Supporting Information

Light-induced stabilization of microtubules by photo-crosslinking of Tau-derived peptide

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Supporting Text

Molecular mechanics calculation

The binding of **DA-TP-TMR** to tubulin was modeled by molecular mechanics calculation using a MacroModel module (Schrödinger, Inc., New York, NY). **DA-TP-TMR** was put to the Taxol-binding pocket of β-tubulin instead of Taxol, and the energy-minimized structure of **DA-TP-TMR** binding to tubulin was obtained as we have reported previously.[1,2] It was estimated that the DA moiety of **DA-TP-TMR** was close to 278R and 284R of β-tubulin at a distance of 11.9 Å and 17.6 Å, respectively (Fig. S5). Because DA is known to react with cationic amino acids,[3] it is expected that these residues have high possibility to react with DA of **DA-TP-TMR**.

Analysis of control peptides

We prepared two peptides as controls of **DA-TP**. One is a peptide in which the 14th valine from the N-terminus of the **TP** sequence (CGGGKKHVPGGGS**V**QIVYKPVDL) was substituted by a phenylalanine with a diazirine moiety (Tdf) (**DA(F)-TP**). Since the 14th valine of **TP** was estimated to be located inside the hydrophobic pocket from molecular mechanics calculation,[1] we evaluated the effect of the position of the diazirine on the binding to microtubules (MTs). The other is an actin-binding peptide Lifeact (MGVADLIKKFESISKEE)[4] modified with diazirine at the N-terminus (**DA-Lifeact**) as a control of **TP**. **DA(F)-TP** and **DA-Lifeact** were synthesized and labeled with TMR (**DA(F)-TP-TMR** and **DA-Lifeact-TMR**) by the same procedure for **DA-TP** and **DA-TP-TMR** (Fig. S1b, S1c, S1e, S1f, S2b, S2c, S2e, S2f). We observed the binding of **DA(F)-TP-TMR** and **DA-Lifeact-TMR** to MTs, but differently from **DA-TP-TMR**, the addition of Taxol did not decrease the TMR fluorescence (Fig. S11). The results indicate that the binding sites of these two peptides were not the Taxol-binding pocket. We also evaluated the effects of **DA(F)-TP-TMR** and **DA-Lifeact-TMR** on the stability of GTP MTs (Fig. S12). In both cases, MT formation was minimally observed and there were no changes after UV light irradiation. These results indicate that binding of **DA-TP** to the Taxol-binding pocket of MTs and stabilization of MTs by UV light irradiation require a **TP** sequence and a diazirine group at a position that does not inhibit binding to the Taxol-binding pocket.

Experimental Section

Equipment and materials.

Reverse-phase high-performance liquid chromatography (RP-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). Microwave-assisted solid-phase peptide synthesis was carried out using an Initiator+ (Biotage). Fully automated solid-phase peptide synthesis was carried out using a Syro I (Biotage). Electrospray ionization (ESI) mass spectra were taken using a Thermo Scientific Exactive. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were taken using a Bruker Daltonics Autoflex TII and UltrafleXtreme with α-cyano-4-hydroxycinnamic acid (α-CHCA) as a matrix. UV-vis spectra were obtained using a Jasco V-630. Confocal laser scanning microscopy (CLSM) measurement was carried out using a FluoView FV10i (Olympus). In the motility assay, samples were illuminated with a LED light source and visualized by using an epi-fluorescence microscope (Eclipse Ti2-E; Nikon) using an oil-coupled Lambda S 60x objective (NA 1.4) (Nikon). Ultracentrifugation was performed using an Optima MAX-TL ultracentrifuge (Beckman Coulter) using TLA 102.2 rotor. Tubulin was purified from bovine brain by a reported procedure.[5] The reagents used were purchased from Watanabe Chemical Ind., Ltd., Tokyo Chemical Industry Co., Dojindo Laboratories Co., Ltd. and FUJIFILM Wako Pure Chemical Co.. All the chemicals were used without further purification.

Synthesis of peptides.

For **DA-TP**, DA-Cys(Trt)-Gly-Gly-Gly-Lys(Boc)-Lys(Boc)-His(Trt)-Val-Pro-Gly-Gly-Gly-Ser(Trt)-Val-Gln(Trt)-Ile-Val-Tyr(tBu)-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 using 4 equiv. Fmoc-amino acids and 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl] benzoic acid (for introduction of DA moiety). *N*-methylpyrrolidone (NMP) solution of (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholinocarbenium hexafluorophosphate (COMU, 4 equiv.) and diisopropylethylamine (DIPEA, 8 equiv.) were used as condensation reagents for the condensation at room temperature and the microwave reaction. *N*,*N*-dimethylformamide (DMF) solution of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, 4 equiv.) and 1-hydroxybenzotriazole monohydrate (HOBt·H_2O, 4 equiv.) were used as condensation reagents for automatic peptide synthesis. Each condensation reaction was performed at room temperature for 2 h or by a microwave reaction with 35 W of microwave power at 75°C for 5 min or using automated peptide synthesizer. The peptide synthesis was performed under dark after the condensation reaction of 4-[3-(Trifluoromethyl)-3*H*-diazirin-

3-yl] benzoic acid. Deprotection of Fmoc groups from the resin was performed using 40% and 20% piperidine in DMF. The peptidyl-resin was washed with NMP, 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)/thioanisole/water/ethanedithiol/triisopropylsilane = 91.5/2.5/2.5/2.5/1, v/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*-butyl methyl ether. After centrifugation, the peptide was washed with *tert*-butyl methyl ether 4 times. The precipitated peptide was dried under vacuum. The crude product was purified by RP-HPLC with elution of a linear gradient of water/acetonitrile containing 0.1% TFA (95/5 to 0/100, v/v over 100 min, 10 mL/min, detected at 220 nm). The isolated yield was 28%. ESI-MS: *m/z* found: 836.8 ($[M+3H]^{3+}$), calcd. 836.9 (Fig. S2a).

For **DA(F)-TP**, Fmoc-Phe[4-{3-diazirin(3-CF_3)}]-OH (Fmoc-Tdf-OH) (Watanabe Chemical Ind., Ltd.) that is phenylalanine with a diazirine group in the side chain was used. H-Cys-Gly-Gly-Gly-Lys-Lys-His-Val-Pro-Gly-Gly-Gly-Ser-Tdf-Gln-Ile-Val-Tyr-Pro-Val-Asp-Leu-OH was prepared by the same procedure described above using Fmoc-Leu-Alko-PEG resin. ESI-MS: *m/z* found: 818.1 ($[M+3H]^{3+}$), calcd. 818.2 (Fig. S2b).

For **DA-Lifeact**, DA-Cys-Met-Gly-Val-Ala-Asp-Leu-Ile-Lys-Lys-Phe-Glu-Ser-Ile-Ser-Lys-Glu-Glu-OH was prepared by the same procedure described above using Fmoc-Glu-Alko-PEG resin. ESI-MS: *m/z* found: 747.3 ($[M+3H]^{3+}$), calcd. 747.5 (Fig. S2c).

UV-vis spectrum measurement.

UV light (CL-1503, ASAHI SPECTRA, 365 nm, 52 mW) was irradiated to the solution of 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl] benzoic acid or **DA-TP** (72.5 μM) in water/acetonitrile (1/1, v/v) with different irradiation time, and then UV-vis spectra were measured.

Estimation of binding affinity of DA-TP to tubulin using intrinsic tryptophan fluorescence of tubulin.

DA-TP (0, 2, 4, 6, 12, 18, 24, 30 μM, 100 μL) in BRB80 was added to a solution containing tubulin (6 μM, 100 μL) in BRB80. The mixture (200 μL) was kept at 25°C for 30 min in the dark. Then fluorescence spectra were measured by exciting at 295 nm and recording the spectra from 310 to 400 nm. As the control, **DA-TP** (0, 2, 4, 6, 12, 18, 24, 30 μM, 100 μL) in BRB80 was added to a BRB80 (100 μL, without tubulin) and then fluorescence spectra were measured. The fluorescence change subtracting the fluorescence of tubulin at 334 nm was calculated as ΔF. ΔF was plotted as a function of **DA-TP** concentration, and the K_d and ΔF_{max} (a saturated fluorescence difference) were determined by fitting to a quadratic binding function to Equation (1) using Excel and Solver.

$$\Delta F = \frac{\Delta F_{max} \times \left\{([T_{total}] + [DA-TP_{total}] + K_d) - \sqrt{([T_{total}] + [DA-TP_{total}] + K_d)^2 - 4 \times [T_{total}] \times [DA-TP_{total}]}\right\}}{2 \times [T_{total}]} \quad \textbf{(1)}$$

where $[T_{total}]$ is final concentration of tubulin (3 μM) and $[DA\text{-}TP_{total}]$ is final concentration of **DA-TP** (0–15 μM).

