*, "TFOS DEWS III: Management and Therapy," 2025. doi: 10.1016/j.ajo.2025.05.039.

STRUCTURAL CHARACTERIZATION OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) GLYCANS FROM *CHLORELLA VULGARIS* USING MALDI-MS AND MS/MS

B. Komazec[1] *and P. Peharec Štefanić*[1]

1) Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia

Extracellular polymeric substances (EPS) produced by microalgae represent structurally complex matrices rich in high-molecular-weight polysaccharides whose detailed characterization remains analytically challenging due to their heterogeneity and broad mass distribution [1]. In this study, we developed and optimized a glycomic workflow for structural characterization of EPS isolated from the freshwater microalga *Chlorella vulgaris* using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and tandem MS (MS/MS). EPS was isolated using ion-exchange resin to enrich high-molecular-weight fractions, followed by controlled partial hydrolysis with trifluoroacetic acid (TFA) to generate oligosaccharides suitable for MS analysis. Reduced sugars were derivatized with phenylhydrazine via on-target derivatization to improve ionization efficiency and reproducibility. Sample clean-up was performed using different solid-phase extraction sorbents, with Strata-X-C providing the most effective removal of interfering components and improved spectral quality. MALDI-MS measurements were conducted using 2,5-dihydroxybenzoic acid and 2-aza-2-thiothymine matrices in both positive and negative ion modes, and structural assignments were confirmed by MS/MS fragmentation. Spectral analysis revealed a consistent series of ions with mass differences corresponding to uronic acid oligomers, while MS/MS fragmentation confirmed the presence of β-D-mannuronic acid and α-L-guluronic acid residues, indicating that the dominant EPS polysaccharide has an alginate-like composition. In addition, signals corresponding to hexoses and pentoses were detected, highlighting the compositional complexity of the EPS. Detailed interpretation enabled the identification of four distinct polysaccharide chain types: uronic acid-rich, oligo-hexose, mixed uronic acid–hexose, and hexose–pentose structures. Consistent results were obtained across independent replicates, indicating good reproducibility of the analytical approach. The identification of alginate-like polysaccharides in *C. vulgaris* EPS represents a novel finding and demonstrates the applicability of MALDI-MS/MS for resolving structurally complex glycan mixtures in microalgal systems.

References

1. Lesco KC, Williams SKR, Laurens LML (2025) Marine algae polysaccharides: an overview of characterization techniques for structural and molecular elucidation. Mar Drugs 23(3):105

INFLUENCE OF PROTEIN SUBSTRATE ON THE INTEGRITY OF AN *IN VITRO* BLOOD-BRAIN BARRIER MODEL

D. Kosturíková[1], K. Bhide[1] and M. Bhide[1,2]

1) Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia
2) Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

The blood-brain barrier (BBB) is a complex structure that plays a key role in maintaining central nervous system (CNS) homeostasis. However, some pathogens are capable of crossing the BBB causing severe neuroinfections. Treatment is difficult due to the problematic drug delivery through the BBB thus numerous *in vitro* BBB models have been developed. A major issue remains insufficient standardization, which is related to the use of different protein substrates for the cultivation of brain microvascular endothelial cells (BMECs). The aim of this study was to compare the integrity of hBMEC/D3 cell monolayers cultured on transwell inserts coated with type I collagen, gelatin, or a mixture of extracellular matrix (ECM) proteins that forms endothelial basement membrane of the BBB. Integrity was evaluated by continuous monitoring of capacitance (Ccl) and transendothelial electrical resistance (TEER) by cellZscope device (nanoAnalytics, Germany), as well as permeability determination using CF770 dextran. In case of gelatin substrate the average Ccl value was lower than 0.5 μF/cm² and the average permeability to CF770 dextran reached 7.55 ± 0.28%, indicating poor integrity of the monolayer. In case of type I collagen the average Ccl value was 0.59 ± 0.09 μF/cm² and permeability for CF770 dextran was 2.82 ± 0.71%, indicating good barrier integrity. The best integrity was observed with ECM protein mixture in which the average Ccl value reached 1.22 ± 0.01 μF/cm² and permeability for CF770 dextran was 0.92 ± 0.11% only. The difference in permeability between cells cultured on inserts coated with ECM protein (of human origine) mixture and gelatin, as well as between type I collagen and gelatin, was statistically significant ($p \leq 0.01$).

The study was funded by projects VEGA 1/0381/23 and APVV-22-0084.

References

1. Kim KS (2008). Mechanisms of microbial traversal of the blood–brain barrier. Nature Reviews Microbiology 6(8):625–634.
2. Pardridge WM (2005). The blood-brain barrier: bottleneck in brain drug development. NeuroRx 2(1):3–14.
3. Du F, Shusta EV, Palecek SP (2023). Extracellular matrix proteins in construction and function of in vitro blood-brain barrier models. Frontiers in Chemical Engineering 5:1130127.

MULTI-OMICS PLASMA PROFILING FOR BIOMARKER PANEL IDENTIFICATION IN MYOCARDIAL INFARCTION AND ISCHEMIC STROKE

Štěpánka Kučková[1], Adéla Mazáčová[1], Jiří Šantrůček[1], Vít Kosek[2], Kamila Bechyňská[2], Pavel Cejnar[3], David Netuka[4], Martin Malý[4]

1) Department of Biochemistry and Microbiology, UCT Prague, Technická 3, 166 28 Prague, Czech Republic
2) Department of Food Analysis and Nutrition, UCT Prague, Technická 3, 166 28 Prague, Czech Republic
3) Department of Mathematics, Informatics and Cybernetics, UCT Prague, Technická 5, 166 28 Praha 6, Czech Republic
4) Military University Hospital, U Vojenské nemocnice 1200, 169 02 Prague, Czech Republic

Atherosclerosis and associated thrombotic complications are major causes of acute coronary syndrome (ACS) and acute ischemic stroke (AIS). The aim of this study was to characterize plasma protein and lipid profiles in patients with myocardial infarction (MI) and ischemic stroke (AIS) and to identify biomarkers enabling their differentiation and improved patient stratification. C-reactive protein (CRP) levels were determined using hsCRP ELISA, while additional candidate biomarkers were analysed by multiplex immunoassay. Untargeted lipidomics and proteomics were performed using high-resolution liquid chromatography – mass spectrometry combined with multivariate statistics and machine learning approaches.

