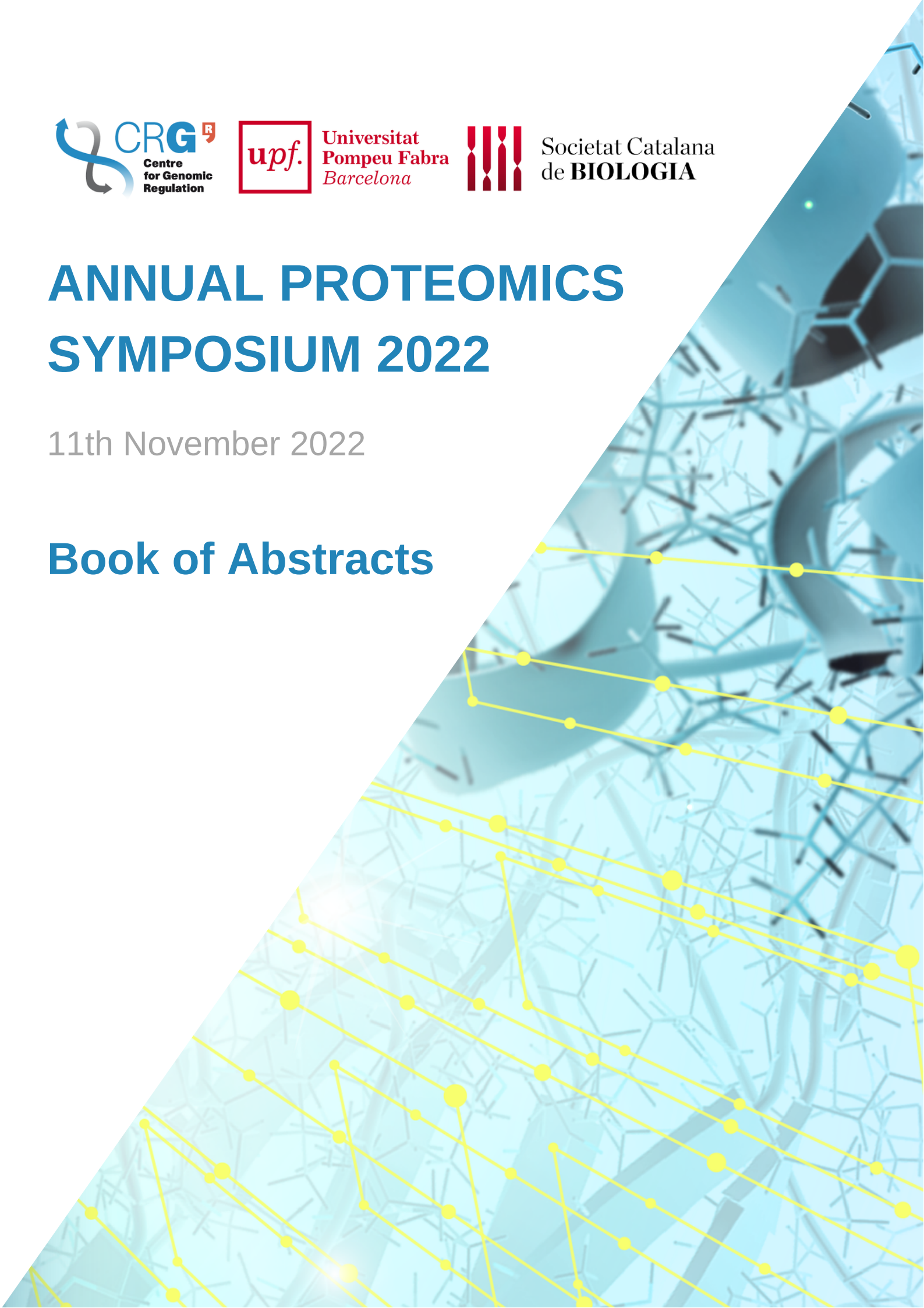
ANNUAL PROTEOMICS SYMPOSIUM 2022

11th November 2022

Book of Abstracts



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PROGRAM

OCTOBER 4th

09:00 Registration

09:15 Morning breakfast & Meet the Speakers

09:45 Welcome by **Eduard Sabidó**, Head of the CRG/UPF Proteomics Unit, Barcelona

10:00 **"Title TBD"**
Lauren Stopfer | BioNTech, US

Short Talks

10:45 **"Identification of conventional and non-canonical tumor antigens through proteogenomics "**
Maria Lozano | Vall d'Hebron Institute of Oncology, Barcelona

11:00 **"Protein biomarkers for an accurate endometrial cancer diagnosis"**
Eva Colàs | Vall d'Hebron Institute of Research, Barcelona

11:15 **"The Human Protein Atlas – the open access resource for human proteins and next generation precision medicine"**
Mathias Uhlén | Royal Istitute of Tecnhology (KTH), Stockholm

12:00 **"BeatBox & iST workflows enable evolutionary in-depth analysis of tissue & cell proteomes" [Sponsored talk]**
Quentin Enjalbert | Preomics

12:30 *Lunch and poster session*

13:15 **"Deciphering signal transduction using PTMScan technology" [Sponsored talk]**
Maxime Jacquet | Cell Signaling Technology & Werfen

