Moc/Bio and Nano/Micro

Lee and Stowell

Biotemplating

Choice of biomaterial

Lipid, protein, nucleic acid

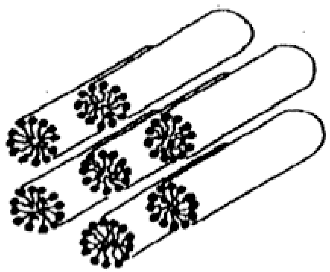
Methods of deposition

Chemical, photochemical

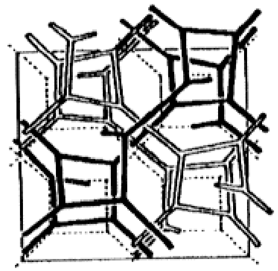
Methods of optimization

Altering the reaction conditions or the biomaterial

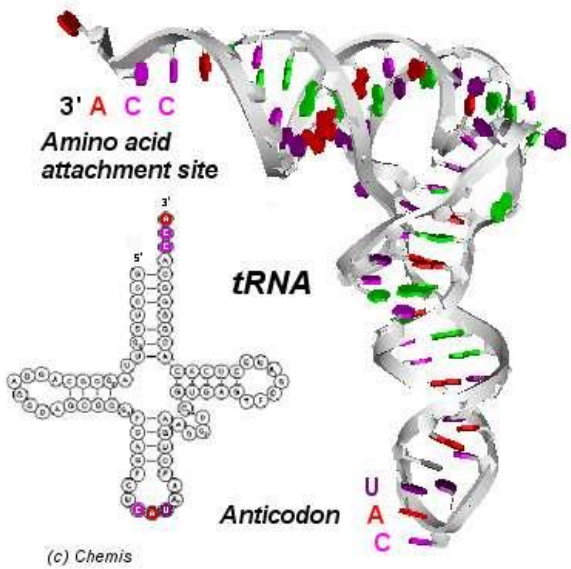
Remember all the choices…

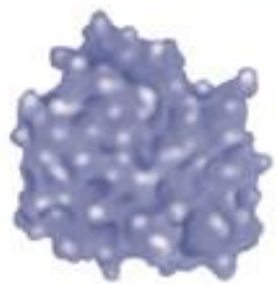


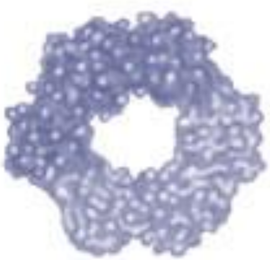
Hexagonal Phase



Cubic Phase (Q^{230})







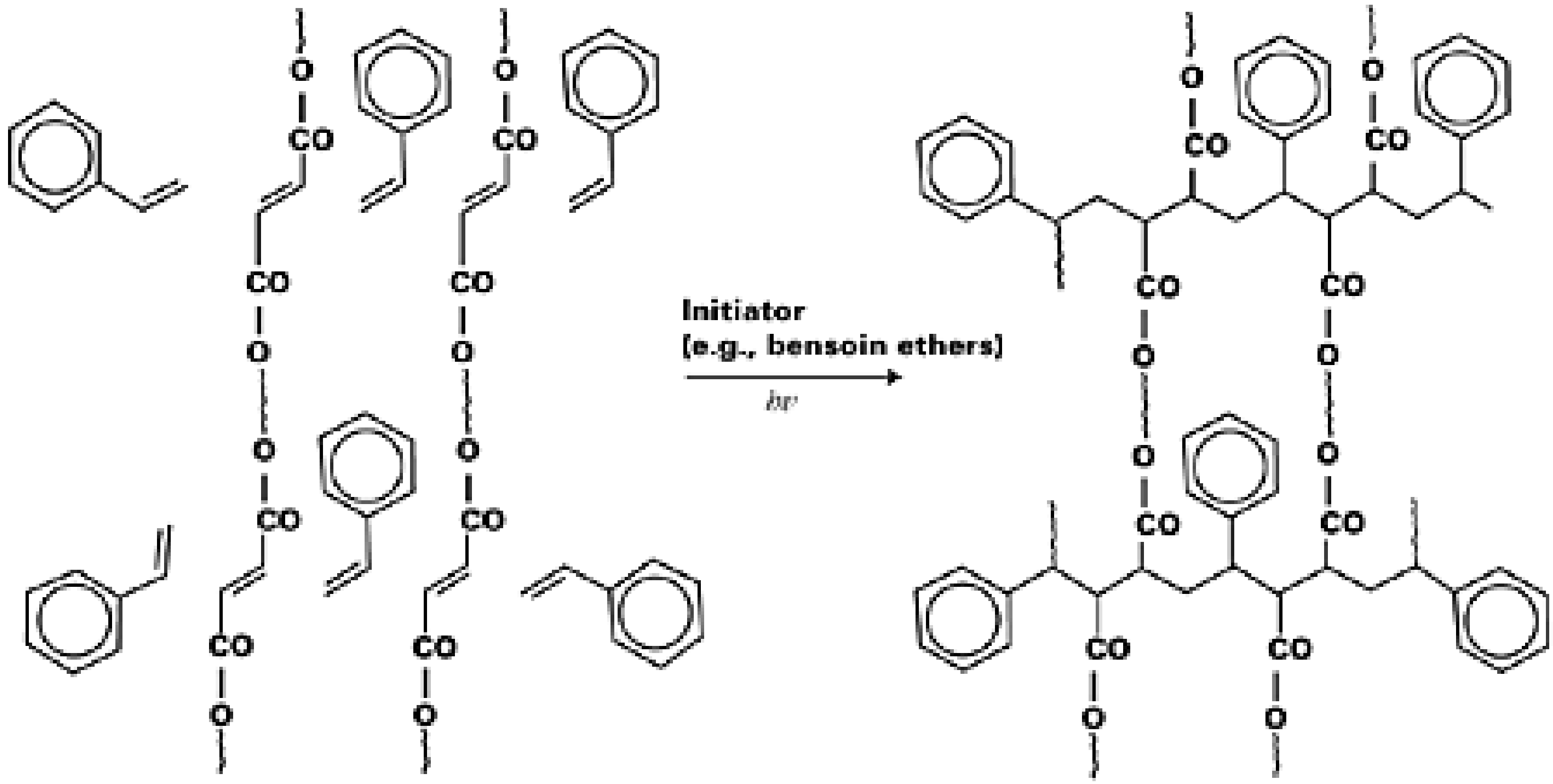
Using bio templates for materials fabrication

- Identify a biomaterial with a desired pattern
- Determine conditions for materials deposition
  - Chemical or photochemical
- Optimize materials formation either chemically or biologically
  - Mutation of biomaterial
  - Optimization of the chemical process

Chemical processes

- Photopolymerization (primarily organic)
  - Light initiated
  - Fast and more easily controlled
  - Clean
- Chemical polymerization (primarily organic)
  - Harder to control
  - Slow
- Mineralization (inorganic)
  - Biomineralization of calcium phosphates, magnetite
  - Slow not well understood or controllable
- Electrochemical (metals)
  - Catalysts assisted metal deposition
  - Somewhat controllable via catalyst doping

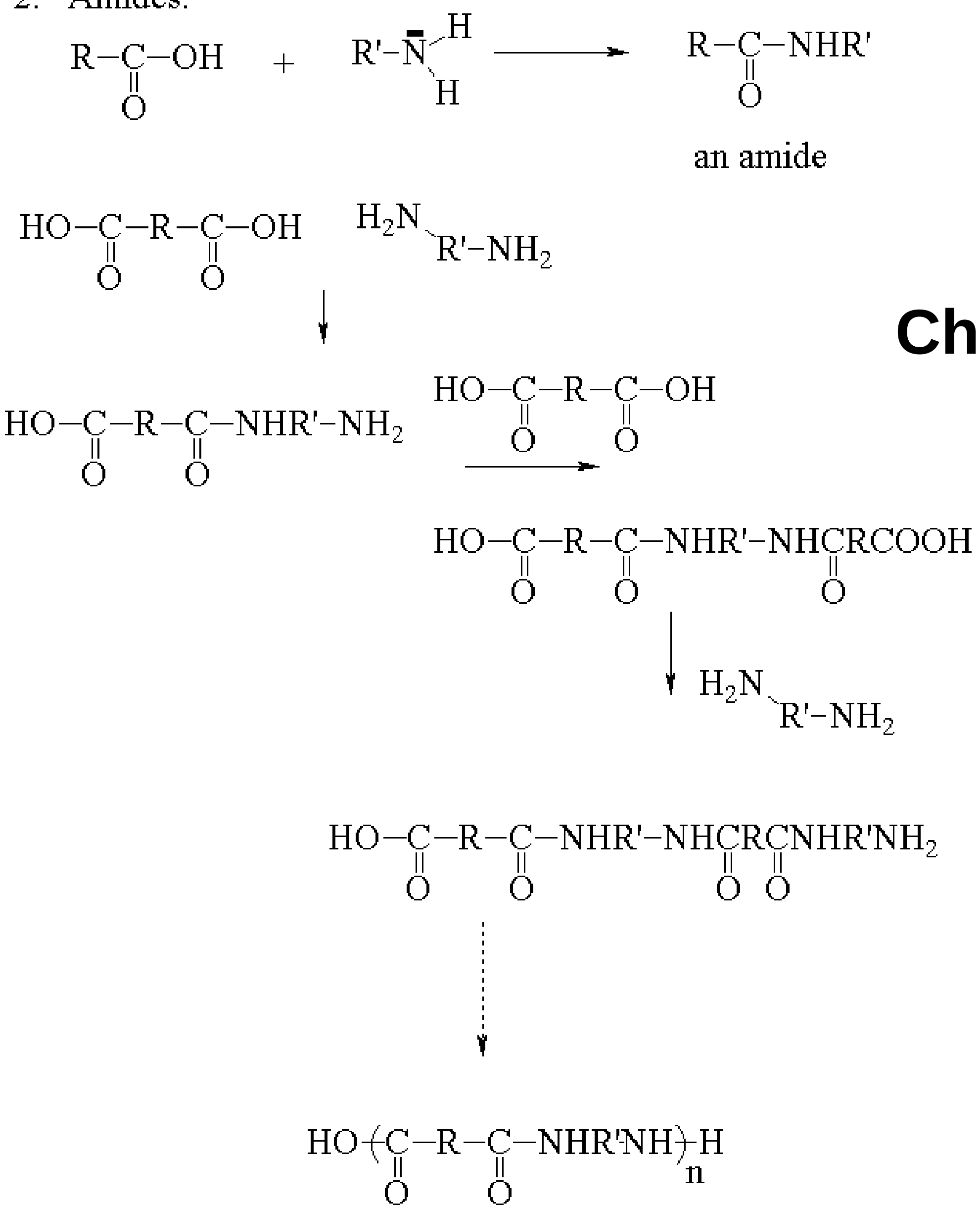
Photochemical polymerization



Chemical polymerization

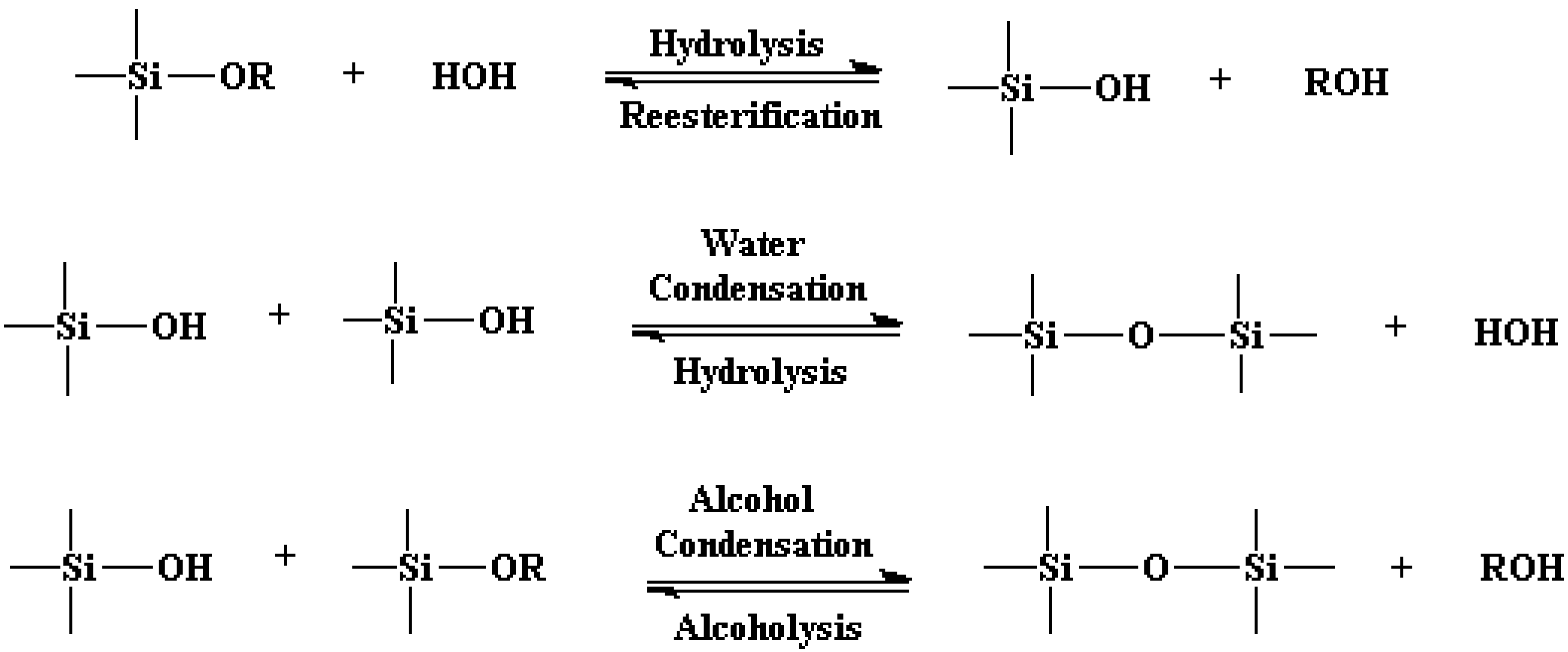
Condensation Polymerization.

2. Amides:



a polyamide (example: nylon)

Chemical polymerization



1

2a

2b

Electrochemical deposition

Activation: $Pd^{2+} \rightarrow Pd^{2+}_{ads}$ [and $Pd^{2+} + 2\,e^{-} \rightarrow Pd$]

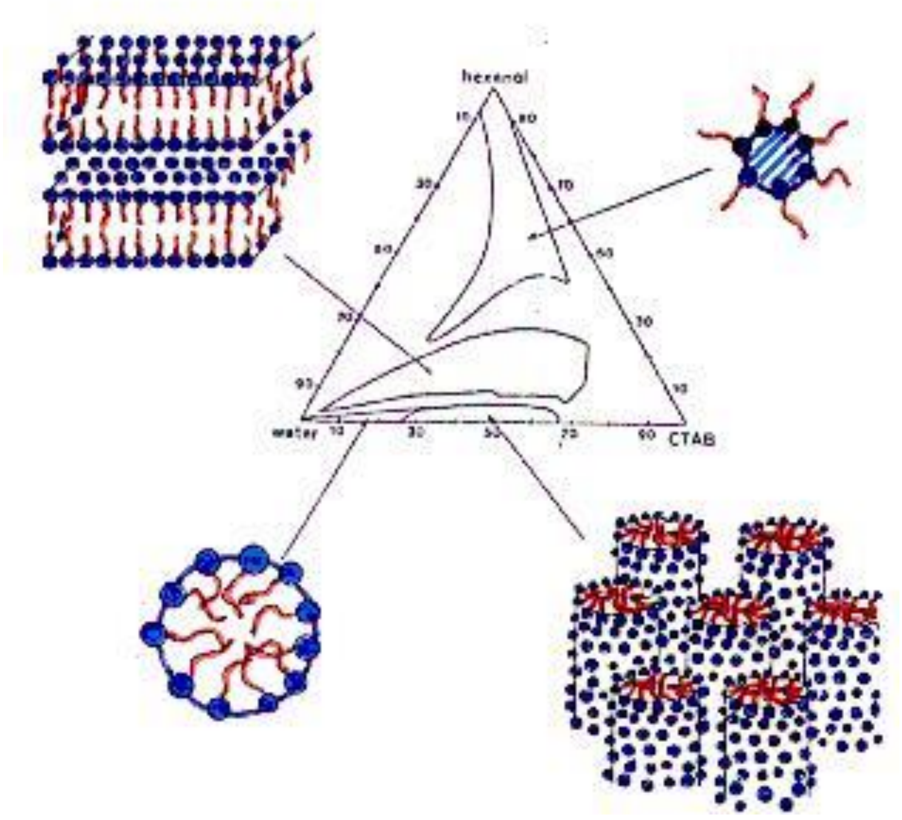
Deposition: $3Ni^{2+} + (CH_3)_2NHBH_3 + 3H_2O \rightarrow$

$3Ni + B(OH)_3 + (CH_3)_2NH_2^{\,+} + 5H^{+}$

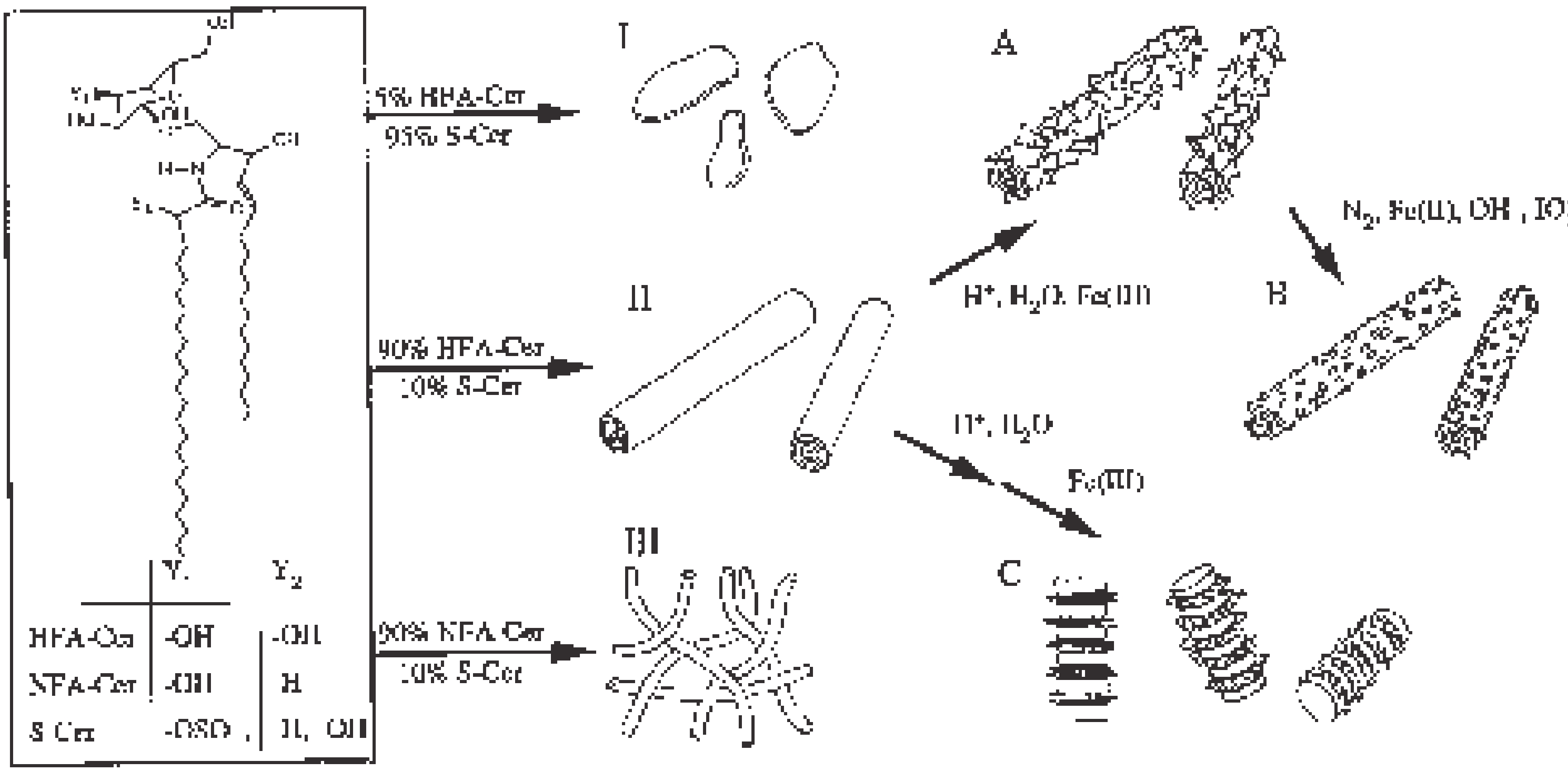
- Template mineralization or photochemical polymerization on lipid structures. Archibald, D.D. and S. Mann, *Template mineralization of self-assembled anisotropic lipid microstructures.* Nature, 1993. **364**: p. 430-433. Evans, E., et al., *Biomembrane Templates for Nanoscale Conduits and Networks.* Science, 1996. **273**: p. 933-935.
- Bacterial fibres. Davis, S.A., et al., *Bacterial templating of ordered macrostructures in silica and silica-surfactant mesophases.* Nature, 1997. **385**: p. 420-423
- Methods for DNA driven nanocrystal **organization.** Livisatos, A.P., et al., *Organization of 'nanocrystal molecules' using DNA.* Nature, 1996. **382**: p. 609-611 ; Chad A. Mirkin, Robert L. Letsinger, Robert C. Mucic & James J. Storhoff DNA-based method for rationally assembling nanoparticles into macroscopic materials Nature 1996, 382, p 607
- S-layer templates for nanoparticle fabrication. Sleytr, U. B. et al. Applications of S-layers. *FEMS Microbiol Rev* **20**, 151-75 (1997).
- Two-dimensional array fabrication using ferritin. Yamashita, I., *fabrication of two-dimensional array of nano-particles using ferritin molecule.* Thin solid films, 2001. **393**: p. 12-18.
- Chemoselective Templates from TMV. 1. Demir, M. & Stowell, M. H. B. A chemoselective biomolecular template for assembling diverse nanotubular materials. *Nanotechnology* **13**, 541-544. (2002). Biotemplate Synthesis of 3-nm Nickel and Cobalt Nanowires Mato Knez et al Nanoletters 2003 Vol. 3, No. 8 1079-1082
- Bacterial Phage virus. Mao, C. et al. Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires. *Science* **303**, 213-7 (2004).
- 3D DNA templates. Shih, W. M., Quispe, J. D. & Joyce, G. F. A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. *Nature* **427**, 618-21 (2004).

