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Spectroscopic biosensors

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INSTITUT DE RECHERCHE EN BIOSCIENCES DE L'UMONS

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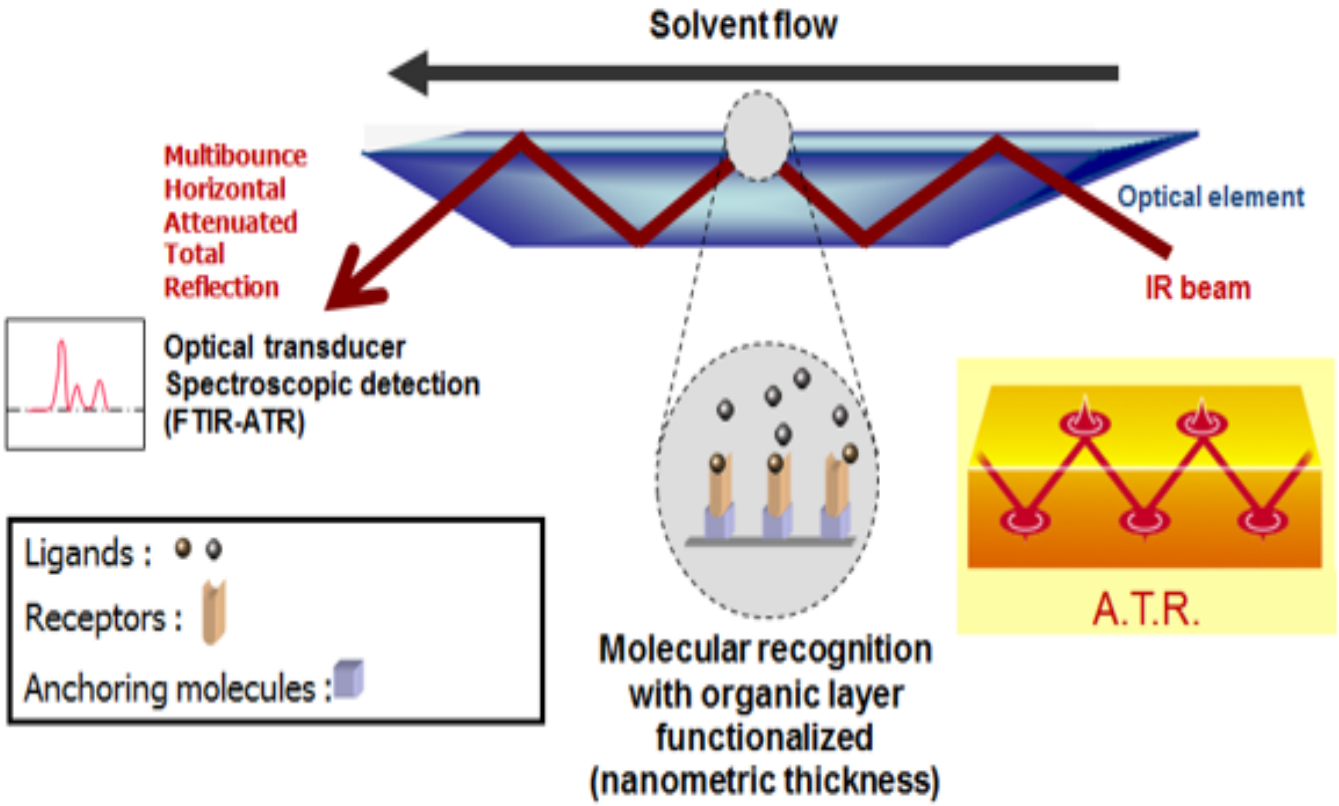
LPSI

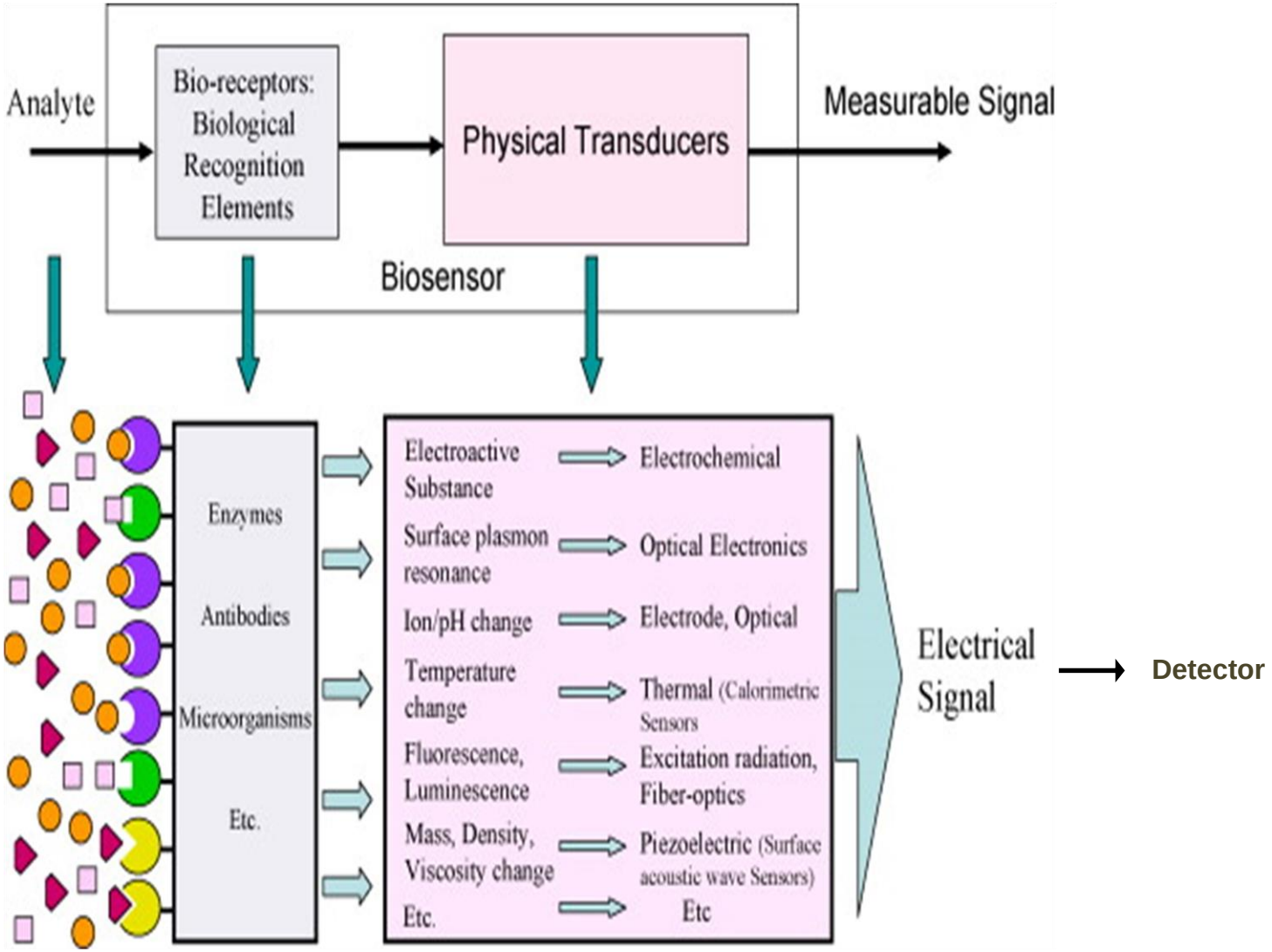
Spectroscopic biosensors



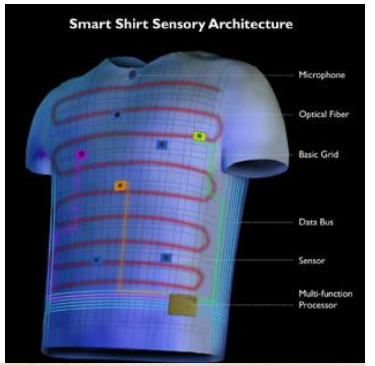
Abstract:
Sensors based on the molecular recognition of biomolecules have already attracted intensive interest in many different fields. Different surface sensitive techniques can be applied to detect these biomolecular interactions. We propose to assess the utility of Fourier Transform Infrared (FTIR) spectroscopy in studying biomolecules attachment to inorganic surfaces in a variety of biosensing applications. We have designed a new generic device suitable for the investigation of ligand–receptor interactions based on successive grafting of a novel silanization reagent and a bifunctional molecular clip directly at the surface of an internal reflection element. These molecular constructions lead to activated transducer substrate ready for the covalent binding of any bioreceptor molecules. Contrarily to SPR or quartz crystal microbalance (QCM) sensors, FTIR sensors provide useful spectroscopic information concerning the chemical nature of the interacting molecules, the amount of bound receptors and ligands, and even possible conformational transitions of the receptor during the interaction with the ligand can also be monitored. Currently, these informations are usually not accessible using standard sensors that are limited to measure physical modifications onto the surface. We will illustrate attachment of biomolecules to such organic surfaces through various systems commonly used in the biosensing field.

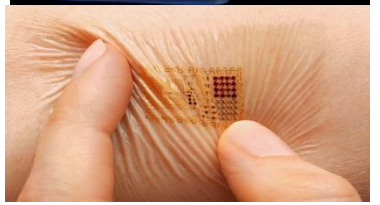
Keywords: Spectroscopy; Biosensor; Biomolecules; FTIR/ATR; grafting.

