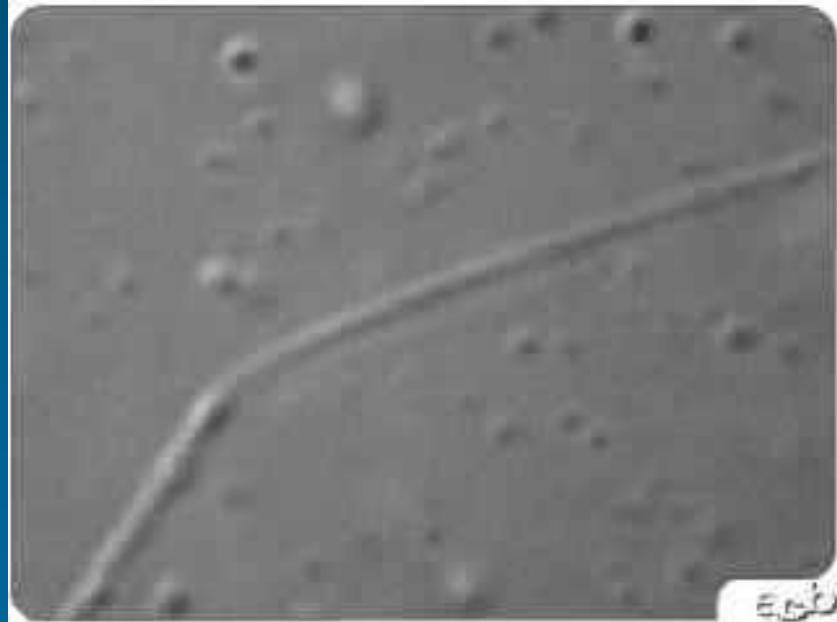
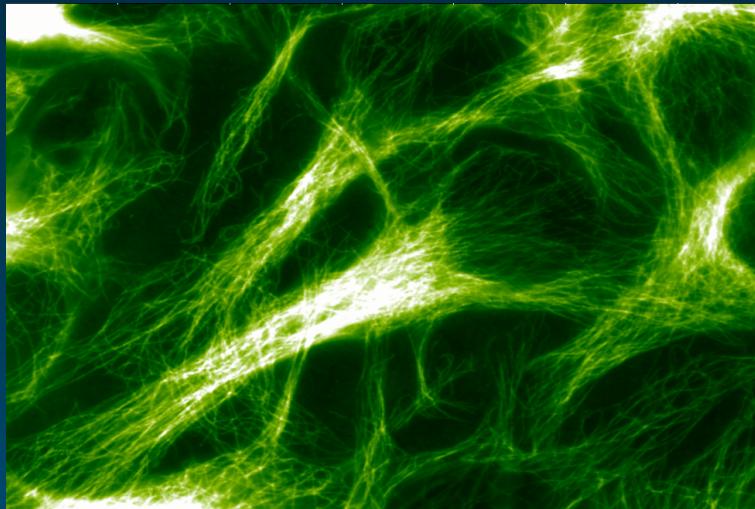


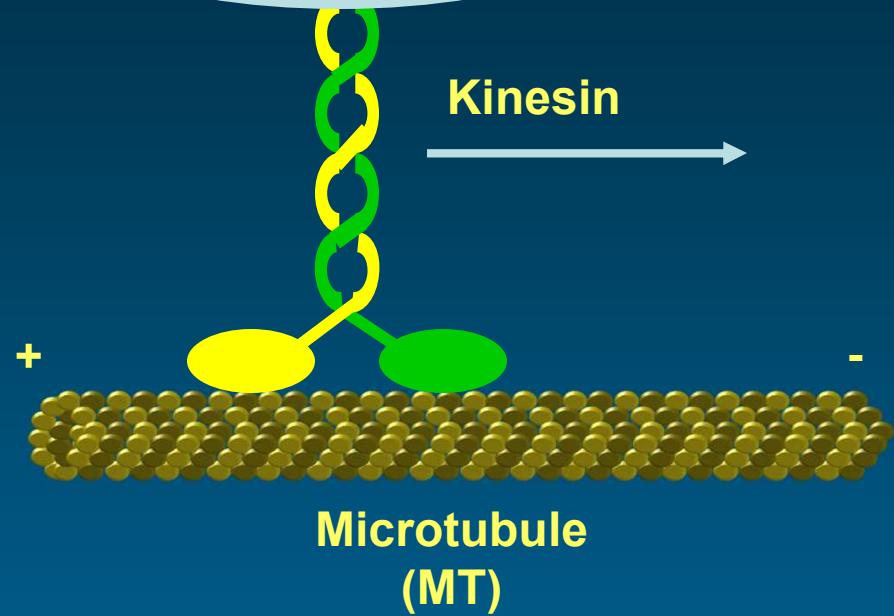
Engineering of a Genetically Modified Motor Protein for Cargo-Specific Transport