Lipid templates

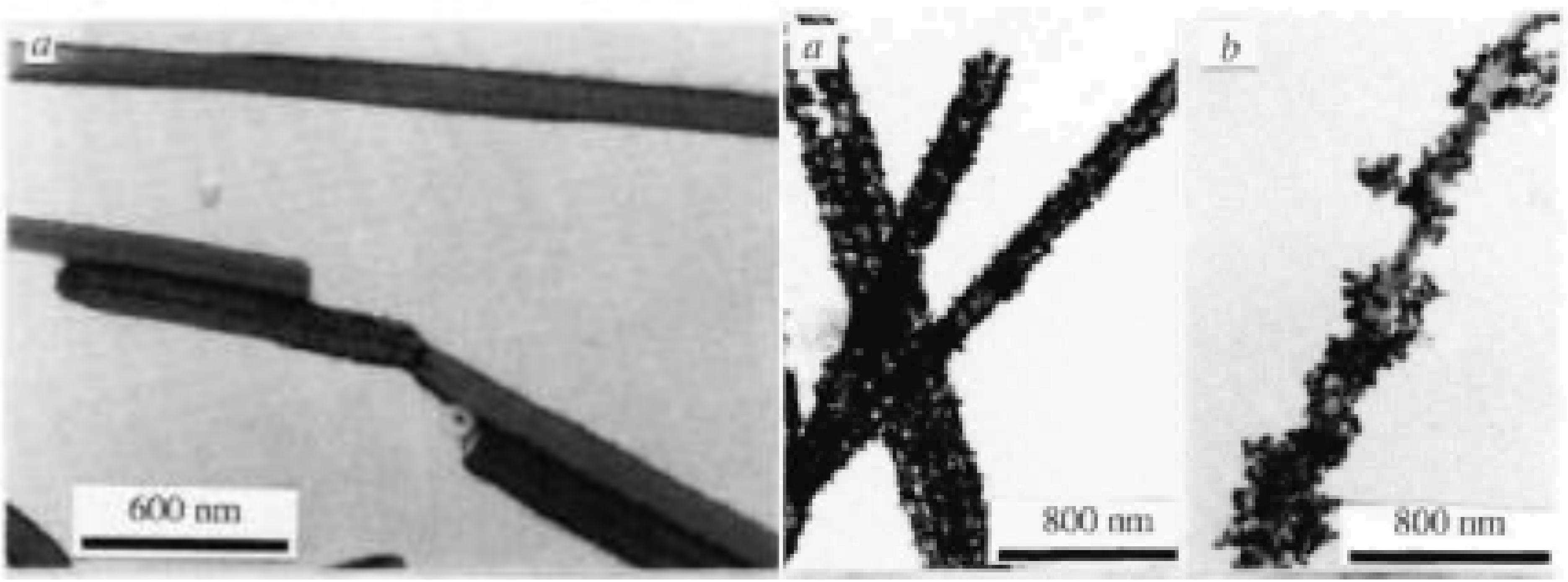
- Recall lipids and their many phases
  - Cubic,hexagonal,etc
- Use stable lipid phases as a template
- Polymerization or mineralization



Cerebrosides (tube forming)



Iron oxide deposition

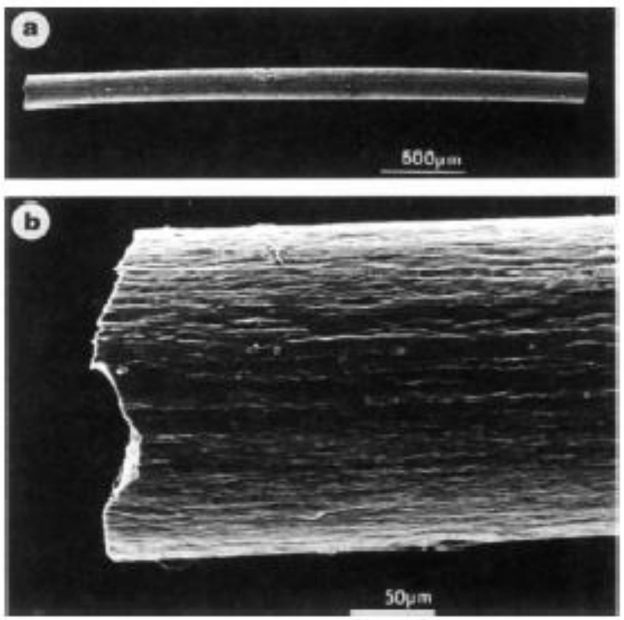


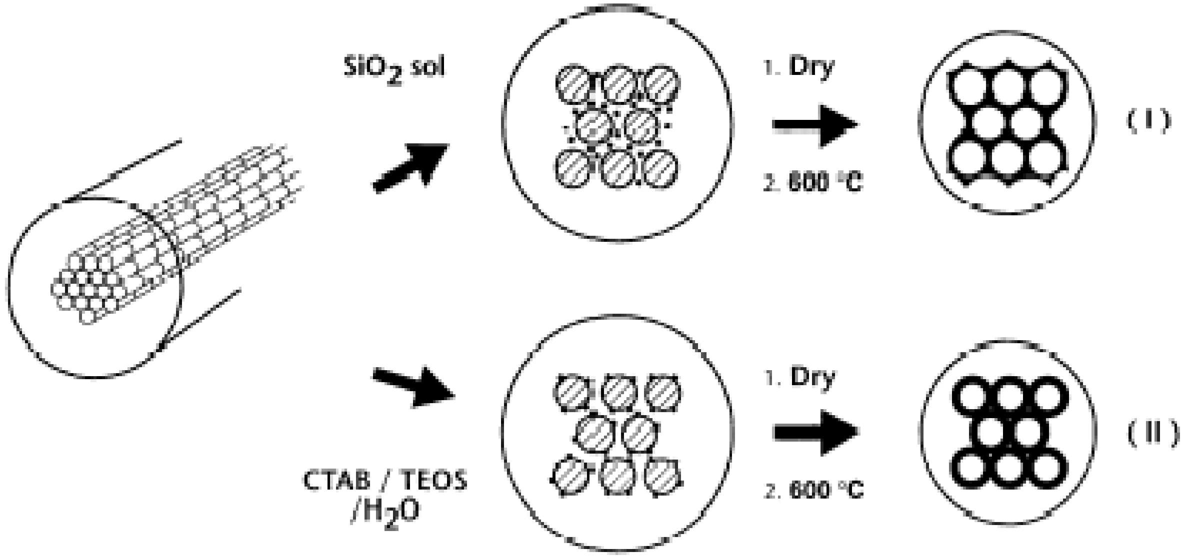
Various forms of Fe oxide were produced

Bacterial Fibres

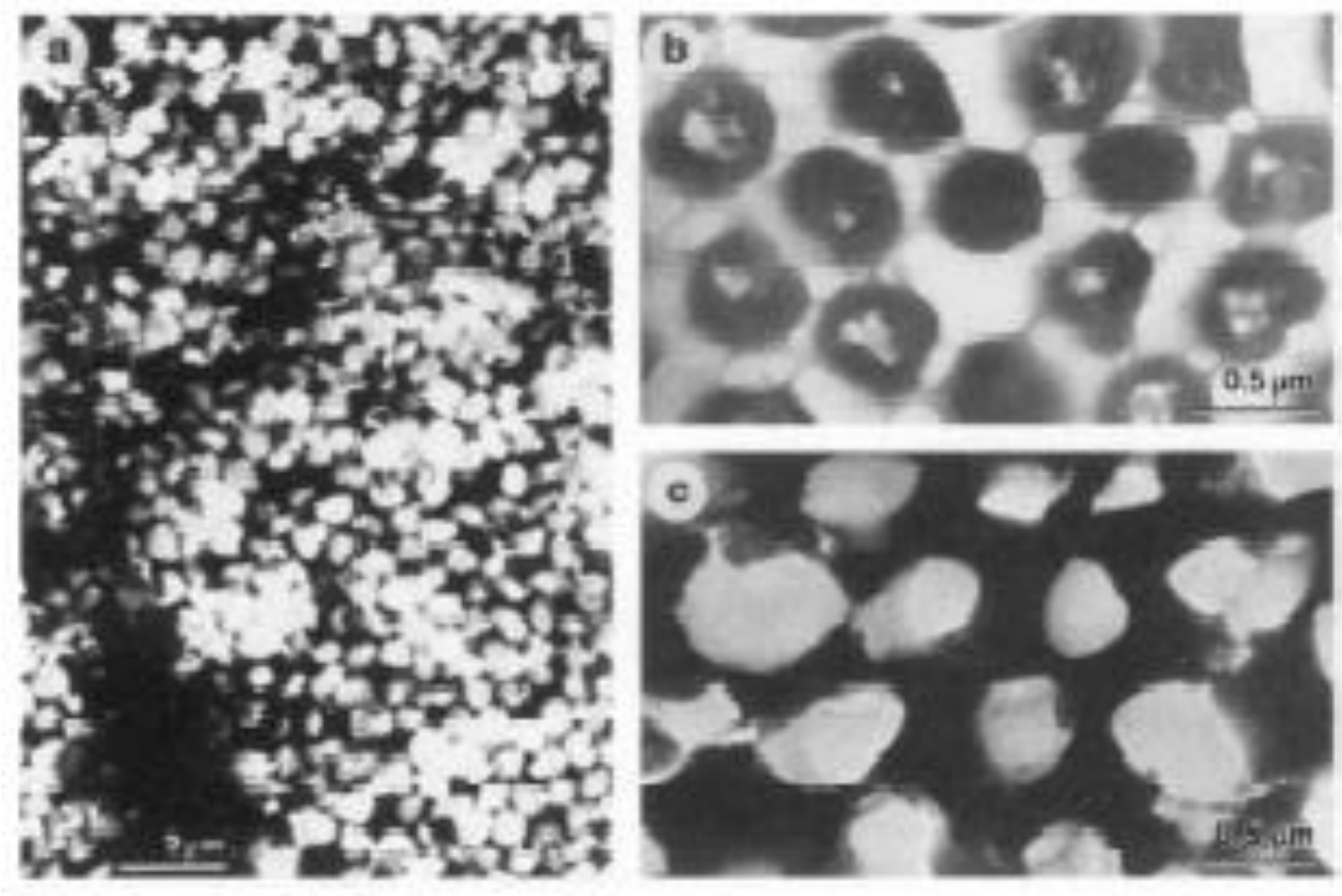
- What are they
  - Like drawn nylon from polymerizing solutions
- How to use them as a template
  - Actually using the interstices between the bacterium

Silicate formation at the interstices



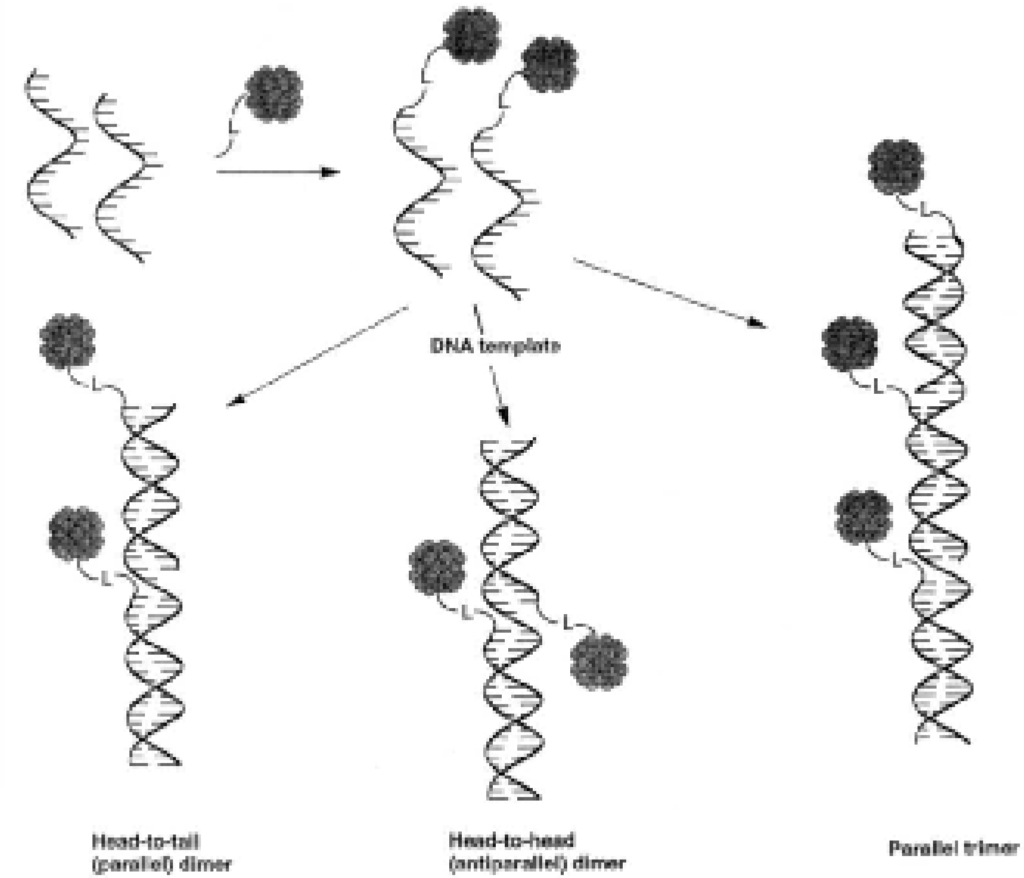


After pyrolysis to remove organic matter

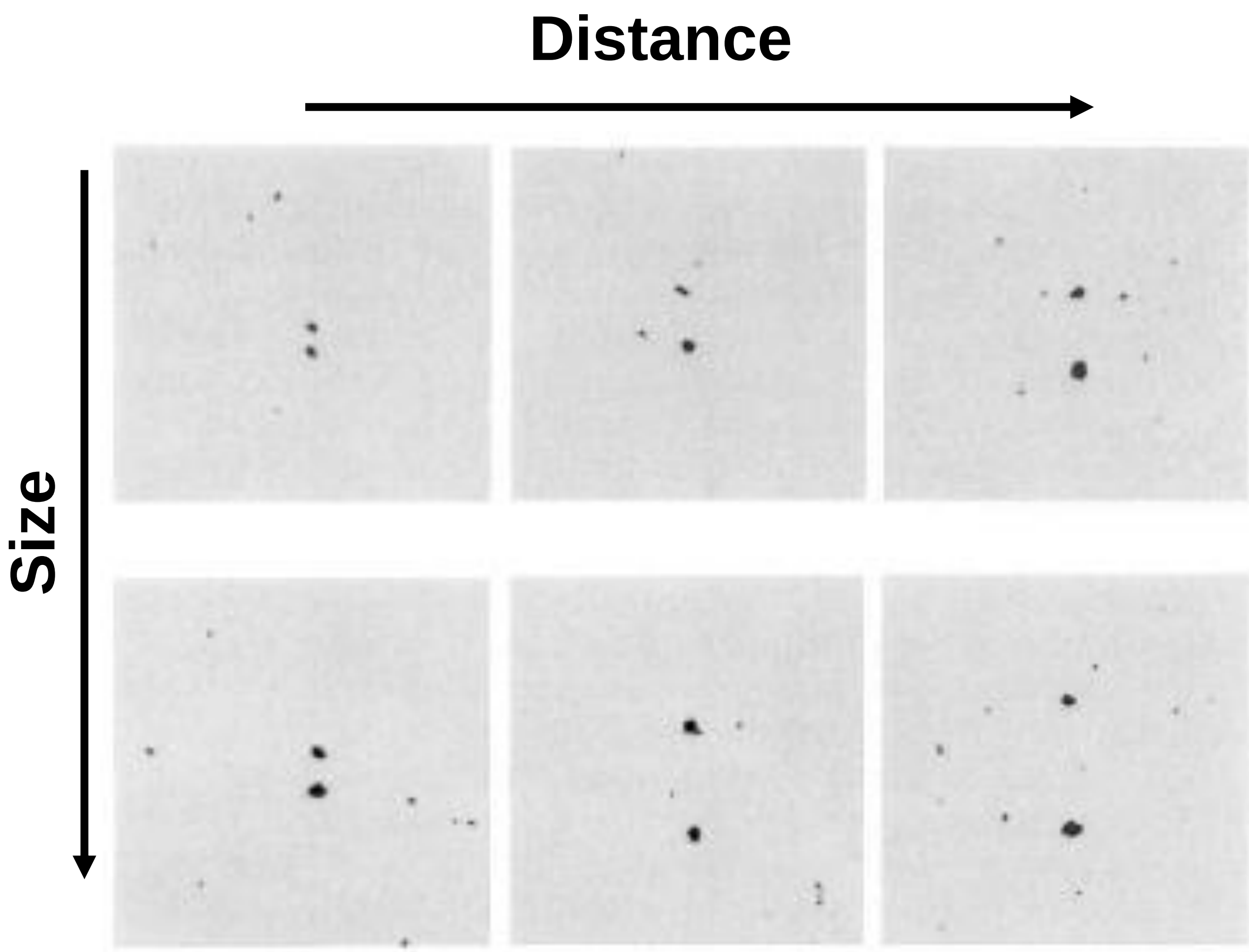


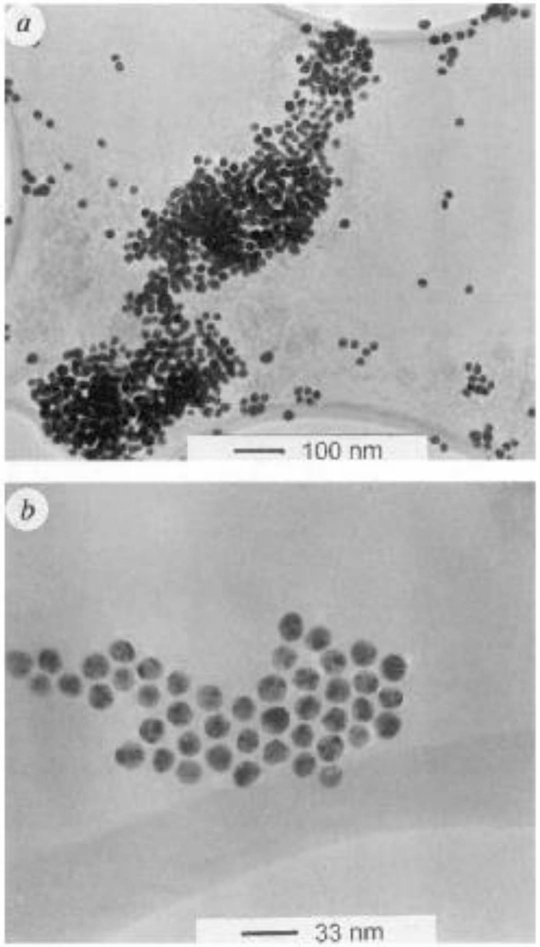
2D-DNA templates

- Linear arrays can be made using gold particles
- 2D arrays possible



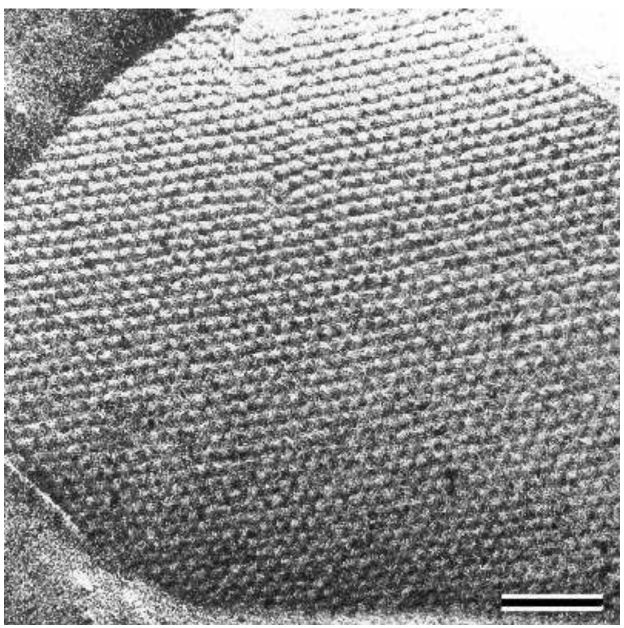
Control of size and spacing

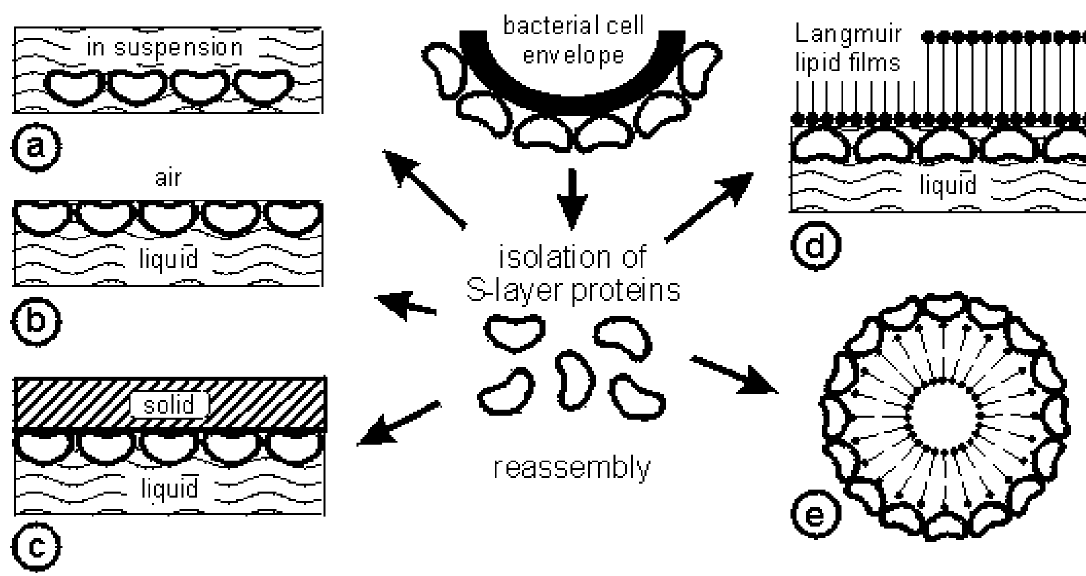




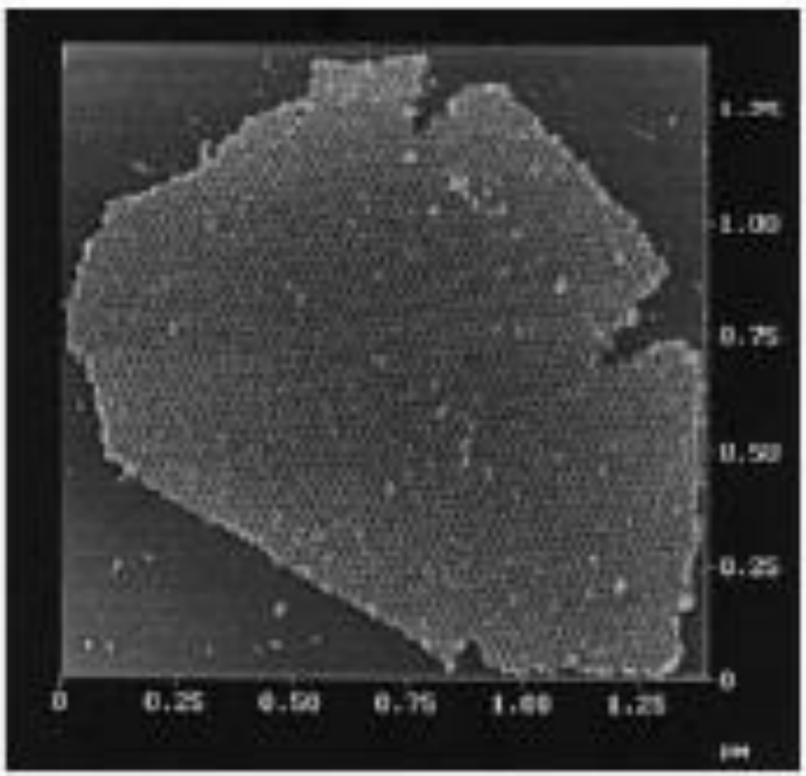
Bacterial S-layers

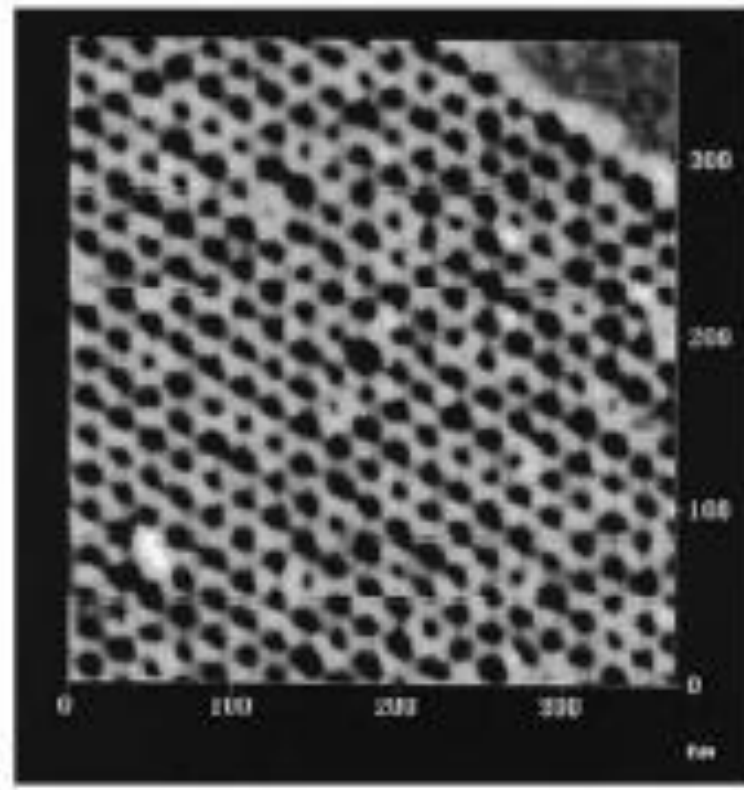
- Outer surface protein layer of bacteria
- Highly regular ordered 2D array



