Contribution of nanotechnology in biosensor field

Nanotechnology will enable us to design sensors that are

- much smaller
- less power comsuming
- more sensitive

Biosensors which allow:

- integrated
- high-throughput measurement

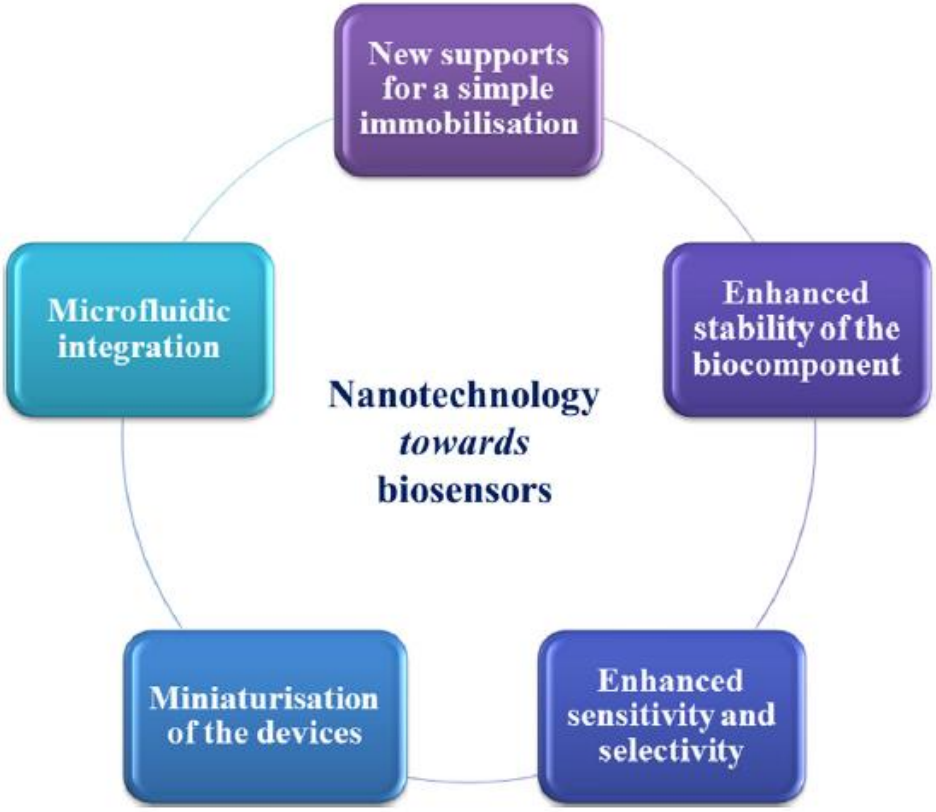
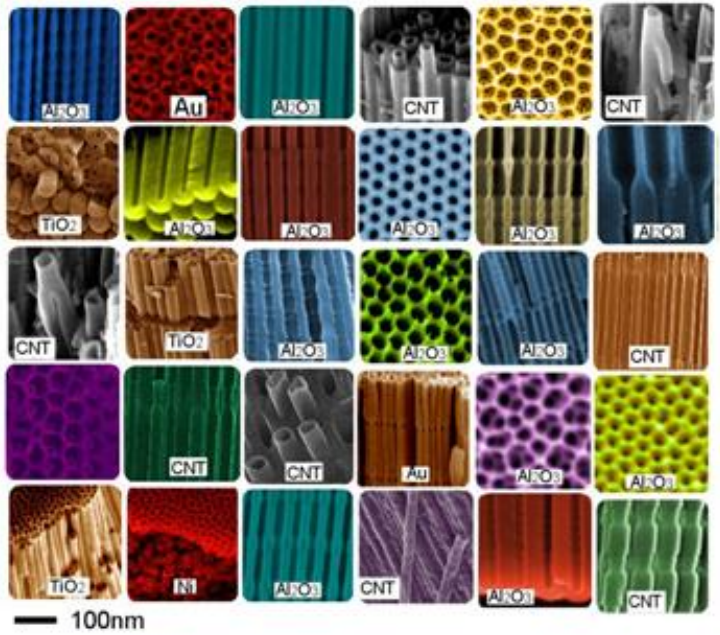


Fig. 1. Nanotechnology contributions for the development of biosensors with commercial promises.

Nanomaterials

- Characteristic dimension at the range of 1–100nm
- Because of extremely small size and high S/V ratio, the physicochemical properties of nanoparticles are quite different to those of the bulk materials:
  - Optical
  - Magnetic
  - Electrochemical
  - Mechanical
  - Catalytic properties



- As surface to volume ratio increases:
  - A greater amount of a substance comes in contact with surrounding material
  - This results in better catalysts, since a greater proportion of the material is exposed for potential reaction

sides = 3
surface = $3^2 \times 6 = 54$
volume = $3^3 = 27$

surface/volume = 2

sides = 2
surface = $2^2 \times 6 = 24$
volume = $2^3 = 3$

surface/volume = 3

sides = 1
surface = $1^2 \times 6 = 6$
volume = $1^3 = 1$

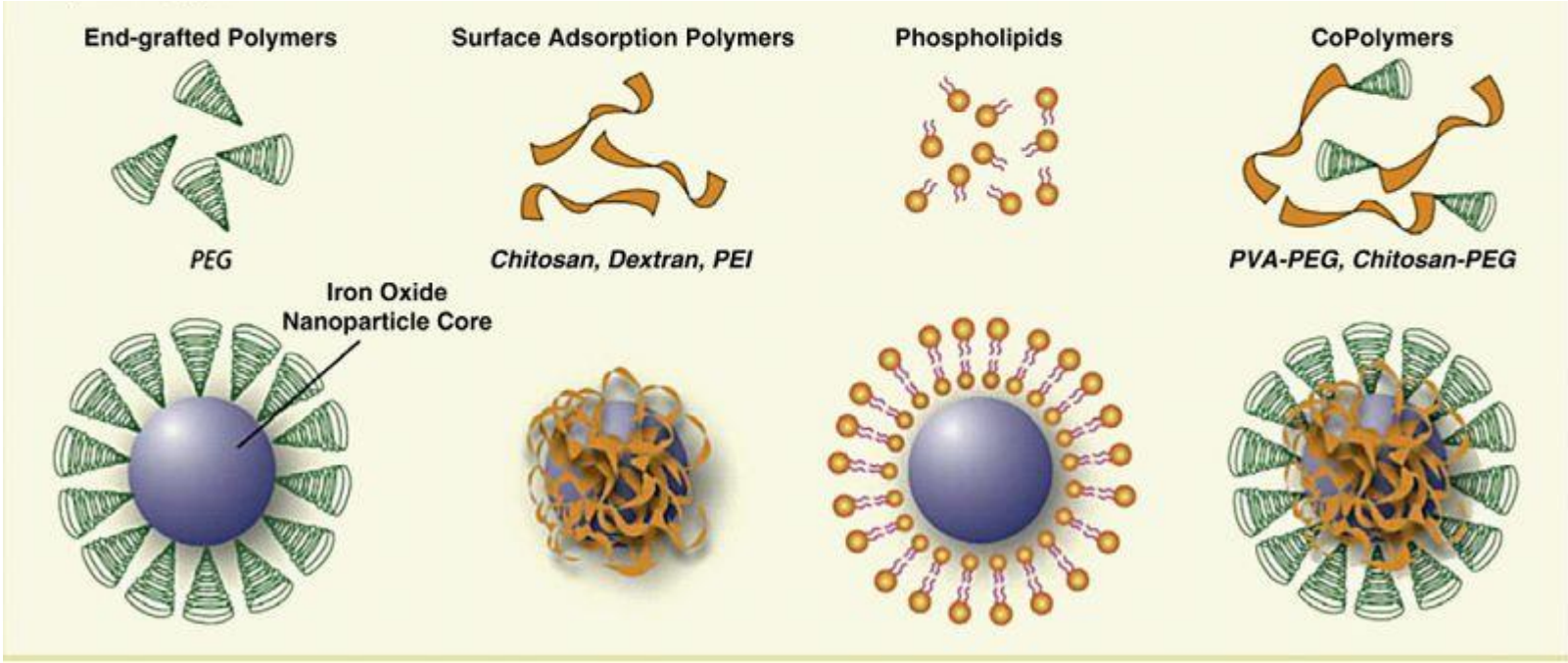
surface/volume = 6

Nano in Biosensors

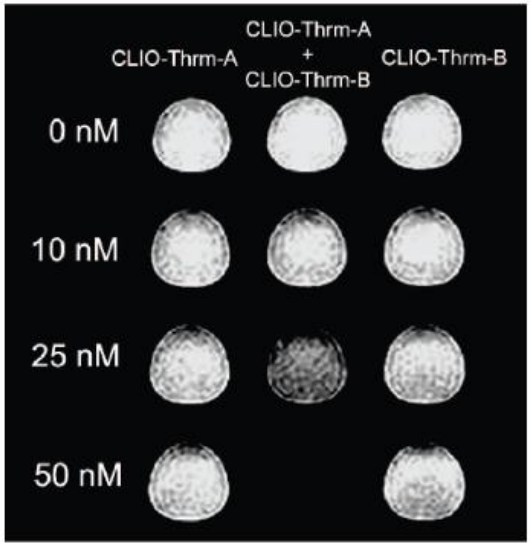
- Carbon Nanotube-Electrodes
- Metallic Nanoparticles-sensor probes and electrodes;
- Magnetic Particles-sensor probes;
- Nanowires-FET sensing system,
- Quantum dot (AsSe, CdSe, etc.)
- Nano/Micro-Electro-Mechanical Systems (N/MEMS) for Sensor Fabrication
- BioMEMS/BioNEMS, Lab-on-Chip, Microfluidic System,
- Sensor Arrays
- etc

