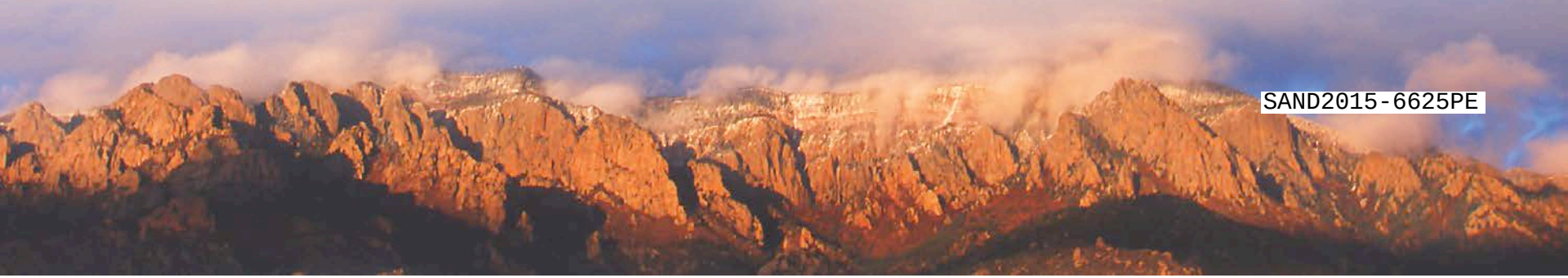

SAND2015-6625PE

Active Assembly of Dynamic and Adaptable Materials

George D. Bachand, Erik D. Spoerke,
Darryl Y. Sasaki, & Mark Stevens

Sandia National Laboratories, Albuquerque, NM

Biomolecular Materials PI Meeting
August 3-5, 2015

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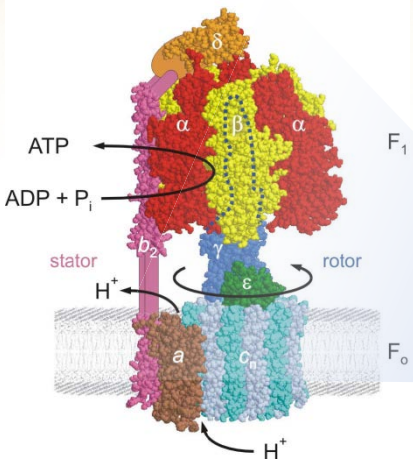
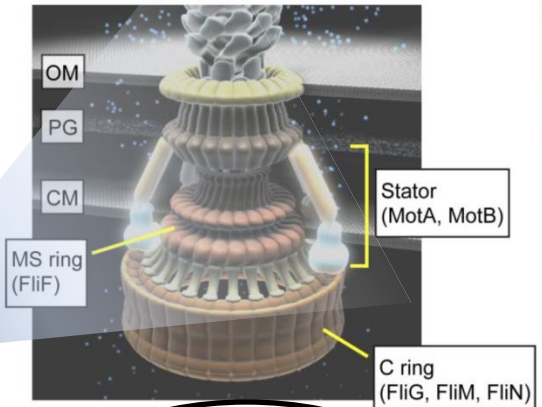
Molecular Motors/Machines in Cells

Ion Pumps

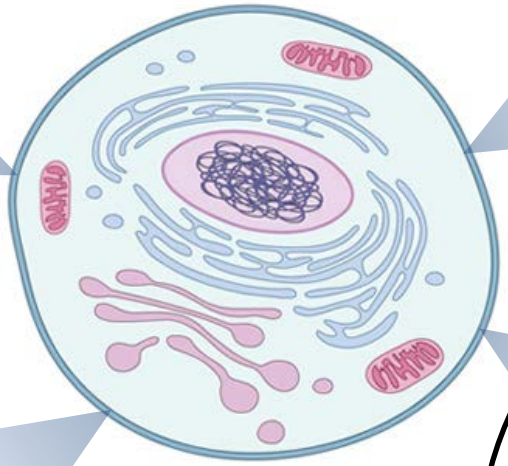


http://www.depts.ttu.edu/chemistry/Faculty/weber

Actuators

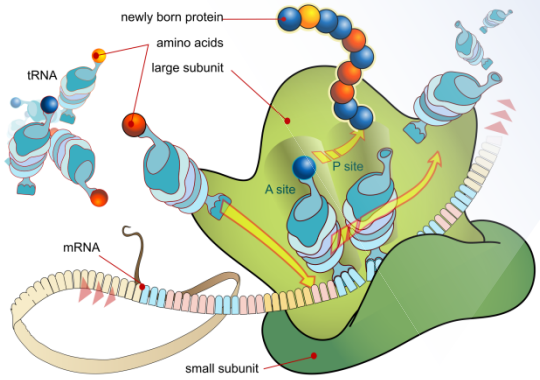


Morimoto & Minamino *Biomolecules* 4, 217 (2014)



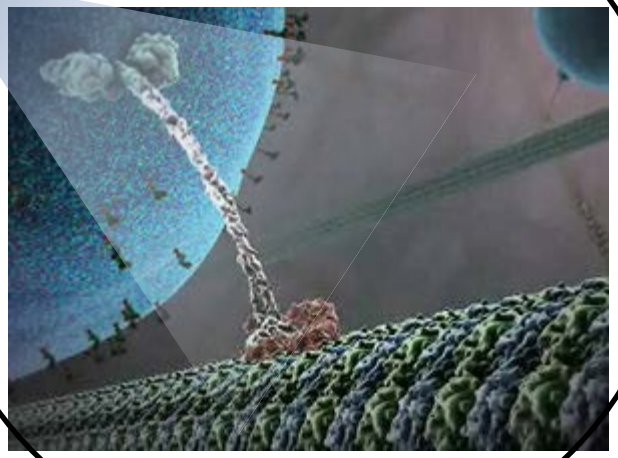
http://www.nature.com/scitable/topicpage/what-is-a-cell-14023083

Molecular Assemblers



http://en.wikipedia.org/wiki/Translation_(biology)

Intracellular Transporters



http://multimedia.mcb.harvard.edu/

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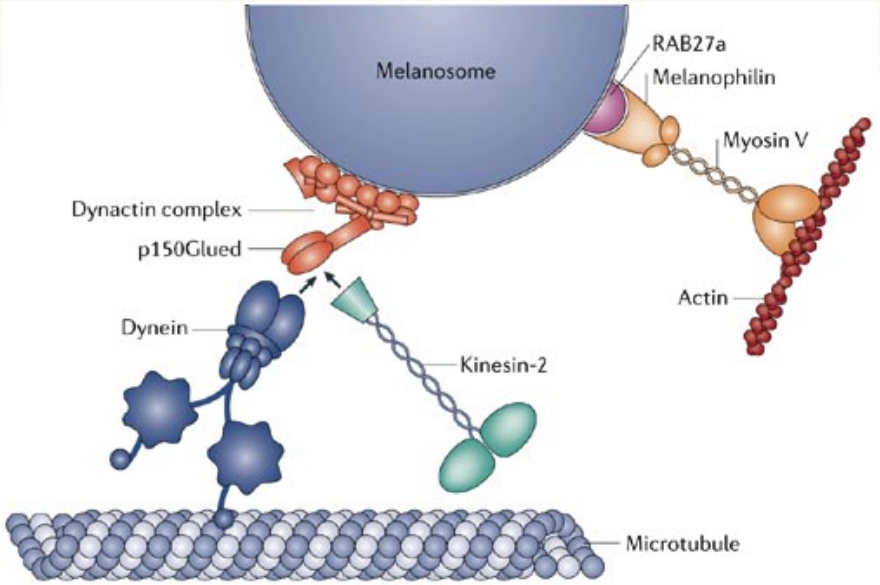
Active Transport & Multiscale Phenomena

Video Credit: Caldwell Lab, UC-Berkeley

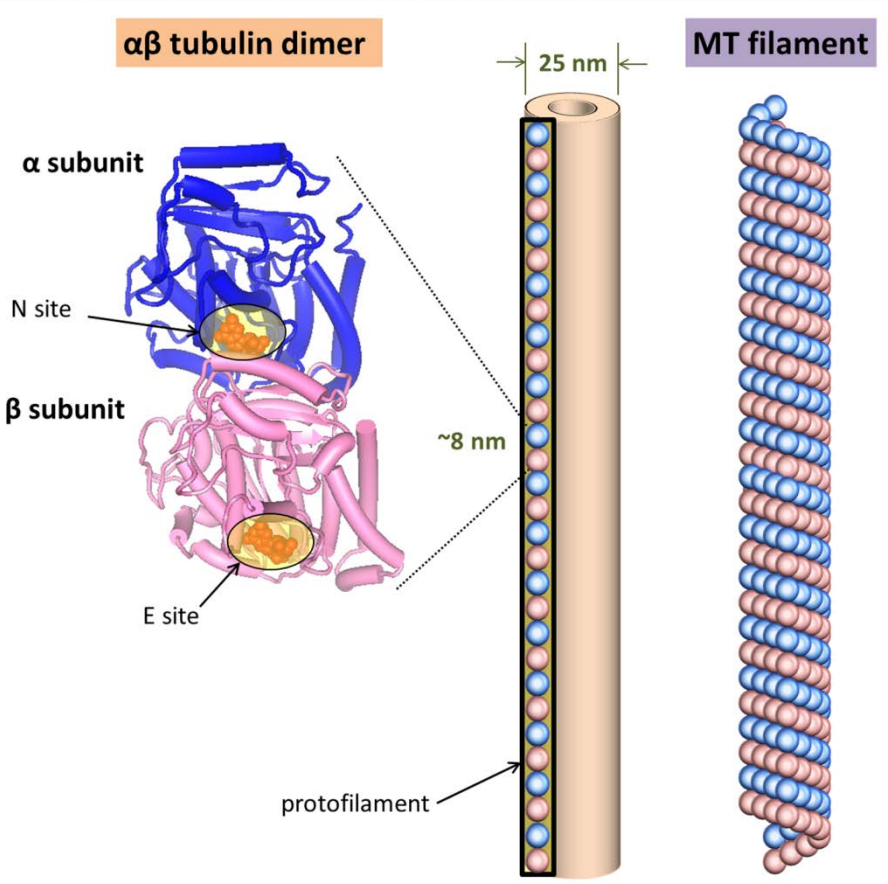


Soldati & Schliwa, 2006, Nat. Rev. Mol. Cell Biol., 7, 897

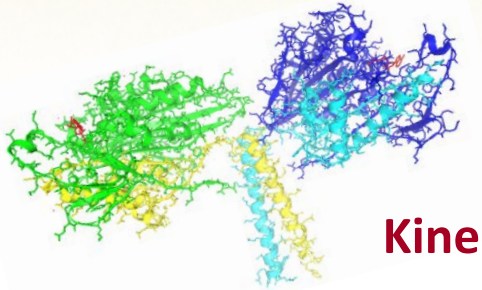
Project goal: to understand how nature's biomolecular machines dynamically assemble materials across multiple lengths scales, and to apply these principles and components in hybrid or composite materials whose assembly and organization can be "self-directed" or autonomously responsive to stimuli

Active Transport Proteins

Microtubules (MTs)



- Consumes guanosine triphosphate (GTP)
- Forces of 40 pN (polymerization) & −15 pN depolymerization



Kinesin Motors

- Dimeric protein
- Consumes adenosine triphosphate (ATP) to produce mechanical force
- Work = ~40 pN•nm; Efficiency = ~50%
- “Walks” along MTs in 8-nm steps
- Transport velocity = 0.5 – 12 μm/sec

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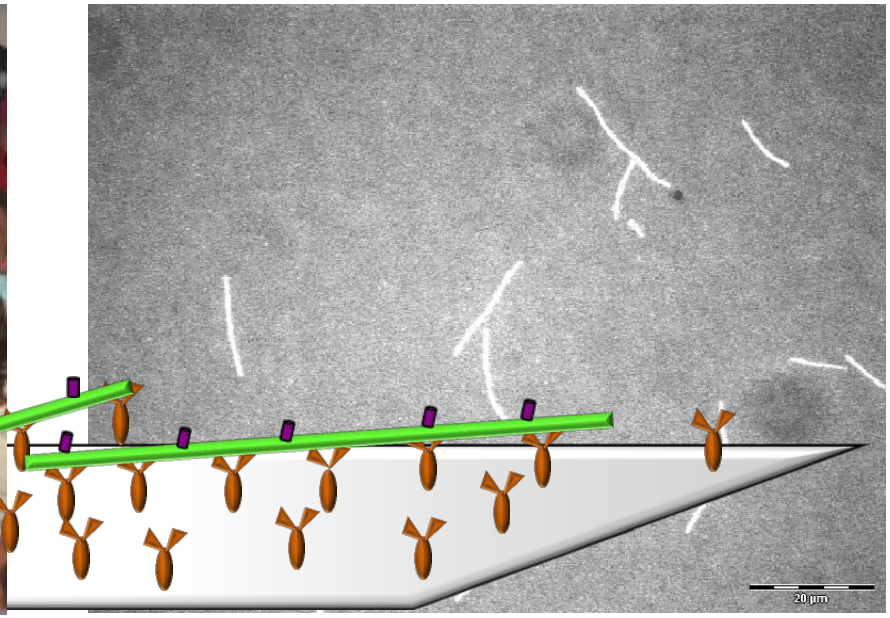
Ex vivo Kinesin Biomolecular Machines

A minimalistic system can be assembled from:

- Solid surface
- Kinesin motor proteins
- Biotinylated microtubules

Transport @ ~750 nm/s, forces >100s pN, near-infinite run length

Streptavidin-functionalized cargo



How can the energy-dissipative nature of these machines exploited to dynamically assemble hybrid/composite materials?

