ESRF	**Experiment title:** Determination of the solution conformation of CD46 by SAXS	**Experiment number**: SC-2529
Beamline: ID2	**Date of experiment**: from: 6th Feb. 2009 to: 9th Feb. 2009	**Date of report**: 25 Feb 2009
Shifts:	**Local contact(s)**: Dr. Anuj Shukla	*Received at ESRF:*

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Report:

This beamtime aimed to study the structure and conformation of CD46 in solution, in addition, since the experiment was going very smoothly, we had a chance to make short tests with samples from related projects. We have measured:

- CD46 in solution with 4 protein concentrations
- ExbB in solution with 6 protein concentrations
- Standard protein BSA in solution in the presence of multivalent cations (yttrium chloride)

In the following, we present the preliminary SAXS results on the proteins measured in this beamtime, but we emphasize that only two weeks after the beamtime the analysis is far from completed.

Membrane cofactor protein (MCP: CD46) is a ubiquitously expressed protein playing a central part in the alternative complement pathway of the innate immunity [1]. CD46 is a member of the Regulators of Complement Activation (RCA) and has high structural homology to other RCA family members, among them complement receptor type 2 (CD21) and decay accelerating factor (CD55). CD46 is expressed on the cell surface of all nucleated cells, where it acts as a cofactor for the serine protease factor I, which in turn proteolytically cleaves and inactivates C3b and C4b.

We recently determined the crystal structure of a complex between CD46 SCR1-SCR2 and the Adenovirus type 11 knob (Ad11 knob) [2]. A notable feature of this complex is that the overall shape of the receptor is very different from that seen in the unliganded structure. In the crystal structure of ligandfree CD46 SCR1-SCR2, a pronounced bend of ~60º was observed between SCR1 and SCR2. As this bend was present in 12 copies of crystallographically independent molecules, we consider it unlikely that the bend is a crystallization artifact. Upon formation of a complex with the Ad11 knob, the bend between the two domains becomes close to zero. We conclude, therefore, that Ad11 knob alters the conformation of CD46 by realigning the two repeats and molding them into a rod-like segment. The change in conformation alters the

relative orientation between Ad11-binding epitopes on SCR1 and SCR2 and exposes residues for binding that were hidden in the unliganded structure of CD46. Viral proteins can sometimes undergo large conformational changes upon receptor binding. However, the structure solved by us is the first example of a virus protein profoundly altering the overall conformation of its receptor. The discovery generated a new set of questions; *how CD46 is situated on the cell surface and how are the domains orientated relative to each other in solution?* Answering these questions is highly relevant for understanding of how the Adenoviruses have evolved to bind CD46, and also for developing an improved gene transduction using adenovirus based vectors for gene therapy.

In the first part of this beamtime, we measured the solution scattering of CD46 at ID2. Protein solutions with 4 concentrations ranging from 1 to 4 mg/mL were measured at two sample-to-detector distances (0.8 and 2 m) in order to cover the whole q-range (0.04 to 8 nm^{-1}) (Figure 1). The scattering intensity at very low q-region (below 0.1 nm^{-1}) shows a clear increase, that may be due to the formation of small clusters of proteins in solution. Another feature of the SAXS profiles is the nearly q^{-2} decay of the scattering intensity in the medium q-range, which indicates the loose packing or polymer-like behaviour of the protein in solution.This is reasonable due to the attached *sugar chains*. The Guinier plots (onset Fig.2) show a very good linear relationship between ln(intensity) and q^2 in the q^2 range of 0.04 to 0.4 nm^{-2}, linear fitting gives the radius of gyration at each protein concentration. The Rg values decrease linearly as protein concentrations increasing (Figure 2), the Rg at c=0 was determined by extroplating to c=0, wich is 2.25 nm.

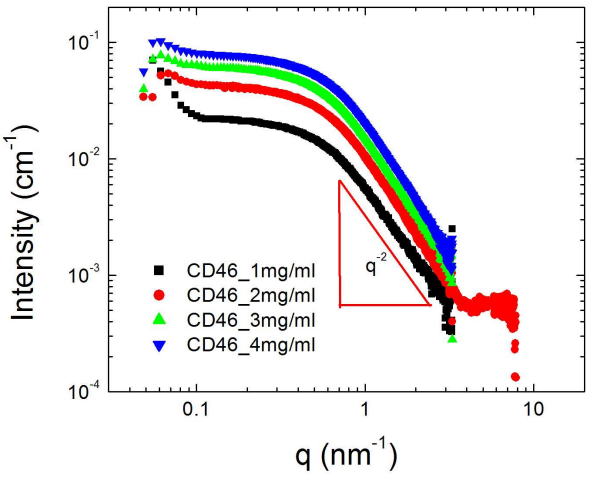

Figure 1 SAXS profiles for CD46 in solution with different protein concentrations. The scattering intensity at the medium q range decays as q^{-2} indicating a polymer like structure of protein.

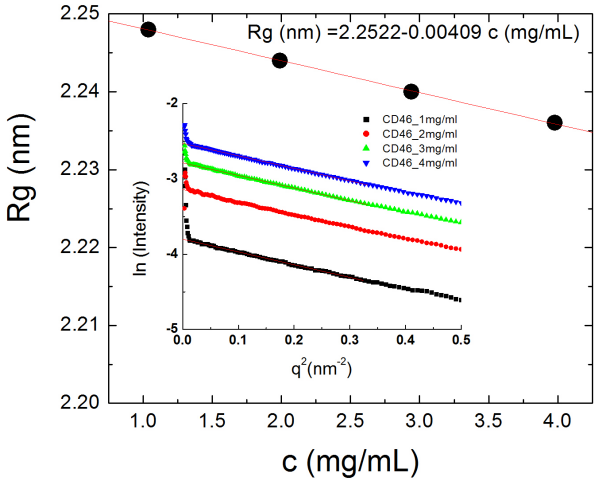

Figure 2 Radius of gyration of CD46 in solutin determined by Guinier plots (insets) and Rg extroplated to zero concentration is 2.252 nm.