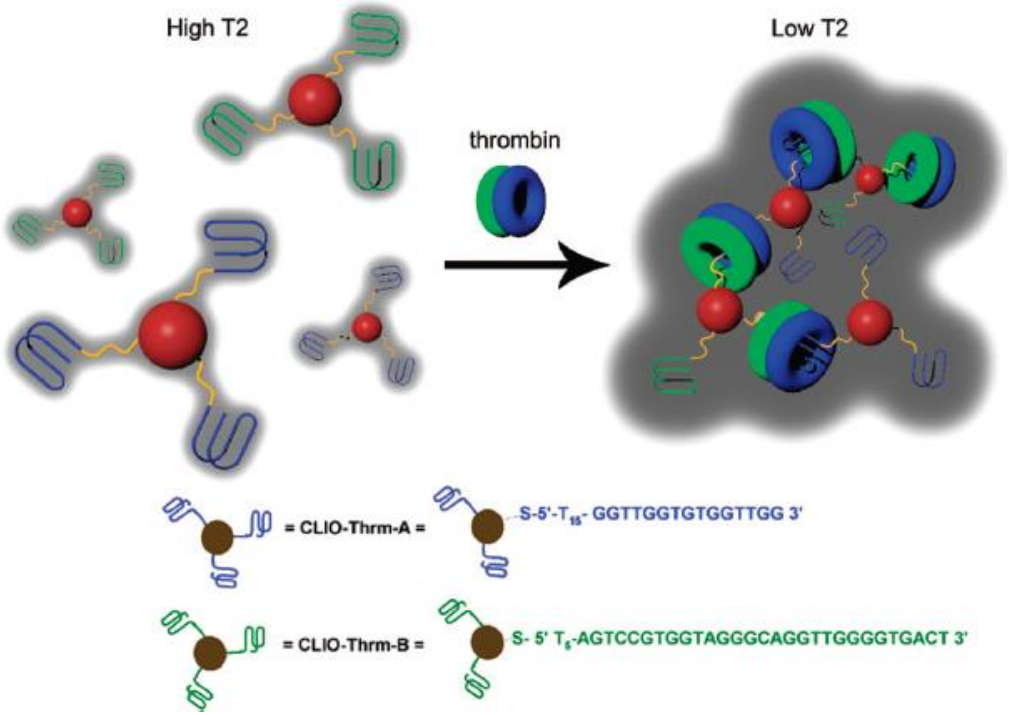
Superparamagnetic NPs

- Inorganic based particles having an iron oxide core coated by either inorganic materials (silica, gold) or organic materials like phospholipids, fatty acids, polysaccharides, peptides, polymers
- Unique property: their induced magnetization, without retaining residual magnetism after the removal of the field.



Superparamagnetic NPs in MRI





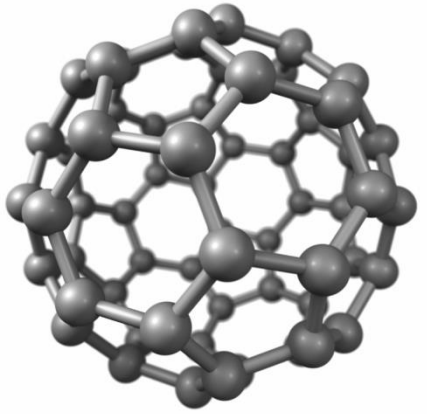
- Xlinked dextran coated spmagnetic NPs (CLIO)
- Trombin detection by aptamer functionalized CLIO in serum

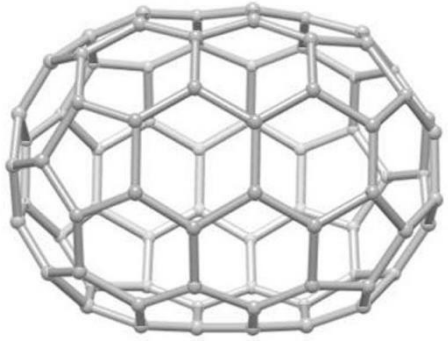
Aggregates → change in magnetic relaxation properties of nearby water protons → low relaxation time (T2) → decrease in brightness

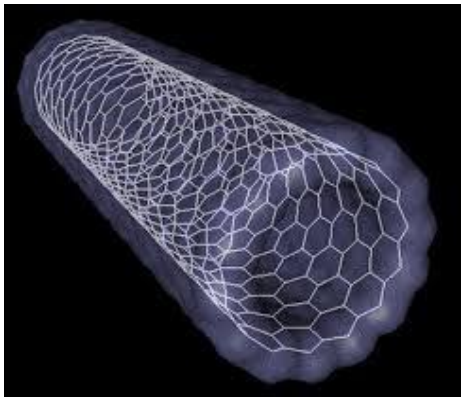
Fullerenes (1996 Nobel Prize in Chemistry)

They are made exclusively of carbon and they exist in different forms such as

- hollow spheres (buckyballs; C60)
- ellipsoids
- tubes (carbon nanotubes (CNTs))







Fullerenes: CNTs

CNTs:

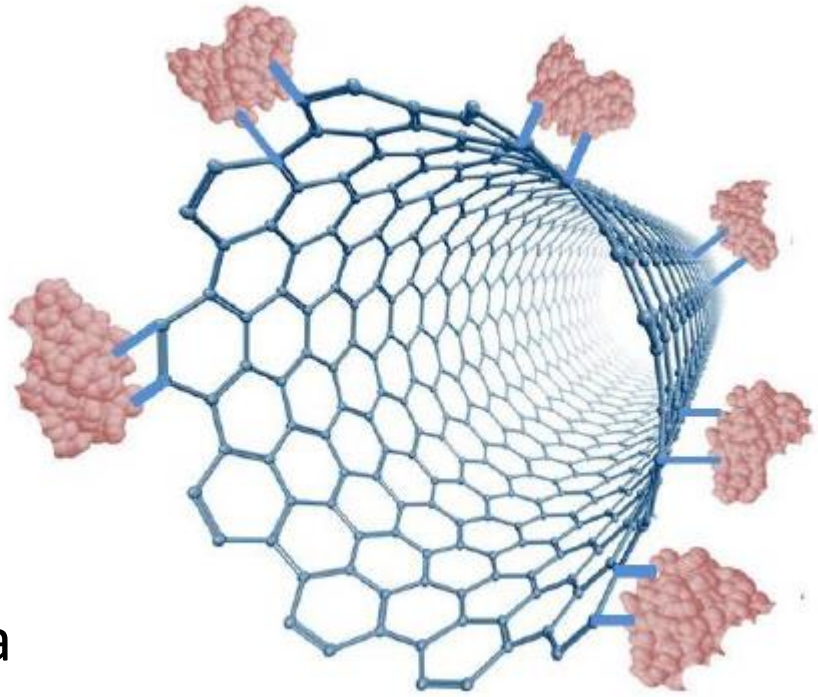
- Exceptional strength (tensile strength ca. 50x more than steel)
- Excellent electrical properties (current carrying capacity is 1,000 x greater than that of copper wire) (especially SWNT preferred)
- Excellent conductors of heat
- Chemical stability
- Hydrophobicity (☹ spontaneous coagulation and lack of solubility in aqueous media)

These properties are favorable for acting as solid contacts in electrochemical sensors to obtain:

- higher sensitivities
- faster response times
- lower detection limits

Fullerenes: CNTs

- For example, in glucose sensors CNT usage effectively lowers the oxidation overpotential for the indirect detection of glucose by H_2O_2 oxidation and eliminate interferance problem.
- Biofunctionalization of CNTs gives additional selectivity of detection on the CNTs.
- 3-D shape and large surface area of CNTs allow large enzyme loading that is accessible within a very thin layer

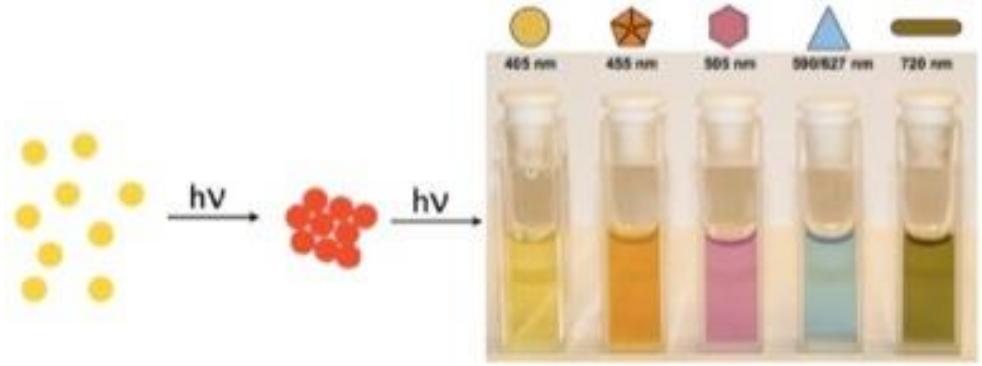


Silver Nanoparticle (Ag-NPs)

- To enhance electrochemical signal:

Ag NPs on MWCNT →enhanced electrocatalycal activity detection for e.g. Glucose (Chen, 2012)