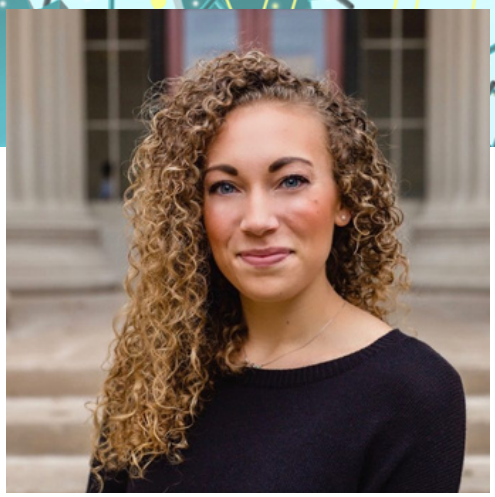
13:45 **"Advances and next frontiers in modelling proteins and their interactions"**
Amelie Stein | University of Copenhagen, Copenhagen

Short talks

14:30 **"Automated structure-based learning to model complex protein-DNA interactions and co-operativity in cis-regulatory modules"**
Baldo Oliva | Universitat Pompeu Fabra, Barcelona

14:45 **"Alternative Splicing Proteoforms of Polycomb Repressive Complex 2"**
Niccolo Arecco | Centre for Genomic Regulation, Barcelona

15:00 **"Post-translational regulation"**
Mikhail Savitski | EMBL Heidelberg



LAUREN STOPFER

BioNTech

"TBD"

TBD

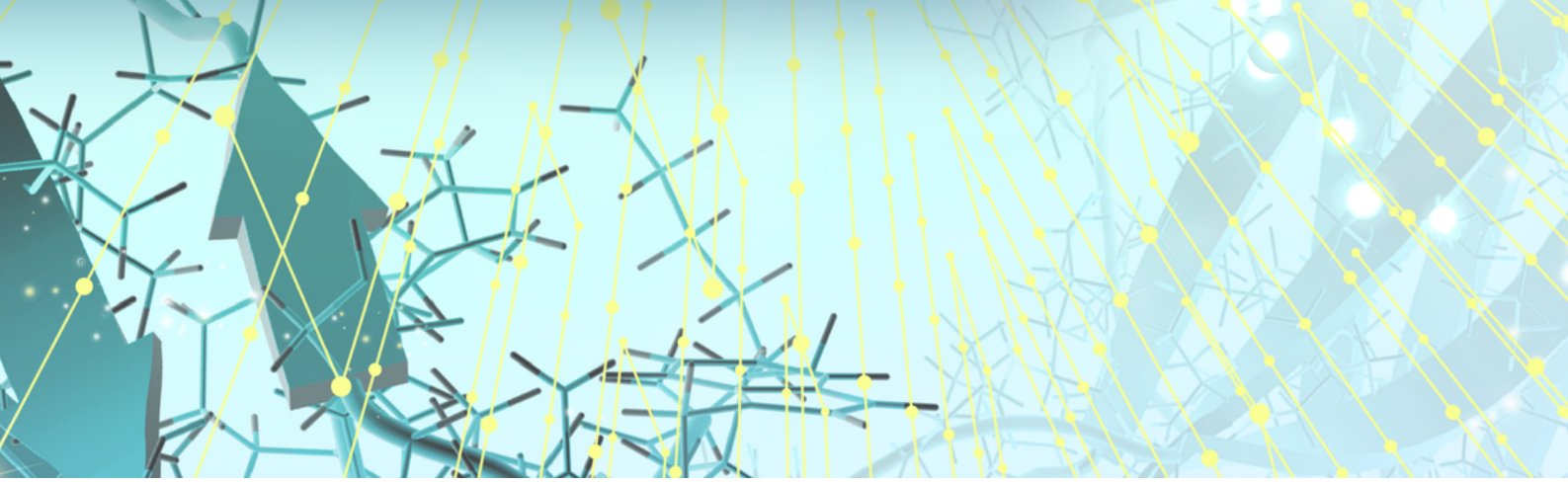
MARIA LOZANO

Vall d'Hebron Institute of Oncology

"Identification of conventional and non-canonical tumor antigens through proteogenomics"

Purpose: Tumor antigens are central to antitumor immunity. Recent evidence suggests that peptides from non-canonical (nonC) aberrantly translated proteins can be presented on HLA-I by tumor cells, thus expanding the targets for cancer immunotherapy. Here we use proteogenomics to identify personalized conventional and non-canonical tumor antigens and to evaluate their contribution to cancer immunosurveillance in patients.

Methods: Whole exome sequencing was performed to identify the non-synonymous somatic mutations and immunopeptidomics to identify the peptides presented on HLA-I in 9 patient-derived tumor cell lines (TCL). Peptide-PRISM proteogenomics pipeline was used to identify both canonical and nonC HLA-I ligands. Peptides derived from conventional tumor antigens including mutations, cancer-germline antigens (CGA) or melanocyte differentiation antigens were selected. For nonC peptides, an immunopeptidomics healthy dataset containing several tissues and HLA-I alleles was used to eliminate those derived from normal ORFs and select nonC HLA-I ligands preferentially expressed in tumor cells (nonC-TL). The selected candidate peptides were synthesized, pulsed onto autologous APCs and co-cultured with tumor-reactive ex vivo expanded lymphocytes to assess immune recognition.



Results: We identified a total of 517 nonC-TL in all TCL studied, being 5'UTR the main source. We found no recognition of the 507 nonC-TL tested by autologous ex vivo expanded tumor reactive T-cell cultures while the same cultures demonstrated reactivity to mutated, CGA, or melanocyte differentiation antigens. However, in vitro sensitization of donor peripheral blood lymphocytes, led to the identification of T-cell receptors (TCRs) specific to three nonC-TL, two of which mapped to the 5' UTR regions of HOXC13 and ZKSCAN1, and one mapping to a non-coding spliced variant of C5orf22C. T cells targeting these nonC-TL recognized multiple cancer cell lines naturally presenting their corresponding antigens.

