Neutrons and life science-Oral

Structure dynamics of energized biological membranes - time-resolved neutron scattering TR-SANS

T. Nawroth[1], R. Gebhardt[1], K. Zwicker[2], I. Grillo[3], R.P. May[3]

(1) Physics Department E17 / E13, TU Muenchen, James-Franck-Str., D-85747 Garching. (2) Zentrum Biol. Chemie ZBC, University Clinics, D-60596, Frankfurt/M. (3) Institut Laue Langevin ILL, Large Scale Structure Group LSS, BP156, F-38042 Grenoble CEDEX9

Biological membranes of energy metabolism get their function by energization, i.e. generation of an electrochemical proton potential difference across a membrane. This couples the energy of respiration, photosynthesis or ion transport to membrane proteins. Those processes can be studied with liposomes as model membranes. Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from *Micrococcus luteus* [1,2] were prepared from DMPC-D54, protein-free SUV from protonated lecithins. The energized membrane state was estimated by TR-SANS of liposomes after a large pH-jump (ΔpH $>$ 1) at the D22-beamline at ILL in frames of logarithmic time resolution. 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). As a novel result we observed a change in the lipid bilayer structure upon membrane energization (ΔpH $>$ 0.5). The thickness of the hydrophobic core shrinked by 1 Å while no swelling was observed. 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 were extended to ATP-synthase-liposomes. In those proteoliposomes the lipid entity was matched by solvent-contrast variation [2]: The liposomes from DMPC-D54 were matched by 85% D2O-buffer, while the lipid contributed 98% of the mass. Subtraction of the scattering of matched protein-free reference liposomes yielded the contribution of the protein *in situ.* It was compared to the neutron scattering of ATP-synthase in detergent solution (5 mM TDOC, glycerol).

[1] H. Freisleben; K.Zwicker; T. Nawroth et al. (1995) Chem.Phys.Lip. 78, 137-147. [2] T. Nawroth; K. Dose; H. Conrad (1989) Physica 156 B, 489-492