- Colorimetric detection

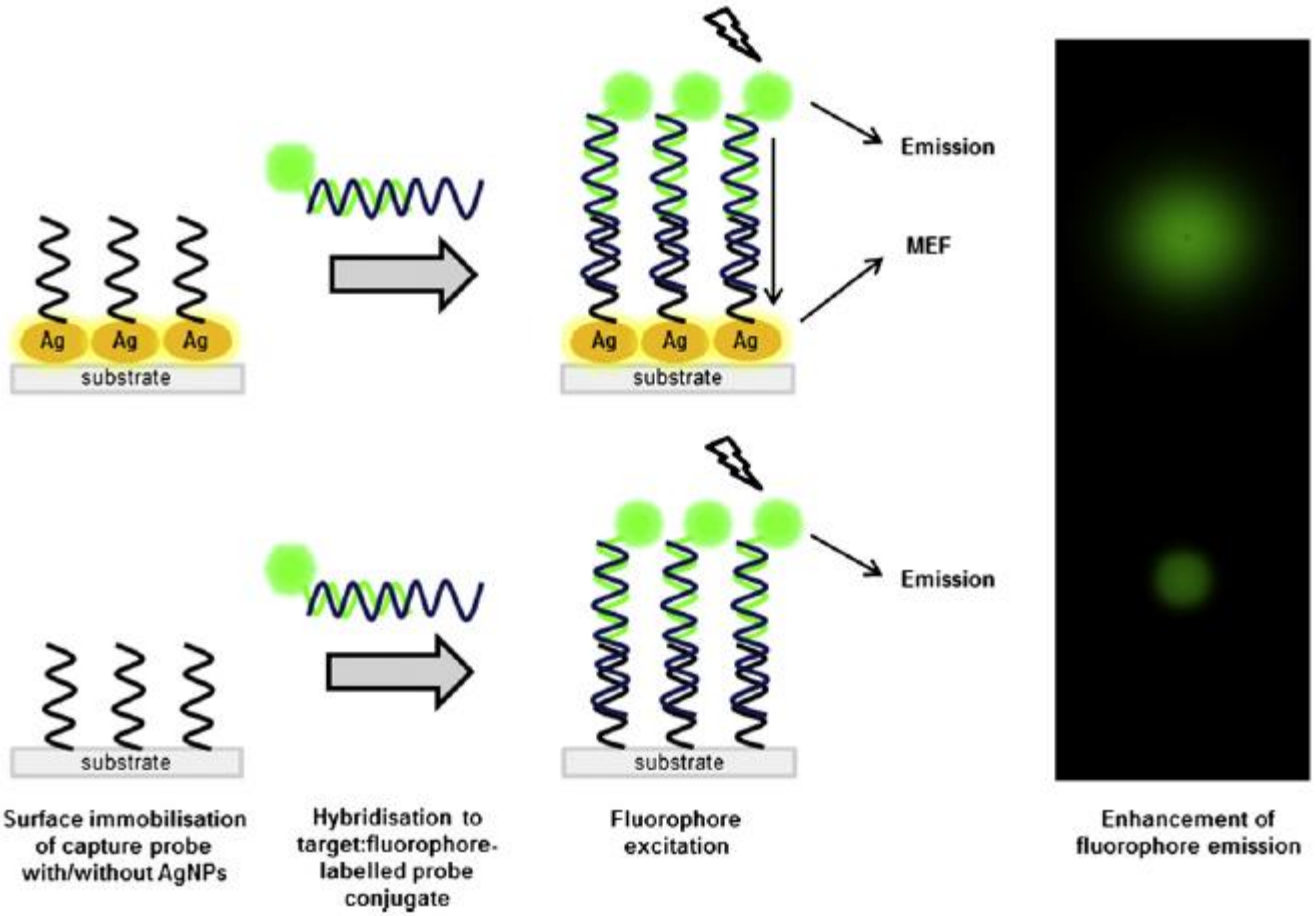


Stamplecoskie and Scaiano, 2010

Ag NPs-Signal Enhancement

Larguinho, 2012

- Metal-enhanced fluorescence signal enhancement may be achieved by deposition of AgNPs onto a surface, before probe immobilisation.



Gold NPs (AuNPs)

(*Li, 2010*)

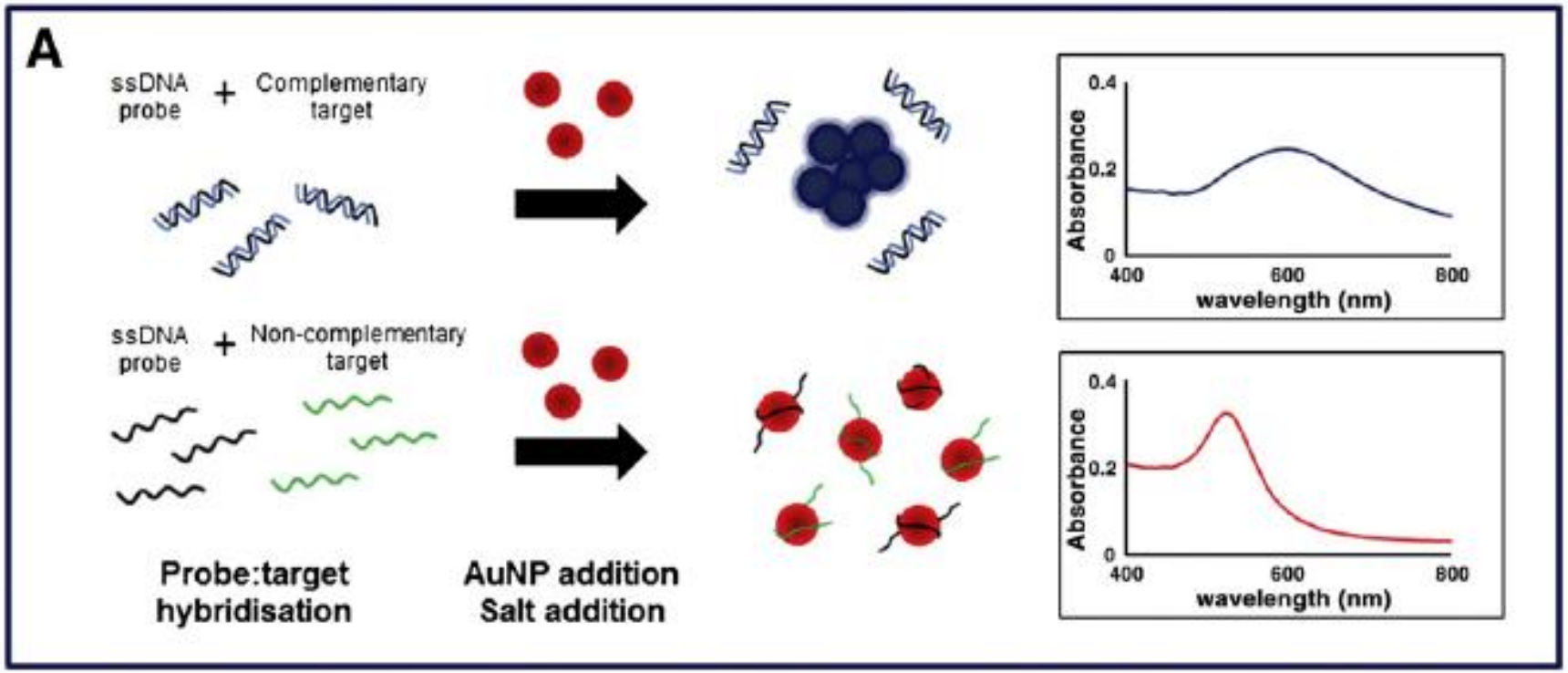
Table 1. Different functions of GNPs in biosensor systems

Types of biosensors	Principle of detection	Functions of GNPs	Properties used	Sensor advantages	Typical examples
Optical biosensor	Changes in optical properties	Enhancement of refractive index changes	large dielectric constant, high density, high molecular weight	Improved sensitivity	DNA sensor with GNPs responses 1000 times more sensitive than without [34].
		Enhancement of electron transfer	Conductivity, quantum dimension	Improved sensitivity	Electron transfer rate of 5000 per second with GNPs, while 700 per second without GNPs [4]
Electrochemical biosensor	Changes in electrical characteristics	Immobilization platform	Biocompatibility, large surface area	Improved sensitivity and stability	Glucose biosensor with GNPs achieves detection limit of 0.18 μM [82].
		Catalysis of reactions	High surface energy, interface-dominated properties	Improved sensitivity and selectivity	NADH sensor based on GNPs shows 780 mV overpotential decrease without any electron transfer mediators [114].
Piezoelectric biosensor	Changes in mass	Biomolecule Immobilization, amplification of mass change	Biocompatibility, high density, Large surface-to-volume ratio,	Improved sensitivity	DNA sensor using GNPs as amplification tags with detection limit of 10^{-16} mol/L [134]

Gold NPs

Larguinho, 2012

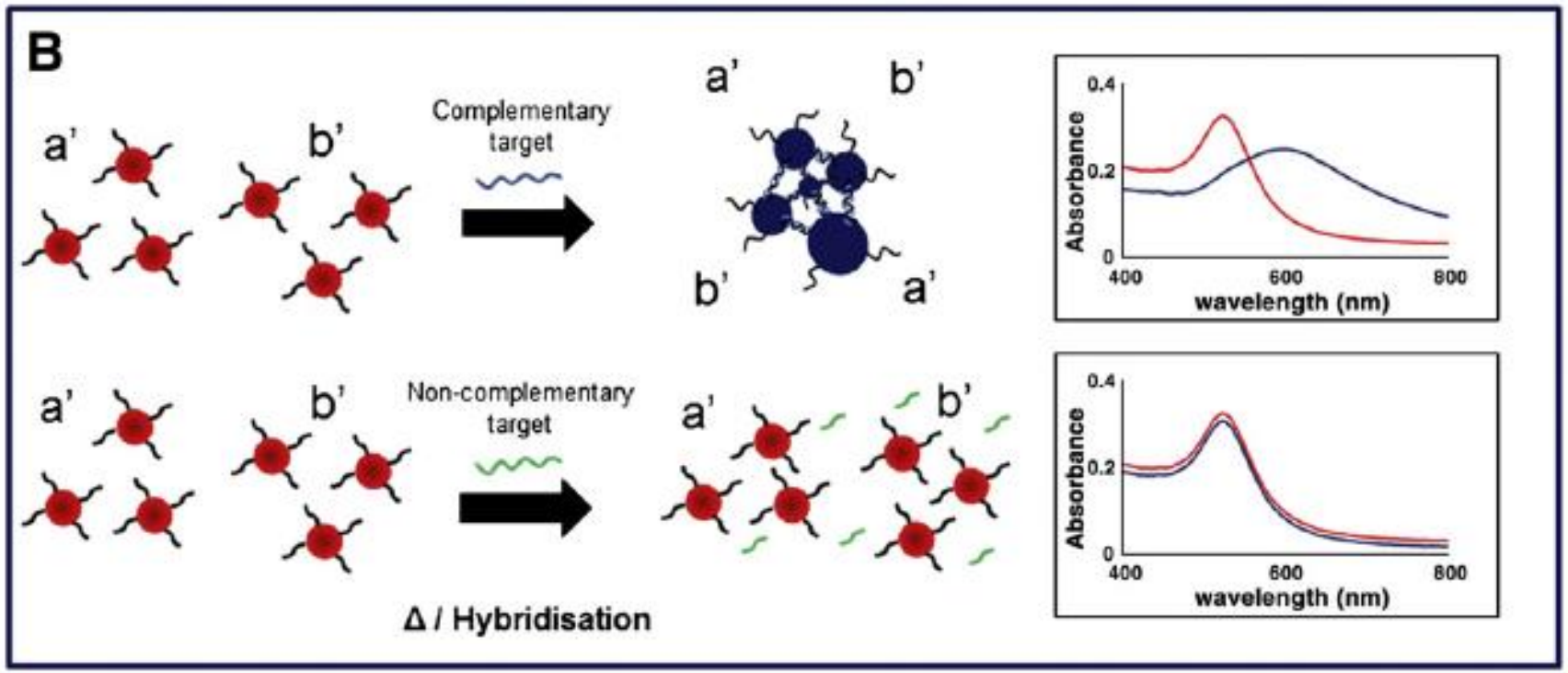
Colorimetric assay based on naked gold nanoparticles (AuNPs) – the presence of ssDNA stabilizes AuNPs against salt induced aggregation, whereas double-strand DNA does not.



