Biomolecular NMR

Professor Gottfried Otting

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 a new 800 MHz NMR spectrometer installed in April 2004.

We discovered that binding of a paramagnetic lanthanide ion at a specific protein site provides a novel route to assign the NMR signals of the protein to its specific amino acids with unprecedented ease and speed.

To follow up on this discovery, we are working on widely applicable methods to attach lanthanide ions to proteins that don't have a natural ion binding site. One such strategy involves the synthesis of chemical compounds which on one side specifically attach to cysteines in proteins and on the other side carry a paramagnetic lanthanide ion. The work includes the production of proteins containing

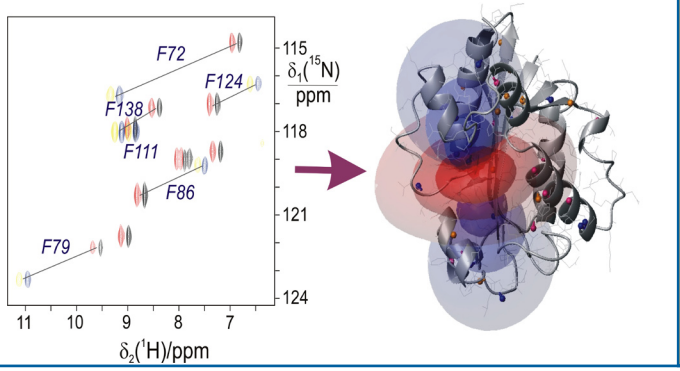
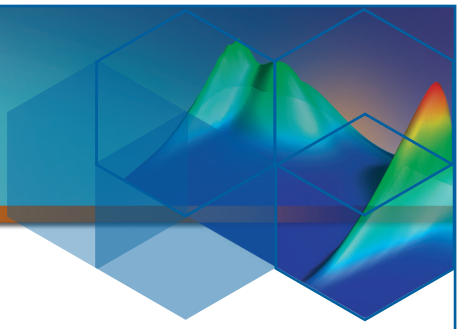


Figure: From NMR spectra to resonance assignment, structure determination and interaction studies of proteins. A wealth of structural information is gained by tagging with a paramagnetic ion. Isosurfaces of the anisotropic magnetic susceptibility (blue and red) are superimposed on the structure of the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

single cysteines at specific sites and the application of high-yield *in vitro* protein expression techniques which are being developed in collaboration with Dr Nicholas Dixon to allow inexpensive residue-selective ^{15}N-labelling of proteins.

Labelling proteins with lanthanide tags opens up a wide range of applications which 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 provide structural information to NMR spectroscopists. The lanthanide tagging approach promises to broaden these applications considerably. For example, it will provide information about the orientation of small chemicals (drug candidates) as they bind to protein targets. Finally, lanthanide labelling will allow the characterisation of large amplitude motions of proteins with unprecedented accuracy.

The highlight of the year was the installation of the 800 MHz NMR facility in the RSC. The system presents the highest magnetic field available for NMR in Australia and will be equipped with a cryoprobe for maximum sensitivity early in 2005. 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 Dr Nicholas Dixon and Dr Max Keniry, Dr Thomas Huber (Queensland U), Drs Edvards Liepinsh, Anatoly Sharipo (Latvian U) and Dr Laszlo Patthy (Hungarian Academy of Sciences).

Fast Algorithm for the Assignment of NMR Spectra

The assignment of NMR resonances to specific protons of a protein is a time-consuming task which can be very much shortened by the use of a novel strategy, if the 3D structure of the protein is known and a lanthanide ion can be bound to the protein at a specific site. The strategy has been verified for a 30 kDa ^{15}N-labelled complex formed between the *E. coli* proteins epsilon and theta. *(With N E Dixon, M A Keniry, A Y Park, and T Huber [U Qld], G Pintacuda [Karolinska Institute, Stockholm])*

In vitro *Expression of Residue-selectively Isotope Labelled Samples*

The cell-free expression system available in Dr Nicholas Dixon's laboratory was used to express samples of selectively ^{15}N-labelled human cyclophilin. The yields were sufficiently high that NMR spectra (^{15}N-HSQC spectra) could be recorded straight from the reaction medium without any protein purification or concentration step. The spectra were analysed for metabolic side reactions of the labelled amino acids that might be catalysed by enzymes present in the reaction medium. The data provide a catalogue of spurious signals which can be encountered in NMR spectra of *in vitro* synthesised and unpurified protein samples. *(With N E Dixon, K Ozawa, M J Headlam)*

Structure Dependent ^{1}H NMR Relaxation Due to Curie-spin/CSA Cross-correlation

^{1}H magnetisation relaxes more quickly if a paramagnetic ion is present in the same molecule. We discovered that this long-held belief can be quite wrong under certain circumstances. In fact, the ^{1}H spins may even relax more slowly in the presence of display enhanced relaxation! This effect is due to cross-correlated relaxation between the chemical shift anisotropy (CSA) of amide protons and the Curie-spin relaxation caused by the presence of paramagnetic ions with rapidly relaxing electron spins. A theoretical study showed that the effect can be experimentally relevant. *(With G Pintacuda, and A Kaikkonen [Karolinska Institute, Stockholm])*

Protein Solvation by NMR and MRD

The residence time of hydration water molecules on the surface of proteins and peptides was investigated by a high-resolution NMR spectroscopy and magnetic resonance dispersion (MRD). A new relaxation model assuming different diffusion coefficients of hydration and bulk water provides a consistent theory which explains the data obtained with both techniques. The result shows that solvent-exposed hydration water molecules have residence times in the picosecond time range even at temperatures near the freezing point of water. *(With B Halle, K Modig [Lund U Sweden], E Liepinsh [Karolinska Institute, Stockholm])*

Protein Structure Determinations

The 3D structure of human CLP was published. The protein binds to 5-lipoxygenase which is an important drug target for the suppression of inflammation. *(With E Liepinsh, O Rådmark [Karolinska Institute, Stockholm])*

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