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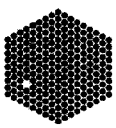
Arbeitsgruppen für Strukturelle Molekularbiologie



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Editorial Note (HASYLAB)
The authors of the individual scientific reports published in the HASYLAB annual report (part I and II) are fully responsible for the contents.
We kindly request your comprehension as pertains the adaption of the layout of contributions to our requirements (without changing the contents). Altough we tried to take care of any errors caused by the electronic submission of the contributions, we cannot fully exclude this possibility.

Contributions with the MPSD group :

Ultra small angle scattering of the detergent solubilized membrane protein ATP-synthase

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Membrane proteins can be structurally investigated after solubilization by weak non-denaturating detergents, e.g. bile acids or alkylated carbohydrates as laurylmaltoside. The detailed three-dimensional structure investigation requires X-ray crystallography whereas the structure dynamics is accessible by small angle scattering of protein - detergent solutions. Both of these complementary methods require monodisperse solutions free of protein agregates. Those aggregates may destroy the scattering profile even if present in the low percent range. They can be impurities or may be formed by slow aggregation after thawing of a frozen protein solution. We have shown earlier by time resolved neutron small angle scattering of ATP-Synthase from *Rhodospirillum rubrum*, a rather sensitive protein of 500,000 mass, at the FRJ-2 reactor, FZ Jülich, that those aggregates can be detected by a break in the Guinier plot at very low scattering angle ($q = 0.001 - 0.02\ A^{-1}$) [1]. A similar problem occurs in the structure investigation of very large protein complexes, e.g chaperonins or heat shock proteins as GroEL (900.000 mass), or the oligomeric oxygen transport proteins Hemocyanins, which can exhibit a mass of up to 50.000.000. Those proteins allow a Guinier approximation of the scattering in the q-range of 0.001 to $0.02\ A^{-1}$ (ultra small angle scattering range). This range of the momentum transfer is only difficult to access with X-ray small angle scattering as protein solutions are weak scatterers and contain typically only 1% protein in an aqueous buffer solution. Present X-ray small angle scattering instruments are to our experience for protein investigations limited by window and slit scattering or background to $q = 0.01\ A^{-1}$ (DESY/ HASYLAB -B1, -X33), $0.015\ A^{-1}$ (ESRF-ID02) and $0.02\ A^{-1}$ (ELETTRA-SAXS 5.2) [4].

At the DESY-HASYLAB we have investigated the structure of the stable ATP-synthase from *Micrococcus luteus* (500,000 mass), its catalytic fragment F_1ATPase (400,000 mass), the chaperonin GroEL from *Escherichia coli* and large Hemocyanins from arthropods, e.g. spiders at the beamlines B1 (JUSIFA, q-range = 0.01 - $0.6\ A^{-1}$) and BW4 (USAX, q-range = 0.001 - $0.03\ A^{-1}$). In case of complete ATP-synthase the protein solutions contained as detergent the bile acid taurodesoxycholate (TDOC; 5 mM = 1.5 * cmc), which was shown earlier to contain no large detergent micelles under those conditions [2]. The first test experiments with protein solutions at the USAX beamline were done with flat cells (2 mm pathlength, 10 mm diameter, 2 mylar windows 6.25 µm each) in an air environment with two capton windows (2 x 50 µm) at the beam tubes. The experiments with 8 keV photons in a 1.5 x 3 mm spot supplying 5×10^{10} ph/s at the sample yielded protein scattering profiles valid down to $q = 0.005\ A^{-1}$ at 12.7 m detector distance. The radius of gyration was obtained by Guinier approximation of the profiles ($R_g = 58$ A) and matched the parallel experiments with the same sample at the JUSIFA beamline ($q > 0.01\ A^{-1}$). In contrast to the enzyme from *Rhodospirillum rubrum* [1], the Guinier plot of the scattering of the *Micrococcus luteus* ATP-synthase showed now break below $0.01\ A^{-1}$. This indicates this enzyme solution to be free of aggregates even after freezing in liquid nitrogen, which is the prerequisite for the present investigation of structure dynamics by comparative and time resolved X-ray small angle scattering.