Preparation of tetramethylrhodamine (TMR)-labeled peptides (Scheme S1).

A DMSO solution of TMR-5-maleimide (1 equiv.) was added to 20 μM **DA-TP**, **DA(F)-TP**, or **DA-Lifeact** in 50 mM sodium phosphate buffer pH 7.0. The reaction mixture was stirred at 25°C for 24 h in the dark. **DA-TP-TMR**, **DA(F)-TP-TMR**, **DA-Lifeact-TMR** were purified by RP-HPLC with elution of a linear gradient of water/acetonitrile containing 0.1% TFA (95/5 to 0/100, v/v over 100 min, 10 mL/min, detected at 220 nm and 551 nm). MALDI-TOF-MS for: **DA-TP-TMR**: *m/z* found: 2979 ($[M–N_2+H_2O]^+$), calcd. 2979 (Fig. S2d), **DA(F)-TP-TMR**: *m/z* found: 2905 ($[M–N_2+H]^+$), calcd. 2906 (Fig. S2e), **DA-Lifeact-TMR**: *m/z* found: 2741 ($[M+Na]^+$), calcd. 2744 (Fig. S2f).

Preparation of Alexa Fluor 488-labeled tubulin (AF-tubulin).

AF-tubulin was prepared using Alexa Fluor™ 488 NHS ester (Thermo Fisher Scientific) according to the standard procedure.[6] The labeling ratio of Alexa Fluor 488 to tubulin was determined by measuring the absorbance of the protein and Alexa Fluor™ 488 at 280 and 488 nm, respectively.

Confocal laser scanning microscopy (CLSM) measurement.

Observation of MTs *in vitro*: 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 1 h, then observed by CLSM. AF-tubulin was excited by 499 nm laser and observed through a 520 nm emission band-pass filter (Green). TMR-labeled peptide was excited by 550 nm laser and observed through a 574 nm emission band-pass filter (Red).

Observation of cells: **DA-TP-TMR** was excited by 550 nm laser and observed through a 574 nm emission band-pass filter (red). Hoechst 33342 was excited by 352 nm laser and observed through a 455 nm emission band-pass filter (cyan). Tubulin Tracker™ Deep Red was excited by 650 nm laser and observed through a 668 nm emission band-pass filter (blue).

Binding analysis of DA-TP-TMR to GMPCPP MTs.

DA-TP-TMR, **DA(F)-TP-TMR**, or **DA-Lifeact-TMR** (70 μM, 4 μL) in BRB80 (80 mM PIPES pH 6.9, 1.0 mM $MgCl_2$, 1.0 mM EGTA) was added to a solution (2 μL) containing tubulin (35 μM) and AF-tubulin (35 μM) in BRB80. In the case of **DA-TP-TMR**, 1% DMSO was added to increase

the solubility. The mixture (6 μL) was kept at 25°C for 30 min in the dark. Then 1 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 the samples with UV light irradiation, UV light (365 nm, 52 mW) was irradiated for 10 min at a distance of 5 cm at 37°C. The mixture was diluted 10-fold by BRB80 and used for CLSM imaging. For inhibition experiments with Taxol, the mixture was diluted 10-fold by BRB80 containing Taxol (111 μM) and used for CLSM imaging. AF488 and TMR fluorescence intensity per MT were measured from the fluorescence images by subtracting the background intensity using ImageJ software. The background-subtracted TMR fluorescence intensity per AF488 fluorescence intensity for each MT (N = 40) was calculated to estimate the binding of TMR-labeled peptide to MTs from at least 4 images.

Evaluation of covalent bonding formation between tubulin and DA-TP-TMR.

DA-TP-TMR (40 μM, 10 μL) in BRB80 was added to a solution containing tubulin (20 μM, 10 μL) in BRB80. The mixture (20 μL) was kept at 25°C for 1 h in the dark. Then UV light (365 nm, 52 mW) was irradiated for 20 min at a distance of 5 cm from liquid surface to avoid thermal effects. The reaction solution was mixed with 5 μL of 5 × sample buffer (pH 6.8, 312.5 mM Tris–HCl, 25% sucrose, 10% SDS, 0.025% bromophenol blue) containing 250 mM DTT and vortexed for 1 h at room temperature. The samples were subjected to SDS-PAGE with a home-made 10% acrylamide gel and analyzed by a FUSION FX (Vilber) with green (530 nm) excitation light source. The protein band was stained with Imperial Protein Stain (Thermo).

Molecular mechanics calculation.

Molecular mechanics calculation was performed using MacroModel 10.4 (Schrödinger, Inc., New York, NY) with optimized potentials for liquid simulations (OPLS) 2005 force field with default setting. The refined structure of the α- and β-tubulin dimer at 3.5 Å resolution (PDB ID: 1JFF) was used for molecular modeling and ligand docking. The model structure of **DA-TP-TMR** was constructed based on the structure of **TP**.[1] The obtained **DA-TP-TMR** was put to the Taxol-binding pocket of β-tubulin instead of Taxol. Firstly, **DA-TP-TMR** with the surrounding residues around 5.0 Å were energy-minimized with a shell of constrained residues located within an additional 5.0 Å. The minimum energy conformation was then used as a starting point for a Monte Carlo multiple minimum conformational search with up to 1000 search steps, an energy window of 200 kJ mol^{-1} for saving structures, the loosened threshold for conformer redundancy with a root mean square deviation (RMSD) cutoff of 1.0 Å. In the calculations, **DA-TP-TMR** with its surrounding residues within 3.0 Å were applied for the conformational search. Finally, the obtained structures were energy-minimized by using the same parameters above.

Evaluation of the effect of DA-TP on MT formation.

DA-TP or Taxol (800 μM, 4 μL) in BRB80 was added to a solution (4.1 μL) containing AF-tubulin (195.1 μM) in BRB80. The mixture (8.1 μL) was kept at 25°C for 30 min in the dark. Then 1.9 μL of GTP premix (5 mM GTP, 80 mM PIPES pH 6.9, 21 mM $MgCl_2$, 1.0 mM EGTA, 25% DMSO) was added to the mixture and kept at 37°C for 30 min in the dark. For the samples with UV light irradiation, UV light (365 nm, 52 mW) was irradiated for 5 min at 37°C. 490 μL of BRB80 was added and each sample was ultracentrifuged at 50000 rpm at 37°C for 5 min. Then the supernatant was collected and the pellet was resuspended in 28 μL of BRB80. The supernatant of each sample was concentrated to 28 μL by ultrafiltration. Each sample solution (10 μL) was mixed with a 2×sample buffer (4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, 0.125 M Tris-HCl pH 6.8, SIGMA-ALDRICH, 10 μL). The samples were incubated for 5 min at 95°C and then loaded to SDS-PAGE (15% acrylamide) and stained with Coomassie brilliant blue. The relative amount of AF-tubulin in the supernatant and in the pellet were estimated by measuring the band intensity. The density of bands in each lane was measured from the gel image by subtracting the background intensity using ImageJ software. The background-subtracted density in pellet lane per sum of the density of the supernatant and pellet bands for each sample was calculated to estimate the ratio of MT formation. The original image is converted to a 32bit image in ImageJ for better visibility and quantification.

Evaluation of the effect of DA-modified peptides on GTP MTs.

DA-TP, **DA(F)-TP-TMR**, or **DA-Lifeact-TMR** (20 μM, 4 μL) in BRB80 was added to a solution (4 μL) containing tubulin (5 μM) and AF-tubulin (5 μM) in BRB80. The mixture (8 μL) was kept at 25°C for 30 min in the dark. Then 2 μL of GTP premix was added to the mixture and kept at 37°C for 30 min in the dark. For the samples with UV light irradiation, UV light (365 nm, 52 mW) was irradiated for 5 min at a distance of 5 cm at 37°C. The mixture (5 μL) was used for CLSM imaging. The remaining solution (5 μL) was kept at 4°C for 15 min and used for CLSM imaging. The fluorescence images of MTs were analyzed to determine the contour length and end-to-end distance of each MT by using ImageJ software. In order to determine persistence length (L_p), both the contour length and the end-to-end distance of the same MT were measured (N = 100). L_p was determined by fitting the data to Equation (2) using Excel and Solver.

