Characterization of Microtubules for Use in Multi-Scale Nanomaterials

Adrienne C. Greene, Marlene Bachand, Andrew Gomez, and George D. Bachand

Center for Integrated Nanotechnologies
Sandia National Laboratories

MRS Fall Meeting

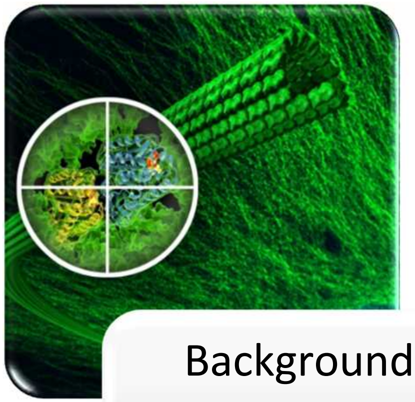
December 1st, 2015

Office of Basic Energy Sciences • Serving the Present, Shaping the Future •

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Sandia National Laboratories

Outline for Today's Talk



Background

- Biopolymers
- Self-assembly at multiple length-scales

Building Microtubule Arrays

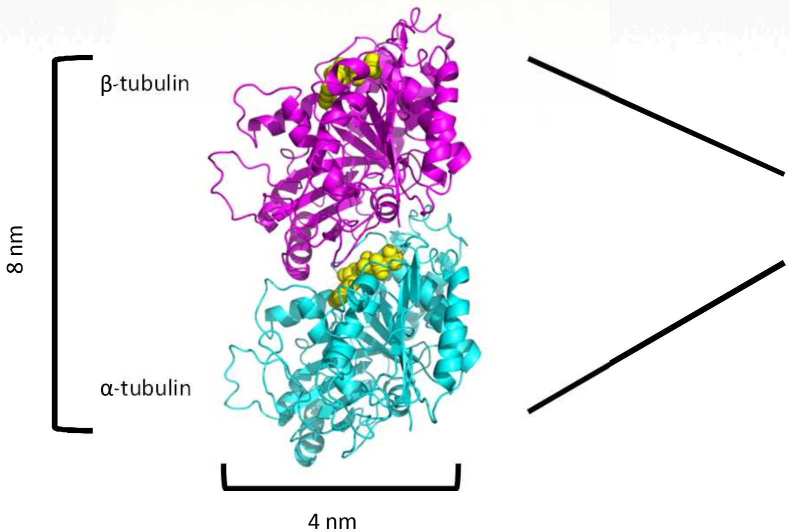
- Characterize assembly

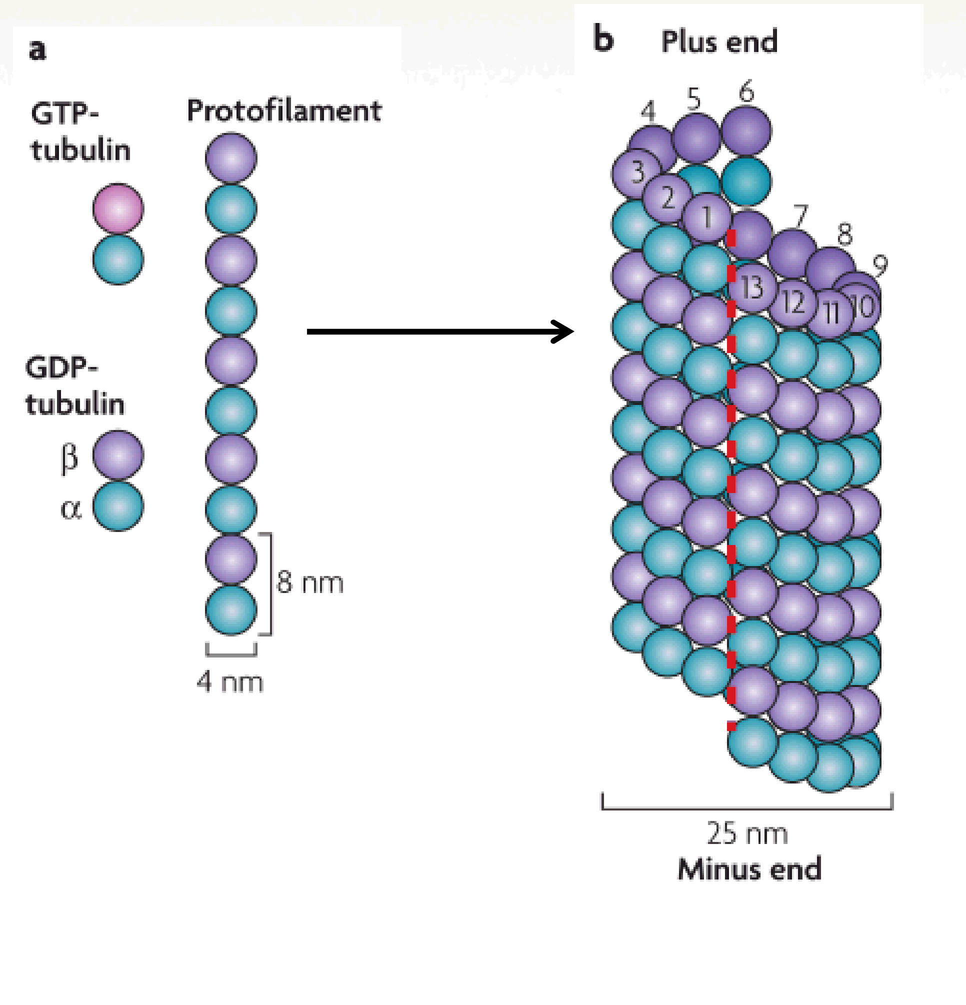
Overall Goal: Use biological components as templates for nanomaterial synthesis

