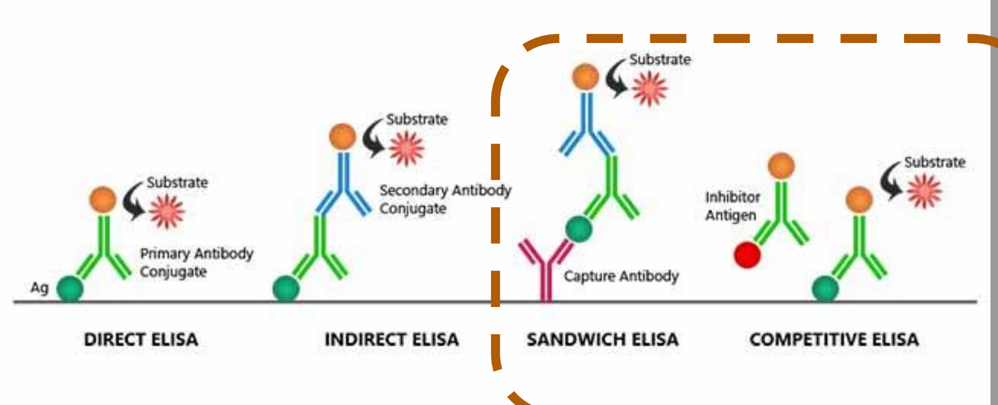
CONCENTRATION MOLES/LITER	METABOLITES/IONS	THERAPEUTIC DRUGS	STEROID AND AMINO ACID HORMONES	PROTEIN/POPLYPEPTIDE HORMONES	ANTIBODIES
10^{-1}	SODIUM				
	CHLORIDE				
10^{-2}		ETHANOL			
	GLUCOSE				
(mM)	UREA				
10^{-3}	CHOLESTEROL				
	CALCIUM	SALICYLATE			IgG (Total)
	TRIGLYCERIDES	ACETAMINOPHEN			
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)
				PARATHYROID HORMONE	
10^{-10}			ALDOSTERONE	HGH (Growth Hormone)	
				LH (Luteinizing Hormone)	
10^{-11}			TSH (Thyroxine Stim.		
			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



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.

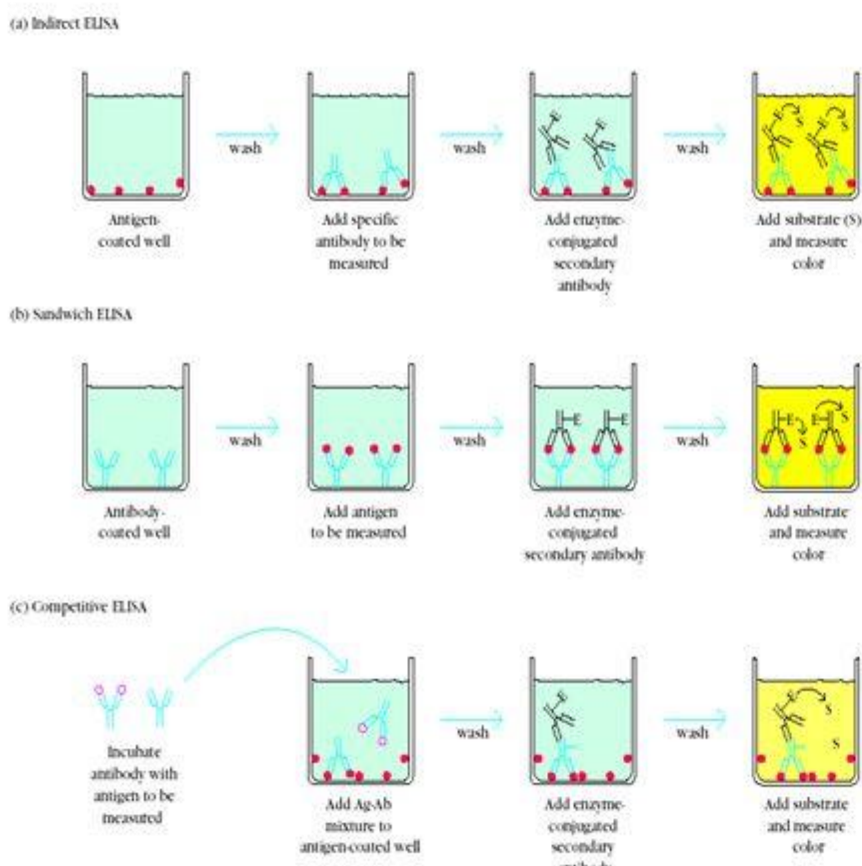
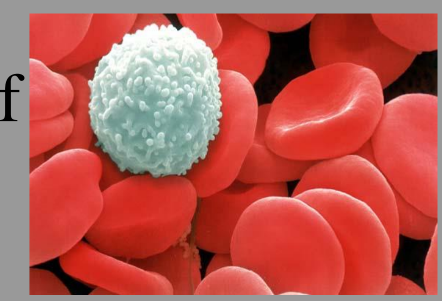


FIGURE 6-10 Variations in the enzyme-linked immunosorbent assay (ELISA) technique allow determination of antibody or antigen. Each assay can be used qualitatively, or quantitatively by comparison with standard curves prepared with known concentrations of antibody or antigen. Antibody can be determined 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.

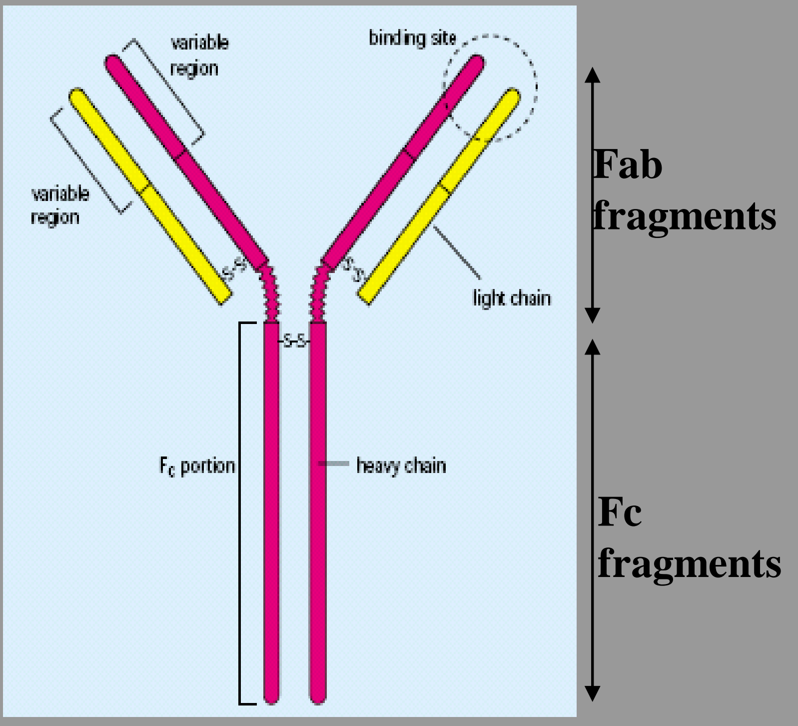
Antibodies



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

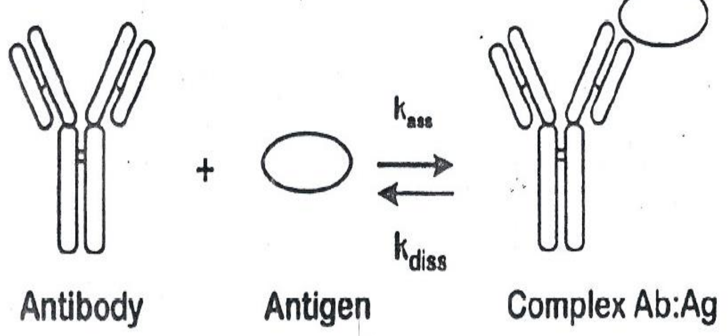
- Recognise and bind to molecules (**antigens**) on foreign particles, marking them for destruction by T-lymphocytes.

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



IgG molecule

Antibody - Antigen interaction

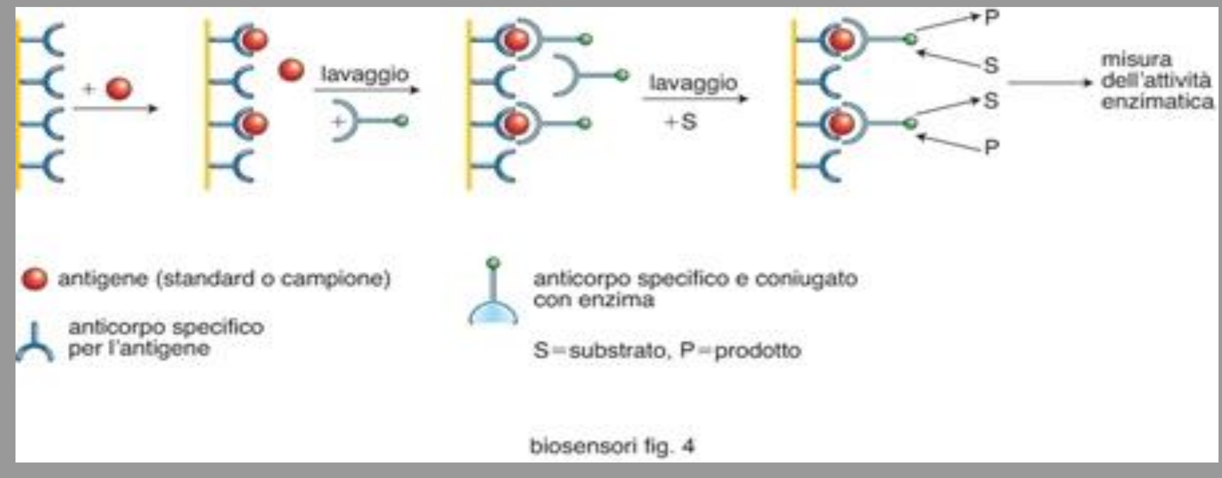


$$v = \frac{d[Ab:Ag]}{dt} = k_{ass}[Ab][Ag] - k_{diss}[Ab:Ag]$$

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

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

Dosaggi
Non competitivi
(sandwich)



Dosaggi
competitivi

con antigene immobilizzato

lavaggio
+S
P
S
S
P
misura dell'attività enzimatica

con anticorpo immobilizzato

lavaggio
+S
P
S
S
P
misura dell'attività enzimatica

antigene (standard o campione)
anticorpo specifico per l'antigene e coniugato con enzima
anticorpo specifico per l'antigene da dosare
antigene coniugato con enzima
S=substrato, P=prodotto

biosensori fig. 3

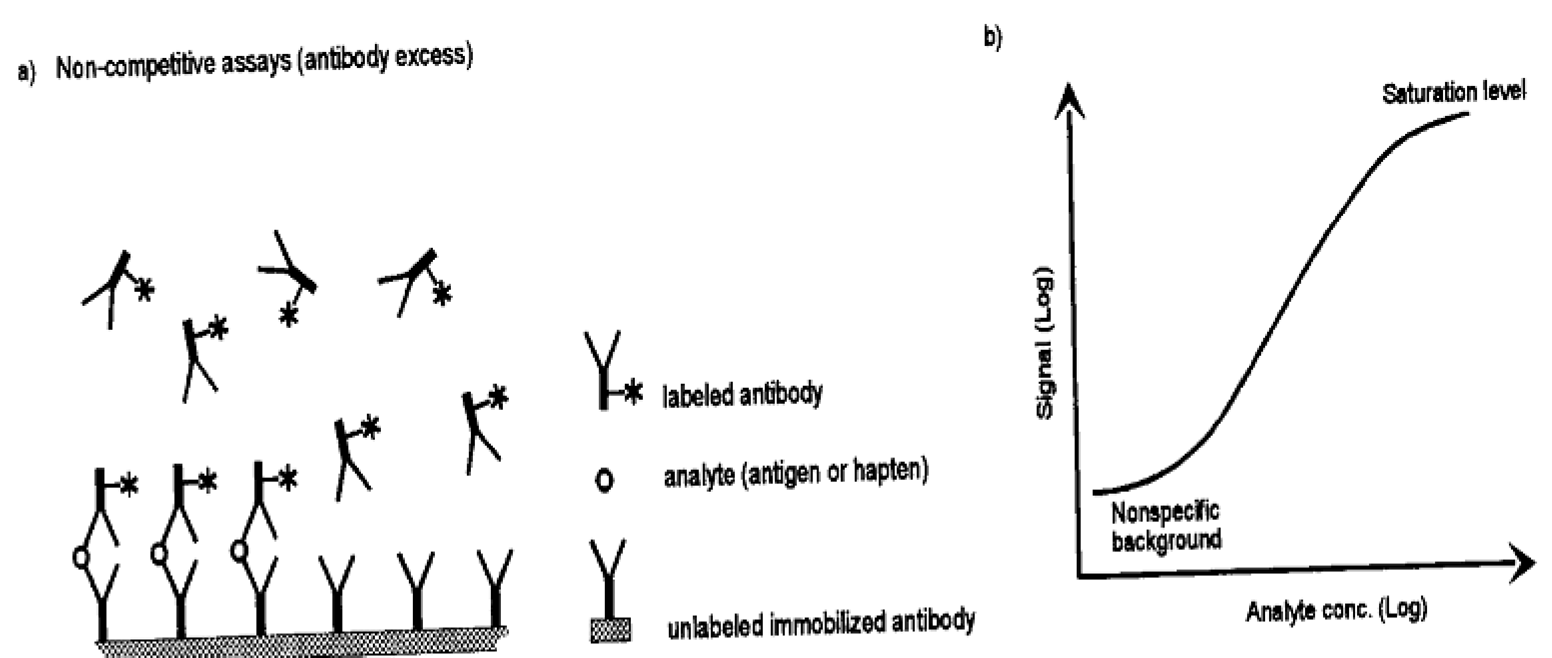


FIGURE 17.3 Schematic drawing of a sandwich immunoassay with typical calibration curve.

