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Supplementary Information

Thermo- and Photo-Enhanced Microtubule Formation from $Ru(bpy)_3^{2+}$-Conjugated Tubulin

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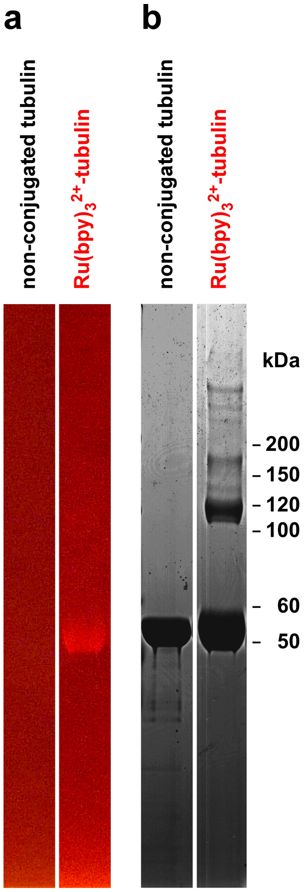

Figure S1. SDS-PAGE of non-conjugated tubulin and $Ru(bpy)_3^{2+}$-conjugated tubulin with an optical band-pass filter ($\lambda = 450 \pm 25$ nm) (a), and with Coomassie Brilliant Blue -stain (b).

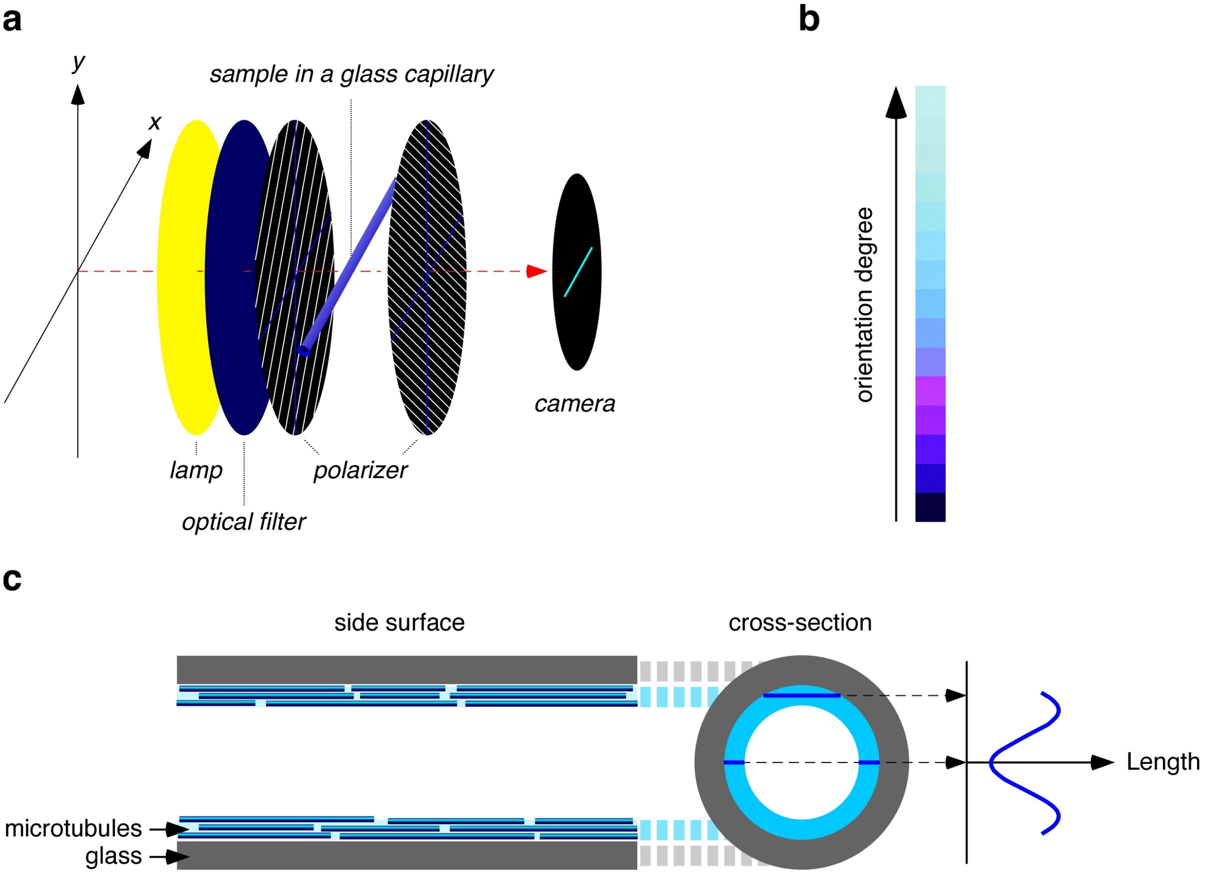

Figure S2. a) Schematic illustration of the experimental setup used for observations under cross-polarized light. The polarizers were adjusted to 45° and 135°. The long axis of the sample was adjusted to 0°, parallel to the *x*-axis. b) Color meter of the orientation degree. c) Schematic illustration of the MT orientation on a glass capillary wall and views from the side surface and cross section. The orientation degree detected by the cross-polarized light observation depends on the length of the light pathway in the oriented MTs.