Sandia National Laboratories

Self-Assembly of Microtubules

Microtubules (MTs) have unique self assembly processes ranging across multiple length scales





- Microtubule filaments are biopolymeric self-assemblies of αβ tubulin dimer units
- Tubulin dimer units polymerize into protofilaments which then assemble into microtubule filaments
  - ~13 protofilaments assemble into hollow filaments (25 nm diameter, 10s of microns in length)

PDB: 1TUB

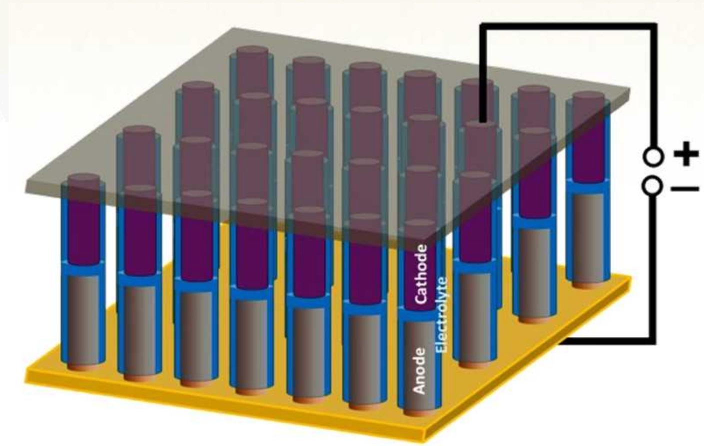
Akhmanova, A. and Steinmetz, M.O. *Nat Rev: Mol Cell Biol*. **9**(4): 309-22.

Microtubules as Templates for Nanomaterial Synthesis

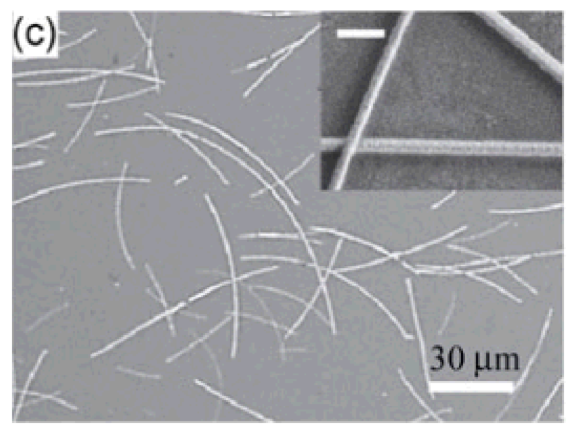
Nanowire synthesis is of increasing interest to the biosensing, MEMS, NEMS and nanobattery fields

Microtubules have attractive properties as templates for synthesis of nanomaterials

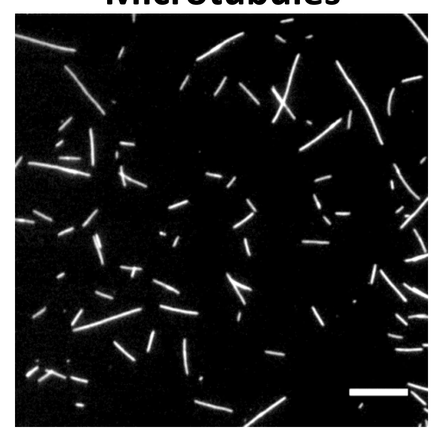
- Similar size scale
- Monodisperse diameter (25nm)
- Tunable length
- Versatility in chemical functionality to different amino acids
- *Simple and quick polymerization dynamics*
    - *Nanowire synthesis typically uses extreme conditions and takes many hours*