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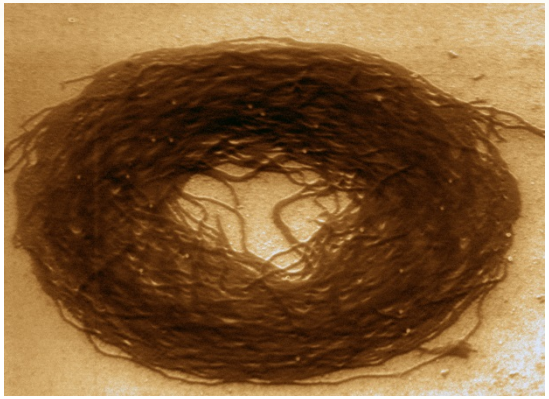
Outline

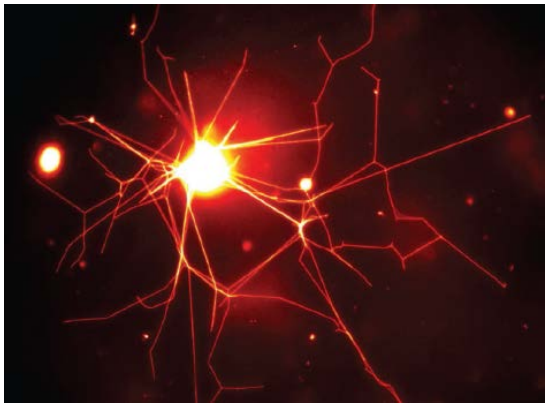
I. DSA of Nanocomposite Rings

- Background
- Mechanism of Assembly
- Regulating Ring Properties

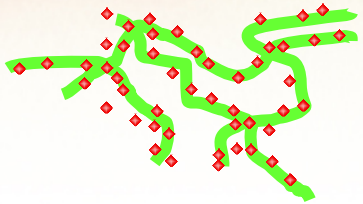


II. DSA of Lipid and Polymer Nanotube Networks

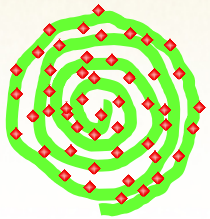
- Background
- Lipid Nanotube Networks
- Polymer Nanotube Networks
- Transport on Nanotubes
- Conclusions & Future Work



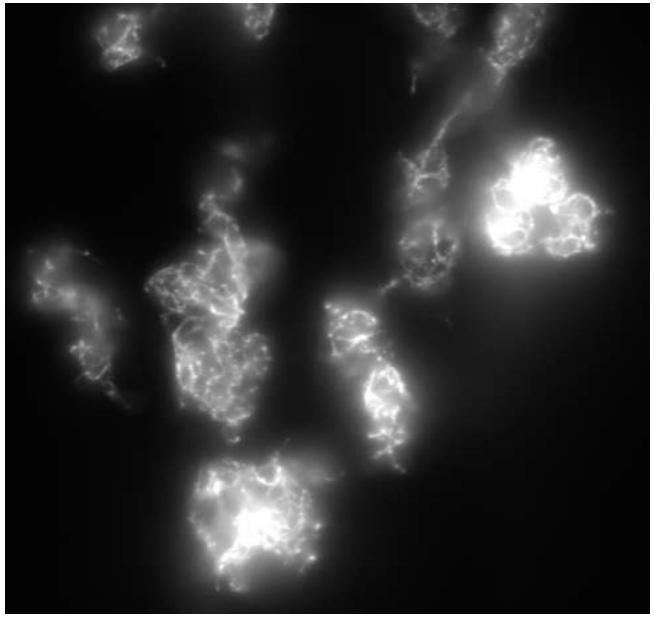
DSA of Nanocomposite Rings



Streptavidin-QDs + biotinylated MTs



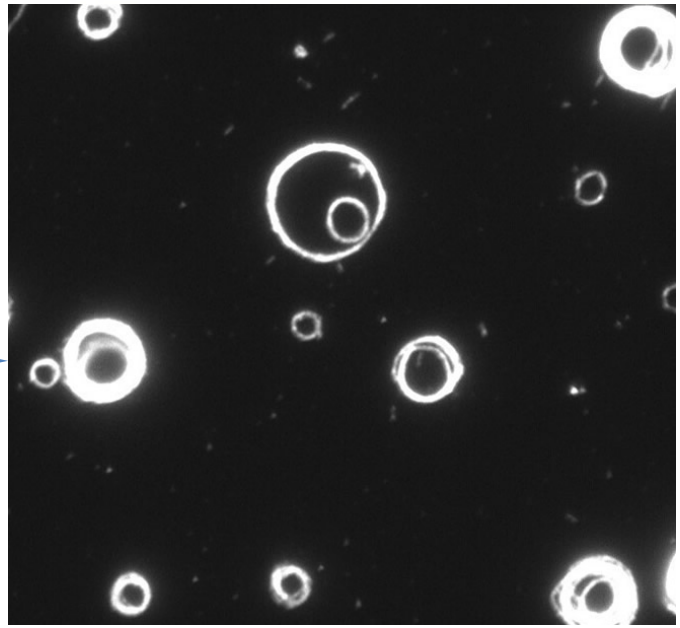
Streptavidin-QDs + gliding biotinylated MTs



Randomly assembled MTs and QDs (equilibrium)

Energy dissipation via ATP hydrolysis

~50 kJ/mol ATP



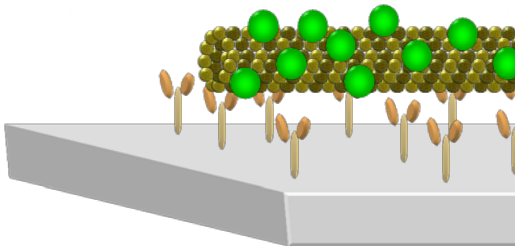
Actively assembled MTs and QDs **(+ work)**

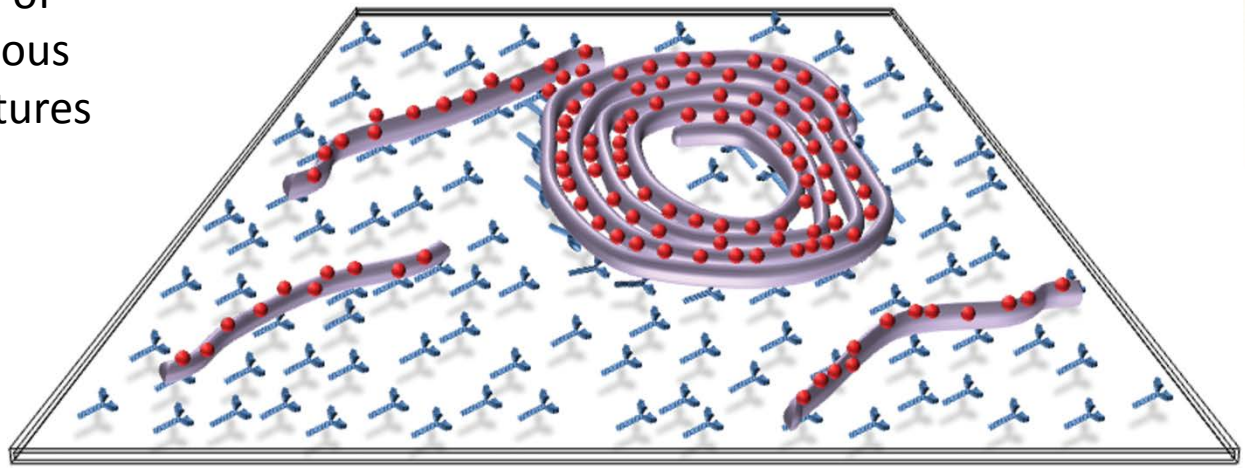
chemical energy → mechanical work → active assembly

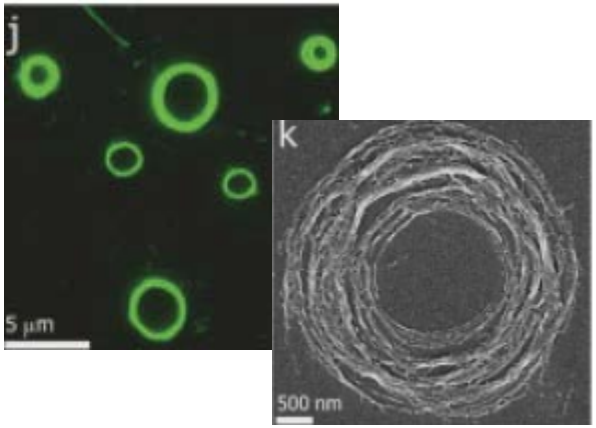
Bachand et al, (2004), *Nano Lett.* **4**, 718; Bachand et al., (2005), *J. Nanosci. Nanotechnol.* **5**, 781.
Liu et al., (2008), *Adv. Mater.* **20**, 4476; Liu et al., (2011) *Soft Matter* **7**, 3087.

DSA of Nanocomposite Rings

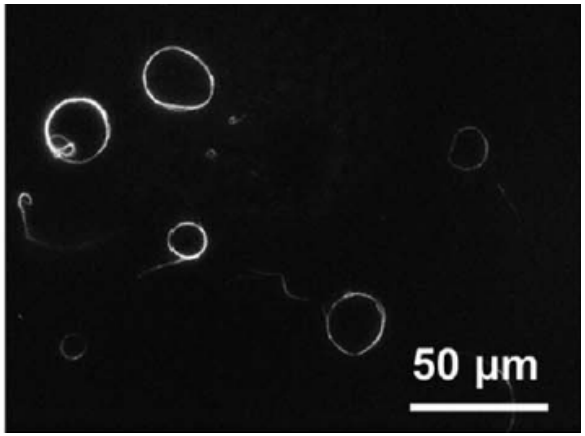
Co-deposition of, addition of Qdots induces the spontaneous DSA of ring composite structures



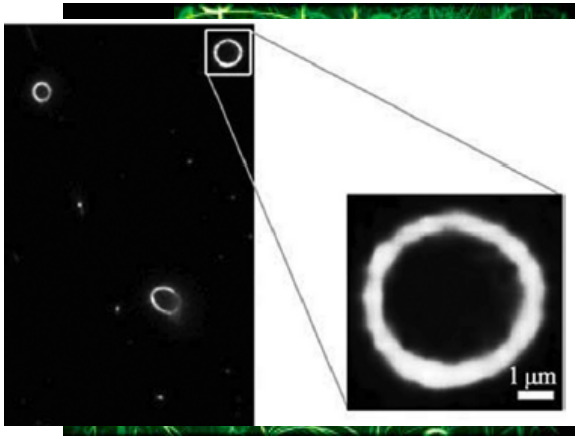




Liu et al. (2008) *Adv. Mater.* **7**, 3087



Lam et al. (2014) *Soft Matter* **10**, 8731



Kukugo et al. (2011) *Biomacromolecules* **12**, 3394

Schaller et al. (2011) *PNAS* **108**, 19183

Mechanisms proposed, but not well-characterized (effects from ROS and photodamage)