**Amanda Carroll-Portillo and
George D. Bachand**

**Sandia National Laboratories
Albuquerque, NM USA**



Transported Molecule

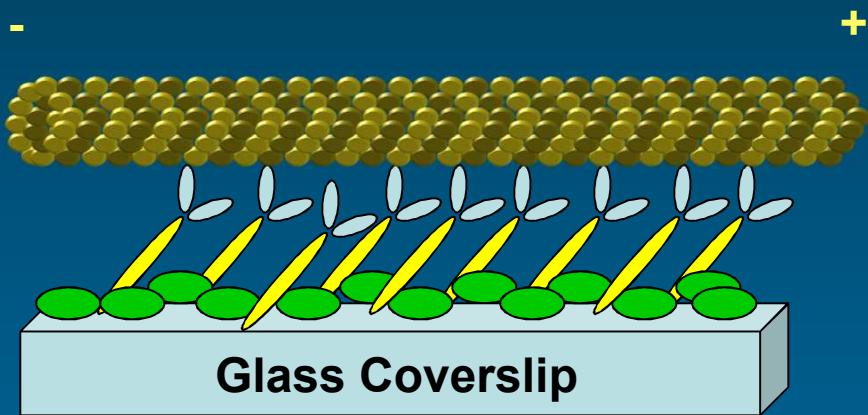


Nature = transport via motor proteins and associated filaments

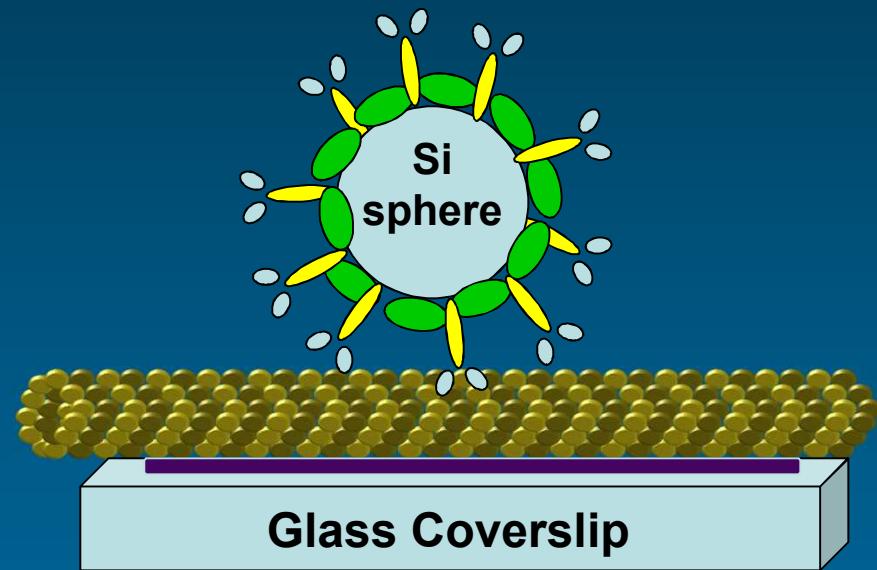
Experimentally = kinesin and microtubules

Two types of kinesin-based *in vitro* motility

Inverted Motility



Standard Motility



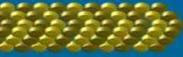
Casein



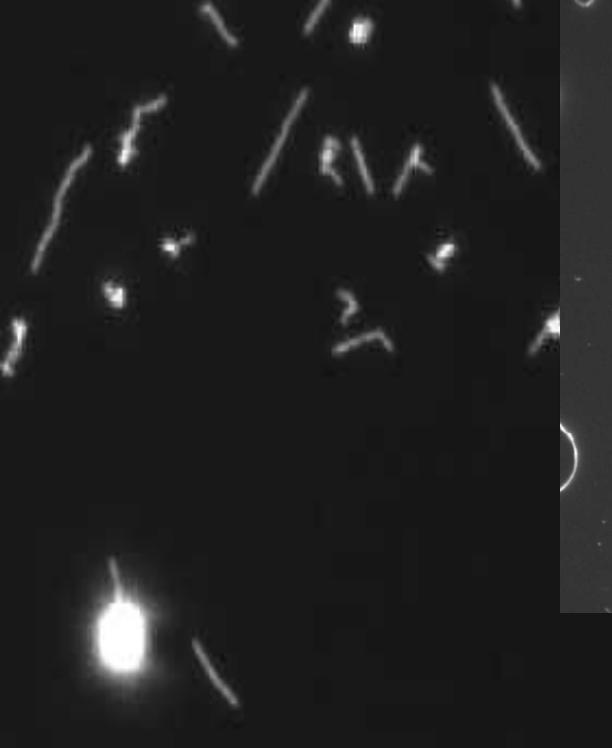
Drosophila kinesin (DK)



