SUPPLEMENTAL INFORMATION

Understanding the role of transport velocity in biomotor-powered microtubule spool assembly

Amanda J. Tan[a], Dail E. Chapman[b], Linda S. Hirst[a], and Jing Xu[a,*]

[a]Physics, University of California, Merced, CA 95343, USA

[b]Developmental and Cell Biology, University of California, Irvine, CA 92697, USA

[*]**Correspondence:** Jing Xu (jxu8@ucmerced.edu)

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Experimental

Proteins and reagents

Biotin-labeled tubulin (T333P) and rhodamine-labeled tubulin (TL590M) were purchased from Cytoskeleton Inc. (Denver, CO, USA). Streptavidin-fluorescein isothiocyanate conjugate (21224) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Recombinant kinesin consisting of the first 560 amino acids of human kinesin-1 was prepared using plasmid pET17_K560_GFP_His[1] (Addgene, Cambridge, MA, USA). Kinesin was bacterially expressed and Ni-NTA purified as previously described.[2]

Microtubule preparation

Microtubules were rhodamine-labeled for fluorescence imaging and biotin-labeled to mediate streptavidin-based lateral interactions between microtubules. Dual-labeled microtubules were prepared by incubating tubulin mix (6:1 biotinylated tubulin to rhodamine-labeled tubulin, 0.47 mg/mL in PEM80 buffer (80 mM PIPES, 1 mM ethylene glycol bis(β-aminoethyl ether), 1 mM $MgSO_4$, pH 6.9)) supplemented with 20 μM taxol and 2 mM GTP for 1 h at 37 °C. Microtubules were kept at room temperature in a dark box and used within four days of preparation.

Microtubule spooling experiments

Microtubule spooling experiments were carried out in flow cells in vitro. Flow cells were constructed using a coverslip (22 mm x 22 mm) and a microscope slide, which were held together with double-sided tape (Fig. 1a). Both the coverslip and the microscope slide were biologically clean (accomplished via sequential washing with acetone and ethanol). The volume of each flow cell was approximately 10 μL.

Kinesin solution (100 nM in PEM80 buffer supplemented with 20 mM dithiothreitol) was flowed into the flow cell to bind to the glass surface (5 min). Excess/unbound kinesin was washed away and the flow cell was blocked with 5 mg/mL bovine serum albumin in PEM80 buffer supplemented with 20 mM dithiothreitol and 50 μM taxol. Dual-labeled microtubules (0.047 mg/mL) were introduced into the flow cell to bind kinesin (3 min). Excess/unbound microtubules were washed away with an anti-fade solution (20 mM dithiothreitol, 8.5 mg/mL glucose, 0.28 mg/mL glucose oxidase, and 210 mM catalase in PEM80 buffer) supplemented with 8 μM ATP. Streptavidin-fluorescein isothiocyanate (0.01 mg/mL in PEM80 buffer supplemented with 10 μM ATP, 20 μM taxol, and 0.2 mg/mL bovine serum albumin) was exchanged into the flow cell and incubated for 3 min. Excess streptavidin-fluorescein isothiocyanate was washed out with anti-fade solution. Finally, anti-fade solution supplemented with ATP (0-1 mM) and an ATP regenerating system[3] (2 mM phosphocreatine and 70 μg/mL creatine phosphokinase) were added to the flow cell to initiate kinesin-based microtubule gliding. The flow cell was then sealed using vacuum grease (Dow Corning, Midland, MI, USA) to prevent drying.

Imaging and analysis

Self-assembly of microtubules was imaged via epifluorescence microscopy using a fluorescence microscope (DM 2500P, Leica Microsystems Inc., Buffalo Grove, IL, USA) equipped with a Retgia Exi camera (QImaging, Surrey, BC, Canada). For each flow cell, five areas were observed; each area was typically imaged for 10.6 min at 40x magnification and at 0.17 Hz (0.5-1 s exposure).

Image analysis was carried out using Image Processing and Analysis in Java (ImageJ, http://imagej.nih.gov/ij/). The gliding velocity of microtubules was measured using the MTrackJ plugin for ImageJ (http://www.imagescience.org/meijering/software/mtrackj/). Microtubules typically assembled into linear bundles prior to spool formation.[4, 5] We tracked the trailing end of each microtubule bundle to determine the microtubule gliding velocity at each ATP concentration. The number of microtubule spools in an image was counted manually. The circumference of microtubule spools in an image was determined manually using the circle tool and the segmented line tool in ImageJ.

Supporting Movie

Movie S1. A representative movie of microtubule spool assembly at 1 mM ATP. The movie is sped up 5-fold.

Supporting Figure

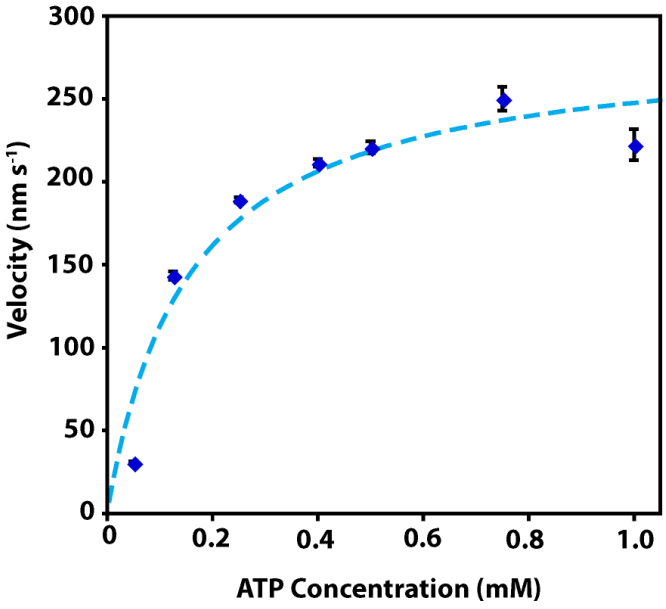

Figure S1. Microtubule gliding velocity as a function of ATP concentration. The dependence of microtubule gliding velocity on ATP concentration is well characterized by Michaelis-Menten kinetics. Error bar, standard error of the mean. Dashed line, Michaelis-Menten kinetics (K_m = 154±54 μM, and V_{max} = 286±28 nm/s). At 1 mM ATP, the velocity of microtubule gliding (~200 nm/s) is consistent with the 100 nm/s reported previously.[6]

Supporting References

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