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

For the different timing of UV light irradiation, **DA-TP** (50 μM, 2 μL) in BRB80 was added to a solution (2 μL) containing tubulin (12.5 μM) and AF-tubulin (12.5 μM) in BRB80. The mixture (4 μL) was kept at 25°C for 30 min in the dark. UV light (365 nm, 52 mW) was irradiated for 5 min,

GTP premix (1 μL) was added to the mixture, and then kept at 37°C for 30 min in the dark.

Concentration dependence of DA-TP on the structures of GTP MTs.

DA-TP (25, 50, 125 μM, 2 μL) in BRB80 was added to a solution (2 μL) containing tubulin (12.5 μM) and AF-tubulin (12.5 μM) in BRB80. The mixture (4 μL) was kept at 25°C for 30 min in the dark. Then 1 μL of GTP premix was added to the mixture and kept at 37°C for 30 min in the dark. For the samples with UV light irradiation, UV light was irradiated for 5 min at a distance of 5 cm at 37°C. The fluorescence images of MTs were analyzed to determine the contour length and end-to-end distance of each MT by using ImageJ software. In order to determine L_p, both the contour length and the end-to-end distance of the same MT were measured ($N = 49$). L_p was determined by fitting the data to Equation (2) using Excel and Solver. There was no significant difference in contour length and L_p without UV irradiation, but UV irradiation caused MTs to become longer and more rigid as the concentration of **DA-TP** increased (Fig. S10).

Motile properties of DA-TP-incorporated GMPCPP MTs.

DA-TP-encapsulated GMPCPP MTs were prepared as above (preparation concentrations: 5 μM tubulin, 5 μM AF-tubulin, 100 μM **DA-TP**). The MTs were diluted 20-fold by BRB80. Flow cells were prepared by making a narrow channel on a 24 mm × 60 mm coverslip covered with 18 mm × 18 mm coverslip (Matsunami, Osaka, Japan) using double-sided tape as a spacer. Firstly, 0.5 mg/mL casein in BRB80 was introduced into 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) containing 1 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 every 10 sec. All the experiments were performed at room temperature. The images obtained by the fluorescence microscopy were analyzed to determine the velocity, end-to-end length, and contour length of each MT by using ImageJ. 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 (2) using Excel and Solver.

Cell culture.

Human hepatoma HepG2 cells (RIKEN BioResource Research Center, Japan) were cultured in Dulbecco's modified Eagle's medium. All medium contained 10% fetal bovine serum (v/v), 50 μg/mL streptomycin, 50 μg/mL penicillin, 100 μg/mL neomycin, 1 mM sodium pyruvate, and 1%

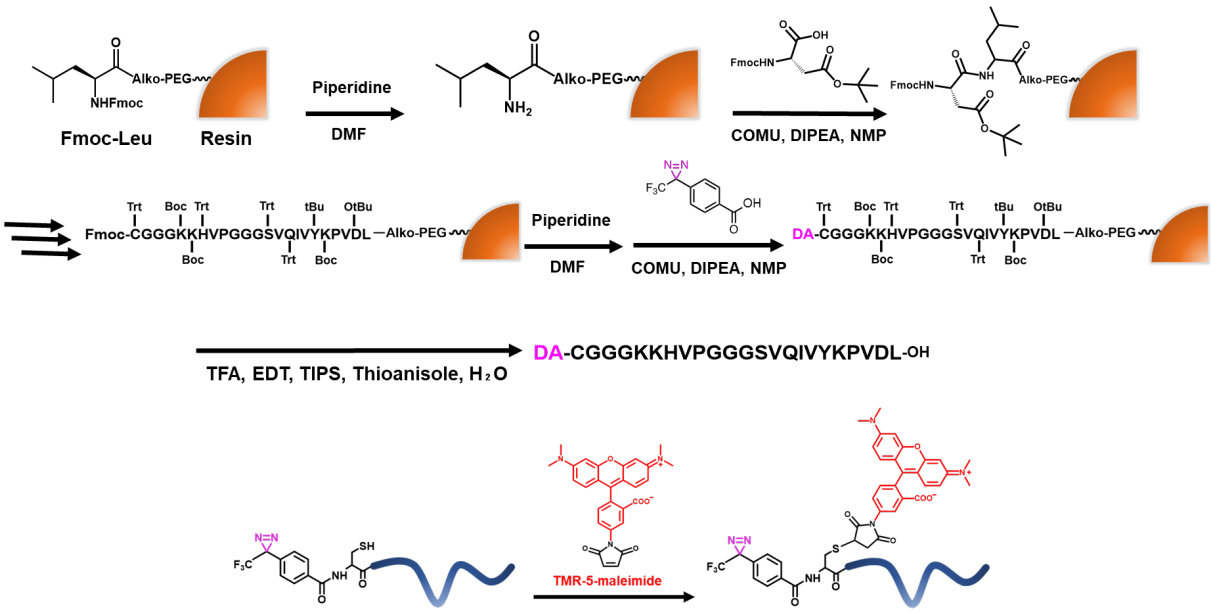
MEM nonessential amino acids (v/v, Sigma M7145). Cells were maintained at 37°C in 5% CO_2 incubator, and a subculture was performed every 3–4 days.

Imaging of DA-TP-TMR in HepG2 cells.

HepG2 cells were seeded onto a single-well glass bottom dish at 2.0×10^4 cells/well in a final volume of 100 μL and incubated at 37°C for 24 h, 5% CO_2. To depolymerize intracellular MTs, the culture medium was replaced with fresh medium (90 μL) and 50 μg/mL demecolcine in MilliQ was added to the cells (10 μL per well) and incubated at 37°C for 4 h, 5% CO_2. After washing with PBS, the mixture of **DA-TP-TMR** (100 μM, 1 μL), 50 × ProteoCarry (4 μL) (Funakoshi Co., Ltd.), and the medium without fetal bovine serum (95 μL) was added to the cells and incubated at 37°C for 30 min, 5% CO_2. After washing with PBS, the cells were incubated with 10 μg/mL Hoechst 33342 and 1 μM Tubulin Tracker™ Deep Red (Thermo Fisher Scientific) in the culture medium at 37°C for 30 min, 5% CO_2. After washing with PBS and 7.7 μg/mL probenecid in HBSS, the medium with 7.7 μg/mL probenecid was added to the cells and CLSM images were acquired. After imaging, UV light was irradiated to the cells for 5 min and the cells were further incubated at 37°C for 15 h, 5% CO_2 and imaged again.

Cell viability assay.

HepG2 cells were plated in 96-well plates at 1.0×10^4 cells/well in a final volume of 100 μL and incubated at 37°C for 24 h, 5% CO_2. The culture medium was replaced with medium containing 5 μg/mL demecolcine and incubated at 37°C for 4 h, 5% CO_2. After washing with PBS, 0.01, 0.1, 1, 10, 100 μM **DA-TP-TMR** and 2 × ProteoCarry or 0.01, 0.1, 1, 10, 100 μM Taxol in the medium without fetal bovine serum were added to the cells and incubated for 30 min. After washing with PBS, the culture medium was replaced with fresh medium (100 μL) and UV light (365 nm, 52 mW) was irradiated to the cells for 1, 5 or 10 min. The cells were further incubated at 37°C for 24 h, 5% CO_2. After washing with PBS, the culture medium was replaced with fresh medium (100 μL), and 10 μL of the Cell Counting Kit-8 reaction solution (Dojindo, Japan) was added to each well. After incubation for 4 h, the absorbance at 450 nm was measured. Cell viability (%) was calculated by setting the absorbance using the demecolcine-treated cells as 100%.