Conclusions: Our findings predict a limited contribution of nonC-TL to cancer immunosurveillance but demonstrate they may be attractive novel targets for widely applicable immunotherapies.

EVA COLÀS

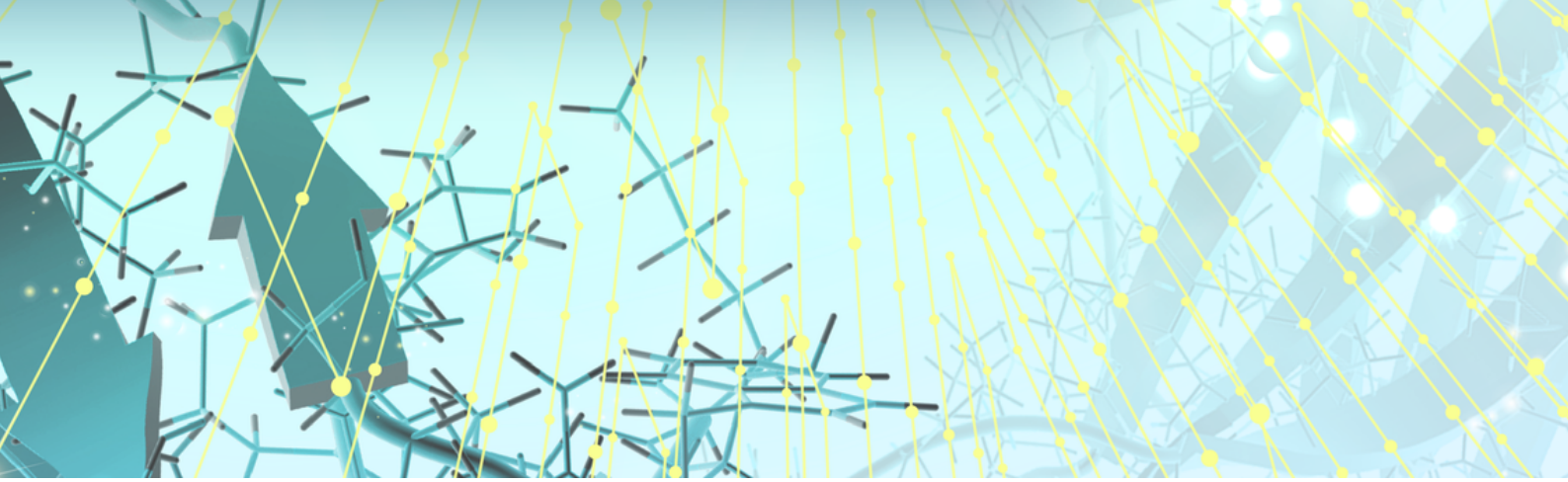
Vall d'Hebron Institute of Oncology

"Protein biomarkers for an accurate endometrial cancer diagnosis"

Background: Endometrial cancer (EC) incidence has been rising over the past 10 years. Early and accurate detection of the disease is an important contributor to survival. Diagnosis of EC patients is favored by the presence of symptoms like abnormal vaginal bleeding in women, which affects 11M women in the EU and US yearly, but only 5-10% of these women will be diagnosed with EC. Current EC diagnostics is based on the observation of cells in an endometrial biopsy, but this fails in 31% patients requiring additional more invasive procedures to be diagnosed, and inaccurately identifies histological type and grade of EC in 55% of patients compromising the treatment-decision making.

Study aims: Elucidate protein biomarkers in gynaecological fluids to accurately diagnose EC.

Materials and Methods: Biomarkers have been discovered through literature review and a discovery phase and validated by using targeted Mass Spectrometry. Biomarkers have been evaluated in five clinical retrospective studies in uterine fluids from 358 patient specimens. A subset of biomarkers are also validated in an independent retrospective clinical study in a cohort of 250 samples using ELISA technology.



Results: Our research has permitted to identify 5 protein biomarkers, which expression in uterine fluid permits a quantitative and accurate diagnosis with 97% negative predictive value, 99% sensitivity, 79% specificity and 87% positive predictive value. Moreover, it provides an objective assessment of the endometriod vs. non-endometrioid histological subtype of cancer to guide treatment planning and optimize surgical intervention.

Conclusion: These results are the basis to develop an in vitro diagnosis for accurate, faster, and minimally invasive diagnosis that will improve patient outcomes, reduce patient morbidity, and lower the cost of care.

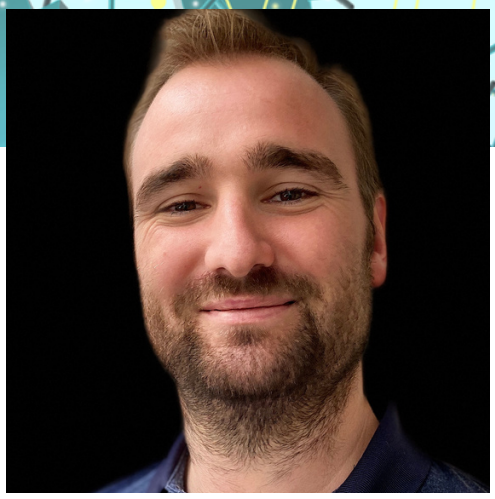
MATHIAS UHLÉN

Royal Institute of Technology (KTH),
Stockholm, Sweden

"The Human Protein Atlas – the open access resource for human proteins and next generation precision medicine"

The correct spatial distribution of proteins is vital for their function and often mis-localization or ectopic expression leads to diseases. For more than a decade, the Human Protein Atlas (HPA) has constituted a valuable tool for researchers studying protein localization and expression in human tissues and cells. The centerpiece of the HPA is its unique antibody collection for mapping the entire human proteome by immunohistochemistry and immunocytochemistry. By these approaches, more than 10 million images showing protein expression patterns at a single-cell level were generated and are publicly available at www.proteinatlas.org. The antibody-based approach is combined with transcriptomics data for an overview of global expression profiles. The present article comprehensively describes the HPA database functions and how users can utilize it for their own research as well as discusses the future path of spatial proteomics.

