Supporting Information

# Stabilization of Microtubules by Encapsulation of GFP Using Tau-Derived Peptide

Hiroshi Inaba,* Takahisa Yamamoto, Takashi Iwasaki, Arif Md. Rashedul Kabir, Akira Kakugo, Kazuki Sada and Kazunori Matsuura*

**Experimental Section**

**Equipment and materials.**

High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-6AD liquid chromatograph with GL Science Inertsil WP300 C18 columns (4.6 × 250 mm for analysis and 20 × 250 mm for purification). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were taken using a Bruker Daltonics Autoflex TII with α-cyano-4-hydroxycinnamic acid (α-CHCA) as a matrix. UV-vis spectra were obtained using a Jasco V-630. Fluorescence measurements were performed using a Jasco FP-8200. Ultracentrifugation was performed using an Optima MAX-TL ultracentrifuge (Beckman Coulter) using TLA 120.2 rotor. Transmission electron microscope (TEM) was measured with a Jeol JEM 1400 Plus with a grid (C-SMART Plus TEM grid, ALLIANCE Biosystems Inc., Osaka, Japan). CD spectra were recorded with a JASCO J-820 spectrophotometer using a 1 mm quartz cell. Confocal laser scanning microscopy (CLSM) measurement was carried out using a FluoView FV10i (Olympus). In the motility assay, samples were illuminated with a 100 W mercury lamp and visualized by using an epi-fluorescence microscope (Eclipse Ti; Nikon) using an oil-coupled Plan Apo 60 × 1.40 objective (Nikon). Tubulin was purified from porcine brain by a reported procedure.[1] Recombinant kinesin-1 consisting of the first 573 amino acid residues of human kinesin-1 was prepared according to a reported procedure.[2] The reagents used were purchased from Watanabe Chemical Ind., Ltd., Tokyo Chemical Industry Co., Dojindo Laboratories Co., Ltd. and Wako Pure Chemical Industries. All the chemicals were used without further purification.

**Synthesis of TP-GFP11 and GFP11 peptides.**

For TP-GFP11 peptide, H-Arg(Pbf)-Asp(OtBu)-His(Trt)-Met-Val-Leu-His(Trt)-Glu(OtBu)-Tyr(Boc)-Val-Asn(Trt)-Ala-Ala-Gly-Ile-Thr(tBu)-Gly-Gly-Gly-Ser(Trt)-Gly-Gly-Gly-Lys(Boc)-Lys(Boc)-His(Trt)-Val-Pro-Gly-Gly-Gly-Ser(Trt)-Val-Gln(Trt)-Ile-Val-Tyr(Boc)-Lys(Boc)-Pro-Val-Asp(OtBu)-Leu-Alko-PEG resin was synthesized on Fmoc-Leu-Alko-PEG resin (Watanabe Chemical Ind. Ltd) using standard Fmoc-based solid phase chemistry (4 equiv. Fmoc-amino acids). *N*-methylpyrrolidone (NMP) solution of 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)] methanaminium hexafluorophosphate (COMU, 4 equiv.) and diisopropylethylamine (DIPEA, 4 equiv.) were used as coupling reagents. Each condensation reaction was performed at room temperature for 90 min. Deprotection of Fmoc groups from the resin was performed using 40% and 20% piperidine in *N*,*N*-dimethylformamide (DMF). The peptidyl-resin was washed with NMP and $CH_2Cl_2$, and then dried under vacuum. The peptide was deprotected and cleaved from the resin by treatment with a cleavage cocktail (trifluoroacetic acid (TFA)/water/ethanedithiol/triisopropylsilane = 94/2.5/2.5/1, v/v/v/v). The mixture was kept at room temperature for 3 h. After filtration, the peptide was precipitated by adding ice-cooled *tert*-

butylmethylether. After centrifugation, the peptide was washed with *tert*-butylmethylether 3 times. The precipitated peptide was dried under vacuum. The crude product was purified by RP-HPLC with water/acetonitrile (both containing 0.1% TFA, 80/20 to 60/40, v/v for 100 min, linear gradient, 10 mL/min, detected at 220 nm). The isolated yield was 56%. MALDI-TOF-MS: *m/z* found: 4256 ($[M+H]^+$), calcd. 4260 (Fig. S1a).

GFP11 peptide (H-Arg-Asp-His-Met-Val-Leu-His-Glu-Tyr-Val-Asn-Ala-Ala-Gly-Ile-Thr-OH) was prepared by the same procedure described above using Fmoc-Thr(tBu)-Alko-PEG resin. The isolated yield was 45%. MALDI-TOF-MS: *m/z* found: 1827 ($[M+H]^+$), calcd. 1827 (Fig. S1b).

**Design of GFP1–10.**

For expression of GFP1–10 in *E. coli* as a soluble fraction, GFP1–10 was fused with a soluble maltose-binding protein (MBP) connected by a cleavable linker by a TEV protease (Fig. S2). The MBP was removed by the treatment with a TEV protease and the resulting GFP1–10 was purified as shown below.

**Construction of MBP-GFP1–10 plasmid.**

A nucleotide fragment of GFP1–10 was amplified using standard PCR methods from the synthesized nucleotide fragment (Thermos Fisher). The GFP1–10 fragment was cloned into the pMal-c2X vector (New England Biolabs) with the MBP located at the N-terminus, followed by a TEV protease cleavage site, using the In-Fusion HD cloning kit (TaKaRa Bio).

**Expression and purification of GFP1–10.**

The pMal-c2X vector coding GFP1–10 was transformed into *E. coli* strains BL21(DE3). Bacterial cells were spread on LBA agar containing 100 μg/mL of ampicillin and grown overnight at 37°C. A single transformant colony was grown in LBA medium at 37°C overnight. The culture was diluted 100-fold by addition to fresh LBA medium and grown to an OD 600 nm of 0.5, and then the culture was incubated with 0.1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG) at 37°C. After 3 h of incubation, cells were harvested by centrifugation at 8000 rpm for 10 min. The cell pellets were suspended in Ni-affinity binding buffer (20 mM Tris-HCl pH 7.4, 500 mM NaCl, 20 mM imidazole, 1% Triton X-100) on ice. The cells were lysed by sonication. After centrifugation at 13000 rpm for 10 min, the supernatant was loaded onto 1 mL Ni-affinity column (GE healthcare). After washing with the same buffer, the protein was eluted from the column by using the Ni-affinity elution buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 500 mM imidazole, 0.1% Triton X-100). The eluted sample was loaded onto 1 mL MBP Trap column (GE healthcare). After washing with the MBP Trap binding buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 0.5 mM EDTA), the protein was eluted from the column by using the MBP Trap elution buffer (20 mM Tris-HCl pH 7.4, 200 mM NaCl, 0.5 mM EDTA, 10 mM maltose). TEV protease was added to the eluted sample as the absorbance ratio of the eluted sample and TEV protease at 280 nm

was 5 to 1. DTT (1 mM) was added to the mixture and incubated at 25°C overnight. Then GFP1–10 was purified by Ni-affinity column as above by using the Ni-affinity binding and elution buffers without Triton X-100. The purity of GFP1–10 was evaluated by SDS-PAGE. The concentration of GFP1–10 was determined by the Bradford method.

