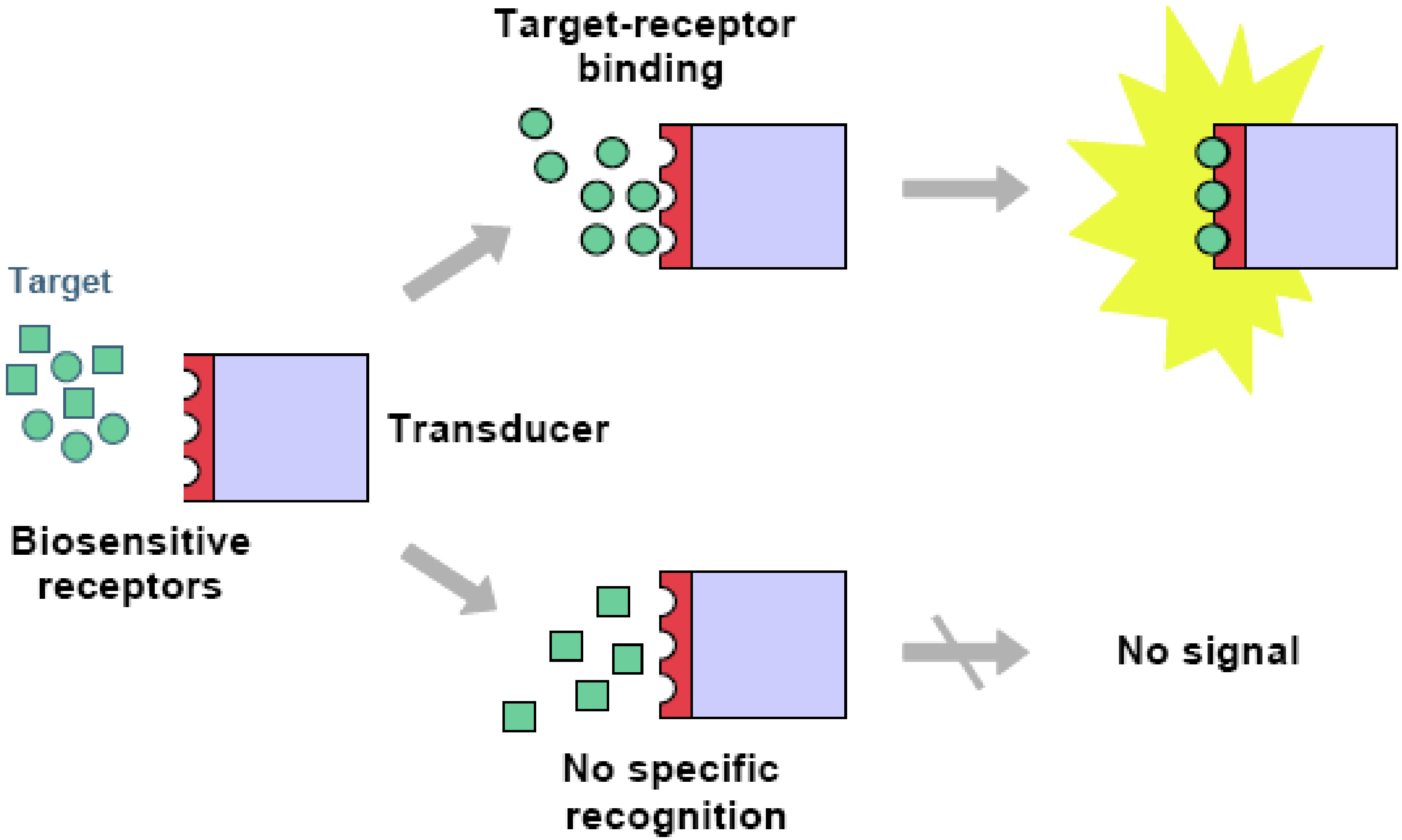
NANOBIOSENSORS

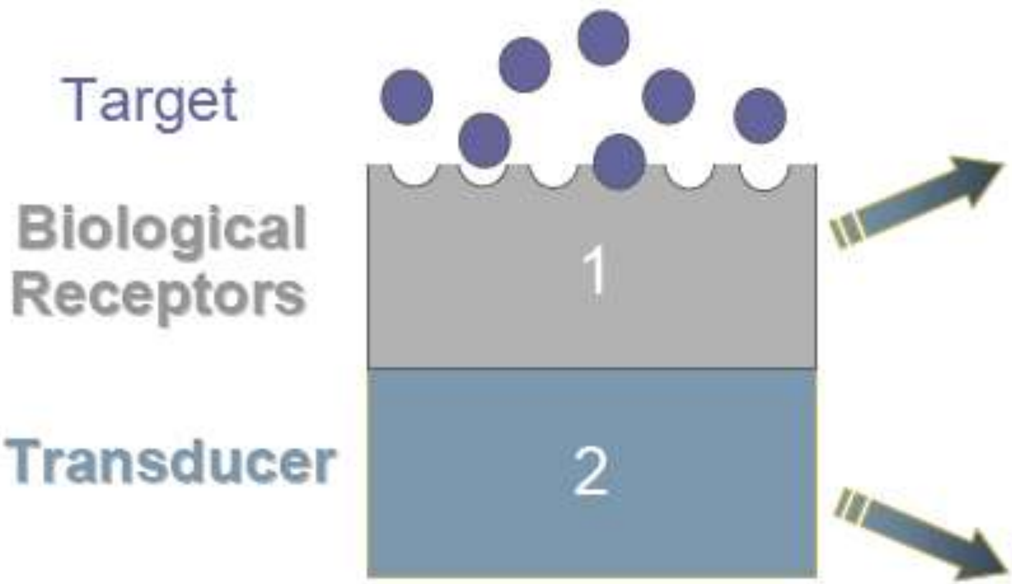
biosensors on the nano-scale size

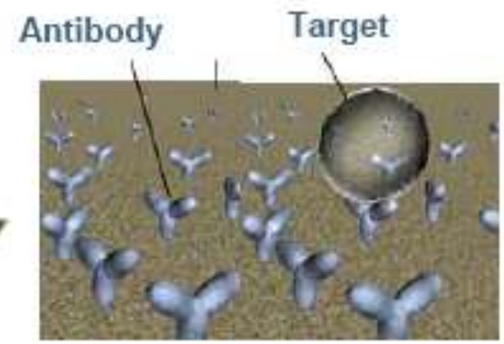
BIOSENSORS

- A device incorporating a biological sensing element either intimately connected to or integrated within a transducer.
- Recognition based on affinity between complementary structures like:
    - ❖enzyme-substrate, antibody-antigen , receptor-hormone complex.
- Selectivity and specificity depend on biological recognition systems connected to a suitable transducer.

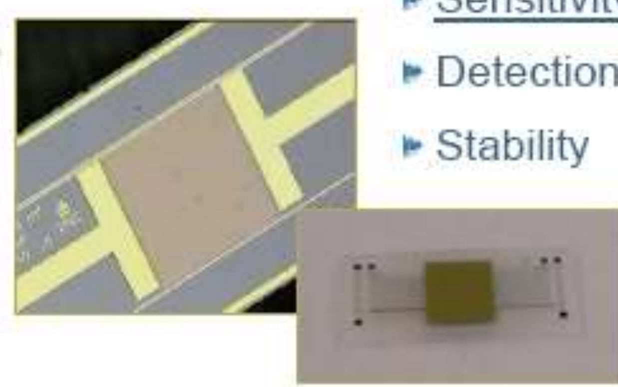

Target-receptor
binding
Target
Transducer
Biosensitive
receptors
No signal
No specific
recognition

The two parts of any biosensor





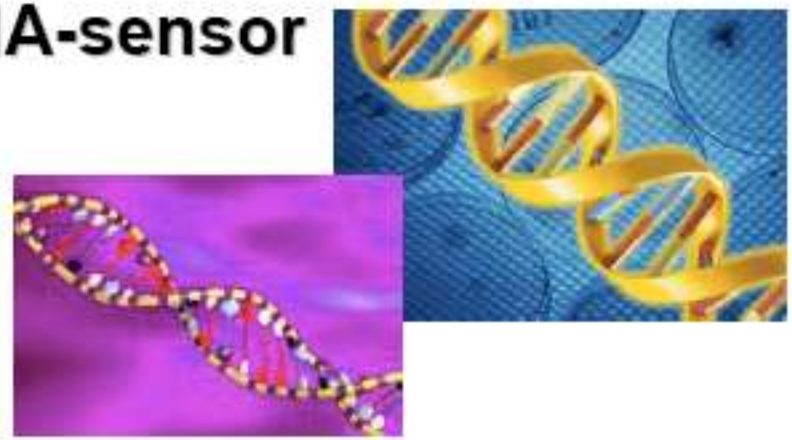
- Specificity
- Sensitivity
- Detection limit
- Stability
- Reproducibility



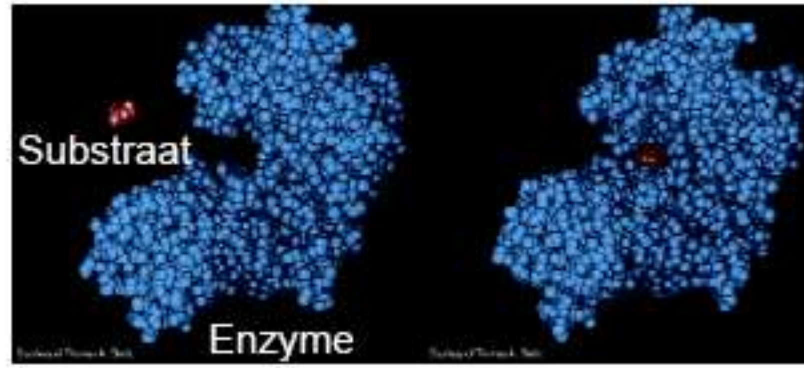
- Sensitivity
- Detection limit
- Stability

Biosensor classification (receptor-based)

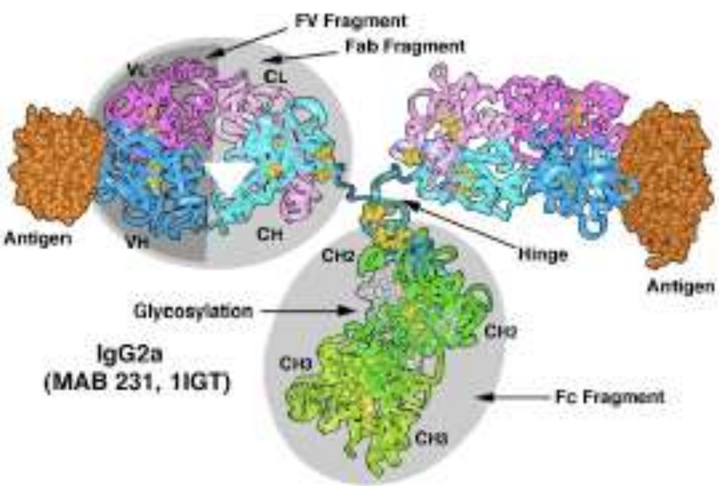
DNA-sensor



Enzyme sensor



Immuno-sensor

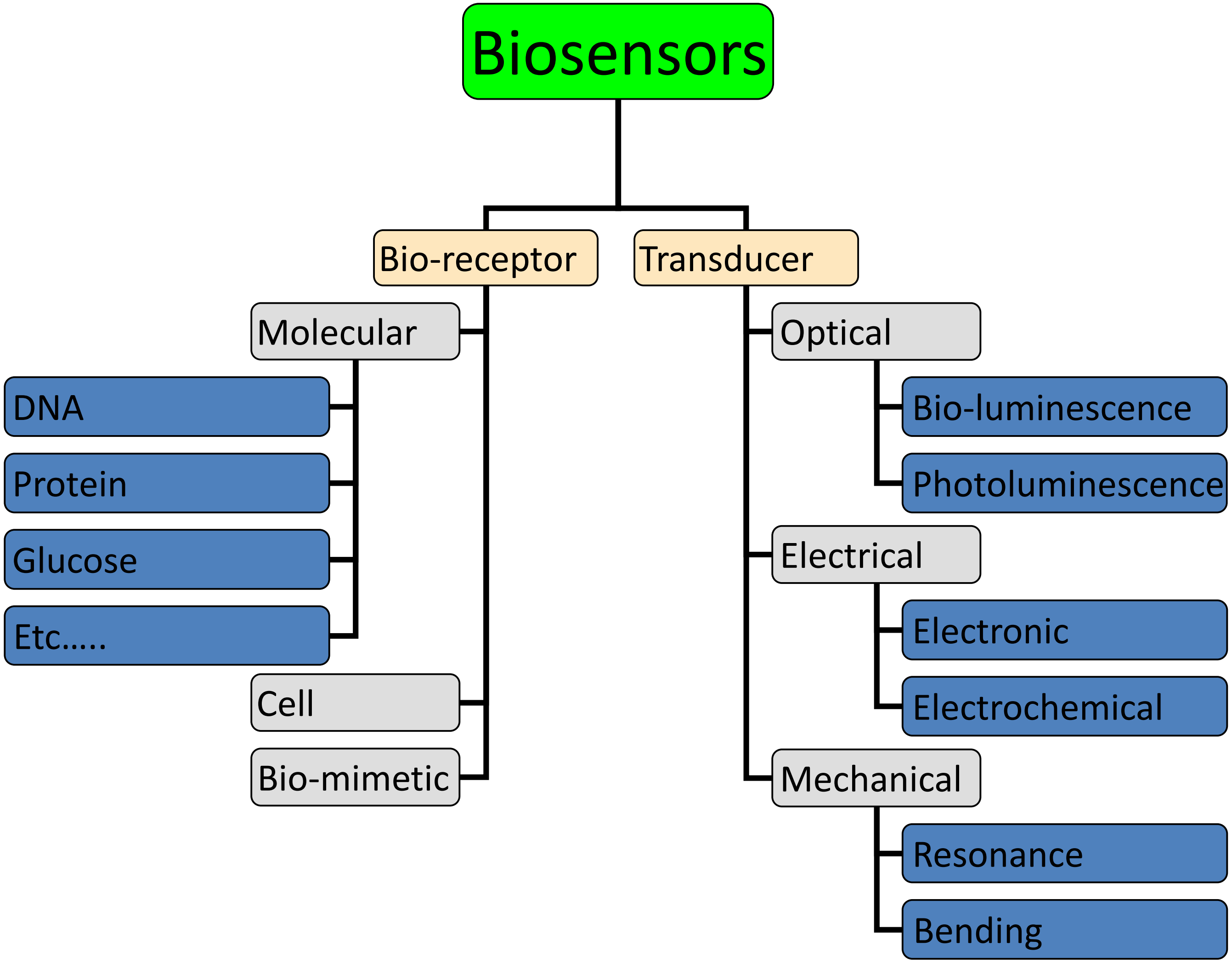


Whole-cell biosensor

Biosensor Development

- **1916** First report on the immobilization of proteins: adsorption of invertase on activated charcoal.
- **1956** Invention of the first oxygen electrode [Leland Clark]
- **1962** First description of a biosensor: an amperometric enzyme electrode for glucose. [Leland Clark, New York Academy of Sciences Symposium]
- **1969** First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea. [Guilbault and Montalvo]
- **1970** Invention of the Ion-Selective Field-Effect Transistor (ISFET).
- **1972/5** First commercial biosensor: Yellow Springs Instruments glucose biosensor.
- **1976** First bedside artificial pancreas [Clemens et al.]
- **1980** First fiber optic pH sensor for in vivo blood gases.
- **1982** First fiber optic-based biosensor for glucose
- **1983** First surface plasmon resonance (SPR) immunosensor.
- **1987** Launch of the blood glucose biosensor[MediSense]

Theory


Biosensors
Bio-receptor
Transducer
Molecular
DNA
Protein
Glucose
Etc.....
Cell
Bio-mimetic
Optical
Bio-luminescence
Photoluminescence
Electrical
Electronic
Electrochemical
Mechanical
Resonance
Bending

Bio-receptor/ analyte complexes

- Antibody/antigen interactions,
- Nucleic acid interactions
- Enzymatic interactions
- Cellular interactions
- Interactions using bio-mimetic materials

(e.g. synthetic bio-receptors).

