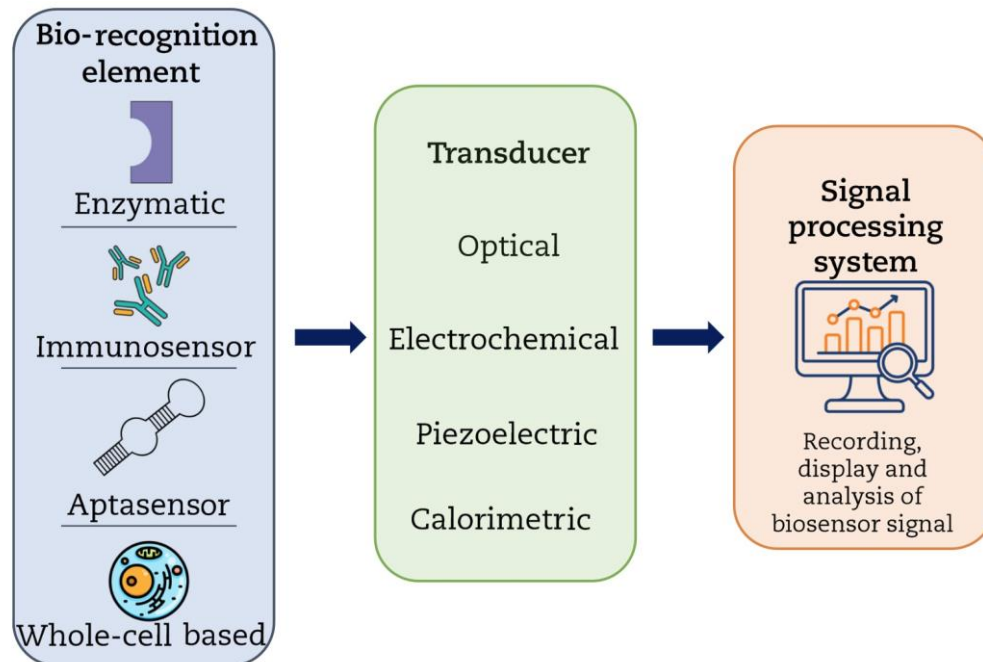


Applicazioni biomediche e
biotecnologiche di biomolecole:

I Biosensori

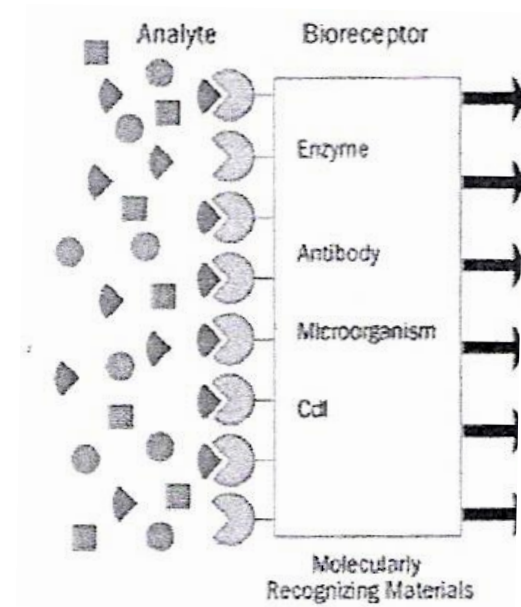
I Biosensori

- Sono strumenti analitici in grado di fornire informazioni quantitative o semiquantitative utilizzando un **elemento di riconoscimento biologico** integrato con un **trasduttore**
- **Convertono un segnale biochimico in un segnale elettrico quantificabile**



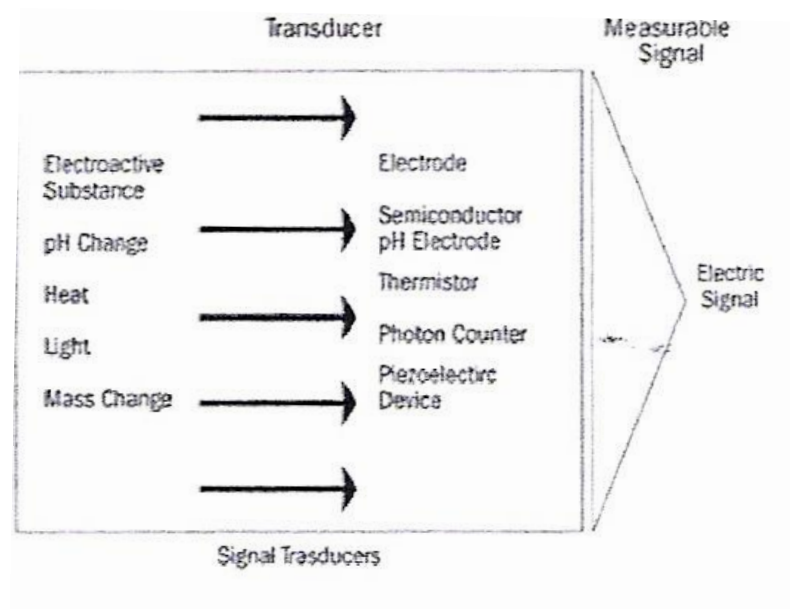
Componente biologico

- Genera il segnale specifico
- Catalitico: Enzimi purificati
Microorganismi
Cellule o tessuti
- Non catalitico: Anticorpi
Recettori
Acidi nucleici



Componente non biologico (trasduttore)

- Converte il segnale biologico in un segnale facilmente quantificabile
- Trasduttori elettrochimici (potenziometrici ed amperometrici)
- Trasduttori ottici
- Trasduttori termici
- Trasduttori acustici



Trasduttori elettrochimici

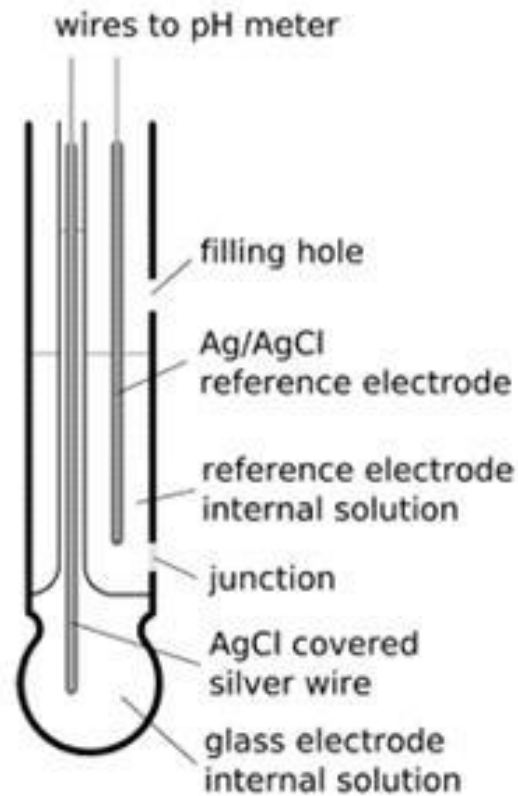
- **Potenziometrici:**

la variazione del potenziale elettrochimico è funzione della concentrazione di ioni (es. elettrodo per la misura del pH, elettrodi iono-selettivi)

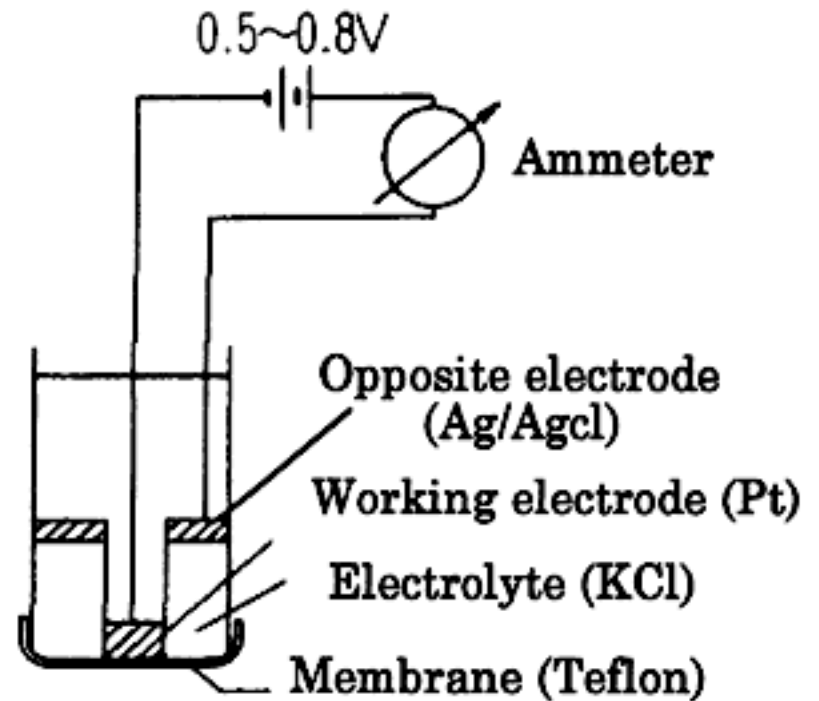
- **Amperometrici:**

il potenziale dell'elettrodo è costante ed è sufficiente ad ossidare o ridurre la specie di interesse (es. elettrodi per la misura di O_2 e H_2O_2)

Elettrodo per la misura del pH

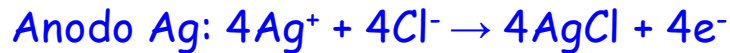


Elettrodo di Clark per la misura dell'ossigeno

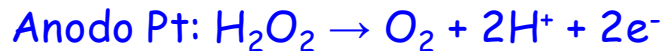


Elettrodi amperometrici per la misura di O_2 e H_2O_2

- L'elettrodo di Clark per la misura di O_2 ha un potenziale catodico di $-0.6V$ al quale l'ossigeno viene ridotto ad acqua



- L'elettrodo per la misura di H_2O_2 ha un potenziale anodico di $+0.68V$ al quale l'acqua ossigenata viene ossidata a ossigeno



L'ossigeno e l'acqua ossigenata sono specie elettrochimiche di interesse biologico perché sono consumate o prodotte nelle reazioni catalizzate da numerosi enzimi

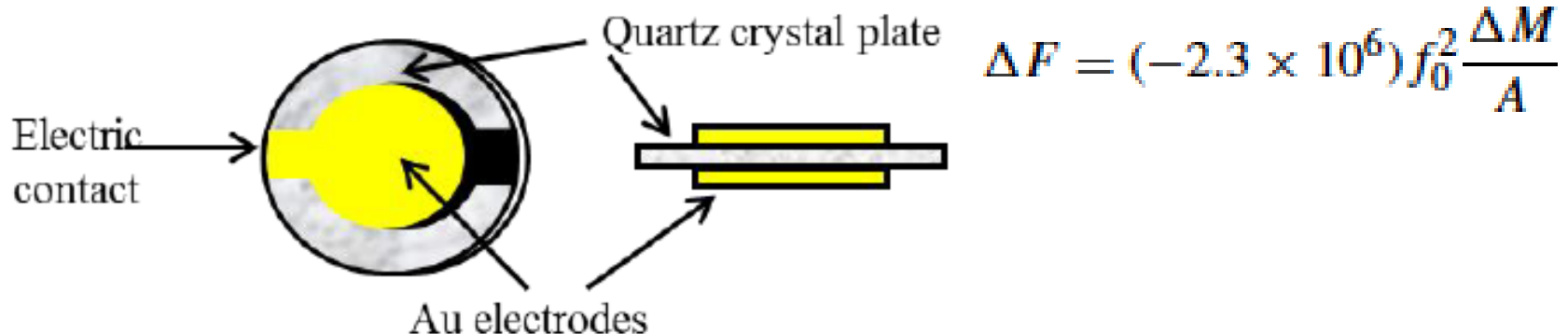
Trasduttori ottici

- Fibre ottiche con il biocomponente ad un'estremità e componenti per l'eccitazione ed il rilevamento dell'assorbimento e/o emissione di luce all'altra estremità
- Utilizzabili quando la reazione catalizzata dall'enzima dà origine a prodotti colorati, fluorescenti o a luminescenza
- Tecnologia SPR

Trasduttori acustici

- Cristalli di quarzo piezoelettrici posti in un campo elettrico alternato sono soggetti a deformazione meccanica che induce risonanza ad una particolare frequenza.
- La frequenza di risonanza dipende dalla **massa** del cristallo: cambiamenti di massa sono rivelati da variazioni nella frequenza di oscillazione del cristallo.
- Il biocomponente (anticorpo, recettore o acido nucleico) viene immobilizzato sulla superficie del cristallo.

