Nanotechnology in the life sciences

A FRONTIS LECTURE SERIES

organized by

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February 13	13:30	Pieter Stroeve-Size, measurement and sensing
	14:30	Mieke Kleijn (WUR)- Surface forces using AFM
February 20	13:30	Pieter Stroeve- (Bio)materials
	14:30	Ernst Sudholter (WUR)- Hybrid organic semiconductor FETs
February 27	13:30	Pieter Stroeve- Self-assembly of molecular structures
	14:30	Richard Schasfoort (U Twente)- Surface modification and microfabrication strategies
Friday, March 5	13:30	Pieter Stroeve- Nanotechnology and the environment
	14:30	Keurentje (TU Eindhoven)- Micellar systems for nanoscale engineering of reaction and separation processes
Friday, March 12	**13:30**	**Pieter Stroeve- Life sciences and medicine**
	14:30	**Ton Visser (WUR)- Single-molecule fluorescence in microfluidic devices**

Nanotechnology in the life sciences

TOPICS

- **Biosensing**
- **Microarrays: genes and proteins**
- **Nanoparticle complexes of DNA and peptides**
- **Drug encapsulation and delivery**
- **Molecular machines and devices**

What do we want to sense?

- toxins in food
- pollutants in air and water
- bioprocess monitoring
- viruses
- bacteria
- metal ions
- biochemicals
- bacterial activity
- intracellular

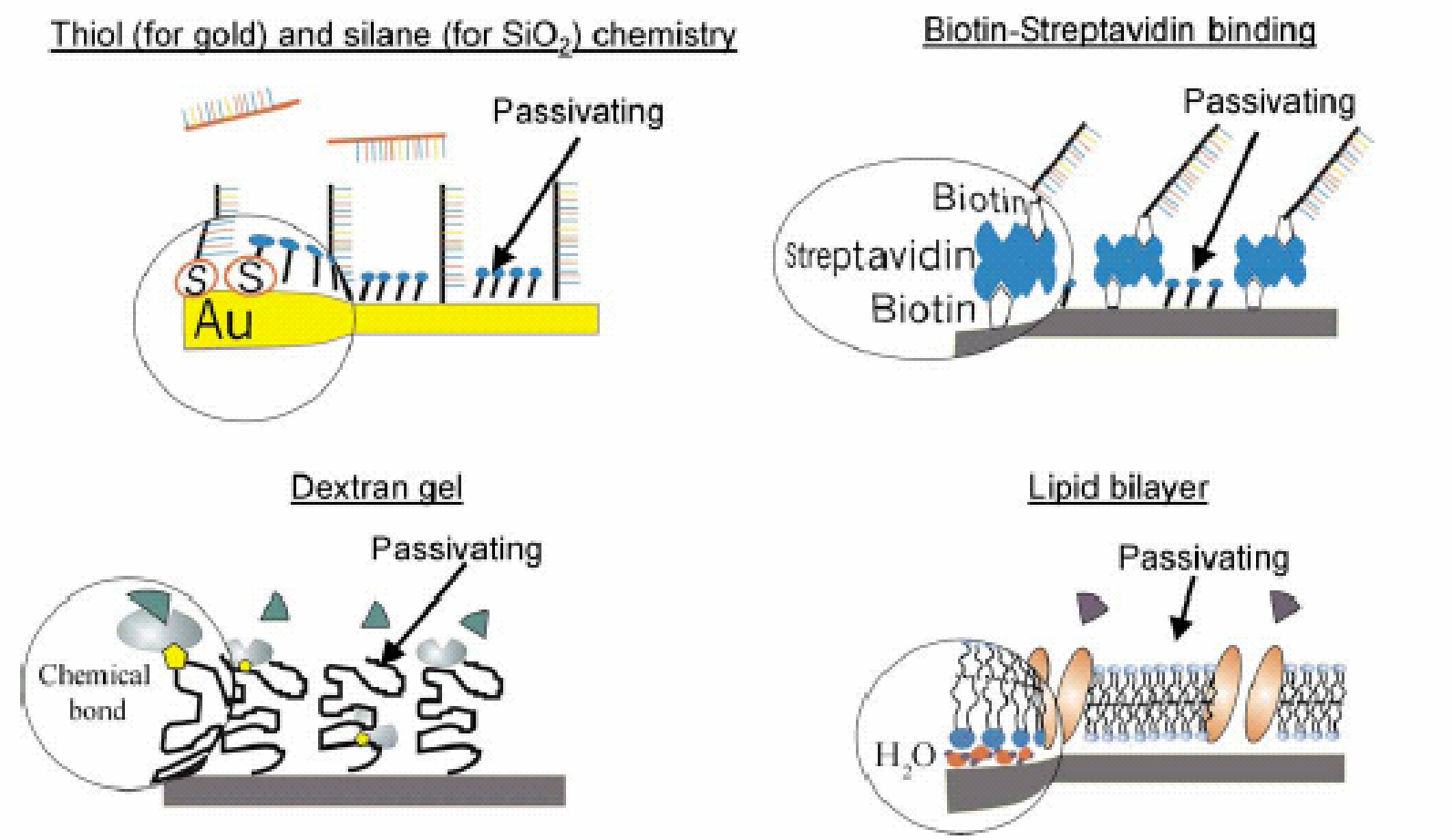
Biological recognition elements for sensors

- Enzymes
  - -transformation of analyte into sensor detectable product
  - -inhibition of enzyme by analyte
  - -detectable characteristic of change of enzyme by analyte
- Antibody-antigens
  - -high affinity binding with tracer to generate a signal
- DNA-ligand binding
- Biomimetic sensors
  - -engineered molecules (single chain antibody fragment)
  - -supported lipid bilayers
  - -molecularly imprinted polymers
- Whole cells or cellular structures
  - -pollutant dependent inhibition of cell respiration
  - -pollution dependent increase in cell respiration
  - -membrane transport proteins
  - -neuroreceptor proteins produce signal through ion channels

Typical sensing techniques for biosensors and biochips

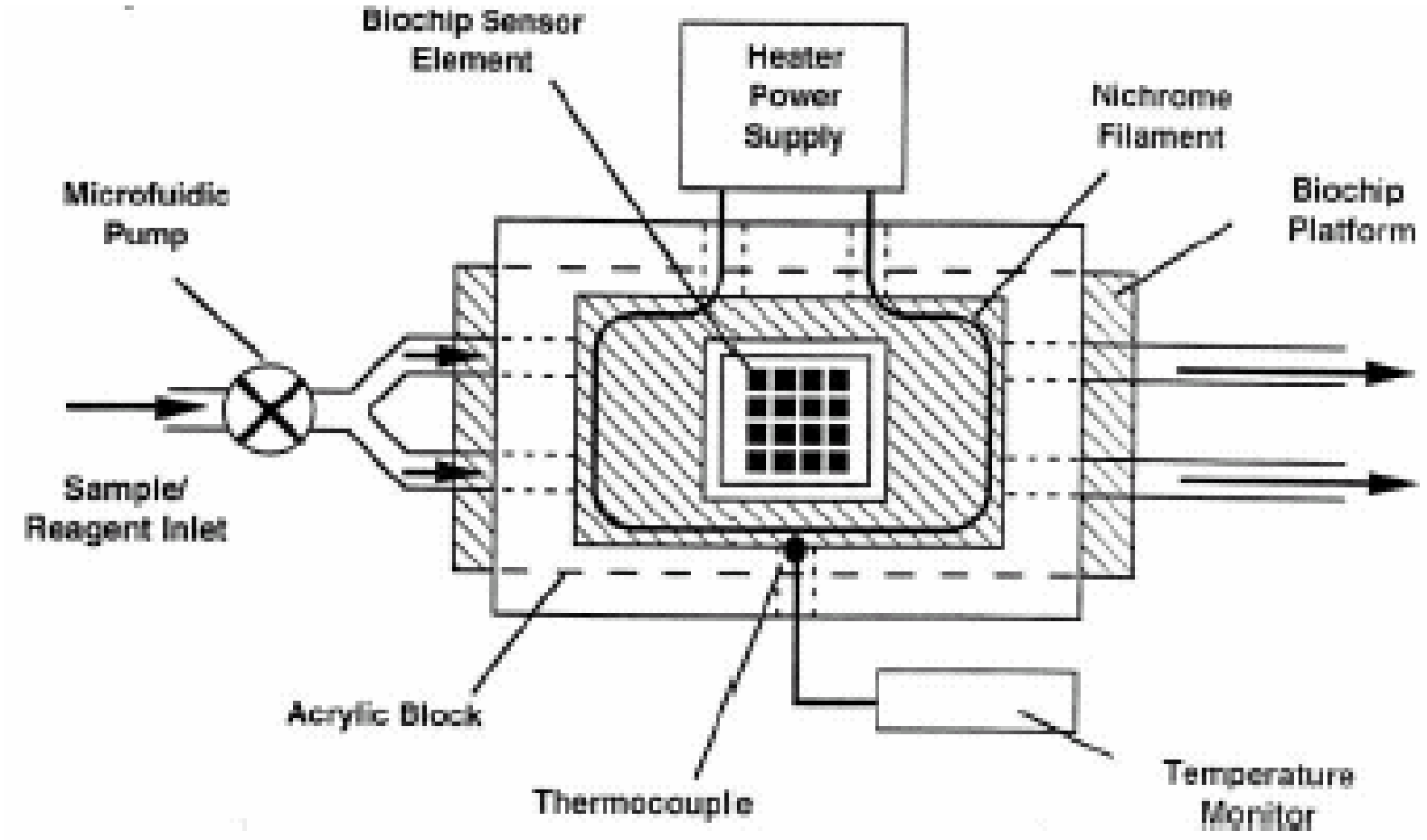
- Fluorescence
- SPR Surface plasmon resonance
- Ellipsometry
- SHG Second harmonic generation
- QCM Quartz crystal microbalance
- SAW Surface acoustic wave
- Impedance spectroscopy
- SPM Scanning probe microscopy
- Electrochemical