Signal transduction methods

1. Optical measurements - luminescence, absorption, surface plasmon resonance
2. Electrochemical - potentiometric, amperometric, etc.
3. Electrical – transistors, nano-wires, conductive gels etc.
4. Mass-sensitive measurements- surface acoustic wave, microcantilever, microbalance, etc.).

Electrical sensing

_Nano-bio interfacing → how to translate the biological information onto electrical signal ?

- Electrochemical
  - Redox reactions
- Electrical
  - FET

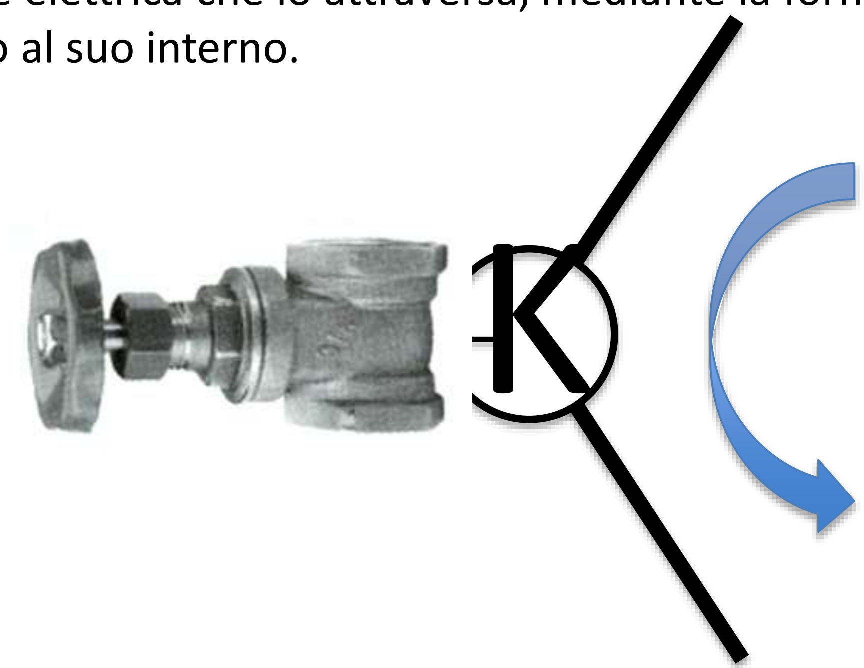
  (Nano wires; Conducting Electro-active Polymers)

Electrical sensors

➢ **FET based methods – FET – Field Effect Transistors**

- ❖ ISFET – Ion Sensitive FET
- ❖ CHEMFET – Chemically Sensitive FET
- ❖ SAM-FET – Self Assembly Monolayer Based FET

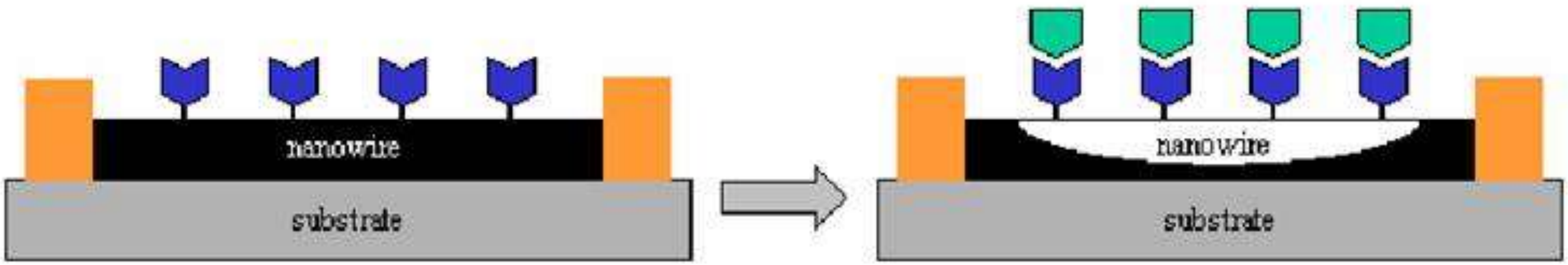
Il principio di funzionamento del transistor a effetto di campo si fonda sulla possibilità di controllare la conduttività elettrica del dispositivo, e quindi la corrente elettrica che lo attraversa, mediante la formazione di un campo elettrico al suo interno.



Corrente elettrica

Amplificazione di un segnale in entrata.
Funzionamento da interruttore (switcher).

Nanowires Biosensors
Field Effect Nanobiosensors (FET)

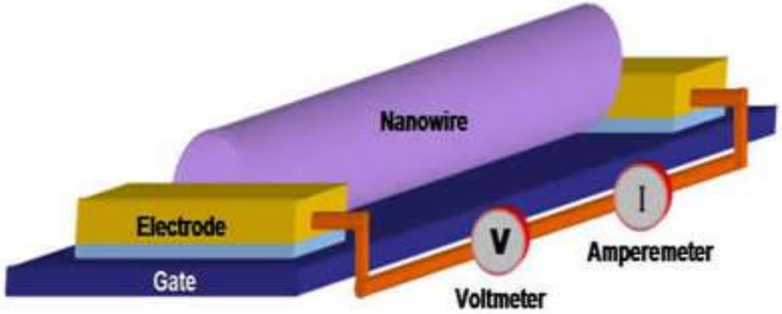


- Functionalize the nano-wires
- Binding to bio-molecules will affect the nano-wires conductivity.

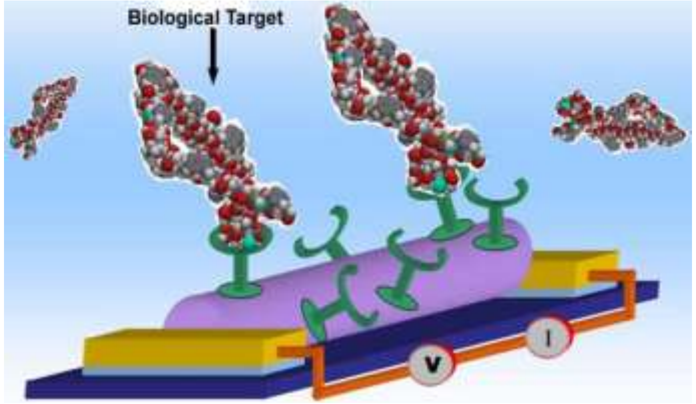
Nanowire Field Effect Nanobiosensors (FET)

Sensing Element

Semiconductor channel transistor.

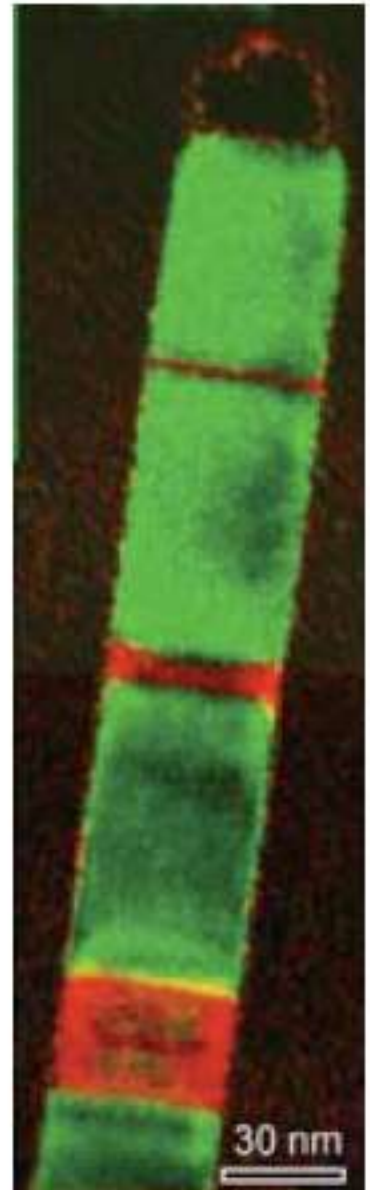


- The semiconductor channel is fabricated using nanomaterials such carbon nanotubes, metal oxide nanowires or Si nanowires.
- Very high surface to volume radio and very large portion of the atoms are located on the surface. Extremely sensitive to environment



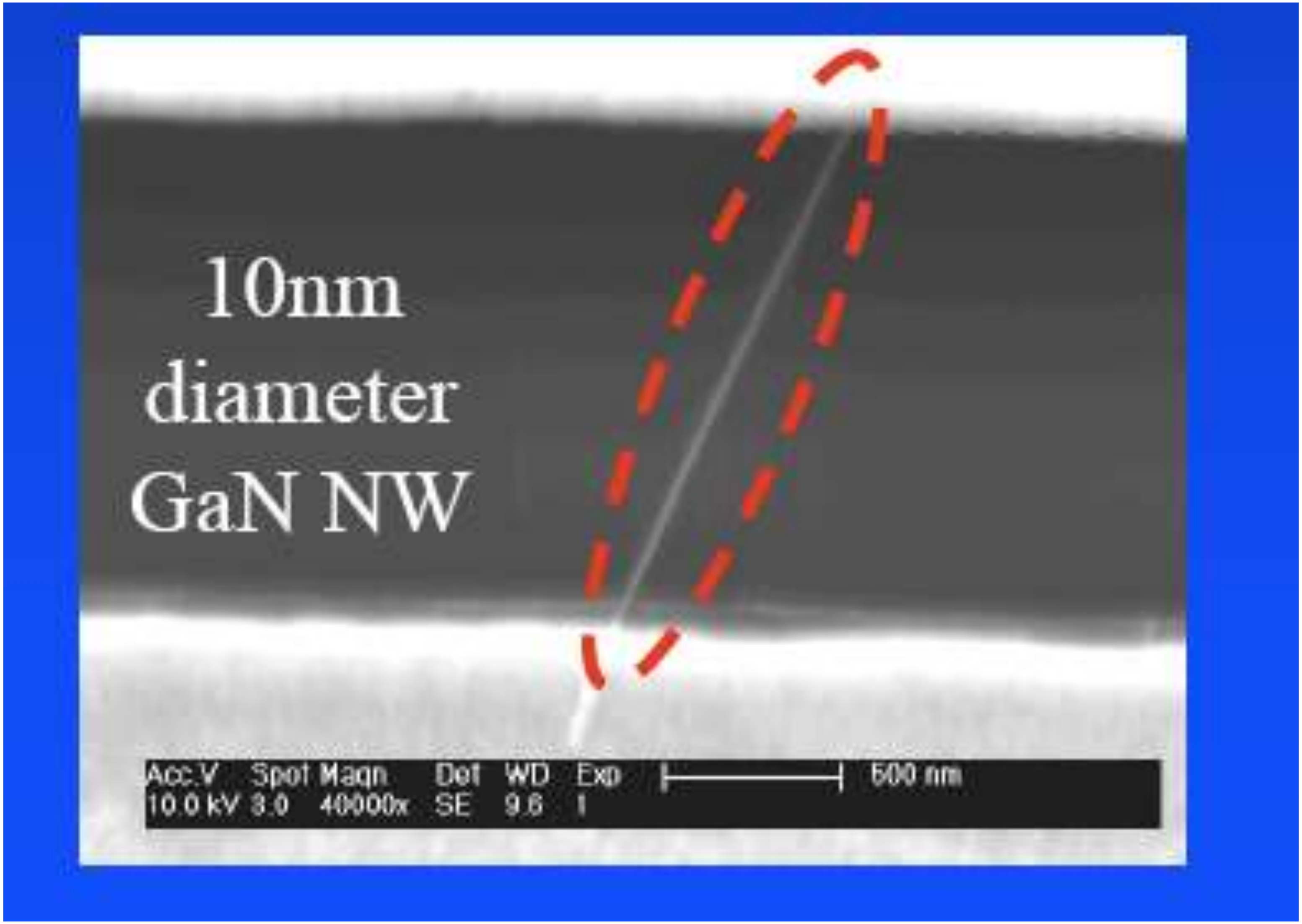
NANOWIRE

- A: Any solid material in the form of wire with diameter smaller than about 100 nm



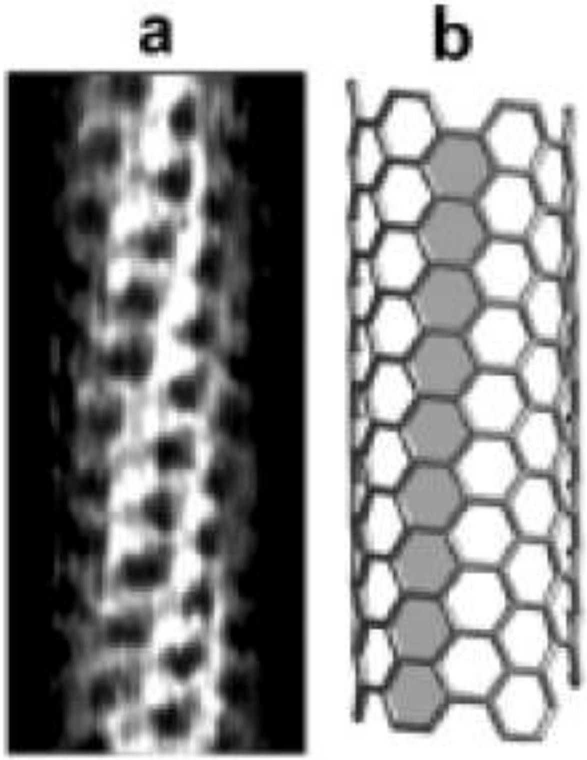
Transmission electron micrograph of an InP/InAs **nanowire**

(M.T. Bjork et. al., Nanoletters, 2:2 2002)


10nm
diameter
GaN NW
Acc.V Spot Magn Det WD Exp
10.0 kV 3.0 40000x SE 9.6 1
500 nm

NANOTUBE

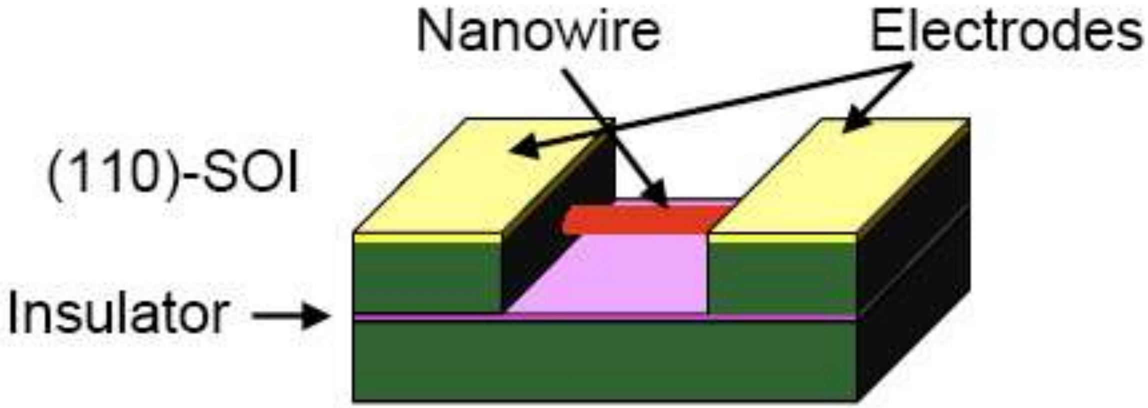
- A: A hollow nanowire, typically with a wall thickness on the order of molecular dimensions
- The smallest (and most interesting) nanotube is the single-walled carbon nanotube (SWNT) consisting of a single graphene sheet rolled up into a tube

