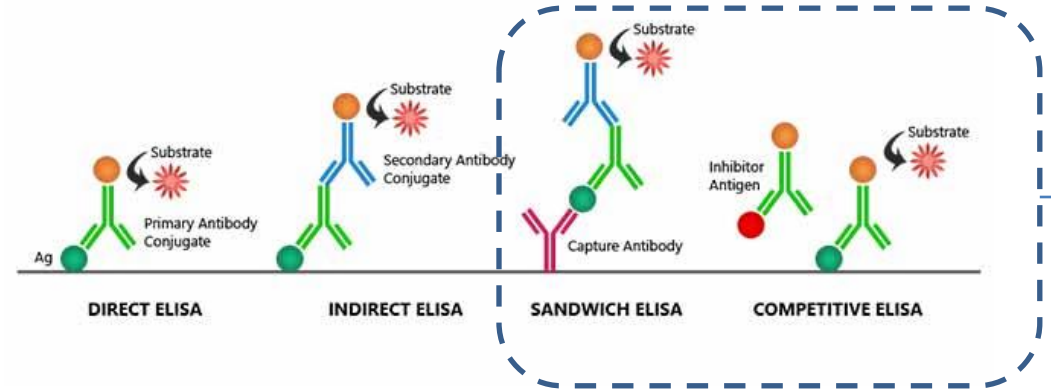
CONCENTRATION MOLES/LITER	METABOLITES/IONS	THERAPEUTIC DRUGS	STEROID AND AMINO ACID HORMONES	PROTEIN/POPLYPEPTIDE HORMONES	ANTIBODIES
10^{-1}	SODIUM				
	CHLORIDE				
10^{-2}		ETHANOL			
	GLUCOSE				
(mM)	UREA				
10^{-3}	CHOLESTEROL				
	CALCIUM	SALICYLATE			IgG (Total)
	TRIGLYCERIDES	ACETAMINOPHEN			
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

Introduction to Antibodies - Enzyme-Linked Immunosorbent Assay (ELISA)

An assay for quantitating either antibody or antigen by use of an enzyme linked antibody and a substrate that forms a colored reaction product.

Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily assayed enzyme that possesses a high turnover number. ELISAs can provide a useful measurement of antigen or antibody concentration.

Sandwich ELISA Assays

To utilize this assay, one antibody (the "capture" antibody) is purified and bound to a solid phase typically attached to the bottom of a plate well. Antigen is then added and allowed to complex with the bound antibody. Unbound products are then removed with a wash, and a labeled second antibody (the "detection" antibody) is allowed to bind to the antigen, thus completing the "sandwich". The assay is then quantitated by measuring the amount of labeled second antibody bound to the matrix, through the use of a colorimetric substrate. Major advantages of this technique are that the antigen does not need to be purified prior to use, and that these assays are very specific. However, one disadvantage is that not all antibodies can be used. Monoclonal antibody combinations must be qualified as "matched pairs", meaning that they can recognize separate epitopes on the antigen so they do not hinder each other's binding

Competitive ELISA Assays

Briefly, an unlabeled purified primary antibody is coated onto the wells of a 96 well microtiter plate. This primary antibody is then incubated with unlabeled standards and unknowns. After this reaction is allowed to go to equilibrium, conjugated immunogen is added. This conjugate will bind to the primary antibody wherever its binding sites are not already occupied by unlabeled immunogen. Thus, the more immunogen in the sample or standard, the lower the amount of conjugated immunogen bound. The plate is then developed with substrate and color change is measured.

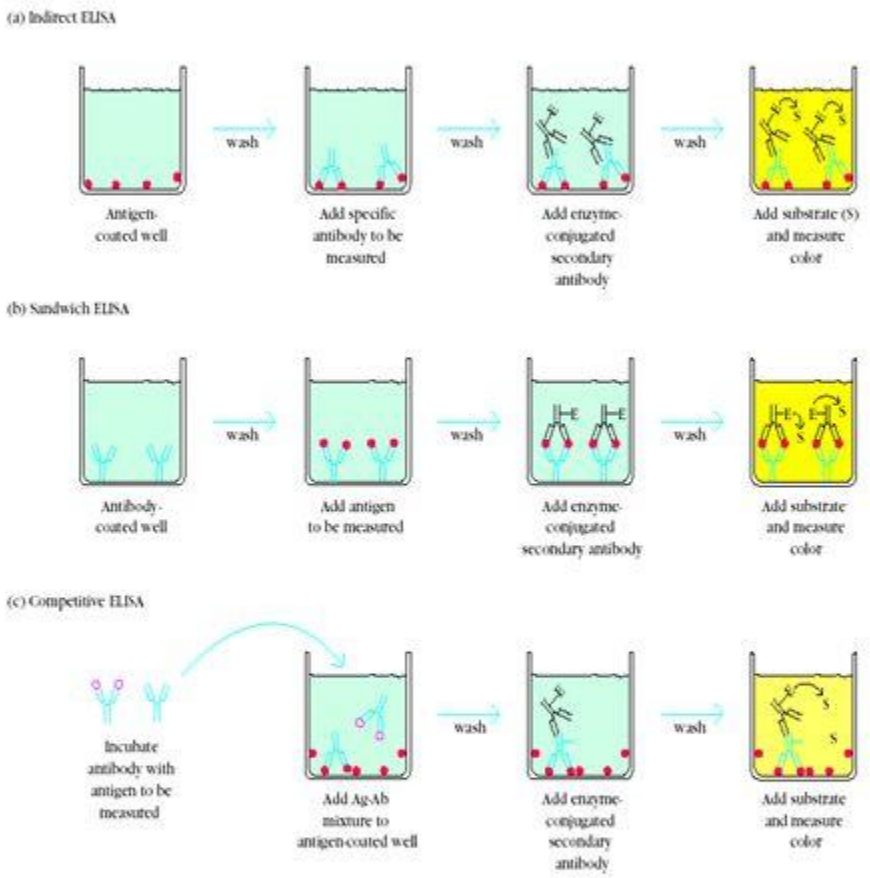


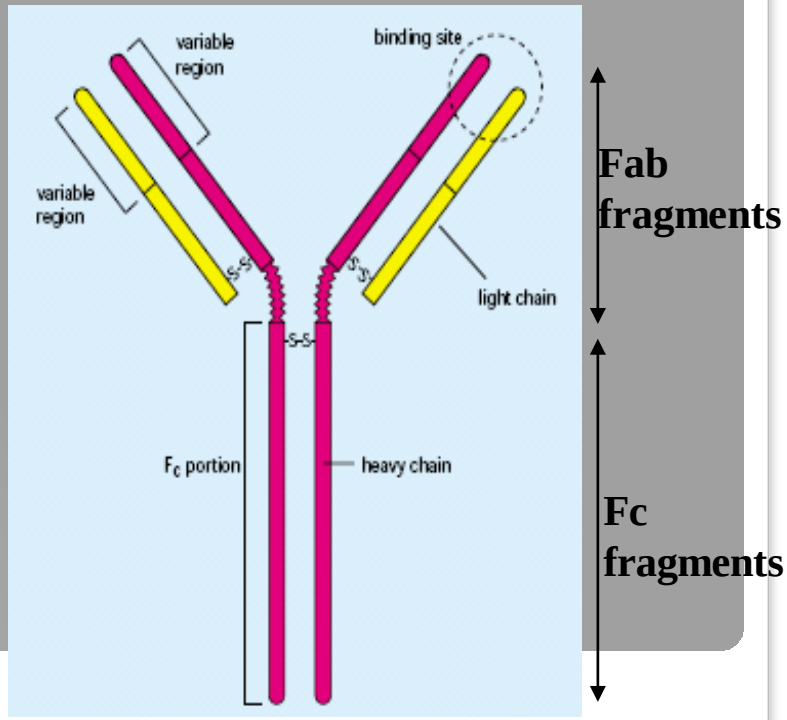
FIGURE 6-10 Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively, or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined with an indirect ELISA (a), whereas antigen can be determined with a sandwich ELISA (b) or competitive ELISA (c). In the competitive ELISA, which is an inhibition-type assay, the concentration of antigen is inversely proportional to the color produced.

Antibodies

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

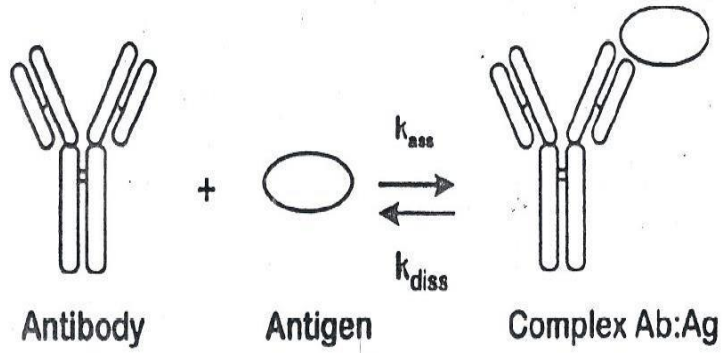
- Recognise and bind to molecules (**antigens**) on foreign particles, marking them for destruction by T-lymphocytes.

- 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 $K_{aff} = \frac{k_{ass}}{k_{diss}} = \frac{[Ab:Ag]}{[Ab][Ag]}$

Immunoassays

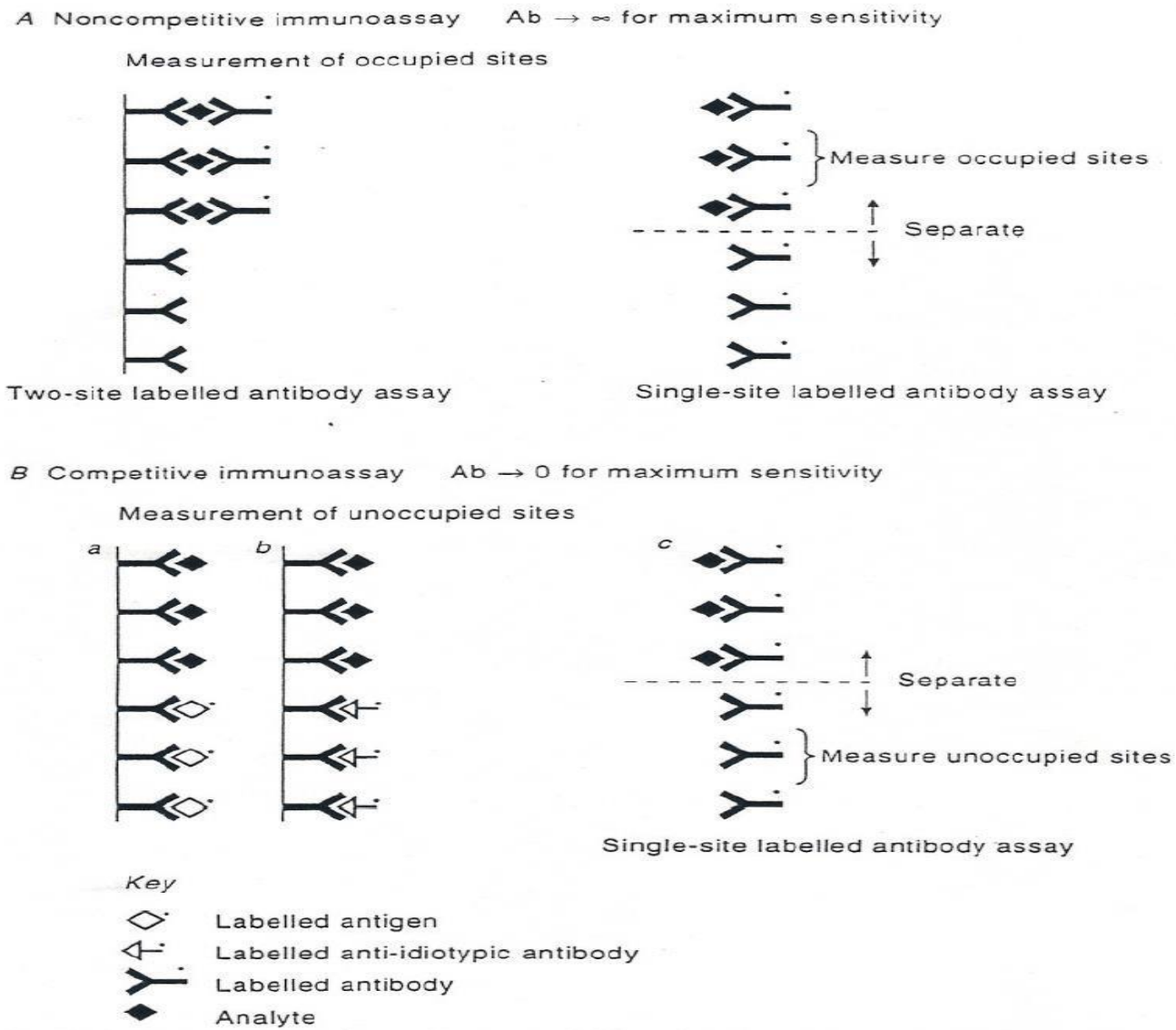


Figure 6 Basic competitive and noncompetitive immunoassay designs. The distinction between noncompetitive (*a*) and competitive immunoassays (*b*) reflects the way in which antibody binding site occupancy is observed. Labelled antibody methods are noncompetitive if occupied sites of the (labelled) antibody 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.

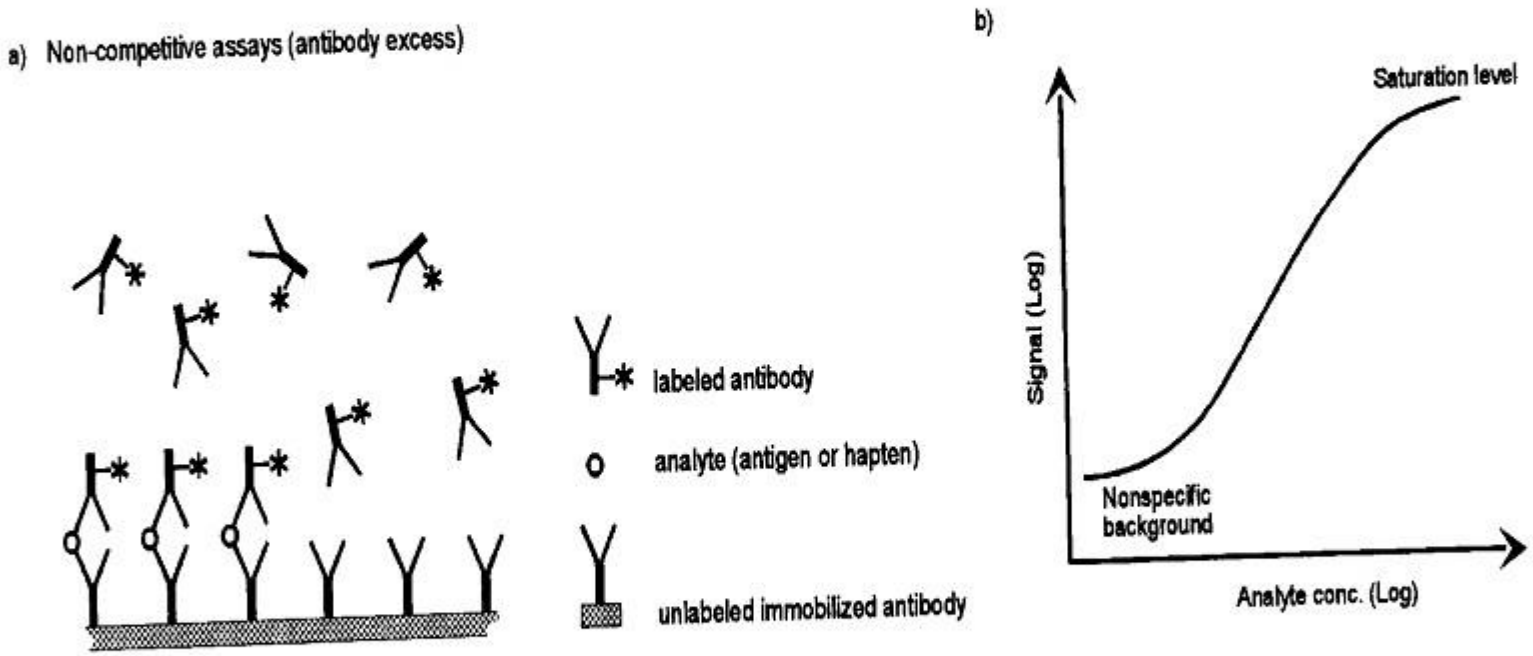


FIGURE 17.3 Schematic drawing of a sandwich immunoassay with typical calibration curve.

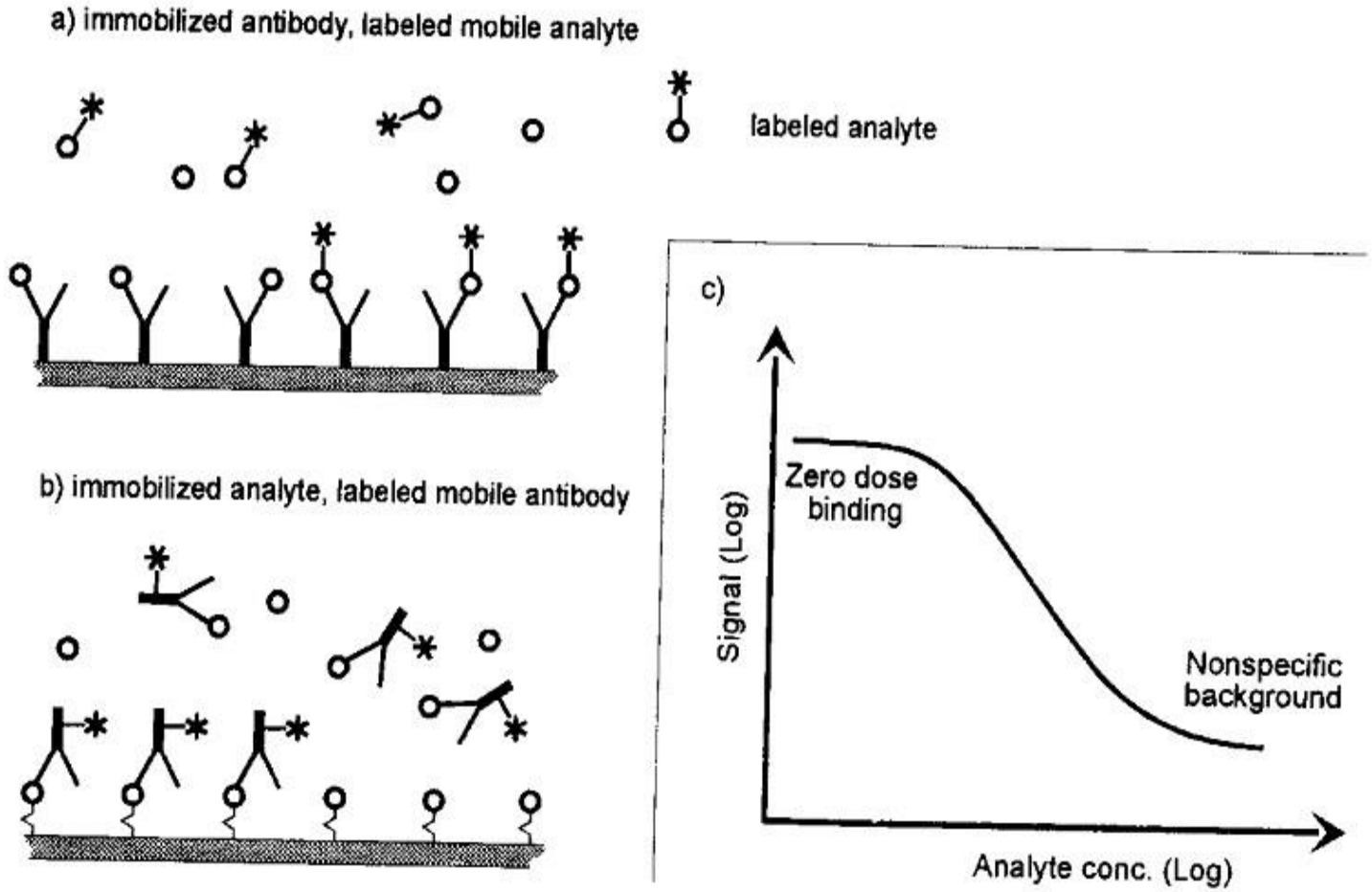


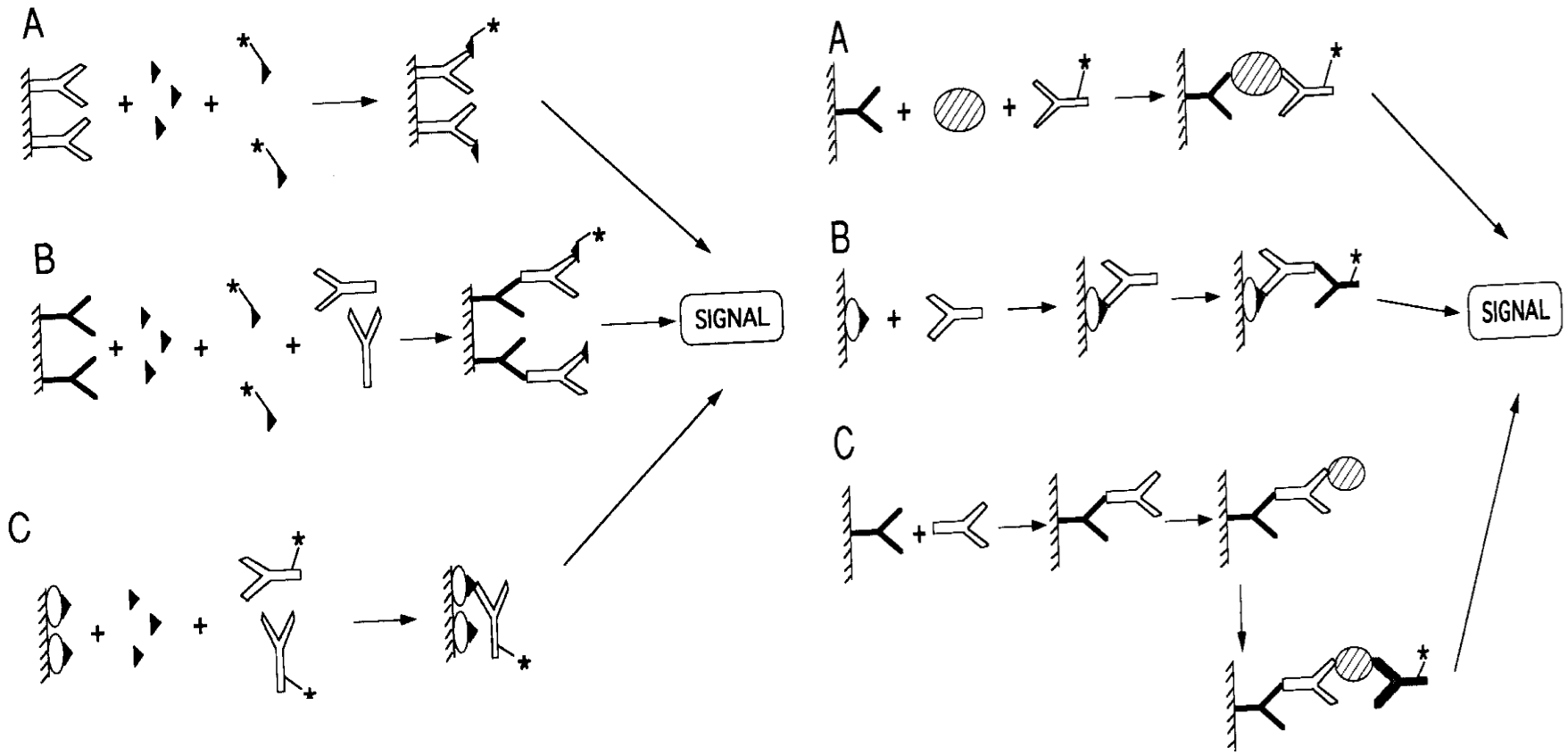
FIGURE 17.4 Competitive immunoassay: (a) competitive binding of analyte and labeled analyte to a limited number of antibody binding sites, (b) immobilized antigen competes with free antigens in solution for binding sites of the labeled antibody, (c) calibration curve obtained with either approach of the competitive assay type.

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	Fluorescence	Fluorimeter
	rhodamines, dansyl chloride, cumarines, phycoerythrin, also liposomes		
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.

possible immunoassays schemes



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

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

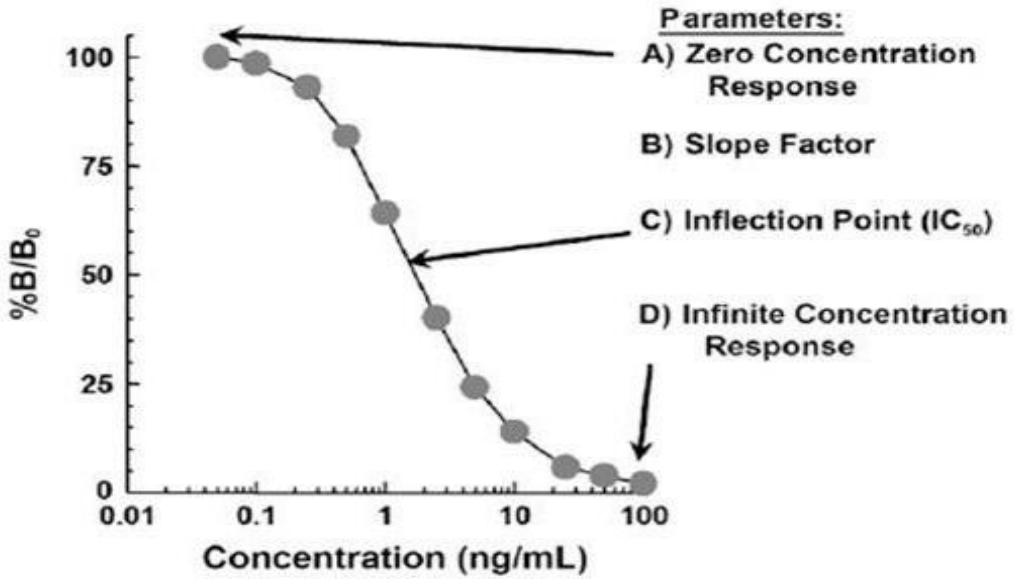
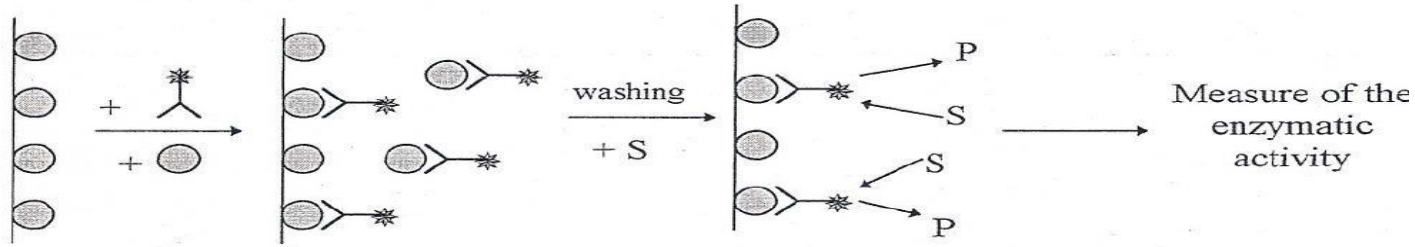


Figure 1. Typical 4-parameter logistic graph for a competitive-format immunoassay.

