Master Student Project

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Heterodimers to study the transport cycle of OpuA

Cell volume regulation in changing osmotic conditions is essential for all organisms. Hyper-osmotic conditions require the influx of ions and compatible solutes to prevent the loss of turgor (or ultimately cell-death). OpuA is a homodimeric, ATP-driven osmoregulatory transporter of the compatible solute glycine betaine in Lactococcus Lactis. This membrane protein helps to restore the cell volume in hyper-osmotic conditions. Its activity can be trigged by increasing intracellular ionic strength and is inhibited by the second messenger cyclic di-AMP [1].

While some structures of OpuA have been solved with cryo-EM, still a lot remains unknown about the order of events and dynamics within the protein. We therefore want to probe these conformations using Single-molecule Förster Resonance Energy Transfer (smFRET), serving as a molecular ruler [2]. With this technique, we can gain information about the distances between parts in the protein by introducing cysteine mutations and labelling it with fluorescent dyes. Additionally, we can also measure how fast these parts move compared to each other.

One problem is that the protein of interest is a homodimer: if we want to make a mutation in one subunit of a protomer it is also replicated in the other. This strongly limits the different combinations of mutations that can be made for smFRET. We would ideally look at the dynamics between two different subunits, which would require a heterodimer where one protomer would include different mutation(s) compared to the other.

Recently, a protocol has been developed in our lab to produce heterodimers of OpuA [3]. In this ambitious project, you will work on the expression, purification and testing mutants of the heterodimeric protein. Experimental skills that can be acquired range from protein overexpression in a pH- and temperature-controlled fermenter, affinity based chromatography, nanodisc reconstitution, size exclusion chromatography, in vitro activity assays (such as an NADH-coupled ATPase assay) to fluorescence spectroscopy and microscopy.

[1] H. R. Sikkema *et al.*, 'Gating by ionic strength and safety check by cyclic-di-AMP in the ABC transporter OpuA', *Sci Adv*, vol. 6, no. 47, p. eabd7697, 2020, [Online]. Available: https://advances.sciencemag.org/lookup/doi/10.1126/sciadv.abd7697

[2] E. Lerner *et al.*, 'Toward dynamic structural biology: Two decades of single-molecule förster resonance energy transfer', *Science (1979)*, vol. 359, no. 6373, 2018, doi: 10.1126/science.aan1133.

[3] P. Alvarez-sieiro, H. R. Sikkema, and B. Poolman, 'Heterodimer formation of the homodimeric ABC transporter OpuA', *Int J Mol Sci*, vol. 22, no. 11, 2021, doi: 10.3390/ijms22115912.