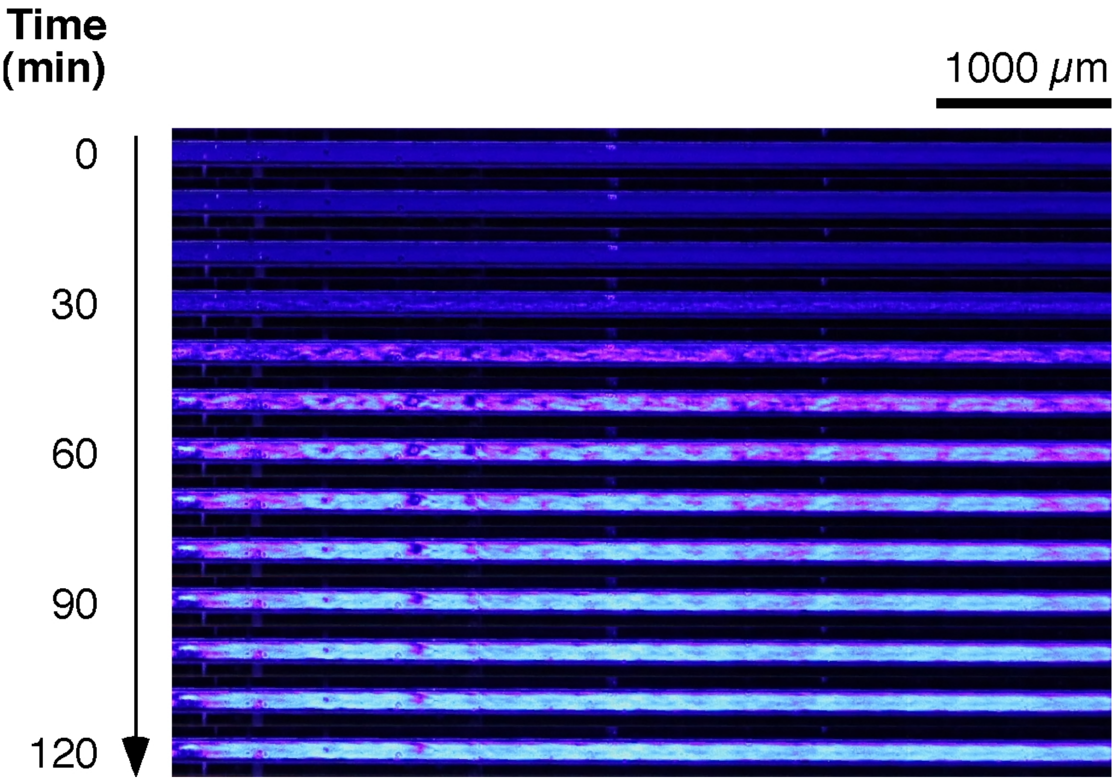

Figure S3. Observation of the MT formation in non-irradiated samples from non-conjugated tubulin solutions at 29 °C in a cylindrical glass capillary (I.D. = 140 μm) under cross-polarized light.

In case of non-conjugated tubulin, formation of MTs took more than 30 min. The gradually grown MTs formed small domains randomly.

