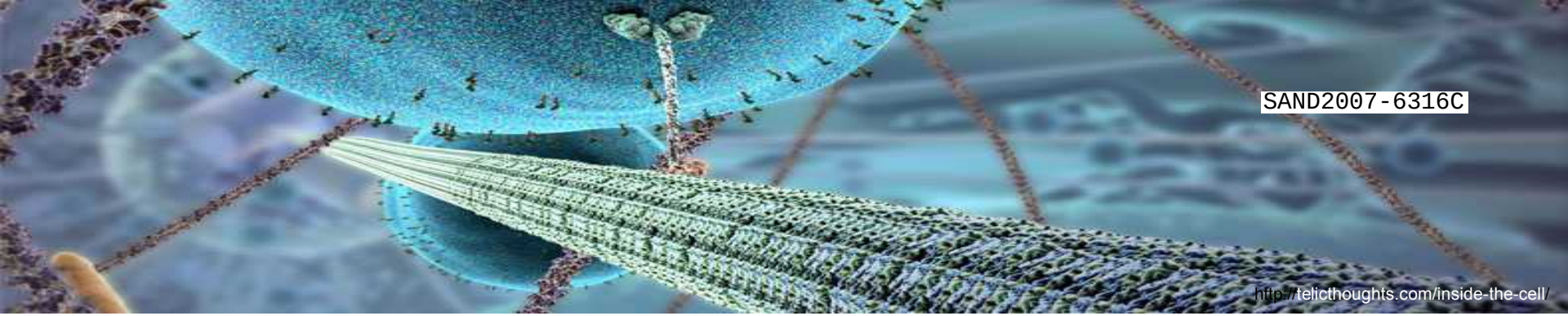

Genetic engineering of a chemically regulated "on/off" switch into the kinesin biomolecular motor for controlling *in vitro* motility

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Sandia National Laboratories
Biomolecular Interfaces and Systems (8331)

Office of Basic Energy Sciences
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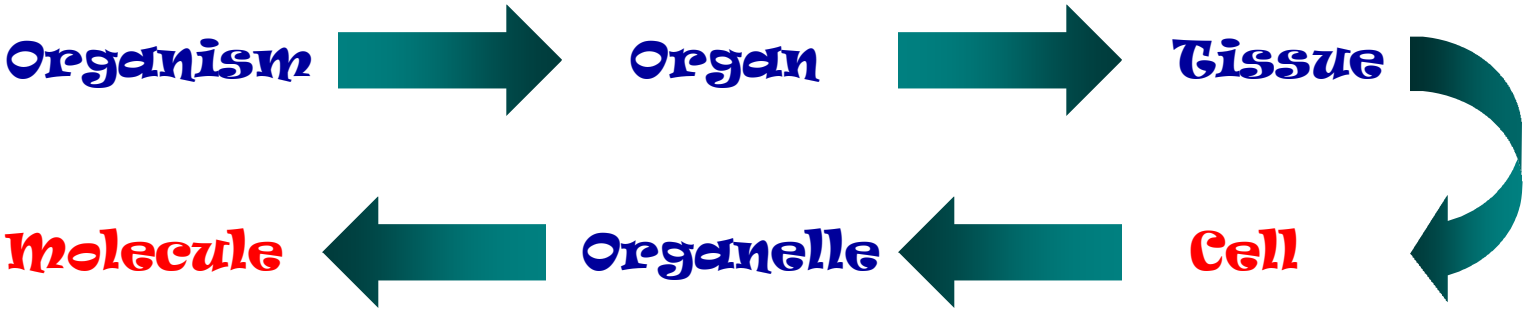
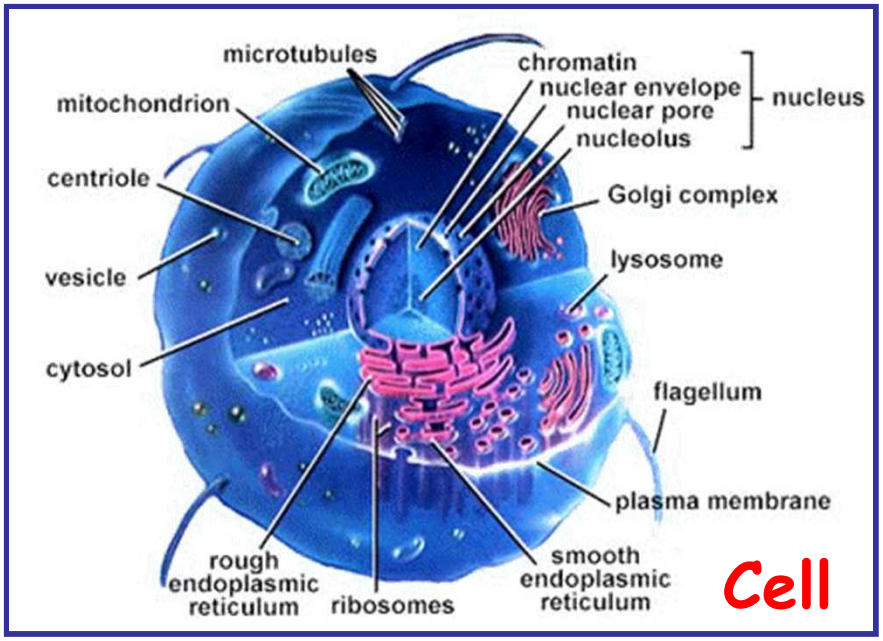
Heirarchy of Living Systems





