Modulating the microtubule assembly and dynamics by altering the chemical environment

S. Bhattacharyya,[a] K. Kim, [a] H. Nakazawa,[b] M. Umetsu, [b] and W. Teizer [a,c,d] *

**E-mail: teizer@physics.tamu.edu*

Supporting Information

Materials and Methods:

Reagents:

Unlabeled tubulin (T290) and rhodamine labeled tubulin (TL590M) were purchased from Cytoskeleton. Taxol (paclitaxel, T7402), adenosine triphosphate (A9187), glucose oxidase (G7141), catalase (C40), calcium chloride (V800104), monosodium glutamate monohydrate (MSG, 28-2800), iron (III) sulfate hydrate (307718) and acetylcholine (A2661) were purchased from Sigma Aldrich. Other materials: casein (037–20815, Wako Pure Chemical Industries), quantum dot (QD655-streptavidin conjugated, Q10123MP, Life Technology), wild-type tau protein (T-1001-1, rPeptide), methylene blue (25908-23, Kanto chemical), glucose (076-05705, Wako), sodium chloride (31320-05, Nacalai Tesque), poly-L-lysine (167-18651, Wako) and zinc chloride (High Purity Chemical).

Kinesin purification

A detailed description of biotinylated kinesin expression and purification is in a previous report.[1] Kinesin plasmid-inserted (Neurospora Crassa's with 400 residue, truncated) E-coli underwent sequential double-step bacterial proliferation at 28 °C in LB medium culture (3 mL/vial) and in 2 × YT medium culture (250 mL/vial).[2] All of the growth media included two antibiotics (ampicillin 0.1 mg/mL, chloramphenicol 34 μg/mL). Kinesin expression was achieved by shaking the E-coli culture at 15 °C for 24 h after adding 1 mM IPTG and 50 μM biotin. E-coli pellets collected by centrifugation were lysed by sonication in the lysis buffer (50 mM Tris, 200 mM NaCl, 40 mM imidazole, 100 μM MgATP, 10% (v/v) glycerol, 5 mM β-mercaptoethanol (BME), pH8-HCl balance). Purification of the histidine-tagged kinesin proteins was performed with a 2 ml Ni-sepharose column. After gel-filtration chromatography, SDS-PAGE was carried out to choose kinesin fractions. Selected fractions were dialyzed (storage buffer: 50 mM imidazole, 100 mM NaCl, 1 mM $MgCl_2$, 2 mM EGTA, 0.1 mM EDTA, 5 % (w/v) sucrose, 5 mM BME, 100 μM MgATP, pH7), concentrated by centrifuge filtration and stored in 5 μl aliquot at -80 °C. The kinesin concentration was estimated by UV-spectroscopy measurements.

MT labeling and Kinesin-QD complex

A stock solution of tubulin subunit proteins was prepared using a polymerization buffer (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$, 1 mM GTP, 6 % (v/v) glycerol) at the tubulin concentration of 5 mg/ml and stored in 1 μl aliquots at − 80 °C. For fluorescence microscopy, we used a mixture of rhodamine labeled tubulin and unlabeled tubulin at a ratio of about 3 to 7. Tubulin polymerization was achieved by incubating one stock aliquot for 1 hr at 37 °C. In order to prepare a motility solution, the resulting MT solution was 200 fold further diluted by adding 10

μM taxol solution (10 μM taxol in PEM buffer). Kinesin-QD complexes were prepared by mixing biotinylated kinesin solution (64 μM) with streptavidin QD solution (1 μM) in PEM buffer and incubating on ice for more than 10 min. Volume ratio between each solution in the mixture was, 1: 1: 8 (kinesin: QD: PEM buffer).

Motility assay: MT gliding on QD kinesin coated surface

All assays were performed using a conventional flow-cell chamber that was assembled by using a glass slide, a glass coverslip and double-sided tape. Fluid exchange (i.e. flow-cell filling) was achieved by using a micropipette for fluid injection and a piece of filter paper for fluid absorption. For the MT gliding assay, a flow cell was first filled with casein solution (2 mg/ml in PEM, filtered using a 0.2 μm syringe filter). After 10 min incubation at room temperature, the cell was flushed with PEM buffer before it was filled with Kinesin/QD complex solution (5 fold further dilution of the starting kinesin-QD mixture described above). After 5 min incubation at room temperature, the cell was flushed by PEM buffer, filled with motility assay buffer that typically contains 20 % (v/v) of the 200 fold diluted MT solution stabilized with 10 μM taxol in PEM including 20 mM glucose, 5 mM ATP, 20 μg/mL glucose oxidize, 8 μg/mL catalase and 0.1 % (v/v) β-mercaptoethanol, and sealed using VALAP (1:1:1 vaseline/lanolin/paraffin). Finally, the flow cell was mounted on a fluorescence microscope for observation. The reagent of interest for the studies of the effects on the bio-motility system (Iron (III) sulfate, MSG, poly-L-lysin, tau protein, and aspirin) was directly added into the motility solution for certain time of pre-incubation at a desired concentration, before it was introduced into the flow cell.

