Supporting Information

Experimental methods

All tubulin was obtained from Cytoskeleton, Inc (Denver, CO). Chemical reagents were provided by Sigma (St. Louis, MO). Silica microspheres were purchased from Bangs Laboratories (Fishers, IN). Fluorescent streptavidin and streptavidin-labeled quantum dots were obtained from Invitrogen, Inc. (Carlsbad, CA).

Monomeric, unlabeled tubulin was dissolved at 5 mg/mL in BRB80 (80 mM piperazinebis(ethanesulfonic acid), 1 mM $MgCl_2$, 1 mM ethylene glycol bis(b-aminoethyl ether-N,N,N,',N'-tetraacetic acid (EGTA), pH 6.9). This tubulin was first reacted with 1 mM N-ethyl-maleimide (NEM), 0.1 mM guanosine 5'-triphosphate (GTP) in water for 10 min at 0^oC before quenching with 8mM β-mercaptoethanol (BME) for 10 min at 0^oC. Excess BME, GTP, and NEM was removed using a Biorad P6 spin column.

Polymerization of "plus" end functionalized MTs was achieved by polymerizing 1 μL of 5 mg/mL rhodamine-labeled tubulin in BRB80P (BRB80 + 10% glycerol + 1 mM GTP) for 10 minutes at 37^oC before adding 1 μL of 5 mg/mL NEM-capped tubulin in BRB80P for an additional 20 minute polymerization. 5 μL 0.5 mg/mL biotinilated tubulin in BRB80P was then added directly to the polymerizing tubes. Following a 20 minute polymerization of this mixture at 37^oC, the polymerized MTs were stabilized by the addition of 200 μL of BRB80T. Tubes were collected by centrifugation at 20,000 x g and resuspended in 10 μL of BRB80TAF (BRB80T in 20mM dextrose + 0.02mg/mL glucose oxidase, 8 μg/mL catalase, and 0.5% β-mercaptoethanol).

Polymerization of “minus” end functionalized MTs was achieved using identical methods, but the order of introducing the biotinylated and rhodamine-labeled tubulin was reversed.

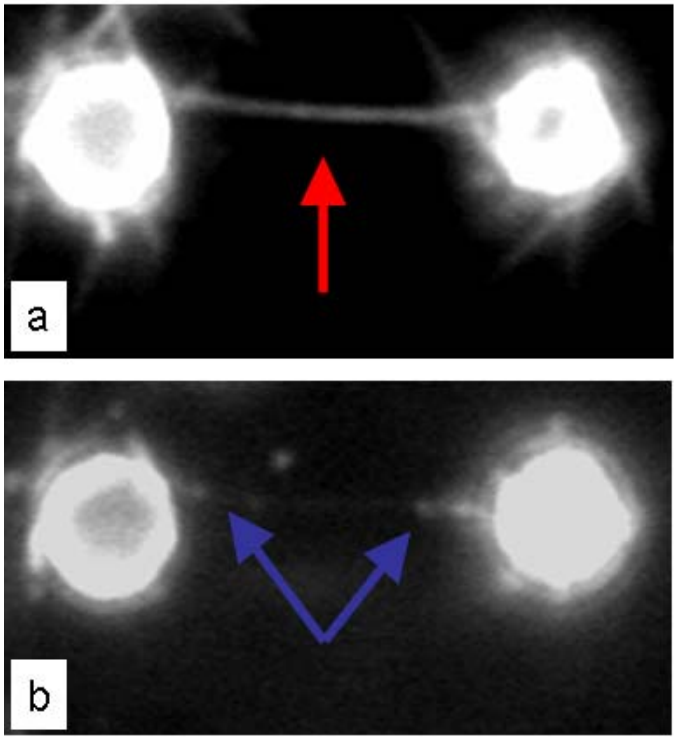
To test the polarity of the functionalized MTs, they were examined using an inverted motility assay. 20 μL of BRB80 containing 0.5 mg/mL of casein was introduced to a blank cover slip flow cell and allowed to incubate at room temperature for 5 minutes. *Drosphilia Melanogaster* dimeric kinesin proteins, expressed and purified from *Escherichia coli*,[26] were flowed into the cell and allowed to incubate another 5 minutes. A suspension of polar MTs and streptavidin-labeled microspheres (dragon green fluorescent, 0.5 μm) in motility buffer (BRB80TAF containing 100 μM adenosine triphosphate (ATP)) was then introduced to the cell and MT motility was imaged by fluorescence microscopy using an Olympus IX71 microscope and a Hamamatsu digital camera.

Functionalization of silica microspheres was achieved by first washing 100 μL of 2.34 μm silica microspheres 3X with BRB80, then 2X with chloroform, collecting beads by centrifugation in a Galaxy benchtop centrifuge. Beads were then treated for 20 min at room temperature in a 10 mM solution of 3-aminopropyltriethoxysilane (APTES) in chloroform. Following silanization, beads were rinsed 3X with chloroform and then dried and annealed dry at 60^{o}C for 1 hour. These amine-functionalized beads were then suspended with a 10 mM Biotin-N-hydroxysuccinimide ester in dimethylformamide (DMF) for 1 hour. Following 3 rinses with DMF and 2 rinses with PBS (phosphate buffered saline (pH 7.4)), beads were incubated with 1 mL of Oregon Green-labeled

streptavidin (10X dilution in PBS) or streptavidin-labeled quantum dots (500X dilution) in1 mL of PBS for at least 10 minutes before use.

The POSMOCs were assembled by first rinsing 1 μL of streptavidin-functionalized microspheres twice in BRB80. Beads were then incubated for 10 minutes in BRB80 containing 0.5 mg/mL casein. Beads were then spun down and resuspended in 10 μL of polar MTs in BRB80TAF as synthesized above. This suspension was allowed to incubate at room temperature for a minimum of 10 minutes before flowing into a cover slip flow cell comprising two glass cover slips adhered to one-another by two pieces of double-sided clear tape, the pieces of tape separated to form a channel approximate 5 mm wide. POSMOCs were then imaged by fluorescence microscopy.

In cases where quantum dots were added to the assembled POSMOCs, streptavidin-labeled quantum dots, diluted 1000X in BRB80TAF, were introduced to the flow cell and allowed to incubate 2 minutes before rinsing with BRB80TAF.



Supplemental Figure 1: Fluorescence micrographs of a microtubule bridging two functionalized beads. The MT was grown in stages to create functionalized segments, but no NEM was used to block the "minus" end of the growing MTs. The resulting MT was biotinylated on both ends, enabling it to form a bridge between the two beads (~2.3 micron bead diameter). In a), the image shows the fluorescence from both the rhodamine-labeled tubulin and the fluorescent quantum dots used to label the biotinylated regions of the MT, revealing the entire MT bridge In b), the image shows only the fluorescence from the quantum dots, revealing the isolation of the quantum dots on the ends of the segmented MT. This result clearly shows that both ends of the MT were biotinylated when no NEM was used.