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Photo-regulated trajectories of gliding microtubules conjugated with DNA

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Materials and Methods

Purification of tubulin and kinesin

Tubulin was purified from porcine brain using high-concentration PIPES buffer (1 M PIPES, 20 mM EGTA, and 10 mM $MgCl_2$) and preserved in BRB80 buffer (80 mM PIPES, 1 mM EGTA, 2 mM $MgCl_2$, pH maintained to 6.8 using KOH) at -80 °C.[1] Recombinant kinesin-1 consisting of the first 573 amino-acid residues of human kinesin-1 was prepared as described in the literature[2]. Azide labeled tubulin was prepared using N_3-PEG_4-NHS following an established protocol of tubulin labeling with a fluorescent dye[3]. The concentration of tubulin was determined by measuring the absorbance at 280 nm using a UV spectrophotometer (Nanodrop 2000c).

Design and preparation of DNA sequences

The sequence of *p*DNA used in this work was TTTTTTTTTTTTTTGZTTGZTTGZTTG ($T_{12}(TTG)_4Z_3$; Z is azobenzene). *p*DNA strand was purchased from Hokkaido System Science Co. Ltd. The *p*DNA was modified at the 5′ end with dibenzocyclooctyne (DBCO). Quality control of all the synthesized DNA was performed by polyacrylamide gel electrophoresis (PAGE). Characterization of the *p*DNA strands were carried out by LC-ESI-MS spectroscopy.

Preparation of microtubules (MTs)

MTs were polymerized from a mixture of azide labeled tubulin and Atto 550 labeled tubulin in which the concentration of tubulin was 70 μM. Molar ratio of azide labeled tubulin and Atto 550 labeled tubulin was 4:1 in polymerization buffer (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM GMPCPP, pH~6.8). Then the tubulin mixture in polymerization buffer was incubated at 37 °C for 30 min to form GMPCPP-MTs. 1 μL of 4×BRB80 buffer and 0.5 μL of 1 mM taxol (in DMSO) were added to the MT solution just after polymerization to stabilize

the MTs. Copper free click reaction was initiated by adding 3.5 µL DBCO conjugated *p*DNA (500 µM) to 5 µL MT solution (56 µM) and incubating at 37 °C for 6 h to allow azide-alkyne cycloaddition reaction.[4] 100 µL of cushion buffer (BRB80 buffer supplemented with 60% glycerol) was used to separate the MTs by centrifugation at 201,000 × g (S55A2-0156 rotor, Hitachi) for 1 h at 37 °C. After removing the supernatant, the pellet of *p*DNA-conjugated MTs was washed with 100 µL BRB80P (BRB80 supplemented with 1 mM taxol) and resuspended in 15 µL of BRB80P buffer.

Measurement of the labeling ratio of *p*DNA to MTs

The *p*DNA conjugated MTs were depolymerized to *p*DNA conjugated tubulins by keeping the MTs on ice overnight. The absorption spectrum of the *p*DNA conjugated tubulin dimers was measured using a spectrophotometer (NanoDrop™ 2000c, Thermo Fisher Scientific Inc.) and deconvoluted using the normal distribution function with Microsoft Excel (Windows Edition, Microsoft Corporation) with peaks at 260 nm and 280 nm. The concentrations of *p*DNA and tubulin dimers were calculated from the Beer-Lambert law using molar extinction coefficient of tubulin dimers (115,000 L mol^{-1} cm^{-1}) and *p*DNA (230,000 L mol^{-1} cm^{-1}) from which the labeling ratio was determined (Figure S1).

Gliding assay of MTs on a kinesin coated substrate

A flowcell was constructed using two glass cover slides of 40×50 mm^2 and 18×18 mm^2 (MATSUNAMI Inc.) adhered together by parafilm as a spacer with a channel pattern cut off. Prior to preparing the channel the glass slides were plasma treated for 3 min by a plasma etcher (SEDE-GE; Meiwafosis) to make it hydrophilic. The channel pattern was designed using Brother Canvas Workspace software on the parafilm and cut by Brother ScanNCut printer. The flow cell was prepared setting the designed parafilm on the large cover glass and then the small