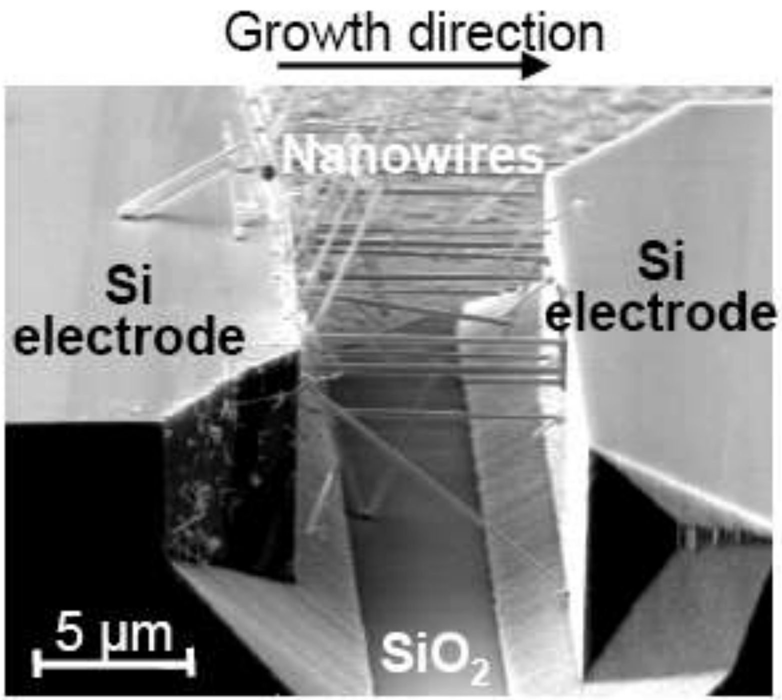


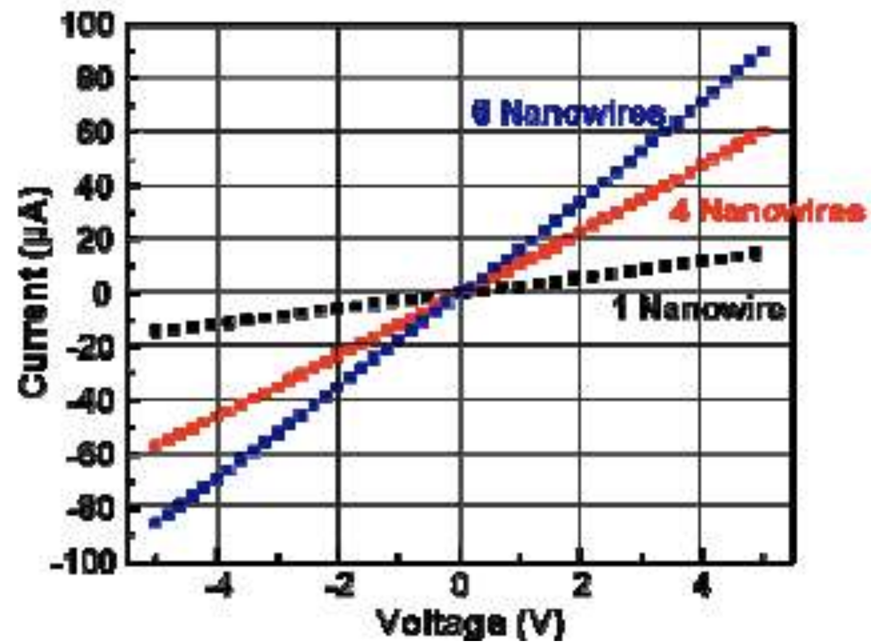
Scanning Tunneling Micrograph of a single-walled carbon **nanotube** and corresponding model (Dekker)

Q: What makes nanowires and nanotubes (scientifically) interesting?

- **Electronic & optical properties**
  - Nanowires and nanotubes are the most confining electrical conductors - puts the squeeze on electrons
  - Can be defect free - electrons move "ballistically"
  - Quantum confinement - tunable optical properties
- **Mechanical properties**
  - Small enough to be defect-free, thus exhibiting ideal strength
- **Thermal properties**
  - Can be designed to conduct heat substantially better (or much worse) than nearly every bulk material
- **Chemical properties**
  - Dominated by large surface-to-volume ratio

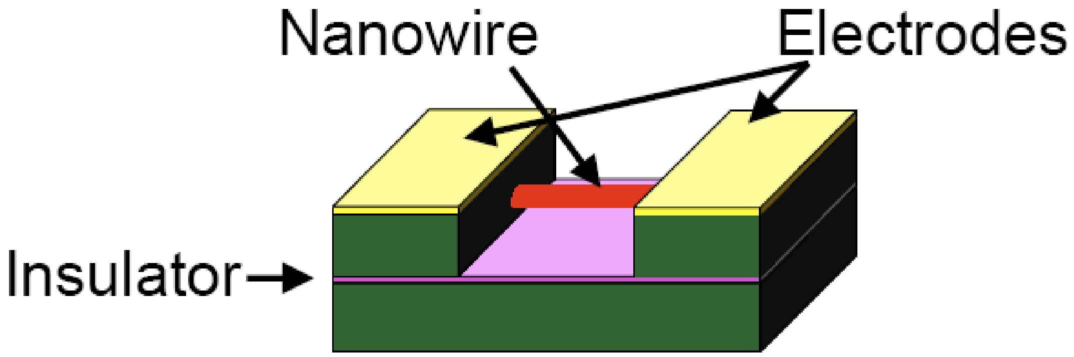

Nanowire
Electrodes
(110)-SOI
Insulator


Growth direction
Nanowires
Si
electrode
Si
electrode
5 µm
SiO_2


6 Nanowires
4 Nanowires
1 Nanowire
Current (µA)
Voltage (V)
100
80
60
40
20
0
-20
-40
-60
-80
-100
-4
-2
0
2
4

Connecting both ends of nanowires

Grow nanowires between pre-formed electrodes
(electrodes formed by conventional lithography)

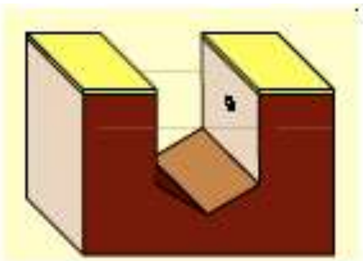


NW grow across gap between electrodes
Growing NW connect to the second electrode

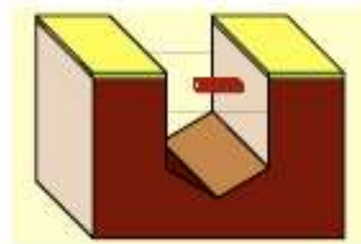
Connecting nanostructures

Bridging nanowires

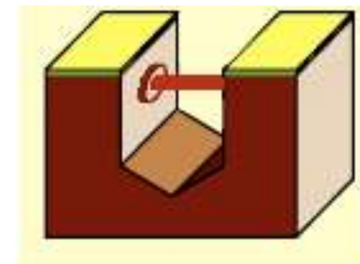
Form trench in Si and
Deposit catalyst

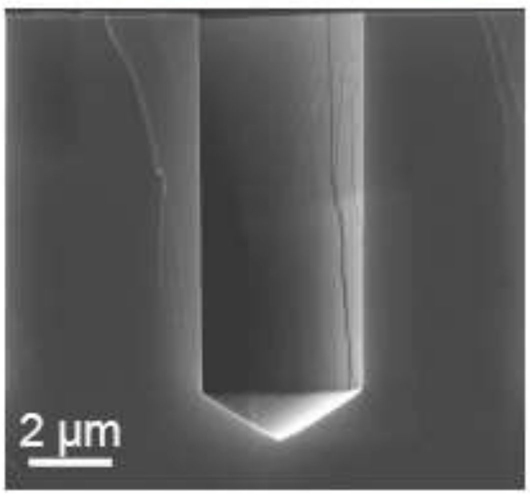


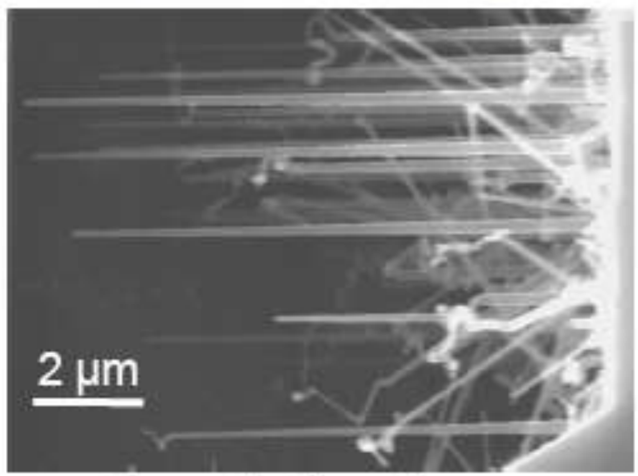
NW grows
perpendicular



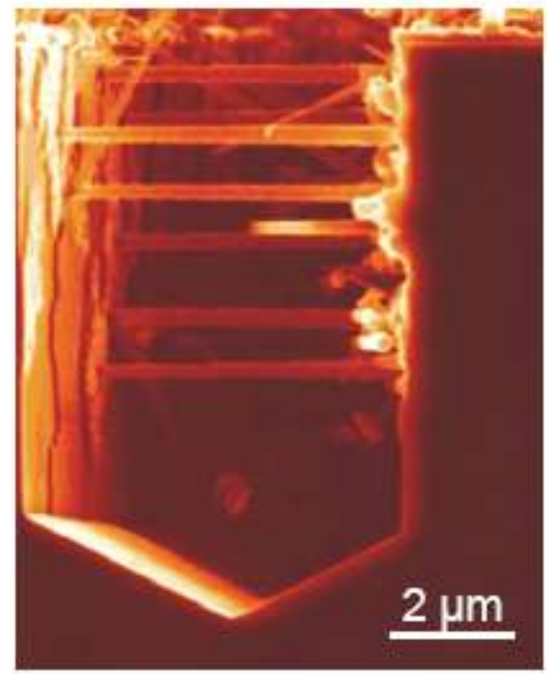
NW connects to opposite
sidewall





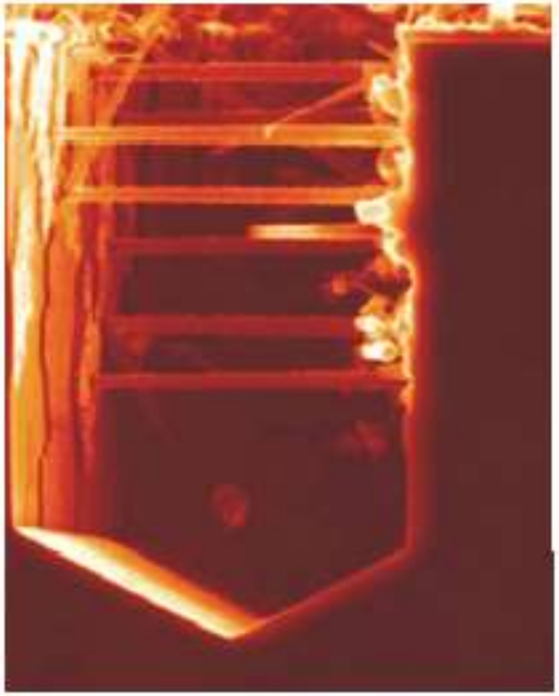


Growth direction



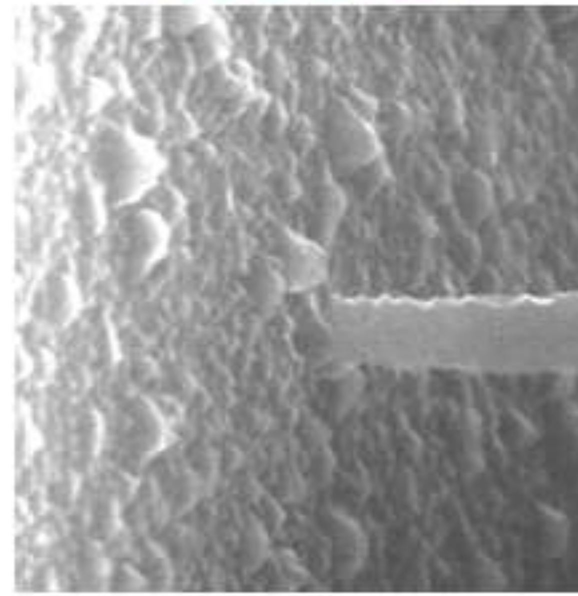
M. Saif Islam, S. Sharma, T. I. Kamins, and R. Stanley Williams, Nanotechnology **15**, L5-L8 (May 2004)

