ESRF	Experiment title: **X-ray fiber diffraction of microtubule: Analysis of structural dynamics of native microtubules on a second time scale**	**Experiment number**: LS 2805
Beamline: BM26B	**Date of experiment**: from: 27/03 to: 29/03, from: 25/06 to: 28/06/2108	**Date of report**: 01/08/2018
Shifts: 12	**Local contact(s)**: Daniel HERMIDA-MERINO	*Received at ESRF:*

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Microtubules are key components of the cytoskeleton in eukaryotic cells. Dynamic conversion between tubulin dimers (free unit protein before assembly in cytoplasm, MW=110,000) and assembled microtubules (polymerized state) occurs in a controlled manner, which modifies intracellular microtubule networks along with the whole cell activities such as cell-migration, shape changes, mitosis, differentiation and so on. Since microtubules are one of the most crucial targets of anti-cancer chemicals (*e.g.*, paclitaxel) that knockout cancer cells, our question is how such tubulin-binding drugs and related derivatives affects the structure of microtubules, and how such binding depends on the chemical states of tubulin dimers during the reaction of GTP-hydrolysis. For these purposes, we applied our original technique for the rapid shear-flow alignment of biological filaments (Sugiyama et al., 2009; Kamimura et al 2016) to observe X-ray fiber-diffraction signals from microtubules at higher resolutions (up to 0.45 nm), the Q-range of which is expected to provide us with crucial signals from the internal structures of tubulin molecules (Fig. 1).

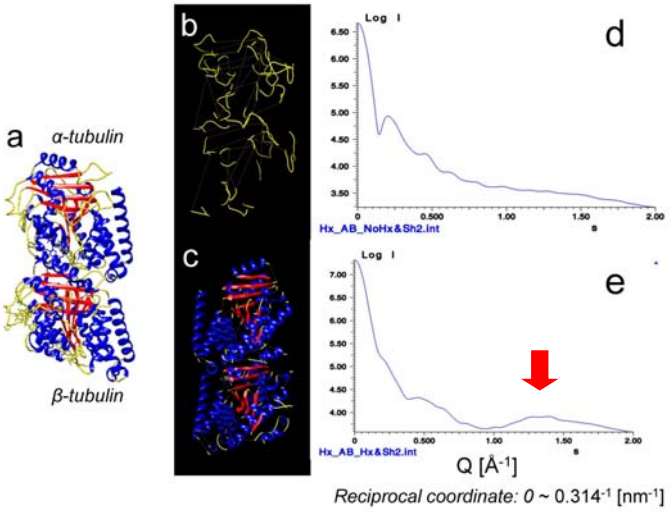

Fig. 1 Solution scattering (SAXS) pattern of tubulin dimer. **a**, Structure of tubulin dimer in microtubules (5syf) determined by cryo-electron microscopy (Kellogg et al., 2017). **b**, structure after removing all the helixes and strands. **c**, helixes and strands that are removed from the original structure (**a**). **d** and **e** indicate SAXS pattern of **b** and **c**, respectively, in a wide range of Q (0~2Å^{-1}) simulated with a software based on Debye scattering equation (program designed by Dr. Alessandro Longo, DUBBLE/ESRF). Red arrow represents the area of SAXS signals derived from the internal structure of tubulin.

RESULTS & DISCUSSION

In the present study, we finihsed the following two improvements in our experimental methods, eventually both improvements worked well as we expected. First, according to the test results obtained during our previous beam time (LS2703, 26-02 852), whch showed a high bachground of scattering at high Q-range, we replaced all the quartz and kapton windows in the apparatus to mica plates. The effects of this improvement were tested during the first session of our beam time (27-29/03/2018). Effects of noise reduction were obvious, in particular, at the Q-range of WAXS signals (>0.5 nm in a reciprocal space). Second, we planned to execute a series of observations of microtubule fiber-diffraction with various tilting angle of specimen (Fig. 2). As shown in Fig.2b, differently from conventional methods of SAXS or powder scattering, diffractin intensity and angle from aligned biological fibers should vary depending on the tilting angle of specimen (β) against the beam axis of X-ray. Therefore, we can expect the signal position in diffraction pattern moved in an expected manner depending on β+α, where α is the off-set of tilting angle of structures within microtubules. The variable α is expected to have a certain specific value in the case of secondary structures of tubulin (helices or strands), but it should be equal to 0 for the structures regularly arranged in the longitudinal direction of microtubule axis. This approach seems to be working well at the high Q-range (1.25 $Å^{-1}$, ~0.5 nm in a reciprocal space) as shown in Fig. 2a (*). Further details analysis, changing β in a wide range should be required to test this hypothesis more exactly, and to obtain definitive conclusion which high-Q signals reflects the structural dynamics within tubulin molecules.

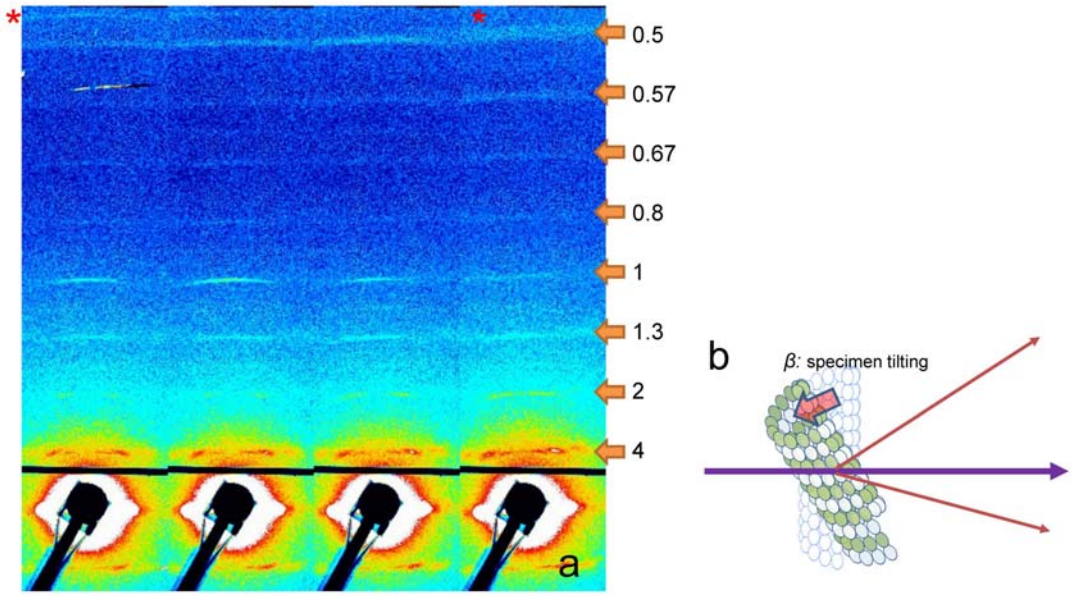

Fig. 2 Observed meridional signals at different tilting angles of microtubules (β) of -8, -4, 0 and +4 degrees. **a**, layer lines due to the longitudinal repeat of tubulin molecules within microtubules are shown with numbers, 4/n [nm] where n is positive integer. Signal positions of these layer lines shifted in an expected β-dependent manner. Marks (*) in the diffraction pattern indicate the signals that showed unexpected irregular dependency on the microtubule tilting (β), i.e., α should have certain specific values in the case of signals observed at a high-Q range. **b**, diagram illustrating specimen tilting with angle, β.

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