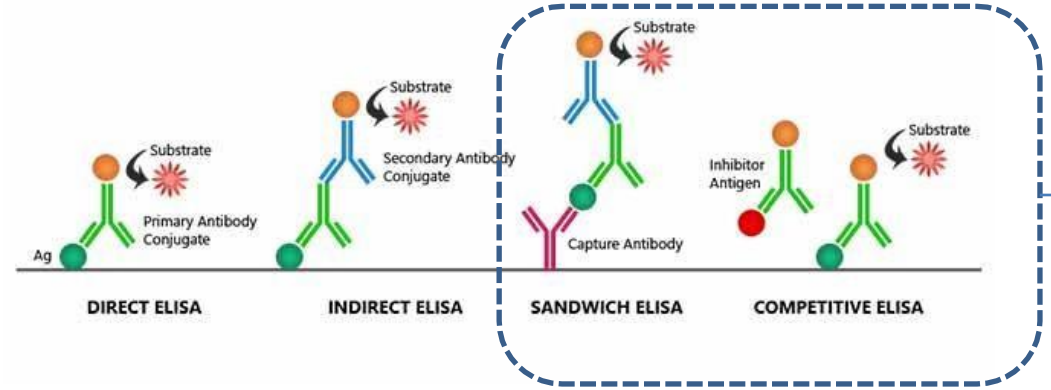
CONCENTRATION MOLES/LITER	METABOLITES/IONS	THERAPEUTIC DRUGS	STEROID AND AMINO ACID HORMONES	PROTEIN/POPLYPEPTIDE HORMONES	ANTIBODIES
10^{-1}	SODIUM				
	CHLORIDE				
10^{-2}		ETHANOL			
	GLUCOSE				
(mM)	UREA				
10^{-3}	CHOLESTEROL				
	CALCIUM	SALICYLATE			IgG (Total)
	TRIGLYCERIDES	ACETAMINOPHEN			
10^{-4}	PHENYLANINE	THEOPHYLLINE			
	AMMONIA	GENTAMICIN			
10^{-5}	IRON				
	BILIRUBIN				
(μM)				THYROXINE BINDING	IgM (Total)
10^{-6}			CORTISOL	GLOBULIN	
			T_4 ESTRIOL	PLACENTAL LACTOGEN	
10^{-7}			(Total)		IgG (SPECIFIC)
10^{-8}		DIGOXIN	CORTICOSTERONE		SYPHILIS
(nM)			T_3 ESTRADIOL	PROLACTIN	RUBELLA
10^{-9}			(Total) PROGESTERONE	HCG	ETC.
			T_4 (Free)	INSULIN	
					IgE (Total)
10^{-10}			ALDOSTERONE	PARATHYROID HORMONE HGH (Growth Hormone)	
10^{-11}			TSH (Thyroxine Stim. Hormone)	LH (Luteinizing Hormone)	
(pM)			ANGIOTENSIN		
10^{-12}			OXYTOCIN		
			VASOPRESSIN		

FIGURE 2. CLASSES OF CLINICALLY SIGNIFICANT ANALYTES AS A FUNCTION OF CONCENTRATION IN THE SAMPLE.

Enzyme Linked Immuno-Sorbent Assay (ELISA)

	ELISA	HPLC	LC-MS/MS
			
Price	Low	Medium	High
No. analytes per run	1 target	Multiple target	Up to 650
Accuracy	Screening	Reference method, highly sensitive and precise	Reference method (accredited results), highly sensitive and precise

Types of ELISA



Used for food and clinical analysis

An Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used laboratory technique designed to detect and quantify soluble substances such as peptides, proteins, antibodies, and hormones. ELISA tests are based on the principle of antigen-antibody interaction and are commonly used in diagnostics, research, and various industries for their sensitivity and specificity.

Key Components of ELISA:

1. **Antigen**: The substance to be detected or measured.
2. **Antibody**: A specific protein that binds to the antigen.
3. **Enzyme**: An enzyme linked to an antibody or antigen that produces a measurable signal, often a color change, when a specific substrate is added.
4. **Substrate**: A chemical that the enzyme acts upon to produce a detectable signal.

Types of ELISA:

1. **Direct ELISA**: Involves the direct attachment of the antigen to a plate, followed by the application of an enzyme-linked antibody.
2. **Indirect ELISA**: Uses a two-step process where a primary antibody binds to the antigen, and a secondary enzyme-linked antibody binds to the primary antibody.
3. **Sandwich ELISA**: Requires the antigen to be captured between two antibodies – a capture antibody bound to the plate and a detection antibody that binds to another site on the antigen.
4. **Competitive ELISA**: Measures the concentration of an antigen by detecting its ability to compete with a labeled antigen for binding to an antibody.

Procedure:

1.Coating: The wells of a microplate are coated with the antigen or antibody.

2.Blocking: A blocking agent is used to prevent nonspecific binding.

3.Incubation: The sample containing the antigen or antibody is added and incubated to allow binding.

4.Washing: Unbound substances are washed away.

5.Detection: An enzyme-linked antibody or antigen is added, followed by a substrate to produce a measurable signal.

6.Measurement: The signal, often a color change, is measured using a spectrophotometer or other appropriate device.

Applications:

•**Medical Diagnostics**: Detection of diseases, including HIV, hepatitis, and COVID-19.

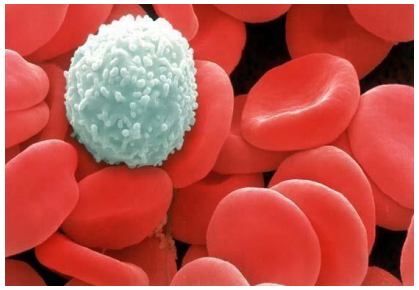
•**Food Industry**: Detection of allergens or pathogens.

•**Research**: Quantification of cytokines, hormones, and other biomarkers.

ELISA is valued for its accuracy, ability to process multiple samples simultaneously, and relatively

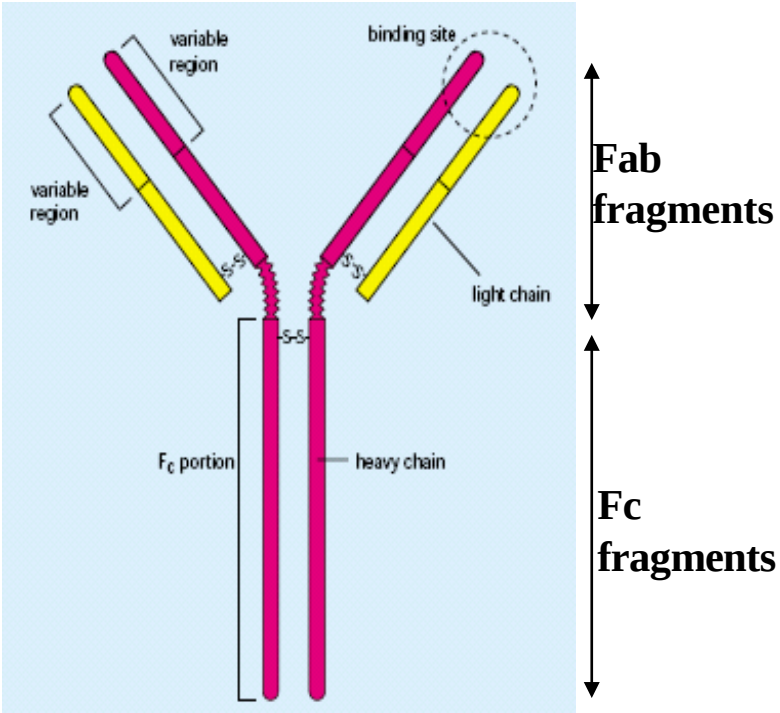
Antibodies

- Proteins secreted by B-lymphocytes (type of white blood cell), in vertebrates.



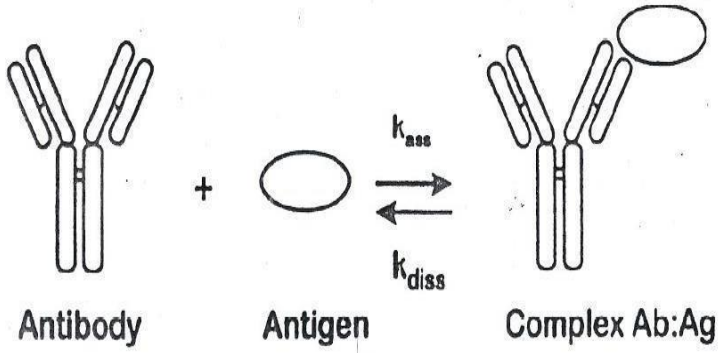
- Recognise and bind to molecules (**antigens**) on foreign particles, marking them for destruction by T-lymphocytes.

- Each antigen may generate several antibodies for different sites (**epitopes**) on antigen.



IgG molecule

Antibody - Antigen interaction



- Non - covalent
- Highly specific
- $k_{ass} \approx 10^6 - 10^8\ M^{-1}s^{-1}$
- $k_{diss} \approx 10 - 10^{-4}\ s^{-1}$
- $K_{aff} \approx 10^6 - 10^{12}\ M^{-1}$

$$v = \frac{d[Ab:Ag]}{dt} = k_{ass}[Ab][Ag] - k_{diss}[Ab:Ag]$$

Equilibrium: $\frac{d[Ab:Ag]}{dt} = 0$ and $K_{aff} = \frac{k_{ass}}{k_{diss}} = \frac{[Ab:Ag]}{[Ab][Ag]}$

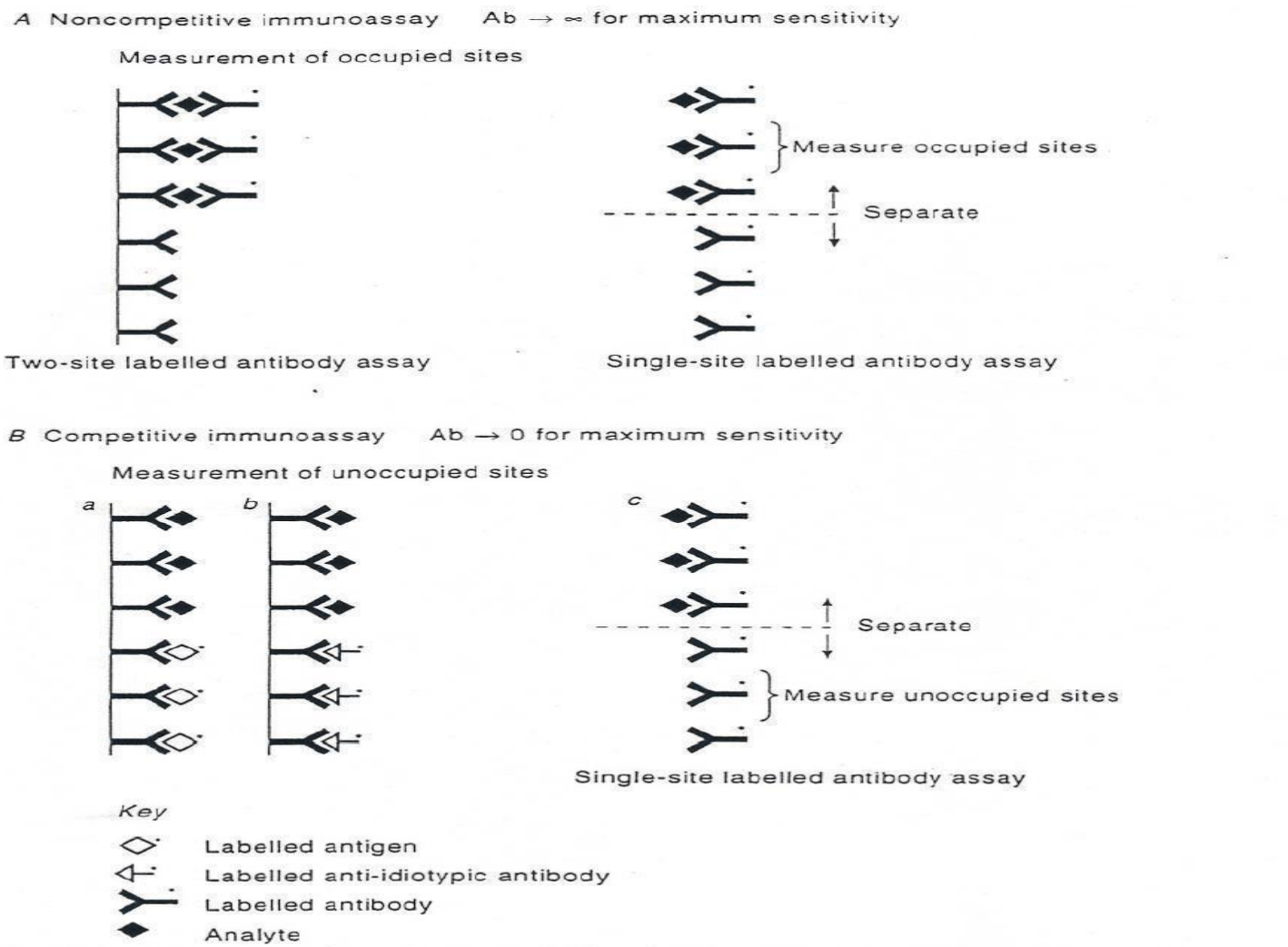
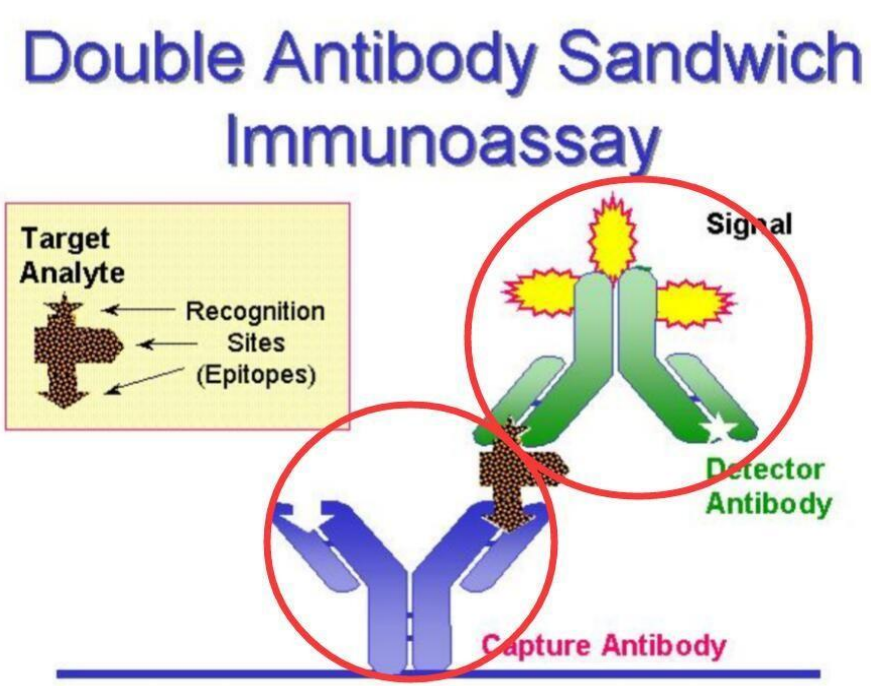
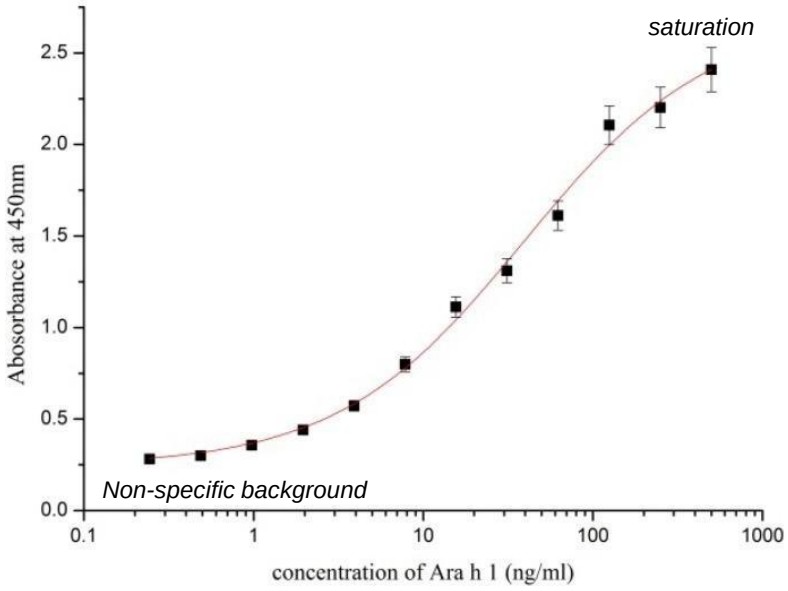


Figure 6 Basic competitive and noncompetitive immunoassay designs. The distinction between noncompetitive (*a*) and competitive immunoassays (*b*) reflects the way in which antibody binding site occupancy is observed. Labelled antibody methods are noncompetitive if occupied sites of the (labelled) antibody are directly measured, but are competitive (*Bc*) when unoccupied sites are measured. Labelled antigen (*Ba*) or labelled anti-idiotypic antibody methods (*Bb*) rely on measurement of sites unoccupied by analyte, and are therefore invariably of competitive design.



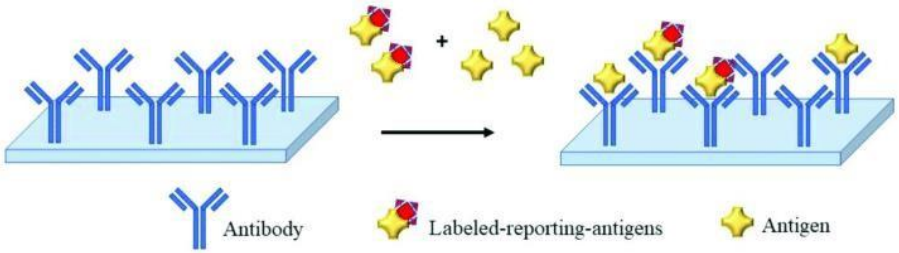
Dosaggio di un allergene (proteina) di arachide



Excess Antibody LOG SCALE!!

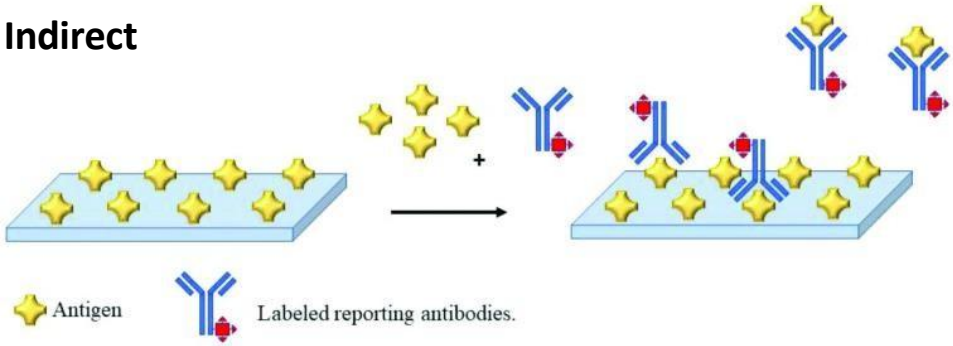
Competitive immunoassays

Direct



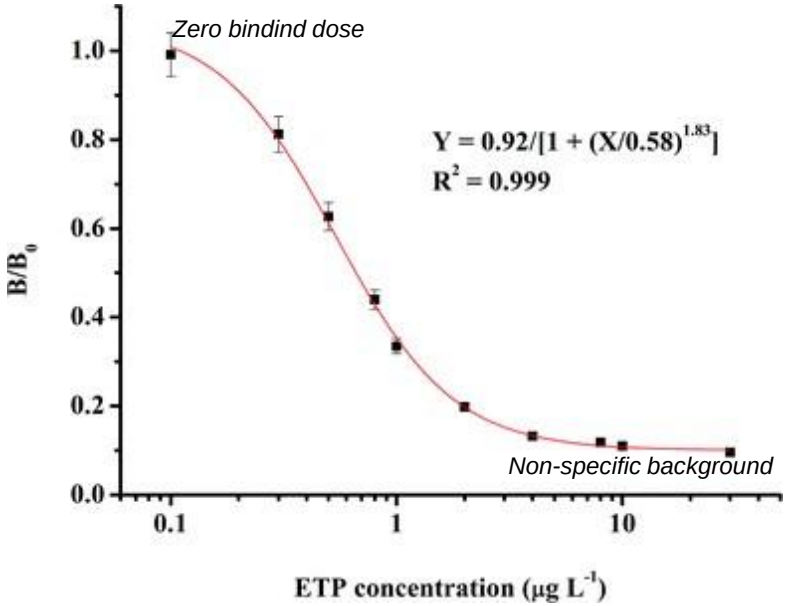
(a)

Indirect



(b)

Calibration curve for ethopabate (veterinarydrug)



Limiting Antibody LOG SCALE!!

TABLE 17.1
Overview of Immunoassay Techniques

Assay method	Label	Detected	Detector
RIA (radioimmunoassay)	^{125}I, ^{3}H, ^{14}C	Radiation	Scintillation counter
EIA (enzyme immunoassay)	HRP	Color change (absorbance)	Photometer
	AP		
	β-D-galactosidase		
	HRP, AP, galactosidase	Fluorescence	Fluorimeter
	HRP	Luminescence	Luminometer
	HRP, AP, GOD, catalase	Current	Amperometric electrode
FrIA (fluoroimmunoassay)	Fluorescein rhodamines, dansyl chloride, cumarines, phycoerythrin, also liposomes	Fluorescence	Fluorimeter
TR-FrIA (time-resolved FIA)	Lanthanoid cations: Eu^{3+}, Tb^{3+}, Sm^{3+}	Delayed fluorescence	Time-resolved fluorimeter
LIA (luminescence immunoassay)	Acridinium esters	Chemi- and bioluminescence	Luminometer
	Dioxetanes		
	Peroxyoxalates		
	Luminol		
	Luciferase/luciferin		
	Peroxidase		
	Pyrene	Electroluminescence	Electrode luminometer
Electrochemical immunoassays	Metallocenes	Current	DPP (differential pulse polarograph)
	Metals		
	GOD, catalase		DPASV (differential pulse anodic stripping voltammetry)
	Urease	Ions (potential change)	Potentiometric electrode
	Liposomes		

Note: AP: alkaline phosphatase, GOD: glucose oxidase, HRP: horseradish peroxidase.

$$y = \frac{a-d}{(1+(x/c)^b)} + d$$

a = (theoretical) response at low concentration/dilution
b = absolute value of the slope at the inflection point
c = value of x at inflection point
d = (theoretical) response at high concentration/dilution
x = concentration or dilution
y = response (OD)

Typical calibration curve for immunoassay

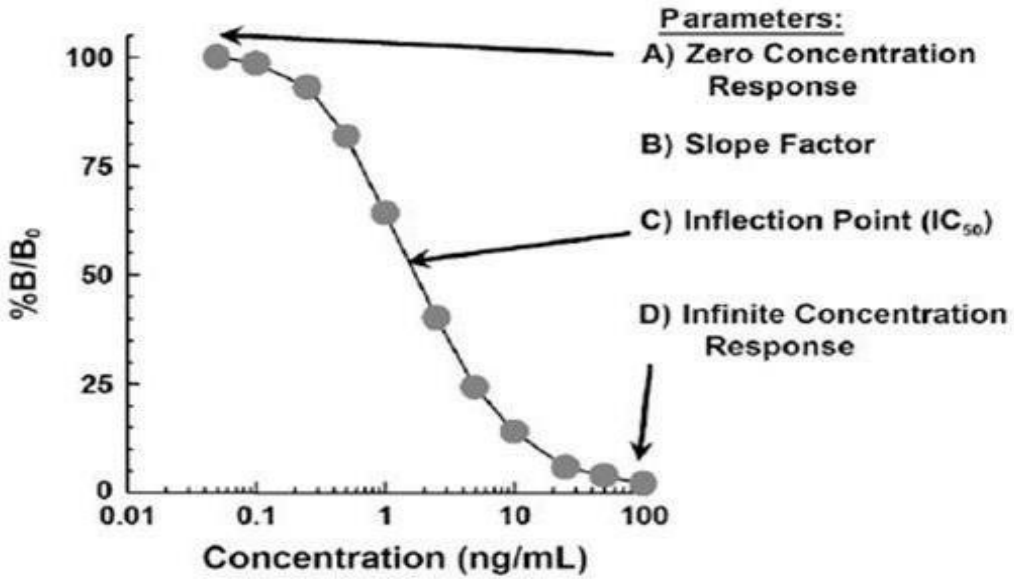
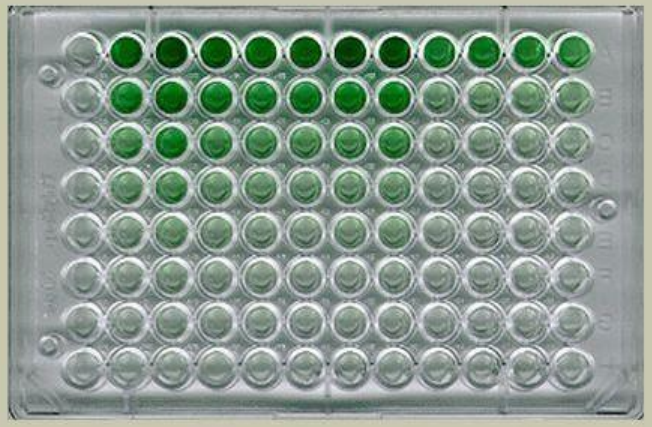


Figure 1. Typical 4-parameter logistic graph for a competitive-format immunoassay.

ELISA

- **Enzyme-linked immunosorbent assay (ELISA)** is a test that uses antibodies and color change to identify a substance.

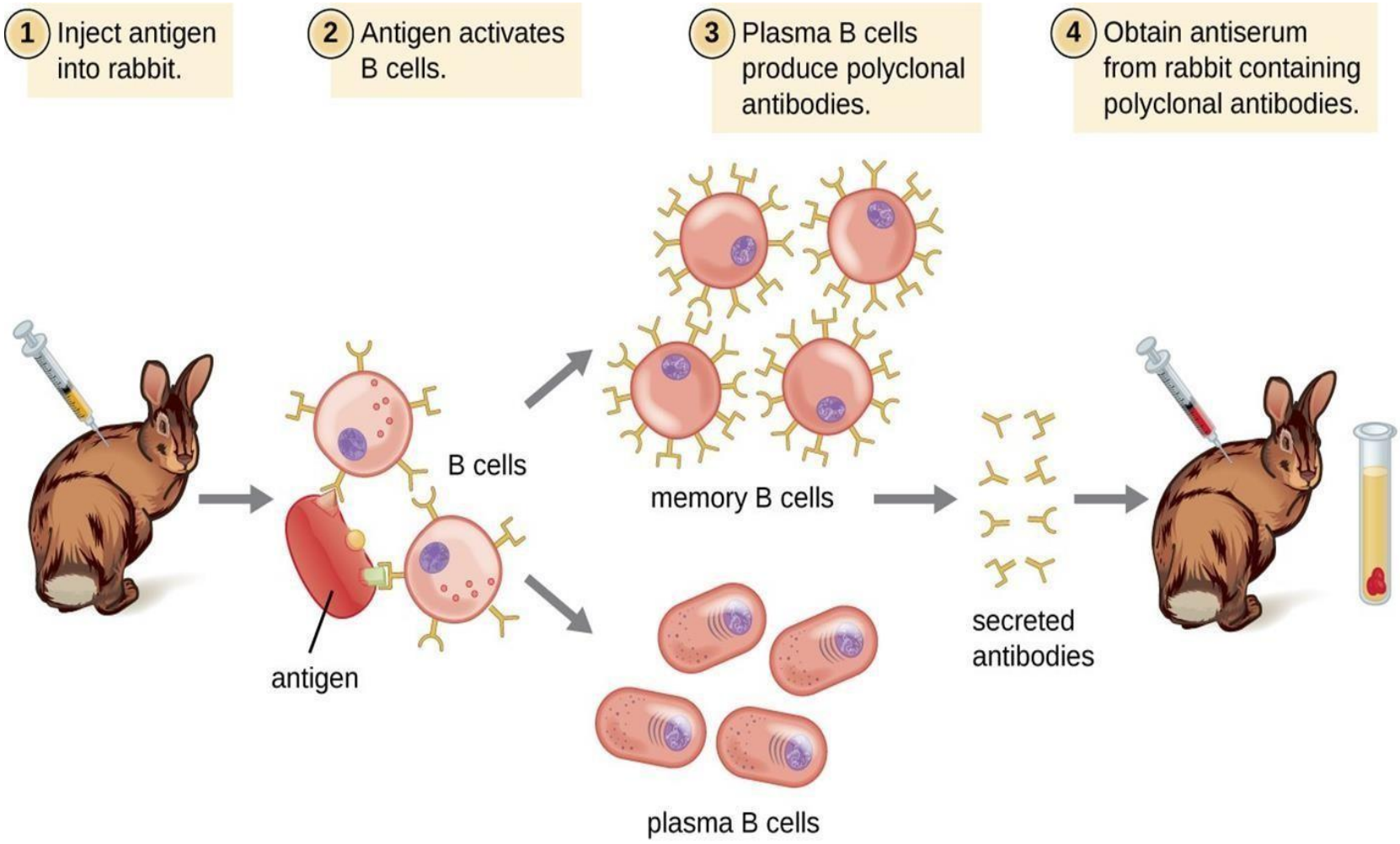



DIAGNOSTIC AUTOMATION, INC.
IMMUNODIAGNOSTICS
AccuDiag™ ELISA
Aflatoxin B1
(In Food)
REF 5120-8
LOT AFB-132
2012-02
96 Tests
2°C
8°C
DIAGNOSTIC AUTOMATION, INC.
23961 Craftsman Road, Ste E/F,Calabasas,CA 91302 U.S.A.
Tel: 1 (818) 591-3030 Fax: 1 (818) 591-8383
Website: http://www.rapidtest.com
Email: onestep@rapidtest.com
Made in USA
961 CRAFTSMAN ROAD, SUITE E/F • CALABASAS, CA 91302,
•TEL (818) 591-3030 • FAX (818) 591-8383.
ebsite: http://www.rapidtest.com • Email: onestep@rapidtest.cc
LOT
0.5 ml
60 ml

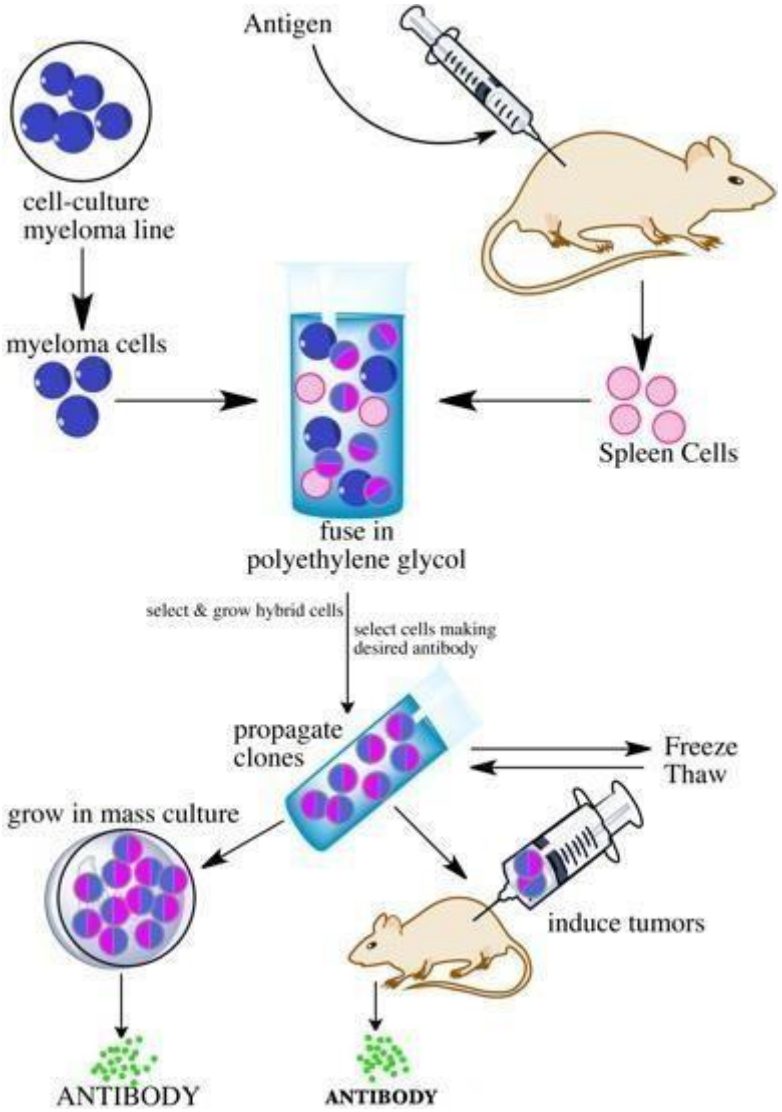
À la carte ELISA Systems

Almond	Beta-Lactoglobulin	Buckwheat	Casein	Crustacean
Egg	Gluten	Hazelnut	Lupin	Mustard
Peanut	Sesame	Soy		

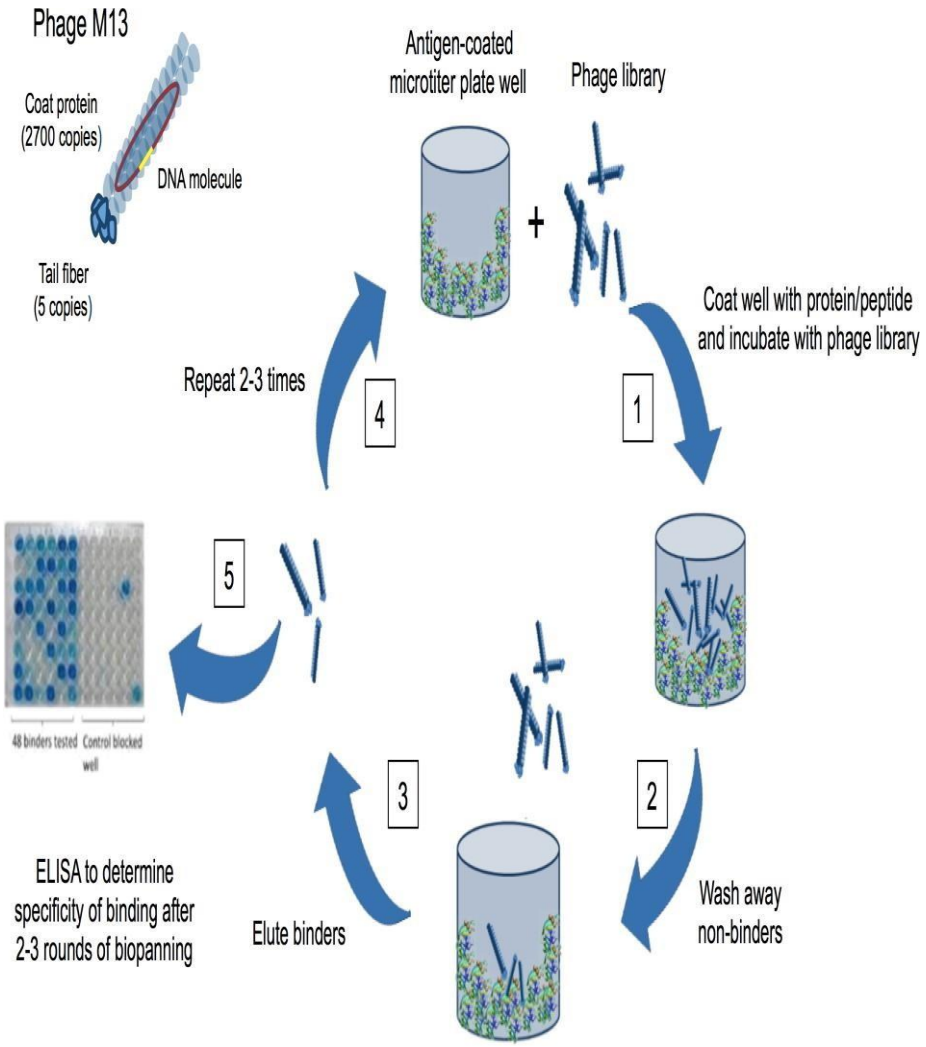
Polyclonal antibodies production



Monoclonal antibodies

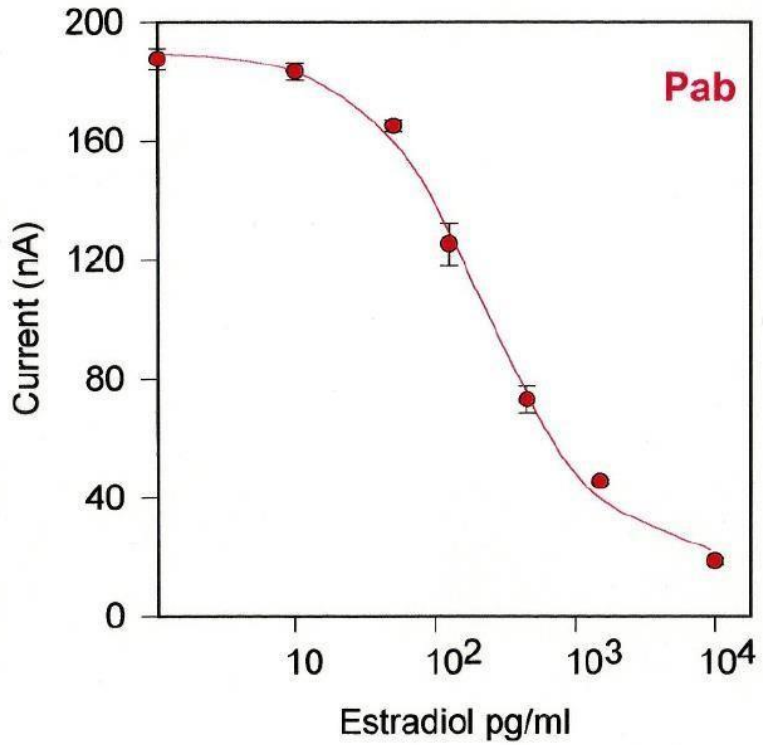


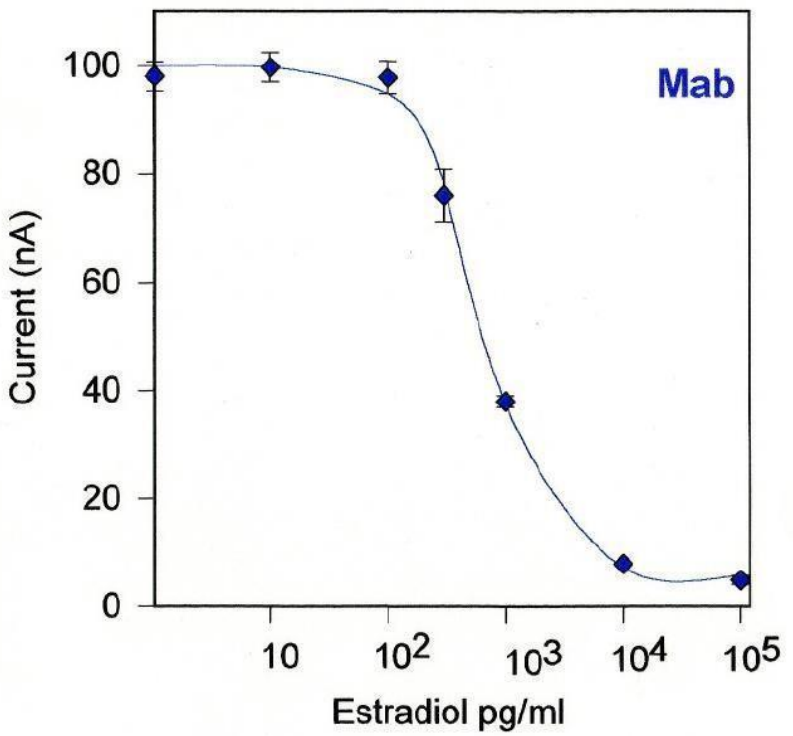
Recombinant antibodies



Enzime Linked Immuno-Sorbent Assay

ELISA elettrochimico





❖ Electrochemical detection:

Chronoamperometry

Differential pulse voltammetry (DPV)

❖ Enzymes and substrates:

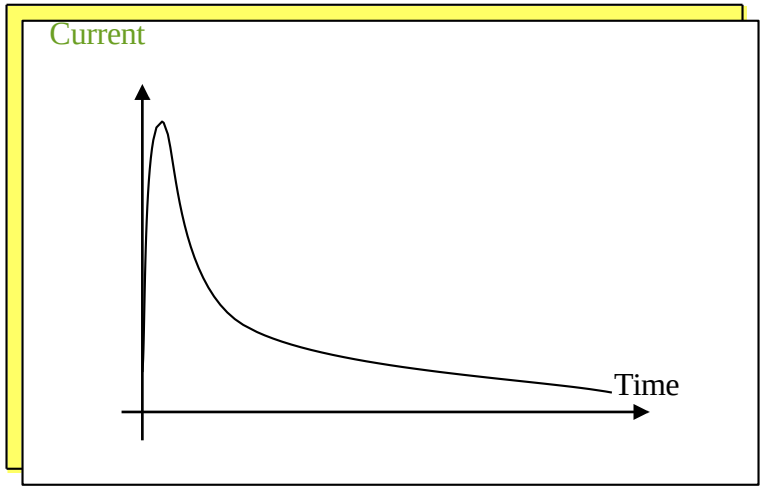
Alkaline phosphatase

1-naphtyl-phosphate

Horseradish peroxidase

- TetramethylBenzidine + H_2O_2
- $[K_4Fe(CN)_6]$ + H_2O_2

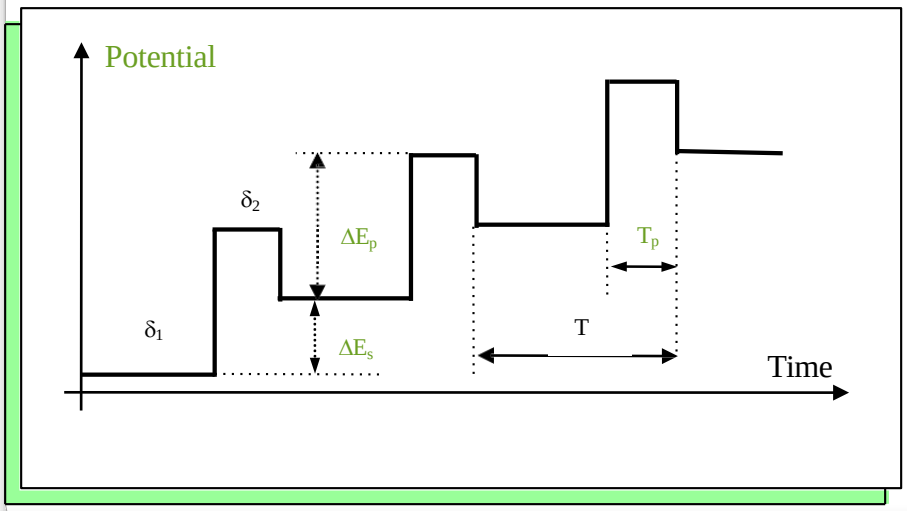
electrochemical detection:
chronoamperometry and differential pulse voltammetry (DPV) :

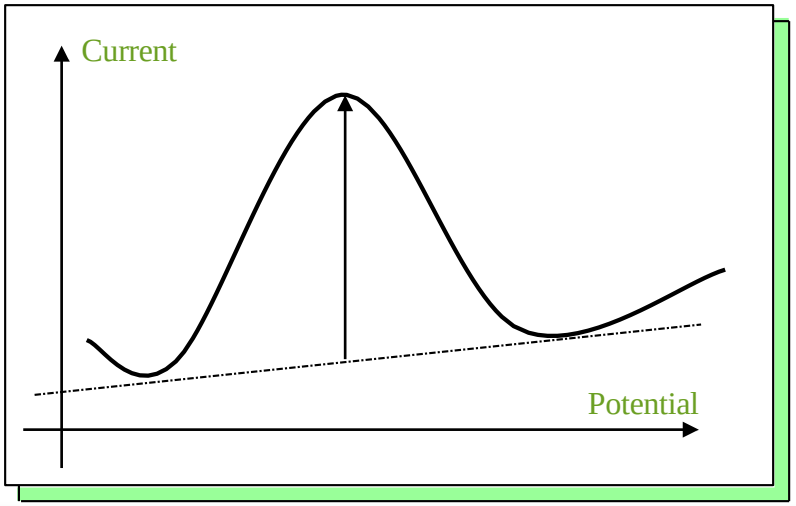


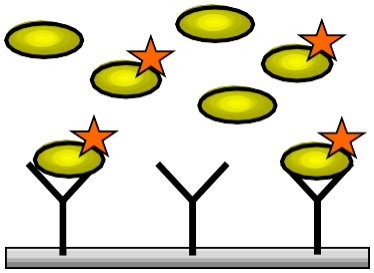
ΔE= const.