Motility assay: QD cargo motion of Kinesin on MT tracks

For the QD-cargo transportation assays, a flow cell was filled with casein solution. After 10 min incubation at room temperature, the cell was filled with 5 fold diluted kinesin stock solution. After 5~10 min incubation at room temperature, 200~400 fold diluted MT solution was injected into the cell. After 10 min incubation at room temperature, the cell was filled with motility solution (Kinesin/QD complex solution (QD final concentration: 200 pM) in PEM including 10 μM taxol, 20 mM glucose, 5 mM ATP, 20 μg/mL glucose oxidize, 8 μg/mL catalase and 0.05 % (v/v) β-mercaptoethanol) followed by VALAP sealing for the fluorescence microscopy. For the study of tau protein treated MT tracks, the flow cell was filled with tau protein solution (in PEM with 10 μM taxol) as an additional step and further incubated at room temperature before the motility solution was introduced.

MT-tau complex formation

Tau-induced MT entanglement was observed when tau protein (in 50 mM MES, pH 6.8, 0.5 mM EGTA, 100 mM NaCl) was added into the MT-motility assay buffer at a molar ratio of tau to tubulin (10:1) and incubated for 30 min. We have used tubulin concentrations of 0.04 μM and tau protein concentrations of 0.4 μM.

Rescue/Reversibility experiment

For rescue/reversibility experiments, we first prepared a motility solution containing tau-induced MT bundles (MT-tau complexes). Then a reagent of interest (MSG, Iron (III) sulphate and sodium chloride) was added into the motility solution at a 200 μM concentration and incubated for 20 min before it was introduced into a flow-cell chamber for the microscope observation.

Fluorescence microscopy

Observations were performed with a fluorescence microscope (IX-71, Olympus) equipped with a CCD camera (ImagEM, Hamamatsu, Adaptor: U-PMTVC4XIR). Two objective lenses, PlanApo 60 × (for QD-cargo assays, Olympus) and UPlanFL 40 × (for all other experiments, Olympus), were used. Two filter-sets, XF204 (for rhodamine, Omega optical) and XF305-1 (for QD655, Omega optical), were used. Note that the filter-set XF305-1 allows observation of MTs and QDs at the same time, particularly for bundled MTs (for an example see **Figure 4 (b-c)**). All movies were acquired with either the time lapse or streaming option in Metamorph (exposure time: 130 ms for time lapse (rate: 1 frame per sec), 100 ms for streaming).

Analysis

Image analysis was performed using ImageJ (available at http:// imagej.nih.gov/ij/) and Matlab (R2015b). MT gliding speeds were measured by tracing a tip of each MT during its gliding motion. Displacements made by the moving tip for an equal time interval (10 sec) were collected. Each sampling defines the short-term speed of the MT motion by dividing the displacement by 10 sec (**Figure S1**). The surface coverage by MTs bound to kinesin coated glass surfaces was estimated by measuring the area fraction of MTs in fluorescence images. This was done after threshold adjustment by ImageJ to avoid MTs floating in solution being counted (Figure S8). 10 different areas (except the case of Tau (1/50) for which 9 different areas were measured) were measured to get the mean values in Figure 1b (error bars indicate +/- standard deviations). For particle tracking analysis, QD-cargo movements on MT tracks were recorded using Metamorph (streaming mode, exposure time for each frame: 100 ms). Supplementary movies, S10 and S11, show the QD-cargo motion on untreated and Tau treated MT tracks, respectively. QDs in the movie frames were further clarified by threshold adjustment (see Figure S9) before we applied the Matlab code for particle tracking (developed by Daniel Blair and Eric Dufresne, http://site.physics.georgetown.edu/matlab/). Resulting traces are shown in Figure 4a and 4b (static description) as well as supplementary movies S10 and S11 (dynamic description in which red circles keep following QDs). MSD was calculated with the particle tracking data. For this, MSD was defined by [3]