Bridging nanowires

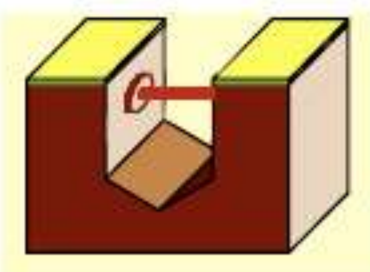


Au catalyst

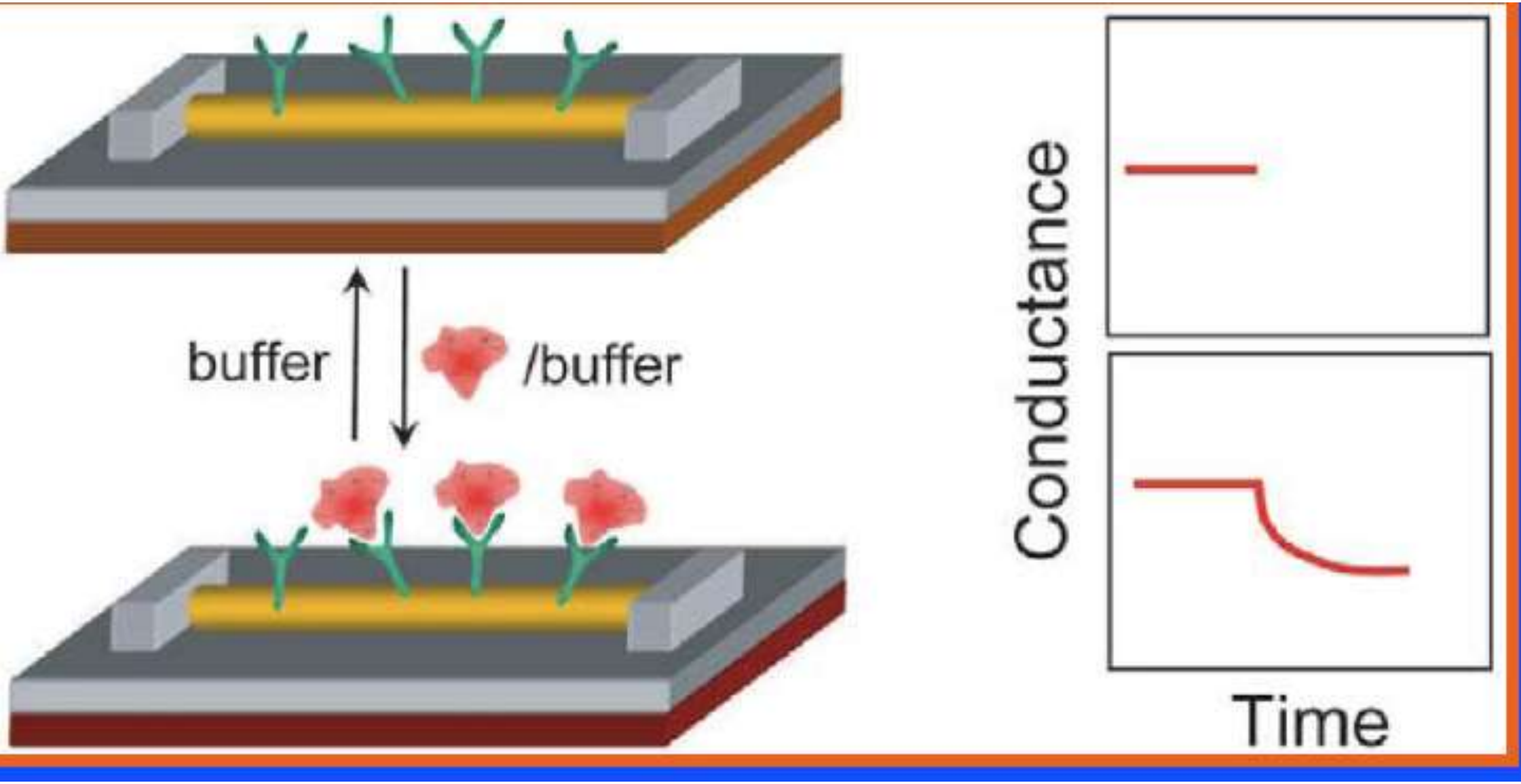
Ti catalyst



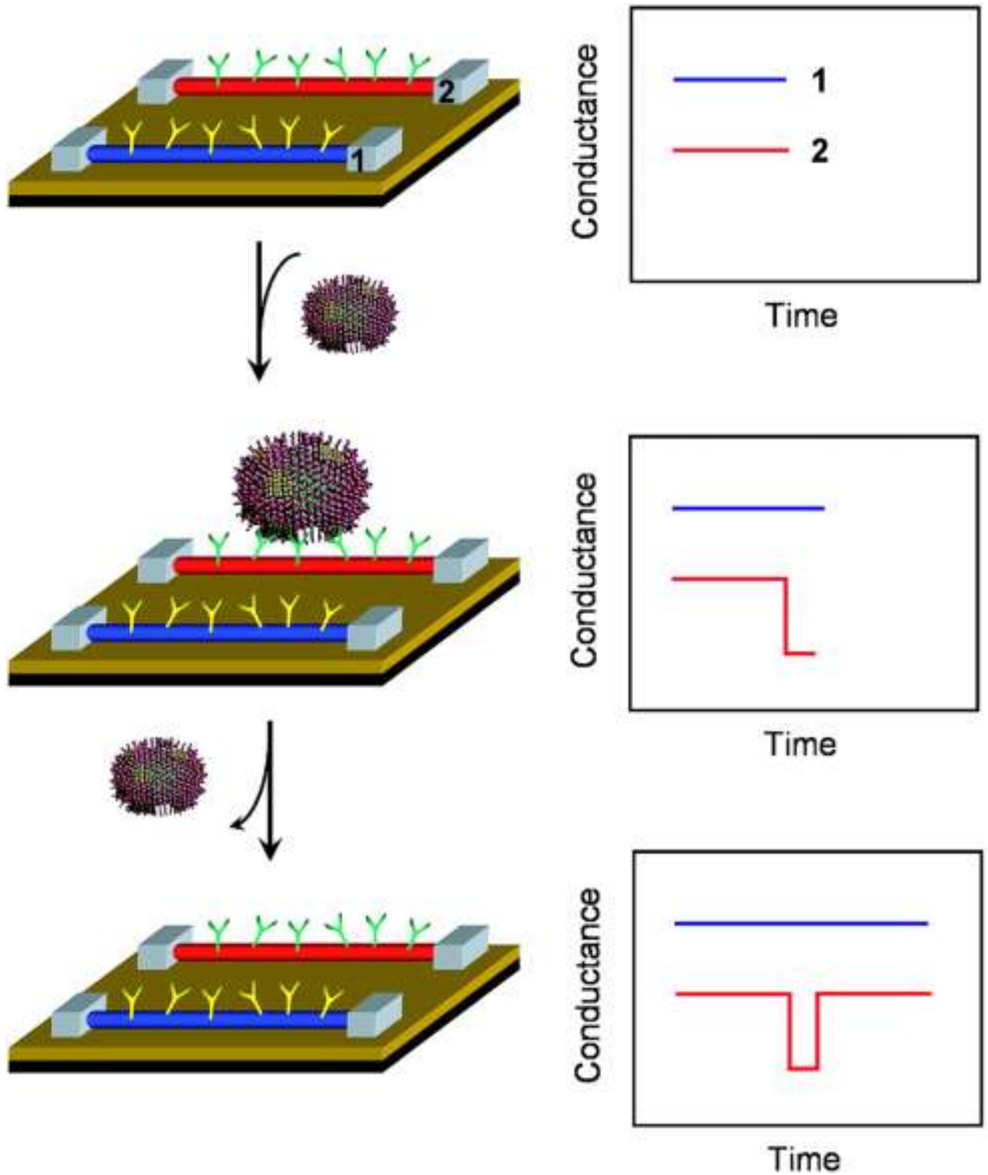
Growth direction



M. Saif Islam, S. Sharma, T. I. Kamins, and R. Stanley Williams, Nanotechnology **15**, L5-L8 (May 2004)


buffer
/buffer
Conductance
Time

Conductive Nanowire Sensors: Detecting Single Recognition Events



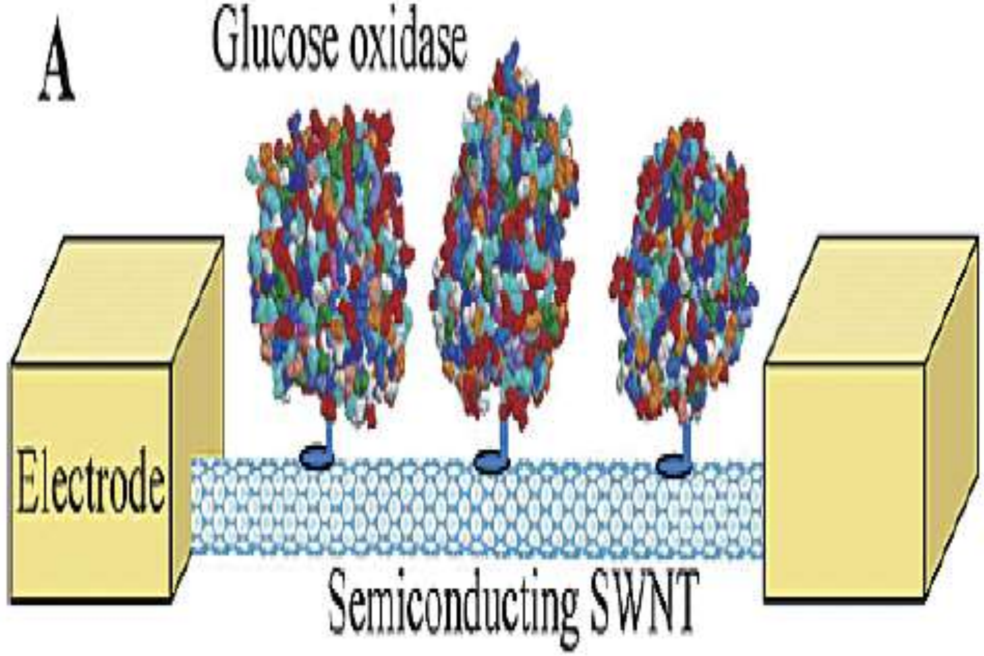
Schematic shows two nanowire devices, 1 and 2, where the nanowires are modified with different antibody receptors. Specific binding of a single virus to the receptors on nanowire 2 produces a conductance change (Right) characteristic of the surface charge of the virus only in nanowire 2. When the virus unbinds from the surface the conductance returns to the baseline value.

Carbon Nanotubes for Glucose Sensing

- Two electrodes connecting NT with glucose oxidase immobilized

SWNT:
chemical vapor deposition

Electrodes:
lithographical patterning

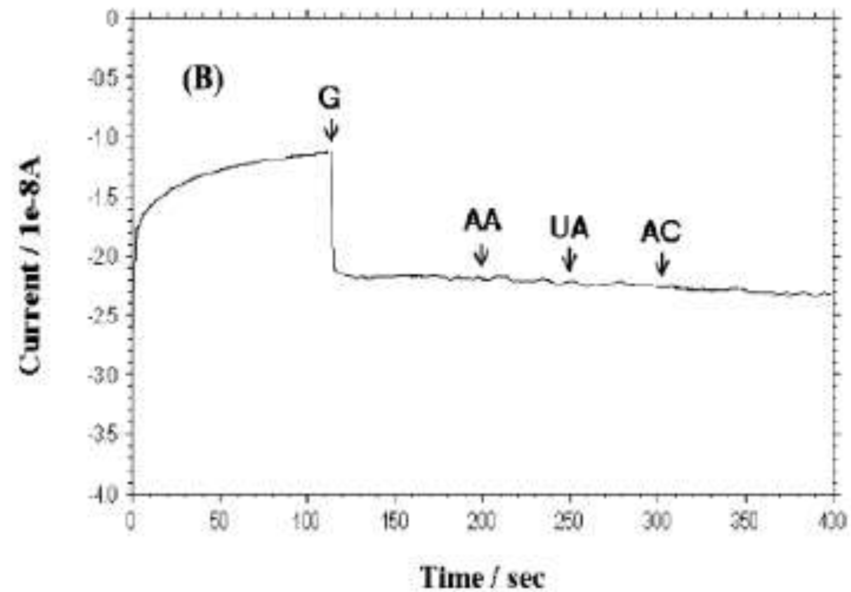


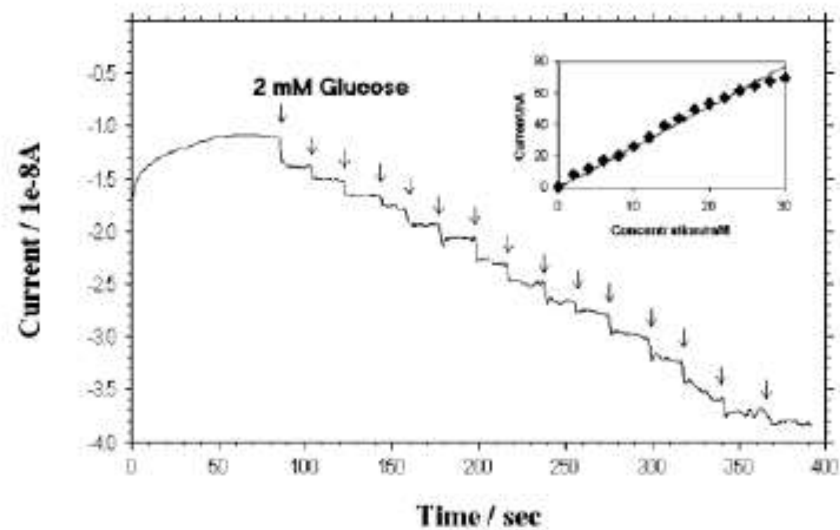
Besteman K., et al *Nano lett.* **3.** 727, 2003

Biosensors

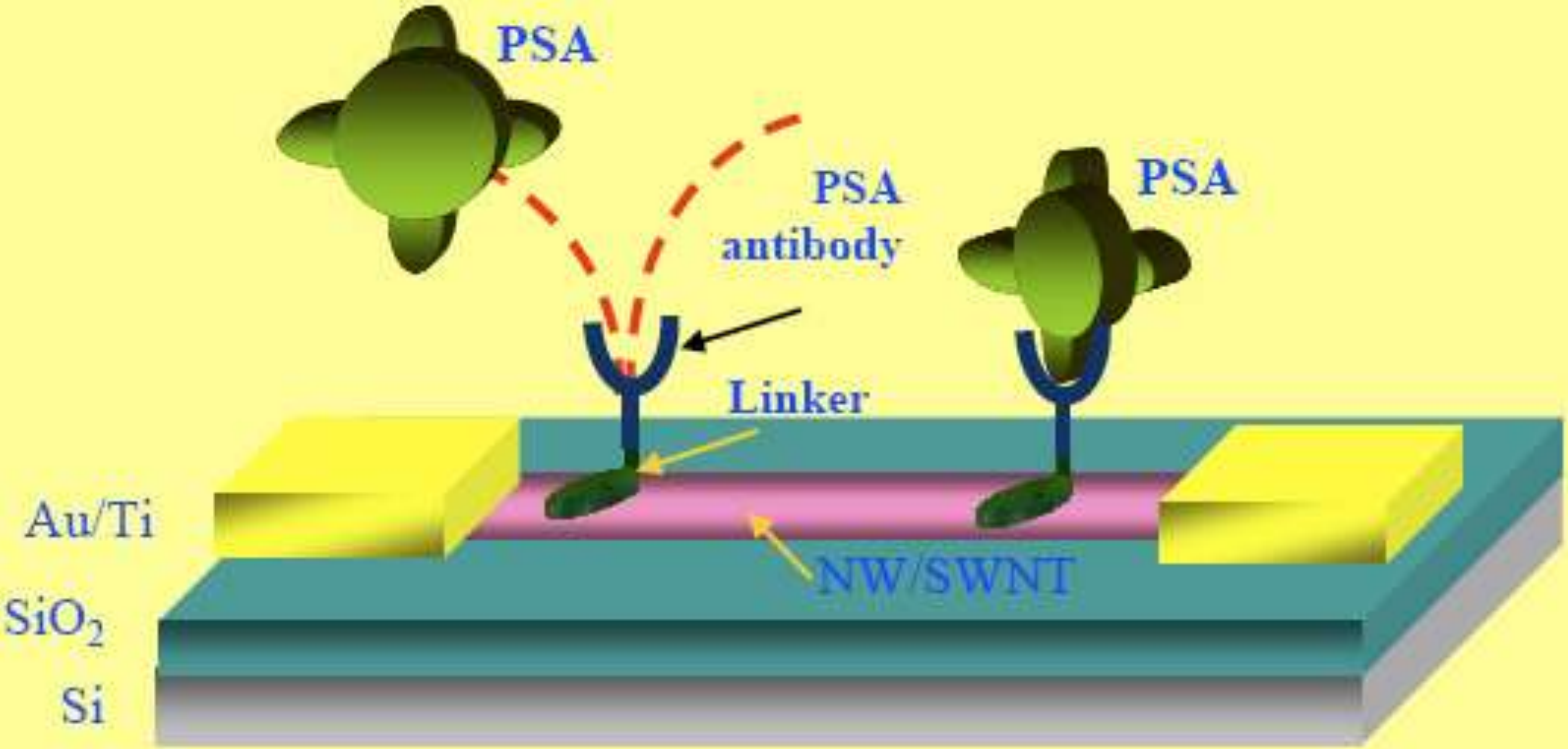
Glucose Detectors

- In this setup, CNT's are grown in nanoelectrode ensembles
- Functionalize the open ends for processing
- Detection limit ~0.08mM

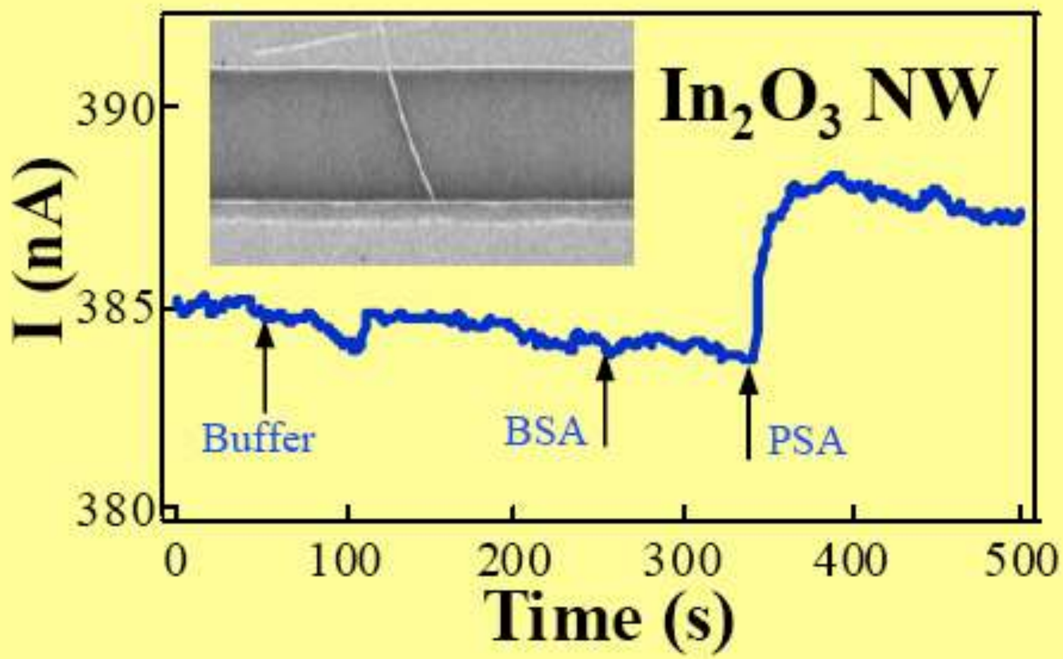




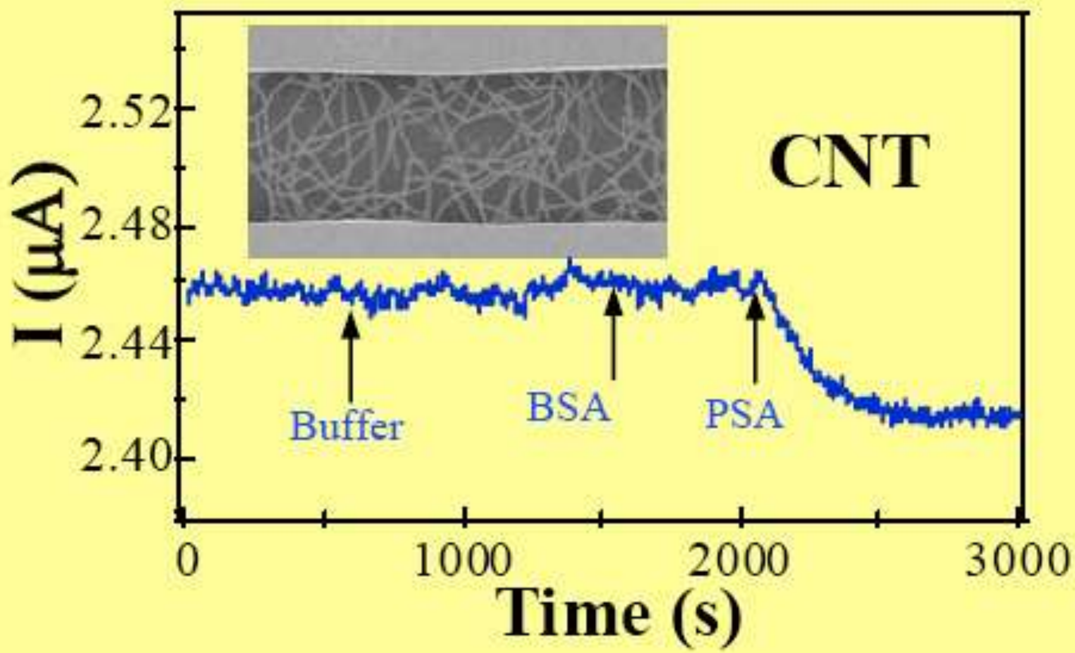
Y. Lin, F. Lu, Y. Tu, and Z. Ren, *Nano Letters*, **4**, 191 (2



