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Biomolecular Nanocomposites: A Dynamic Convergence of Microtubules, Motor Proteins, and Materials Chemistry

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Sandia National Laboratories, Albuquerque, NM

Composites at Lake Louise
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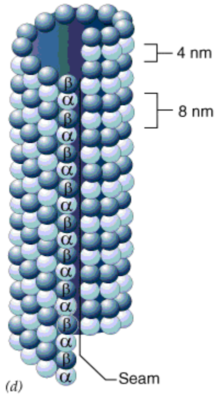
Alberta, Canada

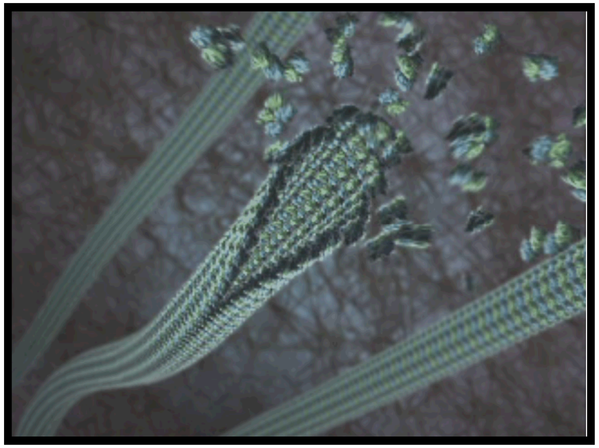
Microtubules: Biological Templates



Polar protein filaments
(~25 nm diameter)

Polymerized from α-tubulin/β-tubulin dimers

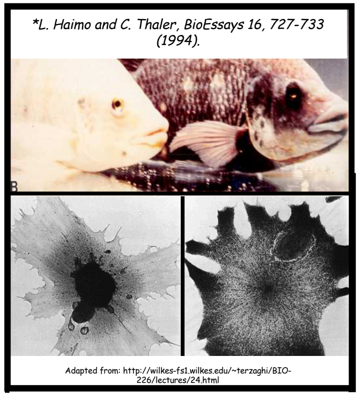
Highly specific interactions with motor proteins (kinesins and dyneins)



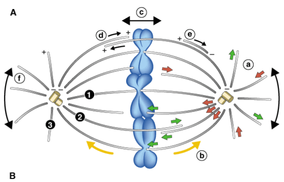
"Inner Life of the Cell," Harvard University

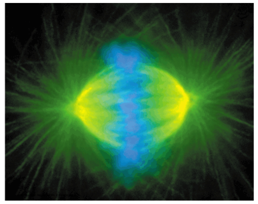
Microtubules (MTs) facilitate a remarkable number of extremely diverse functions throughout biology…

Can we exploit MTs as protein nanowire templates for biomineralization?

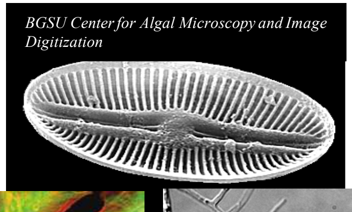


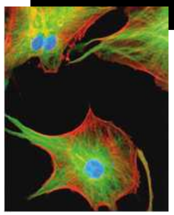
Adaptive reorganization of pigment granules in melanophore cells





Chromosome positioning and separation during cell splitting



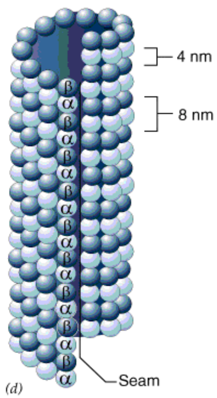


http://probes.invitrogen.com/

Trafficking of vesicles and macromolecule building blocks

Bio-Assemblers:
Microtubules and Motor Proteins

MTs and motor proteins are dynamic agents that facilitate intracellular organization across a wide range of natural systems.



Microtubules (MTs)

Dynamic, polar protein filaments (~25 nm diameter)

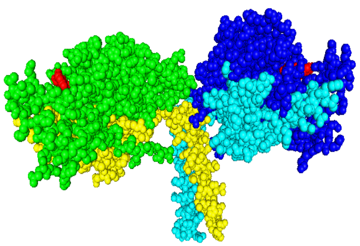
Polymerized from α-tubulin/β-tubulin dimers

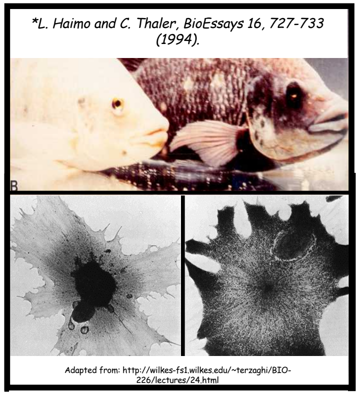
Highly specific interactions with motor proteins (kinesins and dyneins)

Kinesin

A mechanochemical protein (force-generating enzyme)

Fueled by ATP hydrolysis, kinesin latches onto MTs and translocates along them





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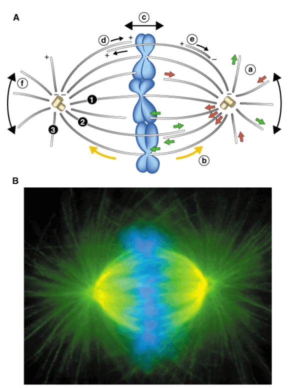

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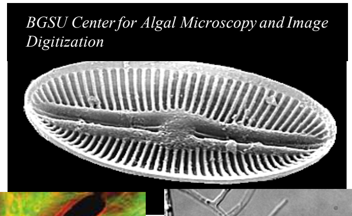
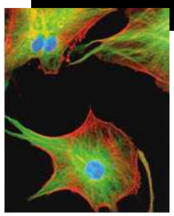




http://probes.invitrogen.com/

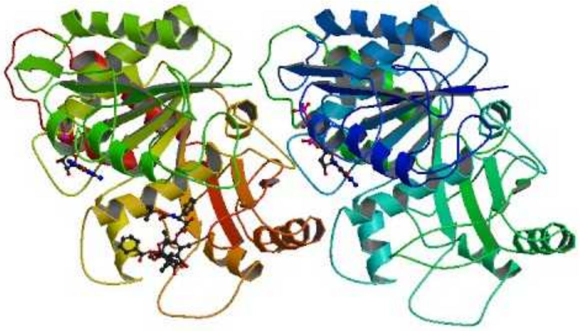
Trafficking of vesicles and macromolecule building blocks

Microtubules as Biomolecular Tools

- These organized, protein nanofilaments are extremely attractive biotemplates:

- Diverse chemistry makes them capable of interacting with a wide range of biomineral precursors

  Positive charges: Lysines, Arginines
  Negative charges: Glutamic acid, aspartic acid
  Chelators: Histidine (e.g. Fe)
  Thiol chemistry: Cysteines



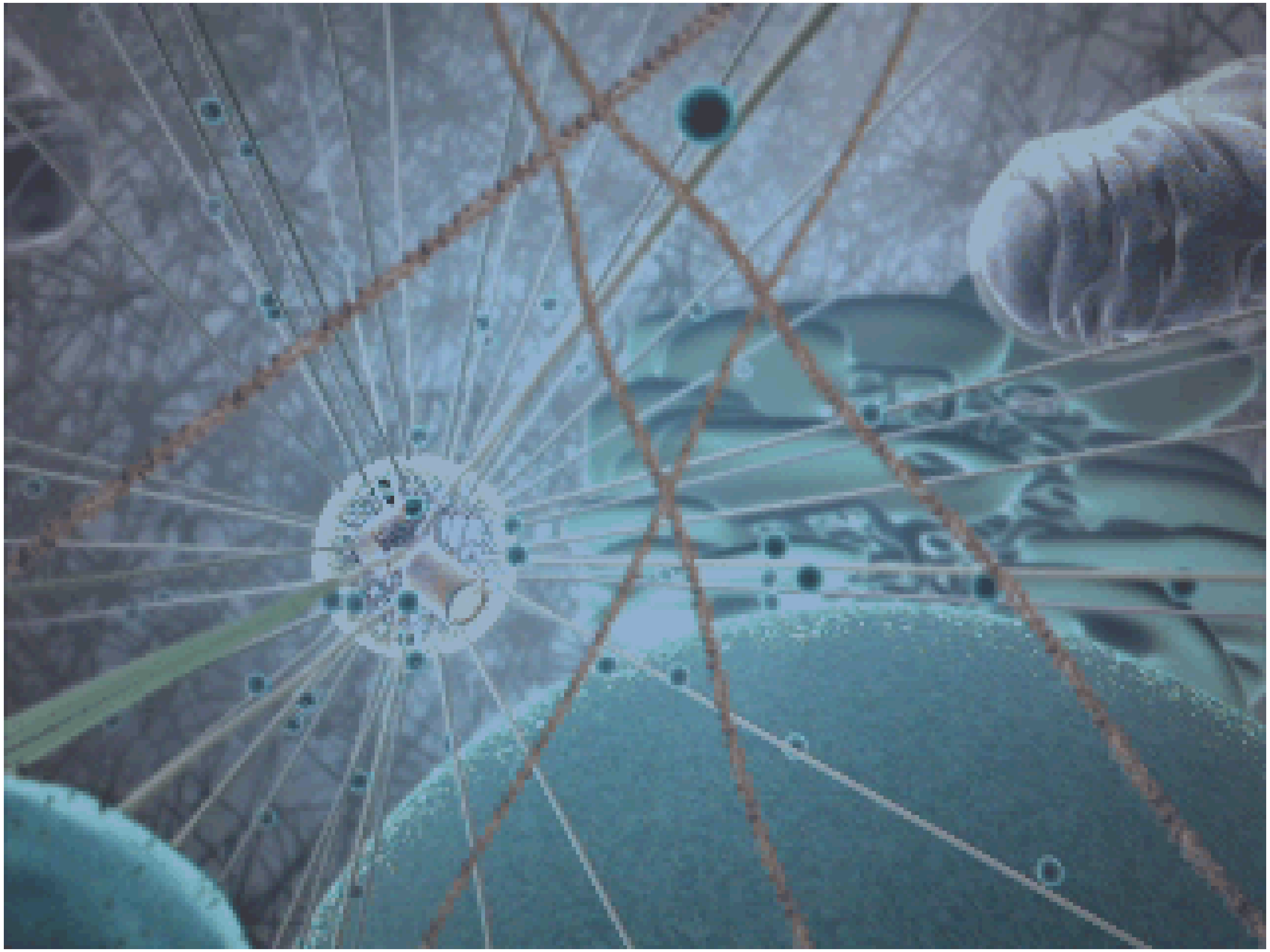
- They exhibit monodispersity in diameter around 25 nm.

- They can be assembled and organized on the nanoscale.

- MT chemistry and assembly can be manipulated for complex templating.

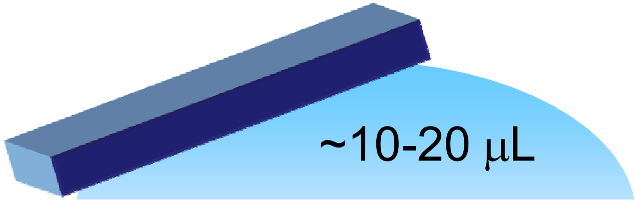
Translating BioAssembly:
MT Organization

Can we adapt of mimic these biological tools?

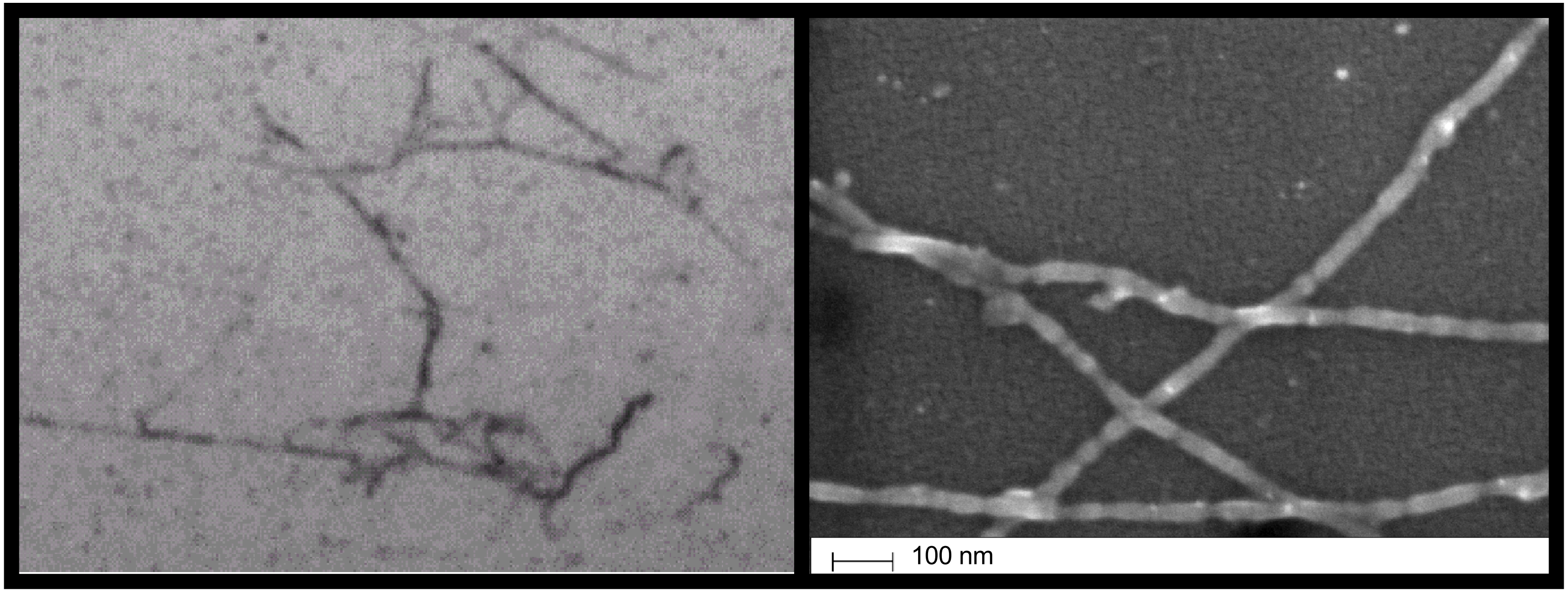


Initial Demonstrations: Metallization

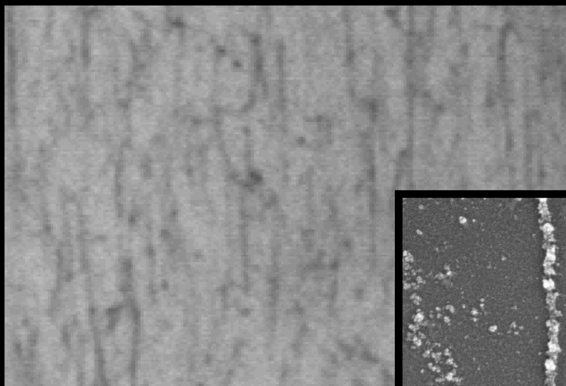
1. Microtubules (MTs) are bound to aminosilane-coated silicon substrates.
2. MTs are incubated in aqueous ionic solutions.
3. Reducing or oxidizing agents added to incubation solution to drive mineralization.



