Supporting information

Localized control of the swarming of kinesin-driven microtubules using light

Mousumi Akter[1], Arif Md. Rashedul Kabir[2], Jakia Jannat Keya[3], Kazuki Sada[2], Hiroyuki Asanuma[4] & Akira Kakugo[*5]

[1]Department of Mechanical Engineering, University of Michigan, Ann Arbor, 48108, Michigan, United States

[2]Faculty of Science, Hokkaido University, Sapporo, 060-0810, Japan.

[3]Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, 48109, United States

[4]Graduate School of Engineering, Nagoya University, Nagoya, 464-8603, Japan.

[5]Department of Physics, Kyoto University, Kyoto 606-8224, Japan

*Correspondence and requests for materials should be addressed to A.K. (email: kakugo.akira.8n@kyoto-u.ac.jp)

Methods

Design and preparation of DNA sequences

Photoresponsive azobenzene tethered DNA (*p*DNA) strands were designed from melting temperature (T_m) simulation using the OligoAnalyzer tool (https://sg.idtdna.com/calc/analyzer) with a T_m between 0 and 70°C for experimental testing. *p*DNA strands were purchased from Hokkaido System Science Co. Ltd. The 5′ end of all the *p*DNAs was modified with dibenzocyclooctyne (DBCO). Polyacrylamide gel electrophoresis was performed to control the quality of the purchased *p*DNA. Characterization of the *p*DNA strands was carried out by LC-ESI-MS (liquid chromatography–electrospray ionization–mass spectrometry). Table S1 presents the sequences of the used *p*DNAs related to the experiments. The molar extinction coefficient was measured from the Beer Lambert law using the absorbance of different concentrations of DNA.

Measurement of the melting temperature (Tm) of the p-DNA duplex

The *p*DNA duplex (2 μM) was dissolved in 10 mM phosphate buffer (pH 7.0) with 100 mM NaCl. The *p*DNA duplex in the cis and trans state was obtained by irradiating with a high-power UV light-emitting diode lamp (SOLIS-365C, Thorlabs) having a dominant wavelength of 365 nm. Before the T_m measurement, either 365- or 450-nm light was irradiated to the *p*DNA duplex solution for 5 min. The melting curves of the duplex in the cis and trans state were obtained with a spectrophotometer (V-760 spectrophotometer, Jasco) at 260 nm in a quartz cell with a 10-mm path length. The heating rate was 0.5°C/min. T_m was determined from the maximum in the first derivative of the melting curve (**Supporting Table S5**).

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

*p*DNA-conjugated MTs were depolymerized to *p*DNA-conjugated tubulins, which were kept 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 and 280 nm. The concentrations of *p*DNA and tubulin dimers were calculated from the Beer–Lambert law using a molar extinction coefficient of tubulin dimers (115,000 liters mol^{-1} cm^{-1}) and *p*DNA from which the labeling ratio was determined (Table S2-S4).

Flow channel preparation for *in vitro* motilityassay

A microfluidic channel was constructed using two glass cover slides of 40 mm by 50 mm and 18 mm by 18 mm (Matsunami Inc.) adhered together by parafilm as a spacer with a channel pattern cut out. The glass slides were plasma-treated for 3 min by a plasma etcher (SEDE-GE; Meiwafosis) to make them hydrophilic. The channel pattern (length of 22 mm and width of 1.8 mm) was designed using Brother Canvas Workspace software on parafilm and cut with a Brother ScanNCut printer. The flow cell was prepared by setting the designed parafilm on the large cover glass and then the small slide on the top and then heating it to 70°C.

Imaging

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 (numerical aperture = 1.4). UV cutoff filter blocks (tetramethyl rhodamine isothiocyanate: EX 540/25, DM565, BA605/55; green fluorescent protein: EX 470/40, DM500, BA535-50; Nikon) were used in the optical path of the microscope. Images were captured using a cooled complementary metal-oxide semiconductor camera (NEO sCMOS, Andor) connected to a PC. UV light was irradiated to observe the dissociation of swarms into single MTs. To observe the effect of UV on the swarming of MTs, a Nikon super high-pressure Hg lamp was used as a light source that passed through a UV1A filter (EX 365/10, DM400, BA390; Nikon). The beam is expanded and steered into the microscopic objective lens. UV light intensity was measured as 0.6 mW/cm^2 by a Thorlabs power meter (PM100). Different ND filters were used to control the intensity of the UV light through the UV irradiation pathway. The intensity of UV light in different ND filters is mentioned in Table S5.