$$MSD(t) = \frac{\sum_{i=1}^{(N-n)} \left\{ \left(X_{i+\frac{t}{\delta}} - X_i \right)^2 + \left(Y_{i+\frac{t}{\delta}} - Y_i \right)^2 \right\}}{(N-n)},$$

where i is the data index for a QD, X and Y are coordinates of the QD (ratio of length to pixel: 0.06667 μm/pixel), δ is the constant time interval (100 ms), N is the maximum index, t is lag time and n is the dimensionless lag time (t/δ). MSD curves for all QDs tracked are plotted in a log-log scale as a function of t (Figure 4c and 4d). As our focus is on the processive movement of QDs along a linear track (dynamic regime close to the case of linear trajectory (ballistic regime), two slopes representing the ballistic mode (~ t^2) and the normal diffusion (~ t, independent of dimension), are added on the plot for comparison.[4,5] Each curve was normalized in a way that the initial value of the MSD is one.

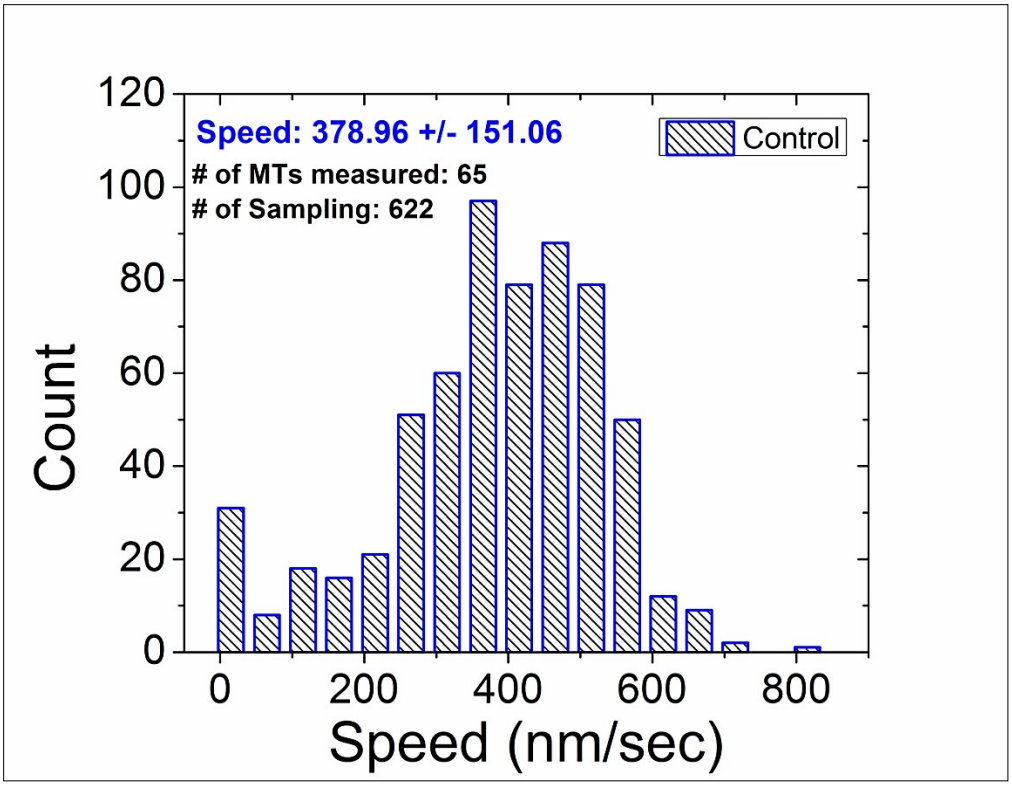

Fig. S1 Histogram for the speed of microtubules gliding on a QD kinesin coated surface without any treatment (control).