http://www.modares.ac.ir/elearning/mnaderi/Genetic%20Engineering%20course%20II/Pages/Lecture1.htm

Organism: any living thing

Organ: Body part with ≥2 tissues

Tissue: a group of similar cells

Cell: fundamental unit of structure and function

Organelle: components that make up cells

Molecule: ≥2atoms

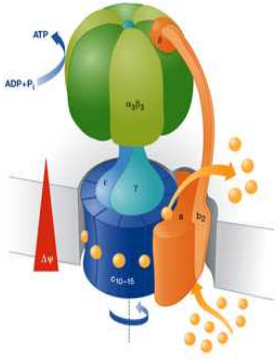
Motor Protein Molecules

What are motor proteins?

- Molecules that convert chemical energy (i.e. ATP) into mechanical work
- Rotary motors
  - Found in bacterial and eukaryotic cells
- Linear motors
  - Found in eukaryotic cells

ATPase

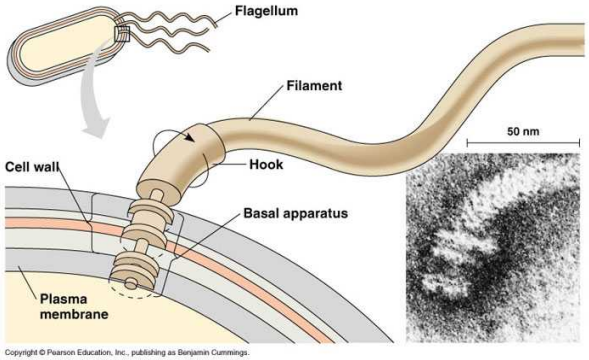
- Synthesizes ATP



http://www.mpibp-frankfurt.mpg.de/meier/

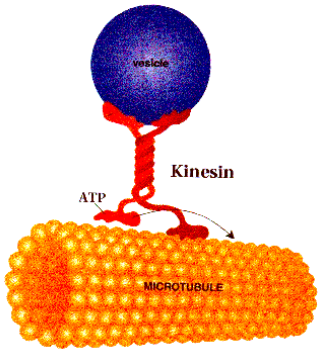
Bacterial Flagellum

- Propels bacteria



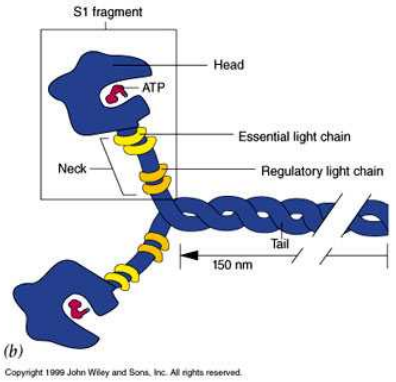
Kinesin

- Microtubule-based motility



Myosin

- Actin-based motility



Cellular Filaments

What are filaments?

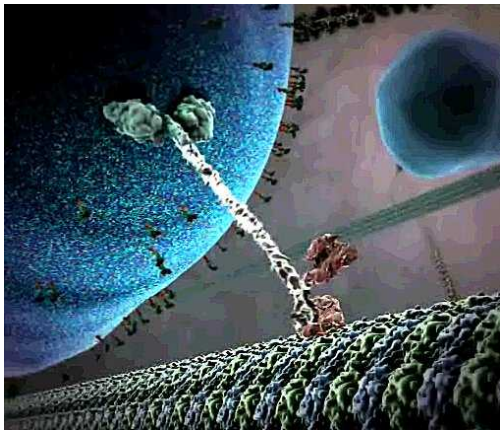
- Cytoskeletal components that provide structure, motor highways, and some protection to the cell
  - Actin
  - Intermediate
  - Microtubules

http://www.oceanexplorer.noaa.gov/explorations/02sab/background/biodiversity/biodiversity.html

Kinesin Motor Protein

What is kinesin?