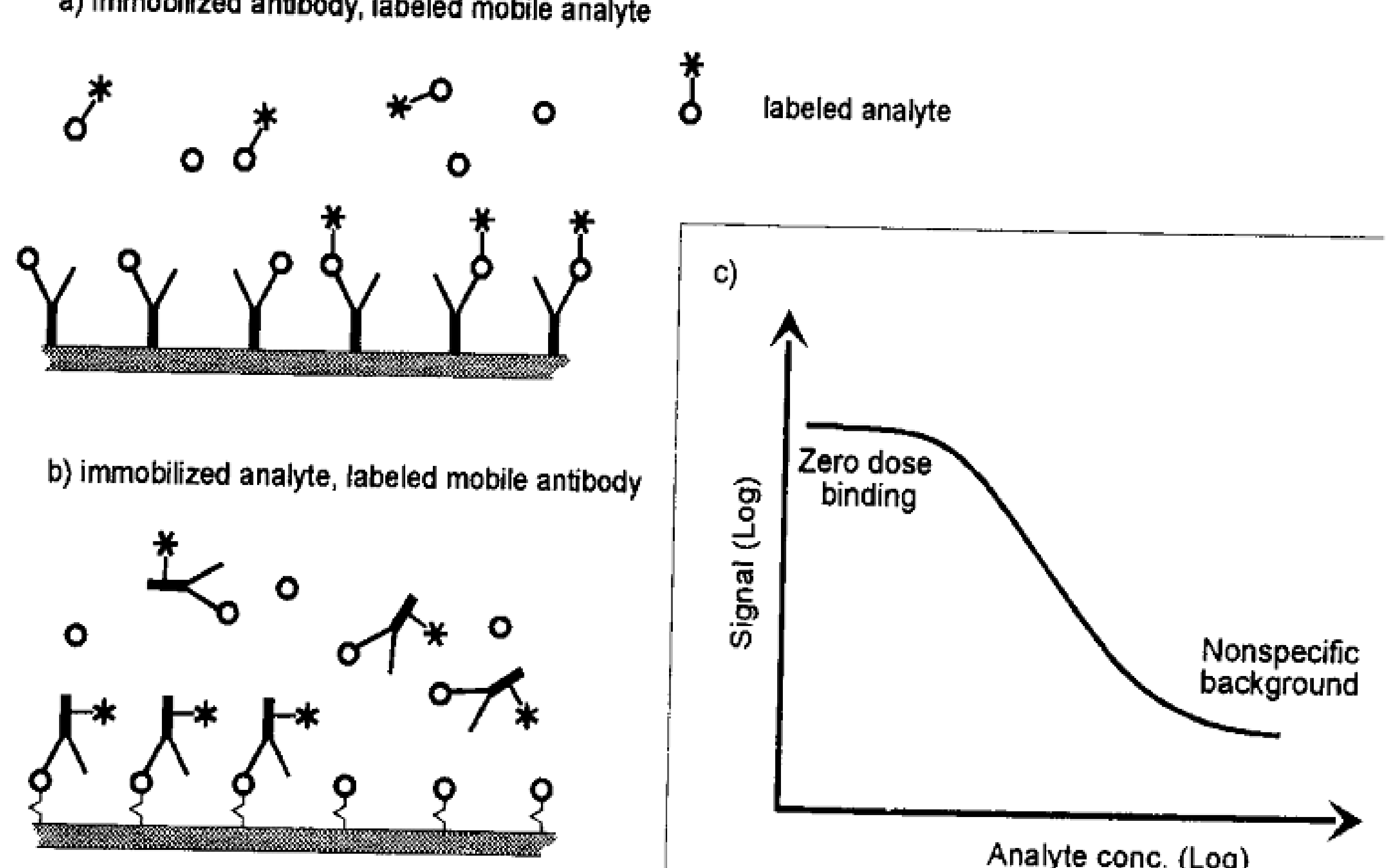


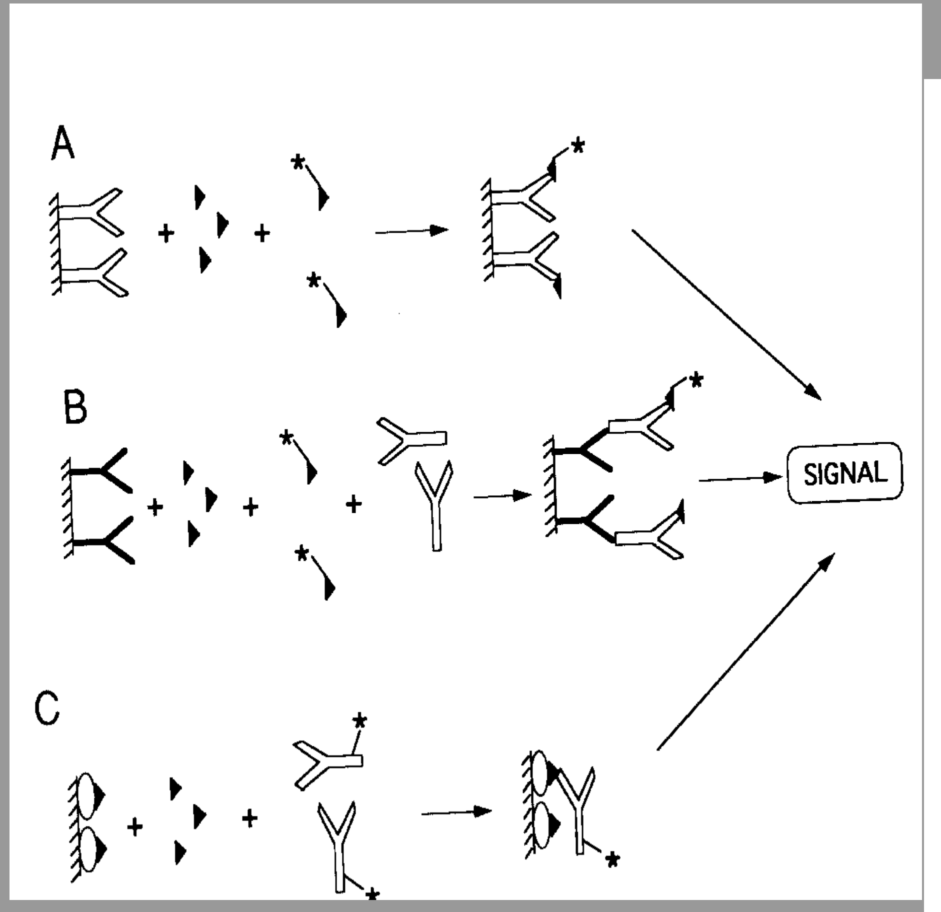
FIGURE 17.4 Competitive immunoassay: (a) competitive binding of analyte and labeled analyte to a limited number of antibody binding sites, (b) immobilized antigen competes with free antigens in solution for binding sites of the labeled antibody, (c) calibration curve obtained with either approach of 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	Sensitive unsafe, high cost
EIA (enzyme immunoassay)	HRP	Color change (absorbance)	Photometer	
	AP			
	β-D-galactosidase			
	HRP, AP, galactosidase	Fluorescence	Fluorimeter	Sensitive Low to medium cost
	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	Very sensitive high cost
LIA (luminescence immunoassay)	Acridinium esters Dioxetanes Peroxyoxalates Luminol Luciferase/luciferin Peroxidase	Chemi- and bioluminescence	Luminometer	
Electrochemical immunoassays	Pyrene	Electroluminescence	Electrode luminometer	Not commonly used
	Metallocenes Metals GOD, catalase	Current	DPP (differential pulse polarograph) DPASV (differential pulse anodic stripping voltammetry)	
	Urease Liposomes	Ions (potential change)	Potentiometric electrode	

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

schemi di immunodosaggi



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

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

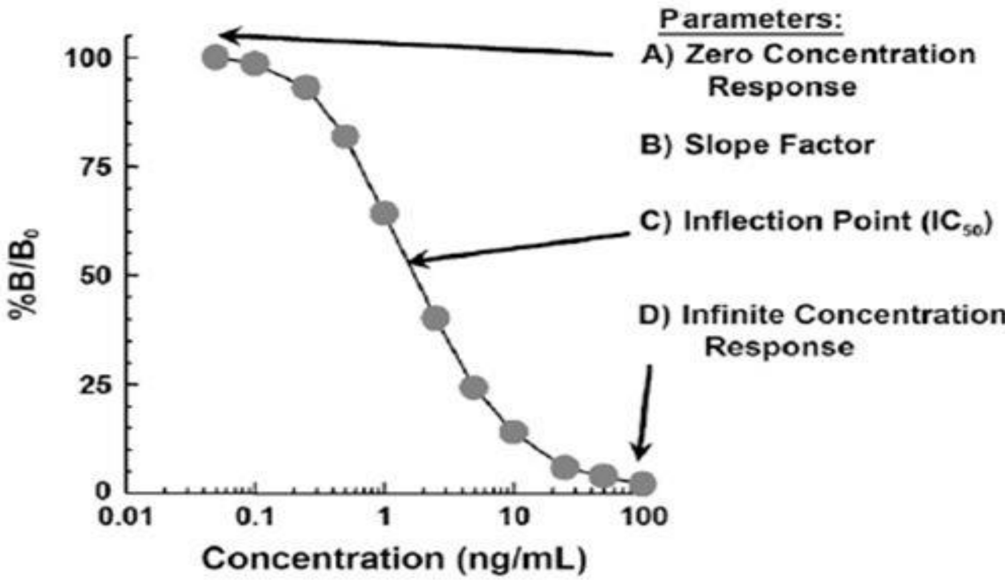
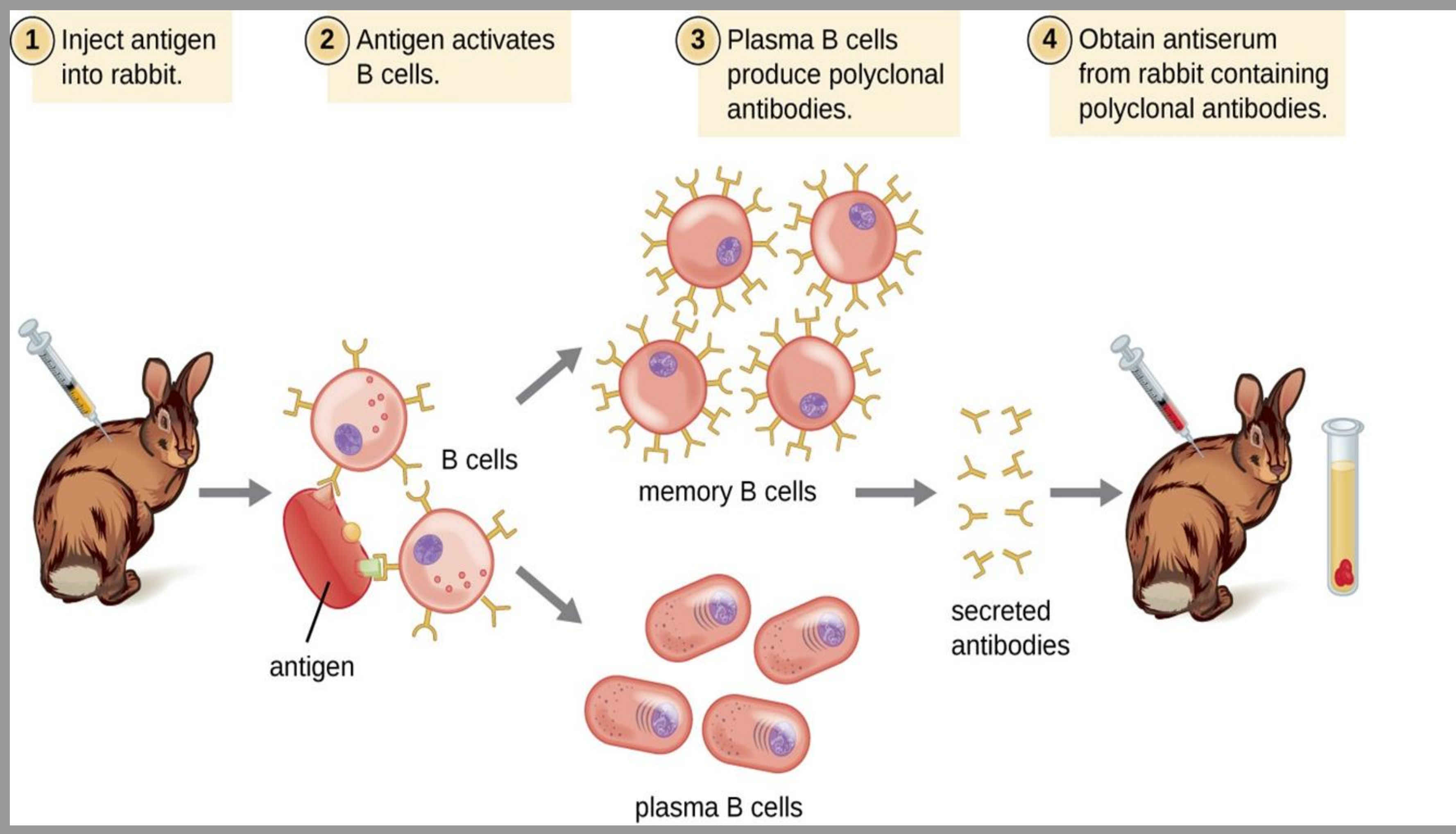
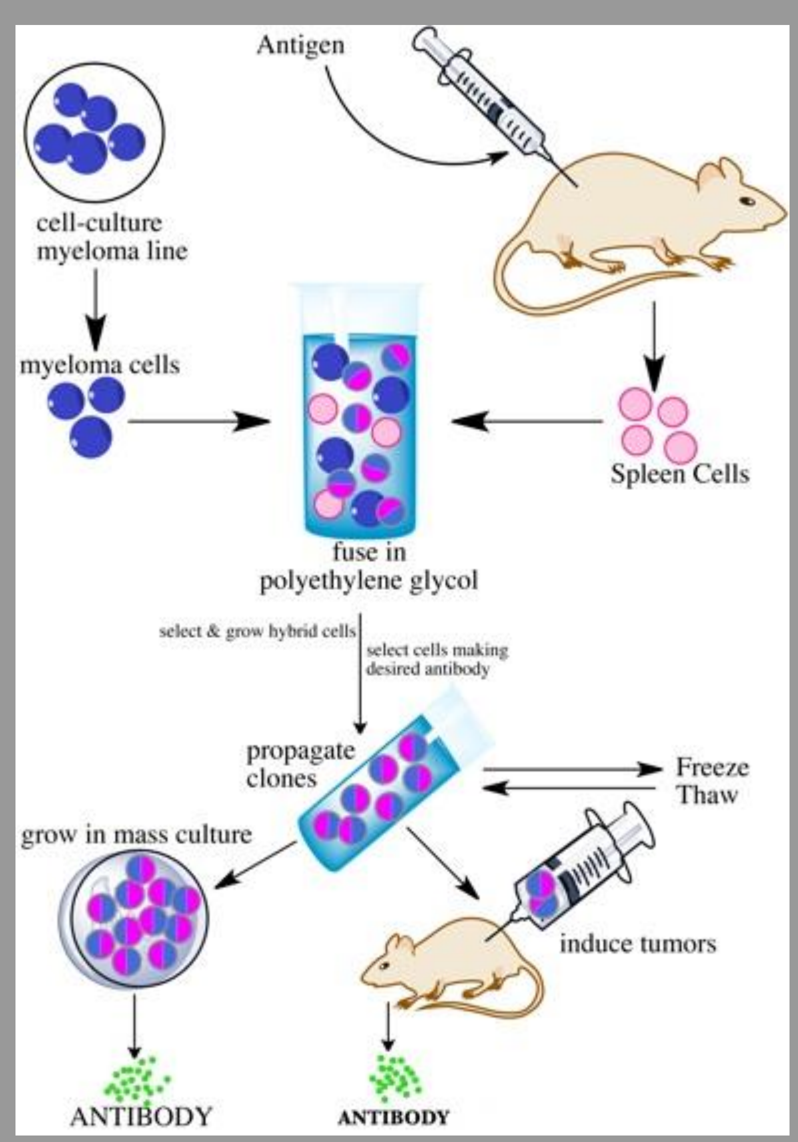


Figure 1. Typical 4-parameter logistic graph for a competitive-format immunoassay.

Polyclonal antibodies production



Monoclonal antibodies

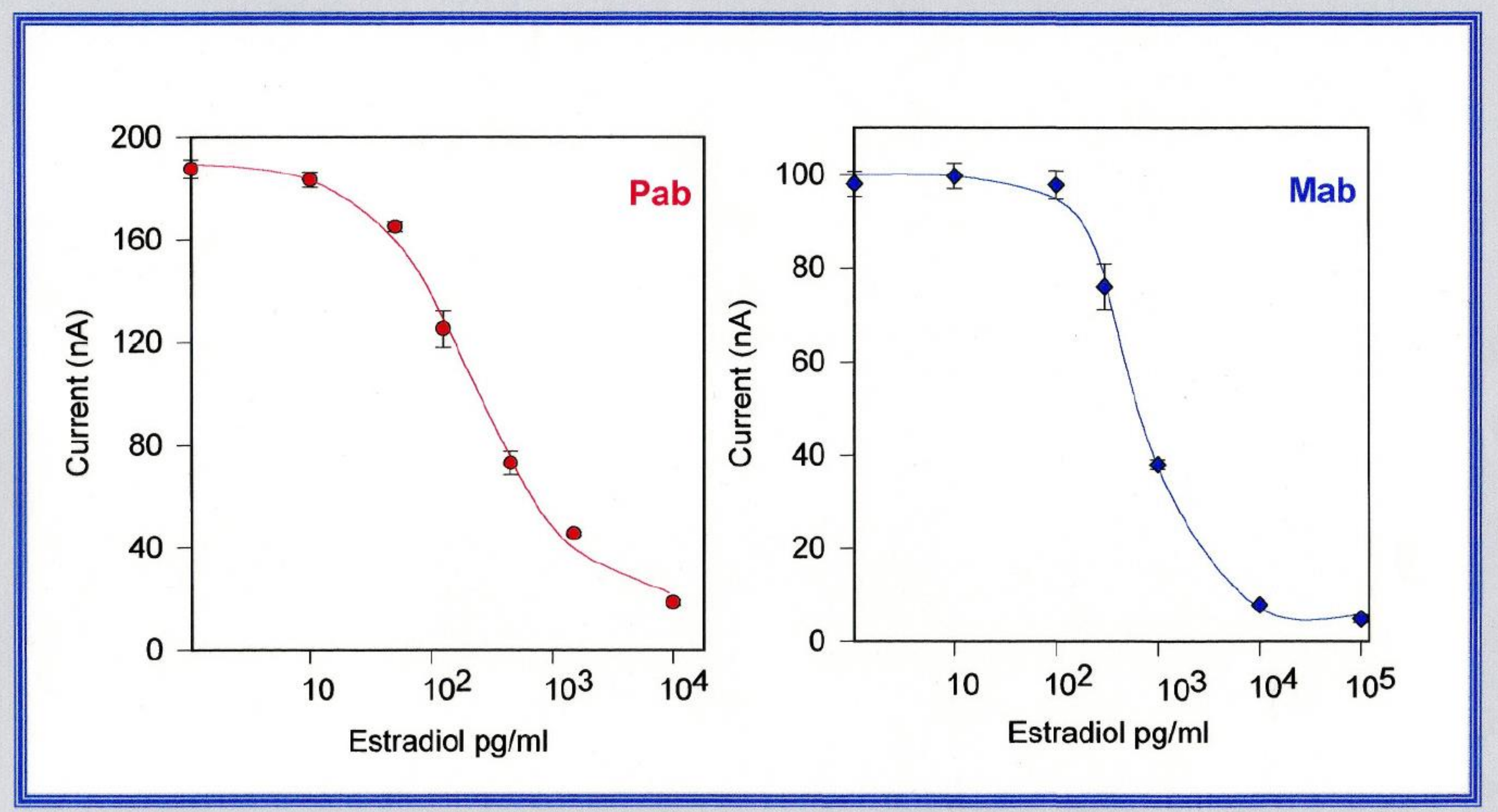


Recombinant antibodies

Phage M13
Coat protein
(2700 copies)
DNA molecule
Tail fiber
(5 copies)
Antigen-coated
microtiter plate well
Phage library
+
Coat well with protein/peptide
and incubate with phage library
1
2
Wash away
non-binders
3
Elute binders
4
Repeat 2-3 times
5
48 binders tested
Control blocked
well
ELISA to determine
specificity of binding after
2-3 rounds of biopanning