Sponsored Talk



QUENTIN ENJALBERT

Preomics

"BeatBox & iST workflows enable evolutionary in-depth analysis of tissue & cell proteomes"

The pertinence of protein analysis through LC-MS/MS technics is highly correlated with the achievement of suitable sample preparation. PreOmics provides for many years reliable and standardized solution for proteomics sample preparation thanks to the user friendly iST solution.

When working on biological samples such as tissue and cell, the protein extraction lyse step is crucial to access components of interest. Despite high progress in proteomic sample preparation during the last ten years, this specific step remains underestimated, leading to low throughput systems prone to heat degradation and cross contaminations. The novel BeatBox homogenization technique has already demonstrated its capability to achieve high-throughput tissue lysis of 96 samples in just 10 minutes. This study indicates that BeatBox is versatile in sample types and that it can perform straightforward protein extraction from various tissue types and cell lines.

The combination of the BeatBox and the iST provide a standardized, user friendly and robust workflow for tissue and cell protein analysis in proteomics.

Sponsored Talk

MAXIME JACQUET

Cell Signaling Tecnology & Werfen

"Deciphering signal transduction using PTMScan technology"

TBD

AMELIE STEIN

Kobenhavn Universitet

"Advances and next frontiers in modelling proteins and their interactions"

TBD

BALDO OLIVA

Universitat Pompeu Fabra

"Automated structure-based learning to model complex protein-DNA interactions and co-operativity in cis-regulatory modules"

TBD

NICCOLO ARECCO

Centre for Genomic Regulation

"Alternative Splicing Proteoforms of Polycomb Repressive Complex 2"

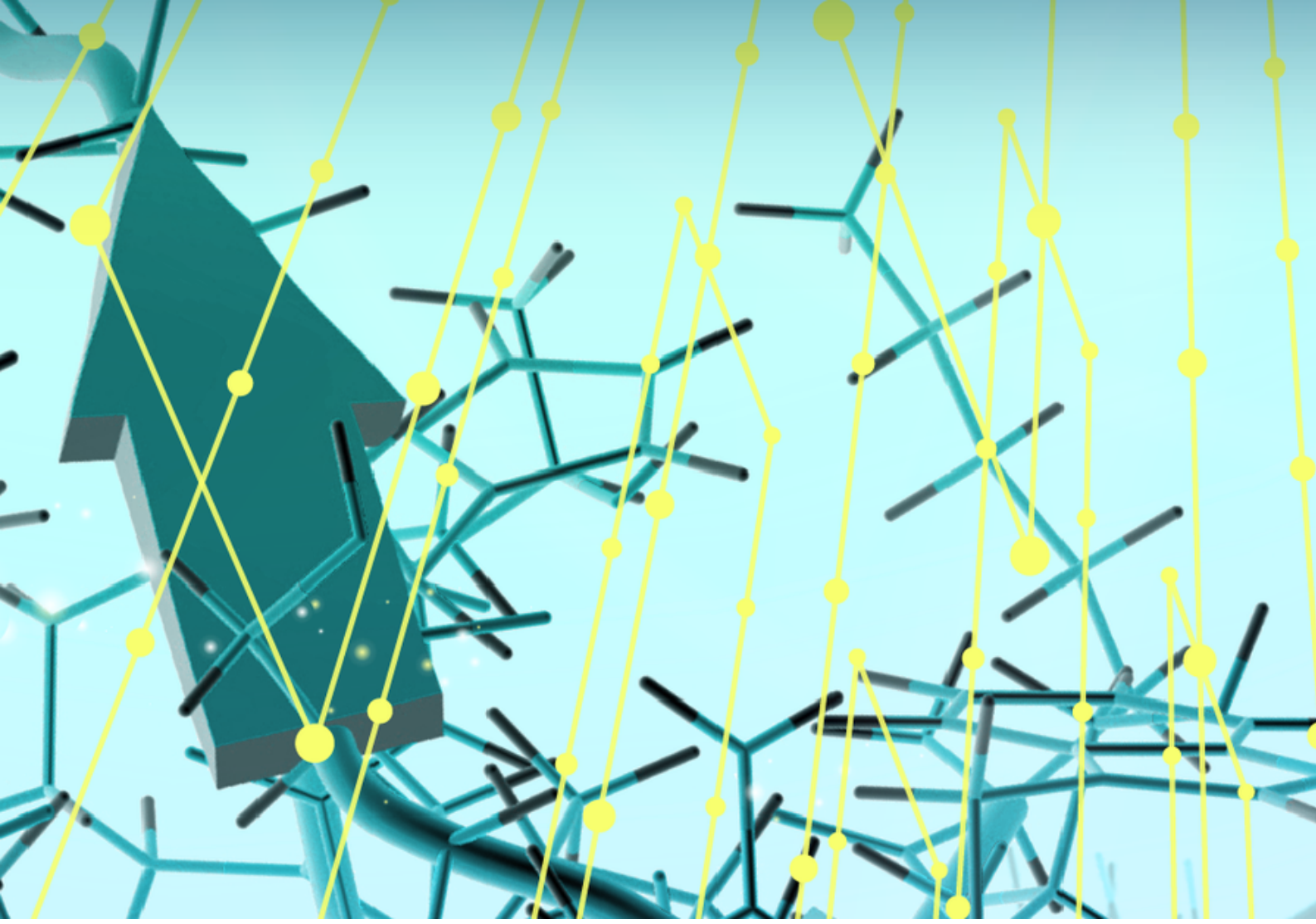
Eukaryotic cells need to generate a complex and specialised proteome from one single genome. In order to diversify gene function capabilities cells use alternative splicing (AS) of exons and introns within a pre-mRNA molecule to increase transcriptome complexity. This phenomenon is widespread in mammals and more than half of the human genes have isoforms that are highly regulated in a cell and tissue type-dependent manner. Interestingly a substantial fraction of chromatin regulators contains AS exons that are not tissue-specific but instead display highly alternatively splicing patterns across many tissues. One such example is the chromatin regulator Polycomb Repressive Complex 2 (PRC2). Using mass-spectrometry based proteomics we show how AS can influence PRC2 assembly, ultimately impacting histones post-translational modifications deposition on chromatin. Our findings suggest a possible cross-talk between splicing and gene expression regulation layers within the cell nucleus.

