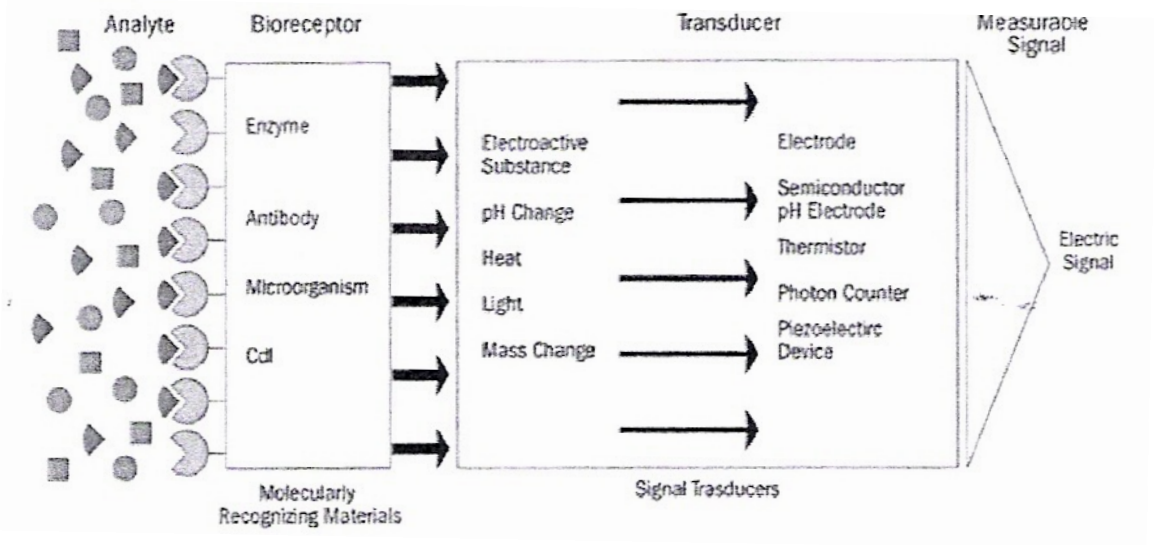
Biomedical and biotechnological applications of biomolecules:

Biosensors

Biosensors

- Biosensors are analytical instruments able to produce quantitative or semiquantitative information using a biological recognition element integrated with a transducer
- Biosensors convert a biochemical signal in a measurable electric signal
- Biosensors are constituted by a biological and a non biological component

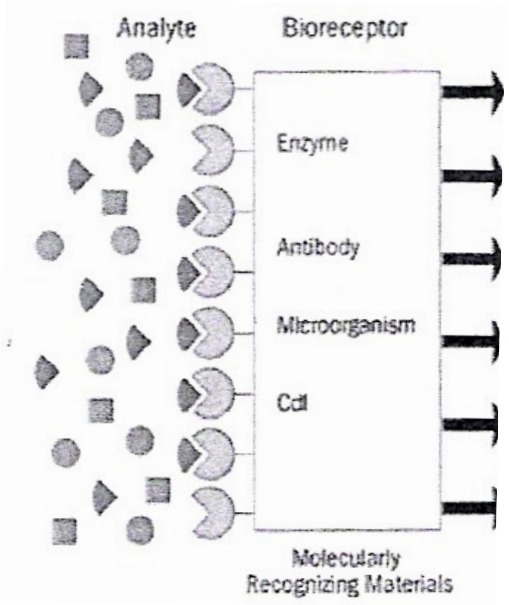


Biological component

- Generates the specific signal

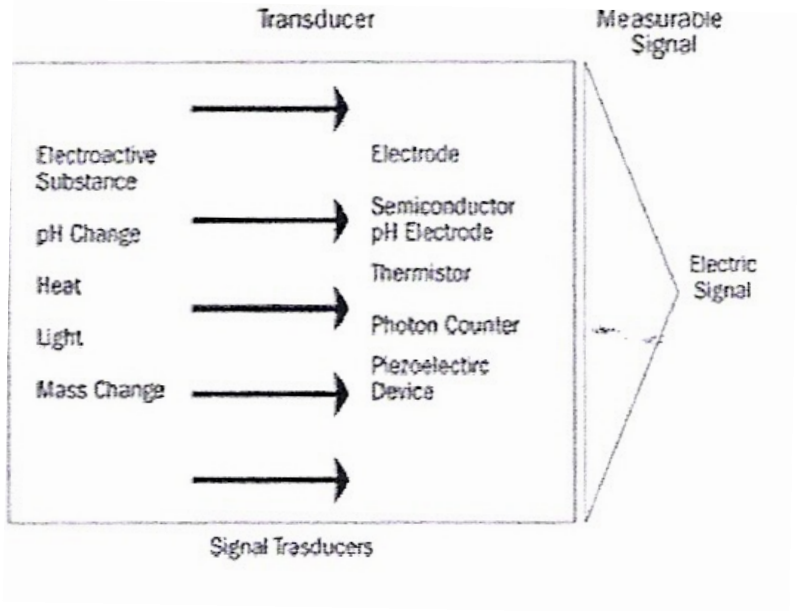
- Catalytic: Purified enzymes
  Microorganisms
  Cells or tissues

- Non catalytic: Antibodies
  Receptors
  Nucleic acids



Non biological component (transducer)

- Converts the biological signal in an easily measurable signal (electronic)
- Electrochemical transducer (potentiometric and amperometric)
- Optical transducer
- Thermal transducer
- Acoustic transducer



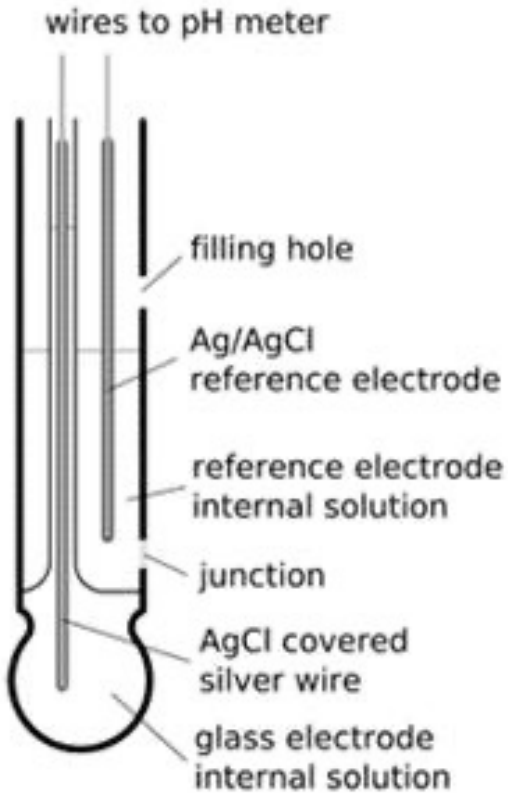
Electrochemical transducers

- Potentiometric:

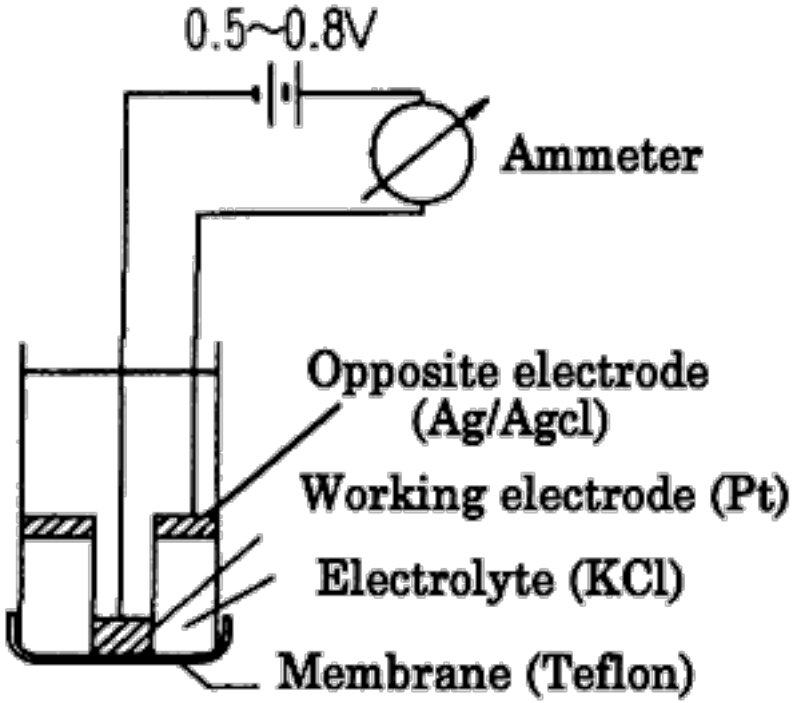
  electrochemical potential varies in function of ion concentration (es. pH electrode, ion-selective electrodes)
- Amperometric:

  the electrode potential is constant and it is sufficient to oxidize or reduce the species of interest (es. O_2 and H_2O_2 electrodes)

pH electrode



Clark electrode
to measure oxygen



Amperometric electrodes to measure O_2 and H_2O_2

- The Clark electrode to measure O_2 has a cathodic potential of -0.6V where oxygen is reduced to water

  Anode Ag: $4Ag^+ + 4Cl^- \rightarrow 4AgCl + 4e^-$

  Cathode Pt: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

- The electrode to measure H_2O_2 has an anodic potential of +0.68V where hydrogen peroxide is oxidized to oxygen

  Anode Pt: $H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$

  Cathode Ag: $2AgCl + 2e^- \rightarrow 2Ag^+ + 2Cl^-$

Oxygen and hydrogen peroxide are electrochemical species of biological interest because they are produced or consumed in many enzymatic reactions

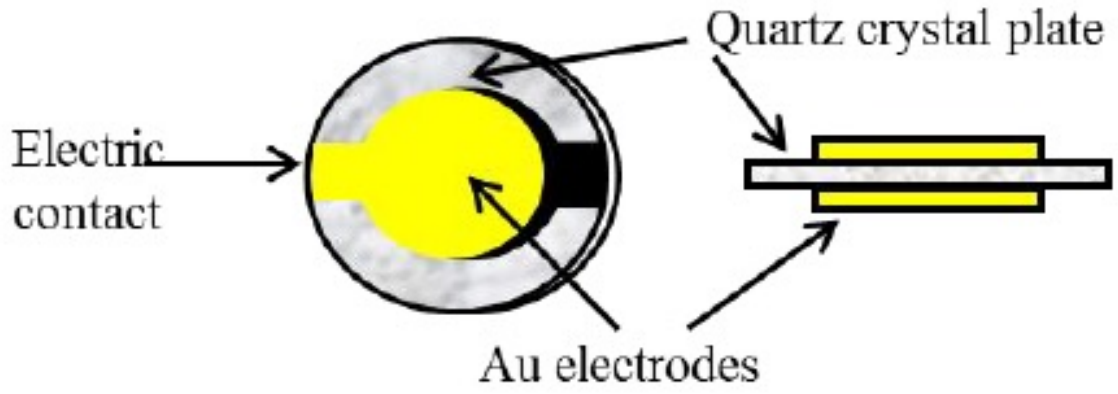
Optical transducer

- Optical fibers with the biocomponent at one end and components for excitation and detection of absorption and/or emission of light at the other end
- Useful when the enzyme reaction produces colored, fluorescent or luminescent products
- SPR technology

Acoustic transducer

- Piezoelectric quartz crystals in an alternating electric field are subject to mechanical deformation that induces resonance at a specific frequency.
- The resonance frequency depends on the mass of the crystal: mass changes are detected as variations in oscillation frequency of the crystal.
- The biocomponent (antibody, receptor or nucleic acid) is immobilized on the surface of the crystal.