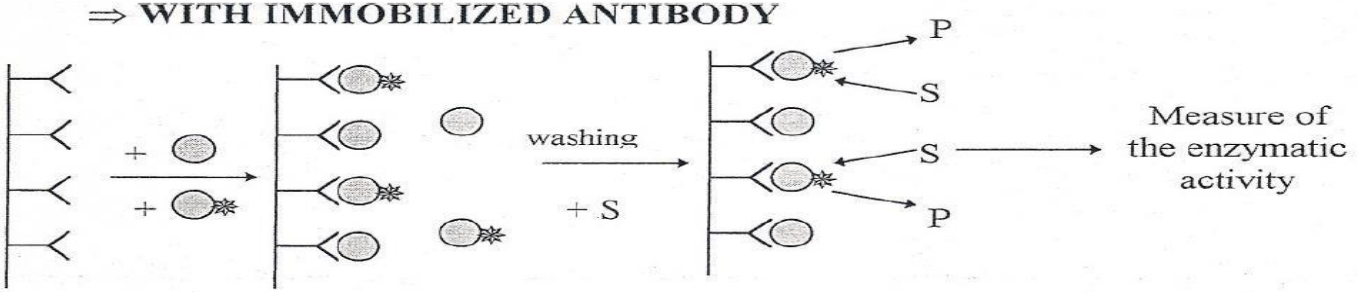
Enzime Linked Immuno-Sorbent Assay (ELISA)

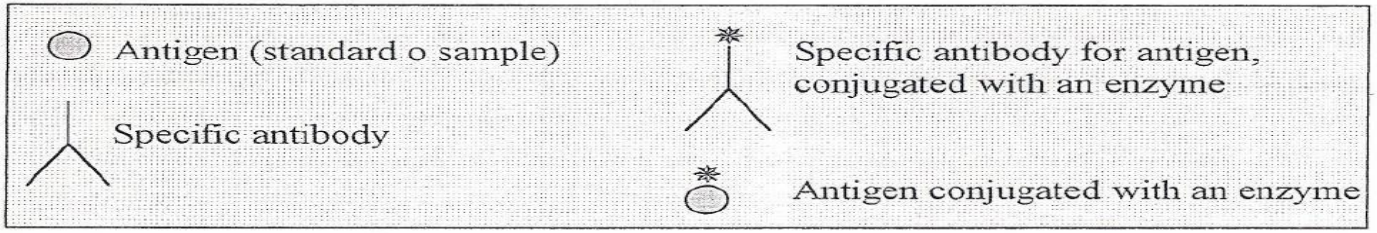
COMPETITIVE TEST

⇒ WITH IMMOBIZED ANTIGEN



⇒ WITH IMMOBILIZED ANTIBODY



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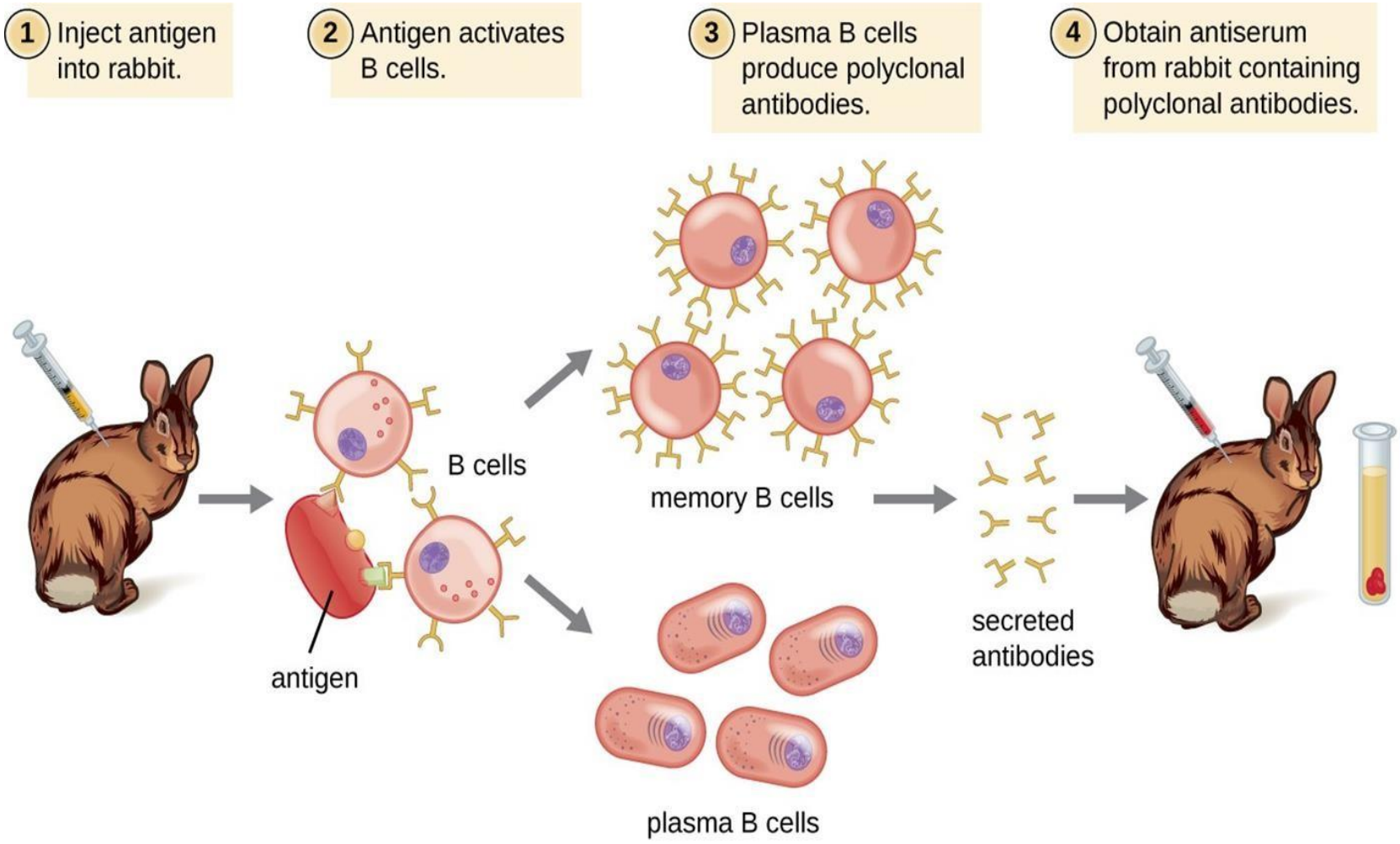
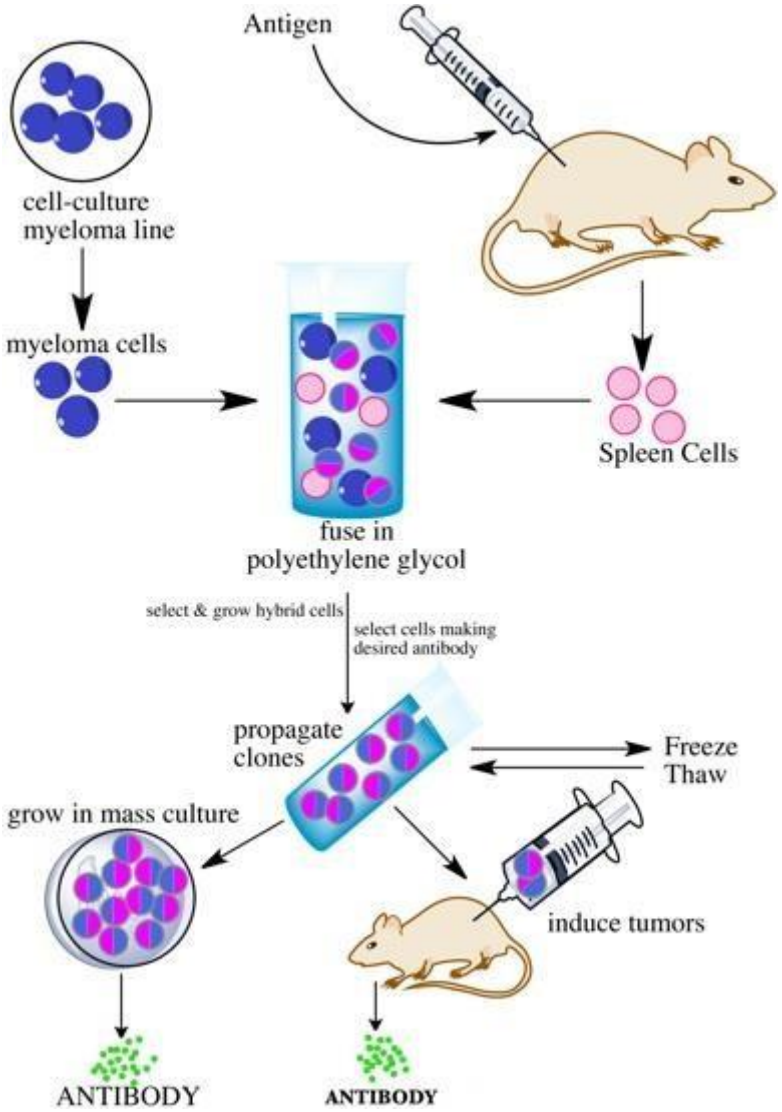
Almond	Beta-Lactoglobulin	Buckwheat	Casein	Crustacean
				
Egg	Gluten	Hazelnut	Lupin	Mustard
				
Peanut	Sesame	Soy		

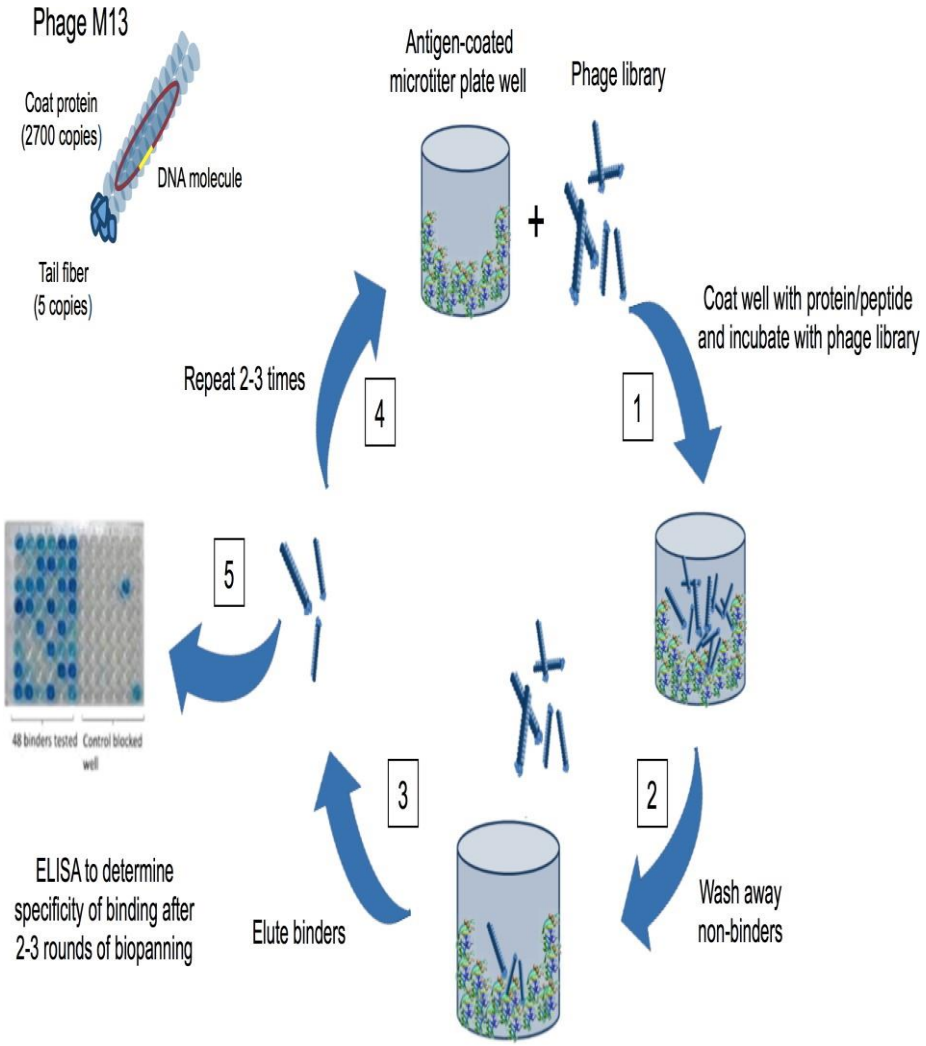
Polyclonal antibodies production



Monoclonal antibodies

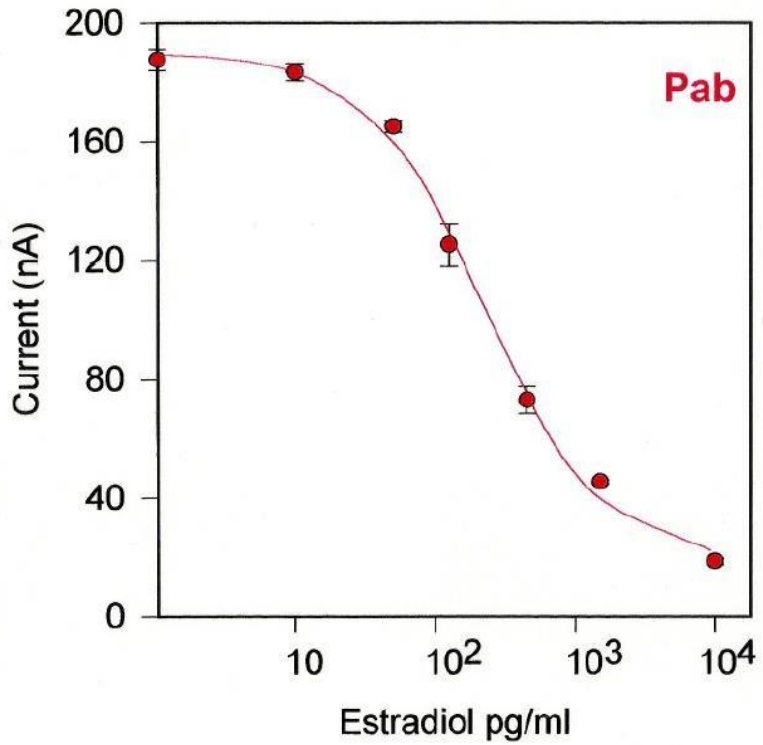


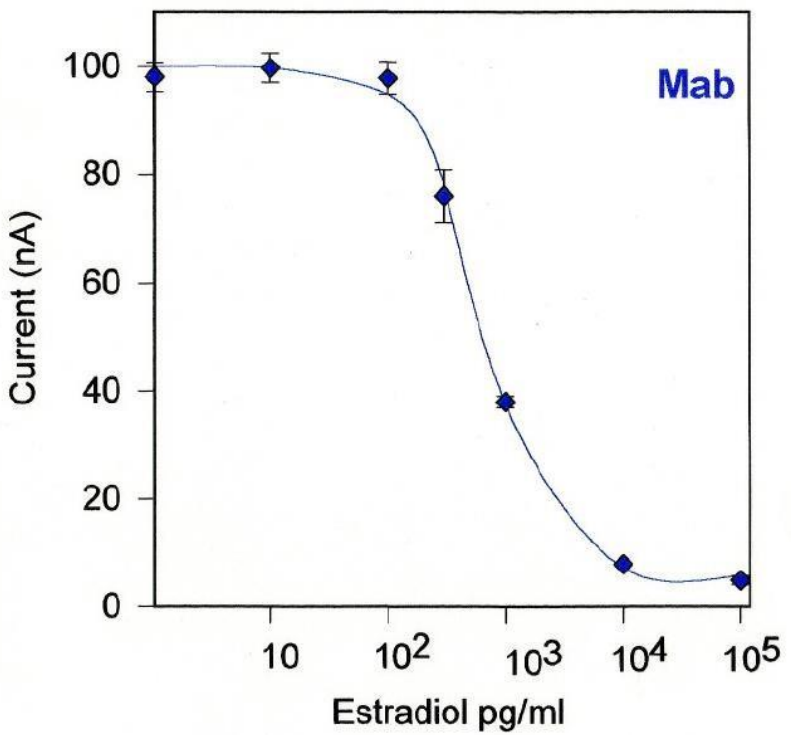
Recombinant antibodies



Enzime Linked Immuno-Sorbent Assay

ELISA elettrochimico





❖ Electrochemical detection:

Chronoamperometry

Differential pulse voltammetry (DPV)

❖ Enzymes and substrates:

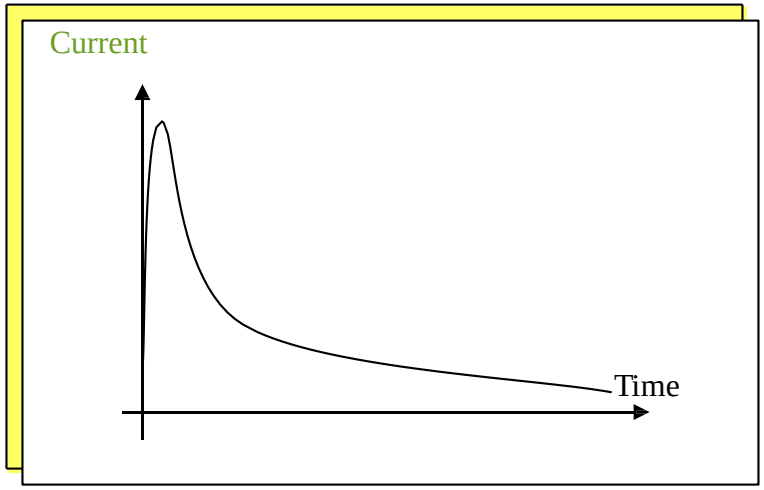
Alkaline phosphatase

1-naphtyl-phosphate

Horseradish peroxidase

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

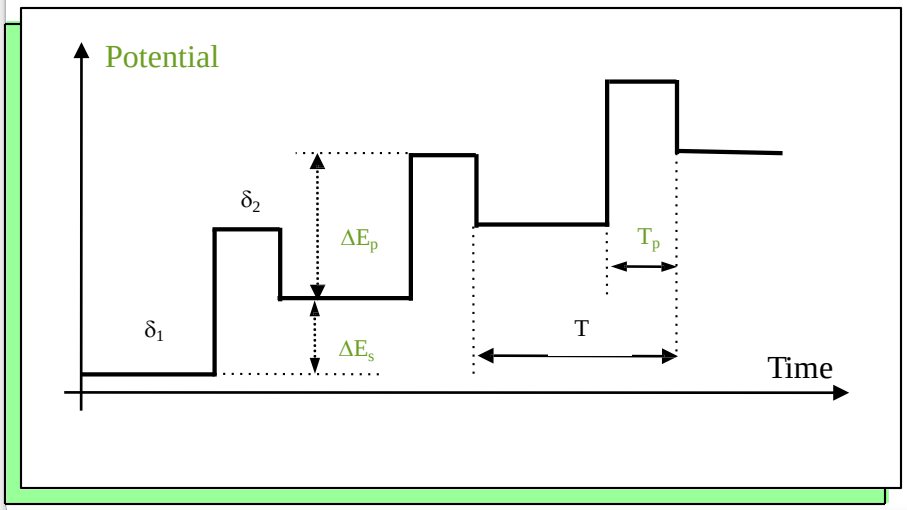
electrochemical detection:
chronoamperometry and differential pulse voltammetry (DPV) :

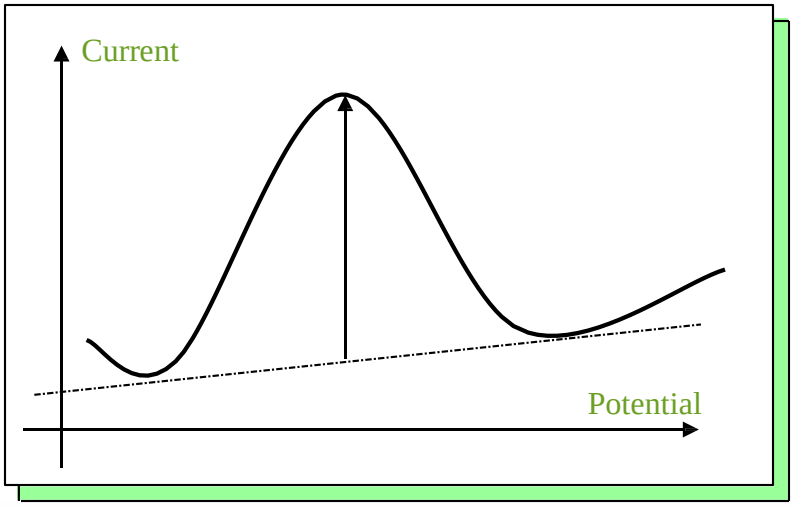


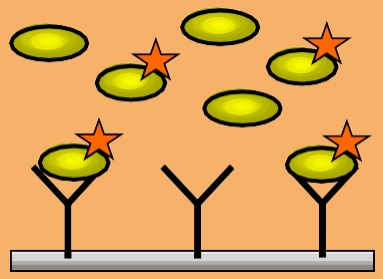
ΔE= const.

δ_2= 60 ms

ΔE_p= 5-100 mV







saggio diretto

Anti OTA-Ab

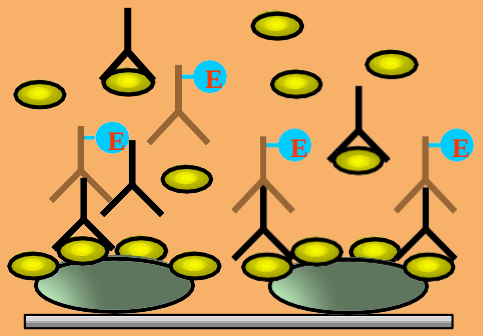
OTA

OTA-ALP

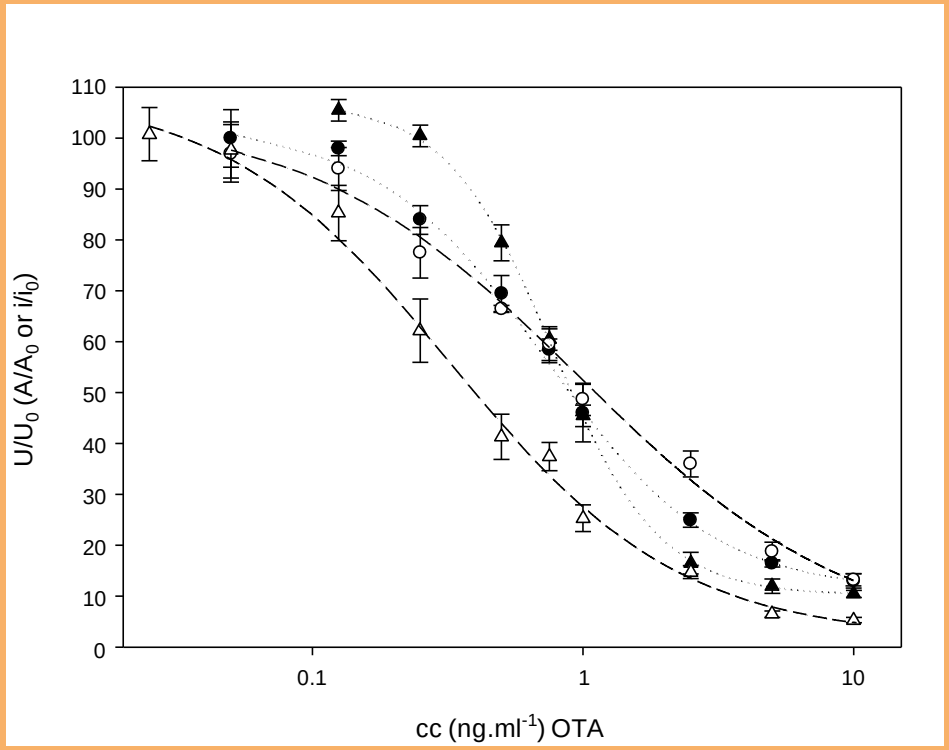
BSA-OTA

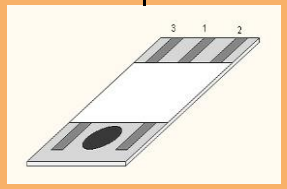
SAb-ALP

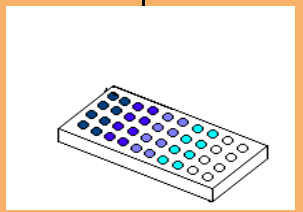
Anti OTA-Ab



saggio indiretto







OTA = ochratoxin A

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

	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)

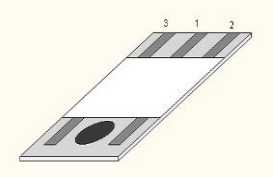
✓Detection: 100 of 5 mg/ml 1-Naphtylphosfate (2 min) + DPV

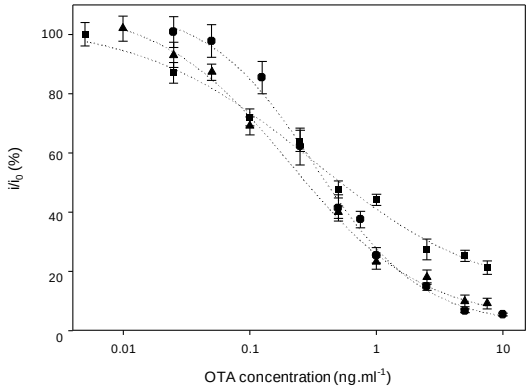
washings: 150 μl phosphate buffer pH 7.4

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

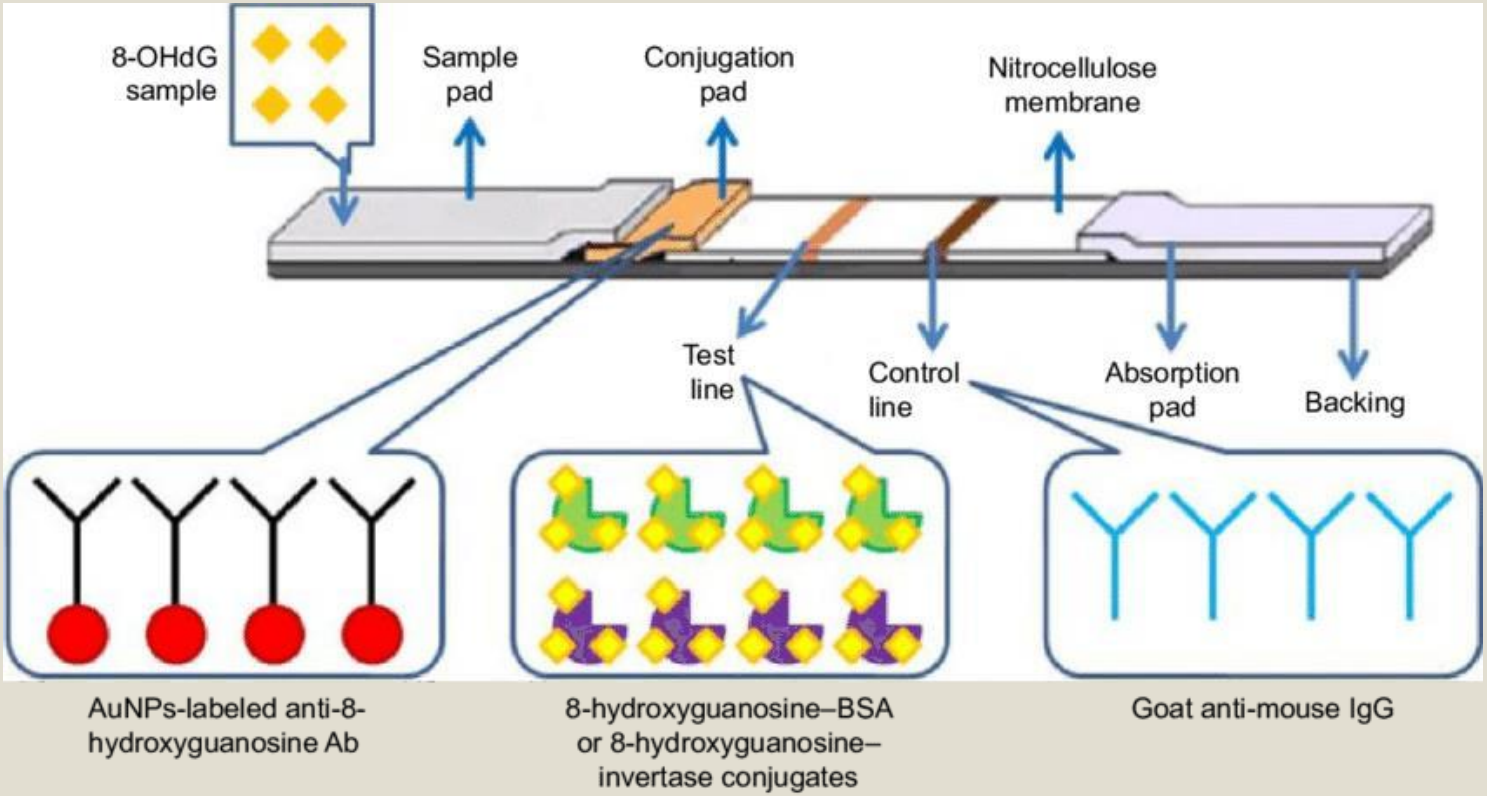
Final dilution 1:8

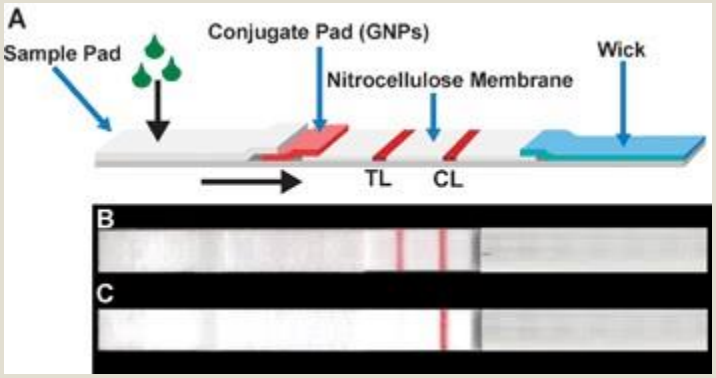
MRL = 3 ng/g

I_{50} = 1.6 ng/g

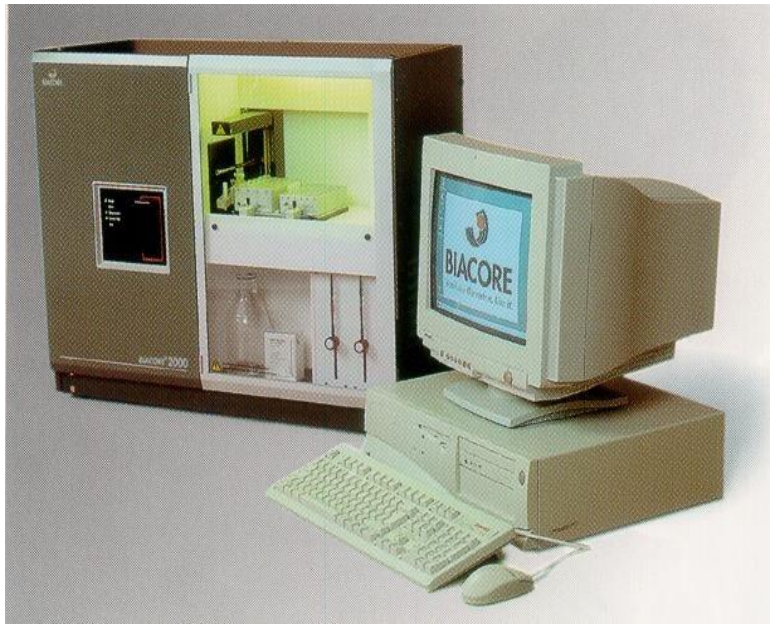
Parameters		**0.1 PBS Buffer ●**	**ACN:H_2O (6:4) ▲**	**Wheat Extract (blank) ■**
a	(nA)	707 (± 56)	260 (± 12)	408 (± 72)
b	(nA.ng.ml^{-1})	1.1 (± 0.1)	0.62 (± 0.03)	0.8 (± 0.1)
***c* (I_{50})**	(ng.ml^{-1})	0.35 (± 0.04)	0.32 (± 0.02)	0.20 (± 0.03)
d	(nA)	16 (± 13)	24 (± 8)	13 (± 15)
w.r.	(ng.ml^{-1})	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*

