Shaping, clustering and sorting of BmrA, a multi-drugs resistance membrane transporter with specific lipids. D. Lévy and P. Bassereau, UMR 168 CNRS UPMC Institut Curie, Paris.

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Cellular compartments are enclosed by membranes composed of transmembrane proteins, membrane associated and lipids (reviewed in [1]). Membrane proteins are involved in major cellular functions including cell communication, adhesion, homeostasis detoxification or bioenergetics and their dysfunction is responsible of several diseases. The knowledge of the structure and function of many individual membrane components has reached a level that allows now focusing on their interactions. Biologists and physicists with their respective approaches have evidenced the crucial role of lipid and protein interactions in the formation of protein clusters in the membrane plane, in the remodeling of lipid domains and in the changes of membrane curvature (reviewed in [2]). These clusters can be transient or not, and are crucial for intracellular signal induction. Here the Teams of D. Lévy (T1) and of P. Bassereau (T2) propose to join their expertise in membrane biology and membrane physics, respectively, to understand the role at the molecular level of the lipid environments on the conformation of membrane proteins and thus on their function and the consequence at the membrane scale on the formation of supramolecular assembly of lipid/protein domains and on the sorting of proteins.

In this context, members of the family of ABC transporters are powerful models to tackle this question. ABC (ATP-Binding Cassette) transporters are involved in the ATP-driven efflux of various substrates including lipids, sterols, hormones, peptides, antibiotic, anticancer drugs (see figure below, Blue Panel, A) [3; 4]. Several ABC transporters are responsible of Multi–Drugs Resistance (MDR) phenotypes, against antibiotic by bacteria or against chemotherapeutic agents in human tumors (Blue Panel, A). Recent publications, including from Team 1, have provided evidences that specific lipids modulate the conformation of the internal protein cavity in which drugs and other translocated substrates are binding [5; 6]. Furthermore, several ABC bacterial and eukaryotic transporters are also involved in the translocation of lipids or sterols with major consequences on the cellular functions. This includes e.g. in the case of Gram-negative bacteria the transport of lipid A, a lipid component of an endotoxin responsible for toxicity or in the case of human cells the formation of lipoprotein complexes (HDL and LDL) or remodeling of cholesterol domains in plasma membrane[7].

We are focusing on BmrA, an ABC transporter that Team 1 purified and biochemically characterized in detail [8; 9]. Team 1 has also published a 3D model of the apo-conformation of BmrA in the absence of drugs, i.e. apo-conformation from cryo-electron microscopy images (cryo-EM) at 2.3 nm resolution, revealing a V-shape conformation of protein and the surrounding lipid leaflet (Blue Panel, B). This V-shape induces a large curvature of the lipid membrane due to an asymmetrical distribution of lipids on both leaflets. Preliminary reconstitution in small liposomes of apo-BmrA resulted in the formation of ribbons (Red panel, A), rings (B) or tubes (C) depending on the lipid environment, suggesting different conformations as a function of the lipid nature. Moreover, when BmrA is in the post-hydrolytic state, a large conformation change occurs that leads to the formation of planar membranes. This strongly suggests a direct link between the lipid composition, the conformations of BmrA and the shape of the membrane. Such link has been widely studied with membrane-associated proteins (reviewed in [10]) but BmrA seems to be the first reported example of a transmembrane protein for which both the lipid environment and the functional state are responsible of changes in membrane curvature. From the physics point of view, team 2 has been able to recently demonstrate the coupling between protein shape and its curvature-dependent distribution, in model membranes [12]. However, different theoretical models predict lipid-protein interactions, depending on protein conformation as well as lipid bilayer properties that can lead to protein clustering or repulsion and also affect protein activity. A direct and quantitative proof of these models is still lacking. We expect this work to bring new understanding on the interplay between lipids and proteins for their function in cell membranes.

The aims of the PhD thesis will be to address the following questions: 1. What are the conformations of BmrA in different lipid environments? 2. What are the consequences of these conformations on the local and large-scale curvature of the membranes? 3. Do these conformations induce the formation of cluster of proteins, the reorganization of the lipids and domain formation or the sorting of proteins in membranes regions of specific curvatures? After studying the protein in fixed conformations, we will investigate these questions in dynamical conditions. This project requires a multidisciplinary approach of structural biology to determine the different protein conformations as well as local membrane deformations and of membrane biophysics to establish large-scale membrane deformation, protein clustering, curvature-induced protein sorting dependent on the conformation and on the activity. The PhD thesis will combine biochemical and structural approaches of cryo-EM and cryo-tomography with in vitro physical studies of protein clustering and sorting after reconstitution in Giant Unilamellar Vesicles (GUV) and formation of membrane nanotube of controlled diameter.

A provisional program of the PhD thesis is the following:

Year 1: 3D reconstructions of BmrA in apo conformation in the presence of specific lipids by cryo-electron microscopy by single particle analysis or cryo-tomography and sub-tomogram averaging associated with images analysis. The goal is to obtain 2-3 different 3D models of apo-BmrA and its lipidic environment at nanometric resolution. This will performed with Team 1. In parallel, functional analysis of BmrA reconstituted in small liposomes with specific lipids by biochemical approaches will be performed in collaboration with J. M. Jault (IBCP, Lyon). The goal is to establish structure/function relationships with specific lipids.

Year 2: Reconstitution in Giant Unilamellar liposomes according to the new method developed by Teams 1 and 2 [11]. Study of lipid and protein clustering by FCS (Fluorescence Correlation Spectroscopy) and membrane morphology by 3D confocal microscopy (see previous reconstitution experiments on an ABC transporter (green), translocating ethidium bromide (red) Blue panel, C). The goal is to correlate the molecular conformation of BmrA and the consequence at large scale on the membrane organization and shape. The effect of protein ATP-ase activity on clustering and membrane shape will be studied. This will performed with Team 1 and 2.

