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The Art of Microtubule Ring Formation

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Abstract:

Biotinylated microtubules have the capability to actively self assemble into ring structures when they are coated with streptavidin quantum dots. There is much debate on the mechanisms by which MTs form these spools. We have found the two primary methods of spooling to be pinning, which forms small loops, and coiled-coil, which forms larger loops. Determining the causes and methods of this self-assembly has important implications for the future development of biomachines and other nano-technological applications. We have also looked at controlling the size of ring diameters by observing MT lengths and how they affect ring size. So far there seems to be no correlation between these two measurements.

Introduction:

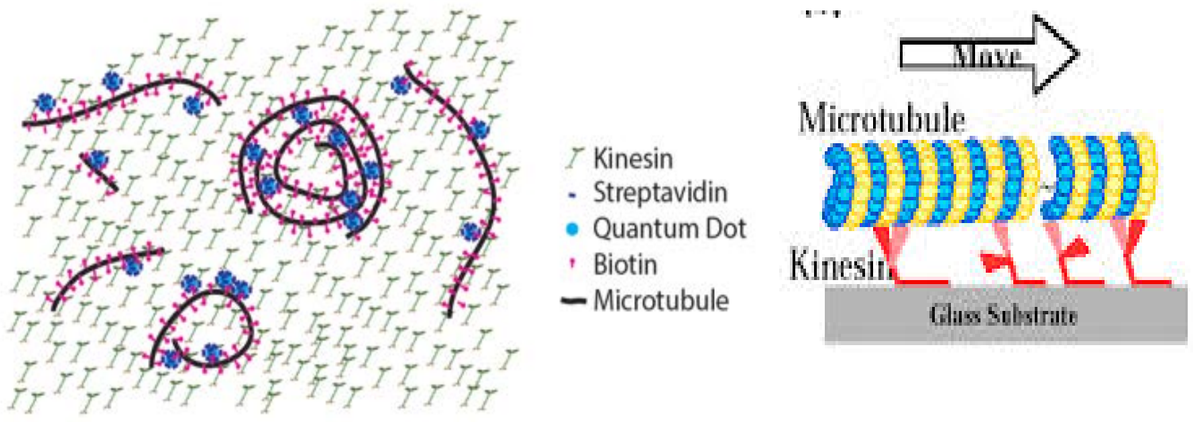
What is a microtubule?
Microtubules are very rigid components of the cell partly responsible for:

- Holding cell structure, allowing cell movement, cell division, and other cellular mechanisms.

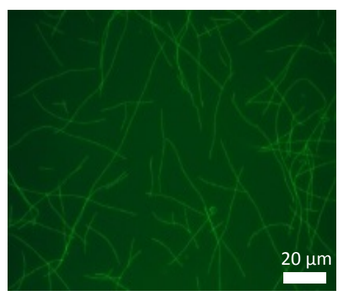
What are streptavidin quantum dots?
Streptavidin is a protein with high binding affinity for biotin; it is covalently bonded to quantum dots, which are fluorescent nanoparticles.

Methods:

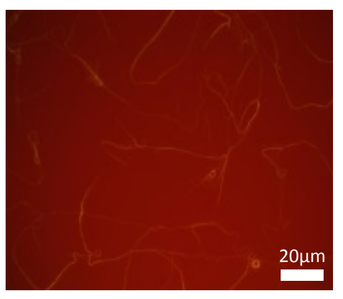
- **Inverted Motility Assay:** MTs move along a surface by the interaction between the MTs and kinesin motors. Kinesin sticks to a glass surface, then binds to the MTs, "walking" along the filaments to move them.



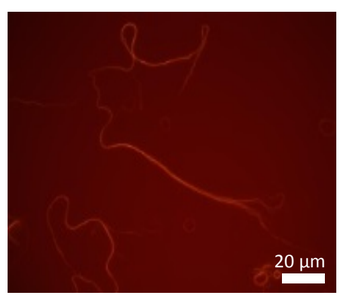
- **Microtubule Interaction:** When streptavidin quantum-dot solution interacts with biotinylated MTs, it creates biotin-streptavidin bonds, causing the MTs to stick to each other.

