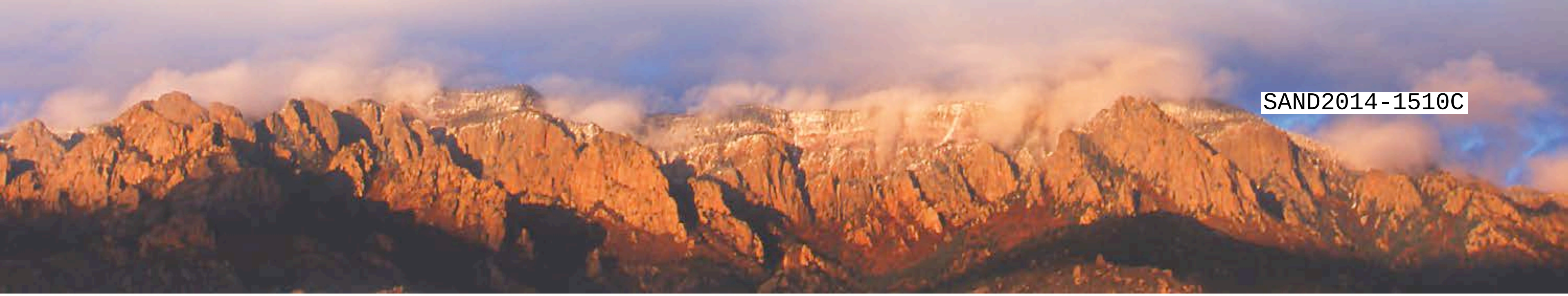

SAND2014-1510C

Motor-Driven Assembly of Dynamic, Self-Healing Lipid Nanotube Networks

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Micro- and Nanotechnology I

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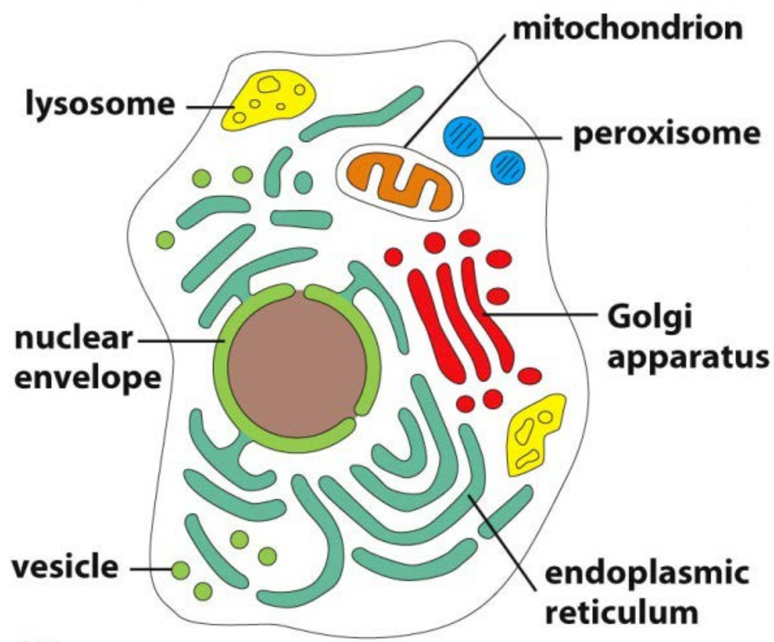
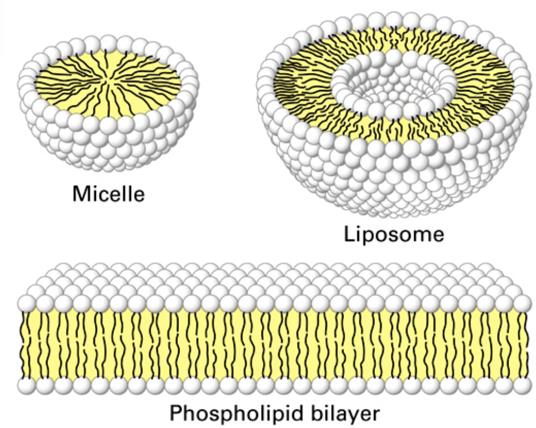
Natural vs. Synthetic Lipid Assemblies

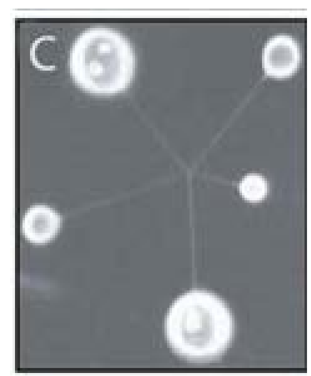
Non-equilibrium lipid assemblies are critical to a wide range of cellular functions (e.g., energy conversion), but difficult to reproduce in artificial environments.



Alberts et al., *Essential Cell Biology* (2010)



