CENTRE NATIONAL
DE LA RECHERCHE
SCIENTIFIQUE

QCM and biosensors

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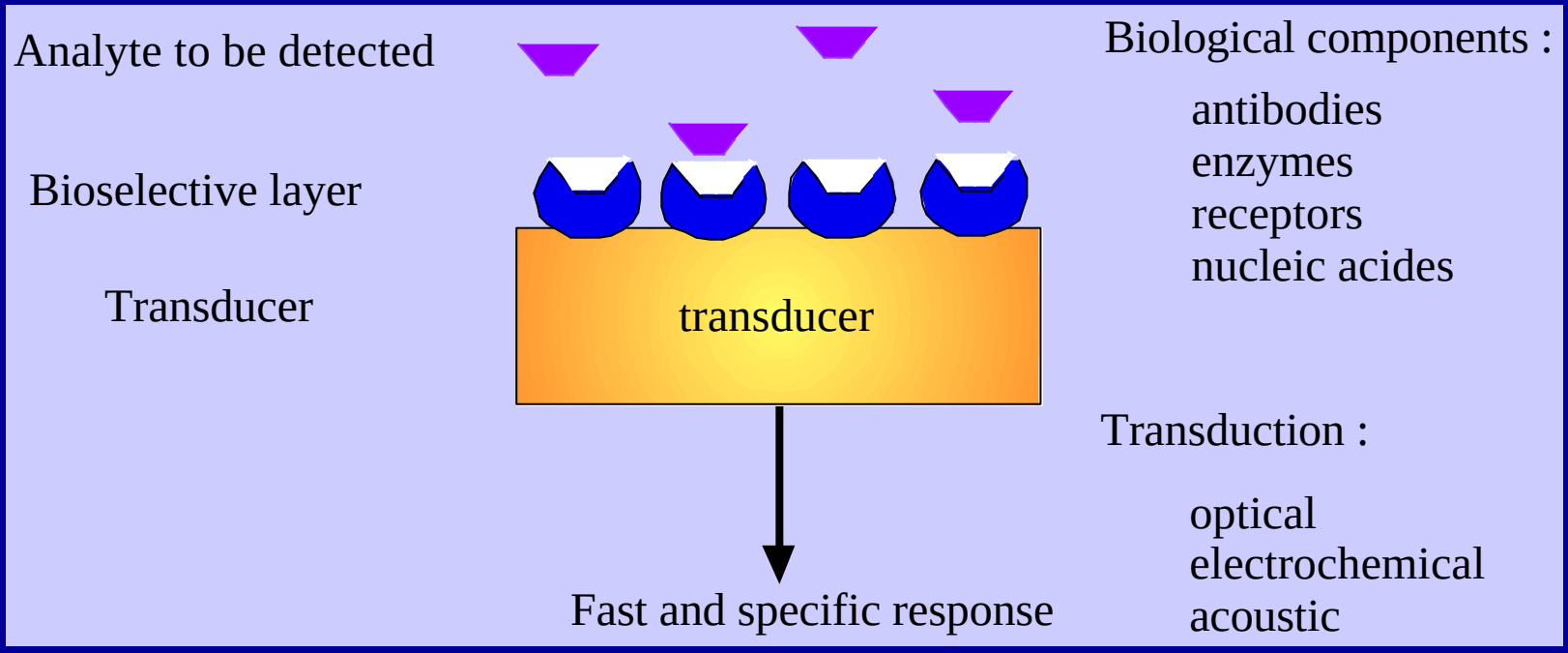
Introduction

What is a biosensor ?



What are the fields of application?

- Medical:
  home tests, fast analyses for biomedical diagnostics

- Industrial or agricultural field:
  quality controls or detection of contamination

- Military applications:
  detection of biological agents

- Research:
  studies of biomolecular interactions

But problems, specific to the QCM devices, remain:

- lack of mass sensitivity for a direct detection: pg range

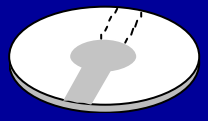
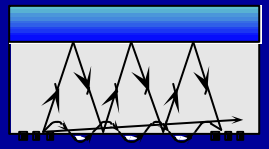
- to get the good biosensitive film

- flow and micro volume cell for using expensive biospecies

- validation of QCM measurements for non purely elastic layers

About the mass sensitivity:

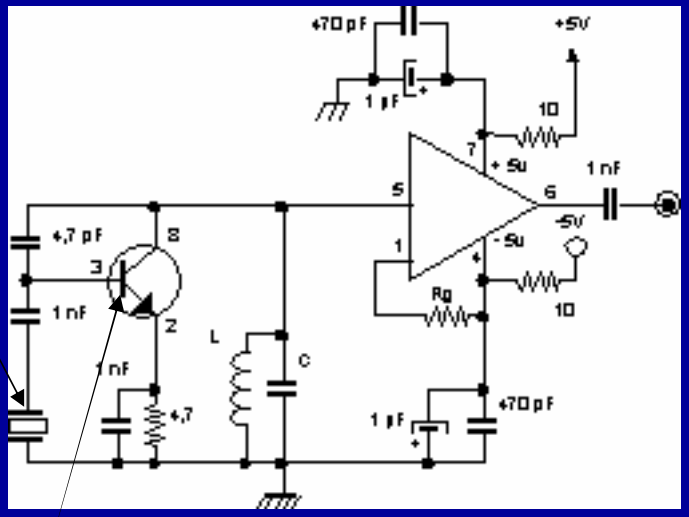
If acoustic transducers were used…

Devices		Mass Senstivity $Hz.cm^2.g^{-1}$	Mass for one Hz according to f_o
QCM		$-2{,}26.10^{-6}\ f_o^2$	6 MHz: 12.2 ng cm^{-2} 27 MHz (P3): 1.8 ng cm^{-2} 50 MHz: 5 pg cm^{-2}
SAW		$-2{,}26.10^{-6}\ f_o^2$	200 MHz: 10 pg cm^{-2}
APM		$-10\ f_o$ (n=0) $-20\ f_o$ (n>0)	104 MHz: 1ng cm^{-2} 104 MHz: 0.5 ng cm^{-2}

High frequency devices for small mass change

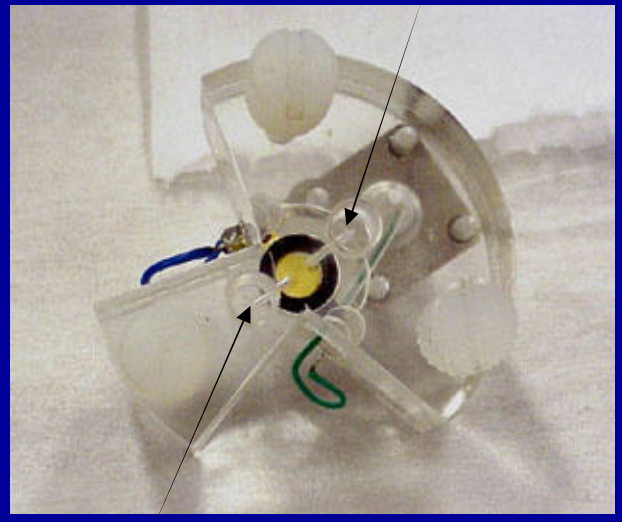
To work at 27 MHz with a BAW resonator included in a miniaturized cell

