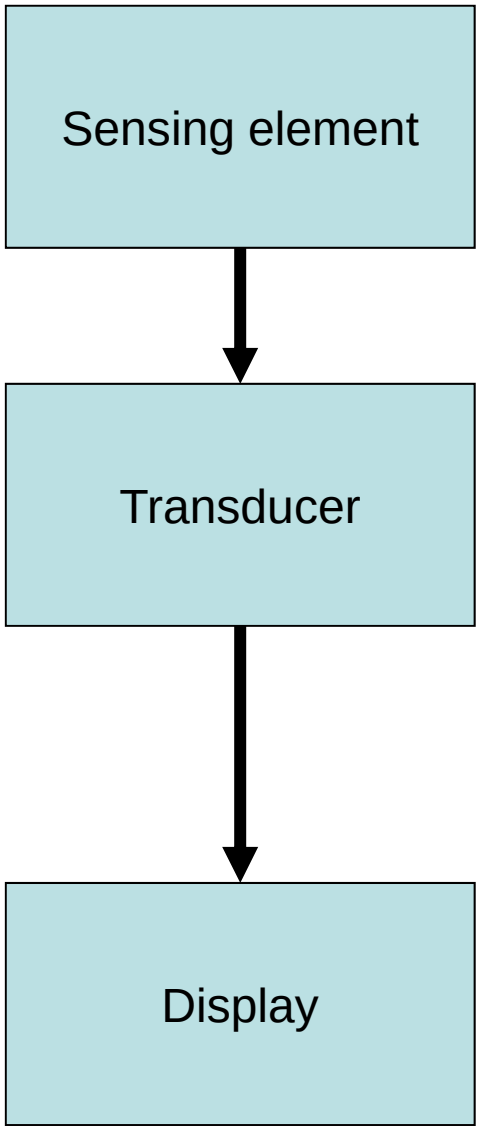
Lecture 9

DNA Arrays.
Reaching single molecule sensitivity:
Patch clamping and Nano-sensors

DNA sensing

- **The Aim:**
  - Detect the presence of a particular DNA sequence in a sample
- **Applications:**
  - Detection of bacteria and viruses
  - Diagnostics of genetic diseases
  - DNA sequencing

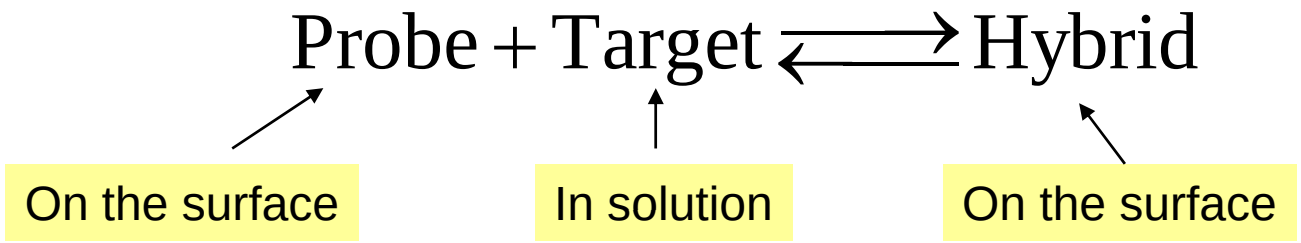
DNA sensing



- Single-stranded DNA (ssDNA) that can hybridize the matching strand

- Any device or process that can discriminate between ssDNA and dsDNA at the interface (e.g. e/chemical, fluorescent sensing etc.

Efficiency of DNA hybridization



- Hybridization efficiency is a measure of a proportion of probe nucleotides that successfully hybridize to the complementary target strand

$$K = \frac{[\text{Hybrid}]}{[\text{Probe}][\text{Target}]} \quad or \quad \nu = \frac{[\text{Hybrid}]}{[\text{Probe}] + [\text{Target}]}$$

Efficiency of DNA hybridization

- Characteristics of surface bound probe
  - surface concentration
  - probe structure and orientation
- Hybridization conditions
  - temperature
  - ionic strength
  - stability of mismatches (number, bp involved, location etc)
- Hybridization kinetics

Surface bound probe

- Probe concentration:
  minimize steric hindrance at highest density

Theoretical estimates of the optimal surface concentration

Table 1 Relating length of probe oligonucleotide in base pairs with maximum close packed ($\sqrt{3}$) surface coverage to minimize nearest neighbor interactions. Roughness is r = (actual surface area)/(geometric area)

Attachment point	Equation	Variables
Terminus	$\frac{1.48 \times 10^{14} r}{b^2}$ molecules per cm^2	b is length in bases and r is roughness factor
Terminus via spacer	$\frac{2.88 \times 10^{15} r}{[(4.42b) + s]^2}$ molecules per cm^2	b is length in bases, r is roughness factor, and s is spacer length in Å
Midpoint	$\frac{3.7 \times 10^{13} r}{b^2}$ molecules per cm^2	b is total length in bases and r is roughness factor
Midpoint via spacer	$\frac{2.88 \times 10^{15} r}{[(4.42b) + s]^2}$ molecules per cm^2	b is length in bases, r is roughness factor, and s is spacer length in Å

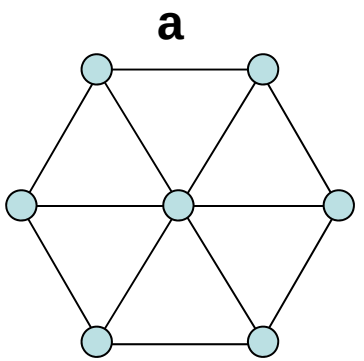
Surface bound probe

- For example:
  - 20 bp ssDNA probe attached via C6-thiol linker

$$\frac{2.88\cdot 10^{15}}{\left(4.42b+s\right)^2}=\frac{2.88\cdot 10^{15}}{\left(4.42\times 20+7.5\text{Å}\right)^2}=\frac{2.88\cdot 10^{15}}{9.2\cdot 10^3}=3.13\cdot 10^{11}\,\text{molecules/cm}^2$$

average distance between the molecules:

$$a=\sqrt{\frac{2}{\sqrt{3}N}}\approx 19\,\text{nm}$$



Practical issues:

- Determining DNA concentration:
  - measure absorbance of DNA solution at 260nm, 280nm, 230 nm and 325nm
  - concentration is given by the following equation:

$$C(pmol/\mu l) = A_{260}\frac{100}{1.5n_A + 0.71n_C + 1.2n_G + 0.84n_T}$$

where n_A, n_C, n_G and n_T are the number of corresponding bp

  - ratios of absorbances should be as below in purified samples:

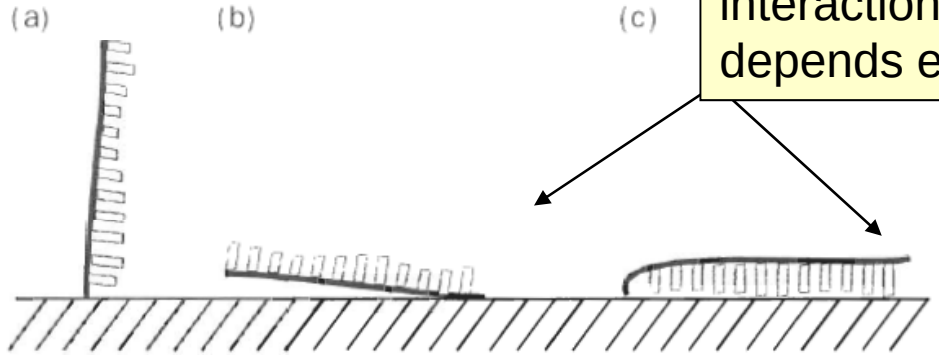
$$A_{260}/A_{280} \approx 1.8 \div 1.9; \quad A_{230}/A_{325} \approx 1.9 \div 2.0;$$

Practical issues

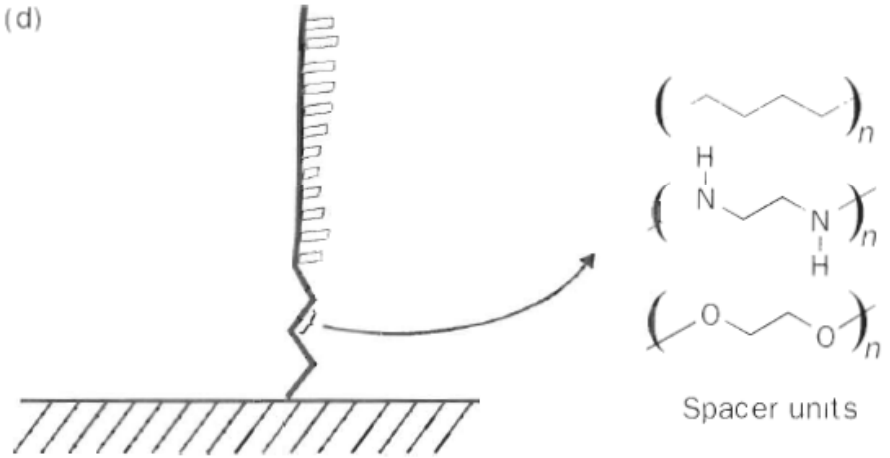
- Attachment of DNA to gold surface:
  - use freshly sputtered gold surface or clean it by repetitive cycling in a weak acid (50mM H_2SO_4)
  - mix DNA and diluent thiol (e.g. cysteamine) in HEPES buffer
  - place solution on the electrode surface and leave for 24h
  - wash thoroughly with DI water

Surface bound probe

- Orientation of probe on the surface
- Important issues:
  - the length of probe and spacer
  - properties of the spacer (hydrophobicity, charge etc.)



interaction with the surface, depends e.g. on charge

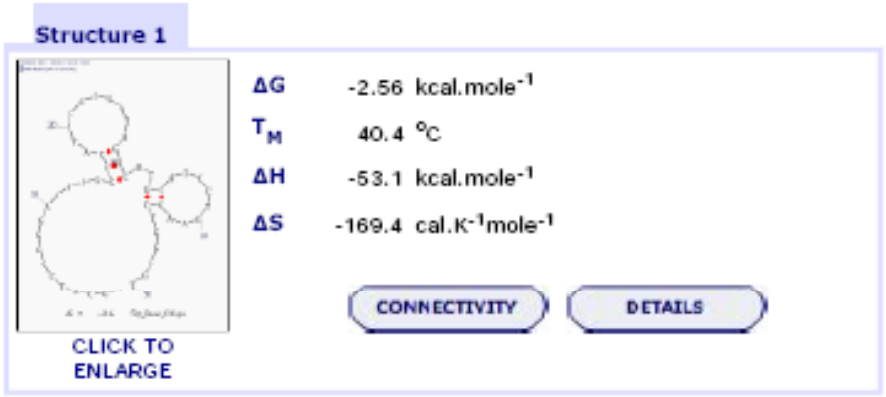


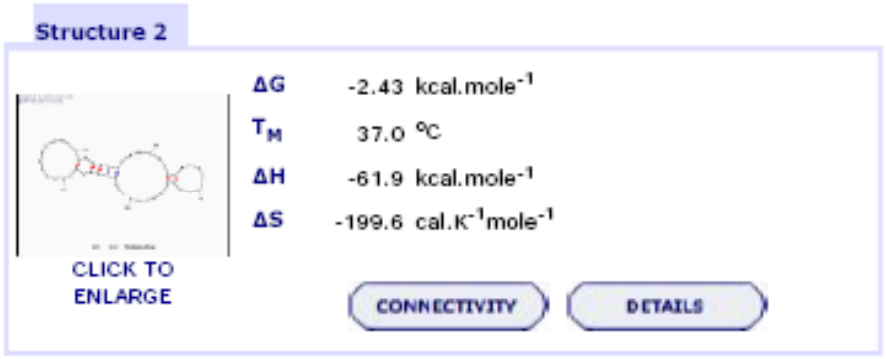
long spacers reduce steric effects, but reduce solubility, amino-based and glycol spacers are more water soluble

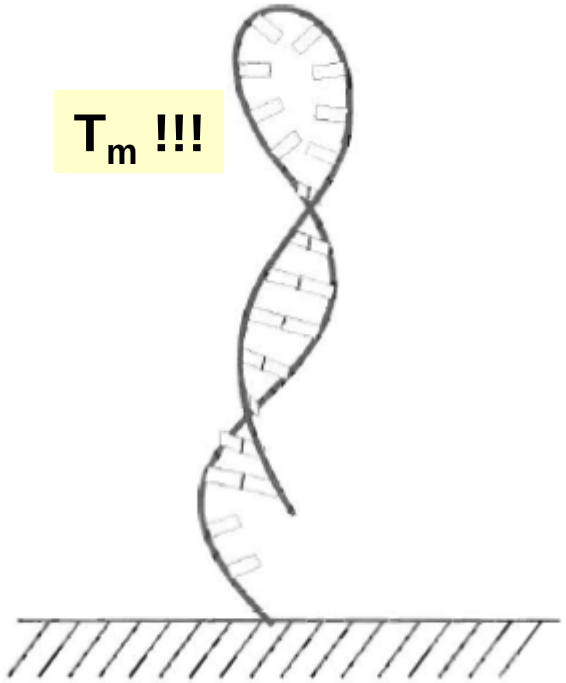
Surface bound probe

- ssDNA on the surface can acquire a secondary structure (e.g. hairpin structure) greatly reducing efficiency of hybridization

- Probe should be optimized using e.g. mFold: http://eu.idtdna.com/Scitools/Applications/mFold/

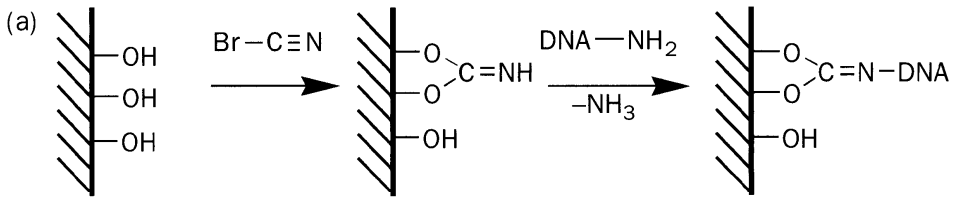






Attachment of DNA

- base-by-base synthesis on the surface of interest (e.g. Affymetrix arrays)
- attachment of ready synthesized ssDNA
  - Physisorption on polymeric membranes (nitrocellulose, polystyrene, nylon) of lond ssDNA (>500bp)
  - Covalent attachment to gold via thiol linker
  - CNBr activated coupling to hydroxilated surface (e.g. oxidized graphite)



  - deposition on polymer support e.g. via electro-polymerization of pyrrole-monomer with attached DNA strands

Hybridization conditions

- Hybridization temperature:
  - defined relatively to T_m: optimally ~T_m-10ºC.
  - melting temperature of an oligonucleotide:

$$T_m\left(^\circ C\right) = 81.5 + 16.6 \cdot \log\left[\mathrm{Na}^+\right] + 0.41\left[\%\left(G+C\right)\right] - 600/L$$

length in bp

the equation is valid for 14 to 72bp at pH=5-9

  - melting temperature can be determined by absorbance as absorbance of melted DNA is higher than a dsDNA. Tm is defined as a middle point of the transition

Hfybridization conditions

- **Ionic strength:**
  - high ionic strength improves stability of the hybrids and increases the rate of hybridization (when not limited by mass transport).
  - Rate increases x4 when [NaCl] increased to 0.4M, no further changes up to 1M; usually 1M NaCL is used for hybridization
  - quaternary ammonium salts (2-3M TEA^+ or TMA^+) lower the melting point and make it independent of the base composition (good for arrays!).
- **Hybridization accelerators:**
  - 10% of dextran sulphate can increase the hybridization rate by a factor of 10 (due volume exclusion).

Hybridization kinetics

- We have a case of one ligand and muiltiple competing analytes with different k_a and k_d.

$$\text{Probe} + \text{Target}_{\text{match}} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \text{Hybrid}$$

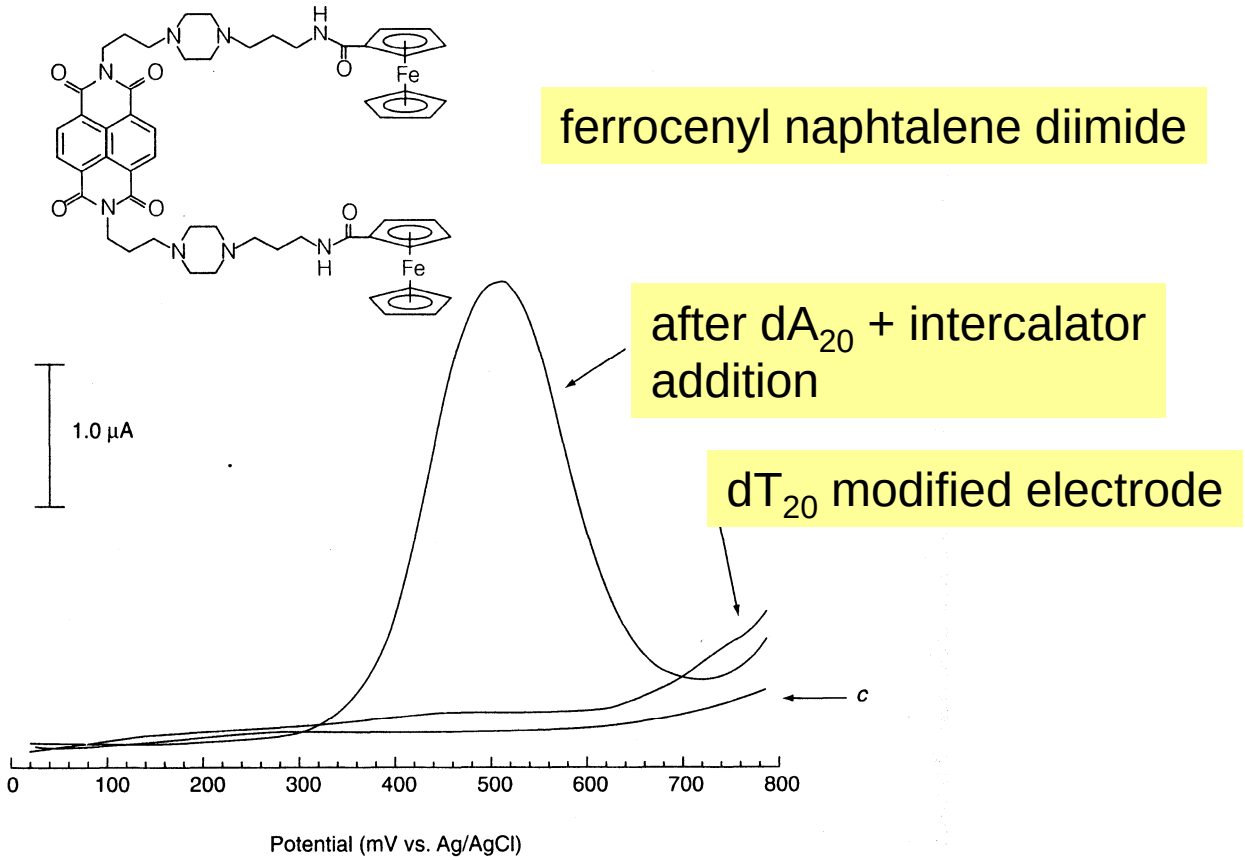
$$\text{Probe} + \text{Target}_{\text{non-match}} \underset{k'_d}{\overset{k'_a}{\rightleftharpoons}} \text{Hybrid}'$$

- Hybridization should be run at T close to Tm ($T_{\text{single mismatch}}<T<T_m$) to achieve selectivity towards the matching strand
  - depends on the position (less important at the ends) and nature of the mismatch (in the order of stability: T-A, A-T > G-T, G-A > A-A, T-T, C-T, C-A)
  - for a mismatched dsDNA an estimate:

$$T_m\left(^\circ C\right) = 81.5 + 16.6 \cdot \log\left[\text{Na}^+\right] + 0.41\left[\%\left(G+C\right)\right]` - 600/L - 50 \times M/L$$

Electrochemical DNA sensors

- Electrochemically active (redox) intercalators

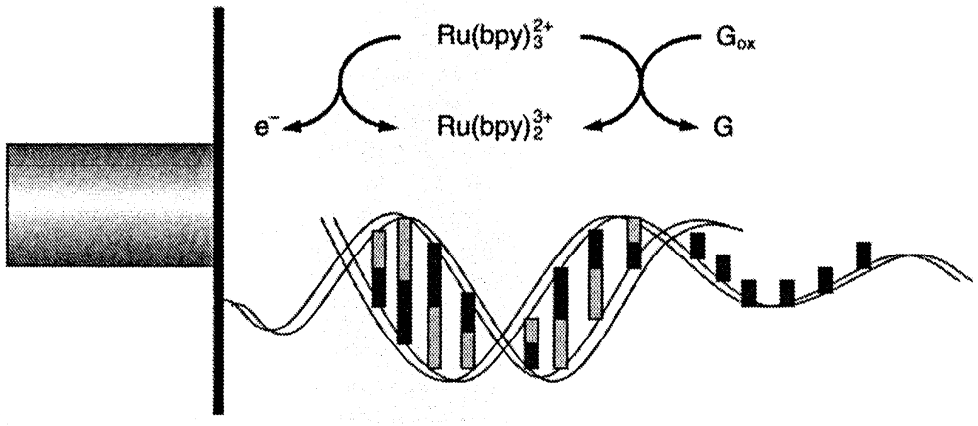


differential pulse voltammogram

Electrochemical DNA sensors

- Different rates of charge transport through ssDNA and dsDNA

- Guanine oxidation using electrocatalytical action of $Ru(bpy)_3^{2+}$



DNA sensors

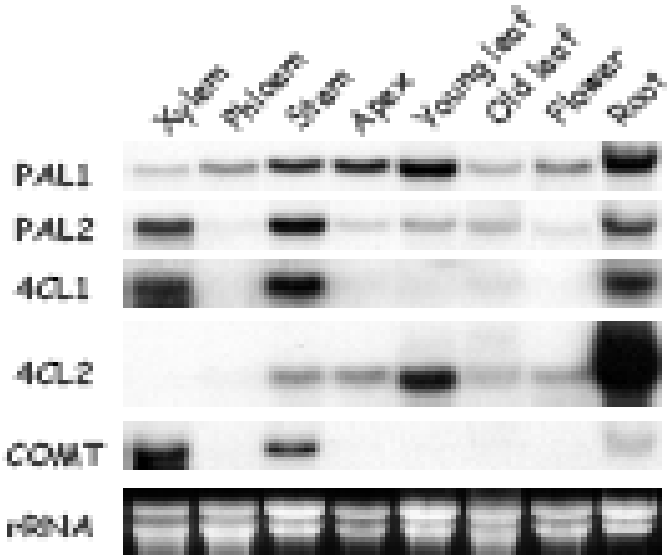
Microarray Gene Profiling

What is it for?