http://andersonlab.qb3.berkeley.edu

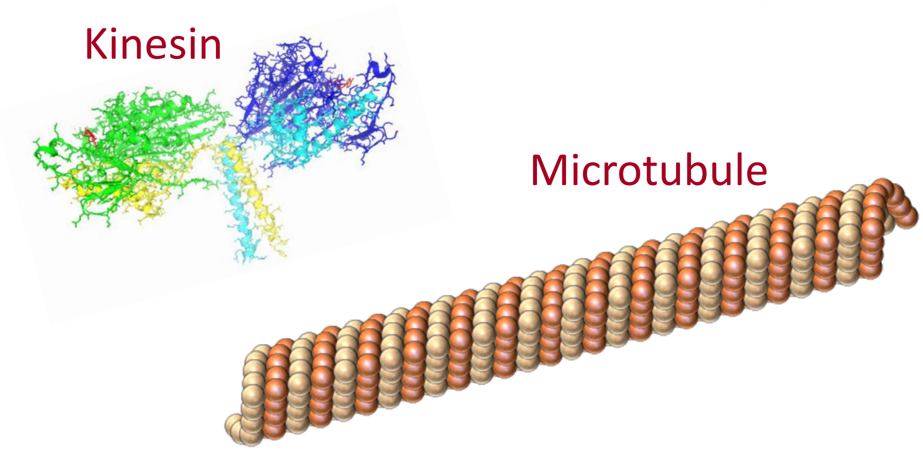


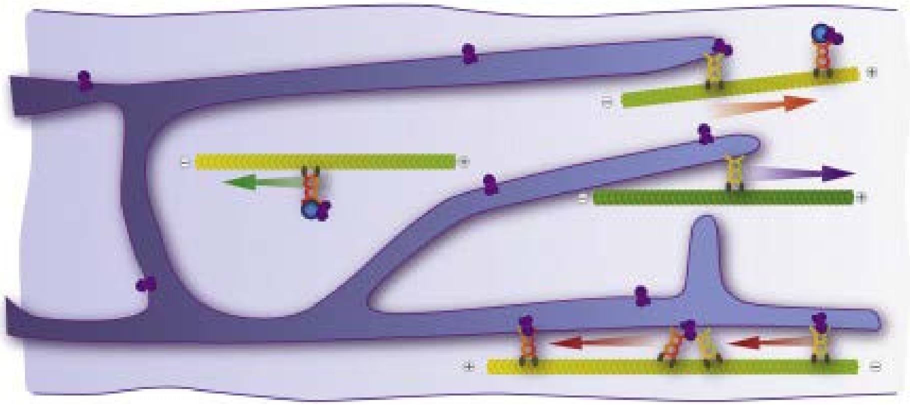
Wegrzyn et al., 2011, *Nano Commun. Netw.*, **2**, 4

How does Nature from these highly complex and functional lipid structures?

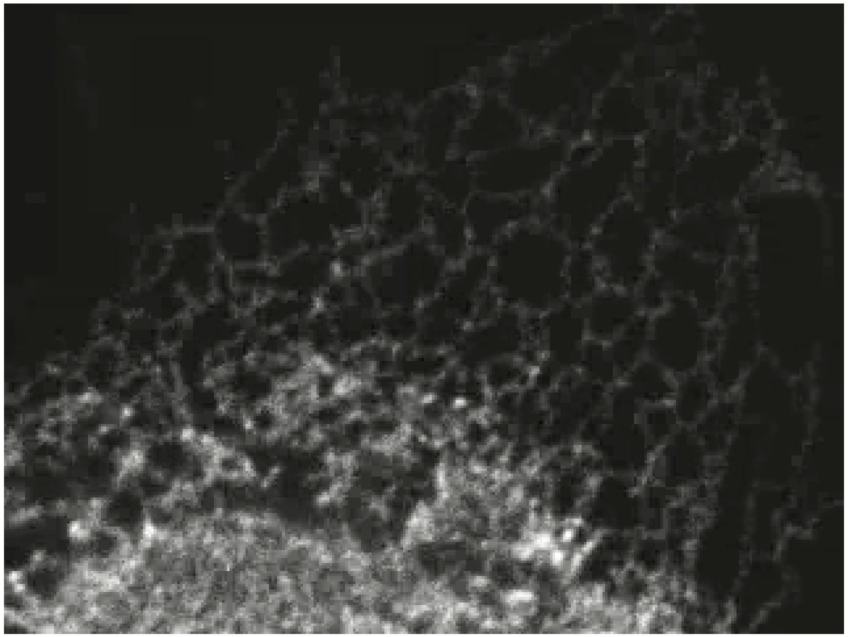
Dynamic Assembly of Lipid Structures

Energy-dissipative, active transport drives the formation and 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



Alberts et al., *Essential Cell Biology* (2010)

Can we reconstruct a similar system in vitro?

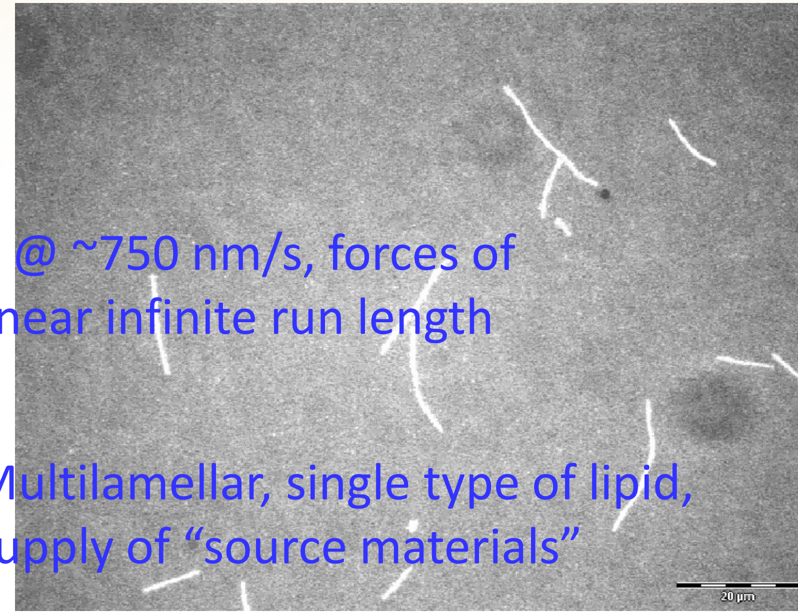
A Synthetic System for Creating Lipid Networks