Introduction



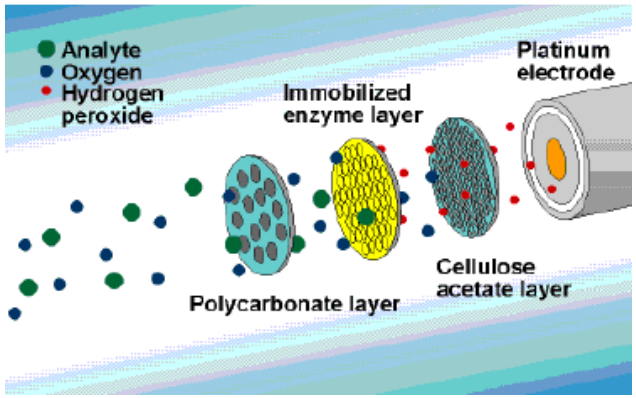
2015





1956

Leland and Clark : « enzyme electrode » for glucose concentration measurement (diabetes patients).

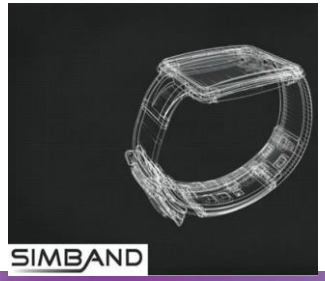


Take part in a sleep study – in the comfort of your own bed.

Sensors to measure motion, heart rate and rhythm, respiratory rate and rhythm, oxygen and carbon dioxide saturation.

The Doctor Can See You Now

New wearable health gadgets on the horizon:

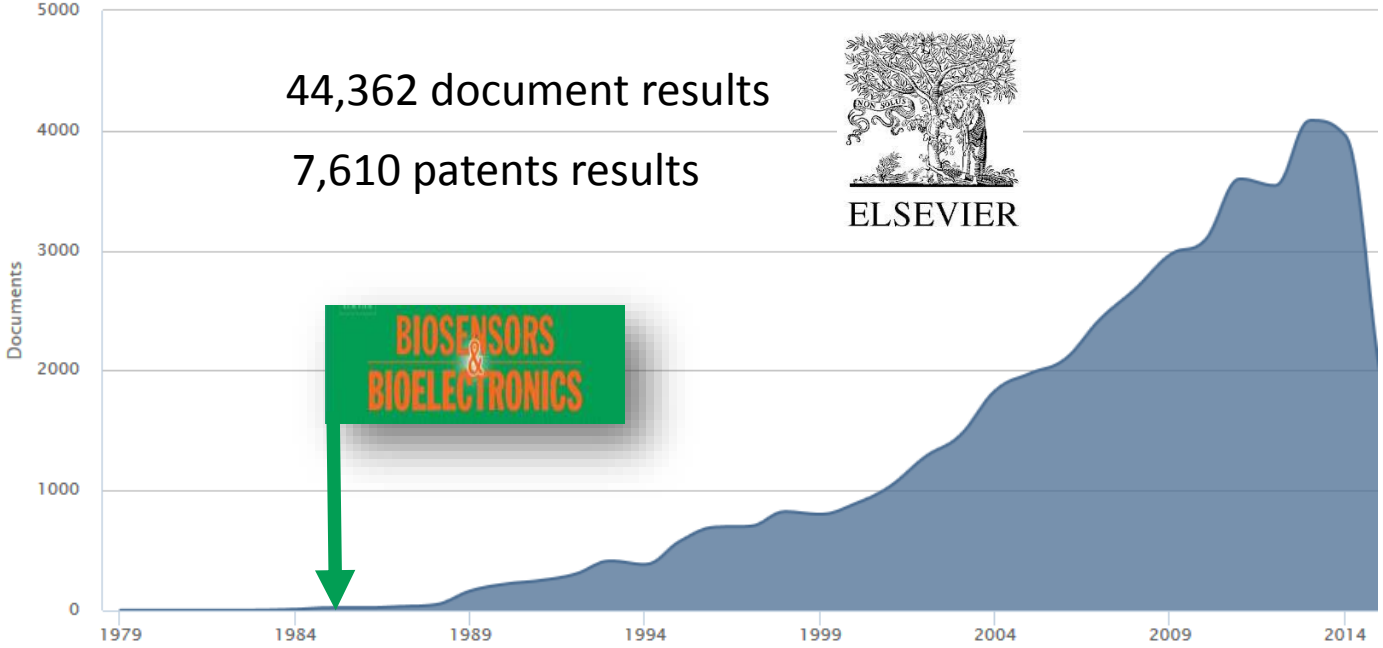


Track what gets you stressed.

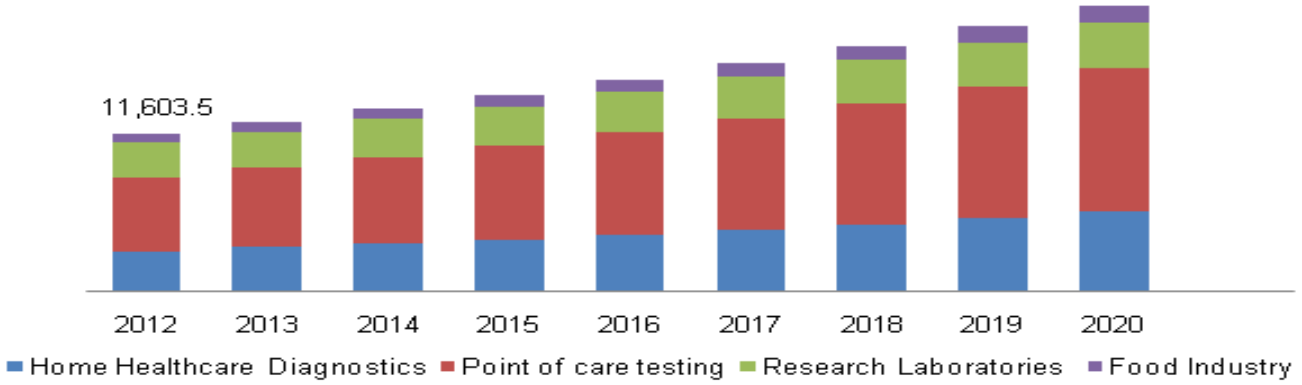
For example, Samsung has partnered with UCSF to develop the Simband, which will measure heart rate, blood pressure, temperature, oxygen level and even signs of stress.