APTES



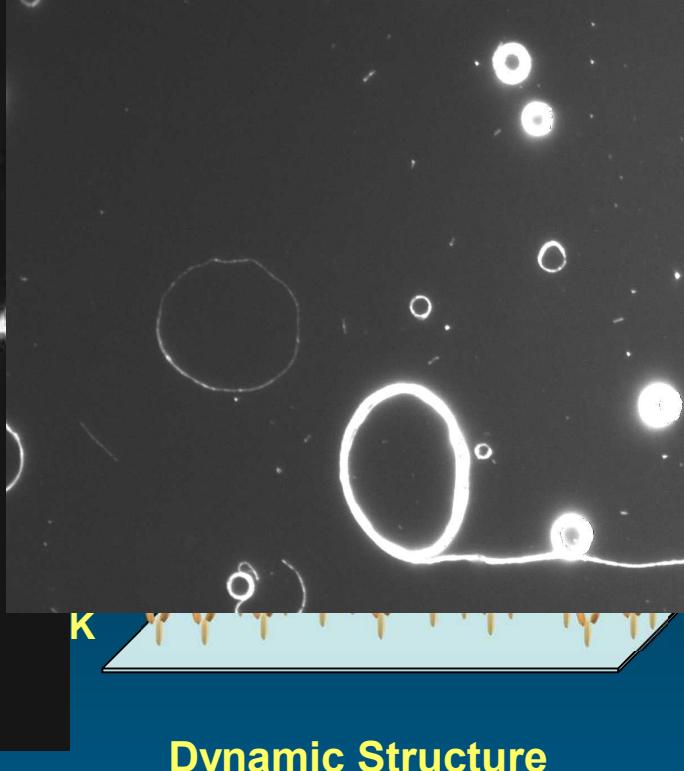
Microtube



Capture and transport

antibody-coated
microtubes and
antibody-coated
fluorescent
microspheres

Bio-detector

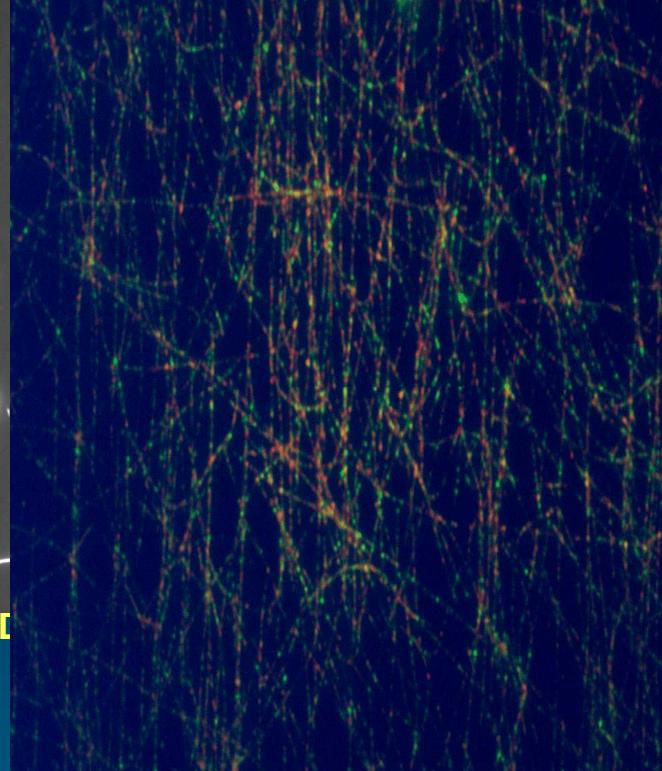


Dynamic Structure Formation

Haiqing Liu

Biotinylated
microtubes and
Streptavidin Qdots

Circles & aggregates



Antigen Detection

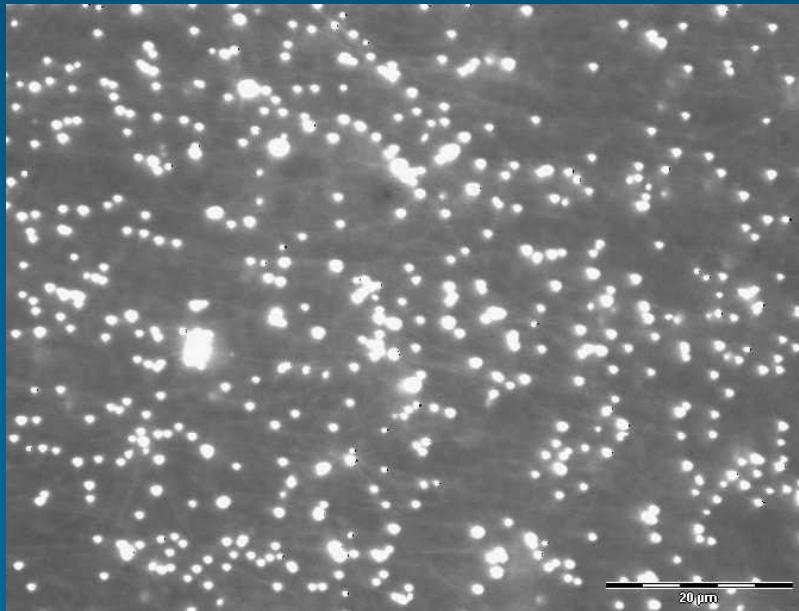