δ_2= 60 ms

ΔE_p= 5-100 mV







Anti OTA-Ab

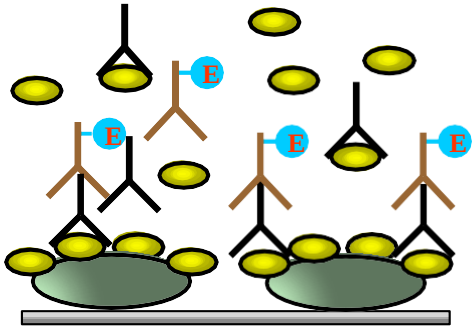
BSA-OTA

OTA

SAb-ALP

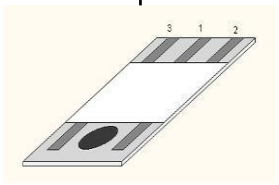
OTA-ALP

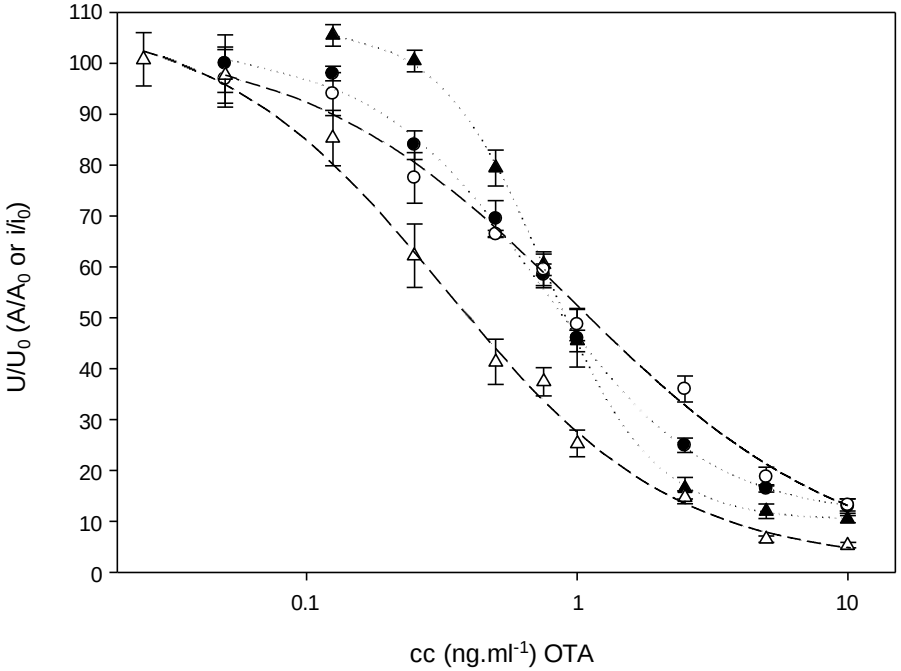
Anti OTA-Ab

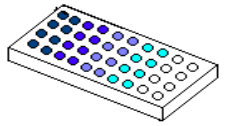


Direct

indirect







$$f(x) = \{(a - d)/[1 + (x/c)b]\} + d$$

	Competition curve parameters				Linear regression
	a (A or nA)	*b* (nA.ng.ml^{-1})	*c* (ng.ml^{-1})	*d* (A or nA)	
ic **spettr**	1.220 ± 0.053	1.40 ± 0.50	0.80 ± 0.22	0.129 ± 0.077	$f(x) = 49.3\ (\pm 0.8) - 57.5\ (\pm 0.1)\ x$ [$r = 0.991$]
ic **amp.**	6019 ± 118	0.90 ± 0.22	0.93 ± 0.10	176 ± 30	$f(x) = 52.5\ (\pm 0.4) - 43.7\ (\pm 0.5)\ x$ [$r = 0.994$]
dc **spettr**	1.392 ± 0.061	2.17 ± 0.15	0.80 ± 0.14	0.132 ± 0.071	$f(x) = 47.4\ (\pm 0.7) - 86.0\ (\pm 0.3)\ x$ [$r = 0.993$]
dc **amp.**	707 ± 56	1.10 ± 0.10	0.35 ± 0.04	16 ± 13	$f(x) = 34.9\ (\pm 0.6) - 52.2\ (\pm 0.9)\ x$ [$r = 0.992$]

Immunoassay	**Working Range** (ng/ml)	**L.O.D.** (Blank – 3 σ) (ng/ml)
ic spettr	0.20 – 2.5	0.150
ic **amp.**	**0.10 – 7.5**	**0.120**
dc **spettr**	0.10 – 10	0.080
dc **amp.**	**0.05 – 2.5**	**0.060**

Immunosensor procedure:

✓Pre-coating: 6 μl of rabbit anti IgG (4° C overnight)

✓Blocking: 6 μl of 1 % PVA (polyvinyl alcohol) (30 min)

✓Coating: 6 μl of anti-OTA Ab (1 h)

✓Competition: 6 μl of OTA-AP + standard/sample (30 min)

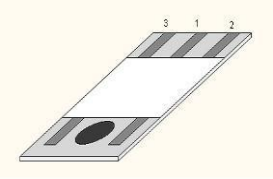
✓Detection: 100 of 5 mg/ml 1-Naphtylphosfate (2 min) + DPV

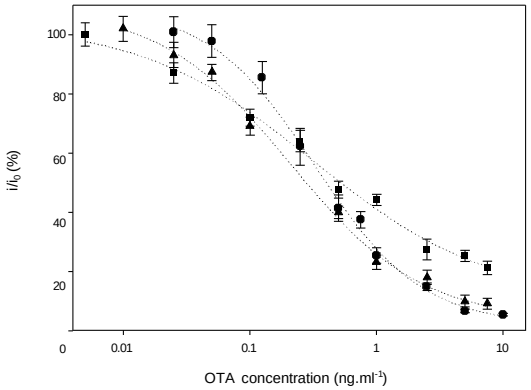
washings: 150 μl phosphate buffer pH 7.4

effect of extraction solvent

Activity of an electrode modified with IgG-ALP after 30 min incubation with 1:9 - 9:1 solutions (1:1 in DPBS) acetonitrile:water → 95-108%

Sensitivity of the calibration curve ~ 50%





25 g in 100 mL di ACN:H_2O

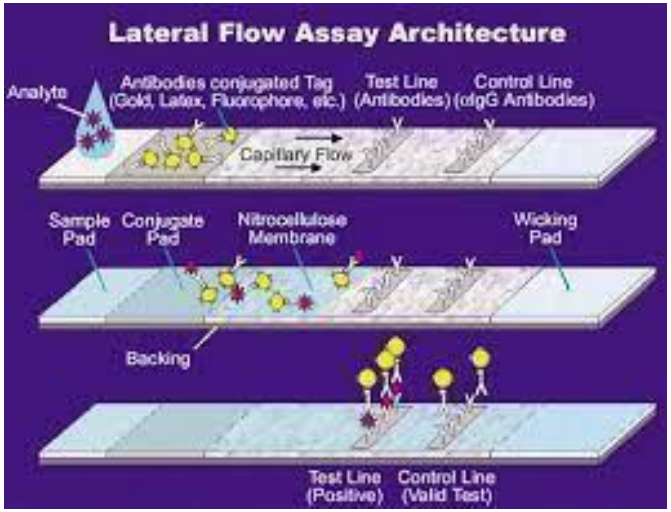
Final dilution 1:8

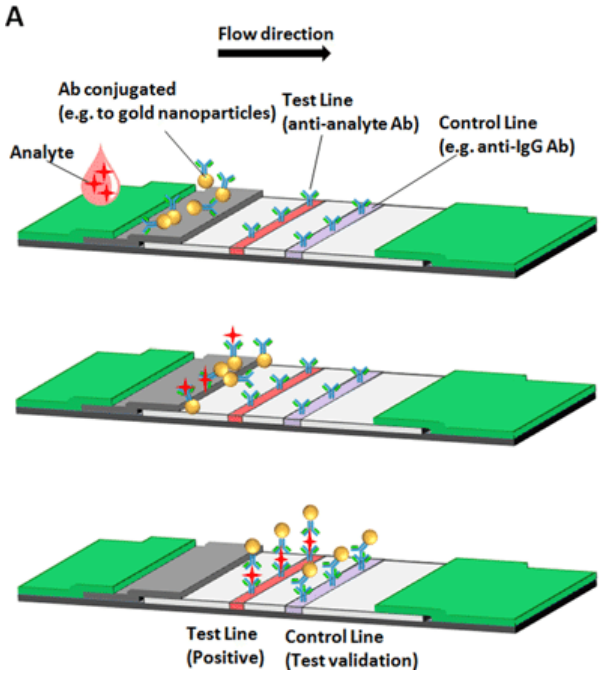
MRL = 3 ng/g

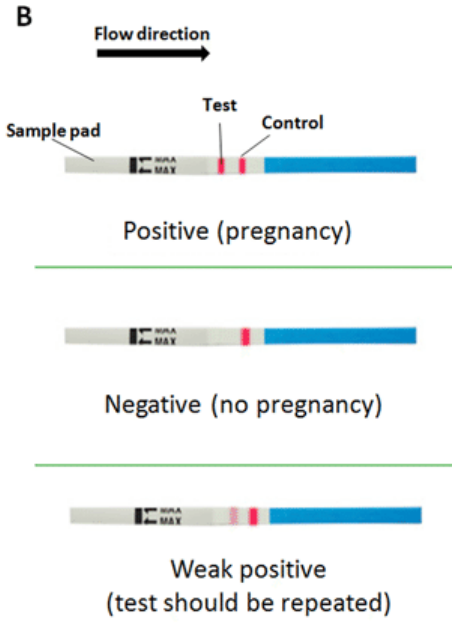
I_{50} = 1.6 ng/g

Parameters		0.1 PBS Buffer ●	ACN:H_2O (6:4) ▲	Wheat Extract (blank) ■
a	(nA)	707 (± 56)	260 (± 12)	408 (± 72)
b	(nA.ng.ml^{-1})	1.1 (± 0.1)	0.62 (± 0.03)	0.8 (± 0.1)
***c* (I_{50})**	(ng.ml^{-1})	0.35 (± 0.04)	0.32 (± 0.02)	0.20 (± 0.03)
d	(nA)	16 (± 13)	24 (± 8)	13 (± 15)
w.r.	(ng.ml^{-1})	0.05 – 2.5	0.02 – 5.0	0.05 – 2.5
L.O.D.	(ng.ml^{-1})	0.06	0.015	0.05
Lin.	**Reg.**	30.9 (± 0.6) – 52.2 (± 0.9) *x*	42.3 (± 0.3) – 25.4 (± 0.6) *x*	23.5 (± 0.1) – 41.1 (± 0.5) *x*

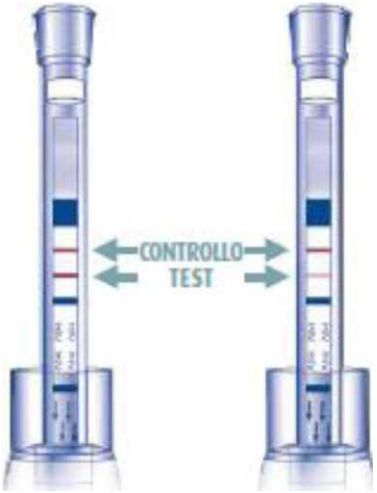
Lateral flow immunoassays





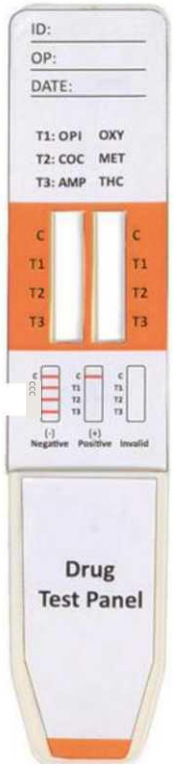


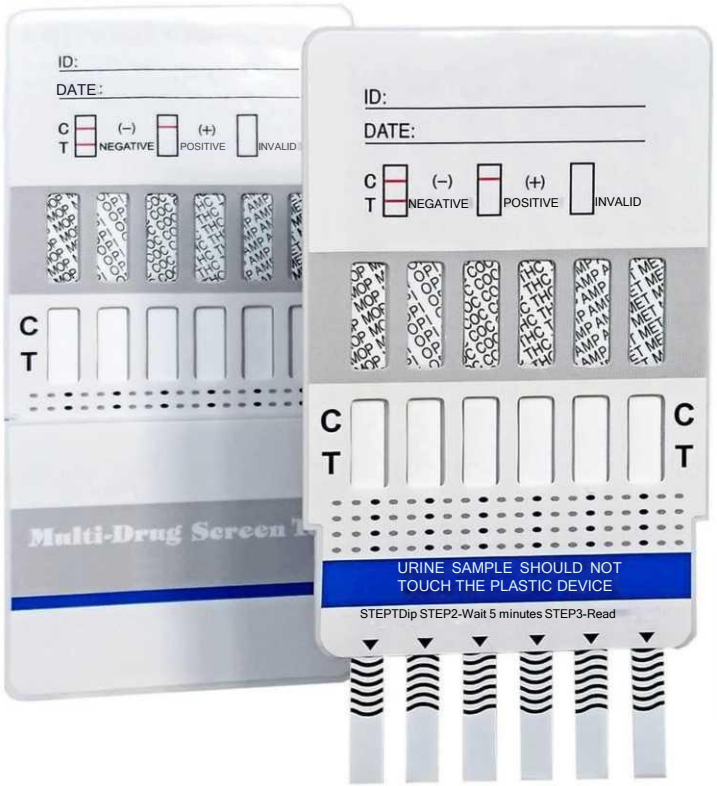
Human corionic gonadotropin in urine assay



First Commercialised LFIA by Unipath, 1988

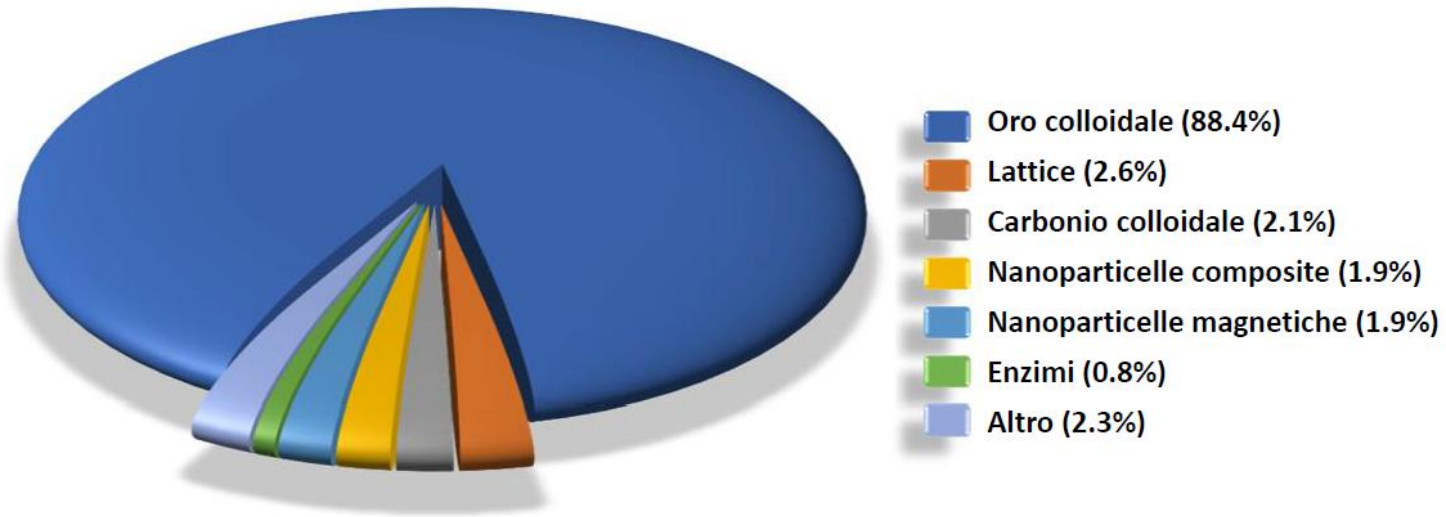
Drugs of Abuse





Marcatori colorimetrici in LFIA

Marcatori colorimetrici più utilizzati in LFIA (pubblicazioni scientifiche dal 2010 al 2019 compresi):



* Di Nardo, F.; Chiarello, M.; Cavalera, S.; Baggiani, C.; Anfossi, L. *Sensors* **2021**, 21, 5185.

LFIA FORMATS

NON-COMPETITIVE

Negative

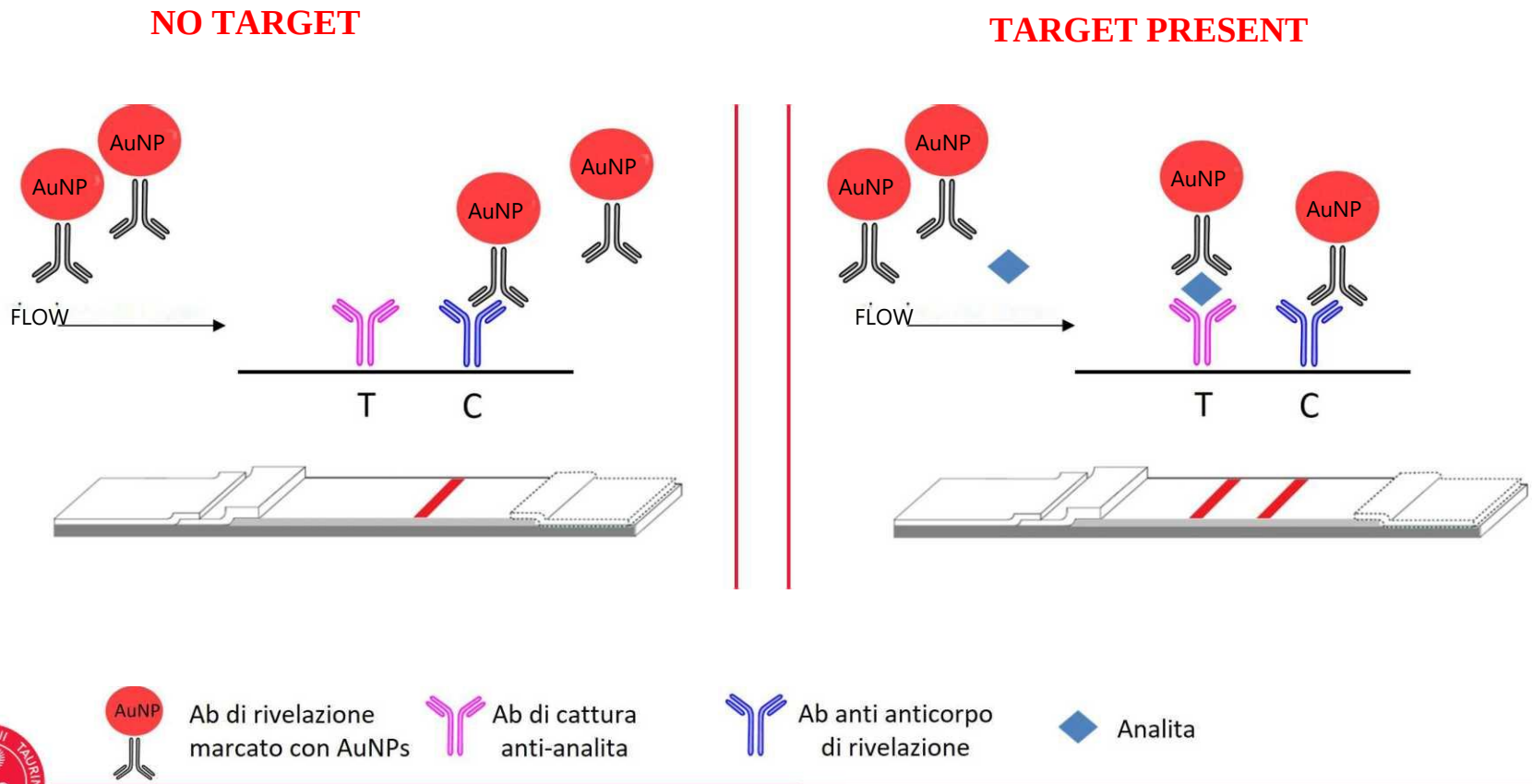
Positive

COMPETITIVE

Negative

Positive

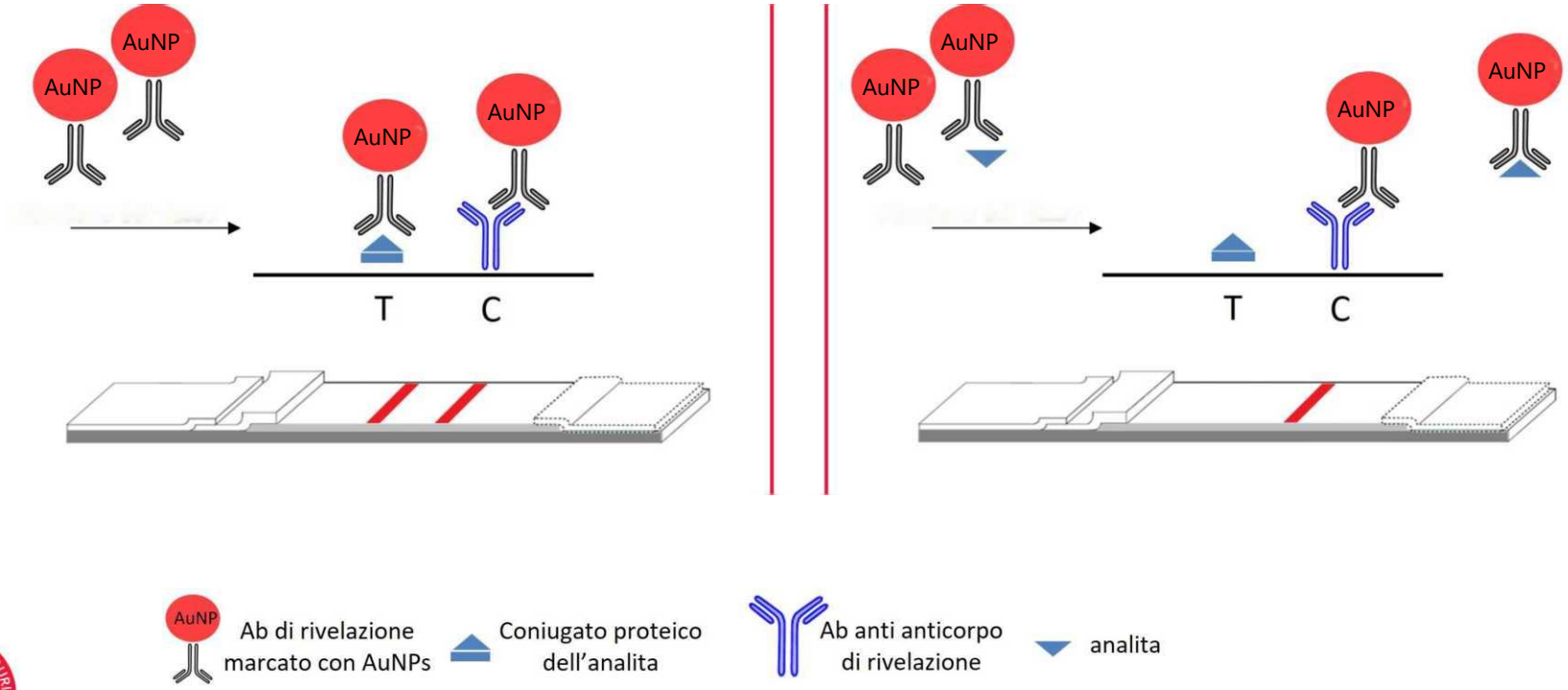
non competitive (sandwich)



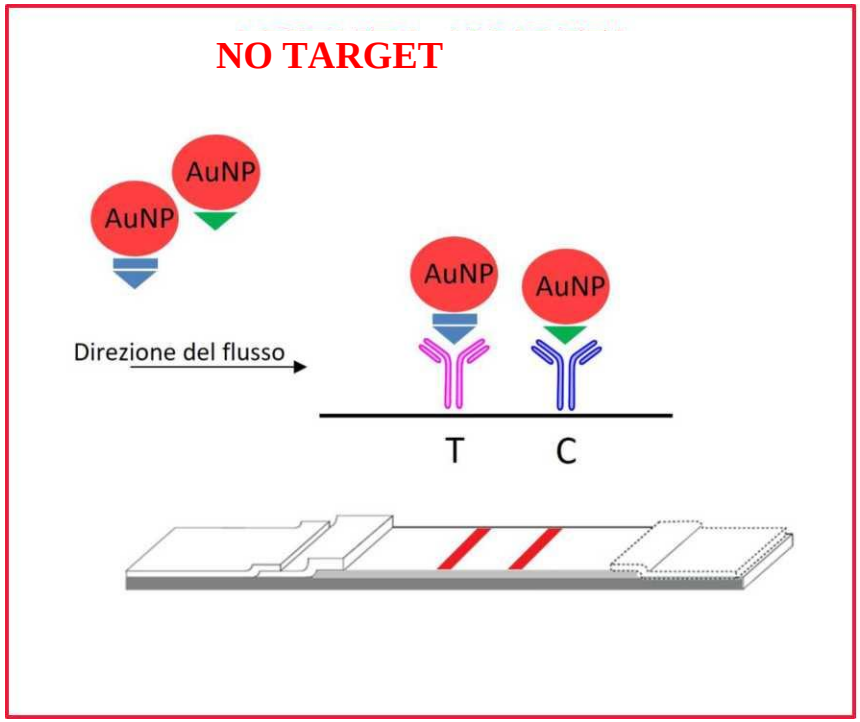
competitive indirect

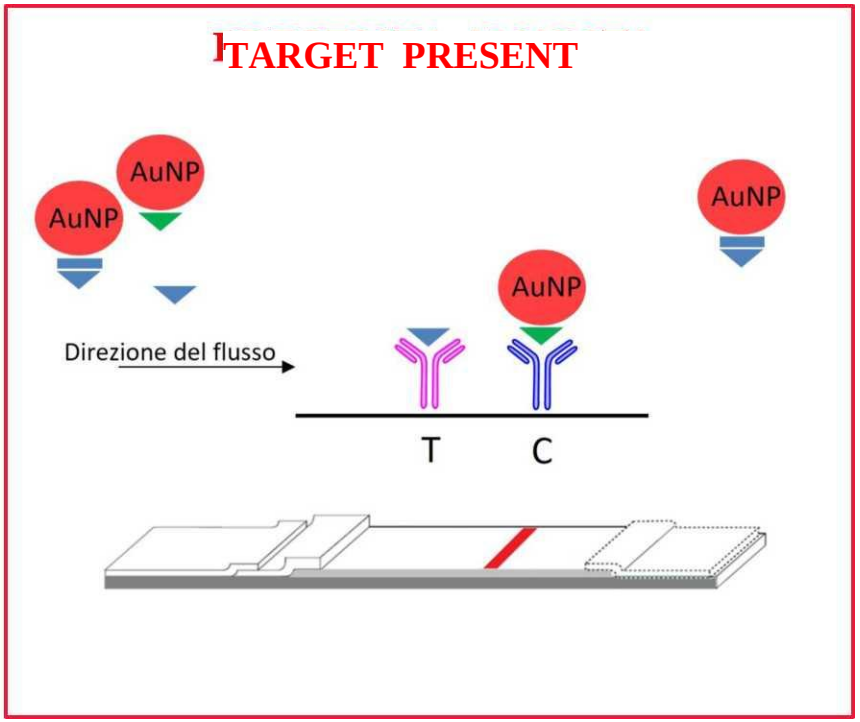
NO TARGET

TARGET PRESENT



competitivo direct





AuNP Omologo dell'analita marcato con AuNPs

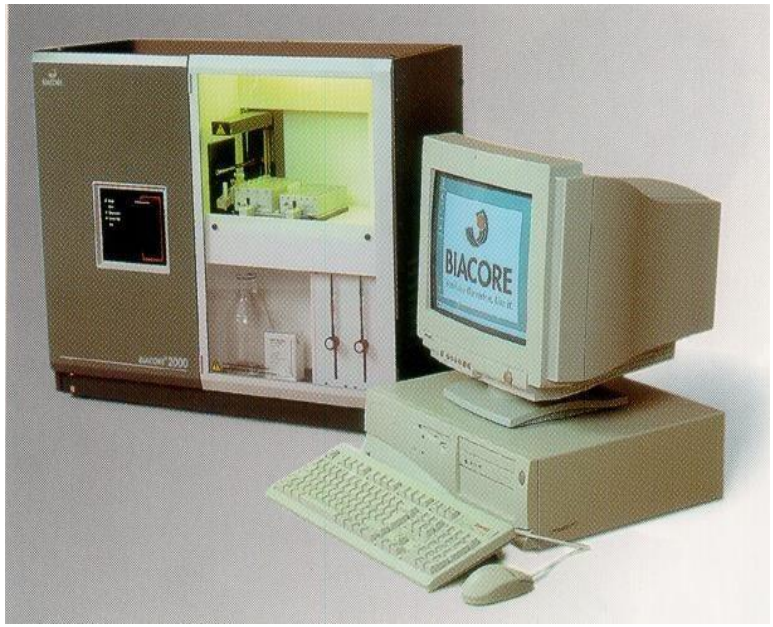
AuNP Antigene non-target marcato con ANPs

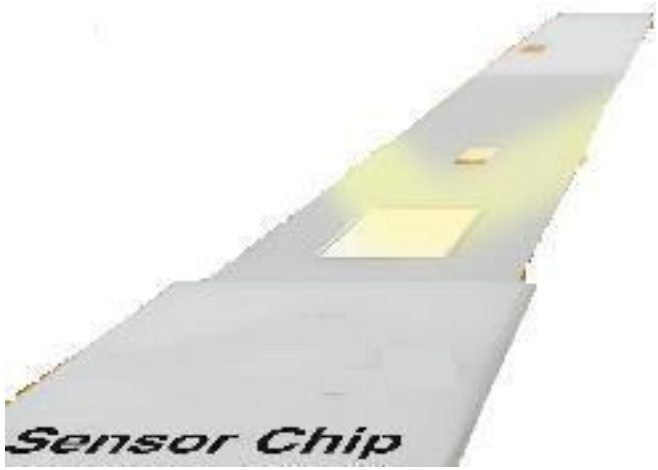
Ab di cattura anti-analita

Ab di cattura anti antigene non target

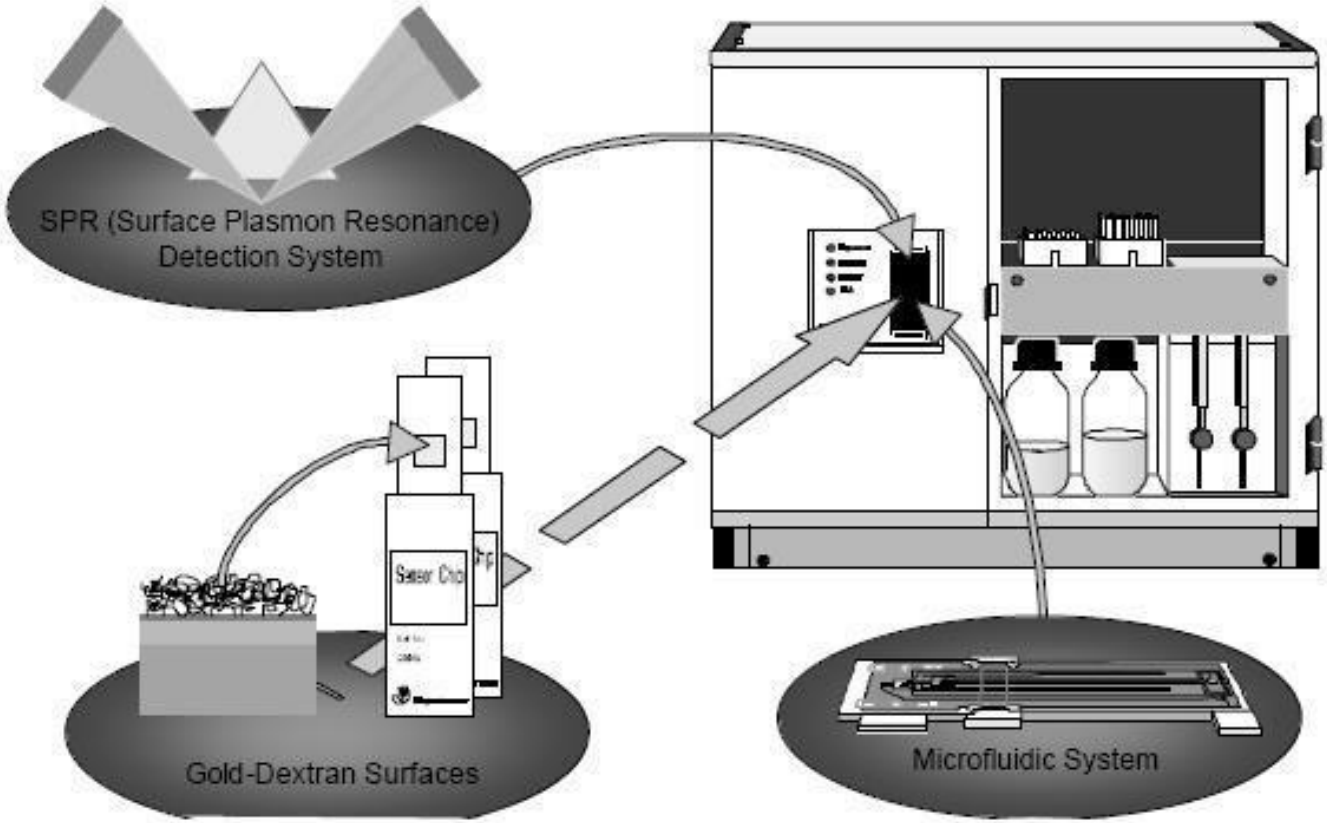
Analita

Biacore

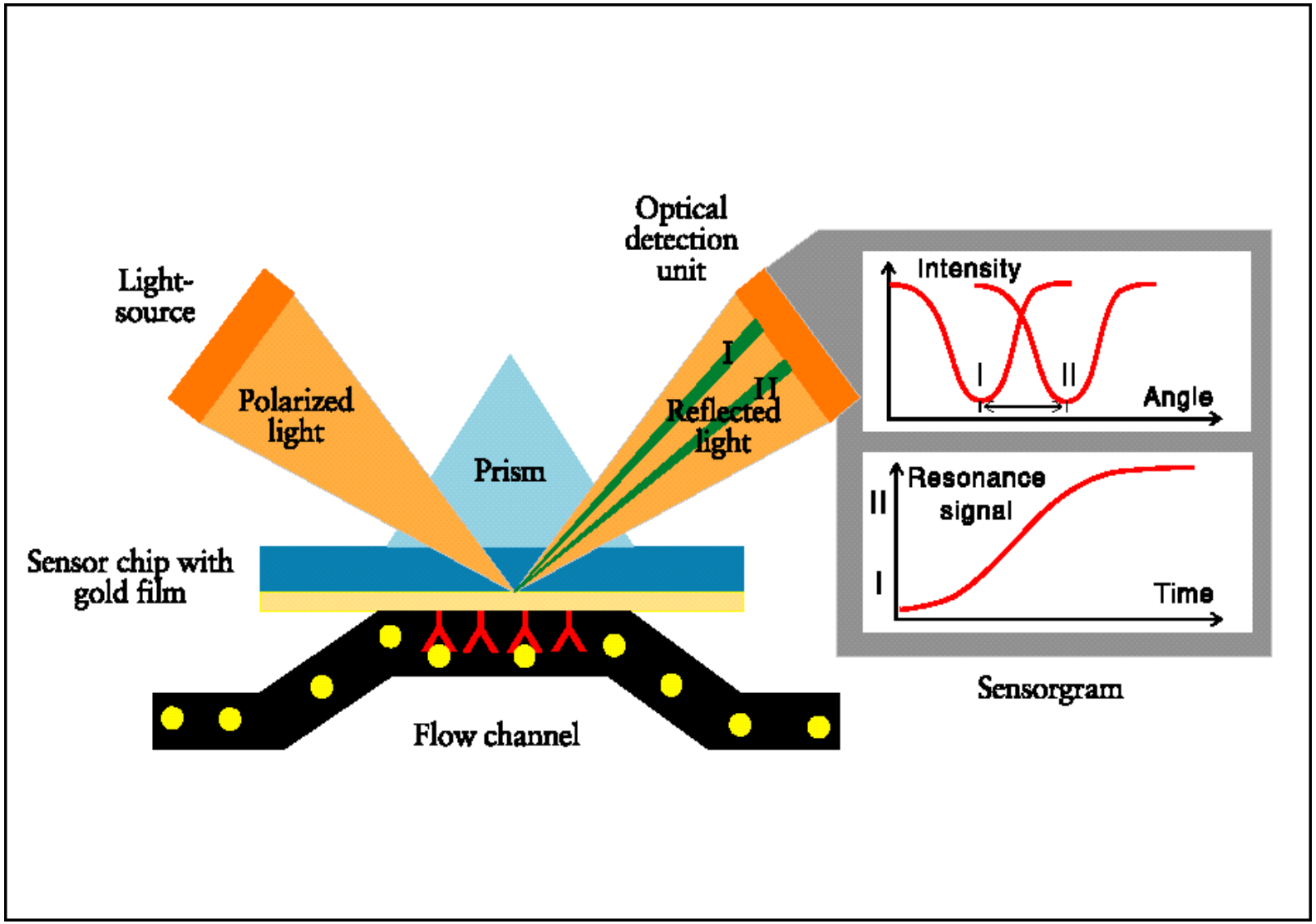




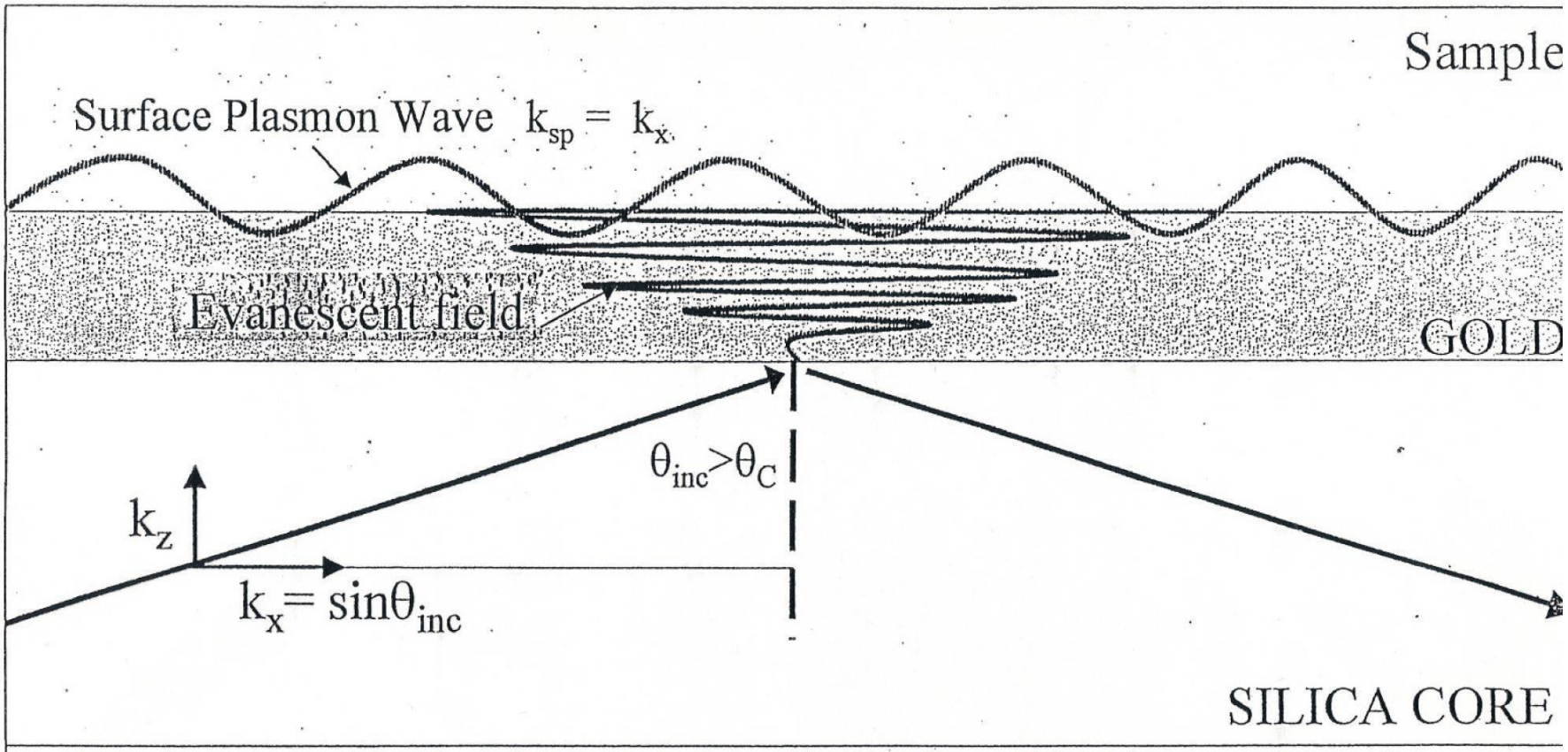

BIACORE


SPR (Surface Plasmon Resonance)
Detection System
Gold-Dextran Surfaces
Microfluidic System