refine your research
SCOPUS™

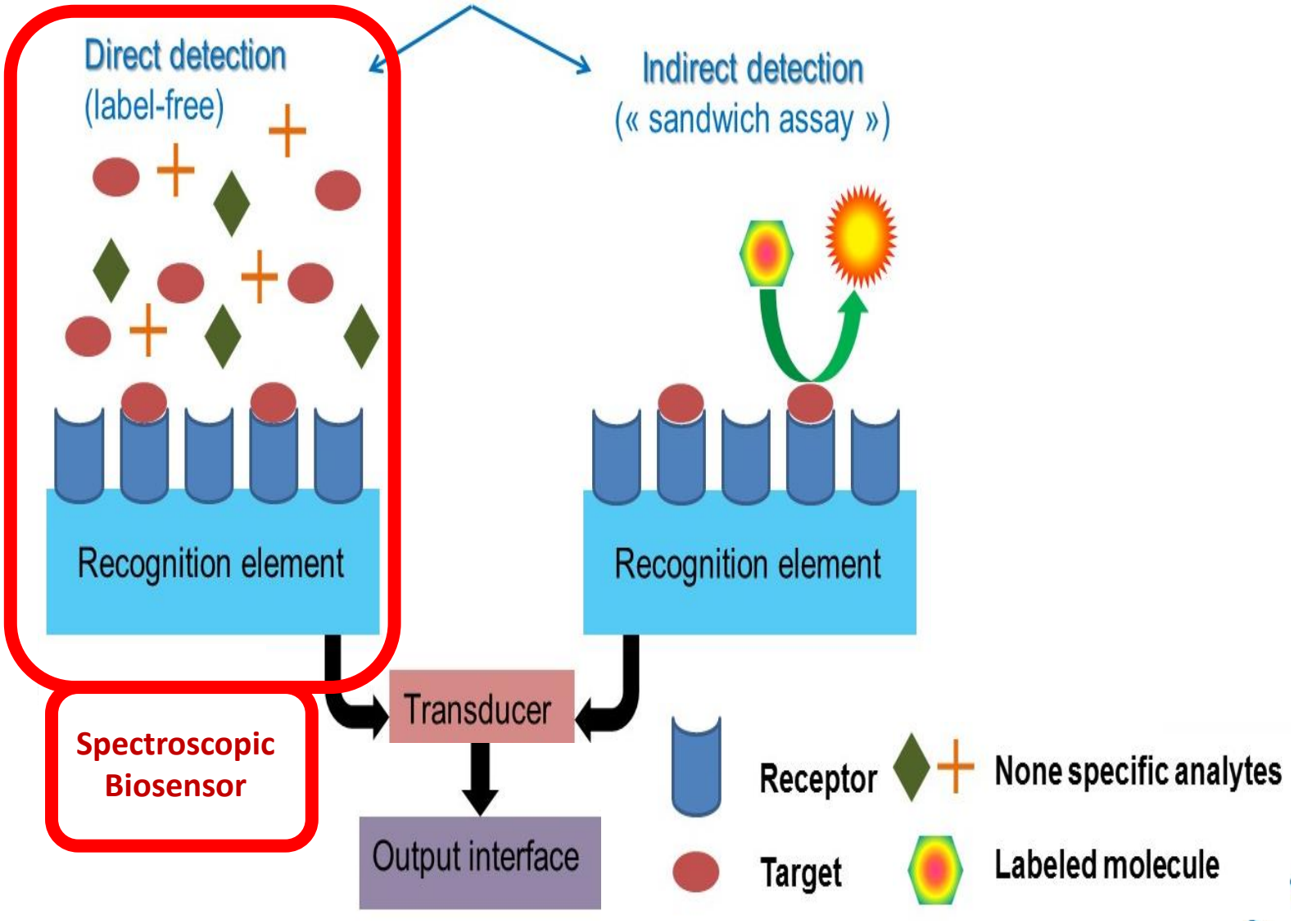
Documents by year

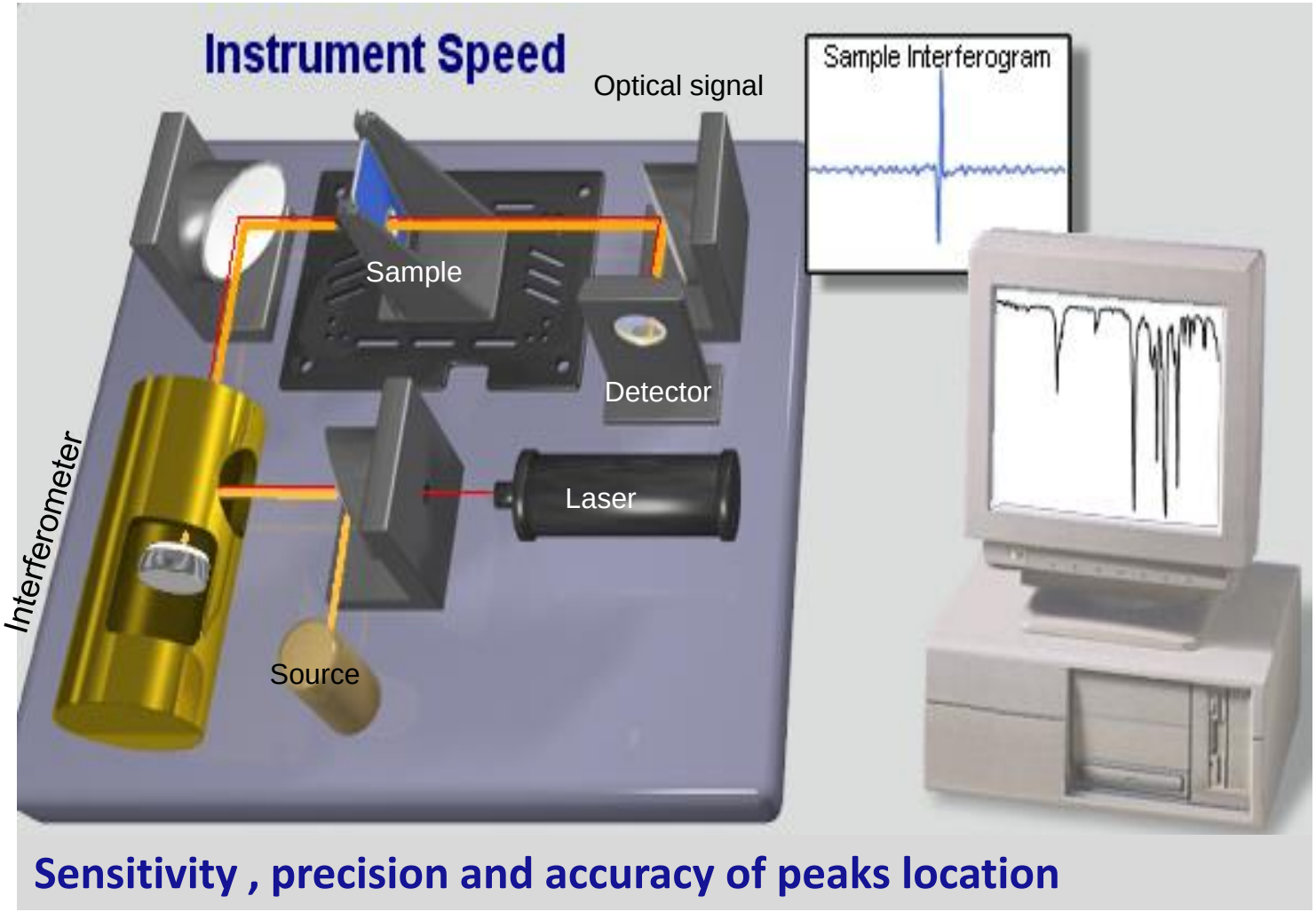


Graph of a search on the term biosensor during the period 1979 to 2015.