Figure 3. Schematic of a piezoelectric quartz crystal.



Metodi di immobilizzazione delle biocomponenti

- Adsorbimento su matrici insolubili
- Intrappolamento in un gel
- Cross-linking con reattivi multifunzionali
- Legame covalente su un supporto solido
- Tecnologia dei circuiti stampati (miniaturizzazione) e nanomateriali

E' necessario preservare l'attività biologica del biocomponente

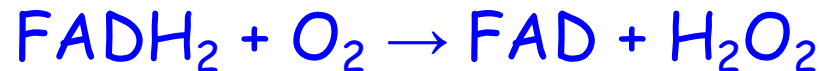
Nanomateriali

I nanomateriali permettono di funzionalizzare gli elettrodi e migliorarne la sensibilità (sono buoni conduttori elettrici)

- Grafene
 - monostrato di atomi di carbonio con ottime proprietà di stabilità meccanica, conducibilità elettrica e termica, biocompatibilità e elevato rapporto superficie/volume
- Nanotubi al carbonio
 - Fogli di grafene arrotolati a formare tubi lunghi da nm a μm
- Nanoparticelle di ossido di zinco (ZnO)
- Nanoparticelle d'oro

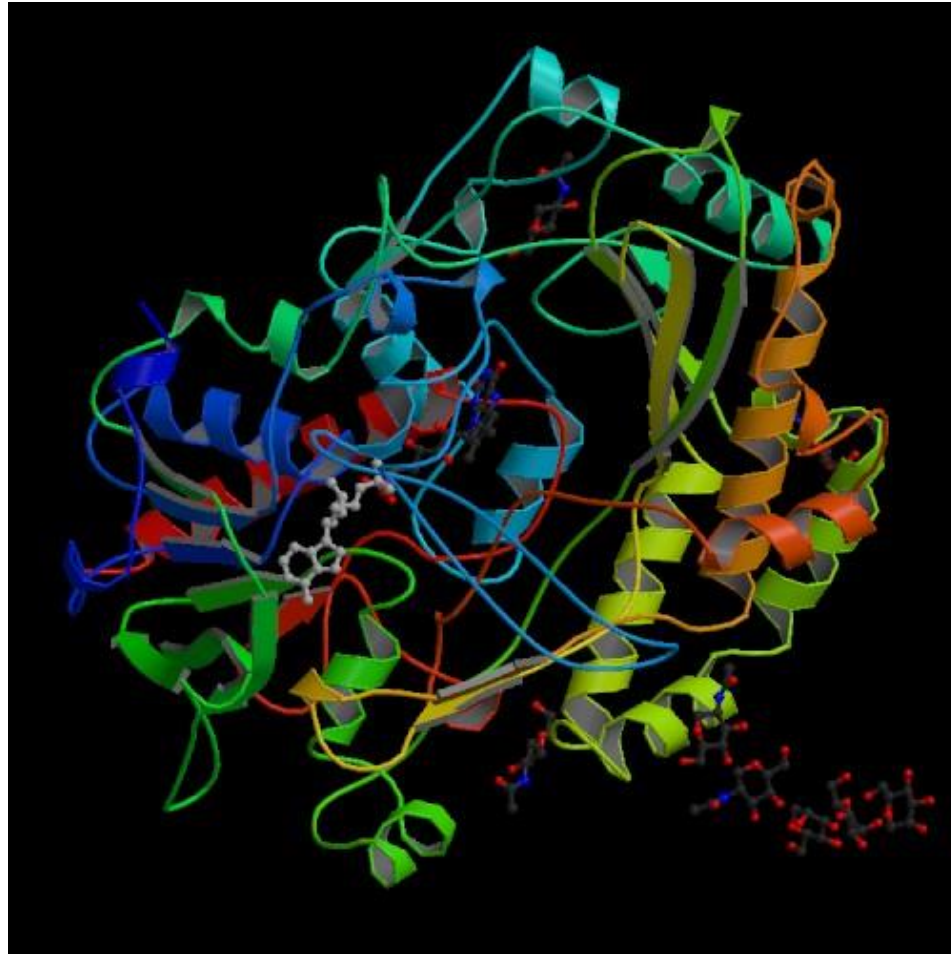
Biosensore per il glucosio

- Il componente biologico è l'enzima **glucosio ossidasi** che catalizza la reazione:



- L'enzima richiede ossigeno per riossidare il coenzima FADH_2 che si produce nella prima fase della reazione

Struttura della glucosio ossidasi di *Aspergillus niger*



Biosensore per il glucosio

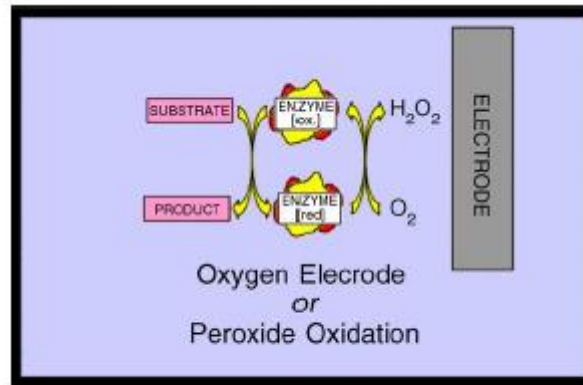
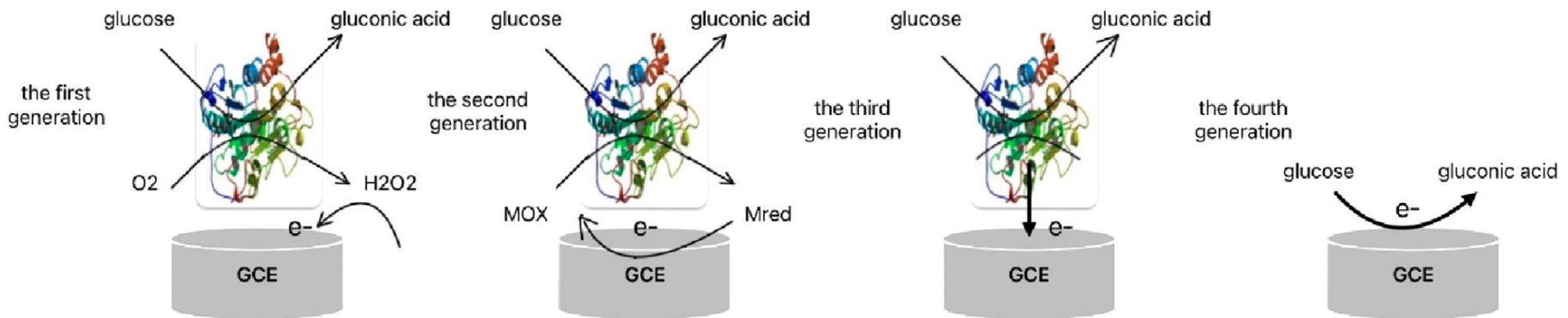


Fig. 2. First generation glucose biosensor schematic.

- **Trasduttori:**
Elettrodo di Clark per la misura di O₂
Elettrodo per la misura di H₂O₂
La corrente sarà proporzionale alla concentrazione di O₂ o H₂O₂ e quindi alla quantità di glucosio nel campione
- La glucosio ossidasi è immobilizzata su una membrana di nylon o di cellulosa posta a contatto con l'elettrodo
- **Problemi:**
limitata diffusione dell'ossigeno
scarsa selettività dell'elettrodo a H₂O₂ a causa dell'elevato potenziale anodico

Evoluzione dei biosensori per il glucosio



Biosensori di seconda generazione

- Uso di mediatori chimici che sostituiscono l'ossigeno nella riossidazione del FADH_2 (E_0 -220 mV) e sono a loro volta riossidati all'elettrodo

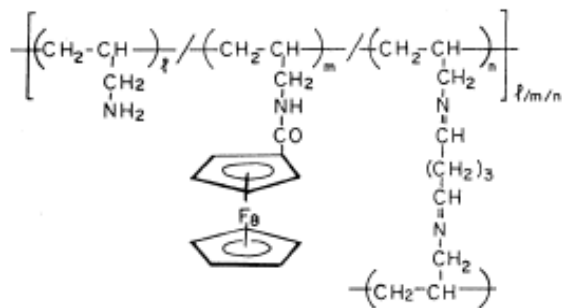


Fig. 7. Ferrocene containing cross-linked polyallylamine.

Enzima	Mediatore	E_0 (mV)
Glucosio ossidasi	1, 1-dimetil ferrocene	100
	ferrocene	165
	idrossimetil ferrocene	185
	vinil ferrocene	250
	ferrocene acido carbossilico	275
	$[\text{Ru}(\text{CN})_6]^{4-}$	685
	TTF (tetrathia fulvalene)	300
	$[\text{Fe}(\text{CN})_6]^{4-}$	180
NMP (N-metil fenazinium)	-161	

Biosensori di seconda generazione

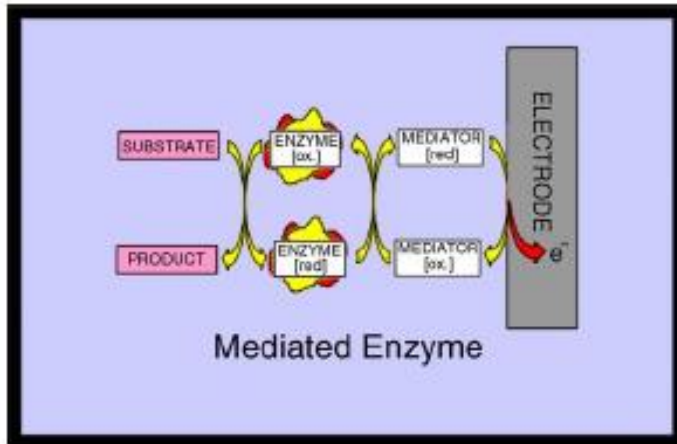


Fig. 4. Mediated biosensor schematic.