- A biomolecular motor protein that utilizes cellular microtubule networks to "walk" on within in the cell
- Involved in active transport of macromolecules and organelles within cellular systems
  - Vesicle/signaling molecule transport
  - Mitosis
  - Melanophore transport (in fish)



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

Experimental Application: Inverted Motility Assays

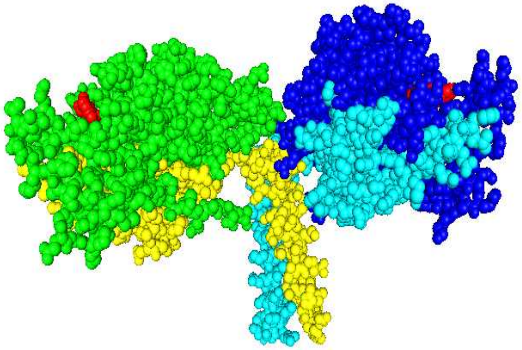
What is an inverted motility assay?

- *In vitro* assembly of kinesin and microtubule filaments

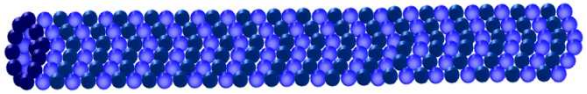
Why use inverted motility assays?

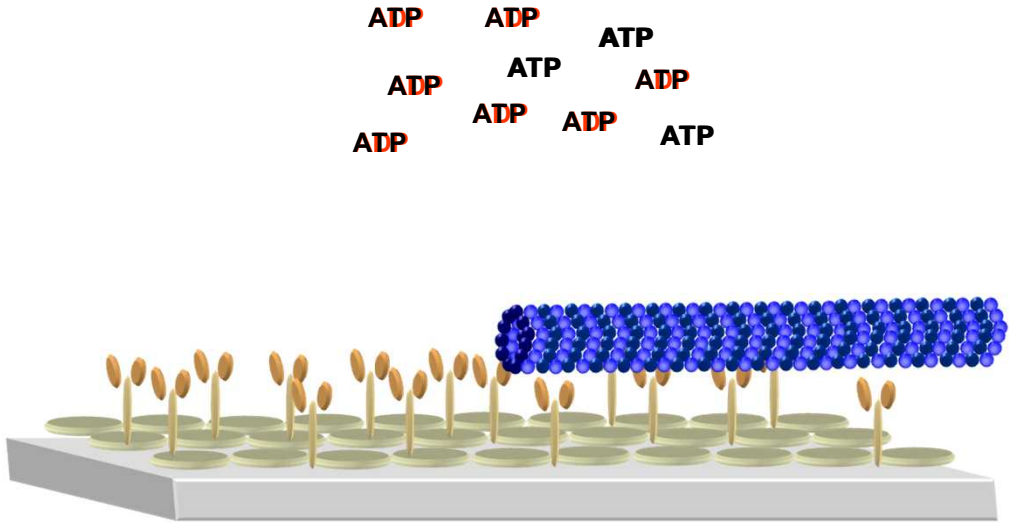
- To be able to mimic Nature's assembly of kinesin and MTs for integration into synthetic nanoscale materials, devices, and systems

Kinesin Motor Protein

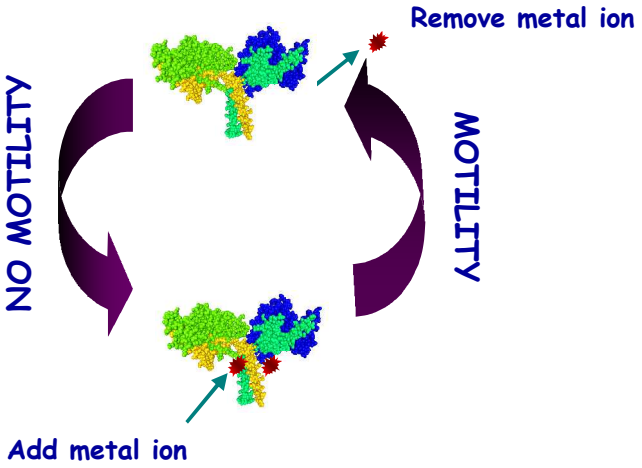


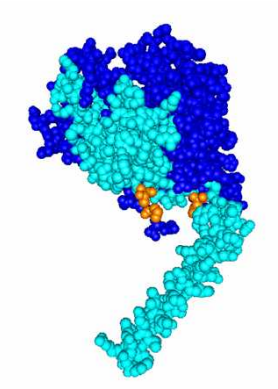
Microtubule Filaments (MTs)





Project Idea: Controlling Kinesin Movement





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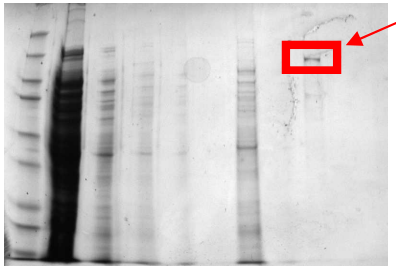


The Goal

- Achieve cyclic, on/off control of the biomolecular motor by using divalent metal ions.