Surface immobilization of molecules for biosensing



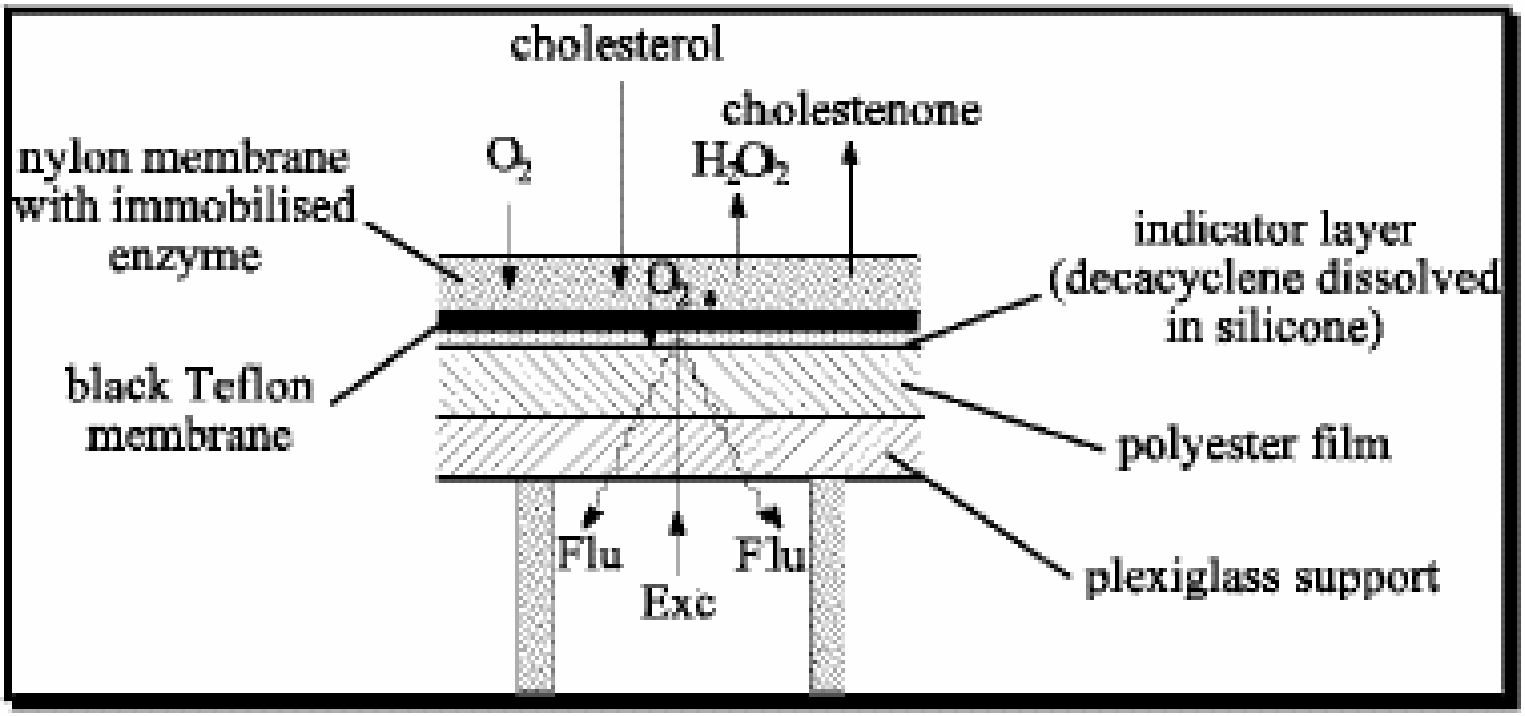
Microfluidics based biochip for sensing

T. Vo-Dingh et al., Sensors and Actuators B, 2001



Fiber-optic cholesterol sensor

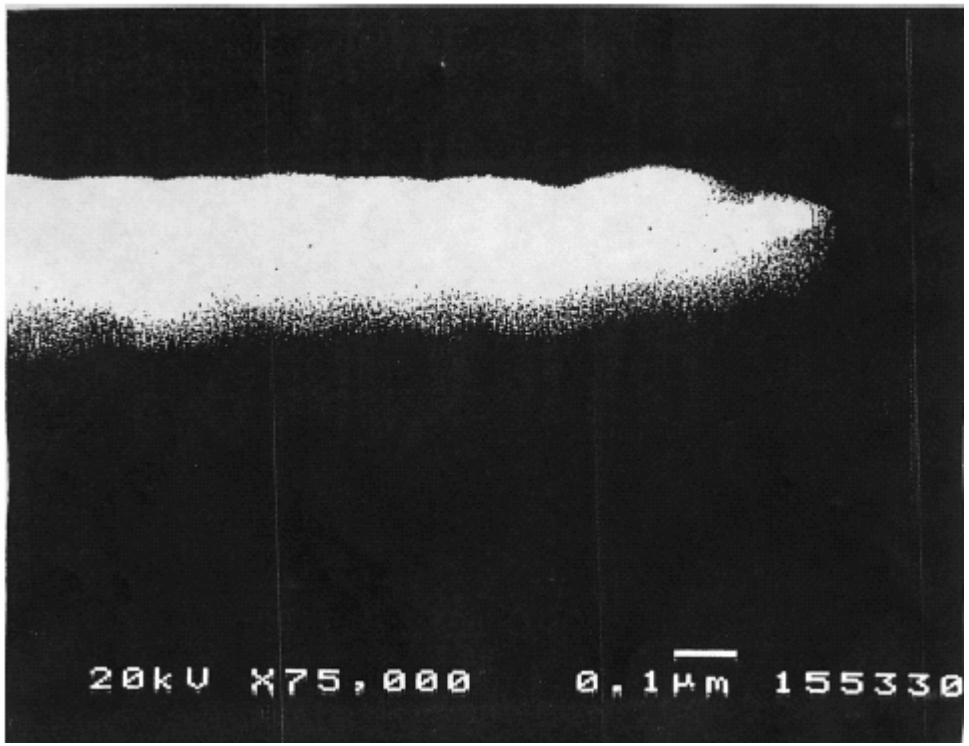
The enzyme cholesterol oxidase converts cholesterol and oxygen to cholestenone and peroxide. The change in oxygen is sensed by the decacyclene fluorescence. **B. Kuswandi et al., Analyst, 2001**



SEM of optical fiber

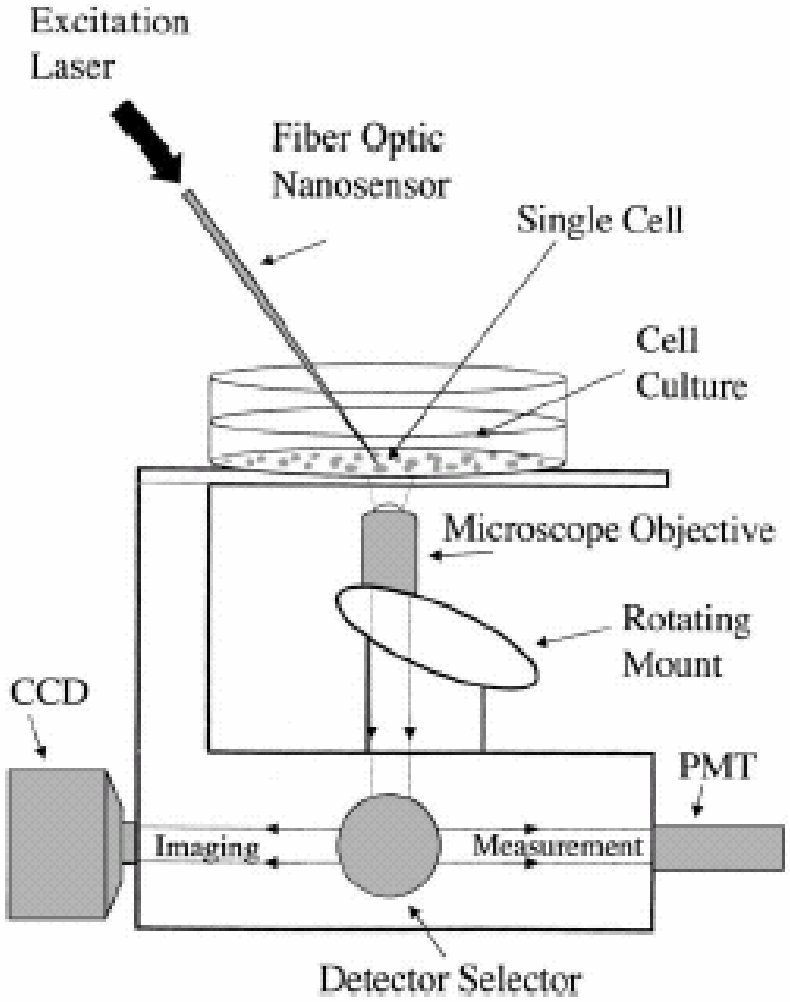
Tip size of optical fibers can be as small as 40 nm.

T. Vo-Dingh et al., Sensors and Actuators B, 2001



Optical system for intracellular measurement

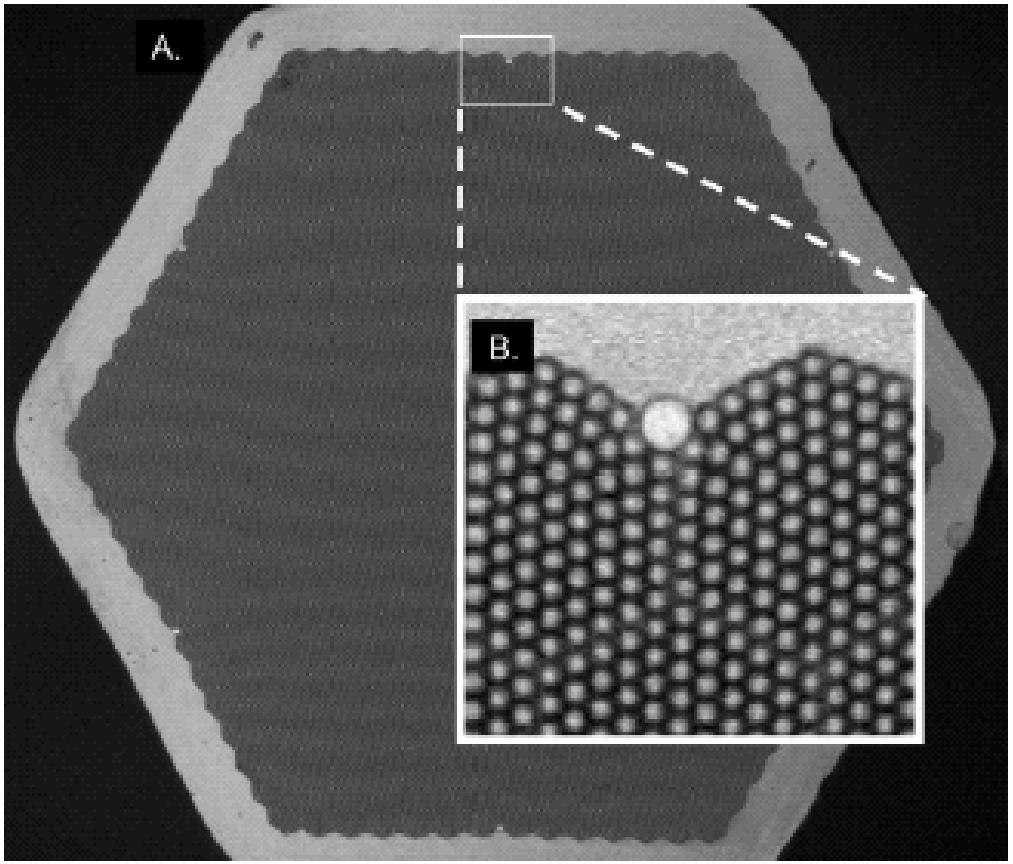
T. Vo-Dingh et al., Sensors and Actuators B, 2001



Optical fiber microarray

Fiber bundle is 1 mm^2 and contains 50,000 individual fibers.

J. R. Epstein and D. R. Walt, Chem. Soc. Rev., 2003



pH sensing by optical fiber microarray: intensity proportional to pH value

J. R. Epstein and D. R. Walt, Chem. Soc. Rev., 2003

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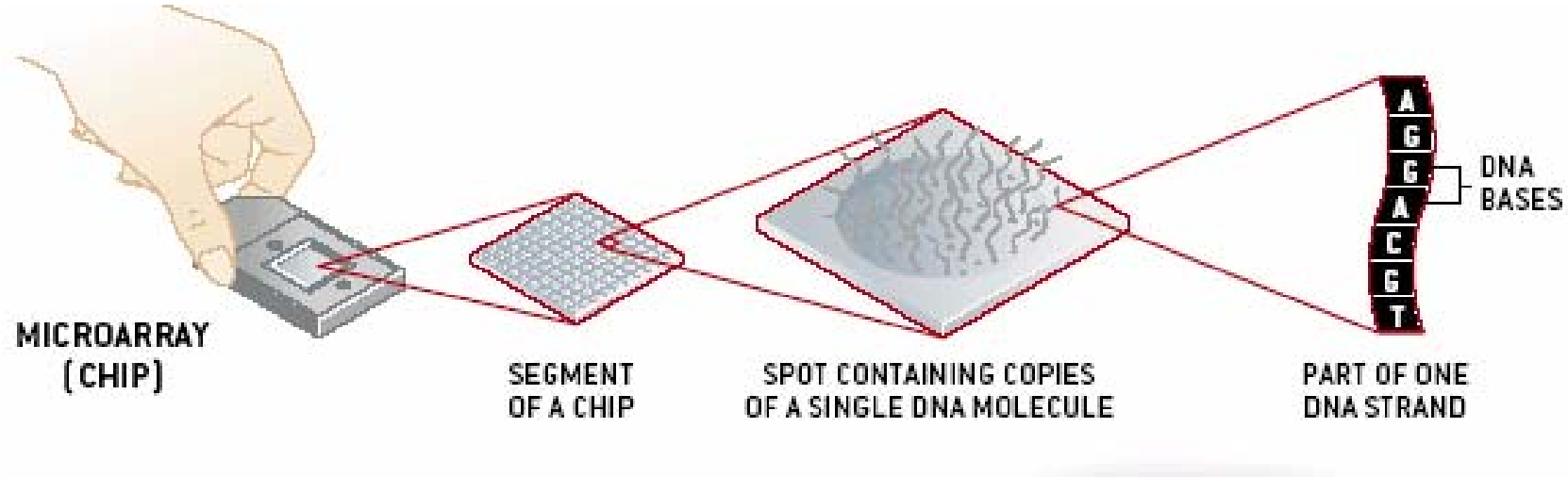
Microarrays or gene chips

- DNA microarrays can track thousands of molecular reactions in parallel on a wafer smaller than a microscope slide. Chips can be designed to detect specific genes or measure gene activity in tissue samples.
- Microarrays are being studied as diagnostic tools.
- Protein arrays are being developed and have great promise as diagnostic devices for proteomics- the study of networks of proteins in cells and tissues. However, proteins are more complex than genes and more difficult to study.
- Identification of proteins and the 3-D structures allows one to find sites where proteins are most vulnerable to drugs.