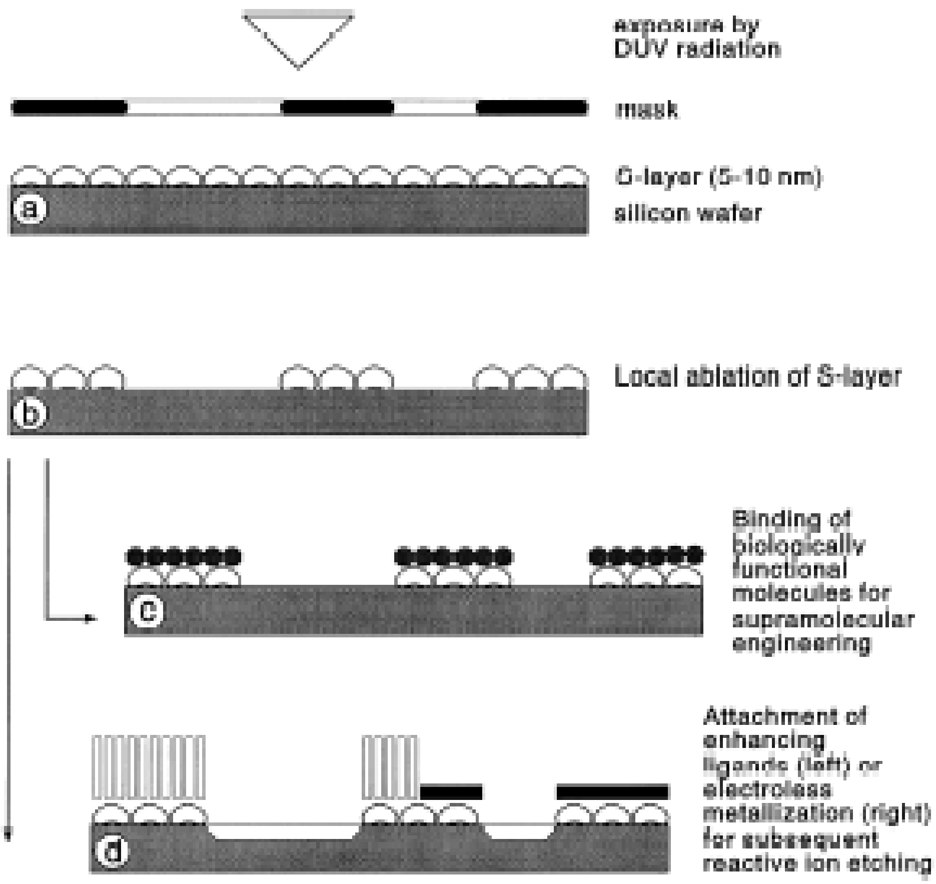


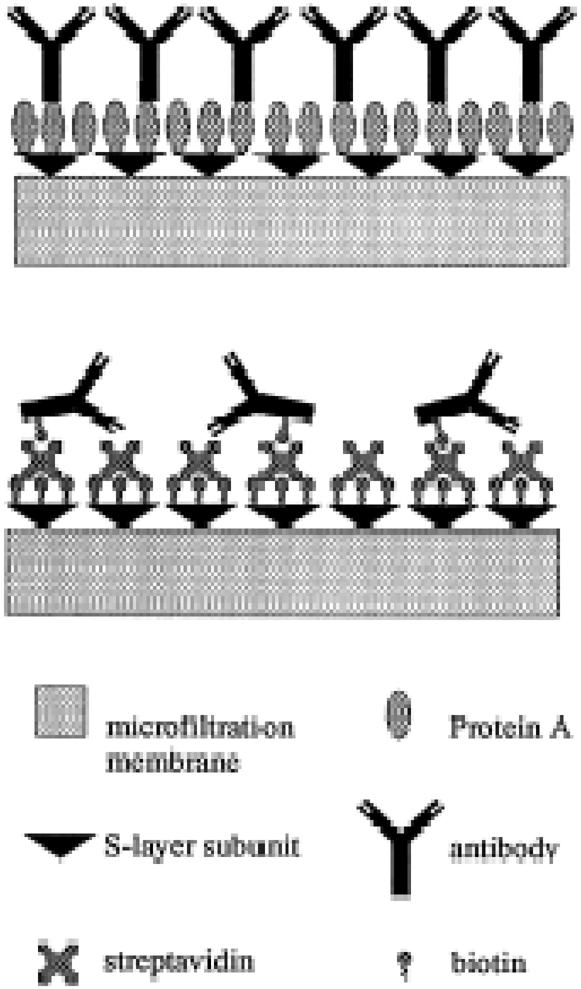
Mineralization using CdS



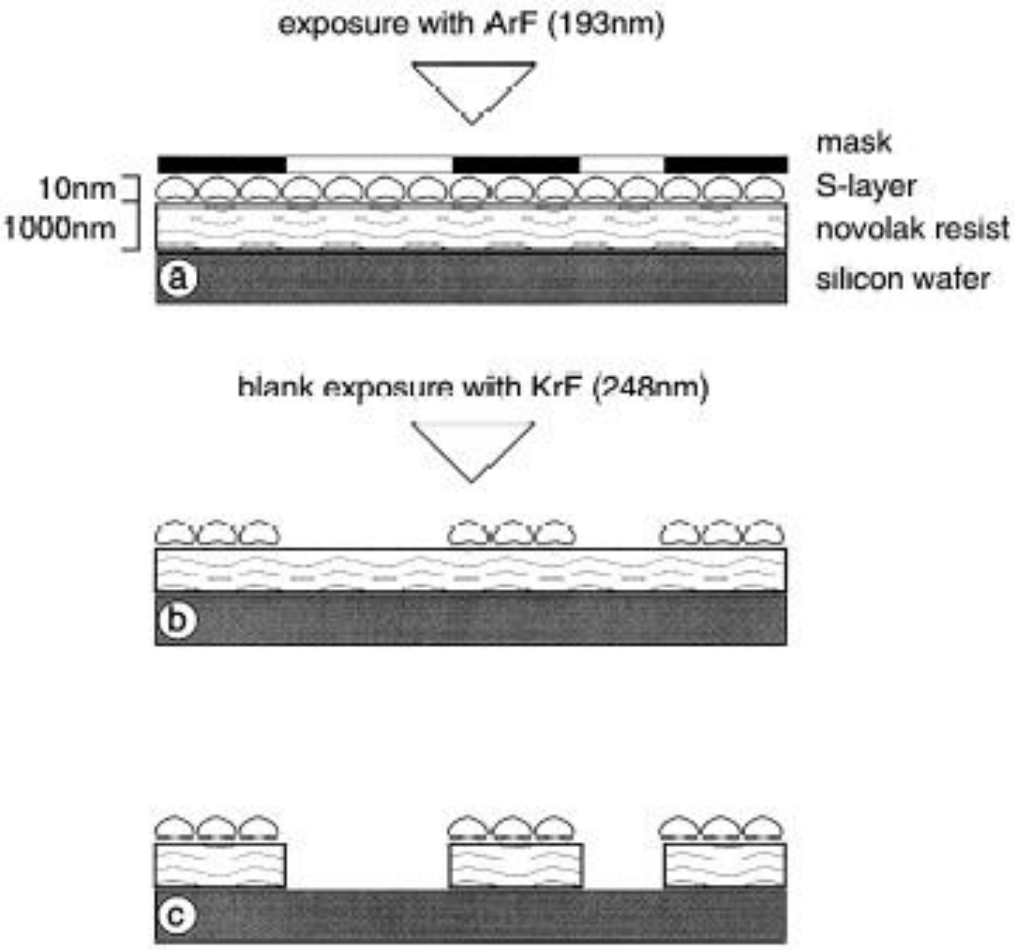


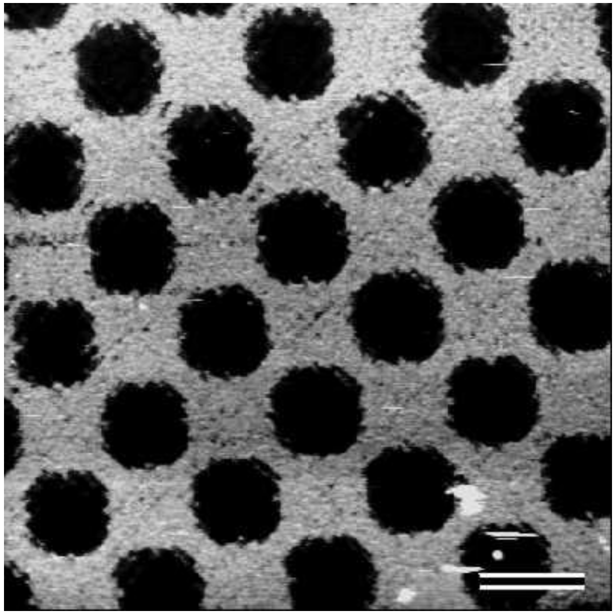
Can also use to pattern and assemble other protein layers

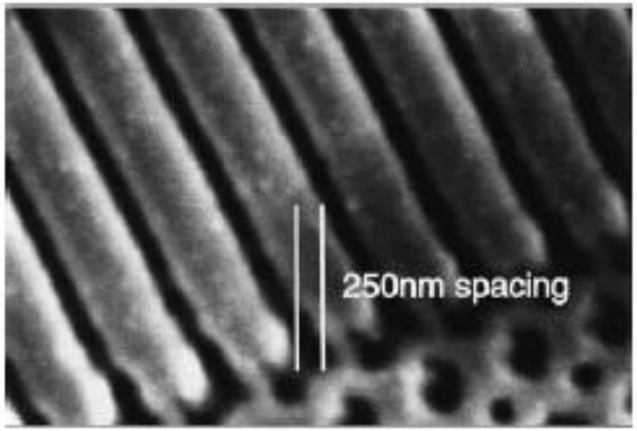




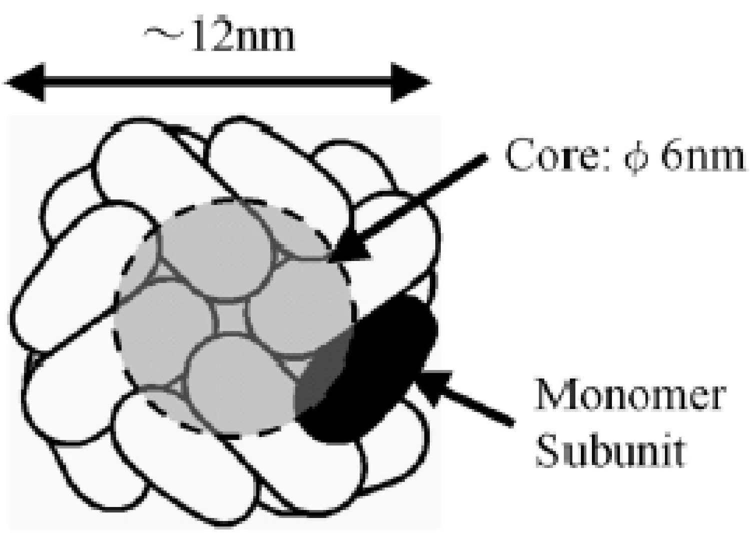
Lithographic methods for nano to micro scale patterning

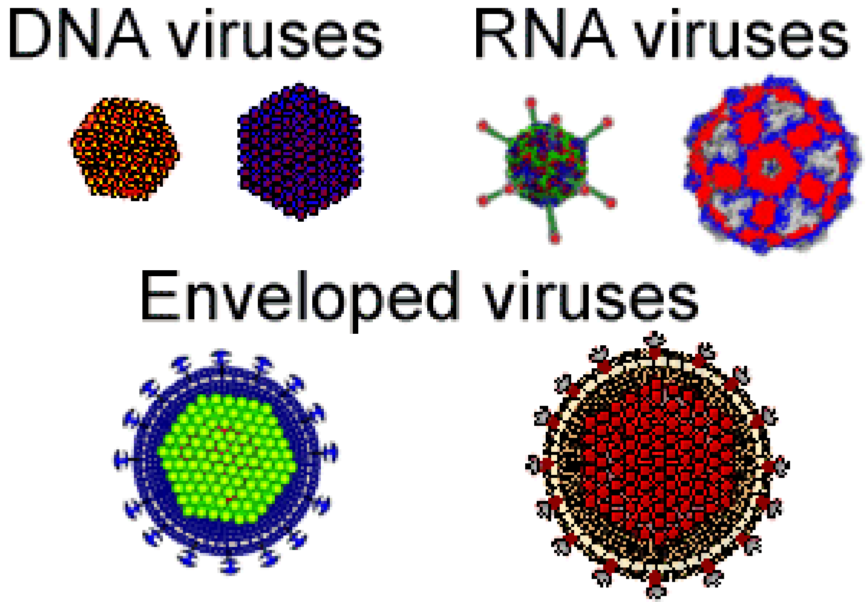


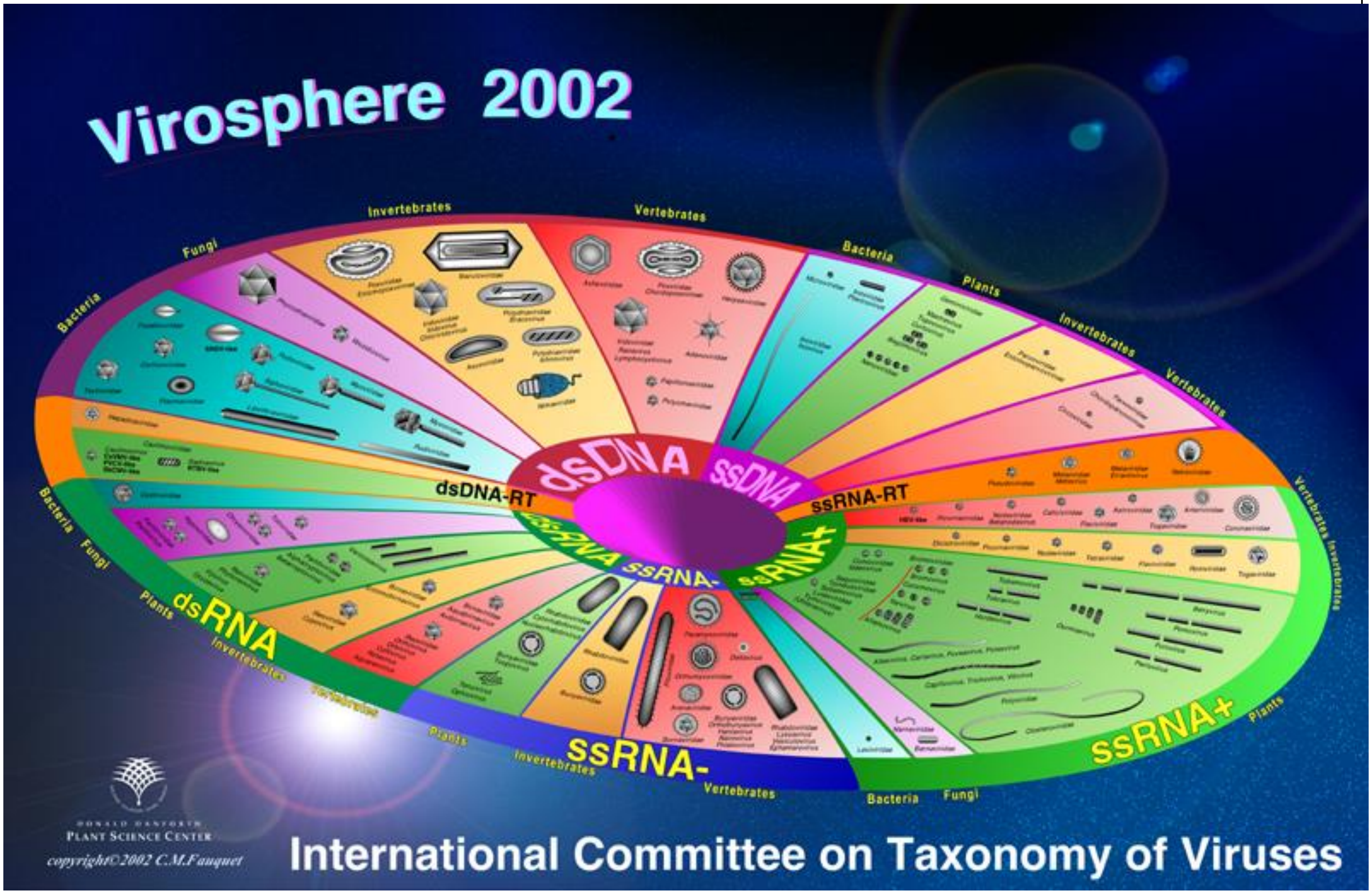




Ferritin and other spherical cages for nanodot formation

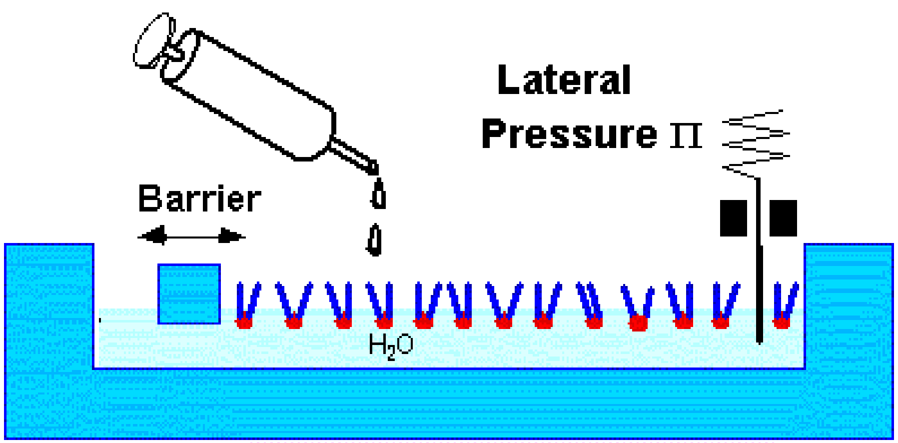


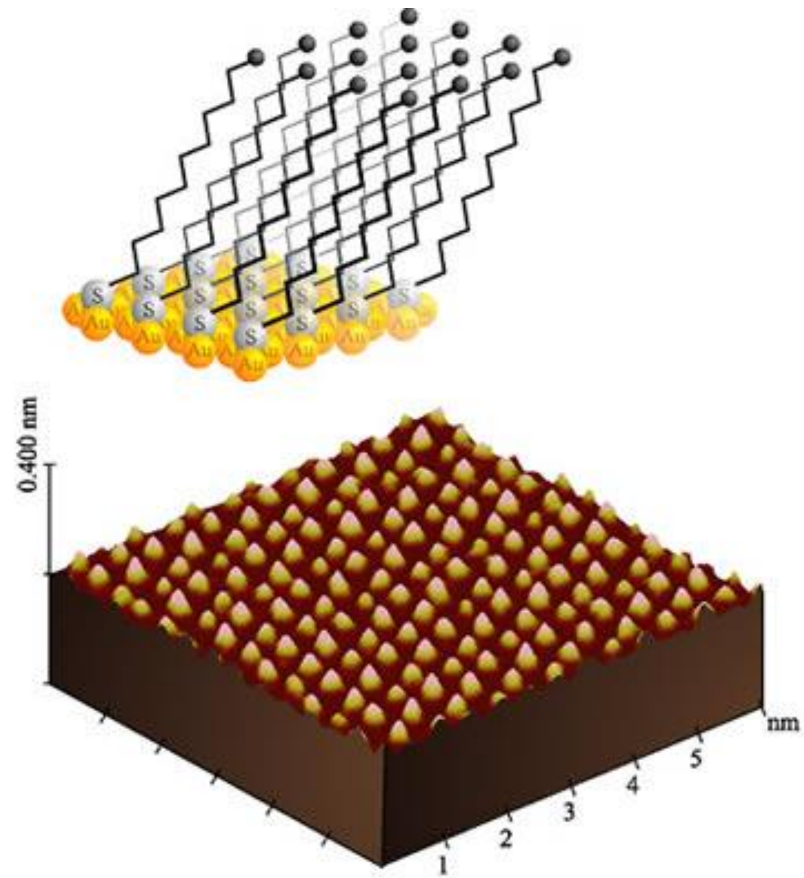




http://www.ncbi.nlm.nih.gov/ICTVdb/

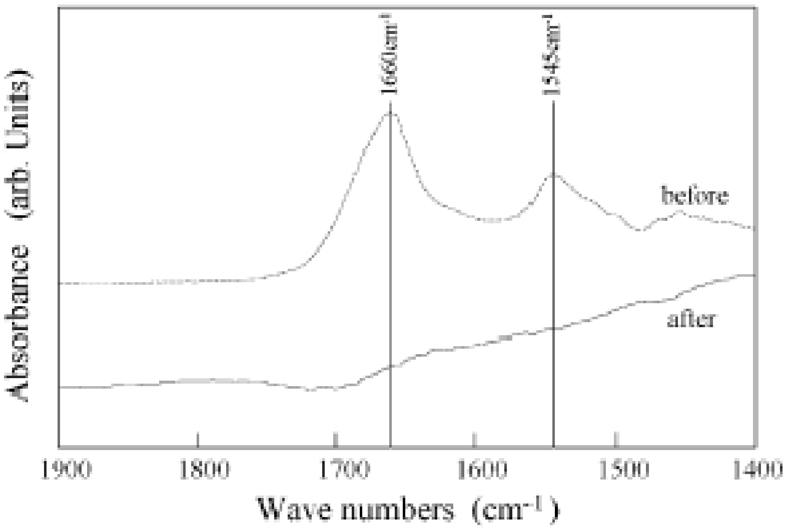
Making a 2D array of nanoparticles using lipids



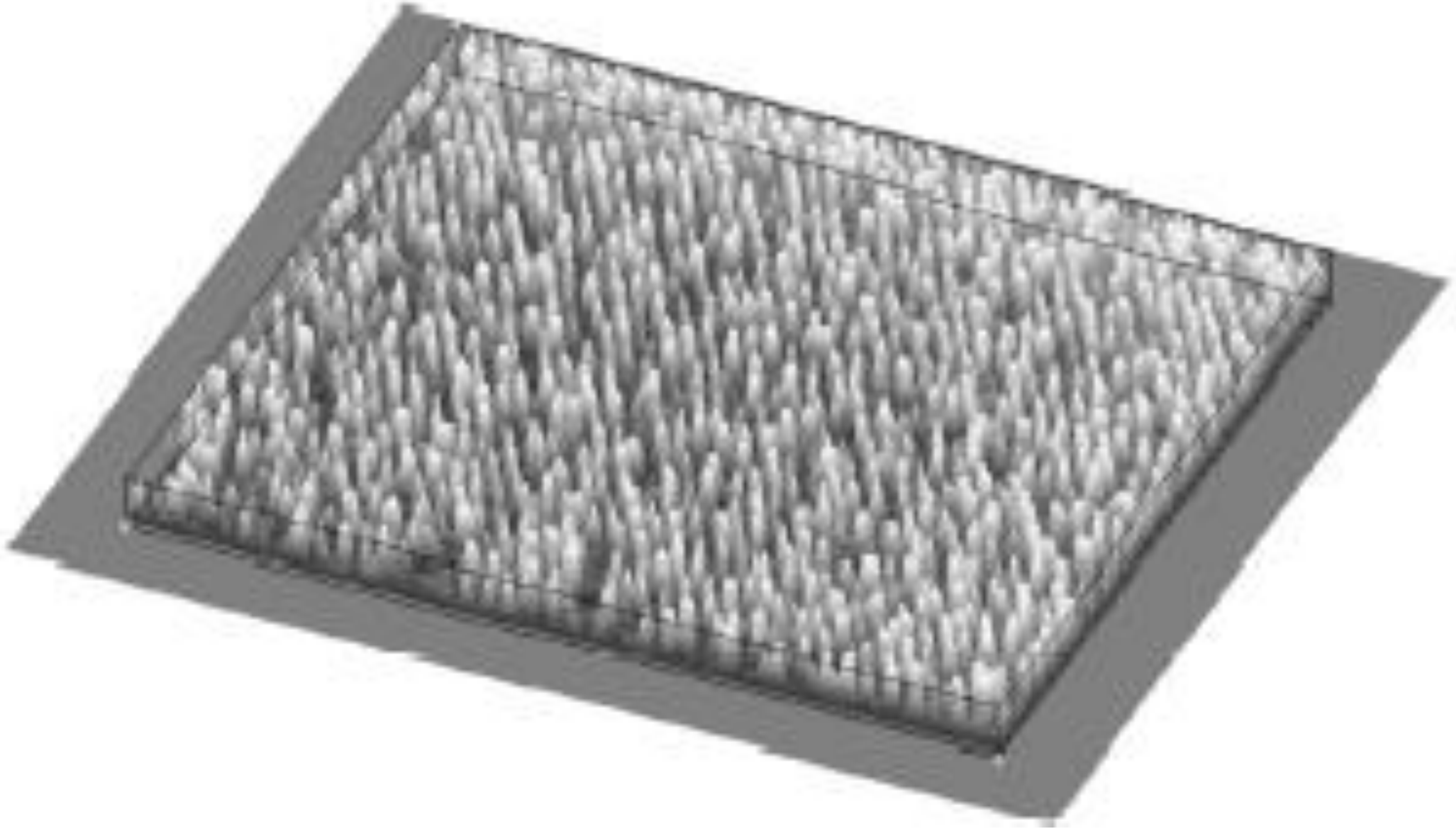


STMによるウンデカンチオール自己組織化単分子膜の観察

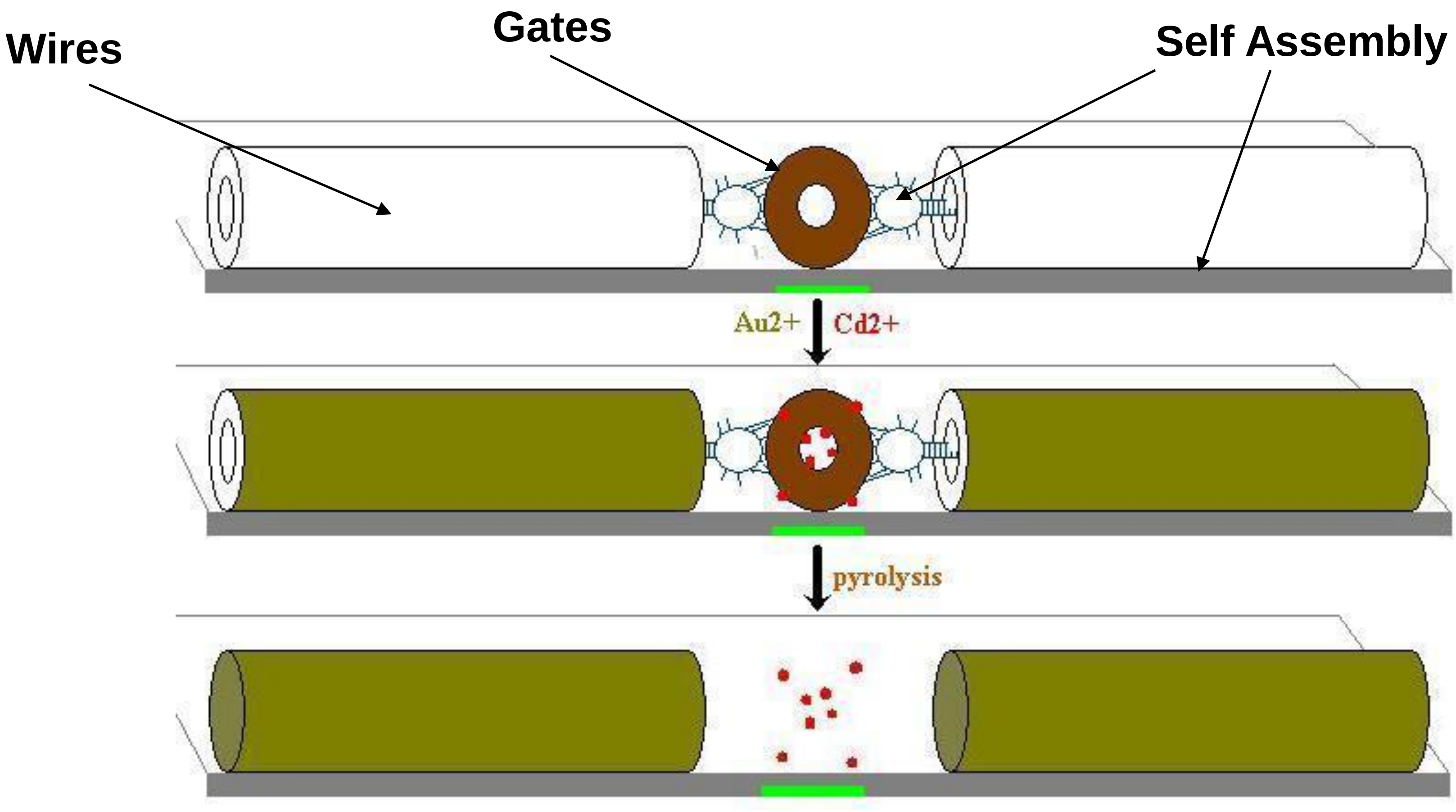
Single Nanodot arrays



Array of 5nm quantum dots from ferritin



Designing a self assembled single electron transistor (SASET)

