Spectroscopy and Nuclear Magnetic Resonance *Core Unit*

Ramón Campos-Olivas

Unit Head

Ramón Campos-Olivas, born in Albacete in 1969, obtained his Bachelor of Science Degree in Chemistry from the University of Strathclyde (Scotland) in 1991, and graduated in Physical Chemistry and Biochemistry from the *Universidad Complutense de Madrid* in 1992 and 1995, respectively.

After obtaining his PhD in Chemistry in 1997 for work on NMR studies of proteins in M. Rico's Group at the *Consejo Superior de Investigaciones Científicas* (CSIC), Madrid, he carried out his postdoctoral research in the USA under the supervision of M. F. Summers at the Howard Hughes Medical Institute, the University of Maryland, Baltimore, and A. M. Gronenborn at the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institutes of Health (NIH), Bethesda, on the application of NMR spectroscopy to characterise diverse proteins of biomedical interest.

Shortly after returning to the *Consejo Superior de Investigaciones Científicas* in 2001, he obtained a *Ramón y Cajal* research contract from the Spanish Government and joined the CNIO's NMR Group in 2002.

Since 2005 he has been an Honorary Professor of Biochemistry at the *Universidad Autónoma de Madrid*. In February 2008 subsequent to the regrouping of NMR activity and focus at the CNIO, he was appointed as Head of the Spectroscopy and Nuclear Magnetic Resonance Core Unit.

Summary

Our Core Unit was created in 2008 to unify the technical and scientific management of nuclear magnetic resonance spectroscopy (NMR) as well as other biophysical instrumentation. It provides CNIO researchers with intrumentation and technical support for a variety of optical spectroscopy and other biophysical techniques, including NMR for the characterisation of biomolecules (proteins in particular) and their interactions with other biomolecules and small molecules that could represent initial hits in the drug discovery process or research compounds for biophysical and functional studies, as well as the NMR characterisation of metabolic profiles of biofluids, cells and intact tissues.

Main Objectives

- Maintain instrumentation for the biophysical characterisation of biomolecules and their interactions, providing all the necessary guidance for their use

- Conduct NMR-based screening to identify and characterise the binding of small molecules to protein targets, potentially providing hits for drug discovery research and chemical tools for biophysical and functional applications

- Support research by offering NMR application to both the structural and dynamic characterisation of proteins as well as the metabolic profiling of biofluids, cells and intact tissues from animal models and humans

Technician: Marco Marenchino.

Highlights

This 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 surface plasmon resonance (BIACORE) machine, analytical ultracentrifugation, as well as an NMR spectrometer of 700 MHz which is well equipped with probes (HR-MAS, dual fluorine/proton, and triple and quadruple resonance).

The NMR spectrometer is equipped with a sample changer capable of running up to 120 samples without human intervention, providing the throughput for NMR-based screening for small molecule protein binders, as well as metabonomics measurements, which are performed in collaboration with the CNIO´s Tumour Markers Group (Molecular Pathology Programme), Tumour Suppression Group (Molecular Oncology Programme) and Breast Cancer Clinical Research Unit (Clinical Research Programme).

An example of this latter collaboration with the Breast Cancer Clinical Research Unit is depicted in the figure which shows comparison of proton NMR spectra of intact tissues from mouse mammary tumours and the corresponding control hearts. Regarding the first NMR application, a collection of ~370 fluorinated small molecule compounds was assembled that have been screened against selected protein targets using ligand-observed ^{19}F NMR methods, and the first hits of this fragment-based approach are in the process of characterisation and follow up.

Lastly, in collaboration with the CNIO´s Computational Biophysics Group, we are applying advanced NMR methods to characterise the internal dynamics of protein kinases implicated in cancer.

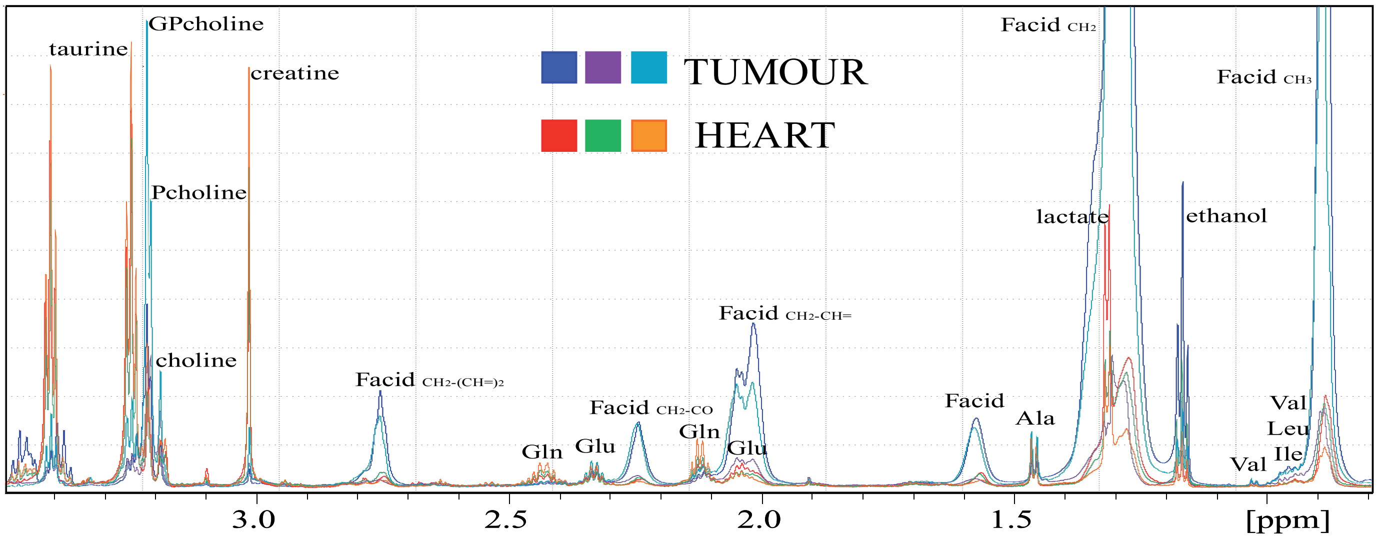

Figure: Comparison of HR-MAS proton NMR spectra of intact tissues from three different mouse mammary tumours with the corresponding control hearts. The most abundant metabolite signals are labelled (Facid= fatty acids, GP=glycerophospho, P=phospho). The spectra shows a dramatic increase in fatty acids, a moderate increase in choline-containing metabolites and the aminoacids leucine, isoleucine and valine, and a clear decrease in taurine and creatinine as well as in some aminoacids such as glutamine, in the tumours with respect to the heart. The ethanol signal is a contaminant of the surgical procedure used for tissue collection.

Publication

Sánchez R, Pantoja-Uceda D, Prieto J, Diercks T, Marcaida MJ, Montoya G, Campos-Olivas R, Blanco FJ (2010). Solution structure of human growth arrest and DNA damage 45alpha (Gadd45alpha) and its interactions with proliferating cell nuclear antigen (PCNA) and Aurora A kinase. *J Biol Chem* 285, 22196-22201.