SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

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OVERVIEW

The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance Spectroscopy (NMR) and of other biophysical instrumentation available through the Structural Biology and Biocomputing Programme. It provides CNIO researchers with instrumentation and technical support for a variety of spectroscopic and biophysical techniques. This includes the application of NMR to the in vitro characterisation of the structure and dynamics of biomolecules (proteins in particular) and their interactions with other biopolymers, as well as with small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical 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 2016, we quantified metabolites from cell media, mice blood and liver extracts, thereby contributing to the understanding of the cellular and physiological metabolic responses to fasting and to oncogene activation, which are important aspects of tumour biology.”

RESEARCH HIGHLIGHTS

Our Core Unit incorporates 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, a multi-angle static light scattering apparatus, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to, the Structural Biology and Biocomputing Programme have extensively used these technologies throughout 2016. For example, we reported the results of a multidisciplinary intra-Programme collaboration this year (with the former Computational Biophysics Group), illustrated in the FIGURE, which combines various NMR and SPR experiments with enhanced sampling molecular dynamics simulations to shed light on the conformational dynamics associated with the binding of Imatinib to the proto-oncogene c-Src. We found that both conformational selection and induced fit play a role in the binding mechanism, reconciling opposing views held in the literature.

The Unit hosts a 700 MHz NMR spectrometer, which is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provided the required throughput for screening small molecule protein binders (together with the CNIO’s Structural Biology and Biocomputing and Experimental Therapeutics -ETP- Programmes), as well as for metanobomics measurements that were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Tumour Suppression Group (from the Molecular Oncology Programme), as well as the Genes, Development and Disease and the Growth Factors, Nutrients and Cancer Groups (from the Cancer Cell Biology Programme). Collectively, with these and previous groups, we implemented sample preparation protocols and developed spectroscopic and analysis technology to characterise the metabolites present in different biological samples, as illustrated by two important publications.

PUBLICATIONS


Figure Free energies for local unfolding represented on the backbone structure of the kinase domain of Src in its free form (left) as well as their variation upon binding to Imatinib (right). The indicated values are coded both in the colour and in the thickness of the backbone coil. They were derived from the rates of exchange of backbone amide protons measured for Imatinib. EPR measurements for all but grey-coloured residues. Red circles indicate α-helices (αD and αE), the regions with increased exposure to the solvent in the bound structure.