Gold NPs

Larguinho, 2012

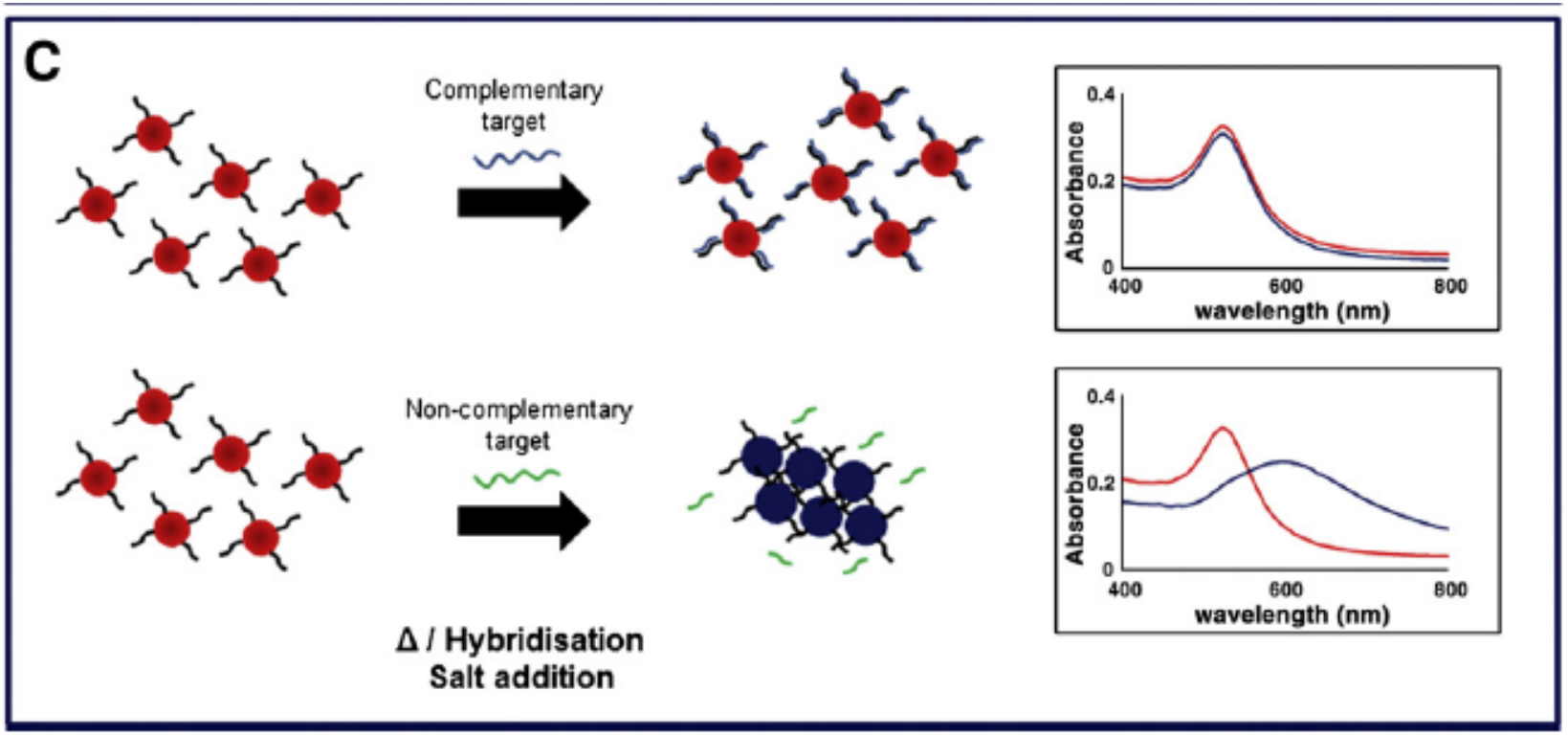
Cross-linking hybridisation assay — hybridisation brings both Au-nanoprobes in close vicinity leading to aggregation and colour change



Gold NPs

Larguinho, 2012

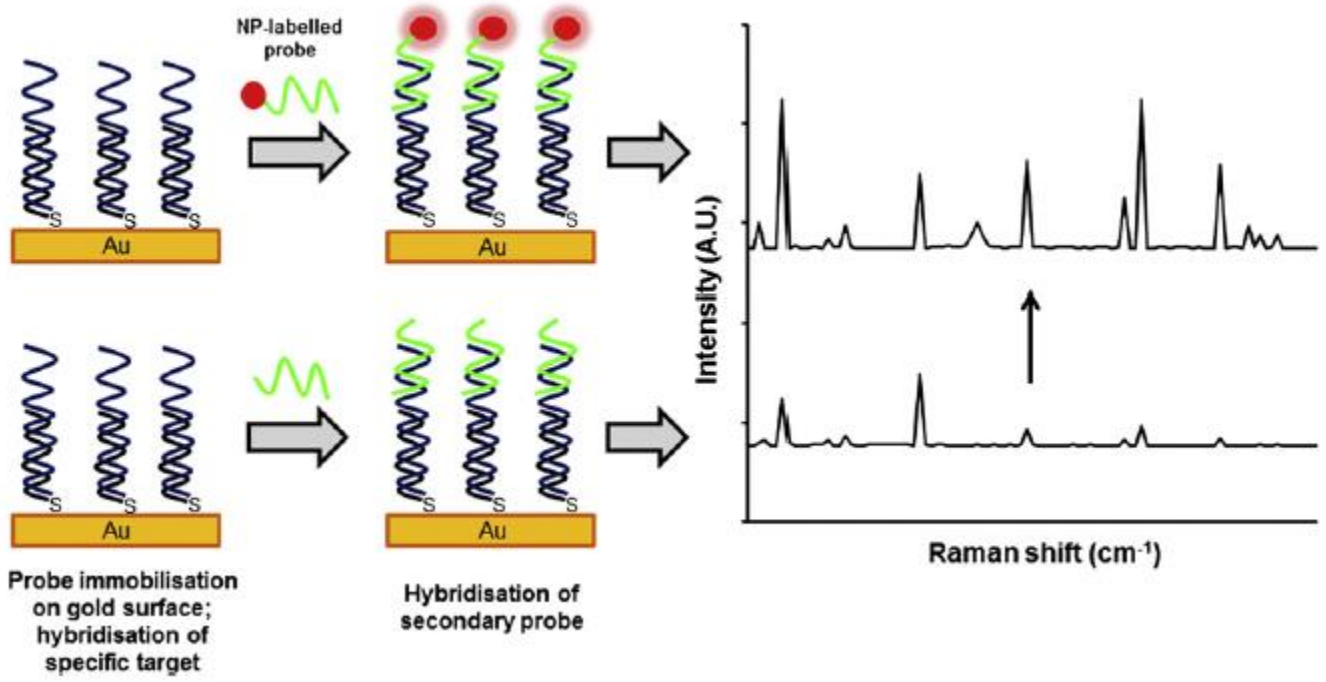
Non-cross-linking hybridisation assay - an increase in ionic strength causes Au-nanoprobe aggregation (blue solution), which is prevented by the presence of the complementary target.



Gold NPs-Signal Enhancement

Larguinho, 2012

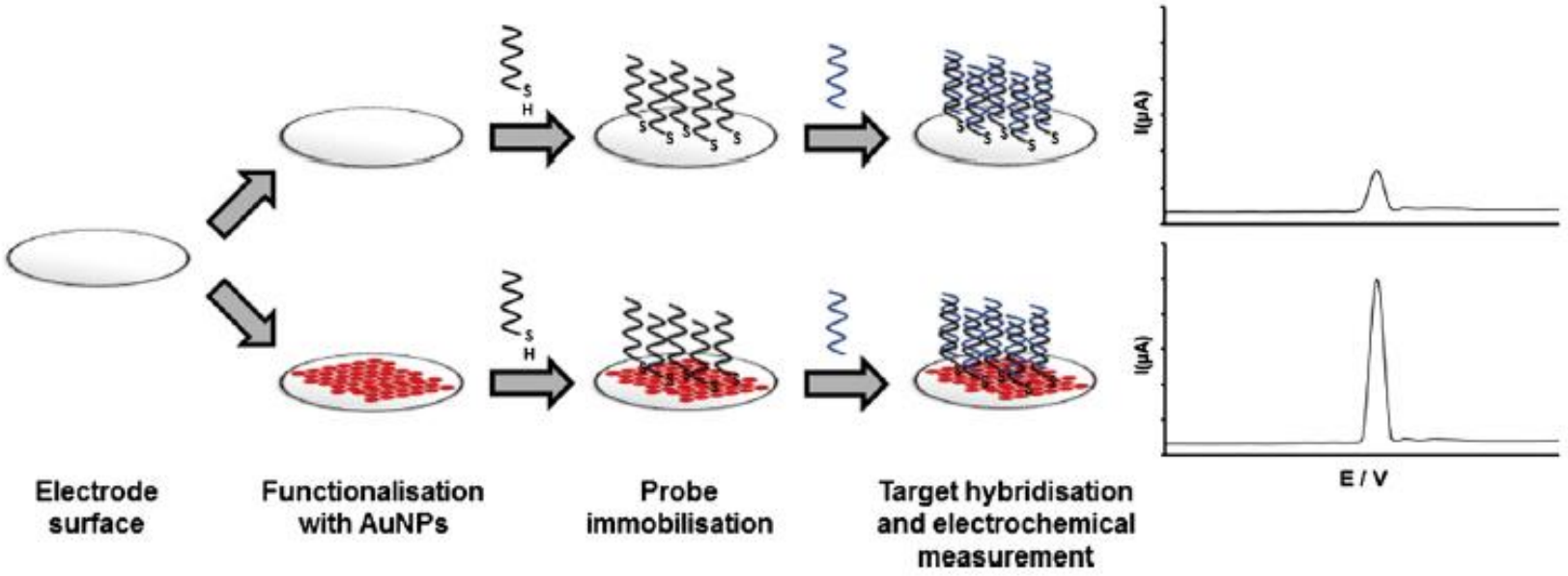
Signal enhancement on a surface-enhanced Raman scattering (SERS) spectrum using noble metal nanoparticles.



