BSPR

EuPA
EUROPEAN PROTEOMICS ASSOCIATION

Programme

BSPR - EuPA Annual Scientific Meeting

Next Generation Proteomics

Newcastle upon Tyne, UK

Platinum Sponsor: BRUKER

Gold Sponsor: ThermoFisher SCIENTIFIC

Silver Sponsor: BIOGNOSYS

Bronze Sponsors: Covaris PREOMICS SCIEX

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The Power of Precision

Join the Zeno revolution!

Zeno Trap and EAD

A high-resolution mass spectrometry solution that combines powerful MS/MS sensitivity, fragmentation technology and a step-change in data independent acquisition.

Meet us at booth 17 to find out more!

YPIC Breakfast
Wednesday 19 | 8:00 am

Lunch Seminar
Thursday 20 | 13:00 pm

BSPR-EUPA Annual Meeting 2023

July 17-20th, 2023

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British Society for Proteome Research

The British Society for Proteome Research (BSPR), is a registered charity that aims to advance the science of proteomics, to further public education therein and to promote study and research work in proteomics and related subjects for the public benefit.

European Proteomics Association

The European Proteomics Association (EuPA) is a non-profit organisation that was founded in 2005 and is a federation of 21 European national proteomics societies. It has been established to coordinate and integrate national initiatives within the proteomics field. The main objectives are to strengthen and promote fundamental research and applications, as well as education and training in all areas of proteomics throughout Europe.

Welcome address

Welcome,

It is with great pleasure, as Presidents of the British Society for Proteome Research (BSPR), and of the European Proteomics Association (EuPA) to welcome you to the annual BSPR meeting, which is this year a joint meeting with the European Proteomics Association (EuPA), and this on behalf of the BSPR, its management committee, EuPA, and the 2023 organising committee.

This year's meeting takes place in the vibrant city of Newcastle upon Tyne, well known for being a powerhouse in the Northeast of England with its eclectic mix of early industrial foundations and modern sciences, alongside the arts and sports. This joint meeting with EuPA will be significantly larger and more diverse than the typical BSPR meeting, and will provide even greater international flair than usual, which already has been a long-standing tradition and hallmark of the BSPR meetings.

The programme of the meeting is a testimony to the diversity and scientific depth of this year's presentations and their underlying research, and to the international character of our field, albeit with a typically British as well as a broader European touch.

It is wonderful to see the focus on support for our Early Career Researchers, for whom the programme includes workshops and seminars that provide expert help in taking the first steps into the vibrant (but sometimes challenging) field of proteomics, from setting up an experiment to achieving a full-fledged career in proteomics.

The numerous talks and posters cover a wide range of fundamental and applied proteomics research, running the gamut from large-scale, bottom-up proteomics, over top-down proteomics, to new technologies and straight into the burgeoning topic of single-cell proteomics.

This truly magnificent programme and organisation of this year's meeting is owed to the work of uncounted hours put in by a small army of volunteers. Therefore, a huge thanks goes to the various BSPR and EuPA committees and helpers who have made this meeting possible, in particular to Matthias Trost and his local team who have truly worked around the clock to make this meeting one of the biggest proteomics meetings in the UK.

We would also like to warmly thank our sponsors and industrial partners who have been extremely supportive of this meeting, and whose technologies and applications continue to inspire and fuel novel discovery in our exciting field. Please do take some time to engage with all sponsors at the exhibition, as there is quite a lot more interesting science and technology to be discovered there than what can be presented in the talks and posters! Moreover, strong and cordial interaction between industry and science forms a fundamental aspect of the field of proteomics, and you will be very pleasantly surprised by the warm welcome that awaits you at our sponsors' booths!

Finally, we hope you will have many engaging and rewarding discussions during the meeting, and that these will provide you with further stimulus to your research, enable you to set up new collaborations, and make a lot of new friends!

Enjoy Newcastle and its great bazaar of cutting-edge proteomics research,

Rainer Cramer
(BSPR President)

Lennart Martens
(EuPA President)

Welcome address

Dear attendees,

A warm and heartfelt welcome to the BSPR-EUPA 2023 Annual Scientific Meeting in the vibrant city of Newcastle! This year's meeting holds a special significance as it showcases the ongoing interconnection between the UK scientific community and Europe, seven years after the Brexit referendum.

Under the theme **Next Generation Proteomics**, this conference will feature the very best in cutting-edge advancements in instruments, technology, methods, and software. But we also will see the **Next Generation *in* Proteomics**: as almost half of the selected presentations from abstract submissions will be delivered by PhD students, allowing these rising stars to shine among their peers.

In keeping with Newcastle's well-deserved reputation as a city that knows how to have a good time, we have organised two exciting evenings to enjoy. On **Monday night**, we invite you to the **Discovery Museum**, a treasure trove of fascinating exhibits highlighting the region's maritime, scientific, and technological contributions to Britain and the world. **Wednesday night**, we will gather at the Wylam Brewery, a 30-Barrel Micro Brewery located in the beautiful Palace of Arts within the Exhibition Park. We will first have a lovely 3-course dinner followed by a conference party (open to every delegate!) with a live band playing Funk & Soul.

We extend our sincerest gratitude to Newcastle University, whose invaluable support, along with that of our numerous sponsors and exhibitors, has made this meeting possible. Thanks to their generous contributions, we were able to keep conference fees at a reasonable level, ensuring accessibility for all.

We hope that you will enjoy Newcastle and the conference.

Warm regards,

The BSPR-EUPA 2023 Organising Committee

Prof Matthias Trost

Dr Maria Dueñas

Amy George

Atakan Nalbant

Frances Sidgwick

Ruth Walker

Exhibitors and sponsors

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BIOGNOSYS
NEXT GENERATION PROTEOMICS

PREOMICS

SCIEX
The Power of Precision

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crb CAMBRIDGE RESEARCH BIOCHEMICALS BIOSYNTH group

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PEAK SCIENTIFIC

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somalogic

Goss Scientific

Cube Biotech

Conference information - Getting here

AIRPORT MAP & TRANSPORT LINKS

Newcastle University

USEFUL CONTACTS

CAMPUS SECURITY
General: 0191 208 6817
Emergency: 0191 208 6666

CITY INFORMATION
newcastlegateshead.com

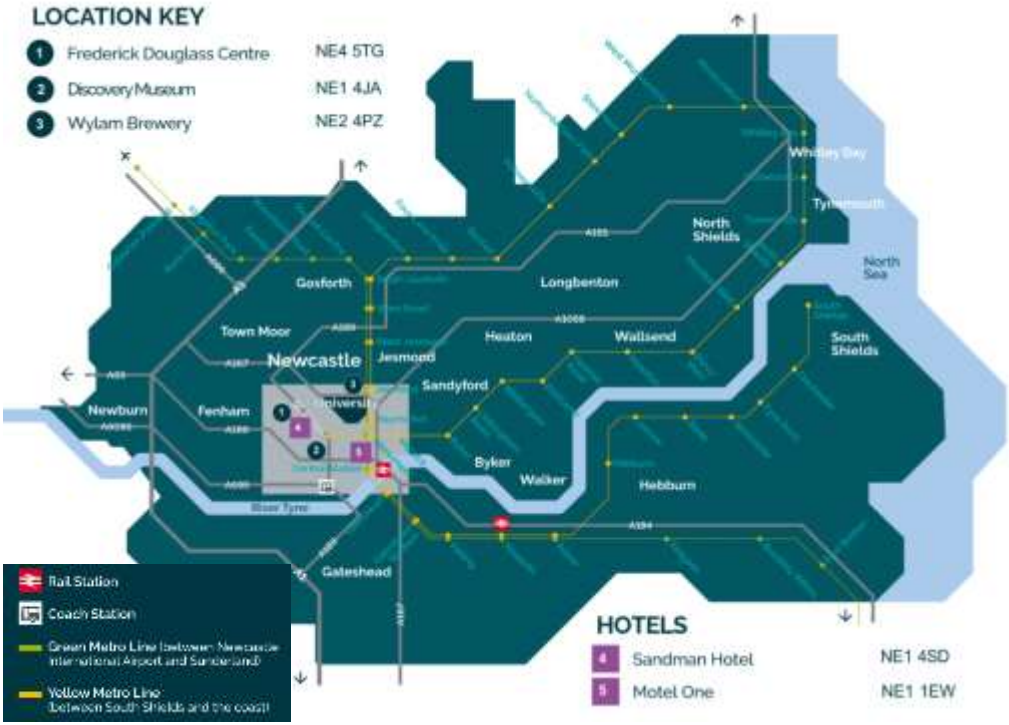
NEWCASTLE COACH STATION
National Express: 08717 818181
nationalexpress.com

NATIONAL RAIL ENQUIRIES
03457 48 49 50
nationalrail.co.uk

NEWCASTLE INTERNATIONAL AIRPORT
0871 882 1121
newcastleairport.com

Newcastle International Airport is only seven miles north west of Newcastle University.
A taxi from the airport will take about 15 minutes, costing approximately £20-£30.
You can also travel by Metro to Haymarket or Monument, which takes about 25 minutes and costs £3.90. From there it is about 10 min walking to the conference centre.

Conference Information –Travel in Newcastle

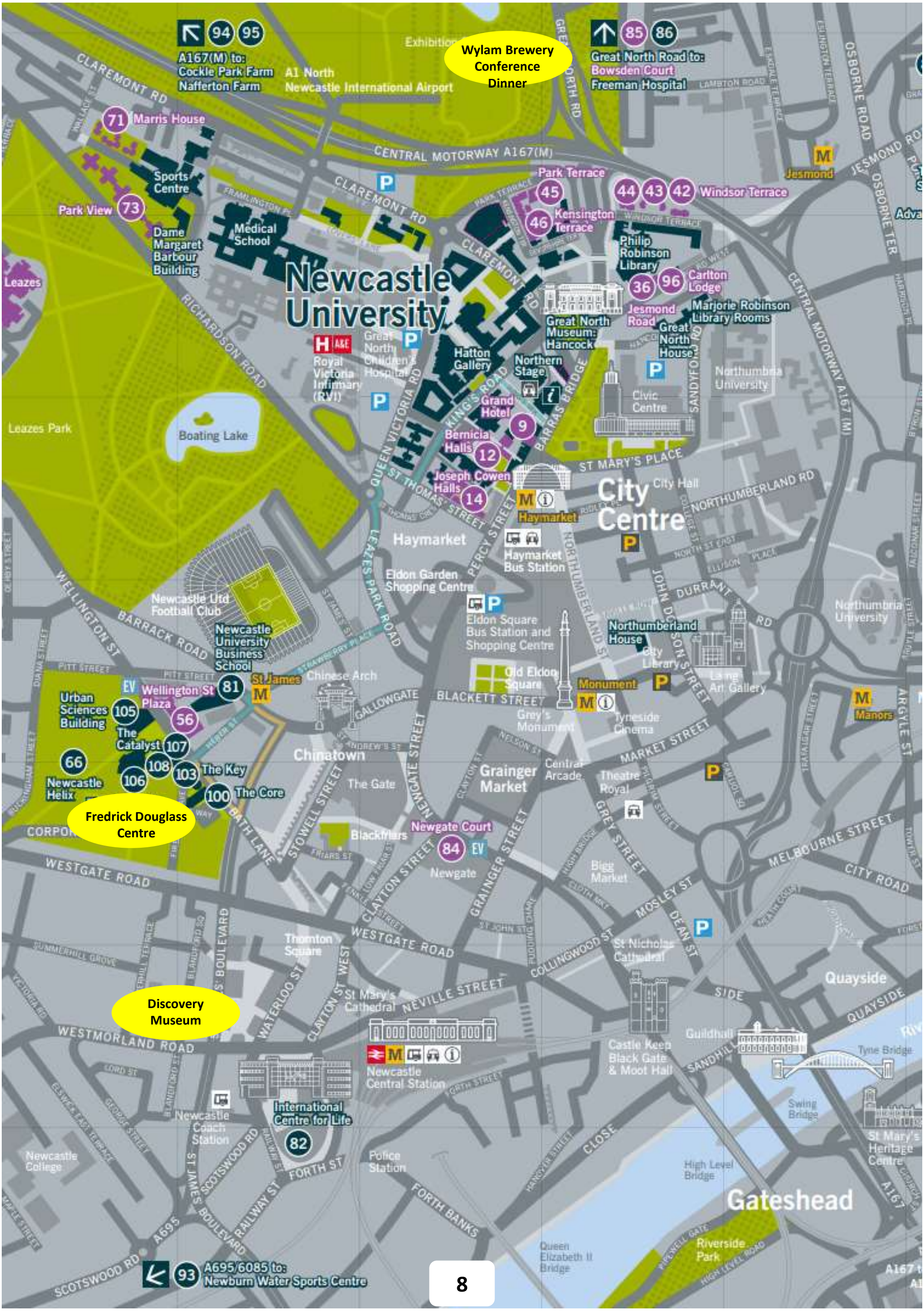


BY TAXI
Our postcode is NE4 5TG. Taxi's can drop off on Wellington Street, just outside The Frederick Douglass Centre.

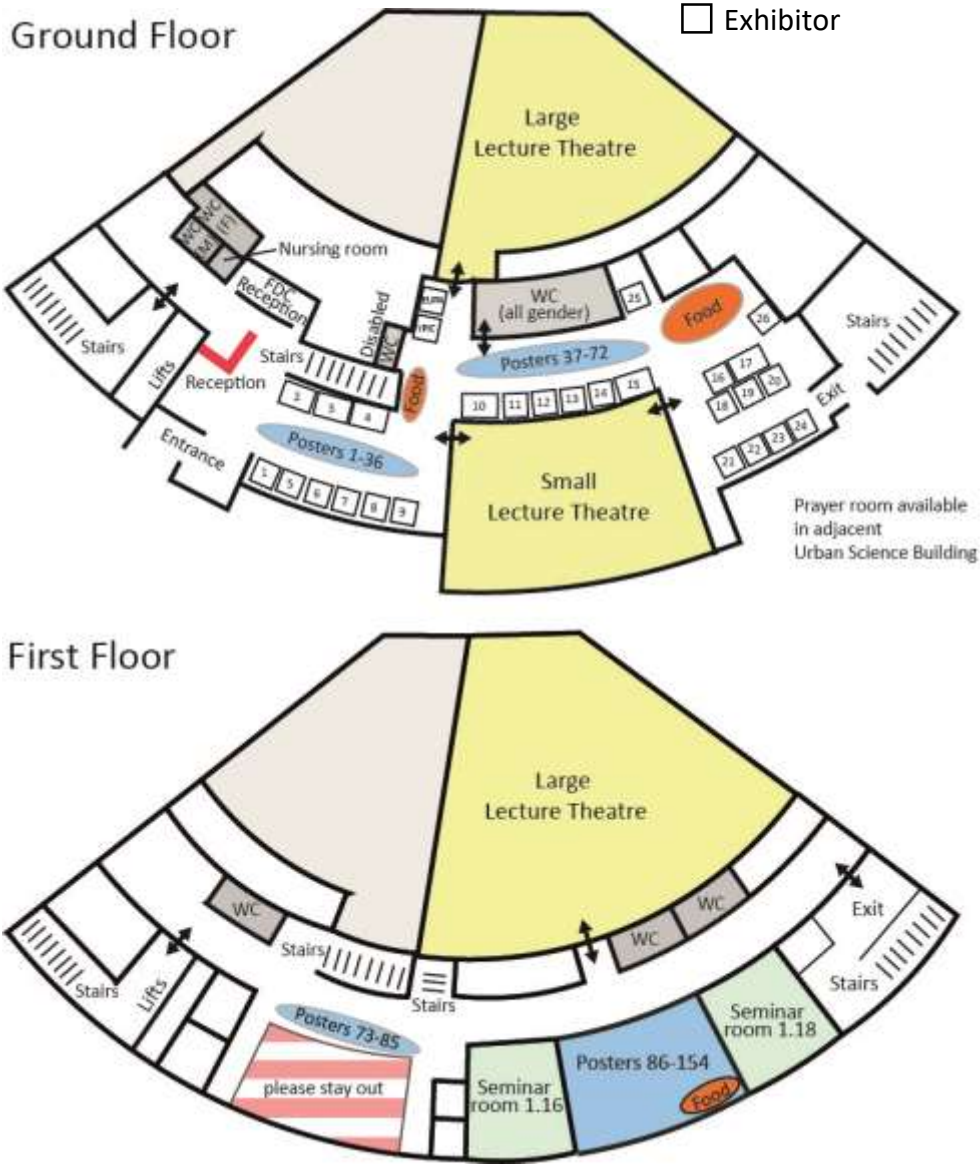
BY METRO (5 minute walk from St James Metro)
From St James Metro Station cross over Gallowgate and walk up Heber Street (with the Sandman Hotel on your left). The building will be in front of you.

BY BUS (5 minute walk from Barrack Road)
The nearest bus stop is Barrack Road-St James Park. Walk down Barrack Road in the direction of the City Centre. Turn right onto Heber Street and continue to Wellington Street. The building will be in front of you.

BY TRAIN (15 minute walk from Central Station)
From Central Station cross the road onto Westgate Road. Turn left and walk up Westgate Road and then right onto Bath Lane. Cross over St James' Boulevard and continue on Bath Lane for 200m. The building is on the left hand side.


Wylam Brewery Conference Dinner
Fredrick Douglass Centre
Discovery Museum
94 95 A167(M) to: Cockle Park Farm Nafferton Farm
A1 North Newcastle International Airport
Exhibition
85 86 Great North Road to: Bowsden Court Freeman Hospital
CLAREMONT RD
71 Marris House
Sports Centre
73 Park View
Dame Margaret Barbour Building
Medical School
CENTRAL MOTORWAY A167(M)
Park Terrace
45
46 Kensington Terrace
44 43 42 Windsor Terrace
Jesmond
OSBORNE ROAD
JESMOND ROAD
Philip Robinson Library
36 96 Carlton Lodge
Jesmond Road
Marjorie Robinson Library Rooms
Great North House
Great North Museum: Hancock
Newcastle University
Leazes
Royal Victoria Infirmary (RVI)
Great North Children's Hospital
Hatton Gallery
Northern Stage
Grand Hotel
9
Bernicia Halls
12
Joseph Cowen Halls
14
Civic Centre
Northumbria University
CENTRAL MOTORWAY A167 (M)
ST MARY'S PLACE
City Hall
City Centre
NORTHUMBERLAND RD
Haymarket
Haymarket Bus Station
Leazes Park
Boating Lake
RICHARDSON ROAD
QUEEN VICTORIA RD
KING'S ROAD
BARRAS BRIDGE
ST THOMAS' STREET
PERCY STREET
Eldon Garden Shopping Centre
Eldon Square Bus Station and Shopping Centre
LEAZES PARK ROAD
NORTHUMBERLAND ST
JOHN DOBSON STREET
DURRANT RD
Northumberland House
City Library
Laing Art Gallery
Northumbria University
Newcastle Utd Football Club
Newcastle University Business School
WELLINGTON ST
BARRACK ROAD
St James
Chinese Arch
GALLOWGATE
BLACKETT STREET
Old Eldon Square
Monument
Grey's Monument
Tyneside Cinema
Manors
ARGYLE ST
EV Wellington St Plaza 81
56
Urban Sciences Building 105
The Catalyst 107
66 Newcastle Helix
108 106 103 The Key
100 The Core
Chinatown
The Gate
NEWGATE STREET
Grainger Market
Central Arcade
MARKET STREET
Theatre Royal
GREY STREET
BATH LANE
STOWELL STREET
Blackfriars
Newgate Court
84 EV
Newgate
CLAYTON STREET
GRAINGER STREET
Bigg Market
MOSLEY ST
DEAN ST
MELBOURNE STREET
CITY ROAD
WESTGATE ROAD
Thornton Square
WESTGATE ROAD
COLLINGWOOD ST
St Nicholas Cathedral
Quayside
QUAYSIDE
St Mary's Cathedral
NEVILLE STREET
WESTMORLAND ROAD
Newcastle Central Station
Castle Keep Black Gate & Moot Hall
Guildhall
Tyne Bridge
SANDHILL
Swing Bridge
St Mary's Heritage Centre
Newcastle Coach Station
International Centre for Life
82
Newcastle College
Police Station
FORTH ST
FORTH BANKS
CLOSE
High Level Bridge
Gateshead
Queen Elizabeth II Bridge
Riverside Park
A167
SCOTSWOOD RD
A695
93 A695/6085 to: Newburn Water Sports Centre

Frederick Douglass Centre (FDC) Map



WiFi Guest Service

Most delegates should be able to connect to the WiFi through Eduroam.

Wireless Guest Service Visitors who are not able to connect to eduroam can use the **free** cloud WiFi network **WiFi Guest** to access the internet using their own computer.
The service is provided free of charge, is simple to use and requires no configuration changes to the visitor's computer. The service is managed by The Cloud Networks Ltd and is the same free public WiFi service available in thousands of locations across the UK.

You will need to create an account to use the service, unless you already have an account for The Cloud.

Creating An Account
Here are the steps to get connected:

1. From your device connect to the network **WiFi Guest**
2. On The Cloud landing page locate the box **Get online at Newcastle University** and click **Go**
3. Scroll down to select **Create Account**
4. Enter your details and the account will be created.
5. The device will then be connected to **WiFi Guest**

How to use the service
Visitors and delegates should connect their device to the open wireless network WiFi Guest and open a web browser. They will be automatically redirected to the The Cloud login page where they should enter their account details. After connecting the first time your device should remember your credentials for next time. You can also download the Sky WiFi app from the App Store or Google Play Store to locate other compatible hotspots outside the University.

Social Events

Networking Reception at Discovery Museum - Sponsored by Thermo-Fisher Scientific.

Networking reception at the Discovery Museum (18:00-21:00 Monday 17th July 2023). Following the Plenary lecture, we will walk to the Discovery Museum (approx. 7 min) where there will be an opportunity to network over drinks and nibbles and learn something about local history and science.

Map location:
https://goo.gl/maps/9GZHxjmDQMVKWKVY9

Conference Dinner and Party at Wylam Brewery - Sponsored by Bruker.

Conference Dinner at Wylam Brewery (18:30-24:00 Wednesday 19th July 2023). - *Attendance by ticket only* -

Followed by **Conference Party** with *The Yo Man Funk Band* (from 21:00). - *Open to all delegates* -

For people with mobility issues, we have organised a minibus leaving at 18:10 from the Sandman Hotel.
For those of you who wish to make their own way there, the postcode is NE2 4PZ and it is a 20 min walk from the conference centre.
For taxis, we recommend Uber or Budget Taxis (+44 191 222 0222).

Map location: https://goo.gl/maps/QsmU1iosUw76bVYK9

Awards and Prizes

EuPA Awards

- EuPA Early Career Researchers Awards
  - Best Doctoral Thesis Award
  - Bioinformatics for Mass Spectrometry Award
  - Vision and Commitment Award
- EuPA Breakthrough in Proteomics Award
- EuPA Technology Award
- EuPA Science Outreach Award
- EuPA Juan-Pablo Albar (JPA) Proteome Pioneer Award

Early Career Researcher (ECR) Talk Awards (sponsored by ResynBio)

All ECRs giving oral presentations at BSPR-EuPA 2023 will be considered. ResynBio kindly sponsored £300 / £200 / £100 prizes for the best three ECR talks.

Poster Awards (sponsored by Promega)

During the poster session on **Tuesday July 18th (17:15-19:30)**, poster judges will come and ask you about your poster.
Promega kindly sponsored £300 / £200 / £100 prizes for the best three posters.

Best Conference Tweet #BSPREUPA2023

A jury will award a £100 Prize for the Best Conference Tweet with the #BSPREUPA2023 hashtag.

Passport to Prizes

In the welcome package, you will have received a **Passport to Prizes**. This encourages delegates to visit the booths of exhibitors and gives them a chance to win one of many amazing prizes provided by our sponsors.

How to complete your passport:
1) Visit every booth, find and tick the correct answer, and collect a stamp/sticker.
2) Hand in your passport by **11 am** on **Thursday, July 20th** at the registration desk for a chance to win amazing prizes, one from each company!
Please note, the passport will need to be filled in correctly with all 26 questions answered and all 26 different stamps/stickers obtained.

Prizes will be awarded on Thursday 20th, 12:10-12:20 in the Large Lecture Theatre.

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For more information please visit www.bruker.com

Innovation with Integrity

Etiquette

COVID-19:

- **If you have symptoms or start feeling unwell, please do not attend any event of BSPR-EuPA 2023.**
- **Please make use of the hand sanitizers provided.**

Talks:

- **Please do not photograph unpublished data slides.**
- **Please do not tweet about unpublished data**
  – check for the no-tweet sign!
- **Please do ask questions!**

Posters:

- **Please do not photograph any poster unless you have the author's explicit approval.**

Lecture Theatres:

- **Please do not take drinks and food into the lecture theatres.**

Scientific Programme

Monday, July 17th 2023

Seminar room 1.16
1000-1130 Meet the EUPA Committee members

Seminar room 2.15
0915-1600 **YPIC Workshops**

0915-1045 **Intro to Bioinformatic Tools**
Dominik Lux & EuBIC

1100-1145 **Intro to Metaproteomics**
Jean Armengaud, Robert Heyer, Celine Henry & Metaporoteomics Initiative

1145-1245 **Mental Health Lunch Seminar**
Marie Chion

1250-1420 **Advanced Proteomics**
Daniel Figeys and Tamar Geiger

Proteomics 101 Session
Mike MacCoss and Éva Csösz

1430-1600 **Career Session**
Jenny Ho (Thermo), Adam Hughes (Bruker), Simon England (VRS)
Anjali Seth (Cellenion), Chiara Francavilla, Christian Moritz

Small Lecture Theatre
1200-1600 **Bruker post-ASMS Workshop**

Seminar room 2.16
1200-1600 **Thermo Fisher Scientific post-ASMS Workshop**

1400-1800 Registrations open
Poster set up

Large Lecture Theatre
1630-1745 **Opening Session**

1630-1645 **Welcome address**

1645-1745 **Plenary: Mike MacCoss (University of Washington)**
Next Generation Translational Proteomics of Alzheimer's Disease

1800-2100 **Social event at Discovery Museum - Sponsored by**

Scientific Programme

Tuesday, July 18th 2023 (morning)

Large Lecture Theatre

0900-1030 Session 1: Clinical Proteomics — Chair: Rodrigo Barderas

0900-0930	**Markus Ralser (Charité Berlin)**	The metabolic and proteomic landscape of genome-scale genetic perturbation
0930-0950	**Sandra Goetze (ETH Zurich)**	*Ex vivo* drug response heterogeneity combined with cell-population-specific proteotyping reveals personalized therapeutic strategies for patients with multiple myeloma
0950-1010	**Pavel Bouchal (Masaryk University)**	Proteotype classification of localized and metastatic renal cell carcinoma for prognosis and therapy response
1010-1030	**Christian Moritz (University Hospital of Saint-Étienne)**	Autoantigenomics: A Systematic Proteomics Approach for Identifying the Repertoire of Autoantibody-Targeted Proteins in Autoimmune Neuropathies

1030-1100 Coffee/Trade Exhibition

Large Lecture Theatre

1100-1210 Session 2: Chemical Proteomics & Drug Discovery — Chair: Maria Dueñas

1100-1130	**Mikhail Savitsky (EMBL)**	Biophysical Proteomics
1130-1150	**Stephan Eckert (TU Munich)**	Dose-dependent and proteome-wide drug response profiling by micro-flow-LC-FAIMS-MS/MS
1150-1210	**Frances Sidgwick (Newcastle University)**	Mass Spectrometry techniques in PROTAC drug discovery

Small Lecture Theatre

1100-1210 Session 3: Structural Proteomics & Protein Complexes — Chair: Stefan Müller

1100-1130	**Aygiris Politis (University of Manchester)**	Uncovering the structural dynamics of membrane proteins and their complexes
1130-1150	**Mariette Matondo (Institut Pasteur)**	The RBPome of influenza A virus mRNA reveals a role for TDP-43 in viral replication
1150-1210	**Frederico Uliana (ETH Zurich)**	Phosphorylation-linked complex profiling identifies assemblies required for Hippo signal integration

1210-1400 Lunch/Posters/Trade Exhibition

Large Lecture Theatre

1300-1345 **Bruker Lunch Seminar** BRUKER

Small Lecture Theatre

1300-1345 **Covaris Lunch Seminar** Covaris

Scientific Programme

Tuesday, July 18th 2023 (afternoon)

Large Lecture Theatre

1400-1530 Session 4: Post-Translational Modifications — Chair: Franck Vandermoere

1400-1430 **Tiziana Bonaldi (European Inst of Oncology, Milan)**
The role of protein methyltransferases in cancer plasticity and resistance to therapy, through the proteomics investigation of ovarian cancer preclinical models and clinical samples

1430-1450 **Stefanie Höfer (TU Munich)**
Proteomic basis for understanding the molecular mode of action of clinical ATR kinase inhibitors and their combination with gemcitabine in pancreatic cancer cells

1450-1510 **Roberta Noberini (European Inst of Oncology, Milan)**
A histone PTM-centered, multi-OMICs approach to dissect aberrant epigenetic mechanisms in triple negative breast cancer

1510-1530 **Kerry Ramsbottom (University of Liverpool)**
PTMeXchange: Globally harmonized re-analysis and sharing of data on post-translational modifications

Small Lecture Theatre

1400-1530 Session 5: Bioinformatics | Data processing — Chair: Alejandro Brenes

1400-1430 **Mathias Wilhelm (TU Munich)**
Deep learning-assisted mass spectrometry-based proteomics

1430-1450 **Witold Wolski (Swiss Institute of Bioinformatics)**
The Role of Deep Learning Spectra Libraries in DIA-based Differential Expression Analysis

1450-1510 **Marie Chion (MRC Biostatistics Unit, Cambridge)**
ProteoBayes: a Bayesian framework for differential proteomics analysis

1510-1530 **Robert Heyer (ISAS)**
Advanced bioinformatics tools for analysing metaproteomics data

1530-1600 Coffee/Trade Exhibition

Large Lecture Theatre

1600-1710 Session 6: Top Down Proteomics & New Technologies/Methods — Chair: Rainer Cramer

1600-1630 **Julia Chamot-Rooke (Institut Pasteur)**
Case Studies of Proteoforms in Human Health and Disease

1630-1650 **Allen Po (University of Liverpool)**
The challenges of top down proteomics and true proteoform characterization

1650-1710 **Jean Armengaud (CEA Marcoule)**
Paleoproteomics & Metaproteomics exploration of unique historical samples

Small Lecture Theatre

1715-1800 BSPR AGM

1715-1930 **Posters / Trade Exhibition / Wine & Nibbles**

Scientific Programme

Wednesday, July 19th 2023 (morning)

0800-0900 **YPIC breakfast**

Large Lecture Theatre

0900-1030 **Session 7: Computational Proteomics & Big Data** Chair: Juan Antonio Vizcaino

0900-0930 **Alexey Nesvizhskii (University of Michigan)**
Open-minded exploration of large proteomics datasets

0930-0950 **Ludwig Lautenbacher (TU Munich)**
Koina: Bringing machine learning to the community

0950-1010 **Wout Bittremieux (University of Antwerp)**
Improved immunopeptidome analysis using timsTOF fragment ion intensity prediction

1010-1030 **BSPR & EuPA Travel Awards**

1030-1100 Coffee/Trade Exhibition

Large Lecture Theatre

1100-1210 **Session 8: Subcellular & Spatial Proteomics** Chair: Kathryn Lilley

1100-1130 **Chiara Francavilla (University of Manchester)**
Spatially resolved phosphoproteomics reveals recycling endosomes as signalling hubs that coordinate Receptor Tyrosine Kinases (RTKs) responses

1130-1150 **Ana Carolina Pinto (University of Aveiro)**
Unveiling the health benefits of muscle contraction: Proteomics of circulating extracellular vesicles

1150-1210 **Luisa Schmidt (CECAD, Cologne)**
Mapping protein profiles of the muscle-tendon junction with spatial proteomics

Small Lecture Theatre

1100-1210 **Session 9: BMSS Session** Chair: Andrew Pitt

1100-1130 **Justin Benesch (University of Oxford)**
Structural proteomics: weighing up structure, dynamics and function

1130-1150 **Leonie Müller (Newcastle University)**
Identifying inhibitors of an aminopeptidase with a high-throughput MALDI-TOF MS workflow

1150-1210 **Rainer Cramer (University of Reading)**
MALDI MS sample analysis at ultrahigh speed and its applications in protein analysis

1210-1400 Lunch/Posters/Trade Exhibition

Large Lecture Theatre

1300-1345 **Thermo Fisher Scientific Lunch Seminar**

Small Lecture Theatre

1300-1345 **Preomics Lunch Seminar**

Scientific Programme

Wednesday, July 19th 2023 (afternoon)

Large Lecture Theatre

1400-1530 Session 10: Human Disease Proteomics Chair: Aida Serra

1400-1430 **Tamar Geiger (Weizmann Institute of Science)**
Immunological impact on internal proteomics cancer heterogeneity

1430-1450 **Emily Gaizley (UCL Cancer Institute)**
Proteomic characterisation of rare quiescent therapy-resistant cancer cells

1450-1510 **Georg Tascher (Goethe University Hospital, Frankfurt)**
Quantitative Translation and Import Proteomics using mePROD

1510-1530 **Maurine Fucito (University of Ferrara)**
Exploring the Brain Proteome Signatures of Multiple Sclerosis-Like Models Using Mass Spectrometry

Small Lecture Theatre

1400-1530 Session 11: Plants | Animals | Microbes | Metaproteomics Chair: Angeliki Katsafadou

1400-1430 **Daniel Figeys (University of Ottawa)**
Metaproteomic applications in pharmacology and clinical research

1430-1450 **Ezgi Aydin (TU Munich)**
The Proteomes that Feed the World

1450-1510 **Emily Clarke (University of Liverpool)**
Proteome and lipidome interrelationship of synovial fluid-derived EVs in Equine osteoarthritis: An exploratory 'multi-omic' study

1510-1530 **Thibaut Dumas (CEA-Li2D)**
Metaproteomics exploration of the gut microbiota of the freshwater sentinel animal *Gammarus fossarum*

1530-1600 Coffee/Trade Exhibition

Large Lecture Theatre

1600-1730 Session 12: Early Career Researcher talks Chair: Eva Czósz

1600-1615 **Yun Chiang (University of Copenhagen)**
Casanovo? Is *de novo* sequencing a pipe dream or an undisputed Casanova?

1615-1630 **Wouter van Bergen (Utrecht University)**
Site-Specific Activity-Based Protein Profiling Using Phosphonate Handles

1630-1645 **Giuliana Siragusa (University of Verona)**
The epigenetic landscape of pancreatic cancer stem cells: target identification and therapeutic potential.

1645-1700 **Savvas Kourtis (University of Pompeu Fabra)**
Automated workflow for proteomic-reanalysis and coregulation-based functional characterisation

1700-1715 **Marek Polak (BioCeV, Prague)**
Fast photochemical oxidation of nucleic acids coupled to high-resolution MS analysis

1715-1730 **Lily Adair (University of Reading)**
Can precursor ion connectivity of different isolation windows improve peptide and protein identification in chimeric MS/MS spectra?

1830-0000 **Conference dinner and party at Wylam Brewery - Sponsored by** BRUKER

Scientific Programme

Thursday, July 20th 2023

Large Lecture Theatre

0900-1030 Session 13: Systems Biology & Medicine Chair: Sandra Goetze

0900-0930 **Nicola Ternette (University of Oxford)**
Off-target identification of TCR-like antibody cancer therapeutics

0930-0950 **Alejandro Brenes (University of Dundee)**
Quantitative analysis of how the immunosuppressive drug rapamycin shapes immune cell proteomes

0950-1010 **Dario Frey (German Cancer Research Center (DKFZ))**
The interplay between EGFR and MET receptor abundance and cross-talk determines the effectiveness of tyrosine kinase inhibitors in lung cancer patients

1010-1030 **Jenny Hansson (Lund University)**
A complex interplay of intra- and extracellular factors regulates the outcome of fetal- and adult-derived MLL-rearranged leukemia

1030-1100 Coffee/Trade Exhibition

Large Lecture Theatre

1100-1210 Session 14: Single Cell Proteomics Chair: Karl Mechtler

1100-1130 **Erwin Schoof (Technical University of Denmark)**
Unleashing the next-generation single-cell proteomics workflows

1130-1150 **Ludwig Sinn (Charité Berlin)**
Slice-PASEF: maximising the sensitivity and speed of DIA

1150-1210 **Ed Emmott (University of Liverpool)**
Single-cell proteomic analysis of RNA virus infection

1210-1220 **Passport to Prizes**

1220-1400 Lunch/Posters/Trade Exhibition

Large Lecture Theatre

1300-1345 **Biognosys Lunch Seminar** BIOGNOSYS NEXT GENERATION PROTEOMICS

Small Lecture Theatre

1300-1345 **SCIEX Lunch Seminar** SCIEX

Large Lecture Theatre

1400-1600 Session 15: Awards and Plenary

1400-1445 **EuPA Awards**

1445-1545 **Sara Zanivan (CRUK Beatson Institute, Glasgow)**
Fibroblasts: bystanders or culprits in cancer?

1545-1600 **ECR & Poster Awards**

1600 **End**

YPIC Programme

BSPR-EuPA2023 : YPIC Event Schedule

Date	Time	Session Title
16th July	Evening	Networking Session
17th July	09:15-10:45	Intro to Bioinformatic Tools – Dominik Lux in cooperation with EuBIC; chairs Maurine Fucito and Erdenetsetseg Nokhoijav
	11:00-11:45	Intro to Metaproteomics - Jean Armengaud, Robert Heyer, and Celine Henry in cooperation with the Metaproteomics Initiative; chairs Maria Bourganou and Vincent Albrecht
	11:50-12:45	Mental Health Lunch Seminar – Marie Chion, Simon Sugár and Angeliki Katsafadou **(Please pre-register so we can sort the lunch order!))**
	12:50-14:20	Proteomics 101 Session - Mike McCoss and Éva Csősz; chairs Megan Ford and Maria Bourganou
	12:50-14:20	Advanced Proteomics - Daniel Figeys and Tamar Geiger; chairs Luisa Schmidt and Alexandre Leytens
	14:30-16:00	Career Session; chairs Christian Moritz and Monique van Schie

14:00 Career advice from Thermo Fisher Scientific - Jenny Ho
14:15 Career advice from BRUKER - Adam Hughes
14:30 Recruitment - Head hunting (VRS) - Simon England
14:45 Tba (Cellenion) - Anjali Seth Winner of the EuPA Technology Award
15:00 How bumpy is the "academic road"? - Chiara Francavilla
15:15 How to optimally use this conference for career purpose - Christian Moritz

18th July	Evening	Networking Session
19th July	8:00-9:00	Meet-the-Experts Breakfast – sponsord by Sciex; chairs Luisa Schmidt and Vincent Albrecht

(Spaces are limited to 8 ECRs per table, pre-registration necessary)

SCIEX

Table 1: Mikhail Savitski
Table 2: Tamar Geiger
Table 3: Sara Zanivan
Table 4: Erwin Schoof and Anjali Seth
Table 5: Kathryn Lilley
Table 6: Rebekah Sayers (Sciex)

DAY 1 – Monday – 17th July 2023
PLENARY

16:45 – 17:45

Next Generation Translational Proteomics of Alzheimer's Disease

Michael MacCoss, Gennifer Merrihew, Jea Park, Deanna Plubell, Brian Searle[1], Dirk Keene, Kathleen Poston[2], Tom Montine[2], and Christine Wu

University of Washington, Seattle, United States; [1]The Ohio State University; [2] Stanford University

Alzheimer's disease (AD) is a looming public health disaster with limited interventions. Alzheimer's is a complex disease that can present with or without causative mutations and can be accompanied by a range of age-related comorbidities. This diverse presentation makes it difficult to study molecular changes specific to AD. To better understand the molecular signatures of disease we constructed a unique human brain sample cohort inclusive of autosomal dominant AD dementia, sporadic AD dementia, and those without dementia but with high AD histopathologic burden, and cognitively normal individuals with no/minimal AD histopathologic burden. All samples are clinically well characterized, and brain tissue was preserved postmortem by rapid autopsy. All data were collected using data independent acquisition-mass spectrometry. Furthermore, we also performed similar analyses in cerebral spinal fluid (CSF) and from plasma extracellular vesicles using a well characterized cohorts containing individuals with AD dementia, Parkinson's disease dementia, Parkinson's disease cognitively normal, and healthy cognitively normal. I will present the considerations made during the experimental design and the process our lab uses to assess data quality using multiple internal and external control strategies.

DAY 2 – Tuesday – 18th July 2023
SESSION 1 – CLINICAL PROTEOMICS

09:00 – 09:30

The metabolic and proteomic landscape of genome-scale genetic perturbation

Markus Ralser
Charité Universitätsmedizin Berlin, Germany
Nuffield Department of Medicine, University of Oxford, UK

Metabolic reactions are vital for keeping cells and organisms growing and alive, and problems with cellular metabolism are implicated in ageing and diseases such as cancer, diabetes and brain disorders. Metabolism in the cell is organised in a genome-spanning network, known as the metabolic network, that connects several hundred enzymes with more than a thousand metabolites. In order to understand metabolism at its scale, novel technologies are required. These need to measure metabolites and proteins at precision, at high throughput, and at costs that facilitate systematic perturbation experiments at large scale. In this lecture, I'll summarise our efforts in using mass spectrometry, yeast as a simple system, as well as human plasma analytics, for conducting hundreds to thousands of analytical proteome measurements, allowing us to study how these complex metabolic processes are controlled/ Technical aspects of the lecture will include the summary of novel mass spectrometric acquisition techniques that centre around high-floware liquid chromatography to measure up to 1,800 samples per week per mass spectrometer, new acquisition schemes, Scanning SWATH, Zeno-SWATH, and SpeedyPASEF as well as new software that centres around the DIA-NN suite, developed in my laboratory. I'll further show new results that i) demonstrate the acquisition of large numbers of plasma proteomes to study human metabolic disease, ii) a study that involved the acquisition of more than a 1,000 proteomes of yeast natural isolates to understand dosage compensation in genomic aneuploidies, and iii) the generation of a proteome for each non-essential yeast gene knock-out, to study the function of so far uncharacterized proteins.

DAY 2 – Tuesday – 18th July 2023
09:30 – 09:50

Ex vivo drug response heterogeneity combined with cell-population-specific proteotyping reveals personalized therapeutic strategies for patients with multiple myeloma

Klara Kropivsek,[1,2] Paul Kachel,[3] **Sandra Goetze,[2,4,5]** Rebekka Wegmann,[1,2] Yasmin Festl,[1,2] Yannik Severin,[1,2] Benjamin D. Hale,[1,2] Julien Mena,[1,2] Audrey van Drogen,[2,4,5] Nadja Dietliker,[5] Joëlle Tchinda,[6] Bernd Wollscheid,[2,4,5] Markus G. Manz,[5] Berend Snijder,[1,2]

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Introduction: Multiple myeloma (MM) is a complex disease characterized by the proliferation of abnormal plasma cells in the bone marrow. In recent years, treatment options for MM patients have increased substantially: instead of standard chemotherapy, patients receive proteasome inhibitors frequently containing immunomodulatory drugs as initial treatment. However, patients respond differently and invariably relapse, which requires successive therapies to control the disease. To understand clinical treatment response and variability within primary patient-derived cells, we combined pharmacoscopy, an image-based ex vivo drug screening strategy, with cellular proteotyping.

Methods: In parallel to a pharmacoscopy screen to assess the *ex vivo* drug responsiveness of malignant MM cells to 61 drugs and immunotherapies, the proteotype of sub-sorted cell populations from the patient-derived samples was analyzed. CD138-positive plasma cells, CD3-positive T-cells and CD14-positive monocytes which support malignant plasma cell growth and survival, were isolated from MM patient bone marrow aspirates. Using a standardized and harmonized DIA-based proteotyping strategy, we generated a data matrix of 5723 human proteins across 294 samples of 102 patients with MM.

Results: The MM patient cohort was stratified into three phenogroups (PGs) based on the cellular composition of the bone marrow biopsies. These PGs corresponded to disease progression, inflammation, and clonality and were strong predictors for *ex vivo* drug sensitivity. Proteotype profiles from CD138-positive plasma cells differed significantly between the different PGs, which was also reflected in CD3-positive and CD14-positive cell populations. Correlation of proteotype patterns across the 61 drugs and combinations revealed well-defined functionally related protein sub-networks, centered around a ribosomal and translational core network. Amongst others, prototyping identified CDK5 and HLA-DRB5 as potential new drug targets.

Conclusion: The integration of proteotyping with pharmacoscopy data enabled the identification of protein signaling networks involved in MM drug responsiveness. The proteotyping data provides a rational basis to better understand the molecular mode of action (MMoA) of selected MM drugs and drug combinations.

DAY 2 – Tuesday – 18th July 2023
09:50 – 10:10

Proteotype classification of localized and metastatic renal cell carcinoma for prognosis and therapy response

Jan Šimoník,[1] Richard Štefaník,[1] Pavla Bouchalová,[1] Petr Lapčík,[1] David Potěšil,[2] Vlad Popovici,[3] Ján Podhorec,[4,5] Milan Hora,[6] Alexandr Poprach,[4,5] Ondřej Fiala[7] and **Pavel Bouchal**[1]

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Renal cell carcinoma (RCC) represents a serious oncological disease with one of the highest incidences in the Czech Republic across the world. Reliable molecular prognostic and predictive biomarkers for RCC are mostly unavailable, namely at protein level. To quantify proteins associated with pro-tumorigenic and pro-metastatic mechanisms in RCC, we first generated a comprehensive RCC-specific spectral library of targeted proteomic assays for 7960 protein groups (FDR=1%) [1]. Second, we have applied data independent acquisition mass spectrometry (DIA-MS) on QExactive HF-X LC-MS system to analyze a well-characterized set of initially localized RCC tumors (n=86) of which a half exhibited a relapse in <5 years after diagnosis. We have identified a single potential biomarker and two protein classifiers able to predict the relapse, for which we have developed selected reaction monitoring assay for further validation and routine quantification. CRISPR/Cas9 knockdown confirmed the role of the key protein in cell migration in 786-0 cells, supporting its role in metastatic potential of RCC. Third, we have analyzed a well-characterized set of metastatic RCC tumors (training set n=53, validation set n=22) and adjacent non-cancerous tissues (n=17) a part of which responded and a part did not respond to tyrosine kinase inhibitor (TKI) treatment. We have identified and validated a single protein biomarker and one classifier associated with a poor response to TKI but not with tumor grade and lymph node status. Functional assays using CRISPR/Cas9 knockdown confirmed its role in metastatic potential of 786-0 cells. In a summary, next generation proteomics based on DIA-MS is a powerful tool to classify RCC tissues, to identify prognostic biomarkers and alternative therapeutic targets. Supported by Ministry of Health of the Czech Republic, project No. NV19-08-00250, all rights reserved. CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2018127, is gratefully acknowledged for the financial support of the measurements at the CEITEC Proteomics Core Facility. Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

DAY 2 – Tuesday – 18th July 2023
10:10 – 10:30

Autoantigenomics: A Systematic Proteomics Approach for Identifying the Repertoire of Autoantibody-Targeted Proteins in Autoimmune Neuropathies

Christian Moritz, Yannick Tholance, Jean-Philippe Camdessanché, Jean-Christophe Antoine

University Hospital of Saint-Étienne, France

Autoantigenomics is a systematic approach that identifies and characterizes the repertoire of autoantibody-targeted proteins, also known as the "autoantigenome," which is a subcategory of the proteome. This promising subdomain of proteomics enables a systemic understanding of a patient's autoimmune reaction. Here, we discuss our recent research examples of using autoantigenomics to study patients with autoimmune-related neuropathies, specifically chronic inflammatory demyelinating polyneuropathy (CIDP) and sensory neuronopathy (SNN).

To obtain the autoantigenome, we utilized protein microarrays containing over 16,000 full-length proteins. We then filtered the lists of significantly targeted and study-group-specific autoantigens, and perform over-representation analyses, cluster analyses, heat maps, and pathway analyses.

We present three examples of using autoantigenomics. In the first example, we found that CIDP patients who responded to intravenous immunoglobulin targeted three times more antigens than non-responders. Furthermore, we identified anchoring junction proteins as a major target in CIDP, which is a recently revealed autoimmune target in this disease. In the second example, we identified the P-body and RISC complex as significantly overrepresented in the SNN autoantigenome, which may serve as potential biomarkers for underlying autoimmune context in sensory neuronopathies. In the third example, we found that immune system pathways were significantly targeted by non-paraneoplastic SNN sera.

We conclude that autoantigenomics is a powerful approach that identifies global events in the repertoire of autoantibodies. It provides important insights into the underlying pathophysiology of autoimmune neuropathies. This may lead to the development of new diagnostic tools and assist treatment decisions.

DAY 2 – Tuesday – 18th July 2023
SESSION 2 – CHEMICAL PROTEOMICS & DRUG DISCOVERY

11:00 – 11:30

Biophysical Proteomics

Mikhail Savitski

EMBL, Heidelberg, Germany

In order to understand cellular phenotypes, it is not sufficient to only look at RNA and protein expression levels since many molecular processes are regulated post-translationally. Mass-spectrometry based proteomics has been instrumental in mapping thousands of post-translational modifications, (PTMs), highlighting the extent and complexity of post-translational regulation. A huge challenge that needs to be tackled now is the understanding of which of the thousands PTMs are functionally relevant and understanding the mechanisms. Our lab has developed biophysical proteomics methodologies for systematically assessing protein thermal stability and solubility in order to map on a proteome-wide scale how these biophysical parameters change upon perturbation or due to addition of post-translational modifications. The has enabled us to detect changes in protein activities which are not accessible to standard protein concentration measurements and also to understand which PTMs have an effect on biophysical properties of proteins and are thus likely functionally relevant. We will present the most recent results on this topic.

DAY 2 – Tuesday – 18th July 2023
11:30 – 11:50
Dose-dependent and proteome-wide drug response profiling by micro-flow-LC-FAIMS-MS/MS

Stephan Eckert, Nicola Berner, Annika Schneider, Severin Lechner, Sarah Brajkovic, Stephanie Wilhelm and Bernhard Kuster

Chair of Proteomics and Bioanalytics, Technical University of Munich, 85354 Freising, Germany; German Cancer Consortium (DKTK), Partner-site Munich and German Cancer Research Center (DKFZ), Heidelberg

Introduction: Mass spectrometry-based proteomics emerges as a powerful read-out for mechanisms of action studies of drugs. Since drugs act in a dose-dependent manner, this dimension must not be neglected in screening approaches and carries valuable information. Acknowledging this fact, we profiled dose resolved drug perturbed proteomes employing a streamlined workflow based on a micro-flow-LC-MS/MS setup incorporating ion mobility (FAIMS).

Methods: For drug profiling, Jurkat cells were treated with five concentrations of the respective drug for 18 hours followed by standard methods to obtain tryptic peptides. Peptides were analyzed using a 60 min gradient at a flow rate of 50 µL/min on a microflow-LC-FAIMS-MS/MS setup applying a single compensation voltage.

Results: Optimizing the coupling of FAIMS to microflow-LC-MS/MS led to the identification of 7,000 proteins in a one-hour single-shot. We employed this setup to the dose-dependent profiling of 144 clinically relevant drugs representing 13 drug classes. Data analysis showed frequent up- and down-regulation of proteins, enabling characterisation of drugs and clustering based on similar elicited cellular effects. The technique could identify molecular phenotypes induced by several drugs, such as different stages of cell cycle arrest, and the corresponding EC50s (i.e., Paclitaxel: ≈5 nM, Colchicine: ≈30 nM (M-phase-arrest)). Moreover, this unveiled exclusive ribosomal stress induction by Oxaliplatin in contrast to other platin drugs.
Comparing the obtained results to dose-dependent transcriptomics experiments highlighted the importance of investigating drugs on the proteome level. For instance, Decitabine, a nucleoside analogue and inhibitor of the DNA-methyltransferase-1, led to the potent degradation of DNTM1 (EC50= 8 nM). This could exclusively be observed for the protein while the transcript stayed unaffected.
Furthermore, our data revealed downregulation for key components of T-cell receptor signalling (TCR, CD3, LAT etc.) in response to the majority of tested histone deacetylase inhibitor (HDACi). The transcriptomics data was in line with these observations, proposing epigenetic remodelling as the root cause. A T-cell activation assay showed dose-dependent reduction of stimulation suggesting a functional implication of these downregulations. Translating these findings into human primary T-cells could recreate this behaviour. Taken together the data raises concerns of detrimental effects of HDACi treatment on the immune response for the therapy of solid tumours. On the other hand, offering a potential treatment for T-cell receptor signalling driven leukaemia. The presented method offers a simple yet powerful option to improve the understanding of drug mode of action by measuring the potency of induced protein expression changes in response to treatment.

DAY 2 – Tuesday – 18th July 2023
11:50 – 12:10

Mass Spectrometry techniques in PROTAC drug discovery

Frances Rachel Sidgwick,[1] Maria Emilia Dueñas,[1] Sandra Kumper,[2] Markus Queisser,[2] Matthias Trost[1]

[1]Newcastle University, Newcastle University Biosciences Institute, Newcastle upon Tyne, United Kingdom
[2]Glaxo Smith Kline, Stevenage, United Kingdom

Introduction: PROteolysis TArgeting Chimeras (PROTACs) are bi-functional small molecules that select target proteins for degradation. PROTACs are exciting novel drugs that allow targeted degradation of disease-causing proteins. To date, there are limited assays available to measure PROTAC mediated ubiquitin transfer in a high-throughput (HT) manner. We have developed a HT matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS) method to screen different PROTACs, E3 ligases, and target protein combinations as potential drug candidates. Using this method, we screened PROTACs that act 'promiscuously' and target several kinases. Selected PROTACs from our MALDI-TOF MS screen, that were able to deplete mono-ubiquitin over time, were analysed in a cellular-based assay and by full proteome analysis. Using data independent acquisition (DIA) analysis, we were able to identify and quantify PROTAC kinase targets.

Methods: A HT MALDI-TOF assay was developed to measure the depletion of mono-ubiquitin over time using a Bruker's RapifleX MALDI Pharma Pulse MS and a Mosquito-TPP Labtech liquid handling robot. A cellular assay was developed using K562 cells. Samples were tryptic digested and analysed using a Thermo Fisher QE Exactive HF Mass Spectrometer in DIA mode, using a µPAC C18 column.

Preliminary data: Using our optimised MALDI-TOF MS method, we screened four different PROTACs that were synthesised to degrade FAK. These PROTACS have been shown to act more promiscuously and have multiple kinase targets. Three of these PROTACs recruit TRAF4 and one recruits Cereblon, both of which are RING domain E3 ligases. We observed an increased rate in the depletion of mono-ubiquitin over time on the addition of TRAF PROTAC 1 and Cereblon PROTAC 1. Contrary, on the addition of TRAF PROTAC 2 and TRAF PROTAC 3, the rate of depletion of mono ubiquitin over time remained unchanged; this is similar to what is observed when the PROTAC is not present. This shows that TRAF PROTAC 1 and Cereblon PROTAC 1 have a positive effect on the rate of mono ubiquitin depletion.
To determine the kinase targets of these PROTACs, a cellular assay using K562 cells was set up. Cells were incubated with 0.1 µM PROTAC for 18 hours. Cells were then harvested, and a tryptic digest was performed using the S-TRAP method. Digests were then analysed via DIA mass spectrometry on a Thermo Fisher QExactive HF mass spectrometer. Data was analysed using DIA-NN software and monitored for the up and down-regulation of protein groups. The data revealed kinase targets for both PROTAC treatments, showing that the Cereblon recruiting PROTAC does indeed act promiscuously and also targets other kinases including FAK2, Aurora kinase AURKA and tyrosine protein kinases; BTK and TEC.

DAY 2 – Tuesday – 18th July 2023
SESSION 3 – STRUCTURAL PROTEOMICS & PROTEIN COMPLEXES

11:00 – 11:30

Uncovering the structural dynamics of membrane proteins and their complexes

Aygiris Politis

University of Manchester, Manchester, UK

Here, we investigate the molecular mechanisms underpinning membrane protein structure and function using the emerging hydrogen deuterium exchange-mass spectrometry (HDX-MS) technology. In my group, we have recently established the study of lipid-mediated conformational dynamics in membrane transporters. Currently our interests lie in exploring how the lipid environment and pharmacologically important ligands shape the conformational landscape of membrane proteins and their complexes, including transporters and G-protein coupled receptors, important for human health and disease. Moreover, we develop associated biophysical and biochemical tools to visualise and interpret HDX-MS data including strategies to dissect co-populated states of membrane proteins identified by HDX-MS.

DAY 2 – Tuesday – 18th July 2023
11:30 – 11:50

The RBPome of influenza A virus mRNA reveals a role for TDP-43 in viral replication

Maud Dupont, Tim Krischuns, Quentin Giai-Gianetto, Sylvain Paisant, Stefano Bonazza, Jean-Baptiste Brault, Thibaut Douché, Joel I Perez-Perri, Matthias W Hentze, Stephen Cusack,Catherine Isel, David G Courtney, Nadia Naffakh, **Mariette Matondo**

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Recent technical advances, particularly in mass spectrometry-based proteomics, have significantly improved our understanding of the RNA-binding protein (RBP) repertoire present within eukaryotic cells, with particular emphasis on RBPs that interact with cellular polyadenylated mRNAs. However, recent studies using the same technologies have distinguished the RBP interactome of viral mRNAs, including SARS-CoV-2, revealing both similarities and differences between the RBP profiles of viral and cellular mRNAs.

In our study, we combined recent protein-RNA crosslinking, RNA interactome capture using a sequence-specific antisense RNA probe with quantitative high-resolution mass spectrometry-based proteomics . Additionally, dedicated bioinformatics and statistical analyses were used to identify the RBP proteins that are associated with the NP mRNA of an influenza A virus (IAV).. Our study allowed us to identify 51 RBP proteins in infected human cells that interact directly with the NP mRNA of an influenza A virus.

By combining our proteomic data with loss-of-function experiments, we discovered cellular factors important for productive viral infection, among which the host protein TDP-43 is recruited by the transcribing viral polymerase through an RNA-independent interaction between FluPol and TDP-43. Overall, our data provide the first insights into the composition and function of influenza mRNPs and reveal a critical role for viral transcriptase in the recruitment of cellular RBPs to viral mRNPs.

DAY 2 – Tuesday – 18th July 2023
11:50 – 12:10

Phosphorylation-linked complex profiling identifies assemblies required for Hippo signal integration

Federico Uliana,*[1] Rodolfo Ciuffa,*[1] Ranjan Mishra [1], Andrea Fossati [1], Fabian Frommelt [1], Sabrina Keller [1], Martin Mehnert [1], Eivind Salmorin Birkeland [1], Frank van Drogen [1], Nevena Srejic [1], Matthias Peter[1], Nicolas Tapon[2], Ruedi Aebersold [1], Matthias Gstaiger [1]

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Introduction
While several computational methods have been developed to predict the functional relevance of phosphorylation sites, experimental analysis of the interdependency between protein phosphorylation and Protein-Protein Interactions (PPIs) remains challenging. Here, we describe an experimental strategy to establish interdependencies between protein phosphorylation and complex formation. For this purpose, we used as model YAP1, a transcriptional co-activator for the control of organ size and tissue homeostasis that is highly phosphorylated and among the most connected proteins in human cells (signaling hub protein).

Methods
The experimental strategy is based on three main steps: (i) systematically charting the phosphorylation landscape of a target protein; (ii) assigning distinct proteoforms of the target protein to different protein complexes by native complex separation (AP-BNPAGE) and protein correlation profiling; and (iii) analyzing proteoforms and complexes in phospho-null mutants and in cells lacking regulators of the target protein.

