Biosensors: biological receptors, and transduction modes

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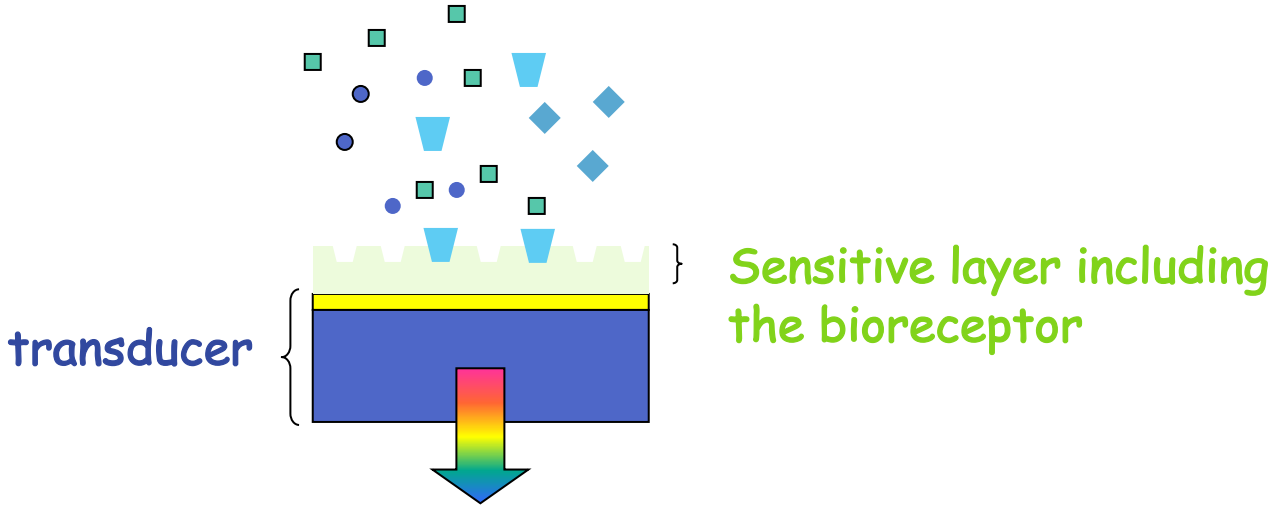
What is a biosensor ?

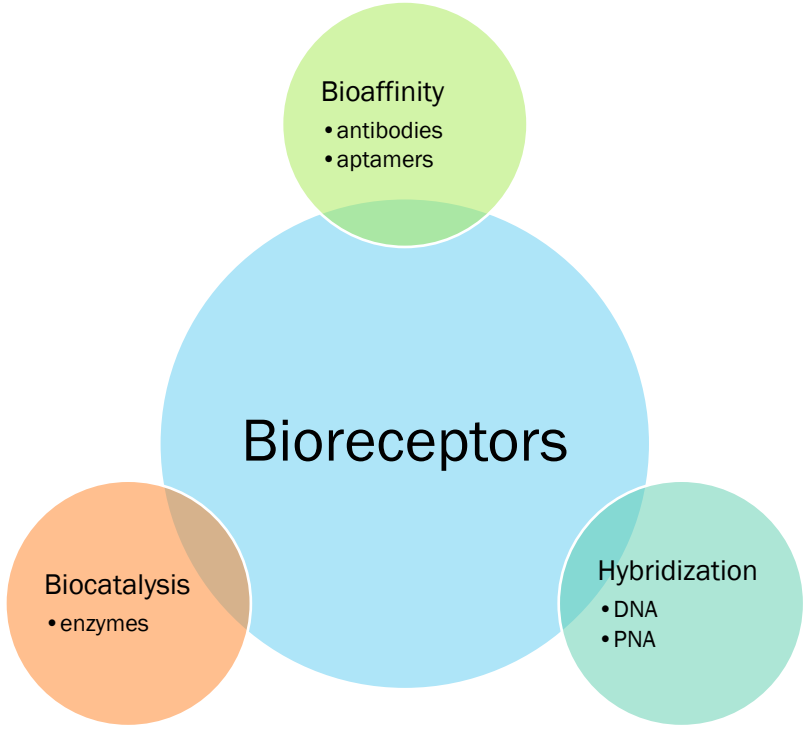
Analytical device enabling the detection and even the **quantitation** of a single species or a family of species (denominated ***the analyte***) present in various ***matrices***

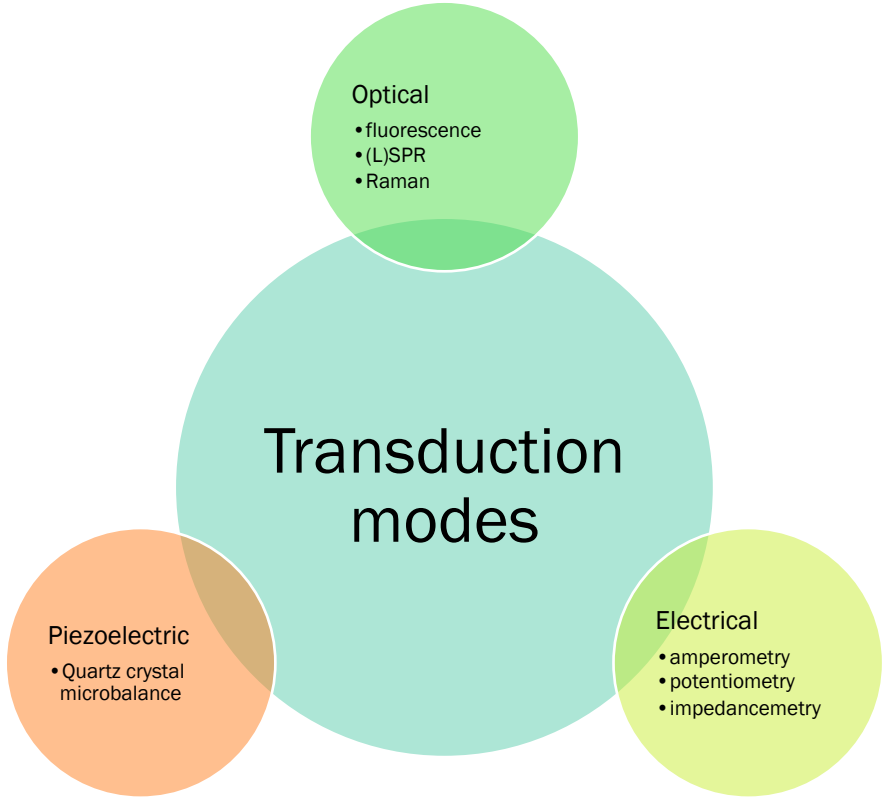
Combination of a ***biological receptor*** (providing analyte recognition specificity) with a ***transducing*** element (or **transducer**) that translates the biological signal into a physical signal

