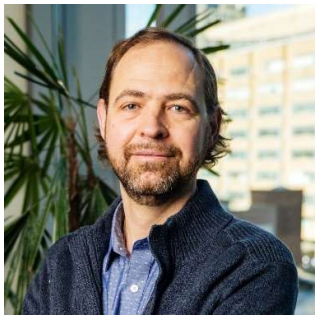
Day1, 22.08.23			
time	name	affiliation	title
09:00 - 09:10	Mechtler Karl & Matzinger Manuel	Head/Deputy Head, Proteomics TechHub, Research Institute of Molecular Pathology, Vienna Austria	Welcome and Introduction
09:10- 09:50	Bogdan Budnik	PI, Wyss Institute, Boston, MA, USA	Single cell proteomics study of drugs responses
09:50 - 10:30	Lennart Martens	Group Leader of the Computational Omics and Systems Biology (CompOmics) group at VIB, and Associate Director of the VIB-UGent Center for Medical Biotechnology, all in Ghent, Belgium	It takes a village to analyse a single cell: where a lot is made of very little
10:30 - 10:45	Teeradon Phlairaharn	Technical University of Munich	Optimizing linear ion trap data independent acquisition towards single cell proteomics
10:45 - 11:10	coffee break		
11:10 - 11:50	Fulcher James	Staff Scientist, Pacific Northwest National Laboratory, Richland, WA, USA	Into the single-cell multiverse through nanodroplet splitting
11:50 - 12:30	Ivanov Alexander	Associate Professor, Chemistry and Chemical Biology, Barnett Institute for Chemical & Biological Analysis, Northeastern University, Boston, MA, USA	Evaluation of top-down proteomic and glycomic profiling of small cell populations and single cells
12:30 - 12:45	Abel Vertesy	Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austra	A single-cell proteomics of human brain organoids
12:45 - 13:40	lunch		
13:40 - 14:20	Seth Anjali	Head of Single Cell Proteomics, Cellenion, Lyon, France	tba
14:20 - 15:00	Kelly Ryan	Full Professor, Department of Chemistry and Biochemistry, Brigham Young University, Povo, UT, USA	Improved Sample Preparation, Separations and Data Acquisition for Label-Free Single-Cell Proteomics
15:00 - 15:15	Anuar Makhmut	Max Delbrück Center for Molecular Medicine, Berlin, Germany	Streamlining ultra-low input spatial tissue proteomics
15:15 - 16:10	cofee break & poster session		
16:10- 16:50	Valdemaras Petrosius	Postdoc, Department of Biotechnology and Biomedicine, DTU, Lyngby, Denmark	Evaluating the capabilities of the Astral mass analyzer for single- cell proteomics
16:50 - 17:05	Suniya Khatun	Institute of Structural and Molecular Biology, Division of Bioscience, University College London, London, UK	Deciphering the molecular pathway driving cell competition using label-free mass spectrometry
17:05 - 18:00	Peter Pichler	PostDoc, Proteomics TechHub, Research Institute of Molecular Pathology, Vienna Austria	Of Urine, Mice and Men: A Time Travel through History of Medicine
from 18:00	BBQ		

Day2, 23.08.2023			
time	name	affiliation	title
09:00 - 09:40	Rose Chris	Director, Microchemistry, Proteomics & Lipidomics, Genentech, San Francisco, CA, USA	Improved Throughput of Single Cell Proteomics with PairQuant Intelligent Data Acquisition
09:40 - 10:20	Schoof Erwin	Associate Professor, Department of Biotechnology and Biomedicine, DTU, Lyngby, Denmark	Unleashing the next-generation single-cell proteomics workflows
10:20 - 10:35	Susmita Ghosh	Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Dortmund, Germany	Profiling of few neutrophils isolated freshly from healthy and diseased species using highly sensitive LC-MS based proteomics
10:35 - 11:00	coffee break		
11:00 - 11:40	Matzinger Manuel	PostDoc & Deputy Head, Proteomics TechHub, Research Institute of Molecular Pathlogy, Vienna Austria	How to reach unprecedented coverage, throughput, and quantitative accuracy in single cell proteomics by improving all workflow steps, from sample preparation to data analysis.
11:40- 12:20	Vegvari Akos	Project Manager, Karolinska Institutet, Stockholm, Sweden	Single Bacterium Proteomics and Analysis of Xenoproteins in Single Cells.
12:20- 12:35	Sabrina Richter	Helmholtz Zentrum München – German Research Center for Environmental Health, Institute of Computational Biology, Neuherberg, Germany	Leveraging Intracellular Protein Measurements to Enhance Velocity-Based Trajectory Inference in Single-Cell Data
12:35 - 13:30	lunch		
13:30- 14:10	Florian Rosenberger	EMBO postdoctoral fellow working with Matthias Mann at the Max Planck Institute of Biochemistry in Martinsried, Germany	Single shape, single cell: diving deep into visual proteomics for unveiling cellular secrets
14:10- 14:50	Orsburn Ben	Department of Pharmacology and Molecular Sciences, The Johns Hopkins School of Medicine, Baltimore, MD, USA	tba
14:50- 15:05	François Berger	BrainTech Lab U1205, INSERM-Grenoble University, Grenoble, France	“Enlightened-Glioblastoma”: a precision medicine clinical trial integrating single cell analysis, tumoroid and artificial intelligence guided multi-drug repositioning.
15:05- 15:30	coffee break		
15:30- 16:10	Caldwell Michael	Scientific Officer, Proteomics Center of Excellence Northwestern University, Evanston, IL, USA	Label-free Detection of Proteoforms in Single Cells using Individual Ion MS
16:10- 16:50	Krisp Christoph	Application Development Specialist Single Cell Proteomics, Bruker Daltronics, Bremen, Germany	Ultra-high sensitivity label-free single cell proteome analysis with the timsTOF Ultra.
16:50- 17:05	Serrano Irene	Associate Editor, Nature Communications, Berlin, Germany	Publishing at Nature Communications: an editor’s perspective
17:05	closing remarks, best short talk award and drinks		

Bogdan Budnik

Principal Scientist at Wyss Institute - Boston, MA, USA



Bogdan has over 20 years of extensive experience in mass spectrometry-based proteomics analysis utilizing a broad range of biological samples with a keen interest in single cell proteomics. After serving as Director of the Proteomics Laboratory at Harvard University for 15 years and developing the popular SCoPE-MS approach together with Nikolai Slavov, he recently joined the Wyss Institute to lead the Multi-omics Discovery Engine (MoDE) with to catalyze innovation in the fields of therapeutics and diagnostics.