**Estimation of binding affinity of TP-GFP11 peptide to GFP1–10.**

Aqueous solution containing 2 μM GFP1–10 and 0, 2, 10, 20, and 50 μM TP-GFP11 or GFP11 peptide (hereafter simply called the Peptide) in 20 mM Tris-HCl buffer pH 7.4, 200 mM NaCl was incubated at 25°C for 5 h in the dark. Then the fluorescence spectra of the resultant solution were measured at 25°C by excitation at 480 nm. From the fluorescence intensity at 510 nm derived from the reassembled GFP, $\Delta I = I - I_0$, was calculated, where $I$ is the fluorescence intensity of the solution in the presence of each concentration of the Peptide, and $I_0$ is the fluorescence intensity in the absence of the Peptide. $\Delta I$ was plotted as a function of the concentration of the Peptide, and the $K_d$ and $\Delta I_{max}$ were determined by fitting to a quadratic binding function to equation **(1)** using Excel and Solver, where $\Delta I_{max}$ is a saturated fluorescence difference.

$$\Delta I = \Delta I_{max} \frac{[\mathrm{GFP1{-}10}] + [\mathrm{Peptide}] + K_d - \sqrt{([\mathrm{GFP1{-}10}] + [\mathrm{Peptide}] + K_d)^2 - 4[\mathrm{GFP1{-}10}][\mathrm{Peptide}]}}{2[\mathrm{GFP1{-}10}]} \quad \textbf{(1)}$$

where [GFP1–10] is initial concentration of GFP1–10 (2 μM) and [Peptide] is initial concentration of TP-GFP11 or GFP11 peptide (0–50 μM).

**Preparation of tetramethylrhodamine (TMR)-labeled tubulin (tubulin-TMR).**

TMR-labeled tubulin (tubulin-TMR) was prepared using 5-Carboxytetramethylrhodamine succinimidyl ester according to the standard procedure.[3] The labeling ratio of TMR-modified tubulin was determined by measuring the absorbance of the protein and TMR at 280 and 555 nm, respectively.

**CLSM measurements.**

The glass bottom dishes (Matsunami, Osaka, Japan) were coated by 1 mg/mL poly-L-lysine (Mw: 30000–70000, Sigma) at room temperature for 1 h, then removed and dried. The MT samples were put on the plate and kept at room temperature for 0.5–1 h, then observed by CLSM. Tubulin-TMR was excited with 550 nm and observed through a 574 nm emission band-pass filter (Red). GFP was excited with 489 nm and observed through a 510 nm emission band-pass filter (Green). ATTO 647N-labeled anti-GFP single domain antibody (Synaptic Systems GmbH) was excited with 647 nm and observed through a 664 nm emission band-pass filter (Magenta). TMR, GFP, and ATTO 647N fluorescence intensity per MT were measured from the fluorescence images by subtracting the background intensity using ImageJ software. The background-subtracted ATTO 647N fluorescence intensity per GFP

fluorescence intensity and GFP fluorescence intensity per TMR fluorescence intensity for each MT (*N* = 20) were calculated at least from 6 images.

**Construction of TP-GFP-bound MTs.**

Typically, **TP-GFP** was prepared by incubating GFP1–10 (25 μM) and 5 equivalents of TP-GFP 11 peptide (125 μM) in 20 mM Tris-HCl buffer pH 7.4, 200 mM NaCl at 25°C for 5 h in the dark. The mixture was used as 25 μM **TP-GFP**. Reassembled GFP without **TP** was prepared by the same method using GFP11 instead of TP-GFP11 peptide. In the typical "Before" method, **TP-GFP** solution (3 μL) was added to a tubulin solution (5 μL) in BRB80 buffer (80 mM PIPES pH 6.9, 1.0 mM $MgCl_2$, 1.0 mM EGTA). The mixture was kept at 25°C for 30 min in the dark. Then 2 μL of GMPCPP premix (1 mM GMPCPP, 80 mM PIPES pH 6.9, 21 mM $MgCl_2$, 1.0 mM EGTA) was added to the mixture and kept at 37°C for 30 min in the dark for polymerization (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM). In the typical "After" method, GMPCPP premix (2 μL) was added to a tubulin solution (5 μL) in BRB80 buffer. The mixture was kept at 37°C for 30 min in the dark for polymerization. Then **TP-GFP** (3 μL) was added to the mixture and kept at 25°C for 30 min in the dark (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM). In both methods, tubulin and tubulin-TMR were used as 4 : 1 ratio and total concentration of 2.5 μM for monitoring MTs.

**TEM measurement.**

**TP-GFP**-MTs were prepared by the "Before" method as above. The solution (5 μL) was put on a positively-charged C-SMART Plus TEM grid (ALLIANCE Biosystems Inc.), allowed to stand for 1 min, and then removed. The grid was exposed to 2% $Gd(CH_3CO_2)_3$ $(H_2O)_n$ aqueous solution (5 μL) for staining, which was allowed to stand for 1 min, and then removed. The resulting grid was dried in vacuo and observed by TEM using an accelerating voltage of 80 kV.

**Evaluation of binding of TP-GFP on MTs.**

Treatment of anti-GFP antibody

**TP-GFP**-bound MTs were prepared by the "Before" or "After" method as above. Then 5 μM ATTO 647N-labeled anti-GFP single domain antibody (2 μL, Nanotag Biotechnologies) was added to the **TP-GFP**-bound MT solution (8 μL) and incubated at 25°C for 1 h in the dark (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM, [Anti-GFP antibody] = 1 μM). The mixture was used for CLSM imaging.