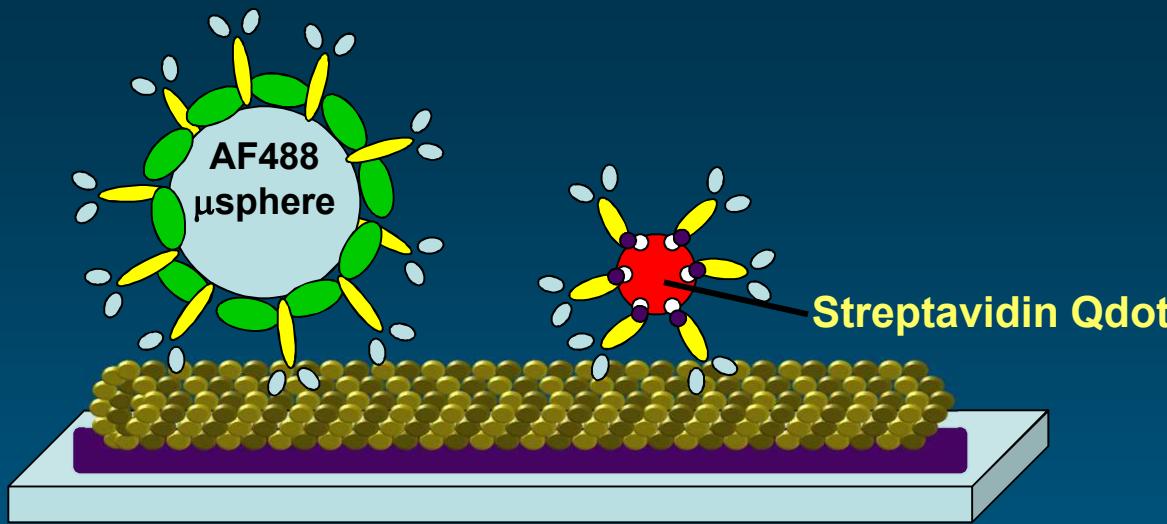
Lynnette Rios

Antibody-coated
microtubes and
quantum dots

Quantitative detection
of cytokines

Functionalized microtubes; kinesin provides energy/motility

Standard Motility

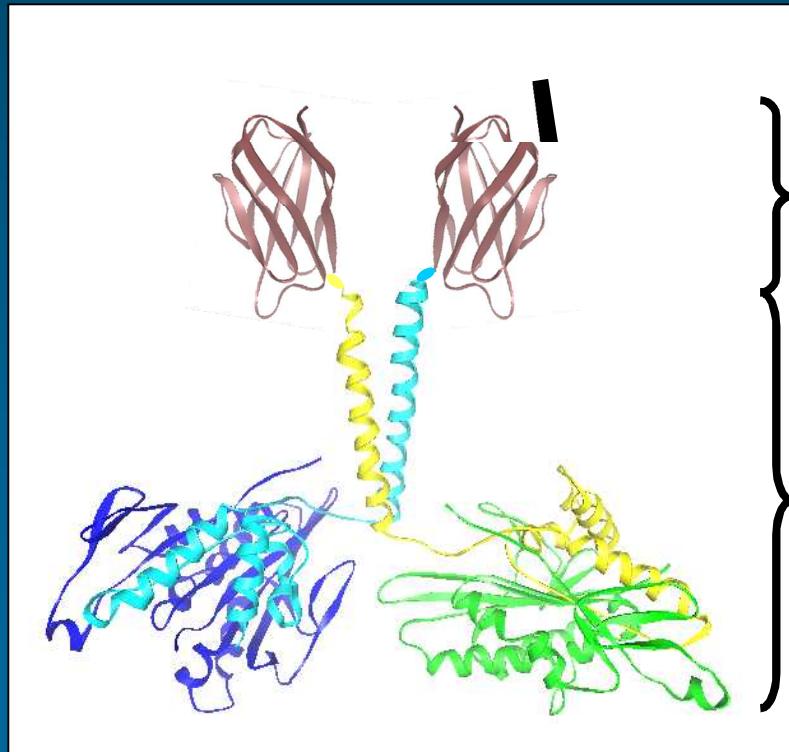


- Mimics natural transport pathways
- Kinesin attached via passive adsorption or biotin-streptavidin interaction