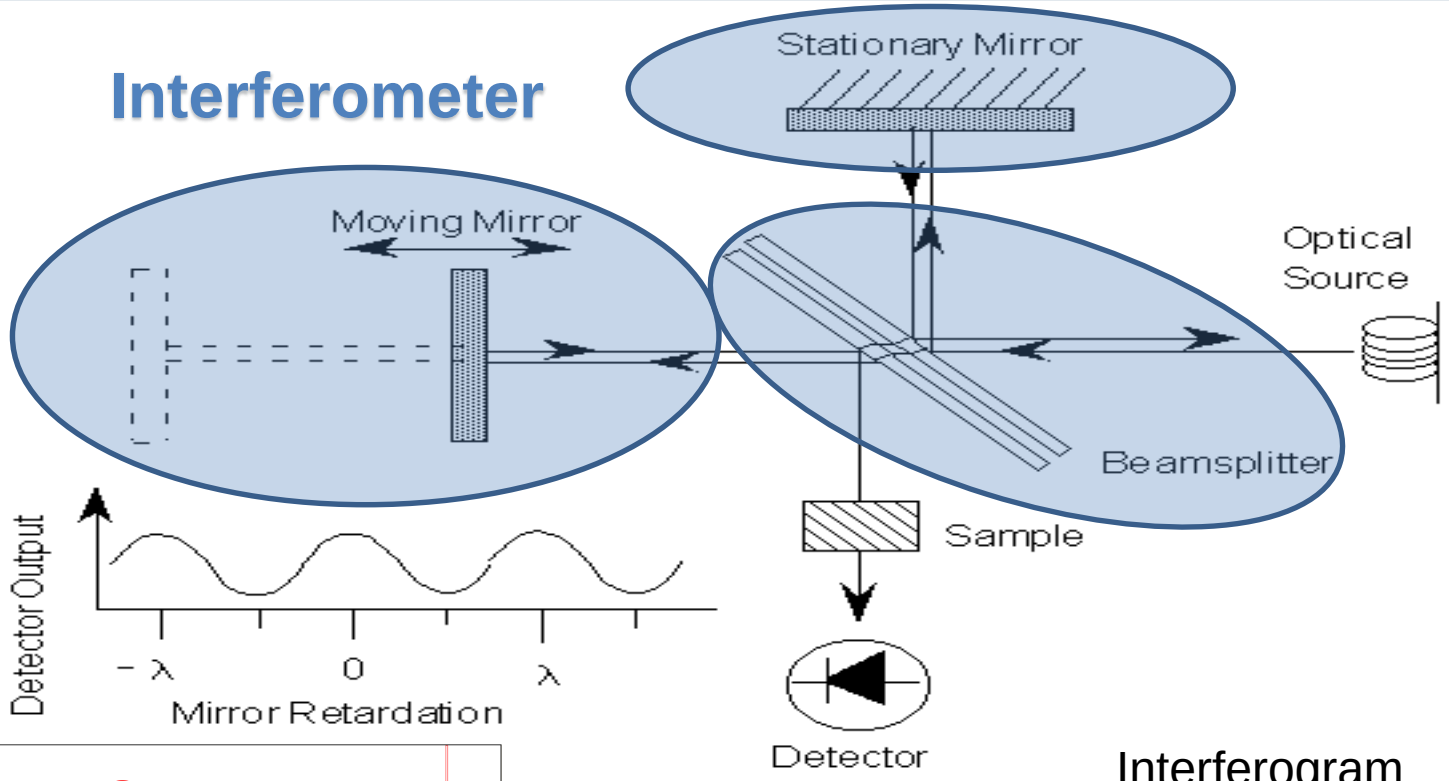
Classification

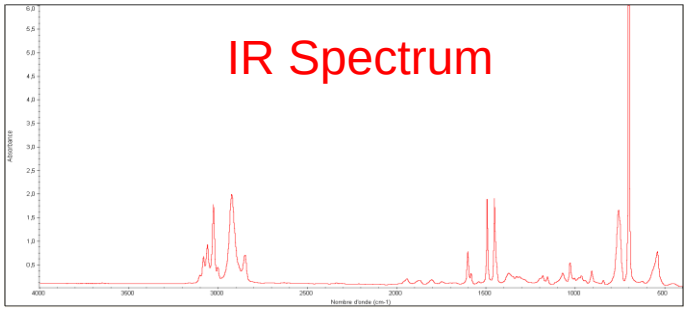




Sensitivity , precision and accuracy of peaks location

This Is What It Looks Like When Running…

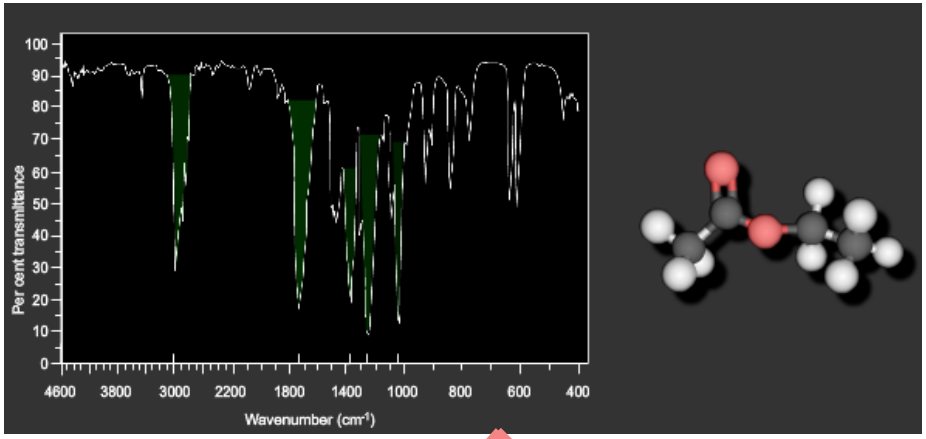


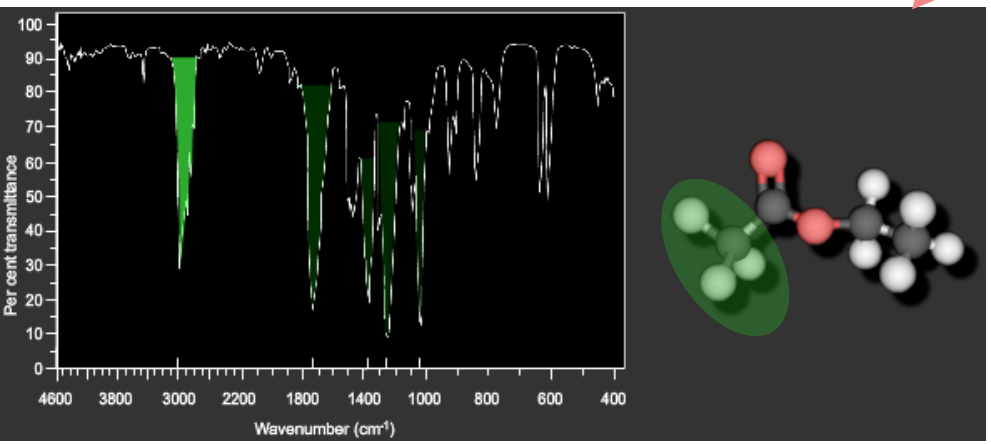


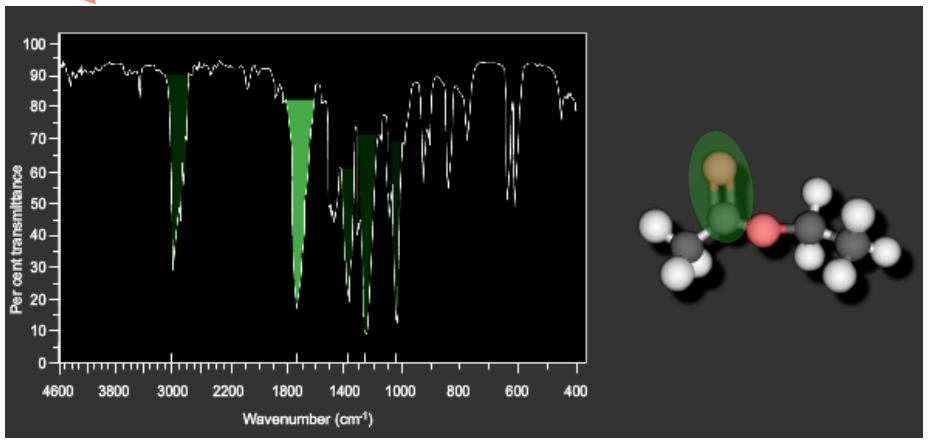
F.T.

Interferogram

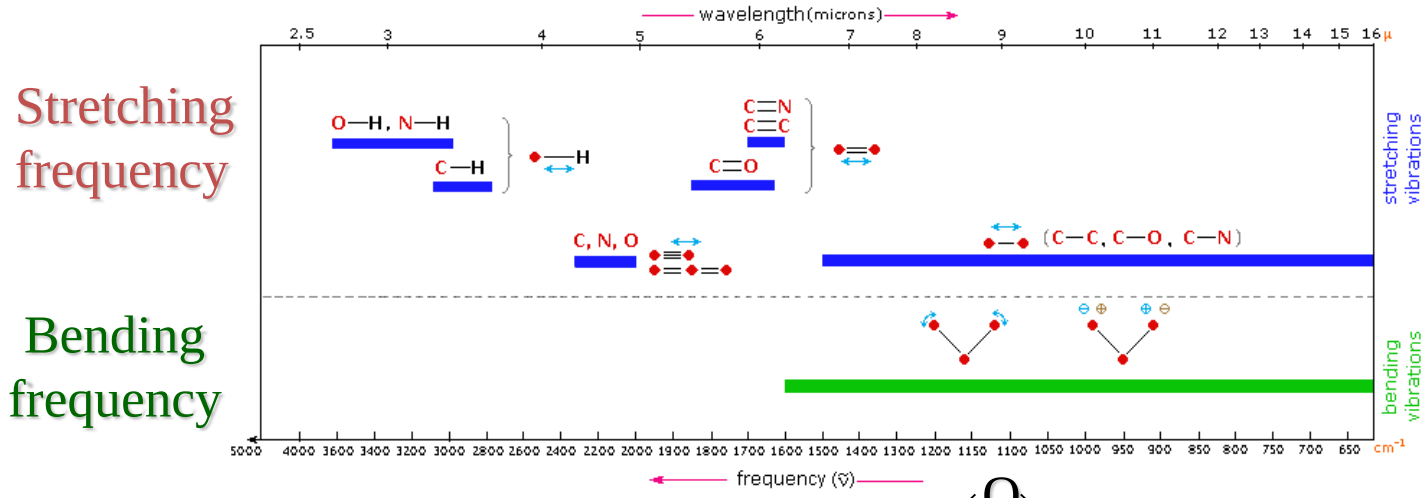
Molecular Vibrations Provide Information





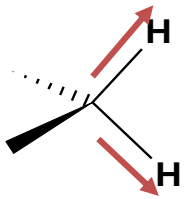


Vibrations

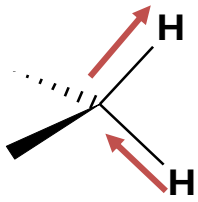


Modes of vibration

Stretching

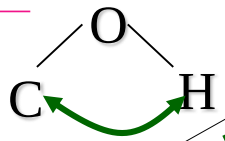


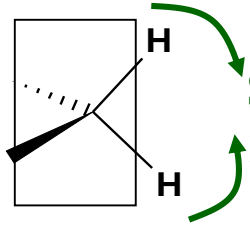
Symmetrical
2853 cm^{-1}



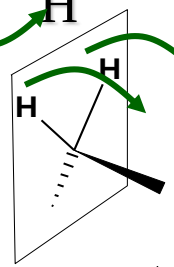
Asymmetrical
2926 cm^{-1}

Bending

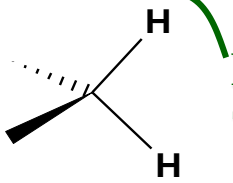




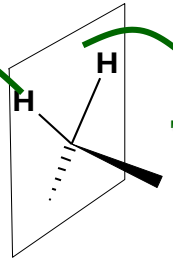
Scissoring
1450 cm^{-1}



Wagging
1350 cm^{-1}



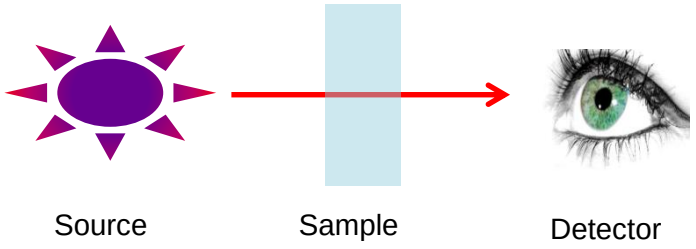
Rocking
720 cm^{-1}



Twisting
1250 cm^{-1}

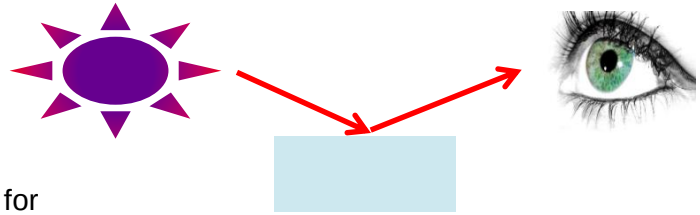
Transmission

Excellent for solids, liquids and gases
The reference method for quantitative analysis
Sample preparation can be difficult**.**

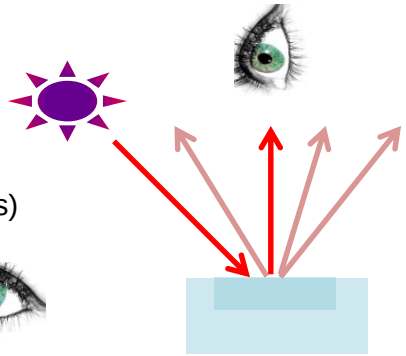


Reflection

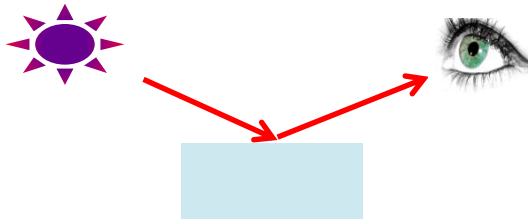
Collect light reflected from an interface air/sample, solid/sample, liquid/sample
Analyze liquids, solids, gels or coatings
Minimal sample preparation
Convenient for qualitative analysis, frequently used for quantitative analysis