1)


MTs on kinesin coated surface

2)


Add streptavidin Q-dots ; MTs stick

3)


MTs bundle together

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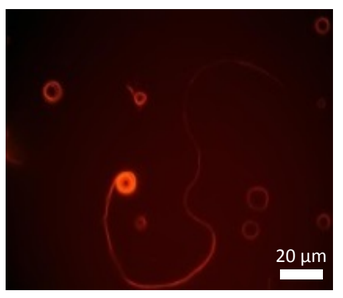
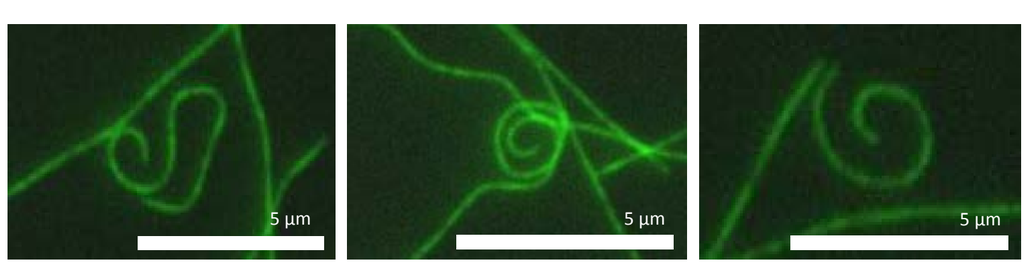


MTs spool into loops

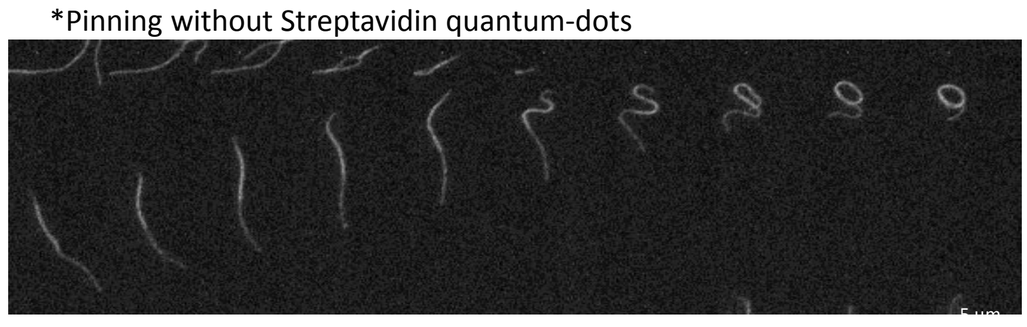
- **Ring Formation:** There have been multiple observed mechanisms by which MTs form into rings or spools: pinning, coiled coil, tip collision, and simultaneous sticking.

Results:

- **Pinning**: The tip of an MT hits a defective motor and gets stuck while the rest of the MT filament keeps travelling in the same direction. A nearby segment of the MT quickly touches the tip and binds to it because of the biotin-streptavidin bonds. This seems to be the cause for the smaller loops.



