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Shifts: 4	**Local contact(s)**: Dr Andrew McCarthy	*Received at ESRF:*

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Report:

B. Gigant, M. Knossow: Molecular mechanisms of tubulin regulation. (1.5 shift)

The αβ tubulin heterodimer is the microtubule (MT) building block. The tubulin/MT cycle is regulated by intracellular proteins. It is also perturbed by exogenous compounds, some of which are useful anticancer drugs.

In this session, we have attempted to get insight into tubulin regulation by intracellular proteins of the Map2/Tau family, which promote microtubule polymerisation. Crystals of tubulin in complex with the stathmin-like domain of the RB3 protein (T2R) have been soaked with either a Tau peptide of 12 residues, or which a longer construct of 115 residues. We have also obtained co-crystals of T2R with both, either in the same crystallization conditions (in the case of the dodecapeptide) or in different conditions (co-crystals T2R-longer tau construct). Overall, 8 dataset have been collected. It turned out that the co-crystals obtained in the different crystallization conditions were of the same space group and cell as the usual T2R crystals. They all diffracted to ca 4 Å resolution, the best crystal was processed to 3.8 Å. The structures were refined with Refmac5, using the T2R structure as a starting model. No signal for the tau fragments was detected in the electron density maps; as a control we had a good signal for ligands of tubulin not included in the model.

In the case of the dodecapeptide, RMN data indicate that the affinity for T2R is rather low, with a Kd estimate around 0.5 mM. But for the long construct, we measured a Kd in the 10 nanomolar range, and we checked by biochemical methods the presence of the tau fragment in the crystal. One explanation is that it is not well ordered in the crystal and higher resolution data will be needed to get a chance to have signal for it.

Jenny Keller*, Julien Henri*: : yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project (2.5 shift)

1) Fibronectin

Spacegroup $P3_221$ a=b=125 Å and c=60.5Å.
Resolution 2.80A.
Completion= 98.4%
Rsym=9.6%

Human fibronectin, a multifunctional protein, mediates a wide variety of cellular interactions with the extracellular matrix and plays important roles in cell adhesion, migration, survival and differentiation. It has a significant role in cancer progression by increasing the migration and proliferation of human carcinoma cells. We have solved the 2.4 Å resolution structure of its 45kDa collagen/gelatin binding fragment. So as to understand the molecular basis responsible for the interaction of fibronectin with collagen, we have grown crystals of this fragment in the presence of a collagen derived peptide. These crystals have been tested during this session and diffracted to 2.8-3A resolution. Two datasets have been collected to 3 and 2.8A, respectively.
Analysis of the residual maps did not reveal the presence of the peptides bound to this fibronectin fragment.

2) *S. cerevisiae* Dcs1-Dcs2.

In yeast, the heterodimer Dcs1-Dcs2 catalyses cleavage of 5′end m^7G-oligoribonucleotide fragments generated by 3′→5′ exonucleolytic decay, and cleavage of m^7GDP generated by Dcp1/Dcp2-mediated decapping in the 5′→3′ decay pathway. We have obtained small crystals of this heterodimer that were tested for diffraction during this run. Spots could be observed beyond 3A resolution but diffraction was weak between 7 and 3A. We have collected dataset but its quality is too low (resolution limit: 4.5A). Further optimisation is currently performed.

3) V28

Spacegroup: $P2_1$ a=50A b=59 c=84A
Resolution: 3.45
Completion= 100%
Rsym=15%

Lately, phages infecting hyperthermophilic and acidophilic archaea (Sulfolobus, Acidianus) have been discovered in geothermally heated hot aquatic environments. They belong to a new class unrelated to other phages. 90% of their genes have no homologues in sequences databases suggesting unknown mechanisms for their biological functions. With the aim of deciphering this new branch of life at a molecular level, we have launched a multidisciplinary project for systematic expression and structure/function determination of all proteins from Acidianus Filamentus phages (AFV) associating structural, biochemical and genetical approaches. The strategy consists in expression, purification and structure determination of 60 proteins from 2 archaeophages. In a situation where little information is deductible from sequence, 3D structure will lead to hypothesis about the molecular function. V28 is a 12kD protein from AFV6 which is homologue to CopG a transcription factor from a plasmid from *E.coli*, involved in copies number control. The protein crystallizes as stacked plates and the diffraction quality is often poor. We collected a 3.45A data set on a SeMet labelled crystal but the weak anomalous signal did not permit the phasing.

Moreover, during this session, diffraction tests have been performed on other new proteins studied in our group. Some of the crystals were salts, others diffract only weakly.