slide glass on the top and then heating at 70 °C. The flow cell was filled with 5 μL casein buffer (BRB80 buffer supplemented with 0.5 mg mL^{-1} casein). After incubating for 3 min, 0.8 μM kinesin solution was introduced into the flow cell and incubated for 5 min. After washing the flow cell with 5 μL of wash buffer (BRB80 buffer supplemented with 1 mM DTT, 10 μM taxol), 5 μL of ATTO550-labeled MT (*p*DNA modified or unmodified) solution was introduced and incubated for 2 min, followed by washing with 10 μL of wash buffer. The motility of MTs was initiated by applying 5 μL ATP buffer (wash buffer supplemented with 5 mM ATP, 4.5 mg mL^{-1} D-glucose, 50 U mL^{-1} glucose oxidase, 50 U mL^{-1} catalase, and 0.2% methylcellulose (w/v). The time of ATP addition was set as 0 h. Soon after the addition of ATP buffer, the flow cell was placed in an inert chamber system (ICS)[5] and the MTs were monitored after 30 min of ATP addition using an epifluorescence microscope at room temperature (25 °C). The samples were illuminated with a 100 W mercury lamp and visualized by an epifluorescence microscope (Eclipse Ti, Nikon) using an oil-coupled Nikon Plan Apo 60× objective (N.A.=1.4). UV cut-off filter blocks (TRITC: EX 540/25, DM565, BA605/55; Nikon) were used in the optical path of the microscope. Images were captured using a cooled-CMOS camera (NEO sCMOS, Andor) connected to a PC. In order to observe the effect of UV on the motility of MTs, Nikon super high-pressure Hg lamp was used as a light source which passed through a UV1A filter (EX 365-10, DM400, BA390; Nikon). The beam is expanded and steered into the microscopic objective lens. The intensity of UV light was 0.13 mW/cm^2 by Thorlabs power meter (PM100). Movie was captured for 100 s taking each frame in 4 s interval. There was rest time of 100 s and then the next movie was captured under UV irradiation. This cycle was repeated twice for both the *p*DNA modified and unmodified MTs.

Estimation of the path persistence lengths of MTs

The path persistence lengths of MTs were estimated using the following equation:

$$R^2 = 2{L_P}^2\left[\left(\frac{L}{L_P} - 1\right) + exp\left(\frac{-L}{L_P}\right)\right]$$

where R is the end-to-end distance, L is the contour length of the trajectories of MTs, and L_p represents the path persistence length of MTs. From the fitting of the data of R and L, L_p was estimated.

Data analysis

The fluorescence microscopy images were analyzed by NIS- Elements BR software (Nikon) and Fiji 1.52J software (National Institutes of Health, USA). Velocity of the gliding MTs was measured using the ImageJ plugin 'MTrackJ' (https://imagej.net/MTrackJ). The temporal color coded projection of the of the gliding MTs in Figure 3a was prepared using the ImageJ plugin 'Temporal color code' (https://imagej.net/Temporal-Color_Code). Statistical analysis and graphs were performed with the software OriginPro Version 2019, OriginLab, USA. Mann-Whitney test or two-tailed Student's t-test was used to compare two groups of data where applicable.

References:

(1) Castoldi, M.; Popov, A. V. Purification of Brain Tubulin through Two Cycles of Polymerization – Depolymerization in a High-Molarity Buffer. **2003**, *32*, 83–88.

(2) Case, R. B.; Pierce, D. W.; Hom-booher, N.; Hart, C. L.; Vale, R. D. The Directional Preference of Kinesin Motors Is Specified by an Element Outside of the Motor Catalytic Domain. **1997**, *90*, 959–966.

(3) Peloquin, J.; Komarova, Y.; Borisy, G. Conjugation of Fluorophores to Tubulin. *Nat. Methods* **2005**, *2* (4), 299–303.

(4) Früh, S. M.; Steuerwald, D.; Simon, U.; Vogel, V. Covalent Cargo Loading to Molecular Shuttles via Copper-Free "Click Chemistry." *Biomacromolecules* **2012**, *13* (12), 3908–3911.

(5) Kabir, A. M. R.; Inoue, D.; Kakugo, A.; Kamei, A.; Gong, J. P. Prolongation of the Active Lifetime of a Biomolecular Motor for in Vitro Motility Assay by Using an Inert Atmosphere. *Langmuir* **2011**, *27* (22), 13659–13668.