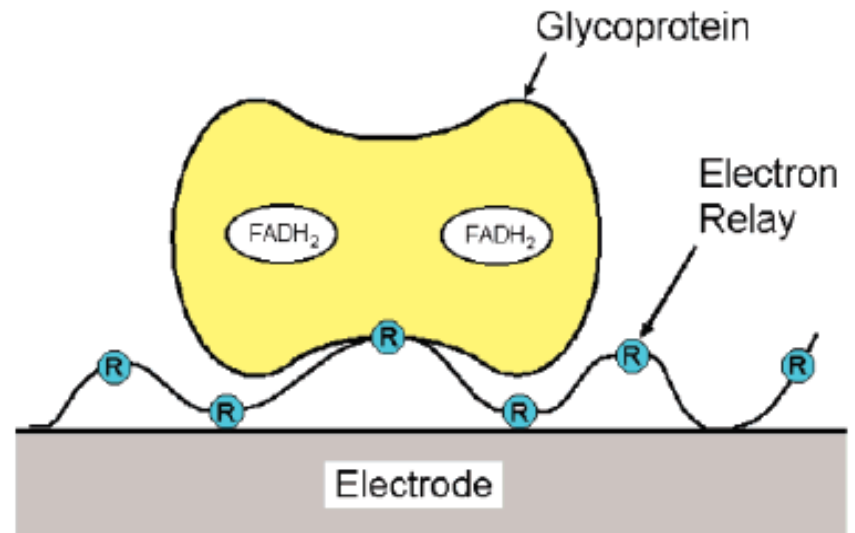


Figure 2. Use of a redox polymer for wiring GOx: efficient electrical communication between the redox center of the enzyme and electrode surfaces.

Biosensori basati su nuovi materiali: nanotubi al carbonio con FAD immobilizzato

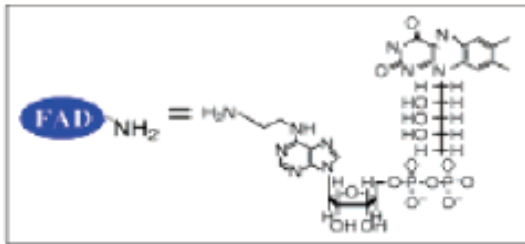
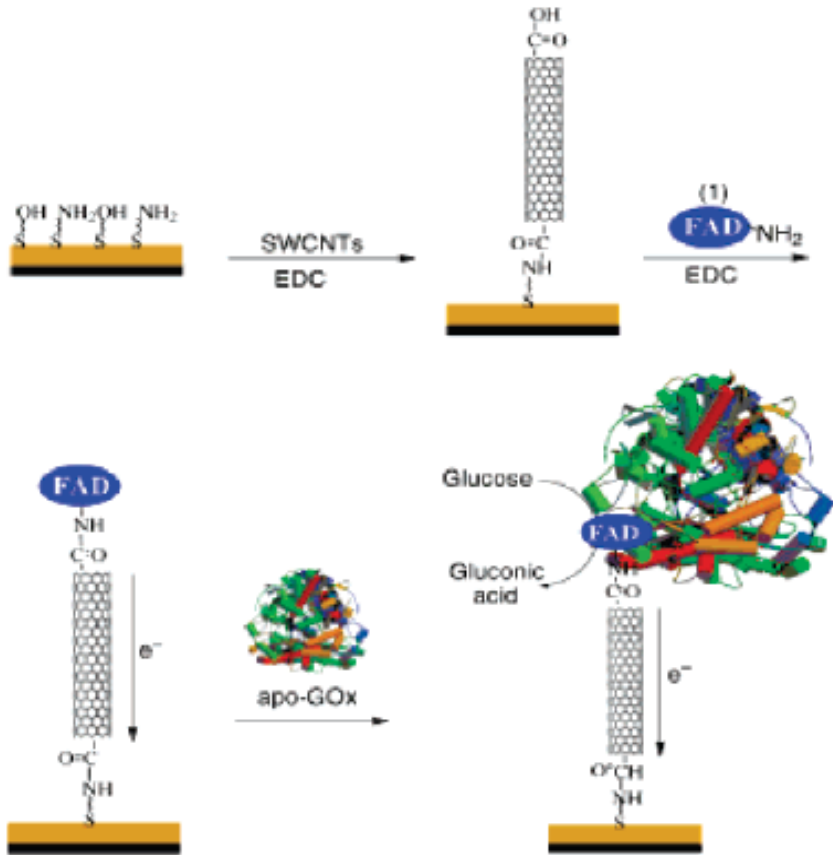


Figure 3. Carbon nanotube (CNT) connectors with long-range electrical contacting. Assembly of the CNT electrically contacted glucose oxidase electrode. (Reprinted with permission from ref 59. Copyright 2004 Wiley-VCH.)

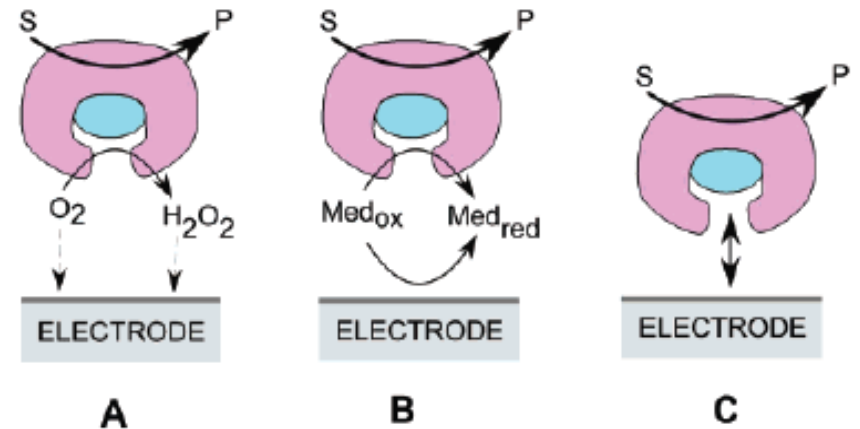
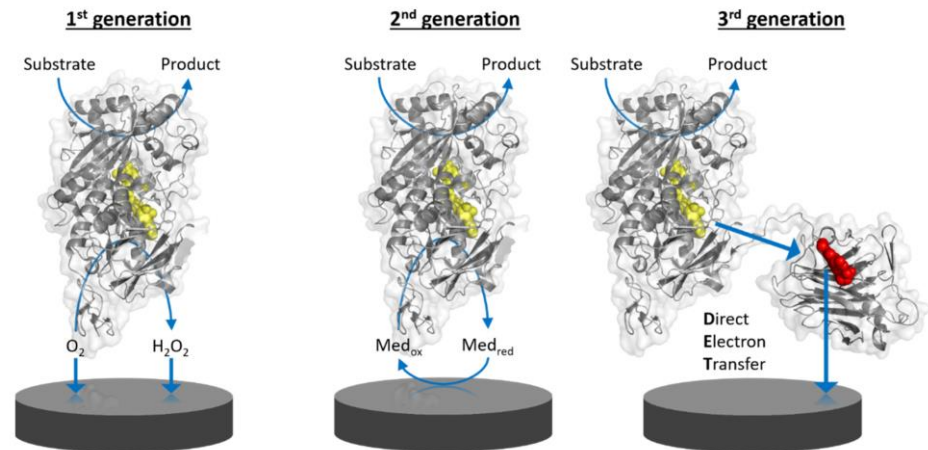
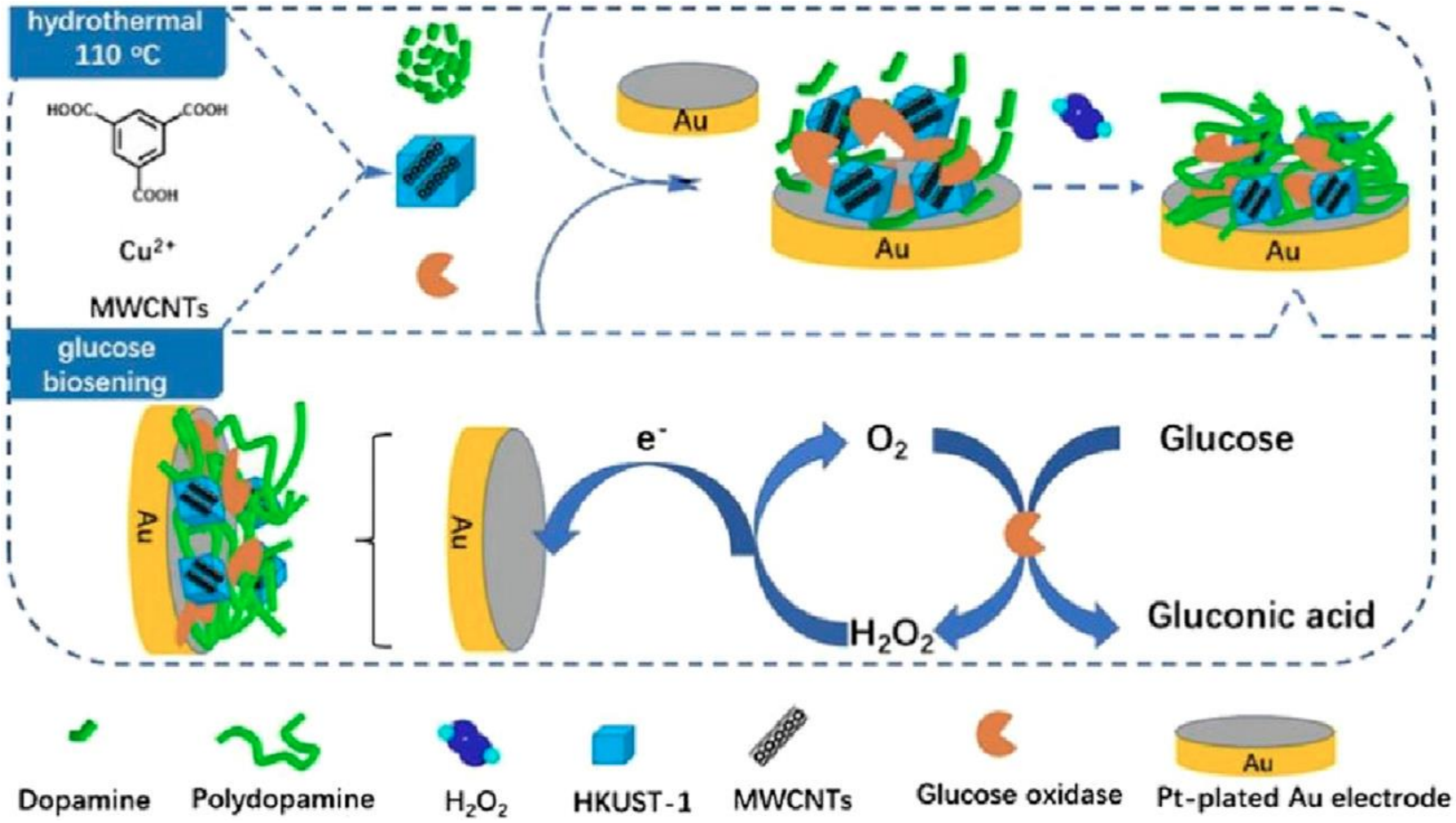


Figure 4. Three generations of amperometric enzyme electrodes for glucose based on the use of natural oxygen cofactor (A), artificial redox mediators (B), or direct electron transfer between GOx and the electrode (C).



