ESRF	**Experiment title:** Molecular characterization of an intermembrane energy transduction complex	**Experiment number**: SC-2806
Beamline: ID2	**Date of experiment**: from: 23rd Nov. 2009 to: 24th Nov. 2009	**Date of report**: 7 Jan 2010
Shifts: 3	**Local contact(s)**: Dr. Shirley Callow	*Received at ESRF:*

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Report:

The outer membrane of E. coli and other gram-negative bacteria contains energy-coupled transport proteins which also function as energy-dependent receptors for viruses and protein toxins. However, there is no energy source in the outer membrane. The proton motive force of the adjacent inner (cytoplasmic) membrane provides the energy. Energy is transmitted from the cytoplasmic membrane into the outer membrane by a protein complex that consists of the proteins TonB, ExbB and ExbD which are localized in the cytoplasmic membrane. This concept rests on studies in which mutations in these proteins abolish outer membrane transport and receptor activity and indicate interactions between these proteins. In addition, crystal structures of outer membrane transporters were determined, two of them with a fragment of TonB bound to the transporters (receptors). The structure of the TonB-ExbB-ExbD complex must be known to devise a concept of how energy is harvested in the cytoplasmic membrane and transmitted to the outer membrane. No such structure has been determined. We have sequenced the ExbB and ExbD genes and determined the location of the ExbB and ExbD proteins in the cytoplasmic membrane and their transmembrane topology. We also isolated inactive mutants of all three proteins. The lab of K. Postle measured the amounts of these proteins in cells and their stoichiometric ratio (TonB:ExbB:ExbD=1:7:2) which does not necessarily reflect the stoichiometry in the predicted complex. We could isolate ExbB as a multimeric protein in pure form and ExbB together with ExbD. ExbB seems to form a scaffold on which ExbD and TonB bind. The aim of the project is to determine the shape and the number of ExbB and ExbD monomers in the isolated complexes. Then isolated TonB will be added to obtain the structure of the entire complex. These data should provide insight into how the complex measures the proton gradient across the cytoplasmic membrane (proton-conducting pore). The system is of general interest since energy is transmitted between two biological membranes.

In this beamtime we also measured the solution scattering of ExbB in solution (Figure 1). 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 linear fit of the Guinier plots was carried out in the q^2 range of 0.01 to 0.2 nm^{-2}. The R_g values for each protein concentration were determined from the slope of the linear fit. 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 forward intensity I(0) linearly increases for samples with low protein concentrations (<10 mg/mL). A linear fit from the first 4 data points gives I(0) = 0.2526 cm^{-1} at c = 1.0 mg/mL [3]. The perfect SAXS profiles will make the 3D protein structure re-construction possible.

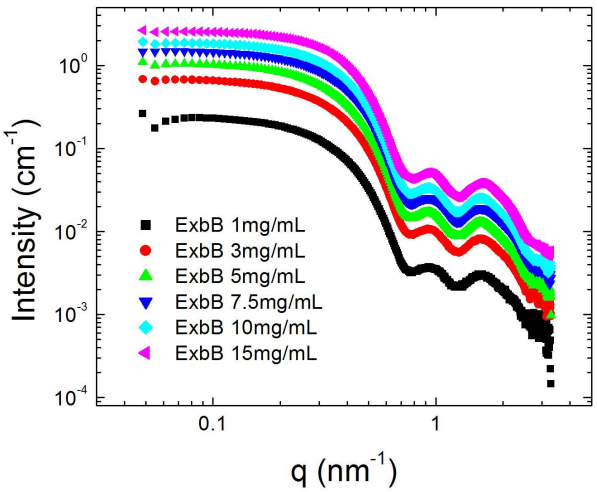

Fig. 1 SAXS profiles for ExbB in solution with different protein concentrations.

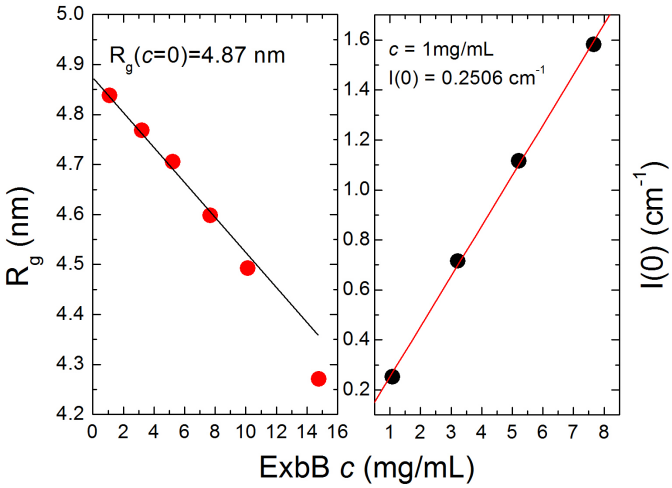

Fig. 2 Plot of Rg and forward intensity I(0) as a function of protein concentration, the linear fit was performed for samples below 5 mg/mL.

References:

[1] Shultis, D.D. et al. Science 312:1396-1399 (2006).
[2] Pawelek, P.D. et al. Science 312:1399-1402 (2006).
[3] Pramanik, A.; Zhang, F.; Schreiber, F.; Braun, V. Manuscript in preparation.