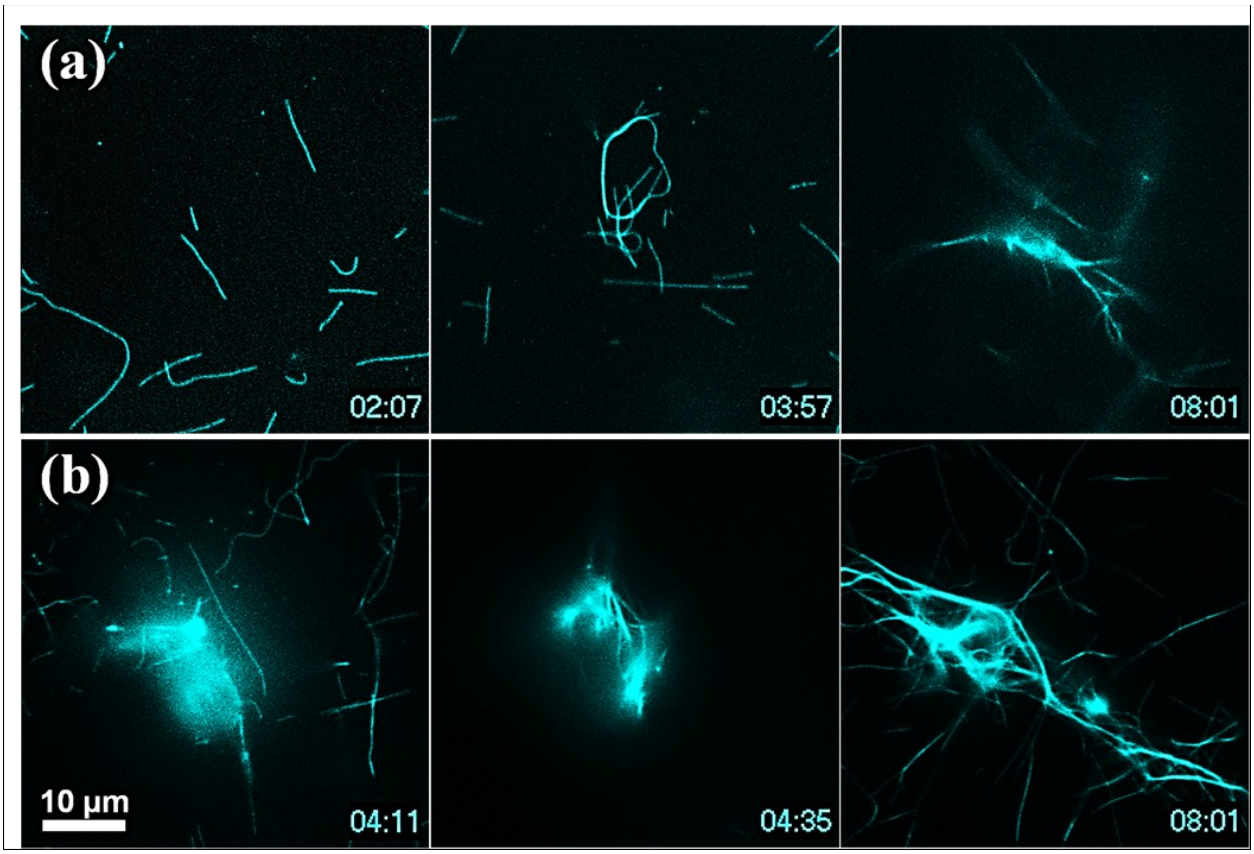

Fig. S2 (a) Time lapse of fluorescence images presenting MTs freely gliding on a kinesin coated surface at a ratio (1:1, tubulin: tau). Some MT-tau complexes show an apparent tug of war, exerted by competing forces on a MT by kinesin motors and tau proteins. At a later time, some population is in solution. (b) Time lapse of fluorescence images presenting diminished gliding of MTs on a kinesin coated surface at a ratio (1:5, tubulin: tau) where a larger population of MT-tau complexes are having a tug of war and some population is floating in solution.

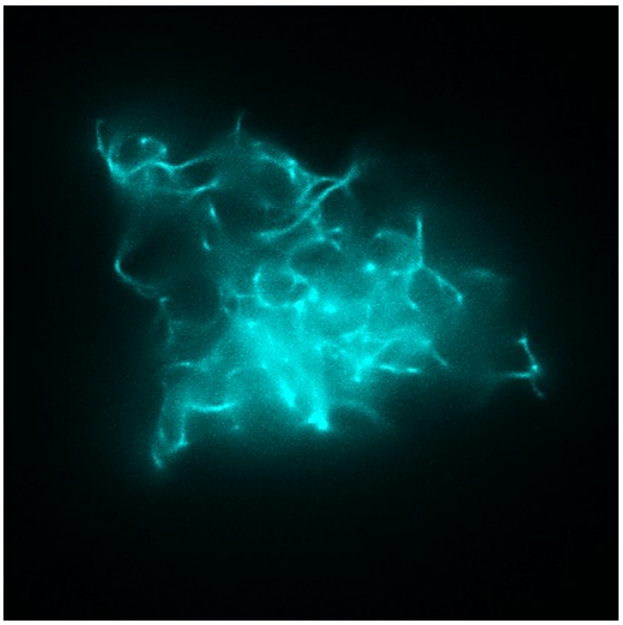

Fig. S3 Abrogation of MT gliding and subsequent MT cluster formation in solution by the treatment with poly-L-lysine, homopolypeptide construct.