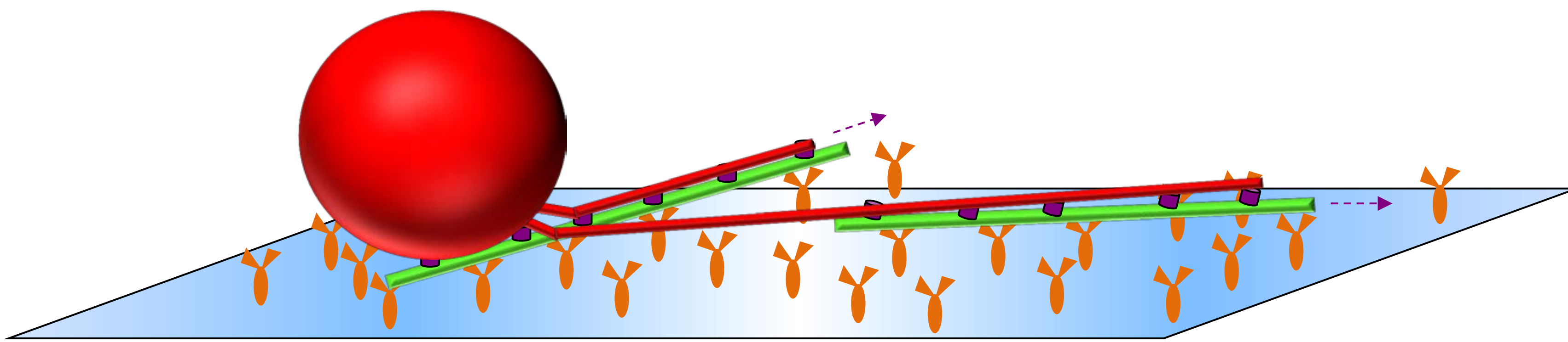
A minimalistic system can be assembled from:

- Solid surface
- Kinesin motor proteins
- Biotinylated microtubules
- Streptavidin bridge
- Biotinylated lipid vesicle

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

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



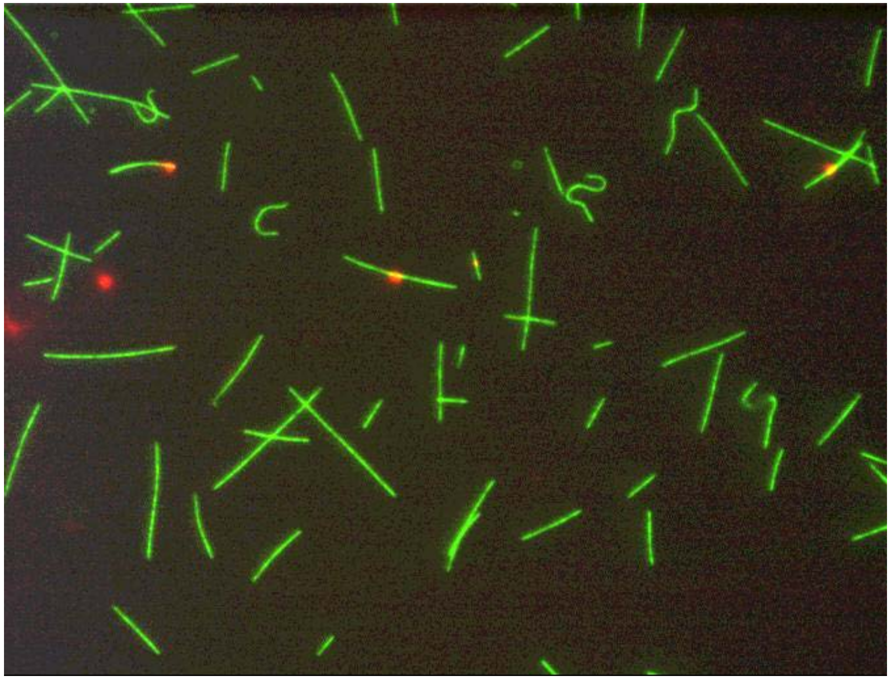


Transport, Nanotube and Network Formation

Motor protein-based transport enables various behavior depending upon the vesicle size (i.e., amount of source material).

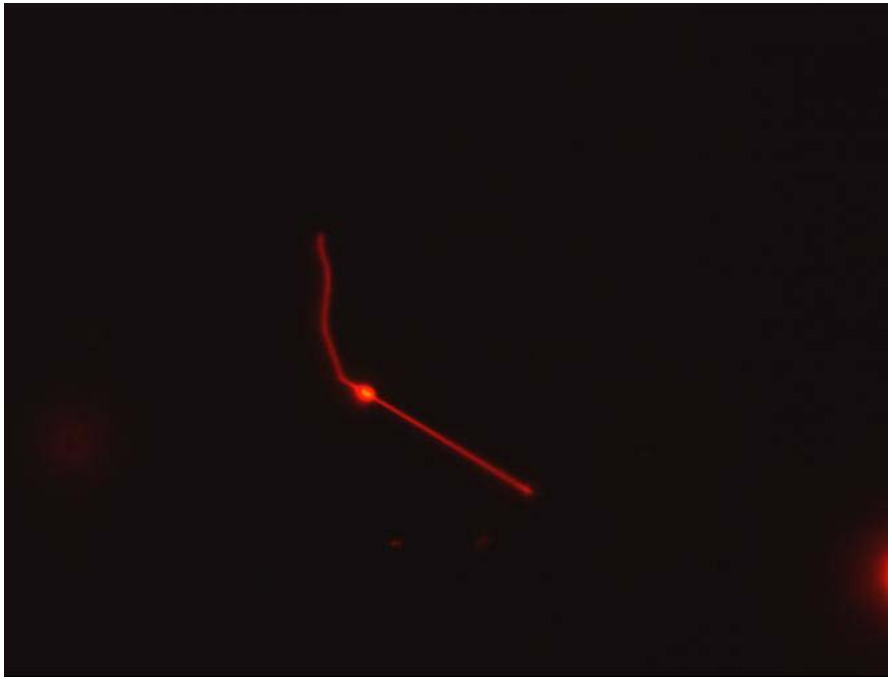
Vesicle Size

Vesicle transport (1-2 μm)



Vesicle = red; MTs = green

Self-limiting networks (2-5 μm)