SPR Biosensors



Surface Plasmon Resonance



θ_{inc} - angle of incident light

λ_{inc} - wavelength of incident light

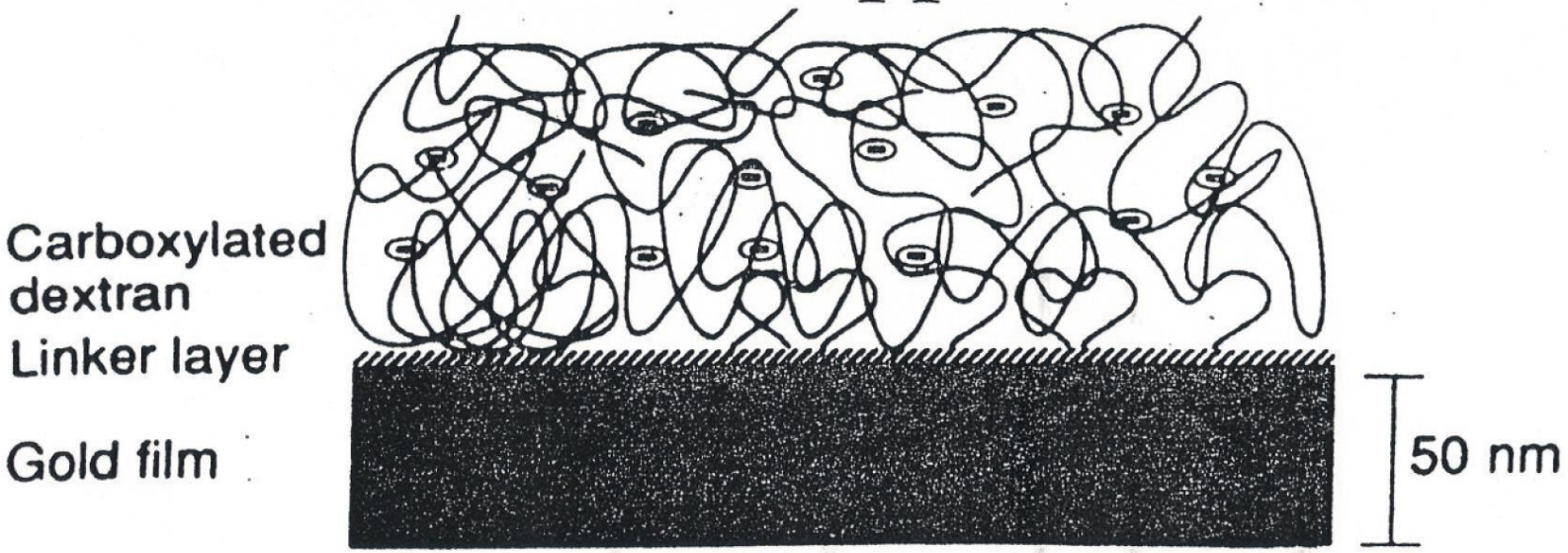
n_{glass} - Refractive Index of glass

n_{metal} - Refractive Index of metal

n_{sample} - Refractive index of sample

t_{metal} - Thickness of metal

BIACORE approach



dextran hydrogel

open structure (good accessibility)

no denaturation

- **enhancement of the capacity of the interaction layer**
- **stagnant layer / mass transport flow needed (µl/min)**
- **negative charge**
- **regenerable (up to 100 x)**

SPR principles

Surface plasmon resonance (SPR) arises when light is reflected under certain conditions from a conducting film at the interface between two media of different refractive index. the media are the sample and the glass of the sensor chip, and the conducting film is a thin layer of gold on the chip surface. SPR causes a reduction in the intensity of reflected light at a specific angle of reflection. This angle varies with the refractive index close to the surface on the side opposite from the reflected light.

When molecules in the sample bind to the sensor surface, the concentration and therefore the refractive index at the surface changes and an SPR response is detected. Plotting the response against time during the course of an interaction provides a quantitative measure of the progress of the interaction. This plot is called a sensorgram.

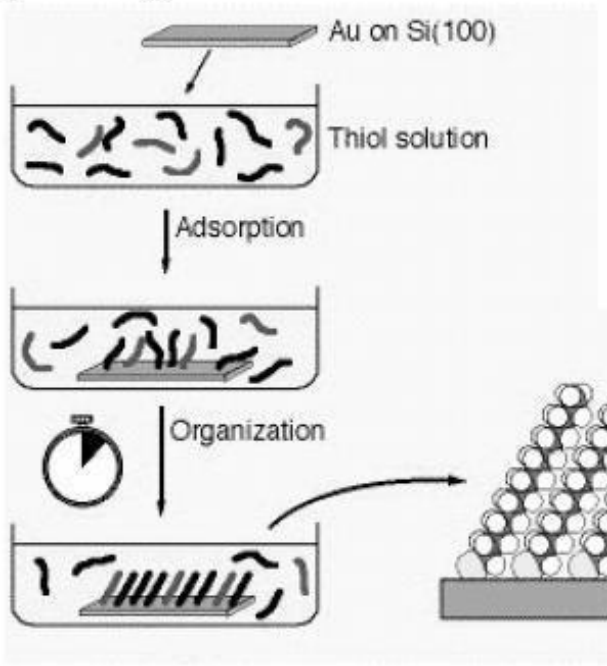
What Biacore actually measures is the angle of minimum reflected light intensity. The light is not absorbed by the sample: instead the light energy is dissipated through SPR in the gold film. Thus the light used to detect interaction processes never enters the sample.

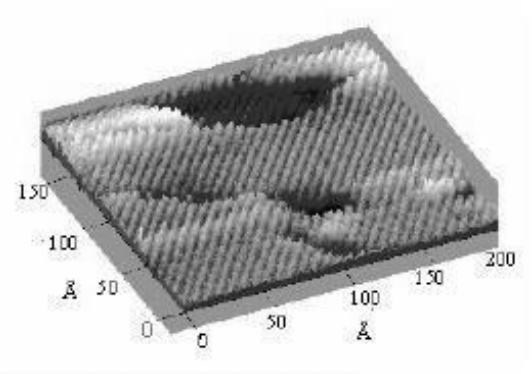
SPR response values are expressed in resonance units (RU). One RU represents a change of 0.0001° in the angle of the intensity minimum. For most proteins, this is roughly equivalent to a change in concentration of about 1 pg/mm2 on the sensor surface. The exact conversion factor between RU and surface concentration depends on properties of the sensor surface and the nature of the molecule responsible for the concentration change.

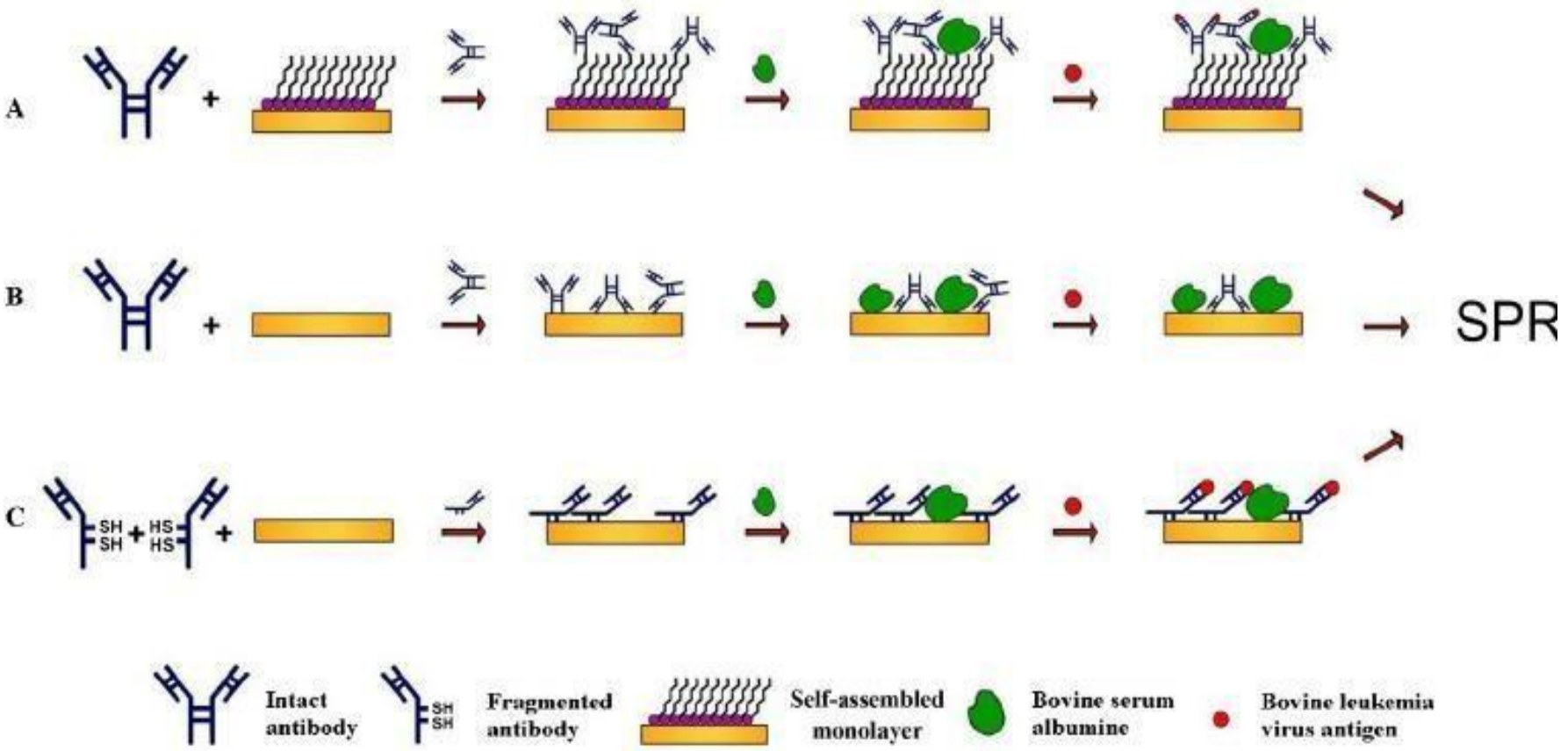
Immobilisation of organic molecules on gold

Self Assembled Monolayers (SAM)

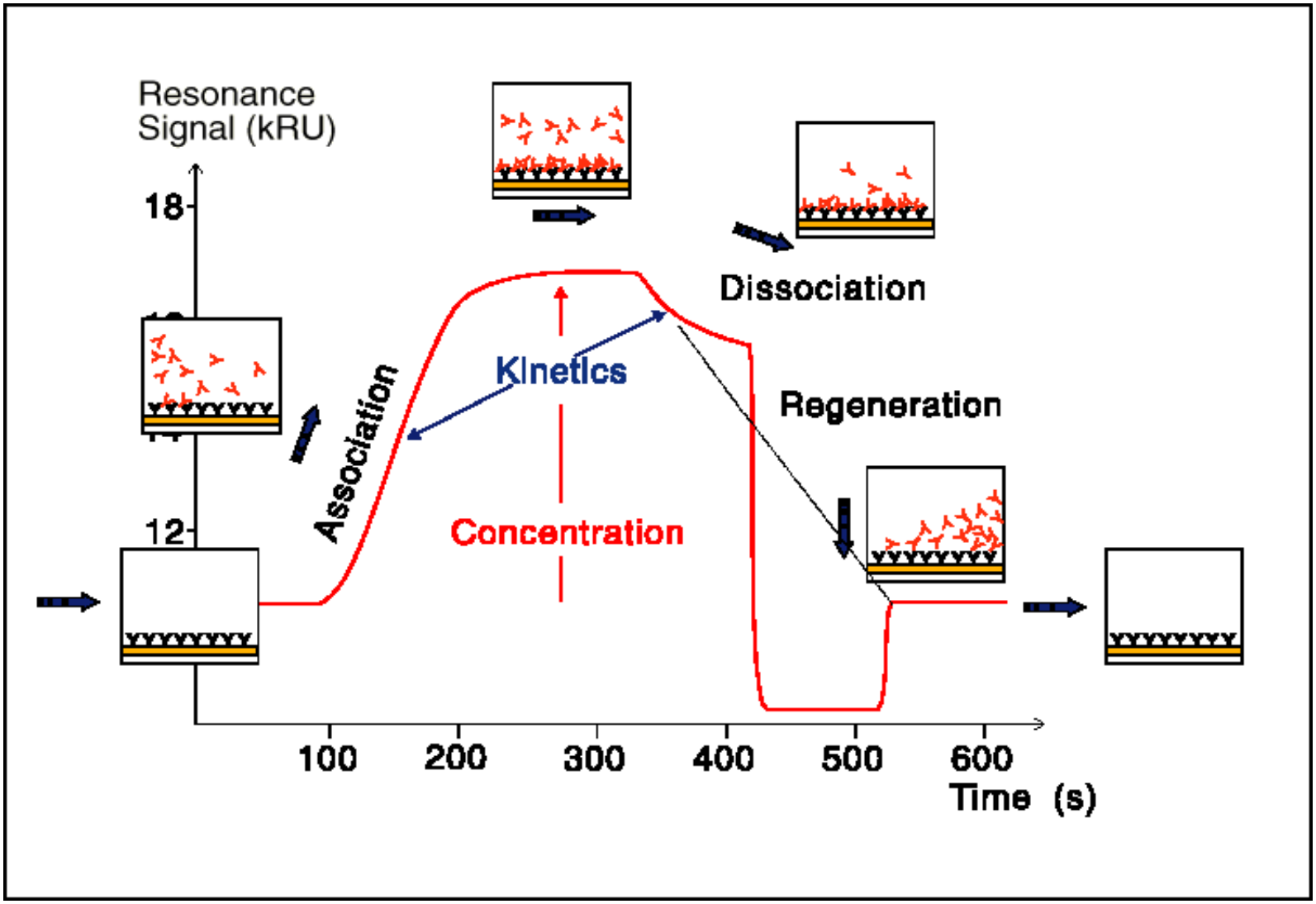
Disulphides (R-S-S-R)
Sulphides (R-S-R)
Thiols (R-SH)

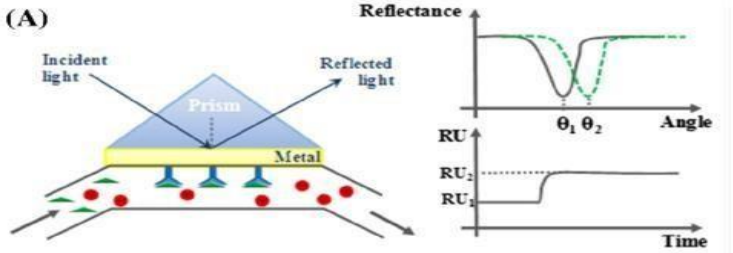


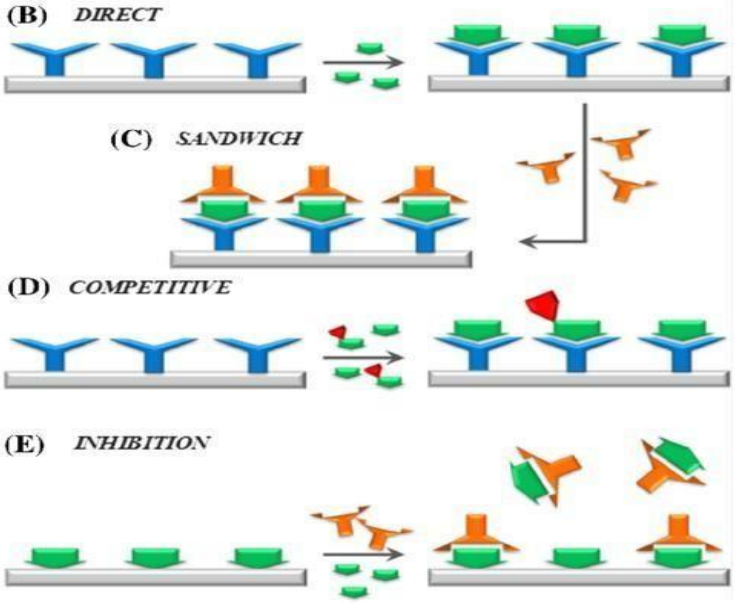



A
B
C
SH
SH
HS
HS
SPR
Intact antibody
Fragmented antibody
Self-assembled monolayer
Bovine serum albumine
Bovine leukemia virus antigen

Sensorgram

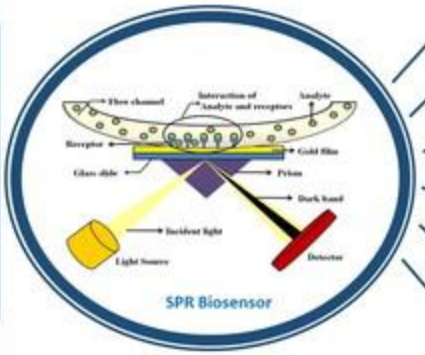

Resonance
Signal (kRU)
18
12
Association
Kinetics
Concentration
Dissociation
Regeneration
100
200
300
400
500
600
Time (s)





Why SPR biosensor

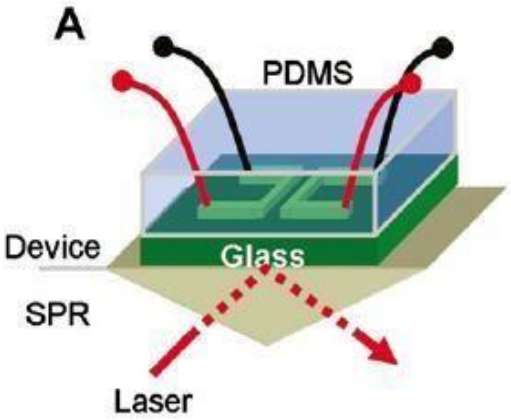
- Optical Biosensor
- High specificity
- Rapid detection
- Real time and label free assay
- Compact design
- Light weight
- Can be coupled with modern technology.

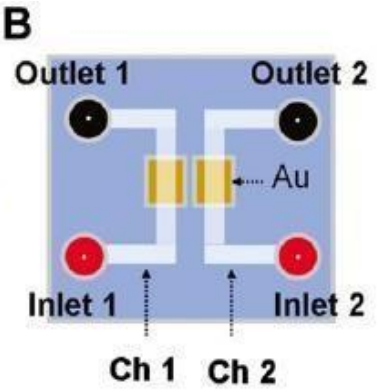


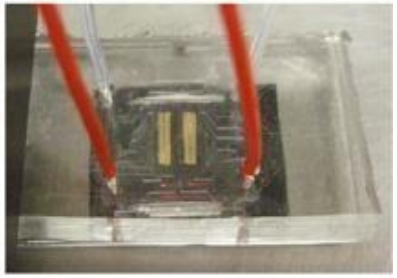
Food Applications

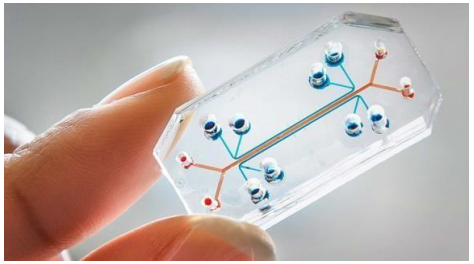
- Antibiotics detection
- Adulteration detection
- Biomolecules detection
- Microbes and Toxins detection
- Pesticide detection
- Genetically modified food detection

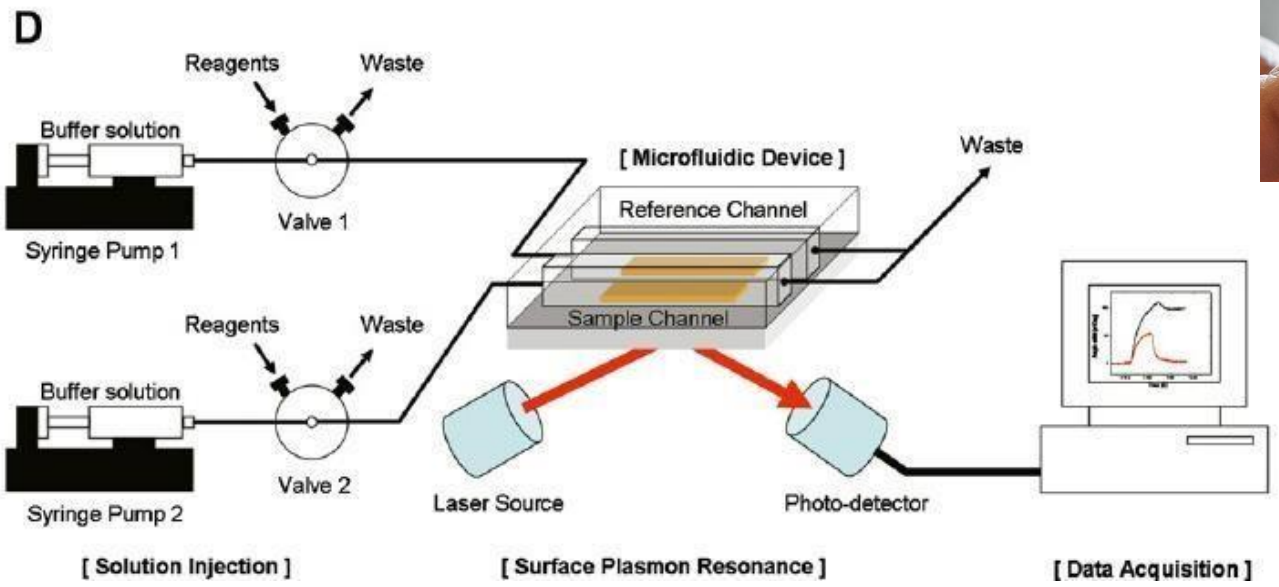
Spr sensors and microfluidics



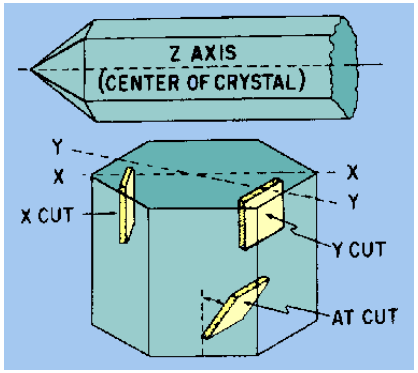


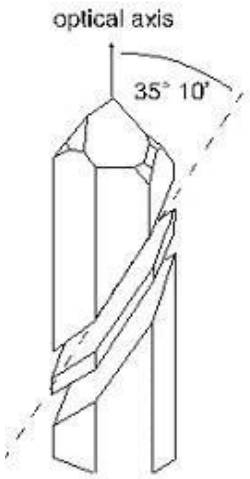


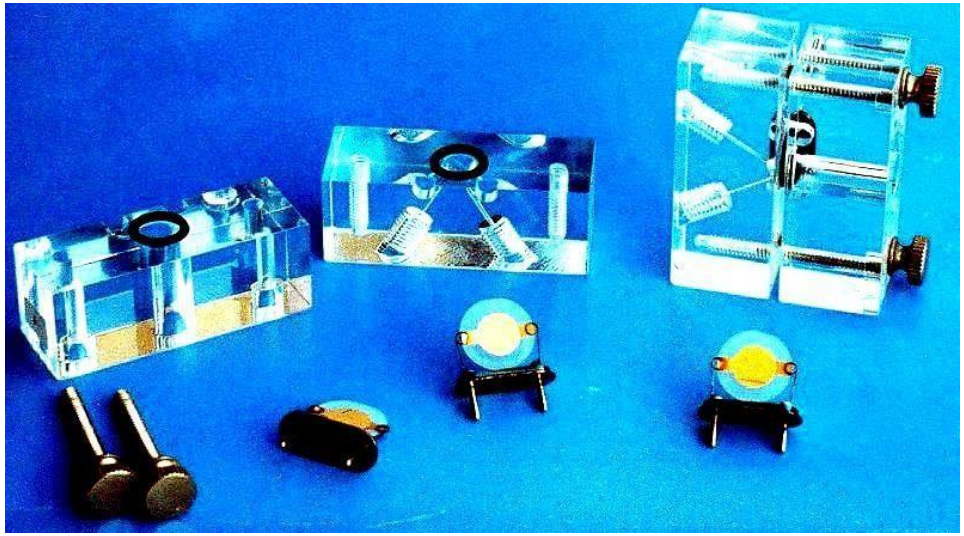


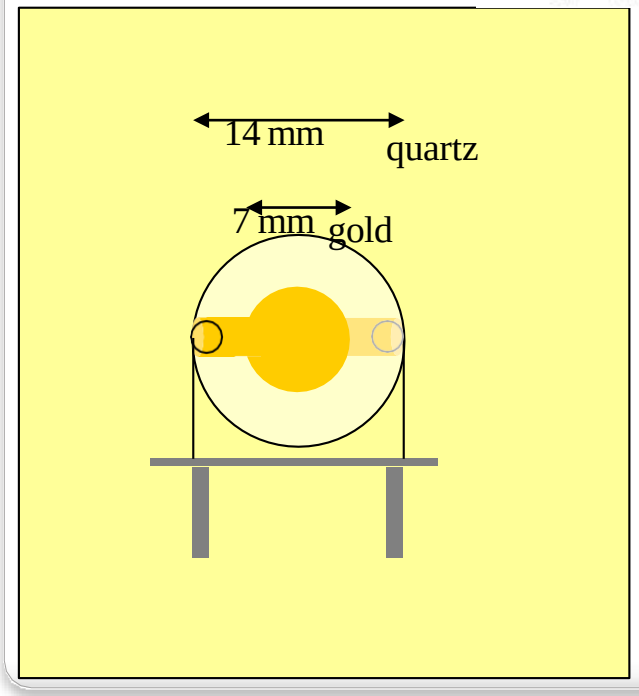


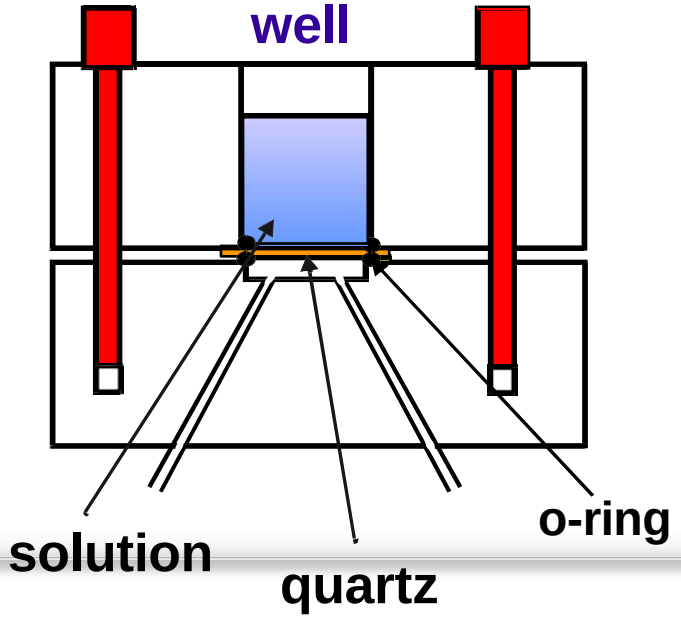
Piezoelectric Biosensors

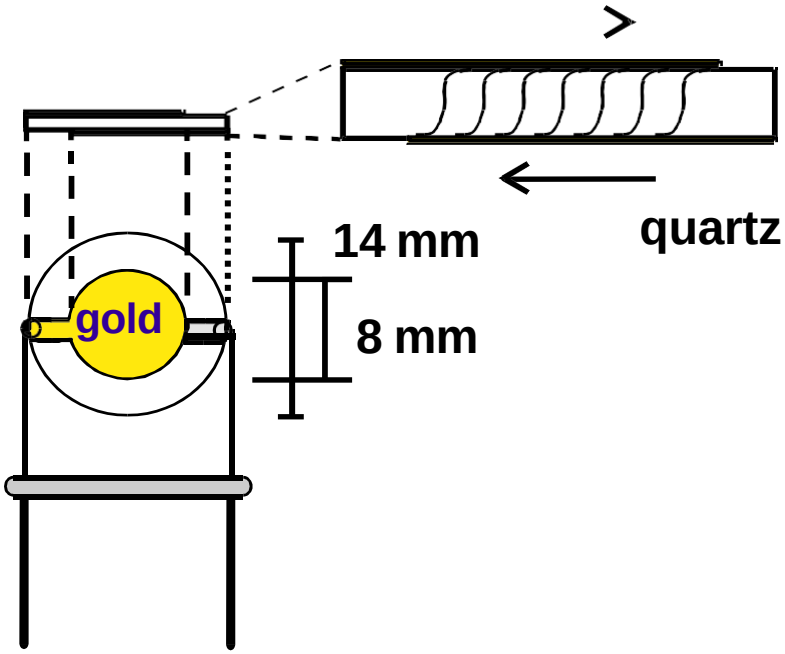






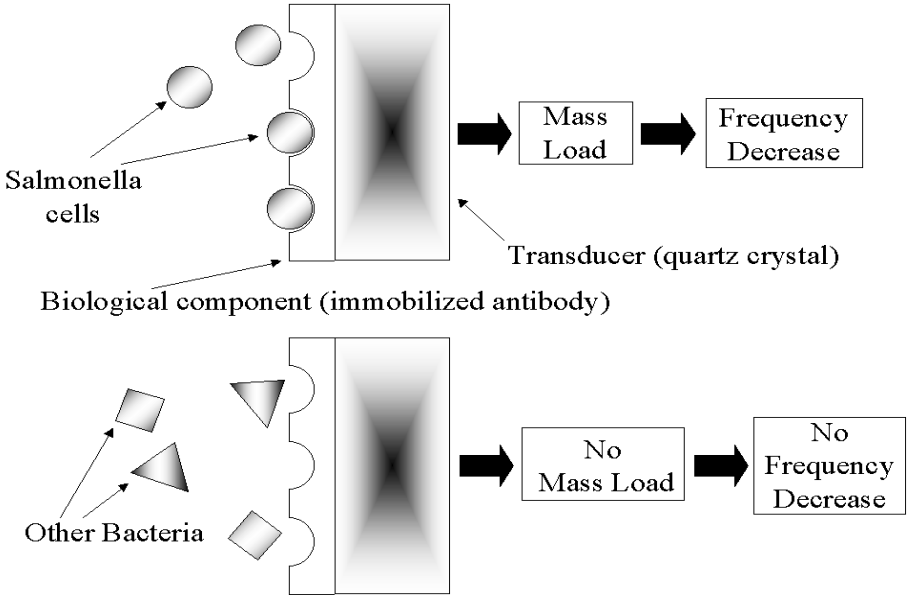






The standard QCM measures the mass of a material deposited on a quartz crystal surface as a linear function of a change in the oscillating crystal resonant frequency.

