Introduction

The goal of the BioSAXS experiment with number MX-1967 was to study the protein KAP1 in solution, as well as two of its interacting partners: an RNA helicase and the Heterochromatin Protein 1 (HP1). 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. Only the HPLC mode was used as we knew from previous experiments that the proteins tend to aggregate. In HPLC mode, the samples were submitted to size exclusion chromatography, using a Superose 6 column (24 ml, GE Healthcare) equilibrated in buffer with different salt concentrations (20 mM Hepes pH 7.5, 100-500 mM NaCl, 10% Glycerol and 2mM TCEP), at a flow rate of 0.5 ml/min. 100 ul of sample were injected at sample concentrations around 9-15 mg/ml. During the chromatography run, data were collected for 50 min, with one frame per second, originating 3000 frames per run.

Data analysis

Most of the proteins behaved well and gave good scattering elution profiles that were analysed using SCÅTTER [3].

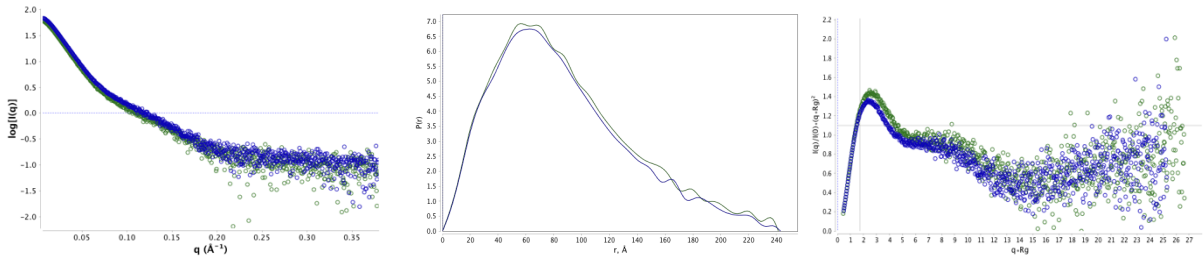

Figure 1: Data collected for the RNA helicase with and without the addition of AMPPNP, blue and green curves, respectively. Left panel, intensity plot for the selected peak area. Central panel, P (r) function showing the maximum dimension of the samples, D_{max} = 240 Å. Right panel, dimensionless Kratky plot which shows the flexible nature of the proteins.

Results and Conclusions

During this shift, we have obtained the following results: 1) We have collected data on KAP1 variants to confirm the experiments from the previous shift (MX-1963). Solution scattering data shows that KAP1 is elongated and flexible. The stimated maximum dimension D_{max} is in agreement with our 3-D models. 2) We have studied whether the addition of AMPPNP to the RNA helicase leads to conformational changes that can be monitored by SEC-SAXS. The Rg changes by 4% and the Dmax is not very different (Figure 1), indicating that the changes are almost neglegible. We plan further experiments with shorter constructs lacking a long unfolded region and with RNA substrate bound. 3) We run the HP1 protein alone and also in complex

with KAP1. We are processing the results and probably need to optimise sample preparation and the choice of column, to improve the separation of the complex from the individual components.

Structural information obtained by SAXS, in combination with our ultracentrifugation and light scattering data, would be very useful to validate the 3-D 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 and will help us separate the complexes from the individual components. Our next objective will be to study the RNA helicase and the KAP1-HP1 complex in more detail. 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.