Treatment of anti-tubulin antibody

**TP-GFP**-bound MTs were prepared by the "Before" or "After" method as above. Then 0.5 mg/mL Anti-β-tubulin, monoclonal antibody (3 μL, Wako) was added to the **TP-GFP**-bound MT solution (7 μL)

and incubated at 25°C for 1 h in the dark (Final concentrations: [Tubulin] = 2 μM, [Tubulin-TMR] = 0.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM, [Anti-tubulin antibody] = 0.15 mg/mL). The mixture was used for CLSM imaging.

**Evaluation of amount of bound TP-GFP on MTs.**

**TP-GFP**-bound MTs were prepared by the "Before" method as above (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 1, 5, 10 μM, [TP-GFP11 peptide] = 5, 25, 50 μM (5 equivalents to GFP1–10)). After ultracentrifugation of **TP-GFP**-bound MTs and free **TP-GFP** at 50000 rpm at 37°C for 5 min, the supernatant was collected and diluted 17-fold by BRB80 buffer. Then the fluorescence spectra of the solution were measured at 25°C by excitation at 480 nm. Concentration of **TP-GFP** bound on MTs was estimated according to equation **(2)**.

$$[\text{TP–GFP}_{\text{MT}}] = [\text{TP–GFP}]\,\frac{I_{\text{free}} - I_{\text{MT}}}{I_{\text{free}}} \qquad \textbf{(2)}$$

where [TP-GFP$_{MT}$] is concentration of **TP-GFP** bound on MTs, [TP-GFP] is initial concentration of **TP-GFP**, $I_{free}$ and $I_{MT}$ are the fluorescence intensity of supernatant after centrifugation of free **TP-GFP** and **TP-GFP**-bound MTs, respectively. Amount of **TP-GFP** bound on MTs per tubulin (Fig. S9d) was calculated by using the concentration **TP-GFP** bound on MTs and tubulin (2.5 μM). The concentration of **TP-GFP** indicates the concentration of GFP1–10 with 5 equivalents of TP-GFP11 peptide. Since it is estimated that the binding of **TP-GFP** to the inside of MTs is stronger than the binding to the exterior, **TP-GFP** selectively binds to the inside at low concentration of **TP-GFP**. By increasing the concentration, **TP-GFP** also binds to the exterior in addition to the inside.

**Motility assay.**

**TP-GFP**-encapsulated MTs were prepared by the "Before" method as above (Final concentrations: [Tubulin] = 2 μM, [Tubulin-TMR] = 0.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM). The concentration of **TP-GFP** was changed according to experimental conditions. The motility assay was performed in a flow cell with dimensions of 5.0 × 5.0 × 0.15 mm$^3$ ($w \times l \times h$), which was assembled from two cover glasses of sizes (5 × 7) mm$^2$ and (40 × 50) mm$^2$ (MATSUNAMI), and a double-sided tape was used as the spacer.[4] Firstly, 0.5 mg/mL Casein in BRB80 buffer was introduced unto the flow cells and incubated for 3 min. Then the solution was exchanged with Wash buffer (0.5 mg/mL Casein, 4.5 mg/mL D-Glucose, 50 U/mL Glucose oxidase, 50 U/mL Catalase, 1.0 mM DTT, 1.0 mM $MgCl_2$ in BRB80 buffer) containing 0.6 μM kinesin and incubated for 3 min. After washing with Wash buffer, the solution was exchanged with MT solution and incubated for 3 min. After washing with Wash buffer, the solution was exchanged with Wash buffer containing 5.0 mM ATP and 1.0 mM Trolox. Then the motility of MTs was imaged. All the experiments were performed at room temperature.

**Image analysis for motility assay.**

Movies of the motility assays of MTs and images obtained by the fluorescence microscopy were analyzed to determine the velocity, end-to-end length and contour length of each MT by using the image analysis software, ImageJ. In order to determine persistence length ($L_p$), both the contour length along each MT and the end-to-end distance of the same MT were measured. $L_p$ was determined by fitting the data to equation **(3)** using Excel and Solver.

$$\langle R^2 \rangle = 2L_p^2 \left[ \frac{L}{L_p} - 1 + \exp\left( -\frac{L}{L_p} \right) \right] \qquad \textbf{(3)}$$

where $<R^2>$ is the mean squared end-to-end distance and $L$ is the contour length.[5]

**Turbidity measurement.**

**TP-GFP** was prepared by incubating GFP1–10 (25 μM) and 5 equivalents of TP-GFP11 peptide (125 μM) in 20 mM Tris-HCl buffer pH 7.4, 200 mM NaCl at 25°C for 5 h in the dark. **TP-GFP** and tubulin in BRB80 buffer were mixed and preincubated at 37°C, then turbidity experiments were performed by adding GTP premix (5 mM GTP, 20 mM $MgCl_2$ in BRB80 buffer, 25% DMSO, 20 μL) to the mixture (80 μL) at 37°C (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM, [GTP] = 1 mM). As a control, taxol and TP-GFP11 peptide (Final concentration: 25 μM) were added instead of **TP-GFP**. Optical density at 350 nm was monitored with a UV-Vis spectrometer for 60 min at 1 min intervals. After 60 min measurements, the samples were cooled at 4°C for 15 min and the optical density was measured again. Since **TP-GFP** has absorbance at 350 nm, the absorbance was subtracted in the turbidity assay when **TP-GFP** was used. The average of two independent measurements is shown in Fig. 4.

**Evaluation of thermal stability of TP-GFP-MTs.**

**TP-GFP**-encapsulated MTs were prepared by the "Before" method as above (Final concentrations: [Tubulin] = 2.5 μM, [GFP 1-10] = 5 μM, [**TP-GFP 11**] = 25 μM). After ultracentrifugation at 50000 rpm at 37°C for 5 min, the supernatant was removed and the resulting pellets were suspended in the BRB80 buffer. The samples were incubated at 25, 40, 50, and 60°C for 1 day. The solution was cooled to 25°C and used for CLSM imaging. Since **TP-GFP** encapsulated in MTs formed aggregates at 50–60°C (Fig. S12), it is estimated that **TP-GFP**-encapsulated MTs were not stable at the high temperature. The results are in good agreement with the previous report that MTs are not stable at 55°C.[6] Thus, the thermal stability of TP-GFP-encapsulated MTs is estimated to be similar to the unbound MTs.