Scheme S1. Synthesis of TMR-labelled **DA-TP** (**DA-TP-TMR**)

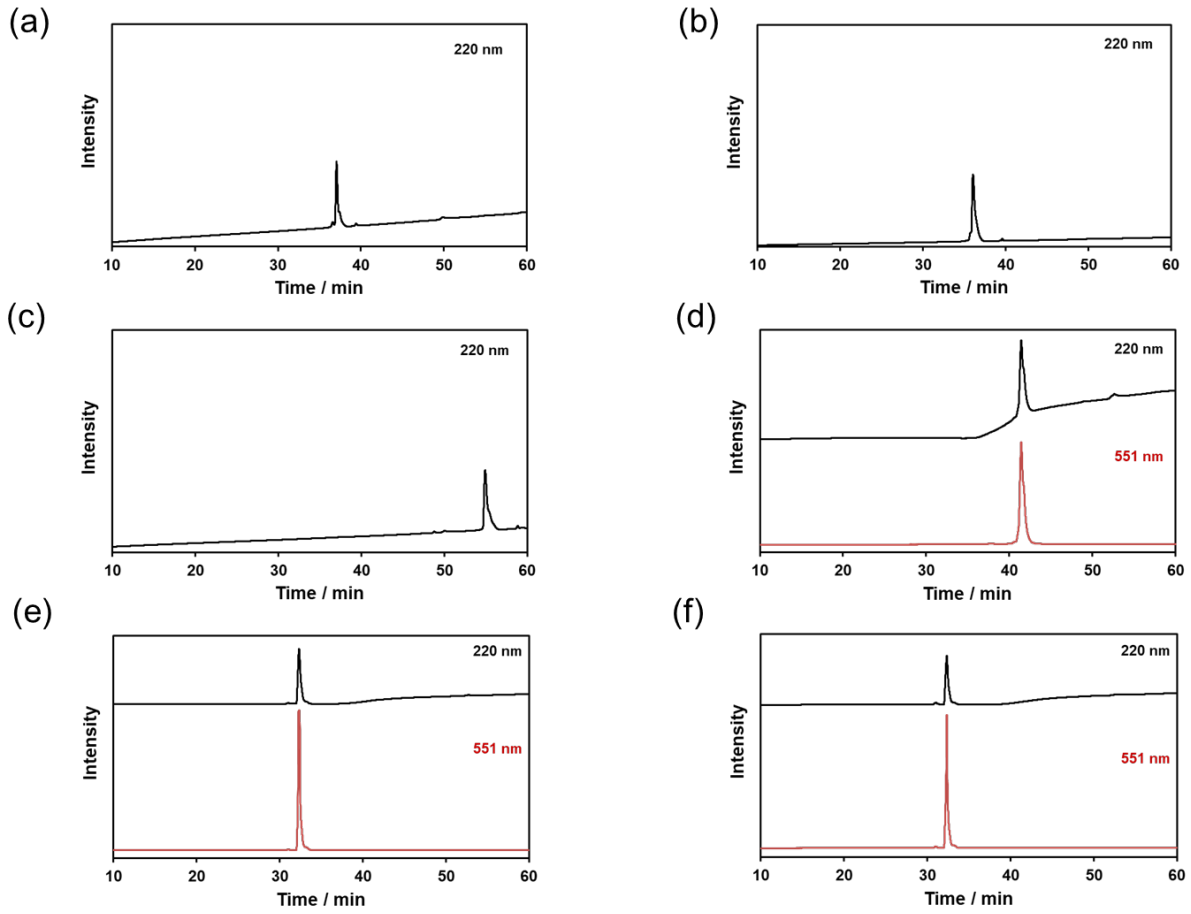


Fig. S1. HPLC charts of (a) **DA-TP**, (b) **DA(F)-TP**, (c) **DA-Lifeact**. (d) **DA-TP-TMR**, (e) **DA(F)-TP-TMR**, (f) **DA-Lifeact-TMR**. The shoulder peaks at (a)–(c) are considered to be oligomers of the peptides, while the small peaks around 39 and 50 min are junk peaks derived from the solvent.

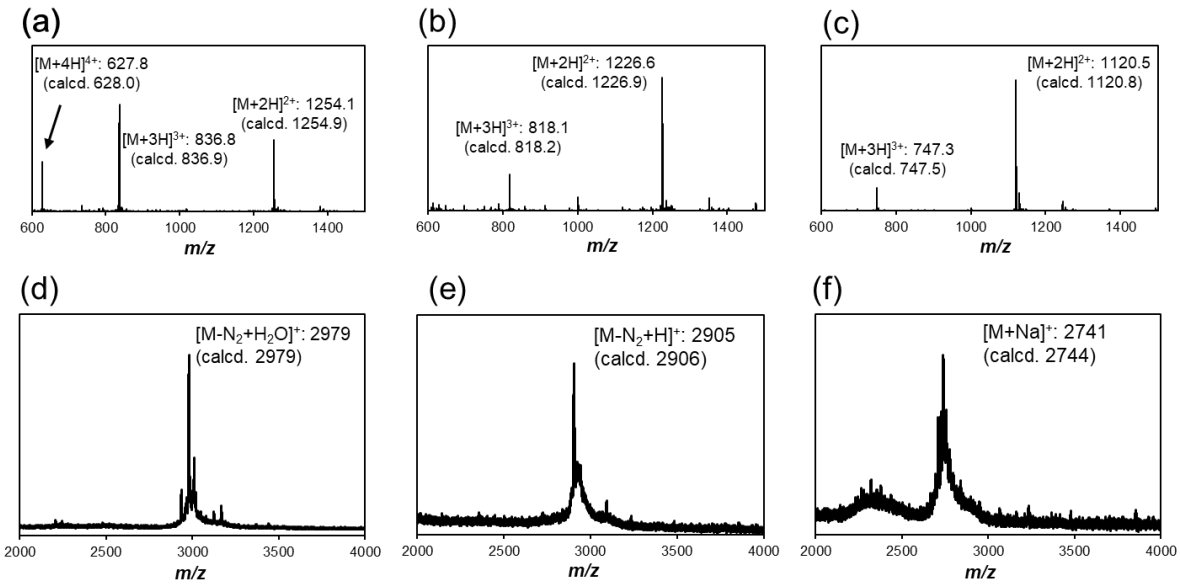


Fig. S2. ESI-MS of (a) **DA-TP**, (b) **DA(F)-TP**, (c) **DA-Lifeact**. MALDI-TOF-MS of (d) **DA-TP-TMR**, (e) **DA(F)-TP-TMR**, (f) **DA-Lifeact-TMR**.

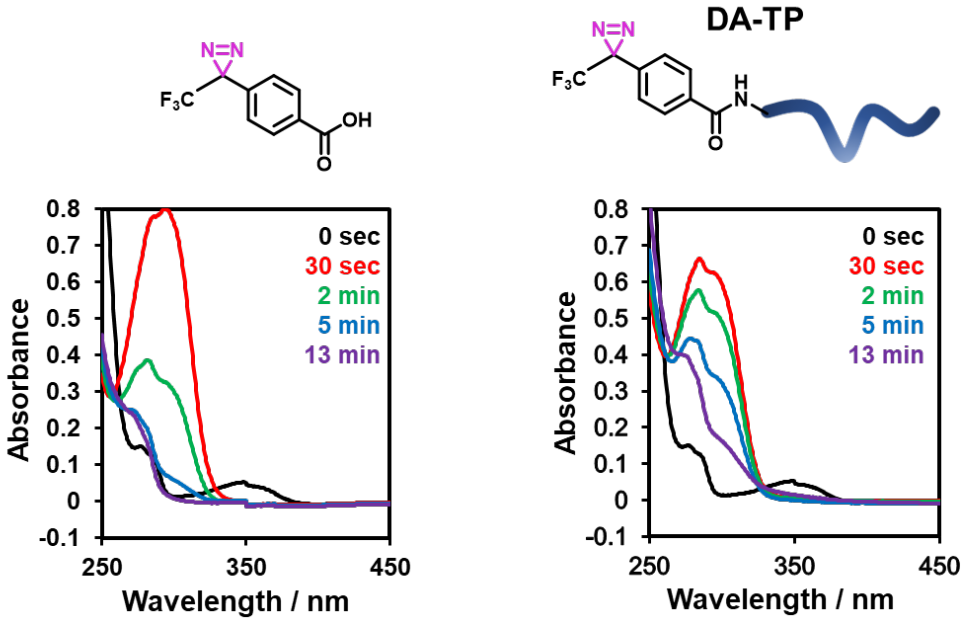


Fig. S3. UV-vis spectra of 4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl] benzoic acid (left) and **DA-TP** (right) (72.5 μM) in water/acetonitrile (1/1, v/v) with UV light irradiation for 0 sec (black), 30 sec (red), 2 min (green) 5 min (blue) and 13 min (purple).