Measurement of the association ratio and dissociation ratio of MTs

The association ratio at a given time *t* was determined by counting the number of single MTs manually and dividing the number at time *t* by the number present initially ($t = 0$).[1-2] The time-dependent association ratio, $A(t)$, of red and green MTs was determined as follows:

$$A(t)=\frac{N(0)-N(t)}{N(0)} \qquad (1)$$

Here, $N(0)$ is the initial number of single MTs, and $N(t)$ is the number of single MTs after time *t*. The dissociation ratio, $R(t)$, was calculated from the ratio of $N(t)$ and $N(0)$.

$$R(t)=\frac{N(t)}{N(0)}=1-A(t) \qquad (2)$$

The mean association ratio and dissociation ratio were obtained from the average of four regions of interest (2500 μm^{-2}).

Data analysis

The fluorescence microscopy images were analyzed by NIS-Elements AR 5.1 (Nikon) and Fiji-ImageJ 1.52J software (National Institutes of Health, USA). The velocity of the gliding MTs and swarms was measured using the ImageJ plugin 'MTrackJ' (https://imagej.net/MTrackJ). Statistical analysis and graphs were performed with the software OriginPro Version 2019, OriginLab, USA and Prism GraphPad. Mann–Whitney test or two-tailed Student's t test was used to compare two groups of data where applicable.

References

1. Akter, M., Keya, J. J., Kayano, K., Kabir, A. M. R., Inoue, D., Hess, H., Sada, K., Kuzuya, A., Asanuma, H. & Kakugo, A. Cooperative cargo transportation by a swarm of molecular machines. *Sci. Robot.* **7,** eabm0677 (2022).

2. Keya, J. J.; Suzuki, R.; Kabir, A. M. R.; Inoue, D.; Asanuma, H.; Sada, K.; Hess, H.; Kuzuya, A.; Kakugo, A. DNA-Assisted Swarm Control in a Biomolecular Motor System. *Nat. Commun.* **2018**, *9* (1), 4–11. https://doi.org/10.1038/s41467-017-02778-5.

