Introduction

The goal of the BioSAXS experiment with number MX-1963 was to study the protein KAP1 in solution. KAP1 or Krüppel associated protein 1, is a multidomain protein that has been linked to the development and differentiation of many adult cell types as well as many other fundamental cellular processes including gene silencing, transcription regulation and DNA damage repair [1, 2]. It acts like a scaffold protein recruiting many different proteins and enzymes to influence the organization of chromatin structure. Because of that, we are interested in finding out the three dimensional structure of KAP1 and understanding the molecular interactions between KAP1 and the proteins it recruits.

Data acquisition

The data were recorded at the ESRF BM29 over a q range of 0.025-5 nm^{-1} and beam wavelength of 0.992 Å. Both sample changer and HPLC modes were used. In sample changer mode, protein samples were exposed under flow through a capillary at a rate of 10 or 8 $\mu L \cdot s^{-1}$ with 10 individual frames collected and averaged to give the final scattering profile. The dilution series was done diluting the protein in buffer: 20 mM Hepes, pH 7.5, 500mM NaCl, 10% glycerol and 2mM TCEP. The blank for each measurement was the corresponding flow through from protein concentration. Scattering from this black alone was collected before and after each sample to correct for fluctuations in beam intensity and to clean the capillary between samples. The temperature was always 20° C and the viscosity was set to low. We used 100% of the beam transmission. Four variants of KAP1 of different lengths were measured: The concentration series ranged from 8 to 0.1 mg/ml.

In HPLC mode, the samples were submitted to size exclusion chromatography, using a Superose 6 column (GE Healthcare) equilibrated in the buffer mentioned above at a flow rate of 0.7 ml/min. 100 ul of sample were injected at concentrations around 9-15 mg/ml. Data was collected for 36 min, with one frame per second, origination 2160 frames.

Data analysis

We focussed on the HPLC runs and these were analysed using SCÅTTER [3].

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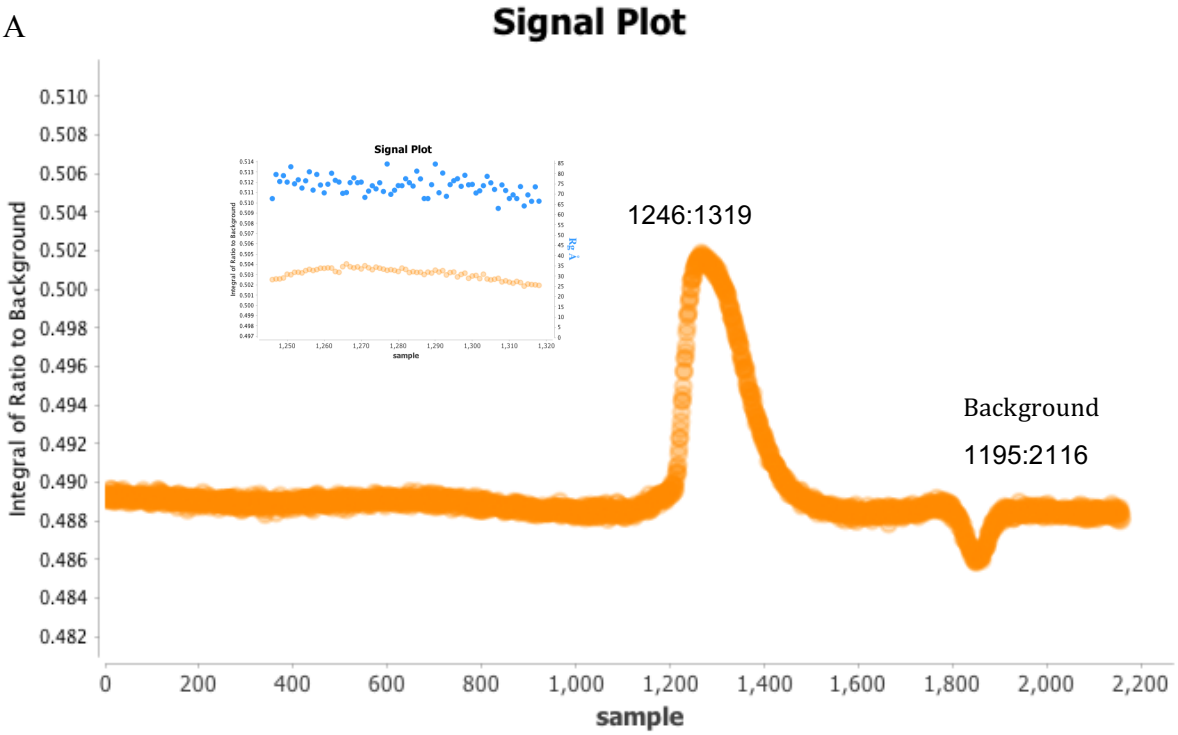