Supplementary Figures and Table

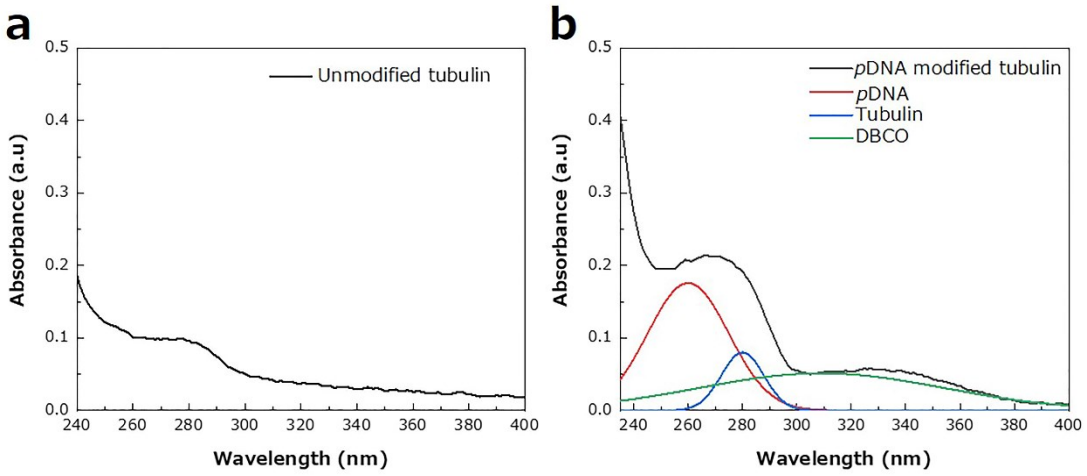

Fig. S1: Estimation of the labeling ratio of *p*DNA to MTs. (a) Representative absorption spectrum of unmodified MTs. (b) Representative deconvoluted absorption spectra of *p*DNA conjugated MTs. The labeling ratio of *p*DNA to tubulin dimers was determined after estimating the concentrations of *p*DNA and tubulin from their absorption maxima.

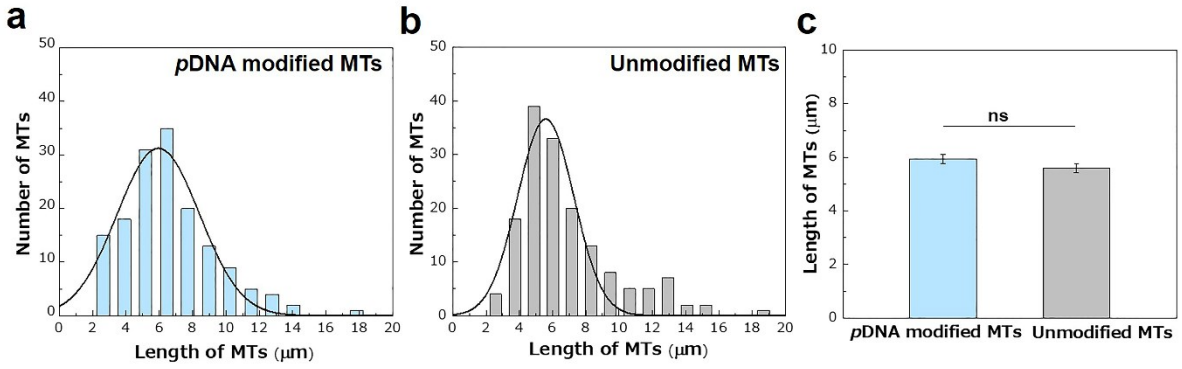

Fig. S2: Distribution of length of (a) *p*DNA modified and (b) unmodified MTs. The number of MTs considered for analysis was 153 and 157 for *p*DNA modified and unmodified MTs respectively. The distributions of MT lengths were fitted to the Gaussian equation ($y = Ae^{\frac{-(x-b)^2}{c^2}}$) for normal distribution. The fitted curves are represented by the solid lines (R^2=0.95 and 0.90 for *p*DNA modified and unmodified MTs respectively). The mean MT length obtained from the fitting was 5.9±0.2 μm and 5.6±0.2 μm for *p*DNA modified and unmodified MTs respectively. (c) Mean lengths of *p*DNA modified and unmodified MTs were not significantly different as obtained from the result of Student's t-test. ns: non-significant. Error bar: standard error.