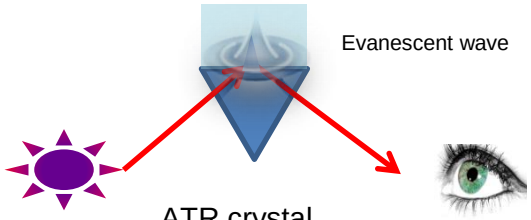
Combination of Internal and External Reflection:
Diffuse Reflection (DRIFTs) (rough surfaces)



External Reflection Spectroscopy:
Specular Reflection (smooth surfaces)



Internal Reflection Spectroscopy:
Attenuated Total Reflection (ATR)



BIA-ATR (WDU)

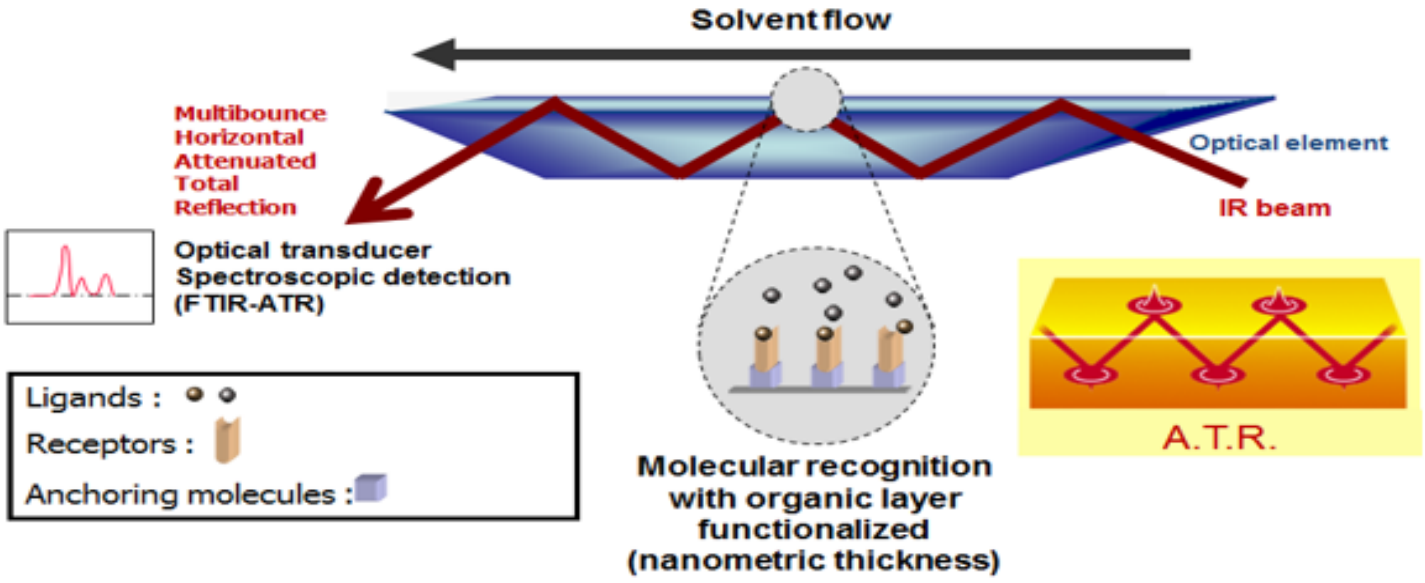
Surface chemical modification of optical element for spectroscopic detection of molecules and organic components.

1999 — 2001 — 2006

Patent — **Biosentech s.a.** — **Article**

Step-by-step construction of the molecular interface using a wet chemistry approach.

Requires a functionalization of the ATR element surface.



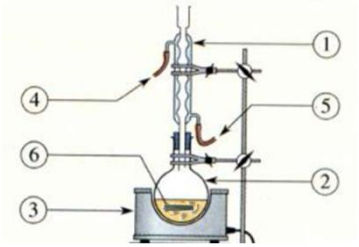
Molecular construction using commercial or synthesis products to create our biosensors

Step 1 : Surface cleaning. → Regenerable crystals by mechanical polishing and chemical cleaning

Step 2 : Surface activation. → Chemical solution (pirhana) or by plasma

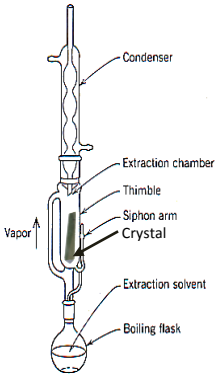
Step 3 : Antifouling coating by chemical grafting.

PEG grafting by wet chemistry



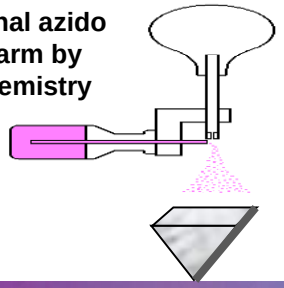
1- Refrigerant.
2- Balloon.
3- Heating mantle.
4- Water outlet.
5- Water inlet.
6- Crystal immerged in reaction mixture.

Rinsing step using soxhlet extractor

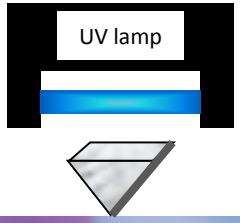


Step 4 : Covalent bonding of the spacer arm.

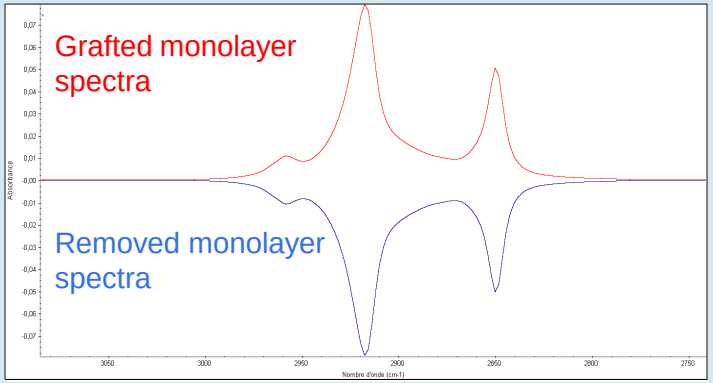
Bifunctional azido spacer arm by photochemistry

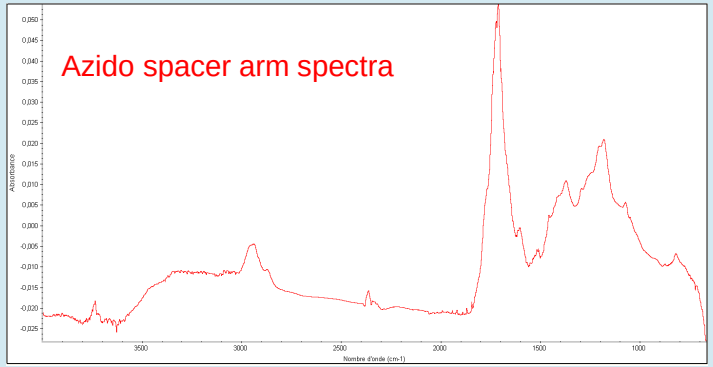


Irradiation at 254nm during 2h and then rinsing in a solvent under ultrasonic bath.

