ESRF	**Experiment title:** A structural insight onto dynamics of two components of bacterial translocon: colicin N and TolA	**Experiment number:** MX-1400
Beamline: BM29	**Date of experiment:** from: 29.06.09 to: 30.06.09	**Date of report:** 07.09.12
Shifts: 2	**Local contact(s):** Dr Adam Round	*Received at ESRF:*

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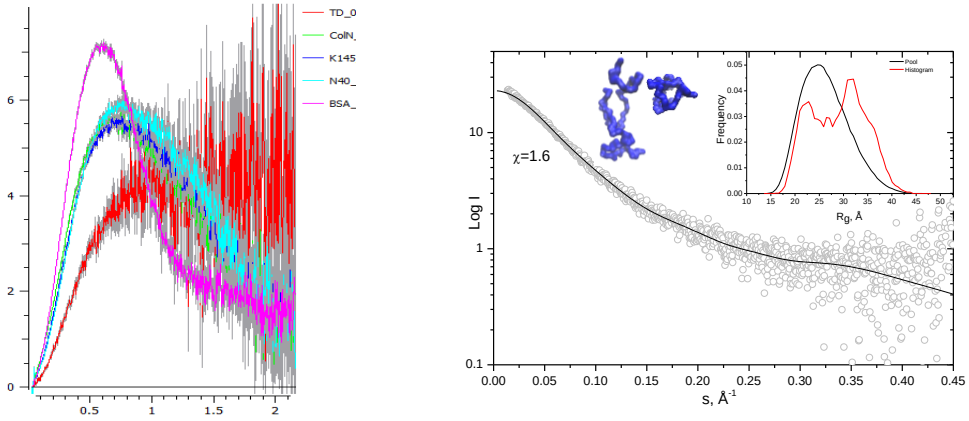
Report:

Introduction.The present SAXS experiment was focused on two proteins involved in bacterial translocon formation: Colicin N (ColN), the smallest pore-forming colicin (a bacterial toxin) and TolA which is essential for colicin function and bacterial viability is the protein. ColN is comprised of three domains R (receptor-binding), P (pore-forming) and T (translocation domain). Since the latter is intrinsically disordered[(1)] and absent from the high-resolution structure the conformation of ColN in solution still undefined. The SAXS data from this experiment produced a models for ColN and its domains and deletion mutants in solution which provides an essential contribution towards understanding the mechanism of membrane translocation.TolA protein is also involved in bacterial translocon formation. The domain I of TolA (first 40 amino acid residues) (TolAI) is embedded in the inner membrane but the long 260 residue TolAII domain strongly helical and very dynamic. The SAXS data collected on our TolAII-III in solution revised the published low-resolution *E coli* data[2] and the high-resolution *P. aeruginosa* structure.

Experimental method

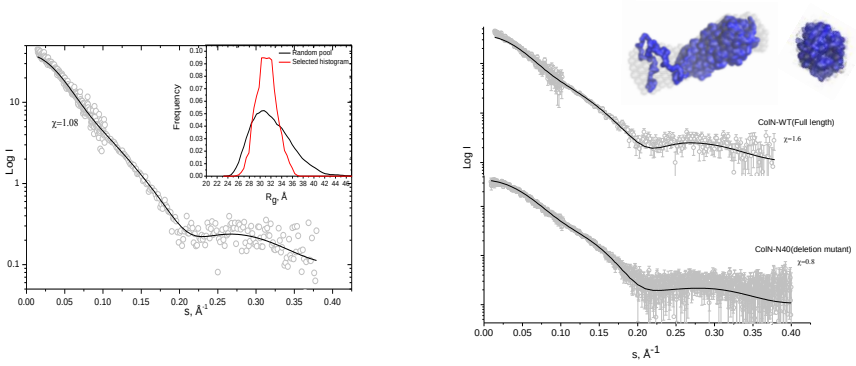
The SAXS data data were collected on beamline BM29 equipped with automatic sample changed at experimental temperature of 4°C. Scattering curves were recorded at a wavelength of 1.5 Å at a sample-detector distance 2.2 m covering the momentum transfer range $0.01 < s < 0.45$ Å^{-1}. Sample concentrations were ranged between 0.5 and 10 mg/ml. Data were normalised to the intensity of the incident beam and corrected for detector response, buffer scattering, scaled for concentration and checked for radiation damage and aggregation during all SAXS experiments.