Lennart Martens

Senior Full Professor at Ghent University, Group Leader and Associate Director CMB at VIB – Ghent, Belgium

Lennart obtained his Ph.D. in Sciences: Biotechnology from Ghent University and served as PRIDE Group Coordinator at EMBL-EBI afterwards before returning to Ghent University and VIB where he currently serves as group leader and senior full professor. Lennart's group, the Computational Omics and Systems Biology group (CompOmics) is focusing on bioinformatics with a focus on the development of bioinformatic tools and peptide centric approaches as well as protein structure and high-throughput omics among many projects.

Abstract

It takes a village to analyse a single cell: where a lot is made of very little.

An exciting new frontier in proteomics concerns the analysis of the proteomes of single cells. Mirroring the rapid developments in single cell transcriptomics, proteomics is now catching up with impressive speed.

Obviously, the challenges in analysing single cells are many, and these span a broad range of expertises. First of all, single cells need to be selected, which is a near-mythical feat in its own right. Then the proteomes of these single cells need to be made accessible, and digested, all while maintaining throughput and adequate recovery. The technical barriers in these steps are profound; indeed, as the sample gets smaller, so do the acceptable limits on the tolerances for sample losses.

Once the single cell proteome has been readied for mass spectrometry analysis, it is vital to possess of the right instrumentation to dig as deeply as possible into these tiny samples, requiring specially adapted sources and a plethora of innovative ion optics to squeeze every last ion out of the sample.

And even then, once the mass spectrometer has recorded its data on the sample, the processing of the resulting fragmentation spectra is far from trivial, with signal to noise often skimming the borderline, and classical informatics tools not ideally suited to interpret the resulting, typically sparser data.

Here, we will look into end-to-end workflow approaches that aim to tackle these many challenges, and that focus on making a lot out of very little, but to do so reliably and robustly.

Some emphasis will be placed on the role of cutting-edge, machine-learning based bioinformatics tools in the optimal processing of single cell proteomics data. Specifically, the impressive capabilities of MS2ReScore (https://github.com/compomics/ms2rescore) will be highlighted, which itself is in turn based on the MS²PIP (https://iomics.ugent.be/ms2pip) and DeepLC (http://compomics.github.io/projects/DeepLC) machine learning models to predict peptide fragmentation and chromatographic retention time, respectively.

Overall, it is clearly an exciting time for proteomics, as new instruments, methods, and machine learning-based informatics tools are unlocking biological information at that most interesting of levels: that of the dynamic, cell-specific proteome! Indeed, by leveraging these innovative analytics, and cutting-edge software tools, the field is now ideally positioned to make a proud entry into, and a profound impact on, the life sciences in general. It is about time!

James Fulcher

Analytical Chemist at PNNL – Richland, WA, USA

James is a chemist in Pacific Northwest National Laboratory's (PNNL's) Environmental Molecular Sciences Division and the Environmental Molecular Sciences Laboratory with more than 10 years of experience in analytical and biological chemistry. He acquired his PhD in biochemistry from the University of Utah after developing new tools for the synthesis of large proteins and peptides. At PNNL, he developed nanoSPLITS to analyze the proteome and trancsriptome of the same single cell and he is currently applying and further developing nanoPOTS single-cell proteomics and nanoSPLITS.

Alexander Ivanov

Associate Professor at Northeastern University - Boston, MA, USA

Alexander earned his Ph.D. in Bioorganic Chemistry at the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow in 2000 before starting a postdoc at Northeastern University. He later joined Harvard University and became Director of the HSPH Proteomic Resource in 2008, before rejoining Northeastern, where he is currently working as Associate Professor at the Department of Chemistry & Chemical Biology Alexander's research interests include characterization of PTMs and protein isoforms, deep proteomics profiling of limited biological samples, ultra-low flow separation techniques such as capillary electrophoresis and extracellular vesicle analysis.

Anjali Seth

Head of Single Cell Proteomics at Cellenion – Lyon, France

Anjali obtained a PhD in 2014 from Pierre and Marie Curie University for her research on magnetic nanoparticles for targeted drug delivery. Next, she underwent a two-year postdoctoral project in Oxford where she used magnetic particles and ultrasound for cancer theranostics. Thereafter, she went back to France, at the Ecole Centrale de Lyon, to work on the creation of cell aggregates by electrophoresis in microfluidic chips. In 2019 she joined Cellenion to develop and lead the single cell proteomics R&D department as Head of Single Cell Proteomics.

Ryan T. Kelly

Full Professor at Brigham Young University - Provo, UT, USA

Ryan received his Ph.D. in analytical chemistry from BYU in 2005 before spending the next 13 years at PNNL, ultimately serving as Senior Research Scientist, Manager and Chief Technologist for EMSL, a national scientific user facility at PNNL, where he developed the popular nanoPOTS platform. Ryan is (co-)author of >100 publications, has won several awards and is named inventor on a number of licensed and commercialized patents. He focuses on technological improvements for ultrasensitive proteomic analyses and is currently Associate Professor at BYU.

Valdemaras Petrosius

Postdoctoral Fellow at Technical University of Denmark - Lyngby, Denmark

Valdemaras completed both MSc and PhD at the University of Copenhagen in the Sørensen lab, where he studied DNA damage response mechanisms and their potential to target cancer therapy. He is currently a postdoc in the Schoof lab and focuses on establishing quantitative proteomics workflows for limited input samples like rare cells populations and single cells. His major interest lies in applying the developed methods to tackle key biological questions in disease pathology and cell development.

Abstract

Evaluating the capabilities of the Astral mass analyzer for single-cell proteomics

The complexity of human physiology arises from well-orchestrated interactions between trillions of single cells in the body. While single-cell RNA sequencing (scRNA-seq) has enhanced our understanding of cell diversity, gene expression alone does not fully characterize cell phenotypes. Additional molecular dimensions, such as proteins, are needed to define cellular states accurately. Mass spectrometry (MS)-based proteomics has emerged as a powerful tool for comprehensive protein analysis, including single-cell applications. However, challenges remain in terms of throughput and proteomic depth, in order to maximize the biological impact of single-cell proteomics by Mass Spectrometry (scp-MS) workflows. This study leverages a novel high-resolution, accurate mass (HRAM) instrument platform, consisting of both an Orbitrap and an innovative HRAM Asymmetric Track Lossless (Astral) analyzer. The Astral analyzer offers high sensitivity and resolution through lossless ion transfer and a unique flight track design. We evaluate the performance of the Thermo Scientific Orbitrap Astral MS using Data-Independent Acquisition (DIA) and assess proteome depth and quantitative precision for ultra-low input samples. Optimal DIA method parameters for single-cell proteomics are identified, and we demonstrate the ability of the instrument to study cell cycle dynamics in Human Embryonic Kidney (HEK293) cells, and cancer cell heterogeneity in a primary Acute Myeloid Leukemia (AML) culture model.