27 MHz
quartz crystal



OPA 660:
large frequency range
high gain

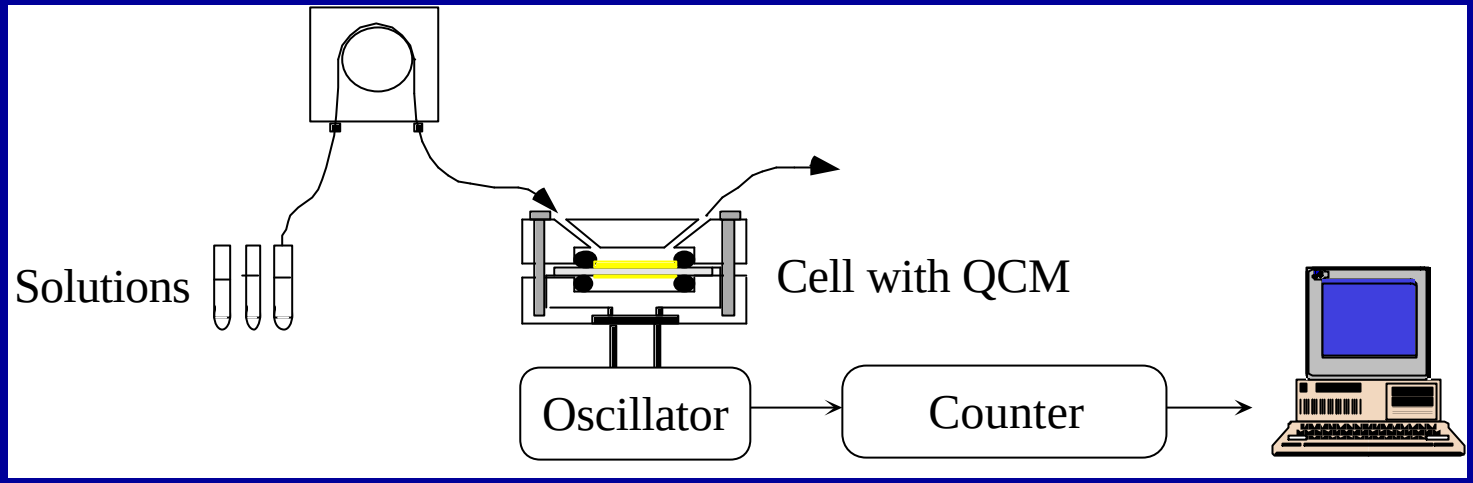
liquid input

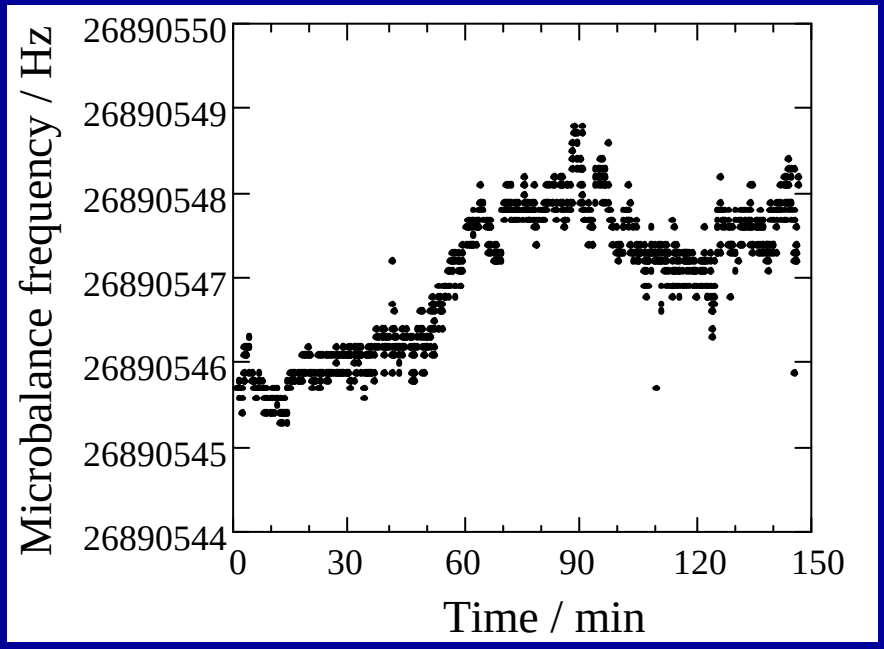


liquid output

inner volume: 50 µl

Complete experimental set up





Output signal for a 27 MHz piezoelectric device:

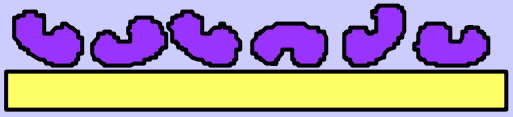
→ signal noise = 2 Hz

→ drift = better than 1Hz/hour

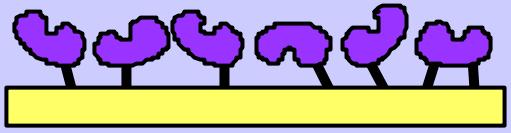
Method of immobilization

In general onto metal (gold, platinum) but also carbon, silicon dioxide…

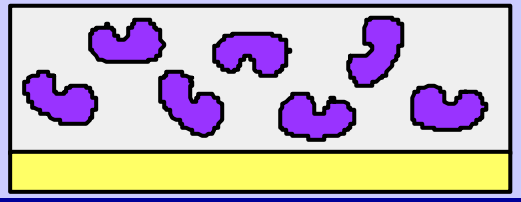
Adsorption



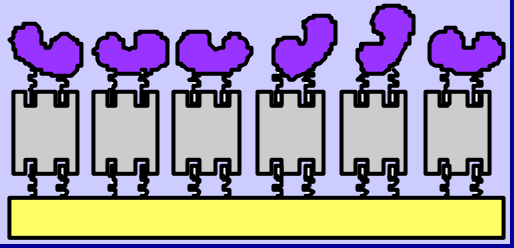
Covalent grafting



Inclusion



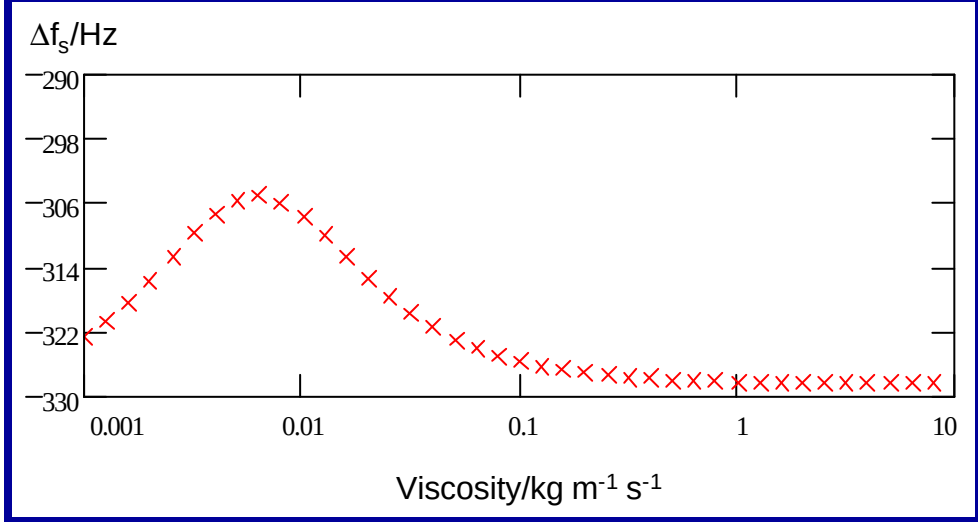
Assembled layers

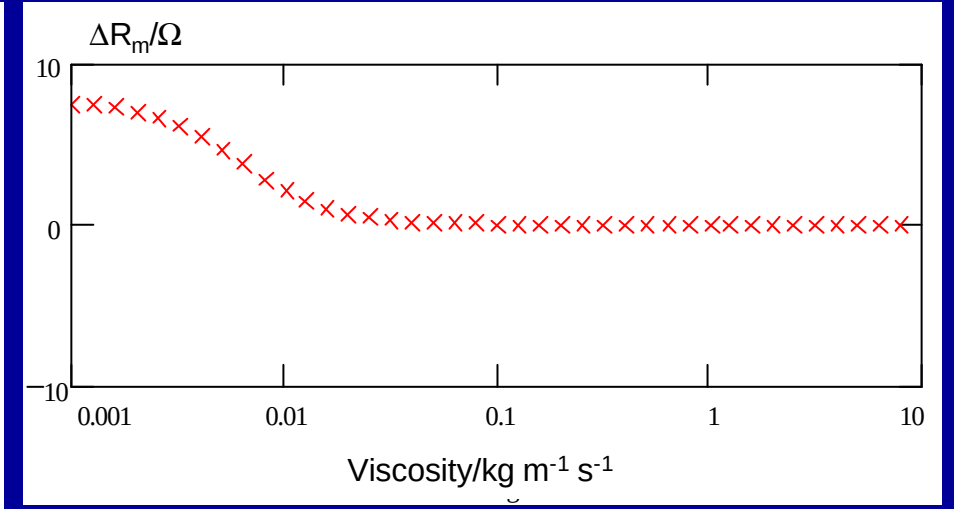


Biosensivite films act as non purely rigid films.
So, the validity of the sauerbrey equation is questionnable during experiments