Figure 3. Schematic of a piezoelectric quartz crystal.



$$\Delta F = (-2.3 \times 10^6) f_0^2 \frac{\Delta M}{A}$$

Methods of immobilization of biocomponents

- Adsorption on insoluble matrices
- Entrapment in a gel
- Cross-linking with multifunctional reagents
- Covalent binding on solid supports
- Screen-printed technology (miniaturization) and nanomaterials

It is necessary to preserve the biological activity of the biocomponent

Nanomaterials

Nanomaterials allow to functionalize electrodes and improve sensitivity (they are good electrical conducers)

- Graphene
  - monostrato di atomi di carbonio con ottime proprietà di stabilità meccanica, conducibilità elettrica e termica, biocompatibilità e elevato rapporto superficie/volume
- Carbon nanotubes
  - Graphene sheets forming tubes long from nm to μm
- Zinc oxide nanoparticles (ZnO)
- Gold nanoparticles

The Glucose biosensor

- DIABETES is a metabolic pathology where pancreas produces no insulin (type 1) or low levels (type 2). Insulin is necessary for glucose absorption by cells.
- High glicemia associated to diabetes causes damage to the vascular system, increased cardio-vascular risk, blindness, kidney damage.
- Over 170 million diabetics are estimated in the world.
- Frequent glicemia monitoring is critical to evaluate when to administer insulin.

Glucose biosensor

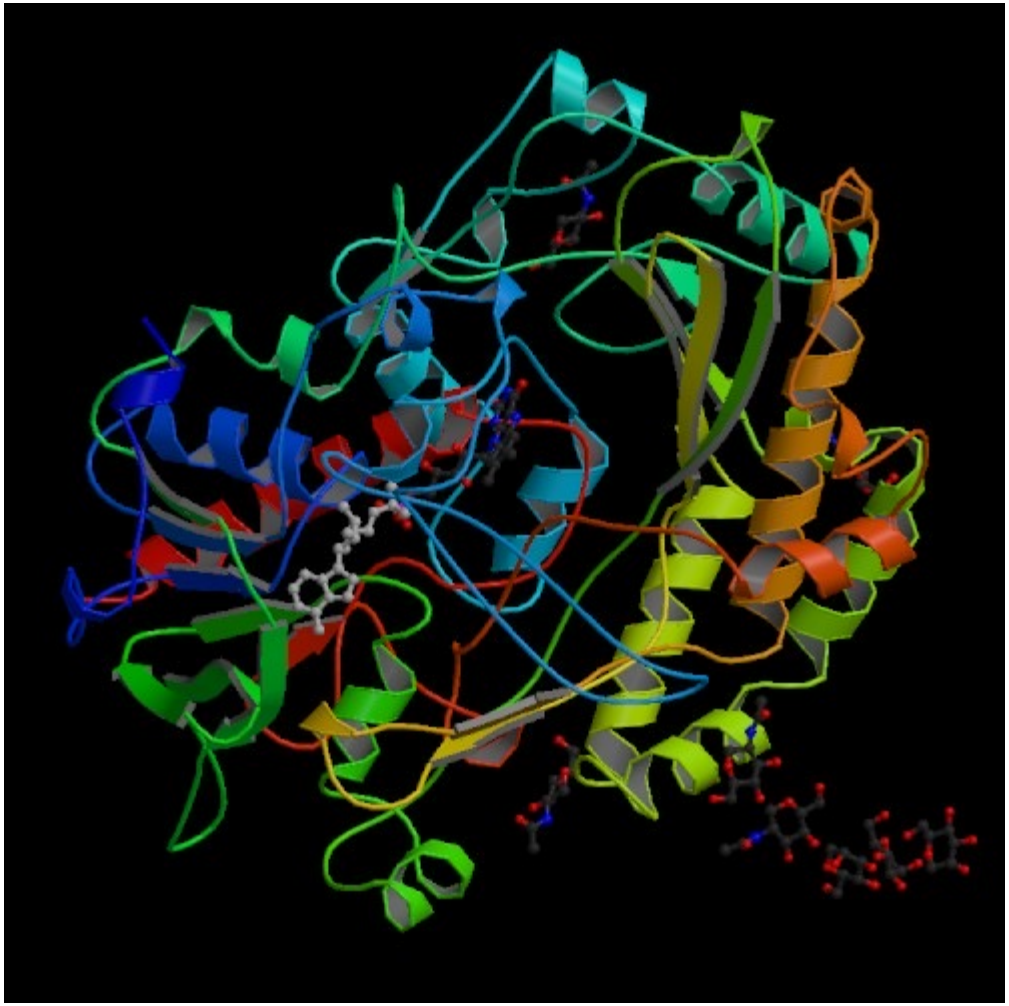
- The biological component is the enzyme glucose oxidase that calyzes the reaction:

$$\text{glucose} + O_2 \rightarrow \text{gluconate} + H_2O_2$$

$$\text{glucose} + FAD \rightarrow \text{gluconate} + FADH_2$$
$$FADH_2 + O_2 \rightarrow FAD + H_2O_2$$

- The enzyme requires oxygen for reoxidation of the coenzyme $FADH_2$ produced in the first phase of the reaction

Structure of glucose oxidase of *Aspergillus niger*



Glucose biosensor

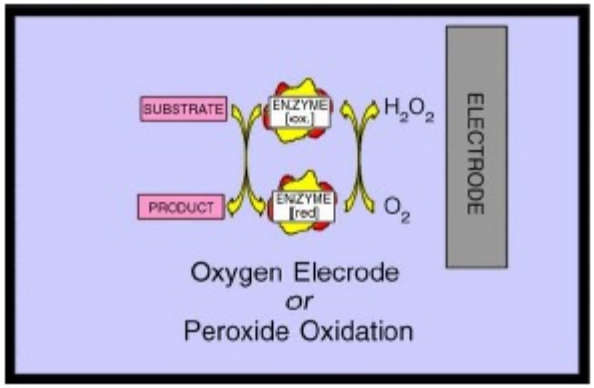


Fig. 2. First generation glucose biosensor schematic.

- **Transducer**:
  Clark electrode to measure O_2
  Electrode to measure H_2O_2
  Current will be proportional to concentration of O_2 or H_2O_2 and thus to the amount of glucose in the sample
- Glucose oxidase is immobilized on a nylon or cellulose membrane in contact with the electrode

- Problems:
  oxygen diffusion
  low selectivity of the H_2O_2 electrode due to high anodic potential

Second generation biosensors

- Use of chemical mediators that replace oxygen for reoxidation of $FADH_2$ (E_0 - 220 mV) and are reoxidized at the electrode

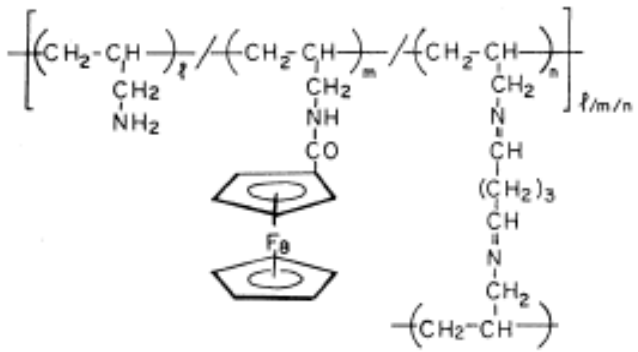


Fig. 7. Ferrocene containing cross-linked polyallylamine.

Enzyme	Mediator	E_0 (mV)
Glucose oxidase	1, 1-dimethyl ferrocene	100
	ferrocene	165
	hydroxymethyl ferrocene	185
	vinyl ferrocene	250
	ferrocene carboxylic acid	275
	$[Ru(CN)_6]^{4-}$	685
	TTF (tetrathia fulvalene)	300
	$[Fe(CN)_6]^{4-}$	180
	NMP (N-methyl fenazinium)	-161

Second generation biosensors

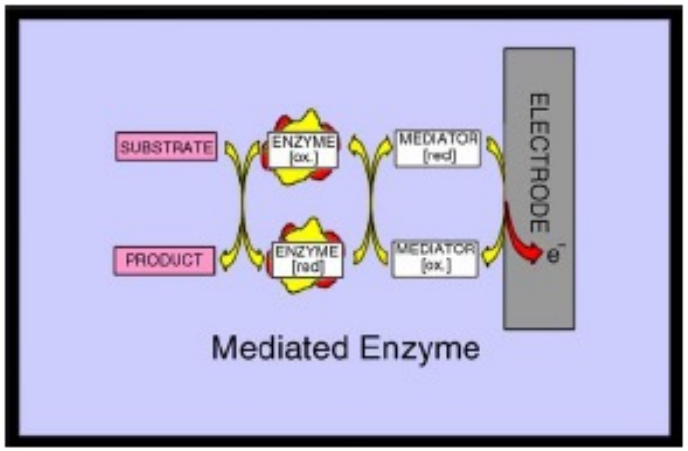


Fig. 4. Mediated biosensor schematic.

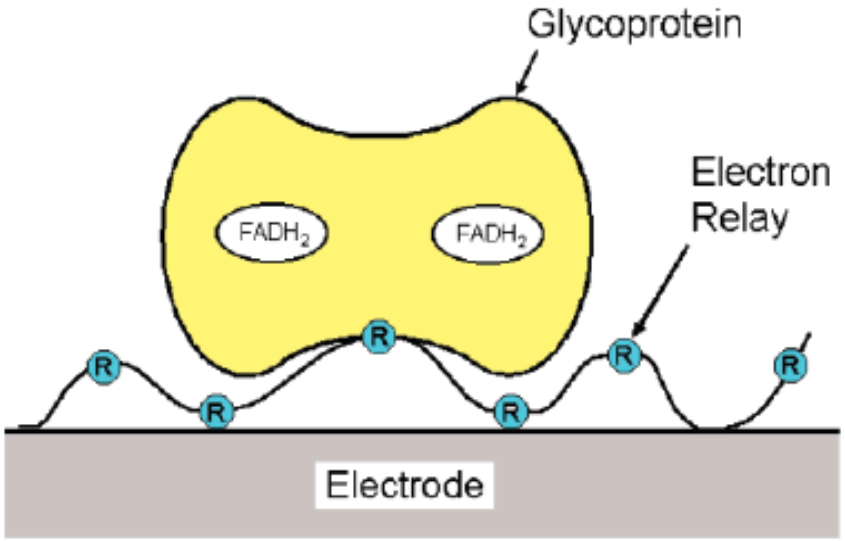
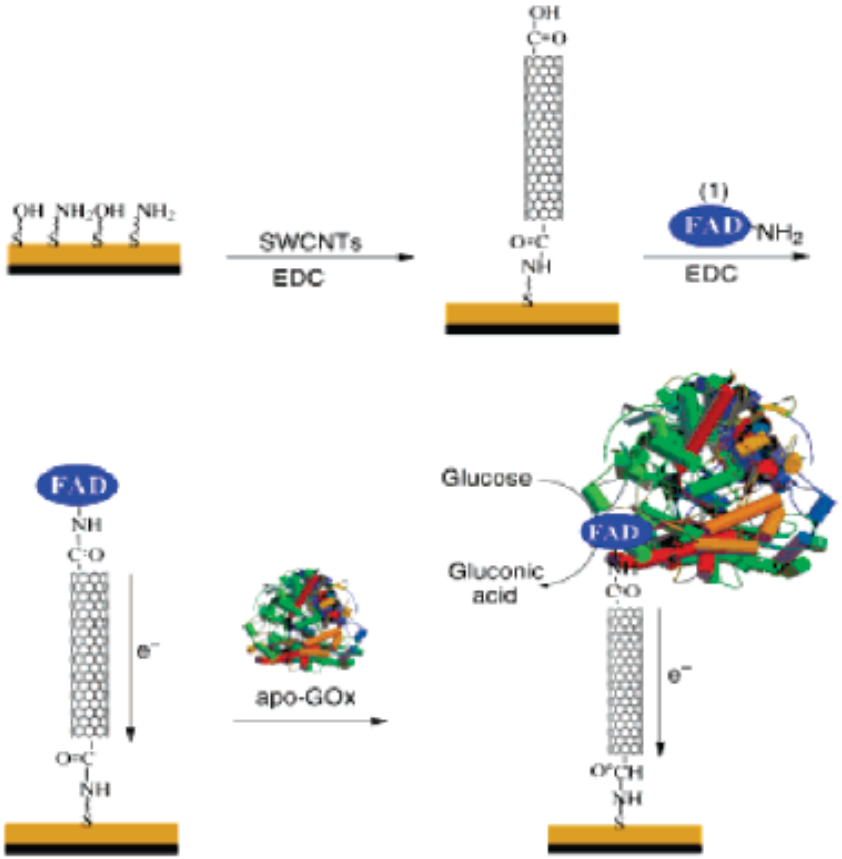


Figure 2. Use of a redox polymer for wiring GOx: efficient electrical communication between the redox center of the enzyme and electrode surfaces.