Biosensore basato su GOx e MOF (metal organic framework) HKUST-1 e nanotubi al carbonio (MWCNT) accoppiati ad un elettrodo d'oro rivestito di Pt.
 La polidopamina (PDA) è utilizzata per stabilizzare l'enzima



Biosensori commerciali per il dosaggio della glicemia



Fig. 6. Original MediSense products.



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

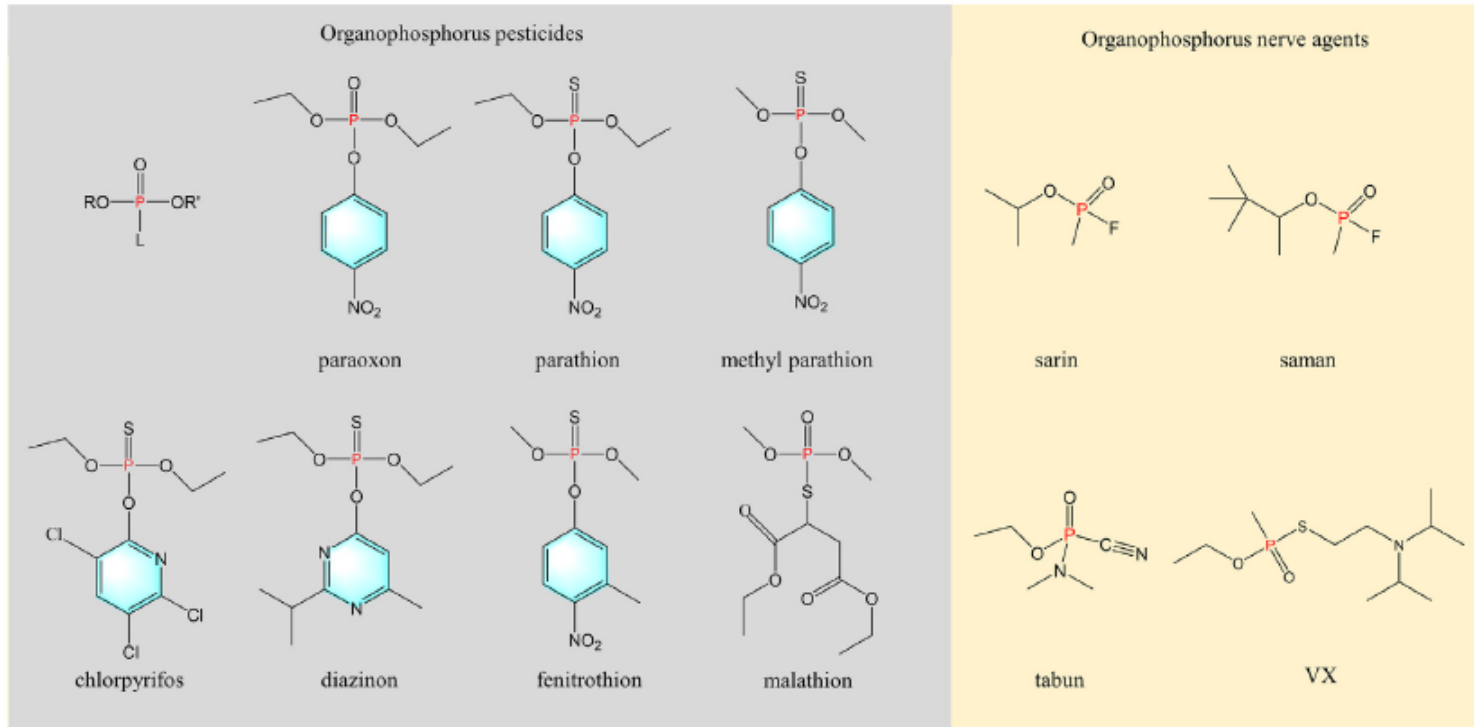


Fig. 13. OneTouch ultra blood glucose biosensor.



Fig. 14. LifeScan UltraSmart system.

Pesticidi organofosforici



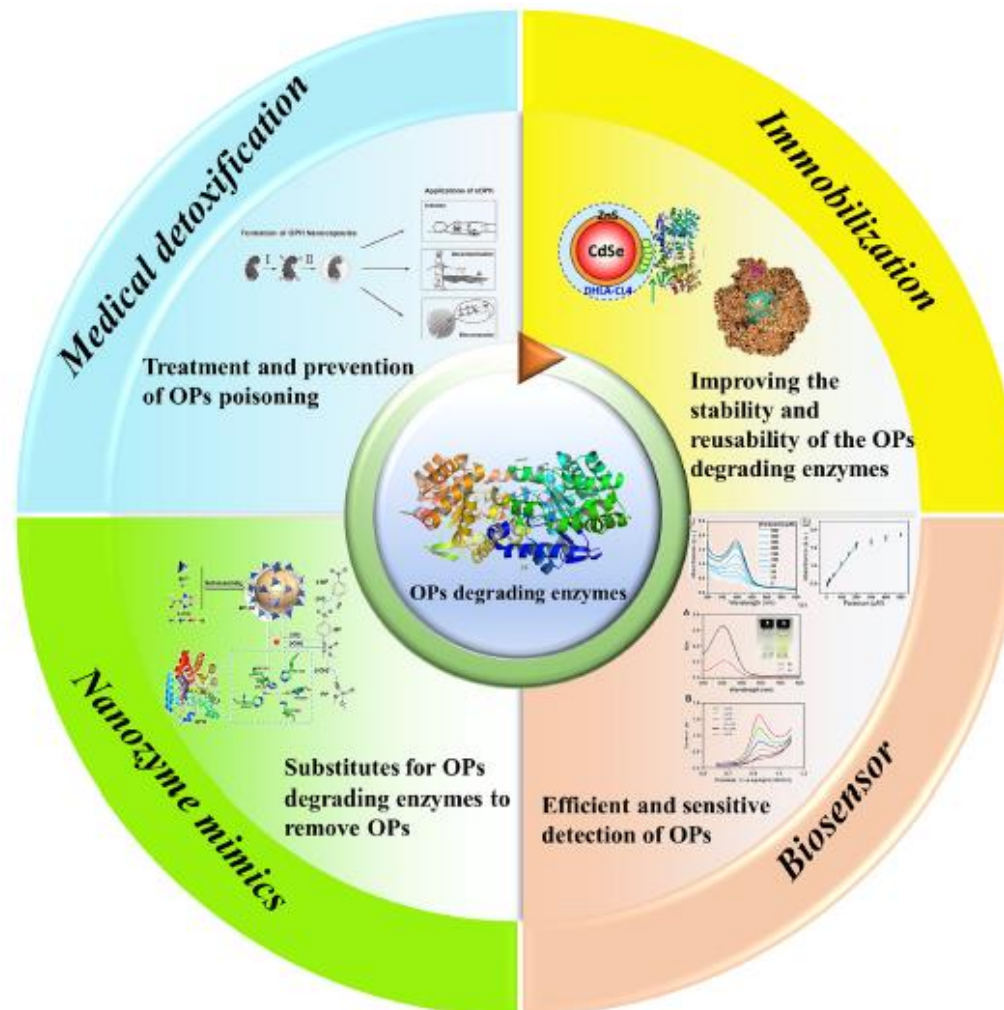
I pesticidi organofosforici
sono inibitori
dell'acetilcolinesterasi

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]
	Fungicides	Carbamates	Aldicarb, Carbaryl Propoxur
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
	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

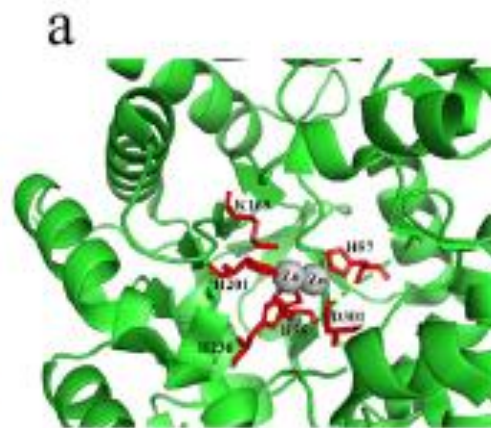
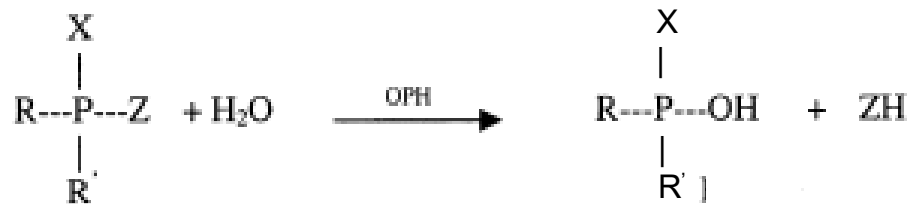
Applicazioni di enzimi che degradano i pesticidi organofosforici



Biosensori per il dosaggio di pesticidi organofosforici

- Biocomponente:

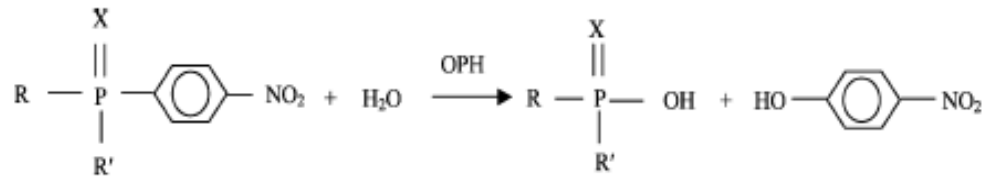
l'enzima **organofosfato idrolasi** (OPH) di *Pseudomonas diminuta*, catalizza la reazione



Biosensori per il dosaggio di pesticidi organofosforici

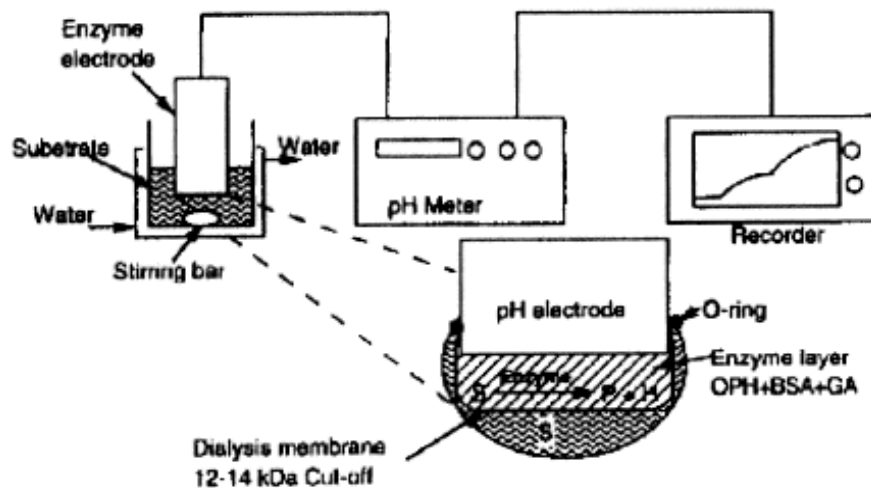
- Biocomponente:
 - cellule di *E. coli* che esprimono l'enzima OPH ricombinante
 - OPH purificato
- Immobilizzazione:
 - criopolimerizzazione in alcool polivinilico
 - legame covalente su membrana di nylon