Supporting figures and Tables

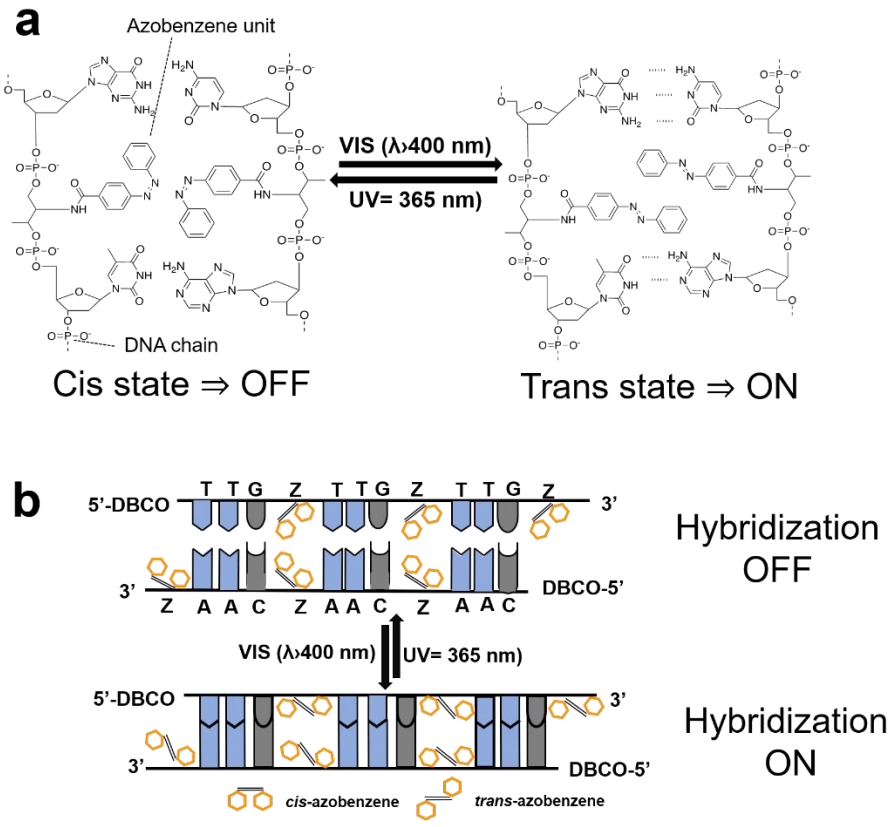

Figure S1 Schematic representation of **(a)** reversible hydrogen bonding of complementary *p*DNA by light-induced *cis-trans* isomerization of azobenzene. **(b)** Schematic representation of the sequence design of photoregulated azobenzene tethered DNAs in which Z denotes the azobenzene to understand how the complementary sequences initiate hybridization under VIS light.

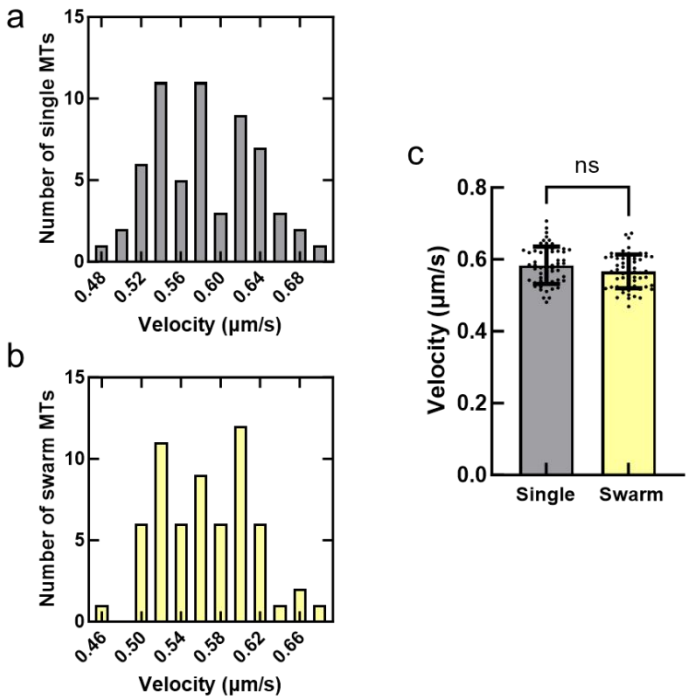

Figure S2 Velocity distributions of (a) single MTs and (b) swarm MTs. The number of MTs considered for analysis was 60. The D'Agostino−Pearson test, at $\alpha < 0.0001$, showed that the velocity data of the MTs passed normality test. (c) Comparison of the fitted mean velocity of single and swarm MTs. Error bar: standard error. The arithmetic means of the velocity of the single MTs and swarm MTs are 0.58±0.05 μm/s and 0.56±0.04 μm/s respectively. Mean velocities of MTs were determined to be not significantly different from the result of Mann-Whitney test at P=0.2955 indicated by ns. ns: non-significant.

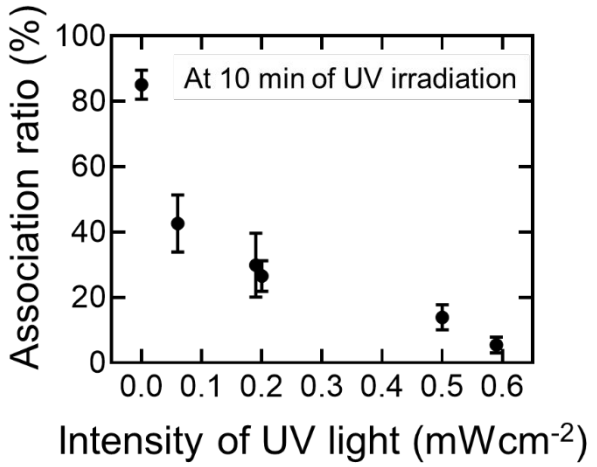

Figure S3 Change in the association ratio of MTs with the intensity of UV light irradiation after 10 min of UV irradiation. Error bar: standard deviation.

