Experimental report for ESRF Expt. MX-987, main proposer Clemens Grimm, University of Wuerzburg, Beamline ID-29

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 a complex of pICln, the SMN complex components SMN and Gemin2 and Sm proteins D1, D2, E, F and G. This complex is particularly 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 the expression constructs, crystallization screening and crystal optimization. The crystals typically show limited diffraction with high anisotropy and high mosaicity (see fig. 1 for a typical diffraction image from this experiment).

During our first session on 31 Aug. 2009 we encountered technical problems; test datasets collected of lysozyme and insulin crystals showed intolerable high R_{sym} values. Therefore, replacement time was granted on 20 Nov. 2009.

Evaluation and results

Initial measurements of the crystals indicated possible 222 point group symmetry; however, this obvious pseudo-symmetry brakes down with the higher resolution and data quality achieved during the current experiment. After careful examination of all data measured so far, the most likely space group of the crystals is C2.

We tested several crystals cooled under different cryo conditions for their diffraction quality. The large plate-like crystals (up to a size of approx. 500 x 200 x 30 microns) have also been probed at different spots and we found huge differences in diffraction quality. Diffraction could be observed up to around 3.4 Å in the 'good' direction of the crystals. Due to the typical high anisotropy, at the same time diffraction was limited to around 4.8 Å resolution in the 'bad' direction (fig. 1). Finally, we selected the most promising crystals for data collection (see table 1 for dataset statistics).

Overall, we see a substantial improvement over data collected in our initial experiments and MAD experiments seem to be sensible at this stage. Therefore, a number of heavy atom soaked crystals were tested, however no derivative crystal with sufficient diffraction quality had been found so far.

Table1. Statistics of best native dataset (space group C2, a=262.6Å, b=72.7 Å, c=153.1Å, β=118.0°)

Resolution Limit	Observed Reflections	Unique Reflections	Possible Reflections	Completeness	R_{sym}	I/sigma(I)
10.00	6140	1476	1509	97.8%	6.6%	15.23
8.00	5555	1333	1346	99.0%	8.7%	12.84
6.00	16798	3803	3819	99.6%	18.1%	6.59
5.00	21182	4729	4747	99.6%	28.2%	4.58
4.70	10126	2257	2262	99.8%	27.9%	4.54
4.30	18341	4113	4127	99.7%	33.4%	3.96
4.10	11931	2682	2691	99.7%	57.9%	2.58
4.00	6728	1525	1529	99.7%	84.4%	1.86
3.90	4241	1063	1726	61.6%	100.8%	1.59
total	**101042**	**22981**	**23756**	**96.7%**	**18.7%**	**5.41**

Figure 1. Diffraction image obtained from native crystal.