Structure Dynamics of Energized Biological Membranes estimated by Time-Resolved Neutron Small Angle Scattering TR-SANS

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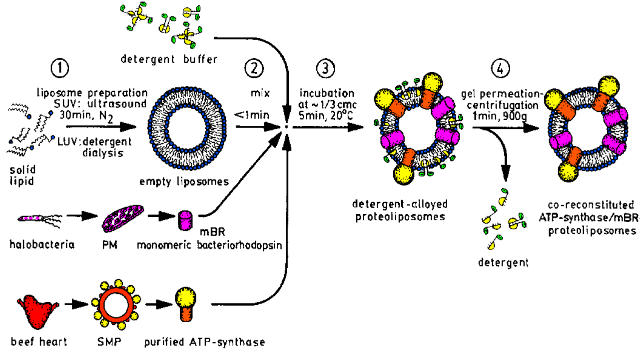
Biological membranes – Energization by the proton potential difference

Biological membranes of energy metabolism in Mitochondria, Chloroplasts and micro-organisms perform their function by membrane-energization, which is the generation of an electrochemical proton potential difference across a membrane. This couples the **energy** of respiration, photosynthesis or ion transport to membrane proteins as ATP-synthase and Cytochrome-Oxidoreductases: **function** by energetic coupling during proton transport.
Furthermore the membrane potential acts as an effector in the molecular regulation of several proteins: **structural regulation** by the physical membrane.
While the biological relevance is clear, the effect of the electrochemical 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



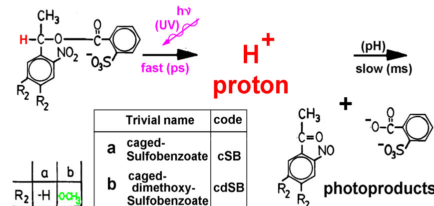
Detergent assisted reconstitution of preformed liposomes (sonified) with purified membrane proteins (ATP-synthase [1,2], monomeric bacteriorhodopsin mBR [3]) : The complex proteoliposomes are used for function investigations [1,2] , while simpler vesicles (only 1 protein molecule/vesicle) and pure lipid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

Materaials and Methods:
Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from *Micrococcus luteus* ATCC4698 were prepared from DMPC-D_{54} and matched by 85% D_2O, while protein-free SUV from protonat Phosphatidyl-Cholins (DMPC, DOePC, SbPC) were investigated in H_2O-buffer (pH8, 10% glycerol).

The **energized membrane state** was estimated by spectroscopy and TR-SANS of liposomes after a large pH-jump (? pH > 1).
The pH-jump was achieved by two techniques:

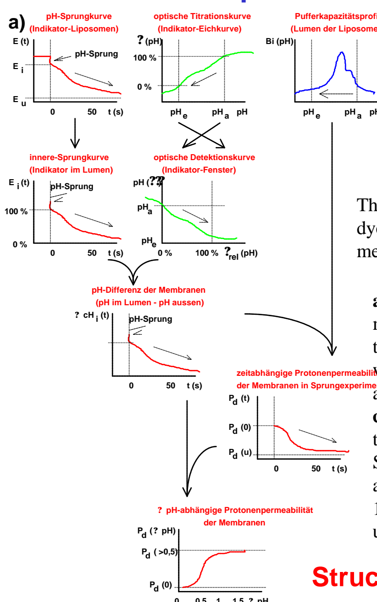
i) by rapid **acid addition** using a **stopped flow** device: acid outside

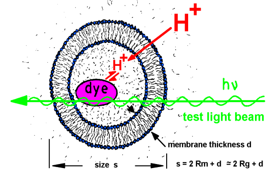
ii) by **flash photolysis** of novel **caged acids** (caged proton, jump-time = 170 µs) : acid inside, as required for ATP-synthase. The caged acid replaces the Bacteriorhodopsin by a small molecule in structure studies.



	Trivial name	code
a	caged-Sulfobenzoate	cSB
b	caged-dimethoxy-Sulfobenzoate	cdSB

Function test : proton tracking - entrapped dye

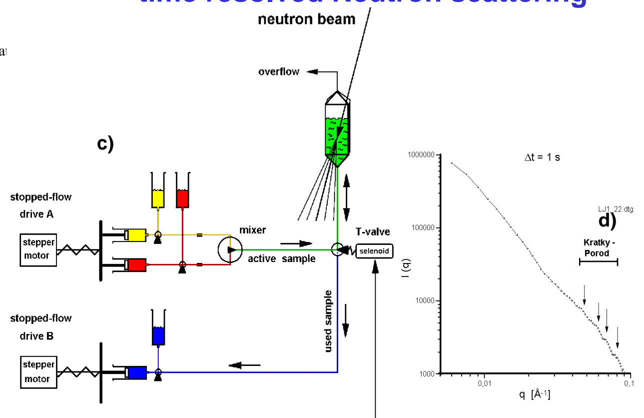




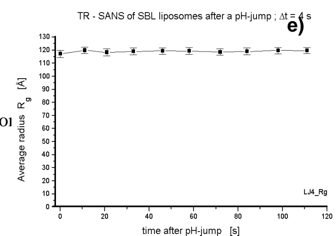
The response of indicator-liposomes, dye and the buffering of lipid membranes was calibrated as shown left.

a,b) 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); **c)** 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 dynamics estimation by time resolved Neutron scattering



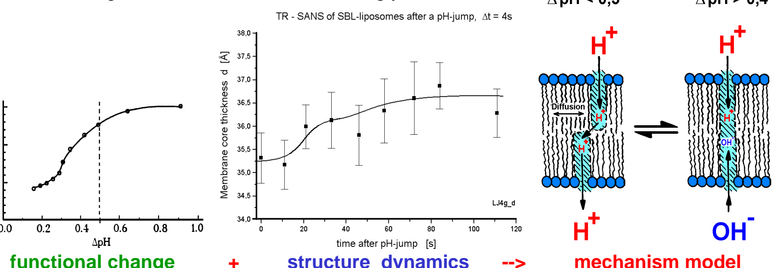
The time resolved scattering was observed with cold neutrons at the D22-beamline at ILL in 65-200 frames of logarithmic time resolution (>500 ms, 5.3% time-increase /frame). Our sample environment resembled that we developed for ESRF& DESY [5]



Structure and proton permeability of membranes changes upon energization (H^+)

As novel **results** we observed a change in lipid bilayer structure upon membrane energization (? pH > 0.5). The thickness of the hydrophobic core shrinked by 1 Angstroem 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 (tHBC) pores of diffusion conntrolled 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 D_2O/H_2O-mixtures as solvent. The liposomes from DMPC-D_{54} were matched by 85% D_2O-buffer, while the lipid contributed 98% of the particle mass. After subtraction of the neutron scattering of matched protein-free reference liposomes, the scattering contribution of the protein *in situ* was obtained and compared to the neutron scattering of purified ATP-synthase in detergent solution (5 mM TDOC, 10% glycerol).



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