Results
Using YAP1 as a model, we integrated multiple proteomics layers to study (i) the role of phosphorylation for complex formation, (ii) how phosphorylation and complex formation are controlled by known pathway effectors, and (iii) how phenotypes could emerge from perturbing these signaling mechanisms.
Through our study, we identify multiple YAP1 phosphosites associated with distinct complexes and inferred how both are controlled by Hippo pathway members. We detected a PTPN14/LATS1/YAP1 complex and suggest a model how PTPN14 inhibits YAP1 via augmenting WW domain dependent complex formation and phosphorylation by LATS1/2.
This proteomics-based approach can be generalized to study functional relationship of two critical mechanisms of cell signaling: PTMs and complex formation. This strategy can be readily adapted to screen the effect of different types of PTMs (i.e., ubiquitination) on a wide range of proteins that partition into multiple complexes.

DAY 2 – Tuesday – 18th July 2023
SESSION 4 – POST-TRANSLATIONAL MODIFICATIONS

14:00 – 14:30

The role of protein methyltransferases in cancer plasticity and resistance to therapy, through the proteomics investigation of ovarian cancer preclinical models and clinical samples

M. Carminati[1,2], M. Maniaci[2], A. Dondi[2], A. Vai[1,2], C. Pozzi,[2], R Noberini,[2] and **Tiziana Bonaldi[1,2,3]**

[1]European School of Molecular Medicine (SEMM), Milano- Italy; [2]Department of Experimental Oncology, European Institute of Oncology IRCCS, Milano- Italy; [3]Department of Oncology and Hematology-Oncology, University of Milan, Milan -Italy

A large number of site-specific post-translational modifications both on histones (hPTMs) and on non-histonic proteins contribute to gene expression plasticity, DNA repair and adaptive response in cancer. Among them, protein K/R-methylation have gained momentum, due to their implications not only in transcription, RNA processing, and signal transduction cascades, but also in many stress response and cancer-immunity processes. Histone- and protein-methyltransferases and de-methylases are generally considered innovative targets for cancer therapy and several inhibitors are currently being tested in clinical trials. Hence, new analytical platforms enabling the functional understanding of histone methyltransferase activity and their outcome in terms of histone and non-histone methylation patters in healthy and cancer samples -and in response to different external cues- are highly needed.

Quantitative mass spectrometry (MS) has become the elective technology both in dissecting the histone code and in modification-proteomic profiling of health and disease states. The unbiased view offered by MS-based proteomics allowed gaining a broader perspective on the extent and function of epigenetic modifications on chromatin and beyond. We have pioneered this field by setting-up distinct MS-proteomics strategies for epigenome mapping of cancer patient samples to identify both novel histone PTMs signatures with potential as biomarker and new mechanisms underpinning cancer plasticity, heterogeneity and response to therapy and for the exploration of the methyl-proteome beyond chromatin, its plasticity upon epigenetic therapy and its functional implication in adaptive response to genotoxic stress in ovarian cancer.

DAY 2 – Tuesday – 18th July 2023
14:30 – 14:50

Proteomic basis for understanding the molecular mode of action of clinical ATR kinase inhibitors and their combination with gemcitabine in pancreatic cancer cells

Stefanie Höfer,[1] Larissa Frasch,[1] Teresa Rogler,[1] Florian Bayer,[1] Matthew The,[1] Kerstin Putzker,[2] Joe Lewis,[2] and Bernhard Kuster[1,3]

[1]Chair of Proteomics and Bioanalytics, Technical University of Munich, Freising, Germany; [2]Chemical Biology Core Facility, EMBL Heidelberg, Heidelberg, Germany; [3]Bavarian Biomolecular Mass Spectrometry Center (BayBioMS), Technical University of Munich, Freising, Germany

Introduction

In pancreatic adenocarcinoma (PDAC), the DNA-damaging agent gemcitabine (GEM) is used as first-line chemotherapy, but chemo-resistance is frequently observed. One potential way to overcome GEM resistance is the combination with kinase inhibitors. To date, the EGFR inhibitor erlotinib is the only kinase inhibitor approved in combination with GEM, but other targeted agents are being clinically investigated. Here, we apply a novel phosphoproteomic approach called decryptM to characterize kinase inhibitors in DNA-damaged PDAC cells to investigate the mechanistic rationale for the use of GEM-based combinations with targeted therapy in PDAC patients.

Methods

To identify active drug combinations, we screened for drug synergy between GEM and 146 targeted agents in a panel of 13 human PDAC cell lines. Dilution series of drugs were tested in combination with two doses of GEM (2x11 dose matrix). Drug synergy was assessed based on shifts in AUC and EC50. For decryptM experiments, AsPC-1 cells were incubated with GEM to induce DNA damage, followed by treatment with increasing concentrations of selected kinase inhibitors. Samples were processed using SP3 magnetic beads, and each drug dose was labeled using tandem mass tags (TMT). The pooled sample was fractionated using basic-reversed phase chromatography and pooled to 12 fractions, followed by IMAC enrichment of phosphopeptides and nano-LC-MS/MS.

Results

From all targeted drugs, the ATR kinase inhibitor (ATRi) elimusertib showed the strongest synergy with GEM in 11 out of 13 PDAC cell lines. ATR is a key regulator of cellular DNA damage response (DDR), and the combination of ATRi and GEM is currently under early clinical investigation. DecryptM experiments with elimusertib resulted in the quantification of >20,000 phosphorylation sites, of which >200 sites were regulated by the ATRi in a dose-dependent fashion. Potential ATR substrates containing the pSQ/pTQ motif were found to be heavily downregulated, indicating direct target engagement and inhibition of DDR in cells. Regulation of these sites occurred at lower doses of ATRi compared to sites involved in other signaling pathways, such as cell cycle control (mean EC50<100nM vs >500nM). Moreover, we found that most, but not all of these sites are inversely regulated by GEM monotherapy, and we hypothesize that this may explain the observed synergy. Taken together, this phosphoproteomic study characterizes ATRi in the context of GEM-induced DNA damage and may help to further rationalize the clinical use of this drug in combination in PDAC patients.

DAY 2 – Tuesday – 18th July 2023
14:50 – 15:10

A histone PTM-centered, multi-OMICs approach to dissect aberrant epigenetic mechanisms in triple negative breast cancer

Noberini R.,[1] Robusti G.,[1] Vai A.,[1] Pece S.,[1] Pavesi G.,[2] and Bonaldi T.[1,3]

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Introduction

Although cancer was long considered a genetic disease, the contribution of epigenetics to various aspects of cancer biology is now being increasingly recognized. Aberrations in the deposition and maintenance of epigenetic features, including histone post-translational modifications (PTMs) and histone variants, can result in the inappropriate expression or silencing of genes, potentially leading to cancer. Histone PTMs can be investigated as biomarkers for patient stratification, and as key players in aberrant epigenetic mechanisms potentially targetable for therapy.

Methods

Here, we applied quantitative mass-spectrometry-based technologies based on a super-SILAC approach to study histone PTMs and variants in different breast cancer subtypes, with a special focus on triple-negative breast cancers (TNBCs), which comprise a heterogeneous group of tumors lacking well-defined molecular targets and targeted therapies. We then investigated the mechanistic role of histone PTM changes through a multi-OMICs approach involving the integration of epigenomics, transcriptomics, and proteomics data from matched breast cancer samples and public repositories.

Results

After developing a battery of mass spectrometry-based approaches that allow the comprehensive and quantitative analysis of up to 105 histone modified peptides from clinical samples, we profiled >200 cancer patient tissues of different origin. By comparing tumor and normal tissues for various cancer models, we identified histone modification changes that represent general hallmarks of cancer, in addition to those previously reported, as well as tumor- and subtype-specific changes. Moreover, the MS- profiling of histone PTMs in different breast cancer subtypes revealed a panel of epigenetic marks that separate TNBCs from the other subtypes, which includes changes in H3K4me2, H3K9me3, and H4K20me3, and that distinguish TNBC patients with different outcomes after adjuvant chemotherapy. Among them, we focused on investigating mechanistic role of H3K4 methylation through a multi-OMICs approach, and found that the presence of H3K4me2 at promoters and super-enhancers in TNBC samples drives the expression of genes associated with the TNBC aggressive phenotype.

DAY 2 – Tuesday – 18th July 2023
15:10 – 15:30

PTMeXchange: Globally harmonized re-analysis and sharing of data on post-translational modifications

Kerry A. Ramsbottom[1], Ananth Prakash[2], Yasset Perez Riverol[2], Oscar Martin Camacho[1], Maria-Jesus Martin[2], Juan Antonio Vizcaíno[2], Eric W. Deutsch[3], and Andrew R. Jones[1]

[1]University of Liverpool, [2]EMBL-European Bioinformatics Institute (EMBL-EBI), [3]Institute for Systems Biology

Introduction: Post-translational modifications (PTMs) play an important role in e.g. cell signalling, protein function and disease processes. Mass spectrometry (MS) and database searching are commonly used to detect and localise modification sites on proteins, with confidence governed by peptide-spectrum match (PSM) and PTM localisation statistics. The aim of the PTMeXchange is to re-analyse public PTM enriched proteomics datasets, focusing on accurate PTM localisation, for human and the main model organisms. Integrating data across studies and disseminating the data to UniProtKB, linking it to the original MS evidence in PRIDE and PeptideAtlas.

Methods: Using an open data analysis pipeline, Trans Proteomic Pipeline (TPP), we developed a statistical method to ensure confident PTM localisation. We tested several decoy amino acids for false localisation rate (FLR) control, allowing us to estimate and threshold global FLR, ensuring that only high-quality PTM data is reported. We also developed an empirical method for controlling FLR inflation when combining multiple datasets. The final step was integrate the "PTM build" data into public databases.

Results: We showed searching for phosphorylation on alanine (pA) gave the best modelled false positives on target amino acids (STY). We therefore propose the "pASTY" method when preforming PTM enrichment studies to estimate and threshold global FLR. When combining multiple datasets to generate a species PTM build, we use a "Gold/Silver/Bronze" (GSB) classification of sites to control FLR inflation. Where the Gold category shows FLR well below 1%, Silver is <3% and Bronze is all sites reported at <5% FLR in any dataset. We have multiple PTM builds completed for phosphorylation in different species; Human, Mouse, Plasmodium, Rice and Arabidopsis. We have also prototyped PTM builds for more challenging modifications including ubiquitin, SUMOylation and lysine acetylation. The first "proof of concept" build, for the rice phosphoproteome, is already available in UniProtKB, with others planned to be included in 2023 releases. This allows users to visualise the PTMs in the Protein Record pages, including the feature viewer, where sites can be observed alongside SNPs, domain and other proteomics evidence and linked to protein structures/AlphaFold2 predictions. Each build is also integrated into PRIDE and/or PeptideAtlas. Universal Spectrum Identifiers are available for all spectra. This infrastructure provides multiple routes for PTM data access with a full evidence trail. We are currently working on community guidelines to allow users to generate similar PTM builds, providing a gold standard for making PTM build data available to the wider community.

DAY 2 – Tuesday – 18th July 2023
SESSION 5 – BIOINFORMATICS & DATA PROCESSING

14:00 – 14:30

Deep learning-assisted mass spectrometry-based proteomics

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Various deep learning-assisted data analysis approaches developed recently have demonstrated to boost the performance of mass spectrometry-based proteomics in recent years. One such model, Prosit, a deep neuronal network trained on synthetic peptides generated in the ProteomeTools project, has been used to predict >9 billion spectra since its release in 2019. Here, we summarize the available Prosit models for peptide property prediction and specifically highlight recent developments to generate a new single model covering tryptic and non-tryptic, modified and unmodified, and labeled and unlabeled peptide fragment intensity prediction for various mass analyzers and fragmentation methods. The application and impact of this model when using it for the reanalysis of public data is shown on various datasets covering different organisms, sample complexities and modifications, demonstrating that deep-learning assisted data analysis is able to increase the confidence and coverage of proteomics experiments. The application of deep learning in proteomics is still in its early stages and holds the potential to boost its performance even more. To facilitate this development, while ensuring reproducibility and FAIRness, we developed a number of open-source packages relevant for the development and application of deep learning models in proteomics. One of which is DLOmix, a python framework tailored towards training and evaluating deep learning models in proteomics. We used DLOmix to train and evaluate Prosit, which can be reused by the community to retrain our models with data from ProteomeTools, to develop custom models or compare the performance of different models in a controlled environment. We believe that such software ecosystems can be a key factor in avoiding an impeding reproducibility crisis.

DAY 2 – Tuesday – 18th July 2023
14:30 – 14:50

The Role of Deep Learning Spectra Libraries in DIA-based Differential Expression Analysis

Witold Wolski, Christian Panse, Ludwig Lautenbacher, Jonas Grossmann, Gerhard Duernberger, Tobias Jumel, Mathias Wilhelm and Ralph Schlappbach

Swiss Institute of Bioinformatics, Swiss Federal Institute of Technology - ETH-Zurich, Technical University of Munich, University of Zurich, Gregor Mendel Institute Vienna, Max Planck Institute of Molecular Cell Biology and Genetics, Technical University of Munich, Functional Genomics Center Zurich

Introduction: Label-free data-independent acquisition (DIA) is the to-go method for label-free proteomics differential expression experiments. New developments in computational analysis of DIA data substantially contributed to the method's popularity. We conducted a comprehensive parameter sensitivity analysis to assess the impact of some of the data analysis components on the performance of differential expression analysis (DEA). For instance, we generated spectra libraries by proteome-wide prediction of peptide tandem mass spectra using deep learning models (DLM) made available by the DLOmix project. The results presented are essential to the users and developers of the software to analyze DIA data.

Methods: The Tripleproteome LFQBench dataset is a complex mixture of proteins from three different organisms: yeast, human, and E. coli. Two combinations of the proteomes result in the ratio “1:2” for yeast and “4:1” for E.coli. Tobias Jumel of the MPI-CBG measured the samples in technical replicates on a QExactive instrument. Furthermore, human and yeast proteomes were mixed in three combinations, resulting in a ratio of 0:3:10 for the yeast proteome. These samples were measured on an Eclipse / FAIMS instrument by the Max Perutz Labs Mass Spectrometry Facility.

For predicting MS2 spectra, we use Koina, a generic, open-source, and online-accessible prediction service(https://github.com/wilhelm-lab/koina), allowing predictions from the Prosit, AlphaPept, and MS2PIP models. We used rawDiag (https://doi.org/10.1021/acs.jproteome.8b00173) to determine the collision energy of the experimental data. As input, we use the tryptic-digested peptide sequences of the Tripleproteome and generate a spectrum library readable by the DIANN and Spectronaut software, drawing upon the expertise obtained when developing the specLib R package (https://doi.org/10.1093/bioinformatics/btv105). To compute the benchmarks, we extended our methods to compare R packages for modeling DEA experiments (https://doi.org/10.1021/acs.jproteome.2c00441). However, this time, we contrasted the upstream software and perturbed their input parameters while we fixed the method to compute the DEA statistics.

Results: We examined how new software developments, specifically spectra libraries generated using DLMs, and differences among the FragPipe, DIANN, and Spectronaut software impact proteomics DEA performance. Furthermore, we investigated how to generate the best spectra libraries from DLMs. To contrast the results, we are developing an R application based on the prolfqua package for benchmarking proteomics DEA software pipelines which creates a dashboard visualizing various benchmark metrics to simplify the comparison of software versions and perturbations of input parameters.

DAY 2 – Tuesday – 18th July 2023
14:50 – 15:10

ProteoBayes: a Bayesian framework for differential proteomics analysis

Marie Chion, Arthur Leroy

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Introduction

Current statistical methods in differential proteomics analysis generally leave aside several challenges, such as missing values, correlations between peptides' intensities and uncertainty quantification. Moreover, they provide point estimates, such as the mean intensity for a given peptide or protein in a given condition. The decision, whether an analyte should be considered as “differential”, is then based on the comparison of the p-value to a significance threshold, usually 5%.

In the state-of-the-art “limma” approach of Smyth (2004) and its extension by Chion et al. (2022) to the multiple imputation framework, a hierarchical model is used to deduce the posterior distribution of the variance estimator for each analyte. The expectation of this distribution is then used as a moderated estimation of variance and is injected directly into the expression of the t-statistic. However, instead of merely relying on the moderated estimates we could provide more powerful and intuitive results by leveraging a fully Bayesian approach and hence allow the quantification of uncertainty (Crook et al., 2022).

Methods

The present work follows a similar idea by taking advantage of standard results from Bayesian inference with conjugate priors in hierarchical models to derive a methodology tailored to handle multiple imputation contexts. Furthermore, we aim to tackle the more general problem of multivariate differential analysis, to account for possible correlations between analytes.

The developed model was a applied to a real dataset from Muller et al. (2016). The experiment involved six peptide mixtures, composed of a constant yeast (Saccharomyces cerevisiae) background, into which increasing amounts of UPS1 standard proteins (48 recombinant human proteins, Merck) were spiked at 0.5, 1, 2.5, 5, 10 and 25 fmol, respectively. Data acquisition was performed on a nanoLC-MS/MS coupling composed of a nanoAcquity UPLC device (Waters) coupled to a Q-Exactive Plus mass spectrometer (Thermo Scientific).

Results

By defining a hierarchical model with prior distributions on both mean and variance parameters, we achieve a global quantification of the uncertainty for differential analysis. Inference is thus performed by computing the posterior distribution for the difference in mean peptide intensities between two experimental conditions.

In contrast to more flexible models that can be achieved with hierarchical structures, our choice of conjugate priors maintains analytical expressions for directly sampling from posterior distributions without requiring expensive MCMC methods.

DAY 2 – Tuesday – 18th July 2023
15:10 – 15:30

Advanced bioinformatics tools for analysing metaproteomics data

Robert Heyer,[1,2] Daniel Walke,[3] Emanuel Lange,[1] Maximilian Wolf,[2] Kay Schallert[1]

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Metaproteomics enables the comprehensive analysis of microbiomes required to study human health as well as many environmental and biotechnological processes. Thanks to continuous advances in experimental workflows and mass spectrometry, the resolution of metaproteomics analysis has improved significantly, generating more protein identifications and, thus, a more complete picture of the microbial phenotypes. Researchers use the protein lists to reconstruct the taxonomic and functional composition of different microbiomes, conduct statistical tests to reveal significant fold changes, and map them to metabolic pathways to elucidate underlying mechanisms. However, due to the large quantity of data and the inherent complexity of microbiomes with hundreds of species, automated analysis of metaproteomics data is challenging and requires sophisticated bioinformatics approaches. The MetaProteomeAnalyzer software was developed in 2015 to enable protein identification from MS data, sophisticated grouping of homologous proteins, and the taxonomic and functional evaluation of results. Recently, we developed a new version of the MetaProteomeAnalyzer using cloud technology. The tool scales with the amount of data generated and is user-friendly accessible via a web interface. It was developed as a web application in Angular, empowered by REST services programmed in Java as well as a neo4J and a Cassandra database for data storage and organization. In addition, we have explored the use of deep learning approaches for PSM validation, which can mitigate shortcomings of the current PSM validation by calculating the false discovery rate.

A further tool, the MPA_Pathway_Tool, addresses the need to reconstruct the metabolic phenotype from metaproteomics data. It allows users to map protein identifications to predefined molecular networks and to modify or create entirely new networks. Another feature of the MPA_Pathway_Tool is to use experimental metaproteomics data to increase the precision of metabolic models, which could increase the precision of an Escherichia coli metabolic model by a factor of five. Based on this approach, it is possible to constrain the microbiome's metabolic phenotype, enabling a more precise microbiome simulation and optimization.

Conclusion: Our tools facilitate and accelerate the data evaluation of (meta)-proteomics data.

DAY 2 – Tuesday – 18th July 2023
SESSION 6 – TOP DOWN PROTEOMICS & NEW TECHNOLOGIES/METHODS

16:00 – 16:30

Case Studies of Proteoforms in Human Health and Disease

Julia Chamot-Rooke

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Proteins are the central intermediaries between genotype and phenotype. It is not possible to understand the functioning of a biological system if one does not know what protein molecules are present, as well as the nature and abundances of their proteoforms. In a recent paper[1], the Consortium for Top-Down Proteomics highlighted five important disease areas illustrating the critical role of proteoforms in disease and health: neurodegeneration, cardiovascular health, infectious disease, cancer and immunology.

After a short introduction on the concept of proteoforms and the challenges associated with their analysis, examples showing how disease-driven research can be advanced by discovery of proteoforms and their post-translational modifications will be highlighted. These examples concern applications in the field of infectious diseases[2] and clinical immunology[3]. Recent results obtained on a new instrumental platform (Q-Exactive HF modified with an Omnitrap[4] and equipped with a FTMS Booster) will also be introduced.

1. Smith, L. M. et al. The Human Proteoform Project: Defining the human proteome. Sci Adv 2021, 7 (46), eabk0734.
2. Dupre, M. et al. Optimization of a Top-Down Proteomics Platform for Closely Related Pathogenic Bacterial Discrimination. J Proteome Res 2021, 20 (1), 202-211.
3. Dupre, M. et al. De Novo Sequencing of Antibody Light Chain Proteoforms from Patients with Multiple Myeloma. Anal Chem 2021, 93 (30), 10627-10634.
4. Papanastasiou, D. et al. The Omnitrap Platform: A Versatile Segmented Linear Ion Trap for Multidimensional Multiple-Stage Tandem Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2022, 33(10), 1990–2007.

DAY 2 – Tuesday – 18th July 2023
16:30 – 16:50

The challenges of top-down proteomics and true proteoform characterization

Allen Po, Claire Eyers

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Introduction

Top-down proteomics is the study of proteins and their proteoforms, a term that describes the complexity of different protein forms that can arise from a single gene, introduced by truncations, post-translational modifications (PTMs), alternative splicing and/or genetic variation. Intact protein analysis in theory can define these proteoforms, providing a holistic view of a proteome. However, such analyses are far from trivial. From the ~20,000 human genes, the number of proteoforms is estimated to be anywhere between 1 million and 6 million different species, considering the combinatorial diversity of different isoforms, single nucleotide polymorphisms (SNPs) and PTMs. While these are unlikely to all co-exist, there is no doubt that proteoform heterogeneity is extensive, with hundreds of thousands of proteoforms being present in a cell at the same time, each having defined, and possibly unique, biological roles. Much progress has been made in the field of top-down proteomics over the last ~20 years, although there remain numerous challenges particularly for the robust characterization of proteoforms larger than ~30 kDa and specifically, in the discrimination and localization of PTMs. To truly understand the physiological and disease-specific roles of distinct proteoforms, such information is essential.

We introduce the multiplexed proteoform challenge: the analytical and computational issues that remain to be overcome to allow true proteoform characterization where both the types and sites of modification can be defined. Specifically, we will highlight the challenges associated with kindred proteoforms that may contain an abundance of potential 'action sites', as they exist in a complex and heterogenous population.

Methods

Direct infusion top-down experiments were conducted on a Thermo Fisher Orbitrap Fusion Lumos mass spectrometer, and data analysis and processing were done using ProSightPD and TDAcquireX. The system model used for the study was the recombinant catalytic subunit of cAMP-dependent protein kinase A (PKAc).

Results

The top-down study of PKAc using direct infusion experiments shows that complete and unambiguous characterisation of the proteoforms that exist within its proteoform population often remains incomplete, and will contain "floating" mass modifications that lack localisation evidence. Due to the combinatorial effects of top-down analyses, mass shifts with no fragmentation evidence of localisation are at best placed in regions that contain modifiable residues, termed "action sites". The increase in number of PTMs and number of action sites adds to the complexity of the combinatorial effects, and further limits our ability to eventually characterise unique proteoforms' functionalities.

DAY 2 – Tuesday – 18th July 2023
16:50 – 17:10

Paleoproteomics & Metaproteomics exploration of unique historical samples

Jean Armengaud, Mélodie Kielbasa, Guylaine Miotello, Olivier Pible

CEA, ProGénoMix platform

Introduction

How microorganisms interact with each other, with their hosts, and with their environment are key questions addressed by metaproteomics (Armengaud 2023). The study of proteins from these particularly complex samples provides four types of answers: 1) identification of the taxa present, 2) quantification of the biomass of these organisms, 3) identification of the proteins of these taxa and their function, and 4) overall functional representation of the biological system. Paleoproteomics is the science of ancient samples. The best of both worlds is needed for characterizing ancient and complex samples that are only available in minute quantities.

Methods

For some historical artifacts, metagenomics cannot be performed due to the limited amount of material and the level of degraded DNA. To circumvent this time-consuming step, the list of organisms actually present in the sample can be established on the basis of the most reliable taxon-specific peptides. To do so, our proposal relies on an unbiased search of an extensive generic database, followed by a peptide search restricted to the most representative organisms.

Results

This forensic style approach will be illustrated by very "unique" samples such as dental remains of monks from the 16th century for which traces of coronavirus were identified (Oumarou Hama et al., 2023), cardiac relics of a blessed woman for which no trace of embalming were evidenced (Bourdin et al., 2023), as well as with samples from very hostile environments such as nuclear storage pools (Pible et al., 2023). The challenges in terms of mass spectrometry, interpretation and exploitation of the results will be discussed.

References

Armengaud J (2023) Metaproteomics to understand how microbiota function: the crystal ball predicts a promising future. Env Microbiol 25(1):115-125.

Oumarou Hama H, Chenal T, Pible O, Miotello G, Armengaud J, Drancourt M (2023) An ancient coronavirus from individuals in France, circa 16th century. International Journal of Infectious Diseases. 131:7-12.

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Bourdin V, Charlier P, Crevat S, Slimani L, Chaussain C, Kielbasa M, Pible O, Armengaud J (2023) Deep paleoproteotyping and microtomography revealed no heart defect nor traces of embalming in the (cardiac relics of Blessed Pauline Jaricot. International Journal of Molecular Sciences, 24(3):3011.

DAY 3 – Wednesday – 19th July 2023
SESSION 7 – COMPUTATIONAL PROTEOMICS & BIG DATA

09:00 – 09:30

Open-minded exploration of large proteomics datasets

Alexey Nesvizhskii

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I have been fascinated with the subject of the “dark proteome” since my entry in the field of proteomics in 2001. What are all those spectra that we cannot identify in a typical search, and how do we design efficient computational strategies to move beyond standard peptide identification searches. To this end, we have developed a highly efficient fragment ion indexing algorithm and implemented it in MSFragger, which has become a widely used tool and the engine behind our FragPipe computational platform. MSFragger “open” and “mass offset” searches have empowered new strategies for faster and more sensitive identification of biologically or chemically modified peptides. Here I will describe new and improved algorithms, including localization-aware open search and new methods for the identification of N- and O-linked glycopeptides, labile PTMs, and chemically labeled peptides in chemoproteomics experiments. I will then illustrate the power of fragment ion indexing in reanalysis of large-scale proteomics and peptidomics datasets. I will also provide an overview of the various quantification workflows (DIA, TMT, LFQ-MBR) available in the latest FragPipe 20 release (June 2023).

DAY 3 – Wednesday – 19th July 2023
09:30 – 09:50

Koina: Bringing machine learning to the community

Ludwig Lautenbacher,[1] Wassim Gabriel,[1] Dulguun Bold,[1] Tobias Schmidt,[2] Marco Schmidt,[3] Tobias Kockmann,[3] Christian Panse,[3,4] Mathias Wilhelm[1]

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Introduction

Recent developments in machine learning (ML) and deep learning (DL) have proven immense potential for applications in proteomics, such as optimized spectral library generation or improving peptide identification. Although new ML/DL models for various applications and peptide properties are frequently published, only few are readily available and thus adopted by the community. Two models that are widely used are Prosit and MS2PIP. We attribute this success not only to the excellent prediction accuracy, but also to the existence of user-friendly web servers. To make full use of state-of-the-art models, ease of use and reusability in existing packages is required. To facilitate this, we developed the model hosting platform Koina, a generic, open-source, and online-accessible prediction service that enables ML/DL model usage in any pipeline by serving ML/DL models.

Methods

Koina is based on Nvidia Triton, a state-of-the-art open-source model hosting platform that can be used to retrieve predictions from ML models trained with a variety of frameworks. Source code for Koina is available at github.com/wilhelm-lab/koina. A Docker image is provided to streamline deployment of new servers.

Results

We developed Koina with a focus on incorporating the FAIR4RS principles, to improve findability, accessibility, interoperability, and reusability of ML models. To improve model interoperability, we defined a common interface based on the ProForma PSI. Requests can be sent via either HTTP for ease of use or gRPC when performance is critical, ensuring optimal accessibility for various use cases. A rich documentation is available summarizing model capabilities and limitations, improving model findability, as well as ensuring that models can be reused effectively within the limitations of their development. Multiple public servers already hosted Koina, whereas koina.proteomicsdb.org serves as the central access point and distributes load across all connected public instances, resulting in an estimated <100 ms latency across Europe.

We believe that the open-source and federated design will lead to widespread community adoption, by developers and users alike, potentially resulting in Koina becoming a common standard for publishing ML models in proteomics and thus facilitating model adoption and supporting model performance comparison. To illustrate its flexibility, we already provide access to popular ML models, such as AlphaPept (PyTorch), DeepLC (TensorFlow), MS2PIP (XGBoost), and Prosit (TensorFlow). We invite all developers of ML models to improve the impact of their work by adding their model to Koina.

DAY 3 – Wednesday – 19th July 2023
09:50 – 10:10

Improved immunopeptidome analysis using timsTOF fragment ion intensity prediction

Charlotte Adams, Wassim Gabriel, Kris Laukens, Mathias Wilhelm, Kurt Boonen, **Wout Bittremieux**

University of Antwerp, Technical University of Munich, University of Antwerp, Technical University of Munich, University of Antwerp, University of Antwerp

Introduction: Immunopeptidomics plays a crucial role in identifying targets for immunotherapy and vaccine development. Because immunopeptides are generated from their parent proteins in an unpredictable manner, rather than being able to use known digestion rules, every possible protein subsequence needs to be considered. This leads to an inflation of the search space and results in a low spectrum identification rate. Rescoring is a powerful enhancement of standard database searching that boosts the spectrum identification sensitivity and accuracy by unlocking the intensity dimension of MS/MS spectra with peptide fragment intensity predictions.
The high sensitivity of a timsTOF instrument makes it ideal for detecting immunopeptides that are present at relatively low abundances. To improve the identification rate and the reliability of immunopeptidomics experiments performed using timsTOF instruments, we developed an optimized fragment ion intensity prediction model based on Prosit.
Methods: We analyzed over 300,000 synthesized non-tryptic peptides from the ProteomeTools project on a TimsTOF-Pro (Bruker, Bremen). The spectra were searched using MaxQuant (version 2.1.2.0) at 1% PSM-level FDR. The 277,781 obtained PSMs (93,227 non-tryptic measured in this study and 184,554 previously published tryptic peptide PSMs) were split into training (80%), validation (10%), and test (10%) sets. The training set was used to fine-tune the existing Prosit fragment intensity prediction model, the validation set to control for overfitting with early stopping, and the test set to evaluate the model.
We reprocessed immunopeptidomics timsTOF data from a recent study using MaxQuant (version 2.0.3.1) and rescored all proposed PSMs by integrating the fragment intensity predictions.
Results: Comparison of the previously published and the here developed Prosit models showed a substantially improved normalized spectral contrast angle (SA) between predicted and experimental spectra for non-tryptic peptides (SA ≥ 0.9 for 2.4% vs 26.3% of spectra, respectively) and for tryptic peptides (SA ≥ 0.9 for 0.2% vs 42.1%). To evaluate whether rescoring with our model is able to increase the identification rate we reprocessed public HLA Class I and Class II immunopeptidome data. Similarly to what was observed previously on Orbitrap instruments, incorporating our model into the database matching process increased the spectrum identification rate of immunopeptides measured on a timsTOF. Compared to MaxQuant, we identified 3.0-fold more HLA class I peptides and 1.7-fold more HLA class II peptides after rescoring. To evaluate the clinical relevance of rescoring, we will look for peptides exclusively presented by tumors. We hypothesize that rescoring results in an increased reliability and identification rate of neoepitopes.

DAY 3 – Wednesday – 19th July 2023
SESSION 8 – SUBCELLULAR & SPACIAL PROTEOMICS

11:00 – 11:30

Spatially resolved phosphoproteomics reveals recycling endosomes as signalling hubs that coordinate Receptor Tyrosine Kinases (RTKs) responses

Michael P. Smith*, Harriet P. Ferguson*, Joanna Watson*, Jennifer Ferguson, Jess Price, Kathy Bexley, Paul Fullwood, Jean-Marc Schwartz, **Chiara Francavilla#**

The University of Manchester, United Kingdom, #DTU Bioengineering, Denmark, *equal contribution

Trafficking routes after internalization regulate the signalling outputs of ligand-bound Receptor Tyrosine Kinases (RTKs), such as Fibroblast Growth Factor and Epidermal Growth Factor Receptors (FGFR2, EGFR). RTK recycling to the plasma membrane counterbalances lysosome-mediated receptor degradation, induces sustained signalling activation, and regulates cell proliferation and motility. Dysregulated recycling and/or signalling are associated with human diseases, such as breast cancer. However, it is not known through which signalling players recycling endosomes fine-tune downstream responses.

Here, we used FGFR2b as a model system to study signalling modules which depend on the presence or on the permanence of RTKs in the recycling endosomes upon ligand stimulation. We combined traditional Mass Spectrometry-based quantitative phosphoproteomics with the Spatially Resolved Phosphoproteomics (SRP) approach - which detects signalling proteins in proximity of FGFR2b during receptor recycling - bioinformatics, imaging, and functional assays in breast cancer cells.

We showed that recycling endosomes integrate the signalling outputs of FGFR2b and of EGFR in response to their respective ligands. Furthermore, FGFR2b signalling partners in the proximity of recycling endosomes which regulated autophagy, cell growth, and stress responses were identified only by the SRP approach. Therefore, SRP could revolutionize the way to study how signalling architecture changes depending on the spatio-temporal regulation of RTKs.

Overall, these findings increase our scarce understanding of recycling endosomes as fine-tune regulators of signalling outputs and reveal how manipulating specific signalling players during receptor recycling maintains cell survival, such as in breast cancer cells.

DAY 3 – Wednesday – 19th July 2023
11:30 – 11:50

Unveiling the health benefits of muscle contraction: Proteomics of circulating extracellular vesicles

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Physical activity has consistently shown to be strongly associated with a lower risk of chronic diseases, including cancer. Among the various mechanisms by which muscle contraction can exert its health effects, the release of extracellular vesicles (EVs), which carry bioactive molecules capable of modifying the behaviour of recipient cells, has gained considerable attention. This study aims to add insights on the health effects of muscle contraction by analysing the proteome of EVs isolated from plasma samples of young male athletes (obtained within 15 minutes after an aerobic exercise session) and sedentary subjects using ultracentrifugation. Proteome profiling of the EVs was performed using GeLC-MS/MS, and data were further analysed using bioinformatic tools such as MetaboAnalyst and String.

According to String, the proteins identified in the EVs were mainly associated with the immune system and lipoprotein assembly. Of these proteins, 14 were identified exclusively in the EVs of the trained subjects, and they were predominantly from the immune system. LRG1, associated with angiogenesis and tumour progression, and APOM, associated with healthy adipose tissue and insulin sensitivity, were two proteins identified only after intense muscle contraction. In addition, higher AGP content was found in the EVs of exercised individuals. AGP has also been associated with several types of cancers, but levels were also found to be elevated immediately after exercise. Nevertheless, the exact physiological role of these proteins in EVs after intense muscle contraction has not been fully elucidated. To better understand the muscle contraction effects on EVs cargo and their health consequences, in vitro studies using cancer models are currently underway. We anticipate that we will ultimately shed more light on the complex interplay between exercise, the molecular composition of EVs, and health promotion, providing valuable insights for the design of novel therapeutic interventions.

DAY 3 – Wednesday – 19th July 2023
11:50 – 12:10

Mapping protein profiles of the muscle-tendon junction with spatial proteomics

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Introduction: An extensive extracellular matrix (ECM) connects and stabilizes skeletal muscle fibers. Moreover, the ECM include the neuromuscular junction (NMJ), where motor neurons innervate skeletal muscle fibers. Degeneration of these neurons leads to severe muscle diseases such as amyotrophic lateral sclerosis (ALS). Another ECM area is the myotendinous junction (MTJ), a transition zone between muscle and tendon. During muscle activity, forces are transmitted from the muscle to the tendon via the MTJ. As the MTJ is the weakest point along the muscle-tendon axis, workload often leads to a tear of the MTJ in humans. Although morphological characterisation of the NMJ and MTJ has provided important insights into the function of these areas, spatial mapping of proteins from central to proximal areas of skeletal muscle is lacking.
Methods: Soleus and diaphragm skeletal muscles from adult wild-type mice were embedded in a cryo-matrix and cut into thin slices using a cryotome. Single slices were transferred to 96-well plates, lysed and digested using the SP3 method. Peptides were measured by LC-MS using short gradients in DIA-PASEF mode. The spectra were analysed using DIA-NN and protein intensities were used to generate spatial protein profiles along the longitudinal skeletal muscle-tendon axis. A delay analysis enabled us to correlate protein abundances across the skeletal muscle unit. Finally, the protein profiles were used to create a network visualised with Cytoscape.
Preliminary Data: In total, we identified almost 10K proteins and generated 3315 protein profiles from central to distal muscle areas. Based on our protein profiling, we identified 282 MTJ-enriched proteins in the soleus and diaphragm muscles, including 58 common proteins that had maximal protein intensity in the MTJ of the soleus and diaphragm compared to the muscle and tendon. Several proteins associated with the de-novo fatty acid synthesis were observed in the MTJ, which is distinct from metabolic activity in muscle. The analysis of the NMJ revealed maximal peak intensities of neurofilaments, CHAT and ACHE. The soleus profiles were used to create a distance-based network to identify connections between muscle fibers and ECM. The resulting network visualises ~ 2900 proteins with over 102,000 connections showing a separation of muscle, MTJ and tendon areas. A high betweenness centrality score indicates that the MTJ has more connections between proteins compared to tendon and muscle. The network helps us to localize and categorize proteins and can be used in the future as ideal for comparison of diseased muscles.

DAY 3 – Wednesday – 19th July 2023
SESSION 9 – BMSS SESSION

11:00 – 11:30

Structural proteomics: weighing up structure, dynamics and function.

Justin L.P. Benesch

University of Oxford, Oxford, UK

We have been developing and applying mass-measurement-based approaches to interrogate directly the structure and dynamics of proteins. Here I will focus on the insights this has enabled in studying the specificity of interactions and assembly of molecular chaperone proteins, specifically small heat-shock proteins. I will also present mass photometry, a new method we have developed that allows the quantitative, label-free interrogation of proteins in solution. The combination of mass measurement approaches provides an unprecedented opportunity to quantify the heterogeneity of protein assembly, elucidate the influence of the proteins' physical chemistry – specifically entropy – on their evolution and function.

DAY 3 – Wednesday – 19th July 2023
11:30 – 11:50

Identifying inhibitors of an aminopeptidase with a high-throughput MALDI-TOF MS workflow

Leonie Müller[1], Simon Peace[2], Melanie Leveridge[2], Matthias Trost[1], Rachel Peltier-Heap[2], Maria Emilia Dueñas[1]

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Introduction

There is an increasing need for fast and label-free readouts for biochemical assays in the high-throughput screening (HTS) environment. I will present the development of a novel, HTS compatible MALDI-TOF MS-based drug discovery workflow which can meet these needs. This workflow is used to identify small molecule inhibitors of the endoplasmic reticulum aminopeptidase 1 (ERAP1) which is an important target in immuno-oncology and for auto-immune diseases. I will also show the validation of the workflow by comparing the MALDI-TOF MS assay performance to an established lower-throughput RapidFire (RF) MS assay.

Methods

The MALDI-TOF MS workflow consists of the following steps: (1) enzyme and compound pre-incubation, (2) reaction start by substrate addition, (3) reaction quench by acidic solution addition which also contains the internal standard, (4) sample mix with matrix (5) sample-matrix spotting on MALDI target plate, (6) data acquisition on rapifleX MALDI-TOF mass spectrometer. Initially, ERAP1 trimming of the substrate peptide YTAFTIPSI into the truncated product peptide TAFTIPSI was monitored as described in the established RF MS assay. The acetylated version of the YTAFTIPSI peptide was used as an internal standard for MS analysis.

Results

In a first step, the buffer conditions and peptide sequences were optimized for MALDI-TOF MS detection to reduce ion suppression effects and increase assay robustness. The ionization efficiency of our analytes and thereby the limit of detection of the peptides was improved by introducing arginine residues into the substrate sequence. We also designed a heavy labelled version of the new product peptide and used it as an internal standard to improve the linearity of detection. The substrate and enzyme concentrations for the MALDI-TOF MS based assay were titrated with these new basic peptides and validated by acquiring dose-response curves for known ERAP1 inhibitors. Assay stability, robustness, hit frequency, and reproducibility, was confirmed by screening multiple analytical replicates of DMSO plates, pilot test and ~9,600 compound sets. With the MALDI-TOF MS workflow, weak and potent inhibitors were reliably determined from a set of confirmed ERAP1 binders in a single concentration screen. The hit reproducibility of the workflow was equal to the established RF MS assay. Full-curve dose-response profiling was conducted with the hits and showed correlation of the pIC50 values between the two platforms.

To summarise, we were able to describe the first MALDI-TOF MS campaign to identify ERAP1 inhibitors, adding to the growing number of targets that can be assessed with this technology.

DAY 3 – Wednesday – 19th July 2023
11:50 – 12:10

MALDI MS sample analysis at ultrahigh speed and its applications in protein analysis

Sophie Lellman,[1] Bob Challen,[1] Cristian Piras,[1,2] Henriette Krenkel,[1] Jeff Brown,[3] Mike Morris,[3] Nick Taylor,[1] Barney Jones,[1] Chris Reynolds,[1] **Rainer Cramer**[1*]

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Introduction

Liquid atmospheric pressure (LAP)-MALDI MS was recently introduced, adding several functionalities to the speed and general toolbox of MALDI MS. Due to the liquid nature of the MALDI sample a highly stable ion beam can be achieved. Apart from improvements in quantitative data analysis, this feature greatly facilitates further advances in sample analysis speed and the accuracy of MALDI MS profiling analysis. LAP-MALDI can provide ESI-like multiply-charged peptide/protein ions, together with singly-charged metabolites and lipids, all in the same mass spectrum of a single acquisition. Here, we will present our latest data with a focus on the high-speed analysis of proteins.

Methods

A commercial Q-TOF instrument was fitted with a heated stainless-steel inlet tube as described previously (https://doi.org/10.1021/acs.analchem.9b05202). Samples were irradiated with a UV laser (up to 2000 Hz) using an extraction potential of ~3kV. Liquid MALDI matrices consisting of α-CHCA (5 mg/mL in 50:50 H2O/MeCN, v/v) and propylene glycol or ethylene glycol (+ 60% v/v) were mixed 1:1 with the sample. To ensure sufficient temporal resolution of TOF scans, SONAR software (Waters Corp.) was modified to store 200 distinct mass spectra per scan while allowing the quadrupole to operate without scanning and voltage ramping.

Preliminary data

Analysis of individual samples were achieved at speeds of up to 60 samples/second with 10 or more desorption events per sample. For direct protein analysis, speeds of up to 10 samples per second with CVs of less than 20% were achieved using a protein mixture. Fast LAP-MALDI MS measurements also allow the acquisition of enzyme kinetics data within a couple of minutes from just one sample in real time and label-free.

Disease classification using LAP-MALDI MS is exemplified by the detection of preclinical bovine mastitis from small amounts of bovine milk samples using a biobank of 12,000 samples from a longitudinal study over six months of sample collection. From this data, bovine mastitis was detected in preclinical samples (up to 2 days before symptoms) with a sensitivity of up to 70% and 100% specificity. Antimicrobial bioactive peptides were identified by MS/MS as important drivers for this classification at the early detection stage. In addition, assays for the detection of antimicrobial resistance (AMR) can be run by using the same low-volume milk samples.

Finally, LAP-MALDI MS analysis of bacterial showed that both lipid and protein profiles can accurately identify bacteria as well as AMR with the added benefit of MS/MS analysis of the multiply charged proteins for further improvements in specificity.

DAY 3 – Wednesday – 19th July 2023
SESSION 10 – HUMAN DISEASE PROTEOMICS

14:00 – 14:30

Immunological impact on internal proteomics cancer heterogeneity

Mariya Mardamshina[1], Shiri Karagach[2], Vishnu Mohan[2], Anjana Shenoy[1,2], Daniela Necula[3], Kateryna Krol[3], Einav Gal-Yam[4], Iris Barshack[3], Ofra Golani[2], **Tamar Geiger[2]**

[1] Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; [2] Weizmann Institute of Science, Rehovot, Israel; [3] Department of Pathology, Sheba Medical Center, Ramat Gan, Israel; [4] Department of Oncology, Sheba Medical Center, Ramat Gan, Israel

Trafficking routes after internalization regulate the signalling outputs of ligand-bound Receptor Tyrosine Kinases (RTKs), such as Fibroblast Growth Factor and Epidermal Growth Factor Receptors (FGFR2, EGFR). RTK recycling to the plasma membrane counterbalances lysosome-mediated receptor degradation, induces sustained signalling activation, and regulates cell proliferation and motility. Dysregulated recycling and/or signalling are associated with human diseases, such as breast cancer. However, it is not known through which signalling players recycling endosomes fine-tune downstream responses.

Here, we used FGFR2b as a model system to study signalling modules which depend on the presence or on the permanence of RTKs in the recycling endosomes upon ligand stimulation. We combined traditional Mass Spectrometry-based quantitative phosphoproteomics with the Spatially Resolved Phosphoproteomics (SRP) approach - which detects signalling proteins in proximity of FGFR2b during receptor recycling - bioinformatics, imaging, and functional assays in breast cancer cells.

We showed that recycling endosomes integrate the signalling outputs of FGFR2b and of EGFR in response to their respective ligands. Furthermore, FGFR2b signalling partners in the proximity of recycling endosomes which regulated autophagy, cell growth, and stress responses were identified only by the SRP approach. Therefore, SRP could revolutionize the way to study how signalling architecture changes depending on the spatio-temporal regulation of RTKs.

Overall, these findings increase our scarce understanding of recycling endosomes as fine-tune regulators of signalling outputs and reveal how manipulating specific signalling players during receptor recycling maintains cell survival, such as in breast cancer cells.

DAY 3 – Wednesday – 19th July 2023
14:30 – 14:50

Proteomic characterisation of rare quiescent therapy-resistant cancer cells

Emily Gaizley, Amandeep Bhamra, Xiuyuan Chen, Anni Poysti, Jason Wray, Simona Parrinello, Silvia Surinova, Tariq Enver

UCL Cancer Institute, University College London, United Kingdom

Introduction

Many cancers are characterised by the presence of a rare subpopulation of quiescent stem-like cells which are a source of heterogeneity, phenotypic therapy resistance, relapse and further disease evolution. These cells are able to evade cytotoxic chemotherapeutics, which selectively target proliferating cells, thus representing a major therapeutic challenge. The mechanisms controlling quiescence remain largely unknown, and transcriptomic analysis of quiescent cells has yielded only limited insights. Proteomic analysis of rare cancer stem cells requires the development of a low-input methodology in order to capture global protein changes and differentially expressed post-translational modifications.

Methods

To identify and isolate rare quiescent cells, we generated and validated a genetically engineered fluorescent label-retaining *in vitro* model using patient-derived glioblastoma cell lines, and used somatic mouse models of glioblastoma with the same inducible reporter. We developed a robust proteomic workflow for rare FACS-sorted cells which has been used for multiplexed isobaric labelling and phosphopeptide enrichment. Together, these workflows enable the processing of protein loads down to the low-microgram/sub-microgram level to identify signalling networks that govern quiescence.

We have optimised and validated a reproducible workflow for low-input proteomic sample preparation and used this technique to show significant changes in the total proteome of quiescent cells, compared to their proliferating counterparts. To further investigate the signalling pathways modulating quiescence, we established a workflow for low-input tandem mass tag multiplexed quantitative phosphoproteomics.

Results

We have demonstrated the presence of a pre-existing, treatment-naïve quiescent state in *in vitro* and *in vivo* models of glioblastoma, and shown that these cells exhibit self-renew capacity and increased therapy resistance. To better understand the interplay between inherent and acquired resistance mechanisms we compared the proteome of treatment-naïve quiescent cells and cells challenged with chemotherapy.

Our findings indicate that the quiescent proteome is conserved across models of glioblastoma, both *in vitro* and *in vivo*. These data have been integrated with transcriptomic data to offer a multi-omic analysis of quiescence in glioblastoma. We have shown that lysosomal function plays a crucial role in regulating quiescence at the protein level and exposed the lysosome as a potential therapeutically targetable vulnerability, which is being functionally validated.

DAY 3 – Wednesday – 19th July 2023
14:50 – 15:10

Quantitative Translation and Import Proteomics using mePROD

Georg Tascher, Kevin Klann, Jonas Michaelis, Jasmin Schäfer, Suleyman Bozkurt, Christian Münch

Institute of Biochemistry II, Goethe University Hospital, Frankfurt am Main, Germany

Introduction
Stress-induced response mechanisms control cellular fate through a multi-layered regulation in order to minimize impairments in homeostasis and cell survival. One essential mechanism to reshape cellular proteomes is the adjustment of protein translation and its attenuation is a rapid cellular response induced by various stresses, such as the induction of the integrated stress response (ISR) and mTOR inhibition. Hence, measuring protein translation is an invaluable tool for the understanding of cellular stress-responses and protein homeostasis in general. One drawback of the current gold standard in MS-based translation measurements, namely pulsed stable isotope labeling with amino acids in cell culture (pSILAC), is the relatively long pulse time required for sufficient incorporation of heavy isotopes into the proteome under study. To this end, we developed multiplexed enhanced protein dynamics mass spectrometry (mePROD) combining pSILAC with Tandem mass tags (TMT), which enables robust quantification of translation on a proteome wide scale in experiments with short labeling times.

Methods
After pulse labeling, the sample preparation is the same as for standard TMT-based experiments, but with the addition of a „booster-channel" containing only heavy-labeled peptides as well as a "noise-channel" containing only light peptides to determine background noise levels and co-isolation interference for each individual peptide. Moreover, we recently adapted the method to study mitochondrial protein import by using a booster-channel comprised of enriched mitochondria from a fully heavy-labeled cell lysate. For benchmarking, we analyzed a dilution series of SILAC-labelled peptides and titrated the relative amount of the booster-channel to maximize identifications but limiting impact on quantification and applied mePROD to several different cellular stressors.

Results
We show that mePROD provides an easy and cost-efficient method to profile proteome-wide translatome changes at a temporal resolution of minutes. The booster strongly increased the identification and thus quantification of newly synthetized proteins. After treating cells with ISR/mTORC1-modulating stressors, extensive translatome modulation was observed with 20% of proteins synthesized at strongly reduced rates. Comparing translation-deficient sub-proteomes revealed an extensive overlap demonstrating that target specificity is achieved on protein level and not by pathway activation. The method already has brought valuable insight into different biomedical contexts, such as SARS-CoV2-Infection and acute myeloid leukemia. Notably, the noise-channel included in mePROD makes ratio compression, caused by co-isolation of non-targeted ions, as typically observed in TMT MS2-based methods, largely negligible.

DAY 3 – Wednesday – 19th July 2023
15:10 – 15:30

Exploring the Brain Proteome Signatures of Multiple Sclerosis-Like Models Using Mass Spectrometry

M. Fucito,[1] R. Guerrero,[2] E. Yilmaz,[2] B. Ojha,[3] S. Albrecht,[2] T. Kuhlmann,[2] R. Khorooshi,[3] T. Owens,[3] A. Nawrocki,[4] M. Røssel Larsen,[4] P. del Boccio,[1] D. Pieragostino[1]

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Multiple sclerosis (MuS) is the most common chronic inflammatory autoimmune neurological disease in young adulthood affecting 2.8 million people worldwide with an estimation of one patient diagnosed every five minutes. The pathological hallmark of multiple sclerosis is the loss of myelin, known as demyelination, around axons of neurons of the central nervous system (CNS). This demyelination impairs the transmission of the electrical signals to and from the brain. In addition to neurons, the oligodendrocytes are particularly interesting to study in the context of MuS because they are the myelinating cells of the CNS. The pathophysiology of this complex and heterogeneous disease is unknown and efforts towards finding molecular signatures of lipids and proteins would lead to more efficient treatments. In this context, we used leading edge mass spectrometry technologies to explore the brain proteome signatures of multiple sclerosis-like models. We used liquid chromatography coupled to tandem mass spectrometry to explore the proteome signatures of a focal experimental autoimmune encephalomyelitis (f-EAE) mouse model and to highlight the protein regulations and function modulations in differentiating human induced pluripotent stem cells (hiPSCs) derived oligodendrocytes and in stressed hiPSCs derived neurons after they had been exposed to four drugs separately (interleukin 4, minocycline, pioglitazone, synthetic preimplantation factor), compared to a dimethyl sulfoxide control. All experiments were conducted in triplicates. Data allowed to shed light on two potential biomarkers of neurodegeneration namely synaptotagmin-7 and fascin, both significantly downregulated in the f-EAE, both compared to the immunised control. It also emphasised the potential role of minocycline in helping the differentiation of the hiPSCs derived oligodendrocytes via the significant downregulation of tenascin C (TNC) and the significant upregulation of sex-determining region Y-box 10 (SOX10), compared to the control, which were both confirmed by western blot. Finally, we showed the potential role of minocycline in the limitation of mitochondrial dysfunction via the significant downregulation of bcl-2-associated x protein (BAX), already known to be regulated by the drug, and via the significant upregulation of 2-oxoglutarate dehydrogenase complex component E1 (OGDH), which could be a new factor, regulated by minocycline, not yet described in literature.

DAY 3 – Wednesday – 19th July 2023
SESSION 11 – PLANTS | ANIMALS | MICROBES | METAPROTEOMICS

14:00 – 14:30

Metaproteomic applications in pharmacology and clinical research

Leyuan Li, Zhongzhi Sun, Xu Zhang, Joeselle Serrana, James Ryan, Wenju Wang, Caitlin Simopoulos, Janice Mayne, Zhibin Ning, David Mack[1], Mathieu Lavallée-Adam, Alain Stintzi and **Daniel Figeys**

School of Pharmaceutical Sciences, Faculty of Medicine, University of Ottawa, Canada; [1]Children Hospital of Eastern Ontario, Ottawa, Canada.

Over the last decades numerous studies have reported a link between the gut microbiome and human health and diseases, including obesity, inflammatory bowel disease, cardiovascular diseases, cancer, gut-brain axis, and other diseases. Metabolites produced by the gut microbiomes are key players in the interactions between gut microbiome on the host. Metabolites secreted by the microbiome are produced by a series of enzymes from one or multiple microbes under different regulatory mechanisms. The field of microbiome has been primarily focused on using genomic tools to assess the composition of the microbiome and then predicting functional changes. Therefore, our understanding of the real functional changes that occur in a microbiome is very limited. Metaproteomics measures the functional changes in the microbiome and is well suited to better understand changes in biological processes and in particular metabolic pathways responsible to produce metabolites. I will present applications of metaproteomics to 1) better understand the role of the microbiome in pediatric inflammatory bowel disease, 2) to understand drug-microbiome interactions and 3) and its application in umbrella clinical intervention studies.

DAY 3 – Wednesday – 19th July 2023
14:30 – 14:50

The Proteomes that Feed the World

Ezgi Aydin, Sarah Brajkovic, Andrea Piller, Armin Soleymaniniya, Cemil Can Saylan, Genc Haljiti, Giorgi Tsiklauri, Guido Giordano, Jiuyue Pan, Lukas Wurstl, Mario Picciani, Patrick Rohrl, Paula Andrade Galan, Sebastian Urzinger, Sophia Hein, Veronica Ramirez, Qussai Abbas, Corinna Dawid, Stephanie Wilhelm, Claus Schwechheimer, Chris Schoen, Josch Pauling, Christina Ludwig, Dmitrij Frishman, Mathias Wilhelm, Caroline Gutjahr, Ralph Hueckelhoven, Brigitte Poppenberger, Bernhard Kuster

Elite Network of Bavaria, School of Life Sciences, Technical University of Munich

Introduction
Plants are the nutritional basis of almost all life on earth; Protein-rich foods from crop plants as an alternative to animal-based protein not only represent a global megatrend but are essential to sustaining a growing human population while tackling climate change. While the genomes of crops are increasingly being elucidated, little is known about crop proteomes – all the proteins that drive and control nearly every aspect of life. Our visionary international doctoral program "LRTN360 - The Proteomes that Feed the World" aims to map the proteomes of the 100 crop plants most important for human nutrition, thus creating the Crop Proteome Atlas which will be valuable for academia and industries. We are still looking for partners interested in joining the initiative by contributing plant material or bioinformatics capabilities. In addition, we will utilize chemical proteomics strategies to elucidate potential enzymatic functions of plant proteins. Functional annotation is still a major bottleneck in plant proteomics and will leverage our understanding of many so far poorly characterized plant proteins.
Methods
The Crop Proteome Atlas project has developed a robust and reproducible protocol for processing and analyzing plant tissues using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to achieve proteomic depth and sample throughput. Peptides and proteins are identified and quantified by using MaxQuant combined with Prosit-based rescoring and Chimerys. To perform chemo proteomic mapping of protein functions, co-factors or small molecule inhibitors will be immobilized on a solid support to generate affinity matrices. These will be used to enrich bona fide protein interactors from complex native plant lysates – hinting towards potential (und mostly unknown) enzymatic functions of these proteins. The project will follow FAIR principles by sharing the data publicly accessible via PRIDE and ProteomicsDB throughout the project.
Results
The optimized Crop Proteome Atlas pipeline has already elucidated the first comprehensive proteomes of quinoa (Quinoa bicolor) covering ~147k peptides and ~20k proteins, and fruits of tomato (Solanum lycopersicum) identifying ~189k peptides and ~10k proteins. Besides, the chemo proteomic workflow for functional annotation is currently being developed. In this regard, we have optimized the extraction of native proteins from plant material by using a combination of bead beating, sonication and an Igepal-based lysis buffer. Several chemo proteomic affinity matrices were generated by targeted immobilization of different small molecule inhibitors (e.g., addressing kinases, histone deacetylases, deubiquitylates). Their interactomes were elucidated by competitive pulldown experiments coupled to a quantitative proteomics readout.