MIKHAIL SAVITSKI

EMBL Heidelberg

"Post-translational regulation"

TBD



SELECTED POSTERS

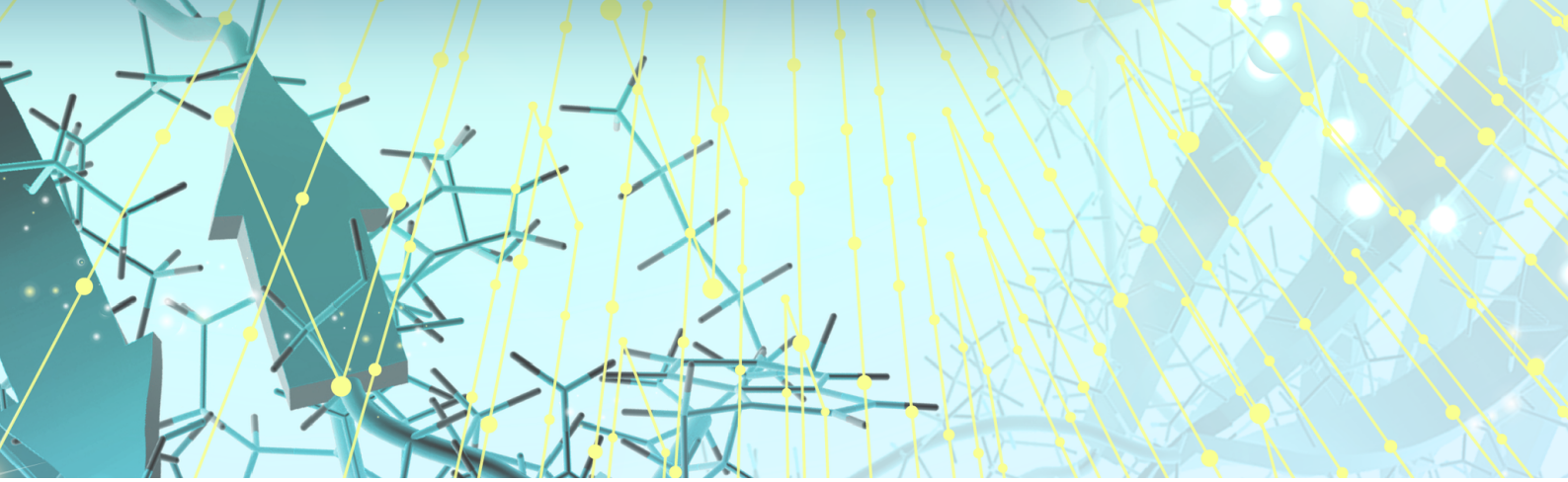
Poster 1

Cristian Munteanu

Institute of Biochemistry of the Romanian Academy

"Monitoring oxidative modifications and glycosite occupancy of melanoma antigens using targeted based mass spectrometry analysis "

Although peptide-based immunotherapy is a viable alternative to the classical chemotherapy still, there are a significant number of patients which show relapse. An interesting strategy is to characterize side-chain modifications (PTMs) of such antigens which could display a superior specific immune response compared to the non-modified version. Here using customized workflows employing targeted-based mass spectrometry (DIA) analysis we examine the presentation of peptide-based oxidative variants in complex with Major Histocompatibility Complex (MHC) class I. We further scrutiny the role of PTMs in peptide based presentation and CD8+ T cell recognition by employing dedicated LC-MS/MS based analytical workflows to investigate the N-glycosylation site occupancy of the melanoma autoantigen tyrosinase, (frequently found as an overexpressed, endoplasmic reticulum-retained, misfolded protein) and its consequence in peptide-MHC class I presentation by targeted relative quantification of one of its most abundant epitopes. Our results suggest that some cell lines can present nonameric peptides with oxidized residues in complex with MHC class I. This in turn implies



that the modified version could also result in a distinct affinity toward immune recognition and response. We also found that two of the glycosylation sites are only partial occupied and that the C-terminus located ones have a greater impact in melanoma cell surface presentation . These delineate the value of DIA based methods to provide novel findings regarding the impact of PTMs in peptide-MHC class I presentation.