Enzyme Linked Immuno-Sorbent Assay

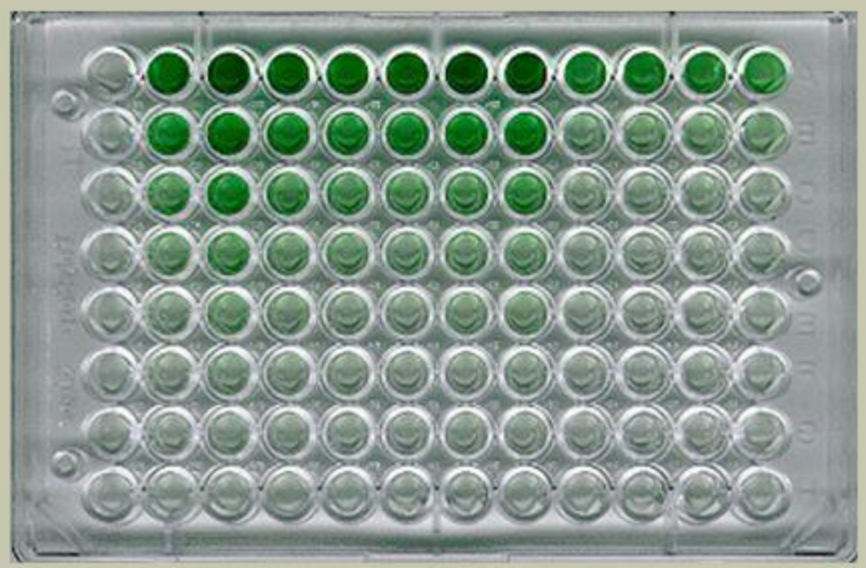
ELISA elettrochimico



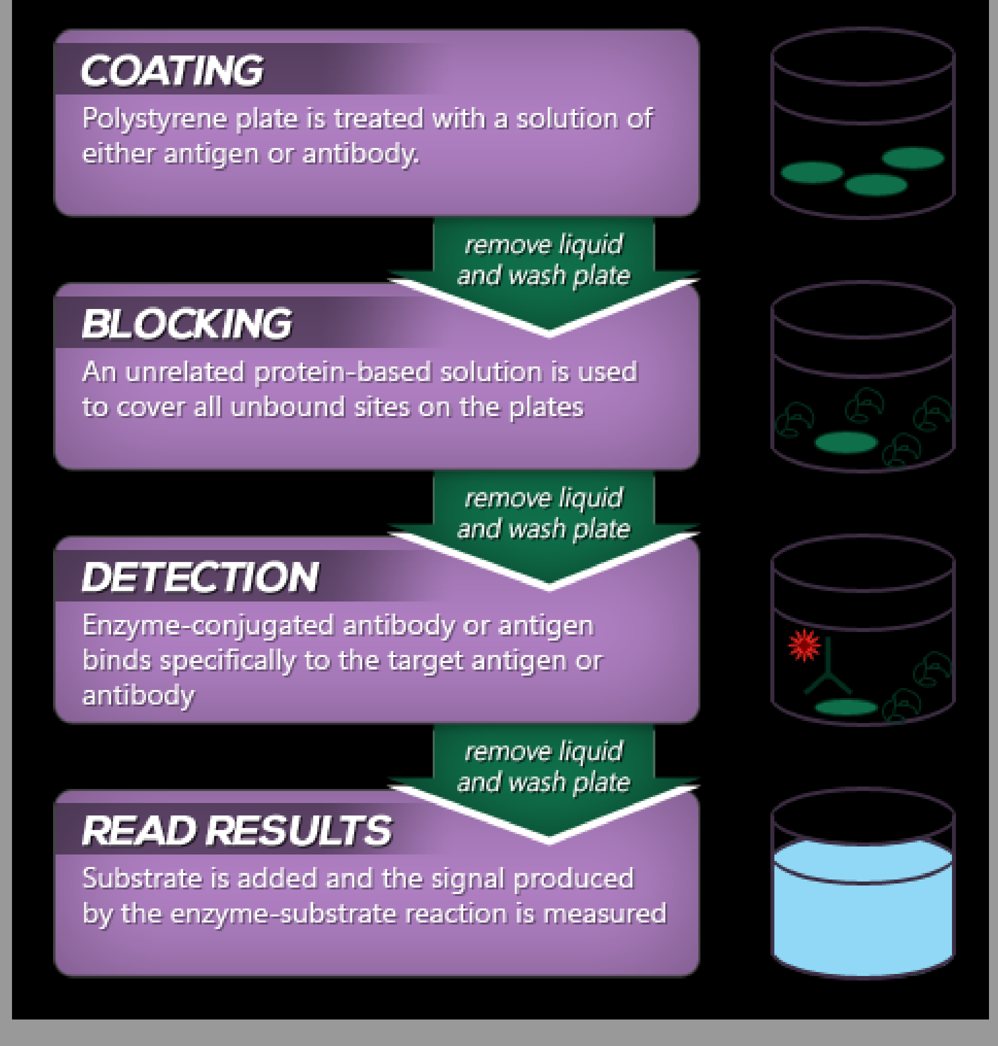
Pab have wider analytical range   sensitivity depends on K_{aff}

ELISA

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



ELISA STEPS



DIAGNOSTIC AUTOMATION, INC.
IMMUNODIAGNOSTICS
AccuDiag™ ELISA
Aflatoxin B1
(In Food)
REF 5120-8
96 Tests
LOT AFB-132
2012-02
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

AgraQuant® Aflatoxin M1
ELISA Tests
NEW
ROMER
Romer Labs®

Food as a source of hidden allergens

How to detect food allergens?

ELISA

Direct Sandwich Assay Principle

Enzyme-labelled analyte-specific antibody

Analyte-specific capture antibody

Analyte

Indirect Sandwich Assay Principle

Enzyme-labelled secondary antibody

Analyte-specific antibodies

Analyte

Direct Competitive Assay Principle

Enzyme-labelled analyte

Analyte-specific capture antibody

Analyte

Indirect Competitive Assay Principle

Analyte

Analyte-specific antibodies

Analyte-bound to protein

Enzyme-labelled secondary antibody

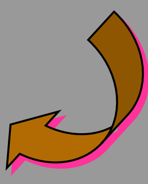
Schubert-Ulrich et al. (2009) Anal. Bioanal. Chem. 395: 69-81

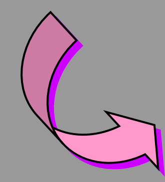
À la carte ELISA Systems

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

❖ Rivelazione elettrochimica per immunosensori:

Cronoamperometria





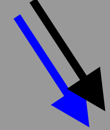
Voltammetria Differenziale a impulsi (DPV)

❖ Enzimi e relativi substrati:

Fosfatasi alcalina

1-naftil fosfato

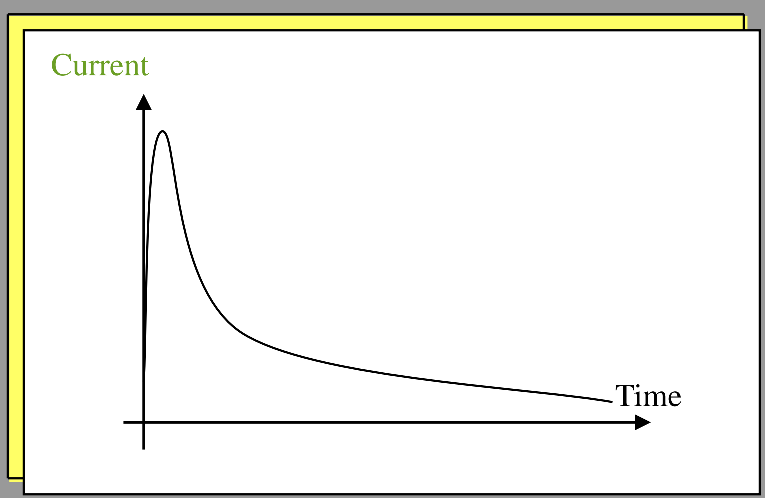
Perossidasi da rafano



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

electrochemical detection:

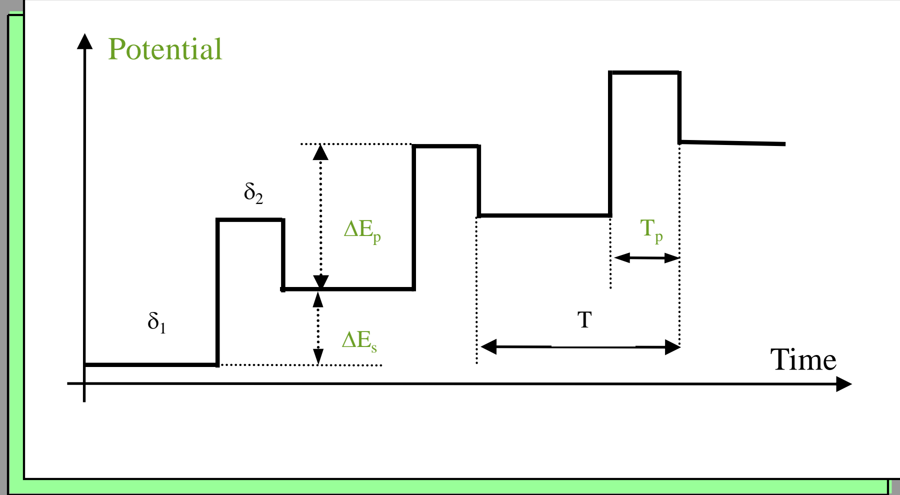
chronoamperometry and differential pulse voltammetry (DPV) :

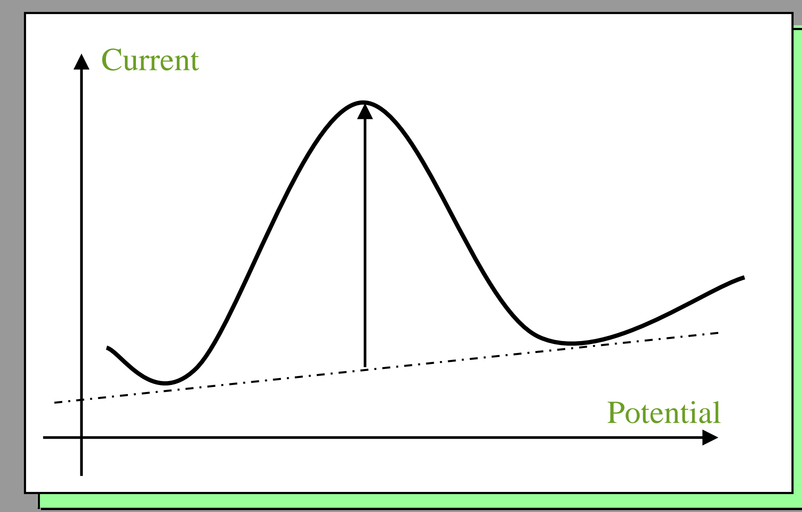


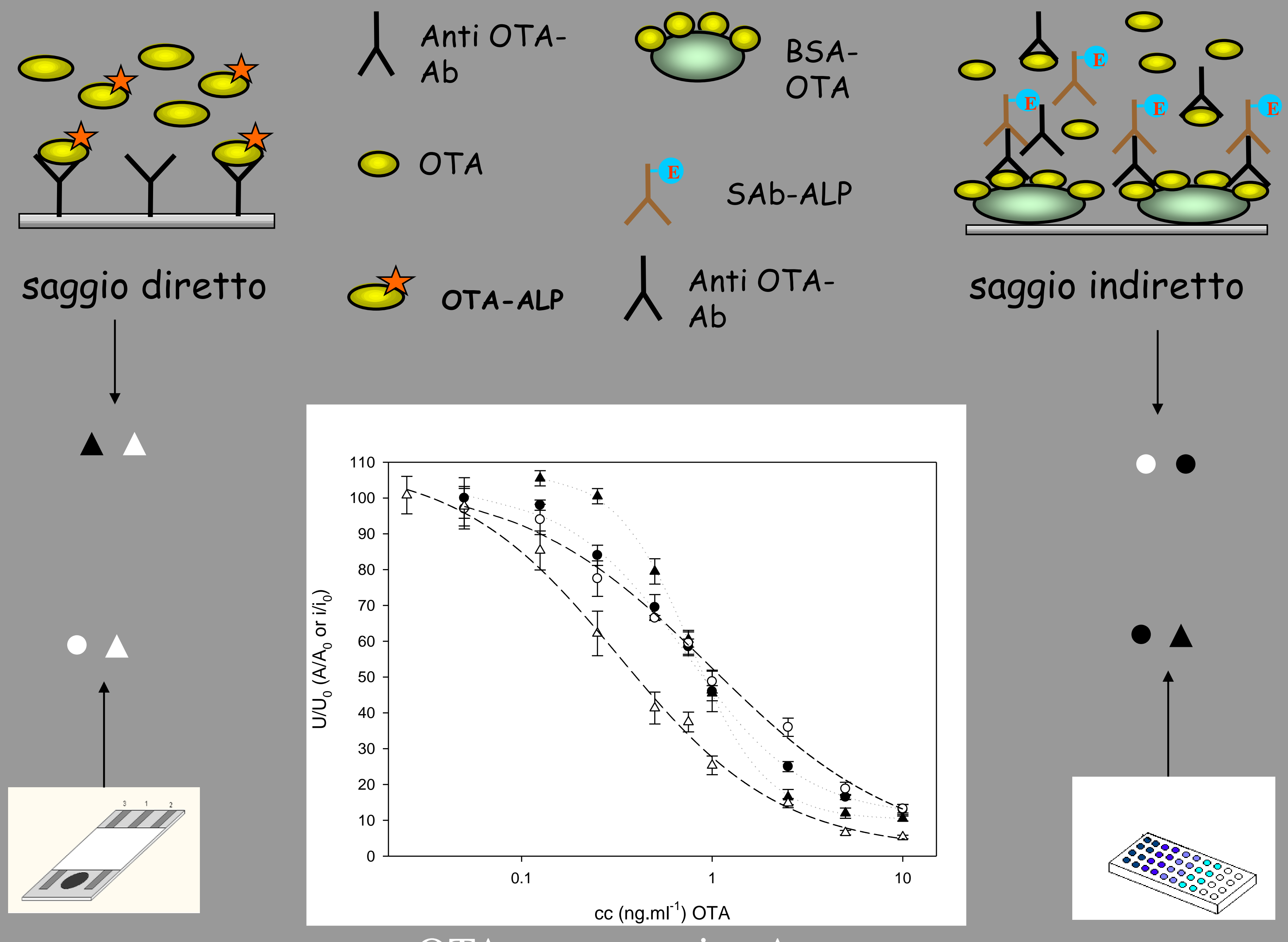
ΔE= const.

δ_2= 60 ms

ΔE_p= 5-100 mV







OTA = ocratossina 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**

Procedura immunosensore:

✓Pre-coating: 6 μl di soluzione di anti IgG di coniglio (4° C overnight)

✓Bloccaggio: 6 μl di soluzione 1 % di alcool polivinilico (30 min)

✓Coating: 6 μl di anti-OTA (1 h)

✓Competizione: 6 μl di OTA-AP + standard/campione (30 min)

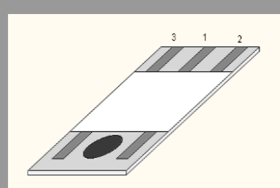
✓Rivelazione: 100 μl di 5 mg/ml 1-Naftilfosfato (2 min) + DPV

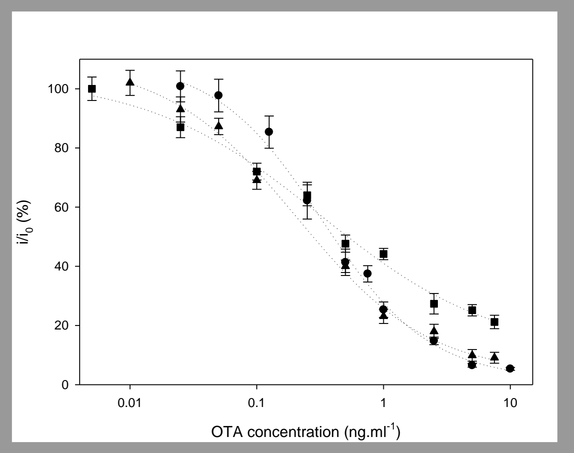
Lavaggi: 150 μl tampone fosfato pH 7.4

Effetto del solvente di estrazione

Attività di un elettrodo modificato con IgG-ALP dopo incubazione per 30 min con soluzioni 1:9 - 9:1 (1:1 in DPBS) acetonitrile:acqua → 95-108%