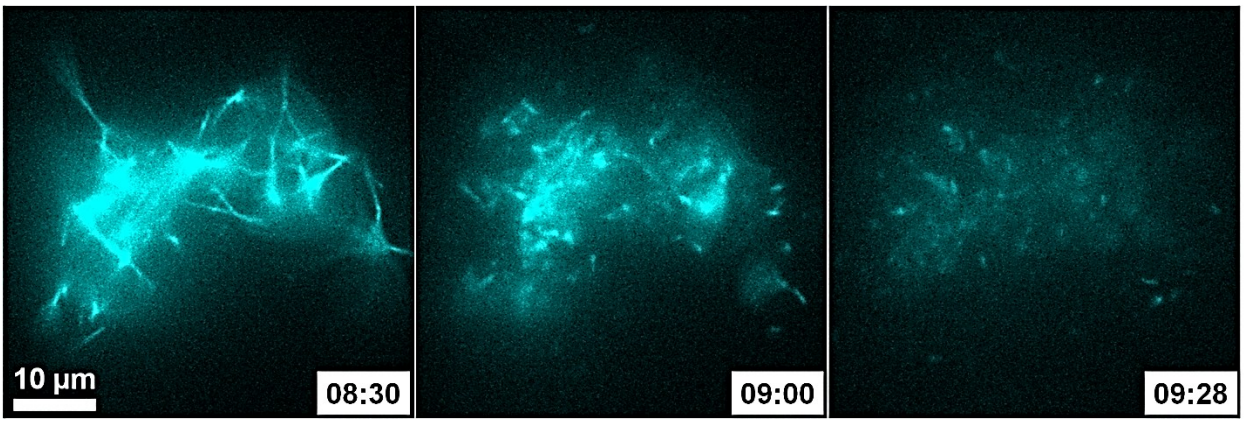

Fig. S4 Time lapse fluorescence images presenting a MT-tau complex collapsing in the presence of MSG (200 µM).

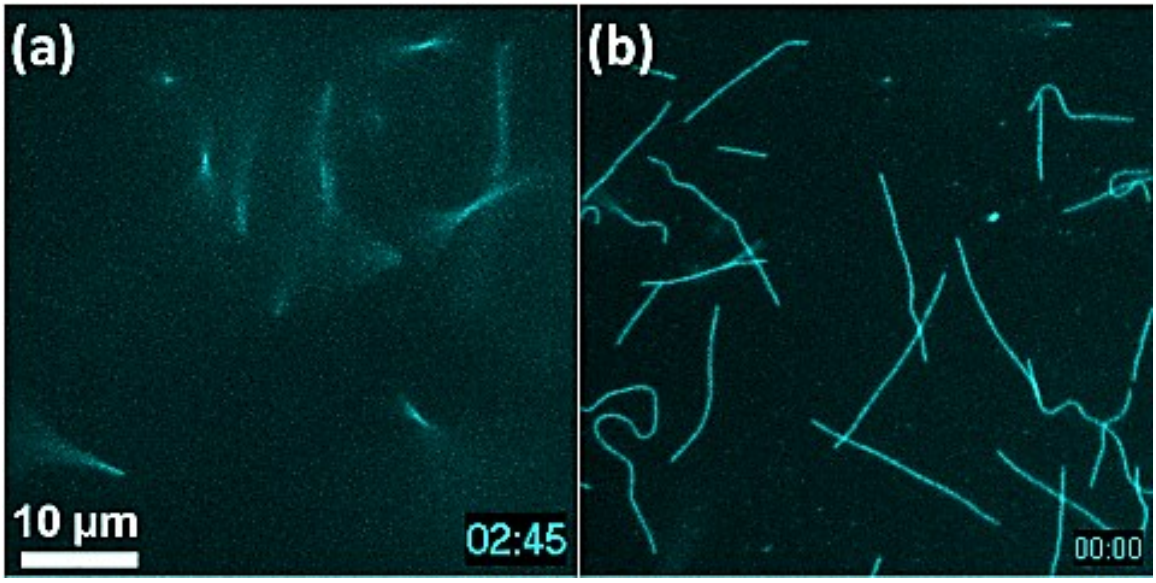

Fig. S5 Control samples showing the effect of (a) Iron (III) (1 mM) and (b) MSG (1 mM) on MT-kinesin shuttle system (Supplementary Movies).

