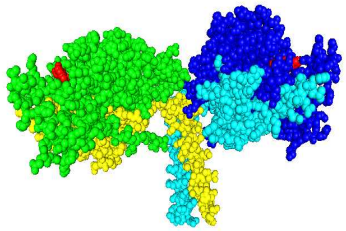


SAND2007-2139P

Kinesin-driven cargo pick up through the unzipping of DNA surface tethers

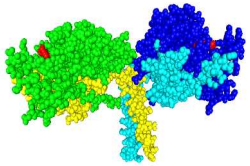
Symposium T: The Nature of Design—
Utilizing Biology's Portfolio
(11:00 am, T9.6)

George D. Bachand
Marlene Bachand
Steve Koch
Brandon Heimer

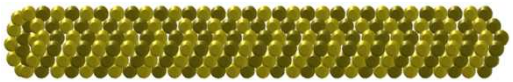
Biomolecular Interfaces & Systems
Sandia National Laboratories

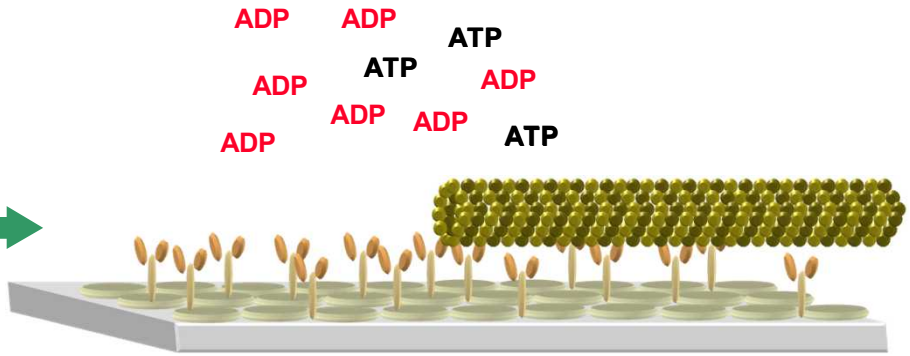
Biomolecular transport systems at nanoscale interfaces

Kinesin Motor Proteins



Microtubules (MTs)

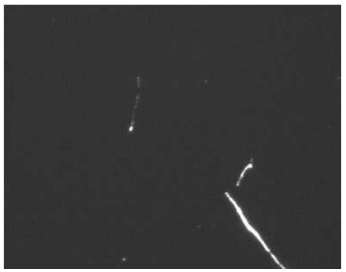




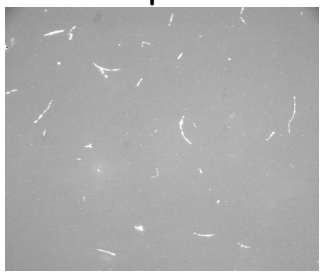
Transport rates 0.1 – 5.5 μm/sec

Synthetic cargo (materials assembly)

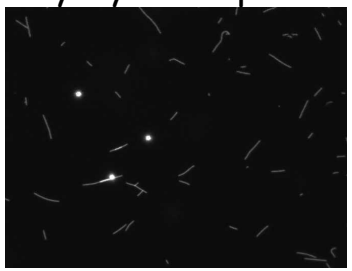
Quantum dots



Au nanoparticles



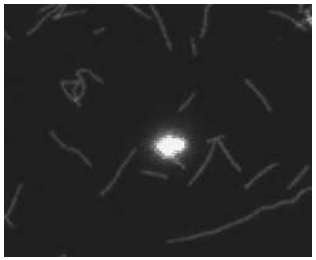
Polystyrene spheres



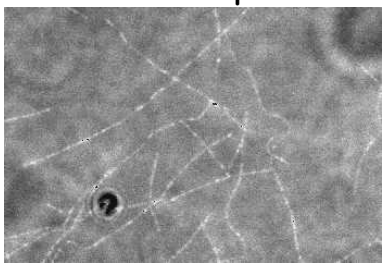
Limitation:
Solution-based adsorption

Biological cargo (sensor applications)