*Pinning without Streptavidin quantum-dots


*Pinning with Streptavidin quantum-dots

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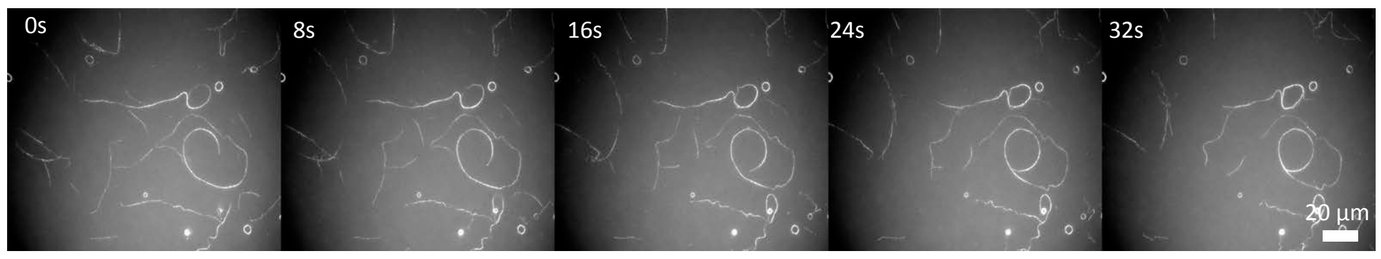
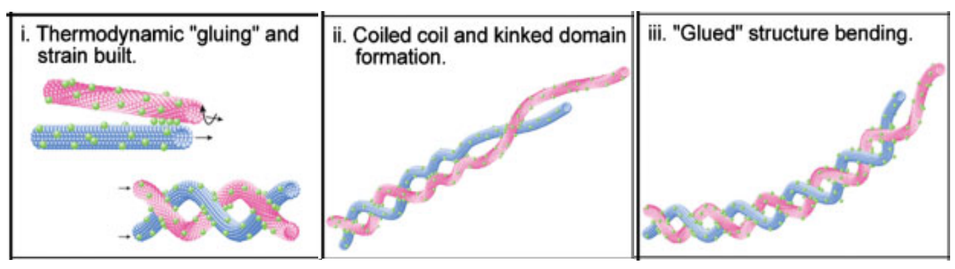
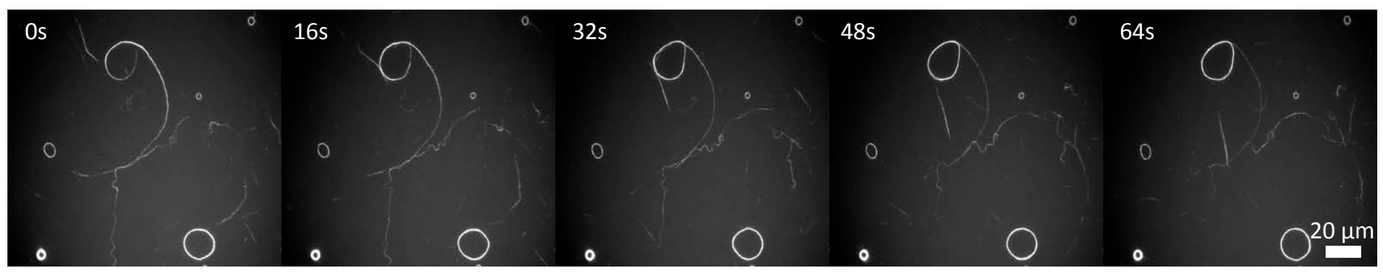
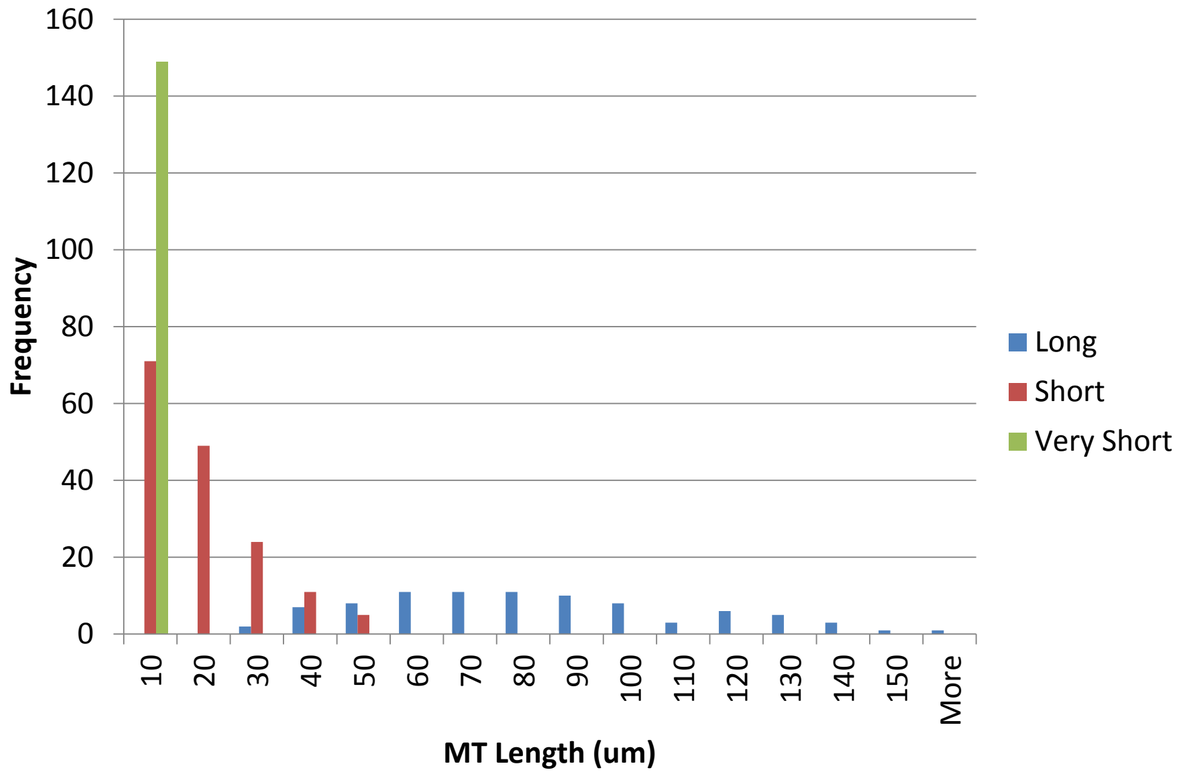
- **Coiled coil:** Two MTs bundle together in a helix. This configuration causes the MT bundle to move in a circular trajectory. Eventually, the tip of the bundle encounters the body of the bundle and sticks to it. This seems to be the cause for the formation of larger loops.