Year 3: Curvature-dependent sorting of BmrA. Fluorescent BmrA will be reconstituted in GUVs made on different lipid compositions. Its distribution between the GUV and a membrane nanotube pulled with an optical tweezers will be measured by confocal microscopy, for the apo-protein and upon activation. Curvature can be tuned by changing membrane tension upon micropipette aspiration. The possible mechanical effect on the membrane will be deduced from the force measurement [12]. This will performed with Team 2. Writing PhD thesis manuscript and publications.

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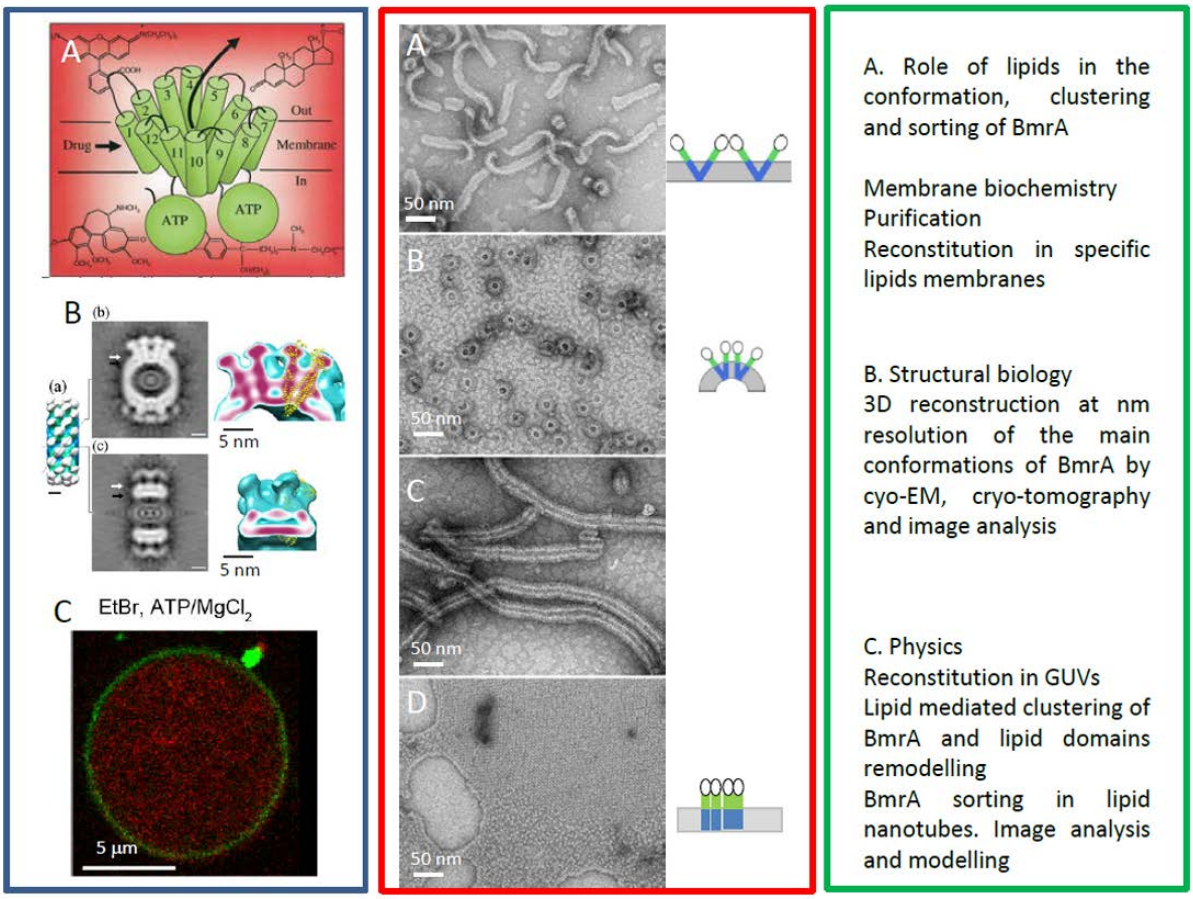

Role of lipids in the conformation, supramolecular assembly and sorting of BmrA, a bacterial ABC membrane transporter. Blue panel:- A. ABC membrane transporter translocate various drugs using ATP hydrolysis. **B.** 3D reconstruction of BmrA, a bacterial ABC transporter reconstituted in lipid bilayer at 2.3 nm resolution computed from cryo-electron microscopy images [6]. The apo-conformation of BmrA is V-shaped. The external envelop of protein reconstituted in lipid membrane is in indigo and interior in purple. a) Apo-BmrA was reconstituted in EPC/EPA lipid in the absence of drugs, as in red panel image (B). b, c slides through reconstituted proteins. The inner and outer lipidic leaflets are resolved (arrows). **C.** Reconstitution and drugs translocation of BmrC/BmrD, an ABC transporter in GUVs. Proteins (green labeled) have translocated ethidium bromide that further bound to encapsulated DNA (red fluorescence). **Red panel.** BmrA was reconstituted in the apo-conformation in various lipid environments, in EPC/EPA (A), DMPC (B) and E. coli lipids (C) and in EPC/EPA but in post-hydrolytic state (D). The shape of the reconstituted membranes is related to the conformation of BmrA. Green panel. Provisional program of the PhD Thesis.