Biological membranes – Energization by the proton potential difference

Biological membranes of energy metabolism in Mitochondria, Chloroplasts and micro-organisms perform their function by membrane-energization, which is the generation of an electrophysical proton potential difference across a membrane. This couples the energy of respiration, photosynthesis or ion transport to membrane proteins as ATP-synthese and Cytochrome-Oxidoreductases: function by energetic coupling during proton transport. Furthermore the membrane potential acts as an effecter in the molecular regulation of several proteins: structural regulation by the physical membrane. While the biological relevance is clear, the effect of the electrophysical membrane potential difference on lipid and protein structure and function has to be investigated at the molecular level. Those processes can be studied with liposomes as homogenous model membranes using time resolved methods (transient states).

**Liposomes as model membranes – Reconstitution and Energization by pH-jump**

The energized membrane state was estimated by spectroscopy and TR-SANS of liposomes after a large pHJump (pH > 1).

The pH-jump was achieved by two techniques:

i) by rapid acid addition using a stopped flow device: acid outside

ii) by flash photolysis of novel caged acids (caged proton, jump-time = 170 µs) : acid inside, as required for ATP-synthese. The caged acid replaces the Bacteriorhodopsin by a small molecule in structure studies.

As novel results we observed a change in lipid bilayer structure upon membrane energization (pH > 0.5). The thickness of the hydrophobic core shrunk by 1 Angstrom while no swelling (liposome size change by water uptake) was observed in the choosen system (10% glycerol-buffer).

Spectroscopic experiments with pH-indicator entrapped liposomes showed an increase of the proton permeability by an order, which is consistent with a transition of transient hydrogen bond chain (HBC) pores of diffusion controlled type-C to type-A of longer lifetime.

The experiments are currently extended to ATP-synthase-liposomes. In those proteoliposomes the lipid entity was matched by contrast variation, i.e. application of D2O/O-mixtures as solvent. The liposomes from DMPC-D2O were matched by 85% D2O, while protein-less SUV from protonat Phosphatidy-Cholins (DMPC, DOPC, SbPC ) were investigated in H2O/O-buffer (pH, 10% glycerol).

**Function test : proton tracking - entrapped dye**

The response of indicator-liposomes, dye and the buffering of lipid membranes was calibrated as shown left.

(a) The proton flux across liposome membranes can be estimated by tracking the light absorption of an indicator dye, while the structure parameters (s, Rg, d) are obtained by neutron scattering (4);

(b) Setup for time resolved scattering at the D22-beamline at ILL: (d) SANS of SBL-liposomes in a single frame of 1s after pH-jump; (e) the evaluation during 120 s reveals that no pH-driven water uptake occurs in this system.

Structure and proton permeability upon energization (H+)

Structure dynamics estimation by time resolved Neutron scattering

The time resolved scattering was observed with cold neutrons at the D22-beamline at ILL in 65-200 frames of logarithmic time resolution (>500 ms, 5.3% time-increase /frame). Our sample environment resembled that we developed for ESRF & DESY [5]

Materials and Methods:

Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from Micrococcus luteus ATCC6069 were prepared from DMPC-Δφ and matched by 85% D2O, while protein-less SUV from protonat Phosphatidy-Cholins (DMPC, DOPC, SbPC ) were investigated in H2O/O-buffer (pH, 10% glycerol).

SBL-liposomes in a single frame of 1s after pH-jump; (e) the evaluation during 120 s reveals that no pH-driven water uptake occurs in this system.


**Structure of Liposomes and Proteoliposomes analyzed by Time-Resolved Neutron Small Angle Scattering TR-SANS**

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**Results**

Detergent assisted reconstitution of preformed liposomes (sonified) with purified membrane proteins (ATP-synthase [1,2], monomeric bacteriorhodopsin mBR [3]):

The complex proteoliposomes are used for function investigations [1,2], while simpler membrane proteins (ATP-synthase [1,2], monomeric bacteriorhodopsin mBR [3]):

Detergent assisted reconstitution of preformed liposomes (sonified) with purified membrane proteins (ATP-synthese [1,2], monomeric-bacteriorhodopsin mBR [3]): The complex proteoliposomes are used for function investigations [1,2], while simpler vesicles (only 1 protein molecule/vesicle) and pure lipid vesicles with entrapped pH-indicator dye are suitable for H-transport and structure dynamics investigations.

**Materials and Methods:**

Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from Micrococcus luteus ATCC6069 were prepared from DMPC-Δφ and matched by 85% D2O, while protein-less SUV from protonat Phosphatidy-Cholins (DMPC, DOPC, SbPC ) were investigated in H2O/O-buffer (pH, 10% glycerol).