Vesicle = red; MTs = not visible

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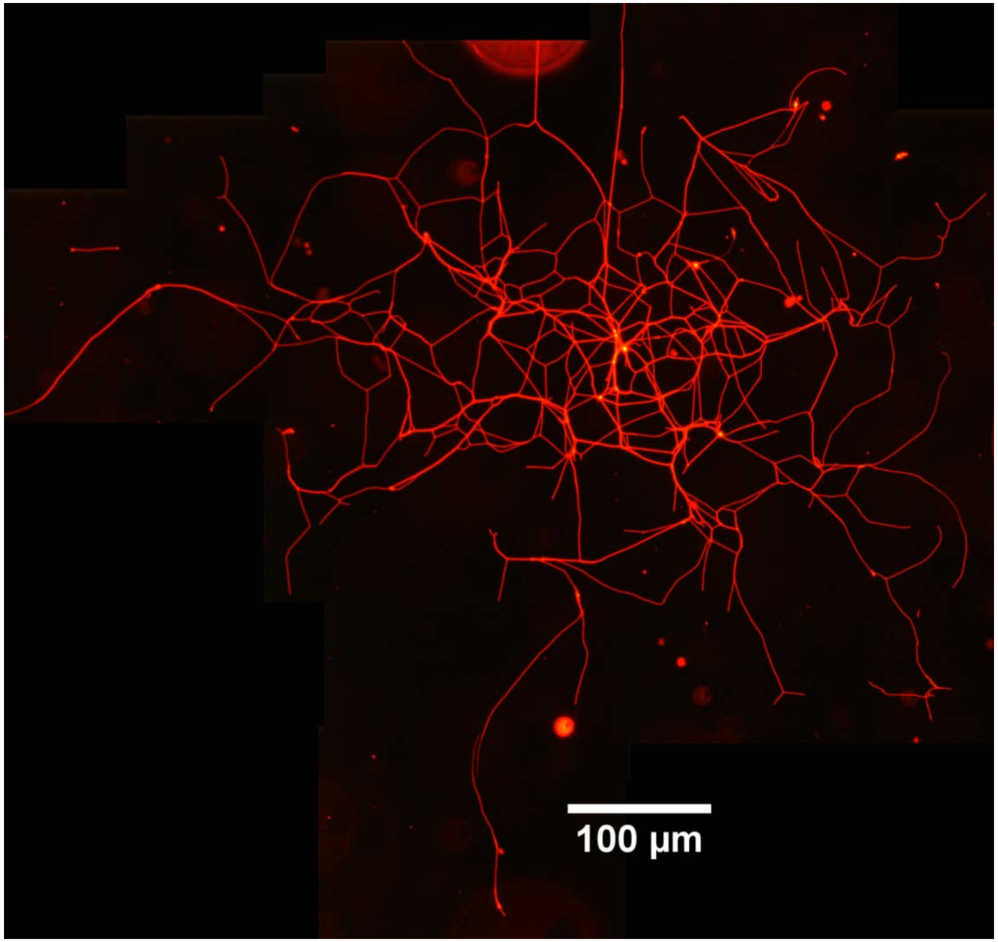
Transport, Nanotube and Network Formation

Motor protein-based transport enables various behavior depending upon the vesicle size.

Large, highly bifurcate networks (10-20 μm)



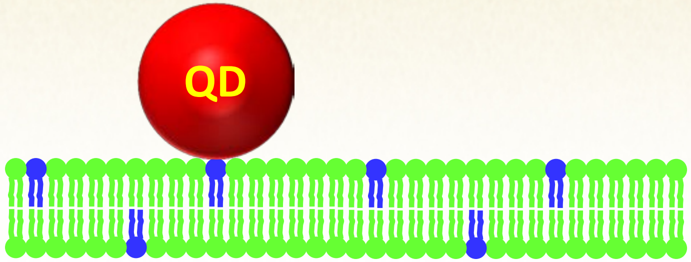
- Total network size <u>>10 mm</u> from a single MLV
- Assembly within 15 min
- Self-healing: networks continue growing, shrinking, moving
- Morphology can be altered by surface density of moving microtubules

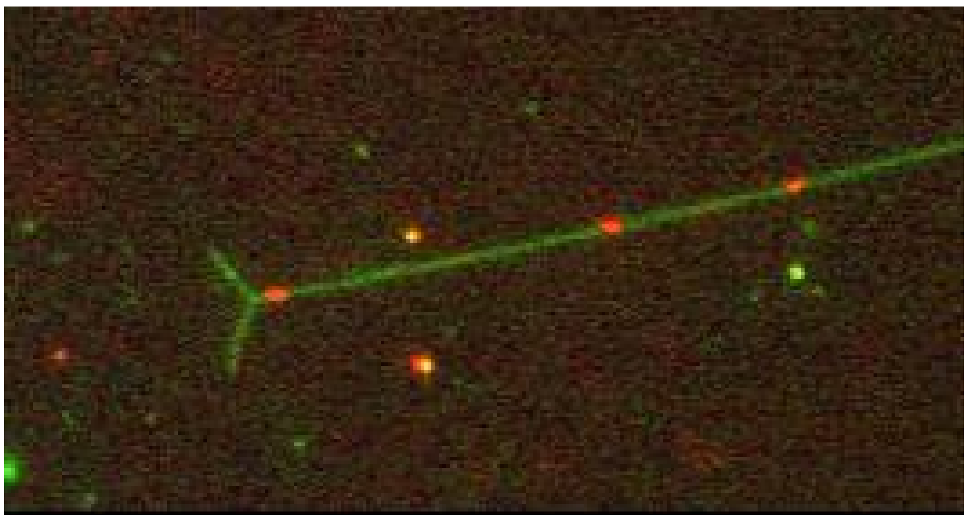
Bouxsein, N. F., Carroll-Portillo, A., Bachand, M., Sasaki, D. Y., & Bachand, G. D. (2013) *Langmuir* **29**, 2992-2999.