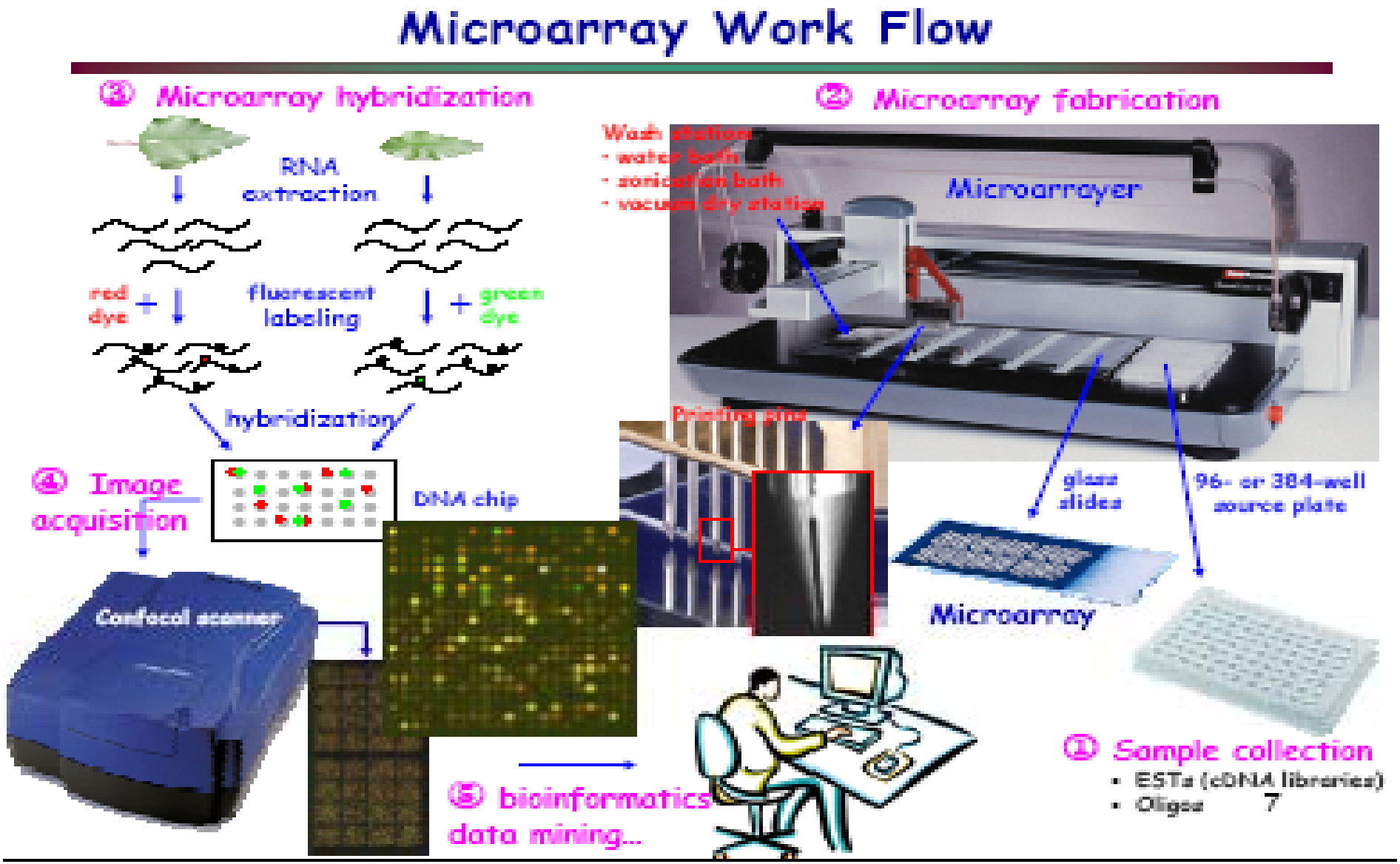
- Gene expression analysis on a large scale

"one-gene-at-a-time" approach:
- slow
- not effective for complex pathways or biological processes

>188 sequenced genomes in public and private databases



DNA sensors


Microarray Work Flow
③ Microarray hybridization
RNA extraction
red dye
fluorescent labeling
green dye
hybridization
④ Image acquisition
DNA chip
Confocal scanner
⑤ bioinformatics data mining...
② Microarray fabrication
Wash station
- water bath
- sonication bath
- vacuum dry station
Microarrayer
Printing pins
glass slides
96- or 384-well source plate
Microarray
① Sample collection
• ESTs (cDNA libraries)
• Oligos
7

DNA sensors

Microarray Gene Profiling

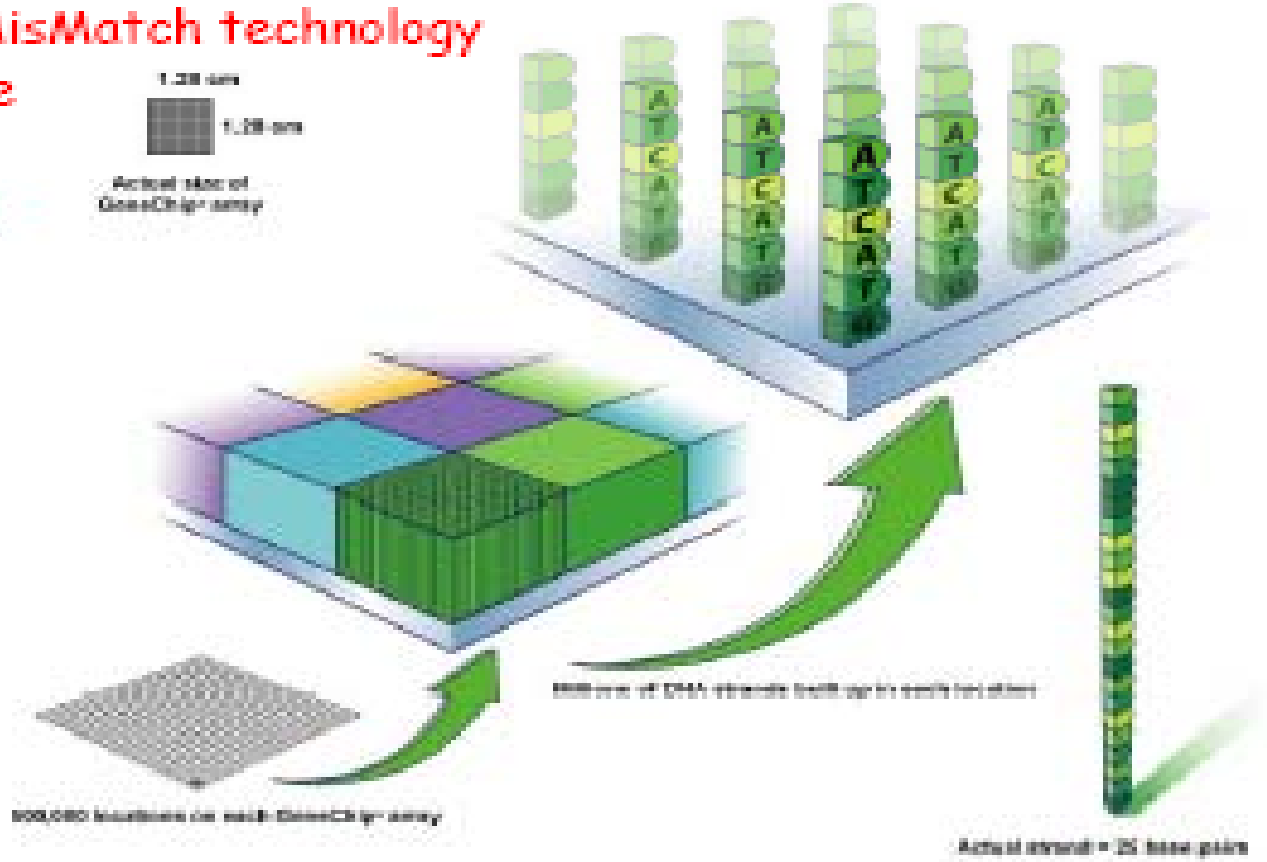
How many different types of arrays are there?

- Spotted DNA arrays
  - cDNA arrays (ESTs): require clone collection
  - Oligo arrays: require oligo synthesis (seq info needed)
- In situ synthesized oligo arrays
  - Affymetrix (GeneChip®): photolithography & solid-phase chemistry (25-mer)
  - NimbleGen: digital light processing technology (maskless) & photo deposition chemistry (24- to 70-mer)
  - Agilent: inkjet, non-contact printing technology & phosphoramidite chemistry (60-mer)

DNA sensors

Affymetrix (GeneChip®)

- 1.3 million feature capacity
- Perfect Match & MisMatch technology
- 11 probe pairs/gene
- 61,200 probe sets
- 11 µm feature size

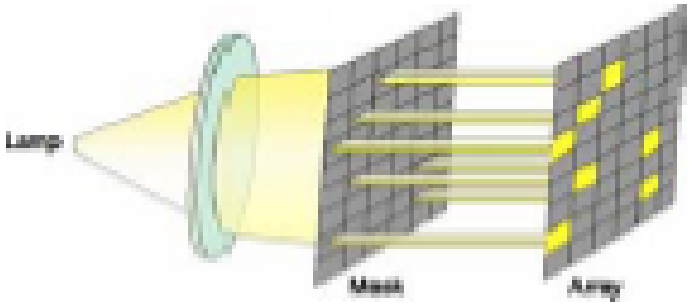


DNA sensors

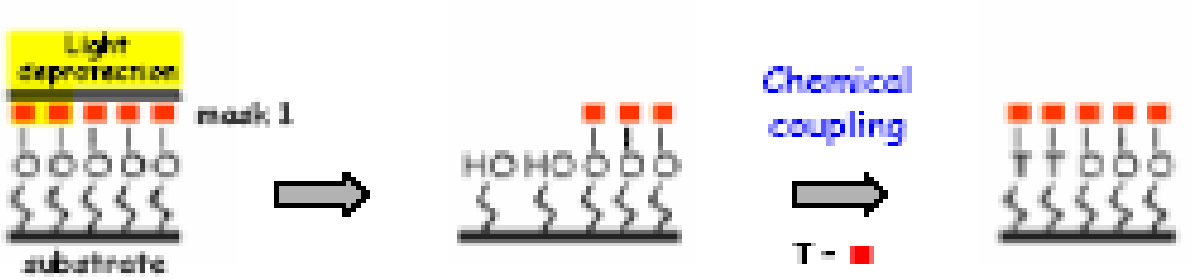
Affymetrix (GeneChip®)

Synthetic linkers modified with photochemically removable protecting groups

glass substrate



- How many masks needed per design?



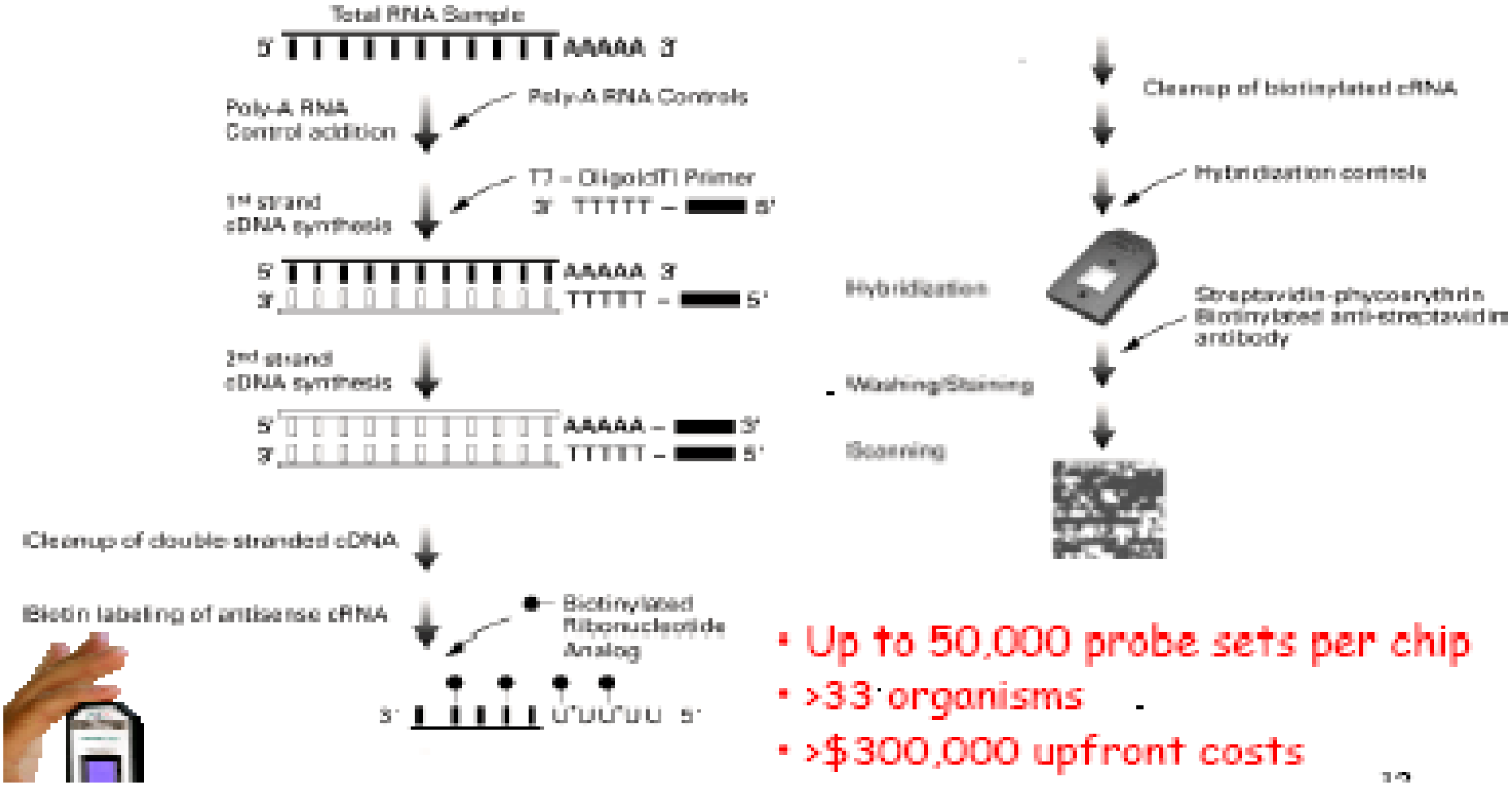
DNA sensors

Affymetrix (GeneChip®)

- Biotin-based labeling, single-channel detection

DNA sensors

Affymetrix (GeneChip®)



- Up to 50,000 probe sets per chip
- >33 organisms
- >$300,000 upfront costs

DNA sensors

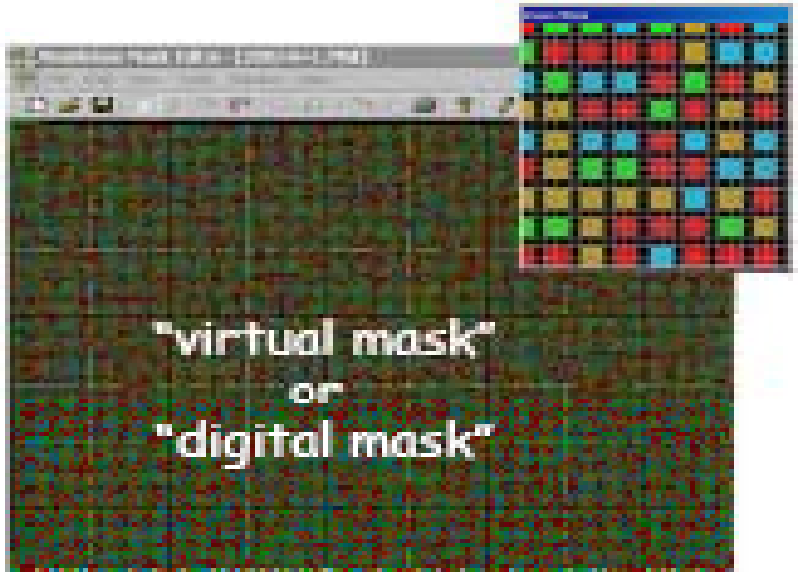
NimbleGen

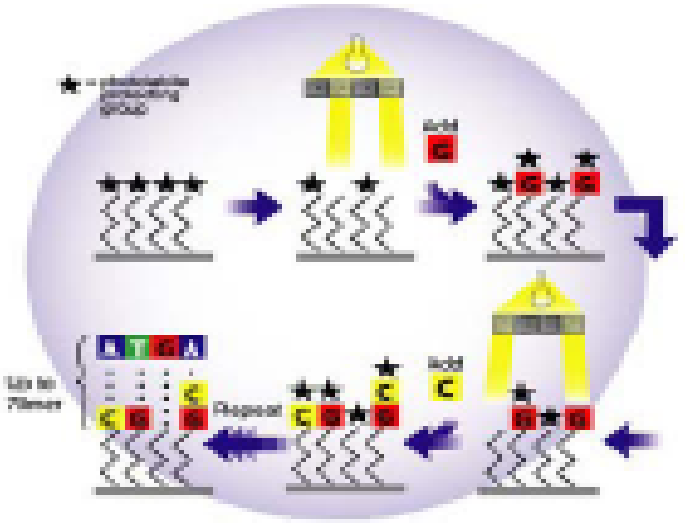
MAS technology: Maskless Array Synthesizer

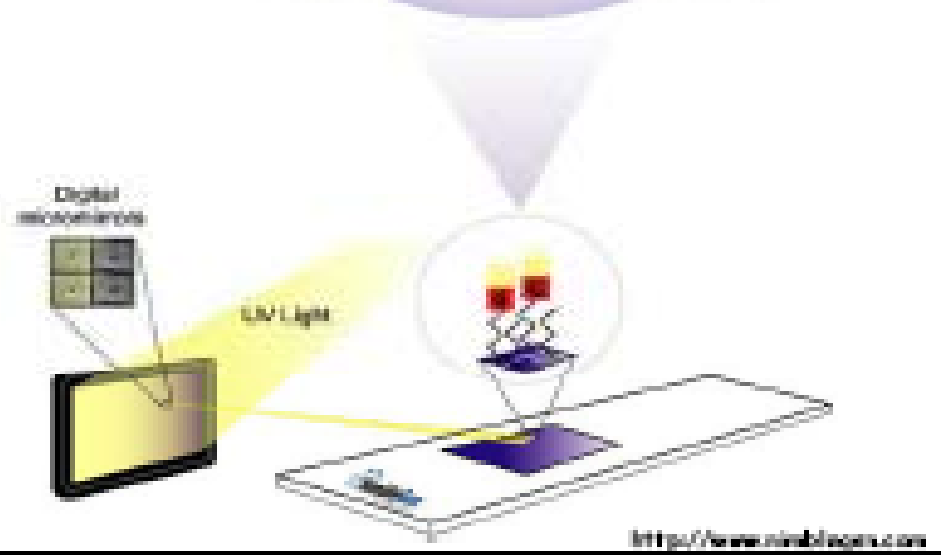
- maskless light projector
- DNA synthesizer

Digital Micromirror Device:

- miniature aluminum mirrors to pattern up to 786,000 individual pixels of light.