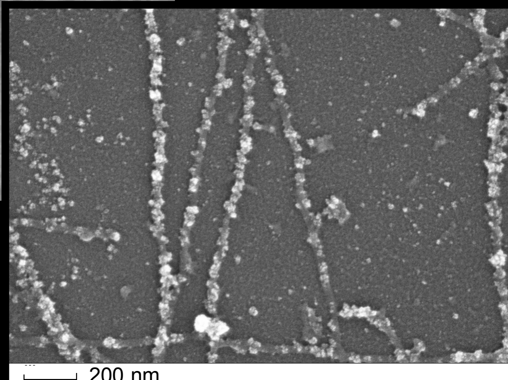
Metallization of microtubules with silver (Ag)



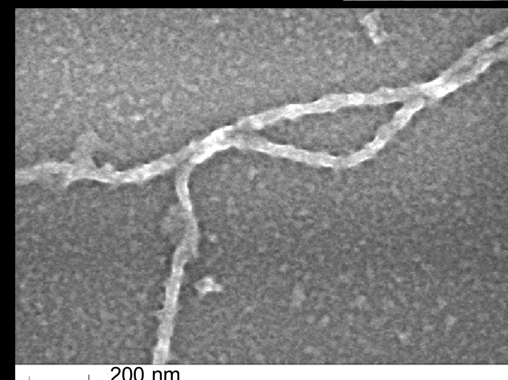
Metallization Diversity

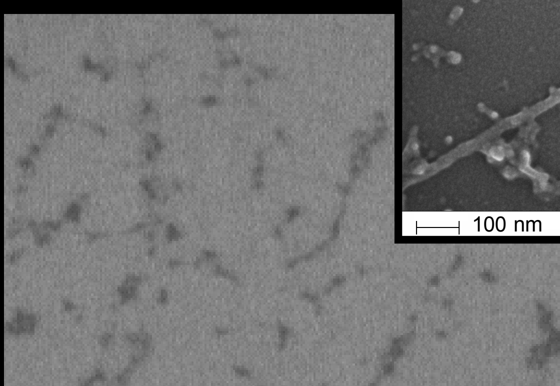


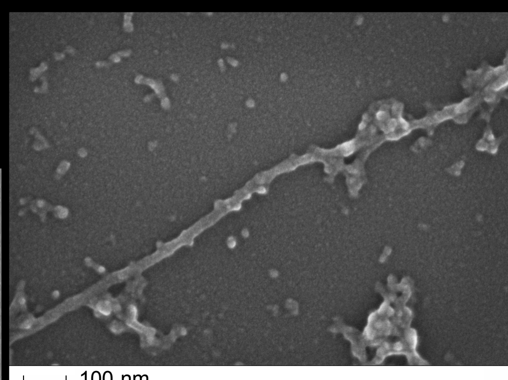
Fe



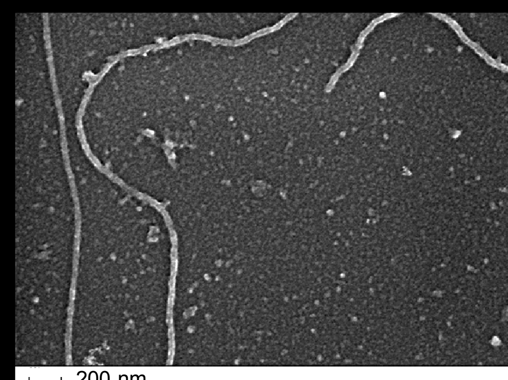
Co



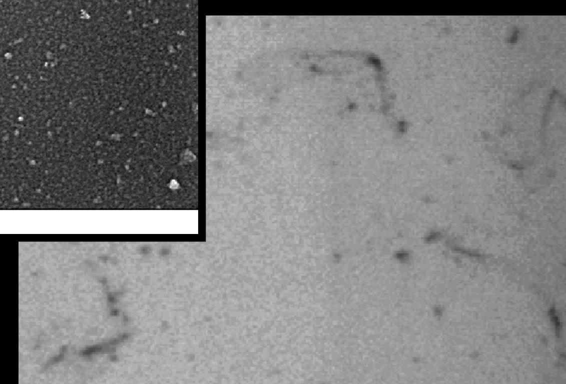




Cu



Mn



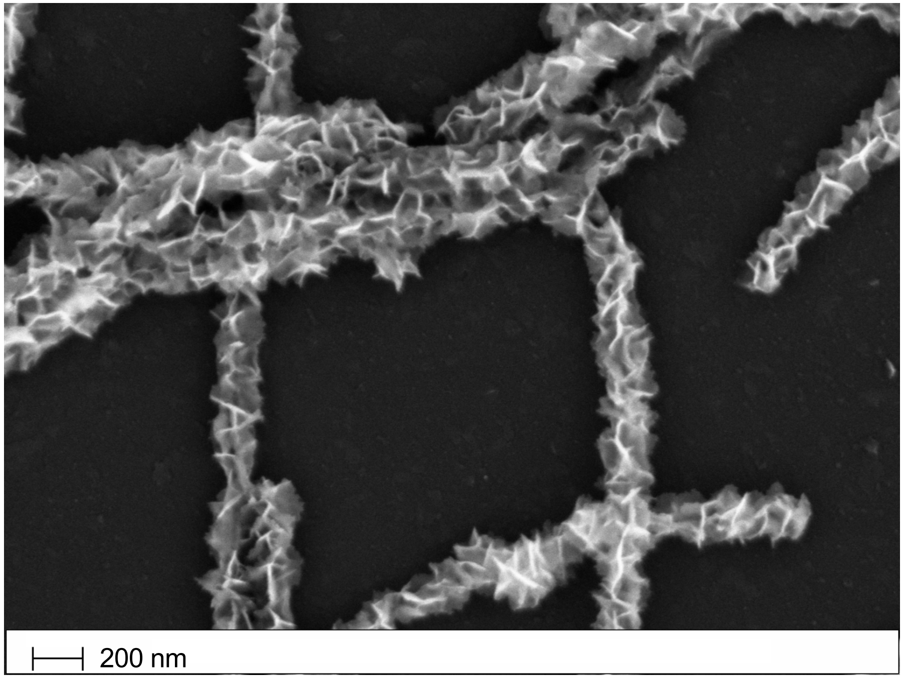
Microtubule Mineralization

In addition to metallization, MTs can serve as templates for mineralization

Iron oxyhydroxide (lepidocrocite)

1. Incubate unlabeled MTs in Fe^{2+}
2. Expose to NH_4OH vapors

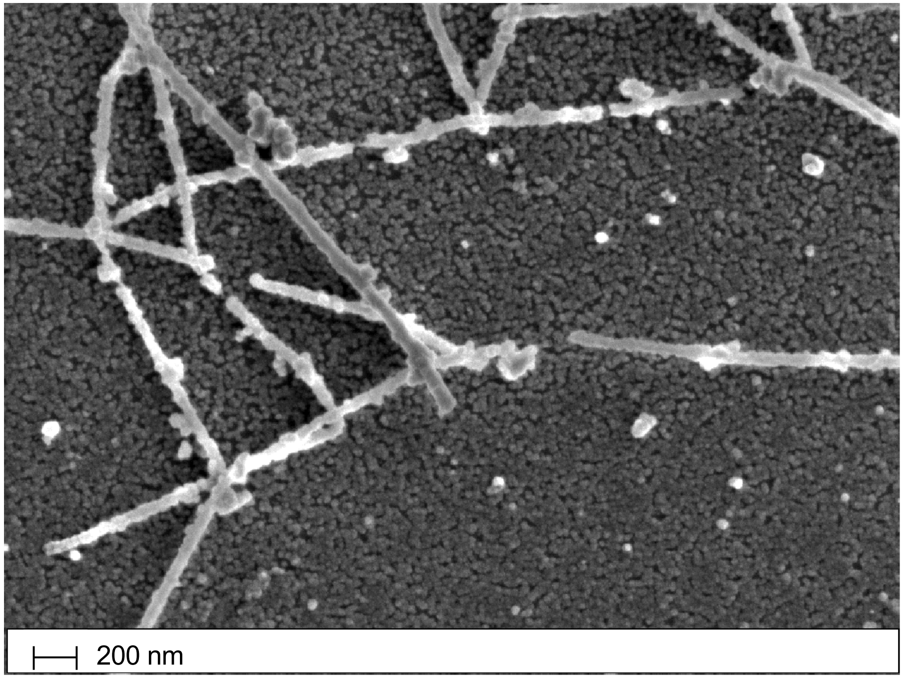
$$4Fe(OH)_2 + 4OH^- + O_2 \longrightarrow 4FeOOH + H_2O + 2O^{2-}$$



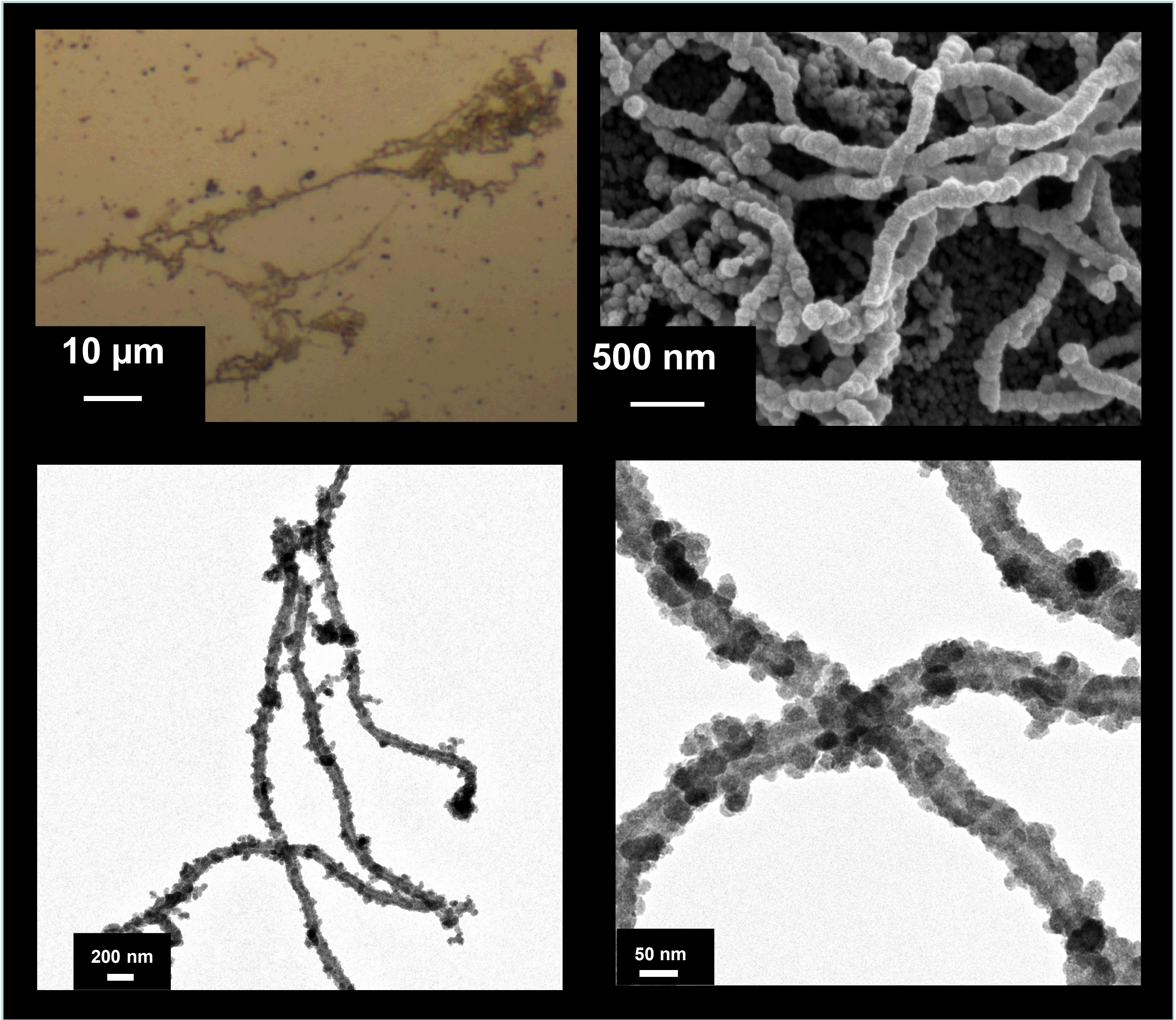
Cadmium sulfide

1. Incubate unlabeled MTs in 10mM Cd
2. Add equimolar thioacetamide
3. Expose to NH_4OH vapors

$$Cd(OH)_2 + CH_3C(S)NH_2 \longrightarrow CdS + CH_3C(O)NH_2 + H_2O$$



A Closer Look at MT-Templated CdS

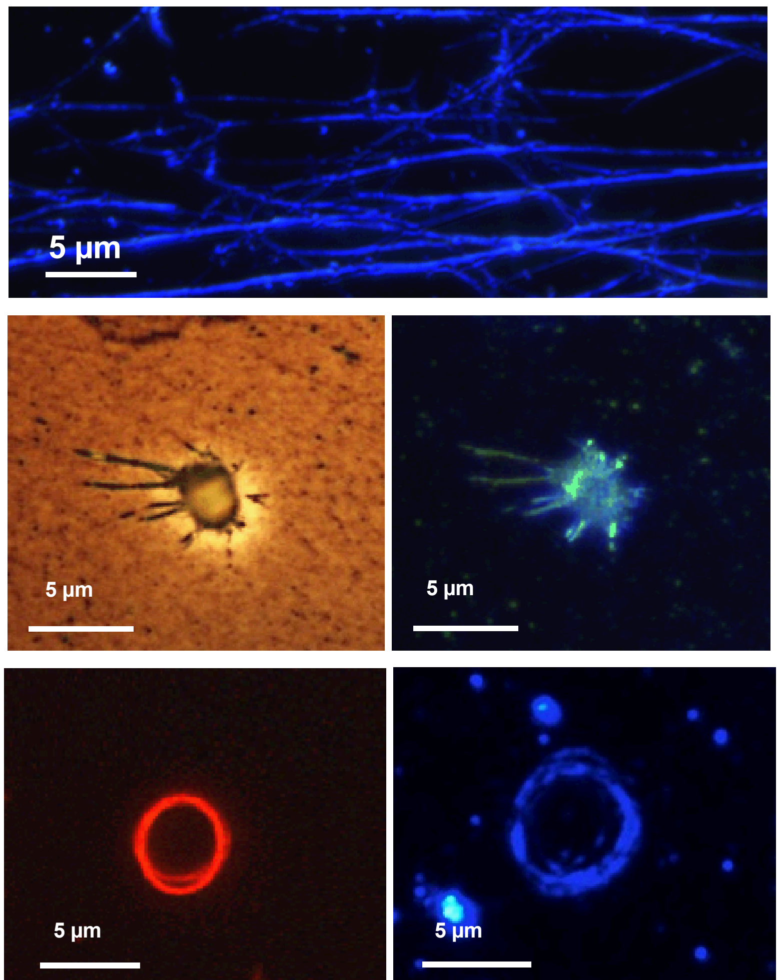


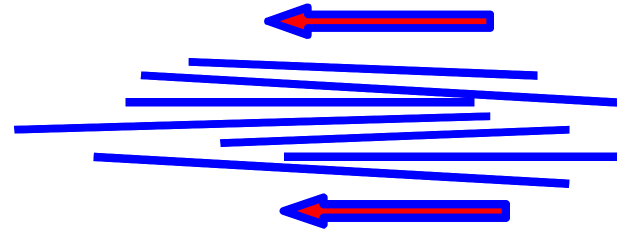
MT surface chemistry promotes dense, conformal CdS mineralization.

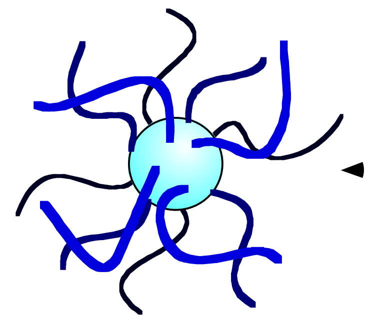
Mineralization is limited to the “bulk” of the protein assemblies – the central pore of the MT is preserved during CdS growth.

