Nanobioelectrochemistry

(Applied) electrochemistry at nanoscale

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Nanobioelectrochemistry

- Electrochemistry at nanoscale
- Broad field of application

- Nanobiosensors
- Nanopores and Nanoscale field effect devices DNA sequencing
- Biological inspiration and Study of electrochemical processes at nanoscale
- Biofuel cells
- SECM

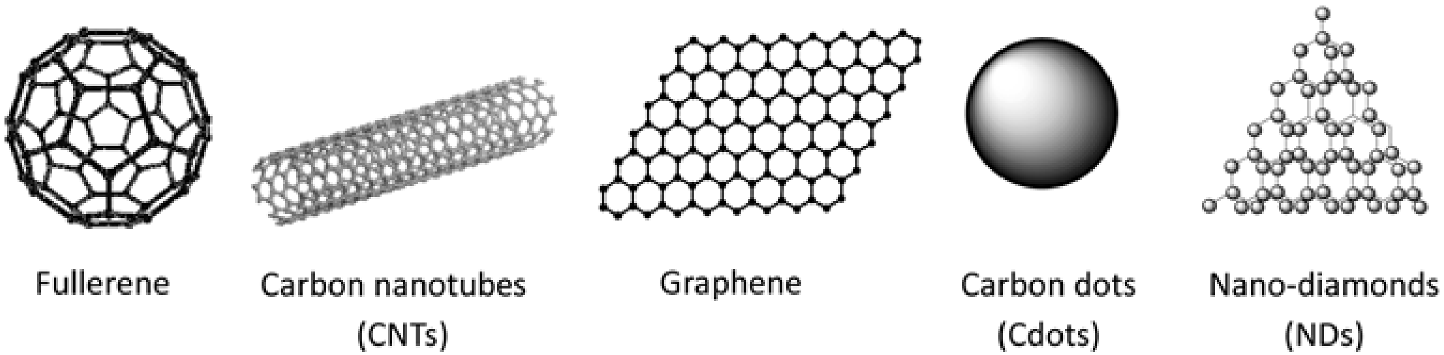
Literature

- Nanobioelectrochemistry, From Implantable Biosensors to Green Power Generation
  – ed. F.N. Crespilho (2013, Springer)

- Internet…

Electrochemistry with Nanoparticles (NPs)

- Au (most used), Ag, Pt, Pd, Cu, Co…
- Nanocrystals
  - Prussian blue,
- Synthesis, enhancement of function, stabilisation
- Carbon nanomaterials



Liu, Liang, Theranostics 2 (2013) 235

- ***Conductive!***

Electrochemistry at nanoscale

- Problem
  - **Noise distorts the accuracy of the measurements**
  - fA or pA and lower values of current are measured

  ($x10^{-15}$ or $x10^{-12}$)

- Instrument demanding

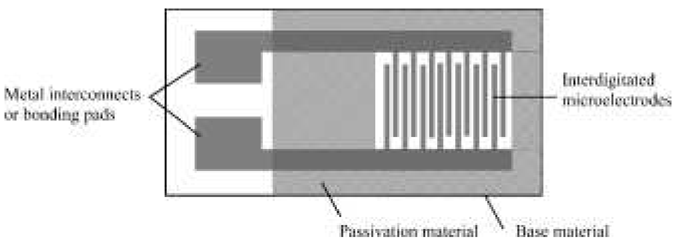
- *Macroscale smoothing of electrochemical signal can not be simply used!*

- Possible solutions are sought - numerical modeling, filters, etc.

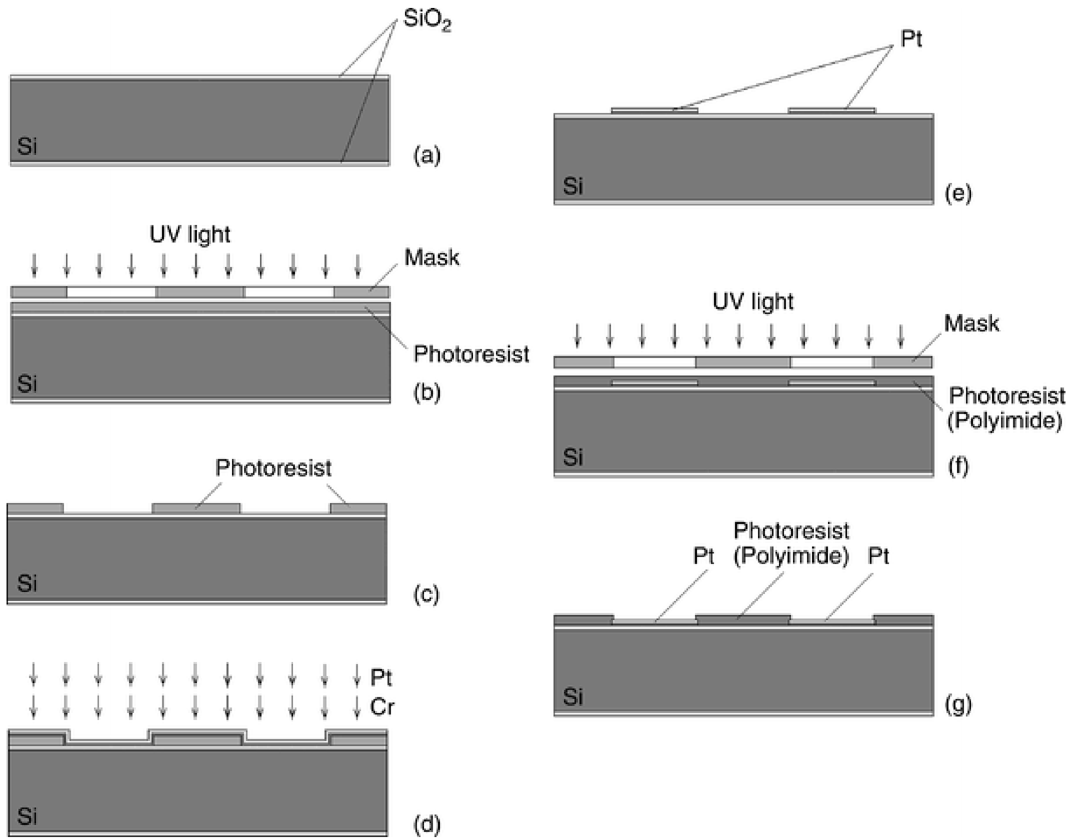
Electrodes

- (Macro)electrodes with nanostructures
  - On polished flat surface in mm range
  - Au, Pt, C …

- Microelectrodes
  - lithography for production of defined structures

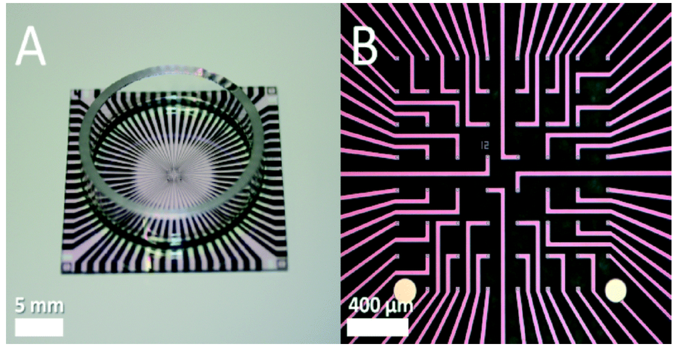


Varshney, Li,
Biosensor Bioelectronic 24 (2009) 2951



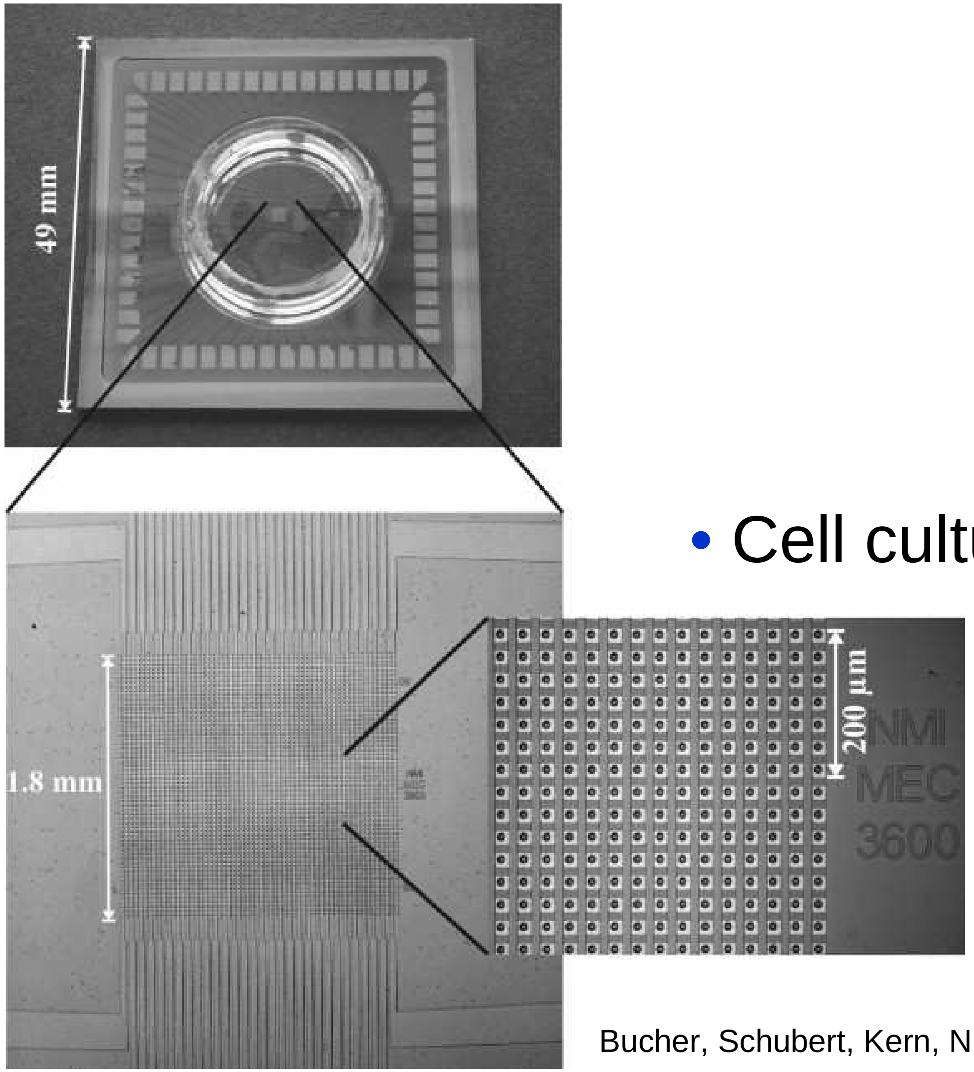
Yu, Wilson, Faraday Discuss 116 (2000) 305

For illustration
Microelectrode Array - MEA Chip



Yakushenko, Mayer, Buitenhuis, Offenhäusser, Wolfrum, Lab Chip 14 (2014) 602

- Cell culture experiments

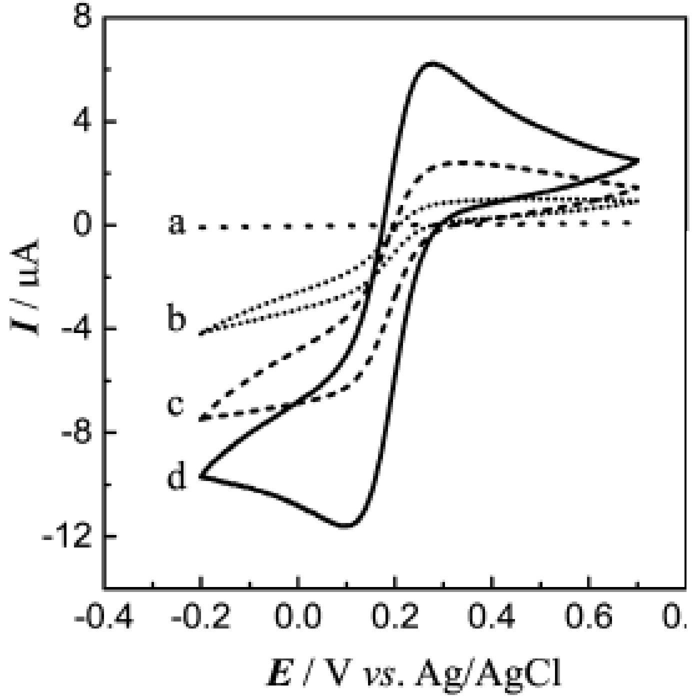


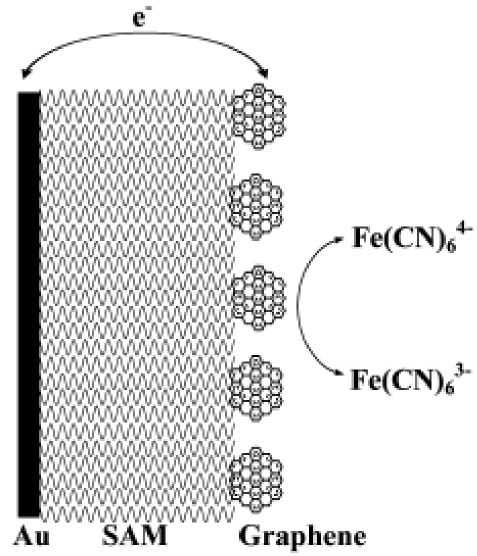
Bucher, Schubert, Kern, Nisch, Microelectron Eng 57-58 (2001) 705

Nanostructured electrodes

- Enhancement of (electro)active x sensing surface
  – NOT ONLY!

- Incubation (min)
  – (a) 0
  – (b) 5
  – (c) 15
  – (d) 120

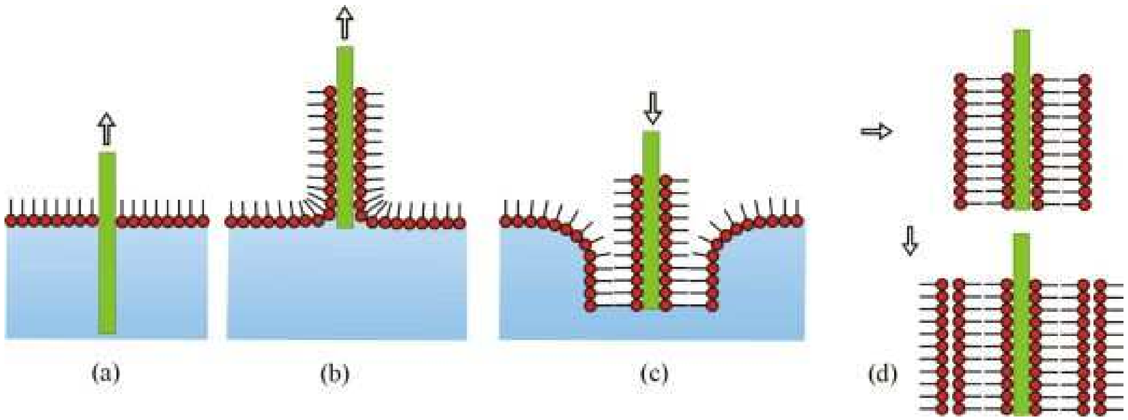




- Valid for all nanoparticles attached to the surface!