Schematic principle of biosensors

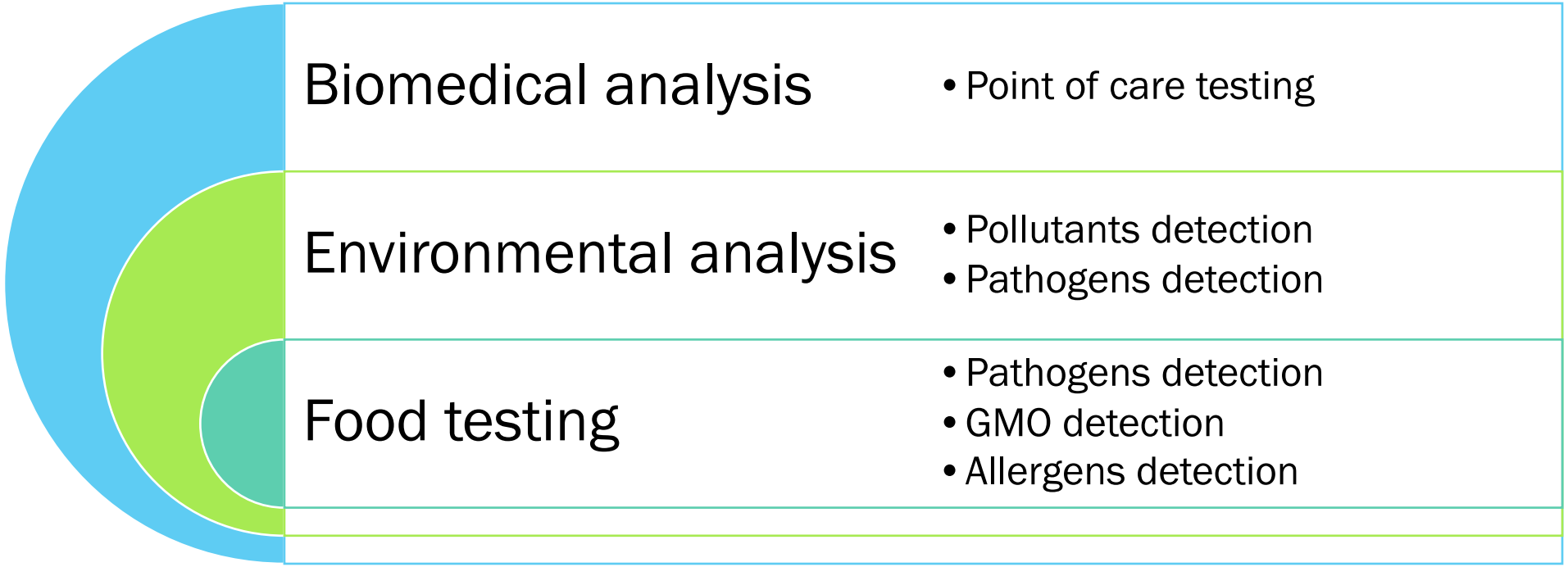


Bioreceptors for analyte detection and transduction modes





Applications



Biological receptors

Enzymes

Enzymes

- Proteins (=polypeptides)
- Catalysts of the living world
- Lock-and-key type mechanism
  (complementarity of structure between substrate(s) and enzyme)
- Specificity of substrate recognition
- Existence of inhibitors (ex: analogues of substrate)

Classification of enzymes

- Oxido-reductases (EC 1.)
- Transferases (EC 2.)
- Hydrolases (EC 3.)
- Lyases (EC 4.)
- Isomerases (EC 5.)
- Ligases or synthetases (EC 6.)

Enzymatic biosensors

Two operating configurations :

1. The analyte is a **substrate** of the enzyme and the **product** of the reaction catalyzed by the enzyme is quantified directly or indirectly at the transducer

Examples : glucose oxidase, urease

2. The analyte is an **inhibitor** of the enzyme and its detection is done by measurement of the residual enzymatic activity (substrate -> product quantified at the transducer)

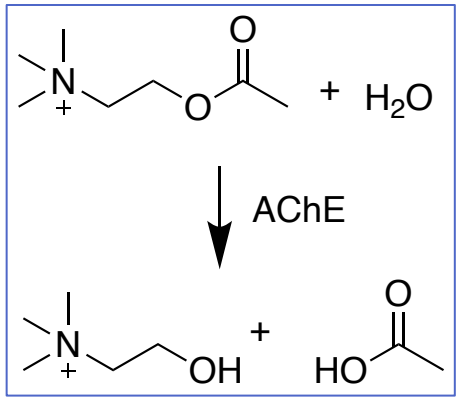
Example : acetylcholinesterase

Acetylcholinesterase (AChE)

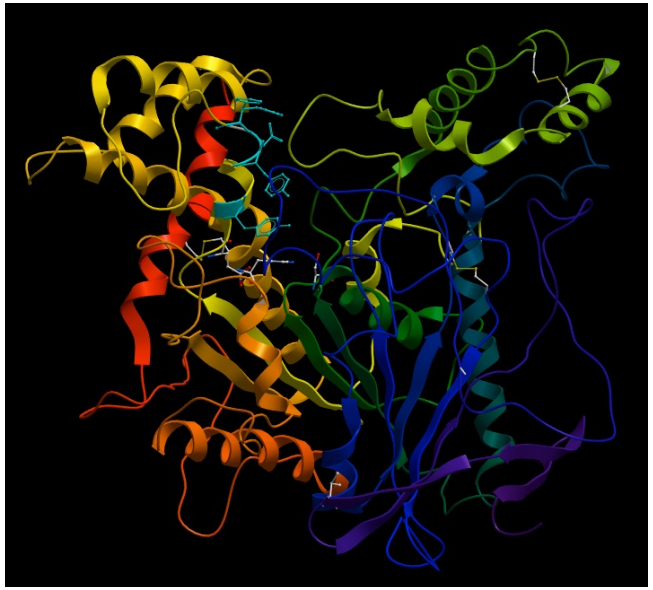
Biological role : termination of the transmission of the nerve impulse at cholinergic synapses

Function : Catalyzes the hydrolysis of the neurotransmittor **acetylcholine** into choline and acetic acid

Type : Serine hydrolase

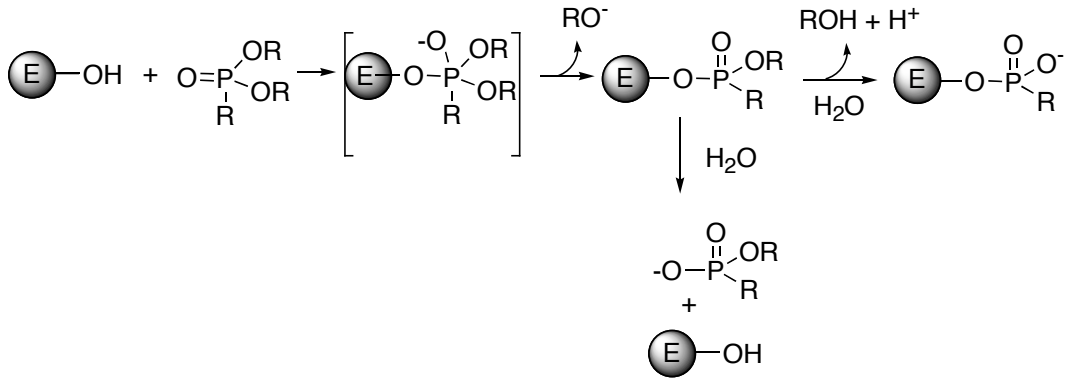


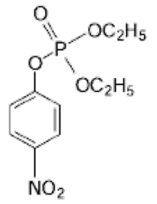
X-ray structure of AChE (drosophilia)