The Technical Approach

- Use site-directed mutagenesis to create a metal binding site in the head-neck linker region of the *Drosophila melanogaster* kinesin

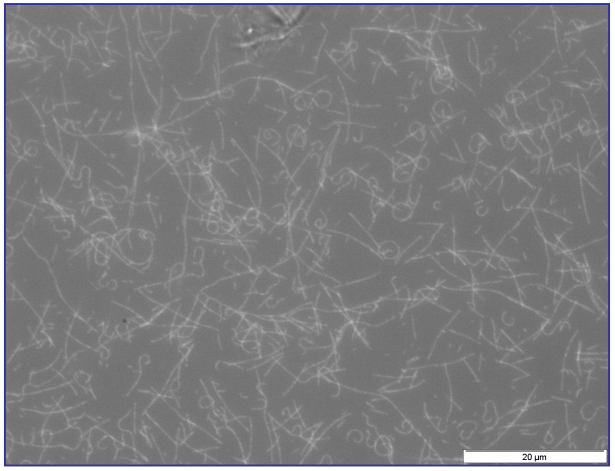
Note

- To increase the stability to metal ion exposure, MTs were crosslinked with 500μM glutaraldehyde

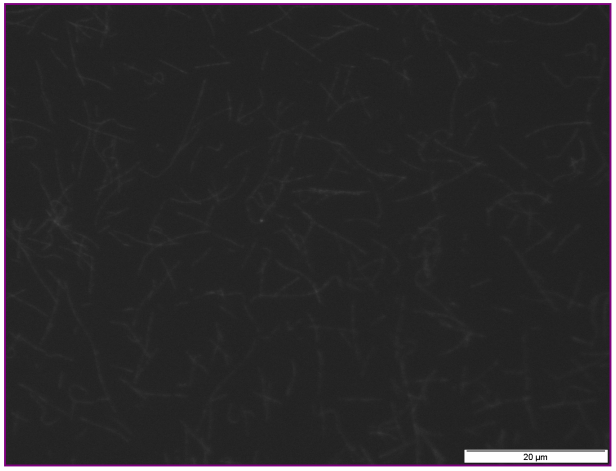
Stopping kinesin movement with Zn^{2+}

- 10μM Zn^{2+} effectively stops the mutant but not the wildtype

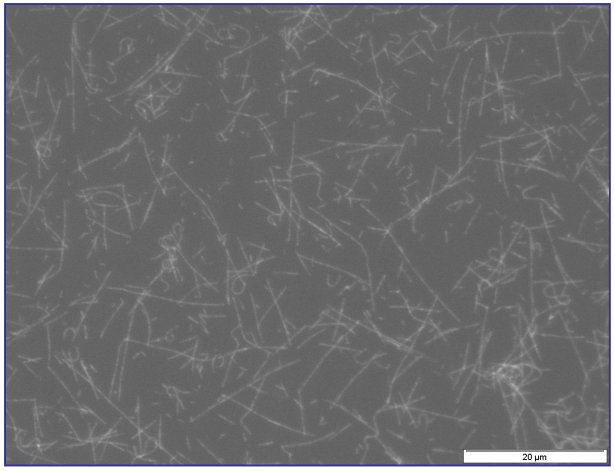
Initial Motility of Control