Silver Nanowires



Microtubules

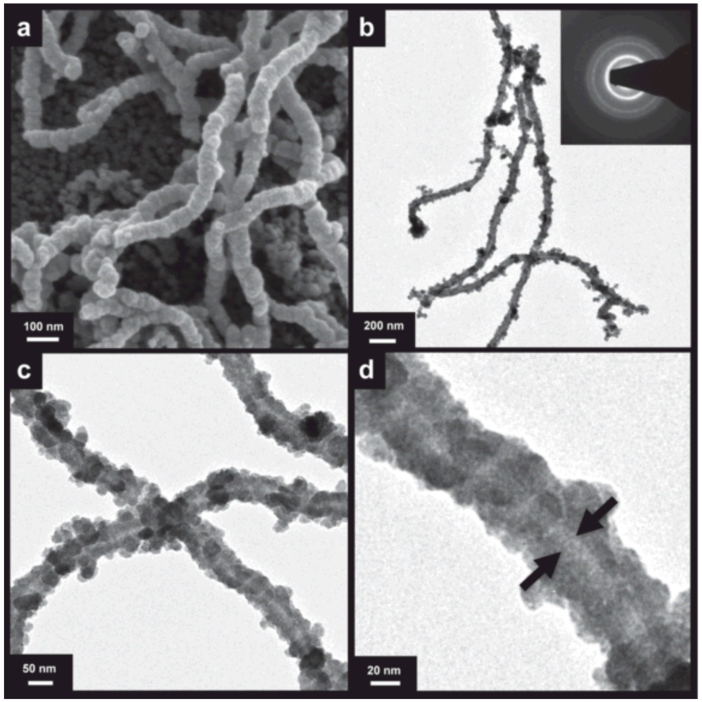


Araki, T., *et al. Nano Res*. **7**(2): 236-245.