Sensibilità della curva di calibrazione ~ 50%





25 g in 100 mL di $ACN:H_2O$

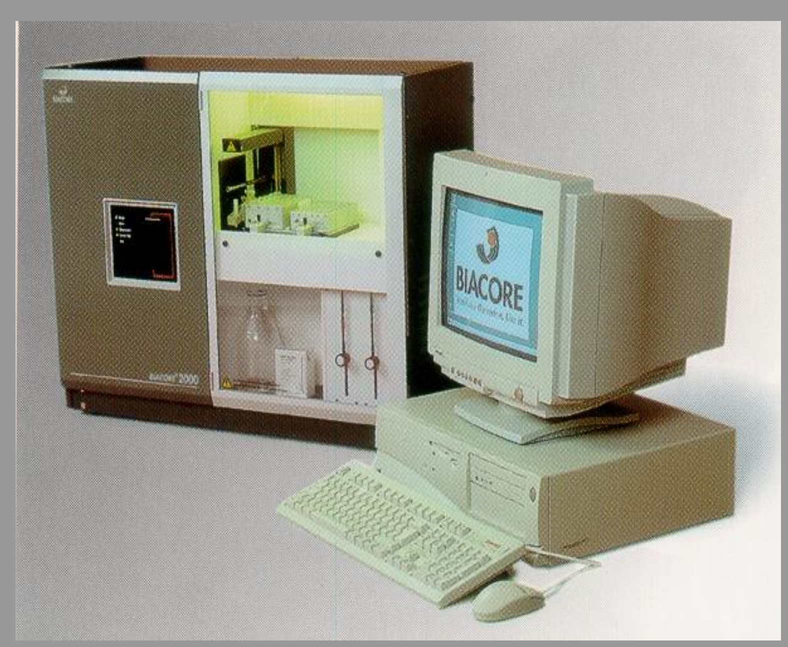
Final dilution 1:8

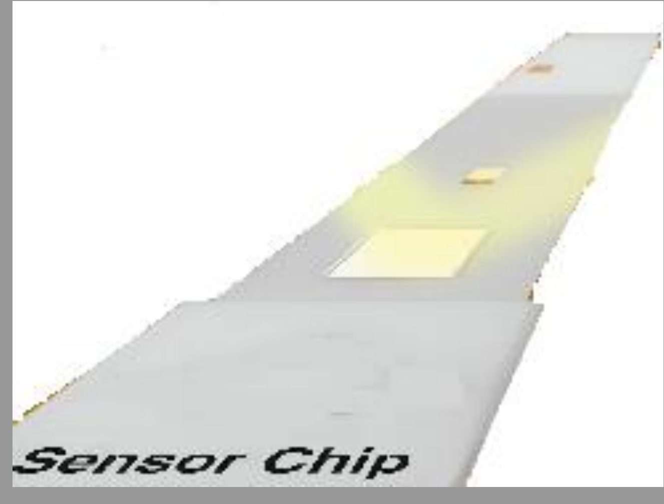
MRL = 3 ng/g

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*

Biacore



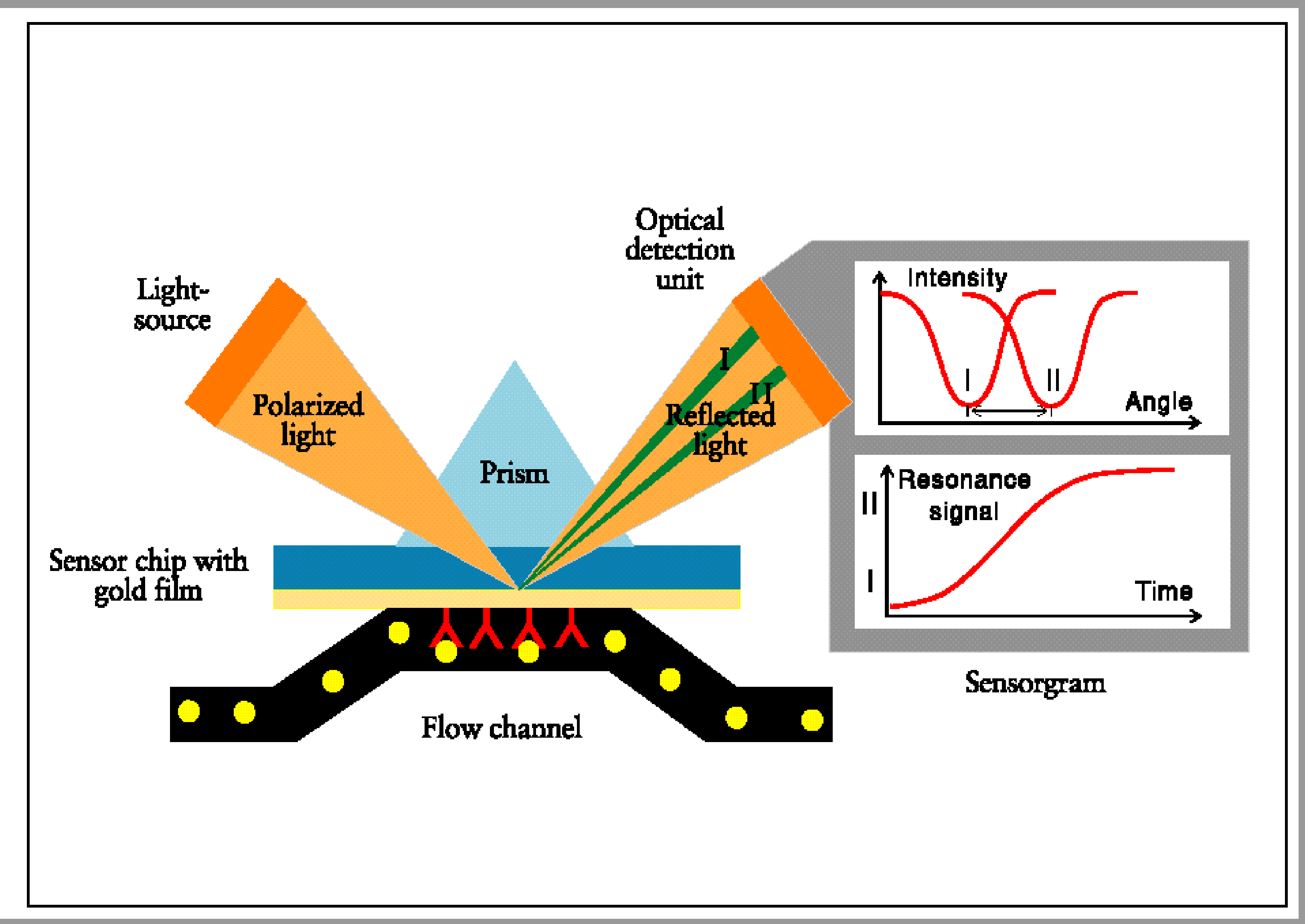


SPR (Surface Plasmon Resonance)
Detection System

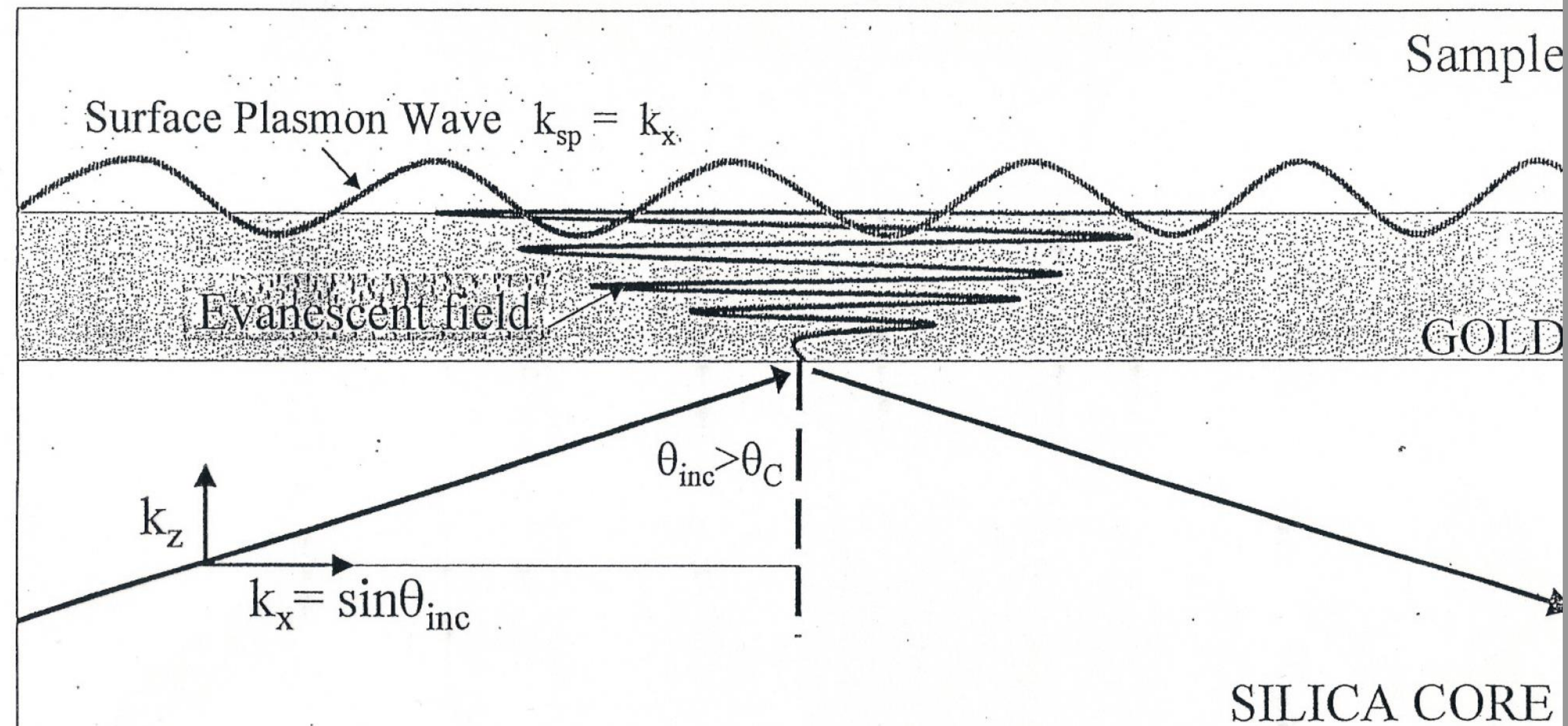
Gold-Dextran Surfaces

Microfluidic System

SPR Biosensors



Surface Plasmon Resonance



θ_{inc} - angle of incident light

λ_{inc} - wavelength of incident light

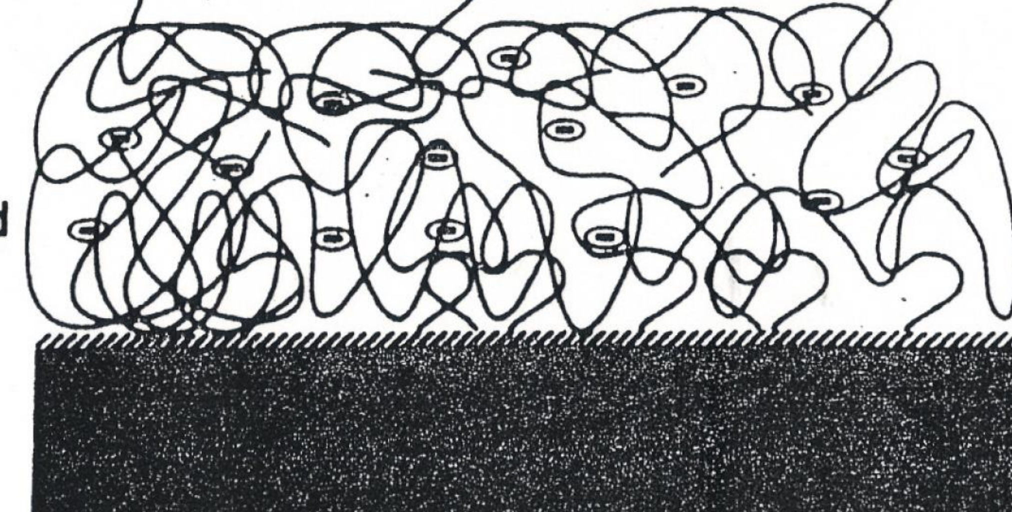
n_{glass} - Refractive Index of glass

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)

Principio della SPR

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.

Immobilizzazione di biomolecole su oro

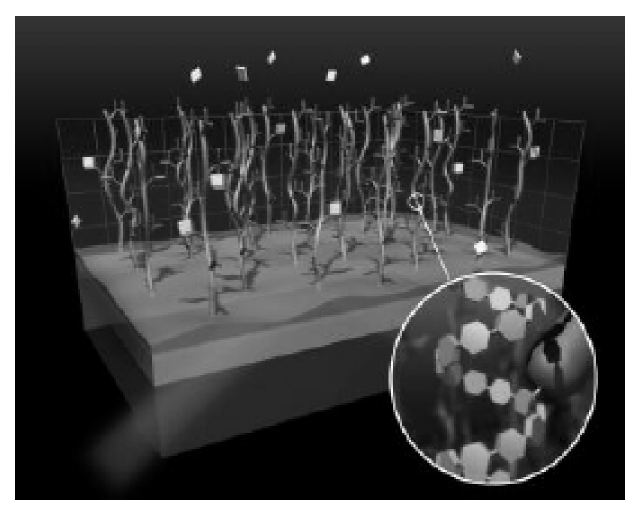
Adsorbimento

Forze elettrostatiche;
Attraverso i disolfuri delle proteine

Vantaggi:
semplicità, economicità, facilità di realizzazione;

Svantaggi:
Perdita del recettore dal supporto;
Legame non-specifico;
Immobilizzazione non orientata (random orientation).

Destrano

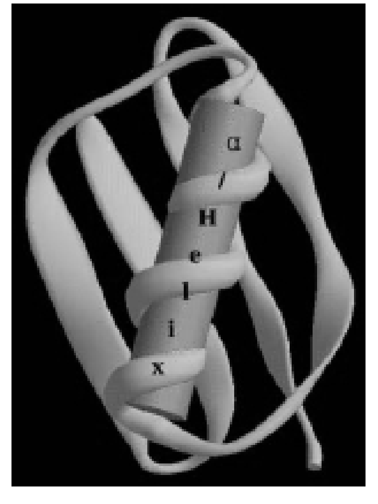


Carboidrato polimerico naturale

Specific layer
Dextran layer
Linker layer
Gold film
Glass

Stabilità
Basso legame aspecifico
Matrice tridimensionale

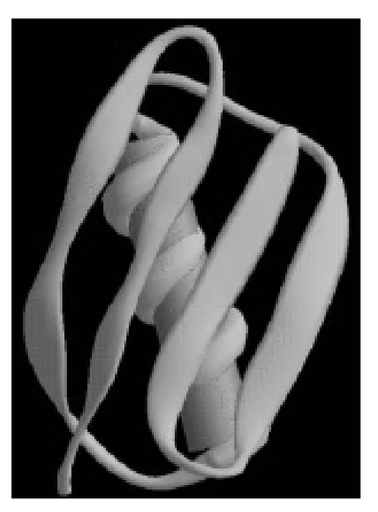
IgG binding proteins

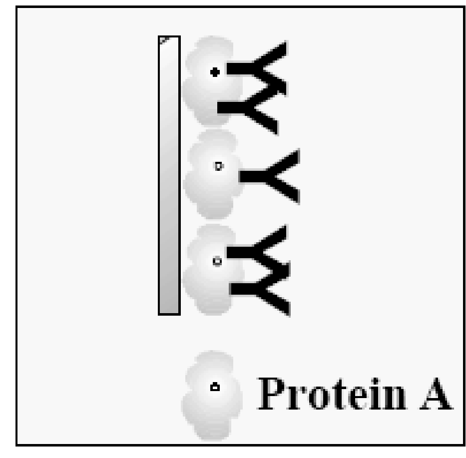


PROTEINA A e PROTEINA G

Proteine batteriche

Contengono siti ad alta affinità di legame per la regione Fc degli anticorpi di varie specie



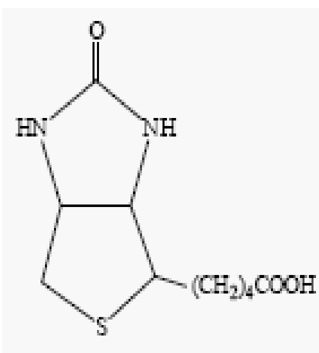


Principali svantaggi:
breve vita del sensore;

Avidina-Biotina

Alta costante d'affinità: 10^{-15} mol^{-1}l

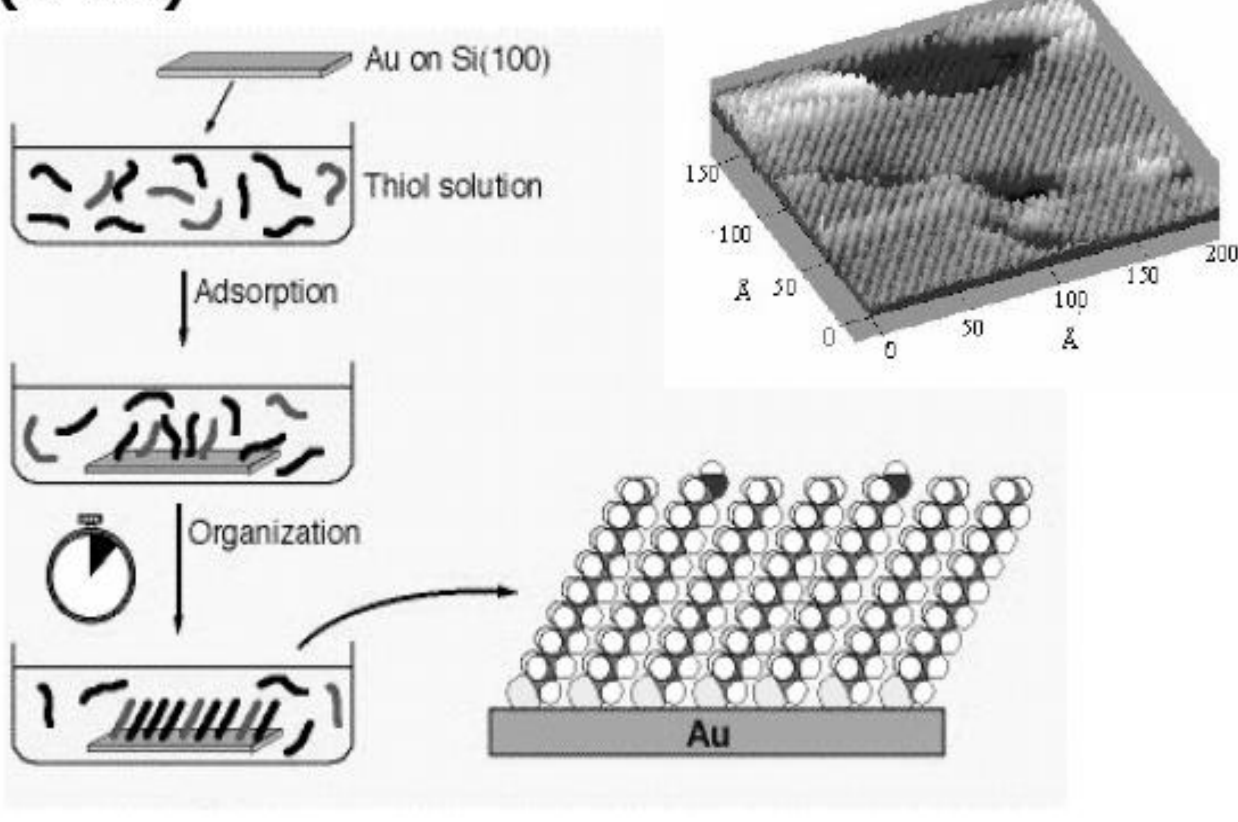
Legame molto stabile (pH, lavaggi)