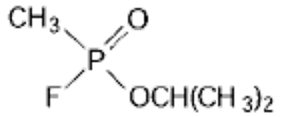


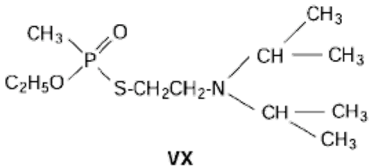
Mechanisms of inhibition of AChE

Irreversible inhibition : organophosphorous derivatives

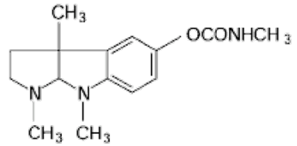



Paraoxon

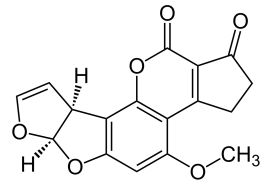

Sarin


VX

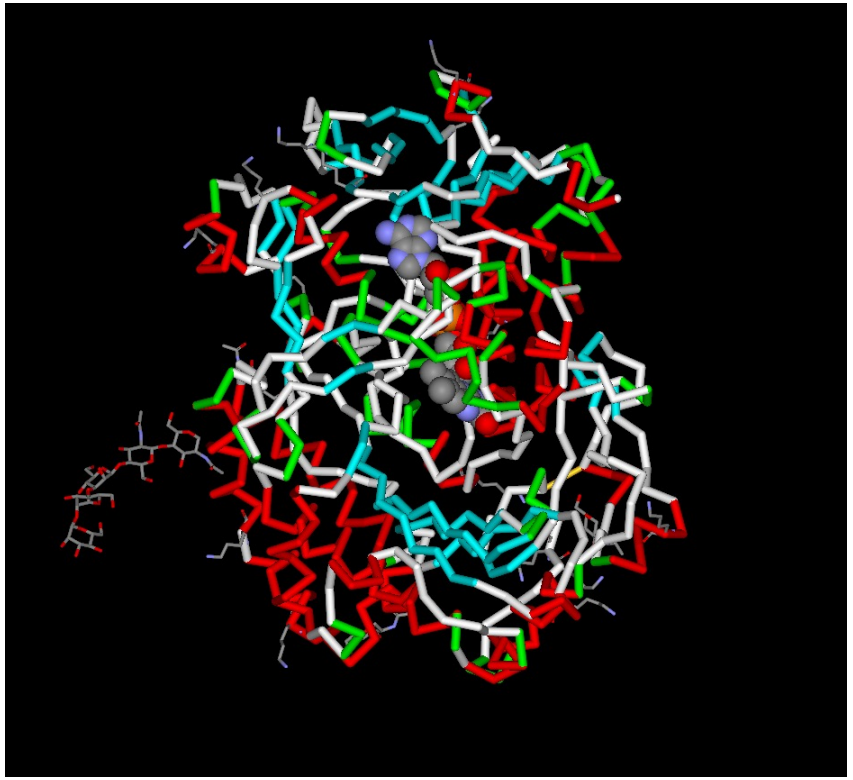
Pseudo-irreversible inhibition : carbamates


Physostigmine

Reversible inhibition : aflatoxin B1 (mycotoxin produced by *Aspergillus flavus* with hepatotoxic and hepatocarcinogenic properties)



Glucose oxidase (GOx)



X-ray structure of GOX from *Aspergillus niger* (flavoenzyme; glycoprotein)

Enzyme relatively specific of glucose, stable to pH, temperature, ionic strength

<u>Glucose oxidation</u>

Glucose + GOx-FAD -> Gluconolactone + GOx-$FADH_2$

<u>FAD regeneration by dioxygen</u>

GOx-$FADH_2$ + O_2 -> GOx-FAD + H_2O_2

H_2O_2 -> 2 H^+ + O_2 + 2 e-

<u>FAD regeneration by mediator</u>

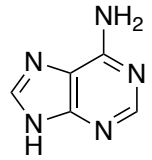
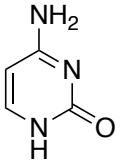
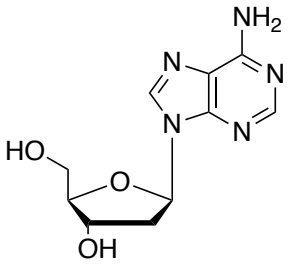
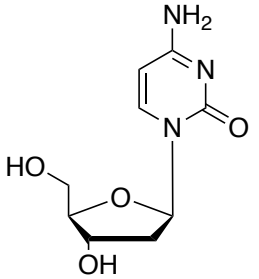
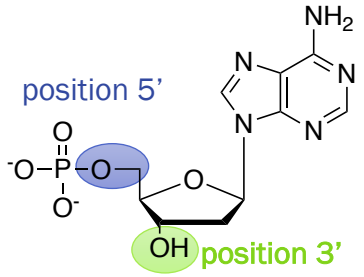
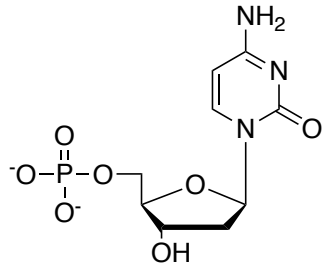
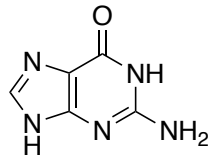
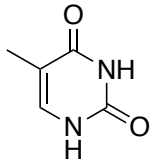
GOx-$FADH_2$ + 2 Med (ox) -> GOx-FAD + 2 Med (red)

2 Med (red) -> 2 Med (ox) + 2 e-

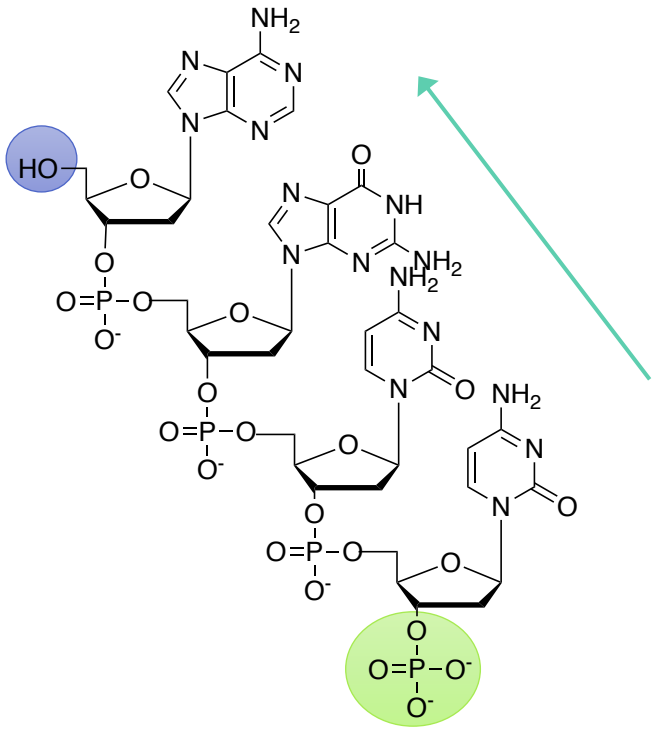
Biological receptors

DNA

Bases, Deoxyribonucleosides and -tides

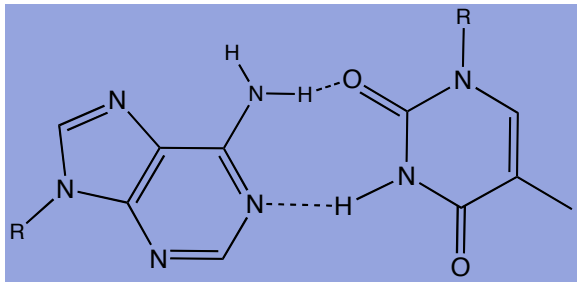
Purines	Pyrimidines
 Adenine (Ade)	 cytosine (Cyt)
 deoxyadenosine (dA)	 deoxycytidine dC
 deoxyadenosine 5'-phosphate (dAMP)	 deoxycytidine 5'-phosphate (dCMP)
 Guanine (Gua)	 Thymine (Thy)