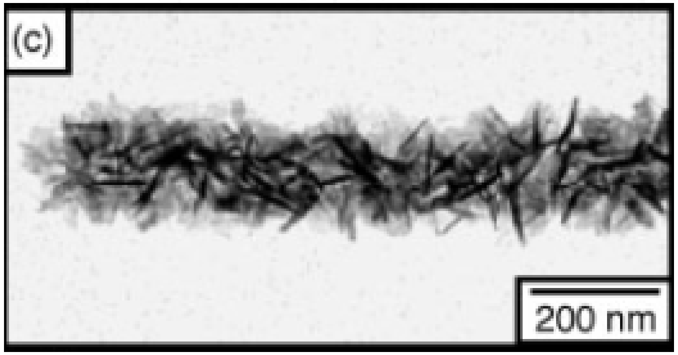
Microtubule-Templated Nanowires

Microtubules have been used as templates for a variety of nanowire synthesis

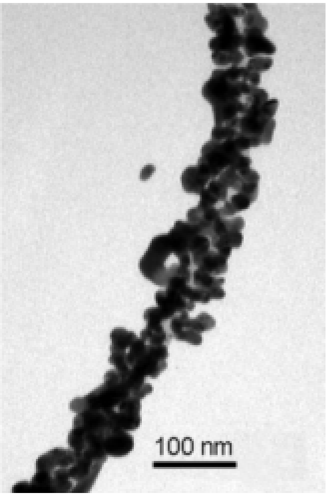
CdS Templated on Microtubules



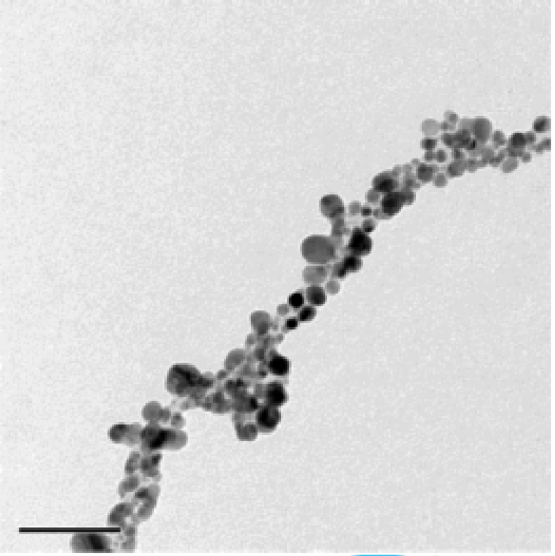
FeO(OH) Templated on Microtubules



Au-Templated on Microtubules

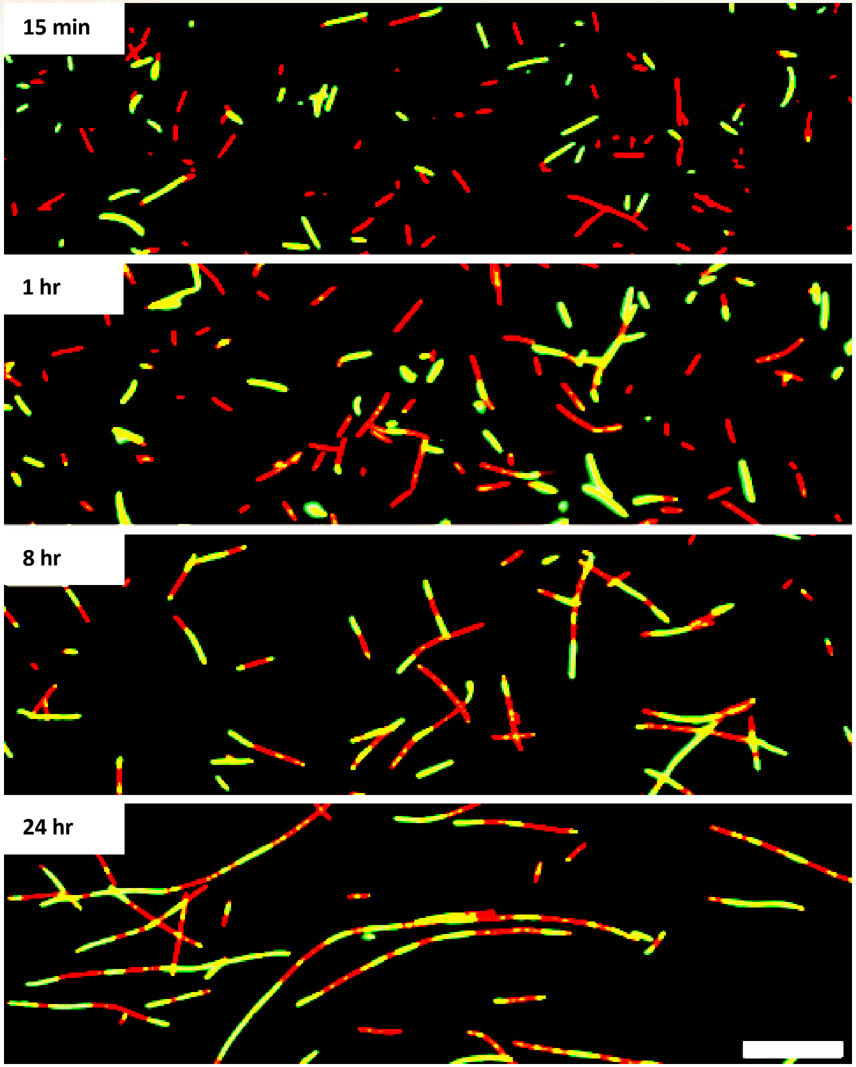


Ag-Templated on Microtubules



Spoerke, E., *et al. Part and Part Syst Characterization*. **31**(8): 863-70.
Boal, A., *et al. Adv Funct Mat*. **14**(1): 19-24.
Zhou, J., *et al. Small*. **4**(9): 1507-1515.
Behrens, S., *et al. Chem Mater*. **16**(16): 3085-90.

MTs Self Assemble Using a Unique Growth Mechanism

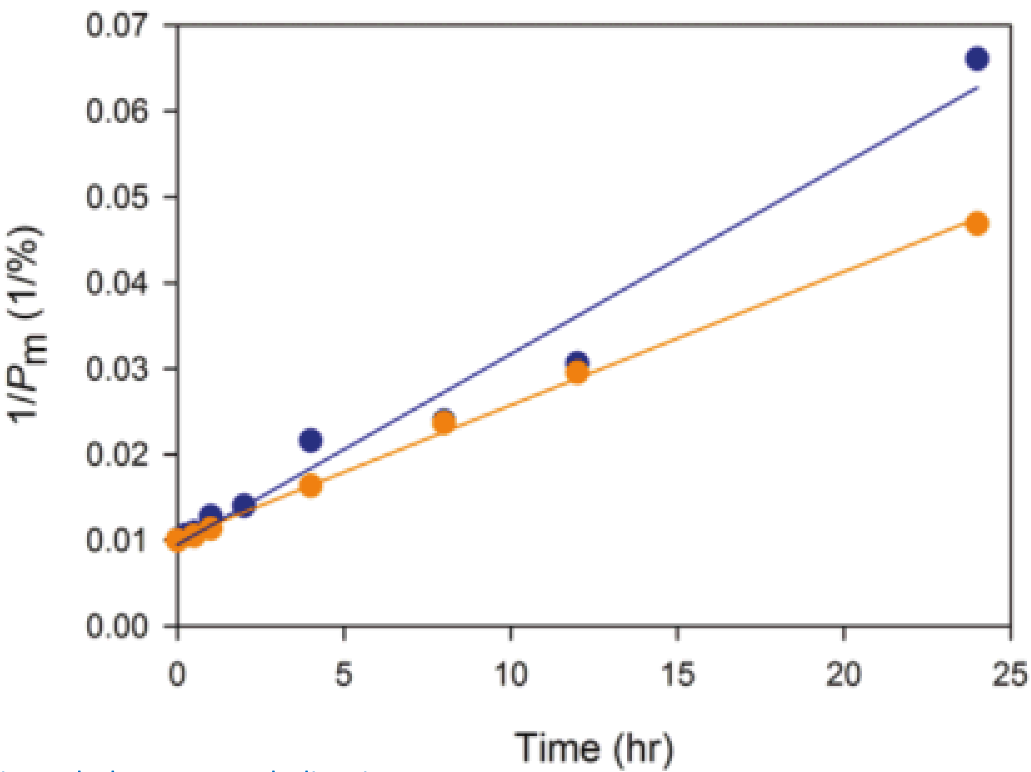


- Microtubule filaments fuse end-to-end as quickly as 15 minutes
- These fusion events increase over time, up to several weeks
- Microtubule fusion occurs using a unique secondary self-assembly
  - This is not a polymerization or nucleated growth mechanism

Bachand, M., *et al. RSC Adv*. **4**(97): 54641-49.

