

Structure Dynamics of Energized Biological Membranes estimated 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 studied with liposomes as model membranes.

Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from *Micrococcus luteus* were prepared from DMPC-D₅₄ and matched by 85% D₂O, while protein-free SUV from protonated Phosphatidyl-Cholins (DMPC, DOePC, SbPC) were investigated in H₂O-buffer. The energized membrane state was estimated by TR-SANS of liposomes after a large pH-jump ($\Delta\text{pH} > 1$). 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, $t\text{-jump} = 170 \text{ micro-s}$). 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 ($>500 \text{ ms}$).

As a novel result we observed a change in lipid bilayer structure upon membrane energization ($\Delta\text{pH} > 0.5$). The thickness of the hydrophobic core shrank by 1 Angstrom while no swelling (liposome size change by water uptake) was observed in the chosen 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.