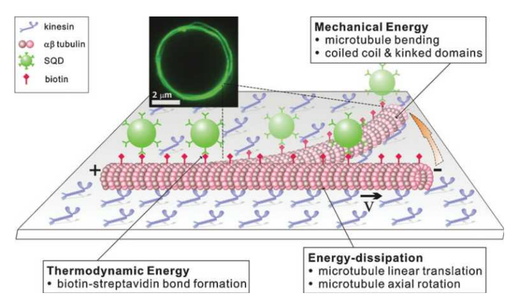
Templating Bio-Mediated Morphologies

The biological character of MTs allows us to template the growth of unique, non-equilibrium CdS nanostructures



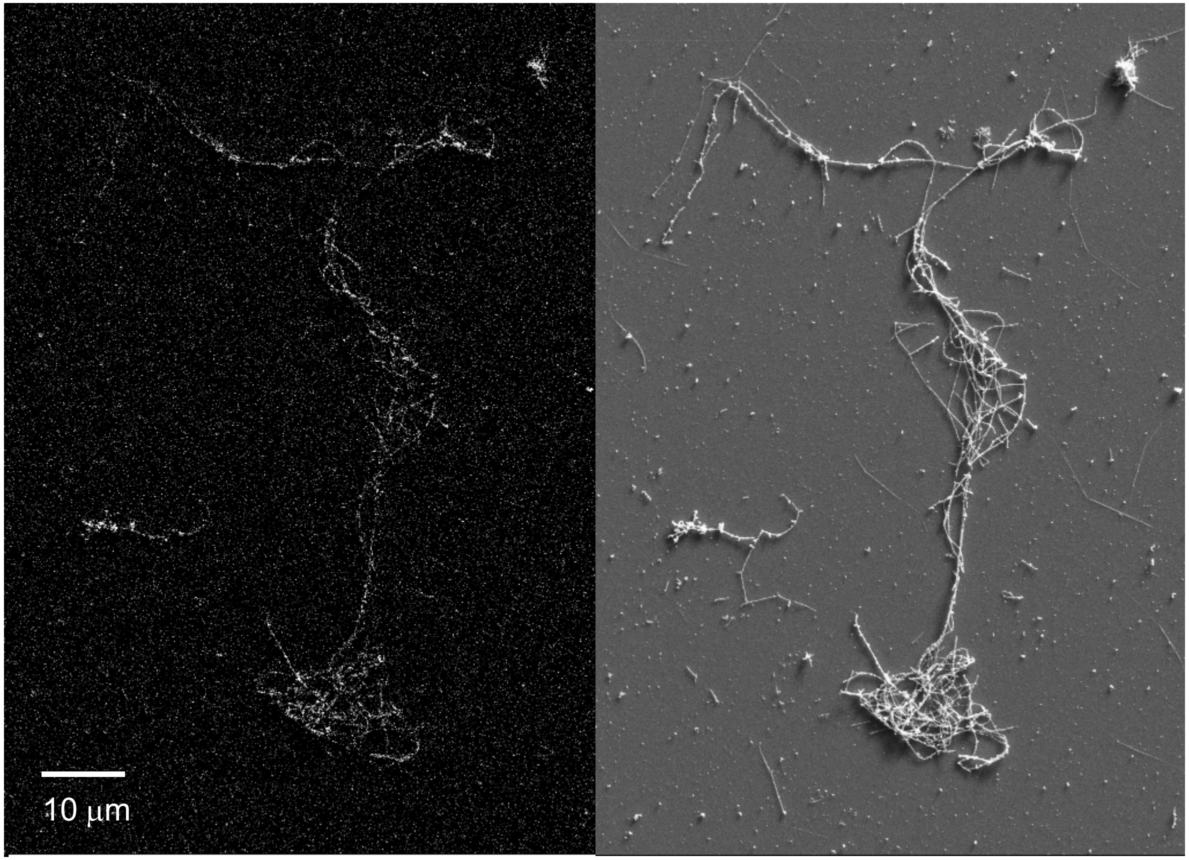






Templating Luminescent Nanostructures

Templating *CdS* not only provides unique architectures, but creates functional (luminescent) structures.



Cathodoluminescent (left) and scanning electron (right) images of CdS-coated MTs

Templating Mixed Materials

Au/Ag

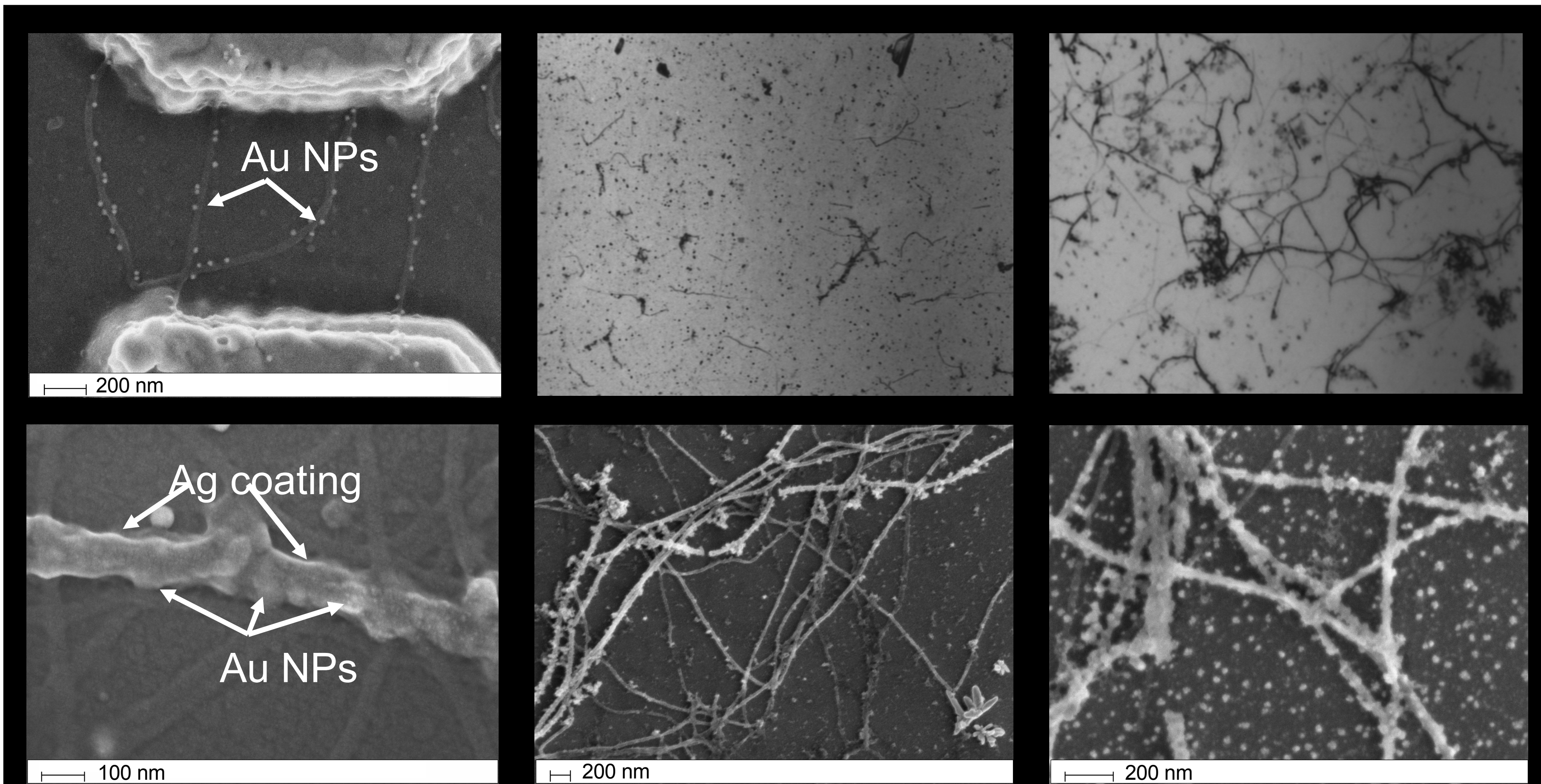
1. Treat biotinylated MTs with anti-biotin Au
2. Incubate with Ag^+
3. Reduce with hydroquinone

Ag/FeOOH

1. Incubate unlabeled MTs with Ag^+ and Fe^{2+}
2. Reduce with hydroquinone
3. Oxidize with NH_4OH vapor

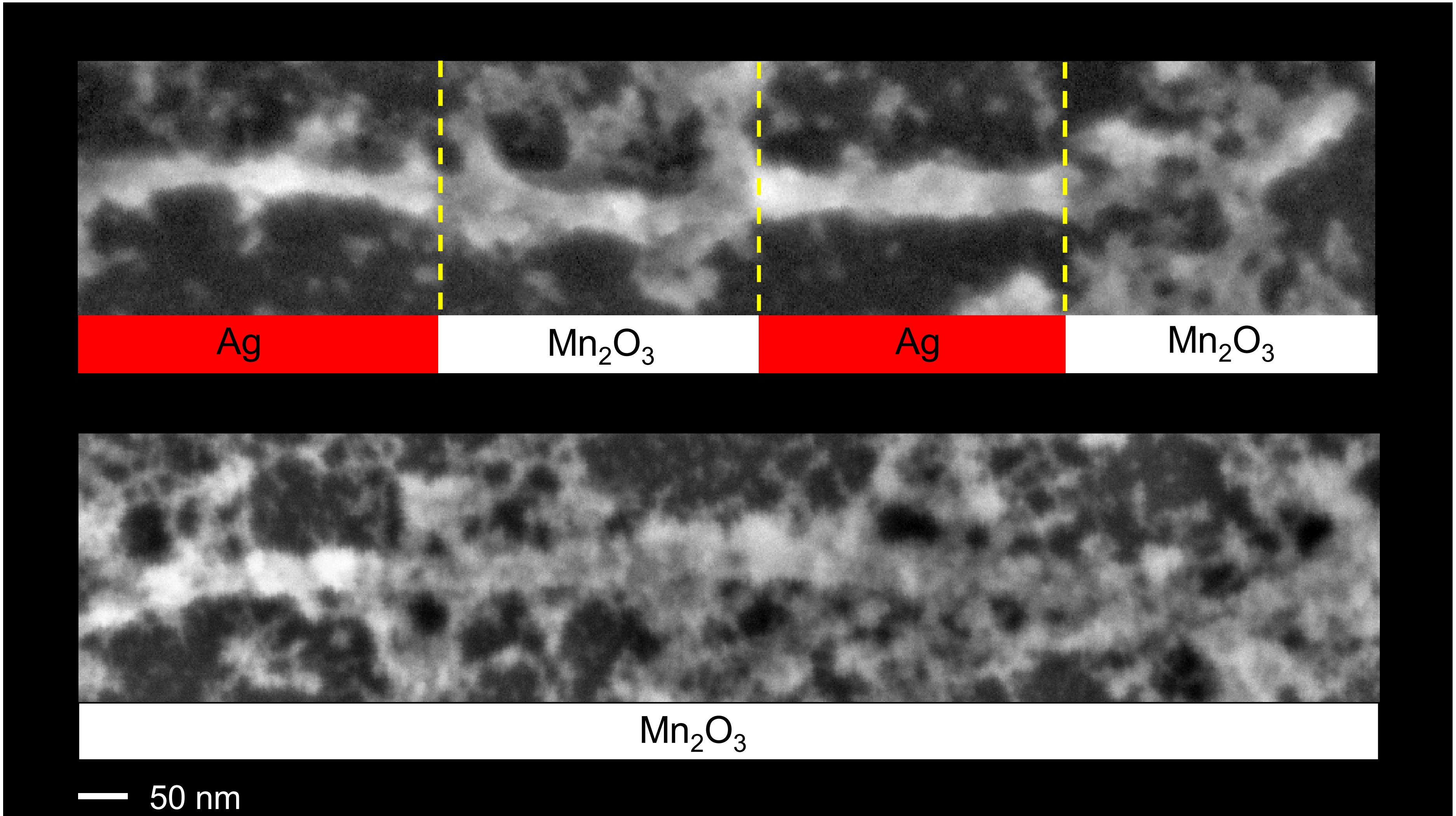
Ag/Mn_2O_3

1. Incubate unlabeled MTs with Ag^+ and MnO_4^-
2. Reduce with hydroquinone

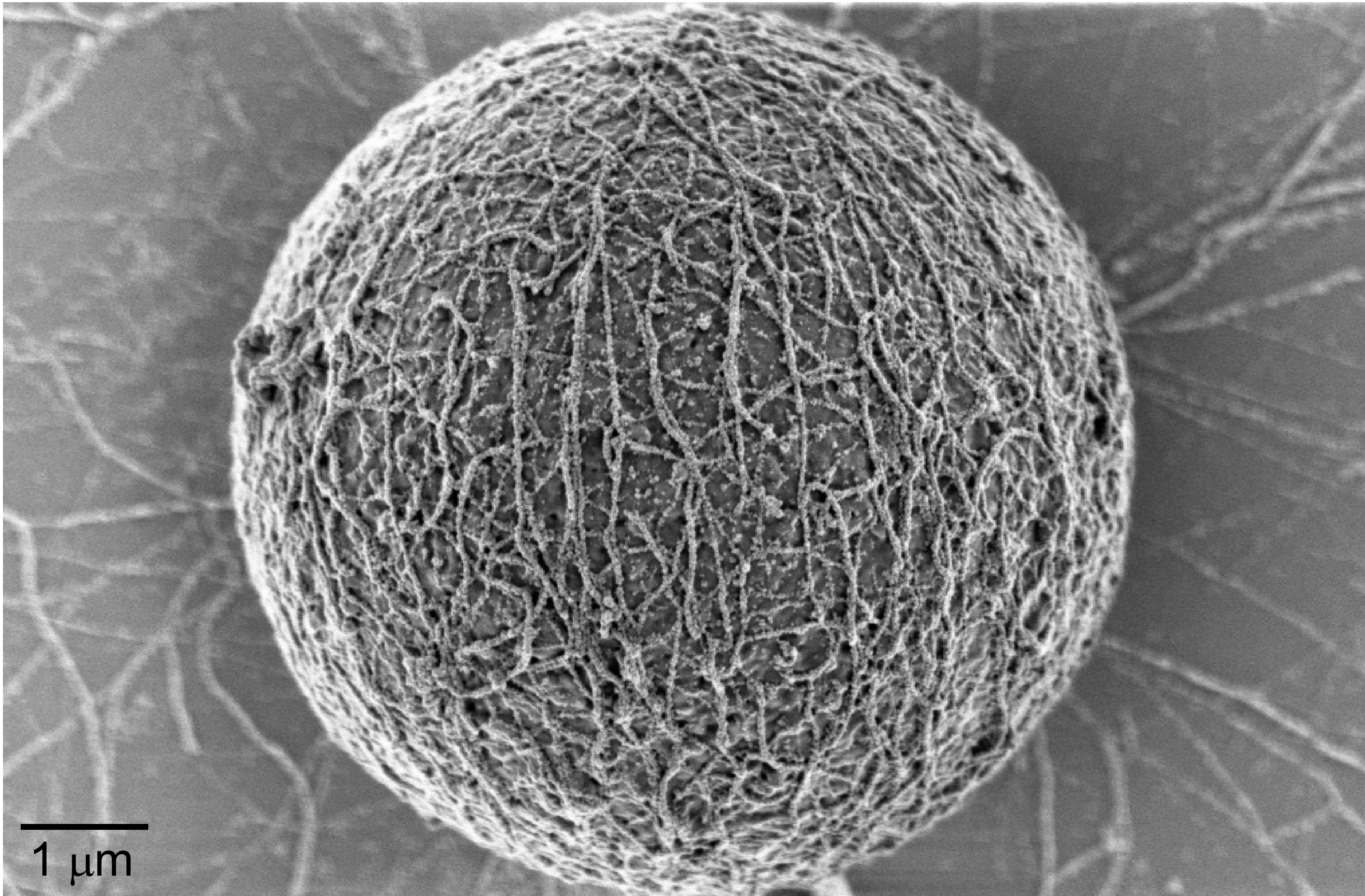


Nanoengineered Hybrid Templates

Functionally segmented MTs produce nanoscale spatially-resolved, selective mineralization