Surface modification

- Langmuir-Blodgett films
  - Well defined monolayers
  - Co-deposition of polyanions and polycations
    - Layer-by-layer



- Nanostructured modifications of electrodes
  - 0D quantum dots, nanoparticles
  - 1D nanowires and carbon nanotubes
  - 2D metallic platelets and graphene sheets

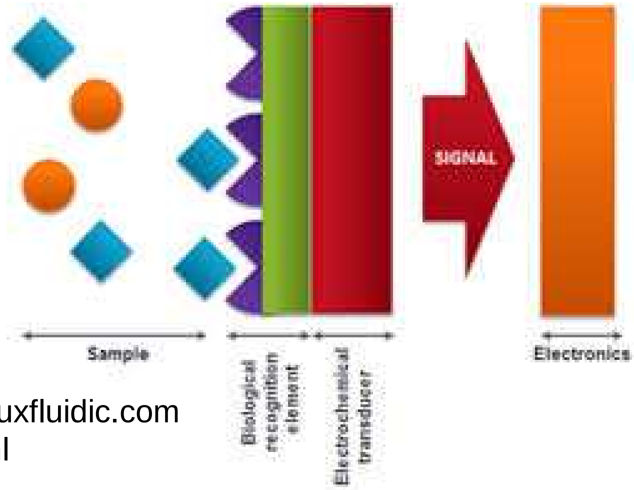
- Depends on particular case of study

Electrochemistry at nanoscale

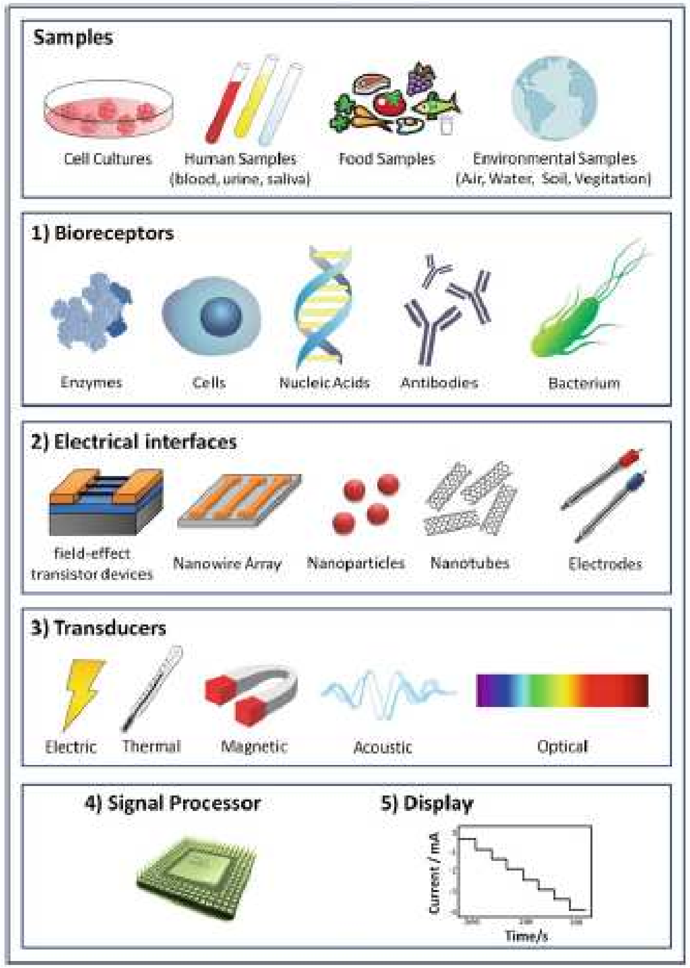
- Electrode modifications
- Current (today’s) functional schemes improved by employment of “nano”
- Nano utilisation results in novel functional schemes
- Electrochemical biosensors using nanoparticles
  - **Enhancement of active surface**

Biosensors at nanoscale

- Usual biosensing schemes using nanotechnology



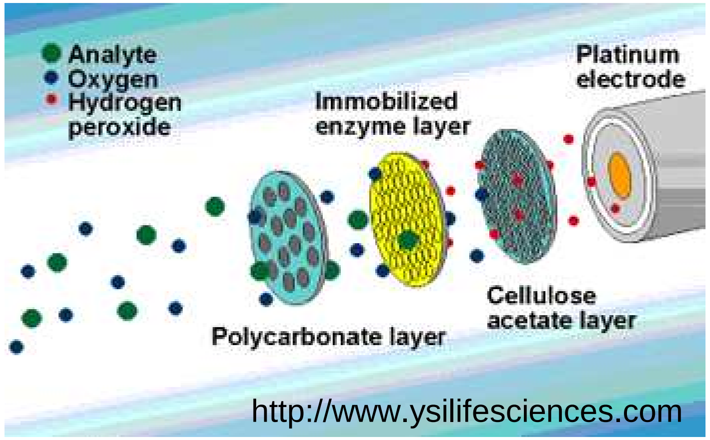
http://www.micruxfluidic.com /technology.html



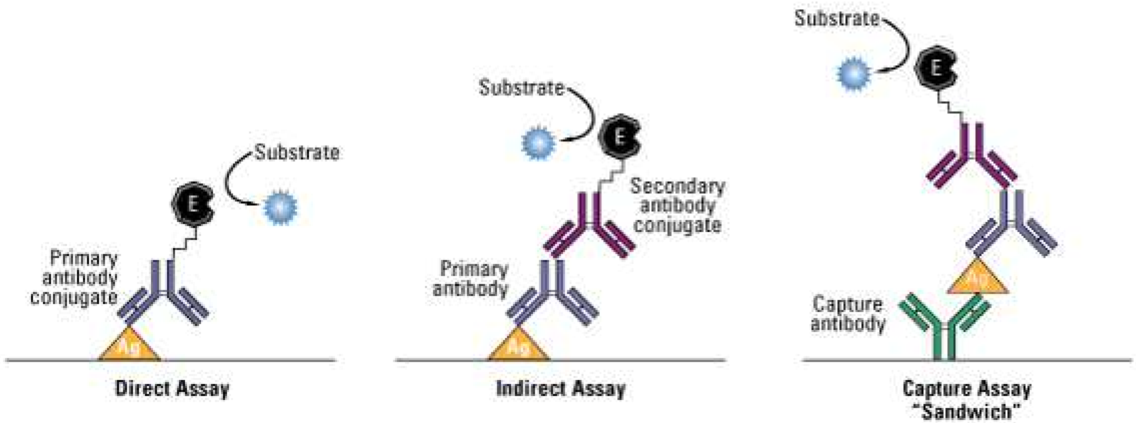
- Improvement in
  - ***Sensitivity***
  - ***New functional schemes***
  - Selectivity ?
  - Instrumental simplicity ?
  - Low cost ?

Biosensing schemes using Electrochemistry

- Enzymatic biosensors



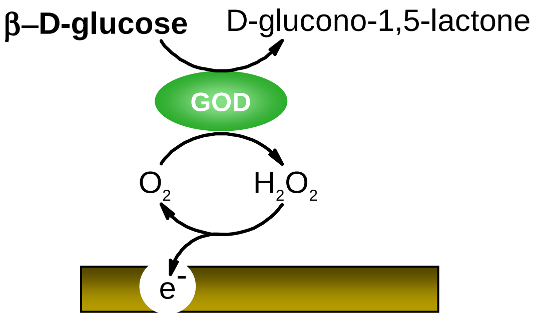
- Immunoassays

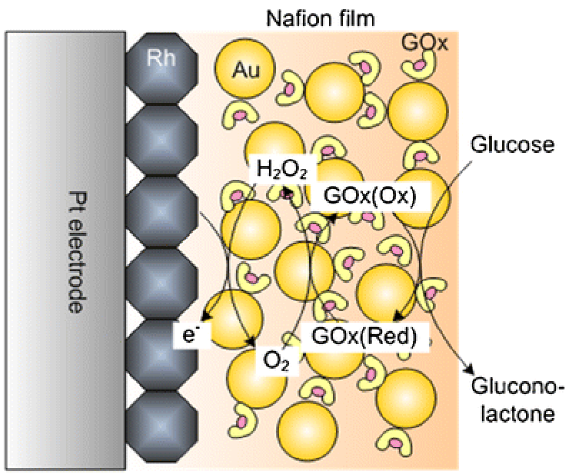


www.lifetechnologies.com

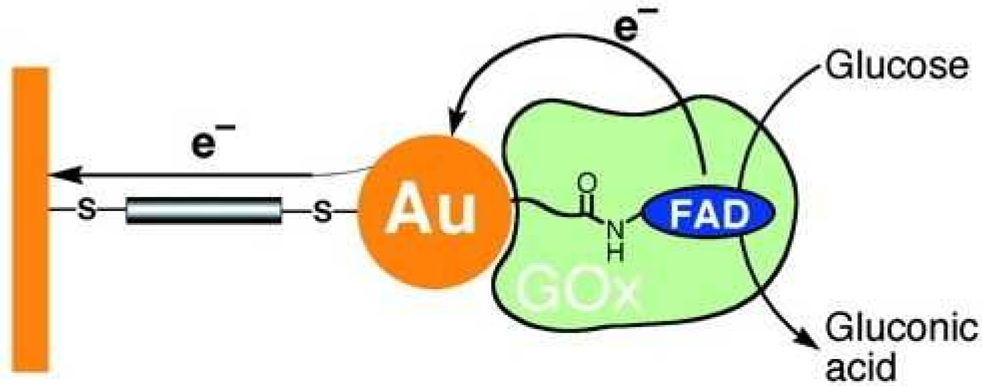
Enzymatic biosensors

- Enhancement of electroactive surface area – higher signal (also non-specific)

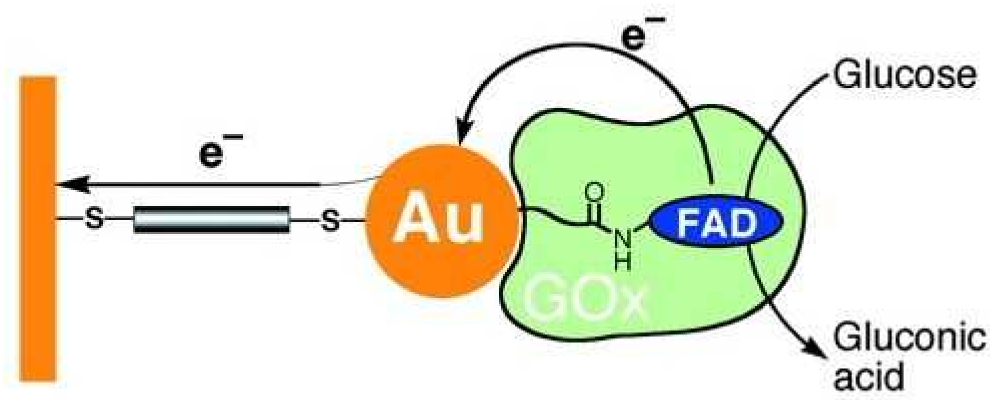


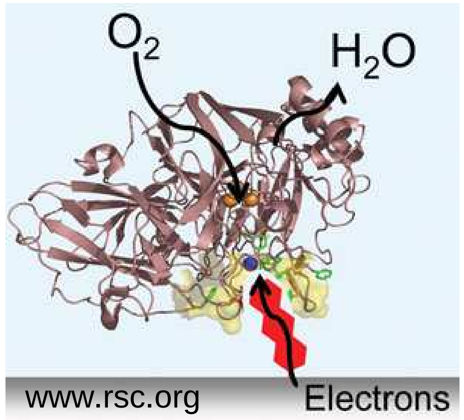


- Direct electron transfer

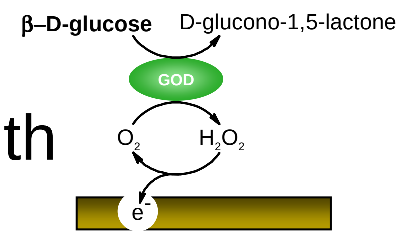


Enzymatic biosensors
Wiring of enzymes





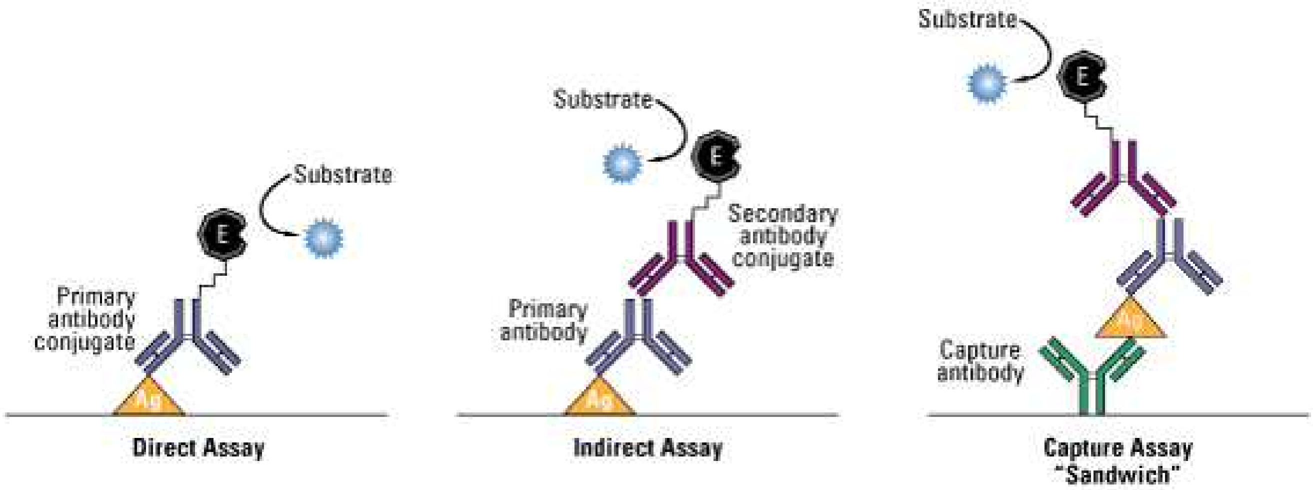
- Connection of enzyme redox (active) site and electrode
- Interference free
- Improved electrode kinetics in comparison with



- Employed also in biofuel cell technology

Electrochemical immunoassays using nanomaterials

- Stemming from ELISA (Enzyme-linked immunosorbent assay)