PSA detection

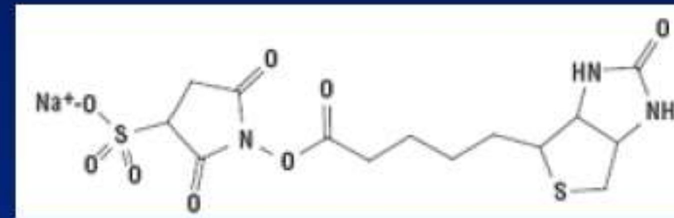


In$_2$O$_3$ NW
I (nA)
390
385
380
Buffer
BSA
PSA
0
100
200
300
400
500
Time (s)

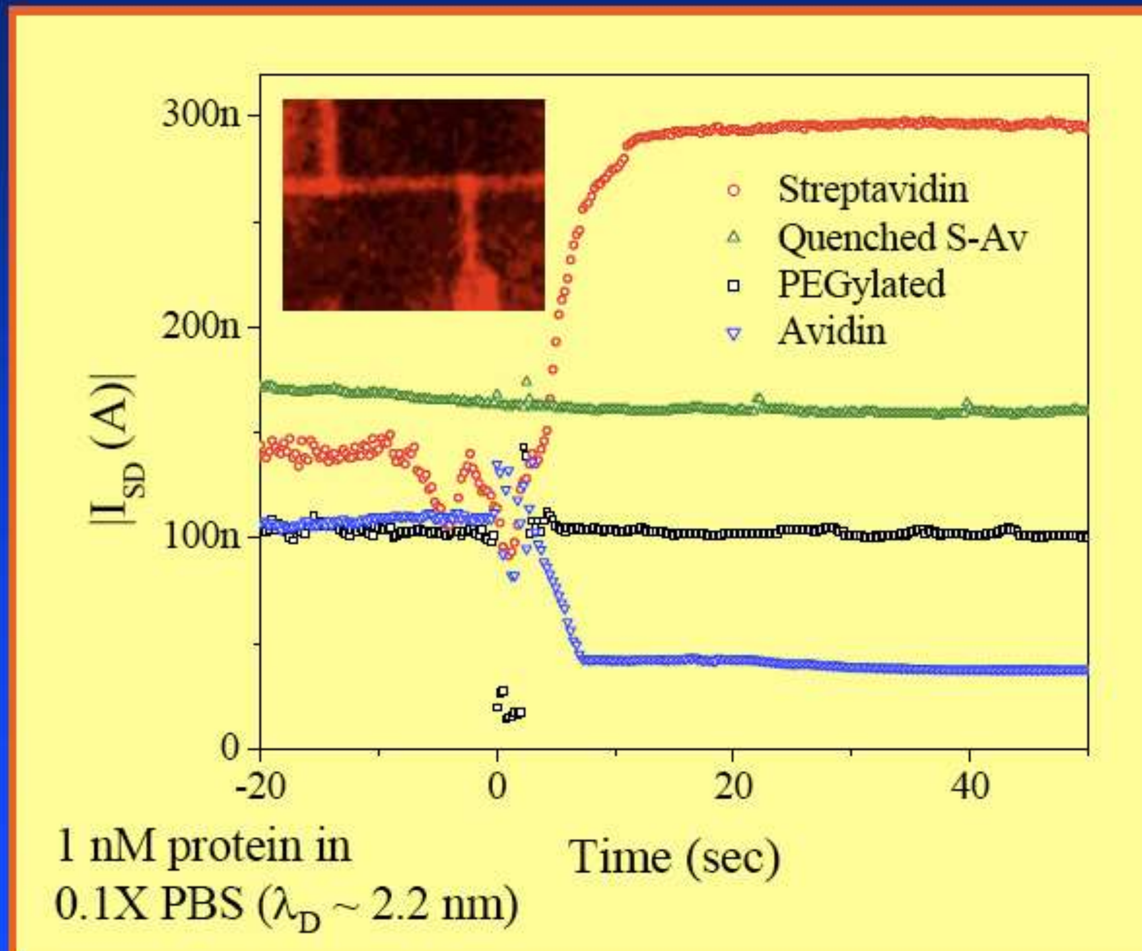


CNT
I (μA)
2.52
2.48
2.44
2.40
Buffer
BSA
PSA
0
1000
2000
3000
Time (s)

Biotin-Avidin & Streptavidin Sensing

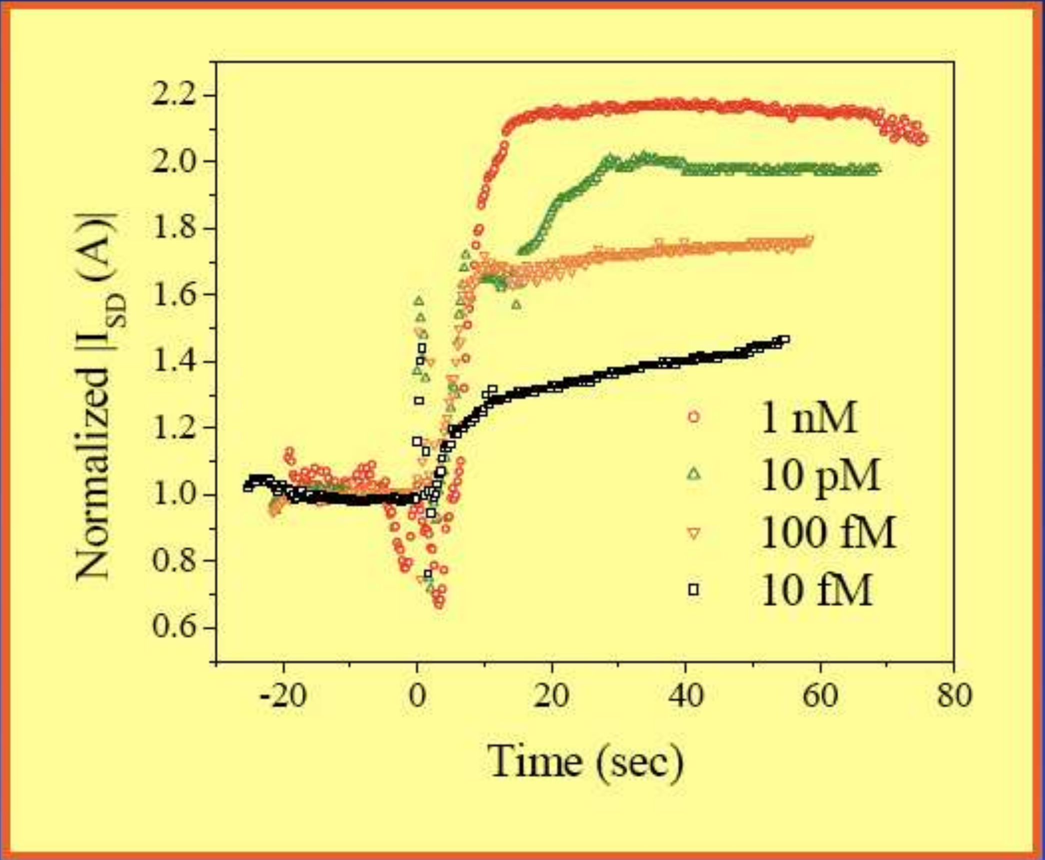


- p-type accumulation mode, biotinylated NW device
- avidin
  - positive charge
  - ⇒ current decrease
- streptavidin
  - negative charge
  - ⇒ current increase
- poly(ethylene glycol) (PEG)-ylated device, quenched avidin controls



Nature, **445**, 519 (2007)

Concentration Dependence: Ultimate Limits



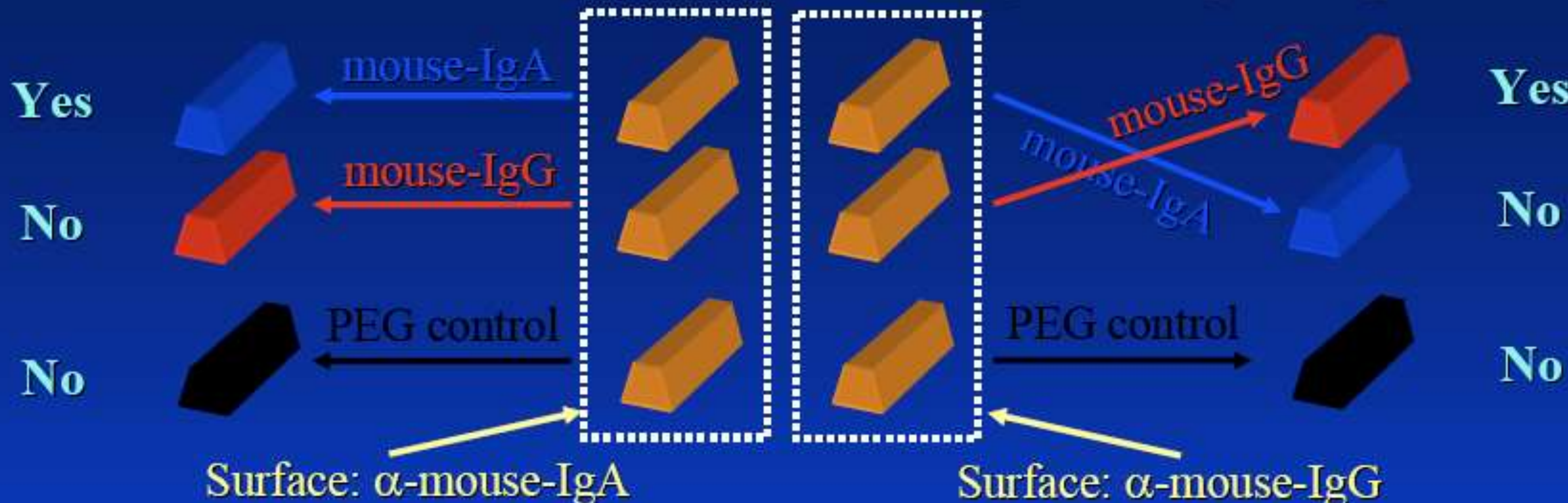
- At lowest concentration, initial S/N ~ 140 for 10fM
  ~ <100 aM limit

(1 aM = 30 molecule per mm^3)

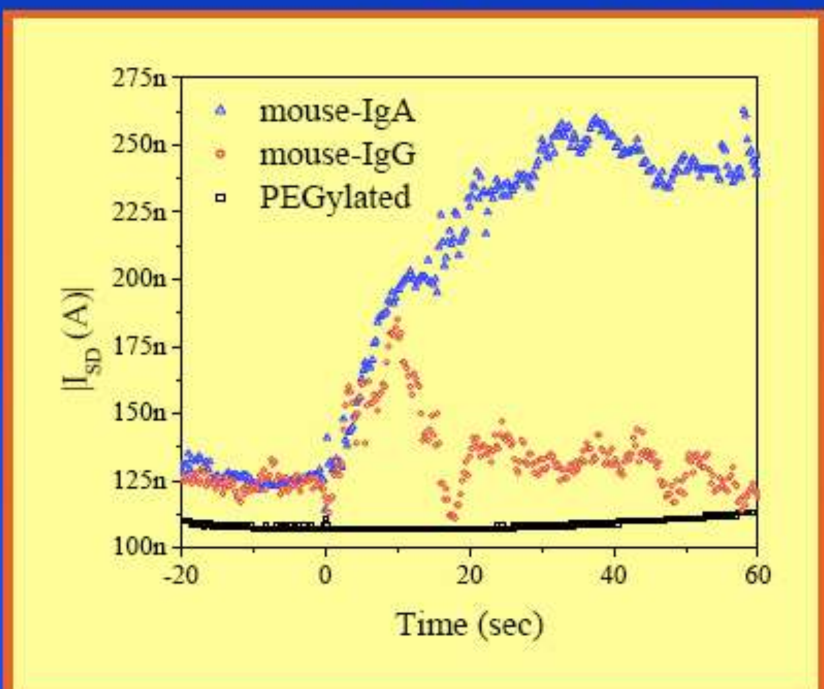
- S/N ~ 500 for saturation
- Strepavidin: 1 molecule/25 nm^2 when closest-packed
- Sensor area ~ 2.0 x 10^6 nm^2
  ⇒ ~ 8 x 10^4 molecules (max)

