ESRF	**Experiment title:** Charge-correlation induced short-ranged attraction in protein-multivalent ion solution	**Experiment number**: SC-2624
Beamline: ID2	**Date of experiment**: from: 13th March. 2009 to: 16th March. 2009	**Date of report**: 04 May 2009
Shifts:	**Local contact(s)**: Dr. Shirley Callow and Dr. Theyencheri Narayanan	*Received at ESRF:*

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Report:

Effective interactions of biological macromolecules in aqueous solution are generally complex and depend on a number of environmental parameters such as concentration and valence of salt ions, pH, and temperature. These complex interactions can give rise to a rather rich phase diagram. The interactions are also crucial, e.g., for protein crystallization and protein aggregation-related physiological diseases [1]. Understanding the relationship between the interactions on the one hand and the phase behaviour on the other is thus an important fundamental issue, which has equally serious implications for applied science.

We recently observed re-entrant phase behaviour of a globular protein in solution induced by multivalent counterions [2]. For a given protein concentration and increasing the salt concentration, the protein solution becomes a turbid, two-phase state by crossing the first critical salt concentration, c*. Upon further increase of the salt concentration, the protein aggregates gradually dissolve, upon crossing the second critical salt concentration, c**, the aggregates dissolve completely and the solution becomes one-phase again.

In this beamtime, we aimed to study protein interactions in solution in the presence of multivalent counterions in order to understand the charge correlation, condensation and phase behavior of protein solution.we measured the protein interactions in solution in the presence of multivalent cations, i.e. yttrium chloride. Three proteins, human serum albumin, ovalbumin, β-Lactoglobulin were used. The phase diagrams of all three proteins with yttrium chloride have beeen determined. In all cases, reentrant phase behavior was observed. In this beamtime, the protein interactions in the reentrant regime were measured.

The SAXS measurements at the ESRF (Grenoble, France) were performed on the beamline ID02 with sample-to-detector distance of 2 m [3]. The wavelength of incoming beam is 1.08 nm (11.5keV), covering a q

range of 0.05 to 2.1 nm^{-1}. The data were collected by a high sensitivity fiber-optic coupled CCD (FReLoN) detector placed in an evacuated flight tube. The protein solutions were loaded using a flow-through capillary cell (diameter ~ 2 mm; wall thickness ~ 50 μm). The radiation damage was checked with 10 successive exposures of 0.1 s. The incident and transmitted beam intensities were simultaneously recorded with each SAXS pattern with exposure of 0.3 s. The 2D data were normalized to an absolute scale and azimuthally averaged to obtain the intensity profiles, and the solvent background was subtracted.

Figure 1 presents the corrected SAXS profiles for HSA, Ovalbumin and β-Lactoglobulin in reentrant regime. It was interesting to see that for HSA, the scattering intensity at low q region decreases with increasing salt concentration. This observation is similar to our previous study on BSA. For ovalbumin, however, the scattering intensities in the low q region show a significant increase, but change slightly with salt concentration. In the case of β-Lactoglobulin, the scattering intensity increases with salt concentration. These three cases present the typical responses of protein-protein interactions in solution in the presence of multivalent counterions. Further detailed data analysis will provide the insights on the charge correlation induced attraction in these systems. The effect of surface charge distribution and charge density may play a crucial role in each case.

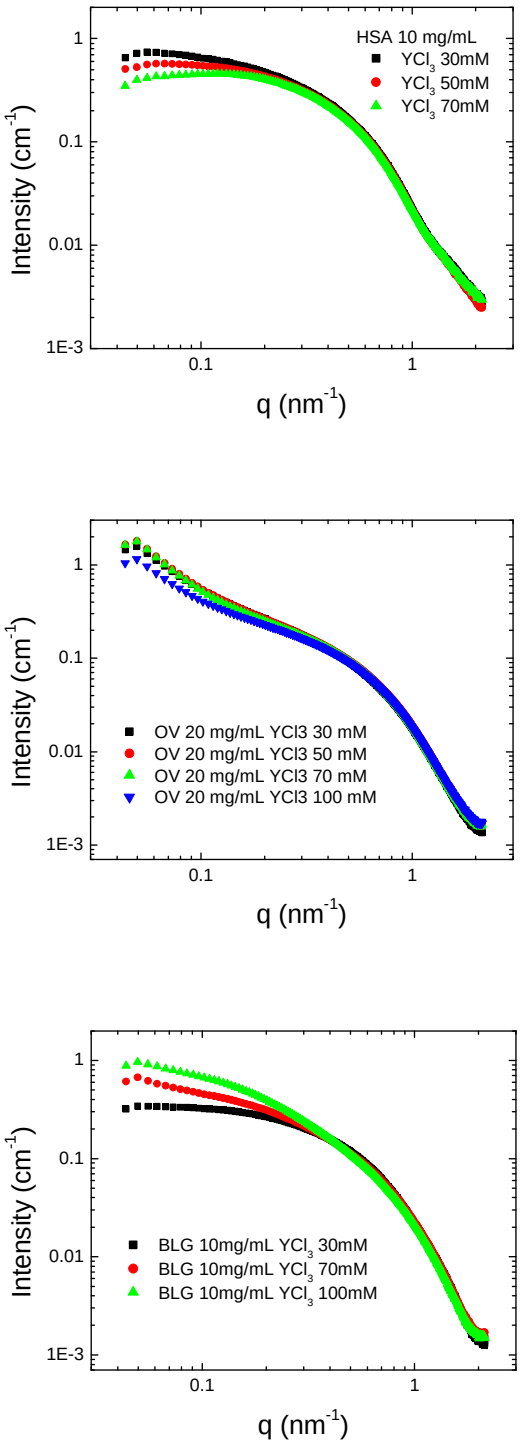

Figure 1. SAXS data for HSA, Ovalubmin and BLG solutions with different ionic strength in the re-entrant regime.

References:

[1] R. Piazza, *Curr. Opin. Colloid Interface Sci*. **2004**, 8, 515.

[2] F. Zhang, M. Skoda, R. Jacobs, F. Schreiber, et al. *Phys. Rev. Lett.* **2008**, 101, 148101.

[3] Sztucki M, Narayanan T, Belina G, Moussaid A, Pignon F, Hoekstra H. *Phys. Rev. E* **2006**, 74, 044507