Microarrays

Microarray with single-stranded DNA representing thousands of different genes, each assigned to a specific spots on a 2.5 by 2.5 cm device. Each spot includes thousands of to millions of copies of a DNA strand.

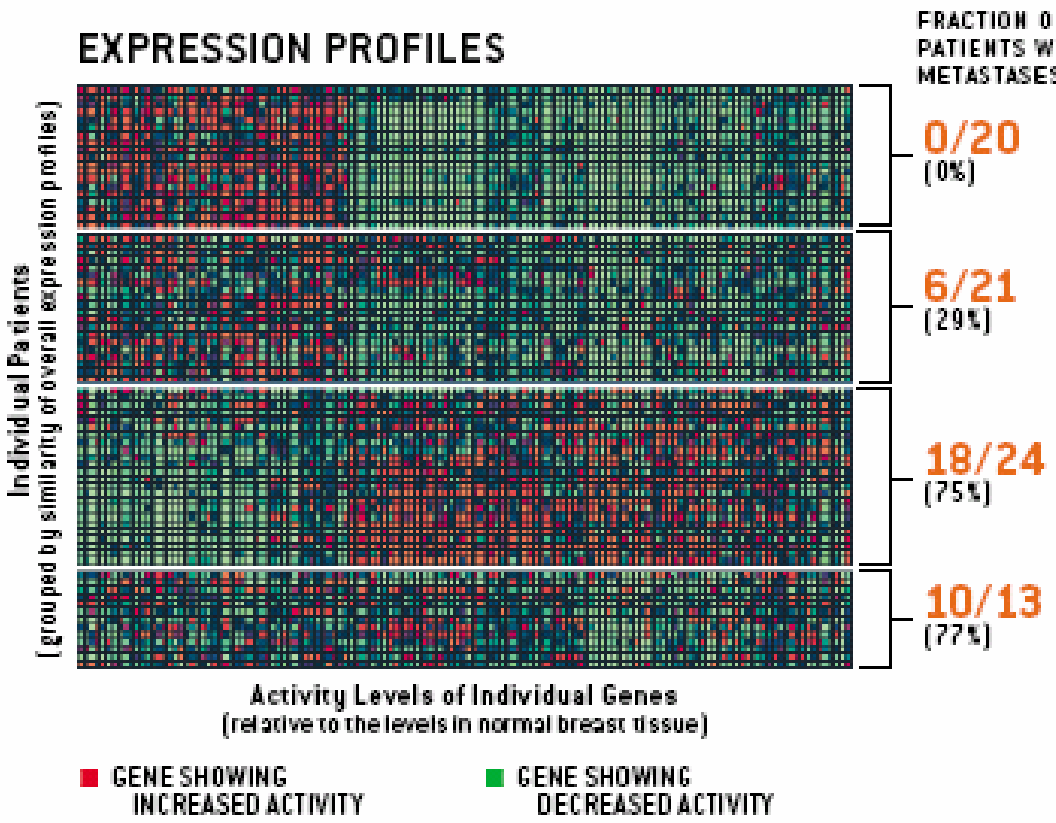


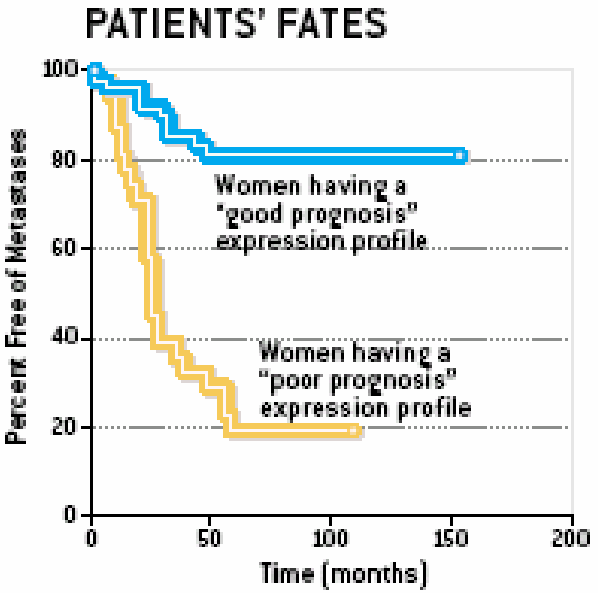
Microarrays for gene diagnostics

S. H. Friend and R.B. Stoughton, Sci. Am., 2002

PREDICTING CANCER'S COURSE

WORK AT ROSETTA INPHARMATICS and the Netherlands Cancer Institute suggests that microarrays can help distinguish cancer patients with different prognoses. After determining the activity (expression) levels of genes in small, localized breast tumors from young women who were followed for at least five years after surgery, the researchers found that the expression profiles—the overall patterns of activity across a selection of genes in the tumors—differed among the patients (*left*). A mathematical analysis (*right*) then revealed that patients whose expression profiles resembled a "poor prognosis" signature (the average pattern in tumors that metastasized) were much more likely to suffer a quick recurrence than were patients whose profiles resembled a "good prognosis" signature (the typical pattern in tumors that did not spread). If such results are confirmed by others, doctors may one day be able to discern which patients need the most intensive therapy based in part on how closely their expression profiles match a standard good or poor prognosis profile.





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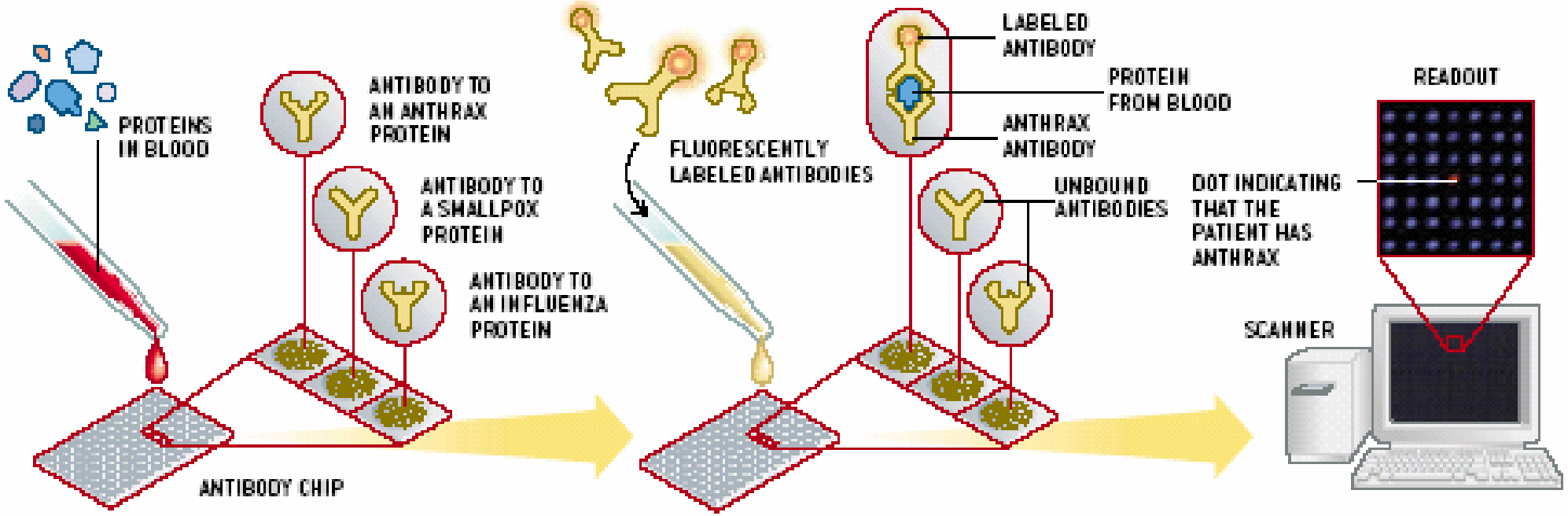
Protein arrays for diagnostics

S. H. Friend and R.B. Stoughton, Sci. Am., 2002

DOCTORS MIGHT ONE DAY use a "sandwich assay" to identify the infectious agent responsible for a patient's illness. Is it a common flu bug or a new, deadly variety? Might the tuberculosis bacterium be at fault—or even anthrax, smallpox or Q fever microorganisms unleashed by bioterrorists? Following the steps below would reveal the answer.



1 Apply blood from a patient to a chip, or array, consisting of antibodies assigned to specific squares on a grid. Each square includes multiple copies of an antibody able to bind to a specific protein from one organism and so represents a distinct disease-causing agent.

2 Apply fluorescently labeled antibodies able to attach to a second site on the proteins recognizable by the antibodies on the chip. If a protein from the blood has bound to the chip, one of these fluorescent antibodies will bind to that protein, enclosing it in an antibody "sandwich."

3 Feed the chip into a scanner to determine which organism is present in the patient's body. In this case, the culprit is shown to be a strain of anthrax.

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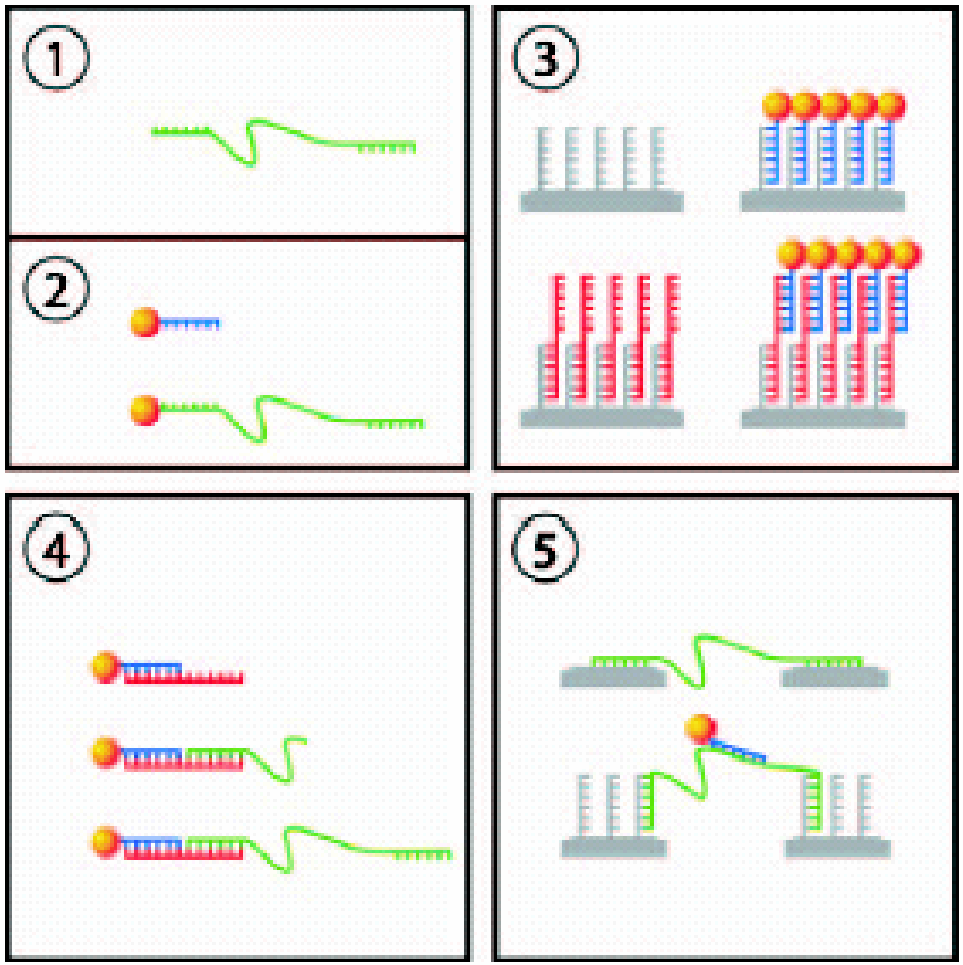
Nanoconstructions of DNA and DNA-nanoparticle complexes

1) DNA molecule; 2) DNA-nanoparticle complexes based on Au-thiol binding; 3) nanoparticle labeling for biochips; 4) labeling of single molecules; 5) devices, e.g. nanoelectronics.