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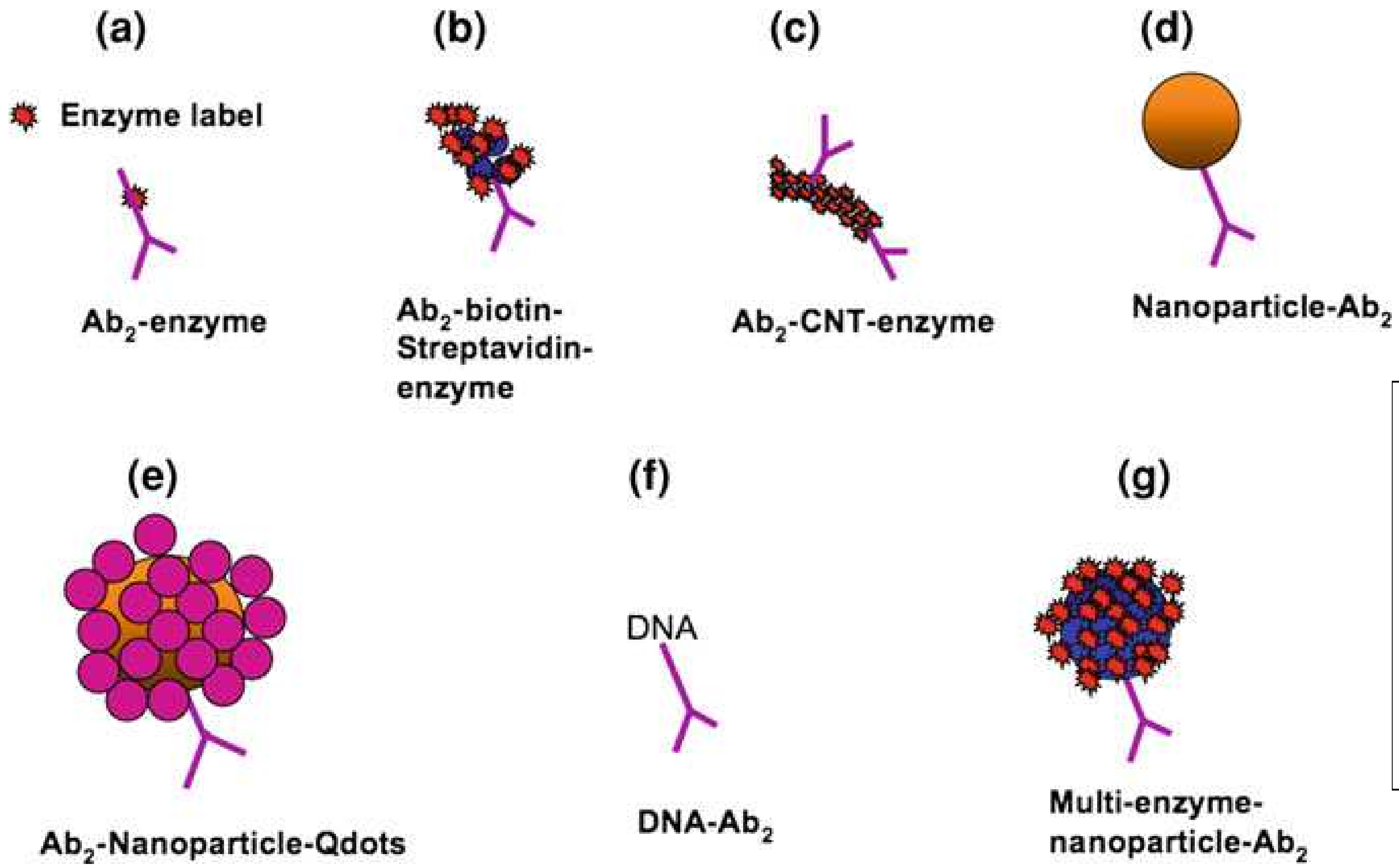
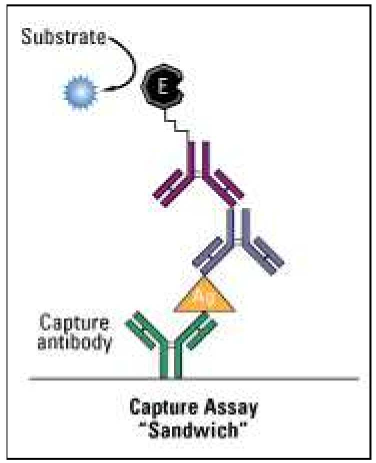
- Employment of
  - Nanostructured electrodes
  - Nanoparticle labels
  - Magnetic nanoparticles
- Protein cancer markers

Possible amplification of signal for electrochemical immunoassay





Nanostructured electrodes in immunoassay

- ***Bigger surface:***
- Enabling the attachment of a large number of capture antibodies on the sensor surface
- Better access of protein analytes to these antibodies
  - carbon nanotubes (single-, multi- walled CN)
  - gold nanoparticles
  - electrodepositing gold

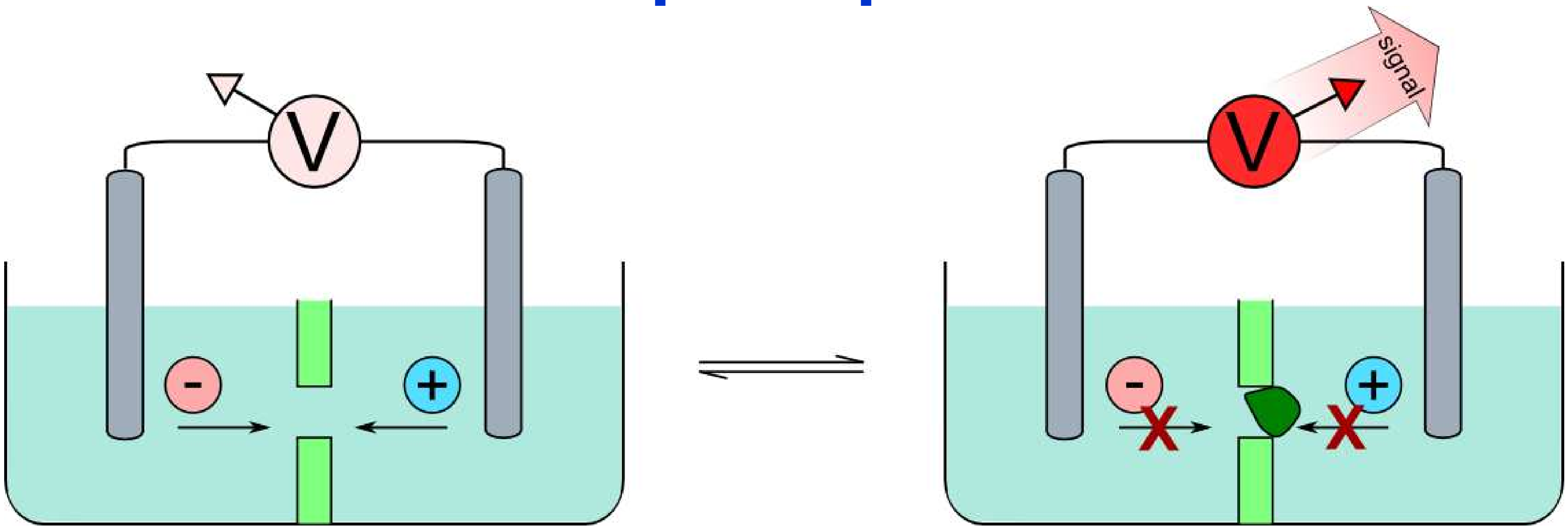
Nano-Immunoassay

- Nanoparticles firstly used in immunoassay in 2000 by Dequaire et. al.

- Amplification by nanoparticles
  - Dissolution to electroactive salts
  - Multi-enzyme NP
  - Quantum dots
  - etc.

- LOD in range of pico (10^{-12}) to femto (10^{-15}) molar concentration

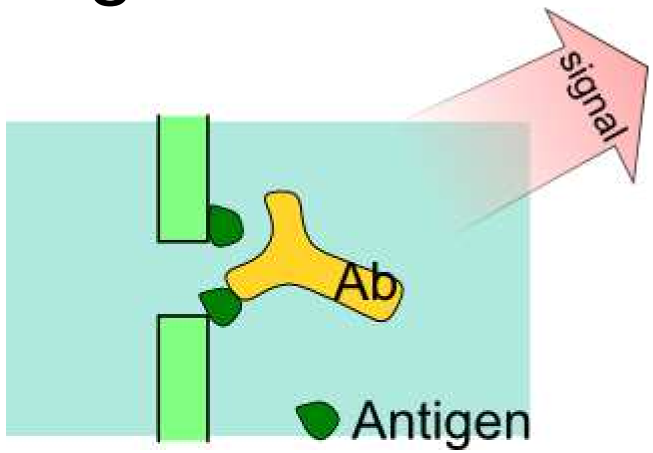
Nanopores
- principle

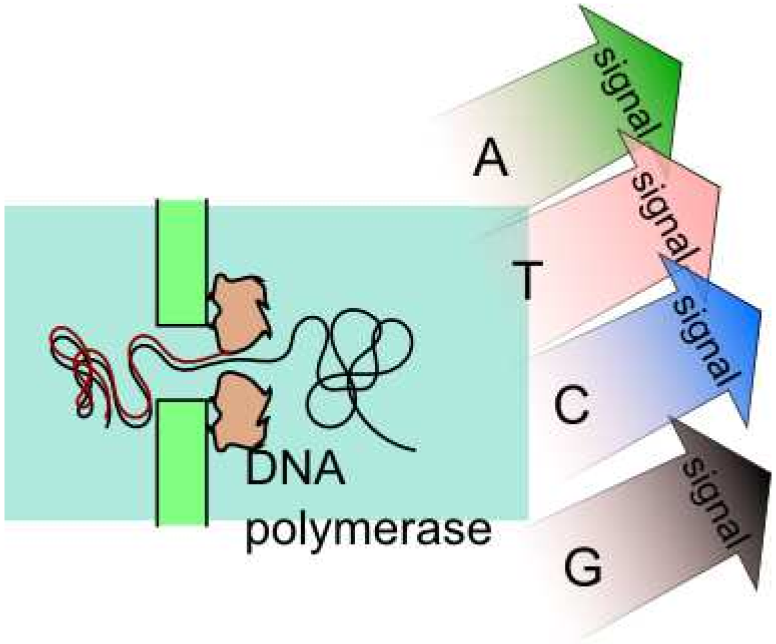


- Monitoring of ionic current
- Blockage = generation of signal
- Nanopore = diameter in range of nm

Nanopores Sensing

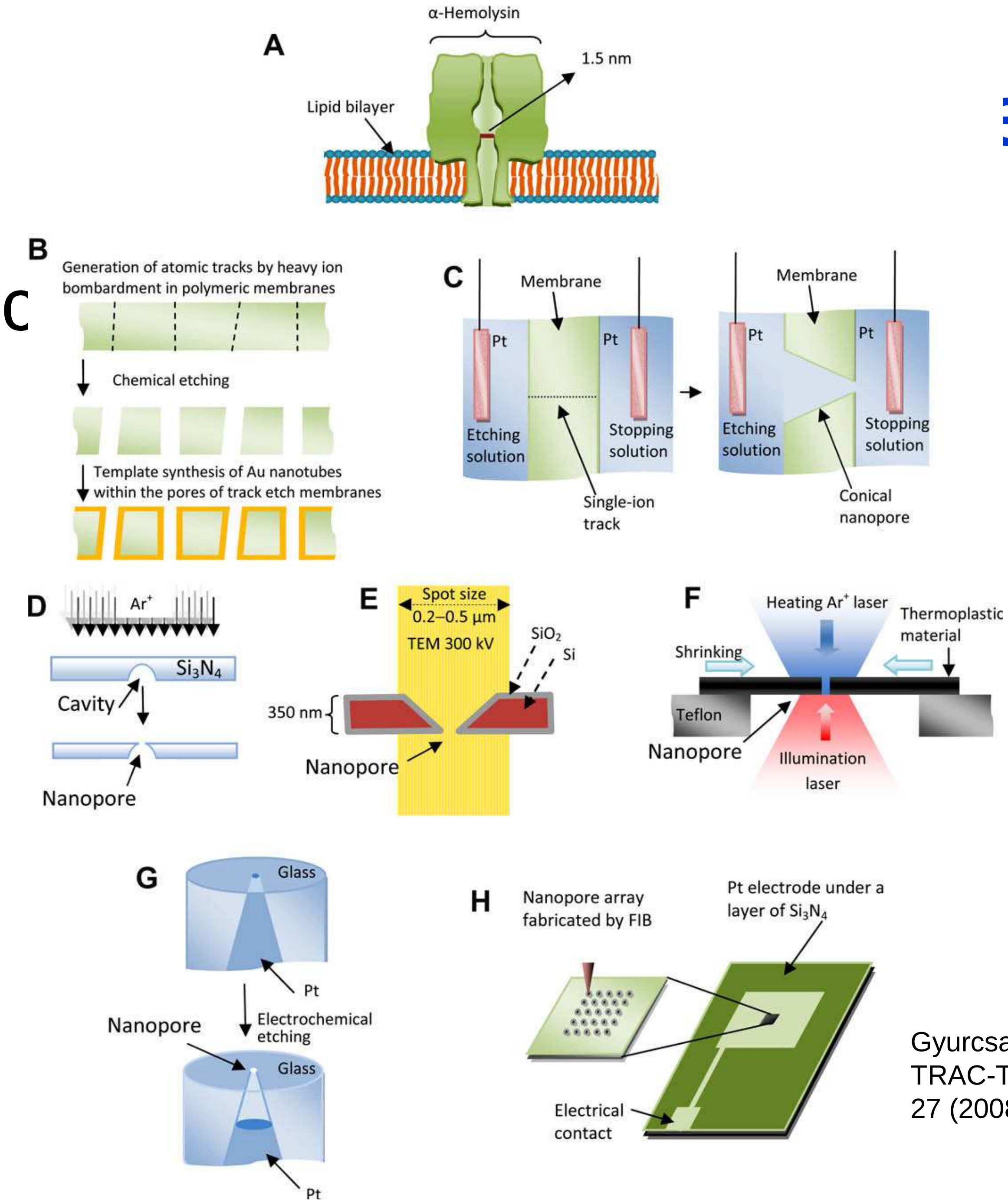
- Nanopore modified with e.g.
  - Antigen
  - DNA polymerase





