The group, started in April 2002, works primarily with biomolecular applications of NMR spectroscopy. Besides 3D structure determinations of proteins and protein domains, methods are developed for rapid identification of ligand binding sites, including protein-protein interaction sites, with the aim of pharmaceutical applications. From the beginning of 2004, projects will be supported by a 800 Mhz NMR spectrometer with cryoprobe.

This goal is approached from several angles:

(i) Development of an algorithm for the rapid resonance assignment of uniformly ^{15}N-labelled proteins for which the 3D structures have been determined by X-ray crystallography. The goal is to assign the ^{15}N-HSQC spectrum of the protein without expensive ^{13}C labelling. High-yield *in vitro* protein expression techniques are developed in collaboration with Dr N.E. Dixon to allow inexpensive residue-selective labelling of proteins.

(ii) Development and implementation of NMR experiments suitable for 3D structure determination of small regions in a protein at high resolution. The goal is to "zoom" in on a region of a protein, selected by segmental isotope labelling or a paramagnetic spin label, and determine its structure quickly and with high accuracy. The approach further relies on residue-selectively labelled protein samples which allow the assignment of NMR signals of strategically chosen amino acids without having to assign or measure the resonances of the rest of the protein.

(iii) Identification of protein-protein and protein-ligand binding surfaces by chemical modification. This includes the development of a variety of experimental strategies based on OH-radical triggered H/D exchange, chemical cross-linking and paramagnetic spin labelling techniques. The aim is to develop practical techniques which identify intermolecular binding surfaces rapidly either by NMR spectroscopy or mass spectrometry.

Highlights of the year were the completion of 3D structure determinations for several proteins and conserved protein domains, and the demonstration of an *in vitro* protein expression system with yields sufficiently high that NMR spectra (^{15}N-HSQC spectra) could be recorded straight from the reaction medium without any protein purification or concentration step.

In September and October Professor Gottfried Otting visited his former laboratory at the Karolinska Institute in Stockholm for experiments on an NMR spectrometer with cryoprobe. Continuing major collaborations are with Dr Nicholas Dixon (in-house), 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).

Protein Structure Determinations

3D structures were completed of *Citrobacter freundii* AmpD, a 187-residue protein involved in the evolution of constitutive antibiotic resistance; the R3H domain from human Sμbp-2, a protein domain conserved in over 100 proteins with specificity for binding to single-stranded 5'-phosphorylated DNA; the PYRIN domain, also called DAPIN or PAAD domain, which turned out to present a fourth

class of death domain type proteins. All three proteins belong to large families of proteins with amino-acid sequence homology. Our structures were the first for any member of these families. An unexpected structural relationship between AmpD and the previously reported structure T7 lysozyme allowed us to predict accurate models of a novel class of proteins, the peptidoglycan recognition proteins (PGRP), and enzymatic activity for several members of these proteins which play important roles in human immune defence. (*with E.! Liepinsh, L.! Guignard [Karolinska Institute, Stockholm], C.! Généreux, D.! Dehareng, B.! Joris [U. Liège, Belgium], A.! Leonchiks, A.! Sharipo [Latvian U.], E.! Staub [metaGen Pharmaceuticals, Berlin]*)

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 and ^{13}C-labelled PpiB, a 163-residue prolyl-*cis-trans* isomerase from *E. coli*. 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 sufficiently clean to enable an interaction study with substrate added to the NMR sample. The system allowed the identification of an amino-acid residue in the active site of the enzyme in less than twenty-four hours, including *in vitro* protein synthesis and NMR data recording and analysis. (*with K.! Ozawa, N.E.! Dixon*)

Dipole-Curie-Spin Cross-correlation

Dipole-CSR cross-correlation effects between the Curie spin of the iron and the amide protons in ^{15}N-labelled myoglobin were measured in a quantitative way for high-spin and low-spin complexes with F^- and CN^-, respectively, by subtraction of the dipole-CSA cross-correlation effect measured with diamagnetic CO-myoglobin. The data contain accurate long-range structural information about the angle formed between the ^{15}N–^{1}H^{N} bond and the vector connecting the ^{1}H^{N} spins with the electron spin of the iron. (*with G.! Pintacuda [Karolinska Institute, Stockholm], K.! Hoenthanner, N. Müller [U. Linz, Austria]*)

http://rsc.anu.edu.au/~go/index.html