Report of Activity of MX-1920 on BM29 (11-12 May 2017)

During the 3 shifts at BM29 we have been able to test several samples of proteins, glycosamino-glycans (GAG) and complexes between proteins and GAG. We have been using both the automatic sample changer (SC) and the HPLC online with the beamline. For the SC we have used 10 exposures of 0.5 seconds to avoid radiation damage and we have measured the samples under flow at full intensity. For the HPLC-SAXS measurements we used a pre-packed column Superdex S200 Increase 5/150 (GE Healthcare) under a 0.4 ml/min flow and 1 frame per sec acquisition.
Among the GAGs we have been able to characterize Heparins (HP) of different length form two different commercial sources (SIGMA and Iduron). All of the HP ranged from 0.5 to 4 mg/ml in concentration and behaved as relatively rigid rod particle in solution. We have analysed HP, dp10 (degree of polymerization), dp16 and dp22. The Guinier plot were proportional to the length of the sugar chain, as already shown in the past by the analysis of dp18, dp24, dp30 and dp36 [Khan S, Gor J, Mulloy B, Perkins SJ. Semi-rigid solution structures of heparin by constrained X-ray scattering modelling: new insight into heparin-protein complexes. J Mol Biol. 2010 Jan 22;395(3):504-21. doi: 10.1016/j.jmb.2009.10.064]. The short segments were more rigid than HP, where some bending was detected. The analysis of the scattering curves resulted in Dmax of 4 nm for dp10, 6.3 nm for dp16, 7.8 nm for dp22, and 17.8 nm for HP. We confirmed the results obtained with the SC by SEC-SAXS for HP and dp16.
We then analysed the solution structure of the propetide of lysyl oxidase (PP-LOX), which is the glycosylated N-terminal part of the enzyme. The glycoprotein was behaving as intrinsically disordered (IDP) with an Rg of 3.2 nm and Dmax of 18 nm. We have also investigated the complexes of PP-LOX with HP, dp16 and with the von Willenbrand Factor 1 of collagen VI (vWF1), both in solution and by SEC-SAXS. The propeptide did not show signs of structuring around the HP rods, confirming our data from CD spectroscopy, while the analysis of the complex with vWF1 is less clear, since the partner is a compact globular domain with flexible N- and C-terminal extensions. The Kratky plot seems a linear combination of the two isolated proteins.
We have also analysed the ectodomains (ED) of 4 isoforms of Syndecans (SDC), a family of membrane glycoproteins involved in cell-cell and cell-extracellular matrix interactions. Since we have produced the ED in bacteria, the proteins were not glycosylated and had a tendency to aggregate, so we screened for the best buffer composition to give a monodisperse sample. In any case ED1 to ED4 were IDP-like and very elongated, with Rg ranging from 4.0 to 4.5 nm and Dmax from 14.0 to 16.0 nm. In the buffer screening we found that adding glycerol or LMNG was increasing the propensity to aggregation, while HEPES was better than phosphate buffer in keeping the samples monodisperse.
Finally the beamtime was very productive, the change from SC to HPLC was not problematic, the online BsXCube software was user-friendly and the backup system synchronised with the data collections very useful, especially during the night shift. We would also like to acknowledge the professional support of the beamline scientist.