…es

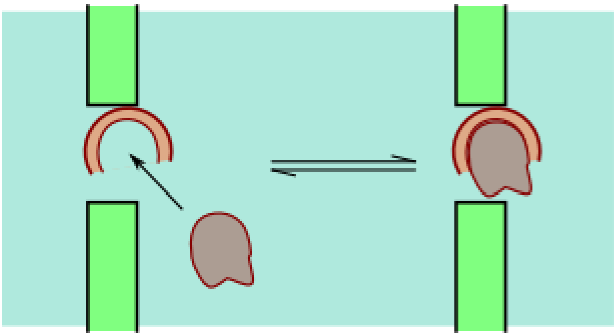
- Cruc…

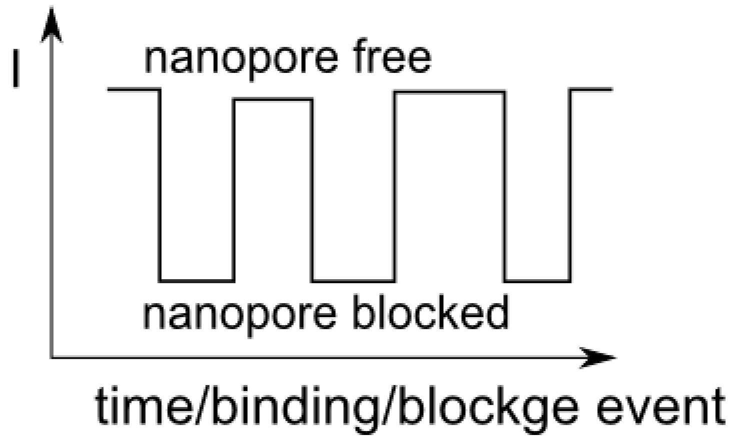


Gyurcsanyi,
TRAC-Trend Anal Chem
27 (2008) 627

Nanopores

- Affinity reactions – equilibrium (Ab-Ag high binding constant)
- “Pulse”-like signal
- Binding and release *etc*….
- Estimation of affinity

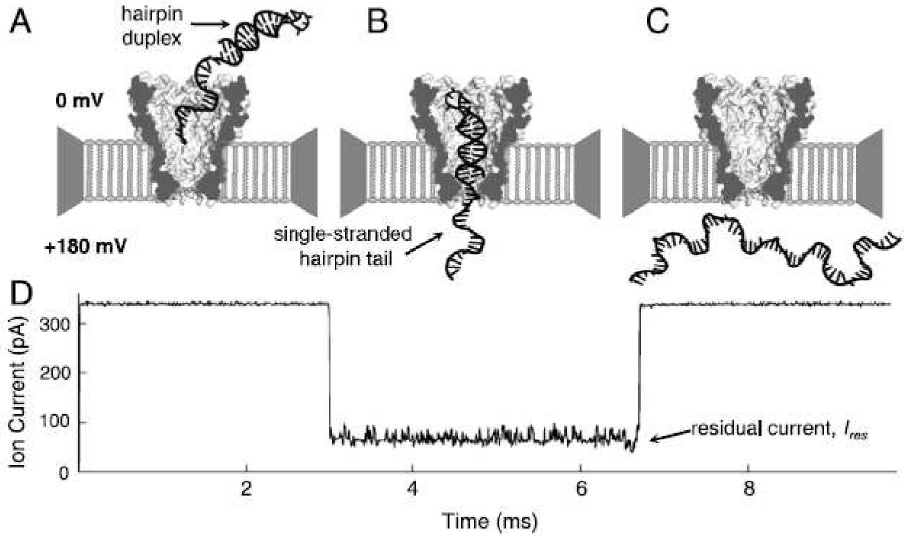


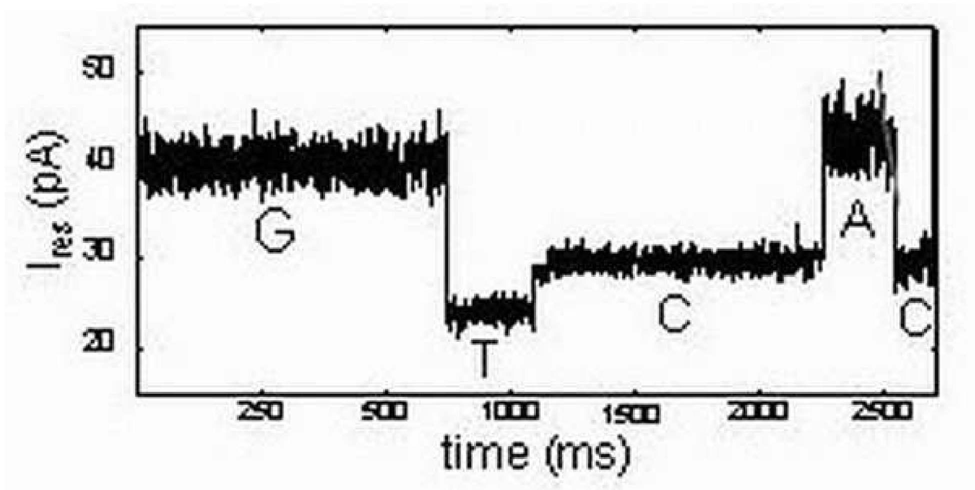


Nanopores
DNA sequencing

- Several sensing schemes:
- Membrane and pore
  - Solid (ion beam milling)
  - Protein pore in bilipid layer
- Detection
  - Charge of single base (A,G,C,T)
  - Number of H^+ released upon DNA polymerisation

Real-time DNA sequencing Example



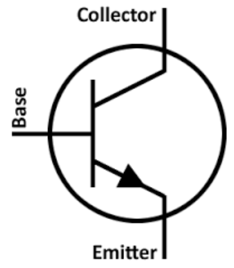


Derrington, Butler, Collins, Manrao, Pavlenok, Niederweis, Gundlach, PNAS 107 (2015) 16060

Field-effect transistor (FET)

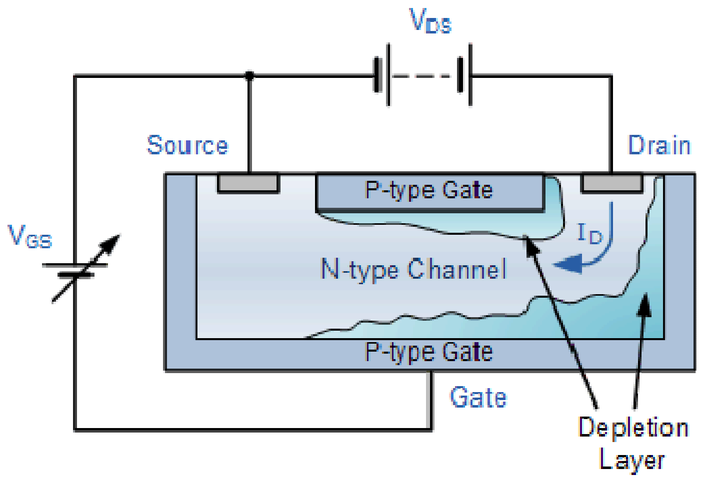
- Semiconductor **micro/nano**technology
- Part of ***Field Effect Devices*** category
  - *ISFET* (ion-sensitive field-effect transistors)
  - *EGFET* (extended gate field effect transistors)
  - capacitive *EIS* sensor (electrolyte-insulator-semiconductor)
  - *LAPS* (light-addressable potentiometric sensors)

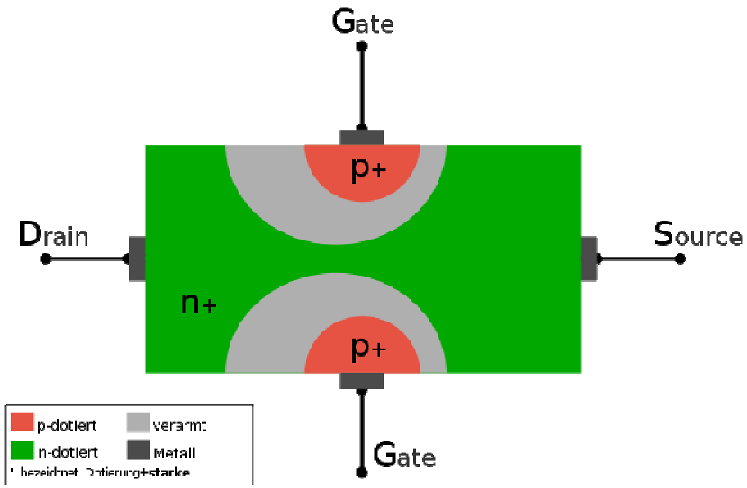
- Transistor
  - Electronic element
  - Amplification of signal



Field-effect transistor

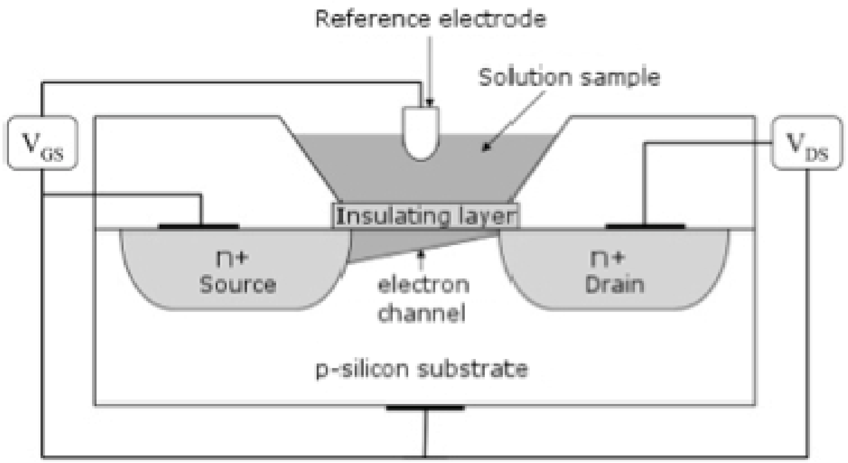
- Principle
  - electric field creates regions of excess charge in a semiconductor substrate





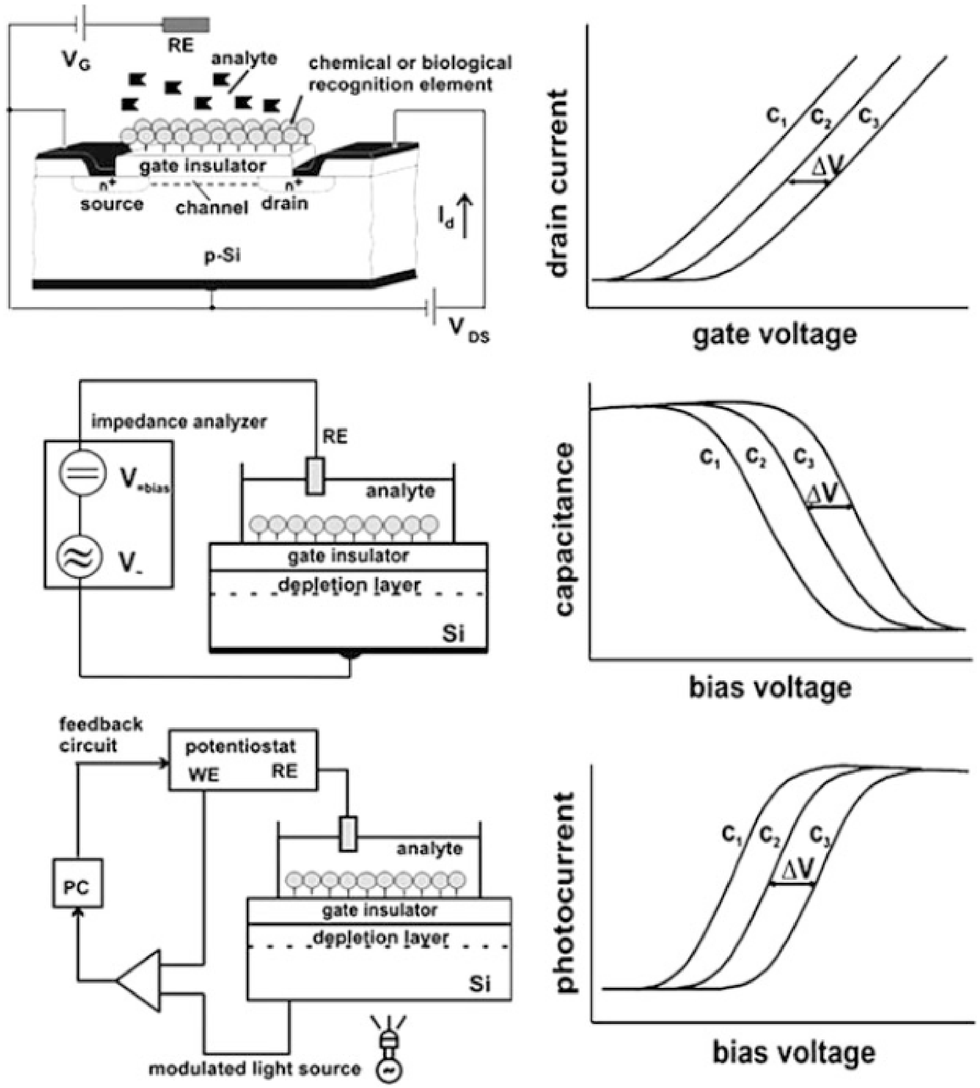
FET sensor principle of operation

- Signal generation (electric field modulation)
  - pH
  - changes of ion-concentration
  - changes of ion-species through enzymatic reaction
  - adsorption of macromolecules
  - affinity binding of molecules (Ab-Ag, DNA hybridization)
  - Changes due to the living systems (e.g. metabolic processes)



Types of Field Effect Devices (FED)

- ISFET
  - Bergveld, 1970
- EIS
  - Simplest senor based on FEDs
- LAPS
  - Modulation of/by photocurrent

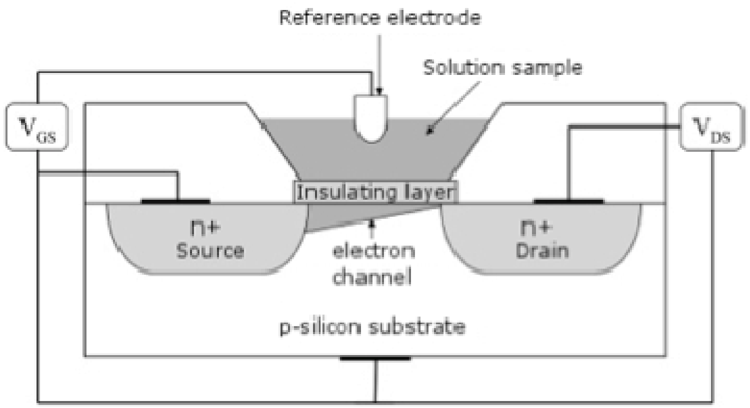


FED based sensors

(Bio-) chemical sensor	Ion/analyte	Sensitive membrane or (bio-) recognition element
pH sensor	H^+, OH^-	Si_3N_4, Al_2O_3, Ta_2O_5 ITO, ZnO, V_2O_5, SnO_2
Ion sensor	K^+, Li^+, Cs^+, Ca^{2+}, Mg^{2+}, NO_3^-, SO_4^{2-}	Polymer membrane & ionophore Dendrimers, silicon nanowires
Enzyme sensor	Glucose, urea, penicillin, Acetylcholine, pesticides, H_2O_2, lactate	Glucose oxidase, urease, penicillinase, acetylcholinesterase, horseradish peroxidase, organophosphorus hydrolase, phthalocyanines, L—lactic dehydrogenase

