Supporting Information

Interactions regulating the head-to-tail directed assembly of biological Janus rods

A.C. Greene,[a] M. Bachand,[b] A. Gomez,[c] M.J. Stevens,[b,c] and G.D. Bachand[b]*

[a.] WMD Threats and Aerosol Science Department, Sandia National Laboratories, Albuquerque, NM 87185 USA.
[b.] Center for Integrated Nanotechnologies, Sandia National Laboratories, PO Box 5800, MS 1303, Albuquerque, NM 87185 USA.
[c.] Center for Materials Science & Engineering, Sandia National Laboratories, Albuquerque, NM 87185 USA.

Experimental

Preparation of MTs and Kinesin Motor Proteins

TRITC and HiLyte488 fluorescently-labeled porcine tubulin was purchased from Cytoskeleton, Inc. (Denver, CO). Tubulin was prepared by rehydrating the lyophilized tubulin protein in BRB80 buffer (80 mM PIPES pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA) supplemented with 1 mM guanosine triphosphate and 10 % glycerol to a concentration of 5 mg mL^{-1} tubulin. Tubulin was polymerized at 37 ˚C for 20 min and stabilized by diluting 100-fold into BRB80 supplemented with 10 µM paclitaxel (BRB80T), resulting in a final concentration of ~0.9 µM.

A histidine-tagged kinesin-1 heavy chain motor protein from *Drosophila melanogaster* was expressed from the pPK113[1] plasmid in *Escherichia coli* BL21 (DE3) pLysS cells. At an OD 600 nm of ~0.7, protein expression was induced through the addition of 0.5 mM isopropylthio-β-galactoside (IPTG). Cells were harvested through centrifugation at 9,000 x g and then stored at -80 ˚C until used. Cells were lysed using a lysis buffer (50 mM $NaPO_4$ pH 8.0, 300 mM NaCl, 40 mM imidazole, 5 mM BME, 10% glycerol and 50 µM MgATP) supplemented with BugBuster®, 100 µM AEBSF and benzonase (all from EMD Millipore, Billerica, MA). Kinesin was then purified using an Ni-NTA gravity column equilibrated in wash buffer (50 mM $NaPO_4$ pH 7.0, 1 M NaCl, 80 mM imidazole, 5 mM BME, and 50 µM MgATP) as previously described.[1,2] Following two washes with wash buffer, kinesin was eluted (50 mM $NaPO_4$ pH 7.0, 300 mM NaCl, 700 mM imidazole, 5 mM BME, and 50 µM MgATP) and protein presence was confirmed using polyacrylamide gel electrophoresis (PAGE). Kinesin concentration was measured using a Bradford assay.

Characterization of MT Self-Assembly

TRITC and HyLyte488 labeled tubulin was polymerized as described above. Following stabilization with BRB80T, the indicated concentration NaCl were added to the differentially-labeled populations. The ionic strength (I) of BRB80 has been estimated to be 176 mM (130 mM monovalent species)[3]. NaCl was added to BRB80 at the following concentrations: 0, 50, 100, 150, 200 or 300 mM. The two separate populations were combined and stored at room temperature in the dark. Flow cells for inverted kinesin-MT assays were constructed by adhering a #1 glass coverslip to double-sided tape on a microscope slide.

A solution containing 1.5 mg mL^{-1} casein, ~5nM kinesin and 1.5 mM adenosine 5'-(β,γ-imido)triphosphate lithium salt hydrate (or AMP-PNP, a non-hydrolyzable form of ATP) was incubated at room temperature in the flow cell for 5 min. MTs were then infused into the flow cell in a motility solution composed in BRB80T containing 0.15 mg mL^{-1} casein, 1 mM AMP-PNP, 0.02 mg mL^{-1} glucose oxidase, 0.08 mg mL^{-1} catalase, 20mM glucose and 1 mM DTT (the last three compounds are anti- fade reagents)[2] and allowed to bind to the kinesin-coated surface for 5 min. After incubation, the flow cell was washed with the motility solution to remove unbound any unbound microtubules. Characterization of the self-assembled MTs was performed by epifluorescence using an Olympus IX71 microscope and Hamamatsu ORCA 3CCD camera. Still-frame images were taken every hour over the course of eight hours.