Reaction Rate of Self-Assembly can be Calculated

End-to-end self-assembly (or fusion) follows a **second-order rate function**, suggesting that there are two "reactants" involved in the assembly.



Microtubules + Free Tubulin Dimers
Microtubules - Free Tubulin Dimers

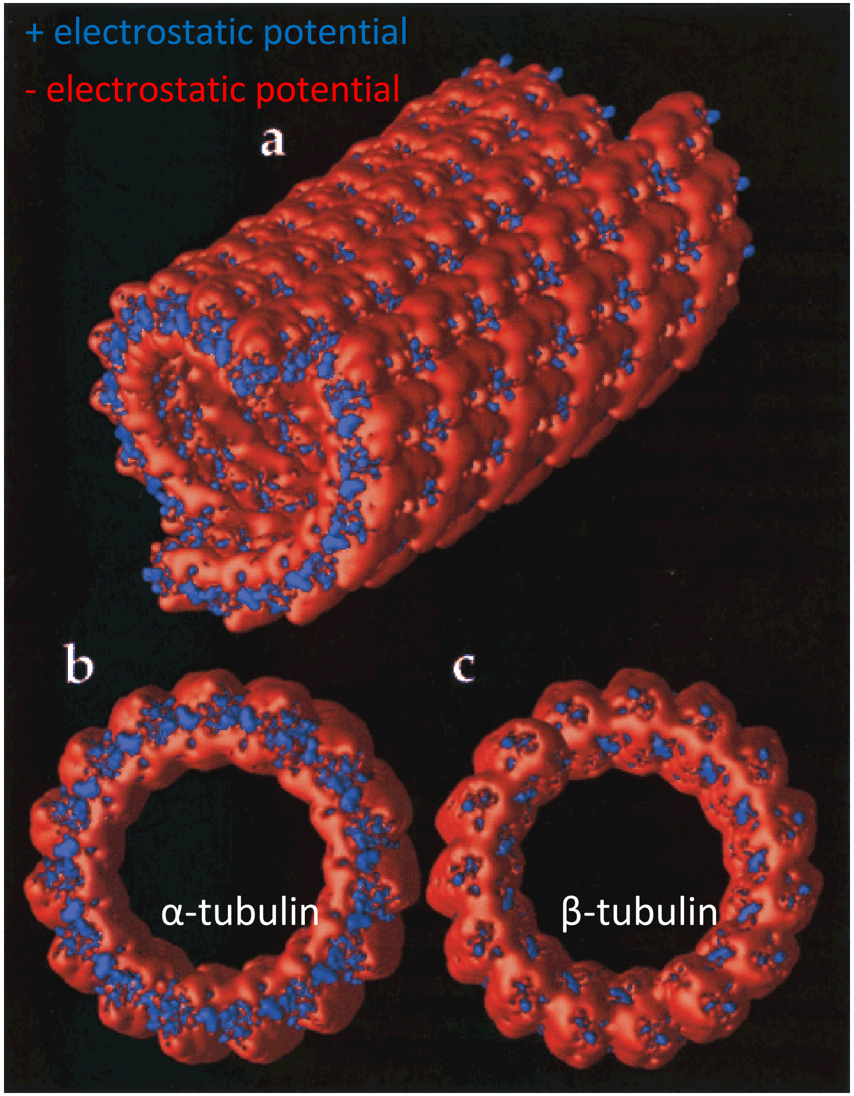
Rates of fusion can be calculated by:

1. Determining the amount of fusion over time
2. Taking the inverse to determine average rates of fusion

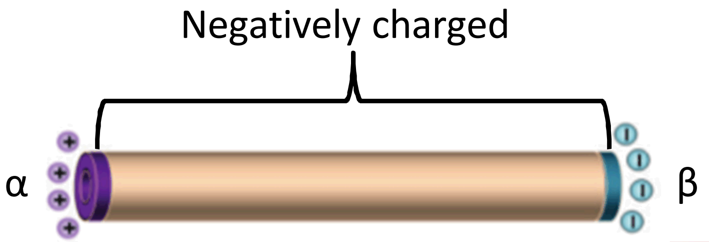
This provides an assay to probe different factors that can alter the rates of fusion

Bachand, M., *et al. RSC Adv*. **4**(97): 54641-49.