CRP concentrations were elevated in both pathological conditions but did not allow their mutual differentiation. Multivariate analysis of multiplex data identified troponin and pentraxin 3 as highly specific markers of MI, whereas follistatin-like protein 1 and tissue factor were key discriminators of AIS. CD31 reflected general vascular injury. Untargeted proteomics differentiated MI and AIS patients with a classification accuracy of 91% and revealed 526 significantly altered proteins out of 4,357 detected proteins. Major contributors to group separation included triglyceride lipase, brain-type creatine kinase, and α-2-antiplasmin. Lipidomic analysis identified 409 plasma lipids, of which 22 species, mainly from LPC, LPE, and triacylglycerol classes, showed the strongest discriminatory potential.

These findings demonstrate that single biomarkers are insufficient for accurate diagnosis and patient stratification. Integration of untargeted proteomics, lipidomics, and multivariate modelling enables the identification of complex biomarker panels with superior discriminatory performance compared with traditional targeted markers.

The work was supported by Ministry of Education, Youth and Sports of the Czech Republic – INTER-EXCELLENCE II grant number LUC23138. This work was also funded by a grant from the Czech Health Research Council, Czech Republic (NU22-04-00389).

PROTEOMIC RESPONSES OF *CHLORELLA VULGARIS* TO COPPER OXIDE NANOPARTICLES AND COPPER IONS

B. Komazec[1], D. Loknar[1], B. Balen[1], R. Biba[2], M. Cindrić[2] and P. Peharec Štefanić[1]

1) Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
2) Laboratory for Bioanalytics, Division of Molecular Medicine, Ruđer Bošković Institute, POB 1016, HR-10000 Zagreb, Croatia

Copper-based nanomaterials are increasingly released into aquatic environments, yet their toxicity mechanisms in primary producers remain insufficiently understood [1]. In this study, we investigated proteomic changes in the freshwater microalga *Chlorella vulgaris* after 72 hours of exposure to copper(II) oxide nanoparticles (CuONPs), copper(I) oxide nanoparticles (Cu_2ONPs), and ionic copper ($CuSO_4$). Algal cultures were treated with concentrations corresponding to 75% survival, and differentially expressed proteins were analyzed by two-dimensional gel electrophoresis followed by in-gel digestion and liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QTOF-MS/MS) identification. A total of 29 differentially expressed protein spots were identified across the three treatments. CuONPs induced a predominantly adaptive response, with 18 of 19 altered proteins showing increased abundance. Most of these proteins were associated with photosynthesis, carbohydrate metabolism, ATP synthesis, signaling, and cytoskeletal organization. In contrast, Cu_2ONPs and $CuSO_4$ caused a distinctly repressive proteomic profile, with 15 of 19 proteins downregulated after both treatments. The most affected proteins were involved in photosynthesis, signal transduction, and translation-related pathways, including carbonic anhydrase, PSI/PSII-associated proteins, elongation factors, and calcium/calmodulin-dependent protein kinases. Only a limited set of proteins, such as actin and aconitate hydratase A, showed increased abundance after all copper treatments. These results indicate that copper toxicity in *C. vulgaris* strongly depends on nanoparticle oxidation state and copper speciation. CuONPs triggered a proteomic pattern consistent with compensatory metabolic adjustment, whereas Cu_2ONPs and $CuSO_4$ caused broader suppression of photosynthetic and biosynthetic functions, suggesting stronger stress associated with lower particle stability and enhanced ion-mediated toxicity. Proteomic profiling therefore provides mechanistic insight into the distinct cellular responses elicited by nanoparticulate and ionic copper.

References

1. Solomonova E, Shoman N, Akimov A, Rylkova O (2023) Impact of copper oxide nanoparticles on the physiology of different microalgal species. Reg Stud Mar Sci 66:103128

DESIGN AND MODIFICATION OF TRUNCATED AURELIN-DERIVED PEPTIDES

Lea Pašalić[1] *and Andreja Jakas*[1]

1) Rudjer Bošković Institute, Division of Organic Chemistry and Biochemistry, Bijenička cesta 54, Zagreb, Croatia

Aurelin is a cationic antimicrobial peptide originally isolated from the jellyfish *Aurelia aurita,* exhibiting activity against both Gram-positive and Gram-negative bacteria.[1] Due to the increasing prevalence of antimicrobial resistance, antimicrobial peptides have attracted significant interest as potential alternatives to conventional antibiotics.[2] Their biological activity is strongly influenced by amino acid composition, charge distribution, hydrophobicity, and conformational flexibility.[3] In this work, selected aurelin-derived peptide fragments (AACSDRAHGH, ICESFKSFCK, DSGRNGVKLR, and ANCKKTCGLC) (Figure 1.) were synthesized using automated solid-phase peptide synthesis (SPPS) to investigate the influence of targeted amino acid substitutions on peptide properties. Cysteine residues were substituted with arginine to further increase the overall cationic character of the peptides and eliminate the possibility of disulfide bond formation. The study is primarily focused on the rational design and synthetic preparation of modified aurelin fragments. The applied modification strategy represents a systematic approach to understanding the relationship between amino acid composition, charge distribution, and peptide behavior. The obtained peptide analogues provide a basis for future structure–activity relationship studies and biological evaluation of aurelin-derived antimicrobial peptides.