La biotinilazione non inficia l'attività biologica della biomolecola (recettore)

Self Assembled Monolayers (SAM)

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



SAM

Thiactic acid on gold + $CH_3CH_2N{-}C{-}N(CH_2)_3\overset{+}{N}H$ (EDC) → $COCNH(CH_2)_3NH$ + NH_2–Y (Antibody) → CNH–Y

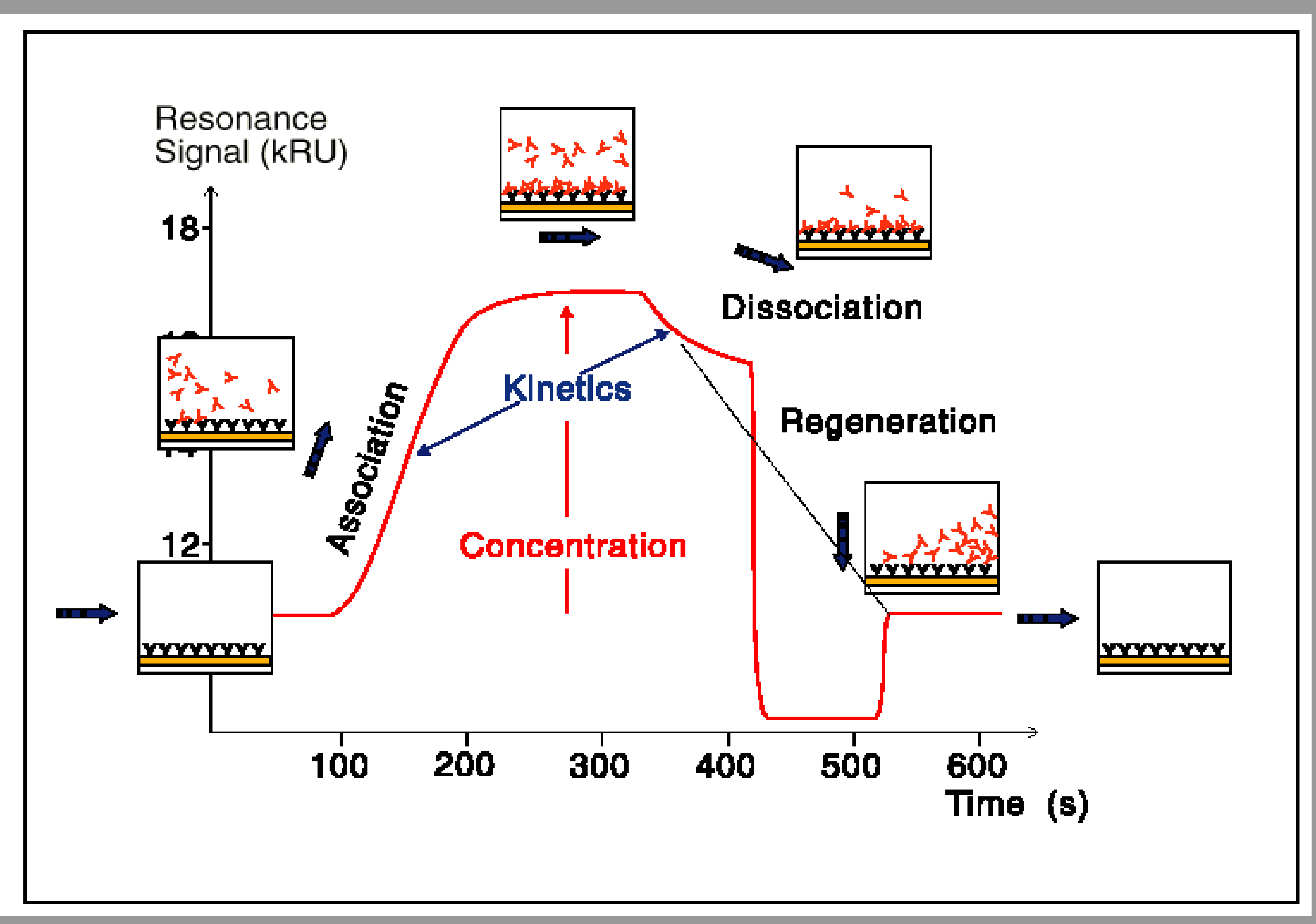
Cysteamine on gold ($S{-}CH_2CH_2{-}NH_2$) + $OCH(CH_2)_3CHO$ (GA) → $NC(CH_2)_3CHO$ + NH_2–Y (Antibody) → $NC(CH_2)_3CN$–Y

Stabilità

Alta densità di superficie

Rigenerazione

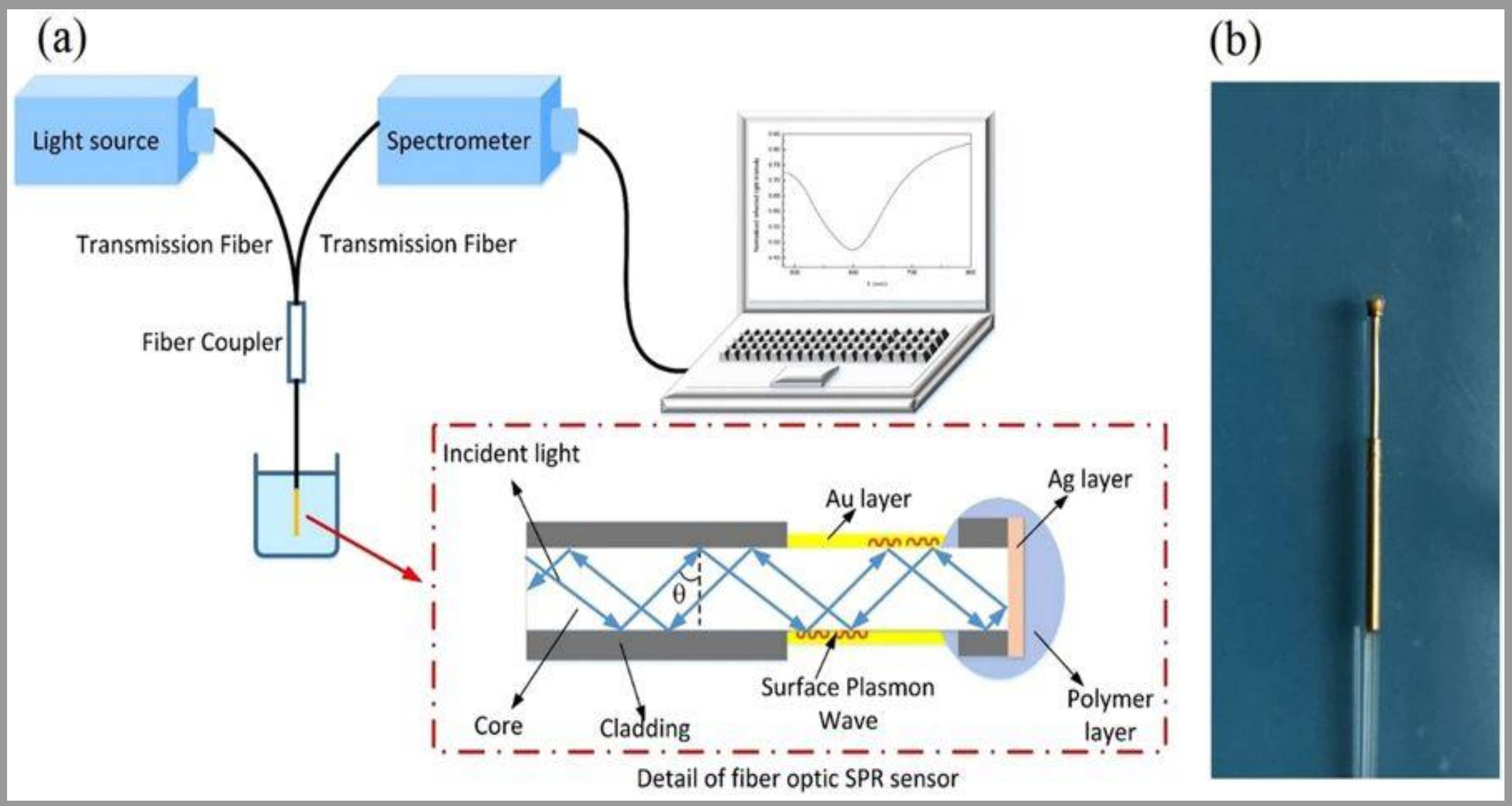
Sensorgram



A label-free fiber optic SPR biosensor for specific detection of C-reactive protein

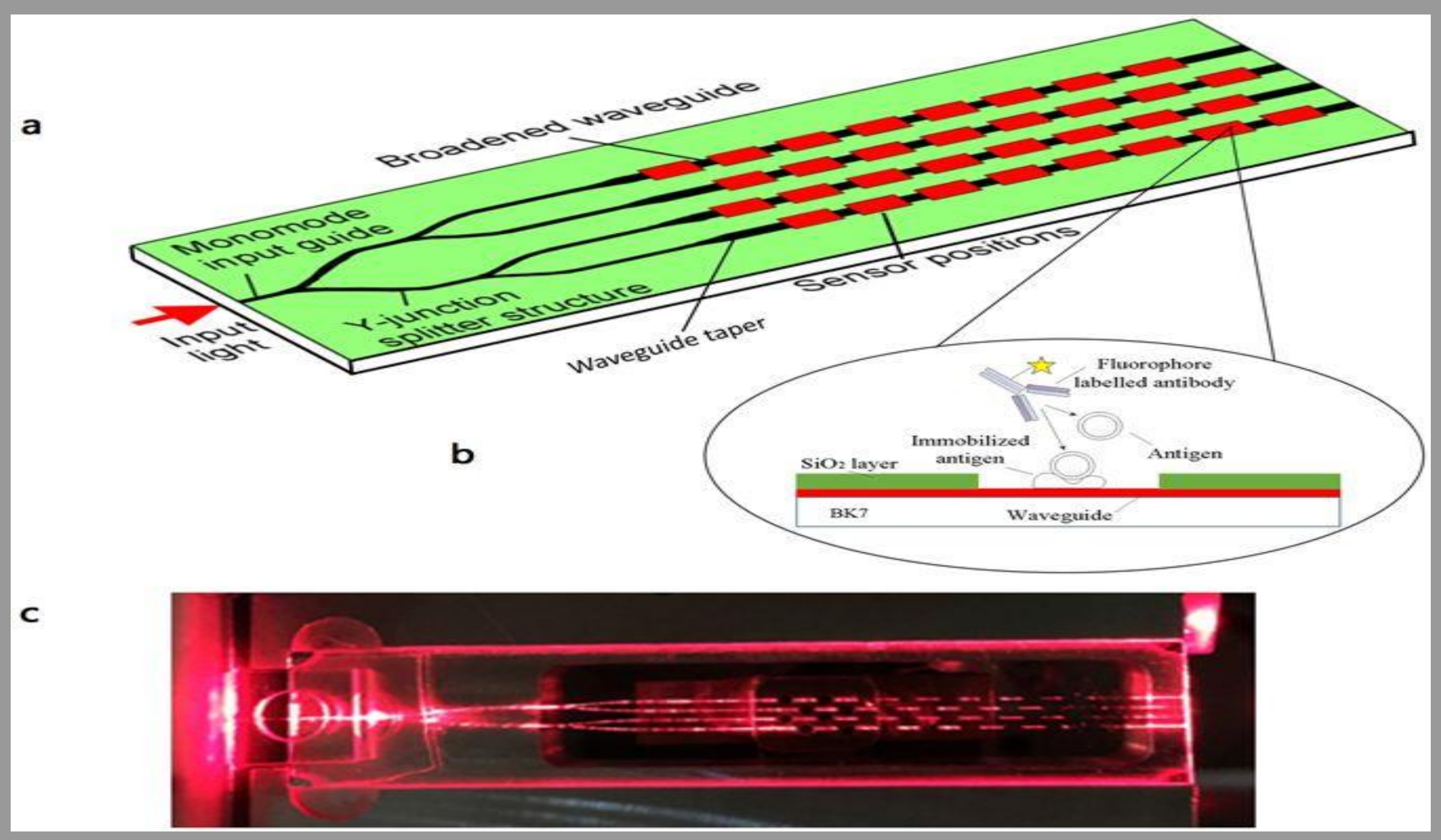
Wenjia Wang, Zhigang Mai, Yuzhi Chen, Jiaqi Wang, Liang Li, Qingning Su, Xuejin Li
Xueming Hong

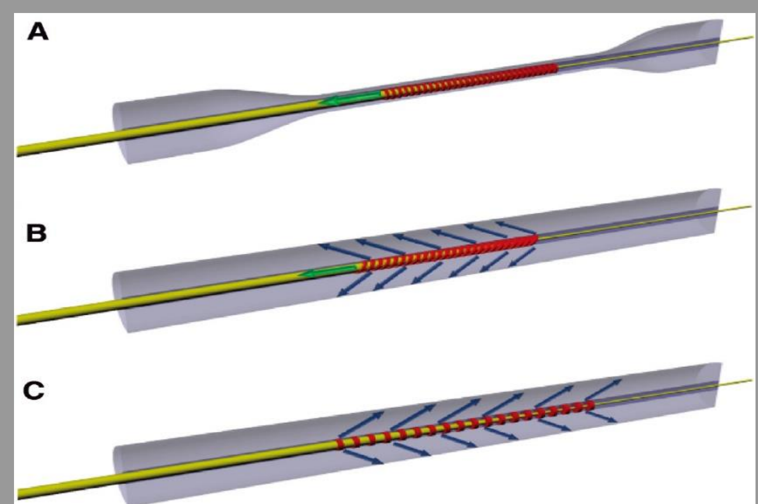
Scientific Reports **volume 7**, Article number: 16904 (2017)



Integrated optical waveguide-based fluorescent immunosensor for fast and sensitive detection of microcystin-LR in lakes: Optimization and Analysis Lanhua Liu, Xiaohong Zhou, James S. Wilkinson, Ping Hua, Baodong Song, Hanchang Shi

Scientific Reports **volume 7**, Article number: 3655 (2017)





Biosensing with optical fiber gratings

Francesco Chiavaioli,Francesco Baldini,Sara Tombelli, Cosimo Trono, Ambra Giannetti

Published Online: 2017-06-07 | **DOI:** https://doi.org/10.1515/nanoph-2016-0178

Review Wiley Online Library ELECTROANALYSIS

DOI: 10.1002/elan.201500237

Lateral Flow Immunoassays – from Paper Strip to Smartphone Technology

Evgeni Eltzov,[a, b, c] Sarah Guttel,[d] Adarina Low Yuen Kei,[b, c] Prima Dewi Sinawang,[b, c] Rodica E. Ionescu,[b, e] and Robert S. Marks*[a, b, c, f, g]

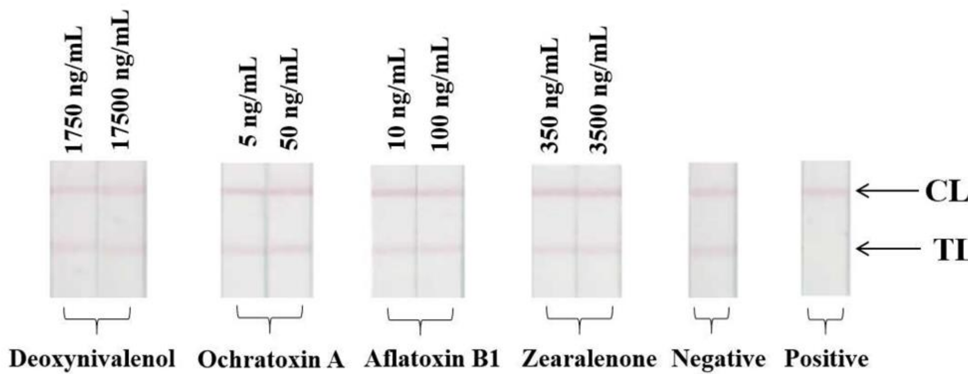
Lateral flow biosensors

Advantages

- Simple operation without pretreatment
- Low cost
- Stable
- Portable
- Fast
- Wide range of the formats
- Simple in constriction, that will allow adaption to the target analyte

Disadvantages

- Only qualitative and not quantitative
- Very limited quantitative dynamic range
- Low multiplexity
- Lack of automation
- Possible error for color blind people