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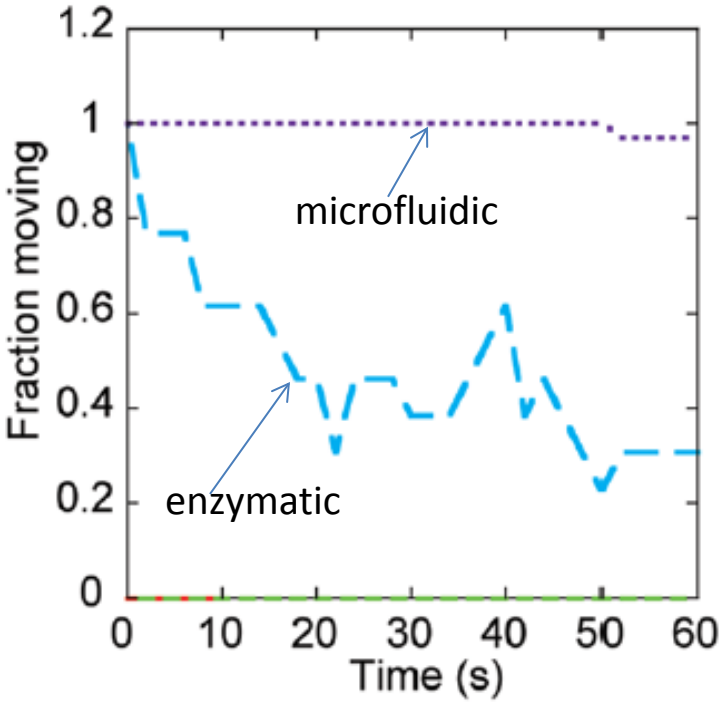
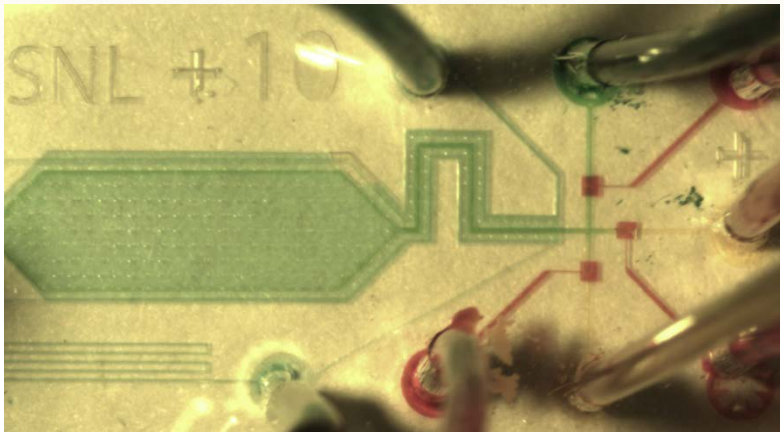
DSA of Nanocomposite Rings

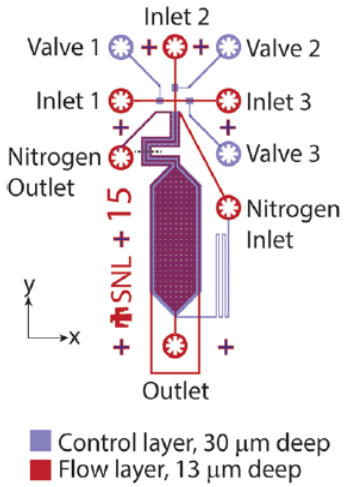
Motor function and/or MT structure damaged by ROS generated during imaging

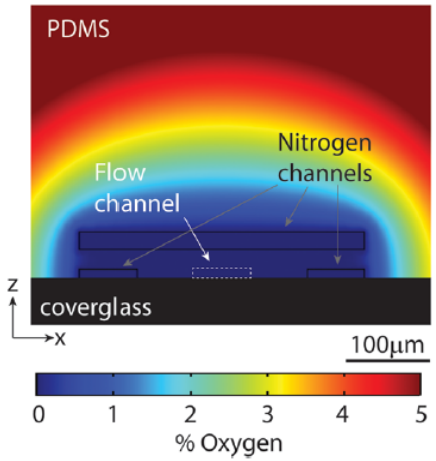


Device enables characterization of DSA without adverse effects due to dye excitation

Microfluidic deoxygenation







VanDelinder & Bachand (2014) *Analytical Chemistry* **86**, 721

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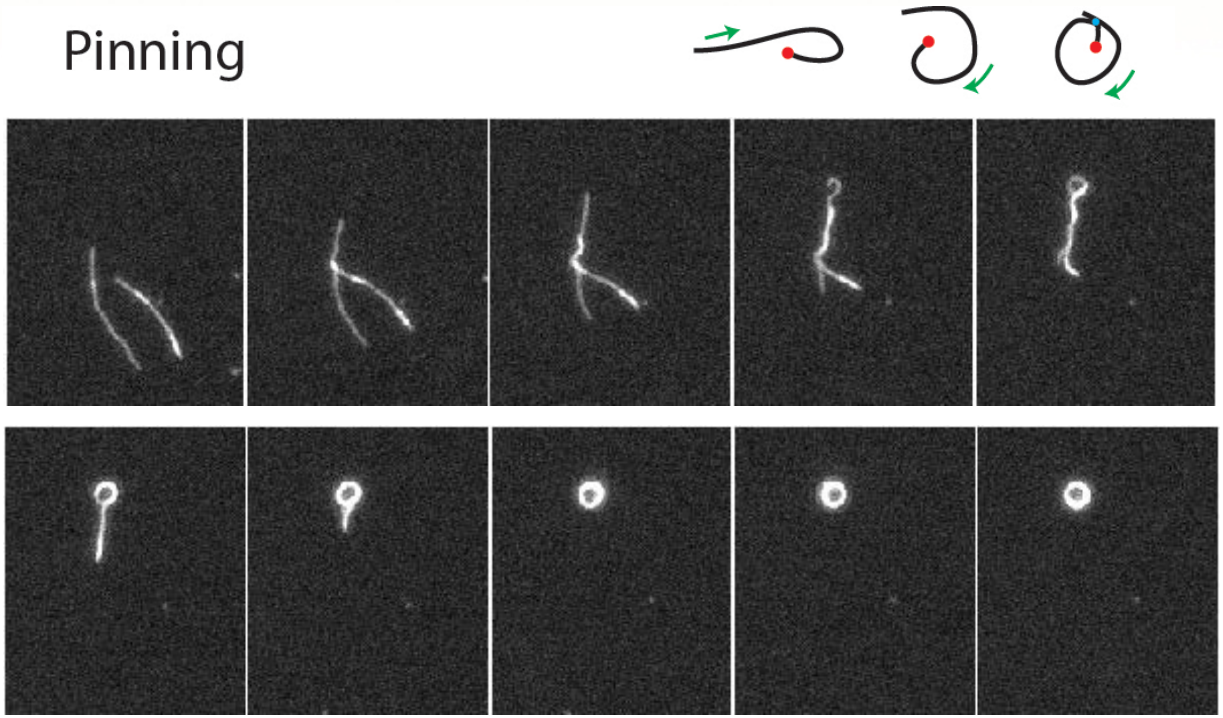
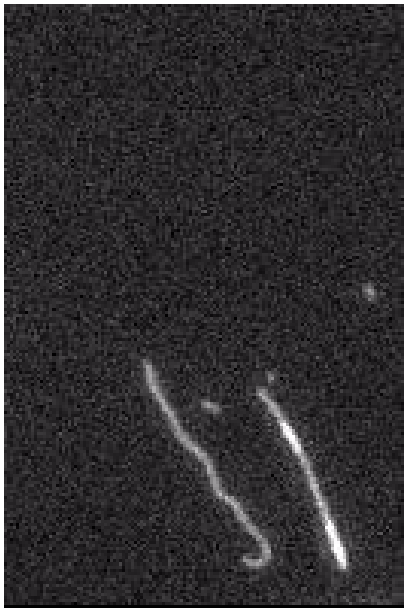
DSA of Nanocomposite Rings

Three mechanisms were observed with differing frequency and resulting ring structures.

Pinning



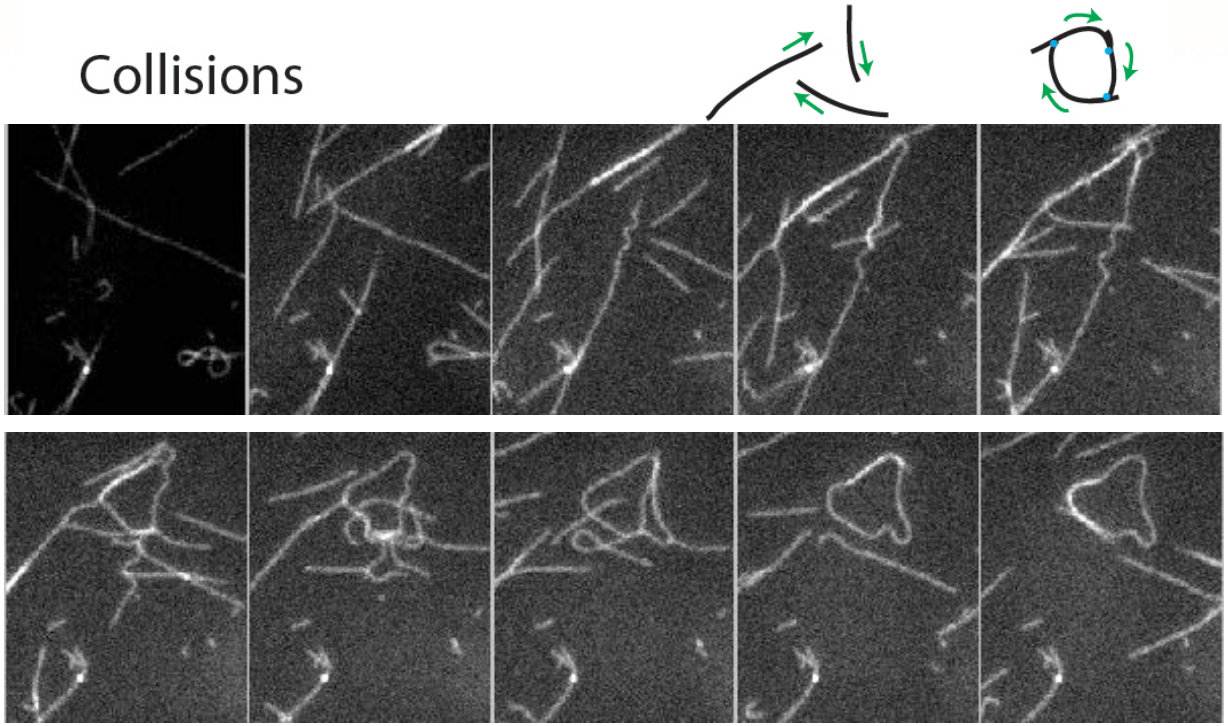


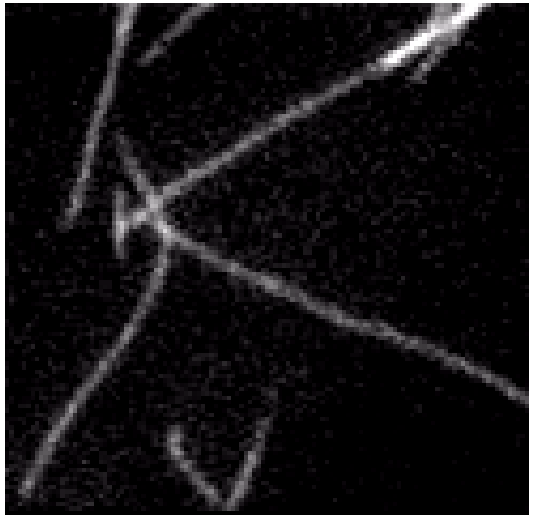
- Occurs when leading tip encounters inactive motor
- Results in ring w/ small inner diameter (<3 μm)
- Rarely observed when ROS are removed

VanDelinder et al., (2015) *Biomacromolecules* (submitted)

DSA of Nanocomposite Rings

Three mechanisms were observed with differing frequency and resulting ring structures.





