Molecular Interactions and NMR

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A brief overview of our 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. Our project proposals cover a wide range of topics and there is something suitable for chemists, biochemists, biologists, biotechnologists…

A) Analysis of protein-ligand interactions by NMR spectroscopy

BACKGROUND: We offer training in NMR spectroscopy and NMR methods, in particular Diffusion NMR and Saturation Transfer Difference spectroscopy –commonly used by the pharmaceutical industry for drug discovery and design [1-3]. The project can be expanded to involve protein expression and purification as part of the sample preparation and/or docking and molecular modeling of the NMR data. We are open to collaboration where you "bring your own samples" from another laboratory.

WORKPLAN: (i) Protein expression and purification will involve cloning and selection, expression and purification. (ii) NMR spectroscopy methods including sample preparation, parameter optimization, processing and analysis. (iii) Docking of ligand structures with existing protein structures. Verification of structures with NMR data.

SUPERVISION: Supervised by P.Groves. Part (i) will be co-supervised by M. Palczewska.

References:

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

BACKGROUND: 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 by our collaborators. Examples of new and modified protocols are given in [4-8]. 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.

WORKPLAN: The student will first work on a short project involving a model target-ligand system to learn the established NMR methods before starting to develop new methodology on a model system. The project is mostly a dry lab project requiring computer skills.

SUPERVISION: Supervised by P.Groves.

References:

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.
7. J.P. Ribeiro, M. Palczewska, S. André, F.J. Cañada, H-J. Gabius, J. Jiménez-Barbero, B. Mellström, J.R. Naranjo, D.J. Scheffers, P. Groves "Diffusion nuclear magnetic resonance spectroscopy detects substoichiometric concentrations of small molecules in protein samples." *Anal. Biochem*. 2010, 396, 117-23.
8. P. Groves**,** M. Webba da Silva "Rapid Stoichiometric Analysis of G-Quadruplexes in Solution." *Chem. Eur. J.* 2010, in press.

C) Determining the pH structural switch in calbindin D28k
BACKGROUND: 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.
WORKPLAN: (i) to take the established expression clones to express and purify a small, 87 residue calbindin domain in isotope-labeled form. (ii) to biochemically characterize the proteins to verify their pH properties are the same as the full-length proteins. (iii) to prepare two sets of samples at pH 7.5 and pH 6.5. (iv) to acquire and process NMR data using established methods. (v) to assign the NMR data, using published data as a guide. (vi) to model the NMR data. (vii) to verify the resulting 3D structures.
SUPERVISION: Overall supervision will be given by P. Groves. M. Palczewska will co-supervise parts (i-iii).
References:
9. 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
BACKGROUND: 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.
WORKPLAN: (i) design, cloning and expression of a series of His-tagged deletion mutants. (ii) Purification of the proteins. (iii) Spectroscopic analysis of mutants by fluorescence, circular dichroism, NMR.
SUPERVISION: Overall supervision will be given by M. Palczewska. Co-supervision will be provided by P. Groves.
References: 7, 1, 4, 5 and:
10. 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 D28k domain I-II (residues 1-93)", Eur. J. Biochem., 2001, 268, 6229-6237.

E) Yeast two-hybrid at low pH
BACKGROUND: Yeast two-hybrid (YTH) 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 and maintained at pH 6.8. In principal, these conditions allow us to screen 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 case, i.e. an interaction at pH 7.4 that will be absent at pH 6.8, namely the interaction between MinC and FtsZ from a Gram-positive bacterium, Bacillus subtilis.
WORKPLAN:
(i) design, cloning and expression of two bait proteins fused with DB domain and a variety of target proteins and/or its fragments as a AD fusion . (ii) Setting up YTH system and testing it in physiological and low pH. (iii) Screening the interaction of two bait proteins and their predicted targets using YTH system in physiological and low pH conditions.
SUPERVISION: Overall supervision will be given by M. Palczewska. Co-supervision will be provided by P. Groves.
References: 9, 10, 11
11 Scheffers DJ "The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA." FEBS Lett. 2008, 582:2601-8.