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Understanding initiation mechanisms and controlling properties of microtubule spools

Virginia VanDelinder, Stephanie Brenner, George Bachand

Center for Integrated Nanotechnology

Sandia National Laboratories

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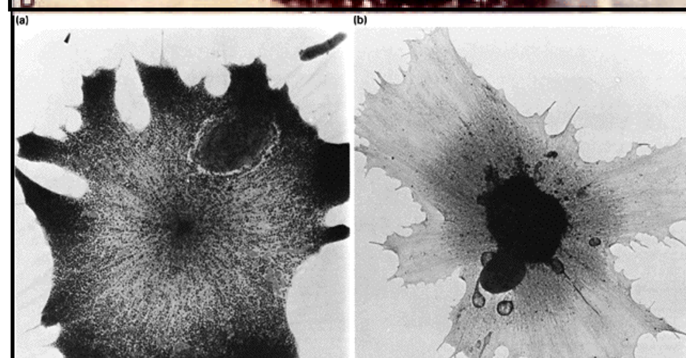
Biological molecular motors

- Active transport systems:
  - Key to many dynamic physiological processes
  - Nature evolved motor proteins to transport materials in static, nanofluidic environments because fluid flow at those length scales becomes problematic (e.g., pressure-driven fluid low is hard in nano-channels)
  - Biomotors do not require external power – rather they convert chemical energy (low mass/weight) into mechanical work with great efficiency
  - Biomotors function autonomously without the need for a "user" to control function
  - Ability to transport in complex solutions (e.g., blood, saliva)

Biology: Adaptive and responsive materials properties

*L. Haimo and C. Thaler, BioEssays 16, 727-733 (1994).



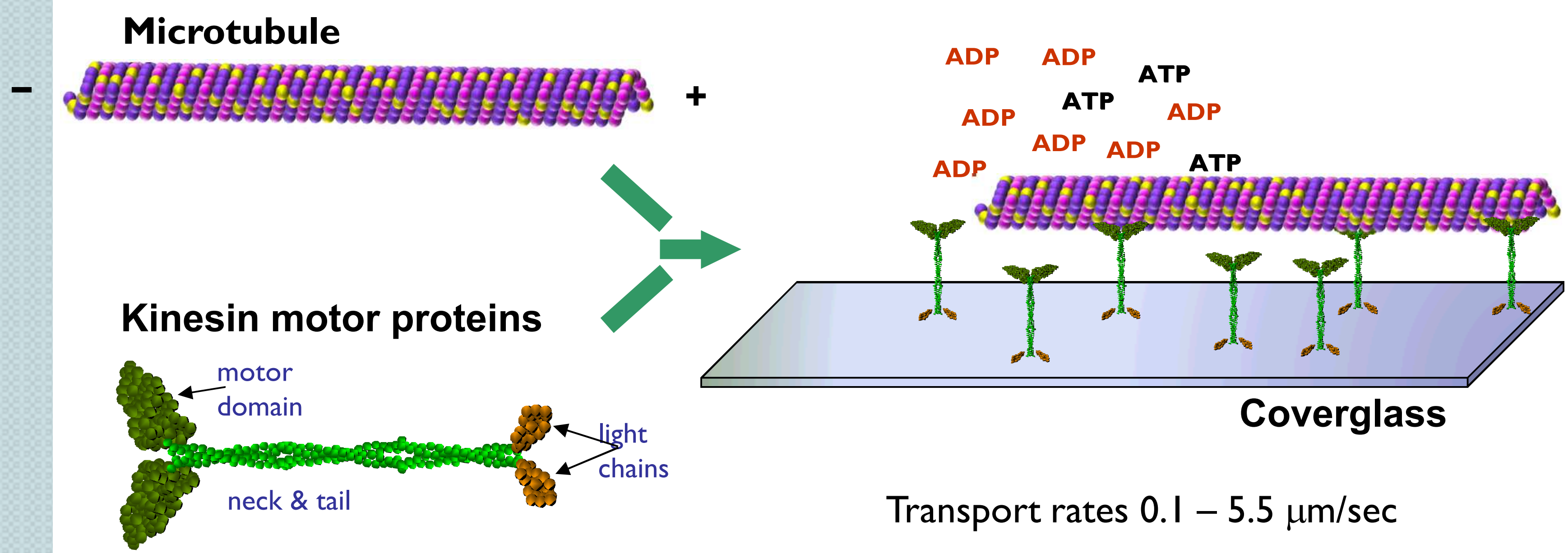
Adapted from: http://wilkes-fs1.wilkes.edu/~terzaghi/BIO-226/lectures/24.html

Nanotechnology: Dynamic materials assembly

Kinesin and Microtubules

Biomolecular motors & active transport:

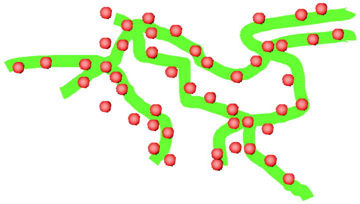
- Convert chemical energy (ATP) to mechanical work (40 pN•nm) with high catalytic efficiency (>50%)

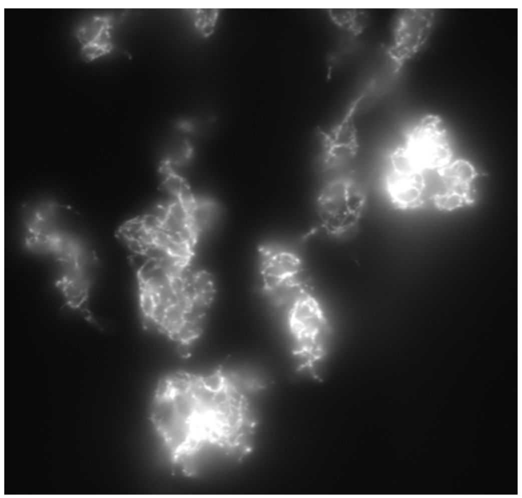


Goal: To utilize biomolecular motor-powered systems to develop novel nanomaterials and nanofluidic systems with biomimetic functionality

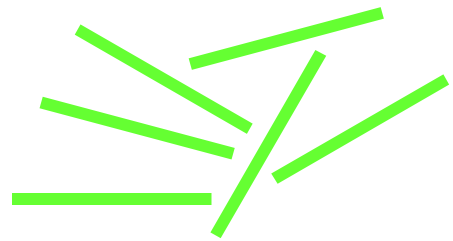
Active self-assembly: spools

QDs + biotinylated MTs



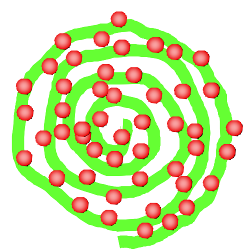


Randomly assembled MTs and QDs (equilibrium)