- Primary mechanism of ring DSA
- Strongly dependent on MT surface density
- Results in rings w/ intermediate inner diameters (3 - 8 μm)

VanDelinder et al., (2015) *Biomacromolecules* (submitted)

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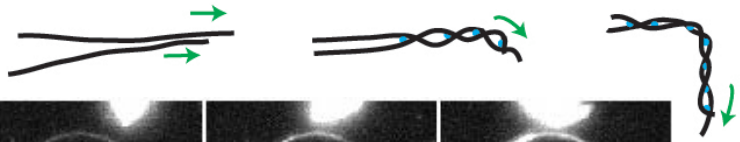
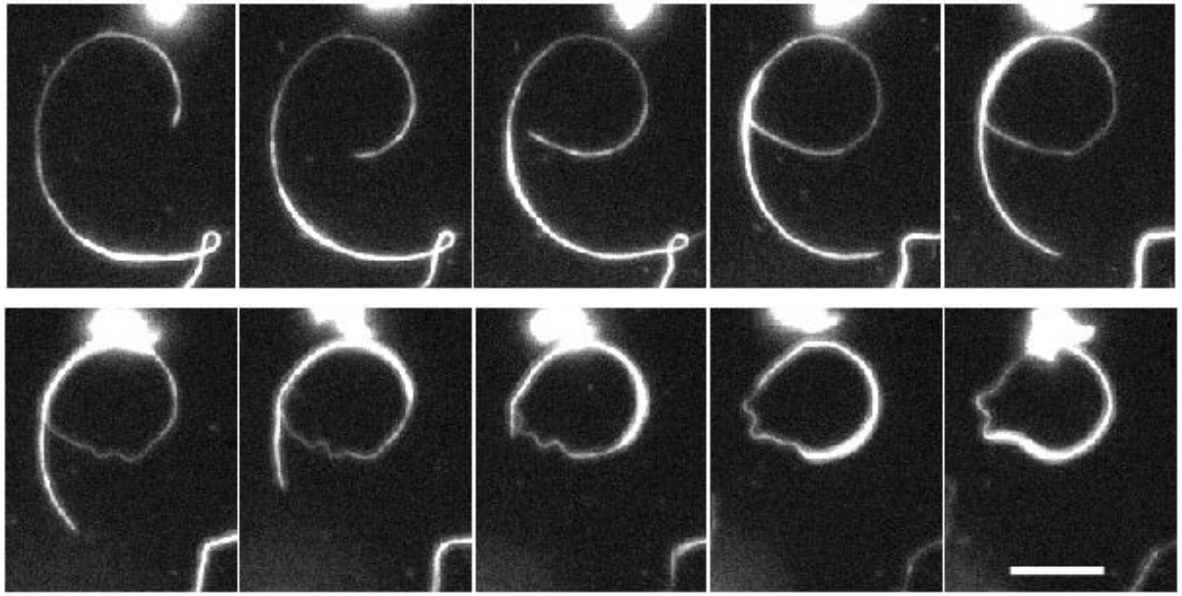
DSA of Nanocomposite Rings

Three mechanisms were observed with differing frequency and resulting ring structures.

Frozen in curvature

Coiling





- Twisted and/or kinked domain – induced curvature
- Results in ring w/ large inner diameter (>8 μm)

VanDelinder et al., (2015) *Biomacromolecules* (submitted)

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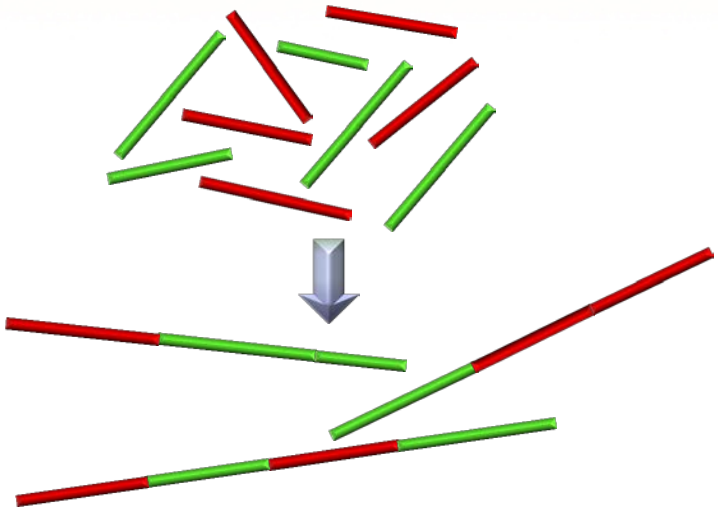
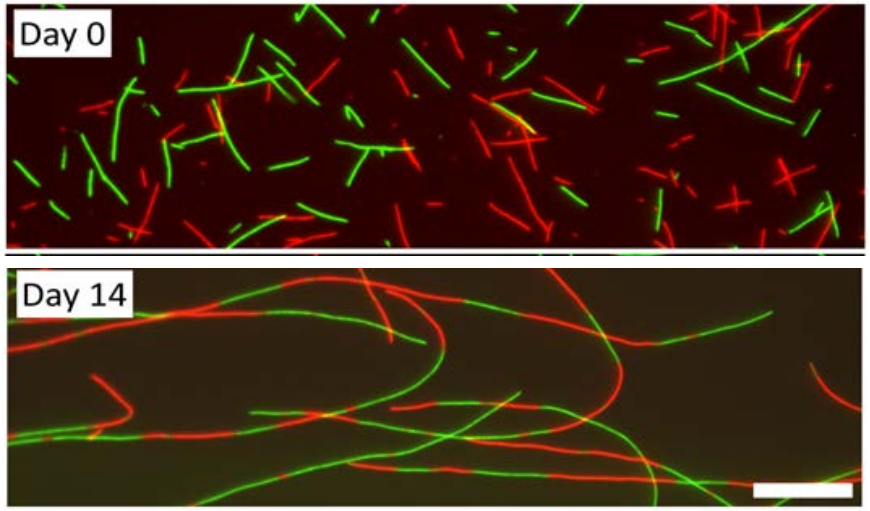
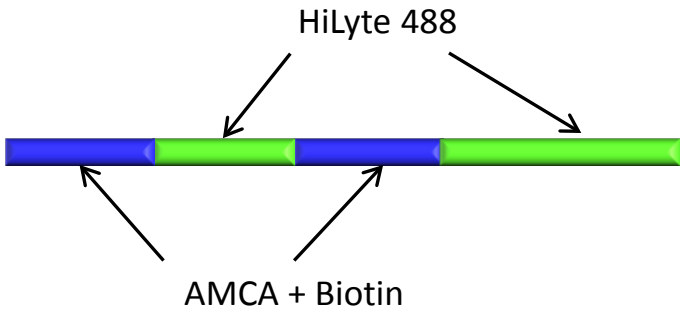
Regulating Ring Properties – Building Blocks

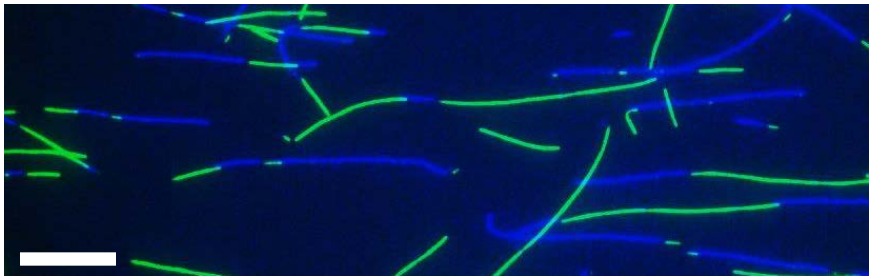
Head-to-tail assembly of MTs → permits the production of segmented MT building blocks with varying functionality, lengths, and frequency.





Bachand et al., (2014) *RSC Adv.* **4**, 51641





How will segmentation affect DSA of rings?

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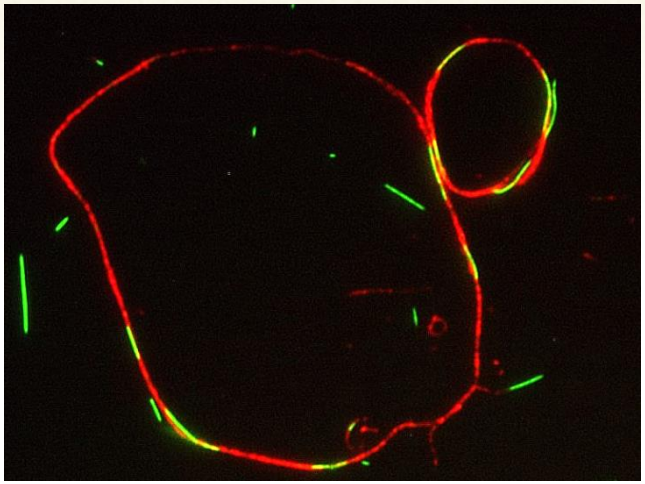
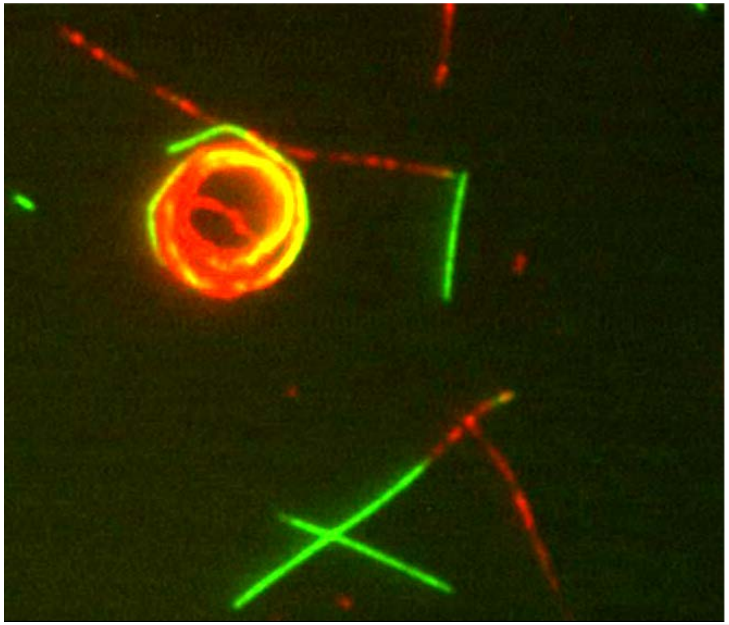
Changing Ring Building Blocks

Segmented MT building blocks dynamically assemble into composite rings that display similar and unique behaviors:

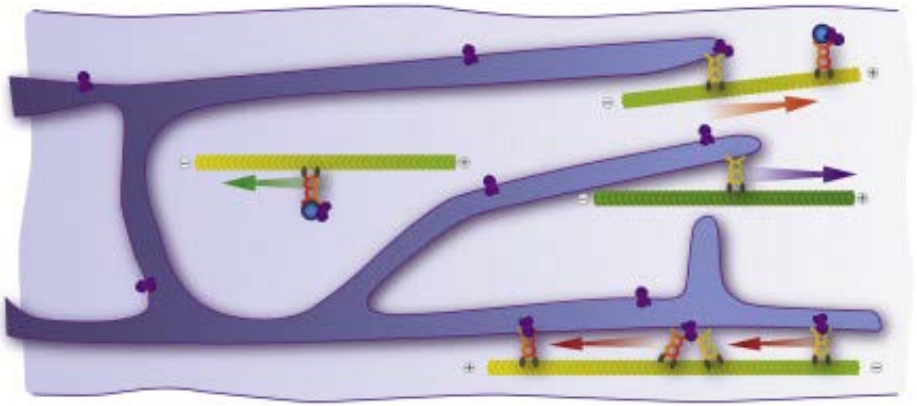
- Rings form through all three mechanisms
- Rings with similar morphology and size
- Rings incorporate and maintain non-biotinylated segments of MT building blocks
- Shearing – primarily QD carrying section break upon collision
- Incorporation of MTs lacking biotin (transiently and stably)
- Unspooling – removal of QD carrying section