The mass-loading frequency effects of the transducer are based upon Sauerbrey's equation



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

ΔF (Hz) = frequency shift of the coated crystal

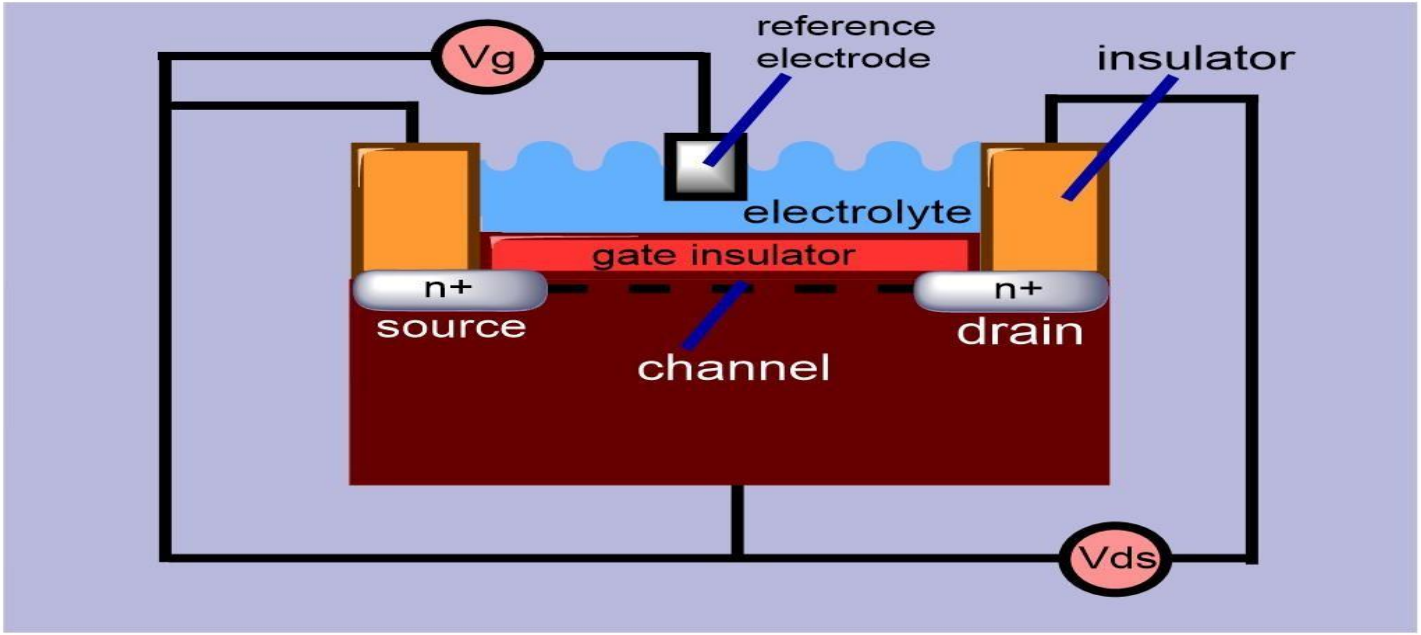
F (Hz) = resonance frequency of the crystal

ΔM (g) = increase in mass loading

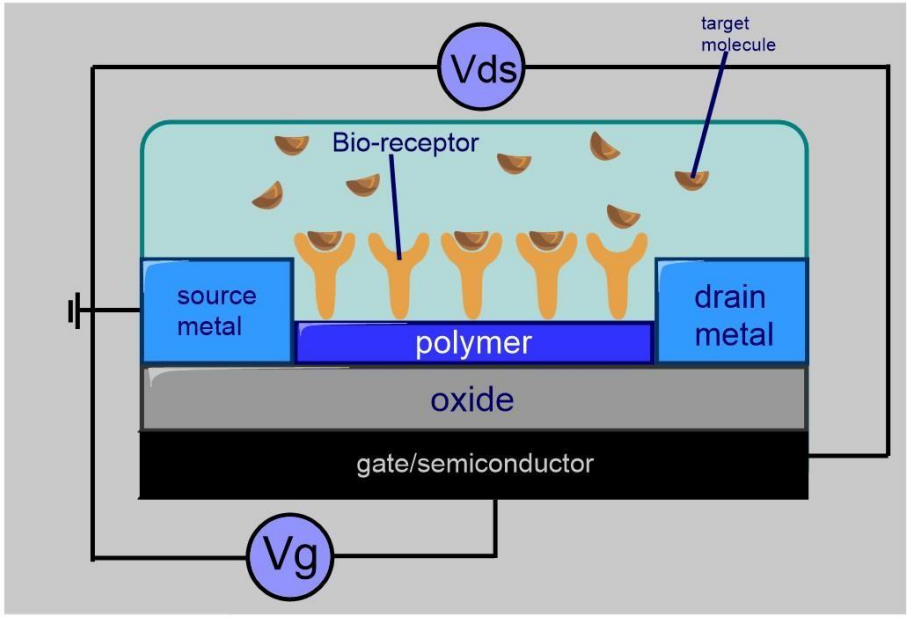
A (cm^2) = area of the coated crystal

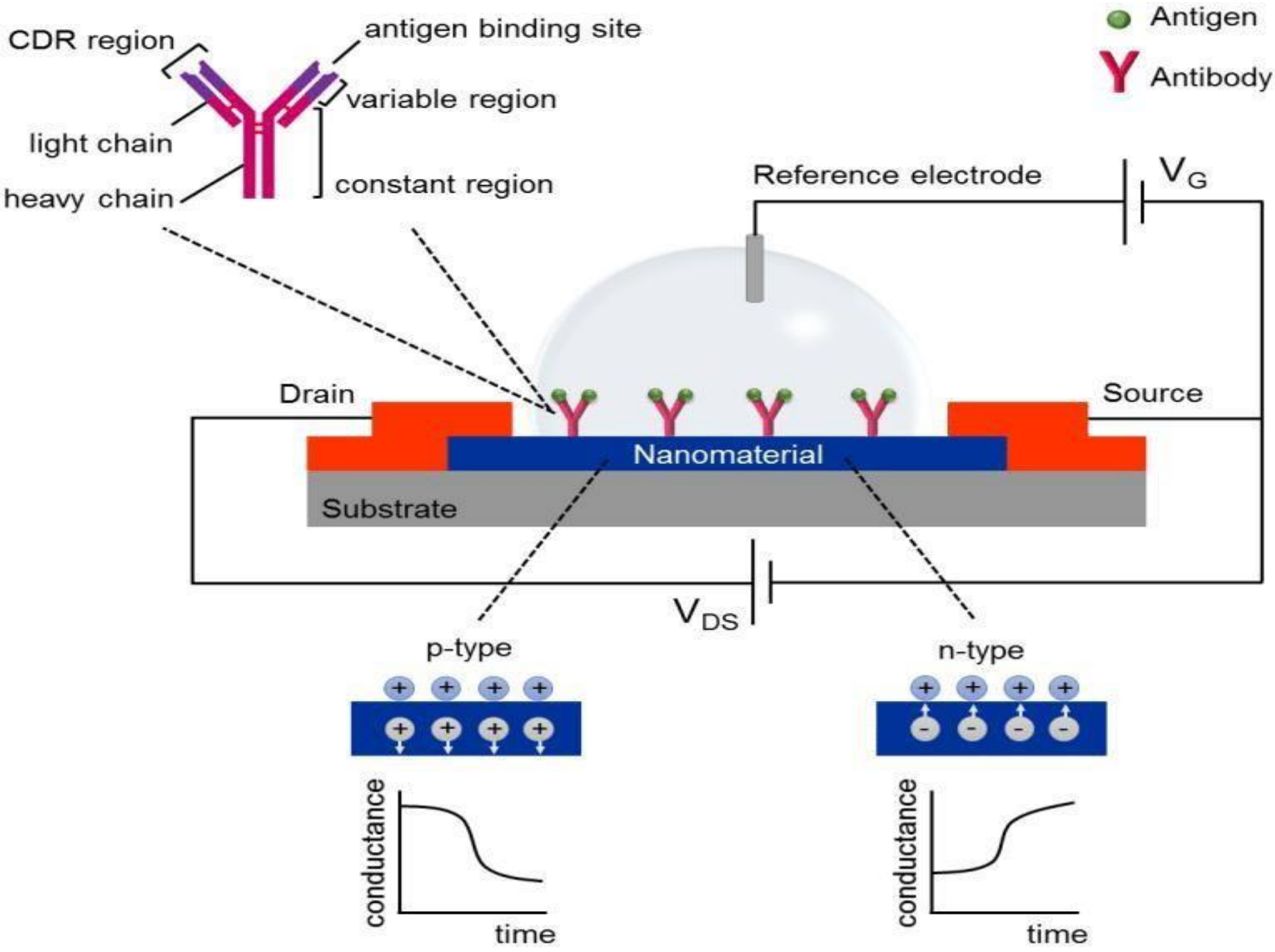
BIOFETs

An **ion-sensitive field-effect transistor** (**ISFET**) is a field-effect transistor used for measuring ion concentrations in solution; when the ion concentration (such as H^+, see pH scale) changes, the current through the transistor will change accordingly. Here, the solution is used as the gate electrode. A voltage between substrate and oxide surfaces arises due to an ion sheath. It is a special type of MOSFET (metal-oxide-semiconductor field-effect transistor),[1] and shares the same basic structure, but with the metal gate replaced by an ion-sensitive membrane, electrolyte solution and reference electrode.[2] Invented in 1970, the ISFET was the first biosensor FET (BioFET) source wikipedia

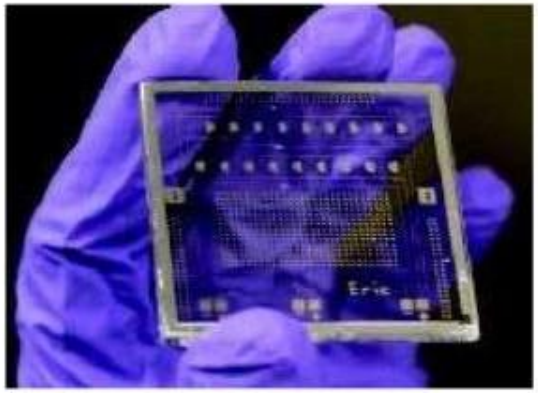


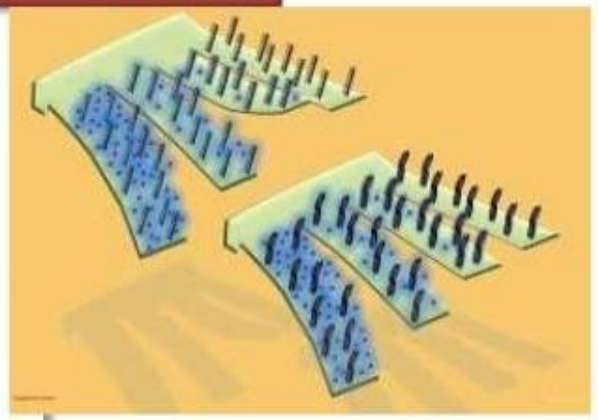
Bio-FETs couple a transistor device with a bio-sensitive layer that can specifically detect bio-molecules such as nucleic acids and proteins. A Bio-FET system consists of a semiconducting field-effect transistor that acts as a transducer separated by an insulator layer (e.g. SiO_2) from the biological recognition element (e.g. receptors or probe molecules) which are selective to the target molecule called analyte.[8] Once the analyte binds to the recognition element, the charge distribution at the surface changes with a corresponding change in the electrostatic surface potential of the semiconductor. This change in the surface potential of the semiconductor acts like a gate voltage would in a traditional MOSFET, i.e. changing the amount of current that can flow between the source and drain electrodes.[9] This change in current (or conductance) can be measured, thus the binding of the analyte can be detected. The precise relationship between the current and analyte concentration depends upon the region of transistor operation (source Wikipedia)

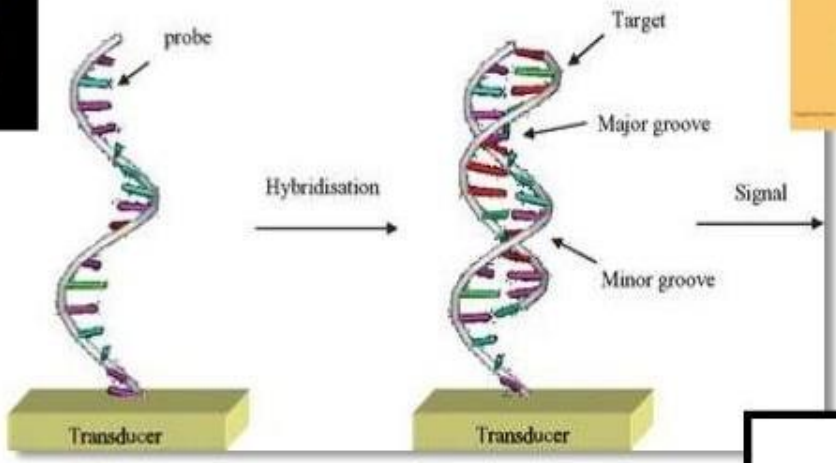


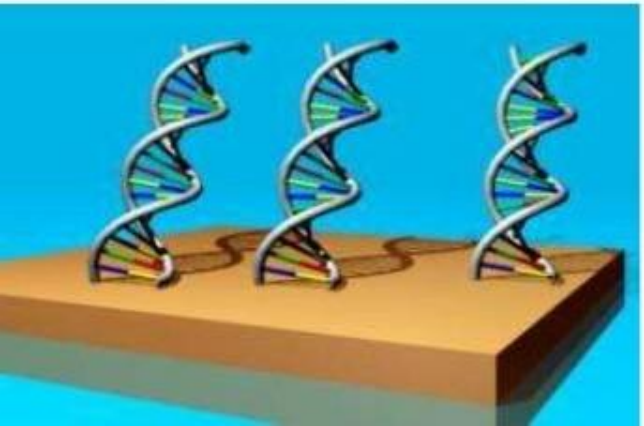

CDR region
antigen binding site
variable region
light chain
constant region
heavy chain
Antigen
Antibody
Reference electrode
V_G
Drain
Source
Nanomaterial
Substrate
V_{DS}
p-type
n-type
conductance
time
conductance
time

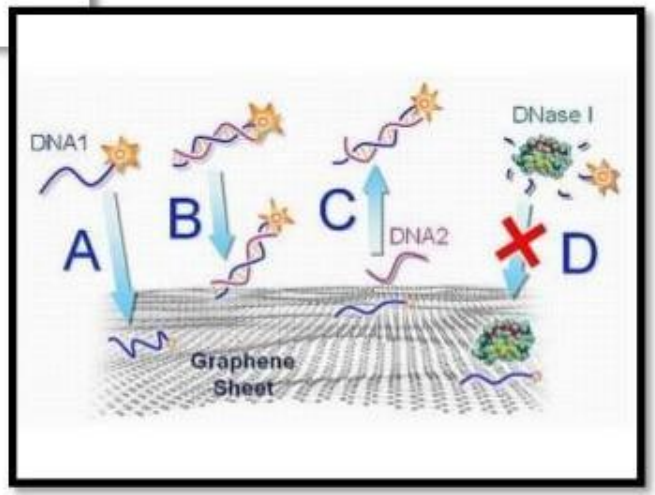
DNA biosensors











Principles of DNA biosensors

❖Nucleic acid hybridization

- **Perfect match**
  stable dsDNA, strong hybridization

- **One or more base mismatches**
  weak hybridization

❖ Forms of DNA Biosensors

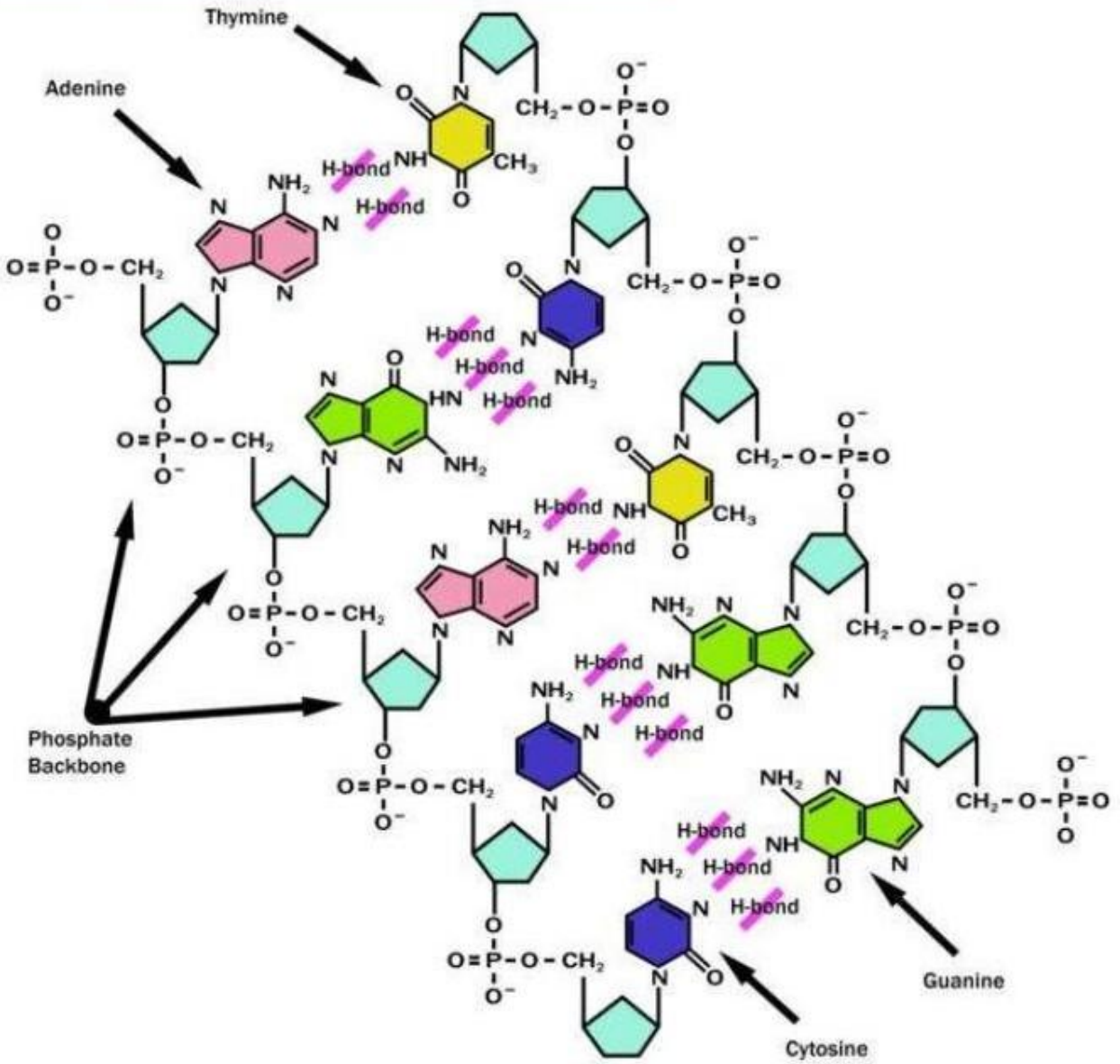
- Electrodes
- Chips
- Crystals

❖ Types of DNA Based Biosensors

- Optical
- Electrochemical
- Piezoelectric

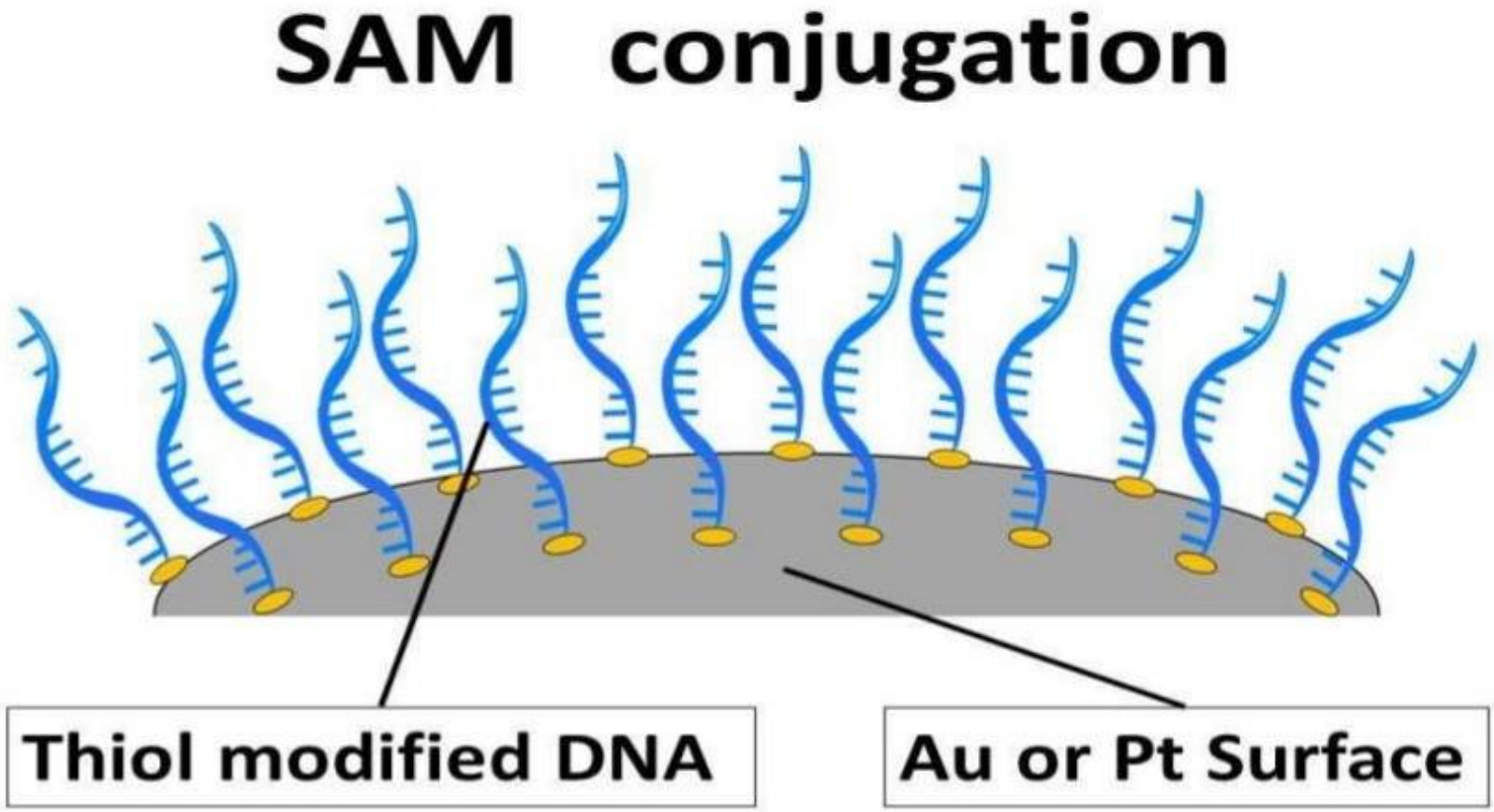
Immobilization of DNA Probe onto Transducer Surface

- **simple adsorption**
  onto carbon surfaces



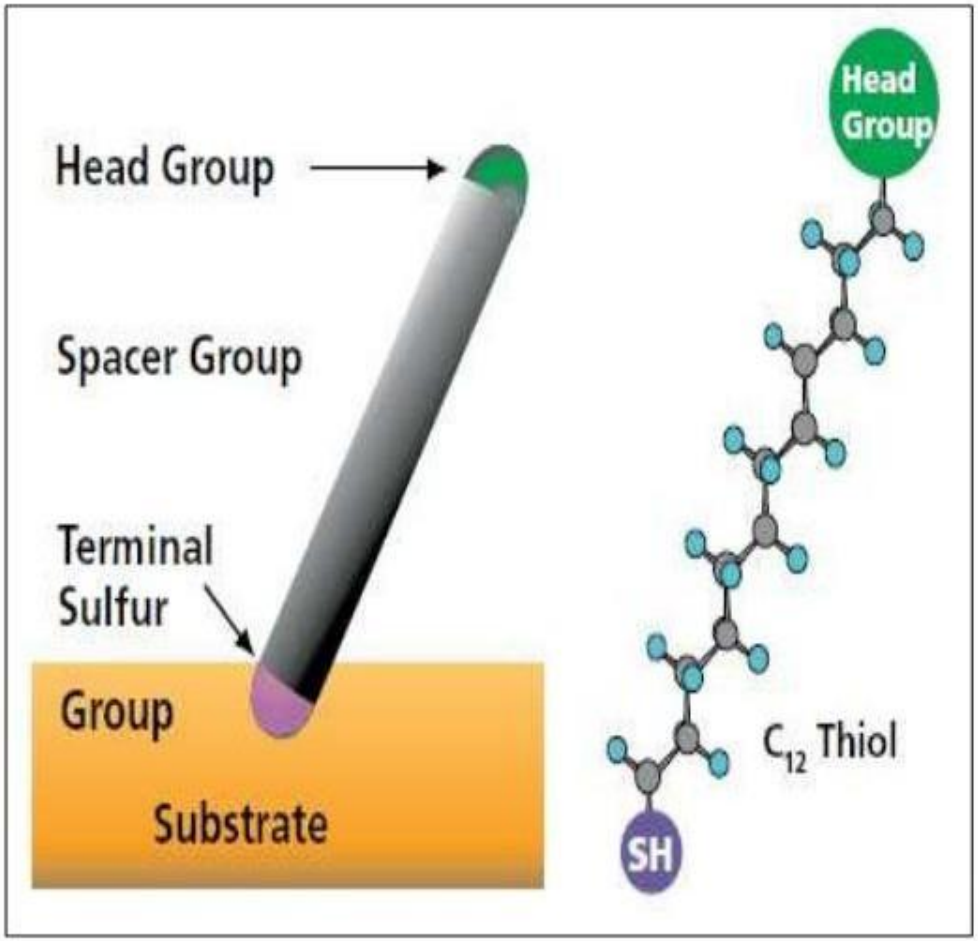
Immobilization of DNA Probe onto Transducer Surface

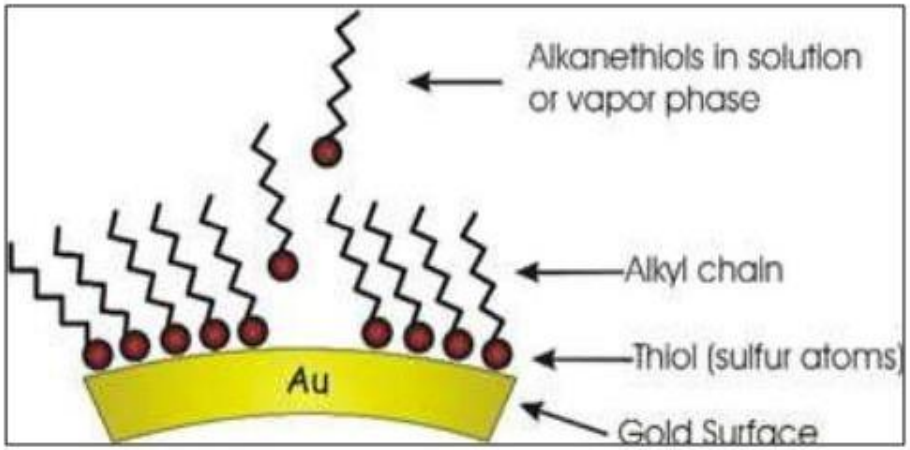
- Thiolated DNA for self assembly onto gold (or platinum) transducers

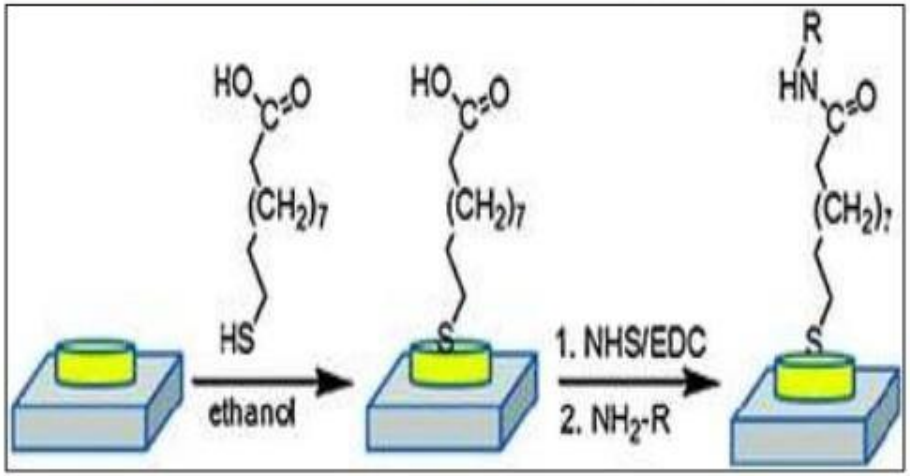


Immobilization of DNA Probe onto Transducer Surface

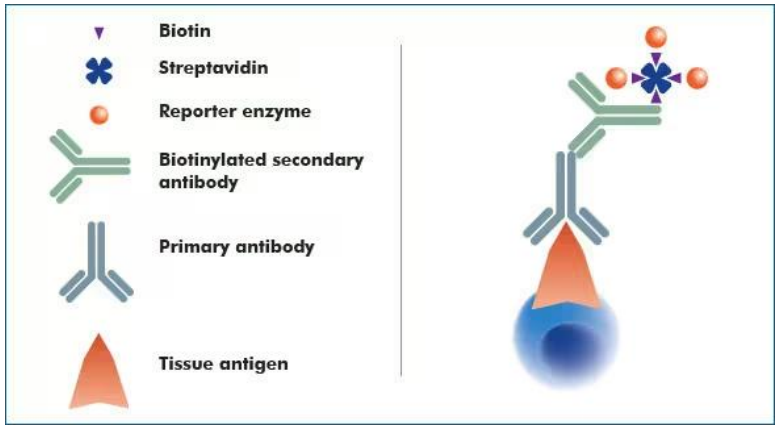
- Covalent linkage to the gold surface via functional **alkanethiol-based** monolayers

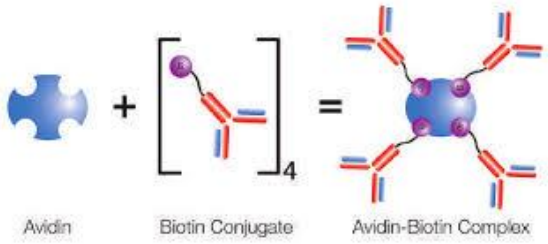


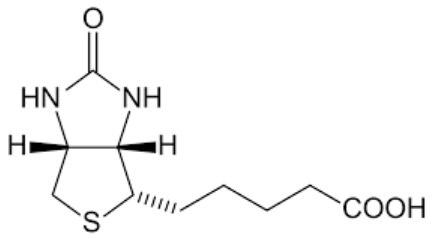


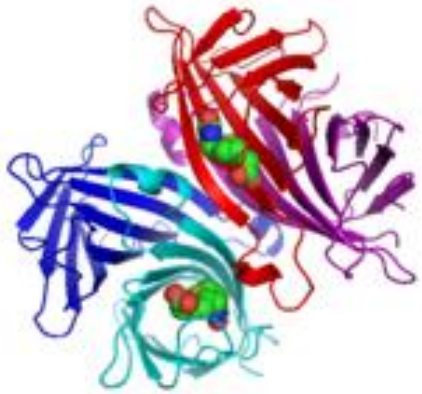


Strept(avidin)- Biotin $K_D = 10^{-15}$



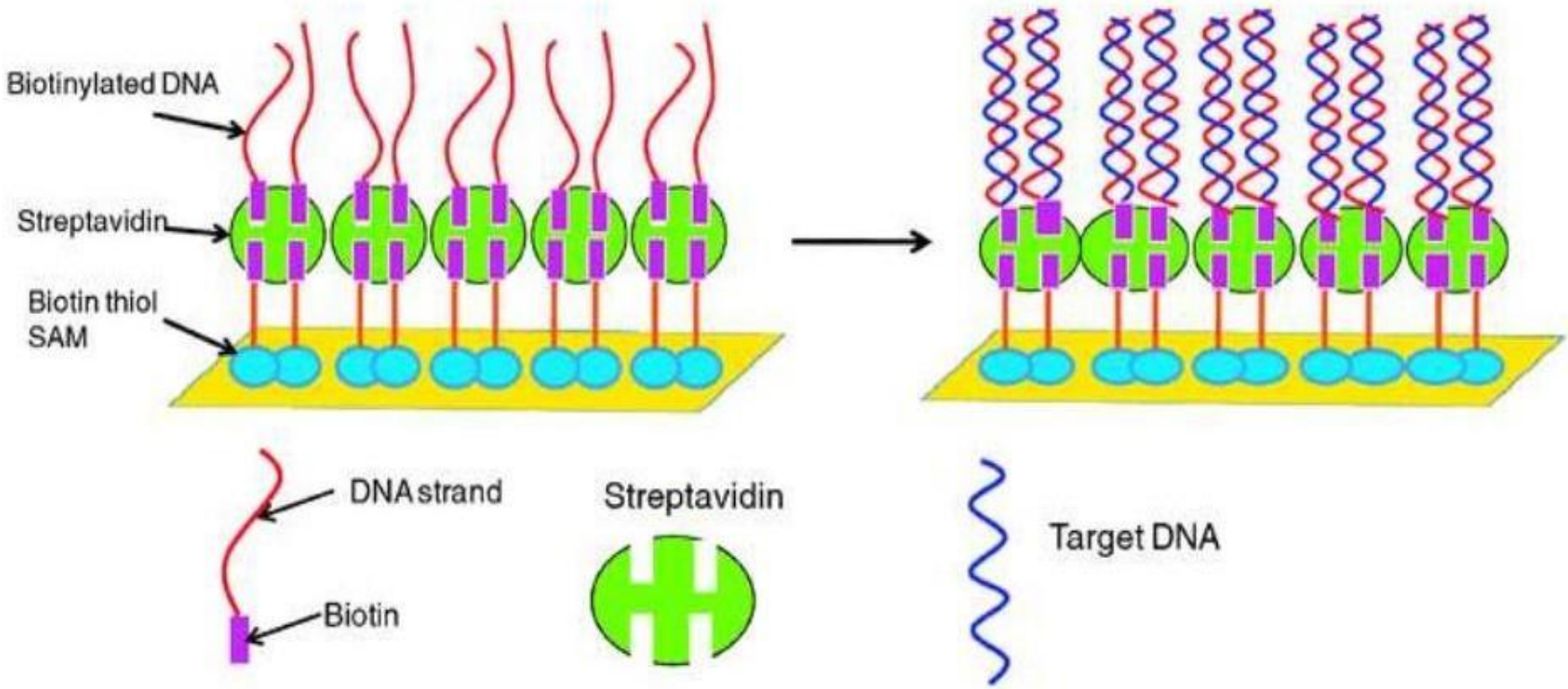






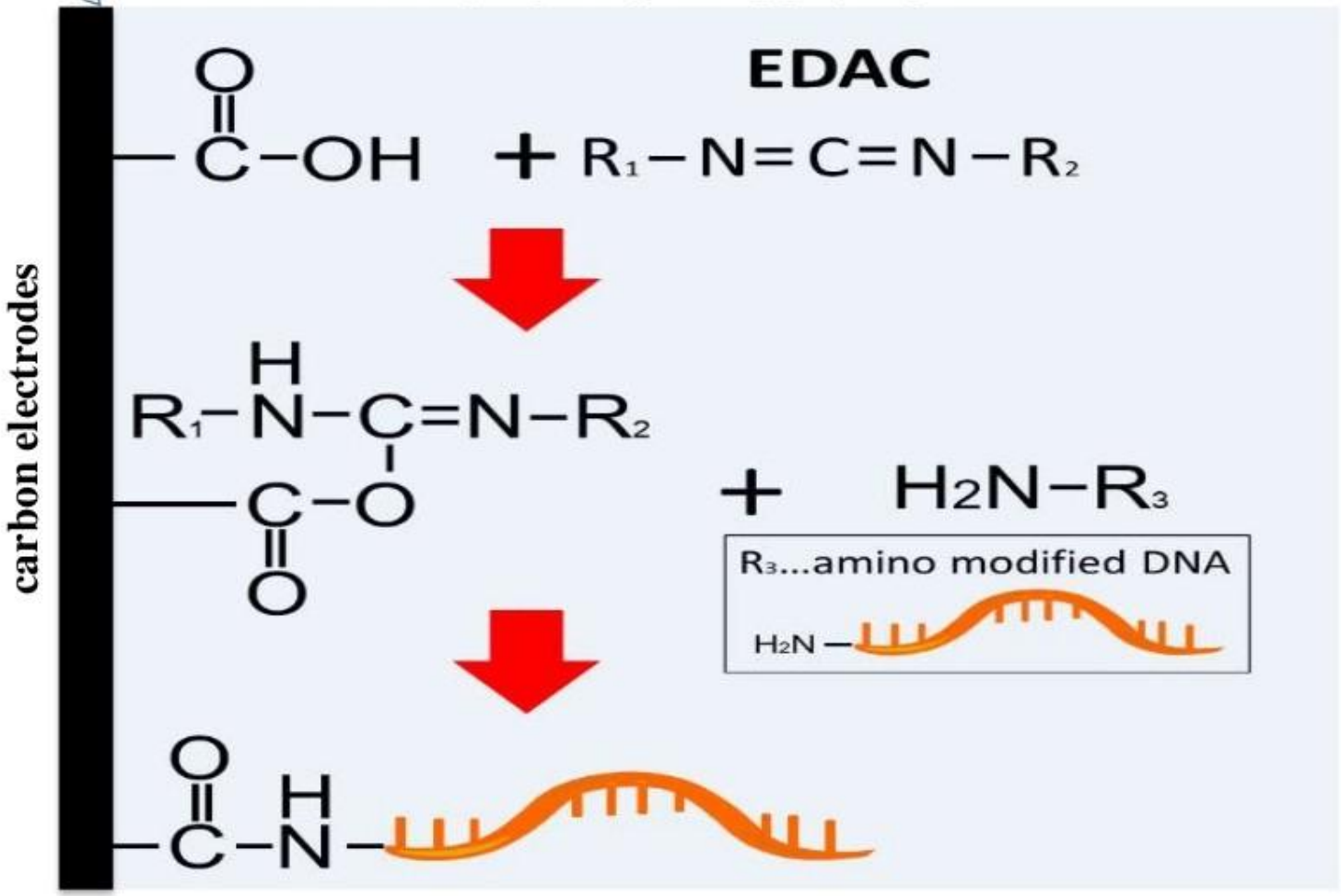
Immobilization of DNA Probe onto Transducer Surface

- Use of biotinylated DNA for complex formation with a surface-confined avidin or streptavidin



Immobilization of DNA Probe onto Transducer Surface

- Covalent (carbodiimide) coupling to functional groups on carbon electrodes for carboxyl groups supplying.

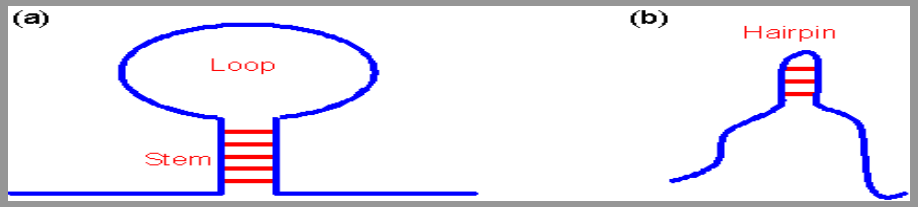


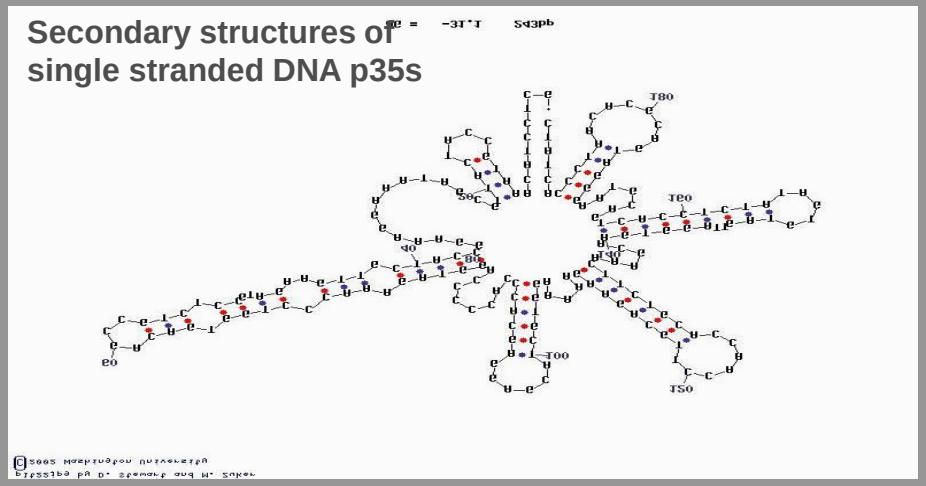
Immobilization method of DNA probes on functionalized surfaces.

Immobilization Method	Interaction or Reaction	Advantages	Drawbacks
Physical Adsorption	Charge-charge interaction or Hydrophobic interaction	Simple	Desorption by change of ionic strength or pH
		Fast	Random orientation
		Direct method (no linker molecules)	Desorption by detergent
		Suitable to DNA, RNA, and PNA	Problem of crowding effect and poor reproducibility
Covalent bonding	Chemical bonding	Good stability	Use of linker molecules
		High binding strength	Slow, Irreversible
		Use during long term	Problem of crowding effect
			Island formation
Streptavidin-Biotin interactions	Specific Streptavindin-Biotin interaction	Improved orientation	Expensive, Slow
		High specificity and functionality	Problem of crowding effect
		Well-controlled	Use of biocompatible linker
		Reversible	Poor reproducibility

ssDNA strands can form intra-strand base pairs and secondary structures

Limitation of the available ssDNA target for hybridisation with the immobilised probe



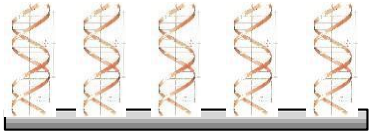


To avoid this: proper denaturation!

Aim: prolongate the ssDNA life time as much is possible, preventing dsDNA formation

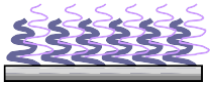
DNA electrochemical biosensors

DNA layer



Electrochemical transducer

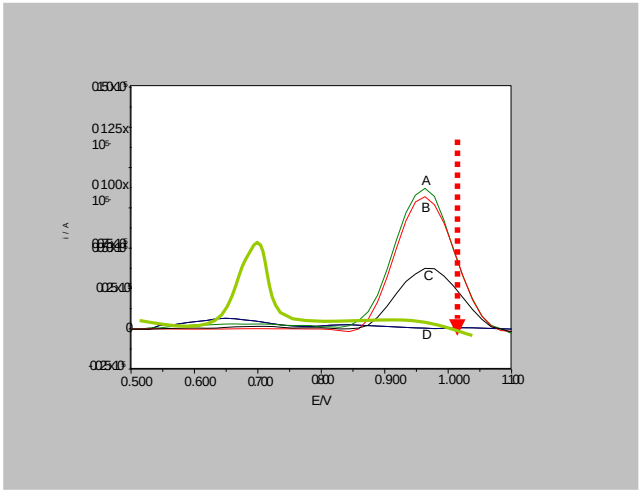
Hybridisation



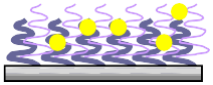
detection

Direct DNA electrochemistry

Use of an electroactive tracer



Intercalation

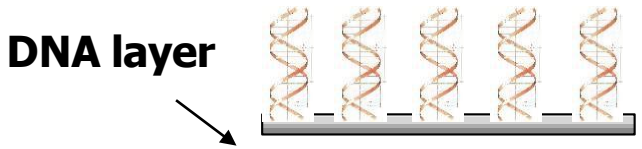


detection

Direct DNA Electrochemistry e.g. reduction of guanine oxidation peak

Appearance of new oxidation peak

DNA electrochemical biosensors application



Electrochemical transducer

Hybridisation:

Molecular diagnostic application:
- microorganism
- gene single-point mutation
- species identification
- GMO

Intercalation

Antitumoral drugs

Genotoxic compounds:
- stable DNA complex
- adduct formation

Sensori elettrochimici a DNA

Biosensors and Bioelectronics 154 (2020) 112069

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

Review: Electrochemical DNA sensing – Principles, commercial systems, and applications

Martin Trotter [a,*], Nadine Borst [a,b], Roland Thewes [c], Felix von Stetten [a,b,**]

[a] Hahn-Schickard, Georges-Koehler-Allee 103, 79110, Freiburg, Germany
[b] Laboratory for MEMS Applications, Department of Microsystems Engineering – IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110, Freiburg, Germany
[c] Faculty of EECS, Chair of Sensor and Actuator Systems, TU Berlin, Einsteinufer 17, 10587, Berlin, Germany

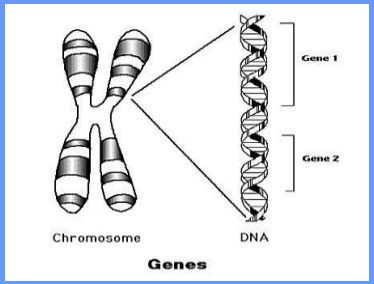
Table 1
Categories of electrochemical NA sensing principles.

	Label-free, reagent-less	Label-free, reagent-dependent	Labeled, reagent-less	Labeled, reagent-dependent
Heterogeneous detection	• Change in capacitance • Change in impedance • Field-effect	• Intercalation • Groove-binding • Electrostatic binding • Electrostatic repulsion	• Labeled capture probes • Labeled signaling probes • Labeled nucleotides	• Enzyme labels
Homogeneous detection	• Detection of NA amplification by-product with ISFET	• Consumption of electroactive molecules by interaction with NA	• Release of electroactive molecules	• No principle known
Advantages	• Cost-effective reagents possible	• Enhanced signals	• Specific signal	• Signal amplification
Drawbacks	• Low signals • Risk of unspecific signal changes	• Risk of unspecific signal changes	• Modification of oligonucleotide increases costs	• Additional process steps complicate automation

Detection of DNA hybridisation in PCR samples

Fig. 1. Images of some of the reviewed commercial electrochemical NA detection systems in the order of their appearance in the text. A: Cubed Laboratories' NESDEP instrument (copyright: Cubed Laboratories) B: Canon's Genelyzer II instrument (copyright: Canon Medical Systems Corp.) C: GenMark's ePlex instrument (four tower version shown – the device can also be equipped with fewer towers, copyright GenMark Diagnostics Inc.) D: Friz Biochem's envisioned Cycle device (copyright: Friz Biochem GmbH) E: CustomArray's ElectraSense reader (copyright: Custom Array Inc.) F: Elice's Leo instrument (copyright: Easy Life Science) G: Binx's io instrument (copyright: Binx Health Inc.). The images are not to scale. All images are published with the permission of the respective companies.