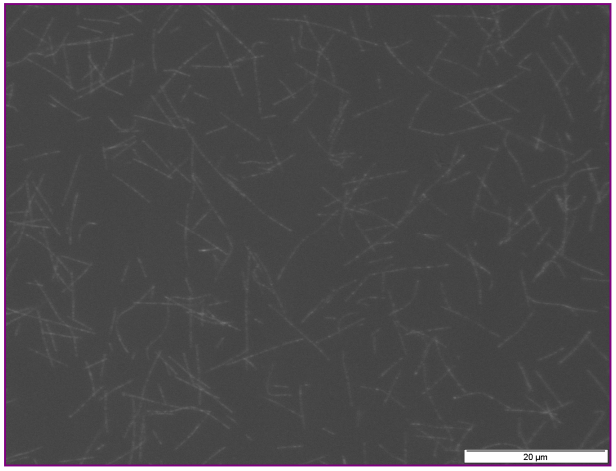
Initial Motility of Mutant



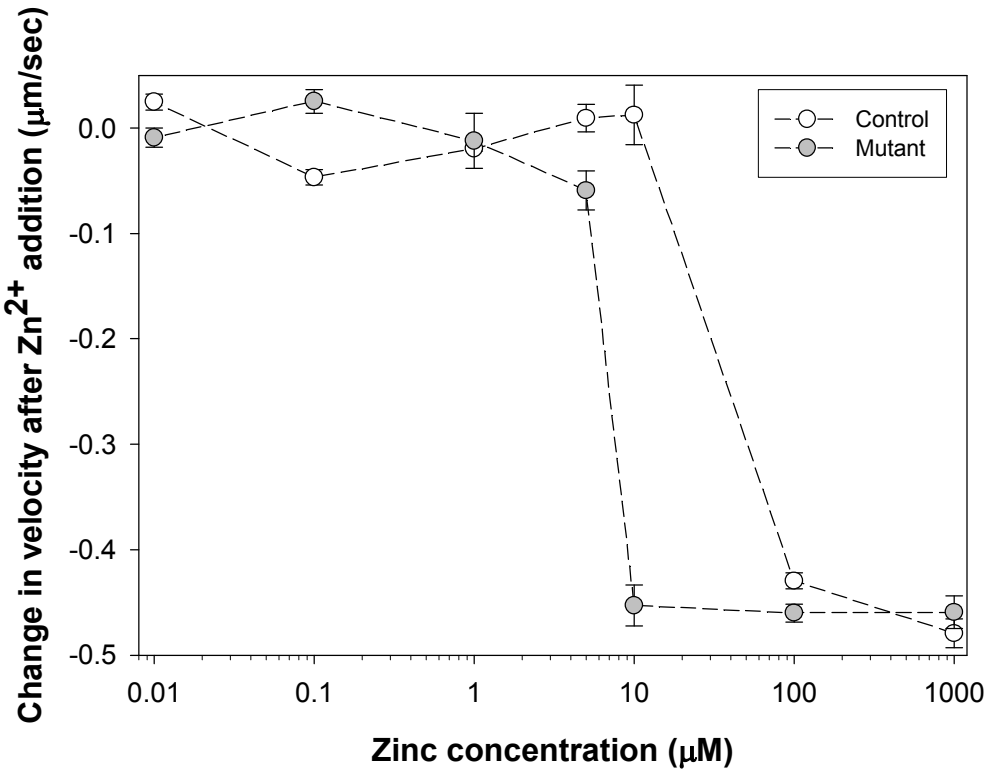
Motility of Control After 10μM Zn^{2+}



Motility of Mutant After 10μM Zn^{2+}



Stopping kinesin movement with Zn^{2+} and other metals



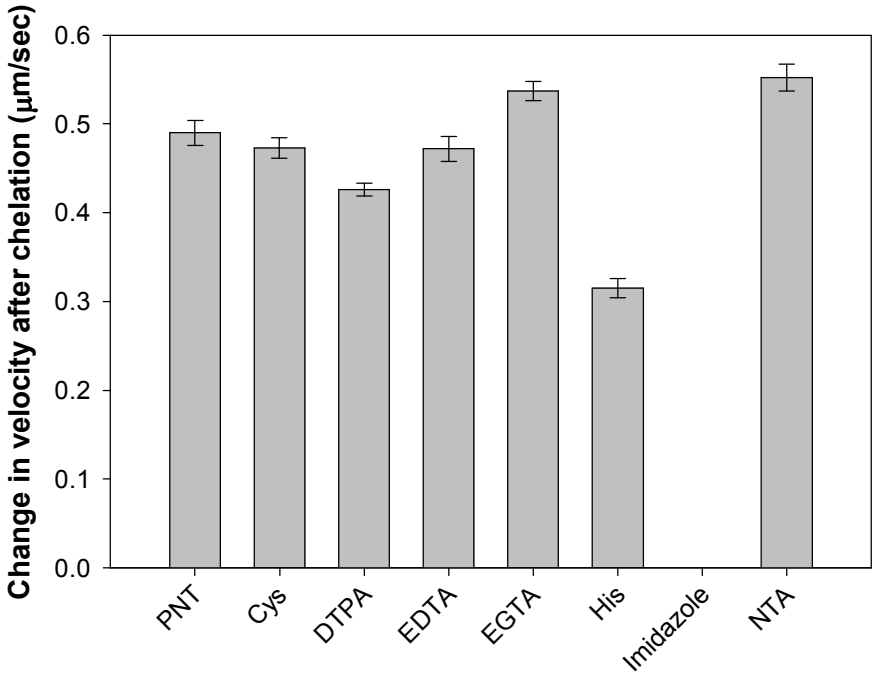
10µM Metal	Kinesin Stopped?
Buffer	No
Cobalt	No
Copper	Yes
Magnesium	No
Manganese	No
Nickel	No

- 100µM and 1000µM Zn2+ stop both the mutant and wildtype kinesin. This is reversible without chelation for the control.