A viscoelastic model allows to estimate the effect of the viscoelastic parameters of the film deposited onto the top of the resonator

→ Thickness film: 50 Å
→ Density film: 1200 kg m^{-3}
→ G'=1.0 10^{+6} N m^{-2}

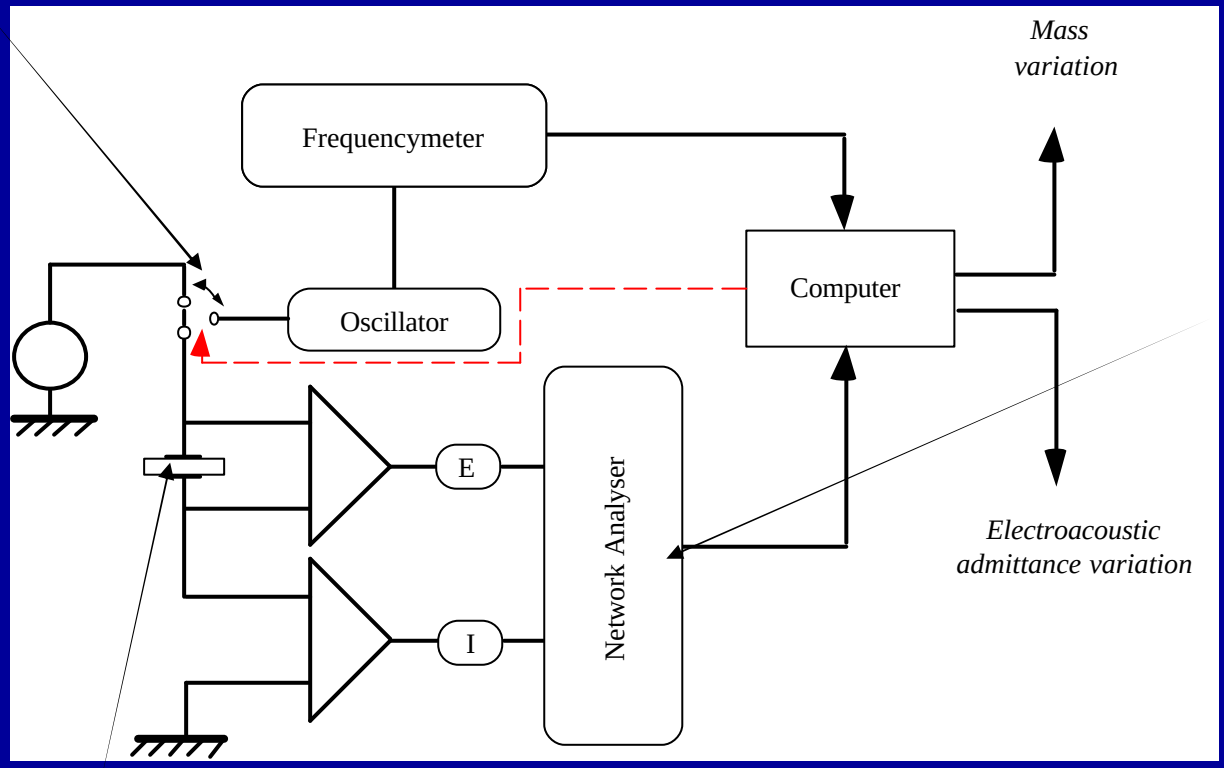




Mass estimation error: 10 %

Electroacoustic measurement

electronic and automatic switch



Network analyzer:
HP4194

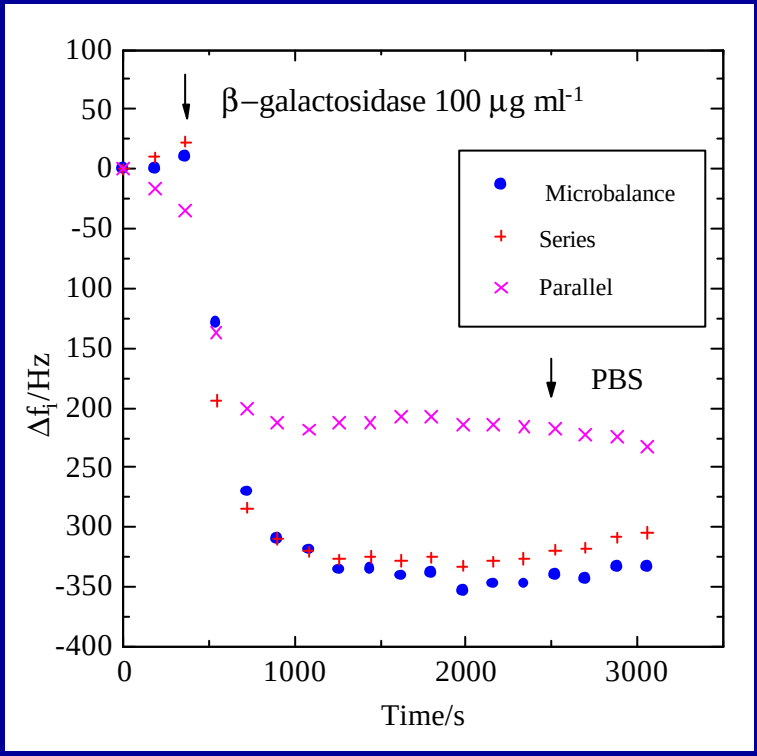
Automatic control:
HPVEE soft

QCM

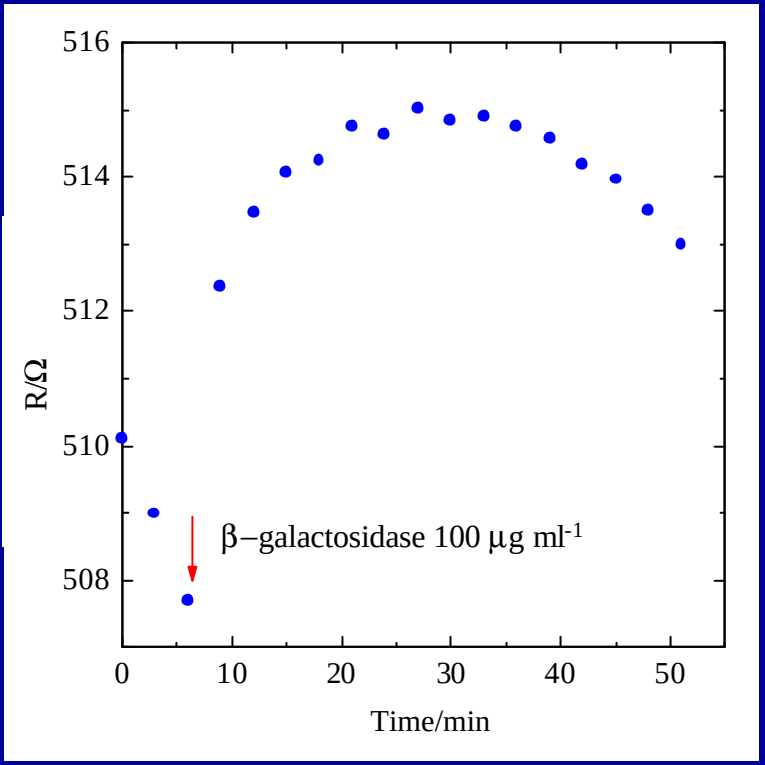
Alternate measurements of $f_{microbalance}$
and electroacoustic admittances
(equivalent circuit, modelling…)