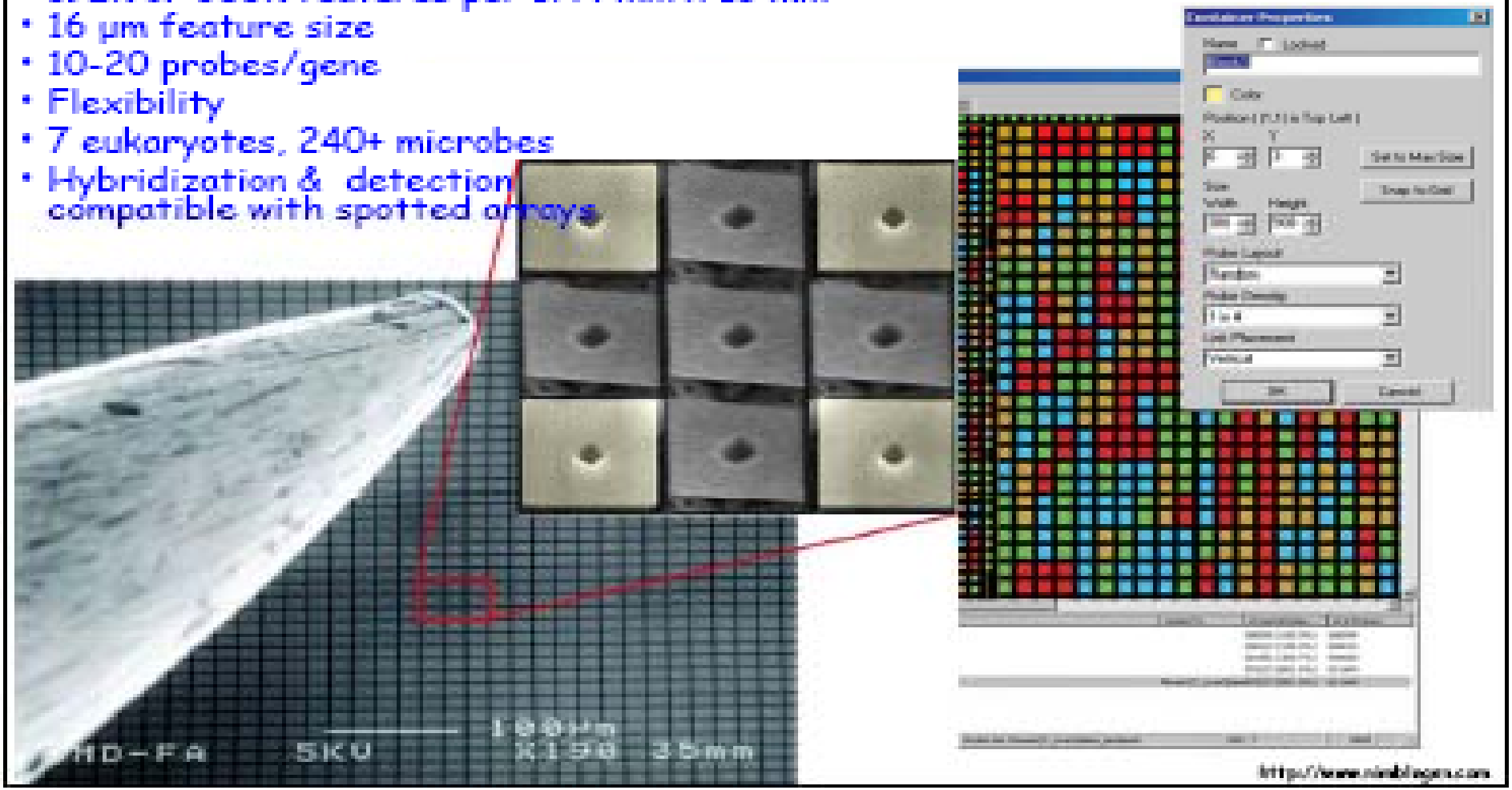




DNA sensors

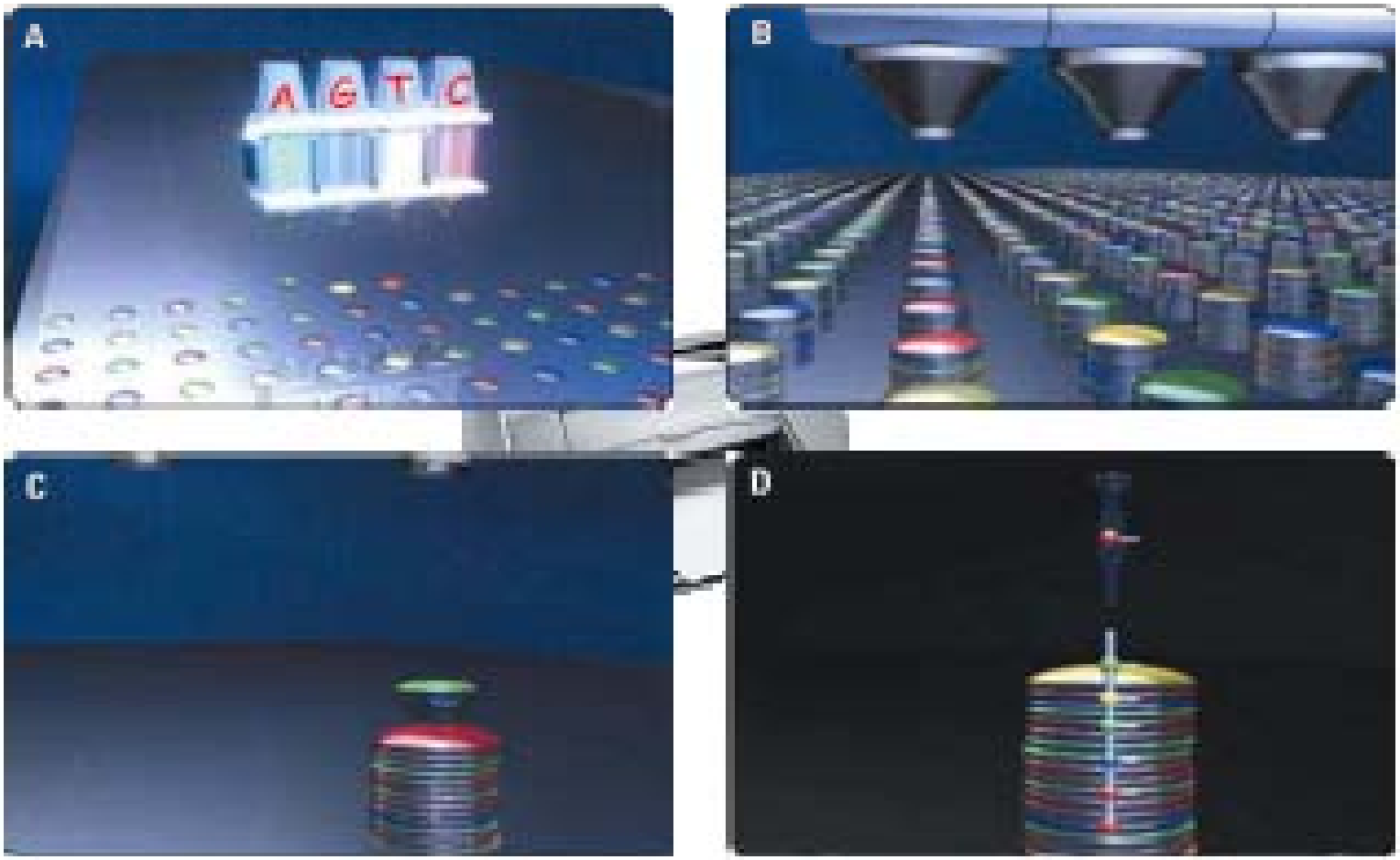
NimbleGen

- 192K or 385K features per 17.4 mm x 13 mm
- 16 µm feature size
- 10-20 probes/gene
- Flexibility
- 7 eukaryotes, 240+ microbes
- Hybridization & detection compatible with spotted arrays



DNA sensors

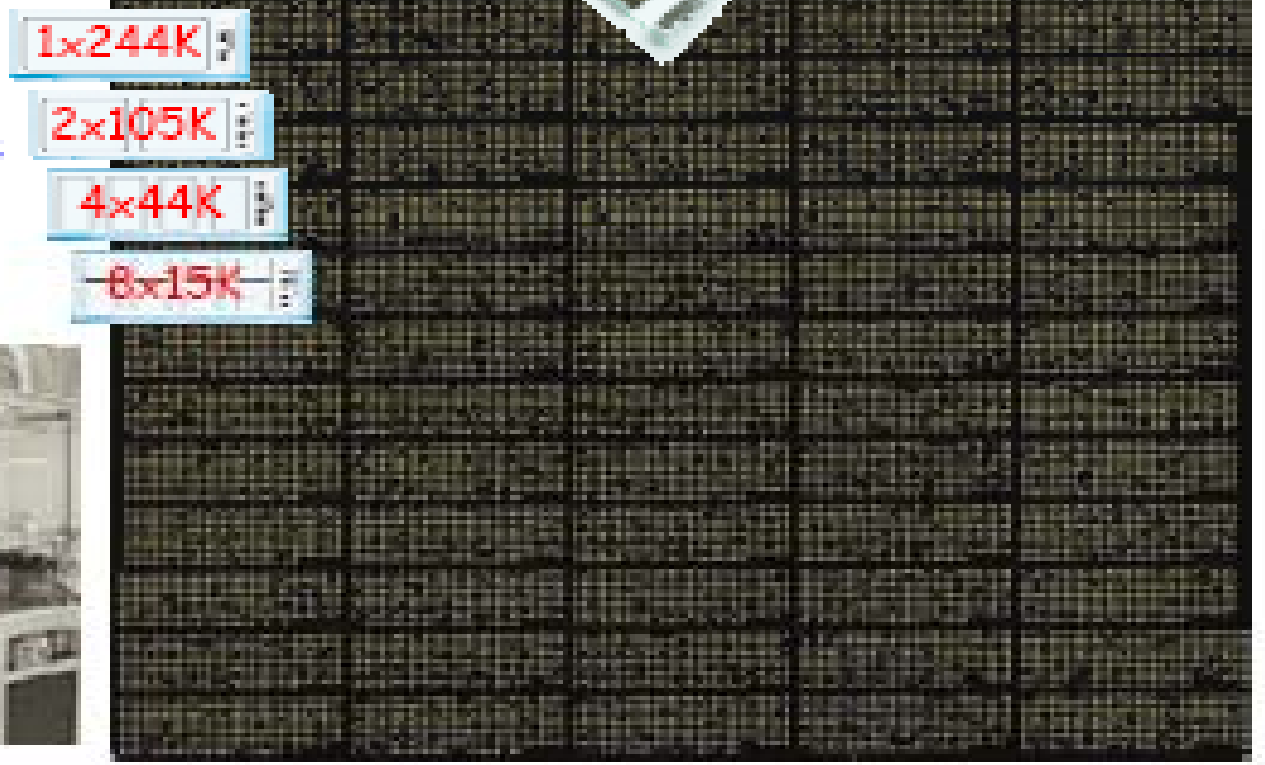
Agilent Inkjet Arrays



DNA sensors

Agilent Inkjet Arrays

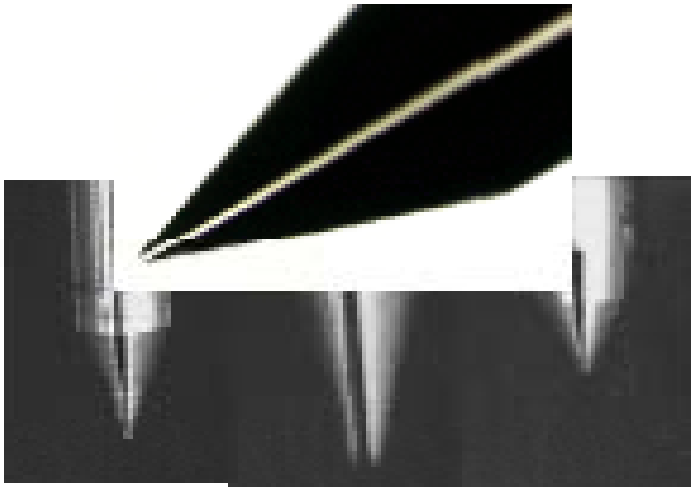
- Non-contact printing: better spot morphology & uniformity
- 244,000 spots per 1x3" slide
- 65 µm feature size
- Flexible
- 12 organisms
- Hybridization and detection compatible with spotted arrays



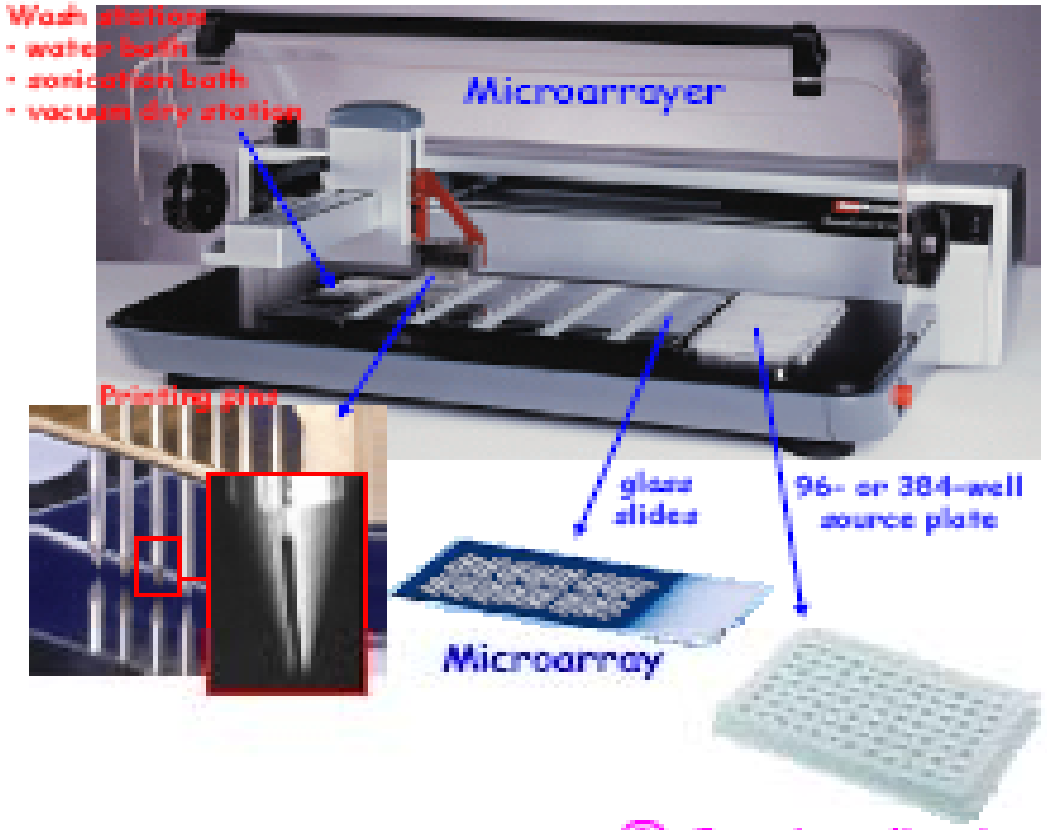
DNA sensors

Spotted DNA Arrays

- Physical clone collection & maintenance: BIG tasks
- QC & tracking
- Inconsistent print quality
  - clone prep
  - print tip
  - printing condition

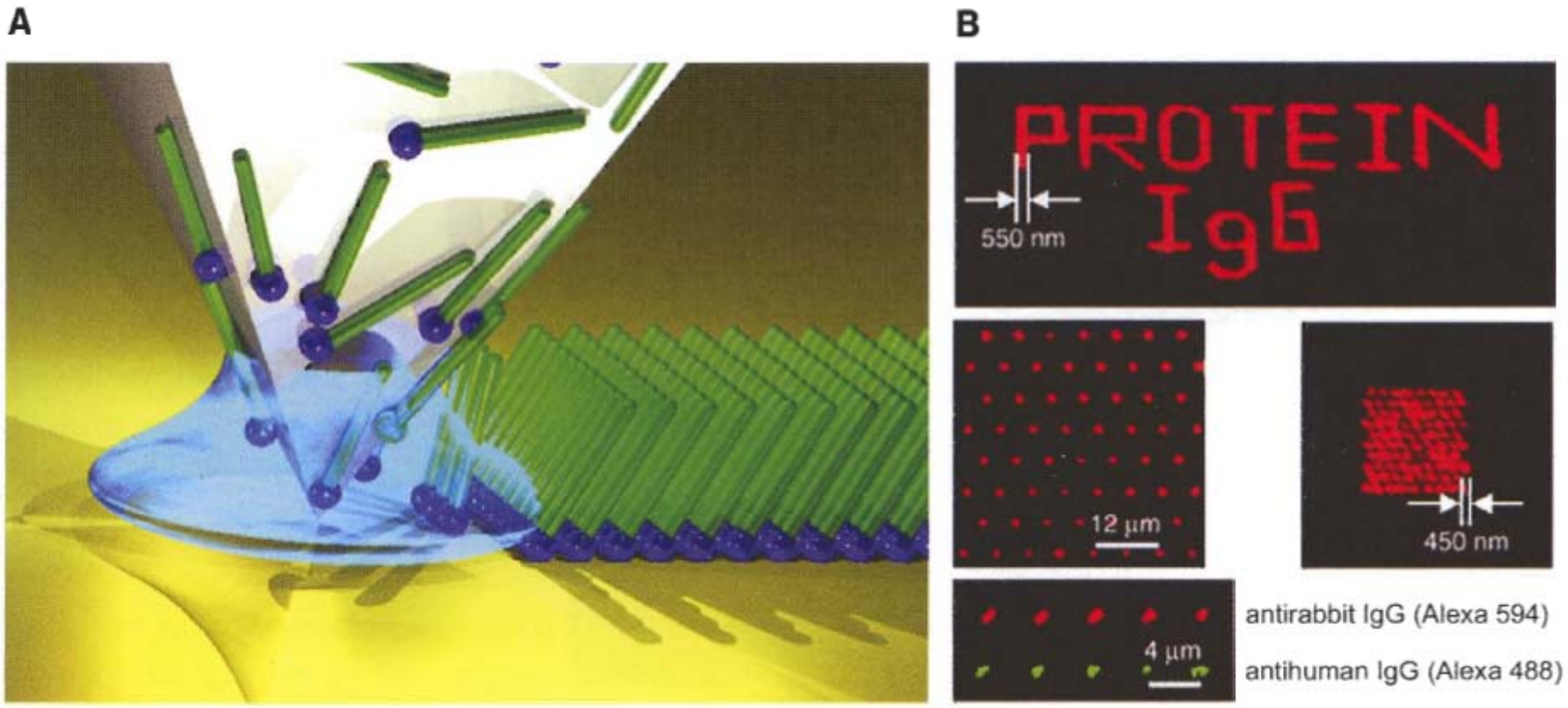


② Microarray fabrication

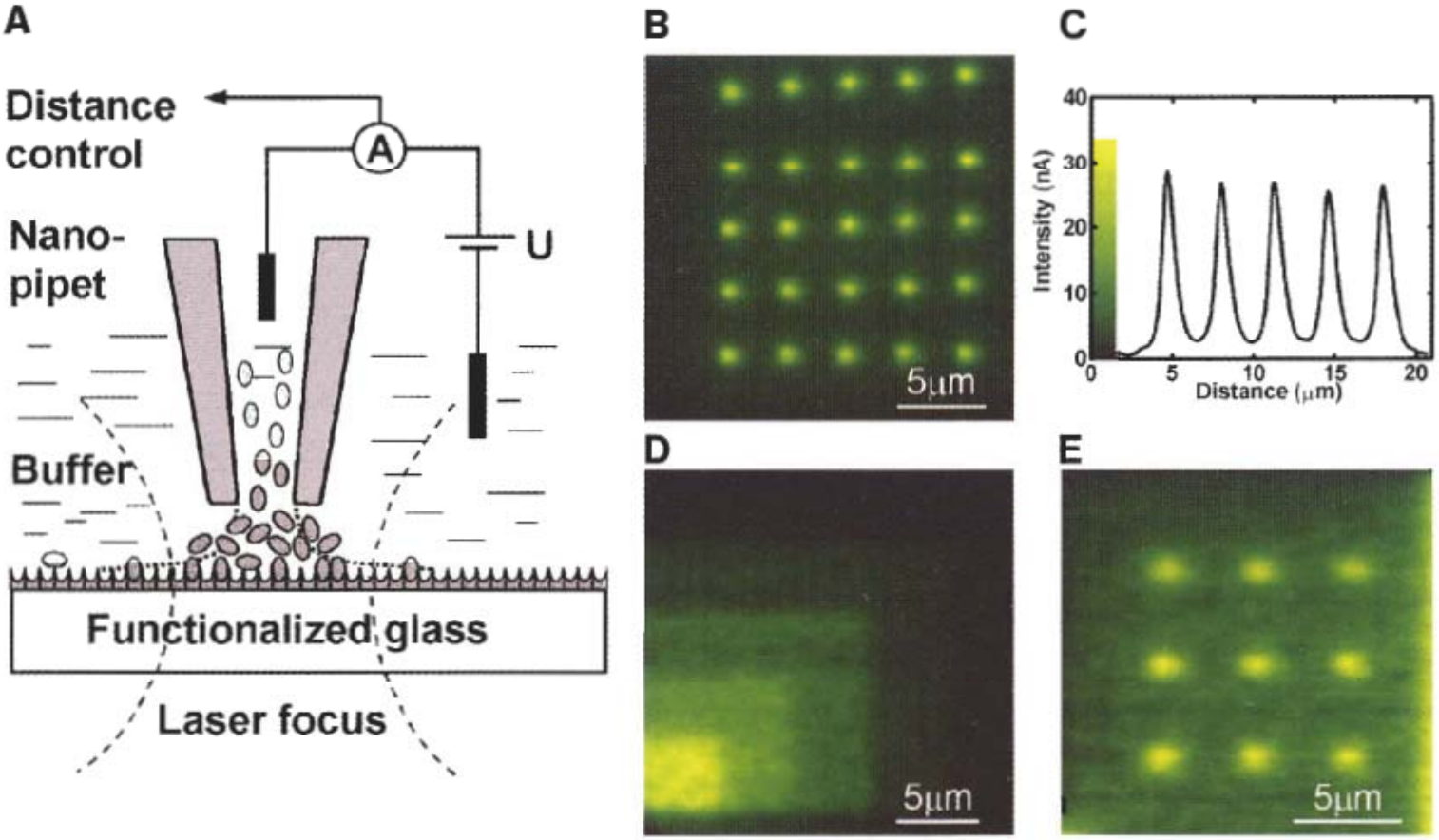


① Sample collection
- ESTs (cDNA libraries)
- Oligos

Bionanoarrays via Dip-Pen Lithography

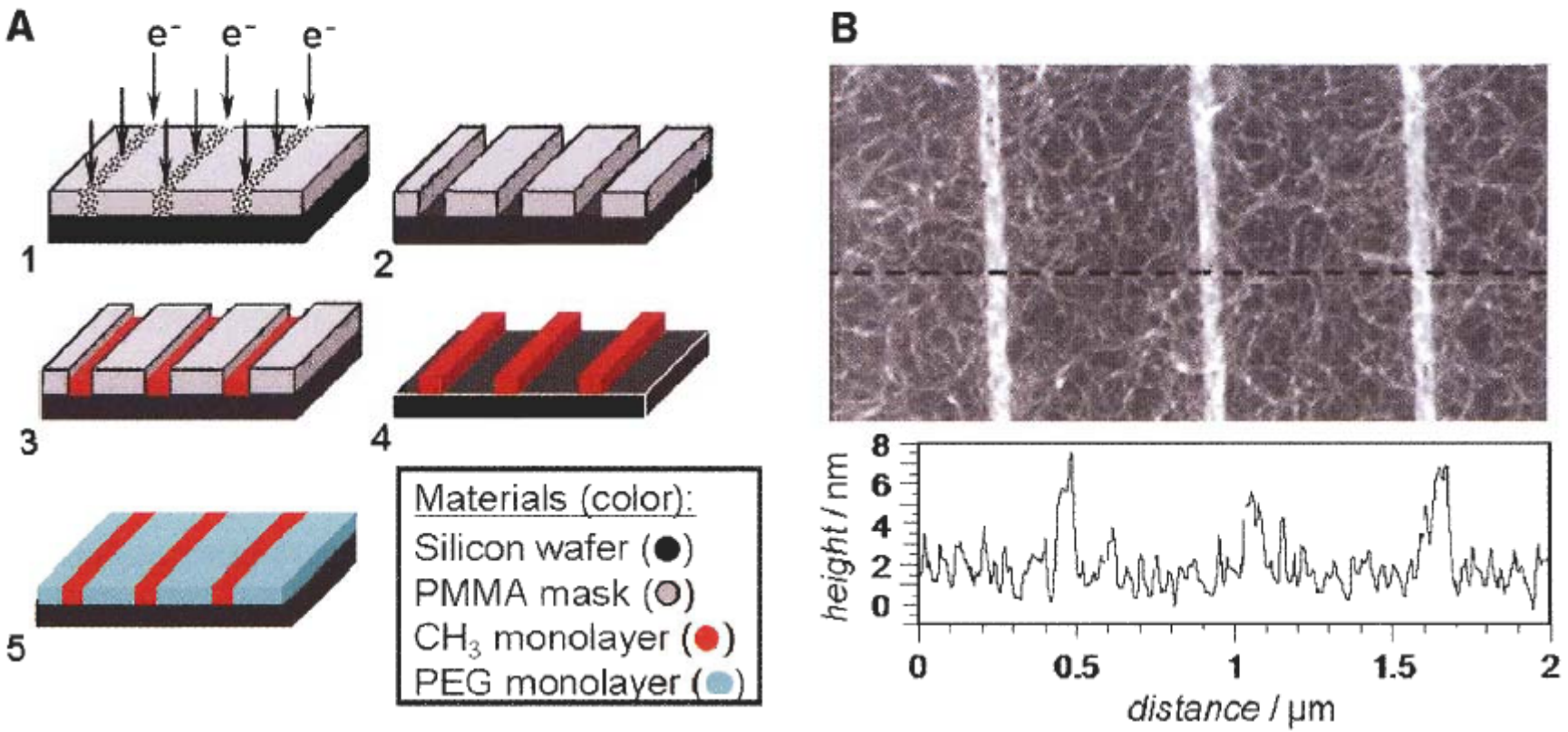


Nanopipet deposition of bioarrays



- based on scanning ion-conductance approach, ion-current is used to control the distance;
- 100-150nm pipette
- number of molecules depends on applied current, electro-osmotic effect, electrophoresis, dielectrophoresis, size, charge and polarizability of molecules
- once left the pipette, the molecules diffuse away (and driven by e/osmotic flow)
- deposits molecules in a buffer solution