Pichler Peter

Postdoctoral Fellow at Research Institute of Molecular Pathology (IMP) – Vienna, Austria

Peter received his M.D. from Vienna Medical School in 2005, after studying medicine and electrical engineering. Inspired by papers on MS-based proteomics, he applied to Austrian proteomics researchers Gustav Ammerer and Karl Mechtler. Surprisingly, he found himself accepted, possibly due to being the only applicant. While accomplishing an analysis of the urinary proteome by measuring 130 iTRAQ 4plex-labeled SCX fractions, Peter realized that a throughput of N=4 per month might not suffice for meaningful clinical studies. He thus decided to change track, turning to clinical training in general medicine and nephrology for a decade. Recent developments in mass spectrometry lured Peter back to Karl's lab at the IMP, where he currently works on label-free quantification of larger sample numbers in urinary and single cell proteomics.

Abstract

Of Urine, Mice and Men: A Time Travel through the History of Medicine

The valued audience will be taken on a journey along some of the winding roads that turned medicine into a science, at least to some extent. We will encounter early approaches to urine analysis, ancient physicians considered saints (by others, not by themselves), transgenic mice models, some more modern views of kidney diseases including reports on drugs that save kidney patients' lives despite nobody knowing why, and finally a glimpse of urine proteomics data that can be generated by currently available methodology.

Christopher Rose

Director & Senior Principal Scientist at Genentech - San Francisco, CA, USA

Chris received his Ph.D at the University of Wisconsin-Madison after his studies in the Coon lab. As a postdoctoral research fellow in the Gygi lab at Harvard Medical School he focused on further developing quantitative MS methods resulting in a multiplexed targeted method TOMAHAQ, and led to the proof of principle implementation of real-time database search for SPS-MS3 data acquisition. Chris is currently Director of Discovery Proteomics within the Microchemistry, Proteomics, and Lipidomics department as well as Senior Principal Scientist with an interest in single cell and low input proteomics as well as immunopeptidomics.

Erwin M. Schoof

Associate Professor at Technical University of Denmark - Lyngby, Denmark

Erwin received his PhD from the Technical University of Denmark (DTU) in 2014 for his work on computational methods to model cell behavior. Since 2017, he has been head of the Proteomics Core Facility at DTU, a facility specialized in high-sensitivity proteomics workflows. He was appointed Associate Professor at the Department of Biotechnology and Biomedicine in 2020. His research focuses on normal and malignant hematopoiesis, which involves comprehensive optimization of single-cell proteomics workflows for deciphering cellular heterogeneity.

Manuel Matzinger

Postdoctoral Fellow at Research Institute of Molecular Pathology (IMP) – Vienna, Austria

Deputy Head for Method Development, Proteomics Technology Hub, IMP, Vienna, Austria

Manuel completed his PhD in pharmaceutical chemistry at the University of Vienna before he joined the Mechtler lab in 2020 as postdoctoral scientist with research focusing on crosslinking mass spectrometry and single cell proteomics. Since 2022 he is deputy head of the group responsible for method development. His research focuses on method development in the field of single cell proteomics and crosslinking mass-spectrometry.

Abstract

How to reach unprecedented coverage, throughput, and quantitative accuracy in single cell proteomics by improving all workflow steps, from sample preparation to data analysis

This talk will give an overview of the efforts in SCP method development within the Mechtler group, involving all steps, from sample preparation to data analysis. We identify up to 6000 proteins from a single cell and improve quantitative accuracy using our optimized methods. In an attempt to increase throughput for label free SCP we manage to analyze up to 100 cells per day without loosing the ability to successfully investigate cellular heterogeneity based on cultured cell lines as model system.

Ákos Végvári

Project Lead at Karolinska institutet - Stockholm, Sweden

Ákos has co-authored more than 100 publications in the field of proteomics and currently focuses on Single Cell Proteomics and method development at the Department of Medical Biochemistry and Biophysics, Karolinska Institutet together with Roman Zubarev. Previously he has worked at Lund University and the University of Texas.

Abstract

Single Bacterium Proteomics and Analysis of Xenoproteins in Single Cells

Bacteria are much smaller than mammalian cells, and while single cell proteomics (SCP) detects and quantifies today several thousand of proteins in a single mammalian cell, it is not at all clear whether conventional SCP methods will be suitable for bacteria. We present on the first attempt to detect proteins from individual *Escherichia coli* bacteria and validating our finding by comparison with bulk proteomics.

Yet another challenge to detect xenoproteins in individual mammalian cells was addressed by investigating the proteomic alterations during reactivation of latently infected HIV in J-Lat10.6 cells induced by prostratin drug. While 1341 human proteins were quantified, HIV gag-polyprotein was also readily identified and quantified across the 128 analyzed single cells. The SCP analysis showed a variation of the abundance of the gag-polyprotein among the J-Lat cells and identified host proteins co-expressed with the virus gag protein. This finding may provide important details on viral mechanisms that bulk proteomic analysis cannot reveal, highlighting the potential of SCP analysis to study HIV infection at the single-cell level.

Matthias Mann

PotsDoc at Max Planck Institute for Biochemistry – Martinsried, Germany

Florian Rosenberger is an EMBO postdoctoral fellow working with Prof. Matthias Mann at the Max Planck Institute of Biochemistry in Martinsried, Germany. He holds a PhD in mitochondrial biology from the Karolinska Institute. Florian's research in the field of MS-based single cell proteomics centers around understanding the metabolic niches in both healthy and diseased contexts. His work primarily focuses on liver biology, encompassing the development of a single-cell proteomics map to unravel the spatially dependent proteome of hepatocytes.

Benjamin C. Orsburn

Instructor and Principal Investigator at Johns Hopkins University School of Medicine - Baltimore, MD, USA

Ben received his Ph.D. from Virginia Tech in 2009 and completed his postdoctoral fellowships at the Johns Hopkins University and National Cancer Institute. In 2020 he rejoined Johns Hopkins University School of Medicine, where he currently works as Instructor and Principal Investigator. Additionally, Ben is the author of the popular “News in Proteomics Research” blog and has founded and serves as CSO for Coral Reef Labs, which provides next-generation cannabis testing solutions. Ben’s group currently applies single cell proteomics to understand resistance in chemotherapy.

