PROTEOMICS CORE UNIT

Javier Muñoz
Core Unit Head

Post-Doctoral Fellow
Francisco J. Sánchez (until June)

Graduate Students
Cristina Sayago

OVERVIEW

Current advances in high-throughput techniques represent a paradigm-shift and have revolutionised biomedical research. Omics technologies provide an unbiased view of a biological system and can be used to test and generate novel hypotheses. Proteins are the molecular effectors of cells and transcriptomics merely represents a proxy to estimate the final protein product. Moreover, genomic data do not provide information about the post-translational modifications of proteins or their interactions. Thus, direct interrogation of proteins is of paramount importance. Proteomics is a discipline that aims to understand the complex regulation of the proteome and its impact on disease. However, global analysis of proteins is challenging owing to their high complexity and high dynamic range. To tackle these analytical challenges, proteomics uses a combination of sample preparation, mass spectrometry (MS) and bioinformatics. The CNIO Proteomics Core Unit provides MS-based proteomics to research groups in order to better understand, at the proteome level, the molecular basis of cancer.

"Direct analysis of proteins through mass spectrometry-based proteomics is essential to fully understand the underlying mechanisms of cancer."

Technicians
Enrique Alonso (TS)*, Fernando García (TS)*, Julia Isabel Morales (since December) (TS)˙, Jana Sánchez (TS)*, Álvaro Soriano (until January) (PEJ, CAM)**, Pilar Ximénez De Embún (TS)*, Eduardo Zarzuela (TS)*

˙*Titulado Superior* (Advanced Degree)
**Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

RESEARCH HIGHLIGHTS

In 2020, the Proteomics Unit participated in multiple projects that demanded advanced proteomics. In collaboration with the Melanoma Group, we investigated the role of MIDKINE in the tumour micro-environment. We analysed the downstream effects (loss-of-function and gain-of-function) of this secreted factor with respect to inflammatory processes. Together with the former Tumour Suppression Group, we showed that inhibition of CDK8, a negative regulator of the Mediator complex, activates super-enhancers of key identity genes and stabilises the so-called naïve pluripotent state. Using phosphoproteomics, we analysed the early effects of a CDK8i to identify downstream effectors that could play a role in this process. Moreover, we collaborated in other projects related to, among others, the identification of biomarkers for lymphatic-promoted disorders (with the Microenvironment and Metastasis Group) and the impact of BCL7A mutations in the interactome of the SWI/SNF complex in diffuse large B-cell lymphoma (DLBCL). Finally, the Unit worked on implementing new technologies to the range of available services. Recent advances in machine learning have made it possible to predict fragmentation spectra from peptide sequences. This has pushed forward the implementation of Data-Independent Acquisition (DIA)-based methods. Unlike Data Dependent Acquisition (DDA), DIA sequentially fragments the entire m/z range using wide isolation windows (8-20 m/z). The deconvolution of high multi-plexing spectra is achieved by software like EncyclopeDIA. Empirically-corrected libraries can be easily generated from a representative pool of samples that is analysed by Gas Phase Fractionation (GPF) using lower isolation windows. This strategy enables 6000-8000 proteins from cell lysates to be identified in 90 minutes of instrument time, representing a promising approach for quantitative proteomic studies. ■

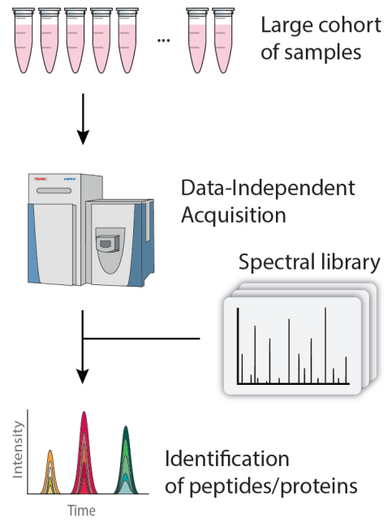

FIGURE Data-Independent Acquisition enables fast and cost-effective profiling of proteomes with a significant coverage depth. This will facilitate studies that require comparisons for a large number of conditions and biological replicates.

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