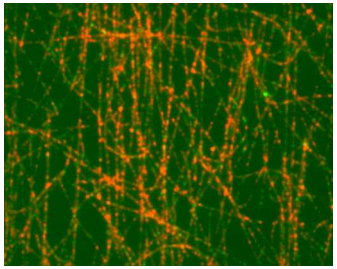
Viruses



Bacterial spores



Proteins



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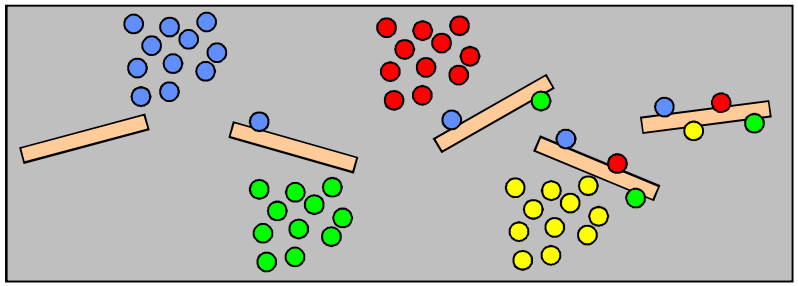
Defining cargo pick up on molecular assembly lines

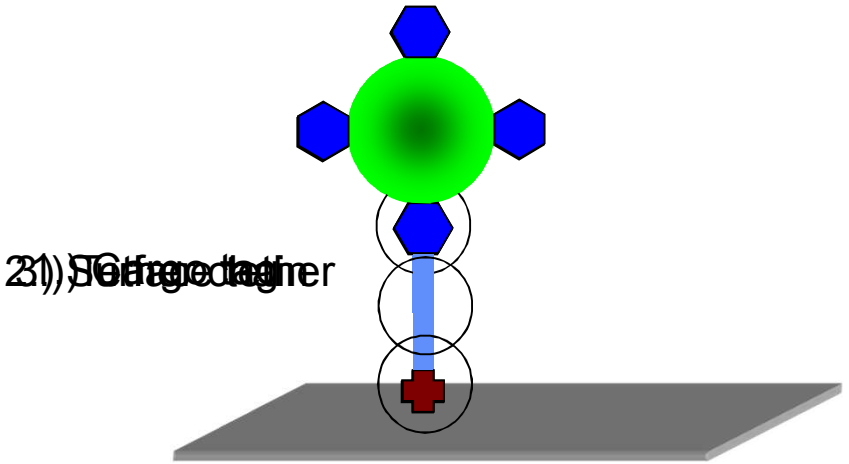
Project goals:

1. To develop a means of tethering nanoparticle cargo at synthetic interfaces
2. To demonstrate cargo pick-up using kinesin-driven microtubule shuttles



Approach:

- Attach nanoparticle cargo to surfaces using bifunctional tethers
- Adhesion of cargo to functionalized microtubules following a collision
- Force generated by kinesin pushing the microtubule (and attached cargo) enables rupture of tether bonds



Designing a cargo pick-up system

Efficient cargo pick up necessitates careful selection and implementation of chemistries that balance the molecular forces.

Motor forces:

Kinesin (one motor) = 6 pN/8 nm step, 62 step/s, ~375 pN/s pulling rate

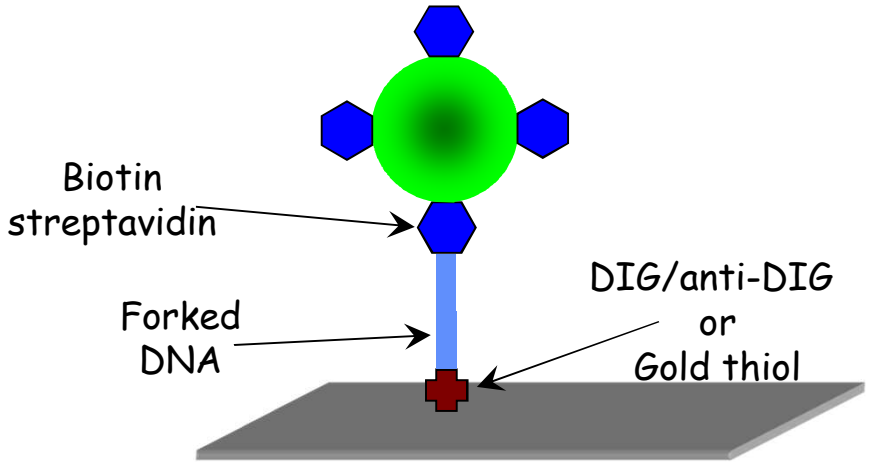
Multiple kinesin:

Max density = 1 motor/10 nm = 700 motors/7-μm MT

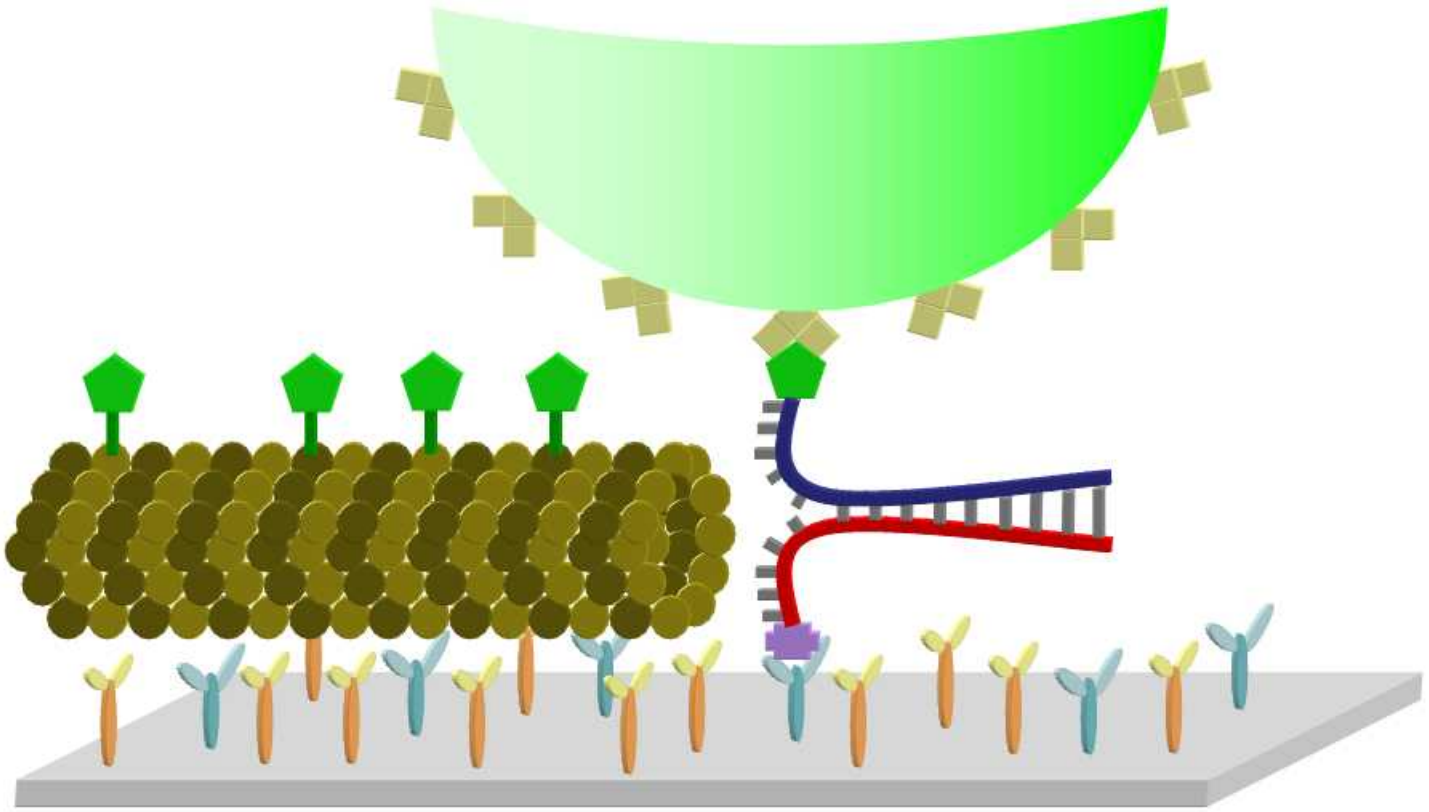
10% occupancy = 70 motors/7-μm MT, 420 pN per step, 26 nN/s pulling rate

Potential tether forces:

Streptavidin-biotin	~75 pN
Antibody-antigen	50-100 pN
Ni-NTA	38±4 pN
Gold-thiol	?
DNA (parallel)	
Short DNA (e.g., 20 bp)	~45 pN
Long DNA (e.g., >1000 bp)	~65 pN
DNA (forked)	
A-T pair	9±3 pN
G-C pair	20±3 pN



Picking up tethered optical tags – Unzipping DNA

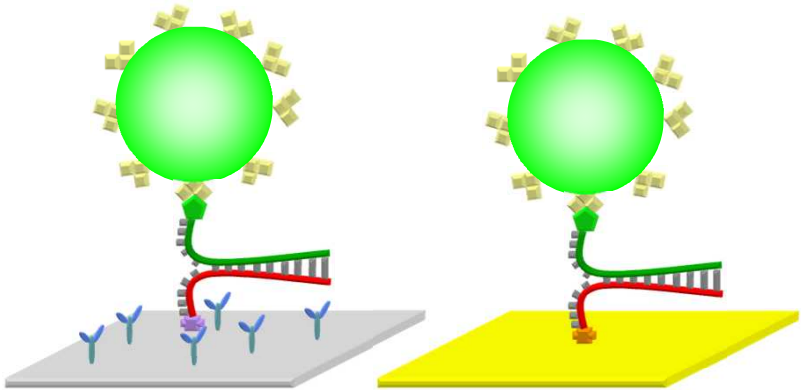


Unzipping forces = 10 pN for polyA-T bond & 20 pN for polyG-C bond

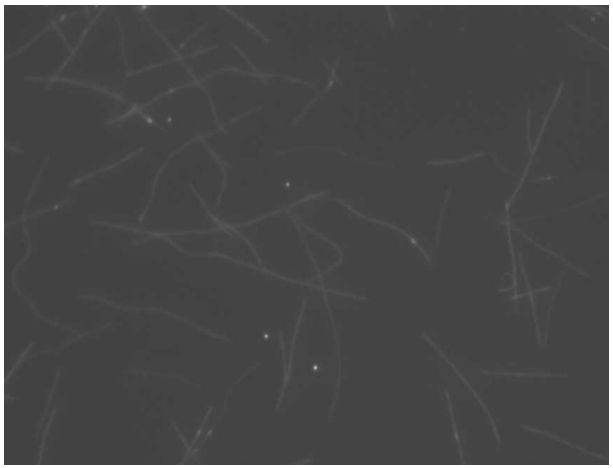
Each bond is independently broken, thus DNA length may be adjusted w/o changing unzipping force.

Tethering particles to surfaces with DNA

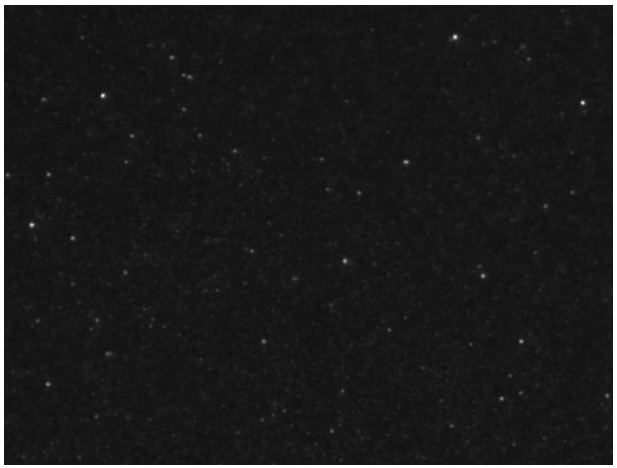
- DNA tethers:
  - 70-bp (50-bp overlap) DNA tether with 5′-biotin and 3′-DIG labels
  - 100-bp (50-bp overlap) DNA tether with 5′-biotin and 3′-thiol labels