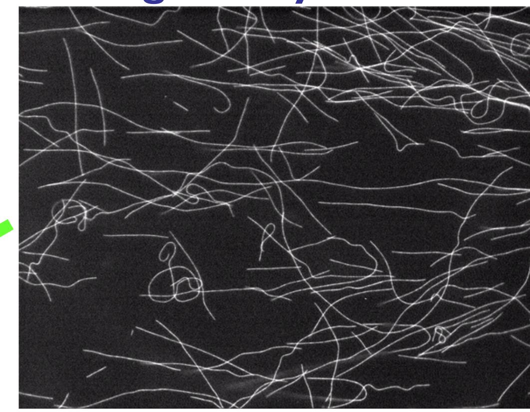


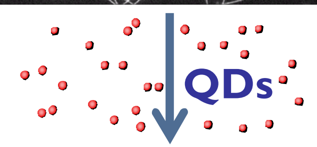
Energy input via ATP hydrolysis

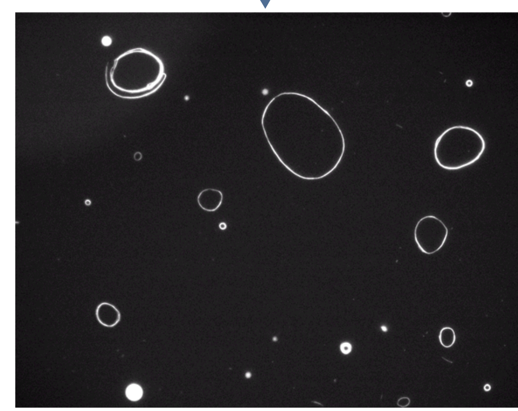
~50 kJ/mol ATP



Gliding biotinylated MTs



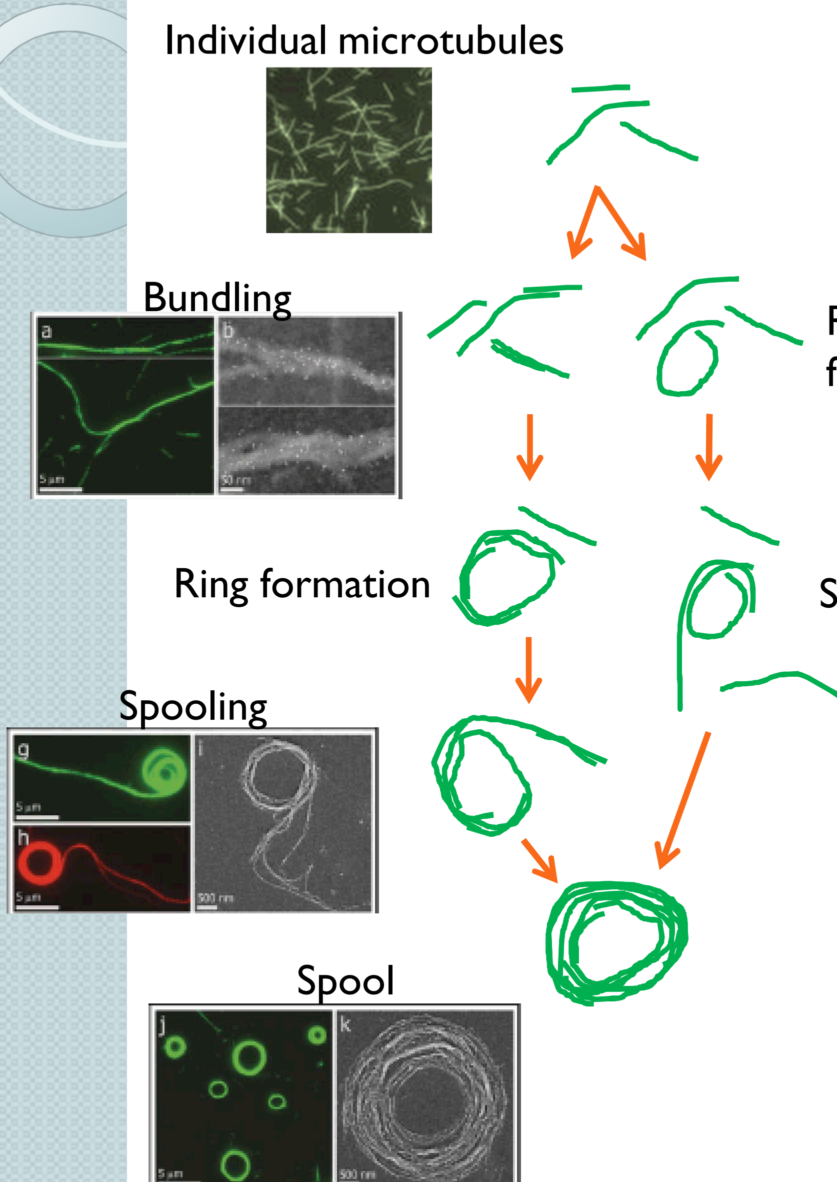


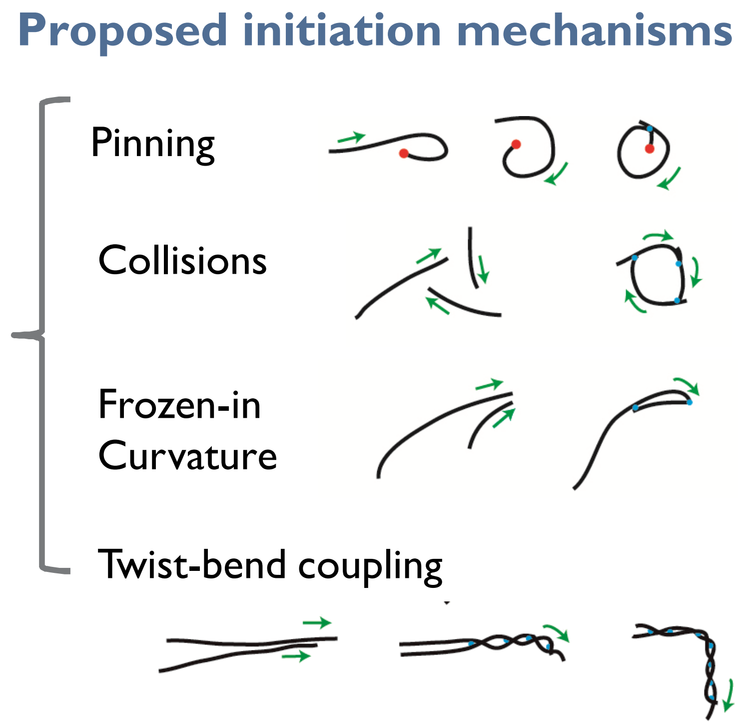


Actively assembled MTs and QDs (+ energy)

chemical energy → mechanical work → active assembly

Spool initiation and growth

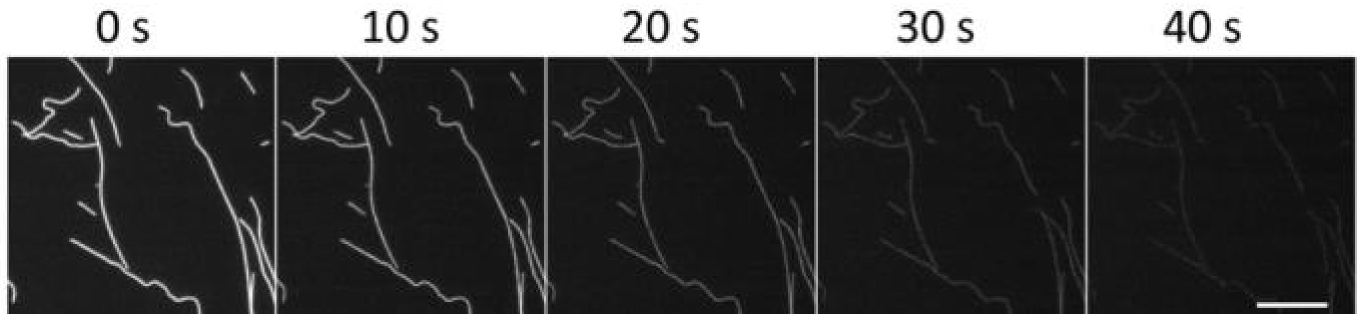




Initiation mechanism studied primarily by changing experiment parameters, observing effect on resultant spools, and then inferring back to implications for formation.

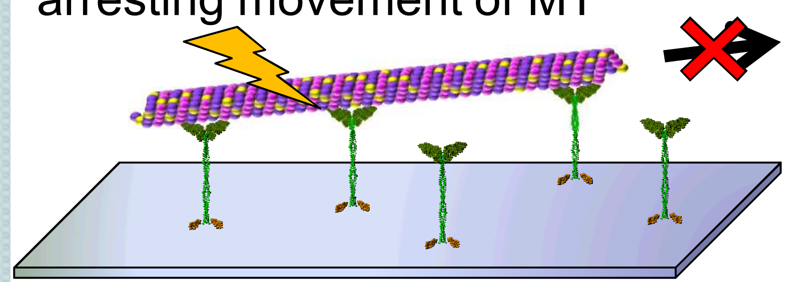
Photodamage

Damage to fluorescent dyes: Decreased brightness

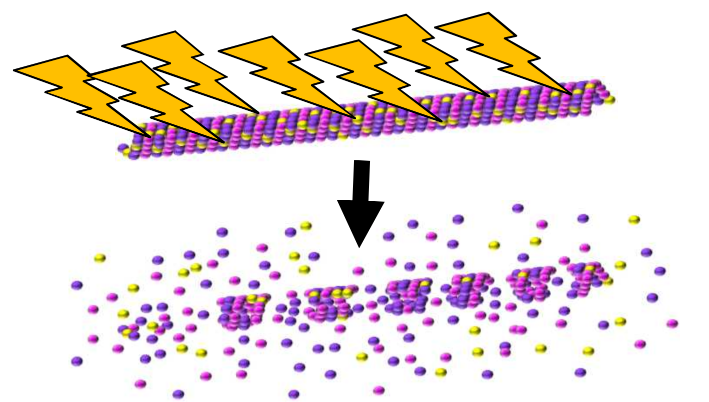


Damage to motors and microtubules

Pinning: fluorophore in excited state or other radical reacts with kinesin, arresting movement of MT

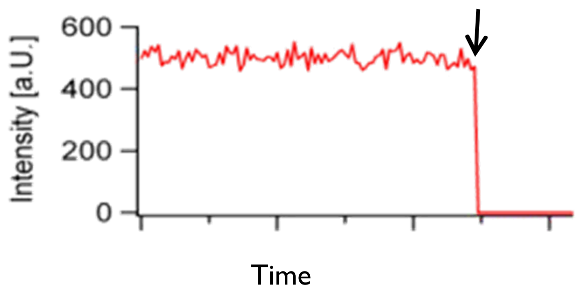


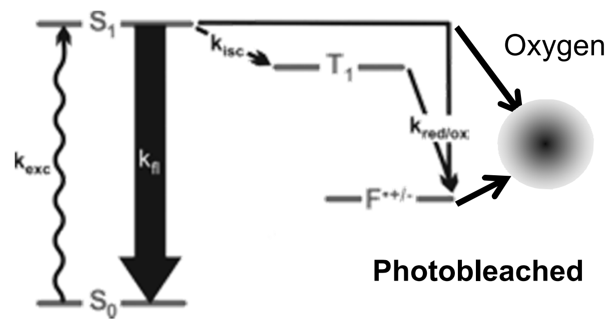
Depolymerization: MT catastrophically disassembles