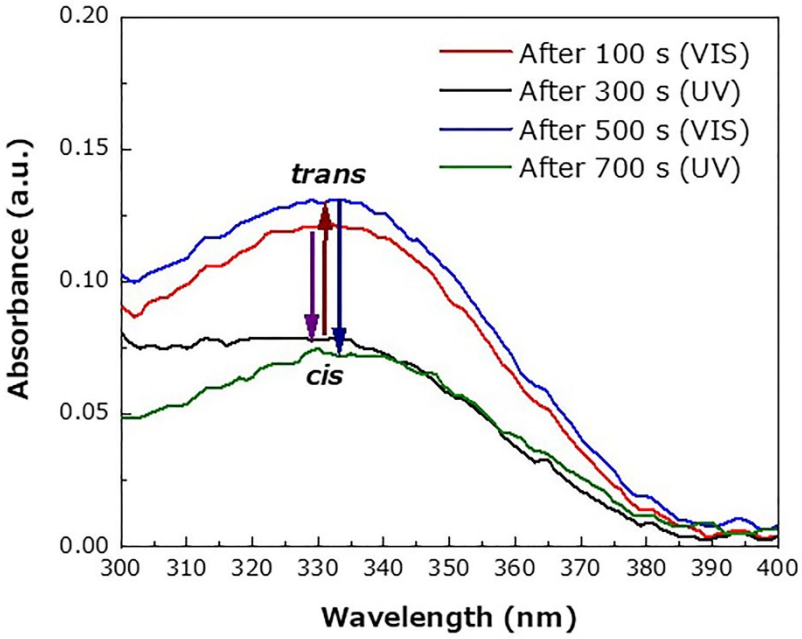

Fig. S3: Absorption spectra of azobenzene in *p*DNA. After 100 s of VIS light irradiation the azobenzene in *p*DNA isomerized into trans state (red line). Upon irradiation with UV light azobenzene returend to the *cis* state (black line). After cycle 1, first VIS and then UV light was irradiated consecutively for 100 s. Experimental conditions: 20 µM *p*DNA, 100 mM NaCl, 10 mM phosphate buffer, pH 7.0.

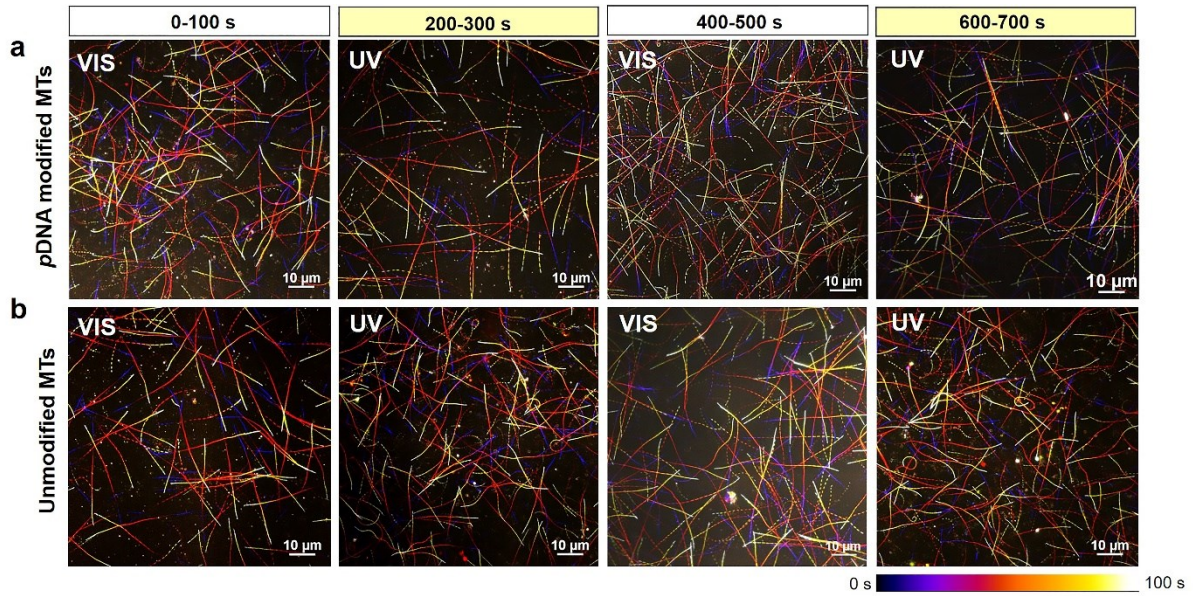

Fig. S4: Temporal color-coded maximum projections of (a) *p*DNA modified and (b) unmodified MTs for 100 second interval frames in the absence (white box) and presence (yellow box) of UV irradiation. The color code denotes the traveled distance of MTs with time.