β-galactosidase adsorption onto gold

Two main frequencies:
-microbalance
-series



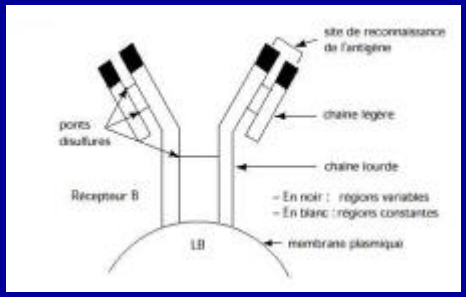
Resistance changes

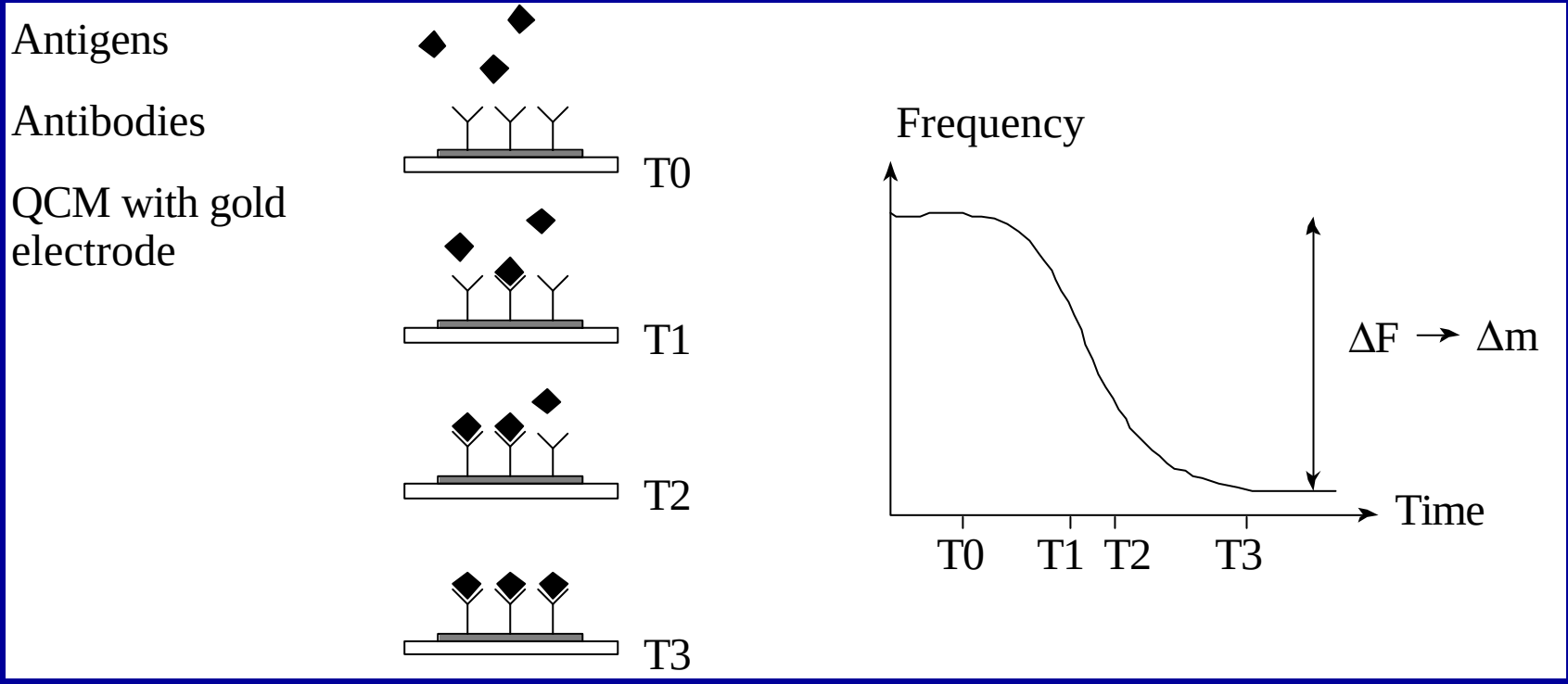


Frequency changes due to enzyme adsorption are similar

QCM and IMMUNODETECTIONS

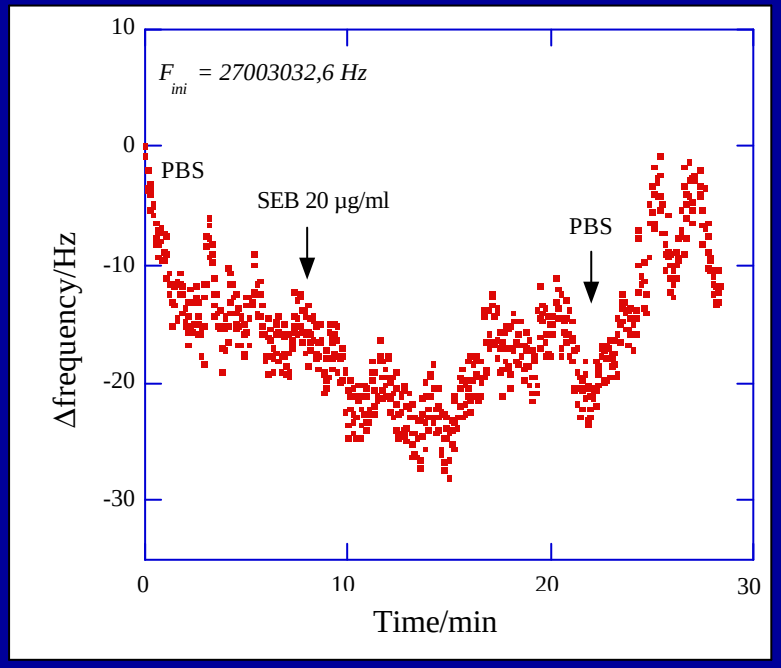
Immunodetection with QCM transducers offers large possibilities due to a direct conversion of the binding event between the detected biospecies and a selective layer.



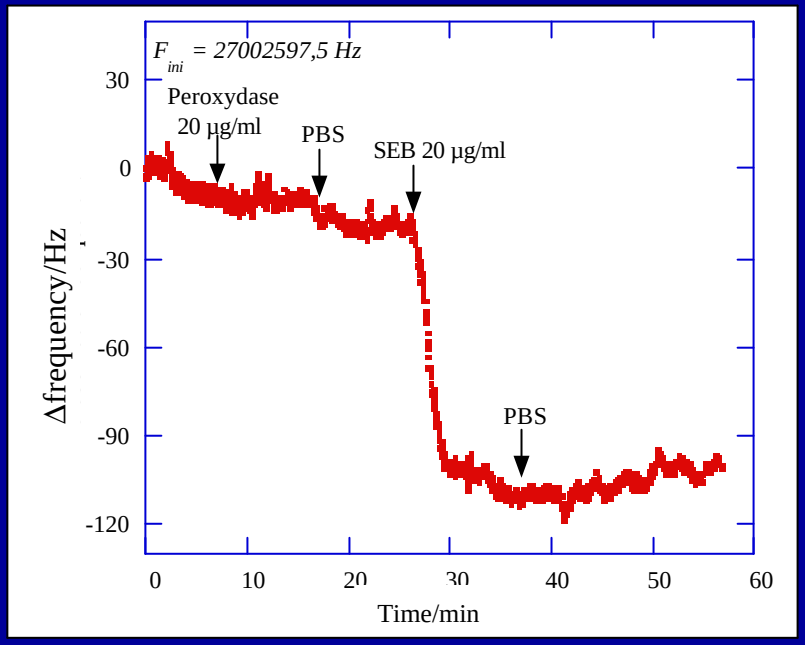


Detection of SEB (Staphylococus Enterotoxin B)