DAY 3 – Wednesday – 19th July 2023
14:50 – 15:10

Proteome and lipidome interrelationship of synovial fluid-derived EVs in Equine osteoarthritis: An exploratory 'multi-omic' study

Emily Clarke,[1ǂ] Laura Varela,[2,3ǂ] Rosalind Jenkins,[4] Estefania Lozano-Andrés,[3,5] Anna Cywińska,[6] Milosz Grabski,[7] Maciej Przewozny,[7] Agnieszka Turlo,[1] P. René van Weeren,[2] Chris H.A. van de Lest,[2,3] Mandy Peffers[1,*] Marca H.M. Wauben[3*]

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Background
Osteoarthritis (OA) impacts both humans and companion animals, including horses, causing chronic joint degeneration and resulting in significant morbidity and decreased mobility. An emerging source for biomarker discovery is extracellular vesicles (EVs). EVs are nanoparticles secreted by cells that facilitate intercellular communication. Here, we aimed to identify potential biomarkers of equine OA in synovial fluid-derived EVs using a novel multi-omics approach.
Methods
Fourteen pooled synovial fluid (SF) samples were used, with three biological replicates from 42 horses (Healthy n=7; OA n=4; Advanced OA n=3). OA was diagnosed based on clinical examination and the severity of radiographic lesions. EVs were isolated from SF following differential ultracentrifugation with a sucrose density gradient. The phospholipidome was analysed by high-resolution mass spectrometry on a Fusion Orbitrap. EV proteins were assessed using a data-independent proteomic approach on a Triple TOF 6600. Data was processed independently, with an analysis of variance performed on proteomic data. Then multi-omic integration was performed using unsupervised sparse Partial Least Squares, and further bioinformatics was performed utilising R or metaboanalyst.
Results and Conclusions
A total of 280 lipid species and 559 proteins were identified across all samples. The lipidome's principal component analysis (PCA) explained 69% of the variation with the principal components -1 and 2 according to disease status. The sphingomyelins represented 35.5% of the lipid profile in OA, 37.5% in advanced OA and 19.9% in the healthy group. The three groups were primarily composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and sphingomyelin. In the proteomics PCA analysis, the first two components accounted for 36.4% of the variation associated with the disease group. There were 40 differentially expressed proteins ($p<0.05$), which were subsequently used in functional enrichment analysis. In both OA and Advanced OA groups, canonical pathways identified included signalling by Rho family GTPases ($p=1.24x10-19$). Additionally, significant diseases and functions included inflammatory disease ($p=1.21x10-14$). Data integration demonstrated an association between lipidome and proteome, with proteins associated with lipids activating Rho family GTPases, known for their inflammatory involvement and promotion of chondrocyte hypertrophy. There was an association between specific lipids and proteins involved in pathways related to chondrocyte dysregulation and inflammatory activation, suggesting a concerted response from proteins and lipids. Thus, SF-EV could be potential biomarkers for OA; however, further exploration in a larger cohort is required.

DAY 3 – Wednesday – 19th July 2023
15:10 – 15:30

Metaproteomics exploration of the gut microbiota of the freshwater sentinel animal Gammarus fossarum

Thibaut Dumas, Lucia Grenga, Olivier Pible, Olivier Geffard, Nicolas Delorme, Davide Degli Espoti, Arnaud Chaumot, Jean Armengaud

CEA ProGénoMix platform Bagnols-sur-Cèze France, CEA ProGénoMix platform Bagnols-sur-Cèze France, CEA ProGénoMix platform Bagnols-sur-Cèze France, INRAe UR RiverLy Laboratoire d'écotoxicologie Villeurbanne France, INRAe UR RiverLy Laboratoire d'écotoxicologie Villeurbanne France, INRAe UR RiverLy Laboratoire d'écotoxicologie Villeurbanne France, INRAe UR RiverLy Laboratoire d'écotoxicologie Villeurbanne France, CEA ProGénoMix platform Bagnols-sur-Cèze France

The amphipod Gammarus fossarum is a sentinel animal used to assess the biological impacts of chemical contamination in rivers using in situ ecotoxicological bioassays. The gut microbiota plays a role in contaminant biotransformation and toxicity-related responses in host organisms, but it also influences host life history traits. However, no data are available on the dynamics of the gut microbiota of G. fossarum despite its important role in ecotoxicology. Among the different meta'omics approaches to assess microbial community and their interactions with host organisms, metaproteomics delivers unbiased quantities of the taxa present in the sample, defines the main metabolic pathways associated with the microbiota, and identifies host proteins.

In this study, we characterized the gut microbial community and its functioning under normal, contaminant-free conditions by examining the effects of different diets (alder leaf, carrot, spinach, and marketed granules) over a 9-day period. This metaproteomics study utilized extensive molecular data obtained from a sentinel species for which no annotated genome has been established yet. We recorded large MS/MS datasets on total proteins extracted from pools of intestines collected from the millimetric animals as well as on the diets themselves. Interpretation of results was based on annotated genomes and RNA-seq derived proteogenomics information from the host, as reference metagenomics data are not yet available.

The gut microbiota analysis revealed the presence of 37 bacterial and 5 fungal genera in at least one dietary condition. The relative biomass of the main phyla consisted of 38% Proteobacteria, 25% Actinobacteria, 12% Firmicutes, and 9% Bacteroidetes. Our study demonstrated that diet had a strong effect on the taxonomic composition of the gut microbiota, with specific taxa associated with particular diets and changes in taxa abundance. The impact of food on the microbial communities in these detritivorous animals was primarily attributed to the ingestion of microorganisms present in the food.

We observed a core microbiota common to all individuals, regardless of diet. This core microbiota comprised 8 bacterial genera and 1 fungal genus, most, but not all, of these genera were also detected in other amphipods. Functional analysis of this core microbiota should provide valuable insights into its key physiological role for the host. Importantly, our findings suggest that the core microbiota is not significantly affected by fluctuations in environmental factors, such as food, which is an important consideration for future ecotoxicological studies.

DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

16:00 – 16:15
Casanovo? Is *de novo* sequencing a pipe dream or an undisputed Casanova?

Yun Chiang, Matthew Collins

The University of Copenhagen, The University of Cambridge/The University of Copenhagen

Mass spectrometry-based approaches have been widely used in archaeology to elucidate palaeodiet, phylogeny, the development of ancient diseases and protein degradation over time. However, the identification rates of archaeological experiments are consistently below 10%; most mass spectra appear to be uncharacterised, and these dark proteomes remain an untapped reservoir of ancient proteins and complex damage patterns. One could only speculate about the sources of these undetected queries; they could be uncanonical genetic variants, extensively damaged peptides containing multiple post-translational modifications (PTMs), or cross-linkers derived from interactions between proteins, carbohydrates and lipids. Conventional database search engines, for example, MaxQuant and Mascot, may not be suitable for these analytical challenges. Searching ancient proteins against Swiss-Prot and curated databases may also result in long processing time, decreased sensitivity and increased false positives. Degraded peptides, complex PTMs and noisy spectra could equally affect computational efficiency. How to unravel novel peptides and detect PTMs securely are outstanding challenges not only in archaeology but also in modern metaproteomics, cancer research, therapeutic designs and ageing studies. One potential strategy is leveraging deep learning models such as Casanovo to shed light on all acquired queries. We have tested Casanovo and ground-truthed it using a combination of ancient and artificial spectra. We would argue that a hybrid approach increases sequence coverage and the identification of PTMs.

DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

16:15 – 16:30

Site-Specific Activity-Based Protein Profiling Using Phosphonate Handles

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Introduction: Comprehensive mapping of small molecule drug target proteins in their native environment can improve our understanding of drug action and potential off-target proteins. Activity-based protein profiling (ABPP) by mass spectrometry is a powerful approach for identification of small molecule inhibitor targets in complex proteomes. ABPP utilizes an activity-based probe (ABP) to interrogate the activity status and occupancy of active site pockets in proteins. The identification of the exact drug binding sites on proteins is essential to understand and predict how drugs affect protein structure and function. However, when using biotin, which is commonly used as an enrichment tag in combination with ABPP, the exact binding site of the ABP in proteins often remains elusive. To address this challenge, we have developed a strategy that uses IMAC-enrichable phosphonate affinity tags for efficient and selective enrichment of peptides bound to an ABP, enabling identification of the exact drug binding sites.[1]
Methods: Intact cells and cell lysates were treated with an alkynylated derivative of the marketed anti-cancer drug Afatinib (PF-06672131). Copper-catalyzed alkyne-azide cycloaddition (CuAAC) with an azide functionalized phosphonate handle allowed specific labeling of probe-targeted sites in native proteomes.[2] After phosphonate tagging, fast and efficient enrichment of probe-labeled peptides was achieved by automated immobilized metal affinity chromatography (IMAC). Enriched probe binding sites were analyzed with LC-MS/MS, and MaxQuant and MSFragger were used for probe binding site identification.
Results: Using the site directed ABPP approach, termed PhosID–ABPP, over 500 unique ABP binding sites of an alkynylated Afatinib derivative were identified. Comparison of probe labeling in intact cells and cell lysates revealed differences in approachable binding sites in intact cells and cell lysates of the same cell line. Interestingly, an alternative protease digestion approach using pepsin led to a significant increase of the number of identified probe binding sites. Moreover, pepsin also allowed capturing the known Afatinib binding site in its main target, the epidermal growth factor receptor (EGFR), which was not identified using solely trypsin as protease. Overall, we find that PhosID–ABPP is highly complementary to biotin-based enrichment strategies in activity-based protein profiling studies. Compared to biotin, PhosID–ABPP provides the advantage of direct ABP-interaction site identification.

1. van Bergen, W., Hevler, J. F., Wu, W., Baggelaar, M. P. & Heck, A. J. R. Site-Specific Activity-Based Protein Profiling Using Phosphonate Handles. Mol. Cell. Proteomics 22, 100455 (2023).
2. Kleinpenning, F., Steigenberger, B., Wu, W. & Heck, A. J. R. Fishing for newly synthesized proteins with phosphonate-handles. Nat. Commun. 11, 3244 (2020).

DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

16:30 – 16:45

The epigenetic landscape of pancreatic cancer stem cells: target identification and therapeutic potential

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related death, with its aggressiveness and resistance to therapies attributed mainly to the presence of pancreatic cancer stem cells (PCSCs). Epigenetics is responsible for the reprogramming, transformation, and de-differentiation of CSCs, and targeting epigenetic modifiers, such as writers and erasers, could represent a strategic therapy against PDAC. Despite this, the characterization of histone PTMs (hPTMs) and of dysregulated proteins that influence specific histone modifications in PCSCs is still missing.

Methods

This work analyzed three biological replicates of parental cells, PCSCs, and adherent-CSCs of two PDAC cell lines (i.e., PaCa3 and PANC-1). For epi-proteomics, a super-SILAC-based LC-MS/MS analysis was carried out on extracted histones. An unsupervised PCA and a supervised OPLS-DA were applied to provide an overview of the hPTMs and to obtain information about the differences between epithelial or stem-like cells, respectively. A total proteome study was conducted with a label-free (SWATH-MS) strategy through a micro-LC 5600+ TripleTOF MS system. Identified deregulated proteins were subjected to bioinformatic analyses. Lastly, some histone marks and dysregulated proteins of PCSCs were validated by immunoblotting.

Results

The epi-proteomic profiling revealed altered hPTMs in PCSCs, related to quiescence, apoptosis, chemoresistance, and EMT. Among them, H4K20me3 and H3K9me3 were found to be more abundant in PCSCs. Interestingly, H4K20me3 is a repressive mark of several key drivers of the epithelial state, while H3K9me3 is correlated to the silencing of pro-apoptotic genes leading to chemoresistant PDAC cells. The comparative proteome analysis of PANC-1 cells allowed the detection of dysregulated proteins in PCSCs specifically influencing some of the identified hPTMs. Among the most interestingly modulated proteins, there are some histone methyltransferases, epigenetic regulators (such as H1.0, and RBBP4), and enzymes involved in 1C- metabolism (such as PHGDH), which affect histone methylation. Further studies are currently ongoing including the investigation of global DNA methylation status. In conclusion, we expect that accomplishing this work will allow the selection of new therapeutical targets, improving PDAC therapies, usually not affecting PCSCs.

DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

16:45 – 17:00

Automated workflow for proteomic-reanalysis and co-regulation-based functional characterisation

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Combining and re-analyzing publically available proteomic studies is a powerful approach to identifying novel proteins and predicting their function. This however is laborious and computationally intensive. We present a workflow that can process and integrate an essentially unlimited number of raw files, while controlling FDR across the combined studies, thus making the results of the different studies comparable. We use this workflow to integrate 150 SILAC PRIDE projects, to produce a new human protein covariation map. We focus this workflow on identifying proteomic evidence for the recently annotated 7,264 human Ribo-seq ORFs, by Mudge et al., and contributing to their functional characterization through our protein co-regulation map.

DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

17:00 – 17:15

Fast photochemical oxidation of nucleic acids coupled to high-resolution MS analysis

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Recent years have seen significant growth in the methods of structural proteomics, which have had had a significant impact in the field of structural and molecular biology. These methods may address questions related to structure and dynamics of protein and protein complexes, making them favorable for studying protein-DNA interactions. One of these methods, radical covalent labelling, is one of the structural techniques that has showed to be an effective analytical tool for characterization of biomolecules. In this study, we applies the Fast Photochemical Oxidation of Proteins (FPOP) approach to study the dynamics of FOXO4 transcription factor and its DNA partner, Insulin Response Element (IRE). To study such complex, FOXO4 in the absence or presence of dsIRE was oxidized by FPOP in a quench-flow capillary reactor. Irradiated samples were further analyzed by classical bottom-up approach. To investigate IRE-FOXO4 binding and to monitor the DNA damage caused by the hydroxyl radicals, IRE alone and in the complex with the protein was exposed to FPOP oxidation. FOXO4 was digested into dipeptides using proteinase-K and IRE fragments were analyzed by LC-MS using high-resolution FT-ICR mass spectrometer operated in negative ion mode.
Analysis of oxidized peptides enabled localization and quantification of residues directly involved in protein-DNA interaction. Analysis of separated IRE fragments revealed that hydroxyl radicals cleave the DNA nonspecifically, creating set of all possible 3'OH, 3'P, 5'OH and 5'P terminal fragment ions. Complementary fragment ions were found in the LC-MS trace and further quantified. Comparison of IRE fragment ions revealed significant protection of IRE by FOXO4 binding, predominantly in both major and minor groove of IRE. Obtaining detailed information about solvent accessibility for IRE and FOXO4 surfaces might enable ab initio design of FOXO4/IRE structural model. This is potentially valuable because the corresponding crystal structure is currently unclear.

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DAY 3 – Wednesday – 19th July 2023
SESSION 12 – EARLY CAREER RESEARCHER TALKS

17:15 – 17:30

Can precursor ion connectivity of different isolation windows improve peptide and protein identification in chimeric MS/MS spectra?

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Introduction: Chimeric MS/MS spectra result from the co-sequencing of two or more precursor ions which have a similar, if not the same m/z ratios. The simultaneous fragmentation of multiple parent ions that fall within the same m/z isolation window complicate the analysis of peptide and protein mixtures due to the production of a greater number of mixed-source fragment ions submitted to sequence database searches. This significantly reduces search scores and lends to less confident peptide and protein identification because of poor discrimination of the co-fragmented peptides. By exploiting multiple isolation windows of the entire charge state distribution, the various MS/MS analyses can be filtered for common ions and used for improving search scores in database searching.

Methods: Common MS peptide and protein standards were selected and a theoretical m/z list for each was produced to determine potential overlapping monoisotopic peaks of parent ions to create chimeric MS/MS spectra. The standard mixtures were analysed using a Synapt G2-Si mass spectrometer (Waters) fitted with an in-house built LAP-MALDI ion source which was operated in sensitivity mode with ion mobility active. LAP-MALDI is known to generate multiply charged analyte ions, producing similar charge-state distributions as ESI. The overlapping precursor ion species alongside other intense charge states present in the MS spectra were sequenced. Common fragment ions specific for each analyte were then separately selected and used for identification. This methodology was then applied to more complex biological samples like *E. coli.*

Preliminary Data: Solely submitting data from the chimeric MS/MS spectra for database searching led to insignificant search scores due to the presence of non-specific ions. Alternatively using the workflow presented, singly charged ions were filtered out by exploiting ion mobility with the help of Driftscope (Waters) before further data processing and analysis using charge-state deconvolution software. It's worth noting that this workflow can be executed without the use of ion mobility filtering. Using an automated script that utilises the knowledge of precursor ion connectivity, common fragment ions from several isolation windows were selected, allowing for the identification of peptides and proteins with a much higher score; identifying ubiquitin from bovine erythrocytes from a two-protein mixture with a significant protein score of 160. Although several solutions for processing chimeric MS/MS spectra exist which allow for the selection of different precursor ions within the same isolation window, here MS/MS data of several precursor isolation windows of the entire charge state distribution are combined.

DAY 4 – Thursday – 20th July 2023
SESSION 13 – SYSTEMS BIOLOGY & MEDICINE

09:00 – 09:30

Off-target identification of TCR-like antibody cancer therapeutics

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Selective binding of TCR-like antibodies that target a single tumour-specific peptide epitope presented by human leukocyte antigens (HLA) is the absolute prerequisite for their therapeutic suitability and patient safety. To date, selectivity assessment has been limited to peptide library screening and predictive computational modelling. We developed the first experimental platform to *de novo* identify interactomes of TCR-like molecules directly in human tissues using mass spectrometry, and conclude that our strategy offers an accurate, scalable route for de-risking TCR-based therapeutics prior to first-in-human clinical application.

DAY 4 – Thursday – 20th July 2023
09:30 – 09:50

Quantitative analysis of how the immunosuppressive drug rapamycin shapes immune cell proteomes

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Introduction
mTORC1 is a protein complex, that includes mTOR a serine /threonine kinase, which controls mRNA translation along with protein synthesis and degradation. mTORC1 is the target for the immunosuppressive drug frequently prescribed to transplantation patients, rapamycin. To understand the effects of rapamycin treatment in immune cells, an understanding of how mTORC1 shapes the proteomes is required. In this context there are many general assumptions made about what mTORC1 does to control cell function which are often based on data from an individual cell type (i.e. U2OS or HeLa cells) and are then extrapolated to all other cells.

Methods
Samples were subjected to SP3 procedure for protein clean-up before elution into digest buffer (0.1% sodium dodecyl sulfate, 50 mM TEAB (pH 8.5) and 1 mM CaCl2) and digested with LysC and Trypsin. TMT labelling and peptide clean-up were performed according to the SP3 protocol. The TMT samples were fractionated using off-line high-pH reverse-phase chromatography. For each fraction, 1 µg was analysed using an Orbitrap Fusion (Thermo Fisher Scientific) equipped with a Dionex ultra-high-pressure liquid chromatography system (RSLCnano) and acquired using SPS-MS3.The data were processed with MaxQuant and protein copy numbers were calculated using the proteomic ruler (Wisniewski et al, 2014) as previously described (Howden et al, 2019).

Results
We comprehensively studied the impact of inhibiting mTORC1, via rapamycin treatment, on the proteomes of different immune cell populations along with embryonic stem cells. The immune cells studied include antigen and cytokine activated CD4 and CD8 T lymphocytes and innate immune cells including mast cells and natural killer cells. We quantified >8000 proteins per population, and revealed some shared, but also clearly divergent outcomes of mTORC1 inhibition in the different immune cell populations. One general result frequently associated with mTORC1 is that it controls cell growth. The present data show that mTORC1 control of cell mass is relatively limited in immune cells, with multiple populations showing no significant effect on cell mass after rapamycin treatment. The data also show that mTORC1 has very selective effects in shaping cellular proteomes and that generalisations based on data generated on cancer cell lines frequently prove to not be applicable to immune cells. This comprehensive analysis is a resource for exploring and understanding contextual effects of mTORC1 signalling pathways and affords new understanding of how mTORC1 acts to control mammalian immune responses, with all processed proteomic data made available via the Immunological Proteome Resource (ImmPRes; Brenes et al. 2023).

DAY 4 – Thursday – 20th July 2023
09:50 – 10:10

The interplay between EGFR and MET receptor abundance and cross-talk determines the effectiveness of tyrosine kinase inhibitors in lung cancer patients

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Non-small cell lung cancer (NSCLC) is a major cause of cancer-related deaths worldwide. While first generation tyrosine kinase inhibitor (TKI) treatments targeting epidermal growth factor receptor (EGFR) have been shown to extend survival for a few months, their effectiveness is temporary due to the emergence of therapy resistance. Although recent studies have identified the hepatocyte growth factor (HGF) receptor MET as a factor contributing to resistance to EGFR-TKIs, the underlying mechanisms are not yet fully understood.

We investigated signal transduction induced by EGF and HGF in four NSCLC cell lines with varying expression levels of EGFR and MET and carrying different EGFR mutations. Our findings suggested that co-stimulation of EGF and HGF activates MET phosphorylation in a cell type-specific manner. According to a dynamic pathway model, the ratio of EGFR and MET abundance regulates their cross-talk, which leads to an increased half-life of the MET receptor. Our hypothesis was confirmed by quantitative immunoblotting, first. We used mass spectrometry with data-independent acquisition (DIA) and targeted proteomics with parallel reaction monitoring (PRM) and established in an extended panel of NSCLC cell lines a wide variation in the receptor abundance ratio.

By applying our proteomics methods to primary patient material, we confirmed an EGFR to MET surface expression ratio dependent effect on EGFR TKI treatment response and independence from the EGFR mutation status. The absolute quantification of the receptors by PRM, in combination with our mathematical model promises to facilitate the rapid stratification of lung cancer patients and identify those that are likely to benefit from treatment with novel TKIs or monoclonal antibodies targeting either EGFR, MET or both.

DAY 4 – Thursday – 20th July 2023
10:10 – 10:30

A complex interplay of intra- and extracellular factors regulates the outcome of fetal- and adult-derived MLL-rearranged leukemia

Sudip Ghosh, Maria Jassinskaja, Mina Davoudi, Melina Claesson Stern, Ugarit Daher, Mohamed Eldeeb, David Bryder and **Jenny Hansson**

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Introduction: Despite decades of research, infant and adult MLL-rearranged (MLLr) acute leukemia remains a disease with few treatment options and a dismal prognosis. The majority of infant leukemias are driven by in utero-acquired MLL rearrangements with no or few cooperating mutations, whereas in adults, MLLr leukemias represent less than 10% of all acute leukemia cases, and rarely present as a de novo disease. In addition, a marked discrepancy exists in the occurrence of different disease phenotypes between infants and adults; MLLr leukemia predominately manifests as acute lymphoblastic leukemia (ALL) in infants, while acute myeloid leukemia (AML) presents at similar frequency as ALL in MLLr leukemia in adults. The lack of detailed molecular understanding on the divergent disease biology and leukemia progression is hampering improvements of current treatment protocols for childhood and adult leukemia.

Methods: Here, to facilitate development of age-tailored therapies, we present an in-depth functional and proteomic characterization of in utero-initiated and adult-onset MLLr leukemia in an inducible mouse model of MLL-ENL-mediated leukemogenesis.

Results: We reveal that fetal MLL-ENL-expressing lymphomyeloid multipotent progenitors (LMPPs) are intrinsically programmed towards a lymphoid fate but give rise to myeloid leukemia in vivo, highlighting a complex interplay of intracellular and niche-associated factors in determining disease subtype. Using mass spectrometry-based quantitative proteomics, we characterize the early proteomic events of MLL-ENL-mediated leukemic transformation in fetal and adult progenitors and reveal that differentiation arrest and alterations to the translational machinery are early events in MLLr leukemia regardless of developmental origin. We show that whereas adult leukemic protein signatures are mainly downregulation of ribosomal and metabolic proteins, MLLr leukemia initiation in fetal hematopoietic progenitor cells is governed by enrichment of translation-associated and HDAC signaling proteins, and a decreased expression of proteins involved in inflammation and myeloid differentiation, indicating a differential vulnerability of fetal and adult leukemic cells to modulation of these processes. Finally, we describe the proteomic composition of the extracellular environment of fetal and adult hematopoietic progenitors at steady state and integrate with the cellular proteome of leukemia initiation. This reveals ligand-receptor pairs with potential regulatory interactions and highlights differential regulation of IGF2 bioavailability, as well as VLA-4 dimer and its ligandome, upon initiation of fetal- and adult-origin leukemia. Our study has uncovered opportunities for targeting ontogeny-specific proteomic vulnerabilities in in utero-initiated and adult-onset MLLr leukemia.

DAY 4 – Thursday – 20th July 2023
SESSION 14 – SINGLE CELL PROTEOMICS

11:00 – 11:30

Unleashing the next-generation single-cell proteomics workflows

Valdemaras Petrosius[1], Pedro Aragón Fernández[1], Benjamin Furtwängler[1,2,3], Nil Üresin[1,2,3], Bo T. Porse[2,3], **Erwin M. Schoof[1]**

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Single-cell proteomics by Mass Spectrometry (scp-MS) can provide valuable insight into distinct cell-states and signalling patterns present in a cell population. However, carrying out proteomics profiling from the limited amount of material encapsulated in an individual cell presents significant challenges. Tremendous efforts have been made to optimize all aspects of scp-MS, with the aim of minimizing losses during sample preparation and maximizing sensitivity of data acquisition. Nevertheless, continuous development is needed to increase both the quantifiable proteome depth per cell, the quantitative accuracy and precision of those measurements, and the number of cells measured per unit time.

Here, we will present recent approaches developed in Erwin Schoof's Cell Diversity Lab. We will cover key aspects of the entire workflow, ranging from sample preparation and chromatographic separation, to advanced data acquisition and data interpretation and visualization methods. We will cover a range of instrumentation, including the newly announced ThermoFisher Orbitrap Astral MS, and span both TMTPro-labeled and label-free approaches. By showcasing the application of our methods to biological questions, we aim to convey possible biomedical implications of scp-MS, and provide an overview of the current technological state of the field.

DAY 4 – Thursday – 20th July 2023
11:30 – 11:50

Slice-PASEF: maximising the sensitivity and speed of DIA

Ludwig Sinn[1], Lukasz Szyrwiel[1], Di Qin[2], Florian Mutschler[3], Luke Khoury[4], Matthias Selbach[3], Martin Steger[5], Nikolai Slavov[4], Markus Ralser[1,5], Fabian Coscia[2], Vadim Demichev[1]

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Recent proteomic applications that deal with low sample amounts, including spatial tissue proteomics and single-cell proteomics, as well as PTM analyses, empower both basic and translational research. However, capabilities of current instrumentation limit both the proteomic depth as well as throughput and scale of such experiments, calling for new proteomic technologies with improved sensitivity and speed.

We present Slice-PASEF, a novel data-independent acquisition technology based on trapped ion mobility (TIMS)-enabled mass spectrometry. Aligning ion release by the TIMS device with quadrupole ion selection for fragmentation and TOF analysis, all peptide ions can be fragmented to achieve up to 100% duty cycle of MS/MS, thus yielding a fold-change sensitivity boost over state-of-the-art approaches. To fully utilize the high information content of Slice-PASEF data and make it readily available to the proteomic community, we implemented a Slice-PASEF module in our automated neural network-based DIA-NN software. Slice-PASEF can be used right away without any modifications of the mass spectrometer, and the required acquisition settings are publicly available.

We demonstrate that Slice-PASEF allows to gain up to 60% in protein identifications from low sample amounts and up to 3.4 improvement in the numbers of proteins quantified with high precision (CV < 10%). Leveraging Slice-PASEF, we show, for the first time, that comprehensive profiling of single cell-level peptide amounts is possible using ultra-fast microflow chromatography at 200 samples per day (SPD) throughput on the Evosep One LC system, allowing precise quantification of 1417 proteins from 200pg of a HeLa cell peptide standard on a Bruker timsTOF Pro 2. We further quantify 750 proteins on average from individual HeLa cells on a timsTOF SCP at 200 SPD, analyzed with a public HeLa spectral library. Applying Slice-PASEF to ubiquitinomics, which previously typically required mg-level protein amounts before enrichment of diglycine-modified (K-ε-GG) peptides, we quantify > 20’000 K-ε-GG peptides from just 50µg of starting protein amount and 3’350 K-ε-GG from just 1µg of starting amount, analyzing on timsTOF HT using a 45-min nanoLC gradient. We envision Slice-PASEF to significantly boost sensitivity and quantitative performance of a wide range of proteomics applications utilizing trapped ion mobility, including in high-throughput workflows. We discuss further promising developments of the technology, including in combination with multiplexing.

DAY 4 – Thursday – 20th July 2023
11:50 – 12:10

Single-cell proteomic analysis of RNA virus infection

Ed Emmott

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RNA viruses are responsible for many common human diseases ranging from the COVID-19 pandemic, to pathogens such as norovirus which result in major disruption for healthcare systems and large economic costs. Many RNA viruses such as norovirus and coronaviruses replicate through mechanisms which rely upon post-translational modifications. This includes proteolytic cleavage of a large viral polyprotein(s) into fully and partially-cleaved intermediates which play distinct roles in virus replication. Further PTMs of interest include covalent RNA:protein linkages between a viral protein and the viral RNAs. To understand viral replication at single-cell level requires the ability to monitor these PTMs to gain a complete understanding of what drives differences in virus replication at the single-cell level resulting in diverging cell fates even in simple systems using clonal virus stocks and cell lines.

He we present SCoPE2/nPOP-based single-cell proteomic analysis of murine norovirus-infected BV-2 cells, a cell culture model for human norovirus which is challenging to study in the laboratory. We offer insight into the events occurring in infected, bystander cells and mock-infected cells, alongside some of the sample preparation and instrument acquisition considerations for analyzing PTMs at single-cell level.

DAY 4 – Thursday – 20th July 2023
SESSION 15 – AWARDS & PLENARY

14:45 – 15:45

Fibroblasts: bystanders or culprits in cancer?

Sara Zanivan

CRUK Beatson Institute, Glasgow, UK

Fibroblasts play a fundamental role in physiology. They are the principal cell component of the connective tissue, where their role is to maintain the structure and homeostasis of tissues and organs. It has been known for a long time that there are many fibroblasts also in solid tumours. However, because of the important physiological function of fibroblasts and because they do not carry the multitude of genetic alterations typically found in the cancer cells, they have been overlooked for many years in cancer research. Work from our lab and others has started unravelling the fascinating biology of fibroblasts in cancer, discovering their plasticity and unique ability to secrete plethora of molecules with which they modify the tumour structure and influence the behaviour of cancer cells and other cell types in tumours. I will discuss our most recent discoveries on the role played by fibroblasts in tumour progression and response to therapy, and the potential of targeting these cells for anti-cancer therapy. I will also discuss how MS proteomics, including approaches that we have developed tailored to study fibroblasts *in vitro* and in *in vivo* models of cancer, have been fundamental to advance our understanding of their role in cancer.

Posters

No	Presenter	Affiliation	Poster Title
1	Andrew Reeder	Verulam Scientific Ltd	Online Electrochemical Reduction of mAbs for Rapid LC-MS Analysis
2	Berfu Nur Yiğit	Koc University	Molecular Analysis of Malformations in the Mammalian Cerebral Cortex using Proteomics Approaches
3	Vivek Sarohi	Indian Institute of Technology Mandi	Prolyl 3-hydroxylase 1 deficiency unhinges collagen post-translational modification patterns far beyond the lack of specific 3-hydroxyproline sites
4	Martha Nikopaschou	National Centre for Scientific Research Demokritos	Effects of allosteric or genetic inhibition of ERAP1 on the immunopeptidome and proteome of A375 melanoma cells.
5	Clemens Grünwald-Gruber	University of Natural Resources and Life Sciences	Site-specific N- and O-glycan Analysis of ACE2, SARS-CoV-2 and RBD proteins
6	Adam Hughes	Bruker	Deeper plasma proteome coverage enables identification of novel biomarkers and classification of diseases
7	Laura Guerrero	National Center for Biotechnology, Madrid	Analysis of the proteome and phosphoproteome of PFIC3 for understanding involved molecular mechanisms and cellular processes
8	Abu Zaid Khan	The University of Edinburgh	Characterising a novel tumour suppressor in Liver cancer
9	Najib Abualetham	Institute of Genetics and Cancer, University of Edinburgh	Characterising Novel off-target effects of a Brain-Penetrant Drug in Glioblastoma Cell Lines using different proteomic approaches
10	Chartinun Chutoe	Institute of Genetics and Cancer	USP18 and its role in mediating IFN response of triple-negative breast cancer
11	Melanie Brunner	University of Fribourg	Phosphoproteomic characterization of GSK-3 in autophagy
12	Sophia Laposchan	Technical University of Munich	Exploring Motif Preferences and Functional Specificity of the Protein Arginine Deiminase Family
13	Alexandre Leytens	University of Fribourg	Monitoring autophagy using parallel reaction monitoring
14	Robert van Ling	Thermo Fisher Scientific	Single-Shot deep DIA LCMS workflow for near-full proteome coverage

Posters

No	Presenter	Affiliation	Poster Title
15	Julian Müller	Chair of Proteomics and Bioanalytics, Technical University of Munich	Exploring Cellular Signaling Networks in ProteomicsDB: Interactive Visualization of Differentially Regulated Post-Translational Modifications in Biochemical Pathways
16	Jean-Baptiste Vincendet	SCIEX	A simplified orthogonal electrospray source setup for robust nanoflow or microflow proteomics analysis
17	Siddharth Jadeja	Faculty of Pharmacy, Charles University	Charged surface stationary phase allows for minimizing formic acid concentration in the mobile phase, enhancing electrospray ionization in LC-MS proteomic experiments
18	Ananth Prakash	EMBL-EBI	Integrated view of baseline protein expression in human, mouse and rat from reanalysis of public DDA and DIA experiments
19	Dan Parnaby	AB SCIEX	Improved DIA and DDA performance on low-level proteomic samples using a novel Zeno trap
20	Jakub Sýs	Czech Academy of Sciences	The single amino acid substitutions in Mason-Pfizer Monkey Virus matrix protein modulate its proteolytic cleavage rate
21	Shengbo Wang	EMBL-EBI	An approach to integrate metagenomics, metatranscriptomics and metaproteomics data found in public resources
22	Stefan Mueller	University of Cologne	Comparison of synthetic co-polymers and classical detergents as reagents for membrane protein solubilization utilizing shotgun proteomics
23	Rebekah Sayers	SCIEX	Exploring the benefits of differential mobility separation (DMS) and SWATH acquisition for complex proteomic sample analysis
24	Periasamy Parthiban	A*-Star	Small open reading frame-encoded protein production is modulated by nonsense-mediated decay in breast cancer
25	Van Kelly	University of Edinburgh	Data Independent Acquisition of Pulse-SILAC Proteomes and its Application to Cell-Based PROTAC Characterisation
26	Jan-Wilm Lackmann	CECAD	Robust and Standardized Workflow for Large Scale Clinical Proteomics
27	Simon Daled	Ghent University	Sexing based on tooth enamel proteins. Can co-selection PRM save the day?
28	Karin Barnouin	UCB BioPharma	Identification of translatable chronic kidney disease biomarkers by comprehensive transcriptomics and proteomics analysis of the rat sub-total nephrectomy SNx model of fibrosis

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29	Joseph Inns	Newcastle University	Data-independent acquisition proteomics reveals a disordered extra cellular matrix in CYLD deficient human skin tumours.
30	Jarrett Egertson	Nautilus Biotechnology	Detection and Quantification of Single-Molecule Proteins Using Protein Identification by Short-epitope Mapping (PrISM)
31	Maria Bourganou	University of Thessaly	Unique Peptides of Cathelicidin-1 in the Early Detection of Mastitis in Sheep
32	Angeliki Katsafadou	University of Thessaly	Detection of Cathelicidin-1 in the Milk as an Early Indicator of Mastitis in Sheep
33	Gina Eagle	SCIEX	Quantifying 1000 protein groups per minute of gradient using Zeno SWATH DIA on the ZenoTOF 7600
34	Luisa Canè	University Federico II of Naples	Targeted proteomics approach for qualitative and quantitative monitoring of human TSLP proteoforms
35	Christoph Schlaffner	Hasso Plattner Institute for Digital Engineering	Tissue deconvolution using cell-type specific protein profiles: The whole is more than the sum of its parts
36	Oliver Crook	University of Oxford	Residue-resolved HDX-MS with ResHDX
37	Amy George	Newcastle University	A Comparison of Quantitative Mass Spectrometric Methods for Drug Target Identification by Thermal Proteome Profiling
38	Nicole Kabella	Technical University Munich	decryptM: Profiling the phosphoproteomic response to Kras inhibition using a novel dose-dependent proteomic approach
39	Ana Montero Calle	Instituto de Salud Carlos III	Identification of novel proteins dysregulated in Alzheimer's Disease patients and related to Amyloid-β Plaques or as blood-based biomarkers of the disease
40	Zuzana Kalaninova	Faculty of Science, Charles University	Utilization of AnPEP in structural proteomic workflows
41	Charlotte Hutchings	University of Cambridge	Investigating the molecular pathways by which recombinant adeno-associated virus (rAAV)6.2 is released from HEK293-VP cells during manufacturing.
42	Eva Csösz	University of Debrecen	Integrated multiomics examinations in the study of obesity and type 2 diabetes coverage
43	Erdenetsetseg Nokhoijav	University of Debrecen	Multi-Omics approach for the examination of grape-derived beverages
44	Petra Magdolna Bertalan	University of Debrecen	Examination of protein-protein interaction networks in obesity and type 2 diabetes mellitus

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45	Balázs Kunkli	University of Debrecen	Validation of Label-Free Proteomics Data Analysis Reproducibility for Early-Phase HIV-1 and HIV-2 Transduction Using WOMBAT-P Pipelines and the Proteomics Metadata Standard SDRF
46	Andrea Guba	University of Debrecen	Proteomics and metabolomics analysis of recombinant protein-expressing CHO cell lines
47	Hamid Hachemi	CEA - Li2D	Metaproteomics allow deep and accurate characterization of cystic fibrosis' patients' respiratory microbiota
48	Roz Jenkins	University of Liverpool	Characterisation of precision-cut tumour slices, a patient-derived model of cholangiocarcinoma
49	Florine Hallez	Affinisep	A fast and efficient method for peptide fractionation at basic pH in proteomic studies
50	Rudolf Kupcik	University Hospital Hradec Kralove	Conventional liquid chromatography and its potential for diagnosis and monitoring of multiple myeloma progression
51	Yvonne Reinders	Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.	Development of a microflow LC-MS-based Peptide Assay to elucidate the coagulation cascade in human plasma.
52	Firas Hamood	Technical University of Munich (TUM)	Combining SIMSI-Transfer and Prosit leads to a synergistic increase in identifications
53	Consuelo Marin-Vicente	Spanish Cardio-vascular Research Centre (CNIC)	Single cell proteomics for discerning adult cardiomyocyte subpopulations.
54	Mostafa Kalhor	Computational Mass Spec, TU Munich	XL-Prosit: Transfer learning for tuning Prosit to predict fragment intensities of cleavable cross-linked peptides
55	Xavier Gallart-Palau	Biomedical Research Institute of LLeida	Brain extracellular vesicles provide novel clues to decipher connectome imbalances in Schizophrenia
56	Aida Serra	University of Lleida/ Biomedical Research Institute of Lleida	Discovery-driven proteomics of brain extracellular vesicles reveals novel molecular clues on the higher prevalence of dementia in psychotic subjects
57	Anas Kamleh	Thermo Fisher Scientific	Enhanced TMT data analysis with AI-driven workflows utilizing CHIMERYS and INFERYS algorithms
58	Sean Burnap	University of Oxford	Cross-linking Mass Spectrometry Uncovers Interactions Between High-density Lipoproteins and the SARS-CoV-2 Spike Glycoprotein
59	Monique van Schie	Wageningen University	Location, location, location: a system-wide assessment of subcellular protein localization in Arabidopsis roots by mass-spectrometry

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60	Jo Kirkpatrick	Thermo Fisher Scientific	High resolution DIA: A workflow for highly accurate relative label-free quantification of microbial proteins in complex cell lysates
61	Nicolas Autret	Covaris	Reproducible and scalable one-day FFPE sample preparation method for LC-MS assays with Adaptive Focused Acoustics
62	Megan Ford	University of Liverpool	Atabecestat and metabolite interactions with the immunopeptidome and atabecestat binding to model proteins in the presence of metabolising systems
63	Scott Adams	University of Southampton	Mechanistic understanding of the relationships between oxidative and electrophilic stress in allergic skin sensitisation
64	Heeyoun Hwang	Korea Basic Science Institute	Advanced assessment through intact glycopeptide analysis of Infliximab's biologics and biosimilar
65	Michał Puchalski	University of Gdansk	Search for peptide markers of salivary gland tumors in patients' saliva samples. Pilot study.
66	Jordan Hartig	Medical University of South Carolina	Glycan and Collagen-ECM Peptide Imaging Mass Spectrometry and Proteomics of FFPE Prostate Tumor Tissues
67	Rebecca Herzog	Medical University of Vienna	Cross-Omics Analysis of Transcriptome, Proteome and Metabolome Dynamics during Peritoneal Dialysis
68	Juan Manuel Sacnun	Medical University of Vienna	Unlocking the Secrets: Exploring Cell-to-Cell Communications through Secretomics Analysis
69	Fred Lamoliatte	University of Dundee	Uncovering the Interactome of endogenous PINK1 during mitophagy through TurboID-based temporal proteomic analysis
70	Luis Coy	University of Southampton	Molecular Phenotyping of 3D Cultured Triple-Negative Breast Cancer Cells Reveals Pathways Influenced by Culture System
71	Dušan Živković	IPBS Toulouse	Mass spectrometry – Swiss Army knife to dissect the spermatoproteasome structure and function
72	Ricardo Carreira	Immunocore Ltd	Identifying targets for immunotherapy with large scale immunopeptidomics: challenges and opportunities
73	Tiaan Heunis	Immunocore Ltd	Immunopeptidomics enable the development of soluble TCR bispecifics to provide a functional cure for HIV
74	Yeji Yang	Korea Basic Science Institute	Flashlight into the Function of Unannotated C11orf52 using Affinity Purification Mass Spectrometry
75	Simon Sugár	Research Centre for Natural Sciences, Budapest, Hungary	Proteomic Analysis of Lung Cancer Types - A Pilot Study
76	Domonkos Pál	Research Centre for Natural Sciences, Budapest, Hungary	Investigating Chondroitin Sulfate and Heparan Sulfate Glycosaminoglycans in Different Lung Cancer Tissues

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77	Barbara Sváková	Slovak Academy of Sciences	Analysis of the dynamics of meiotic proteome and phosphoproteome in *Schizosaccharomyces pombe*
78	Essraa Metwali	University Collage of Dublin	Discovery/development of protein biomarkers for the stratification of triple negative breast cancer (TNBC)
79	Geul Bang	Korea Basic Science Institute	Proteomic and Glycoproteomic Profiling of Organoids Derived from Human Hepatocellular Carcinoma
80	Mark Churchill	Bruker UK Ltd	Pushing DIA proteomics analyses of neat plasma to 1000 protein groups ID/hr
81	Max Hoek	Thermo Fisher Scientific	Predictive Ion Control for a new High-Throughput Proteomics Platform
82	Sira Echevarria	Biognosys	Optimizing Immunopeptide Analysis Sample Preparation in Needle Biopsy Size Tissue Samples with AFA® Technology and Spectronaut
83	Pierre-Olivier Schmit	Bruker	Evaluating the benefit of dia-PASEF approaches and sample-specific database strategies for metaproteomics of very complex microbiomes
84	Payal Nashier	University of Tübingen	HipA-like kinases as regulators of antibiotic tolerance and metabolism in bacteria
85	Renata Blatnik	Bruker Daltonics GmbH & Co. KG	Operating, maintaining, and troubleshooting the sensitivity and robustness of timsTOF platforms for proteomics studies
86	Theo Platt	Seer, Inc	Enabling Deep and Unbiased Proteomics at an Unparalleled Scale with a Cloud-Native Pipeline
87	Fanni Bugyi	Research Centre for Natural Sciences	Phosphoproteomic and Proteomic Analysis of Lung- and Prostate Adenocarcinomas
88	Raja Nirujogi	MRC-PPU, University of Dundee	Development of multi-PTM enrichment to study PTM profile of Lysosomes and Golgi
89	Kish Adoni	UCL	Probing the Host-Virion Protein-Protein Interactome of the Human Cytomegalovirus (HCMV) Assembly Compartment
90	Athanasia Yiapanas	University of Edinburgh - Karolinska Institutet	Quantitative proteomic analysis of adhesion protein localisation in patient-derived glioma stem
91	Linlin Zhang	University of Oxford	Guiding Antiviral Discovery against Filoviruses via Glycosylation Analysis of Viral Spikes
92	Rebecca Rutherford	Seer Inc	A scalable, robust, and sensitive multi-nanoparticle-based label-free mass spectrometry workflow for deep plasma proteomics
93	Ansgar Poetsch	Nanchang University	Effects of ageing and calorie restriction on mitochondrial proteome of brain and heart tissues
94	Anton Neubauer	Hasso Plattner Institute for Digital Engineering	PROTzilla - A novel downstream-analysis platform for proteomics data

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95	Sheon Mary Samji	University of Glasgow	NOX5 promotes vascular smooth muscle cell phenotypic switching in human hypertension
96	Claudia Cavarischia Rega	University of Tübingen	Differential proteomics of somatodendritic and axonal proteins in neurons
97	Dominik Lux	Ruhr-University Bochum	Data-Processing Workflow for Quantifying identified and unidentified Features across measured Stool Samples.
98	Richard Broadhead	Seer	A Cloud-scalable Software Suite for Large-Scale Proteogenomics Data Analysis and Visualization
99	Rosie Maher	University of Liverpool	The influence of highly effective modulator therapies on the sputum proteome in cystic fibrosis
100	Xuan-Tung Trinh	University of Southern Denmark	Data mining antibody sequences for database searching in proteomics data
101	Gianluca Sigismondo	Heidelberg University Hospital and DKFZ	Ion-mobility separation deciphers epigenetic complexity
102	Jenny Ho	Thermo Fisher Scientific	A Benchmarking Workflow for High-Throughput DIA Label-Free Quantification using a Novel High-Resolution Accurate Mass Platform
103	Emmanuel Okwelogu	Nanomedicine Lab	Exploitation of the graphene oxide biomolecule corona in 2D and 3D cancer cell secretome-based biomarker discovery
104	Pedro Casado	Barts Cancer Institute	Proteomically distinct groups of KMT2A-rearranged leukaemia are in different stages of hematopoietic maturation
105	Leandro Neves	University of Liverpool	Mapping Proteolysis During Murine Norovirus Infection Using Multi-Protease N-Terminomics
106	Armin Soleymaniniya	Technical University of Munich	The Proteomes that Feed the World
107	Simon Davis	University of Oxford	Spatial Proteomics of a Human Brain Tumour
108	Marc Guender	Thermo Fisher Scientific	Characterization of Adeno-associated viral proteins and related proteoforms using top-down approach on a LC-Orbitrap Tribrid MS platform
109	Olga Tereszkowska-Kaminska	University of Liverpool	Application of capillary electrophoresis-mass spectrometry to identify histone non-canonical phosphorylation and other post-translational modifications
110	Stephen Holmes	University of Liverpool	Establishing nPOP-based single-cell proteomics at the University of Liverpool

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111	Frédéric Fontaine	ThermoFisher Scientific	An in-depth plasma proteomics workflow powered by a Novel HRAM mass spectrometer
112	Maksym Danchenko	Plant Science and Biodiversity Centre	Consequences of chronic radiation exposure on the proteome, protein carbonylation, and antioxidants: Implications for biotic stress resistance of aquatic plants
113	Petr Halada	BioCeV, Czech Academy of Sciences	MALDI-TOF mass spectrometry on the track of species diversity and bloodmeal sources of phlebotomine sand flies
114	Aristotelis Kotronoulas	Volition RX	Nu.Q® Capture-MS as a novel methodology for profiling the post translational modifications of circulating nucleosomes in colorectal cancer and non-Hodgkin lymphoma patients
115	Sebastian Wolf	German Federal Institute for Risk Assessment	Transient modifications of lysosomal proteases by cosmetic preservative methylisothiazolinone in human contact allergy
116	Jose Luis Marin-Rubio	Newcastle University	Proteomic-based characterization of Trilaciclib in Chronic Myeloid Leukemia (CML)
117	Stephanie Kaspar-Schoenefeld	Bruker Daltonik GmbH & Co KG	Improved dia-PASEF isolation window schemes for proteomics measurements
118	Ece Aydin	Thermo Fisher Scientific	High-throughput nano LC-MS for sample-limited proteomics
119	Rupert Mayer	Research Institute of Molecular Pathology (IMP)	Wide Window Acquisition and AI-based data analysis to reach deep proteome coverage for a wide sample range, including single cell proteomic inputs
120	Clarissa Braccia	San Raffaele Scientific Institute	Large-scale phosphoproteomics reveals divergent kinase-activity profiles in different groups of mouse medulloblastoma.
121	Harendra Guturu	Seer, Inc.	Deep Plasma Proteome Landscape of Alzheimer's Disease: An 1800-Sample Cohort Study
122	Nefeli Boni-Kazantzidou	University of Liverpool	Global phospho-proteomics analysis of EGFRi drug tolerance and resistance mechanisms in cell-based models of non-small cell lung cancer
123	Elisabeth Lang	Medical University of Vienna	Distinct phosphorylation patterns induced by stimulation of mesothelial cells with high glucose containing PD fluid vs. heat stress induction
124	Joel Vej-Nielsen	Evosep	A complete and automated end-to-end sample preparation strategy for high-throughput and standardized proteomics with high sensitivity
125	Colleen Maxwell	University of Leicester	The Edge Effect in High Throughput Proteomics: A Cautionary Tale

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126	Claire Jennings	National Horizons Centre, Teesside University	Conformational investigation of charge heterogeneity through native analysis of monoclonal antibody fragments using High-Resolution Cyclic IM-MS
127	Klara Brozova	Medical University of Vienna	MALDI-MSI of breast cancers reveals intratumoral heterogeneity induced by the tumor microenvironment.
128	Rodrigo Barderas	Instituto de Salud Carlos III	Mass spectrometry analysis of paraffin-embedded tissue samples from CRC patients revealed novel key proteins for the study of the disease
129	Benjamin Neuditschko	IMC University of Applied Sciences Krems	An Anticancer Rhenium Tricarbonyl Targets Fe–S Cluster Biogenesis in Ovarian Cancer Cells
130	Till Möcklinghoff	German Cancer Research Center (DKFZ) Heidelberg	A Quantitative Proteomics Approach to Interferon Signal Transduction – Bridging from the Receptor to the Intracellular Level
131	Xiaoke Yin	King's College London	Poor Quantitative Concordance of Proteomic Data between Autopsy Samples and Fresh Frozen Samples from Human Coronary Arteries
132	Gajanan Sathe	MRC-PPU, University of Dundee	Ubiquitinome Profiling: Optimizing the enrichment and Data-Independent Analysis for Deeper Insights into Targeted Protein Degradation
133	Busra Ergun	Acibadem Mehmet Ali Aydinlar University	Evaluation of Peptide Profiles of OipA Gene On and Off Status on Ulcer Tissues by MALDI Mass Spectrometry Imaging
134	Mario Picciani	TU Munich	Oktoberfest: A search engine and prediction model agnostic rescoring pipeline
135	Kieran McCaskie	University of Cambridge	Fractionation methods for subcellular spatial proteomics: a direct comparison of resolution and reproducibility.
136	Hugo Amedei	University Medical Center Göttingen	Targeted Detection of Protein Complexes by Mini-Complexome Profiling (mCP)
137	Sümeyye Akçelik Deveci	Acıbadem Mehmet Ali Aydınlar University	Comparison of Helicobacter pylori G27 and G27-ΔoipA Infected Gastric Organoid Model Protein Profile ; Understanding the Effect Of Oipa Protein On Helicobacter pylori Infection
138	Louise Ulrich Kurt	Fiocruz - Carlos Chagas Institute	QUIN-XL 2.0: A computational framework for large-scale protein-protein interaction quantification
139	Angela Paul	Bruker UK Ltd	Ultra-high sensitivity for single cell proteome analysis

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140	Irep Uras	Acibadem Mehmet Ali Aydinlar University	Investigation of the Bystander effect in the neuronal culture of 5xFAD Alzheimer's disease transgenic neonatal mouse model
141	Michelle Dubuke	Seer	A Novel Proteomics Workflow for Sensitive and Scalable Analysis of Limited Plasma Samples from Model Organisms
142	Zoltan Szabo	University of Szeged	Tear fluid proteomics: issues of protein sub-populations
143	Sara Gomes	MRC PPU, Dundee	Development of an automated sample processing workflow for mass spectrometry-based assessment of LRRK2 kinase activity in clinical samples
144	Vittoria Monaco	University of Naples "Federico II"	Activity-based Protein Profiling to investigate the interactome of the antimalarial early lead Plasmodione
145	Andrew Frey	Newcastle University	Investigating the Macrophage proteome and PTM assessment by Parallel Accumulation and Serial Fragmentation (PASEF)
146	Lydie Lane	University of Geneva	The neXtProt function prediction project for uncharacterized human proteins
147	Matthias Fahrner	Universitätsklinikum Freiburg	Integrating proteomics into diagnostic molecular pathology reports for molecular tumor board decisions
148	Lisa McGowan	MSVision	Compact, high-resolution time of flight mass spectrometer based on an electrostatic analyser
149	Daniel Hermanson	Thermo Fisher Scientific	Pushing the frontiers of high throughput and high sensitivity analyses: Orbitrap technology unites with a new star
150	Katharina Limm	Preomics	BeatBox and iST for streamlined FFPE tissue processing: A xylene-free, robust, and high-throughput sample preparation for proteomic analysis
151	Patrícia Tavares	CIAFEL, University of Porto	Revisiting the impact of exercise on the proteome of circulating extracellular vesicles
152	Pathmanaban Ramasamy	VIB-UGent Center for Medical Biotechnology	A tissue specific post-translational modification (PTM) map of human proteome
153	Simran Aulakh	University of Oxford	Genome-scale analysis unveils the eukaryotic metal-responsive biochemical network
154	Cristian Piras	Magna Graecia University of Catanzaro	LAP-MALDI and proteomics profiling of milk from different Calabrian bovine breeds

Posters

P1. Online Electrochemical Reduction of mAbs for Rapid LC-MS Analysis

Andrew Reeder, Hendrik-Jan BrouwerP,[1] Tomos Morgan,[2] Jonathan Bones,[2] Craig Jakes,[2] Ken Cook,[3] Jean- Pierre Chervet[1]

Verulam Scientific Ltd

Introduction

In this poster, we will demonstrate the successful reduction of inter- and intrachain disulfide bonds of various monoclonal antibodies (mAbs) with an inline electrochemical flow cell coupled to a liquid chromatography-mass spectrometry (LC-MS) system. The addition of a trap/release column in the chromatographic set-up allowed the analytical separation and mass spectrometry analysis to be unmodified with run times of only 23 min. The study demonstrates the complete reduction of intact mAbs to the corresponding light and heavy chain (Lc, and Hc) subunits. Middle-up subunit analysis by electrochemical reduction coupled to HRAM LC-MS can be carried out from intact antibodies without the need for enzymatic digestion, specific reducing agents, or specific denaturing agents.

Methods

A μ-PrepCell SS cell with a ROXY Exceed Potentiostat (Antec Scientific), was used for the reduction, controlled by Chromeleon software (Thermo Scientific). The temperature was 20 °C for partially and 60 °C for the fully reduced experiments. 1 μL of sample was injected onto the μ-PrepCell with products trapped on a MAbPac column (50 mm length), after column switching samples were washed onto a MAbPac column (100 mm length). Separation occurred with a linear gradient. LC-MS analysis was acquired on a Vanquish Flex Duo UHPLC system coupled to a Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific).

Preliminary data (results)

Electrochemical reduction of both the intra- and interchain disulfide bonds in different mAbs such as Rituximab, Nivolumab, Denosumab, and Cetuximab, could be carried out using electrochemical reduction inline with an LC-MS system. Increasing the electrochemical potential of the electrochemical cell resulted in more complete disulphide bond reduction. Tertiary structure of mAbs was shown to reduce electrochemical efficiency but denaturing the antibodies by termostating the electrochemical flow cell at 60 °C, using the oven of the ROXY Potentiostat, increased the reduction efficiency. The LC-MS system required no modification to the separation and mass spectrometry methods other than the introduction of the electrochemical cell. The reduction of antibodies can be carried out inline with the addition of an electrochemical cell into the chromatographic flow path reducing the intact mAbs. The developed workflow reduces an antibody down to light chain and heavy chain subunits without the need for addition of enzymes or specific denaturing agents. The electrochemical reduction workflow can be used for the analysis of previously digested fabricator samples as well as intact antibody species. Glycoforms of each antibody were shown to be unaffected, even at the maximum level of reduction. Overall, the data showed completely reduced light and heavy chain formation for the online analysis of intact mAbs, and completely reduced light chain, Fd, and Fc/2 subunits when coupled with offline IdeS digestion. Selectivity of the disulfide bond electrochemical reduction by tandem MS could yield useful information about the formation of non-uniform disulfide bonding structures within antibodies.

P2. Molecular Analysis of Malformations in the Mammalian Cerebral Cortex using Proteomics Approaches

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The layered structure of the cerebral cortex is formed through a complicated sequence of highly controlled stages during corticogenesis. The perturbation of neuronal migration and cell division during this process can result a rare disorder called as cortical heterotopia. Patients with heterotopia can have recurrent epileptic seizures, developmental delay, mild intellectual disabilities, and the disorder is not well understood because the human mutations associated with the disease have often failed to give rise to the phenotype in mouse models. EML1 is a heterotopia associated gene where the perturbations cause heterotopia formation in human as well as in mouse. In this project we aim to understand the pathological mechanisms involved in the formation of EML1 related heterotopia using proteomics approaches.

In this project, BioID proximity labeling, microtubulome and live-cell imaging approaches are used to understand mechanisms giving rise to EML1 related heterotopia, using mouse primary neuronal progenitor cells and a neural cell line.

Protein interaction partners of wild-type and mutant EML1 were identified by BioID proximity labeling approach with LC-MS/MS. According to these results, EML1 interacts with many microtubule, cytoskeletal organization proteins including microtubule associated proteins. Strikingly the interaction of many proteins related to cell division and the microtubule cytoskeleton are significantly impacted by a heterotopia causing mutation in EML1. To determine differentially expressed proteins in mouse Eml1 conditional knockout (cKO) primary neuronal progenitor cells, the dimethyl labeling based quantitative proteomic approach is used in three biological replicates to compare the proteome of wild-type and Eml1 cKO mouse models. RNA processing, cell adhesion, chromosome organization, response to ER stress, coating of Golgi vesicle and endocytic recycling are among the pathways found to be downregulated in the Eml1 cKO cells.

This study identified numerous novel interactors along with the known interaction partners of EML1 in neuronal cells. The interaction partner analysis revealed that the heterotopia associated mutant form of EML1 loses many interactions in the cells. Our quantitative proteomics approach defined EML1 associated signaling pathways that provide important resources to study the underlying mechanism in brain development and heterotopia formation.

P3. Prolyl 3-hydroxylase 1 deficiency unhinges collagen post-translational modification patterns far beyond the lack of specific 3-hydroxyproline sites

Vivek Sarohi,[1] Ceylan Onursal,[2] Natalia Cabeza-Boeddinghaus,[2] Elisabeth Hennen,[2] Juliane Merl-Pham,[3] Hans Peter Bächinger,[4] Stefanie Hauck,[3] Roberto Vanacore,[5] Trayambak Basak,[1] Claudia A. Staab-Weijnitz[2]

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Introduction

Collagens, the predominant proteins of extracellular matrix (ECM) play important roles in the maintenance of tissue structure, tissue-specific functions, cell-to-cell communication, cell migration, and cell growth. Among collagens, collagen I is the most abundant. During collagen I biosynthesis, it gets heavily post-translationally modified with hydroxylations (on prolines and lysines) and O-glycosylation (on hydroxylysines) prior to helix formation. Prolyl 4-hydroxylation in collagens contributes to thermodynamic stability and lysyl hydroxylation along with O-glycosylation in collagen plays a pivotal role in collagen cross-linking and fibril formation. However, the role of prolyl 3-hydroxylation in collagen chains is yet to be well understood. Prolyl 3-hydroxylation of collagen I is primarily catalyzed by prolyl 3-hydroxylase 1 (P3H1). Deletion (including mutations) of P3H1 leads to several deformities in muscles and bones. Here, we aimed to comprehensively map the site-specific identification and quantitation of collagen PTM changes upon P3H1 deletion using high-resolution MS.