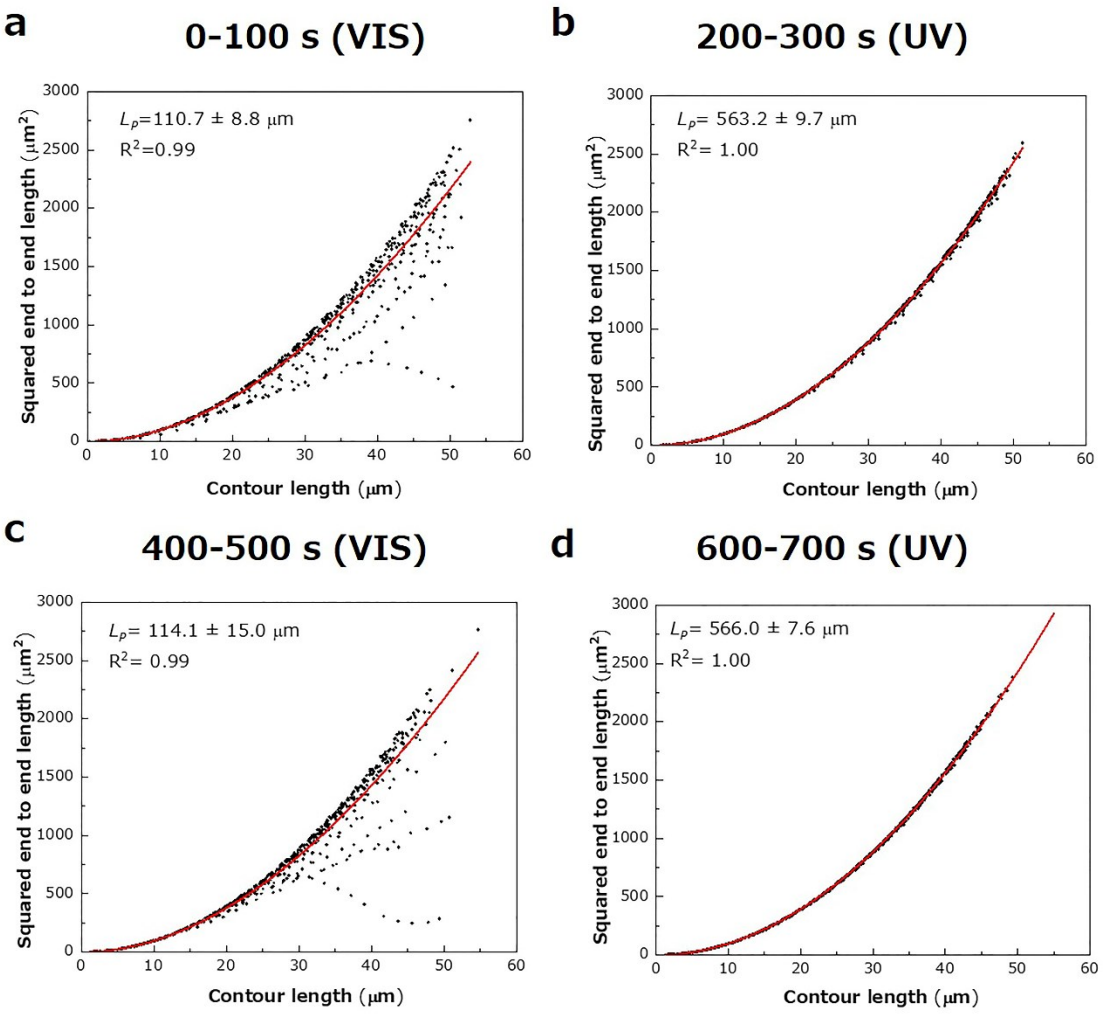

Fig. S5: Estimation of the path persistence length (*Lp*) of *p*DNA modified MTs where the MTs were irradiated with: (a) VIS irradiation (0-100 s); (b) UV irradiation (200-300 s); (c) VIS irradiation (400-500 s) and (d) UV irradiation (600-700 s). The data were fitted using equation 1 shown in the article.

