EXPERIMENTAL REPORT

EXPERIMENT N° **8-03-302** INSTRUMENT **D22**

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TITLE **In situ structure of membrane bound ATP-synthase from *Micrococcus luteus* in contrast-matched liposomes**

EXPERIMENTAL TEAM (names and affiliation)

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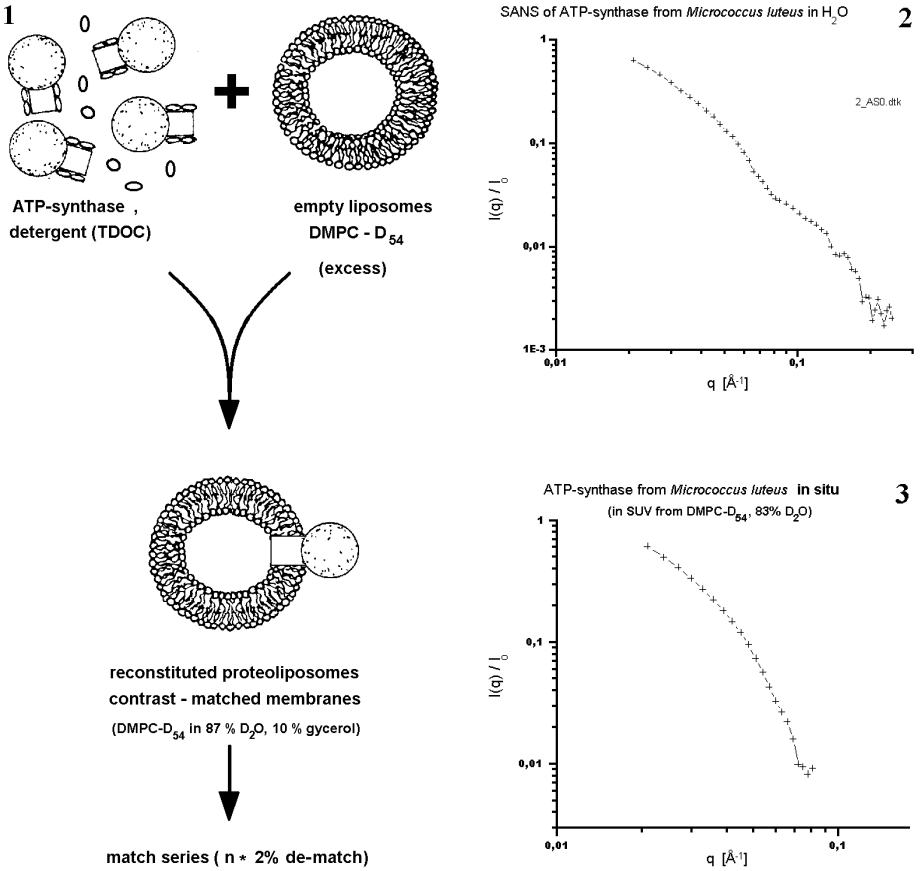
The structure of membrane proteins in situ, i.e. bound to membranes, can be exclusively studied by neutron small angle scattering of contrast matched reconstituted proteo-liposomes [1,2]. In this study we were successful in improving the method by application to the remarkable stable ATP-synthase from *Micrococcus luteus* in DMPC-D_{54} liposomes and match variation series (systematic de-matching in 2% steps).

In the experiments three short subexperiment series with each of the particle types in the reconstitution process were done: As shown in fig.1 the proteoliposomes were formed by reconstitution of preformed liposomes from DMPC-D_{54}, ATP-synthase and detergent solution by our liposome - detergent incubation method [1]. The very small contribution of 5 mM TDOC detergent micelles [3] was eliminated by subtracting detergent reference buffer, which yielded the SANS of free ATP-synthase in H_2O (fig.2). The Guinier approximation (q= 0.021-0.027 $Å^{-1}$) yielded a radius of gyration of R_g= 55.21 ±0.13 Å (in D_2O: 62.3 ±0.5 Å). The semi-direct Fourier transformation (extrapolation of the missing regions only [by Guinier q<0.021 $Å^{-1}$; by Porod 0.25<q<0.6 $Å^{-1}$], then discrete sinus FT) yielded the maximum dimension r_{max}= 180 ± 2 Å. By contrast variation (0, 68, 97% D_2O content in 10% H-glycerol buffer) the protein scattering length density ρ = 2.24 ± 0.03 * 10^{-10} cm^{-2} was obtained, which is required for the evaluation of the proteoliposome subexperiment.

Pure (protein-free) liposomes (sonicated SUV) from DMPC, SBL and DMPC-D_{54} were investigated. With SUV from DMPC and SBL time resolved SANS was done as reported in the TEST-261 and TEST-252 experiments. As important improvement we were successful in testing the novel ILL-time-frame controller, which allowed TR-SANS without time-gaps.

With ATP-synthase/DMDC-D_{54} liposomes and pure reference vesicles match variation series were done by systematic de-matching of slightly over-matched SUV (87% D_2O, 10% H-glycerol). The detergent content (1.67 mM) was not removed for SANS because it stabilizes the liposomes [4]. The subtraction of the scattering at the lipid match point yielded the SANS of ATP-synthase from *Micrococcus luteus* in situ (fig.3): R_g= 60.64



Figures:

1) Proteoliposome reconstitution by incubation of preformed DMPC-D_{54} liposomes (41 g/l), ATP-synthase (4.74 g/l) and detergent (TDOC; finally 1.67 mM = 1/2 cmc); **2)** SANS of 3.74g/l ATP-synthase in 5 mM TDOC buffer pH8 (H_2O; 1h); **3)** SANS of ATP-synthase of *Micrococcus luteus* (1.58 g/l) in contrast-matched liposomes, i.e. in situ (t = 1h). The valid range will be increased by a factor of 2 by subtraction of match-extrapolated datasets instead of direct experiment files after completion of the KINEX2 evaluation program.

References1) Nawroth, T.; Conrad, H.; Vienken, J.; Dose, K. (1983) Hoppe Seyler's Z. Physiol. Chemie 364, 923-931
2) Nawroth, T.; Dose, K.; Conrad, H. (1989) Physica B 156 & 157, 489-492
3) Conrad, H.; Dose, K.; Nawroth, T. (1989) Physica B 156 & 157, 474-476
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