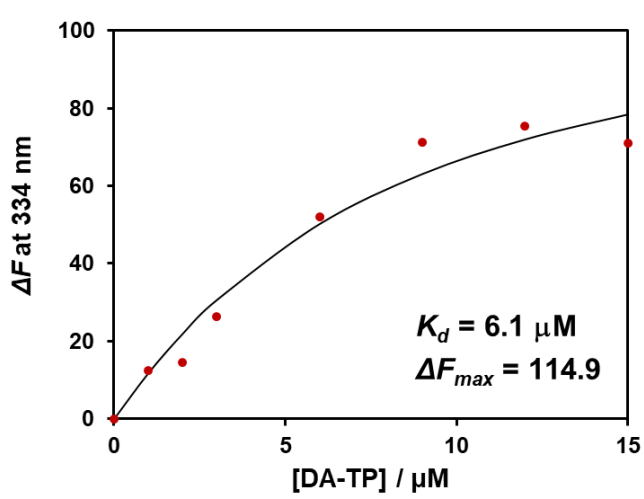


Fig. S4. Analysis of binding affinity of **DA-TP** to tubulin by measuring changes of intrinsic tryptophan fluorescence of tubulin. Closed circles show experimental values, while the solid line is theoretical curve obtained by fitting the K_d and ΔF_{max} at 334 nm.

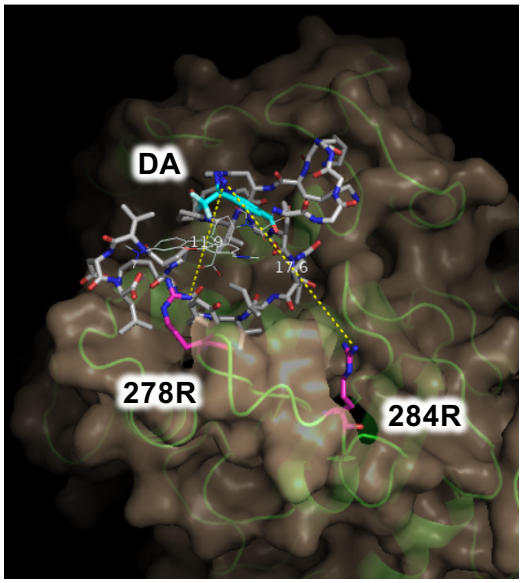


Fig. S5. Model of the binding of **DA-TP-TMR** (stick representation) to tubulin obtained by molecular mechanics calculation. Cyan indicates the DA moiety of **DA-TP-TMR** and magenta indicate 278R and 284R of β-tubulin. The yellow dashes indicate approximate distances (Å) between DA moiety and 278R or 284R.

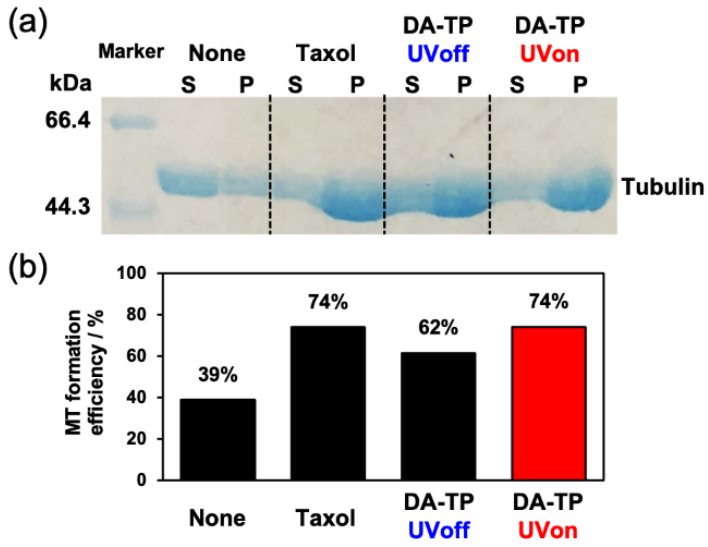


Fig. S6. Effects of **DA-TP** on the formation of GTP MTs with and without UV light irradiation (5 min). (a) SDS-PAGE of the supernatants (S) and pellets (P) obtained by centrifugation of each sample. Preparation concentrations: 80 μM AF-tubulin, 320 μM **DA-TP**. (b) Formation efficiency of MTs determined based on the SDS-PAGE band density.

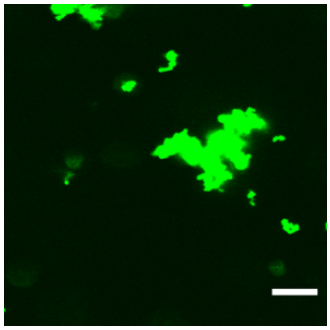


Fig. S7. CLSM image of tubulin treated with **DA-TP**, UV light irradiation (5 min), and then GTP. Preparation concentrations: 5 μM tubulin, 5 μM AF-tubulin, 20 μM **DA-TP**. Scale bar, 10 μm.

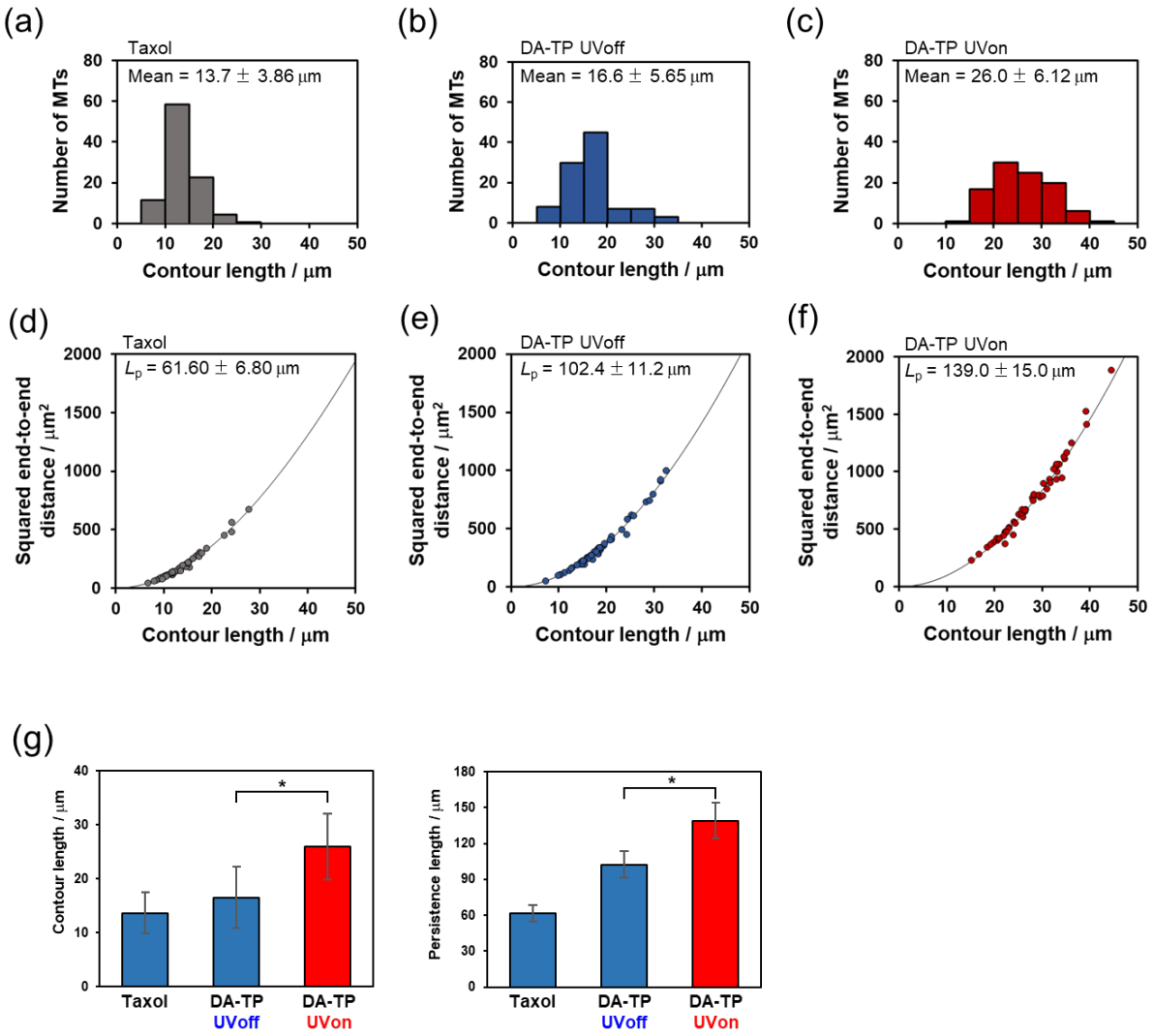


Fig. S8. (a–c) Distribution of contour length and (e–f) persistence length (L_p) of MTs treated with (a, d) Taxol, (b, e) **DA-TP** without UV light irradiation, and (c, f) **DA-TP** with UV light irradiation (5 min) (N = 100). Black curves indicate the statistically estimated results. Data presented as average ± standard deviation. (g) Comparison of contour length and L_p of MTs treated with Taxol, DA-TP with or without UV light irradiation. *P < 0.0001, two-tailed Student's t-test. Error bars represent the standard deviation (N = 100). Preparation concentrations: 2 μM tubulin, 2 μM AF-tubulin, 8 μM **DA-TP** or Taxol.