ExbB is part of the protein complex consists of the protein TonB, ExbB and ExbD which are localized in the cytoplasmic membrane [3,4]. The protein complex serve as the channel for energy transmission from the cytoplasmic membrane into the outer membrane. In this beamtime we also measured the solution scattering of ExbB in solution (Figure 3, 4). It has shown that 1.8% DM in the solution, which is slightly above the CMC value of DM in water. Due to the absorption of DM on protein surface, the bulk DM concentration is lower than CMC and no significant contribution from DM micelles was observed in the SAXS profiles (Figure 1). The SAXS profiles for a wide range of protein concentrations from 1 to 15 mg/mL indicate that above 5 mg/mL clearly deviation from non-interacting solution could be observed. This is also shown in the plot of Rg vs protein concentrations (Figure 2), deviation from linear relationship above 5 mg/mL is obvious, from the Rg values determined at low protein concentrations, Rg at c=0 was determined as 4.87 nm. The perfect SAXS profiles will make the 3D protein structure re-construction possible.

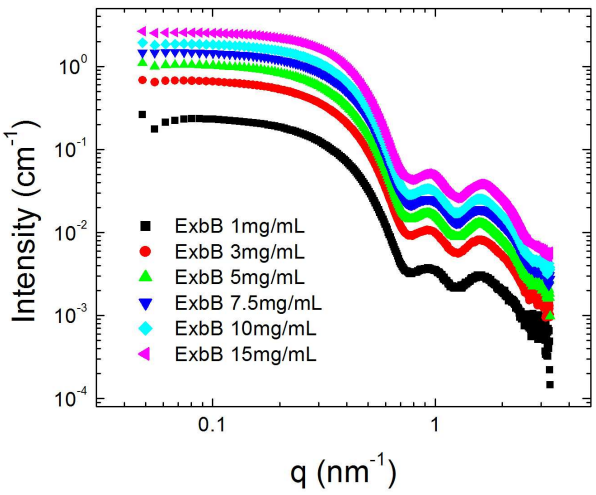

Fig. 3 SAXS profiles for ExbB in solution with different protein concentrations.

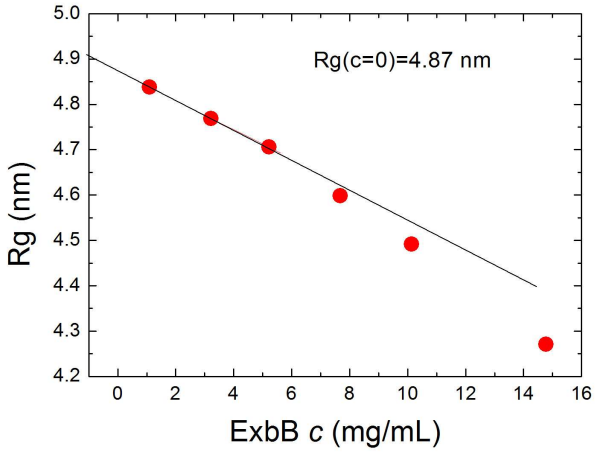

Fig. 4 Plot of Rg as a function of protein concentration, the Rg (c=0) was determined below 5 mg/mL.

We also measure the scattering of BSA in the presence of multivalent counterions. We have reported that in the presence of multivalent cations, globular proteins undergo a reentrant condesation [5]. But the effective protein-protein interactions in the reentrant regime are not clear. In this beamtime, we measured BSA solutions with a wide range of protein concentrations in the reentrant regime. The aim is to determine the form factor of the protein and the effective interactions of them in this regime. Typical SAXS profiles for BSA solutions from 2 to 100 mg/mL with 50 mM yttrium chloride was shown in Figure 5. Compared to the scattering profiles at low protein concentration (<5 mg/mL), increasing protein concentration, the increasing of scattering intensity at low q region indicates the rising of attractive interaction between the ion-bound proteins.

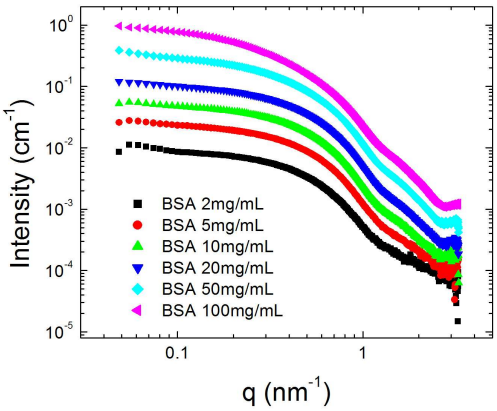

Figure 5. SAXS data for BSA solutions at YCl_3 50 mM in the re-entrant regime [5].

This measurement encourages us to study the ion binding and distribution on protein surface using anomalous small-angle X-ray scattering (ASAXS). This re-entrant phase behavior has been explained by a charge inversion theory, which predicts a condensation layer of counterions on a charged surface and the strong correlation between bound ions induces a strong short-ranged attraction between macroions (proteins). Both predictions can be demostrated by using anomalous (A)SAXS and SAXS measurements. This application of anomalous scattering has been used in similar configurations for related problems [6-8].

References:

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