Michael A. Caldwell

Scientific Officer at Northwestern University

Mike received his Ph.D. in 2019 from Northwestern University for conducting work on Lanthanide magnetism and chemical synthesis using a collaborative, transdisciplinary approach at the interface of chemistry and biology. He then went on to Tempus Lab, Inc. to coordinate and manage a plenitude of NGS scientific collaborations. Since 2022, Mike is Scientific Officer for Northwestern Proteomics, an umbrella covering the Proteomics Center of Excellence and proteomics core facility at Northwestern supporting center operations and plan as well as execute R&D and application projects.

Christop Krisp

Application Development Specialist Proteomics at Bruker Daltonics - Bremen, Germany

Christoph obtained his PhD in Chemistry and Biomolecular Sciences in 2013 jointly from the Ruhr University Bochum, and the Macquarie University in Sydney on wound healing processes in diabetics followed by postdoctoral stays at Macquarie University in Sydney and University Medical Center Hamburg Eppendorf. Since 2021, he is Application Development Specialist Proteomics at Bruker Daltonics focusing on Single Cell Proteomics workflow development.

Abstract

Ultra-high sensitivity label-free single cell proteome analysis with the timsTOF Ultra

For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key to reach proteome coverages necessary for understanding the cellular heterogeneity on a cell-by-cell level. Latest enhancements in ion transfer with a larger transfer capillary, an additional higher-pressure segment for more effective ion collection and two orthogonal deflections, to maintain robustness, and high-capacity trapped ion mobility spectrometry (TIMS) pushes the limits of detection to single cell level.

K562 cell digest (Promega) dilution series from 16 ng to 15 pg, in 2-fold dilution increments, was prepared. One, five, ten and twenty HeLa cells were isolated with a cellenONE and prepared in a proteoCHIP. Tryptic peptides were loaded onto Aurora Elite column (IonOpticks) separated with a 22 min active gradient (32 SPD) or an Aurora Rapid column with a 10 min active gradient (80 SPD) using a nanoElute2. Eluting peptides were detected with a timsTOF Ultra mass spectrometer in dia-PASEF and analyzed using Spectronaut 18.

First, we assessed the sensitivity of a timsTOF Ultra mass spectrometer using a dilution series of K562 cell digest showing excellent identification rates, reproducibility, and quantification accuracy per concentration replicates for both column setups. Processing of the dia-PASEF data identified >1,000 protein groups out of 15 pg, with 80SPD, and 32SPD. More than 6000 protein groups for 80SPD and >7,000 protein groups for 32 SPD were detected out of 16 ng K562 peptides loaded on column. Quantitative accuracy improved inversely with loaded peptide amounts with around 20% at 15 pg to 4 - 6% at loads of 4, 8 and 16 ng. Identification rates on protein group level for K562 peptide loads < 500 pg were comparable between the 80 SPD and 32

SPD, peptide identification rates were higher in the 32 SPD setup. Analysis of the isolated HeLa cells resulted in good identification rates and good reproducibility per individual cell count group with expected increase in protein abundance from the single cells with about 4000 protein groups to 6000 protein groups for 20 cells in 80 SPD and 32 SPD.

The timsTOF Ultra combined with automated single cell isolation and sample preparation using the cellenONE® platform for protein-loss reduced preparation and transfer with the proteoCHIP format leads to deep proteome coverage and high reproducibility with fast chromatographic separation at 32 and 80 SPD.

Irene Serrano

Associate Editor at Nature Communications – Berlin, Germany

Irene obtained her PhD in plant biology at University of Granada, combining cell biology with biochemistry methods to study self-incompatibility. During her postdoctoral training at Indiana University, the Laboratory for Plant-Microbe-Environment Interactions in Toulouse and University of Göttingen her research focused on translational and post-transcriptional regulation of plant immunity. Irene joined Nature Communications in September 2022. She handles papers on post-translational modifications, as well as general biochemistry papers and is based in the Berlin office.

SHORT TALKS

A single-cell proteomics of human brain organoids

Ábel Vértesy-1, Balint Doleschall-1, Elisabeth Müller-2, Gerhard Dürnberger-3, Manuel Matzinger-2, Ramsey K Najm-1, Karl Mechtler-2*, Juergen Knoblich-1*
(Authors listed alphabetically by first name, except group leaders authors)

Recent advances in single-cell proteomics now make it possible to analyze 100's of cells per day, up to 1000 proteins recovered per cell. So far, biological applications of these techniques have been limited to simple comparisons between 2D cultured cell lines or other simple systems . However, complex developmental programs, such as neurogenesis are not well recapitulated in 2D and are poorly understood at the protein level. Recently, our laboratory has used a complex in vitro model of the human brain, stem cell derived-cerebral organoids, to reveal translational regulation as a key component of neural differentiation and disease. Here, we set out to understand the relationship between the transcriptome and proteome throughout human neuronal development with cell-type resolution by a novel single-cell proteomics workflow applied to cerebral organoids. We successfully implemented an automated label free workflow to address challenges of low protein content and cell isolation from complex 3D tissue that enables us to quantify roughly 500 peptides per single cell. We have prepared libraries from individual FACS sorted cells and compared fresh and frozen samples at different MS acquisition modes. Our work has the potential to not only provide insight into single-cell complex tissue proteomics as a technique, but also may reveal the interplay of transcriptome and proteome throughout human neuronal development.

Streamlining ultra-low input spatial tissue proteomics

Anuar Makhmut, Di Qin, David Hartlmayr, Anjali Seth, Fabian Coscia

Formalin fixation and paraffin-embedding (FFPE) is the most prevalent method to preserve human samples for clinical studies. FFPE tissues can be archived for long periods of time and the proteins they contain are largely stable, making them ideal analytes for global mass spectrometry (MS) - based proteomics studies. Traditional proteomic approaches focused on analyzing tissues as a whole (bulk approaches), which causes the loss of spatial and cell type information. We recently co-developed a new spatial tissue proteomics concept, Deep Visual Proteomics, combining microscopy-based cell phenotyping with ultrasensitive MS-based proteomics for the study of cell function and heterogeneity. However, optimized and automated sample preparation workflows that not only preserve morphological information for image-based phenotype discovery, but also maximize proteome coverage of few or even single cells from laser microdissected archival tissue, are currently lacking. We therefore developed an ultrasensitive, robust and scalable protocol for the proteomic analysis of FFPE tissues on the basis of standard laboratory equipment. We benchmarked our protocol in murine liver and human tonsil tissues, covering known biomarkers, cytokines and transcription factors, while also highlighting spatial proteome organizations. Finally, we integrated our workflow with the cellenONE robotic system, which allowed us to reduce sample preparation time to 3 hours for 96 samples per single batch. We believe our framework provide important insights into future spatial proteomics applications.

