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Contributions of the MPSD group :

Part1, 865-866 : Nawroth et al. : XANES and EXAFS of Metalloproteins

Part1, 979-980 : Gebhardt et al. : pH dependent SAXS of Hemocyanin

XANES and EXAFS of Metalloproteins by subtraction of true reference spectra obtained by a flow through cell

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Metalloproteins are essential components of cells, namely in redox processes, e.g. in electron transfer reactions in respiration and photosynthesis, and in oxygen transport and storage functions. Most metallo-proteins exhibit several structural states, which may occur as substates during function, e.g. redox catalysis or ligand binding, or in the molecular regulation (allosteric conformational changes). The accompanied metal ligand and distance changes can be estimated by XANES, EXAFS and ASAXS of protein solutions. The dispersive estimation of the metal environment structure is difficult with metal protein solutions because of two reasons: i) a protein solution consists mainly of water (>80%) and ii) the specific metal content in the protein entity is low because of the high molecular mass of the protein, e.g. n*70,000 in case of oligomeric Hemocyanin oxygen transport proteins. Thus the metal content is below 20 mM/l, i.e. 0.03%. This makes the usual background correction by fit functions (Victoreen fit etc.) at least difficult.

We have developped a procedure for background subtraction by estimation of a true reference spectrum of a metal free sample under identical conditions as in the metal protein experiment at the JUSIFA (B1) ASAXS - beamline at HASYLAB [1]. As shown in fig.1, the setup contains of a quartz flow-through capillary (30x1 mm), which has been established as favorite sample cell for solution small angle scattering (SAXS/ASAXS) static experiments at the B1-beamline, as well as for time resolved SAXS at ESRF (ID2A) and ELETTRA (SAXS-5.2) [2,3]. The sample is supplied by a syringe and Teflon tubing (0.5 mm i.d.) without breaking the instrument vacuum or recalibration. Thus sample and reference solutions can be investigated under identical conditions (location, irradiated volume). The incoming beam I_1 is monitored by an ionization chamber and a NaJ-scintillation detector in parallel. The transmitted beam I_2 is measured with a photodiode of 10 x 10 mm size (Hamamatsu S2387-1010N, special windowless version). The complex X-ray fluorescence+SAXS +ASAXS signal of the sample was estimated additionally by a 180 x 180 mm gas detector (2D, Ar/CO_2, 918 mm distance to sample). The beam size 5 x 0.6 mm yielded a flux of $\approx 5*10^8$ ph/s ($dE/E = 10^{-4}$).

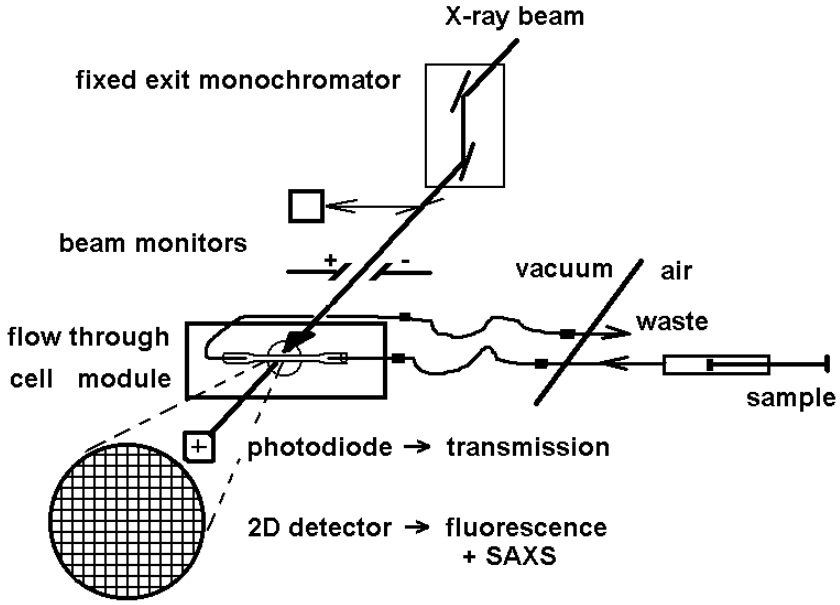

Figure 1: Setup for XANES, EXAFS and ASAXS of metalloprotein solutions with a flow through cell at the JUSIFA beamline (B1). The stable quartz capillary allows the estimation of true reference spectra.

