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Engineering functional selectivity into kinesin/microtubule-based transport systems

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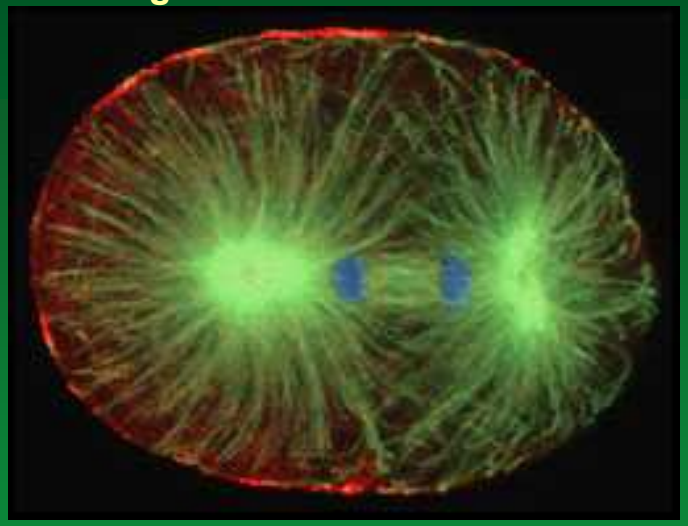
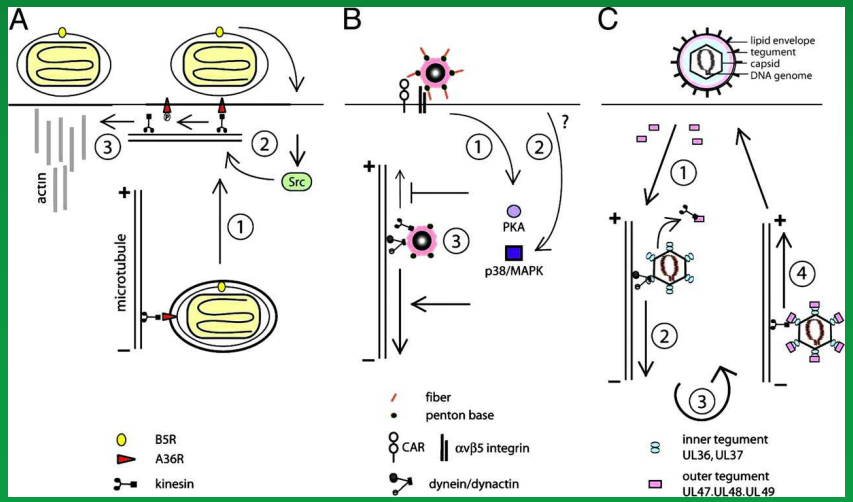
Intracellular kinesin-based transport

- Important for cellular activities: chromo-some segregation, vesicular transport, and signaling events
- Pathways hijacked by intracellular pathogens (viruses, bacteria, etc.)

Dr. Bruce Bowerman
Morel.uoregon.edu/home97/bowerman.html





Dr. Greber-- PNAS 102(16): 5639-5640

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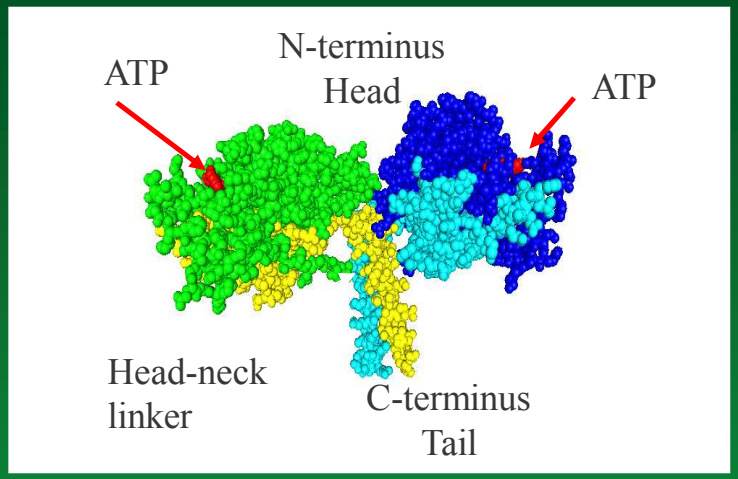
Kinesin & Microtubules – Nanofluidic Transport

