ESRF	**Experiment title:** Multivalent cation binding and distribution around proteins in the re-entrant regime	**Experiment number**: SC-2805
Beamline: ID2	**Date of experiment**: from: 20th Nov. 2009 to: 23th Nov. 2009	**Date of report**: 07 Jan. 2009
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Report:

For the interaction of proteins, the distribution of charges is crucial. In fact, salt ions are ubiquitous in biological media, and the folding of DNA as well as protein is intimately coupled to the counterions that neutralise these macroions. Thus, a detailed understanding of the counterion distribution is essential for many biological systems. However, little is known experimentally about the counterion distribution around charged proteins. ASAXS provides the only way to study this issue by selecting the energies away and near the absorption edge of the target ions, which has been proved by recent successful studies [1-4]. The present work is devoted to the characterization of the counterion distribution around globular proteins in aqueous solution using ASAXS.

In the present experiment, we intend to study the ion distribution around proteins as a function of protein and salt concentrations. We recently observed a reentrant condensation phase behavior for globular proteins in the presence of multivalent cations [5], as shown in Figure 1. Negatively charged globular protein in solution undergoes a phase-separation upon adding trivalent counterions up to a critical concentration C*. Further increasing the salt concentration above a second critical value, C**, causes the precipitate to dissolve and the system turns back to a homogeneous solution [4]. The reentrant phase behavior ensure the studied systems are stable at high salt concentrations, which is important for ASAXS measurements. During this beamtime, we use trivalent salt, yttrium chloride, in solution. Our goal is to get reliable ASAXS signals for the counterions by optimizing the sample and measurement parameters, such as protein/salt concentration, measuring time and number of energies, etc. and establish the data analysis and fitting protocol for a direct description on the ion distribution around proteins in solution. Further, this method will be extended to different proteins and salts of different valency.

Anomalous small-angle X-ray scattering (ASAXS) measurements were carried out at station ID2, ESRF, Grenoble. The experimental absorption K-edge of yttrium in solution was determined using a concentrated salt solution (1M) by an energy scan. The absorption edge is 17048 eV, with ΔE= 10 eV compared to the theoretical value (17038 eV). 12 energies were selected for following ASAXS measurements: with ΔE = -1000, -300, -100, -36, -12, -6, -4, -2, 0, 2, 6 and 36 eV.

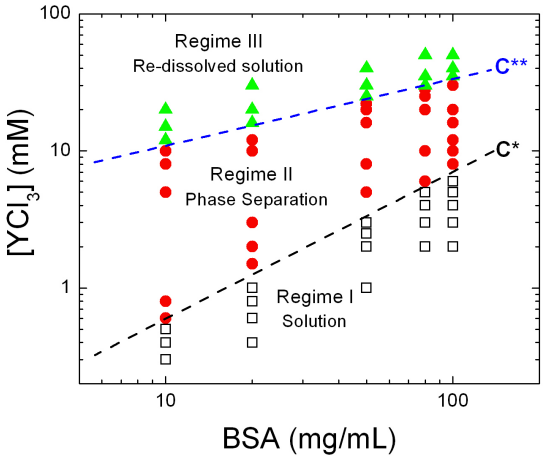

Figure 1 Phase diagram of protein solution in the presence of multivalent salt [4].

Protein solution were filled into 2mm quartz capillarys. The scattering of a salt solution was measured as the background, in exactly the same way as the protein solutions and was subtracted from the sample scattering. All measurements were carried out at room temperature. The raw data were corrected for transmission, fluctuation of primary beam intensity, exposure time, and the response of the detector. Bovine serum albumin (BSA) and β-lactoglobulin with protein concentration of 5, 10, 20 and 50 mg/mL and salt concentration (YCl_3) from 1 to 50 mM were measured at 12 energies with q-range from 0.06 to 4.5 nm^{-1}.

It has been estimated that in order to get a good ASAXS signal, about 0.2% of electrons participating is necessary. Indeed, we found that when using salt concentratnion below 5 mM, i.e. in the regime I of the phase diagram, no systematic shift of the scattering intensity could be observed in a full ASAXS run (data not shown). ASAXS signals become visible at higher salt concentrations in the reentrant regime III. Figure 2 presents the ASAXS data of BLG 20 mg/mL with 20 mM YCl3 and BSA 20 mg/mL with 50 mM yttrium chloride. In both cases, the pure resonant term can be successfully deduced, which is about 1% of the total SAXS signal. The pure resonant term was calculated from the following equation [3]:

$$S_{ion}(q) = \frac{1}{F(E_1,E_2,E_3)}\left[\frac{\Delta I(q,E_1,E_2)}{f'(E_1)-f'(E_2)} - \frac{\Delta I(q,E_1,E_3)}{f'(E_1)-f'(E_3)}\right] \qquad (1)$$

Where $F(E_1,E_2,E_3) = f'(E_2)-f'(E_3)+\frac{f''^2(E_1)-f''^2(E_2)}{f'(E_1)-f'(E_2)} - \frac{f''^2(E_1)-f''^2(E_3)}{f'(E_1)-f'(E_3)}$

In this study, the energies were selected such that the imagery part of the scattering factor, f" remains constant for all energies. Hence the observed ASAXS signal will be determined mainly by the real part f'. The successful separation of pure resonant signal for the multivalent ion makes it possible for further understanding the binding number and thickness of counterion around charged protein molecules. Detailed data analysis and model fitting will be carried out imminently.

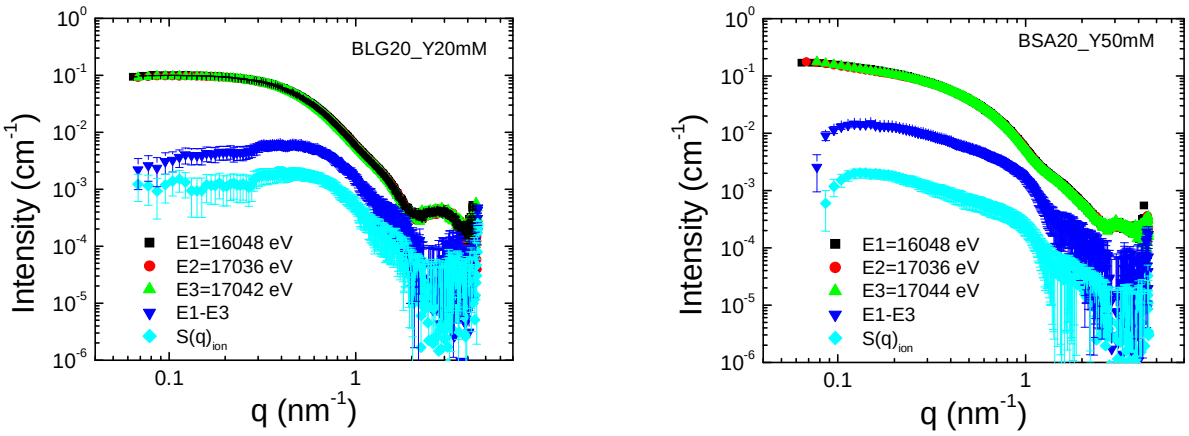

Figure 2 Typical ASAXS curves measured at different energies and the separated ASAXS curve from two energies. The pure resonant term deduced from the separated forms according to Eq. 1.

Nevertheless, we have demonstrated that the ASAXS measurements on the counterion distribution around charged protein molecules are feasible and that information on the ionic cloud can be obtained. The present results encourage us to continue our research on proteins with other counterions, such as Br and Rb.

References

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