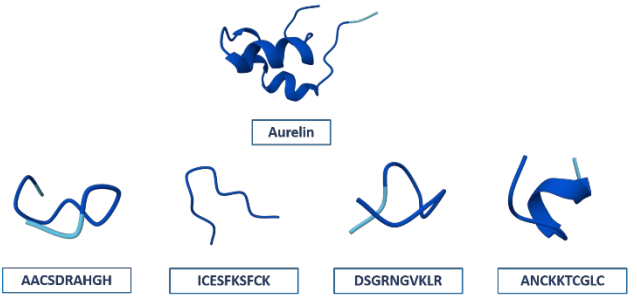


Figure 1. Aurelin and aurelin-derived fragments

References

1. Tatiana V. Ovchinnikova, Sergey V. Balandin, Galina M. Aleshina et al. (2006) Aurelin, a novel antimicrobial peptide from jellyfish Aurelia aurita with structural features of defensins and channel-blocking toxins, *Biochem. Biophys. Res. Commun.* 348 (2): 514-523

2. Berger, I.; Loewy, Z.G. (2024) Antimicrobial Resistance and Novel Alternative Approaches to Conventional Antibiotics. *Bacteria* , 3, 171-182.

3. Poluri, K.M., Gulati, K., Sarkar, S. (2021). Structural and Functional Properties of Proteins. In: Protein-Protein Interactions. Springer, Singapore. 1-60.

SURFACE FUNCTIONALIZATION SHAPES THE PROTEOMIC RESPONSE OF *CHLORELLA VULGARIS* TO POLYSTYRENE NANOPARTICLES

P. Peharec Štefanić[1], B. Komazec[1], D. Vuković[1], B. Balen[1], R. Biba[2] and M. Cindrić[2]

1) Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia
2) Laboratory for Bioanalytics, Division of Molecular Medicine, Ruđer Bošković Institute, POB 1016, HR-10000 Zagreb, Croatia

Polystyrene nanoparticles are an emerging class of aquatic contaminants whose biological effects depend strongly on surface chemistry [1]. To assess how nanoparticle functionalization influences algal responses, we analyzed proteomic changes in the freshwater microalga *Chlorella vulgaris* after 72 hours of exposure to non-functionalized polystyrene nanoparticles (PS-NPs) and amino- or carboxyl-modified particles (PS-NH_2-NPs and PS-COOH-NPs). Total soluble proteins were separated by two-dimensional electrophoresis and differentially expressed spots were identified by liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry (LC-ESI-QTOF-MS/MS). After all treatments, 29 proteins with altered abundance were identified. Proteome changes were dominated by downregulation, particularly after exposure to PS-NPs and PS-COOH-NPs. PS-NPs altered 25 proteins, of which 21 were decreased, while PS-COOH-NPs affected 26 proteins, with 24 showing lower abundance. PS-NH_2-NPs produced a less extensive but still clear response, with 11 of 13 differential proteins downregulated. The most consistently affected functional category was photosynthesis, including carbonic anhydrase, RuBisCO small subunit, chlorophyll *a*/*b*-binding proteins, PSI/PSII-associated proteins, and ATP synthase subunit beta. In parallel, proteins involved in stress defense, signal transduction, carbohydrate metabolism, and translation were frequently reduced. In contrast, only a few proteins, such as actin and the dehydration-related protein PCC13-62, showed increased abundance in all or most treatments. The observed proteomic signatures indicate that polystyrene nanoparticles primarily impair photosynthetic and regulatory processes in *C. vulgaris*, with the strongest overall repression caused by unmodified and carboxylated particles. These findings support the view that nanoparticle surface functionalization is a key determinant of algal stress responses and should be considered when evaluating the ecological risk of nanoplastics in freshwater systems.

References

1. Natarajan L, Soupam D, Dey S, Chandrasekaran N, Kundu R, Paul S, Mukherjee A (2022) Toxicity of polystyrene microplastics in freshwater algae *Scenedesmus obliquus*: effects of particle size and surface charge. Toxicol Rep 9:1953-1961

SERUM PROTEOME PREDICTS RESPONSE TO ANTI-TNFA THERAPY IN INFLAMMATORY BOWEL DISEASE

M. Petek[1], Y.-I. Kim[2], B. Gole[1] and U. Potočnik[1]

1) Faculty of Medicine, University of Maribor, Taborska ulica 8, 2000 Maribor, Slovenia
2) Cambridge Centre for Proteomics, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK

Inflammatory bowel disease (IBD) is a chronic, relapsing and progressive disorder of the gut driven by tumour necrosis factor alpha (TNFα)-mediated inflammation. Severe IBD cases are treated with anti-TNFα biologics (infliximab, adalimumab, golimumab) or biologics targeting alternative pathways. Predicting patient response to anti-TNFα agents remains challenging: up to 30% of patients show no primary response and a further 30-40% lose response within one year[1]. We investigated whether the serum proteome differs between responders and non-responders to anti-TNFα therapy, either at treatment initiation or during the first six months of treatment.

Serum samples were obtained from 19 IBD patients eligible for anti-TNFα therapy (11 responders, 7 non-responders, 1 excluded). Samples were collected at treatment initiation and after three and six months of treatment. Non-responders were switched to an alternative biologic following enrolment. Serum proteins (50 µg) were precipitated with acetone, and cysteine-carbamidomethylated tryptic peptides were prepared. Peptides (1.25 µg) were separated using a 30-min LC gradient and analysed by LC-MS/MS on a timsTOF HT mass spectrometer in diaPASEF mode with ion mobility separation. Proteins were identified with DIA-NN against a spectral library derived from extracellular vesicle samples.