Biosensori per il dosaggio di pesticidi organofosforici

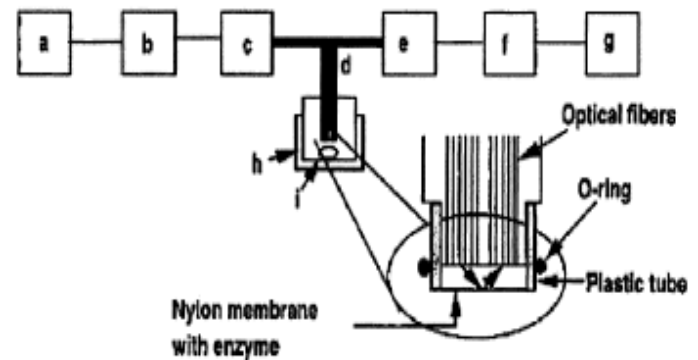


- Trasduttori:

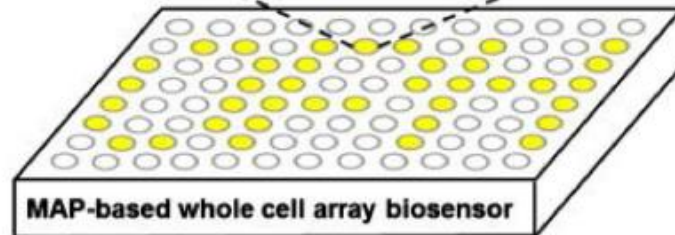
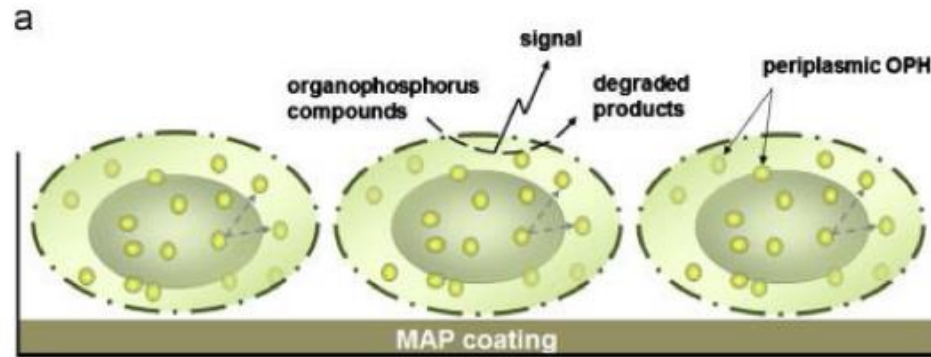
Elettrodo per la misura del pH



Fibra ottica



Sensore OPH per il dosaggio di pesticidi organofosforici



MAP: mussel adhesive protein



Biosensori per il dosaggio di pesticidi organofosforici

- Biocomponente:

L'enzima **acetilcolinesterasi** catalizza la reazione:

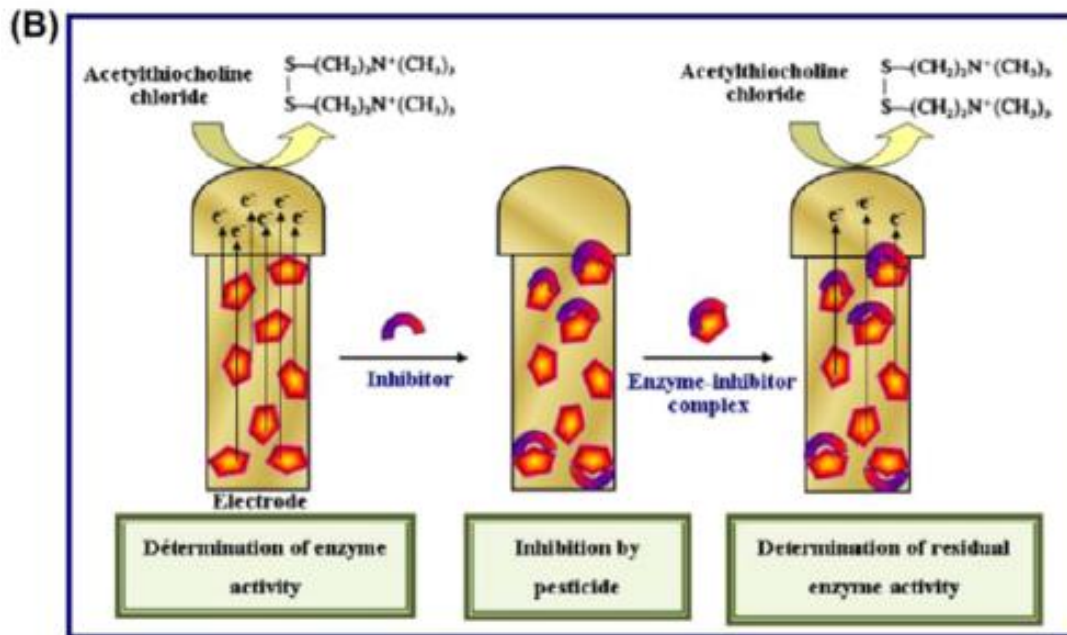
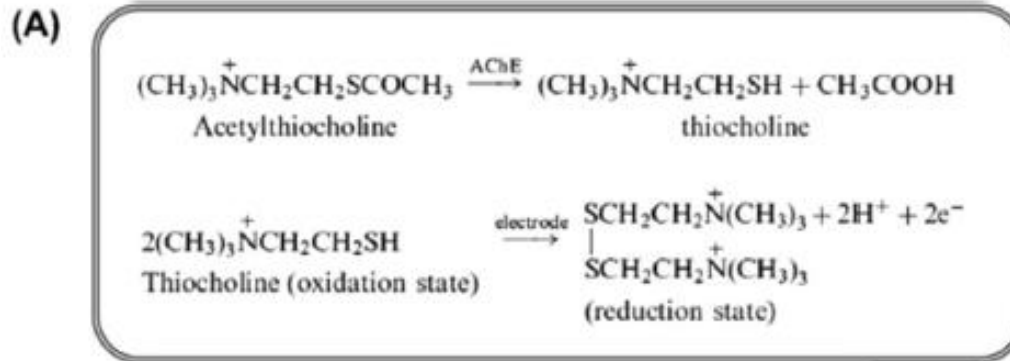


- Trasduttore:

Elettrodo amperometrico che misura la corrente prodotta dall'ossidazione della tio-colina

Il biosensore viene immerso in una soluzione contenente il pesticida e incubato 10-30 min, successivamente viene misurata l'attività residua dell'acetilcolinesterasi

Principio di funzionamento del biosensore basato sull'inibizione dell'AChE



Biosensori basati su AChE

- Per migliorare sensibilità e selettività
 - Enzimi da fonti diverse (*Torpedo*, *Drosophila*...)
 - Mutagenesi tasca riconoscimento del substrato

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
<i>dm</i> AChE (E69Y Y71D)	AChE/Carbon pellet	Amperometry-FIA	$\sim 1 \times 10^{-17}$	Dichlorvos	10 min
<i>ee</i> AChE	AChE/Carbon pellet	Amperometry-FIA	1×10^{-8}	Dichlorvos	10 min
<i>dm</i> AChE (E69Y Y71D)	AChE/Carbon pellet	Amperometry-FIA	1×10^{-12}	Paraoxon	10 min
<i>dm</i> AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-17}$	Dichlorvos	NR
<i>dm</i> AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-16}$	Parathion	NR
<i>dm</i> AChE	AChE/CoPC/SPE	Electrochemical-FIA	$\sim 1 \times 10^{-16}$	Azinphos	NR
<i>dm</i> AChE	AChE/PVA-SbQ/SPE	Amperometry	7×10^{-11}	Dichlorvos	10 min
<i>ee</i> AChE	AChE/PVA-SbQ/SPE	Amperometry	6×10^{-7}	Dichlorvos	10 min

Note: NR, not reported.



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

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^b Uniscan Instruments, Sigma House, Burlow Road, Buxton, Derbyshire, SK17 9JB, UK

^c CCFRA, Chipping Campden, Gloucestershire, GL55 6LD, UK

^d Gwent Electronic Materials Ltd., Monmouth House, Mamhilad Park, Pontypool, Gwent, NP4 0HZ, UK

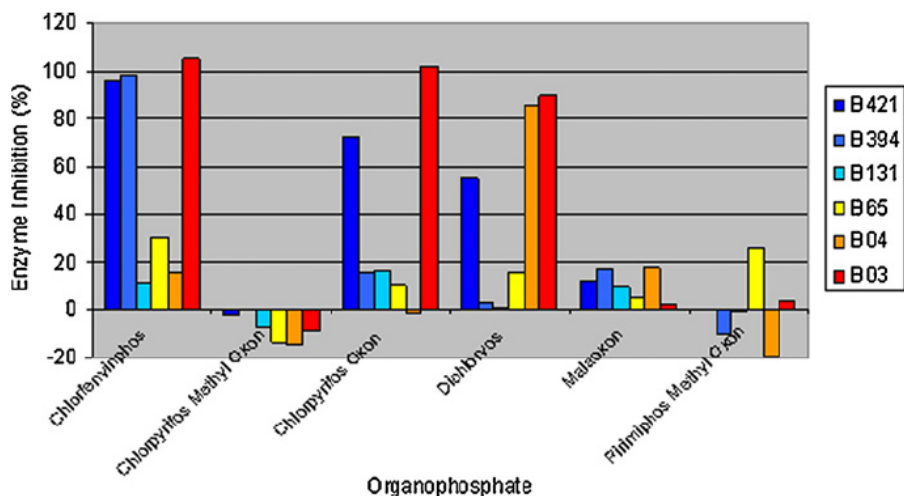


Fig. 5. Inhibition profiles constructed from the inhibition of each of the enzymes by each of the OPs at a concentration of 10^{-6} M.

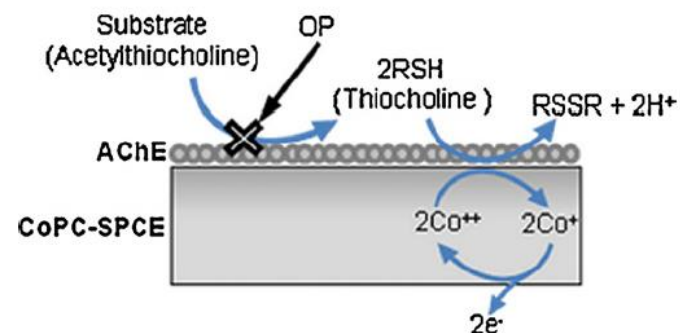


Fig. 2. Schematic diagram showing the reactions taking place during the operation of the proposed amperometric biosensor.