**CD measurement.**

**TP-GFP** solution was added to a tubulin solution in BRB80 buffer and the mixture was kept at 25°C for 30 min in the dark (Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11

peptide] = 25 μM). CD spectrum of the complex of tubulin and **TP-GFP** was recorded at 25°C. Only **TP-GFP** and only tubulin were used as controls at the same condition. A difference CD spectrum subtracting tubulin and **TP-GFP** from a complex of tubulin and **TP-GFP** showed weak intensity without significant peak (Fig. S13), indicating that the secondary structures of tubulin and **TP-GFP** were minimally affected by their binding.

**Molecular modeling.**

Molecular mechanics calculations were performed using MacroModel 10.4 (Schrödinger, Inc., New York, NY) using optimized potentials for liquid simulations (OPLS) 2005 force field with default setting. As ligands, **TP-GFP** was prepared as below. The model structure of TP-GFP11 peptide was prepared manually by connecting GFP11 structure extracted from superfolder GFP (PDB ID: 2B3P)[7] and **TP** structure[8] by a linker (GGGS). The linker moiety of TP-GFP11 was energy-minimized using Maestro interface ver. 10.4 (Schrödinger). Then TP-GFP11 was put to GFP1–10 extracted from superfolder GFP (PDB ID: 2B3P)[7] and energy-minimized. The complex of TP-GFP11 and GFP1–10 was used as **TP-GFP**. The structure of three adjacent tubulins of GMPCPP-stabilized MT (PDB ID: 3J6E)[9] was used for molecular modeling and ligand docking. Addition of missing hydrogen atoms to the model was carried out based on an explicit all atom model. **TP-GFP** was put to the taxol-binding pocket of central β-tubulin of the three adjacent tubulins. **TP-GFP** with the surrounding residues around 10.0 Å were energy-minimized. One of the energy-minimized structures was shown in Fig. S14.

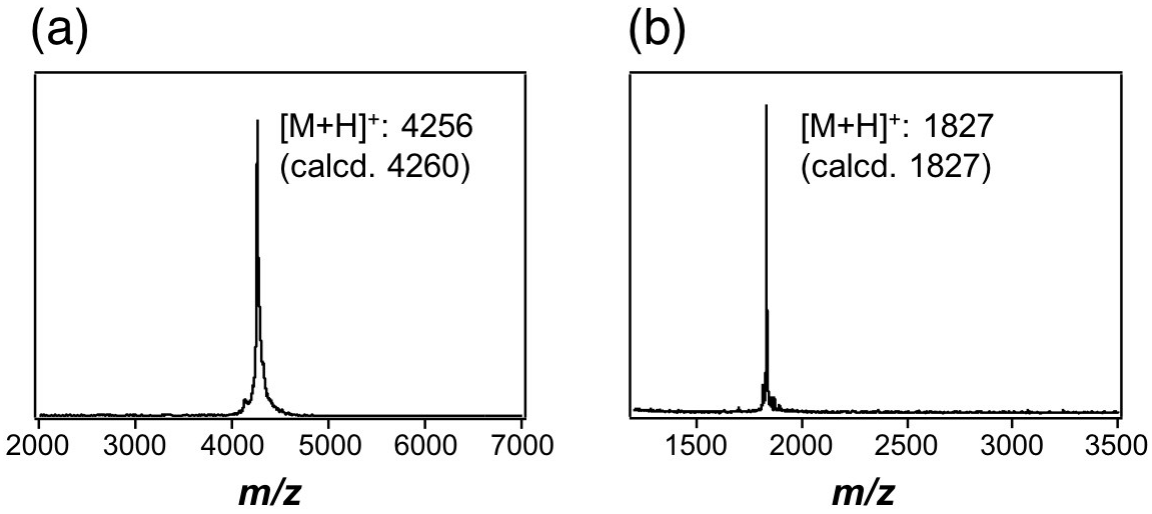



**Figure S1.** MALDI-TOF-MS of (a) TP-GFP11 and (b) GFP11 peptides.

[MBP]
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAH
DRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTW
EEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLT
FLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKP
FVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATME
NAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLG

[TEV cleavage site]
IDTTENLYFQG

[GFP1–10]
MHHHHHHGSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATIGKLTLKFICTTGKLPVP
WPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGKYKTRAVVKFEGDTL
VNRIELKGTDFKEDGNILGHKLEYNFNSHNVYITADKQKNGIKANFTVRHNVEDGSVQLADHYQ
QNTPIGDGPVLLPDNHYLSTQTVLSKDPNEK