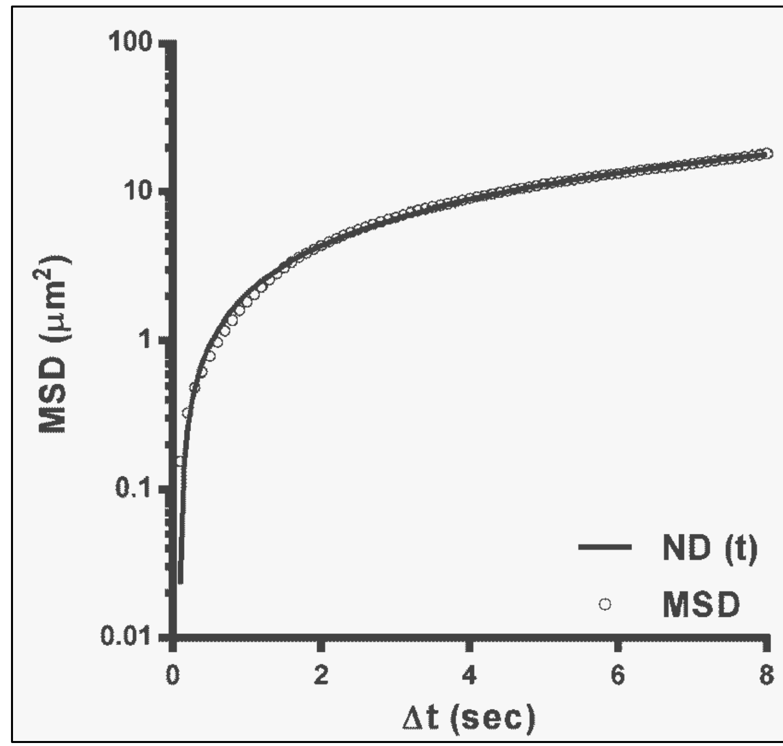
Materials Transport on Lipid Nanotube Highways

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

- Fluidity across junctions







Transport follows normal 1D diffusion

D_{QD} = 2.3 μm^2 sec^{-1}

(D_{DOPC} = 9.32 μm^2 s^{-1})

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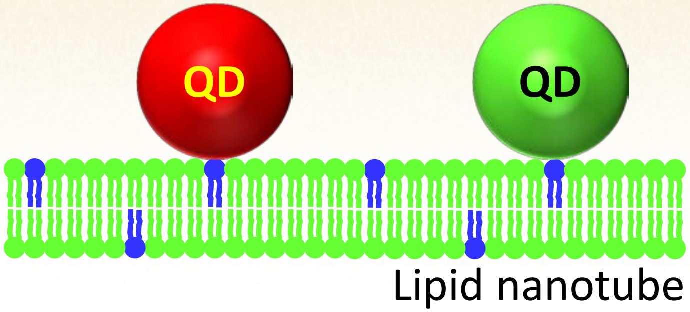
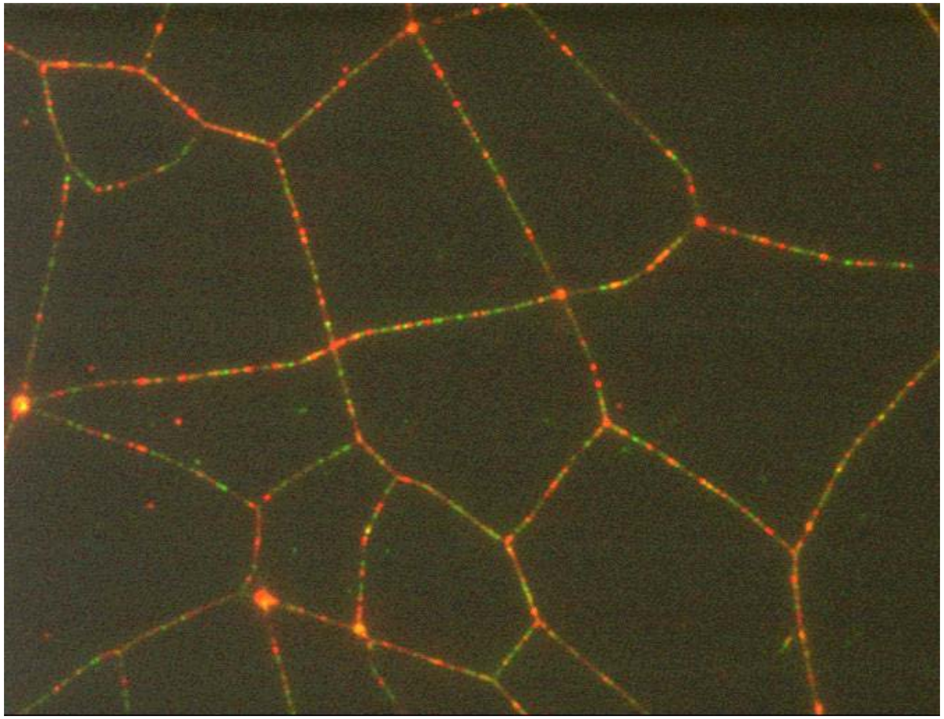
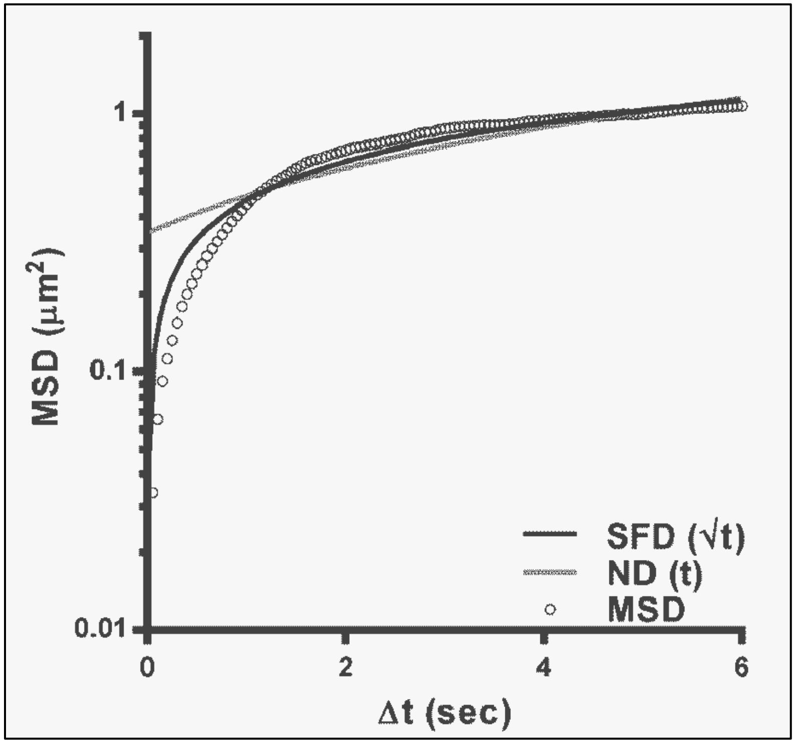
Lipid Nanotube Highways – Effects of Traffic