“Enlightened-Glioblastoma”: a precision medicine clinical trial integrating single cell analysis, tumoroid and artificial intelligence guided multi-drug repositioning

F Berger, W Bourgeois, A bouamrani-INSERM U1205- A Seth, F Izaguirre, D Hartlmayr -Cellenion- D Argenti- MoreHisto- C Boyaux- Reckonect.

Glioblastoma (GBM) is a devasting disease, with a 15-month median survival. This is contrasting with the incredible amount of molecular data provided by last generation sequencing and connected technologies from DNA to proteomic and metabolomic investigations. GBM only poorly respond to classical radio-chemotherapy as well as to last generation targeted therapies and immunotherapies. Main bottlenecks are tumor heterogeneity, peritumoral microenvironment inaccessibility (“driver compartment” not removed by surgery also protected by the blood-brain-barrier), molecular and cellular adaptation, invasion, and immunosuppression. A major bottleneck is also our inability to integrate the exponentially growing amount of multi-level data (clinical data, multimodal imaging, biology, multi-level sequencing …) to extract the relevant targets for therapy. Single cell analysis is a key investigation to solve tumor heterogeneity not mixing “the tea towel and the napkins” but also increasing dramatically data complexity. Moreover, it is a research technology warranting yet many developments for translation at the bedside. Aiming to implement a personalized trial benefiting from single cell analysis, we developed an innovative technology for robust fast-track single cell/tumoroid implementation at the bedside. It is based on nanoporous tissue imprint, also providing the opportunity to explore peritumoral brain area not compatible with biopsy. Cellenion single cell technology was associated with success to this innovative brain sampling technology followed by mRNA and proteomic sequencing. An innovative artificial intelligence strategy was developed for single cell data and connected tumoroid analysis using a digital avatar of GBM (MoreHisto and Reckonect companies). Driving pathways will be identified from transcriptomic and proteomic expression data providing a list of repositioned drugs with the prediction of side effects. Some optimizations need to be done and responses to crucial questions such as the optimal number of singe cell analysis. The validation of the best methodology to Extract immunogenic candidate for mRNA vaccine is also crucial. The principle objective of the clinical trial will be the proof of concept validation of this translational single cell precision medicine strategy in 10 GBM patients, comforting multicenter dissemination.

Leveraging Intracellular Protein Measurements to Enhance Velocity-Based Trajectory Inference in Single-Cell Data

Sabrina Richter [5,6], Benjamin Furtwängler [1,2,3], Nil Üresin [1,2,3], Despoina Barmpouri [1,2,3], Henrietta Holze [4], Anne Wenzel [1,2], Erwin M. Schoof 4, Bo T. Porse 1,2,3 & Fabian Theis [5,6]

[1] The Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark
[2] Biotech Research and Innovation Centre (BRIC), Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3] Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, University of Copenhagen, Copenhagen, Denmark
[4] Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark
[5] Helmholtz Zentrum München – German Research Center for Environmental Health, Institute of Computational Biology, Neuherberg, Germany
[6] TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

The analysis of single-cell RNA sequencing (scRNAseq) data has revolutionized our understanding of cellular dynamics, offering valuable insights into differentiation processes and cellular responses to perturbations. One widely used trajectory development method is RNA velocity, which utilizes changes in gene expression to infer the directionality of cellular differentiation or response. Specifically, it relies on detecting shifts in the abundance of unspliced RNA counts preceding changes in spliced RNA abundance.

However, the efficacy of RNA velocity is hindered in certain scenarios due to its dependency on capturing relatively small time shifts in gene expression. To address this limitation, we propose to leverage the more pronounced delay between RNA and protein expression dynamics. Previous efforts in this direction have been constrained to CITEseq data, but our study stands as the first attempt to apply this strategy to intracellular protein measurements based on mass spectrometry.

In this talk, we present our novel methodology for incorporating intracellular protein measurements to enhance velocity-based trajectory inference. We hope that modeling translation dynamics instead of splicing might overcome the limitations of conventional RNA velocity methods and analyzing the inferred translation and protein degradation rates might augment our understanding of cellular behavior.

Deciphering the molecular pathway driving cell competition using label-free mass spectrometry

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Riccardo Zenezini Chiozzi, Email: r.chiozzi@ucl.ac.uk Affiliations: Institute of Structural and Molecular Biology, Division of Bioscience, University College London, London, UK.
Manasi Kelker, Email: m.kelkar@ucl.ac.uk, Affiliations: London Centre for Nanotechnology, University College London, London, UK.
Guillaume Charras: Email: g.charras@ucl.ac.uk, Affiliations: Institute for the Physics of Living Systems, University College London, London, UK. Department of Cell and Developmental Biology, University College London, London, UK. London Centre for Nanotechnology, University College London, London, UK.
Alan Lowe, Email: a.lowe@ucl.ac.uk, Affiliations: Institute for the Physics of Living Systems, University College London, London, UK. Department of Cell and Developmental Biology, University College London, London, UK. London Centre for Nanotechnology, University College London, London, UK. The Alan Turing Institute, London, UK
Konstantinos Thalassinos: Email: k.thalassinos@ucl.ac.uk, Affiliations: Institute of Structural and Molecular Biology, Division of Bioscience, University College London, London, UK. Institute of Structural and Molecular Biology, Birkbeck College, University of London, London, UK.

Cell competition is phenomenon that results in the elimination of sub-optimal cells from tissue. This process is crucial for development and maintaining tissue homeostasis. It is believed that the fate of cells in competition is determined by their local cellular neighbourhood as sub-optimal cells are only eliminated in the presence of fit cells. To gain a deeper understanding of this elimination process, we employed a mass spectrometry-based proteomics approach to investigate the molecular mechanisms involved in cell competition at both bulk and single-cell level. Specifically, we focused on studying the molecular pathways that determine the fate of MDCKWT (Madin-Darby Canine Kidney) cells and MDCK scribble depleted cells (scribblekd) during competitive interactions in a 50:50 co-culture. Our analysis revealed significant alterations in proteins associated with cellular stress response, Wnt, NF-kB, Hippo and MAPK signalling pathways. Additionally, we observed dysregulation of cell adhesion, cell-cell communication, perturbation of cytoskeletal organisation, cell migration, as well as disruption of mitochondrial function and metabolism in scribblekd when in competitive condition. Our findings suggest that cells engaged in competition undergo complex molecular and cellular changes, employing diverse mechanisms for growth and survival in order to gain a competitive advantage.