**Figure S2.** Amino acid sequence of MBP-GFP1–10. The black arrow indicates the position of cleavage by TEV protease.

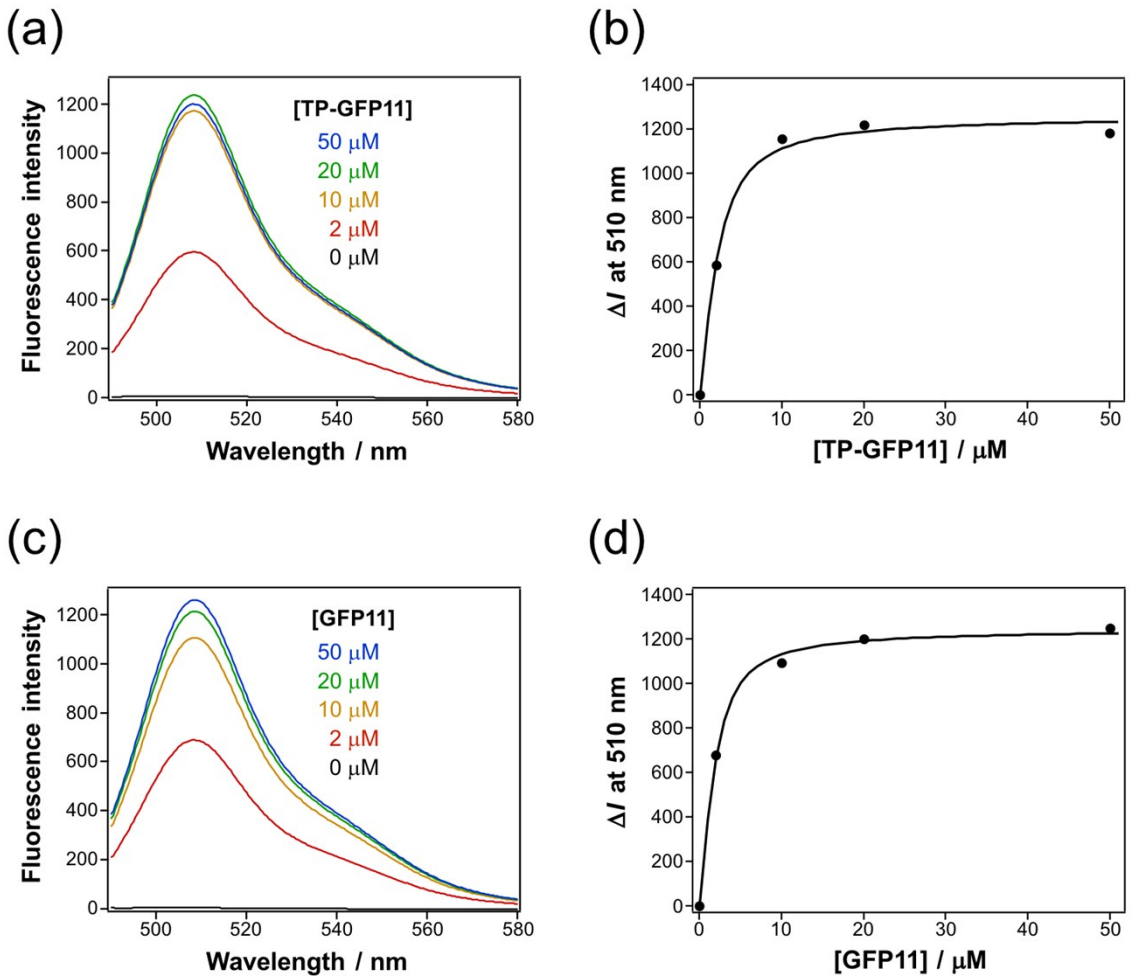



**Figure S3.** Fluorescence spectra of 2 μM GFP1–10 after incubation with 0-50 μM (a) TP-GFP11 and (c) GFP11 peptides in 20 mM Tris-HCl buffer pH 7.4, 200 mM NaCl at 25°C for 5 h. Excitation at 480 nm. Binding parameters of (b) TP-GFP11 and (d) GFP11 peptides to GFP1–10 from (a) and (c), respectively. Closed circles are experimental values and the solid lines are the theoretical curves obtained by fitting $\Delta I_{max}$ at 510 nm and $K_d$ using equation **(1)**.

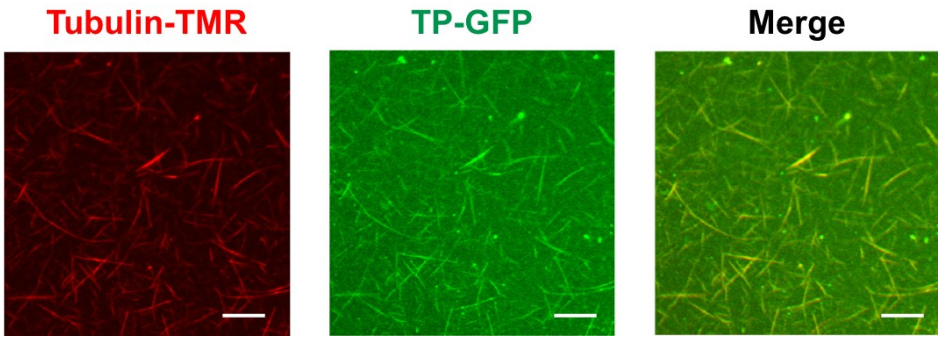



**Figure S4.** CLSM images of MTs incubated with **TP-GFP** by the "After" method (scale bar: 10 μm). Final concentrations: [Tubulin] = 2.0 μM, [Tubulin-TMR] = 0.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM.

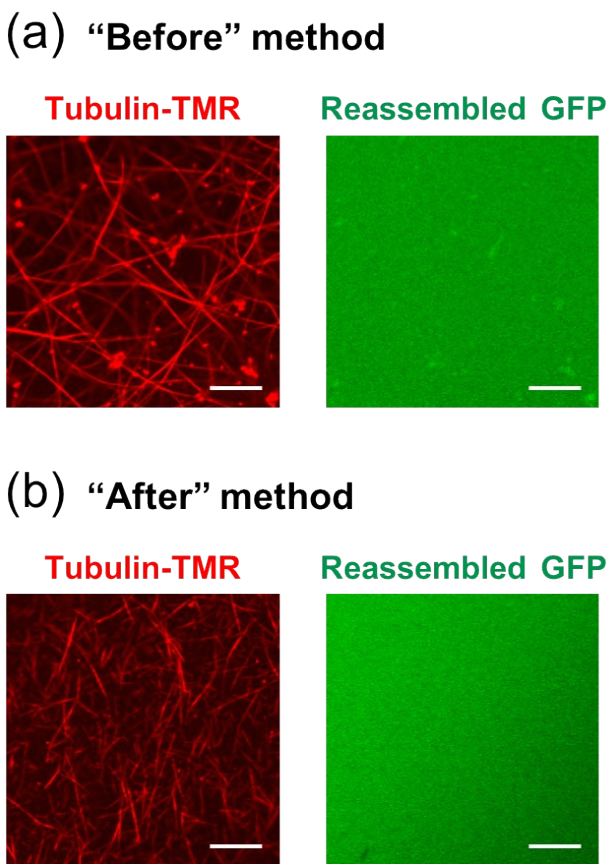



**Figure S5.** CLSM images of MTs incubated with reassembled GFP consisting of GFP1–10 and GFP11 peptide by the (a) "Before" and (b) "After" methods (scale bar: 10 μm). No binding of reassembled GFP to MTs was observed. Final concentrations: [Tubulin] = 4 μM, [Tubulin-TMR] = 1.0 μM, [GFP1–10] = 10 μM, [GFP11 peptide] = 50 μM.

