Experimental report for ESRF Expt. MX-1390, proposer Clemens Grimm, University of Wuerzburg, Beamline ID14-4

Overview

The multi-component SMN (Survival of Motor Neuron) complex promotes ribonucleoprotein (RNP) formation by catalyzing the joining of U snRNP proteins (termed Sm proteins) with U snRNA. Furthermore, this assembly process is controlled and assisted by pICln that acts as a chaperone. To shed light onto the fundamental mechanisms of the snRNP assembly machinery, we have reconstituted and crystallized an 8S complex of pICln, the SMN complex components SMN and Gemin2 and Sm proteins D1, D2, E, F and G. as well as a 6S variant lacking SMN and Gemin2. The 8S complex is mechanistically interesting as it can be regarded as a snapshot of the cellular snRNP assembly process.

This is a challenging project where already quite a lot of work has been invested in optimization of expression constructs, crystallization screening and crystal optimization. During this session, we evaluated contact-engineered mutants of the 6S complex for improved diffraction.

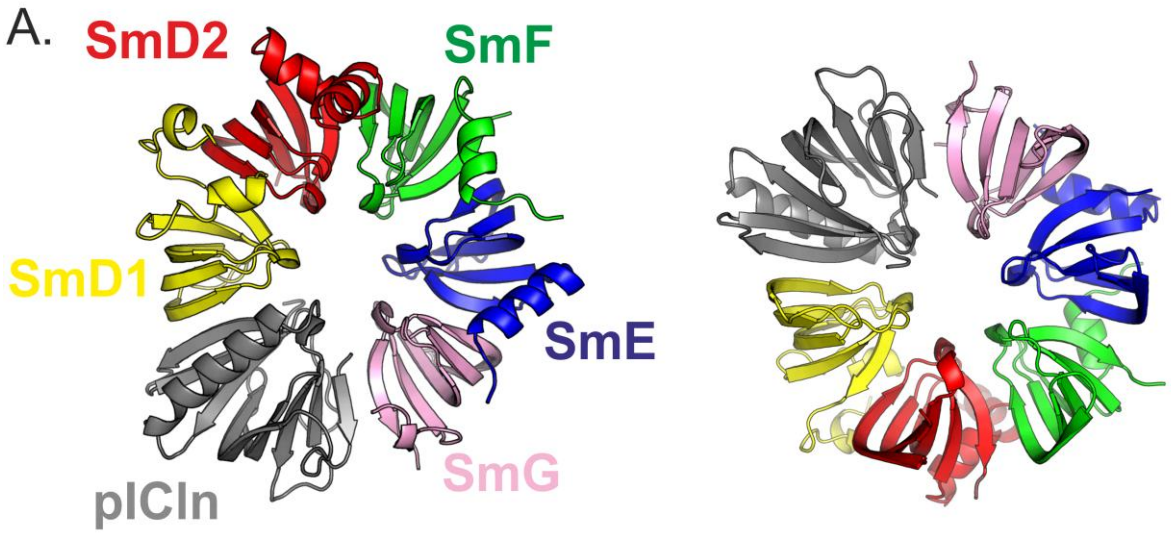
Evaluation and results

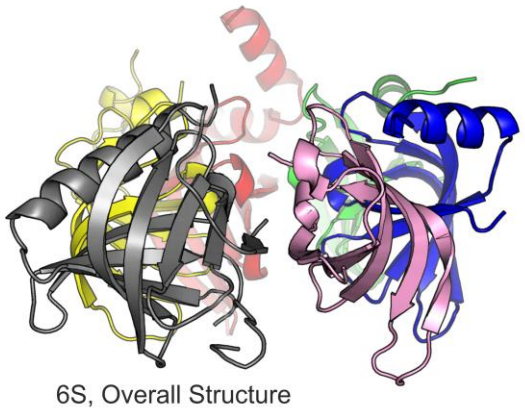
Out of four 6S complex variants that produced suitable crystals, a single one showed an increase in diffraction limit from 4 Å to 1.9 Å. We collected a dataset and solved the structure by molecular replacement. Subsequent refinement including water building and modeling of alternative side chain and loop conformations resulted in R / Rfree factors of 0.178 / 0.221. See table 1 for data collection and refinement statistics and figure 1A for the overall structure of the ring-shaped 6S complex structure in three orthogonal views. Fig. 1B shows a close-up of the chaperone pICln. Surprisingly, its pleckstrin homology (PH) topology is extended by an additional strand β0 residing on the very N-terminus.
In summary, the data collected during the experiment will be extremely useful to solve the puzzle of how Sm proteins are assembled into snRNP particles.

Table1: Data collection and refinement statistics of the 6S complex crystal

Data Collection	
Beamline	ID14-4
Wavelength (Å)	0.9393
Space group	C2
Cell dimensions a, b, c (Å) α, β, γ (°)	 180.67, 65.22, 99.22 90.0, 92.5, 90.0
No. molecules in asymmetric unit	2
Resolution (Å)	49.6 -1.9 (2.01-1.9)
Unique reflections	88058
Rsym (%)	4.4 (58.2)
Mean I/σ(I)	14.3 (1.84)
Completeness (%)	96.2 (96.2)
Redundancy	2.69 (2.71)
Refinement	
Resolution (Å)	49.6 - 1.9
No. reflections	88058
R / R_{free}	0.178 / 0.221
No. atoms Protein Ligand/ion Water	 8140 31 372
RMS deviations Bond lengths (Å) Bond angles (°)	 0.012 1.520
Ramachandran Favoured, Allowed, Outliers [%]	 98.3, 1.8, 0.0
PDB Accession number	4F7U

Fig 1: 6S and pICln




6S, Overall Structure