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

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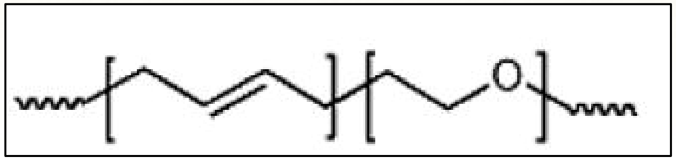
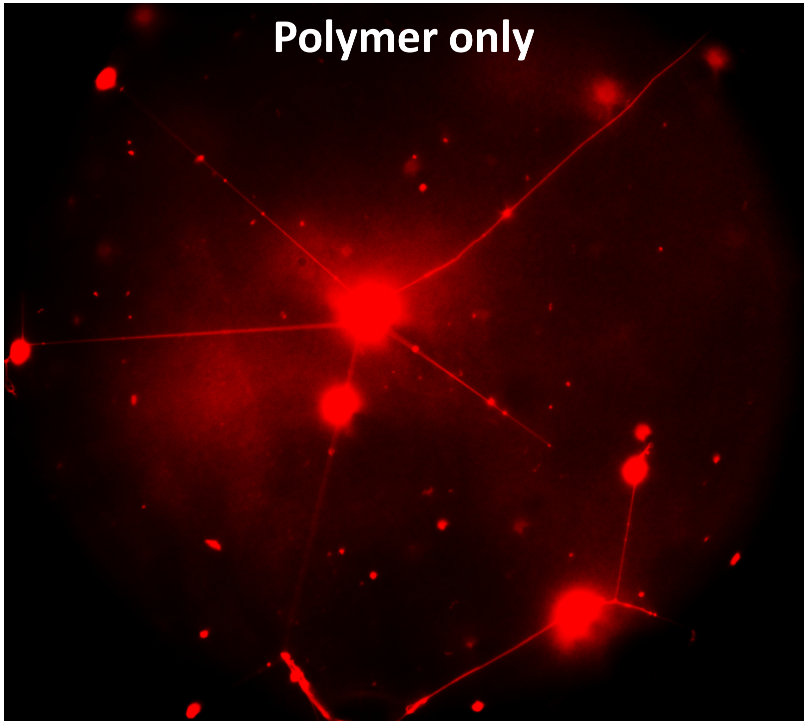
Assembly of Polymer Nanotube Networks

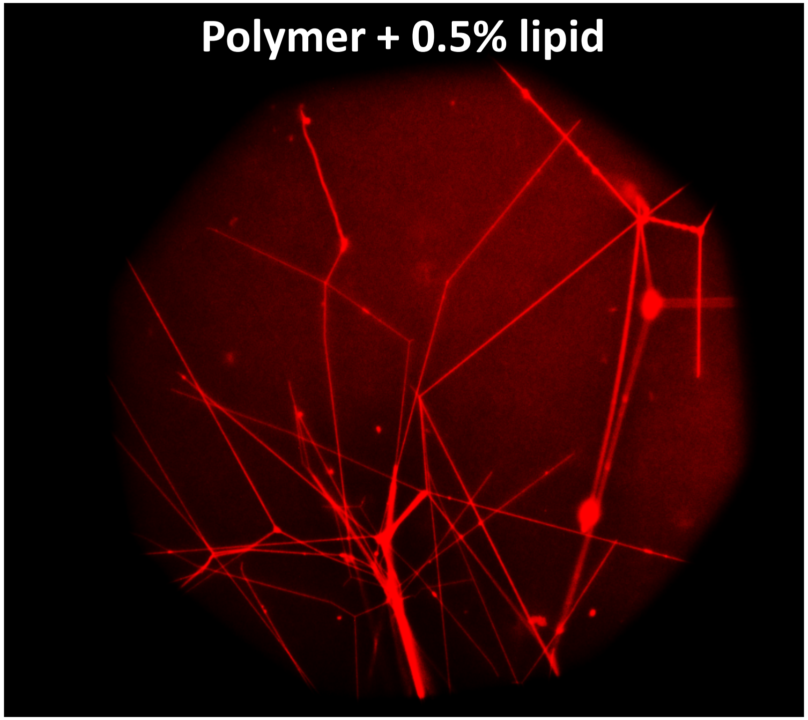
Can motor proteins actively assemble similar nanotube networks from polymersomes?

Yes, but…

PBD-PEO diblock copolymer







Formation of large extended networks requires the addition of a lipid to the polymersomes. What is the lipid's role?

Mobility in Polymer Nanotube Networks

Addition of lipid significantly affects the diffusivity of the polymers:

- Increase in 1D diffusivity of polymer
- Decrease in half-time of recovery (τ) of polymer
- Fraction recovered (mobile fraction) of polymer is unchanged