Photostability of fluorescent dyes

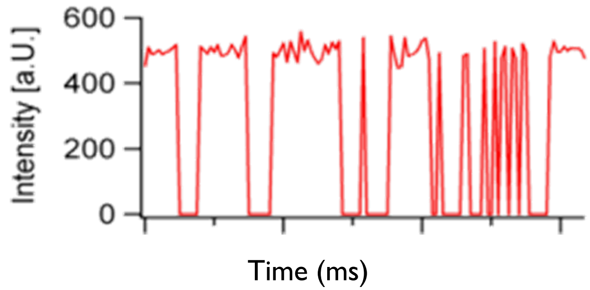
Bleaching

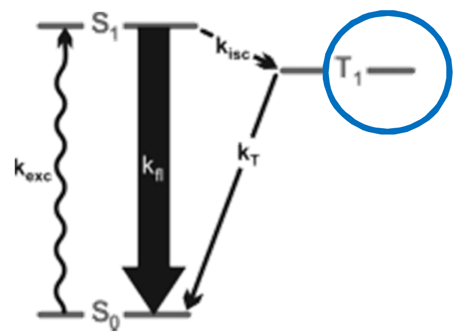




Oxygen radicals react with the fluorophore in an excited state and cause irreversible loss of fluorescence.

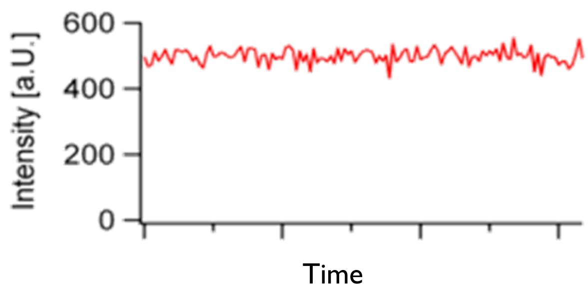
Blinking

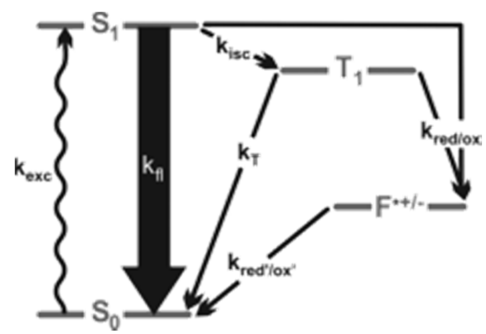




Once every ~1000 excitations, the fluorophore goes from excited state (S_1) to long-lived triplet state (T_1) and stays "dark" for milliseconds before returning to the ground state. These undesired intensity fluctuations decrease the ensemble intensity.

Photostable



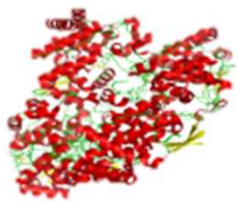


Fluorophore is rapidly rescued from the triplet state by a reducing and oxidizing system.

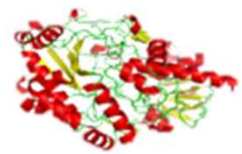
Oxygen scavenging system

Remove oxygen to ameliorate bleaching:

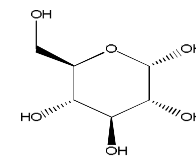
Enzymatic oxygen scavenger system



glucose oxidase



catalase

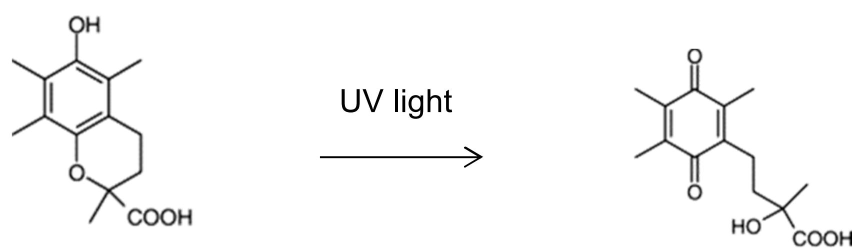


glucose

Reducing and Oxidizing System to ameliorate blinking:

- Rescues fluorophore from dark triplet state, reducing blinking.
- Reduces bleaching by shortening the time the fluorophore spends in reactive excited states.

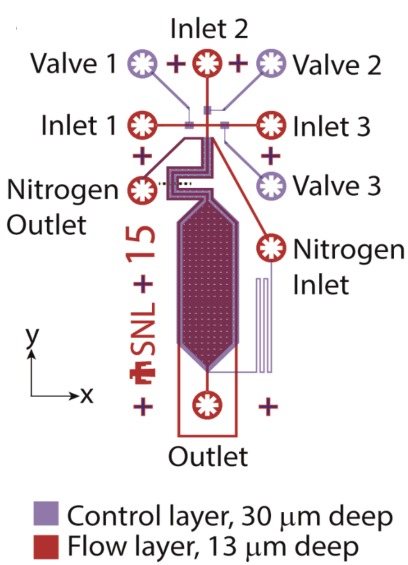
Reductant:
Trolox
(900 μM)

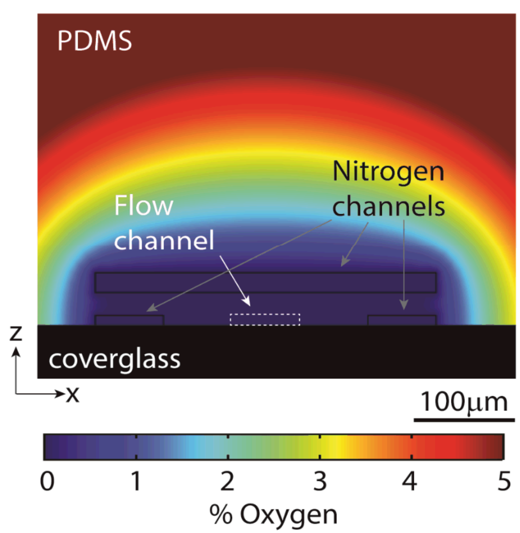


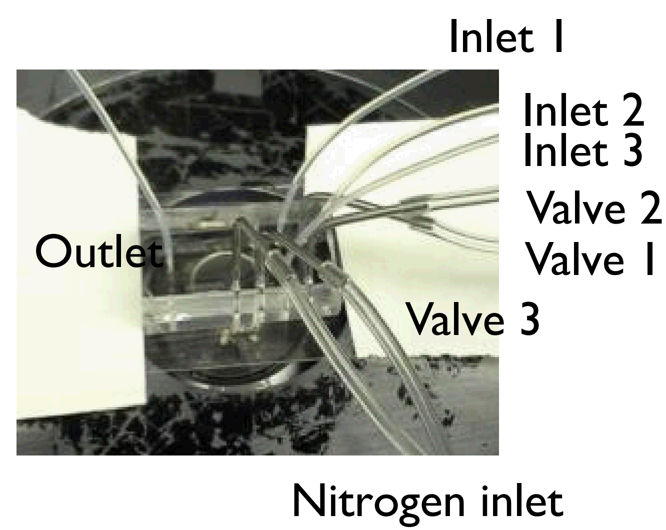
Oxidant:
Trolox Quinone
(100 μM)

Integrating biomotors into microfluidic devices

- PDMS devices made via soft lithography and molding
  - Rapid prototyping compared to hard materials
  - Multi-layer devices enable integrated valves
  - Permeable to gases
- For lab use to control experiment parameters in the study of biomotors





