Supporting Information

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

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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 MgCl2, 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 pPK1131 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-  

Characterization of MT Self-Assembly

TRITC and HiLyte488 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.

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).\(^4\)

\[
1 = k^* [\text{ends}]^2
\]
To determine the rates of assembly, the relative concentration (percentage) of single-coloured microtubules ("monomers") over time was determined by Equation 2.5

\[ C_{MT} = \frac{MT_t - MT_{m}}{MT_t} \]

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

![Graph showing the percentage of monomeric microtubules over time for different NaCl concentrations.](image)

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.

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

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