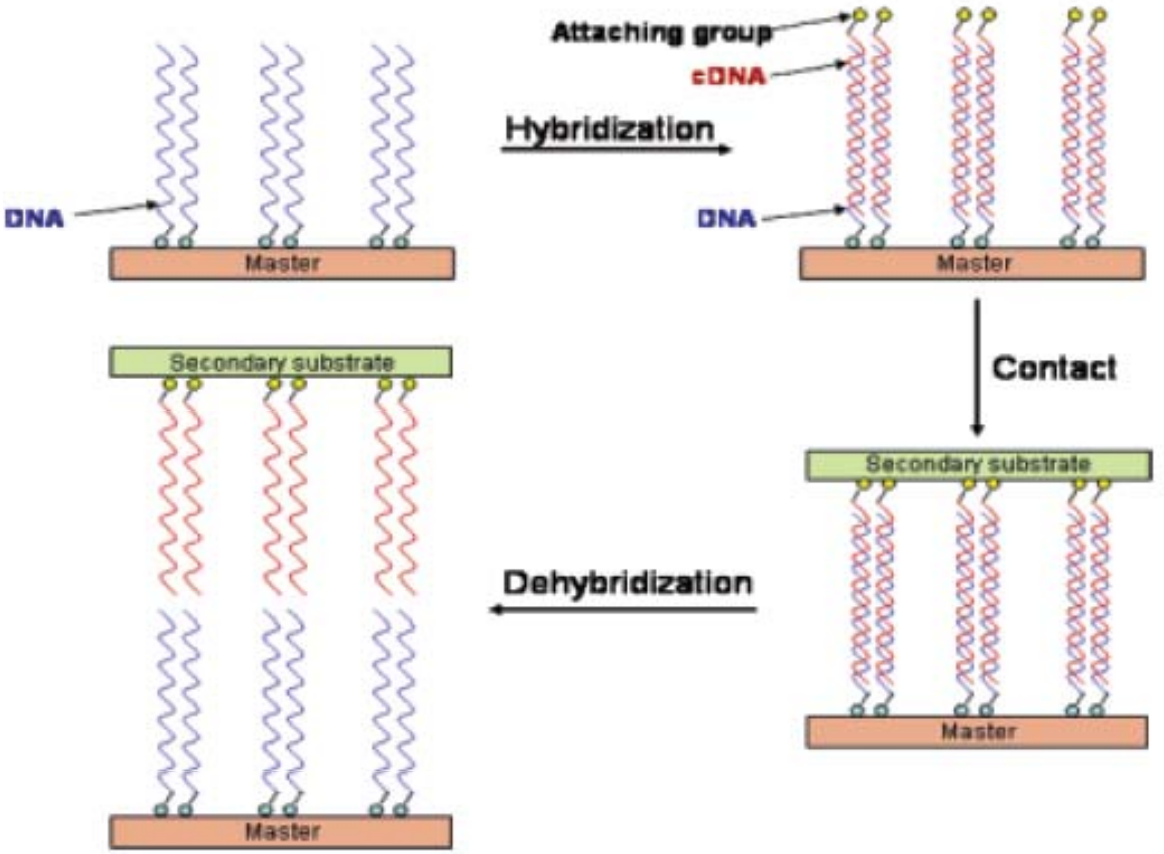
Beam based techniques



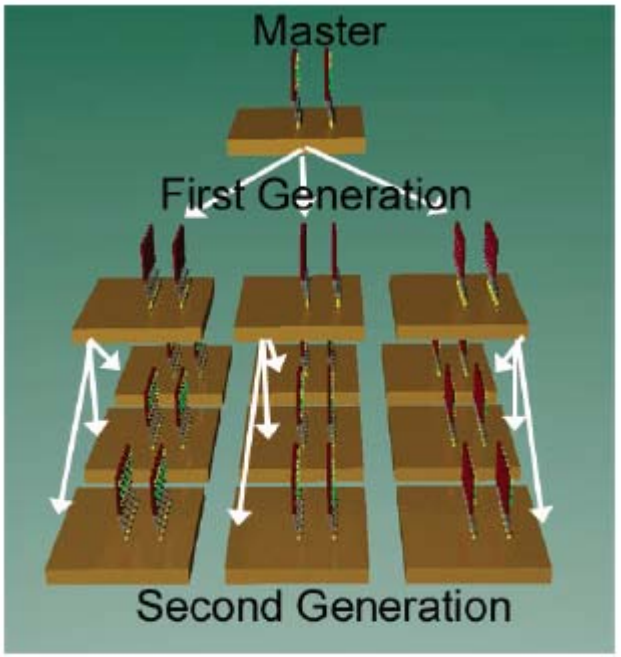
- gas-phase grafting of CH_3-alkylsilanes on silicon using PMMA mask

DNA array printing (SuNS)

Supramolecular NanoStamping technique



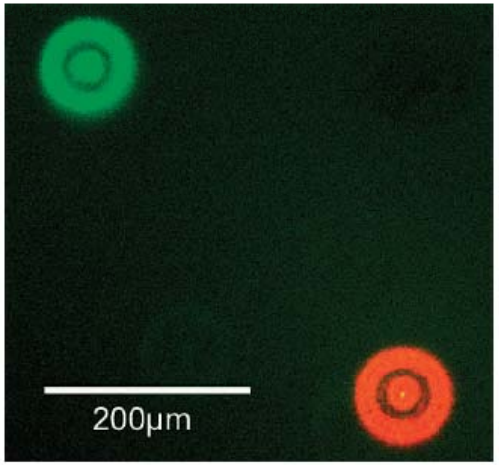
- **Thiol group for gold;**
- **hexylamine for aldehyde-modified PMMA**



- F. Stellacci et al., “High Resolution Printing of DNA Feature on Poly(methyl methacrylate)”, JACS 127, 16774 (2005

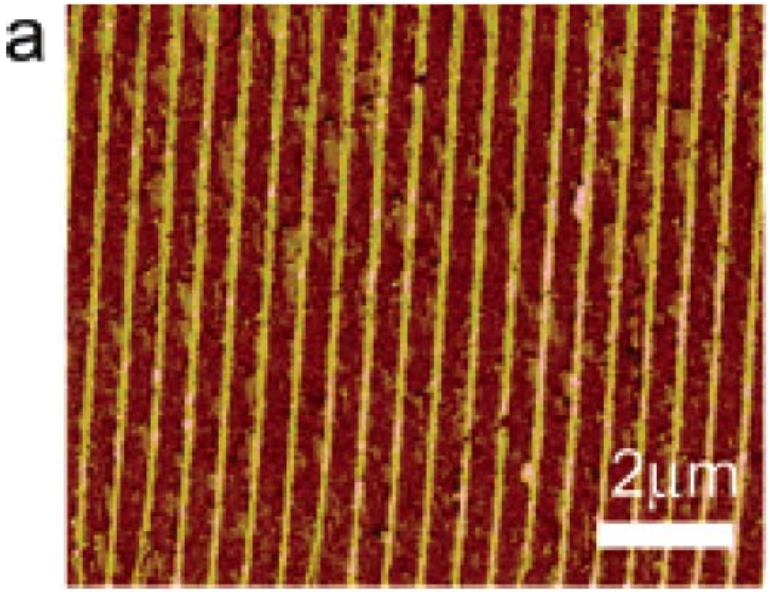
The process can be repeated over and over…

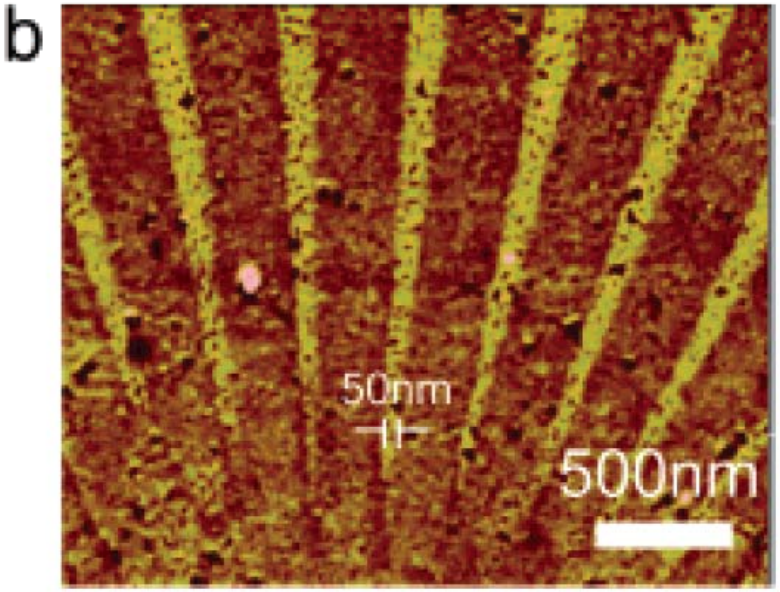
DNA array printing (SuNS)



DNA printing on gold: Both spatial (i.e., spot size, shape, and position) and chemical (i.e., DNA sequence) information were printed at the same time. The printed dots show features typical of the drying of a water droplet containing DNA and are due to the master used.

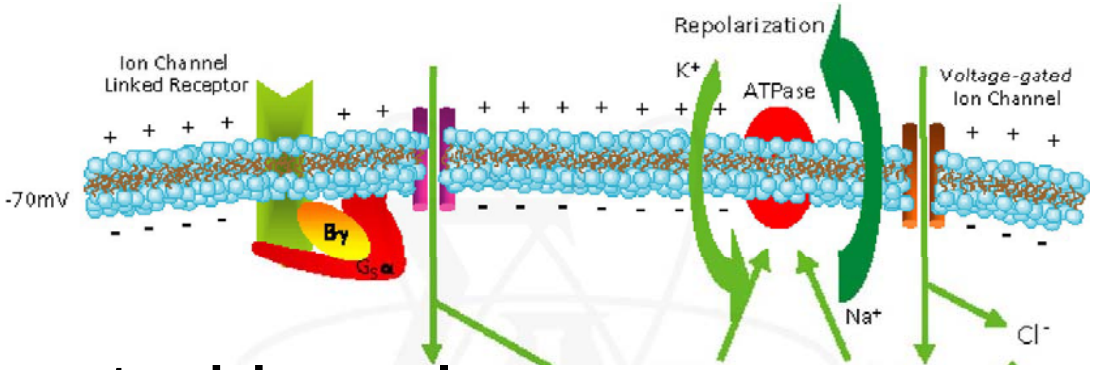
- AFM images of DNA wires printed on a PMMA substrate. The arrows in (b) indicate the thinnest continuous part of the wire that was successfully printed. In an isolated case, could be printed down to a thickness of 25 nm.





Patch clamping techniques

- Ion channels are essential for:
  - maintenance of membrane potential
  - osmoregulation
  - energy generation
  - signal transduction



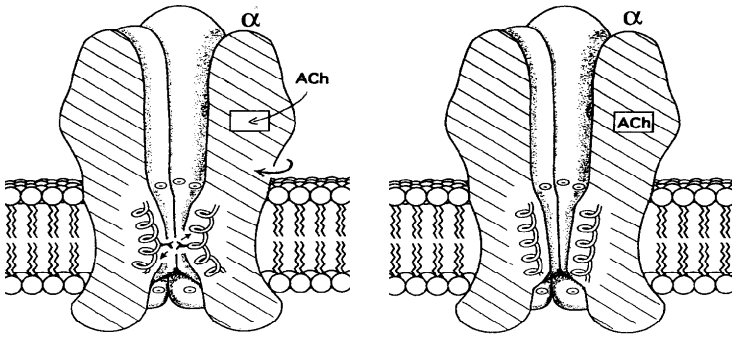
- Ion channels are targeted by a large number of drugs
- Several drugs act through pore-forming in cell membranes (some antibiotics, AMP)

Patch Clamping technique

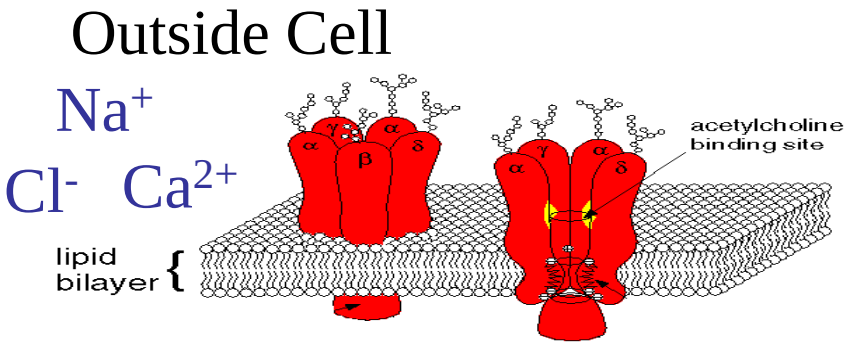
- Patch clamp techniques gives a direct way to study passage of ions through a single ion channel. Nobel Price 1991 (E. Neher and B. Sakmann)
- Gave rise to a new area in life science, **electrophysiology**

Ion channels

- Can be subdivided into several groups according to the actuation:
  - voltage-gated
  - ligand gated (extra- or intra-cellular)
  - rectifier etc.

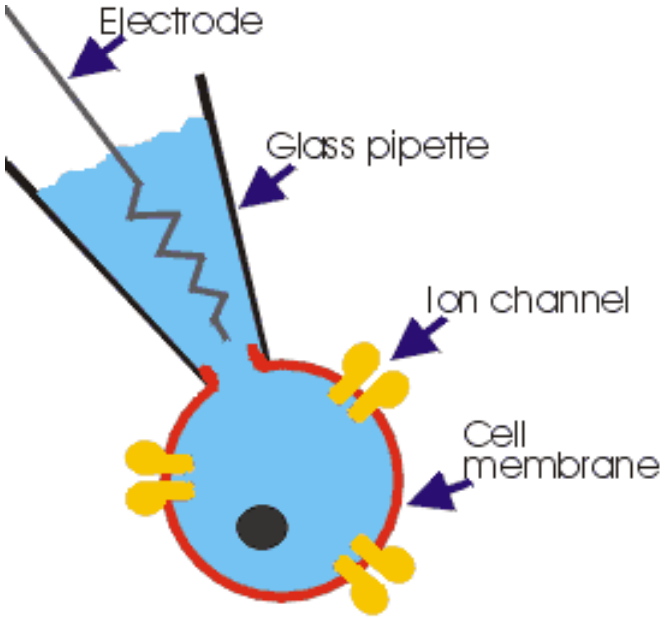


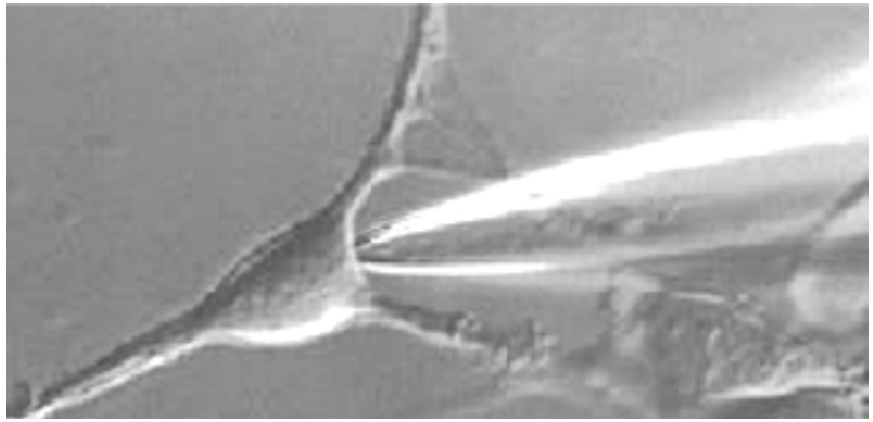
Closed Open



Patch Clamp Recording Technique

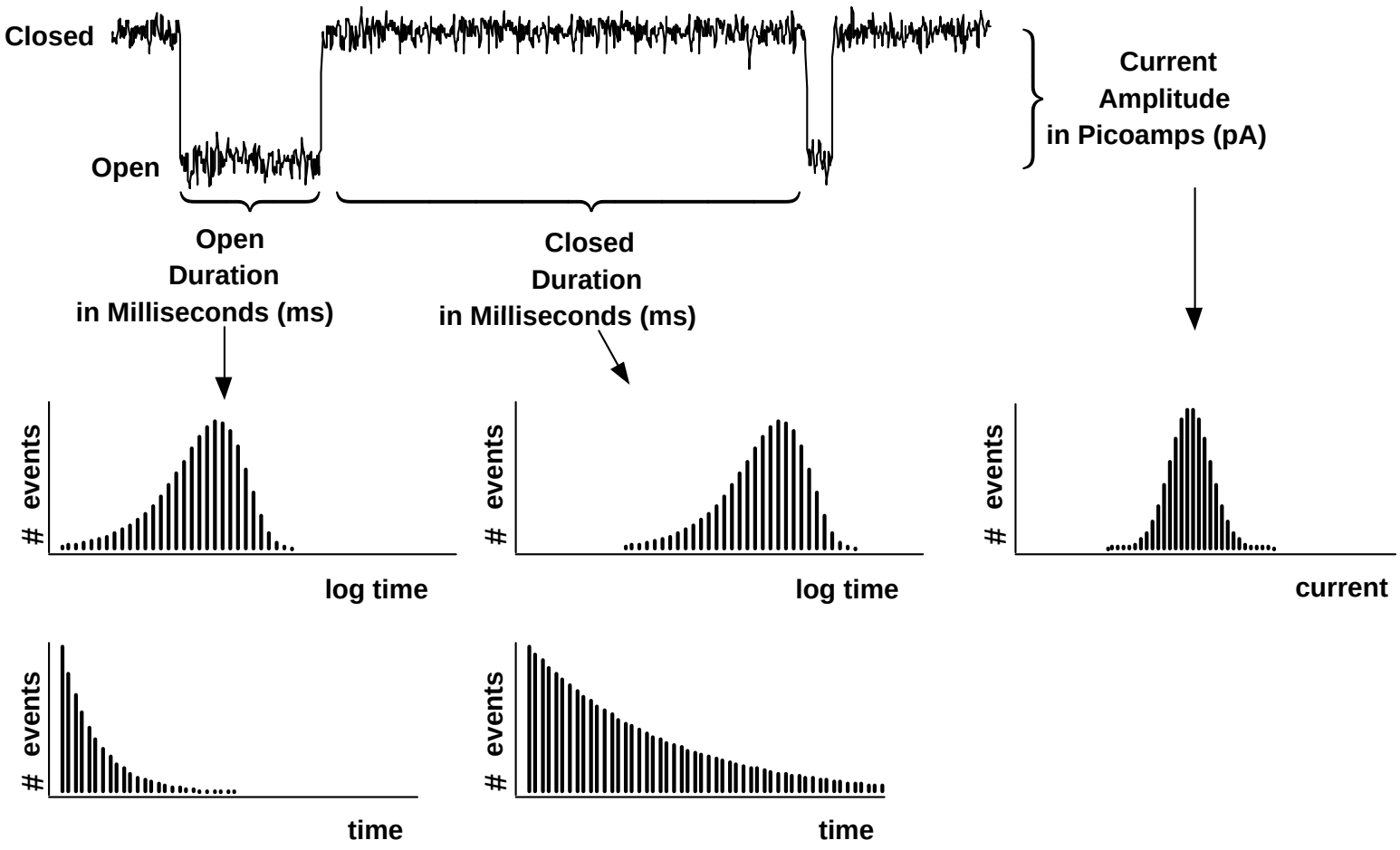
- Using pipette for patch clamping





Current through a channel

- Typically a statistics is made:

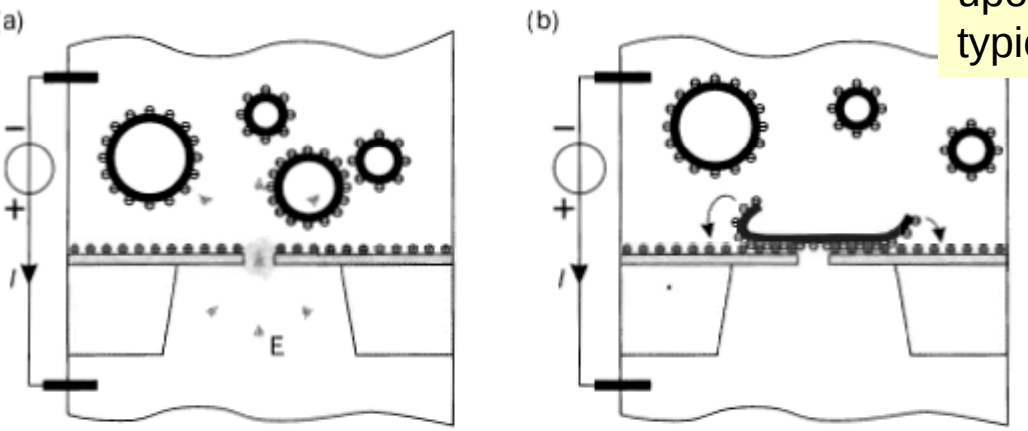


Planar Patch clamping

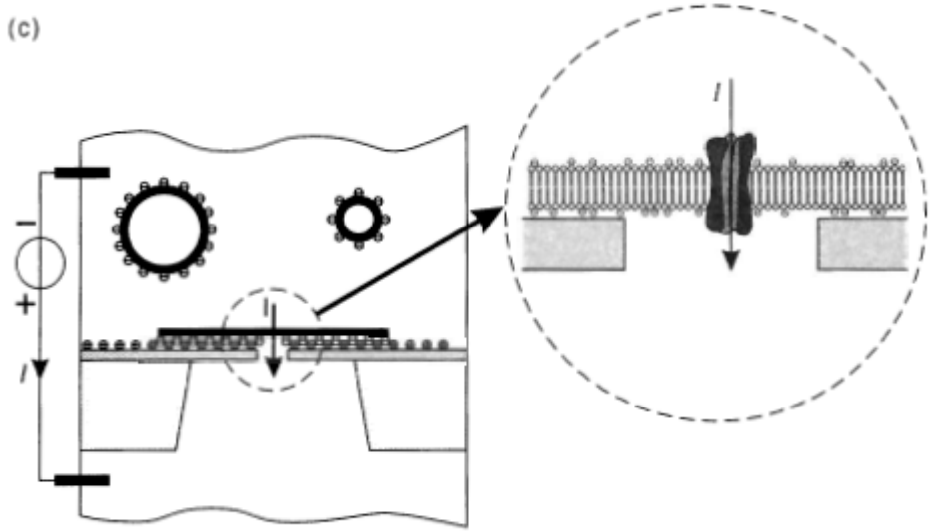
- The principle of planar patch clamping

Electric field attracts negatively charged vesicles

vesicle spreads upon the contact, typical R~200GOhm

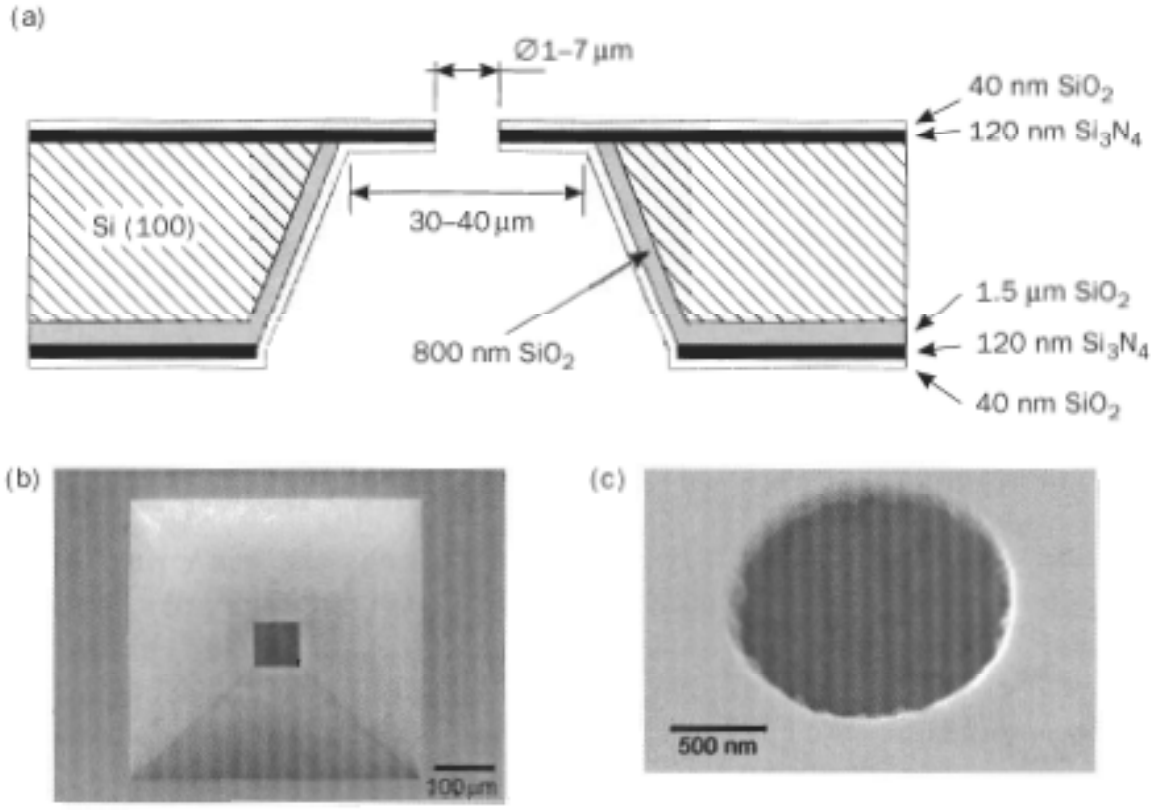


ion current can be now monitored through a single channel



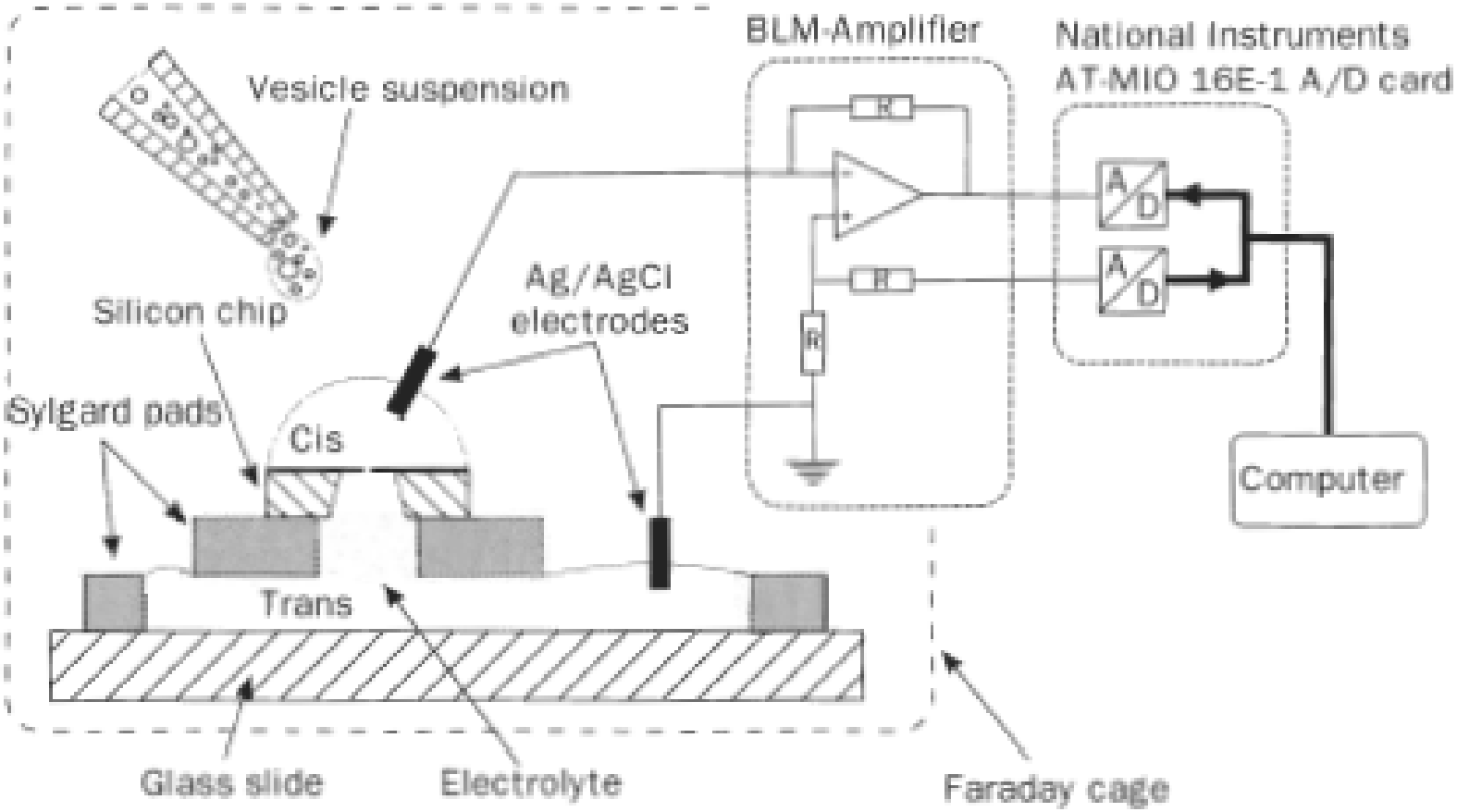
Planar Patch clamping

- Silicon chip for patch clamping
- Fabrication steps
  - start with a DS Si-wafer
  - grow low-stress SiNx
  - photolithography on the back side
  - anysotropic wet etching
  - deposition of LTO SiO2
  - photolithography on the front side (aperture pattern)
  - protection and sawing
  - oxygen plasma cleaning and modification with poly-L-lysine (0.01-0.1%, 5 min)



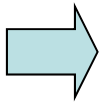
Planar Patch Clamping

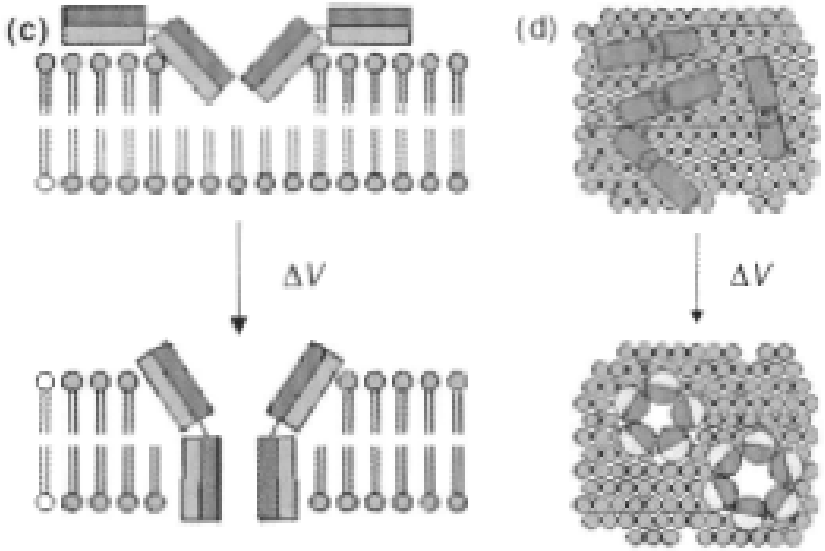
- Measurements scheme



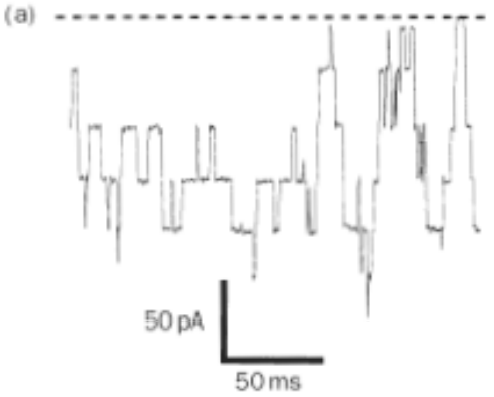
Example: measurement on Alamethicin pores

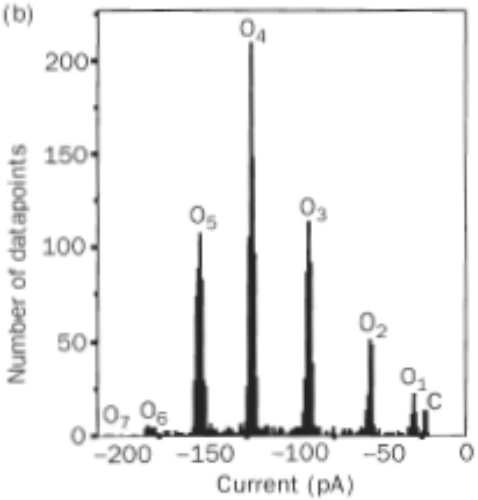
- Alamethicin is a pore forming antibiotic, activated by membrane potential





upon applying activation voltage the protein integrates into the membrane and forms pores





HTS Patch Clamping

- Commercial devices produced by several manufacturers incl. Sophion A/S (DTU spin-off) www.sophion.dk
- Sophion QPatch HTS allows parallel measurement on 48 channels, designed for screening drug candidates.

Nanosensors

Advantages of Nanosensors

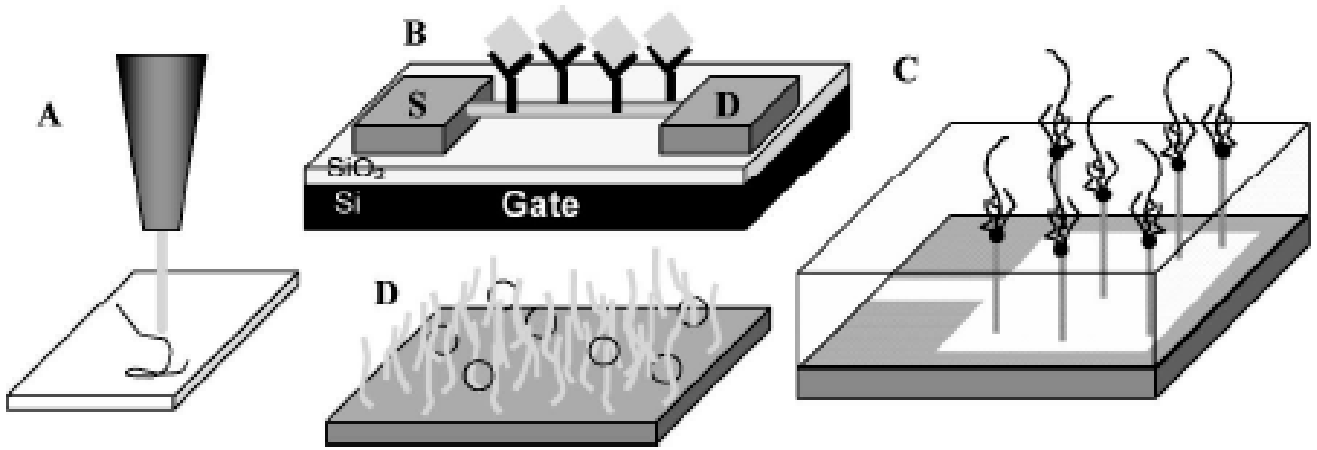
- nano-tube and nano-wires sensors could provide means to probe behavior of molecules on a level of single molecules or very small ensemble;
- presence of a very small amount, potentially single molecules can be detected

Nanotube/wire devices

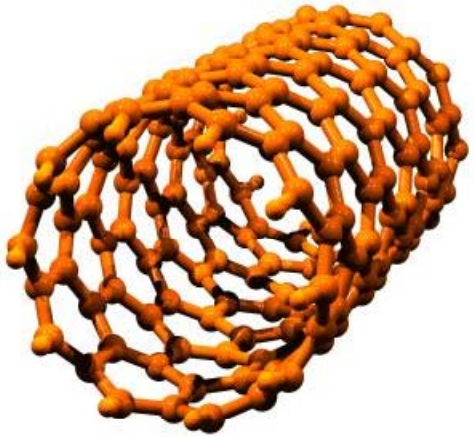
- Ultra sharp probe

- Electrochemical electrode coatings and arrays

- nano- FET

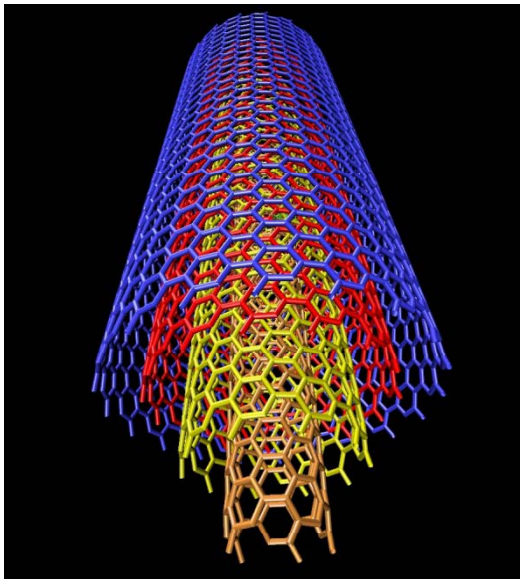


Nanotubes



SWNT

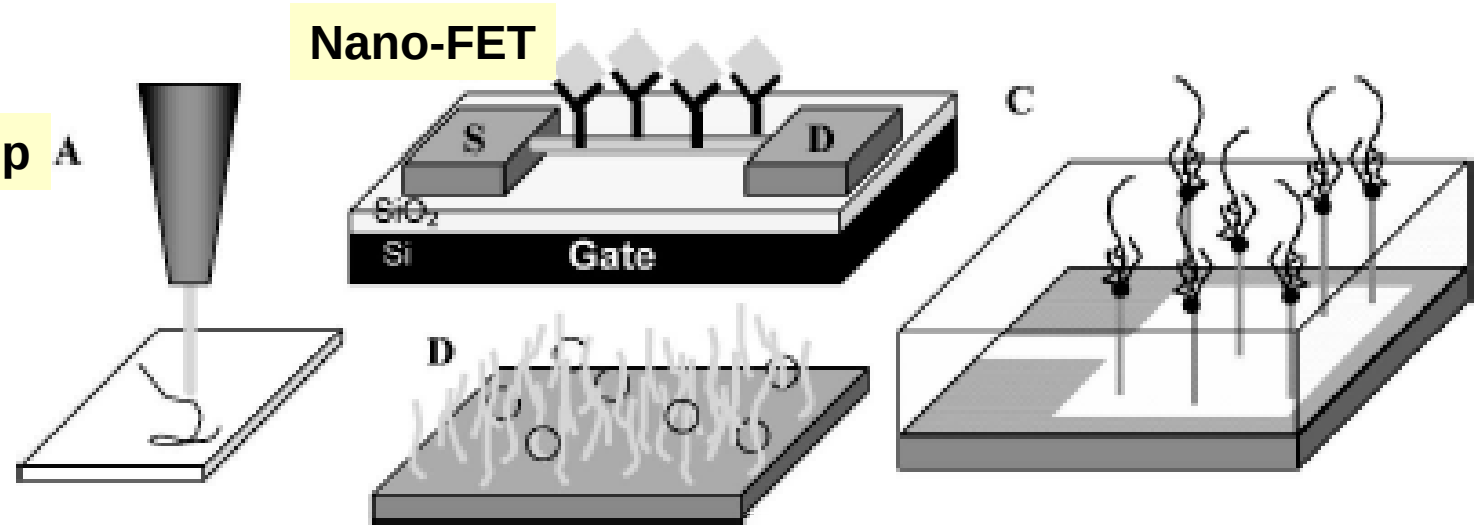
MWNT



From electrochemical prospective:

- promote wide range of electron transfer reaction (redox) due to high electrical conductivity, proper electronic structure and redox active sites
- have a wide useful potential range due to slow carbon oxidation
- have large surface/volume ratio
- can form 3D immobilization matrix

Main approaches to CNT/Nanowire devices

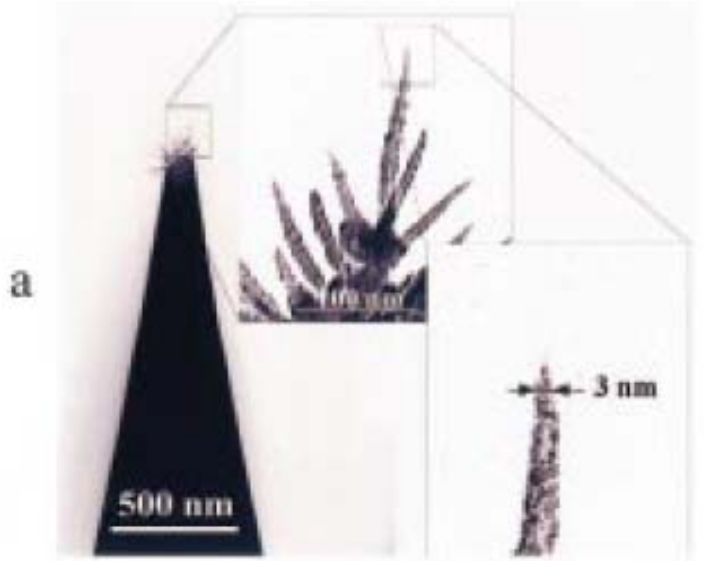


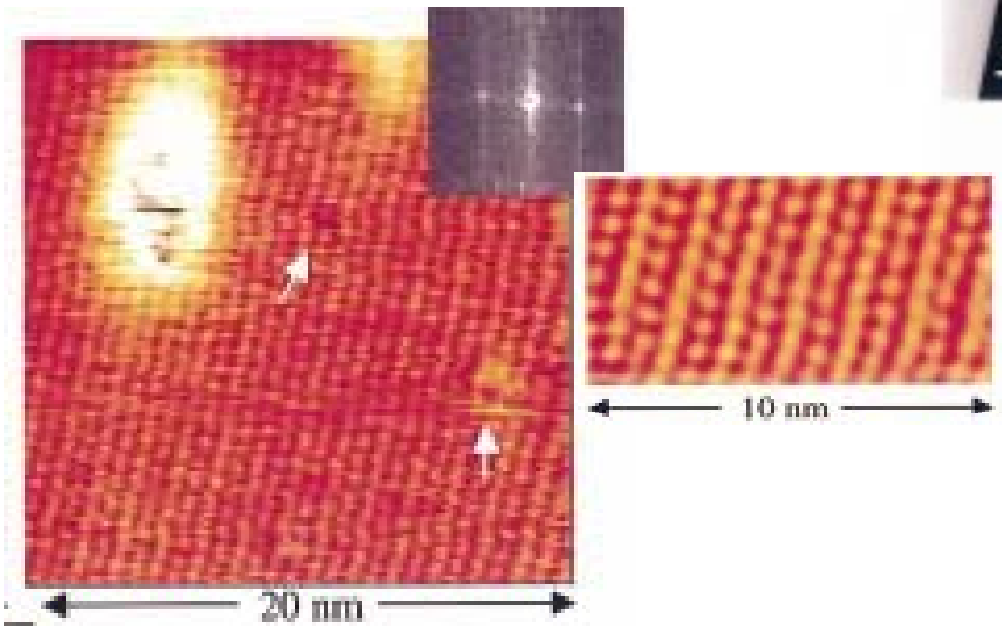
NT modified electrodes

Ultrasharp AFM tips

- Ultrahsarp spikes achieved via CVD growth of diamont like carbon on Si tips

- Image of polydiacetylene crystal





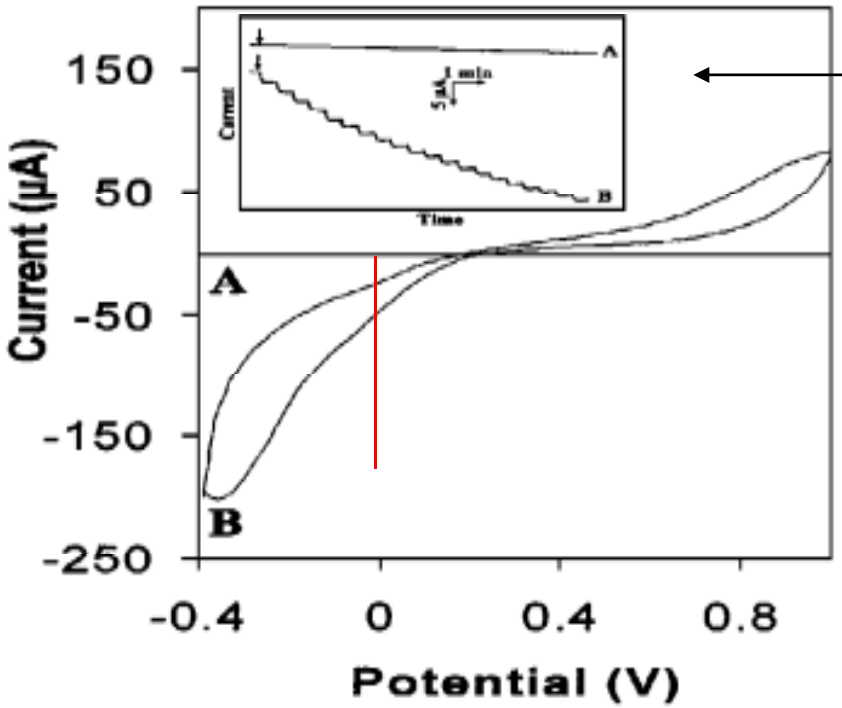
Nanotube modified electrodes

- Carbon nanotube electrodes exhibit usually a featureless cyclic voltammogram (CV) over a rather large potential range in aqueous and nonaqueous solutions.
- Carbon nanotubes show electrocatalytic properties promoting reversible electron transfer reactions in other electrochemical systems
- Due to small size, carbon nanotubes offer large surface/size ratio.