Biosensors based on novel materials: carbon nanotubes with immobilized FAD



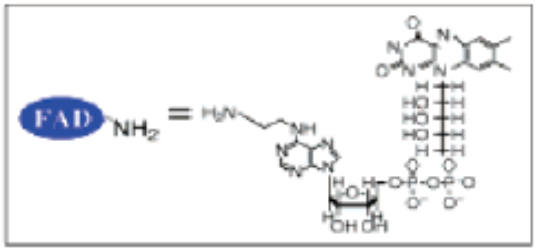


Figure 3. Carbon nanotube (CNT) connectors with long-range electrical contacting. Assembly of the CNT electrically contacted glucose oxidase electrode. (Reprinted with permission from ref 59. Copyright 2004 Wiley-VCH.)

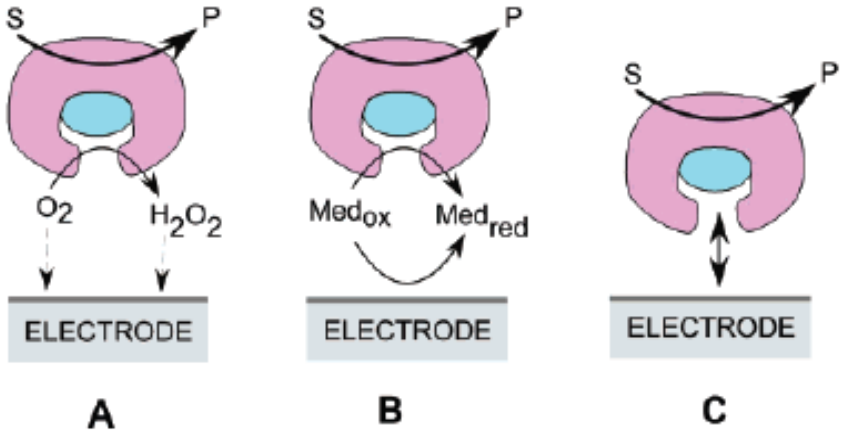


Figure 4. Three generations of amperometric enzyme electrodes for glucose based on the use of natural oxygen cofactor (A), artificial redox mediators (B), or direct electron transfer between GOx and the electrode (C).

Commercially available biosensors for glicemia monitoring

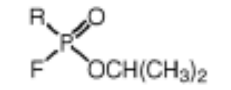
Fig. 6. Original MediSense products.

Fig. 9. Precision Xtra™ glucose/ketone monitor.

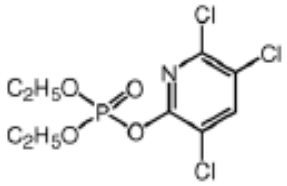
Fig. 13. OneTouch ultra blood glucose biosensor.

Fig. 14. LifeScan UltraSmart system.

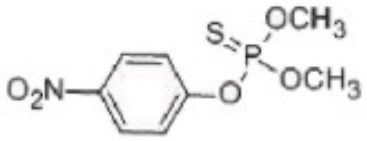
Organophosphate pesticides



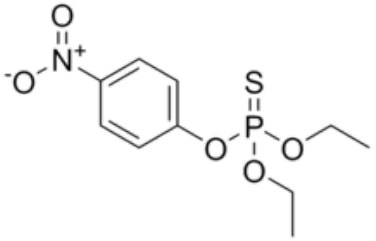
DFP [R=$(CH_3)_2CHO$]
sarin (R=CH_3)



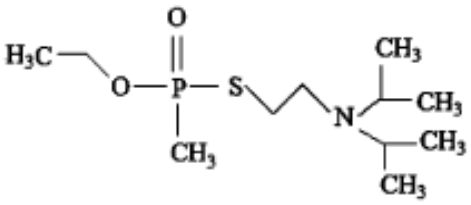
chlorpyrifos oxon (CPO)

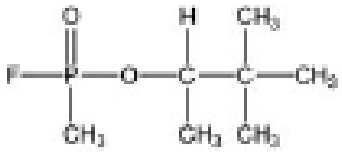


Methyl parathion (OPs)

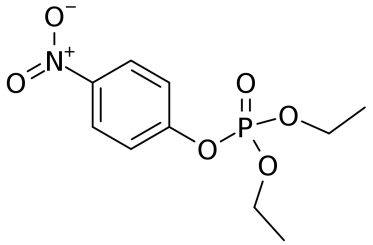


VX

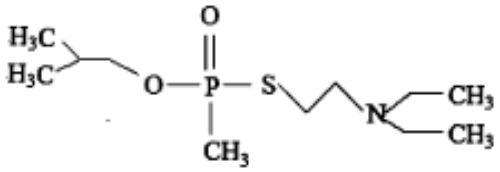




Soman



R-VX



Organophosphate pesticides are inhibitors of acetylcholinesterase

Table 1 Classification of pesticides with their biological effects

Class	Group	Chemical Name	Target sites/effects
Insecticides	Organochlorines	DDT	Liver and lungs [57] Reproductive system [58] Immune system [59]
		Hexachlorohexane	Immune system [60] Liver [57] Blood dyscrasias anemia [61] Reproductive system [62]
	Organophosphates	Chlorpyrifos	Immune system [63] AChE activity in developing fetus [64] Mammalian cell cultures [65] Neurodevelopmental disorders [66]
		Methyl parathion	Neurotoxic effects (CNS) [21, 67, 68]
		Malathion	Reproductive system [69] AChE activity [70] Lipid peroxidation [71] Genotoxic effects [72]
	Pyrethroids	Allethrin, Permethrin	Neurotoxic effects and NA^{+}-K^{+} ions channels [73, 74]
	Carbamates	Aldicarb, Carbaryl Propoxur	Nervous system [75, 76]
Fungicides	Dicarboximide	Mancozeb	Endocrine disruptor [77]
	Dithiocarbamates	Vinclozolin, Asomate, Amobam,	Antiandrogenic effects [78]
	Organomercuricals	Methyl mercury, Phenyl mercuric acetate	Central nervous system [79]
Herbicides	Sulfonylureas	Chlorosulfuran	Embryo development [80]
	Chlorophenoxy compounds	MCPA, MCPP	Human carcinogens [81]
		2,4-D	Gastrointestinal and peripheral neuromuscular systems [82]

DDT dichlorodiphenyltrichloroethane, *AChE* acetylcholinesterase, *CNS* central nervous system, *MCPA* 4-chloro-2-methyl phenoxyacetic acid, *MCPP* 2-(4-chloro-2 methylphenoxy) propionic acid, *2,4-D* 2,4-dichlorophenoxy acetic acid

Biosensors for organophosphate pesticides

- Biocomponent:

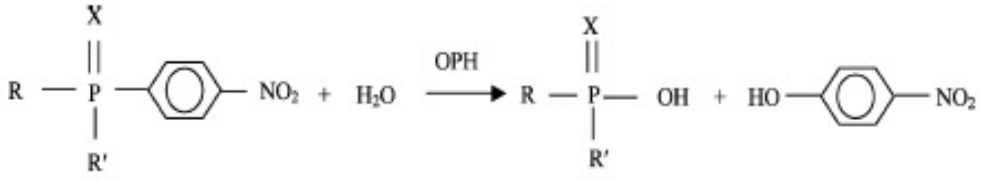
  the enzyme organophosphate hydrolase (OPH) of *Pseudomonas diminuta*, catalyzes the reaction

- *E. coli* cells expressing recombinant OPH / purified OPH
- Immobilization:

  cryopolymerization in polyvinyl alcohol

  covalent binding on nylon membranes

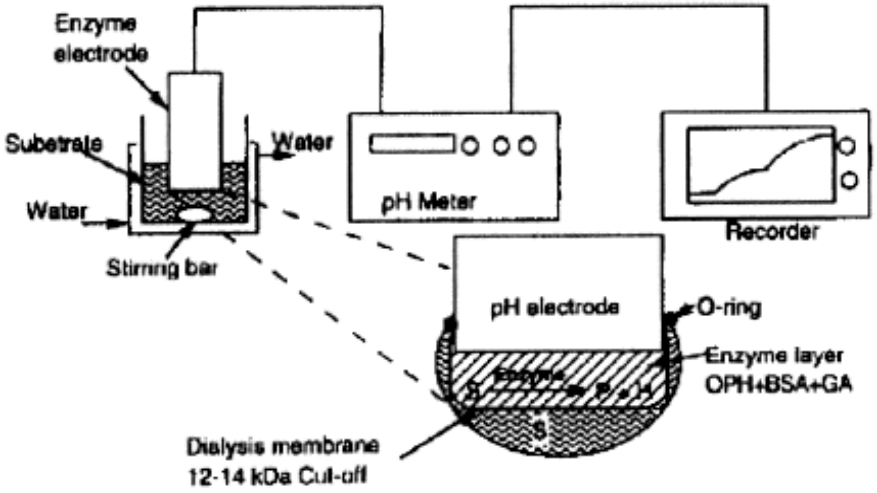
Biosensors for organophosphate pesticides