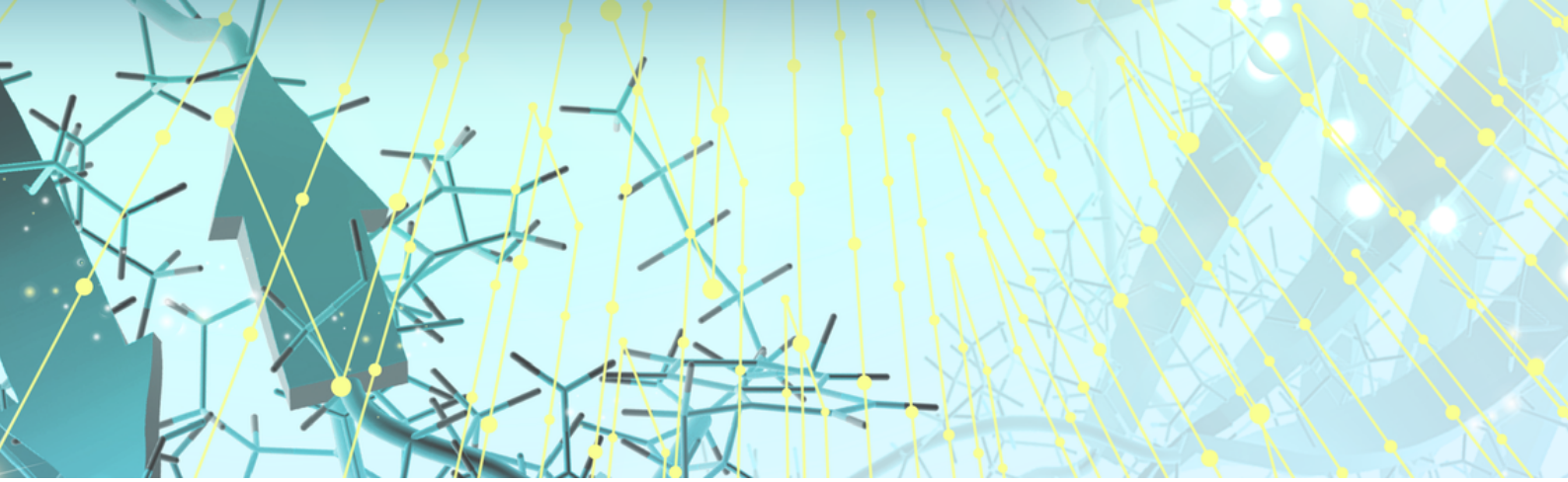
Poster 2

Gabriela Chirițoiu

Institute of Biochemistry of the Romanian Academy

"Targeted proteomics for the assessment of new potential melanoma antigens"

Cancer immunotherapy is a non-invasive treatment which improved the outcome of patients, although there are still clinical cases which develop resistance resulting in ineffective treatment in the end. It is currently widely accepted that this involves a mechanism in which cancer cells downregulate the level of cell surface antigens and thus become “invisible” and escape the immune surveillance of cytotoxic T cells. Thus, the ability to restore and increase the level of cell surface antigens would result in a potential increase of the immune response to eradicate the tumor. The restoration process is based on specific intracellular events, so modulation of such events coupled with proteome and peptidome screening could lead to the enrichment of antigens repertoire. Using a discovery-based strategy for data acquisition we analyzed the MHC1 peptide repertoire of melanoma cells with down regulated cytosolic antigen processing. We validated our workflow using a targeted based mass spectrometry method by analyzing known melanoma antigens such as gp100 and Tyrp1. We also searched for peptides in alternative routes of antigenic peptide presentation such as endosomal pathway in WT or TAP1-deficient cell lines, which is a key-component in the classical antigen presentation pathway. Using a targeted proteomic approach we validated our



workflow by confirming the presentation of gp100 antigen by alternative endocytic pathways and processing by cytosolic peptidases. By discovery based strategies of data acquisition we delineate other new potential immunogenic sequences dependent on cytosolic processing and presented by the alternative endosomal pathway.

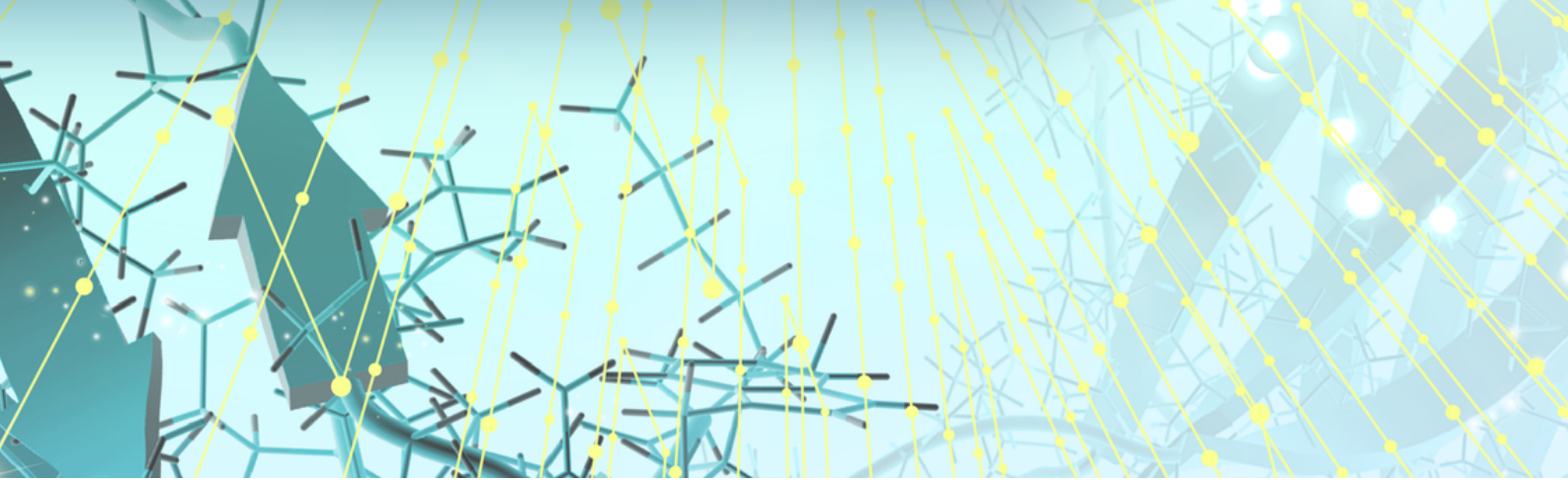
Poster 3

Yago A. Arribas

Insitut Curie Paris

"Transposable elements and non-canonical splicing represent a source of recurrent neoantigens in cancer patients"

