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)

Masters projects

A) Analysis of protein-ligand interactions by NMR spectroscopy

Training in NMR spectroscopy will start immediately for a largely computer based project. One of the groups' tasks is to analyze samples, brought to us by collaborators, using TR-NMR methods. TR-NMR methods include diffusion NMR and STD – methods commonly used by the pharmaceutical industry for drug design. The projects involve the preparation of suitable NMR samples from the supplied materials, acquisition of data, processing and analysis of data. In some cases, it is possible to AutoDock the ligands with available protein structures to better illustrate the binding site. Some recent examples of such collaborative work include:

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) Analysis of protein-ligand interactions by NMR spectroscopy

Training in NMR spectroscopy will start immediately for a largely computer based project. One of the groups' tasks is to analyze samples, brought to us by collaborators, using TR-NMR methods. TR-NMR methods include diffusion NMR and STD – methods commonly used by the pharmaceutical industry for drug design. Although we have many established protocols, 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, 5]. 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.

4. K.E. Kövér, P. Groves, J. Jiménez-Barbero and G. Batta "Molecular recognition and screening using STD NMR: ^{15}N-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.

C) Determining the pH structural switch in calretinin

We have previously characterized the pH/Ca^{2+} switch of calretinin, a calcium-binding, EF-hand protein, at the biochemical level [6]. Understanding the dual action of pH and Ca^{2+} at the structural level gives us clues as to how this protein provides neuronal protection against Alzheimers and Parkinsons disease. Calcium usually activates EF-hand proteins (like calmodulin) but it seems that calcium plus low pH is needed to activate calretinin. If this is true, it makes it difficult to design cell biology experiments to test this hypothesis as most protocols are designed to test the effect of one factor at a time.

1) Expression and purification of the GST-calretinin I-II (CR12) domain that contains the pH switch, using established clones.
2) Analysis of CR12 by Tris-tricine PAGE, UV and fluorescence spectroscopy
3) Production of labeled protein
4) pH titrations of CR12 followed by NMR and as a function of temperature
5) NMR analysis of the different CR12 structures at low and high pH
6) Collecting and analyzing RDC-NMR data to refine the structures

Training in NMR spectroscopy will start during the wet-lab work (steps 1-3). Steps 5-6 are the most time consuming requiring data acquisition, processing and the analysis of large datasets on the computer.

6. 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.