We quantified 751 proteins from 31 non-depleted, non-fractionated human serum samples using minimal sample preparation and achieved good reproducibility. After correction for multiple hypothesis testing, significant differences in protein expression were observed between responders and non-responders prior to treatment initiation. Responders showed significantly higher levels of 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase eta-1 (PLCH1), apolipoprotein C-III, CD5 antigen-like protein, selenoprotein P, and multiple immunoglobulin protein groups. Non-responders showed higher levels of apolipoprotein A and haptoglobin proteins. Most proteomic differences remained stable over time.

The study was supported by the Ministry of Higher Education Science and EU (grant MN-0014–2334) and Innovation and the European Regional Development Fund OP20.05187 RI-SI-EATRIS.

References

1. Katsanos KH, Papamichael K, Feuerstein JD, Christodoulou DK,Cheifetz AS (2019) Biological therapies in inflammatory bowel disease: Beyond anti-TNF therapies. Clinical Immunology 206, 9–14

COMPARISON OF THE TARGETING BEHAVIOR OF TWO EXCHANGEABLE PERILIPINS, PLIN3 AND PLIN4 TO LIPID DROPLET

Bayane Sabbagh[1], Cyril Moulin[1], Céline Franckhauser[1], Nicolas Fuggetta[1], Amel El-Bahloul[1], Alenka Čopič[1]

1) Montpellier Cell Biology Research Center (CRBM), CNRS & Université de Montpellier, Montpellier, France

Due to the particular structure of lipid droplets (LDs), the physico-chemical properties of their lipid surface are more diverse compared to other organelles [1]. This diversity is exploited by LD-targeting proteins; such as the perilipin family [1]. While most perilipins share common structural features—including a conserved PAT domain, an amphipathic helix (AH), and a C-terminal four-helix bundle, perilipin 4 (Plin4) is unique: its AH is more than 10 times longer and it lacks the PAT domain [2]. In this study, we focus on PLIN3 and PLIN4, both of which target LDs from the cytosol using their amphipathic helices and require increased surface tension for efficient binding, yet differ in their LD-binding properties [2,3]. We explore how different domains of PLIN3 and PLIN4 contribute to LD binding. Studying Plin4 in its full-length is particularly challenging due to its large size inherently complex nature, and highly repetitive sequence. To achieve this, I analyzed individual Plin4 domains to understand their specific contributions to LD interactions. Furthermore, mutations in PLIN4 that lead to the expansion of its repetitive region have been associated with pathological aggregation in skeletal muscle and degeneration [2]. During my PhD, I have studied the properties of the different regions of Plin4 to understand how they contribute to Plin4 binding to LDs. In this study, we show that small variations in the Plin4 AH sequence influence its aggregation and potentially toxic behavior observed in myopathy patients [2] .

References

1. Bersuker K, Olzmann JA (2017) Establishing the lipid droplet proteome: mechanisms of lipid droplet protein targeting and degradation. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1862(10,Part B):1166–1177.

2. Araujo ARD, Bello AA, Bigay J, Franckhauser C, Gautier R, Cazareth J, Kovacs D, Brau F, Fuggetta N, Copic A, Antonny B (2024) Surface tension-driven sorting of human perilipins on lipid droplets. bioRxiv :2024.02.12.579497.

3. Giménez-Andrés M, Emeršič T, Antoine-Bally S, D'Ambrosio JM, Antonny B, Derganc J, Copic A (2021) Exceptional stability of a perilipin on lipid droplets depends on its polar residues, suggesting multimeric assembly. eLife 10:e61401.

THE POTENTIAL PROTEIN MARKERS OF BIPOLAR DISORDER

Liudmila Smirnova[1], Alexander Seregin[1], Evgeny Ermakov[1,2], Maria Zavialova[3,4], Elena Dmitrieva[1], Svetlana Novikova[4], and Svetlana Ivanova[1,5]

1) Mental Health Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, 634014, Aleutskaya street 4, Tomsk, Russia
2) Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, 630090, Lavrentiev Avenue 8, Novosibirsk, Russia
3) Skolkovo Institute of Science and Technology, 121205, Bolshoy Boulevard 30, bld. 1, Moscow, Russia
4) Institute of Biomedical Chemistry, 119121, Pogodinskaya Street 10, bld. 8, Moscow, Russia
5) Siberian State Medical University, 634050, Moskovskiy Trakt 2, Tomsk, Russia

Bipolar disorder (BD) are major mental disorder with complex genetic and pathophysiological underpinnings. There is a growing need for peripheral markers that can support diagnosis and treatment outcome prediction (theranostics) in mental disorders. Modern high-throughput methods, particularly proteomic analysis, enable the identification of protein biomarkers associated with functional impairments involved in disease pathophysiology. Following initial mass spectrometric profiling (MS/MS), five minor proteins (NMDAR1, ANK3, ANKRD12, LRP2, DCD) and anti-NMDAR antibodies were quantified by ELISA in an expanded cohort (BD n=50, control n=70). NMDAR1 was also quantified by SRM mass spectrometry.