Results.. SAXS data demonstrated that T-domain is flexible and disordered (Kratky plot) (Fig1A). The Ensemble Oprimisation Method (EOM) shows the presence of two populations different from a random pool (Fig2B) more compact pre-molten/molten globula and more extended



A **B**

Figure 1. SAXS study of isolated ColN-T domain Panel A: Kratky plot for ColN mutants and isolated ColN T-domain. Bovine Serum Albumin data are shown for comparison. Panel B. EOM applied to isolated ColN-T-domain: experimental data fit, R_g histogram and the most compact model of T-domain

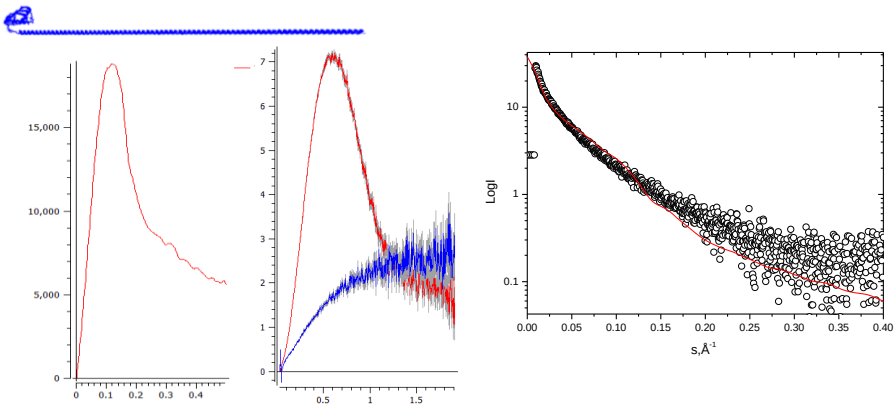


A B

Figure.2. SAXS data and modelling of ColN full length
Panel A The EOM results on ColN full length: the data fit and R_g histogram.
Panel B BUNCH global data fit for ColN full length and its deletion mutant N40. The final BUNCH model is superimposed with averaged rigid body model (30 DAMs were averaged)

Full length ColN and its mutants form relatively compact and be presented in solution by a single population according to the Kratky plot (Fig.1) and EOM (Fig.2). EOM seems to be a more effective tool in characterising these dynamic proteins than rigid body modelling approach, even for compact particles which have a flexible fragments

The estimative tropomyosin-like model of TolAII-III (Fig.3, A) was created in attempt to interpret our experimental SAXS data . Nevertheless it did not fit our experimental data (Fig.3, B). The TolAII-III seems to be flexible or disordered molecule as Kratky plot suggests (Fig.3A left).



A B

Figure.3 Interpretation of TolAII-III scattering data using extimative tropomyosin-like model
Panel A: The estimative tropomyosin-like model of TolAII-III (top) and its simulated Kratky plot (right) along with experimental Kratky plot of TolAII-III (in blue), the BSA data (in red) are shown as an example of folded protein.
Panel (B) CRYSON fit of the experimental TolAII-III data against tropomyosin-like model

Our SAXS data treated as a flexible system show that the most probable maximal length of TolAII-III in solution is significantly smaller than tropomyosin-like structure. It seems that the molecule has a well-defined conformation, since its R_g histogram is distinctively different from the random pool (Fig.4). The published TolAII-III solution structure[2] exhibits the most compact population amongst other conformations

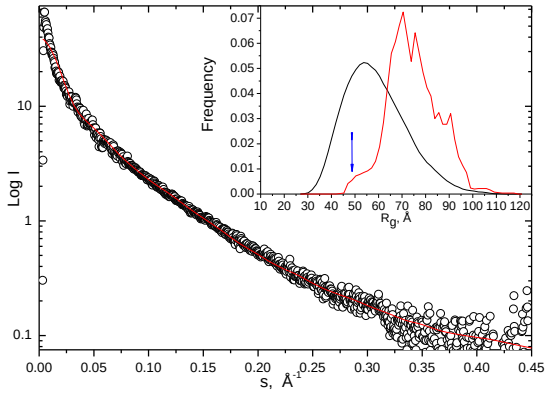


Figure.4 EOM. applied to TolAII-III scattering data
Main panel: EOM data fit
Insert: R_g histogram; the population with the Rg/Dmax parameters reported before was clearly seen on distribution plot (marked with an arrow)

References:(1)Hecht O, Ridley H, Boetzel R, Lewin A, Cull N, et al. 2008. *FEBS Letters* 582: 2673-7 (2) Witty M SC, Shah A, Grossmann JG, Mizuguchi K, Perham RN, Luisi B. 2002. *EMBO J.* 21: 11