The nature of the antigens that mediate anti-tumor immune responses in cancer patients, while sparing healthy tissues, is still unclear. The vast majority of known tumor antigens, including mutated neo-epitopes, are derived from canonical coding genomic regions. Recently, tumor specific, non-canonical open reading frames (ORFs), including non-coding genome and novel protein isoforms, were characterized, and peptides derived from these ORFs have been shown to be presented on the MHC-I molecules of tumor cells. Because defects in both splicing and transposable element (TE) silencing are common in human cancer, we investigate here the tumor-specificity of 2 different sources of TE-derived MHC-I peptides: 1) autonomous TE transcription and translation, and 2) splicing events between coding exons and transposable elements (JETs). To explore this hypothesis, we coupled RNA sequencing, ribosome profiling (RiboSeq) and mass spectrometry-based proteomics to define a subset of TE-containing transcripts that are translated recurrently in tumors and absent in healthy tissues. In addition, to investigate whether TE-derived peptides are presented on tumor MHC-I



molecules, we used immunopeptidomics (wherein MHC-I molecules are immunoprecipitated and bound peptides are analyzed by mass spectrometry). Immunopeptidomics reliably identified around 400 tumor specific TE-derived peptides in tumors and cancer cell lines. We also showed that TE-derived peptides are immunogenic, and they induce specific anti-tumor T cell responses in cancer patients. Our results contribute to the characterization of a new class of recurrent and immunogenic tumor-specific antigens, opening multiple possible applications in the fields of therapeutic vaccination and TCR-based cell therapies

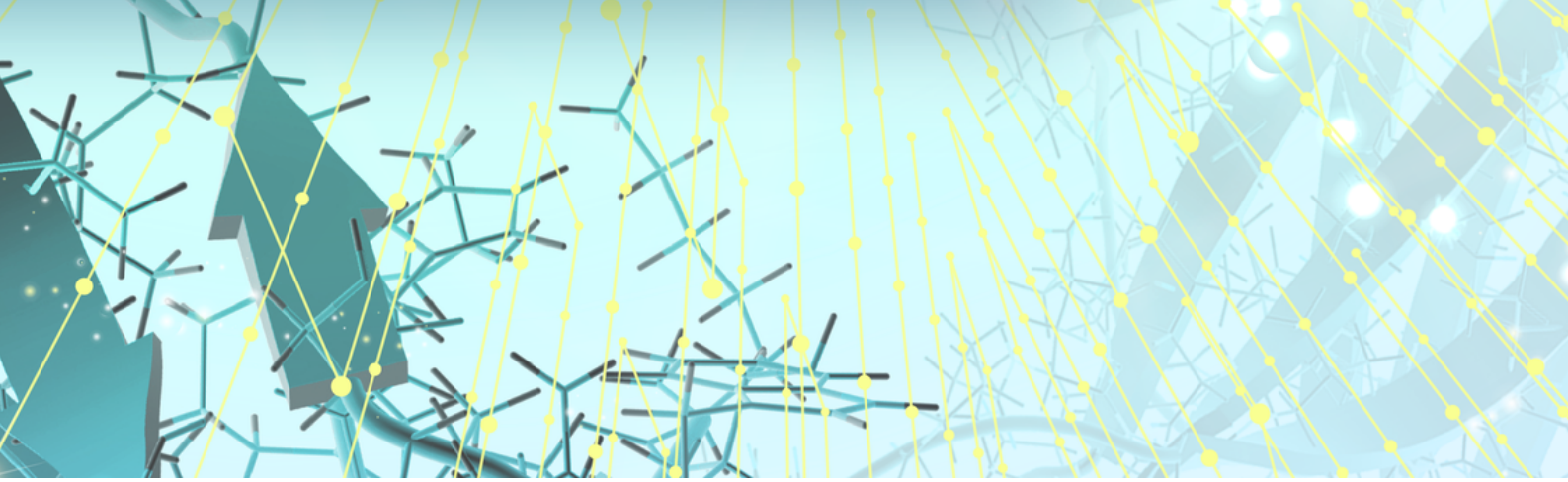
Poster 4

Laura Gadea Salom

Institut Universitari de Biotecnologia i Biomedicina
(BIOTECMED), Universitat de València

"Spatial and temporal characterization of an Influenza A infection by quantitative proteomics"

Influenza A virus (IAV) is a highly contagious disease responsible for annual epidemics that affect millions of people worldwide. Furthermore, IAV had caused several pandemics with disastrous consequences. Despite being one of the most studied viruses, many nuances of the viral infection remain unapprehended. Thus, we decided to study how viral and host proteins change their subcellular localization during the infection and the functional implications of these changes for both the virus and the cell. Since influenza viruses mainly infect the epithelial cells of the upper respiratory tract, we chose to infect human lung carcinoma A549 cells with the Influenza A/WSN/33 strain. Then, to investigate changes in protein subcellular localization, we performed a two-step proteomic based analysis at several infection times. First, using differential centrifugations we obtained a set of cellular fractions. Next, these fractions were analyzed through SWATH-MS obtaining the relative protein abundance (RPA) profiles of each protein. Finally, utilizing a list of subcellular markers, we analyzed the consensus profile describing each subcellular niche, and through machine learning assigned the



non-marker proteins to a subcellular niche. Our results demonstrate that a considerable portion of the host proteins change their subcellular localization during an infection. By means of bioinformatic analysis and RNA silencing assays, we are studying how these localization shifts may alter protein functionality, a necessary step to fully understand how IAV uses the host cellular networks to achieve a successful infection