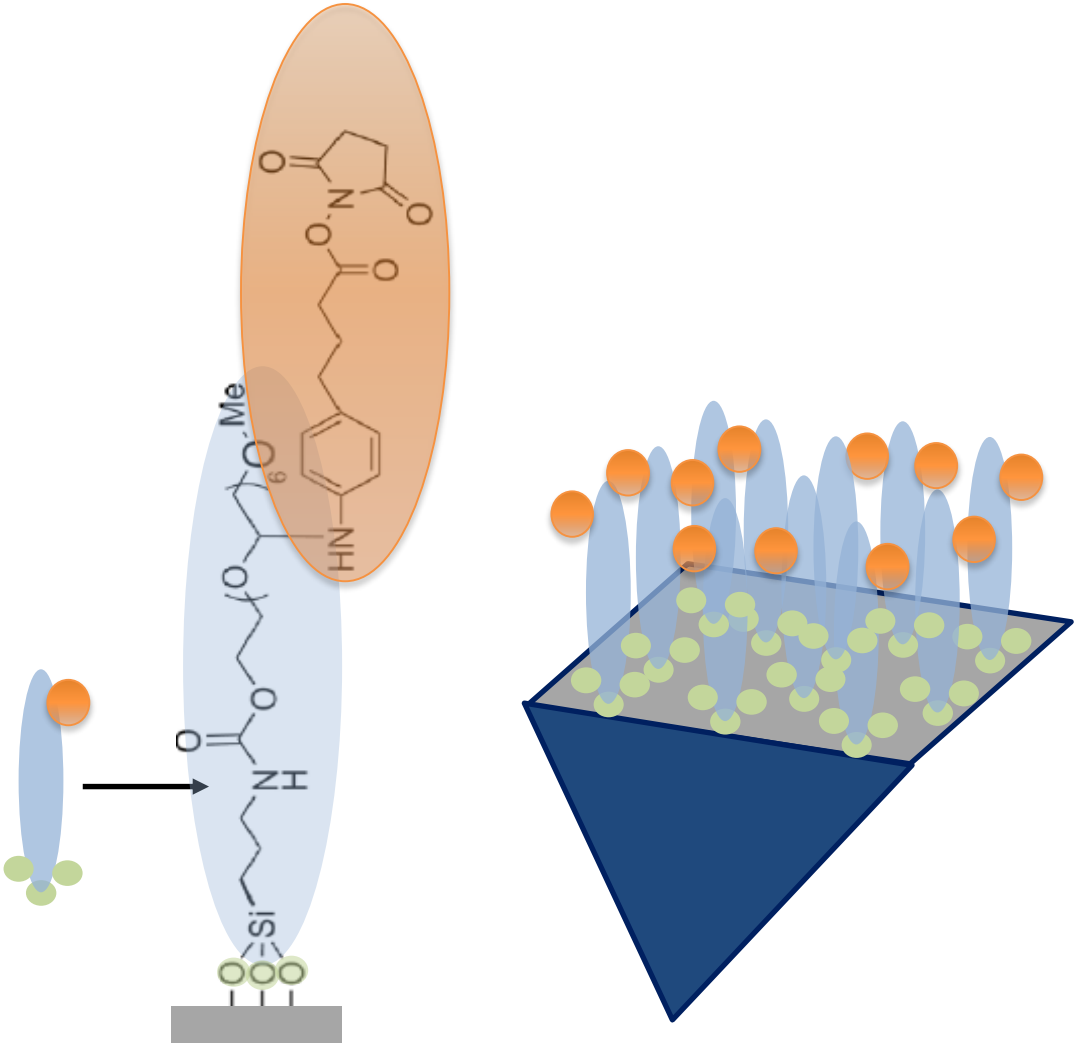


Control quality of our molecular construction using infrared spectroscope.