Lateral flow immunoassays

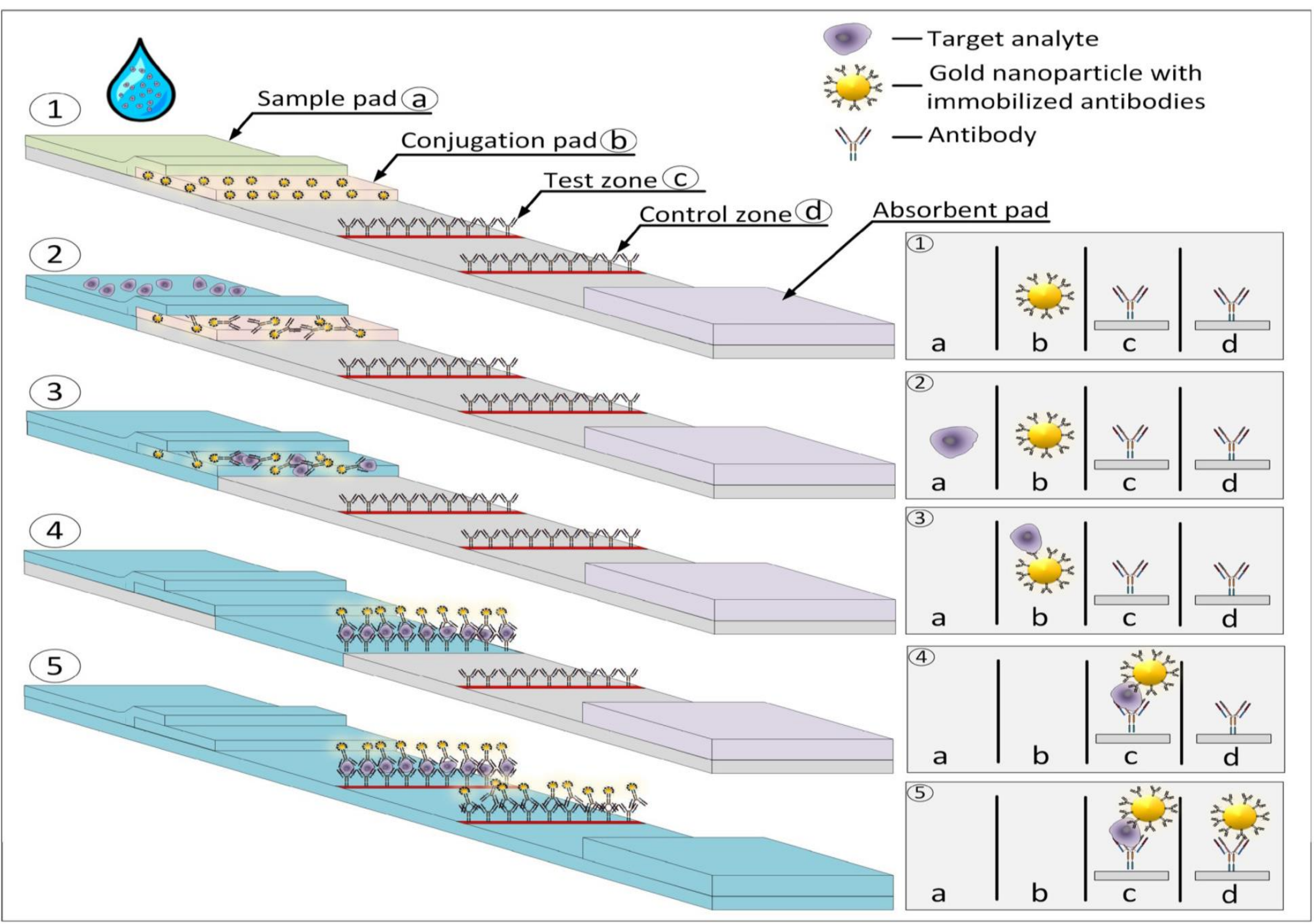


Fig. 1. Schematic presentation of lateral flow assay and measurement process. 1 and 2. Water sample placed above sampling pad. 3. Target analyte migrates through sample pad to conjugation. 3. It will bind to conjugated labeled antibodies. All these clusters are moved to the test zone (4) and control zone (5).

Labels used in lateral flow assays

Label type	Advantages	Disadvantages
Latex	• Low price • Appropriate for multiple components assays • Variety of colors • Variety of available surface chemistries • Good control of antibodies orientation and their activity	• Large and not very intensely colored particles • Reduced parking density on test lines and less sensitivity than gold in many instances • Requirement for the use of high surfactant due to hydrophobic characteristics and size
Gold	• Price • Different sizes • Generate relatively high sensitivity results • User friendly format • Inert	• Only qualitative and not quantitative • Very limited quantitative dynamic range • Low multiplexity • Lack of automation • Sensitive to changes in pH, salt and organics • Single color choice, thus can not be used in multiplexing environments
Colloidal carbon	• Increased surface area • Don't aggregate • Inexpensive • Easily may be scale up • High color contrast • Stable	• Longer immobilization procedures • Only a few vendors for well-characterized carbon particles available and the use of colloidal carbon for lateral flow assays requires a licensing agreement from the vendors
Magnetic beads	• Measuring of particles throughout the membrane • High stability • Aggregation hardly changes the absorption properties • Not affected by the sample matrix • Lables stable thus may be reanalyzed if required	• Identification of the population of multiple markers must be done in series, rather than concurrently, increasing the time of the testing
Fluorescent nanoparticles	• Sensitive • Broad absorption bands • Commercially available	• Quenching • Photobleaching • Extensive modification of the protein frequently cause physicochemical changes that can lead to changes in hydrophobicity, conformational changes, and steric hindrance

Top Lid

Main Unit

Bottom reservoir

"Main Unit after assembling of different sub-components"

Specifically formulated chemical reagents A, B, C and D

Pipette tips

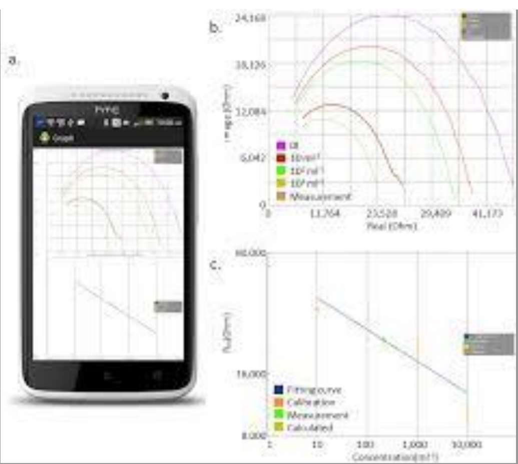
Mobile Water Kit

Millex syringe filter units with 0.45 µm pore

Sterile syringe (100 ml / 60 ml)

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

Smartphone detection of E.coli in water



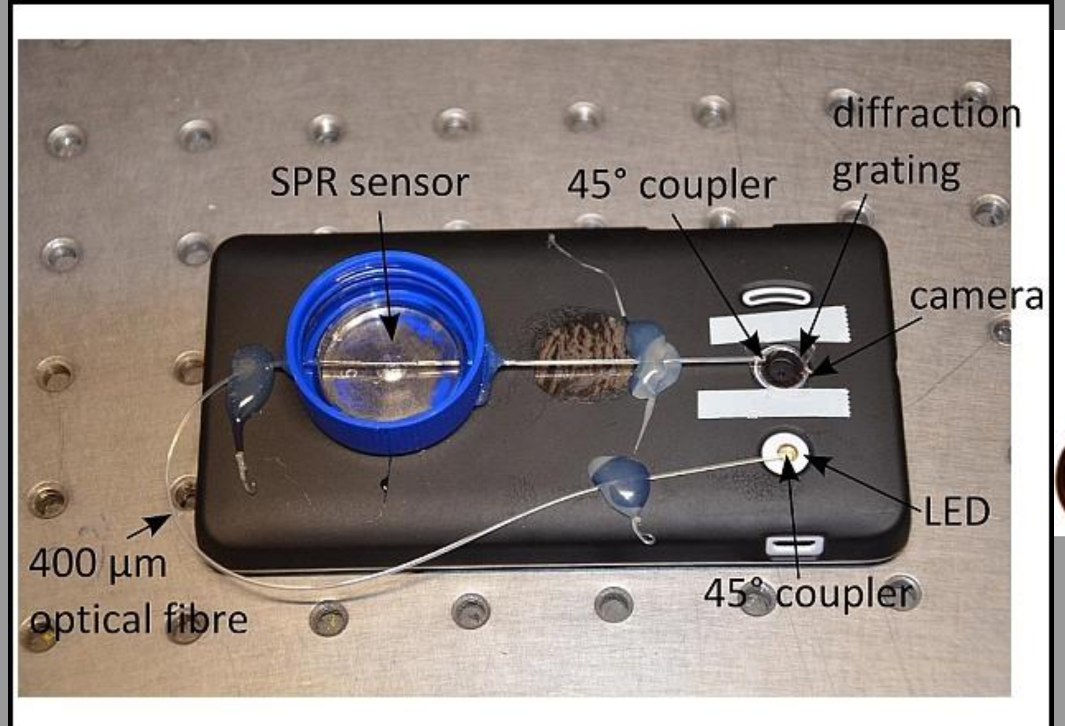
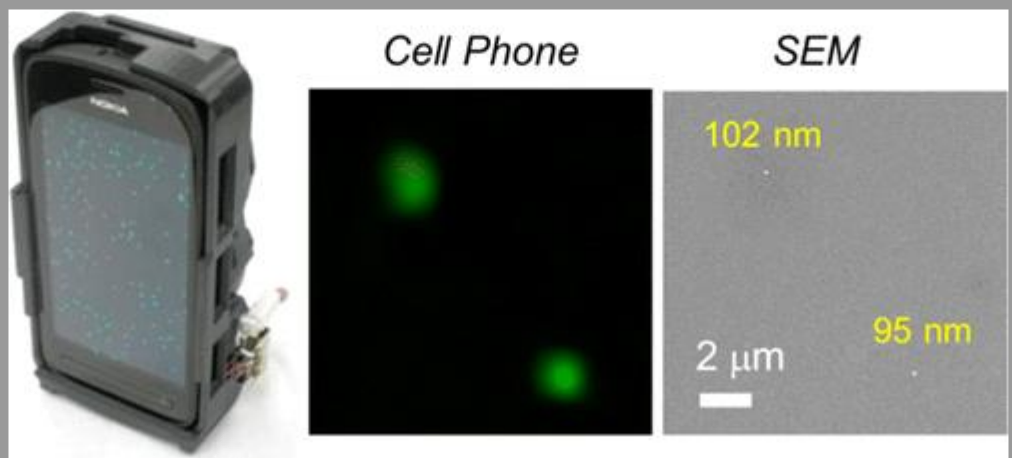


Image: Kort Bremer, Hanover Centre for Optical Technologies



Miscoscopio basato su smart detection di virus

Fig. 1

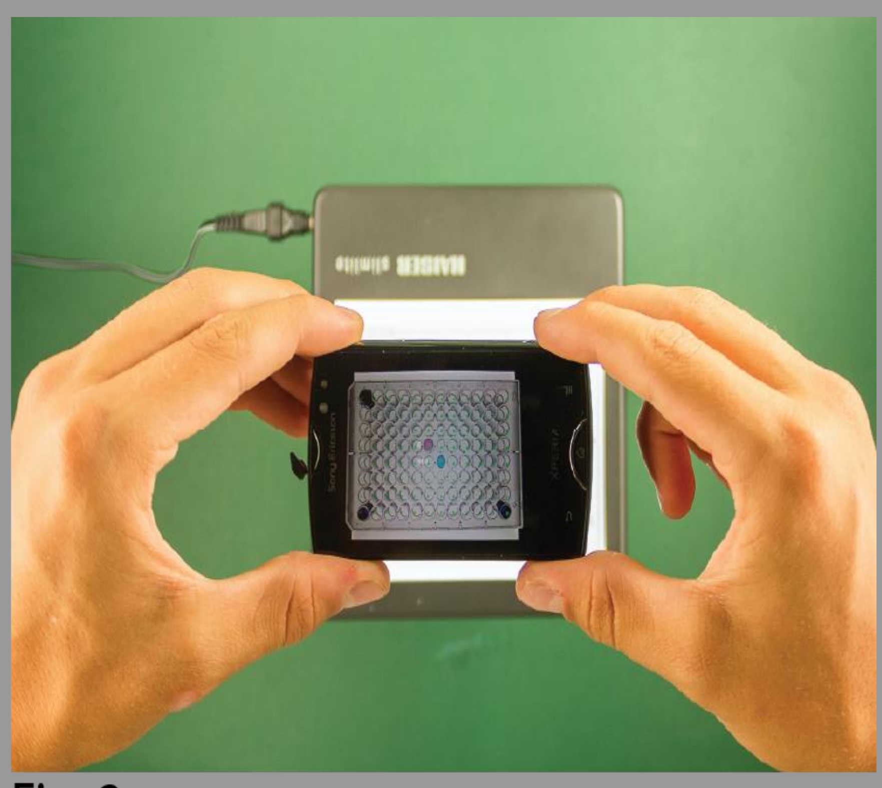
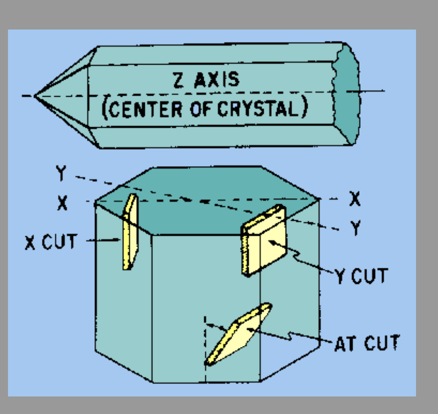
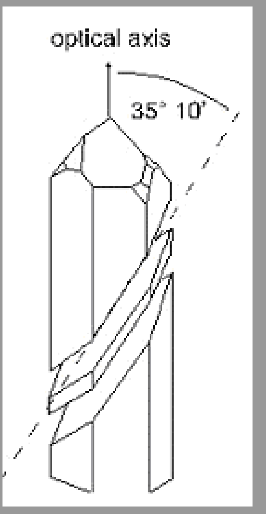