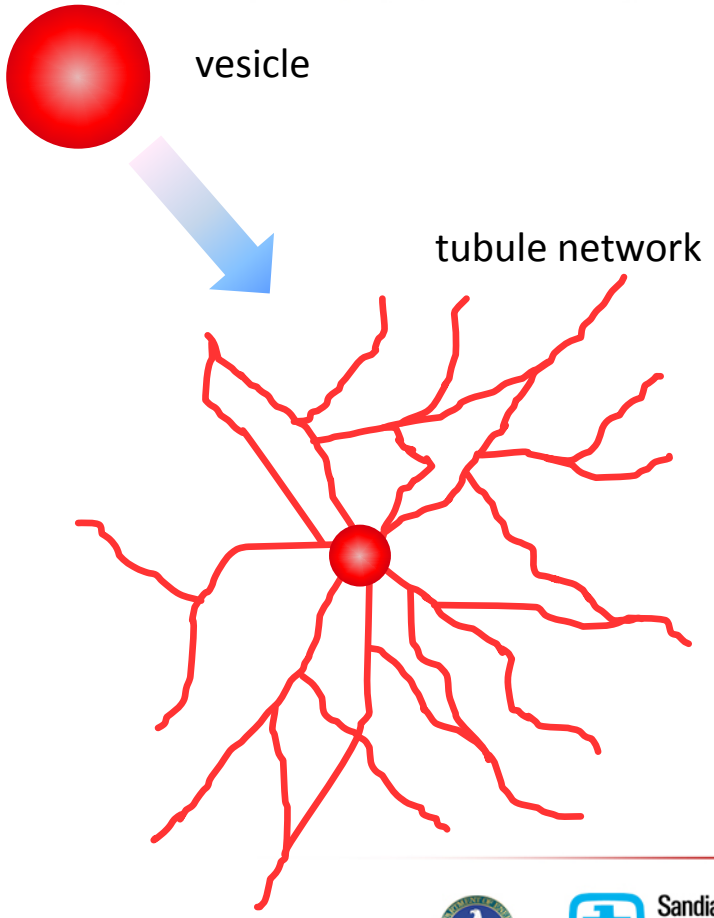


DSA of Lipid & Polymer Nanotube Networks

Energy-dissipative, active transport drives the dynamic assembly and reorganization of lipid-based organelles into complex structures such as the endoplasmic reticulum (ER) and Golgi apparatus.



Valenzuela et al. (2011) *Mol. Cell. Neurosci.*, 48, 269



Can biomolecular motors dynamically assemble complex lipid structures *ex vivo*?

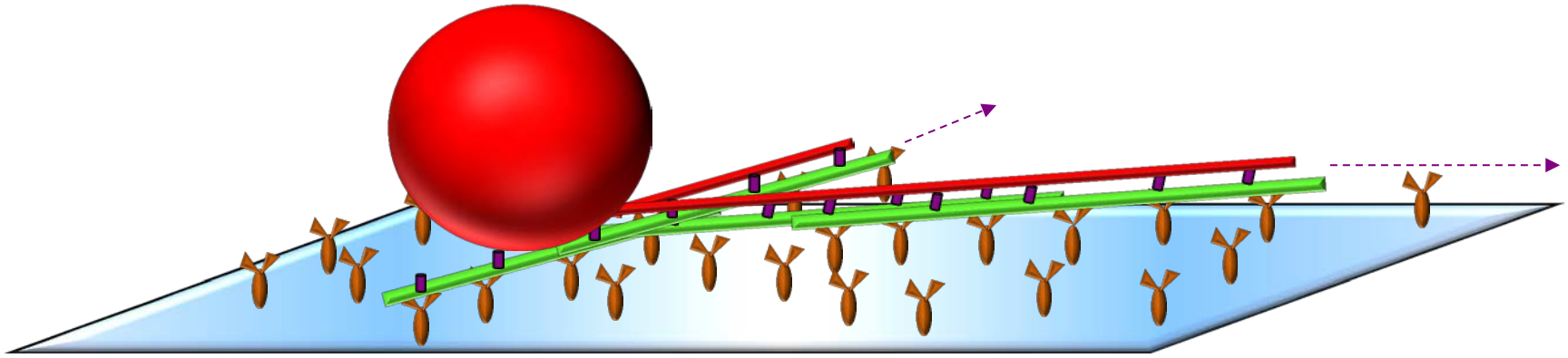
A Synthetic System for Creating Lipid Networks

A minimalistic system can be assembled from:

- Inverted kinesin motility
- Biotinylated microtubules
- Streptavidin bridge
- Biotinylated lipid vesicle

Multilamellar, single type of lipid, supply of "source materials"

microtubule motility → dynamic tug-of-war → lipid nanotube formation



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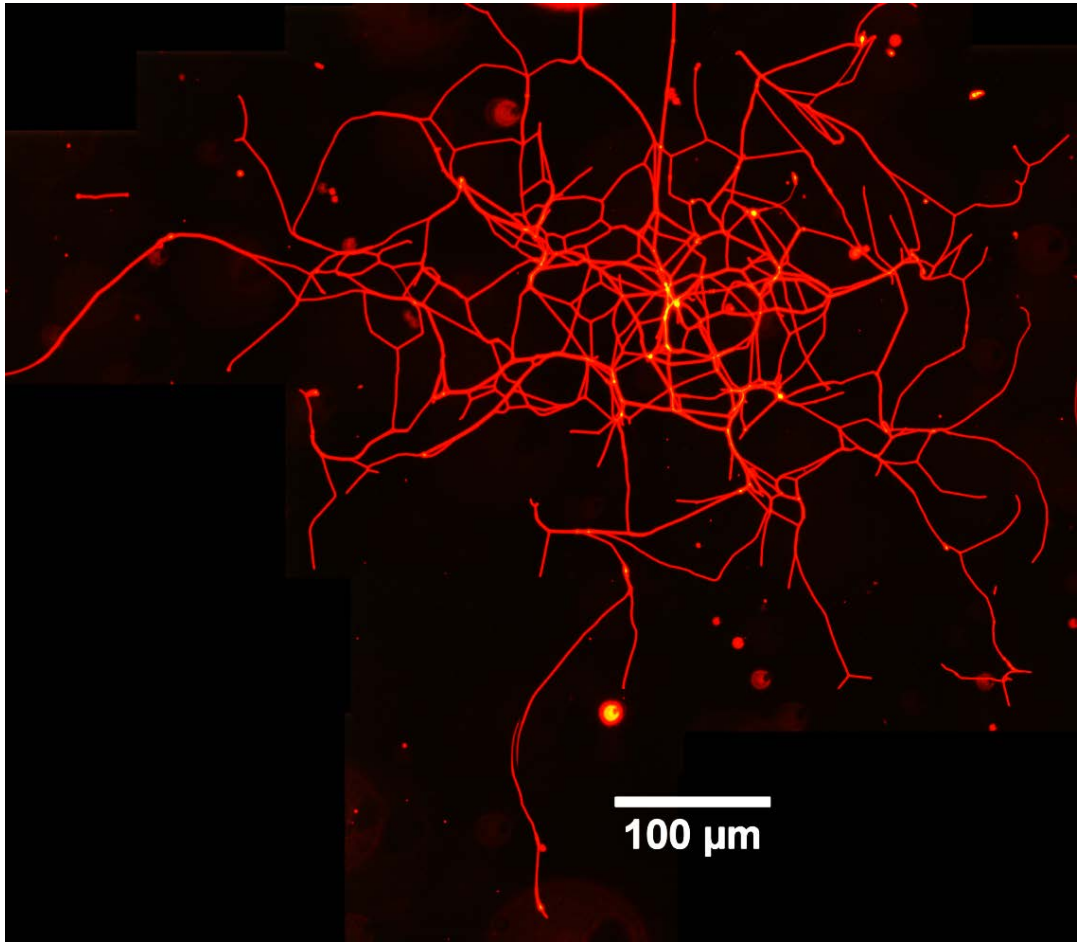
Transport, Nanotube and Network Formation

Motor protein-based transport enables DSA of multiscale networks of lipid nanotubes.

Large, highly bifurcate networks



- Total network size >10 mm from a single MLV
- Assembly <15 min
- Self-healing: networks continue growing, shrinking, moving
- Morphology can be altered by surface density of moving MTs

Bouxsein et al. (2013) *Langmuir* **29,** 2992

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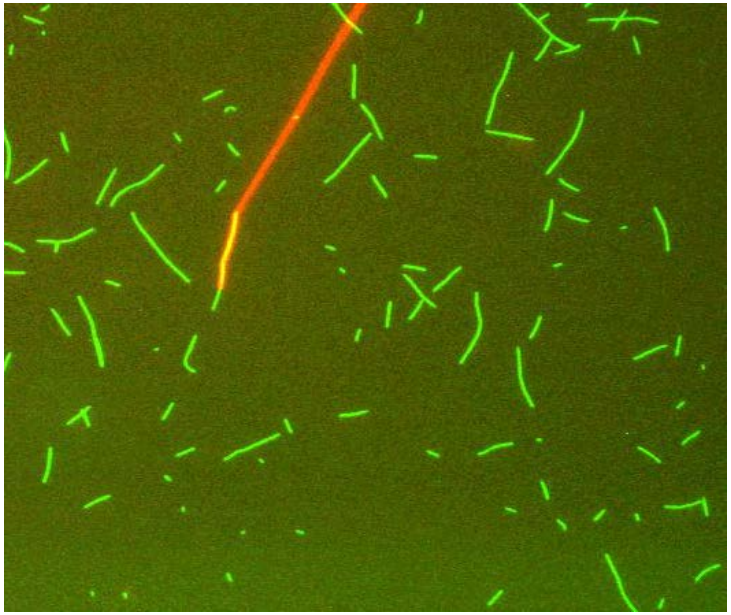
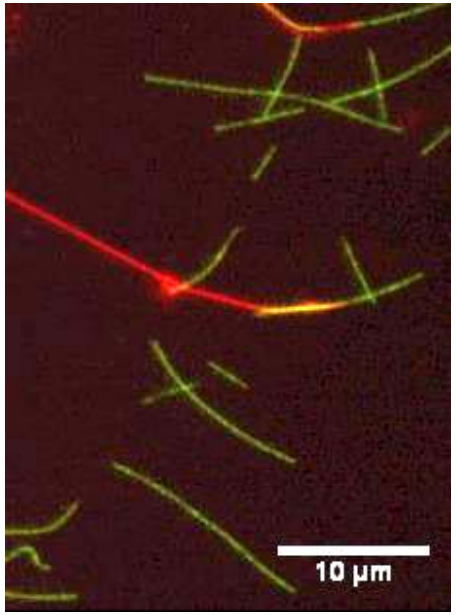
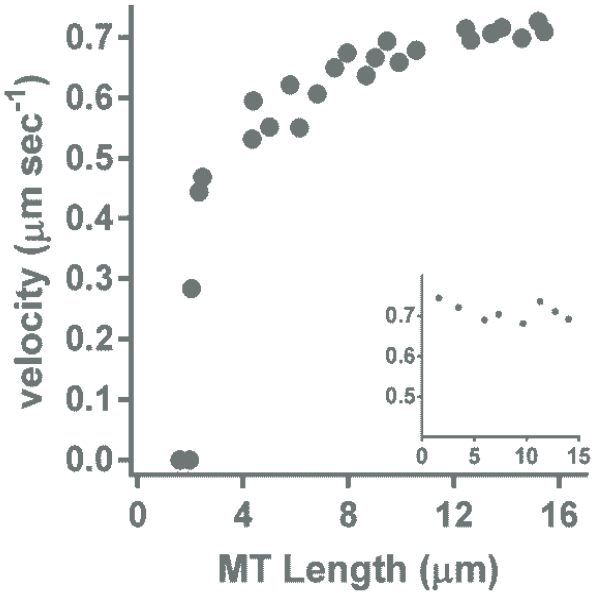
Living Lipid Networks

Collective action of the kinesin motors enables extraction of nanotubes from a variety of lipid phase lipids.