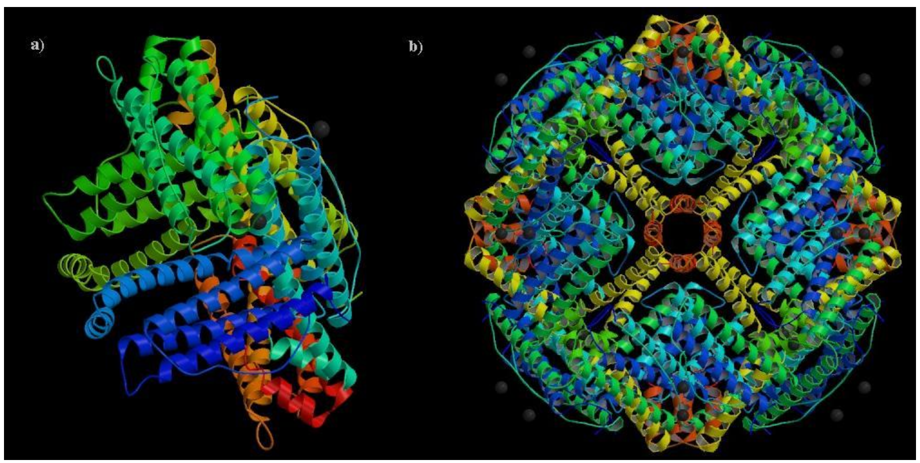


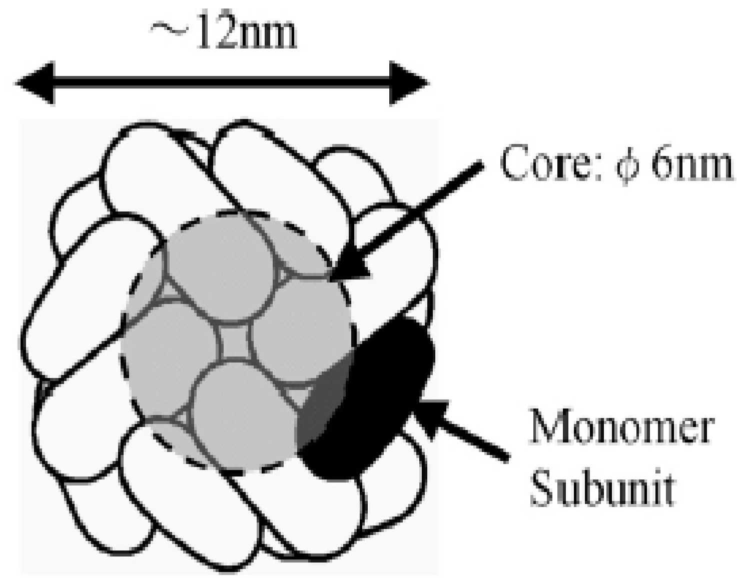
7nm gate and 4 nm interconnects

Designing a self assembled single electron transistor (SASET)

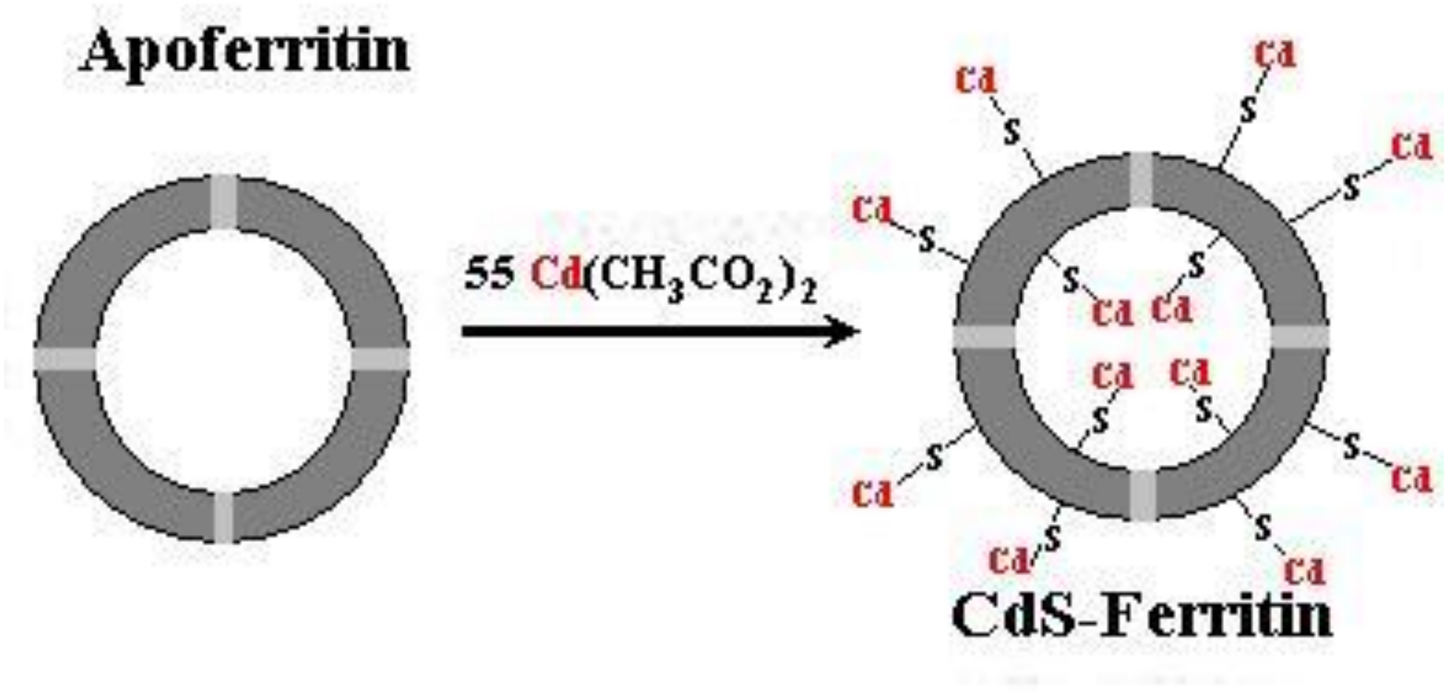
- Nanodots
- Nanowires
- Self assembly

Ferritin

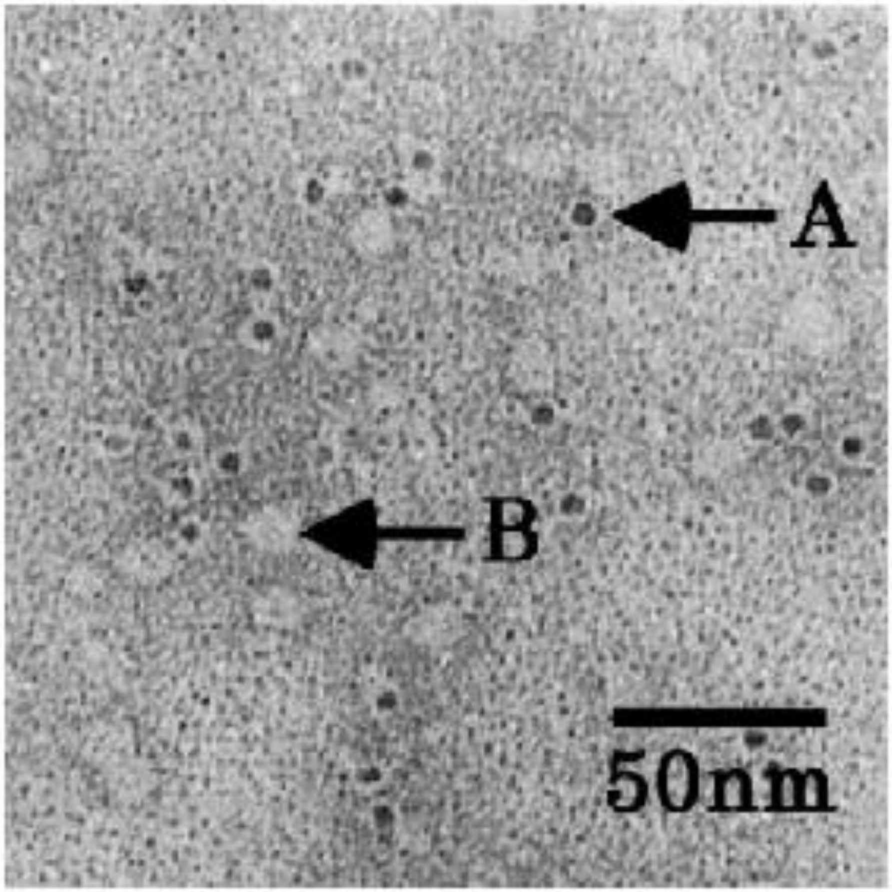


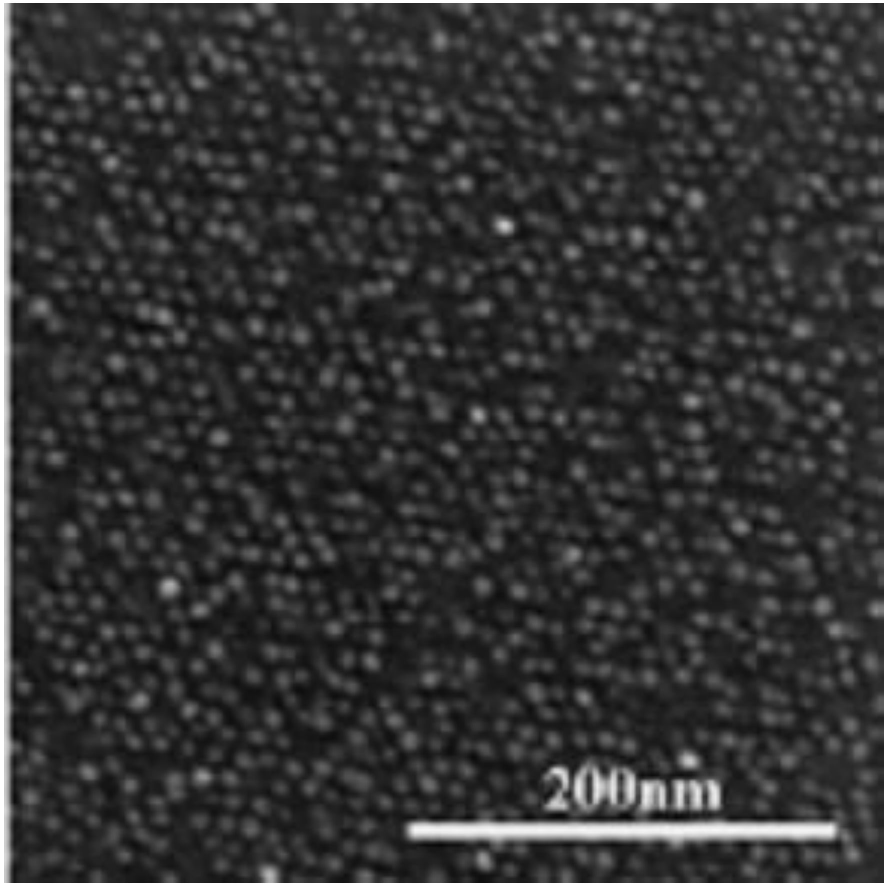


Biomineralization



TEM following mineralization



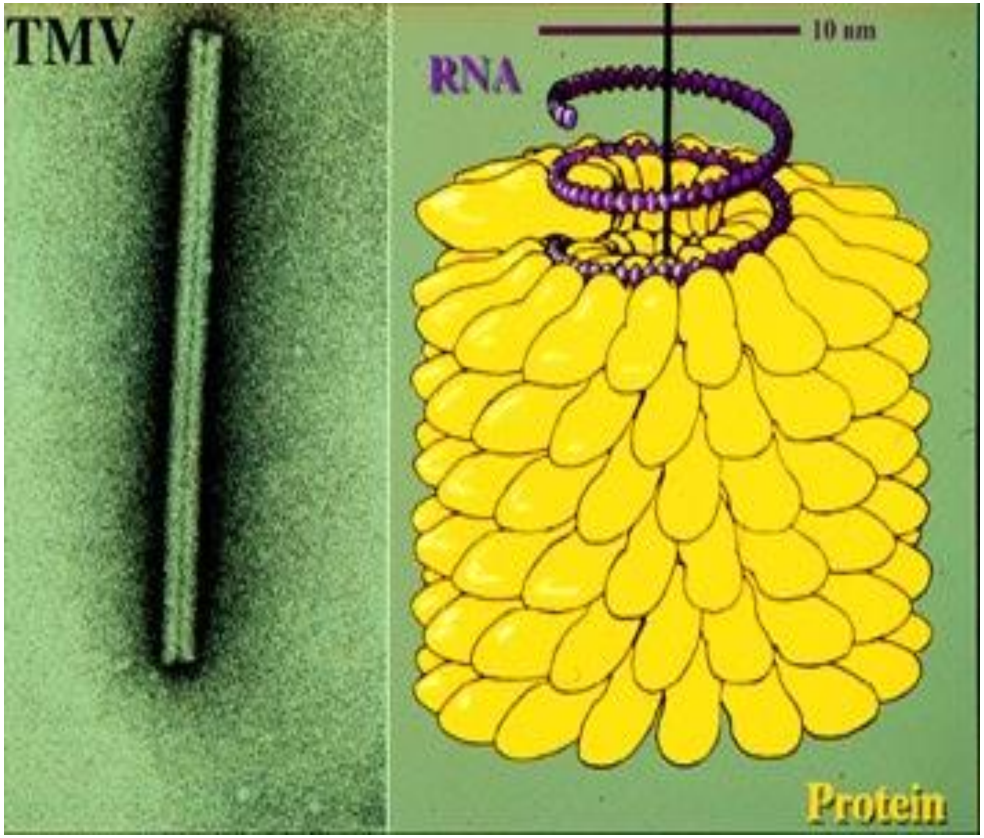


Designing a self assembled single electron transistor (SASET)

- Nanodots
- Nanowires
- Self assembly

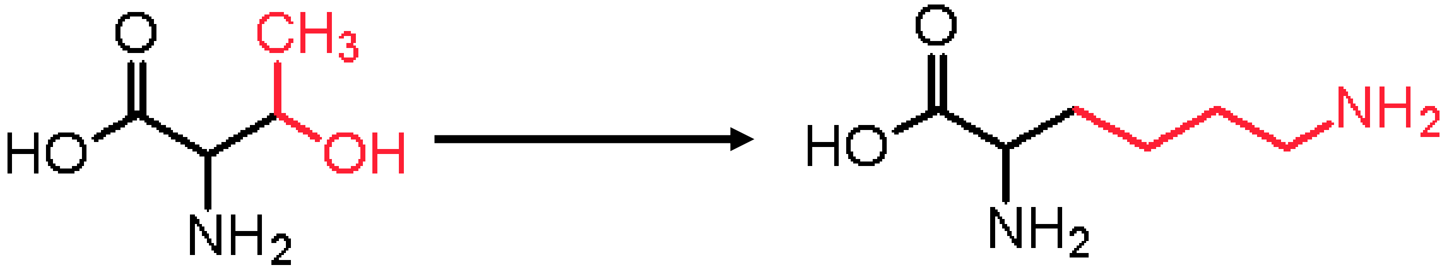
Strategy for wires

- Prototypical tobamovirus
- 18nm diameter 300nm length
- ~2100 identical protein monomers
- 17.5 kD coat protein monomer
- Very stable to a variety of conditions
- Genetically accessible
- Structure of monomer available



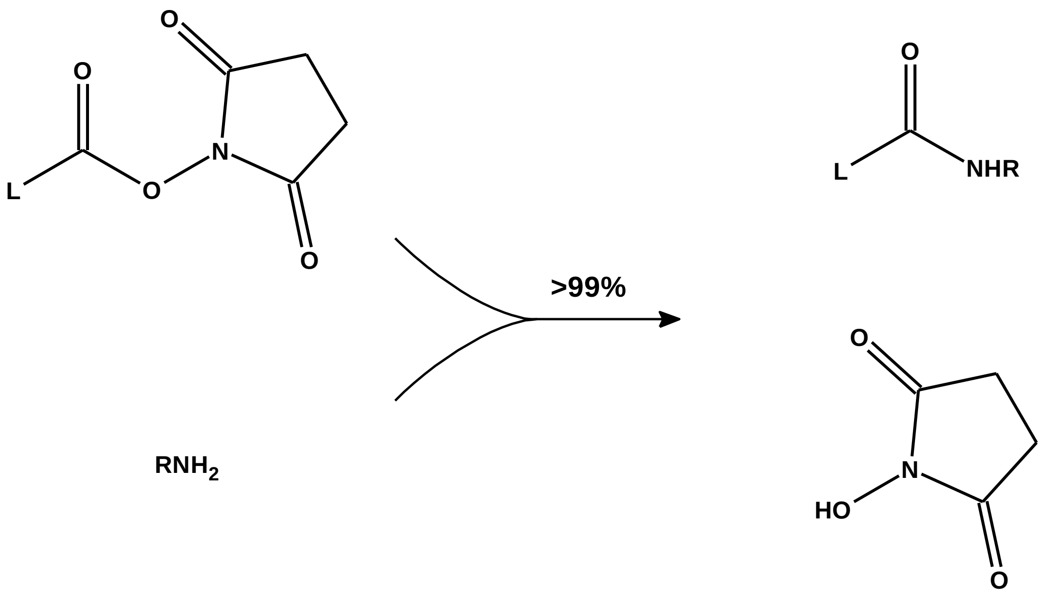
Genetic modification TMV to achieve a chemoselective template.

- **The three potential external labeling positions**
  - **N-terminus**
  - **C-terminus**
  - **63-66 loop**
- **Conversion of Threonine to Lysine**

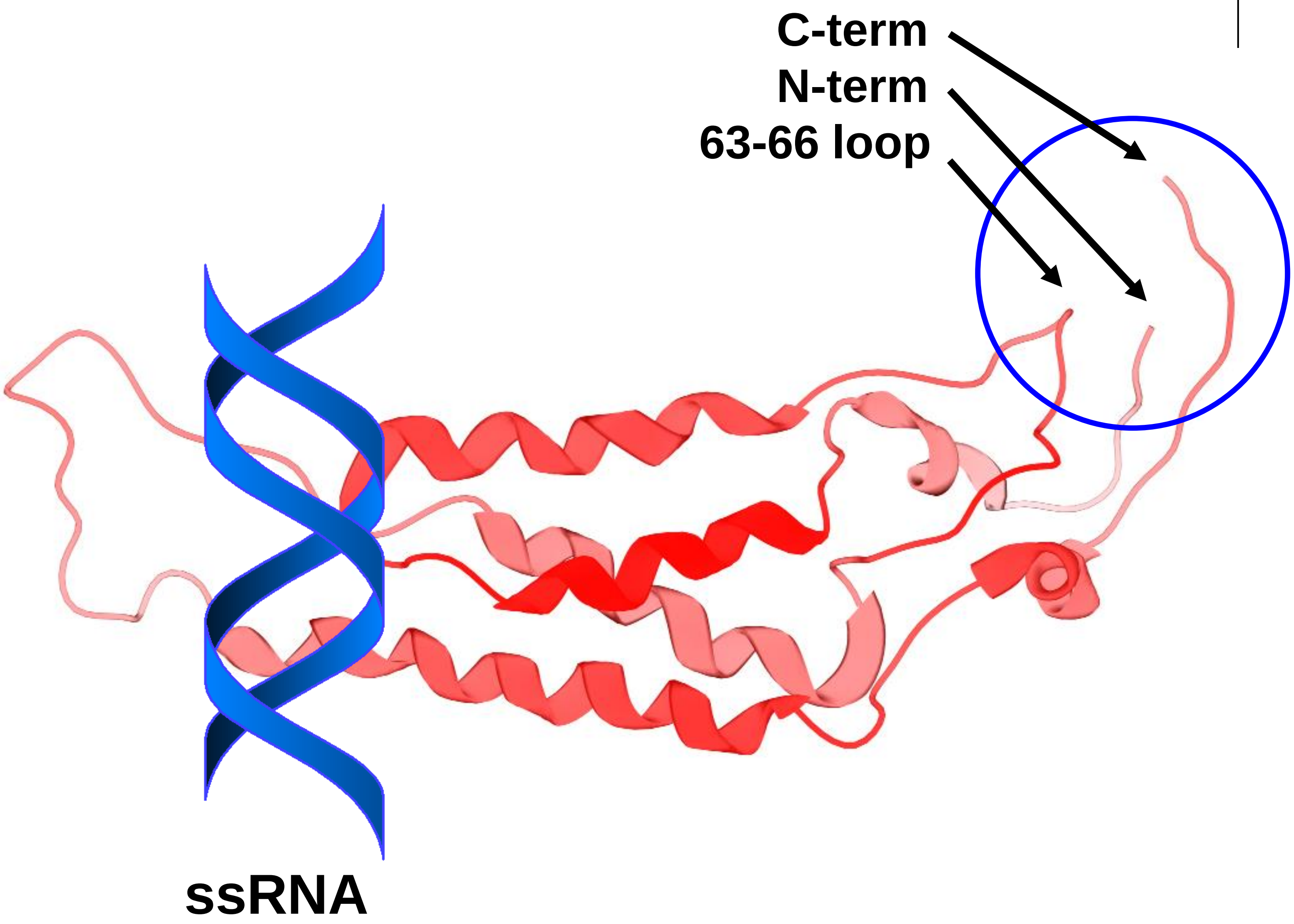


N-Hydroxy-Succinamide

- **Facile**
- **Specific primary amine reactive**
- **Inexpensive**
- **Mild and biocompatible products**
- **Variety of solvent conditions**
  - **Aqueous or non-aqueous**

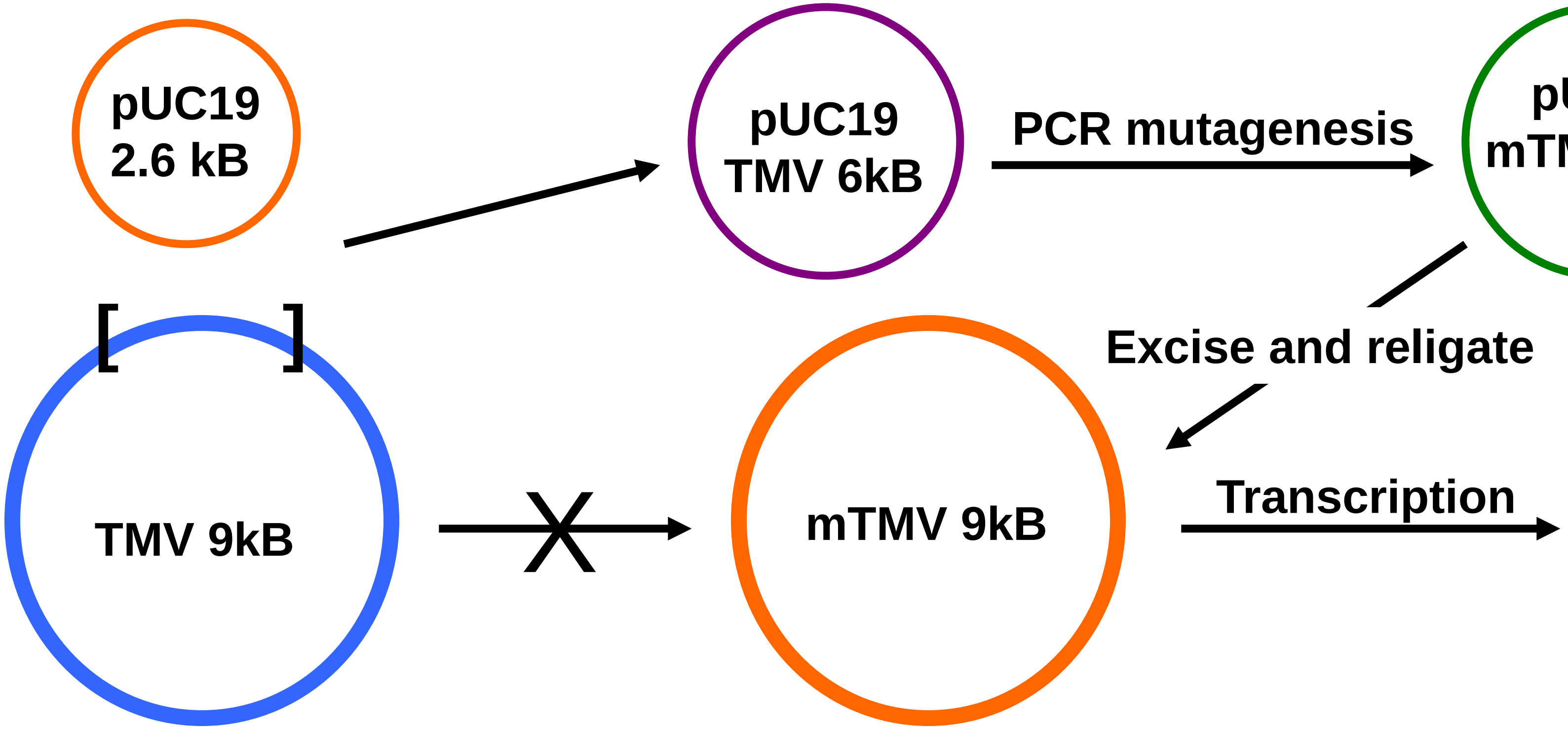


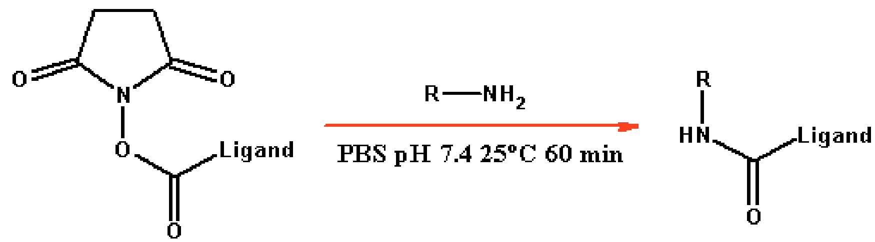
Rational for exterior labeling

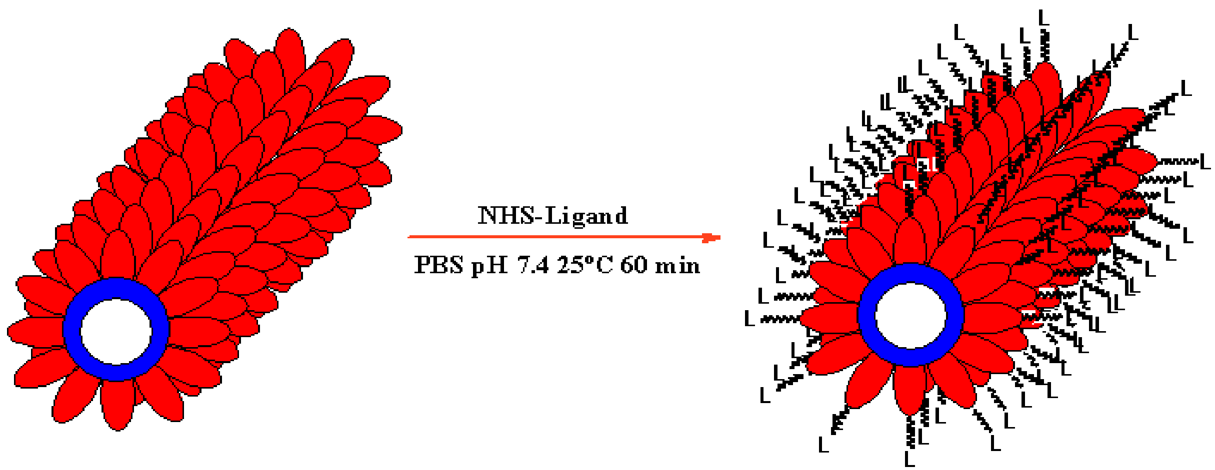


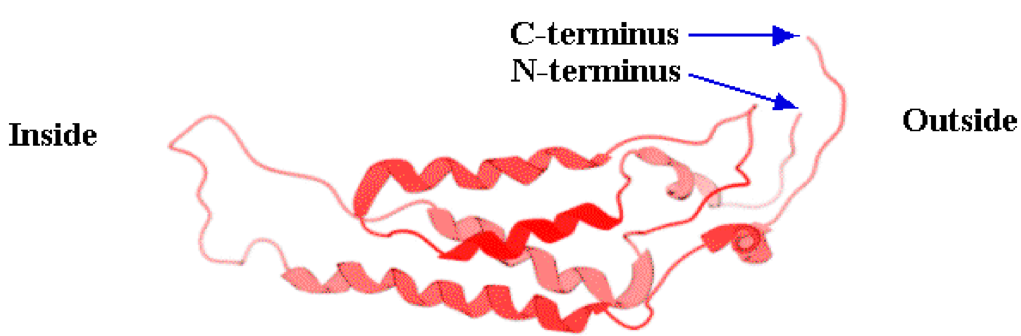
Mutagenesis and expression

- Subcloned coat coding region
- PCR mutagenesis
- Re-ligate back mutated fragment
- Transcription and plant dusting