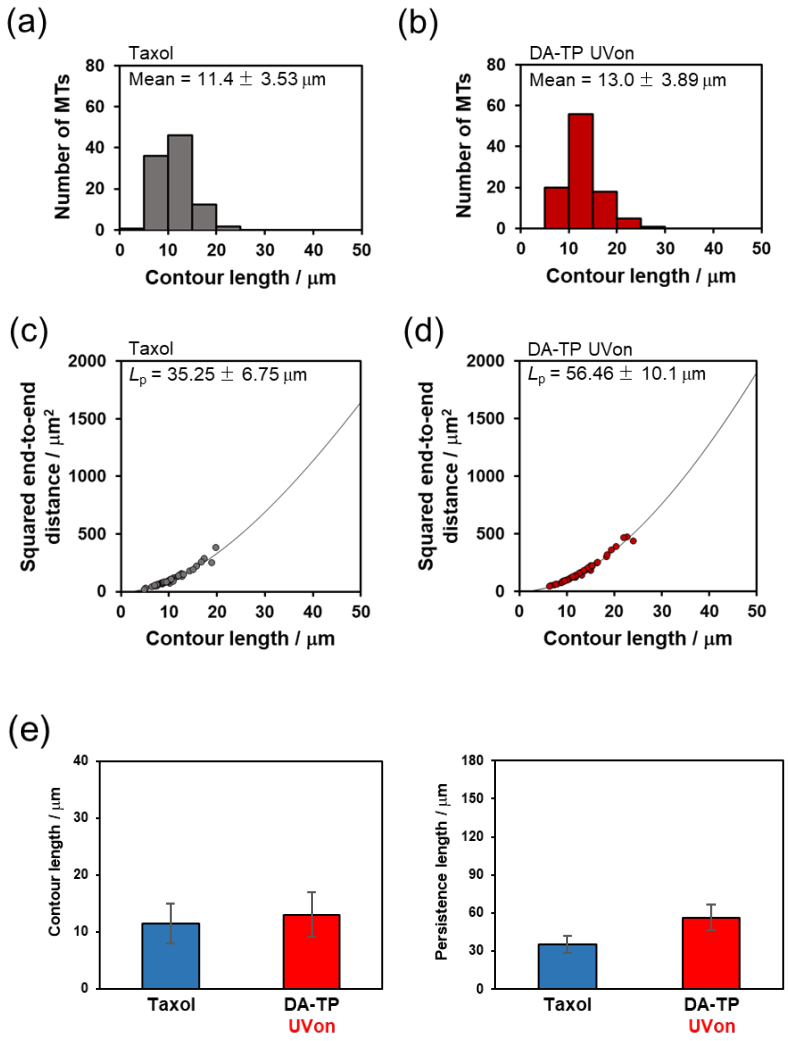


Fig. S9. (a, b) Distribution of contour length and (c, d) L_p of MTs treated with (a, c) Taxol and (b, d) **DA-TP** with UV light irradiation after incubation at 4°C for 15 min (N = 100). Black curves indicate the statistically estimated results. Data presented as average ± standard deviation. (e) Comparison of contour length and L_p of MTs treated with Taxol or DA-TP with UV light irradiation. Error bars represent the standard deviation (N = 100). Preparation concentrations: 2 μM tubulin, 2 μM AF-tubulin, 8 μM **DA-TP** or Taxol.

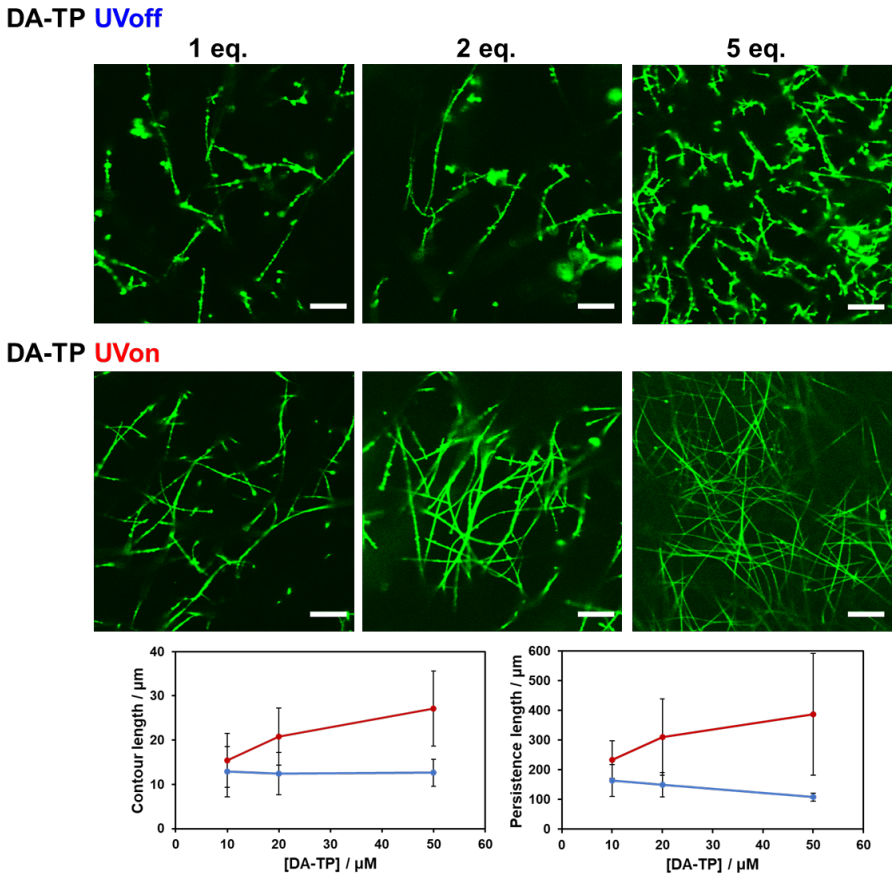


Fig. S10. CLSM images of concentration dependence of **DA-TP** on GTP MTs with and without UV light irradiation (5 min). Error bars represent the standard deviation (N = 49). Preparation concentrations: 5 µM tubulin, 5 µM AF-tubulin, 10 µM, 20 µM, 50 µM **DA-TP**. Scale bars: 10 µm.