FET based sensors using nanotechnology

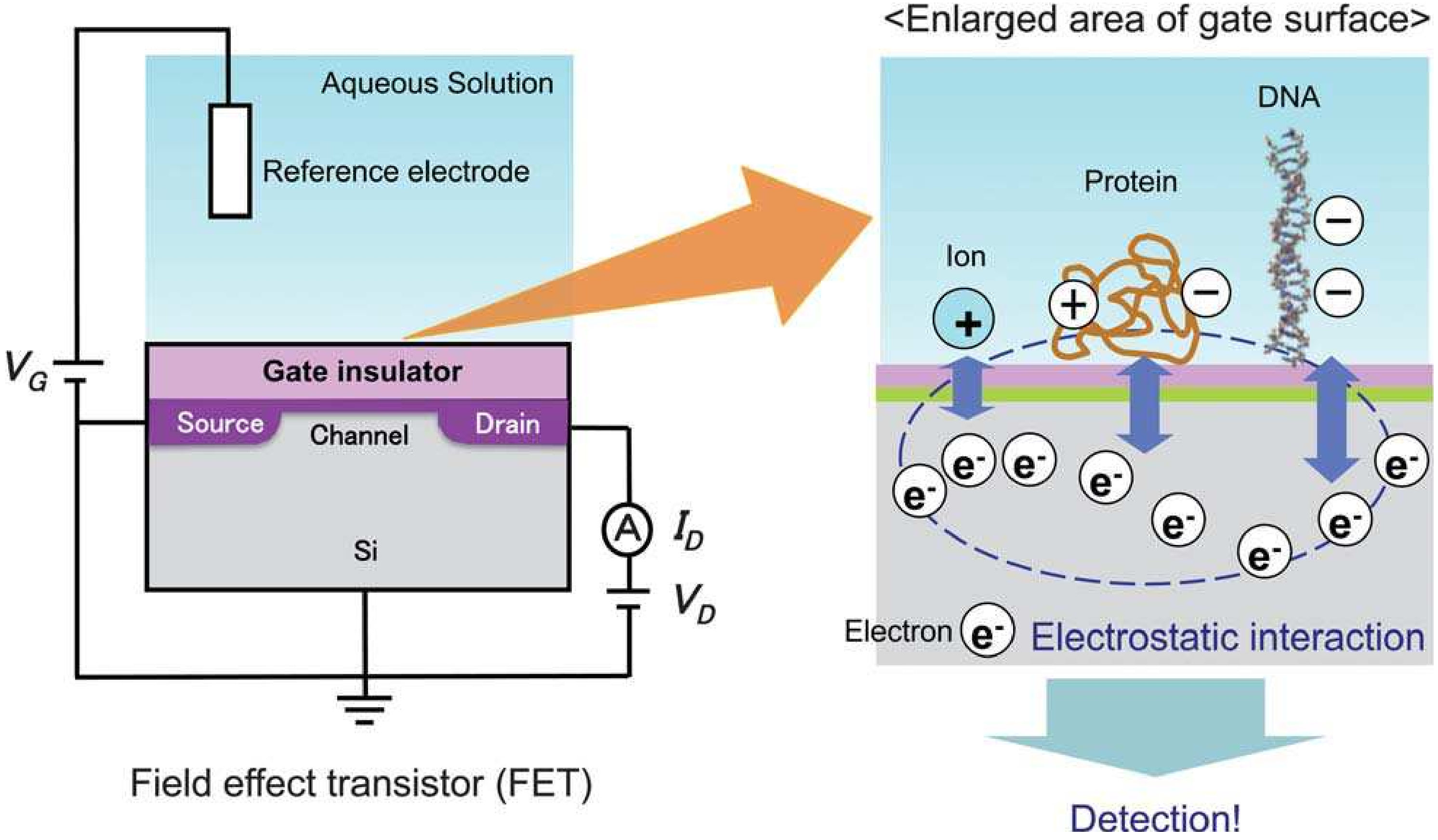
- FET coupled with biorecognition element (e.g. enzyme, antibody)



http://lsi.epfl.ch

- SWCNT
  - Uniform and enhanced adsorption of enzyme
  - Enhanced porosity facilitates the ion permeation

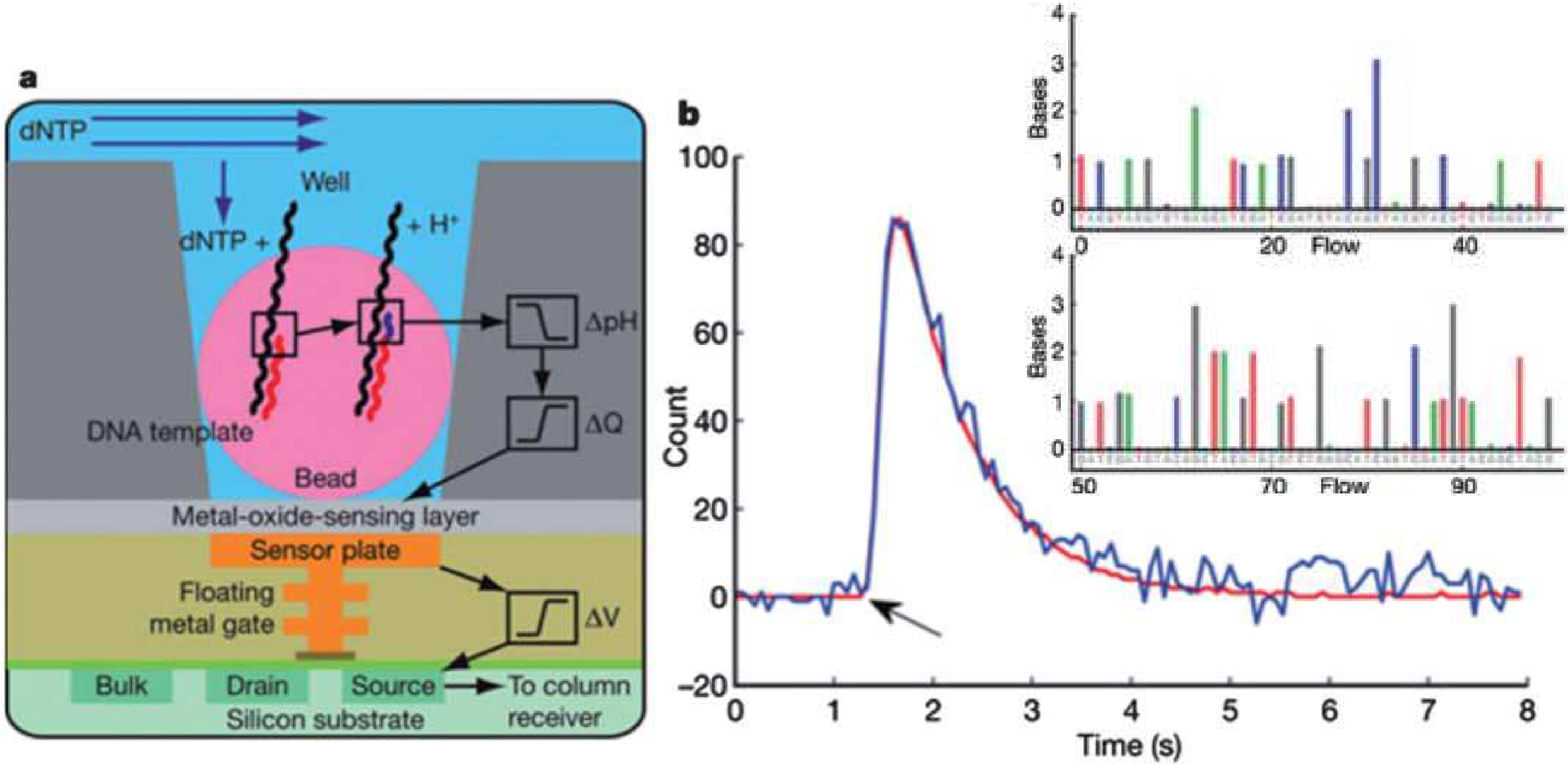
Field-effect transistor (FET)



Field effect transistor (FET)

Matsumoto, Miyahara, Nanoscale 5 (2013) 10702

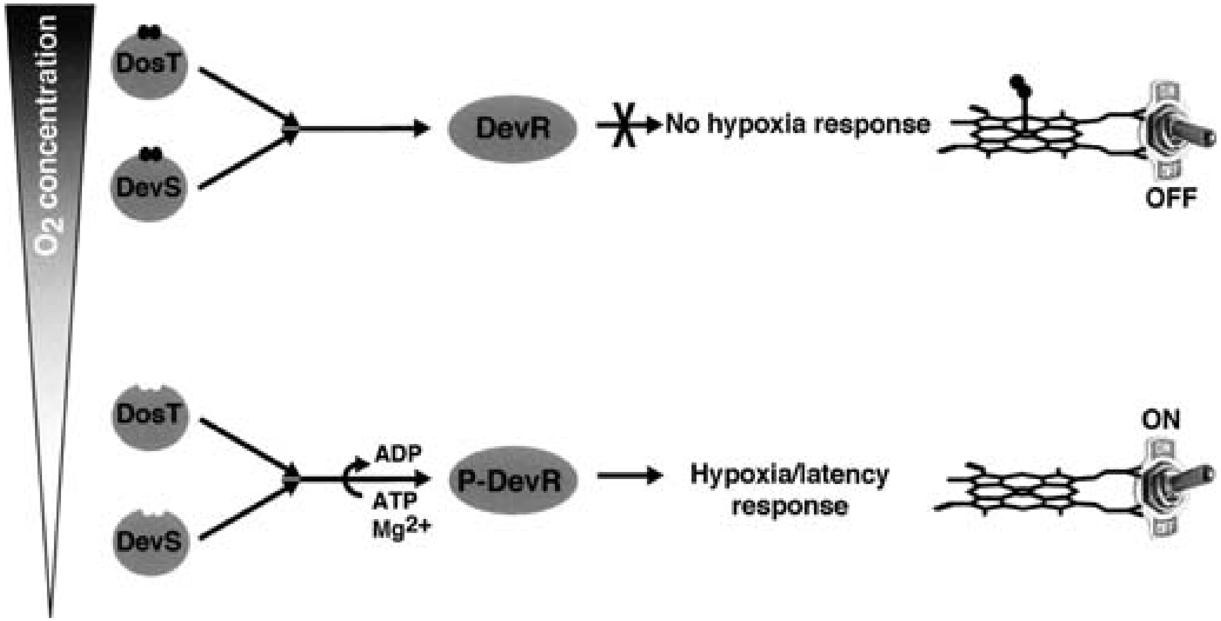
Combination of FET with Nanopores DNA sequencing



Matsumoto, Miyahara, Nanoscale 5 (2013) 10702

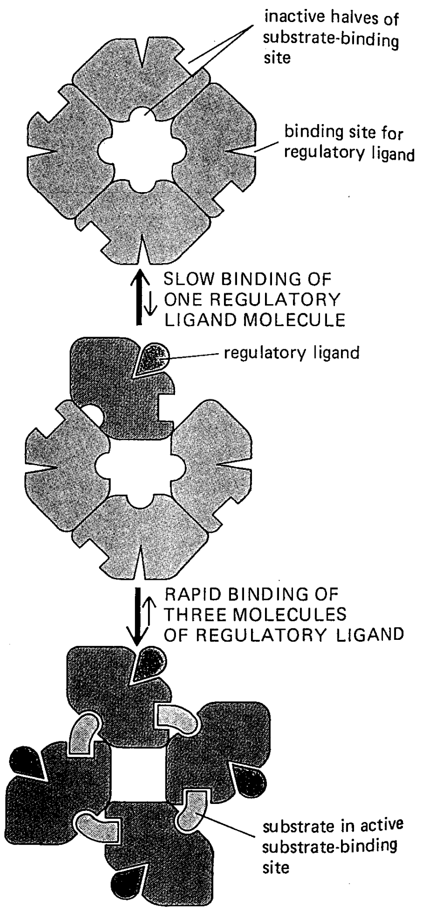
Biological (nano)sensors

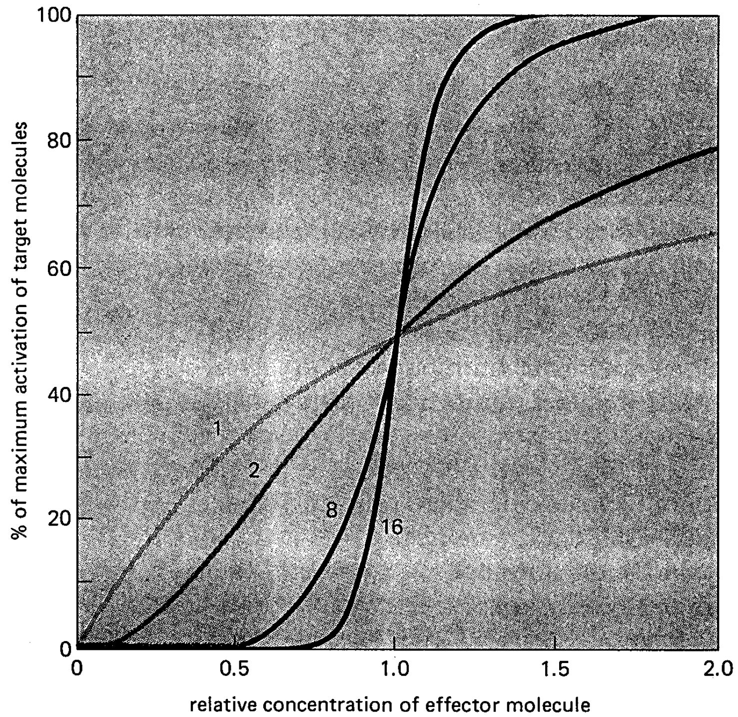
- Biological demand
- Not only human development – e.g. plants, bacteria
- Feedback loop regulation
- Regulation of metabolism