A comparative proteomic analysis of serum proteins from patients with bipolar disorder and healthy individuals identified a total of 3,036 proteins. Of these, 452 unique proteins were identified in patients with bipolar disorder, while 249 proteins not found in patients with bipolar disorder were identified in healthy individuals. SRM analysis revealed a significant increase in protein levels in BD compared to controls ($p=0.021$). ELISA confirmed a significant increase in NMDAR1 in BD patients with a current depressive episode compared to a mixed episode. Quantitative analysis of other proteins showed a significant decrease in ANK3 in BD ($p=0.03$) compared to controls. In contrast, LRP2 levels increased 2-fold in BD. Elevated levels of NMDAR antibodies in high titers were most often observed in mixed episodes of BD (28.6%). The frequency of DCD detection was higher in the depressive episode BD compared to the mixed one ($p=0.008$). Conclusions: The findings suggest a unified mechanism of "synaptopathy" involving destabilization of the ANK3-NMDAR scaffold and a compensatory neuroprotective response (LRP2, DCD). The specific association of ANKRD12 with negative symptoms and anti-NMDAR antibodies with mixed episodes highlights their potential for differential diagnosis and phase-specific monitoring.

This study was supported by a grant from the Russian Science Foundation (No. 23-75-00023).

ALTERNATIVE SPLICING OF *ARABIDOPSIS THALIANA* BPM2 TRANSCRIPTS: A POSSIBILITY OF FUNCTIONAL DIVERSIFICATION OR PROTEIN DOWNREGULATION

Emma Stepinac, Gaj Keresteš, Mateja Jagić, Sandra Vitko, Paula Štancl, Karlo Miškec, Marta Frlin, Rosa Karlić, Željka Vidaković-Cifrek, Dunja Leljak Levanić, Nataša Bauer

Division of Biology, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia

MATH-BTB protein family is a component of the ubiquitin–proteasome system, acting as substrate adaptors in the CUL3 complex. The N-terminal MATH domain of MATH-BTB proteins binds the protein targeted for degradation, while the C-terminal BTB domain binds the ubiquitin E3 ligase Cullin-3, which in turn, through ubiquitin attachment, directs the target protein towards proteasomal degradation[1]. MATH-BTB proteins have diverse developmental and stress-related roles in eukaryotes. In Arabidopsis thaliana there are six such BTB/POZ AND MATH DOMAIN genes (BPM1-6)[1] which may be, according to the latest transcriptome database AtRTD3, transcribed and spliced into 56 transcript variants, and could encode for 38 different BPM proteins. We particularly focused on BPM2 gene as the candidate with the highest number of splice variants, all of which encode putative protein isoforms with an intact MATH domain but a truncated BTB domain, suggesting distinct functional properties. Following the mRNA isolation and RT-PCR, we selected several of the most widely expressed BPM2 splice variants and analyzed their subcellular co-localization in planta with some of the BPM-interacting proteins, like CUL3, DREB2A and WRI1. While DREB2A is a key transcription factor involved in drought and heat stress response, WRI1 is a transcription factor involved in seed development. Our co-localization analysis indicates that, unlike the canonical BPM2 variant that co-localizes with the targets to the nucleus, BPM2 isoforms with a truncated C-terminal BTB domain alter the intracellular distribution of target proteins by retaining them to the cytoplasm. Whether this might be interfering with the function of transcriptional regulators and to which extent remains to be investigated. Moreover, the question arises whether alternative splicing of BPM2 gene expands the functional repertoire of MATH-BTB proteins by not only enhancing but sometimes repressing the degradation of its target proteins, affecting the plant development and stress response regulation.

References

1. Derek J. Gingerich, Jennifer M. Gagne, Donald W. Salter, Hanjo Hellmann, Mark Estelle, Ligeng Ma, Richard D. Vierstra (2005) Cullins 3a and 3b Assemble with Members of the Broad Complex/Tramtrack/Bric-a-Brac (BTB) Protein Family to Form Essential Ubiquitin-Protein Ligases (E3s) in Arabidopsis. Journal of Biological Chemistry (280):18810-18821

QUANTITATIVE PROTEOMIC MAPPING OF BACTERIAL PHENOTYPE SWITHCING AFTER BACTERIOPHAGE INFECTION

Z. Szabó[1], G. Kecskeméti[1], R. Csordás[1] and D. Virók[2]

1) Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary
2) Department of Medical Microbiology, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Antibiotic resistant bacterial infections is an ever-growing problem of modern human health. Combination of antibiotic and bacteriophage treatment is a promising method of fighting against this problem. Bacteriophages are viruses that selectively infect bacteria and may induce biochemical processes in their bacterial hosts, which may increase the effectiveness of antibiotics treatment.

In this work we present our methods to achieve the most comprehensive proteome coverage of the isolated bacterial strains and to determine protein abundance changes induced by bacteriophage induced phenotype changes in two different human pathological bacteria (*E. Coli* and *S. Aureus*) using data independent acquisition (DIA) mass spectrometric methods. At the same time, we have evaluated the possibility of proteomic identification of the effectively infecting bacteriophage strain from phage cocktails, which may be a challenge considering the suppressing presence of the host bacterial proteome and the small overlapping (200-300 proteins) of bacteriophage proteomes.

Using our approaches we have identified sensitive marker proteins and peptides which may reflect bacterial phenotype switching and successful infection by specific bacteriophage strains.