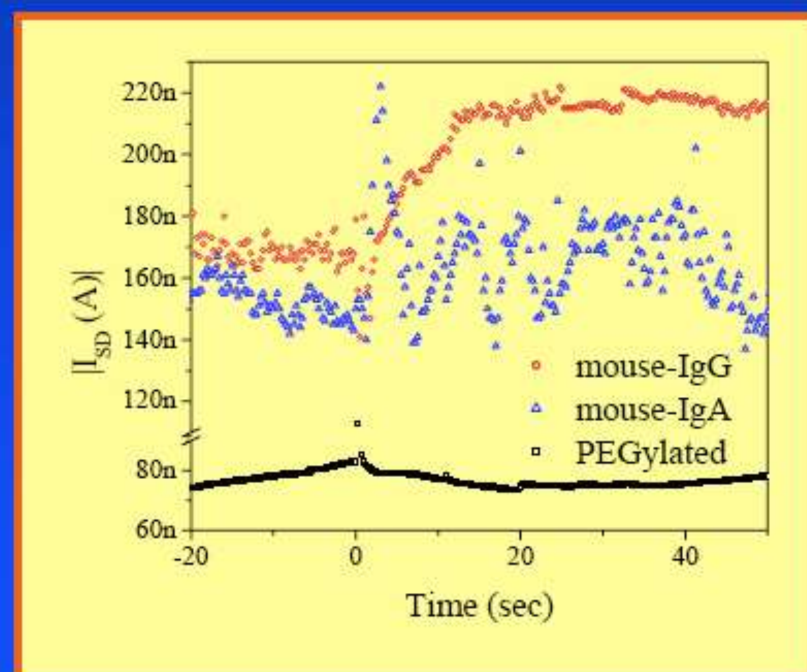
- ⇒ Detection limit ~ 15 molecules

Crisscross Protein Assay: Antibody-Antigen Specificity

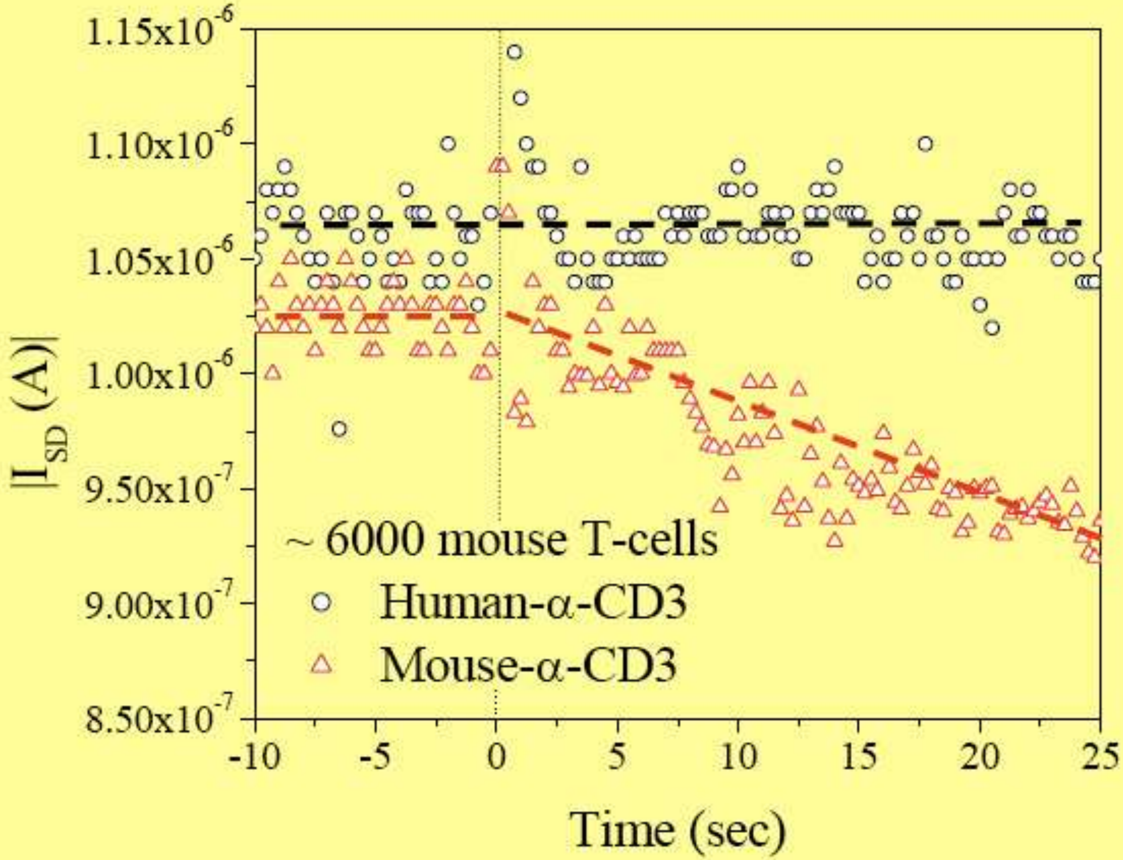


100 fM mouse-IgG/IgA in 1.5 mM bicarbonate ($\lambda_D \sim 6.8$ nm)

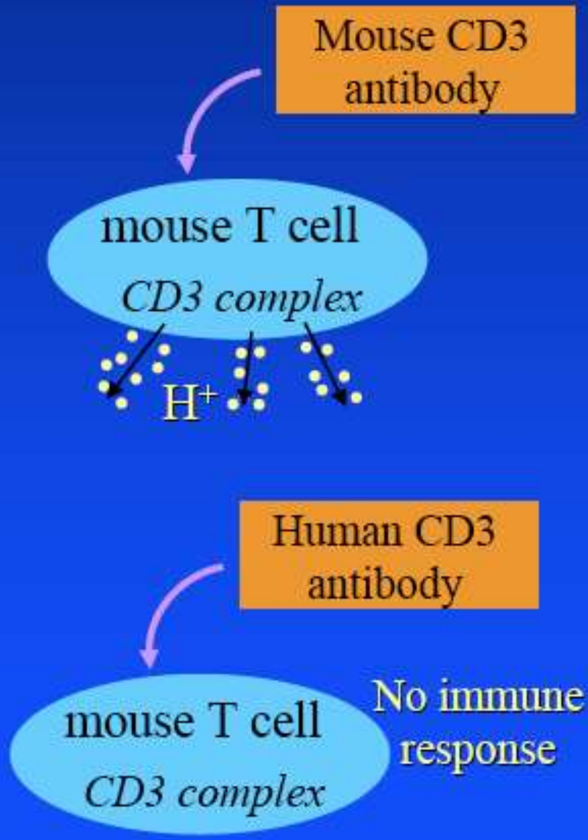




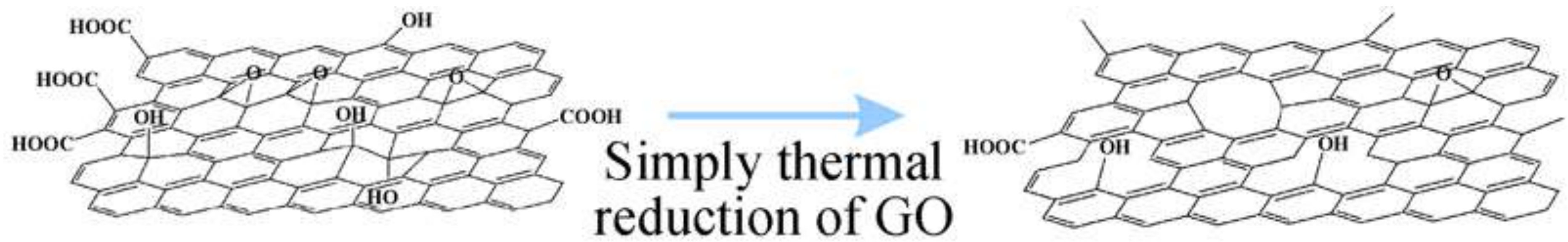
First real-time live cellular response – species-specific T-lymphocyte activation



Mouse splenocyte T cells

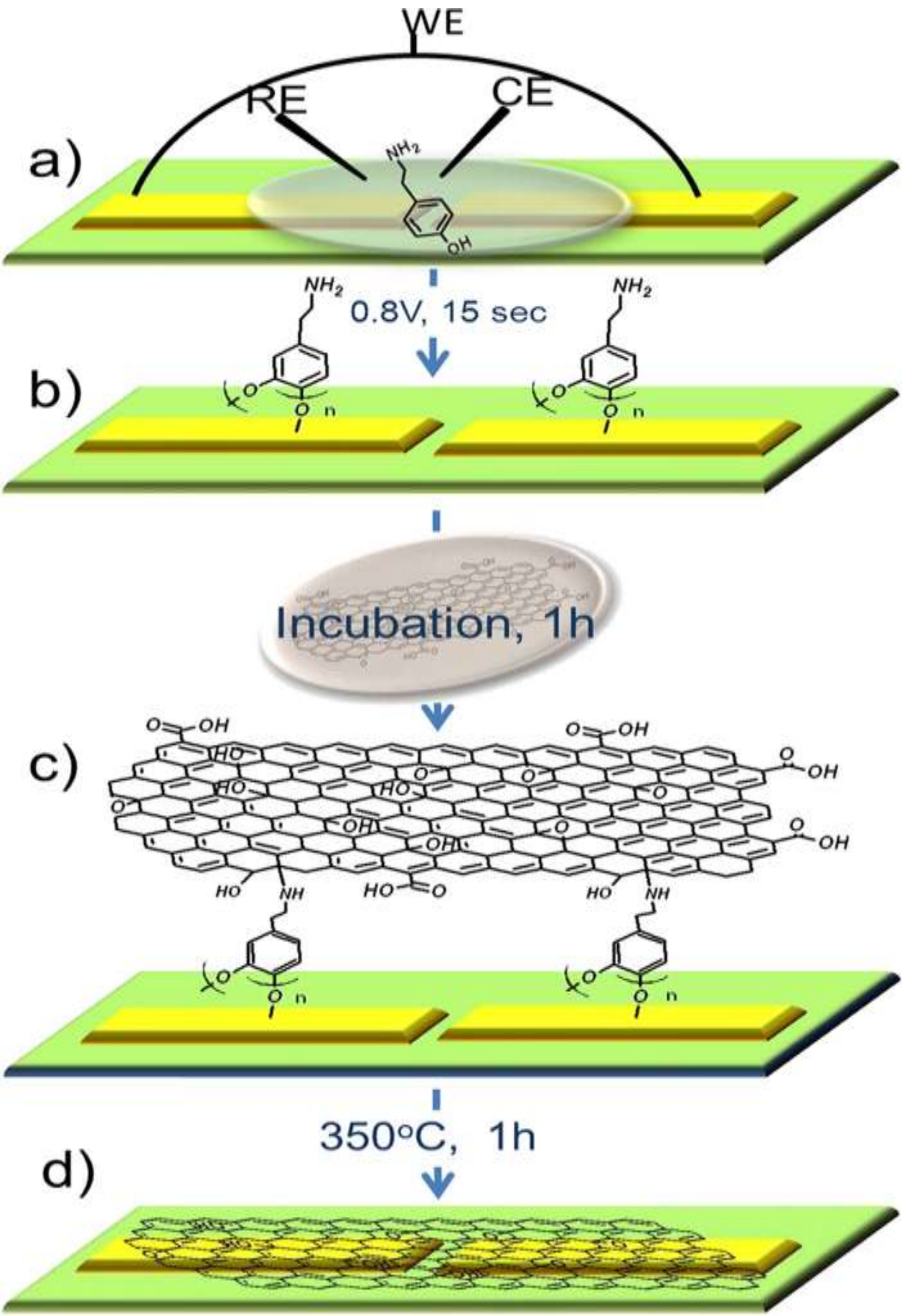


Self-Assembled Electrical Biodetector Based on Reduced Graphene Oxide

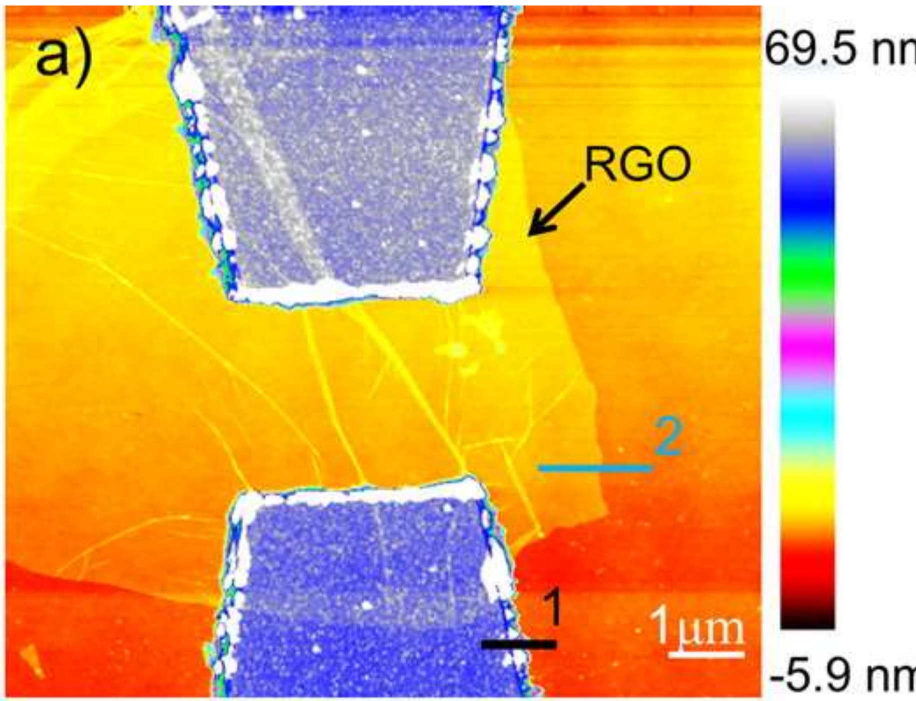


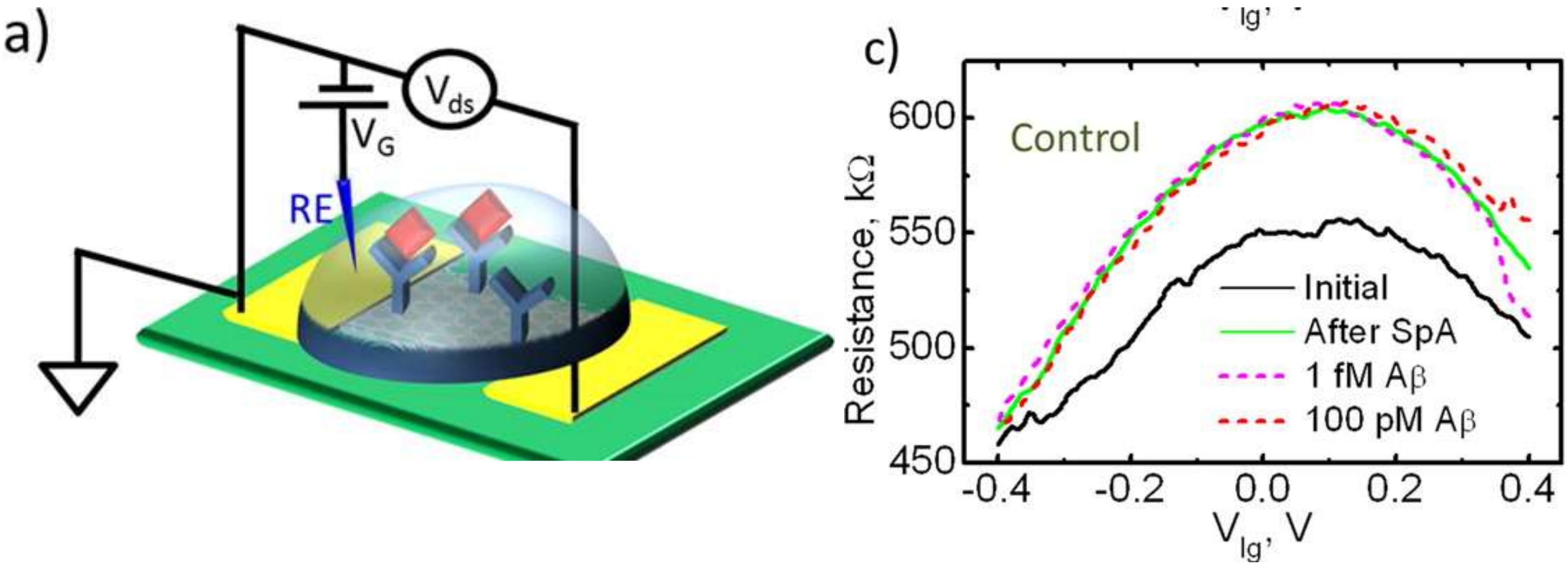
Due to the presence of polar groups such as hydroxyl, carboxyl, and epoxy groups, GO is very hydrophilic in comparison to graphene. For the purpose of device applications, the drawback when using GO is that it is an insulating material. This can be overcome by the use of a reduction procedure, which improves its conductivity, yielding reduced graphene oxide (RGO).

Published in: Tetiana Kurkina; Subramanian Sundaram; Ravi Shankar Sundaram; Francesca Re; Massimo Masserini; Klaus Kern; Kannan Balasubramanian; *ACS Nano* **2012,** 6, 5514-5520.



(a) electrochemical functionalization of Pt electrodes with tyramine (b) coating of polytyramine on the electrode; (c) coupling of GO to polytyramine; (d) removal of polytyramine layer and reduction of oxygen-containing groups in argon at 350 °C . WE = working electrode, CE = counter electrode, RE = reference electrode.