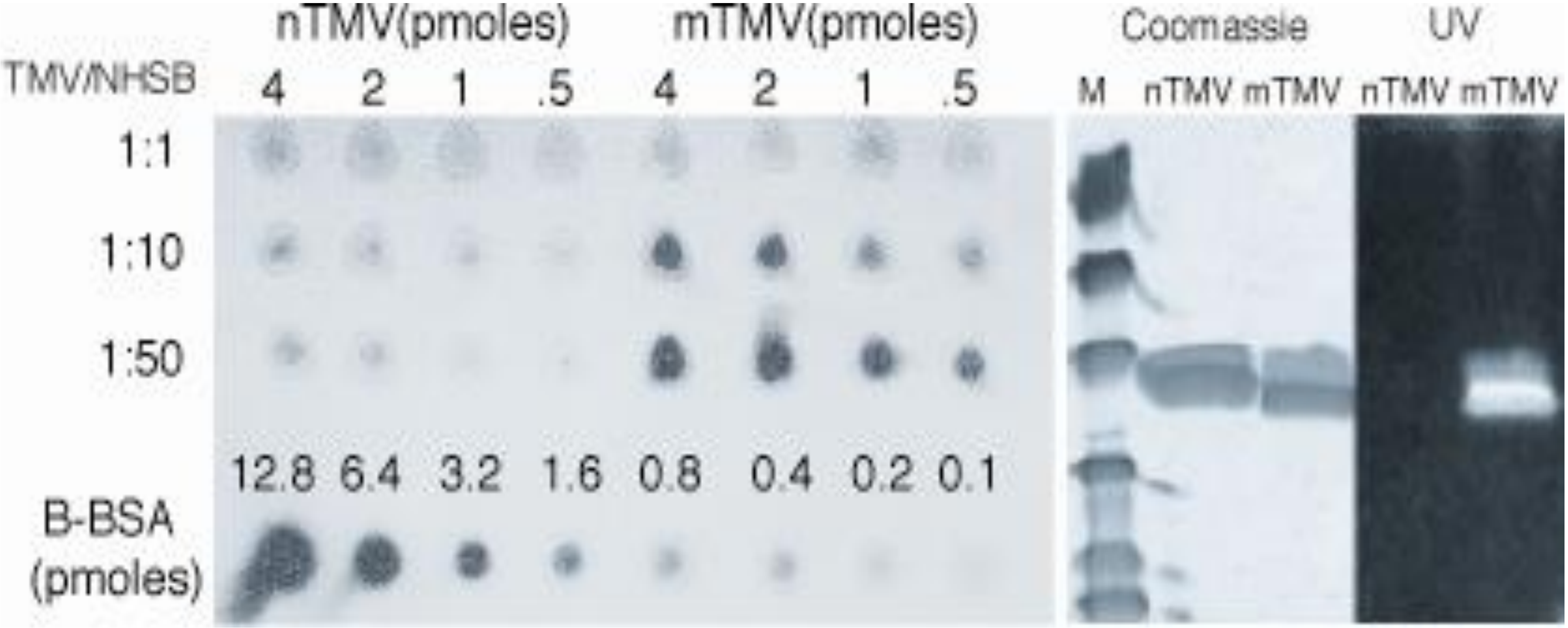



O
O
N
O
Ligand
O
R—NH2
PBS pH 7.4 25°C 60 min
R
HN
Ligand
O


NHS-Ligand
PBS pH 7.4 25°C 60 min


C-terminus
N-terminus
Inside
Outside

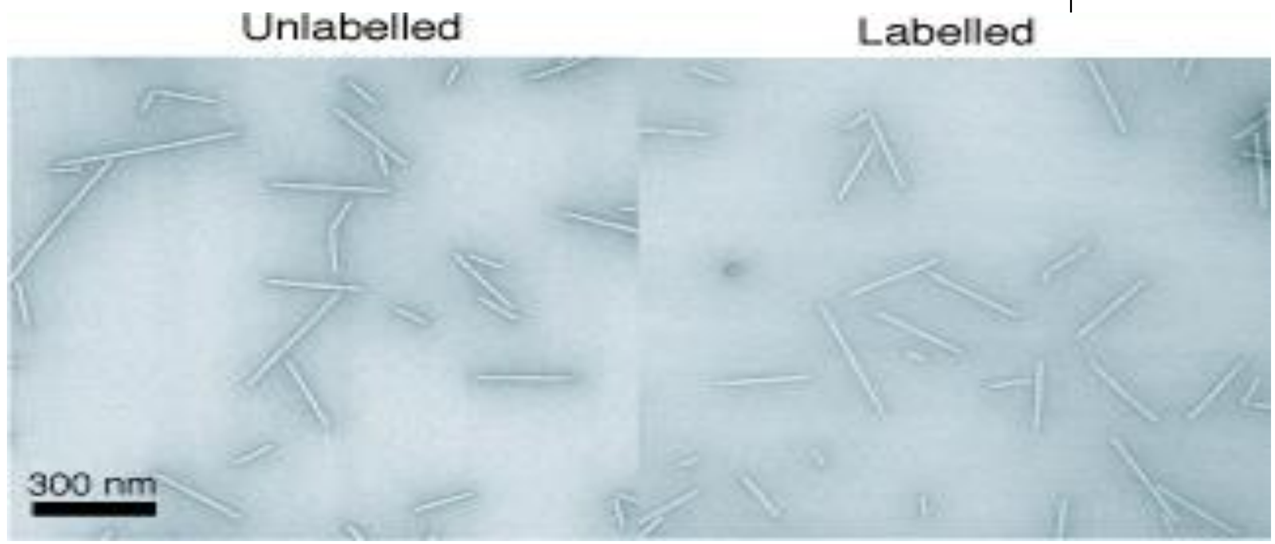
Quantification of labeling



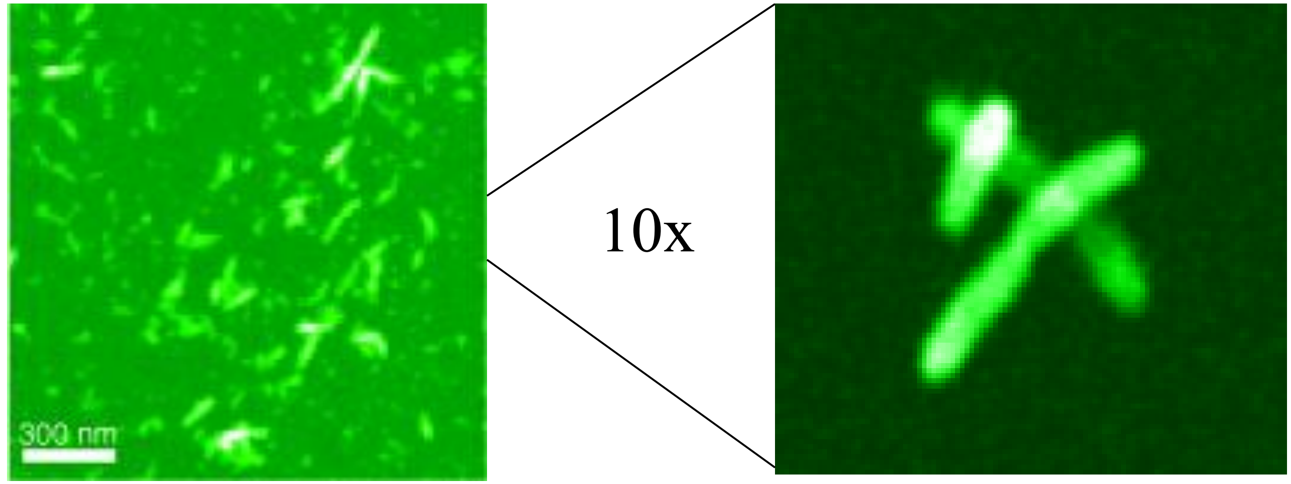
- **Quantitative labeling of mutant TMV (mTMV) 106+/- 12%**
- **No labeling of native TMV (nTMV)**

Fluorescent labeling

EM

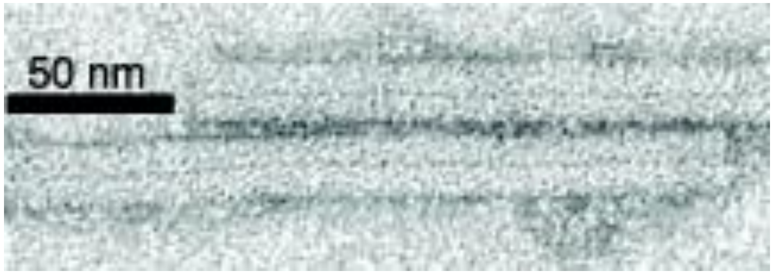


Confocal



Ordered assembly of avidin on biotin TMV

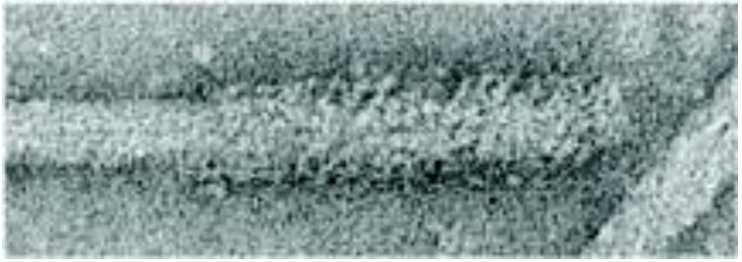
Biotin labeled



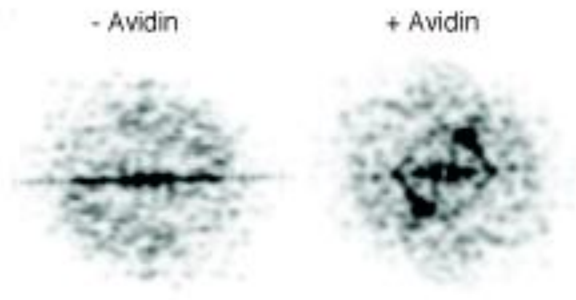
+Avidin

+Avidin

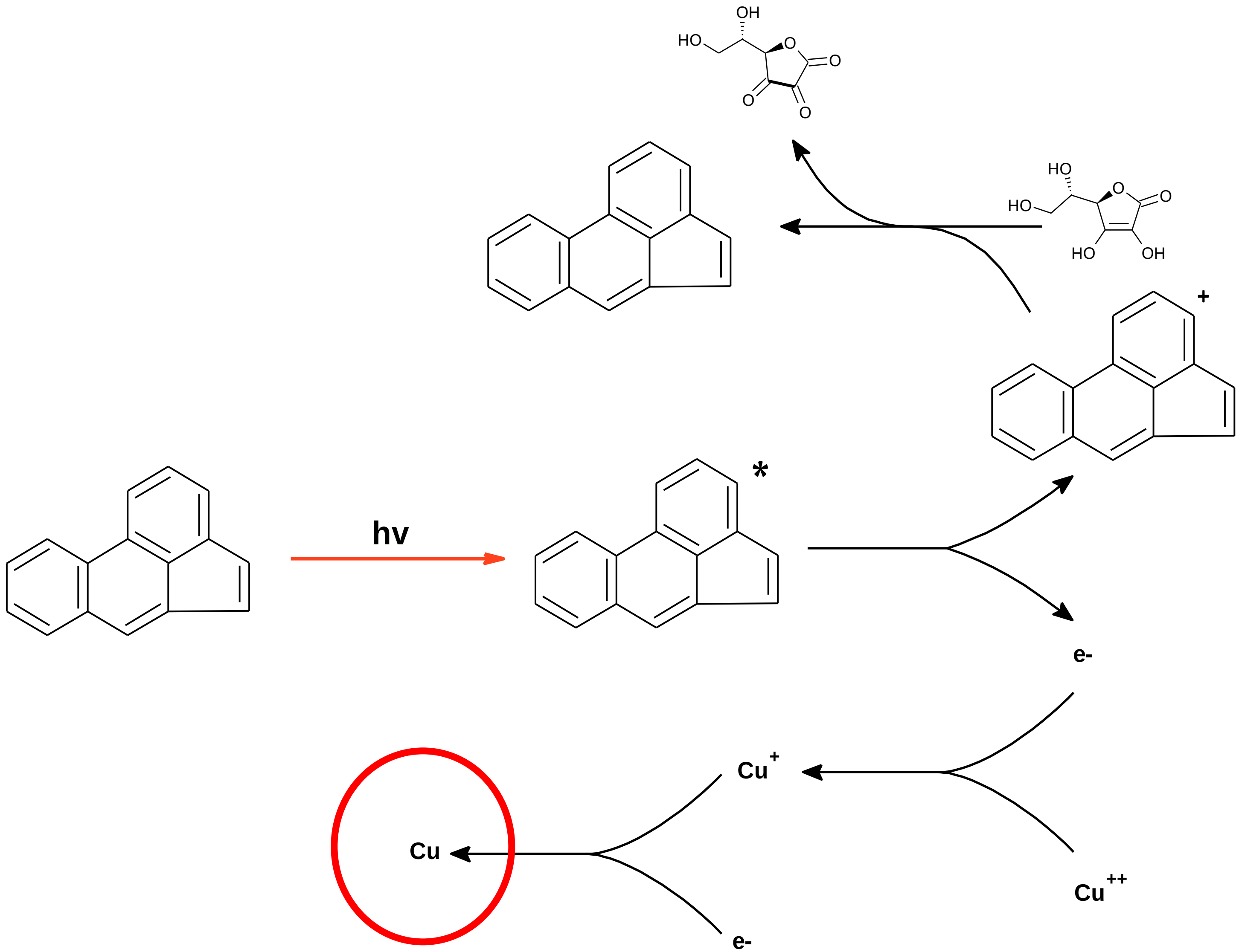
+Avidin



Computed diffraction pattern from avidin treated biotin labeled TMV

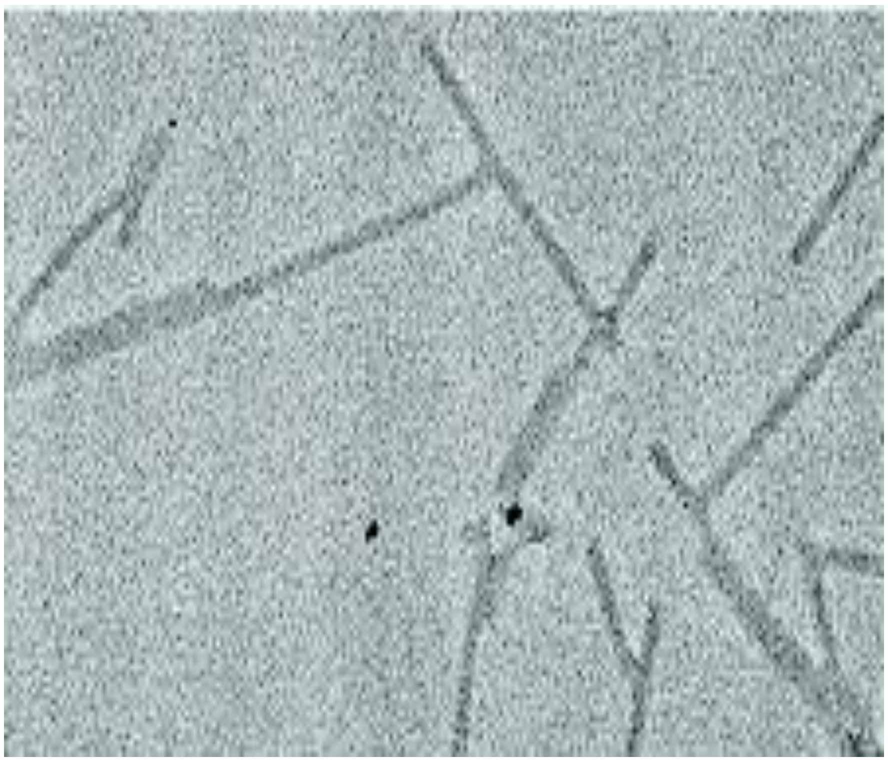


Metal coating via in situ photoreduction

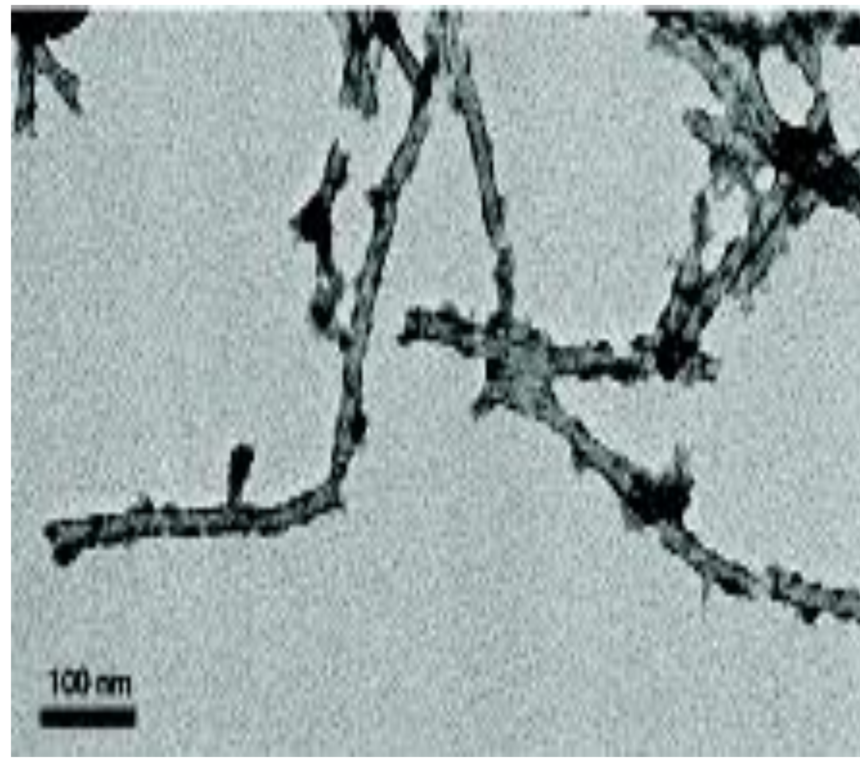


Metal coated TMV

-hν

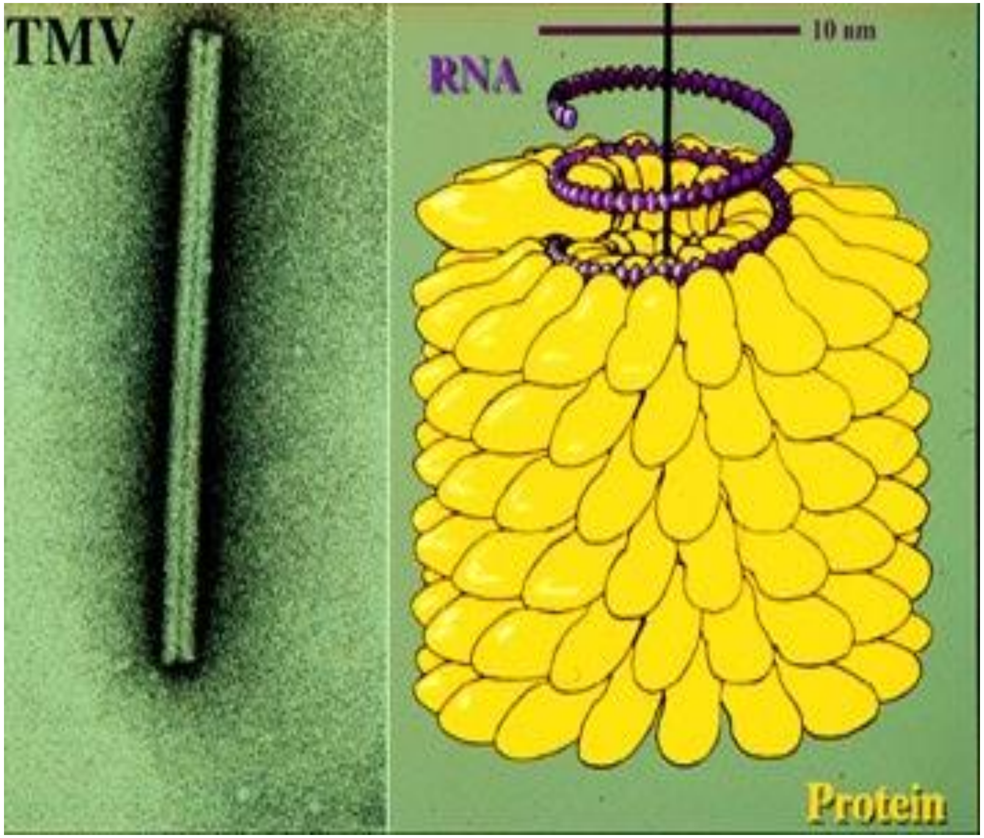


+hν



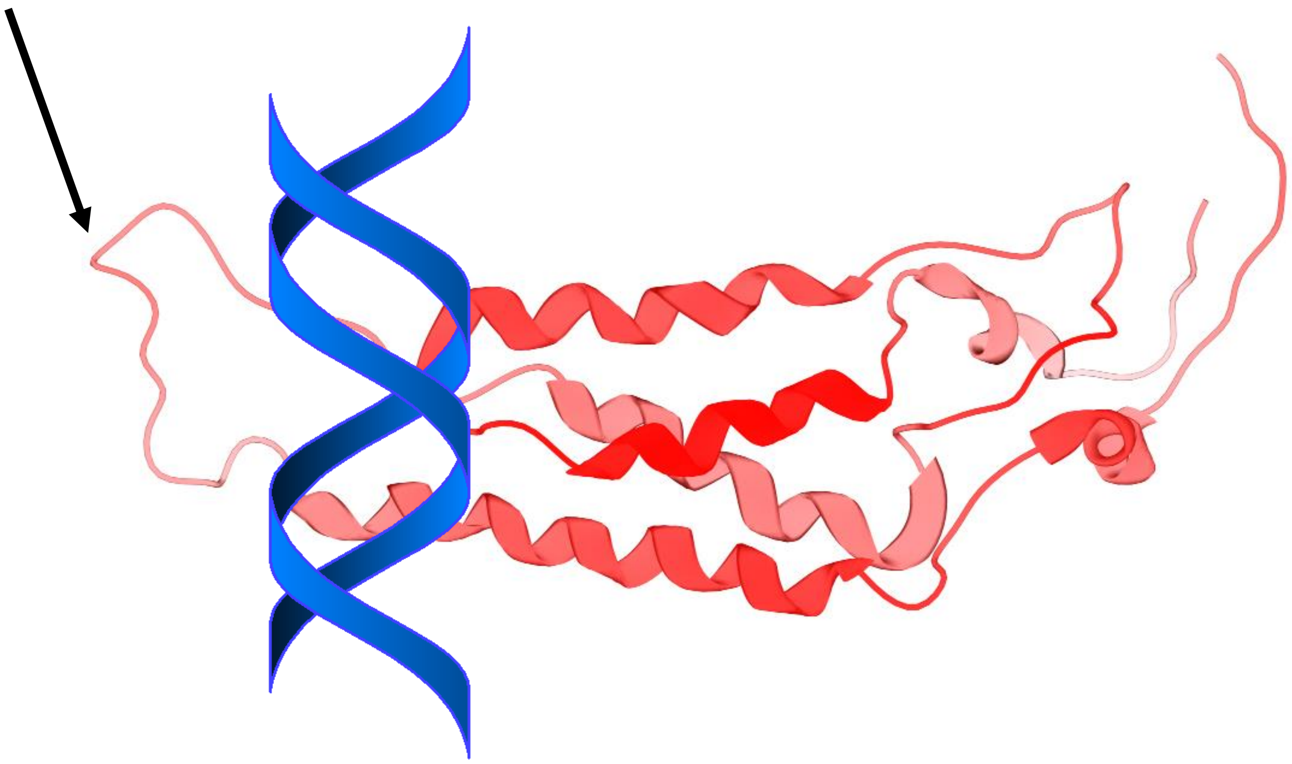
Tobacco Mosaic Virus

- Prototypical tobamovirus
- 18nm diameter 300nm length
- ~2100 identical protein monomers
- 17.5 kD coat protein monomer
- Very stable to a variety of conditions
- Genetically accessible
- Structure of monomer available
- Internal diameter ~4nm



