Patrick Groves
Molecular Interactions and NMR

A brief overview of my research interests are given in the Institute's webpages (link to: http://www.itqb.unl.pt/research/biological-chemistry/molecular-interactions-and-nmr)

The group's main focus is on method development for structural and systems biology, with a personal interest in calcium-binding, EF-hand proteins. We have a number of topics on offer. and can adapt them towards BII/Masters students.

A) Analysis of protein-ligand interactions by NMR spectroscopy
We offer training in NMR methods, in particular Diffusion NMR and Saturation Transfer Difference spectroscopy –commonly used by the pharmaceutical industry for drug discovery and design. The projects will involve the preparation of suitable NMR samples, acquisition of data, processing and analysis of data. The project can be expanded backwards to involve protein expression and purification as part of the sample preparation and forwards to molecular modeling of the NMR data. Suitable for anyone with a chemistry / biology / physics background, with P.Groves.

1. M. Politi, J. Alvaro-Blanco, P. Groves, A. Prieto, J.A. Leal, F.J. Cañada and J. Jiménez-Barbero "Screening garlic water extract for binding activity with Cholera Toxin B pentamer by NMR. An old remedy giving a new surprise", Eur. J. Org. Chem., 2006, 2067-73.
2. A. Bastida, A. Hidalgo, J.L. Chiara, M. Torrado, F. Corzana, J.M. Cañadillas, P. Groves, E. Garcia-Junceda, J. Jimenez-Barbero and J.L. Asensio "Exploring the use of conformationally locked amino-glycosides as a new strategy to overcome bacterial resistance", J. Am. Chem. Soc., 2006, 126, 100-16.
3. F. Chevalier, J. Lopez-Prados, P. Groves, S. Perez, M. Martín-Lomas and P.M. Nieto "Structure and dynamics of the conserved protein GPI anchor core inserted into detergent micelles", Glycobiol., 2006, 16, 969-980.

B) New methods for the analysis of protein-ligand interactions by NMR spectroscopy
Although we have many established NMR protocols suitable for the project described above, we still need to develop new tools to solve some of the problems brought to us. Examples of new and modified protocols are given in [4-6]. Future challenges include the use of DNA or in situ membrane proteins present in live mammalian cells as target molecules in STD experiments, as well as the application of the methods in [4] to investigate protein-protein interactions. The student will first work on a short project using established methods before starting to develop new methodology. Apart from preparing samples, this will essentially be a dry lab project requiring computer skills. Suitable for anyone with a physics / chemistry background with P. Groves.

4. K.E. Kövér, P. Groves, J. Jiménez-Barbero and G. Batta "Molecular recognition and screening using STD NMR: 15N-group selective STD NMR experiment to study intermolecular interactions in heavily overlapped spectra", J. Am. Chem. Soc., 2007, 129, 11579-82.
5. P. Groves, K.E. Kövér, S. André, J. Bandorowicz-Pikula, G. Batta, M. Bruix, R. Buchet, A. Canales, F.J. Cañada, H-J. Gabius, D.V. Laurents, J.R. Naranjo, M. Palczewska, S. Pikula, E. Rial, A. Strzelecka-Kiliszek, and J. Jiménez-Barbero "Effect of temperature in Saturation Transfer Difference NMR experiments", Magn. Reson. Chem., 2007, 45, 745-8.
6. K, Fehér, P. Groves, G. Batta, J. Jiménez Barbero, C. Muhle-Goll, K.E. Kövér "Application of isotope edited and filtered STD NMR experiments for ligands with overlapping signals", J. Am. Chem. Soc., 2008, 130, 17148-53.

C) Determining the pH structural switch in calbindin D28k

Closely related calretinin and calbindin D28k are neuronal proteins that offer protection against intracellular calcium insults. We believe these proteins also contain a pH switch that turns them into dual sensors, only becoming activated in the presence of elevated concentrations of both calcium and protons. We will express and purify a small, 87 residue calbindin domain to prove this by solving NMR structures at low and high pH. We will use standard NMR methods to collect, assign and model the structures. We will also use advanced NMR methods to collect complementary data to improve the structures. This longer project first needs the expression / purification system to be established (cloning, electrophoresis, chromatography) before biochemical characterization can take place (calcium-binding measurements, 1D NMR, fluorescence and UV). Suitable for someone with a biology/biochemical background with M. Palczewska (and P. Groves). The follow-up structural project will be carried out with P. Groves.

7. M. Palczewska, G. Batta, P. Groves, S. Linse, and J. Kuźnicki "Localization of the Ca(2+)- and H(+)-dependent hydrophobic properties of calretinin", Protein Sci., 2005, 14, 1879-87.

D) Characterization of a new EF-hand protein from Medicago truncatula

The lab of Prof. Julie Cullimore (INRA, Toulouse) has discovered a new EF-hand protein related to the signaling pathway between symbiotic rhizobia and legumes, leading to nitrogen fixation. Our task is to express and purify this protein, its deletion mutants and to characterize the interaction of these proteins with a peptide derived from the interacting protein by spectroscopic and biochemical methods. This longer project first needs the expression / purification system to be established (cloning, electrophoresis, chromatography) before biochemical characterization (fluorescence, circular dichroism, nmr spectroscopy). Suitable for someone with a biology/biochemical background with M. Palczewska and P. Groves.

References 7, 1, 4, 5 and:

8. M. Palczewska, P. Groves, A. Ambrus, A. Kaleta, K. E. Kövér, G. Batta and J. Kuźnicki "Structural and biochemical characterization of neuronal calretinin domain I-II (residues 1-100); comparison to homologous calbindin D_{28k} domain I-II (residues 1-93)", Eur. J. Biochem., 2001, 268, 6229-6237.

E) Yeast two-hybrid at low pH

Yeast two-hybrid techniques are at the forefront of systems biology efforts to define protein-protein interactors. A recent paper describes the conditions where the intracellular pH of yeast can be lowered from pH 7.4 to pH 6.8. In principal, these conditions allow us to screen for protein-protein interactions at low pH. In this project, we will set up a defined library to compare protein-protein interactions at the two different pH. The library will include neuronal calbindin D28k and three known binding partners. We expect at least one of them will only interact at low pH. A second test system from Dr D.J. Scheffers' lab will test the reverse, i.e. an interaction at pH 7.4 that will be absent at pH 6.8. Suitable for someone with a biological background with M. Palczewska.

F) Functional foods against cholera toxin

The effect of cholera toxin can be blocked by plants extracts. We will express and purify cholera toxin B and test several plant extracts by fluorescent and NMR methods. Suitable for someone with a biology / chemistry background with M. Palczewska and P. Groves.

Reference 1