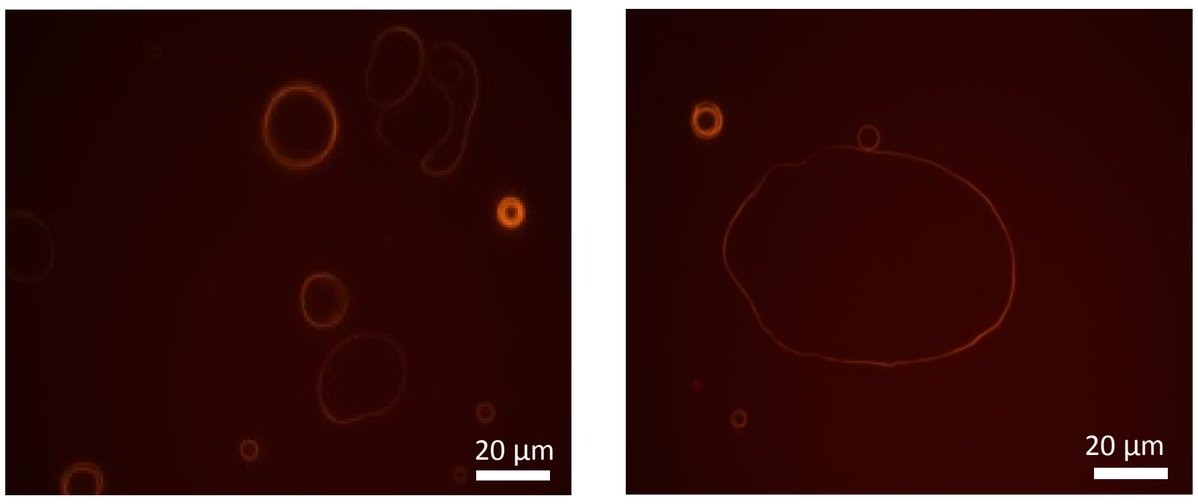


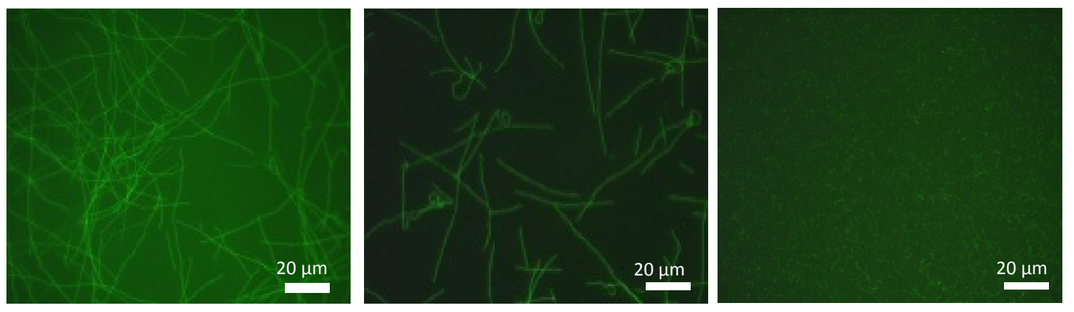


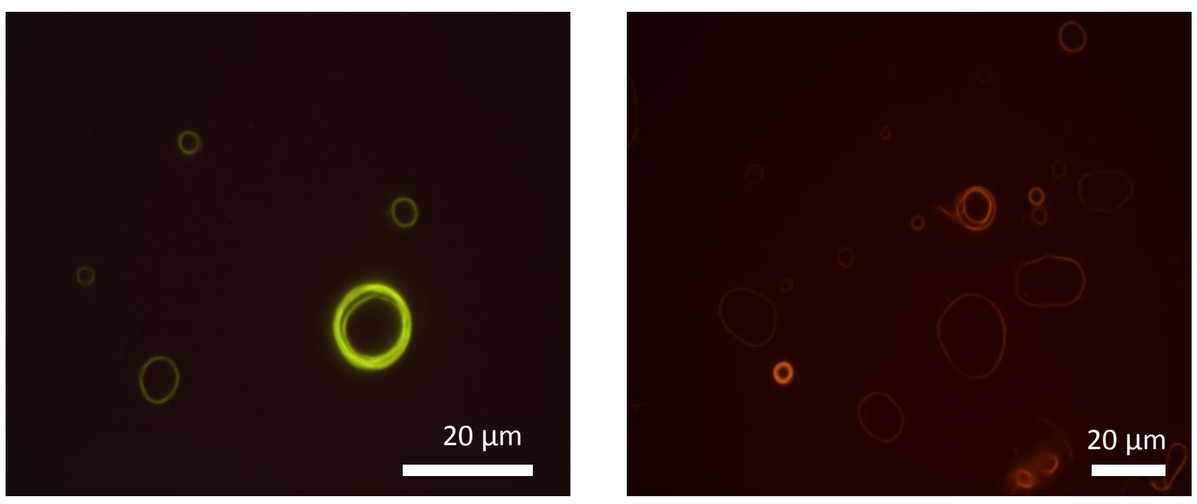


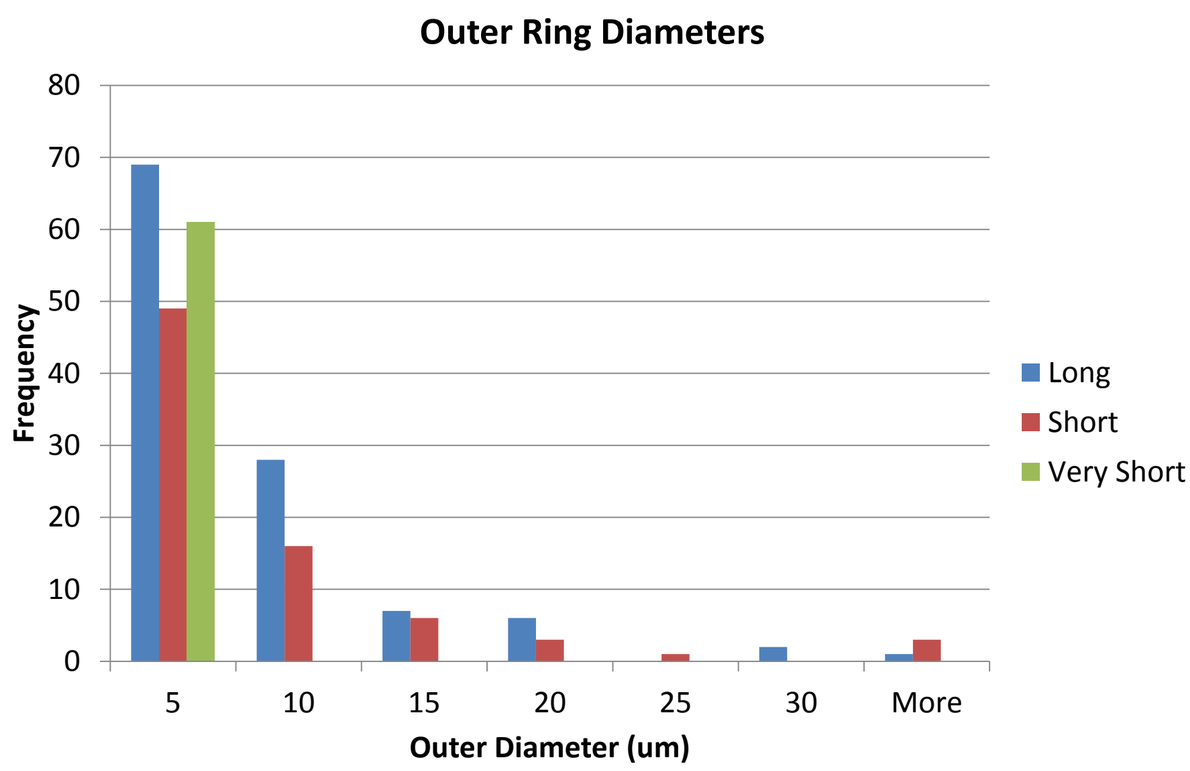
- **MT Length vs. Ring Diameter:** We observed that with very long MTs, very large loops formed. Does MT length correlate to ring size? We ran inverted motility assays with short MTs and very short MTs to see if they would yield smaller rings. Surprisingly, both long and short MTs yielded the same size distribution of rings. However, MTs less than 5µm seem to cross a threshold yielding smaller or no rings.