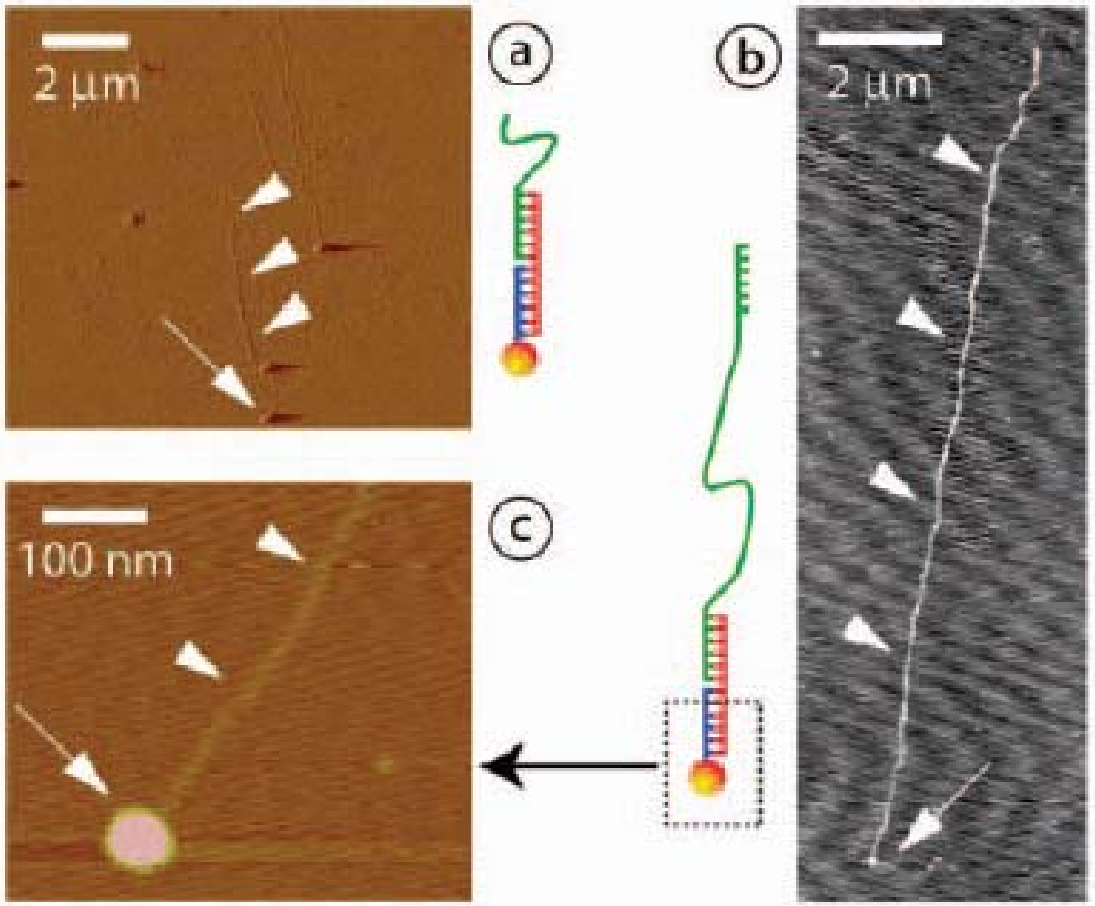
A. Csaki et al., Single Mol., 2003



Nanoparticles as labels for DNA

a) nanoparticle (arrows) and DNA fragment (arrow head); b) nanoparticle with complete DNA; c) zoom of b).

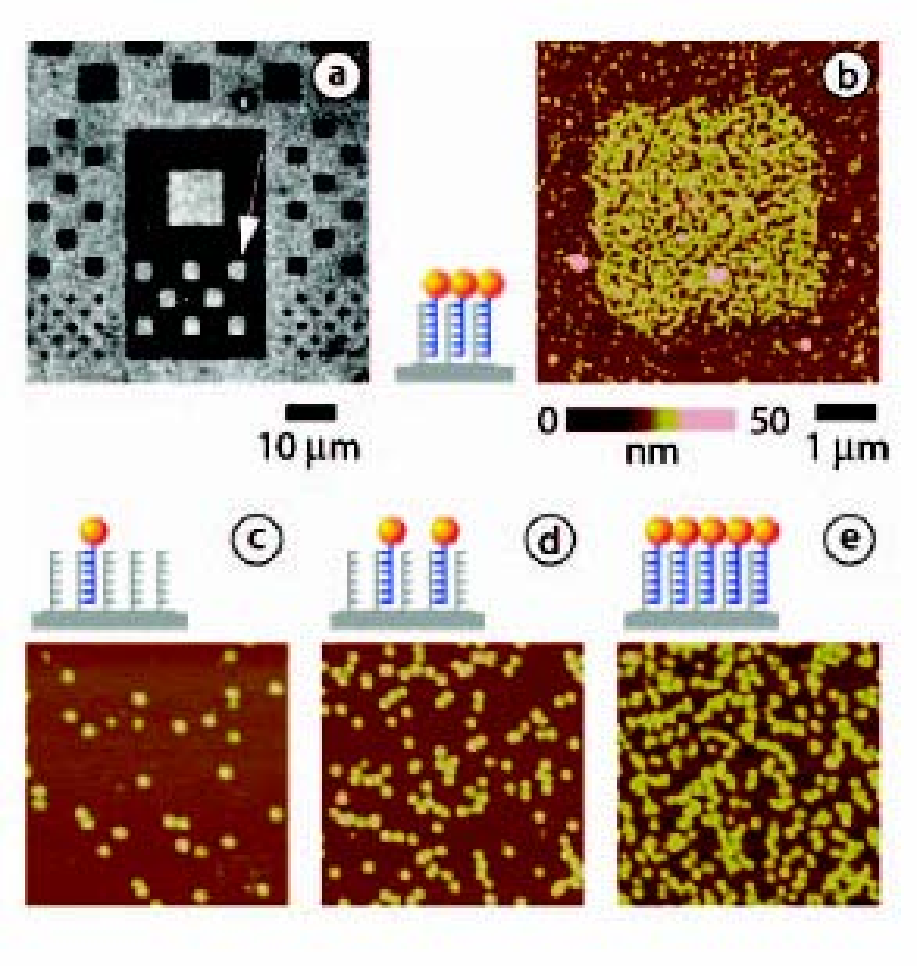
A. Csaki et al., Single Mol., 2002



Nanoparticles for DNA-chip labeling

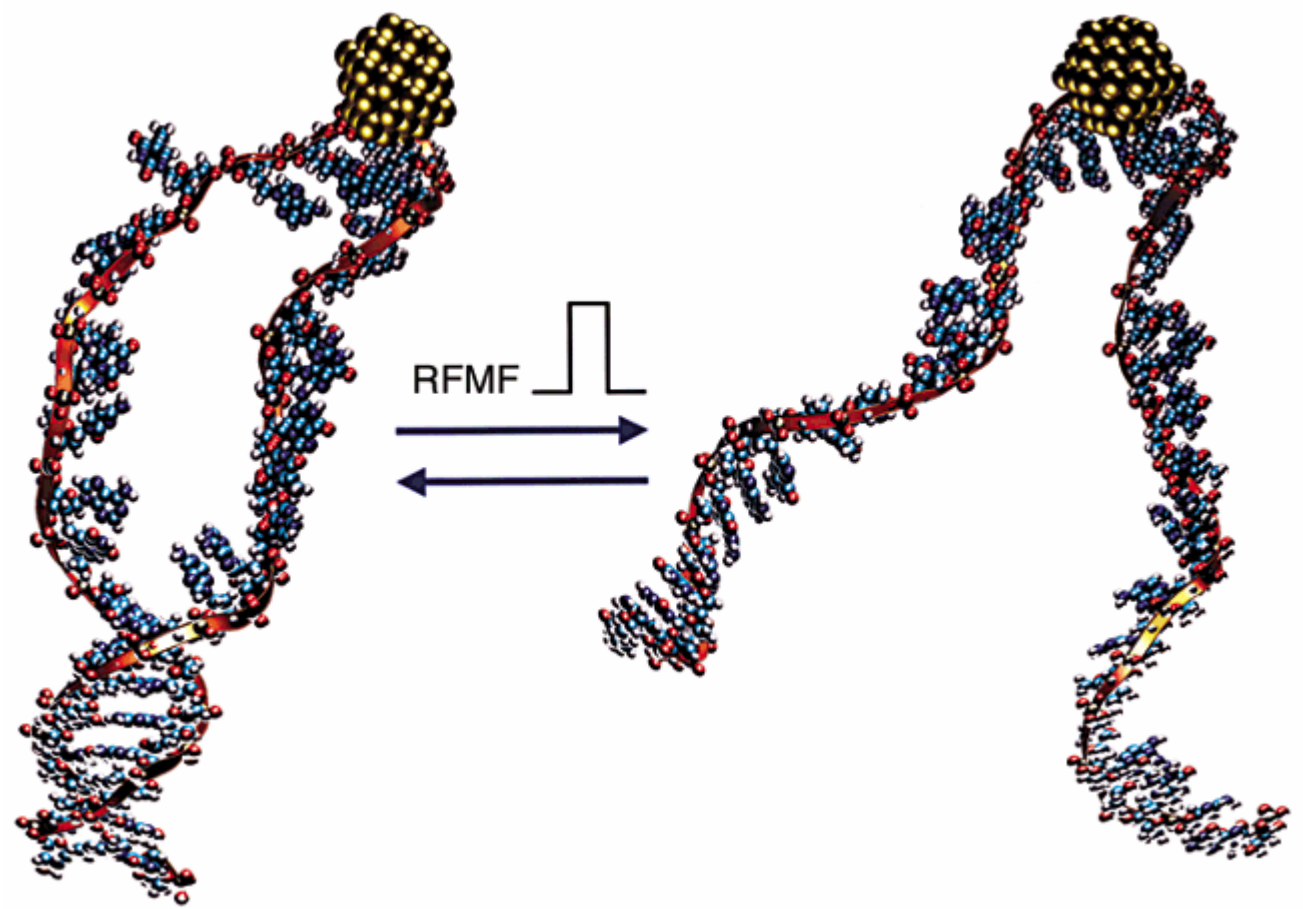
a) optical reflection picture of nanoparticle-labeled DNA chip; b) AFM zoom of one square of a); c-e) concentration-dependence of surface coverage (height range 50 nm, scan size 2 x 2 μm)