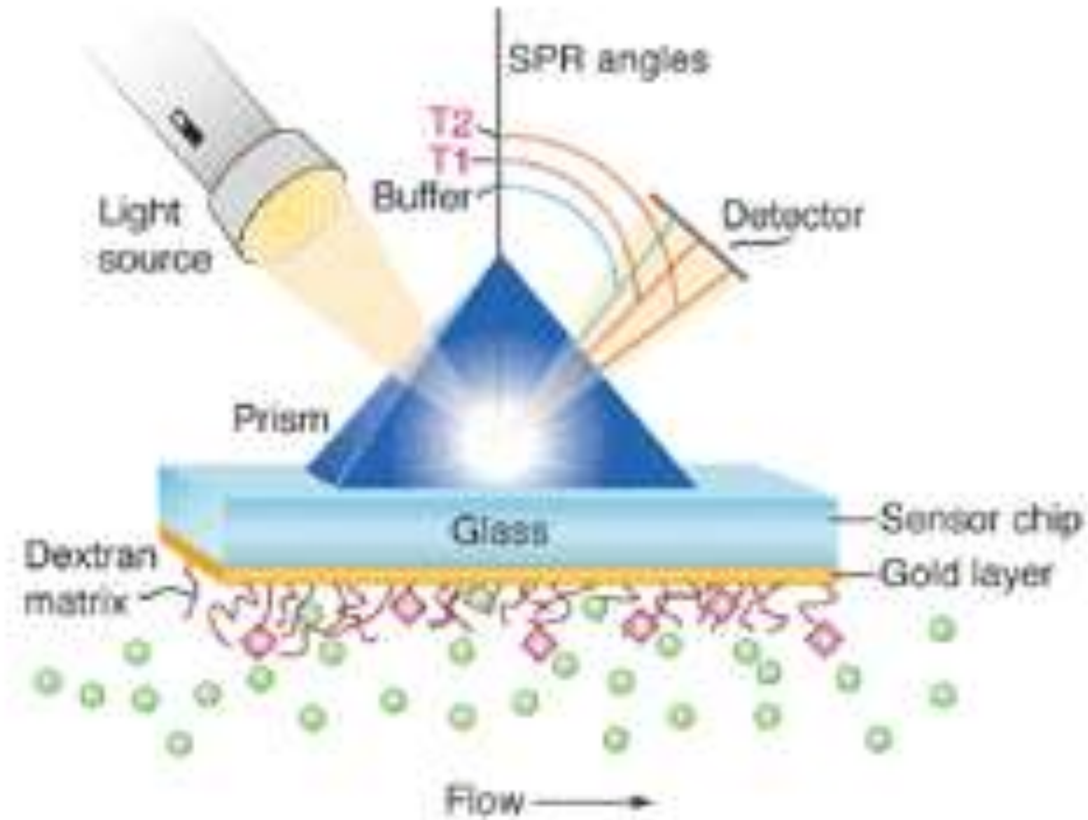
Sensing of amyloid beta peptide using RGO immunosensor. (a) Schematic of RGO-FET immunosensor; RE = reference electrode. (b) Field-effect characteristics of the RGO immunosensor before and after functionalization and after the exposure of antibody-functionalized device to 1 fM Aβ. (c) Control device showing the field-effect characteristics of another RGO device without the antibody.

RGO was covered with Staphylococcus aureus protein A (SpA) through carbodiimide coupling. SpA ensures a proper orientation of the subsequently immobilized antibodies since it has high specificity to the Fc fragments. In a second step, SpA-RGO was modified with anti-Aβ-antibodies by incubating the samples in a 50 mM antibody solution.

optical methods

- Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR)



Mechanical sensing

Cantilever-based sensing

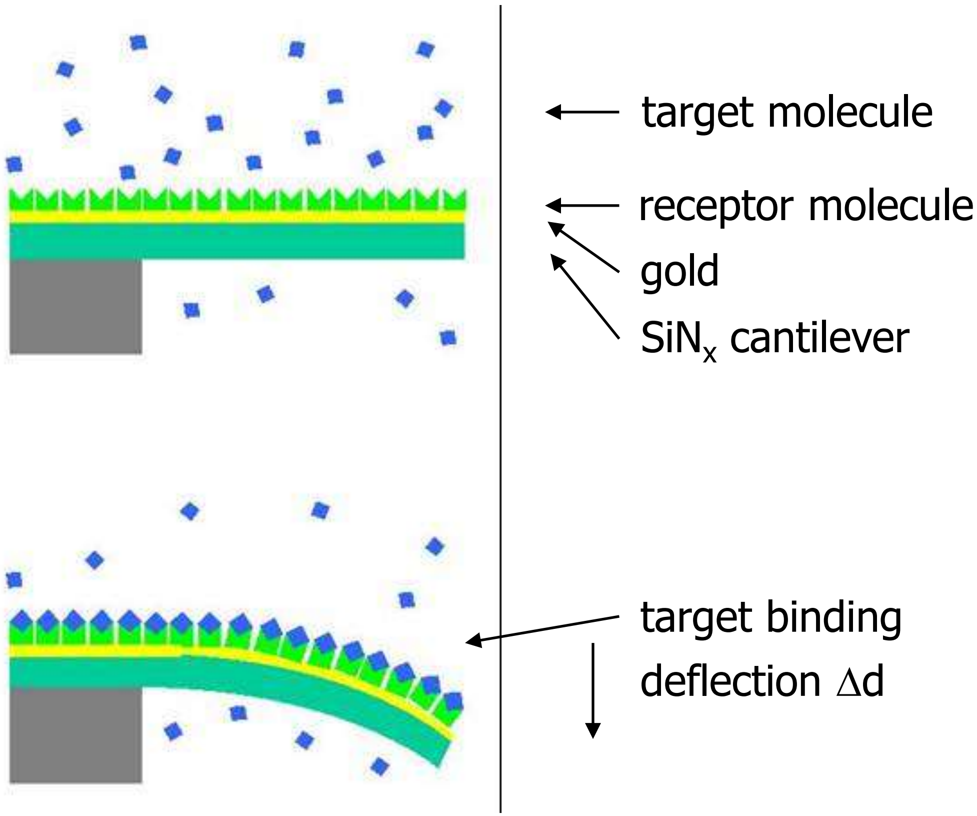
Nanoscale - cantilevers

Nanoscale cantilevers - microscopic, flexible beams resembling a row of diving boards - are built using semiconductor lithographic techniques. These can be coated with molecules capable of binding specific substrates-DNA complementary to a specific gene sequence, for example. Such micron-sized devices, comprising many nanometer-sized cantilevers, can detect single molecules of DNA or protein.

As a cancer cell secretes its molecular products, the antibodies coated on the cantilever fingers selectively bind to these secreted proteins. These antibodies have been designed to pick up one or more different, specific molecular expressions from a cancer cell. The physical properties of the cantilevers change as a result of the binding event. Researchers can read this change in real time and provide not only information about the presence and the absence but also the concentration of different molecular expressions. Nanoscale cantilevers, constructed as part of a larger diagnostic device, can provide rapid and sensitive detection of cancer-related molecules.

Source: US National Cancer Institute
Reference: Arun Majumdar, UC Berkeley

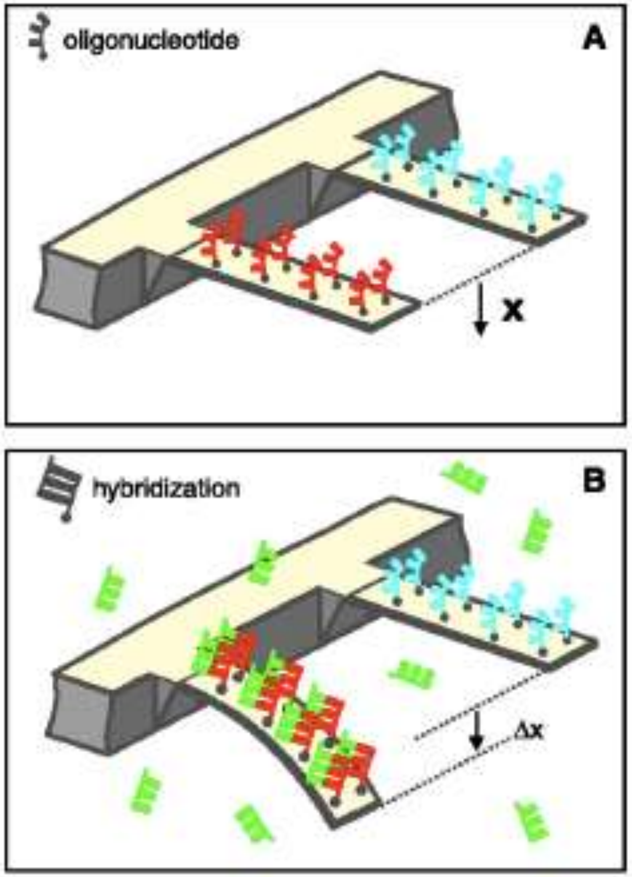
Bio-molecule sensing

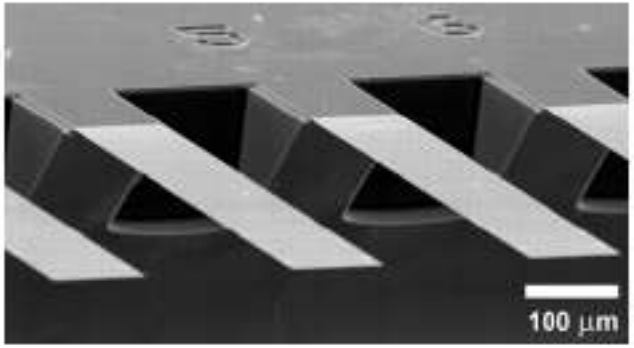


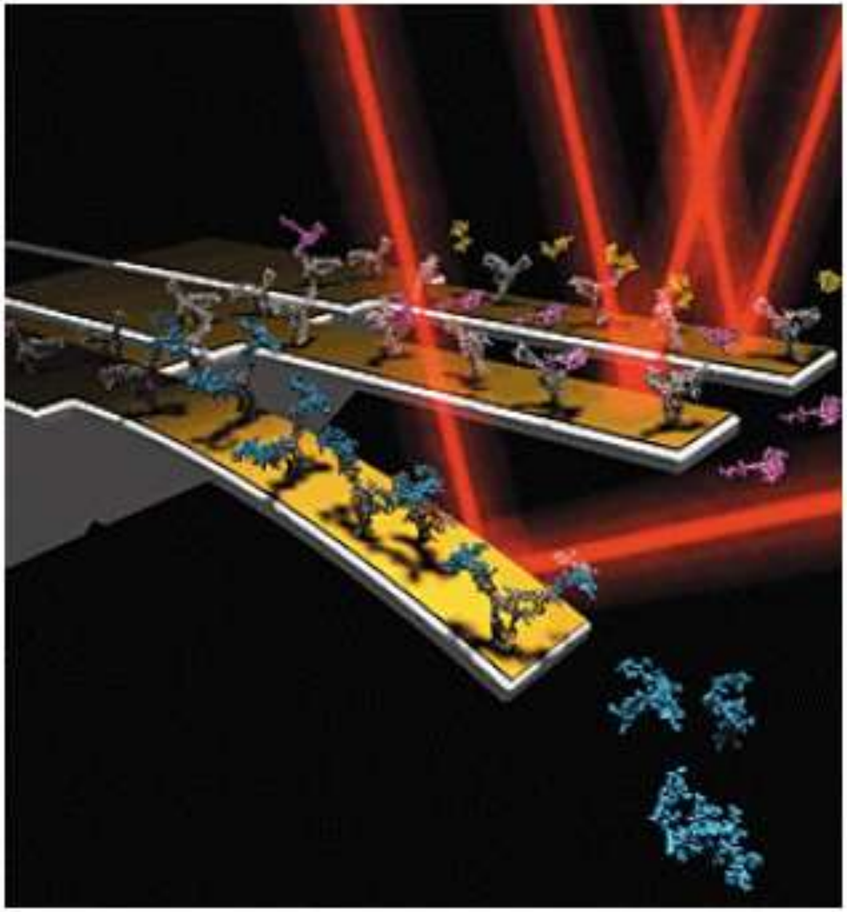
Detection of biomolecules by simple mechanical transduction:

- cantilever surface is covered by receptor layer (functionalization)
- biomolecular interaction between receptor and target molecules (molecular recognition)
- interaction between adsorbed molecules induces surface stress change
  $\Rightarrow$ bending of cantilever

Cantilever Nanosensors

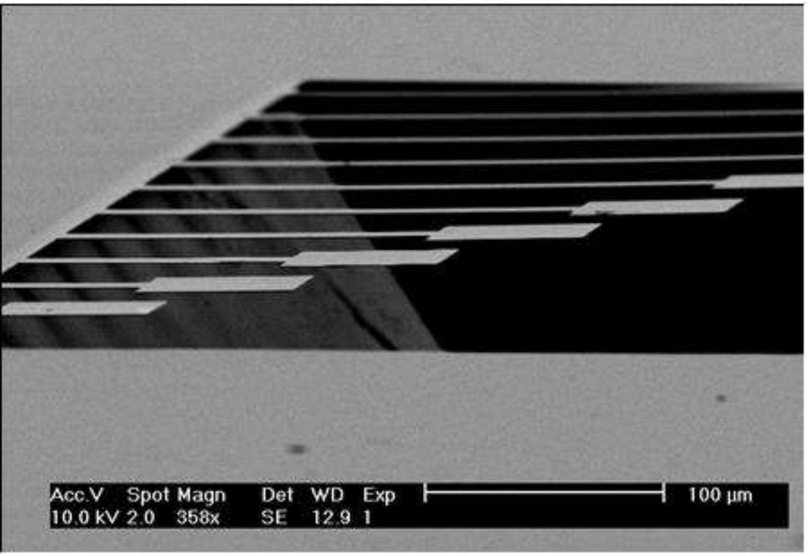


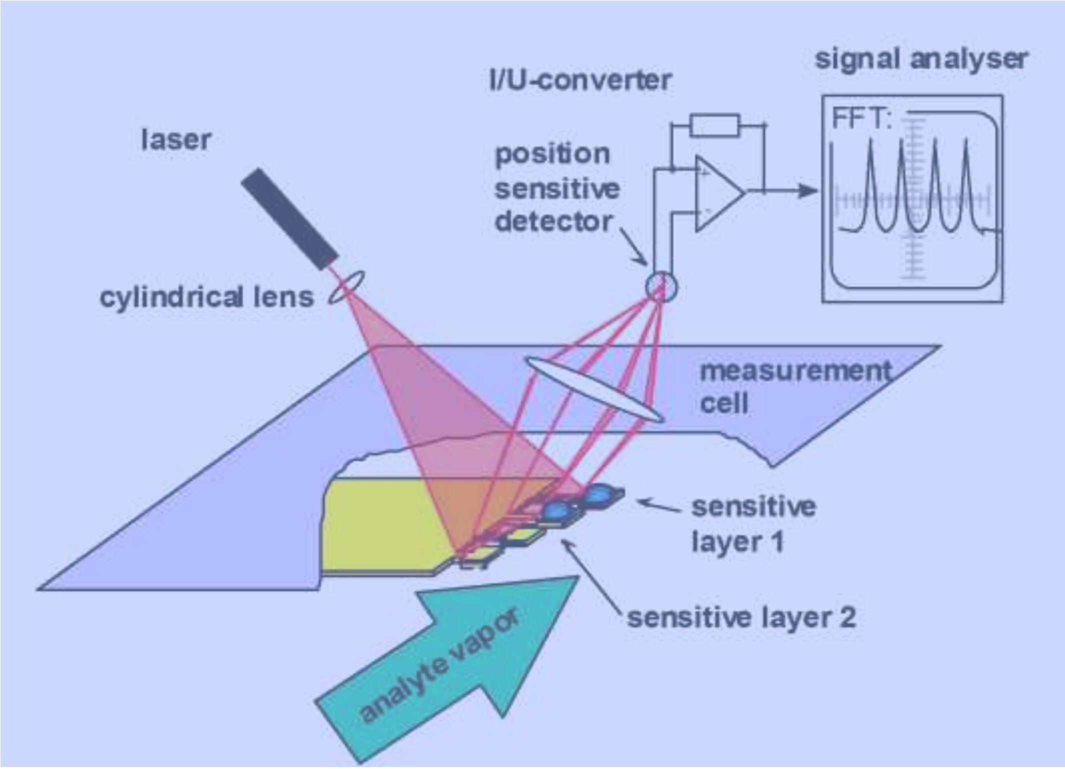




Wu, G.; Thundat, T.; Majumdar, A. et al. *Nat. Biotechnol.* **2001**, *19*, 856-860.
Fritz, J.; Baller, M. K.; Lang, H. P.; Rothuizen, Vettiger, H. P.; Meyer, E.; Guntherodt, H.-J.; Gerber, Ch.; Gimzewski, J. K. *Science*, **2000**, *288*, 316-318.