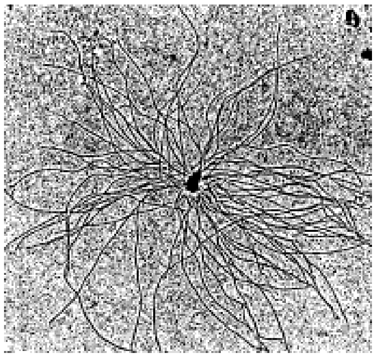


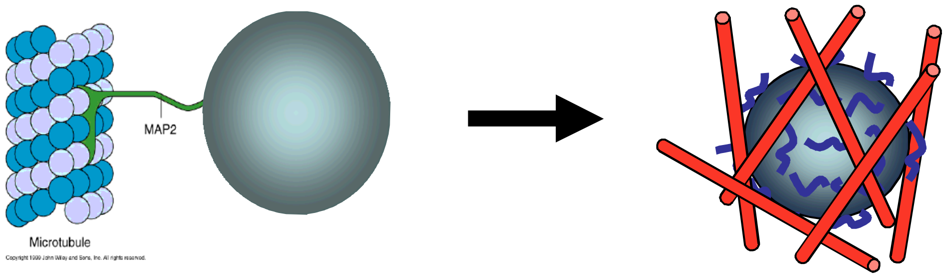
Metallized Architectures



Artificial MT Asters

AMOCs:Artificial Microtubule Organizing Centers

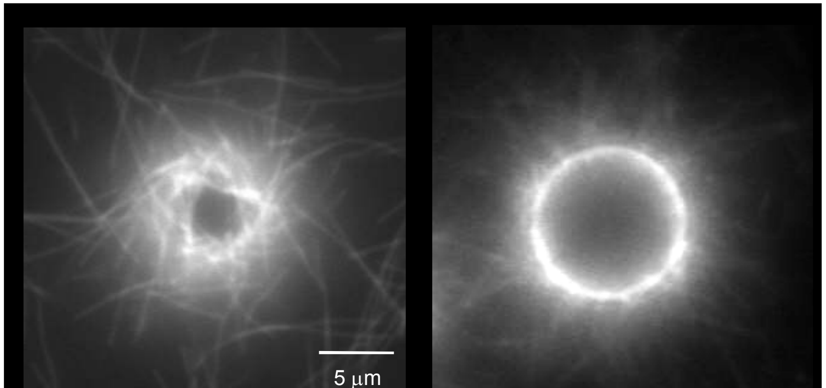




MAPs (1,2, Tau) serve to nucleate and stabilize the MT growth around a central particle.

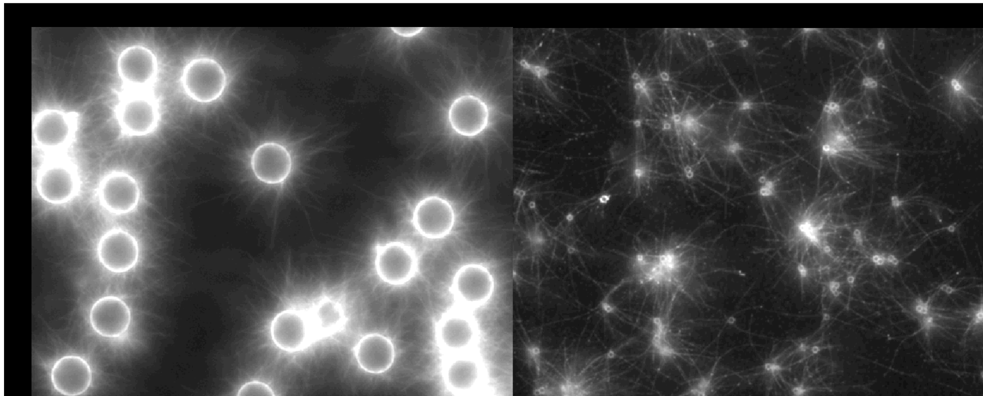
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Cytoskeleton.html#centrosome

MTs assemble in 3D around particles of variable sizes



Near AMOC base

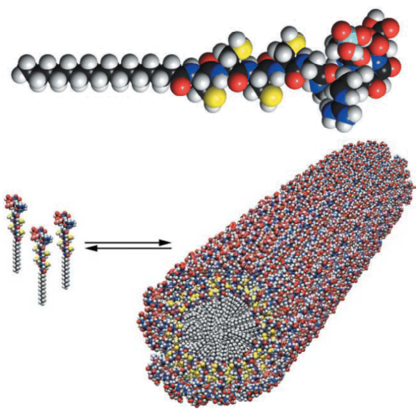
Near AMOC equator



Bead diameter ~7 µm

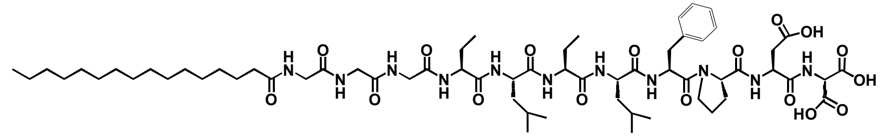
Bead diameter ~1 µm

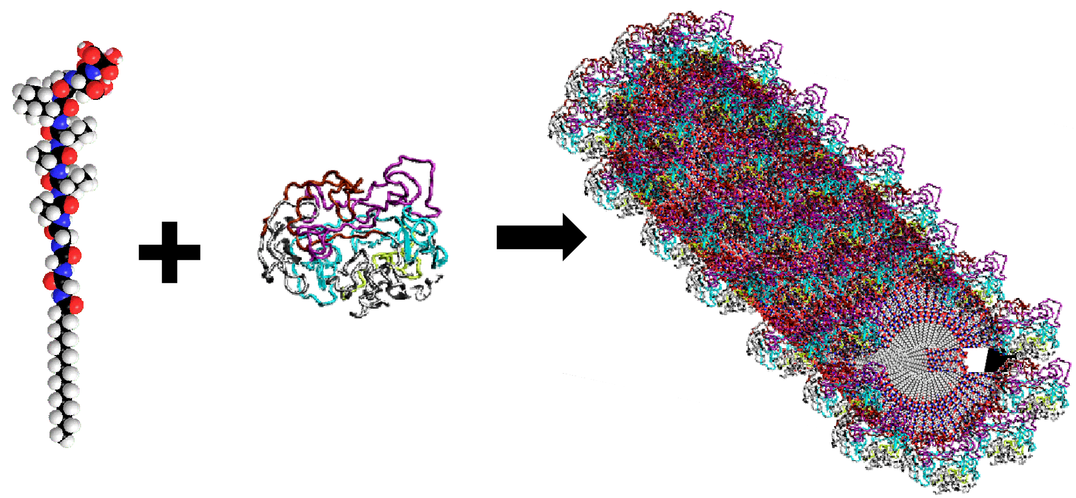
Biomediated Nanofiber Formation



Anionic peptide amphiphiles (PAs) self assemble to form 3D nanofiber gels in the presence of divalent cations (e.g. Ca^{2+}, Mg^{2+}) or at low pH.

Hartgerink, et al. *Science*, **294**. 2001.
Hartgerink, et al. *PNAS*, **99**. 2002.
Spoerke, et al. *Adv Mater*, **21**. 2008.

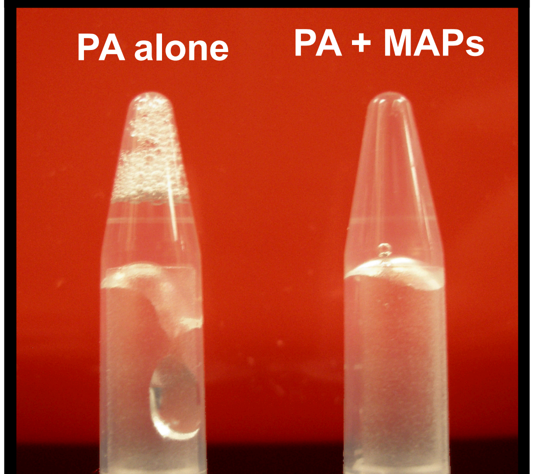


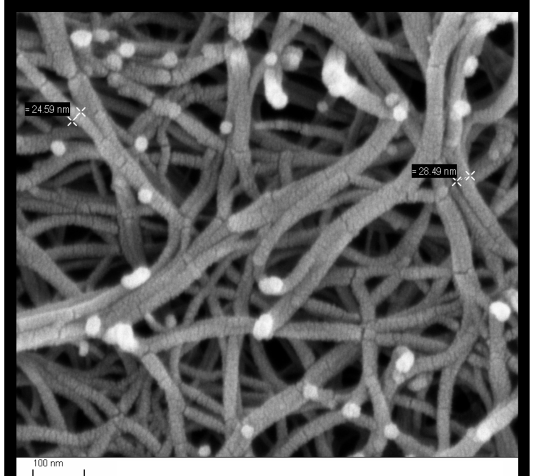


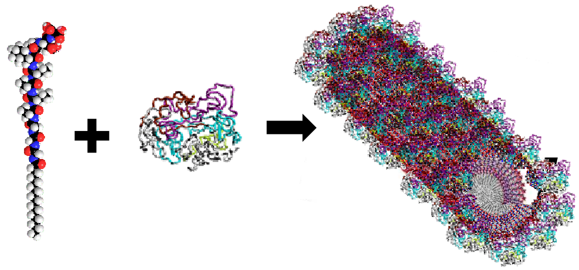
Objective: Combine a highly anionic PA with a cationic microtubule associated protein (MAP) to drive nanofiber gel assembly.

3D Nanofiber Matrix Templating

MAP-mediated assembly produces gel, comprising MAP-covered nanofiber bundles



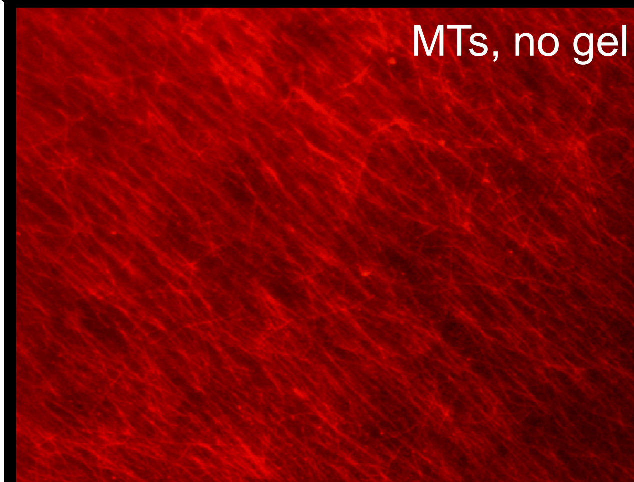


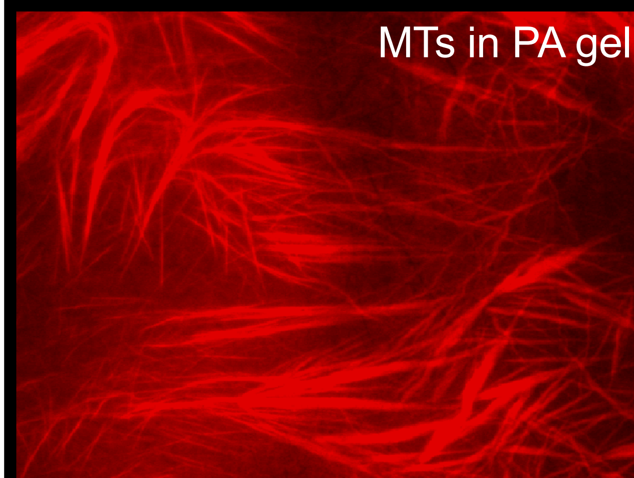


MAP-decorated nanofiber gels serve as 3D templates for MT polymerization

(red fluorescence from MTs polymerized within nanofiber gel)

Microtubules grown in Nanofiber gels show3D texture from gel morphology



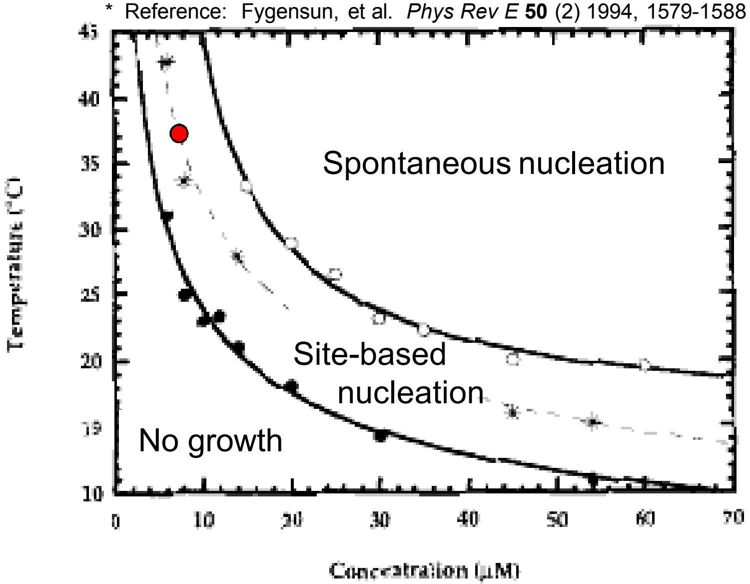


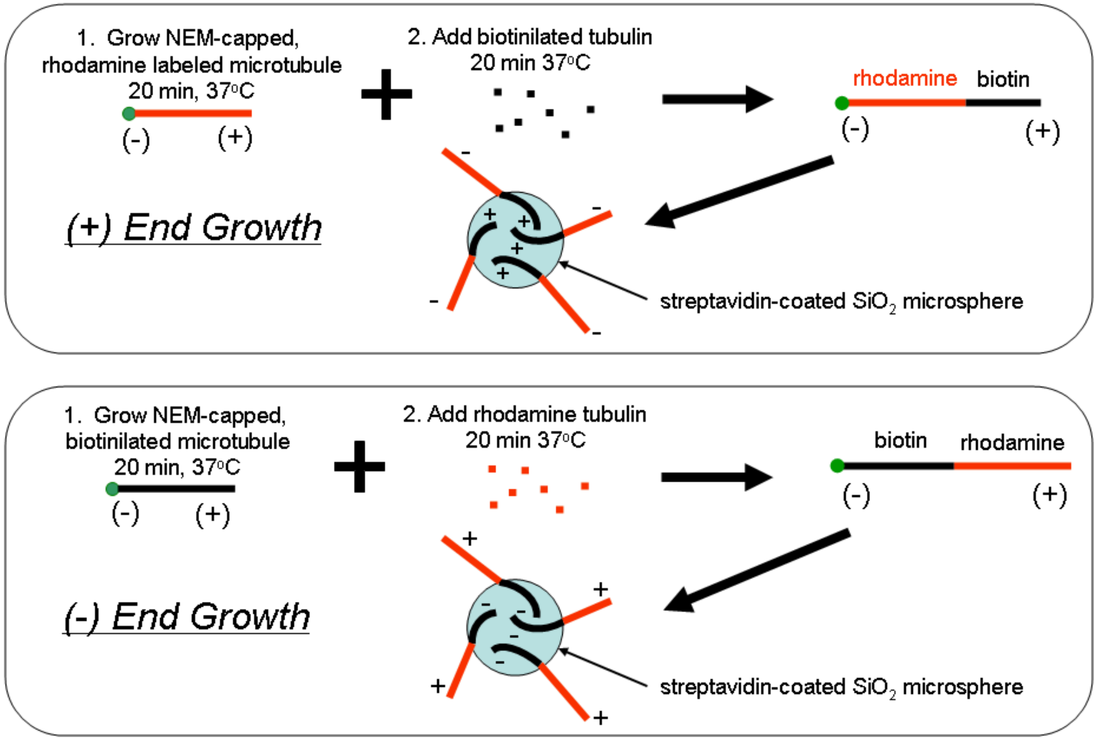
Engineering Polar Microtubules

Sequential polymerization is used to produce functionally-segmented MTs

Microtubule nucleation is a concentration-dependent event with parallels to crystal growth