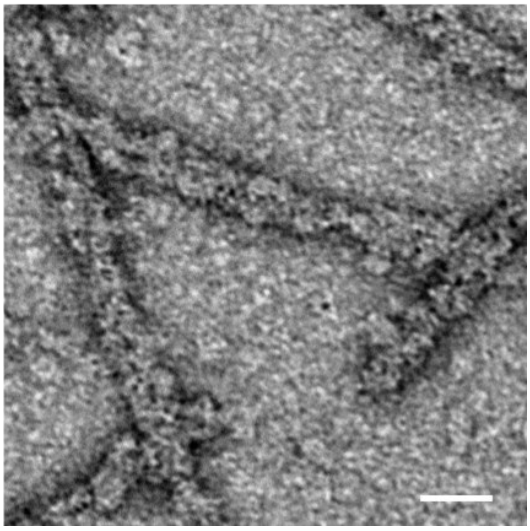


**Figure S6.** A transmission electron microscopy (TEM) image of **TP-GFP**-MTs (scale bar: 50 nm).

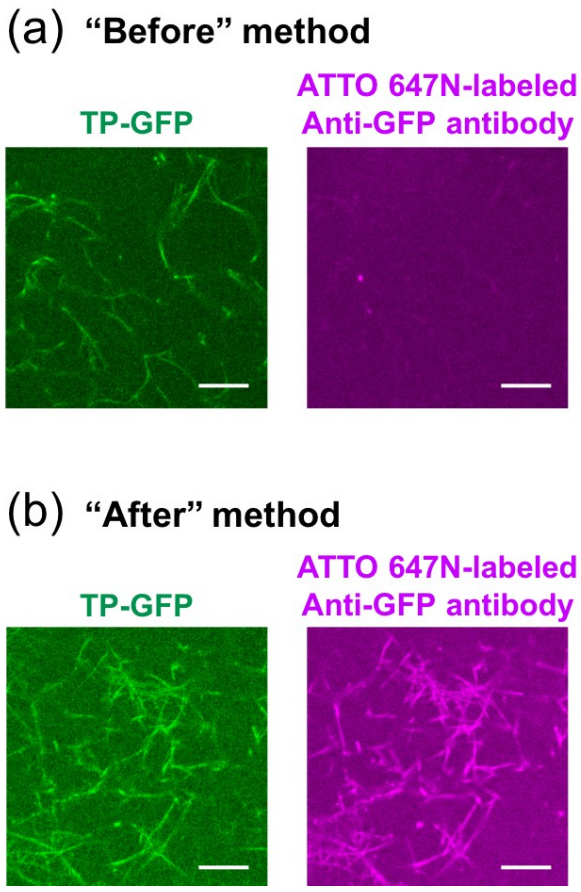



**Figure S7.** Binding of the ATTO 647N-labeled anti-GFP antibody to **TP-GFP**-bound MTs prepared by the (a) "Before" and (b) "After" methods (scale bar: 10 μm). Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM, and [anti-GFP antibody] = 1 μM.

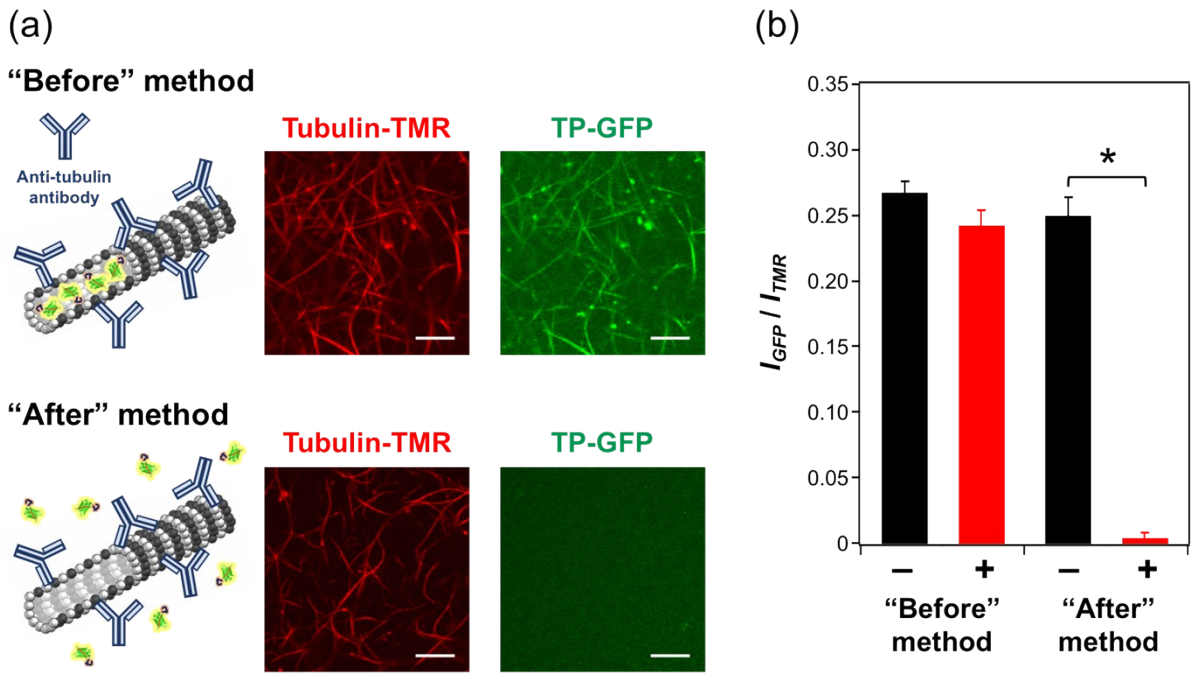
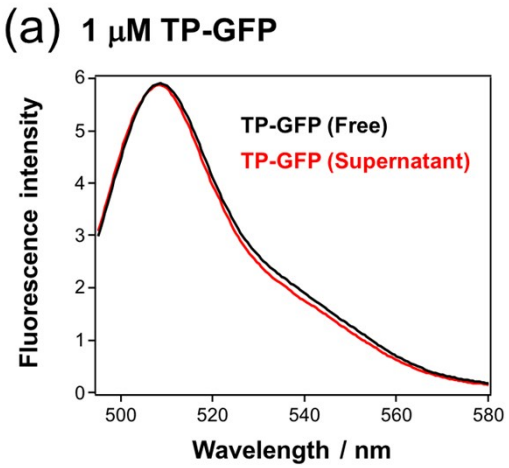
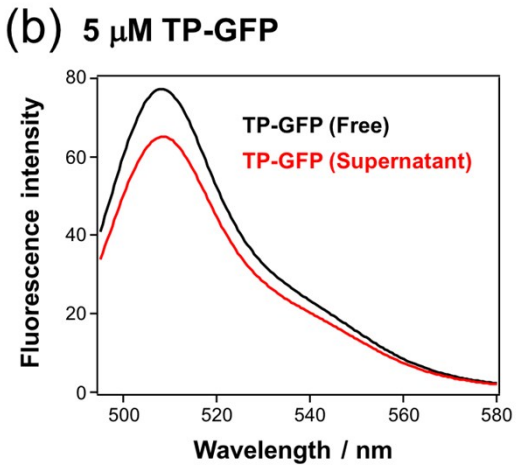
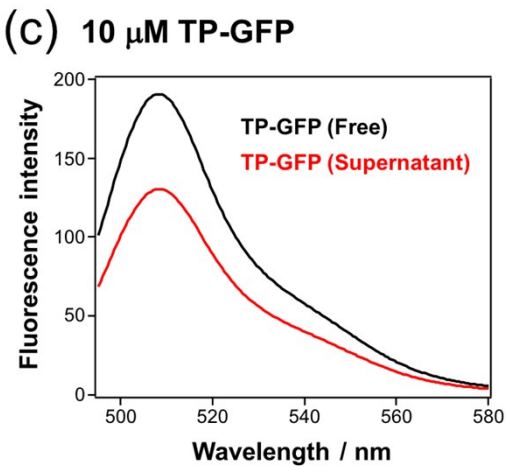