Optical detection of analyte binding

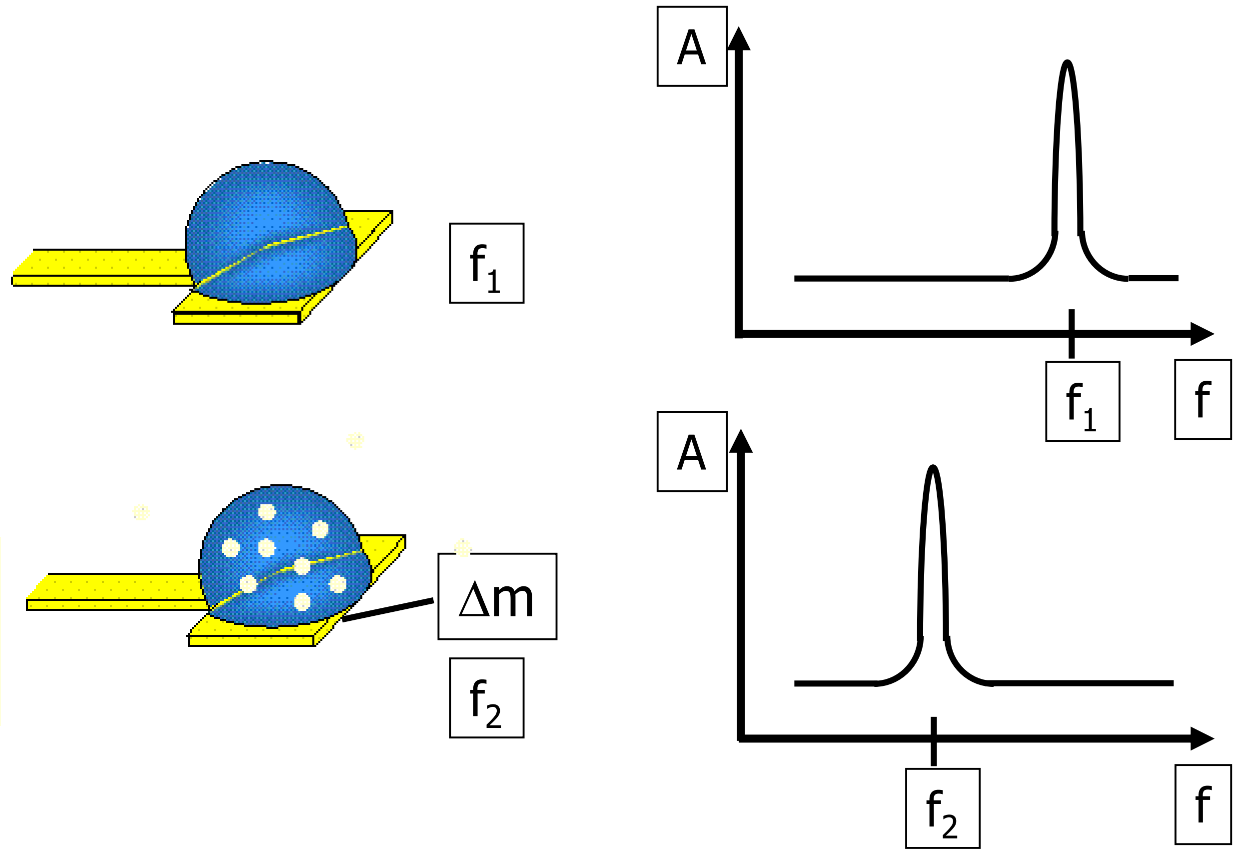




Quantitative assay: resonance frequency mass-sensitive detector

$$f_1 = \frac{1}{2\pi}\sqrt{\frac{k}{m_{eff}}}$$

$$f_2 = \frac{1}{2\pi}\sqrt{\frac{k}{m_{eff} + \Delta m}}$$



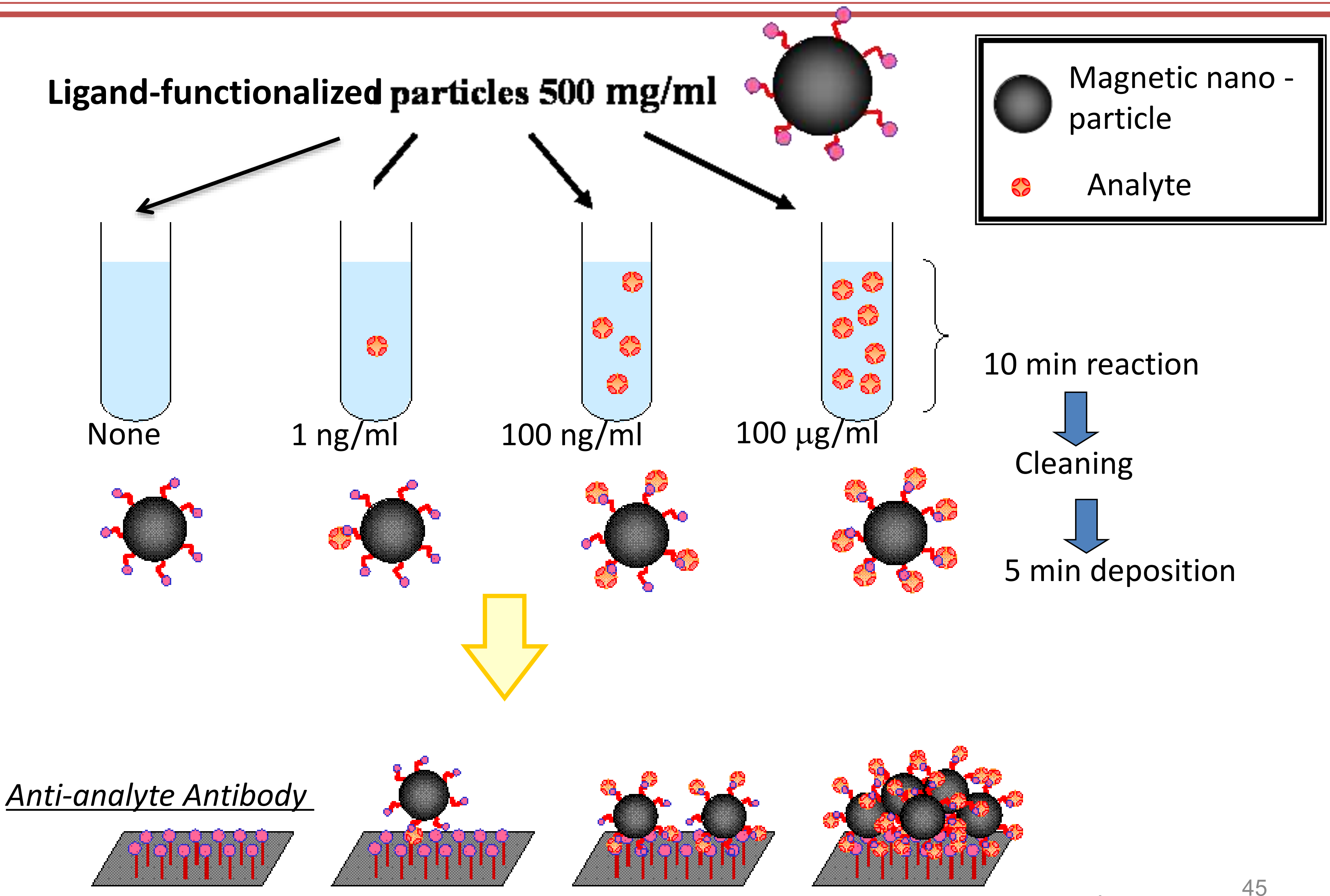
A mass sensitive resonator transforms an additional mass loading into a resonance frequency shift $\Rightarrow$ mass sensor

B. Kim et al, Institut für Angewandte Physik - Universität Tübingen

Magnetic sensing

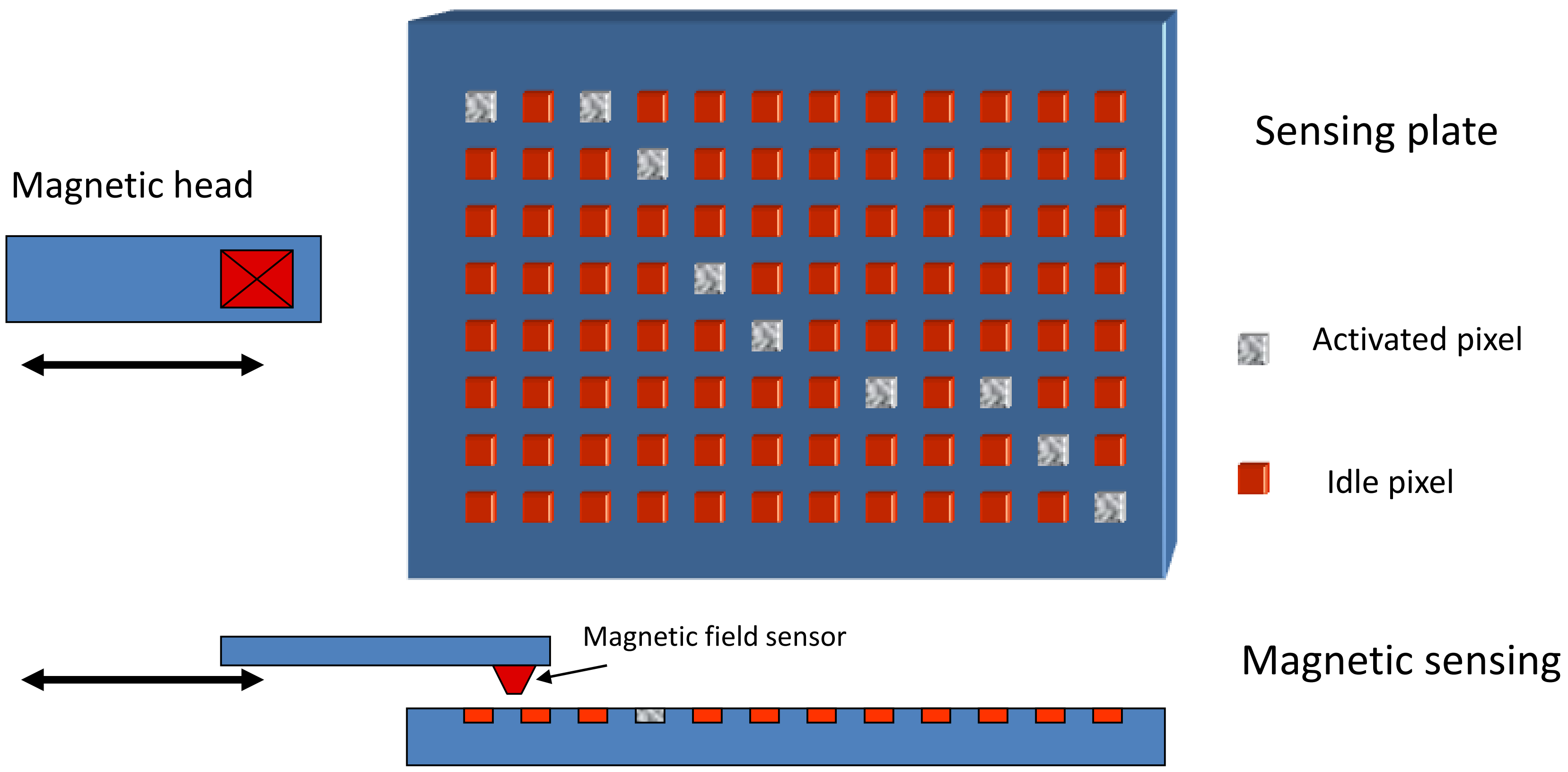
- magnetic fields to sense magnetic nano-particles that have been attached to biological molecules.

Stabilization of Magnetic Particles with various Streptavidin Conc.

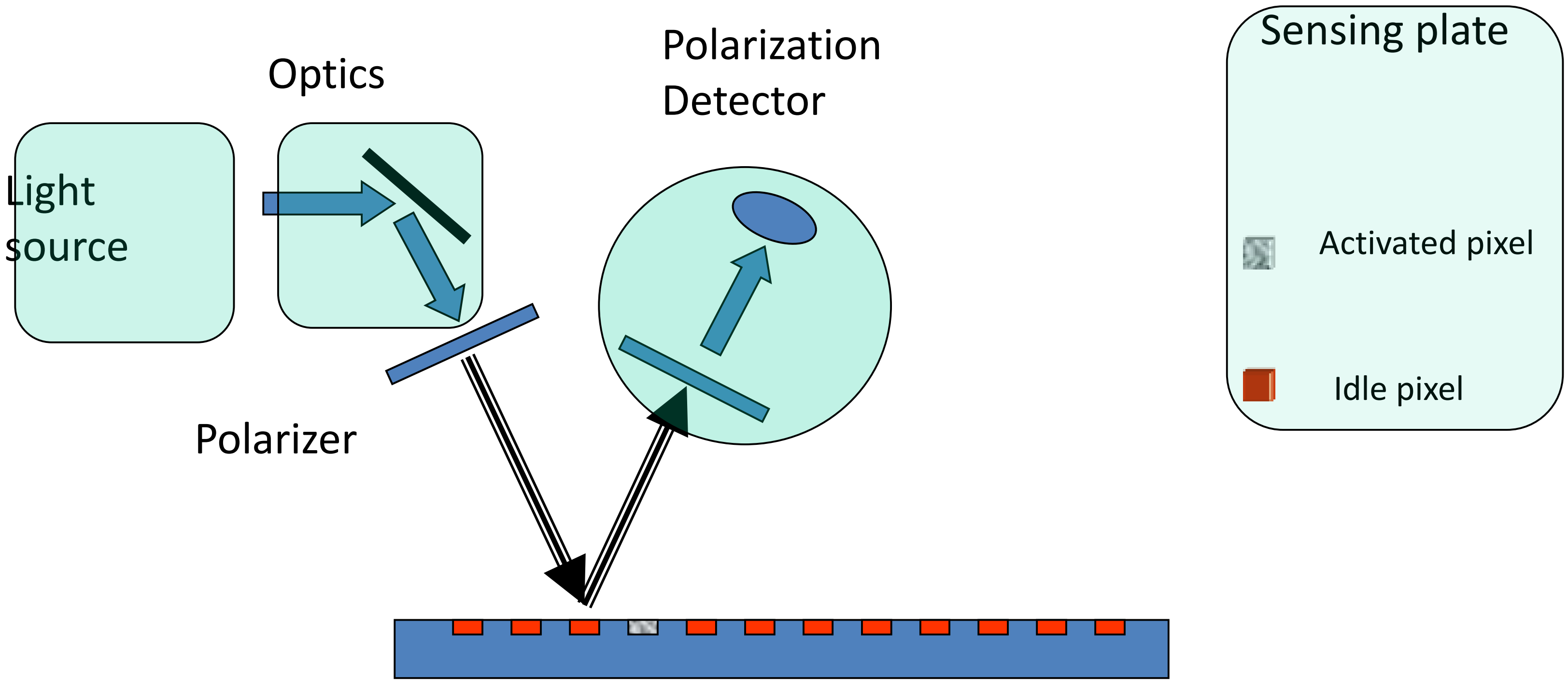


T. Osaka, 2006

Magnetic sensing



Magnetic-optic sensing



Applications of Nanobiosensors

Biological Applications

- DNA Sensors; Genetic monitoring, disease
- Immunosensors; HIV, Hepatitis,other viral diseas, drug testing, environmental monitoring…
- Cell-based Sensors; functional sensors, drug testing…
- Point-of-care sensors; blood, urine, electrolytes, gases, steroids, drugs, hormones, proteins, other…
- Bacteria Sensors; (E-coli, streptococcus, other): food industry, medicine, environmental, other.
- Enzyme sensors; diabetics, drug testing, other.

Environmental Applications

- Detection of environmental pollution and toxicity
- Agricultural monitoring
- Ground water screening
- Ocean monitoring