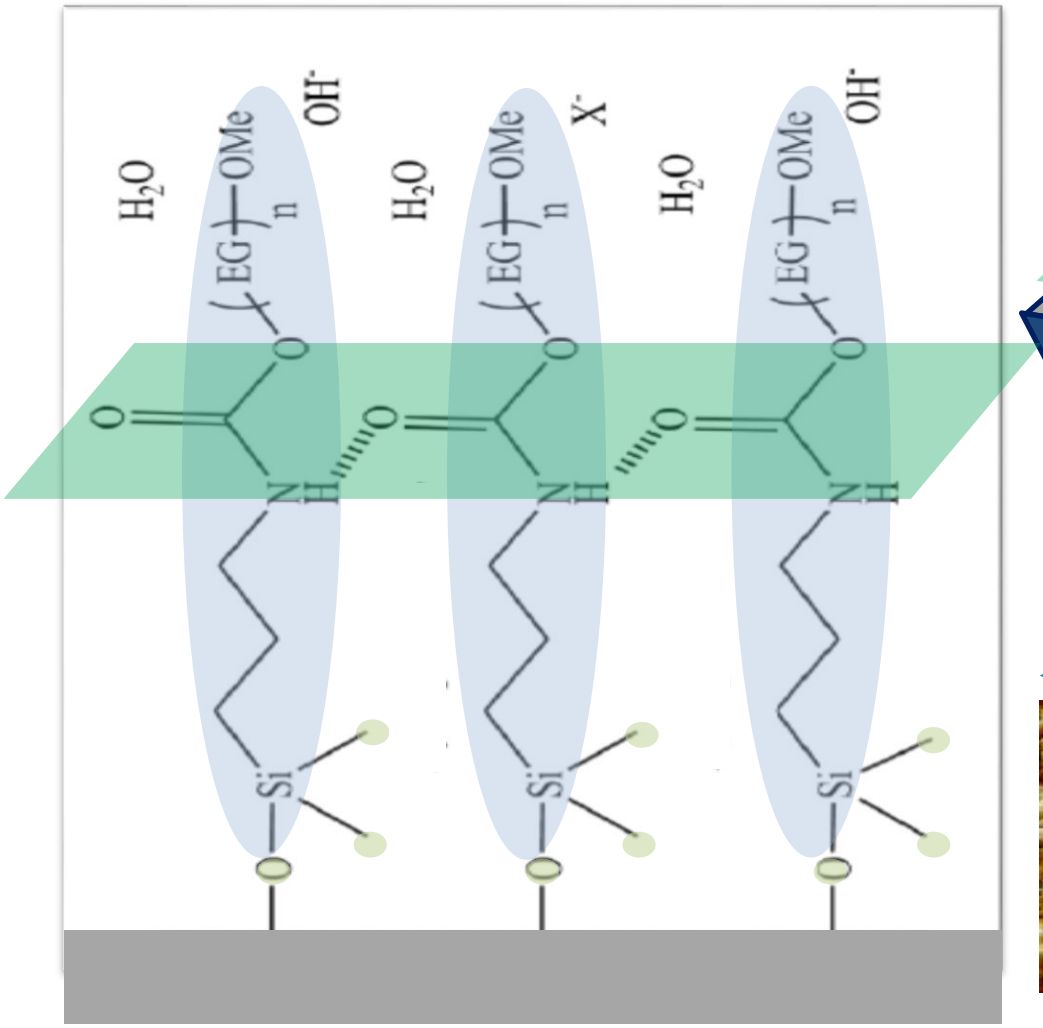




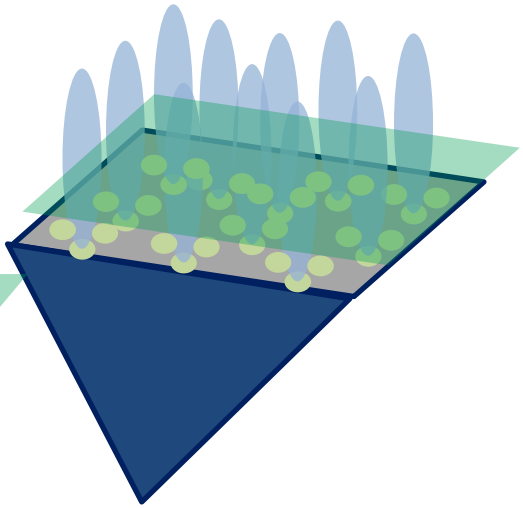
Molecular construction



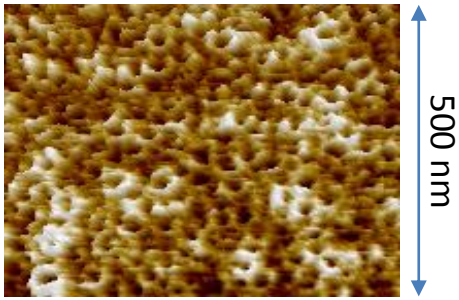
Key feature



Hydrophobic barrier

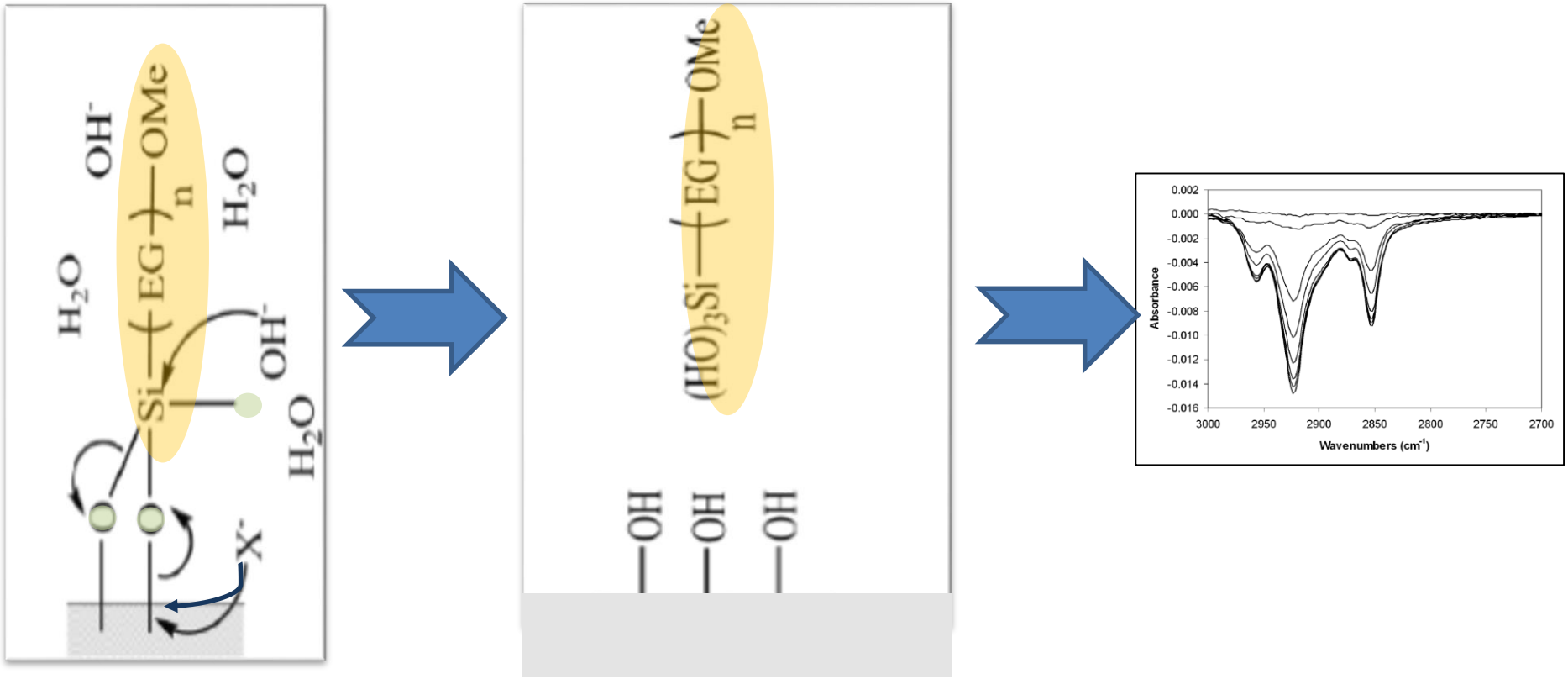


AFM



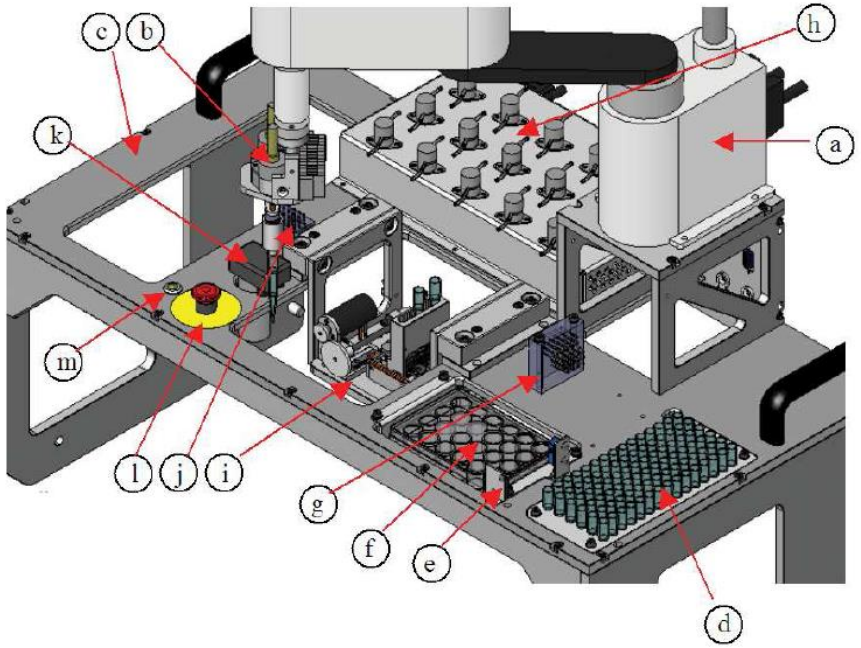
Surface functionalization of germanium ATR devices for use in FTIR – Biosensors

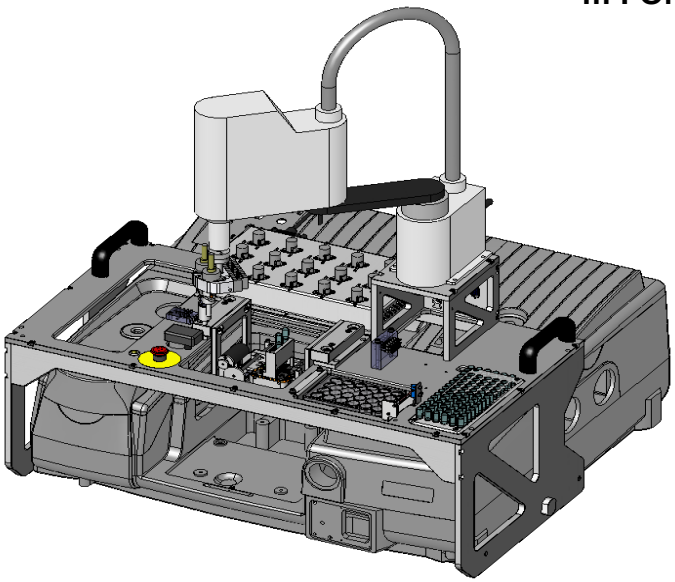
S. Devouge, J. Conti, A. Goldsztein, E. Gosselin, A. Brans, M. Voué, J. De Coninck, F. Homblé, E. Goormaghtigh, J. Marchand-Brynaert,

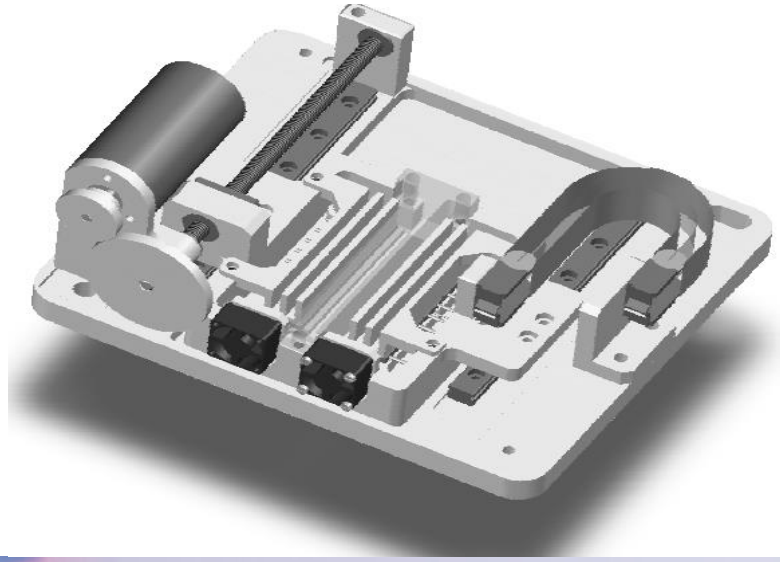


Robotized setup

a : robot
b : gripper
c : framework
d : store pipettes
e : barrier of presence
f : multiwell plate
g : 15/15 junction block
h : Block 15 valves
i : Floor Indexing
j : Junction Block 15 / 1
k : Peristaltic pump
l : Emergency Stop
m : ON/OFF LED

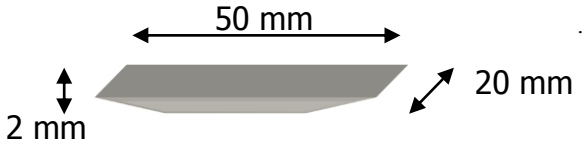


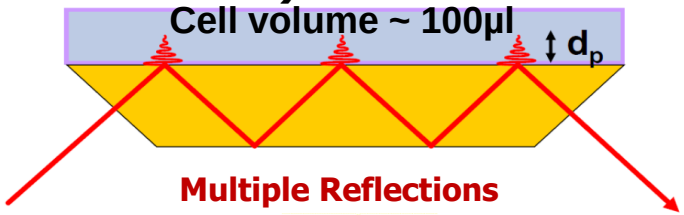




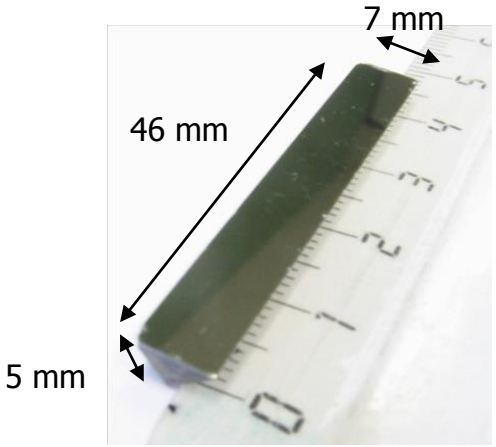
Miniaturization

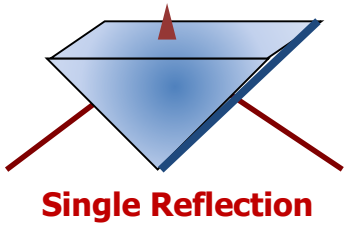
Old system : Ge and Si cristals (50 x 20 x 2 mm³)





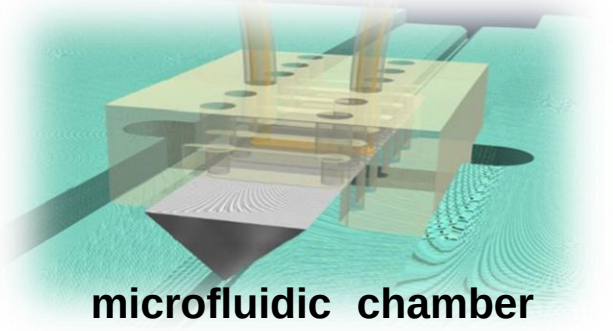
New system : « toblerone » Ge and Si





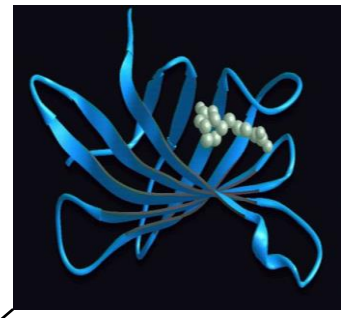
Multi-lanes sensor :

