# SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

Ramón Campos-Olivas
Unit Head

Technician
Clara M. Santiveri (TS)*

**Titulado Superior* (Advanced Degree)

## 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 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 metabonomics 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. ■

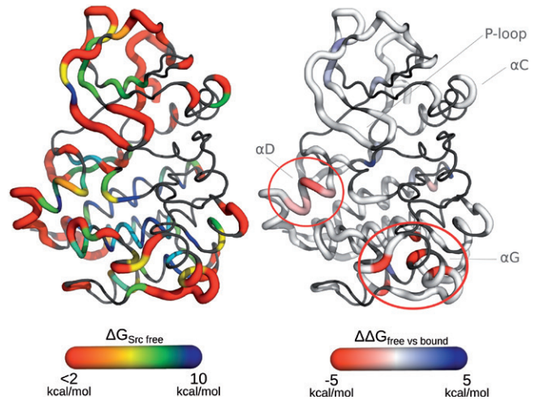


**Figure** Free energies for local unfolding represented on the backbone structure of the kinase domain of Src in its free form (left) as well as their variation upon binding to Imatinib (right). The indicated values are coded both in the colour and in the thickness of the backbone coil. They were derived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

### PUBLICATIONS


- Burén S, Gomes AL, Teijeiro A, Fawal MA, Yilmaz M, Tummala KS, Perez M, Rodriguez-Justo M, Campos-Olivas R, Megías D, Djouder N (2016). Regulation of OGT by URI in Response to Glucose Confers c-MYC-Dependent Survival Mechanisms. *Cancer Cell* 30, 290-307.
- Fernández FJ, Ardá A, López-Estepa M, Aranda J, Peña-Soler E, Garces F, Round A, Campos-Olivas R, Bruix M, Coll M, Tuñón I, Jiménez-Barbero J, Vega MC (2016). Mechanism of sulfur transfer across protein-protein interfaces: The cysteine desulfurase model system. *ACS Catal*, 6, 3975–3984.
- Assi HA, Harkness RW 5th, Martin-Pintado N, Wilds CJ, Campos-Olivas R, Mittermaier AK, González C, Damha MJ. (2016). Stabilization of i-motif structures by 2'-ß-fluorination of DNA. *Nucleic Acids Res* 44, 4998-5009.
- López-Guadamillas E, Fernández-Marcos PJ, Pantoja C, Muñoz-Martin M, Martínez D, Gómez-López G, Campos-Olivas R, Valverde AM, Serrano M (2016). p21Cip1 plays a critical role in the physiological adaptation to fasting through activation of PPARα. *Sci Rep* 6, 34542.
- Morando MA, Saladino G, D'Amelio N, Pucheta-Martinez E, Lovera S, Lelli M, López-Méndez B, Marenchino M, Campos-Olivas R, Gervasio FL (2016). Conformational Selection and Induced Fit Mechanisms in the Binding of an Anticancer Drug to the c-Src Kinase. *Sci Rep* 6, 24439.
- Bayó-Puxan N, Rodríguez-Mias R, Goldflam M, Kotev M, Ciudad S, Hipolito CJ, Varese M, Suga H, Campos-Olivas R, Barril X, Guallar V, Teixidó M, García J, Giralt E. (2016). Combined Use of Oligopeptides, Fragment Libraries, and Natural Compounds: A Comprehensive Approach To Sample the Druggability of Vascular Endothelial Growth Factor. *ChemMedChem* 11, 928-939.
- Barbero N, Marenchino M, Campos-Olivas R, Oliaro-Bosso S, Bonandini L, Boskovic J, Viscardi G, Visentin S (2016). Nanomaterials-protein interactions: the case of pristine and functionalized carbon nanotubes and porcine gastric mucin. *J Nanopart Res* 18, 84.


### Book Chapter


- Santiveri CM, López-Méndez B, Huecas S, Alfonso C, Luque-Ortega, JR, Campos-Olivas R. A biophysical toolkit for molecular interactions. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net/ In press (A27015).