Methods

Collagen I was extracted from tail tendon of P3H1 knock-out (KO) and littermate wild-type (WT) mice. Tryptic peptides were subjected to LC-MS/MS analysis in a data-dependent (Top10) manner on Q-Exactive HF mass spectrometer. We utilized our optimized proteome-informatics pipeline using a MyriMatch-based strategy for in-depth collagen post-translational modification (PTM) analysis.

Results

We identified 30 3- hydroxyproline (HyP) and 8 O-glycosylation sites in collagen I alpha 1 (COL1A1) from the tail tendon of WT mice and 30 3-HyP and 11 O-glycosylation sites were identified in COL1A1 from of P3H1 KO mice. In COL1A2, we identified 15 3-HyP sites and 8 O-glycosylation sites similar in WT and P3H1 KO samples. Further, PTM occupancy analysis revealed reduced 3-HyP occupancy on P1153 in COL1A1 and P803 in COL1A2 in P3H1 KO samples. We found a significant ($p<0.05$) reduction in 3-HyP occupancy on COL1A1 P760 in P3H1 KO samples (~25%) compared to WT samples (~52%). We also found a reduction (ns) in 3-HyP occupancy on COL1A1 P364 in P3H1 KO (~20%) compared to WT (~33%). On COL1A1 and COL1A2, we also detected increased 3-HyP occupancy in 27 sites, due to the compensatory effect of P3H2 isoform along with increased O-glycosylation on 7 sites in P3H1 KO samples compared to WT.

Conclusion

We report P760 and P364 as novel P3H1-specific sites in COL1A1. Our results indicate that P3H1 deficiency is just not limited to the loss of 3-hydroxylation on selected proline sites, but it can also lead to compensatory induction of numerous changes in collagen I PTM network.

P4. Effects of allosteric or genetic inhibition of ERAP1 on the immunopeptidome and proteome of A375 melanoma cells

Martha Nikopaschou,[1,2] Martina Samiotaki,[3] George Panayotou,[3] Efstratios Stratikos[1,2]

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Introduction

Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an ER-resident enzyme that regulates adaptive immune responses by trimming N-terminal amino acids from antigenic peptide precursors thus preparing them for loading onto Major Histocompatibility Complex I molecules (MHC-I). While ERAP1 activity is necessary for the presentation of many antigenic peptides, it can also over-trim others thus blocking their MHC-I-mediated presentation. Over-trimming can be a mechanism of immune evasion in cancer since the destruction of cancer-specific antigens can reduce cytotoxic T-cell responses. Subsequently, ERAP1 inhibition is an emerging strategy for cancer immunotherapy. Modulation of ERAP1 can be modeled in vitro by two distinct methods: genetic knock-out or pharmacological inhibition. While efforts for the development of ERAP1 inhibitors were initially focused on the enzyme's active site, concerns for off-target effects have shifted the focus to allosteric sites.

Methods

In this study, we used a library-free data-independent acquisition (DIA) strategy to explore the effects of allosteric inhibition of ERAP1 focusing on the B3P site. To do so, we used a modulator that targets this site on A375 melanoma cells and compared the obtained immunopeptidome with that of wild-type and ERAP1 KO cells. While immunopeptidome shifts are expected as a direct outcome of ERAP1 inhibition, indirect changes in the cells' proteome may also contribute, which led us to the utilization of DIA proteomics to explore changes in expressed proteins.

Results

Immunopeptidomic analysis identified 800 presented peptides (FDR=0.05), with all conditions presenting peptides of high-affinity and with appropriate sequence motifs for the HLA expressed by A375 cells. Notably, the shifts in peptide composition were not identical between the two methods of ERAP1 functional disruption. Proteomic analysis identified 5575 proteins, of which 1583 were differentially expressed (FDR=0.01, S0=0.1). Subsequent pathway analysis indicated statistically significant proteomic changes in pathways relating to RNA metabolism, cellular response to stress and translation under both methods of ERAP1 functional disruption with these effects being more prominent in the KO cells. Despite substantial changes in the cells' proteome, less than 6% of these proteins were represented in the immunopeptidome. Nevertheless, peptides from differentially expressed proteins constituted 19% and 13% of the KO- and inhibitor-altered immunopeptidome, respectively. Taken together, our findings suggest that allosteric inhibition of ERAP1 can generate unique immunopeptidomes, primarily due to altered peptide processing in the ER, and that ERAP1 functional disruption may affect the cellular proteome by indirect mechanisms possibly related to the contribution of ERAP1 to ER homeostasis.

P5. Site-specific N- and O-glycan Analysis of ACE2, SARS-CoV-2 and RBD proteins

Clemens Grünwald-Gruber, Daniel Maresch, Rudolf Figl, Johannes Stadlmann

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Introduction

The viral entry of SARS-CoV-2 is dependend on the binding of its Spike protein to angiotensin converting enzyme 2 (ACE2) of the host cell. Both proteins are heavily glycosylated and the type and site occupancy of the glycosylation is crucial for the interaction. The comprehensive evaluation of N-glycan profiles of several biotechnological produced proteins (spike, nucleocapsid, RBD, ACE2) is presented in this study to shed light on the differences of the individual platforms.

Method

Recombinant expressed and purified proteins were carbamidomethylated and digested with various proteases to obtain site specific (glyco-)peptides, which were analysed using a QTOF (Bruker maXis 4G) or Orbitrap (Exploris 480) MS system in the positive DDA mode.

Glycopeptides were identified as sets of peaks consisting of the peptide moiety and the attached N-glycan varying in the number of HexNAc units, hexose, deoxyhexose and pentose residues. Verification of putative glycopeptides was done by MS/MS.

Results

Analysis of ACE2:

ACE2 produced in CHO cells and plants was analysed. The eight individual glycosylation sites were identified site specific as glycopeptides performing a combined chymotryptic/tryptic digest. Both expression systems showed the expected glycoforms and additionally differed in the site occupancy of some glycosites, which affected the binding properties of the spike protein to ACE2.

Analysis of Spike:

The 22 N-glycan sites of Spike remain highly conserved among all SARS-CoV-2 variants identified, showing the importance of the glycosylation. In our study we were able to identify all 22 sites in three different production systems, allowing an interesting comparison of the biotechnological platforms used. To achieve a complete glycosite coverage, five different proteases were used (trypsin, pepsin, Chyotrypsin, GluC, LysC)

P6. Deeper plasma proteome coverage enables identification of novel biomarkers and classification of diseases

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Introduction

Blood plasma is one of the least invasive biopsies and a valuable specimen for clinical research and patient health monitoring. Since almost all tissues are sustained by the constant blood flow and proteins are constantly being actively secreted or leaked into the blood, plasma provides comprehensive information about health or disease state. However, access to proteome information is limited by the highly dynamic nature of protein abundance in plasma, which spans approximately 10 orders of magnitude and with only 22 proteins accounting for 99% of the whole protein mass. To address this challenge, we developed a novel workflow for LC-MS-based plasma proteomics that enriches low abundant proteins and enables an improved coverage of the plasma proteome.

Methods

Starting from 20 µl plasma per sample, the described workflow achieves efficient dynamic range compression by capturing and thus enriching low abundant proteins on paramagnetic particles using dedicated buffer conditions (called ENRICH technology, PreOmics). The particles were separated from the residual plasma and briefly washed to remove residual low-affinity interactors. For subsequent LC-MS sample preparation, samples were processed with our iST-BCT protocol (PreOmics) including on-bead reduction and alkylation, digestion and peptide clean-up. Resulting peptides were analyzed by on-line nanoC18 separation on a nanoElute LC coupled to a TimsTOF HT mass spectrometer using a DIA-PASEF acquisition cycle (Bruker). Measured peptides are identified and quantified by Spectronaut 17 software suit (Biognosys) against a plasma proteome database.

Preliminary Data

We applied our novel workflow to a set of plasma samples derived from lung cancer patients and age matched healthy donors. First, samples (N = 10 healthy, 10 lung cancer) were prepared with the published iST protocol and spiked with the PQ500 reference panel, secondly identical plasma samples were processed with the ENRICH technology coupled to iST-BCT sample preparation. For each analysis, 300 ng of peptides were injected. From neat plasma samples, we recovered 350 protein groups per sample, covering 405 protein groups represented in the PQ500 panel. From ENRICH-processed samples, we obtained on average 1000 protein groups per sample and were able to recover 800 protein groups in the majority of the two plasma conditions. Statistical analysis of quantified proteins demonstrated a clear separation of healthy donors from patients with lung cancer diagnosis. Strongly upregulated factors in lung cancer samples include S110A8 and S100A9 which have previously been reported to be upregulated in lung cancer and other types of cancer (Gebhardt, et al.) Gebhardt C. et al., Biochem.Pharmacol. 2006; 72(11):1622-31

P7. Analysis of the proteome and phosphoproteome of PFIC3 for understanding involved molecular mechanisms and cellular processes

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Progressive familiar intrahepatic cholestasis type 3 (PFIC3) is a severe rare liver disease which affects between 1/50,000 to 1/100,000 children. Under physiological conditions, bile is produced by hepatocytes in the liver and bile is subsequently stored in the gallbladder, then it flows to the duodenum to play its role in fat digestion. Bile acids are stored in fatty acid micelles to prevent tissue damage. PFIC3 is due to mutations in phosphatidyl choline transporter ABCB4 (MDR3) that lead to intrahepatic accumulation of free bile acids, resulting in liver injury. PFIC3 typically manifests at a young age, progresses rapidly, and has a poor prognosis. The only available treatment for this illness currently, other from the palliative use of ursodeoxycholate, is liver transplantation, which is extremely difficult for young patients.

We conducted an integrative proteome and phosphoproteomics analysis in human liver tissues to gain insight on the pathophysiology of PFIC3, and we subsequently validated the developing functional hypotheses in a PFIC3 mouse model. 324 proteins out of the 6,246 protein groups that we identified exhibit differential expression between the control and PFIC3 samples. The phosphoproteomic analysis allowed the identification of 5,090 phosphopeptides, of which 215 were differently phosphorylated in PFIC3. These 215 peptides correspond to 157 protein groups, including MDR3.

The main processes identified as drivers of this disease were inflammation, metabolic reprograming, cytoskeleton and extracellular matrix remodeling and cell proliferation. Furthermore, regulation of several sORF (short open reading frames) of relatively unknown function were also detected in PFIC3 patients. Our findings provide a solid molecular background that significantly contributes to a better understanding of PFIC3 and provides new concepts that might prove useful in the clinical management of patients.

P8. Characterising a novel tumour suppressor in Liver cancer

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KDM8/JMJD5 is and under-investigated 2-oxoglutarate dependent dioxygenase with alleged lysine demethylase activity. KDM8-loss is embryonically-lethal as it is involved in embryonic development, cell cycle regulation, transcriptional regulation and in DNA damage repair pathways. In adult tissues, it is expressed almost exclusively in liver cells and is actively involved in liver metabolism and hepatocyte differentiation. Patient data from the cancer genome atlas (TCGA) has shown that the expression of KDM8 is significantly downregulated in all liver hepatocellular carcinoma cohorts (LIHC), suggesting its activity as a tumour suppressor. While it was previously found to be a prognostic gene in pancreatic adenocarcinoma cohorts, research on KDM8 in the context of liver cancer is lacking.

In this project we try to uncover the consequences of silencing KDM8 in liver cancer. We have performed bottom-up proteomics analysis on peptides extracted from hepatocellular carcinoma cell lines (Hep-G2) where KDM8 was either wild-type, knocked -out, re-expressed or re-expressed with catalytically inactive mutation.

The data reveals a significant disruption in the proteome upon knocking out KDM8. Over 300 different proteins were significantly up or downregulated. Loss of KDM8 led to an increased expression of metabolic proteins, notably the ones involved in drug metabolism, bile secretion, fatty acid metabolism and glycolysis. Proteins encoded by GSTA family of gene, which are involved in chemical carcinogenesis were upregulated. PPM1M and PPM1D which are negative regulators of p53 were also upregulated. Notable tumour suppressors like HEPACAM and ARID1A were downregulated. Proteins involved in NF-κB activation like PEG3, MYD88 and P62 were downregulated in the catalytically inactive mutant and knock-out cell lines. Gene set enrichment analysis revealed downregulation of mTORC1 signalling and MYC targets V2 which are common cancer hallmarks. This data was also corroborated in patients. mRNA expression data of LIHC cohorts from TCGA was corelated with KMD8 expression and we see upregulation of bile acid and fatty acid metabolism and downregulation of MYC targets V2 and mTORC1 signalling. This was a clear reflection of what we observed in our proteomics data.

P9. Characterising Novel off-target effects of a Brain-Penetrant Drug in Glioblastoma Cell Lines using different proteomic approaches

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Introduction

Glioblastoma Multiforme (GBM) is an aggressive brain tumour with poor prognosis, accounting for up to 50% of all gliomas. For decades, treatment of this tumour has been limited to surgery with concurrent radiation and chemotherapy with temozolomide. However, recurrence is inevitable and resistance develops rapidly to current treatments, leading to a high mortality with a median survival of approximately 15 months. Over the past decades no new treatments have been licenced for GBM. Therefore, new approaches to drug discovery are particularly pertinent to facilitate treatment strategies for GBM. Drug repurposing, through identification of underappreciated off-target actions in existing drugs approved for other indications, is an attractive strategy for identifying new treatment options for cancer patients, and reduces the extremely long and arduous drug discovery process.

Methods and Results

We characterised off-target effects of an antipsychotic drug arising from a screen of FDA-approved compounds designed to identify drugs that reduce proliferation of glioma neural stem cells. To characterise the previously unrecognised off-target actions for this class of antipsychotics we used a combination of expression and chemical proteomics methods and identified that the disruption of lipid metabolism, leading to autophagy block and mTORC1 inhibition as early biological phenotypes. Proteomic approaches such as bead-affinity pull down helped in generating hypotheses regarding the spectrum of targets of the drug and subsequently played an integral part in drug target deconvolution and discovery of off-target effects. The magnitude and reproducibility of observed phenotypes suggests this class of agents as promising candidates for future use in GBM. Further analysis will identify the precise molecular mechanism underpinning these phenotypic changes. Following full characterisation of this novel mechanism of drug action, these agents will be accelerated toward in vivo testing in proof-of-concept studies using animal GBM models.

P10. USP18 and its role in mediating IFN response of triple-negative breast cancer

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Introduction

Triple-negative breast cancers (TNBC) are amongst of the most aggressive tumours displaying high invasiveness and high recurrence rate. In TNBC treatment, immune checkpoint blockade (ICB) can stimulate immune activation and increase the anticancer immune response. The response rate for ICB is highly variable and it is more likely that highly immunogenic, "hot" tumours respond successfully. Type I interferon (IFNs) can enhance cancer immunogenicity and promote immune cell infiltration. Accordingly, modulation of IFN signalling is being investigated for transforming “cold” into “hot” tumours. However, the IFN response signalling pathway is frequently dampened by negative feedback inhibitors such as USP18. Consequently, we hypothesise that USP18 inhibition may potentiate the IFN response and synergise with ICB. Understanding how USP18 regulates the immune system and microenvironment, specifically in highly aggressive and metastatic TNBC, is essential to determine if USP18 inhibition is a viable strategy. There is, however, scarce data on how USP18 inhibition affects immune resistance and cancer metastases.

Method

To investigate the role of USP18 in breast cancer progression, the CRISPR-Cas9 system was used to deplete of USP18 in a human TNBC cell line (MDA-MB-231). Then, characteristics of USP18-depletion were then studied in vitro, including proliferation, cell migration as measures of tumour aggressiveness. Additionally, we determined how USP18 loss altered the proteome under basal and IFN-stimulated conditions.

Results

To investigate the IFN response of human TNBC, MDA-MB-231 cells were treated with IFN-α2b between 0-10,000 U/ml. Cell viability was decreased by IFN-α2b treatment in a concentration dependent manner. Moreover, we found that USP18-KO sensitized IFN response by ~50-fold compared to WT MDA-MB-231 cells, suggesting a critical role for USP18 in promoting cell viability when challenged by IFN-α2b. Furthermore, we found that cell migration was decreased upon loss of USP18. To characterise the proteome between WT and USP18-KO MDA-MB-231, proteins were extracted and analysed using LC-MS/MS. The proteomic results demonstrated that the absence of USP18 enhanced IFN-response pathways in MDA-MB-231, exemplified by increased expression levels of downstream signalling molecules including STAT1, STAT2, IFITM1, and other interferon-stimulating response element gene products (ISGs). In addition, we found that human-leucocyte antigens (HLAs) were broadly upregulated in USP18-KO which suggests an increase in antigen presentation. This could promote an anti-cancer immune response. In contrast, cell adhesion molecules were downregulated in USP18-KO cells which could be related to cell migration defects. In summary, here we show that USP18 could be a potential target for anti-cancer immune response in TNBC.

P11. Phosphoproteomic characterization of GSK-3 in autophagy

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Macroautophagy, hereafter referred to as autophagy, is a highly conserved cellular process that allows cells to degrade and recycle their own components including organelles, macromolecules and proteins (Yu, Chen et al. 2018). Autophagy is essential for maintaining cellular homeostasis as well as responding to a variety of stressors. Dysregulation has been implicated in several diseases including cancer and neurodegenerative diseases (Mizushima and Levine 2020). Various kinase complexes are associated with autophagy regulation including mTOR complex 1 (mTORC1), a major suppressor of autophagy by phosphorylating and thereby inhibiting UNC-51-like kinase (ULK1) complex, one of the components to initiate autophagy. However, recent studies also implied glycogen synthase kinase-3 (GSK-3), to be involved in the negative regulation of autophagy on various levels. Whether this is only upstream of mTORC1 is currently under debate.

In the present study, we aim to gain a deeper understanding of the functions of GSK-3 in autophagy by investigating the molecular mechanisms and determining the impact of GSK-3 dysregulation on autophagic processes. Taking into consideration that ULK1 is a direct mTORC1 target, we compared the effects of mTORC1 and GSK-3 inhibition under growth conditions on the phosphoproteome using SILAC-based quantitative mass spectrometry. Proteins of various pathways were identified as carrying significantly regulated phosphosites. Among those, phosphosites on known autophagy relevant proteins were found to be downregulated. Additionally, phosphosites on cytoskeleton proteins were highly regulated, with most proteins either associated with actin or tubulin. These findings, together with functional analyses let us to hypothesize that the vesicular transport could potentially be impaired upon inhibition of GSK-3. To test this hypothesis, both pharmacological inhibition and gene knockouts of GSK-3 are used to study vesicle trafficking in different autophagy-inducing conditions.

P12. Exploring Motif Preferences and Functional Specificity of the Protein Arginine Deiminase Family

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Introduction

The protein arginine deiminase (PAD) family catalyzes the conversion of arginine to citrulline in proteins, a process known as citrullination. Citrullination affects protein conformation, function, and interactions, playing a significant role in gene regulation, cell differentiation, and immune responses. Dysregulation of PAD activity contributes to the pathogenesis of several diseases, like rheumatoid arthritis, multiple sclerosis, and cancer. Among the five human PAD isozymes, only PAD1-4 are catalytically active in vitro. Previous studies have demonstrated their distinct substrate protein profiles, but it is unclear whether this arises from cellular location, substrate accessibility, or unique motif preferences. However, no comprehensive study has explored this motif selectivity in complex protein backgrounds thus far. This study aims to unravel the motif preferences of PAD isozymes, assessing the impact of cellular location and substrate accessibility on their specificity. By integrating proteomic analyses and enzymatic assays, we will characterize PAD enzyme motif selectivity and its relevance to disease. Understanding these mechanisms will inform targeted therapies for diseases associated with dysregulated PAD activity.

Methods

To characterize the target sequence motif of the PAD enzymes, the lysates of three cell lines (HeLa, H4 and HEK298) with no intrinsic PAD expression were incubated with PAD1-4 to induce extensive citrullination in vitro. Successful citrullination was confirmed by western blots analysis using a custom pan-citrullination antibody. Following sample clean-up and digestion, the samples were fractionated and finally analyzed using micro-LC-MS/MS. Citrullination sites were tentatively assigned by MaxQuant and curated using customized tools to achieve confident identifications. The amino acid sequence of citrullinated peptides were examined in the context of the human background proteome to assess the presence of specific motifs favored by PAD enzymes in general and to identify potential differences in substrate preferences among the enzymes.

Results

Consistent with previous studies, western blot analysis of citrullinated lysates demonstrated distinct substrate protein profiles among the PAD isozymes. Mass spectrometric data analysis provided initial insights into the preferred target sequences of PAD enzymes. Specifically, PAD4 exhibited a preference for Asp at the -1 position relative to the citrullinated arginine, as well as for Asp, Glu, Lys, and Gly at the +1 position. In comparison, PAD2 displayed a more permissive target specificity, with a wider range of tolerated amino acids surrounding the citrullination site. These findings suggest that PAD4 exhibits a more restricted target specificity than PAD2.

P13. Monitoring autophagy using parallel reaction monitoring

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Introduction

Autophagy is a major catabolic pathway in eukaryotes, delivering damaged or superfluous cellular material to the lysosome for enzymatic digestion and recycling of their basic components. This pathway is constitutively active at low basal levels but greatly enhanced by a variety of stressors including starvation or organellar damage. While autophagy is often considered a non-selective process in which random cytoplasmic material is delivered to the lysosome, autophagy can also be extremely selective specifically targeting specific cellular component depending on cellular needs. Selectivity is ensured by a variety of "cargo receptor proteins", bridging the autophagic core machinery and the specific cargo.

Common methods for monitoring autophagy usually involve immuno-detection of specific proteins or fluorescence labeling followed by microscopy. While these are well established and efficient methods, performing them in a high throughput fashion or automating them remains challenging. Additionally, several of these methods rely on the quantification of single markers, which probably underestimates the complexity of the whole process.

Methods

Here we describe a parallel reaction monitoring (PRM) assay for accurate quantification of proteins involved in human autophagy. Targeted proteins include components of the core autophagy machinery as well as specific cargo receptors in order to enable distinction between different autophagic pathways.

Results

Using this assay, we can monitor the accumulation of autophagy receptors and other known markers upon lysosomal hydrolase inhibition, a common method to measure autophagy. We identified a set of core autophagy proteins that are being turned over under basal conditions but appear to be spared from degradation upon stress, when these become needed for efficient autophagy.

P14. Single-Shot deep DIA LCMS workflow for near-full proteome coverage

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Introduction

Comprehensive identification and quantification of all proteoforms present in biological samples has been the ultimate ambition of LC-MS based proteomics. 2DLC approaches, based on fractionation of digested proteins using orthogonal separation mechanisms (SCX, high-pH C18, HILIC) followed by low-pH reversed phase LC-MS analysis of isobaric labeled peptide samples provided deepest coverage. Recent innovations in LC-MS instrumentation have leveraged significant increases in analysis speed, resolution and sensitivity, allowing similar coverage using single-shot label-free analyses with ultra-high peak capacity LC-MS, significantly reducing labor-intensiveness, overall cost and required analytical skills needed to achieve near-full proteome coverage.

Methods

Protein digest samples were analyzed using a Vanquish Neo UHPLC and Orbitrap MS set-up, utilizing a 110cm µPAC Neo column with gradient lengths ranging from 67 to 240min.

Lyophilized human cell digest standards (Pierce HeLa) were resuspended in 0.1% FA to a final stock solution of 1µg/µl. For the three-proteome mix, E. coli protein digest (Waters) and yeast protein digest (Promega) were added to a fixed amount of HeLa digest (2µg) at amounts of 400 to 800, and 600 to 200ng, respectively. Yielding an E. coli peptide ratio of 1:0.5 and a yeast peptide ratio of 1:3.. Both DDA and DIA acquisition strategies were used to collect data. LC-MS data were searched using Spectronaut 17 or a beta version of Thermo Scientific Proteome Discoverer 3.1.

Results

Single-shot deep dive proteomics requires LC columns delivering highest peak capacities to reduce sample complexity as much as possible prior to MS/MS analysis. Typically, long nanoLC columns (up to 75cm) packed with sub-2µm C18 silica particles are used. Operated at pressures near the maximum of current state-of-the-art UHPLC system, peak capacities close to 1200 have been reported on packed bed nanoLC columns.

The 110cm long µPAC Neo column, uniformly filled with 2.5µm pillars, results in increased separation performance, delivering peak capacities well above 1600 in single-shot analyses, with median peak width as low as 4.6 and 7.2s for gradient lengths of 90 and 180min.

Here, we evaluated the effect of increased separation performance on proteome depth for single and multiple species tryptic digests. Using library-free data processing to analyze single shot 180 min gradient separations, up to 10.300 protein groups could consistently be identified from a single species (human) protein digest sample (no matching between runs). Using the same method but injecting the three-proteome mix, more than 15.000 protein groups could be identified, near-full coverage of all three species.

P15. Exploring Cellular Signaling Networks in ProteomicsDB: Interactive Visualization of Differentially Regulated Post-Translational Modifications in Biochemical Pathways

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Introduction

A central element of cellular signaling mechanisms are protein post-translational modifications (PTMs). Upon a perturbation such as a drug treatment, many cellular proteins undergo a change on PTM level, which can have fundamental consequences for the phenotype. While large-scale PTM data nowadays is routinely acquired by proteomics labs, it is still hard to 'connect the dots', i.e. to interpret the up- and down-regulated PTMs in the context of signaling cascades. Here, we present a web-based software tool to explore proteomics datasets from a pathway-centric viewpoint. We collected hundreds of manually curated pathways for 10 different species from publicly available databases (KEGG, WikiPathways). Our software is able to render these pathways in an interactive fashion and project experimental PTM data onto them. The tool will soon be hosted on ProteomicsDB (www.proteomicsdb.org), and users can either browse through the data available there or upload their own experimental results.

Methods

KEGG and WikiPathways provide pathway definitions in the form of XML files that describe which proteins are part of a pathway and how they are connected. We implemented a Python package that converts these files into a uniform internal representation. Then we developed a Vue.js application that can read these and combine these representations with a list of modified peptides measured in a perturbation experiment. After uploading a dataset, the user can select from the list of available pathways. The data is then projected onto the graphical representation of the pathway using the library D3.js as a programmatic framework. The visualization is dynamic, meaning that users can move the nodes of the graph around, highlight interesting subnetworks and switch back and forth between different pathways and datasets. Our software is open source and we intend it be reused outside of ProteomicsDB as well.

Results

Using a number of recently published datasets, we demonstrate some applications of our software. For example, a common, but superficial way of interpreting PTM data from a pathway perspective is an overrepresentation analysis, which yields a list of pathways that are possibly relevant for the interpretation of the data. Our tool can give the user a deeper look into the results of such an analysis and help to confirm or deny whether the suggested pathways are actually interesting. Other use cases include generating hypotheses about the functions of phosphosites and studying signaling downstream and upstream of the primary targets of a drug, which we illustrate with several examples.

P16. A simplified orthogonal electrospray source setup for robust nanoflow or microflow proteomics analysis

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Most bottom-up proteomics analysis workflows are based on nanoliter-per-minute flow rates (nanoflow) during the chromatographic separation of analyzed peptides. This flow regime typically increases the sensitivity of the assay compared to higher flow rate regimes. However, there are significant challenges with nanoflow that impact productivity, such as the fragility of nanoflow spray emitters, their susceptibility to clogging and leaks in the plumbing connections. The OptiFlow TurboV ion source on the SCIEX ZenoTOF 7600 system gives users the capability to do electrospray ionization through either nanoflow (≤ 1 µL/min) or microflow (≥ 1 µL/min, ≤ 50 µL/min) LC connections through 2 separate dedicated electrospray probes/electrodes designed specifically for each of these workflows. In this work, we describe the application of a single probe/electrode for either flow rate during proteomics analysis. Here, the dedicated microflow probe/electrode was tested at flow rates commonly used for nanoflow. This setup has the advantages of (1) spraying orthogonally to the source inlet, thereby minimizing eventual contamination of the system through extended use, (2) not requiring any additional hardware or interface changes to the MS system and (3) simultaneous use of the built-in Calibrant Delivery System (CDS) for fast, easy calibration of the MS system. The performance of the MS system using the microflow probe at nanoflow flow rates was determined using both simple peptide mixtures and complex cell lysate digests, with different LC column types and on-column peptide loadings. At typical nanoflow flow rates (300 nL/min), the microflow probe yielded equivalent performance to when using the dedicated nanoflow probe on the OptiFlow TurboV ion source. The use of the microflow probe/electrode therefore offers users the option for a simplified setup while maintaining the high sensitivity performance required for impactful nanoflow proteomics workflows.

P17. Charged surface stationary phase allows for minimizing formic acid concentration in the mobile phase, enhancing electrospray ionization in LC-MS proteomic experiments

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Introduction

The default choice of mobile phase acidifier for bottom-up LC/MS proteomics analysis is 0.1% formic acid because of its decent ion pairing ability and low MS signal suppression. Despite the inception of state-of-the-art columns, designed specially to provide efficient separation even when using an MS-friendly mobile phase of low ionic strength, no attempts have been made to exploit the maximum sensitivity of the analytical methods by reducing the amount of formic acid to its optimum minimal concentration.

Methods

This study used analytical columns packed with a charged surface hybrid (CSH) stationary phase to evaluate the MS sensitivity gain obtained by reducing the formic acid concentration in the mobile phase. The effect of the reduction in the acidifier concentration on the chromatographic separation of peptides was also evaluated.

Results

The application of 0.01% formic acid in the mobile phase maintained excellent chromatographic performance and led to a ≈2× increase in MS signal intensity. The increased retention of peptides by ≈0.4% ACN at reduced formic acid concentration was directly proportional to the number of acidic residues on the sequence. The increased MS sensitivity translated to ≈50% improved identification depending on the complexity and amount of sample injected. The effect of reduced formic acid concentration in the mobile phase was evaluated using peptide samples of varied complexity (simple peptide mixture, trastuzumab digest, bacterial cells digest, and eukaryotic cells digest) and on microflow and nanoflow regimes to expand the applicability in routine practice.

P18. Integrated view of baseline protein expression in human, mouse and rat from reanalysis of public DDA and DIA experiments

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Introduction

The availability of proteomics datasets in the public domain, and in the PRIDE database in particular, has increased dramatically in recent years. This unprecedented large-scale availability of data provides an opportunity for combined analyses of datasets to get organism-wide protein expression data in a consistent manner.

Method

We have reanalysed 24 public proteomics datasets from healthy human individuals, 14 datasets from mouse and 9 datasets from rat, to assess baseline protein abundances. Overall, the aggregated human dataset contains 67 healthy tissues from 31 organs, corresponding to 3,119 mass spectrometry runs covering 498 samples, coming from 489 individuals, whereas the aggregated mouse and rat dataset contains 211 samples coming from 34 different tissues across 14 organs, comprising 9 mouse and 3 rat strains, respectively.

Results

We compared protein expression between the different organs and studied the distribution of proteins across organs. We also compared the results with data generated in analogous studies. We performed gene ontology and pathway enrichment analyses to identify organ-specific enriched biological processes and pathways. We carried out a comparative analysis of protein expression between mouse, rat and human tissues. We observed a high level of correlation of protein expression among orthologs between all three species in brain, kidney, heart and liver samples. As a key point, we have integrated the protein expression results into the resource Expression Atlas, where it can be accessed and visualised either individually or together with gene expression data coming from transcriptomics datasets. We believe this is a good mechanism to make proteomics data more accessible for scientists, especially those non-experts in proteomics.

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P19. Improved DIA and DDA performance on low-level proteomic samples using a novel Zeno trap

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SCIEX, Canada

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 6483% 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.

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P20. The single amino acid substitutions in Mason-Pfizer Monkey Virus matrix protein modulate its proteolytic cleavage rate

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N-terminal domain of polyprotein Gag of Mason-Pfizer Monkey Virus (M-PMV) Matrix protein (MA) is naturally myristoylated on its N-terminus. When MA is a part of polyprotein Gag, the myristate moiety is buried inside hydrophobic pocket and exposed probably upon interaction with host cell plasma membrane. This mechanism called myristoyl switch does not occur in M-PMV as readily as it does in HIV-1, suggesting that it may have an important role in M-PMV maturation by regulating the MA cleavage from Gag polyprotein. To address this hypothesis, we have tracked the cleavage rate of MA from several protein constructs on artificial liposomes mimicking plasma membrane. To support the observations, we also performed the proteins structural analysis.

Proteolytic digestion of myristoylated (myrMAPPHis) and nonmyristoylated MAPPHis (nonmyrMAPPHis) M-PMV protein constructs, bearing the cleavage site for M-PMV viral protease (Pr13), was performed in solution and also on liposomes with treating of proteins by Pr13. The resulted products were then visualized by SDS-PAGE. The hydrogen-deuterium exchange coupled with mass spectrometric detection of deuterium incorporation (HDX-MS) was applied to reveal the structural differences in MAPPHis protein forms (liposome free).

In contrast to rapidly degraded nonmyrMAPPHis even without liposomes, the myrMAPPHis, surprisingly, become cleaved more frequently only after addition of liposomes indicating the possible exposure of myristate upon interaction with plasma membrane.
To support our findings, we have designed and examined also four MAPPHis mutants with single amino acid substitutions with expectation to block or, conversely, facilitate the myristoyl switch by stabilization (A79V, A79L) or destabilization (I51A, I86A) of MA M PMV hydrophobic pocket. The mutants A79V and A79L were cleaved even less effectively than myrMAPPHis as well as the degradation of mutants I51A and I86A was faster compared to nonmyrMAPPHis. The different cleavage rates of proteins were confirmed also by using HDX MS method. In contrast to myrMAPPHis wild type, the HDX-MS analysis of proteins I51A and I86A shows protease cleavage site more accessible even with the indication of formation of oligomers, thus at least their partial myr-switch event already in solution. Unexpectedly, the destabilisation effect was confirmed also for mutant A79L. Contrarily, the mutant A79V showed the clear evidence of stabilisation of hydrophobic pocket as an effect of A79V mutation. The data show that the protease cleavage site has different dynamics in proteins as a result of disruption/stabilisation of hydrophobic pocket.

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P21. An approach to integrate metagenomics, metatranscriptomics and metaproteomics data found in public resources

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The amount of public metaproteomics, metagenomics, and metatranscriptomics data available in resources like MGnify and the PRIDE database is increasing, offering opportunities to gain a more comprehensive understanding of the microbiome's structure and functional expression when these techniques are applied to the same sample. In this pilot study, we integrate public multi-meta-omics datasets from three studies, two based on human gut samples and one on marine samples.

We created reference search databases for the proteomics analysis using assembled metagenomic (and metatranscriptomic, where available) sequence data and de novo gene calling, utilizing both data from the same sampling event and independent samples. We evaluated the resulting protein sequence databases' effectiveness in metaproteomics analysis using SearchGUI (search engines) and PeptideShaker for post-processing. Consistent with previous results, we found the highest proportion of peptide identifications when using reference search databases created from the same samples.

We extended the MGnify website to visualize the resulting peptide/protein information from the three reanalyses of the metaproteomic datasets, linking to the original datasets in PRIDE. We also developed a publicly available open workflow (https://github.com/PRIDE-reanalysis/MetaPUF) to allow researchers to perform equivalent multi-omics integration using paired multi-omics datasets. To our knowledge, this is the first implementation of a data integration approach for multi-omics datasets in MGnify and PRIDE.

P22. Comparison of synthetic co-polymers and classical detergents as reagents for membrane protein solubilization utilizing shotgun proteomics

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The solubilization of native and structurally intact proteins is essential for the proteomic characterization of protein structures and the interaction of proteins with biological molecules, including nucleic acids, metabolites, and drugs. While soluble proteins are easily extracted in aqueous buffers, the addition of detergents is essential for extraction and stabilization of membrane proteins in solution. Non-ionic detergents, such as Digitonin or Dodecyl-ß-D-maltoside, combine low denaturation potential with sufficient solubilization efficiency and are commonly used for mild membrane protein extraction. Recently a novel class of synthetic polymer-based reagents has been demonstrated to allow single step solubilization and stabilization of native membrane proteins in near native quality suitable for structure determination by Cryo Electron Microscopy or interaction analysis by surface plasmon resonance. Here, we present a global characterization of transmembrane protein extraction properties of novel, non-denaturing synthetic co-polymers, namely SMA, DIBMA, AASTY, and Amphipol, in comparison to classical ionic and non-ionic detergents. Using LC-MS based proteomic analysis of HEK293T membrane pellets, we were able to show that synthetic co-polymers extract membrane proteins in a similar efficiency compared to detergents and that protein abundances in the extracts roughly followed the RNA expression levels. However, despite this trend, we hardly observed any additional correlation between solubilization efficiency and other physicochemical features such as number of transmembrane domains, hydrophobicity, isoelectric point, or charge, neither for the detergents, nor for any of the co-polymers. Overall, synthetic co-polymers are promising reagents for many applications, including the structural analysis of membrane proteins, affinity-based proteomics or generally as components of native lysis and solubilization buffers.

P23. Exploring the benefits of differential mobility separation (DMS) and SWATH acquisition for complex proteomic sample analysis

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The advent of SWATH DIA in the past decade has revolutionized LC-MS analysis in proteomics applications. The ability to select a wide precursor window in combination with high-resolution MS/MS detection allows the selective detection of multiple peptides simultaneously on an LC time scale. With the ability to control the range of precursors subjected to MS/MS, it is possible to maintain the analytical selectivity when LC analysis time is reduced, thus enabling faster analysis. However, under fast LC separation (<10min), smaller precursor windows are frequently required to maintain selectivity. Here we propose using differential mobility separation to improve the selective detection of peptides from larger SWATH windows. This involves optimizing the transfer from the DMS (differential mobility separation) cell to the TOF detector to maximize coverage of the CoV (compensation voltage) space, while providing sufficient data points across the LC peak for quantitative analysis. Tryptic digest from human K562 cell lysate were analyzed using an Evosep One LC system using the 100, 200 and 300 samples/day (SPD) workflow representing 20, 10, 5 and 3 min gradient elutions, respectively. Analysis was performed on a TripleTOF 6600 system with a SelexION device for DMS separation. Using a fixed precursor SWATH windows of 25 amu size, replicate analysis (n=3) of K562 digest were performed at different compensation voltages (CoV) ranging from 10 V to 33 V, at a fixed SV (separation voltage) of 3800V. The data showed that when all DMS-enabled acquisitions are combined and compared to data acquired without the DMS active, the number of unique peptides detected increased by 75%, while the number of unique proteins increased by 48%. Using this information, an acquisition method combining different SWATH windows operated at different CoV values was built to maximize the peptide coverage while minimizing the number of injections to obtain the total information. With this approach, similar increases in the number of peptides and proteins were observed relative to data collected with the DMS turned off. To assist in data collection and processing, research software tools were developed to simplify the building of acquisition methods, and tools to parse the collected data into independent SWATH-CoV combinations enabled DIA-NN processing. When the DMS-SWATH approach was applied to fast LC analysis (5min, 200 SPD), the number of unique peptides detected increased by ~ 80% whereas the number of unique proteins increased by 55% when compared to the acquisition with DMS off.

P24. Small open reading frame-encoded protein production is modulated by nonsense-mediated decay in breast cancer

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Peptides from low abundance non-canonical proteins encoded by the human genome are presented by the major histocompatibility complex and serve as potential neoantigens or therapeutic targets. However, their prevalence in the genome is unclear.

We identified several non-canonical proteins produced by breast cancer cell lines using proteogenomics approach. Although these proteins were detectable, the transcripts and corresponding proteins showed low abundance and inconsistent expression pattern.

Targeting the nonsense-mediated decay pathway by UPF1-knockdown increased the levels of both non-coding transcripts and non-canonical proteins, suggesting they are subjected to degradation by conserved quality control mechanisms in cells. We also observed increased expression of unannotated transcripts and human leukocyte antigen transcripts associated with antigen presentation. These observations suggest that UPF1 has a role in regulating or suppressing transcriptional noise and that modulating the expression level of UPF1 could expand the reservoir of neoantigens and increase neoantigen presentation, potentially augmenting immunotherapeutic responses in cancers.

P25. Data Independent Acquisition of Pulse-SILAC Proteomes and its Application to Cell-Based PROTAC Characterisation

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Introduction

Unlike a conventional SILAC analysis, which only reveals protein abundance changes, a pulse-SILAC (pSILAC) analysis is able to distinguish between protein synthesis and degradation, thus providing valuable mechanistic insights into proteome remodelling. Distinguishing between degradation and synthesis is also key to elucidating target and off-target proteins of synthesis and degradation specific drugs, such as Antisense oligonucleotides (ASOs) and proteolysis targeting chimeras (PROTACs). With improvements in MS hardware and software innovations, data-independent acquisition (DIA) now surpasses data-dependent acquisition (DDA) for the MS analysis of complex proteomes, without the need for sample fractionation. This throughput potential makes DIA ideal for temporally sampled pSILAC protein turnover experiments. However, DIA analysis softwares have only recently implemented support for isotope labelling. This work utilises the recently implemented isotope support of the open-source software DIA-NN and compares to conventional DDA for the analysis of unfractionated SILAC proteomes. The application of this DIA pSILAC workflow for the analysis of cellular responses to PROTACs is presented.

Methods

A fractionated peptide library and SILAC standards were generated to benchmark the performance of DIA and DDA workflows (analysed with DIA-NN and MaxQuant, respectively). RPE1 cells were temporally sampled during pSILAC labelling and treatment with PROTACs targeting BET domain family of proteins.

Results

An experimental design using a deep learning-based predicted spectral library conveniently outperformed an empirical DDA library. DIA also outperformed DDA for the analysis of unfractionated SILAC samples, particularly for the LFQ-based quantification required for pSILAC analyses. Target protein degradation was observed within 30 minutes of PROTAC treatment in cell culture, in addition to non-target protein degradation. Broad proteome remodelling was evident in the nascent proteome from 6hrs. DIA MS of pSILAC is a powerful technique for the analysis of rapid responses within mature and nascent proteomes.

P26. Robust and Standardized Workflow for Large Scale Clinical Proteomics

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Proteomic analyses have been used for more and larger clinical studies over the last years. In addition, patient samples are inherently heterogenous and therefore large cohorts are required to obtain meaningful and significant data. Clinical studies are often spread out over several years due to patient availability and/or longitudinal tracing of patients over longer periods of time to monitor illnesses or treatment efficacies. Proteomic analyses, however, are affected by technical variations, such as sample preparation, calibrations, and maintenance of the instruments. Here, we present a workflow designed to optimize robustness and minimize variations of technical batches. While multiple batches that cannot be prevented, such as replacement of columns and system calibrations, the presented workflow is suited to track them. The presented workflow includes an automated SP3 digestion [1] protocol on a Hamilton Star M liquid handling platform, followed by a data-independent analysis in 21 min gradients approach on an Orbitrap Exploris 480 coupled to an Evosep One. Serum samples from several healthy donors were mixed together creating one master pool, which was analyzed in several batches over a total time frame of 16 month. Overall, we demonstrate the impact of batch effects and the increase in reproducibility through fully automated sample processing.

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P27. Sexing based on tooth enamel proteins. Can co-selection PRM save the day?

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Introduction

Palaeoproteomics is an emerging research field focused on the analysis of proteins derived from ancient remains in an archaeological context using (mainly) shotgun mass spectrometry approaches. Compared to DNA, proteins are highly stable biomolecules, which makes them suitable analytical targets in remains that are thousands of years old. However, these specific , often mineralised, matrices (such as bone and teeth) require significant changes to the current proteomic workflows.

One of the most crucial factors in reconstructing the past of a given society is the sex determination of human remains. The current two most common approaches are visual determination and ancient DNA analysis, both based on sexual dimorphism of either the skeletal remains or the sex chromosomes. However, these require intact, non-juvenile remains or the presence of DNA, which is often not the case.

Methods

More recently sex determination of human remains based on the presence of amelogenin Y protein in male enamel has been introduced. However, the biggest hurdle here is to “prove” or rather evaluate the absence of amelogenin Y in female individuals, since absence of evidence is not evidence of absence. Therefore we have developed a PRM based quantitative assay with quadrupole co-selection of light and heavy labelled target peptides not only including amelogenin Y, but also amelogenin X and ameloblastin as matrix proteins for thresholding the absence of amelogenin Y. The heavy labelled standards enable us to accurately quantify the enamel matrix proteins and correct for taphonomic and sampling differences of the enamel samples. This quantitative data can be translated into a logistic regression model that models the chance of detecting amelogenin Y (if present) given the abundance of certain matrix proteins in the sample. This in turn reflects the chance of the individual being female in absence of amelogenin Y.

Results

To develop the PRM assay the quadrupole selection window was varied from 6 to 12 Da and the selected m/z was shifted between the light and heavy-labelled target m/z to ensure equal transmission efficiency. The non-labelled target combined with a 12Da transmission window ensured an equal transmission of both targets with little interference. The logistic regression model will be trained and tested on data analysed with this method from 400 individuals, including both contemporary, post-medieval and juvenile individuals.

P28. Identification of translatable chronic kidney disease biomarkers by comprehensive transcriptomics and proteomics analysis of the rat sub-total nephrectomy SNx model of fibrosis

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Introduction

The rat sub-total nephrectomy (SNx) is a functional model of chronic kidney disease (CKD), where the main pathological driver is glomerular hypertension. Renal function can be monitored through proteinuria and serum creatinine, and hence provides a translational model for the identification of functionally relevant mechanistic CKD biomarkers. Comprehensive transcriptomics and proteomics analyses on the rat SNx model were performed to identify biomarkers in plasma or urine that correlate with both kidney disease and functional kidney loss.

Methods

Two SNx studies were completed at two independent locations. When rats reached predetermined levels of disease (serum creatinine >2-fold increase, proteinuria >3-5-fold increase over sham-operated), plasma, urine and kidneys were collected. Kidneys were subjected to collagen staining for fibrosis scoring, SWATH proteomics and bulk RNA-sequencing transcriptomics (RNA-seq), with SWATH also performed on plasma and urine. Differential expression analysis was performed on both protein and RNAseq datasets using the Limma framework. Statistical significance threshold was set at FDR adjusted p-value < 0.05 in each dataset and split by directionality for cross comparison and triaging potential CKD markers. Downstream analysis of functional enrichment was performed using the gene ontology (GO) framework.

Results and Discussion

Each SNx study took 7.5-16.5 weeks to reach the pre-defined disease thresholds and displayed variable degrees of fibrosis. RNA-seq and SWATH proteomics demonstrated significant dysregulation of genes/proteins involved in fibrosis, metabolism, and immune response in the SNx rats compared to controls. Gene ontology analysis of the intersecting genes and proteins from both studies demonstrated common biology between animal cohorts that had reached the kidney disease thresholds. Thirteen significantly differentially regulated molecules were detected (six up-regulated, seven down-regulated), with consistent directional changes in both transcriptomics and proteomics assays. These molecules were detected independently in kidney (both assays) and urine (proteomics), but not in plasma. Of the up-regulated proteins Lumican and Col3A1 were identified as relevant translatable mechanistic CKD biomarkers.

This study is the first in depth and direct comparison of the transcriptome and proteome for the kidney and biofluids in the rat SNx model. A comprehensive bioinformatic analysis of differentially regulated genes and proteins enabled the identification of translatable mechanistic CKD biomarkers including lumican and Col3A1, whose co-expression has previously been both implicated in fibrosis and detected in urine in CKD.

P29. Data-independent acquisition proteomics reveals a disordered extra cellular matrix in CYLD deficient human skin tumours

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Introduction

Patients with CYLD cutaneous syndrome (CCS) develop hair follicle skin tumours, associated with loss of function mutations in CYLD. CYLD is a tumour suppressor gene that encodes a de-ubiquitinase, targeting substrates with K63 and M1 linked ubiquitin chains. An unbiased proteomic analysis of CCS tumours stands to offer novel insights into the cellular mechanisms and origins involved in the formation of these rare tumours, a prerequisite to the development of preventative treatments for CCS patients.

Methods

Consented fresh CCS tumour from genotyped patients and control healthy skin samples were used (n=6 each). Snap frozen tissue was homogenised, and trypsin digested in preparation for data independent acquisition (DIA) on an Orbitrap tribrid mass spectrometer. Label free quantification (LFQ) using DIA-NN software was employed to determine protein abundance. In parallel, we analysed the transcriptome of flow sorted CD45- CCS tumour cells and normal skin (n=5 each) to investigate tumour cell specific alterations in transcript levels. Gene set enrichment analysis was performed to identify enriched gene ontology terms in differentially expressed genes and proteins.

Results

Proteomics analysis identified 5243 proteins of which 1568 were differentially expressed in the whole lysate samples, while transcriptome analysis revealed 2183 genes that were differentially expressed in the CD45- CCS tumour cells, both compared to normal skin (FC >2 and adjusted p-value of <0.05). Increased protein expression of hair follicle keratins 5,13 and 75 was corroborated in the transcriptomic data, with additional hair keratins 15,17,74 transcripts detected. Collagen 7, 4 and 18 and laminin 332, and beta-6 integrin protein were increased in tumour samples, supplanted with increased expression of transcripts of collagen 9 and 17. Matrix metalloproteinase (MMP) 7 was increased in CCS tumours whilst conversely, a decreased abundance of MMP2, MMP8 and collagen 5 was seen compared to normal skin. Alignment of proteome and transcriptome data revealed a set of 228 genes/proteins which gene ontology analysis revealed were enriched for ECM protein assembly, organisation, and remodelling processes.

Together these datasets add to the debate that hair follicle stem cells rather than eccrine gland cells are the cell of origin of CCS skin tumours, as evidenced by retention of a repertoire of hair keratins and basement membrane proteins.

P30. Detection and Quantification of Single-Molecule Proteins Using Protein Identification by Short-epitope Mapping (PrISM)

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Introduction

The combination of single-molecule resolution together with comprehensive proteome coverage has potential to improve sensitivity and reproducibility of protein analysis. Here, we demonstrate Protein Identification by Short-epitope Mapping (PrISM), which aims to provide comprehensive proteome analysis with broad dynamic range at single-molecule sensitivity by interrogating immobilized, full-length proteins in parallel using multi-affinity probes.

Methods

PrISM uses non-traditional, multi-affinity reagents with high affinity and low specificity that bind to short epitopes in multiple proteins. Sample proteins were conjugated to DNA nanoparticles and deposited on a high-density patterned flow cell at optically resolvable locations. Multi-affinity probes were applied to sample proteins over multiple cycles to generate binding patterns for each single-molecule protein, which are translated to protein identifications and quantities using a machine learning approach. We acquired PrISM data on biological samples using dozens of multi-affinity probes targeting trimer or tetramer sequences.

Results

We report single-molecule deposition of over 1 billion DNA nanoparticle-protein complexes on a flow cell. We demonstrate how the PrISM methodology identifies individual protein molecules through iterative probing with our multi-affinity probes. Further, we provide an analytical assessment of the sensitivity and specificity of PrISM and present progress toward the estimation of false identification rate of these proteins using a target-decoy based statistical approach.

Conclusions

Combining single-molecule high-density protein nanoarrays with iterative multiaffinity reagent probing, PrISM provides a new tool for quantitative proteomics. We demonstrate identification of proteins using PrISM, enabling detection of low abundance proteins in native protein and control samples. The ability to make comprehensive measurements of intact proteins at single-molecule resolution will accelerate proteomic research.

P31. Unique Peptides of Cathelicidin-1 in the Early Detection of Mastitis in Sheep

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Objective

The study refers to the investigation of the sequence of cathelicidin-1 present in ewes' milk, with the aim to identify its unique peptides and core unique peptides, which would reveal potential targets for accurate detection of the protein for early diagnosis of mastitis in ewes.

Methods

Potential uniqueness of each peptide of cathelicidin-1 was investigated against those in all proteins reported to have been detected in sheep, goats or cattle, thus creating a set of Core Unique Peptides (CUP). The analysis was performed by using a bioinformatics tool, built on a big-data algorithm. Thereafter, Composite Core Unique Peptides (CCUP) that were constructed based on a sequence of two or more consecutive or overlapping core unique peptides, were also searched. Finally, the three-dimensional (3D) structure of the protein was analyzed, using the AlphaFold predicted 3D model. In addition, the detection of unique sequences among the tryptic digest peptides of cathelicidin-1 was achieved, which would improve accuracy of identification of the protein during targeted MS-based proteomics.

Results

In total, 59 CUPs and four CCUPs were detected in cathelicidin-1 of sheep origin. During the 3D structure analysis of the protein, 35 CUPs were found on the core of the protein and, of these, 29 CUPs were located on amino acids in regions of the protein with 'Very high' or 'Confident' estimates of confidence of the structure. Finally, it was found that six CUPs were located entirely on loop or α-helix motifs of the secondary structure of the protein and were considered to be suitable as potential antigenic targets for accurate detection of the protein. Among tryptic digest peptides, there were six unique peptides in that protein.

Conclusion

The detection of unique peptides in cathelicidin-1 provided potential targets for accurate detection of the protein as biomarker. The selection of six CUPs as potential antigenic targets may be used to improve early diagnosis of mastitis in sheep. Moreover, another six unique peptides were detected in tryptic digests and may offer novel mass tags, which would facilitate the detection of cathelicidin-1 during MS-based diagnostics.

P32. Detection of Cathelicidin-1 in the Milk as an Early Indicator of Mastitis in Sheep

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Objective

The objective was the investigation of the presence of cathelicidin-1 in milk, after experimental mammary infection with two important bacterial pathogens, for potential use as an early indicator (biomarker) for the diagnosis of mastitis in sheep.

Methods

In two experiments, after bacterial inoculation into the mammary gland of ewes (experiment 1: Mannheimia haemolytica, experiment 2: M. haemolytica and Staphylococcus chromogenes), conventional bacteriological and cytological examinations of milk samples were performed to monitor the establishment and the course of the infection. Proteomics examinations, specifically two-dimensional gel electrophoresis analysis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) analysis, were performed sequentially in the collected milk samples. Cathelicidin-1 was detected and spot densities obtained from PDQuest v.8.0 were recorded. Associations were calculated between leucocyte counts and spot densities, as well as between the presence of mastitis in a mammary gland at a given time-point and the detection of cathelicidin-1 in the respective milk sample.

Results

All inoculated mammary glands developed mastitis, as confirmed by the consistent bacterial isolation from the mammary secretion samples and the increased leucocyte counts in these samples. The spot density of cathelicidin-1 on gels produced from mammary secretion samples from the inoculated glands increased as soon as 3 h post-inoculation. The spot density of cathelicidin-1 on gels produced from samples from the inoculated glands was higher than in samples from the uninfected control mammary glands. There was evidence of correlation between leucocyte counts in mammary secretion samples and spot densities of cathelicidin-1 on gels from the same samples. There was also significant association between presence of mastitis in a mammary gland and detection of cathelicidin-1 in the respective mammary secretion sample. The overall accuracy was 81.8%, and was higher during the initial 24 h after challenge (90.3%) than after the first day (70.4%).

Conclusion

Presence of cathelicidin-1 was detected in mammary secretion samples earlier than the increased leucocyte counts and was also highly associated with development of mastitis in the ewes. The associations were stronger during the initial 24 h after infection. The results support the potential use of cathelicidin-1 as a biomarker for mastitis in sheep. Cathelicidin-1 also has the advantage that it can be a non-specific biomarker, as simply a ‘positive’ / ‘negative’ assessment would be sufficient, which is simpler that the measurement (i.e., quantification) required for somatic cell counts.

P33. Quantifying 1000 protein groups per minute of gradient using Zeno SWATH DIA on the ZenoTOF 7600

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High-throughput proteomic analysis has long been the goal of many researchers, and this workflow has been shown to work well using the Evosep One HPLC system when coupled to a variety of mass spectrometry systems. Data-independent acquisition (DIA) has become the standard method of analysis with many researchers. Using DIA on a hybrid quadrupole time-of-flight (QTOF) system gives incredible speed and sensitivity with very high quantitative precision, especially when the quantitation is performed on the MS2 transitions. It has been previously shown using a combination of the Evosep One and a QTOF system that impressive numbers of proteins can be identified and quantified from standard cell lysate digests using DIA. Here, we present this workflow using a conventional UPLC system at 5 uL/min. The gradients used were designed to approximate the active gradient used on the Evosep One system, and cell lysate digests were separated on a 150 mm x 0.3 mm microflow column in trap/elute mode. A trap/elute method was designed using a 5-minute active gradient and 11 minutes total run time to mimic the Evosep 100 SPD method. Using human cell lysates of K562 and HeLa and a 200 ng on-column load, approximately 5,000 protein groups could be quantified using DIA, which is approximately 1,000 protein groups per minute of active gradient. These analyses were very robust with about 90%-95% of proteins being quantified with a CV <20%. These analyses were not restricted to human cell lines, as impressive data were also obtained with a yeast proteome sample where over 3,100 protein groups could be quantified in 5 minutes. To visualize the data obtained from DIA-NN software, we used a cloud-based software suite to import the .tsv output file, followed by statistical analyses to show differential protein expression.

P34. Targeted proteomics approach for qualitative and quantitative monitoring of human TSLP proteoforms

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Thymic stromal lymphopoietin (TSLP) is a pleiotropic cytokine highly expressed by epithelial cells and several cells of the innate and adaptive immune system. TSLP exerts its biological roles by binding to the heterodimeric receptor TSLP-IL-7R present on a plethora of cells of the immune system. The TSLP/TSLP receptor axis plays a central role in several inflammatory disorders and cancers. Overexpression of TSLP has been involved in orchestrating allergic inflammation in asthma and other atopic diseases. There are two proteoforms of TSLP: the short form (sfTSLP), which is constitutively expressed, and the long form (lfTSLP), which is upregulated in inflammatory condition. LfTSLP consists of 159 amino acids, while sfTSLP comprises the last 96 amino acids of the C-terminal portion of the protein (1). TSLP has been involved in orchestrating the inflammation in asthma and other atopic diseases. Recent clinical and experimental investigations suggest that lfTSLP may represent a reliable biomarker of airway inflammation in humans. However, to date there aren't antibodies directed specifically towards the two TSLP isoforms (2). We are investigating the existence of both TSLP proteoforms (short and full length) in unstimulated and stimulated human peripheral blood monocytes and lung-resident macrophages, by using targeted (MRM) and untargeted proteomics approaches. We are also evaluating whether lfTSLP can be cleaved by tryptase and chymase, proteases secreted by activated human mast cells, by limited proteolysis and MALDI-MS analysis.

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P35. Tissue deconvolution using cell-type specific protein profiles: The whole is more than the sum of its parts

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Introduction

In proteomics analyses, determining condition-specific protein expression is key and achieved by contrasting tissue samples. However, any protein abundance changes can also result from altered tissue compositions through immune cell invasion or apoptosis. This can lead to false positive results of biomarkers or drug targets. One option to account for these differences is to assess the composition in the laboratory with one sample half, while using the other half for mass-spectrometry analysis. This is cumbersome and bears additional costs. Computational methods are a cost-efficient alternative. Transcriptomics uses single-cell RNA-sequencing to assess the composition of bulk tissue analyses. Here, we provide a proof-of-concept method for computational tissue deconvolution at the protein level.

Methods

Protein quantification of publicly available FACS-sorted skin cell-types was downloaded from the human skin atlas, proteins quantified in less than 50% of samples were removes, and remaining missing values were imputed. Finally, all pairwise protein ratios were computed within each sample and low dimensionality clustering was used to group samples by cell-types using both cell-type specific and common proteins. Furthermore, a computational model using non-negative least squares (NNLS) was constructed based on the cell-type specific profiles to estimate mixture proportions of tissue samples. This method was compared to deconvolution methods developed for RNA-sequencing deconvolution.