The surface is saturated with only BSA (Bovin Serum Albumin)



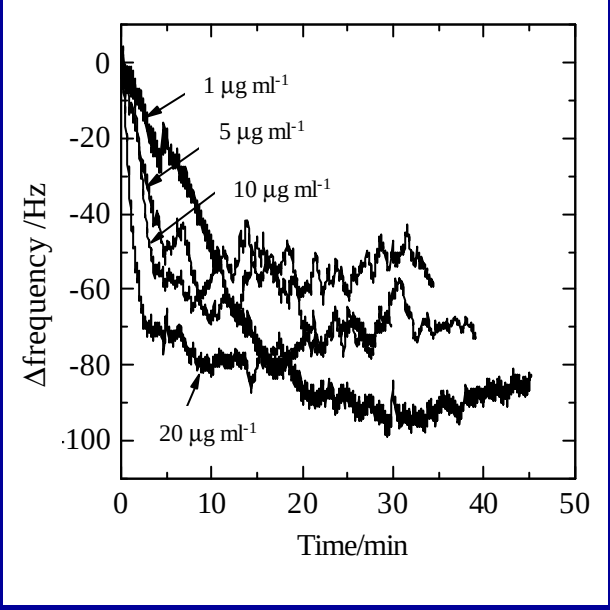
Sensitive layer: adsorption of antibodies against SEB



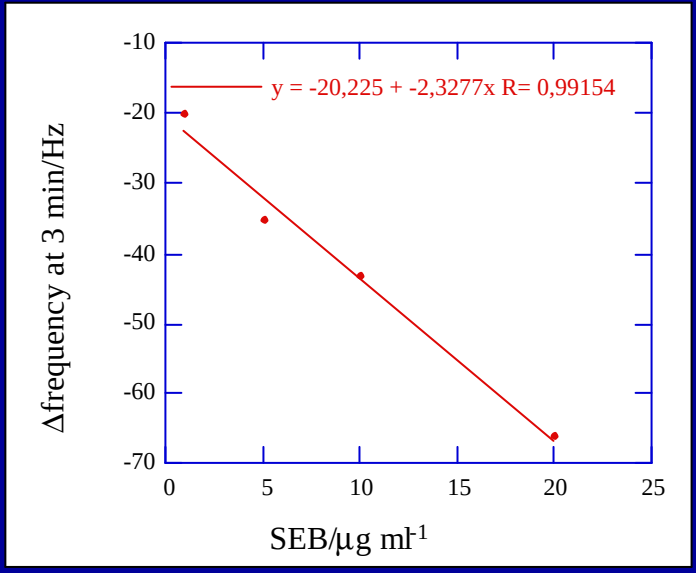
Nothing significant…

☺ Direct response
☺ Detection limit: 10^{-10} M
☺ Response time: min

By changing the SEB concentrations, the microbalance frequency evolution is plotted:



By selecting a good time, a calibration curve can be drawn:
⇒ direct detection of an unknown [SEB]



Kinetic of antigen/antibody interactions

$$Ag + Ab \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AgAb$$

Kinetic law:

$$\frac{d[AgAb]}{dt} = k_a [Ag][Ab] - k_d [AgAb]$$

In term of resonant frequencies:

$$\frac{df}{dt} = k_a c(f_m - f) - k_d f$$

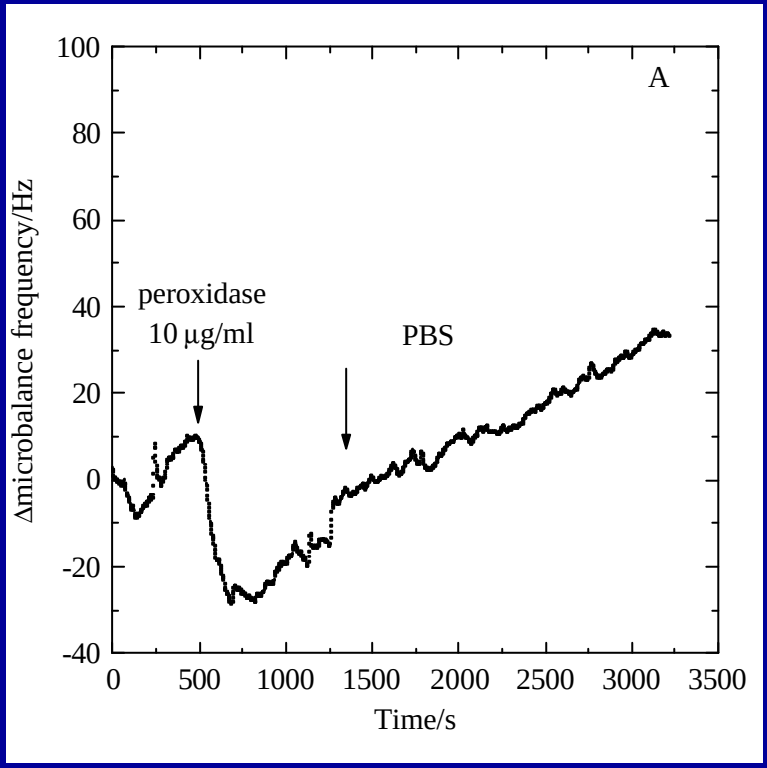
where c is the concentration of free antigens

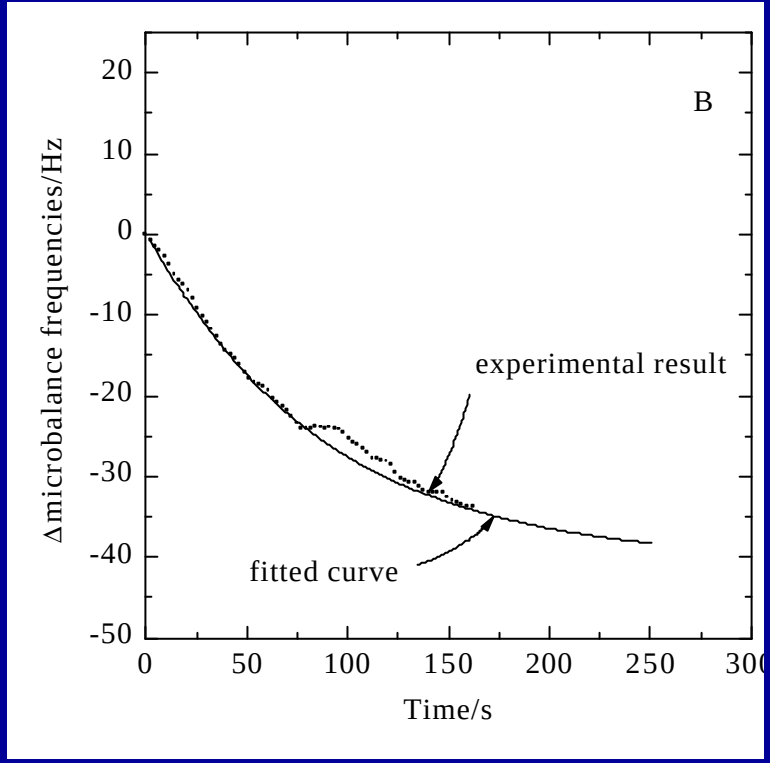
After solving this differential equation, it comes:

$$f(c,t) = \frac{k_a c f_m + k_d f_i}{k_a c + k_d} + (f_i - f_m)\exp[-(k_a c + k_d)t] - f_i$$

Kinetic of interaction and estimation with the QCM of the affinity constant

Sensitive layer is realized by a direct adsorption of the corresponding antibody (anti-peroxidase) onto the QCM gold electrode.