- Elongation & bifurcation of lipid nanotubes
- Plucking – sub-critical extraction force
- Velocity of elongation is dependent on MT length







Bouxsein et al. (2013) *Langmuir* **29,** 2992

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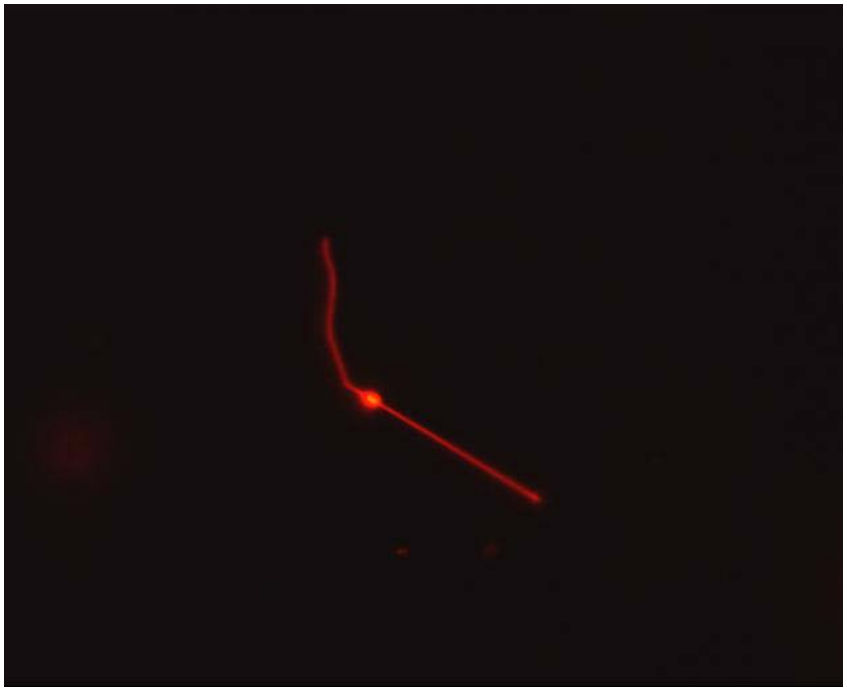
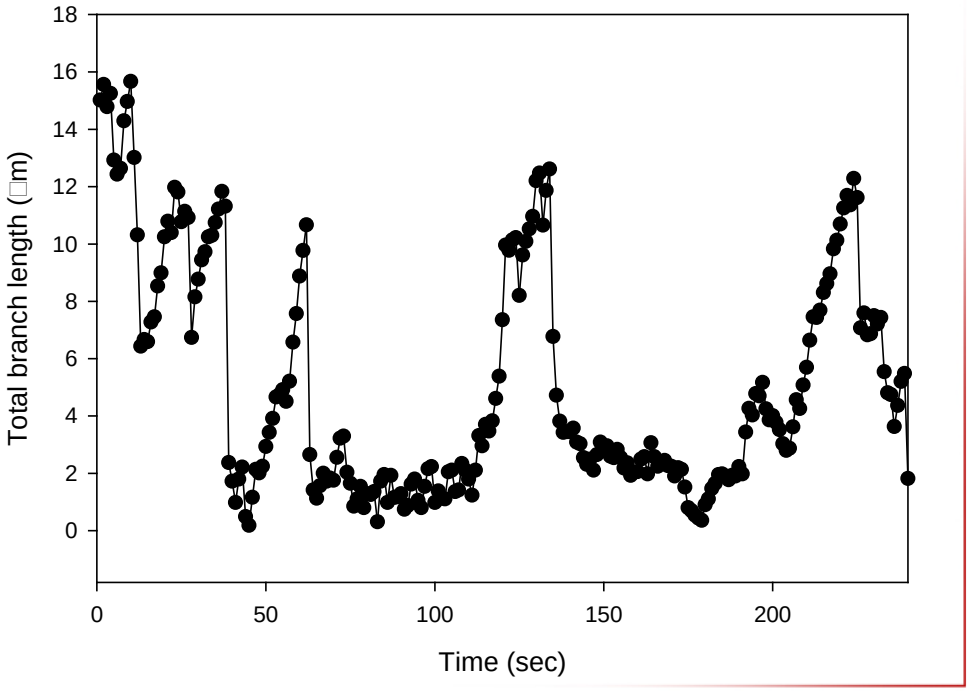
Self-Healing Networks

Dissipation of energy (i.e., motor transport) results in highly dynamic or self-healing networks capable of morphological self-healing:

- Branch collapse – bond rupture
- Action of motors generate new “replacement” nanotube



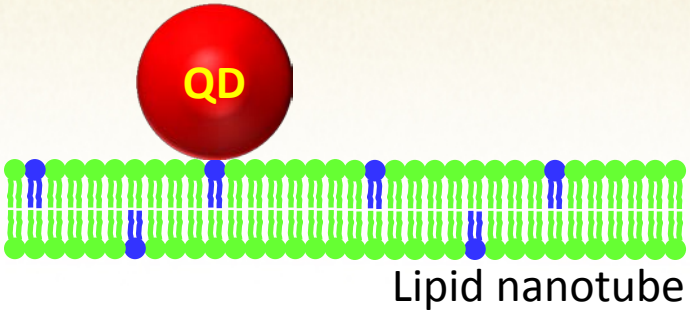
Vesicle = red; MTs = not visible

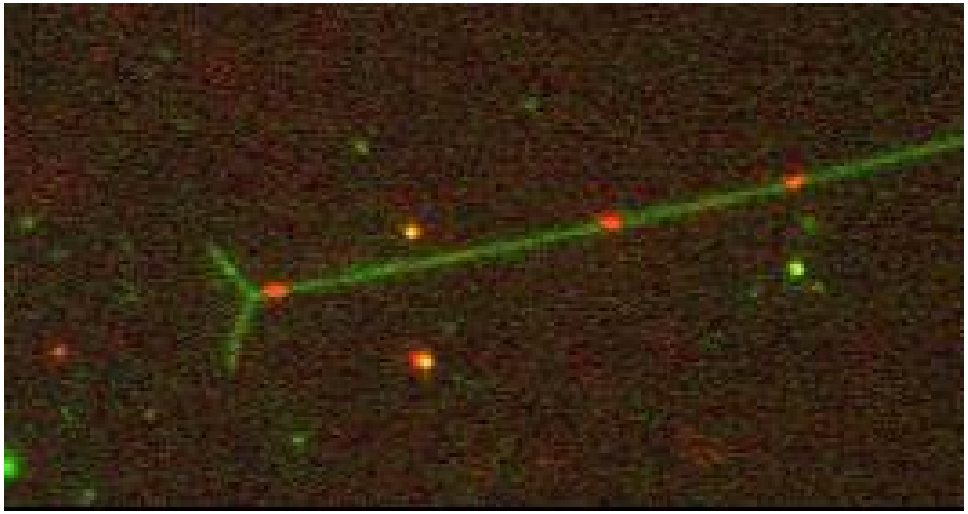


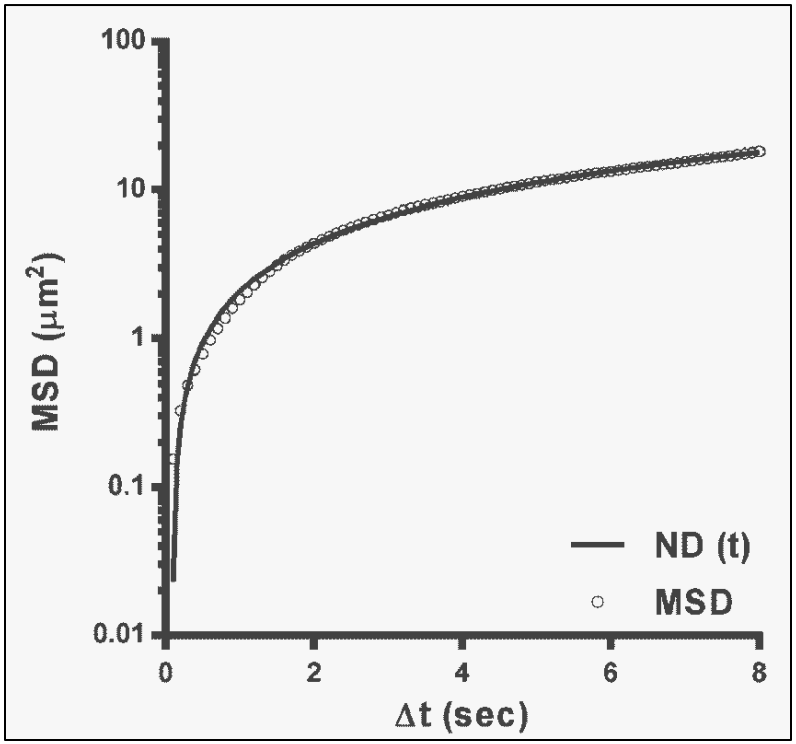
Materials Transport on Lipid Nanotube Highways

Nanoparticle (red) "surfing" – transport of materials on outer leaflet of lipid nanotubes (green) via thermal motion.

- Fluidity across junctions







Transport – 1D diffusion

D_{QD} = 2.3 μm² sec⁻¹

(D_{DOPC} = 9.32 μm² s⁻¹)

Bouxsein et al. (2013) *Langmuir* **29,** 2992

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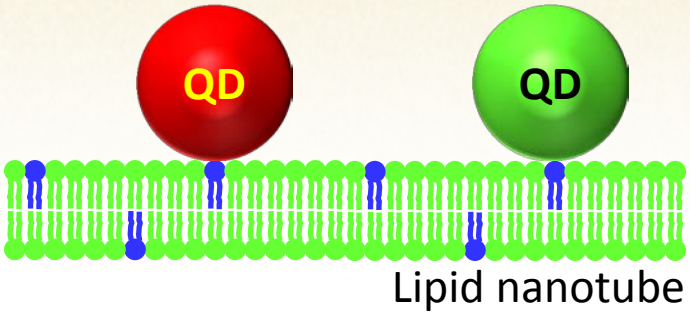
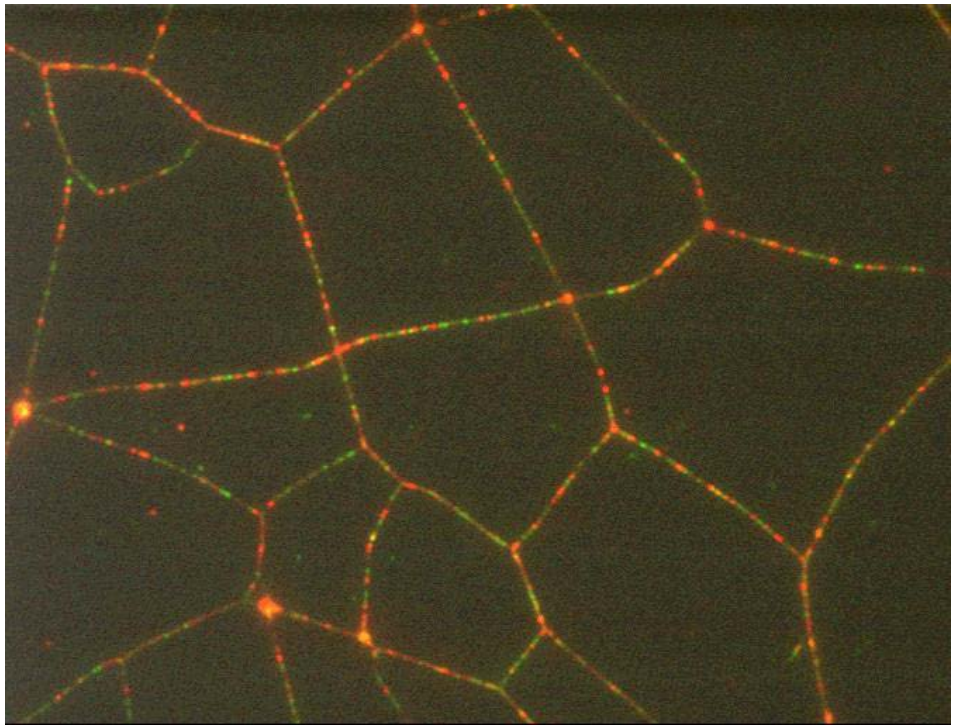
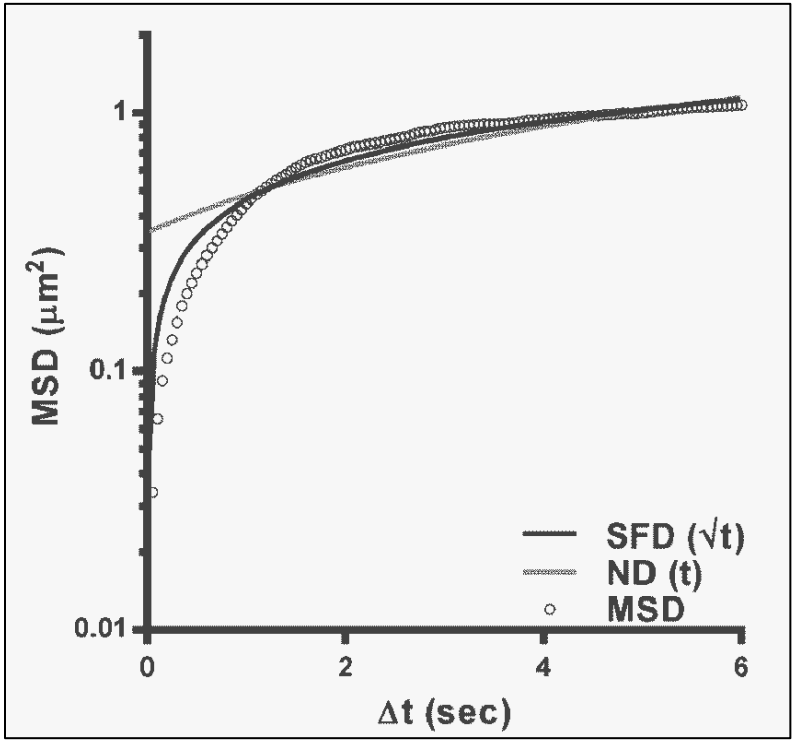
Lipid Nanotube Highways – Effects of Traffic

At high capture densities, nanoparticle surfing experiences significant traffic effects (red and green QDs).







