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Application for beam time at ESRF – Experimental Method

X-ray fiber diffraction of microtubules: New application of modified active flow chamber for the recording of microtubule diffraction signals with higher intensities

Proposal Summary (should state the aims and scientific basis of the proposal) :

In a series of the e fiber diffraction experiments of microtubules, we used a rheometer type flow chamber that are composed of two parallel plates (Figure 1). With the apparatus we could accomplish the rapid shear-flow alignment of biological filaments [1-4]. In the case of microtubules in solution, that could be aligned with small angular deviations (<5º) within a few second, thus enables us to collect the structure information from microtubules in axial (meridional) and diameter (equatorial) directions. Although the major advantage of the method is the sample volume of 0.1 mL which enabled us to apply to many other different type of biological materials (DNA, virus, actin, collagen and Fitz proteins), the signal intensity of diffraction is low due to the thickness of specimen due to the narrow gap (0.35 mm) between two plates in the rheometer. Simple modification of the thickness > 0.5 mm, we have tried, was impossible to maintain stable shear flow of solution in the chamber without dispersing. To solve the problem, in this proposal, we are going to use a different designs of flow chamber that is directly driven by a vibrating plate immersed in the experimental solutions (Figure 2).

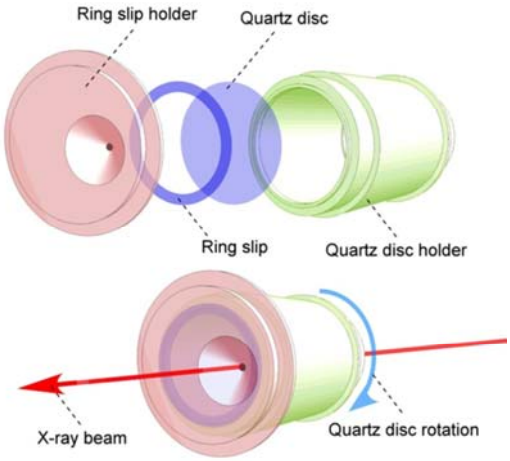

Figure 1. Schematic illustration of the rheometer-type apparatus used for shear-flow alignment of microtubules. The chamber used for shear-flow alignment included a quartz disc (glued on a tube, right side) and a ring-shaped cover slip (glued on a copper plate, left side). These were placed face-to-face with a narrow gap of 0.32 mm. After assembled as shown in the lower figure, the quartz disc was spun at 10-30 rps (blue arrow).

The main advantage of the new design is the thick ness of the specimen (>1 mm) keeping the volume of specimen low enough (<0.1 mL), which is crucial for many biological materials where enough amount of pure materials is usually difficult to prepare. Using the new design of flow chamber, we are going to try the collection of diffraction signals with higher time resolution at SAXS. The expected time resolution should be shorter than 1 sec as we have already executed the recording of diffraction signals within 5 sec from the aligned microtubules (equatorial signals reflecting diameter change of filaments). Another advantage of the new design is that we can easily increase the chamber thickness up to several mm, thus, we are going to try to find out optimal specimen thickness by compromising between X-ray absorption and suitable S/N ratios. The smaller size of chamber is also suitable for quick mixing with other chemicals that changes the structure of microtubules. Temperature control is also simpler comparing with our previous model.

Scientific background :

MTs are key components of the cytoskeleton in eukaryotic cells. Dynamic conversions between tubulin dimers (free unit molecules in cytoplasm) and assembled microtubules (polymerized state) are closely related to the formation of intracellular microtubule networks, which is occurring concomitantly with the whole cell activities such as cell-migration, shape changes, mitosis and differentiation. One of the most fundamental questions of us is how such microtubule dynamics is associated with the molecular configuration of tubulin dimers, which is expected to be influenced by various factors, e.g., GTP-hydrolysis, inter-tubulin interactions, chemical modifications, structural heterogeneity among tubulin

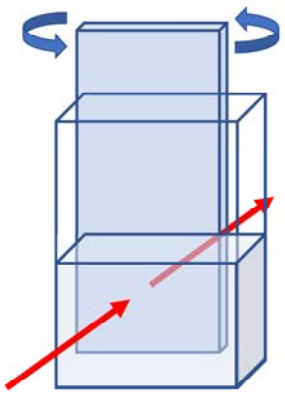

Figure 2. A new type of shear-flow machine. In a chamber containing 0.05 – 0.1 mL (7x7x2 mm) solution, a quartz plate is placed and vibrated in a circular orbit, making active shear flows between the two chamber walls.

species, interaction with molecular motors, and the decoration by other associated proteins). In addition, tubulin-targeting chemicals, such as paclitaxel and laulimalide that can be used as an anti-cancer agent, are expected to have strong effects on the microtubule dynamics. Our approach is to address this issue, how the tubulin-binding chemicals are affecting on the structural stability and molecular dynamics of tubulin within native micro-tubules. For such purposes, fibre diffraction is expected as one of the most powerful techniques, since we can directly acquire the information of microtubule stability. In the present proposal, we are going to collect diffraction signals with higher time base (<1 sec) in order to distinguish the quick diameter changes of microtubules due to configuration changes of tubulin molecules within microtubules (expected to be occurring within 1 sec) from the slower incorporation of new tubulin molecules into microtubules (expected to be occurring 10-30 sec). For such purposes, improving a new design of shear-flow chamber is indispensable.

Experimental technique(s), required set-up(s), measurement strategy, sample details:

Diffraction signals from microtubules are very weak due to the low electron density of biological materials and we cannot keep assembled microtubules under the same conditions for a long time in suitable solution, therefore a high X-ray flux, only available at synchrotron beam lines, is needed to obtain enough signals to analyse the structural dynamics. To know the time course of diffraction changes, which should reflect the dynamic structural changes of tubulin, before and after the addition of tubulin drugs, we are going to modify the method by Kamimura et al. [4] for higher time-scale analysis (<1 sec). We will design our apparatus to control specimen temperature from 5 to 40°C. Off-site control to apply chemicals is also possible.

Beamline(s) and beam time requested with justification :

We are planning to collect diffraction signals under two main conditions, GTP- and GTPγS-micro-tubules that are reflecting GDP- and GTP-tubulin states, respectively. To investigate the structures with and without two different types of tubulin-binding chemical (paclitaxel and laulimalide [16-18]) at different temperature (5-40ºC [4]), we estimate roughly, 40 experimental conditions in total, 6-shift of beam time will be required. In addition, three more shift we will need for the machine-setting to optimize our setting for efficient data acquisition.

Results expected and their significance in the respective field of research :

1) Showing the new direct evidence of quick protein conformational changes observed in native filaments in solution
2) Understanding the effects of various tubulin-binding chemicals on the tubulin structures
3) Understanding the physical conditions (temperature etc) for chemicals to show the effects
4) Clarifying how the effects of anti-cancer drugs depend on the chemical states of tubulin dimers

References

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