* Reference: Fygensun, et al. *Phys Rev E* **50** (2) 1994, 1579-1588

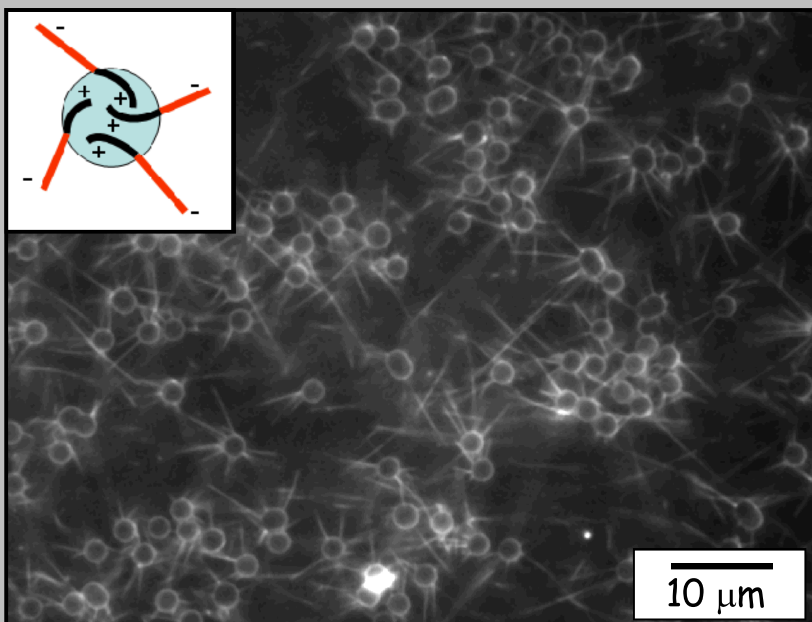




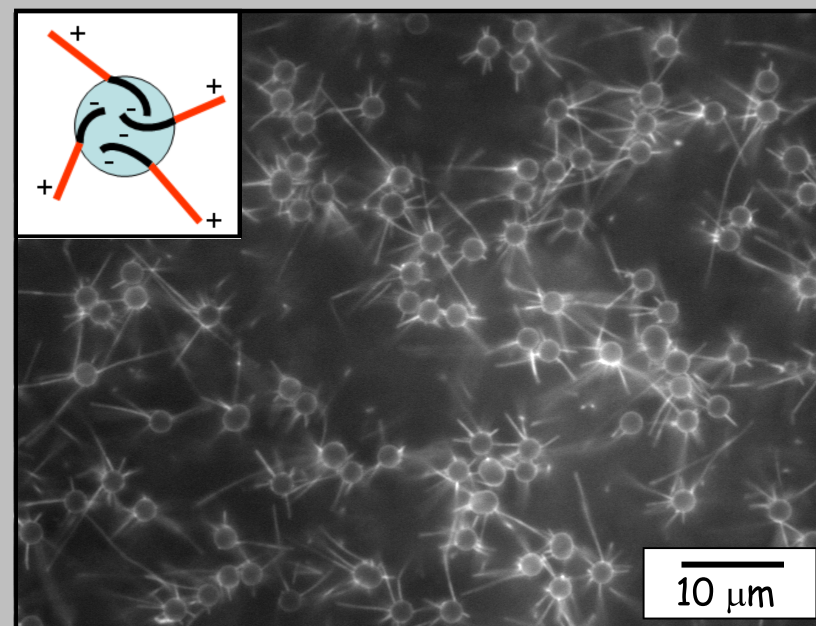
E.D. Spoerke, et al. *Langmuir*. In press.

Polar Microtubule Organizing Centers

Functionally-segmented MTs will assemble around microspheres to create aster-shaped polar-organized structures



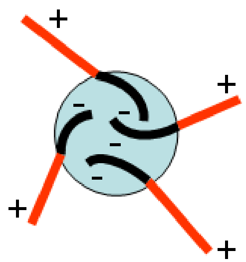
“Plus” end in



“Plus” end out

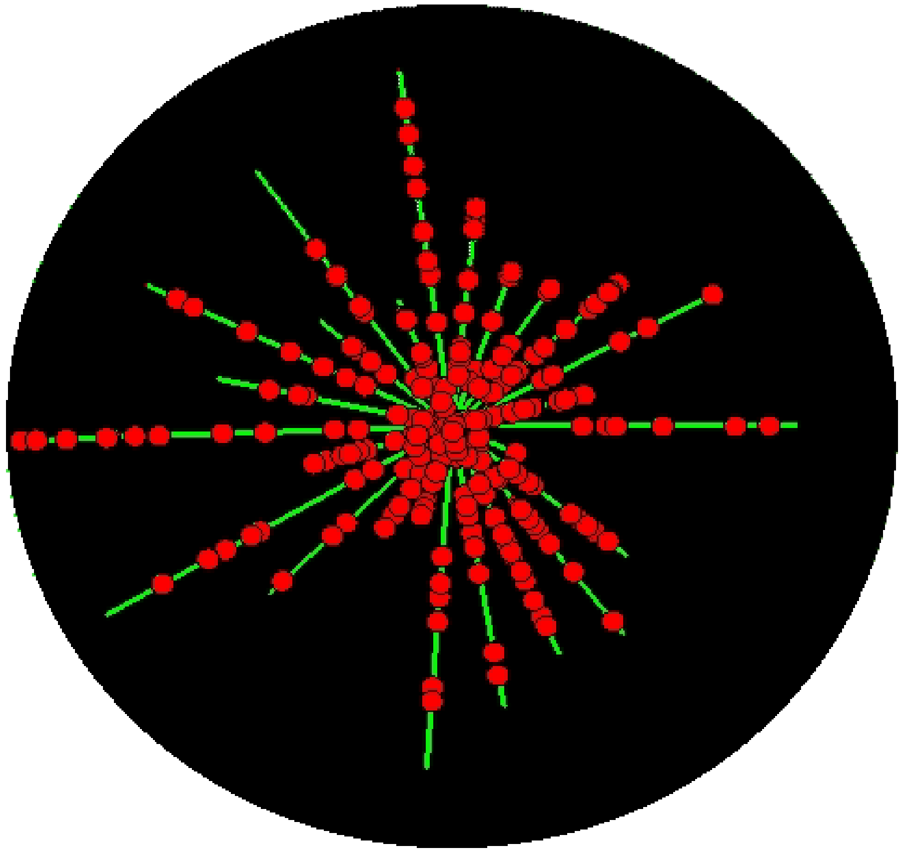
Simulating Kinesin Transport on POSMOCs

Computer models simulate nanocargo concentration and dispersion on polar microtubule asters (POSMOCs)



- Simulated centrosomes created with MTs (green) organized "plus-out"
- Two types of motors are loaded onto red cargo

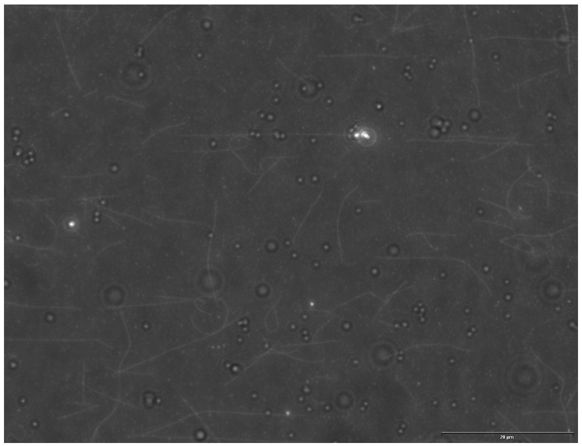
1. Fast, outward-walking motor
   D. melanogaster KHC
2. Slower, inward-walking motor
   S. cerevisiae Kar3



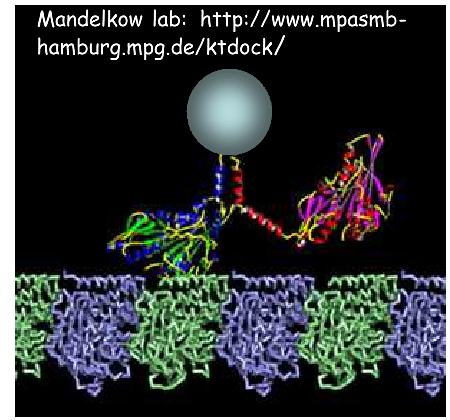
Christina Warrender, Ann Bouchard, Gordon Osbourn

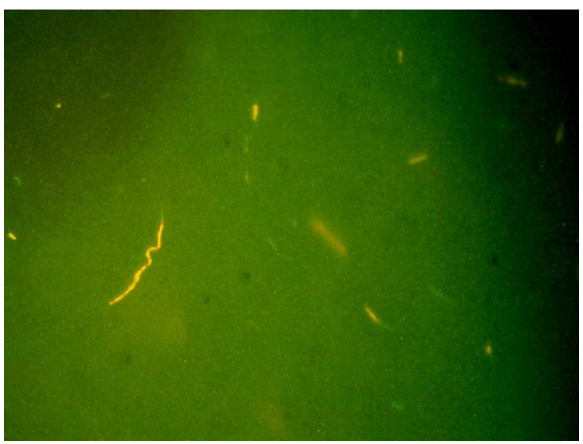
Translating BioAssembly:
Motility

Motor proteins and microtubules can interact dynamically in “standard” or “inverted” configurations

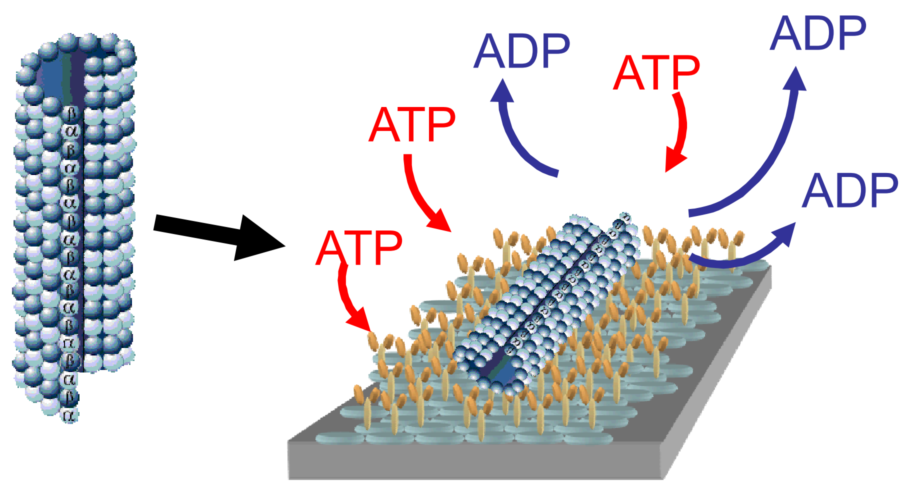


“Standard Motility” involves motor proteins moving, often carrying cargo, over an array of surface-bound MTs



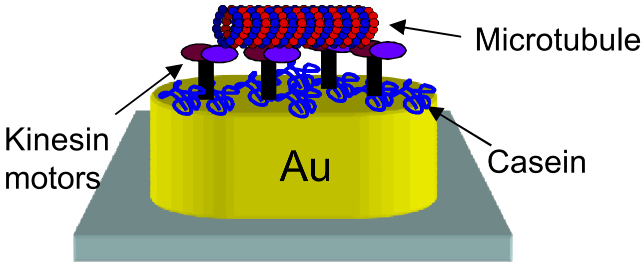


“Inverted (Gliding) Motility” relies on array of surface-bound inverted kinesins to capture and transport MTs over a surface

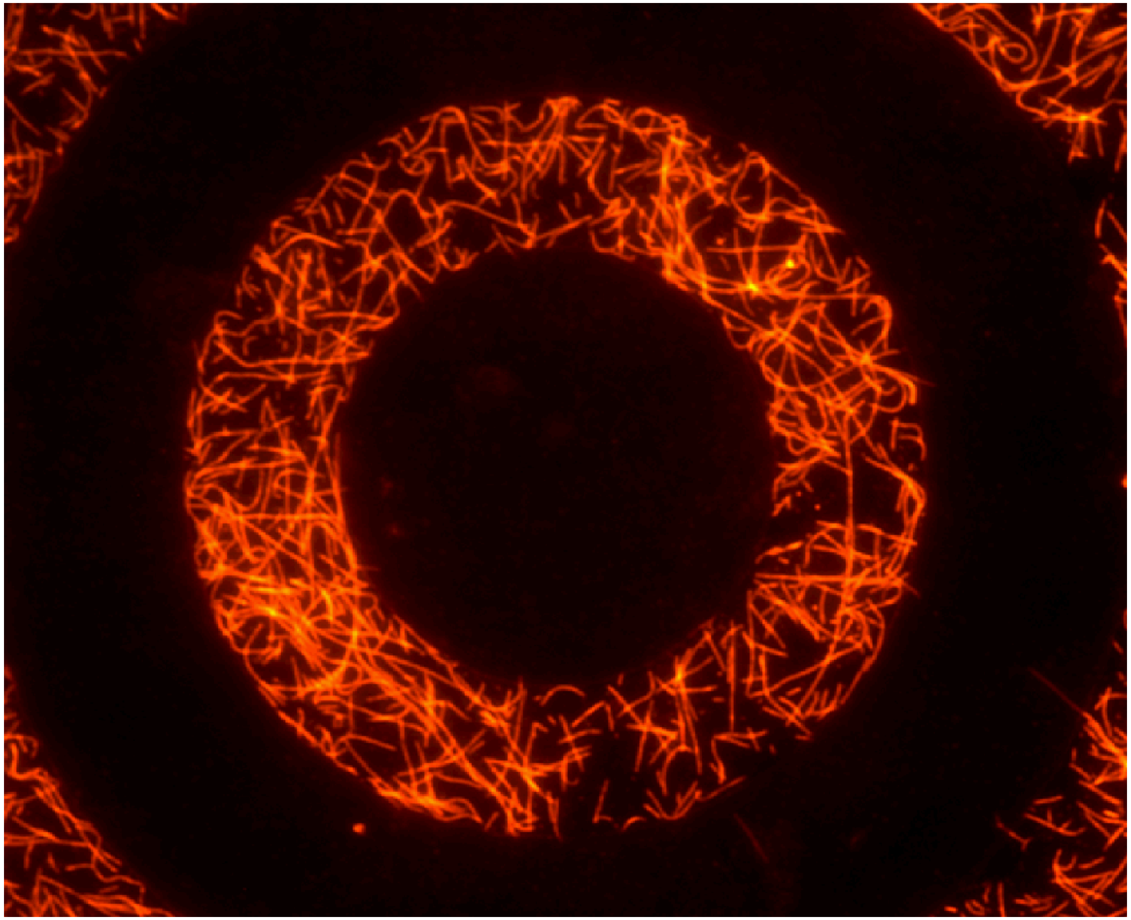


George Bachand, Haiqing Liu

Selective MT Capture



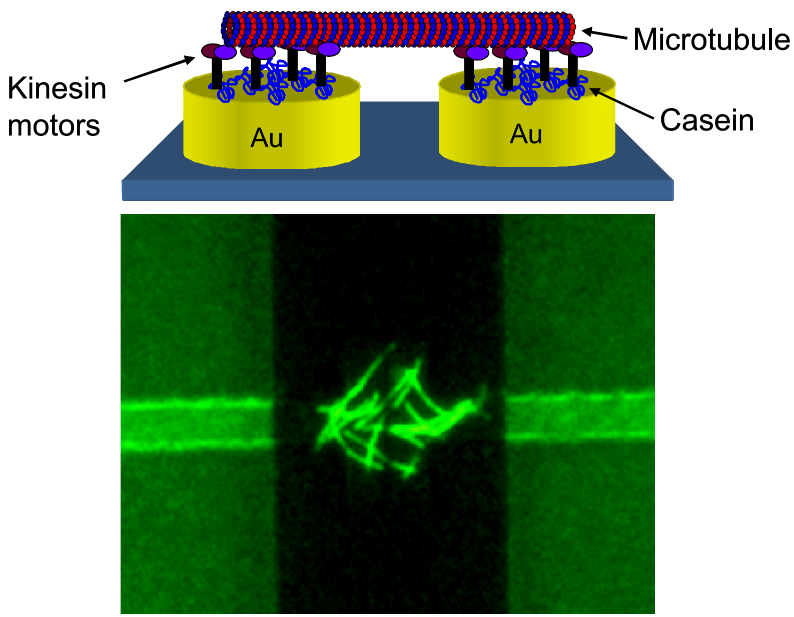
When ATP is replaced with AMPPNP, kinesins bind MTs statically



Applying Biometallization

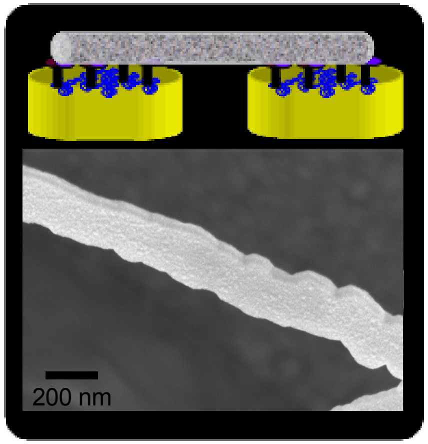
Motor proteins can be selectively bound to gold surfaces and used to capture MT bridges between gold electrodes.