Microtubules are Polar and Overall (-) Charged

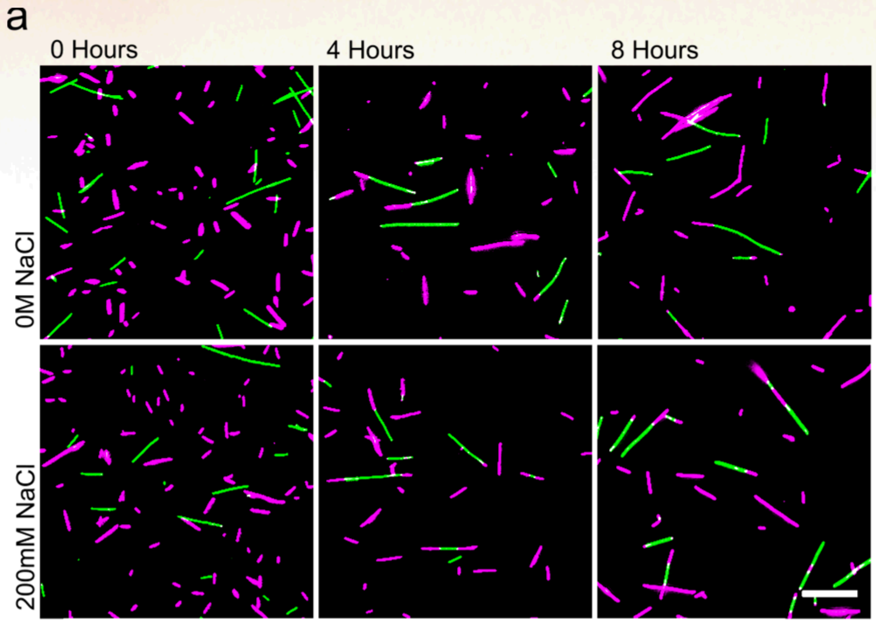


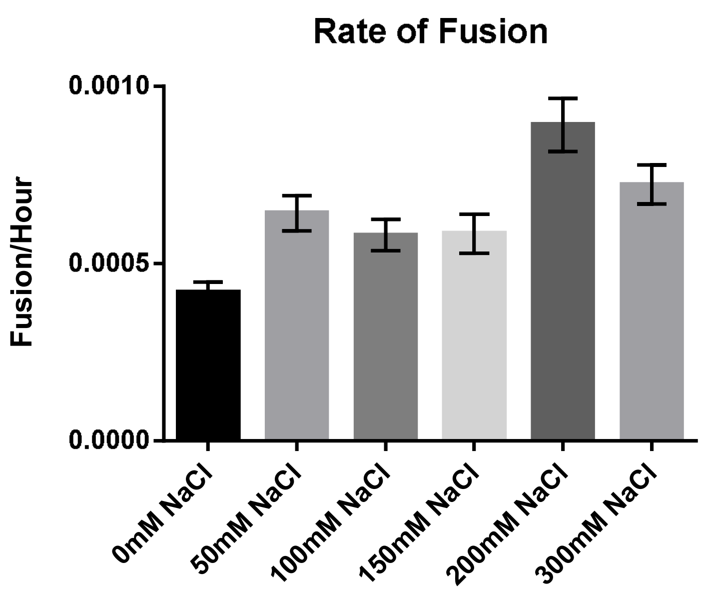
- **How do electrostatics regulate fusion?**
- The overall charge of a microtubule is highly negative
  - Decreasing electrostatics decreases excluded volume effect, thus increasing probability of microtubules interacting with each other
  - ***A positive effect on fusion***
- Microtubules have electrostatically attractive ends (+ and -)
  - Decreasing electrostatics decreases electrostatically attractive ends
  - ***A negative effect on fusion***



Baker, N., *et al. PNAS*. **98**(19): 10037-41.