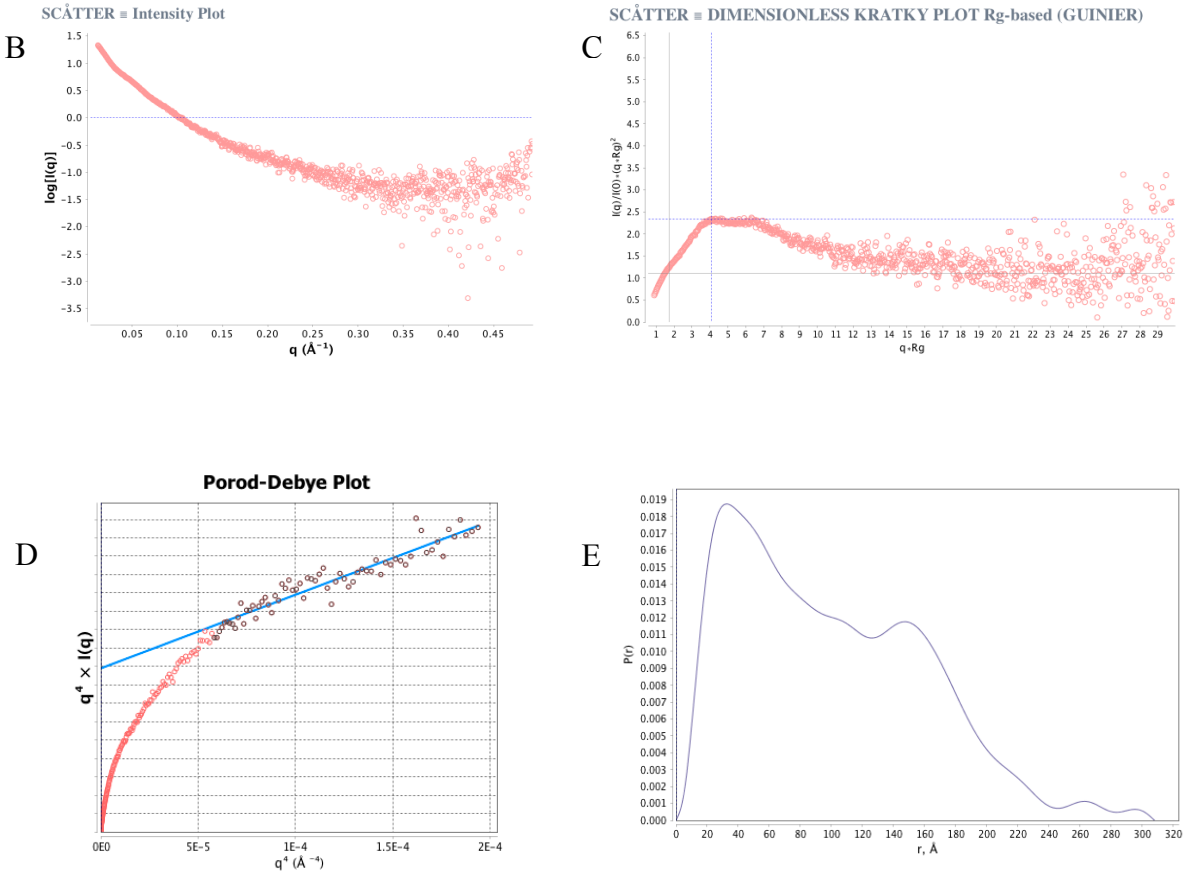

Figure 1: One example of the HPLC mode experiments for one of the KAP1 constructs tested. A) Signal plot from the complete run, with a zoom-in in the peak area, plotting also the Rg. B) Intensity plot for the selected peak area. C) Kratky plot shows the flexible nature of the protein. D) Porod –Debye Plot fit giving a Porod exponent of 2.7, indicating a flexible and elongated molecule. E) P (r) function showing the maximum dimension of the sample, D_{max} = 310 Å and the presence of two small globular domains separated by approximately 160Å.

Results and Conclusions

Preliminary analysis of the data collected shows that KAP1 is elongated and flexible. The stimated maximum dimension D_{max} is in agreement with our 3D models. Structural information obtained by SAXS, in combination with our ultracentrifugation and light scattering data, would be very useful to validate the 3D models and assess the sample heterogeneity and suitability to perform further crystallographic and electron microscopy experiments. Performing SEC-SAXS has clearly removed problems due to the aggregates that were present in the sample changer mode experiments from the previous shift. Out next objective will be to study KAP1 in complex with interacting partners, also using SEC-SAXS. Please see the application for the next shift.

References

1. Iyengar, S. and P.J. Farnham, *KAP1 protein: an enigmatic master regulator of the genome.* J Biol Chem, 2011. **286**(30): p. 26267-76.
2. Cheng, C.T., C.Y. Kuo, and D.K. Ann, *KAPtain in charge of multiple missions: Emerging roles of KAP1.* World J Biol Chem, 2014. **5**(3): p. 308-20.
3. ScÅtter - bioisis.net - Rambo RP and Tainer JA. Biopolymers (2011):p. 559-571.