Polynucleotides and base pairing

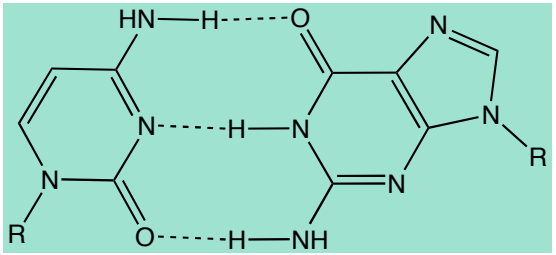


dCdCdGdA

Base complementarity



A = T



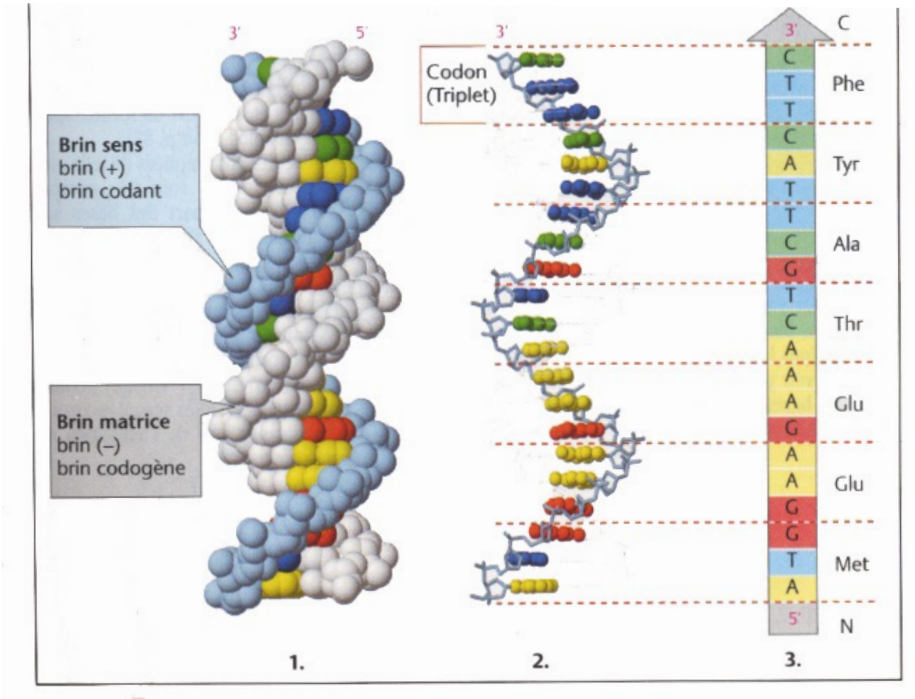
C ≡ G

DNA structure

Double helix : the antisense strand is read during transcription of DNA to mRNA) The sense strand (or coding strand) has the sequence identical to mRNA produced during transcription

The 2 strands are made of complementary bases and associate to form the double helix in an antiparallel fashion

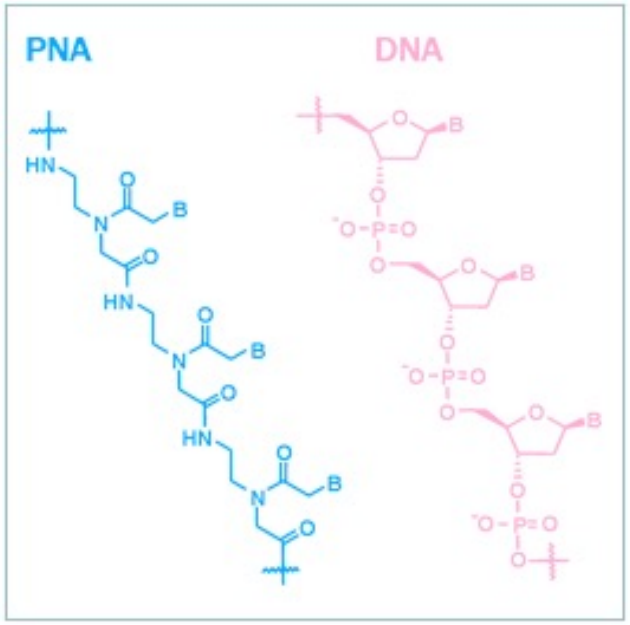
By convention, the gene sequence is defined as the sequence of the sense strand read in the 5’ to 3’ direction



The 2 strands can be separated by heating (denaturation; the melting temperature Tm is defined as the temperature at which 50% of DNA is present as single strands

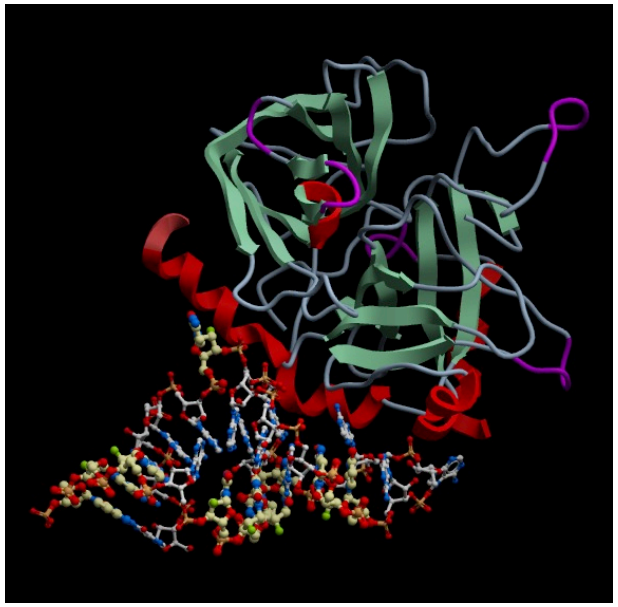
Bioreceptors related to DNA

Peptide nucleic acid (PNA)



Phosphodiester backbone replaced by polypeptidic chain
PNA is neutral, forms a duplex with complementary DNA
PNA/DNA duplex is more stable than the DNA duplex

Aptamers



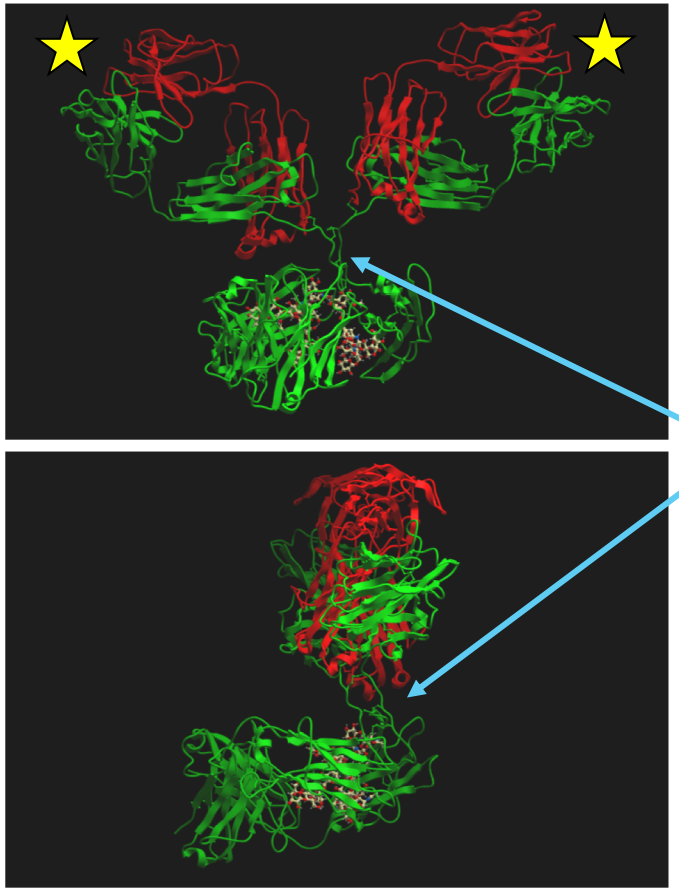
X-ray structure of aptamer / thrombin complex

