PROTEOMICS CORE UNIT

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OVERVIEW

Proteins catalyse and control almost all cellular processes in a living cell. The levels of protein abundance, together with their modification states and interactions, adapt dynamically to external or internal (genetic) stimuli and thus define the cell's functional state and determine its phenotype. Recent developments in sample preparation, liquid chromatography, mass spectrometry and data analysis have enabled researchers to investigate diverse proteomic facets in a systematic high-throughput manner, currently comparable to next-generation sequencing platforms. As a result, proteomics is positioned as one of the most powerful technologies to study, at the protein level, complex cellular processes. This vast amount of data is providing new insights into the molecular mechanisms underlying diverse human pathologies such as cancer.

"Mass spectrometry-based technologies enable probing the composition, structure, function and regulation of the proteome, providing new insights into the underlying mechanisms of cancer."

Technicians
Fernando García (TS)*, Nuria Ibarz (TS)*, Ailyn Martínez (since April), M. Isabel Ruppen (TS)*,

Pilar Ximénez de Embún (TS)*, Eduardo Zarzuela (TS)* (PEJ-L)**

**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

Student in Practice
Julia Beltran (since October)

RESEARCH HIGHLIGHTS

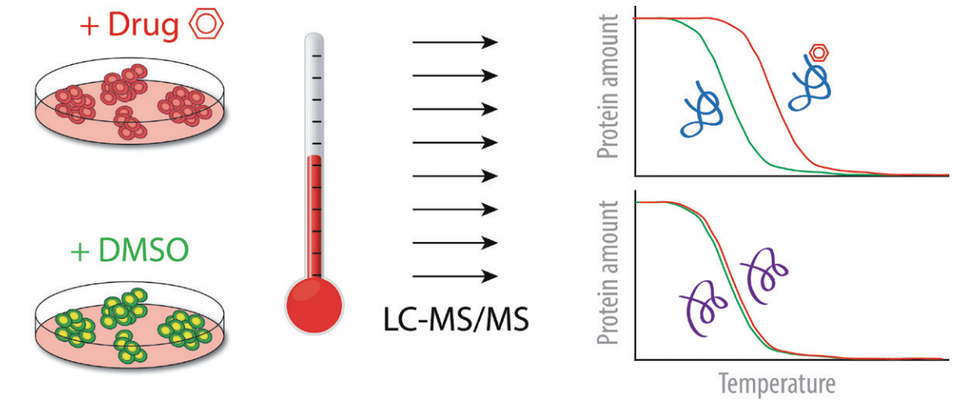

Figure Thermal proteome profiling (TPP). Cells are treated either with the compound of interest or the vehicle. Cells are subjected to increasing temperatures, and denatured proteins are discarded by centrifugation. Supernatants are analysed by LC-MS/MS enabling the reconstruction of the melting curves for all identified proteins. Proteins showing a difference in their melting point between drug and vehicle might be potential targets.

Throughout 2016, the Unit continued its mission of implementing and optimising quantitative proteomic strategies. More specifically, we have introduced a new fractionation method using high pH reverse phase micro columns, which minimises sample loss and thus is highly suitable for low amounts of material. We used this approach to post-fractionate samples enriched in phosphopeptides, substantially increasing the number of identifications. This workflow was used to determine phosphorylation dynamics upon activation of WT and kinase-mutant platelets (in collaboration with the Cell Division and Cancer Group), as well as to identify potential substrates of CDK8 involved in the establishment of ground state pluripotency (in collaboration with the CNIO Tumour Suppression Group). More recently, in collaboration with the Metabolism and Cell Signalling Group at the CNIO, we also used phosphoproteomics to better understand the molecular mechanism of the mTOR pathway. Together with the CNIO Genomic Instability Group, we are using a recent approach, named Thermal Proteome Profiling (Savitski *et al.*, see FIGURE), to identify protein targets of certain inhibitors (e.g. target deconvolution). We have also performed several AP-MS/MS experiments for different proteins (STAG1, STAG2, PDS5A, PDS5B) belonging to the cohesion complex (with CNIO's Chromosome Dynamics Group). Likewise, we have identified a large protein network (more than 300 proteins) that interacts with the RNA pol II complex (in collaboration with the Tumour Suppression Group). Over the last few years, the analysis of the protein content of exosomes has received great interest in the context of metastasis and the pre-metastatic niche. Along this line, we are conducting several proteomic analyses of exosomes from different origins in collaboration with CNIO's Microenvironment and Metastasis Group, the Gastrointestinal Cancer Clinical Research Unit and the Melanoma Group. ■

PUBLICATIONS

- Lecona E, Rodriguez-Acebes S, Specks J, Lopez-Contreras AJ, Ruppen I, Murga M, Muñoz J, Mendez J, Fernandez-Capetillo O (2016). USP7 is a SUMO deubiquitinase essential for DNA replication. *Nat Struct Mol Biol* 23, 270-277.
- Navarro P, Bueno MJ, Zagorac I, Mondejar T, Sanchez J, Mourón S, Muñoz J, Gómez-López G, Jimenez-Renard V, Mulero F, Chandel NS, Quintela-Fandino M (2016). Targeting Tumor Mitochondrial Metabolism Overcomes Resistance to Antiangiogenics. *Cell Rep* 15, 2705-2718.
- Martinez-Val A, Garcia F, Ximénez-Embún P, Ibarz N, Zarzuela E, Ruppen I, Mohammed S, Munoz J (2016). On the Statistical Significance of Compressed Ratios in Isobaric Labeling: A Cross-Platform Comparison. *J Proteome Res* 15, 3029-3038.