Lipid Nanotubes for Biomimetic Structures and Materials Transport

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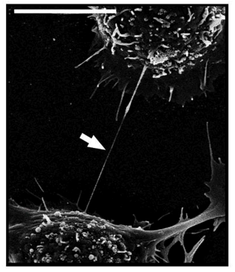
Motivation

Many higher order biological structures are formed from the active processes of bimolecular motors operating on their respective filamentous proteins (e.g. Kinesin/Microtubules and Myosin/Actin). One common example is the formation of lipid tubules, characteristically found in the endoplasmic reticulum, neuronal processes and between cells as tunneling nanotubes. These structures have a variety of functions as sites for metabolic and protein processing and in intracellular communication and transport

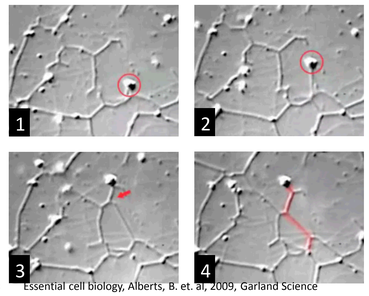
Here, we mimic these lipid nanotubule (LNT) structures in a cell free environment using large multilamellar lipid vesicles (MLVs) in combination with motile microtubules (MT) under the power of surface fixed kinesin. We create large network structures that resemble neuronal networks and demonstrate their ability to capture and transport quantum dot nanoparticles. Network features, such as segment length, are tunable and the total network length can reach values > 10mm.

Intercellular Tunneling Nanotubes

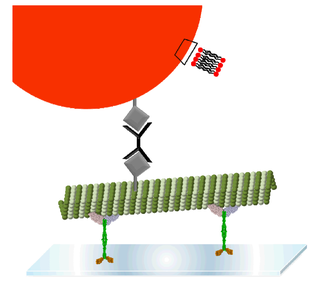


SEM showing a tunneling nanotube connecting two cultured PC12 cells. Bar, 10 mm.
Rustom A, et. al (2004) Nanotubular highways for intercellular organelle transport. *Science* 303:1007-10.

Dynamic formation of Endoplasmic Reticulum



Essential cell biology, Alberts, B. et. al, 2009, Garland Science



Multilamellar Lipid Vesicle
(95% DOPC 5% Biotin-PE)

Biotin-Streptavidin-Biotin Bridge

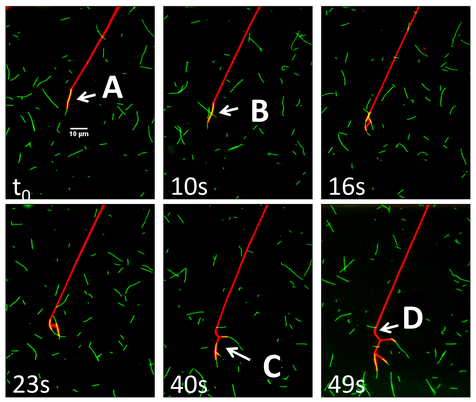
Polymerized MT (5% Biotin Tubulin)

Drosophila Kinesin (ATP)

Glass Substrate (flow cell)

1. Flow cell (.03 mm x 15 mm x 4mm) – add casein then Kinesin
2. Incubate with polymerized microtubules and energy source (ATP)
3. Incubate with streptavidin
4. Incubate with MLV – tube formation depends on vesicle sedimentation

Tubule Characteristics



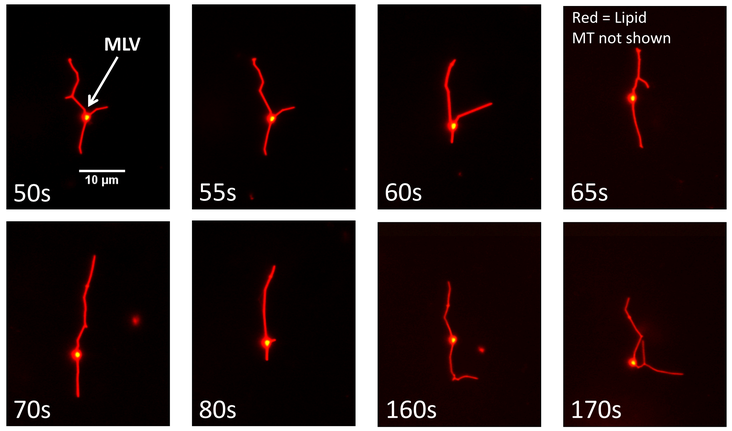
Growing Lipid Nanotubes

A. Velocity of MT bound to LNT equivalent to free MT
   - $V_0^{MT} = V_{LNT}^{MT} = 0.545 \pm 0.08$ µm·sec^{-1}
B. Collision with secondary unbound MT
   - If B-S-B bridge forms then:
C. Bifurcated "Y" junctions
D. Surface pinning of lipid nanotube
   - "Kinked" junctions

