Masters projects at ITQB-UNL (www.itqb.unl.pt)

Under the Orientation of Dr. Patrick Groves and/or Dr. Malgorzata Palczewska

Molecular Interactions and NMR (room 3.19)

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The following three Masters projects (to accommodate 3-4 students) require 6-12 months, full-time research. All candidates are required to speak English. Details regarding the projects, including workplans and training, are given below. Further details and copies of the references can be obtained by email.

**TITLE: Characterization of a new protein purification tag**

**BACKGROUND:** The double-lanthanide-binding tag (dLBT) has been developed as a useful biochemical, biophysical and structural tool. Our lab recognizes the potential for the dLBT to also function as a purification tag. We have shown the potential one-step purification of a soluble protein and a urea-denatured protein. We aim to purify three other proteins to show the all-round abilities of the dLBT purification tag. (1) dLBT-lysozyme. We will produce the protein for G. Batta/K.E.Kover, Debrecen (Hungary) who will use it for interaction studies. Purification of lysozyme may require the presence of reducing agents. (2) Hevein is a small, model carbohydrate-binding protein used by J. Jimenez-Barbero, Madrid (Spain). This tricky protein will need to be purified in the presence of urea and reducing agents before refolding. (3) Rustacyanin is a copper-binding protein studied by A. Donaire, Murcia (Spain). The challenge will be to place the lanthanide and copper cations in the correct binding sites during purification.

**WORKPLAN:** (i) All three proteins have been transformed into E.coli and express > 10 mg/L quantities. PCR, transformation, cloning, expression, (ii) purification, polyacrylamide gel electrophoresis (PAGE), protein quantitation, spectroscopic analyses (fluorescence, circular dichroism, NMR).

**SUPERVISION:** Part (i) will be supervised by M. Palczewska. Part (ii) will be supervised by P.Groves. Practical training in NMR spectroscopy and FPLC (both 1 week, 1ECTS courses) will be provided.

**REFERENCES:**

L.J. Martin, M.J. Hähnke, M. Nitz, J. Wöhnert, N.R. Silvaggi, K.N. Allen, H. Schwalbe and B. Imperiali “Double-lanthanide-binding tags: design, photophysical properties, and NMR applications” J. Am. Chem. Soc. 2007 (129) 7106-13.

Sara Fernandes, BII lab report, ITQB-UNL, 2009

Ewa Krol, Masters thesis, University of Lodz, 2010.

**CANDIDATE**: Should have a biological (or analytical/organic chemistry) background and be able to work in English. Candidates should be prepared to work within an International team.

**TITLE: Analysis of protein‐protein interactions by NMR spectroscopy**

**BACKGROUND:** To analyze several protein-protein complexes involved in tuberculosis signaling pathways. Individual protein structures have been determined by crystallography in the lab of Prof. Shekhar Mande (CDFD, Hyderabad, India). Molecular modeling will show how the proteins fit together, and allow the rational design of drugs that can interfere with specific protein-protein interactions. Our task is to provide experimental evidence to confirm the model structures and refinement in cases where modeling has failed.

**WORKPLAN:** (i) Protein expression and purification will involve cloning and selection, expression and purification of His-tagged proteins as established in India. (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. Note, protein samples can be provided by our collaborators resulting in a “dry-lab” / computer-based project consisting of Parts (ii) and (iii).

**SUPERVISION:** Supervised by P.Groves. Part (i) will be co-supervised by M. Palczewska. Practical training in NMR spectroscopy and FPLC (both 1 week, 1ECTS courses) will be provided.

**REFERENCES:**

C.M. Kumar, G. Khare, C.V. Srikanth, A.K. Tyagi, A.A. Sardesai and S.C. Mande “Facilitated oligomerization of mycobacterial GroEL: evidence for phosphorylation-mediated oligomerization” J Bacteriol. 2009 (191) 6525-38.

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.

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.

**CANDIDATE:** Biological background, able to work in English. Candidates should be prepared to work within an International team.

**TITLE: Determining the pH structural switch in calbindin D28k**

**BACKGROUND:** Two closely related proteins, 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 small, 87 residue protein domains in isotope-labeled forms. (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). Practical training in NMR spectroscopy and FPLC (both 1 week, 1ECTS courses) will be provided.

**REFERENCES:**

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.

**CANDIDATE:** Biology / Chemistry background, able to work in English. Each protein constitutes a single project allowing two students to work side-by-side.