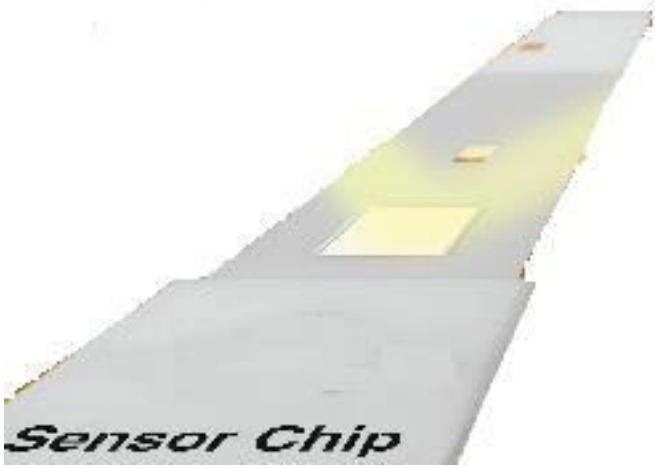
LATERAL FLOW IMMUNOASSAYS



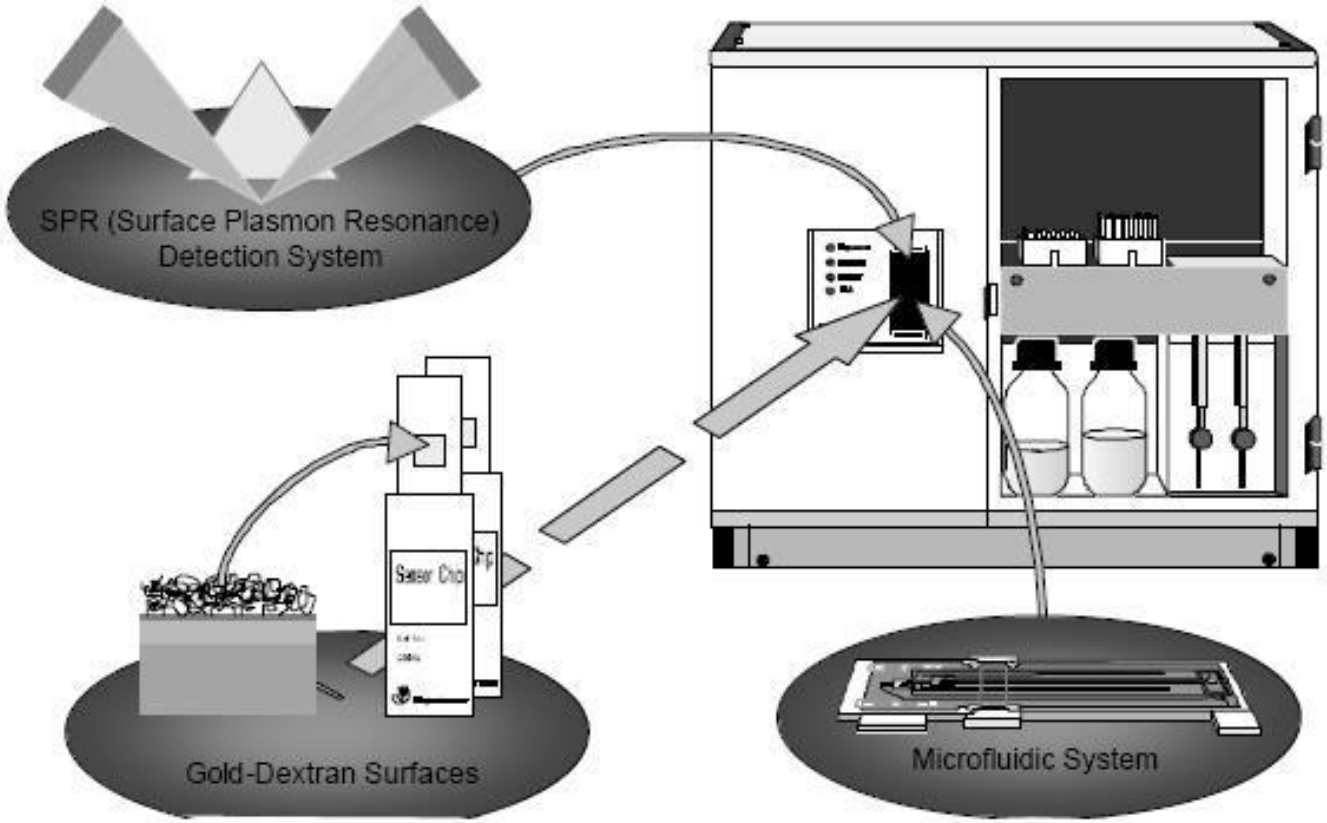


Biacore

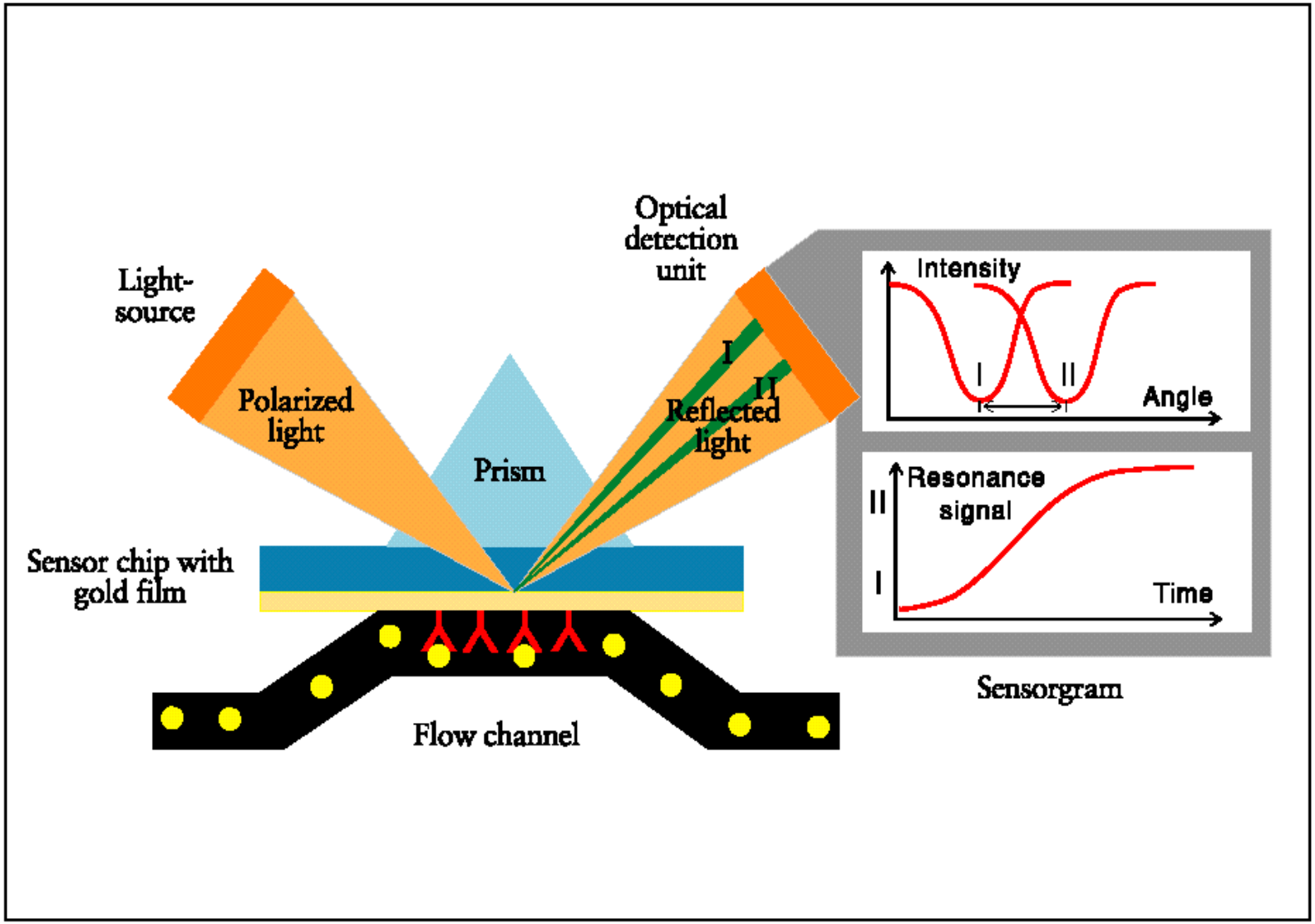




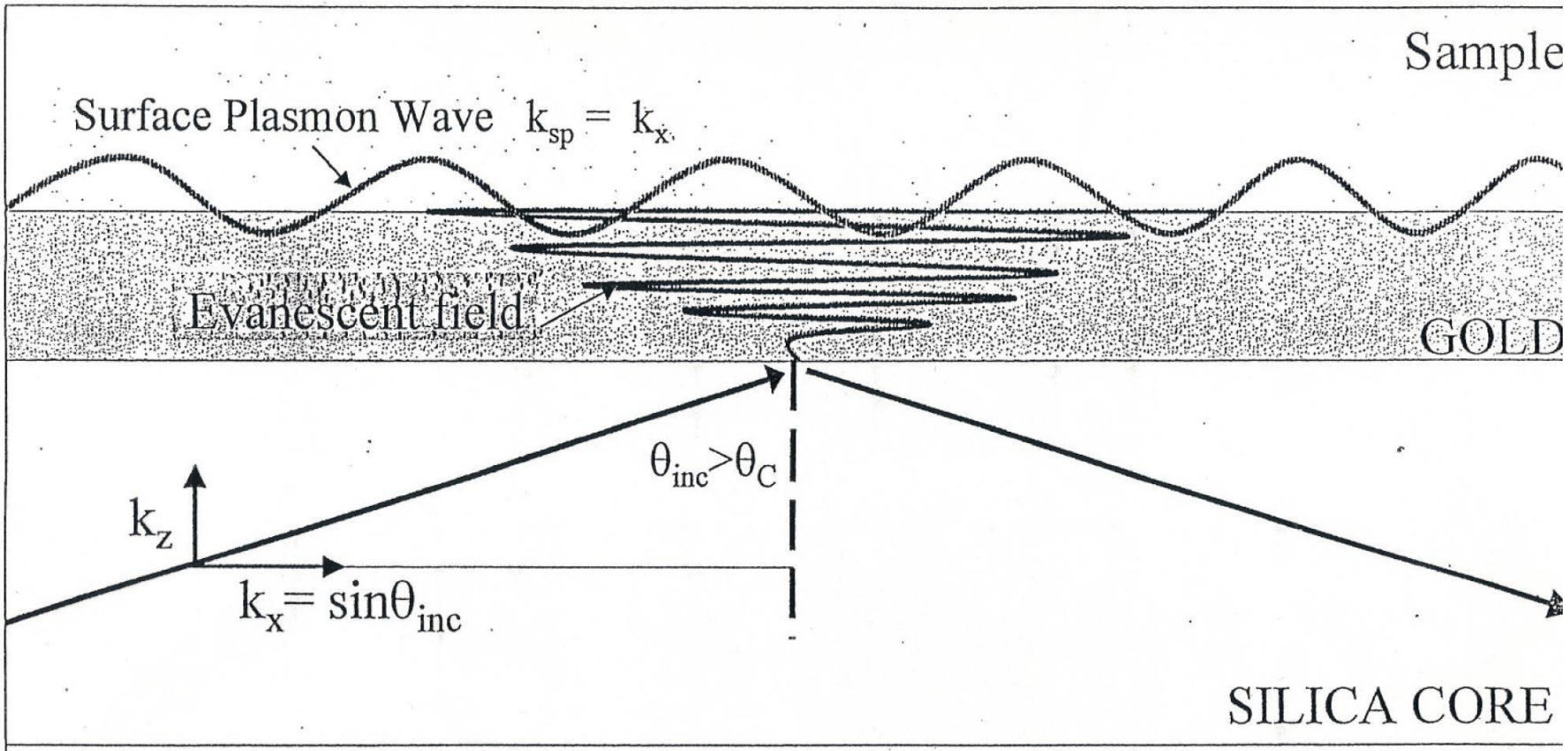

BIACORE


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

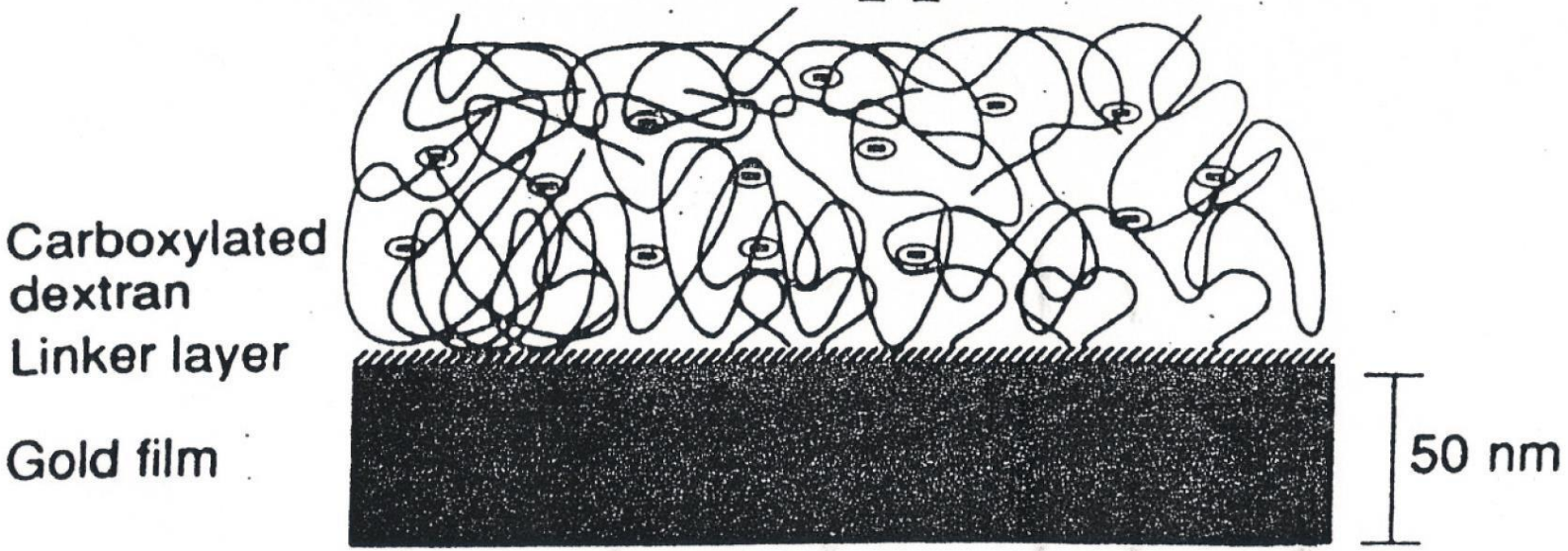
n_{glass} - Refractive Index of glass

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.

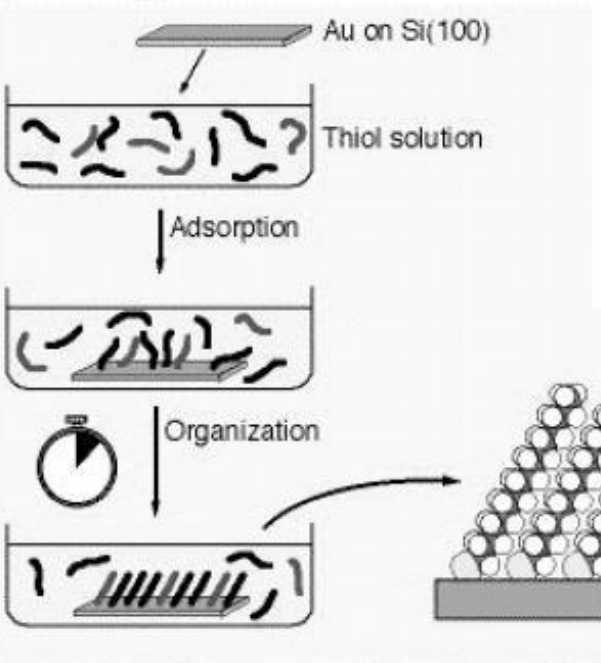
What Biacore actually measures is the angle of minimum reflected light intensity. The light is not absorbed by the sample: instead the light energy is dissipated through SPR in the gold film. Thus the light used to detect interaction processes never enters the sample.

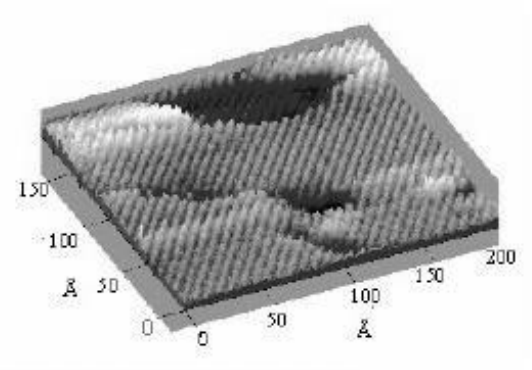
SPR response values are expressed in resonance units (RU). One RU represents a change of 0.0001° in the angle of the intensity minimum. For most proteins, this is roughly equivalent to a change in concentration of about 1 pg/mm2 on the sensor surface. The exact conversion factor between RU and surface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

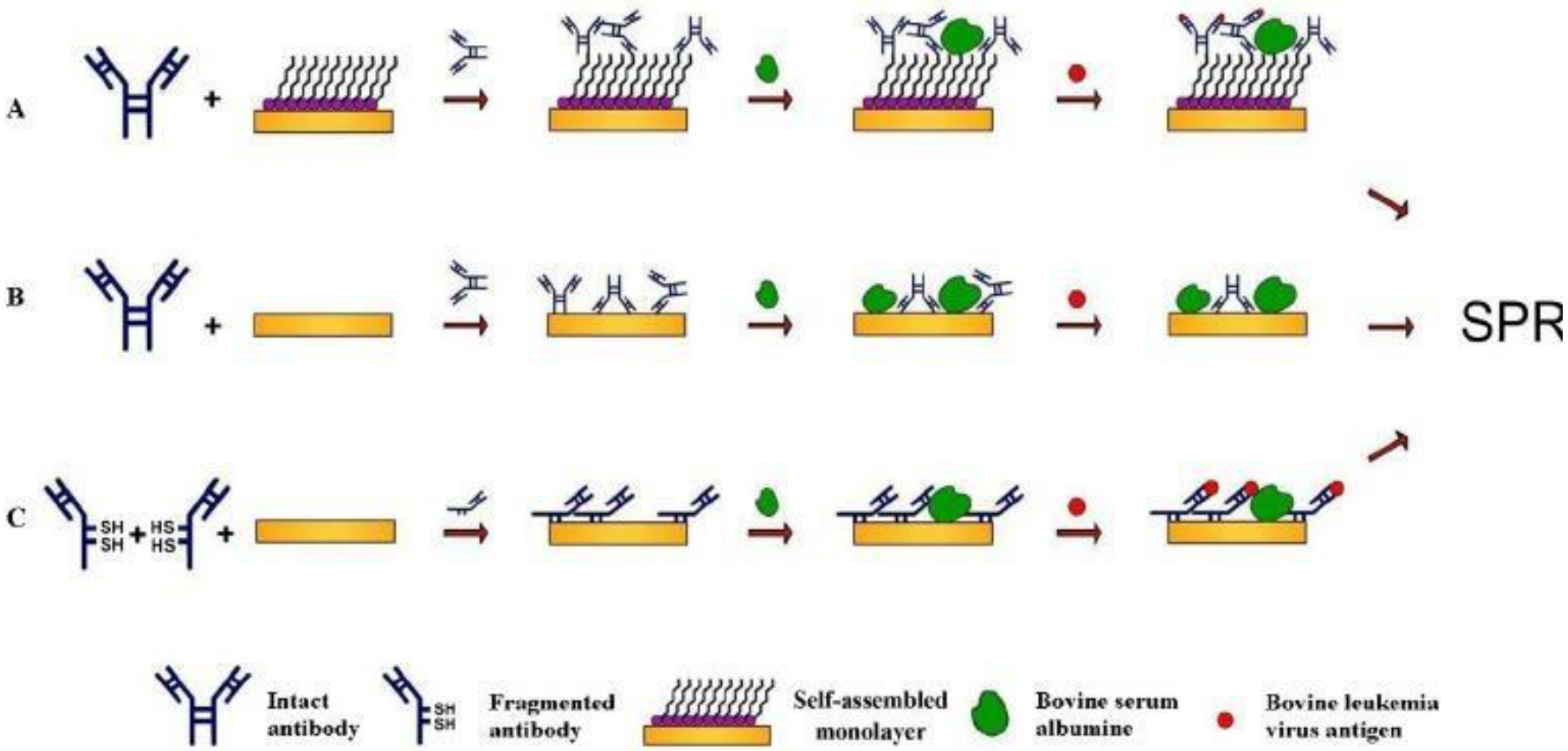
Immobilisation of organic molecules on gold

Self Assembled Monolayers (SAM)

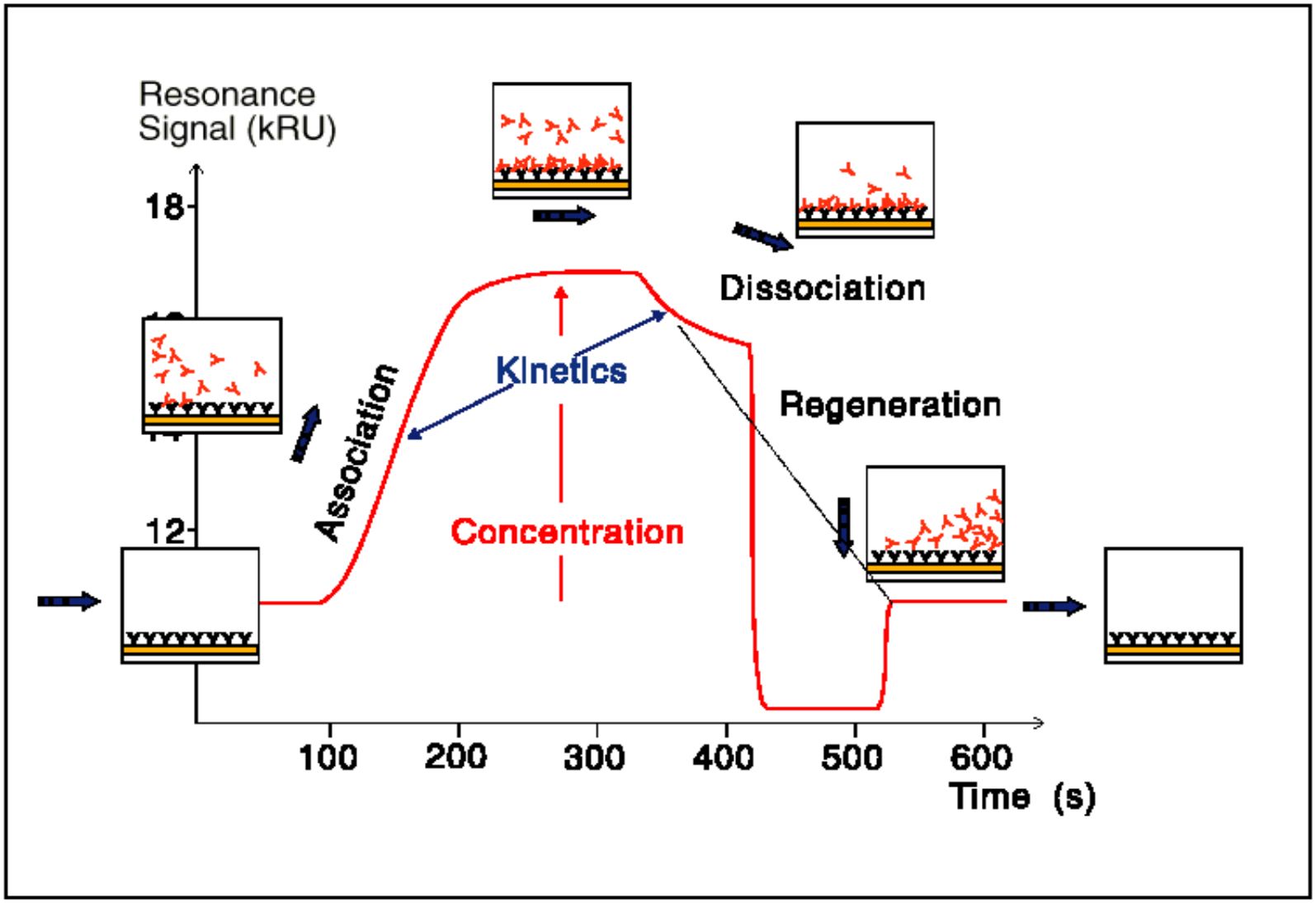
Disulphides (R-S-S-R)
Sulphides (R-S-R)
Thiols (R-SH)

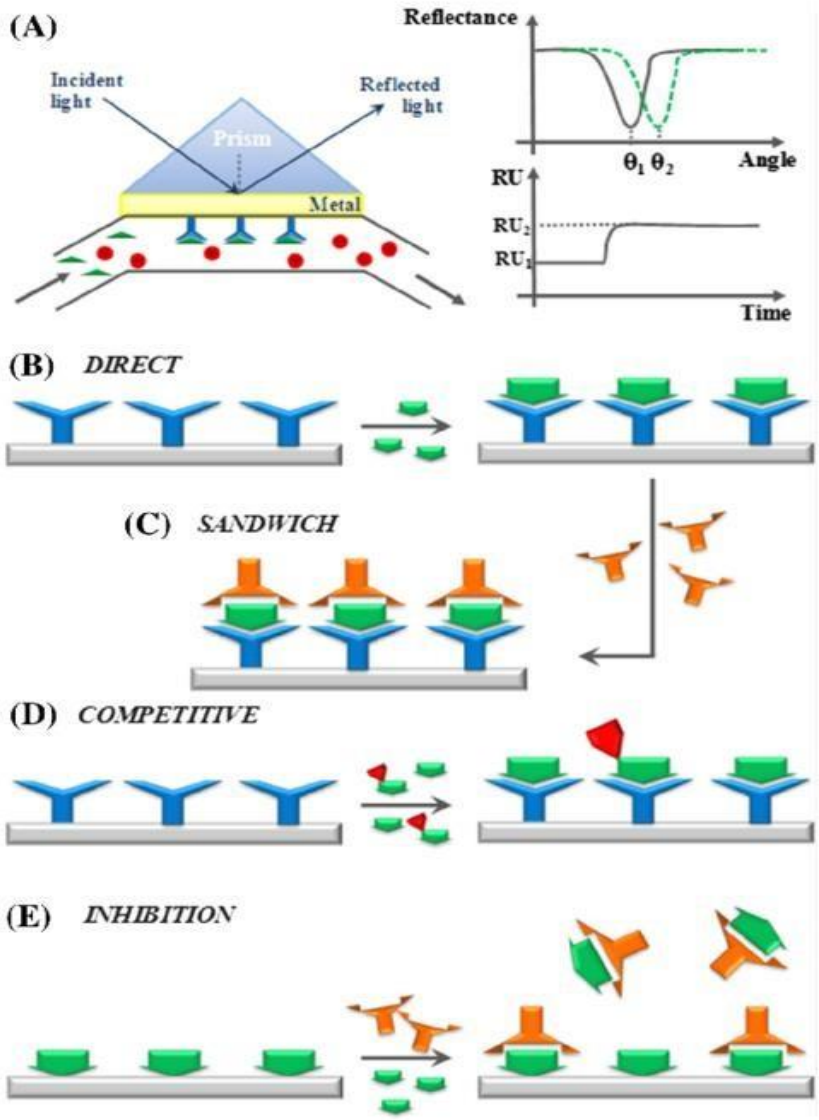




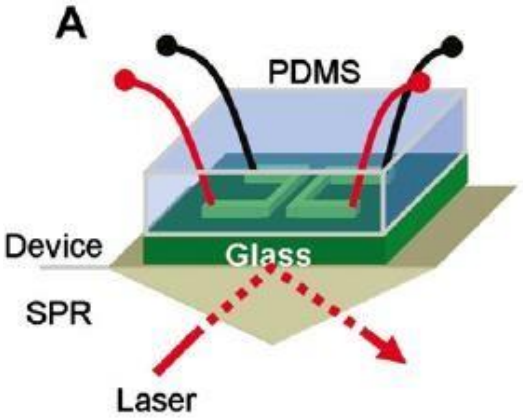

A
B
C
SH
SH
HS
HS
SPR
Intact antibody
Fragmented antibody
Self-assembled monolayer
Bovine serum albumine
Bovine leukemia virus antigen

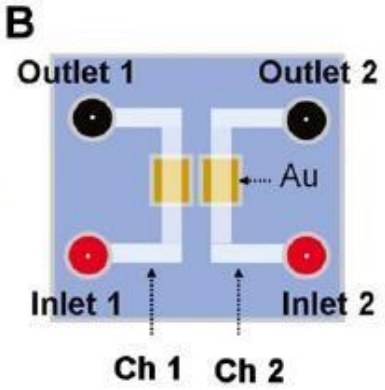
Sensorgram

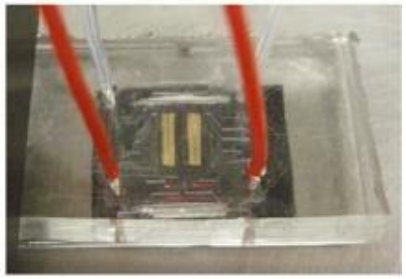

Resonance
Signal (kRU)
18
12
Association
Kinetics
Concentration
Dissociation
Regeneration
100
200
300
400
500
600
Time (s)

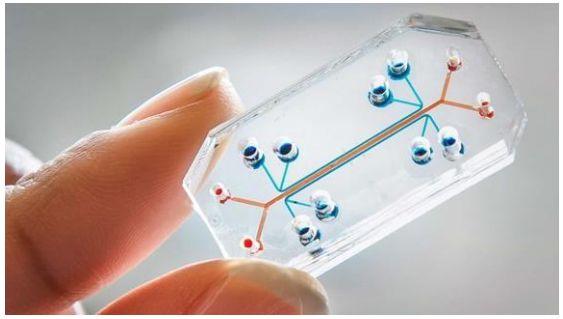

(A)
Reflectance
Incident light
Reflected light
Prism
Metal
θ1 θ2
Angle
RU
RU2
RU1
Time
(B) DIRECT
(C) SANDWICH
(D) COMPETITIVE
(E) INHIBITION