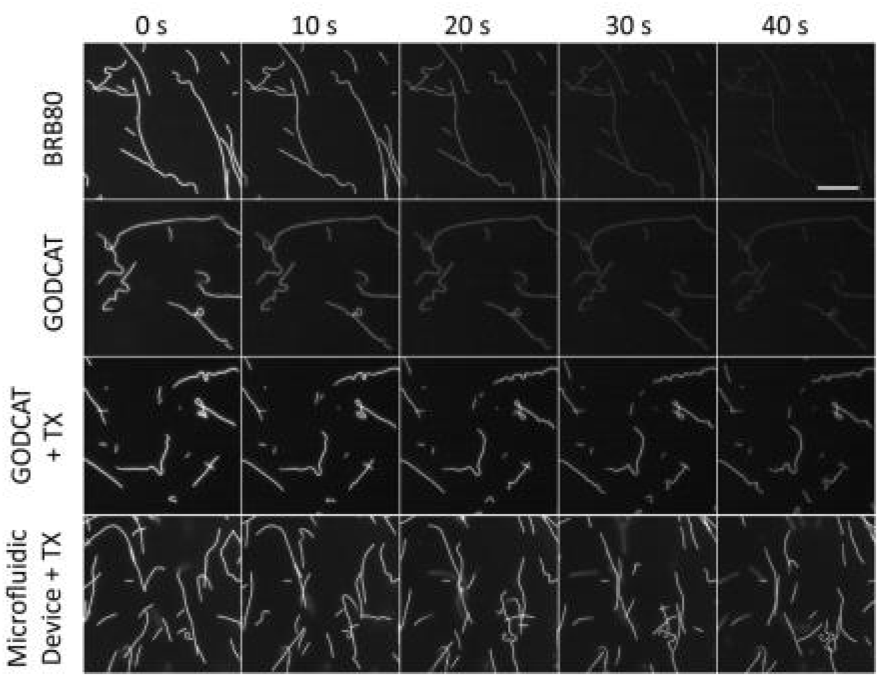


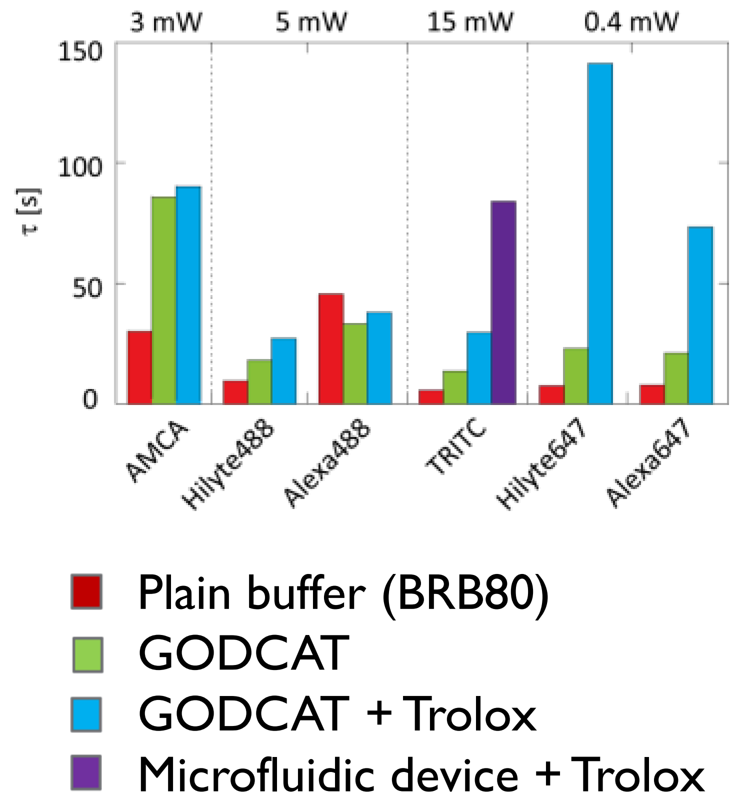
VanDelinder, *Anal. Chem.*, 2013

Photodamage

Photodamage to dyes → loss of brightness

Photodamage to motors → loss of motion

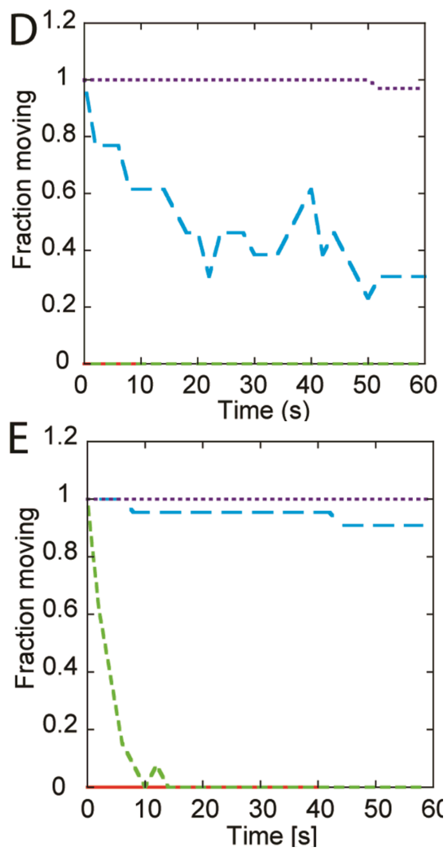


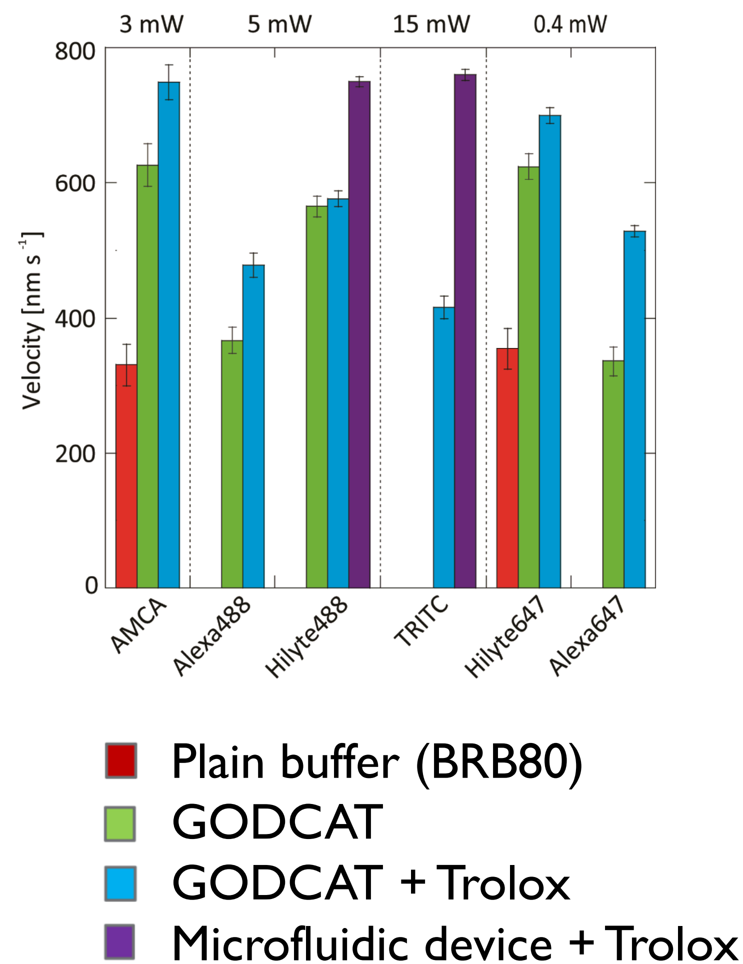


Photodamage

Photodamage to dyes → loss of brightness

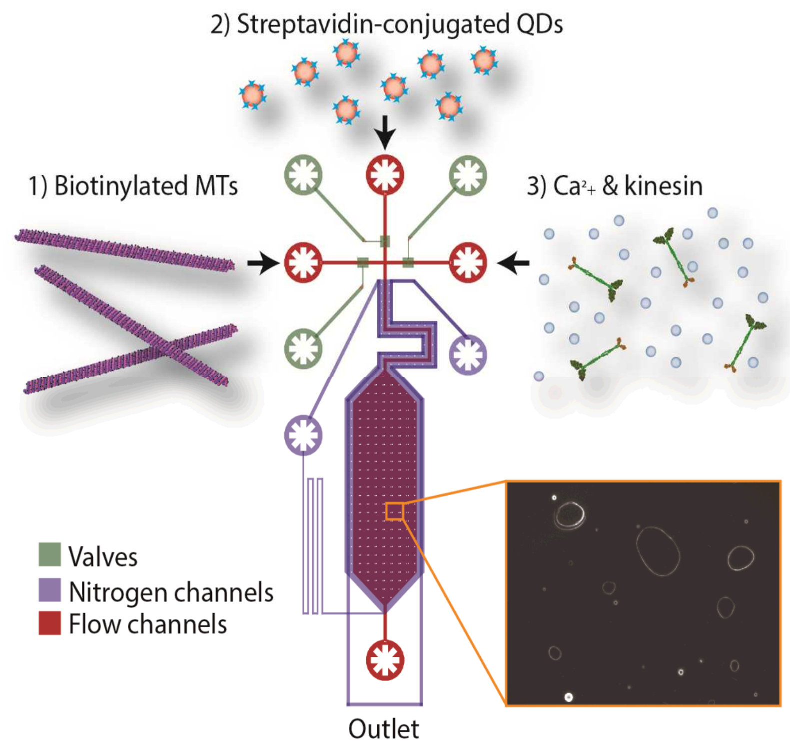
Photodamage to motors → loss of motion



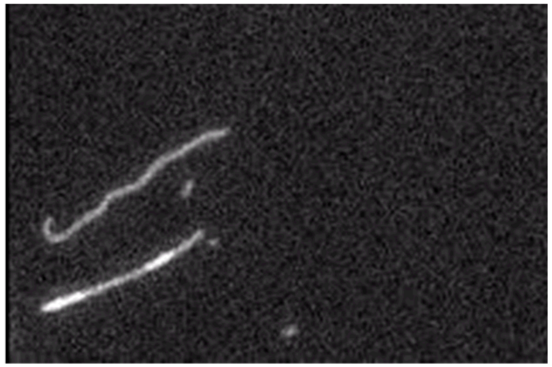


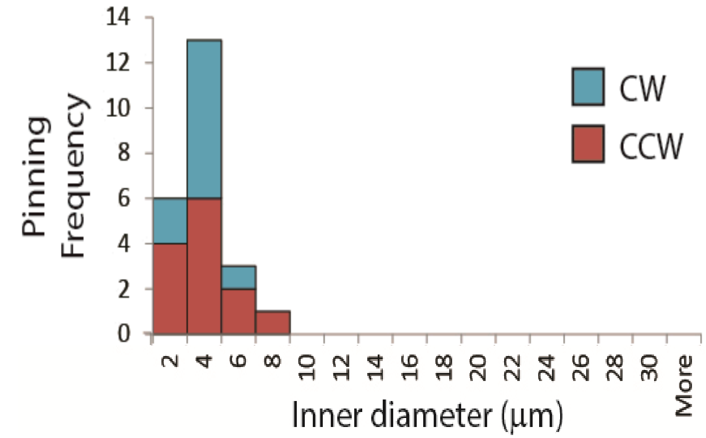
Direct observation of spool formation via microfluidics