Sousa, Tuckerman, Gonzalez, Gilles-Gonzalez,
Protein Sci 16 (2007) 1708–1719

Allosteric regulation





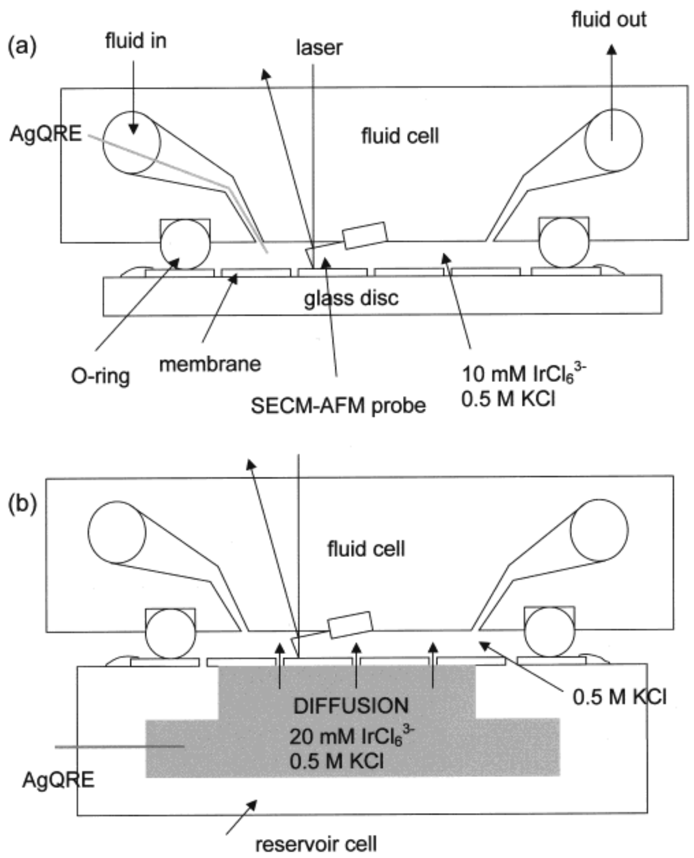
- Hemoglobin – pH dependent O_2 binding and its release

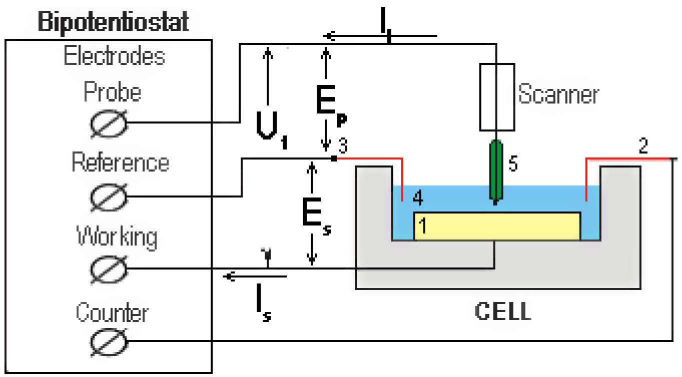
Combined techniques

- Electrochemical methods combined with
  - AFM (Atomic Force Microscopy)
  - SPR (Surface Plasmon Resonance)
- Complementary and additional information

Electrochemistry and AFM

- Possible modes:
  - Discrete AFM and electrochemical characterisation
  - **EAFM – Electrochemical AFM**, AFM tip is conductive and used as working electrode (similar to SECM - overlap)

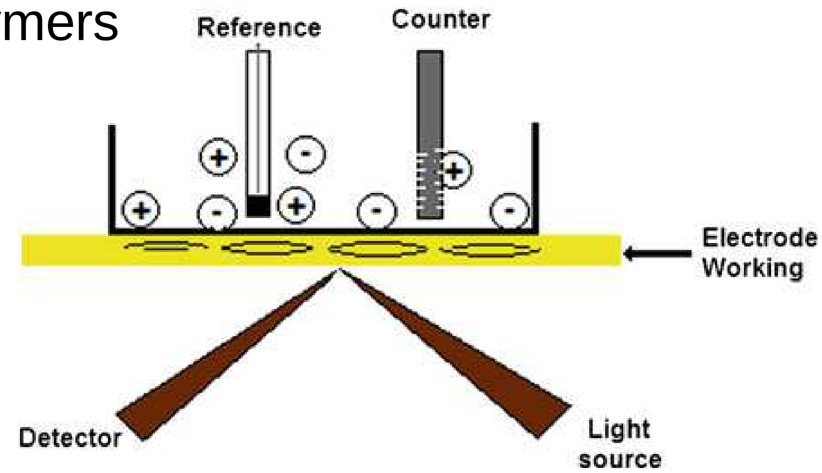




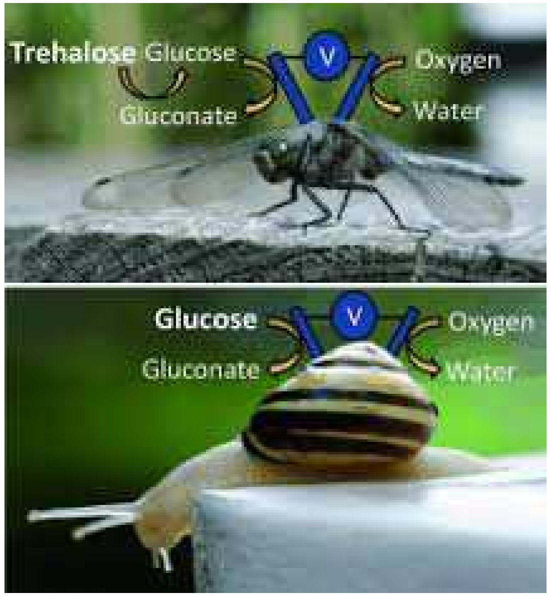
Macpherson, Unwin, Anal Chem 72 (2000) 276

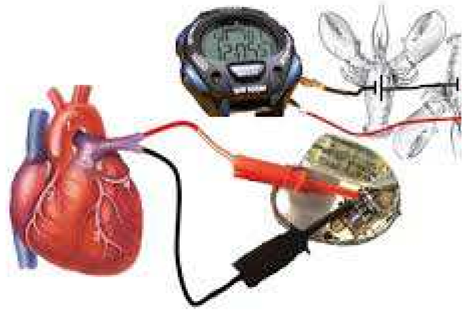
Electrochemistry and SPR

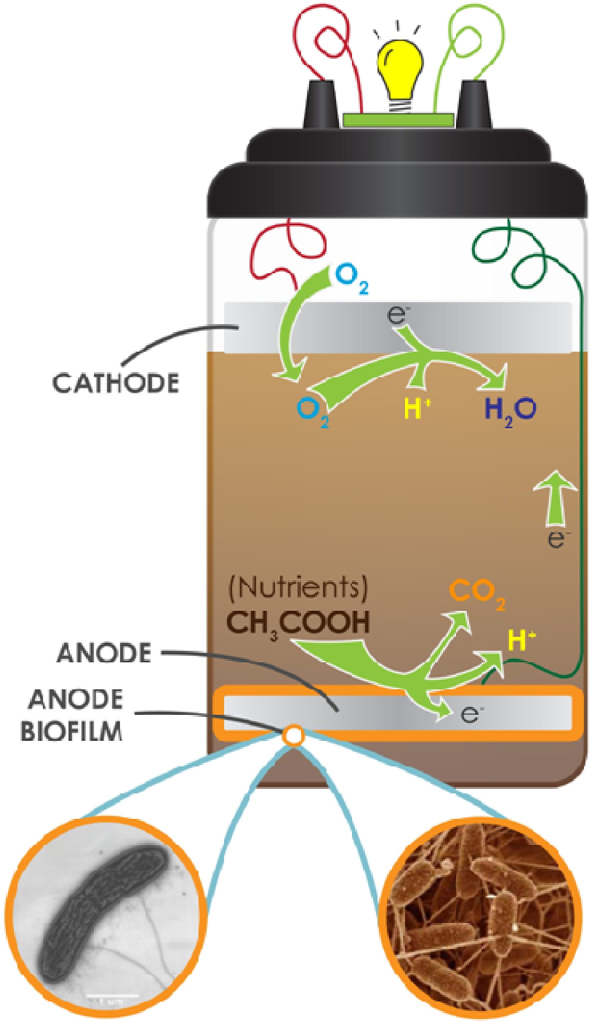
- Simultaneous information about the (electrochemical) processes at the surface
- Nanometer range layer
- antigen–antibody, nucleic acids, cells, enzymes, micro-organisms, etc.
- Characterisation of
  - self-assembly and electro-polymerisation
  - ultra-thin film and conducting polymers
  - redox transformations
  - electrochemically catalysed processes



Biofuel cell





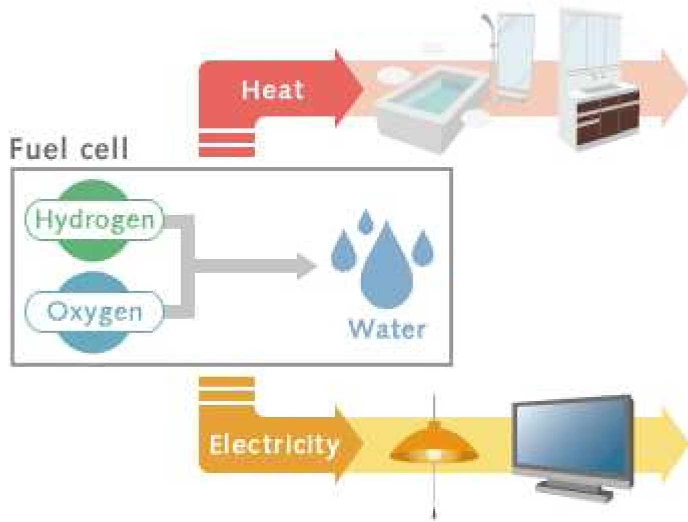


Biofuel cells

- Enzymatic fuel cell
- Interface between redox enzymes and electrical circuitry
- efficient immobilisation and wiring of enzymes
  - carbon nanotubes, inorganic and polymer nanoparticles

Fuel cell

- transforming chemical energy into electrical energy



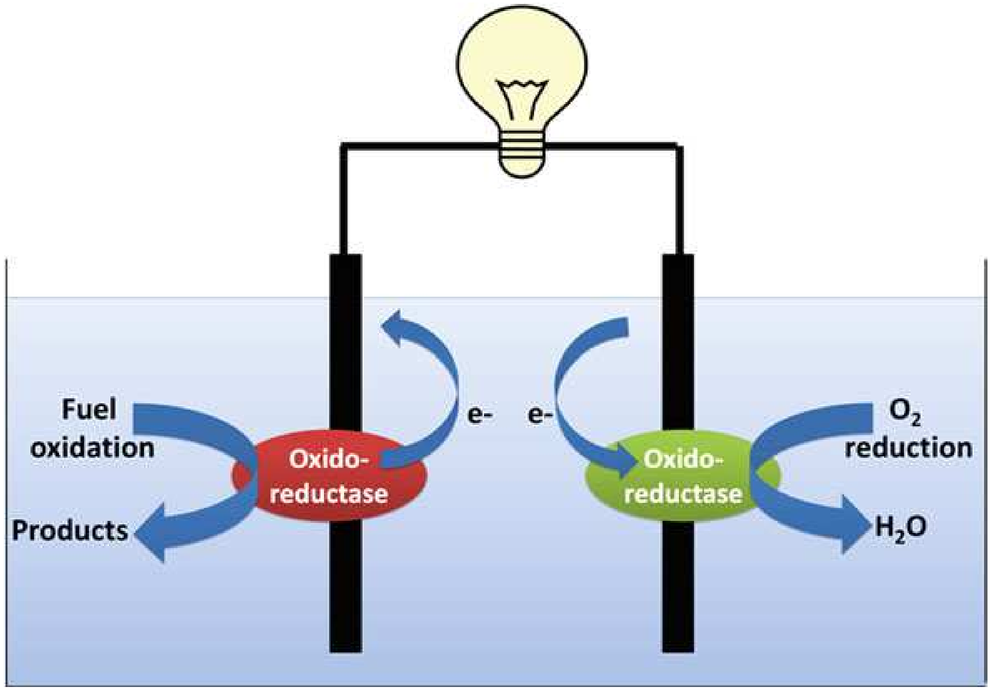
http://www.toto.co.jp

BioFuel cell

- “Fuel cell” using the bio-catalytic reaction of enzymes or living organisms

Biofuel cell

- Redox enzymes
- Oxidation at anode – sugars, alcohol
- Reduction at cathode – O_2, H_2O_2



Biofuel cell

- Firstly described in 1964
- Advantages – “ecofrendly”
  - No metal catalyst (platinum, palladium, iridium,…)
  - Biodegradable

- Motivation
  - Power supply for pacemakers ,micro machines, micro-pumps, sensors
  - Utilisation of glucose and O_2 – present in our body fluids

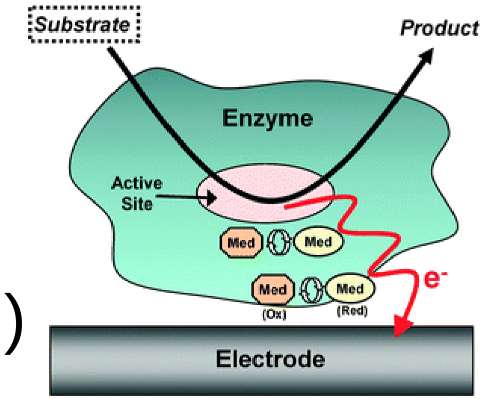
Biofuel cell
Principle of function

- Majority
  - oxidation of glucose by glucose oxidase
  - Reduction of oxygen by laccase (bilirubin and ascorbate oxidase)

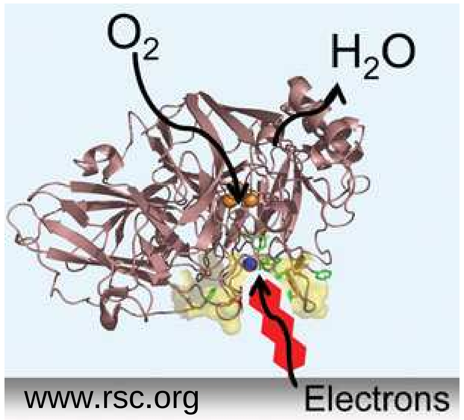
- Mediated or direct electron transfer
  - Between enzyme redox centre (active site) and electrode