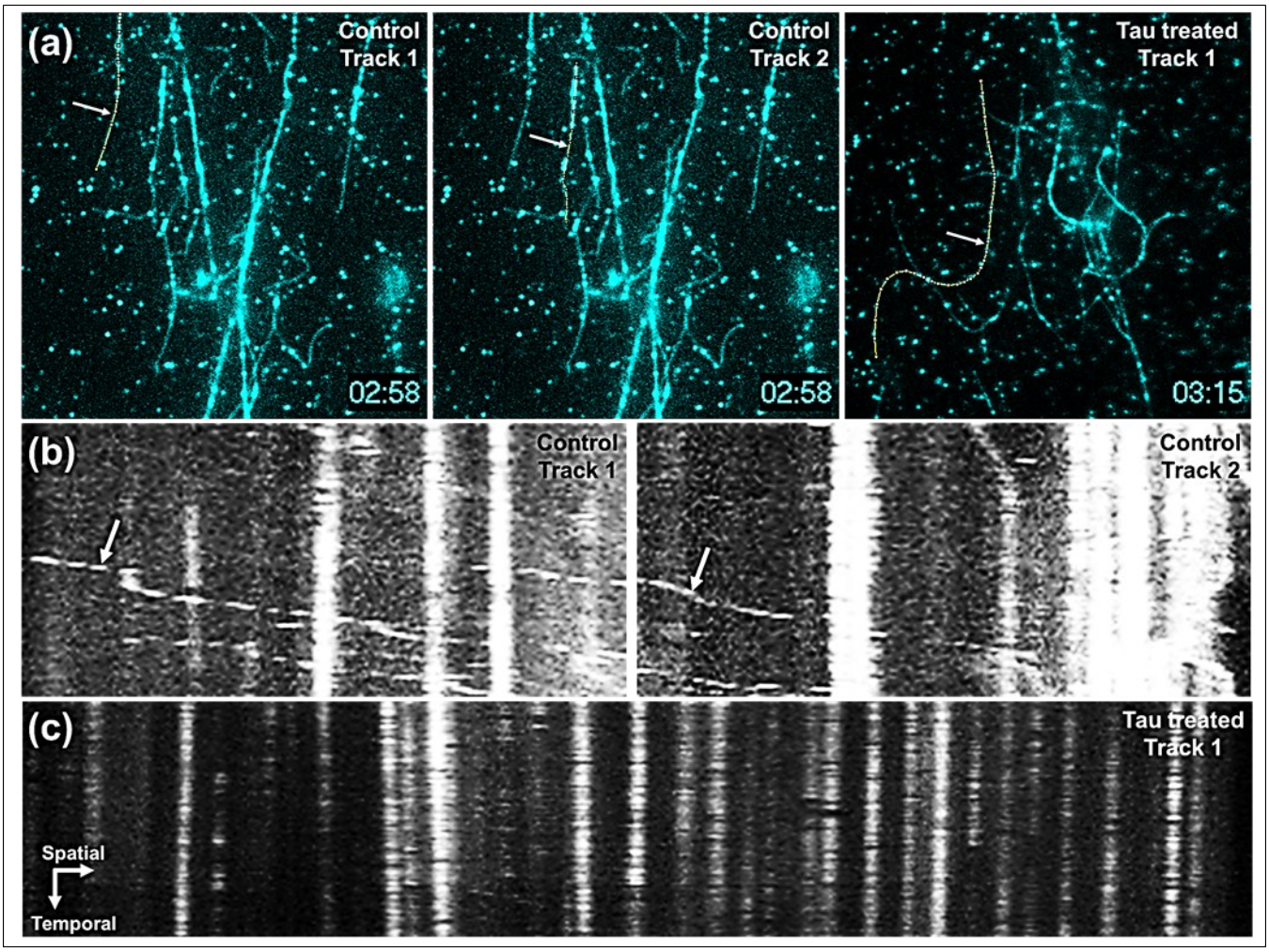

Fig. S6 (a) MT contours defined for kymographs to identify QD motion on MT tracks that have not been (left and middle)/have been treated (right) with tau protein. Frame size: ~ 34 μm × 34 μm. (b) Kymographs presenting kinesin driven QD transportations along MT tracks defined in (a). Arrows indicate kinesin driven QD cargo motion while vertical lines indicate immobile QDs. Frame sizes are 13.3 μm × 178 sec (left) and 13.5 μm × 178 sec (right), respectively. (**c**) Kymograph revealing only the immobile QDs on a MT track after tau protein treatment. Frame size: 31.5 μm × 178 sec.