PRELIMINARY LC-MS/MS PROTEOMIC PROFILING OF HUMAN DEEP FASCIA REVEALS SHARED AND REGION-DEPENDENT EXTRACELLULAR MATRIX SIGNATURES

*Chiedozie K. Ugwoke[1], Craig Pattinson[2], Kate Burgoyne[2], Renata Biba[3], Mario Cindrić[3], Denis Korady[1], Nejc Umek[1]**

[1] Institute of Anatomy, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[2] Aberdeen Proteomics, Rowett Institute, University of Aberdeen, Foresterhill, Aberdeen, United Kingdom
[3] Ruđer Bošković Institute, Zagreb, Croatia
* Corresponding author: Nejc Umek, nejc.umek@mf.uni-lj.si

Human deep fasciae are mechanically specialised connective tissues, yet their regional proteomic architecture remains incompletely defined, in part because fascia is an extracellular-matrix (ECM)-rich and technically challenging tissue for proteomic analysis. We performed a pilot LC–MS/MS proteomic study of fascia lata, plantar fascia and thoracolumbar fascia from two donors, complemented by an independent fascia lata technical replicate. Samples were processed by liquid-nitrogen tissue grinding, homogenisation in RIPA buffer with protease inhibition and sonication, followed by SDS-PAGE-assisted protein cleanup, proteolytic digestion, peptide cleanup and fractionation, and LC-MS/MS-based protein identification with label-free abundance estimation. The regional dataset yielded 977 protein groups, with 813–865 quantified proteins per sample. Within-region donor profiles showed high reproducibility (log10 abundance r = 0.94–0.95). PCA of 687 complete-case quantified proteins separated plantar fascia from fascia lata and thoracolumbar fascia along PC1, whereas PC2 mainly reflected donor effects. The detected fascia proteome included a prominent ECM-associated component, including collagens (COL6A3, COL12A1, COL6A1/2), proteoglycans (DCN, LUM, BGN, PRELP), and matricellular proteins (TNXB, TNC, THBS1/4 and COMP). Plantar fascia showed a proteoglycan- and cartilage-like matrix signature enriched in ACAN, CHAD, PRG4, COMP, CILP, VCAN and BGN. Fascia lata and thoracolumbar fascia clustered more closely with each other than with plantar fascia and, relative to plantar fascia, showed higher abundance of selected collagen-associated and matricellular matrix proteins, including COL14A1, TNC, COL1A1/2 and THBS1. The independent fascia lata acquisition identified 873 protein entries and supported a collagen/proteoglycan-rich ECM signature, but also showed prominent detection of blood-, vesicle- and muscle-associated proteins, consistent with residual vascular/plasma content and workflow-sensitive recovery of soluble proteins. Together, these data support the feasibility of LC-MS/MS-based human deep fascia proteomics and suggest region-dependent extracellular-matrix specialisation. The findings remain hypothesis-generating because of the small sample size and potential contributions from residual blood/ plasma-associated proteins and adjacent-tissue carry-over.

THE ALLIANCE4LIFE VIRTUAL RESEARCH CENTER: BUILDING CEE COLLABORATION IN MOLECULAR BIOMEDICINE

Nejc Umek[1,2], Chiedozie Kenneth Ugwoke[1,2], Denis Korady[1,2], Sergej Pirkmajer[1,3*]*

[1] Alliance4Life Virtual Research Center, Department of Metabolism and Endocrinology of Skeletal Muscle

[2] Institute of Anatomy, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[3] Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

[*] Corresponding authors: Nejc Umek, nejc.umek@mf.uni-lj.si, Sergej Pirkmajer, sergej.pirkmajer@mf.uni-lj.si

The Alliance4Life Virtual Research Center (A4L VRC) provides a structured environment for connecting biomedical expertise across Central and Eastern Europe. Its Department of Metabolism and Endocrinology of Skeletal Muscle focuses on skeletal muscle as a metabolically active tissue, an endocrine target and a source of signalling molecules that contribute to whole-body physiology. The department brings together complementary expertise in muscle metabolism, physical inactivity, ageing, diabetes, obesity, sarcopenia and related disorders. These research areas increasingly require study designs that connect biological questions with appropriate tissue sampling, functional phenotyping, molecular profiling and computational interpretation. This is particularly relevant for proteomics and other molecular technologies, where the scientific value of a dataset depends not only on analytical performance, but also on pre-analytical quality, biological context and joint interpretation. At CEEPC, we present the A4L VRC skeletal muscle department as a collaboration hub for biomedical researchers, proteomics laboratories, core facilities and data-analysis teams. The proposed model includes shared expertise, harmonised sample-processing strategies, co-developed pilot studies, joint interpretation of molecular datasets, early-career researcher exchange and training visits. Its purpose is to make molecular profiling an integrated part of biomedical research design rather than a downstream analytical service. By linking skeletal muscle biology with enabling molecular technologies, the A4L VRC can strengthen Central and Eastern European research in biomedicine. The expected outcome is a practical collaboration route for multi-site pilot studies, future competitive funding applications and longer-term research capacity building across the region.

Acknowledgement: The A4L_BRIDGE project is funded by the European Union under Grant Agreement No. 101136453. The views and opinions expressed, however, are those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

SIMULTANEOUS MONITORING OF GENE AND PROTEIN EXPRESSION IN HIV PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY AND AT INCREASED RISK OF AIDS-DEFINING CANCERS

D. Vangelov[1], R. Emilova[1], Y. Todorova[1], S.J. Gadher[2], M. Nikolova[1]

1) National Centre of Infectious and Parasitic Diseases, 1504 Sofia, Bulgaria.

2) Thermo Fisher Scientific, 5781 Van Allen Way, Carlsbad, CA 92008 USA.

Keywords: HIV, ART, immune monitoring, viral reactivation, chronic inflammation

The ability to simultaneously measure multiple secreted proteins and the corresponding gene expression levels of HIV patients receiving continuous antiretroviral therapy (cART) is prerequisite to ensuring patient benefits and long-term effectiveness of ART treatment. Interrogation of such patient samples for molecular signature of the disease together with clinical parameters such as mitochondrial mass, incomplete immune reconstitution, and CD4+ T-cell counts in people living with HIV (PLWH), may help better understand disease progression and efficacy of treatment.