Addition of NaCl Increases Rates of Fusion

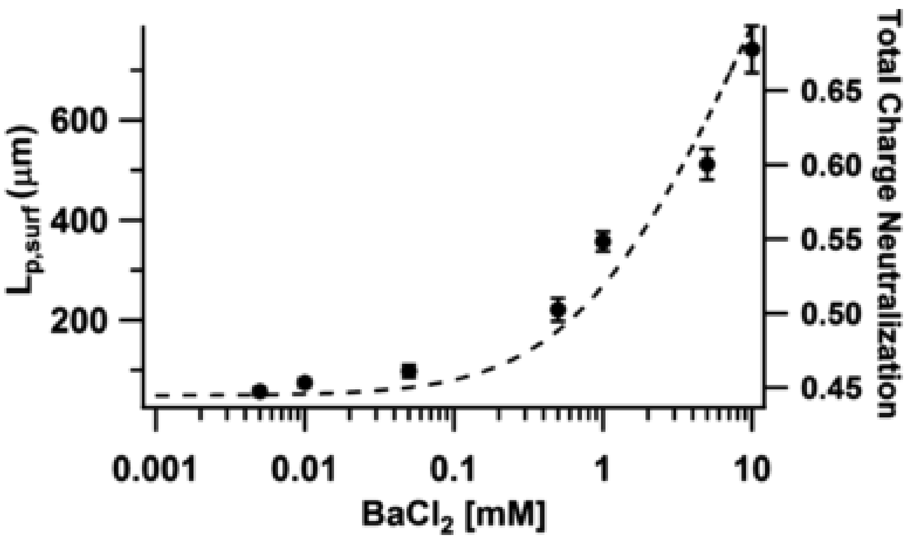


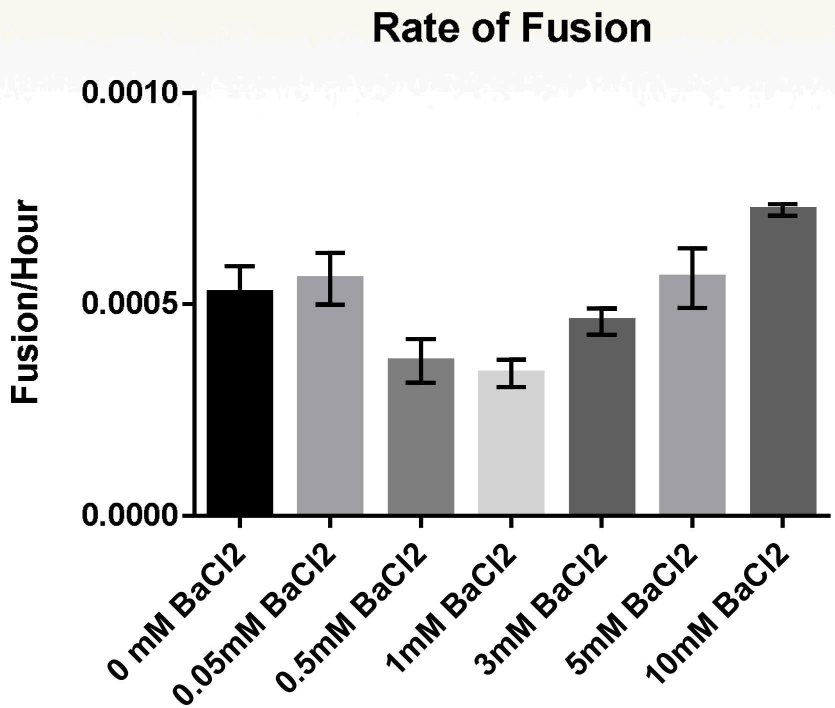


- Addition of NaCl shields the overall negative charge of the microtubule filaments
  - Decreases the excluded volume of interaction
  - Increases probability of interaction
- Addition of NaCl increases the rate of fusion, almost in a step-wise function at **~200mM**
- Model calculations determined that microtubules are fully charge neutralized with **~217 mM NaCl**
- End-to-end fusion of microtubules must be dominated by hydrophobic interactions

Sandia National Laboratories

Addition of $BaCl_2$ Alters Rates of Fusion





- Addition of $BaCl_2$ increases the persistence length and charge neutralization of microtubules
- Addition of $BaCl_2$ alters rates of fusion following charge neutralization trends

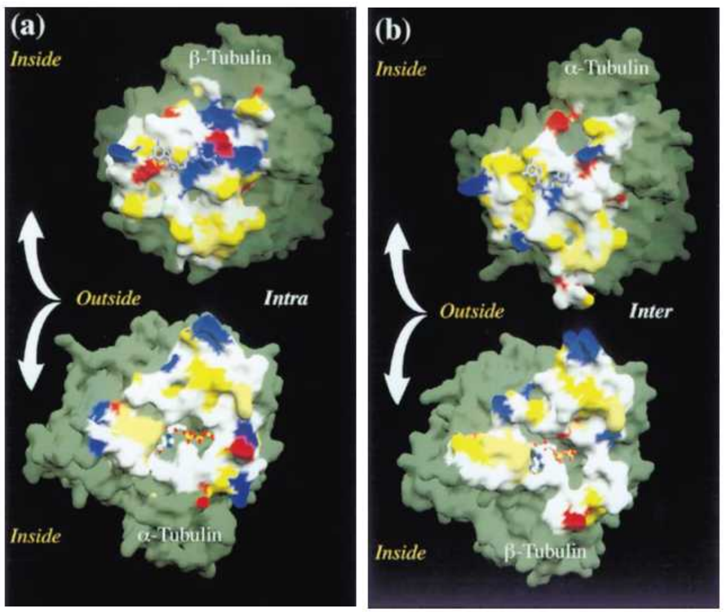
Bouxsein, N., *et al. Biomacro*. **15**: 3696-05.

Hydrophobic Interactions Drive Longitudinal Contacts

Structural studies suggest that:

- Hydrophobicity drives monomer and dimer longitudinal contacts
- Electrostatics drives lateral protofilament interactions