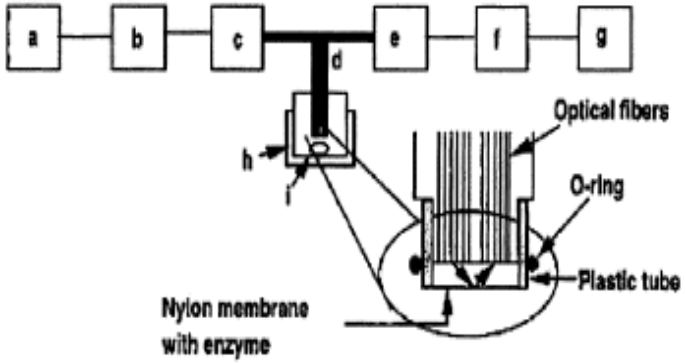


- Transducer:

  pH electrode

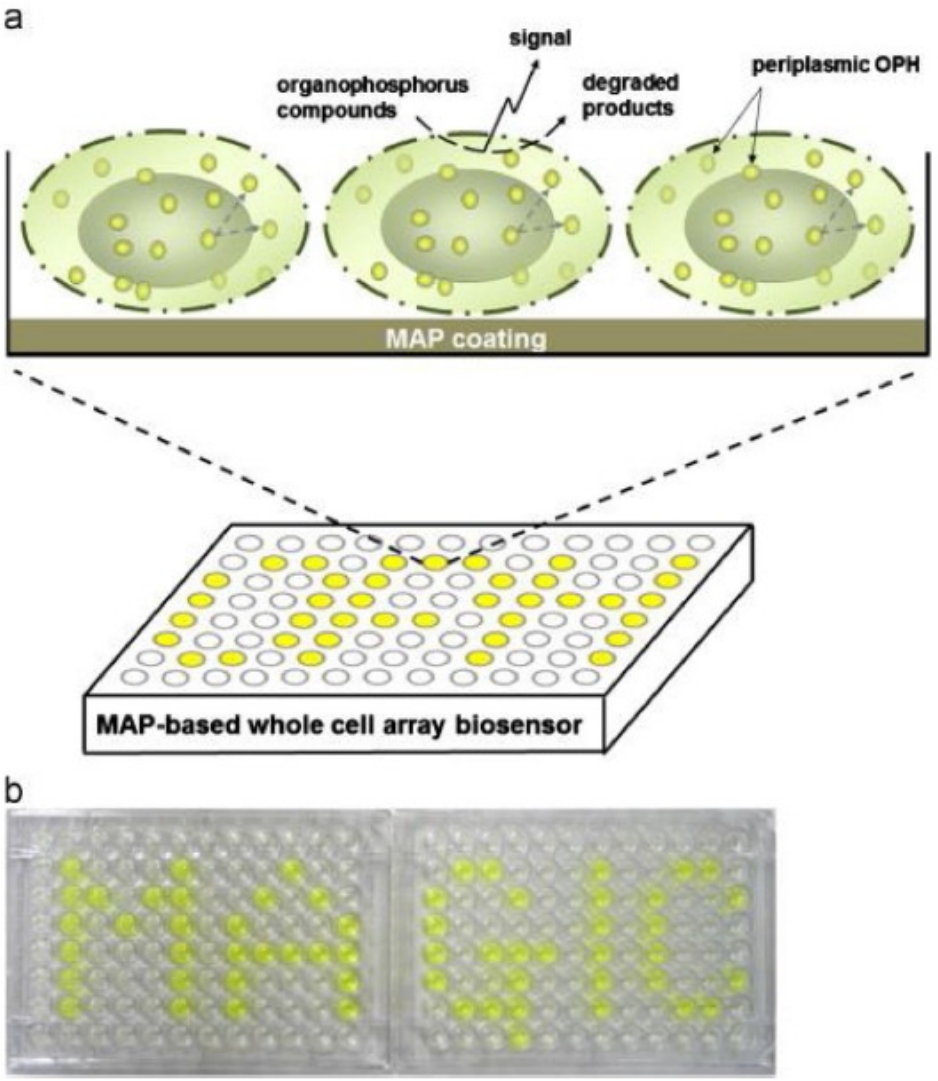
  optical fiber





Whole cell OPH sensor for organophosphate pesticides

MAP: mussel adhesive protein



Biosensors for organophosphate pesticides

- Biocomponent:

The enzyme acetylcholinesterase catalyzes the reaction:

Acetyl-(thio)-choline ➔ (thio)-choline + acetate

- Transducer:

Amperometric electrode that measures current produced by oxidation of thio-choline

The biosensor is immersed in a solution containing the pesticide and incubated 10-30 min, then residual activity of acetylcholinesterase is measured

Principle of analysis of biosensors based on AChE inhibition

(A)

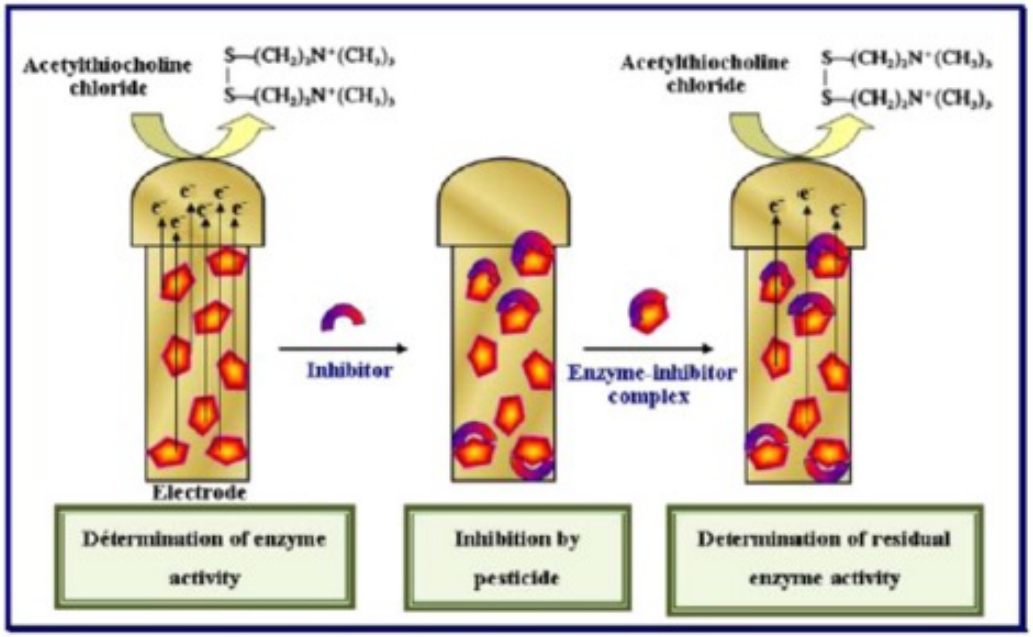
$(CH_3)_3\overset{+}{N}CH_2CH_2SCOCH_3 \xrightarrow{AChE} (CH_3)_3\overset{+}{N}CH_2CH_2SH + CH_3COOH$

Acetylthiocholine — thiocholine

$2(CH_3)_3\overset{+}{N}CH_2CH_2SH \xrightarrow{electrode} \begin{matrix} SCH_2CH_2\overset{+}{N}(CH_3)_3 \\ | \\ SCH_2CH_2\overset{+}{N}(CH_3)_3 \end{matrix} + 2H^+ + 2e^-$

Thiocholine (oxidation state) — (reduction state)

(B)



Biosensors and Bioelectronics 30 (2011) 43–48

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Site-specific immobilization of a (His)6-tagged acetylcholinesterase on nickel nanoparticles for highly sensitive toxicity biosensors

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[b] Université de Perpignan Via Domitia, IMAGES EA4218, 52 Av Paul ALDUY 66860 Perpignan Cedex, France

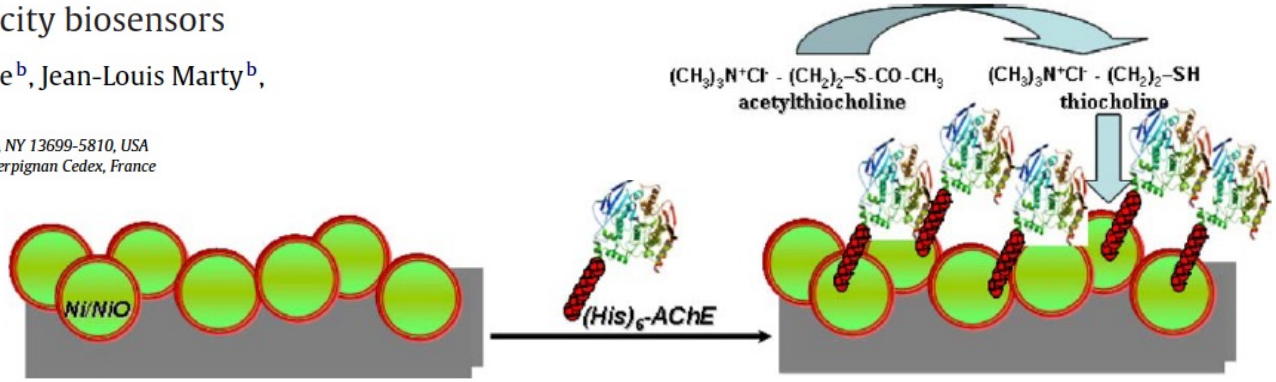

Fig. 1. Schematic representation of the oriented immobilization of the AChE enzyme onto the SPE-Ni/NiO modified electrode though the (His)6 residue.

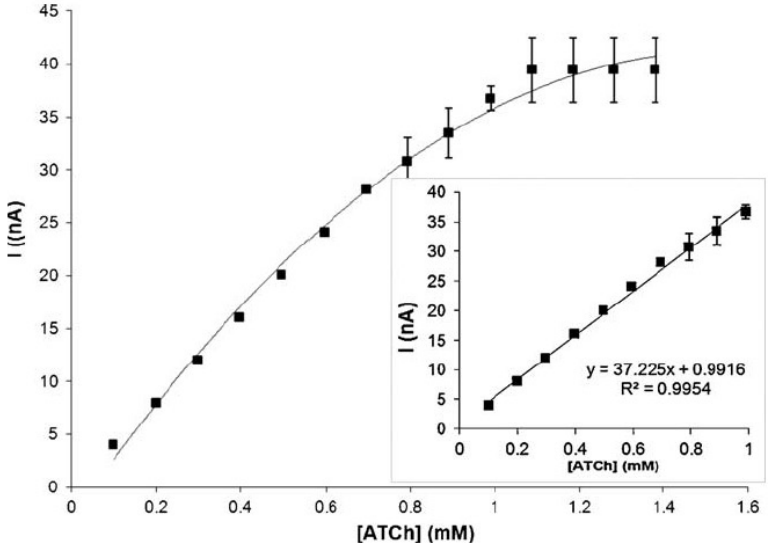

Fig. 4. Calibration curve of the (His)6-AChE biosensor to acetylthiocholine (ATCh) substrate.

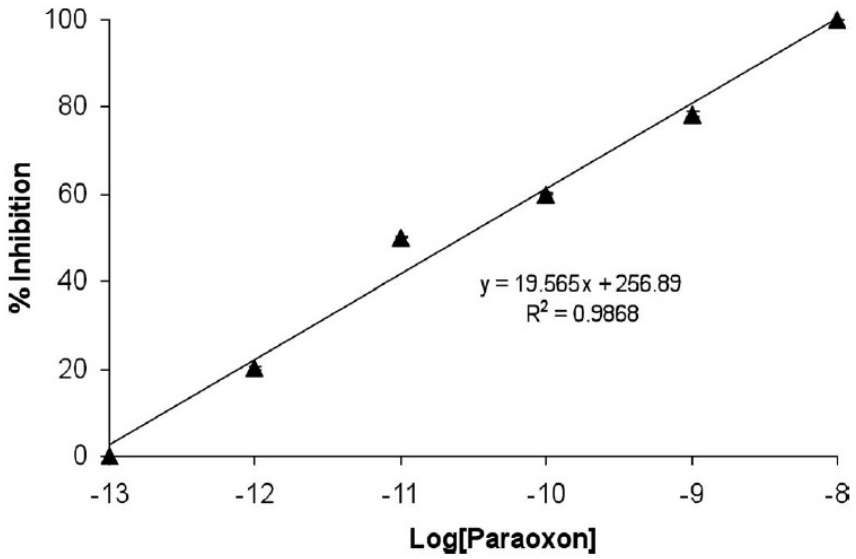

Fig. 5. Inhibition curves of the immobilized (His)6-AChE onto Ni/NiO modified screen-printed electrodes by paraoxon after 20 min incubation.

Biosensors based on AChE

- To improve sensitivity and selectivity
  - Enzymes from different sources (*Torpedo*, *Drosophila*…)
  - Mutagenesis of the substrate binding pocket

Table 1
Comparison of limits of detection for AChEs (from different sources) when applied to OP analytes.

