Proposition de stage de M2 et de thèse
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Physical mechanisms controlling Cdc42 binding to membranes

Cdc42 is an evolutionary conserved small G protein controlling many cellular processes, such as cell polarity, cytoskeleton dynamics and membrane trafficking, in response to a wide variety of extracellular signals. While inactive Cdc42 localizes in the cytosol, its regulators and effectors are generally **membrane-associated**. The regulation and functions of Cdc42 at the cell plasma membrane have been extensively studied. However, a significant pool of Cdc42 localizes **at the Golgi apparatus** where its specific regulation and functions remain elusive. Previous studies have focused on only one isoform of Cdc42, known as the **ubiquitous isoform** (Cdc42u), whereas in vertebrates two isoforms of Cdc42, that are alternative splice variants, are expressed. The second isoform, known as **the brain isoform** (Cdc42b), mainly localizes to intracellular membranes, including the Golgi apparatus.

The goal of this M2/PhD project is to identify the physical mechanisms controlling the binding of Cdc42 isoforms to membranes. We have reconstituted the binding of fluorescently-tagged Cdc42u and Cdc42b *in vitro* using **Giant Unilamellar Vesicles (GUVs)** and purified GFP-Cdc42 constructs. Using this assay, we have previously shown that the membrane lipid composition is a crucial parameter controlling the binding of Cdc42 isoforms to membranes (Figure). During this project, we want to 1) characterize the **dynamics of Cdc42** binding to membranes; 2) study the potential role of Cdc42 as a **curvature sensor and/or inducer**; and 3) determine if Cdc42 impacts on lipid **phase separation**. To achieve these goals, Fluorescence Recovery After Photobleaching (FRAP) experiments will be performed on GFP-Cdc42 isoforms bound to GUVs. The candidate will perform **FRAP** on small regions on the GUV membranes or on whole GUVs to measure the diffusion and binding kinetics of Cdc42 respectively. Highly curved membrane **nanotubes** will be pulled from GUVs using kinesin molecular motors moving on *in vitro* reconstituted microtubule networks to test the potential curvature sensitivity of the Cdc42 constructs. Finally, the candidate will use **ternary mixtures** of phosphocholine, cholesterol and sphingomyelin close to the liquid ordered (Lo) / liquid disordered (Ld) phase transition to test whether the presence of Cdc42 modifies the phase behaviour.

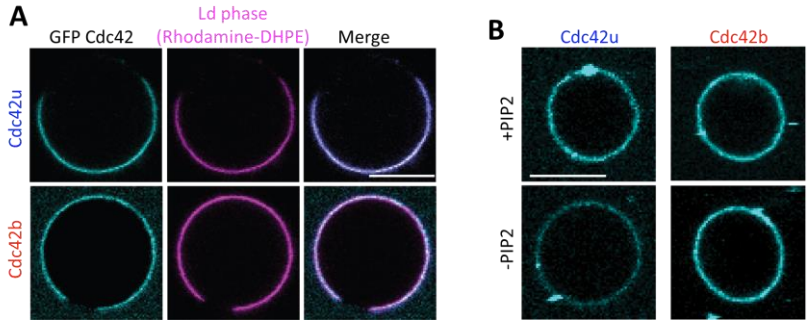

Figure: Membrane composition controls the binding of Cdc42 to model membranes. A. Cdc42u (top, cyan) and Cdc42b (bottom, cyan) only bind to the liquid disordered (Ld) phase (purple) in phase separated GUVs. B. The binding of Cdc42u but not Cdc42b depends on the presence of the charged lipid PIP2.

Key words: membranes; RhoGTPases; Golgi apparatus; FRAP; optical tweezers; membrane curvature; nanotubes; kinesin; microtubules; phase separation; Lo Ld.

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