Master Student Project

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Engineering riboflavin binding proteins to develop a vitamin sensor

Substrate binding proteins (SBDs) are part of the ATP-binding cassette (ABC) transporters. They are soluble proteins capturing substrates and allowing their translocation by docking on the transmembrane part of the ABC transporter. SBDs are composed of two domains which are closing upon substrate binding in a Venus flytrap like motion. By engineering fluorophores on each domain, we can use fluorescence recordings (FRET) to monitor this motion and thus detect the presence of substrate. This approach has already been used in the lab notably with the binding protein for glutamine, glutamic acid and asparagine from the ABC transporter GlnPQ (*Gouridis et al., 2015*). Such sensors will be very useful to perform transport assays to unravel the dynamics of membrane transporters.

In this project, we aim to expand our tool box by developing sensors for different substrates, in this particular case the vitamin riboflavin. We already identified potential protein candidates from *Streptomyces davaonensis* and *Chloroflexus aurantiacus* that we would like to investigate in the framework of this internship. The student will be involved in all the experimental steps from molecular biology to protein expression, purification and substrate binding assay. If the candidate proteins are promising and the length of the internship allows, the student will be involved in the design of cysteine positions in order to label the protein with fluorophores. Ultimately, fluorescence recordings can be performed on the labeled protein either using a fluorometer (bulk measurements) or with a confocal microscope (single molecule measurements) with the protein in solution or encapsulated in proteoliposomes.

This ambitious project call for a motivated student, in exchange he/she will develop his/her professional experience in project design and project management, as well as acquire a broad experimental skill set including cloning, protein expression, affinity purification, size exclusion chromatography, SDS page electrophoresis, fluorescence spectroscopy and microscopy and data analysis.

Gouridis G, Schuurman-Wolters GK, Ploetz E, Husada F, Vietrov R, De Boer M, Cordes T and Poolman B. 2015. 'Conformational dynamics in substrate-binding domains influences transport in the ABC importer GlnPQ'. Nature Structural and Molecular Biology, 22(1), pp. 57–64. doi: 10.1038/nsmb.2929.