Master 2 internship project

Nano-ions as tools for membrane protein research

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Membrane proteins (MPs) are involved in numerous cell functions, such as cell signaling, metabolite/ion/lipid transport, and cell detoxification. It is therefore of major interest to characterize MPs at a molecular level. Such characterization generally requires extraction of MPs from biological membranes using detergents, for subsequent purification (Fig. 1). Owing to the inherent ability of detergent to denature MPs, solubilization of MPs remains challenging and empirical and new techniques are now emerging as alternatives to classical detergents.

In the frame of a collaborative project between the ICSM in Marcoule and the IBS in Grenoble, we aim at testing the potential of metallacarboranes (MCs), which are ions of nanometric size, for the solubilization as well as the functional and structural investigation of MPs. Indeed, the ability of MCs to solubilize model membranes has recently been demonstrated by our collaborators in Marcoule. Our project should help understanding interactions between nano-ions and biological membranes. Furthermore, our approach aims to identify a potential stabilizing effect of nano-ions toward MPs, to facilitate determination of their three-dimensional structure. As MPs represent the therapeutic of about 60 % of the currently used drugs, determination of their high-resolution structure is of critical importance.

The student hired on this project will examine the effect of COSAN, a reference MC that has been characterized in depth from a physico-chemical perspective, on four different target MPs of eukaryotic origin: i) the Ca^{2+}-transporter SERCA1a, which is essential for uptake of Ca^{2+} into the endoplasmic reticulum lumen and therefore for muscle relaxation, ii) the lipid transporter ('flippase') Drs2p/Cdc50p, which controls transbilayer lipid asymmetry in eukaryotic cells, iii) P-glycoprotein, an ABC transporter involved in multidrug resistance, and iv) Caveolin, a MP which controls shear-induced stress in cell membranes. The diversity of the chosen targets should allow to provide a proof of principle using different membrane systems. Solubilization of membranes with COSAN will be investigated by centrifugation and wester-blotting techniques, by measuring light scattering, and will be compared to solubilization of the same MPs with classical detergents used routinely in our lab. The function and stability of detergent- and MC-solubilized MPs will be assessed thanks to ATPase activity assays or phosphorylations assays using radioactive tracers. The oligomeric state and monodispersity of solubilized proteins may also be investigated using size-exclusion chromatography. In case COSAN would not exhibit any ability to solubilize MPs, we plan to test its possible use as a stabilizing additive during purification or crystallization of MPs.

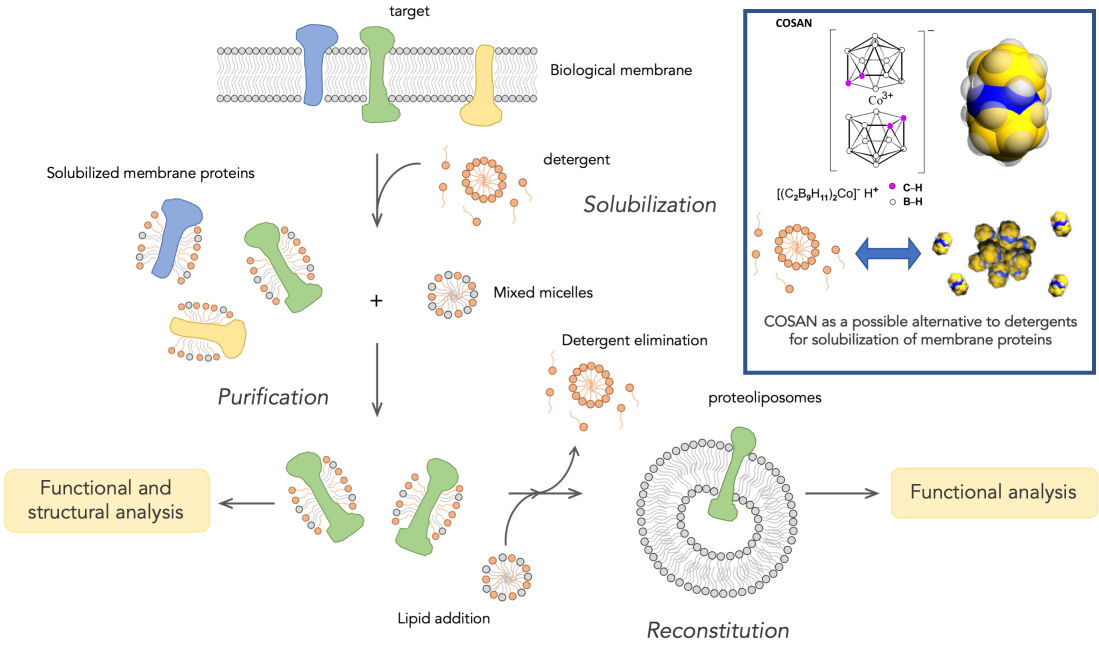

Figure 1: schematic of the various steps required for the functional and structural analysis of membrane proteins (solubilization, purification, and reconstitution). COSAN, an inorganic nano-ion which proved able to solubilize model membranes, appears as an alternative to detergents.

Selected references of the team on this topic:

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