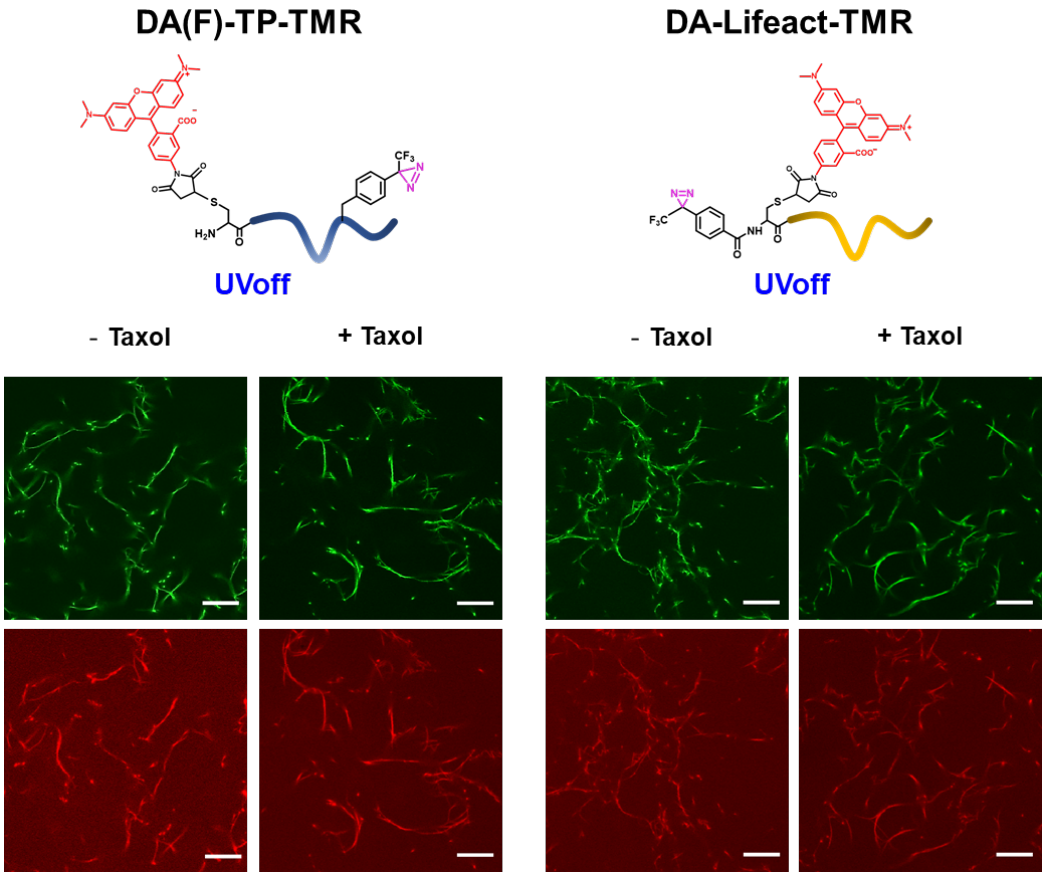


Fig. S11. CLSM images of **DA(F)-TP-TMR** or **DA-Lifeact-TMR**-incorporated GMPCPP MTs without and with treatment of Taxol. Preparation concentrations: 5 μM tubulin, 5 μM AF-tubulin, 20 μM **DA(F)-TP-TMR** or **DA-Lifeact-TMR**, 100 μM Taxol. Scale bars: 10 μm.

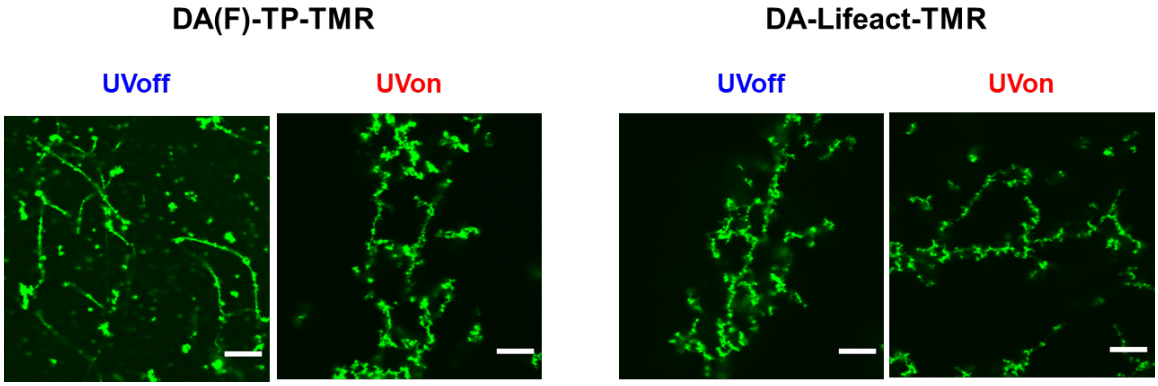


Fig. S12. CLSM images of GTP MTs treated with **DA(F)-TP-TMR** or **DA-Lifeact-TMR** with and without UV light irradiation (5 min). Preparation concentration: 2 μM tubulin, 2 μM AF-tubulin, 8 μM **DA(F)-TP-TMR** or **DA-Lifeact-TMR**. Scale bars: 10 μm. No clear MTs were observed in all samples.

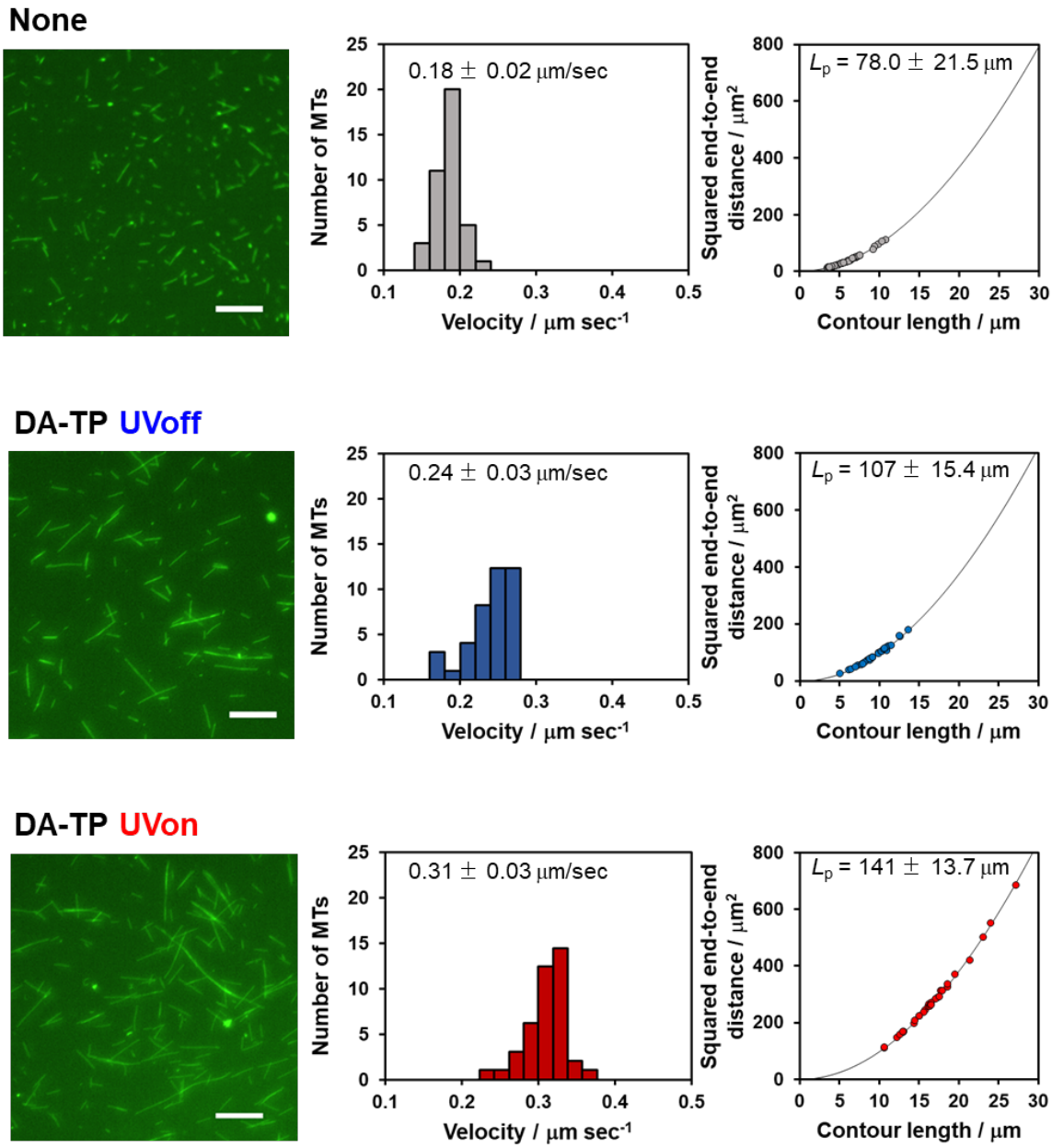


Fig. S13. Effect of **DA-TP** on the velocity and L_p of GMPCPP MTs on the kinesin-coated substrates. Fluorescence image (left), histogram of the velocity of the MTs (center, $N = 40$), and distribution of L_p (right). Black curves indicate the statistically estimated results. Data presented as average ± standard deviation. Preparation concentrations: 5 μM tubulin, 5 μM AF-tubulin, 100 μM **DA-TP**.