Nanotube modified electrodes

- deposition of solubilized nanotubes (e.g CNT/PVP or CNT/Nafion) on electrode (e.g. glassy carbon)
- casting CNT/Teflon composites

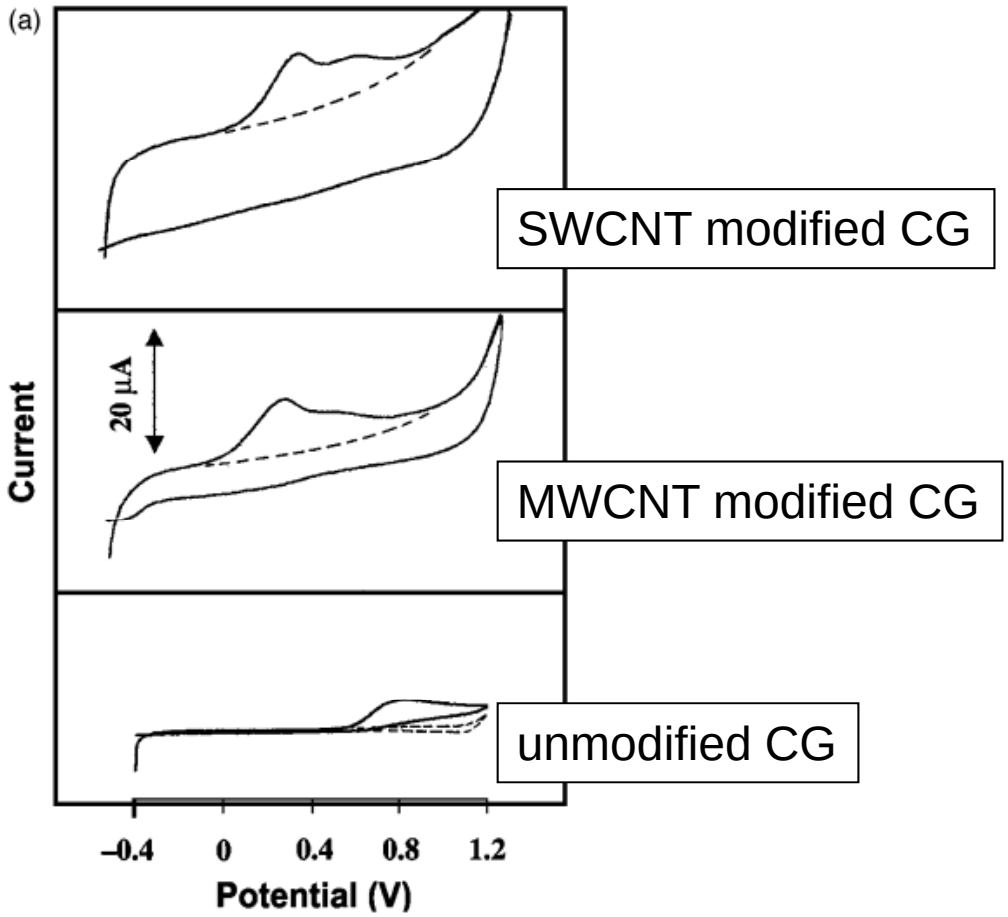
Hydrogen Peroxide oxidation on GC electrode: unmodified (A) and CNT modified (B)

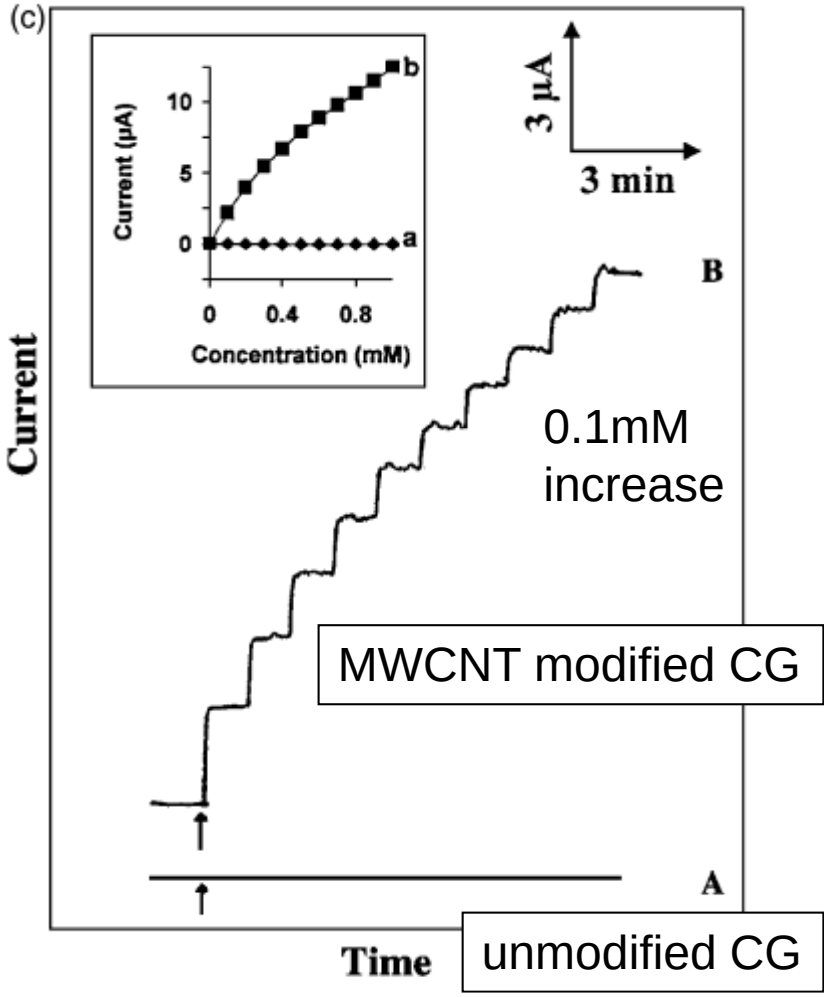


response to increasing concentration, 1mM step

Nanotube modified electrodes

- Redox reaction on NADH (Nicotinamide adenine dinucleotide)



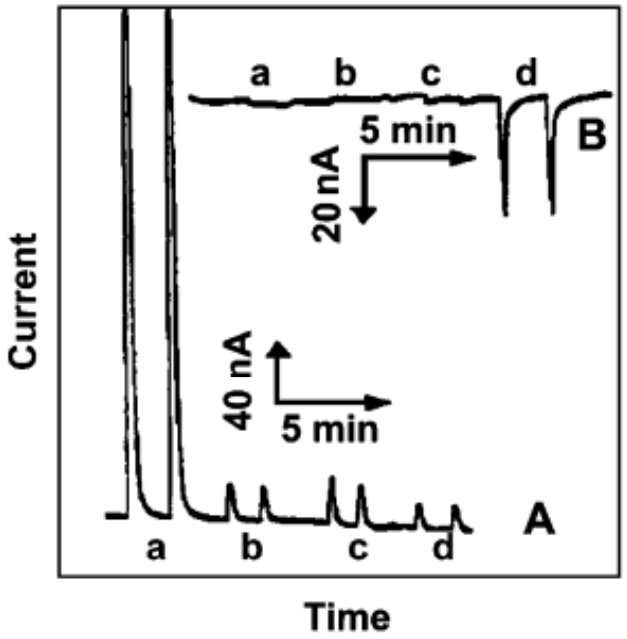


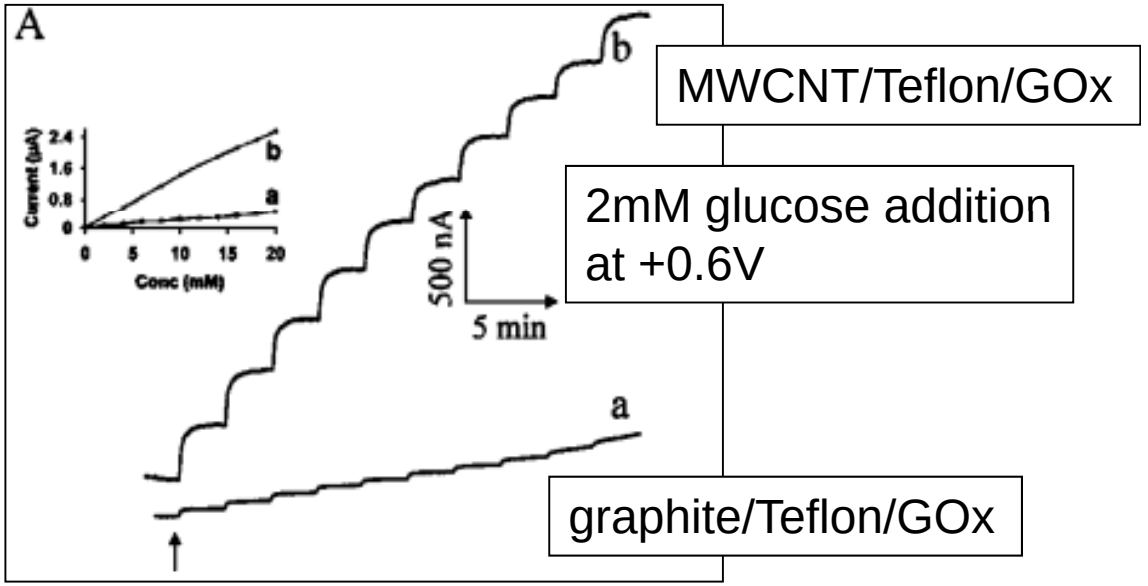
M. Musameh et al, Electrochem.Comm. 4, 743 (2002)

Nanotube modified electrodes

- Oxidase based amperometric sensors measurements at very low overpotential:
  - interfering reactions are minimized therefore leading to **high selectivity**
  - very linear response
  - mediators are not required

CNT/Nafion/GOx deposited on GC





Flow-injection signals for $2x10^{-4}$ M acetaminophen (a), $2x10^{-4}$ M ascorbic acid (b), $2x10^{-4}$ M uric acid (c), and $1x10^{-2}$ M glucose (d), at the Nafion/GOx-modified GC electrode (A) at +0.8 V, and the MWCNT/Nafion/GOx-modified GC electrode (B) at 0.05 V

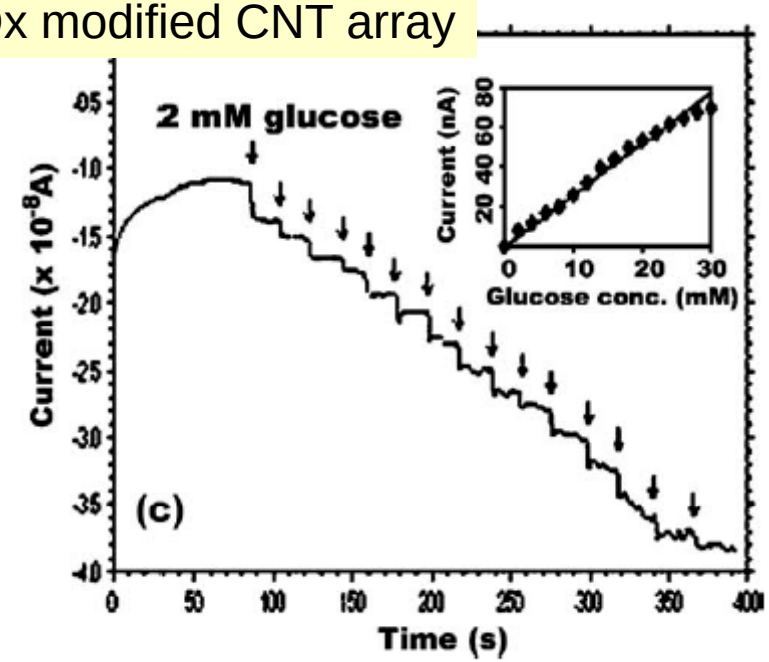
J. Wang et al, JACS 75, 2075 (2003

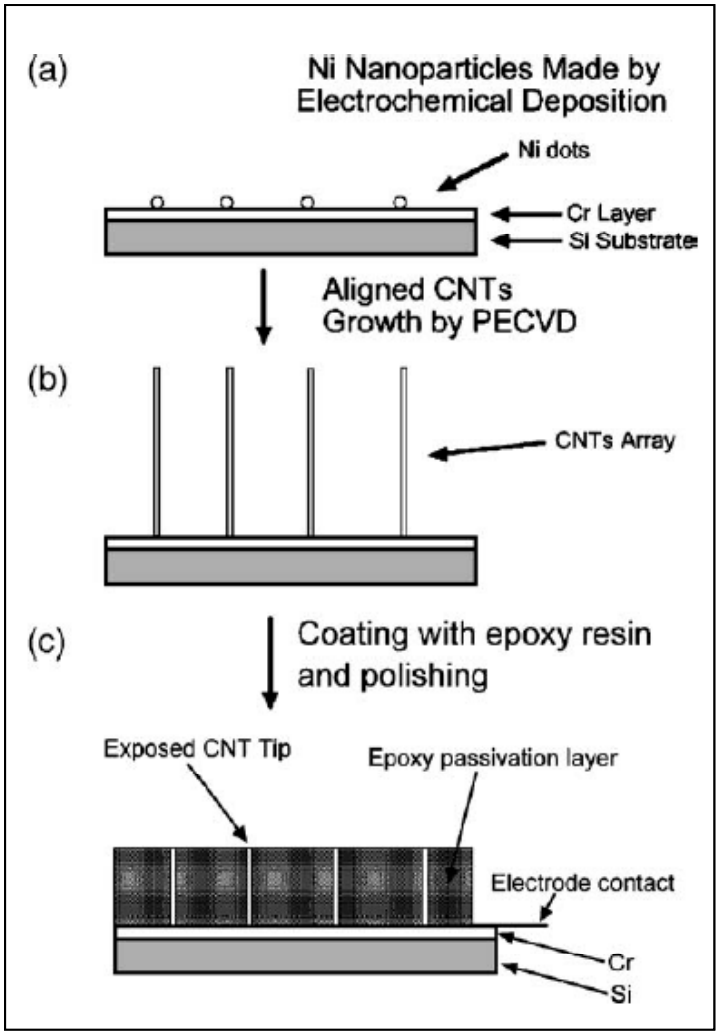
Nanotube modified electrodes

- Controlled density aligned nanotube electrodes

- density is optimized to prevent diffusion layer overlap (interspacing ~1um)
- improved signal to noise ratio and detection limit
- faster response

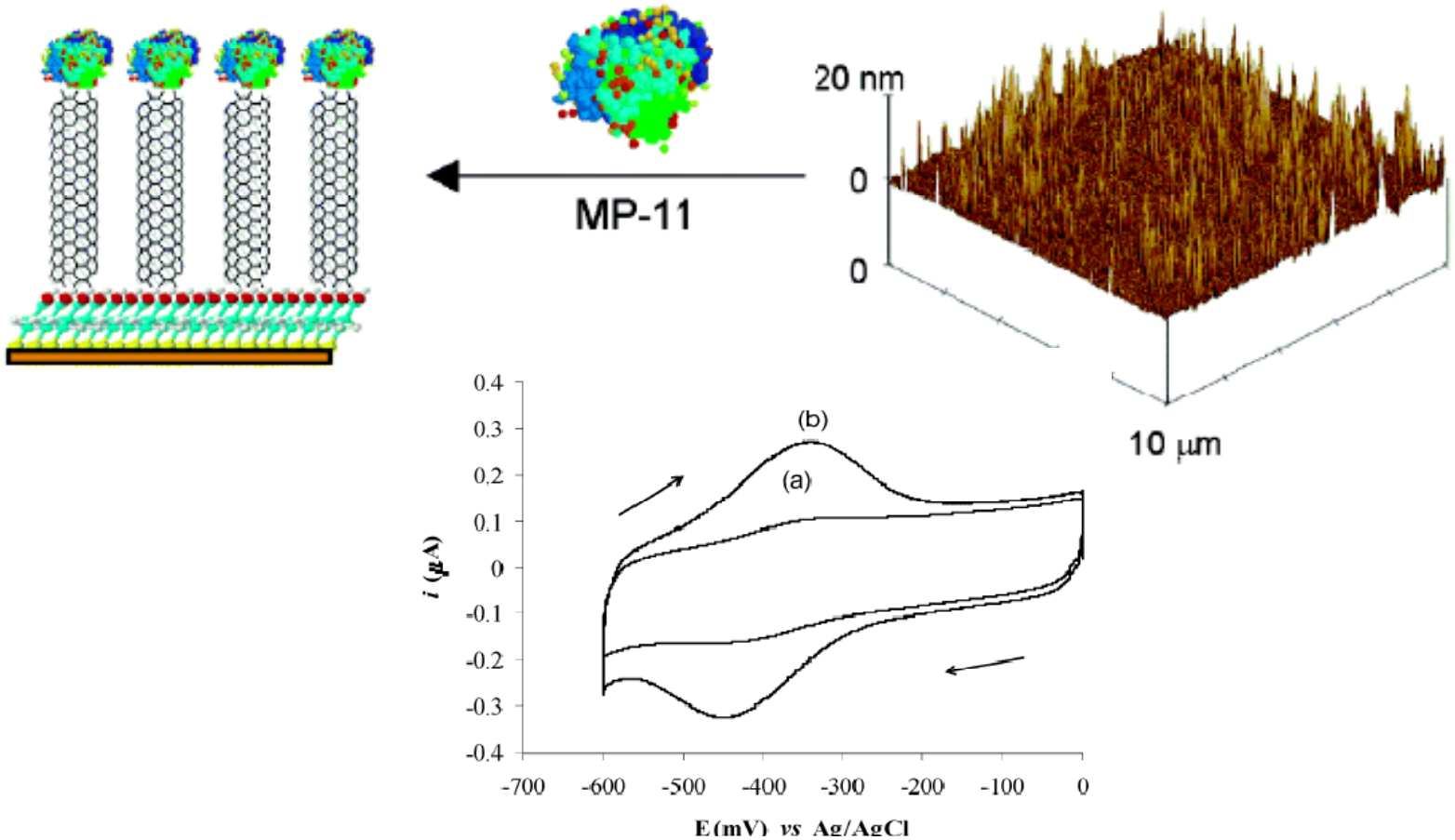
GOx modified CNT array





Nanotube-modified electrodes

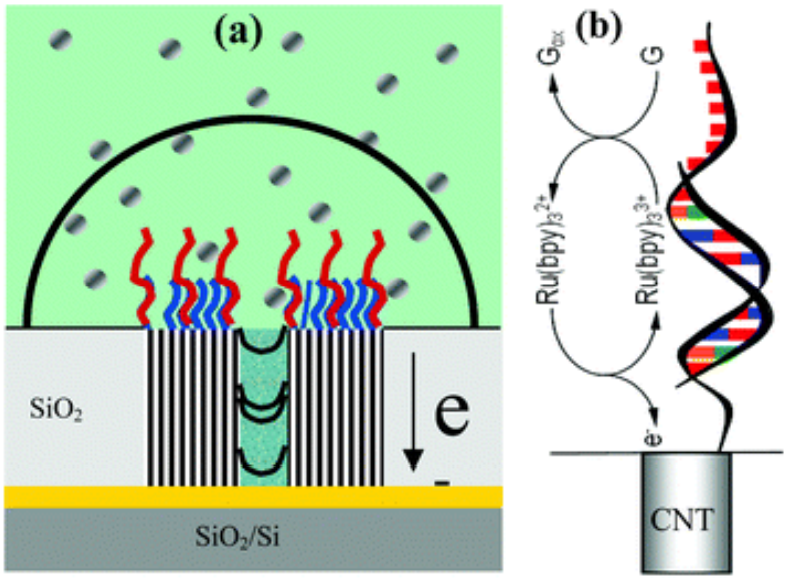
- gold electrodes are modified with shortened nanotubes (~120nm long)
- direct electron transfer between an enzyme (microperoxidase MP11) and the electrode demonstrated, largely independent on the SWNT length



J.J. Gooding et al, JACS 125, 9006 (2003)