**Figure S8.** Effect of the anti-tubulin antibody for binding of **TP-GFP** to MTs. (a) CLSM images of **TP-GFP**-bound MTs prepared by the "Before" and "After" methods with treatment of the anti-tubulin antibody (scale bar: 10 μm). Final concentrations: [Tubulin] = 2 μM, [Tubulin-TMR] = 0.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM, and [anti-tubulin antibody] = 0.15 mg/mL. (b) $I_{GFP}/I_{TMR}$ of each **TP-GFP**-bound MT in the absence (black bar) and presence (red bar) of the anti-tubulin antibody, analyzed from CLSM images. Error bars represent the standard error of the mean ($N = 20$). *$P < 0.01$, t-test.




(d)

| [TP-GFP] (μM) | Bound TP-GFP/tubulin |
|---|---|
| 1 | 0.0034 |
| 5 | 0.31 |
| 10 | 1.26 |

**Figure S9.** Evaluation of amount of **TP-GFP** bound to MTs. Fluorescence spectra of supernatant of (a) 1 μM, (b) 5 μM, (c) 10 μM **TP-GFP** upon ultracentrifugation in the absence of MTs (black) or after conjugation with MTs by the "Before" method. (d) Table of **TP-GFP** bound on MTs per tubulin calculated from the fluorescence difference in (a)-(c) and concentrations of **TP-GFP** and tubulin. The concentration of **TP-GFP** indicates the concentration of GFP1–10 with 5 equivalents of TP-GFP11 peptide.

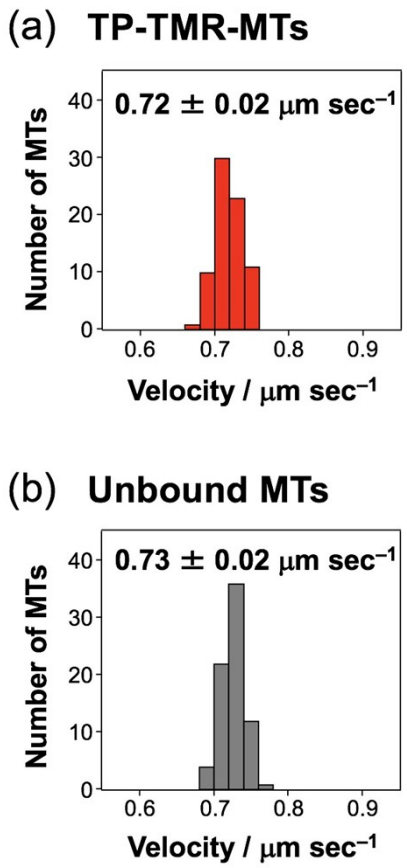



**Figure S10.** Distribution of velocity ($N$ = 75) of **TP-TMR**-encapsulated Alexa488-labeled MTs (2.5 μM) prepared by the "Before" method using (a) 25 μM **TP-TMR** and (b) unbound MTs on kinesin-coated substrates.

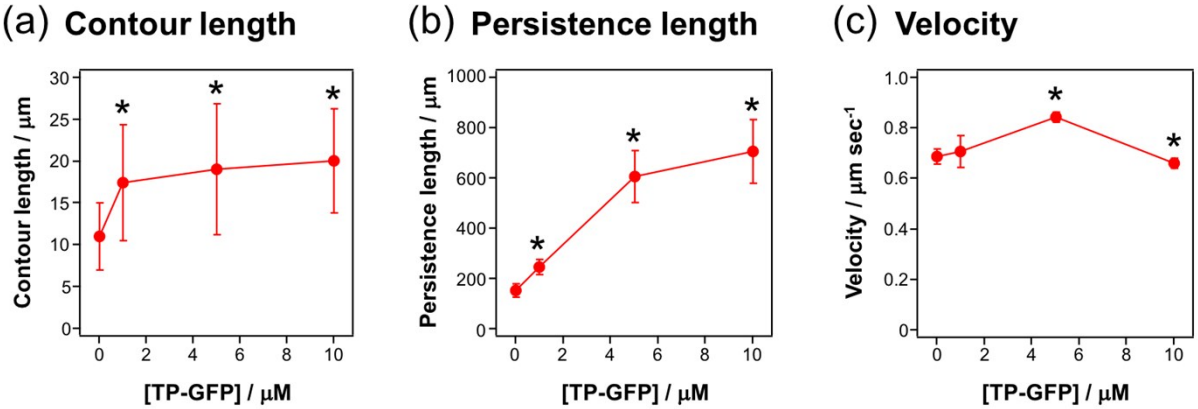



**Figure S11.** Concentration dependence of **TP-GFP** on the (a) contour length, (b) persistence length, and (c) velocity of **TP-GFP**-encapsulated MTs. *$P$ < 0.01 compared to unbound MTs, t-test. [**TP-GFP**] indicates the concentration of GFP1–10 containing 5 equivalents of TP-GFP11 peptide.