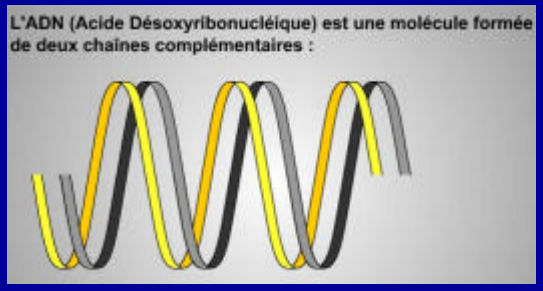
In this case by direct fitting of the QCM response:

QCM: $\frac{k_a}{k_d} = 1\,10^8 M^{-1}$

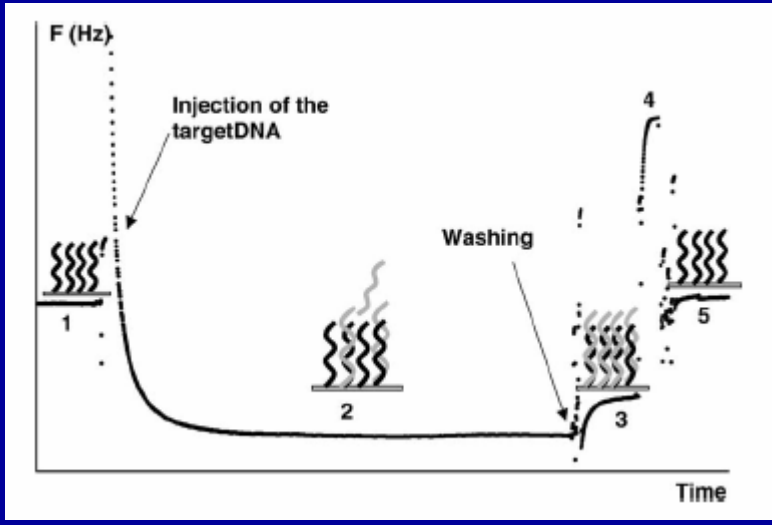
SPR system (Pharmacia): $\frac{k_a}{k_d} = 5\,10^8 M^{-1}$

DNA detection through QCM measurements

To record the transducer signal resulting from the hybridisation between the probe and the complementary strand in solution called target.



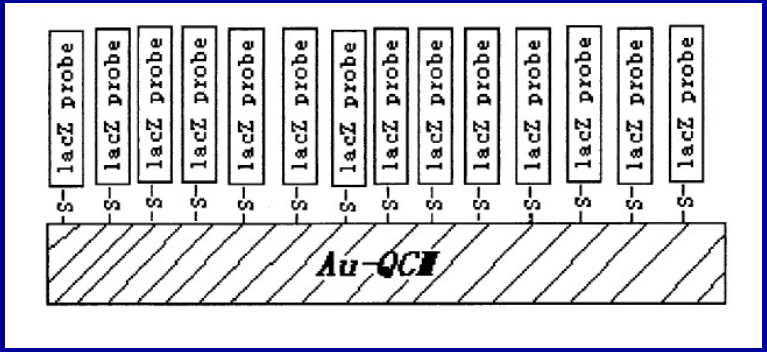
1. Free probe
2. DNA target in contact with the free probe
3. Washing
4. Regenerating agent
5. Free probe (initial step)



Theoretical advantages: real time without any label

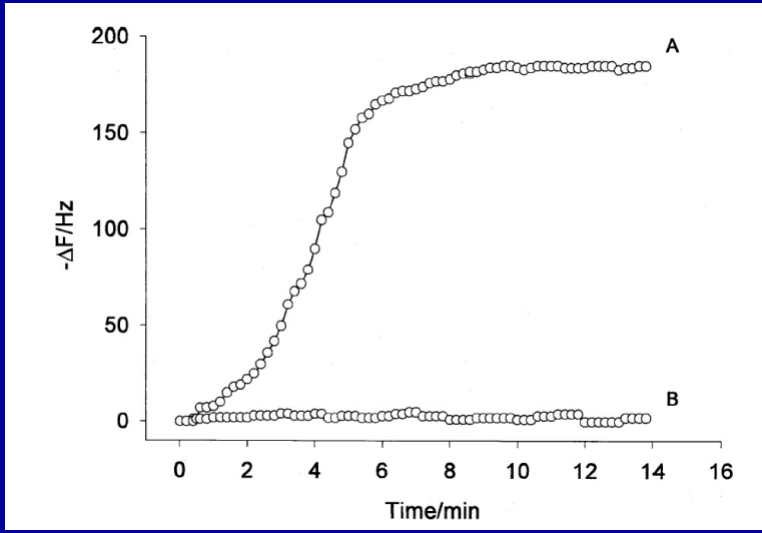
Direct grafting onto the gold electrode

- detected species (bacteria): E. Coli et P. Pulida
- identification of the DNA
- making of a synthetic probe with PCR technique
- chemical modification of the DNA branch by a thiol group
- direct chemical grafting of the DNA probe



Difficulties: to keep the activity of the probe after immobilisation

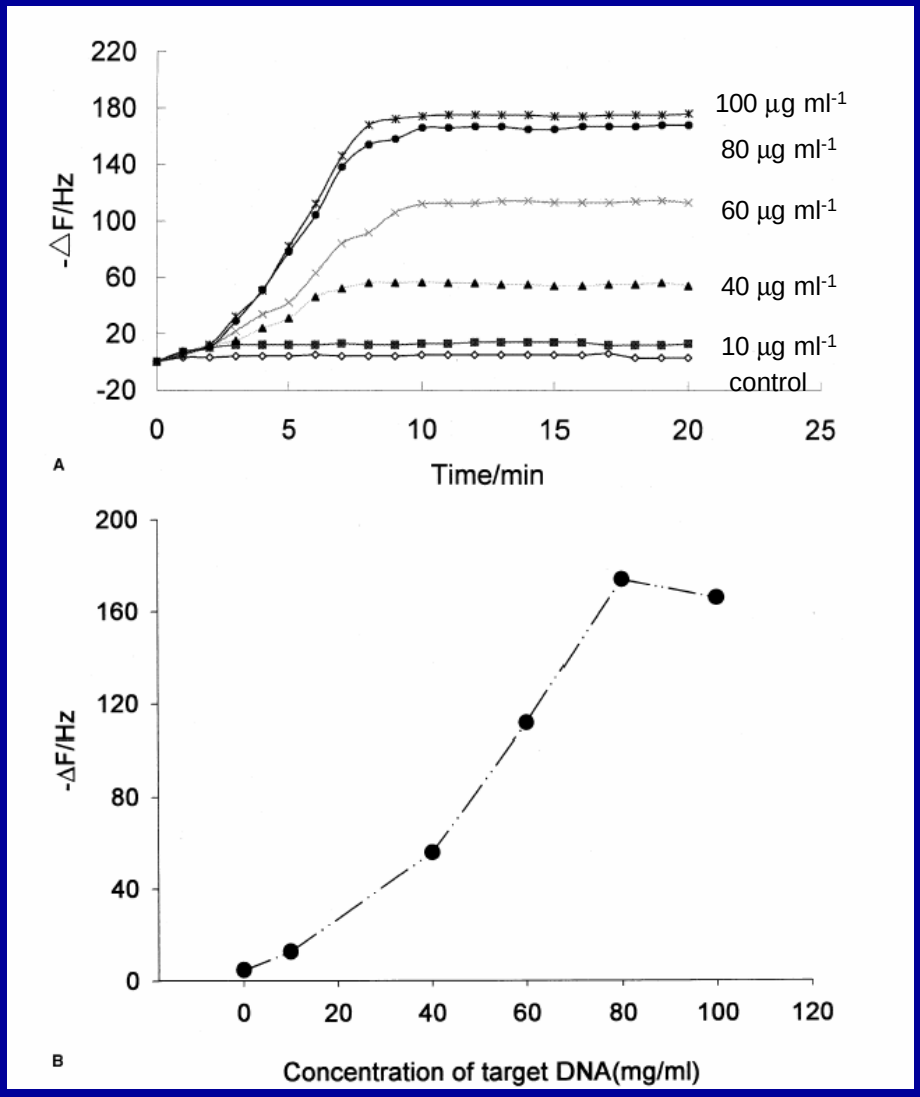
Microbalance working at 10 MHz+cell
Medium of test: Tris-HCl, EDTA and NaCl at 30°C