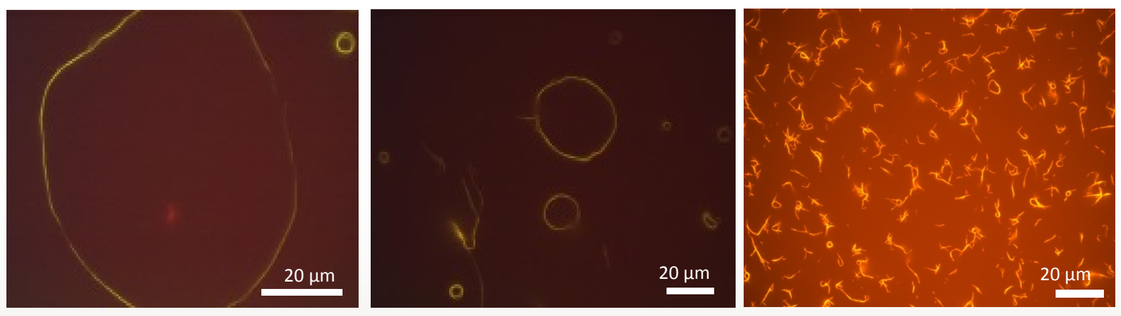






Further study:

- Is density a factor in ring size? Is there any further correlation between MT length and ring diameter?
- Observe more closely the coiled coil mechanism. Do the large rings start forming because of helical coiling of MT bundles or by some other initial step?
- Can we observe the other previously seen ring formation methods?
- Can another compound, like Tau or $BaCl_2$, cause the same looping effect on the MTs?



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