Transport follows singe file 1D diffusion

i.e., Qdots cannot pass each other

Bouxsein et al. (2013) *Langmuir* **29,** 2992

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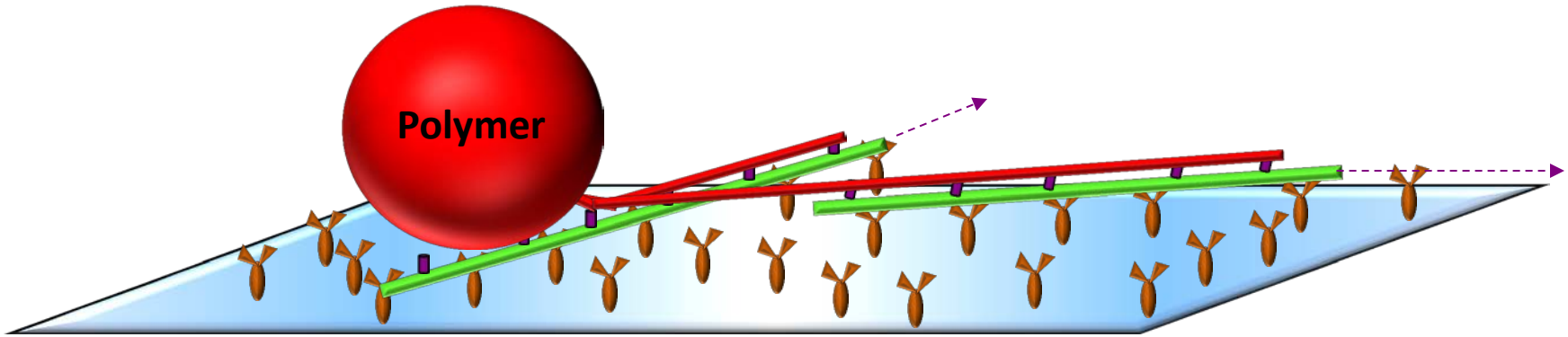
Lipids vs. Polymers

Lipids are inherently unstable, and thus poorly suited for long-lived (i.e., > a few hours) nanofluidic networks for materials transport.

Block copolymers

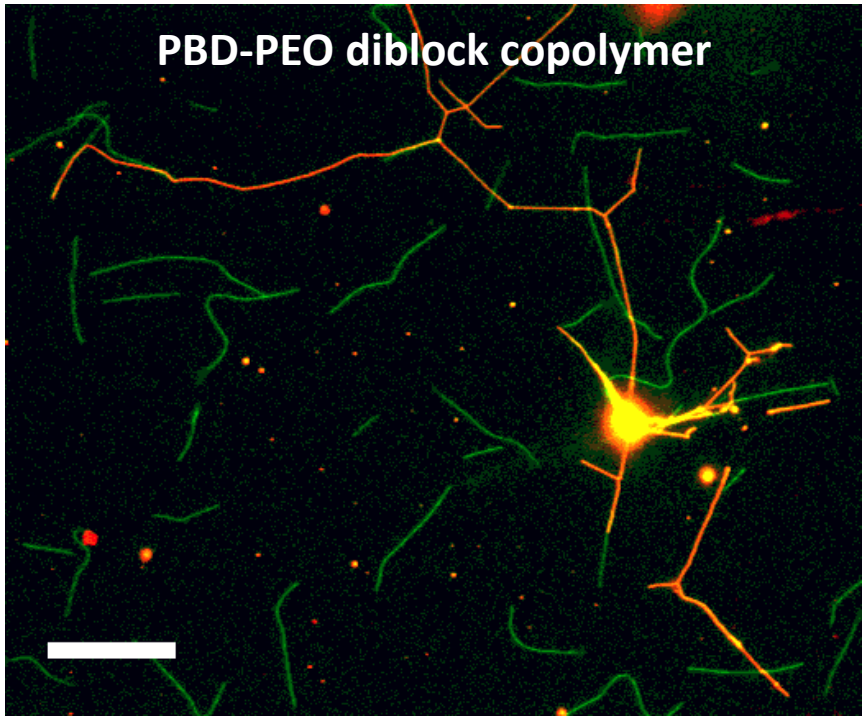
- synthetic analogues of lipid-based materials
- ≈physical behaviors
- >>stability

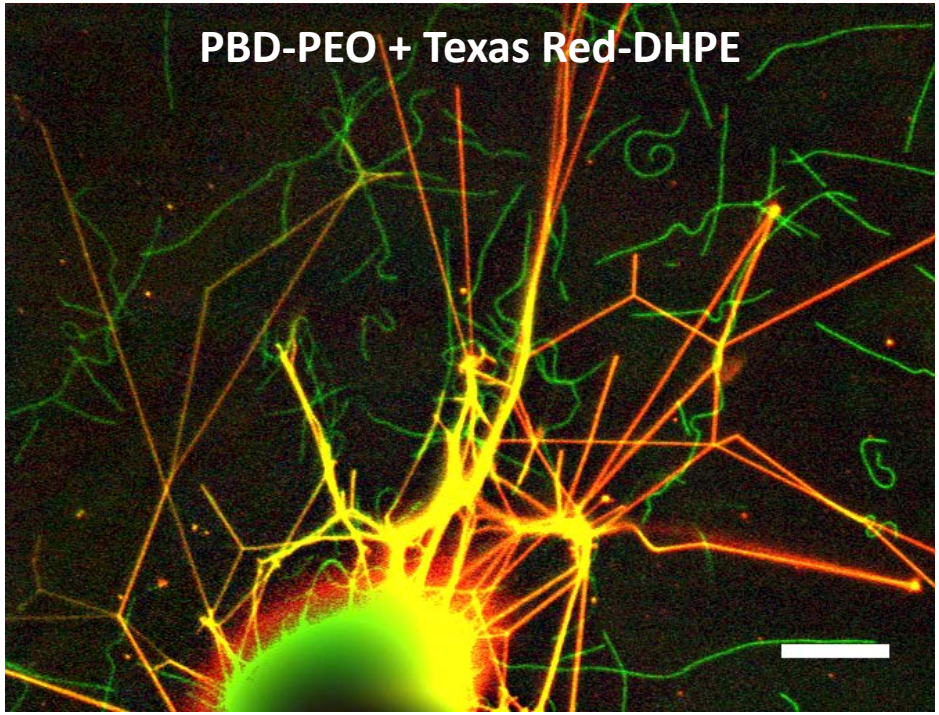
Can the collective force from kinesin motors extract nanotubes from polymersomes?



Assembly of Polymer Nanotube Networks

Yes, but…addition of lipid (0.5 mol %) enhances the formation of large extended networks from polymersomes





Will polymer nanotubes also support Qdot surfing?

Paxton et al. (2015) *Nanoscale* **7**, 10998

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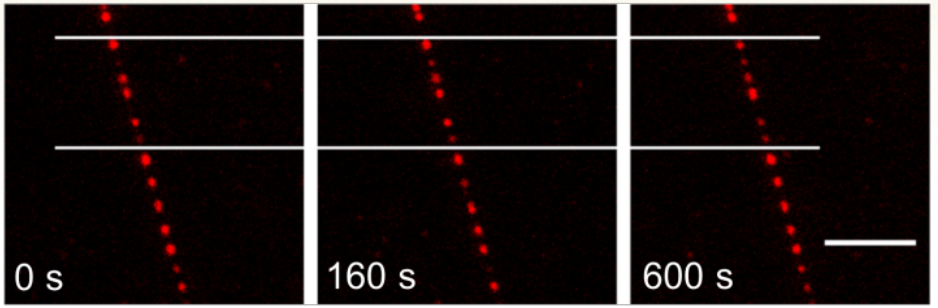
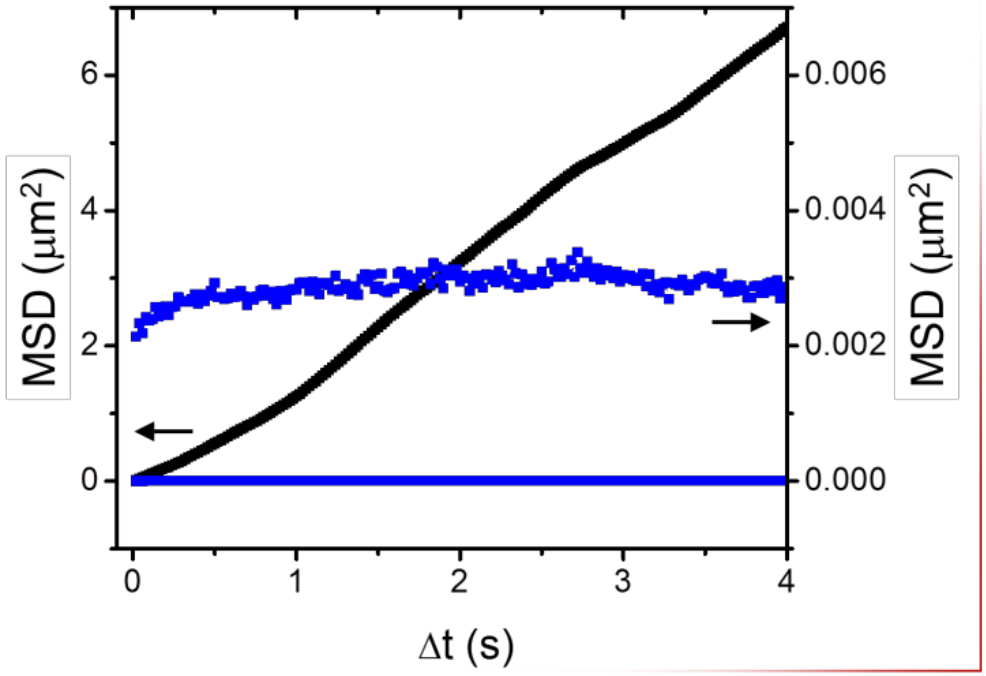
Qdot Surfing Along Polymer Nanotubes

Streptavidin Qdots adhered to polymer nanotubes do not diffuse as previously observed with lipid nanotubes.



- Black line = MSD of Qdots on lipid nanotubes
- Blue line = MSD of Qdots on polymer nanotubes
- Blue squares = MSD of Qdots on polymer nanotubes (right axis)



Immobile islands of polymer generated by multivalent binding of Qdots to polymers?