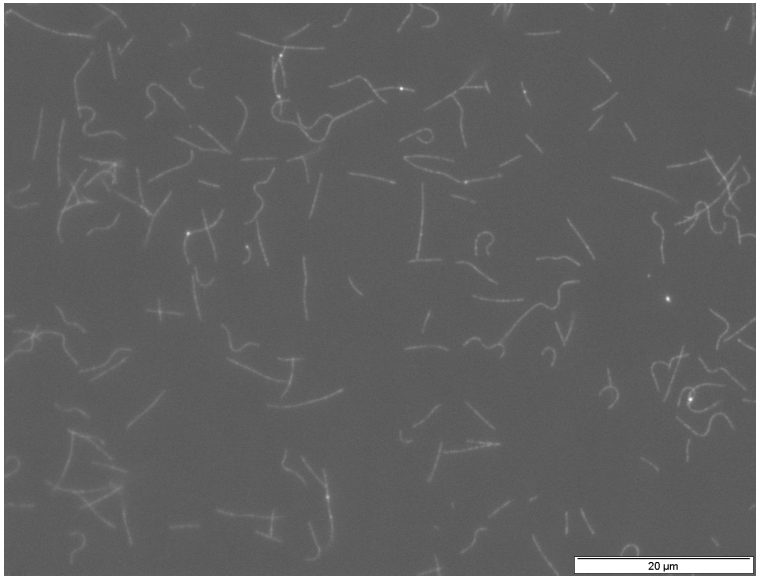
- Cu2+ was the only other metal to fully stop the mutant at 10um.
- Motility buffer (containing ATP energy) did not significantly affect the kinesin motility.

Restarting kinesin movement

- The majority of the chelators successfully restarted the kinesin motor at 1mM.
- Histidine and imidazole, however, did not fully restart the mutant motor.
- This could be due to the high pH range required to fully deprotonate the chelators.

