SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

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OVERVIEW

This Unit focuses on the technical and scientific management of Nuclear Magnetic Resonance (NMR) Spectroscopy and molecular biophysics instrumentation available at the Structural Biology Programme. It provides CNIO researchers with equipment and experimental support for a variety of techniques used in biophysical studies of molecules involved in cancer. This includes the *in vitro* characterisation of the structure and dynamics of proteins by NMR, and characterisation of the affinity and kinetics of the interactions of proteins with other biopolymers and small molecules that could represent initial hits in the drug discovery process, or serve as research compounds for biophysical and functional studies. Furthermore, we use NMR to characterise the metabolic profiles of biofluids, cell growth media, and cell and tissue extracts from both animal models of cancer and human samples. In addition, in 2020, we adopted a mass spectrometer for the characterisation of intact proteins and for metabolite studies using HPLC-MS methods.

"In 2020 we initiated work with a QTOF mass spectrometer that will allow the quality control of purified proteins from their intact mass spectrum, as well as the targeted characterisation or profiling of metabolites in liquid samples of cancer model systems."

RESEARCH HIGHLIGHTS

The Unit provides a broad range of instrumentation for the biophysical characterisation of biomolecules and their interactions, including spectrophotometers, a fluorimeter, isothermal titration and differential scanning calorimeters, a circular dichrograph, dynamic and multi-angle static light scattering devices, and two biosensor instruments: surface plasmon resonance (SPR), and biolayer interferometry (BLI). Research Groups mostly from, but not limited to, the Structural Biology Programme used these technologies throughout 2020 (i.e., the Haematological Malignancies Clinical Research Unit, the Monoclonal Antibodies Unit, and the Experimental Therapeutics Programme – ETP).

The Unit hosts a 700 MHz NMR spectrometer that is equipped with probes and a sample changer to run up to 120 samples automatically. This provides medium throughput for the screening of small molecule protein binders (together with ETP), as well as for metabolite quantification that, in 2020, was done in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (ETP), and the Growth Factors, Nutrients and Cancer and Metabolism and Cell Signalling Groups (Molecular Oncology Programme). During 2020, we incorporated a QTOF mass spectrometer that will complement our battery of techniques for the quality control of purified proteins with the information contained in their intact mass spectra. For example, we examined several reference proteins (see FIGURE), verifying that the instrument can determine the mass with high precision and accuracy employing nanogram amounts. In addition, HPLC-MS measurements of biofluids were also initiated. Collectively, with our client groups, we will continue implementing sample preparation protocols and developing spectroscopic and analytical tools to characterise metabolites present in different biological samples. ■

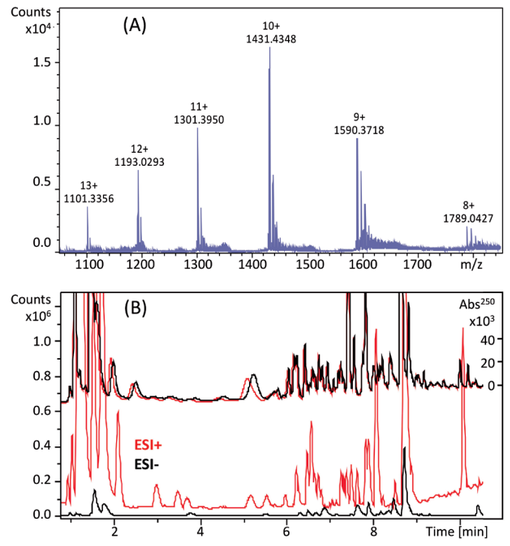

FIGURE (**A**) Mass spectrum of perfused lysozyme (20 μg/mL at 3 μl/min) with indication of the identified cationic forms (+13 to +8), which allows determination of a mass of 14304 Da (expected 14313 Da). (**B**) HPLC-MS chromatograms of human urine injected in a reverse-phase column as followed by base peak ion counts (lower traces, left scale) and UV absorbance (upper traces, right scale). Two consecutive runs of the same sample measured in positive (red) and negative (black) Electro Spray Ionization mode are shown.

PUBLICATIONS

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- Sanz-Castillo B, Hurtado B, El Bakkali A, Salvador B, Martínez D, Santiveri CM, Campos-Olivas R, Ximénez P, Muñoz J, Álvarez-Fernández M, Malumbres M (2020). A cell cycle kinase-phosphatase module restrains PI3K-Akt activity in an mTORC1-dependent manner. *BioRxiv*. https://doi.org/10.1101/2020.11.26.399915doi.