Towards Single Molecule Detection of SEB

A Mobile Sandwich Immunoassay on Gliding Microtubules

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Objectives:

Build micro-devices for single molecule detection.

Approach:

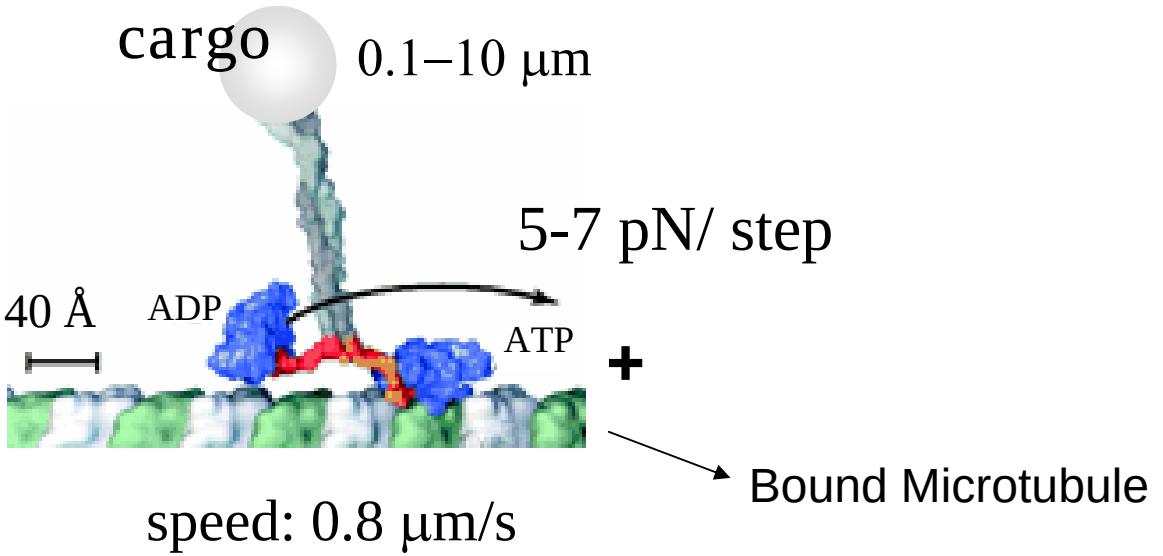
Use kinesin-microtubule nano-machinery to trap toxins in a microfluidic format for immunoassays detection. Toxins are trapped via antibodies that are chemically coupled to the microtubules. Detection is performed in a sandwich assay format in which the tracer is a viral nanoparticle containing proper antibodies and a large (~60 dyes/virus) number of reporter dyes for signal output enhancement.

Kinesin walks along MTs tracks *in vitro*

Chemical Energy into Motion

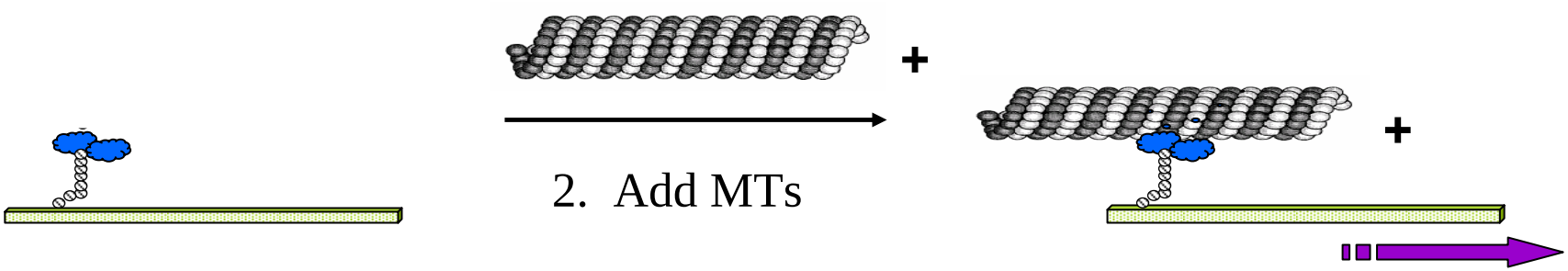
Involved in transport of:

- Organelles
- Protein complexes
- mRNA



Vale, R. D., Milligan, R. A. Science **2000**, *288*, 88.