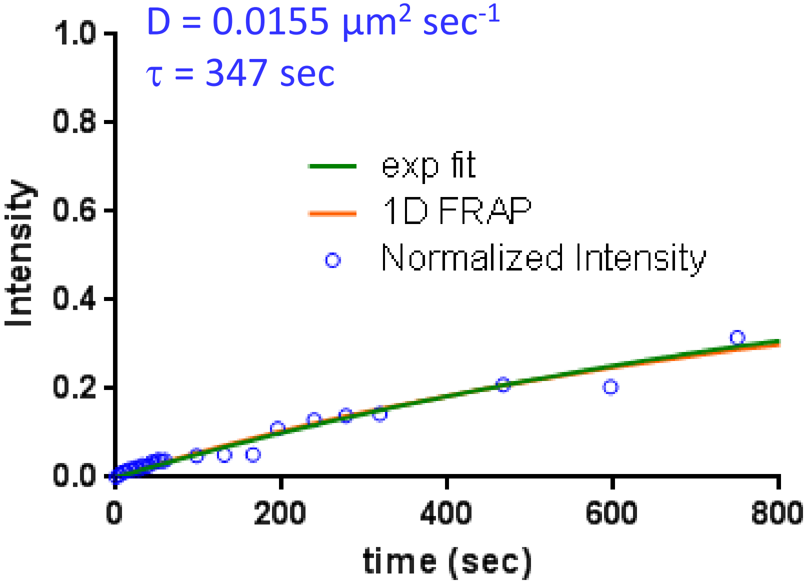
FRAP of Fluorescent Polymer Alone

TRITC-polymer

Mobile fraction = ~30%

D = 0.0155 $\mu m^2\ sec^{-1}$

τ = 347 sec



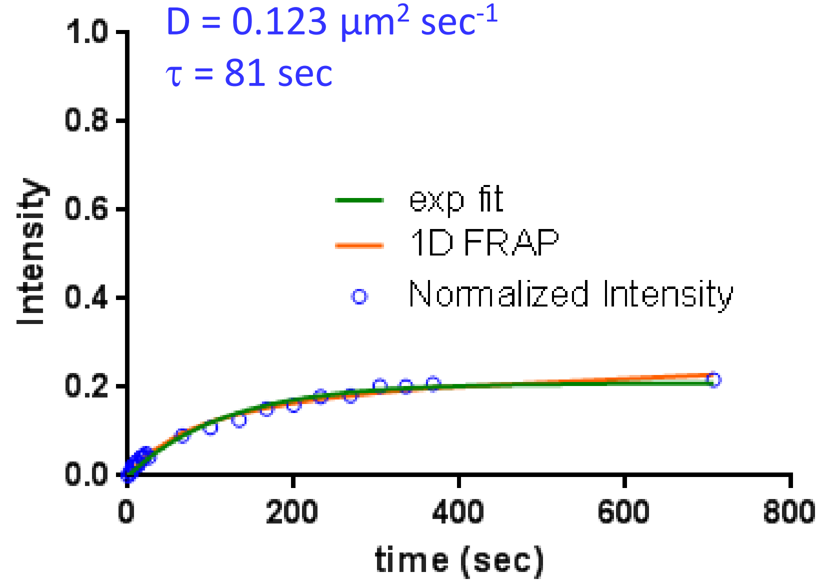
FRAP of Fluorescent Polymer + Lipid

TRITC-polymer

Mobile fraction = ~20%

D = 0.123 $\mu m^2\ sec^{-1}$

τ = 81 sec



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Mobility in Polymer Nanotube Networks

Diffusivity and half life of recovery of lipid and mobile polymer are relatively equivalent, but…