Results

Overall, 4,068 proteins remained after filtering and 34,308 missing values were imputed. These proteins resulted in over 8 million ratios representing the relationships of proteins to one another in each sample. Using only these ratios for low dimensionality clustering of samples created crisp and well-separated clusters of the nine cell-types. A hierarchical clustering for each sample revealed that different groups of protein ratios are characteristic for each kind of cell. Similar cell-types, like dermal and epidermal T-cells, share the majority of these characteristic ratios, while distinct cell-types share few characteristic protein-protein relationships. Building on the discriminating potential of protein ratios the NNLS approach proved effective in estimating cell-type contributions from bulk skin samples, while RNA-seq based approaches outperformed NNLS for simulated admixtures. Overall, the results of the NNLS approach are encouraging and highlight the necessity for continued research, a larger variety of benchmark datasets, and large sets of cell-type reference protein profiles.

P36. Residue-resolved HDX-MS with ResHDX

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Introduction
Hydrogen-Deuterium Exchange mass-spectrometry (HDX-MS) has emerged as a powerful technique to explore the conformational dynamics of proteins and protein complexes in solution. In the bottom-up approach to MS, deuterium uptake is reported at the level of peptides, which complicates interpretation and means ad-hoc approaches are used to resolve contradictions between overlapping peptides.

Methods
Here we propose to leverage the overlap in peptides, the temporal component of the data and the correlation along the sequence dimension to infer residue-level uptake patterns. Our model treats HDX-MS as a multiple change-point problem - inferring at which residues HDX has changed. Fitting our model in the Bayesian non-parametric framework allows inference of the number of parameters, quantitative assessments of the confidence of differential HDX and uncertainty estimates of the temporal kinetics.

Results
We benchmark our approach against others using a three-way proteolytic digestion experiment and find that it out-performs other available methods. We illustrate our approach on a number of case-studies, including small molecule binding to BRD4.

P37. A Comparison of Quantitative Mass Spectrometric Methods for Drug Target Identification by Thermal Proteome Profiling

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Thermal proteome profiling (TPP) provides a powerful approach for investigating interactions between therapeutic molecules and proteins, complementing phenotypic-based drug screens. Precise detection of target engagement-induced deviations in thermal stability requires consistent and accurate quantification. Isobaric tandem mass tags (TMT) enable sample multiplexing, enhancing quantification precision in TPP analysis via data-dependent acquisition (DDA). Yet, recent progress in data-independent acquisition (DIA) offers comparable sensitivity, protein coverage, and reduced costs and sample preparation steps. These advancements make DIA an appealing option for TPP analysis.

In this study, we conducted a comparison between various library-free and library-based DIA pipelines in a TPP workflow, and benchmark these with traditional TMT-DDA for thermal shift quantitation. We utilized losmapimod, a known MAPK14 inhibitor, to treat acute myeloid leukaemia cells. The label-free DIA approaches, especially the library-free mode in DIA-NN, exhibited comparable performance to TMT-DDA in reliably detecting the engagement of losmapimod with MAPK14 and one of its downstream targets, MAPKAPK3. Employing DIA for thermal shift quantification presents a cost-effective alternative to labelled quantification in the TPP pipeline.

P38. decryptM: Profiling the phosphoproteomic response to Kras inhibition using a novel dose-dependent proteomic approach

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Introduction

Kras is a small GTPase that links proliferation signals at the cell surface with downstream signaling cascades involved in cell proliferation. It functions in the MAPK pathway where its activation status is controlled by a GTP/GDP mediated nucleotide exchange mechanism. However, in around 30 % of all cancer patients Kras is mutationally overactivated, resulting in continuous activity of the downstream cascade. The development of the first clinical Kras inhibitors Sotorasib and Adagrasib provides a new treatment option for these patients. However, the establishment of resistance in clinical trials proves that the mechanisms of Kras driven cell proliferation and the effects of its drug-induced inhibition are not fully understood. Since KRAS downstream signaling is mediated by phosphorylation, we can use phospho proteomics to understand the effects of Kras inhibition in Kras driven cancer cells.

Methods

The clinical KRAS G12C inhibitors Sotorasib and Adagrasib and the preclinical Kras G12D inhibitor Mrtx1133 were investigated in MiaPaCa2 (KrasG12C), NCI-H23 (KrasG12C) and ASPC1 (KrasG12D) using the decryptM approach. Cells were treated in a dose-dependent manner and proteins were digested using trypsin. Peptides were encoded by tandem mass tags (TMT) and pooled, following separation into 12 fractions by high-pH reversed-phase HPLC and IMAC enrichment. Additionally, Cysteine profiling was performed for Sotorasib and Adagrasib in a dose-dependent fashion adapted by Gygi et al. Peptides were analyzed by LC-MS3 on an Orbitrap Eclipse and quantified using MaxQuant and Proteome Discoverer.

Results

We identified around 20,000 phospho-peptides (p-peptides) for each decryptM experiment of which over 300 were regulated. For each p-peptide, EC50s values were derived representing the in cellulo potency. The majority of the sites were regulated in the lower nanomolar range for both Adagrasib and Sotorasib in NCI-H23 and MiaPaCa2 cells. Additionally, both clinical drugs displayed high selectivity for KrasG12C in the Cysteine profiling and calculated EC50s were in line with the phosphoproteomic data, highlighting that effects are a result of Kras inhibition and not off-target binding. Besides several MAPK pathway players, we identified multiple novel sites on proteins involved in ubiquitination, Rho signaling, and RNA metabolism that we observed in a time span of 1 to 16 hours and places hitherto functionally unannotated p-peptides into the functional context of Kras-MAPK signaling. In conclusion, this study provides information on the mode of action of KRAS inhibitors, cellular drug response and novel insight into KRAS downstream signaling.

P39. Identification of novel proteins dysregulated in Alzheimer's Disease patients and related to Amyloid-β Plaques or as blood-based biomarkers of the disease

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Introduction and objective

Alzheimer's disease (AD) is a progressive, chronic, and neurodegenerative disease, and the most common cause of dementia worldwide. The mechanisms underlying AD are still undefined. Its diagnosis at early stages may help to delay the progression of the disease. Thus, the study of proteins involved in AD pathogenesis would allow getting further insights into the disease and identifying new potential biomarkers. We here aimed to analyze protein dysregulation in AD tissues in comparison to healthy individuals and other dementia patients' tissue by quantitative proteomics to identify key proteins in the pathogenesis of AD.

Methods

TMT (Tandem Mass Tags) 10-Plex quantitative proteomics was performed using frozen tissue samples from the left prefrontal cortex of AD patients at Braak IV-VI, and from healthy individuals and patients with other dementias as controls (CT). LC-MS/MS analyses were performed on a Q Exactive, whereas data analysis was performed using MaxQuant and Perseus to identify differentially expressed proteins. In addition, amyloidβ peptides (Aβ40 and Aβ42) were used for the identification of novel Amyloidβ interactors by pull-down assays.

Results and discussion

Among the 3281 proteins identified and quantified in the left prefrontal cortex, 15 and 154 proteins were found statistically (p-value < 0.05) upregulated and downregulated, respectively, in AD patients in comparison to CT, with an expression ratio ≥ 1.5. After bioinformatics analysis, 10 dysregulated proteins in AD were selected for further validation by qPCR, WB, IHC, IF, and pull-down assays using tissue and plasma samples of AD patients, and patients with other dementias and healthy individuals as controls.

The dysregulation of these proteins at mRNA and protein levels was confirmed in AD patients in comparison to controls. In addition, HECTD1 and SLC12A5 proteins were found at a high concentration in the plasma of AD patients than in healthy individuals' plasma samples, with high potential as blood-based clinical diagnostic biomarkers of AD. Additionally, PMP2 and SCRN3 proteins were identified as novel Amyloidβ plaques interactors, highlighting a major role of these proteins in AD development.

P40. Utilization of AnPEP in structural proteomic workflows

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Proteolysis is an indispensable tool of the structural proteomic workflow. In hydrogen/deuterium exchange (HDX) it provides spatial resolution while in cross-linking (XL) or fast photochemical oxidation of proteins (FPOP) it enables precise localization of the modification sites. In HDX the non-specific cleavage pattern provided by acid protease is considered as fine, but FPOP and XL better rely on specific and predictable proteolysis by e.g., trypsin. However, for both techniques the search for new cleavage tools is of high importance, simply because there are proteins not easily amenable by the currently used enzymes. Recent papers highlighted the utility of proline semi-specific enzymes such as neprosin or Aspergillus niger prolylendopeptidase (AnPEP).

Here we used the industrial grade AnPEP and tested it under conditions described for research grade ProAlanase that are leading to (semi)specific digestion. In addition, we immobilized the AnPEP and tested it under both – specific (extremely low pH) and HDX-compatible (pH 2.5) conditions. For HDX-MS we show the utility of including AnPEP in combination with other proteases for e.g., analysis of prion proteins. Under the specific conditions we discovered previously overlooked cleavage dependencies and showed that the industrial grade AnPEP represents a good source of enzyme which is available in quantities suitable for generation of protease columns. In order to facilitate cross-comparions of numerous digestion conditions and speed-up the extraction of cleavage specificities and other digestion-related metric, we created a Java-based application DigDig.

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P41. Investigating the molecular pathways by which recombinant adeno-associated virus (rAAV)6.2 is released from HEK293-VP cells during manufacturing.

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Introduction

The potential of recombinant adeno-associated viruses (rAAVs) as therapeutic DNA delivery agents has been established, and three such therapies are currently commercially market. Unfortunately, the wider application of these vehicles is hindered by low yields and recovery during manufacturing. This is in part due to trapping of the rAAVs inside of their producing cells, most commonly HEK293 cells. Cellular retention of rAAV necessitates cell lysis followed by extensive purification, a process which is costly with respect to time, money and material. One proposed solution is to collect the viruses from the cell culture medium without lysis, but this would require a high proportion of rAAVs to be released into the extracellular environment. Hence, to apply such a solution will require a more thorough understanding of the mechanism(s) by which rAAVs are retained within or released from producing cells.

Methods

In this project, rAAV6.2 was produced in HEK293-VP cells using a standard transient triple transfection method. The rAAV yield was determined based on viral genome and capsid counts, as quantified by qPCR and ELISA assays, respectively. To determine the proportion of rAAV released from the cells, yield was monitored in both the cell pellet (lysate) and supernatant (media). The global abundance of cellular and secreted proteins was quantified and compared between control (mock transfected) and rAAV6.2-producing HEK293-VP samples at 96-hours post-transfection. Cellular proteins were quantified via a TMT workflow whilst secreted proteins were studied using label-free quantitation. Global protein localization of control and rAAV6.2-producing HEK293-VP cells was compared using Localization of Organellar Proteins by Isotope Tagging after Differential Centrifugation (LOPIT-DC).

Results

The production of rAAV6.2 yielded 1.80 × 103 (± 3.58 × 102) viral genomes and 2.17 × 104 (± 9.46 × 102) viral capsids per cell at 96-hours post-transfection. Of these, 30.7 % (± 3.43 %) of genomes and 13.7 % (± 1.92 %) of capsids were located outside of the producing HEK293-VP cells, in the supernatant. I will compare global cellular and secreted protein abundance between control and rAAV6.2-producing HEK293-VP cells to reveal the presence of differentially abundant proteins. Functional gene ontology and Reactome pathway analyses of these proteins will indicate molecular pathways which are activated and/or suppressed by the production of rAAV6.2. Data from LOPIT-DC will be used to map the spatial proteomes of control and rAAV6.2-producing HEK293-VP cells. I will then determine the cellular location of rAAV6.2 proteins and identify differentially localized producing cell proteins to infer potential intracellular trafficking pathways used by the rAAV.

P42. Integrated multiomics examinations in the study of obesity and type 2 diabetes coverage

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Obesity and type 2 diabetes (T2D) can be considered as major health-impairing conditions affecting millions of people worldwide. It would be of high importance to acquire information that could make possible patient stratification or can predict the appearance of disease-related complications with an emphasis on cardiovascular complications.

Serum and plasma samples collected from patients with obesity, and/or T2D were examined using liquid chromatography-mass spectrometry (LC-MS). In parallel to this we examined with proximity extension assay (PEA) the relative changes of 366 proteins related to cardiovascular diseases. Along with sample collection a thorough clinical examination was carried out, including classical laboratory tests (lipid profile, liver, and kidney metabolism, etc.), some gene polymorphism analyses, and imaging. Using our data, we were able to construct a combined big data-based repository integrating clinical and laboratory analysis and imaging data, demographics, and other medical records. This database allows us to do a complex analysis of clinical information, metabolomics, and proteomics data as well as patient data.

Classical statistical analysis along with correlation and network analysis was performed to map biological processes relevant to obesity and T2D. The complex, multi-layer analysis of the data is still in progress, but the readout from proteomics and metabolomics data indicate pathways that are characteristic to either or common to both conditions as novel Amyloidβ plaques interactors, highlighting a major role of these proteins in AD development.

P43. Multi-Omics approach for the examination of grape-derived beverages

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Introduction

Aszú and Furmint wines are well-known white wines of Hungary. The presence of amino acids, biogenic amines, and other nutritious components as well as proteins in grape-derived products are mostly related to the grape variety, environmental conditions, and production process. Before or during the fermentation of wine and wine vinegar may come in contact with different microbial agents such as B.cinerea, S.cerevisiae and Acetobacter sp. used in their production.

In our study, we applied ultra-performance liquid chromatography (UPLC) and mass spectrometry (MS) analyses to examine the amino acids, biogenic amines and proteins of grape-derived beverages, including white wine (Furmint), botrytized wine (Aszú), and wine vinegar.

Methods

We examined Aszú, Furmint and wine vinegars from the Hungarian "Tokaj" wine region, a UNESCO world heritage site. 200 µl of the sample was derivatized with an AccQ-Tag Ultra derivatization kit (Waters) after filtering with a 3kDa Nanosep tube. The concentration of 23 amino acids, and 10 biogenic amines was measured using H-class UPLC (Waters)-5500QTRAP (Sciex) mass spectrometry system. The data were processed and integrated by Empower (v3.0, Waters) and Skyline (v.20.2, www.maccosslab.org). The digested proteomes were analyzed using Easy nLC 1200-Orbitrap Fusion (ThermoScientific) system and the raw data was processed with DIA-NN (v18.2). The statistical analysis and illustrations were performed by GraphPad Prism (v.8.0).

Results

All examined amino acids could be detected and quantified in Aszú and Furmint samples. Amino acids, except glutamate, citrulline, and tryptophan, were quantified in wine vinegar. 18 amino acids showed a significant difference between the analyzed samples, and we found no statistically significant differences between the samples for arginine, aspartate, tyrosine, valine, and isoleucine.

For biogenic amines, all examined biogenic amines except histamine were detected. The proteomics and metabolomics analyses revealed differences between examined fermented beverages. To the best of our knowledge, this is the first multi-omics analysis to compare these sample types.

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P44. Examination of protein-protein interaction networks in obesity and type 2 diabetes mellitus

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Introduction

Many people suffer from diabetes mellitus, which is considered one of the most common metabolic disorders in the world and it shows an increasing tendency. The increasingly common obesity can be the cause, which has the highest risk factor in the development of T2D. Most T2D cases are diagnosed in adulthood, however, due to the unhealthy lifestyle it can appear at younger ages as well. This means most of the T2D patients are suffering from obesity, but on the other hand, not every obese patient has this disease. The aim of our study was the identification of blood proteins characteristic to T2D and obesity using liquid chromatography-mass spectrometry (LC-MS). In addition, network analyses were performed in order to map biological processes to understand these pathological conditions.

Method

Serum samples were collected from patients suffering from T2D (46), and obesity (43), as well as age- and sex-matched controls (45) from the Department of Internal Medicine, University of Debrecen. For protein concentration measurement BCA method (Thermo Scientific) was used and the top 14 most abundant proteins were depleted (Thermo Scientific). The digestion of the depleted samples was performed using trypsin and LysC (PreOmics). In the final step, the digested samples were desalted (Thermo Scientific) and two fractions were collected. The analysis of the prepared samples was performed on an Easy-nLC 1200 coupled to Orbitrap Tribrid (Thermo Scientific) instrument and data-independent acquisition (DIA) method was used. A throughout network and gene ontology (GO) analysis was performed using data from the available protein-protein interaction databases.

Results

Pathways and protein clusters characteristic to the disease conditions were identified. Examination of the differences can help to better understand the molecular background of these conditions.

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P45. Validation of Label-Free Proteomics Data Analysis Reproducibility for Early-Phase HIV-1 and HIV-2 Transduction Using WOMBAT-P Pipelines and the Proteomics Metadata Standard SDRF

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Introduction

Proteomics data repositories are essential to advance research in the field, but reproducibility remains a challenge due to the lack of standardized metadata. Metadata captures important information about a data set, such as the sample to data file relationships and standard data file formats, which are necessary to interpret and reanalyze deposited data sets. The Sample to Data file format for Proteomics project created by the European Bioinformatics Community for Mass Spectrometry (EuBIC) aims to standardize sample metadata of public proteomics data sets. Here, we highlight the significance of validating and comparing the results of an automated pipeline that utilizes the standardized metadata format with the original study, in order to enhance the reproducibility and facilitate the reanalysis of label-free proteomics data from early-phase HIV-1 and HIV-2 transduction experiments.

Methods

In this analysis, we used the Wombat-P pipelines to validate a proteomics data set from a study that examined proteomic changes in the early phases of lentiviral transduction expressing HIV-1 and HIV-2 genes in HEK293 cells. Annotation of sample metadata and the links to the corresponding data files were provided in the tab-delimited sample and data relationship file format (SDRF). Specifically, we focused on the proteomic changes that occurred at 2, 8, 12, and 26 hours after transduction. We only detected significant alterations in the proteomic profile at 2 and 26 hours.

Results

Reproducing original results is crucial for validating and advancing proteomics research. However, to do so, it is important to have access to study design details and analysis parameters provided in the SDRF file. By using a standardized metadata format and an automated pipeline, we can improve reproducibility and facilitate proteomics data analysis. Our findings highlight the need for standardization and validation of metadata to advance the field of proteomics research.

P46. Proteomics and metabolomics analysis of recombinant protein-expressing CHO cell lines

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Introduction

Heterologous expression systems are widely used for the production of different proteins, enzymes, or antibodies for various applications. While the appropriate transfection and cellular machinery are important for recombinant protein production, the changes in the concentration of the amino acids in the media also affect the protein production yield. In this study, recombinant GFP protein was produced in two different CHO cell lines with different FBS concentrations. The utilization of the amino acids from the cell culture media was monitored and the changes in the protein content of the cells after the expression was examined as well.

Methods

The 20 proteinogenic amino acids were analyzed by an Acquity H-Class UPLC system (Waters) and a 5500 QTRAP (ABSciex) mass spectrometer. We have developed/utilized an 11 minutes chromatographic gradient including re-equilibration. The samples were derivatized with AccQ-Tag derivatization kit (Waters) that converts both primary and secondary amino groups to stable fluorescent derivatives. The derivatized amino acids can be separated by liquid chromatography and the fluorescent derivates can be detected photometrically at 260 nm. We have also examined the changes in the protein composition of the CHO cells after the recombinant protein expression by shotgun proteomics using an Orbitrap Fusion system (Thermo Scientific).

Results

We have compared the changes in the utilization of amino acids by the two different CHO cell lines cultured in the presence of different FBS concentrations. With shotgun proteomics analysis, we have examined the changes in the proteome of the CHO cells after the recombinant protein expression. The multi-omics analysis of heterologous protein expression systems can reveal new aspects that may improve the yield of recombinant protein production.

P47. Metaproteomics allow deep and accurate characterization of cystic fibrosis' patients' respiratory microbiota

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Introduction

Cystic fibrosis (CF) is a hereditary disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, triggering dysfunction of the anion channel in several organs including the lung and gut. This dysfunction results in thick and dehydrated mucus, leading to polymicrobial infections by several microbes (Pseudomonas, Staphylococcus, Mycobacterium, …). Respiratory infections account for over 80% of deaths in CF patients, thus highlighting the critical role of the microbiota and the need to better understand it to better treat patients. In light of this need, metaproteomics is rising, in recent years, as a key analysis to deeply and accurately characterize the taxonomical and functional dynamics of the respiratory microbiota. In this context, tandem mass spectrometry (MS/MS) and powerful bioinformatics tools associated, to analyse the huge amount of data produced by MS/MS, stand out as very effective in accounting for the complexity of the respiratory microbiota. Thus, this allows a better understanding of the roles of the microbiota components, their contribution to disease progression and host response; and could help in identification of new targets for potential antimicrobial therapy or microbiota-based intervention. Currently, characterization of CF microbiotas is essentially based on metagenomics approaches. Here we demonstrate the ability to get more insight in the respiratory microbiota in two CF's patients chronically infected with Mycobacterium using metaproteomics.

Methods

To investigate the dynamics of the co-evolved respiratory microbiota in the presence of NTM, proteins were extracted from sputum samples and analyzed by high-resolution tandem mass spectrometry. Collected data were interpreted to identify and quantify the microbial genera in the samples, their functions, and the host response. The metaproteomics data were integrated within the clinical and microbiological context.

Results

Our analysis reveals a Mycobacterium's associated host's response following an anti-mycobacteria treatment. Indeed, our data show that it enter a dormant state after getting internalized by macrophages (acidified and devoid of important metal ions). Thus, it suggest persistence of Mycobacterium despite non-detection by conventional clinical examination.

These results highlight the benefit of metaproteomics in characterisation of the taxonomical and functional dynamics of the respiratory microbiota, leading the way to potential improvement in patients' treatment.

P48. Characterisation of precision-cut tumour slices, a patient-derived model of cholangiocarcinoma

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Introduction

Cholangiocarcinoma (CCA) is an aggressive malignancy with increasing incidence and persistently poor prognosis. Unfortunately, the majority of patients present at an advanced-stage precluding a potentially curative resection. Even with surgery, outcomes remain sub-optimal with many patients succumbing to disease recurrence. Current treatments options for CCA are limited and chemoresistance is common. New chemotherapeutic strategies and a greater understanding of the mechanisms related to treatment response/resistance are needed. Precision-cut tumour slices are patient-derived and can be cultured ex-vivo, recapitulating critical aspects of cancer biology and crucially retaining all aspects of the tumour microenvironment. Here we sought to evaluate the phenotypic stability of the tumour slices in culture.

Methods

Resected intrahepatic and perihilar CCA tumours were placed in organ preservation buffer for transfer from the operating theatre to the lab. 5mm cores were sectioned on a Krumdieck reciprocating tissue slicer to generate multiple 250µm slices. These were incubated in 24-well plates containing supplemented William E medium. Viability was assessed by MTS assay and by immunohistochemistry for markers of apoptosis. A spectral library was generated using a pool of bulk CCA tissue, prefractionation of the tryptic digests by strong cation exchange chromatography and data-dependent acquisition on a Triple TOF 6600. Tissue slices from day 0, 3, 7 and 15 were harvested and processed for SWATH/DIA. Data analysis was performed using DIA-NN, Partek Genomics Suite and Ingenuity Pathway Analysis.

Results

SWATH/DIA revealed proteome differences between intrahepatic and perihilar CCA, and preliminary analysis appeared to show that this was associated with increased metastatic potential and increased immune cell infiltration in the latter. Temporal changes were observed at 3 days in culture compared to day 0, but the proteome then stabilised with few further changes up to 15 days. These data suggest that the tumour slices retain good viability in culture and have potential as a platform for novel drug discovery.

P49. A fast and efficient method for peptide fractionation at basic pH in proteomic studies

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Affinisep, Affinisep, Affinisep, INSERM – US24 SFR Necker Proteome, INSERM – US24 SFR Necker Proteome, Affinisep

Introduction

Peptide fractionation plays an important role in bottom-up proteomic approaches, since it simplifies the peptide pool for easier analysis by mass spectrometry, to allow for more accurate interpretation. Indeed, full proteome characterization is often needed for complex biological matrices and, with growing interest in post-translational modifications, an extended protein sequence coverage is required. However, efficient peptide fractionation is very challenging, especially in the case of complex samples. The objective of this study was therefore to develop a simplified procedure for the efficient and fast fractionation of peptides at basic pH, to contribute to the simplification of peptide separation and analysis.

Methods

A new reversed-phase sorbent, based on small sorbent particles tightly embedded in a soft, uniform and mechanically stable monolithic membrane packed in SPE StageTips, was used for the fractionation of peptides resulting from the enzymatic proteolysis of HEK293 cell lysate, and the results were compared to a reference commercial fractionation kit.

Eight fractions were performed on both the commercial column and the SPE StageTips, with an acetonitrile gradient, and each fraction was then evaporated to dryness before being resuspended in an appropriate solvent for nanoLC-MS/MS analysis.

Results

If the total number of proteins identified and the percentage of peptides eluting in only one fraction (50%) were similar for both sorbents, with a good distribution of peptides over the eight fractions, it appeared that the fractionation on the newly developed sorbent presented several advantages compared to the reference kit. Indeed, if the commercial columns have to be stored at 4°C in a storage buffer, the new sorbent has no storage constraints since it can be stored dry at room temperature over several years, without observing degradation. Moreover, due to the SPE StageTips format, the time required for the evaporation of each fraction is almost halved compared to the commercial columns.

Thus, the new sorbent appears as a promising solution for the fractionation of complex samples such as plasma, or the generation of spectral libraries, since it leads to an increase of more than 25% in the number of proteins identified, compared to unfractionated samples. Finally, this new sorbent offers a real flexibility of format and capacity, since it is also available as spin columns, for high amounts of peptides, or as 96 SPE well plates, for high throughput experiments, and it can be used to fractionate peptides samples, from few ng to several mg.

P50. Conventional liquid chromatography and its potential for diagnosis and monitoring of multiple myeloma progression

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Background
Multiple myeloma (MM) is a cancer of plasma cells that produce monoclonal immunoglobulins (mAb). Despite the development of new therapeutics, MM is associated with a high probability of persistent minimal residual disease (MRD) and relapse. Relapse in MM is driven by clonal heterogeneity and evolution that can lead to selection of drug-resistant malignant plasma cell (PC) clone(s) that contribute to treatment failure. Detection of minor clones is currently limited due to the invasiveness of bone marrow biopsy and very low sampling frequency. Therefore, the main goal of our work was to develop a method for sensitive detection of these clones based on mAb and free light chains (FLC) analysis in human serum, allowing early and considerate detection of malignant clone progression.

Methods
The developed method combines selective affinity isolation of mAb and FLC and their analysis by high performance liquid chromatography with UV detection (af-HPLC/UV). Af-HPLC/UV also served as the first step for targeted detection of the variable (CDR3) region of mAb by high-resolution mass spectrometry after prior sequencing of the corresponding DNA regions. The performance of the method was tested on three types of mAb standards spiked to human sera. Patient samples before and after treatment were also included in this study.

Results
The af-HPLC/UV workflow was able to detect mAb in serum with high sensitivity (≤100 mg/L). Selective isolation of mAb and FLC allowed quantitative separation of mAb by light chain types, which has great potential for the diagnosis of oligoclonality. HPLC with UV detection is then suitable for accurate LC and partial HC classification, as well as for the determination of therapeutic mAb, which usually complicate immunofixation electrophoresis analysis. In addition, the sensitivity of the entire workflow is significantly enhanced when combined with LC-MS/MS analysis of the unique CDR3 region.

Conclusions
Thanks to these findings, it would be possible in the future to carefully monitor the patient in short time intervals and to detect not only clones from the time of diagnosis but also newly formed clones with high sensitivity.
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P51. Development of a microflow LC-MS-based Peptide Assay to elucidate the coagulation cascade in human plasma.

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Blood coagulation is a complex process driven by platelet activation and is additionally intensified by the secretion of modulators like thromboxane A2 or ADP. In order to understand the dynamics and underlying mechanism of thromboembolic events in patients, it is mandatory to quantify key protein markers of the coagulation cascade. Thereby, one of the greatest challenges for the analysis is the wide dynamic range of protein amount, varying from nanogram to milligram. Therefore, we developed an MS-based peptide assay using selected reaction monitoring to determine absolute amounts of these proteins in plasma.
The respective biomarkers known from literature and clinical studies were selected based on their presence in a spectral library generated by fractionation of trypsin digested human serum to determine the approximately 40 proteins.
Selected markers cover the intrinsic and extrinsic coagulation cascade, activating factors as well as scavenger proteins, which bind extracellular hemoglobin and heme, respectively.

We synthesized, purified and quantified these peptide standards in-house using stable isotope labels (SIL) and natural isotopes (NAT) using the Fmoc solid-phase peptide synthesis strategy. Peptides for synthesis were selected based on their uniqueness, fully tryptic with no missed cleavage sites, and a length of 8-25 amino acid residues, to ensure reliable protein quantification. 97 out of 120 selected peptide pairs could be synthesized as well as quantified by amino acid analysis. These 97 peptide pairs were tested via LC-MS using NIST human plasma standard. Afterwards, 86 peptide pairs are present in the preliminary assay, representing 28 proteins.

Additionally, we tested and optimized the protocol for plasma digestion. In order to achieve the required throughput and to ensure the utmost compatibility with clinical reality, a triple quadrupole mass spectrometer was coupled to a microflow chromatography system. The retention times of SIL peptides were determined, and the initial concentration of coagulation cascade proteins was estimated in human plasma standard. Thereby, calibration curves had to be generated for each peptide in order to calculate the limit of detection (LOD) and the limit of quantification (LOQ) values.

The development of this LC-MS-based peptide assay enables the estimation of the risk of thrombosis, such as in anaemic patients treated with erythropoiesis-stimulating agents, who have an increased risk of thrombosis due to the latter. Thus, absolute amounts of the intrinsic proteins as well as therapeutically applied recombinant clotting factors can be determined by this assay.

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P52. Combining SIMSI-Transfer and Prosit leads to a synergistic increase in identifications

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Introduction

While missing values are rare in single-batch TMT experiments, combining multiple TMT experiments causes a rapid increase in missing values. To address this challenge, SIMSI-Transfer was published in 2022, which employs MS2 spectrum clustering to group spectra based on similarity and transfer peptide identifications across TMT batches. SIMSI-Transfer only transfers identifications across batches and is therefore unable to generate new identifications. Conversely, the fragment spectrum intensity prediction tool Prosit is capable of generating new IDs compared to an ordinary MaxQuant run, making it an ideal complement to SIMSI-Transfer. Combining SIMSI-Transfer with Prosit proved to be an effective way to benefit from the distinct methodologies of the two tools, and we are therefore aiming to find the best workflow combining the two to minimize missing values in multi-batch TMT experiments.

Methods

To evaluate and compare the performance of SIMSI-Transfer and Prosit rescoring, we conducted individual runs of both tools on an identical dataset of three TMT batches. We analyzed where the two tools agree on the same peptide-spectrum matches (PSMs), where they disagree, and where only one of the two tools managed to identify a spectrum. Additionally, we assessed the combined workflow of SIMSI-Transfer and Prosit by applying Prosit to re-score a 100% FDR MaxQuant search and subsequently using the results as input for SIMSI-Transfer, evaluating changes and increases in the identifications gained.

Results

Applying SIMSI-Transfer and Prosit using the recommended parameters on the test dataset resulted in similar gains, yielding an overall PSM increase of 13% with SIMSI-Transfer and 15% with Prosit. Notably, the difference in identified spectra indicates an orthogonal identification, with only 20% of the spectra identified by SIMSI-transfer also being identified by Prosit and vice versa. Among the spectra identified by both tools, approximately 75% were identified as the same peptide, while the remaining 25% showed strong indications of chimerism, with each tool identifying one of the two peptides. Moreover, in cases where the identification varied between the two tools, the top 5 MaxQuant peptides did not include the peptide identified by SIMSI-Transfer 95% of the time, making it impossible for Prosit to generate the same identification. Running SIMSI-Transfer after Prosit enhanced the PSMs gained by using each tool individually; although 6% of the exclusive IDs from SIMSI-Transfer were lost due to the generation of ambiguous clusters, the combination with Prosit yielded a 20% increase in PSMs acquired by SIMSI-Transfer.

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P53. Single cell proteomics for discerning adult cardiomyocyte subpopulations

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Introduction

High-throughput single cell strategies have emerged as a powerful, resolutive and informative tool to discern subpopulations of interest in various biological contexts. Nowadays, single cell transcriptomics is the most popular approach for defining single cell characteristics and mapping cellular groups based on transcript information. However, other techniques such as proteomics may provide complementary levels of information for population clustering on the basis of protein expression or post-translational modifications. In mass spectrometry-based proteomics, progression in instrument sensitivity and in multiplexed approaches are improving the capacity to study the heterogeneity of single cell populations in a high-throughput manner. Adult cardiomyocytes are a kind of cells of the highest interest in the cardiovascular field. However, their high size unable their isolation for accurate single cell analysis. As a proof of concept for cardiomyocyte isolation and single cell proteomics analyses, we compare control adult cardiomyocytes with myc-overexpressing adult cardiomyocytes. Myc-overexpressing cardiomyocytes may have an advantage in situations of heart injury.

Methods

In this work we show the application of single cell proteomics to isolated adult cardiomyocytes. We apply manual and FACS sort isolation methods. The isolated cardiomyocytes are submitted to Scope sample preparation workflow and TMT labelled. The samples are analysed in an Ultimate 3000 HLPLC coupled to Eclipse MS instrument supported by FAIMS. Data have been analysed in a real time search and close-out mode. We analyse data following our iSanxot in-house developed software for FDR, normalization and quantification of data. SEURAT workflow has been applied for clustering analysis.

Results

We show the importance of properly isolation of cells for an accurate quantification at the single cell level. Besides, normalization method allows us the comparison of data not biased by batches or differential morphological characteristics of each cell type. Subpopulations of cells in control and myc overexpressing cardiomyocytes are detected.

P54. XL-Prosit: Transfer learning for tuning Prosit to predict fragment intensities of cleavable cross-linked peptides

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Introduction

Chemical cross-linking (XL) mass spectrometry (MS) is an effective tool for analyzing protein structure and protein-protein interactions. However, due to the large search space, identifying cross-linked peptides remains a challenging task. Prior research has shown that incorporating fragment intensity information into the matching process can circumvent this problem. Here, we extend Prosit to predict the fragment ion intensities for cleavable cross-linked peptides using minimal data.

Methods

One of the main challenges is the limited number of training data. For this reason, we systematically evaluated methods to fine-tune a pre-trained model of Prosit to allow the prediction for unknown modifications using the concept of transfer learning. In addition, we improved the accuracy of the prediction model by calibrating collision energy across datasets and augmenting training data by swapping the position of two cross-linked peptides in the context of a model that focuses on the prediction of the intensity pattern of one.

Results

To collect training and test datasets, raw data were downloaded from PRIDE and analyzed using Plink 2 and XlinkX. At the time of writing, 130k MS2 and 37k MS3 spectra for cleavable and 80k MS2 spectra for non-cleavable cross-linked peptides were collected. We fine-tuned Prosit with the available MS3 spectra covering cleavable cross-linked peptides. The final model achieves very high accuracy (median spectral angle of 0.85 and Pearson's correlation of ~ 0.95). In order to determine the optimal deep learning model for MS2 spectrum prediction of cleavable cross-linked peptides, we explored various 20 architectures, which incorporated a second peptide sequence as input to the model. In the final XL-Prosit architecture, the input data (sequences of peptide A and B, precursor charge state, calibrated collision energy) are encoded into a latent space This representation is then decoded to predict normal and XL fragment ion intensities. Preliminary results show that this model achieves similar performance for MS2 as for MS3 cleavable cross-linked data (median spectral angle of ~ 0.82 and Pearson's correlation (R>0.9)). The next step is to integrate XL-Prosit into XL-MS search engines to improve the sensitivity and specificity of cross-linked peptide identifications by utilizing the rescoring approach. This approach is also expected to be transferable to non-cleavable cross-linkers.

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P55. Brain extracellular vesicles provide novel clues to decipher connectome imbalances in Schizophrenia

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Background

Chaotic connectome has been found, in neuroimaging studies analyzing resting state parameters, affecting the brain of schizophrenia (SCHZ) subjects [1]. However, the molecular basis of these network imbalances remains obscure. Similarly, extracellular vesicles (EVs) are known to mediate brain intercellular communication in health and disease conditions [2], though the role(s) of that intercellular communication system in the context of the brain connectome in SCHZ remains unexplored.

Materials and Methods

Brain EVs from 30 subjects were obtained by PROSPR [3] from three brain regions centrally implicated in resting state connectome imbalances in SCHZ: prefrontal cortex, caudate and hippocampus. Discovery-driven next-generation proteomics was subsequently implemented to characterize the obtained brain EVs proteomes, as we indicated previously [4]. Finally, the obtained proteomics data were analyzed by parametric and non-parametric variance and correlation analyses ($p<0.05$).

Results and Discussion

Our results indicate abnormal modulation of specific synaptic proteins linked to EVs in the brain of SCHZ subjects. Similarly, we found that the proteins myelin basic protein (MBP), Collapsin Response Mediator Protein 2 (DPYSL2) and glial fibrillary acidic protein (GFAP) belong to an active network of molecular exchange (Fischer's correlation coefficient $p<0.001$), mediated by EVs and centralized to the caudate region, that becomes imbalanced in SCHZ.

Conclusions

Our study indicates that brain EVs may be implicated in active brain networks of molecular exchange, at intercellular level, able to reflect connectome imbalances linked to brain pathology and SCHZ. Furthermore, our data generated by Systems Biology proteomics reinforces the notion that the molecular basis of psychotic mental disorders can be identified and thus it needs to be further explored in benefit of these clinical populations.

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P56. Discovery-driven proteomics of brain extracellular vesicles reveals novel molecular clues on the higher prevalence of dementia in psychotic subjects

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Background and aims

Schizophrenia (SCHZ) is a mental disorder associated with a large array of co-morbidities including higher prevalence of age-associated dementia. Extracellular vesicles (EVs) on the other hand have been implicated in brain pathology, neurodegeneration and dementia [1] but their role(s) in SCHZ are mostly unknown and any potential role(s) on brain EVs in the apparition and progression of dementia in this clinical population remains undefined.

Methods

Discovery-driven proteomics was used to investigate the proteome-wide the compositions of brain EVs in post-mortem brain tissues (prefrontal cortex, BA9) of subjects with SCHZ and subjects with preclinical Alzheimer's disease (n=40). Brain EVs were obtained by PROSPR [2] and samples were analyzed by advanced bioinformatics to uncover common dysregulated molecular pathways between both disease conditions.

Results

Proteins in brain EVs that have previously been significantly involved in crucial aging-associated neurodegenerative processes such as Glial Fibrillary Acidic Protein (GFAP), Immunoglobulins, the white matter protein myelin basic protein (MBP) or the microtubule protein TAU show striking consistent patterns of regulation in brain EVs between both diseases.

Conclusions

Our data provides novel insight on the potential role(s) of brain EVs in the higher prevalence of aging-associated dementia in psychotic subjects and may contribute to uncover novel molecular targets for the disease.

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P57. Enhanced TMT data analysis with AI-driven workflows utilizing CHIMERYS and INFERYS algorithms

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Multiplexed proteomics quantitation using Thermo Scientific™ Tandem Mass Tags™ (TMT™) is a powerful tool to measure differences in cellular states. Much effort has been applied to instrument development and development of smart acquisition strategies such as real time search (RTS) and synchronous precursor selection MS3 (SPS-MS3) to improve the quantitative performance of TMT workflows. At the same time, there has been significant progress leveraging deep-learning and AI to enhance the depth of proteomics data analysis. However, few studies exist which examine the use of cutting-edge AI-driven algorithms for labeled proteomics data analysis. Here we demonstrate the application of deep learning algorithms to increase identified and quantified TMT labelled peptides.

Data from Fürtwangler et al, MCP, 2022 available via PRIDE (PXD029320) were used for this evaluation. Briefly the authors labeled single cell digests with TMTPro reagents and compared three acquisition methods: MS2, SPS MS3 and RETICLE (real time search triggered MS2.) CHIMERYS™ and INFERYS™ algorithms were implemented as nodes in the Thermo Scientific™ Proteome Discoverer™ 3.0 software (PD). CHIMERYS was deployed in the cloud and received prepared data from a local instance of Proteome Discoverer 3.0. Once processed by the CHIMERYS service, results were transmitted to the local instance for final processing and results viewing.

MS2 and MS3 TMT labeled workflows data were analyzed in the PD using INFERYS rescoring and CHIMERYS searching. For the single cell SPS MS3 data SEQUEST HT identified 4863 peptide groups and 1347 protein groups and quantified 3490 peptides and 1111 proteins. Addition of INFERYS rescoring resulted 1667 protein groups identified and 6164 peptide groups. INFERYS rescoring improved quantified peptides to 4086 and quantified proteins to 1229. Using CHIMERYS resulted in 1632 protein groups and 5824 peptide groups identified. CHIMERYS quantified 3662 peptides and 1169 proteins. For single cell MS3 analysis SEQUEST HT with INFERYS rescoring generated the most identified and quantified peptides and proteins. For the single cell MS2 data, SEQUEST HT alone identified 4073 peptide groups and 1135 protein groups, while quantifying 3854 peptides and 1081 proteins. Addition of INFERYS rescoring results in 1150 protein groups identified and 4008 peptide groups identified. Overall quantified peptides fell to 3769 with the addition of INFERYS while quantified protein groups rose to 1085. Finally, CHYMERIS identified 1658 protein groups and 5037 peptides groups while quantifying 3518 peptides and 1168 proteins

Deep-learning based algorithms increased identified and quantified TMT labeled peptides for both MS2 and MS3 based approaches.

P58. Cross-linking Mass Spectrometry Uncovers Interactions Between High-density Lipoproteins and the SARS-CoV-2 Spike Glycoprotein

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Introduction

High-density lipoprotein (HDL) levels are reduced in patients with coronavirus disease 2019 (COVID-19), and the extent of this reduction is associated with poor clinical outcomes. While lipoproteins are known to play a key role during the lifecycle of hepatitis C virus, their influence upon coronavirus (CoV) infections is poorly understood. In this study we utilise cross-linking mass spectrometry (XL-MS) to determine circulating protein interactors of the severe acute respiratory syndrome (SARS)-CoV-2 spike glycoprotein.

Methods

A combination of XL-MS, affinity purification-MS (AP-MS) and computational modelling were used to determine circulating interactors of the SARS-CoV-2 spike glycoprotein. Cell-based assays were utilised on candidate SARS-CoV-2 spike interaction partners to unravel their influence on viral infectivity and replication capacity.

Results

XL-MS of plasma isolated from COVID-19 patients uncovered HDL protein interaction networks, dominated by acute phase serum amyloid proteins, whereby serum amyloid A2 was shown to bind to apolipoprotein (Apo) D. XL-MS on isolated HDL confirmed ApoD to interact with SARS-CoV-2 spike, but not SARS-CoV-1 spike. Other direct interactions of SARS-CoV-2 spike upon HDL included ApoA1 and ApoC3. The interaction between ApoD and spike was further validated in cells using immunoprecipitation-MS, which uncovered a novel interaction between both ApoD and spike with membrane-associated progesterone receptor component 1. Mechanistically, XL-MS coupled with data-driven structural modelling determined that ApoD may interact within the receptor-binding domain of spike. However, ApoD overexpression in multiple cell-based assays had no effect upon viral replication or infectivity. Thus, SARS-CoV-2 spike can bind to apolipoproteins on HDL, but these interactions do not appear to alter infectivity.

P59. Location, location, location: a system-wide assessment of subcellular protein localization in Arabidopsis roots by mass-spectrometry

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Introduction

The development of organelles is an important hallmark of evolution. Organelles offer unique micro-environments in the cell where a broader range of proteins can function, and provide an additional layer of regulation by separating or concentrating proteins. Hence, many cellular processes involve movement of proteins between organelles and subcellular localization of proteins can often be linked to their functions. The systematic assessment of the localization of proteins is therefore insightful, but also technically challenging. In plants specifically, the presence of a rigid cell wall and specific organelles such as chloroplasts and vacuoles add to the challenges.

Methods

To tackle this, we sought inspiration from the broader spatial proteomics field and adapted a method called Dynamic Organellar Maps (DOMS) for use on Arabidopsis roots. In short, plants are sown on solid medium and grown for a week before the roots are harvested. Proteins are extracted by homogenizing the tissue and fractionated by differential centrifugation. All fractions are prepared using PAC beads followed by C18 stagetipping, before measuring on an Exploris 480 in DDA mode. During data analysis, the distribution profiles of marker proteins are used to predict the subcellular localization of all measured proteins. This approach not only offers static maps of protein subcellular localization, but also the possibility to compare maps to detect proteins that move between organelles under the influence of treatments or in specific genetic backgrounds, such as hormone treatments, biotic and abiotic stresses, knock-out and overexpression lines.

Results

In this poster presentation I will describe the progress and challenges of implementing the method to Arabidopsis, such as homogenization of the plant tissue without damaging the organelles, selection of marker proteins and reproducibility.

P60. High resolution DIA: A workflow for highly accurate relative label-free quantification of microbial proteins in complex cell lysates

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Introduction
Relative quantification of proteins in complex samples raises a demand for high sensitivity and reproducibility throughout large sample sets to gain meaningful insights on biological processes. Data-independent analysis has emerged as a powerful technique enabling quantification of thousands of proteins because it avoids the intensity bias and missing value problem that typically limit data-dependent methods. DIA in principle interrogates all peptides that are present in a sample and therefore is especially suitable for high-throughput and large-cohort studies.

Methods
Different microbial proteomes were spiked into a human proteome background at different ratios, yielding two proteome and three proteome mixtures with varying total protein amounts. Samples were separated on a 50 cm µPAC™ HPLC columns in direct injection setup on a Vanquish™ Neo system under nano-flow conditions. DIA experiments were run on an Orbitrap Exploris™ 240 mass spectrometer. Data was analyzed by Spectronaut™ 16 using a library-free approach.

Results
Using micropillar array-based column technology under nano-flow conditions for separation of peptides gives optimal peak shapes and intensities reproducibly over a long-term acquired data set with minimal performance loss. In connection with the high-resolution DIA methodology, this enables for wide proteome coverage in two- and three-proteome mixtures as well as quantification accuracy below 10 % at high sample throughput. The uncomplicated and easily implemented library-free data analysis yields similar performance as low-key library-based approaches.

Conclusions
Micropillar array-based separation technology and high-resolution data-independent analysis enable for a wide proteome coverage at high throughput, while maintaining excellent quantification accuracy of relative protein ratios in complex cell lysates.

P61. Reproducible and scalable one-day FFPE sample preparation method for LC-MS assays with Adaptive Focused Acoustics ® (AFA)

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Introduction

Translational or clinical laboratories require automated, hands-off solutions for protein sample preparation to provide reproducibility, increased efficiency, higher quality results, and faster turnaround time. This communication highlights a simultaneous, one-day, multi sample processing from formalin-fixed and paraffin-embedded (FFPE) tissue for liquid chromatography-mass spectrometry-based (LC-MS) analysis, using focused ultrasonication (AFA).

Methods

AFA was used to efficiently extract proteins from FFPE and to allow a robust single pot sample preparation: this highly reproducible method works with short or long LC-MS gradients, and with every clean-up and digestion method. When working with bead-based protein aggregation capture, the workflow can include an accelerated trypsin digestion step (3 hours).

Results

AFA makes FFPE tissue proteomics compatible with the clinical setting, through a non-toxic deparaffinization and rehydration approach. Typically, short gradients (5 min) allow for ~2,500 protein IDs and longer gradients (60-90 min) for 6,000 to 9,000 IDs depending on the tissue type. Accelerated trypsin treatment yields similar quality compared to overnight digestion (below 10% miscleavages), in a more controlled way, and leads to 96 samples fully processed in 7 to 8 hours.

AFA simplifies workflows by allowing protein extraction through digestion in a single well with an automation compatible high-throughput (HTP) approach, decreases turn-around-time, makes one-day sample prep also fully applicable to laser capture microdissections (LCM) and fresh/frozen tissue preparation.

1. Automated Protein Extraction from Clinical Samples with the Covaris ML230 Focused-ultrasonicator. Staeber et al
2. AFA-sonication Followed by Modified Protein Aggregation Capture (APAC) Enables Direct, Reproducible and Non-toxic Sample Preparation of FFPE Tissue for Mass Spectrometry based Proteomics. Schweitzer et al.,
3. Scalable FFPE Sample Preparation Method for Clinical Proteomics LC-MS Assays with Laser capture microdissection coupled mass spectrometry (LCM-MS) for spatially resolved analysis of formalin-fixed and stained human lung tissues. Herrera et al.,
4. Automated sample preparation with SP3 for low-input clinical proteomics. Mueller et al
5. Adaptive Focused Acoustics ® Dr Jessica Chapman, MSKCC – Covaris Seminar
6. Proteome analysis of formalin-fixed paraffin-embedded colorectal adenomas reveals the heterogeneous nature of traditional serrated adenomas compared to other colorectal adenomas. Sohier et al
7. Novel Laser Capture Microdissection-Proteomic Analysis Identifies Spatially Distinct Extracellular Matrix Signatures in the Core and Infiltrating Edge of Human Glioblastoma. Pedley et al
8. Dietary-challenged mice with Alzheimer-like pathology show increased energy expenditure and reduced adipocyte hypertrophy and steatosis. Schreyer et al

P62. Atabecestat and metabolite interactions with the immunopeptidome and atabecestat binding to model proteins in the presence of metabolising systems

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Introduction

Atabecestat (BACEi) was developed to treat Alzheimer's disease. However, clinical trials were halted due to drug induced liver injury (DILI). T-lymphocyte infiltration in the liver and the detection of drug-responsive T-cells in patients and healthy donors suggested the involvement of the adaptive immune system in DILI. The aim of this study was to (i) identify haptenic structures that trigger T-cell responses (ii) investigate the immunopeptidome in the presence of atabecestat, metabolites and SMXNO, a positive control for detecting haptenated HLA ligands.

Methods

Atabecestat was incubated with metabolising systems (HLMs, CYP3A4, HepG2), model proteins (GST-a1, GST-Pi), and glutathione. Proteins were digested by trypsin and analysed by LC-MS/MS. Antigen presenting cells (Epstein-barr virus transformed B-cells) from homozygote HLA-DRB1*15:01 healthy donors were dosed with atabecestat, DIAT metabolite and SMXNO. HLA-DR peptides from cell lysates were eluted using anti-HLA-DR antibody. Peptides were purified and analysed by LC-MS/MS.

Results

Atabecestat-glutathione conjugates were formed in the presence of CYP3A4, indicating that a thiol reactive intermediate was formed. Atabecestat was found to form covalent adducts with a lysine residue on GST-A1 in the presence of GSH and CYP3A4, and a cysteine residue on GSTPi, with HLMs. HLA-DRB1*15:01 peptides were identified with verified anchor residues. The analysis of HLA-DR peptides presented in the presence of the three drugs compared to the control is still undergoing.

Discussion

These drug-protein conjugates could be processed, presented and interact with T-cell receptors in patients that developed DILI. T-cell clones generated were HLA-DRB1*15:01 restricted. We therefore investigated unique peptides presented in the presence of drugs/metabolites. Thousands of HLA class II ligands have been identified using immunopeptidomics. Further investigation of the immunogenicity of drug associated HLA antigens would allow better understanding of the mechanisms of atabecestat-DILI.

P63. Mechanistic understanding of the relationships between oxidative and electrophilic stress in allergic skin sensitisation

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Lipid peroxidation is a process that occurs during a state of oxidative stress resulting in formation of reactive aldehydes malondialdehyde (MDA), acrolein, and 4-hydroxy-2-nonenal (4-HNE) that can modify proteins by carbonylation. These aldehyde-protein adducts are considered a marker of lipid peroxidation. Carbonylation is considered one of the most damaging oxidative protein modifications. Allergic skin sensitisation is an immune response to chemically modified skin proteins and manifests clinically as allergic contact dermatitis. Oxidative stress is linked to skin sensitisation though the mechanisms of their relationship are incompletely understood. Using a proteomic approach, we examine the effect of skin sensitisers on formation of lipid peroxidation products and their ability to carbonylate proteins to help understand the relationship between sensitisation and oxidative stress.

HaCaT cells are treated with vehicle dimethyl sulfoxide (DMSO) (0.1%), the known electrophilic sensitiser 2,4-dinitrochlorobenzene (DNCB) (10µM) in the presence and absence of oxidative stressor H_2O_2 (200µM) for 4 hours. Positive controls include treatments of acrolein (10µM) and 4-HNE (10µM) in vehicle DMSO. Separately, HaCaT cells treated with DNCB ± H_2O_2 are labelled with carbonyl-reactive aminoxyTMT™sixplex reagents (Thermo). This Tandem Mass Tag system allows enrichment of carbonyl modifications of peptides. LC-MS/MS was performed with Thermo Scientific™ OrbiTrap Fusion™ Tribrid™ Mass Spectrometer. Quantitative proteomic analysis performed using PEAKS (Bioinformatics Solutions Inc.). PEAKS data cleaned up with R. Gene Set Enrichment Analysis (GSEA) and KEGG pathway analysis performed with clusterProfiler for R 4.2.2.

GSEA and KEGG pathway analysis revealed that combination DNCB + H_2O_2 treatment increased expression of immune system pathway-associated proteins signal transducer and activator of transcriptions 1 & 2 (STAT1/2), Rac, Syk & fatty acid synthase (FAS), affecting multiple immune system pathways including B cell receptor signalling, osteoclast differentiation, FcεRI signalling pathways. Upregulation of 1-aminocyclopropane-1-carboxylate synthase (ACS), carnitine palmitoyl transferase I (CPT-1), and downregulation of apolipoprotein A-I (ApoA-I) in combined DNCB + H_2O_2 treatment affect peroxisome proliferator-activated receptors (PPARs) pathway likely leading to increased fatty acid oxidation and decreased lipid transportation. Upregulation of long-chain-fatty-acid CoA ligase and CPT-1 occurred in combined DNCB + H_2O_2 and H_2O_2-only treatments, likely increasing activity of fatty acid degradation. Fatty acid degradation and oxidation are key pathways in lipid peroxidation. This implicates lipid peroxidation as potentially affecting sensitisation. Analysis of aminoxyTMT-labelled enriched carbonylated peptides is ongoing.

This preliminary data suggest lipid peroxidation is likely involved with allergic skin sensitisation.

P64. Advanced assessment through intact glycopeptide analysis of Infliximab's biologics and biosimilar

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Introduction

Originator and biosimilar products of antibody drugs represent heterogeneous variants characterized by differences in glycosylation, oxidation, glycation, aggregation state, and deamidation, where the glycosylation is an important post-translational protein modification that alters protein properties, including the pharmacokinetics (PK), pharmacodynamics (PD), effector functions, solubility, and stability. Infliximab (brand name, Remicade ®) is a representative mAbs that interacts with target tumor necrosis factor α (TNF-α) for treating autoimmune diseases, such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, adult plaque psoriasis, pediatric and adult ulcerative colitis and Crohn's disease. In this study, N-linked glycosylation of Remicade ® and Remsima ® , the originator and biosimilar of infliximab, respectively, were analyzed at the glycopeptide level with a focus on profiling the glycan composition through tandem MS spectra.

Methods

In order to analyze the glycosylation of infliximab, five batches in each originator and biosimilar were used. Based on the number of identified glycopeptide-spectrum matches (GSMs), the originator and biosimilar of Infliximab were compared quantitatively and the similarity was evaluated. Here, we demonstrated high-resolution tandem mass spectrometry with an ultra-high-performance liquid chromatography for characterization and comparison between originator and biosimilar mAbs at an advanced level. Focus on the infliximab, specifically, we compare the N- and O-glycopeptides profiles of originator and biosimilar products of Remicade ® and Remsima ® , respectively. Five batches each of originator and biosimilar were prepared to evaluate the similarity using the Pearson correlation coefficient based on the number of GSMs identified by LC-MS/MS analysis coupled with the GPA software.

Results

A total of 49 and 54 glycopeptides were identified in Remicade ® and Remsima ® , respectively, at two sites of N300 and N41ively. We also identified NeuGc and NeuAc-containing glycopeptides, 12 and 18 glycopeptides (618 and 678 spectra) with NeuGc were identified, and 4 and 3 glycopeptides (23 and 34 spectra) containing NeuAc were identified in Remicade ® and Remsima ® , respectively. At N41site, only hybrid type and high mannose type glycosylation were expressed, unlike the N300 site. All of the top 10 peptides were expressed at the N300 site. The overall qualitative profiles for both the originator and biosimilar are very similar. The most frequently expressed glycan were G0F(3_4_1_0_0) and G1F (4_4_1_0_0) in N300, and is in agreement with the results of previous studies. 8 out of 10 glycopeptides were fucosylated, and 4 out of 10 sialylated glycopeptides were attached with NeuGc. To evaluate the similarity between the originator and biosimilar and among different batches, the Pearson correlation coefficient was used based on the relative abundance. A value close to +1.0 indicates a better correlation. The similarities between batches in the same mAbs (0.93-0.97) were higher than the similarities between batches of originator and biosimilar (0.84–0.94). Therefore, these results confirmed that the similarity between the originator and biosimilar of infliximab and between different batches was significant.

P65. Search for peptide markers of salivary gland tumors in patients' saliva samples. Pilot study.

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Introduction

Salivary gland tumours are highly diverse in clinical manifestation and histology. The World Health Organization (WHO) classification distinguishes 22 types of malignant and 11 benign tumours originating from the salivary glands. Diagnostics of salivary gland tumours are based on imaging (ultrasound, magnetic resonance imaging) and fine-needle aspiration biopsy; however, the final diagnosis is based on the histopathological examination of the removed tumour. In this pilot study, we test a new approach for identification of peptide biomarkers in saliva that can be used in diagnosing tumours of the salivary glands. As a research material for peptidomic studies, we use extracts from washing neoplastic tissues and healthy tissues (control samples). At the same time, samples of saliva from patients, as well as from healthy individuals, will be analyzed. Comparison of the peptidome composition of tissue extracts and saliva samples may allow the identification of potential peptide markers of a salivary gland tumor in patients' saliva.

Methods

We analyzed the peptidome composition extracted from 18 tumour and 18 healthy tissue samples using LC-MS tandem mass spectrometry. PBS and 0.1% trifluoroacetic acid were used to extract the peptide fraction from the tissue samples. We also investigated the peptidome compositions of patients' saliva (11 samples) and healthy individuals (8 samples).

Results

We selected a group of peptides (109 peptides) that were present only in extracts from tumour tissues and in samples of the patient's saliva. Some of the identified peptides come from proteins that were previously indicated as potential biomarkers of salivary gland tumours (ANXA1, BPIFA2, FGB, GAPDH, HSPB1, IGHG1, VIM) or tumours of other tissues or organs (SERPINA1, APOA2, CSTB, GSTP1, S100A8, S100A9, TPI1). Unfortunately, in individual samples only a few peptides characteristic only of the cancer tissue and the patient's saliva appeared, which does not allow to identify a universal biomarker. Perhaps the reason for this situation is the high heterogeneity of this type of cancer. The surprising result was that extracts derived from tumor tissues did not contain peptides derived from salivary gland specific proteins (STATH, SMR3B, HTN1, HTN3). These results suggest that the developing tumor suppresses the production of proteins that are essential components of saliva, however, confirmation of this hypothesis requires further additional research.

P66. Glycan and Collagen-ECM Peptide Imaging Mass Spectrometry and Proteomics of FFPE Prostate Tumor Tissues

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Introduction

Alterations in cell surface N-glycosylation and emergence of reactive stroma in tumors are now recognized as hallmarks of cancer oncogenesis, signaling and metastasis. Imaging mass spectrometry (IMS) analysis of N-glycans and collagen-ECM peptide proteomics is being used to characterize glycoproteins and ECM proteins in formalin-fixed human prostate tumor tissues. A novel cohort of prostate tissues reflecting disease progression were assessed for prognostic marker discovery.