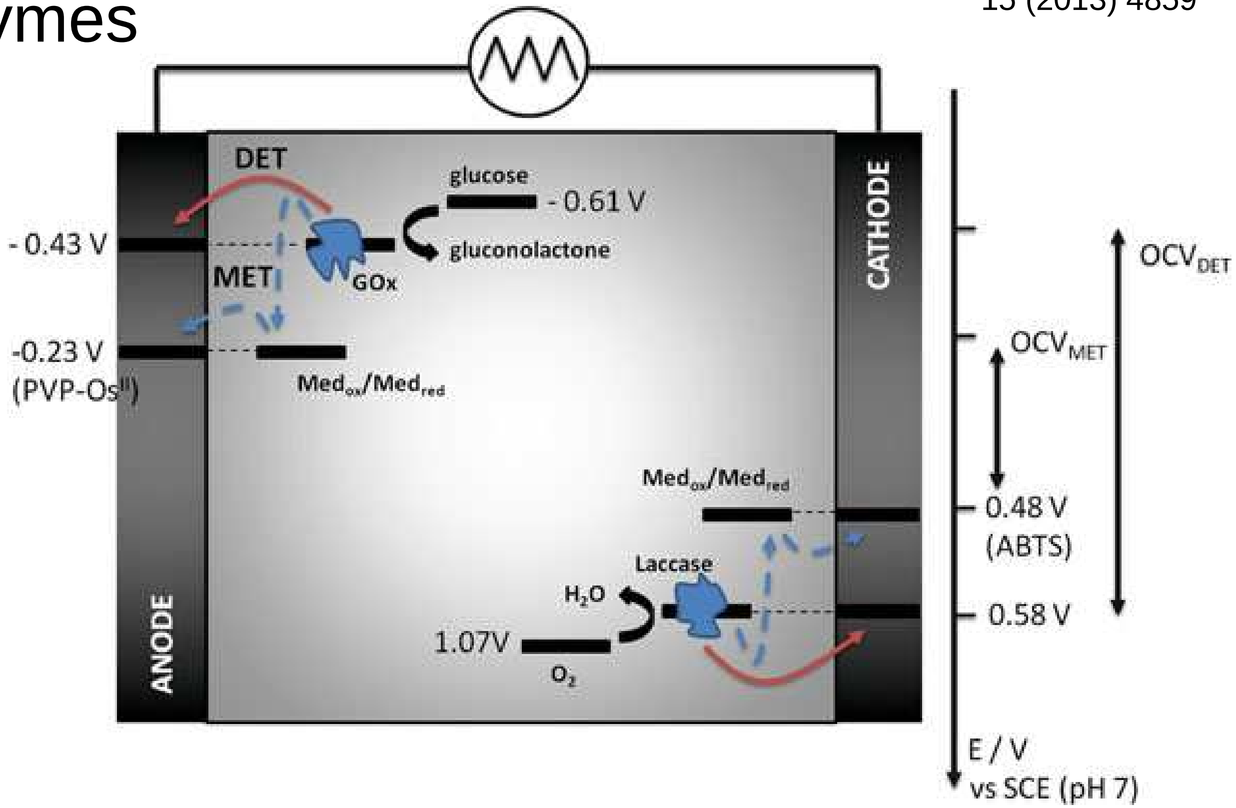
Biofuel cell
Principle of function

- Mediated ET
  - Electron mediator (ferrocene, Os(bipy))
- Direct ET
  - Wiring of enzymes



Kavanagh, Leech,
Phys Chem Chem Phys
15 (2013) 4859



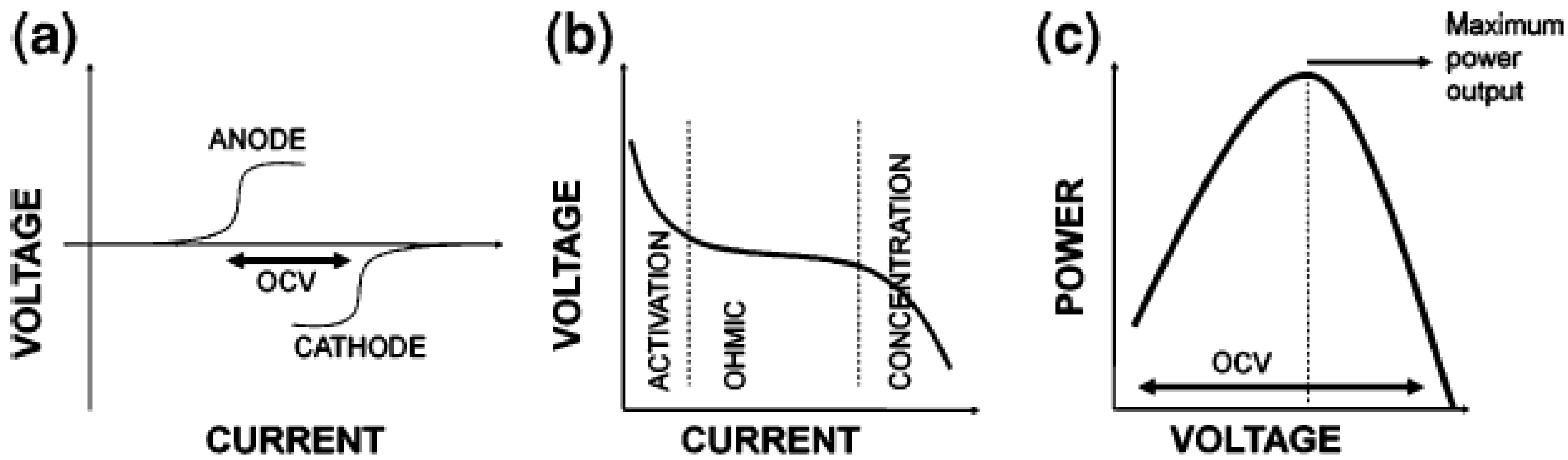


Biofuel cell performance

- Maximum power density
- Maximum current density
- Open circuit potential

- Operational stability (time characteristic of power supply by biofuel cell)
- Storage stability

- Measured by polarization curves



Biofuel cells using nanotechnology

- Nanoparticles for enhanced immobilisation of biomolecules (increased surface coverage) and ***wiring of enzymes***
- Carbon nanotubes
  - nanowire morphology
  - biocompatibility
  - excellent conductivity
  - well described functionalization
- Graphene (carbon nanomaterials)
- Clay nanomaterials

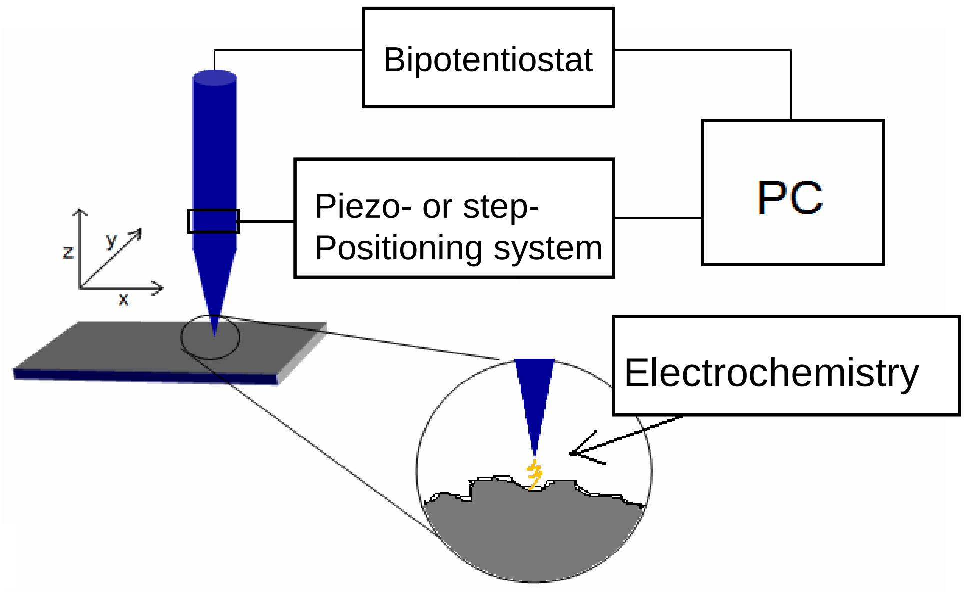
Clay

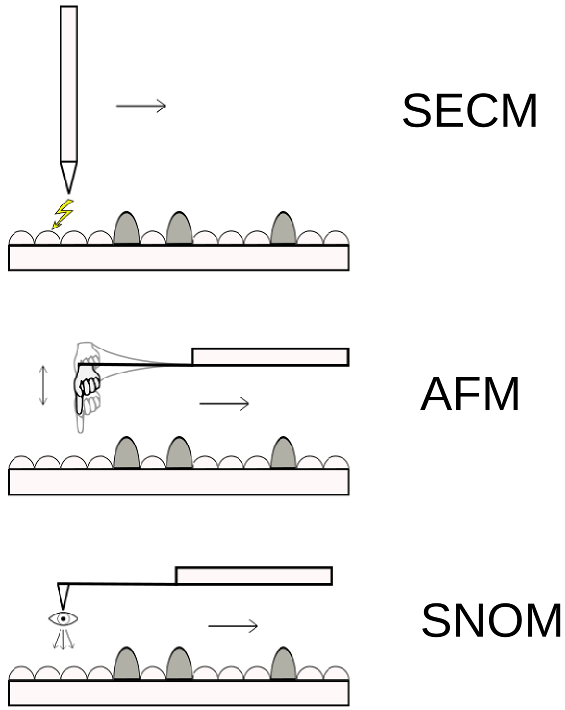
- Metal nanoparticles (gold)
- Polymer materials (functionalization, enzyme entrapment)

Scanning electrochemical microscopy (SECM)

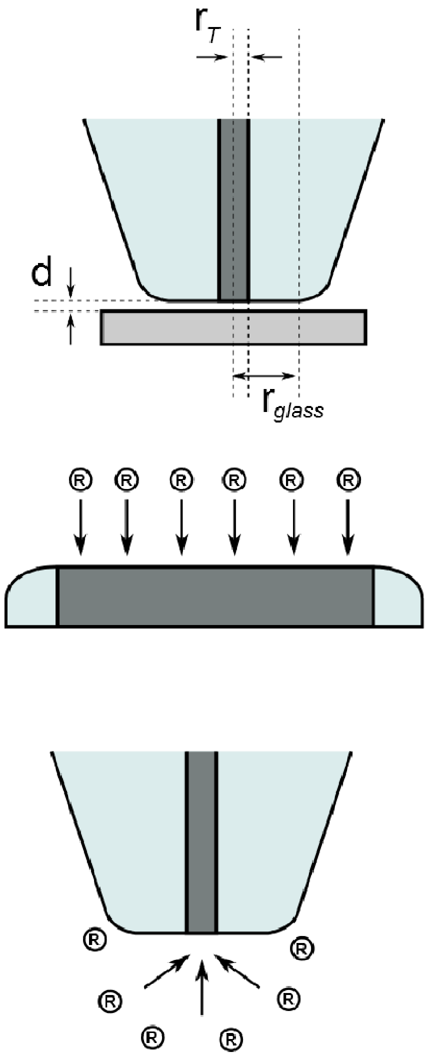
- **Belongs to Scanning Probe Microscopies (STM, AFM, SNOM *etc*.)**
- **Probe = microelectrode**

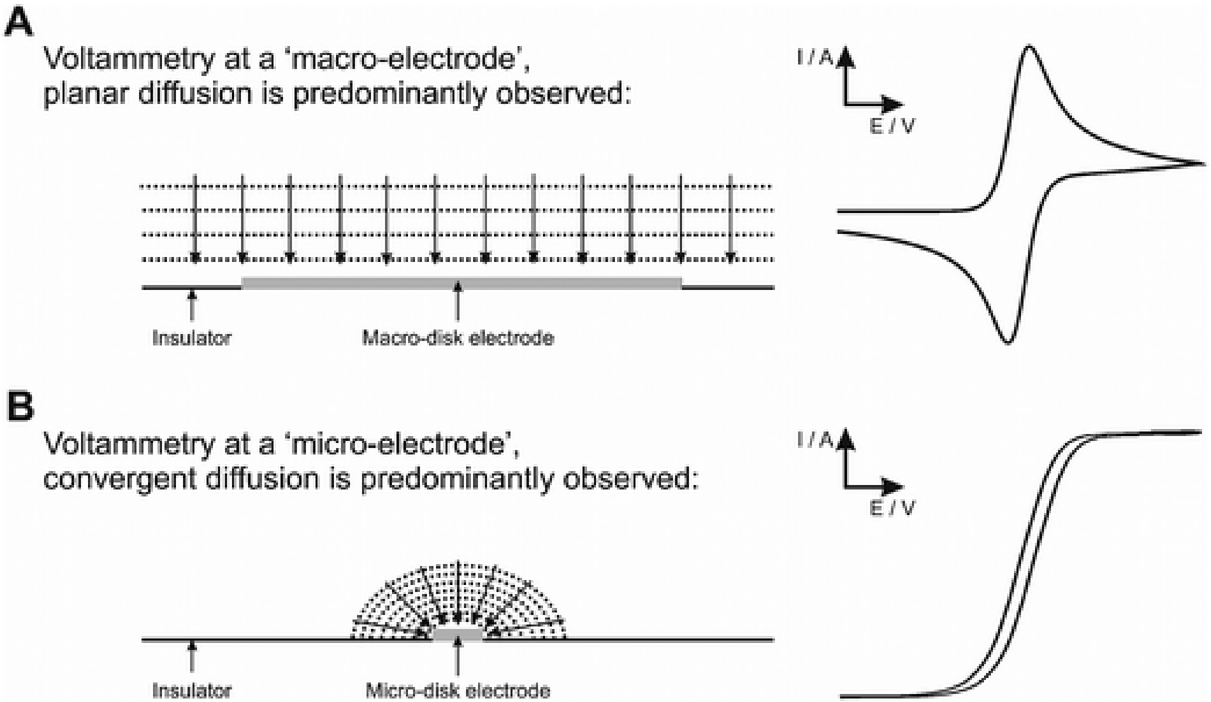
1 dimension in the range of micrometer (x10^{-6} m)





Microelectrodes
Electrode kinetics

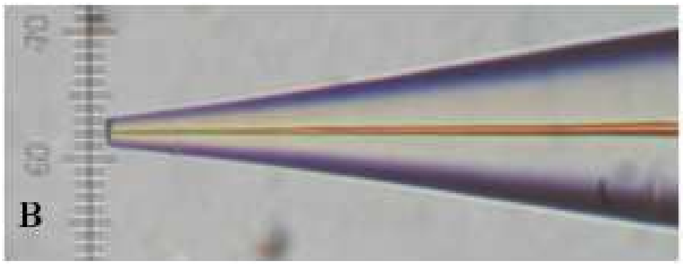


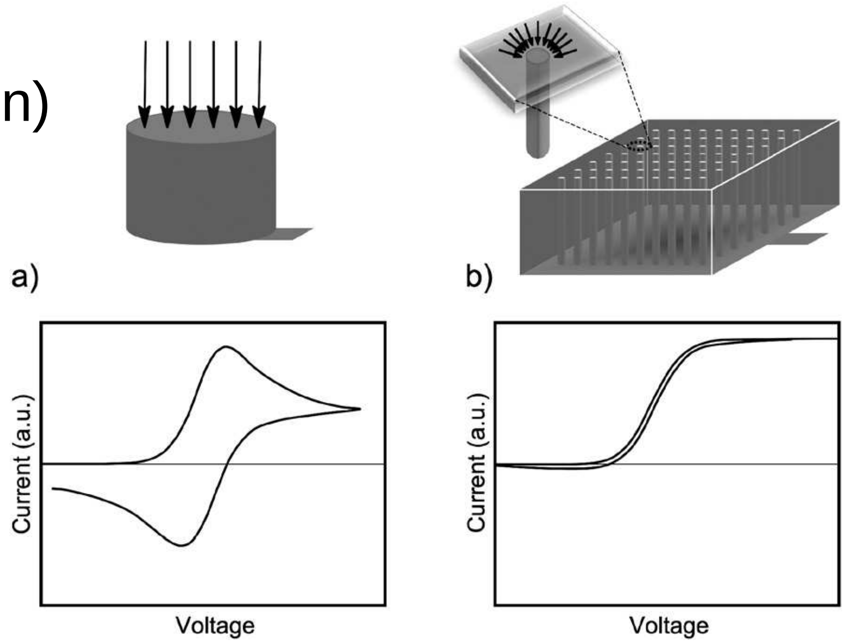


Brownson, Kampouris, Banks,
Chem Soc Rev 41 (2012) 6944

Microelectrodes

- Melting of Pt wire (25 µm) into glass capillary
- Electronic connection
- Grinding and polishing of the tip depending of the application
  - feedback mode
  - G/C mode (generation/collection)
  - *in-vivo* measurement