Calculating Rates of MT Self-Assembly

As previously shown for actin filaments, the time course of end-to-end assembly between two filaments is consistent with a simple biomolecular reaction (Equation 1).[4]

1 $$R = k^{+}[ends]^2$$

To determine the rates of assembly, the relative concentration (percentage) of single-coloured microtubules ("monomers") over time was determined by Equation 2:[5]

2 $$C_{MT} = \frac{MT_t - MT_m}{MT_t}$$

The number of single-colored (or monomeric) microtubules (denoted as MT_m) and the total number of microtubules (denoted as MT_t) per field of view for five fields of view was determined for all treatments at each time point. Based on the second order kinetics, these data were then plotted as 1 / P_m and fit using linear regression analysis in GraphPad Prism (La Jolla, CA) to derive the slope, k^+.

Results

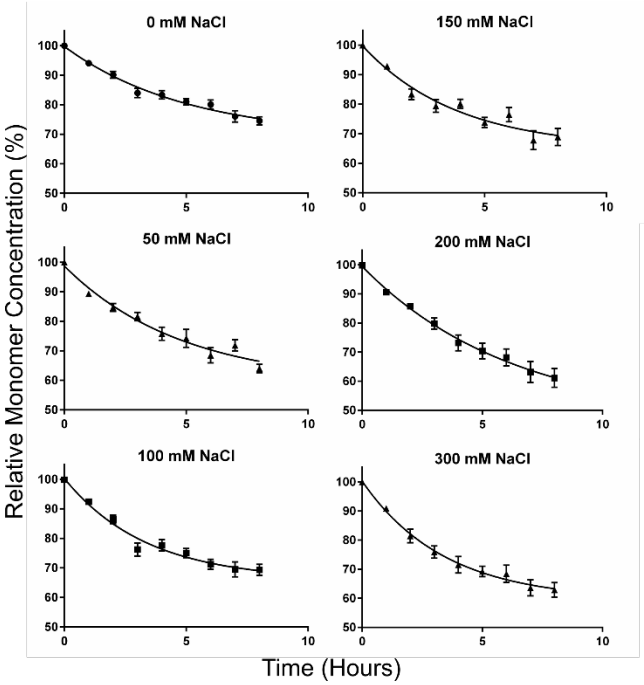

Figure S1. The percentage of monomeric (single-colored) microtubules over time in the presence of added NaCl.

S1 Table. Summary of assembly rates (i.e., slope), standard deviations, and linear regression fits for different added NaCl concentrations**.**

Concentration NaCl (mM)	Assembly Rate* (1/h)	Standard Deviation	R^2
0	0.0004186	2.93e-005	0.9669
50	0.0006422	5.82e-005	0.9457
100	0.0005802	6.55e-005	0.9182
150	0.0005842	6.50e-005	0.9203
200	0.0008912	3.04e-005	0.9919
300	0.0007821	5.94e-005	0.9611

***** Rates reported in the paper were converted to units of s^{-1}.

References

1 D. L. Coy, M. Wagenbach and J. Howard, *J. Biol. Chem.*, 1999, **274**, 3667–3671.

2 G. D. Bachand, S. B. Rivera, A. K. Boal, J. Gaudioso, J. Liu and B. C. Bunker, *Nano Lett.*, 2004, **4**, 817–821.

3 N. F. Bouxsein and G. D. Bachand, *Biomacromolecules*, 2014, **15**, 3696–3705.

4 D. B. Murphy, R. O. Gray, W. A. Grasser and T. D. Pollard, *J. Cell Biol.*, 1988, **106**, 1947–1954.

5 M. Bachand, N. F. Bouxsein, S. Cheng, S. J. von Hoyningen-Huene, M. J. Stevens and G. D. Bachand, *RSC Adv.*, 2014, **4**, 54641–54649.