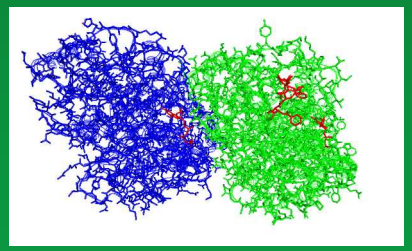
Kinesin Motor Proteins

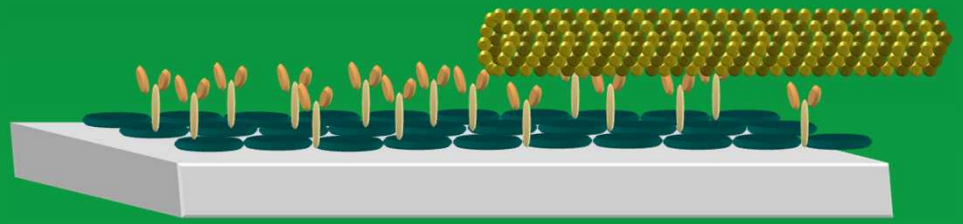


Drosophila Kinesin (50% rhodamine MTs)

Microtubules (MTs)

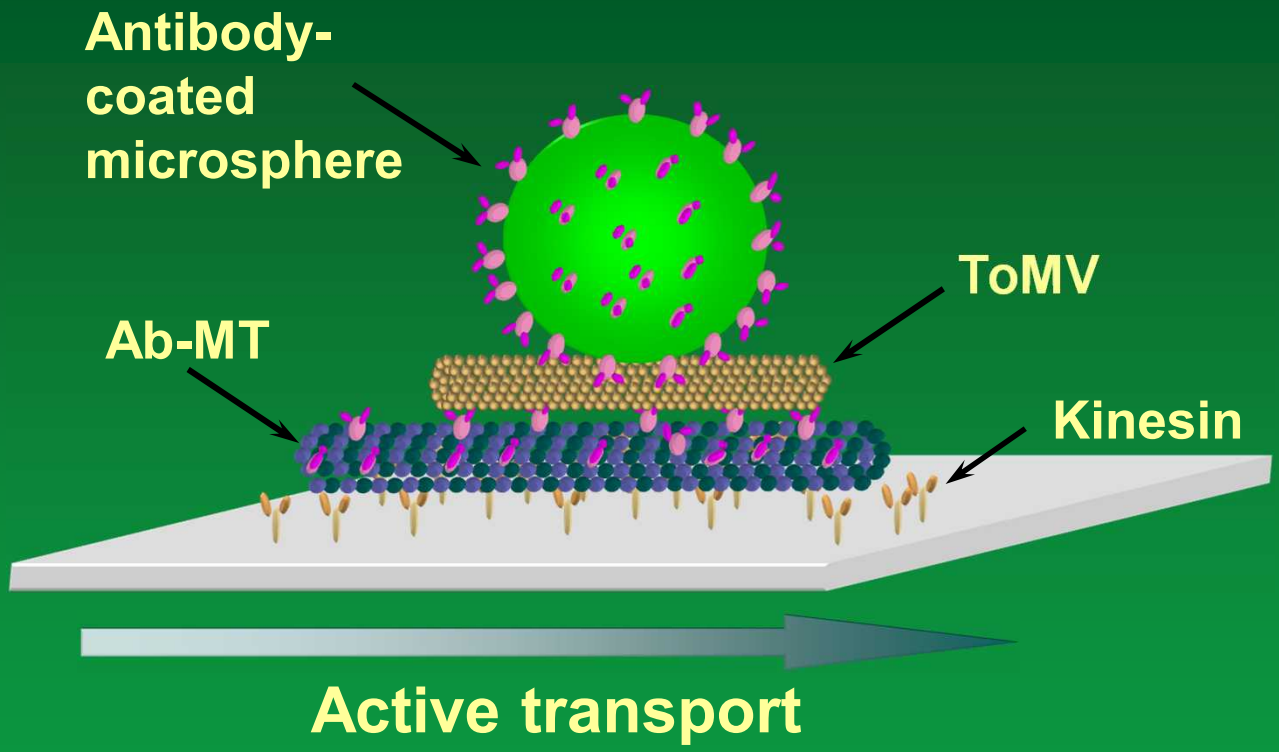
αβ tubulin dimer





Gliding motility geometry

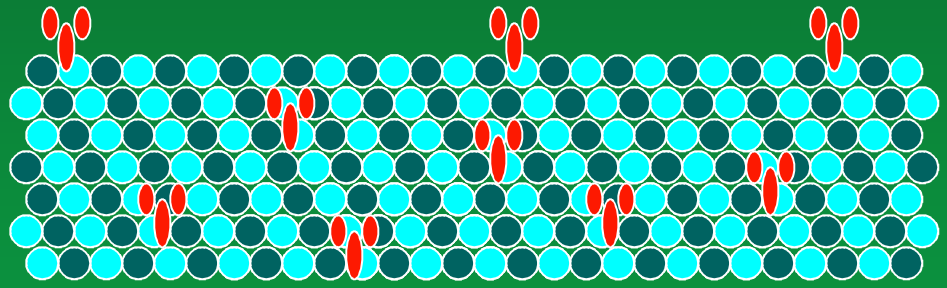
ToMV Transport Assay



***In vitro* assay for antigen (ToMV) capture, transport, and detection**

Project Goal:

Develop a crosslinking strategy for maximal antigen capture and transport by antibody-microtubule complexes along kinesin tracks



Key points:

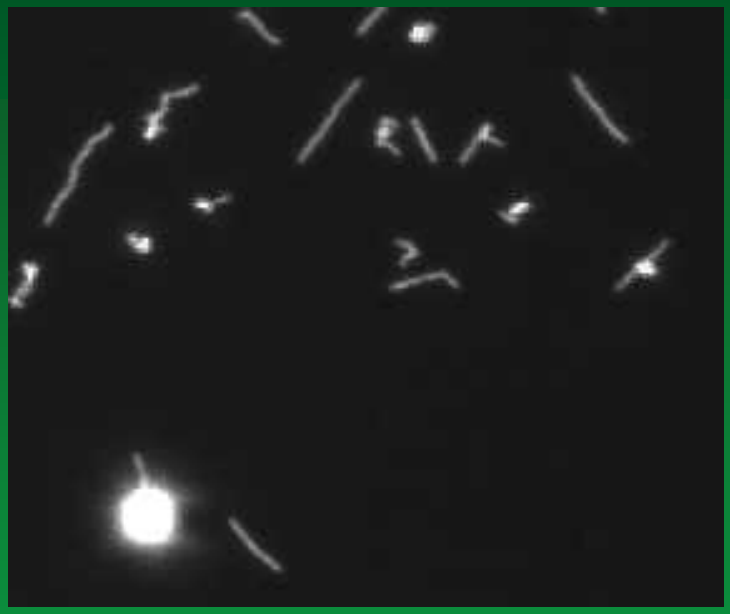
-Antigen binding site consistently available

- Area for kinesin to bind microtubules for motility

-Area for taxol to bind to stabilize microtubules

Homobifunctional Crosslinkers

- Glutaraldehyde (amines)[a]
  - Advantages:
    - Simple protocol
    - Microtubules last days-weeks
  - Disadvantages:
    - Undesirable intermediates
    - Excessive amounts of glutaraldehyde (≥5mM) result in deformed microtubules
- EGS (amines)
  - Advantages & disadvantages similar to glutaraldehyde
    - Longer crosslinking arm



Glutaraldehyde Ab-MTs

- Detection within flow cell <1%
- Able to detect 1ng/mL ToMV by ELISA analysis

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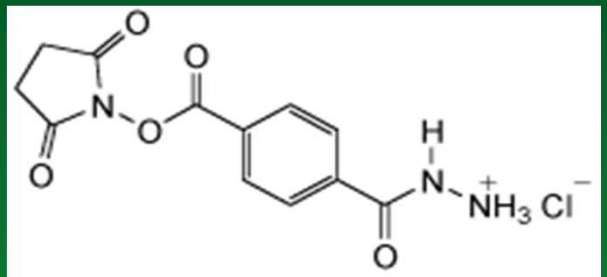
[a] see Bachand et al., 2006, Small (*in press*)