- Can visualize microtubules while adding QDs
- Controlled solution exchange and can image during exchange
- Iterate experiment repeatedly
- Continuous supply of ATP
- Improved photostability
  - Less photodamage to motors (less pinning)
  - No enzymatic oxygen scavenger system needed (which stops working and acidifies solution after 1-2 hrs)



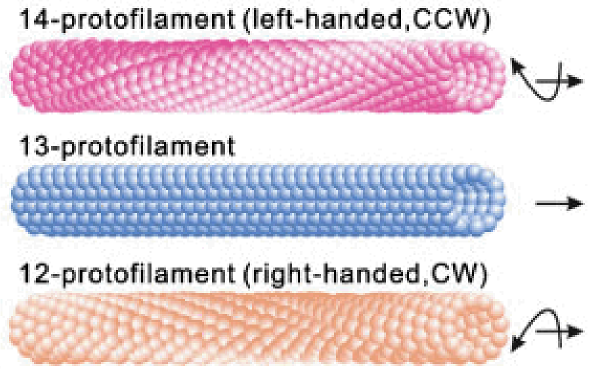
Formation mechanism: pinning



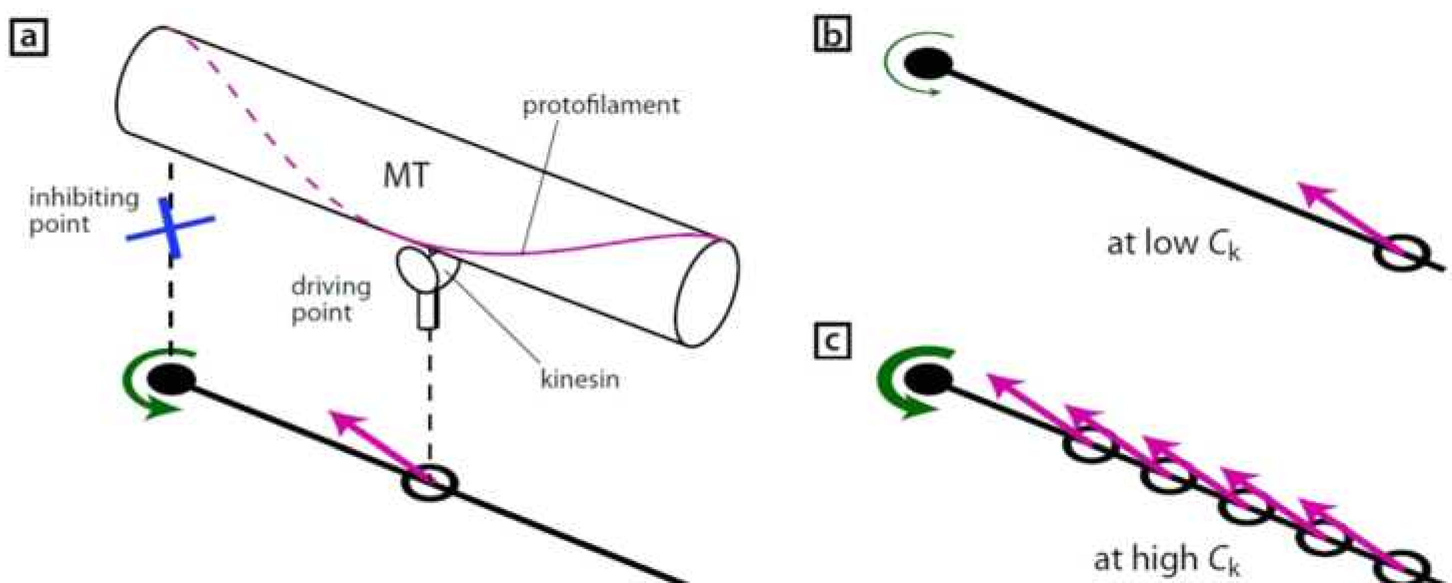


- Diameter 2.7 ± 1.5 µm (mean ± SD)
- The diameter of rings formed by pinning is determined the buckling radius of a MT
  - contributes to the uniformity of size of rings formed by this mechanism
- Rate at which microtubule encounters a dead motor is a stochastic process
- Pinning is greatly increased by photodamage to motors
- The majority of rings formed by pinning rotated in the CCW direction (62%)

Biased rotation direction

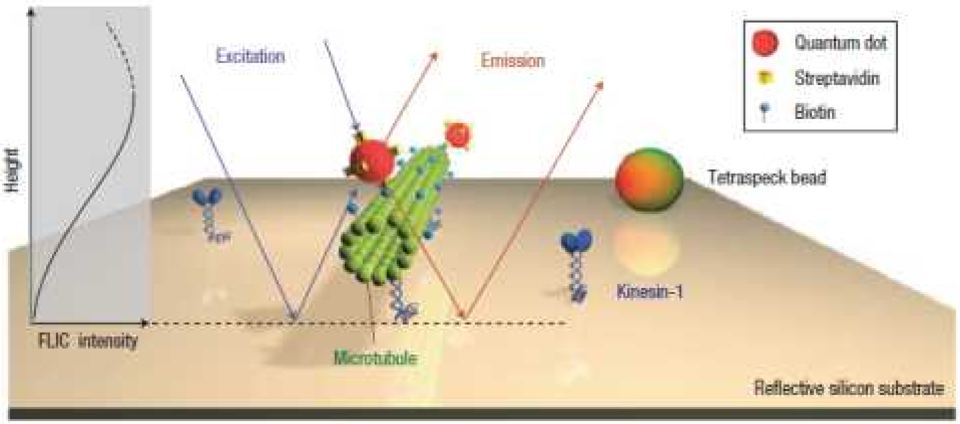


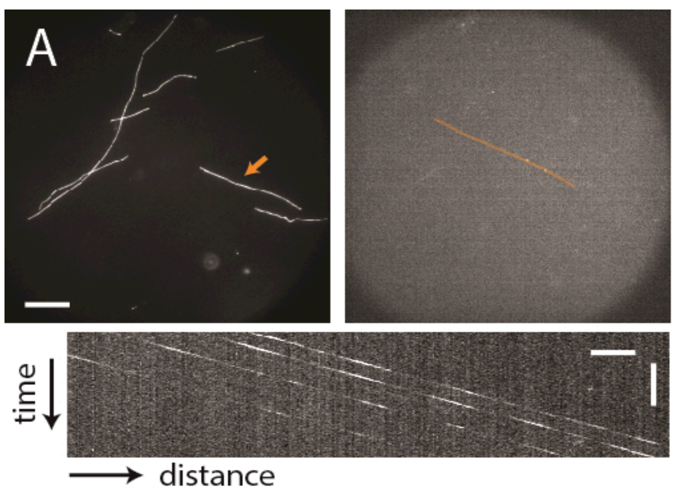
- Microtubules rotate axially depending on protofilament #
- Protofilament # has been observed to affect ring rotation direction
- Wada et al proposed mechanism by which an inhibiting point would produce directional bias in ring rotation direction
  - Amount of bias is dependent on kinesin surface density