Enzyme Source	Electrode material	Detection technique	Limit of Detection (mol L^{-1})	Analyte	Incubation time
*dm*AChE (E69Y Y71D)	AChE/Carbon pellet	Amperometry-FIA	$\sim 1 \times 10^{-17}$	Dichlorvos	10 min
*ee*AChE	AChE/Carbon pellet	Amperometry-FIA	1×10^{-8}	Dichlorvos	10 min
*dm*AChE (E69Y Y71D)	AChE/Carbon pellet	Amperometry-FIA	1×10^{-12}	Paraoxon	10 min
*dm*AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-17}$	Dichlorvos	NR
*dm*AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-16}$	Parathion	NR
*dm*AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-16}$	Azinphos	NR
*dm*AChE	AChE/PVA-SbQ/SPE	Amperometry	7×10^{-11}	Dichlorvos	10 min
*ee*AChE	AChE/PVA-SbQ/SPE	Amperometry	6×10^{-7}	Dichlorvos	10 min

Note: NR, not reported.

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A screen-printed, amperometric biosensor array incorporated into a novel automated system for the simultaneous determination of organophosphate pesticides☆

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[c] CCFRA, Chipping Campden, Gloucestershire, GL55 6LD, UK
[d] Gwent Electronic Materials Ltd., Monmouth House, Mamhilad Park, Pontypool, Gwent, NP4 0HZ, UK

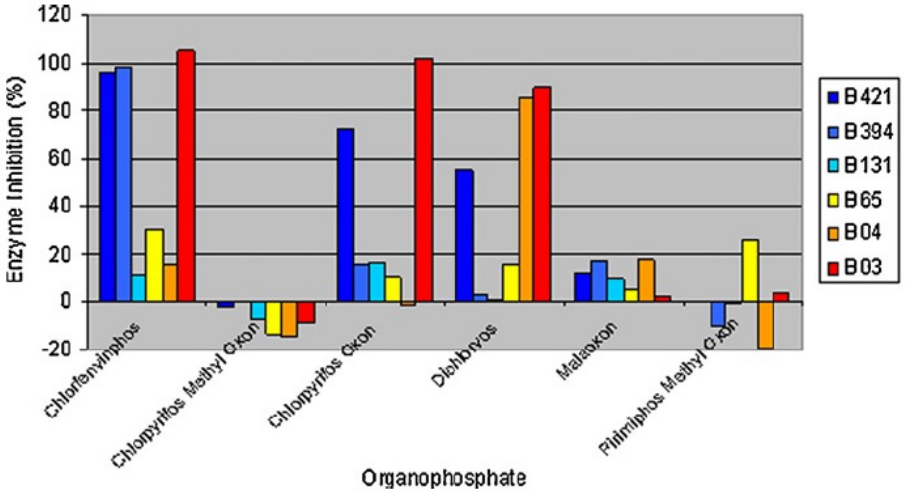

Fig. 5. Inhibition profiles constructed from the inhibition of each of the enzymes by each of the OPs at a concentration of 10^{-6} M.

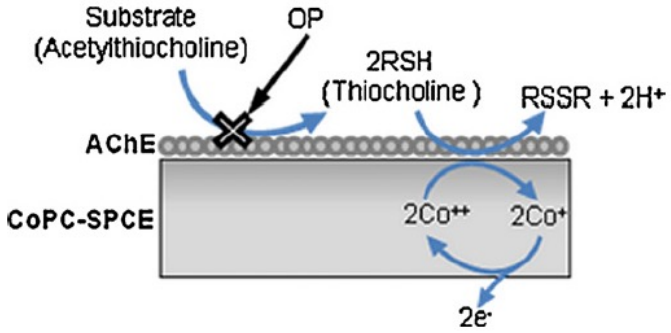

Fig. 2. Schematic diagram showing the reactions taking place during the operation of the proposed amperometric biosensor.

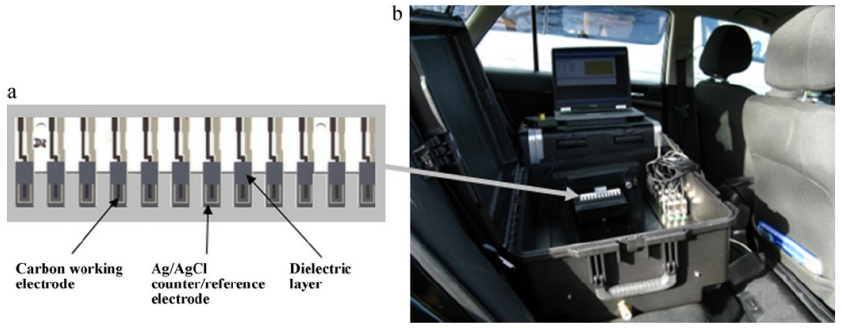

Fig. 1. (a) Electrode array comprising 12 screen-printed carbon electrodes modified with CoPC and an Ag/AgCl counter/reference electrode printed on an alumina substrate (b) array in the prototype biosensor system operating in the field powered from a car battery via the lighter socket.

Research Article

Portable Bioactive Paper-Based Sensor for Quantification of Pesticides

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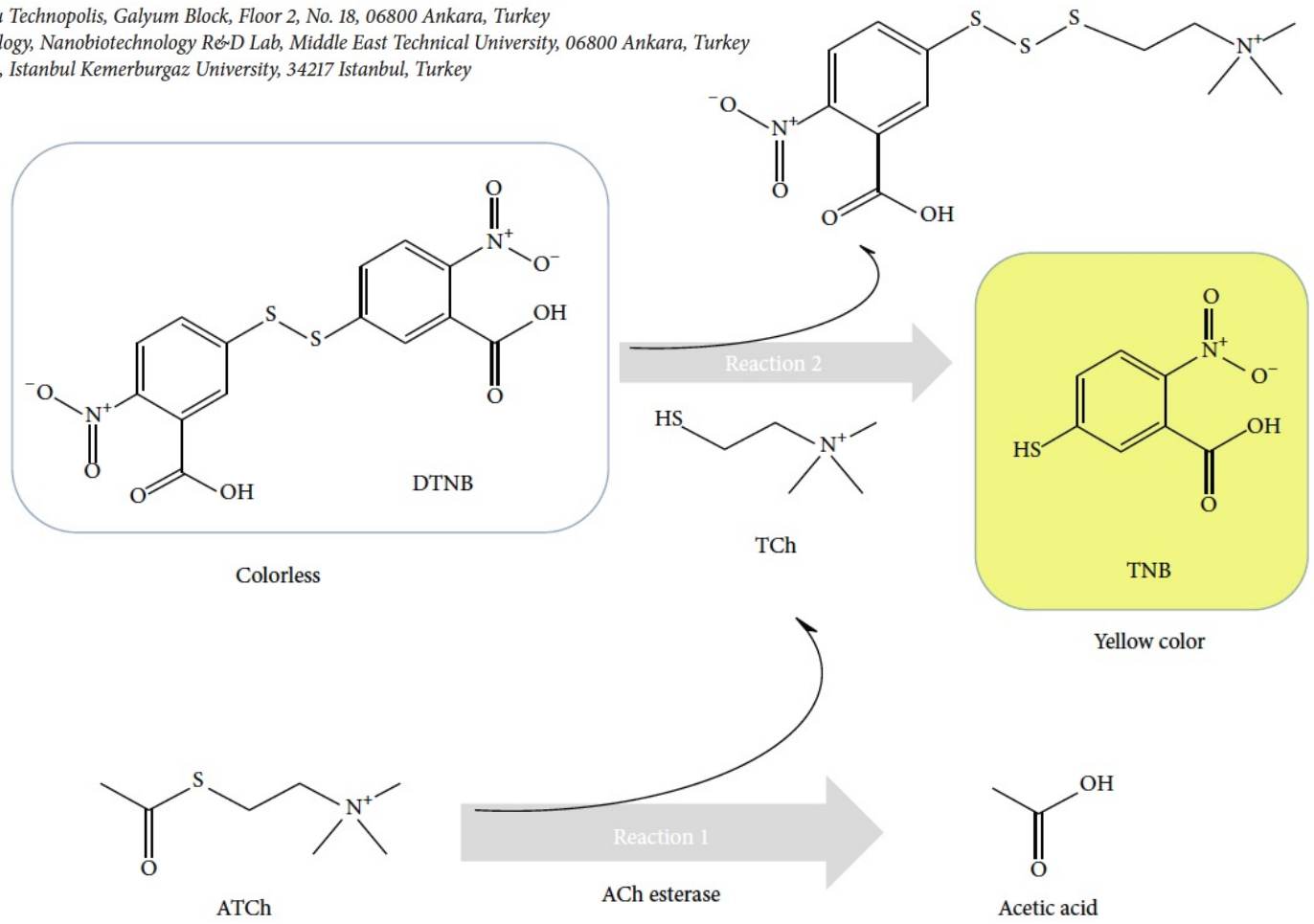


FIGURE 2: Two-step sequential reactions of acetylthiocholine (ATCh) for production of yellow colored TNB. In reaction 1, ATCh is broken to acetic acid and thiocholine (TCH). The free sulfhydryl group of TCh is quantified through Ellman's method in reaction 2. The resulting TNB is used in a direct determination of the activity of ACh esterase.

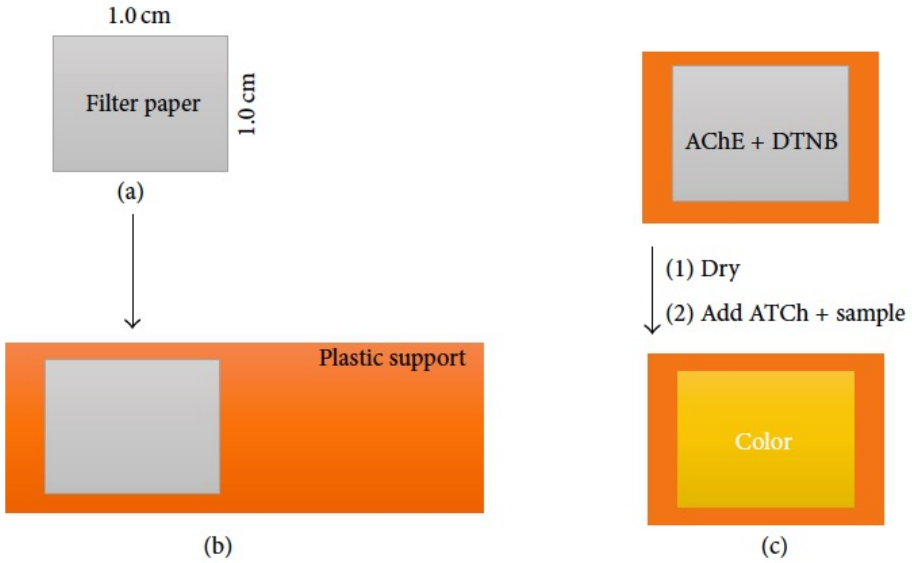
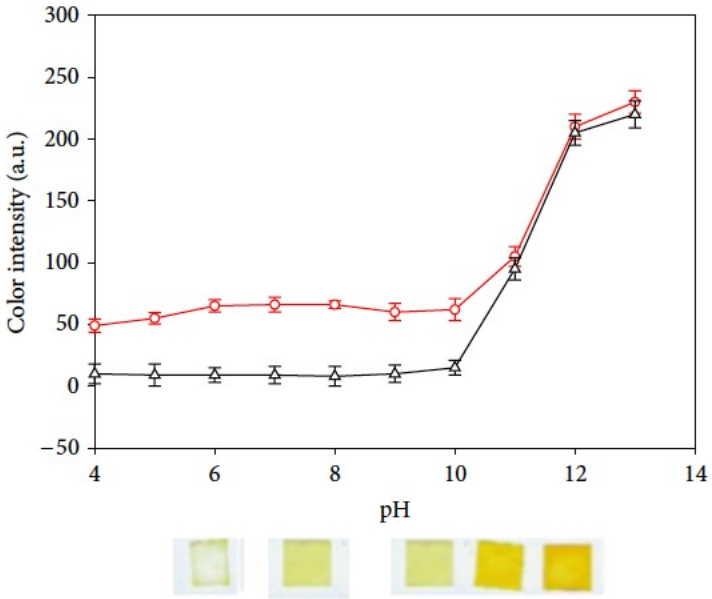


FIGURE 1: Schematic representation of biosensor support construction. (a) Munktell filter papers were cut and (b) fixed on a plastic support. (c) The enzyme mixture (AChE and DTNB) was directly applied on the fixed paper and dried. The samples with ATCh were directly applied on dried paper strips for color formation.



