SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE UNIT

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OVERVIEW

The Unit unifies the technical and scientific management of Nuclear Magnetic Resonance (NMR) Spectroscopy and other molecular biophysics instrumentation available through the Structural Biology Programme. It provides CNIO researchers with equipment and technical support for a variety of techniques used in biophysical studies of molecules involved in cancer. 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 2018, we identified and quantified interactions of small molecule compounds with tumour-relevant proteins and DNA, thereby contributing to the discovery of possible macromolecular inhibitors, as well as to the understanding of the molecular bases of the cell activity of those compounds."

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, dynamic and multi-angle static light scattering devices, and a surface plasmon resonance (SPR) instrument. Research groups mostly from, but not limited to, the Structural Biology Programme extensively used these technologies throughout 2018. For example, in collaboration with the Experimental Therapeutics -ETP- Programme, we conducted quantitative binding measurements using NMR (see FIGURE) to establish that a cell-active small molecule compound interacts weakly with telomeric double stranded DNA. Thus, telomeric DNA binding appears not to be a significant mode of action of the compound to explain its cellular activity.

The Unit hosts a 700 MHz NMR spectrometer that is well equipped with probes, and a sample changer for running up to 120 samples automatically. This provides the required throughput for the screening of small molecule protein binders (together with the CNIO's Structural Biology and ETP Programmes), as well as for metabonomics measurements that, this year, were performed in collaboration with the CNIO-Lilly Cell Signalling Therapies Section (from the ETP), the Cell Division and Cancer Group (-CDC- Group, from the Molecular Oncology Programme), as well as the Growth Factors, Nutrients and Cancer and the Epithelial Carcinogenesis Groups (from the Cancer Cell Biology Programme). For example, in collaboration with the CDC Group, we conducted cell media and intracellular metabolite measurements to characterise the metabolic changes associated to the silencing of the *Mastl* gene. Collectively, with these and other groups, we implemented sample preparation protocols and developed spectroscopic and analytical tools to characterise the metabolites present in different biological samples. ■

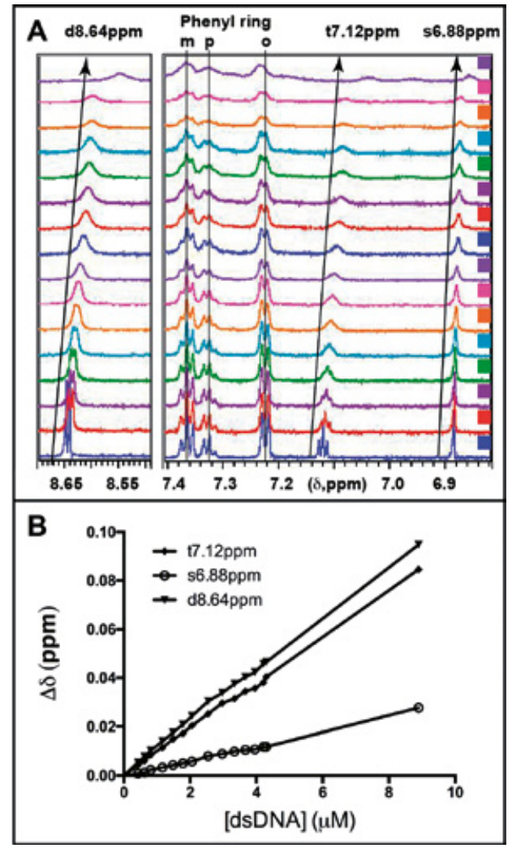

Figure NMR study of the interaction of a small molecule–compound with a DNA duplex containing 7 telomeric repeats. (**A**) Superposition of the aromatic region of the ^{1}H NMR spectrum of the compound (100 μM) recorded after addition of increasing amounts of dsDNA (from bottom to top: 0-8.9 μM). The tilted arrows mark signals changing position upon dsDNA addition, identifying the chemical moieties directly involved in dsDNA binding. In contrast, the 3 signals-from the phenyl ring of the compound only experience broadening as a result of complex formation, but not change of position, as the phenyl moiety does not directly contact the dsDNA. (**B**) Linear variations in the chemical shift (spectral positions) of three signals as a function of added dsDNA indicate weak binding (K_D >> 10 μM).