Metallization or mineralization of these bridges forms engineered nanointerconnects.



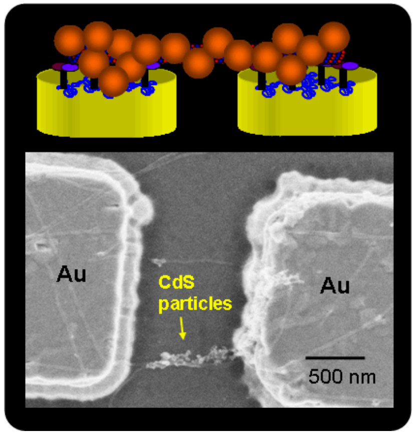
Ag metallization

electrical interconnects (<100Ω)

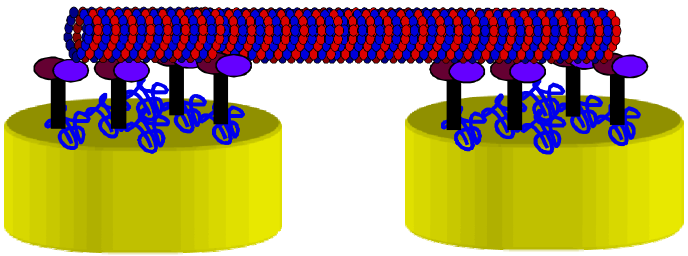


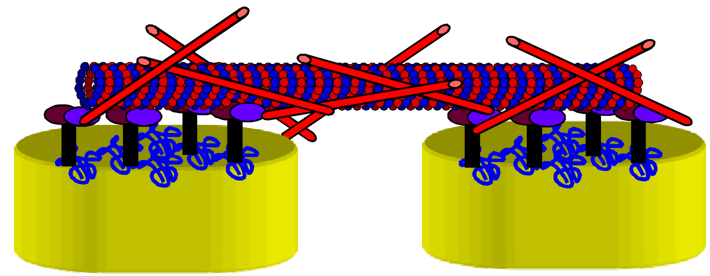
CdS mineralization

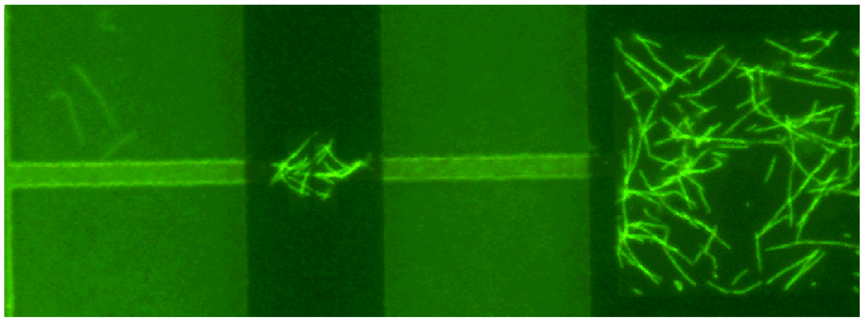
metal/semiconductor junctions

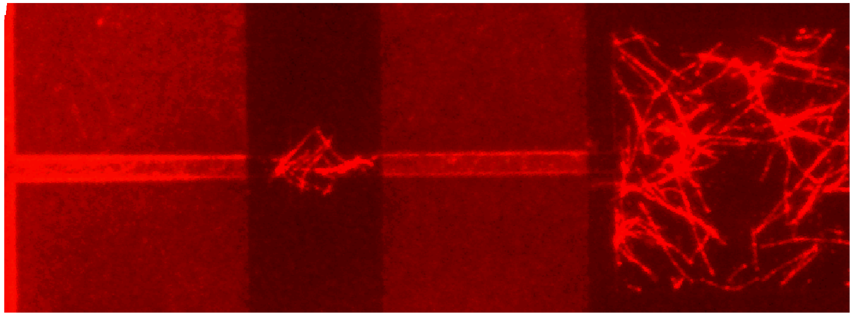


Nanomaterials Templating







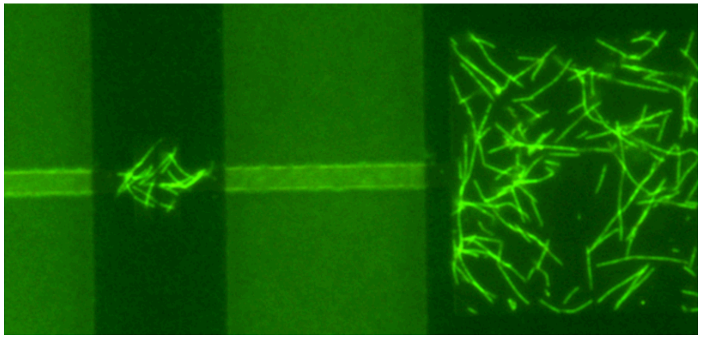


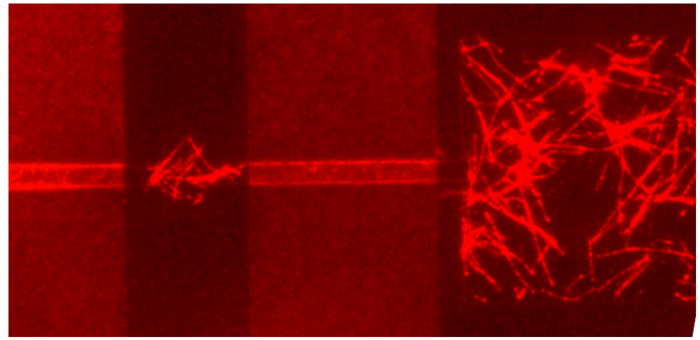
Kinesin-captured MTs will selectively bind to gold platforms, forming bridges between gold electrodes

Using Biotin-Streptavidin linkages, SWNTs may be templated on MT bridges *without degrading MT/kinesin interactions*

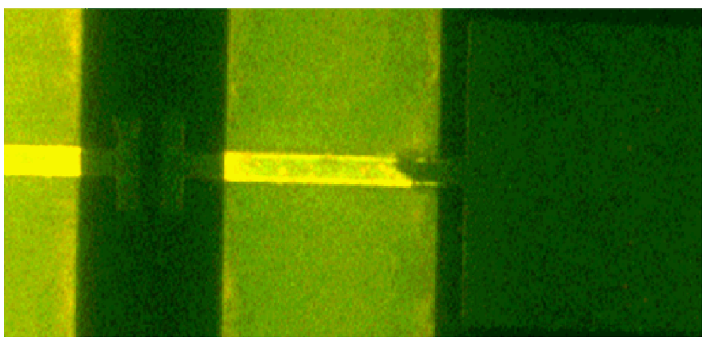
Assembly "Thermo-dynamics"

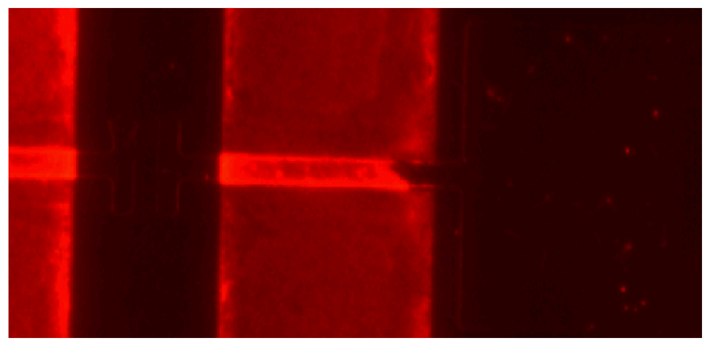
Fluorescent MTs | Fluorescent SWNTs on MTs





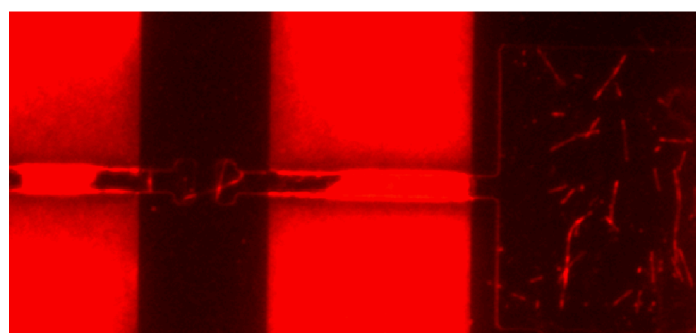
As assembled





After 4^oC treatment

Thermal disassembly imitates the dynamic function of MTs in Nature, introducing dynamic character to this assembly.

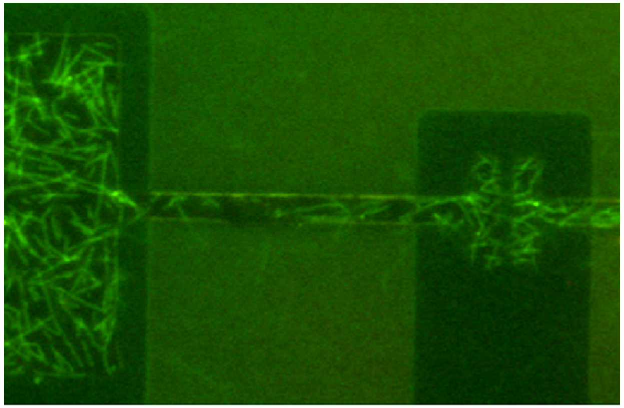


After reassembly

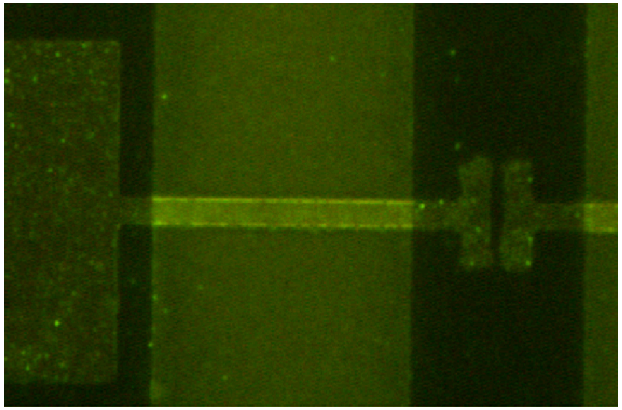
“Motor-cycling”

The motor proteins used to capture the MTs on the gold platforms can be activated to “erase” the MT templates, but the motors retain their bioselective function

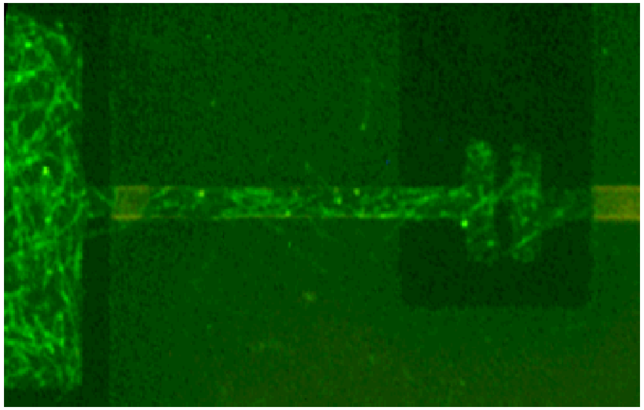
As assembled



After adding ATP



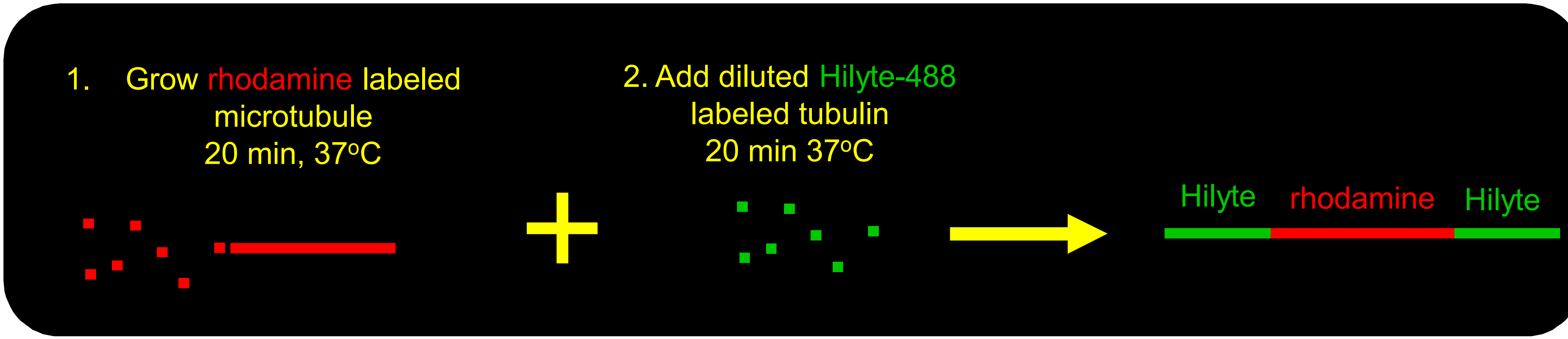
After adding fresh MTs and AMPPNP



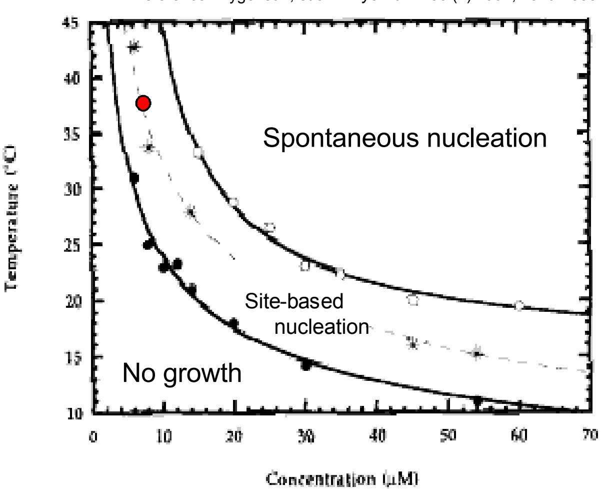
“Cyclability” of these dynamic structures is a key differentiating element of this nanoassembly scheme.