Gliding Assay
Bound kinesin transports MTs

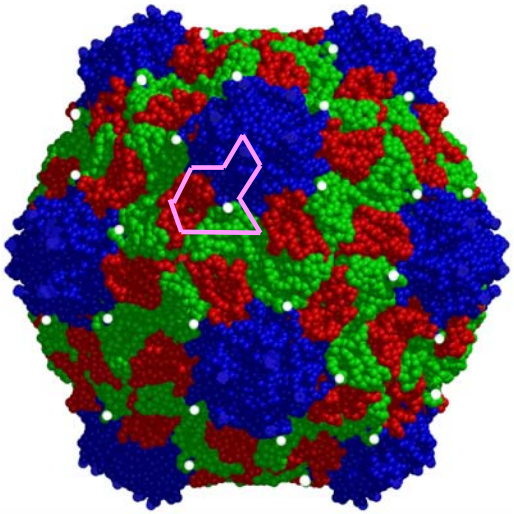


- Reaction driven by ATP.
- Results in movement of MTs.
- Surface pre-treated with Casein.
- MTs labeled with dye for easy imaging.
- Buffer components to prevent MT depolymerization and fluorophore photobleaching.

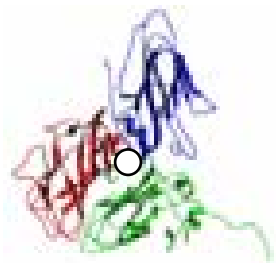
Genetically Engineered Cowpea Mosaic Virus

30 nm particle of icosahedral symmetry

60 subunits form the capsid



A domain
small subunit 24 kDa



B & C domains
large subunit 42 kDa

60 cysteines per virus

EF-CPMV

Wang, Q.; Lin, T.; Tang, L.; Johnson, J. E.; Finn, M. G. *Angew.Chem., Int. Ed.* **2002**, *41*, 459-462.

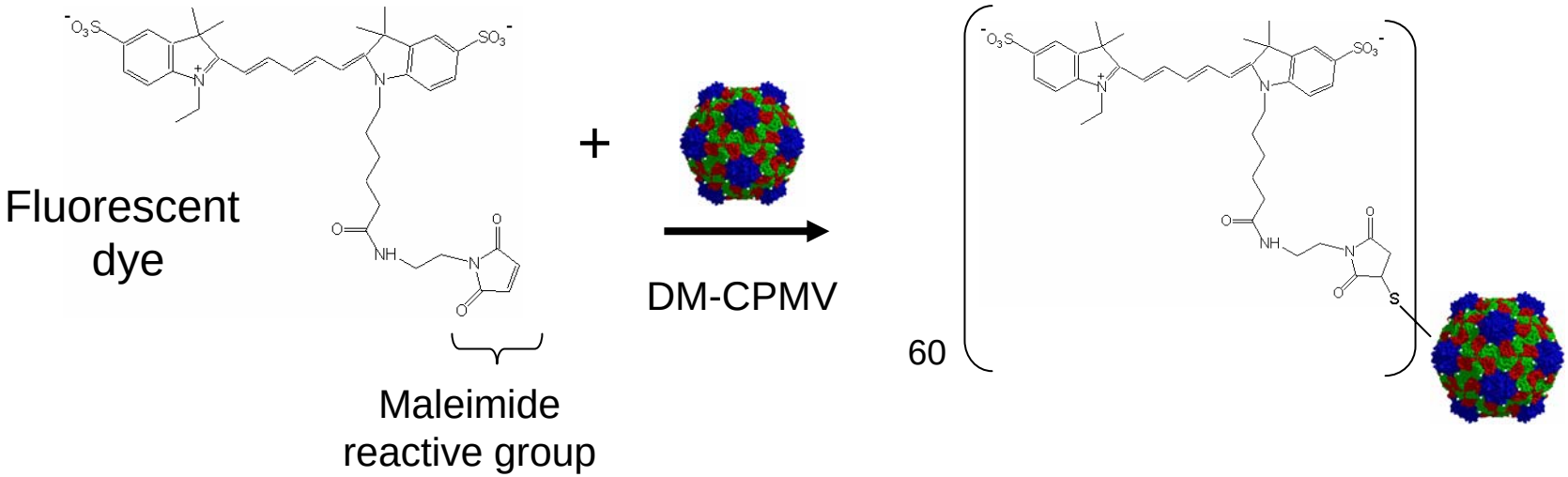
Genetically Engineered Virus

- More than 20 mutants available: His, Lys, Cys
- Provides a wide range of possibilities to couple proteins, dyes, and nanoparticles at specific locations with control of neighbor distances.
- Colored mutants have been used as scaffolds for gold nanoparticles, fluorophores and quantum dots.