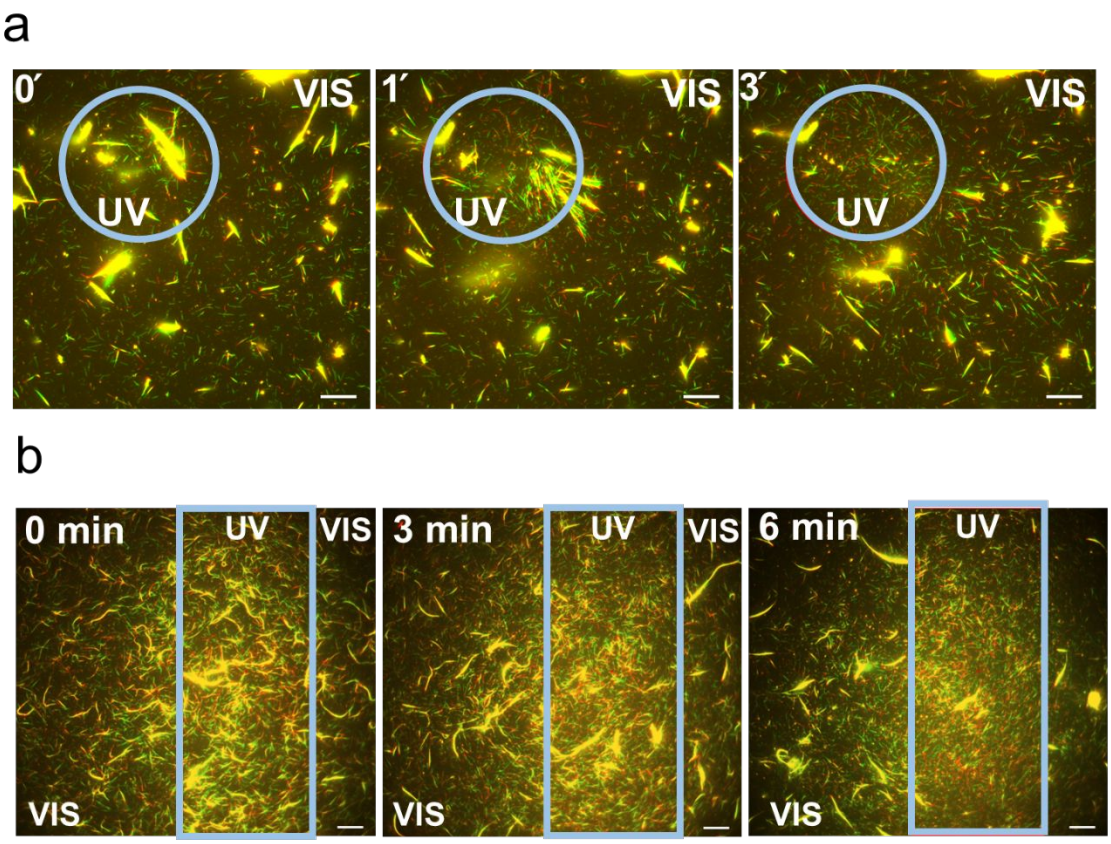

Figure S4 Spatiotemporal control of MT swarms under UV light. **(a)** Time-lapse fluorescence microscopy images of the dissociation of MT swarms in a circular-shaped UV irradiation area. Here, UV light was irradiated through the light blue circle, and visible light was irradiated in the rest of the area. Scale bar: 10 μm. **(b)** Time lapse fluorescence microscopy images of the dissociation of MT swarms at a rectangular-shaped UV irradiation area. Here, UV light was irradiated through the light blue rectangle, and visible light was irradiated in the rest of the area. Scale bar: 10 μm. In both cases, the intensity of the UV light was 0.5 mW/cm^2.

Supporting Tables

Table S1 Sequences of the DNA used to confirm that azobenzene facilitates the reversible swarming of MTs. The labeling ratio of DNA to tubulin was calculated from the ratio of the concentration of DNA and the concentration of tubulin in the DNA-modified tubulin solution.