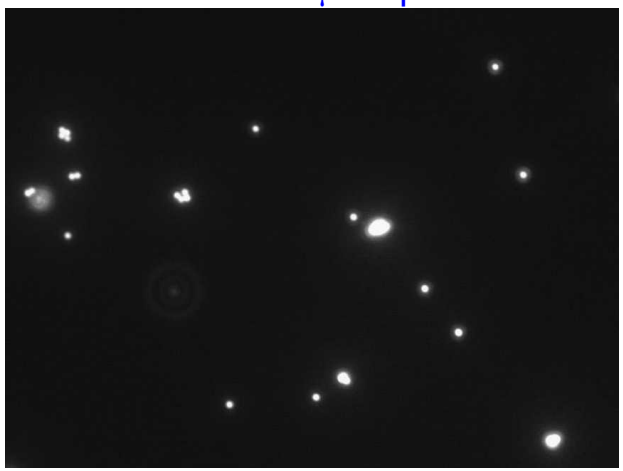
DIG/DIG Ab – Qdots



Au/thiol – Qdots



Au/thiol – 0.5 μm spheres

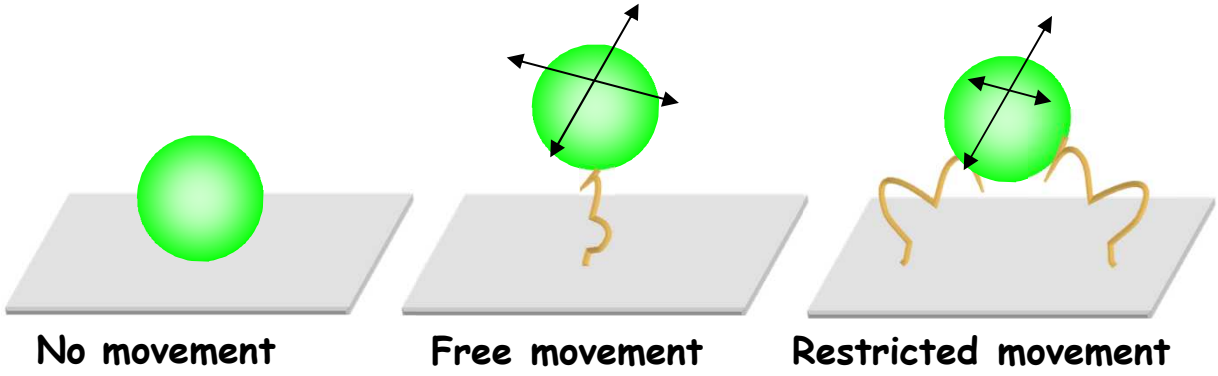


Reducing agent present

- Non-specific adsorption on gold surface mitigated by reducing agents
- Tethering reduced following addition of kinesin/MTs

Thermal motion of tethered particles

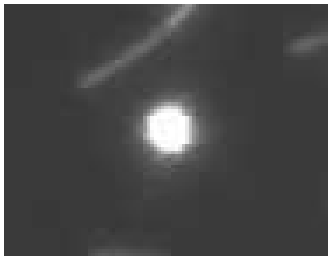
Tethering by single DNA molecules may be confirmed by tracking the motion of microsphere and nanoparticles.