Virus	Description
Wt CPMV	wildtype icosahedral scaffold
BC-cys	unique cys at aa 22-23 in the small subunit
CC-cys	unique cys at aa 44-45 in the small subunit
EF-cys	unique cys at aa 98-99 in the large subunit
228	cys at the indicated residue
252	cys at the indicated residue
2102	cys at the indicated residue
2159	cys at the indicated residue
DM 235/2319	double cys at the indicated residues
DM 237/2319	double cys at the indicated residues
DM 228/252	double cys at the indicated residues
DM 228/2102	**double cys at the indicated residues**
Vk38	unique lys at residue 38 in the small subunit
Vk299	unique lys at residue 99 in the large subunit
Vk2199	unique lys at residue 199 in the large subunit
CC-HIS	6xHIS at aa 44-45 in the small subunit
EF-HIS	6xHIS at aa 98-99 in the large subunit
C-term.trun-HIS	6xHIS at aa 189-190 in the small subunit
C-term.FL-HIS	6xHIS before the stop codon in the small subunit

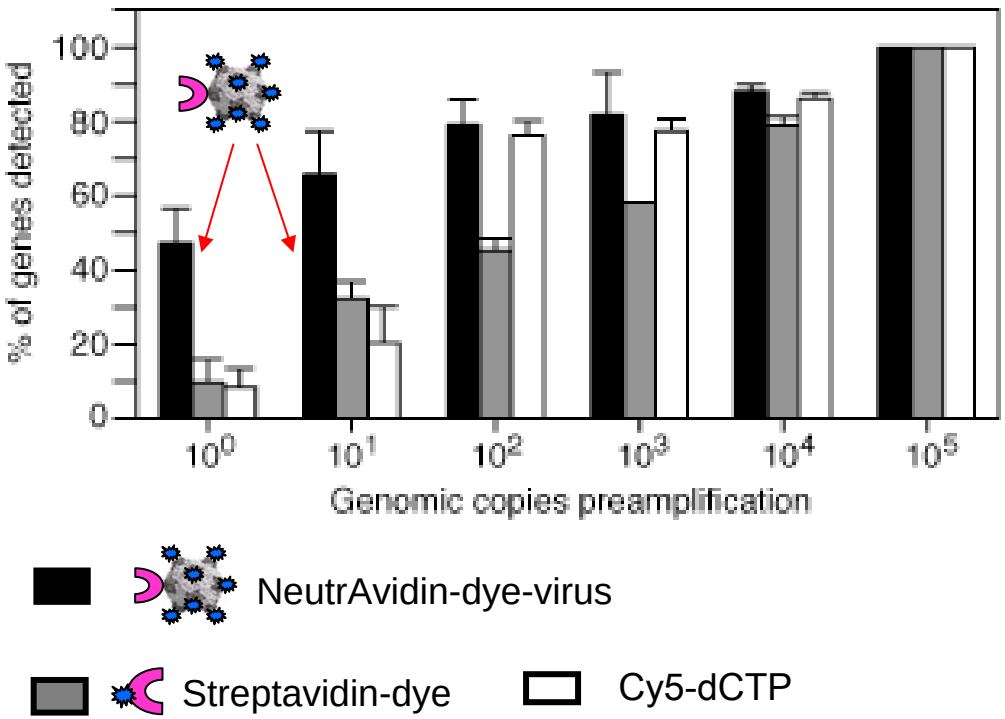
Fluorescent dye at fixed positions

Potential for higher signal throughput per binding event
60 dyes/capsid



Optimum detection at lower amount of starting DNA material

Percentage of targeted genes detected as a function of pre-amplification genomic DNA copy number



Impact

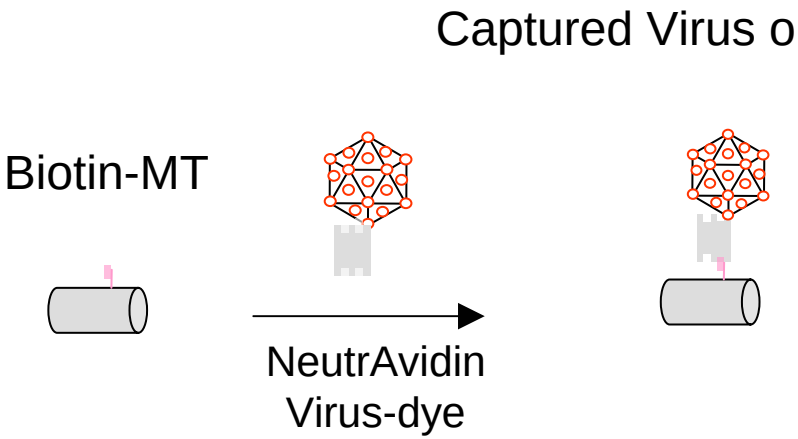
- Control over fluorophore-fluorophore distance to avoid self-quenching.
- Enhanced detection capability at low concentrations of target DNA.
- Improve detection sensitivity which may result in reduction of false negatives.