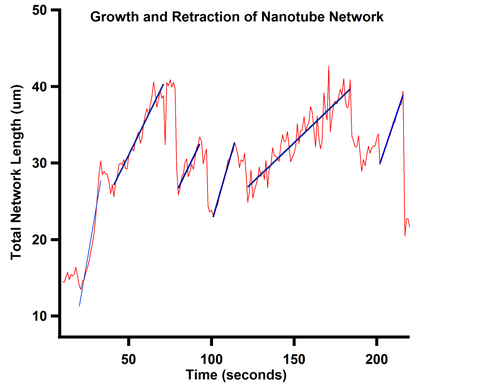
MT velocity appears to always be unloaded. Fluidity of lipid from source MLV to ends of growing LNT prevents buildup of tension

Green = Fluorescent MT
Red = Fluorescent lipid nanotubes
MLV off screen

Small MLVs limit LNT network length



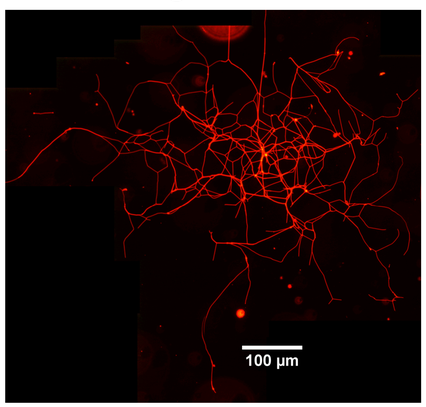
Lipid nanotubes extracted from small MLVs are susceptible to tension buildup due to limited lipid flow
Max total network length ∝ MLV size



Length limited dynamic growth and spontaneous retraction
Lipid adhesion force 30pN < B-S bond 160pN; retraction occurs when tension exceeds force required to remove single biotin-lipid from LNT bilayer

Synthetic "Living" Networks

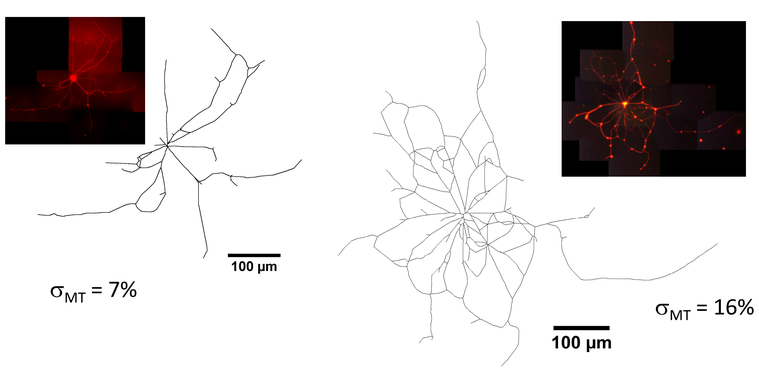
Energy-Dissipation = Self-Healing Networks



Stochastic nature of underlying MT motility used to <u>generate >10 mm</u> networks of lipid nanotubes

MT Surface Density (σ_{MT}) can Tune Network Parameters

Skeletonized Network Analysis		
	σ_{MT} = 7%	σ_{MT} = 16%
# of junctions	30	104
# of branches	62	182
Mean branch length	37.4 µm	31.8 µm

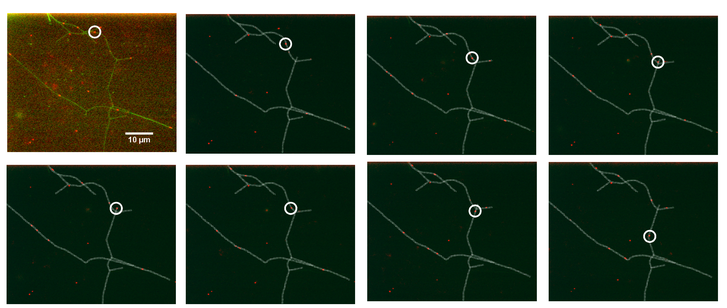


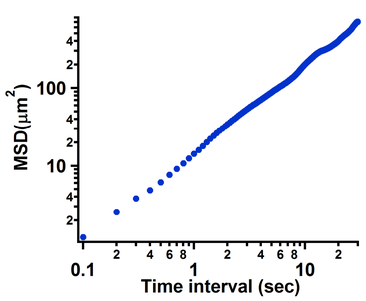
σ_{MT} = 7%

σ_{MT} = 16%

Path Specific Transport of Nanomaterials

Qdot bound via biotin-streptavidin to LNT surface





Qdots locally *confined* to 1D lipid nanotube surface
Seamless diffusive transport though junction points
Underlying lipid fluidity (DOPC @ T=25°C) -> entire network can participate in transport

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