Name of DNA	**DNA sequences**	**Concentra-tion of DNA (μM)**	**Concentr-ation of tubulin dimers (μM)**	**DNA concentration in tubulin dimers (μM)**	**Labeling ratio of DNA to tubulin dimers (%)**
Control DNA 1	$T_{12}(TTG)_6$	100	3	1	39
Control DNA 2	$(CAA)_8$	100	3	1	43
*p*DNA1	$T12(TTG)_6Z_5$	200	5	1	33
*p*DNA2	$(CAA)_8Z_7$	200	5	2	39

Table S2 Sequences of *p*DNA with different lengths used to regulate swarming. Here, Z is azobenzene. All DNA strands were modified with DBCO at the 5' end.

Name of the *p*DNA	**Number of azobenzene number (n)**	**5'-3'**	**Molar extinction coefficient ($Lmol^{-1}cm^{-1}$)**
*p*DNA1: **$T_{12}(TTG)_{n+1}Z_n$**	1	TTTTTTTTTTTTTTG**Z**TTG (**$T_{12}(TTG)_2Z_1$**)	178,900
	2	TTTTTTTTTTTTTTG**Z**TTG**Z**TTG (**$T_{12}(TTG)_3Z_2$**)	205,500
	3	TTTTTTTTTTTTTTG**Z**TTG**Z**TTG**Z**TTG (**$T_{12}(TTG)_4Z_3$**)	230,000
	4	TTTTTTTTTTTTTTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG **($T_{12}(TTG)_5Z_4$)**	245,400
	5	TTTTTTTTTTTTTTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG **($T_{12}(TTG)_6Z_5$)**	253,000
	6	TTTTTTTTTTTTTTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG**Z**TTG **($T_{12}(TTG)_7Z_6$)**	268,200

	7	TTTTTTTTTTTTTTG**Z**TTG**Z** TTG**Z**TTG**Z**TTG**Z**TTG **Z**TTG **(T_{12}(TTG)$_8$Z$_7$)**	296,700
*p*DNA2: **(CAA)$_n$Z$_{(n-1)}$**	7	CAA**Z**CAA**Z**CAA**Z**CAA**Z**CA AZCAA**Z**CAA**Z**CAA **((CAA)$_8$Z$_7$)**	367,800

Table S3 Optimized labeling ratio of pDNA to MT DNA to tubulin for the construction of a molecular swarm system

Name of the *p*DNA	**Number of TTG base (n)**	**Infeed conc of *p*DNA (μM)**	**Final conc of tubulin dimers (μM)**	**Final conc of *p*DNA in tubulin dimers (μM)**	**Labeling ratio of *p*DNA to tubulin dimers (%)**
*p*DNA1: $\mathbf{T_{12}(TTG)_nZ_{(n-1)}}$	2	10	5	2	46
	3	20	15	6	41
	4	100	10	4	40
	5	200	4	2	48
	6	200	5	2	39
	7	300	8	3	38
	8	400	11	4	37
*p*DNA2: $\mathbf{(CAA)_nZ_{(n-1)}}$	8	200	5	2	41

Table S4 Percentage of light transmittance and UV intensity of different ND filters.

Optical density or neutral density or ND	**Denotation of ND**	**Fractional Transmittance (%)**	**Intensity of UV light (mW/cm^2)**
0 (no ND is used)	ND1	100	0.59
0.1		79	0.50
0.3	ND2	50	0.20
0.6	ND4	25	0.19
0.9	ND8	12.5	0.06

Table S5 The melting temperature (Tm) of *p*DNA1 and *p*DNA2 strands at its *trans* state and *cis* state.

Name of the *p*DNA	**Number of Azobenzene, Z (n)**	**Tm_{trans}**	**Tm_{cis}**
*p*DNA1: **$T_{12}(TTG)_{(n+1)}Z_n$**	3	53	37
	4	63	44
*p*DNA2: **CAA_8Z_7**	5	70	30
	6	78	42

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: The effect of UV light intensity on the microtubule swarm. Scale bar: 20 μm. The movie is 100 times faster than the original speed.

File Name: Supplementary Movie S2

Description: Localized control of a microtubule swarm under UV light. UV light was irradiated through the red colored square box. Scale bar: 20 μm. The movie is 100 times faster than the original speed.