Au/thiol

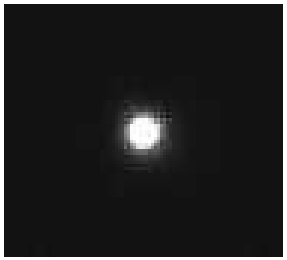
Qdots

DIG/DIG Ab

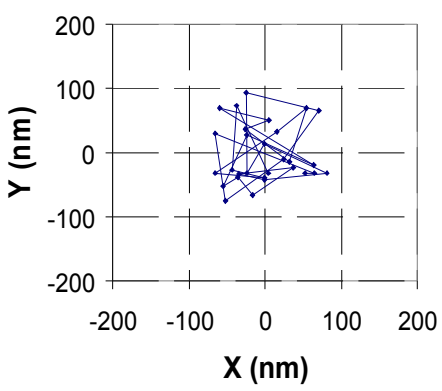


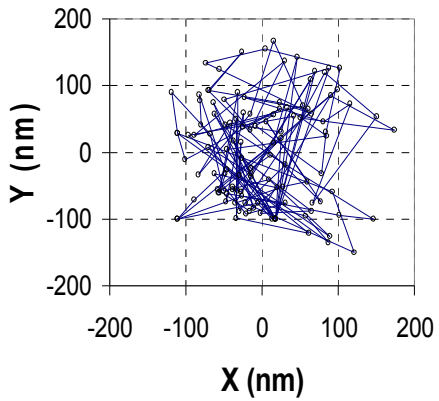
Spheres

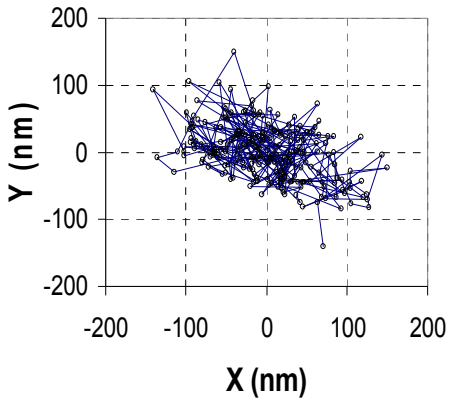
DIG/DIG Ab



Spheres







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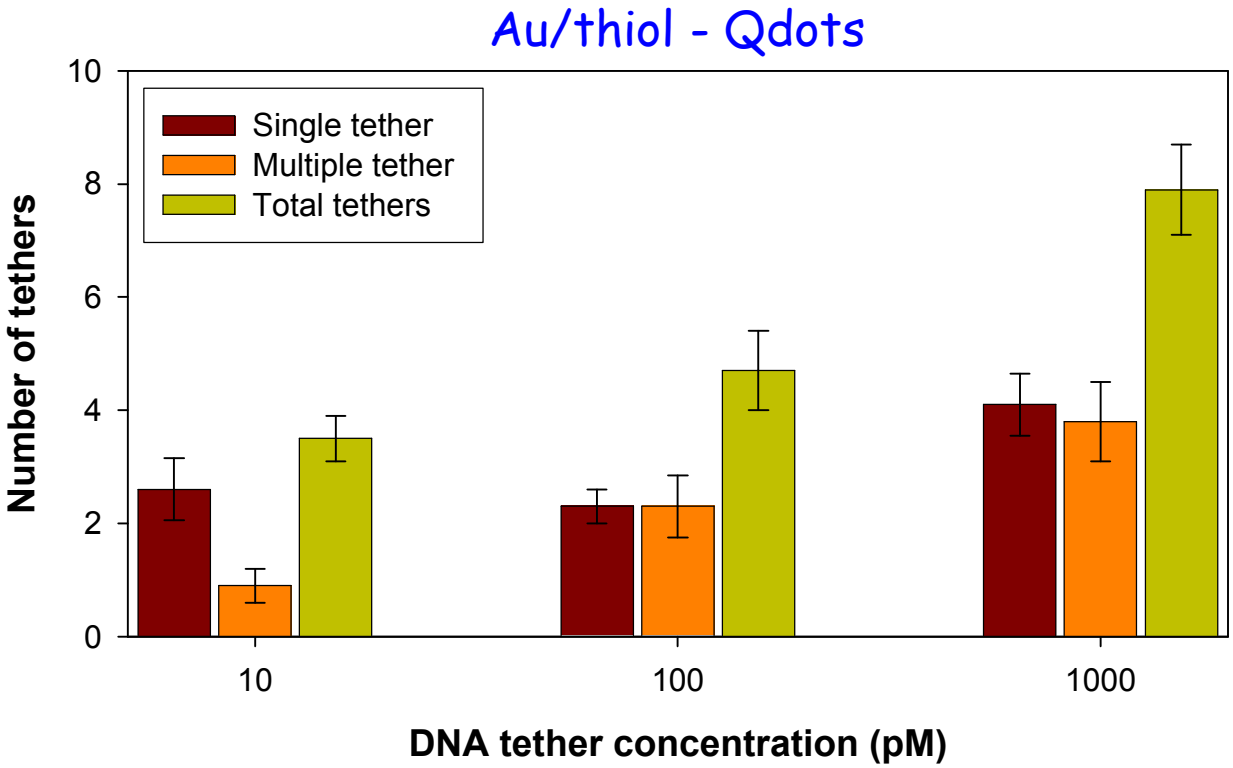
Optimizing tether density

- Non-specific binding is not affected greatly by the DNA tethering density
- Number of cargo molecules increases as a function of increasing DNA tether concentration
- The number of multiple tethers also increases as a function of increasing tether concentration

