Directed Head-to-Tail Self-Assembly of Biological Janus Rods

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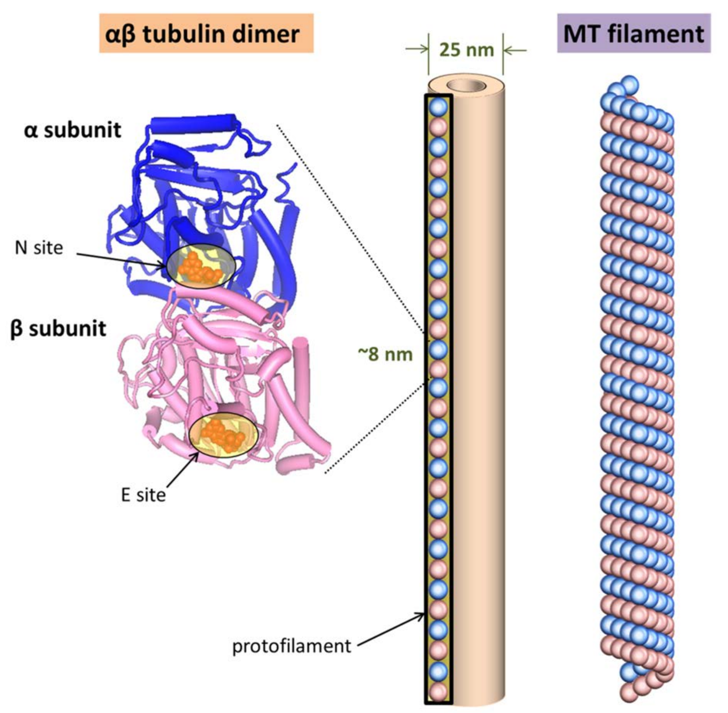
Microtubules – cellular biopolymers

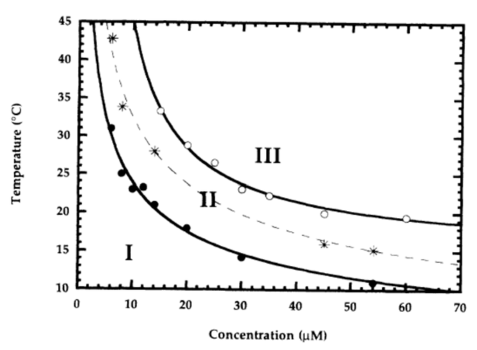
Microtubules (MTs) are one of three protein filaments that compose the cytoskeleton of eukaryotic cells. They play critical roles in common physiological processes (e.g., mitosis), as well as cancer and neurodegenerative disorders.

MTs assemble from αβ tubulin dimers, forming protofilaments (i.e., chains of dimers) that subsequently associate to form a closed tubular filament.

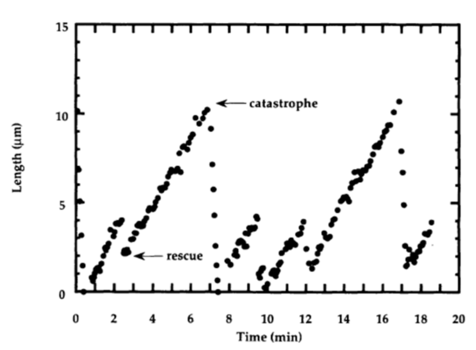
The mature MT is ~25 nm in diameter, 10s of microns in length, and has a persistence length on the order of 1-10 mm.