Synthetic RNA or DNA obtained by chemical synthesis (15-40 bases) that displays high affinity for various analytes thanks to their folding

Biological receptors

Antibodies

Molecular structure of IgG



X-ray structure of mouse IgG 1

Antibodies are glycoproteins containing oligosaccharides (3-12% weight)

MW = 150 000

Symmetrical molecule - Y shape

4 polypeptide chains : 2 light (L, red) and 2 heavy (H, green)

Heavy chains linked at “hinge region” by disulfide bridges

Heavy chains linked to light chains by disulfide bridge

Constant fragment (Fc) typical of the animal and variable part

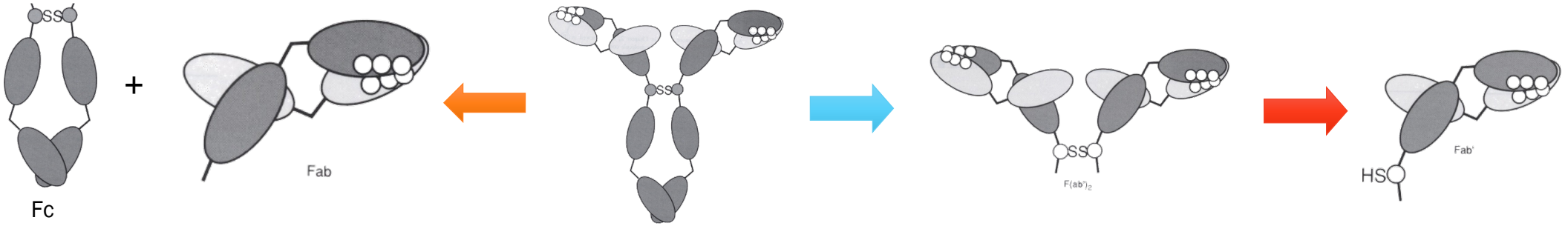
The antigen binding site (paratope) is located on the variable part ★

IgG fragments

Antibody fragments keep their ability to recognize the antigen and are obtained by enzymatic treatment with **proteases**

Pepsin : cleaves the antibody below the S-S bridges of the hinge region to give the **F(ab')$_2$** fragment

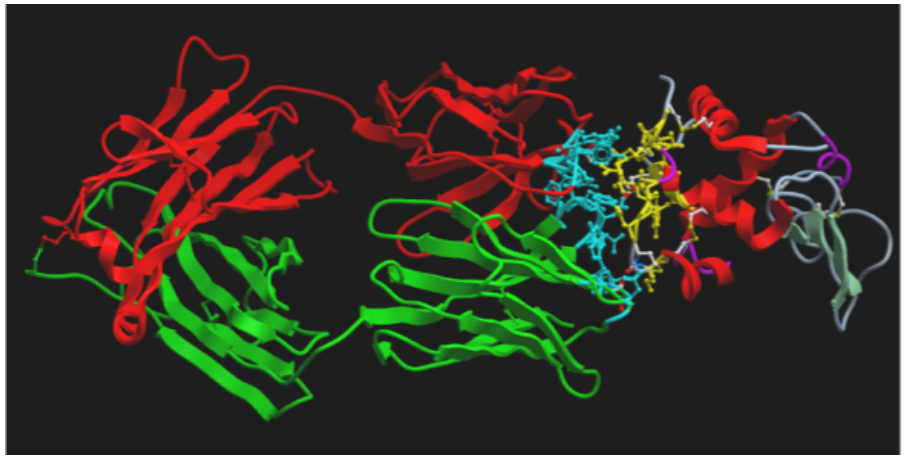
It can be further cleaved to **F$_{ab}$'** by selective reduction of S-S bridges of the hinge region



Papain : cleaves the antibody above the S-S bridges of the hinge region to give 2 **F$_{ab}$** fragments and the **F$_c$** fragment

Examples of Ab-Ag interaction

Interaction between D1.3 (F_{ab}) and antigen (HEWL); $K_a = 2x10^8\ M^{-1}$



15 AA (in blue) of H and L chains (includes 9 hydrophobic AA) in contact with 13 discontinuous AA of HEWL (in yellow)

Interaction between HyHEL-5 (F_{ab}) and same antigen (HEWL); $K_a = 2x10^{10}\ M^{-1}$

8 AA of H and L chains (in blue) in contact with 10 AA of HEWL (in yellow)

≠ epitopes (region rich in alpha helices for D1.3 and region containing the beta sheets for HyHEWL-5) paratopes partially similar

Production of polyclonal antibodies

- Animal : rabbit, goat, sheep, lama (!!)
- Immunogen (purified, 40 µg) with an adjuvant
- 2 injections in subcutaneous, intra-peritoneal or IV every 10 days
- Blood sample taken 2-4 weeks after immunization, serum separation and titer measurement

- Non standardized reagent : the immune response varies from one animal to the other and the taking
- Variable specificity and affinity
- Variable reproducibility
- Availability depends on the size of the animal and its life time

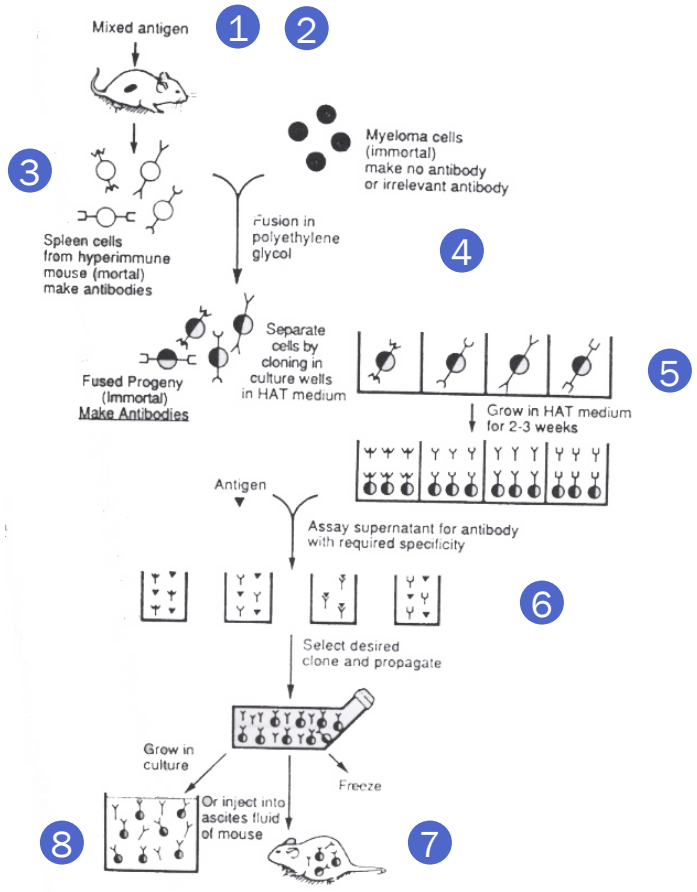
Monoclonal antibodies

Aim: Continuous production of antibodies of defined specificity

- Concept introduced by Köhler & Milstein in 1975
- Each lymphocyte possesses a single type of receptor and recognizes a single type of antigen, leads to the production of a single strain of antibodies.
- Immortality of lymphocytes by fusion with myelome cells + cloning by physical separation of **hybridomes**
- Hybridomes combine properties of cells from which they derive; easy culture as cancer cells and ability to produce antibodies indefinitely