Response to one branch of E. Coli (A) and P. Putida (B)

Performances:

- response time: few minutes
- limit of detection: 10 µg ml^{-1}
- calibration curve: 10 µg ml^{-1} à 100 µg ml^{-1}



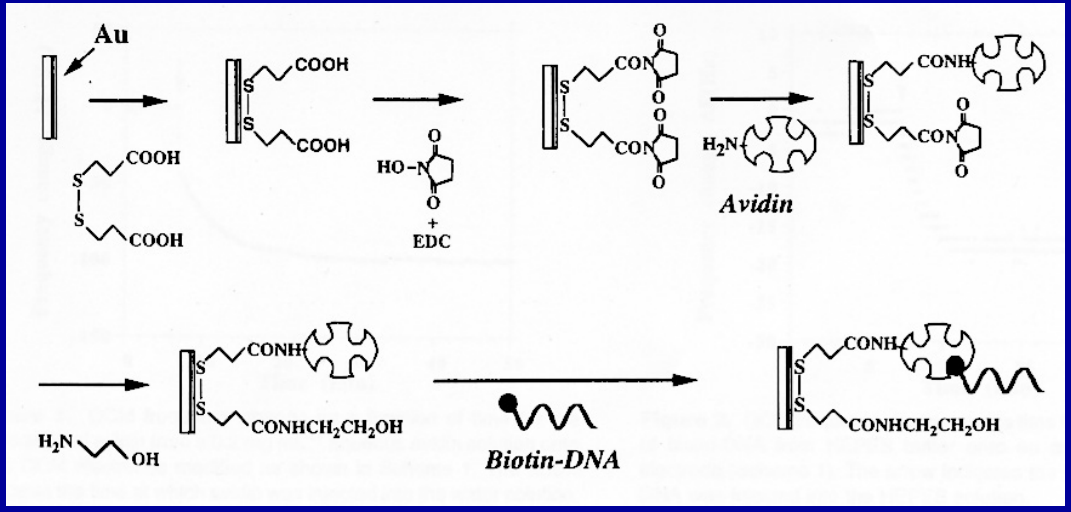
Frequency time response changes due to the binding and for different target concentration

Saturation response vs concentration in the range 10-20 min.

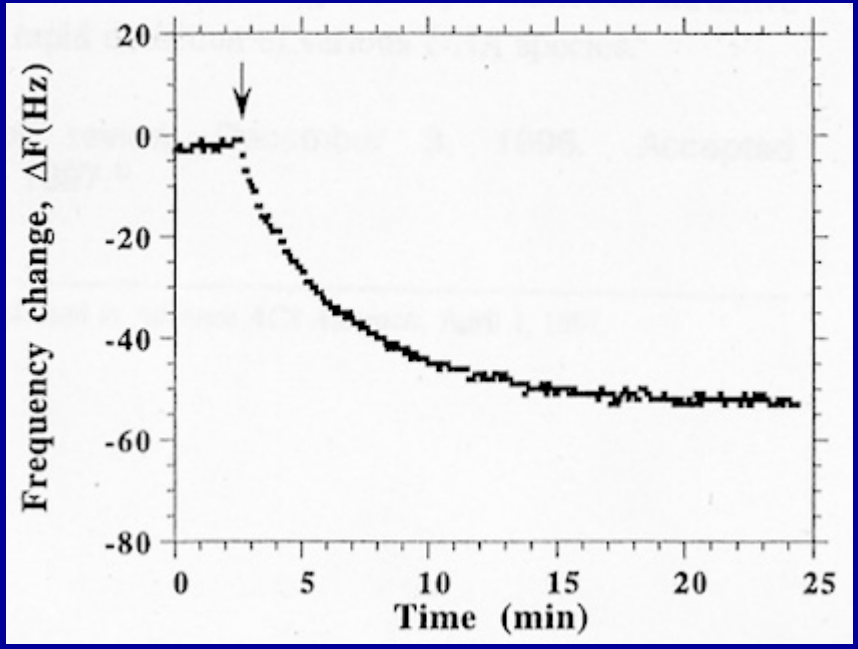
Immobilisation with SAM + Avidin/Biotin system

Method of immobilisation:

- dithiols carboxylique groups
- chemical coupling NHS/EDC
- reaction with avidin
- immobilisation of Biot-DNA (1 µg ml^{-1})
- all the steps were driven with the QCM



Microbalance working at 9 MHz, in situ measurements
Reaction in HEPES/NaCl buffer
Complementary DNA, 0.5 μg ml^{-1}