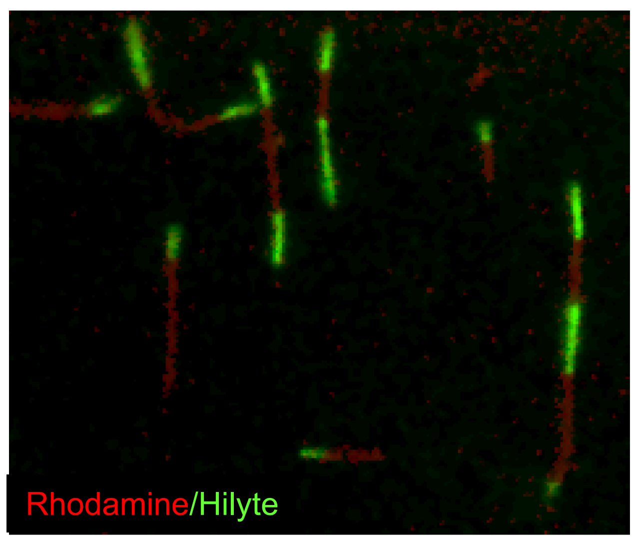
Multifunctional Templates

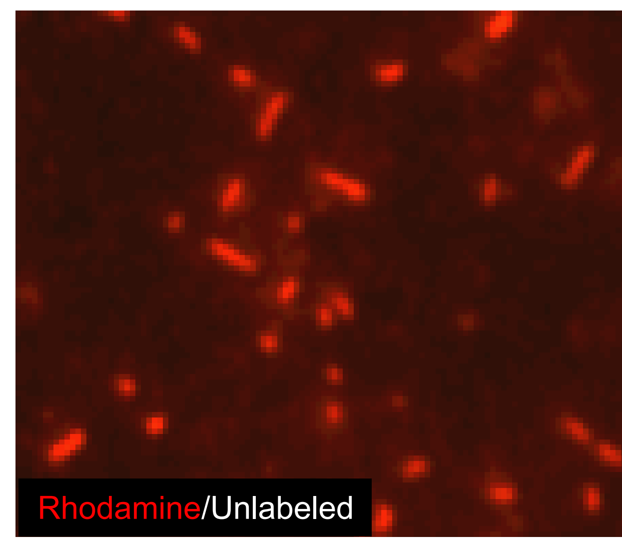
Controlling MT polymerization allows the formation of functionally segmented MTs



* Reference: Fygensun, et al. *Phys Rev E* **50** (2) 1994, 1579-1588



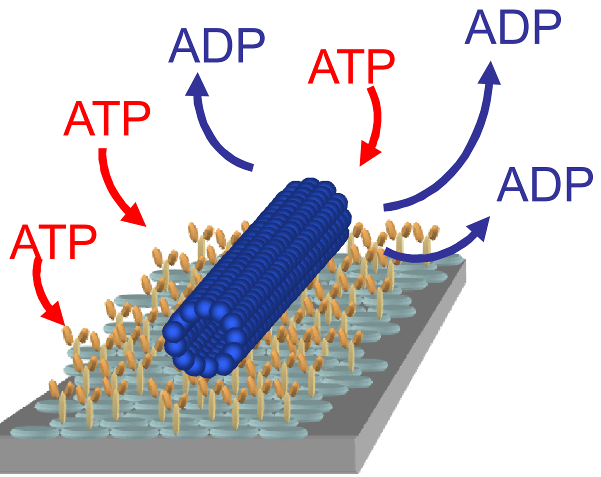




Translating BioAssembly:

Non-biological Assemblies

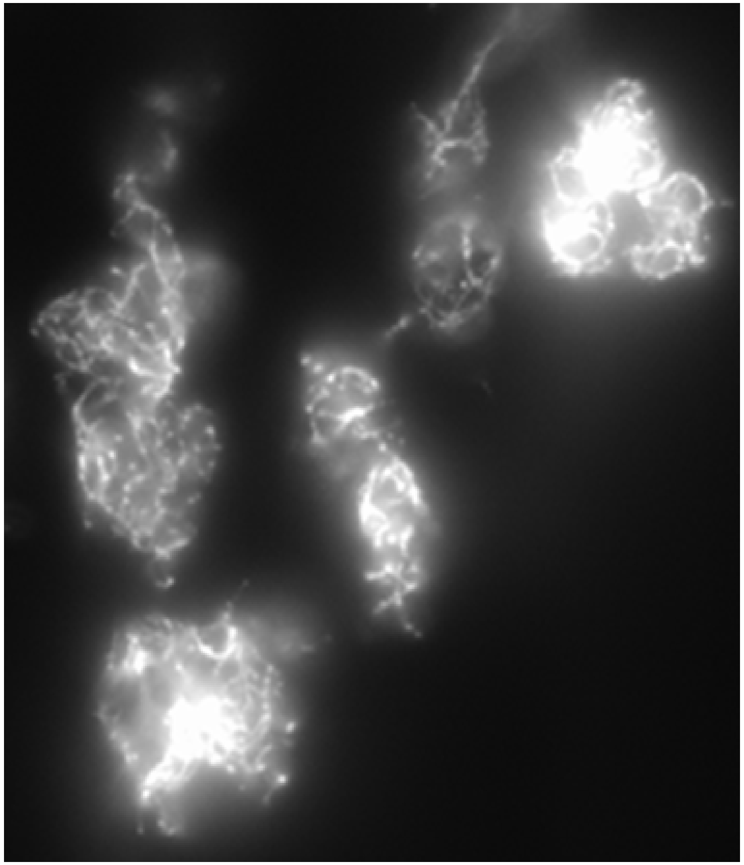
How can we use active, dynamic nature of MTs and motor proteins to create artificial assemblies?



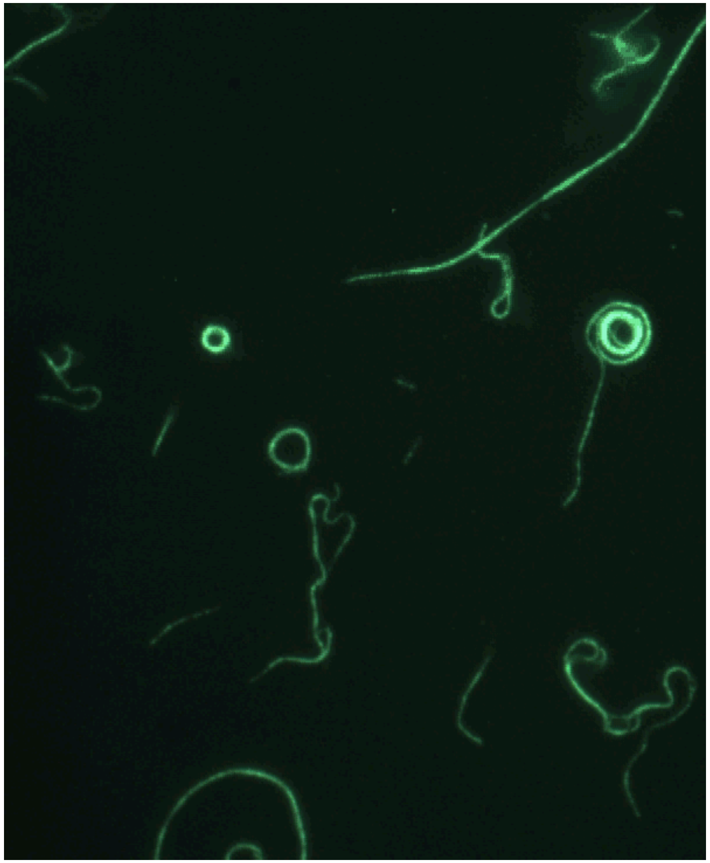
Influence of *Active* Assembly

When biotinylated MTs are combined with streptavidin linkers, active assembly has a profound impact on materials structure

Random Assembly



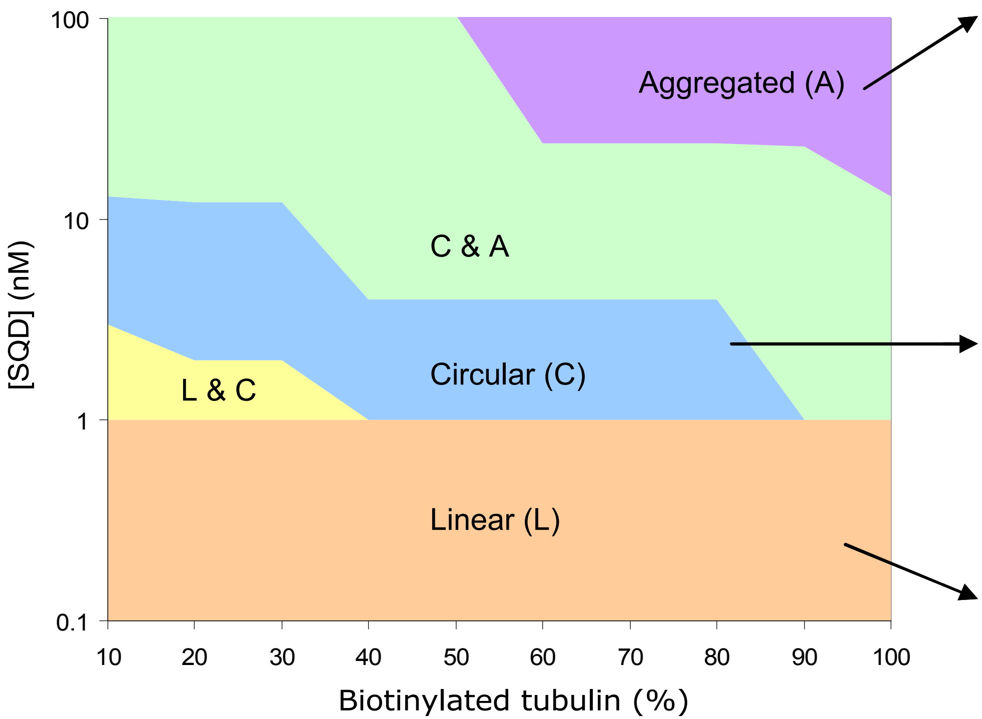
Kinesin-driven Active Assembly

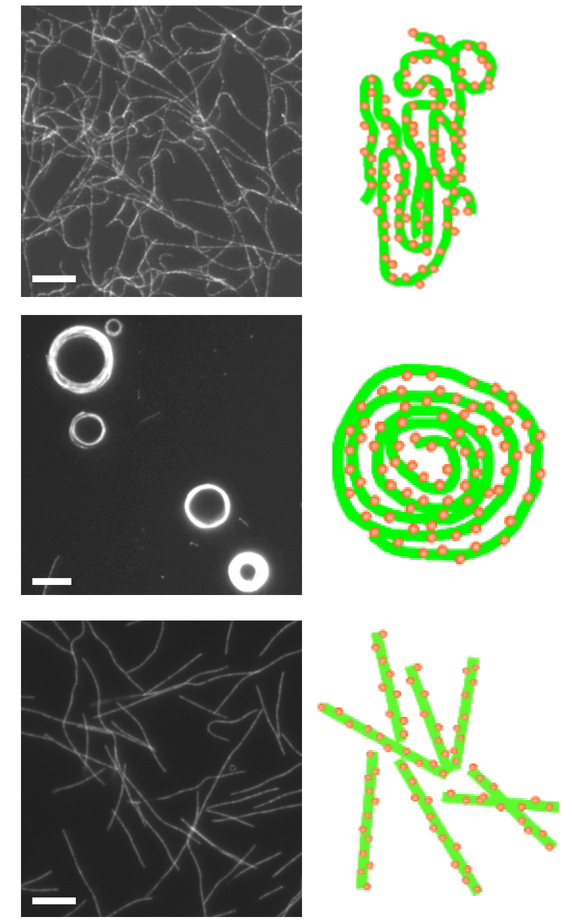


Andrew Boal and George Bachand

A Dynamic Nanocomposite Phase Diagram

Variation in streptavidin and biotin determine nanocomposite “phase”

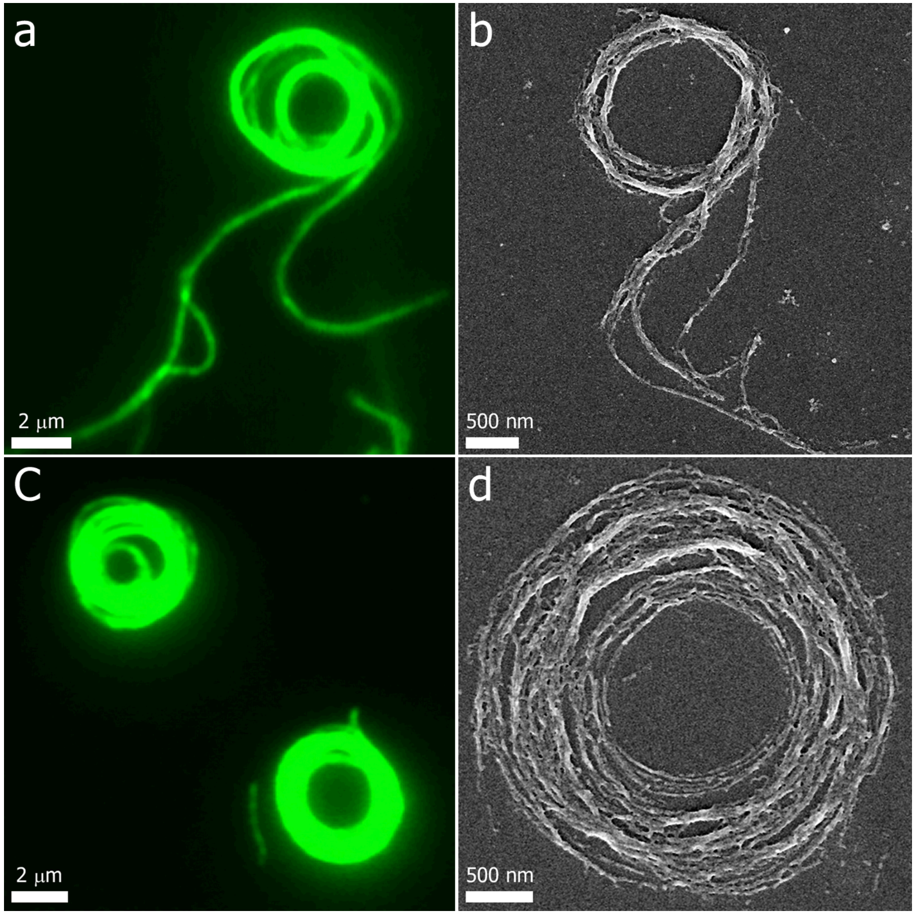


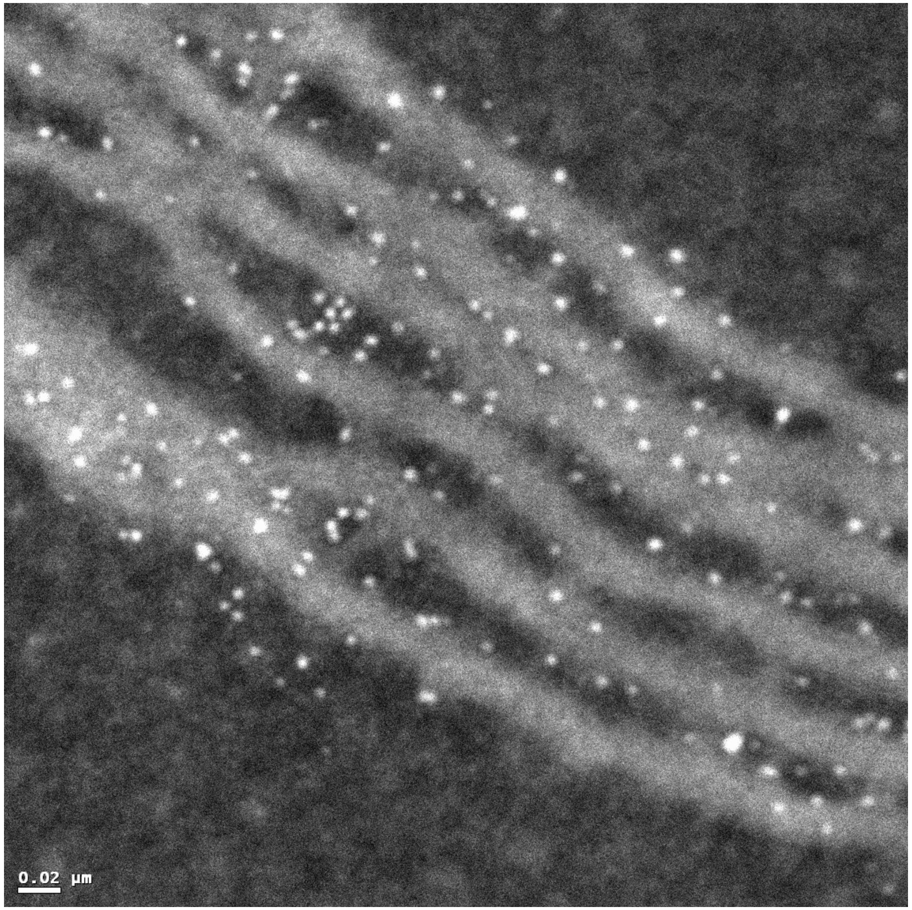


Haiqing Liu and George Bachand

Uncovering Nanocomposite Structure

Electron microscopy reveals the local structure of MTs and SQDs in nanocomposite circles