Heterobifunctional Crosslinkers

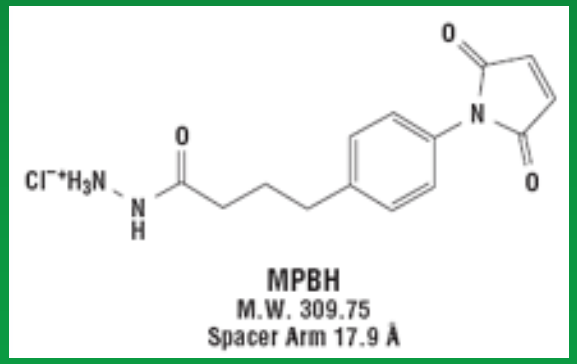
SHTH (amine/aldehyde)

- Advantages:
  - Proper orientation of Ab to MT (no intermediates)
  - Protocol performed within same tube
- Disadvantages:
  - Lengthy protocol
  - Over crosslinking = non-motile tubes



MPBH (sulfhydryl/carbohydrate)

- Advantages:
  - Alleviation of intermediates
- Disadvantages:
  - Complicated, lengthy protocol



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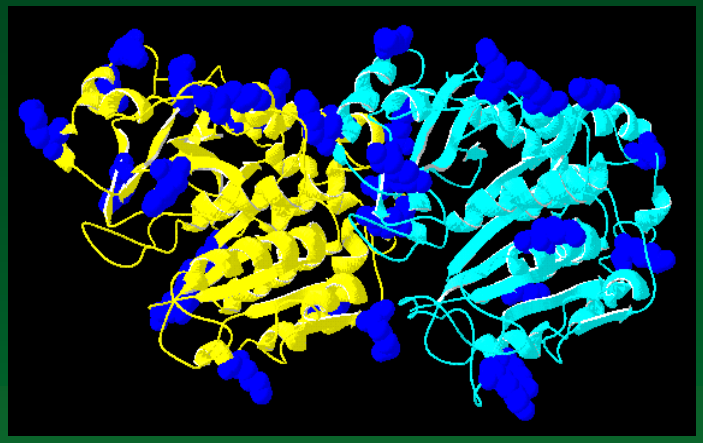
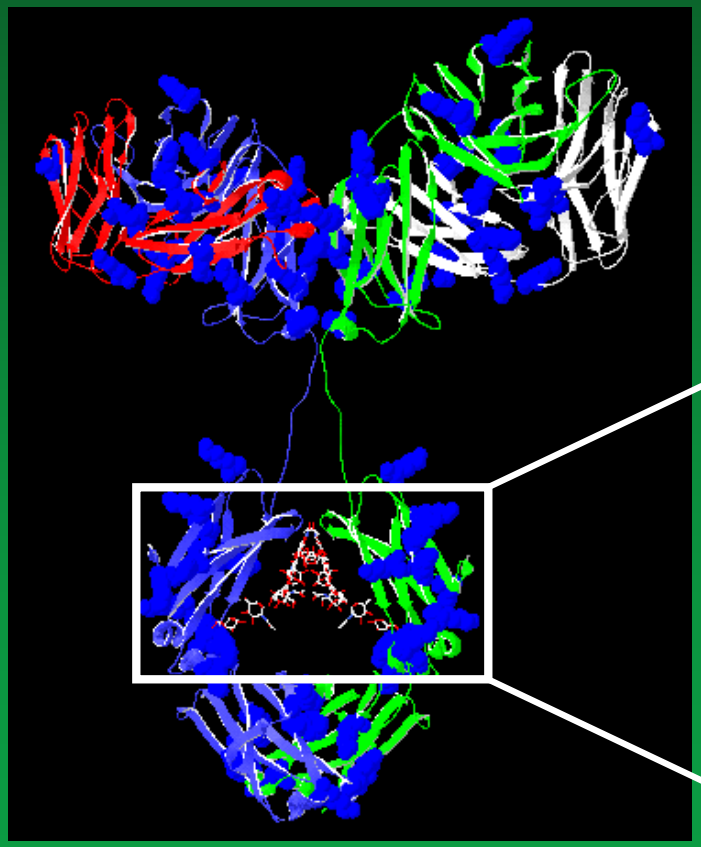
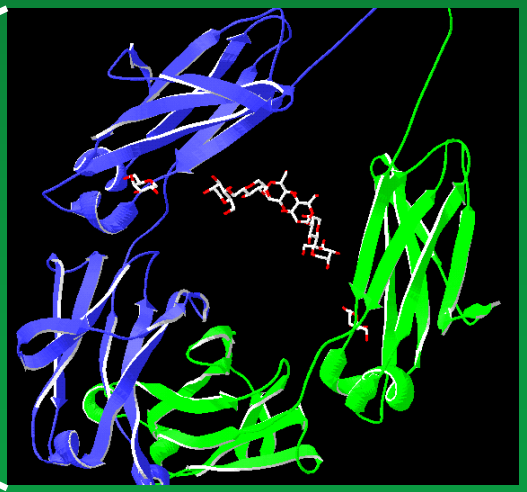
Pictures courtesy of Pierce– www.piercenet.com







