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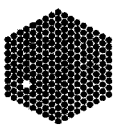

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The authors of the individual scientific reports published in the HASYLAB annual report (part I and II) are fully responsible for the contents.
We kindly request your comprehension as pertains the adaption of the layout of contributions to our requirements (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 :

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X-Ray Small-Angle Scattering of KLH1 in different Oxygenation States

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The oxygen storage protein Keyhole Limpet Hemocyanin (KLH) from the marine gastropod *Megathura crenulata* is often used as an immunostimulating reagent and therefore under clinical investigation, e.g. as a therapeutic in the treatment of bladder cancer. KLH occurs in two different species, KLH1 and KLH2 [1], with molecular masses of about 8 MD. KLH1 is a di-decameric molecule with eight domains per subunit. By electron microscopy with stained specimens it was found that the quaternary structure of KLH1 is a hollow cylinder with a length of 400Å and an outer diameter of 360Å [2]. In a recent structure determination of oxygenated KLH1 by SAXS significant deviations from the electron microscopic model were found [3]. In the present study we investigated the small angle X-ray scattering of KLH1 in both, the oxygenated and deoxygenated form.

KLH1 was purified as described in [1]. The deoxygenated sample was prepared in an atmosphere of pure nitrogen (pO_2<1Torr). Small-angle data were obtained in the momentum transfer range from 0.08Å^{-1} to 0.27Å^{-1} at the beamline B1 (Jusifa) at distances of 3.618m and 1.818m. A wavelength of 1.3795Å was used, calibrated with the Cu-K_α edge. The protein solutions (6.3 mg/ml for the oxy- and the deoxy samples) and the buffer were measured in a flow-capillary at a temperature stabilized to 20^{o}C. Intensities were recorded on a multi-wire proportional counter with a size of 20x20 cm^2 and 256x256 pixels electronic resolution. The actual resolution of the detector and the conversion factor from pixel to length units was measured by placing a metallic plate with 30x30 pinholes in front of the detector and irradiating it with an isotropic scattering sample. The sensitivity of the detector was determined by fluorescent scattering of a Cu sample. After correction for noise, transmission and buffer, the scattered intensities were de-convoluted with the resolution function by the method of indirect Fourier transformation [4,5] (Fig 1). For the resolution function (detector resolution plus beam size) a Gaussian was used with a FWHM of 3 pixels. The different time frames recorded give no indication of a systematic change of the SAXS intensities caused for example by radiation damage.

Indirect Fourier transformation of the intensities gives distance distribution functions with a maximum distance of about 450Å for both oxygenation states of KLH1. This value is about 15% smaller than the maximum distance calculated from the electron-microscopic model [2]. For the radius of gyration we determined values of 163.9±0.4 Å for the oxygenated protein and 164.7±0.4 Å for the deoxy form. The radius of gyration calculated from the electron-microscopic model is about 185Å. Both the maximum distance as well as the radius of gyration indicate that the KLH1 molecule is more compact in solution than measured by electron microscopy.

For the protein KLH which cooperatively binds oxygen different conformations are expected for the oxygenated and de-oxygenated state. Comparison of the smeared intensities (see Fig.2) for KLH1(deoxy) and KLH1 (oxy) indicate changes in the quartenary structure upon oxygenation. The same features are seen comparing the de-smeared intensities (data not shown). The KLH1 molecule seems to be slightly more compact in the oxygenated than in the deoxygenated state.

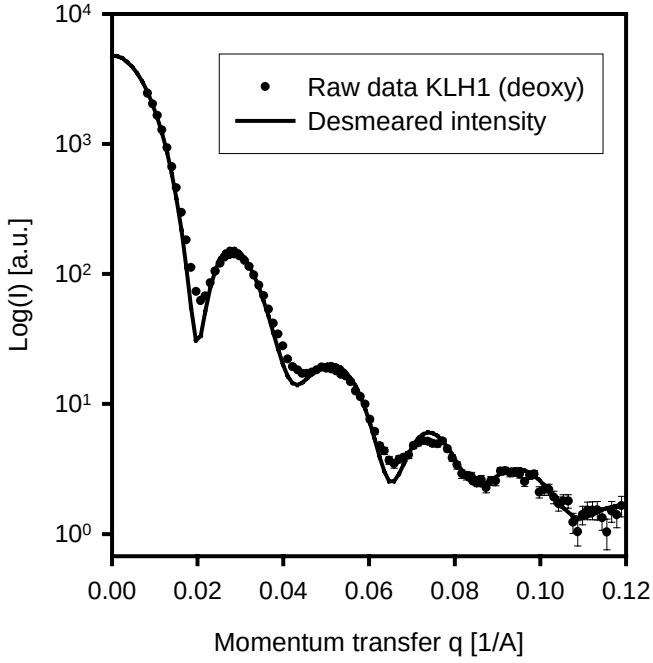

Fig.1 Raw and desmeard intensities of de-oxygenated KLH1.

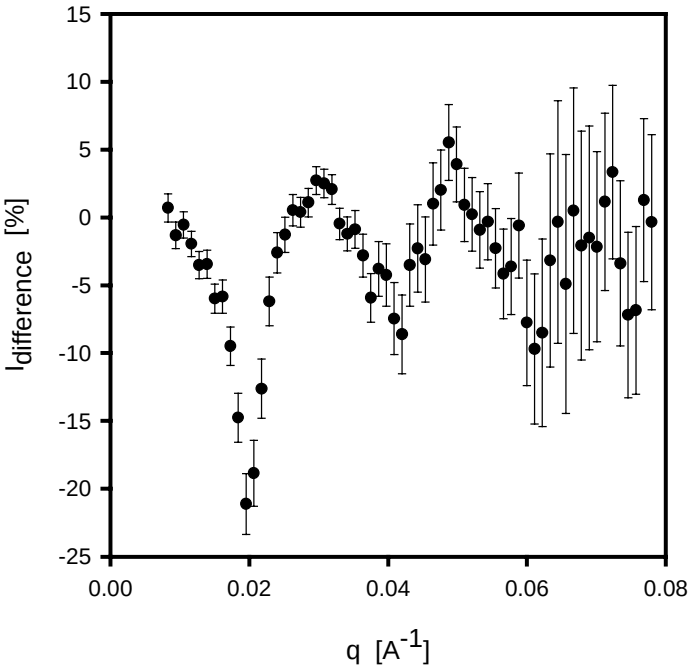

Fig. 2 Difference of scattered intensities between $KLH1_{deoxy}$ and $KLH1_{oxy}$.
$I_{diff} = 2*(I_{deoxy} - I_{oxy})/(I_{deoxy} + I_{oxy})$
$\sigma = \mathrm{sqrt}\,(\sigma^2_{deoxy} + \sigma^2_{oxy})$

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