Development and analysis of biomimetic systems to understand major cellular functions including e.g. cell detoxication, ion homeostasis, bioenergetics and membrane shape with tools and concepts of membrane biochemistry, physical-chemistry and cryo-electron microscopy have been an important approach of the Team since its creation. It involves the identification and purification of the minimal molecular components required to mimic a function, to control and modify the membrane determinants of the function that are not accessible in a more complex system within cells, to understand and propose new mechanisms and to reveal emerging physical properties. It has led to important information about lipid–protein and protein–protein interactions as well as topological and topographical features of different classes of membrane proteins.

We have developed efficient methods of incorporation of any type of transmembrane proteins in Small Unilamellar Vesicles (50-300 nm), Giant Unilamellar Vesicle (5-20 microns), in planar lipid bilayer or at the air/water interface. The strength of these methods are the complete characterisation of the reconstituted systems (protein function, incorporation rate, protein orientation, membrane permeability and liposome lamelarity) to allow accurate quantitative measurements.

Reconstitution of transmembrane proteins in liposomes

Pioneer researches of J.L. Rigaud and colleagues including D. Lévy on the mechanisms of proteins insertion into liposomes during reconstitution by detergent removal led to propose experimental guidelines for the reconstitution of any type of membrane proteins in well-defined proteoliposomes. Direct incorporation into preformed liposomes as the use of BioBeads to remove detergents derived from these studies are widely used today.

Three mechanisms of protein incorporation into proteoliposomes during reconstitution involving detergent removal. (1) Direct and unidirectional incorporation of micellar protein into detergent destabilized liposomes. (2) Transfer of micellar proteins into mixtures of lipidic micelles and vesicles. (3) Incorporation by coalescence of mixed micelles of lipids, proteins and detergent.

Reviews on reconstitution:
in vitro membrane systems
Molecular Microscopy of Membranes


**Detergent removal with Bio-Beads**


**Reconstitution of specific proteins**


Formation of 2D crystals by detergent removal


Reconstitution of membrane proteins in GUVs

Giant unilamellar vesicles (GUVs) are convenient biomimetic systems of the same size as cells that are increasing used to quantitatively address biophysical and biochemical processes related to cell functions. We developed a method to incorporate transmembrane proteins in GUVs, based on concepts developed for detergent-mediated reconstitution in large unilamellar vesicles. Reconstitution is performed either by direct incorporation from proteins purified in detergent micelles or by fusion of purified native vesicles or proteoliposomes in preformed GUVs.
Lipid compositions of the membrane and the ionic, protein, or DNA compositions in the internal and external volumes of GUVs can be controlled. Using confocal microscopy and functional assays, we show that proteins are unidirectionally incorporated in the GUVs and keep their functionality. We have successfully tested our method with three types of transmembrane proteins. GUVs containing bacteriorhodopsin, a photoactivable proton pump, can generate large transmembrane pH and potential gradients that are light-switchable and stable for hours. GUVs with FhuA, a bacterial porin, were used to follow the DNA injection by T5 phage upon binding to its transmembrane receptor. GUVs incorporating BmrC/BmrD, a bacterial heterodimeric ATP-binding cassette efflux transporter, were used to demonstrate the protein-dependent translocation of drugs and their interactions with encapsulated DNA. Our method should thus apply to a wide variety of membrane or peripheral proteins for producing more complex biomimetic GUVs.

Related Publication: Dezi, M. PNAS 2013.
We developed a new method of incorporation of transmembrane proteins in planar lipid bilayer starting from 1 pmol of solubilized proteins. The principle relies on the direct incorporation of solubilized proteins into a preformed planar lipid bilayer destabilized by dodecyl-b-maltoside or dodecyl-b-thiomaltoside, two detergents widely used in membrane biochemistry. Successful incorporations are reported at 20°C and at 4°C with three bacterial photosynthetic multisubunit membrane proteins. Height measurements by atomic force microscopy (AFM) of the extramembraneous domains protruding from the bilayer demonstrate that proteins are unidirectionally incorporated within the lipid bilayer through their more hydrophobic domains. Proteins are incorporated at high density into the bilayer and on incubation diffuse and segregate in protein close-packing areas. The high protein density allows high-resolution AFM topographs to be recorded and protein subunits organization delineated. This approach provides an alternative experimental platform to the classical methods of twodimensional crystallization of membrane proteins for the structural analysis by AFM. Furthermore, the versatility and simplicity of the method are important intrinsic properties for the conception of biosensors and nanobiomaterials involving membrane proteins.


2D crystallization of membrane proteins at the lipid layer

2D crystallization of membrane proteins at the lipid layer has been originally developed by our Team in 1999.
The principle derived from both 2D crystallization of soluble proteins at the lipid layer and 2D crystallization of membrane proteins in bulk. The principle relies on the specific binding of ternary micelles of lipid/protein/detergent to a functionalized lipid layer present at the air/water interface. After binding to the lipid surface, detergent is removed with Bio-Bead or cyclodextrins leading to the reconstitution of membrane protein in a lipid bilayer. Protein/Protein interaction can occur leading to the formation of 2D crystals. Surface can be transferred to an EM grid for analysis.

Main interest of the method: 1) the specific recognition of the surface, e.g. a His-protein to a Ni-NTA DOGS lipid layer, lead to decrease the protein working concentration to less than 10 microg/ml. 2) Due to the specific binding, proteins have a single orientation within the lipid layer while reconstitution is often symmetrical when performed in bulk (Levy, D. J. Struct. Biol., 1999). This allowed to crystallized proteins with bulky extramembraneous domain, like ATPsynthase or ABC transporters, that can not be crystallized in bulk.

Main improvements: 1) we developed an optical set-up to in situ screen the binding and the formation of the reconstituted lipid layer (Dezi, J. Struct. Biol. 2011). 2) We developed a method to transfer the reconstituted lipid bilayer to a hydrophobic wafer for the analysis by Atomic Force Microscopy (Seentier, B. J. Mol. Recong. 2011). 3) We obtained similar 2D crystals of BmrA, an ABC transporter after specific binding of fully hydrogenated Ni-NTA-DOGS lipid and to fully fluorinated Ni-NTA lipid (Hussein, J Org Chem. 2009). 4) The functionalized lipid layer can also be used for the purification of membrane proteins from solubilized extract and single particle analysis (Dezi, BBA, 2011).

Proteins crystallized by the method by us * or other groups:

- E. coli FIFO (Arechaga, Struct. Biol., 2007)
- ADT/ATP carrier (Lévy, D. J. S. Biol., 2001)*
- Bacteriorhodopsin (Lévy, D. J. S. Biol., 2001)*
- Ryanodine receptor (Yin CC, J. Struct. Biol. 2005)
- OprN (Chami, M. Pers. Com)
- Aqp1 (S. Scheuring, Pers. Com)
- LH1-RC PufX deleted (Busselez, Pers. Com)*
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