Here, we demonstrate messenger RNA (mRNA) expression using QuantiGene™ assay on a multiplexing platform. Human peripheral blood mononuclear cells (hPBMCs) stimulated with phytohemagglutinin (PHA) were harvested at 24 hours and assayed for specific mRNA targets on a QunatiGene Human 8plex panel. Quantification of CCL2, CLDN5, CSF2, CXCL8, IL17F, IL25, IL6 and OCLN genes were performed on a multiplexing system.

Additionally, whole blood samples from PLWH on continuous ART (A, n=15), with CD4AC >500 cells/µl and CD4/CD8 ratio > 0.9, or untreated (B, n=10) were collected during routine immune monitoring. Mitochondrial mass (MM), mitochondrial membrane potential (MMP) and mitochondrial superoxide levels (MSL) in CD4 and CD8 T cells were determined using flow cytometry

Conclusion

Increased MSL in T lymphocytes of cART- PLWH suggested elevated oxidative stress and possible mitochondrial damage. Additionally, higher MM found in cART- suggested mitochondrial health disruption as well as on-going biogenesis. Complementing whole blood assays with hPBMCs based Quantigene mRNA assay, provided a more holistic view of the antiretroviral therapy for HIV by helping to screen for molecular signature of the disease at gene level. Such a combined approach can elevate patient blood sample screening to a 'multi-omics' level, providing a high-level interrogation of clinical parameters concurrently with gene expression networks of the disease.

SYNTHESIS AND IN VITRO BBB EVALUATION OF CNS-HOMING PEPTIDE CONJUGATES

V.Sivabalan[1], L. Žid[2], K. Diana[2], K. Bhide[2], K. Kuckova[2], and M. Bhide[1,2]

1) Institute of Neuroimmunology, Slovak Academy of Sciences v. v. i., Dúbravská cesta 9, 845 10 Bratislava, Slovakia
2) Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

Lyme neuroborreliosis caused by *Borrelia* species remains challenging to treat due to the limited penetration of therapeutics across the blood–brain barrier (BBB). CNS-homing peptides have emerged as promising carriers for targeted delivery into the central nervous system. Therefore, CNS-targeting peptides combined with anti-borrelial therapeutics may improve drug delivery across the BBB for neuroborreliosis treatment. In this study, we synthesized and evaluated multiple CNS-targeting peptide conjugates for BBB penetration and biocompatibility. Eight azide-functionalized CNS-homing peptides, including RGD, PepH3, E13, Clone 8, TGN, SynB3, TAT, and Angiopep-2, were synthesized by solid-phase peptide synthesis (SPPS) and purified by reverse-phase chromatography. Molecular weights of the purified peptides were confirmed using MALDI-TOF mass spectrometry, showing values ranging from 847 Da to 2615 Da. The peptides were subsequently conjugated with Sulfo-Cyanine 5.5 DBCO fluorescent dye using strain-promoted azide–alkyne cycloaddition (SPAAC). Successful conjugation was confirmed by MALDI-TOF analysis, demonstrating characteristic molecular weight shifts corresponding to dye incorporation. Subsequently, Cytotoxicity was evaluated using the XTT assay in VERO E6 cell lines, and the results demonstrated no cytotoxicity. Furthermore, BBB penetration efficiency of the fluorescent peptide conjugates was evaluated using an in vitro BBB model composed of hCMEC/D3 endothelial cells cultured on transwell inserts. Among the tested conjugates, RGD-Cy5.5, TGN-Cy5.5, and E13-Cy5.5 demonstrated higher BBB penetration efficiencies with average crossing values of 51%, 42%, and 36%, respectively. PepH3-Cy5.5 (21%) and Clone 8-Cy5.5 (17%) showed moderate BBB transport efficiencies, while SynB3-Cy5.5 (2%) and TAT-Cy5.5 (0%) exhibited minimal BBB penetration. The free dye control showed negligible transport across (0%) the BBB model, confirming peptide-mediated translocation of the conjugates. Based on the high BBB penetration efficiency and low cytotoxicity, RGD, TGN, and E13 represent promising candidates for targeted CNS drug delivery and further anti-borrelial evaluation against *Borrelia* infections.

References

1. Bertrand Y, Currie JC, Poirier J, Demeule M, Abulrob A, Fatehi D, Stanimirovic D, Sartelet H, Castaigne JP, Béliveau R (2010) Transport characteristics of a novel peptide platform for CNS drug delivery. J Cell Mol Med 14(12):2827–2839

SARS-COV-2 NUCLEOCAPSID PROTEIN ACTS AS VIRAL COMPLEMENT MODULATOR

J. Víglaský[1], K, Bhide[1], P. Baráth[2], J. Bellová[2] and M. Bhide[1,3]

1) Laboratory of Biomedical Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovakia

2) Institute of Chemistry, Slovak Academy of Sciences v. v. i., Dúbravská cesta 9, 845 10 Bratislava, Slovakia

3) Institute of Neuroimmunology, Slovak Academy of Sciences v. v. i., Dúbravská cesta 9, 845 10 Bratislava, Slovakia

The involvement of complement system in pathogenesis of COVID19 is well known, thanks to international research effort during pandemic. The hyperactivation of complement cascade has been extensively studied and several mechanisms of hyperactivation have been proposed. However, complement evasion by SARS-CoV-2 received less attention. Incorporation of host membrane-bound complement regulatory proteins (CRPs) CD55 and CD59 and involvement of ORF8 in complement evasion has been demonstrated by others. Our previous results demonstrated that SARS-CoV-2 nucleocapsid (N) protein, which is present in circulation during infection, binds soluble CRPs (C1 inhibitor, C4-binding protein, factor H and vitronectin) and can form CRP-N-virion complexes[1]. That led us to hypothesize that N protein might act as a complement evasion/modulation protein extending its immunomodulatory role during infection. Here we present our recent findings obtained using LS-MS/MS analysis. Briefly, virions purified by gradient ultracentrifugation were incubated in normal human serum (NHS) with or without N protein. After incubation, samples were ultracentrifuged again and virions decorated with complement were analyzed. We demonstrate that N protein in NHS influences the complement activation as seen by decrease in deposition of complement components C3-C9. Decreased deposition of complement was confirmed on the same samples using dot blot and ELISA. Finaly, complement-mediated neutralization test revealed that N protein can protect the virus from complement-mediated neutralization. We report that N protein influences complement activation and deposition on SARS-CoV-2 virions and protects the virus from neutralization.

