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

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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. Those processes can be studies with liposomes as model membranes.
Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from *Micrococcus luteus* were prepared from DMPC-D_{54} and matched by 85% D_2O, while protein-free SUV from protonated Phosphatidyl-Cholins (DMPC, DOePC, SbPC) were investigated in H_2O-buffer. The energized membrane state was estimated by TR-SANS of liposomes after a large pH-jump (delta-pH > 1). The pH-jump was achieved by two techniques:
i) by rapid acid addition using a stopped flow device and
ii) by flash photolysis of novel caged acids (caged proton, t-jump = 170 micro-s).
The time resolved scattering was observed with 0.8 nm neutrons at the D22-beamline at ILL in 65-200 frames of logarithmic time resolution (>500 ms).
As a novel result we observed a change in lipid bilayer structure upon membrane energization (delta-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 type-C to type-A.
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 ATP-synthase in detergent solution (5 mM TDOC).