Master Student Project

Supervisors: Marco van den Noort and Bert Poolman

smFRET to study protein dynamics

Proteins are dynamic molecules that can sample a range of different conformations. In fact, their 'flexibility' is an essential property for proper functioning. However, it remains challenging to study the dynamical behavior of proteins. Single-molecule Förster Resonance Energy Transfer, or smFRET, is an exciting technique to study the dynamics of single proteins under native-like conditions. Here, we use the information from static structures to design two spots in the protein which we label with two different fluorophores. By means of confocal-microscopy we can measure 1-dimensional distances between the two sites in single proteins and see how they change under different conditions [1].

This project mainly focusses on the membrane-embedded transporter OpuA which is of vital importance for a wide range of bacteria. When a bacterium enters a high-salt (i.e. hyperosmotic) environment, it has to prevent excessive cell shrinkage, which will ultimately lead to cell-death. Only in these hyperosmotic conditions OpuA gets activated and starts transporting the molecule glycine betaine to counteract the osmotic disbalance. Recently, we solved the structure of OpuA and found out how this on-off switch works on a molecular level [2]. We also observed that dysregulation of the switch can be lethal for bacteria, because it leads to uncontrolled transport and ultimately cell lysis.

Now it is time for the next step. We would like to uncover the order of events in which transport happens and how the dynamics play a role in the activation profile of OpuA. For this we need your help! In this project, you will get the change to learn and use a range of widely applicable, biochemical techniques:

- Protein overexpression in a pH- and temperature-controlled fermenter
- His-tag based purification of a membrane protein
- Size exclusion chromatography
- Nanodisc reconstitution
- Solution-based smFRET, using a confocal microscope
- *In vitro* activity assays, such as ATPase or transport assays

Besides these specific biochemical competencies, you will be guided in the development of an independent working attitude. If you are motivated, there will be a lot of freedom in the decision of your own experiments.

[1] Lerner, E., Cordes, T., Ingargiola, A., Alhadid, Y., Chung, S., Michalet, X., & Weiss, S. (2018). Toward dynamic structural biology: Two decades of single-molecule Förster resonance energy transfer. *Science*, 359(6373).

[2] Sikkema, H. R., van den Noort, M., Rheinberger, J., de Boer, M., Krepel, S. T., Schuurman-Wolters, G. K., ... & Poolman, B. (2020). Gating by ionic strength and safety check by cyclic-di-AMP in the ABC transporter OpuA. *Science advances*, 6(47), eabd7697.