SHTH crosslinks primary amines of microtubules to sodium *meta*-periodate modified carbohydrate of IgG

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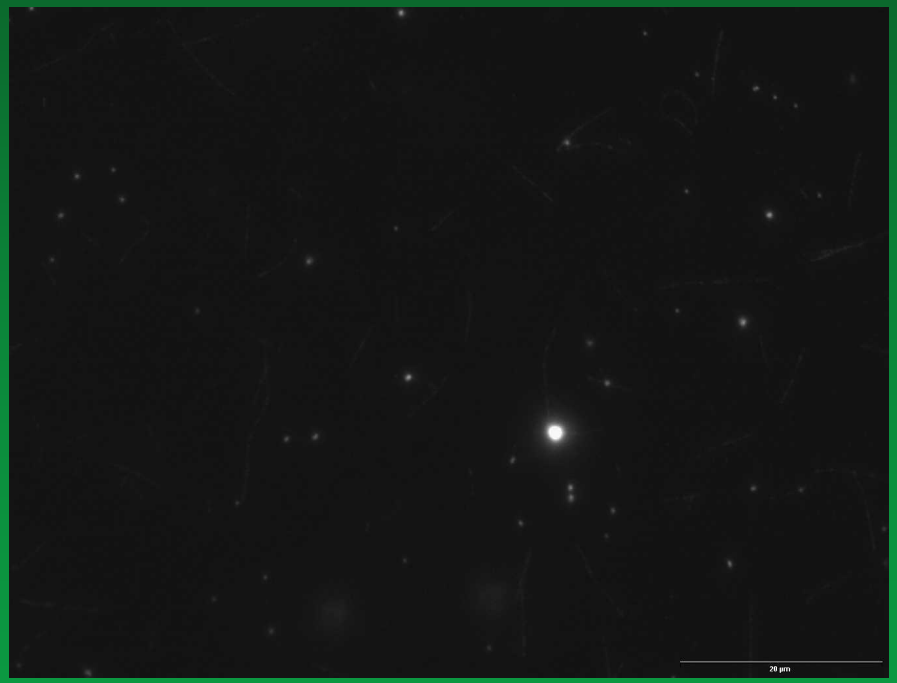
Based on RCSB models: 1jff.pdb and Igg1-All.pdb

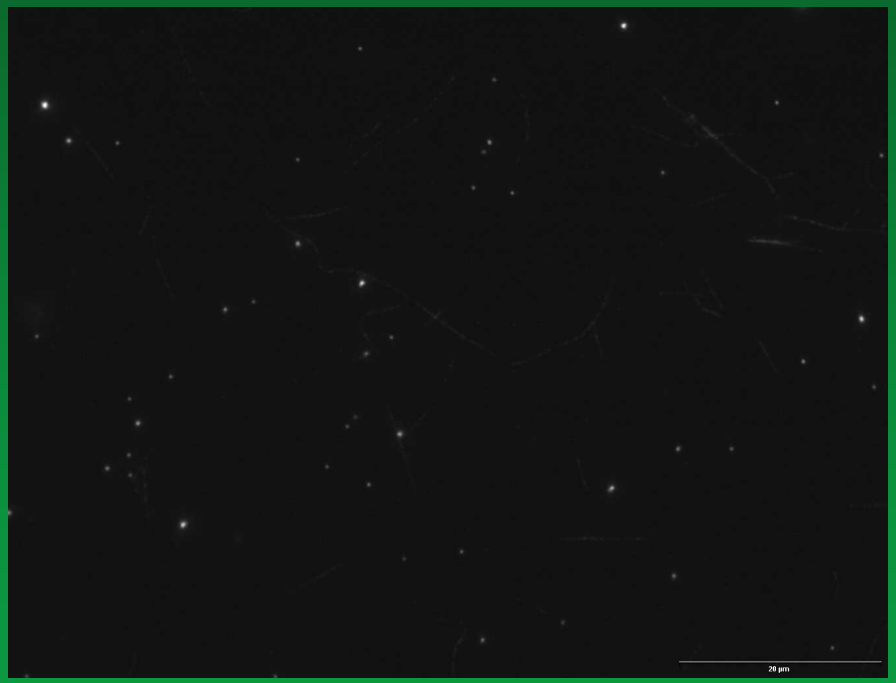
Molar excess of SHTH	ELISA	Motility (Inverted)	Bead/QD Capture and Transport*	Fold excess Ab
1M	--	Motile	None	10x α-ToMV
2M	--	Motile	None	10x α-ToMV
3M	--	Motile	None	10x α-ToMV
4M	--	Motile	Temporary	10x α-ToMV
5M	--	Motile	Yes (QDs/1μm & 0.2μm beads)	5x α-B.g. 10x α-ToMV
6M	10ng/mL in microbial soup	Motile	Yes (0.2 μm beads)	10x α-ToMV
8M	No signal	Non-Motile	None	10x α-ToMV
10M	--	Non-Motile; Occasion non-adherence	None	10x α-ToMV
SHTH alone (6M)	No higher than background	Motile	None	--