Profiling of few neutrophils isolated freshly from healthy and diseased species using highly sensitive LC-MS based proteomics

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Neutrophils, crucial and abundant immune cells in the circulatory system, exert significant impact on microbial infections as well as tumor biology, inflammatory disorders, and autoimmune diseases. Their phenotypic heterogeneity driven by environmental cues, plays critical roles in health and disease. Although neutrophil heterogeneity has been characterized extensively using RNA sequencing, their molecular makeup in terms of protein composition needs to be explored. The burgeoning of proteomics technologies would provide a comprehensive insight into their structural and functional architecture. However, neutrophils obtained from the inflammatory site or tumors are too low to perform routine proteomics analysis. To address this challenge, we employed an integrated sample preparation pipeline with state-of-the art LC-MS methods on ~1,000 neutrophils (~50 ng protein), freshly isolated from human and murine blood. For this study, we isolated and immune-magnetically purified neutrophils from 1ml murine and human blood with 93% purity. Here, we present a S-trap micro column-based protocol (Protifi) with slight modifications as an alternative to existing methods (e.g., SP3) for efficient protein digestion at the nanogram level. To get a comprehensive proteome depth, we first generated species-specific neutrophil spectral libraries covering ~8,000 mouse and ~6,000 human proteins using high pH fractionation of the bulk digest (~30 µg) followed by DDA PASEF analysis. Next, we employed a py-diAID approach to ensure optimal DIA-PASEF acquisition over the ion mobility-mass-to-charge ratio (IM-m/z) plane. The integration of the modified S-trap with DIA PASEF yielded a proteome coverage of >4,000 and ~3,000 proteins from ~1,000 isolated murine and human neutrophils, respectively with high reproducibility (R>0.9) among five biological replicates each. Our method enabled identification of proteins with a broad dynamic range (108-102) of estimated copy numbers per cell across all biological replicates and showed a good overlap with standard input (105 cells). Based on our data, neutrophil granule proteins like- ELANE, MPO and cytoskeletal proteins were highly abundant (> 106 copies per cell) whereas, less than ten thousand copies were found for transcription factors, proteins associated with mRNA processing and DNA repair. Comparing our data with the existing repositories revealed a strong correlation (R>0.8) despite using 10-fold less starting material. Furthermore, we applied our method to evaluate the proteome changes in brain infiltrated neutrophils (~2,000 isolated cells) upon stroke induction compared to sham controls. Thereby, we found that post-stroke brain-infiltrated

neutrophils exhibited a strongly distinct proteome compared to circulatory neutrophils in the same host or from sham controls. Overall, this approach demonstrates a significant leap in understanding intricate proteome-wide neutrophil biology from minute sample amounts. We further envisage to investigate neutrophil at single-cell resolution to reveal the heterogeneity and functional diversity of these intriguing immune cells.

Optimizing linear ion trap data independent acquisition towards single cell proteomics

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Low-input proteomics is emerging as a powerful mass-spectrometry-based technique for studying biochemistry. In these experiments, researchers typically analyze flow-cytometry-sorted samples ranging from 10 ng down to single-cell using high-resolution orbitrap and time-of-flight mass spectrometers. Low-resolution linear ion traps (LITs) have interesting characteristics that make them ideally suited for this task: they are extremely sensitive while also being robust and inexpensive, two qualities necessary to analyze thousands of single cells in parallel from each biological sample. In this study, we sought to determine the analytical characteristics of using LITs as standalone mass spectrometers for single-cell and low-input proteomics.

Our work describes a new data-independent acquisition (DIA) approach for LITs that we test with single cells. Unlike other LIT-based works that required additional high-resolution measurements, our approach uses the LIT at all stages of data acquisition, including spectrum library generation. We have optimized three sets of DIA methods for < 100 ng, < 10 ng and < 1 ng of input protein and we show that low-resolution data are linearly quantitative down to 0.5 ng using matched-matrix calibration curves. We found that spectrum libraries are essential for successful low-input DIA analysis and we demonstrate approaches to build libraries with LIT using as few as 40 total cells. Finally, we test these methods with either single erythroblast cells or pools of up to 10 cells, where we find that low-input libraries outperform libraries generated from larger samples.

The work represents the first effort to analyze the proteomes of single cells using entirely low-resolution mass spectrometry. We believe that it will help democratize single-cell proteomics by demonstrating that high-resolution instruments are not necessary to produce quantitative low-input analysis.

POSTERS

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The process of cell division is one of the most fundamental events in life. During division, significant changes occur rapidly, which are regulated by protein phosphorylation and ubiquitin-based protein degradation mechanisms. Based on our preliminary data and results from a TÜBİTAK-funded project, we hypothesize that palmitoylation, another post-translational modification (PTM), plays an essential role in cell division (during mitosis and cytokinesis). Palmitoylation is a reversible PTM that involves adding a 16-carbon saturated fatty acid palmitate to protein cysteine residues via a thioester bond, which increases the hydrophobicity of proteins. In this research project, we aim to systematically analyze the regulatory role of palmitoylation during cell division for the first time in the literature.

We plan to use synchronized cells at different stages of the cell cycle, combined with the stable isotope labeling by amino acids in cell culture (SILAC) and metabolic labeling-based palmitoylation isolation protocols, to reveal the cell cycle-dependent palmitoylation profile, particularly mitosis and cytokinesis-specific palmitoylation, through mass spectrometry. Additionally, the roles of the DHHC (Asp-His-His-Cys) family of palmitoyl acyltransferases (PATs), the group of enzymes that carry out palmitoylation, in cell division will be systematically investigated using siRNA screening methods and microscopic techniques. Subsequently, to identify the targets of the PATs that play a role during mitosis, we plan to detect proteins with altered palmitoylation in cells in which PAT expression is silenced using shRNA. Thus, we aim to determine the molecular mechanisms in which palmitoylation modification is involved during cell division by establishing the cell cycle-dependent profile of palmitoylation, identifying the PATs that play a role in this process, and identifying proteins that are targeted by PATs during mitosis.