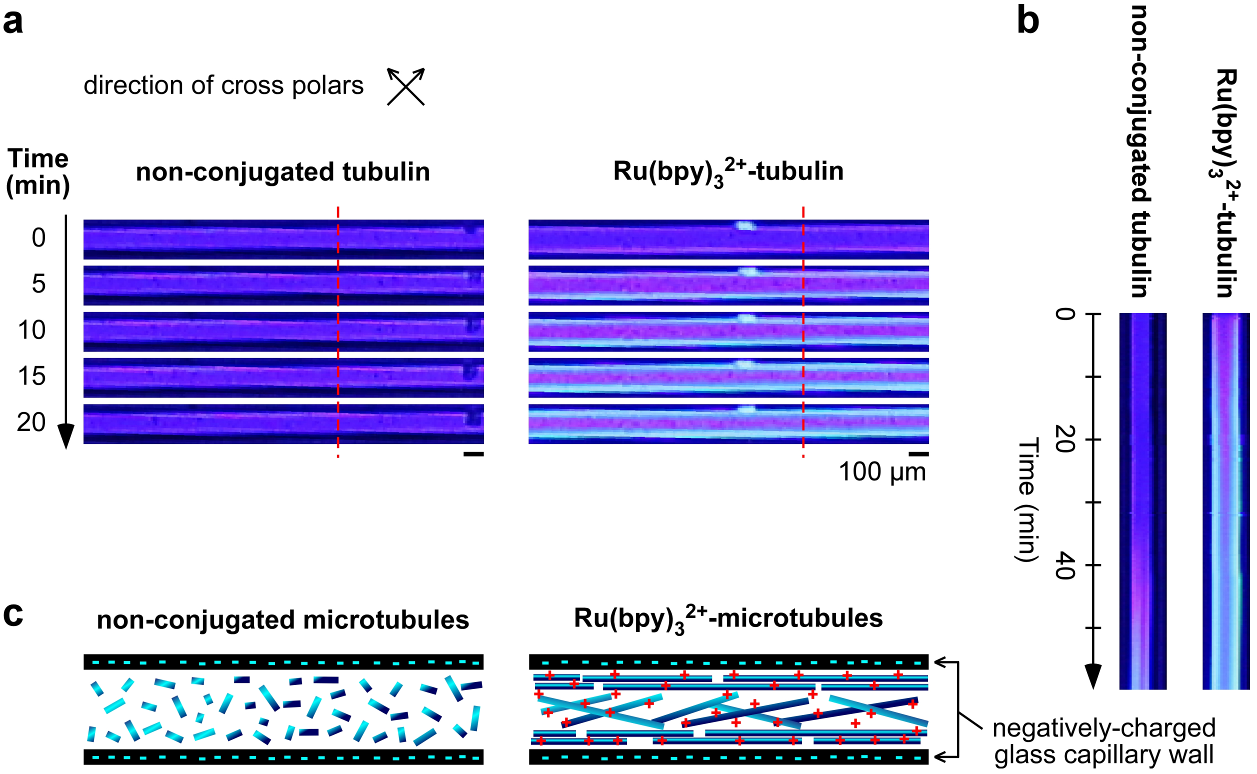

Figure S4. a) Observation of MT formation from tubulin solutions or Ru(bpy)$_3^{2+}$-tubulin solutions at 27°C in non-irradiated samples in a cylindrical glass capillary (I.D. = 140 µm) under cross-polarized light. [Tubulin] = 64 µM and [Ru(bpy)$_3^{2+}$] = 0 (left) and 22 (right) µM. b) The spatio-temporal analysis constructed from the sequential line-up of the dotted line in (a). c) Schematic illustration of MTs in a negatively charged glass capillary.

Figure S4a shows the color changes of the non-conjugated tubulin and Ru(bpy)$_3^{2+}$-tubulin samples under cross-polarized light at 27 °C in the non-irradiated state. In contrast to non-conjugated tubulin, Ru(bpy)$_3^{2+}$-tubulin clearly showed color changes from dark violet to bright aqua blue on the glass capillary wall and the color changed to pink on the central axis. In addition, the thickness of the layer with the changed color increased with time, suggesting that the MTs were formed from the wall to the central axis. Figure S4b shows the spatio-temporal analysis of the sequential images shown in Figure S4a. It is clear that the Ru(bpy)$_3^{2+}$-MT formation began from the wall and proceeded to the central axis. In contrast to non-conjugated tubulin, which showed no MT formation, MT formation on the wall from Ru(bpy)$_3^{2+}$-tubulin was complete within 40 min.

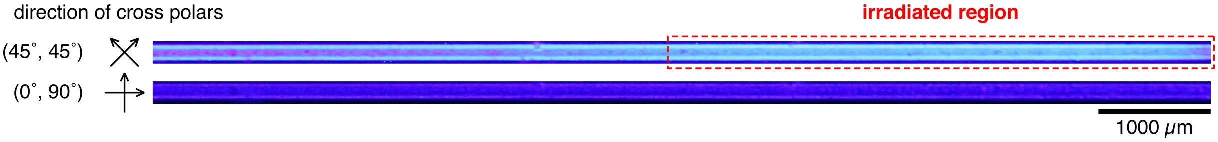

Figure S5. Cross-polarized light observation of $Ru(bpy)_3{}^{2+}$-MT at 240 min after partial photo-irradiation of the cylindrical glass capillary (I.D. = 140 μm).

The maximum orientation degree was at 45° to the cross polars, and the minimum orientation degree was at 0° and 90°. This result clearly shows that the MTs were oriented along the long axis of the glass capillary.

The orientations of the MTs both in the irradiated and non-irradiated regions were stable for more than 240 min.