• *Development of an Hybridisation sensor*



Synthesis of a DNA fragment (probe, bioreceptor) containing the sequence of interest (analytical problem)

Immobilisation of the probe onto the solid support of the sensor (surface)
(thiol/dextran/streptavidin/biotinylated probe)

Extraction of the DNA from the real sample (blood, water, food) and amplification of the sequence of interest (sample pretreatment)

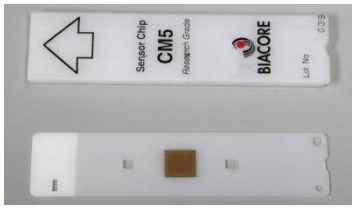
Denaturation of the dsDNA (amplified fragment or genomic) to obtain a single-stranded DNA (sample pretreatment)

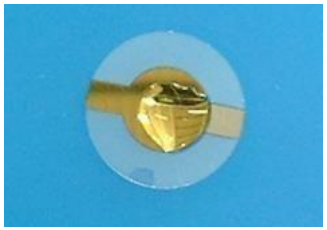
Hybridisation of the obtained ssDNA with the immobilised probe

Changes in the physicochemical parameters of the layer formed on the transducer (quartz crystal or gold –glass chip)

Probe immobilisation on gold film

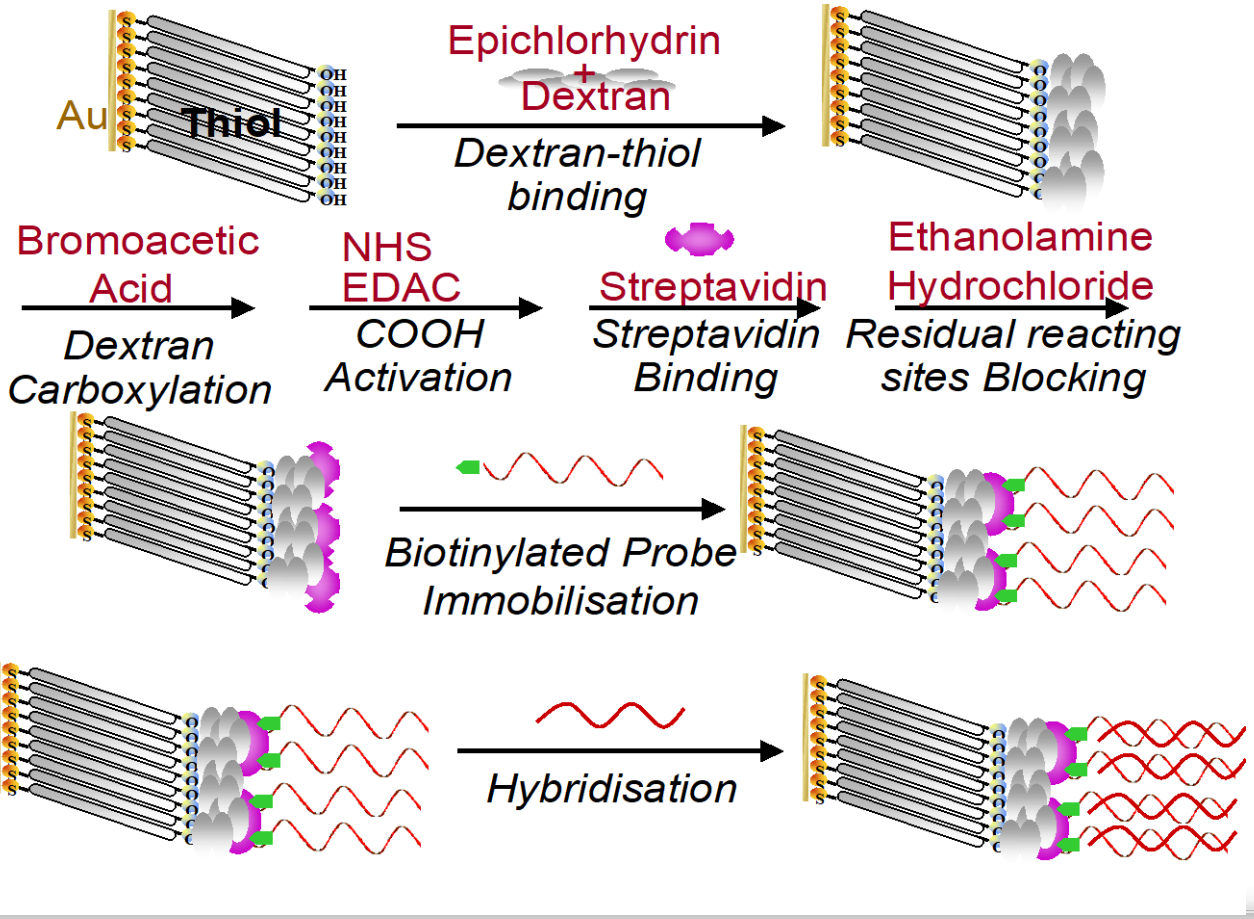
optical





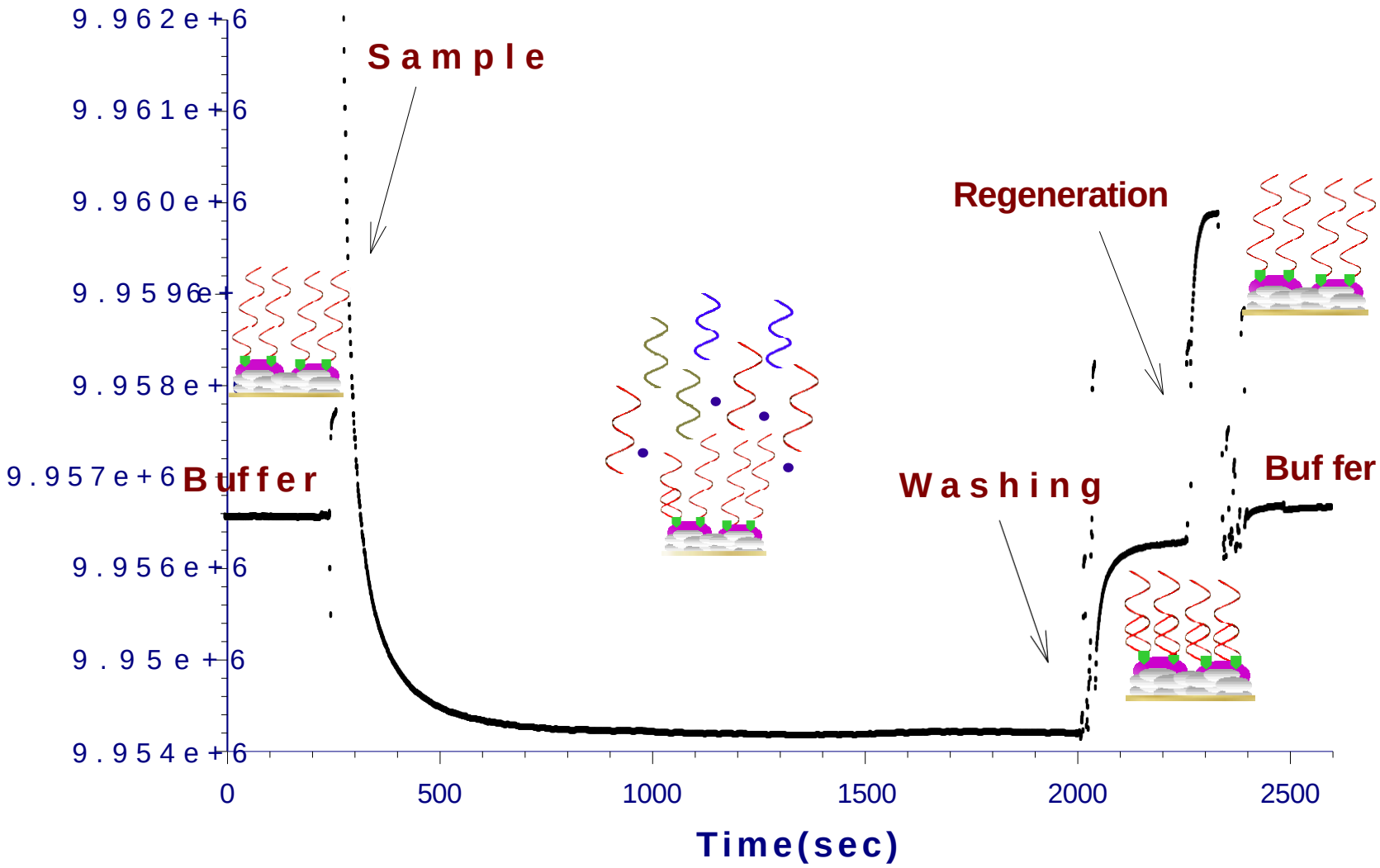
piezoeletric

thiol/dextran/streptavidin/biotinylated probe



Specificity,
no aspecific
adsorption,
stability,
multi-use

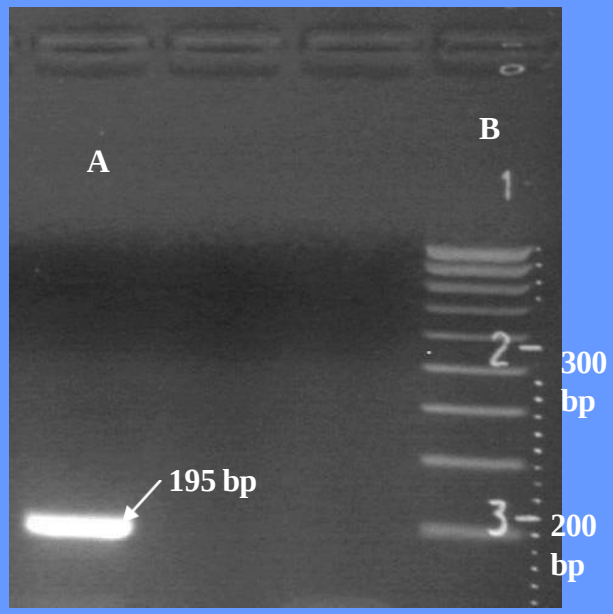
Hybridation-Regenation Cycle



Detection of DNA target sequence in real matrices

- Certified Reference Materials (CRM) -Fluka
- Processed food (dietetic snaks, soy crackers, soft drinks)

Control: Post PCR Electrophoresis



A: amplified fragment (Promoter 35S 195 bp)

B: Standard length fragments

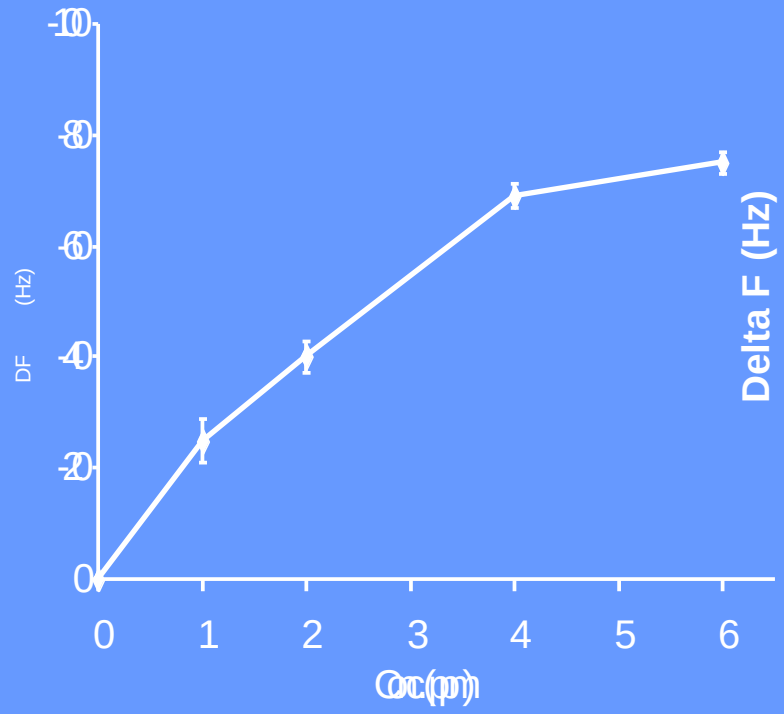
1. PCR amplified DNA

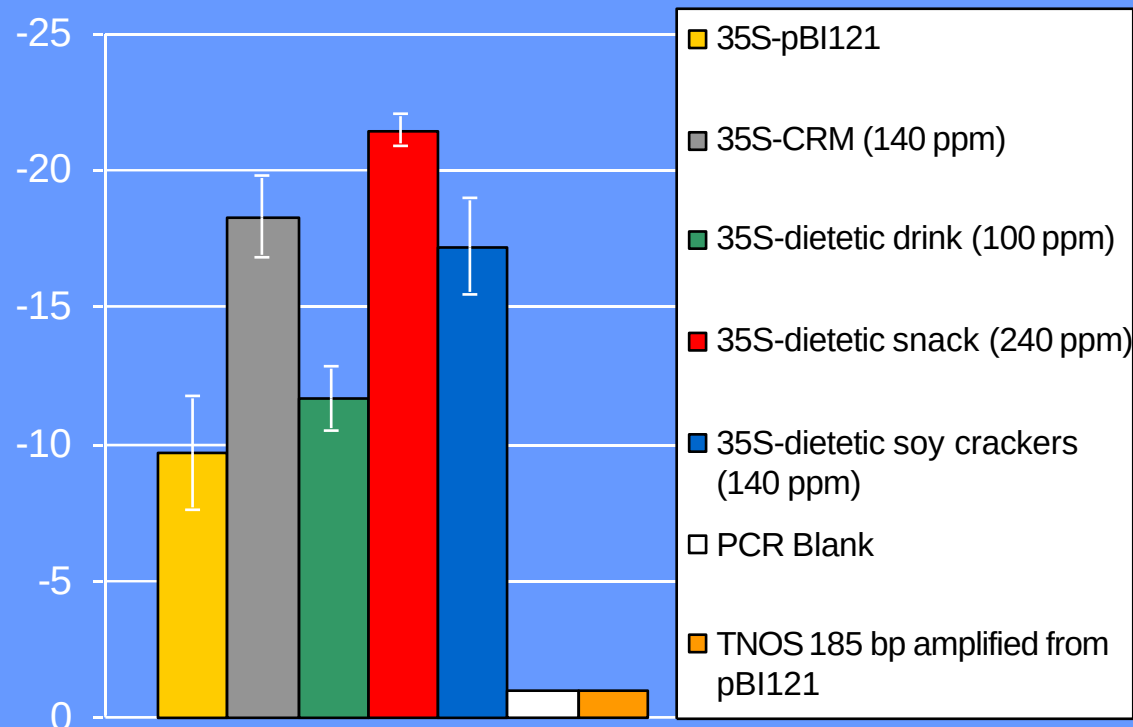
Processing the sample

- Extraction of DNA from samples (CTAB method)
- Amplification of DNA by PCR (Pietsh K. et al. 1997)
- Dilution with *Hybridisation buffer*: NaCl 150 mM, Na2HPO4 20 mM, EDTA 0.1 mM, pH 7.4
- Denaturation to obtain ssDNA from amplified dsDNA

Piezoelectric sensor, CRM 2% samples and processed food samples

Sample pre-treatment: PCR amplified DNA, thermal denaturation





DL: 0,3 ppm

CV% 6 (n=3)

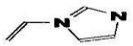
5'-BIOT-ggc cat cgt tga aga tgc ctc tgc c-3' probe 35S
3'- ccg gat gca act tct acg gag acg g-5 target 35S

Biomimetic receptors

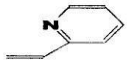
Obtained via combinatorial chemistry and/or molecular modelling

- MIP (Molecularly imprinted polymers)
- Peptides
- Aptamers

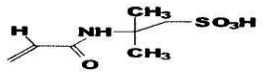
Functional Monomer Database



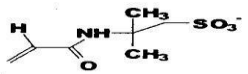
F1 1-VINYLIMIDAZOLE



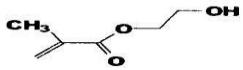
F2 2-VINYLPYRIDINE



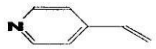
F3 ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)



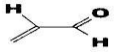
F3a ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)



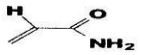
F4 2-HYDROXYETHYL METHACRYLATE



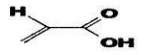
F5 4-VINYLPYRIDINE



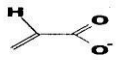
F6 ACROLEIN



F7 ACRYLAMIDE



F8 ACRYLIC ACID



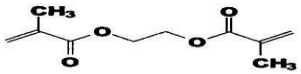
F8A ACRYLIC ACID

F9 ACRYLONITRILE

F10 ALLYAMINE

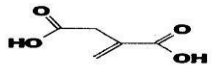
F10A ALLYLAMINE

F11 p-DIVINYLBENZENE

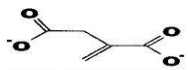


F12 ETHYLENE GLYCOL DIMETHACRYLATE (EGDMA)

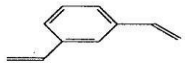
F13 UROCANIC ACID ETHYL ESTER



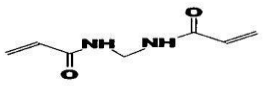
F14 ITACONIC ACID



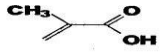
F14A ITACONIC ACID



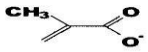
F15 m-DIVINYLBENZENE



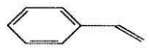
F16 N,N-METHYLENE BIS ACRYLAMIDE



F17 METHACRYLIC ACID



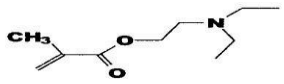
F17A METHACRYLIC ACID



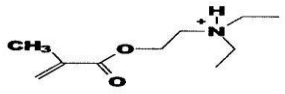
F18 STYRENE

F19 UROCANIC ACID

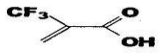
F19A UROCANIC ACID



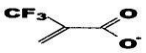
F20 N,N-DIETHYLAMINO ETHYL METHACRYLATE (DEAEM)



F20A N,N-DIETHYLAMINO METHACRYLAMIDE (DEAEM)

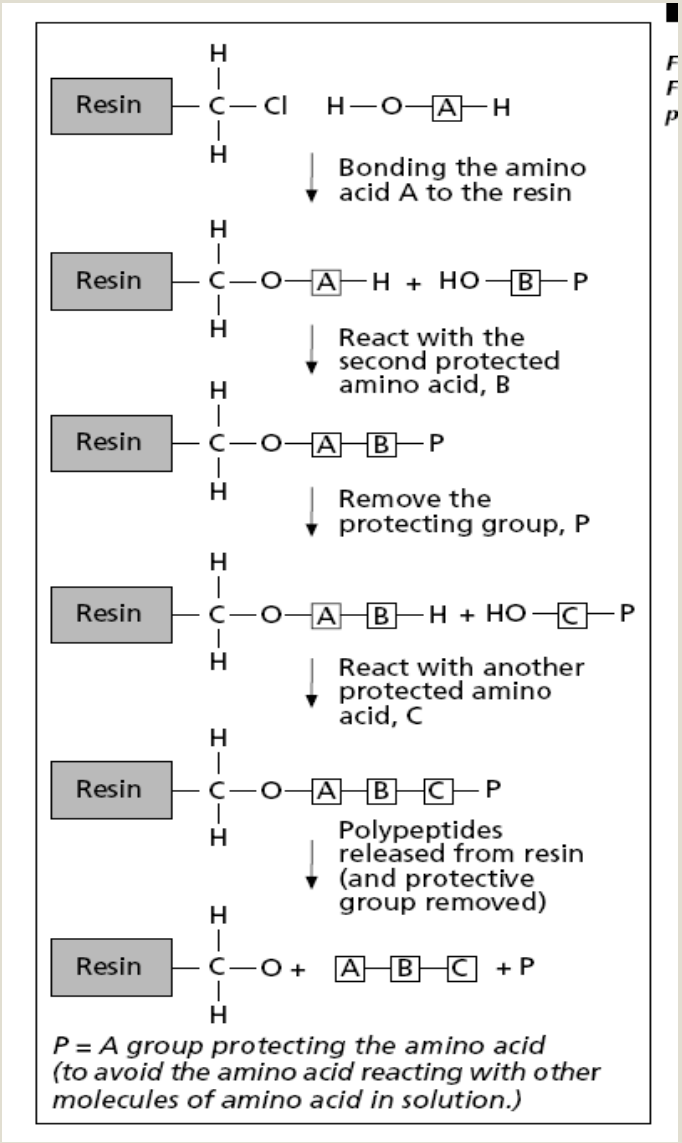


F17 TRIFLUOROMETHACRYLIC ACID



F17A TRIFLOUROMETHACRYLIC ACID

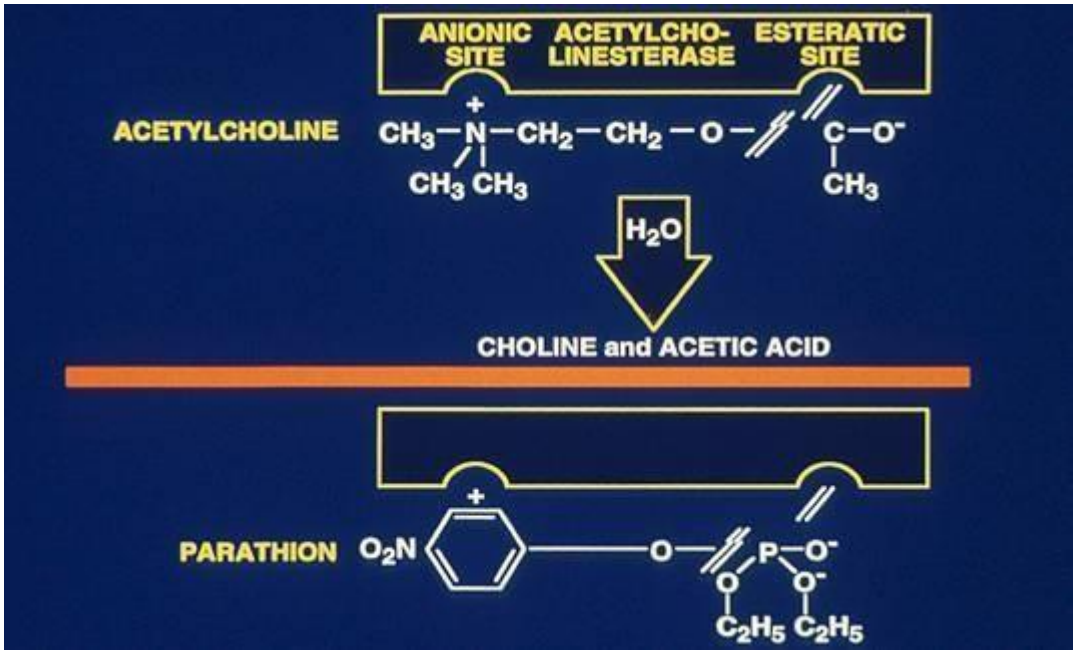
Combinatorial chemistry approach: Synthesys of aminocids via split and mix

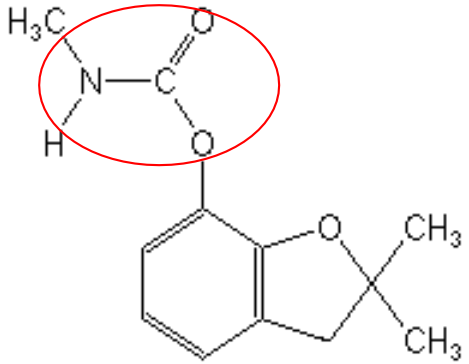


Split synthesis

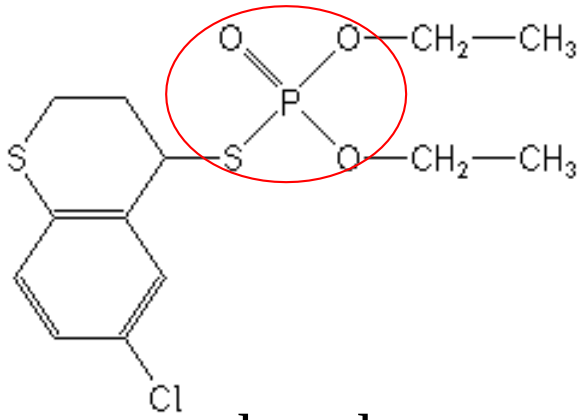
Stage	Reaction vessel 1 (A)	Reaction vessel 1 (B)	Reaction vessel 1 (C)	
1	Resin + A	Resin + B	Resin + C	3 compounds
		MIX		
2	Resin-A+A Resin-B+A Resin-C+A	Resin-A+B Resin-B+B Resin-C+B	?	9 compounds
		MIX		
3	Resin-A-A+A Resin-B-A+A Resin-C-A+A Resin-A-B+A Resin-B-B+A Resin-C-B+A Resin-A-C+A Resin-B-C+A Resin-C-C+A	Resin-A-A+B Resin-B-A+B Resin-C-A+B Resin-A-B+B Resin-B-B+B Resin-C-B+B Resin-A-C+B Resin-B-C+B Resin-C-C+B	Resin-A-A+C Resin-B-A+C Resin-C-A+C Resin-A-B+C Resin-B-B+C Resin-C-B+C Resin-A-C+C Resin-B-C+C Resin-C-C+C	27 compounds
		MIX		

BIOMIMETIC RECEPTORS FOR PESTICIDES





Carbamate



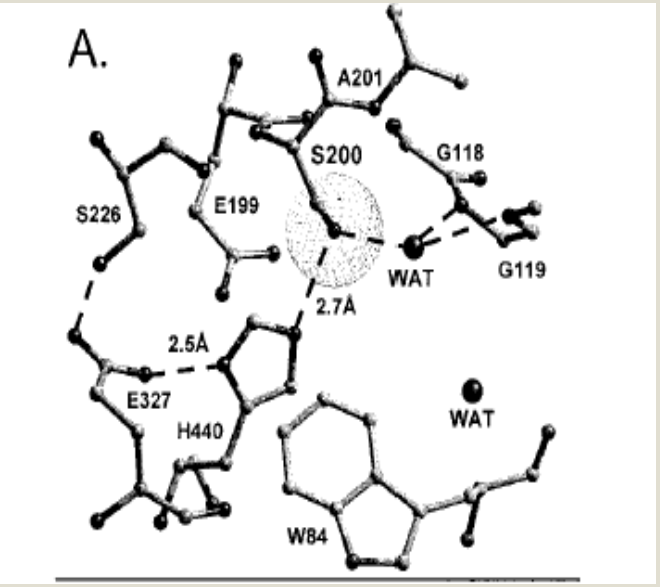
organophosphate

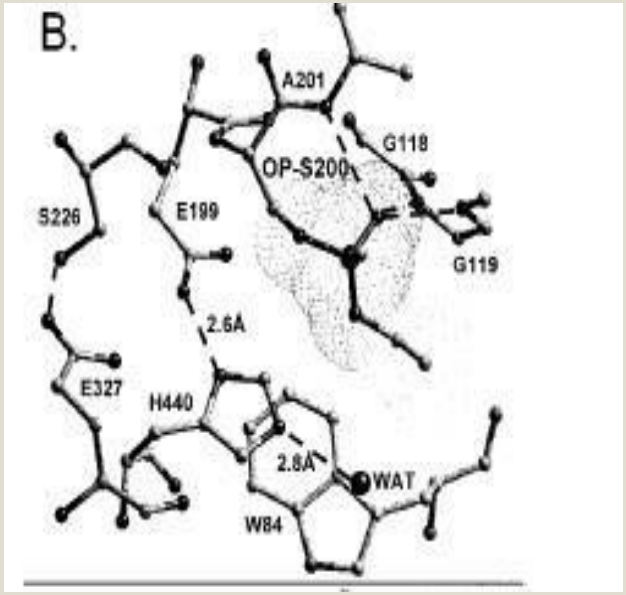
✓Mechanism of AChE inhibition

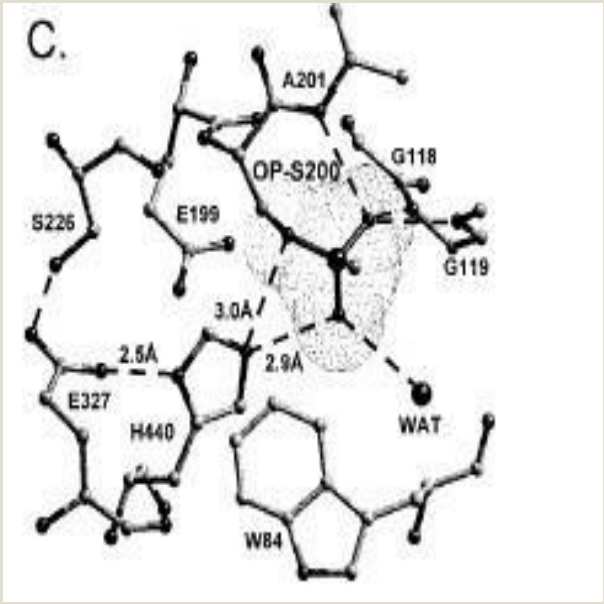
AChE, the target enzyme of pesticides, **is an efficient serine hydrolase** that catalyzes the breakdown of acetylcholine (ACh)

Acetylcholine + H_2O → choline + acetic acid

How pesticides work







Native structure: the active site, including the catalytic triad (S200-H440-E327) and the oxyanion hole (-NH of G118, G119, and A201)

Pro-aged structure: Phosphonylation triggers a conformational change for H440 that disrupts the H-bond to E327

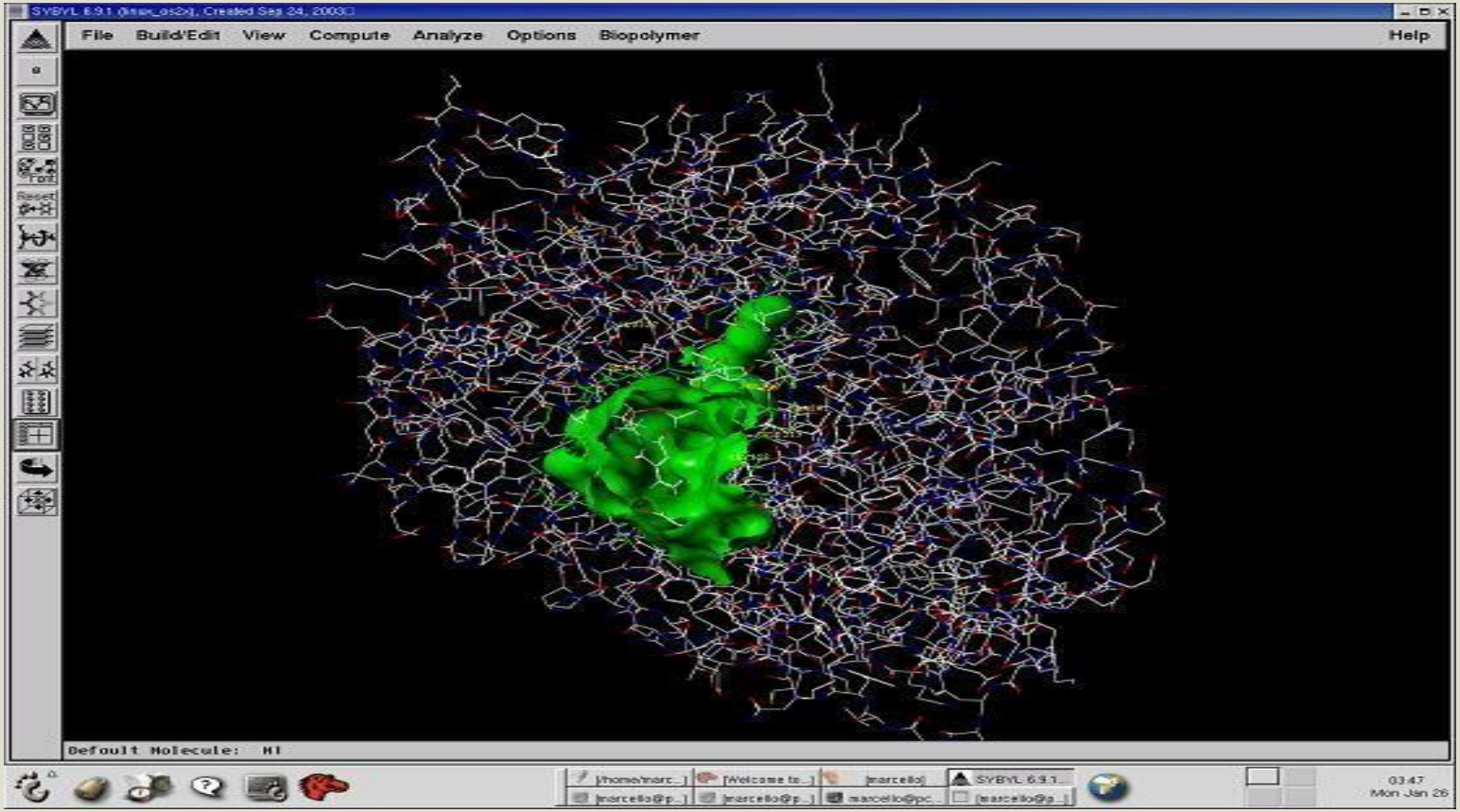
Aged structure: For reaction of AChE with VX and most phosphonates, aging predominates, and dealkylation results in movement of H440 to the negatively charged pocket formed by E327 Ox, S200 Ox, and one anionic oxygen of the dealkylated OP

From *Millard et al J.Am.Chem.Soc. 121, (1999)*

❖ Computational screening

✓ AChE-OP crystallographic structure (PDB ID: 1VXO)

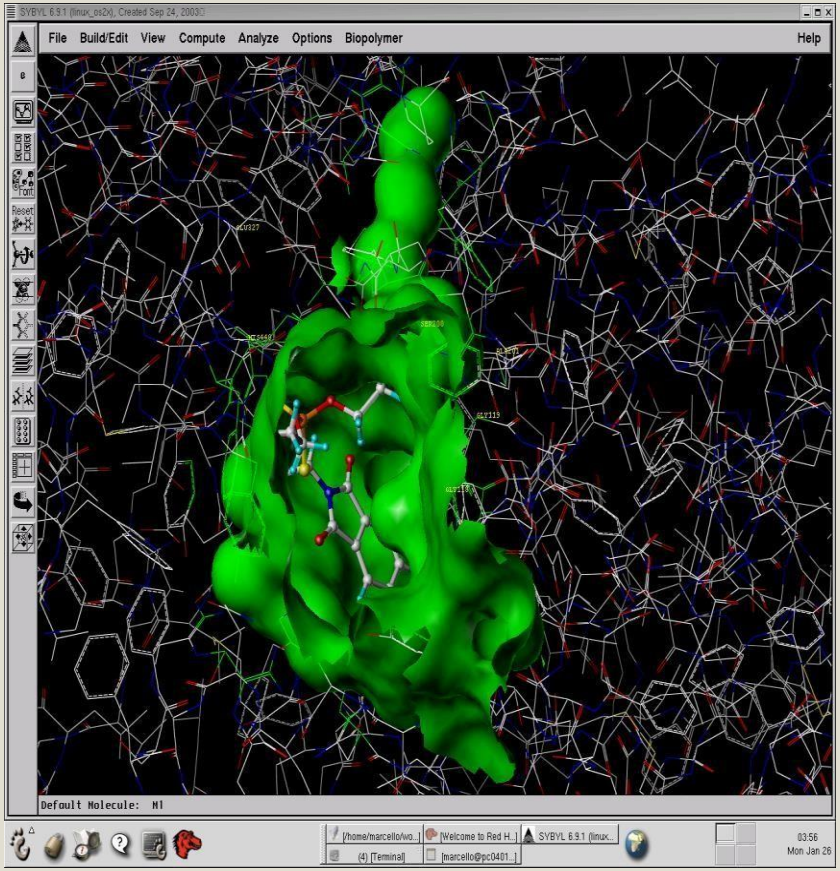
Methylphosphonylated Acetylcholinesterase (Aged) Obtained By Reaction With O-Ethyl-S-[2-[Bis(1-Methylethyl) Amino]Ethyl] Methylphosphonothioate (Vx) conventional X-ray crystallography resolution [Å]: 2.40

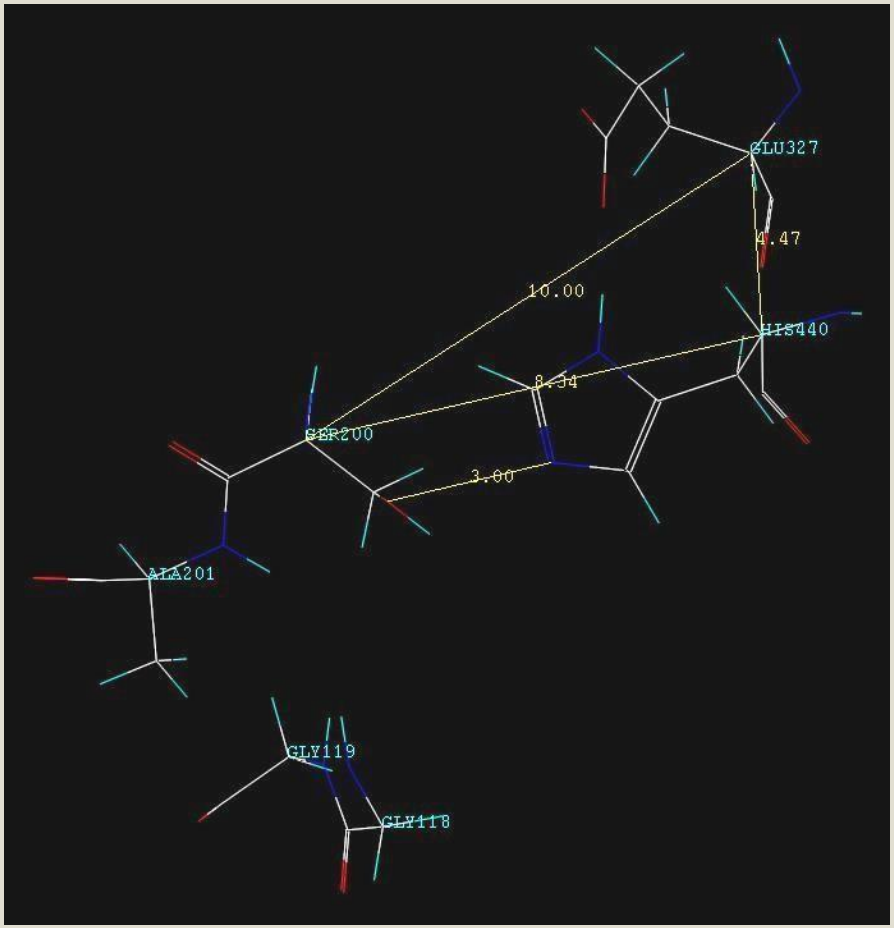


In green the molecular electrostatic potential distribution on the surface of the enzyme binding pocket

✓Design of the oligopeptides library as possible receptors

The geometry of the binding pocket was investigated to create oligopeptides library





Three dimensional coordinates of the asymmetric carbon (Cα) of each aminoacid involved in the binding pocket were calculated in order to reproduce the geometry observed

✓Tetrapeptides library

➢easy to synthesise

➢more possibility to preserve in solution the secondary structure predicted

•A series of tetrapeptides, containing the possible combinations of the catalytic triad (SER 200, HIS 440, GLU 327) and the catalytic oxyanion hole (GLY 118 GLY 119 ALA 201) was drawn

•The proper geometry of binding pocket was achieved using alternatively a GLY or a PRO residue

Library (24 tetrapeptides)

Ser-Gly-His-Glu	Glu-Gly-Ser-Ala
Ser-Gly-Glu-His	His-Gly-Ser-Ala
His-Glu-Gly-Ser	Gly-Pro-Ser-Ala
Glu-His-Gly-Ser	Ser-Ala-Pro-Glu
Ser-Pro-His-Glu	Ser-Ala-Pro-His
Ser-Pro-Glu-His	Ser-Ala-Pro-Gly
His-Glu-Pro-Ser	Glu-Pro-Ser-Ala
Glu-His-Pro-Ser	His-Pro-Ser-Ala
Gly-Gly-Ser-Ala	Gly-Ser-Gly-Ala
Ser-Ala-Gly-Glu	Ala-Gly-Ser-Gly
Ser-Ala-Gly-His	Ser-Gly-Pro-Ala
Ser-Ala-Gly-Gly	Ala-Pro-Gly-Ser

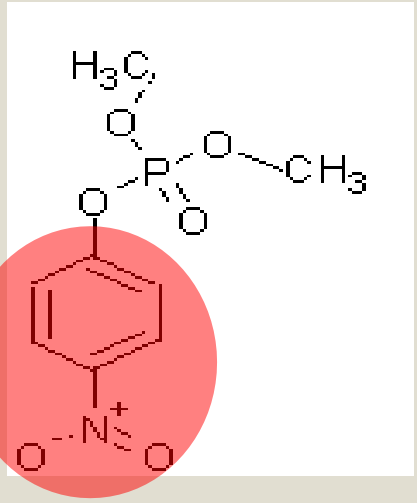
✓Simulated binding results vs paraoxon of the tetrapeptides selected for experimental screening

	A Ser-Ala-Gly-Glu	B His-Gly-Ser-Ala	C Glu-Pro-Ser-Ala	D His-Glu-Pro-Ser
Binding Score (KJ/mol)	38	73	21	93

Negative control (**NC**): **Glu-His-Ser-Gly**

Primary sequence of AChE catalytic triad

PARAOXON



→ **A. Ser-Ala-Gly-Glu**

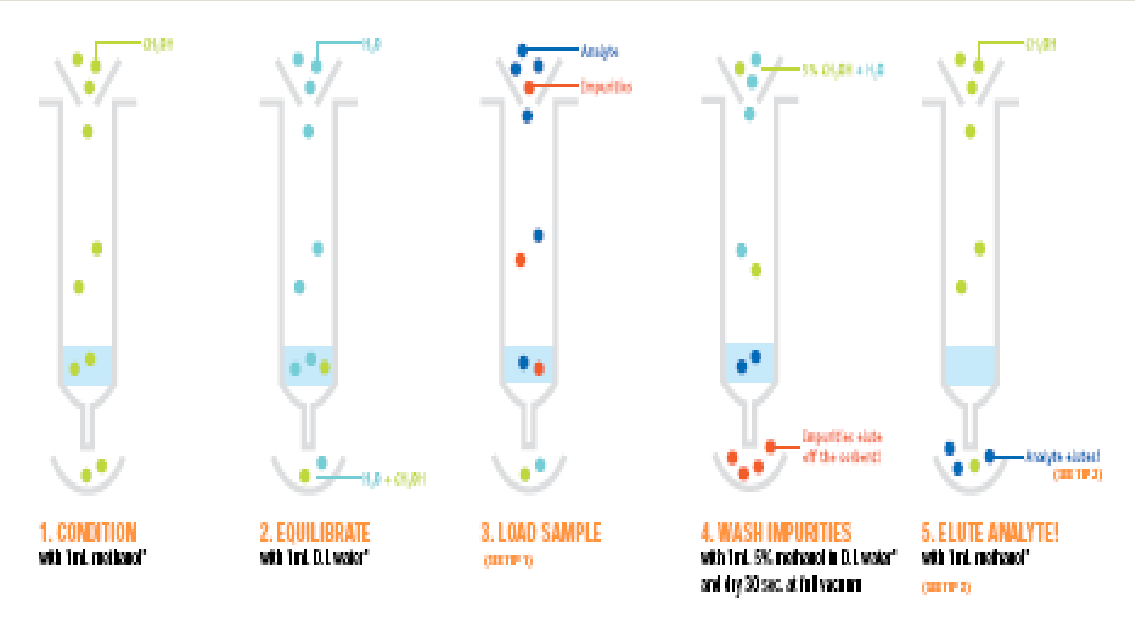
→ **B. His-Gly-Ser-Ala**

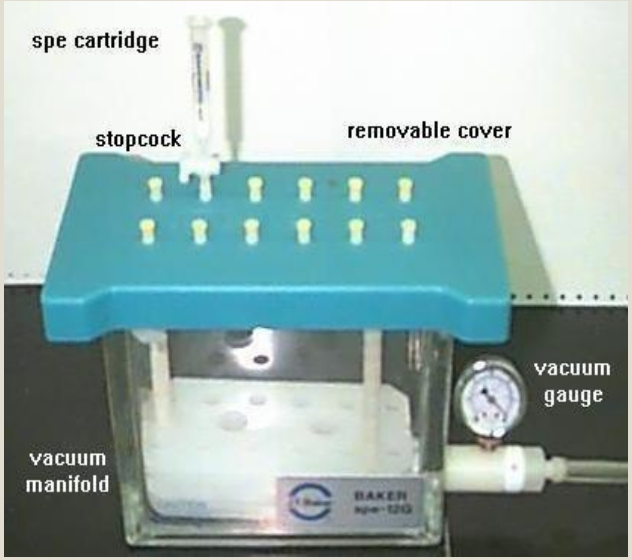
→ **C. Glu-Pro-Ser-Ala**

→ **D. His-Glu-Pro-Ser**

NC **Glu-His-Ser-Gly**

✓Pre-analytical applications: selective affinity columns (Extraction or purification)



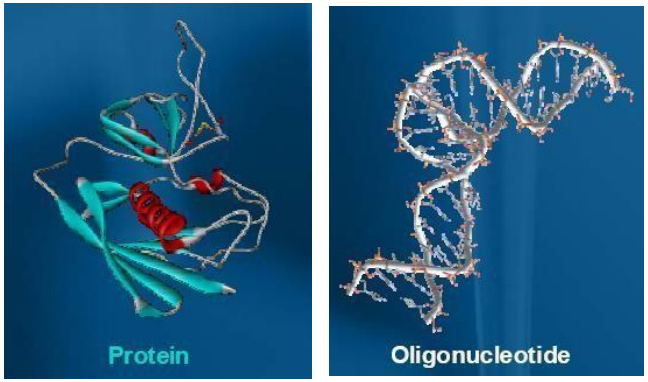


is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure. The substance to be purified is specifically and reversibly adsorbed to a ligand (binding substance), immobilized by a covalent bond to a chromatographic bed material (matrix). Samples are applied under favourable conditions for their specific binding to the ligand. Substances of interest are consequently bound to the ligand while unbound substances are washed away. Recovery of molecules of interest can be achieved by changing experimental conditions to favour desorption.