Methods

Tissue microarrays (TMA) and full tissues from prostate cancer patients who had no cancer recurrence following prostatectomy (NED) or had recurrence of prostate cancer (Met) were evaluated with peptide N-glycosidase F and collagenase type III digestions for multiple IMS studies on a Bruker MALDI-QTOF fleX instrument. Images were visualized with SCiLS Lab software. Collagen-ECM peptides from collagenase digestion were sequenced using a Thermo Lumos Fusion Orbitrap instrument.

Results

N-glycan and collagen-ECM IMS analysis was done sequentially on five prostate cancer TMA slides, 86 cores of NED tumors and 42 cores of MET tumors. Following imaging, detected N-glycan species (n = 169) were categorized into different groups based on structural classes. Multi-fucosylated branched N-glycans, N-glycans containing bisected structures, and N-glycans containing poly-lactosamine chains were all found to be upregulated in the MET cohort. Pauci-mannose and biantennary N-glycans were found to be upregulated in the NED cohort. Following N-glycan analysis, tissues underwent on-tissue collagenase III digestion for IMS analysis of collagen peptide distribution. In combination with untargeted proteomics studies, IMS of each TMAs identified 44 unique collagen-ECM peptides found within the cohort, 5 of which were exclusively found in the MET cohort and 12 found exclusively in the NED cohort. Following TMA analysis, the same workflows were applied to a cohort of prostate cancer tissues containing a larger surface area of tumors and surrounding tumor microenvironments to better spatially define the N-glycans and collagen peptides of interest. N-glycans and collagen peptides consistent with the NED category of TMAs were generally localized to the tumor within the larger tissues while those consistent with the MET cohort of TMAs were generally localized to the surrounding tumor microenvironment and stroma. Ongoing characterization include the use of additional enzymes and reagents for sialic acids for further N-glycan characterization and direct glycopeptide analysis following collagenase digestions.

P67. Cross-Omics Analysis of Transcriptome, Proteome and Metabolome Dynamics during Peritoneal Dialysis

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Background

Peritoneal dialysis (PD) as life-saving renal replacement therapy offers patients a home-based continuous therapy and higher quality of life compared to conventional center-based haemodialysis. PD uses the peritoneal membrane and an highly osmotic PD-fluid instilled into the peritoneal cavity to remove toxins and water from the patient. The resulting waste product (PD effluent) is not only a rich source of markers for therapy monitoring and investigation of deregulated processes during PD it is also surprisingly underexplored and therefore poorly defined. For understanding PD transport dynamics and pathomechanisms on a systemic level, a multi-level omics approach is particularly attractive.

Method

Samples were obtained from stable patients chronically treated with PD at different time-points of standard 4h peritoneal equilibration tests (PETs). Effluent was collected after the pre-PET (overnight) dwell and at 0h, 1h and 4h dwells and plasma samples were also taken during this period. Effluent was separated into a cellular and cell-free component. Soluble metabolites in the cell-free compartment were processed using a targeted LC-MS workflow, proteins were enriched using equalizer beads, digested using the SP3 protocol and analysed using 36 off-line fractions and TMT-18plex with internal standard channel. The cellular material was subjected to RNA sequencing. The plasma proteome database was used for referencing plasma proteins and estimating plasma concentration. A bioinformatic workflow conjoined information from the datasets to reveal novel insights into the "PD-effluentome", especially unravelling the origin of proteins and metabolites in PD effluent.

Results

207 unique metabolites were detected in cell-free PD effluent. A mixed-effect ANOVA of all metabolites demonstrated dwell time-dependent concentration changes in 173 metabolites. Post-hoc testing revealed most metabolites to be changed between 1h and overnight time points, followed by 114 and 46 differently concentrated metabolites between 4h and overnight and 1h and 4h, respectively. We quantified 9,797 transcripts in PD-effluent cells and 2,729 proteins in PD effluent. 342 proteins were filtered from plasma, while 800 proteins were attributable to local origin or production. A quantitative analysis of the interaction proteome and cellular transcripts of ~1700 protein-transcript pairs showed clusters of proteins explained by over-expression in peritoneal cells compared to plasma concentrations.

Conclusion

Cross-omic profiling of PD effluent can be a valuable approach for revealing small molecule related changes during PD. The exploitation of PD effluent on multiple levels could improve the understanding of pathophysiological molecular processes and transport dynamics in the peritoneal cavity and their role in development of PD complications.

P68. Unlocking the Secrets: Exploring Cell-to-Cell Communications through Secretomics Analysis

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Background

Peritoneal Dialysis (PD) is a life-saving renal replacement therapy. The composition of the hyperosmotic PD-fluids triggers morphological and functional changes in the peritoneal membrane eventually leading to reduced ultrafiltration capacity and failure of the technique. Cross-talk among different cell types forming the peritoneal membrane modulates PD-associated deterioration. Currently, there is no in-vitro model system available to study the interactions of these cells in close proximity. Here, we aimed to develop a co-culture model for investigating cell-to-cell communication by analysing the cellular proteome and secretome.

Methods

For modelling the peritoneal membrane, mesothelial and primary endothelial cells were co-cultured in transwell plates. Cells were cultured under optimized conditions for simultaneous culturing under non-starving conditions (5% FCS) and each cell type was differently labelled (SILAC). Cells were exposed to PD-fluids in either co-culture or single culture conditions. To overcome current limitation on secretomics analysis in non-starvation conditions, the secretome was analyzed using an equalizer approach to deplete high abundant proteins from the FCS in combination with a SILAC strategy to identify the cell origin of the secreted proteins. For quantitative analysis of cellular and secreted protein abundances, LC-MS (36 off-line fractions, TMT-18plex) was performed. Multiple runs were combined via an internal pooled standard channel in all runs.

Results

Co-cultured cells yielded differently regulated pathways following PD-fluid exposure compared to individual cultures. Combined proteome and secretome analysis showed different ligand-receptors pairs expressed uniquely in co-culture conditions regulating specific signaling pathways. In the co-culture ~1900 cell-secreted proteins were identified. Protein-protein interaction analysis revealed 11 functional clusters in the secretome interacting with different receptors presented by the cells. The resulting interactome between cells through the secreted proteins in combination with differentially expressed cellular and secreted proteins revealed novel candidates affected by PD-fluids regulating signaling pathways related to angiogenesis, TGFb, NOTCH, and hippo signaling.

Conclusion

This study shows that harmful effects of PD-fluid exposure on mesothelial cells also affect endothelial cells. Interestingly, both cell types react differently when co-cultured compared to the individual culture, showing the importance of models that allow multiple cell types to interact for mimicking the in vivo situation. We further identified potential signaling axes between the cell types explaining pathophysiological changes of the peritoneal membrane during PD. Characterization of PD-induced perturbations may allow identifying mechanisms linking the peritoneal and cardiovascular system, offering therapeutic targets to reduce current limitations of PD, and ultimately decreasing cardiovascular risk of dialysis patients

P69. Uncovering the interactome of endogenous PINK1 during mitophagy through TurboID-based temporal proteomic analysis

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Introduction

Loss-of-function mutations in the human PINK1 kinase are linked to the onset of early-stage Parkinson's disease. In healthy mitochondria, PINK1 is constitutively imported into mitochondria through the translocase of the outer mitochondrial membrane (TOMM) complex. Disruption in the mitochondrial membrane stabilises PINK1 on to the TOMM complex, leading to phosphorylation of ubiquitin and recruitment of the E3 ubiquitin ligase Parkin to the damaged mitochondria. This result in wide scale ubiquitylation of mitochondrial proteins inducing mitophagy. However, the additional factors that complement the upstream PINK1 activation events in response to mitochondrial damage remain poorly characterised. Likewise, very little is known about the repertoire of PINK1's interactors that orchestrate the downstream mitophagy signalling pathways. In this study, we have undertaken a TurboID-based temporal proteomics analysis to precisely capture both the dynamic and direct interactome of endogenously expressed PINK1 during mitophagy.

Methods

WT cells and PINK1-GFP CRISPR/Cas9 KI cells stably expressing a TurboID biotin ligase fused with a destabilised anti-GFP nanobody were treated with mitochondrial depolarising agent oligomycin/antimycin for up to 12h. Cells were stimulated with 500uM biotin 15 mins before being harvested and lysed in native conditions. Total cell extracts were enriched for mitochondrial fractions and lysed in a hypertonic mitochondrial fractionation buffer with 1% SDS. Biotinylated proteins in the lysate were isolated by Streptavidin-magnetic beads. Enriched proteins were then digested in solution and resulting peptides were injected on an Ultimate 3000 coupled to an Exploris 480 operating in Data Independent Acquisition mode. Proteins were identified with DIA-NN and kinetic profiling was carried out using a two-sample Hotelling T2 test and Fuzzy C-means clustering in Python using in-house scripts.

Results

In Total we identified 1,395 proteins groups specifically enriched with the Streptavidin beads, including 279 differentially regulated in the PINK1-GFP KI cells. To remove noisy profiles, the coefficient of determination for a third order polynomial was calculated for each profile and only those with a R2 greater than 0.90 were kept, leaving 133 proteins. Fuzzy C-means revealed 42 proteins clustering with PINK1, including 30 known mitochondrial proteins, suggesting a potential interaction between PINK1 and these proteins. These 42 proteins include previously characterized TOMM complex proteins, such as USP30, RHOT1, RHOT2 or MFN2, thus corroborating the robustness of this analysis. We are now in the process of validating some of the newly discovered hits, such as MAVS, PPP1R15B and MFF and identify their functions.

P70. Molecular Phenotyping of 3D Cultured Triple-Negative Breast Cancer Cells Reveals Pathways Influenced by Culture System

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Breast cancer has the highest incidence and mortality rate of female cancers globally, with 10-15% of cases classified as triple-negative breast cancer (TNBC), which has a worse prognosis and fewer treatment options. 3D culture systems as an in vitro platform for cancer cell modelling are considered more clinically representative and have the potential for ultra-high-throughput therapeutic research. Here we employed proteomic analysis to further the understanding of the biological pathways involved in 3D TNBC spheroid models.

MDA-MB-231 and HCC1143 cell lines were cultured with complete medium for 4-days using standard monolayer or scaffold-free ultra-low adherent culture. Protein extracts were reduced, alkylated and digested using trypsin. Proteomic profiling was performed using a Dionex nano-LC system coupled to an Orbitrap Fusion mass spectrometer with raw data processed and searched against the human SwissProt database. Data were further analysed by Pathway analysis conducted using the R package clusterProfiler. Cell viability was assessed with Celltiter-glo 3D after exposure to the chemotherapeutic agents doxorubicin or paclitaxel for 48 hours.

In scaffold-free culture, both cell lines displayed a distinct proteomic signature compared to traditional monolayer culture. In comparison to MDA-MB-231, HCC1143 exhibited more significant differences with 2098 DEPs out of a total of 4309 proteins, while MDA-MB-231 had 1041 DEPs out of a total of 4498. This may reflect their contrasting morphologies with HCC1143 exhibiting spheroid formation, while MDA-MB-231 formed loose aggregates. Pathway analysis highlighted several pathways that were enriched in scaffold-free culture including DNA repair, cell-cell junctions and metabolic reprogramming. The evaluation of cell viability post-treatment indicated that scaffold-free culture displayed greater resistance to doxorubicin and paclitaxel compared to monolayer culture.

This study identified key cancer pathways enriched in 3D TNBC spheroid models compared to 2D monolayer models and highlights the importance of 3D spheroid models in advancing our mechanistic insight of therapeutic response.

P71. Mass spectrometry – Swiss Army knife to dissect the spermatoproteasome structure and function

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Introduction

The proteasome is a complex molecular machinery whose role is to degrade proteins. Catalytic subunits can be replaced by tissue-specific subunits, making subtypes with particular functions (e.g. constitutive proteasome c20S, immunoproteasome i20S). Proteasome activity is regulated by different protein complexes and it has been shown that these regulators can bind preferentially to certain proteasome types. During spermatogenesis, spermatogonia undergo a series of mitotic and meiotic divisions on their path to spermatozoa, requiring important proteolytic activity, partly orchestrated by the proteasome. The spermatoproteasome (s20S) is specific to the developing gametes, in which the gamete-specific α4s subunit replaces the α4 isoform found in the constitutive proteasome (c20S). Although the s20S is conserved across species and was shown to be crucial for germ cell development, its mechanism, function and structure remain uncharacterized.

Characterizing the differences between the c20S and s20S could help understand why the α4s is crucial for spermatogenesis and help understand how the proteasome activity shapes germ cell differentiation.

Methods

We approached these questions in two ways: 1- exploring the dynamics of proteasome composition and interacting proteins throughout spermatogenesis using affinity purification strategies and shotgun proteomics and 2- looking at structural differences between the c20S and s20S complexes using structural MS technique called Hydrogen-Deuterium eXchange (HDX) as well as Top-Down Proteomics. Moreover, we used biochemical and enzymatic assays to probe for regulator preferences towards different proteasome types and compared the different complexes' proteolytic activity.

Results

After analyzing the germ cells at different stages of differentiation, we observed that the s20S becomes highly activated as germ cells enter meiosis, mainly through extensive 19S activation and PA200 binding. The proteasome population shifts from predominantly c20S (98%) to predominantly s20S (>82-92%) during differentiation, presumably due to the shift from α4 to α4s expression. We demonstrated that s20S, but not c20S, interacts with components of the synaptonemal complex. In vitro, s20S preferentially binds to 19S, and displays higher trypsin- and chymotrypsin-like activities, with and without PA200 activation. Using HDX-MS methods to monitor protein dynamics, we identified significant differences in domain flexibility between α4 and α4s paralogues. With this set of MS-based analyses, we present some key differences between c20S and s20S. Our results imply a more complex process of s20S regulation than previously suggested. We propose that these differences induced by α4s incorporation result in significant changes in the way the s20S interacts with its partners, and dictate its role in germ cell differentiation.

P72. Identifying targets for immunotherapy with large scale immunopeptidomics: challenges and opportunities

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Introduction

The Human Leucocyte Antigen (HLA) complex presents peptides derived from intracellular and extracellular proteins on the cell surface for sampling by T cells, via their T cell receptors (TCRs). Amongst the repertoire of HLA-peptides are several attractive targets for immunotherapy, including peptides derived from cancer-associated, viral, overexpressed, or mutated proteins. Identifying and validating viable HLA-peptide targets is a crucial step in the development of TCR-based immunotherapies. Our immunopeptidomics workflow focuses on the immunopurification and characterisation of HLA-peptides from immortalised cell lines and fresh frozen tissue. Here, we highlight the challenges faced in seeking to decode the immunopeptidome within a tissue microenvironment and demonstrate that leveraging large scale immunopeptidomics libraries and data-led experimental approaches can unravel the complex relationship between mRNA, protein, and HLA-peptide.

Methods

HLA-class I peptide complexes were enriched from immortalized cancer cell lines and fresh frozen tumour tissue lysates by affinity chromatography using a variety of anti-HLA antibodies, fractionated, and analysed by LC-MS/MS (Orbitrap Tribrid and 6600 QTOF instruments). Mass spectra were searched against the human proteome database (Uniprot) with a variety of search engines (PEAKS 7.5; Protein Pilot). Peptide sequences identified were filtered to 5% FDR and integrated in our in-house database.

Results

We have performed thousands of mass spectrometry-based immunopeptidomics experiments in ca. 200 immortalised cell lines and more than 350 tissue samples. We have dramatically increased the depth of the HLA ligandome captured, with up to 100,000 peptides identified from a single sample, and more than 6 million HLA peptide identifications from tissue. This enabled us to achieve near total coverage of the protein-coding genome, with over 90% of the proteome captured for HLA-A*02:01. The pool of HLA-A*02 peptides recovered from tumour tissue correlates better with data obtained from cell line models than that from prediction algorithms. The peptide novelty rate in tissue is low, mostly attributable to genes that are not represented in cell lines. These findings suggest that in vitro cell models are broadly representative of the in-vivo HLA-class I environment and highlight the power of using cell lines to form a reference that enables maximal value to be derived from the more challenging tissue environment. By combining matched immunopeptidomics and transcriptomic experiments, and by leveraging public data we can explore the relationship between the transcriptome and the immunopeptidome to predict the likelihood of peptides being presented by HLA complexes and prioritize target HLA-peptides during the early stages of target validation.

P73. Immunopeptidomics enable the development of soluble TCR bispecifics to provide a functional cure for HIV

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Introduction

An estimated 38 million people were living with human immunodeficiency virus (HIV) infection in 2021 and depend on lifelong antiretroviral therapy to suppress viral replication and opportunistic infections. Elimination of CD4+ T cells harbouring integrated proviruses ('reservoirs') will be required to achieve a cure. Immunopeptidomics is currently the only approach to directly identify viral peptides presented on human leukocyte antigen (HLA) complexes, which can serve as targets for immunotherapies. T cell receptor (TCR)-based therapies engineered to bind viral peptide-HLA complexes with high affinity have the potential to eliminate HIV reservoirs and provide a functional cure. In this study we analysed the immunopeptidome of HIV-infected human cells by mass spectrometry to facilitate the generation of TCR bispecific molecules as a functional cure for HIV.

Methods

Immortalized human T cell lines (C8166 and Jurkat) were transduced with HLA-A*02:01 and infected with HIV-1 strain IIIB. Immunopeptides were enriched using a sequential immunopurification strategy, fractionated, and analysed by LC-MS/MS. PEAKS was used to identify peptides from MS/MS spectra using a combined human and HIV proteome database. Peptide identifications were filtered to 1% false discovery rate at peptide-spectrum match level before downstream analysis. Stable isotope-labelled peptides were used for validation and absolute peptide quantification by targeted proteomics. TCR discovery was performed against prioritised HIV peptides and select TCRs were affinity-enhanced for target HIV peptide-HLA-A*02:01 complexes using phage display. Potency and specificity of the molecules were assessed using HIV-infected target cells and antigen-negative cells, respectively.

Results

Immunopeptidome profiling of HLA-A*02:01-expressing C8166 and Jurkat cells infected with HIV-1 IIIB resulted in the identification of 36,942 and 43,767 peptides, respectively. Most peptides (97%) were 8-14 mers and mapped to HLA alleles present in C8166 and Jurkat cells. In total, 153 and 102 unique HIV peptide sequences were identified in C8166 and Jurkat cells, respectively, that spanned 4 orders of magnitude in intensity. Targeted proteomics assays confirmed peptide abundance rankings and enabled validation of peptide sequences. Conserved and immunodominant peptides were prioritised for TCR bispecific molecule development. Wild type TCRs were successfully affinity-enhanced, achieving ~ 10^6-fold improvement (μM to pM) in affinity. The lead molecule mediated killing of HIV-infected cells in vitro at low picomolar concentrations whilst maintaining an acceptable therapeutic window based on testing against antigen-negative cells. A combined immunopeptidomics and TCR engineering pipeline was successfully applied to generate a highly potent molecule targeting HIV-infected cells, demonstrating the potential of this approach to address unmet medical needs in infectious diseases.

P74. Flashlight into the Function of Unannotated C11orf52 using Affinity Purification Mass Spectrometry

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Introduction

For an enhanced understanding of the biological mechanisms of human disease, it is essential to investigate protein functions. In a previous study, we developed a prediction method of gene ontology (GO) terms by the I-TASSER/COFACTOR result, and we applied this to uPE1 in chromosome 11. Here, to validate the bioinformatics prediction of C11orf52, we utilized affinity purification and mass spectrometry to identify interacting partners of C11orf52.

Methods

To determine C11orf52 interacting partners, we designed an AP-MS analysis and a validation experiment. First, we prepared an empty vector and two plasmids for the expression of C11orf52 and the epitope tags. These plasmids were transiently transfected in HEK 293T cells, and the cell lysates were immunoprecipitated with Myc, Flag, and 2B8 tags. Second, the immunopurified complexes were digested with trypsin following the S-trap digestion method. C11orf52 binding candidates were identified by LC-MS/MS and, with the IP2 search engine, and were selected by a bioinformatic analysis with the DAVID functional annotation tool and the STRING DB. Finally, the interacting candidates were validated with co-IF staining.

Results

Using immunoprecipitation methods with three different peptide tags (Myc, Flag, and 2B8) in HEK 293T cell lines, we identified 79 candidate proteins that are expected to interact with C11orf52. The results of a pathway analysis of the GO and STRING database with candidate proteins showed that C11orf52 could be related to signaling receptor binding, cell–cell adhesion, and ribosome biogenesis. Then, we selected three partner candidates of DSG1, JUP, and PTPN11 for verification of the interaction with C11orf52 and confirmed them by colocalization at the cell–cell junctions by coimmunofluorescence experiments. On the basis of this study, we expect that C11orf52 is related to the Wnt signaling pathway via DSG1 from the protein–protein interactions, given the results of a comprehensive analysis of the bioinformatic predictions.

P75. Proteomic analysis of lung cancer types

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Introduction

Lung cancer is the leading cause of tumor-related mortality worldwide. Therefore, studies focusing on a better understanding of molecular alterations occurring at the origin of the disease are especially important to improve current treatment options. Our aim was the proteomic characterization of formalin-fixed paraffin-embedded tissue sections from patients with small cell (n = 9) and non-small cell lung carcinoma (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, n = 10, 9, 10 respectively).

Methods

On-surface digestion was performed on cancerous and tumor-adjacent normal tissue regions which was followed by the identification of differentially expressed proteins based on label-free quantitative mass spectrometry analysis. Following the proteomics analysis, Gene Set Enrichment Analysis was used to reveal several dysregulated biological processes that were either shared between multiple, or specific to a single lung cancer type.

Results

Principal component analysis revealed the markedly different molecular characteristics between cancerous and cancer adjacent samples, while hierarchical clustering showed clear lung cancer type-specific differences. The gene sets shared between lung cancer phenotypes were primarily involved in extracellular matrix remodeling, altered adhesion, signaling cascades, immune response, coagulation, protein biosynthetic, metabolic, and vesicular transport processes. The results correlated well with previous studies analyzing individual NSCLC types and tumor adjacent tissue. The specific molecular signatures identified in this study might be attractive targets for further in-depth investigations and can also bear potential diagnostic and prognostic value.

Acknowledgments

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P76. Investigating Chondroitin Sulfate and Heparan Sulfate Glycosaminoglycans in Different Lung Cancer Tissues

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Introduction

Lung cancer is the second most frequently diagnosed cancer type and has the highest mortality of any type of tumor. Carbohydrates are used as diagnostic biomarkers in various cancers. Glycosaminoglycans (GAGs) have been shown to undergo changes in their abundance and structure during tumor development. The aim of our work was to perform a quantitative and qualitative glycomic (chondroitin sulfate, CS, and heparan sulfate, HS) study of samples belonging to small cell lung cancer and different non small cell lung cancer subtypes. Both tumorous and tumor adjacent regions were analyzed.

Methods

Our study was performed through disaccharide analysis following tissue surface lyase digestion.The CS and HS disaccharides extracted from the tissue surface were desalted and then separated using self-packed capillary columns packed with hydrophilic interaction liquid chromatography and weak anion exchange (HILIC-WAX) mixed mode resin and detected using negative ionization mass spectrometry. CS and HS disaccharides were analyzed using separate ammonium formate salt gradient HPLC-MS methods developed recently.

Results

Several significant changes were observed in the content and sulfation patterns of GAGs between tumor and tumor adjacent regions. Although the alterations between the lung tumor phenotypes were not as remarkable, some differences could be identified. The quantity of CS was doubled in tumor samples, while the total content of HS did not show significant changes. Additionally, the average degree of sulfation significantly increased in all investigated tumor phenotypes. Comparing adenocarcinoma samples to other lung tumor phenotypes, the 6-O-/4-O-sulfation ratio was higher. O-sulfated HS components were elevated in the tumor samples. These findings emphasize the importance of exploring the role of GAGs in lung cancer development, as several modifications were identified between tumor and tumor-adjacent tissue samples, as well as among different lung tumor phenotypes. Therefore, future large-scale studies of HS and CS GAGs could provide significant correlations between cancer-related structural and quantitative changes.

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P77. Analysis of the dynamics of meiotic proteome and phosphoproteome in *Schizosaccharomyces pombe*

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Many biological processes including cell division are regulated through posttranslational modifications of proteins, mainly by phosphorylation. Effective tool for studying protein phosphorylation represents techniques of affinity chromatography and fractionation coupled with identification by mass spectrometry [1]. In our study, we analyzed dynamic changes in the proteome and phosphoproteome during meiotic division. For this purpose, we used two mutant strains of Pat1 kinase of the yeast *S. pombe*. Inhibition of this kinase by elevated temperature, or an ATP analog induces meiotic division in synchronized yeast culture [2]. In a recent study, Perea et al. presented a protocol using TMT quantification that enables deep proteome and phosphoproteome analysis [3]. Similarly, our approach combines TMT 18plex for quantification with TiO_2 and FeNTA columns for sequential enrichment of phosphopeptide, high pH RP for fractionation, and LC-MS for identification. This integrated phosphoproteomic strategy allows us to identify and quantify 4673 proteins and more than 7100 phosphorylation sites.

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P78. Discovery/development of protein biomarkers for the stratification of triple negative breast cancer (TNBC)

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Introduction

Breast cancer (BC) is the most frequent malignancy in women worldwide. Breast cancer af-fects roughly 3,000,000 people worldwide, with 10-15% of cases being triple negative breast cancer (TNBC). In comparison to other types of breast cancer, TNBC stands out for its heterogenies disease, poor prognosis and aggressive behaviour. In the early stages of TNBC, neoadjuvant chemotherapy (NAC), which is given to the patient before surgery, has been considered a viable treatment strategy. It's been associated with 30% of patients achieving a pathological complete response (pCR), which has been proven to improve dis-ease-free, distant recurrence-free, and overall survival estimates. So, the objective of this study is to apply Mass Spectrometry-based proteomics to serum samples from TNBC pa-tients (both those who achieved pCR and those who did not) who have received NAC to identify i) biomarkers that might measure response to NAC, ii) biomarkers that might cor-relate with the extent of residual disease. These biomarkers will potentially inform treat-ment decisions such as the escalation/de-escalation of chemotherapy dosage for individu-als with early TNBC.

Method

In this study, there are two essential proteomic approaches to identify biomarkers: unbiased discovery proteomics and targeted proteomics. In the discovery approach dentification of proteins present in serum samples of TNBC patients post-NAC. The second method will involve designing and developing multiple reaction monitoring (MRM) assays using synthetic peptides of candidates discovered through discovery LC/MS-MS analysis.

Results

Our finding from the discovery approach has identified number of candidates, a total of 17 unique proteins and 118 unique peptides, that would be beneficial for classifying TNBC pa-tients who would achieve residual disease after the NAC. These candidate biomarkers panel, candidates identified in literature review and candidates selected from tissue-based gene expression will require assay development for multiple reaction monitoring (MRM) based on targeted proteomics. This assay should serve as a valuable resource for on-going research and to the BC research community internationally.

Key words: triple negative breast cancer (TNBC), neoadjuvant chemotherapy (NAC) and
Mass Spectrometry - multiple reaction monitoring (MS-MRM).

P79. Proteomic and Glycoproteomic Profiling of Organoids Derived from Human Hepatocellular Carcinoma

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Organoids have emerged as a novel tool and promising ex vivo 3D culture model in medicinal research. However, the compositions of organoids have not been thoroughly studied, and their proteome and glycoproteome have yet to be compared to the original tissue. In addition, the limited sample amount of organoids can be challenging to obtain high-quality mass spectrometric data. In this study, we generated proteomic and glycoproteomic profiles at the microgram scale (1-5 μg) of protein amount from hepatocellular carcinoma organoids. We identified 2,099 proteins in global proteomics, which were involved in the metabolism of RNA, nucleobase-containing small molecule metabolic process, and carbon metabolism. These functions were consistent with the primary metabolic functions of liver tissue. In the glycoproteomic approach, we identified 41 glycoproteins from 159 glycopeptides, including important therapeutic targets and biomarkers of hepatocellular carcinoma (HCC), such as EGFR and AFP. In addition, we compared glycan compositions between HCC tissue and HCC-derived organoids and found that they were similar. This finding suggests that organoids share identical characteristics with actual tissues.

P80. Pushing DIA proteomics analyses of neat plasma to 1000 protein groups ID/hr

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Introduction

Direct proteomics analysis of neat plasma remains a challenge because of the huge dynamic range of the plasma proteins, but is also very appealing because it requires very small volumes and is unexpensive compared to new depletions technology. We focused on workflow optimization of neat plasma analysis using dia-PASEF® approach to maximize the number of proteins groups (PG) identified and quantified, while minimizing both gradient time and missing value levels. Here we describe the comparisons of different LC settings, and the optimization of data analysis using DiaNN, PaSER and Spectronaut® 17.

Methods

The plasma sample cohort consisted in 15 patients affected by a rare genetic disease and 18 age-matched controls. Samples were digested with trypsin using STRAP columns, separated either by nano-HPLC (nanoElute, Bruker Daltonics) using a 25cm column on a 30min gradient (IonOpticks, Australia), or with an EvosepOne ran with the 60 Samples Per Day (60SPD) method (Endurance column, 8cm). Both LC's were connected to a timsTOF Pro™ instrument (Bruker Daltonics) via its Captive Spray source. The timsTOF Pro was in dia-PASEF acquisition mode: a 19-windows method using 33da windows covering a 0.65-1.3 V/cm2 mobility ranges over a 400-1050m/z range. DiaNN1.8.1 (Demichev group), a GPU-based version of diaNN (TIMS-DIA-NN on PaSER, Bruker) and Spectronaut®17 (Biognosys) were used for data processing.

Results

To understand the key factors in obtaining higher number of proteins we evaluated two LC systems and three DIA library-free data analysis workflows. We found that the number of identifications correlated well with the number of samples simultaneously searched. Using the nanoElute, a single plasma analysis leads to the identification of ca450 proteins groups, the analysis of 4 samples (enabling MBR), leads to 507+/-6 proteins (2% missing values, MV hereafter). Searching 33 samples simultaneously led to 811 +/-78 protein groups (33% MV). Finally, 828 +/-78 (35% MV) protein groups could be identified when searching simultaneously for 110 neat plasma samples, and up to 990 +/-137 (68% MV) if depleted plasma files from independent samples were also added to the analysis. We show a significant effect of the MBR step, as the same analyses run without MBR do not lead to the same improvement in number of IDs (20% increase instead of 70% for the 33-plasma search, and 47% instead of 74% for the 110 neat plasma runs). Analysis on PaSER lead to 376 +/- 102 (with 45% MV) on 33 runs. Using Spectronaut®17, we obtained around 460 proteins on single files, 537+/-4 (with 5% MV) for 4 plasma search, and 731+/-50 (23% MV) on 33 runs. The analysis of the same cohort using 60SPD EvosepOne methods was 2.7x faster than nanoElute, but led to 2x lower number of IDs using both DiaNN and Spectronaut® 17. We obtained 394+/-37 (27% MV) and 426+/-29 (with 20% MV) on the 33 samples analysis using DiaNN and Spectronaut®17 respectively.

P81. Predictive Ion Control for a new High-Throughput Proteomics Platform

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Introduction

All mass spectrometers suffer some limitation in their dynamic range to measure both high and low abundant analytes in complex samples. Therefore, control of the analysed ion population based on understanding of the incoming ion current, often termed automatic gain control (AGC), is an essential feature.

The general challenge with AGC is how to optimise the ion population admitted to the mass analyser or ion processing region. Most critically to prevent saturation effects from the overall population or individual intense species, but also to minimise wasted ion beam time.

We have developed a new predictive ion control (PIC) algorithm that takes advantage of the new features provided by the novel high resolution accuracy mass (HRAM) mass spectrometer, which combines an OrbitrapTM analyser with a novel open electrostatic trap AstralTM analyser. The new platform allows acquisition of Astral scans in parallel with the Orbitrap analyser. This not only provides recording of HRAM MS2 spectra at up to 200 Hz, but also provides high-speed accurate ion current measurements for improved injection time regulation.

Methods

The ion current measurements on the novel mass analyser, termed flux-scans, can be recorded at the same acquisition rate as the HRAM MS2 scans. These flux-scans provide accurate ion current measurements several times per second, dozens of times per LC peak width.

The flux-scans are then used to accurately predict the ion current for wide isolation Orbitrap MS1 scans and also narrow isolation MS2 scans, calculating the injection times required to hit the user-defined ion targets. This is made possible because of the improved sensitivity, high mass accuracy, resolving power, and high single-ion detection efficiency of the Astral analyser.

Results

The new predictive ion control enables accurate control of injection times during data acquisition. LC-MS/MS data such as DIA and DDA were acquired on human plasma protein samples. This data shows shifts in injection times as the ion flux changes with the LC elution. However, the overall number of charges per scan remains quite stable. The quick and accurate regulation of the ion current facilitates in-depth proteomics analysis, allowing identification of >8000 proteins at 180 samples-per-day (SPD), and >10,000 identified proteins at 60 SPD to be acquired on the novel HRAM mass spectrometer.

P82. Optimizing Immunopeptide Analysis Sample Preparation in Needle Biopsy Size Tissue Samples with AFA® Technology and Spectronaut

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Introduction

Immunopeptides play an essential role in adaptive immunity by activating and ensuring T-cell specificity. Mass spectrometry is currently the only technology that can reliably measure and identify the immunopeptide profiles of biological samples at a large scale. However, studies are frequently limited by sample input and poor scalability. Here, we introduce a semi-automated workflow requiring low sample input to robustly identify immunopeptides from cultured cells and tissue samples and apply it to a cohort of colorectal cancer samples for immunopeptide profiling and neoantigen identification.

Methods

Native lysis with AFA-based ultrasonication and the immunoprecipitation workflow were optimized while ensuring scalability and reproducibility. 15 mg of fresh frozen tissue was processed for sequential class-I/class-II immunopeptide enrichment. FAIMS Data-Independent-Acquisition was performed and supported by a high-pH-reversed-phase FAIMS Data-Dependent-Acquisition library. Data analysis was performed with SpectroMine and Spectronaut (Biognosys) with 1% FDR at the PSM, peptide and protein group level. Whole genome sequencing on both tumor and associated normal tissue was used for high-confidence somatic variation calling (Indivumed) and neoantigen definition.

Results

With the optimized workflow, we can now identify over 2,800 class-I immunopeptides with 2.5 mg healthy lung tissue and >11,000 class-I and >10,000 class-II immunopeptides with 15 mg fresh frozen tissue. Overall, we have established a scalable, efficient pipeline for cell line and tissue immunopeptidomics for both class-I and II immunopeptides.

Conclusions

The workflow described here will demonstrate generation of high-quality identifications from minimal starting material and can be deployed to help shed light on immunopeptidomics heterogeneity through large-scale profiling of patients as it will be exemplified in the case of neoantigen identification in MSI-high colorectal cancer.

P83. Evaluating the benefit of dia-PASEF approaches and sample-specific database strategies for metaproteomics of very complex microbiomes

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Introduction

Understanding interaction of microorganisms with their host is crucial in microbiology. The identification and quantification of proteins from a complex mixture of a large variety of organisms, known as Metaproteomics, has emerged recently as a unique tool to get functional and taxonomical insights into microbiota and is currently one of the most challenging areas in proteomics. Species-specific signals can be low, highly diverse, and the search space is extremely large. For these reasons, the increased selectivity potential of LC-IMS-MS based approaches might prove beneficial for metaproteomics analyses. In this communication, we are using a specific sample representative of gut microbiome to benchmark dia-PASEF approaches against PASEF approaches and investigate the benefit of sample-specific database strategies.

Methods

A gut microbiome tryptic digest (200 or 800 ng) was injected on a 25cm X 75µm pulled emitter column (IonOptiks). Nano-HPLC separation was performed with a 36, 66 or 100 min gradient using a nanoElute (Bruker) connected to a timsTOF HT mass spectrometer (Bruker). LCMSMS data were acquired in PASEF or dia-PASEF acquisition mode. PASEF data have been processed in real-time on PaSER (Bruker) or Mascot (MatrixScience), searching against an NCBI database. dia-PASEF data have been searched against a reduced protein sequence database using TIMS DIA-NN on PaSER (Bruker) or Spectronaut 17 (Biognosys). The protein sequence reduced database was constructed after confidently proteotyping the most abundant organisms present in the standard sample. It comprised 893,451 protein entries from 57 taxa.

Results

2110 protein groups could be identified from a 200ng injection and a 66min gradient with the initial Mascot search. On average, 4 peptide sequences were identified for each protein Group ID's. Using the ProLucid Algorithm on PaSER while allowing to take the peptides's collisional cross section (CCS) value into account for the scoring process (TIMScore), a high stringency search allowed to double the number of identified protein groups, while the number of sequences/proteins was down to 3. In the best conditions (800ng-100min), up to 6,537 protein group corresponding to 19,962 peptide sequence could be identified. The use of dia-PASEF with the restricted database allowed to boost the number of protein group ID's: 8,827 for 35,516 peptides for the 200ng/66 min injection (+35% protein group ID's, +78% peptide sequences). Meanwhile, the average number of peptides per protein group was 4, giving high confidence in identification.

P84. aHipA-like kinases as regulators of antibiotic tolerance and metabolism in bacteria

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Protein Ser/Thr kinases (STK) are post-translational regulators of key molecular processes in bacteria, such as virulence, cell division, and antibiotic tolerance. The latter is a major contributor to relapse in many chronic infections and frequently results in antibiotic overuse and the development of antibiotic resistance. One of the best-studied drivers of antibiotic tolerance is the HipA toxin, an STK, first characterized in E. coli. A recent study has identified homologs of the hipA gene in many bacterial species (1). Here we aimed to characterize a HipA-like kinase, YjjJ (HipH) in E. coli and a hipA homolog in Klebsiella pneumoniae, focusing primarily on the identification of their substrates by mass spectrometry-based proteomics.

We used over-expression of kinases in WT E. coli and ΔhipBA E. coli to study their effect on proteome and phosphoproteome. Phosphopeptide enrichment was done using Sachtopore TiO2 beads for 5 consecutive rounds. All the samples were measured on Orbitrap Exploris 480 (Thermo). We confirmed the phosphoproteomics results using in-vitro kinase assay and different molecular biology methodologies.

We found that YjjJ is a Ser/Thr protein kinase that impacted ribosome assembly, cell division, and central carbon metabolism by phosphorylating RpmE and CsrA but, unlike HipA, did not increase antibiotic tolerance. Intriguingly, the overproduction of YjjJ and its kinase-deficient variant could activate HipA and other kinases, pointing to a cross-talk between Ser/Thr kinases in E. coli (2). HipA-homolog in K. pneumoniae (HipAkp) has 70% sequence similarity to HipA from E. coli (HipAec). Overproduction of HipAkp in E. coli had a similar toxic effect on growth and viability as the HipAec. Among several targets of HipAkp, we detected GltX which is known to be involved in antibiotic tolerance in E. coli. We are currently characterizing HipAkp in K. pneumoniae to confirm its role in antibiotic tolerance in this important ESKAPE pathogen.

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P85. Operating, maintaining, and troubleshooting the sensitivity and robustness of timsTOF platforms for proteomics studies

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Introduction

Over the last few years, proteomics applications going from sample preparation to data analysis have significantly improved the sensitivity and robustness. The development of the Parallel Accumulation Serial Fragmentation (PASEF) technology increases peak capacity, sensitivity, and acquisition speed for confident identification and quantification, and developing epiproteomics workflows for post-translational modifications (PTM) studies with maximum duty cycle. However, high throughput sensitive proteomics analysis requires a reliable quality control (QC) approach to maintaining the best performance and minimize down time. Here we study the factors that affect instrument performance and methods to monitor for these effects.

Methods

Commercial LC/MS-compatible Human protein extracts (HeLa and K562) were analyzed by coupling either the nanoElute I or Evosep One to 3 different trapped ion mobility – QTOF mass spectrometers (timsTOF Pro2, timsTOF SCP, and timsTOF HT). Several LC columns (PepSep and IonOpticks), quantities ranging from 60pg to 1µg on column, separation methods, and MS methods were evaluated. Raw data were processed with PaSER, DIA-NN, or FragPipe to estimate the number of peptides and proteins. DataAnalysis and Skyline were used to extract selected "target peptides".

Results

Users mostly rely on the number of identified peptides and proteins to monitor the performance of the LC/MS platform. Using an Evosep One connected to a timsTOF SCP allows for the identification of more than 5,000 proteins and 40,000 peptides in dia-PASEF from 4ng of K562 injected on an IonOpticks column. Additionally, more than 100,000 peptides from 200ng of K562 were identified with a timsTOF HT. Based on these results, we have generated a list of 12 "target peptides" which are detected in both K562 and HeLa across the whole gradient time and the ion mobility range. Then, we tested 8 different MS settings to lower the QC performance compared to a reference method. For each method, we evaluated the total number of proteins and peptides but also the quantitative effect from the "target peptides". For example, decreasing (n=4) or increasing (n=20) the number of PASEF ramps allows for decreasing the number of peptides to respectively 17% and 30% compared to the reference method. Furthermore, setting a 6% duty cycle resulted in the lowest number of peptides and proteins as expected. However, this method demonstrated the highest intensity of the ion chromatograms and mobilograms extracted from the "target peptides" compared to the reference method. Additionally, the influence of chromatographic performance on the number of peptides and proteins will be demonstrated.

P86. Enabling Deep and Unbiased Proteomics at an Unparalleled Scale with a Cloud-Native Pipeline

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Seer Inc,

Liquid Chromatography coupled to Mass Spectrometry (LCMS) is a ubiquitous proteomics technology due to its speed, sensitivity, and flexibility. While instrumentation hardware continues to improve, corresponding increases in translating LC-MS data to insight have not developed. Although substantial progress has been made in data processing algorithms, all common tools are incapable of supporting experiments larger than a few dozen to a few hundred LC-MS injections. This limitation significantly limits statistical power of studies and prevents interrogation of large proteomic data repositories. Significant development and optimization efforts have been invested in the last two decades to ensure reliability and performance of existing tools, but these optimizations unintentionally limit adaptation of these solutions to handle extremely large datasets.

P87. Phosphoproteomic and Proteomic Analysis of Lung- and Prostate Adenocarcinomas

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Introduction

Lung cancer and prostate cancer are two of the most frequently diagnosed cancers, and lung cancer is the leading cause of cancer-related mortality. Most of the prostate tumors, and approximately 40 % of the lung tumors are adenocarcinomas. To gain a better understanding of tumor biology, it is important to uncover common and distinct biological processes and phosphorylation events in these adenocarcinomas.

Methods

In this pilot study, a simultaneous proteomic and phosphoproteomic analysis was performed on a total of 71 formalin-fixed paraffin-embedded tissue sections derived from patients with lung- or prostate adenocarcinoma. On-surface digestion was performed on small cancerous and tumor adjacent tissue regions, resulting in four sample groups. The tryptic digest of each sample extracted from the tissue surface was subjected to phosphopeptide enrichment. Phosphopeptides were separated from the non-phosphorylated peptides, and the two fractions were analyzed in separate mass spectrometry runs. Differentially expressed proteins, and phosphopeptides were identified using label-free quantitative mass spectrometry and subsequent statistical analysis.

Results

Lung cancer, prostate cancer, and respective tumor adjacent samples had distinct proteomic profiles. The proteins identified as differentially expressed between lung and prostate cancer were mainly metabolic interconversion enzymes and cytoskeletal proteins. Most of the dysregulated biological processes were similar in both types of adenocarcinomas, while actin-based and immune response-related biological processes, as well as gene sets related to cardiomyopathy were highly suppressed in prostate cancer. Kinase-substrate enrichment analysis revealed 13 kinases with significantly altered activities. The activities of three kinases (MAPK8, CDK5, and GSK3B) were elevated in lung cancer and suppressed in prostate cancer. Several members of the PAK family, involved in cytoskeletal signaling, showed heavily suppressed activities in both adenocarcinomas. Moreover, the activities of PRKACB, PRKACG, and AURKB were suppressed in lung cancer, while PRKCI activity was elevated in prostate cancer. Results of this pilot study highlight the importance of further validation of the cancer specificity of the significantly altered proteins, and comparing adenocarcinomas of additional tissues would be an important step in uncovering possible novel tissue-specific intervention points.

P88. Development of multi-PTM enrichment to study PTM profile of Lysosomes and Golgi

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The recent development of rapid immunoprecipitation of Organelle (Golgi-IP, Lysosome-IP, mitochondria-IP and Endosome-IP) has allowed to study molecular content of intact Organelles at high-resolution. When combined with High-resolution mass spectrometry analysis this approach readily reveals the molecular content of proteome, metabolome and lipidome at a greater depth. Post translational modifications (PTMs) such as protein phosphorylation and Ubiquitylation are being studied at a global scale by quantitative mass spectrometry to identify, quantify and localize modified peptides precisely, this is often carried out from a milligram amount of starting material derived from whole cell extracts. Here we propose to develop highly sensitive a sequential Phosphopeptide and Ubiquitylation site enrichment of tryptic peptides derived from lysosome-IP and Golgi-IP enrichments by combining Ultra-sensitive data independent acquisition and parallel accumulation and serial fragmentation (dia-PASEF) on a timsTOF SCP mass spectrometer. This could be powerful particularly to identify organelle specific PTMs as well as substrates that are transiently recruited to lysosomes and Golgi which are otherwise could not be detectable using whole cell extracts. Our preliminary analysis revealed an identification and quantification of >10,000 phosphosites from <10 µg of Lysosome enrichment material in A549 cells. We identified phosphosites of several lysosomal proteins as well as phosphosites of non-lysosomal proteins that are enriched. This technology provides a great opportunity to study transient interactors within these organelles that are otherwise not been able to detect using conventional enrichment techniques. We will apply our Organelle-PTM approach to study Parkinson's disease linked LRRK2 kinase signalling in Golgi and Lysosomes immunoprecipitations derived from pathogenic LRRK2 G2019S and R1441C and VPS35[D620N] cells and mouse tissues. We believe our workflow could open new avenues in studying Organelle specific signalling in health and disease.

P89. Probing the Host-Virion Protein-Protein Interactome of the Human Cytomegalovirus (HCMV) Assembly Compartment

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Introduction

Human Cytomegalovirus (HCMV) represents the leading cause of global congenital birth defects. Whilst the life cycle and epidemiology of this viral infection has been investigated in detail, a mechanistic understanding of the ordered assembly dynamics relating to HCMV particles within the host cell remains elusive. As such, analysis of the biochemical machinery that drives virion assembly would be useful for proposing more effective therapeutic solutions. The Assembly Compartment (AC), a make-shift virion assembly site that wraps around the juxtanuclear compartment of the host-cell nucleus, forms from extensive remodeling of the host's secretory apparatus. We applied crosslinking mass spectrometry and PTM-proteomics methodologies to investigate host-protein recruitment into the viral particle, spatiotemporal host-virion protein modification during particle assembly and host-virion protein-protein interaction dynamics as the virion particles are assembled within the host cell AC.

Methods

Human fibroblast cells were infected with HCMV before cells were subjected to multiple purification and lysis techniques to extract infected whole fibroblast host-cells, viral nucleocapsid particles within infected cells and secreted extracellular virion particles. Crosslinking mass spectrometry, with DSSO and tBu-PhoX, were used in tandem with offline SCX fractionation, online High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) gas-phase fractionation before analysis with the Thermo Orbitrap Eclipse Tribrid Mass Spectrometer. Multiple data processing pipelines were utilized to generate information relating to spatiotemporal protein-PTM regulation for ordered virion assembly, host-virion protein-protein interaction dynamics and host-protein recruitment into the viral particle, within the AC of HCMV infected host cells.

Conclusions

Within the Assembly Compartment of host cells, HCMV nucleocapsid formation is accompanied by, and may rely upon, the recruitment of specific host-cell cytoplasmic proteins that are then entrapped within the tegument layer of the viral particle before ejection from the host cell. Further, the identification of multiple host-virus protein-protein interactions within the AC, with this heteromeric complex formation reliant upon ordered protein regulation via PTMs, may represent a therapeutically targetable regulatory mechanism of viral particle assembly.

P90. Quantitative proteomic analysis of adhesion protein localisation in patient-derived glioma stem

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Glioblastoma multiforme (GBM) is an aggressive type of brain cancer with a high mortality rate. Treatment options for GBM remain very limited, so research is needed to identify new biomarkers and therapies for this disease. Understanding how cancer cells survive, invade and migrate is important for the development of approaches to inhibit invasion and metastasis in patients. The tumour microenvironment plays a central role in tumorigenesis, and cancer cells interact with the extracellular matrix using cell-surface receptors, which recruit adhesion proteins to mediate signalling networks that control cancer cell behaviour. Several studies have identified that some adhesion proteins can localise to unexpected parts of cancer cells, which can influence tumour growth. We hypothesised that dysregulation of adhesion protein localisation could affect GBM cell behaviour and reveal vulnerabilities in signalling networks that could be targeted therapeutically.

We set out to identify the adhesion proteins that translocate to the nucleus in disease-relevant cellular models of GBM using quantitative proteomics. Using biochemically purified subcellular fractions, we investigated the nuclear localisation of selected adhesion proteins in glioma stem cells from the mesenchymal and classical GBM subtypes. To enable a global assessment of adhesion protein localisation networks, we next used quantitative mass spectrometry and computational approaches to characterise the nuclear localisation of adhesion proteins in these cells, which will be validated using microscopy. The identification of adhesion protein localisation signatures may provide candidates for future investigation as biomarkers or drug targets in GBM.

P91. Guiding Antiviral Discovery against Filoviruses via Glycosylation Analysis of Viral Spikes

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Introduction

Ebola virus (EBOV) is among the most pathogenic and fatal viruses known to humans. Glycosylation of spike fusion glycoproteins is an important functional feature of enveloped viruses, and their glycans play pivotal roles in evading host immune systems by hiding their surface proteins under a dense 'glycan shield' and communication of the host receptors. The EBOV GP is composed of two disulphide-linked subunits: GP1 and GP2. It has 17 N-linked glycosylation sites, and high-dense O-glycans in the mucin like domain (MLD) in GP1. The N-linked site-specific glycosylation has been proposed to modulate the receptor binding, and we speculated that differences in site-specific glycosylation profile may contribute to variations in lectins binding. Unquestionably, understanding the site-specific glycan structures of EBOV GP and their influence on the properties and interactions of GP to the lectins they bind to is incredibly important for revealing viral pathobiology mechanism and antiviral development.

Methods

Glycoproteomics: recombinant GP timers were digested with trypsin, chymotrypsin and alpha-lytic protease followed by glycopeptide purification and analysis by LC-MS/MS. N-linked glycans of GP were prepared via PNGase F digestion, and analysed by UHPLC. The binding of the EBOV GP to lectins were measured using mass photometry (MP).

Results

Site-specific glycosylation analysis across GP +/- MLD from Makona, Mayinga, Sudan species revealed complex glycosylation at most N-linked sites, while two sites (N257, N563 in GP1 and GP2 respectively) are enriched in unprocessed oligomannose type N-glycans. We also profiled global differences in glycosylation processing with UHPLC, which provides a quantitative readout of complex verses oligomannose type N-glycans. Minor differences were detected when comparing individual peaks of GP across species, but differences from GP +/- MLD were apparent, suggesting that the MLD domain may affect glycan processing. MP results showed that EBOV GP has weak binding affinity with DC-SIGN, which agrees with glycoprotemics data confirming low content of oligomannose glycans on EBOV GP as compared to the HIV spike Env (positive control). DC-SIGN binds HIV Env with high affinity (in nM range) due to its abundance of high-mannose N-glycans clusters. To confirm this DC-SIGN binding potential to EBOV, a glycoengineered GP trimer with nearly complete oligomannose structures was prepared and interaction with DC-SIGN were observed by MP, confirming the view that there is a disconnect between known glycosylation of GP and it purported interactions with at least five host attachment lectins.

P92. A scalable, robust, and sensitive multi-nanoparticle-based label-free mass spectrometry workflow for deep plasma proteomics

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Seer Inc.

Analyzing human plasma proteome to evaluate health status and early detection of life-threating diseases like cancer is a topic of significant interest. However, the large dynamic range of circulating proteins and diversity of proteoforms present in plasma have limited the comprehensive characterization of the proteome in a high-throughput manner. To address this challenge, a multi-nanoparticle-based platform is developed that facilitates deep and broad plasma proteomic measurement at scale. This approach quantifies thousands of proteins from plasma without compromising depth, throughput, or reproducibility, creating a unique opportunity to detect protein biomarkers in an unbiased and robust manner. Here, we evaluate the performance of this workflow using control plasma samples, highlighting the reproducibility and depth of proteomic coverage provided by the platform.

Control pooled human plasma samples were processed with two SP100 automation instruments with Proteograph XT Assay Kits, across two days, resulting in a total of 4 batches. The samples were processed with multiple nanoparticles panels and tryptic digested peptides were generated for downstream LC-MS analysis using Data Independent Acquisition (DIA) LC-MS method on an Orbitrap Exploris 480 MS with a 30-minute LC gradient. Data visualization and statistical assessment of the performance and reproducibility of the assay were performed including quantified peptide mass, peptide intensity CVs, peptide and protein group counts, and Jaccard Index (JI) overlap across replicate sample analysis.

Based on preliminary data, the Proteograph workflow resulted in >13,000 unique peptides and >3,200 unique protein groups covering proteins broadly and deeply across the Human Plasma Proteome Project (HPPP) database in a high throughput manner. Protein group JI overlap between replicate pairs were typically >0.8 for both intra and inter-batch comparisons, and peptide intensity CVs were <15% and <18% for intra and inter-batch comparisons, respectively. These results demonstrate advancement in enabling large-scale plasma proteomics without compromising depth, throughput, or reproducibility.

P93. Effects of ageing and calorie restriction on mitochondrial proteome of brain and heart tissues

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Introduction

Ageing is a currently irreversible, progressive, and complex biological process. Despite progress in its understanding, most aspects are enigmatic and the relevance of the myriad of existing theories and contributing factors are a matter of active research and dispute. Several theories attribute mitochondria a key role to ageing, given their central function as producer of energy and reactive oxidative species. The prominent mitochondrial free radical theory of ageing (MRFA) postulates an increase of (oxidatively) damaged biomolecules in mitochondria as driver for cellular ageing. Moreover, influencing cellular metabolism and signaling by caloric restriction is known to impact ageing. By leveraging proteomics, the impact and interplay of aging and caloric restriction were studied for heart and brain (cerebrum) tissue.

Methods

Crude mitochondrial fractions were isolated from the heart and brain tissues of same rats (Rattus norvegicus), ages 6.5 months (young) and 27 months (old), fed ad libitum or on a CR diet (CR: intake about 60 % of ad libitum fed animals). Mitochondria from each animal group were digested with Lys-C/Trypsin protease mix using the slightly modified iFASP protocol. iTRAQ-labelled samples and the spike-in internal standard were equally mixed to one experiment. Each experiment was analyzed on a LTQ Orbitrap Elite with HCD fragmentation. PTMs were identified and quantified using MaxQuant.

Results

Tissue-specific crude mitochondrial proteomes revealed more than 2000 protein species across all four average animal groups (n=3) from cerebrum and more than 1000 proteins from heart. According to PCA analysis, both ageing and diet separated the proteomes; diet separation was larger in cerebrum than in heart. Due to their significant changes under all conditions, it is speculated that abundances of Immt, Atp5j2, Slc25a11, Mtnd3, Mt-atp8, Slc25a20, and Adck3 are affected in heart tissue after CR treatment; for brain, these were Atad3, Armc10, and Higd1a. We speculated that integral membrane protein abundance may be affected more significantly due to their proximity to reactive oxygen sources and their overall lower turnover according to literature. However, there was no striking difference relative changes between proteins with and without transmembrane domains observed for heart and cerebrum. Interestingly, when comparing total number of significant protein abundance changes among young and old animals, it was lower under CR. Therefore, CR might support mitochondrial proteome homeostasis during ageing.

P94. PROTzilla - A novel downstream-analysis platform for proteomics data

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Introduction

The increasing amount of high-dimensional proteomics mass spectrometry (MS) data requires complex processing to gather insights into the underlying biology. Currently, researchers either rely on custom analysis pipelines, necessitating programming knowledge, or resort to platforms that focus on individual analysis tasks or have historically grown in complexity requiring developer guidance to use. This introduces challenges with analysis, analysis sharing and accessibility for researchers.

Methods

To address these challenges, there is a need for a user-friendly tool that enables downstream proteomics analysis without requiring programming experience. This tool should also be intuitive, offer flexibility to customize data processing and ensure the reproducible sharing of analysis workflows and results. In response, we have developed an open-source tool named PROTzilla, a comprehensive downstream platform for proteomics MS analysis.

Results

PROTzilla comprises a Python package with preprocessing, analysis, and high-quality plotting capabilities, and integration knowledge bases alongside a user-friendly, browser-based interface. Utilizing PROTzilla researchers can effortlessly perform analysis tasks and generate publication-ready visualizations; eliminating the need for programming knowledge. Importantly, analysis workflows in PROTzilla are shareable, reproducible, and automatable. The framework is easily extensible and community members can contribute to future functionality. By introducing PROTzilla, we aim to simplify proteomics data analysis, enhance flexibility, and ensure the transparency and reproducibility of analysis and reporting in the field. We anticipate that this open-source tool will help researchers to generate knowledge from their proteomics experiments and improve current practices of analysis sharing and reporting.

P95. NOX5 promotes vascular smooth muscle cell phenotypic switching in human hypertension

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Background
Phenotypic switching of vascular smooth muscle cells (VSMCs) is a fundamental pro-cess in vascular dysfunction associated with hypertension through mechanisms that involve reac-tive oxygen species (ROS). We identified Nox5 as the main ROS generating enzyme in VSMC in hy-pertension. However, the effect of Nox5-derived ROS on the proteome and phenotype of VSMCs in human hypertension is unknown. We aimed to characterize the global and oxidative proteome profiles of VSMC and the role of Nox5 in VSMC phenotypic switching in human hypertension.

Methods
VSMC from resistance arteries from normotensive (NT) and hypertensive (HT) subjects were studied. High-fidelity proteomic analysis was performed using isobaric tandem mass tag pro-tein labelling and liquid chromatography-tandem mass spectrometry. The oxidative proteome was assessed using stable isotope-labelled iodoacetamide to target cysteine thiols. Nox5 silencing was performed by siRNA. Protein expression was assessed by western blotting. Pro-inflammatory cy-tokines (IL-6, IL-8) and pro-collagen I were detected in the VSMC culture medium by ELISA.

Results
Proteomic analysis revealed the upregulation of extracellular matrix (ECM) and plasma membrane proteins in HT subjects (fold change>1.5, $p<0.05$). Gene ontology enrichment of biolog-ical processes showed that most proteins upregulated in HT were involved in ECM organization, immune response, and cell proliferation. These results suggest that, in HT, VSMC switch to a syn-thetic and pro-inflammatory phenotype. Oxidative proteome analysis identified 130 significant cys-teine-containing peptides, and 88 showed increased oxidation in HT (fold change>1.5, $p<0.05$). Among the highly oxidized proteins in HT were ECM proteins COL11A1, COL16A1, FBLN1, and FBLN2. In HT subjects, expression of the VSMC markers myocardin, α-smooth muscle actin (α-SMA), and smooth muscle specific protein SM22 was reduced ($p<0.05$), while the expression of proliferation markers PCNA and pro-collagen I was increased ($p<0.05$). The release of pro-inflammatory cytokines IL-6 and IL-8 was increased in the HT group ($p<0.05$). Nox5 silencing in HT subjects reduced PCNA expression, pro-collagen I release, and baseline and LPS-induced IL-6 and IL-8 release ($p<0.05$). Furthermore, Nox5 silencing increased the expression of myocardin, α-SMA, and SM22 in HT subjects.

Conclusions
Our study highlights the changes in the global and oxidative proteome of VSMC in human hypertension and demonstrates that Nox5 is a key player in VSMC phenotypic switching associated with vascular injury and remodelling in hypertension.