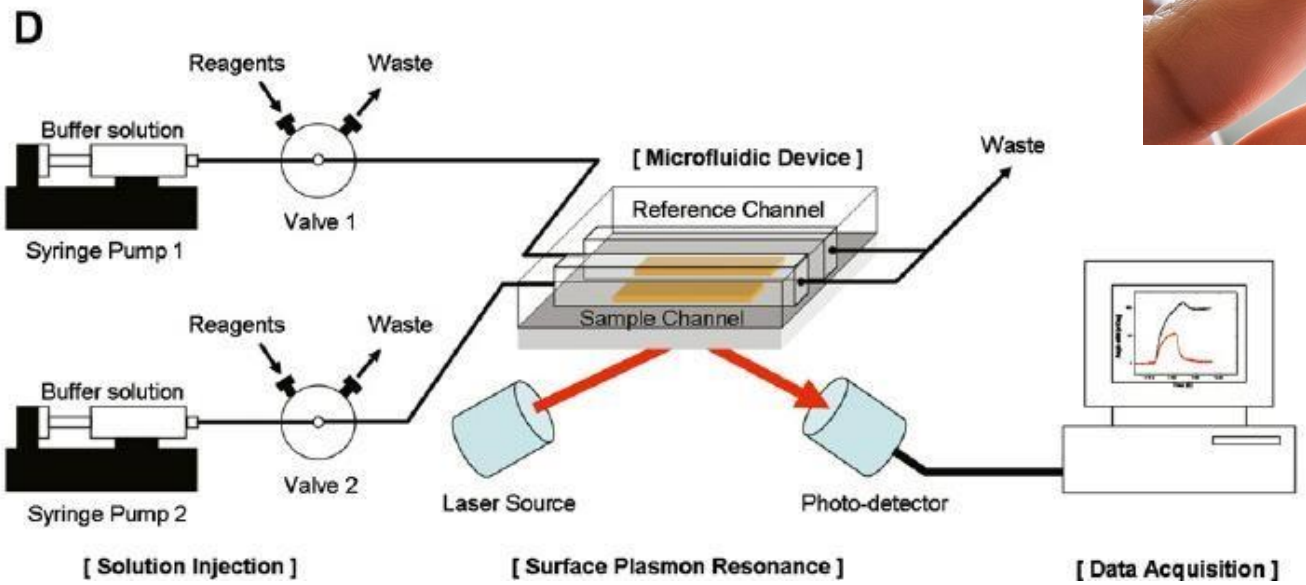
Spr sensors and microfluidics

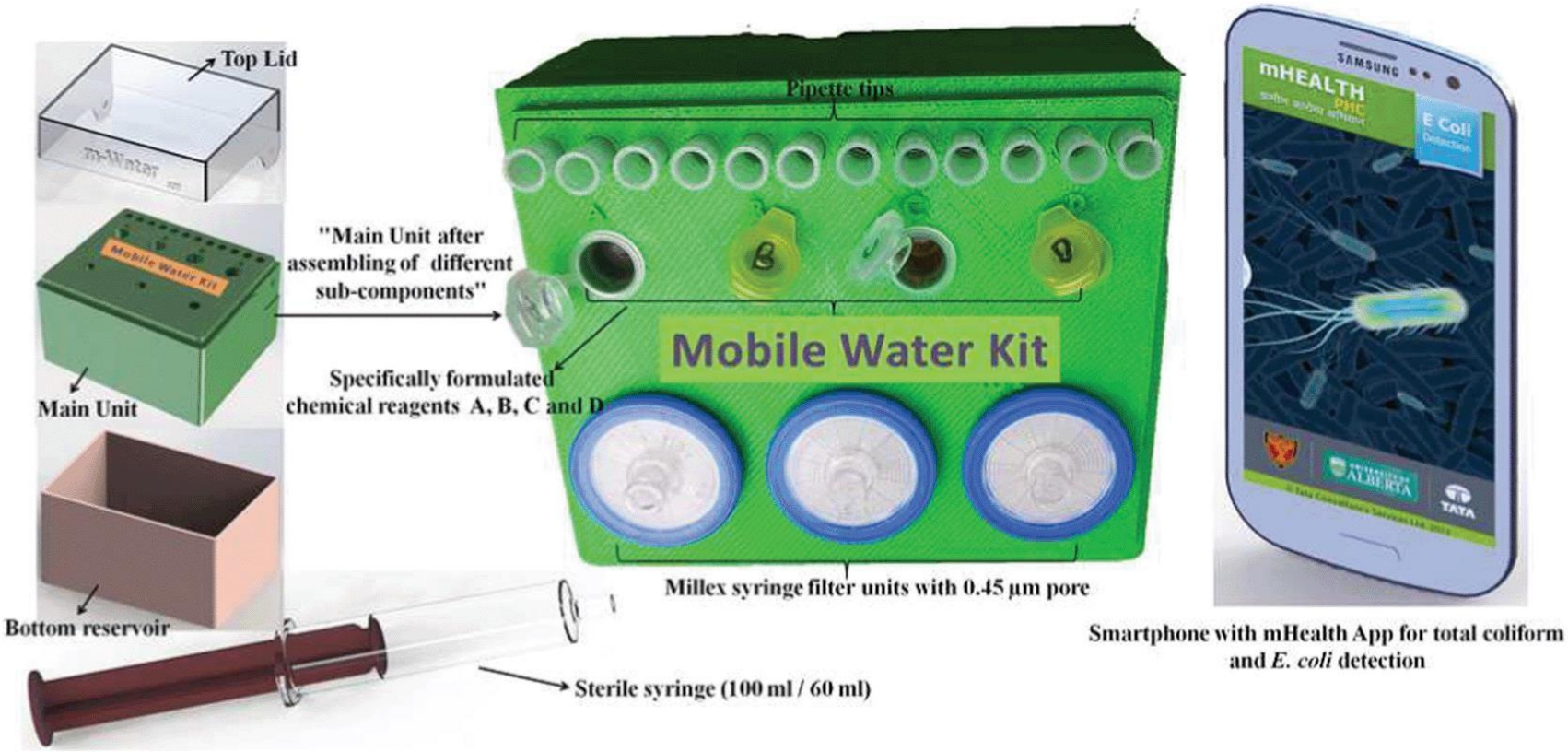






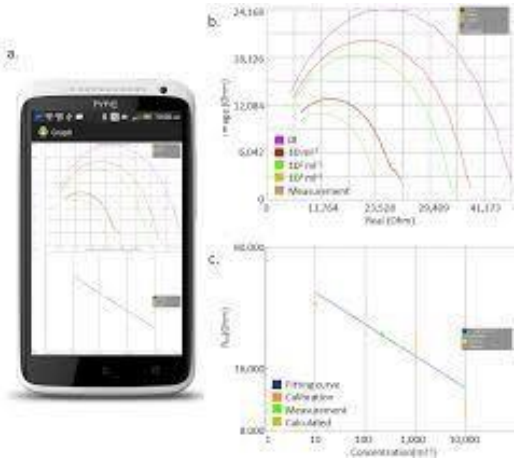






Smartphone with mHealth App for total coliform and *E. coli* detection

Smartphone detection of E.coli in water



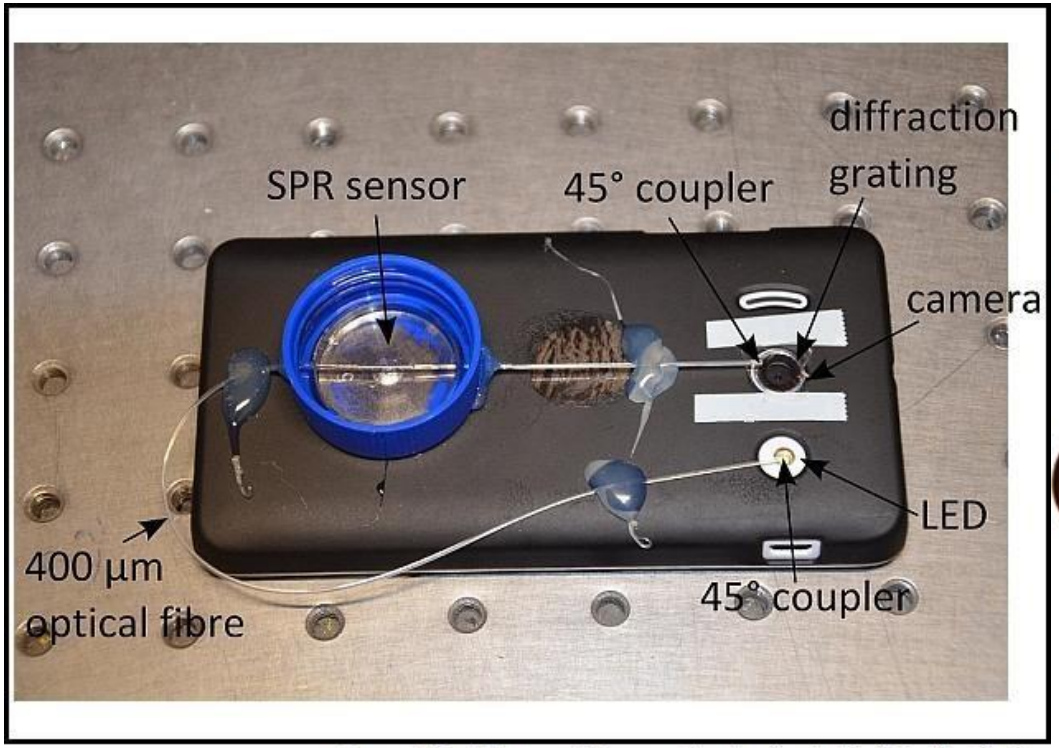
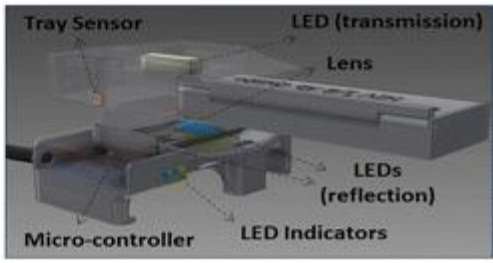
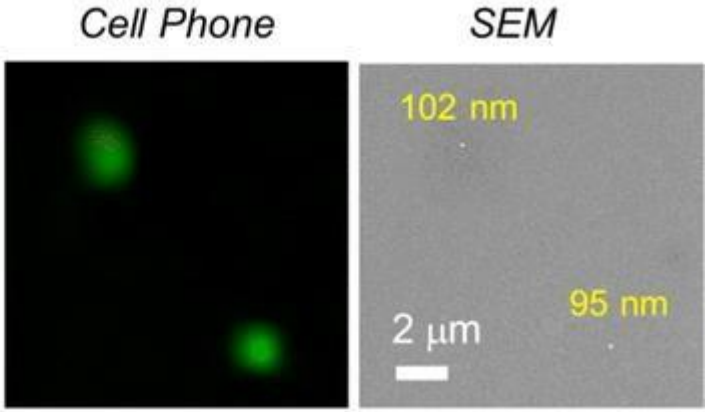


Image: Kort Bremer, Hanover Centre for Optical Technologies





Miscoscope based on smartphone for virus detection

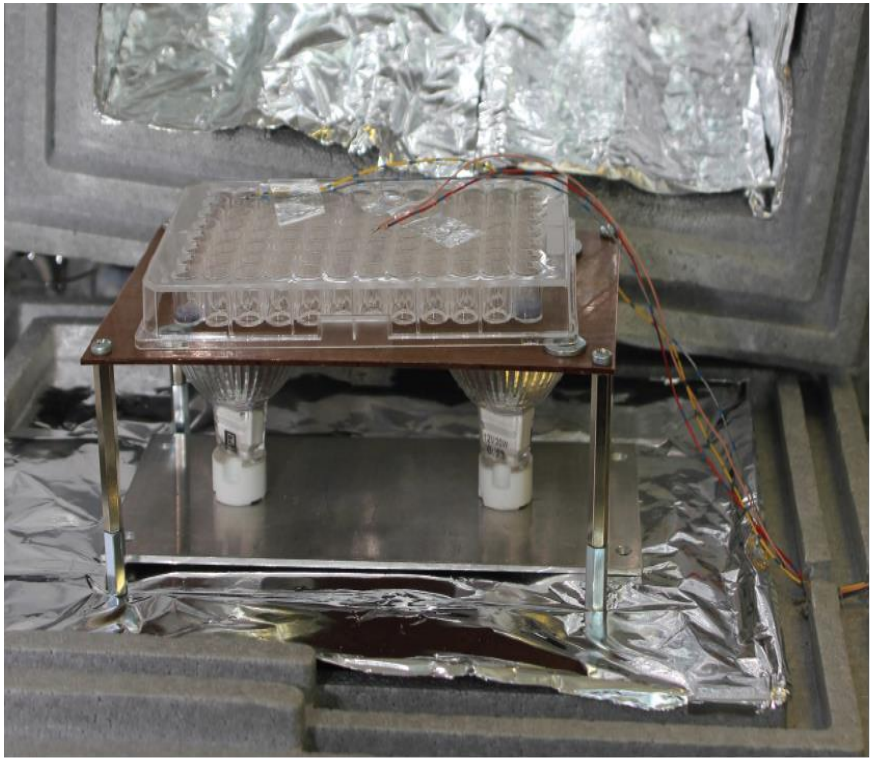


Fig. 1

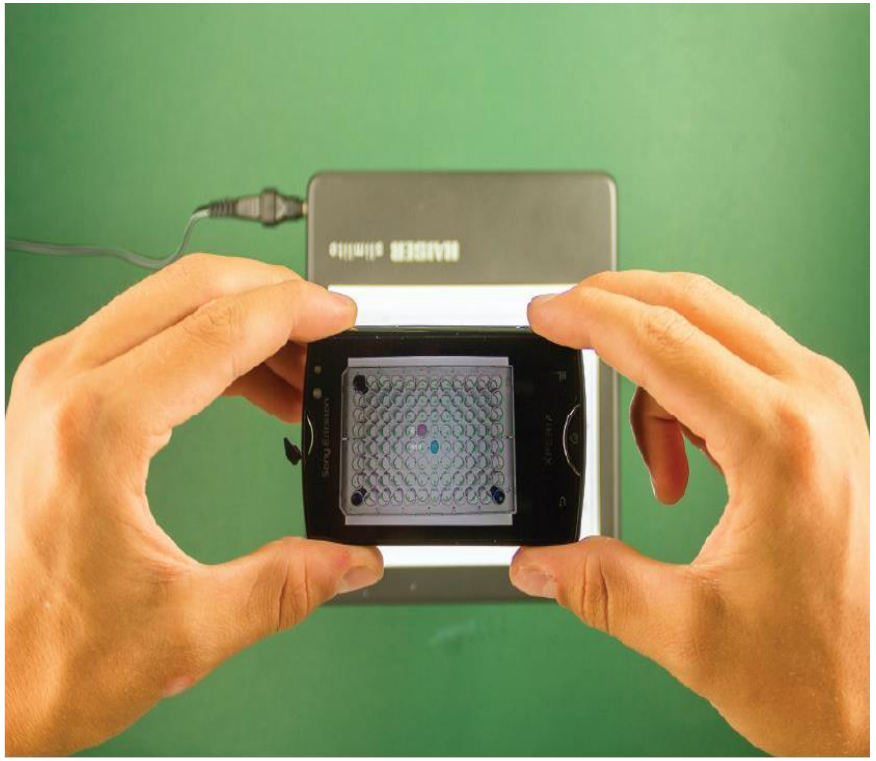
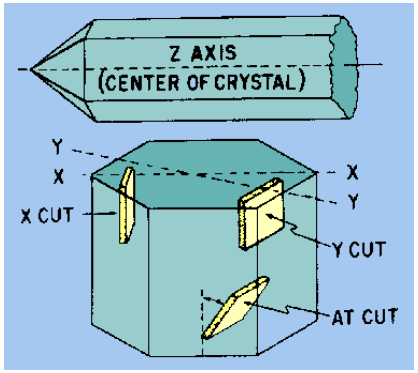
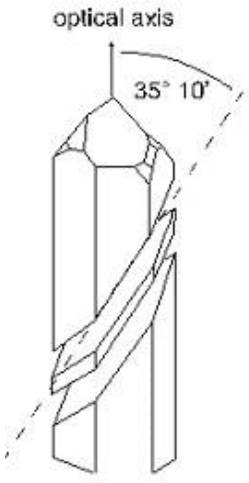


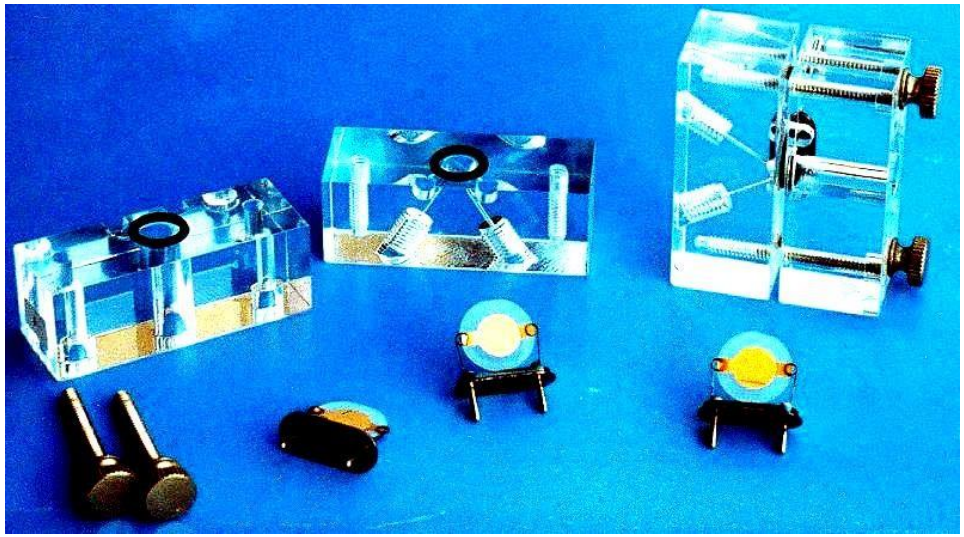
Fig. 2

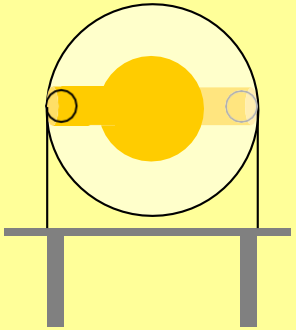
ELISA reading using a Smartphone

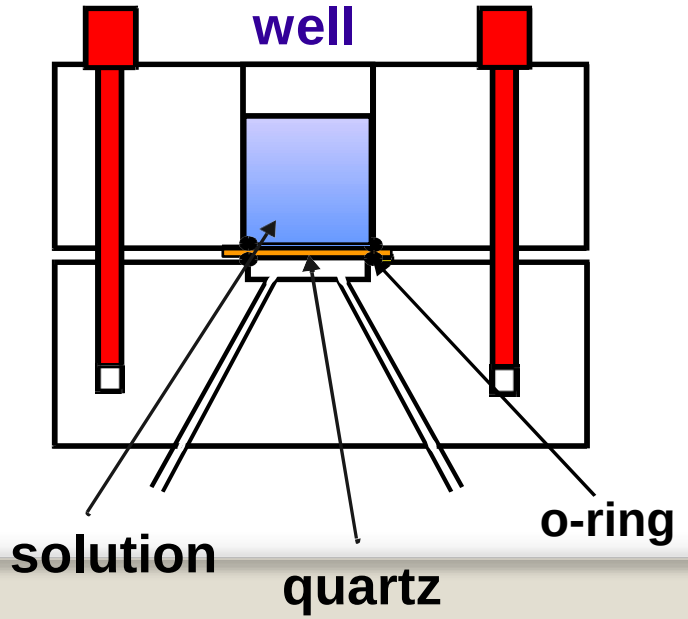
Piezoelectric Biosensors

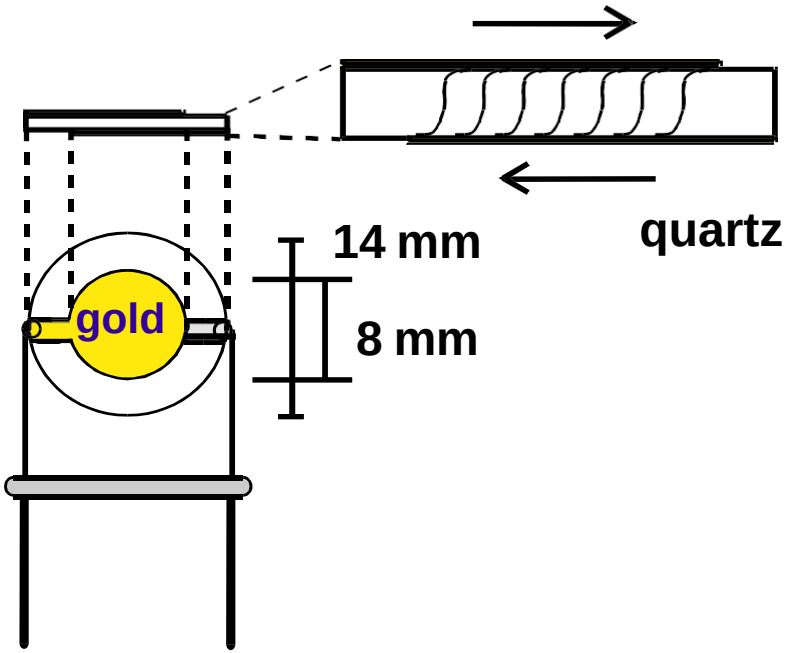




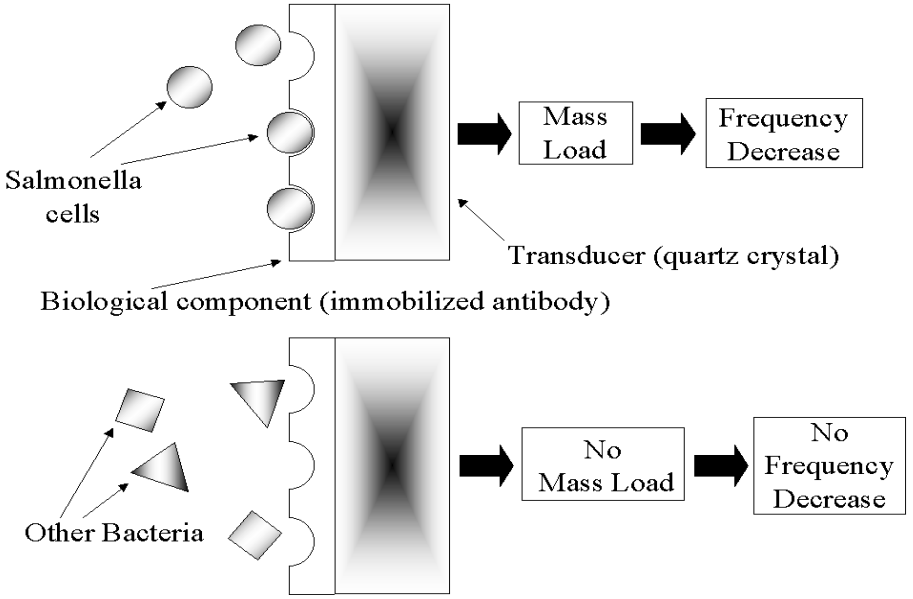








The mass-loading frequency effects of the transducer are based upon Sauerbrey's equation



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.

QCM-Mass

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

ΔF (Hz) = frequency shift of the coated crystal

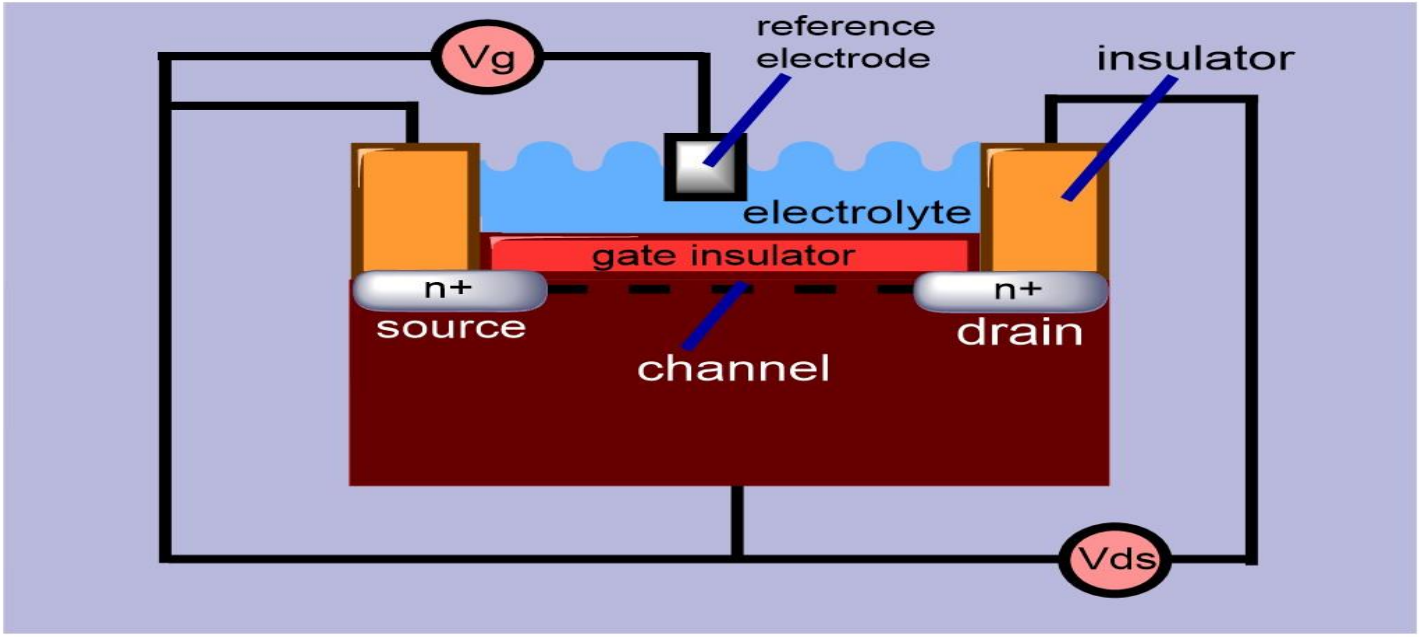
F (Hz) = resonance frequency of the crystal

ΔM (g) = increase in mass loading

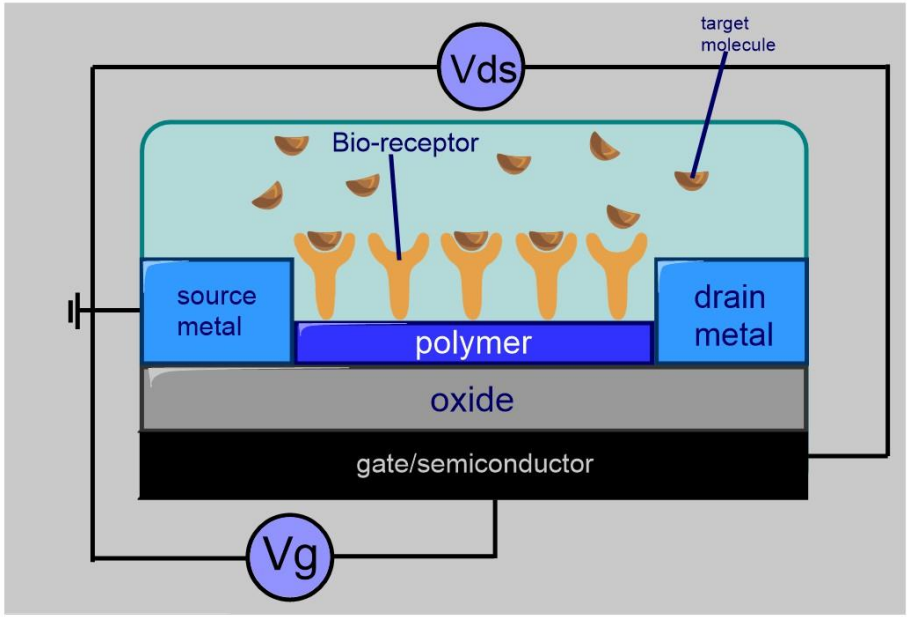
A (cm^2) = area of the coated crystal

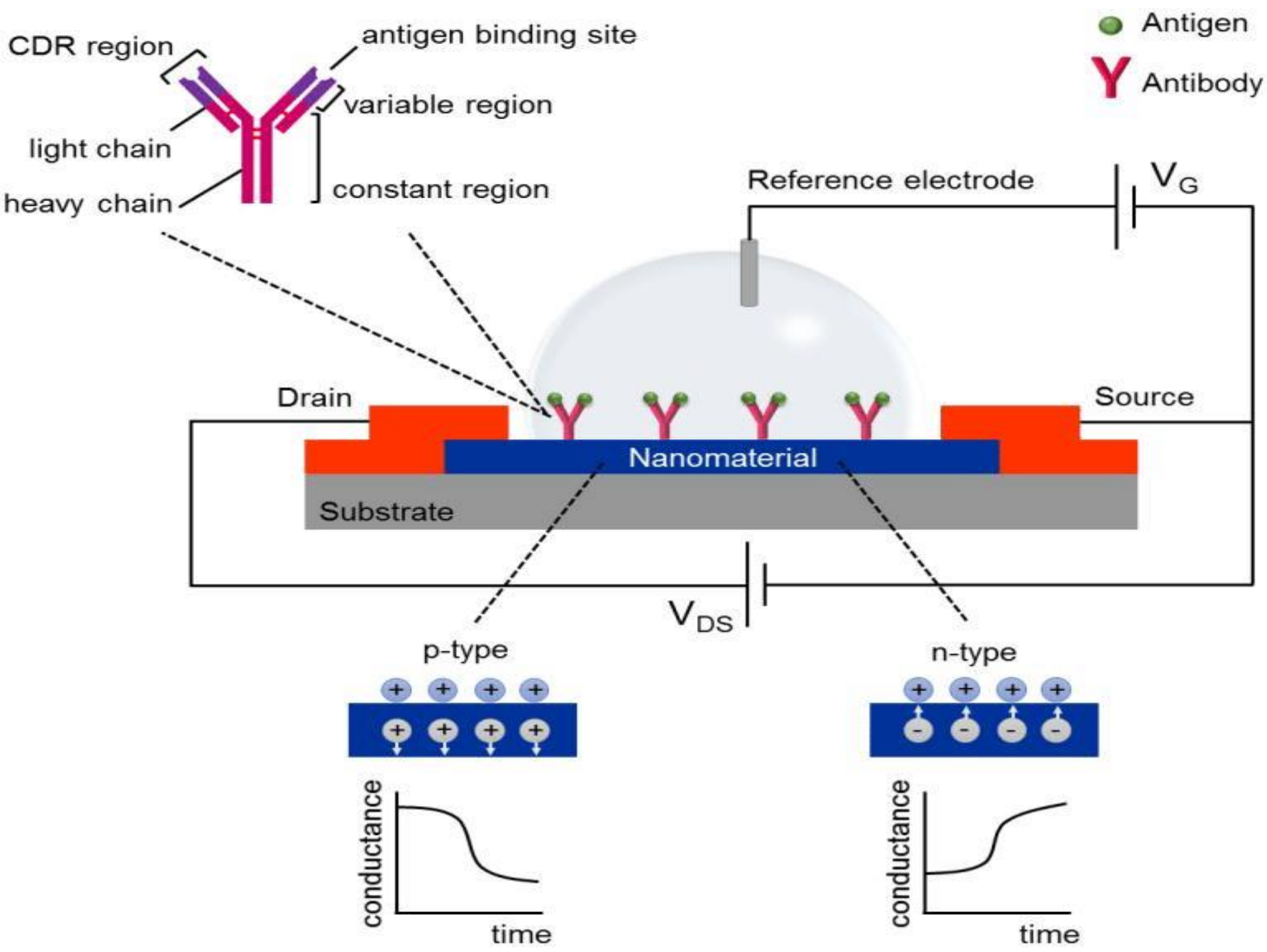
BIOFETs

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

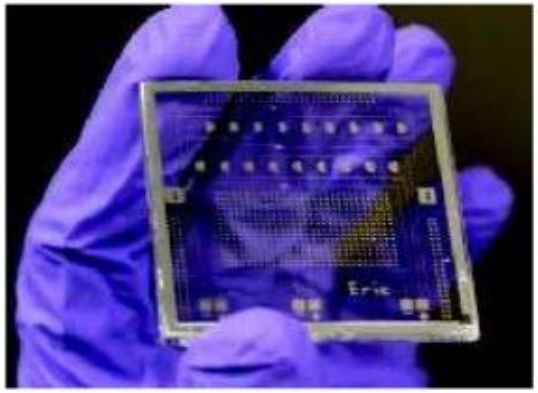


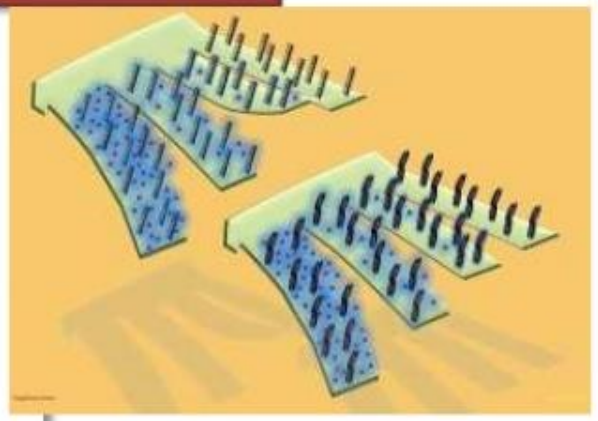
Bio-FETs couple a transistor device with a bio-sensitive layer that can specifically detect bio-molecules such as nucleic acids and proteins. A Bio-FET system consists of a semiconducting field-effect transistor that acts as a transducer separated by an insulator layer (e.g. SiO_2) from the biological 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)

