CRG Proteomics Symposium:
"Applying proteomics to life sciences: from ions to biology"

13 November 2015
PRBB Auditorium, Barcelona

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An integrated multi-layered proteomics approach reveals molecular switches controlling biased ligand-dependent EGF receptor trafficking and cellular responses

Quantitative phosphoproteomics is a powerful technology for unbiased analysis of cell signaling networks. One of the most intriguing and unanswered questions in the field of cell signaling concerns how stimulation of the same receptor with distinct ligands generates specific cellular outputs. The concept of biased ligand signaling is well-established in the field of G-protein coupled receptors, where different ligands binding to the same receptor activates full or only partial downstream signaling networks. This notion of functional selectivity by ligands activating the same receptor is now emerging as an equally important concept in receptor RTK signaling, as we have recently shown for FGF receptor family. Here we performed quantitative interaction proteomics, phosphoproteomics, and to delineate EGF receptor signaling dynamics activated by the recycling ligand TGF-α or by EGF, a ligand that induces receptor degradation. Although the majority of regulated phosphoproteins and interaction partners are shared between the two ligands, their dynamics are often different. We found that EGF induce transient phospho-signaling, whereas TGF-α activates sustained phospho-signaling, which leads to increased cell proliferation and migration in several EGF receptor-positive cancer cell lines. In particular, proteins that are part of the endocytic machinery were differentially regulated and from these we identified and functionally validated 'cellular switches' that control the endocytic trafficking of the EGF receptor and ultimately decide the cellular outcome. By manipulating the protein level or tyrosine phosphorylation of these endocytic proteins we can switch the cellular TGF-α response to an EGF-like response and vice versa. These results, based on a multidisciplinary approach, which combines multi-layered proteomics and functional assays, identify ligand-dependent mechanisms for the control of EGFR intracellular fate and for the specification of long-term responses.

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Progesterone receptor interactome in breast cancer cells

We are interested in understanding the role of chromatin dynamics and the mechanisms governing gene regulation in the progesterone responsive T47D breast cancer cell line. Within this context, this project focuses specifically on identifying changes of the progesterone receptor (PR) interacting proteins in response to hormone. Using T47D cells exposed to progestins for different time periods followed by crosslinking with formaldehyde, chromatin immunoprecipitation with a PR antibody and LC-MS/MS, we detected peptides from more than 2000 proteins. We used SAINT analysis software to filter the high-confidence protein hits, and found several of the already known interactors of PR, as well as many novel interacting partners.
We are currently analysing these results, both with respect to function and dynamics, to gain further insight into the sequence and hierarchy of the PR interacting complexes. Our final goal is to understand how the PR regulates gene expression upon hormone exposure in breast cancer cells, ultimately to identify new potential targets for combinatorial breast cancer treatment.

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High-throughput proteomics reveals the unicellular roots of animal phosphosignaling and cell differentiation

Cell-specific regulation of protein levels and activity is essential for the distribution of functions among multiple cell types in animals. The finding that many genes involved in these regulatory processes have a premetazoan origin raises the intriguing possibility that the mechanisms required for spatially regulated cell differentiation evolved prior to the appearance of animals. Here, we use high-throughput proteomics in Capsaspora owczarzaki, a close unicellular relative of animals, to characterize the dynamic proteome and phosphoproteome profiles of three temporally distinct cell types in this premetazoan species. We show that life-cycle transitions are linked to extensive proteome and phosphoproteome remodeling and that they affect key genes involved in animal multicellularity, such as transcription factors and tyrosine kinases. The observation of shared features between Capsaspora and metazoans indicates that elaborate and conserved phosphosignaling and proteome regulation supported temporal cell-type differentiation in the unicellular ancestor of animals.

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A physical map of a human cell

Compartmentalization is essential for all complex forms of life. In eukaryotic cells, membrane-bound organelles, as well as a multitude of protein- and nucleic acid-rich subcellular structures, maintain boundaries and serve as enrichment zones to promote and regulate protein function. Consistent with the critical importance of these boundaries, alterations in the machinery that mediate protein transport between these compartments has been implicated in a number of diverse diseases. Understanding the composition of each cellular "compartment" (be it a classical organelle or a large protein complex) remains a challenging task. For soluble protein complexes, approaches such as affinity purification or other biochemical fractionation methods coupled with mass spectrometry can provide important insights, but this is not the case for detergent-insoluble components. Classically, both microscopy and organellar purifications have been employed for identifying the composition of these structures, but these approaches have limitations, notably in resolution for standard high-throughput fluorescence microscopy and in the difficulty in purifying some of the structures (e.g. p-bodies) for approaches based on biochemical isolations. Prompted by the recent implementation of *in vivo* biotinylation approaches such as BioID, we have begun the systematic mapping of the composition of various subcellular structures, using well-characterized markers for specific locations. We report here our low-resolution map of a human cell, but also a higher resolution map of RNA-containing cellular structures, including the p-bodies and stress granules that regulate mRNA stability.

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Deconstructing the pluripotency of embryonic stem cells by proteomic approaches

Two major requisites in cell replacement-based therapies are the preservation of cell identity during *ex vivo* expansion of pluripotent embryonic stem cells (ESCs) and the differentiation towards a specific and clinically relevant cell type. The self-renewing nature of ESCs is a consequence of their ability to proliferate indefinitely while maintaining pluripotency, which is capacity to differentiate into all adult cell types. Despite the remarkable scientific advances in our knowledge about the mechanisms controlling ESCs pluripotency, several fundamental questions remain unresolved: How is ESCs cell division coordinated with self-renewal and differentiation? How is the epigenetic state of ESCs restored across cell division? How is differentiation plasticity achieved by an epigenetically equivalent group of cells during early embryo development? By combining unbiased-exploratory and hypothesis-driven approaches, we are addressing these questions in order to gain insights into the biology of stem cells and to develop protocols to manipulate ESCs towards a specific cellular outcome, which is a major goal in regenerative medicine and drug discovery.

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Mass spectrometry assay predicts patient conversion to multiple sclerosis

In the last few years mass spectrometry has emerged as a powerful analytical technique to discover protein biomarkers for neurological diseases. Multiple sclerosis is a chronic inflammatory disease of the central nervous system caused by the interaction of genetic and environmental factors. In most patients, the disease initiates with an episode of neurological disturbance referred to as clinically isolated syndrome. However, not all patients with this syndrome develop multiple sclerosis over time. Here we used both discovery and targeted mass spectrometry to identify protein combinations able to classify patients into those that will eventually develop multiple sclerosis and those that won't.

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Translating big data coming from spatial metabolomics into molecular knowledge

Spatial metabolomics is emerging as a powerful approach to localize hundreds of metabolites directly from sections of biological samples or on biological surfaces. The grand challenges are in the visualization and molecular interpretation of big data generated to provide a mechanistic and systemic understanding of the analyzed biological systems. Existing bioinformatics tools cannot be applied directly because of the sheer size and high complexity of the data reaching hundreds of gigabytes per sample or individual. We will present new bioinformatics tools we developed in two projects. In the first project recently published in PNAS, we created molecular and microbial maps of the human skin surface by combining both mass spectrometry and 16S rRNA sequencing approaches. This allowed us to reveal that the molecular composition of skin has diverse distributions and that the composition is defined not only by skin cells and microbes but also by our daily routines, including the application of hygiene products. The developed software is available as a Google Chrome app `ili. In the European project METASPACE, we challenged ourselves with creating FDR-controlled metabolite annotation of HR imaging MS data. We will show preliminary results combining novel algorithms and modern big data technologies applied to 3D cell spheroids, microbial agar plates, and biological tissues.