Using our expertise in SILAC-based proteomic analysis and our experience in cell division mechanisms from our past successful studies, we aim to investigate the role of palmitoylation and palmitoyl acyltransferase enzymes in the cell cycle, which is a yet-to-be-explored area. The data obtained will shed light on unknown aspects of cell biology and serve as a milestone in understanding the regulatory roles and interactions of post-translational modifications during cell division. Furthermore, the first catalog of palmitoylated proteins dependent on the cell cycle will provide new targets for anti-mitotic chemotherapies commonly used in cancer treatment and serve as an important resource for other critical cellular events, such as viral infections, in which palmitoylation plays a role.

Developments of Earth Materials on behalf of Water Treatment Applications

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Water pollution has been identified as an impact on the environmental equilibrium including the ecosystems. According to the nature of the pollutants, the treatment method is selected such as the chemical treatment, physical treatment or biological treatment. The investigation and development of some cost effective earth materials for the water treatment applications is becoming a huge chapter in the modern research world. Clay is a dominant earth material that having multi-purpose characteristics as the solutions for most of science and technological issues and currently different clays are being experimented for different water treatment uses. In the existing research, there were expected to chemically characterize three selected clay types based upon the disclosing of their important behaviors. The clay samples were collected from three different regions in Sri Lanka and those clays were named as anthill clay, brick clay and roof tile clay based upon their uses. The clay samples were chemically characterized using X-ray fluorescence (XRF) spectrometer, Fourier transform infrared (FT-IR) spectrometer and X-ray diffraction (XRD) spectrometer. As the outcomes of the research, there were found the presence of at least 75% of Fe contents in each clay, at most 6% of Ti in each clay, at most 5.30% of Ba in each clay, at most 13% of K only in both anthill and roof tile clays and only 7.5% of Ca in brick clay with respect to the X-ray fluorescence (XRF) spectroscopic results, presence of kaolinite, muscovite and quartz as the minerals in each of clay according to the available bonds with respect to the Fourier transform infrared (FT-IR) spectroscopic results and also that mineralogy was confirmed through the X-ray diffraction (XRD) spectroscopic results. According to the recent researches there were found kaolinite and muscovite as the strong adsorbing agents for some other metals such as heavy metals and also there were investigated the K^+ and Ca^{2+} are strong exchangeable ions. Therefore, it is possible to recommend these clays for the experimentation and applications for the waste water treatments based upon the tasks of the removal of heavy metals, removal of some pathogens and removal of some unnecessary dissolved cations.

Improved data-independent acquisition (DIA) and data-dependent acquisition (DDA) performance on low-level proteomic samples using a novel Zeno trap

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We used a ZenoTOF7600 system in-line with a Waters M-Class LC system to determine protein identifications across varying commercial K562 tryptic digest loads in either Zeno SWATH DIA or Zeno DDA modes. Using Zeno SWATH DIA at sample loads of 0.25, 0.5 and 1 ng loads, more than 900-1100, 1400-1500 and 2100-2300 protein groups were identified, respectively, and 45-55% of these identifications had a CV less than 20% when searched against a spectral library. At the peptide precursor level for the same loads, there were 2900-4100, 5000-5700 and 8700-12200 corresponding precursors for the 0.25, 0.5 and 1 ng loads. We tested higher loads and identified 4200, 5000 and 6100 protein groups for 5, 10 and 25 ng loads, respectively, and 64-83% of these identifications satisfied the 20% CV cutoff. For a 50 ng load, more than 6300 protein groups were identified, of which 90% had less than 20% CV, and 56000 precursors were identified. When the data were searched against a FASTA library in library-free mode, the overall number of identifications and those at 20% CV cutoffs approach those achieved when processed using the spectral library approach. A 200 ng and 500 ng load of K562 tryptic digest was tested in Zeno DDA mode. From these experiments, we were able to identify 4600 and 5100 protein groups for the 200 and 400 ng loads, respectively, with 43000 and 56000 peptides for each load.

Antimicrobial peptides in pyelonephritis

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Pyelonephritis (PN) is a frequently occurring inflammatory disorder of the renal parenchyma, which is associated with severe morbidity, especially among elderly and immunodeficient patients. The most common treatment is antibiotics, however, multi-drug-resistant strains of uropathogenic bacteria necessitates optimized treatment strategies. To unravel the molecular and cellular heterogeneity in patients with PN, biopsies from nephrectomized patients with histopathological PN were analyzed by label-free LC-MS/MS-based proteomics and compared with healthy regions of renal cell carcinoma (RCC) resections as a control. The measurements were performed on a quadrupole-ion-trap-orbitrap MS (Orbitrap Fusion, Thermo Fisher) coupled to a nano-UPLC (Dionex Ultimate 3000 UPLC system, Thermo Fisher. Ennrichment analysis using Gene Ontology Biological Process (GOBP) disclosed clear upregulation of biological processes involved in the immune and defense response, including significant upregulation of proteins with antimicrobial function. Among nineteen antimicrobial peptides (AMPs), detected in the kidney tissue, twelve were found to be significantly regulated. Upregulation of AMPs was validated in urine through enzyme-linked immunosorbent assay (ELISA) and complemented by assessing the clinical parameters of the urine and plasma samples of the additional PN cohort. Since AMPs are an integral part of the immune response of urinary tract with a variety of microbicidal and immunoregulatory functions, our data extends the current understanding on the secretion and distribution of AMPs in the PN.

Proteomic analysis of the mucus of the photosynthetic sea slug Elysia crispata

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Elysia crispata is a tropical sea slug that can retain intracellular functional chloroplasts from its algae prey, a mechanism termed kleptoplasty. This sea slug, as other gastropods, produce mucus, a viscous secretion with multiple functions, such as lubrication, protection, and locomotion. This study presents the first comprehensive analysis of the mucus proteome of the sea slug E. crispata using gel electrophoresis and HPLC-MS/MS. We identified 306 proteins in the mucus secretions of this animal, despite the limited entries for E. crispata in the Uniprot database. The functional annotation of the mucus proteome using Gene Ontology identified proteins involved in different functions such as hydrolase activity (molecular function), carbohydrate-derived metabolic processes (biological processes) and cytoskeletal organization (cell component). Moreover, a high proportion of proteins with enzymatic activity in the mucus of E. crispata suggests potential biotechnological applications including antimicrobial and antitumor activities. Putative antimicrobial properties are reinforced by the high abundance of hydrolases. This study also identified proteins common in mucus samples from various species, supporting a common mechanism of mucus in protecting cells and tissues while facilitating animal movement.