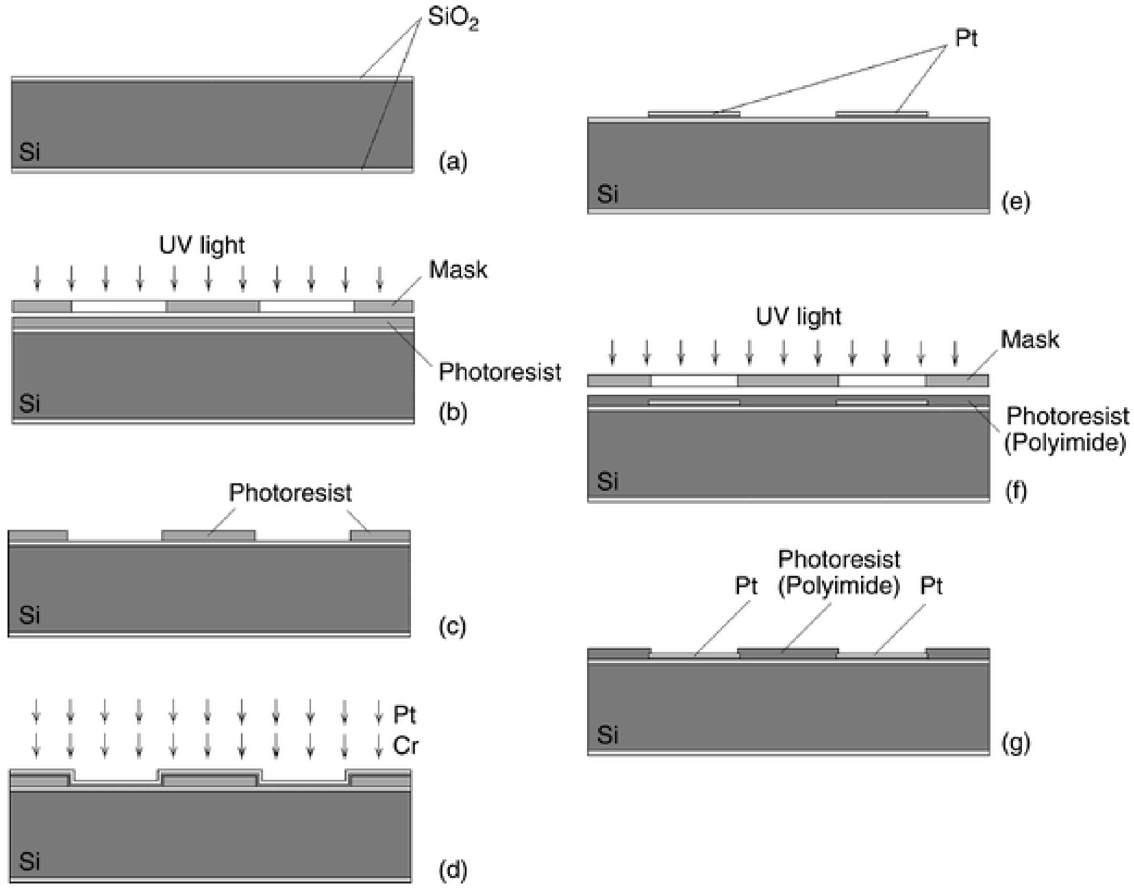




Wei, Bailey, Andrew, Ryhanen, Lab Chip 9 (2009) 2123

Microelectrodes - litography

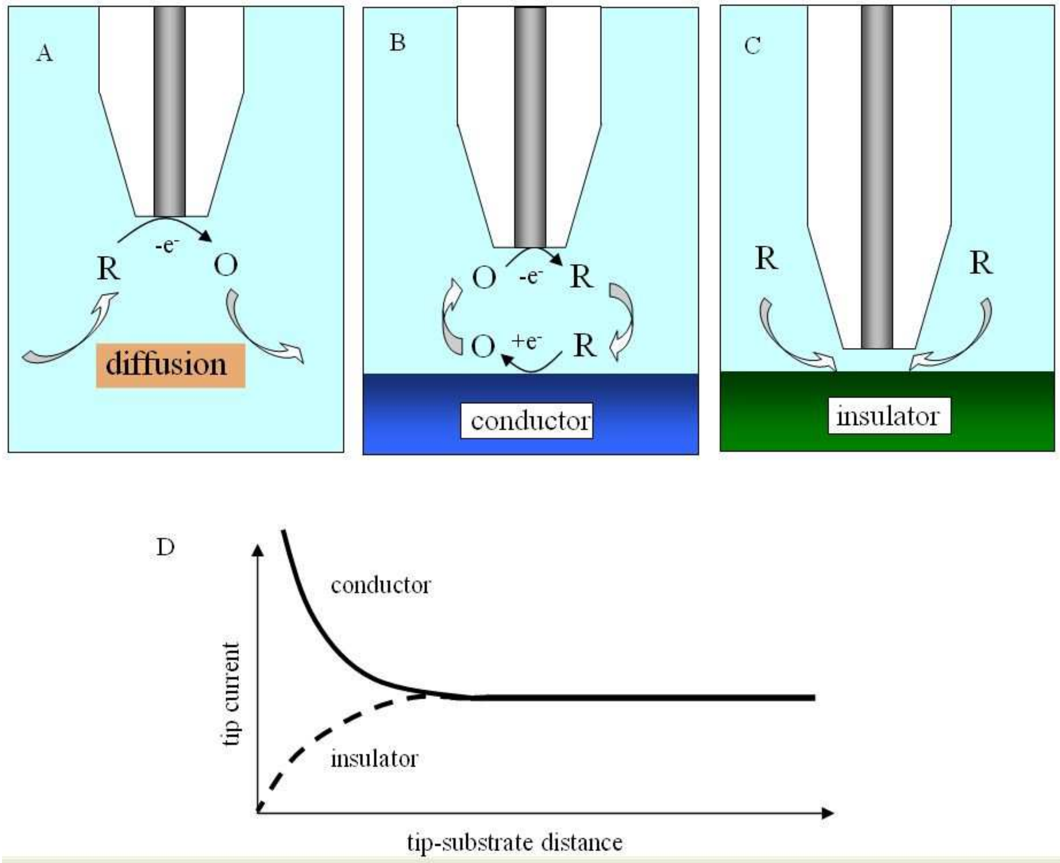
- NOT USABLE for SECM technology

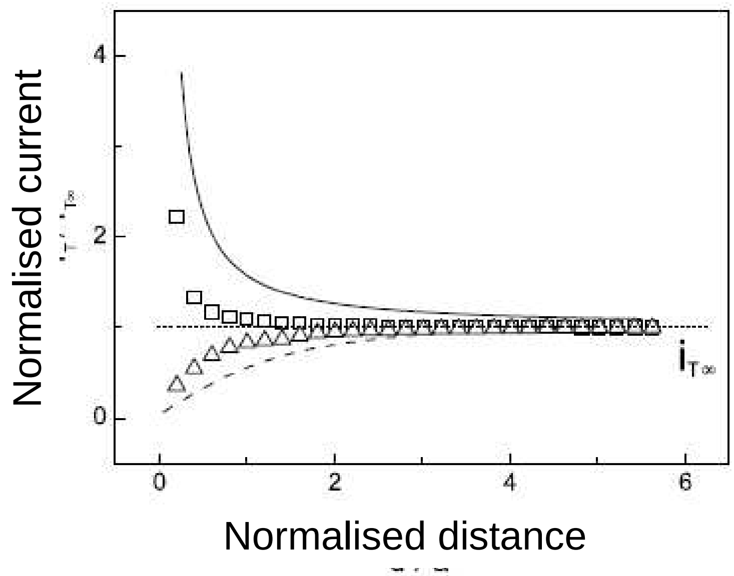


SECM - fundamentals

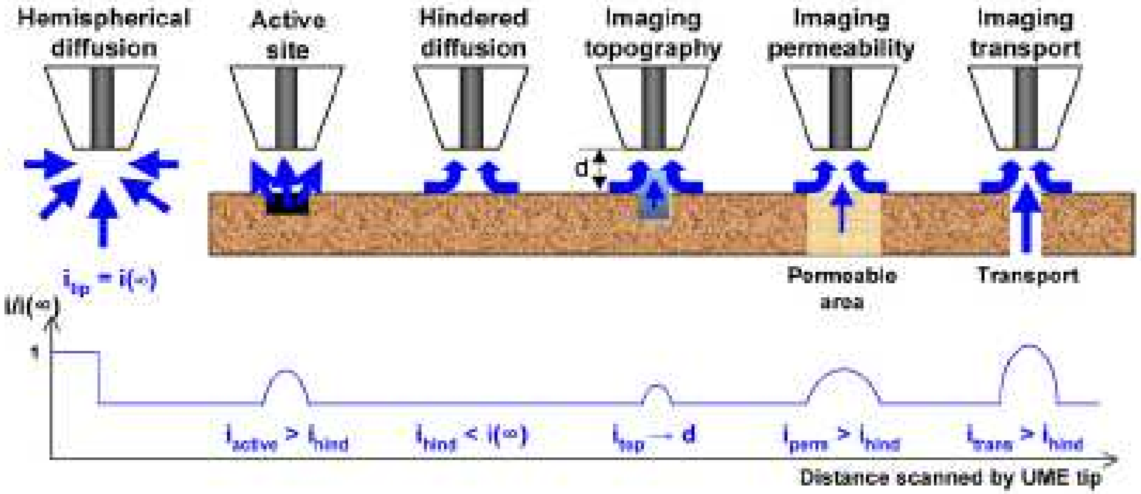
- Positive feedback
- Negative feedback

- Approaching curve





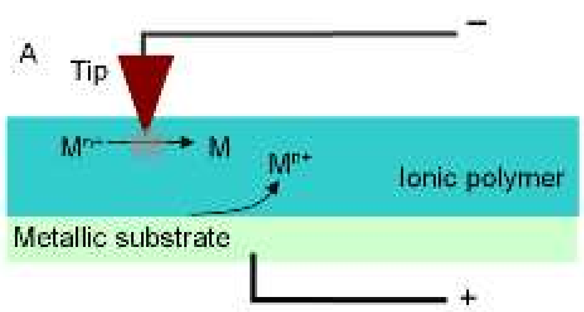
SECM - fundamentals

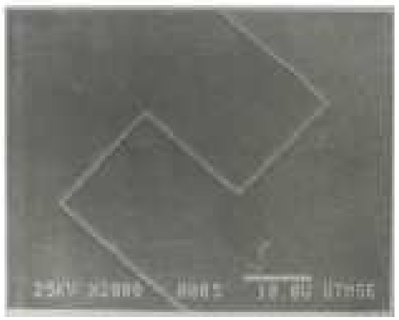


Edwards, Martin, Whitworth, Macpherson, Unwin, Physiol Meas 27 (2006) 63

SECM
Measuring applications

- Surface topography and chemical properties
- Activity of protein (enzymes, mediators)
- Permeability of membranes and channels
- Activity of individual cells and cell
- *In vivo* measurements
- …



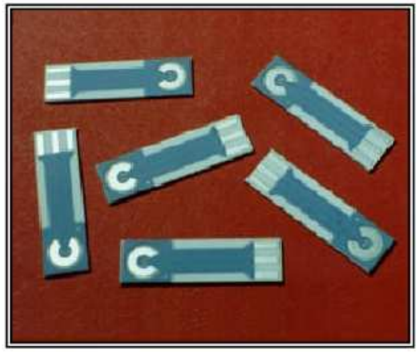


W. Schumann et. al.

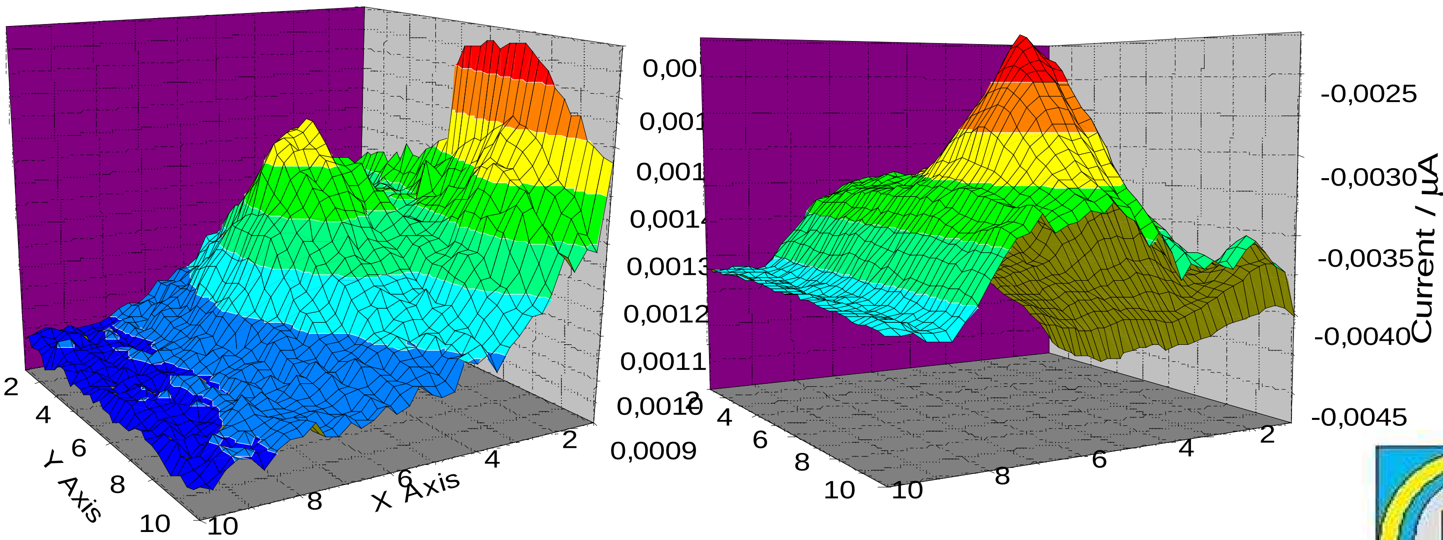
- Surfaces can be modified!!!

F. Laforge

Activity of immobilised enzymes
Glucose oxidase



- Glucose oxidase (GOD) immobilised
- 50 mM glucose
- Production of H_2O_2 or consumption of O_2



(a) (b)

Lacina, Skladal, Nagy, Chem Listy 106 (2012) 253