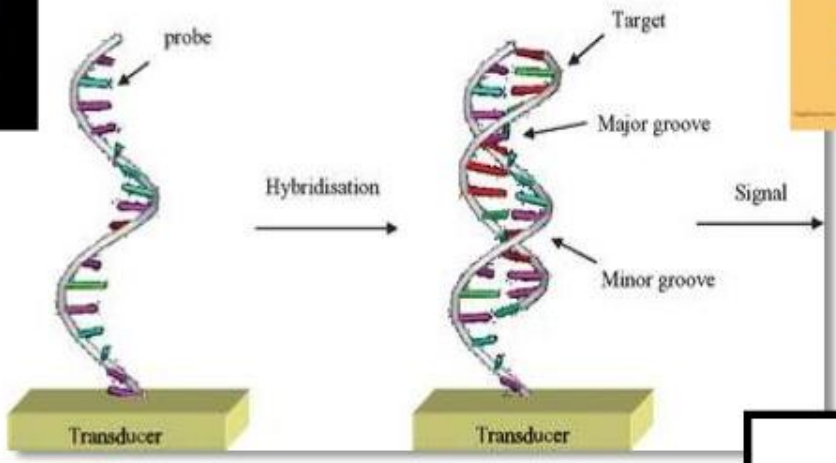


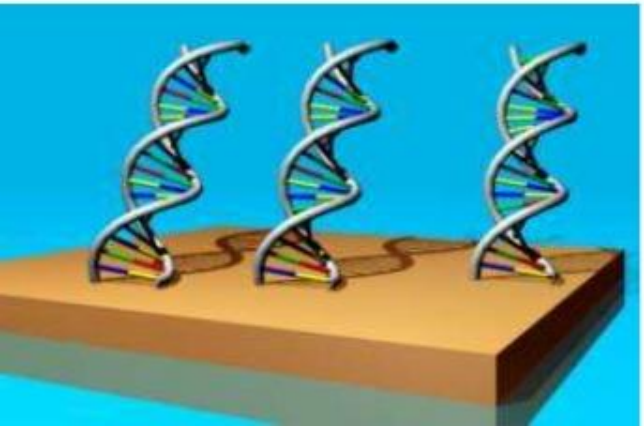

CDR region
antigen binding site
variable region
light chain
heavy chain
constant region
Antigen
Antibody
Reference electrode
V_G
Drain
Source
Nanomaterial
Substrate
V_{DS}
p-type
n-type
conductance
time
conductance
time

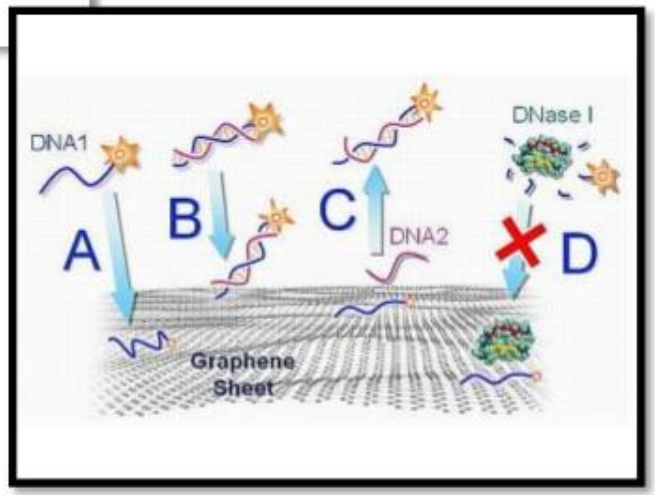
DNA biosensors









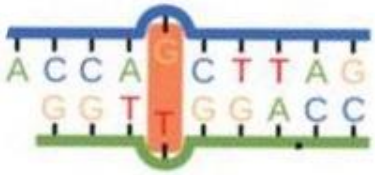


Principles of DNA biosensors

❖Nucleic acid hybridization

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

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



❖ Forms of DNA Biosensors

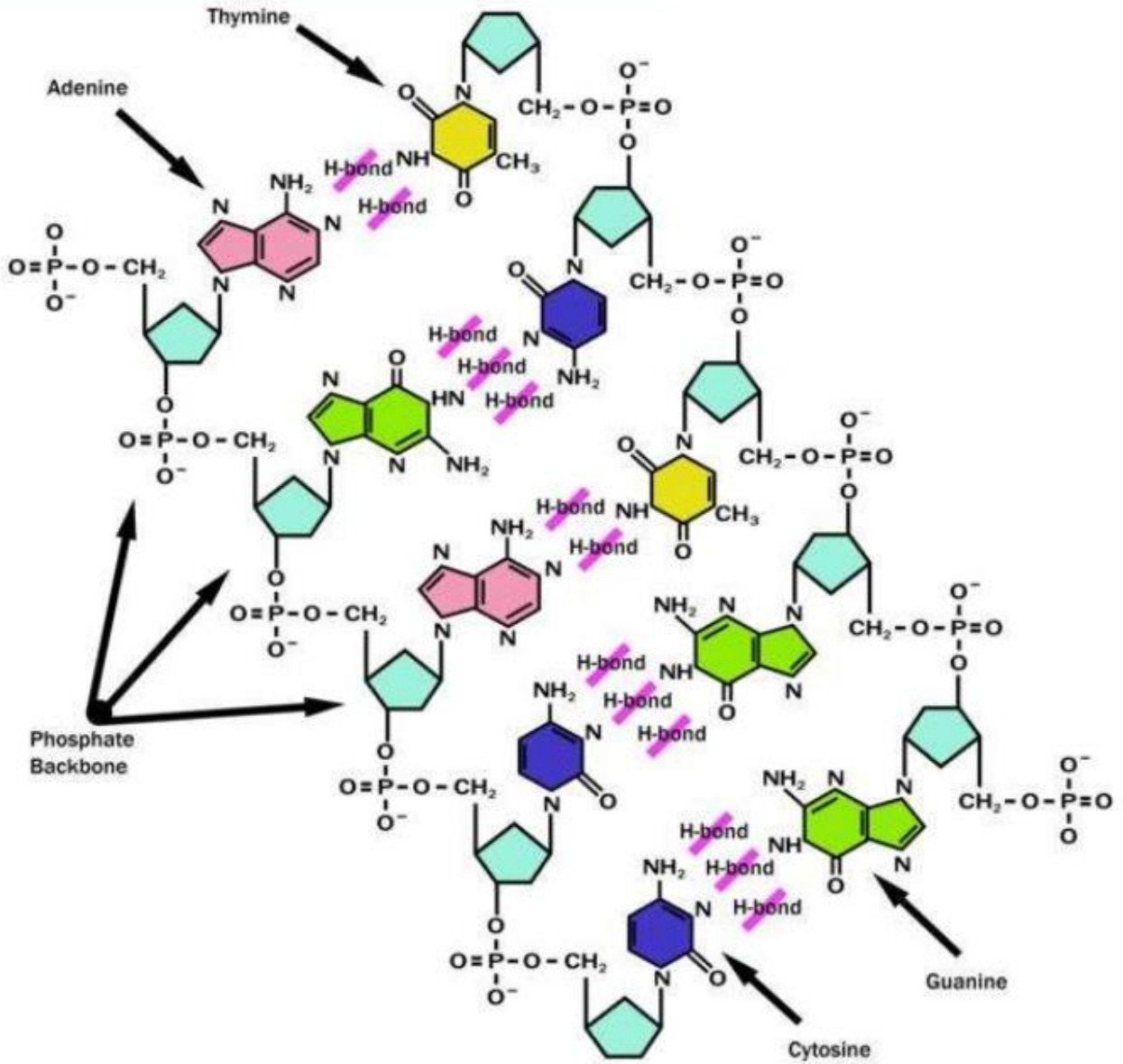
- Electrodes
- Chips
- Crystals

❖ Types of DNA Based Biosensors

- Optical
- Electrochemical
- Piezoelectric

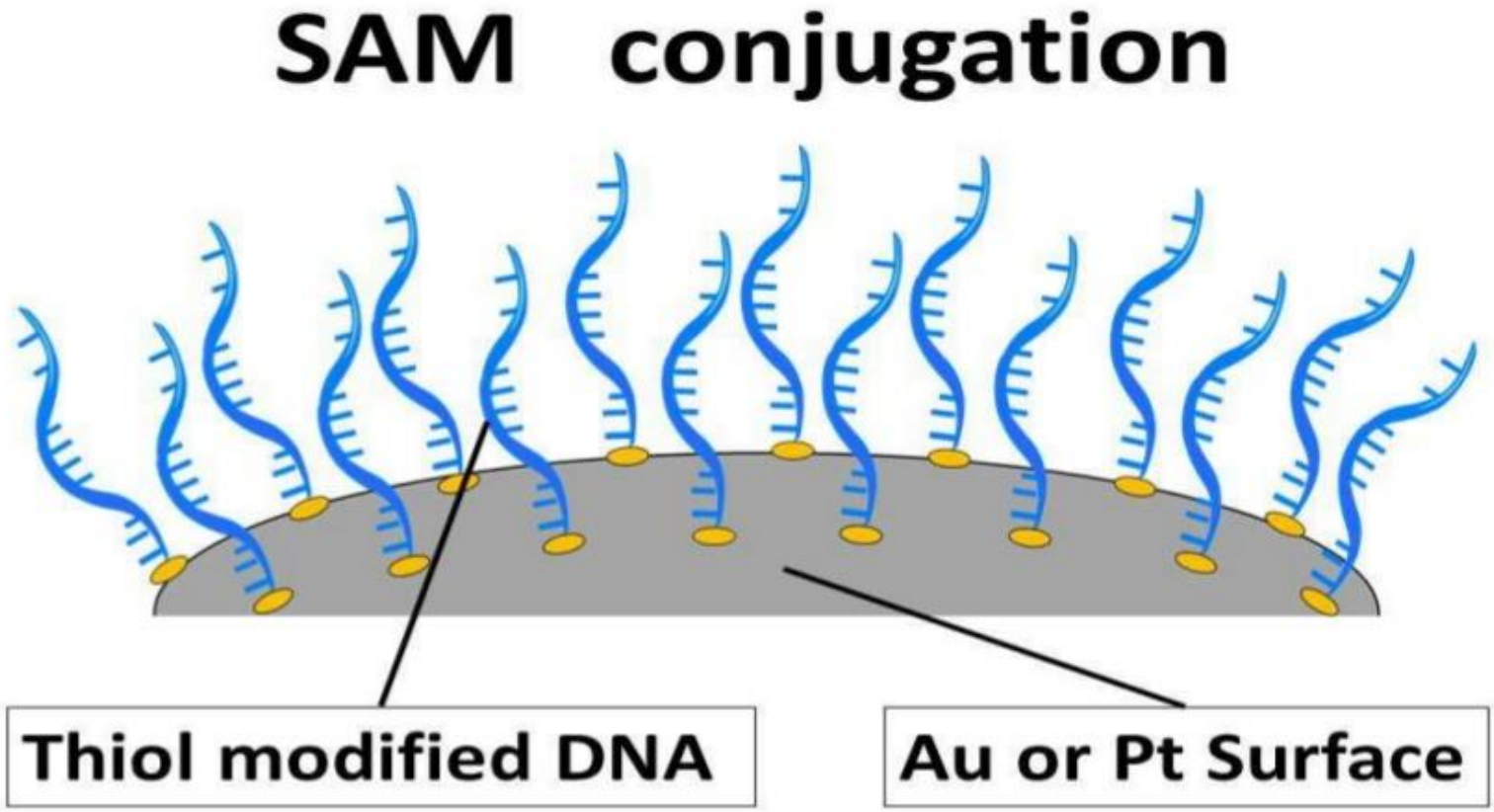
Immobilization of DNA Probe onto Transducer Surface

- **simple adsorption**
  onto carbon surfaces



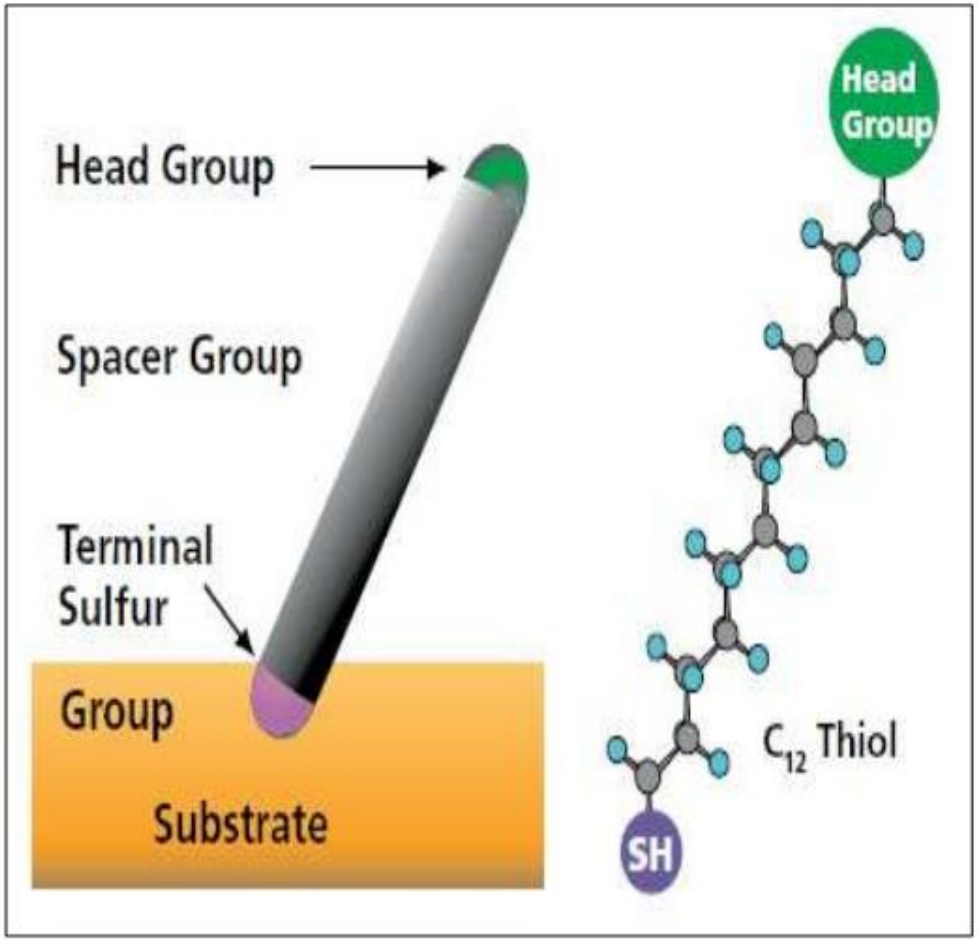
Immobilization of DNA Probe onto Transducer Surface

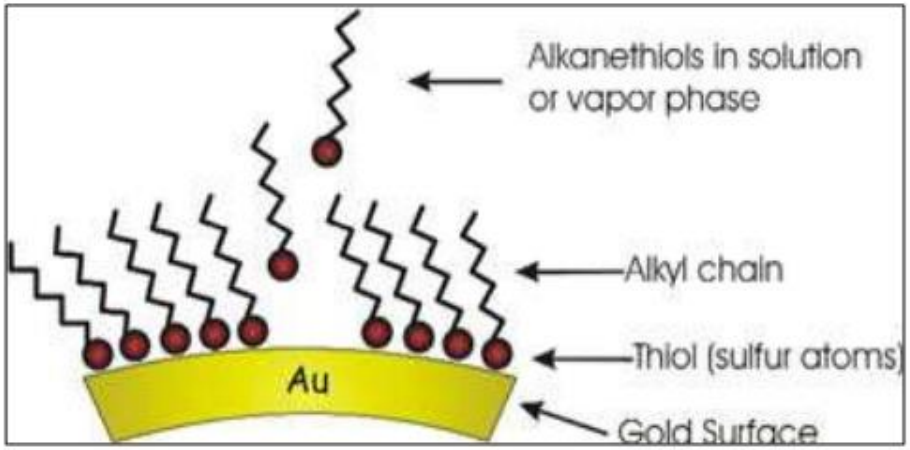
- Thiolated DNA for self assembly onto gold (or platinum) transducers

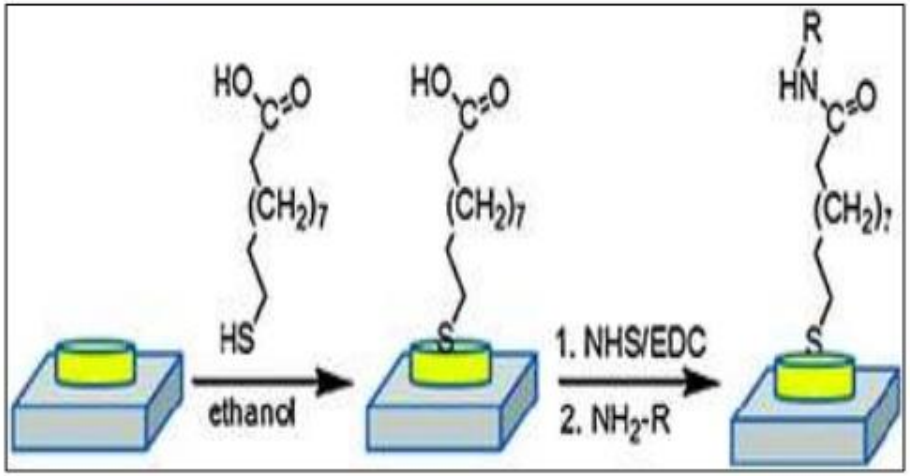


Immobilization of DNA Probe onto Transducer Surface

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

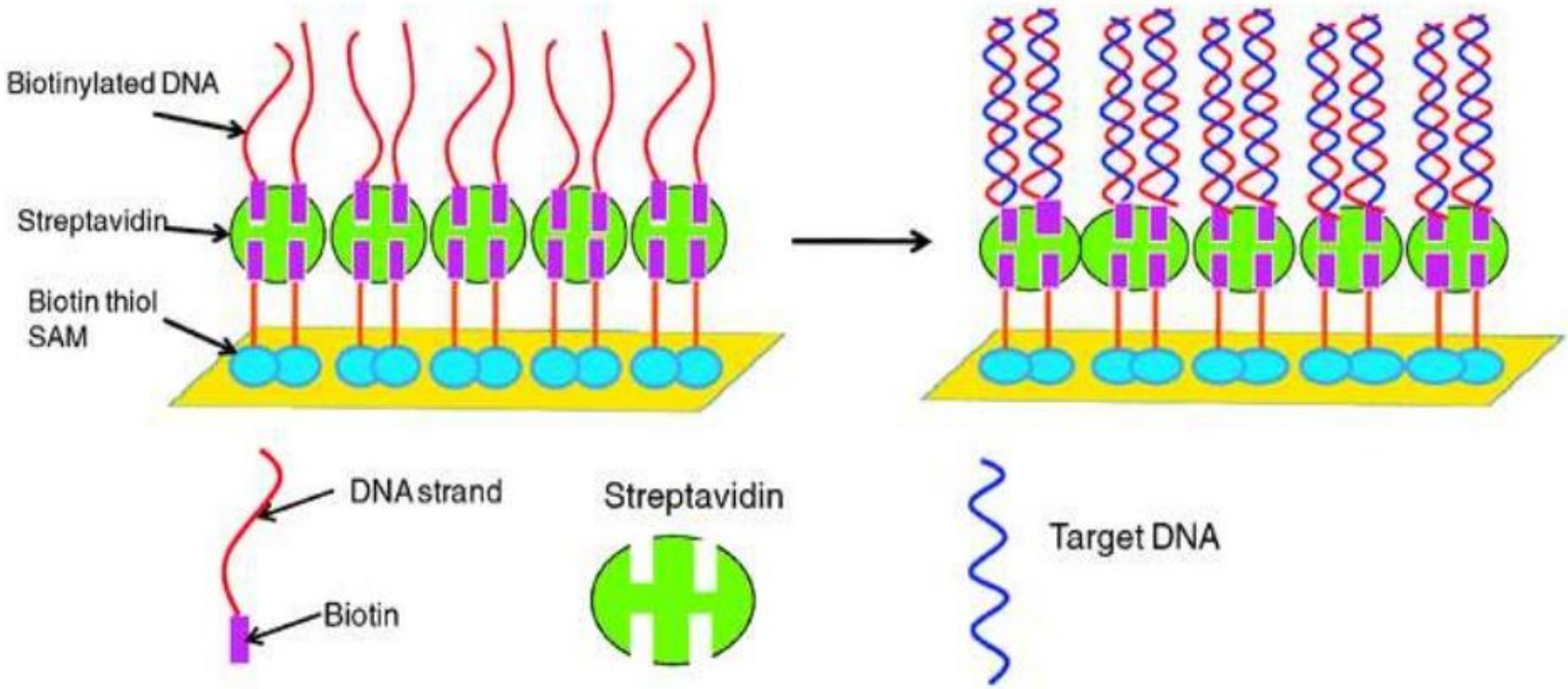






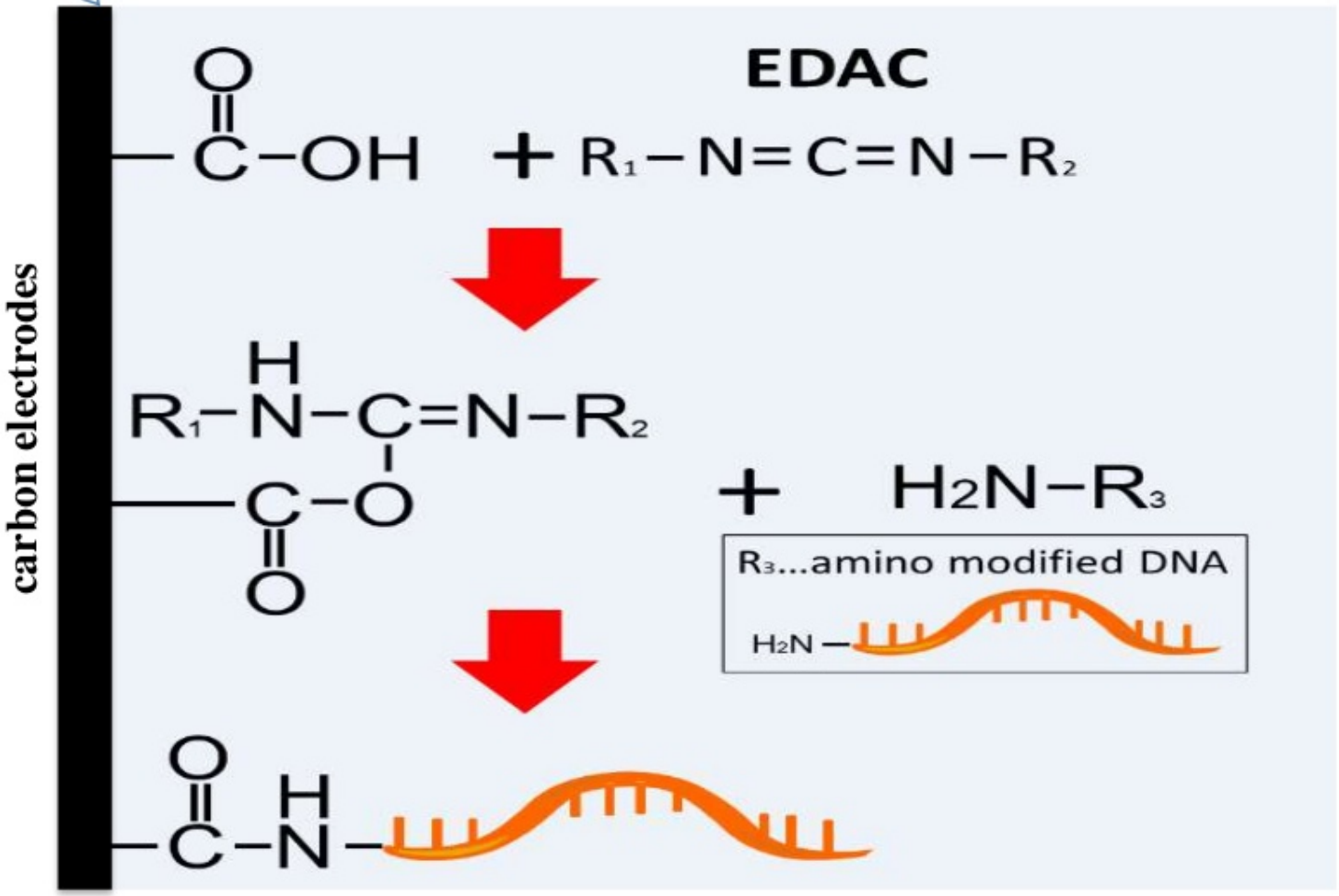
Immobilization of DNA Probe onto Transducer Surface

- 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.

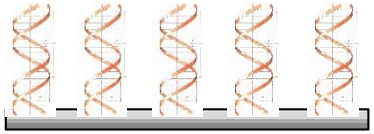


Immobilization method of DNA probes on functionalized surfaces.

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

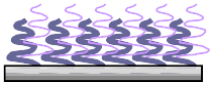
DNA electrochemical biosensors

DNA layer



Electrochemical transducer

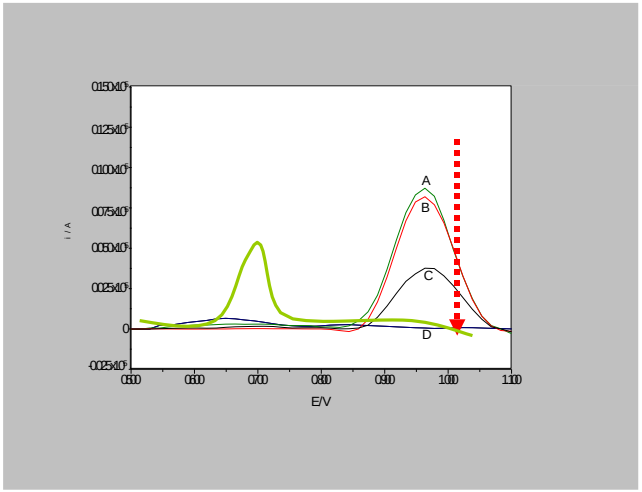
Hybridisation



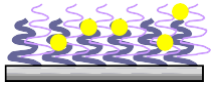
detection

Direct DNA electrochemistry

Use of an electroactive tracer



Intercalation

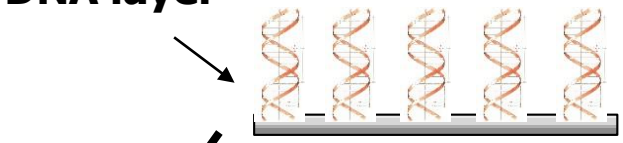


detection

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

Appearance of new oxidation peak

DNA electrochemical biosensors application



Electrochemical transducer

Hybridisation:

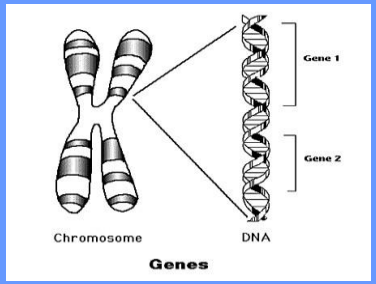
Molecular diagnostic application:
- microorganism
- gene single-point mutation
- species identification
- GMO

Intercalation

Antitumoral drugs

Genotoxic compounds:
- stable DNA complex
- adduct formation

• 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)

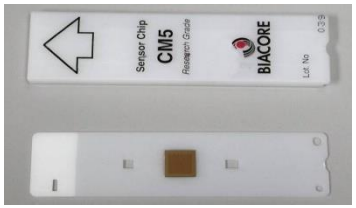
Denaturation of the dsDNA (amplified fragment or genomic) to obtain a single-stranded DNA (sample pretreatment)

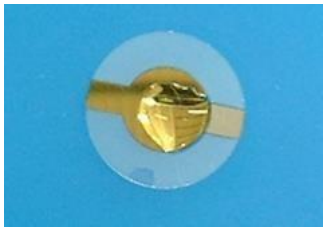
Hybridisation of the obtained ssDNA with the immobilised probe