mobile fraction of lipid >> mobile fraction of polymer

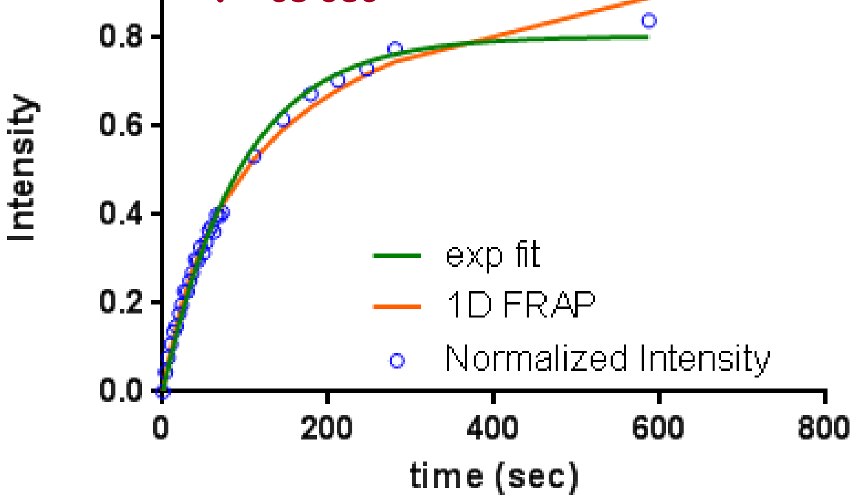
<u>FRAP of Fluorescent Lipid</u>

0.5% Texas Red-DOPE

Mobile fraction = 80%

D = 0.084 $\mu m^2\ sec^{-1}$

τ = 65 sec



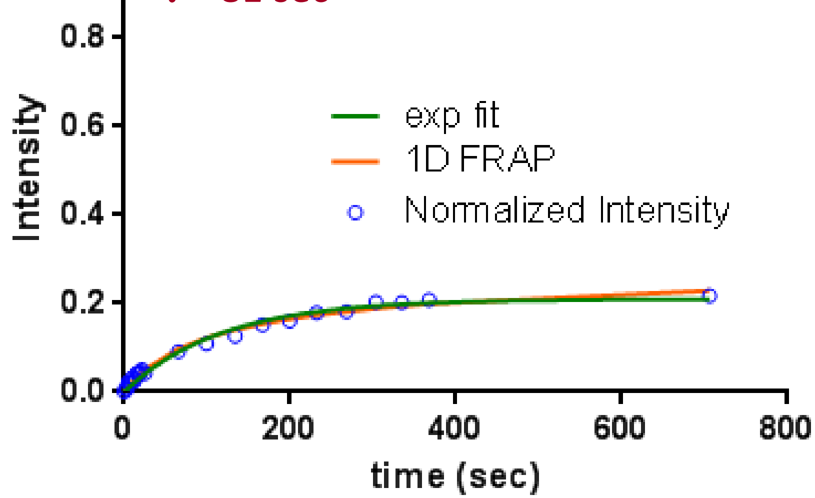
<u>FRAP of Fluorescent Polymer</u>

TRITC-polymer

Mobile fraction = 20%

D = 0.123 $\mu m^2\ sec^{-1}$

τ = 81 sec



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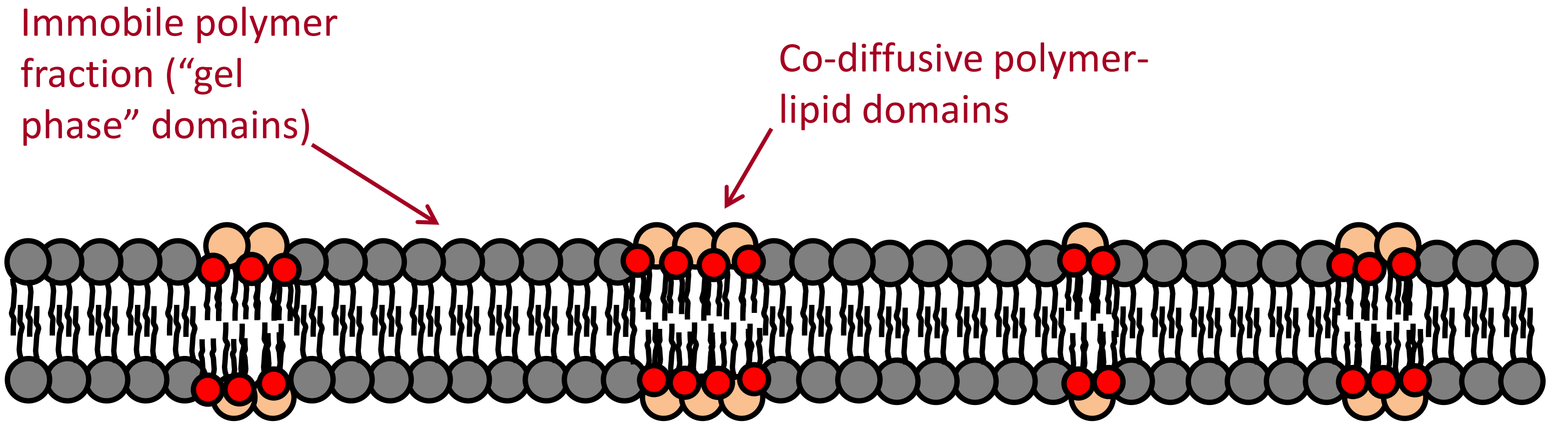
Mobility in Polymer Nanotube Networks

Hypothesized mechanism of mobility in PNTs:

- Majority of polymer immobile due to entanglement of hydrophobic tails, forming raft-like domains
- Majority of lipid is mobile
- Lipid co-localizes with fraction of polymer, changing interactions between tails
- Polymer and lipid co-diffuse as a unified domain
- Exchange of mobile polymers with immobile raft domains (?)



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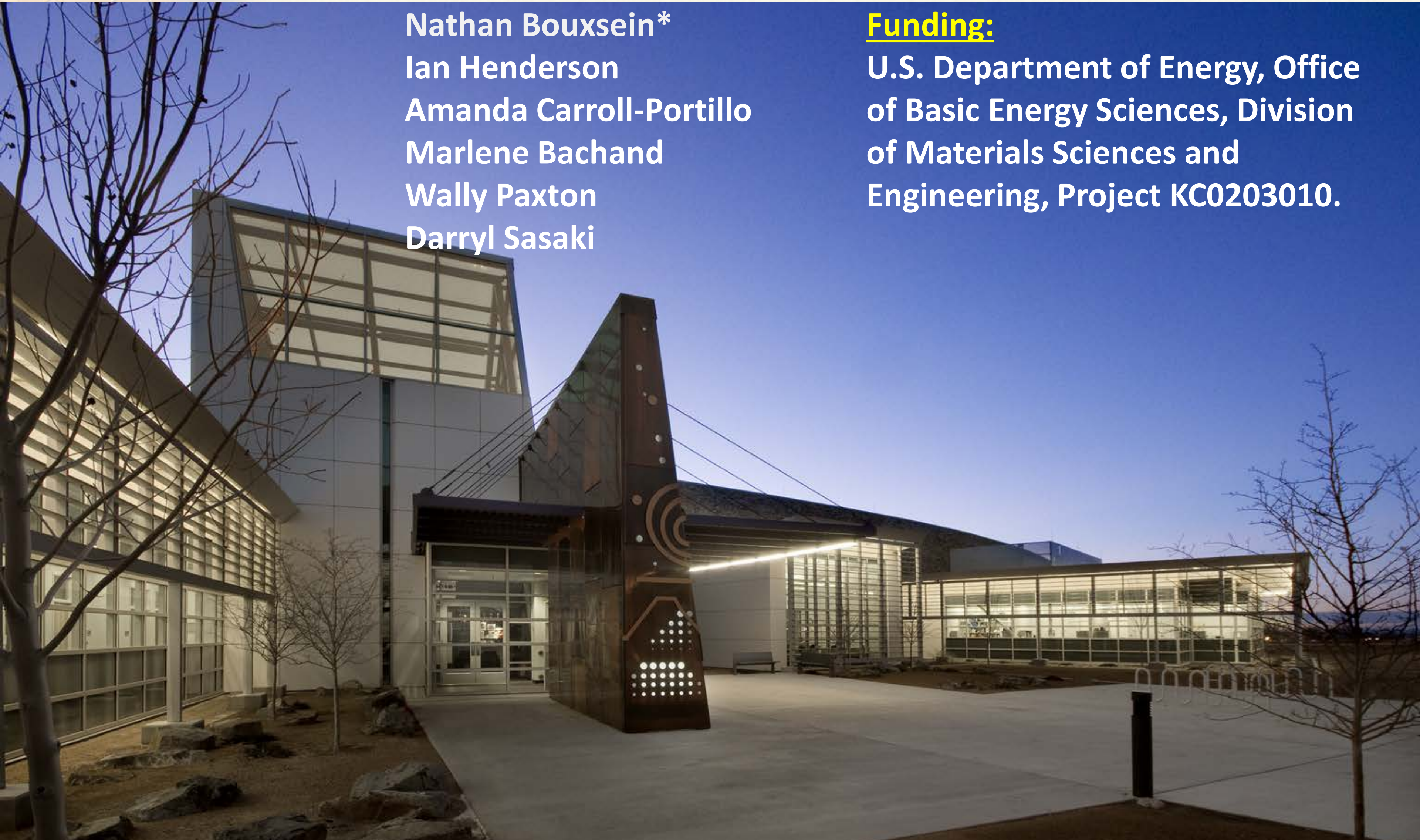

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