Experiment title: The structural cycle of tubulin.

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Report:

1) Project 1: The structural cycle of tubulin

In previous synchrotron sessions, we have obtained data on a new crystal form of tubulin that diffracts to up to 2.1 Å (space group $P2_12_12_1$, cell ~65, 128, 250 $Å^3$). These crystals can be obtained either with GTP or GDP. They open the way for the elucidation of the structural cycle of tubulin linked to its cycle of assembly in microtubule and disassembly and to its nucleotide cycle. In this session, we collected data from crystals of tubulin in complex with GTP, or with GDP and in presence of aluminium or beryllium fluoride. The aim was to record structural snapshots along the nucleotide cycle of tubulin. We also exploited the higher diffracting crystals to gain further details of the interaction of tubulin with small molecule inhibitors of the microtubule dynamics. Two of them, named nocodazole and TN16, were investigated during this session. Statistics for the relevant dataset are summarized in the following table.

Ligands	Resolution (Å)	completeness (%)	redundancy	I/sig(I)	Rmeas (%)
Al fluoride	2.45	98.4 (88.2)	8.5 (5.6)	13.6 (1.9)	10.3 (72)
Be fluoride	2.4	97 (81)	8.8 (5.8)	16.7 (2.1)	9.1 (80)
GTP	2.74	99 (95)	3.9 (3.6)	11.1 (1.9)	9 (76)
Nocodazole	2.5	99 (96)	3.9 (3.8)	11 (1.9)	9 (77)
TN16	2.7	99 (93)	3.9 (3.6)	10 (2)	10 (68)

After refinement, it appeared that the signal for Al or Be fluoride was very low. Nocodazole, which has a low solubility in aqueous buffers, was not detected either, whereas TN16 was clearly visible in the electron density maps. Finally, we also have signal for the GTP, but some hydrolysis takes place during crystallization resulting in a mix of GTP and GDP in the crystal.

2) Project 2: we collected data from crystals of M2-1, a protein from the RVS virus that interferes with microtubule dynamics. A 3.8 dataset was collected but with an overall Rmeas of 30%. Obviously, better crystals will be needed for this project.