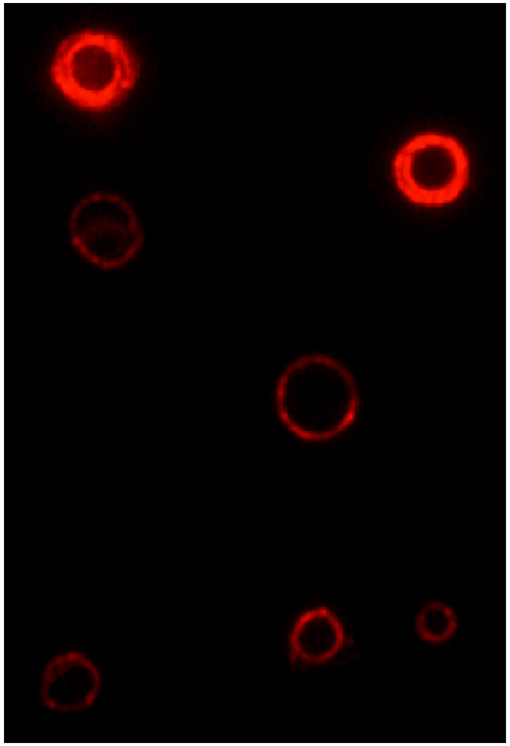




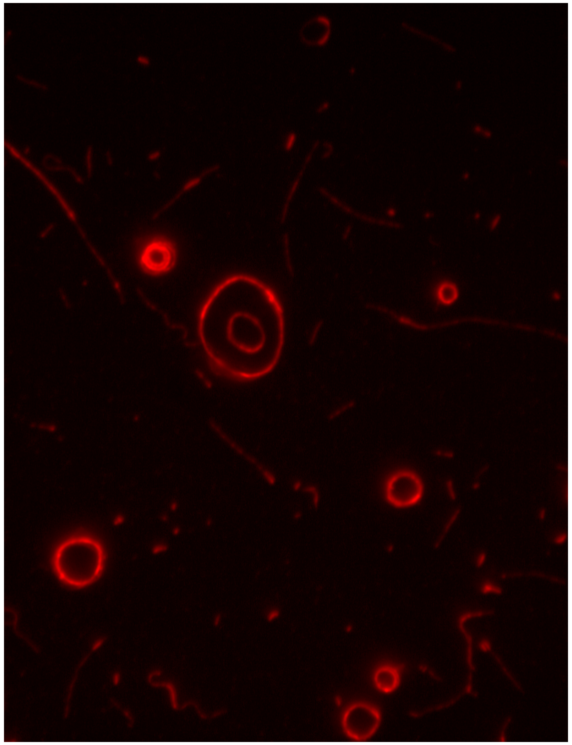
Dynamic Nanocomposite Diversity

Different materials/chemistries may be used to form nanocomposites

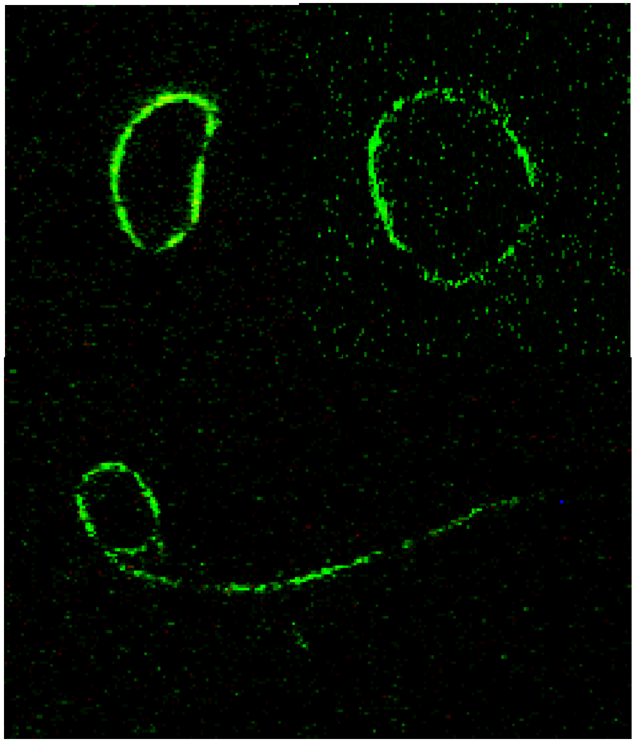
Streptavidin-QDs



Streptavidin-SWNTs



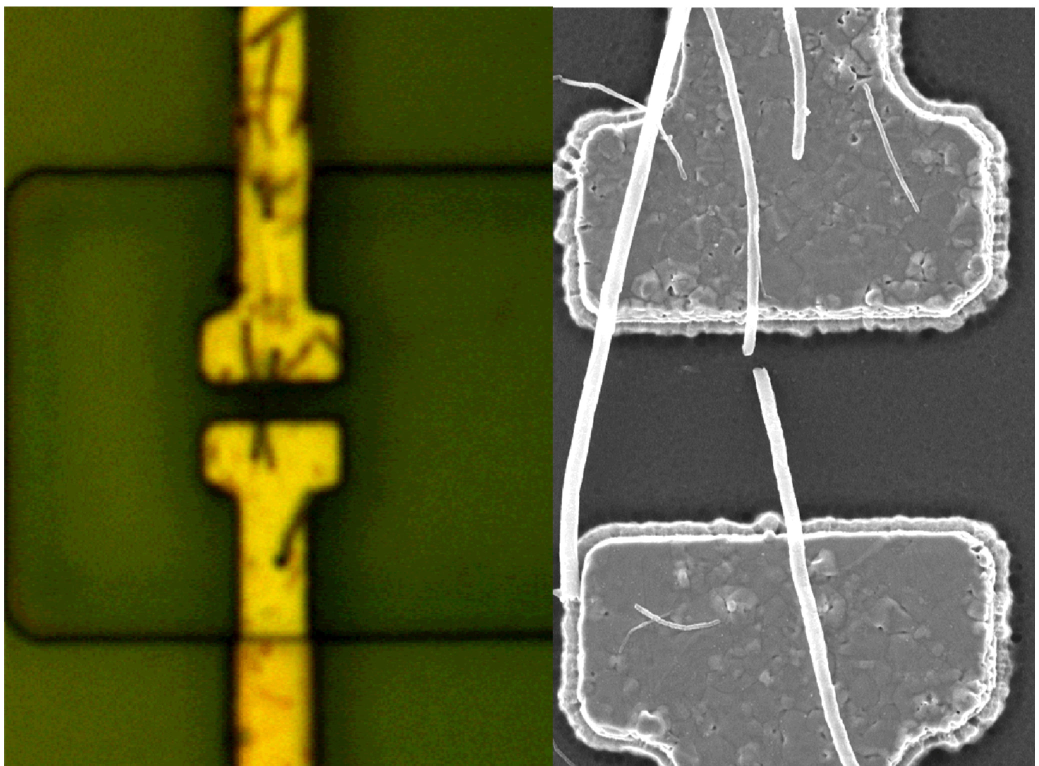
Anti-biotin gold



Conductive Templating: MWNTs

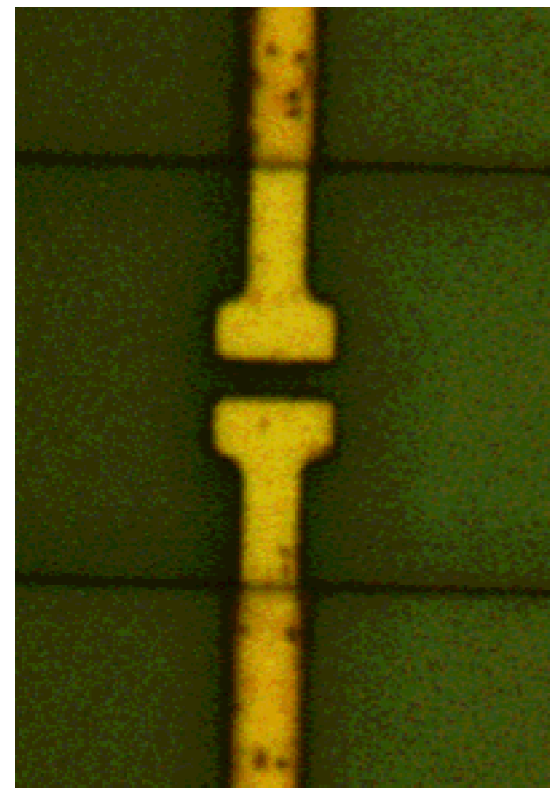
Using biotinylated MTs as templates, MWNTs can be selectively templated onto gold interconnects

MT templated MWNT interconnects



Measures ~15kΩ to 20kΩ!

No MTs = No MWNTs



Summary and Conclusions

✓ MTs are versatile biological nanowires in Nature.

✓ Taking advantage of their diverse biofunctional character we can use these structures to template a wide range of metals, oxides, sulfides, and hybrid materials.

✓ Microtubule templates can be integrated and assembled to form complex, bio-enabled architectures.

✓ Manipulating the polymerization and multifunctional character of microtubules, we can create unique, spatially-resolved hybrid nanowires.

Take home message:

Creative uses of biological tools can facilitate the development of new strategies for nanocomposite synthesis and assembly

This work wouldn't be possible without…

Teamwork

Judy Hendricks (Nano-interconnect work)

Dr. George Bachand (Kinesin work, MT-circles)

Adrienne Greene (Kinesin switch)

Dr. Haiqing Liu (MT-circles)

Dr. Andrew Boal (Organizing Centers)

Dr. Christina Warrender, Dr. Ann Bouchard, and
Dr. Gordon Osbourn (Computer simulations)

Dr. Bruce Bunker (Bio-Integration program head)

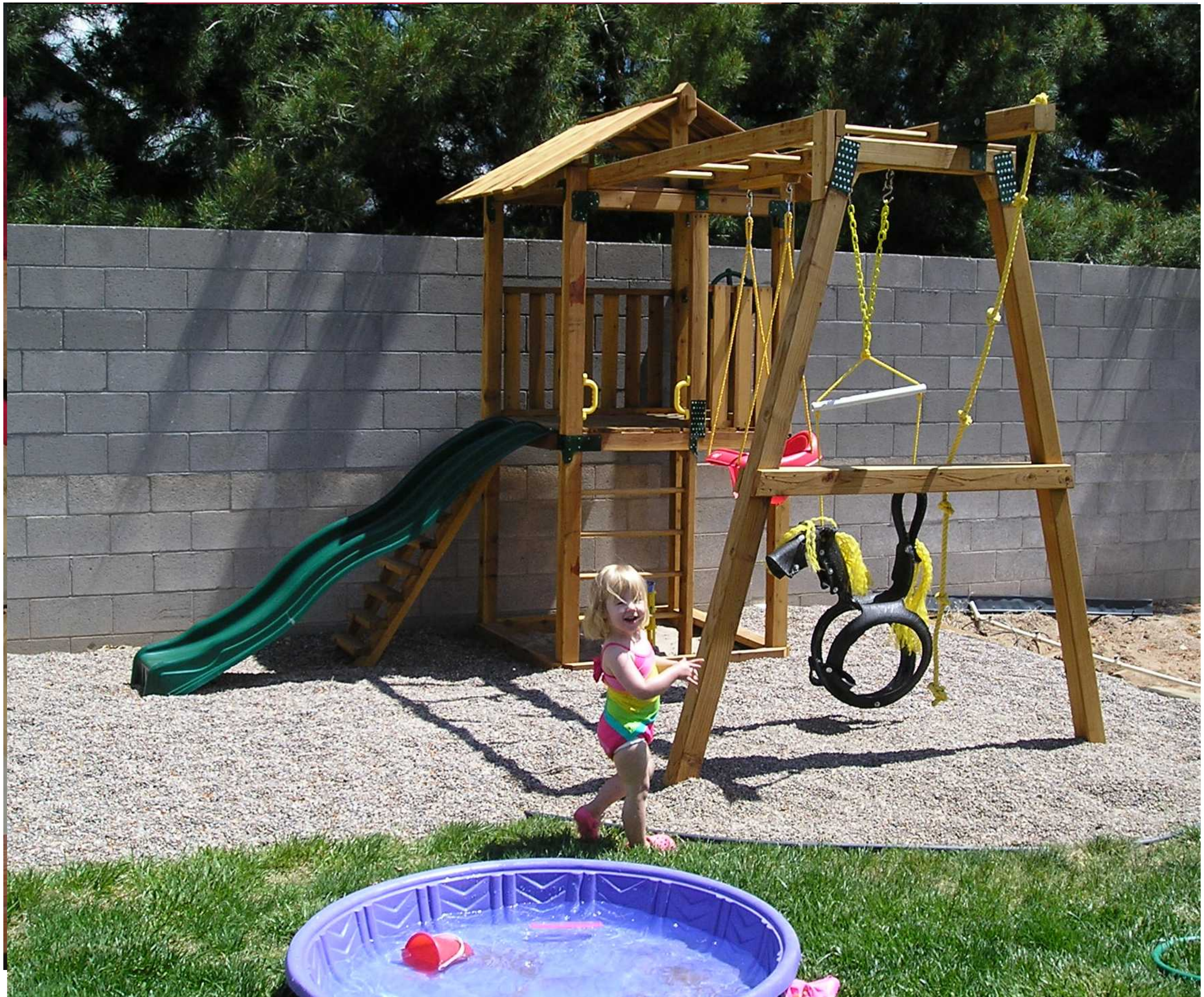
Video Credits: "Inner Life of the Cell"

Conceptualized by *Dr. Alain Viel Ph.D.*, and *Dr. Robert Lue Ph.D.*, Molecular and Cellular Biology, Harvard University

Animated by *John Lieber* of **XVIVO**, Inc.

Funded by the *Howard Hughes Medical Institute*

Fundamentals of Active Assembly



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Sandia National Laboratories

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