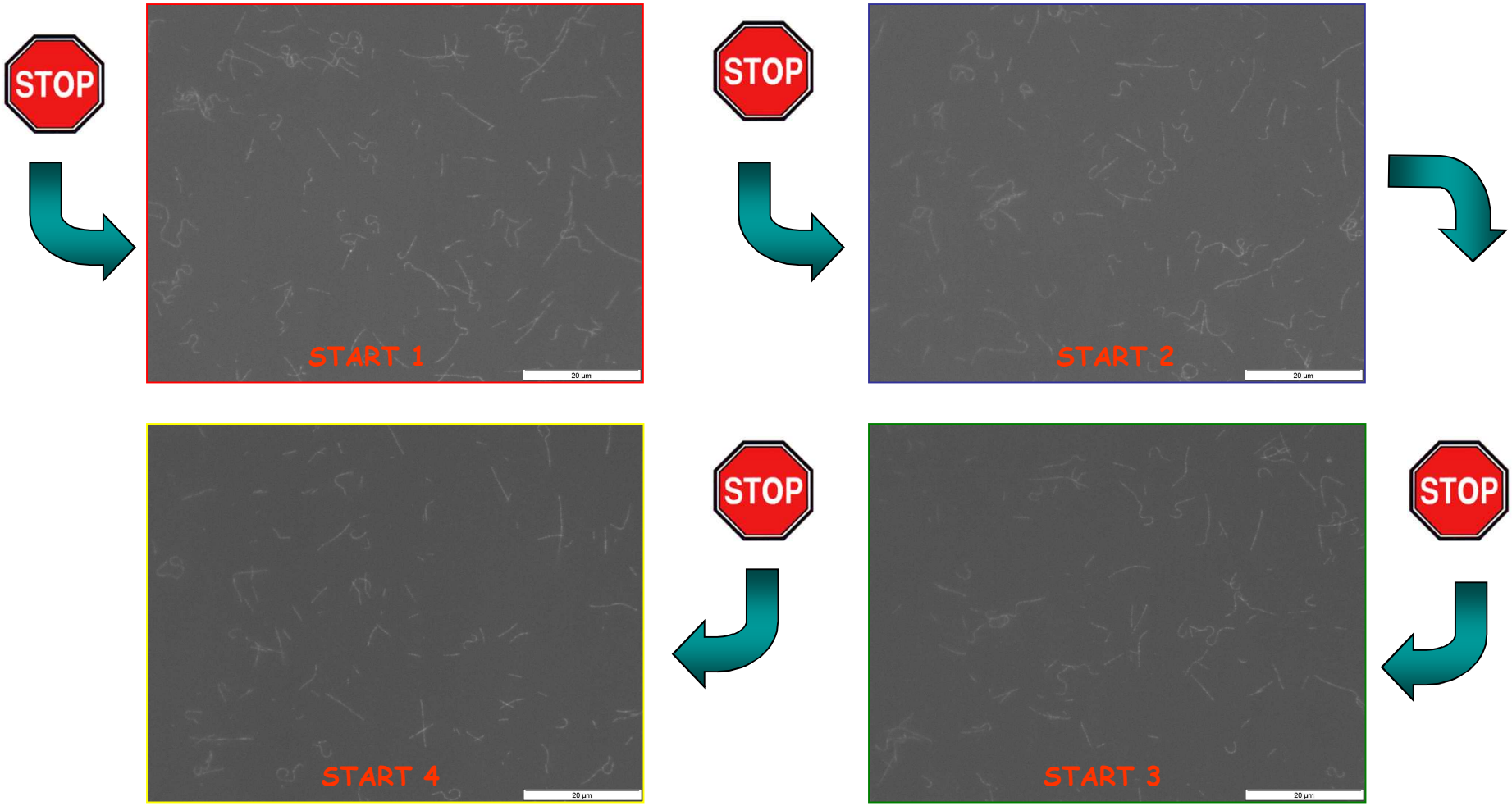


Restarting the mutant with 1mM NTA



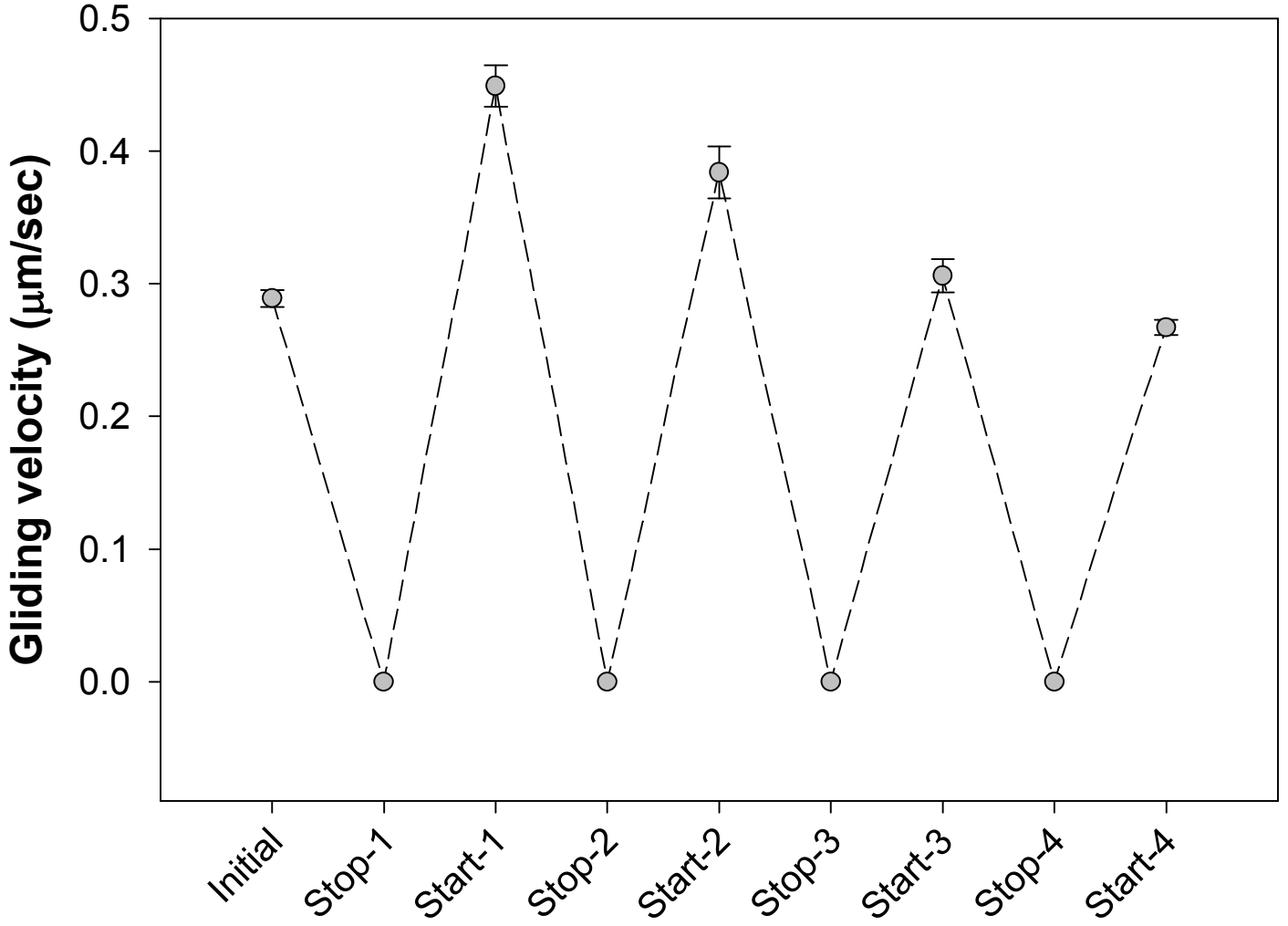
Cyclic nature of the switch kinesin

- The mutant kinesin was able to withstand four on/off cycles
- The wildtype kinesin, however, remained stable through only one cycle.



