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Report for Experiment MX-1915 on beamline BM29: Oligomerization properties of higher order SMN complex particles

Proposal Summary
The SMN complex is a macromolecular machine that orchestrates the assembly of U snRNP particles *in vivo*. We have recently discovered the SMN complex from the fission yeast *Schistosaccharomyces pombe*, which in comparison to its mammalian counterpart features reduced complexity and may hence be preferable for structural studies. We have been able to overexpress and reconstitute the entire five-membered *S. pombe* SMN complex which assembles into higher-order, high molecular weight particles. Small deletions in a disordered region of the SMN protein drastically reduce the molecular weight of the SMN particles. Here, we used SAXS to elucidate the oligomeric properties of these particles and to devise a model of the native SMN complex.

Scientific background :

Spliceosomal U-rich small ribonucleoprotein particles (U snRNPs) are the major building blocks of the pre-mRNA processing spliceosome [1]. Even though these particles can form spontaneously *in vitro*, their assembly *in vivo* requires a plethora of trans-acting assembly factors united in SMN- and PRMT5-complexes. The assembly pathway of U snRNPs can be divided into two distinct temporal phases. The early phase is dominated by the assembly chaperone pICln. pICln binds newly-synthesized Sm proteins and delivers them in a pICln-bound form to the PRMT5 complex. The late phase of snRNP formation is dominated by the SMN complex, which resolves this kinetic trap by dissociating pICln from the pre-organized Sm proteins and subsequently catalyzes the joining of Sm proteins with snRNA [1-3]. We have recently solved the crystal structures of two key intermediates of the U snRNP assembly pathway [2]. With data collected during our last experiment, MX-1826, we were able to solve the crystal structure of a minimal version of the *S. pombe* SMN (Yab8) protein (Fig. 1, unpublished results). This crystal structure reveals that, apart from the previously known oligomerization interface [4] in the YG box region, a second one exists which explains the formation of high-molecular weight entities of the SMN complex. Systematic shortening of a disordered region within the SMN protein leads to a reduction of the order of the resulting SMN complex oligomers (Fig. 2). In the proposed SAXS experiment, we want to determine the exact nature of these complexes and how the SMN protein orchestrates the formation of these particles.

Experiments performed and interpretation of the obtained results :

During this session, we have collected nearly two dozen SAXS datasets of different SMN complexes containing *S. pombe* SMN (and an internal deletion constructs thereof), each in varying complexes with Gemins 2, 6, 7 and 8. We have evaluated the oligomeric behavior as a function of (1) SMN deletions restraining overall complex flexibility, (2) number of complex subunits and (3) protein concentration (Fig. 1 A). The data interpretation (Fig, 1 B, C) allowed us to devise a model of the native SMN complex that explains its flexibility and unique oligomeric properties (Fig. 1D).
The data has substantially contributed to a recent publication that has been selected as a “Breakthrough Article” in the journal Nucleic Acids Research [5].

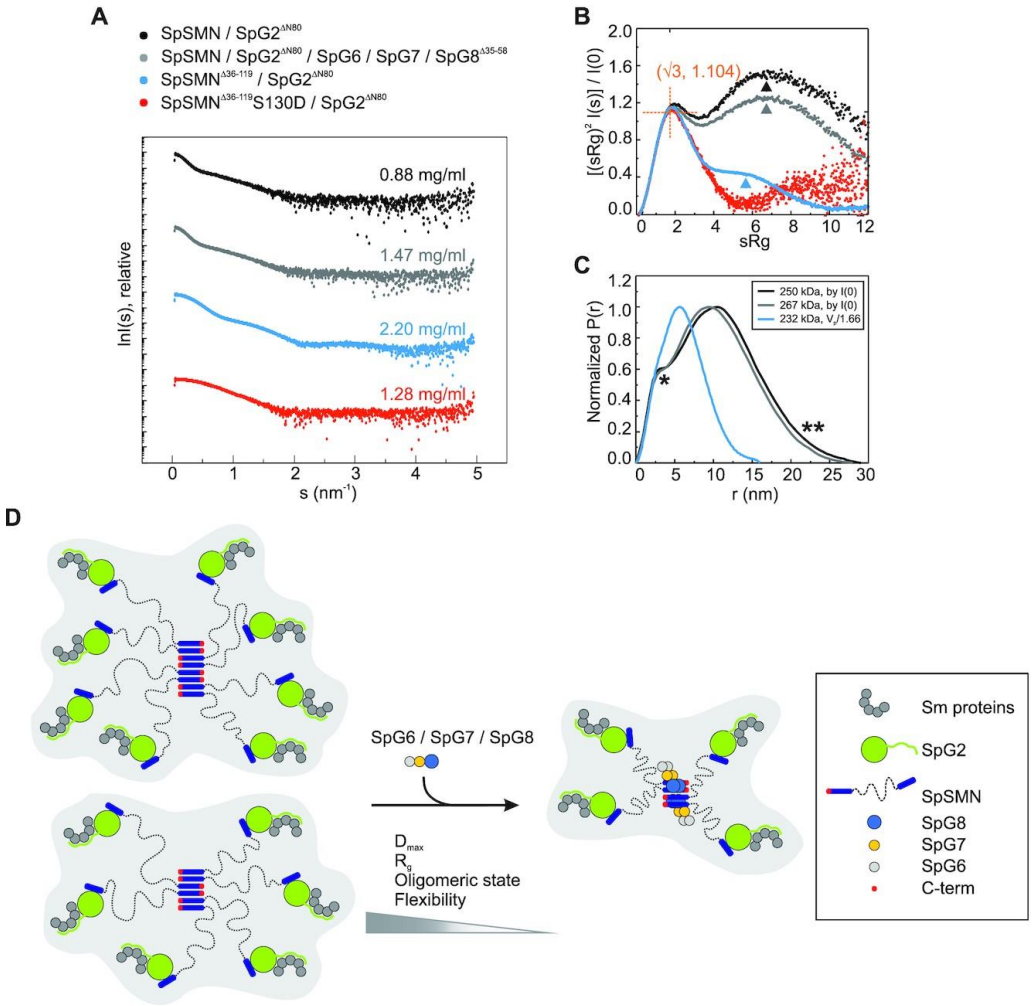

Fig. 1. SAXS analysis and model of the SpSMN complex. (**A**) Small angle X-ray scattering curves of respective complexes at indicated concentrations represented as [I(s) vs s]. The scattering data have been deposited to SASBDB under the following accession codes: SASDKZ4, SASDK85, SASDK66, and SASDKF5. (**B**) Dimensionless Kratky plots [$(sRg)^2 I(s)/I(0)$ versus sRg]. The expected maximum at (√3, 1.104) for globular entities is indicated by an orange crosshair. Deviation from globularity is indicated by arrowheads. (**C**) Normalized pairwise distance distribution functions represented as [Normalized P(r) vs r]. Molecular weights, either calculated from I(0) or from Porod volume (Vp/1.66) are indicated in the inset. For full length SpSMN complexes, * and ** represent shoulder and extended tail, respectively. (**D**) An integrative model of the SpSMN complex. The core of the SpSMN complex is formed by antiparallel multimerization (indicated by alternating SMN C-terminus) of glycine–zipper YG-domain dimeric units. The flexible N-terminal extensions of SpSMN (dotted lines) facilitate the capture of Sm proteins via the SpG2 subunit. The overall shape, flexibility, and oligomeric state of SpSMN is influenced by the SpG8/SpG7/SpG6 sub-complex.

References

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