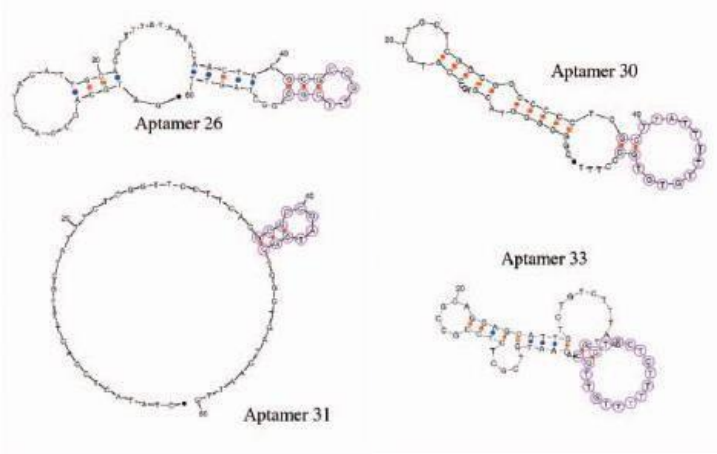
Aptamers are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules (proteins, peptides, drugs, vitamins and other organic or inorganic compounds).

They were "discovered" in 1990 by the development of an in vitro selection and amplification technique, known as SELEX (Systematic Evolution of Ligands by Exponential enrichment).
(Ellington et al., **Nature** 346, 818; Tuerk and Gold, **Science** 249, 505)

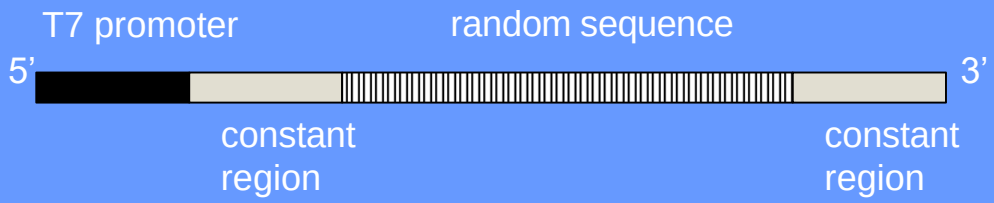
Their name is derived from the Latin word "**aptus**" which means "to fit".



Similar to proteins short oligonucleotides can adopt complex three-dimensional structures



Starting point: Combinatorial oligonucleotide library



A library containing a 25-nucleotide random region is represented by 4^{25} (~10^{15}) individual sequences available for partitioning.
Normally, the starting round contains **10^{14}-10^{15} individual sequences**.

A, **G**, **C**, **U**(**T**)

$4^1 = 4$
$4^2 = 16$
$4^3 = 64$
$4^4 = 256$
$4^5 = 1024$
……
……
……
$4^{25} = 1125899906842624$

Pool of randomized DNA or RNA

10^{15} different sequences!!!!

Biomedicine & Pharmacotherapy 132 (2020) 110902

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

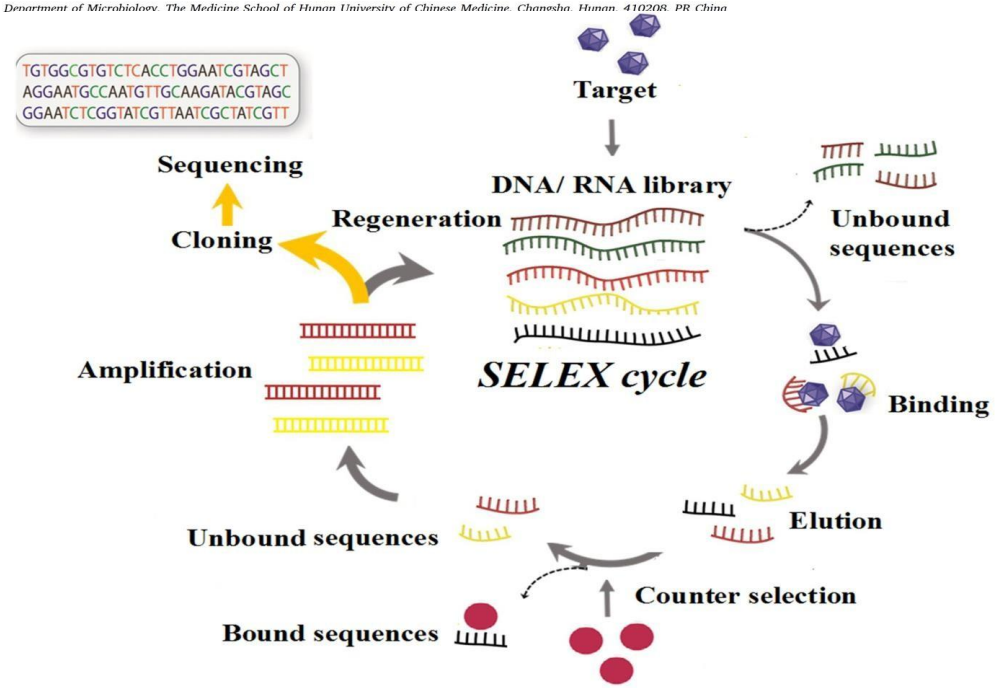
journal homepage: www.elsevier.com/locate/biopha

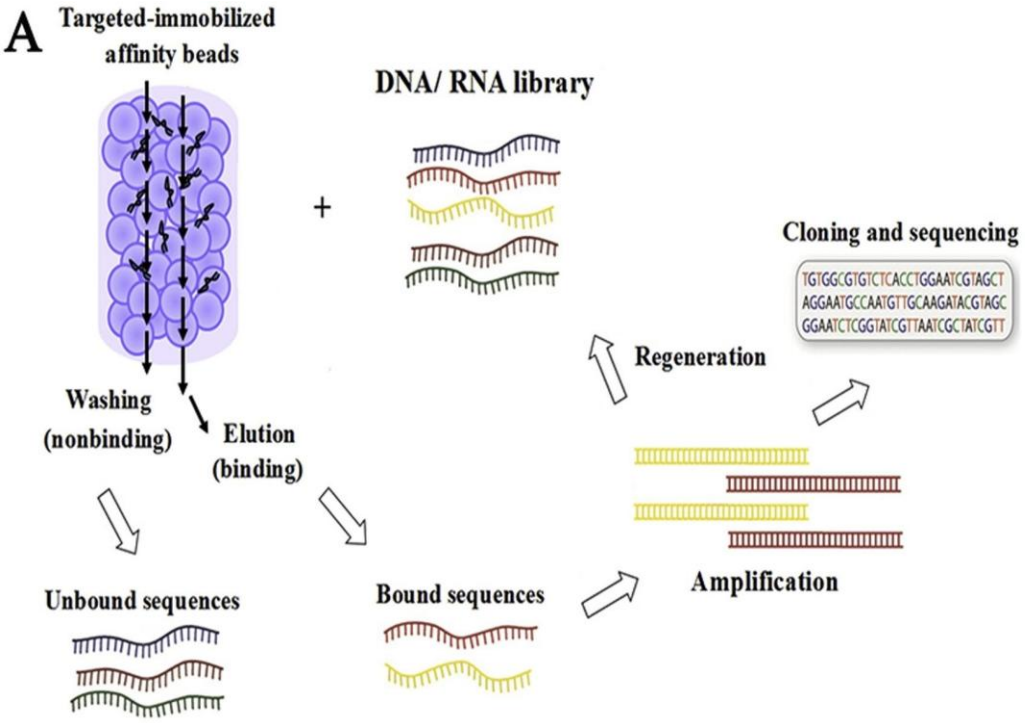
Review

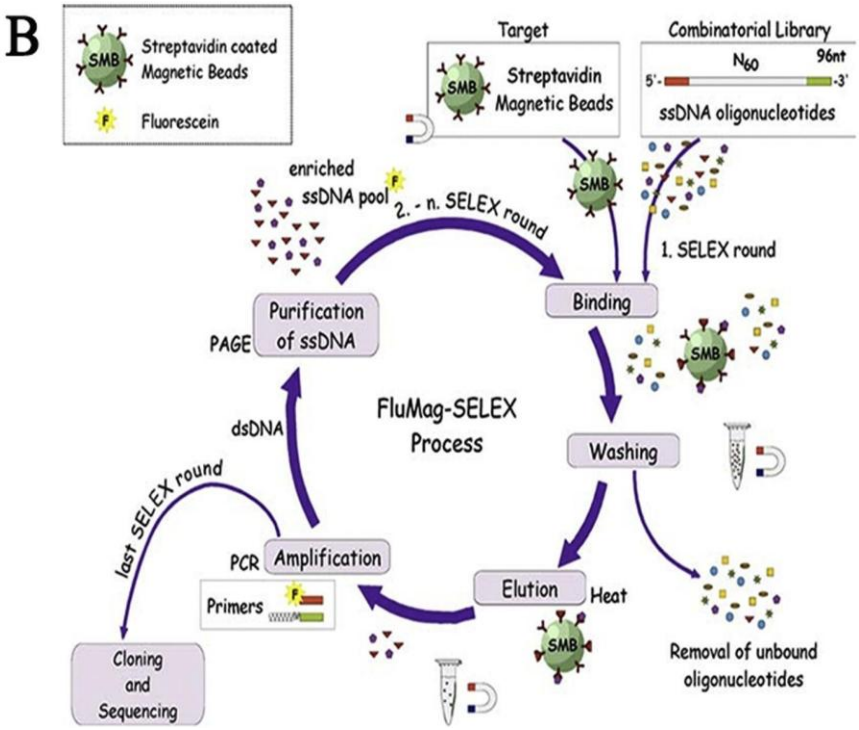
Aptamers used for biosensors and targeted therapy

Yi Ning, Jue Hu, Fangguo Lu *

Department of Microbiology, The Medicine School of Hunan University of Chinese Medicine, Changsha, Hunan, 410208, PR China




A
Targeted-immobilized affinity beads
DNA/ RNA library
+
Washing (nonbinding)
Elution (binding)
Unbound sequences
Bound sequences
Regeneration
Amplification
Cloning and sequencing
TGTGGCGTGTCTCACCTGGAATCGTAGCT
AGGAATGCCAATGTTGCAAGATACGTAGC
GGAATCTCGGTATCGTTAATCGCTATCGTT


B
Streptavidin coated Magnetic Beads
Fluorescein
Target
Streptavidin Magnetic Beads
Combinatorial Library
N60
96nt
5'-
-3'
ssDNA oligonucleotides
enriched ssDNA pool
2.- n. SELEX round
1. SELEX round
Binding
Purification of ssDNA
PAGE
FluMag-SELEX Process
Washing
dsDNA
last SELEX round
PCR
Amplification
Primers
Elution
Heat
Cloning and Sequencing
Removal of unbound oligonucleotides

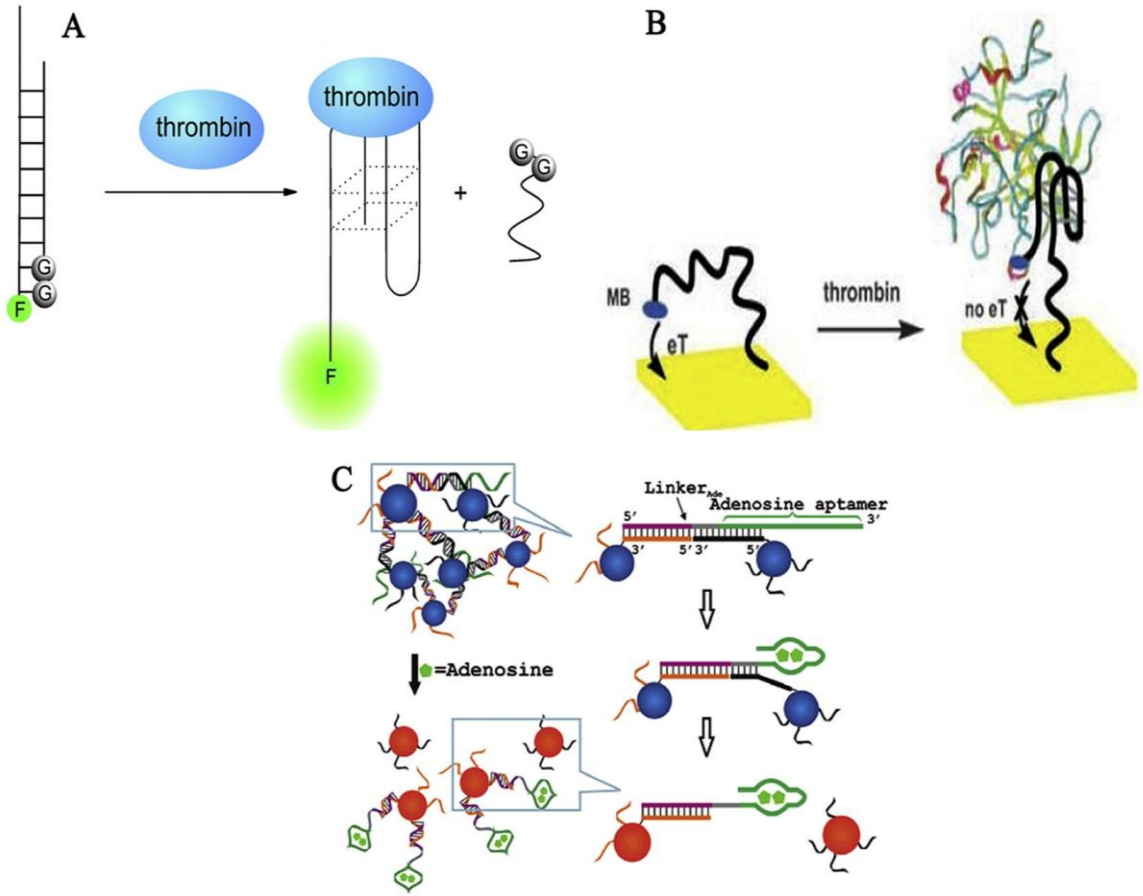
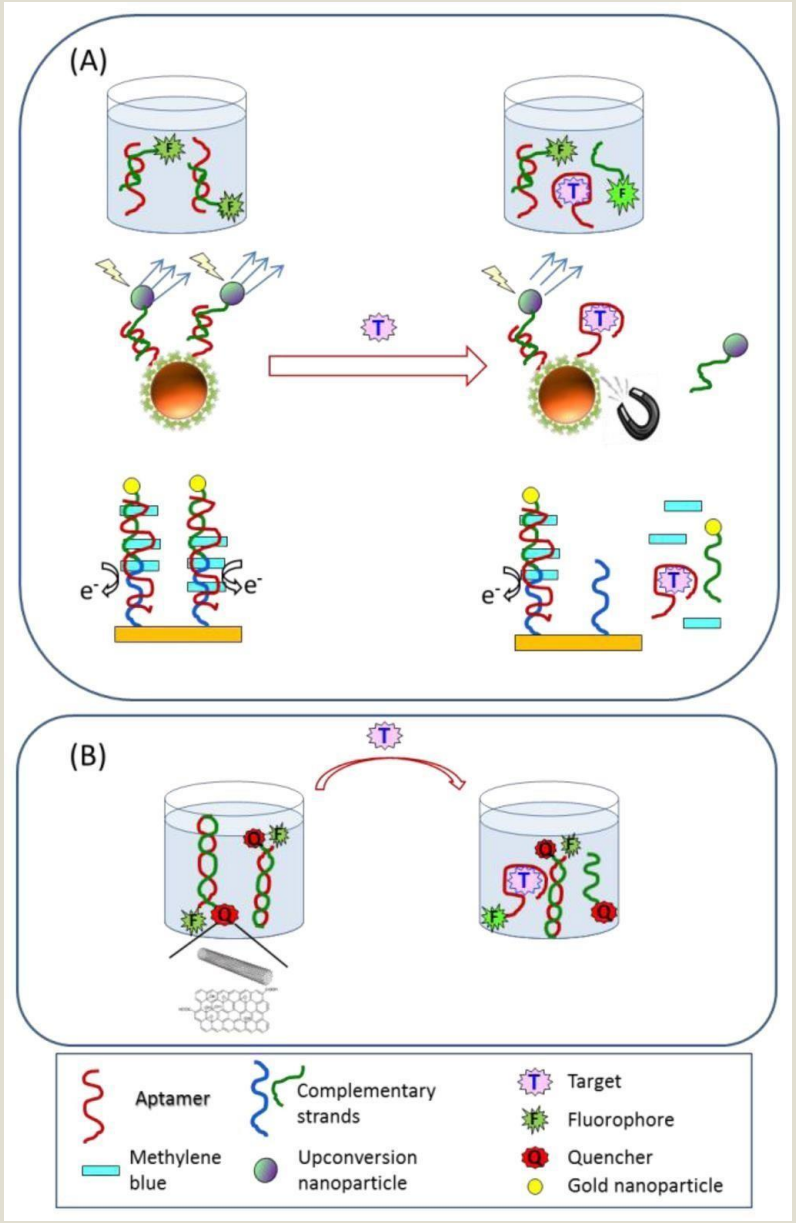


Fig. 5. Various signals generated by aptasensor based on structure-switching designs. (A) A schematic representation of the fluorescent aptasensor for thrombin assay. Thrombin-induced structure change of the aptamer from quenching-state into G-quartet structure could lead to fluorescence enhancement. Fig. 5A adapted from ref. [100]; (B) A schematic representation of the electrochemical aptasensor for thrombin assay. Before adding the thrombin, MB covalently labeled onto aptamer could transfer electron with the electrode surface due to the flexible conformation of the aptamer. Upon adding the thrombin A, G-quaduplex structure was formed and the MB moiety was far away from the electrode surface, resulting in the electrochemical signal-off. Fig. 5B adapted from ref. [105]; (C) A schematic representation of the colorimetric aptasensor for adenosine assay. Gold nanoparticles are functionalized with aptamer. Addition of the adenosine results in nanoparticles linking together and aggregating, thus causing the change in color. Fig. 5C adapted from ref. [107]. Copyright (2007) American Chemical Society.

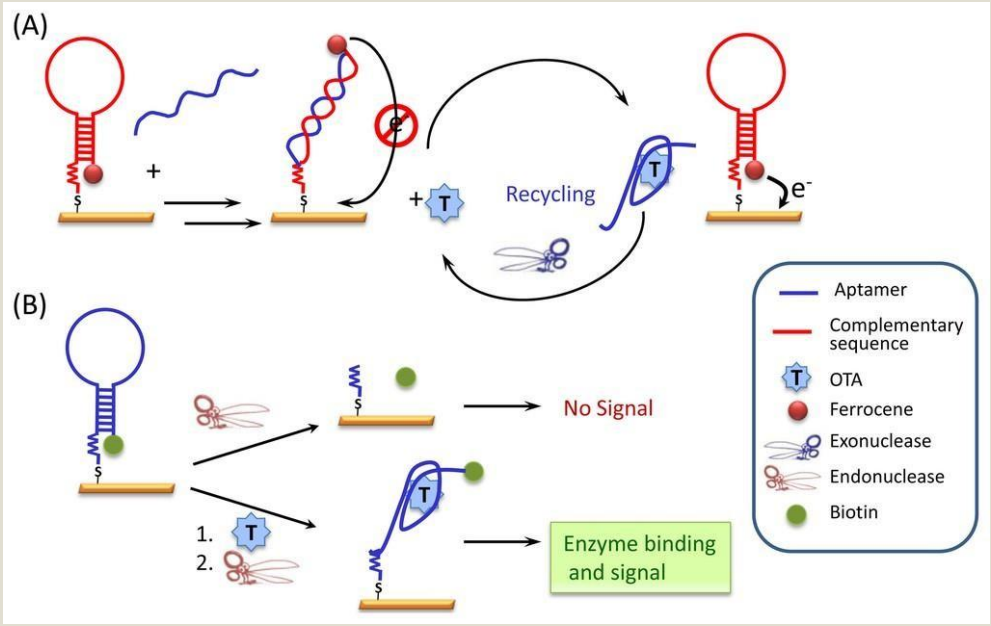


Sensors **2013**, *13*(12), 16292-16311; doi:10.3390/s131216292

Review

Aptamer-Based Analysis: A Promising Alternative for Food Safety Control

Sonia Amaya-González, Noemí de-los-Santos-Álvarez, Arturo J. Miranda-Ordieres and Maria Jesús Lobo-Castañón



Materials Advances

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Mater. Adv.*, 2020, 1, 2663

Detection and beyond: challenges and advances in aptamer-based biosensors

Hyebin Yoo,†[a] Hyesung Jo†[a] and Seung Soo Oh *[ab]

Received 24th August 2020,
Accepted 19th October 2020

DOI: 10.1039/d0ma00639d

rsc.li/materials-advances

Beyond traditional needs of biosensors such as high sensitivity and selectivity for analyte detection, newly emerging requirements including a real-time detection ability and in-field applicability have been gradually emphasized to address clinical and environmental availability. Highly programmable, synthetic aptamers that can specifically recognize a broad range of targets have the potential to fulfill these requirements; cooperative binding to target molecules achieves a significant increase in sensitivity, and binding-induced structure-switching enables target detection even in complex mixtures. Due to the availability of chemical synthesis and functional modifications, these artificial ligand materials are easily installed in many devices, and the amenability to modularization allows the aptamer-based biosensors to diversify detectable targets and signaling processes. In this review, we highlight current progress in the development of aptamer-based, next-generation biosensors including new types of field-effect transistors, electrochemical detectors, and microfluidic devices. As the nucleic acid aptamers have been rapidly generated by various *in vitro* selection techniques, the use of the versatile nanostructures is expected to expand further to include in-field and real-time biosensors.

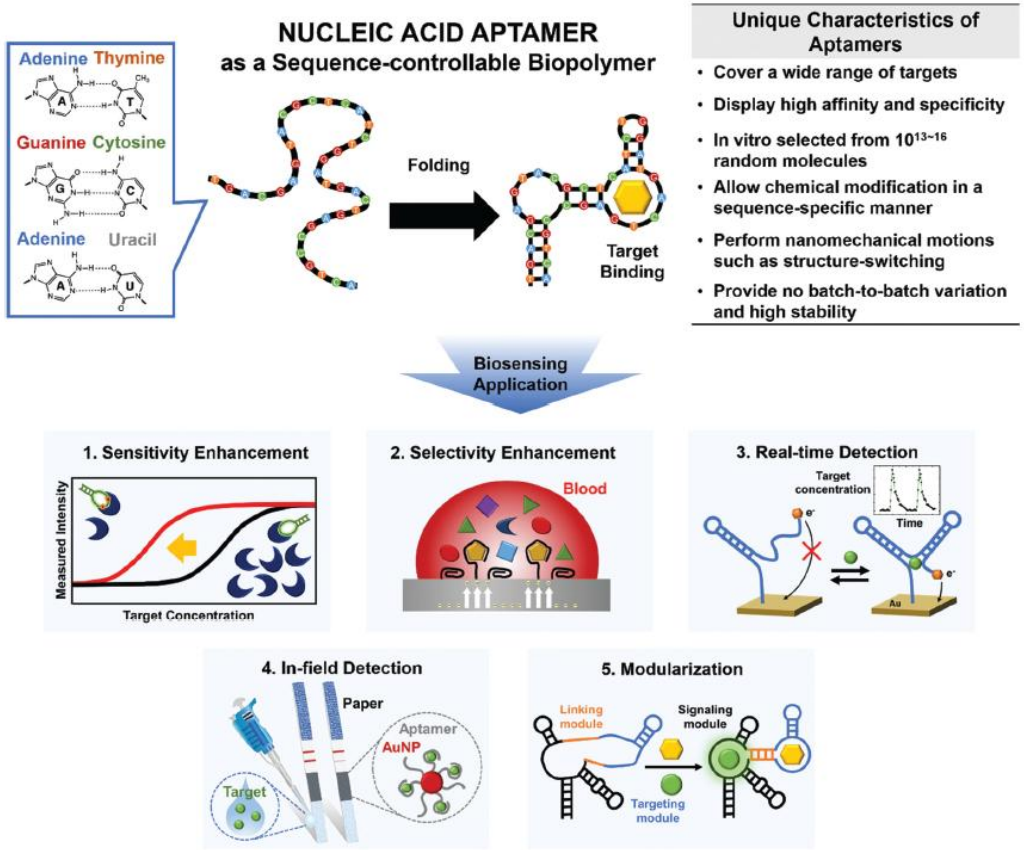


Fig. 1 Main characteristics and functionalities of nucleic acid aptamers to overcome various limitations of conventional biosensors. Specific base pairing (top, left) folds the sequence-controllable biopolymers into thermodynamically-favored 3D nanostructures that enable molecular recognition (top, middle). The synthetic aptamers have unique features that can facilitate the development of next-generation biosensors (top, right). Here, we review technical advances in the development of aptamer-based biosensors, such as increases in sensitivity and selectivity, and actualization of newly emerging real-time and in-field detection applications, along with aptameric biosensors' interesting properties, such as amenability to modularization (bottom).

The sensitivity and selectivity can be improved usign different strategies

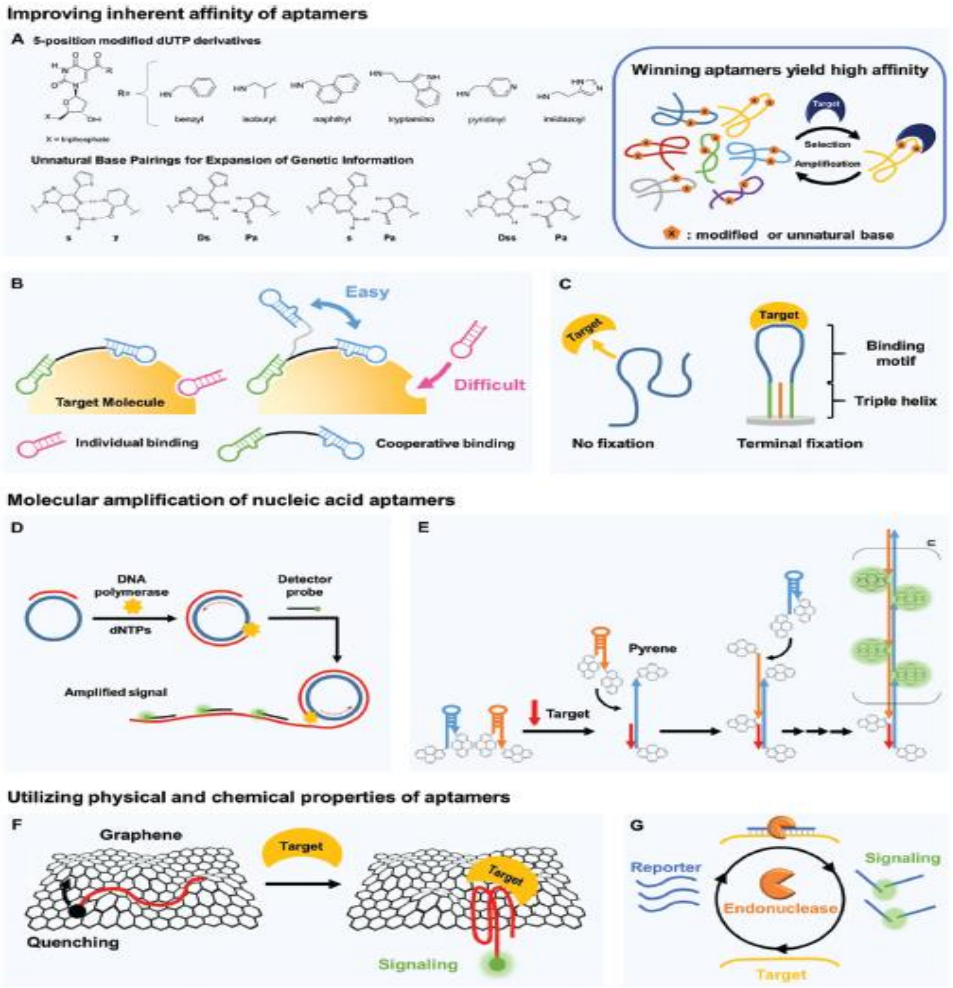


Fig. 2 Aptamers can be key components of biosensors to increase sensitivity in target detection. (A) The inherent binding affinity of aptamers can be strengthened by chemical modification of nucleic acids, e.g., by attaching hydrophobic moieties to nucleotides and by adding non-canonical pairing bases. (B) Multivalency by cooperative binding of multiple aptamers and (C) terminal fixation of folding structures can be also effective to improve the target binding capability of aptamers. (D) Molecular amplification techniques such as rolling circle amplification (RCA) can be useful to develop ultra-sensitive biosensors by significantly increasing detectable signals. In RCA, primers are bound to circular templates; polymerases extend the primers to yield long single-stranded concatemers with tandem repeat structures, and the repeated hybridization of dye-labeled strands with tandem repeats produces amplified fluorescent signals. (E) Hybridization chain reaction (HCR) can also be used to increase sensitivity. In HCR, introduction of DNA targets can trigger a hybridization cascade of signaling probes such as pyrene-conjugated hairpin probes and thereby facilitate ultra-sensitive target detection. (F) Unique physical properties of nucleic acid aptamers can contribute to highly sensitive target detection. By $\pi-\pi$ stacking, single-stranded nucleic acids bind well to graphene surfaces, whereas the target-bound aptamers are released due to folding in tertiary structures. This folding change of aptamers yields changes in fluorescent or electrical signals, which can be easily detected. (G) Target-bound aptamers are less vulnerable to nuclease digestion than their target-free forms, and this feature can be applied to signal accumulation; the exonuclease-based, enzyme-assisted target recycling (EATR) technique can significantly decrease the limit of detection by summing fluorescence signals.

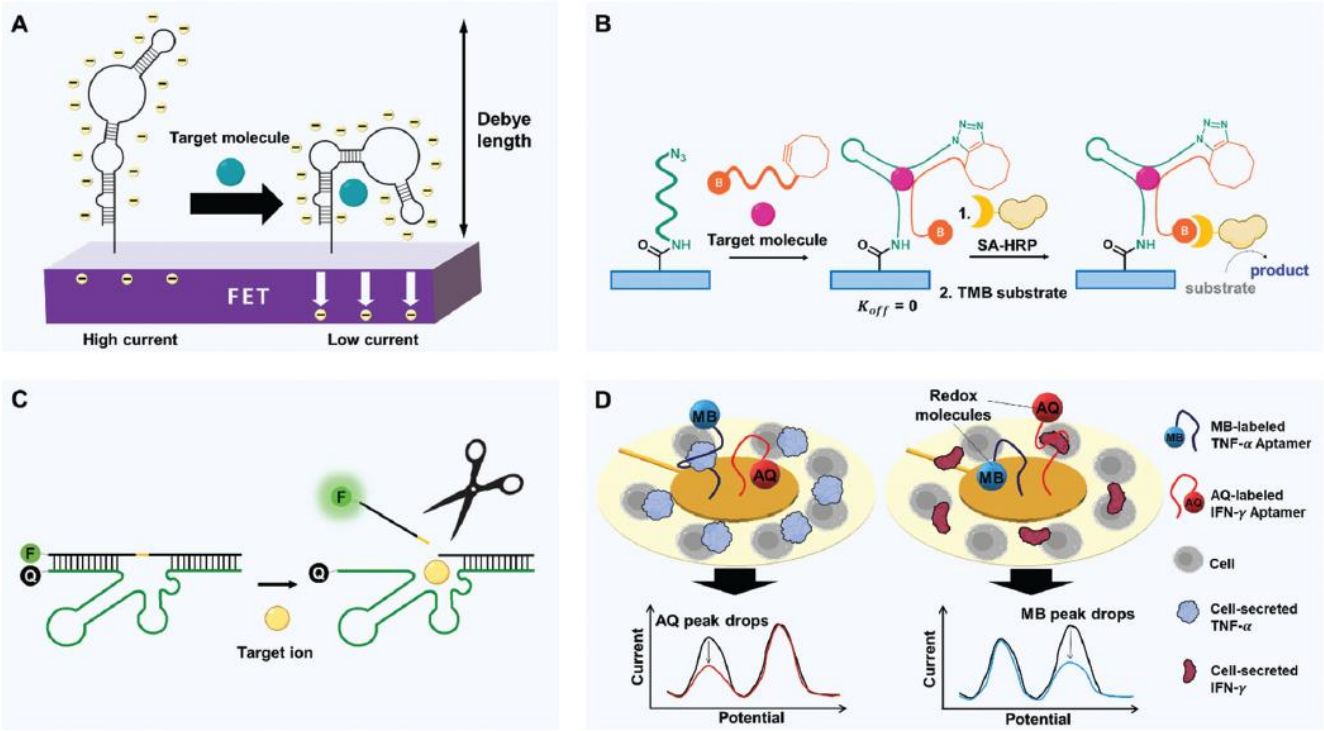
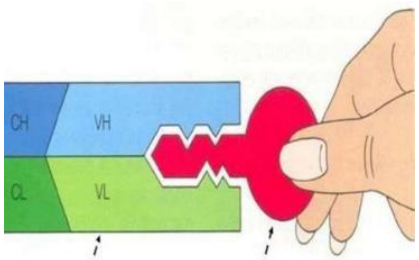


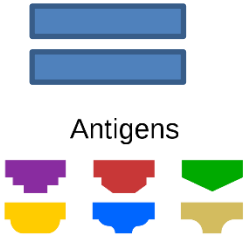
Fig. 3 Diverse strategies with aptamers to improve selectivity of biosensors. (A) Binding-induced self-conformational change of aptamers. FETs modified with target-specific structure-switching aptamers enable selective electronic target detection. Within or near the Debye length, target-induced reorientations of stem-loop aptamers near semiconductor channels deplete the channels electrostatically and thereby decrease transconductance. (B) Binding-induced hetero-conformational change of split aptamers. Upon target binding, split aptamers can be covalently linked to each other by click chemistry. When biotinylated aptamer fragments recruit streptavidin-horseradish peroxidase (SA-HRP), chromogenic substrates such as TMB can be oxidated to emit detectable signals. (C) Binding-activated catalytic reaction. By target binding-induced cleavage of aptazymes, the release of fluorophore-linked fragments can be activated to emit highly target-specific fluorescence by reducing physisorption-derived signaling. (D) High selectivity-driven multiplexing. A multiplex analysis can be conducted by aptamers that are linked to redox molecules. Surrounded by cells, Au electrodes can be modified with different aptamers-redox reporter constructs. Binding to cytokines (TNF-α and IFN-γ) causes target-dependent conformation changes that decrease electron-transfer efficiency and thereby decrease the current. Redox molecules with different potential enable the simultaneous detection of multiple targets.