Gold NPs-Signal Enhancement

Larguinho, 2012

Signal enhancement in electrochemisty: Different types of electrodes (e.g. gold, glassy carbon, etc.) have been combined with AuNP-based protocols with or without silver enhancement or electrode surface modification



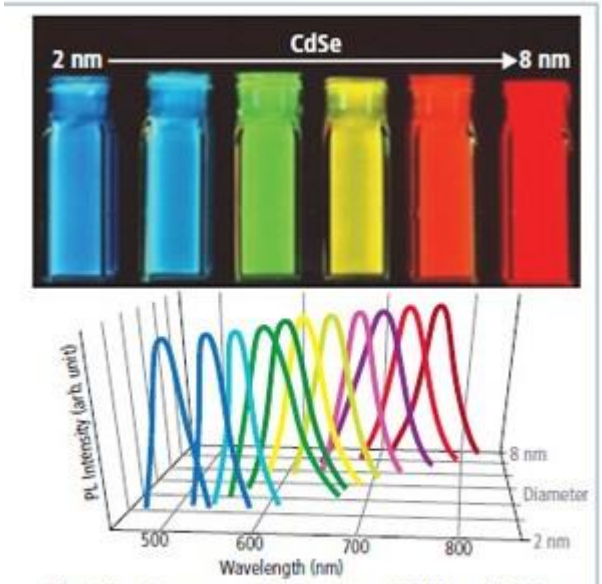
Quantum dots (QDs)

QDs or nanocrystals are semiconductor nanoparticles (1-20 nm) that can emit light in all colours of the spectrum depending on their size

- High quantum yield
- Size-tuneable fluorescence spectra
- Broad absorption spectra BUT narrow and symmetric emission bands (30–50nm),
- High resistance to photobleaching and chemical degradation

Eg. CdSe/ZnS

- CdSe and CdTe can leach heavy metals into the tissue.
  - A non-heavy metal shell, such as ZnS, is used as a barrier.
- Most QDs are only stable in organic solvents
  - QDs are usually encapsulated in a polymer shell or a micelle to make them soluble in aqueous solvents.



Photoluminescence (PL) Spectra of CdSe and CdTe Quantum Dots

QDs

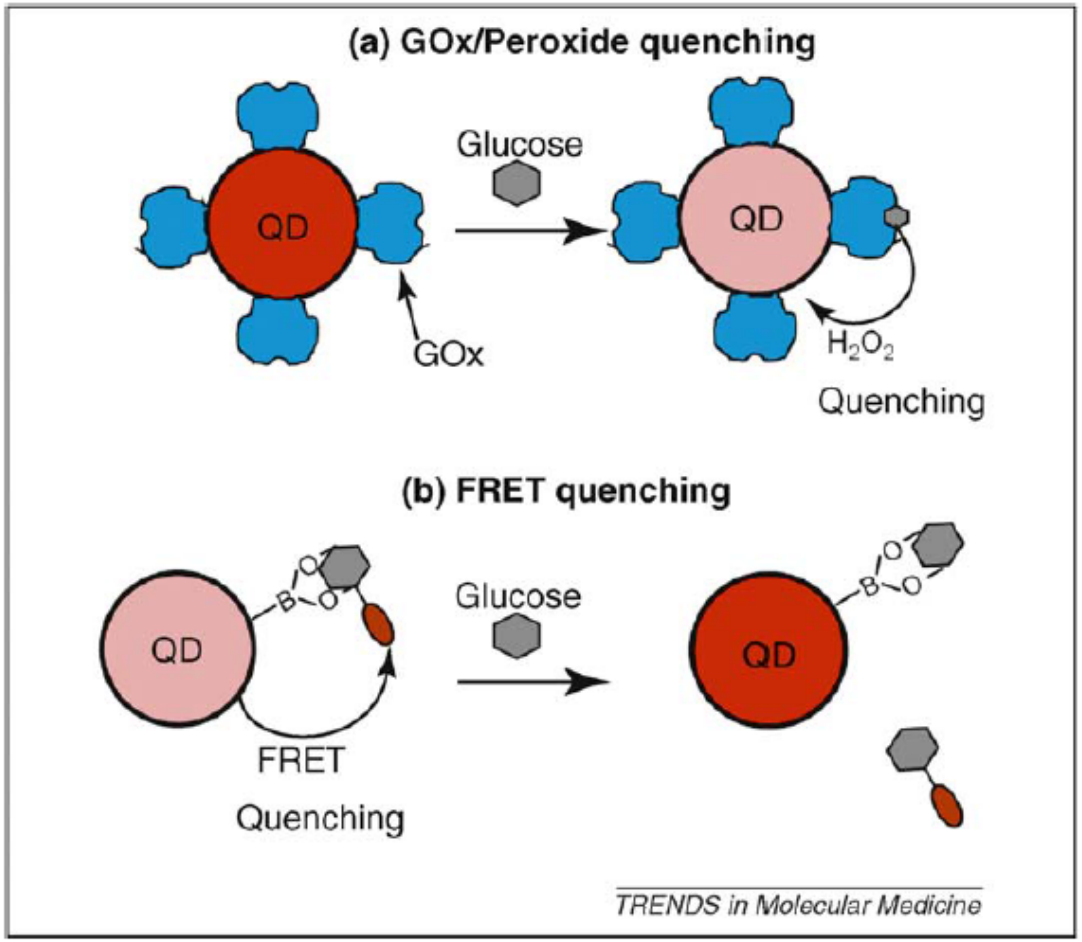
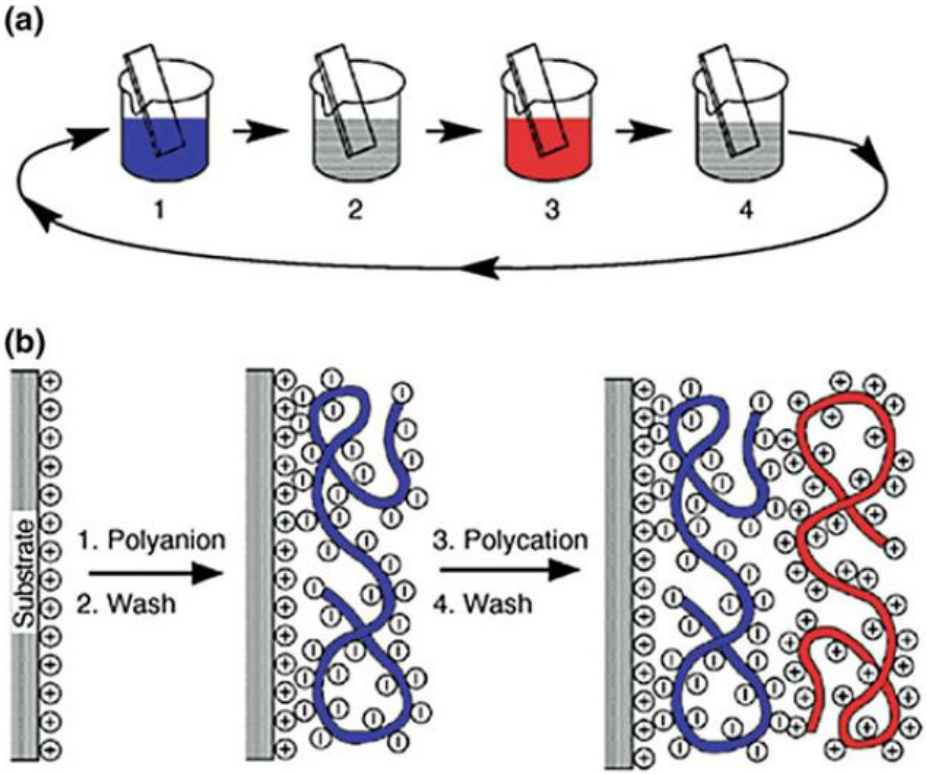


