Structural Biology and Biophysics by NMR

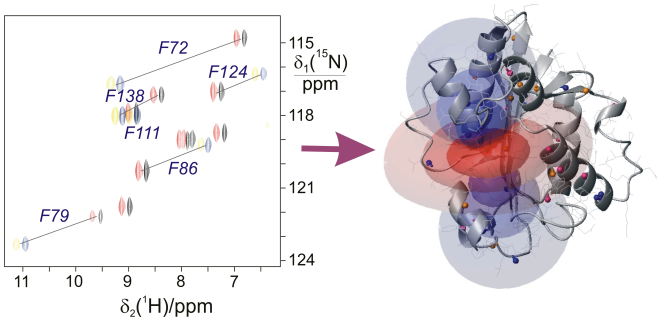
Professor Gottfried Otting

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 characterization of ligand binding sites, including protein-protein and protein-DNA interactions. In addition, NMR is used to determine the three-dimensional structures of proteins and protein domains. This research is supported by an 800 MHz NMR spectrometer to be installed at the beginning of 2004.

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

Labelling proteins with lanthanide tags opens up a wide range of applications which where 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 analyze 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 characterization of large amplitude motions of proteins with unprecedented accuracy.



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.

Highlights of the year were the development of an algorithm to determine sequence-specific resonance assignments of selectively stable-isotope labelled and lanthanide-tagged proteins by comparison with data predicted from the 3D structure of the protein, a study of the residence times of water molecules on protein surfaces which reconciles NMR results with the results from molecular dynamics calculations, and the completion of a 3D structure determination for CLP.

Professor Gottfried Otting continues to supervise his former laboratory at the Karolinska Institute in Stockholm. Continuing major collaborations are with Dr Nicholas Dixon and Dr Max Keniry (in-house), Dr Thomas Huber (Queensland University) Dr Edvards Liepinsh (Karolinska Institute), Dr Anatoly Sharipo (Latvian University), Dr Laszlo Patthy (Hungarian Academy of Sciences) and an EU network on cross-correlation effects in NMR led by Professor Geoffrey Bodenhausen (Paris).

New Algorithm for 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 three-dimensional 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 between the *E. coli* proteins epsilon and theta. *(with N. Dixon, M. Keniry, A. Park, and T. Huber [U. Queensland], 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 catalyzed 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* synthesized and unpurified protein samples. *(with N.E. Dixon, K. Ozawa*)

Homonuclear CSA/DD Cross-Correlated Relaxation in COSY

The cross-correlated relaxation between the chemical shift anisotropy (CSA) of amide protons and the dipolar field from the α protons in the same amino acid was investigated. Experimental results disagree with predictions from DFT simulations *in vacuo*, indicating that solvation significantly affects the amide proton CSA tensor. *(with P. Wu)*

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 completed. 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