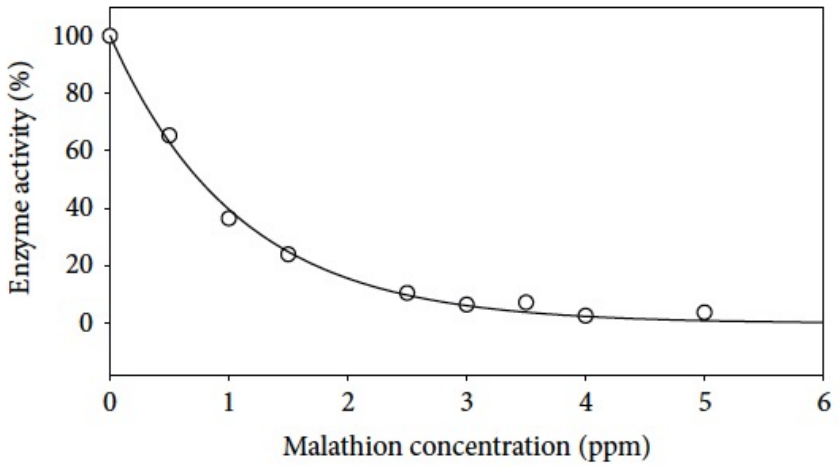
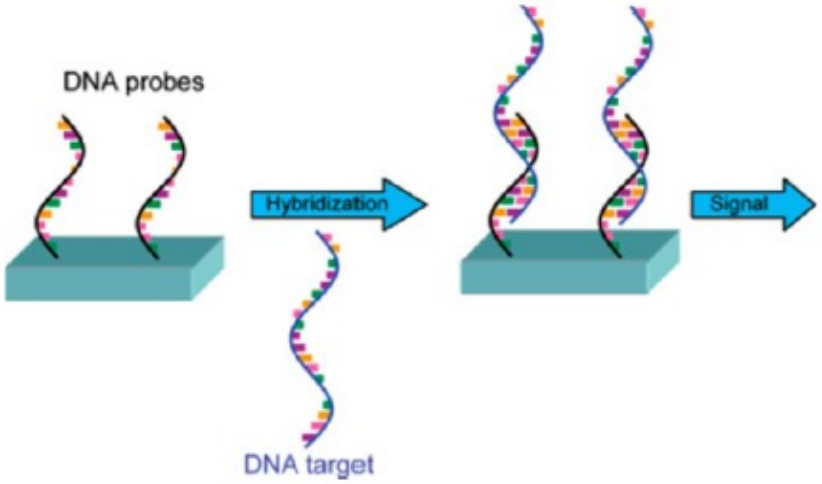


FIGURE 6: Relationship between inhibition of AChE activity and malathion.

Nucleic acid-based biosensors

- Exploit the interaction between DNA fragments used as probes and complementary sequences present in the sample
- Higher stability and immobilization efficiency compared to protein probes
- Hybridization between probe and complementary DNA must generate a measurable signal



Detection of genetically modified organisms (GMO)

- GMO are defined as organisms with a genome modified by introduction of an exogenous gene that produces a protein that confers new properties (es. resistance to herbicides, antibiotics, virus or insects)
- Exogenous DNA is inserted in a gene 'cassette' that contains all elements necessary for expression: a promoter and a terminator
- The cauliflower mosaic virus 35S promoter (CaMV) and the *Agrobacterium tumefaciens* Tnos terminator are used for production of most commercial transgenic plants

Detection of genetically modified organisms (GMO)

Table 2
A labeling system and threshold level of GM crops/products in major countries.
Source: (EC), 1829/2003 and 1830/2003 (Regulation (EC) 1829/2003).

Country	Labeling type	Threshold level	Product/process	Country	Labeling type	Threshold level	Product/process
China	Mandatory	0%	Process	Indonesia	Mandatory	5%	Product
EU	Mandatory	0.9%	Process	Taiwan	Mandatory	5%	Product
Russia	Mandatory	0.9%	Product	Thailand	Mandatory	5%	Product
Australia–New Zealand	Mandatory	1%	Product	Canada	Voluntary	5%	Product
Brazil	Mandatory	1%	Process	Hong-Kong	Voluntary	5%	Product
Saudi Arabia	Mandatory	1%	Product	Japan	Mandatory	5%	Product
Israel	Mandatory	1%	Product	Philippine	Mandatory	5%	Product
Korea	Mandatory	3%	Product	South Africa	Voluntary	–	Product
Chile	Mandatory	2%	Product	USA	Voluntary	–	Product
Philippines	Mandatory	5%	Product	Argentina	Voluntary	–	Product

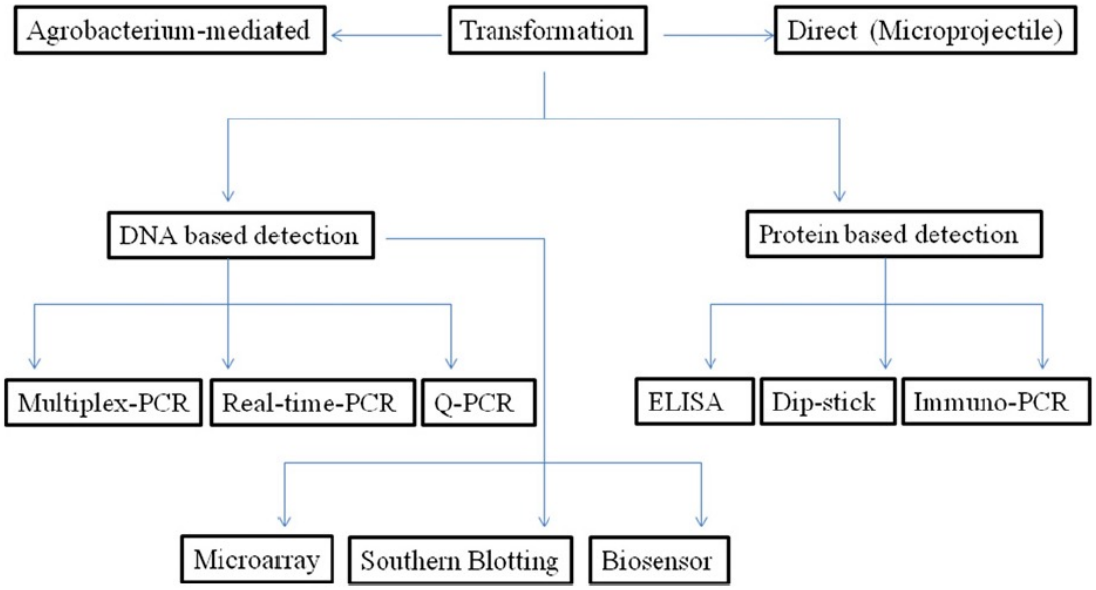


Fig. 5. A schematic view of detection methods for GM crops.

Biosensors for detection of genetically modified organisms (GMO)

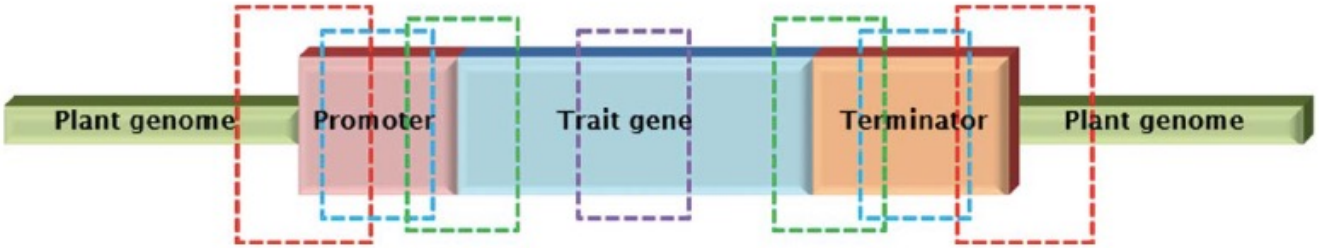


Figure 1. Levels of specificity of GMO methods based on the targeted DNA region: screening (blue-dashed square), gene-specific (purple square), construct-specific (green), and event-specific (red). Adapted from Holst-Jensen et al. [16].

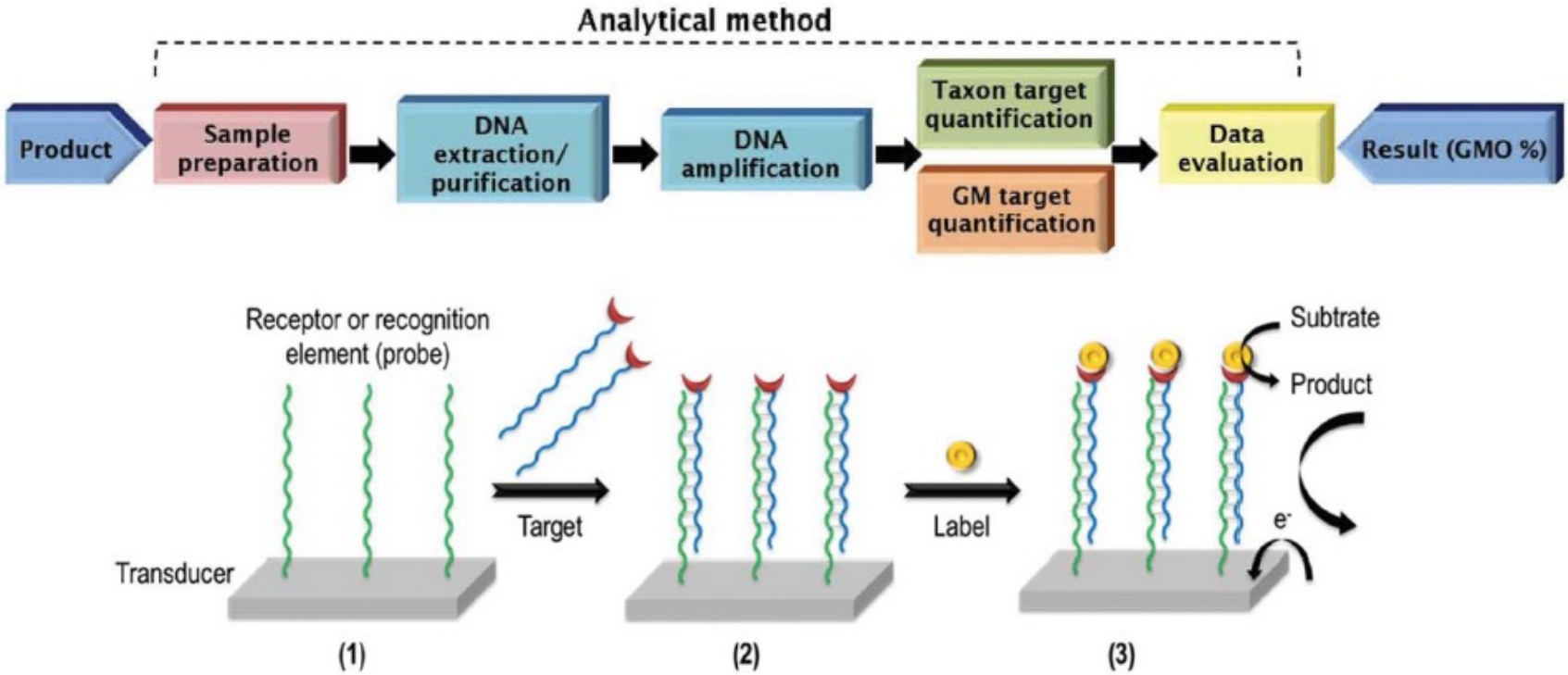


Figure 3. Steps in the design of a genosensor with a labeled target.