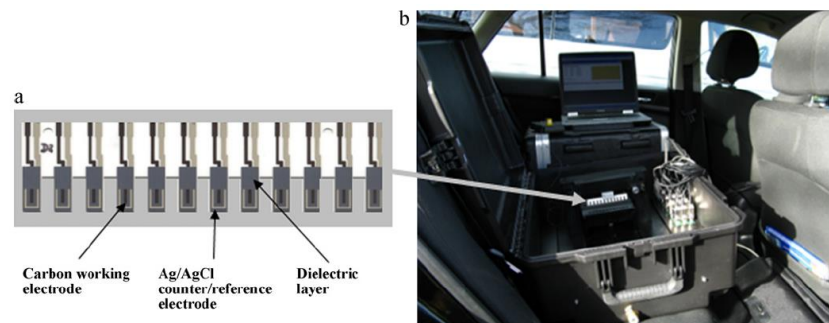
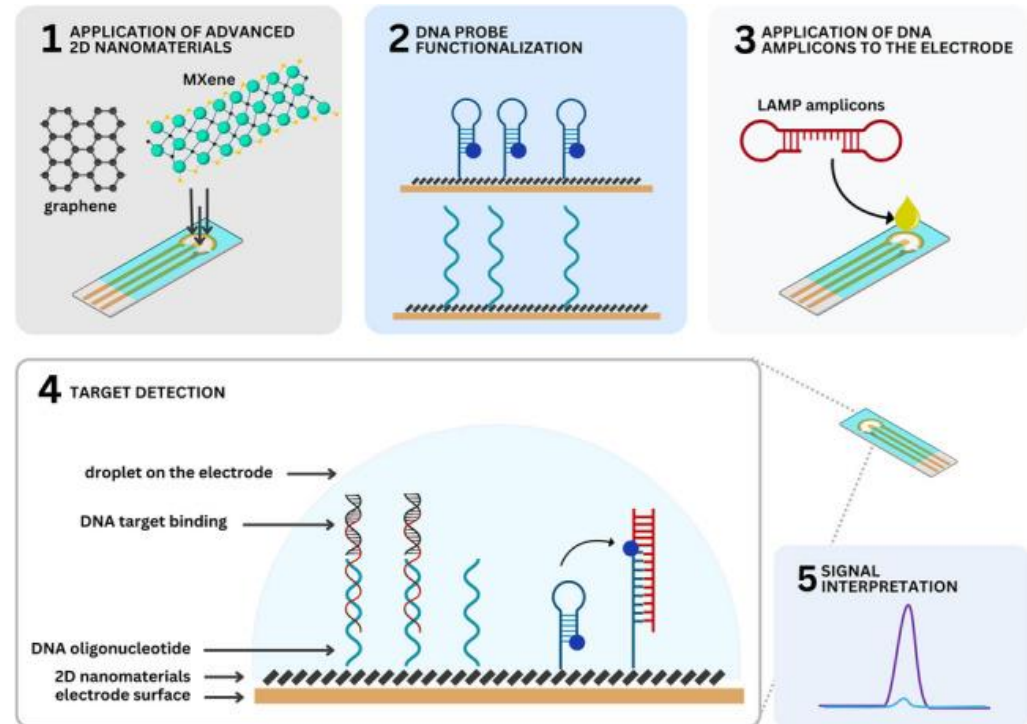


Fig. 1. (a) Electrode array comprising 12 screen-printed carbon electrodes modified with CoPC and an Ag/AgCl counter/reference electrode printed on an alumina substrate; (b) array in the prototype biosensor system operating in the field powered from a car battery via the lighter socket.

Biosensori basati sugli acidi nucleici

- Sfruttano l'interazione tra frammenti di DNA usati come sonda e sequenze complementari ad esse, presenti nel campione da analizzare
- Migliore stabilità ed efficienza di immobilizzazione rispetto a sonde proteiche
- L'ibridazione tra la sonda ed il DNA complementare fa sì che si generi un **segnale** misurabile



Determinazione di organismi geneticamente modificati (OGM)

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

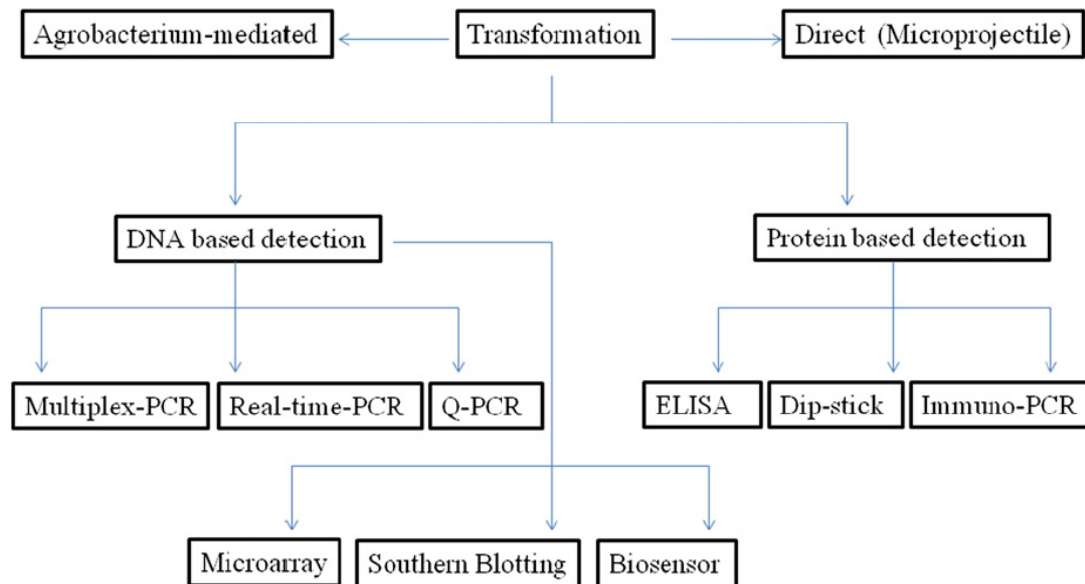


Fig. 5. A schematic view of detection methods for GM crops.

Biosensore per la determinazione di organismi geneticamente modificati (OGM)

- Gli OGM sono definiti come organismi il cui genoma è stato modificato con l'introduzione di un gene esogeno che esprime una proteina che conferisce nuove caratteristiche (es. resistenza a erbicidi, antibiotici, virus o insetti)
- Il DNA esogeno è inserito in una 'cassetta' genica che contiene gli elementi necessari per l'espressione: un promotore ed un terminatore
- Il promotore 35S del virus del mosaico del cavolfiore (CaMV) e il terminatore Tnos di *Agrobacterium tumefaciens* sono usati per la produzione della maggior parte delle piante transgeniche commerciali

Biosensore per la determinazione di organismi geneticamente modificati (OGM)

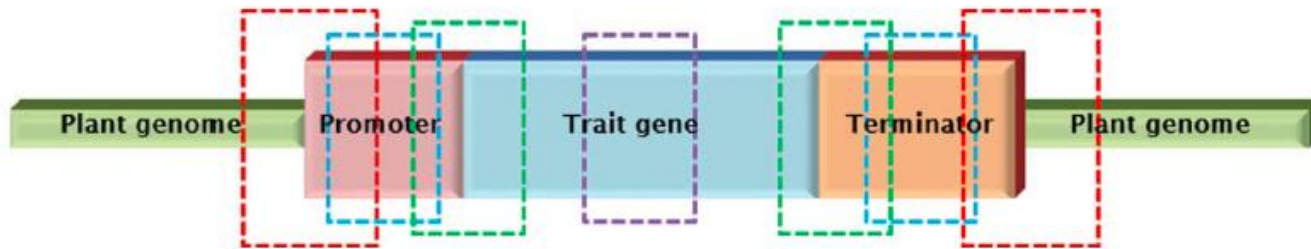


Figure 1. Levels of specificity of GMO methods based on the targeted DNA region: screening (blue-dashed square), gene-specific (purple square), construct-specific (green), and event-specific (red). Adapted from Holst-Jensen et al. [16].

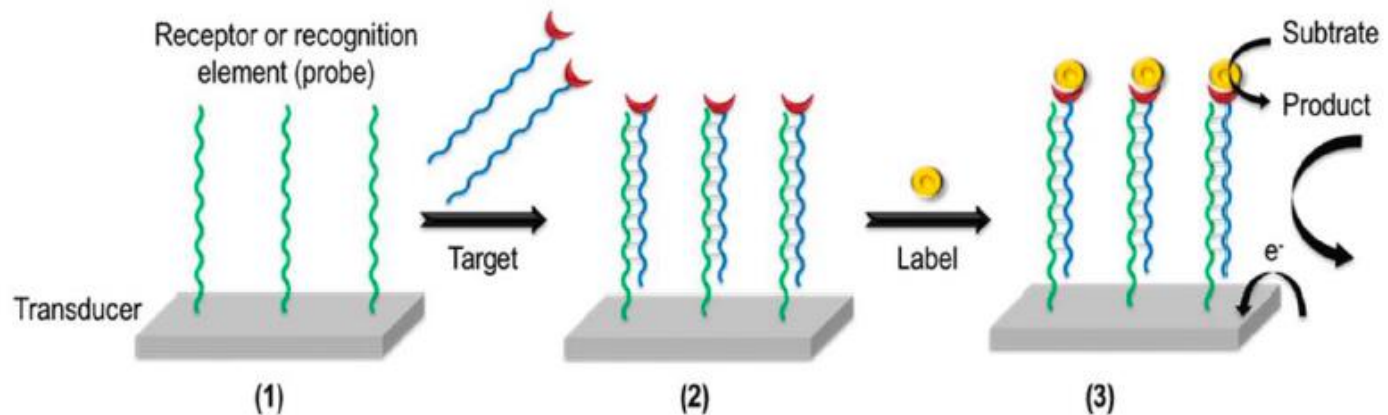
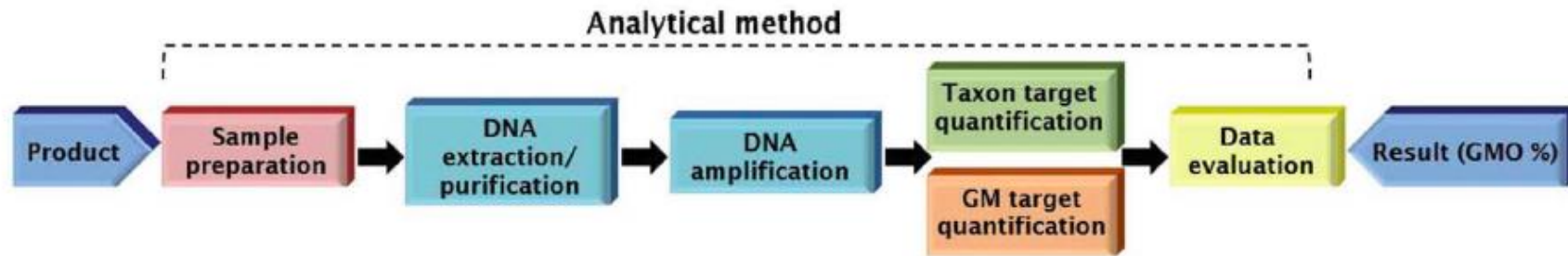


Figure 3. Steps in the design of a genosensor with a labeled target.