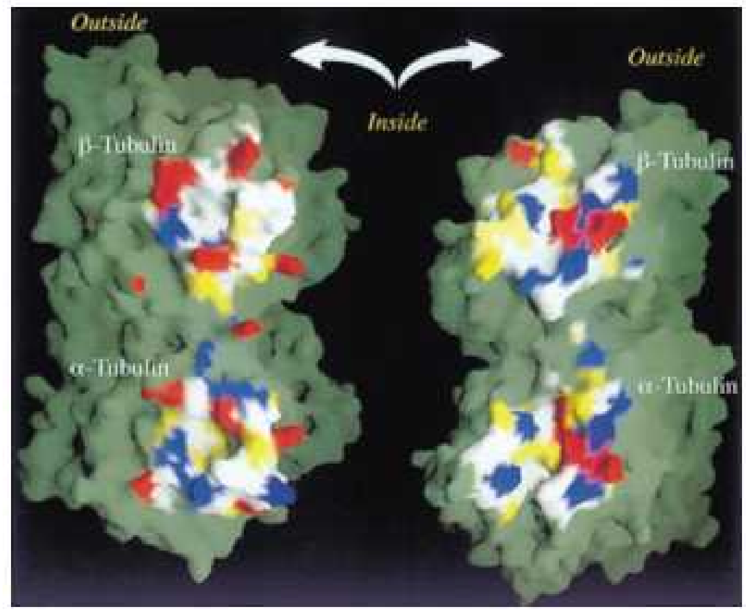
Hydrophobic interactions



Between monomers

Between dimer units

Electrostatics



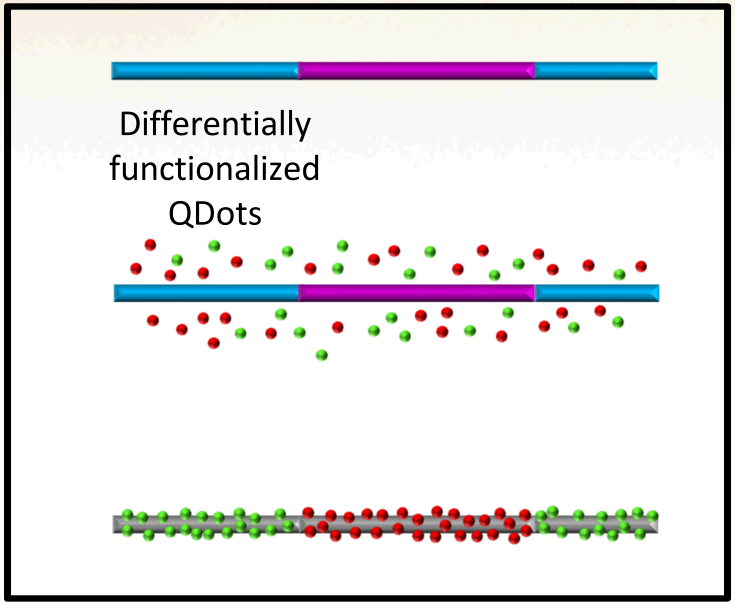
Blue: + charged a.a.
Red: - charged a.a.
White: polar a.a.
Yellow: hydrophobic a.a.
Pale yellow: Trp and Tyr

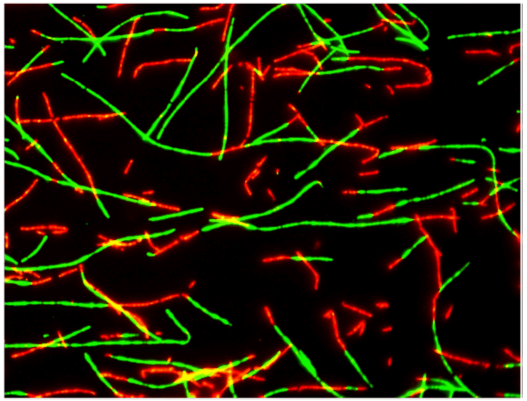
Sandia National Laboratories

Nogales, E., *et al. Cell*. **96**: 79-88.

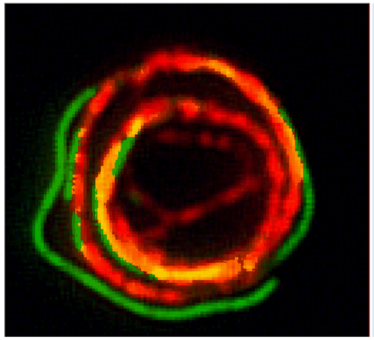
Using Microtubule Arrays to Form More Complex Structures

Morphology of these nanowires arrays can be tuned by altering the assembly process.

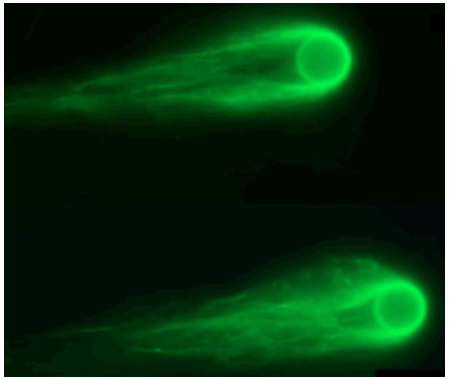




Linear Microtubule Arrays



Spooled Microtubule Arrays



Aster Microtubule Arrays

Spoerke, E., *et al. ACS Nano*. **7**(3): 2012-19.

Acknowledgments

Dr. George D. Bachand
Marlene Bachand
Andrew Gomez

U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering, Project KC0203010.

Office of Basic Energy Sciences • Serving the Present, Shaping the Future •

U.S. DEPARTMENT OF ENERGY

Sandia National Laboratories