A. Csaki et al., Single Mol., 2002



Metal nanocrystal-coupled DNA as a switch

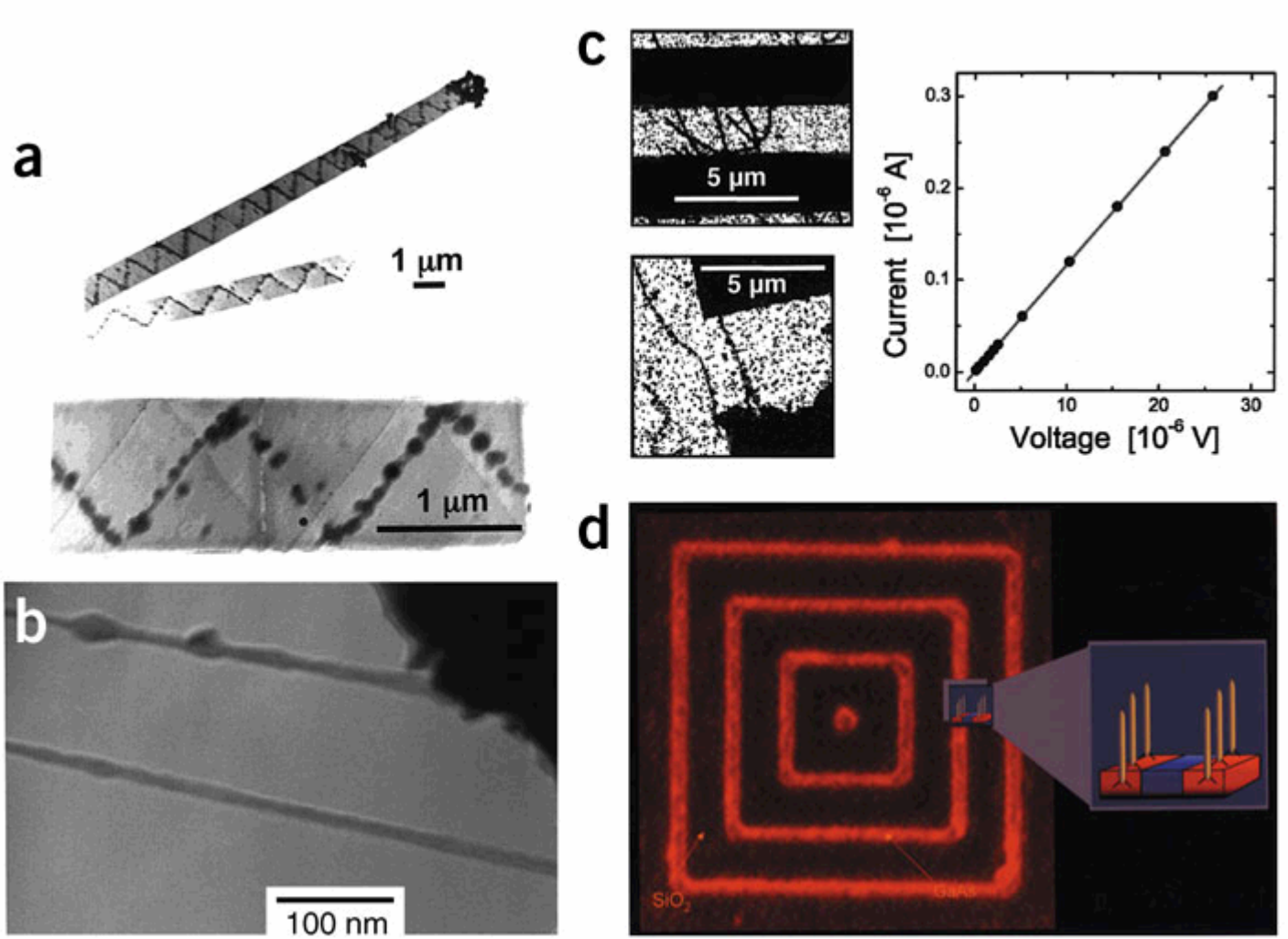
S. Zhang, Nature Biotechnology, 2003



Lipid, peptide and protein scaffolds

a) Nanoparticles coated on left-handed lipid tubules. b) silver ions fill a tubule from a peptide. The silver can form a wire after removal of the peptide scaffold. c) yeast protein forms bridges to gold electrodes. The fibers can pass electric current. d) electronic/peptide device by binding peptide to GaAs pattern on SiO_2 .

Zhang, Nature Biotechnology, 2003



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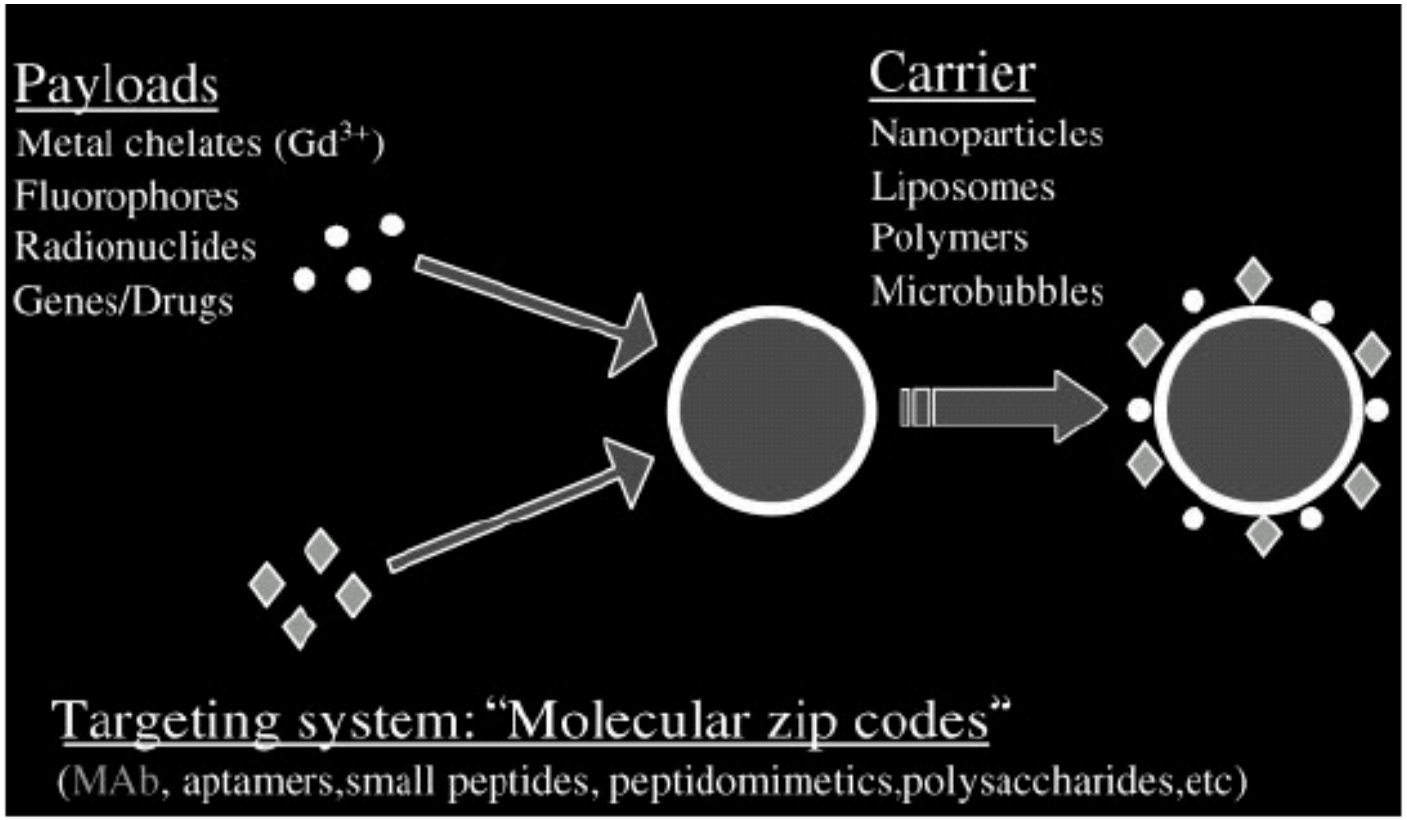
Drug encapsulation and delivery with nanoparticles: vehicles for delivery

- coated solid particles
- vesicles
- liposomes
- micelles
- polymers
- solid lipid nanoparticles

A paradigm for nanoparticle delivery for controlled release of drugs or genes or for tissue and cell imaging

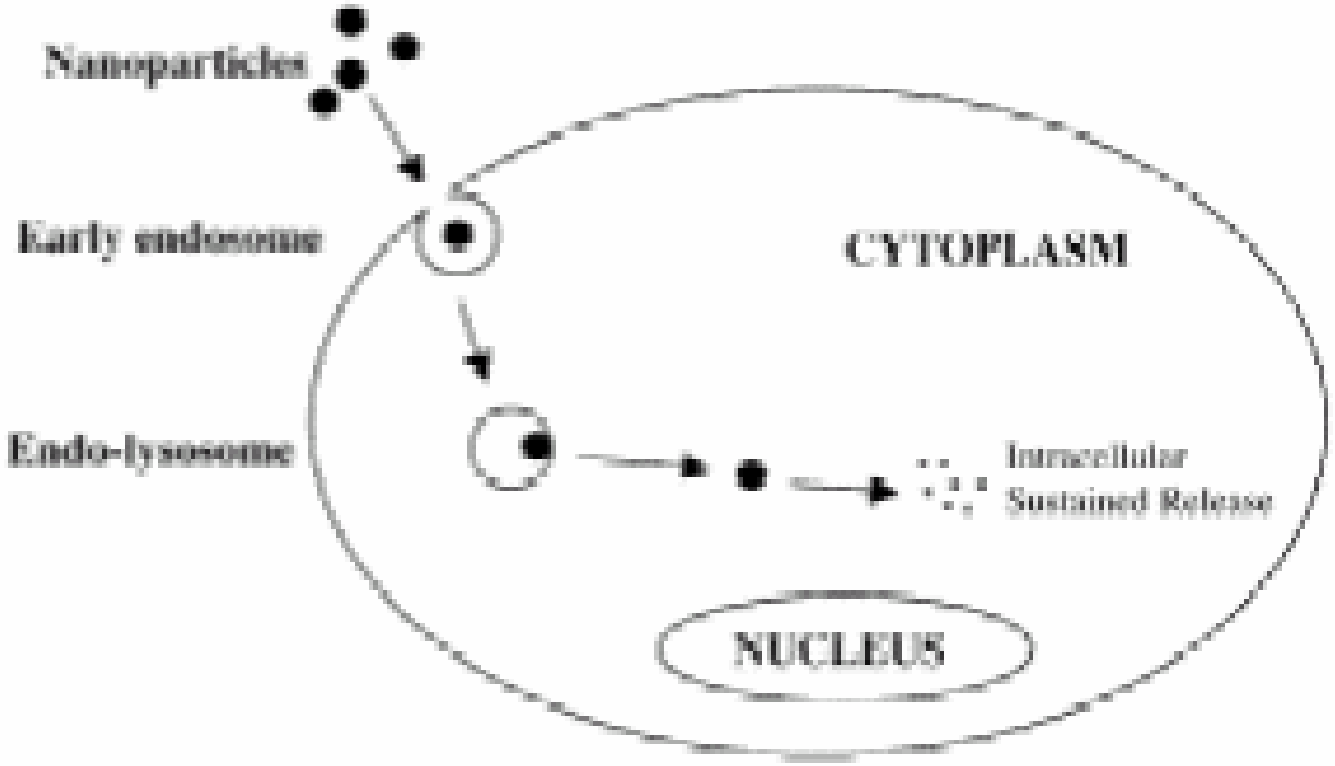
S.A. Wickline and G. M. Lanza, J. Cell. Biochem., 2002



Intracellular trafficking of nanoparticles

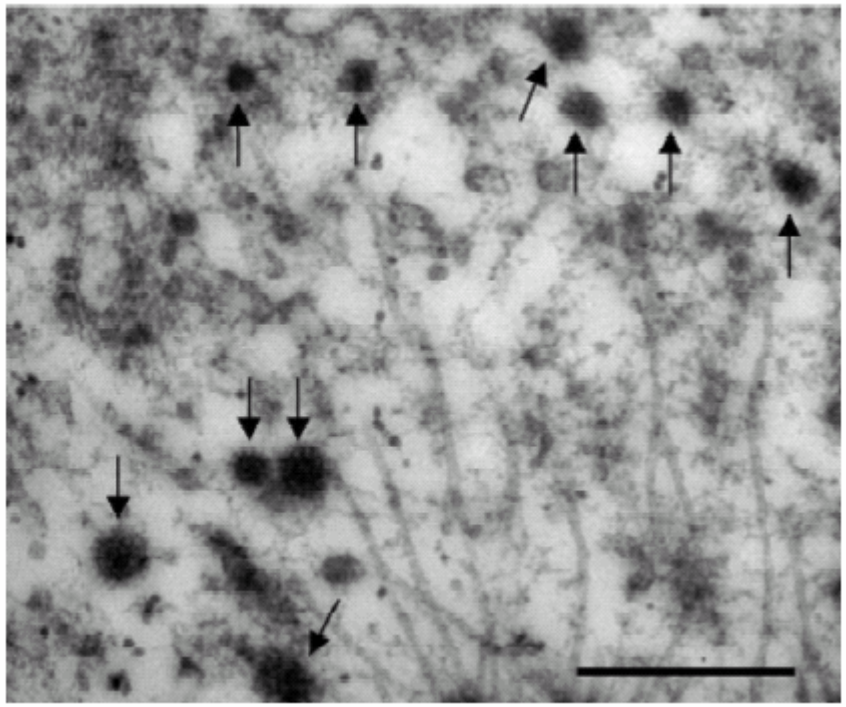
Nanoparticles eventually act as intracellular reservoirs for sustained release of encapsulated therapeutic agent.

