SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

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Titulado Superior (Advanced Degree)

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 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 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 2021, we successfully installed a multiple well microplate reader that includes numerous detectors (absorbance, fluorescence intensity, polarisation and time resolved modes, luminescence, alphascreen, etc.) with excellent sensitivity for both in-solution and adherent cells measurements.

"In 2021 we installed a new and versatile multiple well microplate reader that will allow, among other HT applications, quantification, inhibitor discovery, affinity determination and enzyme kinetics on liquid samples, as well as viability and other functional assays on cell samples."

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, two biosensor instruments — surface plasmon resonance (SPR) and biolayer interferometry (BLI) — and a multiple well microplate reader with numerous detectors. Research groups mostly from, but not limited to (i.e., Haematological Malignancies Clinical Research Unit, Monoclonal Antibodies Unit, Molecular Oncology Group and the Experimental Therapeutics Programme – ETP) the Structural Biology Programme used these technologies throughout the year.

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 the Experimental Therapeutics Programme), as well as for metabolite quantification that in 2021 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). In collaboration with the latter group, we also implemented protocols to detect intracellular metabolites derived from the chemotherapeutic drugs 5-F-uracil and 5-F-uridine, using ^{19}F-NMR spectroscopy. For example, FIGURE 1 shows representative spectra that enable characterisation of the metabolic conversion of the drug into different nucleotides and activated sugars, and how it is affected by overexpressing an enzyme involved in purine metabolism and a mutant thereof, as well as the effect of chemical inhibitors acting upstream in the metabolic pathway. 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. ■

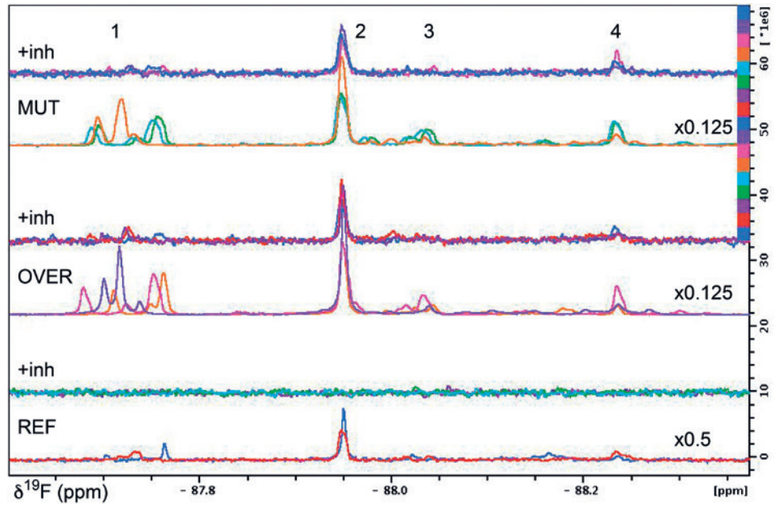

FIGURE 1 Superposition of the ^{19}F-NMR spectra of polar cell extracts following treatment with 100 μM 5-F-uridine for 6 hours. From bottom to top, samples correspond (in triplicate) to control (REF), overexpression of a purine metabolism gene (OVER), and overexpression of an inactive, mutant form of that gene product (MUT). In each of these 3 groups, alternating spectra of control cells (bottom) and of those treated with a chemical inhibitor (+inh, top) of an upstream positive regulator enzyme in the pathway are displayed. The 3 different groups of control spectra (non-treated with inhibitor) are vertically scaled as indicated to facilitate comparison. Tentative assignments of the signals to specific metabolites derived from 5-F-uridine are numbered: 5-F-UDP-hexoses (1), 5-F-UTP (2), 5-F-UDP (3), unknown (4).

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