Dynamic, multiscale assembly and disassembly



Fygenson et al. (1994)Phys. Rev. E, 50, 1579

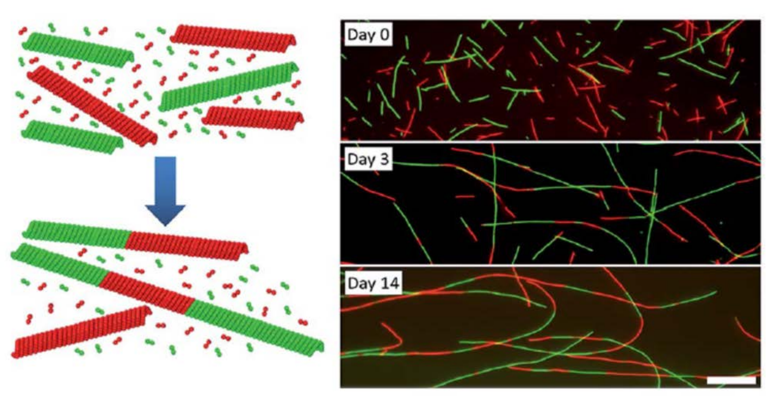


MT polymerization is based on a nucleation and growth process. Above a critical concentration (region III) tubulin will spontaneously nucleate in solution and form protofilaments and MT filaments. Region II is defined by growth from nucleated seeds, whereas both nucleation and growth are absent in region I.

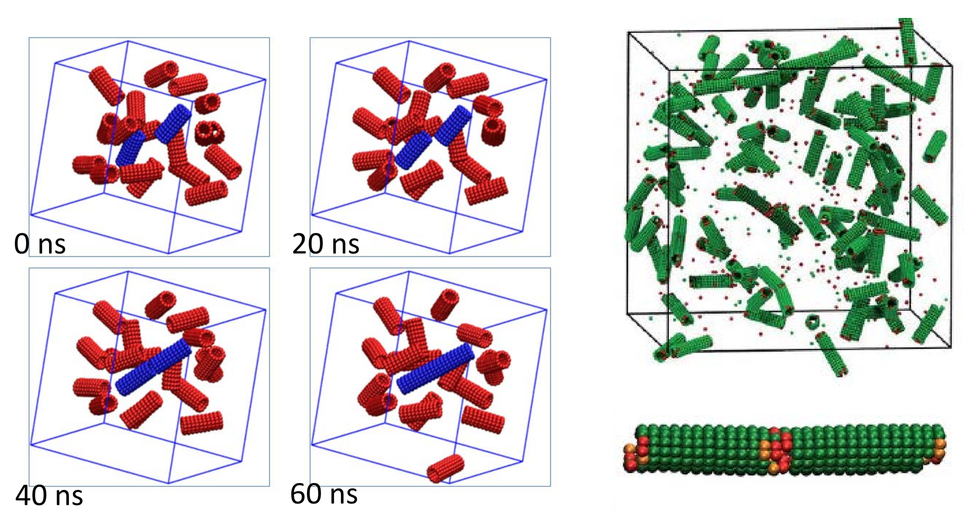
Polymerization involves the hydrolysis of guanosine triphosphate (GTP). The free energy of hydrolysis induces conformation changes in αβ tubulin that ultimately drive the catastrophic depolymerization of MTs. Rescue (i.e., re-polymerization) occurs when the tubulin concentration enters either region II or III. This cyclic process of polymerization and depolymerization is known as dynamic instability.

Directed, self-assembly (DSA) of MT filaments

MTs, stabilized against depolymerization with paclitaxel (Taxol®), will assemble, head-to-tail, over extended time periods, days to months. DSA was observed by fluorescence microscopy (right) using a mixed population of red (Rhodamine) and green (HiLyte® 488) MTs containing free tubulin dimers.



Molecular dynamics (MD) simulations (right) support the hypothesized process of DSA. MD also indicates that free tubulin dimers are able to incorporate at junctions, filling lattice vacancies that result from annealing of imperfect and/or jagged ends.



Bachand et al. (2014), RSC Adv. **4**, 54651

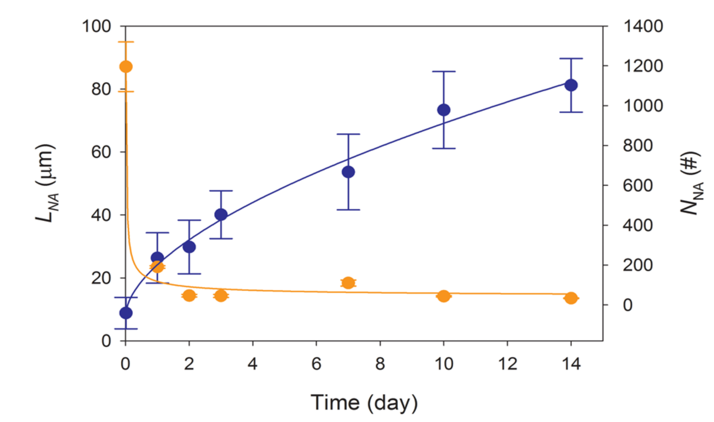
DSA is diffusion-limited, second-order rate process