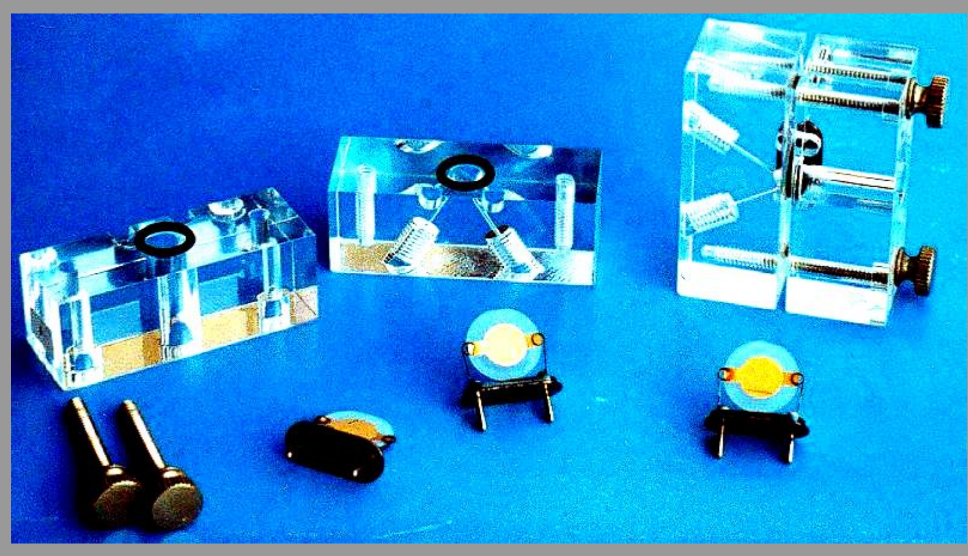
Fig. 2

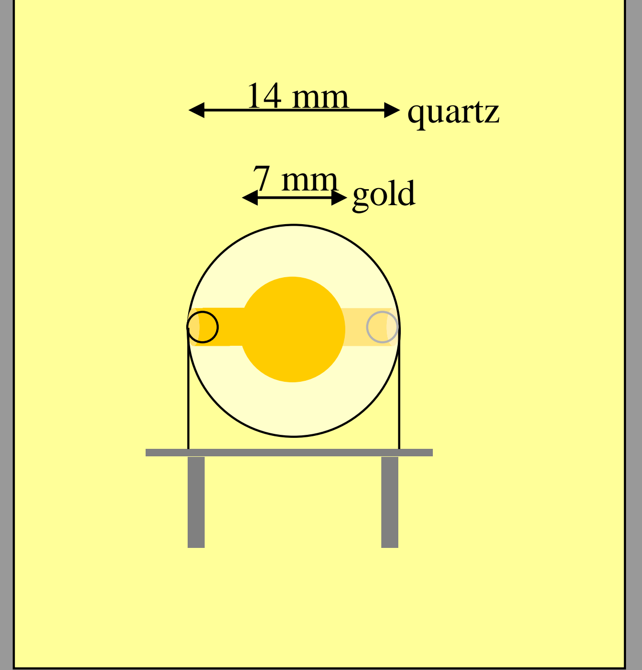
Lettore ELISA adattabile a Smartphone

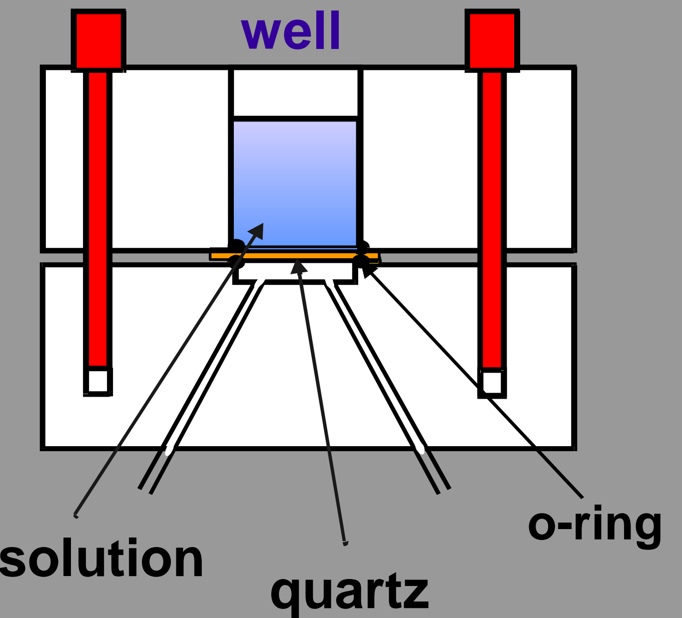
Piezoelectric Biosensors











Sensori piezoelettrici

usati per tutti i biosensori di affinità e in fase gassosa

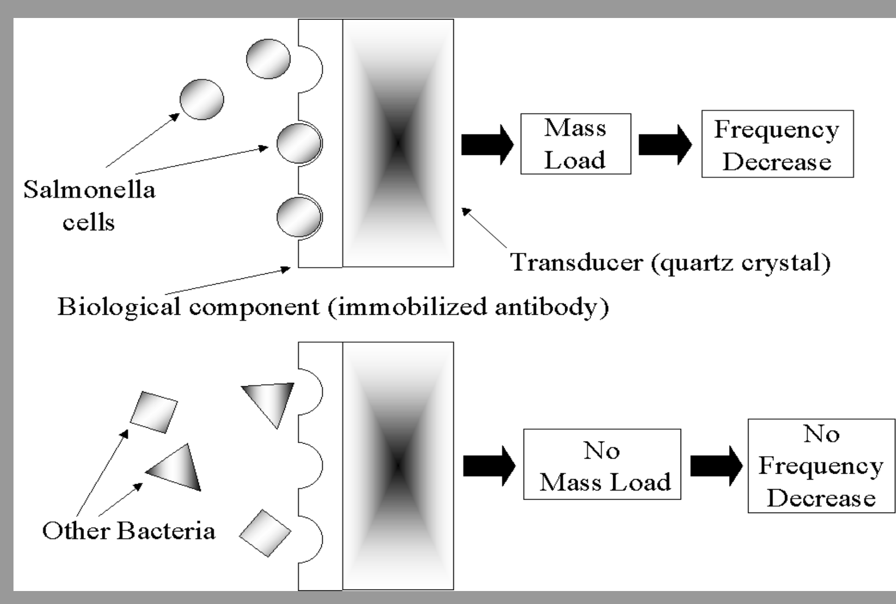
gold

14 mm

8 mm

quartz

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

$$\Delta F = (-2.3\times10^{-6})\, F^2\, \Delta M/A$$

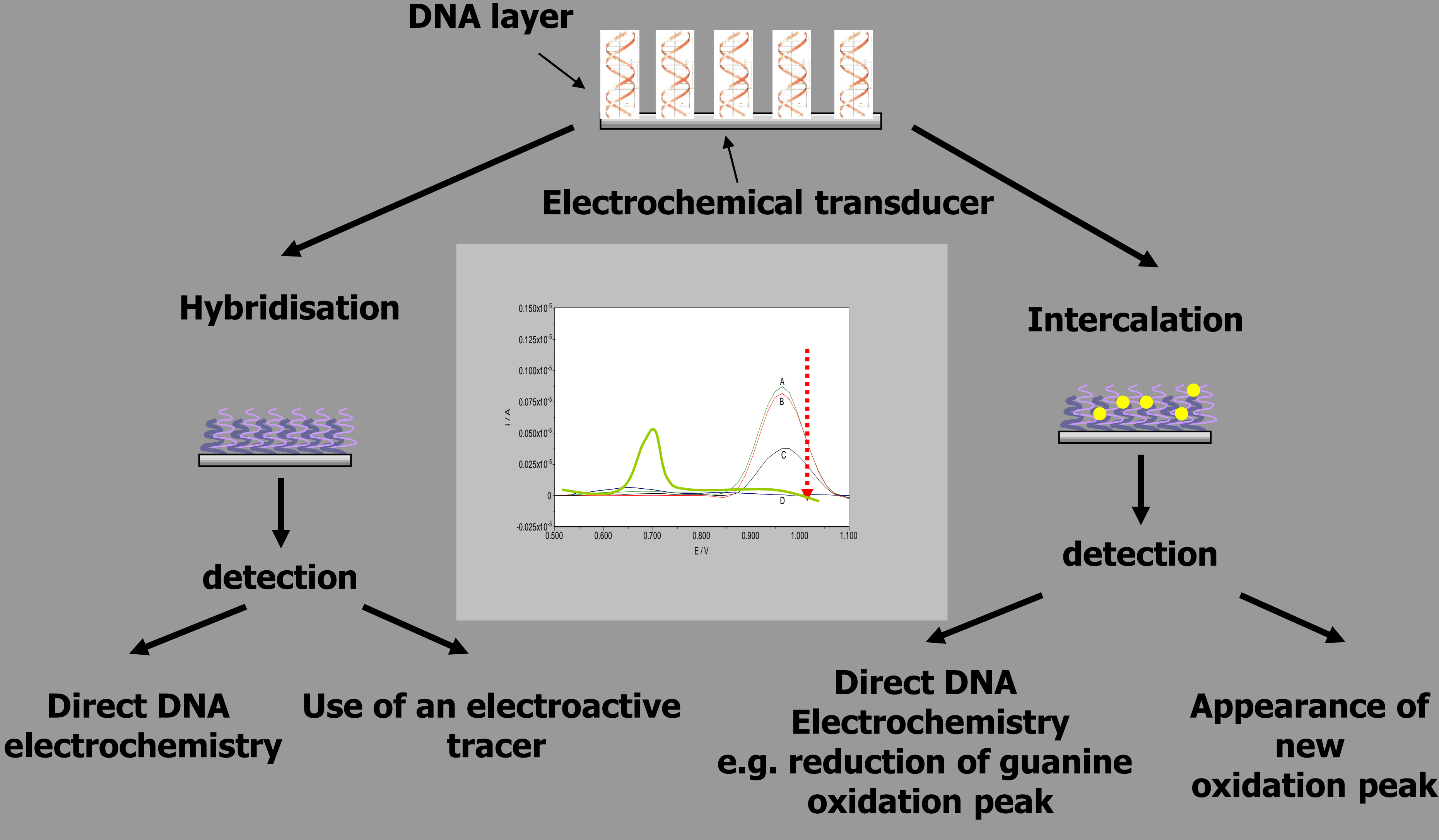
ΔF (Hz) = frequency shift of the coated crystal

F (Hz) = resonance frequency of the crystal

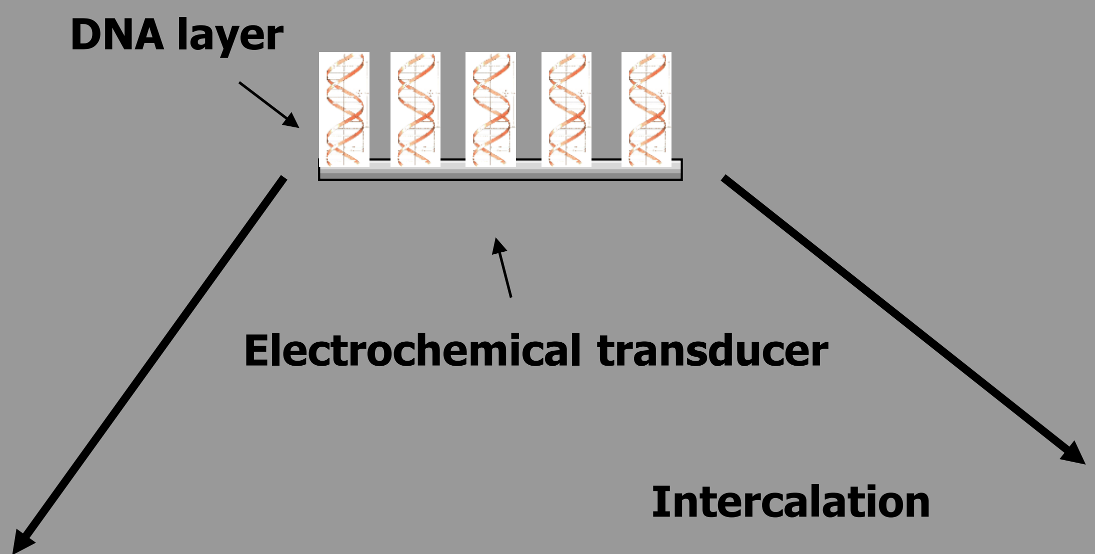
ΔM (g) = increase in mass loading

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

DNA electrochemical biosensors



DNA electrochemical biosensors application



Hybridisation:

Molecular diagnostic application:

microorganism
gene single-point mutation
species identification
GMO

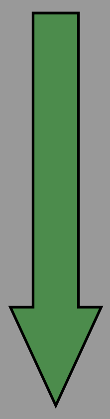
Intercalation

Antitumoral drugs

Genotoxic compounds:

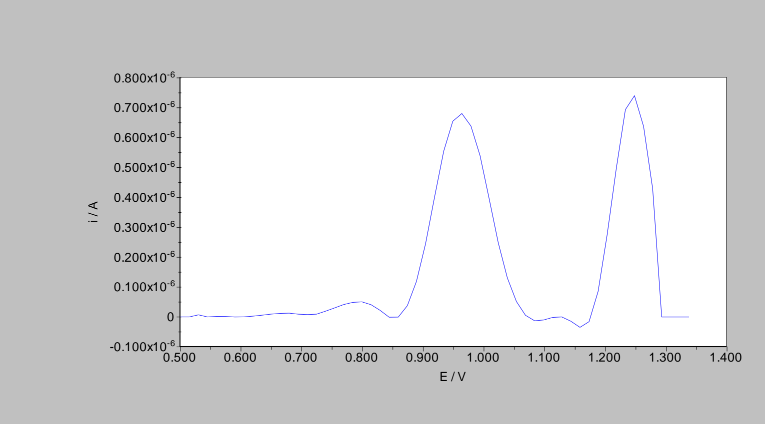
stable DNA complex
adduct formation

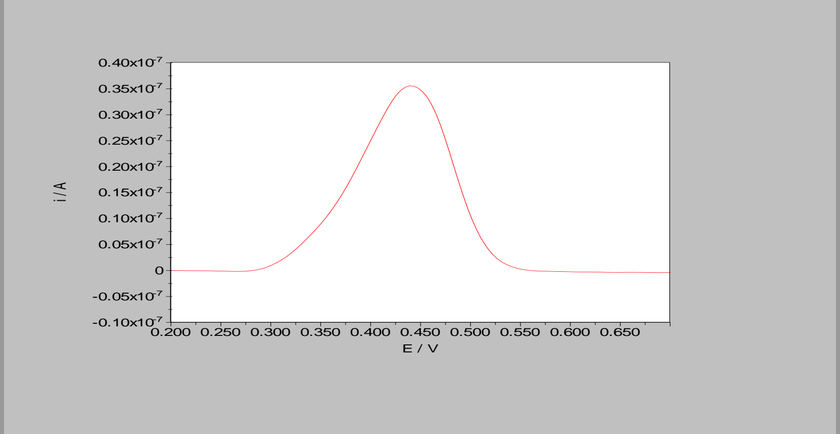
Obiettivo



Ottimizzare una procedura di
monitoraggio rapido basata
sull' utilizzo di sensori elettrochimici a DNA per
rivelare
la presenza di ceppi potenzialmente tossinogenici
di *Fusarium culmorum*

Voltammetria ad onda quadra





Guanine oxidation

+ H_2O → + $2H^+$ + 2 e^-

+ H_2O → + $2H^+$ + 2 e^-

Daunomycin oxidation

→ + $2H^+$ + 2 e^-

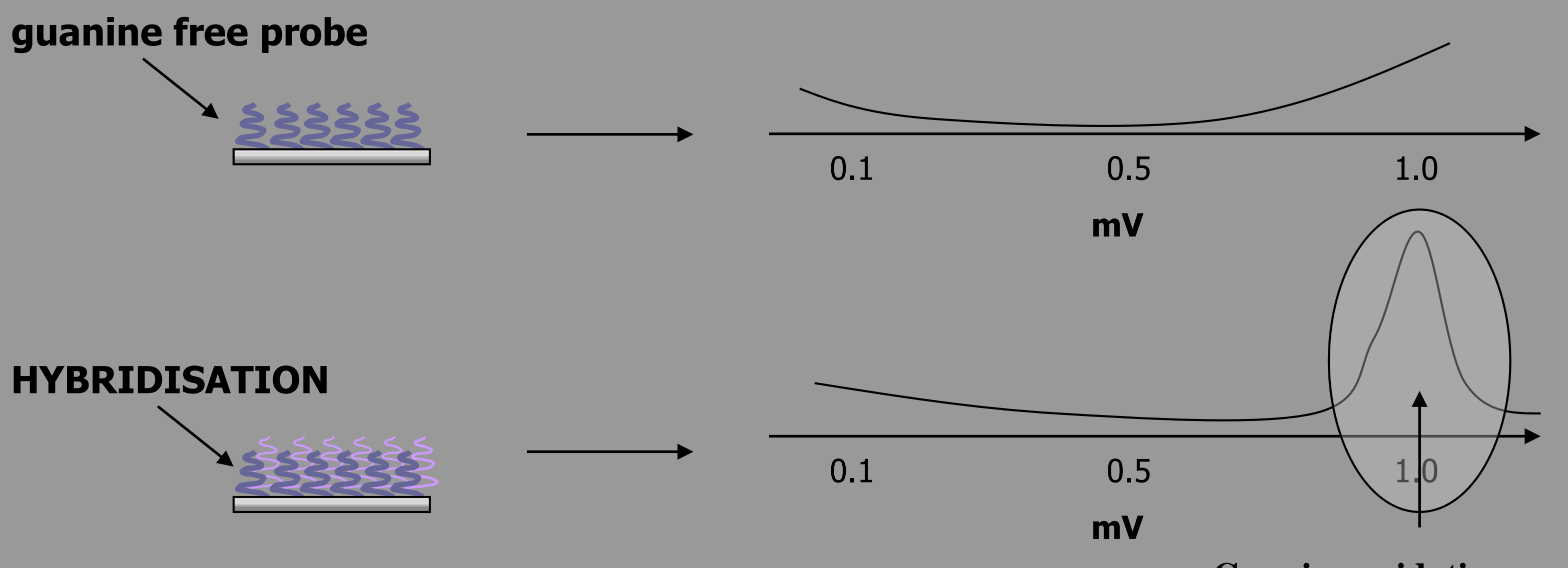
DNA from *Fusarium culmorum*

Aim:

- **to develop a DNA electrochemical biosensor able to specifically detect specific sequences of *Fusarium Culmorum* in PCR amplified samples.**

Sensing principle:

- **direct electrochemical detection of guanine bases upon hybridisation of a *guanine free* probe.**



GACATGAGTCTCTATGGAAAGAAGATTTATATTGAGACTCCTGTGCAGAGATGGGTTG
AAGTGACTGTTGTGGACTCAACCGATGGTGGTATTGATGAGAACTATTTAGACTTGTCT
GTGGCTGCTTTCAAGGTTCTTGCTAGGGTTGAGGATGGCGTTTCGCAGATTACTTGGAA
CTACATGTAGGCTTGTCGTGTCTGAAGCTCTGTTAAGTTTCCAGAGTAAAGCTGATAGC
AGTATTAAATCATACATATGAAAAAAATGGGATGCAAGAAGCTCAAAGTCAAGGTCCA
TTCTTCAATGGACGCCATTTGCTTTACCCCTCTGTTACTAAACTATCACCCAAGACGGG
AATGAATGAACGCTTGGATATCTTTCCAAGCTTGAGGGCAGCGCTCCCCGTGTTGGCG
AAAAAAGGAATCAACCCCGTCCAGAAGCGTCTCAACCTCCCGTGAGTCAAGGTTCTCG
TTTCTGAGTAGAACTGAATTGATCGCAAGCGGTCCCGGGTCTCTGATATGG

Probe ***fusarium* 1**: 5’ TIA-**C**TI-TTI-TII-A**C**T-**C**AA-**CC** 3’ (20 basi; 5 citosine)

Comp *Fusarium* 1: 5’ GG-TTG-AGT-CCA-CAA-CAG-TCA 3’

Probe ***fusarium* 2**: 5’ **C**T**C**-TIT-TAA-ITT-T**CC**-AIA-ITA-A 3’ (22 basi; 4 citosine)

Comp *Fusarium* 2: 5’ T-TAC-TCT-GGA-AAC-TTA-ACA-GAG 3’

Probe ***fusarium* 3**: 5’ **C**TT-TA**C**-**CCC**-T**C**T-ITT-A**C**T-AAA-**C**TA-T 3’(25 basi; 8 citosine)

Comp *Fusarium* 3: 5’ A-TAG-TTT-AGT-AAC-AGA-GGG-GTA-AAG 3’