Nanotube modified electrodes

- Nanoelectrode array for DNA detection



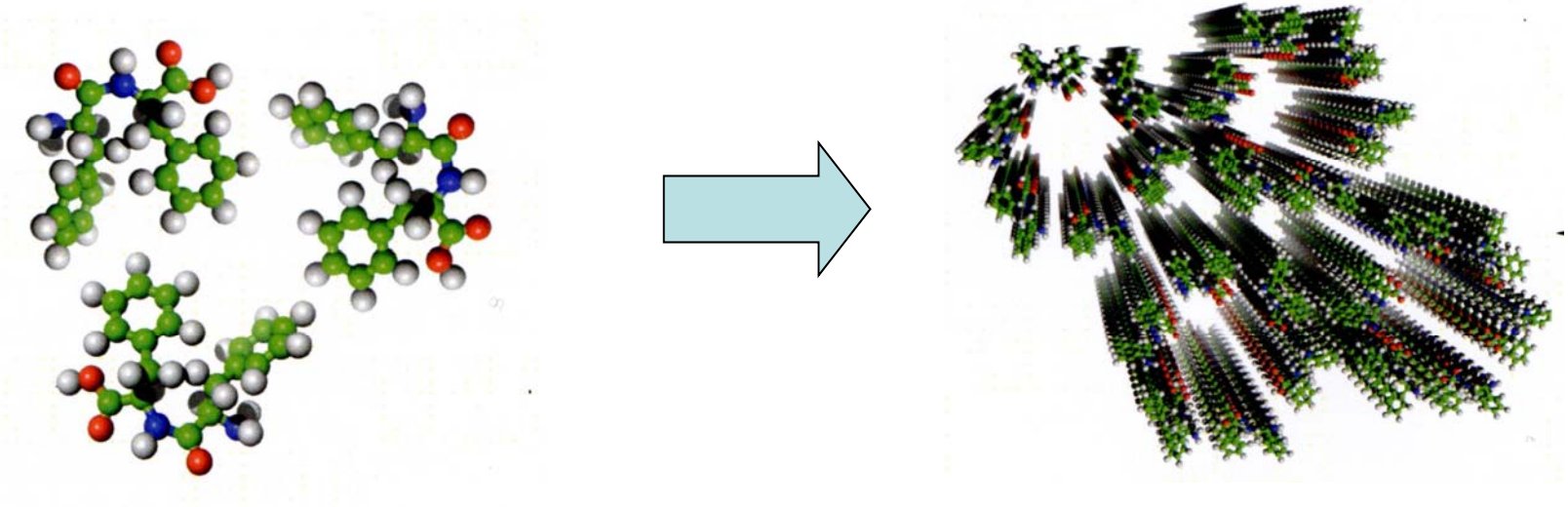
[Cy5]5-AGGACCTGCGAAATCCAGGGGGGGGGGG-3

[Cy3]5-CTIIATTTCICAIITCCT-3[AmC7-Q] attached to the MWNT

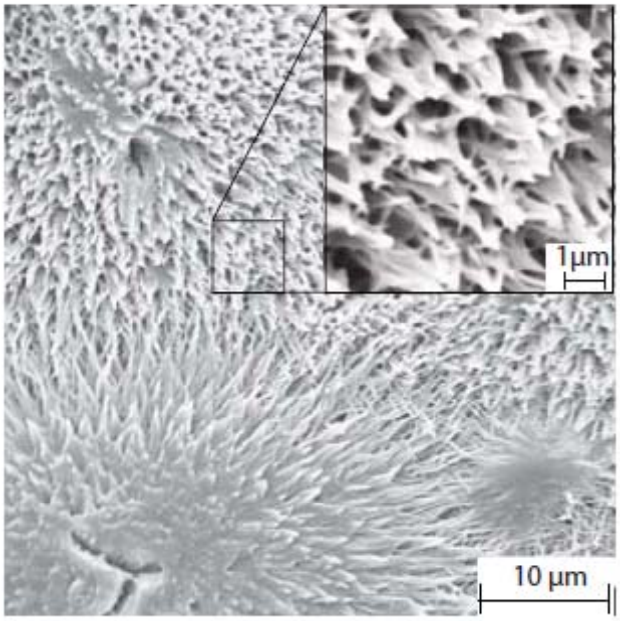
binding of ~ 1000 DNA molecules can be detected

No labeling required, sensitivity approaches fluorescent detection limit!

Diphenylalanine Nanotubes modification for electrochemical sensors

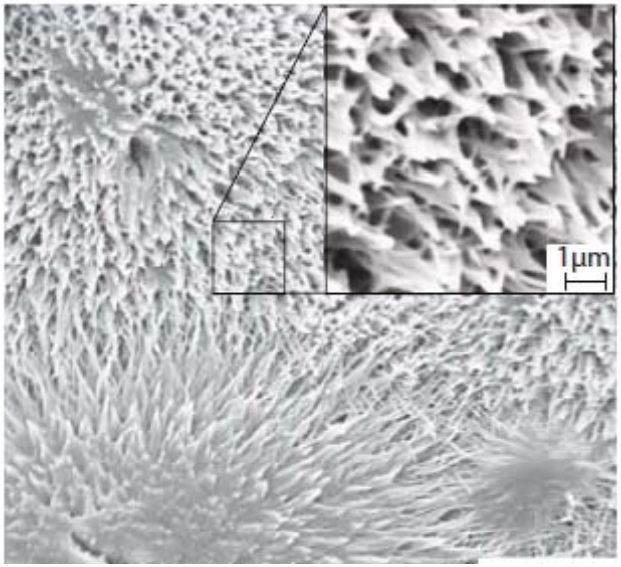


- diphenylalanine peptides self-assemble into nanotube arrays on surface upon solvent evaporation
- stable against water based solution

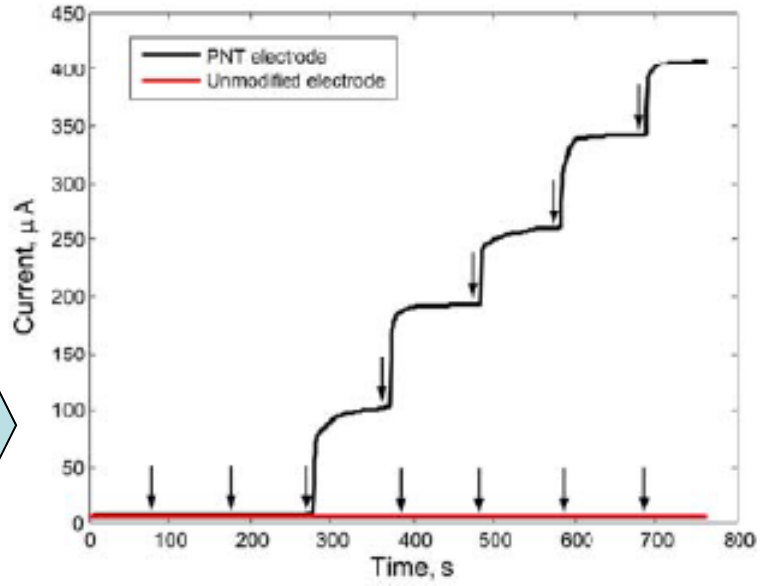


Diphenylalanine Nanotubes modification for electrochemical sensors

- diphenylalanine PNT formation leads to rise of surface area and therefore to tremendous increase in sensitivity of electrochemical assays
- PNT provide binding sites for enzymes (e.g. glucose oxidase) with direct charge transfer between electrode and enzyme
- antifouling biocompartible surface



Chronoamperometric response to successive additions of 20mM H_2O_2 at the applied potential of 0.4V

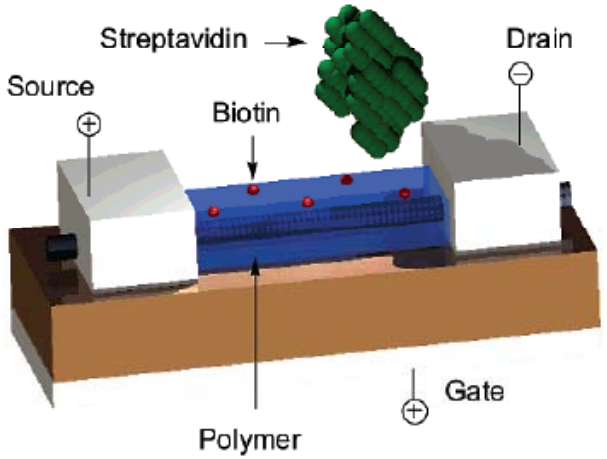


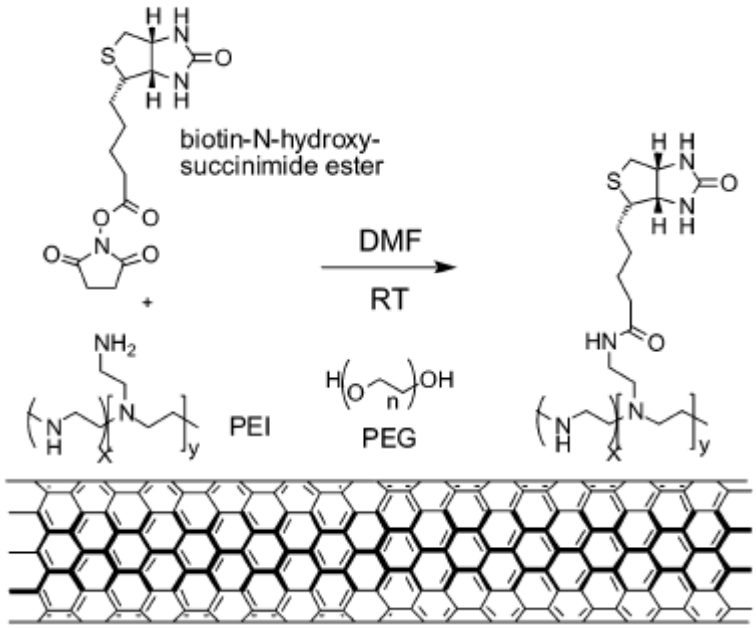
Nano-FET

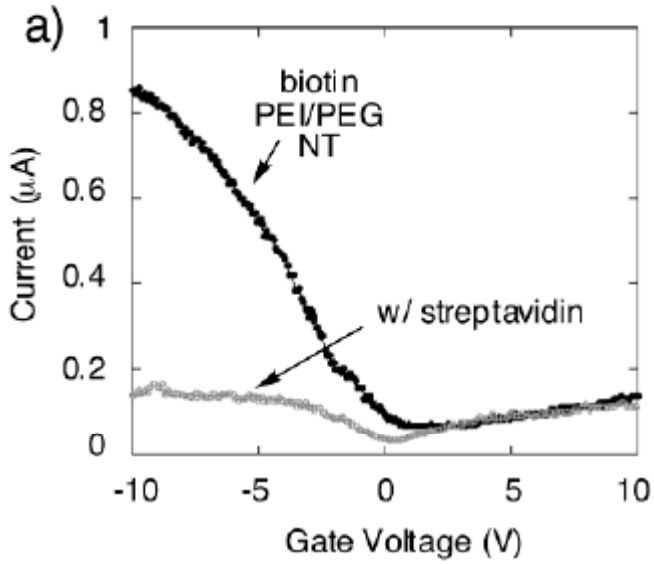
- large surface to volume ratio: dramatically increased sensitivity as even small partial charge induced by chemisorption is sufficient to produce dramatic conductance change
- high density arrays can be prepared due to small size
- very miniature sensors (and therefore less-invasive) can be developed

Nanotube Chem-FET

- sensing biotin-streptavidin binding



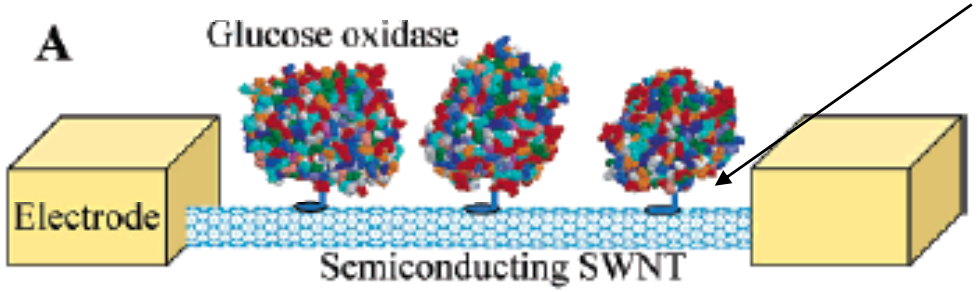




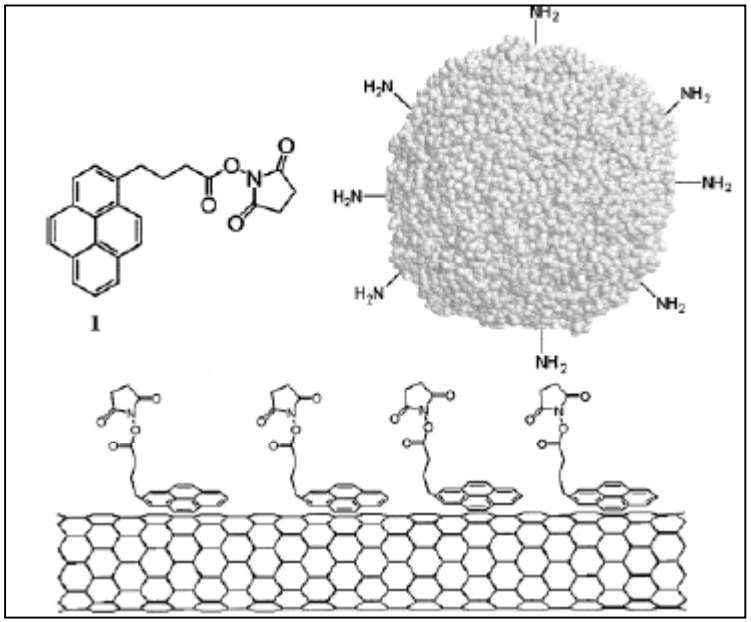
A. Star et al, Nanoletters 3, 459 (2003)

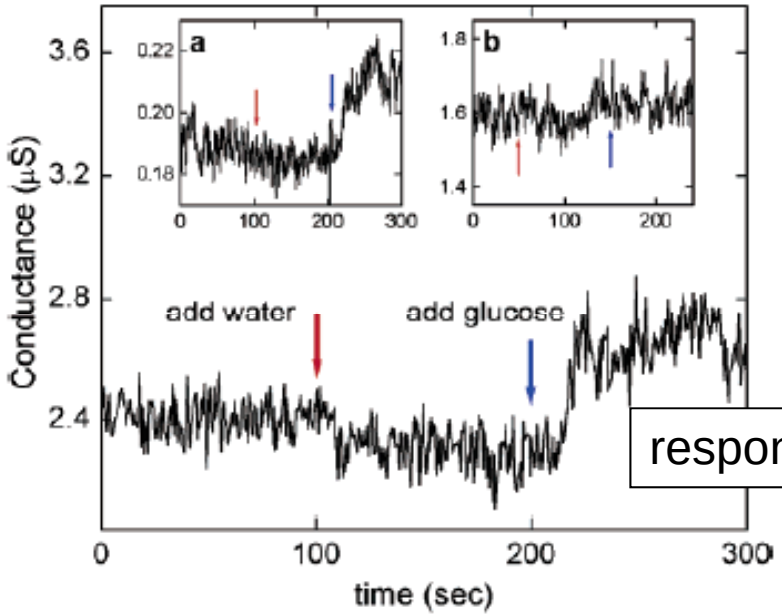
Nanotube Chem-FET

- Oxidase-based sensor



attached using adsorbed 1-pyrenebutanoic acid succinimidyl ester (via pyrene group)



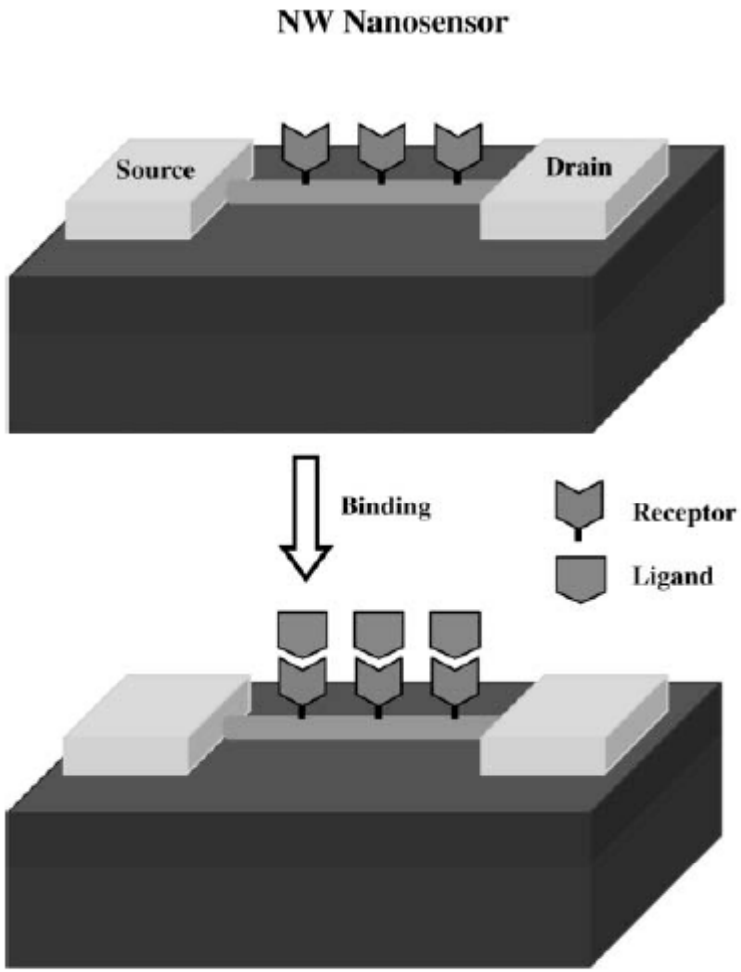


response to addition of 0.1mM glucose

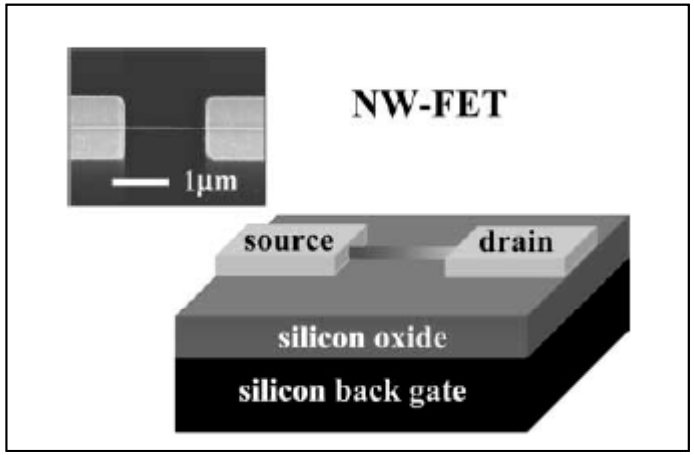
K.Besteman et al, Nanolett. 3, 727 (2003)

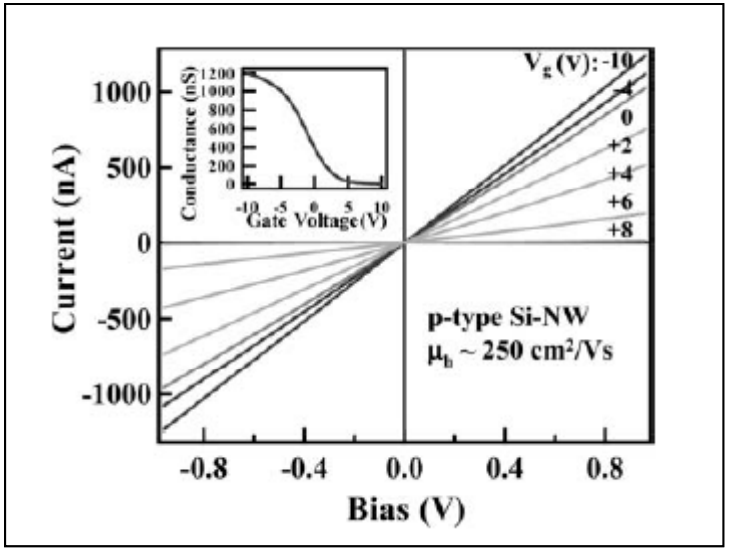
Nanowire-based sensors

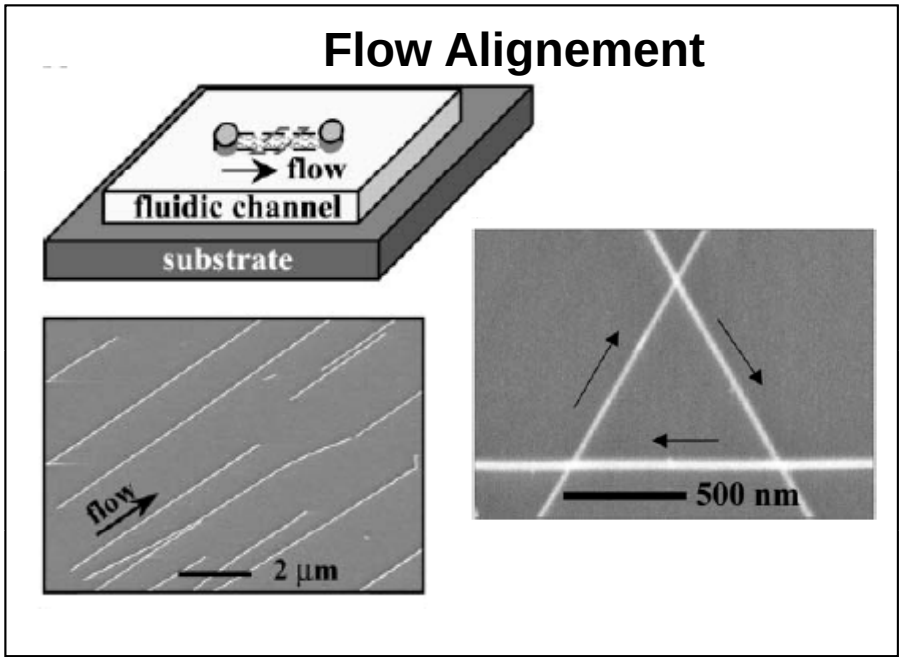
- Nanowires can be produced as purely semiconducting with required doping level
- chemical modification of semiconductors oxide surface is well studied (e.g. silicon), rich fictionalization chemistry exists.

