ESRF	**Experiment title:** Determination of the Cohesin Complex Structure Using Small Angle X-ray Scattering	**Experiment number**: SC-3139
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Report:

In eukaryotic cells sister chromatids are held together, i.e., “cohesed”, from their generation during DNA replication until their segregation to daughter cells during cell division. Sister chromatid cohesin is an essential pre-requisite for proper chromatid segregation and plays an important role in double strand break repair, prevention of unequal crossovers and other aspects of DNA metabolism. Sister chromatids are believed to be trapped inside proteinaceous cohesin rings formed of SMC1 and SMC3 proteins [1-3]. The SMC proteins fold into a 50 nm long intramolecular antiparallel coiled coil “arm” which is flanked on one end by a “head” domain formed by N- and C-terminal regions of protein and on another end by a central “hinge” domain. SMC1 and SMC3 heads bind to each other, a process believed to be facilitated by their ability to “sandwich” two molecules of ATP between them. SCC1 protein further connects the two heads thus closing the ring. The interface between the two heads serves as an “exit” point for DNAs during mitosis when SCC1 is cleaved by a specific protease. SMC1 and SMC3 hinges dimerize as well and an interface between them serves as an entry gate for the DNA. Opening of the hinge interface and DNA import inside the ring are dependent on the ability of the heads to hydrolyse ATP [4, 5]. An important question that remains unanswered is how the heads interact with hinges if they are separated by a very long and flexible coiled coil. One of the models predicts that the coiled coil “arms” can bend thus allowing direct interaction between the heads and the hinges however this remains to be demonstrated [6].

In order to elucidate the conformation of the native cohesin complex we purified cohesin complexes from yeast *Saccharomyces cerevisiae* without over expression. The aim of the SAXS study is to investigate whether the “collapsed” rings exist in solution or if this conformation is the result of their adsorption to a solid support during sample preparation for electron microscopy. If the collapsed rings are indeed observed in solution, it would be important to determine if the SMC arms supercoil or simply align along each other.

In this beamtime we measured the solution scattering of Cohesin solutions with different protein concentrations. Small-angle X-ray scattering (SAXS) measurements were carried out at station ID2, ESRF,

Grenoble. The energy of incoming beam was 16 keV, two sample-to-detector distances (2 m and 5 m) were used in order to cover a large *q* range from 0.02 to 7.8 nm^{-1}. Protein solutions were filled into 2 mm quartz capillaries. The same buffer solution without protein was measured as the background, in exactly the same way as the protein solutions and was subtracted from the sample scattering. All measurements were carried out at room temperature. The raw data were corrected for transmission, fluctuation of primary beam intensity, exposure time, and the response of the detector.

Fig.1 shows the correct SAXS data for Cohesin 0.5 mg/mL and 0.25 mg/mL in the full q range. The data are merged from two SD setups. Visible change of scattering intensity in the low q region can be seen: with 0.5 mg/mL protein, the scattering intensity starts to level off below q~0.1 nm^{-1}; above this, intensity follows $I(q) \sim q^{-3}$ indicating a relatively compact structure. Upon diluting the solution to 0.25 mg/mL, the scattering intensity in the same region follows the relation of $I(q) \sim q^{-2}$, this is the sign of a polymer like (coil-like) structure. These results clearly demonstrate that the conformation/packing density of Cohesin strongly depends on the protein concentration. The structure of Cohesin was further analyzed by calculating the pair-distance distribution function, P(r). The difficulty for the P(r) calculation is that the size of the Cohesin open structure is relatively large compared to the q range of the current experiments. For 0.5 mg/mL, where the Cohesin molecules are relative packed, it gives a radius of gyration of 18 to 19 nm and D_{max} is about 50 nm, which is consistent with the TEM observation. The data for 0.25 mg/mL do not converge properly as expected. Here the SAXS data show clearly the conformation change suggesting the shrinking and opening of the structure with protein concentration.

In summary, we have shown that the SAXS measurements of Cohesin solutions provided clear evidence of the conformation/packing density change of Cohesin molecules upon diluting. These results can help to clarify the different observations on the morphology of Cohesin reported in literature.

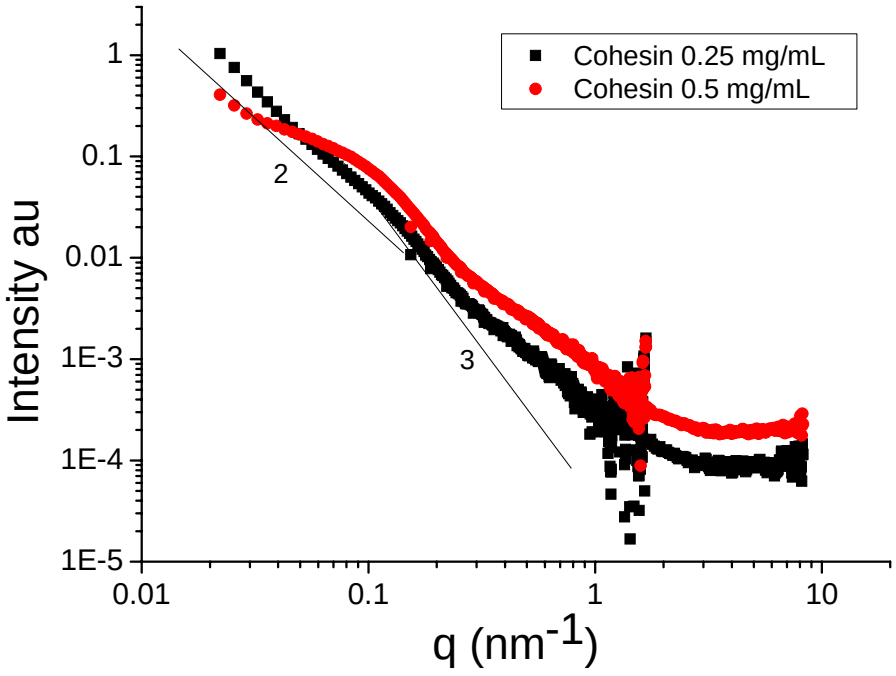

Figure 1 SAXS profiles of Cohesin solutions with different protein concentrations. Data are collected with two SD configurations (2 and 5 m).

References

[1] Haering, C.H., et al., Mol Cell, 2002. **9**(4): p. 773-88.
[2] Anderson, D.E., et al., J Cell Biol, 2002. **156**(3): p. 419-24.
[3] Ivanov, D. and K. Nasmyth, Cell, 2005. **122**(6): p. 849-60.
[4] Arumugam, P., et al., Curr Biol, 2003. **13**(22): p. 1941-53.
[5] Weitzer, S., C. Lehane, and F. Uhlmann, Curr Biol, 2003. **13**(22): p. 1930-40.
[6] Gruber, S., et al., Cell, 2006. **127**(3): p. 523-37.