Reconstitution of Transmembrane Proteins into Pore-Spanning Membranes

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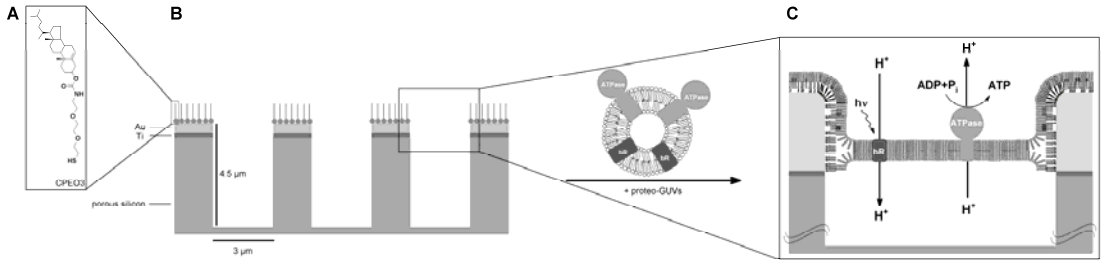

Schematic presentation of the functionalized porous substrates. A) Chemical structure of (cholesterylpolyethylenoxy)thiol (CPEO3); B) Porous substrate with titanium, gold and CPEO3functionalization; C) addition of giant unilamellar vesicles (GUVs) an dformation of planar pore-suspending membrane. Arrows indicate light-induced proton pumping of bacteriorhodopsin (bR) and degradation of the proton gradient coupled with ATP generation by ATPase.

Pore-spanning membranes have been shown to combine the stability of solid-supported membranes (SSM) with the accessibility to aqueous compartments on both membrane sides, which so far has only been achieved by free standing membranes, obtained by the Müller-Rudin- technique [1]. Thus, they are suited for investigating the activity of transmembrane proteins with a broad variety of biophysical methods, such as impedance spectroscopy, scanning ion conductance microscopy (SICM) or confocal laser scanning microscopy (CLSM).

This model-system allows the co-incorporation of ATP-Synthase, a transmembrane protein, which converts ADP and inorganic phosphate into ATP when translocating protons across the membrane and bacteriorhodopsin, a light-driven proton pump, yielding to overall conversion of light energy into chemical energy on a microarray-based setup.

Bacteriorhodpsin (bR) has been reconstituted into giant unilamellar vesicles (GUVs) according to a modified reverse-phase method described by Girard et al. [2]. Reconstitution was verified by means of confocal laser scanning microscopy (CLSM) after labeling the protein with a fluorescence dye. Photocurrent measurements on the one hand and encapsulation of the pH-sensitive dye pyranine on the other hand have been used as tools to prove bR proton-pumping activity. Spreading of proteo-GUVs onto porous silicon yields pore-spanning membranes with diameters in the micrometer-range.

By irradiation of reconstituted bR a proton gradient across pore-spanning membranes can be obtained, which allows an ATP-Synthase induced generation of ATP, the main energy source for in vivo biochemical reactions.

[1] D Weiskopf, E K Schmitt, M H Klühr, S K Dertinger, C Steinem, Micro-BLMs on Highly Ordered Porous Silicon Substrates: Rupture Process and Lateral Mobility, Langmuir, 2007, 23, 9134-9139

[2] P Girard, J Pécréaux, G Lenoir, P Falson, J L Rigaud, P Bassereau, A New Method for the Reconstitution of Membrane Proteins into Giant Unilamellar Vesicles, Biophys J, 2004, 87, 419-429