V. Panyam and V. Labhasetwar, Adv. Drug Deliv. Rev., 2003



TEM micrograph of PLGA nano particles in cytoplasm of vascular smooth muscle cells

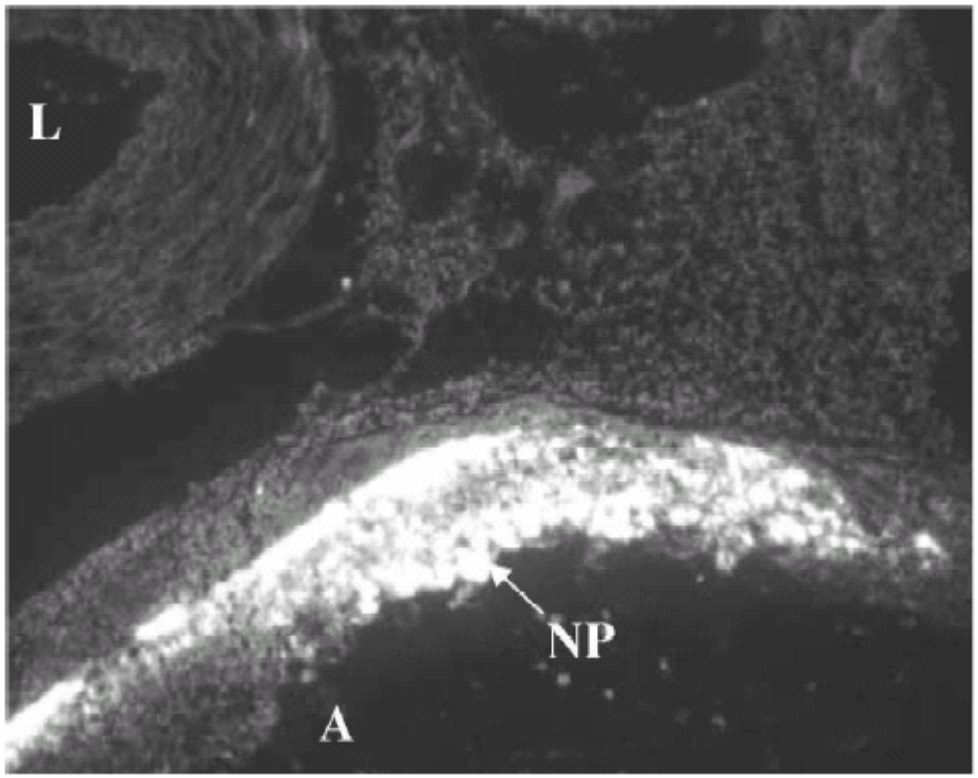
PLGA poly(D,L-lactide-co-glycolide) is a biodegradable polymer. Bar is 250 nm. **V. Panyam and V. Labhasetwar, Adv. Drug Deliv. Rev., 2003**



Tissue targeting of nanoparticles

Cross section of pig coronary artery infused with rhodamine B containing PLGA nanoparticles. Intense fluorescence indicates deposition of nanoparticles in the arterial wall. L=lumen, NP=nanoparticles, A= adventitia.

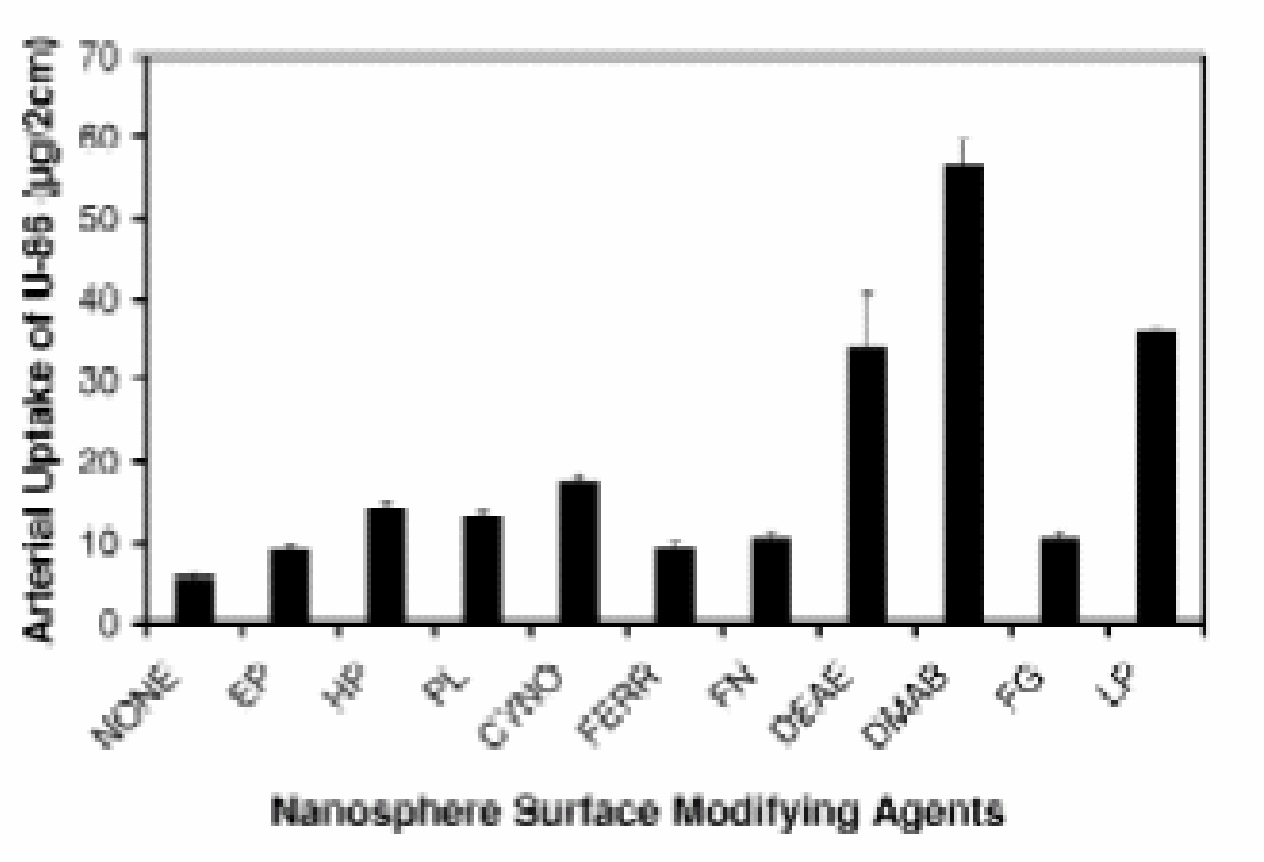
L.labhasetwar et al., Adv. Drug Deliv. Rev., 1997



Tissue targeting with surface modification: U-86 drug levels in an arterial vivo model

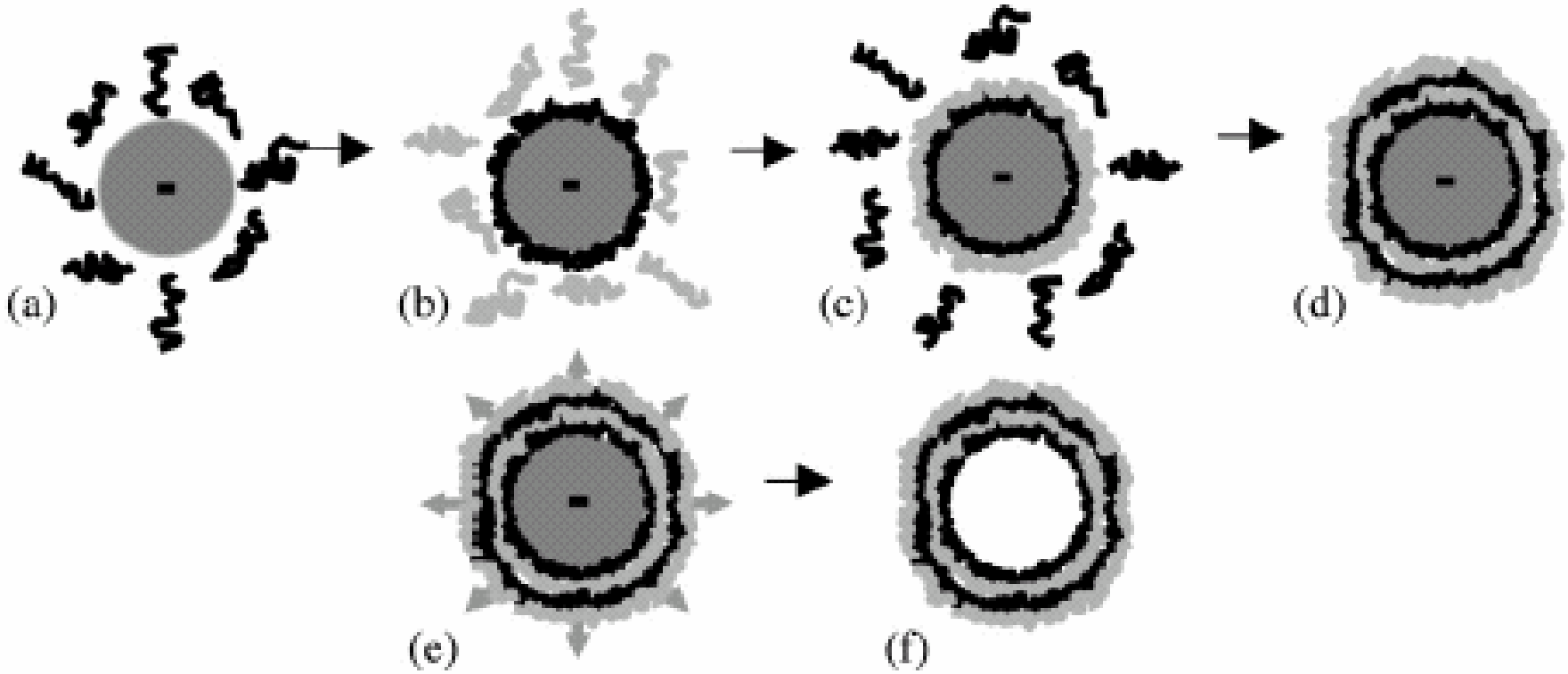
EP=epoxide, HP=heparin, PL=lipofectin, CYNO=cyanoacrylate, FERR=ferritin, FN=fibronectin, DEAE=DEAE-dextran, DMAB=didodecyldimethyl ammonium bromide, FG=fibrinogen, and LP=L-α-phosphatidylethanolamine.