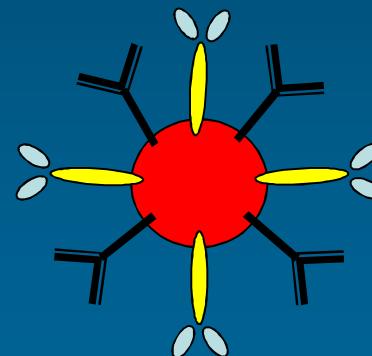
Kinesin-based nano-harvesters: Kinesin conjugates with antigen-recognition capability

Bidirectional Approach

Kinesin-ScFv fusion protein

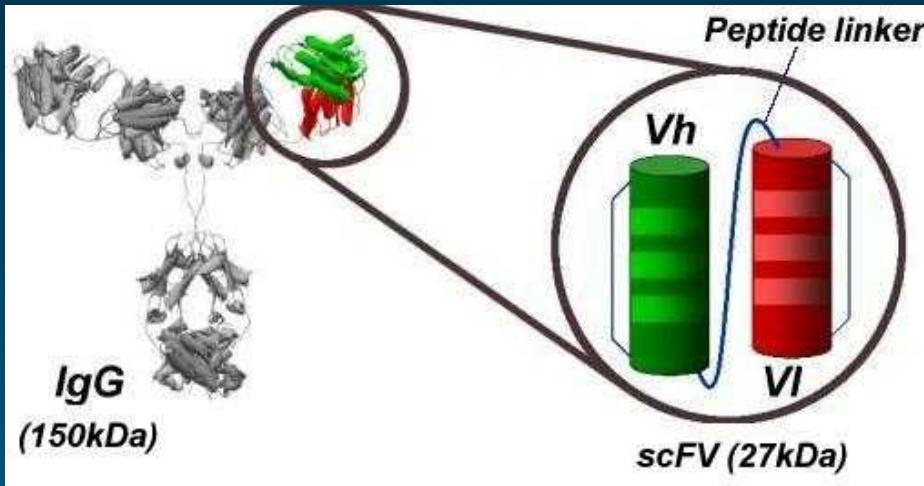


Streptavidin QDot:Biotinylated
Kinesin:Biotinylated Antibody
Conjugate



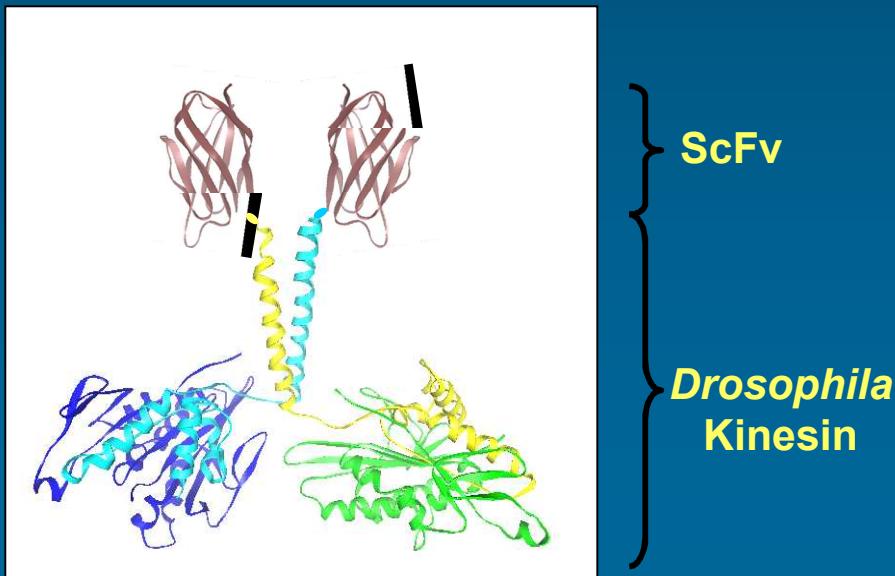
Kinesin-ScFv Fusion Protein

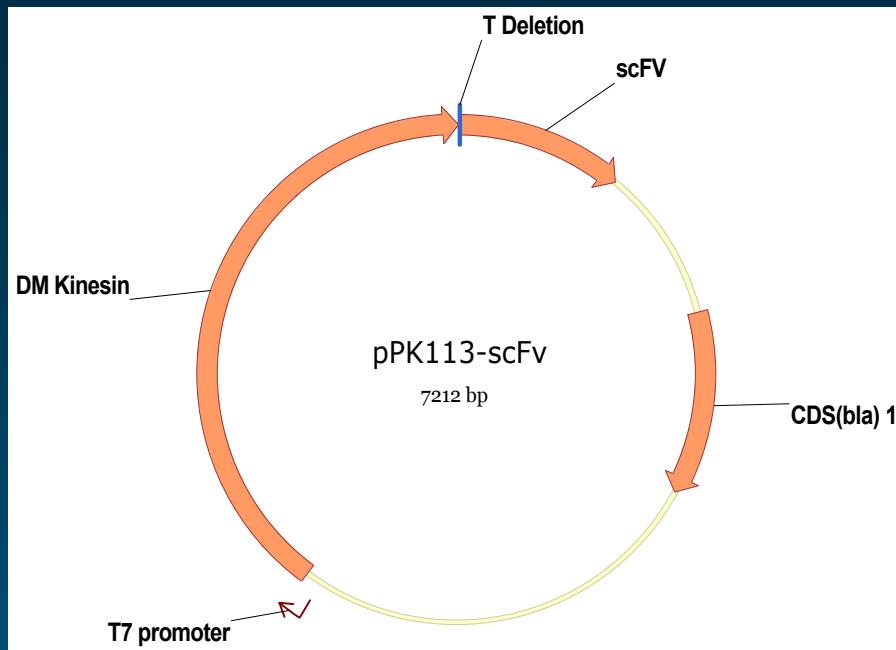
Genetic engineering approach



<http://www3.unifi.it/scibio/pers/deglinnocenti/scFv.jpg>

- Potential to functionalize kinesin with:
 - antibody recognition elements (ScFvs to a variety of antigens)
 - reactive peptides (Au, Pt, Ag, CNTs, etc.)



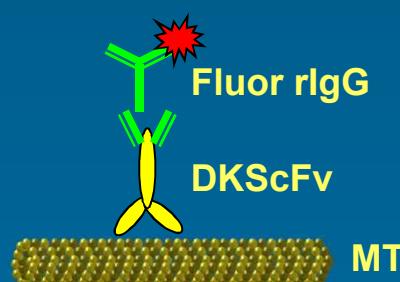


Fusion Protein Functionality:

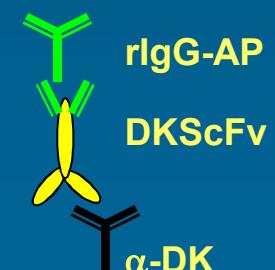
- Kinesin- ATP hydrolysis assays; standard motility assays
- ScFv - ELISA