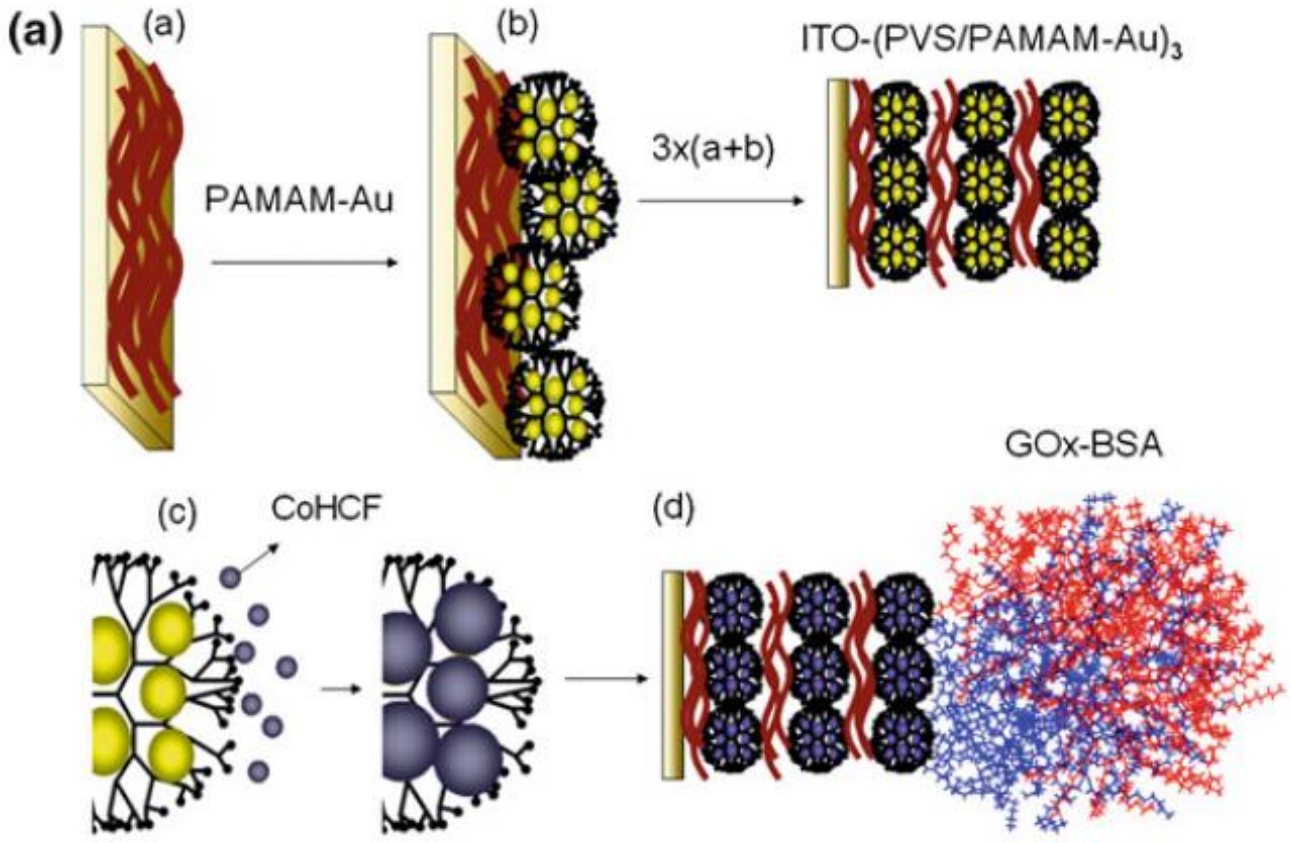
Figure 3. QD-based glucose sensors. The fluorescence or phosphorescence of QDs can be quenched by hydrogen peroxide. Glucose sensors are fabricated through attachment of GOx to a QD reporter (**a**). In the presence of glucose, the enzyme generates hydrogen peroxide, which quenches the QD, providing an optical signal change proportional to glucose concentrations. For examples, see [76–79]. QDs can also be quenched by chromophores through FRET (**b**). Glucose sensors can be fabricated through the attachment of a recognition element to the QD and attaching a FRET quencher to a glucose analog. Without glucose, these molecules are bound together, quenching the QD fluorescence. Glucose displaces the quencher, increasing QD fluorescence. For examples, see [80,81].

Nanostructured Thin Films for Biosensing

Layer-by-Layer (LbL) Assembly: a large range of materials could be incorporated: organic and inorganic materials, hybrids formed by materials at nanoscale and biological components



e.g. LbL: hybrids of PAMAM-AuNPs in multilayer LBL layers to enhance charge transfer in modified electrodes → electroactive nanostructured membranes (ENM)

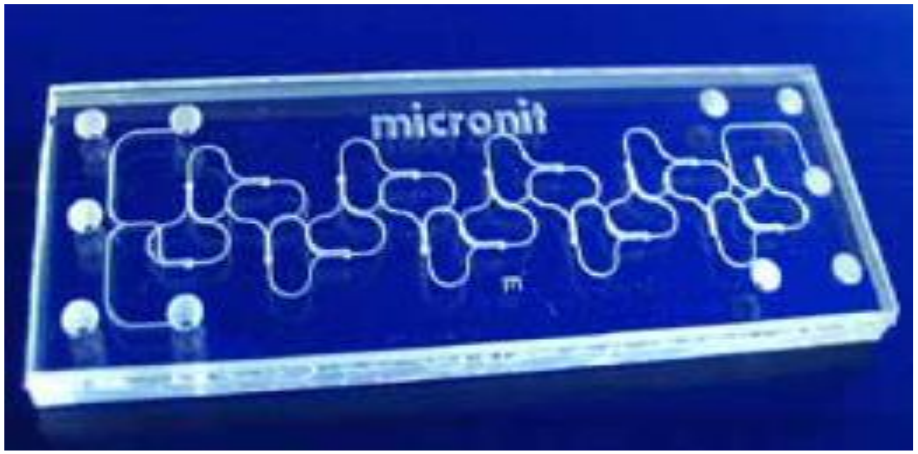


Crespilho, 2006

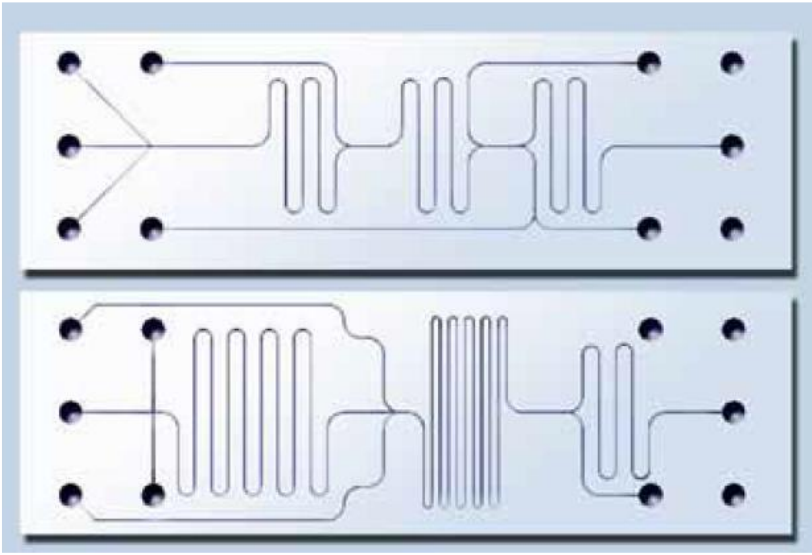
MEMS & BioMEMS

- The fabrication of MEMS (**M**icro**E**lectro**M**echanical **S**ystems) evolved from semiconductor device fabrication, i.e. the basic techniques are deposition of material layers, patterning by photolithography and etching to produce the required shapes
- Poly(dimethylsiloxane) (PDMS) is by far the most popular material for the fabrication of microfluidic devices due in most part to easy fabrication and low cost
- Flow control, mixing, pumping, valves in micro/nano scale !
- BioMEMS (**Bio**medicalMEMS), has emerged as a subset of MEMS devices for applications in biomedical research and medical microdevices:
  - For MicroTotalAnalysis (μTAS) (Lab-On-a-Chip)
  - Embedded in medical devices e.g. stents