Production of mouse monoclonal antibodies

1. Same immunisation protocol
2. 4 days before fusion, new injection
3. Spleen is taken
4. Mixture with myelome cells in HAT medium in the presence of PEG
5. Dilution and distribution in 96-well plates
6. After 2-4 weeks , supernatants are tested
7. After other steps, selected clones are extended in vivo by injection to mice treated with pristane and the ascite liquid is taken in the peritoneal cavity : it contains a large concentration of specific antibodies (10 -20 mg/ml) in addition to non-specific mouse IgGs
8. Alternatively, production by cell culture



Antibodies as analytical reagents

The use of antibodies as biological receptors in biosensors (= immunosensors) derives from their use in immunoanalysis.

Immunoanalysis is a classical example of a discovery based on a natural phenomenon (immunity) that is employed in a totally different context.

To this end, antibodies produced by mammals in response to antigens (virus, bacteria and parasites) are used as analytical reagents thanks for their extraordinary binding affinity and specificity for their corresponding antigens.

Antibodies as analytical reagents

- ✓ Exceptional specificity
- ✓ Stability
- ✓ Nearly universal application
- ✓ Large variety of analytes

- Specificity is not always perfect : an antibody cannot sometimes differentiate molecules with closely related structures : **cross-reactivity**
- Antibody features depend of the immune response of the animal : **reproducibility** and **durability** issues
- Certain antigens can be toxic or non immunogenic
- Issues related to the manipulation of biological material

Immunoanalysis

The use of an antibody for the assay of an analyte was introduced in the early 60's by Yalow & Berson for the assay of insulin (Nobel price in 1977) and by Ekins for the assay of thyroxine.

Its principle is based on the observation that, in a system containing the analyte (Ag) and a specific antibody (Ab), the distribution of the analyte between the bound and free forms quantitatively depends on the total concentration of analyte

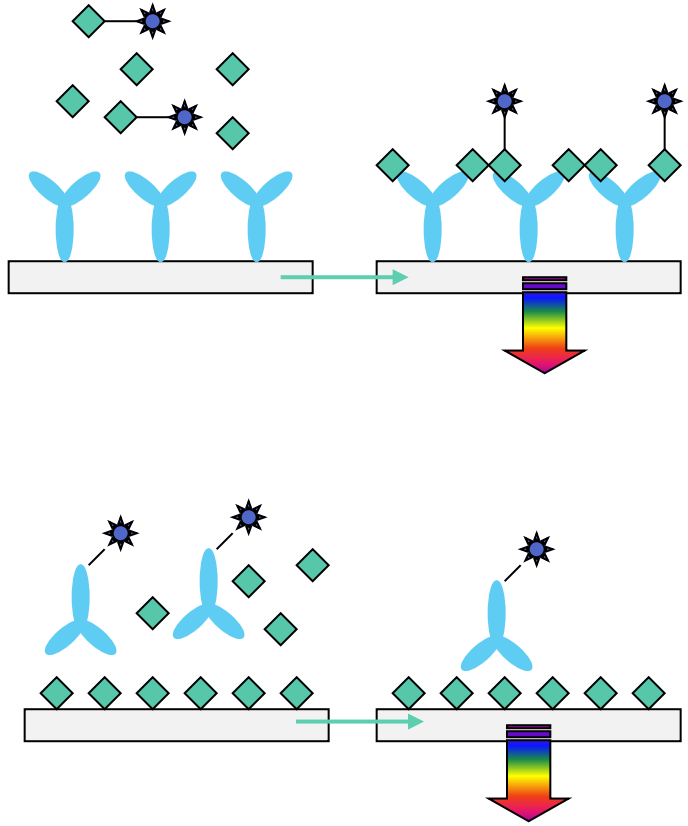
$$Ag + Ab \Leftrightarrow Ag\text{-}Ab$$

Immunoassay configurations

1) Limited reagent methods : competitive assays

A) The anti-analyte antibody is immobilized on a solid phase. The sample is added together with a tracer (labeled analyte) (simultaneously or after preincubation). Free species are discarded by washing and the bound tracer signal is measured. This configuration should be used with an affinity-purified antibody

B) The antigen or the hapten-protein conjugate is immobilized on a solid phase. The sample is added together with labeled antibody. Free species are discarded by washing and the signal of the labeled antibody is measured. If labelled antibody is not available, a secondary labeled anti-IgG antibody can be used afterwards.



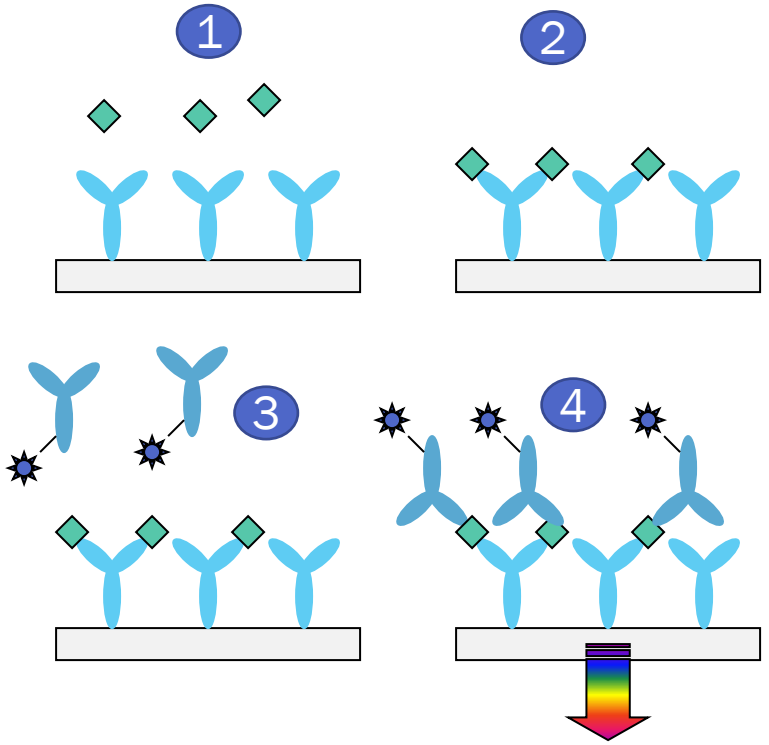
Immunoassay configurations

2) Excess reagent method : non competitive assay

Only for **macromolecular antigens (with at least 2 epitopes)** for which simultaneous binding of 2 antibodies is possible without steric hindrance issues

The capture antibody in excess is immobilized on a solid phase. The sample is added (1). After incubation and washing (2), excess labeled antibody is added (3). After incubation and washing, the signal of the revelation antibody is measured (4)

This format generally uses 2 monoclonal antibodies against 2 different epitopes, or a combination of polyclonal + monoclonal antibodies