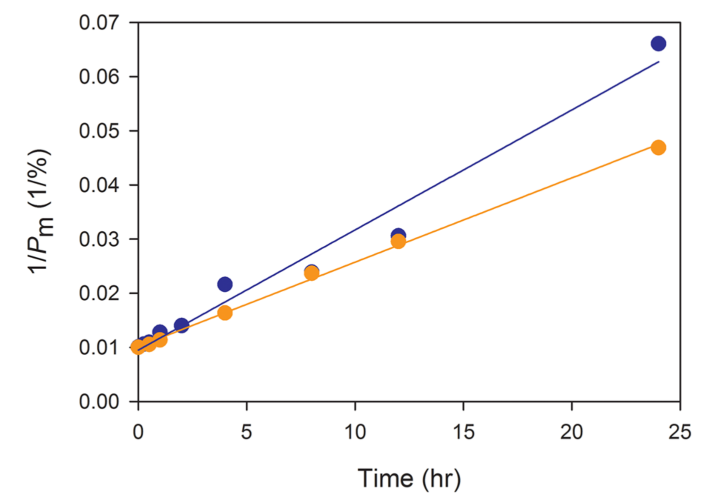
The DSA of MTs, as measured by an increase in length (L_{NA}) over time (●), follows a power law function (right) suggesting that assembly is diffusion limited.

$$L_{NA} = 8.2 + 16t^{0.58}$$

Change is L_{NA} is inversely proportional to the number of MTs (N_{NA}) (●), demonstrating that the increase is length is not due to nucleated polymerization.

Kinetic rate of DSA characterized by change in "monomer" (single color MTs) concentration over time. Second order rate kinetics observed, with the rate significantly enhanced by the presence of free GTP-tubulin in solution (●), when compared against MTs alone (●).



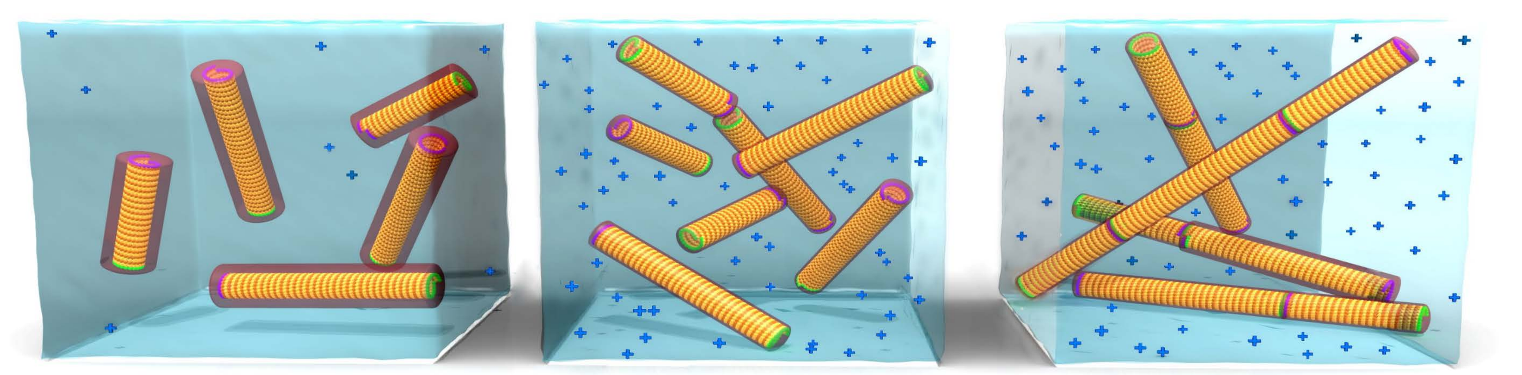


Bachand et al. (2014), RSC Adv. **4**, 54651

Microtubules – biological Janus rod

MTs generalized as biological Janus rods:

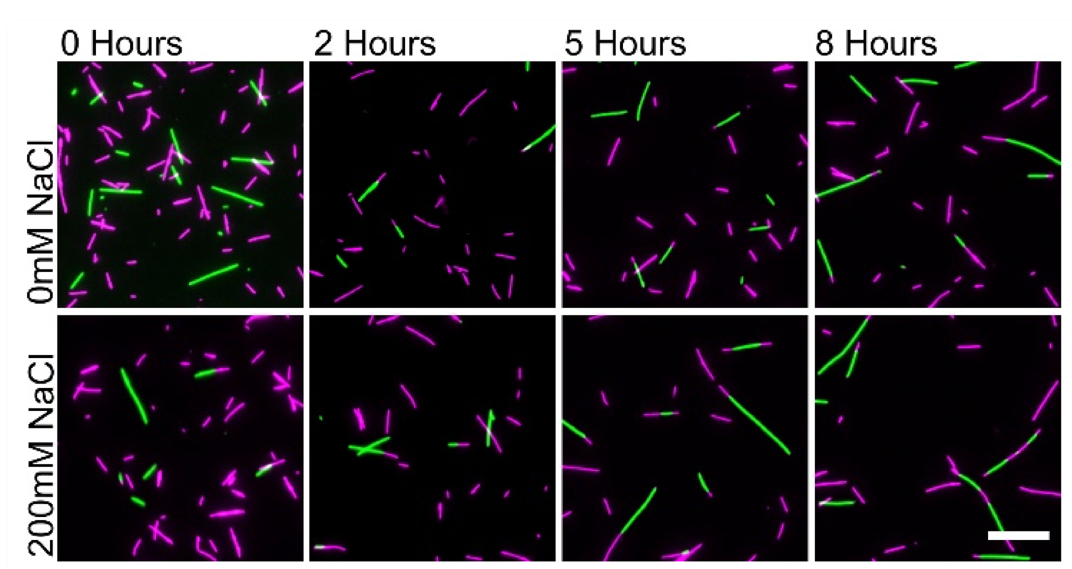
- High aspect ratio (>100:1)
- Rigid: persistence length 1 – 10 mm
- Electrostatically repulsive (~260 e⁻ μm⁻¹)
- Anisotropic end-end interactions



Hypothesis: Addition of monovalent counterion salts will decrease the long-range repulsion and excluded volume exhibited by the MT filaments, leading to an increase in the rate of MT DSA.

Counterion-enhanced DSA

Fluorescence microscopy of red (Rhodamine) and green (HiLyte® 488) MTs in the absence and presence of added NaCl (counterion). Qualitative difference in the DSA rate with the addition of 200 mM NaCl, evidenced by greater number of segmented MTs.



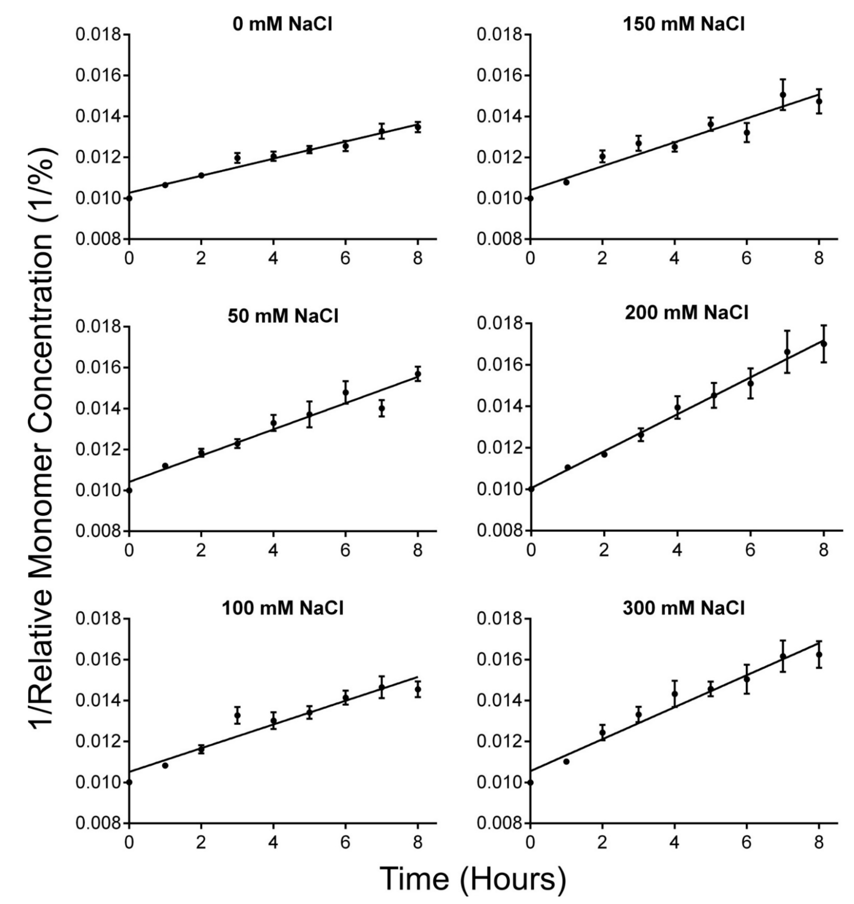
Greene et al. (2017) Chem. Comm., 52, 4493