As shown in fig.2, the stability of sample environment and beamline allowed the estimation of XANES / EXAFS spectra of dilute metal protein solutions in transmission and fluorescence mode in parallel. The metal content was 2 mM/l in case of Hemocyanin (75 g/l) and 10 mM in case of horse Myoglobin (178 g/l), the spectra were estimated between 8.7 - 9.5 keV (Cu-edge in Hemocyanin) and 7.07-7.9 keV (Fe-edge in myoglobin). The metal free reference protein BSA (bovine serum albumin) was used for the "true reference spectra" experiments. The figure shows the original data, normalized to the beam monitor (ionization chamber) without any corrections in order to demonstrate the small level of the metal signal and the high reproducibility of the direct background estimation with the metal free reference protein or buffer. The results show that the estimation of metal ligands in proteins and their dynamics is possible down to 1 mM/l metal content in the transmission mode. This is free of energy dependent scattering and background perturbations and the favorite mode for time resolved XANES/EXAFS, which is planed with a dispersive setup at the ESRF (ID24).

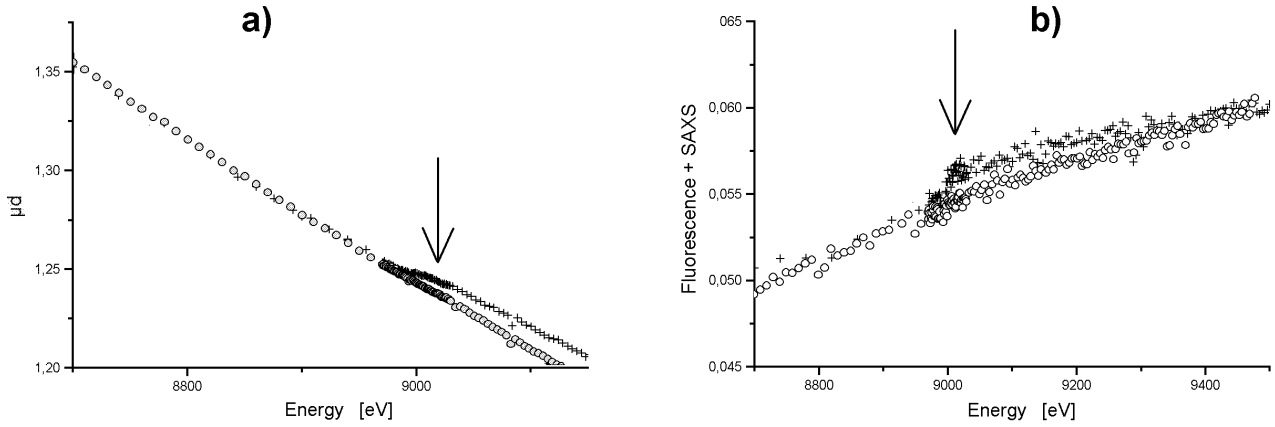

Figure 2: The stable flow through capillary allows the detection of dilute metal protein solutions: **a)** in the transmission mode (linear absorption µd vs. energy) and **b)** as fluorescence+SAXS+ASAXS complex signal in parallel (arbitrary units). The example shows the difficult experiment with a Hemocyanin solution (75 g/l), which contained only 2 mM Copper (upper lane, (+)), and the estimated "true reference spectrum" of a metal free reference protein (BSA; 75 g/l; lower lane, (o)). The arrow indicates the copper absorption edge.

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