Molecularly imprinted polymers (MIPs) are synthetic receptors for a targeted molecule. As such, they are analogues of the natural antibody–antigen systems

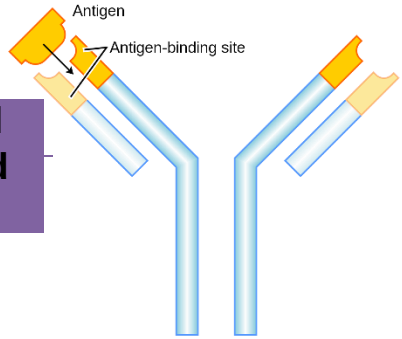
DOI: 10.1021/acs.chemrev.8b00171 Chem. Rev. 2019, 119, 94−119

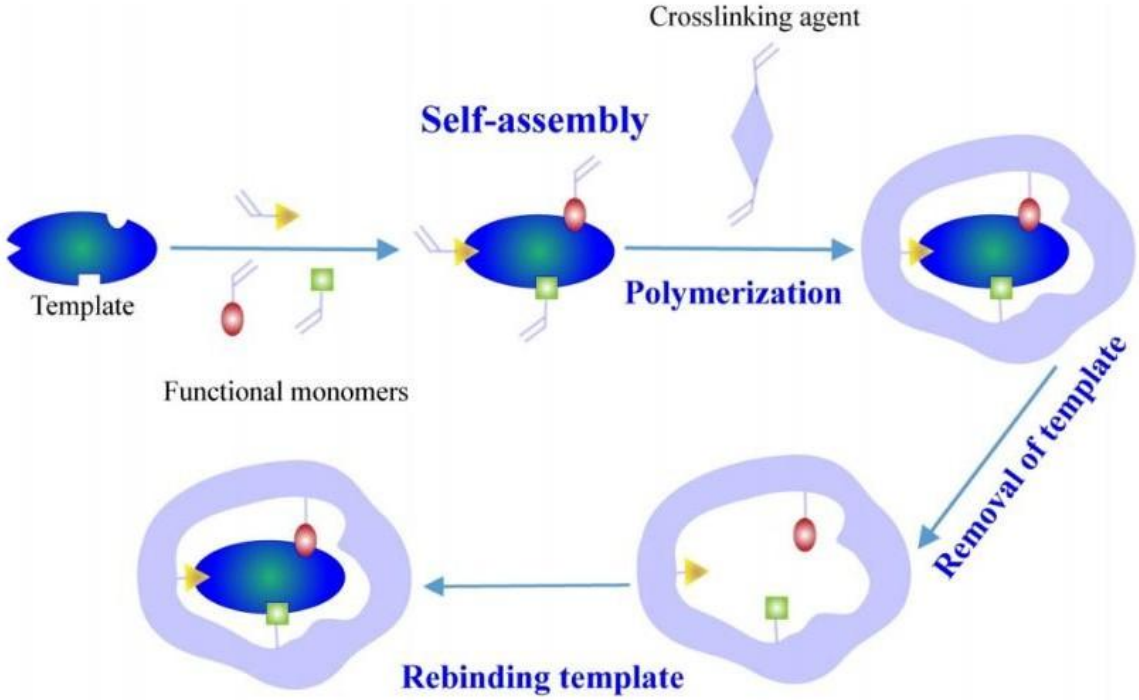
Plastic Antibody





Natural Antibody





Scheme 1. Schematic representation of the synthesis of molecu-larly Imprinted Polymers (MIPs).

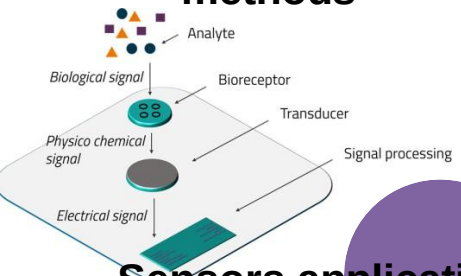
Abdellatif Ait Lahcen[a] and Aziz Amine[a],2018*

MIP-State of the art

MIPs Applications

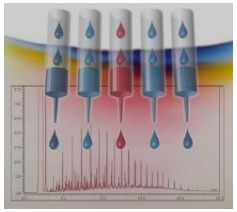
MIPs are excellent materials with high selectivity and are widely used for:

Sample preparation in bio analytical methods



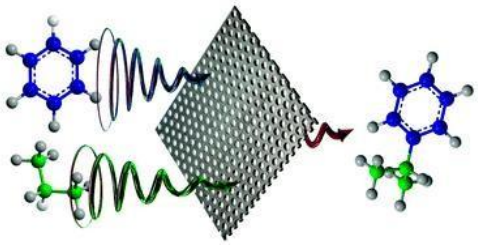
Sensors applications

Chromatography

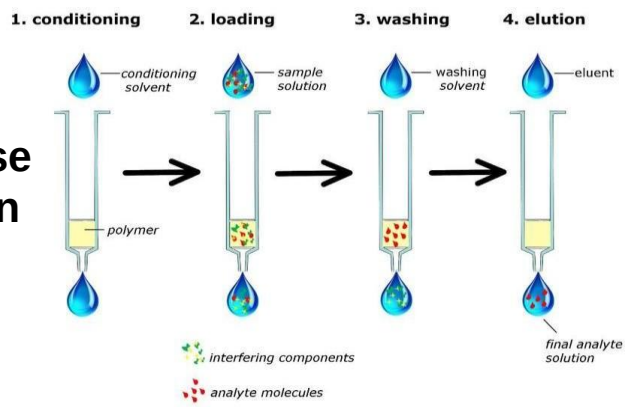


Drug delivery

Catalysis



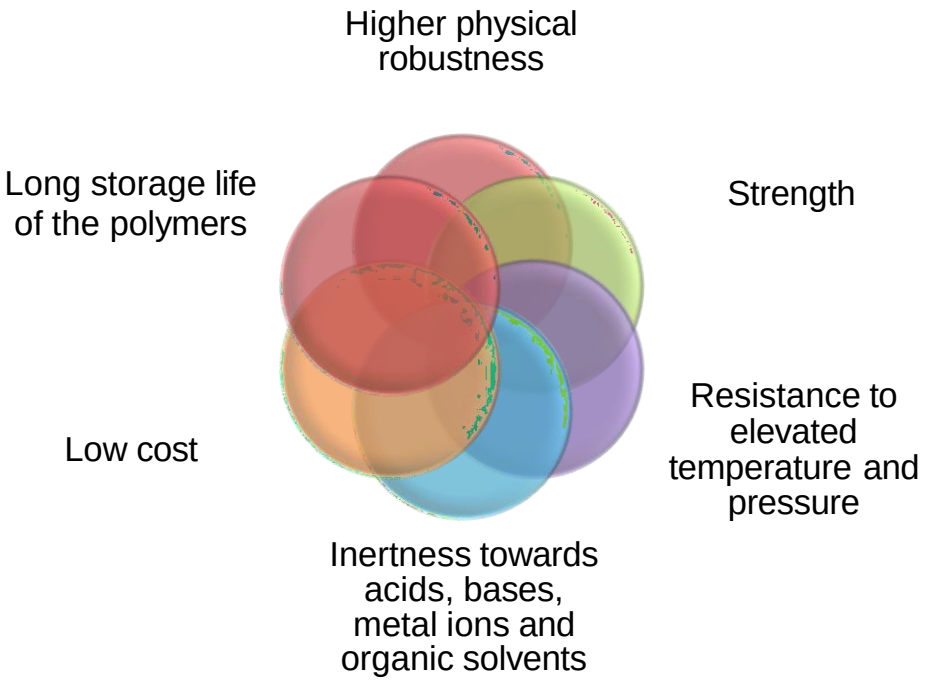
Solid phase extraction



Advantages of MIPs

❖ High **selectivity** and **affinity** for the **target molecule** used in the imprinting procedure.

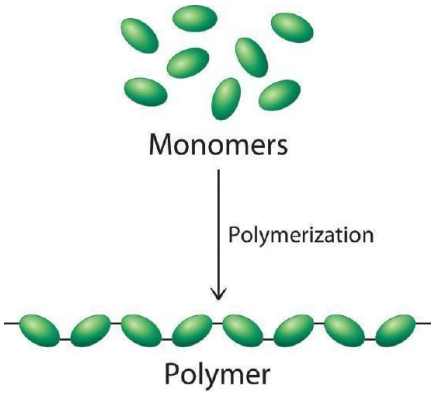
Compared to biological systems such as proteins and nucleic acids MIP has:

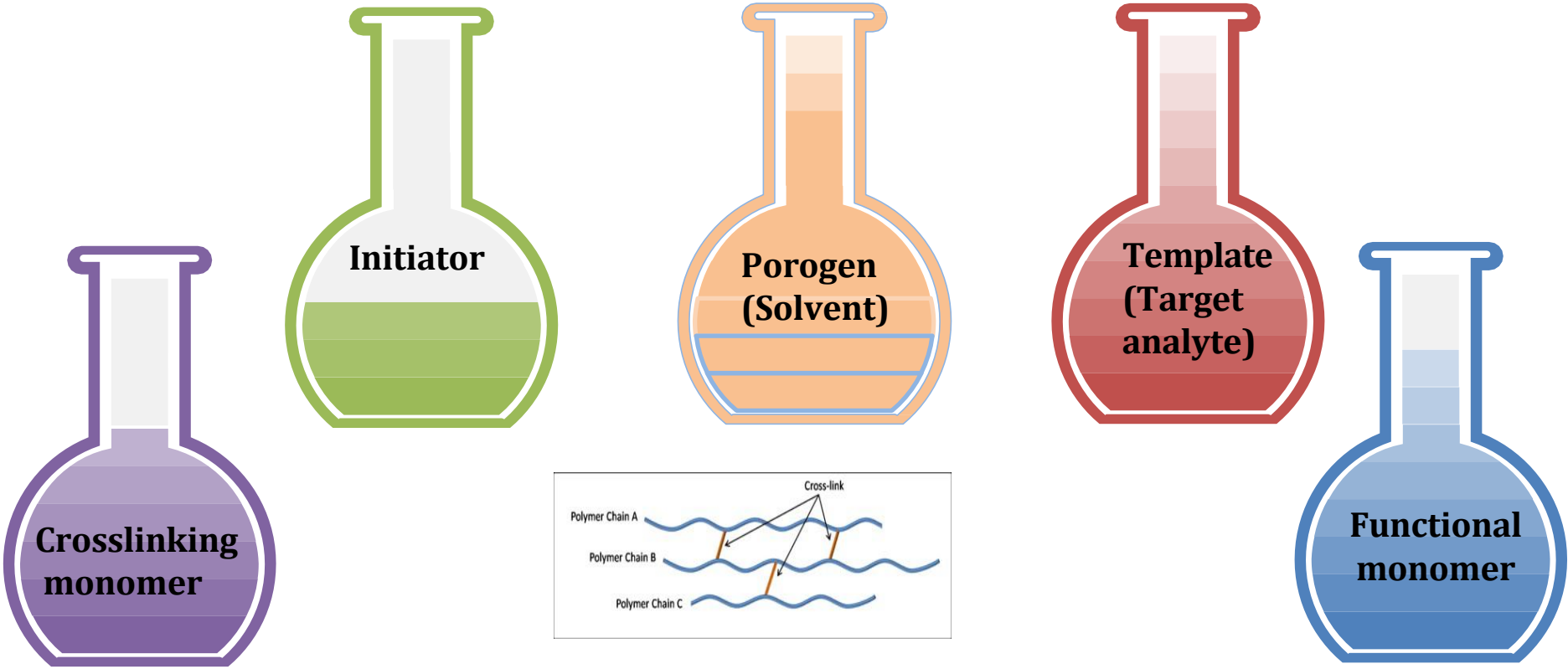


Int J Mol Sci. 2011; 12(9): 5908–5945
***Chem. Rev.* 2000, *100,* 2495-2504**

MIPs Synthesis

→ **Components of MIP Mixture**





MIPs Synthesis

General procedure

Self-assembly step

Polymerization

Extraction

MIPs

Porogen solvent

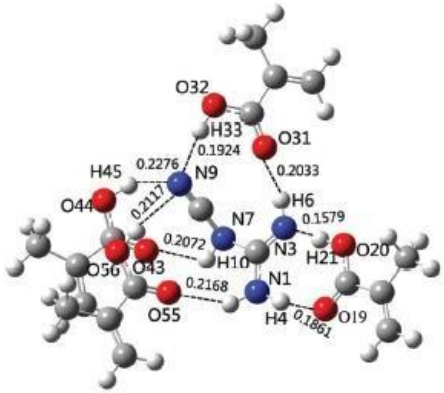
Monomer

Template

Cross-linker

Initiator

2:00



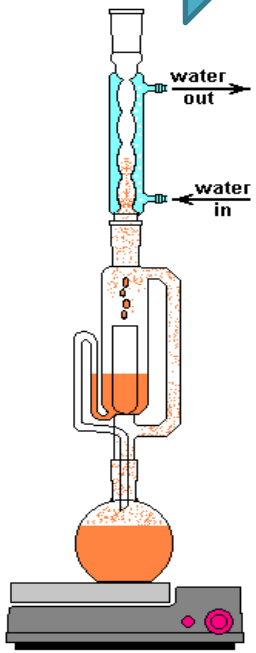
Nitrogen in

Water out

Water in

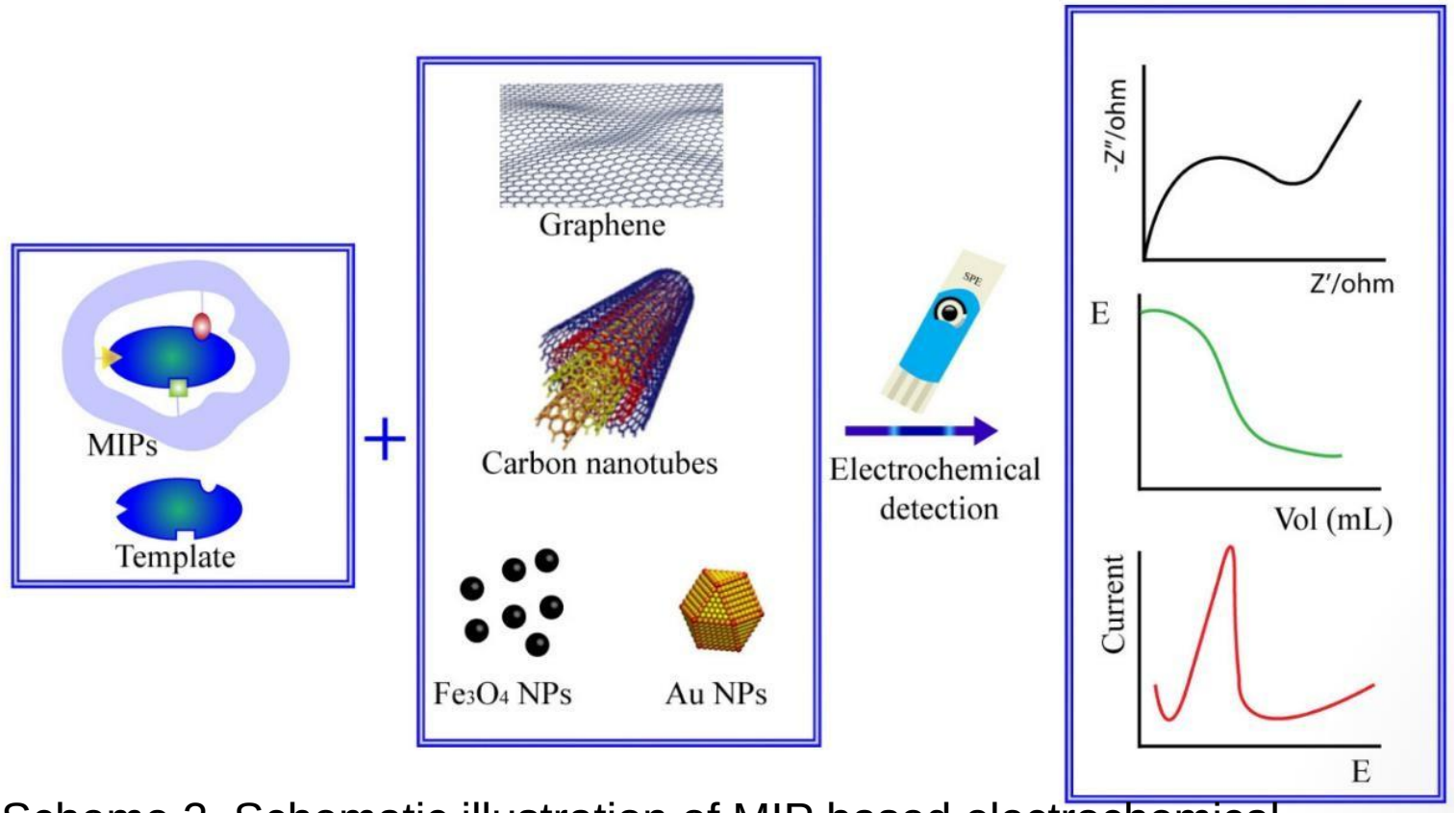
60 °C

24:00



Soxhlet extractor

MIP based electrochemical sensors and nanomaterials

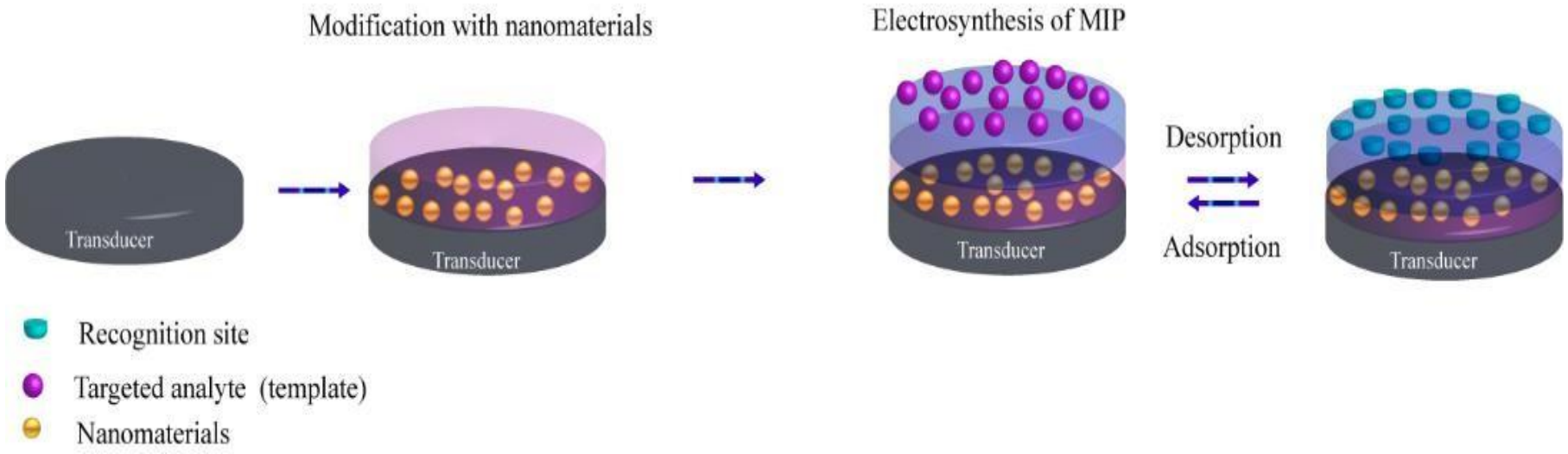


Scheme 2. Schematic illustration of MIP based electrochemical sensors and nanomaterials.

Abdellatif Ait Lahcen[a] and Aziz Amine[a],2018*

MIP based electrochemical sensors and nanomaterials

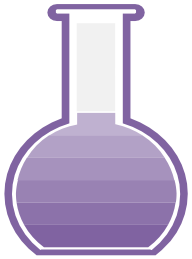
Electrosynthesis of MIPs



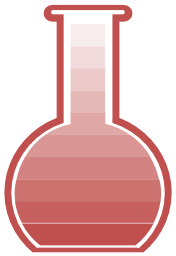
~~**Initiator**~~

~~**Crosslinking monomer**~~

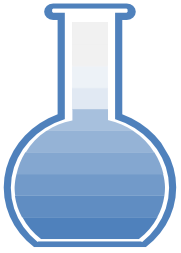
Template (Target analyte)



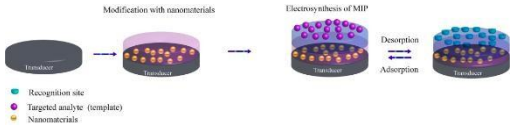
Porogen (Solvent) Buffer



Functional monomer



MIP based electrochemical sensors and nanomaterials



Food contaminant

Template

Electrochemical behaviour

Non-electroactive

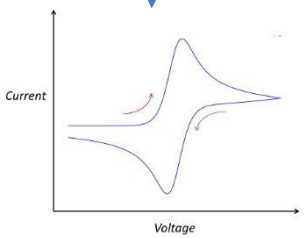
Electroactive

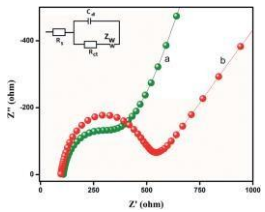
Detection

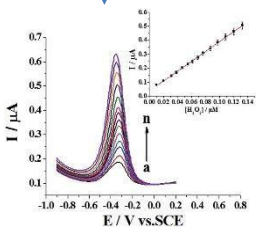
Voltametric measurement of redox marker ferricyanide

Impedance spectroscopy

Direct electrochemical detection



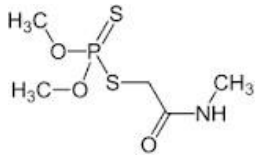


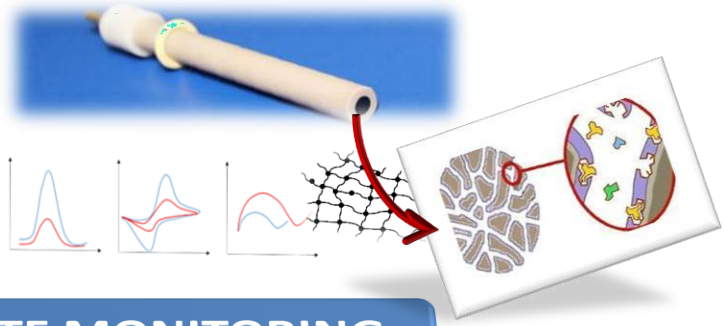


	Thermal heating	Ultrasound		Electro-polymerization
		Bath	Probe	
Complexity	medium	medium	easy	Medium
Time of synthesis	24 h	2-4 h	10 min	10 min
Temperature	60-70 °C	60-70 °C	60-70 °C	Room temperature
Synthesis of high amount	high	medium	medium	Low
Template	All templates except those sensible to high temperature such as proteins, bacterial cells,etc.	All templates except those sensible to high temperature and ultrasonic waves such as proteins, bacterial cells,etc.		It is preferred that templates are soluble in water

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

UNITE UNIVERSITÀ DEGLI STUDI DI TERAMO





DIMETHOATE MONITORING IN WHEAT FLOUR

SAMPLE PREPARATION

ANALYTE DETECTION

MICROEXTRACTION BY PACKED SORBENT (MEPS)

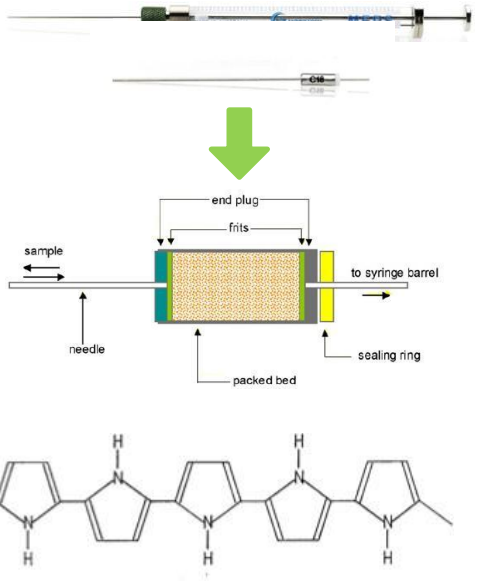
MIP-GLASSY CARBON ELECTRODE


Talanta 174 (2017) 599–604
Contents lists available at ScienceDirect
Talanta
journal homepage: www.elsevier.com/locate/talanta

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

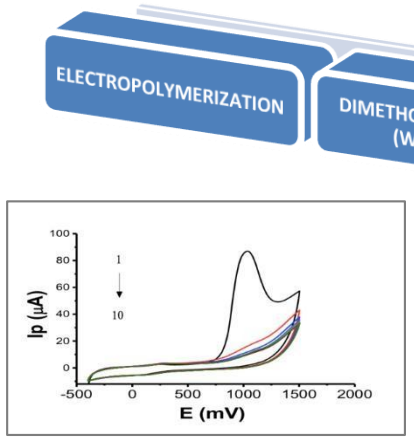
D. Capoferri[a], M. Del Carlo[a,1], N. Ntshongontshi[b], E.I. Iwuoha[b], M. Sergi[a], F. Di Ottavio[a], D. Compagnone[a,*,1]

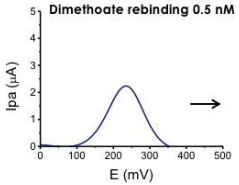
[a] Faculty of Biosciences and Technologies for Food, Agriculture and Environment, University of Teramo, via R. Balzarini 1, 64100 Teramo, Italy
[b] SensorLab, Department of Chemistry, University of the Western Cape, Bellville 7535, South Africa

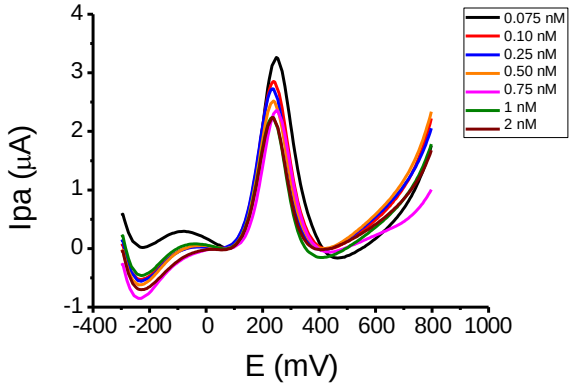


MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

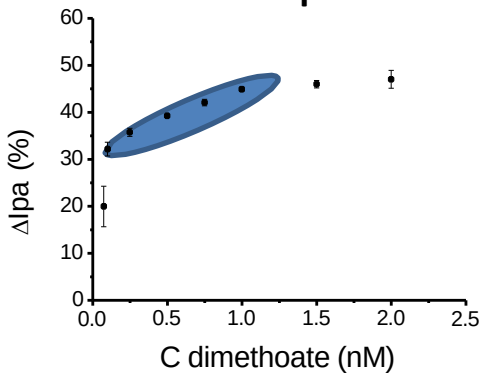
UNIVERSITÀ DEGLI STUDI DI TERAMO





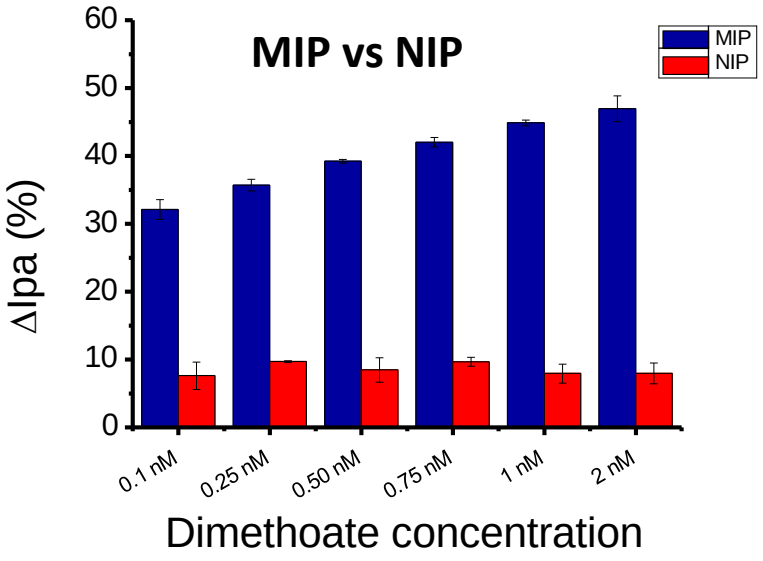


Dose-response curve

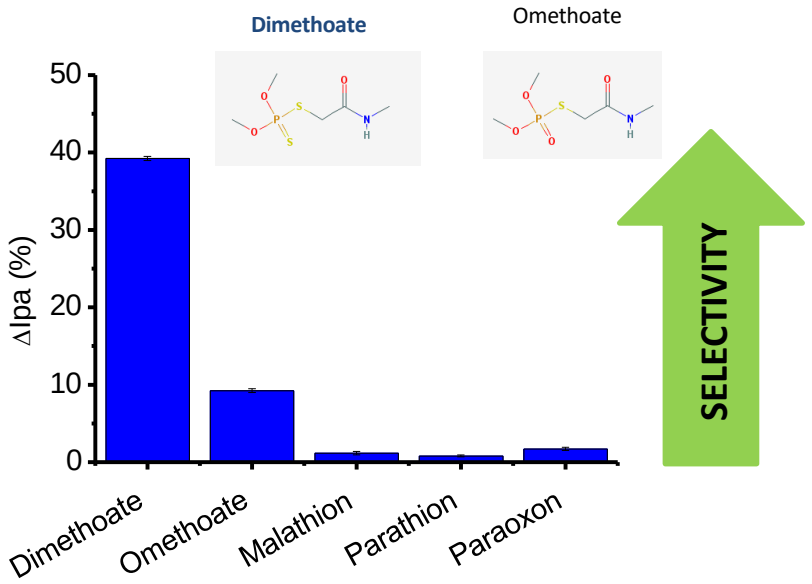


MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

ΔIpa (%)	Repeatability (RSD %)	Reproducibility (RSD %)
0.5 nM dimethoate (n=3)	0.68	2.72
1 nM dimethoate (n=3)	0.95	5.51



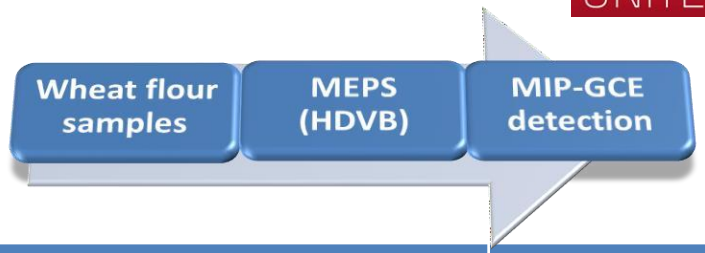
ΔIpa (%) for malathion, parathion and paraoxon after the rebinding step was negligible; **omethoate** gave a response of **23%**.



MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

UNITE UNIVERSITÀ DEGLI STUDI DI TERAMO

Wheat flour samples: MIP vs. UHPLC-MS/MS

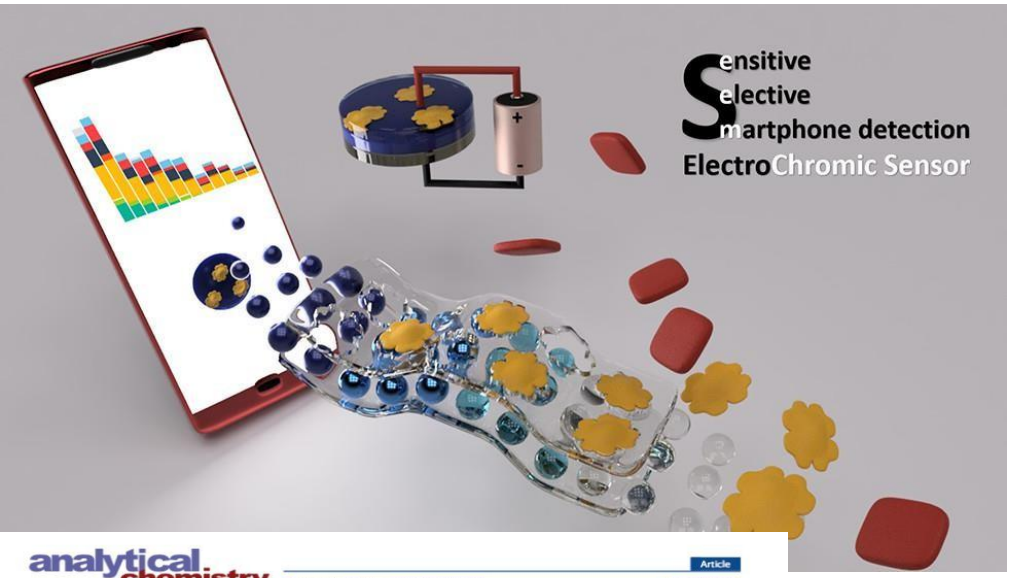


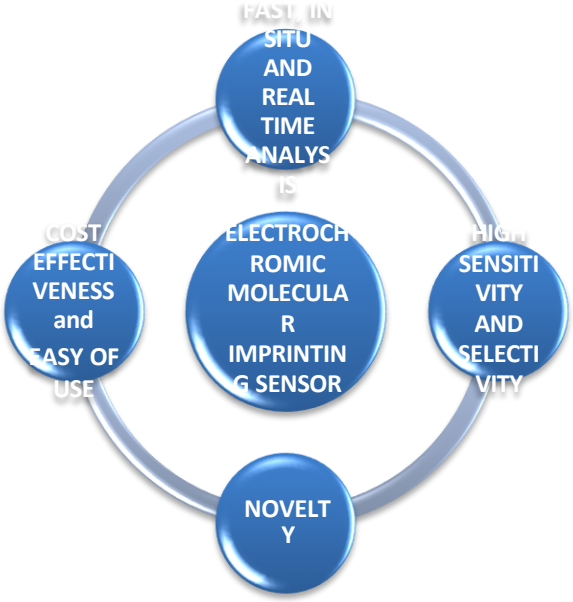
Samples1	MIP-GCE RELATIVE ERROR (%) of dimethoate concentration (µg kg^{-1})	MIP-GCE SD of dimethoate concentration (µg kg^{-1})
Wheat flour spiked with dimethoate 0.5 MRL	+13.5	0.52
Wheat flour spiked with dimethoate 0.5 MRL + mix	+4.6	2.37
Wheat flour spiked with dimethoate MRL	-21.1	1.24
Wheat flour spiked with dimethoate MRL + mix	-21.2	1.36
Wheat flour spiked with dimethoate 1.5 MRL	+16.7	0.74
Wheat flour spiked with dimethoate 1.5 MRL + mix	-0.4	1.69
Wheat flour spiked with dimethoate MRL + omethoate (1:1)	+3.5	2.70
Wheat flour spiked with dimethoate MRL + omethoate (1:10)	-15.5	0.86

Chlorpyriphos

Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

UNIVERSITÀ DEGLI STUDI DI TERAMO





analytical chemistry
Article
Cite This: Anal. Chem. XXXX, XXX, XXX−XXX
pubs.acs.org/ac

Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

Denise Capoferri,[†,‡,§] Ruslan Álvarez-Diduk,[†,§] Michele Del Carlo,[‡] Dario Compagnone,[‡] and Arben Merkoçi*[,†,∥]

[†]Nanobioelectronics and Biosensor Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC, The Barcelona Institute of Science and Technology, Campus UAB, Bellaterra, 08193, Barcelona, Spain
[‡]Faculty of Biosciences and Technologies for Food, Agriculture and Environment, University of Teramo, via R. Balzarini 1, 64100 Teramo, Italy
[∥]Catalan Institution for Research and Advanced Studies (ICREA), Pg. Lluís Companys 23, 08010 Barcelona, Spain

Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

UNIVERSITÀ DEGLI STUDI DI TERAMO

Electrochromism

Strategy

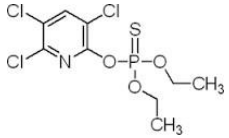
MIP

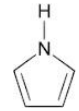
Screen-printing technology

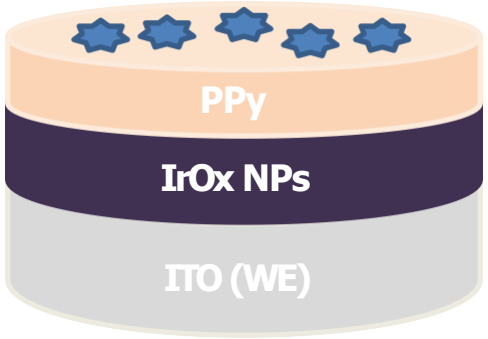
Smartphones

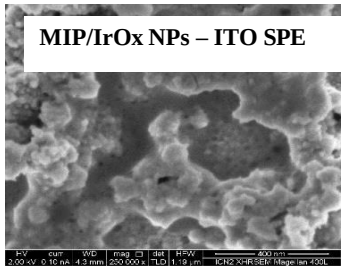
CPF (chlorpyrifos)

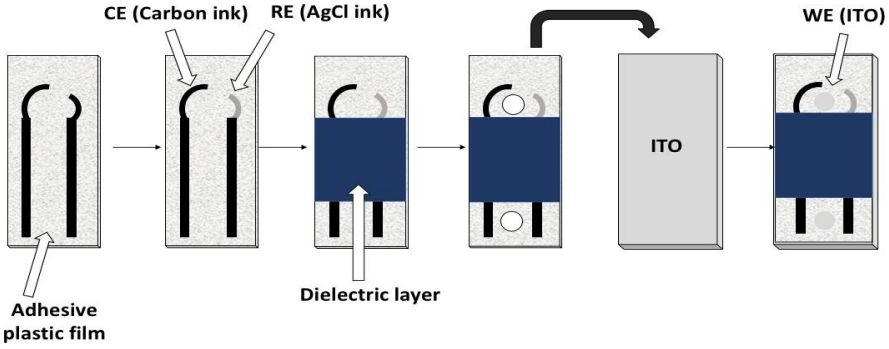
Py









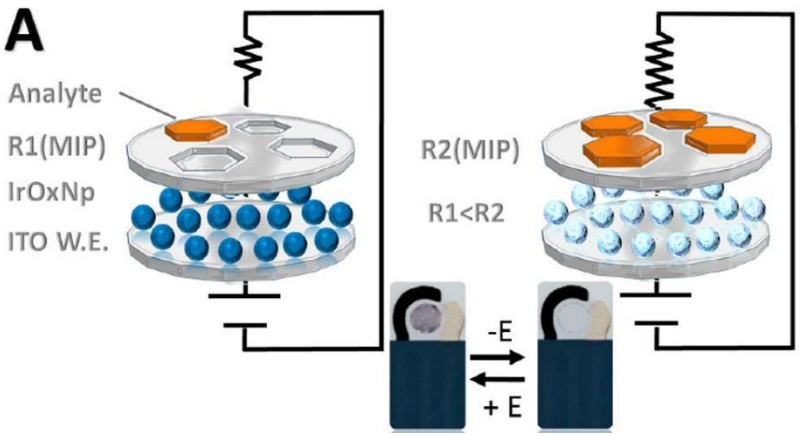


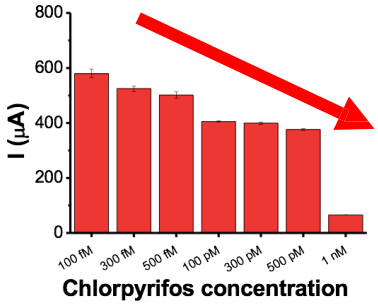
ELECTROCHROMIC MOLECULAR IMPRINTING SENSOR for an ORGANOPHOSPHATE PESTICIDE

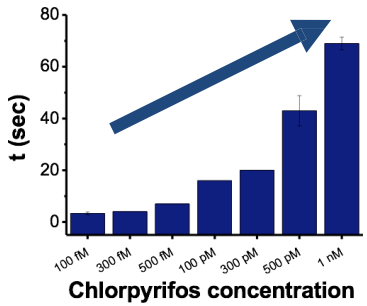
Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

