Master project : Systèmes Biologiques et Concepts Physiques

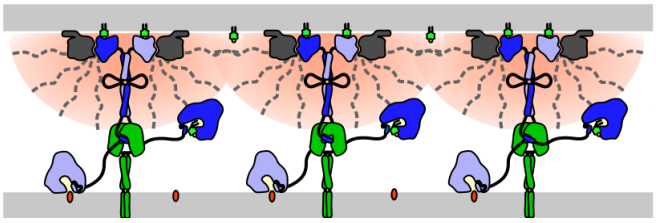
Entropic barriers to control protein movements at membrane contact sites

Person in charge - biology : Dr Bruno Antonny – antonny@ipmc.cnrs.fr
Institut de Pharmacologie Moléculaire et Cellulaire, CNRS et Université Côte d'Azur, UMR7275, 660 Route des Lucioles, SOPHIA ANTIPOLIS, 06560 VALBONNE
Person in charge - chemistry : Professor Alain Burger - Alain.Burger@unice.fr
Université Nice Sophia Antipolis, U.F.R Sciences, Institut de Chimie de Nice, UMR CNRS 7272, 28 avenue de Valrose, 06108 Nice, Cedex 2.

This project has been selected by the program 'Masters Environnés' of the Université Côte d'Azur. Financial support for 2 master students at the Biology/Chemistry interface will be provided.

Summary

Lipid transfer proteins acting at membrane contact sites (MCS) between membrane-bound organelles combine domains for membrane tethering and domains for lipid extraction. This division of labor allows LTPs to act as bridge-and-shuttle, bridging two organelles at an inter-distance of a few tens of nanometers and exchanging lipids between the gathered membranes.
We recently noticed that Oxysterol Binding Protein (OSBP) as well as many proteins of the same family contain a long (≈ 100 amino acids) N-terminal extension (N-ter) of low amino-acid complexity. Using in vitro and cellular assays, we showed that this region plays a critical role in OSBP dynamics at MCS. Specifically, our biochemical and cellular observations suggest a model in which the N-ter acts as an entropic tail that prevents OSBP and similar proteins from being too concentrated at MCS, thereby favoring their lateral movements within the crowded and narrow environment of MCS.



Scheme of a membrane contact site for cholesterol transport formed by OSBP (blue), VAP-A (green) and Arf (grey). Using a chemical biology approach, we will test the involvement of the intrinsically disordered region (red shaded region) in OSBP dynamics.

The aim of this proposal is to test the entropic chain model through a synthetic biology approach. The abundance (> 70%) of Gly, Ala and Pro residues in OSBP N-ter makes this region intrinsically unstructured. We will replace the N-ter by a polyethylene glycol (PEG) chain of defined length using a click-chemistry approach. Expression plasmids of the tethering region of OSBP (PH-FFAT) will be modified to incorporate a N-terminal pyrrolysine analogue (N3-Lys). This unnatural amino-acid will react with an Alkyne PEG chain. Owing to their high flexibility and solubility, PEG chains should impart hydrodynamic properties to OSBP similar to authentic N-ter. Increasing the size of the PEG chain will allow us to quantitatively assess the impact of the chain length on the hydrodynamic and diffusion properties of the protein. PEG-OSBP constructs will be tested using the following approaches: (1) size-exclusion chromatography to determine their hydrodynamic volume; (2) liposome reconstitution approaches to assess their membrane tethering activity; (3) FRAP experiments *in vivo* and *in vitro* to test their velocity in reconstituted or natural MCS. This project should help defining the function of intrinsically disordered sequences in proteins acting at MCS.
The two master students would mutually benefit from this inter-disciplinary project that aims at deciphering how a pure physicochemical principle – disorder – is used in a cellular context.

Related Publications:
- Mesmin B., Bigay J. et al. A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP. *Cell*. 2013 Nov 7;155(4):830-43.
- Mesmin, B., Bigay, J. et al. Sterol transfer, PI4P consumption, and control of membrane lipid order by endogenous OSBP. *The EMBO Journal* 2017 36, 3156–3174.
- Antonny B., Bigay, J., Mesmin, B. The Oxysterol-Binding Protein Cycle: Burning Off PI(4)P to Transport Cholesterol. *Annu Rev Biochem*. 2018 Jun 20;87:809-837
- Jamecna D., Polidori J., et al. An Intrinsically Disordered Region in OSBP Acts as an Entropic Barrier to Control Protein Dynamics and Orientation at Membrane Contact Sites. *Dev Cell*. Apr 2019, 22;49(2):220-234.