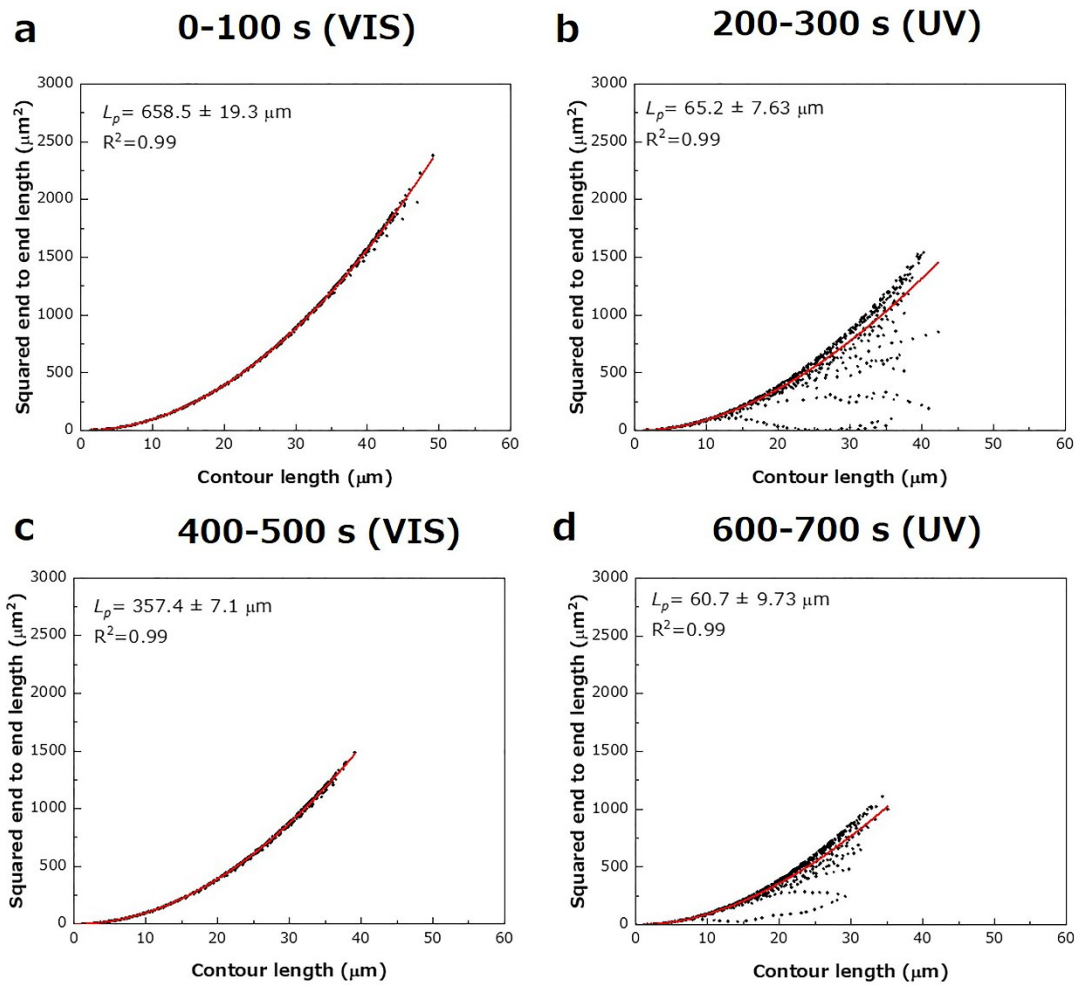

Fig. S6: Estimation of the path persistence length (*Lp*) of unmodified MTs where the MTs were irradiated with: (a) VIS irradiation (0-100 s); (b) UV irradiation (200-300 s); (c) VIS irradiation (400-500 s) and (d) UV irradiation (600-700 s). The data were fitted using equation 1 shown in the article.

Table S1: Estimation of the labeling ratio of *p*DNA to tubulin dimers.

In feed concentration of *p*DNA (µM)	**Concentration of tubulin dimers (µM)**	**Concentration of *p*DNA in tubulin dimers (µM)**	**Labeling ratio of *p*DNA to tubulin dimers (%)**
500	14	13	91