V. Labhasetwar et al., J. Pharm. Sci., 1998



Layer-by-layer polyelectrolyte coating of nanoparticles

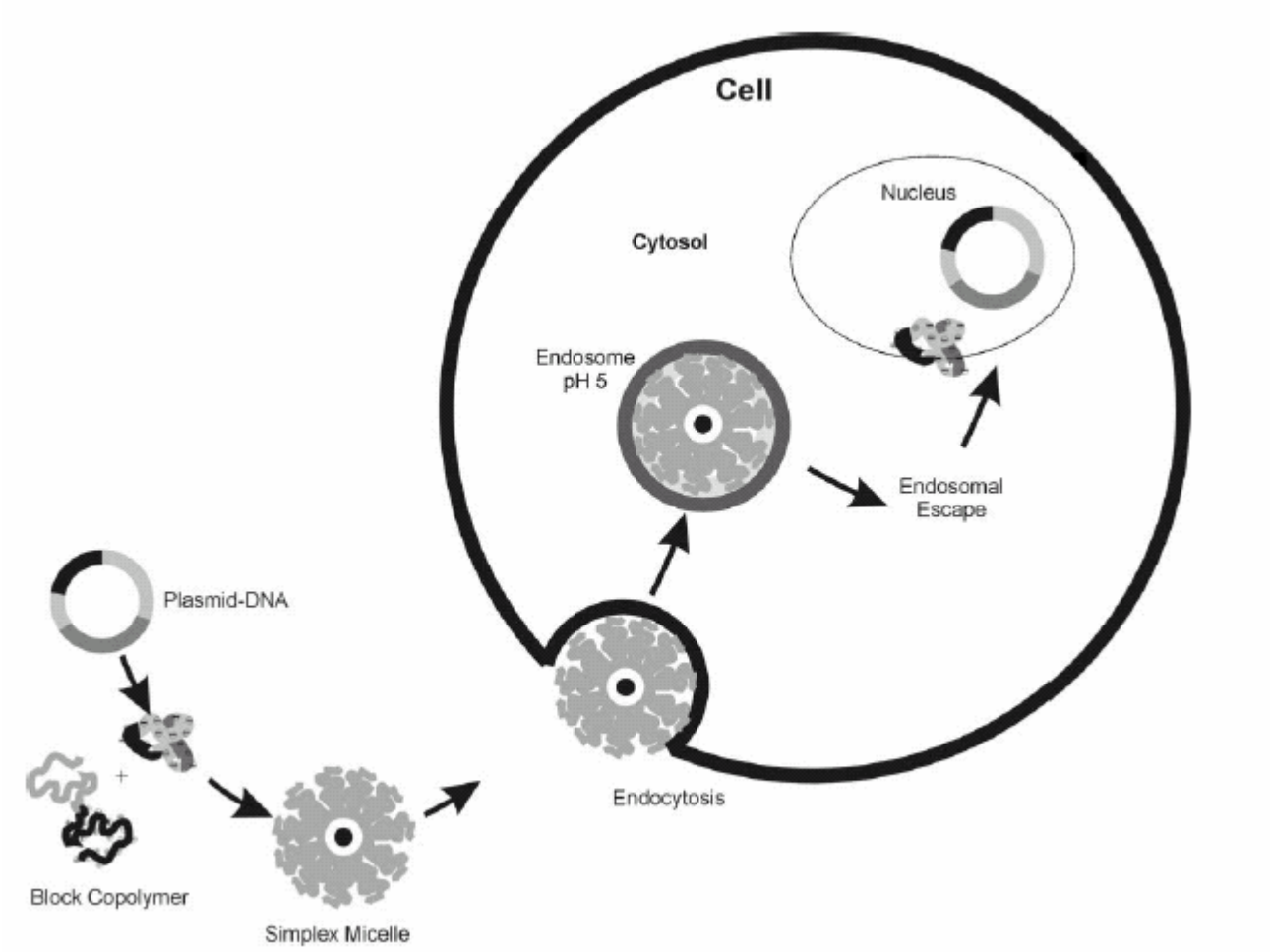
M. Schonhoff, Curr. Op. Coll. Surf. Sci., 2003



Block copolymer micelles for gene therapy

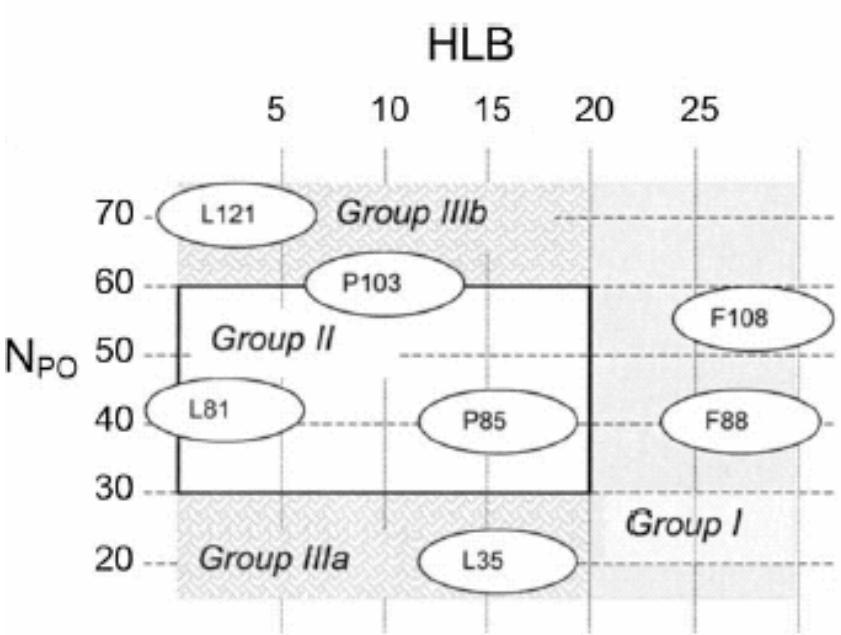
Transfection of plasmid DNA using diblock copolymer. DNA is released inside the cytosol and appears in the nucleus to express a desired protein.

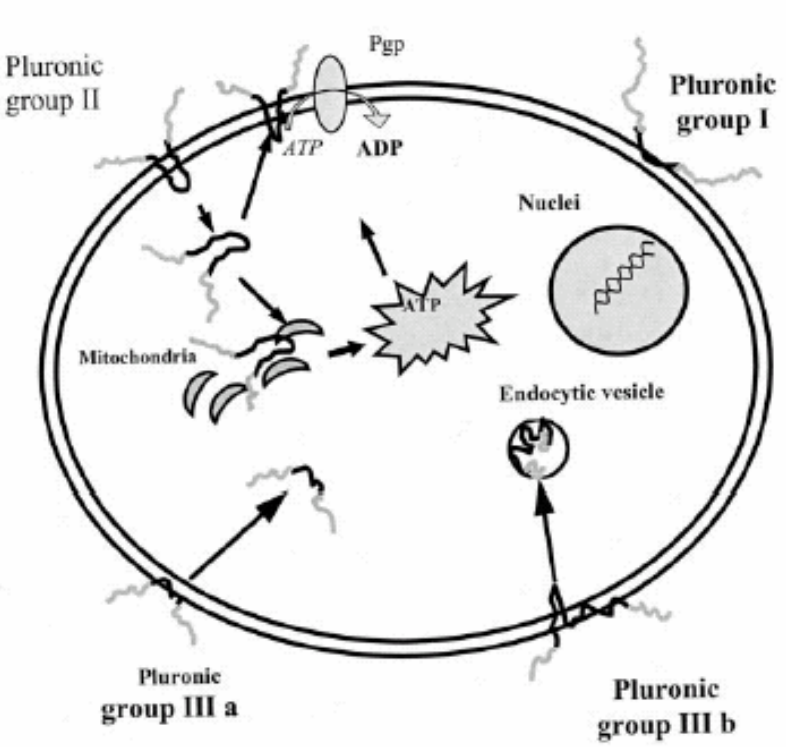
Forster and M. Konrad, J. Mater. Chem., 2003



Pluronic (triblock copolymer) grid and transport into cells: polymer structure

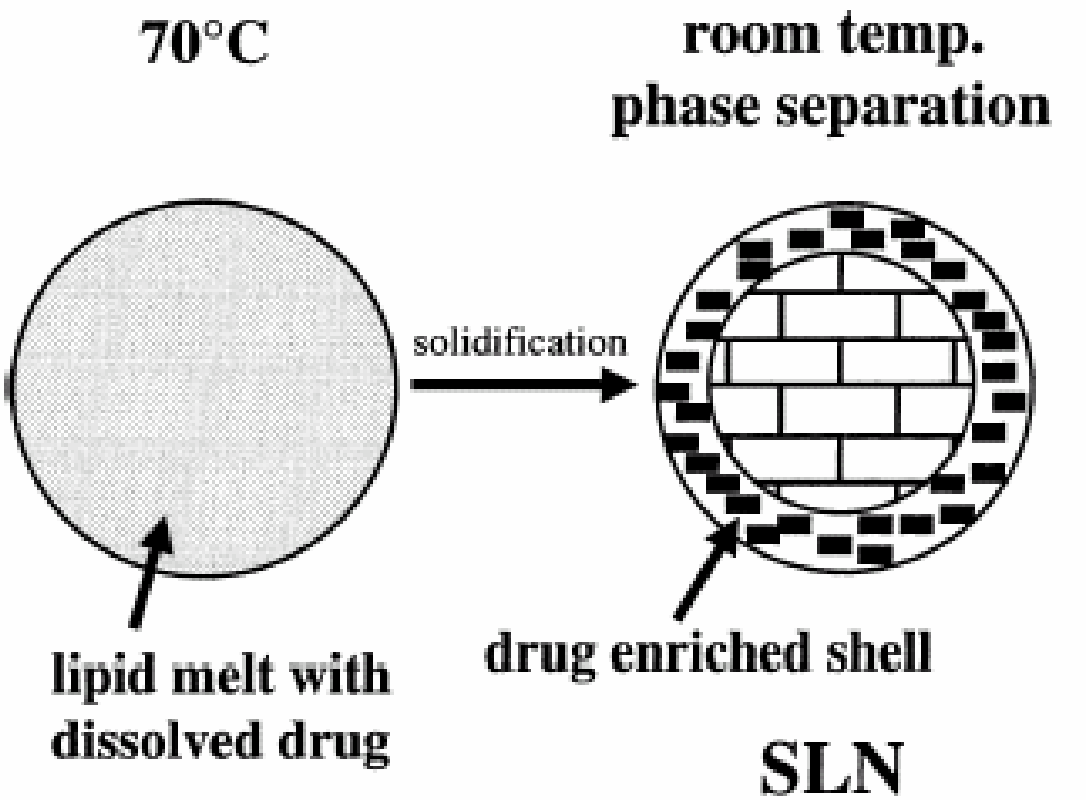
E.V. Batrakova et al., J. Pharm. Exp. Therapeutics, 2003





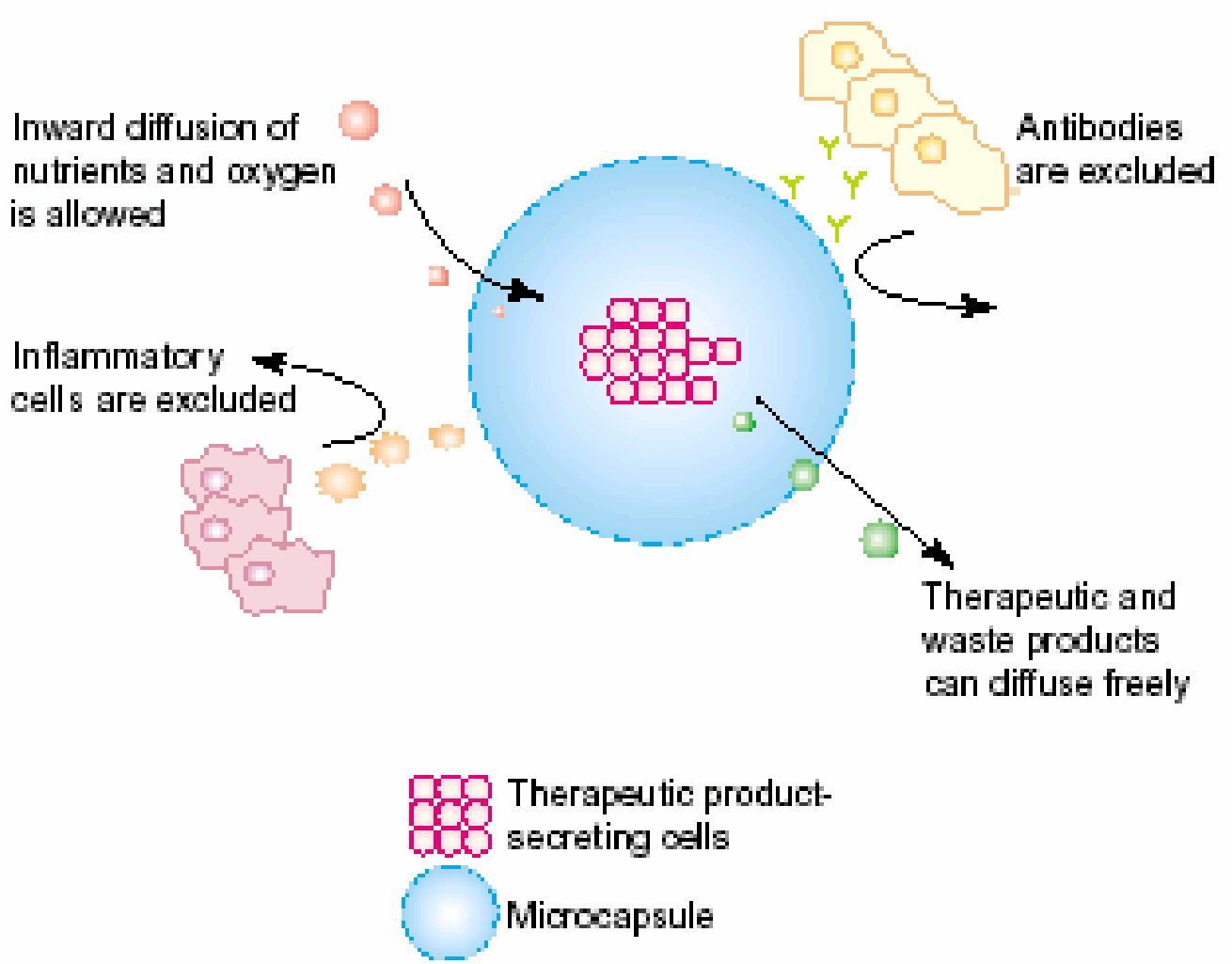
Nanostructured lipid carriers

Phase separation process during cooling in solid lipid nanoparticle (SLN) production leading to a drug enriched shell and consequently leads to a drug burst release upon use. **R.H. Muller et al., Int. J. Pharmaceut., 2002**