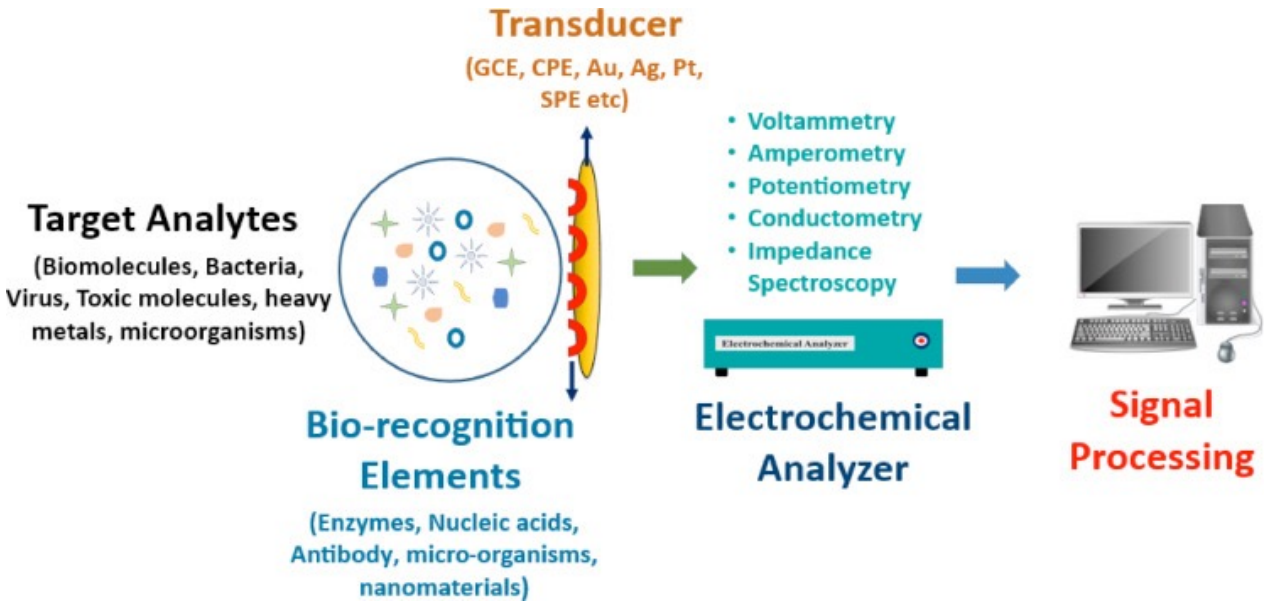


Transduction techniques

Electrical, Optical and Piezoelectric techniques

Electrochemical transduction

General principle: measurement of charge transfer occuring at the electrode / analyte interface
Requires a conductive transducer



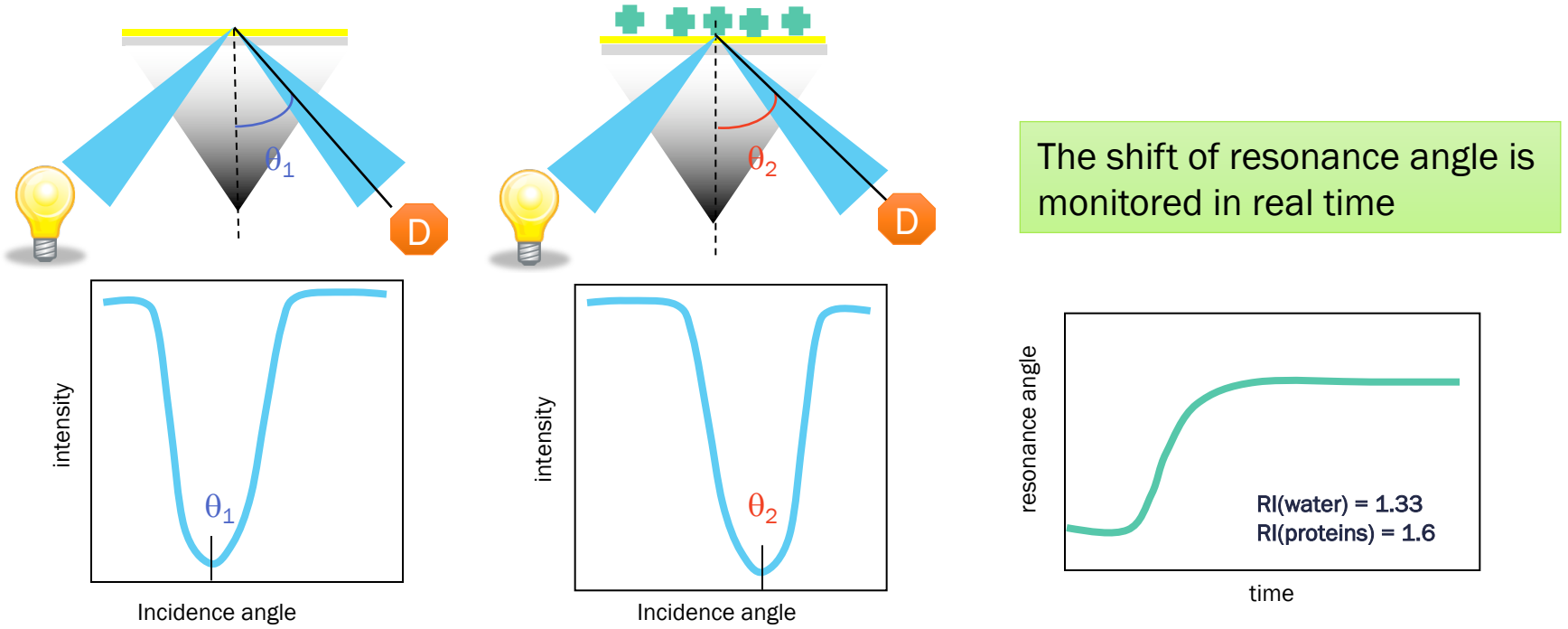
Voltammetry
current and potential are measured while the potential is scanned over a given range

Amperometry
Current resulting from oxidation of reduction of species is measured at a fixed potential

Electrochemical impedance spectroscopy
Measurement of current resulting from sinusoidal potential of increasing frequency

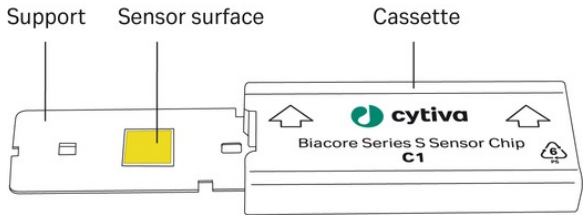
Surface Plasmon Resonance (SPR)

A p-polarized monochromatic light beam covering a range of incidence angles is directed towards the surface of the transducer (40-50 nm thick gold film laid on glass prism). At a certain angle θ (of resonance), the intensity of reflected light is minimal. Variation of the local refractive index (RI) at the vicinity of the transducer (100 – 300 nm) results in a variation of the resonance angle