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

Probe immobilisation on gold film

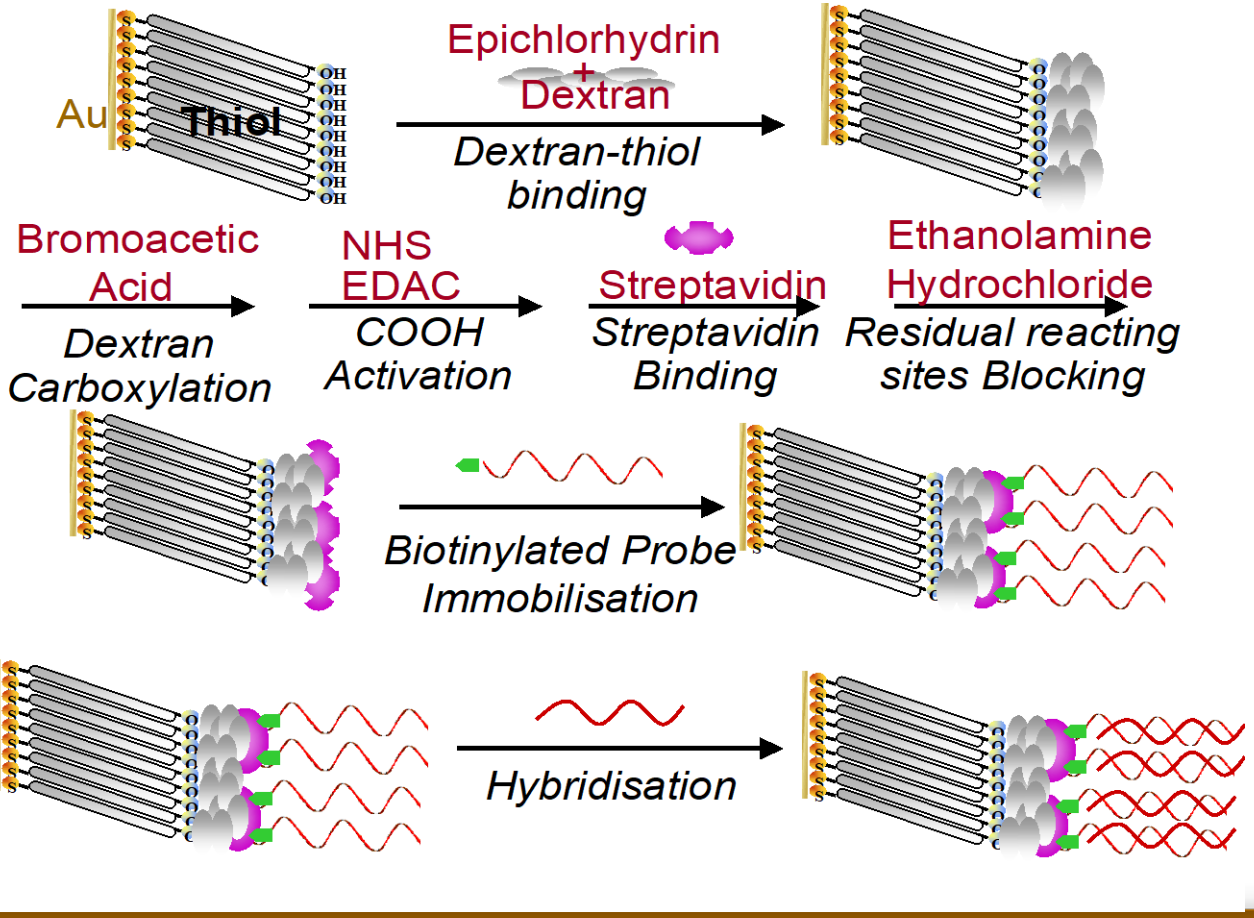
optical





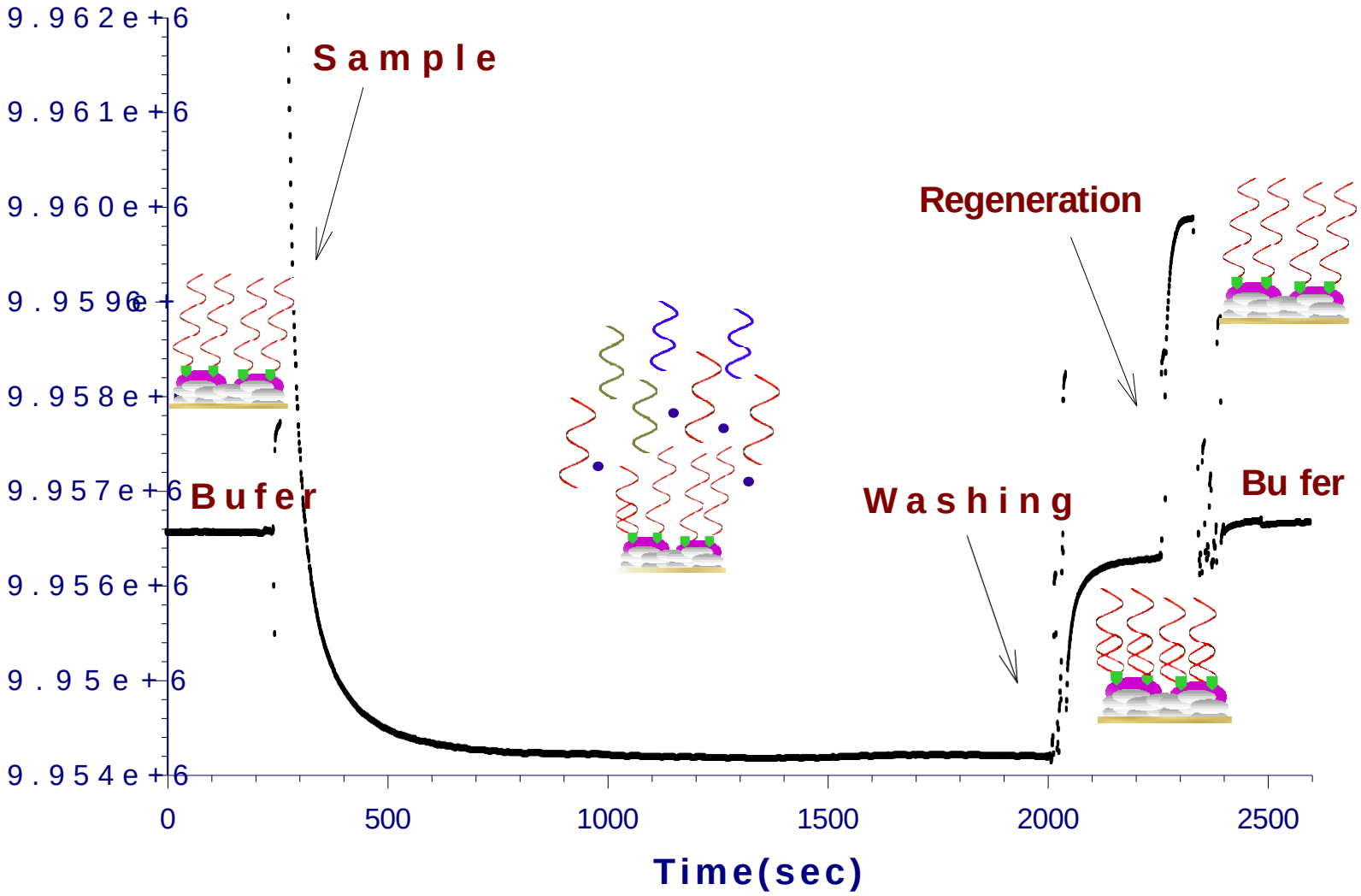
piezoeletric

thiol/dextran/streptavidin/biotinylated probe



Specificity,
no aspecific
adsorption,
stability,
multi-use

Hybridation-Regenation Cycle



Detection of DNA target sequence in real matrices

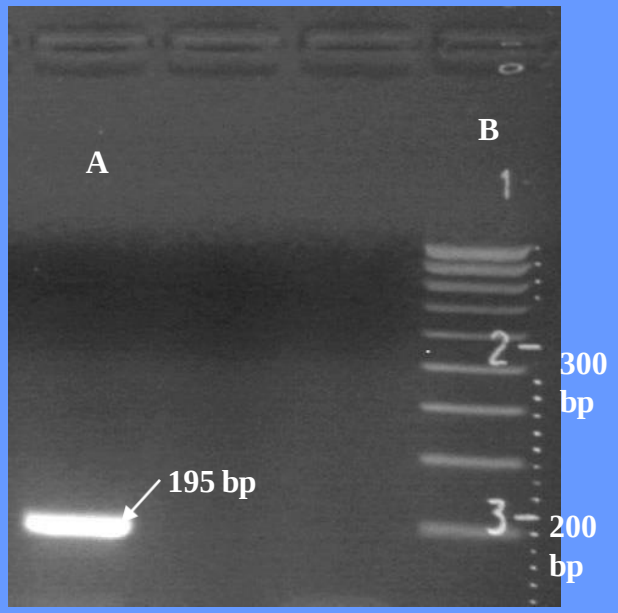
- Certified Reference Materials (CRM) -Fluka
- Processed food (dietetic snaks, soy crackers, soft drinks)

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

Control: Post PCR Electrophoresis

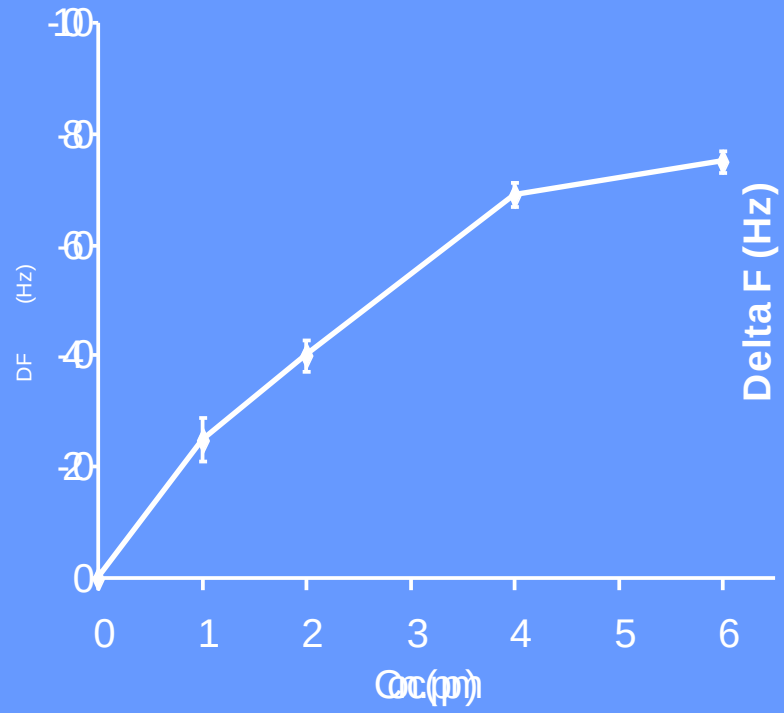


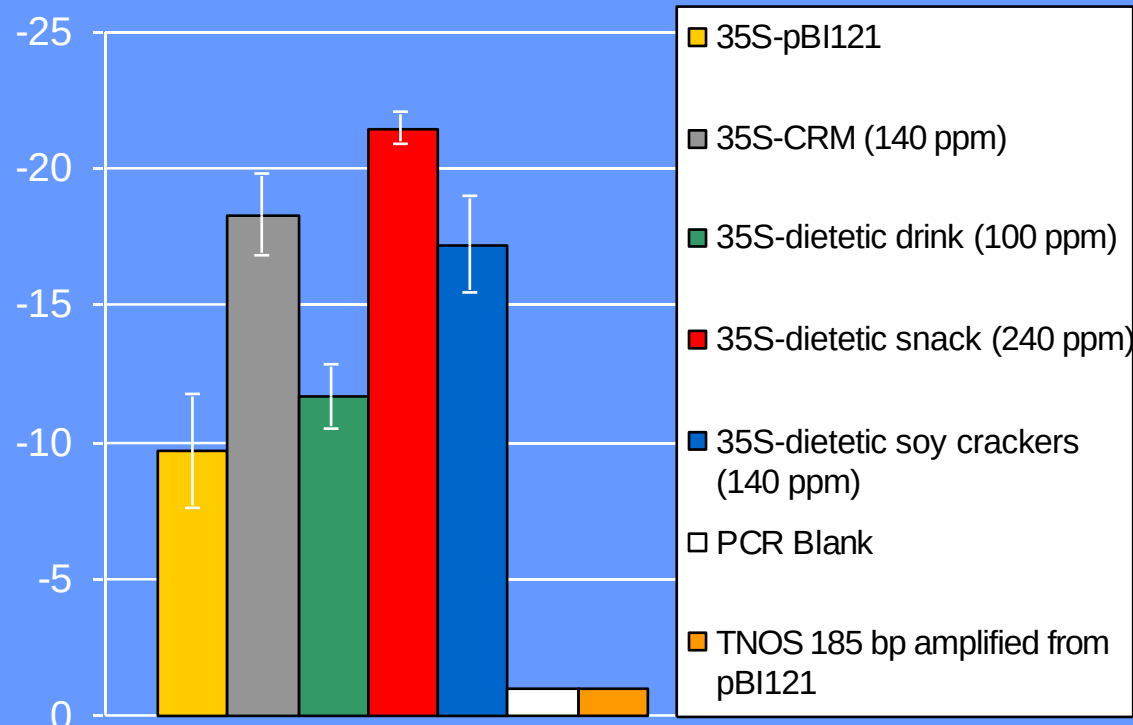
A: amplified fragment (Promoter 35S 195 bp)

B: Standard length fragments

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

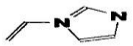
3'- ccg gat gca act tct acg gag acg g-5 target 35S

Biomimetic receptors

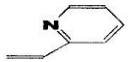
Obtained via combinatorial chemistry and/or molecular modelling

- MIP (Molecularly imprinted polymers)
- Peptides
- Aptamers

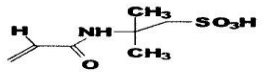
Functional Monomer Database



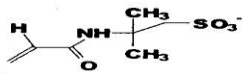
F1 1-VINYLIMIDAZOLE



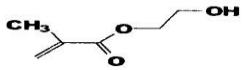
F2 2-VINYLPYRIDINE



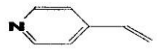
F3 ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)



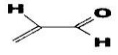
F3a ACRYLAMIDO-2-METHYL-1-PROPANESULFONIC ACID (AMPSA)



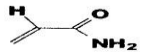
F4 2-HYDROXYETHYL METHACRYLATE



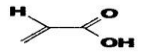
F5 4-VINYLPYRIDINE



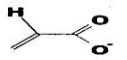
F6 ACROLEIN



F7 ACRYLAMIDE



F8 ACRYLIC ACID



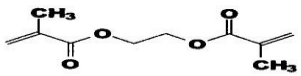
F8A ACRYLIC ACID

F9 ACRYLONITRILE

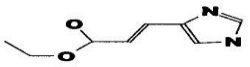
F10 ALLYAMINE

F10A ALLYLAMINE

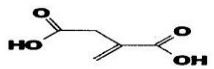
F11 p-DIVINYLBENZENE



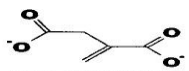
F12 ETHYLENE GLYCOL DIMETHACRYLATE (EGDMA)



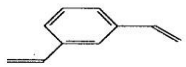
F13 UROCANIC ACID ETHYL ESTER



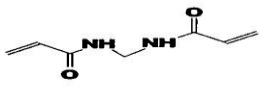
F14 ITACONIC ACID



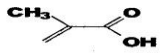
F14A ITACONIC ACID



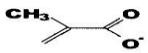
F15 m-DIVINYLBENZENE



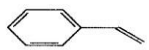
F16 N,N-METHYLENE BIS ACRYLAMIDE



F17 METHACRYLIC ACID



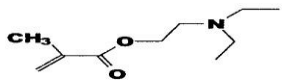
F17A METHACRYLIC ACID



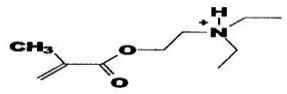
F18 STYRENE

F19 UROCANIC ACID

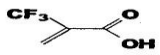
F19A UROCANIC ACID



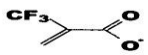
F20 N,N-DIETHYLAMINO ETHYL METHACRYLATE (DEAEM)



F20A N,N-DIETHYLAMINO METHACRYLAMIDE (DEAEM)

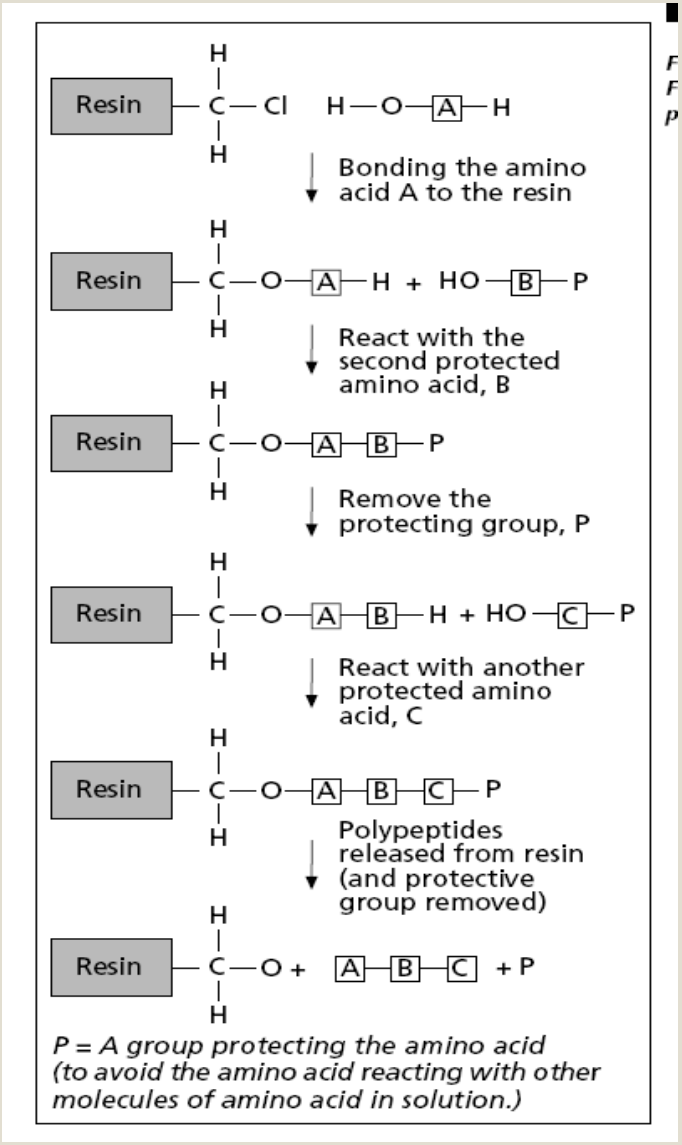


F17 TRIFLUOROMETHACRYLIC ACID



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		

Molecular modelling

Creatio of ligands on the basis of info present in databases, e.g. Crystallographic structures, primary sequenques etc.

Biomimetic Approach

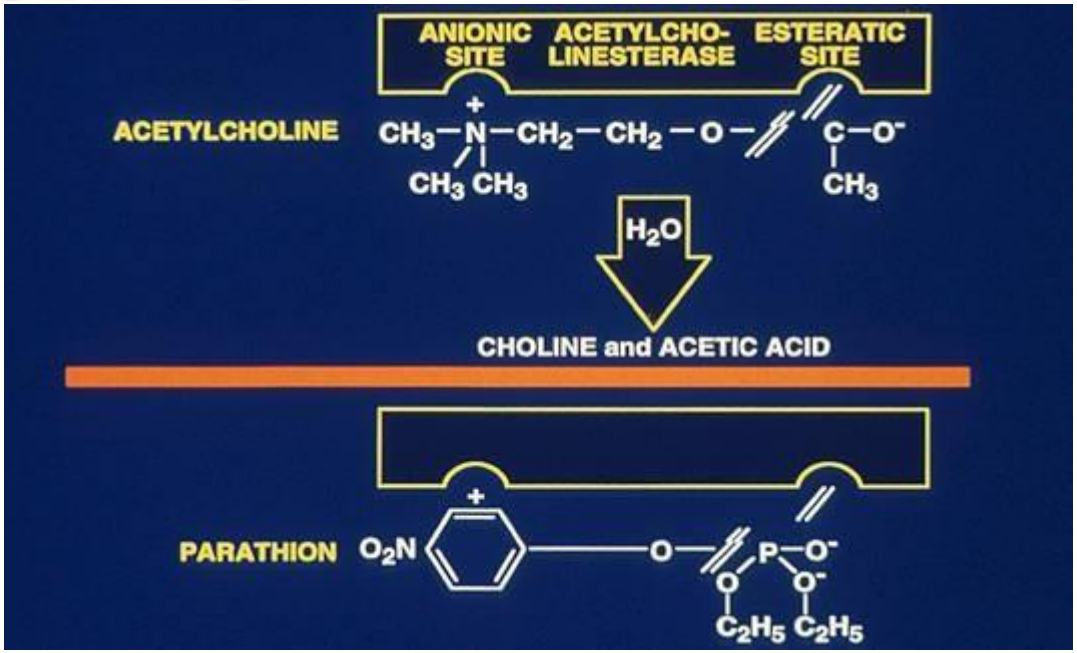
- Starting from the biological structure we thought to reproduce with natural amino acids the proper shape of binding dock

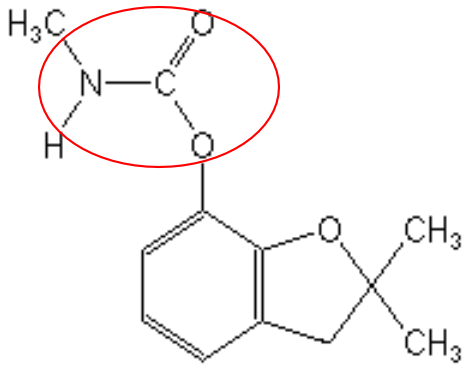
- The biomimetic approach relies on the design and development of artificial oligopeptides as a mimic of the biological binding site by using molecular modeling

✓Why oligopeptides?

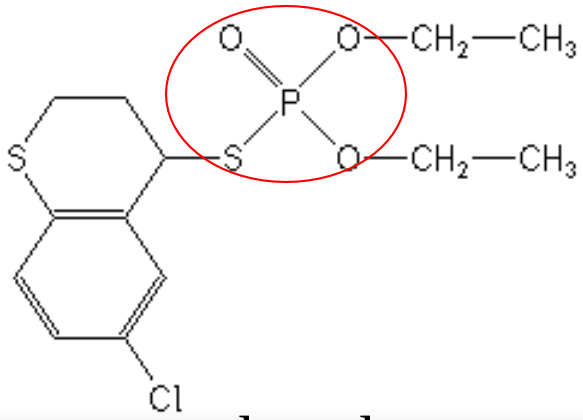
- Nature exploited aminoacids structures to obtain the most of receptors
- Oligopeptides have the advantage of informatics help from the point of the crystallographic informations from native proteins
- Great number of combinations using 20 aminoacids which can do any binding traps

BIOMIMETIC RECEPTORS FOR PESTICIDES





Carbamate



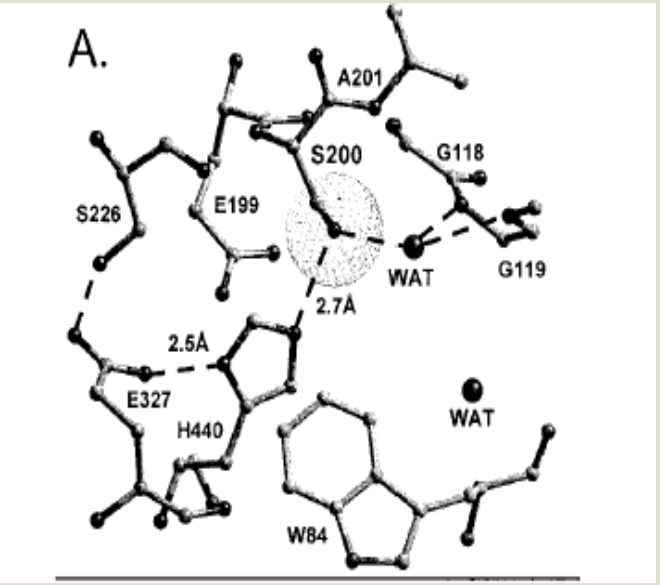
organophosphate

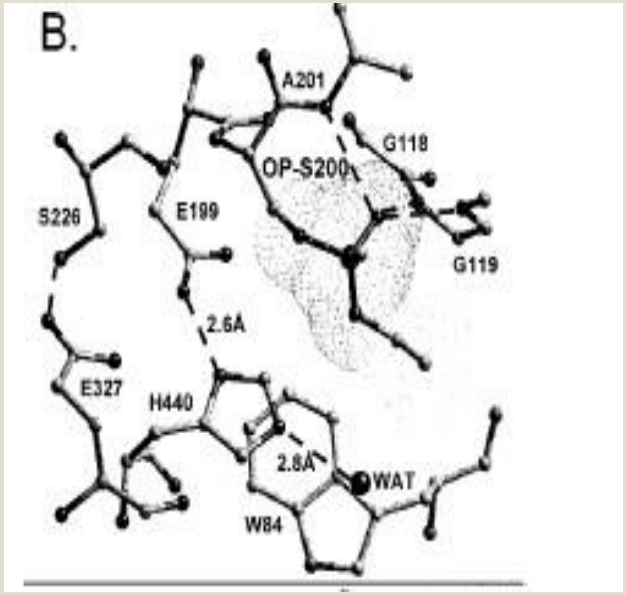
✓Mechanism of AChE inhibition

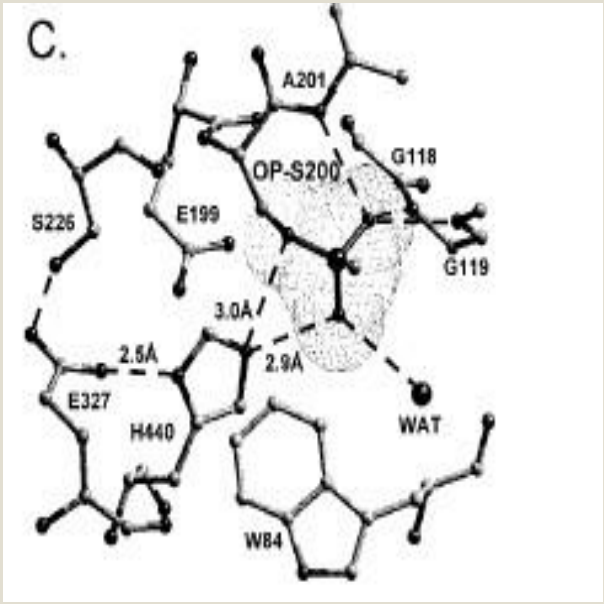
AChE, the target enzyme of pesticides, **is an efficient serine hydrolase** that catalyzes the breakdown of acetylcholine (ACh)

Acetylcholine + $H_2O \rightarrow$ 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

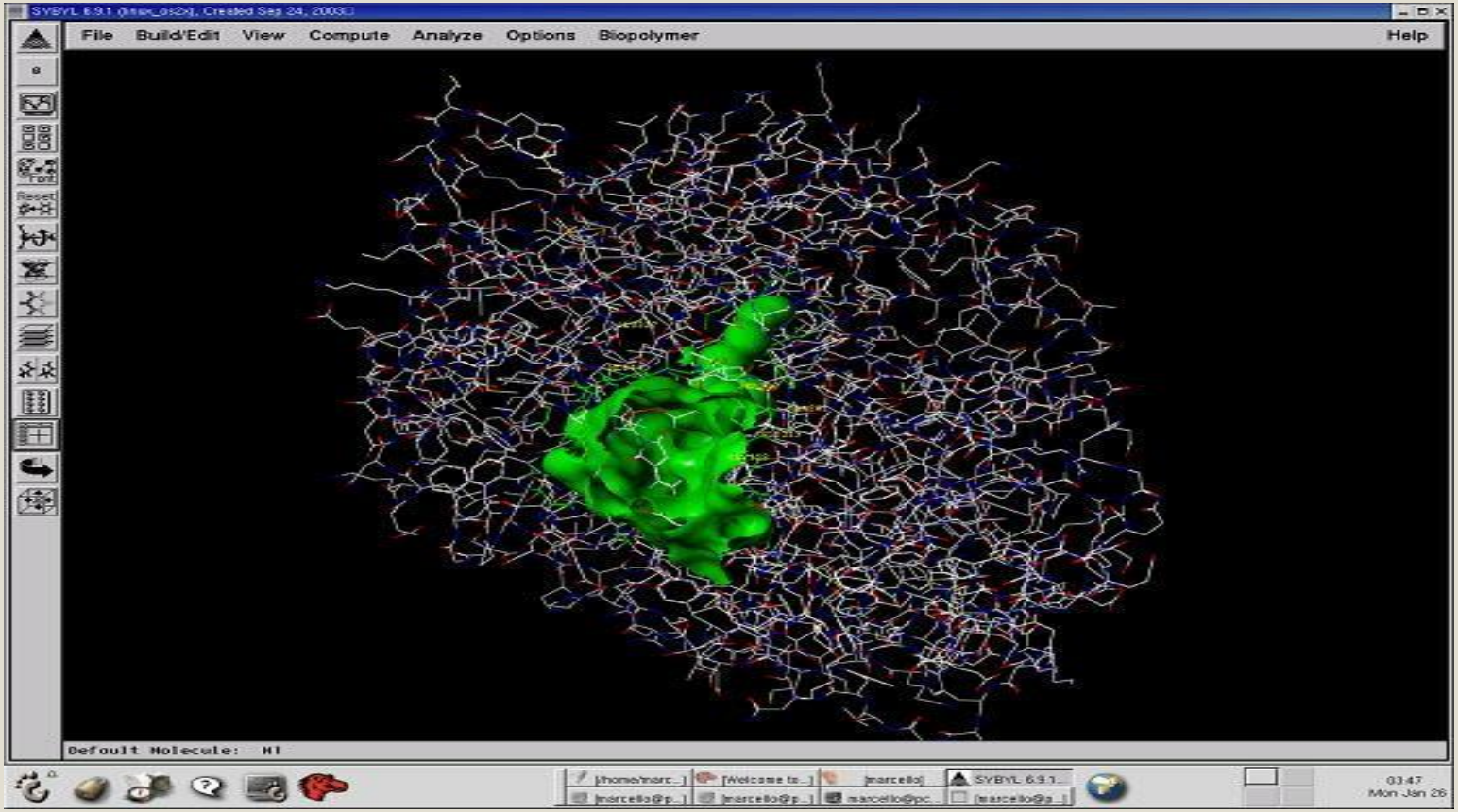
Aged structure: For reaction of AChE with VX and most phosphonates, aging predominates, and dealkylation results in movement of H440 to the negatively charged pocket formed by E327 Ox, S200 Ox, and one anionic oxygen of the dealkylated OP

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

❖Computational screening

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

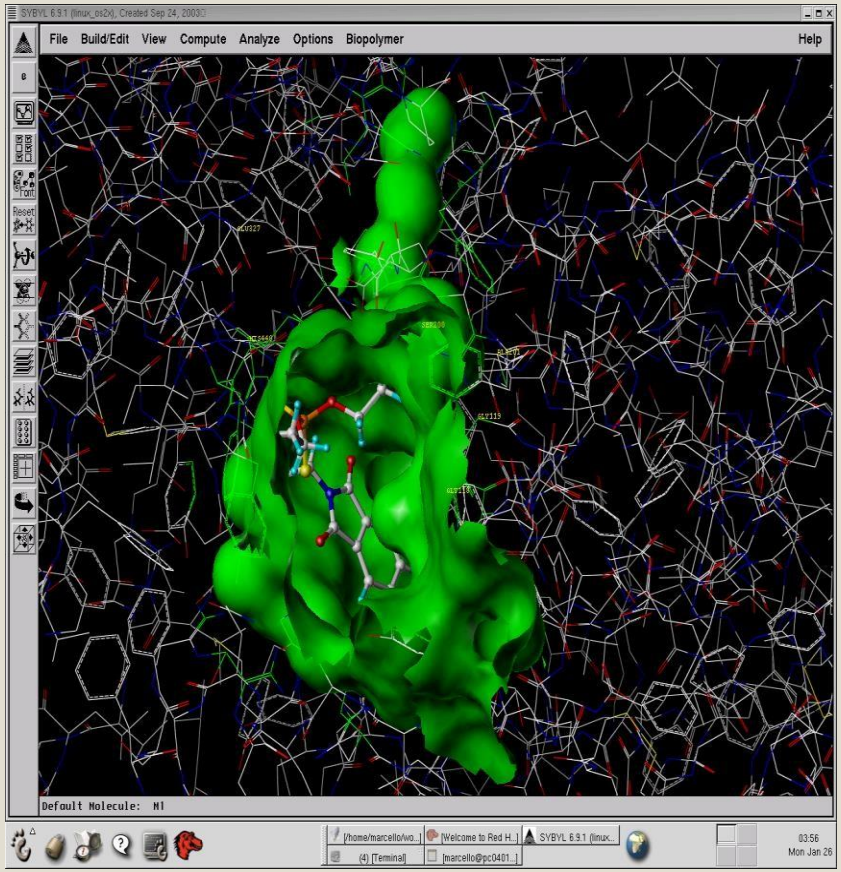
Methylphosphonylated Acetylcholinesterase (Aged) Obtained By Reaction With O-Ethyl-S-[2-[Bis(1-Methylethyl) Amino]Ethyl] Methylphosphonothioate (Vx) conventional X-ray crystallography resolution [Å]: 2.40