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

SULAN BAI,^{†,||} JIE ZHANG,^{‡,||} SHUCHENG LI,^{†,||} HAODONG CHEN,^{#,||} WILLIAM TERZAGHI,[⊥]
XIN ZHANG,[†] XIURONG CHI,[‡] JIN TIAN,[‡] HONGXIA LUO,[‡] WENSHENG HUANG,[§]
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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.

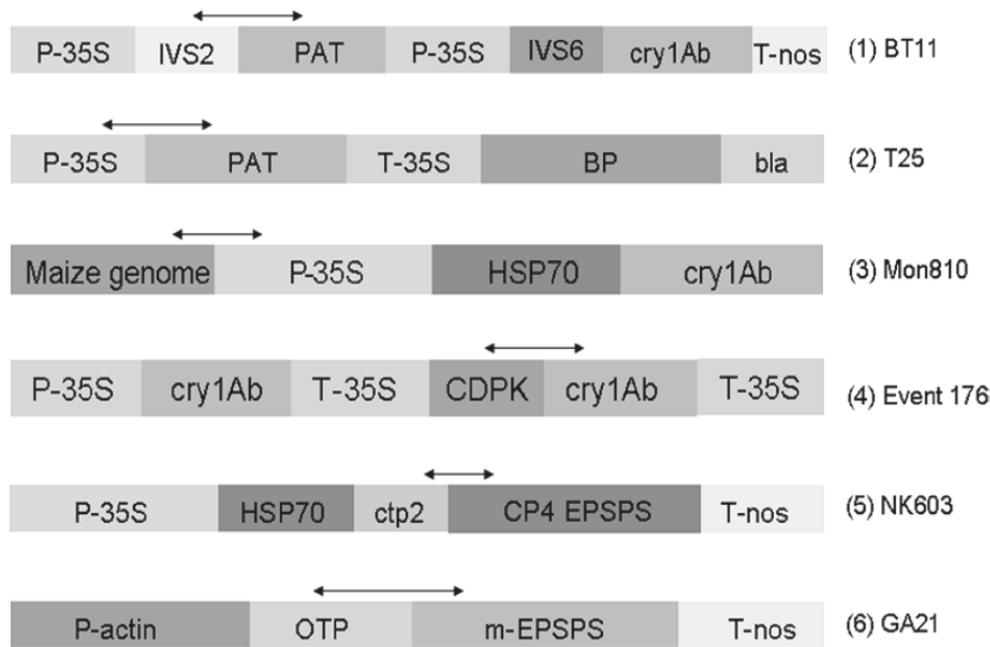


Figure 1. Schematic diagrams of the PCR primer pairs designed to detect six GM maize lines, (1) BT11, (2) T25, (3) MON810, (4) Event 176, (5) NK603, and (6) GA21. Arrows indicate the junctions that the primer pairs amplify.

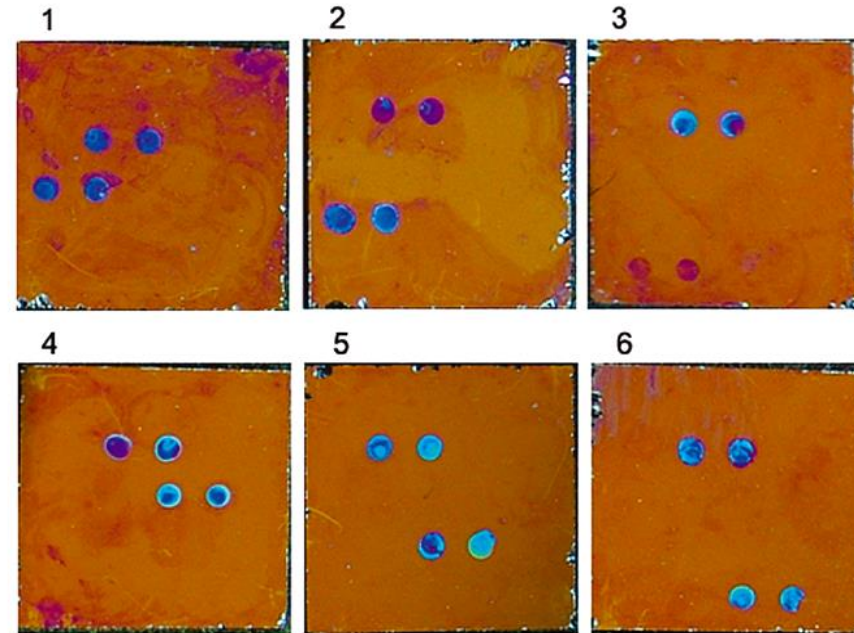
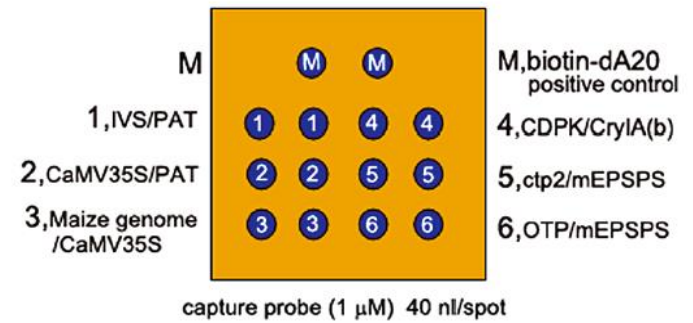


Figure 4. GM maize detection on a chip with capture probes spotted by a computer-controlled dispenser. Each spot comprised 40 nL of 1 μ M probe solution. (Upper panel) Capture probes were printed in the order M, biotin-dA20 (positive control and marker); spots 1–6, specific integration junction regions [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.

Biosensori basati su cellule

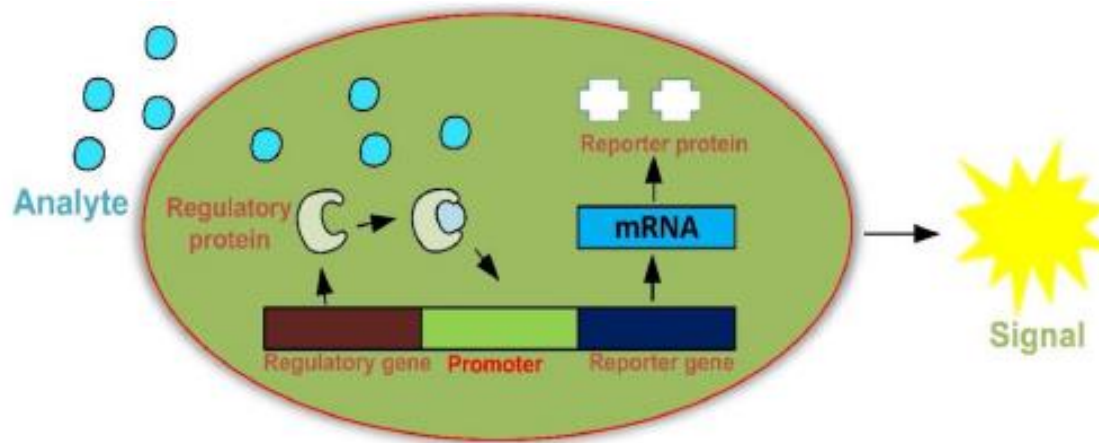


Fig. 2. A schematic illustration of a whole cell biosensor.

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
-

Development of Genetically Encoded Biosensors for Reporting the Methyltransferase-Dependent Biosynthesis of Semisynthetic Macrolide Antibiotics

Yiwei Li, Megan Reed, H. Tonie Wright, T. Ashton Cropp, and Gavin J. Williams*



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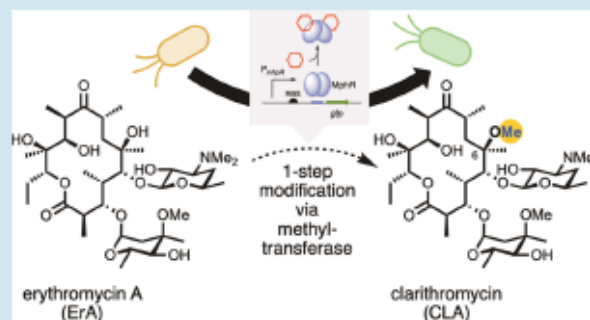


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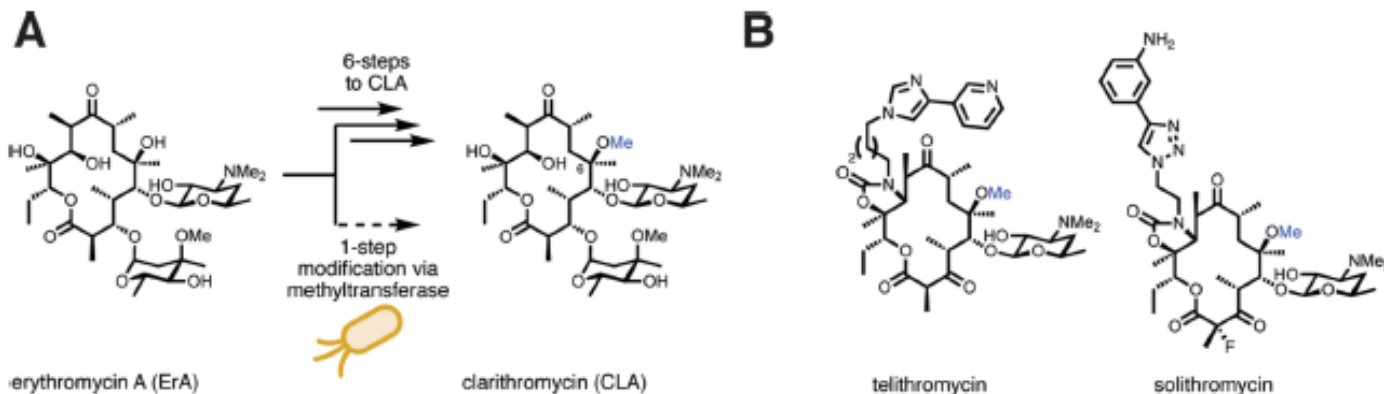


Supporting Information

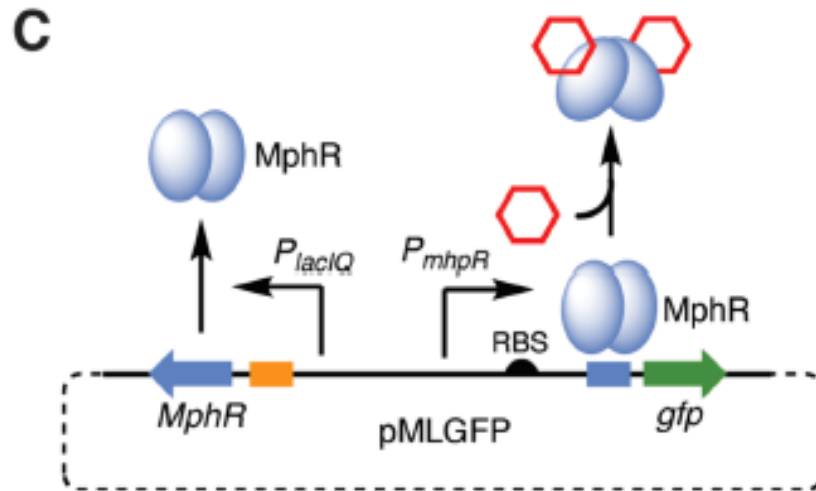
ABSTRACT: Clarithromycin is an improved semisynthetic analogue of the naturally occurring macrolide, erythromycin. The subtle modification of a methyl group on the C-6 hydroxyl group endows the molecule with improved acid stability and results in a clinically useful antibiotic. Here, we show that the effector specificity of the biosensor protein, MphR, can be evolved to selectively recognize clarithromycin and therefore report on the production of this molecule *in vivo*. In addition, a crystal structure of the evolved variant reveals the molecular basis for selectivity and provides a guide for the evolution of a new metabolic function using this biosensor.