Standard Motility

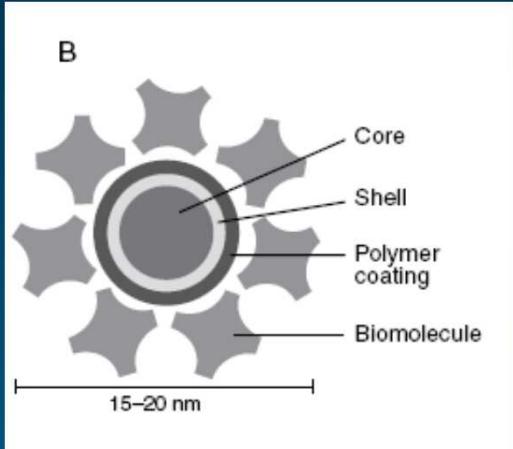


ELISA



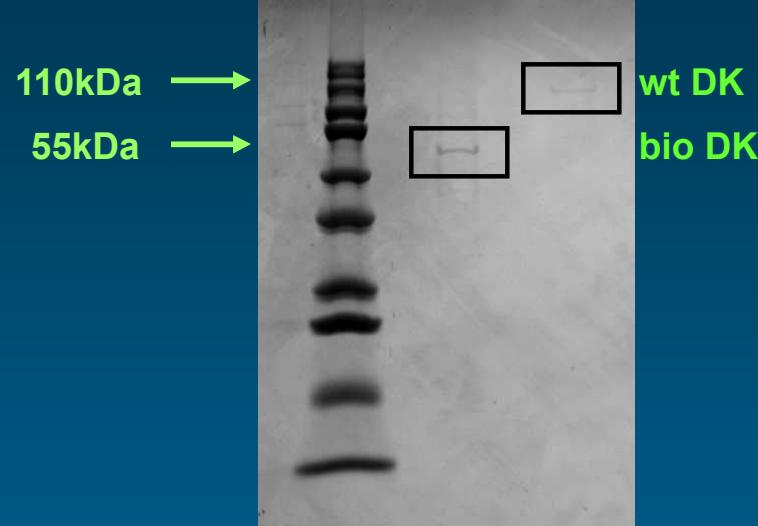
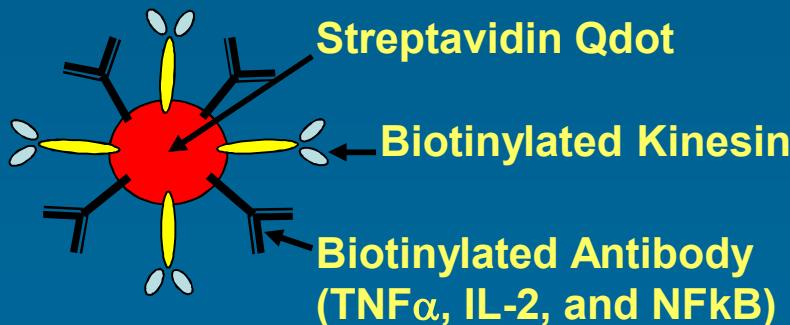
Quantum dot:Kinesin:Antibody Conjugates

www.probes.invitrogen.com



5-10 streptavidins/Qdot

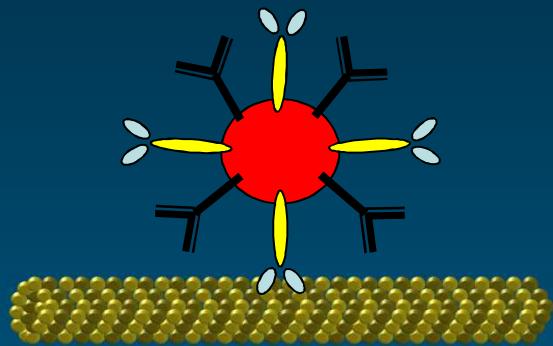
