MEP/BEP project: *In vitro* characterization of membrane binding of yeast polarity proteins

Goal: Characterize the dynamics of polarity proteins binding to membranes of different compositions.

Methods: TIRF microscopy, quartz crystal microbalance, conceptual modelling

Introduction

One of the core components of our minimal in vitro system for yeast cell polarity is the artificial membrane. Its composition influences everything in the system. Without charge, proteins won't bind and without fluidity, the "diffusion" part of reaction-diffusion pattern formation is eliminated.

When characterizing our proteins, we observe a lot of large structures. Bem1, one of the polarity proteins we are interested in, is able to polymerize and multimerize in solution. A next step is to see the influence of the membrane on these structures. We have observed that the binding dynamics of Bem1, both spatially and temporally, differ a lot with membrane composition.

By understanding how our polarity proteins bind and unbind the membrane and what effect this binding has on higher order protein structures, we aim to understand how polymerization and multimerization affect micro and macroscopic dynamics of membrane localization of Cdc42 and its regulators.

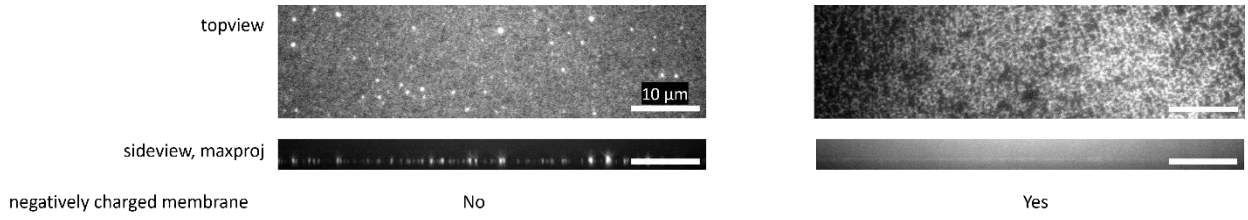

Figure 1. Bem1 on membranes with different charge. Acquired with TIRF microscopy. Bem1 on membranes with no charged lipids show 3D condensates that land on the membrane. Bem1 on membranes with negatively charged lipids bind in a flat, inhomogeneous gel-like way.

Project description

For this project, you would work on the characterization of binding dynamics of mainly Bem1 to membranes of different compositions. You will work on both a qualitative as well as quantitative ways to describe the dynamics. Techniques we will be using are total internal reflection fluorescence (TIRF) microscopy combined with fluorescent recovery after photobleaching (FRAP) and quartz crystal

microbalance. The challenge in this project will be to combine and compare data at different length and timescales to get to a conceptual model of how the structures and dynamics we observe contribute to a robust polarity establishment.

Requirements

This project is suitable for both bachelor and master students with a background or interest in nanobiology, physics or biophysics. Some wetlab experience is nice, but not a must. We are looking for a student that likes precise labwork and is interested in combining different datatypes to get to a full characterization.

Contact

If you're interested in this project or if you have questions/suggestions, feel free to contact me, Nynke Hettema, at n.m.hettema@tudelft.nl .