*Association of beads with microtubules occurs in some instances- transport required

- 10% rhodamine MTs crosslinked to α-ToMV Ab with 6M excess SHTH
- Analysis of ToMV transport with sandwich motility assay



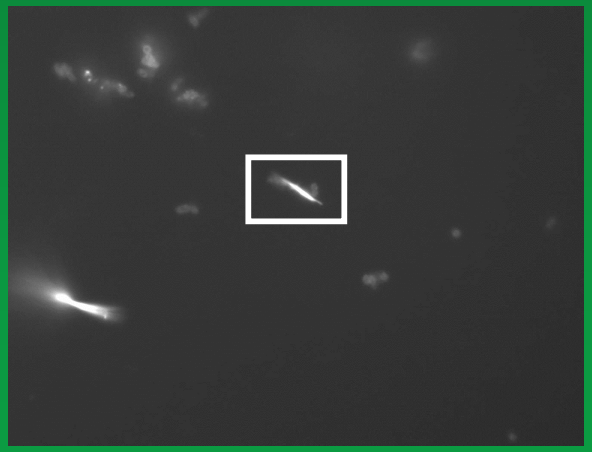
1µm beads coated with α–ToMV Ab

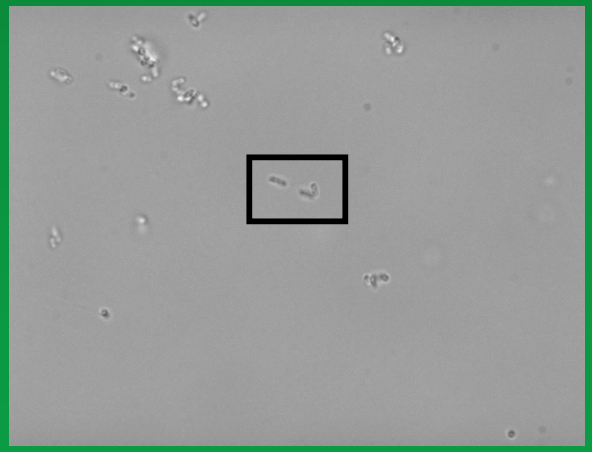


0.2µm beads coated with α–ToMV Ab

Antigen Selectivity

- *B. atrophaeus* spores
  - Ab-coated quantum dots
- *E. coli*





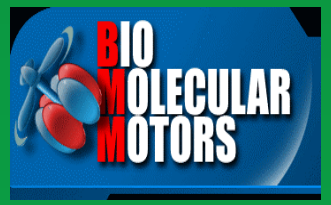
Advantages

- Capture of small quantities (nanogram amounts) of antigen from a large volume
- No need to label antigen of interest prior to assay
- Conversion of chemical energy into active transport enables receptors to “search” sample volume (in two dimensions)
- Assay can be completed within 30 min (ELISA = 6-48 hrs)

Acknowledgements

- George D. Bachand
  - Marlene Bachand
  - Amanda Trent

- Funded by DARPA



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