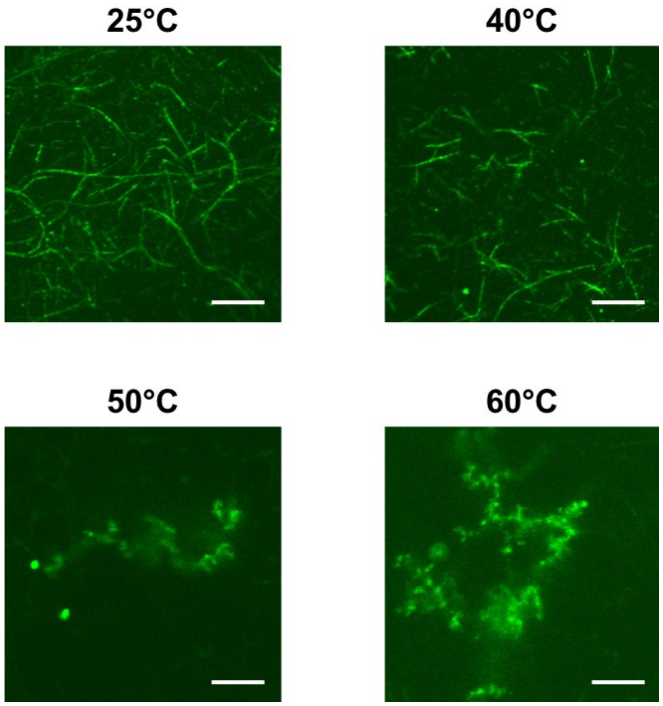



**Figure S12.** CLSM images of **TP-GFP**-MTs incubated at various temperature for 1 day (scale bar: 10 μm).

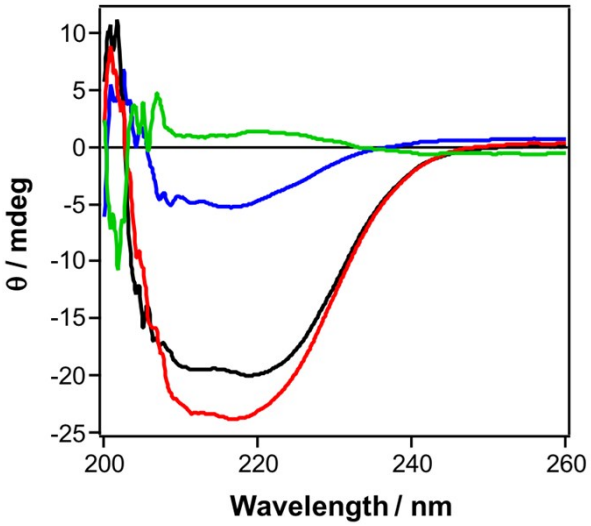



**Figure S13.** Circular dichroism (CD) spectra of tubulin (black), **TP-GFP** (blue), a complex of tubulin and **TP-GFP** (red), and a difference spectrum subtracting tubulin and **TP-GFP** from a complex of tubulin and **TP-GFP** (green). Final concentrations: [Tubulin] = 2.5 μM, [GFP1–10] = 5 μM, [TP-GFP11 peptide] = 25 μM. Each spectrum represents the average of 8 scans.

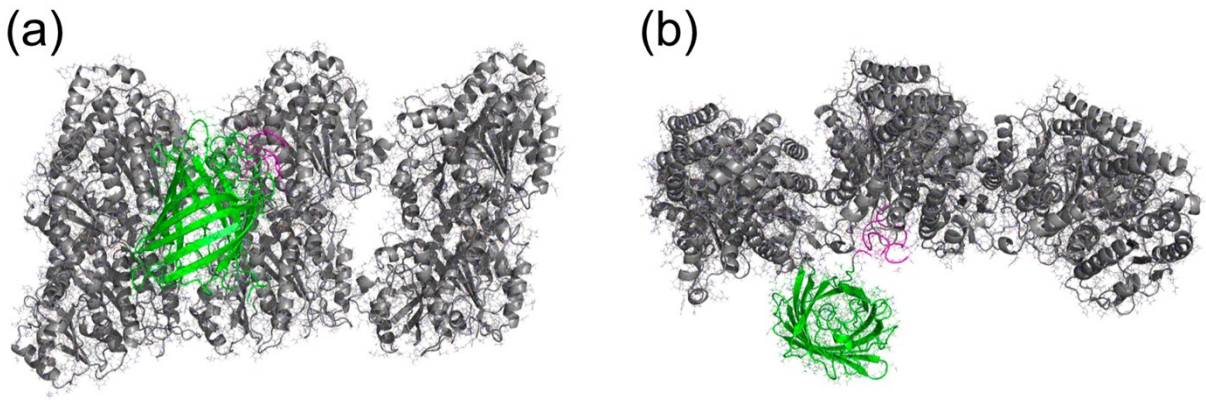



**Figure S14.** (a) Side and (b) top view of model of the binding of **TP-GFP** (magenta for **TP** moiety and green for GFP moiety) to the inside of MT (black), obtained by molecular mechanics (MM) calculations.

**Movie S1.** A movie that illustrates motility of **TP-GFP**-MTs and unbound MTs. Scale bar: 10 μm. The movie is 100 times faster than the original speed.

**References**


1 M. Castoldi and A. V. Popov, *Protein Expr. Purif.*, 2003, **32**, 83.

2 R. B. Case, D. W. Pierce, N. Hom-Booher, C. L. Hart and R. D. Vale, *Cell*, 1997, **90**, 959.

3 J. Peloquin, Y. Komarova and G. Borisy, *Nat. Methods*, 2005, **2**, 299.

4 A. Saito, T. I. Farhana, A. M. R. Kabir, D. Inoue, A. Konagaya, K. Sada and A. Kakugo, *RSC Adv.*, 2017, **7**, 13191.

5 Y. Arii and K. Hatori, *Biochem. Biophys. Res. Commun.*, 2008, **371**, 772.

6 G. D. Bachand, R. Jain, R. Ko, N. F. Bouxsein and V. VanDelinder, *Biomacromolecules*, 2018, **19**, 2401.

7 J. D. Pédelacq, S. Cabantous, T. Tran, T. C. Terwilliger and G. S. Waldo, *Nat. Biotechnol.*, 2006, **24**, 79.

8 H. Inaba, T. Yamamoto, A. M. R. Kabir, A. Kakugo, K. Sada and K. Matsuura, *Chem. Eur. J.*, 2018, **24**, 14958.

9 G. M. Alushin, G. C. Lander, E. H. Kellogg, R. Zhang, D. Baker and E. Nogales, *Cell*, 2014, **157**, 1117.