KEYWORDS: macrolide, polyketide, biosensor, transcription factor, synthetic biology



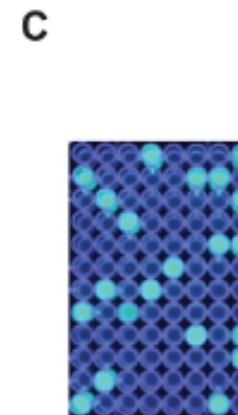
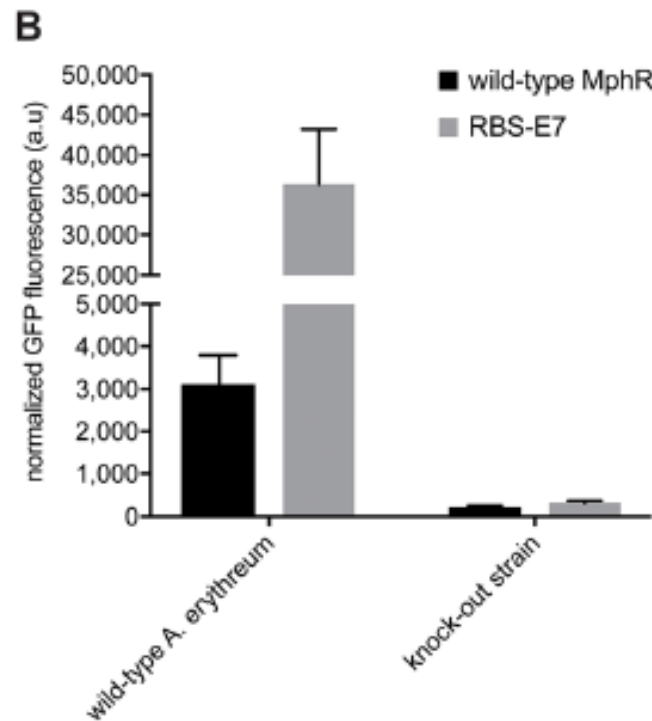
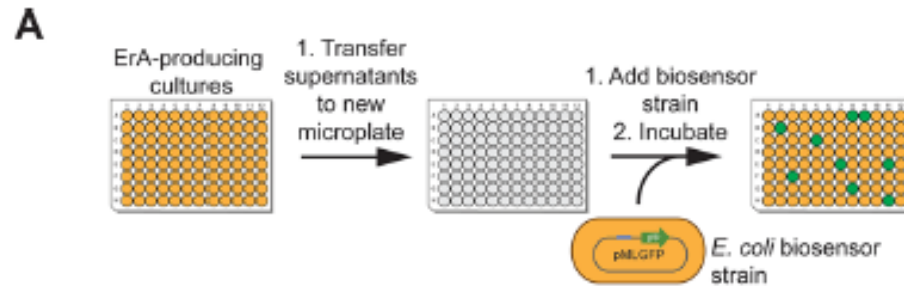
Schema del biosensore MphR



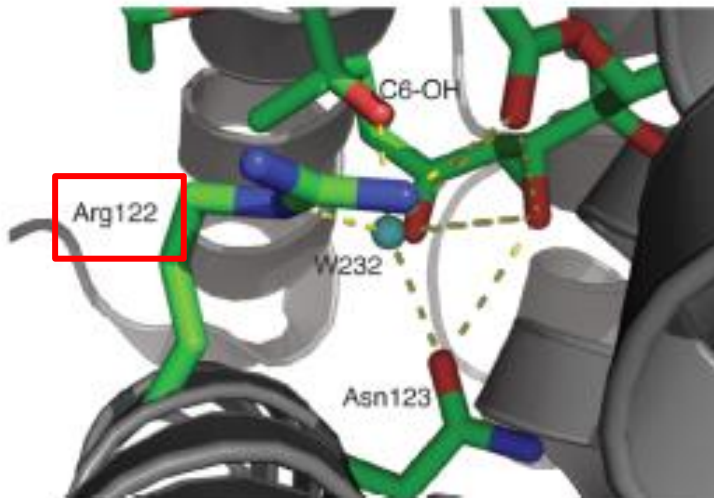
Sistema a due plasmidi

1. pJZ12 (p15A ori, 10 copie per cellula):
espressione di MphA macrolide fosfotrasferasi
2. pMLGFP (pBR322 ori, 10-15 copie per cellula):
espressione del sensore MphR e del reporter **GFP**

Schema del biosensore MphR

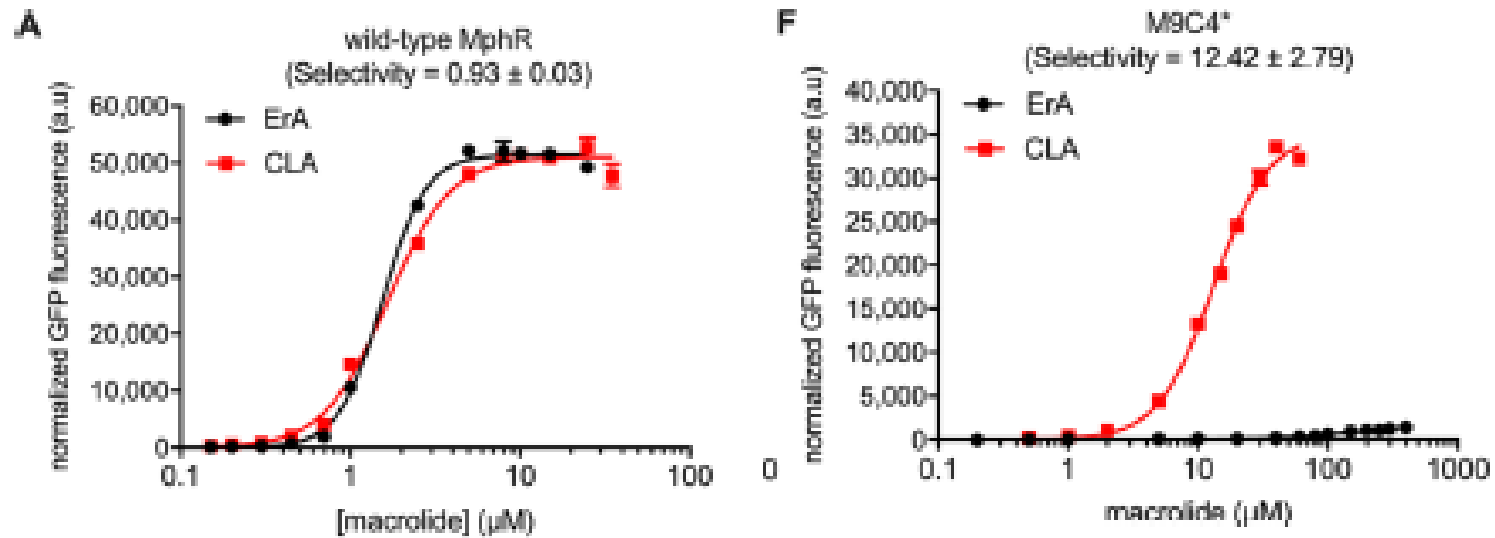


Mutagenesi razionale e mutagenesi random di MphR



PDB 3FRQ

Conversione della selettività di MphR da eryA a Cla



Mutante M9C4: R122T, K132N, A151T, H184Q

Da una libreria da error-prone PCR basata su R122T identificato da mutagenesi razionale

M9C4*: la sostituzione R122I migliora la selettività, diminuendo leggermente la sensibilità

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)

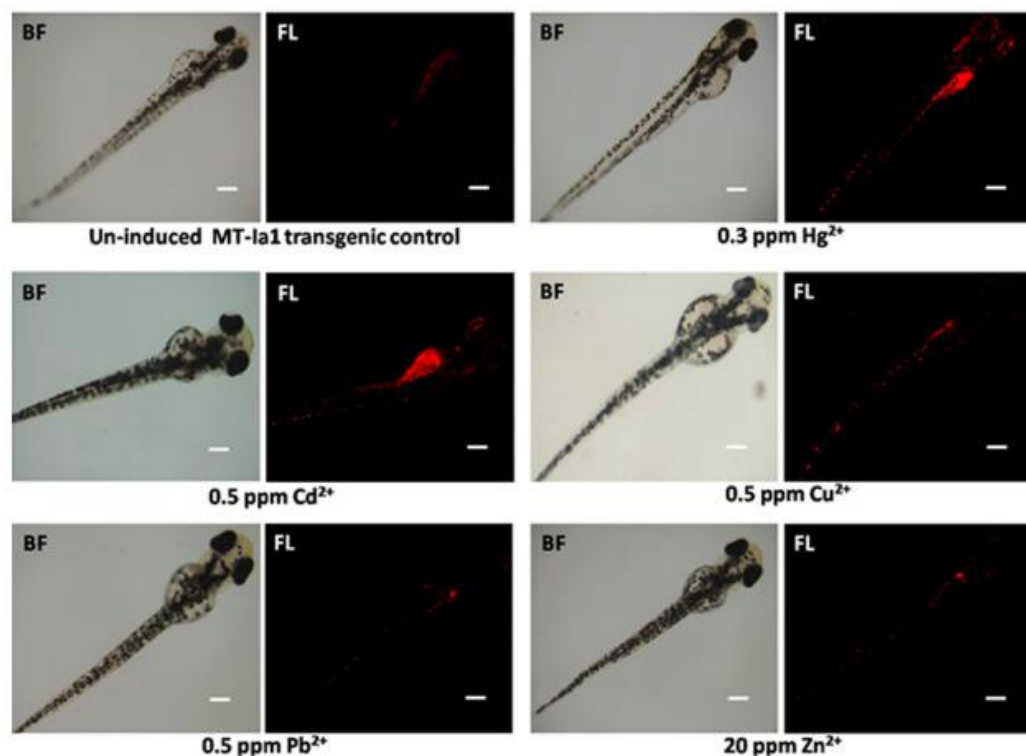


Fig. 2 Fluorescence imaging of F1 transgenic zebrafish (*D. rerio*) larvae (48 h post-fertilization) exposed for 8 h to Hg²⁺, Cd²⁺, Cu²⁺, Pb²⁺ or Zn²⁺ at doses that gave maximum fluorescence. Fluorescence expression is observed only in the

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 μ m