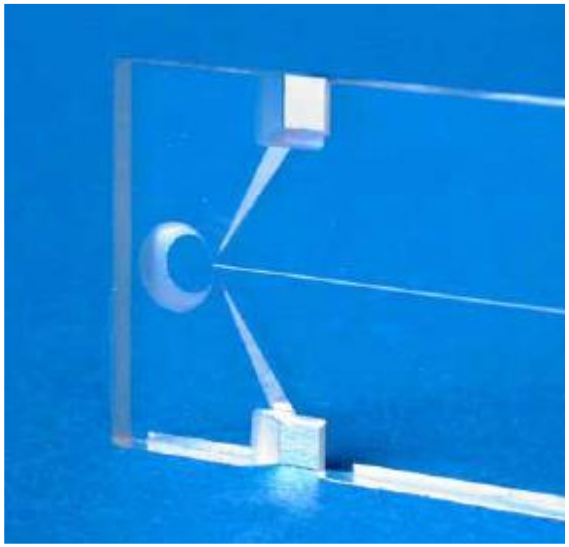
MEMS & BioMEMS



Mixer chip

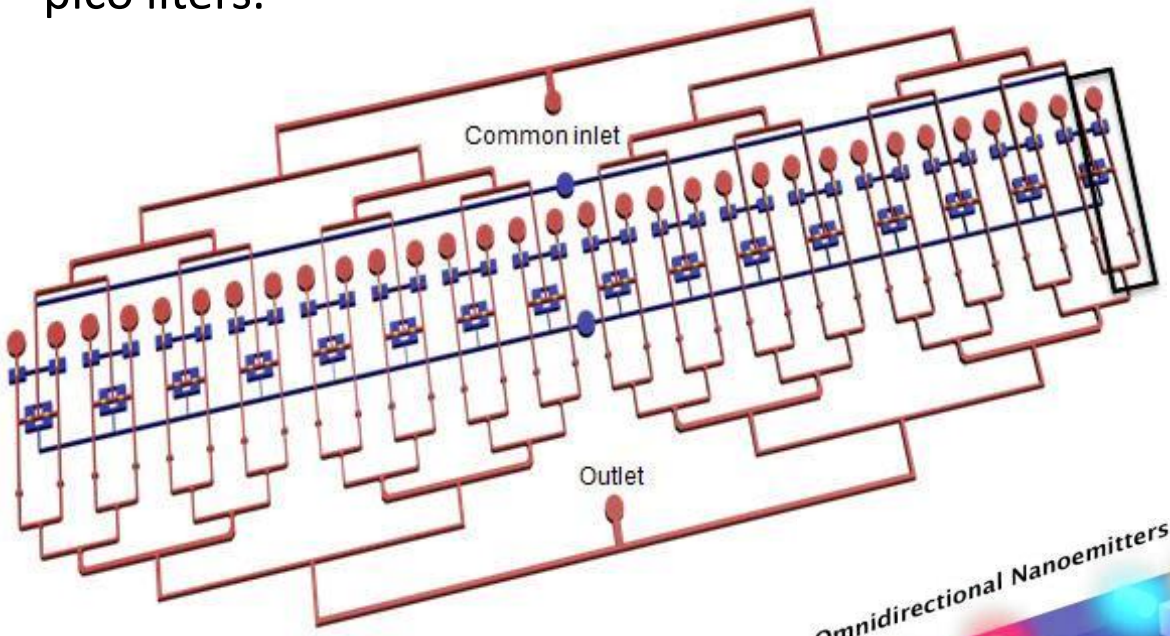


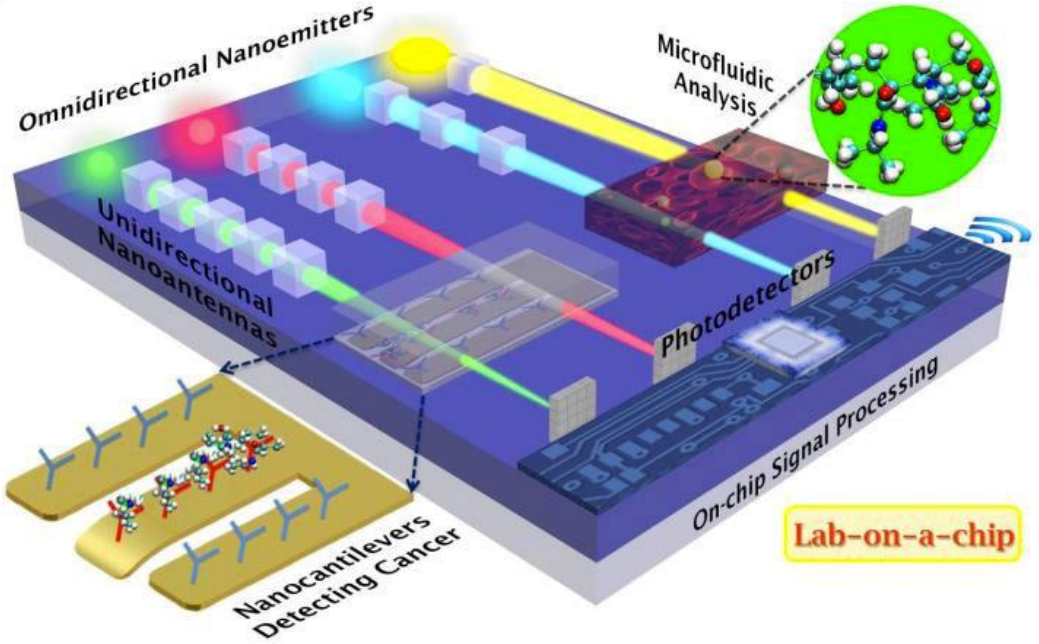
Reactor chip



Integrated electrode

- A lab-on-a-chip (LOC) is a device that integrates one or several laboratory functions on a single chip of only millimeters to a few square centimeters in size
- LOCs deal with the handling of extremely small fluid volumes down to less than pico liters.

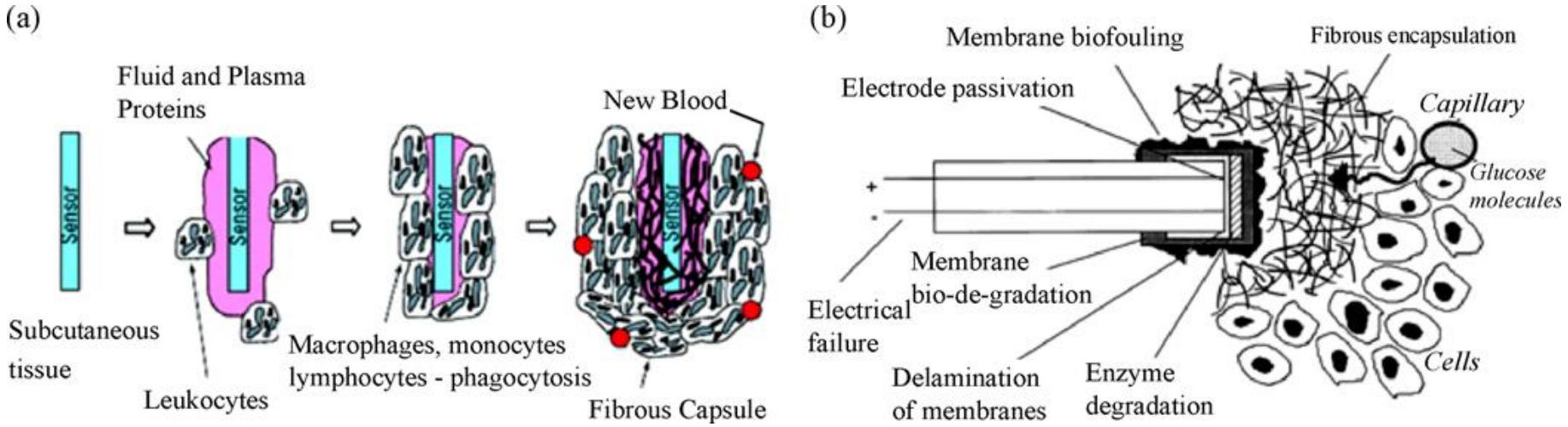




Towards Implantable Biosensors

Vaddiraju, *2010*

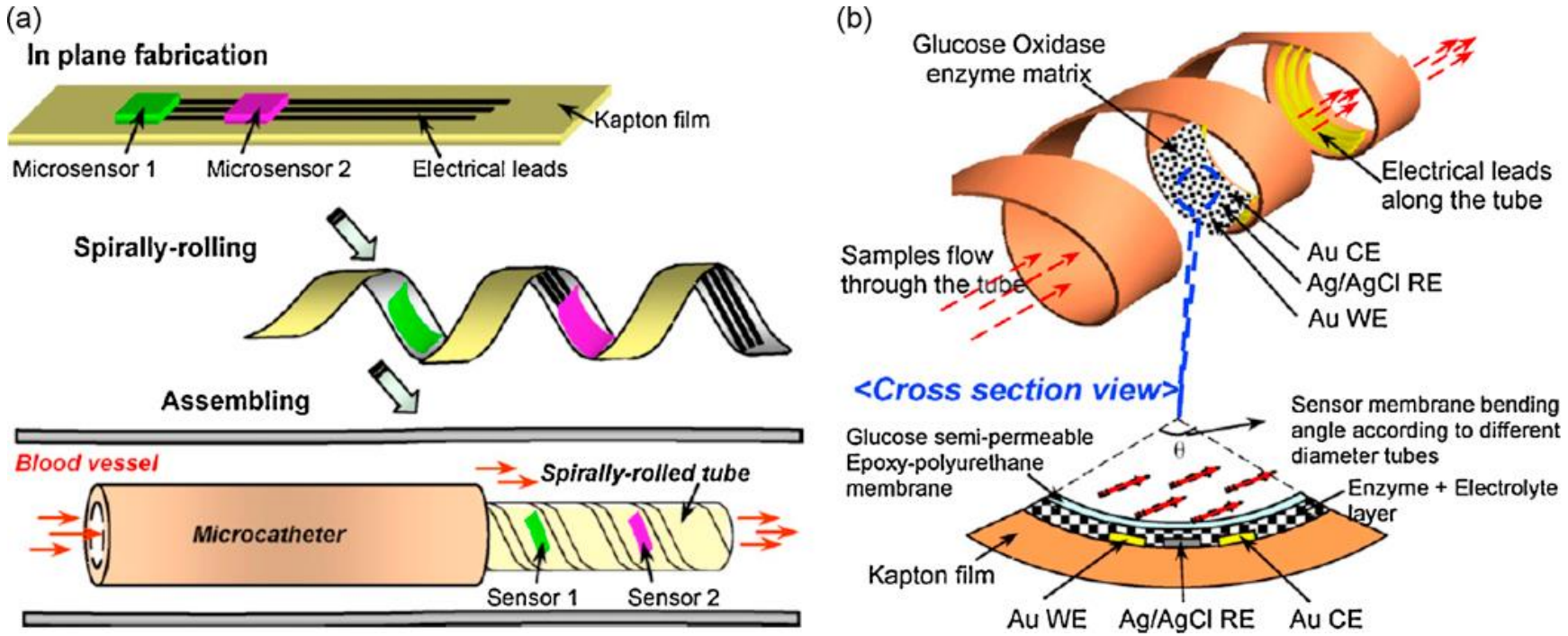
- Implantable biosensors to provide continuous metabolite(s) level(s) monitoring w/o the need for patient intervention and regardless of the patient's physiological state (rest, sleep, exercise etc.)
- Challenges:
  - biofouling
  - foreign body response
  - sensor drifts
  - the response is subject to changes from variations in the local oxygen concentration
  - toxicity of nanomaterials



Towards Implantable Biosensors

Vaddiraju, *2010*

(a) Illustration of the proof-of-concept BioMEMS sensors based on **polymeric substrates** rolled up to form a catheter, wherein the sensing element is located on the inner walls of the rolled-up catheter. (b) Structure and working principle of glucose sensor



Towards Implantable Biosensors

Hoeben, 2008

Au nanoelectrodes with dimensions down to ca. 70 x 70 nm, successfully measured a distinct catalytic response from less than 50 enzymes

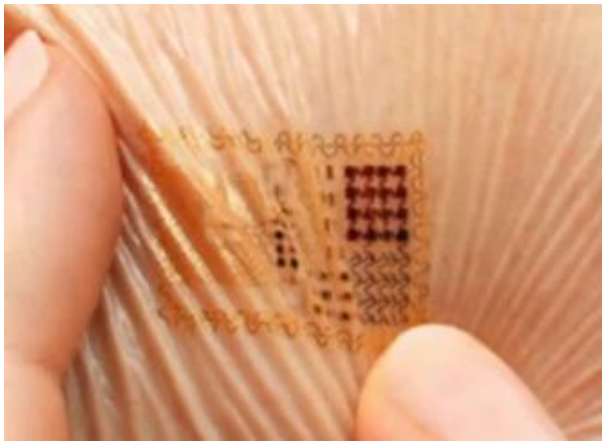
☹ when an electrode with nanometer dimensions is used, various types of noises can affect the measurements and compromise the interpretation of the results