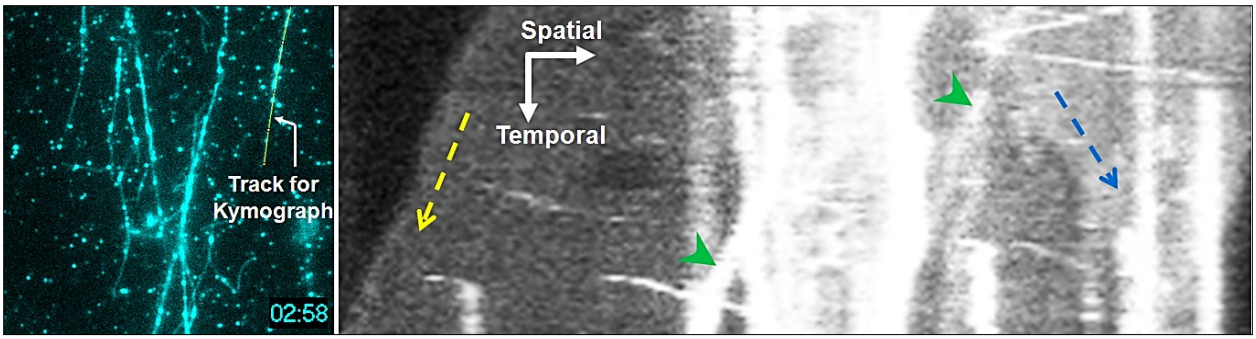

Fig. S7 A MT track chosen (left panel) for a kymograph (right panel, frame size: 16.6 μm 178 sec) to illustrate the kinesin driven QD transport. Thin white diagonal lines appear as indications of the kinesin driven QD motion along the MT track while vertical lines indicate immobile QDs. Zigzag motion (upper-right corner) indicate that the track was a bundle of MTs, consisting of two MTs with opposite polarity orientations, allowing the kinesin to change MT track from one to the other. Two intensity domain boundaries (two dotted arrows put along the boundaries) revealed in the kymograph display two microtubules gliding opposite to each other as evidence. Thicker white lines (see green arrows) mark QDs that were fixed on a gliding MT as the lines are almost parallel to one of the boundary (yellow arrow).

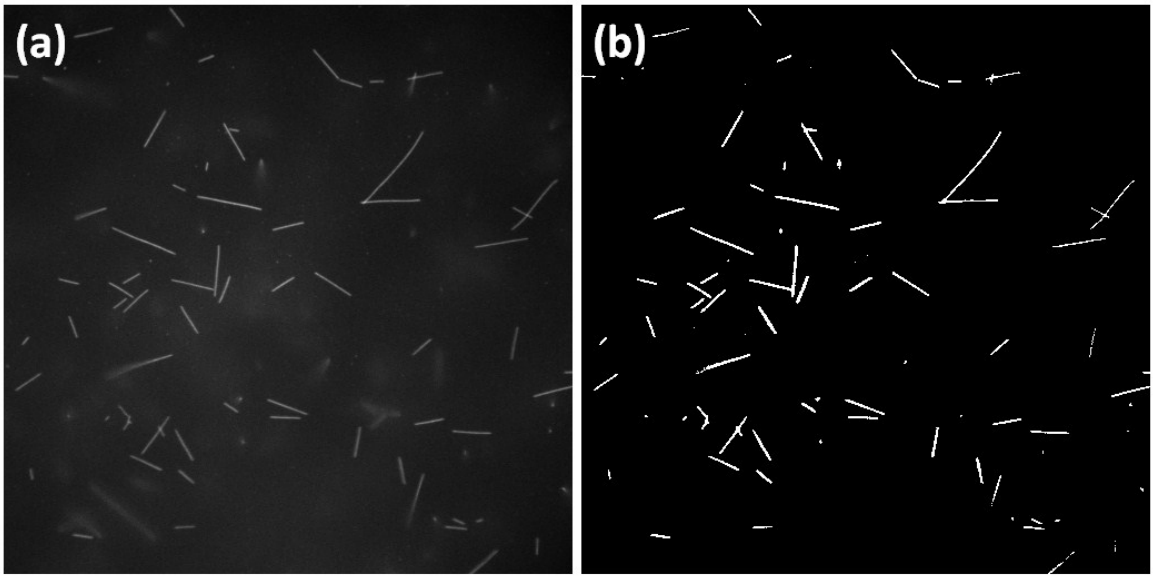

Fig. S8: MT surface coverage estimation. (a) A fluorescence image of MTs on a kinesin coated glass surface. MTs floating either near the surface or in the solution can be recognized. (b) The same image after threshold adjustment to only count MTs bound on the surface for the area fraction measurement in Figure 1b.