Poster 5

Zahra Elhamraoui

Centre for Genomic Regulation

Stability assessment and prediction of the human proteotypic peptides for targeted proteomics quantification

Personalized medicine projects involve large cohorts of patients, in which hundreds of samples are measured by targeted proteomics over several weeks. In targeted proteomics experiments a few peptides are selected to be used as surrogates for protein quantification, and therefore, their stability becomes crucial for high accuracy and precision protein quantification. Here we experimentally assessed the stability of the human proteotypic proteome, including the measurement by LC-MS/MS of over 100,000 different tryptic peptides. Finally, we used the generated dataset to train a deep neural network to predict the stability for any amino acid sequence, extending the utility of the model to any other species and tryptic peptide of interest

Poster 6

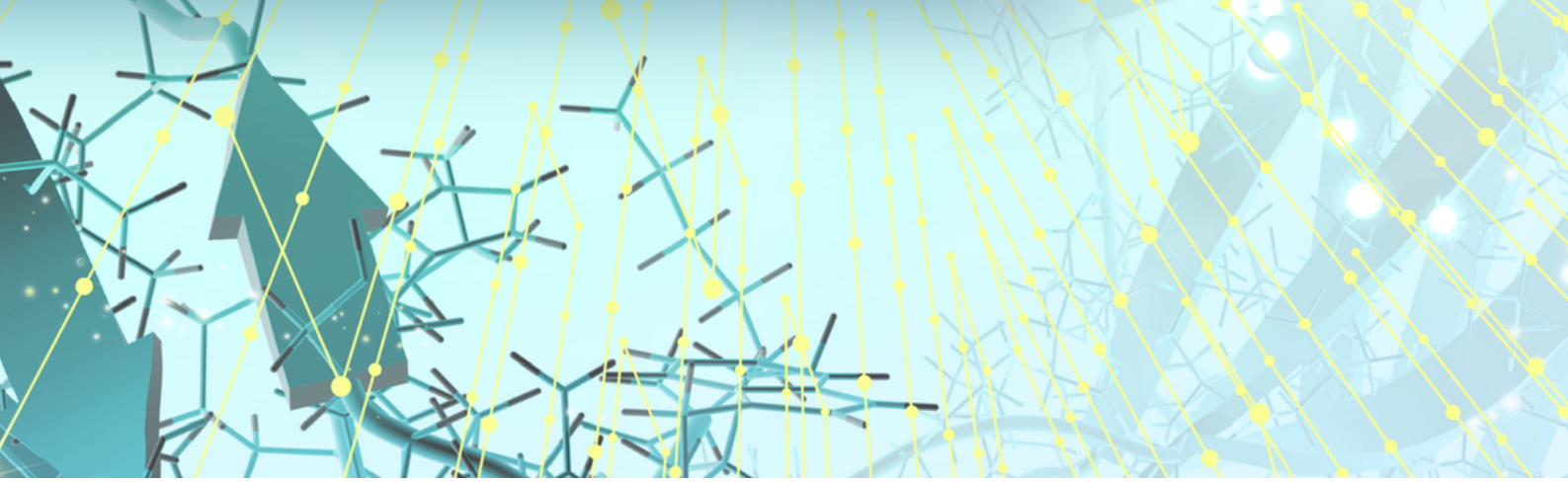
Federica Anastasi

BarcelonaBeta Brain Research Centre

"Discovery of plasma modulators of brain ageing as a novel therapeutic target for Alzheimer's disease using DIA proteomics"

PParabiosis experiments in mice identified circulating blood proteins that have an effect on brain ageing and thus exhibit a great potential as Alzheimer's disease therapeutic target in humans. We aim to identify human circulating proteins able to modulate brain ageing.

Using magnetic resonance imaging data from cognitively unimpaired individuals of the ALFA cohort (N=1414) we computed the brain-age of these subjects. Individual brain-age is estimated and subtracted from chronological age (DeltaAge) to identify individuals with extreme phenotypes (accelerated/decelerated brain aging) stratified by sex (n=85 each group). We also recruited healthy individuals with extreme chronological age [30 young (18-25yo) and 30 old (70-84yo)]. We then optimized a high-throughput proteomics workflow for the analysis of plasma based on StageTip protein digestion followed by a 60 minute LC gradient in a 15 cm column and data-independent-acquisition (DIA) in a tribrid mass spectrometer (Orbitrap Eclipse, Thermo). Data analysis was performed with DIA-NN. To define the differences in the plasma proteome related to age we initially applied t



he optimized DIA workflow to the analysis of plasma samples of the extreme chronological age individuals. Proteins whose expression is linked to ageing are identified by comparing their relative abundances among the extreme groups.

We quantified around 3500 precursors and 300 unique proteins consistently in all samples and identified 52 proteins that significantly change with chronological age. Extreme brain-age groups plasma samples are also being analysed to identify proteins exhibiting brain age related changes.

We are establishing the plasma proteome that changes according to chronological and brain ageing by applying a DIA proteomics workflow. To achieve this, we used both an extreme chronological and an extreme brain-age groups. These analyses will be integrated with plasma metabolomics of the same subjects to better unravel the mechanisms associated with variability in brain aging.