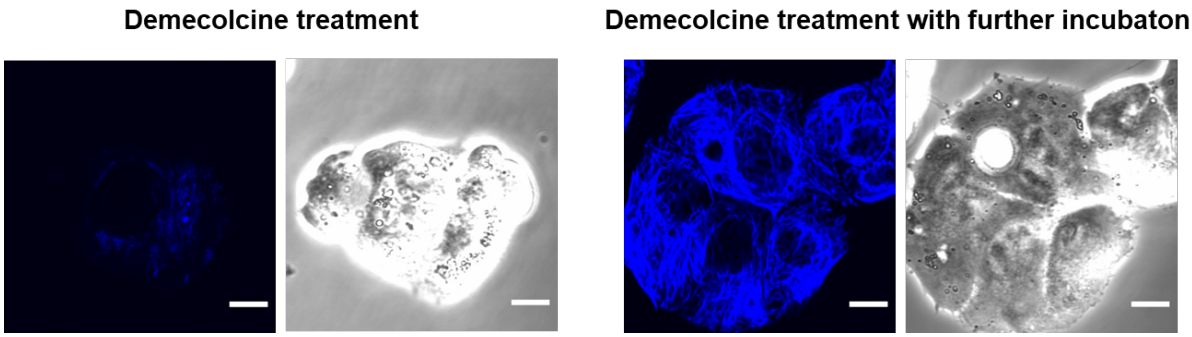


Fig. S14. CLSM images of HepG2 cells after treatment of 5 μg/mL demecolcine for 4 h and washing (left) and further incubation at 37°C for 1.5 h (right). MTs were stained by Tubulin Tracker Deep Red. Scale bars: 10 μm. Depolymerization of MTs by the demecolcine treatment (left) and repolymerization (right) were observed.

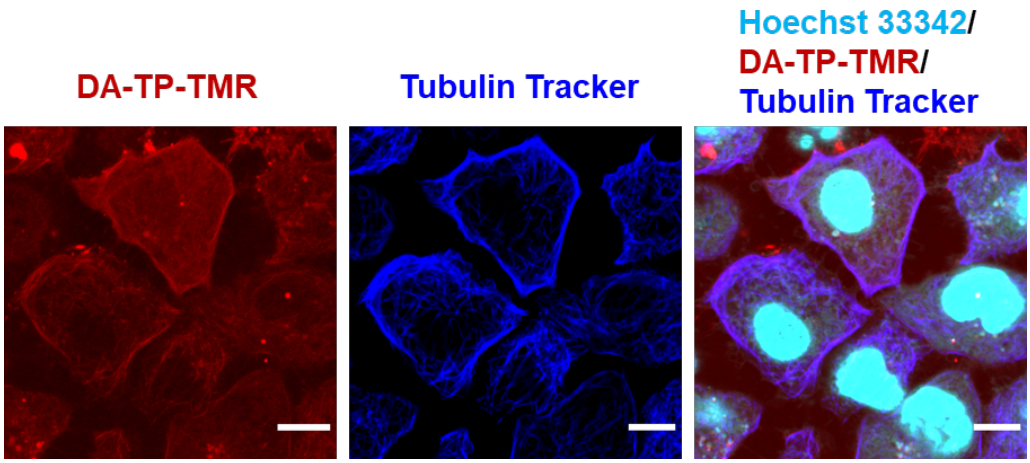


Fig. S15. CLSM images of **DA-TP-TMR**-incorporated HepG2 cells immediately after UV light irradiation for 5 min. Scale bars: 10 μm.

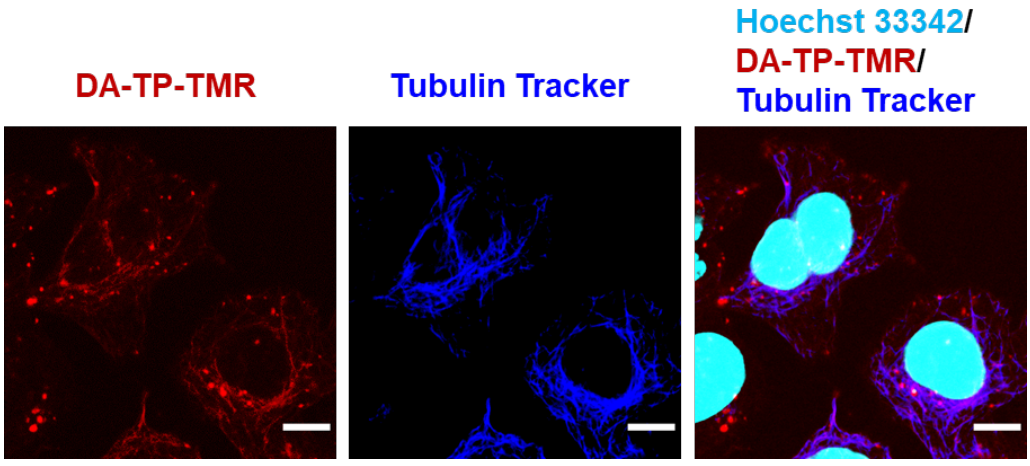


Fig. S16. CLSM images of **DA-TP-TMR**-incorporated HepG2 cells without UV light irradiation and further incubation for 15 h. Scale bars: 10 μm.

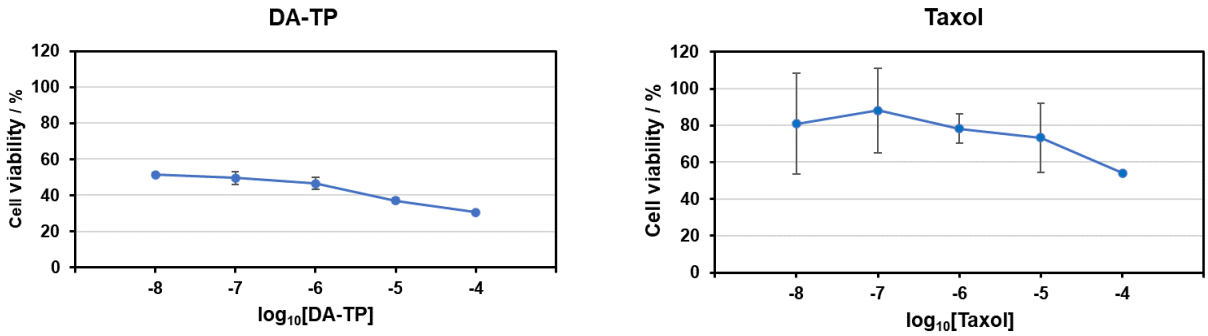


Fig. S17. Concentration dependence of **DA-TP** and Taxol on cell viability. Data presented as average ± standard deviation (N = 3). UV light (1 min) was irradiated for **DA-TP**.

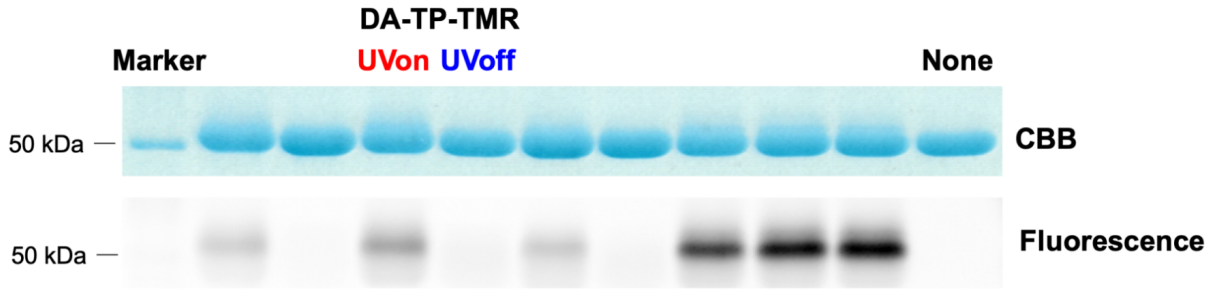


Fig. S18. Raw data of SDS-PAGE and fluorescent scanning results used for Fig. 2d.

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