EXPERIMENTAL REPORT

EXPERIMENT N° Test-261 INSTRUMENT D22

DATES OF EXPERIMENT 21./22. 11. 1997

TITLE **Time resolved neutron small angle scattering (TR-SANS) of liposomes - feasibility test**

EXPERIMENTAL TEAM (names and affiliation)

T. Nawroth, Biochemistry Institute, Gutenberg-University, Becherweg 30, D-55099 Mainz

Tel.: 0 049 6131 395702; http://www.uni-mainz.de/FB/Chemie/Biochemie/MPSD/TNa.html

M. Rössle, Max-Planck Institut für Biochemie, Am Klopferspitz, D-82152 Martinsried

F. Descamps, R.P. May, ILL, Grenoble

LOCAL CONTACT Roland P. May

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Transient structural changes occur in biological systems in working proteins and at membranes during the development of an electrochemical proton potential (energization), e.g. at liposomes after a pH-jump. In the present work we have tested a novel setup for time resolved neutron scattering (TR-SANS) at the D22 beamline of the ILL and investigated the feasibility limits with protein free lecithine liposomes.

The active sample, liposomes after a pH jump at pH6.4, was formed by rapid mixing (< 1s) of lipsomes (SUV from SBL; 24 g/l, 30 mM Tris-MES pH8, D_2O) and acid (0,25 M MES in D_2O) using the TR-SANS setup reported in the experiment TEST-252 (1997) and open flow-through quartz cuvettes (Hellma, d = 1 mm). As depicted in fig.1 the structure was observed during proton flux into the liposomes, i.e. development of the membrane proton potential from ΔpH = 1.6 to 0 during 100 s [3]. With a 6x6 mm neutron beam (8 Å), 1mm cell and 1:1 diluted sample (12 g/l) we obtained 82000 cts. Because of the detector count rate limit a 10 mm cell could only be used with neutron attenuators - a challenge for future detector improvements. The scattering in a single 1s frame of a TR-SANS series in fig.2 consists of a Guinier and a Kratky-Porod region (q= 0.045-0.088 $Å^{-1}$), which was disturbed by side maxima from hollow sphere and aggregate scattering (arrows). The evaluation (fig.3) yielded the average radius R_g = 121.5 ±3.0 Å and the maximal radius R_g = 237.6 ±5.4 Å (largest vesicle subpopulation). The TR-analysis (fig.4) showed that no water uptake occurs with SUV after a pH-jump (important for spectroscopic TR-analysis [3]). The Kratky-Porod analysis (fig.5,6) yielded a final membrane core thickness d= 36.6±0.5 Å. From these results the average liposome size s= 279.8 ±6.8 Å was calulated as shown earlier [1,2]. As an important result we observed a contraction of the lipid bilayer at high proton potential (fig.6).

1) Nawroth, T.; Conrad, H.; Dose, K. (1989) Physica B 156 & 157, 477-480

2) Nawroth, T.; Dose,K.; Conrad, H.; Koch, H., Ringsdorf, H. (1989) Physica B 156, 496-8

3) a) Nawroth et al (1998) DGfB German Biophysics Soc. y conference proceedings, 108

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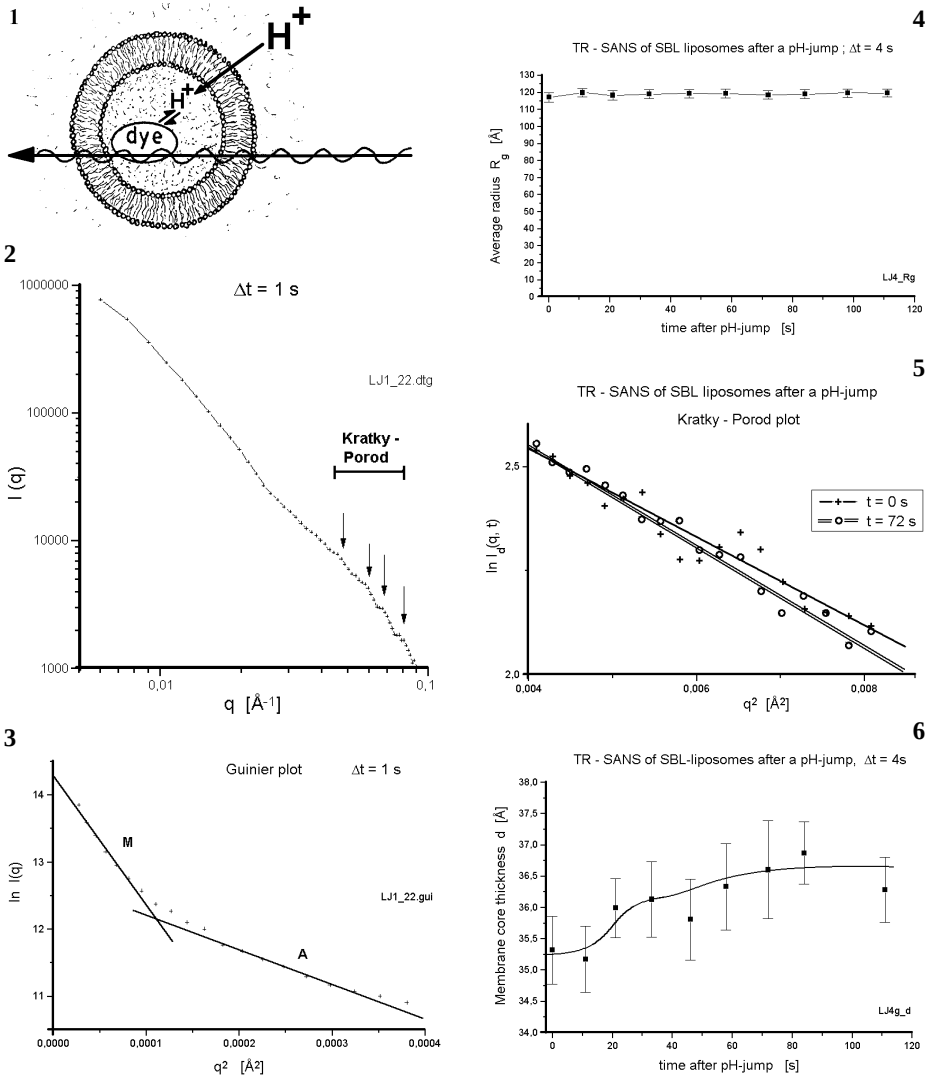

Figures: 1) The proton flow into liposomes is followed by TR-SANS and light; **2)** A single TR-SANS frame of 1 s; **3)** The Guinier region **A** yields the average liposome radius, wheras the maximum radius is obtained by the range **M**; **4)** The constant R_g in TR-SANS indicates that no water uptake occurs after a pH-jump; **5)** The Kratky-Porod plot yields at t=0 and 72s different thickness radius R_d and diameter d; **6)** Immediately after the pH-jump of 1.6 a contraction of the lipid bilayer is indicated by the decreased membrane core

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