UNIVERSITÀ DEGLI STUDI DI TERAMO

WORKING PRINCIPLE



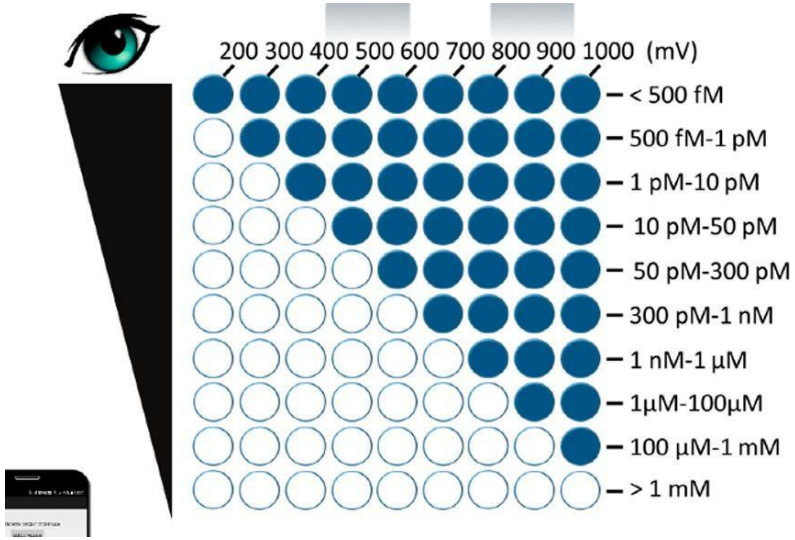




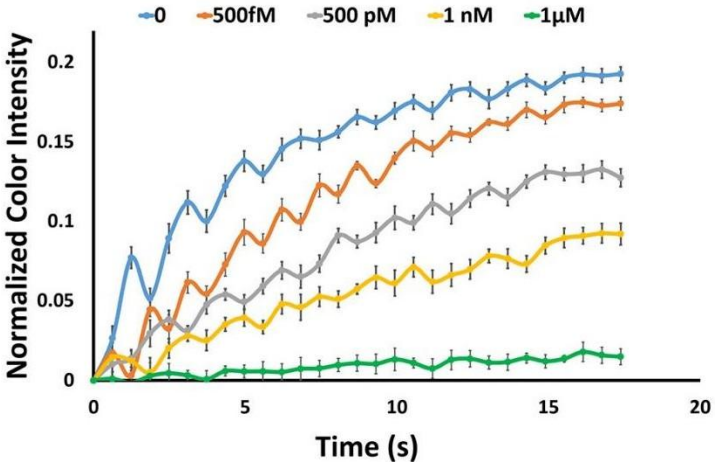
Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

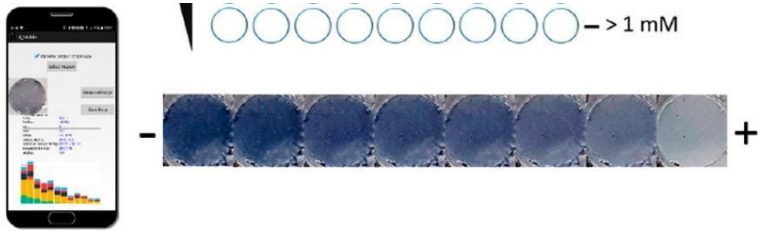
UNIVERSITÀ DEGLI STUDI DI TERAMO

VISUAL APPROACH



SMARTPHONE APPROACH

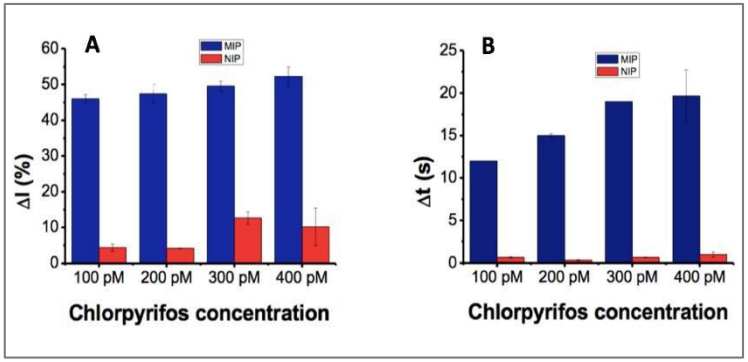




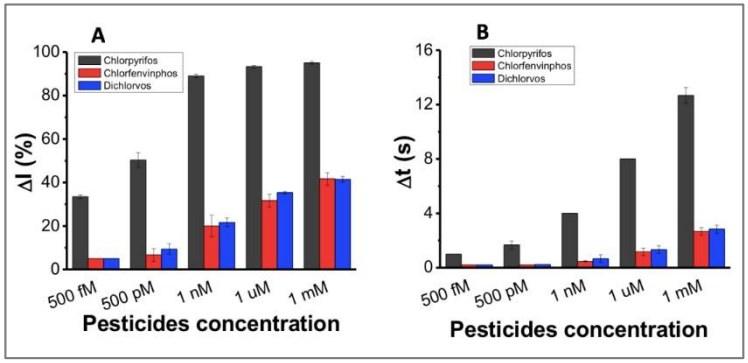
Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

UNIVERSITÀ DEGLI STUDI DI TERAMO

MIP vs NIP



SELECTIVITY (500 mV-1000 mV)

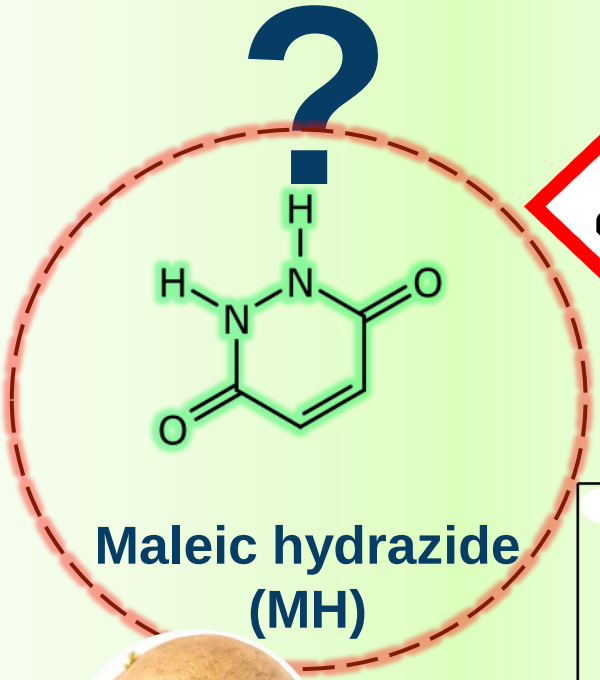


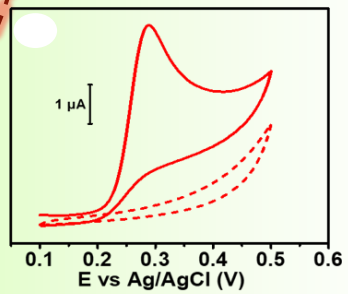
Recovery values of chlorpyrifos in spiked drinking water samples (n = 3) using the current response

Added (Spiked)	Found	Recovery (%)	RSD (%)
500 fM	517.19 fM	**103.44 ± 16.14**	15.60
500 pM	471.45 pM	**94.29 ± 17.92**	19.00
1 nM	0.99 nM	**99.50 ± 19.90**	20.00
1 µM	0.98 µM	**97.55 ± 25.87**	26.52
1 mM	1.07 mM	**106.57 ± 15.30**	14.36

02 MIP-Pesticides : Maleic hydrazide

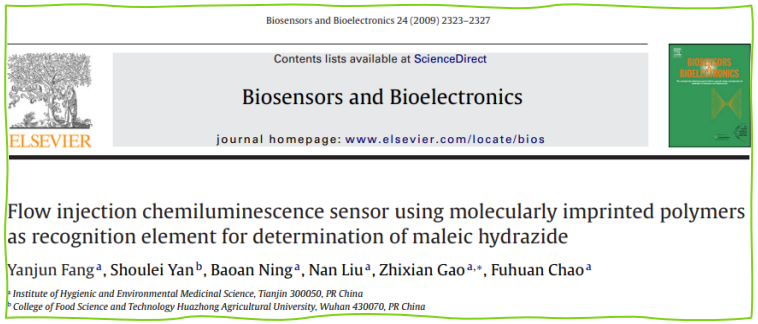
Why herbicide (Maleic hydrazide)?





Maximum residue limits (MRLs)

Sparking idea



Biosensors and Bioelectronics 24 (2009) 2323–2327

Contents lists available at ScienceDirect

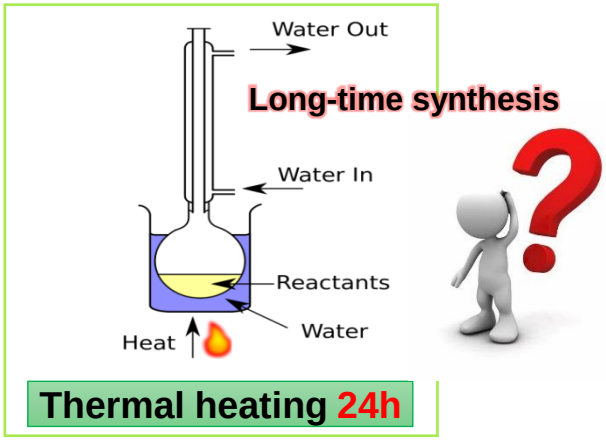
Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Flow injection chemiluminescence sensor using molecularly imprinted polymers as recognition element for determination of maleic hydrazide

Yanjun Fang[a], Shoulei Yan[b], Baoan Ning[a], Nan Liu[a], Zhixian Gao[a,*], Fuhuan Chao[a]

[a] Institute of Hygienic and Environmental Medicinal Science, Tianjin 300050, PR China
[b] College of Food Science and Technology Huazhong Agricultural University, Wuhan 430070, PR China

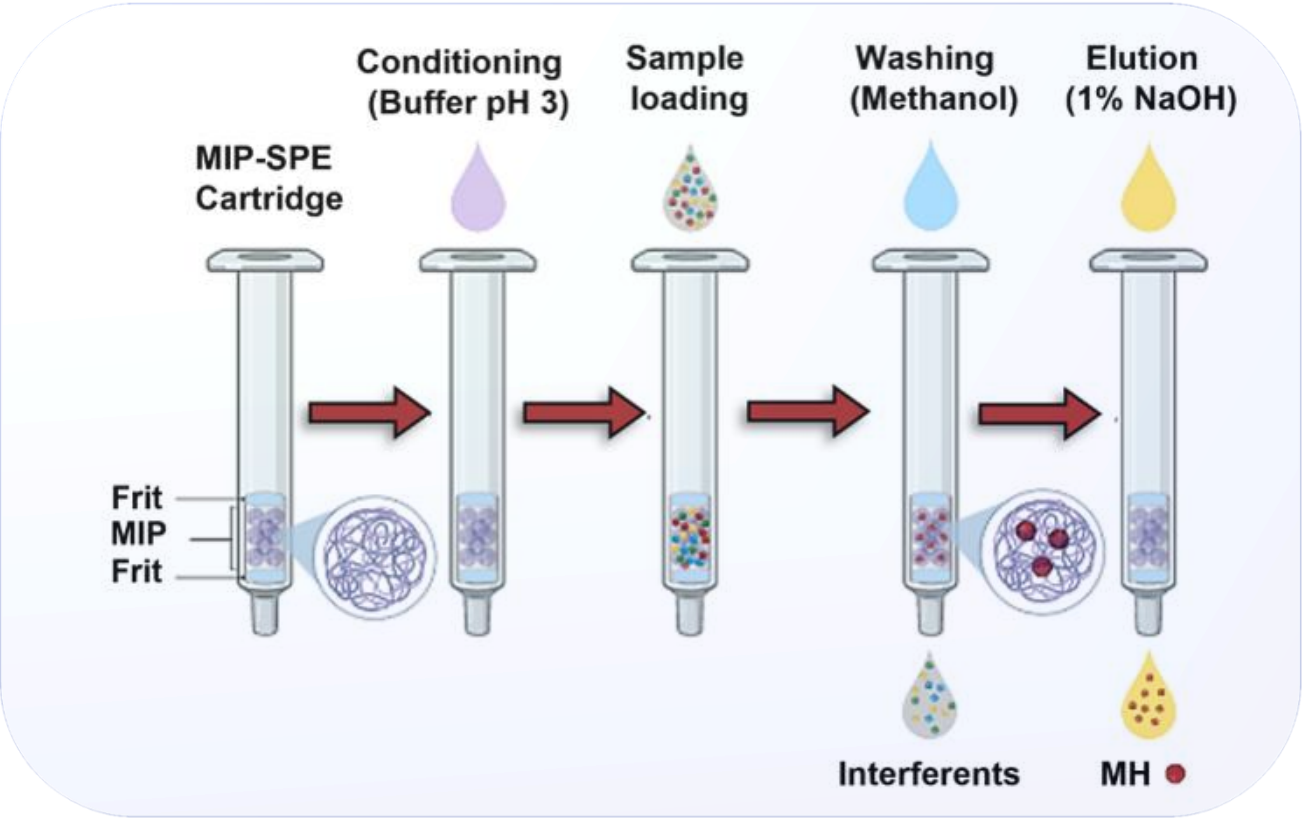


Objective

Development of a fast synthesis strategy based on a high-power ultrasound probe to synthesize effective MIPs for maleic hydrazide

02 MIP-MH based solid phase extraction

Solid phase extraction



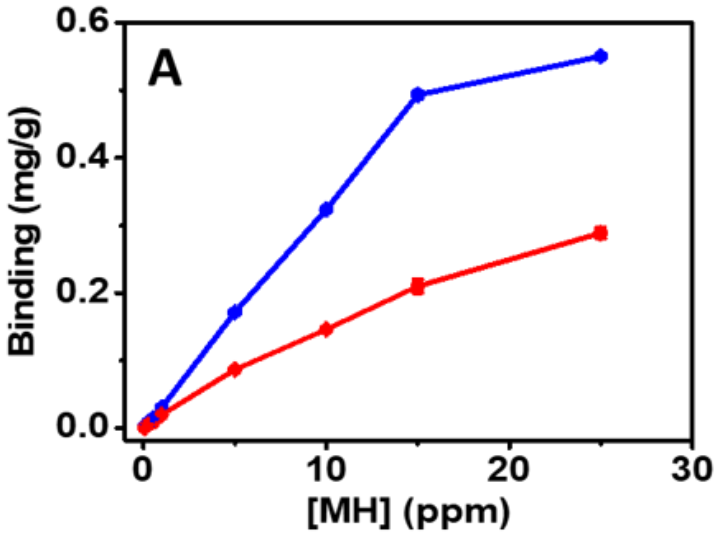
Parameters
A) Adsorption Solvent (1mL)
Water
Phosphate buffer pH 3
B) Amount of polymer (mg)
5
10
15
C) Desorption Solvent
NaOH 1 %
PBS (pH 7)
MeOH
H_20

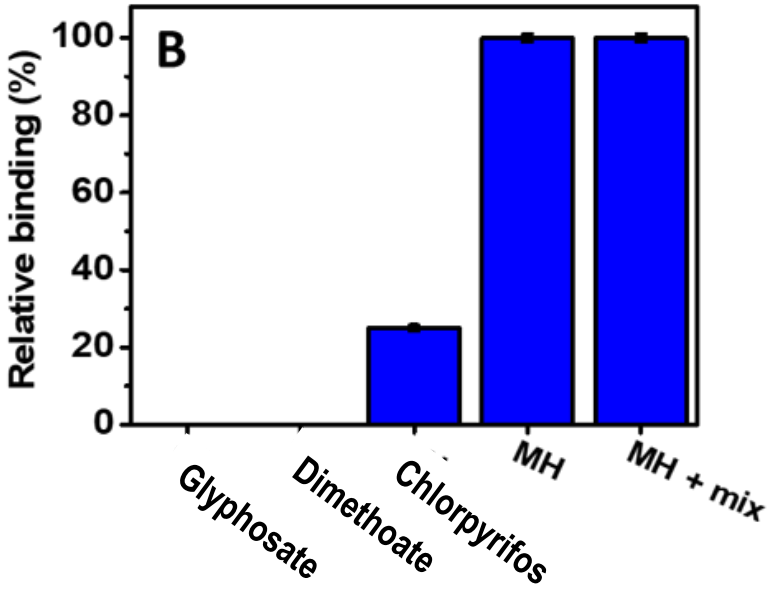
Scheme . Graphical scheme of the solid-phase extraction procedure based on the cartridge containing the MIP.

02 MIP-MH based solid phase extraction

Binding capacity

Selectivity





$$Q = \frac{(Ci - Cf)}{m} * V$$

$$IF = \frac{Q(MIP)}{Q(NIP)}$$

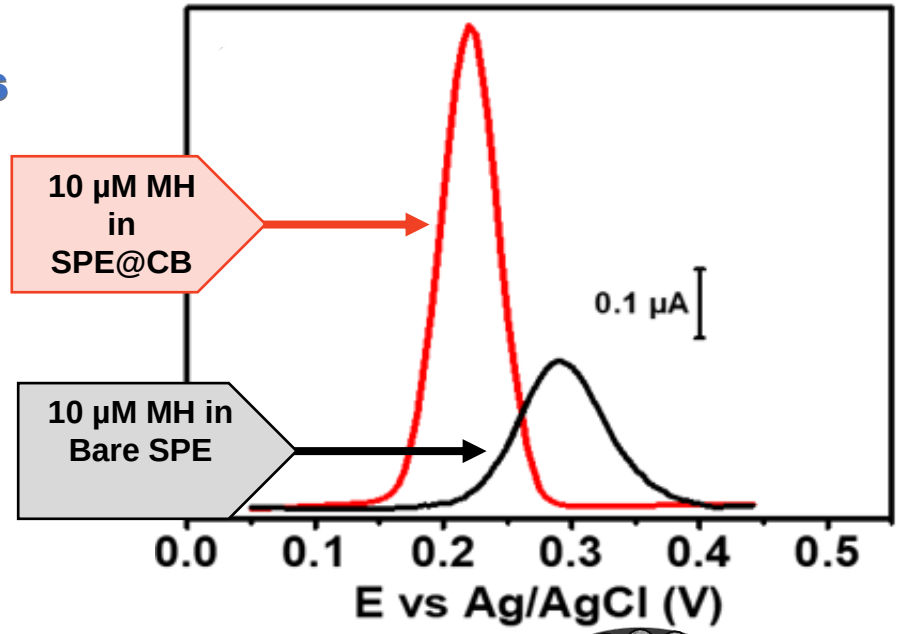
IF=2.30 ± 0.02 (n=3)

-Imprinting factor (IF)

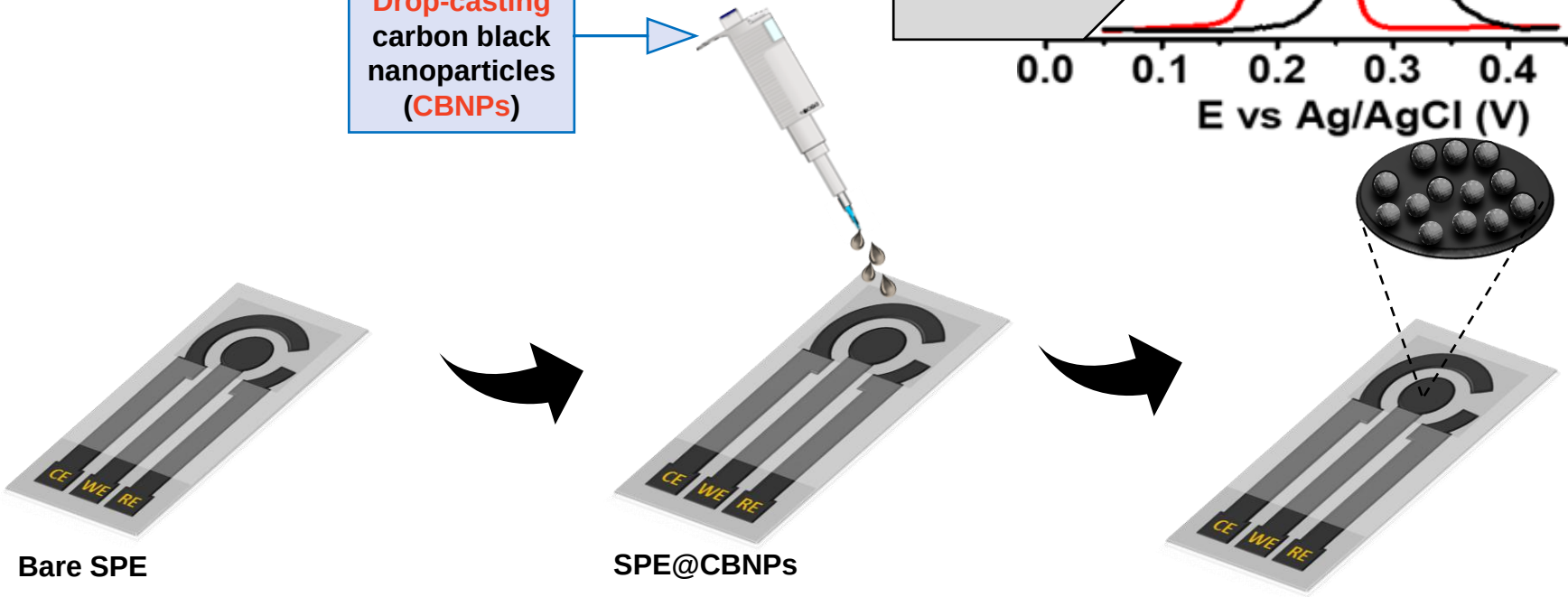
-Non-Imprinted Polymer (NIP)

03 MIP-MH combined EC sensor

Electrochemical measurements



3X2 µL Drop-casting carbon black nanoparticles (CBNPs)



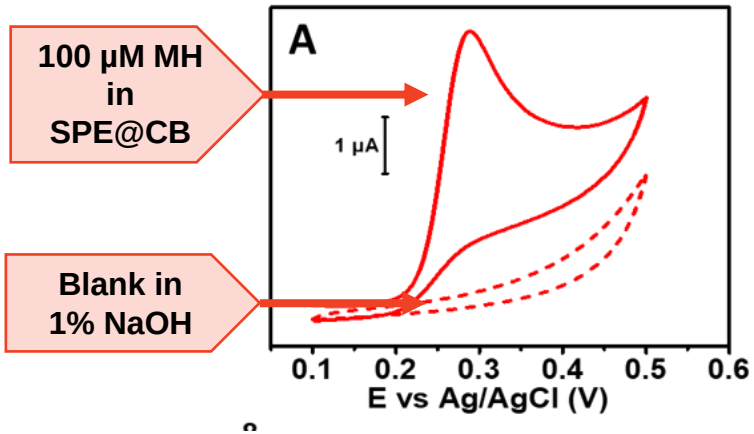
Bare SPE

SPE@CBNPs

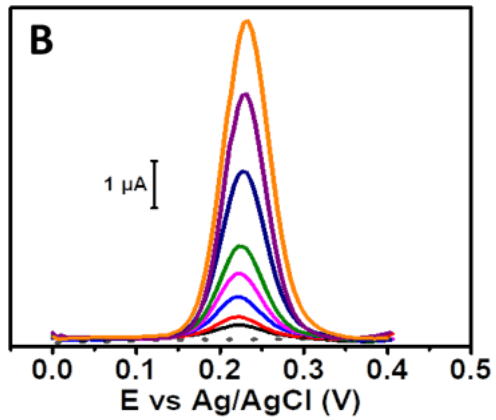
03 MIP-MH combined EC sensor

Electrochemical measurements

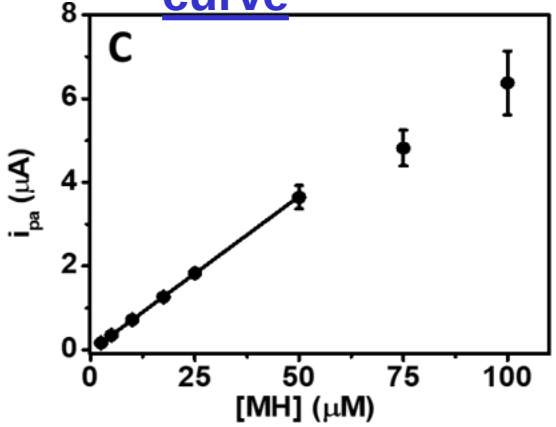
Cyclic voltammetry



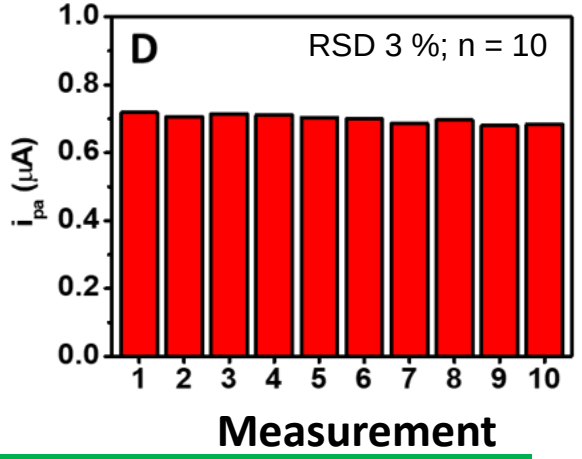
DPV, 2.5-100 µM [MH]



Dose-response curve



Repeatability



LOD=0.4 µM (40 ppb)

LR= 2.5 to 50 µM

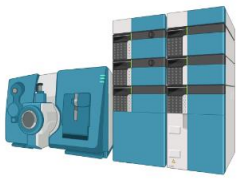
R^2 = 0.9993

02 MIP-Maleic hydrazide

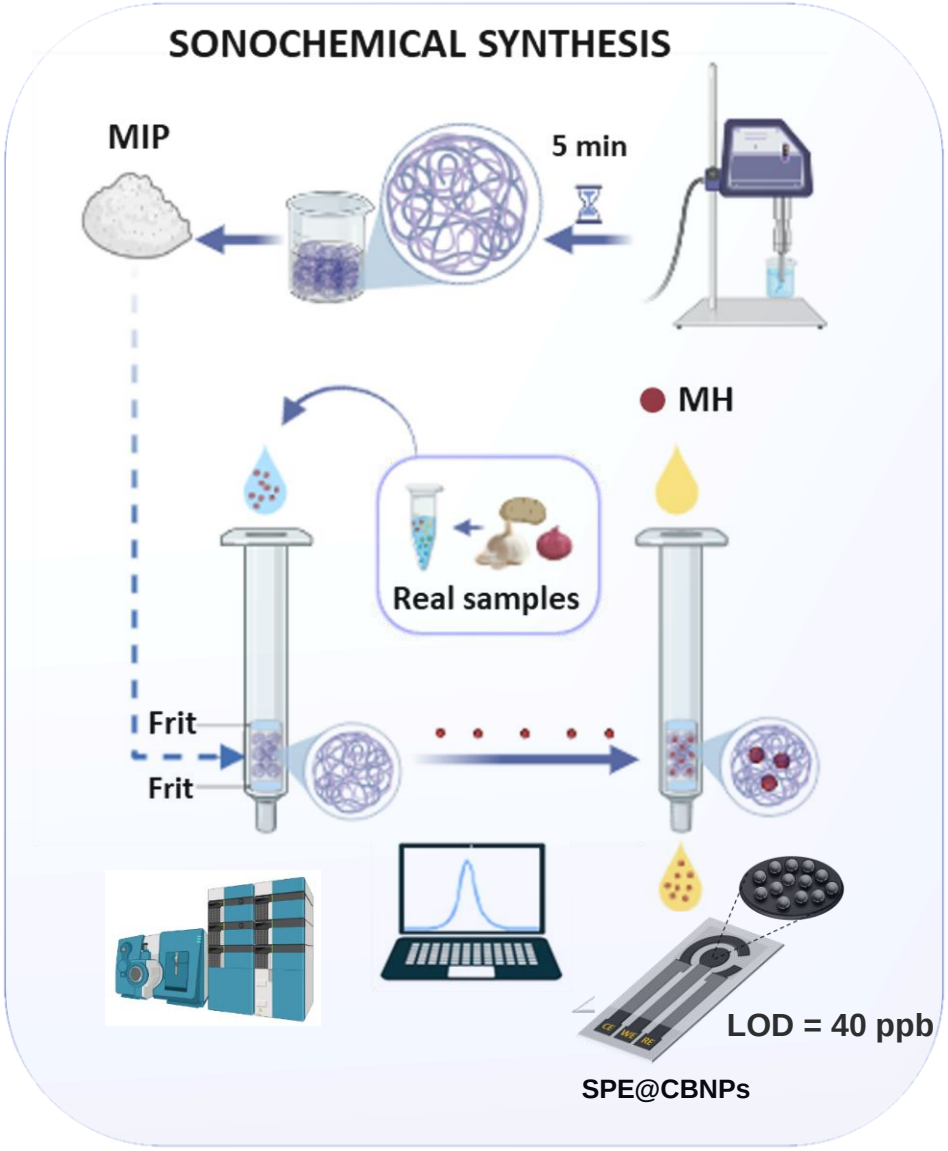
MIP-SPE and sample analysis

Table . Results of MH MIPs-SPE combined with CB-based electrochemical determination applied in food samples

MH added (ppm)	EC found (ppm)	Recovery (%)	LC-MS/MS found (ppm)	Relative Error (%)
		Onion		
5	4.6±0.2	92	4.8±0.02	-4.2
10	8.9±0.5	88.5	8.5±0.9	4.3
15	14.2±0.7	94.5	14.2±0.8	0.2
		Garlic		
5	4.1±0.1	82.2	4.5±0.4	-8.0
10	10.5±0.4	105.1	9.6±0.3	9.4
15	15.3±0.6	102.1	14.6±0.2	5.0
		Potato		
5	5.3±0.3	106	5.5±0.3	-3.6
10	9.7±0.3	97	10.0±0.3	-3.6
15	13±0.2	80.0	14.2±0.6	-8.5

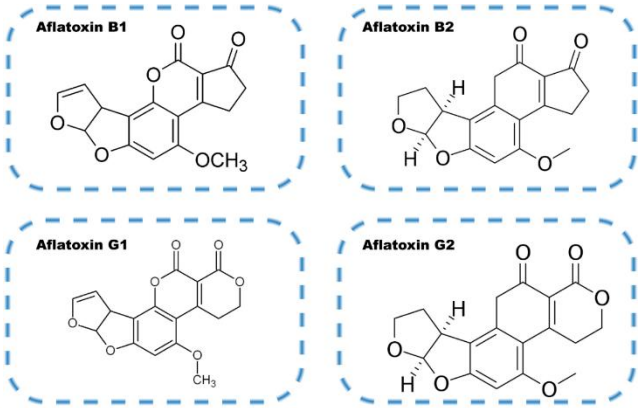


In Europe, the maximum residue limits (MRLs) for MH in **potatoes**, **garlic**, and **onions** are **60**, **40**, and **15 ppm**, respectively.

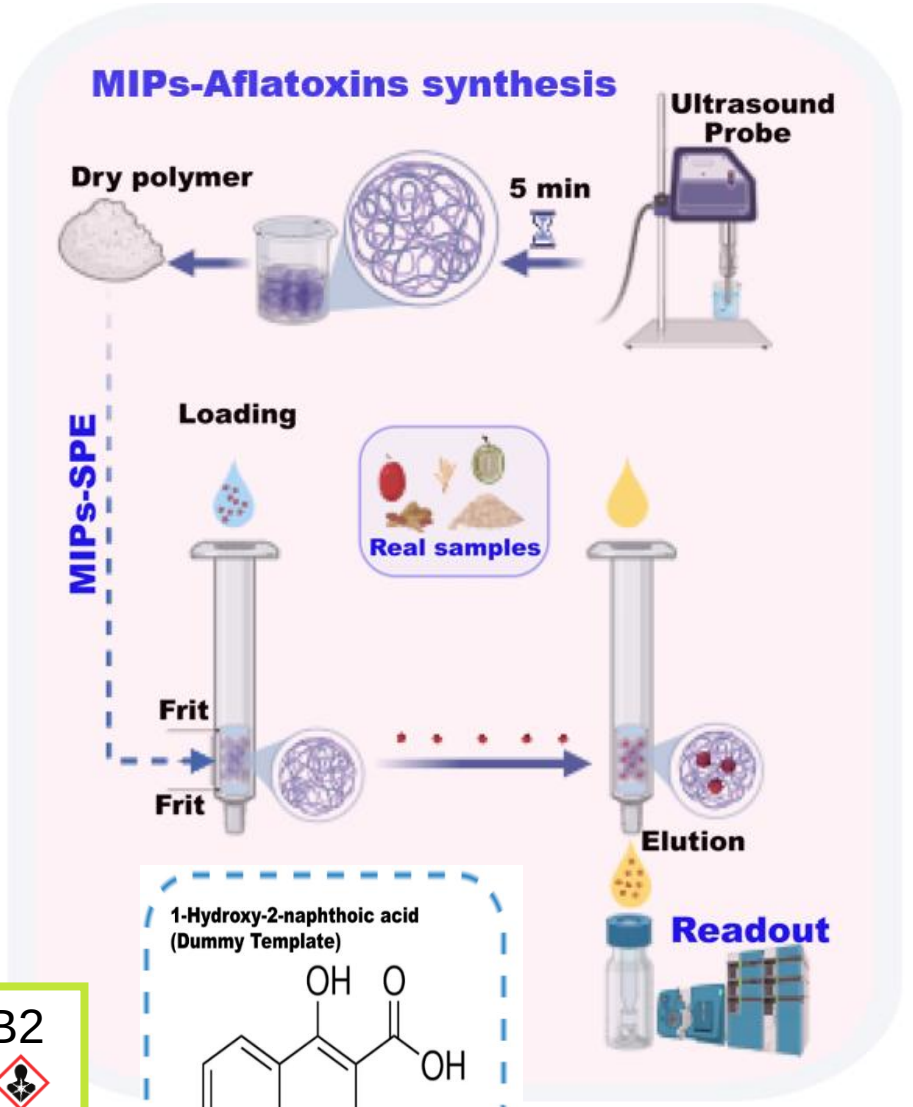


03 MIP-Mycotoxins : Aflatoxins

Commercial immunoaffinity column for Aflatoxins



Vs



Aflatoxins

MRL of AFG1, AFG2, AFB1, and AFB2 for processed food at **2 µg/kg**

03 MIP-Mycotoxins : Aflatoxins

Adsorption performance

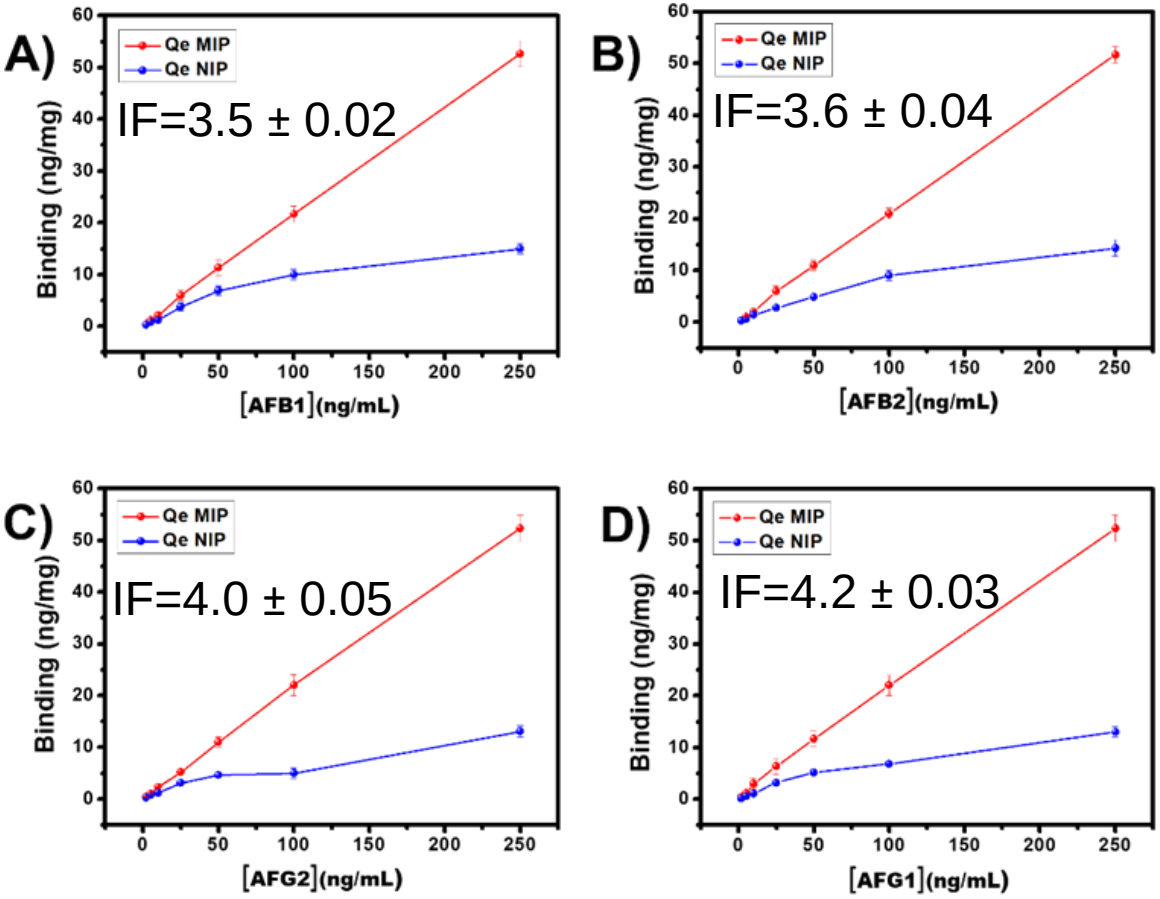


Figure..Adsorption capacity (%) of the MIPs (red) and NIPs (blue) obtained analyzing different aflatoxin amounts (from 2 to 250 ng/mL). Graphs (A–D) correspond to aflatoxins B1, B2, G1, and G2, respectively.

Selectivity

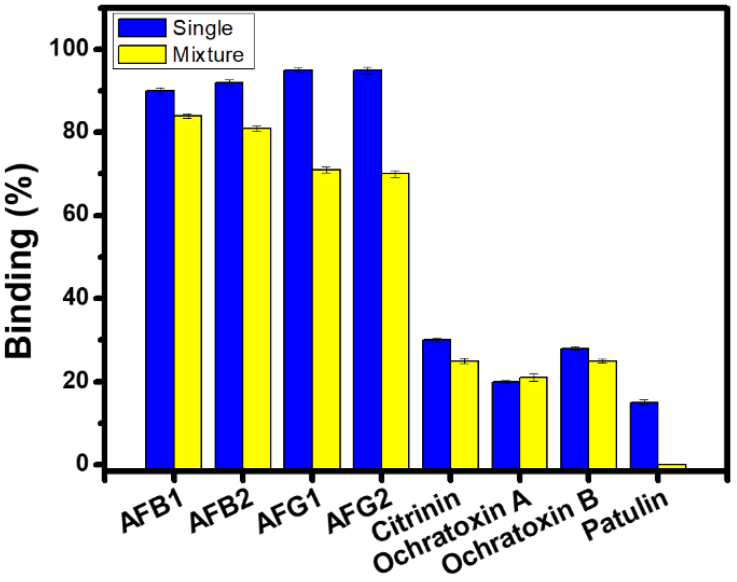


Figure. Selective adsorption of MIPs at a concentration of 5 ng/mL.

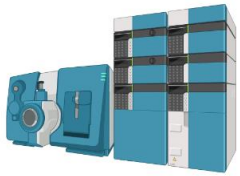
03 MIP-Mycotoxins : Aflatoxins

Table. Data obtained analyzing food samples using the MIPs-based and IAC-based procedures.

Sample	AFG1				AFG2				AFB1				AFB2			
	MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)		MIPs–SPE (%)		IAC (%)	
	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME	RC	ME
Ginger	60	+9	50	−30	63	−6	48	+28	64	−1	50	+25	81	−14	52	+30
Echinacea purpurea	83	+9	73	+15	66	+10	75	+15	90	−1	80	+10	60	−8	72	+12
Ginseng	75	+16	66	−26	60	+1	46	−23	90	+7	67	−28	78	+15	61	−29
Hypericum	50	+15	50	27	60	+15	38	+30	53	+11	52	+25	72	+1	50	+30
Red elm	77	+10	64	+5	70	+7	63	+15	90	−4	80	+10	74	−6	80	+8
Saffron	61	+16	60	+12	69	+11	62	+20	60	+15	60	+17	68	+9	64	+15
Mango	67	+10	65	+15	65	+7	60	+15	65	+10	70	+15	65	+10	68	+12
Red rice	76	+11	50	+25	65	+15	55	+20	89	+3	65	+20	70	+9	66	+20
Parsley	60	+9	50	+30	60	+10	40	+30	76	+1	45	+28	60	+4	43	+19
Red fruits	60	+7	55	+20	60	+10	56	+21	79	+6	60	+18	60	+10	58	+22
Grapefruit	61	+11	65	+15	69	+11	70	+12	68	+12	70	+15	71	+4	68	+16
Magnolia	61	−7	50	+30	64	+12	50	+20	64	+15	50	+18	77	+8	54	+20
Tilia cordata	60	+1	50	+20	62	+12	45	+18	62%	+11	50	+19	72	+2	50	+20
Salsapariglia root	62	+3	55	+20	67	+6	52	+18	72	+15	50	+20	69	+14	70	+17
Hop	60	+2	50	+20	72	+12	51	+20	74	+15	60	+17	57	+12	67	+18
Verbena officinalis	72	+5	56	+18	60	+15	55	+21	69	−8	67	+18	65	+13	66	+16
Galega officinalis	78	+4	64	+15	77	+17	65	+20	73	+14	65	+20	75	+3	74	+20

Appreciable recoveries (65–90%; RSD < 6%, n = 3)

low matrix effect (ME < 15%)



RC: Recovery
ME: Matrix effect
IAC: Commercial immunoaffinity column