Systematic experiments showed that the q-limitation of the instrument with weak scatterers (q = 0.005 A^{-1}) resulted mainly from the beam tube windows and sample cell windows (capton, mylar). Additional tests of several window materials (capton, mylar, mica, Muskovite) in vacuo at the JUSIFA beamline indicated that the mica mineral Muskovite is best suitable for future improvements. The results are used for the construction of a special flat sample environment for scattering experiments with weak scattering solutions (proteins, polymers). As our sample cell for anomalous scattering [3] and our environment for time resolved X-ray scattering at ESRF and ELETTRA [4] this device consists of a thermostated flow-trough sample cell in a helium atmosphere and two additional vacuum windows (10, 20 µm). The quartz capillary used at JUSIFA, ESRF and ELETTRA [4] (1 mm diameter) is replaced by a larger flat cell (7 mm; 2 windows, 10µm). An assessment showed, that under those conditions at the USAX beamline the smallest q-vector of a protein solution can be < 0.002 A^{-1}. Thus very large protein or polymer complexes in the range of 1.000.000 to 50.000.000 molecular mass can be exclusively instestigated at the USAX beamline in the momentum transfer range q = 0.002 - 0.01 A^{-1}, e.g. oligomeric protein complexes, extracellular matrix components and chaperonins.

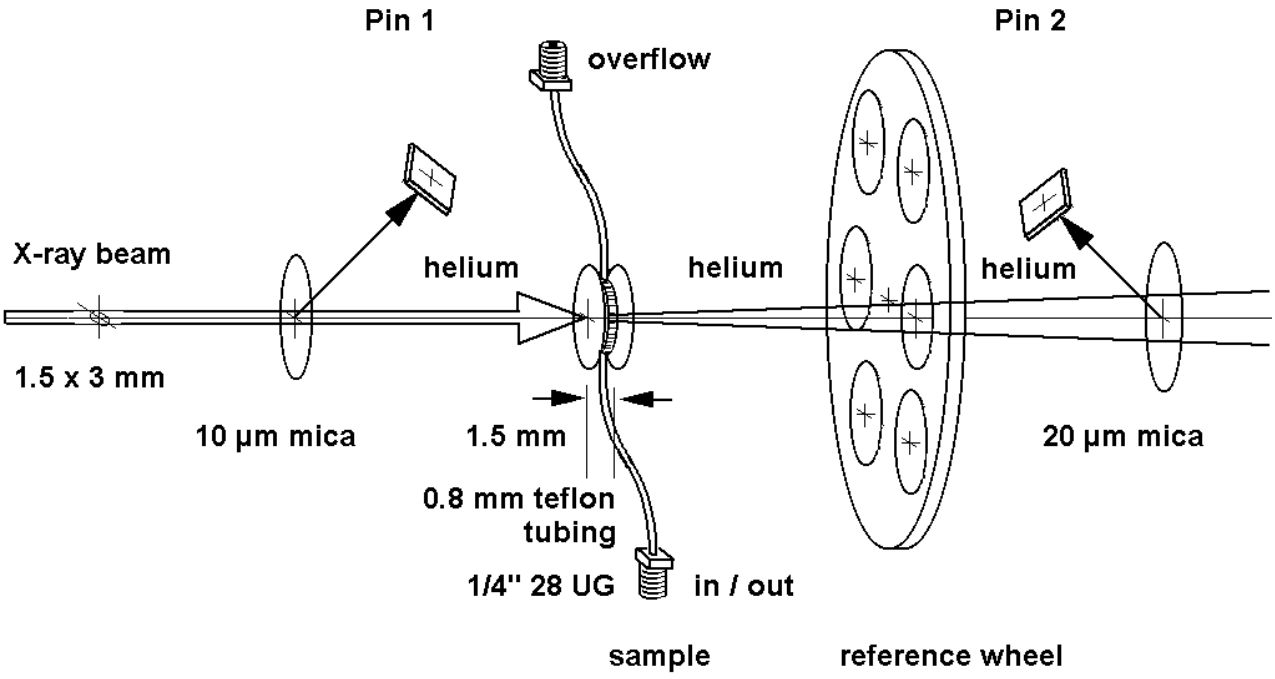

Figure 1: Sample environment for SAXS with protein or polymer solutions in a flat mica flow-through cell.

References

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