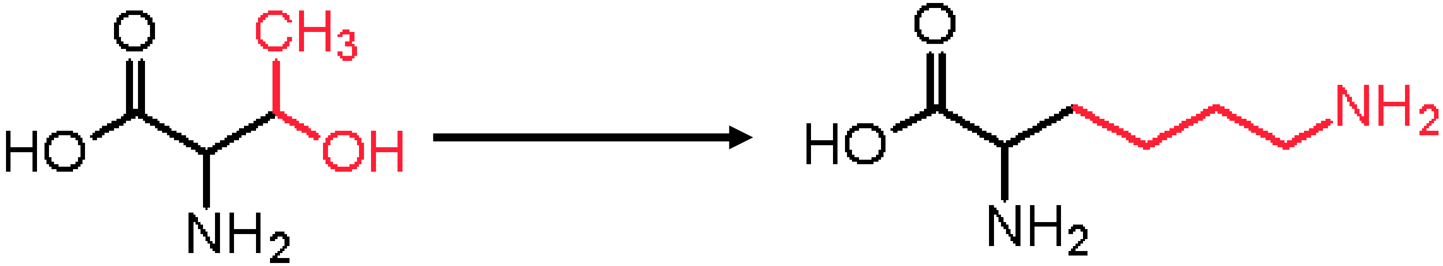
Can we apply a similar strategy for labeling the interior of TMV (4nm)

2 internal Threonines



ssRNA

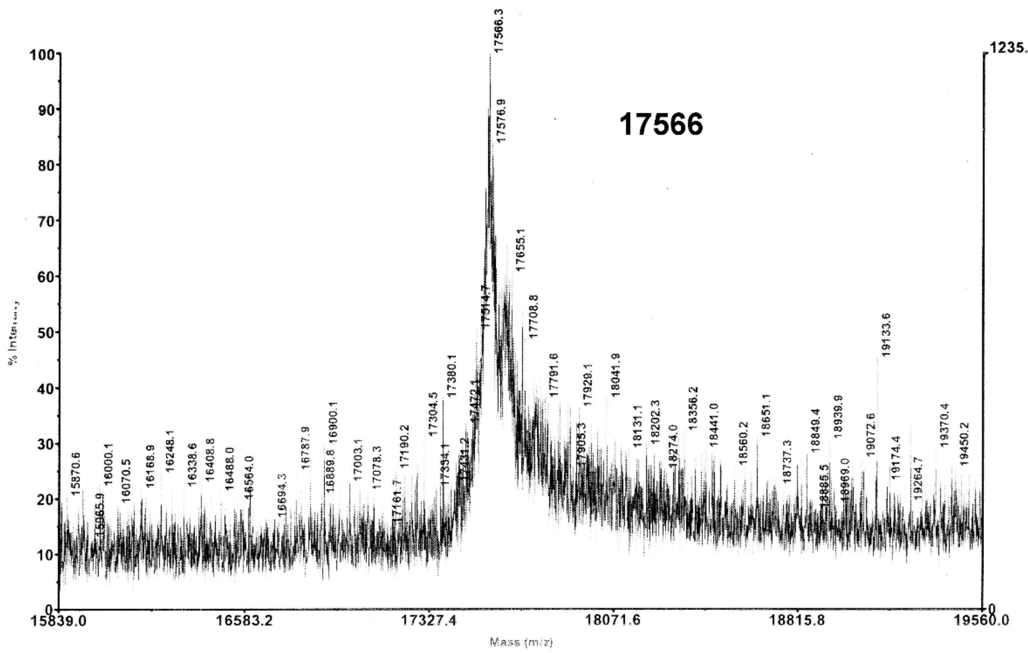
Repeat the similar mutation strategy for internal Threonines

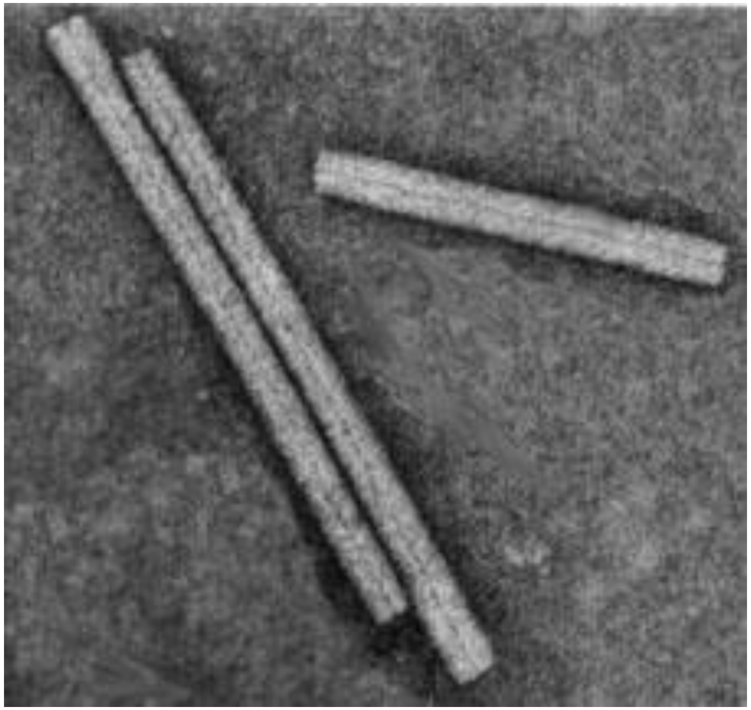


Mass difference 27.1

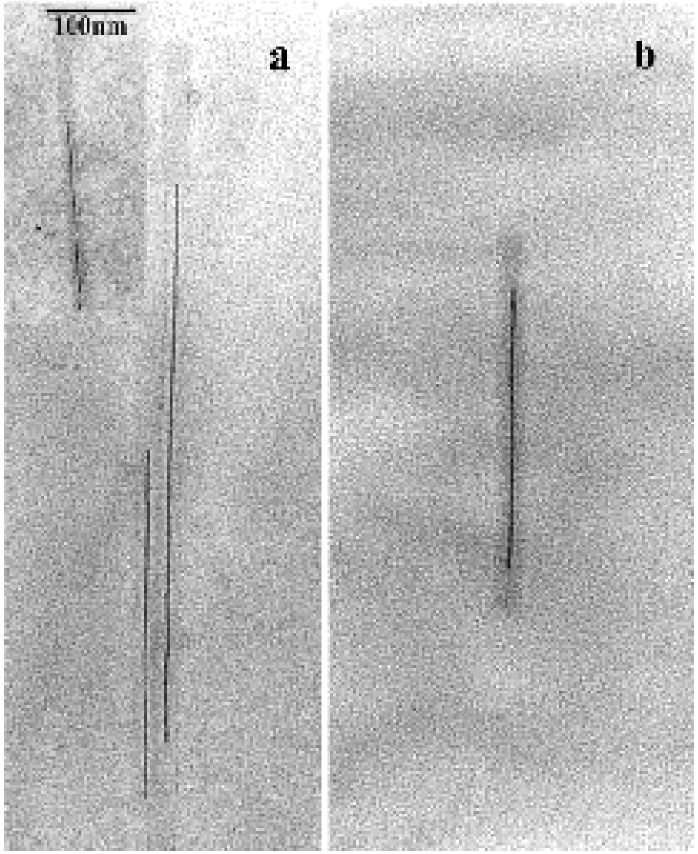
Confirmed by MALDI-TOF

Native expect 17529
Mutant expect 17566





3nm wires on the inside of TMV



Activation: $Pd^{2+} \rightarrow Pd^{2+}_{ads}$ [and $Pd^{2+} + 2\,e^{-} \rightarrow Pd$]

Deposition: $3Ni^{2+} + (CH_3)_2NHBH_3 + 3H_2O \rightarrow$

$3Ni + B(OH)_3 + (CH_3)_2NH_2^{+} + 5H^{+}$

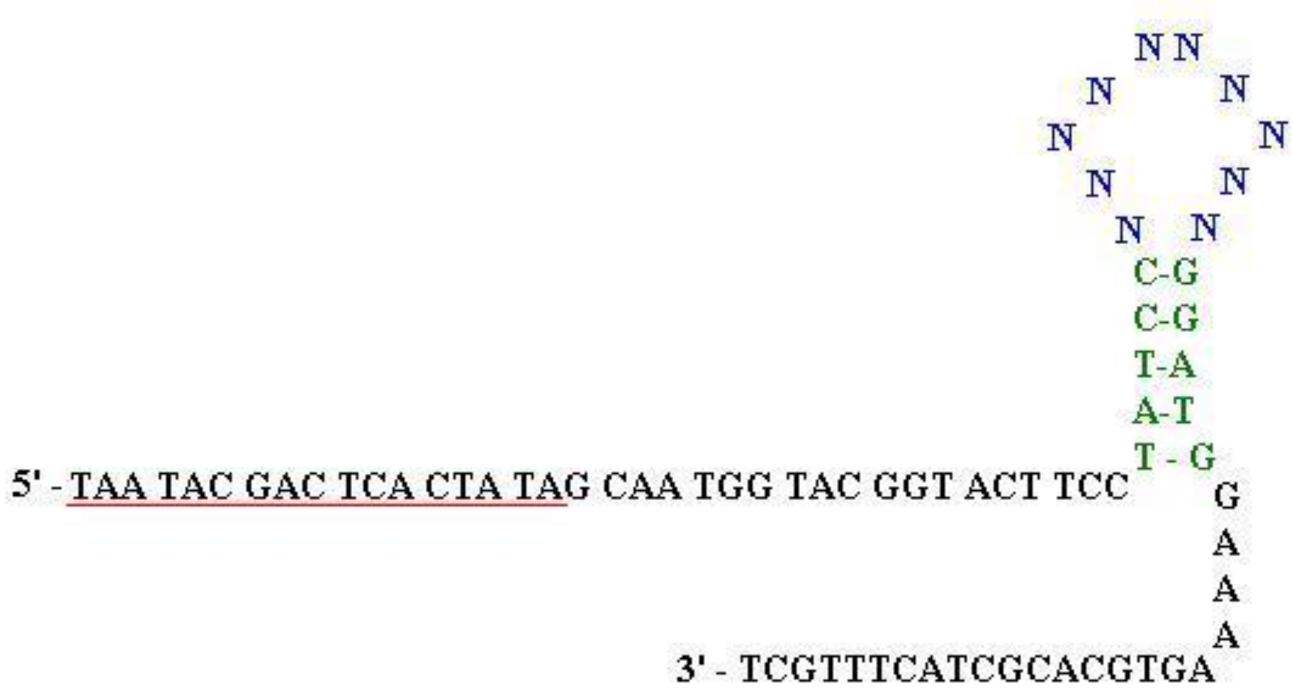
Designing a self assembled single electron transistor (SASET)

- Nanodots
- Nanowires
- Self assembly

Strategy

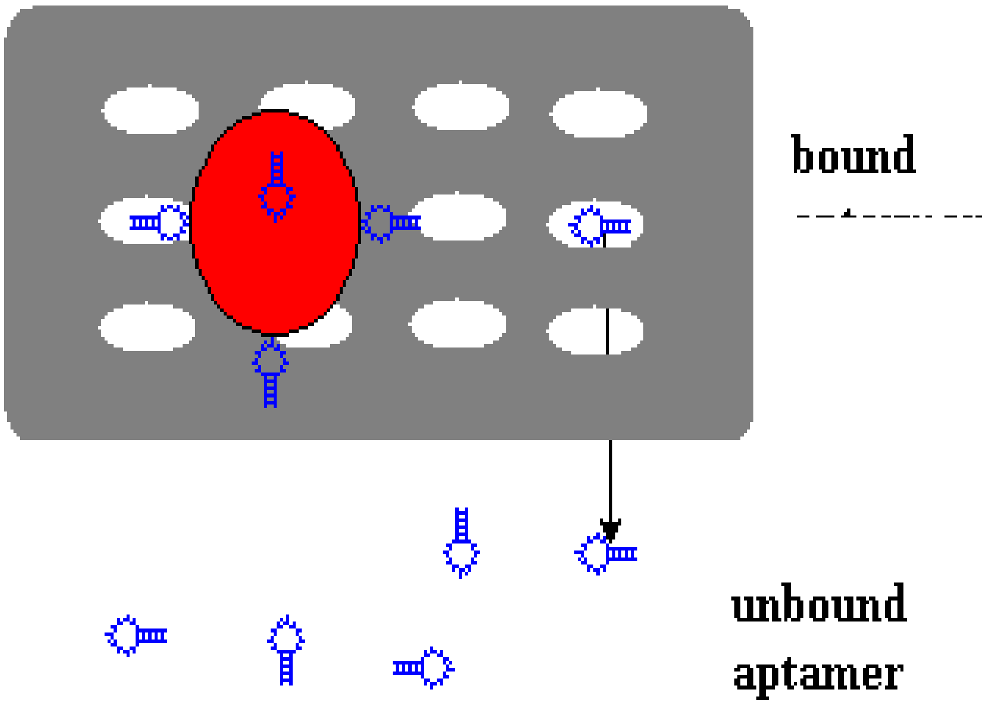
- Use SELEX to determine RNAs with high affinity to Ferritin
- Introduce such sequences into TMV RNA
- Test directed assembly

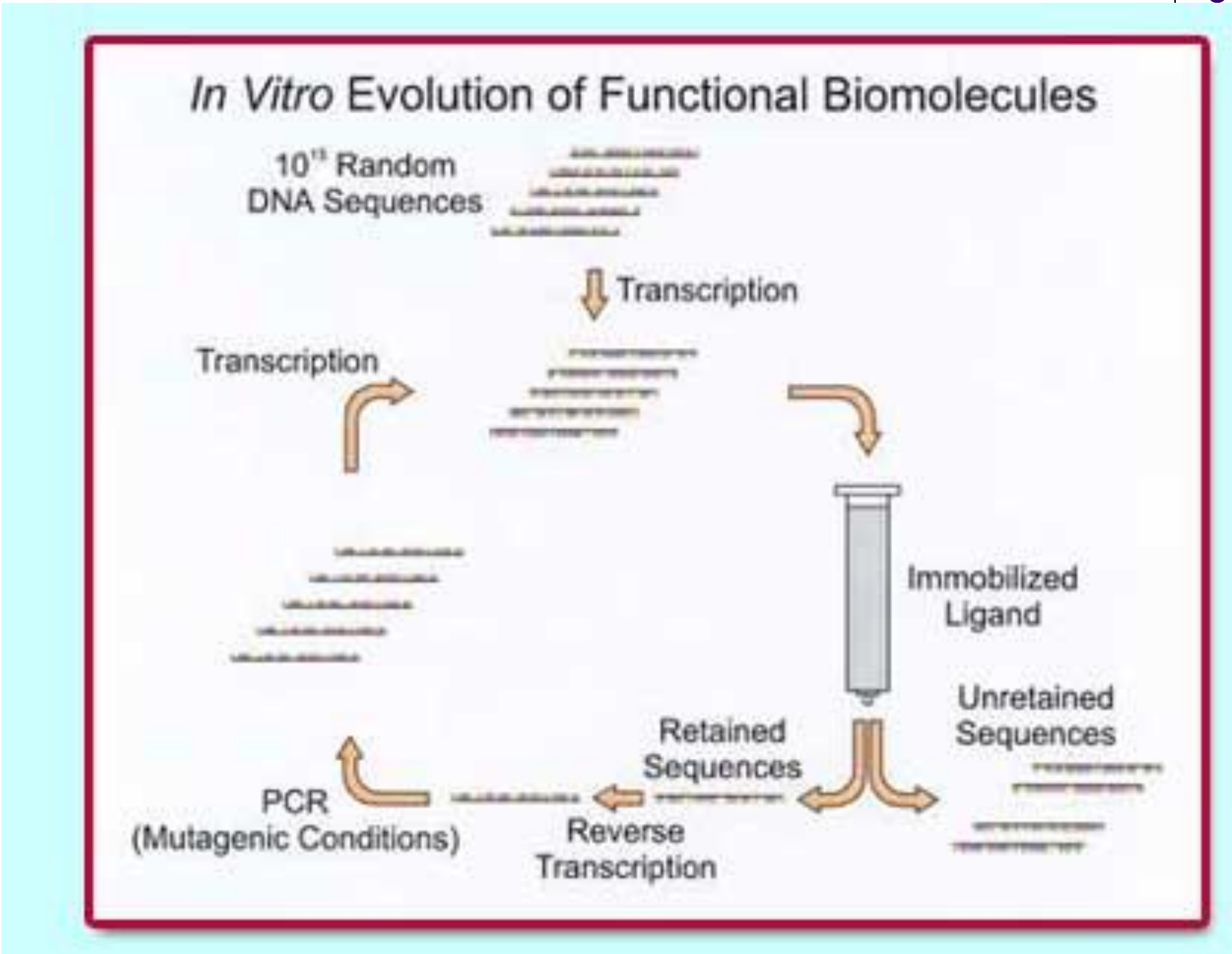
SELEX Systematic Evolution of Ligands by EXponential enrichment” (Tuerk and Gold, 1990)



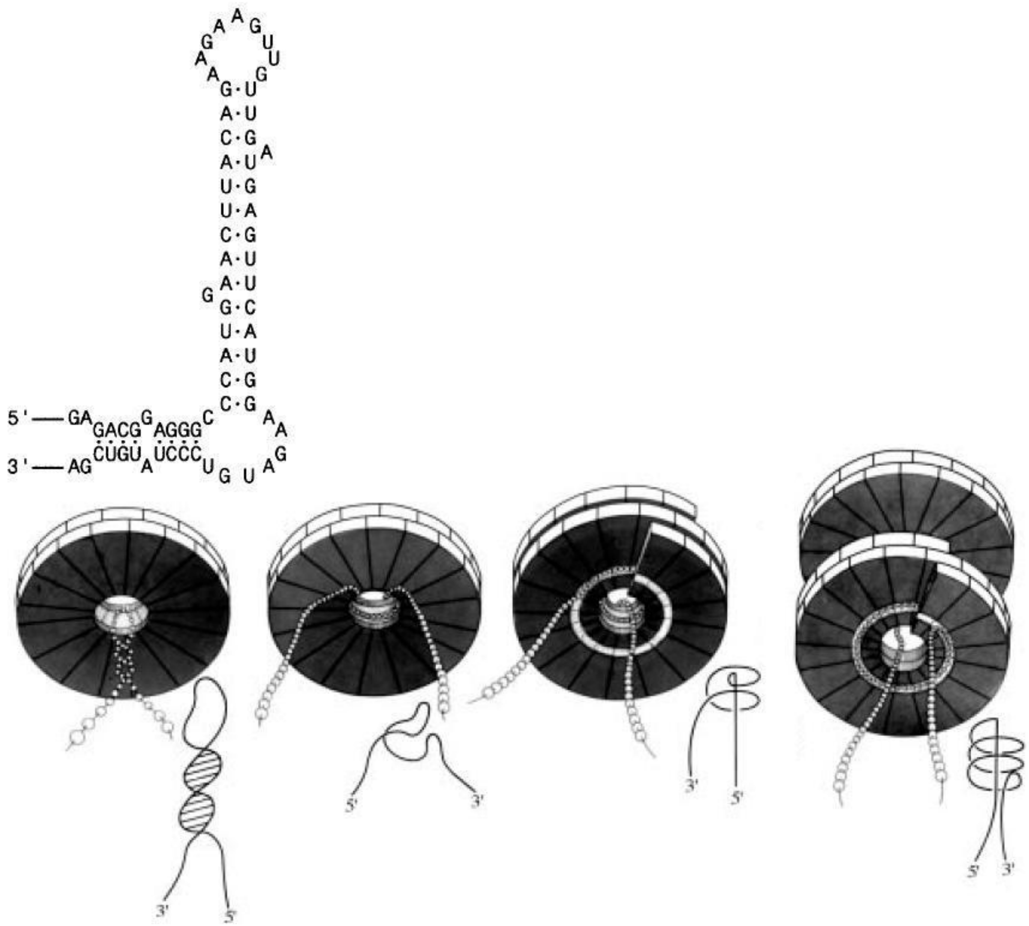
10 random positions and 4 possible bases 4^{10} or > 10^6 different sequences

Panning and selection

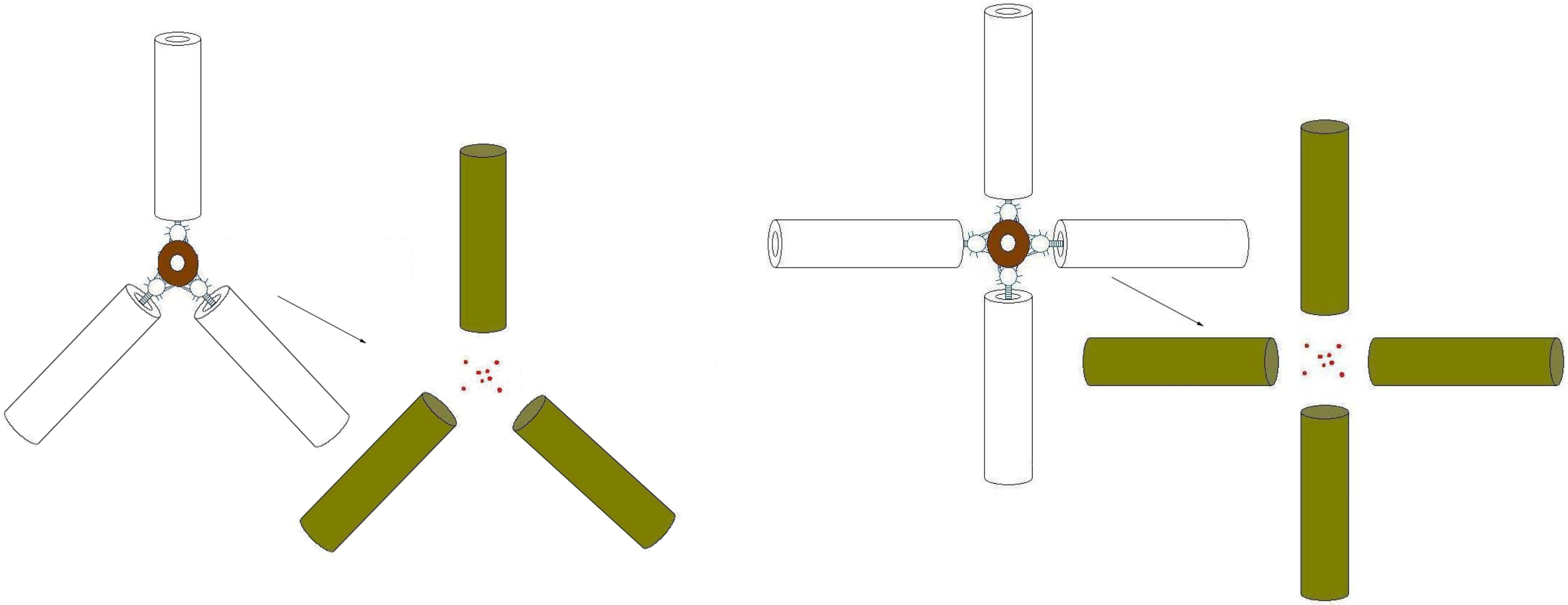



In Vitro Evolution of Functional Biomolecules
10^{13} Random DNA Sequences
Transcription
Transcription
Immobilized Ligand
Unretained Sequences
Retained Sequences
PCR
(Mutagenic Conditions)
Reverse Transcription