Cell microencapsulation in polymer matrix surrounded by semipermeable membrane

G. Orive et al., Trends Pharmacol. Sci., 2003



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Machines and molecular machines

S. Zhang, Nature Biotechnology, 2003

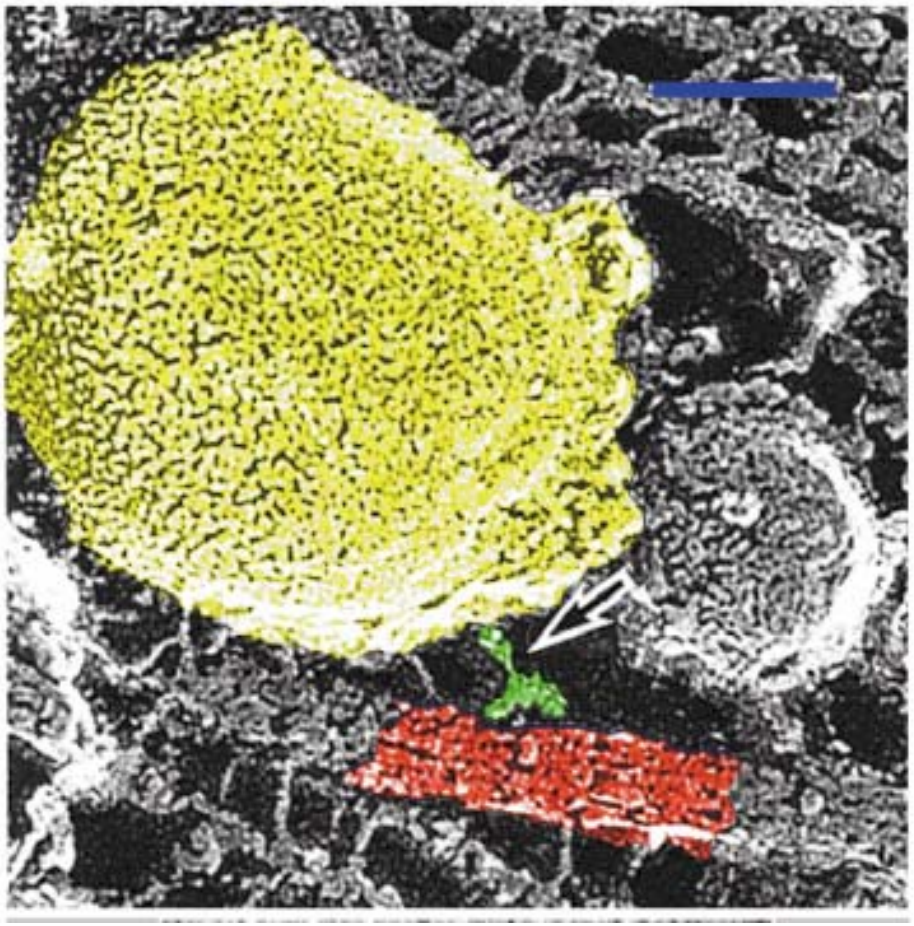
Table 1 What do they have in common? Machines and molecular machines

Machines	Molecular machines
Vehicles	Hemoglobin
Assembly lines	Ribosomes
Motors, generators	ATP synthases
Train tracks	Actin filament network
Train controlling center	Centrosome
Digital databases	Nucleosomes
Copy machines	Polymerases
Chain couplers	Ligases
Bulldozer, destroyer	Proteases, proteosomes
Mail-sorting machines	Protein sorting mechanisms
Electric fences	Membranes
Gates, keys, passes	Ion channels
Internet nodes	Neuron synapses

Motor protein in-vivo

A vesicle-carrying kinesin bound to a microtubule

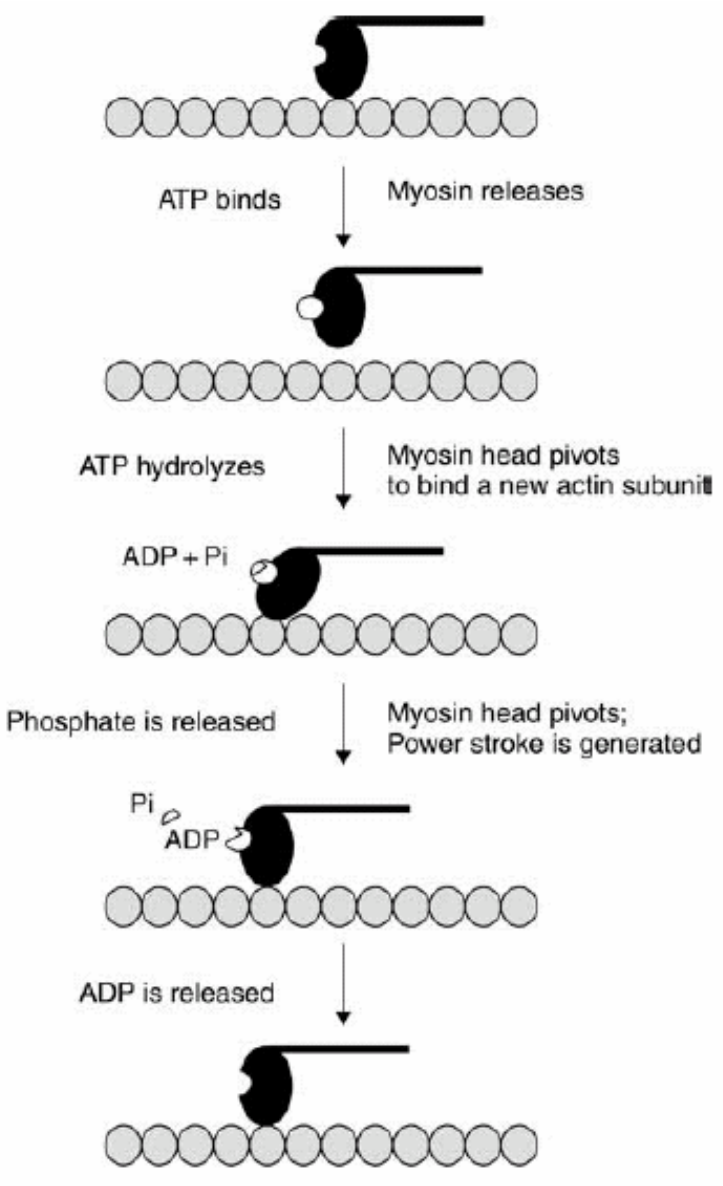
Hirokawa, Science, 1998; Hess and Vogel, Rev. Mol. Biotechnology 2001



Motor protein: myosin on actin filament

Simplified cartoon of the myosin power stroke.

B.S. Lee et al., Biomed. Microdevices, 2003



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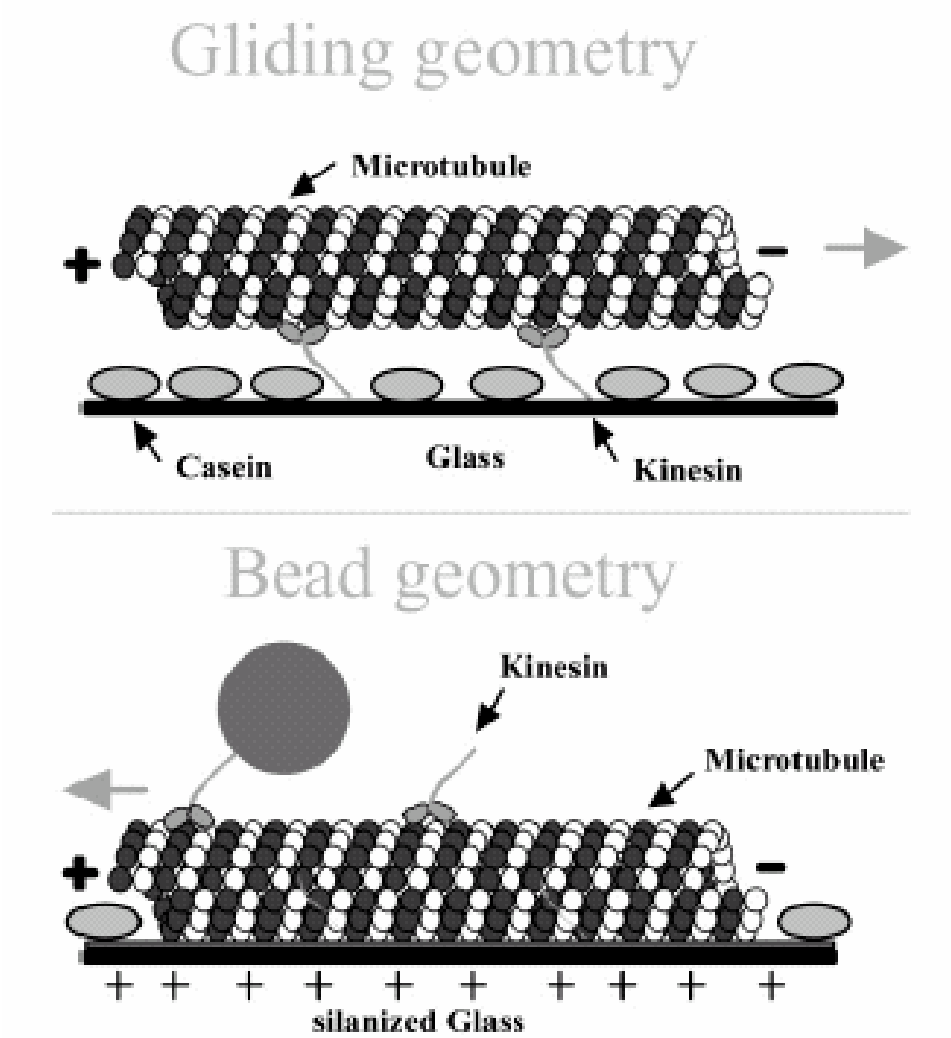
Molecular machines in-vitro

Hess and Vogel, Rev. Mol. Biotechnology 2001



Molecular machines and devices: what can we learn from biology and what machines and devices can we create that have useful biological functions?

- Power generators
- Locomotion systems
- Sensor systems
- Switches
- Control systems
- Assembly systems
- Disposal systems

Nanotechnology challenges in the life sciences

- Making materials and products bottom-up by building them up from atoms and molecules.
- Molecularly engineering of new molecules for bottom-up structures
- Understanding the forces that stabilize and maintain supermacromolecular structures.
- Developing nanocomposite materials that are stronger than steel, but a fraction of the weight (e.g. for implantable materials)
- Using gene and drug delivery to detect and treat cancerous cells or diseases
- Developing nanosensors for pollutants, viruses, toxins, bacteria, cellular activity, monitoring bioprocesses, etc.
- Removing toxins to promote a cleaner environment.
- Developing molecular machines for biological functions.