Contrasting grain proteome changes in bread wheat (Triticum aestivum) cultivars differing in their drought tolerance

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Water shortage at the beginning of anthesis considerably impacts the production of bread wheat. In this study, we estimated the relation to the drought of two bread wheat cultivars by quantifying photosynthesis, water status, and oxidative stress-related parameters at the flowering stage of development after a transient drought. The sensitive cultivar (Darunok Podillia) showed ineffective water management and a more severe decline in photosynthesis. Apparently, the tolerant genotype (Odeska 267) used photorespiration to dissipate excessive light energy. The tolerant cultivar sooner activated superoxide dismutase and showed less inhibited photosynthesis. Such protective effect resulted in less affected yield and seed proteome profile. Proteomic analysis revealed a more stable composition of grain proteins with nutrient reservoir activities in the tolerant genotype accompanied by a lower magnitude of differential accumulation of allergenic proteins. Water deficit caused the accumulation of medically relevant proteins—mainly components of gluten in the sensitive cultivar and metabolic proteins in the tolerant cultivar. We suggest specific proteins as indicators of drought tolerance for guiding effective breeding for more sustainable bread wheat production: thaumatin-like protein, 1-Cys peroxiredoxin, 14-3-3 protein, peroxidase, FBD domain protein, and Ap2/ERF plus B3 domain protein. They should be screened on multiple wheat genotypes using targeted assays, such as immunodetection or targeted mass spectrometry.

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Development of Spatial and Temporal Proteomics Workflows for Pig Hearts

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Background: Many cardiomyopathies, such as myocardial infarction, dilated or hypertrophic cardiomyopathies (CM) and heart failure (HF) are associated with cellular defects such as protein aggregation or fibrosis. Understanding these complex phenotypes and dissecting the underlying cellular and molecular mechanisms requires high-precision systems-biology and multi-omics approaches. For most cardiomyopathies, transgenic mouse lines and human induced-pluripotent stem-cell-derived cardiomyocytes (hiPSC-CMs) represent the state-of-the-art model systems; however, both differ significantly from the native-human cardiac biology. Pig models offer the advantage of human-like physiology, as well as the possibility of conducting spatially-resolved proteomics studies. Workflows for reproducible, comprehensive, medium-throughput proteome analysis by mass spectrometry, however, have been critically lacking (MS)-based proteomics workflows. Here, we highlight recent developments by our group aimed at improving proteomics analysis from small pig heart biopsies.

Methods: We implemented an automated pressure-cycling technology (PCT) single-pot solid-phase-enhanced sample preparation (SP3) workflow for data-independent analysis (DIA)-MS. Cardiac biopsies obtained from live WT pigs were lysed by PCT. Different chaotropes and detergents (8M urea, 2% SDS, 1% SDC) were used for protein extraction. Protein cleanup and digestion was achieved using an automated SP3 protocol. Peptide mixtures were separated and analyzed using a hybrid ion mobility/quadrupole/time-of-flight mass spectrometer. Data analysis was performed using directDIA processing in Spectronaut v16. Overall recovery of protein groups, as well as recovery of specific cardiac proteins of interest were assessed.

Results: Our PCT-SP3-DIA proteomics workflow enables quantitative analysis of approximately 6,000 protein groups from a 0.5 mm3 tissue volume, with a throughput of 12 samples per day. Reproducibility and protein recovery was significantly improved compared to legacy workflows. Detergent-based lysis was found to provide superior results over established urea-based workflows, increasing recovery by up to 90% for selected cardiac proteins of interest such as PLN and SERCA2A.

Conclusion: Our findings demonstrate the capabilities of the PCT-SP3-DIA workflow for high-throughput pig-cardiac proteome analysis in cardiac tissues from human biopsies, animal models, and engineered heart muscle. Notably, reproducible proteomics data could be obtained from biopsies as small as 0.5mm3, opening up the possibility of developing a spatially-resolved proteome mapping. We are currently in the process of applying these techniques to a novel pig disease model.

Realtime Interaction Cytometry: Measuring binding kinetics on live cells

Ralf Strasser - Dynamic Biosensors GmbH

We have developed a novel biosensor that allows the measurement of binding kinetics in real time on living cells by Real-Time Interaction Cytometry (RT-IC). Since many common therapeutic antibody targets (PD-(L)1, CD3, HER2, etc.) are transmembrane proteins, their binding kinetics are influenced by their density and mobility within the membrane, their transmembrane domain folding or the presence of coreceptors. Molecular interactions of therapeutic antibodies with their targets should therefore be characterized within their native environment to obtain physiologically relevant kinetic data with high in vivo predictability. RT-IC enables the physical retention of cells on the biosensor chip surface using flow-permeable cell traps. The feasibility of RT-IC is demonstrated using application examples ranging from immune checkpoint inhibitors to multivalent peptide constructs to GPCRs.

A spectrum-centric search algorithm for any acquisition method

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The intensity dimension of tandem mass spectra carries valuable information for the identification of peptide precursors, especially in the case of sparse spectra from low abundant analytes. Spectral library-based approaches and machine learning models for the prediction of fragment ion intensities unlock this dimension by enabling the calculation of intensity-based figures of merit such as the normalized spectral contrast angle. Recently, we introduced the intelligent search algorithm CHIMERYS, which utilizes accurate predictions of peptide properties for the spectrum-centric deconvolution of chimeric tandem mass spectra. In its initial implementation, CHIMERYS was limited to the analysis of (ww)DDA data and relied on intact b-/y-ions for the calculation of its scores. Here, we present an update to the CHIMERYS algorithm that facilitates the spectrum-centric processing of data from any acquisition method (DDA, wwDDA, DIA and PRM), thereby unifying the analysis of tandem mass spectra. With these new capabilities, the choice of the data acquisition method becomes an optimization parameter that can be permuted during experimental setup while the search engine remains constant. In addition, a new prediction model enhances the sensitivity of the analysis through the addition of relevant neutral loss ions, providing corroborating evidence for identifications in sparse spectra frequently encountered in single cell proteomics data. To showcase the benefits of our improved algorithm, we reprocessed DDA data from a recent publication from Truong et. al. (Angew. Chem. Int. Ed. 2023, e202303415) with the old and the new CHIMERYS version. The improved sensitivity results in 10% more peptide and 4% more protein group identifications, which can be attributed to the increased amount of explained experimental intensity by the new CHIMERYS version. We then demonstrate that CHIMERYS can analyze data from any acquisition method and contrast DDA, wide and DIA data from the same sample. For the first-time, this enabled a fair comparison of DDA and DIA data using the same error control and protein grouping.