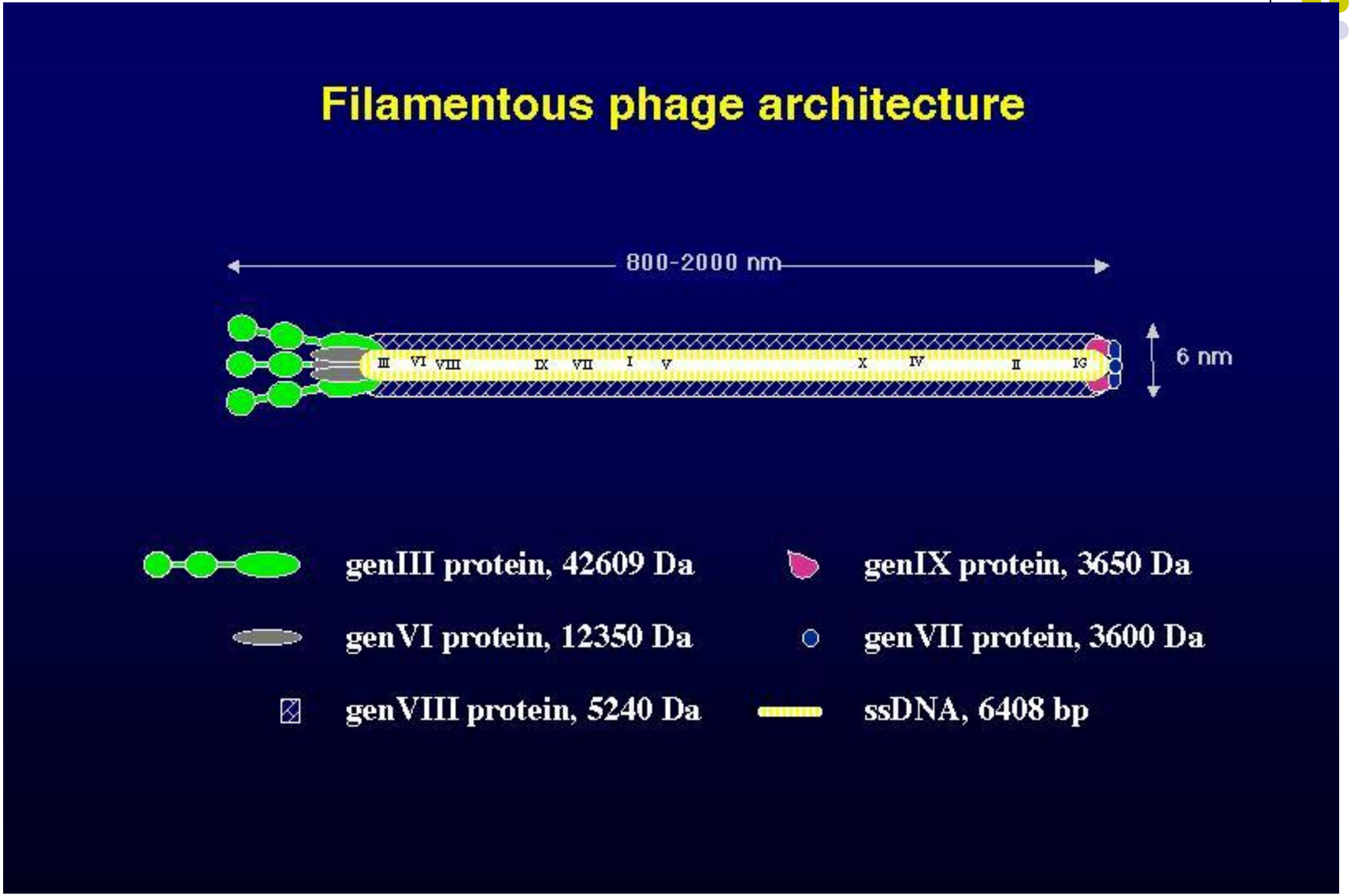
Assembly of virus



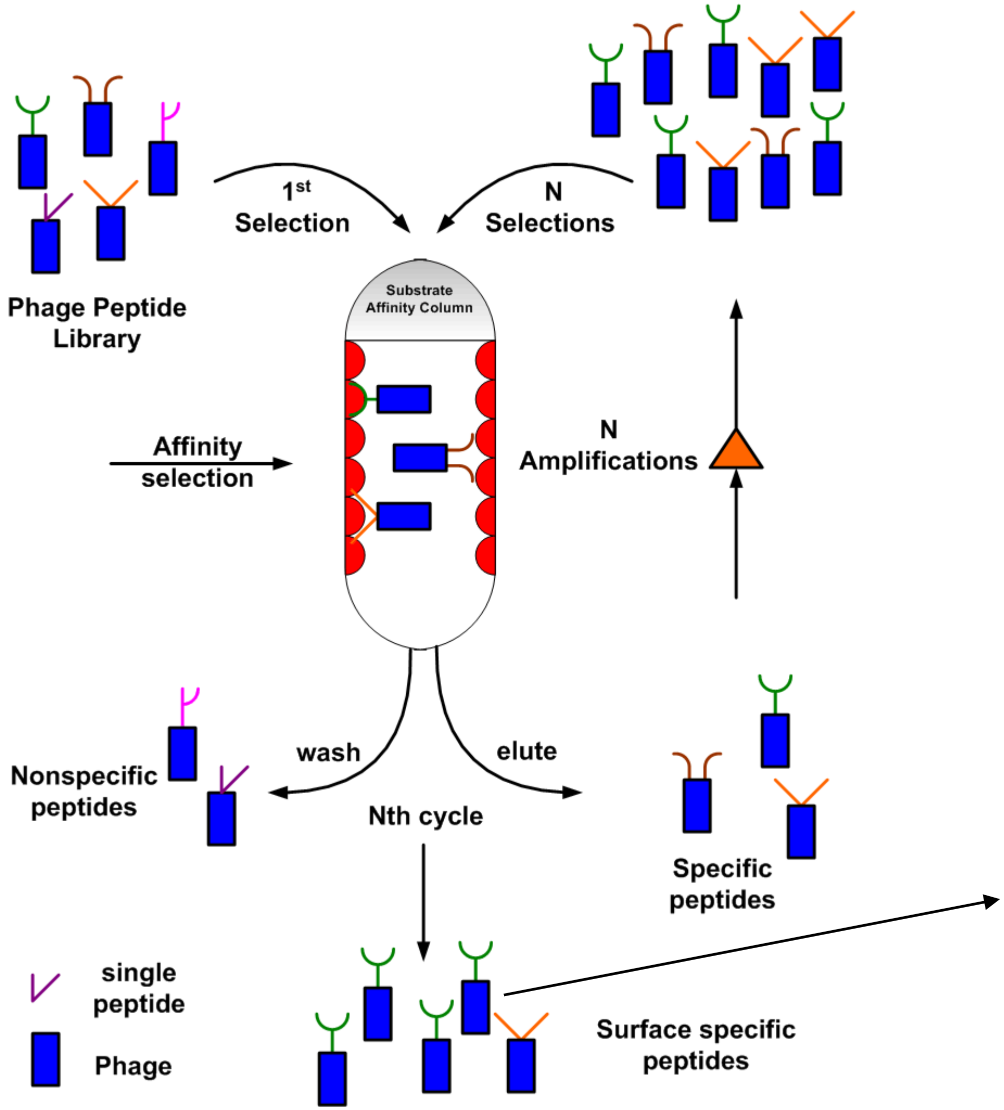
Use of ferritin symmetry sites (432)



Bacterial phages

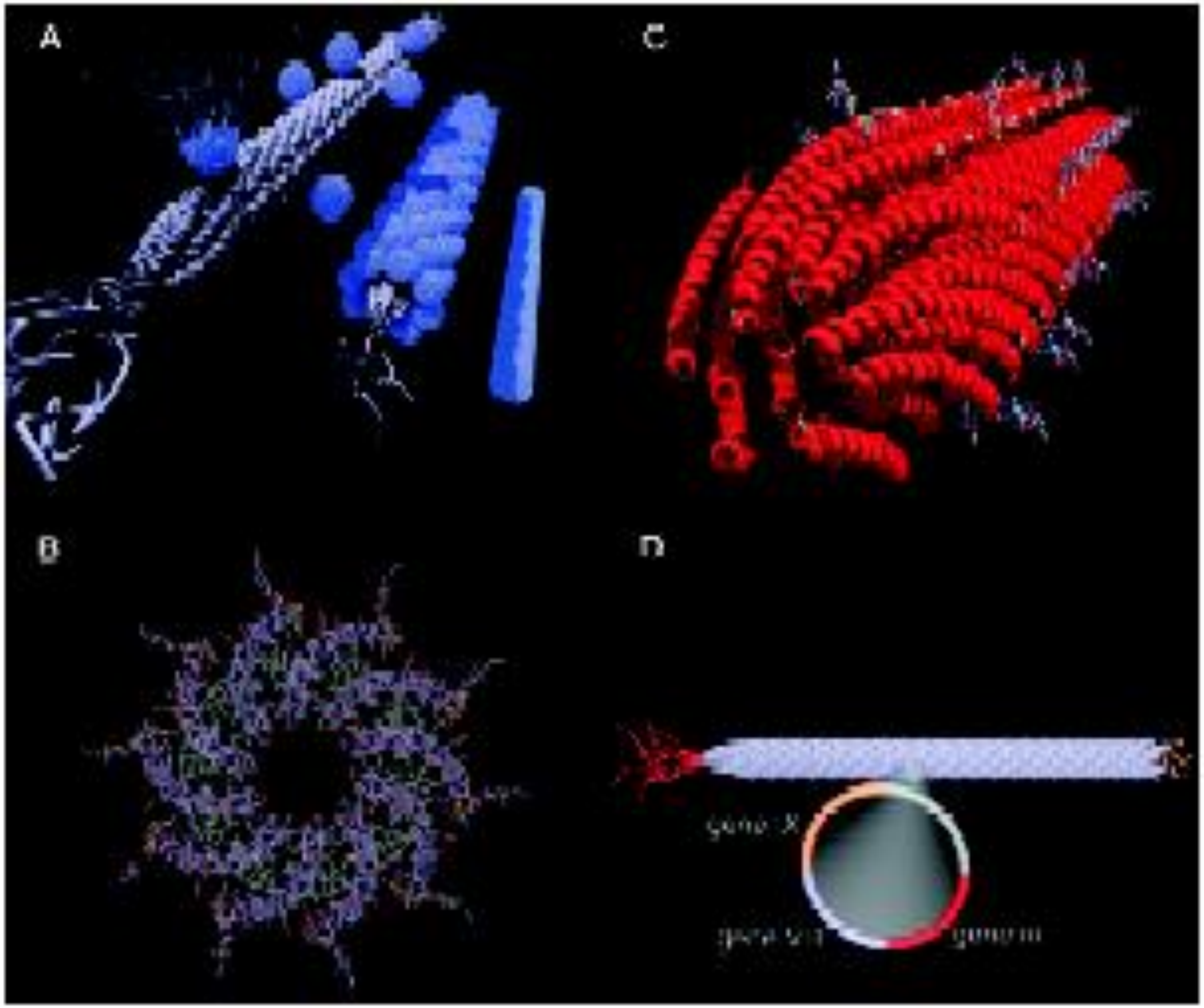


Mutations based on peptides selected to bind materials

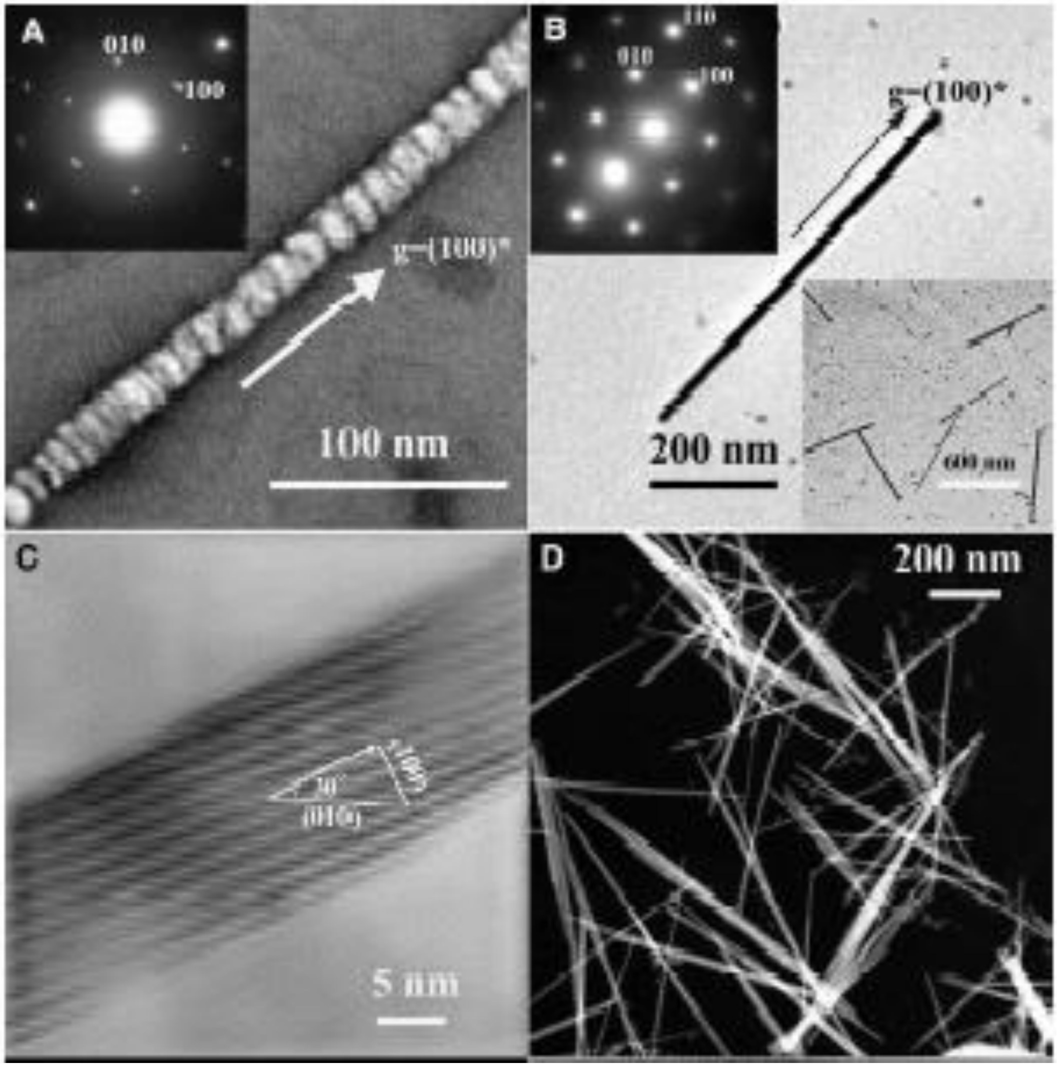


Insert peptide sequence into phage gene

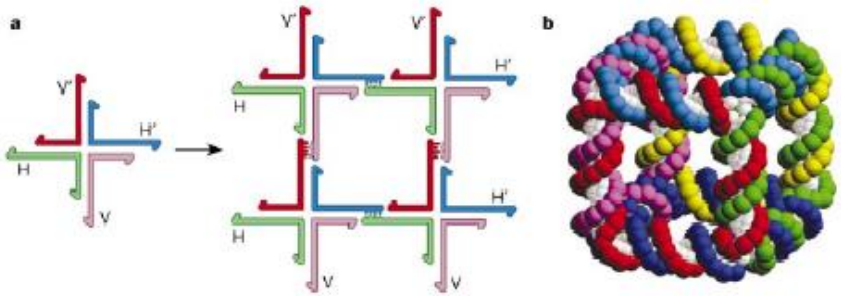
Phage M13

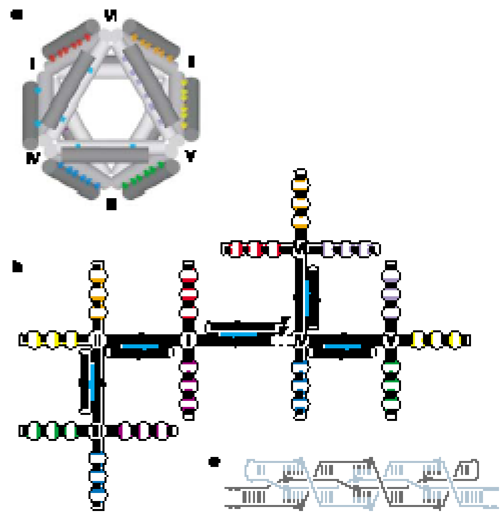


Nanowires after annealing



3D-DNA templates.

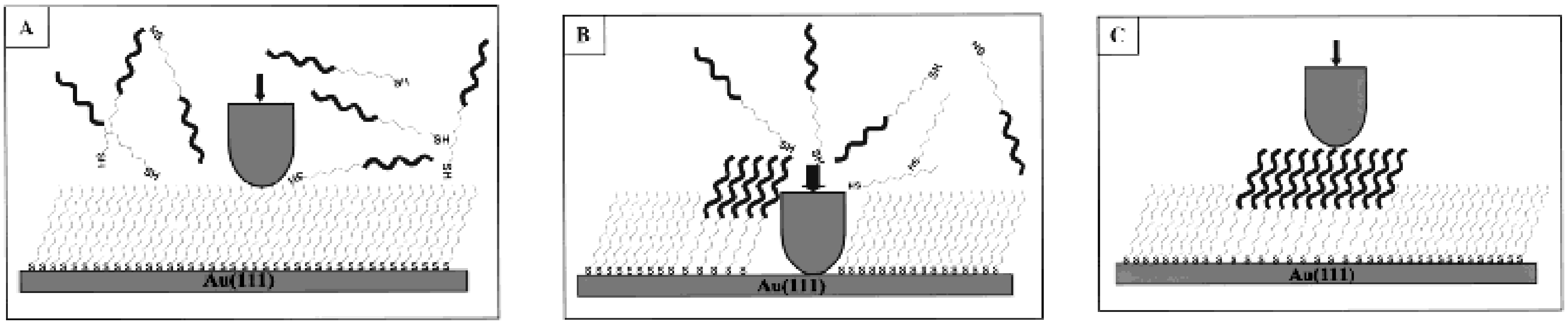


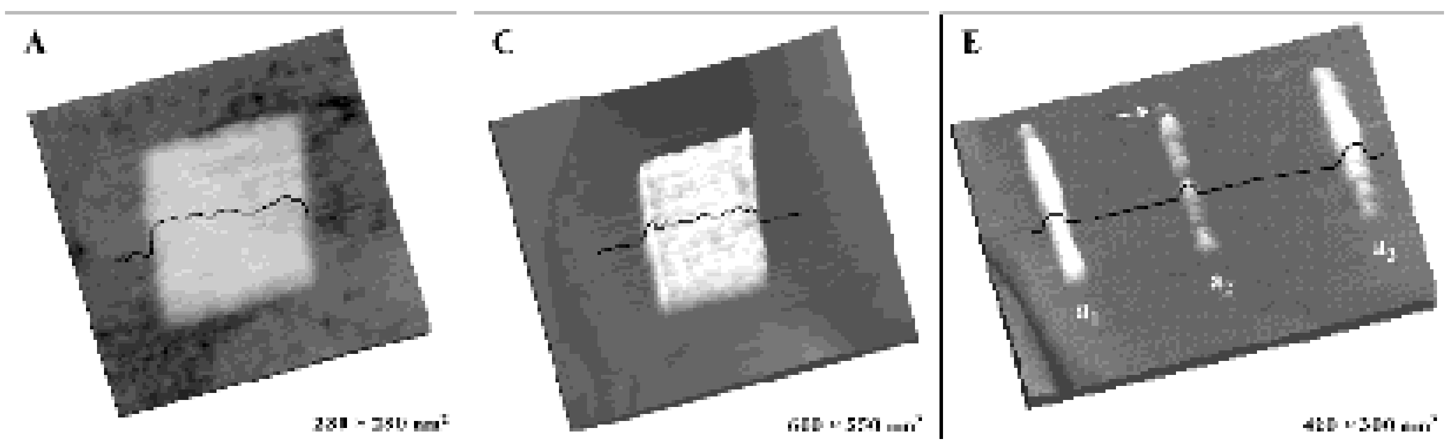


Chen, J. H. & Seeman,N. C. Synthesis from DNAof a molecule with the connectivity of a cube. Nature 350, 631–633 (1991).

Shih, W. M., Quispe, J. D. & Joyce, G. F. A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. *Nature* 427, 618-21 (2004).{Shih, 2004 #849}

DNA surface nanostructures





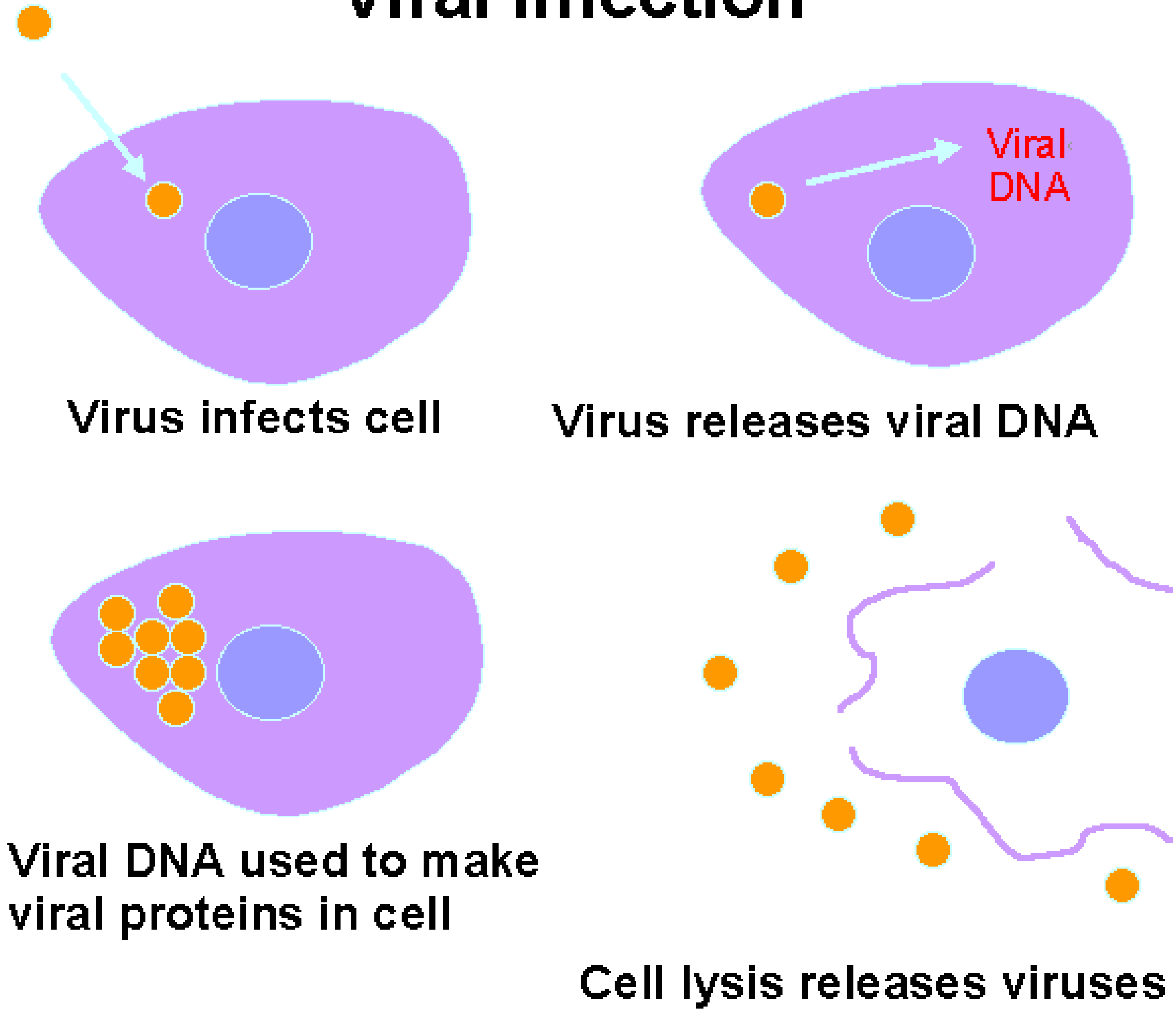
Lui et al Nanolet 2002, Vol 2, 863

Virus templates for Gold Cell Sensors

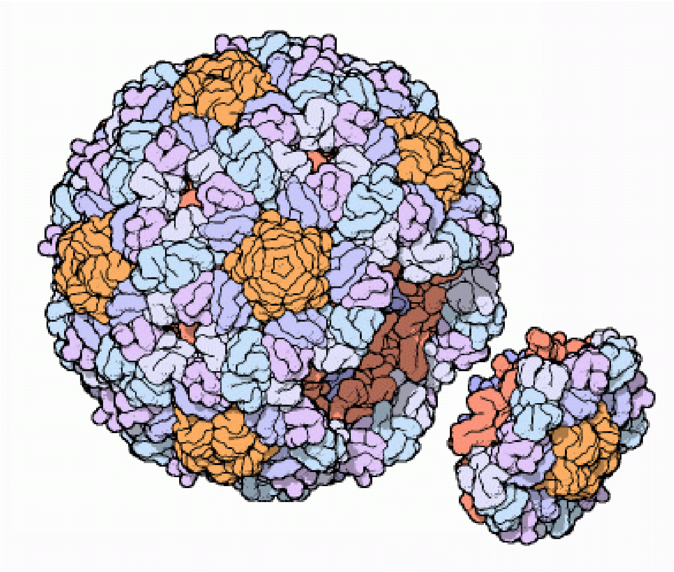
- Introduce gold particles in to viruses
- Infect cells with the virus and perform raman imaging of the cell
  - ~30nm resolution
  - Image cellular conditions
    - pH
    - Metabolites
    - etc

