ABSTRACTS

# CRG Core Facilities Technology Symposium: "Applying proteomics to life sciences: from ions to biology"

16 November 2012
PRBB Auditorium, Barcelona


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## Mass cytometry to comprehensively study single cell signaling in biology and disease


Cancer cells and their signaling networks are highly heterogeneous, thereby driving and maintaining the disease. The structure of these networks and their change during disease or therapy is often unknown. Therefore the definition and analyses of the signaling networks between cell subsets and states and how these respond to drug treatment and other perturbations at the single-cell is highly desirable. Here a novel high throughput workflow based on a next-generation single cell mass cytometry instrument is presented. Mass cytometry allows simultaneous quantification of up to 100 proteins and phosphorylation sites on the single-cell level, thereby the signaling and cellular state can be measured within accurately defined cell types and subpopulations. To add high-throughput capacity to mass cytometry, a cell-based multiplexing technique was developed, called mass-tag cellular barcoding (MCB). Here each cell sample is labeled with a unique combination of mass-tags, mixed with other samples before antibody staining, and then deconvoluted after measurement. This strategy now allows measuring thousands of samples per day, strongly reduces antibody costs and greatly increases the quality of the data due to the homogenous cell labeling. Potential applications for high-content MCB analysis range from drug and RNAi screens to biological discovery. To illustrate the power of the approach for screening applications, the effects of kinase inhibitors on peripheral blood mononuclear cells signaling networks was comprehensively profiled. Each inhibitor was tested at eight dilutions against 12 conditions and levels of 14 intracellular signaling molecules in 14 cell types were evaluated, resulting in 18,816 individual quantified cell populations from a single tube of multiplexed cells. These analyses revealed complex signaling network responses and correlations, enabling to classify each cell population and inhibitor with unprecedented accuracy.

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## ALBERT HECK

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## Enabling technologies in (phospho)proteomics

In this lecture I will first describe the development and applications of a new enrichment method in phosphoproteomics based on polymer-based Ti4+-IMAC, that outperforms TiO2 by a factor of three and is very efficient and ultimately suitable for usage with low sample amounts.

Next I address label free quantitation methods based on spectral counts and/or ion intensities describing how they may be compromised when solely using trypsin for protein digestion. I also will discuss how this may effect other trypsin-only based assays like SRM/MRM.

I will describe the use of dimethyl chemical labeling based quantification methods in MS-based proteomics to profile in-depth difference in protein expression in between FACS sorted adult stem and their daughter cells. In combination with deep transcriptome profiling this work has lead to a definition of the intestinal stem cell proteome.

I will describe a new Orbitrap enabling the detection of single ions of megaDa complexes at very high resolving power opening up was for top-down proteomics and detailed analysis of intact proteins, protein complexes and biopharmaceuticals.

Finally, a modified Orbitrap enabling us to use HCD and ETD on single ion populations will be described, showing the huge benefit of such a platform in for instance de novo sequencing.

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## PAOLA PICOTTI

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## Studying protein networks in health and disease

Protein networks control virtually all cellular processes and respond to external stimuli by changing the abundance, localization, association state, conformational properties or modification degree of their components. The capability to capture such responses is of crucial importance to model the properties of protein networks and predict their behavior. Selected Reaction Monitoring (SRM) is a targeted mass spectrometry (MS) technique which emerged in the field of systems biology as a tool for the targeted, multiplexed analysis of all the components of a protein network, throughout a variety of perturbing conditions. The technology is however still in its infancy for protein analysis and several challenges need to be addressed to demonstrate its power in biological and biomedical research. Recent expansions of the SRM concept, including complete proteome maps of MS coordinates and new MS methods based on data-independent acquisition and targeted data analysis appear to be promising directions to increase the widespread application of SRM-based approaches in non-specialized laboratories.

In this presentation, the applicability of targeted proteomic tools to studying the dynamics of biological protein networks will be presented. The potential and limitations of the approach will be discussed, using examples of protein networks responding to physiological and pathological stimulations. I will also show how we are currently applying such tools to studying the responses of cells to the formation of intracellular protein aggregates and the possibility to modulate such (toxic) process.

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## JUDITH VILLÉN

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## Protein phosphorylation in cellular signaling and degradation

Protein posttranslational modifications (PTMs) function as highly versatile signaling units integral to almost every cellular process. Among all PTMs, protein phosphorylation plays a prominent role in regulating the execution of discrete cellular functions and is the main driver of signaling networks. Our research focuses on the development of mass spectrometry-based proteomics technologies to study protein phosphorylation on a largescale and the application of these technologies into signaling biology. Current proteomic methods can identify thousands of individual phosphorylation events per experiment and allow answering key signaling questions on a systems level.

We are currently investigating the biology of cellular information processing. Towards this goal, we leverage state-of-the-art proteomics, pharmacology, and network control theory to systematically to systematically interrogate the signaling networks. In particular, we are focusing our studies in the insulin/PI3K/Akt signaling pathway. We use kinase inhibitors to perturb this signaling pathway, and interrogate the effects on the globalphosphoproteome. In these studies, we are identifying novel protein effectors of the insulin/PI3K/Akt pathway, and learning about the topology of the signaling network.

On a separate project, we are studying the effects of protein phosphorylation on the attachment of other modifications. PTMs do not exist in isolation crosstalk between different types of modifications on the same protein molecule is utilized to add specificity and combinatorial logic to signal processing. Proteomics has been a powerful tool to characterize a PTMs; however, little is known about the occupancy of multiple modification types on the same protein. I will present two new methods to analyze proteins comodified with ubiquitin and phosphorylation, and the application of these methods to study the effects of phosphorylation on protein degradation by the ubiquitinproteasome system.