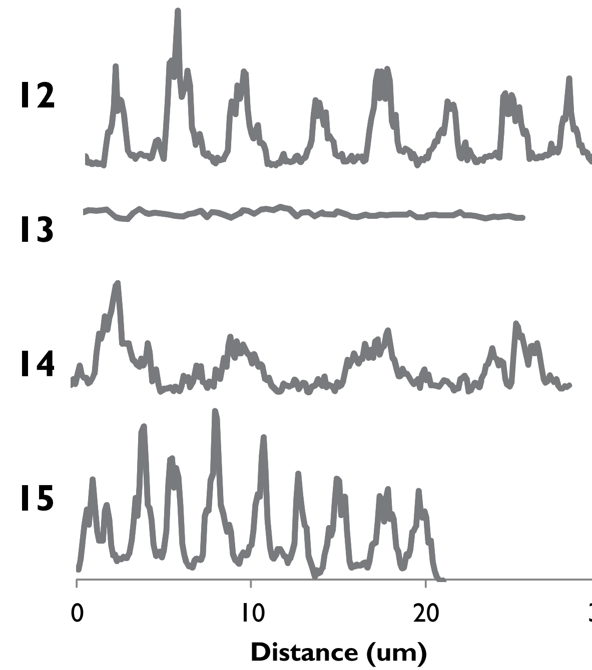
Wada 2015

FLIC of individual MTs





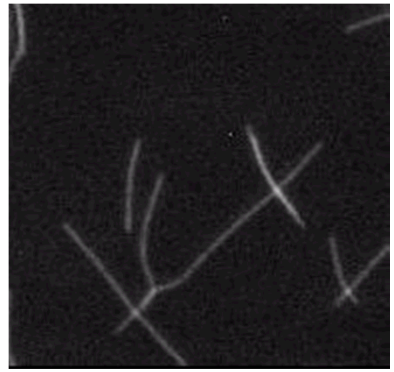
QD intensity traces for various protofilament #'s

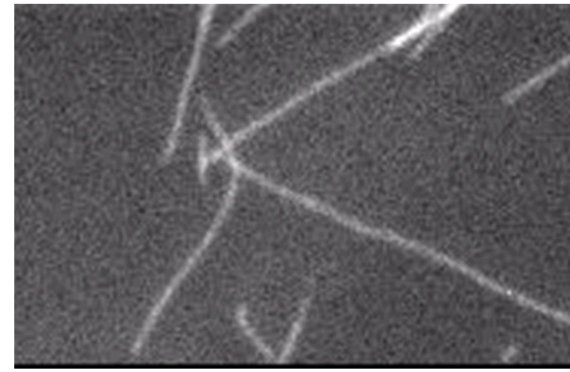


Protofilament #	13	14	15	16
Us	37	51	14	0
Ray 1993 JCB	14	72	11	3
Kakugo 2011 BMM	18	40	30	6

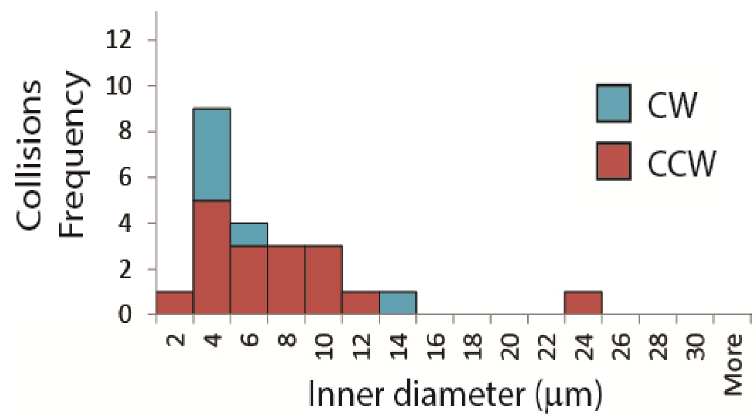
Nitzsche 2008

Formation mechanism: collisions

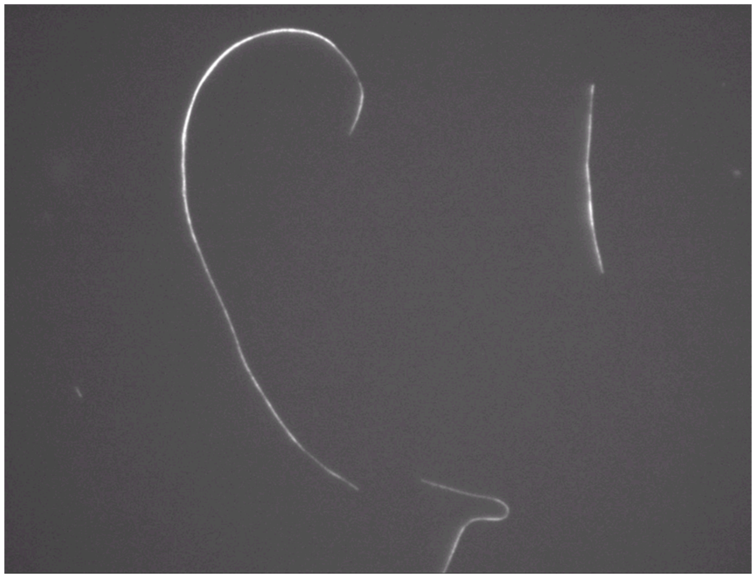




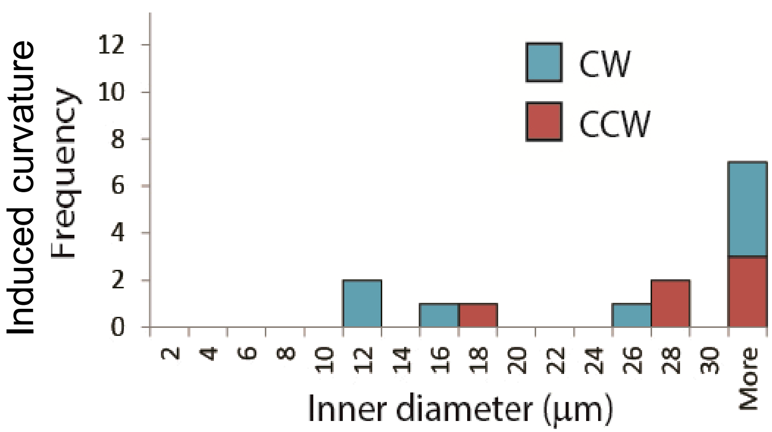
- Diameter 6.2 ± 4.8 μm
- Spools formed via collisions displayed a biased rotation direction (74% CCW)
    - Getting stuck on another MT can serve as an inhibiting point

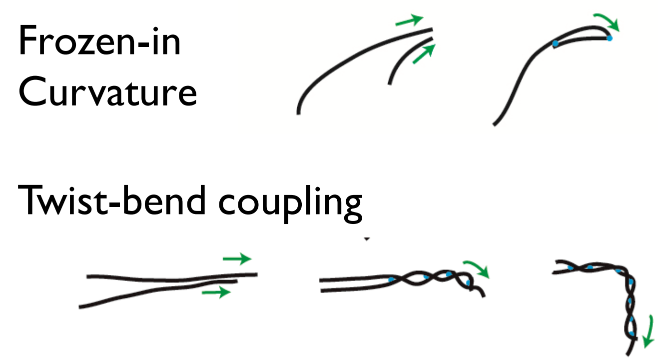


Formation mechanism: induced curvature



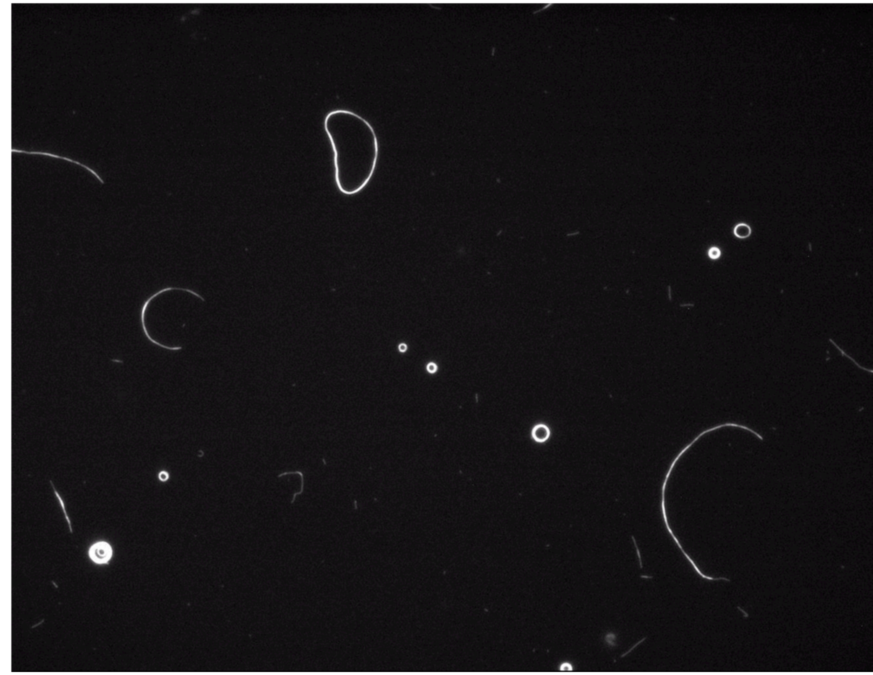
- Closing events were rare and thus difficult to capture
- This mechanism wasn't previously observed
- Diameter 33 ± 20 μm
- 58% CCW
- Two different mechanisms could underlie induced curvature:

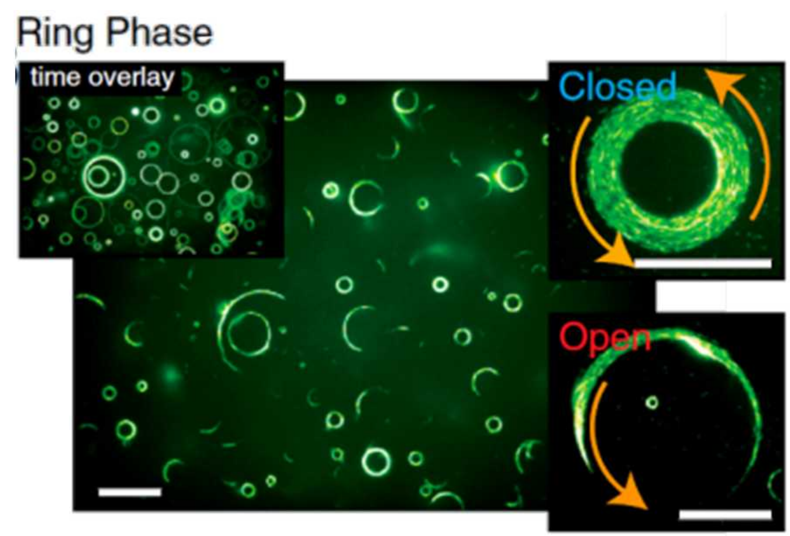




Induced curvature of bundles

- Observed many bundles that followed persistent curved trajectories
- Similar to what was previously seen for myosin-driven actin filaments crosslinked by facsin

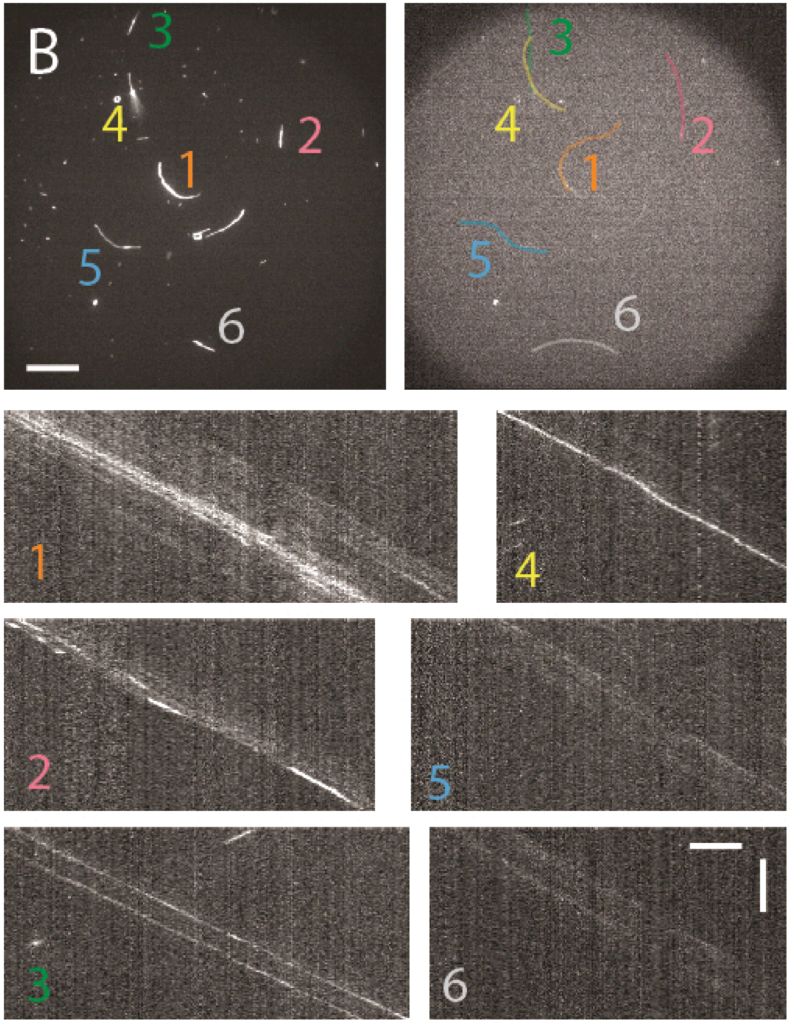




Schaller 2011

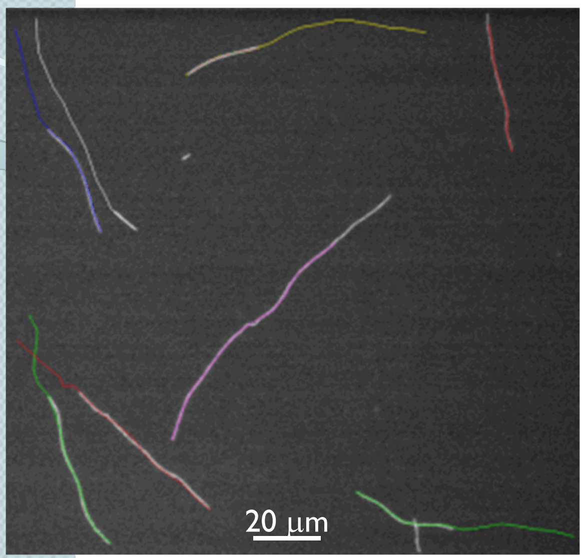
FLIC of bundles

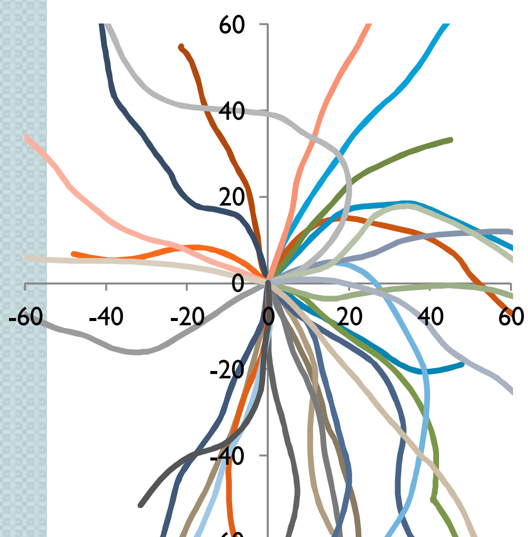
- If twist-bend coupling is responsible to curved trajectory of MT bundles, then bundles should rotate axially as they translate across the surface
- Only ~20% of bundles appear to be rotating
- Does not support twist-bend coupling hypothesis