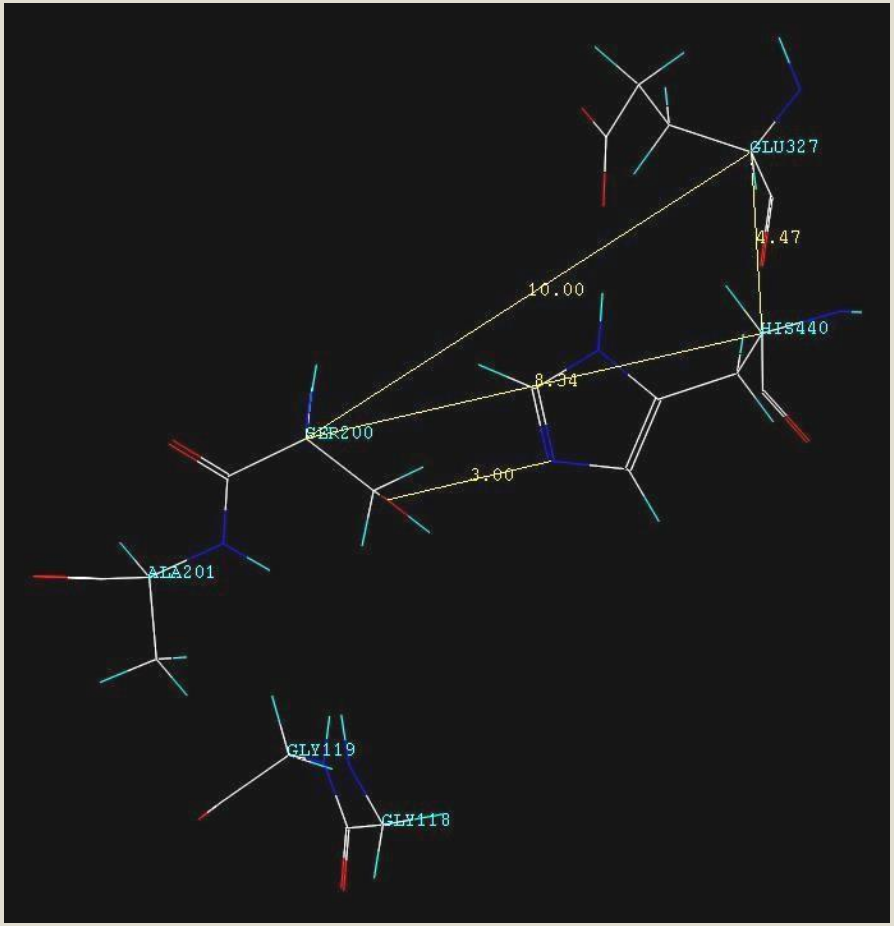


In green the molecular electrostatic potential distribution on the surface of the enzyme binding pocket

✓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

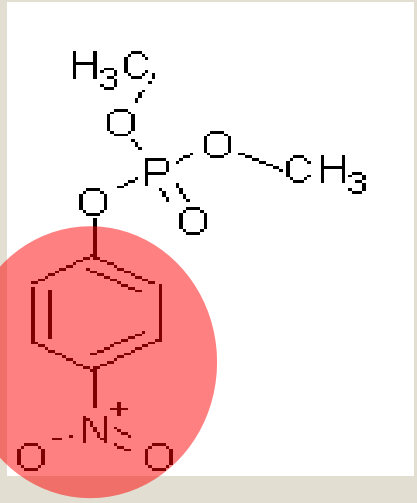
✓Simulated binding results vs paraoxon of the tetrapeptides selected for experimental screening

	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**

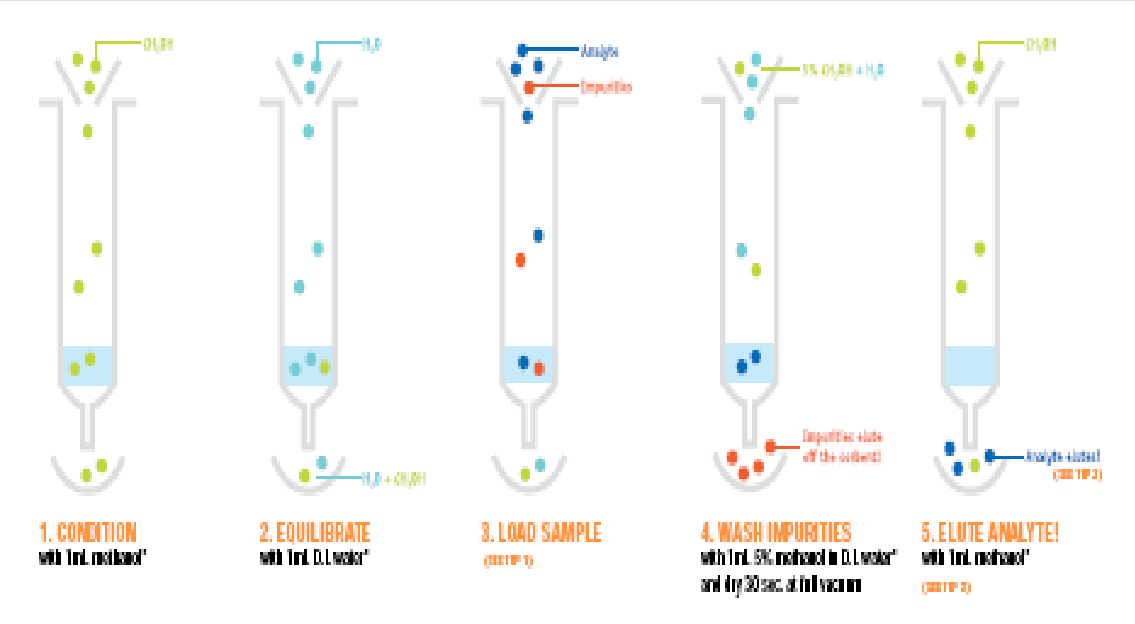
→ **B** **His-Gly-Ser-Ala**

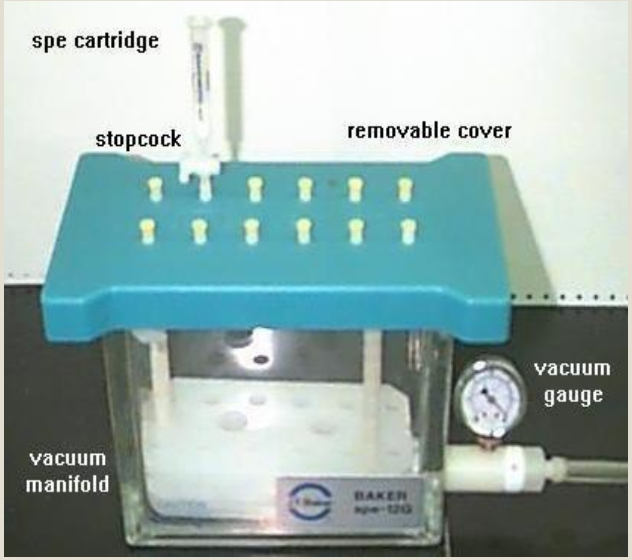
→ **C** **Glu-Pro-Ser-Ala**

→ **D** **His-Glu-Pro-Ser**

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

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





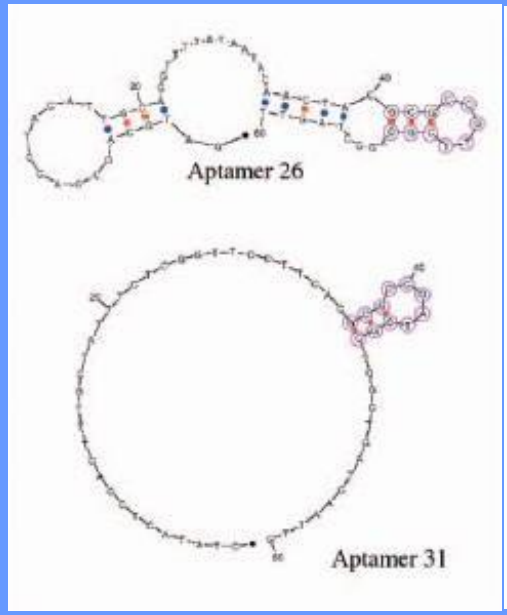
is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure. The substance to be purified is specifically and reversibly adsorbed to a ligand (binding substance), immobilized by a covalent bond to a chromatographic bed material (matrix). 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.

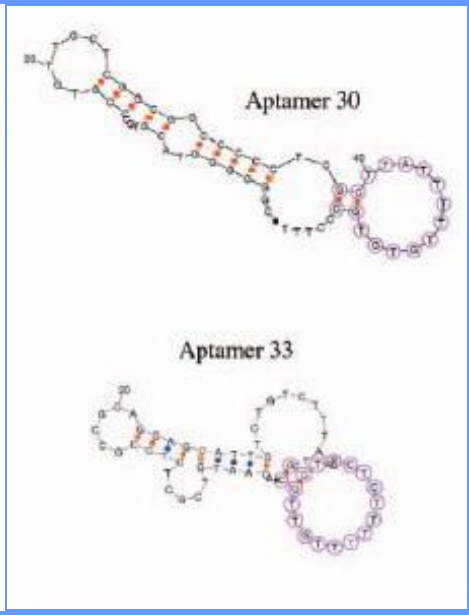
Aptamers are oligonucleotides (DNA or RNA molecules) that can bind with high affinity and specificity to a wide range of target molecules (proteins, peptides, drugs, vitamins and other organic or inorganic compounds).

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)

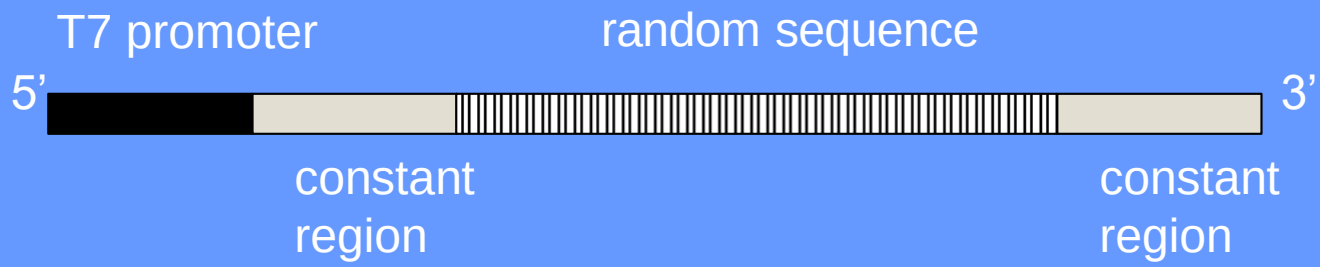
Their name is derived from the Latin word "**aptus**" which means "to fit".

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!!!!

SELEX

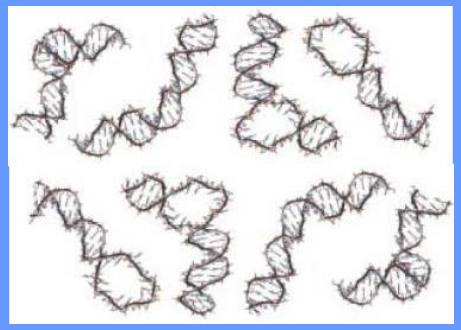
random sequence

5’ 3’

constant region

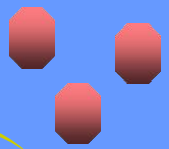
constant region

PCR

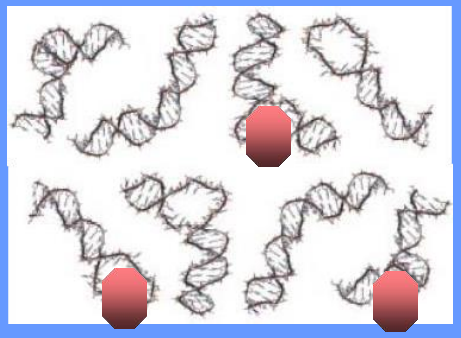


DNA or RNA pool

Amplification

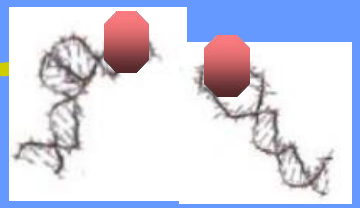


Target



Incubation of target and RNA or DNA

Elution of RNA or DNA



Partition of binding from non-binding species

Target molecules

PROTEINS

Syrian golden hamster prion
Escherichia coli SelB
L-selectin
Tyrosine phosphatase
Ff gene 5
Thrombin
HIV-1 Tat
HIV-1 Rev
Vascular endothelial growth factor
Prostate specific antigen
Human IgE
Taq DNA polymerase
Iron regulatory protein
Human oncostatin M
Human neutrophil elastase
Human CD4 antigen
Lysozyme
C-reactive protein
Tumor necrosis factor a
NF-kB
Acetylcholine receptor
Thyroid transcription factor

INORGANIC COMPOUNDS

Malachite green
Mg^{2+}

ORGANIC COMPOUNDS

ATP
FMN
Theophylline
Organic dyes
Cocaine

VITAMINS

Cyanocobalamin
Biotin

DRUGS

Neomycin B
Streptomycin
Tobramycin
Tetracyclin
Kanamycine A
Dopamine

TOXINS

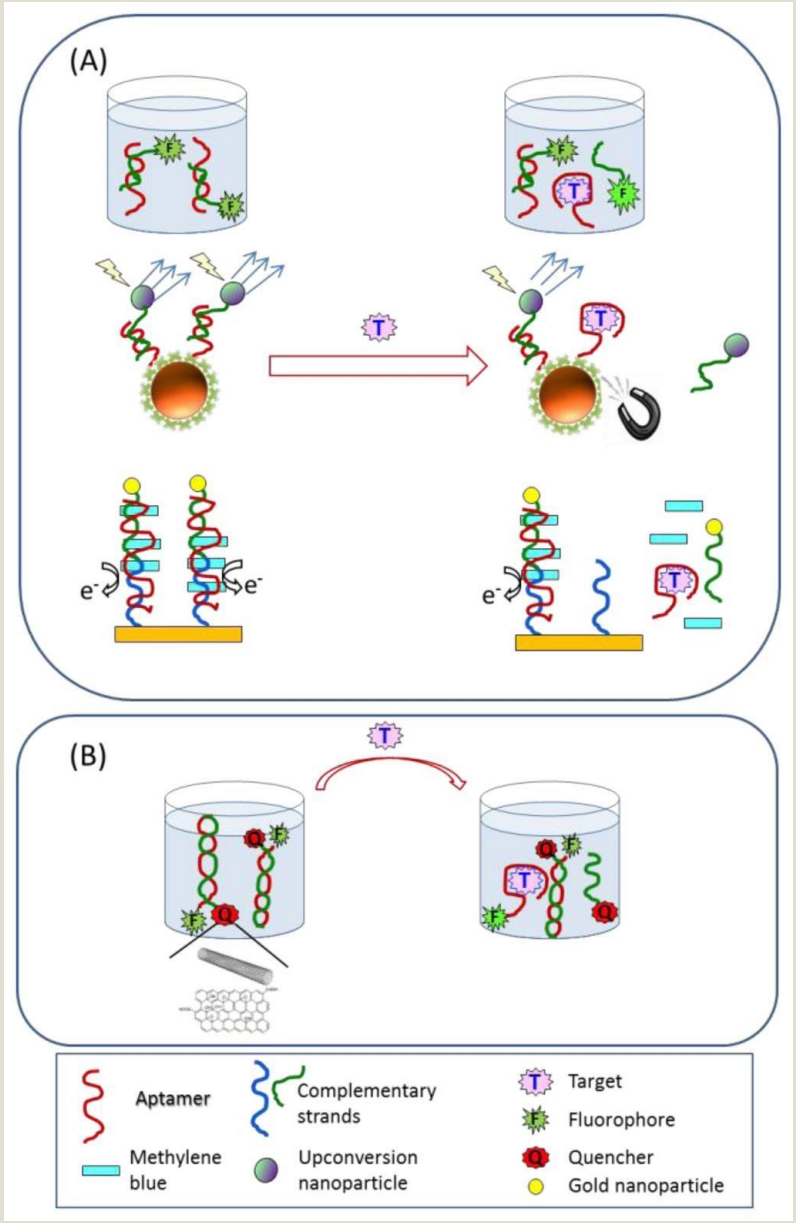
Cholera toxin
Staphylococcal enterotoxin B

POLLUTANTS AND CARCINOGENIC COMPOUNDS

4-chloroaniline
2,4,6-trichloroaniline
Pentachlorophenol
Methylenedianiline

OTHERS

Bacillus anthracis spores

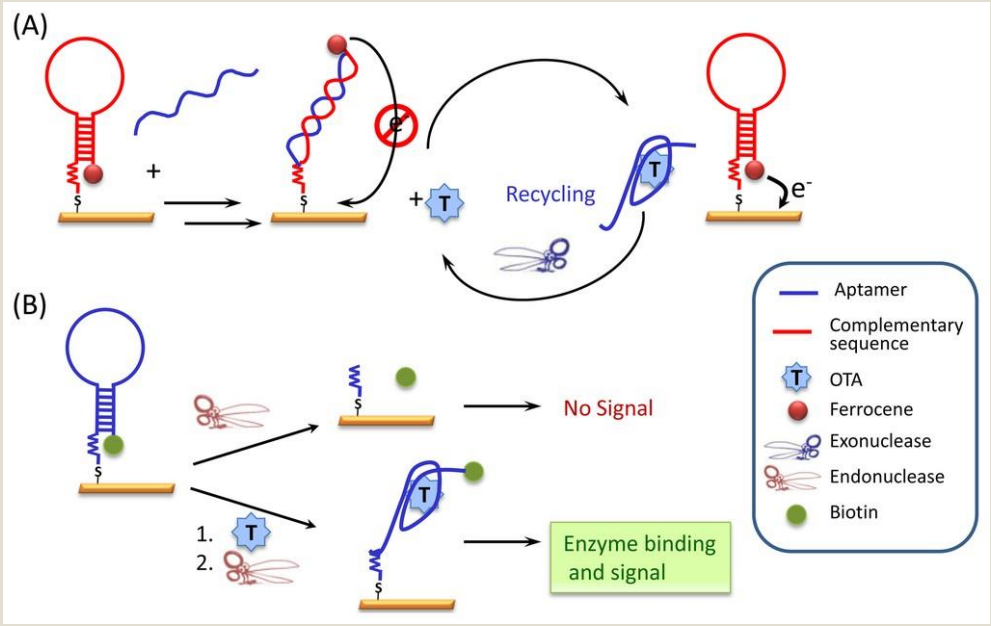


Sensors **2013**, *13*(12), 16292-16311; doi:10.3390/s131216292

Review

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

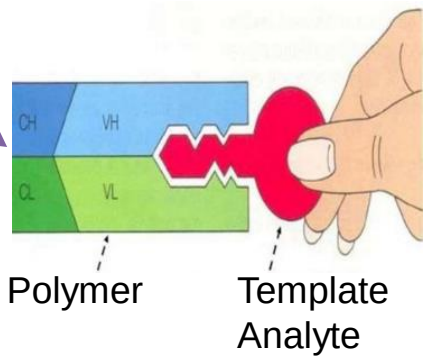
Sonia Amaya-González, Noemí de-los-Santos-Álvarez, Arturo J. Miranda-Ordieres and Maria Jesús Lobo-Castañón



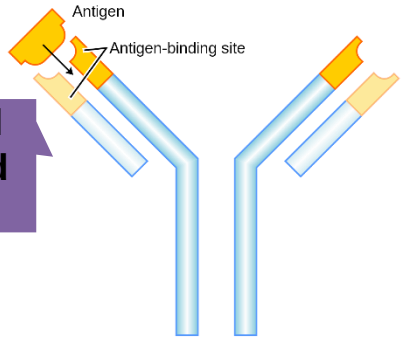
Molecularly imprinted polymers (MIPs) are synthetic receptors for a targeted molecule. As such, they are analogues of the natural antibody–antigen systems

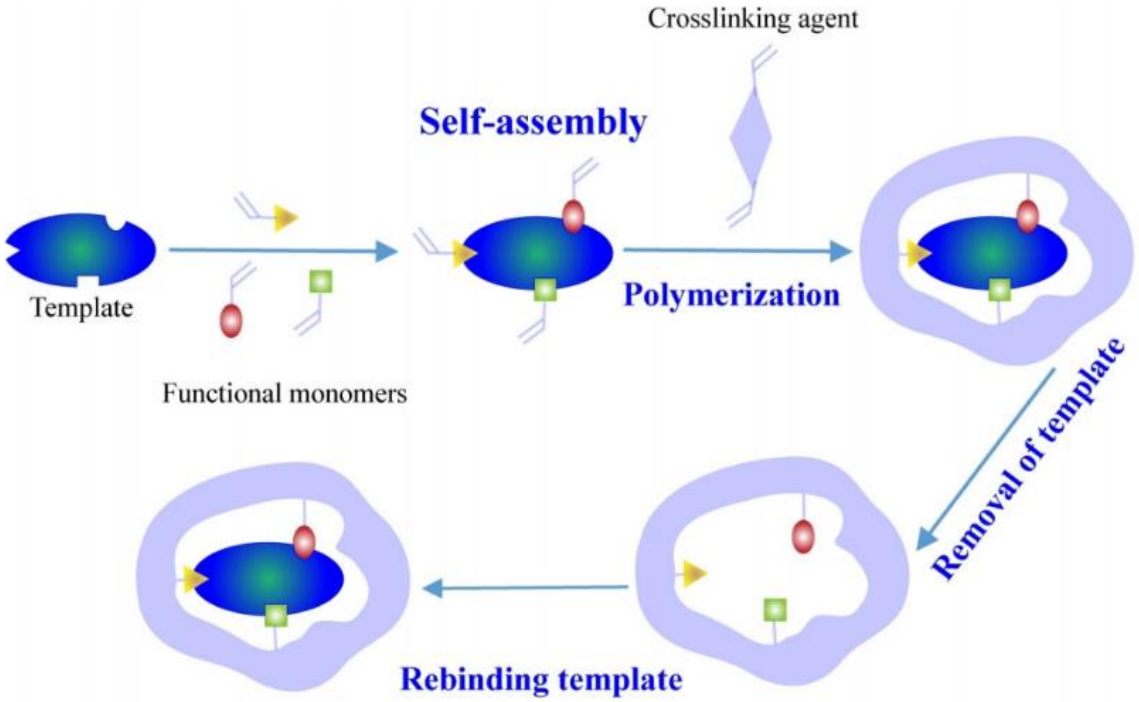
DOI: 10.1021/acs.chemrev.8b00171 Chem. Rev. 2019, 119, 94−119

Plastic Antibody



Natural Antibody





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

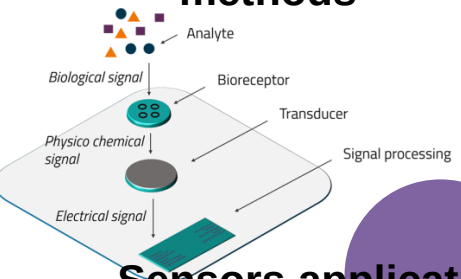
Abdellatif Ait Lahcen[a] and Aziz Amine[a],2018*

MIP-State of the art

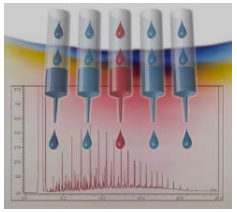
MIPs Applications

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

Sample preparation in bio analytical methods



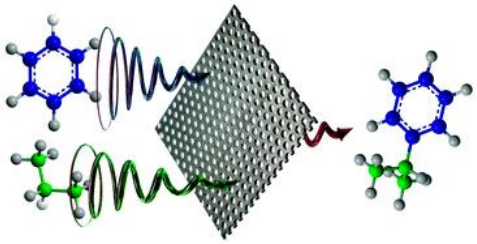
Chromatography



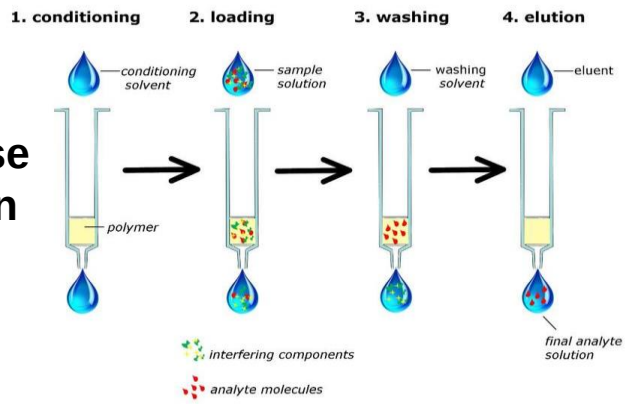
Sensors applications

Drug delivery

Catalysis



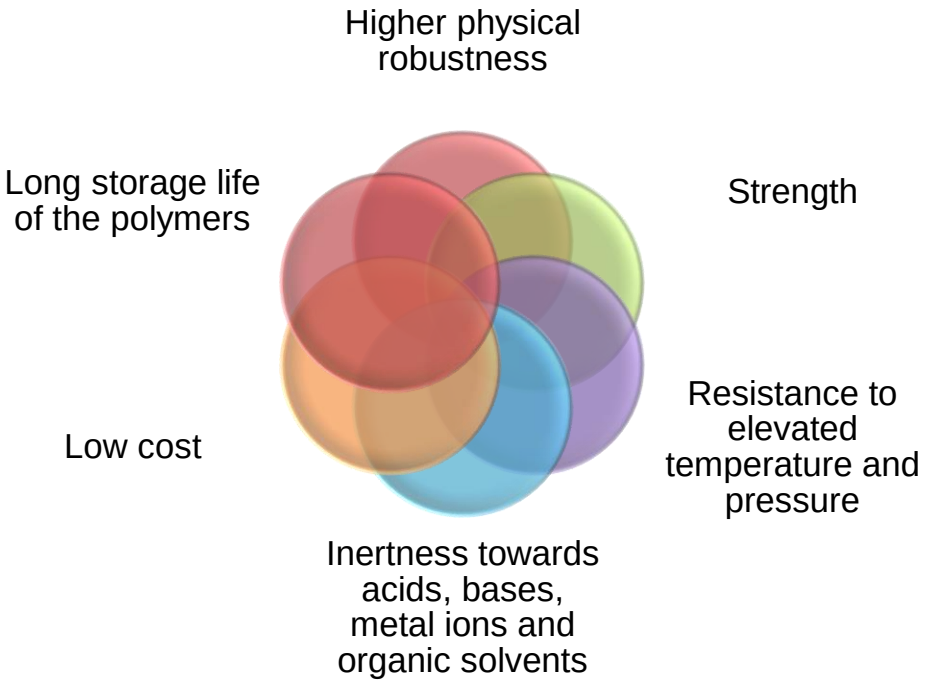
Solid phase extraction



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:

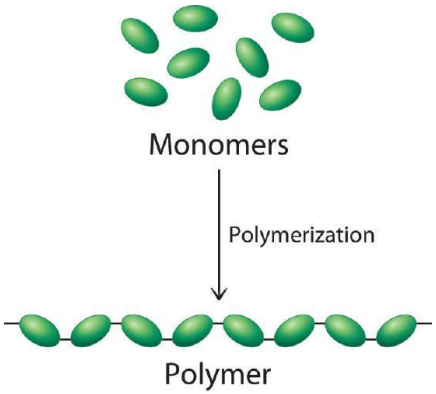


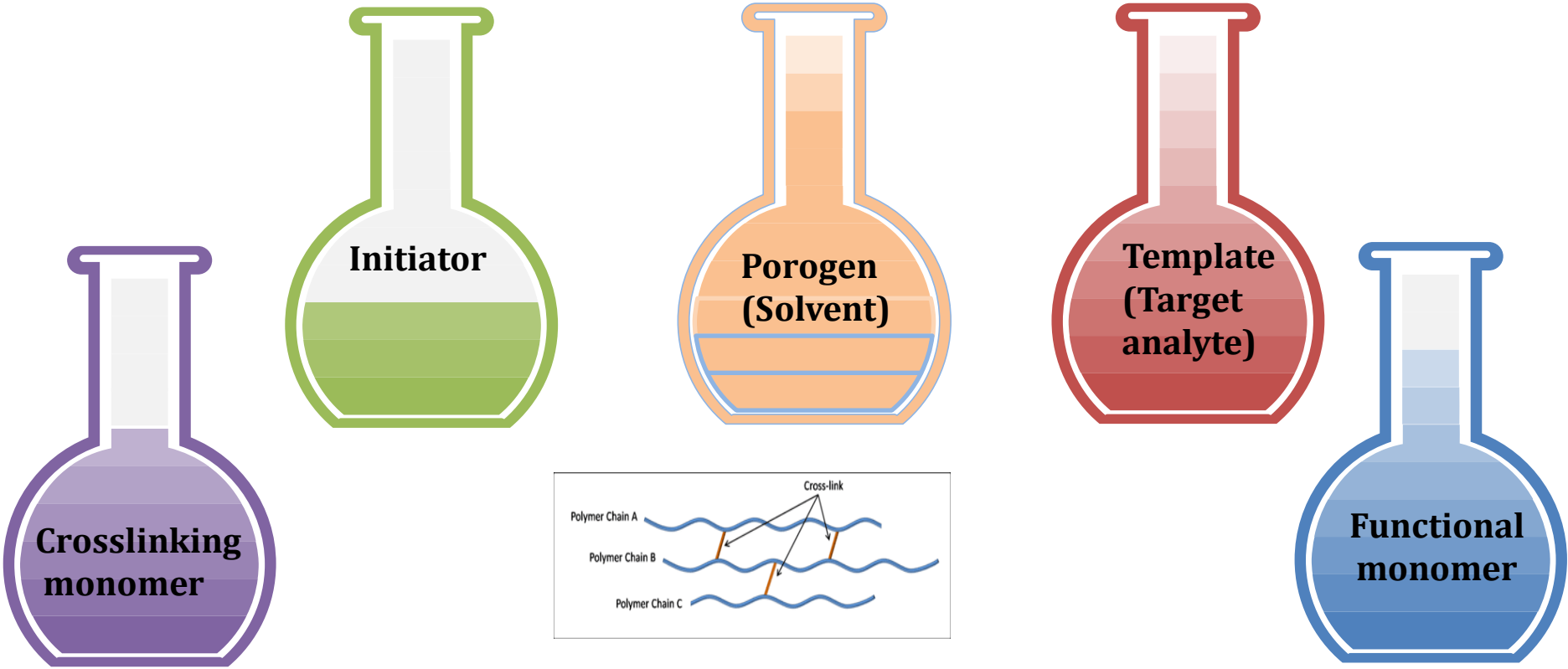
Int J Mol Sci. 2011; 12(9): 5908–5945
***Chem. Rev.* 2000, *100,* 2495-2504**