P96. Differential proteomics of somatodendritic and axonal proteins in neurons

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Axons have highly diverse reservoir of mRNAs that are translated locally upon different stimuli. Regulation of local translation of mRNAs and protein degradation allows spatial and temporal control of the axonal proteome. However, local synthesis, turnover and transport of axonal proteins are still largely unexplored. Our goal is to study local protein synthesis in the axons and, separately, in the soma of cultured neuronal cells.

Differentiated dopaminergic neurons were be seeded in a two-well microfluidic device (Xona Microfluidics) and pulsed with heavy amino acids in both wells simultaneously: Lys4+Arg6 in the somatodendritic well and Lys8+Arg10 the axonal well. Samples were be harvested after different time points (12-120 hours), allowing temporal analyses of label incorporation. MS measurements were performed on an Orbitrap Exploris 480 mass spectrometer coupled to nano-UHPLC.
In preliminary experiments, we were able to identify around 2900 somatodendritic proteins and 300 axonal proteins. After 72h, differential SILAC labeling revealed about 100 proteins synthesized in the axon and transported towards the soma. These results will be confirmed in further experiments. With this approach we will be able to study the specific incorporation dynamics (synthesis and turnover) of the somatodendritic and the axonal proteome, and possibly protein traffic between the two parts of the cell.

P97. Data-Processing Workflow for Quantifying identified and unidentified Features across measured Stool Samples.

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Introduction

Mass spectrometry based stool proteomics enables the quantitative analysis of host and microbial proteins, providing a better understanding of host microbe interactions and the mechanisms involved in the development of various diseases. However, the high complexity and heterogeneity of stool make both sample processing and analysis of the obtained MS-data non-trivial. Thus, the complexity of the samples requires large or specially developed databases for spectra identification, which can lead to an overestimation of the FDR and a low identification yield. The high biological heterogeneity also leads to unreliable quantification results.

Method

With our work, we describe a data-processing workflow for quantification of stool samples, using already well-established tools. To achieve this, we use Nextflow as the workflow engine, converting measured samples into an open-data-format with ThermoRawFileParser, searching spectra using Comet and applying the feature detection and matching between samples, using identified precursors with OpenMS. To counter the FDR-overestimation and to increase the ID yield, we designed a peptide-FASTA-File containing only unique peptides of around 1000 species which have been found in the human gut.

Results

Preliminary results show, that a matching between features in datasets is possible, using only the identified precursors. Further, intensities of identified as well as of unidentified features can be extracted and used for quantification across measured samples. Since this workflow is in an early state, it remains of special interest to verify each step to check for its reliability. But since it provides quantification information of identified and unidentified features, such a workflow could prove itself very useful for complex and heterogeneous samples in metaproteomics like stool where the identification yield is low.

P98. A Cloud-scalable Software Suite for Large-Scale Proteogenomics Data Analysis and Visualization

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Introduction

Integration of large-scale proteomics and genomics data requires complex workflows spanning multiple data types and requires expertise across scientific domains, which may act as a barrier for researchers to perform multi-omics data analyses. The Proteograph™ Analysis Suite (PAS), a cloud-based, proteomics and proteogenomics data analysis software, enables the identification and exploration of proteins and peptide variants arising from allelic variation or other user-defined protein sequence altering genetic variants not captured in standard canonical reference databases through the integration of Proteograph proteomics data with NGS variant information.

Methods

PAS features integration of stablished proteomics database search algorithms, experiment data management system, analysis protocols, result visualizations, and setup wizards for seamless generation of results. PAS can support both Data Independent Acquisition and Data Dependent Acquisition workflows and is compatible with variant call format files. Using the Proteogenomics module in PAS, we performed a customized, sample-specific database search to identify variant peptides, including results interpretation using the Variant Peptide Browser and Proteogenomics Data Explorer.

Results

We demonstrate the Proteogenomic features of PAS by analyzing plasma proteome data generated from Proteograph™ Product Suite and corresponding variant data from healthy controls and Alzheimer's Disease (AD) patients. We highlight an interactive Variant Peptide Browser tool for examining peptide variation in the samples. These results include filtering and grouping features to identify qualitative and quantitative trends in single samples or healthy/disease groups. Further, we demonstrate the Proteogenomics Data Explorer tool, which provides a view of the genomic variant coordinates in relationship to the peptide/protein data. Detected variants can be reviewed in the context of gene structure, protein structure, and functional domains so biological insight can easily be gained from the proteogenomic data.

Conclusions

PAS provides an easy-to-use and efficient suite of tools for seamless and fast proteomics and proteogenomics data analysis enabling novel biological insights.

P99. The influence of highly effective modulator therapies on the sputum proteome in cystic fibrosis

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Introduction

Cystic fibrosis (CF) is caused by dysregulation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein; a ubiquitous ion channel important for epithelial hydration. A direct consequence of this dysfunction is impaired mucociliary clearance, chronic airway infection and a dysregulated inflammatory response that results in progressive loss of lung function and development of respiratory failure. We have previously presented data from proteomic analysis of CF sputum, highlighting protein candidates that correlated with lung function changes, suggesting that proteomics may provide translational insights for CF disease stratification and monitoring (DOI: 10.1016/j.rmed.2022.107002). Since, the landscape of CF lung disease has changed dramatically. Partial restoration of CFTR function is now possible for > 90% of CF patients through mutation specific CFTR modulators, such as elexacaftor/tezacaftor/ivacaftor (ETI), which produce dramatic clinical improvements, particularly by improving lung function. Therefore, using the same proteomic methodologies, we aimed to identify proteins/ protein pathways that are regulated by ETI therapy.

Methods

Spontaneous sputa were collected pre- and post-ETI therapy (n=25) and compared to CF control (n=6), and stored healthy control (n=15) sputum. Samples were analysed by bottom-up shotgun proteomics using liquid-chromatography/mass spectrometry. Clinical data were obtained from medical records.

Results

Obvious sputum proteome changes were seen post-ETI using principal component analysis and hierarchical clustering, with the proteome more closely resembling health. Using Gene Ontology these changes appear driven by dysregulated immune responses with reductions in neutrophil activity and restoration of counterregulatory responses. In most subjects, the post-ETI proteome remained distinct from healthy, even in those achieving normal lung function.

Conclusion

Reduction in neutrophilic airway inflammation may contribute to the clinical response seen with ETI-therapy. However, despite lung function being classified as "normal" post-ETI, proteome abnormalities still persist. Because of this, we suggest a potential role for (1) inflammatory biomarkers to complement existing clinical measure to assess lung disease severity in response to CFTR modulation, or more broadly in clinical care (2) a possible role for anti-inflammatory therapies for patients with persisting inflammation post-ETI, but also for patient's ineligible for modulator therapy.

P100. Data mining antibody sequences for database searching in proteomics data

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The human antibody, an essential immune system component, is a protein composed of two heavy and two light polypeptide chains interconnected by disulfide bonds. Mass spectrometry (MS)-based proteomics is an important method for identifying and quantifying antibodies but suffers from challenges due to their vast variety. Here, common bottom-up proteomics approaches rely on database searches that compare experimental values of peptides and their fragments to theoretical values derived from protein sequences in a database. Increasing the database size to millions of entries inflates the search space leading to very long search times and considerable difficulties with controlling the false discovery rate. Thus, large databases of millions of antibodies produced by our immune system are unfeasible to date. This impedes antibody detection in complex samples like human plasma. In addition, the number of antibody sequences in current human protein databases is limited. Recent genomic studies have compiled millions of human antibody sequences publicly accessible through the Observed Antibody Space (OAS) database. However, these data have yet to be exploited to confirm these antibodies in human samples. In this study, we employed the extensive collection of antibody sequences available in the OAS database for conducting database searches in proteomics-based mass spectrometry. After collecting 30.96 million heavy antibody sequences of 146 SARS-COV-2 patients from the OAS database, we performed in silico digestion (trypsin) and removed the peptides overlapping with reviewed UniProt human proteome (canonical and isoform) to obtain 18.42 million unique peptides. We then extracted a portion of the most common OAS peptides (i.e., peptides that appear in most of the antibodies) and combined them with UniProt human proteome (2023 March) and cRAP (a database of contaminant proteins) to form databases. We conducted tests using those databases and publicly available SARS-COV-2 human plasma samples in the proteomics identification database (PRIDE). To avoid false positives of antibody peptide identification, we tested the database search against negative controls (brain samples) and used different database sizes containing 100, 10^4, and 10^6 OAS peptides. We expect that the newly discovered antibody peptides in the SARS-COV-2 samples can be further employed to develop therapeutic antibodies. The method will be broadly applicable to find characteristic antibodies for other diseases.

P101. Ion-mobility separation deciphers epigenetic complexity

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The functional unit of chromatin is the nucleosome, a DNA-protein complex composed of 147bp of DNA wrapped around an histone octamer. Post translational modification of histone proteins (hPTMs) is mainly responsible for the epigenetic regulation of fundamental biological processes, ranging from DNA replication and repair to tight control of cell cycle and gene transcription program. The N-terminal regions of histones are known to be hypermodified, with multiple classes of simultaneous PTMs either activating or inhibiting each other in cis or trans. Histones are very rich in arginine and lysine residues and among the different modifications, acetylation and methylation of lysines and arginine methylation are therefore highly frequent in the octamer. In contrast to targeted approaches, liquid chromatography coupled to mass spectrometry (or LC-MS) is well suitable for the comprehensive analysis of hPTMs. However, in line with the amino acid composition of histones, the characterization of hPTMs via bottom-up LC-MS requires alternative protocols to the canonical tryptic digestion. Among the possibilities, lysine chemical acylation promotes an ArgC-like in-gel digestion, thereby generating peptides with multiple modified residues and of ideal length for the investigation via LC-MS. Despite recent advancement in sample preparation and separation protocols, the co-elution of nearly isobaric peptides still represents a major limitation in the extensive analysis of hPTMs and in our understanding of the regulation of fundamental biological processes.

Recent improvements in mass spectrometry technology take advantage of differential ion mobility (IM) in gas-phase as function of the structure, for the separation of ions co-eluting from the chromatographic column. This technology has recently boosted peptide identification rate and further expanded the dynamic range of MS, while been efficiently applied to top-down and cross-linking MS.

We therefore explored the potential benefit of trapped and high field asymmetric IM-MS (e.g. Tims and FAIMS, respectively) in separating nearly isobaric hypermodified histone peptides, with the aim of expanding the characterization of hPTMs. In particular, ArgC-like digested histones from U2OS cells were analyzed in parallel on the Exploris 480 with or without FAIMS, on TimsTOF where funnel separation was set to either 100 or 10%. Acquired data were benchmarked against QE-HF. Our results show how IM-MS allows for decreasing sample amount while still recapitulating the hPTMs identified by the QE-HF. In general, IM-MS identifies more hypermodified features in comparison with acquisitions without mobility separation. Between the two IM-MS, Tims seems to be particularly suitable for the identification of isobaric histone peptides.

P102. A Benchmarking Workflow for High-Throughput DIA Label-Free Quantification using a Novel High-Resolution Accurate Mass Platform

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Bottom-up proteomics has proven to be the most suitable technology for the analysis of complex biological samples in biomedical research. However, there are several analytical challenges which include sample concentration/amount, through-put and the data analysis. An integrated workflow for label-free quantitative proteomic studies has been developed, to meet all these challenges. A multi-organism dataset of proteomes mixed in known ratios was analyzed in DIA mode using a novel HRAM platform and compared to a similar dataset acquired on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.

Yeast and E. coli proteomes were spiked into a human proteome background at different ratios, yielding three proteome mixtures with total protein loads from 100 to 500 ng/injection. Peptides were separated on a 5.5 cm long µPAC Neo High Throughput column in a Trap-and-Elute workflow, using a Vanquish™ Neo UHPLC system under sub-microflow conditions. Different gradient lengths were employed, ensuring throughput from 24 to 180 samples per day, depending on the sample amount and complexity. DIA experiments were run on the new HRAM mass spectrometer and on an Orbitrap Exploris 480 MS system. Data were analyzed using Thermo Scientific Proteome Discoverer 3.1 software with Chimerys™ search algorithm.

In this work, we performed ultra-fast (up to 200Hz), narrow windows (2 Th) DIA experiments using a novel HRAM analyzer which was integrated with quadrupole and Orbitrap analyzers into a single instrument. MS1 scans were collected at 240,000 resolution using the Orbitrap analyzer in parallel with MS2 scans at a resolution of 100,000 using the new analyzer. The multi-organism datasets revealed that the novel HRAM platform enabled the quantification of >12,000 protein groups, representing 2-3-fold increase in the number of protein groups, compared to the Orbitrap Exploris 480 MS system, while keeping similar precision. This increase was achieved using ~2-fold shorter gradient (24 min for the new mass spectrometer and 45 min for the Orbitrap Exploris 480 mass spectrometer). In summary, the workflow developed for DIA label-free quantification using the novel HRAM mass spectrometer enables wide proteome coverage at high throughput, while maintaining very high quantification accuracy of relative protein ratios in complex cell lysates.

P103. Exploitation of the graphene oxide biomolecule corona in 2D and 3D cancer cell secretome-based biomarker discovery

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Introduction

Nanotechnology has emerged as a promising tool for cancer biomarker discovery. Nanoparticles (NPs) form a ""biomolecule corona"" when they come into contact with the biological milieu. Analysis of the biomolecule corona by mass spectrometry-based proteomics has shown enhanced discovery of low-abundant proteins and has attracted significant interest as a promising technology in cancer biomarker discovery.

In this study, the biomolecule corona formed around graphene oxide nanosheets, a two-dimensional material with high surface reactivity, is exploited to provide an in-depth analysis of the secretome obtained from mouse glioma cancer cells. The cancer cell secretome consists of proteins released by cancer cells and provides a highly specific analyte for biomarker discovery. However, the utility of the secretome is limited by the low abundance of secreted proteins within the milieu of the culture medium, which mainly contains highly abundant fetal bovine serum (FBS) proteins. Therefore, this study aims to utilize the graphene oxide biomolecule corona to enrich low-abundance secretome proteins in both 2D and 3D cell culture-conditioned media containing FBS.

Methods

The secretome of the 3D-growing mouse glioma (GL261) cell line was obtained by culturing cells in a hollow fiber bioreactor, which provides in vivo-like cell cultures, while the 2D secretome was obtained by conventional cell culture in 150-mm culture dishes. The collected secretome was incubated with graphene oxide nanosheets to form the biomolecule corona. Using a 2-step NanoOmics purification protocol, the graphene oxide biomolecule corona was isolated via a combination of size exclusion chromatography and membrane ultrafiltration.

Results

Proteomic mass spectrometry analysis of the isolated biomolecule corona showed a significant 2-4 fold increase in the number of identified secretome proteins in the corona-processed samples in both 3D and 2D cultures. The relative abundance ratio showed a significant increase in low-abundance proteins and a simultaneous reduction in high-abundance FBS proteins, particularly serum-albumin, due to corona processing. Ingenious pathway analysis showed that the identified secretome proteins were involved in pathways related to the organization of the extracellular matrix and cancer progression. Interestingly, the corona processing protocol also showed significant enrichment of the exosomal protein markers CD9 and CD63 from the 3D secretome, indicating the presence of extracellular vesicles attached to the graphene oxide biomolecule corona.

In the future, we plan to utilize the graphene oxide biomolecule corona platform to identify cancer-specific proteomic fingerprints by comparing the secretomes of matched cancer and non-cancer cell lines.

P104. Proteomically distinct groups of KMT2A-rearranged leukaemia are in different stages of hematopoietic maturation

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Introduction

Acute myeloid leukemia (AML) is a disease of unmet clinical need, especially for patients harbouring certain chromosomal abnormalities (poor risk karyotypes). We previously profiled the phosphoproteomes of 74 of such poor risk karyotype patients and identified a signature that stratified patients with KMT2A (also known as MLL) rearrangements in two functionally different groups that we named MLLGA and MLLGB.

Methods

Here, integrate proteomics data in AML primary cells with single cell transcriptomics data in from healthy blood cells to derive scores for 35 blood cell populations in our cohort of 74 patients.

Results and Conclusion

We found that stem and precursor cell populations scored higher in cases associated with low differentiation (M0, M1 and M2 morphology), while monocyte and neutrophil populations scored higher in cases associated with myeloid and monocyte morphologies (M4 and M5) and the erythroblast population scored higher in cases linked to erythroid morphology. Monocyte and neutrophil populations also scored higher in cases immunophenotipically classified as mature by CyTof analysis, while stem and precursor populations scored higher in primitive cases. More relevantly, MLLGB cases presented higher scores for monocyte and neutrophil populations than MLLGA, indicating that leukemic cells in MLLGB cases are more mature than in MLLGA. Furthermore, MLLGA cases were more sensitive to the IMPDH inhibitor AVN499 and expressed higher levels if IMPDH2 than MLLGB and the scores for the neutrophil population negatively correlated with the response to AVN499 and the expression of IMPDH2. We found that RNA expression of IMPDH2 in healthy blood cells is higher in hematipoietic stem cell populations than in monocyte and neutrophil populations. In addition, treatment of two AML cell lines with the LSD1 inhibitor GSK2879552 increased the scores for monocyte and neutrophil populations and reduced the expression of IMPDH2, further suggesting that the expression of this enzyme is regulated during the differentiation and maturation of myeloid cells.

In summary, we defined a score system for 35 blood cell populations using single cell transcriptomics data, and found that AML cases with KMT2A rearrangements classified as MLLGB showed a greater extent of myelomonocytic maturation than cases classified as MLLGA. We also found that expression of IMPDH2 and sensitivity to IMPDH inhibitors is associated to myeloid maturation in KMT2A rearranged cases. This data could help the implementation of precision medicine in AML patients with KMT2A rearrangements.

P105. Mapping Proteolysis During Murine Norovirus Infection Using Multi-Protease N-Terminomics

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Human noroviruses are the leading global cause of viral gastroenteritis accounting to >200,000 deaths per annum, and major disruption to healthcare systems worldwide. Compared to other single-stranded positive-sense RNA viruses (+ssRNA), our knowledge of human norovirus biology is limited given difficulties in propagating the virus life cycle in vitro. Alternatively, Murine Norovirus (MNV) represents a useful cell culture model for studying infection and replication of noroviruses. As in many +ssRNA viruses, noroviruses encode proteases responsible for cleaving the polyproteins translated from their genome, thus releasing functional structural/non-structural proteins required for viral replication. Nonetheless, mounting evidence suggests these viral proteases also target host proteins and play a major role in subverting host cells and causing disease. Here, we applied an expanded TMTpro 18plex HUNTER methodology for quantitative N-terminomics characterisation of substrates targeted by proteolysis in the course of MNV infection in the BV-2 cell line. Our expanded methodology employed the use of multiple proteases (trypsin, chymotrypsin, AspN and ProAlanase) for sample digestion prior to enrichment of N-terminal peptides for increased coverage of proteolytic cleavage sites. We determined the quantitative robustness of N-terminomics employing multiple proteases using dual-labelled SILAC proteomes and analysing experimental versus theorical light to heavy ratios. Our data represents a first high-throughput analysis of viral protease activity for this important virus family.

P106. The Proteomes that Feed the World

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Introduction

Plants play a significant role in supporting the human population and mitigating climate change. However, their proteomes, which control nearly every aspect of life, are not well understood. To fill this knowledge gap, the international doctoral program ""The Proteomes that Feed the World"" launched at the Technical University of Munich and funded by the Elite Network of Bavaria aims to map the proteomes of the 100 crop plants most important for human nutrition, creating a valuable Crop Proteome Atlas for academia and industries. We seek partners to leverage the potential of this data. The computational solutions developed in this project will be tailored to crop plant research, enabling the discovery of novel biological insights that will benefit academia and industries. Here, we show the optimized pipeline, the bioinformatics strategy and exemplify data usage on a new visualization developed for ProteomicsDB showing protein expression across many organisms.

Methods

To achieve proteomic depth and sample throughput, the Crop Proteome Engine requires optimized and balanced sample preparation, LC-MS/MS measurements, and informatics workflows. The Crop Proteome Atlas project has developed a robust and reproducible protocol for processing and analyzing plant tissues using liquid chromatography-tandem mass-spectrometry (LC-MS/MS). Peptide and protein identification and quantification is achieved by using MaxQuant combined with Prosit-based rescoring and Chimerys. The project will follow FAIR principles by sharing the data publicly accessible via PRIDE and ProteomicsDB throughout the project.

Preliminary Results

Our optimized pipeline resulted in the identification of ~147k peptides and ~10k protein groups in fruits of tomato (Solanum lycopersicum) and ~189k peptides and ~18k protein groups in quinoa (Quinoa bicolor), searching with MaxQuant and rescoring with Prosit. The extensive dataset generated will be used for various computational analyses, including improvements in genome annotations, homology inference, and protein function prediction. As an example, we systematically evaluated multiple homology inference tools based on their performance on published reference orthogroups and a set of in-house metrics. Based on this analysis, the orthogroups of OrthoFinder were imported into ProteomicsDB, which enabled the development of a new visualization module allowing the comparison of protein expression patterns across multiple organisms to facilitate cross-species plant research. Overall, ""The Proteomes that Feed the World"" is an ambitious international initiative that will map the proteomes of the most important crop plants for human nutrition, thereby creating a valuable Crop Proteome Atlas."

P107. Spatial Proteomics of a Human Brain Tumour

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Introduction
The spatial protein abundance profiles within tissues are a key factor in understanding disease pathology and cellular function. These profiles are typically studied by antibody-based imaging techniques that provide high spatial resolution, but can only probe a limited number of proteins. In order to more precisely define molecular phenotypes in disease, there is a need for unbiased, quantitative technology capable of mapping the expression of many hundreds to thousands of proteins within tissue structures. Laser capture microdissection (LCM) in combination with high-throughput LC-MS/MS-based proteomics is well placed to meet this need.

Methods
Tissue voxels were isolated from 10 µm-thick sections of a human brain tumour in a gridded pattern. Captured tissue was processed using an SP3-based protocol prior to analysis by LC-MS/MS on a timsTOF Pro using 17-minute gradients or on an Orbitrap Fusion Lumos using 60-minute gradients. Moran's I tests were used to investigate spatial autocorrelation of quantified proteins. The spatial protein profiles were used for unbiased spatial clustering by affinity network fusion, ANOVA significance testing and pathway analysis between spatial clusters. Spatial protein abundance profiles were validated by immunohistochemistry.

Results
Proteomic depth was comparable between the two platforms, with approximately 5,000 proteins detected across the experiment. The higher throughput of the timsTOF Pro platform allowed for mapping of a complete 20 x 15 mm tissue section at ~800 µm resolution. As the spatial sampling information is retained, quantitative protein measurements can be plotted in their spatial context to generate proteomic tissue maps. 3,212 proteins show significant evidence of spatial autocorrelation, respectively (Moran's I test, $q \leq 0.05$). Immunohistochemistry staining for three highly spatially autocorrelated proteins is consistent with the proteomic dataset. Unbiased spatial clustering generates clusters of samples which co-cluster in space, reflecting the prominent pathology visible in H&E-stained sections along with further nuances.

These and other spatially-resolved proteomics methods which spatially profile thousands of proteins will push the boundaries of understanding tissue biology and pathology at the molecular level. Recent mass spectrometry technology developments were key to achieving this throughput and depth, and this approach stands to benefit from further technological developments.

P108. Characterization of Adeno-associated viral proteins and related proteoforms using top-down approach on a LC-Orbitrap Tribrid MS platform

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AAV capsid viral proteins (VPs) are important constituents of AAV product and play an important role for immunogenicity and tissue tropism in gene therapy. In-depth characterization of viral proteins including their truncated forms and PTMs is critical for developing and manufacturing of AAV products to ensure their safety, quality, and efficacy. However, the AAV drug developers are facing a considerable manufacturing and characterization challenge based on their molecular size, complexity, highly homologous sequences, and general availability in limited quantities. In this study, we used top-down approach on an Oribtrap Tribrid MS for direct characterization of intact VPs, their truncated forms and PTMs. Unique multiple fragmentation capability of the Oribtrap Tribrid MS platform enabled high sequence coverage for the unambitious characterization.

The AAV6 serotype denatured sample was separated using a C4 stationary phase column and detected on a Orbitrap Ascend Tribrid mass spectrometer coupled with a Vanquish Horizon UHPLC system. Targeted top-down analysis with multiple dissociation methods (EThcD, HCD and UVPD) were used for unambitious PTM localization and sequence verification of truncated form of VP3. Top-down mass analysis workflow in the BioPharma Finder 5.1 software was used for data processing.

Intact mass analysis allows direct measurement of molecular weights of VPs and offers a great analytical tool to identity AAV serotype and monitor potential capsid protein heterogeneity including PTMs and truncated forms. However, the molecular weight information does not provide sequence related information and cannot localize the PTM site. Top-down analysis provides in-depth sequencing information without the digestion step and was used in this study for rapid sequence conformation of VPs, sequence conformation of truncated proteoforms and characterization of PTMs. In our previous work, we applied multiple LC-MS/MS approaches (Intact, top-down and peptide mapping) for AAV6 viral protein characterization on a Orbitrap Exploris 480 MS and identified multiple potential phosphorylation sites with peptide mapping approach. However, it was difficult to localize the accurate site of the phosphorylation with the peptide mapping approach only because of the high sequence overlaps of the VP1 and VP2. In this study, we performed targeted top-down analyses using EThcD, HCD and UVPD on the HPLC-Orbitrap Tribrid MS platform for the phosphosite localization of VP2 and protein sequence conformation of VP3 clip.

P109. Application of capillary electrophoresis-mass spectrometry to identify histone non-canonical phosphorylation and other post-translational modifications

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Post-translational modification (PTM) of histones, such as phosphorylation, acetylation and methylation, is known to direct chromatin structure and subsequently gene transcription, with the “histone code” (PTM combinations on different histones) permitting specific regions of the genome to either be “silenced” or made accessible for transcription. Specific histone codes have also been identified as markers of cancer and other diseases. Histone phosphorylation, a dynamic PTM largely directed towards serine, threonine and tyrosine, has been extensively characterised. This project focuses on the characterisation and dynamics of novel sites of non-canonical phosphorylation (NCP) of e.g., histidine, lysine and arginine, which to date, have not been considered as part of the histone code, yet have been shown by us and others to be present. To identify and localise these PTMs, we have used sheath-less capillary electrophoresis coupled to mass spectrometry (CE-MS). For this we have used top-down proteomics (TDP), however bottom-up (BUP) analysis has also been explored by other groups. TDP is beneficial for defining a PTM code on intact proteins and avoiding missing peptides, although lower solubility of intact proteins compared to peptides and multiply charged proteins and product ions can potentially challenge TDP analyses. Using top-down proteomics via CE-MS, we have analysed bovine histone standards and histidine phosphorylated myoglobin, looking at how different analytical parameters change histone separation, and the preservation of sites of phosphohistidine. We have explored parameters such as changes in background electrolyte (BGE) acid content, BGE organic content, capillary temperature and separation voltage. Phosphohistidine has been preserved on phosphorylated myoglobin analysed through these conditions, suggesting successful preservation of NCP. Overall, our data shows that a positively coated capillary with a separation voltage of 10 kV is optimal for separation of different histone proteoforms, potentially including those containing sites of NCP. Since some histone codes have already been identified as markers of disease or normal biological functions, newly discovered NCPs could have similar or novel downstream effects.

P110. Establishing nPOP-based single-cell proteomics at the University of Liverpool

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Single cell proteomics by mass spectrometry (SCOPE2) is a method for studying the proteomes of thousands of single cells. SCoPE2 allows for high through-put analysis of single cells through TMT multiplexing, enabling analysis of up to 14 single cells per run. Multiplexing also allows for the inclusion of carrier or reference channels, and can support improved detection of low-abundance peptides. Our lab is especially interested in applying single-cell proteomics to understand variability in viral infection at single-cell level, which for RNA viruses is typically regulated at the protein or PTM-level.

In support of this research, we established the nPOP-based method for SCoPE2 sample preparation on a CellenONE instrument to perform single-cell proteomics at the Centre of Proteome Research, University of Liverpool. Our adaptations include adjusting cell spacing to avoid droplet merging during sample processing and humidity issues, as well as optimising sample pickup from the glass slide and downstream data analysis. Here we present our SCP data, initially comparing populations of A549 and HEK-239 cell lines and downstream optimisation to permit analysis of post-translational modifications. Overall, this workflow confidently identifies upwards of 3000 peptides per LC-MS/MS run.

P111. An in-depth plasma proteomics workflow powered by a Novel HRAM mass spectrometer

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Mass spectrometry-based plasma proteomics remains the promising method of understanding human molecular pathophysiology and the discovery of disease biomarkers. However, it has been a challenging workflow for many years due to the large dynamic range in protein expression and the current capabilities of analytical methods, especially regarding the throughput and depth of proteome coverage. Here we present a label-free plasma proteomics workflow using a novel high resolution accurate mass spectrometer as a robust analytical setup for in-depth analysis of plasma proteins. Two different types of samples were used, a neat plasma sample and a plasma sample prepared with Seer's Proteograph Product Suite, utilizing a multi-nanoparticle-based approach.

The neat plasma sample was prepared using Accelerome and the enriched plasma sample was prepared on Seer's Proteograph Product Suite. Both were analyzed using two different workflows: (i) a Max-ID method using a 75cm EasySpray column on a 60min gradient for deep proteome coverage (14 SPD) and (ii) a short, 5.5min gradient method using a 15cm EasySpray column for high-throughput (180 SPD for the neat and 36 SPD for the enriched samples). A Vanquish Neo UHPLC system was used at 250nl/min for the Max-ID and 1.3ul/min for the high-throughput method. A new high-resolution accurate mass platform was used with a DIA method. Data analysis was done using a beta version of Proteome Discoverer software 3.1.

To tackle the plasma dynamic range issue, a novel high-resolution accurate mass analyzer utilizing a label-free proteomics workflow in DIA mode was used as a robust analytical setup for both high-throughput and in-depth analysis of plasma samples. Analysis of 500 ng of the neat plasma sample using the 15cm EasySpray column on a 5.5min active gradient resulted in ~600 protein groups. Increasing the gradient length for deeper coverage with a 75cm EasySpray column on a 60min active gradient improved the number of protein groups to ~1000. Analysis of the plasma sample enriched using Seer's Proteograph Product Suite resulted in ~3000 protein groups when the high-throughput method was used with 500 ng peptide loaded on the column for each nanoparticle fraction (5 individual injections of the 5 different nanoparticles separately). Similar to the neat plasma results, when the Max-ID method was used with a onger gradient, a significant improvement in the number of proteins and peptides was observed with a total of ~5000 protein groups from 2 ug sample load of a pool of all 5 nanoparticle fractions.

P112. Consequences of chronic radiation exposure on the proteome, protein carbonylation, and antioxidants: Implications for biotic stress resistance of aquatic plants

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Introduction
Ionising radiation is a ubiquitous challenging factor that induces molecular and cellular changes in plants. Such stress exposure may cause water radiolysis, consequently generating excessive reactive oxygen species. Plants adapt by synthesising defence proteins and metabolites, including antioxidants. Moreover, chronic irradiation may affect plant resistance to simultaneous biotic stress.

Methods
Our study focused on revealing biochemical mechanisms responsible for the reaction of chronically irradiated wild aquatic plants (common reed—Phragmites australis) challenged by pest or pathogen attack. Mature leaves collected from the contaminated (primary with radionuclides Cs-137 and Sr-90) and reference lakes in the Chernobyl Exclusion Zone were used as experimental material.

Results
Protein profiling using ultrahigh-performance liquid chromatography and mass spectrometry quantified 1340 proteins. Among them, 174 proteins were differentially accumulated between control and contaminated locations. Principal component analysis indicated that sampling variables had a higher impact on proteome than contamination with radionuclides. Nevertheless, samples from contaminated lakes were more susceptible to fungal infection in the leaf-sheath assay performed in the laboratory. On the other hand, the level of antioxidants was not affected in reed leaves exposed to chronic ionising radiation. Next, we focus on revealing changes of site-specific protein carbonylations using affinity enrichment and mass spectrometry. The outcomes of our research will bridge fundamental radiobiology and relevant management practices for contaminated lakes.

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P113. MALDI-TOF mass spectrometry on the track of species diversity and bloodmeal sources of phlebotomine sand flies

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Leishmaniases caused by Leishmania parasites and transmitted by females of phlebotomine sand flies belong among important and yet still neglected vector-borne diseases. In order to understand the transmission cycles of leishmaniases between vectors and their hosts it is essential to precisely identify sand flies, especially those occurring in new endemic areas, and to determine their trophic preferences towards reservoir hosts as this knowledge is crucial to assess the significance of particular species (both vectors and hosts) in the local epidemiological context.

A morphological analysis of species-specific characters that has been used for decades remains a golden standard for sand fly typing. However, it demands laborious sample preparation and expertise in character assessment and often fails to produce conclusive results in field samples that may be compromised in crucial decisive morphological features. DNA-based methods offer excellent alternative, however, may become laborious and expensive when high numbers of field-collected specimens from large-scale studies need to be processed. MALDI-TOF MS protein profiling, nowadays being a method of choice for arthropod vector identification, was therefore applied for species determination of sand flies as it requires cheap and simple sample preparation and quick analysis. For bloodmeal source identification, we employed a promising approach based on peptide mass mapping MALDI-TOF MS analysis targeting host-specific haemoglobin peptides generated by trypsin digestion of the engorged blood.

MALDI-TOF protein profiling was proved to be a suitable method for species identification of large sets of field-caught sand flies from various endemic regions of the Old World including the Mediterranean, East Africa or south-east Asia, creating a reference database that currently covers 37 sand fly species. Standardized protocol of specimen trapping, storage and sample preparation ensures to acquire species-specific protein profiles and utilize a single sand fly specimen for other purposes (DNA-based assays, pathogen detection, bloodmeal identification, morphological analysis). MALDI-TOF peptide mass mapping of host haemoglobin in engorged sand fly females successfully identified the host blood, including mixed bloodmeals, up to 48 hours post feeding, providing longer reliable blood source determination than other currently used methods where performance is quickly affected by progression of blood digestion and degradation. Beside sand flies, the approach was proved also on Culex mosquitoes and may be applied universally to other hematophagous insects as well.

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P114. Nu.Q® Capture-MS as a novel methodology for profiling the post translational modifications of circulating nucleosomes in colorectal cancer and non-Hodgkin lymphoma patients

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Introduction

Liquid biopsy is considered a valuable tool for cancer diagnosis and to help to guide treatment decision. Compared to tissue biopsy, it may offer a non-invasive and easily repeatable alternative. Recently, circulating nucleosomes in blood plasma have become a promising cancer biomarker. Alterations of epigenetic modifications, including the dysregulation of histone post-translational modifications (hPTM), play an important role in cancer but their signature on circulating nucleosomes is not yet clearly described.

Methods

In our laboratory we have developed a novel method for the detection and the quantification of circulating nucleosomes and their associated hPTMs in human plasma samples. The method is based on the capture of the nucleosomes using an in-house developed immunoprecipitation followed by liquid chromatography coupled to mass spectrometry (Nu.Q® Capture-MS). The strategy was recently applied to quantify the hPTMs alterations in plasma of CRC (n=9) and NHL (n=9) patients and compared to healthy donors (n=9 and n=5 respectively). In both cases, the abundances of the possible hPTMs were analyzed and compared between groups to determine changes in their profile. The capability of the identified hPTMs to differentiate between cancer and healthy plasma was further confirmed by two independent clinical studies (n=256 and n=59 individuals for CRC and for NHL respectively) using available quantitative Nu.Q® immunoassays (Belgian Volition SRL).

Results

For the CRC study, among 54 identified and quantified histone proteoforms, thirteen distinct histone hPTMs were significantly different in CRC. Notably, methylation of histone H3K9 and H3K27, acetylation of histone H3 and citrullination of histone H2A1R3 were upregulated in plasma of CRC patients. Immunoassay results of the clinical study confirmed the increased levels of circulating H3.1-nucleosomes, H3K27Me2/3- and H3K9Me3-nucleosomes but not the elevated levels of circulating H3K14Ac- and H3K27Ac- nucleosomes. Regarding the NHL study, we identified 56 histone peptides by Nu.Q® Capture-MS. Our analysis demonstrated increased levels of H3.1-nucleosome as well as the lysine acetylation and methylations of the histone H3 in plasma from NHL patients. In addition, the clinical validation of the hPTMs pattern by Nu.Q® immunoassays identified a significant increase in the levels of H3.1-nucleosomes as well as of 6 hPTMs (H3K9Me1-, H3K27Me3-, H3K36Me3-, H3K9Ac-, H3K14Ac-, H3K18Ac-nucleosomes) in NHL patients when compared to healthy donors.

Our results indicate that levels of circulating nucleosome are particularly elevated in cancer patients and highlight the crucial role of the epigenetic marks present on circulating nucleosomes to detect and monitor diseases such as CRC and NHL.

P115. Transient modifications of lysosomal proteases by cosmetic preservative methylisothiazolinone in human contact allergy

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Introduction

Human contact allergy is a worldwide prevalent health problem and affects millions of patients in their daily quality of life. Methylisothiazolinone (MI) is an electrophilic reactive small chemical that is broadly used as a preservative e.g. in hand soaps, varnishes, wall paints and during paper production. Due to its allergy inducing potency and high incidence rates, it is partially restricted in consumer products in the EU. As a molecular initiating event (MIE) of human contact allergy, MI is known to form disulfide bonds with cysteine thiols of self-proteins (haptenation), which are considered to be processed (antigen processing) resulting in immunogenic T cell epitopes on human antigen presenting cells.

In this study, we examined (i) MI modifications of lysosomal cysteine-proteases of the cathepsin family, which play a crucial role in antigen processing and presentation; and (ii) cathepsin enzyme activity and enzyme kinetics, potentially affected by transient or persistent MI-protein modifications.
Methods: Classical Orbi-Trap LC-MS/MS analyses were applied to identify position-specific MI-modified cysteine residues on antigen processing model cathepsin. Data were analyzed in more detail by Peaks software. Complementary functional enzyme assays were performed and kinetic characteristics were assessed comparing activities in presence and absence of allergen specific MI-protein modifications.

Results

(1) LC-MS/MS studies revealed several binding sites for MI, apart from the catalytic center. (2) MI-protein modification demonstrated significant cathepsin B enzyme inhibition, which was not neutralized by physiological concentrations of reducing agent glutathione (GSH). (3) Enzyme kinetics with moderate MI concentrations (MI-protein modification) indicate a non-competitive inhibition of cathepsin B by MI, which supported the LC-MS/MS observation (1).

MI-protein modifications of antigen processing enzymes may induce novel T cell epitopes independent of initially modified self-proteins by interfering with the homoeostatic antigen processing machinery in human immune cells.

P116. Proteomic-based characterization of Trilaciclib in Chronic Myeloid Leukemia (CML)

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Introduction
Chronic Myeloid Leukemia (CML) is a type of blood cancer characterised by the presence of the Philadelphia chromosome and the constitutively active BCR-ABL fusion protein. The use of tyrosine kinase inhibitors (TKIs) has significantly improved the management of CML. However, TKIs can cause chemotherapy-induced myelosuppression, resulting in reduced blood cell counts, as well as induce a state of stable growth arrest known as senescence. Senescence has both positive and negative effects, including limiting cell proliferation and facilitating immune clearance of leukemic cells, but it can also contribute to chronic inflammation and a microenvironment that supports tumor growth. While trilaciclib, a CDK4/6 inhibitor, has been approved as a myeloprotective agent for extensive-stage small cell lung cancer, its therapeutic potential in CML remains uncertain. In this study, our objective was to comprehensively analyse the proteomic changes induced by trilaciclib in CML cells.

Methods
We employed mass spectrometry-based proteomics to analyse the proteome of K562 cells (CML) treated with trilaciclib. K562 cells were cultured and treated with trilaciclib, and protein samples were extracted, trypsin-digested, and subjected to liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis using Data-Independent Acquisition (DIA). The acquired data were processed using DIA-NN software for protein identification and quantification. Additionally, to further characterise senescent cells, we assessed β-galactosidase activity, performed viability and cell cycle analysis through flow cytometry, and analysed energy metabolism using a Seahorse assay.

Results
The proteomic analysis revealed significant alterations in the proteome of CML cells upon trilaciclib treatment. We identified numerous differentially expressed proteins and investigated their functional roles. Functional enrichment analysis highlighted several key pathways affected by trilaciclib, including cell cycle regulation, DNA damage response, and cellular senescence. Moreover, we observed that trilaciclib effectively inhibited cell proliferation while preserving cell viability. Notably, trilaciclib induced G0/G1 cell cycle arrest and increased beta-galactosidase activity, indicative of senescence. These findings suggest that trilaciclib exerts its anti-leukemic effects through diverse cellular mechanisms, including the induction of senescence in K562 cells.

P117. Improved dia-PASEF isolation window schemes for proteomics measurements

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Data-Independent Acquisition (DIA) is widely used for proteomics as it promises reproducible and accurate protein identification and quantitation. dia-PASEF is both more sensitive and selective than traditional DIA approaches as it combines the advantages of DIA with the inherent ion-usage efficiency of PASEF. Making use of the correlation of molecular weight and CCS coded information, dia-PASEF enables highly confident identification. The two-dimensional mass and mobility space enables method creation with extensively different window schemes. Here, a variety of fixed-width as well as more advanced window schemes were evaluated to determine optimal conditions for speed, sensitivity, and selectivity.

Dilution series of tryptic digests from human cell lines (HeLa and K562) from 125pg up to 200ng were separated using different nanoLC gradients (7 to 60min, nanoElute, Bruker). Depending on gradient length columns of 25cm, 15cm and 8cm length with internal diameters of 0.075mm or 0.15mm (PepSep, Bruker) were chosen. Isolation windows of 12, 25 and 50Da width, combined with one and two quadrupole isolation switches during TIMS separation were compared to more sophisticated approaches like schemes with variable window widths based on precursor density (py_diAID). Data were processed using Spectronaut 18 (Biognosys) in library-free mode.

In the presented study, we limited the dia-PASEF windows to the mass and mobility range of highest precursor density, i.e. 400 to 1200 Da in mass and 0.7 to 1.4 Vs/cm^{2}in mobility (1/K0) dimension. For sample amounts in the 10-50 ng range identifications were remarkable similar among the different tested acquisition schemes using either fixed or variable window widths showing a variation of less than 10%. Lower sample amounts benefit from a lower number of broader windows as each individual precursor is fragmented more frequently. Gradient lengths above 15 min did not show many benefits for these low sample amounts, also indicating that ion abundance is the limiting aspect. For higher sample loads, acquisition schemes of more narrow isolation windows resulted in improved identifications due to their higher specificity. However, such narrow isolation widths require a higher number of windows to cover the same mass/mobility range. The resulting slower duty cycle led to reduced quantitative accuracy for very short gradients. Optimal methods for a broad range of sample amounts and gradient lengths could be determined.

P118. High-throughput nano LC-MS for sample-limited proteomics

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Introduction
Nano-flow liquid chromatography-mass spectrometry (nLC-MS) is often utilized for bottom-up proteomics of extremely limited samples, down to single cells. This is due to the large increase in ionization efficiency achieved at LC flow rates < 300 nL/min. Generating reproducible and timely results at ultra-low flow rates requires state-of-the-art LC instrumentation and optimization of LC-MS parameters, particularly with respect to the separation and electrospray ionization (ESI) interface.

Methods
Considering multiple factors for balancing the sensitivity and sample throughput in a low-nano flow LC-MS application, we established a novel configuration to run gradients at 100 nL/min on Thermo Scientific™ Vanquish™ Neo UHPLC system with an Acclaim™ PepMap™ 100 C18 50 µm ID x 15 cm column (2 um dp) in the direct injection and trap-and-elute workflows. Peptide ionization is carried out via a 10 µm ID glass emitter into a FAIMS Pro™ interface operated at a single compensation voltage to reduce background ion interference, followed by data acquisition on an Orbitrap Exploris™ 480 mass Spectrometer in data-dependent (DDA) and data-independent (DIA) acquisition strategies.

Results
Ultimately, we developed six high-performance methods to permit fast sample loading, efficient column washing, and equilibration while maintaining a stable flow of 100 nL/min. For example, it enables up to 100 samples/day analysis with a 10-min elution window, offering extraordinary sensitivity for routine sample-limited proteomics analysis. Using this optimized method, we identified ~2,200 protein groups from 250 pg of standard protein digest using data-independent acquisition (DIA) in a library-free search. A proof of principle study indicated that >1,700 protein groups were identified from single-cell samples in a label-free quantification (LFQ) experiment with neglectable carryover in a 100 cells/day (CPD) throughput.

In conclusion, we demonstrate a high-sensitivity LC-MS/MS configuration that serves the needs for sample-limited analysis, permitting 100 CPD throughput for single-cell proteomics using LFQ-DIA."

P119. Wide Window Acquisition and AI-based data analysis to reach deep proteome coverage for a wide sample range, including single cell proteomic inputs

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Introduction

A comprehensive proteome map is essential to elucidate molecular pathways and protein functions. Despite tremendous progress in the field of proteomics, current studies still suffer from limited proteomic coverage and dynamic range. Here, we present and benchmark an optimized platform utilizing prototype micro pillar array columns (µPAC) in combination with wide-window acquisition (WWA) and the AI-based CHIMERYS search engine to achieve outstanding proteomic comprehensiveness in bulk and single cell proteomics as well as and affinity-purification mass spectrometry.

Methods

Benchmarking of different columns and acquisition strategies was carried out on an Exploris 480 system equipped with a FAIMS Pro device and hyphenated to either an UltiMate 3000 RSLCnano or a Vanquish Neo (all Thermo Fisher Scientific). Different chromatographic columns (two packed bed and one µPAC column) were compared for the analysis of a K562 QC mix using a 30 min gradient. Three different µPAC columns (a 5.5cm, a 50cm and a 110cm column) were benchmarked using various gradient lengths and input amounts of a triple proteome mix (human, yeast, E. coli).

The AI-based CHIMERYS search engine was compared to a classical search engine (MS Amanda 2.0). Different isolation widths up to 56 m/z were tested to assess the optimal settings for further data analysis with CHIMERYS to fully utilize its resolving power for highly chimeric MS2 spectra. Low input and single cell samples were analyzed using narrow (1 m/z) and wide (12 m/z) isolation widths to assess the benefits of wide-window acquisition over typical narrow DDA windows. The entire optimized workflow comprising µPAC, WWA and CHIMERYS was benchmarked against a classical analytical workflow by analyzing a affinity purification experiment.

Results

Our data show that µPAC allow to identify up to 50% more peptides and 24% more proteins, while offering improved throughput, which is critical for single cell proteomics. Combining wide precursor isolation widths of 4-12 m/z with the CHIMERYS search engine identified 51-74% more proteins and 59-150% more peptides for single cell, co-immunoprecipitation, and multi-species samples in comparison to a standard proteomics workflow at a well-controlled false discovery rate. As opposed to a conventional workflow, our entire optimized platform discovered 141% more proteins for a protein-protein interaction study on the chromatin remodeler Smarca5/Snf2h, resulting in 92% more potential interactors. These include previously described Smarca5 binding partners and undescribed ones including Arid1a, which is also involved in chromatin remodeling with a key role in neurodevelopmental and malignant disorders.

P120. Large-scale phosphoproteomics reveals divergent kinase-activity profiles in different groups of mouse medulloblastoma

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Medulloblastoma (MB) is the most malignant brain tumor in children. Despite the significant clinical progresses of the last decades, the long-term outlook and survival of patients remain very poor. To date, four main groups of MB have been recognized (wingless (WNT)-activated, sonic hedgehog (SHH)-activated, group 3 and group 4) and have been further subclassified into 7 to 12 different subtypes. While the four main MB groups have been extensively characterized at both genome and transcriptome level, the proteome remain to be comprehensively explored. In particular, phosphoproteomics can provide insight on active oncogenic signalling that could help to elucidate specifically activated and therapeutically actionable pathways. Quantitative phosphoproteomics analysis has already described distinct post-translational profiles in specific groups of human MB. In our experiment, we aim at studying how the phosphoproteome changes in different preclinical mouse models for the different subgroups of MB in order to define key regulators of the acquisition of highly malignant features. We characterized the phosphoproteome of three subtypes of MB murine models: Ptch1+/-p53+/+ MBs (henceforth referred to as HetWT, representing a subgroup of SHH-activated group); Ptch1+/-p53-/- MBs (indicated as HetNULL, mimicking the high-risk subgroup of SHH-activated group) and Myc amplified/p53 mutant MBs (referred as MYC, corresponding to the group 3 of MB). Due to the limited amount of tissue available, we developed a sample preparation protocol based on two phospho-enrichment strategies (MOAC, Metal oxide affinity-chromatography and IMAC, Immobilized metal affinity-chromatography) that enabled us to use only 1 mg of proteins without any need of labeling. By this protocol, we were able to identify and quantify 4570 phosphosites in the three studied groups. In combination with a recently-developed R package (called PhosR), we were also able to predict the activity of the kinases responsible for the phosphosites dysregulated in the different groups of MB. Our study describes distinctive protein phosphorylation landscapes of different MB groups that could be helpful in clinical decision-making and treatment.

P121. Deep Plasma Proteome Landscape of Alzheimer's Disease: An 1800-Sample Cohort Study

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Introduction

The mechanisms and biomarkers of Alzheimer's Disease (AD) development and progression are still not fully understood. However, recent advances in proteomics have made it possible to study protein level changes in biofluids, such as serum and plasma for early detection of AD. The wide dynamic range of protein concentrations in plasma can make it difficult to analyze all proteins simultaneously, so researchers often need to make trade-offs between protein coverage and analysis throughput.

Methods

Here we have used the ProteographTM Product Suite (Seer, Inc.) in combination with the liquid-chromatography coupled to Data Independent Acquisition mass-spectrometry analysis (DIA LC-MS) to study approximately 1,800 samples from individuals representing healthy controls, AD-affected group and a group affected by other neurodegenerative disease, and some longitudinal samples from the same individuals. The plasma samples were randomized according to their status. Samples are processed with the Proteograph Product Suite and the resulting peptides were analyzed with a Data DIA LC-MS method on a Thermo Fisher Scientific Orbitrap Exploris 480 MS.

Results

The DIA LC-MS data was acquired on 1,790 samples with several samples not being acquired due to insufficient plasma volume. We investigated several DIA spectral library strategies including library-free analysis with match between runs and a project-specific gas-phase fractionation (GPF) library. Across the 1,790 samples we find 5,253 protein groups using the library-free search and 4,007 using the GPF library at false discovery rate of 1%. Our preliminary analysis shows a strong biological signal demonstrated the statistical power of the data to classify individuals. Using 10-fold cross validation and a customized autoML (Seer, Inc.) framework, we can separate controls from AD-affected (area under the receiver-operator curve, AUC-ROC 0.624), controls from MCI-affected (AUC-ROC 0.655), and controls from Dementia-affected (AUC-ROC 0.779). We are at early stage of data analysis, and we are already seeing the potential of this approach to deepen our understanding of AD. In the coming months, we plan to conduct more analysis to better understand the trajectory of the changes over time using the longitudinal data in this dataset as well as the biological pathways underpinning the differences that we observe."

P122. Global phospho-proteomics analysis of EGFRi drug tolerance and resistance mechanisms in cell-based models of non-small cell lung cancer

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Introduction

Lung cancer is the leading cause of cancer mortality in the world, a key target for pharmacological intervention. A significant percentage of tumours belonging to the non-small cell lung cancer (NSCLC) subtype possess 'activating' genetic mutations in the Epidermal Growth Factor Receptor (EGFR) gene, which make it susceptible to small-molecule EGFR inhibitor treatment. Continued development of EGFR inhibitors is necessitated by the development of cellular resistance mechanisms which allow tumours to escape the effects of EGFR inhibition. The third-generation covalent inhibitor osimertinib (Tagrisso ®) was designed to target the common T790M 'gatekeeper' EGFR mutation. This drug is now first-line treatment for EGFR-mutant NSCLC, but is also susceptible to tumour resistance.

Methods

Our aim was to investigate both acute and longer-term adaptation mechanisms in osimertinib-exposed cells with a global quantitative proteomics and phospho-proteomics analysis. The cell-model was the osimertinib-sensitive PC9 lung adenocarcinoma cell line (EGFR Exon 19del activating mutation). We employed a TMT-based quantification strategy for total proteome and TiO2 phospho-enriched samples of acute response (6 timepoints, 0-24h) and long-term treatment (up to 70 days of continuous, fixed-dose drug treatment). Analysis was performed with nano-LC coupled to FAIMS interface and MS/MS analysis at 60.000/50.000 resolution (200 m/z) on an Orbitrap Fusion Lumos. MS data analysis was performed with Mascot (version 2.7), phospho-site localisation with ptmRS in Proteome Discoverer 2.4.

Results

Quantification of EGFR autophosphorylation confirmed persistent blockade of the receptor during the course of treatment. Key changes in the phosphorylation profile during the first 24h of treatment were observed in components of the Rho-GTPase cycle and proteins involved in cell organisation, apoptosis, cell cycle and transcriptional control. These changes in phosphorylation dynamics were accompanied by corresponding changes in cell morphology, growth and attachment. Interestingly, components of the sumoylation pathway were also found to be differentially phosphorylated, leading us to confirm changes in the sumoylation profile in osimertinib resistance. Protein-level changes, starting at 4h post-treatment, showed metabolic remodelling and differences in ECM-interactions and integrin signalling. Regulation of phosphorylation and protein levels in calcium signalling components indicates that osimertinib-tolerance in PC9 cells is calcium-dependent.

This longitudinal study enabled us to discover a signature of adaptive changes occurring during early response that were consolidated into long-term differentiation between parental and drug-tolerant cells, conferring a survival advantage. In addition to confirming previously observed resistance mechanisms, including upregulation of receptor tyrosine kinases, we are investigating the role of potential novel pharmacological targets in osimertinib resistance.

P123. Distinct phosphorylation patterns induced by stimulation of mesothelial cells with high glucose containing PD fluid vs. heat stress induction

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Peritoneal dialysis (PD) is a life-saving renal replacement therapy, where the peritoneum is used as a semi-permeable membrane to remove water and uremic toxins from patients by a glucose-based PD-fluid. Exposure to PD-fluid triggers changes in peritoneal mesothelial cells but has recently been shown to impair cellular stress response (CSR) mechanisms, such as the highly conserved heat shock response. Glucose degradation products (GDPs) in the PD-fluids not only lead to diabetes-like vasculopathy but are also speculated to block critical elements of the CSR which is highly regulated via PTMs including phosphorylation. This study aims to identify system-wide protein phosphorylation impairing the CSR leading to deterioration of the peritoneal membrane and poor clinical outcome of PD.

Human mesothelial cells exposed to heat (42°C, positive control), PD-fluids or individual GDPs were analyzed with a quantitative (phospho)proteomics approach. Single-pot solid-phase-enhanced sample preparation (SP3) was used for digestion (300 µg) and 68 samples were labeled with isobaric tags (TMTpro 18-plex). Phosphopeptides were enriched in a sequential approach, first using TiO2, followed by FeNTA enrichment of the flow-through. The two phospho-peptide enriched eluates were combined and samples were high-pH RP offline-fractionated (12 fractions for phospho-peptides, 36 fractions for total proteome analysis). Both sample types were analyzed by nano-LC coupled to an Orbitrap Exploris 480 with FAIMSpro. An internal pooled standard consisting of equal parts of all samples was used to link multiple TMT-runs. Database search and quantification were performed using Proteome Discoverer.

The approach enabled identification of >10,100 proteins (>9,000 with abundance in all runs), 46,848 phospho-peptides and 38,336 unique phospho-sites in the phospho-enriched samples. Of the detected phospho-sites 83% were located on serine residues, 16% on threonines and 1% on tyrosines. Less than 1% of phospho-peptides were found in the total proteome and over 70% after enrichment. The analysis of the total proteome showed a shift to biological processes related to chaperone activity and stress responses in heat but not in PD-fluid conditions. The quantitative phosphoproteomics approach revealed differentially de/activated players in the HSF-1 activation pathway and its effector kinases depending on the presence of GDPs.

Taken together, this approach enables high coverage of the total and phosphoproteome from the same sample and thereby enabling analysis of relevant processes of the mesothelial reaction to PD-fluid induced stress. New insights into the regulation of stressor-specific induction and dysregulation of the CSR can lead to identification of new therapeutic targets and potentially improve PD-therapy.

P124. A complete and automated end-to-end sample preparation strategy for high-throughput and standardized proteomics with high sensitivity

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Introduction

Proteomics is going through a significant paradigm shift towards higher throughput and robust user-friendly technologies. The use of short LC gradients combined with data-independent acquisition (DIA) has been a key aspect in this development. The Evosep One is designed for high-throughput applications, and standardized methods with increasing robustness that enable routine analysis of hundreds of proteomes per instrument per day. This has shifted the bottleneck for realizing high-throughput proteomics towards sample preparation creating a need for rapid, robust, and reproducible end-to-end automated workflows. Here, we combine our fully automated loading strategy for Evotips with fully automated sample handling functionalities in a modular setup with protein aggregation capture (PAC) assisted digestion in an end-to-end standardized workflow.

Methods

HeLa cells were harvested in boiling 5% sodium dodecyl sulfate (SDS) buffer. The automated sample preparation workflow was implemented on an Opentrons OT-2 liquid handling robot utilizing PAC on magnetic microparticles, followed by 4h on-bead trypsin digestion, and automatic loading of the resulting peptides onto Evotips using a layered sandwich approach with defined airgaps. The layers are pushed through Evotips with the OT-2 pipette for 100 seconds leaving them ready for injection on the Evosep One. The control software for the automated sample handling protocol has been converted into an easy-to-use HTML format, generating a complete python script for loading in the Opentrons app.

Results

Here, we present an automated end-to-end workflow for proteomics sample preparation of up to 192 protein lysates that results in purified tryptic peptides on Evotips ready for LC-MS analysis.

The protocol is optimized for speed and simplicity, resulting in a complete 'one-touch' protocol for digestion and Evotip loading. The reproducibility and digestion of the protocol is comparable to protocols using overnight digestion at 37 °C. Moreover, the protocol exhibits high sensitivity and can efficiently digest protein amounts down to nanogram of input material.

As peptides are loaded directly onto Evotips, the sample can be stored for up to several weeks before LC/MS analysis with no further preparation required. This bypasses the need for sample evaporation and resuspension of the sample, which improves cost efficiency and reproducibility.

The workflow was benchmarked using the Evosep One in combination with DIA workflows on high-end mass spectrometers. The Evosep One standard methods were used to benchmark performance with a throughput of up to 500 samples per day, whereas the Whisper methods were used for input amounts down to 1 ng.

P125. The Edge Effect in High Throughput Proteomics: A Cautionary Tale

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Introduction

The throughput required for large cohort clinical validation in proteomics biomarker discovery and diagnostic screening has driven the growth of multiplexed targeted liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) assays paired with sample preparation and analysis in multiwell plates. However, large scale MS-based proteomics studies are often plagued by batch effects: sources of technical variation in the data which can confound biological signals and compromise data quality. Here we present our recently published findings on an intra-plate batch effect termed the edge effect arising from temperature gradients in multiwell plates, which had not yet been reported in a bottom-up proteomics setting.(1)

Methods

Plasma was collected from healthy donors with informed consent and prepared following the bottom-up plasma proteomics protocol described by Mbasu et al. with volumes adjusted for use in multiwell plates.(2) The extent of the edge effect was assessed for a variety of multiwell plates, plate sealing techniques, and plate heating techniques. Peptide intensities across the plate were assessed for 46 human peptides by LC-MS/MS analysis described in Maxwell et al. Thermal images were obtained of the plates after each heating step to measure temperature gradients using an FLIR SC600 series infrared (IR) camera (Teledyne FLIR LLC, Kent, UK).