Paxton et al. (2015) *Nanoscale* **7**, 10998

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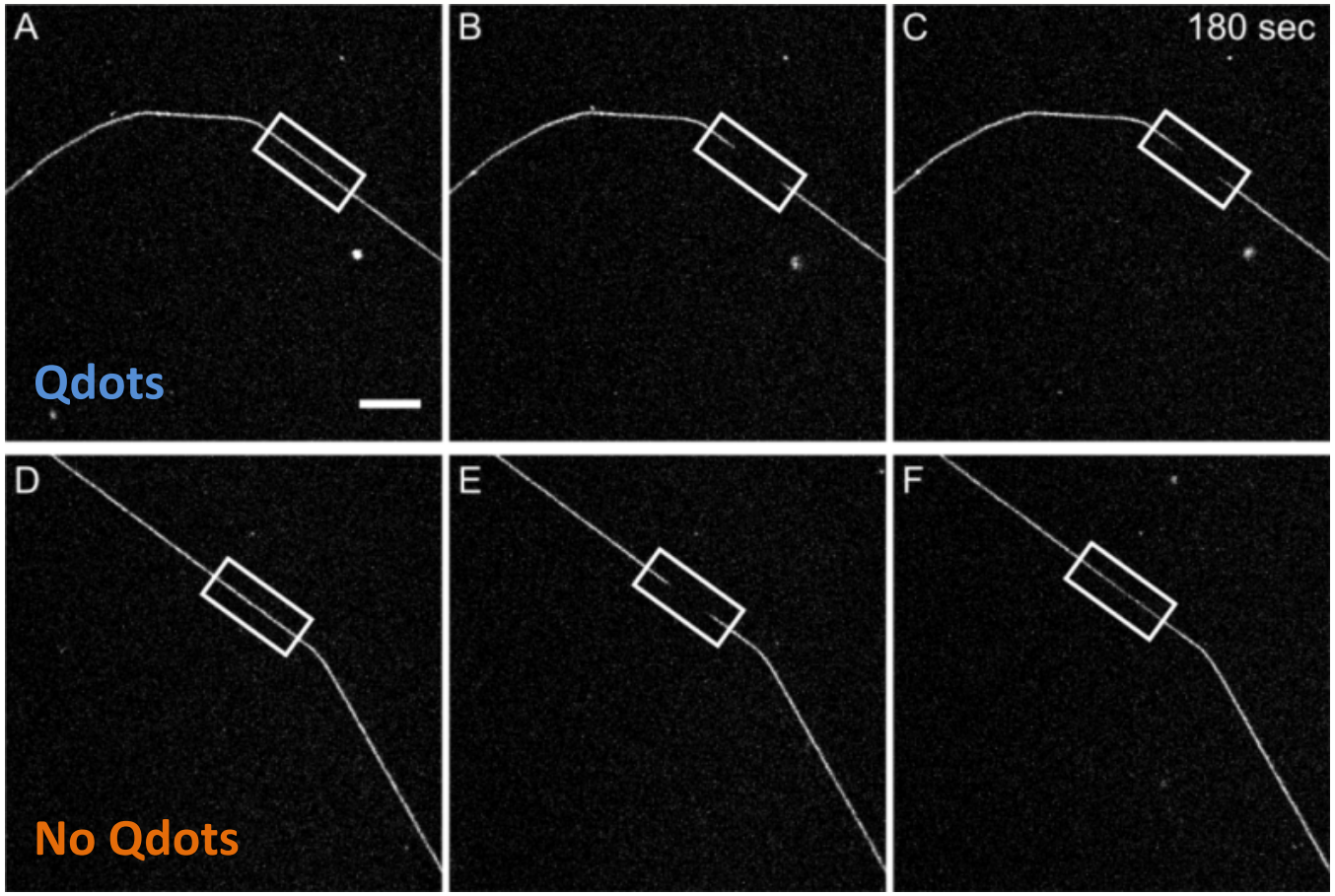
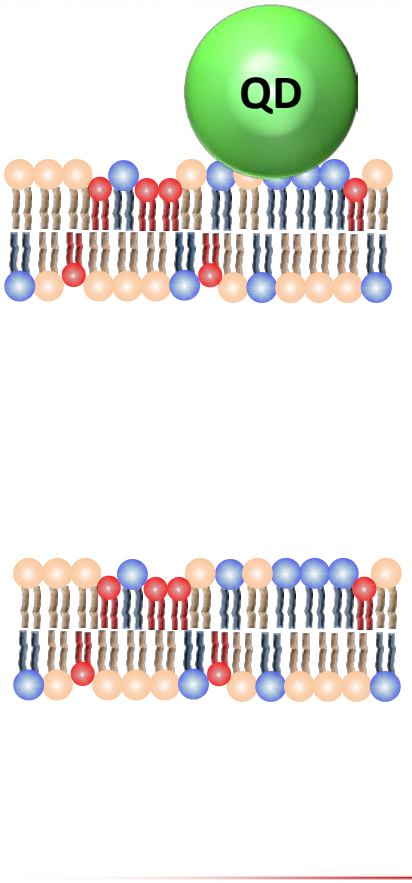
Biomolecular Crosslinking & Mobility

Binding of streptavidin-Qdots to polymer nanotubes alters the diffusivity of the fluorescent lipids, suggesting the formation of larger immobile islands.





Paxton et al. (2015) *Nanoscale* **7**, 10998.

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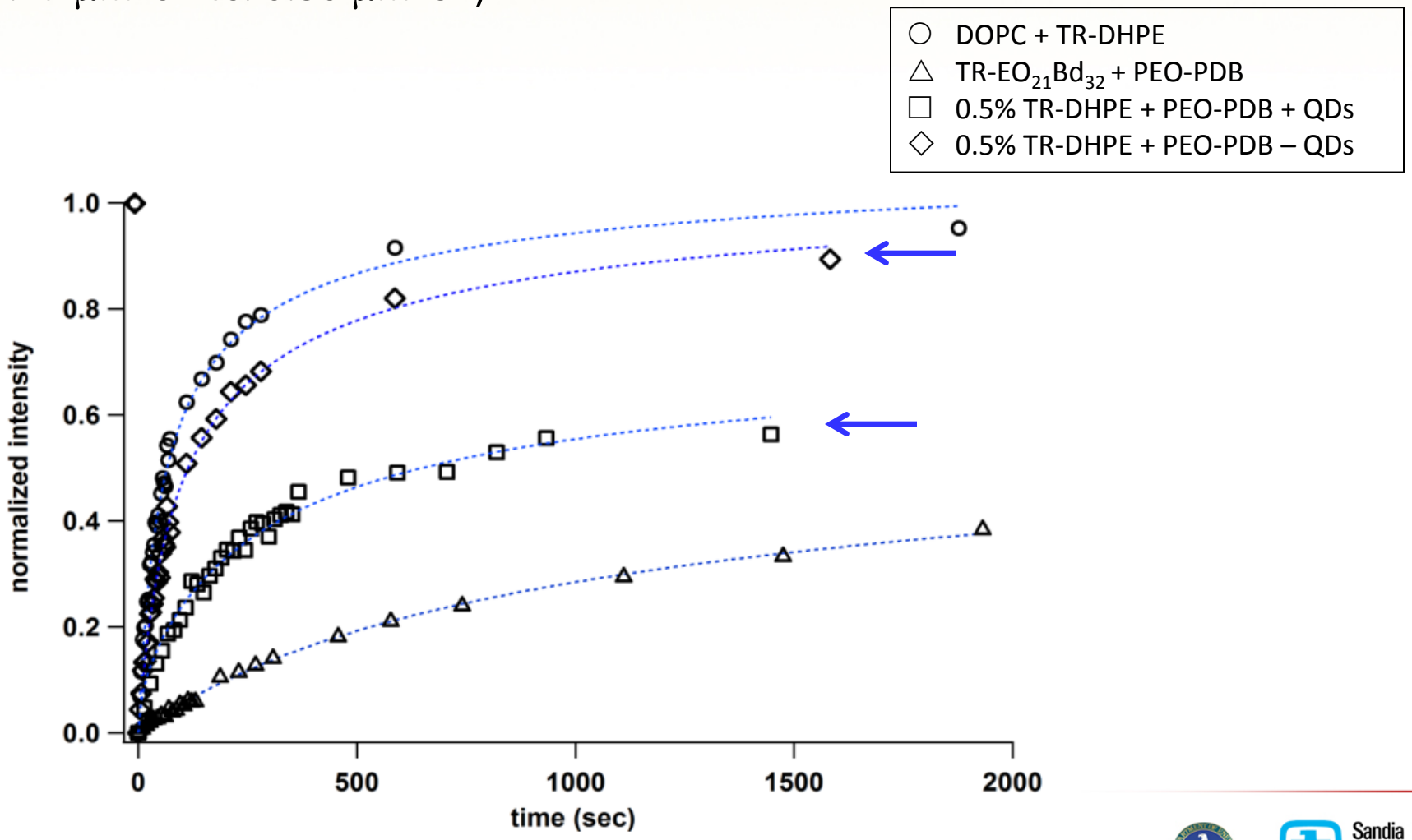
Biomolecular Crosslinking & Mobility

Lipid diffusivity ~50% slower when Qdots adhered to polymer nanotubes (0.17 $\mu m^2 s^{-1}$ vs. 0.30 $\mu m^2 s^{-1}$)



Paxton et al. (2015) *Nanoscale* **7**, 10998.

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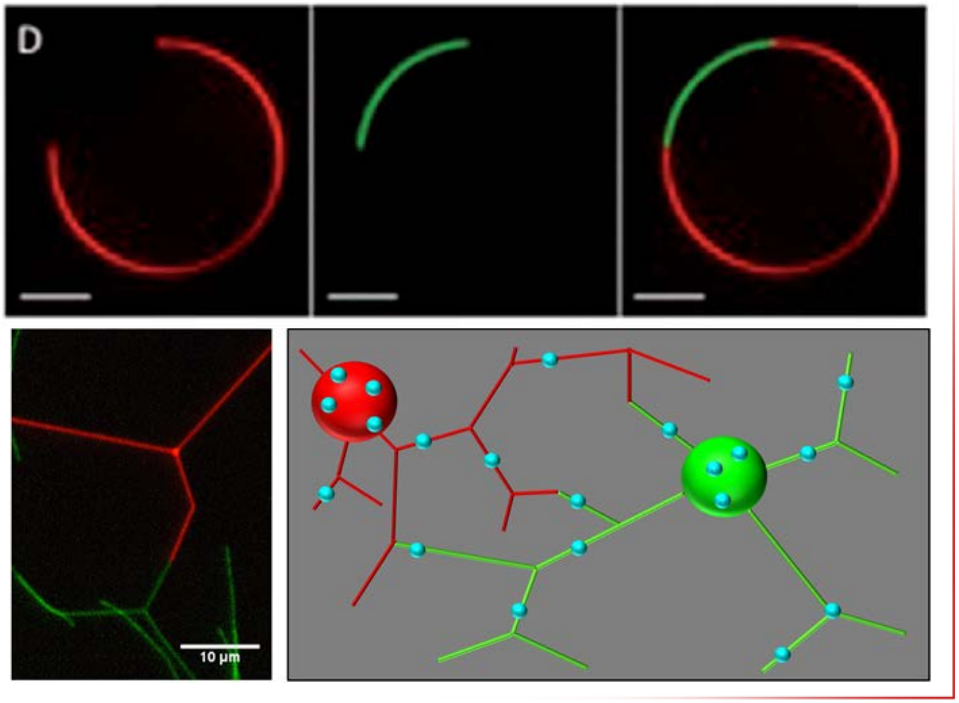
Conclusions & Future Directions

Collective force of biomolecular motors drives the assembly and dynamic reorganization of complex polymer and lipid nanotube networks.

Differences in lipid/polymer properties result in different diffusive behaviors and materials transport along nanotubes.

- Nanotube and vesicle extraction from biphasic liposomes & polymersomes
- Materials transport (e.g., nanoparticles) in interstitial space of lipid and polymer nanotubes
- Multi-network connection and communication

Momin et al. *Soft Matter* (2015); DOI: 10.1039/c4sm02856b



Acknowledgments

Post-docs
Nathan Bouxsein
Virginia VanDelinder
Ian Henderson
Adrienne Greene
Amanda Carroll-Portillo
Haiqing Liu

Students
Haneen Martinez
Stephanie Brener
Rishi Jain

Staff
Wally Paxton
Marlene Bachand

Co-PIs
Erik Spoerke
Mark Stevens
Darryl Sasaki

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