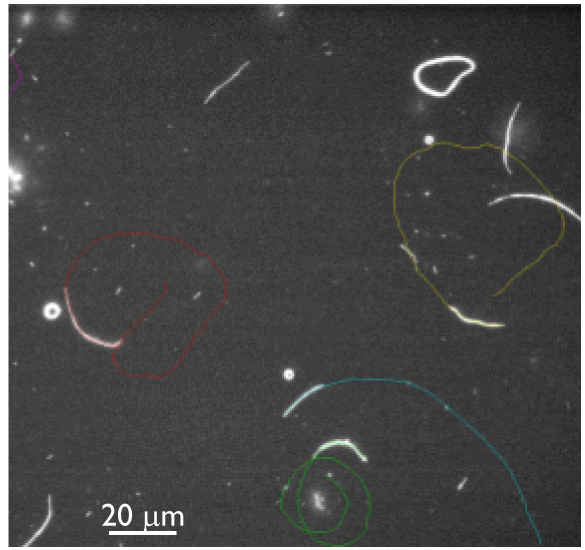
Trajectories

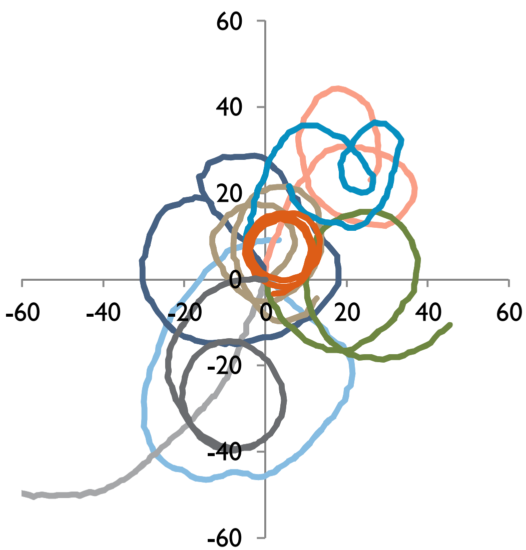
Single MTs



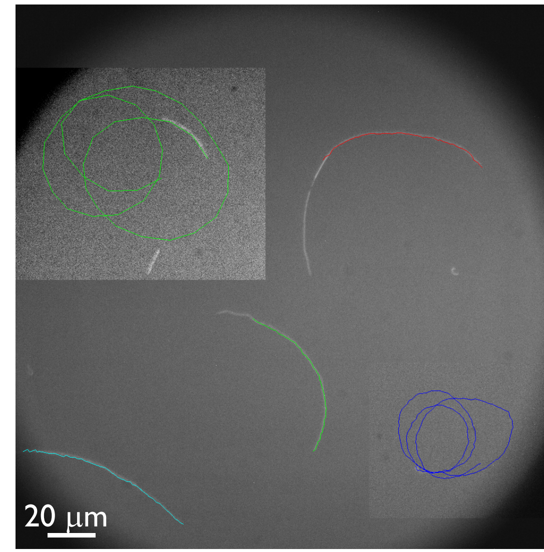


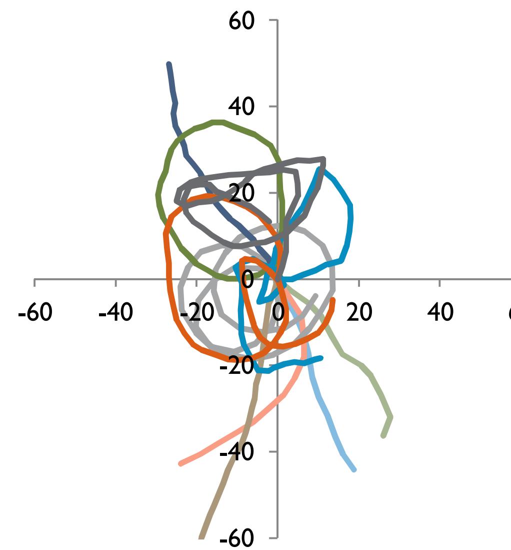
Bundles





Single MTs with QDs

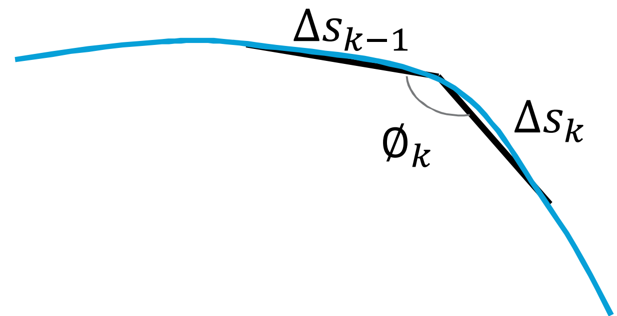


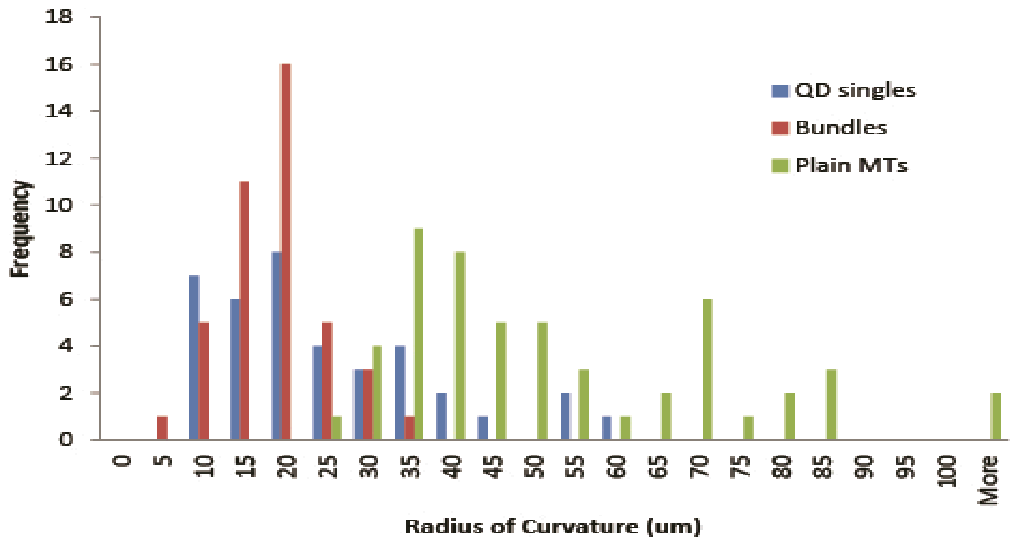


Curvature analysis

Curvature $K \sim \left|\frac{2\emptyset_k}{\Delta s_{k-1} + \Delta s_k}\right|$

Radius of curvature = $^1/_K$



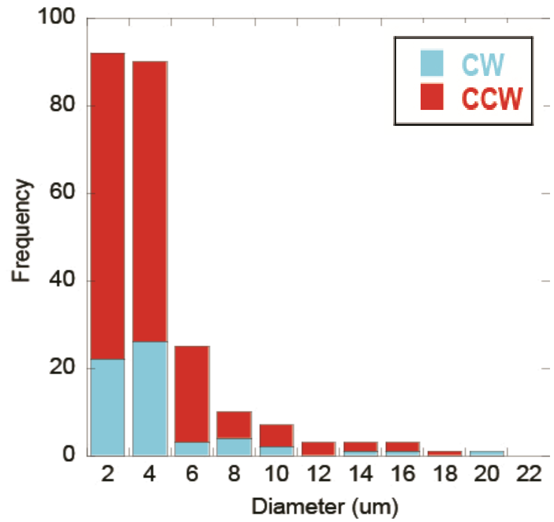


	Average radius of curvature
Individual MTs	51 ± 23 μm
Bundles	17 ± 6 μm
Individual MTs with QDs	22 ± 13 μm

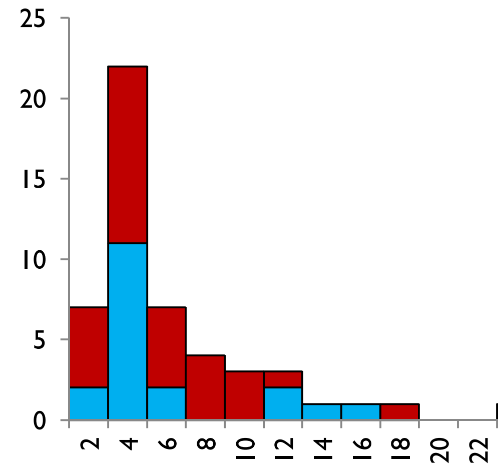
Odde

Comparison between device and flow cell

Conventional glass flow cell



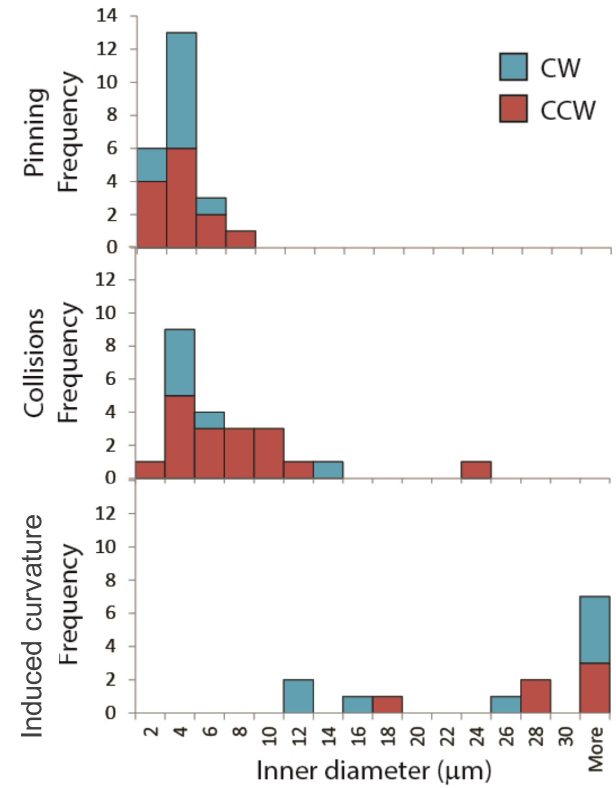
Microfluidic device



- On aggregate, the diameters and rotation direction were consistent in the device and flow cell
  - Device had fewer small (0-2 μm) diameter rings, as expected from decreased photodamage in device

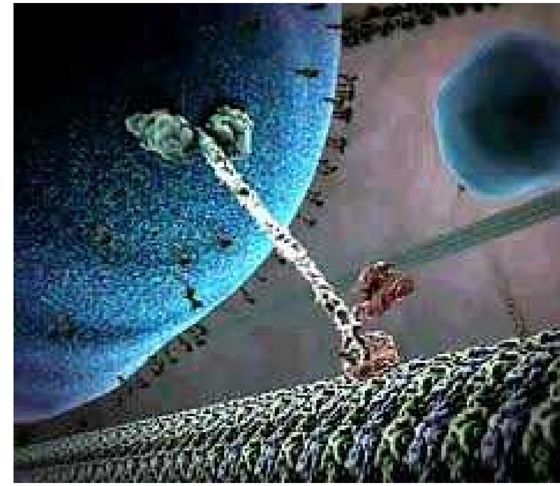
Effect of formation mechanism on spool properties

- Formation mechanism affects properties of spools
- Can tune spool by biasing which formation mechanism dominates
  - Oxidative damage to motors » more pinning and small rings
  - High surface density of MTs » more collisions and medium-sized rings



	Pinning	Collision	Induced Curvature
Inner Diameter (μm)	2.7	6.2	31.6
Std dev	1.5	4.8	19.4
%CCW	62	74	58

Acknowledgements



George Bachand

Stephanie Brenner

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Applications

Macroscale visual readout