Biosensors for detection of genetically modified organisms (GMO)

- Biocomponent: oligonucleotides with P35S and Tnos complementary sequence
- Transducer: 10 MHz piezoelectric quartz crystals sandwiched between two gold electrodes
- Principle of analysis:

  PCR on the sample to amplify 35S and Tnos DNA regions

  hybridization of the PCR product with the probe immobilized on the crystal

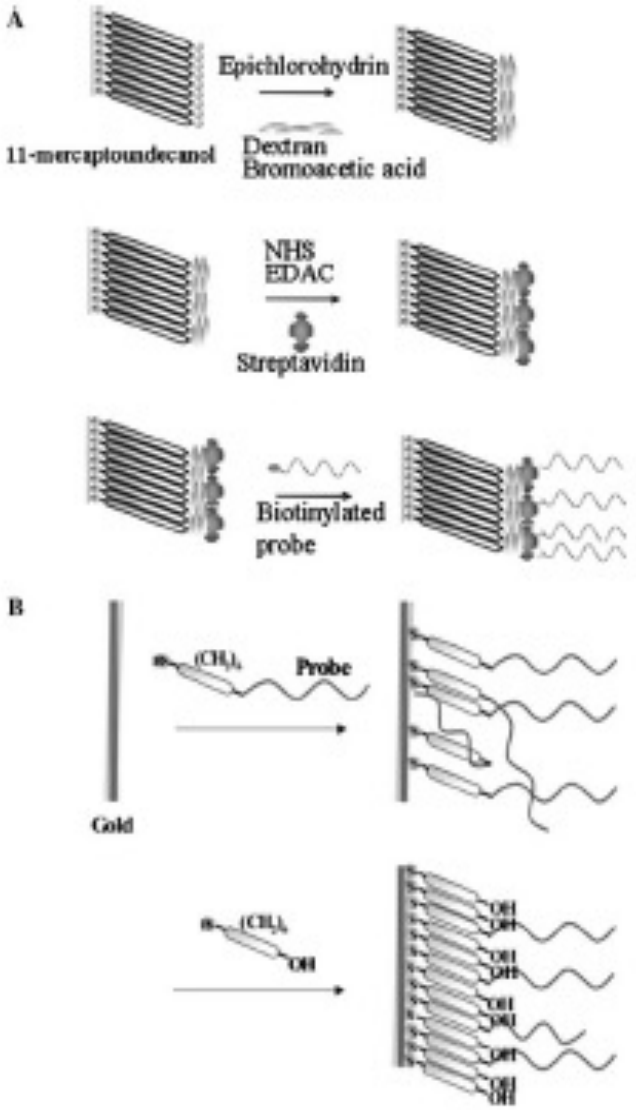
  variations in oscillation frequency indicate that the sample contained 35S and Tnos DNA

Immobilization of probes

Probes are immobilized on gold electrodes

A) 11-mercaptoundecanol and carboxylated dextran to immobilize avidin (via NHS-EDC coupling) that will bind biotinylated probes

B) Probe derivatized with a C6-SH group is immobilized directly on the gold surface



Structure of avidin

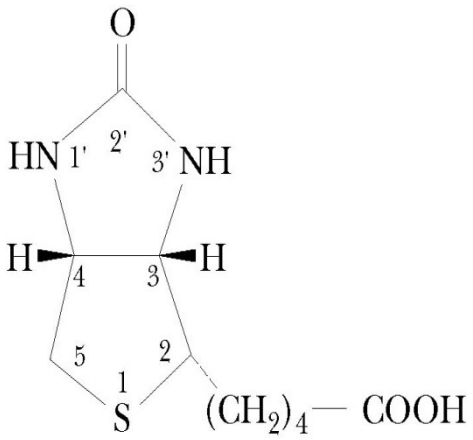
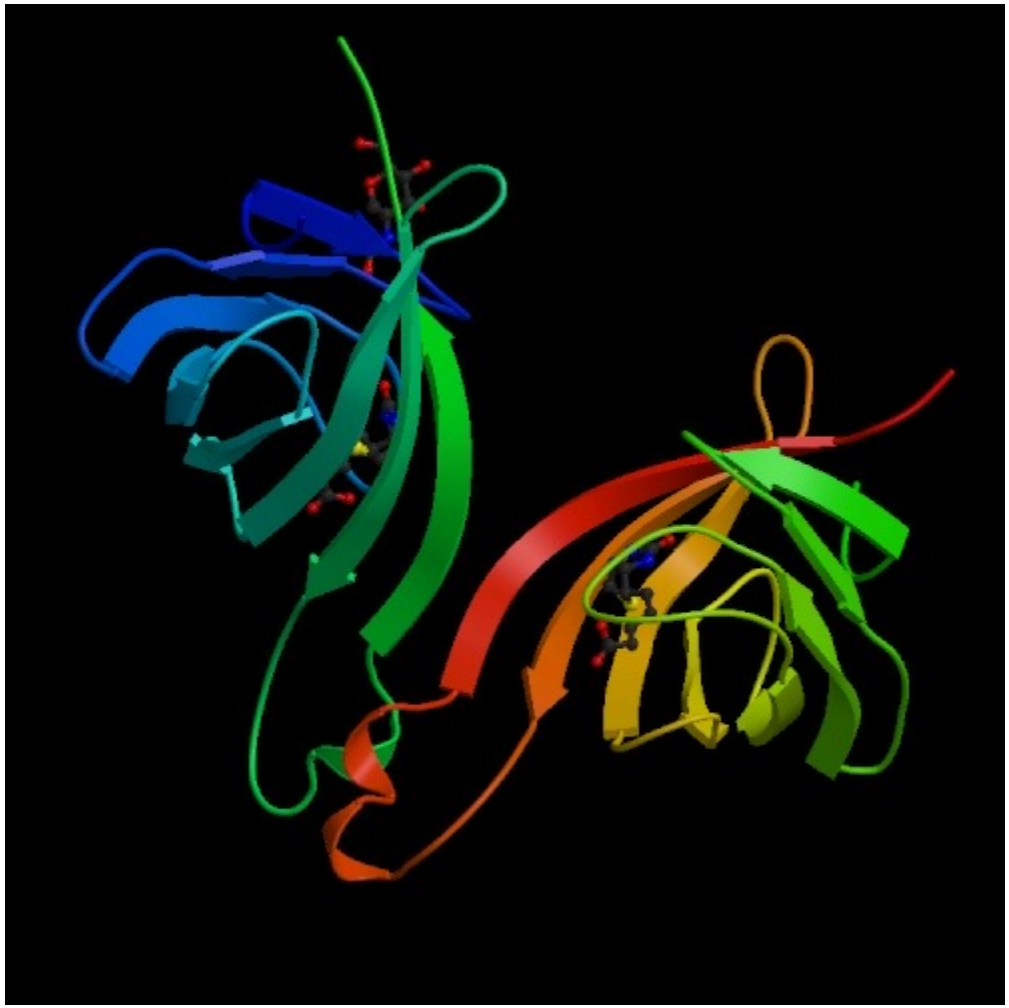


Figura 14.26 Struttura della biotina.

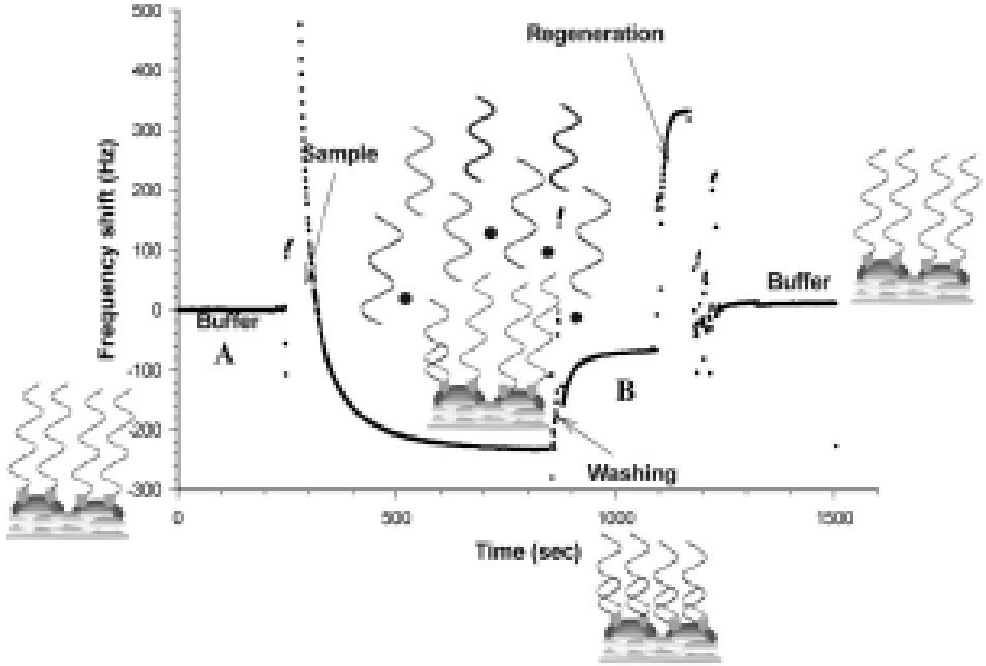


Separation of DNA strands obtained by PCR

- It is necessary to separate the two DNA strands to allow hybridization with the probe
- Thermal denaturation at 95° C for 5 minutes

Scheme of the biosensor

- PCR (60-90 min)
- Denaturation (6 min)
- Hybridization (10-20 min)
- Wash (10 min)
- Regeneration (10 min)



Mannelli et al. (2003) Quartz crystal microbalance (QCM) affinity biosensor for genetically modified organisms (GMOs) detection. *Biosensors and Bioelectronics* 18, 129-140.

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Detection of Six Genetically Modified Maize Lines Using Optical Thin-Film Biosensor Chips

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As more and more genetically modified organisms (GMO) are commercialized, efficient and inexpensive assays are required for their quick detection. An event-specific detection strategy based on the unique and specific sequences of integration junctions is useful because of its high specificity. This study developed a system for detecting six GM maize lines (Bt11, Bt176, GA21, MON810, NK603, and T25) using optical silicon thin-film biosensor chips. Aldehyde-labeled probes were arrayed and covalently attached to a hydrazine-derivatized chip surface. Biotinylated PCR amplicons were then hybridized with the probes. After washing and brief incubation with an anti-biotin IgG horseradish peroxidase conjugate and a precipitable horseradish peroxidase substrate, biotinylated PCR amplicons perfectly matched with the probes can be visualized by the color change on the chip surface (gold to blue/purple). This assay is extremely robust, exhibits high sensitivity and specificity, and is flexible from low through moderate to high throughput.