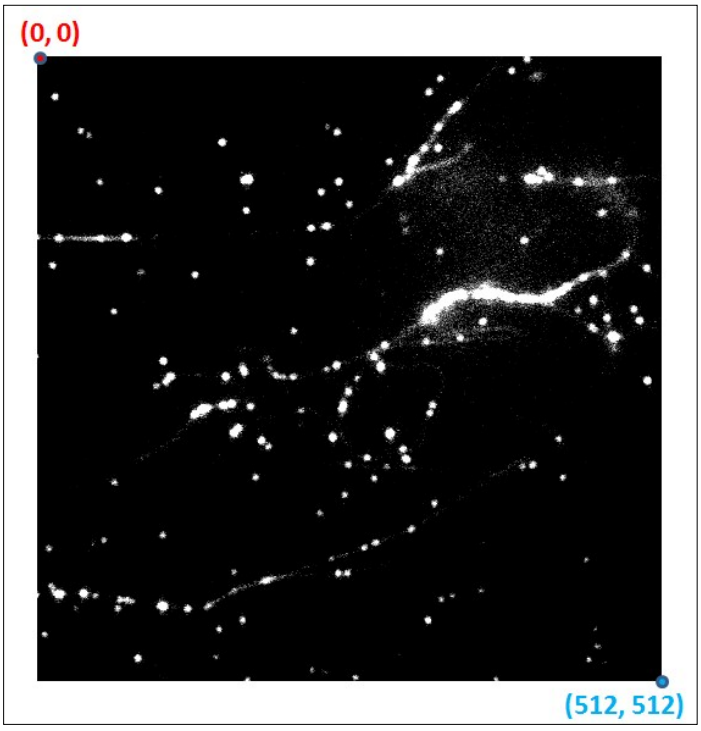

Fig. S9: Example of a threshold adjustment of fluorescence image displaying QD-cargos on a surface where MT tracks are bound. The two points at the top-left and bottom-right are added to indicate the x, y coordinates in pixels. Note that this coordinate system is flipped upside down in Figure 4a.

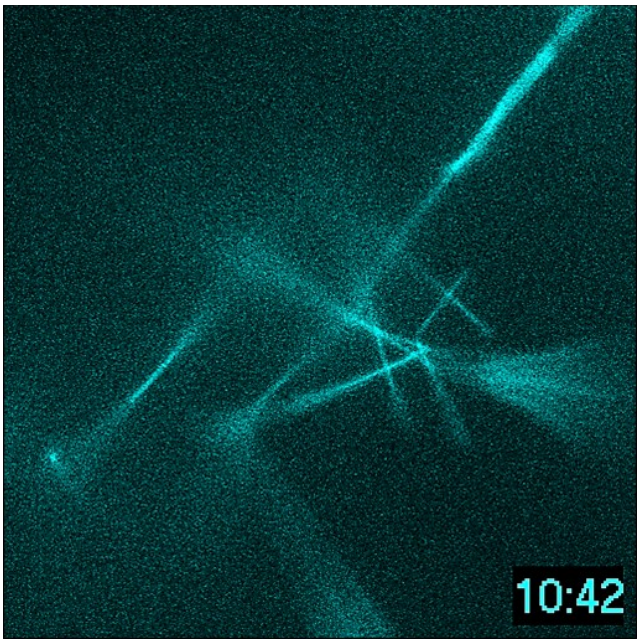

Fig. S10: Effects on the stability of MT-tau protein complex upon treatment with aspirin (100μM).

Table 1:

Treatment	Effect on MT	Effect on MT-tau complex
Fe^{3+} (100 μM)	Impaired gliding	Unlock/release microtubule from bundle
Fe^{3+} (1 mM)	Abrogation of biding and gliding	ND
MSG (200 μM)	Same as control	Demolish the MT structure from MT-tau complex
Aspirin (100 μM)	No effect	No effect
Tau protein (0.4 μM)	Impaired gliding and filament/bundle formation	ND

Table 1 summarizing the effect of various treatments to MT gliding and MT-tau oligomer stability; ND (not determined)

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