Report of the Grenoble ITM (29-30 August, 2013)

The course aimed at developing a solid, basic understanding of NMR spectroscopy and giving a first hands on experience for students within the ITN who themselves do not perform NMR experiments in the course of their everyday work.

During the first day of the course the participating students attended two lectures about the basic principles of NMR spectroscopy. The first speaker, Paul Schanda, summarized the physical background of NMR spectroscopy, covering the topics of nuclear magnetic moments, gyromagnetic ratio, Zeemann splitting, Boltzmann distribution and sensitivity, chemical shifts, scalar couplings and dipolar interaction, interaction with RF fields. In the second part of his talk he gave an introduction on the theoretical background of protein dynamics studies, discussing correlation function, spectral density function, T1 and T2 relaxation, model-free approach, chemical exchange and CPMG sequences.

The second speaker, Dominique Marion explained the principles of multidimensional NMR spectroscopy together with the historical aspects of the discovery of these kind of experiments. The aim that everybody understands how to obtain the second (and further) indirect dimension(s) was successfully achieved, following an interactive discussion. In the second part of his talk he discussed different NMR techniques for the study of protein-protein interactions.

In the afternoon practicals session the students formed two groups (instructors: Jaka Kragelj and Zsofia Solyom) and gained hands-on experience at the 600MHz NMR spectrometers of the IBS. The students could perform the setup of the experiment themselves. The spectra of a folded protein and a disordered protein were measured ad compared. The discussions at the spectrometers about optimal setup of the experiments helped deepening the understanding of the subjects learned during the morning talks.

The spectrometer practicals were followed by a tutorial of the CCPNMR Analysis software (instructor: Zsofia Solyom). This tutorial aimed at teaching how to handle 3D spectra and teaching how backbone assignment of a protein can be obtained by correlating resonances of neighbouring amino acids using sequential and intraresidual pairs of 3D experiments. The students installed the software on their own computers and received the data. After understanding the basic features of the program and playing with the visualization of the spectra they learned how to do assignment by hand and afterwards they used the semi-automated assignment of Analysis and with its help successfully assigned four residues of the protein.

During the second day the participants were introduced to NMR parameters that inform us about the structural propensities of IDPs. In the afternoon's practical session they learned how to use the distributed version of flexible meccano that is available online.

In the first talk, Malene Ringkjøbing Jensen presented the relation between chemical shifts, RDCs and secondary structure propensities. Use of PREs for determining long range interactions was presented by her too. The different options of fitting the NMR data were presented through examples based on work done in the last few years in the group. Emphasis was put on audience speaker interaction and talk was organized in such a way that that questions could be asked during the lecture.

In the second talk, Valery Ozenne presented the distributed version of flexible meccano that is available online. He presented the concept behind this software: how the data is generated, organized and plotted, the capabilities and limitations of the software. By presenting how to run simulations and how to plot the data the participants were better prepared to proceed to the practical session.

The afternoon's practical session was guided by Jaka Kragelj. He helped the students to start a set of simulations of RDC data. The goal of this first exercise was to show the slow convergence of RDC data. In the second exercise the participants examined the scripts for calculating chemical shifts that are included in the distributed flexible meccano. A set of simulated chemical shift data was examined with the goal of comparing the chemical shifts convergence to convergence of RDC data. In the third exercise the participants were acquainted to the possibility of changing the conformational sampling of selected regions of protein in the flexible meccano program and with an example of how can such an option allow us to fit RDC data.

Program of the Course

Thursday 29 August

9:00 – 10:15 Paul Schanda: Principles: NMR and energy levels, the vector model, chemical shifts and couplings, principles of relaxation
10:15 – 10:30 Coffee break
10:30 – 11:45 Dominique Marion: Going from 1D to 2D and multidimensional spectra, protein-protein interactions by NMR
11:45 – 12:00 Discussion, questions
12:00 – 13:30 Lunch
13:30 – 15:40 Practicals at the spectrometer (Zsofia Solyom, Jaka Kragelj)
15:40 – 16:00 Coffee break
16:00 – 18:30 Assignment practicals with CCPNMR (Zsofia Solyom)

Friday 30 August

9:00 – 10:15 Malene Ringkjøbing Jensen:Structure generation and selection: Chemical shifts and paramagnetic relaxation enhancement
10:15 – 10:30 Coffee break
10:30 – 11:30 Malene Ringkjøbing Jensen: Residual dipolar couplings
11:30 – 12:00 Valery Ozenne: Introduction to the Flexible meccano software
12:00 – 13:30 Lunch
13:30 – 15:40 Flexible meccano practicals (Jaka Kragelj)
15:40 – 16:00 Coffee break
16.00 – 18.00 Flexible meccano with asteroids practicals