This work was supported from NextGenerationEU through the Recovery and Resilience Plan for Slovakia project No. 09I03-03-V05-00017 and APVV-22-0084, VEGA1/0381/23.

References

1. Víglaský, J., Bhide, K., Talpasova, L., Fialová, Ľ, Bhide, M (2026) SARS-CoV-2 nucleocapsid protein forms complexes with soluble complement regulatory proteins that can bind to the virion. Sci Rep 16, 6599. https://doi.org/10.1038/s41598-026-37866-4

MITHRAMYCIN DISRUPTS THE SP1–UFMYLATION AXIS TO SUPPRESS HEAD AND NECK CANCER STEM CELLS

Kristina Vukovic Derfi[1], *Tea Vasiljevic*[1], *Tea Dragicevic*[1] *and Tanja Matijevic Glavan*[1]

1) Divison of Molecular Medicine, Ruder Boskovic Institute, Zagreb, Croatia

Background: Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy frequently characterized by recurrence and therapeutic resistance, characteristics largely attributed to the presence of cancer stem cells (CSCs). Using a proteomic approach, we identified UFMylation as a key pathway in HNSCC CSCs. Given the urgent need for novel therapeutic strategies, this study investigates the potential of targeting CSCs with mithramycin through inhibition of the Sp1 transcription factor, UFMylation, and CSC maintenance.

Methods: HNSCC-derived cell lines (Detroit 562, FaDu, and Cal27) and tumor spheres were used as a model for CSCs. Proteomic profiling identified the UFMylation pathway as an important regulator in CSCs. Its role was further evaluated by bioinformatic analyses, Western blotting, immunocytochemistry, gene silencing, and cytotoxicity assays.

Results: Proteomic analysis revealed significant upregulation of the UFMylation-associated proteins UFSP2 and DDRGK1 in tumor spheres. Bioinformatic analyses demonstrated that elevated UFM1 expression was associated with poorer overall and disease-free survival in HNSCC patients, and positively correlated with key epithelial–mesenchymal transition (EMT) markers, including ZEB, TWIST, and fibronectin. UFM1 expression was markedly increased in tumor spheres compared with adherent cells. UFM1 silencing significantly reduced sphere number, size, and stemness properties. Based on the bioinformatic analysis identifying the transcription factor Sp1 as a regulator of nearly half of the altered proteins, we investigated whether its inhibitor, mithramycin, could effectively eradicate cancer stem cells. Mithramycin reduced CSC viability, induced apoptosis, and suppressed both UFMylation and stemness-associated features.

Conclusions: UFMylation plays a critical role in maintaining CSC properties in HNSCC. Targeting this pathway through Sp1 inhibition with mithramycin, or its less toxic analogs, represents a promising therapeutic strategy for eliminating CSCs and overcoming treatment resistance in HNSCC.

PEPTIDE ANTIBIOTIC BIOCONJUGATE AGAINST LYME NEUROBORRELIOSIS

L. Žid[1], S. Vairamuthu[2], K. Kucková[1], D. Kosturíková[1] and M Bhide[1,2]

1) Laboratory of biomedical microbiology and immunology, University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia
2) Institute of neuroimmunology, Slovak academy of Sciences, Bratislava, Slovakia

Infection caused by neuroinvasive *Borrelia* often manifests long-lasting neurological disorders and is difficult to treat, as most antibiotics fail to attain an effective concentration within the brain or cannot kill the persister forms of *Borrelia* (cysts and round bodies). Neuroborreliosis has a favorable prognosis if treated early; however the long-term administration of antibiotics over many weeks for chronic neuroborreliosis carries the risk of serious adverse effects[1]. In the present work, we're using one of the C7C phage-displayed peptide that we obtained after 3 rounds of panning, fused with a BBB homing peptide and conjugating it to antibiotic. Thus, using solid-phase peptide synthesis, we synthesized a fusion peptide comprised of C7C, K(N_3) linker, and TGN, the CNS homing moiety. C7C-K(N_3)-TGN was purified by reverse-phase chromatography, and the quality was assessed by MALDI-TOF mass spectrometry. A peak at 3095 Da confirmed the synthesis of the fusion construct. C7C- K(N_3)-TGN was tested for cell toxicity on the human brain microvascular endothelial cells (D3 cells), which showed no signs of toxicity. C7C- K(N_3)-TGN was bioconjugated to minocycline by SPAAC reaction using BCN-PNP linker. The final construct was purified by gel chromatography and successfully tested for its borreliacidal activity. C7C-K(N_3-minocycline)-TGN holds merit for further development for its translation into the drug for in vivo trials. Research supported by APVV-22-0084 and VEGA 1/0381/23. Post-doc positions of LŽ and KK are supported by OPENMED-NFP313010V455.

References

1. Rauer, S.; Kastenbauer, S.; Fingerle, V.; Hunfeld, K. P.; Huppertz, H. I.; Dersch, R.; Grp, G. (2010) Lyme Neuroborreliosis. Dtsch Arztebl Int, 115 (45), 751-756.