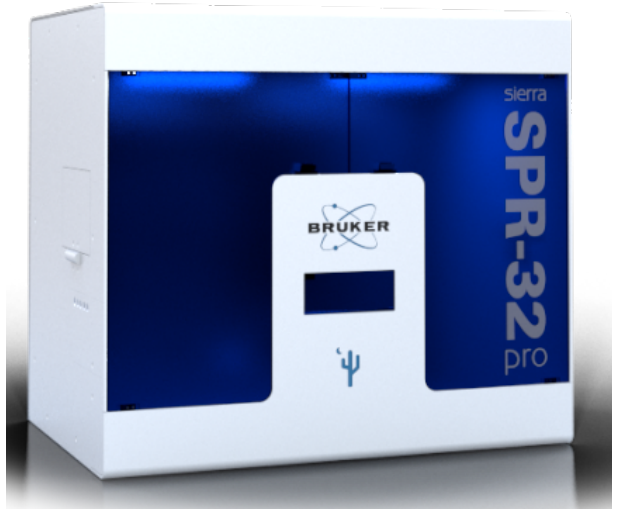


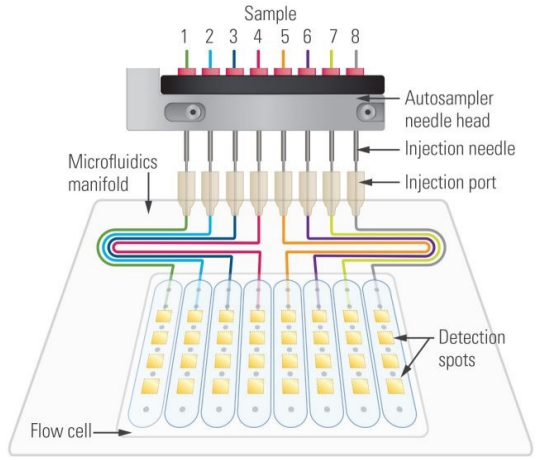
Benchtop SPR instruments

Biacore (Cytiva)



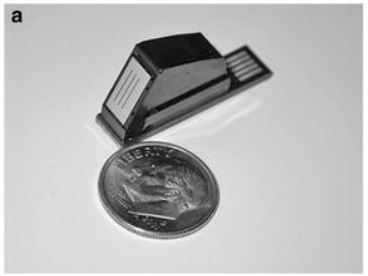
SPR-32 (Bruker)

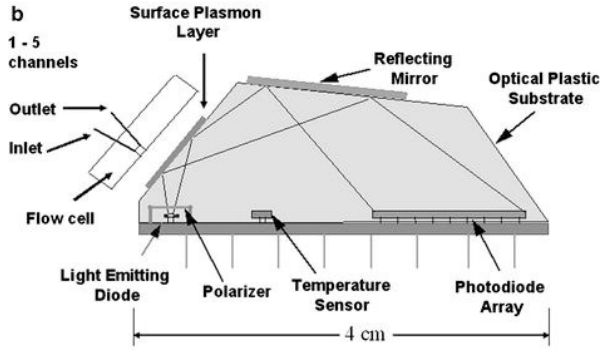




8 channels and 4 detection spots

Miniaturized SPR: Spreeta





3-zone sensor

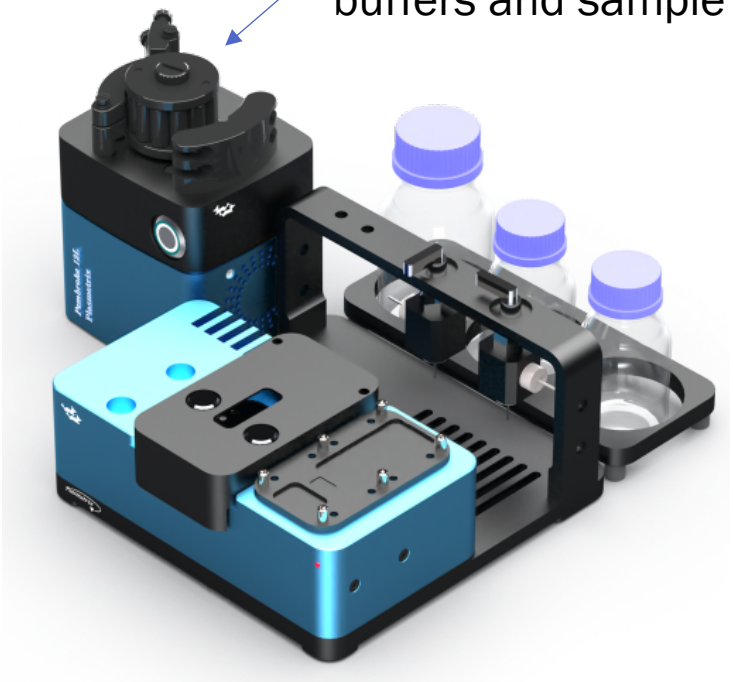
A near IR light beam (830 nm) is emitted by a LED then polarized. It is reflected at the back of the gold-coated surface and directed towards a linear array of 256 silicon photodiodes. The angle of resonance is determined from the diode receiving the minimal reflected intensity

$1° <-> 11x10^{-3}$ RIU
$1x10^{-6}$ RIU <-> 1 pg/mm^2

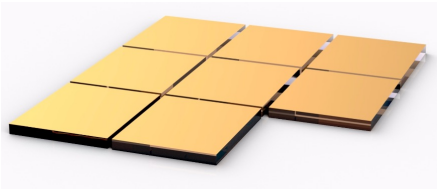
A multi-channel (≥2) circulation cell is sticked to the gold surface. The device is placed in a temperature-controlled cell; The device also includes a peristaltic pump, a vacuum pump and an analog – to digital converter

Miniaturized SPR: CORGI IIF (Plasmetrix)

peristaltic pump to deliver buffers and sample



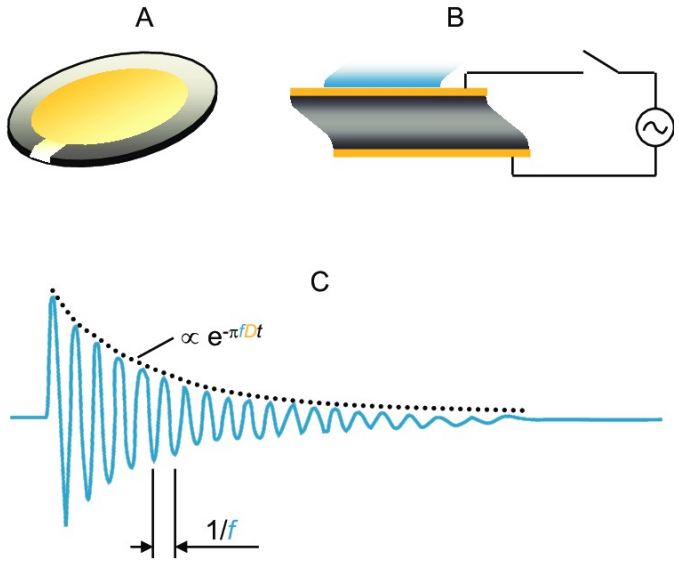
Sensor chips



Other optical transduction techniques

- Localized Surface Plasmon Resonance (LSPR)
- Surface Plasmon Resonance Imaging (SPRi)
- Raman spectroscopy (SERS)

Piezoelectric transduction



The core of the technology is the oscillating unit - a thin quartz crystal disk, which has electrodes deposited on each side. Via an applied voltage, the crystal can be excited to resonance, and the resonance frequency is related to the thickness (mass) of the disk. If the thickness changes, so will the resonance frequency, *f*. By monitoring changes of the resonance frequency over time, Δ*f*, it is possible to detect small changes of the crystal thickness (mass).

The measurement makes it possible to detect nanoscale mass changes such as adsorption or binding of molecules to the surface, which will be detected as mass increase, whereas mass decrease will indicate mass removal, for example via molecular desorption or etching of the surface.

QCM-D measures an additional parameter, the dissipation, Δ*D*.
The dissipation gives information about the energy losses in the system and are particularly useful in the study of soft layers, where this information is used for quantification of the layer properties.

For rigid layers, mass change Δm is proportional to the variation of resonance frequence ΔF according to the Sauerbrey equation.

$\Delta F = - C_f/\Delta m$ (C_f = 17.7 ng/cm^2 for a 5 MHz quartz crystal)