4 biotin binding sites/streptavidin



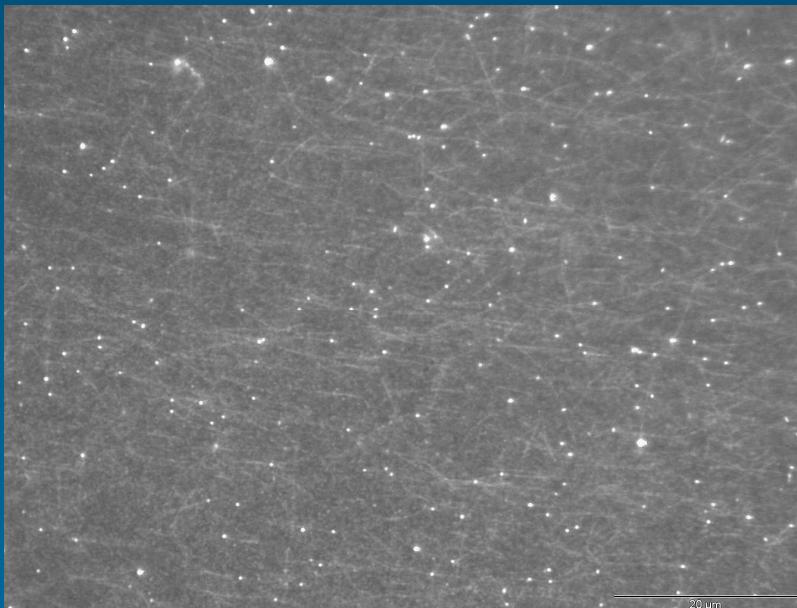
Conjugate Functionality:

- Kinesin – Standard motility assay; ATP hydrolysis assay
- Antibody – ELISA; standard motility assay

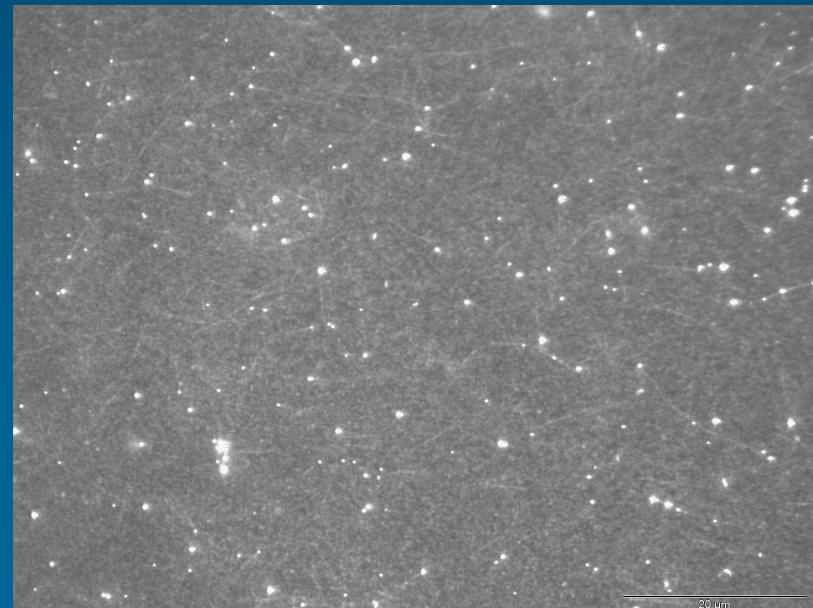
Kinesin Functionality:



1:10 Qdot:BioDK ratio

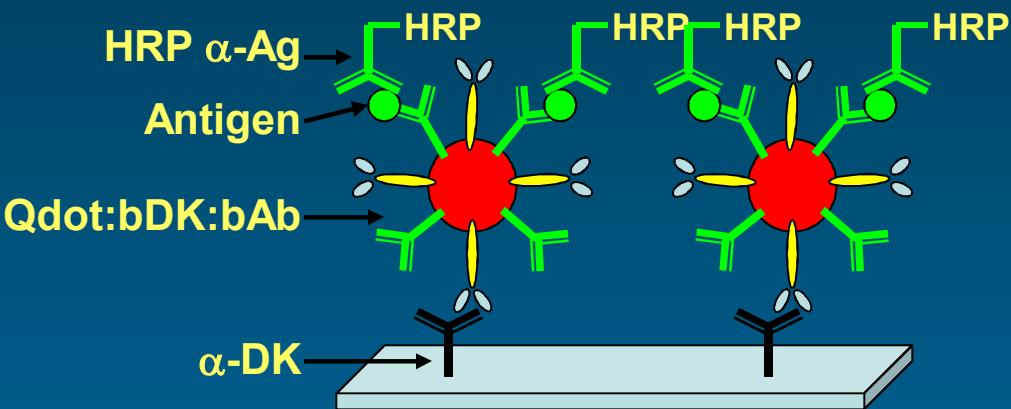


1:10:5 Qdot:BioDK:Antibody ratio

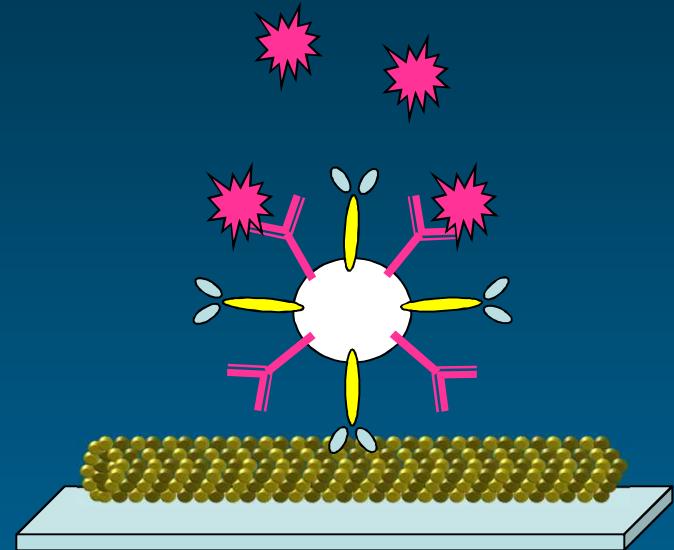


1:10:9 Qdot:BioDK:Antibody ratio

Antibody Functionality



Streptavidin Qdots
Biotinylated Kinesin/Antibody
Antigen
Readout = HRP signal



Streptavidin non-fluorescent
spheres
Biotinylated Kinesin/Antibody
Readout = Transport of
fluorescent protein (Ag)

Applications

- *In vitro*:
 - Antigen capture within biosensor – disease determinants, cytokines, etc.
 - Large scale material organization along microtubule pattern
- *In vivo*:
 - Capture and retention of molecules within a cell (infectious agents, cellular proteins, etc.)
“Tracking individual kinesin motors in living cells using single-quantum dot imaging”

Courty S., et al. Nanoletters 6(7): 1491-1495, 2006

Acknowledgements

- **Bachand Lab**
 - George D. Bachand
 - Marlene Bachand
 - Haiqing Liu
 - Lynnette Rios
 - Adrienne Greene
- **Mernaugh Lab**
 - Vanderbilt University
ScFv plasmid
- **Gelles Lab**
 - Brandeis University
Biotinylated Kinesin
Expression plasmid
- **Ratna Lab**
 - Naval Research
Laboratory
Expression and
Purification Protocols