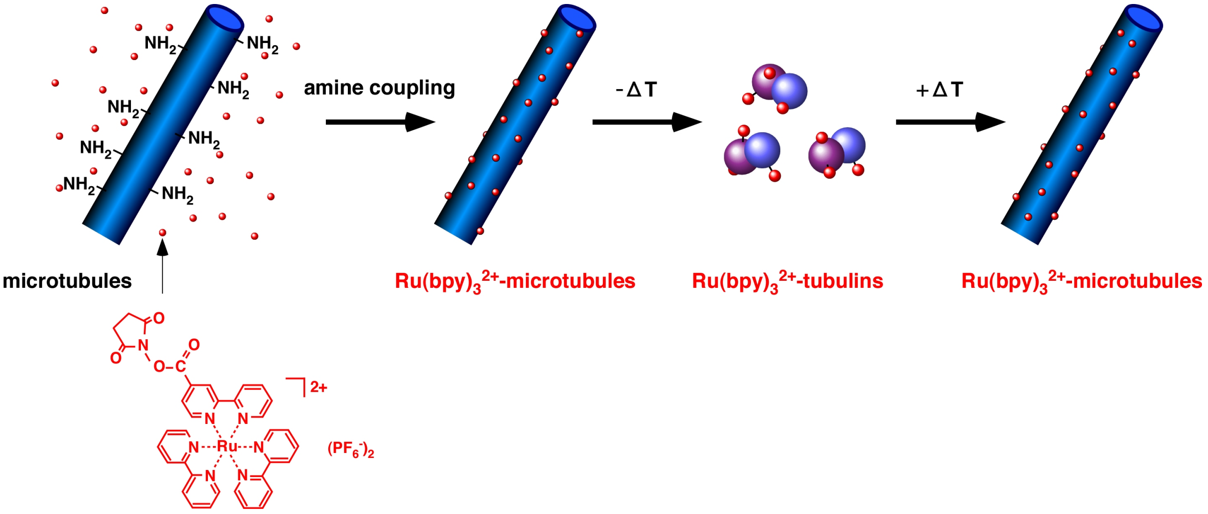

Figure S6. Preparation of Ru(bpy)$_3^{2+}$-conjugated tubulin by amine coupling.

Ru(bpy)$_3^{2+}$-conjugated tubulin was prepared by amine coupling of Ru(bpy)$_3^{2+}$-succinimidyl ester with primary amine groups on the MT surface.

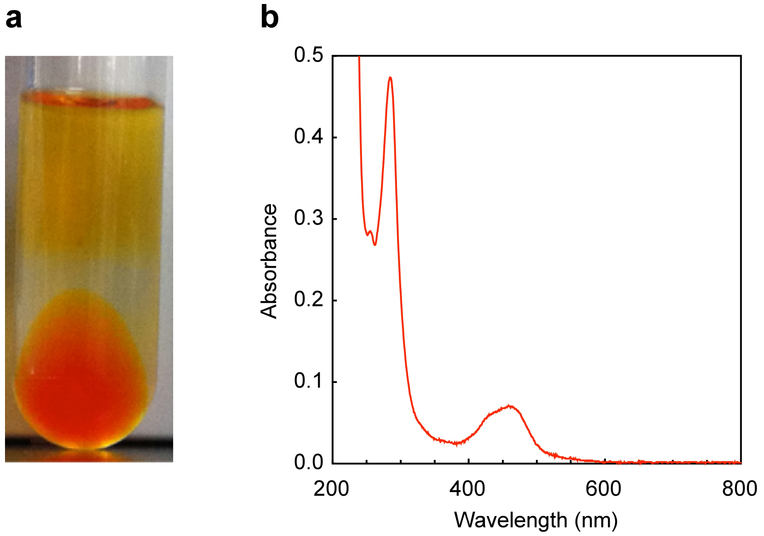

Figure S7. a) Purification of Ru(bpy)$_3^{2+}$-MTs by ultracentrifugation. b) Absorption spectrum of the Ru(bpy)$_3^{2+}$-conjugated tubulin.

The purification buffer contained the following reagents - [PIPES] = 80 mM, [$MgCl_2$] = 1 mM, [EGTA] = 1 mM, [GTP] = 5 mM, and glycerol = 60%. As shown in Figure S7a, the Ru(bpy)$_3^{2+}$-MTs were purified by ultracentrifugation and were recovered from the bottom of a centrifuge tube. The upper liquid phase contained free Ru(bpy)$_3^{2+}$ or free tubulin. After three cycles of purification, the absorbance of the cooled suspension was measured and the original peak from Ru(bpy)$_3^{2+}$ (~460 nm) was confirmed (Figure S7b). By measuring the absorbance of the diluted solution of Ru(bpy)$_3^{2+}$-tubulin stock solution, the Ru(bpy)$_3^{2+}$ concentration of each of the solutions was determined by calculating the dilution ratio.