Results

We show that the edge effect, extensively reported in preclinical cell culture studies, is also a potential pitfall of using multiwell plates in LC-MS/MS based bottom-up proteomics. For deep well plates heated in an oven incubator, total peak areas of the 46 peptides exhibited unacceptably high intra-plate variation with an average relative standard deviation (RSD) across the 96 wells for all peptides of 38.7%. An assessment of plate sealing and heating techniques coupled with thermal imaging of the plates confirmed that temperature gradients during the reduction and tryptic digestion steps were causing variable peptide abundances, with particularly high variation in the edge and corner wells. The use of PCR-style plates and heaters helped to ameliorate the edge effect with additional incorporation of surrogate standards spiked into the sample prior to digestion to normalise for digestion inefficiencies further reducing RSD to < 5%. We propose that all laboratories performing high throughput bottom-up proteomics carefully consider conditions for heating multiwell plates (and indeed other vessels) and call for vendors to address the demand for plates and heaters which have been assessed for even heating.

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P126. Conformational investigation of charge heterogeneity through native analysis of monoclonal antibody fragments using High-Resolution Cyclic IM-MS

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Introduction

Charge variant analysis is widely performed to assess changes in product quality during the manufacture of monoclonal antibodies (mAbs) and can be achieved using techniques such as capillary isoelectric focusing (cIEF), cation exchange chromatography (CEX) and mass spectrometry (MS). As large therapeutic biomolecules (~150kDa), mAbs can be challenging to characterise due to their heterogeneity, which can result from post-translational modifications and alternative disulphide bond connectivities, which alter higher order structure. Coupling of ion mobility to high resolution native mass spectrometry (IM-MS) presents a powerful tool for conformational characterisation of intact mAbs, mAb subunits and associated fragments. In this work, Cyclic IM-MS (cIM-MS) technology was used to compare the ion mobility behaviour and conformation of F(ab')2 fragments from IgG1 preparations which had exhibited heterogeneity during charge variant analysis.

Methods

IgG1 samples were separated and fractionated into lower acidic and higher acidic species using CEX chromatography. Enriched fractions were cleaved using FabRICATOR protease (Genovis AB, Sweden) below the hinge region to generate F(ab')2 and Fc/2 fragments. F(ab')2 fragments were isolated by subtractive affinity purification with Protein A magnetic beads (Promega, UK) and were desalted and buffer exchanged into 100 mM ammonium acetate, pH 6.8 using Zeba™ Spin Desalting Columns 7K MWCO, 0.5ml, (ThermoScientific, UK). Native MS and IM-MS experiments were performed using off-line nanoflow-ESI delivery to directly infuse the desalted samples onto a SELECT SERIESTM Cyclic IMS (Waters Corporation, UK), with subsequent analysis of the data within MassLynxTM v4.2.

Results

Comparative native MS analysis of F(ab)'2 samples showed that higher, less protonated m/z species were detected for the higher acidic F(ab)'2 sample, whilst lower, more protonated m/z species were observed with the lower acidic F(ab)'2 sample. In principle, the degree of protonation was consistent with the charge characteristics that the samples exhibited during prior CEX analysis.

Examination of ion mobility peaks for given charge states within the native MS spectra demonstrated that the higher acidic F(ab)'2 sample had faster drift times, reflecting a more compact conformation in the gas phase. Conversely the lower acidic F(ab)'2 species had slower drift times, indicative of a less compact conformation. These results demonstrate the capability of cIM-MS to resolve mAb fragment conformers, providing a novel analytical tool for investigation of structural differences relating to charge heterogeneity of mAbs.

P127. MALDI-MSI of breast cancers reveals intratumoral heterogeneity induced by the tumor microenvironment.

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Breast cancer (BC) is a global health issue, affecting a high proportion of the female population. Spatial proteomics enables studying the proteome within tissue context, therefore offering a promising approach to unravel the biological processes contributing to heterogeneity within BC tissue that leads to ineffective therapies. The combination of MALDI imaging (MSI) with LC-MS/MS may aid the identification of diagnostic and prognostic biomarkers so far masked by tissue heterogeneity and diversity in the tumor microenvironment. Despite the progress of MSI, a tissue specific systematic optimization of sample preparation and analysis is needed to increase molecular information. Here, we established an optimized protocol for sample preparation of gelatin and paraffin-embedded BC tissue.

Human BC cell lines (MCF-7, SKBR-3, MDA-MB-231) were inoculated into female athymic Balb/c-nude mice. Excised tumors (5-10mm) were embedded in gelatin, paraffin or stored in liquid nitrogen. The parameters optimized regarding the impact on the resulting signal-to-noise (S/N) ratio were as follows: (i) thickness of the section; (ii) digestion enzyme, solvents, deposition method, incubation time, incubation temperature; (iii) matrix concentration, solvents, deposition method; (iv) internal standard selection, concentration, deposition method; (v) MSI measurement parameters. Following MSI, samples were stained for standard histological evaluation. Proteins were identified by LC-MS/MS from snap frozen homogenized tissue and subsequent matching to peptide data from MALDI-MSI.

We compared the results of different pre-analytical and analytical parameters for the identification of peptides and their spatial localization in BC xenografts. After the MSI protocol establishment, peptide signals were analyzed by image segmentation, principal component analysis (PCA) and uniform manifold approximation and projection (U-MAP) plots. Segmentation maps based on MSI were in agreement with expert-based histological assessment. Unsupervised PCA and U-MAP plots allowed visualization of the multidimensional information and enabled segmentation of different tumor, stroma and necrotic tissue regions. Molecular BC subtypes were clearly distinguishable by MSI. LC-MS/MS analysis identified ~7000 proteins based on ~150000 peptides in SKBR-3 and MDA-MB-231 tumors, providing a rich source for charge-state adjusted matching of peptide signals from MSI.

The developed protocol for MSI enables higher sensitivity and reproducibility of BC tissue analysis. Classification of complex cancer tissues by MSI is feasible and opens new avenues for specific in-situ biomarker identification and analysis. Using the established workflow, peptide signatures not only differentiate histologically confirmed tumor regions, but also reveal sub-regions which are not distinguishable by standard methods, demonstrating the potential of MSI as a tool for translational BC research.

P128. Mass spectrometry analysis of paraffin-embedded tissue samples from CRC patients revealed novel key proteins for the study of the disease

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide. A better understanding of the biology of CRC would help to identify specific markers of the disease that could be used as diagnostic and/or prognostic biomarkers or become potential targets of intervention. Our objective is to analyze the differential protein expression of FFPE tissue of colorectal adenocarcinoma and adenoma in comparison to the surrounded healthy adjacent tissues by quantitative proteomics to identify dysregulated proteins at the very early stages of the disease.

Methods

Two independent quantitative 10-plex tandem mass tags (TMT) experiments were separately performed using paired FFPE tissue samples from six different sporadic CRC patients. After protein extraction, trypsin digestion, and peptide labeling, proteins were identified and quantified by mass spectrometry using a Q Exactive. Subsequent data analysis and bioinformatics were performed with MaxQuant and the R program to identify proteins differentially expressed in the adenoma-to-adenocarcinoma transition. The dysregulation of candidate proteins was confirmed by WB and immunohistochemistry, and their role in CRC was analyzed by ELISA and in vitro and in vivo functional assays.

Results

More than 2500 proteins were identified and quantified, with 277 and 283 significantly upregulated or downregulated proteins (expression ratio ≥1.5 or ≤0.67, p-value ≤ 0.05), respectively, in adenoma and/or adenocarcinoma in comparison to healthy tissue. Following bioinformatics analysis, we selected 10 altered proteins to study their role in CRC by western blot, immunohistochemistry, tissue microarrays, and ELISA using tissue and plasma samples from CRC patients, individuals with premalignant lesions (low- and high-grade colorectal adenomas), and healthy individuals (controls). Additionally, loss-of-function in vitro and in vivo assays were performed using three CRC cell lines with different metastatic properties to assess their role in CRC progression and metastasis.

Conclusion

We found two proteins upregulated at early stages of CRC development, associated to CRC survival and liver metastasis, with high diagnostic potential as blood-based biomarkers of the disease.

P129. An Anticancer Rhenium Tricarbonyl Targets Fe–S Cluster Biogenesis in Ovarian Cancer Cells

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Target identification remains a critical challenge in inorganic drug discovery to deconvolute potential polypharmacology. Herein, we describe an improved approach to prioritize candidate protein targets based on a combination of dose-dependent chemoproteomics and treatment effects in living cancer cells for the rhenium tricarbonyl compound TRIP. Chemoproteomics revealed 89 distinct dose-dependent targets with concentrations of competitive saturation between 0.1 and 32 µM despite the broad proteotoxic effects of TRIP. Target-response networks revealed two highly probable targets of which the Fe–S cluster biogenesis factor NUBP2 was competitively saturated by free TRIP at nanomolar concentrations. Importantly, TRIP treatment led to a down-regulation of Fe–S cluster containing proteins and upregulated ferritin. Fe–S cluster depletion was further verified by assessing mitochondrial bioenergetics. Consequently, TRIP emerges as a first-in-class modulator of the scaffold protein NUBP2, which disturbs Fe–S cluster biogenesis at sub-cytotoxic concentrations in ovarian cancer cells.

P130. A Quantitative Proteomics Approach to Interferon Signal Transduction – Bridging from the Receptor to the Intracellular Level

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Interferon alpha (IFNα) is a key component of the innate immune system. Upon binding to two cell surface receptors, IFNAR1 and IFNAR2, IFNα triggers the activation of an intracellular network that results in the induction of interferon-stimulated proteins. Among these are multiple proteins that contribute to the elimination of a viral infection. Our mathematical modeling approach demonstrated the importance of reactions at the receptor level to regulated the dynamics of antiviral responses. However, the necessary tools to reliably assess the IFNα-receptors have been missing and the dynamics of the induction of antiviral proteins has not been characterized.
For the absolute quantification of IFNAR1 and IFNAR2, a targeted mass spectrometry-based approach using parallel reaction monitoring (PRM) was developed achieving limits of quantification in the lower attomolar range for two peptides per receptor subunit and basal amounts of IFNAR1 and IFNAR2 in the hepatoma cell line HepG2 were determined. Surprisingly, we noted a significant discrepancy between the molar amounts of peptides from the same receptor subunit suggesting the presence of multiple protein isoforms, unknown post-translation modifications, or proteolytic cleavage. To resolve this conundrum, the PRM assay was combined with strong cation exchange pre-fractionation, thereby increasing sequence coverage. Of key interest will be to identify the peptides that are informative about the amount of functional receptor at the cell surface and those that allow to distinguish different isoforms.

To monitor the interferon-induced protein dynamics at a global scale, we employed data independent acquisition and obtained time- and dose-resolved data after IFNα stimulation of HepG2 cells. Overall, we quantified over 7500 proteins without missing values with low standard deviation across replicates. To systematically explore the comprehensive time-resolved dataset, a novel data analysis pipeline was developed that combines a two-way ANOVA considering time and stimulus as factors, along with effect size determination based on the difference in the area under the curves between two conditions. This approach confirmed the induction of a multitude of known interferon-stimulated protein and led to the discovery of novel candidates.

Finally, we integrated the uncovered interferon-stimulated proteins into our mathematical model describing the regulation of the IFNα signal transduction pathway in hepatoma cells. This in silico linkage provides a valuable tool for fine-tuning the timing (tmax) and maximum concentration (cmax) of individual interferon-stimulated proteins through personalized IFNs dosing regimens tailored to the specific disease type and patient.

P131. Poor Quantitative Concordance of Proteomic Data between Autopsy Samples and Fresh Frozen Samples from Human Coronary Arteries

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Introduction
Comprehensive mapping initiatives such as the Human Protein Atlas project have not incorporated large blood vessels, and proteomic analyses of human coronary plaques have been either limited in scope or conducted using autopsy samples. No comparison of proteomic data from autopsy samples and fresh frozen samples has been made, nor has there been an evaluation of the extent to which the post-mortem interval (PMI) impacts protein quantification.

Methods
Autopsy samples were collected from the left anterior descending (LAD) coronary artery of deceased patients (n=47), while fresh samples were obtained from patients undergoing coronary artery bypass graft surgery (n=65). For proteomics, protein extracts were deglycosylated before analysis by nanoflow liquid chromatography tandem mass spectrometry on an Orbitrap Q Exactive HF MS. Proteomics data were processed with Mascot, using precursor intensity for quantitation. DAVID software was used to perform pathway and Gene Ontology annotation enrichment analyses. Protein networks were constructed utilizing our proprietary DiREC-AP method.

Results
Approximately 40% of the protein abundances demonstrated a statistically significant correlation with PMI, with the preponderance of these relationships being inverse. This was particularly noticeable for smooth muscle cell markers, which exhibited the most substantial reduction with increased PMI. Conversely, positive correlations with PMI were observed for immunoglobulins, coagulation and complement factors. The observed variations in protein abundances were linear within a PMI ranging from 5 to 70 hours. When contrasting post-mortem and fresh specimens, concordance in protein identification was less than 50%, with a significant reduction in the identification of extracellular matrix proteins in post-mortem samples. Comparative analyses based on sex between these two sample types did not yield any correlation. However, a robust correlation was observed within a sex comparison conducted between two cohorts consisting entirely of fresh samples. Similarly, the correlation was weak when comparing LAD autopsy samples with a previously published dataset of autopsy LAD samples. The examination of extracellular proteins identified in the post-mortem LAD samples unveiled a highly interconnected network, chiefly composed of major serum proteins (42%) and other secreted factors (25%). An autopsy-specific subnetwork highlighted the presence of platelet-specific proteins and complement factors in LAD samples.

Conclusion
This is, to our knowledge, the first proteomics study to examine the impact of the PMI on the protein composition within blood vessels. Our findings highlight potential inconsistencies in the quantitative precision of proteomics data from autopsy samples. Thus, results from post-mortem specimens may not be reliably replicated in fresh-frozen samples.

P132. Ubiquitinome Profiling: Optimizing the enrichment and Data-Independent Analysis for Deeper Insights into Targeted Protein Degradation

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Targeted protein degradation has emerged as a highly promising therapeutic strategy for a wide range of diseases, including cancer and neurodegenerative disorders. The ubiquitin-proteasome system, which is responsible for protein degradation, plays a critical role in this process. Gaining comprehensive insights into the landscape of ubiquitination events is essential for the development of effective targeted protein degradation approaches. In recent years, data-independent analysis (DIA) has gained significant popularity as a robust and unbiased approach for quantitative proteomics. By leveraging on this method, we have developed a cutting-edge workflow that combines diGly antibody-based enrichment with an optimized Orbitrap-based data-independent acquisition method for the identification of ubiquitylated peptides. Through the implementation of our workflow, we have identified over 40,000 diGly peptides corresponding to more than 7,000 proteins in a single measurement from proteasome inhibitor-treated cells, highlighting an exceptional throughput and efficiency. We have applied our optimized workflow to identify ubiquitination sites on substrate proteins targeted for degradation, helping establish mode of action for target protein degradation. In conclusion, we are confident that our workflow holds tremendous potential for rapidly establishing mode of action for PROTAC- and molecular glue-mediated target protein degradation. By precisely and efficiently profiling ubiquitin sites on target protein, our approach facilitates a comprehensive understanding of the mechanisms of how small molecular degraders work.

P133. Evaluation of Peptide Profiles of OipA Gene On and Off Status on Ulcer Tissues by MALDI Mass Spectrometry Imaging

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Introduction

Outer membrane virulence factors are important for Helicobacter Pylori (H. pylori) to attach to the gastric epithelial surface and cause infection. Outer membrane inflammatory protein A (OipA) H. pylori's attachment to the gastric epithelial surface and its involvement in inflammation have been demonstrated by studies on ulcer, gastritis, gastric cancer. Investigation of protein profiles caused by H. pylori, which is the cause of infections, is important because these proteins have the potential to be biomarkers that can be used to diagnose the infection and determine its prognosis. Formalin-fixed paraffin-embedded (FFPE) tissues are valuable in proteomic studies as they allow retrospective studies in relation to patient data. One of the proteomics techniques, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) combines histological information with high resolution and accuracy and determines the spatial distributions of analytes on tissue. The aim of this study is to evaluate the peptide profiles of FFPE ulcer tissues in which H. pylori OipA gene is on and off status by MALDI-MSI.

Methods

Sections of 3 µm thickness from FFPE ulcer tissues were taken on indium- tin-oxide coated slides by microtome. The slides were washed with xylene and decreasing alcohol concentrations. Antigen retrieval was performed with 10 mM Citrate buffer (pH 6) solution. 100 ng/µl trypsin enzyme was used to digestion proteins into peptides and then coated with 7 mg/ml α-CHCA matrix in 70% acetonitrile and 1% trifluoroacetic acid. Mass spectra were acquired in positive ion mode (m/z 900-3500) using Rapiflex MALDI Tissue Typer (Bruker Daltonics, Germany). The number of monoisotopic peptides, peptide localizations associated with histological and hierarchical clustering analyzes were used to evaluate the results.

Results

The peptides obtained as a result of the analysis of ulcer tissues in which the H. pylori OipA gene is on and off status were evaluated in terms of peak numbers and signal intensities. The highest number of peptides were obtained from tissues with H. pylori OipA gene on group. However, S/N ratios and relative intensity values of 3 peptides (m/z 933.8, m/z 1044.3 ve m/z 1144.6) with the highest signal intensity were higher in tissues with the H. pylori OipA gene on group. Peptide identifications were analyzed by LC-MS/MS method and 450 proteins were identified. Proteins identified according to the results of MALDI-MSI and LC-MS/MS analysis were matched.

Conclusion

This is, to our knowledge, the first proteomics study to examine the impact of the PMI on the protein composition within blood vessels. Our findings highlight potential inconsistencies in the quantitative precision of proteomics data from autopsy samples. Thus, results from post-mortem specimens may not be reliably replicated in fresh-frozen samples.

P134. Oktoberfest: A search engine and prediction model agnostic rescoring pipeline

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Introduction
Machine learning (ML) and deep learning (DL) models such as Prosit for peptide properties prediction have enabled the creation of high quality in silico spectral libraries. These libraries are used in various applications, ranging from DIA data analysis to data-driven rescoring of search engine results. We present Oktoberfest, an open source Python package of our spectral library generation and rescoring pipeline originally only available online via ProteomicsDB. Oktoberfest is search engine and prediction model agnostic, promoting the adoption of state-of-the-art ML/DL models in proteomics analysis pipelines. We demonstrate the impact of Oktoberfest on diverse use cases, covering different biologies, organisms, proteases, fragmentation methods, mass analyzers, search engines and prediction models.

Methods
Rescoring relies on initially searching raw mass spectrometry data without applying a false discovery rate filter. For each proposed peptide spectrum match, fragment ion intensities and retention time is predicted and compared to experimentally acquired data. Various intensity-based similarity scores are calculated to distinguish correct from incorrect matches using Percolator or Mokapot. Oktoberfest retrieves predictions from a publicly hosted Koina instance, our new online prediction service delivering Prosit, DeepLC, MS2PIP and AlphaPeptDeep predictions.

Results
Oktoberfest excels when analyzing big search spaces, challenging datasets or high dynamic range samples, such as immunopeptidomics data, full proteome analysis using non-tryptic proteases and metaproteomics data. It confidently identifies a substantially higher number of peptides than classic search engines even if they fail to identify any peptides. We show the benefits of Oktoberfest-based rescoring for datasets obtained from different fragmentation methods, mass analyzers, search engines and rescoring workflows. Across all use cases, Oktoberfest increases peptide coverage by an average of 50% (up to 700%) and protein coverage by an average of 25% (up to 100%).
Additionally, Oktoberfest was applied on data from the "Proteomes that Feed the World" project, mapping the proteomes of the 100 crop plants most important for human nutrition. Reanalysis using Oktoberfest increased peptide coverage by 40% and protein coverage by 25% across 18 tissues for a draft tomato and quinoa proteome.
Oktoberfest is fast and scalable, rescoring 8 raw files on 8 cores with 2.6M PSMs in 59 minutes. It supports all major platforms and is extensively tested using continuous integration / deployment standards. Leveraging Koina reduces hardware requirements and enables users and developers to utilize any combination of prediction models for spectral library generation and rescoring and systematically test and integrate new models into this existing pipeline.

P135. Fractionation methods for subcellular spatial proteomics: a direct comparison of resolution and reproducibility.

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Introduction

Compartmentalisation of eukaryotic cells into organelles allows separate biochemical processes to occur simultaneously. Subcellular localisation of a protein therefore influences its function by defining both the surrounding physical conditions and the biomolecules available for interaction. Movement of proteins between organelles is essential for normal cellular activity, allowing multiple roles to be fulfilled in different environments, and mislocalisation is linked with an increasingly wide range of diseases. Optimisation of techniques for profiling the spatial proteome is imperative, to gain new insight into both normal cellular function and responses to perturbation, especially in cases where overall protein abundance stays constant but localisation changes. Significant recent advancements include the hyperLOPIT and LOPIT-DC protocols, which utilise either density or differential ultracentrifugation fractionation, before isotope tagging, mass spectrometry and correlation profiling based analysis, to simultaneously map the subcellular localisation of thousands of proteins in a single experiment. Here we compare these established LOPIT fractionation methods to a detergent-based fractionation approach within the same experimental pipeline, for a direct comparison of resolution and reproducibility.

Methods

The experimental workflow includes fractionation of the cell to give distinct organellar distributions, then reduction, alkylation and trypsinisation to linearise proteins and generate peptides before TMT labelling, UPLC and LC-SPS-MS3. Proteome discoverer was used to process the raw data through a database search and the pRoloc package was used for protein localisation prediction using a Bayesian framework and t-SNE visualisation. Two fractionation methods were trialled separately on U2OS cells: differential ultracentrifugation based upon sedimentation rate and sequential detergent addition based upon membrane solubility.

Results

Preliminary data suggests that the reproducibility of each fractionation method is comparable. Initial analysis of the resolution suggests that LOPIT-DC is more effective at distinguishing proteins from key secretory pathway organelles including the golgi and ER, and both methodologies seem effective at resolving mitochondrial markers. Both LOPIT-DC and detergent-based fractionation take similar amounts of time to perform, with comparable levels of technical difficulty. Since fewer fractions are generated by the detergent method, a lower number of initial cells can be used, if required.

P136. Targeted Detection of Protein Complexes by Mini-Complexome Profiling (mCP)

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Living organisms are sophisticated machinery that carries out intricate procedures to alter their surroundings, translate, and maintain homeostasis. On the molecular level, the majority of cellular functions, including energy generation, cell division, and replication, rely on the actions of protein complexes (PPCs). During the last decade, the co-fractionation mass spectrometry experiment was an emerging method to detect PPCs from a system biology perspective. Much effort has been made to detect PPCs in a system biology approach1, which usually includes big datasets from a thousand fractions2 to 81 fractions up to 546 PPCs detected in model cells like Hek2933.

Here we present a novel and systematic workflow, called mCP, for wide targeted detection of PPCs. It includes mild extraction of PPCs, fractionation by mini-Blue Native PAGE, in-gel digestion, and Mass spectrometry Data Independent Acquisition detection. Mass spectrometry measurements were done in a high-throughput mode of about 70 samples per day. Data analysis was done by DIANN software. PPCs detection was done using bioinformatics targeted analysis by ourmCP custom-developed R package with a controlled False Discovery Rate (FDR) approach based on Monte-Carlo simulation.

We applied our protocol as a proof of principle on an established cell type for PPCs detection, Hek293 cells. About 7489 protein groups and 362 annotated PPCs were detected. Then, we challenged mCP by investigating changes in the complexome of different heart compartments in isolated cardiomyocytes from a single 12 weeks mouse. A total of about 5900 protein groups and around 30 annotated PPCs per compartment were detected. The resulting data provides a comparison of PPCs coming from heart compartments of a single experimental unit for the first time. Taken together, a considerable reduction of measurement time was achieved in comparison to traditional approaches3, i.e. 1day per cell-type vs to 2 months from previous studies3. A successful mini complexome profiling, with only 35 fractions and controlled FDR detection, was achieved. The reduced material requirements open the possibility of applying this workflow to sparse/rare samples, i.e. patient biopsies.

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P137. Comparison of Helicobacter pylori G27 and G27-ΔoipA Infected Gastric Organoid Model Protein Profile; Understanding the Effect Of Oipa Protein On *Helicobacter pylori* Infection

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Introduction

Helicobacter pylori (*H. pylori*) is a pathogenic bacterium, that infects about half of the world's population and causes inflammation in the stomach. H. pylori infection is a significant risk for gastric diseases such as chronic gastritis, peptic ulcer, MALT, and gastric adenocarcinoma. A crucial step in *H. pylori* infection is the attachment of bacteria to the stomach epithelial cells, which is facilitated by outer membrane proteins. Among these proteins, Outer membrane inflammatory protein A (OipA) is a virulence factor of *H.pylori* which assists the bacterial colonization on the stomach and is closely related to the bacterial infection. However, our knowledge of the effects of this protein on host cells is insufficient. The current study focuses on the role of OipA in *H. pylori* pathogenesis by post-infection proteomic analysis of human gastric organoid model infected with *H. pylori* strains; wild type G27 and mutated G27-ΔoipA.

Methods

The gastric organoid model was developed by using *H. pylori*-free control patient's corpus tissue. The constructed gastric organoid model was infected with *H. pylori* G27 and G27-ΔoipA strain for 8 hours. The proteins were isolated from infected organoid cultures and uninfected control wells. Isolated protein samples were identified by LC-MS/MS analysis. The amounts of identified proteins in the sample were calculated using the label-free quantification (LFQ) method. The protein amount differences between groups were analysed by the ratio of infection samples to control, fold differences were determined (more or less than 1.3 fold) and pathway enrichment analysis was conducted for these proteins.

Results

The 1921 proteins were identified and quantified by LC-MS/MS data analysis. The results of the LFQ analysis showed that out of the proteins identified in the infection groups, while 233 (153 up-regulated, 80 down-regulated) proteins exhibited significantly different quantities compared to the control in the G27 infected group, 223 (136 up-regulated, 87 down-regulated) proteins were identified with significantly different amounts in the G27-ΔoipA infected group. After conducting pathway analysis, it was found that the G27 infection group participated in 22 pathways, while the ΔoipA-G27 infection group participated in 26 pathways. Of these pathways, 20 were found to be common to both infections, with two being specific to the G27 infection and six being specific to the ΔoipA-G27 infection. This study examined the effect of OipA protein on gastric cells during *H. pylori* infection and identified intracellular target proteins and pathways of the oipA protein in the prognosis of *H. pylori*-related diseases.

P138. QUIN-XL 2.0: A computational framework for large-scale protein-protein interaction quantification

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Introduction

Protein-protein interaction elucidation is paramount to advancements in biotechnological applications, such as drug development. The integration of chemical crosslinking with mass spectrometry (XLMS), a prevalent technique for capturing interaction dynamics, facilitates this by covalently stabilizing protein complexes and pinpointing linked peptides via specialized bioinformatics tools. Innovations in cleavable crosslinker design have expedited large-scale in vivo investigations, thanks to their asymmetric cleavage facilitating peptide identification.

We have previously presented QUIN-XL (PMID: 33681984), a software that uses quantitative information of non-cleavable crosslinks to recognize patterns and characterize protein conformers. Here we showcase QUIN-XL 2.0, our computational platform that expands this idea and takes advantage of cleavable crosslinker data by uncovering comprehensive protein interaction maps and mining insightful biological data through quantitative differential interactome analysis, marking a novel milestone in the field.

Methods

QUIN-XL 2.0, our software in development, integrates analyses from both labelled and label-free methodologies. Labelled approaches include isotopic (e.g., SILAC) and isobaric (e.g., TMT) tagging, while label-free techniques rely on extracted ion chromatograms (XIC). QUIN-XL 2.0 provides customizable options for normalization, imputation, and spectra deconvolution, facilitating user interaction and simplifying complexities associated with different isotopes and charges in XIC. Once quantification is achieved, QUIN-XL 2.0 handles all subsequent statistical analyses, including fold changes and p-value assignment. Our software further enriches data interpretation by showcasing interaction maps with quantitative values, thus providing an intuitive visual representation of system dynamics under varying conditions.

Results

QUIN-XL 2.0 is in an advanced developmental stage. Rigorous validation of the XIC algorithms using both technical and biological replicates of identical experiments has affirmed its performance; observed fold changes in crosslinks have consistently approximated zero. Modifications are underway to enable the extraction algorithms to handle ion mobility data, including FAIMS. The foundations of SILAC and TMT modules are established, with current efforts primarily aimed at integration with the Scout cXL-MS search engine and optimizing the graphical user interface.

P139. Ultra-high sensitivity for single cell proteome analysis

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For single cell proteome analysis, ultra-high sensitivity mass spectrometry is a key to reach a proteome coverage necessary for understanding the cellular heterogeneity on a cell-by-cell level. The 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, pushes the limits of detection to the single cell level. 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.

K562 cell digest (Promega) dilution series from 16 ng to 15 pg, in 2-fold dilution increments, was prepared. HeLa cells were isolated with a cellenONE into wells of a proteoCHIP Evo-96 prototype at cell counts of 1, 5, 10 and 20 cells per well, lysed and digested at 50 °C and 85% humidity for 2 h with automated rehydration cycles during incubation. Obtained peptides were transferred by centrifugation into a 96 well plate and placed into the nanoElute2 autosampler. Total volume was picked up and loaded directly onto an Aurora Elite column (250 mm x 75 µm, IonOpticks), separated with a 22 min active MeCN gradient (5% – 35%, total run time 30 min) and detected on a timsTOF series mass spectrometer in dia-PASEF and analyzed using Spectronaut 17 in directDIA+ method evaluation mode for each condition or cell count group.

Here, we assessed the sensitivity of a timsTOF series mass spectrometer using a dilution series of K562 cell digest showing excellent identification rate reproducibility and quantification accuracy per concentration replicates. Processing of the dia-PASEF data identified 1,000 protein groups out of 15 pg, 3,500 protein groups out of 250 pg and 7,000 protein groups out of 16 ng K562 peptides loaded on column. The quantitative accuracy improved inversely with loaded peptide amounts with 19% at 15 pg, 10% at 250 pg and 4% at loads of 4, 8 and 16 ng. Isolated HeLa cells at cell counts of 1, 5, 10 and 20 cells per well resulted in identification rates of 4300 protein groups per single cell, 5,200 protein groups for 5 cells, increasing to 5,500 protein groups for 10 cells and reaching 6000 protein groups for 20 HeLa cells with good reproducibility in the individual cell count groups and a steady increase in protein abundance from the single cells to 20 cells.

P140. Investigation of the Bystander effect in the neuronal culture of 5xFAD Alzheimer's disease transgenic neonatal mouse model

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Introduction

Alzheimer's disease (AD), the most common form of dementia and cognitive impairment, is a progressive neurodegenerative disorder mainly effecting the elderly population. Although the studies that cover the late stage of the disease revealed many unknown, the fact that at which point the AD pathology begins and how it starts has not been elucidated. The aim of this study is to investigate the role of Bystander effect in early degeneration. Bystander effect is a hypothesis which claims that degenerated/senescent neurons cells have a detrimental effect on the healthy neuron cells. It is suggested that this hypothesis will help explaining the progress of the AD pathology and indirectly help clinical studies by giving them a new point of view for further studies. For this purpose, co-culture experiments are performed in order to identify the effect of the hereditarily damaged 5XFAD neuronal culture on the healthy non-transgenic neuronal culture.

Methods

In this study, the 5xFAD, a widely used (~10% of all AD studies) early-onset AD mouse model expressing five FAD mutations is used. Primary neuronal cell cultures are derived from cortical and cerebral tissue of neonatal (24 hours) 5XFAD mice and are cultured as two separate groups. The tissues (cortex and cerebellum) are processed with the NeuroCult Enzymatic Dissociation kit (Stemcell Technologies, 05715) per manufacturer's instructions and are plated onto poly-d-lysine coated plates in three groups: 5xFAD, non-transgenic (control) and 5xFAD-non-transgenic co-culture. Then, the cells are harvested/collected and homogenized. The tryptic peptides are obtained according to the Filter Aided Sample Preparation (FASP) protocol for LC-MS/MS analyses. LC-MS/MS experiments were performed using a nanoACQUITY UPLC coupled to a SYNAPT G2-Si HDMS system (Waters, USA).

Results

As a result of the LC-MS/MS analyses, 1294 and 1194 proteins are identified in the cortex and cerebellum region, respectively. In the cortical culture, 65 proteins (30 upregulated, 35 downregulated) are altered with statistically significant changes ($p \leq 0.05$, fold change ≥ 1.3, unique peptide > 2) in the 5xFAD group. 20 proteins (8 upregulated, 12 downregulated) are observed with statistically significant changes in the co-culture group and 15 of these proteins are common to both groups. In the cerebral culture, 17 (7 upregulated, 10 downregulated) proteins in the 5xFAD group and 16 proteins (7 upregulated, 9 downregulated) in the co-culture group were statistically significant. 9 of the proteins are common to both groups.

Acknowledgement

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P141. A Novel Proteomics Workflow for Sensitive and Scalable Analysis of Limited Plasma Samples from Model Organisms

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Model organisms like mice are studied to unveil insights to human biology research. However, due to their size the available blood plasma volume is often limited, compared to sampling from human donors. Blood plasma and serum are rich, readily available sources of protein that are commonly used in biomarker and clinical research studies. However, the large dynamic range in the plasma/serum proteome has hindered large scale plasma/serum proteomic research. The recently introduced Proteograph™ Product Suite (Seer Inc.) enables high-throughput in-depth plasma proteome quantification, employing a panel of engineered nanoparticles (NPs) with distinct physicochemical properties. This collective panel of NPs provides enhanced protein identifications in terms of depth and breadth along with precise quantification compared to neat plasma/serum digestion.

For analyzing mouse plasma samples with lower than Proteograph™ workflow standard volume, the volume was diluted to yield a final volume of 250 µL. Mouse serum samples were thawed in an ice-water bath, spun down to remove air bubbles, and triplicates of 250, 125, 50, 25, and 10 µL of mouse serum diluted to achieve 250 µL of starting volume. Samples are processed with the Proteograph Product Suite SP100 Automation Instrument, including protein corona formation, digestion and peptide desalting. Tryptic peptides were analyzed on Orbitrap Exploris™ 480 Mass Spectrometer (Thermo Fisher Scientific) with a 30 min DIA method. LC-MS data were analyzed with Proteograph™ Analysis Suite with DIA-NN in library-free mode.

Here we evaluate the current performance of Proteograph Product Suite coupled with Orbitrap Exploris 480 MS when a limited sample volume is utilized. We evaluated 250, 125, 50, 25, and 10 ul of starting volume for (1) human plasma, and (2) mouse serum using Proteograph Product. We investigated the Proteograph workflow performance using 30-minute Liquid Chromatography (LC) methods using data-independent-acquisition strategies (DIA) and analyzed the data via Proteograph™ Analysis Suite 2.0, evaluating depth of proteome coverage, dynamic range, assay yield, and reproducibility of Proteograph workflow for unbiased proteome profiling. Notably, with 5-fold lower than standard input volume (50 uL vs 250 µL), the Proteograph obtained ~3.5-fold increase in protein group IDs (2129 v.s. 627, n=3) compared with traditional neat digestion workflow. This reduced volume workflow improved coverage of low abundance proteins compared to neat digestion protocol. In summary, proteograph workflow, for any given plasma and serum volume (10-250µL) identify significantly higher protein groups compared to neat digestion results.

P142. Tear fluid proteomics: issues of protein sub-populations

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Introduction

Different body fluids are considered as easily available possible sources of biomarkers for systemic and local diseases. Some “external” fluids (e.g., tears, urine and sweat) can be collected using non-invasive methods, while collection others (e.g., serum, plasma, cerebrospinal fluid/CSF) require methods with low or medium invasiveness. Proteins in these fluids are variable combinations from different sources, and methods and circumstances of sample collection/processing adds another level variance. The “external” body fluids show generally higher intrapersonal variance in composition and are more affected by the collection method and environment. These variances can alter the abundance of different sub-populations of proteins, including highly abundant proteins in different ways, which can cause difficulties in application of normalization methods and may introduce some level of bias in quantitative results. In this work we have analyzed human tear samples collected using different methods and replicate samples collected at different time points. Levels of variance were determined, and possible normalization and quality control (QC) methods were evaluated to decrease those. Identification of different proteome sub-populations was performed using different correlation, clustering and feature selection methods based on time and sampling method dependent variability.

Methods

Tear samples were collected using three different methods (capillary, Schirmer’s strip and the novel phenol red thread) from 10 healthy subjects, and at 5 consecutive days using glass capillary method from 3 subjects. Samples were processed using either by an on-pellet digestion or an optimized in solution digestion protocol. Sample specific spectral libraries were created by either data dependent acquisition (DDA) of 1D gel separated pooled samples, or with gas phase fractionation DIA (GPF-DIA) analysis of pooled samples. Proteomes of samples were quantified using data independent acquisition (DIA) protocol. Statistical data analysis was performed using different software packages both on protein and peptide level.

Results

Based on correlation, intra- and interpersonal variances in tear, we have identified clusters of proteins which were found to be mainly related to origin of them. By the comparison of the proteomes detected using different sampling methods we could identify the core tear fluid proteome and those proteins which originate from the eye surface cellular debris. Knowledge of the sources of variations and the affected protein clusters helps the application of cluster based normalization approaches to efficiently decrease the observed variances, and the selection of representative/outlier samples. Our observations may help the analysis of other body fluids with similar issues.

P143. Development of an automated sample processing workflow for mass spectrometry-based assessment of LRRK2 kinase activity in clinical samples

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Introduction: The discovery of monogenic forms of Parkinson's disease (PD) has greatly contributed to improve our understanding of underlying pathomechanisms, while also creating opportunities for targeted disease-modifying treatments and patient stratification. LRRK2 mutations, associated with both familial and sporadic PD cases, lead to increased kinase activity and subsequent hyperphosphorylation of endogenous Rab-GTPase substrates [1]. We recently reported a targeted mass spectrometry assay that enables simultaneous measurement of 6 LRRK2-phosphorylated Rab substrates (Rab1/3/8/10/35/43), and phosphorylated LRRK2 at Ser910/Ser935 biomarker sites [2]. Here we report the development of an automated sample processing workflow for application of this mass spectrometry assay to large sample cohorts.

Methods: All sample processing steps were performed in an automated fashion using an Agilent Bravo liquid handling platform. Tryptic digestion of protein extracts was performed by SP3 method. Peptides were spiked in with heavy-labelled synthetic peptides, followed by immunoprecipitation-based peptide enrichment, using magnetic protein G beads coupled to pan-pThr-Rab, pSer910-LRRK2, pSer935-LRRK2, and total LRRK2 antibodies. A semi-automated workflow was used for loading of samples onto Evotips, followed by LC-MS/MS-PRM analysis, using an Evosep One LC system and Orbitrap Exploris™ 240.

Results: By using a liquid handling platform, we developed a robust solution to achieve high throughput while reducing technical variability inherent to human sample handling. We have been able to successfully measure LRRK2-mediated phosphorylation of Rabs, as well as phospho- and total LRRK2 levels in different biomatrices, including cultured cells, mouse tissues, and human blood cells. We are currently applying this workflow to hundreds of peripheral blood mononuclear cells, neutrophils, and monocytes, from individuals with PD-associated mutations in LRRK2, VPS35 and GBA, to establish a direct comparison between different peripheral blood biomatrices and investigate potential disease- and genotype-specific LRRK2-dependent Rab phosphorylation signatures.
We expect our assay may be useful to others in the field as it can easily be used for analysis of non-clinical samples, such as human or mouse cells or tissue protein extracts. From a clinical translation standpoint, we envision our assay could potentially be a useful tool for mechanistic patient stratification patients and target engagement studies, crucial for clinical trials of emerging LRRK2-targeted therapies.

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[2] R. S. Nirujogi et al., Biochem. J., 2021, doi: 10.1042/BCJ20200930.

P144. Activity-based Protein Profiling to investigate the interactome of the antimalarial early lead Plasmodione

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In 2021, malaria caused an estimated 247 million clinical episodes and 619,000 deaths. Parasites belonging to the Plasmodium genus are the causative agents of malaria. During years, several antimalarial drugs have been developed but the parasite quickly produces drug-resistances to all of them. Plasmodione is a novel antimalarial early lead drug that is highly effective in limiting the proliferation of malaria parasites in vitro in the nM range with very moderate toxicity in the host cells. Literature data demonstrate that this early lead drug, could exhibit several modes of action via its metabolite, depending on the parasitic protein target. The objective of the activities is to identify putative plasmodione targets in the P. falciparum proteome. The methodologies used will be those of the affinity-based protein profiling (ABPP) strategy. The approach is aimed at labelling plasmodione protein targets and identifying them by mass spectrometry approaches. The general ABPP method consists in several steps: 1) UV-irradiation of the ABPP probe (structure based on its major metabolite PDO) incubated with the cell lysate, 2) conjugation of biotin through a click chemistry-based reaction, 3) enrichment of the biotinylated proteins through pulldown from streptavidin beads, 4) enriched protein digestion and liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Activity-based probes were first tested on a model protein, the recombinant glutathione reductase. Each step was optimized: photoaffinity labeling, tagging photolabeled proteins by click chemistry with a biotin probe and enrichment of biotin tagged proteins using streptavidin beads. The optimization involved various conditions to screen: from UV-irradiation under oxygen-free conditions and then, CuAAC reaction under oxygen-free conditions with a biotin azide and pull down of the adducts through biotin-streptavidin beads. The whole procedure will be applied to E. coli (WT, and a strain overexpressing PfNDH2) and S. cerevisiae cell systems (WT and a strain k.o. nde1 gene) to validate the experimental workflow. The two cell systems consist of the wild-type strains and the E. coli and S. cerevisiae mutated ones in which two potential plasmodione targets have been overexpressed or silenced, respectively. After the method validation, it will be applied to P. falciparum cell extracts.

P145. Investigating the macrophage proteome and PTM assessment by Parallel Accumulation and Serial Fragmentation (PASEF)

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Introduction

Data independent acquisition and PASEF (DIA-PASEF) is increasingly reported for its use in sensitive measurements, with identification of ~1000-3000 proteins from picogram amounts of mammalian cell-derived peptides now possible. DIA-PASEF typically utilizes equal-sized IM-m/z windows, in series, to cover the typical range of tryptic precursors, where IM correlates in a roughly linear fashion with m/z. Adaptations to DIA-PASEF have been reported. These include automated selection of variable PASEF windows based on known precursor density in the IM-m/z plane (py_diAID), and using high numbers of PASEF windows covering narrow m/z-1/K0 ranges to separate precursors derived from co-eluting peptides as extensively as possible (slice-PASEF). Such approaches have been shown to improve identification of PTMs, and increase peptide identification for low amounts of sample. PASEF also complements parallel reaction monitoring (PRM) for specified peptides, where serial fragmentation within different IM and m/z windows enables high numbers of precursors to be selected for MS2 without compromising duty cycle time. Here, we apply PASEF approaches to the analysis of an innate immune model (murine macrophages), with a particular emphasis on the ubiquitylation PTM, aiming to maximise proteome depth and quantify ubiquitylation without utilising sample enrichment methods.

Methods

A variety of murine macrophage cell lines and primary cells were cultured as previously reported. Their proteins were extracted and digested by trypsin using suspension trapping (S-Trap). To produce precursor libraries, a pool of peptides was made from these tryptic digests, which underwent high pH reverse phase fractionation into 72 fractions concatenated to 24. LC-MS/MS was performed using an Evosep One HPLC system on-line with a TIMS-ToF HT (Bruker). Data were acquired by DDA-PASEF and analysed using FragPipe for precursor library generation, and a variety of PASEF approaches used to assess the proteome and ubiquitylome in single shot experiments, and analysed using DIA-NN.

Results

DDA-libraries of ~150,000 precursors were produced. These included derivatives of >200 peptides possessing a ubiquitin remnant motif (KεGG). Those expected to be most abundant (e.g. peptides derived from polyubiquitin) were present and among the greatest in abundance. Slice-PASEF and DIA-PASEF (py_diAID) methods were designed to target KεGG-peptides, with variable efficacy.

P146. The neXtProt function prediction project for uncharacterized human proteins

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Introduction

Currently, about 8% of the human protein-coding genes have no function annotated in databases. Yet, protein products have been confidently identified for many of these genes, and they might play important roles in human biology. Several initiatives were launched recently to fill this knowledge gap by encouraging experimental characterization efforts and the development of more reliable function prediction methods. However, the lack of functional hypotheses, the lack of reagents or suitable models, and the lack of motivation and funding for such risky projects are major obstacles. In addition, while protein structure prediction has become highly accurate, predicting protein functions remains very challenging.

NeXtProt standardizes and integrates information on human proteins and provides users with an advanced search capability built around semantic technologies. To support the scientific community in its efforts to complete the human functional proteome, it maintains a list of uncharacterized proteins and proposes community pages where manually curated functional predictions are available.

Methods

Proteins lacking functional annotations and those that are solely annotated with broad Gene Ontology (GO) terms are considered uncharacterized. They can be retrieved using the query https://www.nextprot.org/proteins/search?mode=advanced&queryId=NXQ_00022.

Functional hypotheses for uncharacterized proteins were either retrieved from the literature or manually generated by mining experimental data (subcellular location, expression, interactions with other proteins, phenotypes in model organisms …) and results of sequence-based prediction tools (structure, domains, PTM, phylogenetic profiles …). They were standardized using GO molecular function (MF) or biological process (BP) terms and are displayed in the Function prediction pages located in the Community section of the corresponding entries. For full traceability, the underlying data and method used are indicated (as ECO terms), as well as the organism in which the primary data was obtained, the reference of the publication and/or the ORCID of the submitter.

Results.

In the current neXtProt release (2023-04-18), there are 1521 uncharacterized proteins. Manually reviewed function predictions are provided for 212 of them. The list is available here: https://www.nextprot.org/proteins/search?listId=B39DL206. Out of the 212 proteins with function predictions, only 12 are still awaiting validation by mass spectrometry.

Most of the functional predictions (90%) are GO BP terms. The data types that mostly contributed to the predictions were phenotypes, expression, subcellular location and interaction, in decreasing order. We invite all scientists to refine these predictions using their data and to submit their own predictions, obtained by manual or automated approaches. Let’s complete the human functional proteome together!

P147. Integrating proteomics into diagnostic molecular pathology reports to support molecular tumor board decisions

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Introduction

Molecular pathology largely relies on genomic and transcriptomic approaches for comprehensive and sensitive molecular diagnostics. Continuous instrument and method development in quantitative mass spectrometry (MS)-based proteomics enable reproducible in-depth proteome investigations in various patient-derived samples, including body fluids and tissue specimens. Thus, we set out to integrate clinical proteomics into molecular diagnostics, complementing and expanding the existing molecular diagnostic routine and providing another layer of biological information.

Methods

We applied automated MS-based quantitative proteomic workflows to perform reproducible and robust in-depth proteomics of formalin-fixed paraffin-embedded (FFPE) tissue from patients that were included in the molecular tumor board. All patients also received molecular diagnostic routines including Gene-Panel sequencing and RNA Fusion analysis.

Results

With our robust, reproducible, and automated sample preparation approach, we identified and quantified reliably more than 4000 proteins in over 50 MTB-derived samples. In 26 cases the proteomic analysis has contributed to the molecular pathology reports, providing additional insights into the underlying molecular mechanisms in the respective malignancies. In addition to the full proteome approach, we established a phosphoproteomic approach, which has yielded functional insights regarding the activity of pathways of interest in selected MTB cases. Furthermore, we established a proteogenomic analysis workflow that enables the integration of proteomics and genomics. Exemplary contribution of proteomics includes the detection of CDK4 and CDK6 in cases with a CDKN2A loss-of-function mutation, supporting the efficiency of a CDK inhibition treatment, as well as the detection of ERK signaling in a patient with a BRAF-KIAA1549 fusion, providing a rationale for MEK inhibition.

Conclusion

We demonstrate the added value and potential of such integrated analysis in the molecular characterization of particularly rare and challenging tumors that are investigated and discussed in the interdisciplinary molecular tumor board.

P148. Compact, high-resolution time of flight mass spectrometer based on an electrostatic analyser

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Introduction

Time-of-flight (TOF) mass spectrometry is one of the cornerstones of mass spectrometry, combining a rapid analysis speed with high resolution and an unlimited mass range. The resolution of a TOF is determined by the flight time of the ions. This means that the highest resolution TOFs will have flight tubes several metres long. The use of a reflectron can increase the flight time without significantly increasing the footprint. This means that high resolution, compact TOF mass spectrometers based on a reflectron is not feasible. Here, we present the design and theoretical performance of a TOF mass spectrometer based on an electrostatic analyser (ESA). This instrument was conceived to provide a resolution of greater than 10,000 with a footprint of 450x190x180 mm (lxhxw).

Methods

The conceptual design was converted into a 3D CAD drawing which can be mounted into the SPIDOC chamber to be used as a diagnostic TOF. The design of the instrument was evaluated by ion optics simulations using a combination of the SIMION and SIMAX programs. The model was first tested for ultimate performance using different idealised ion populations. Subsequently, realistic ion populations expected from the SPIDOC experiment were modelled to indicate expected real work performance.

Preliminary data (results)

The design was conceived to provide a compact, high resolution diagnostic TOF within the SPIDOC project. The footprint available was incompatible with a TOF with high enough resolution. The design, based on that of Poteshin et al.[1], using orthogonal acceleration coupled to an ESA. By choosing the correct geometry of pusher, flight tube and ESA allows to perform second order time focusing, providing the high resolution required. This led to the TOF design shown in Figure 1. The design was validated using SIMION and SIMAX simulations. The model was validated by calculating the expected dependences of flight time and resolution on m/z. The performance of the TOF was then explored. The dependence of the resolution on the initial energy spread of the ions in all 3 dimensions was explored, finding that resolutions as high as 40,000 was found for idealised ion distributions. The sensitivity of initial ion position was also explored with similar results. These provided a baseline for ideal performance. Real world performance as tested by simulating ions entering the TOF from the interaction region of the SPIDOC experiment, providing a stringent test as ion focusing into the pusher is difficult, giving values between 5,000 and 9,000. These values will be higher in a design with proper pusher optics, with simulations suggesting resolutions as high as 20,000 can be achieved.

[1] Poteshin, S.S., Chernyshev D.M., Sysoev, A.A., Sysoev, A.A., Physics Procedia, 72, 2015, 266-273. DOI: 10.1016/j.phpro.2015.09.084

P149. Pushing the frontiers of high throughput and high sensitivity analyses: Orbitrap technology unites with a new star

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Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissues are an invaluable resource for retrospective clinical studies to investigate molecular mechanisms or discover novel biomarkers. However, formalin fixation makes FFPE sample preparation for proteomic analysis extremely challenging and harsh conditions must be applied to reverse cross-linking and extract proteins efficiently. In addition, paraffin interferes with liquid chromatography-mass spectrometry (LC-MS) analysis, so most protocols require an upstream xylene-based deparaffinization step, which is time-consuming, toxic, and carries the risk of sample loss. To address these challenges, an optimized workflow combining the BeatBox tissue homogenizer and the iST kit for proteomic sample preparation has been developed. It eliminates the need for xylene-based deparaffinization and allows efficient and robust processing of 96 samples in parallel from FFPE tissue to clean peptides in one working day.

Method

Snap-frozen mouse organs (1-2 mg tissue pieces) and matching FFPE samples (10 µm curls) were processed in 96-well format using BeatBox homogenization coupled to iST sample preparation. For FFPE samples, an optimized workflow was established: FFPE curls were homogenized for 10 minutes with high-power mode in the BeatBox, followed by an one-hour incubation at 80-95°C, 1000rpm to de-crosslink, extract, reduce and alkylate proteins in one step. After cooling to room temperature, the samples were transferred to fresh plasticware while the solidified paraffin remained in the primary plasticware. Applying the iST sample preparation protocol, tryptic digestion was followed by an optimized peptide clean-up with an additional washing step designed to effectively remove last traces of paraffin. Peptides were analyzed on a nano-LC coupled to a timsTOF mass spectrometer in DIA mode.

Results

A step-by-step benchmark of this innovative workflow against a conventional xylene-based deparaffinization and sonication workflow was performed using mouse heart muscle, kidney and liver. The same mouse tissues were preserved either fresh frozen or in FFPE format to evaluate the performance of the two tissue preservation techniques for proteomic analysis.

For FFPE tissues, BeatBox outperformed sonication for both xylene-based and xylene-free methods revealing >10% increase in protein IDs for all tissue types. Comparing proteins extracted from fresh frozen and FFPE tissues, both treated with the BeatBox + iST workflow without xylene, yielded a high overlap of up to 87% of shared proteins and a similar dynamic range.

In conclusion, the PreOmics´ BeatBox-iST workflow for FFPE tissues enables thorough proteomic analyses, while being easy-to-use and eliminating the need for xylene-based deparaffinization, which makes it suitable for large-scale retrospective studies.

P150. BeatBox and iST for streamlined FFPE tissue processing: A xylene-free, robust, and high-throughput sample preparation for proteomic analysis

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Introduction
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In conclusion, the PreOmics´ BeatBox-iST workflow for FFPE tissues enables thorough proteomic analyses, while being easy-to-use and eliminating the need for xylene-based deparaffinization, which makes it suitable for large-scale retrospective studies.

P151. Revisiting the impact of exercise on the proteome of circulating extracellular vesicles

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Introduction

Bioinformatics analysis, particularly functional proteomics, has revolutionized biomedical research by enabling novel ways to (re)trace and validate experimental findings. In this study, we analyzed datasets from the PRIDE database (https://www.ebi.ac.uk/pride) to explore the effect of exercise on the proteome of circulating extracellular vesicles (EVs). Our investigation aims to add insights on how exercise impacts whole body homeostasis, highlighting EVs as a source of biomarkers to trace the systemic adaptations to exercise and provide the molecular basis for the development of exercise mimetics.

Methods

For this purpose, the database PRIDE was selected to query the information of datasets focused on the proteome analysis of circulating EVs after exercise. We used Perseus v2.0.9.0 and Metaboanalyst v5.0 software to process, handle, statistically analyze the data. FunRich and STRING were used for enrichment/pathway/network analysis.

Results

Our analysis highlights the impact of exercise intensity and duration in shaping the protein cargo of EVs. Indeed, distinct protein profiles were observed in circulating EVs following different exercise protocols. After a session of high-intensity interval training (HIIT), 312 proteins were identified in EVs, whereas 188 proteins were observed after an incremental exercise protocol, with 284 and 175 of them listed in Vesiclepedia, respectively. From these, 109 proteins were common to both exercise programs. Considering the proteins known to be present in vesicles, HIIT appears to promote the regulation of norepinephrine uptake, skeletal muscle tissue regeneration, and triglyceride transport. On the other hand, aerobic exercise protocol modulated platelet degranulation and response to stress. Surprisingly, a resistance exercise session did not significantly impact the protein cargo of EVs when compared to the baseline. Collectively, our findings emphasize the critical influence of exercise type, intensity, and duration on the composition of the proteome carried by EVs. This, in turn, has implications for whole-body homeostasis. Future proteomic studies should go deeper into the intricate relationship between exercise programs and the composition of EVs.

P152. A tissue specific post-translational modification (PTM) map of human proteome

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Protein functions are closely linked to their spatial distribution in cells and tissues. Post-translational modifications are the key regulators of protein functions and their spatial distribution. Phosphorylation plays a crucial role in many cellular processes and in disease state. Several studies have demonstrated the tissue specific expression of proteins and in particular kinases, clearly indicates differential phosphorylation across tissues. We performed a proteome wide identification and functional analysis of tissue specific as well as global phosphoproteins and phosphosites (P-sites) in human using large scale re-processed proteomics experiments from PRIDE. We compared the identified tissue specificity of proteins and P-sites to the functional domains, structural, biophysical and disorder annotations. Our preliminary results shows that the protein instances of the same domain family have different tissue specificity. P-sites were most frequently found in inter-domain region of proteins compared to domain regions. Domains involved in more generic functions like structural support (actin, tubulin) and involved in multiple functions were associated with more non-tissue specific P-sites. Most or all of the P-sites identified in domains that are involved in very specific functions, such as trypsin, tyrosine and Serine/Threonine kinase, and heat shock proteins were tissue specific sites. Based on our preliminary results, we hypothesize that, though these proteins have similar biological functions, there are some inherent properties of these protein instances in their disordered and functional domain regions that differentiate the phosphorylation patterns and tissue specificity. In summary, our work on identifying tissue specific phosphoproteins and P-sites show our initial steps towards understanding differential phosphorylation across different tissues which can be easily extended to other common PTM types such as acetylation, methylation and ubiquitination.

P153. Genome-scale analysis unveils the eukaryotic metal-responsive biochemical network

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Metal ions are essential for cells and function as cofactors in ~80% of all metabolic pathways. Although metal ion concentration can exhibit substantial variability in natural environments, metal ion composition has, so far, been explicitly defined and altered only in a small subset of laboratory experiments. This limited investigation into the involvement of metal ions across all biological processes has resulted in a relatively incomplete understanding of the networks that regulate and mediate their crucial biological functions. Herein, we systematically varied the concentration of essential metals over several orders of magnitude to determine the buffering capacity for each metal, interactions between metals and quantified cellular responses to perturbations in metal availability. Using a combination of metallomics, proteomics and phenotypic screening of a genome-scale knock-out collection in budding yeast, we discovered that over half the coding genome is involved in the cellular network that regulates and responds to essential metal availability. By identifying protein-level associations for 67 previously uncharacterised proteins that are part of this network, we exemplify how exploring the metal homeostasis network can unveil novel gene functions. Our data revealed previously unknown interactions between metal ion biology and the metabolic network, corroborating prior evidence for the critical role of metals in metabolism. By demonstrating the profound influence of essential metal ions on the biochemical network, our work advocates for a revision of current laboratory practices and the study of all biological processes in the context of essential metal ion availability.

P154. LAP-MALDI and proteomics profiling of milk from different Calabrian bovine breeds

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Introduction

The Podolica is a cattle breed widespread in southern Italy, its productivity is characterized by low yields compensated by an extraordinary quality of milk and meats. Most of the milk produced is used to be transformed into "Caciocavallo Podolico" cheese which is made with 100% Podolica milk. Considering the high commercial value of this product and the threat of its substitution with milk from other cows, it is relevant to develop rapid and cost-effective methods that may be used for its correct classification. In this study we used LAP-MALDI profiling coupled with machine-learning data analysis to attempt its recognition among milk from other species and bottom-up proteomics/metaproteomics for a deeper characterization of bacterial consortia and proteins involved in antimicrobial resistance.

Methods

LAP-MALDI profiling was used to discriminate 100% Podolica milk from milk of other Calabrian cattle breeds. For this analysis, milk samples were subjected to a TCA precipitation. The pellet was resuspended in water:acetonitrile:isopropanol (1:1:1; v:v:v) and then analysed as previously described (doi: 10.1039/d1sc05171g.). Bottom-up proteomics was carried out after bacterial enrichment through centrifugation and lysis using bead beating. The obtained protein extract was cleaned before the protein digestion with a 10kDa Mw cut off filter and analysed via LC/MS-MS as previously described (doi: 10.3390/ani10122378.)

Results

LAP-MALDI analysis on the test set (16 samples analysed in blind) yielded a correct classification percentage of 87.5%. Among the 8 non-Podolica samples in the test set, 1 was misclassified and recognized as Podolica milk even though it was milk from other species. In the same way, of the 8 Podolica samples in the test set, 1 was mistakenly recognized as non-Podolica. The loading plot highlighted the presence of a protein ion that is the main driver of the classification whose Mw is around 14186 Da. Bottom-up proteomics analysis is still ongoing.

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