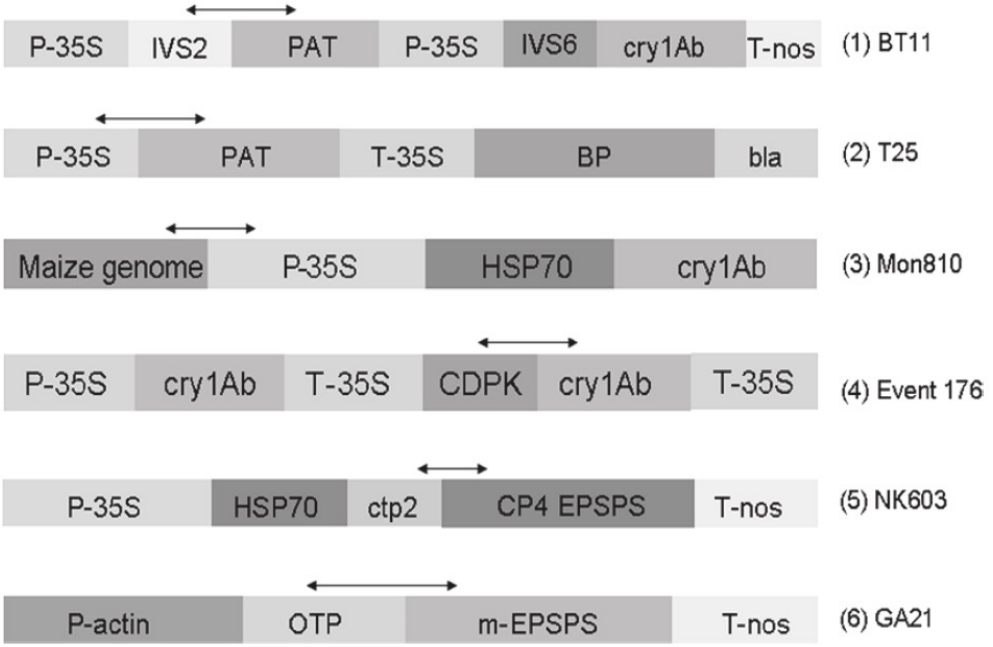

Figure 1. Schematic diagrams of the PCR primer pairs designed to detect six GM maize lines, (1) BT11, (2) T25, (3) MON810, (4) Event 176, (5) NK603, and (6) GA21. Arrows indicate the junctions that the primer pairs amplify.

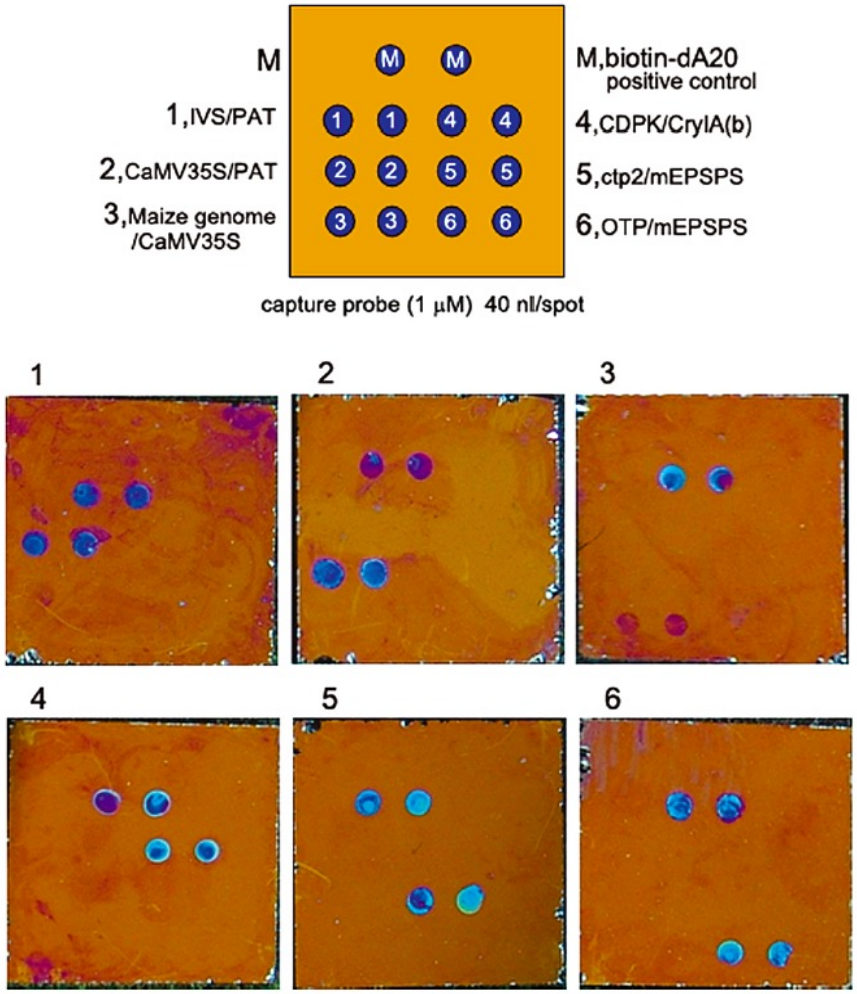

Figure 4. GM maize detection on a chip with capture probes spotted by a computer-controlled dispenser. Each spot comprised 40 nL of 1 μM probe solution. (Upper panel) Capture probes were printed in the order M, biotin-dA20 (positive control and marker); spots 1−6, specific integration junction regions [spot 1, IVS2/PAT; spot 2, CaMV35S/PAT; spot 3, maize genome/CaMV35S; spot 4, CDPK/CryIA(b); spot 5, ctp2/EPSPS; spot 6, OTP/mEPSPS]. (Lower panel) Detection of foreign genes in GM maize products on thin-film biosensor chips. 1−6 represent the detection results of six integration junction regions from Bt11, T25, Mon810, Event 176, NK603, and GA21, respectively.

Cell-based biosensors

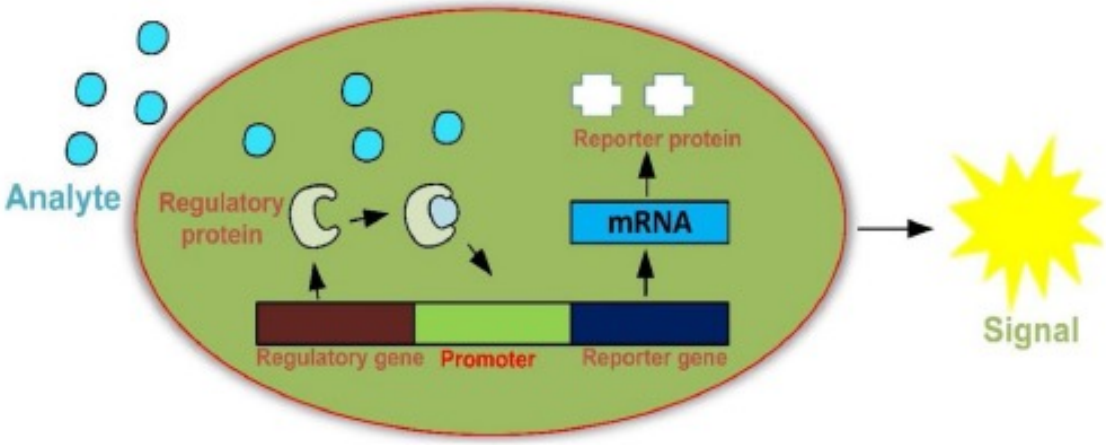

Fig. 2. A schematic illustration of a whole cell biosensor.

Table 3
Advantages and disadvantages of microbial-derived biosensors.

Advantages
✓ Fast and specific detection of compounds
✓ Concurrent monitoring of multiple compounds
✓ High sensitivity monitoring of bioavailable fraction of pollutant
✓ Cost-effective and less labor intensive than conventional sensing methods
Disadvantages
× Prolonged response time
× Difficult maintenance of cell viability and activity
× Lack of durable genetic stability of engineered system
× Technical and societal limitations for using genetically modified strains
× Slow substrates and products diffusion across cell membrane into cells
× Influence of environmental variables (pH, temperature, nutrient availability) on biosensor functionality

Transgenic Res
DOI 10.1007/s11248-016-9959-z

ORIGINAL PAPER

Development of a fluorescent transgenic zebrafish biosensor for sensing aquatic heavy metal pollution

Nilambari Pawar · P. Gireesh-Babu · Supriya Sabnis · Kiran Rasal · Renuka Murthy · S. G. S. Zaidi · Sridhar Sivasubbu · Aparna Chaudhari

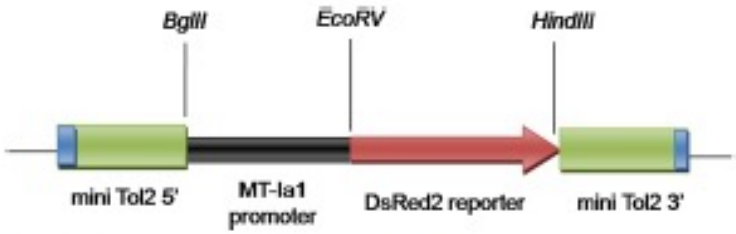

Fig. 1 Schematic representation of the biosensor construct in mini Tol2 transposon vector. The construct comprises the metallothionein Ia1 (MT-Ia1) promoter from *green* mussel, *P. viridis*, the DsRed2 reporter gene, and the mini Tol2 terminal inverted repeat sequences required for transposition. (Color figure online)

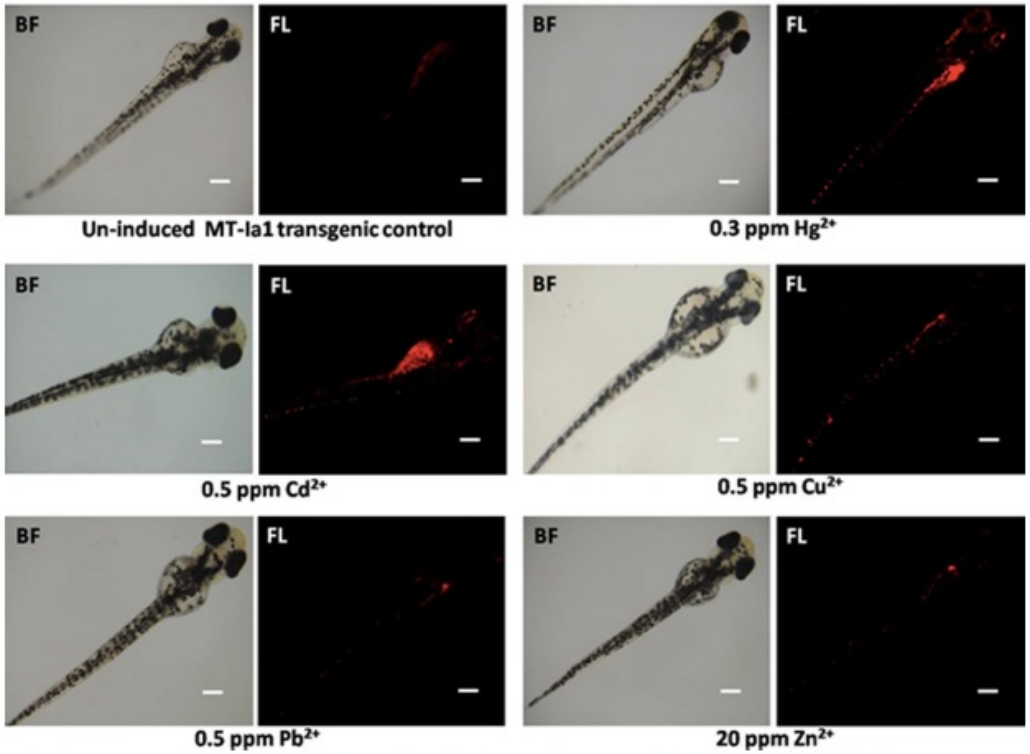

Fig. 2 Fluorescence imaging of F1 transgenic zebrafish (*D. rerio*) larvae (48 h post-fertilization) exposed for 8 h to Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+} or Zn^{2+} at doses that gave maximum fluorescence. Fluorescence expression is observed only in the yolk sac of un-induced transgenic control, while it is present in other body parts in the induced larvae. *BL* bright field, *FL* fluorescence. *Scale bar* 200 μm