BIOMOLECULAR NMR

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The group develops novel tools for biomolecular applications of NMR spectroscopy. Emphasis is placed on extending the range of protein targets that can be investigated by NMR in pharmaceutical drug development. Thus, methods are developed for rapid identification and characterisation of ligand binding sites, including protein-protein and protein-DNA interactions. In addition, NMR is used to determine the three-dimensional (3D) structures of proteins and protein domains. This research is supported by an 800 MHz NMR spectrometer with cryoprobe.

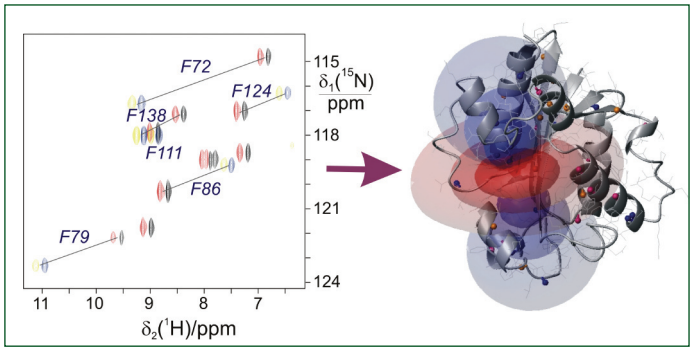

Figure 1: From NMR spectra to resonance assignment, structure determination and interaction studies of proteins. A wealth of structural information is gained by labelling with a paramagnetic ion. Isosurfaces of the anisotropic magnetic susceptibility (blue and red) are superimposed on the structure of the *N*-terminal domain of the proofreading exonuclease epsilon.

We discovered that site-specific labelling of a protein with a lanthanide ion provides access to the 3D structures of protein-protein and protein-ligand complexes with unprecedented ease and speed. Furthermore, if the 3D structures of the individual components in the complex are known, the lanthanide label makes the assignment of NMR spectra straightforward.

We are currently synthesising different compounds that allow the site-specific labelling of proteins with lanthanide ions. These will allow us to attach lanthanide ions to proteins that don't have a natural ion binding site. In particular we are aiming for ways of attaching lanthanide ions to proteins synthesised in cell-free extracts. Cell-free protein synthesis techniques are being refined in collaboration with Professor Nicholas Dixon. It provides fast and inexpensive access to ^{15}N-labelled proteins that we can analyse by NMR spectroscopy without prior purification.

Labelling proteins with lanthanide tags opens up a wide range of applications that were hitherto difficult or impossible to address by NMR or other methods. For example, they will provide a tool for 3D structure determination of small regions in large proteins, *i.e.* to "zoom" in on a region of a protein and study its structure without having to analyse the rest of the protein. It has long been known that lanthanides confer structural information. The lanthanide tagging approach promises to broaden these applications considerably. As one of the most exciting applications, we are currently evaluating the possibility of using lanthanides to gain information about the orientation of small chemicals (drug candidates) as they bind to protein targets. In a different project, we are investigating the use of lanthanide labels to characterise large amplitude motions of proteins with unprecedented accuracy.

The highlight of the year was the installation of the cryoprobe on the 800 MHz NMR facility. This completed the installation of the system that presents the highest magnetic field available for NMR in Australia. Purchased with support

from the Australian Research Council, ANU, U Sydney, UNSW, U Wollongong, U Newcastle and UNSW College at ADFA, the spectrometer is set up for remote operation so that it can be operated by interstate users from their desktop computers *via* the internet.

Continuing major collaborators are Professor Nicholas Dixon and Dr Max Keniry, Dr Thomas Huber (U Queensland), Drs Edvards Liepinsh, Anatoly Sharipo (Latvian U), Dr Guido Pintacuda (École Normale Superieure de Lyon) and Dr Laszlo Patthy (Hungarian Academy of Sciences).

Docking of Proteins of Known 3D Structure Using Paramagnetic NMR

The 3D structure of the 30 kDa ^{15}N-labelled complex formed between the *E. coli* proteins epsilon and theta was determined. The 3D structures of epsilon and theta were known from crystallography and NMR, respectively, and the paramagnetic NMR data generated by different lanthanide ions bound to epsilon were used to dock the two protein molecules. The structure of the complex was verified by conventional NMR methods. *(With N E Dixon, M A Keniry, A-Y Park, and G Pintacuda [École Normale Superieure de Lyon])*

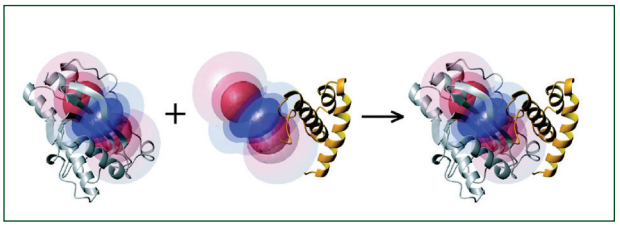

Figure 2: Isosurfaces of the anisotropic magnetic susceptibility (blue and red) are superimposed on the structures of the *N*-terminal domain of ε (left) and of θ (middle). The isosurfaces originate from the same lanthanide ion, *i.e.* their superposition yields the complex (right). It's simple!

Cell-free Synthesis of Residue-selectively Isotope Labelled Proteins

The cell-free expression system available in Professor Nicholas Dixon's laboratory was used to express samples of a number of selectively ^{15}N-labelled proteins. NMR spectra were recorded straight from the reaction medium.

A combinatorial ^{15}N-labelling scheme was developed that allows the identification of all ^{15}N-HSQC cross-peaks by their amino acid type from no more than five samples. In a separate project, the nascent state of the proteins produced *in vitro* was shown to allow the formation of correctly folded complexes with soluble binding partners. This presents access to complexes, where only one partner is isotope labelled. *(With N E Dixon, K Ozawa, P S C Wu, M J Headlam, S Jergic)*

Structure Dependent Modulation of Paramagnetic Shifts

Paramagnetic ions bound to proteins weakly align the proteins in the magnetic field. This gives rise to residual dipolar couplings that contain valuable structural information. For nuclear spins with pronounced chemical shift anisotropy (CSA), however, this effect results in paramagnetic shifts that depend not only on their position with respect to the paramagnetic ion (Figure 1), but also on the orientation of their CSA tensor with respect to the alignment tensor. A careful evaluation showed that the CSA correction is noticeable at 800 MHz, but negligible at 600 MHz and lower magnetic fields. *(With M John, A-Y Park, N E Dixon, and G Pintacuda [École Normale Superieure de Lyon])*

Protein Structure Determinations

The 3D structures of the *C*-terminal domain of the subunit τ of the *E. coli* DNA replisome, of the subunit θ in complex with the *N*-terminal domain of the subunit ε and of the ε-θ complex were determined. *(With X-C Su, S Jergic, A-Y Park, N E Dixon, M Keniry, and G Pintacuda [École Normale Superieure de Lyon])*

http://rsc.anu.edu.au/research/otting.php