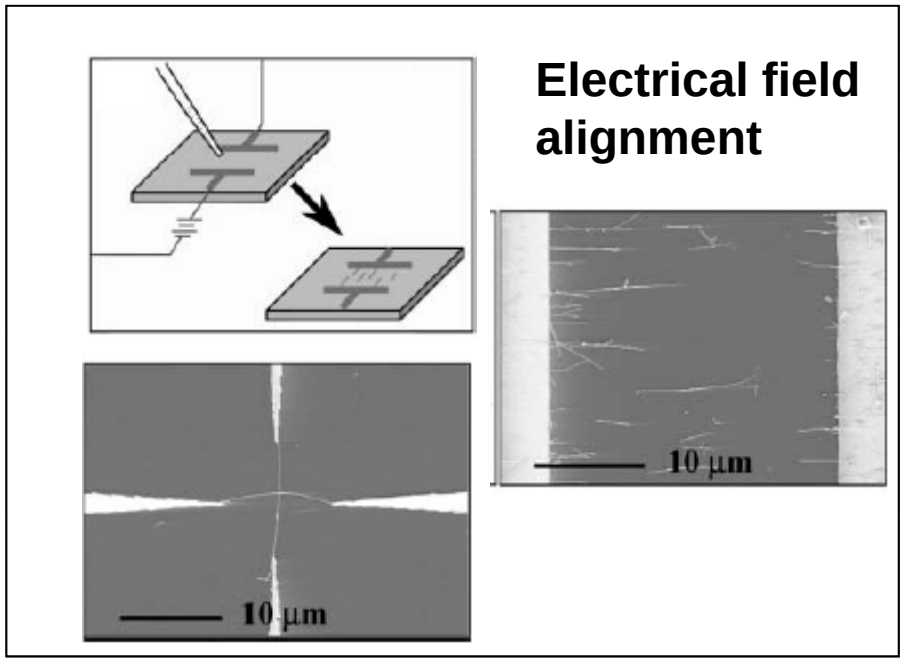


constructing nano-wire devices









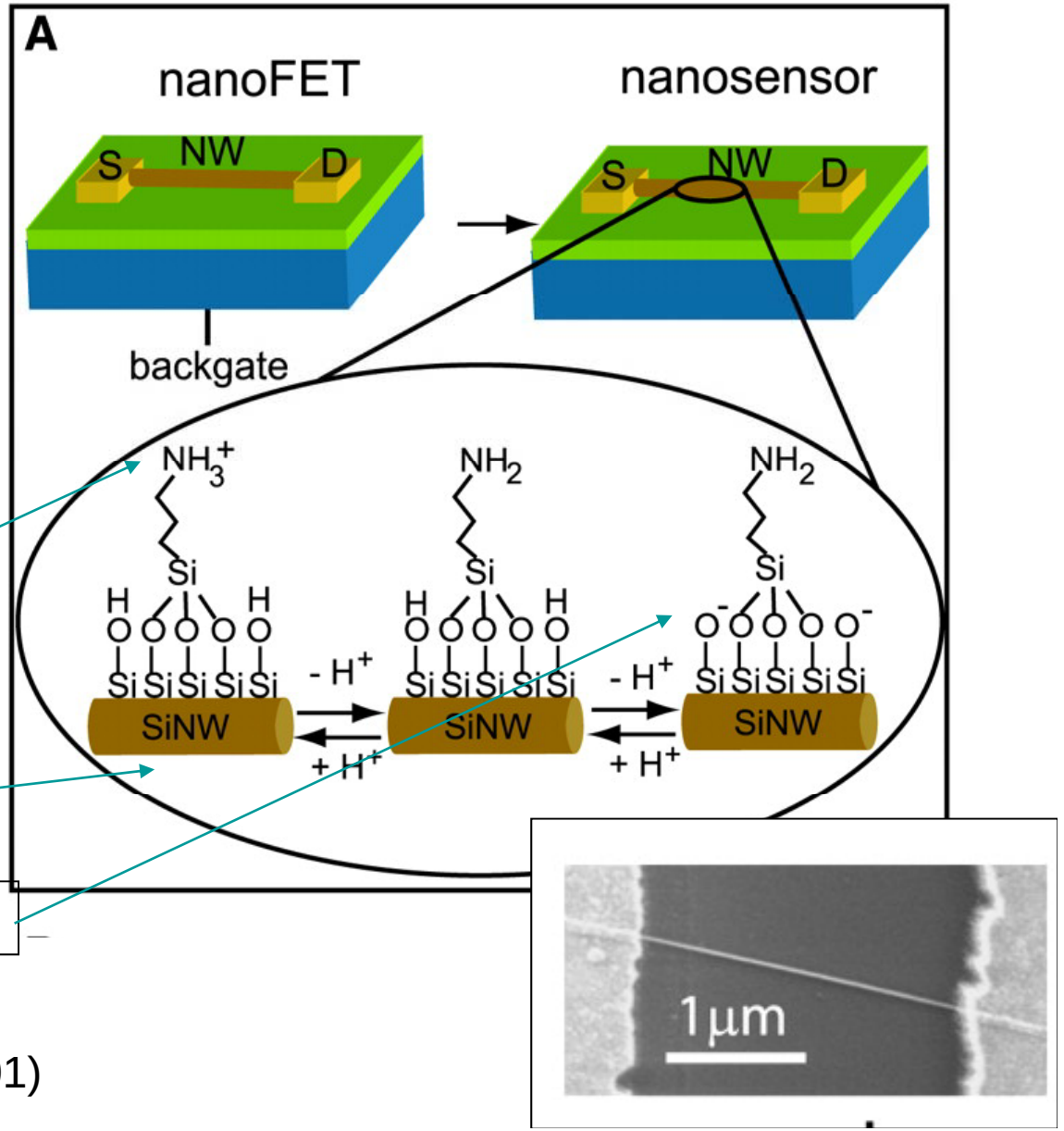
pH-sensing with a Nanowire FET

- APTES modified SiNT;
- change in pH produces gating effect on the nano-wire

low pH: NH_2 group is protonated

p-type Si nanowire

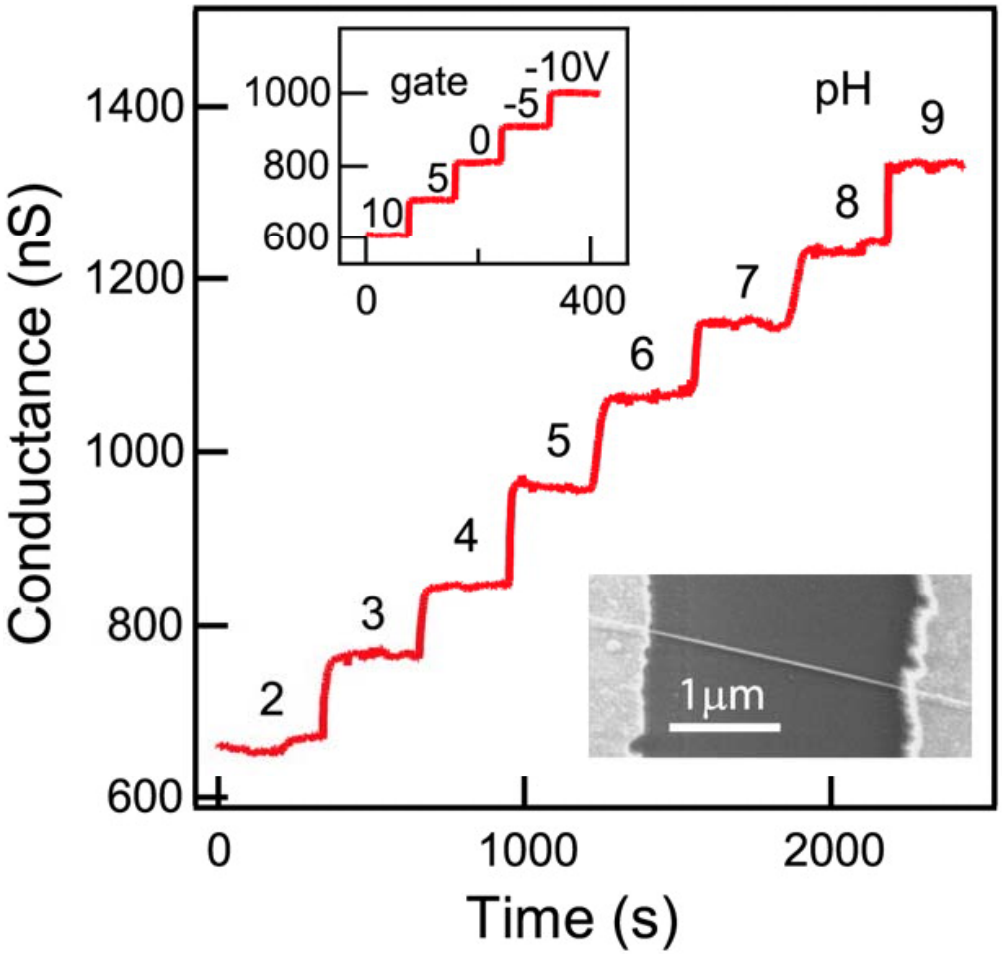
high pH: OH group is deprotonated

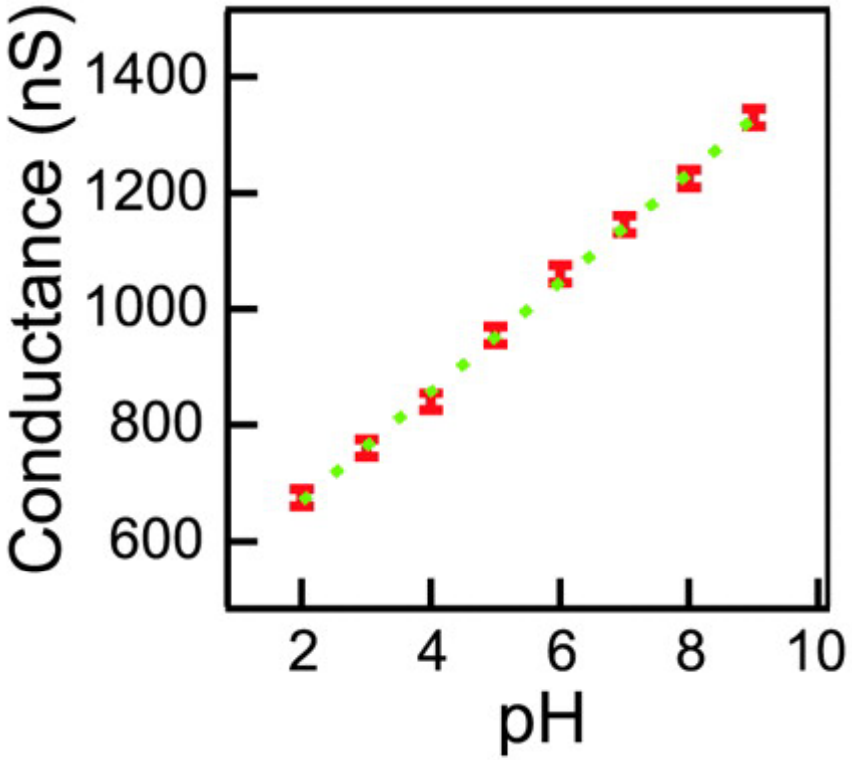


Cui et al, Science293,1289 (2001)

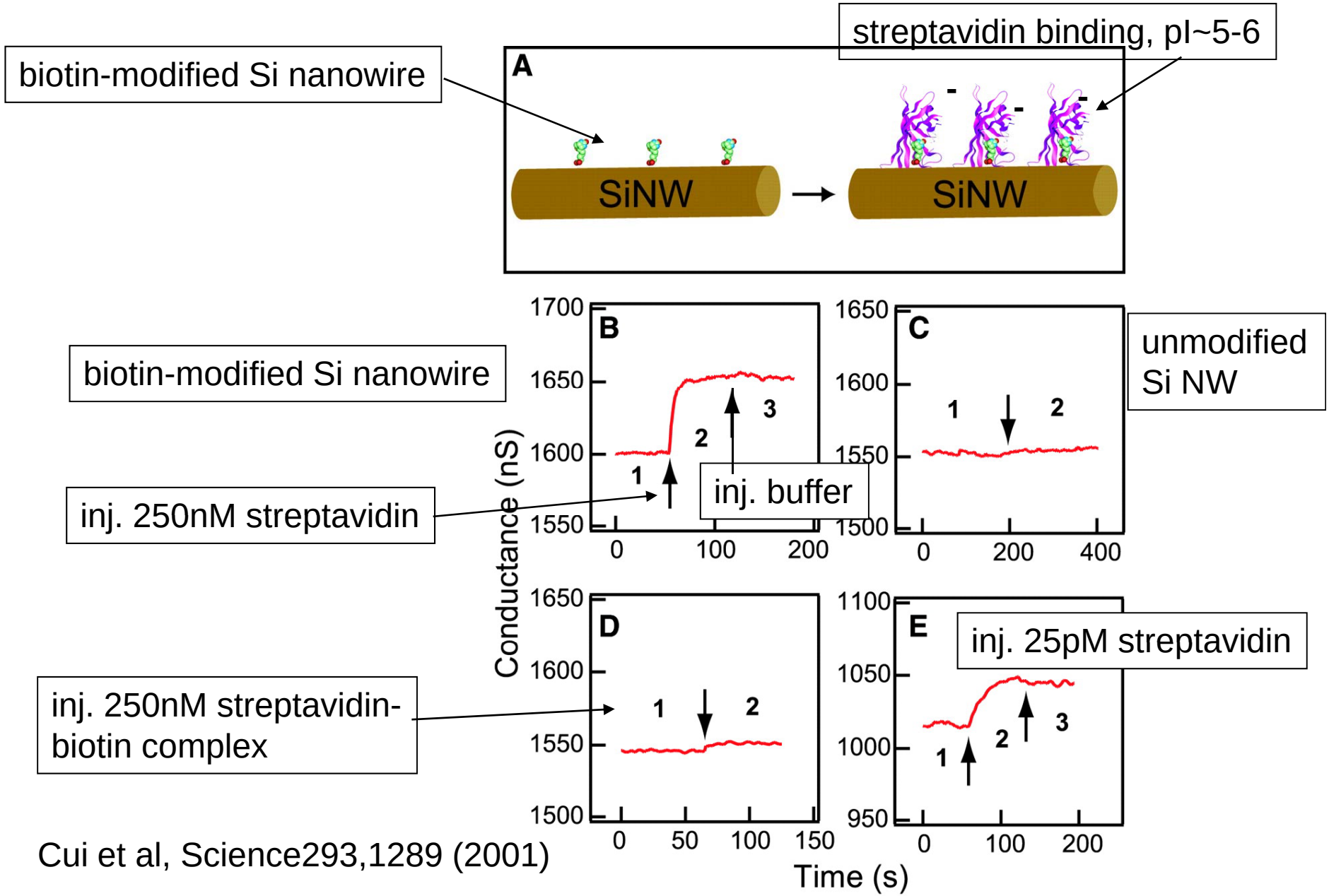
pH-sensing with a Nanowire FET

- change in pH is reversible and linear in the range pH=2 – 9.





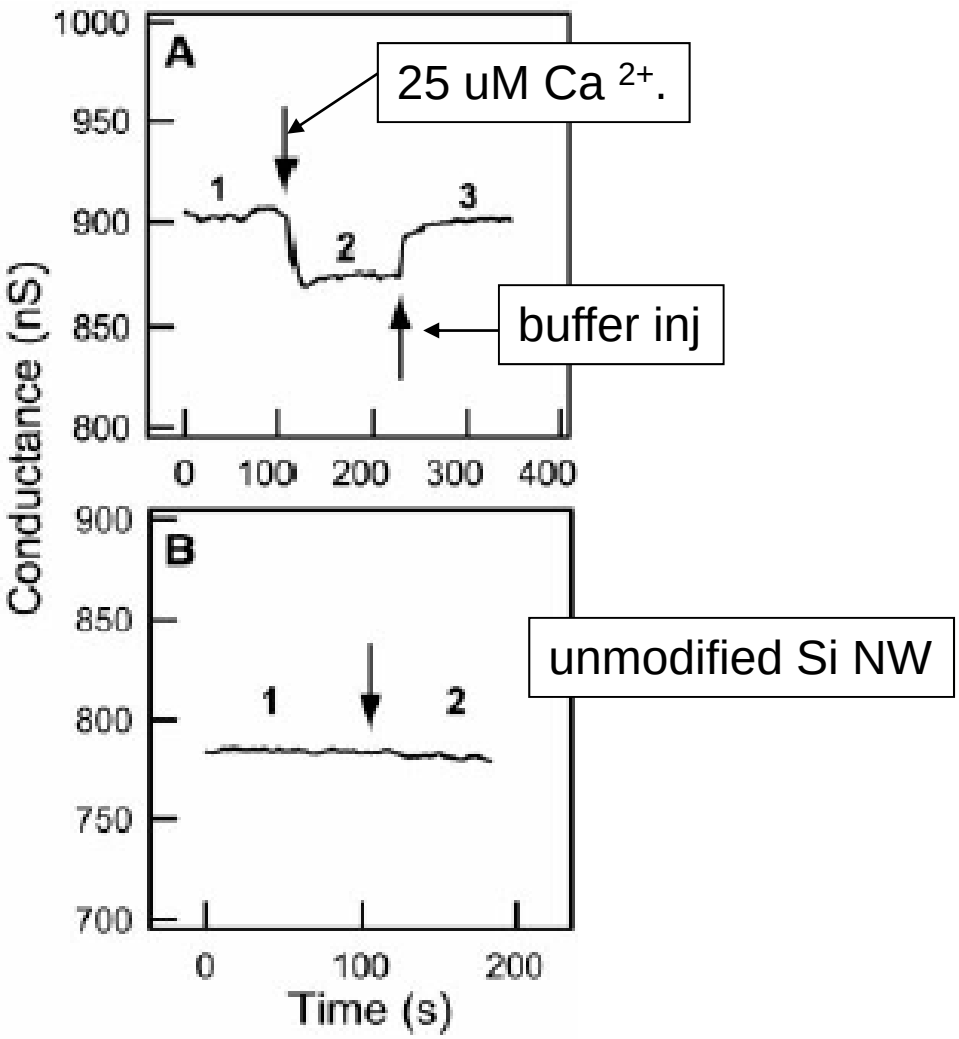
Detection of protein binding with Nanowire FET



Cui et al, Science293,1289 (2001)

Detection of protein binding with Nanowire FET

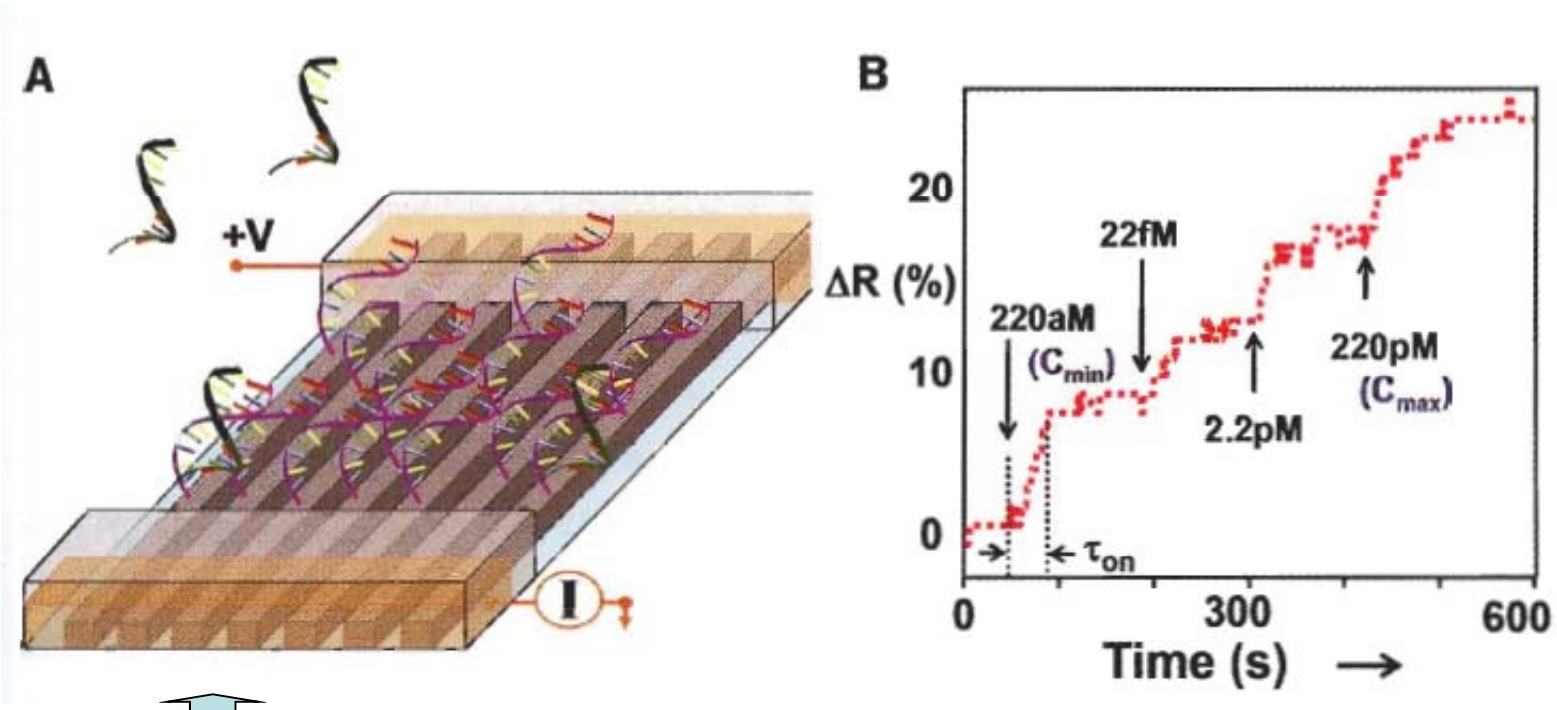
- Ca^{2+} binding to calmodulin-modified SiNT-FET (physisorbed)



Cui et al, Science293,1289 (2001)

Oligonucleotide binding detection with NW

- response of n-type Si-NW coated with ssDNA to cDNA injection (16-mer overlap, 0.154M electrolyte)



Gold electrodes capped with dielectric (gray)

NW Arrays: Current State of Art