Kinesin mutant switch cycles

- The mutant exhibited successful stability to four consecutive cycles



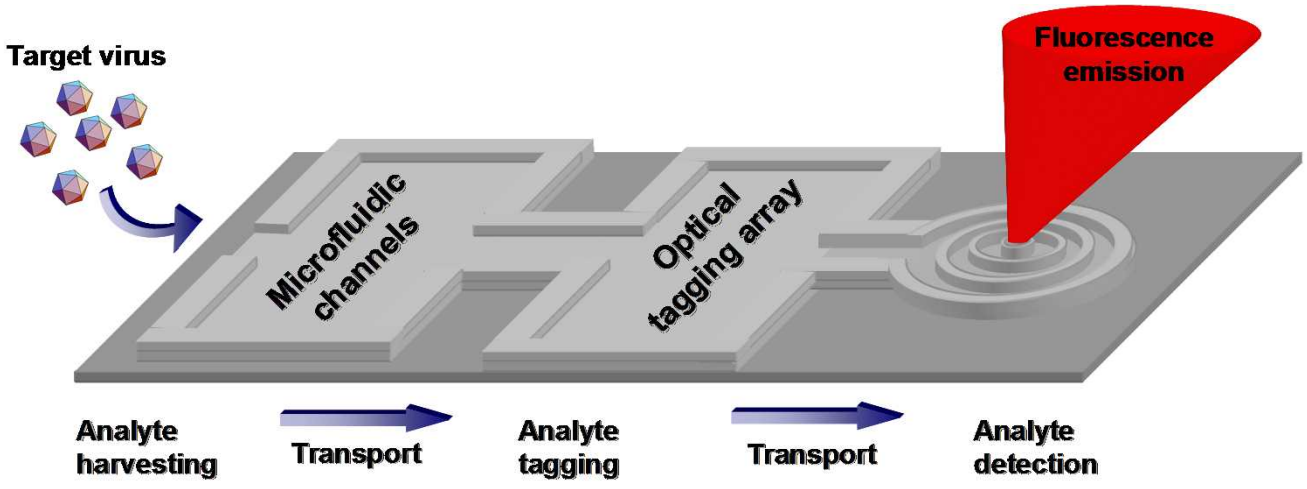
Summary and future work

Accomplishments:

- Successful control of biomolecular motor function
- Reversible inhibition of motor motility

Advantages:

- Chemical regulation of motor protein transport allows for the energy supply (i.e. ATP) to remain in the system
- Controlling motor function allows for integration of controllable active transport systems in nanoscale materials, devices, and systems



Summary and future work

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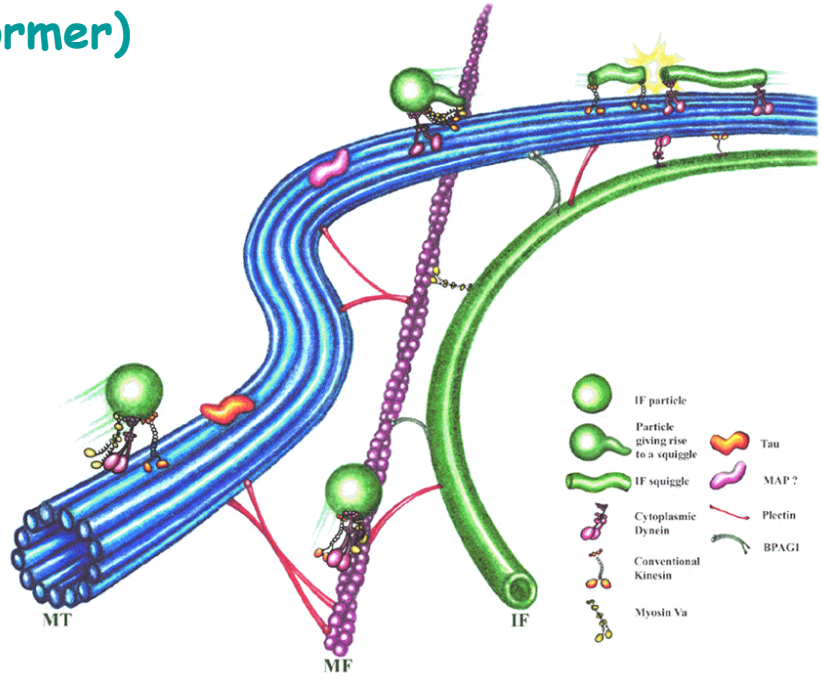

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