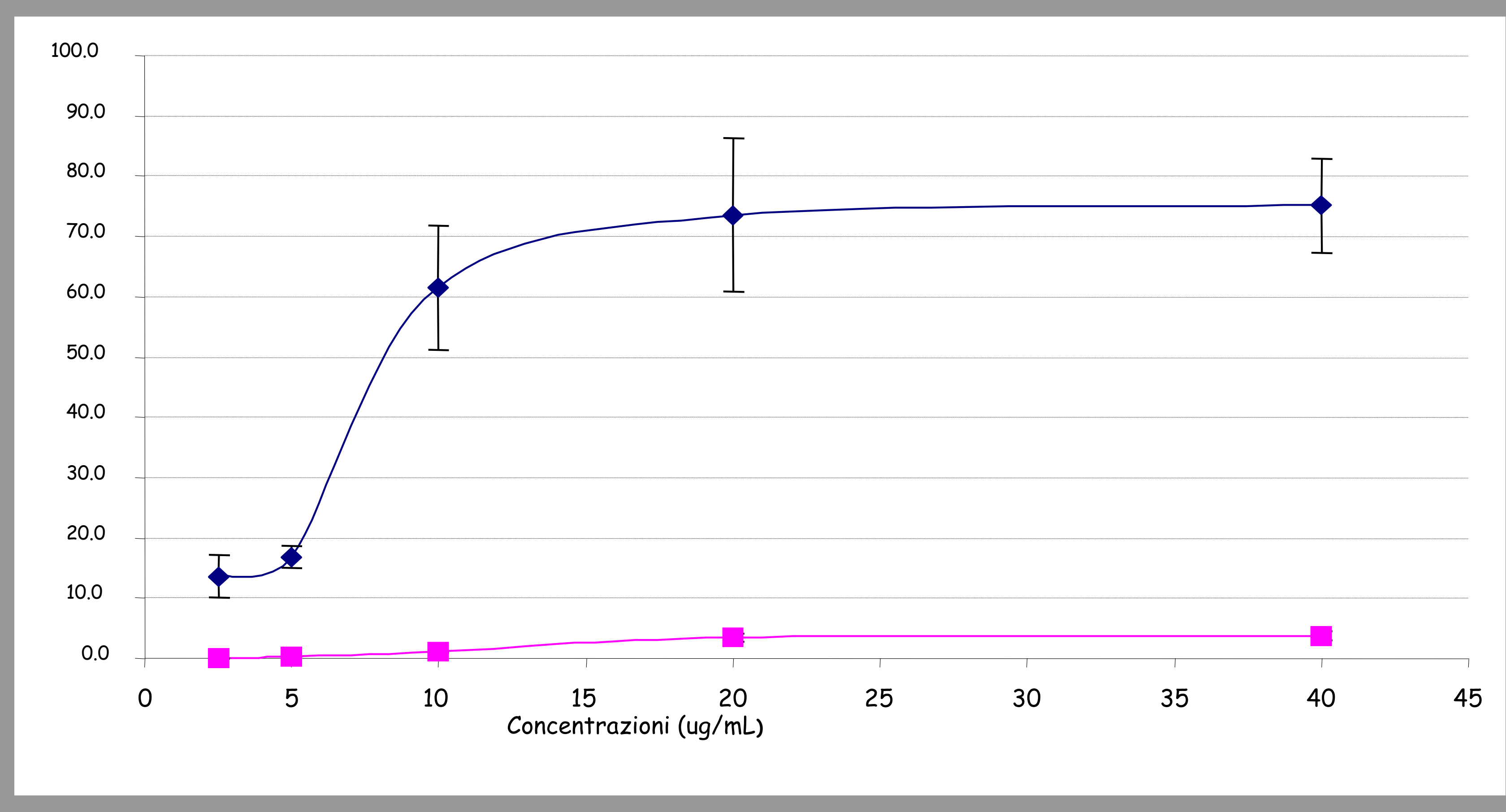
Probe ***Fusarium*4**: 5’**C**II-T**CC**-**C**II-IT**C**-T**C**T-IAT-ATI-I 3’ (22 basi; 6 citosine)

Comp ***Fusarium*** 4: 5’C-CAT-ATC-AGA-GAC-CCG-GGA-CCG 3’

1. PRETRATTAMENTO (+1.6Vx120s - +1.8Vx60s)
2. IMMOBILIZZAZIONE (+0.5Vx300s)
3. IBRIDAZIONE (10min)

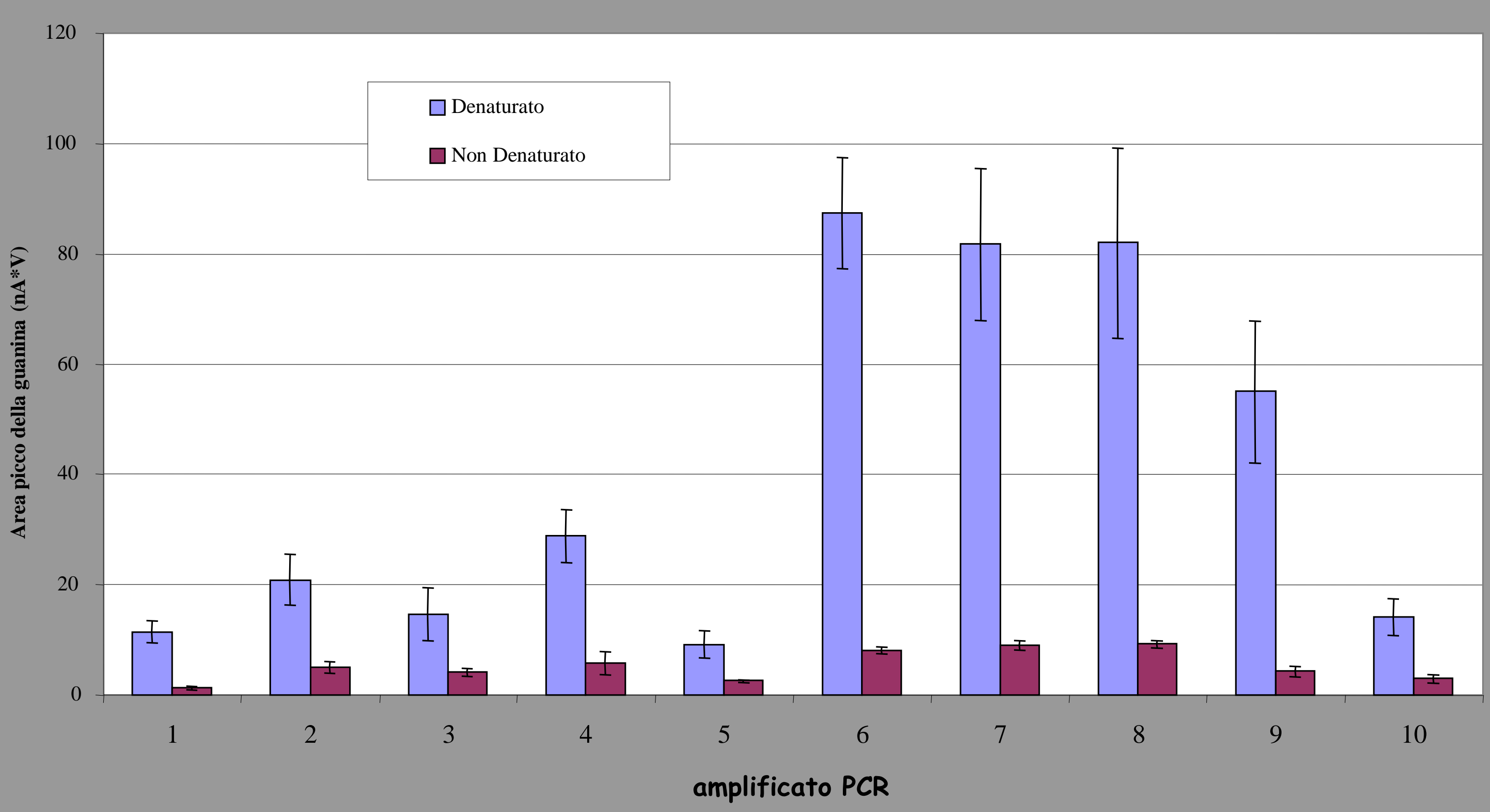
FASI DELLA PROCEDURA ANALITICA

3. IBRIDAZIONE



Risposta del sensore a varie concentrazioni del complementare e del non complementare del probe 3

risposta denaturato/non denaturato per 10 ceppi europei di F.Culmorum



Risposta del probe 3 agli amplificati denaturati e non denaturati

Genetically modified organisms (GMO)

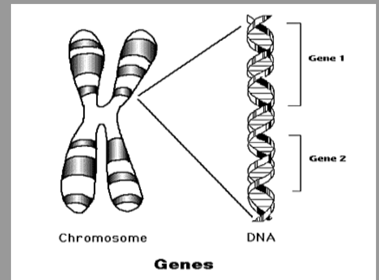
- Determination of specific DNA sequences obtained by a genetic modification in plant materials (the matrix for food production)
- Screening methods are based on the detection of the two regulatory sequences by Polymerase Chain Reaction:

35S promoter
NOS terminator

These sequences are introduced in most of GMO (Except China)

for the expression of transgenes.

- *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)

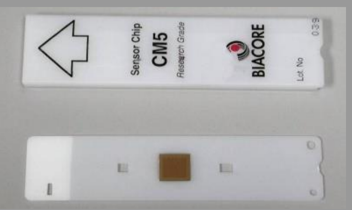
Denaturation of the dsDNA (amplified fragment or genomic) to obtain a single-stranded DNA (sample pretreatment)

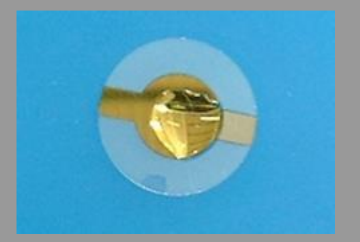
Hybridisation of the obtained ssDNA with the immobilised probe

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

Au

Thiol

Epichlorhydrin
+
Dextran

Dextran-thiol binding

Bromoacetic Acid

Dextran Carboxylation

NHS
EDAC

COOH Activation

Streptavidin

Streptavidin Binding

Ethanolamine Hydrochloride

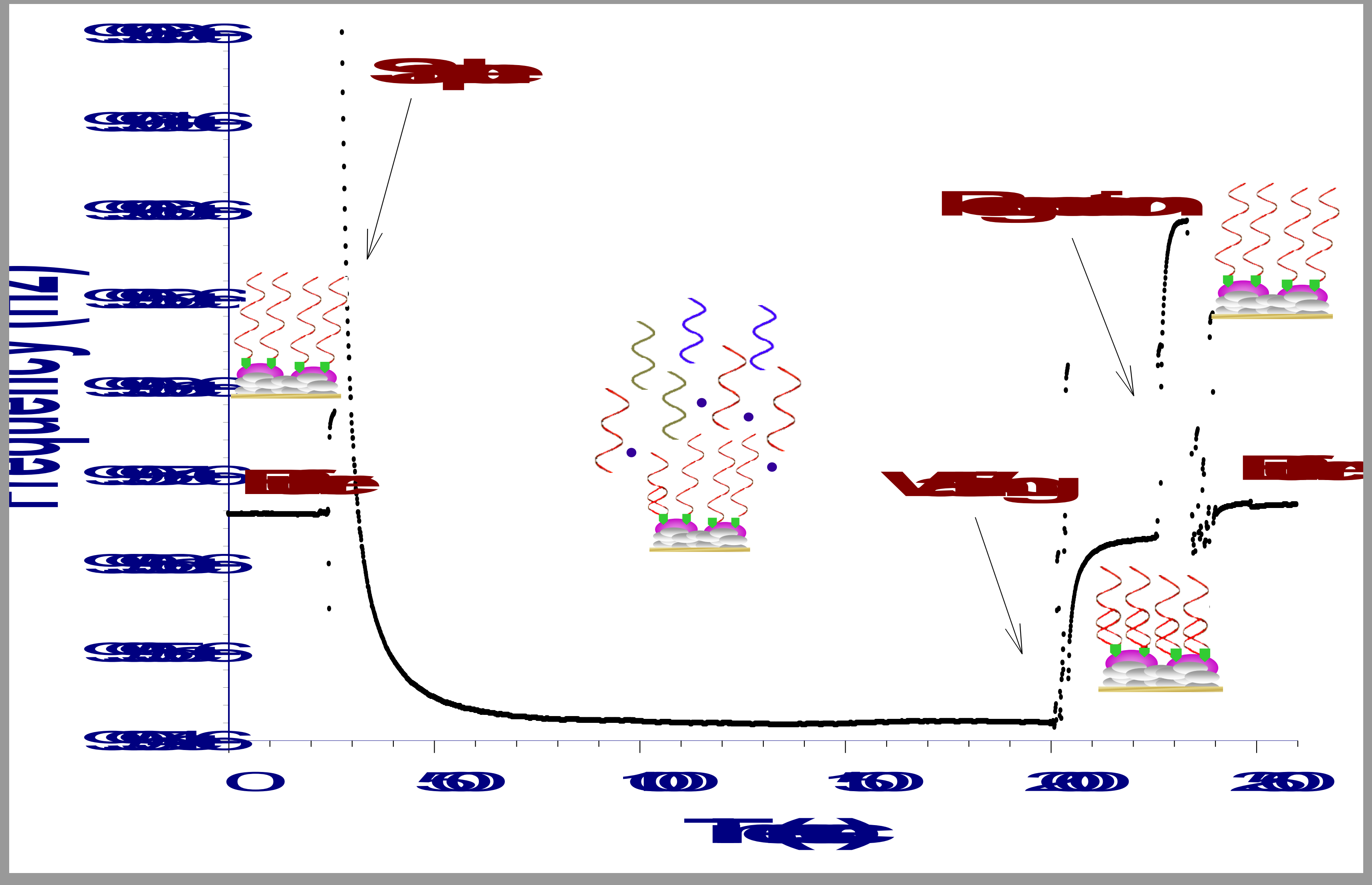
Residual reacting sites Blocking

Biotinylated Probe Immobilisation

Hybridisation

- Specificity, no aspecific adsorption, stability, multi-use

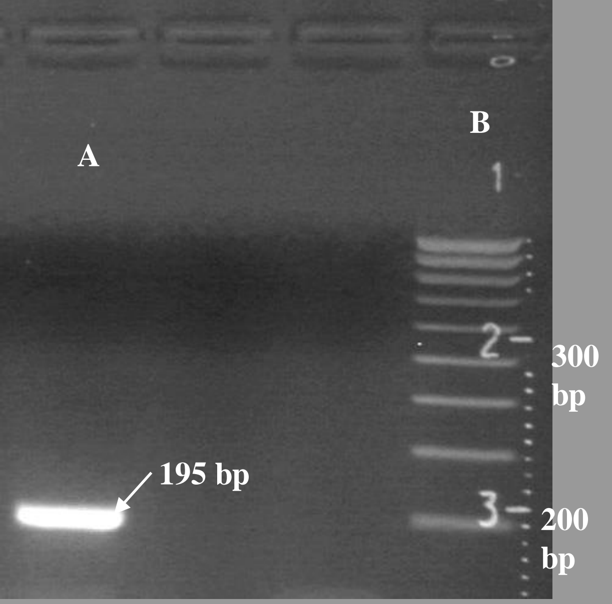
Hybridation-Regenation Cycle



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

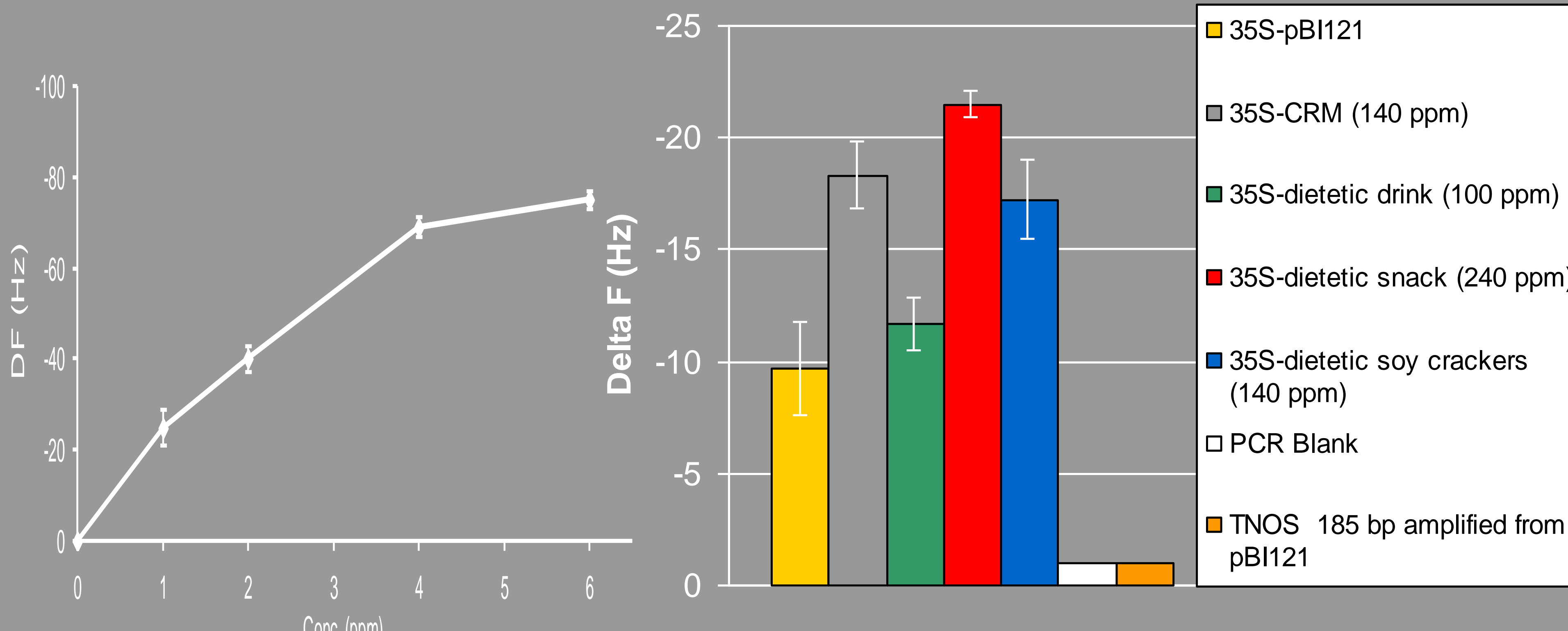
1. PCR amplified DNA

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