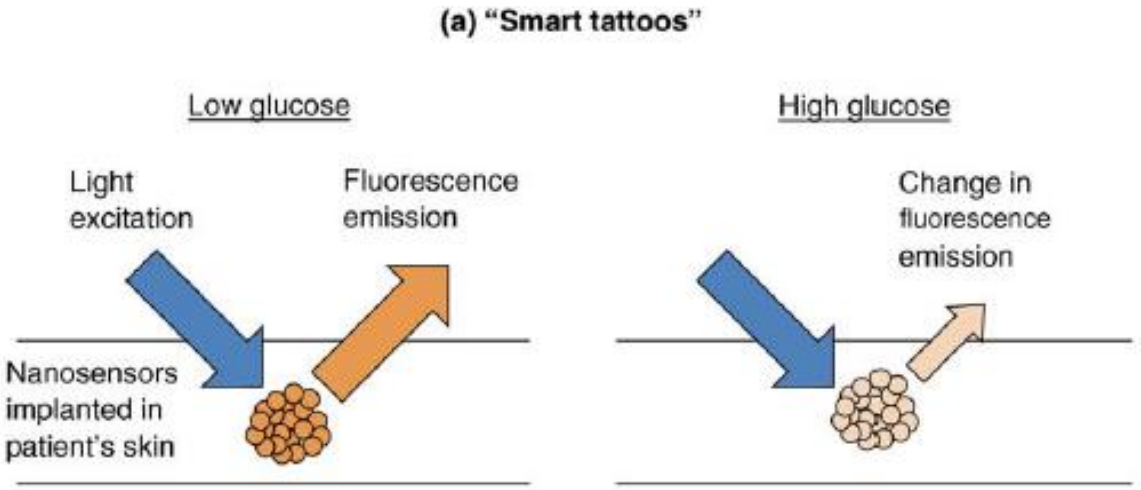
Towards Implantable Biosensors

Zenkl, 2009; Heo, 2012

like a temporary tattoo, an electronic sensor that attaches like a "second skin" to your body and monitors your health.

↓

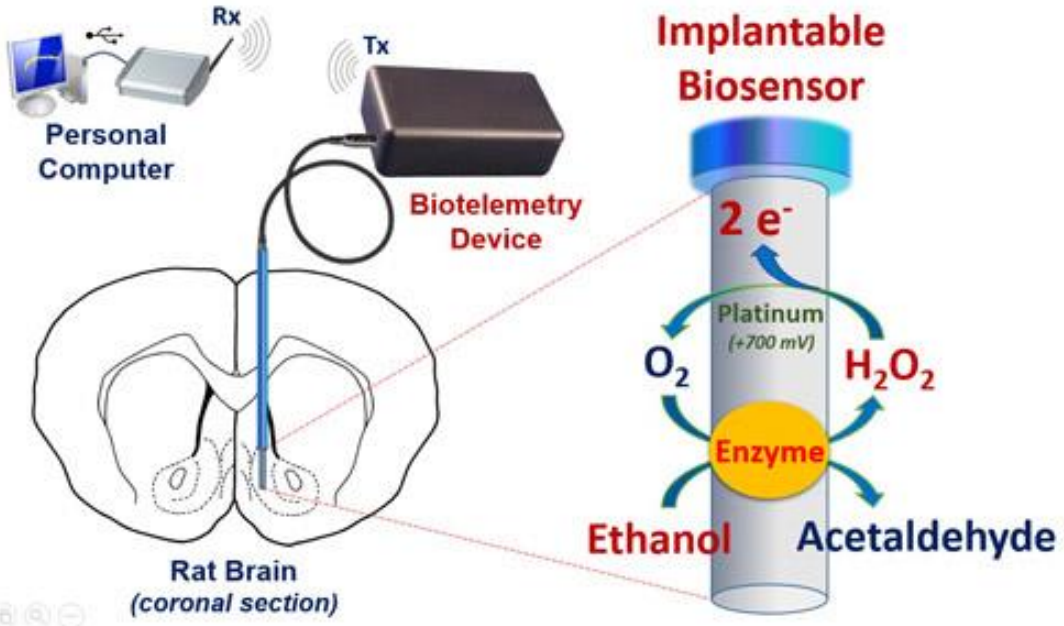




Towards Implantable Biosensors

(Rocchitta, 2012)

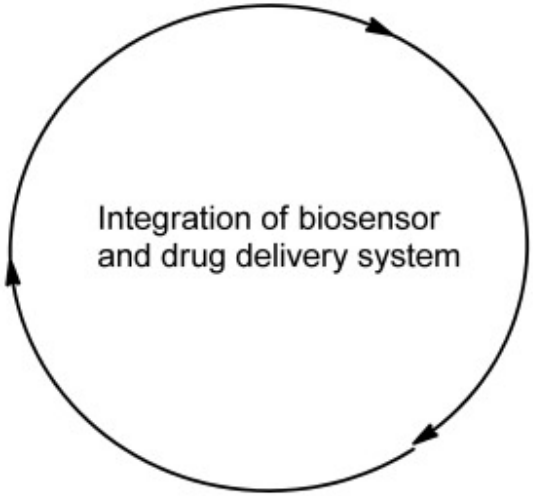
- Short-term monitoring of exogenous ethanol
- A new generation of analytical tools for studying ethanol pharmacokinetics and the effect of drugs on ethanol levels in real time.



Integrated Biosensor-Controlled Drug Delivery

Microchips- microfibrication technology for production of microscale features in or on materials by means of etching, deposition, photolithography and micromolding. Efficient in sustained drug delivery in relation to biomarker (BioMEMS)

Microneedle - has minimally invasive interface with the body as they act as artificial pathways across the skin barrier (transdermal). Highly effective application in drug delivery and biosensing



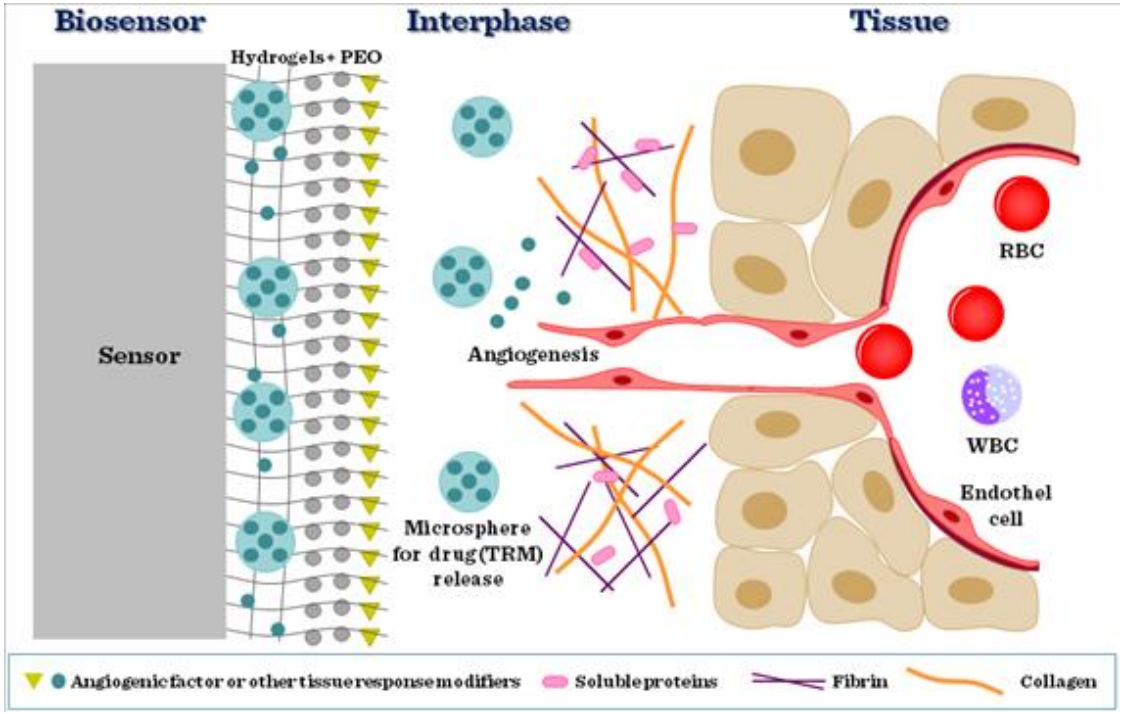
Microfluidics-manipulation and analysis of fluid flow in structures of sub-millimeter dimensions. Micropumps and microvalves.

Implantable-preferential for therapies that require many injections daily or weekly. Implanted into the human body or placed under the skin, consequently reducing the risk of infection by eliminating the need for frequent injections

Integrated
Biosensor-Controlled Drug Delivery

http://www.tankonyvtar.hu/hu/tartalom/tamop425/0011_1A_3D_en_book/ch01s10.html

" A great deal of interest has been focused to glucose-responsive insulin delivery since the development of pH responsive polymeric hydrogels that swell in response to glucose. The "intelligent" system consists of immobilized glucose oxidase in a pH-responsive polymeric hydrogel, enclosing a saturated insulin solution. As glucose diffuses into the hydrogel, glucose oxidase catalyzes its conversion to gluconic acid, thereby lowering the pH in the microenvironment of the membrane, causing swelling and insulin release."



Glucose-responsive insulin delivery:

1. Novel electrodes are required to decrease invasiveness of the implantable glucose biosensor;
2. Improvement of in vivo life of the implantable glucose sensor;
3. Angiogenesis around the glucose sensor could be increased to improve detection potential of glucose levels

Challenges and Qs in commercialization

The cost and effort of the large-scale manufacture of new sensor approaches must provide either extreme improvements in accuracy with minimal additional new cost or an improvement in patient quality of life.

- Can nanomaterials be designed to help improve biocompatibility and sensor lifetime for implantable sensors?
- Are there additional considerations for the biocompatibility of nanoscale materials? (foreign body response??)
- Can nanoscale sensors minimize tissue encapsulation and protein fouling?
- Can nanosensors be administered in locations to minimize glucose transport time lag?