Counterion-enhanced DSA

Rates of DSA determined by measuring the change in relative monomer concentration (i.e., single color MTs) over time.

Inverse concentration values were plotted as a function of varying concentrations of added NaCl ranging from 0 to 300 mM (right). Rates were derived from linear curve fitting to the data.

Data confirm the second-order rate kinetics as was observed in prior experiments, but demonstrate clear differences based on the addition of NaCl (below).



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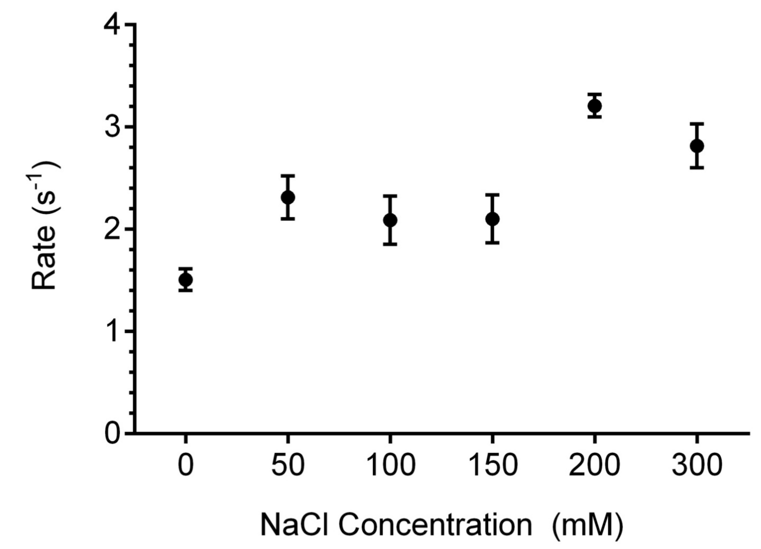

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ANOVA indicates that that the addition of NaCl significant affects the rate of MT assembly (P = 0.002).

DSA rate nearly doubled in the presence of added 200 and 300 mM NaCl, compared to 0 mM NaCl (left).

Increase in ionic strength of the buffer results in counterion condensation, effectively reducing the net charge of the MT.

Screening long-range electrostatic repulsive forces increases the probability that opposing MTs collide and interact. Short-range hydrophobic interactions at the MT ends enhance the rate of DSA, ultimately allowing for the formation of stable junctions

Conclusions & Future Work

DSA of MT filaments is governed by a balance of long- and short-range interactions in a manner analogous to that reported for the assembly of synthetic Janus particles. Our studies establish a deeper understanding of the multiscale assembly of tubulin and MT filaments, as well as a broader perspective of how the balance between long- and short range interactions may be tuned to direct the assembly of Janus colloids and rods. Future work will focus on applying the DSA of MTs as multifunctional templates for the synthesis and assembly of composite nanomaterials, such as heterostructured nanowires.

Acknowledgements

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