02 MIP-State of the art

MIPs Synthesis

Components of MIP Mixture





02 MIP-State of the art

MIPs Synthesis

General procedure

Self-assembly step

Polymerization

Extraction

MIPs

Porogen solvent

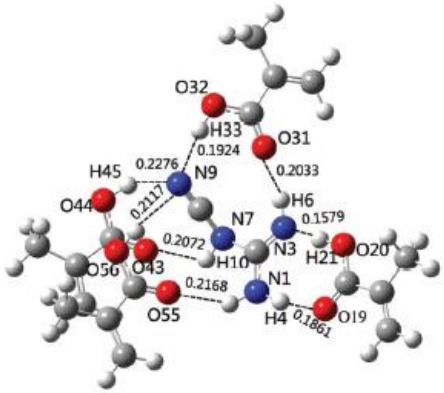
Monomer

Template

Cross-linker

Initiator

2:00



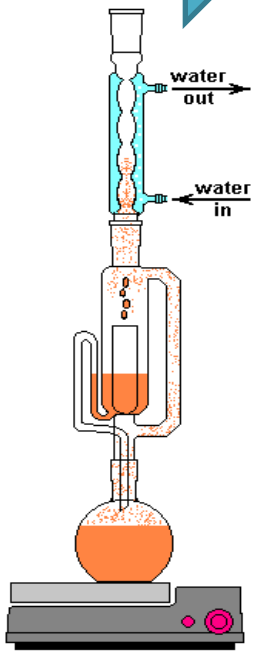
Nitrogen in

Water out

Water in

60 °C

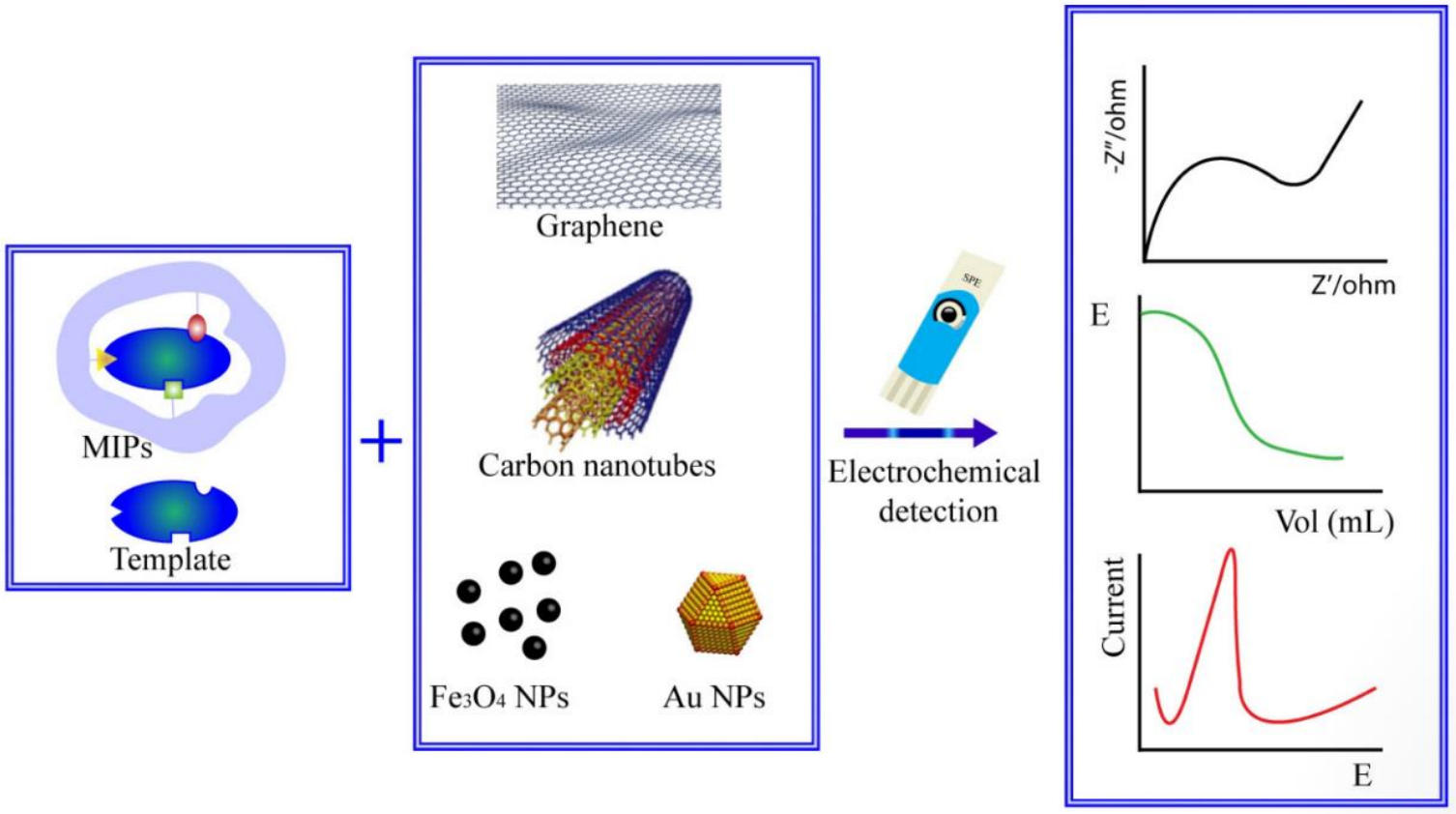
24:00



Soxhlet extractor

02 MIP-State of the art

MIP based electrochemical sensors and



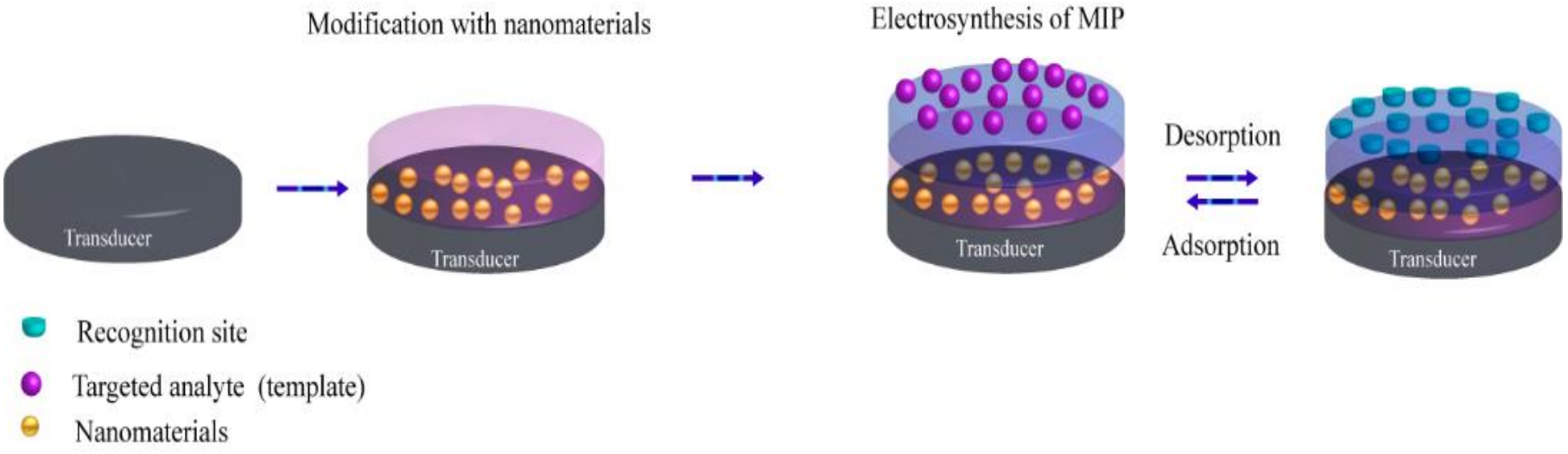
Scheme 2. Schematic illustration of MIP based electrochemical sensors and nanomaterials.

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

02 MIP-State of the art

MIP based electrochemical sensors and nanomaterials

Electrosynthesis of MIPs



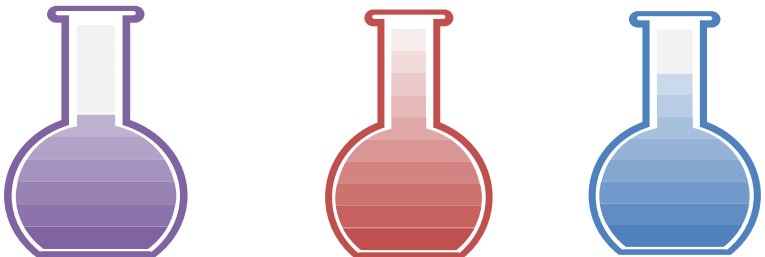
~~**Initiator**~~

~~**Crosslinking monomer**~~

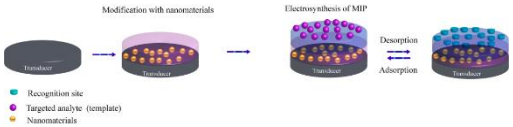
Template (Target analyte)

Porogen (Solvent) Buffer

Functional monomer



02 MIP-State of the art



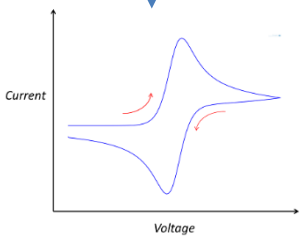
MIP based electrochemical sensors and nanomaterials

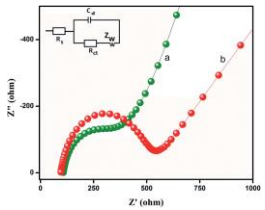
Food contaminant

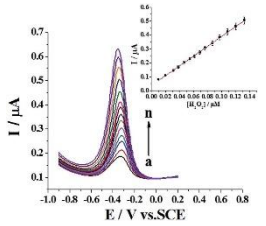
Electrochemical behaviour

Detection

- Template
  - Non-electroactive
    - Voltametric measurement of redox marker ferricyanide
    - Impedance spectroscopy
  - Electroactive
    - Direct electrochemical detection







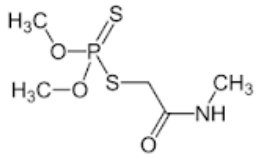
02 MIP-State of the art

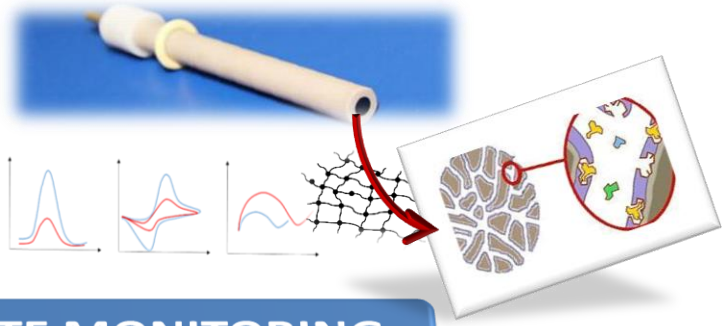
Conclusions

	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

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

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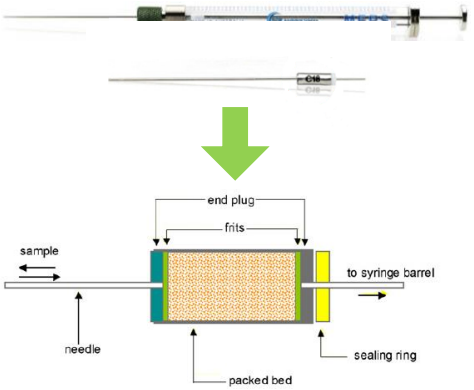

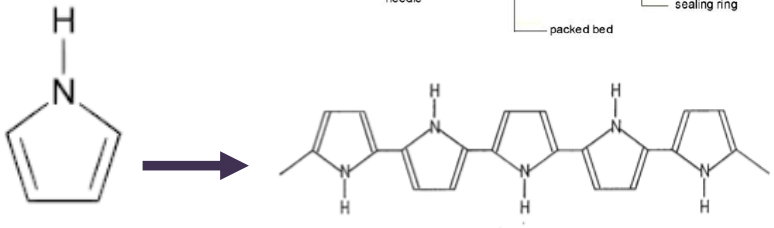

MIP-MEPS based sensing strategy for the selective assay of dimethoate. Application to wheat flour samples

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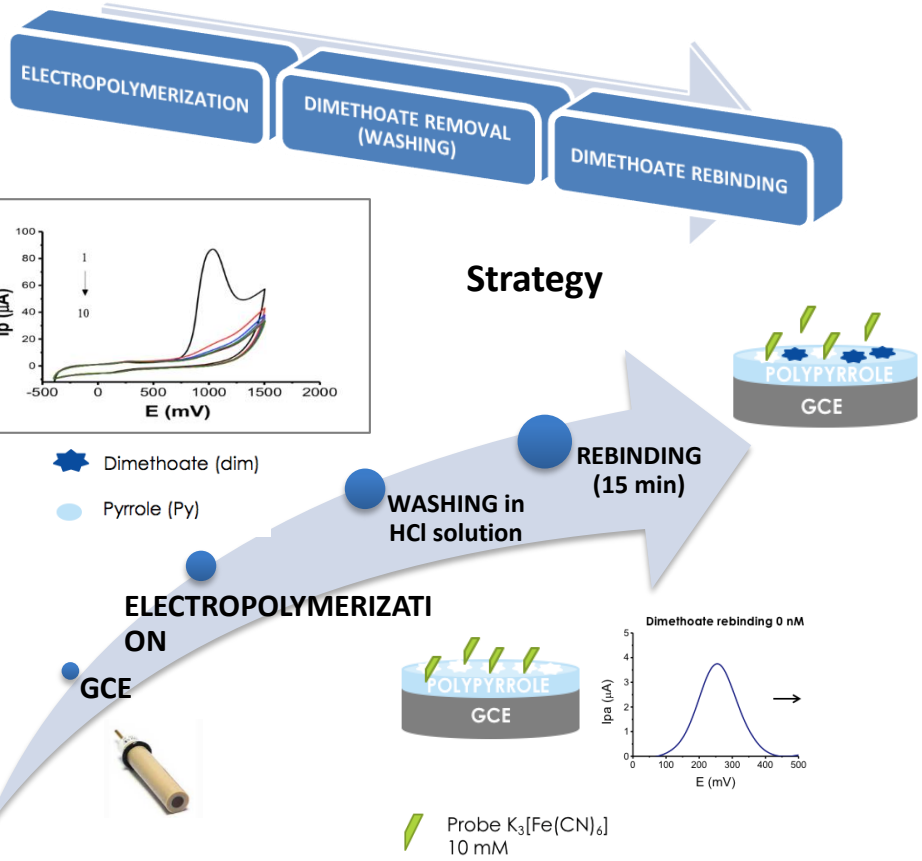
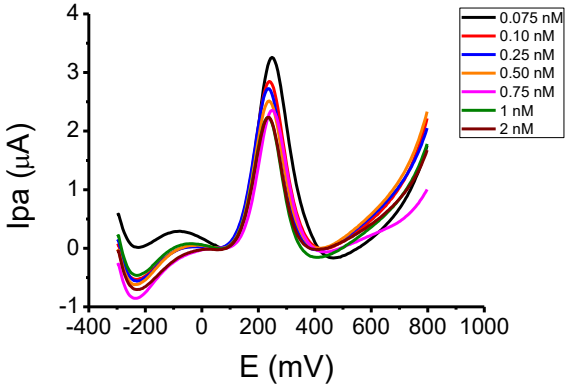
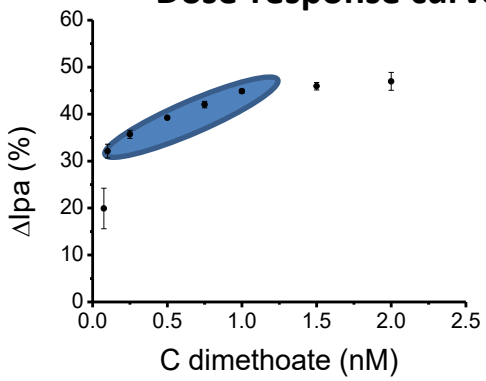




Dose-response curve



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

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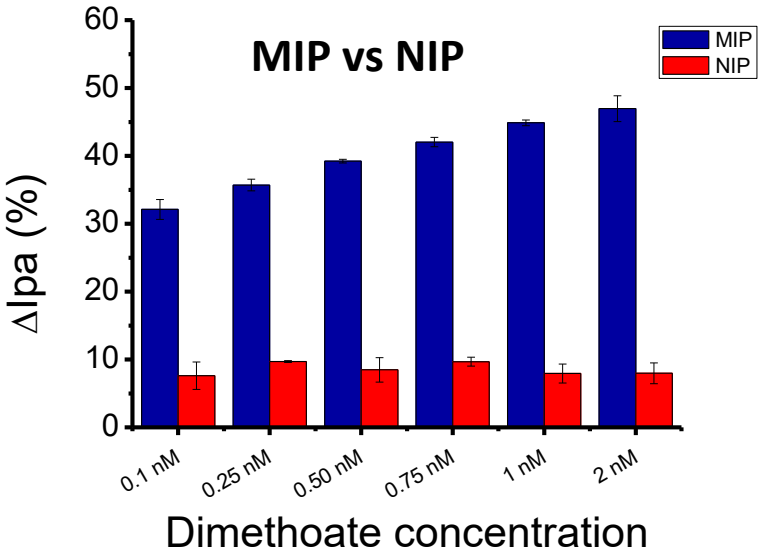
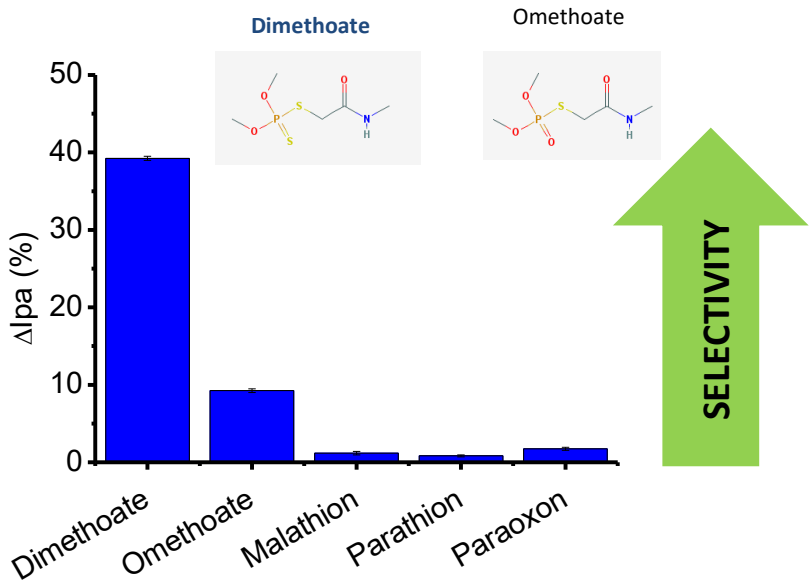
ΔIpa (%)	Repeatability (RSD %)	Reproducibility (RSD %)
0.5 nM dimethoate (n=3)	0.68	2.72
1 nM dimethoate (n=3)	0.95	5.51



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

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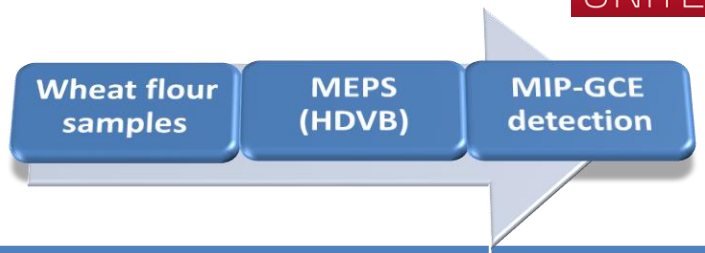
Wheat flour samples: MIP vs. UHPLC-MS/MS



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

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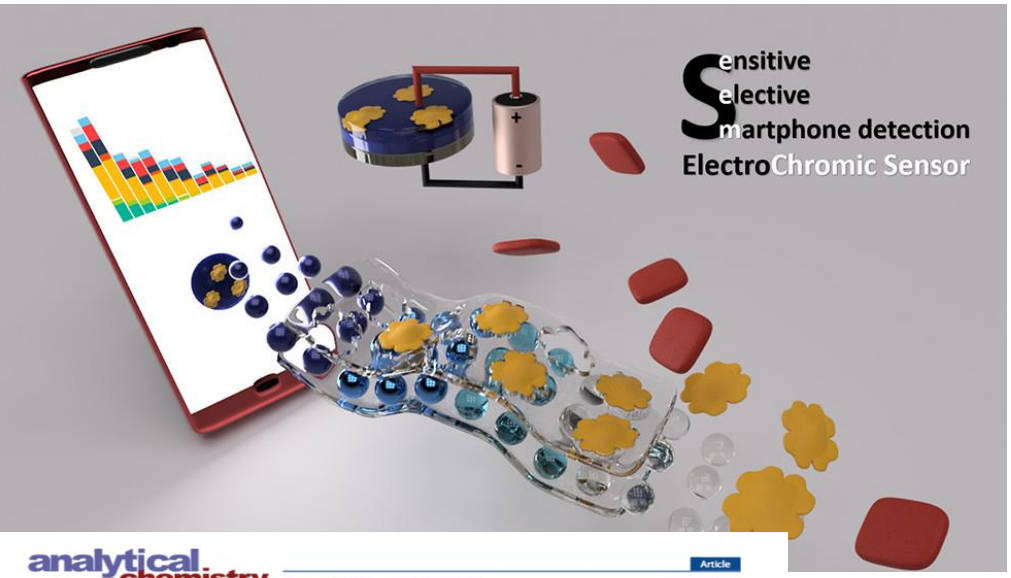

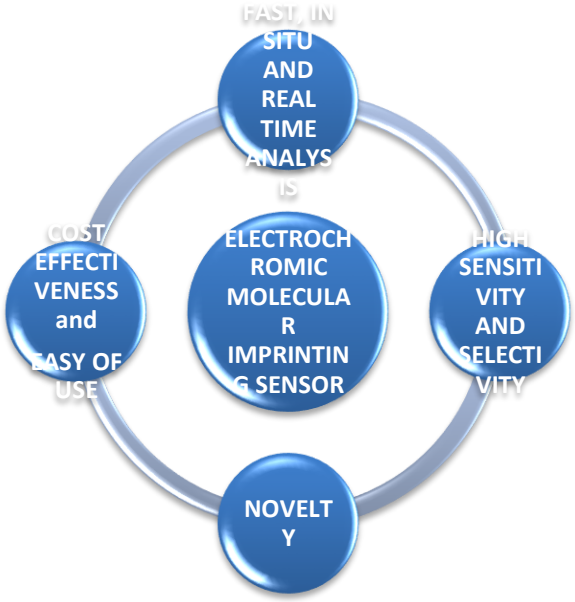

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Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

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Electrochromic Molecular Imprinting Sensor for Visual and Smartphone-Based Detections

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Electrochromism

Strategy

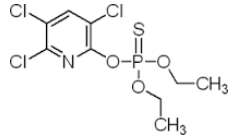
MIP

Screen-printing technology

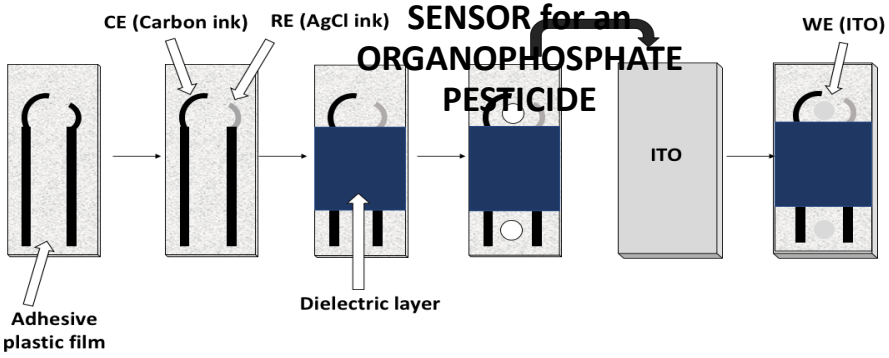
Smartphones

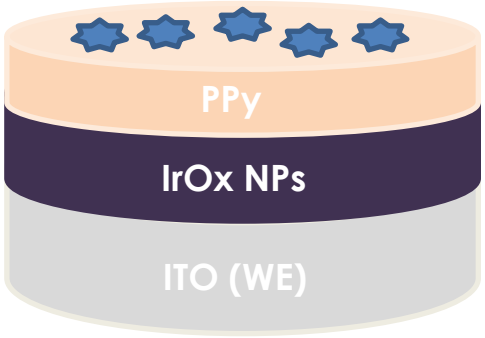
CPF (chlorpyrifos)

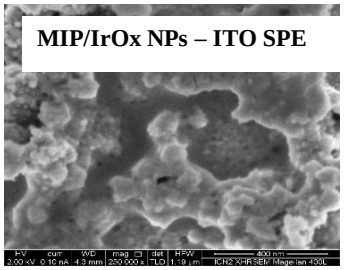
Py



ELECTROCHROMIC MOLECULAR IMPRINTING SENSOR for an ORGANOPHOSPHATE PESTICIDE







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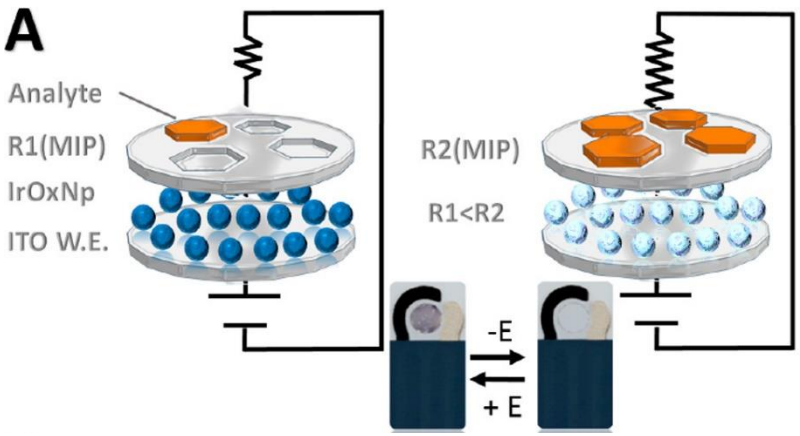
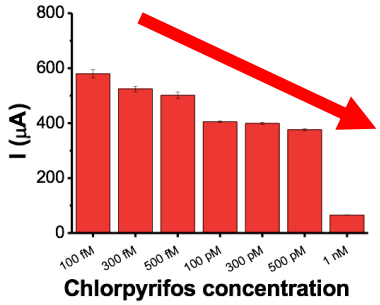
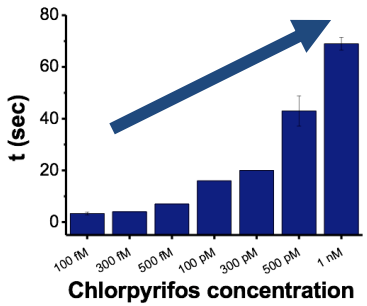
WORKING PRINCIPLE







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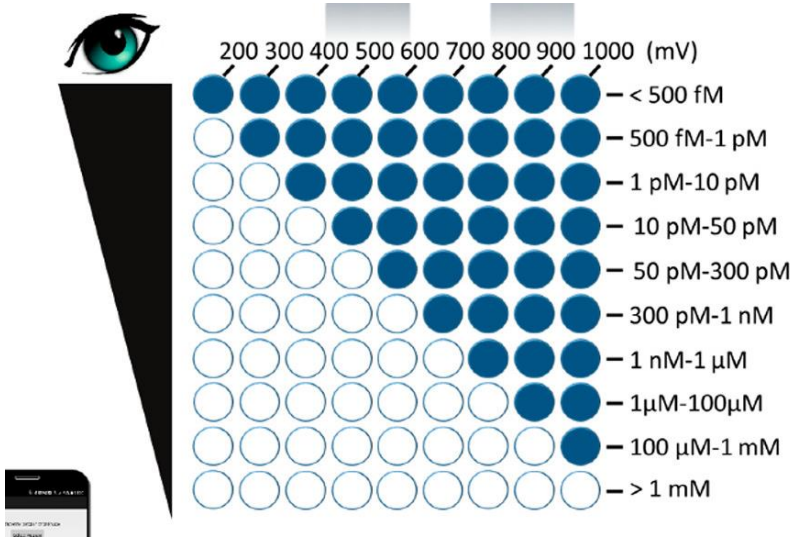
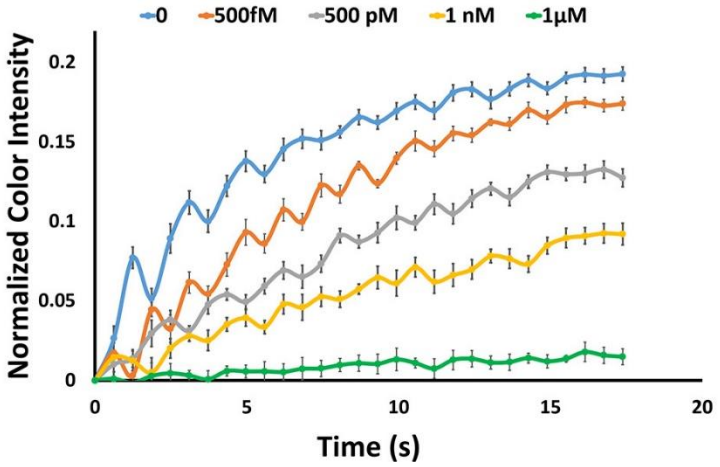
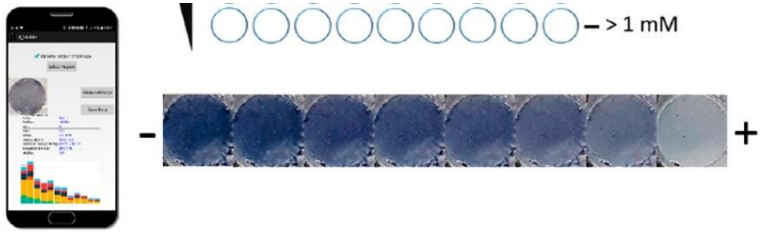
VISUAL APPROACH



SMARTPHONE APPROACH





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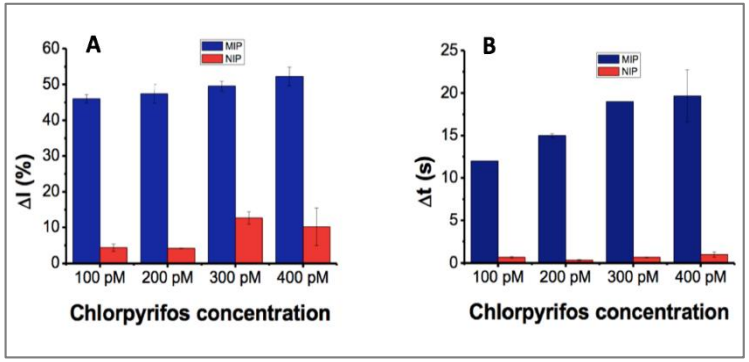
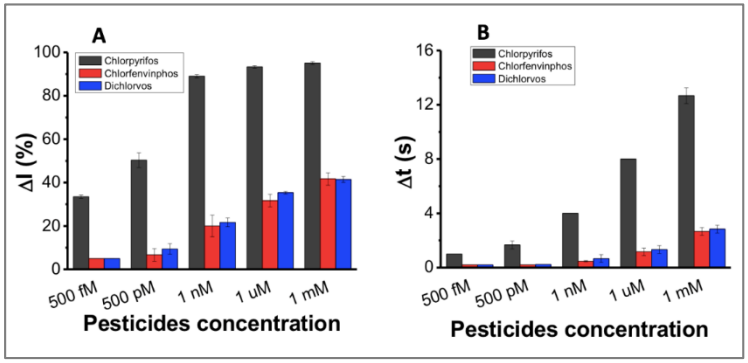
MIP vs NIP



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