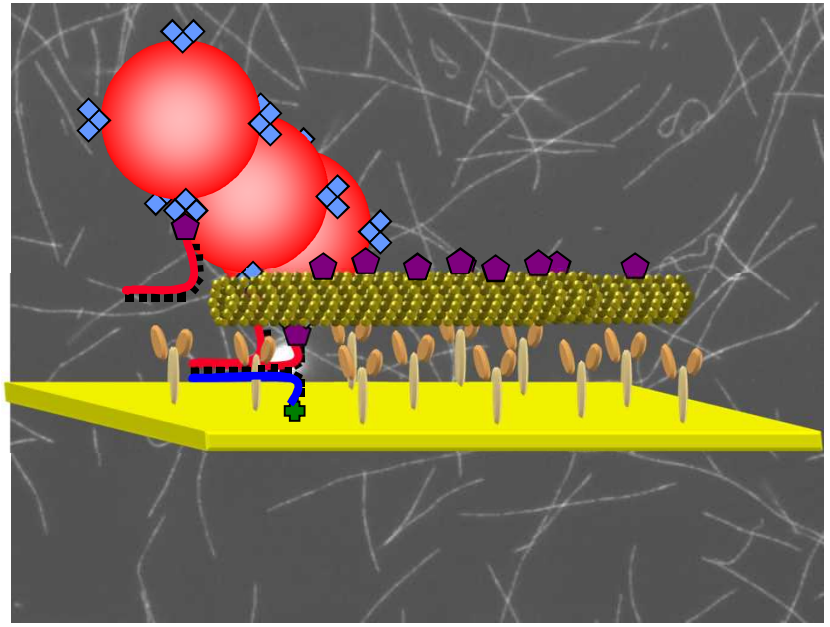


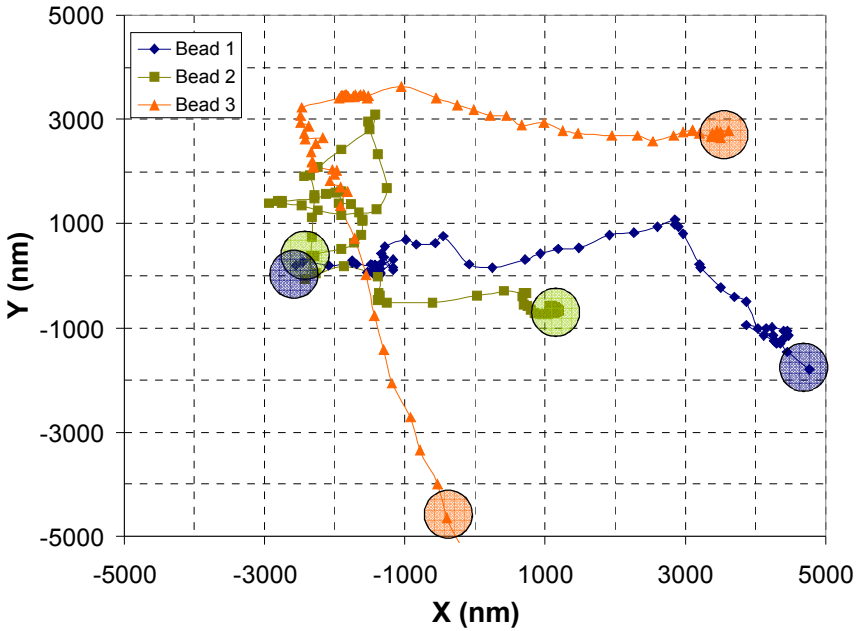
Unzipping and pick-up of tethered microspheres

- No pick-ups for non-specifically bound beads
- ~80% of beads are lost after the addition of kinesin and microtubules (change in ionic strength of buffers)
- ~50% of the tethered spheres were picked up during observation period
- A percentage of beads only travel short distances on microtubules before either:

  1) being transferred to another microtubule, or

  2) dislodged from (by) the microtubule, and bound to the surface again

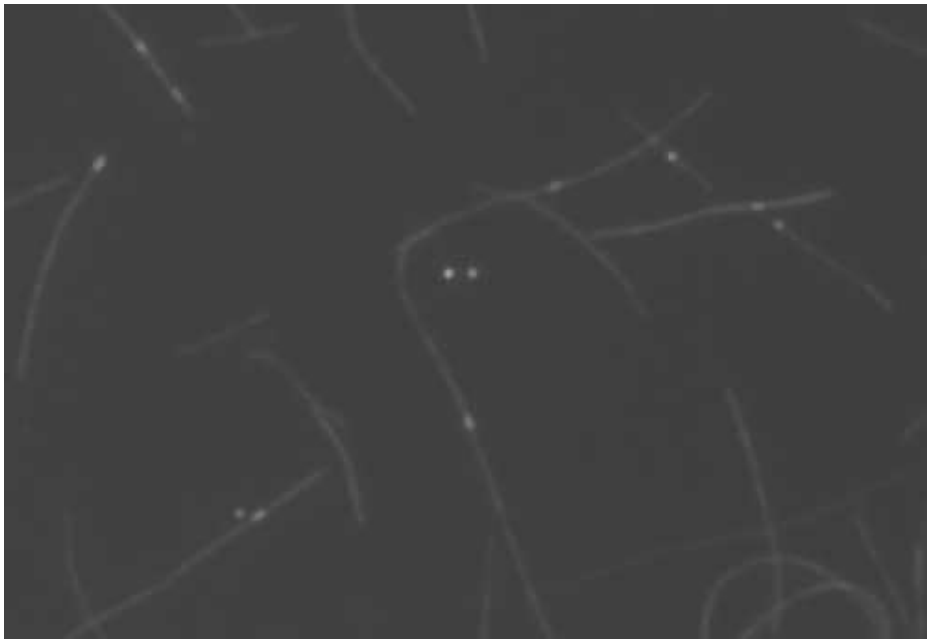


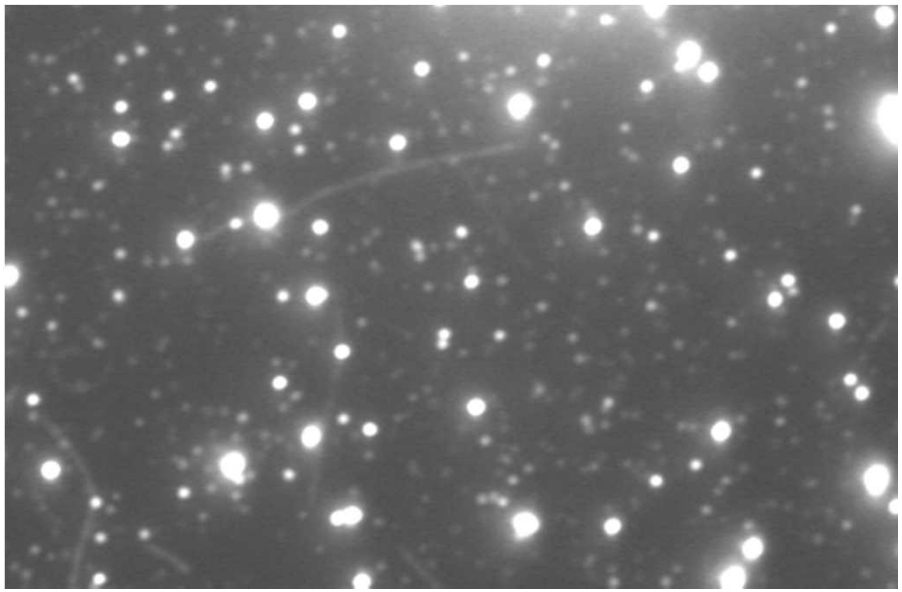


Unzipping and pick-up of tethered quantum dots

Pick-up of surface-tethered QDs was successfully achieved through unzipping DNA.

- Longer transport distances were observed (>100 μm without loss)
- Microtubule-to-microtubule cargo transfer was not observed
- Majority of cargo pick-up was observed within 10-15 min of microtubule addition
- Re-attachment to the surface was not observed
- Individual microtubules able to pick-up and transport several cargo





Summary & Future Work

- Bifunctional DNA molecules can be used to tether nanoparticles to synthetic surfaces
- Cargo attachment can be achieved through pick-up of tethered particles, as compared to solution-based adsorption

Remaining Questions:

- What is the overall pick-up efficiency (collisions vs. pick-up)?
- How does transport velocity affect the pick-up efficiency?
- Does size particle/shape affect pick-up?
- What geometries are optimal for achieving efficient loading stations?

Acknowledgments

Marlene Bachand, Steve Koch, Brandon Heimer