http://www.indiana.edu/~bdlab/

Viral infection



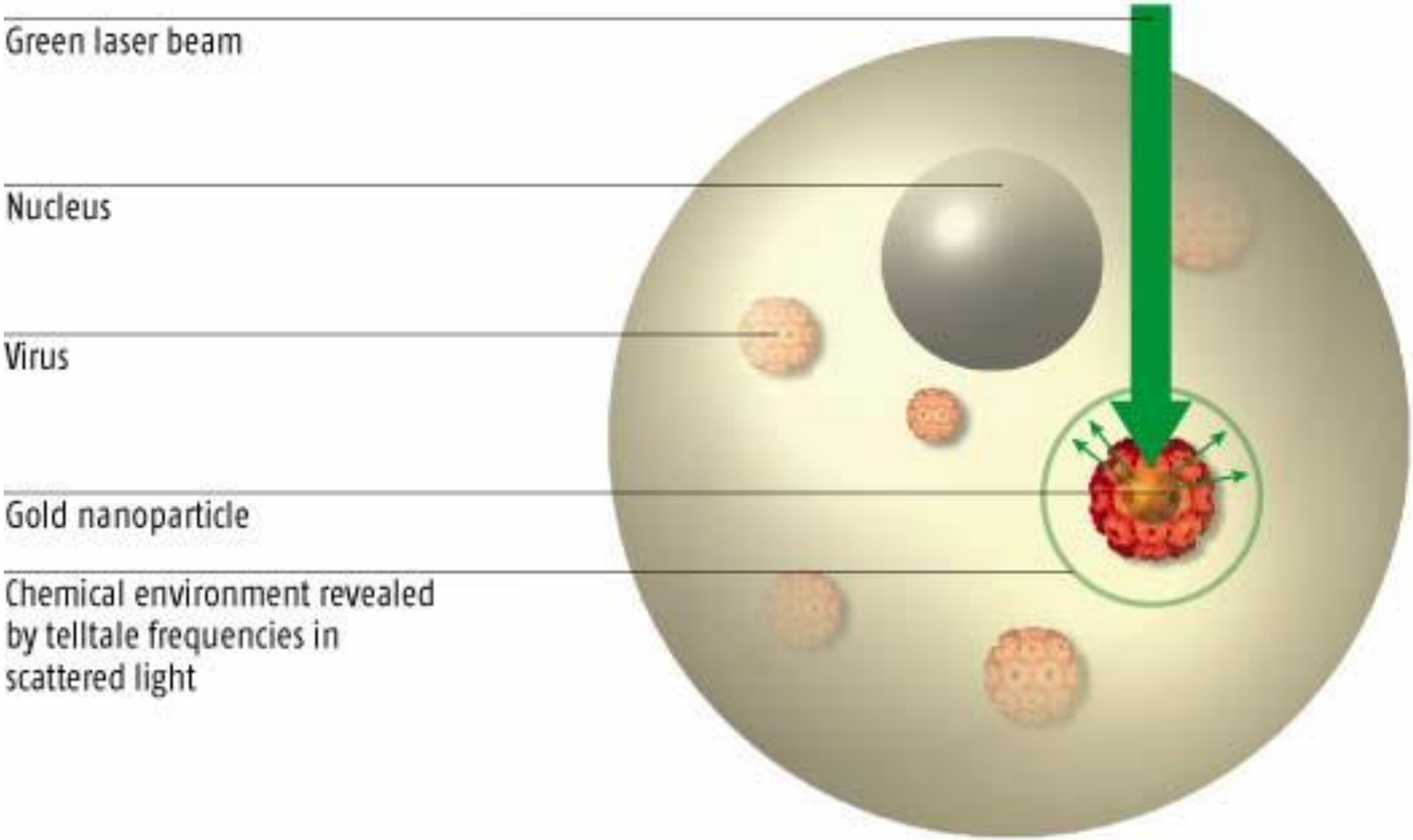
Addition of gold particles



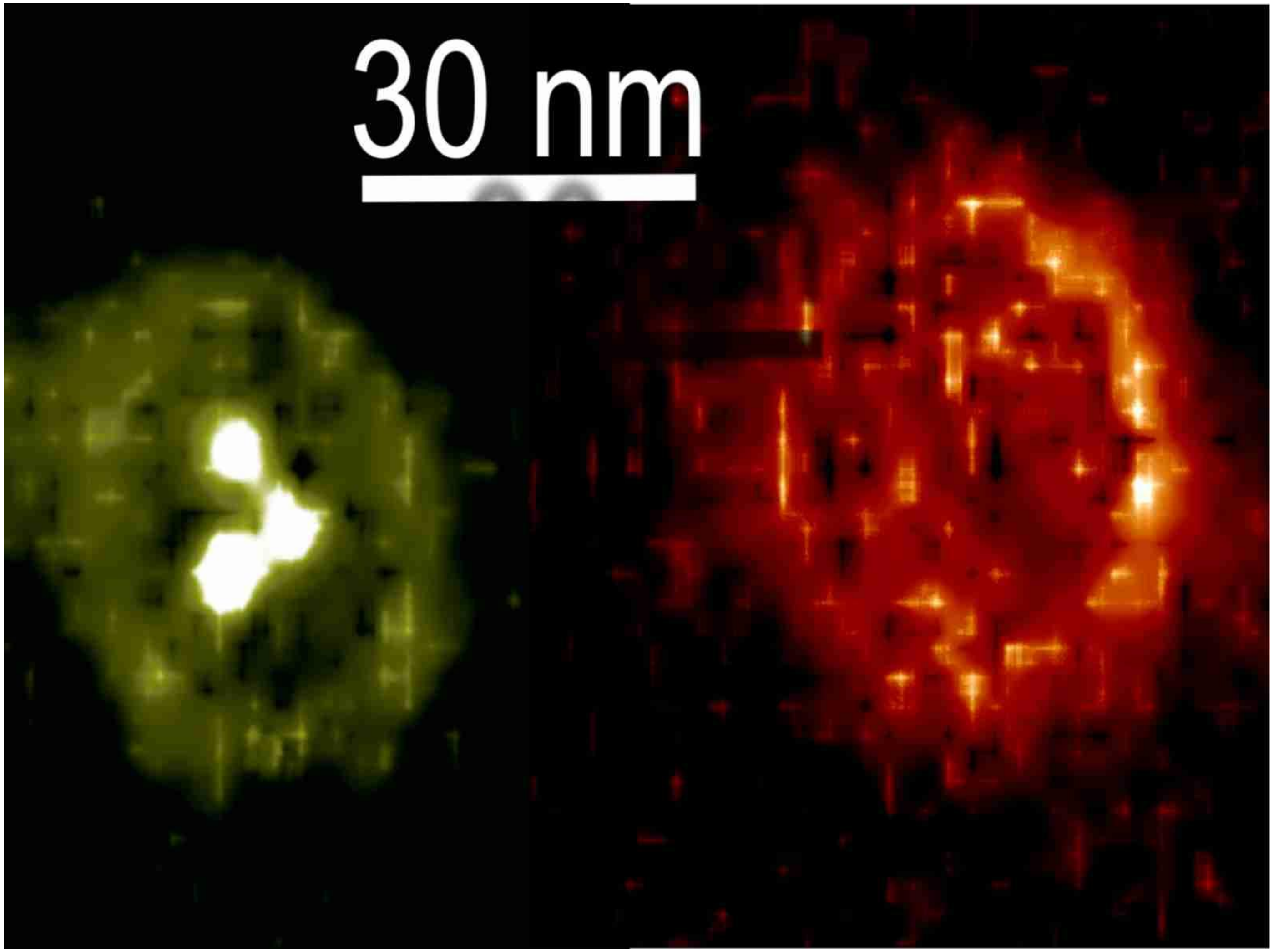
Dissociate viral shell proteins at low pH and then reassemble in the presence of 5 nm gold particles

LOOKING INSIDE A CELL

A gold nanoparticle is introduced into a plant virus, which infects a living cell. As green laser light hits the gold, it scatters and gives a snapshot of the cell's internal chemistry

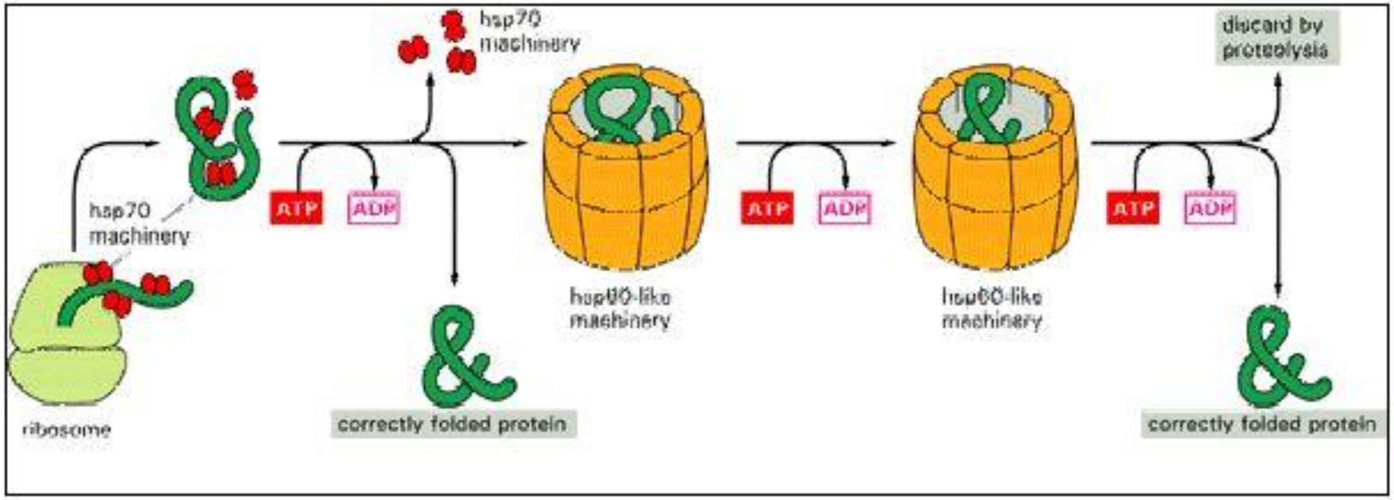


High resolution IR imaging of cell interior



Chaperonins as nanodot depots

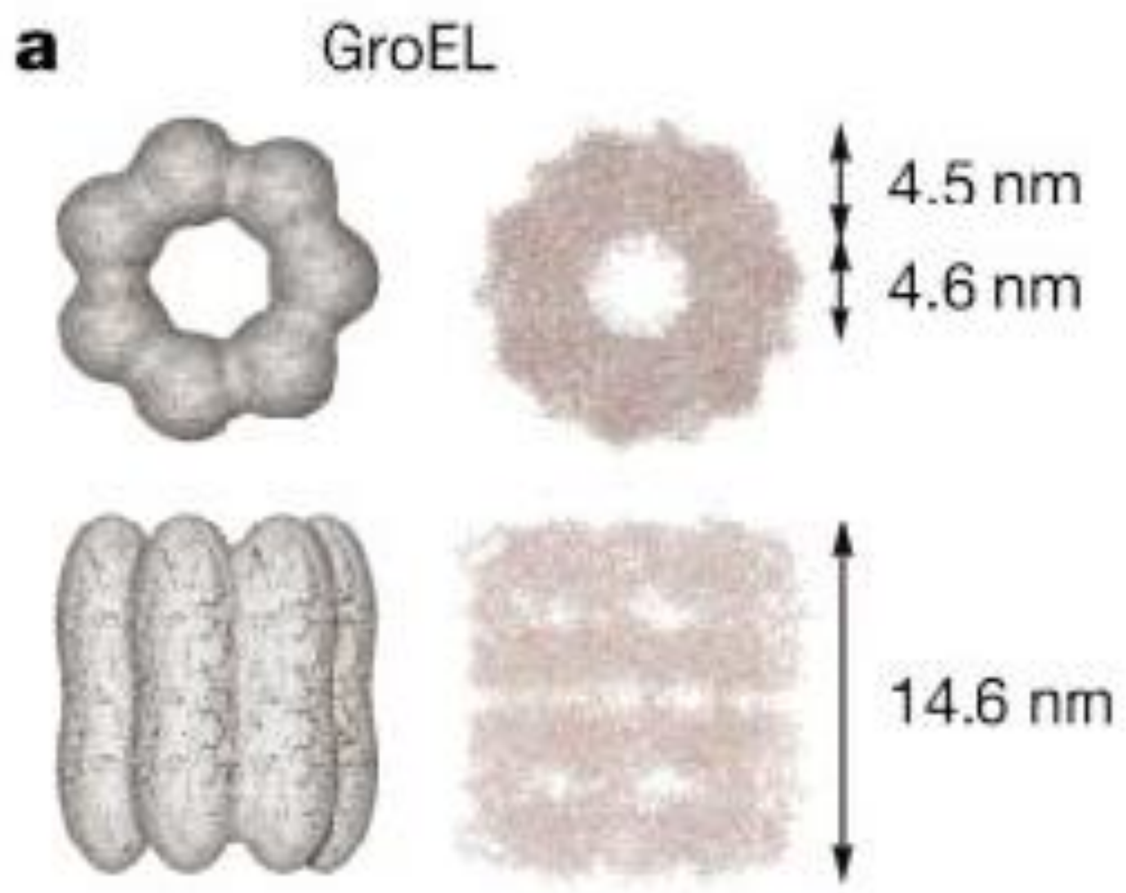
Protein Folding *in vivo*; involvement of Chaperonins



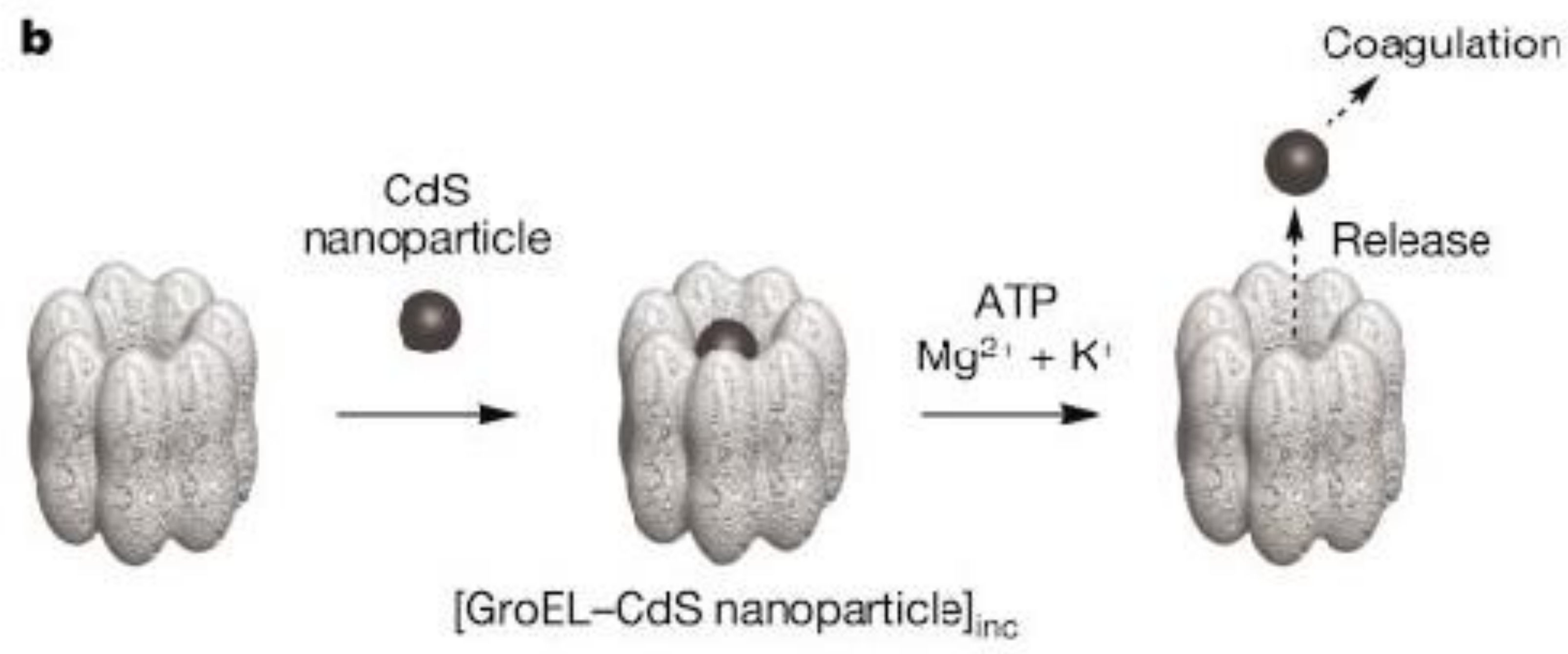
Two families of molecular chaperones.
The hsp70 proteins act early, recognizing small patches on a protein's surface. The hsp60-like proteins appear to act later and form a container into which proteins that have still failed to fold are transferred. In both cases repeated cycles of ATP hydrolysis by the hsp proteins contribute to a cycle of binding and release of the client protein that helps this protein to fold.

Ishi et al, Nature 2003, Vol 423, 628

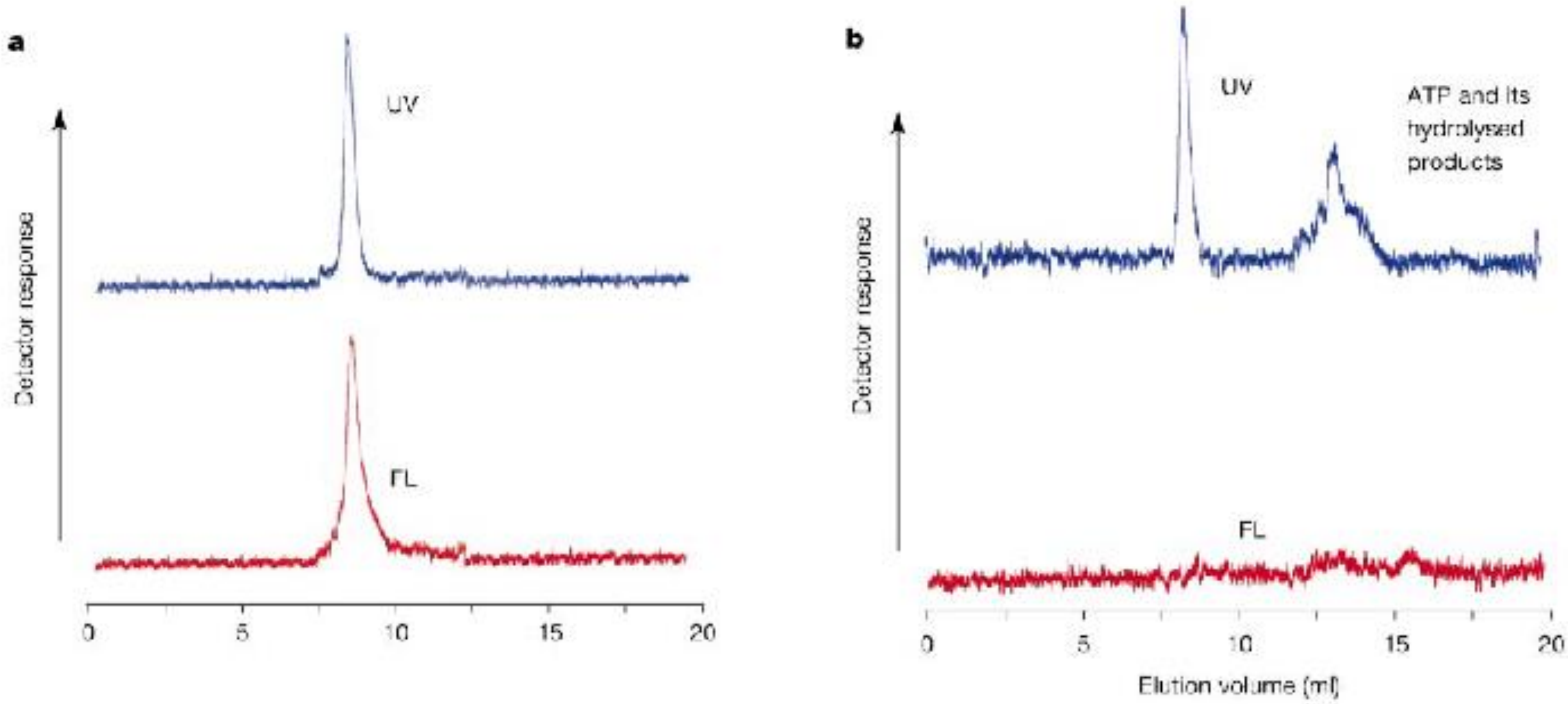
Some dimensions



Uptake of CdS particles



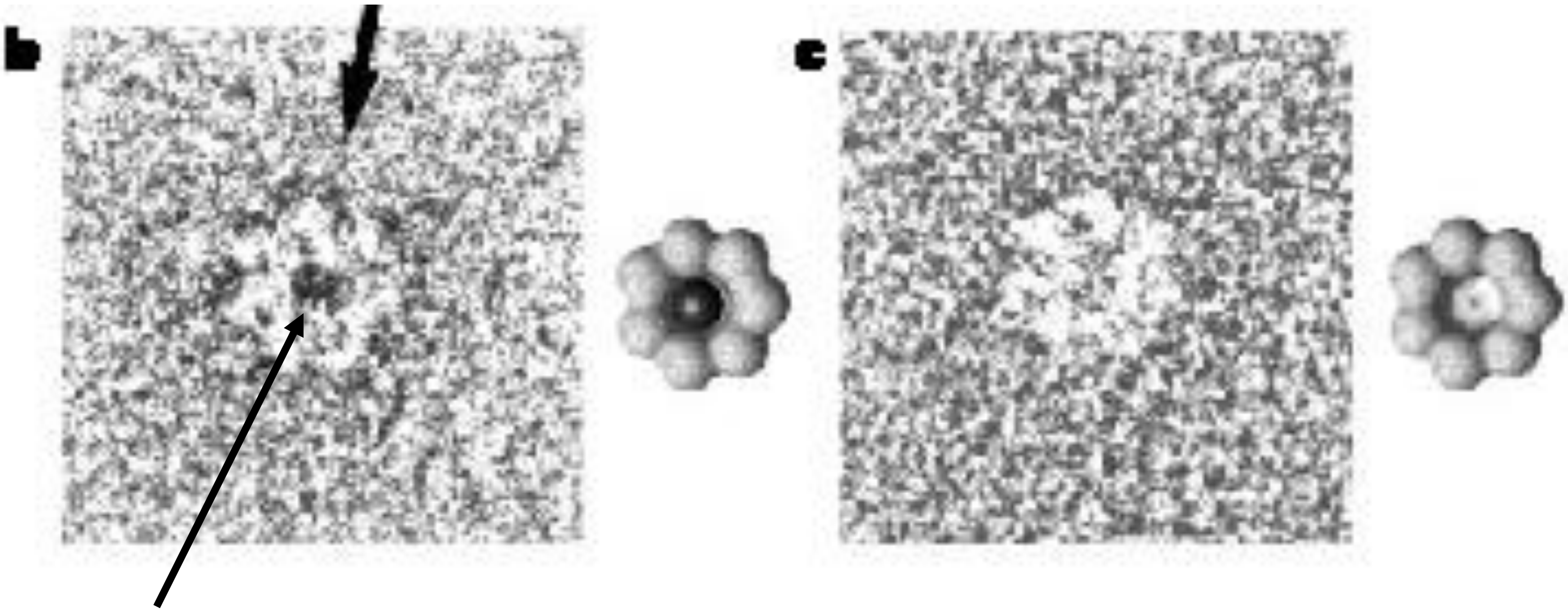
Demonstration of complete release of the CdS particles



Size exclusion chromatography, UV:protein F:CdS.

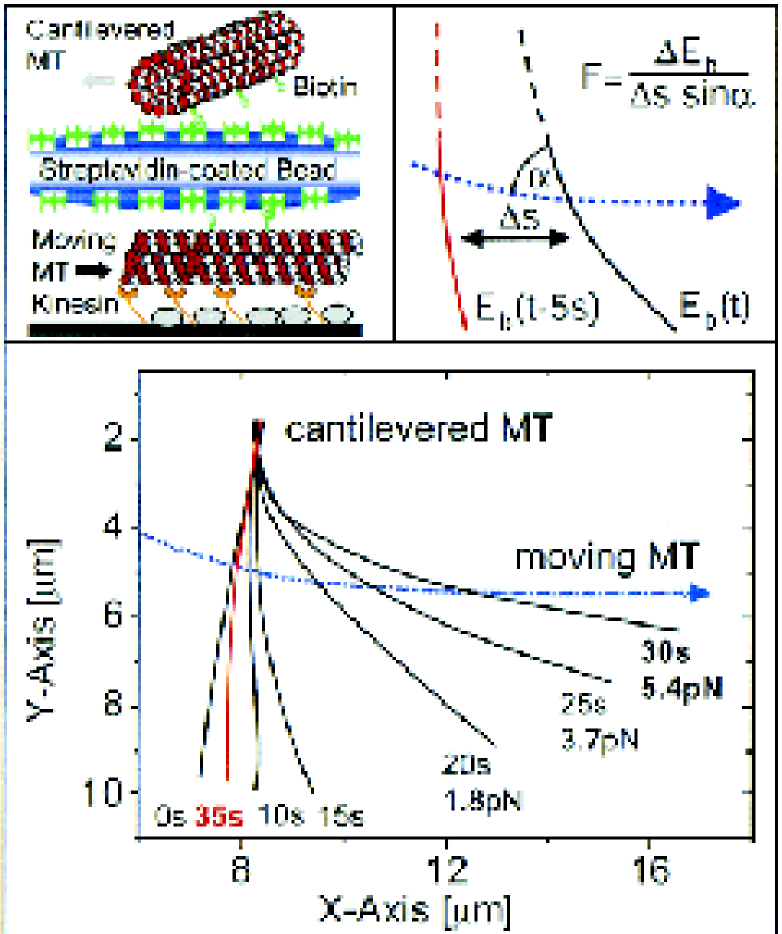
EM analysis in absence and presence of ATP

Without ATP **With ATP**

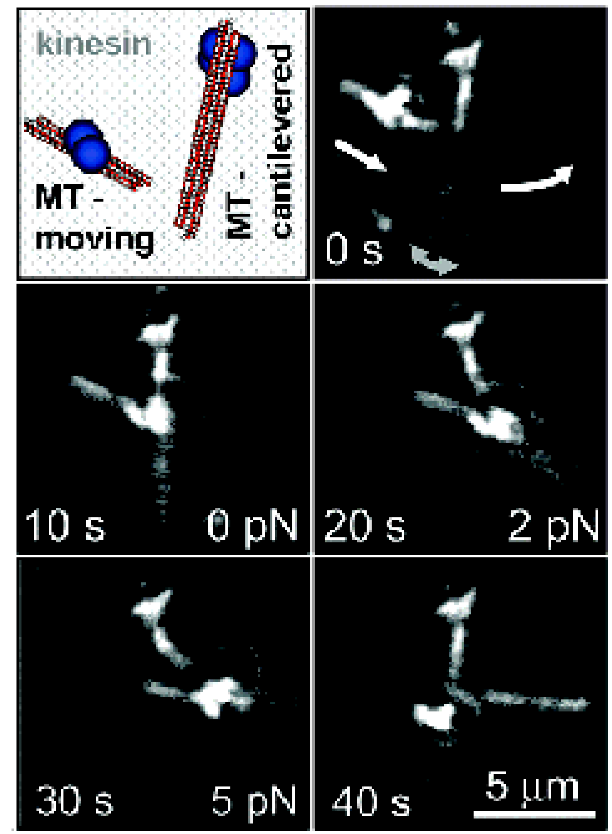


CdS qdot

A piconewton force meter



Tool for measuring pN to nN forces.



Hess et al , Nanolet, 2002, Vol 2 , 1113.

Conclusions

- **Biological systems offer a direct route into highly ordered well defined templates for nanomolecular assembly.**
- **Many such systems can be genetically altered to optimize a variety of desired properties**
- **Can be used for nanoscale pattering and enhanced lithography**
- **Such system open the way for highly efficient, inexpensive self assembled systems for materials fabrication at the nanoscale.**