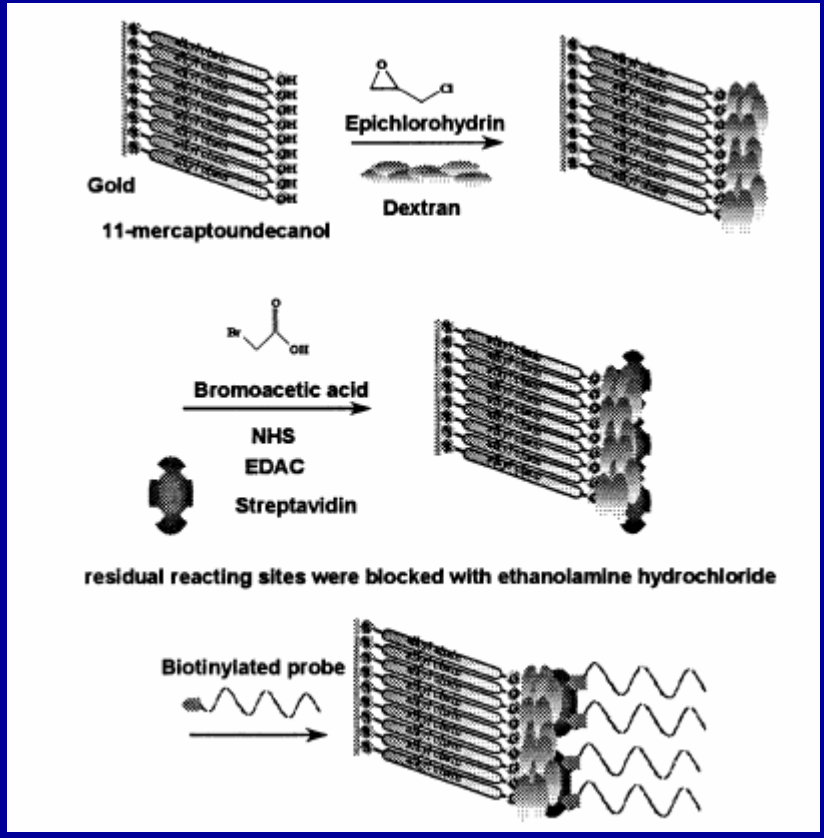


Response time < 5 min
Efficiency of the DNA reactions estimated:100%

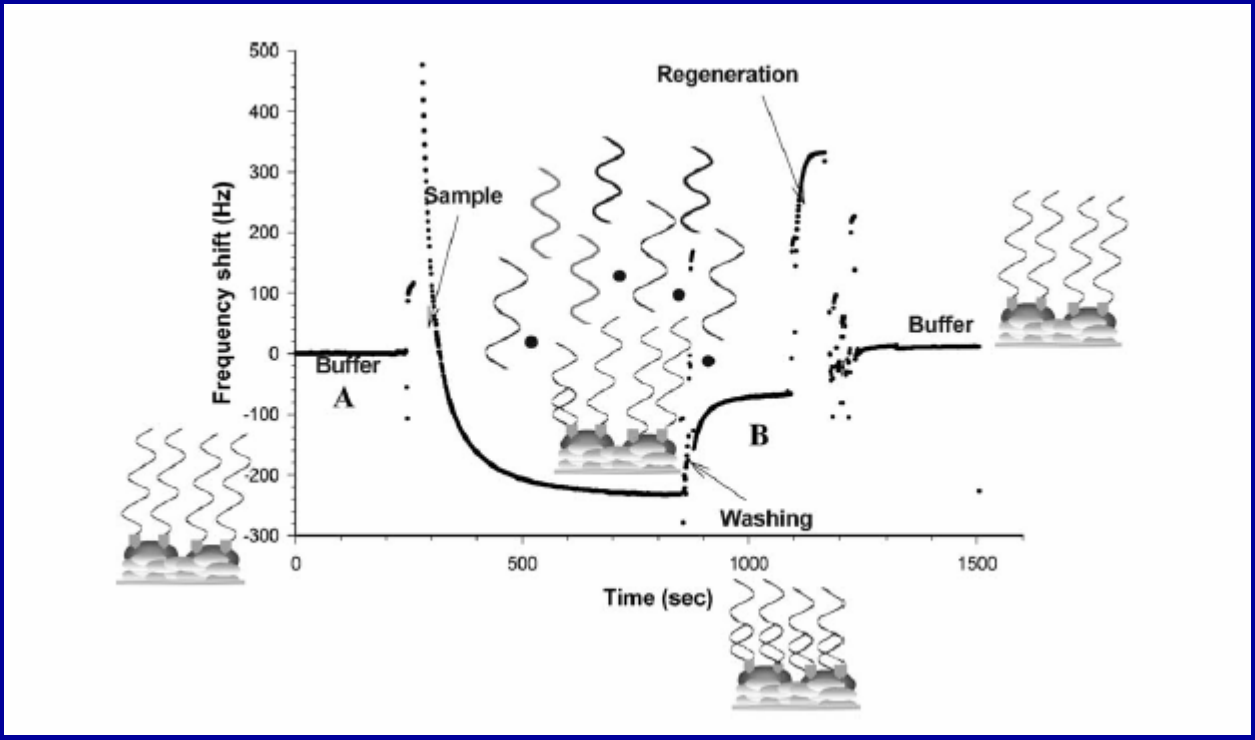
Immobilisation with DEXTRAN + Avidin/Biotin

Method of immobilisation:

- Thiols with -OH groups grafted onto the gold electrode
- Dextran (polymer) is attached onto the modified surface
- Avidin chemically grafted in the Dextran with NHS (200 μg ml^{-1})
- Endly, the biotinylated probe is added



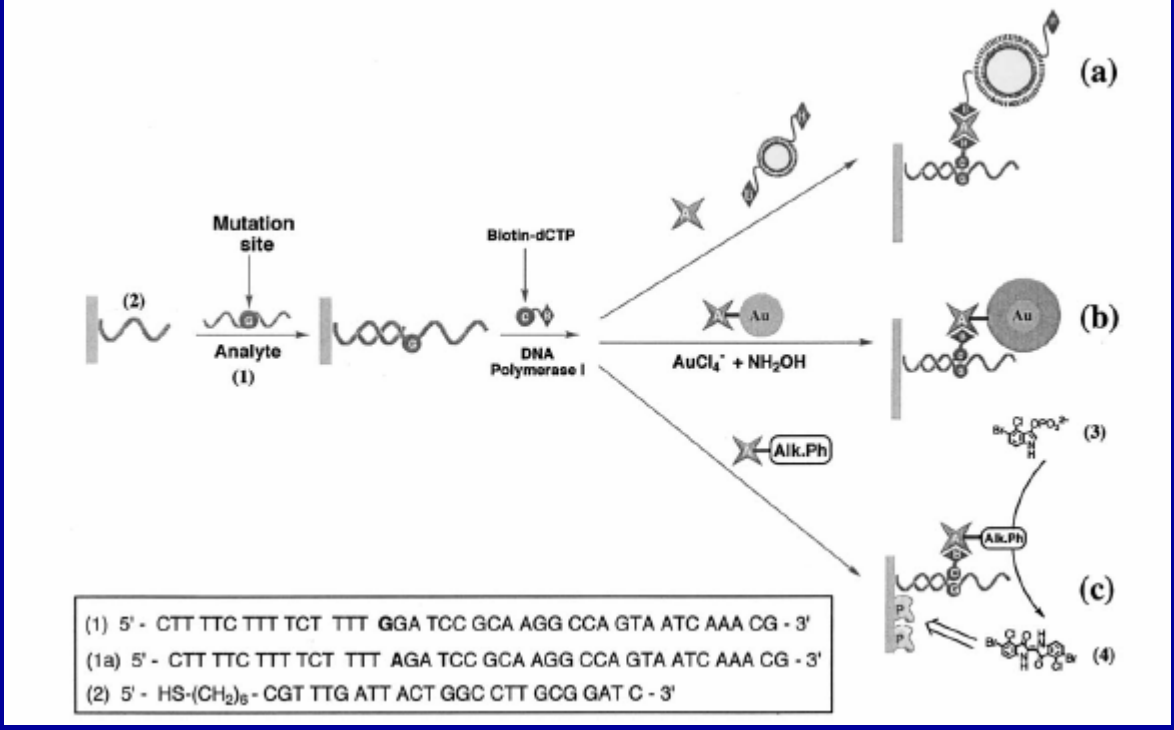
Microbalance working at 10 MHz (Seiko) included in a cell



Frequency changes vs time for the hybridisation of complementary 25-mer DNA oligonucleotides.

Amplified detection paths

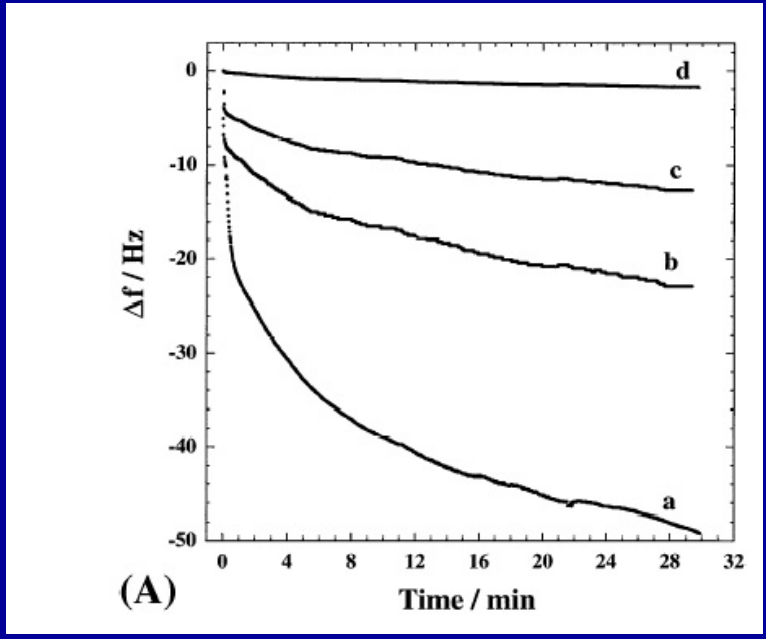
Immobilisation method of the probe:
DNA probe is modified with a thiol group



(a) biotin labeled liposomes
(b) avidin Au nanoparticle
(c) Avidin alkaline phosphatase

Performances:
Microbalance working at 9 MHz (Seiko)
Detection microbalance with gold nanoparticules

Limit of detection: 10^{-15} M



(a) 3 10^{-9} M…(d) 10 $^{-15}$ M