5 fold improvement in the number of genes detected starting with **single** genomic DNA copy.

C. M. Soto, A. S. Blum, G. J. Vora, N. Lebedev,
C. E. Meador, A. P. Won, A. Chatterji, J. E. Johnson, B. R. Ratna

J. Am. Chem. Soc. **2006**, *128*, 5184-5189.

Capture of NA-CPMV-dye by gliding MT



- Capture and transport occurred at CPMV concentrations as high as 1.36 nM.
- Scaffold/tag caused only slight decreases in MT gliding speeds.
- CPMV-dye appears to be superior to fluorescent polystyrene spheres because of its greater biocompatibility.

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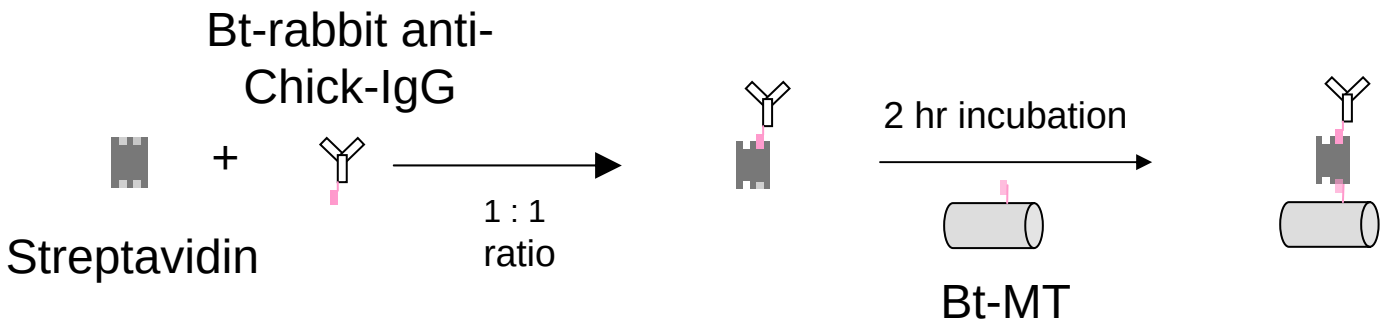
J. L. Whitley, J. E. Johnson, A. Chatterji, B. R. Ratna

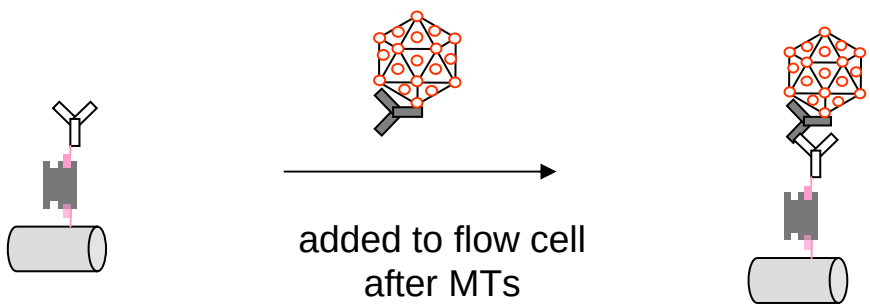
J. Nanosci. Nanotechnol. **2006**, *6*, 1–10.

Direct Assay on a gliding microtubule

Attachment of rabbit anti-Chicken-IgG to MTs *via* streptavidin linkage



Capture of CPMV-dye containing covalently-bound chicken IgG



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Advantages
Covalent attachment of antibodies

- Simplify functionalization of the MTs with capture antibodies.
- Ability to perform sandwich assay for detection of toxins.
- Controlled coupling by adjusting reaction conditions.
- Able to quantify amount of antibodies on the MTs.
- Easier to find conditions in which gliding properties are maintained.

Concluding Remarks

- MT's can be modified in a controlled way with anti-SEB.
- The amount of anti-SEB has to be limited and free thiols capped to maintain MTs activity.
- Antibody-MTs have immuno-detection capabilities comparable to control samples.

Impact:

- Antibody-MTs are used for the detection of SEB in a sandwich assay format down to LOD's of **0.5 ng/mL** in a 30 min assay.
- This system has potential applications in lab-on-a-chip type of technologies for detection of target analyte in a complex solution.

Acknowledgements

- John E. Johnson for CPMV mutants
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