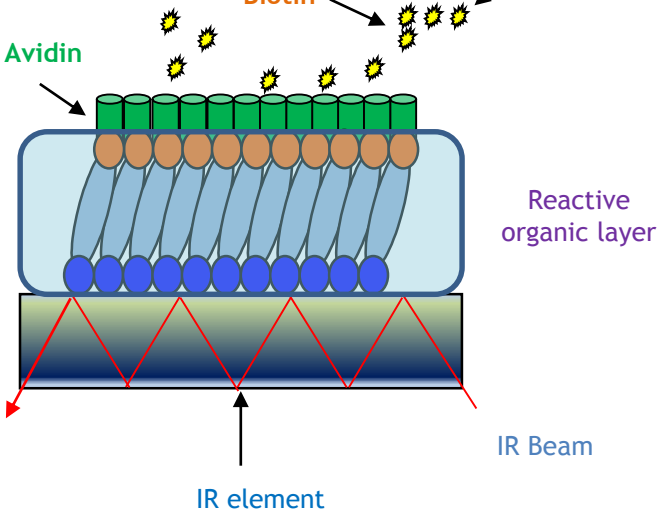
Flow : few µl/min ~ ml/min
Volume : few µl ~ few ml
15 lanes per crystal

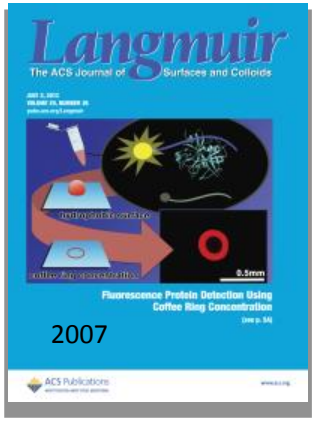


Biochemical Interaction Analysis on ATR Devices: A Wet Chemistry Approach for Surface Functionalization

M. Voue,*,† E. Goormaghtigh,‡ F. Homble,‡ J. Marchand-Brynaert,§ J. Conti,† S. Devouge,§ and J. De Coninck†

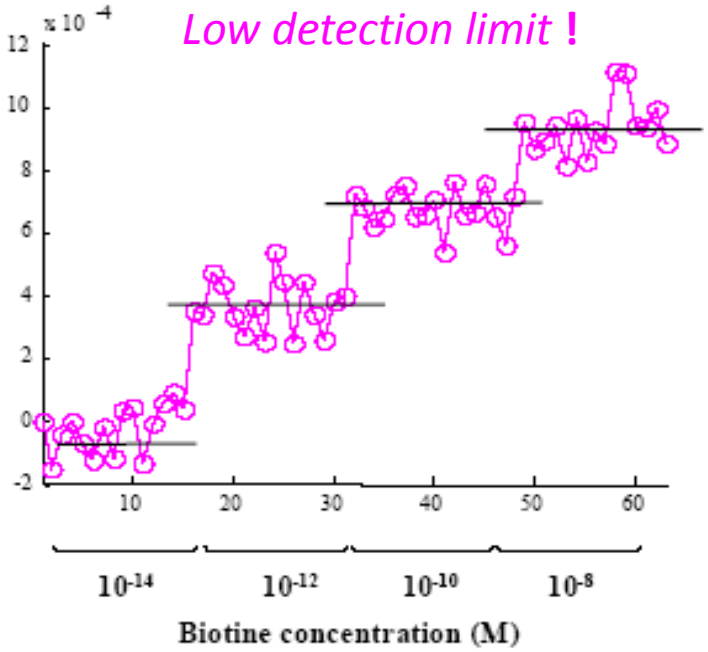






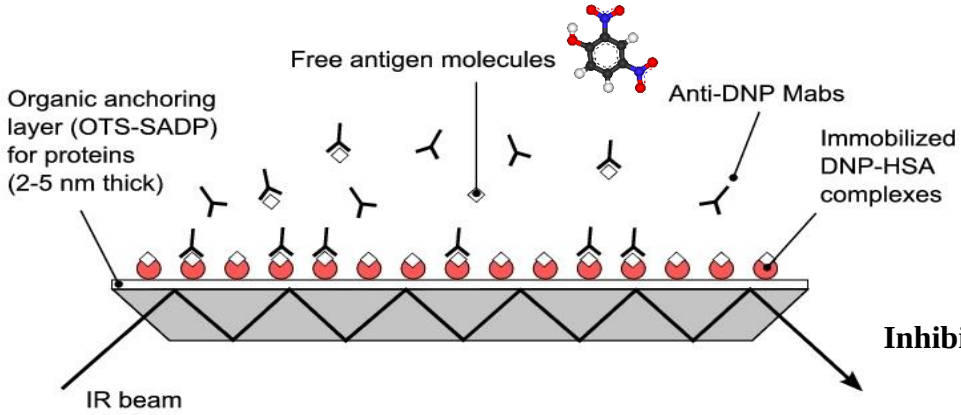
2007

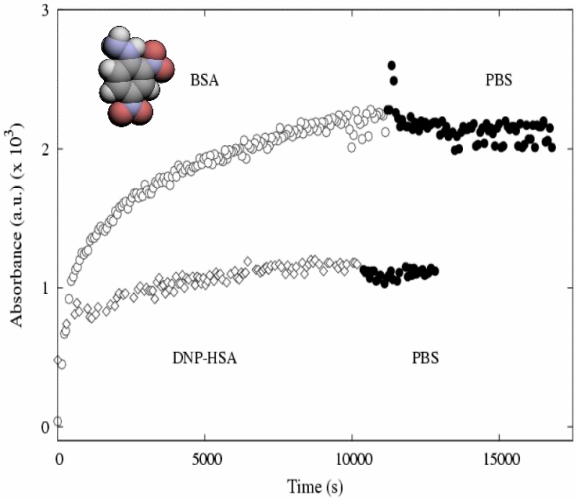
Low detection limit !



Fourier transform infrared immunosensors for model hapten molecules

E. Gosselin, M. Gorez, M. Voué, O. Denis, J. Conti, N. Popovic, A. Van Cauwenberge, E. Noel, J. De Coninck



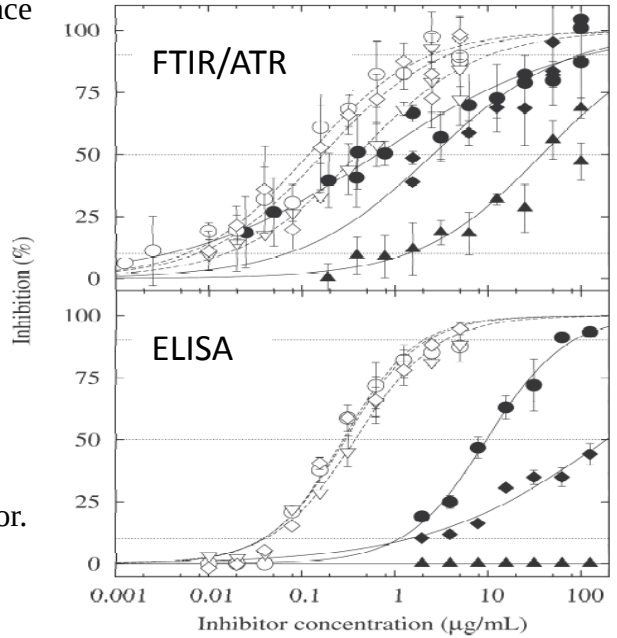


1/ Binding the coupled protein to the sensor surface
2/ Injection of Mabs + inhibitors after 20 min of incubation.
3 / Absorbance of the sample is converted in percentage of inhibition

$$I = 100\left(1 - \frac{A_i - A_0}{A_{max} - A_0}\right)$$

A_i: absorbance of the sample
A_0: absorbance measured after the binding of the protein and the subsequent rinsing with PBS
A_{max}:absorbance measured in the absence of inhibitor.

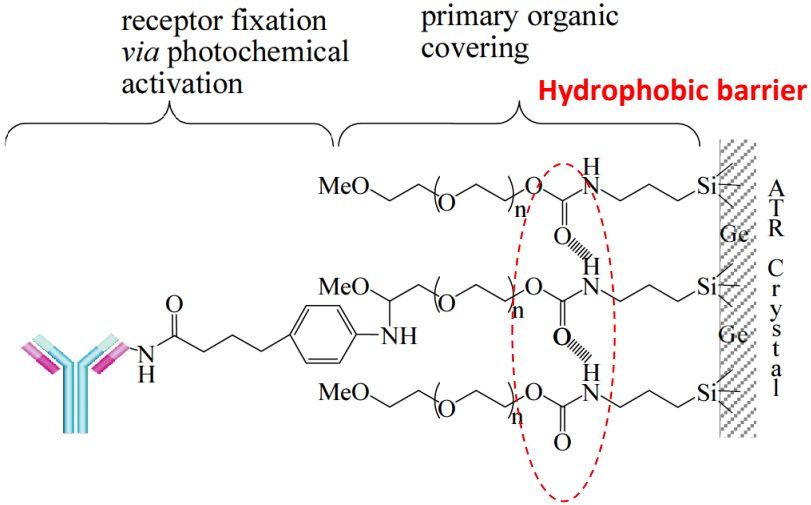
Inhibitors : coupled (open symbols) **or free DNP** (filled symbols)
3 Mabs anti-DNP



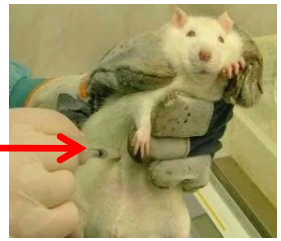
5 ~15 ng/mL for the coupled DNP
≅ 5 ng/mL for the free DNP molecules.

Quantification of the trichothecene Verrucarin-A in environmental samples using an antibody-based spectroscopic biosensor.

E. Gosselin, O. Denis, A. Van Cauwenberge , J. Conti, J.J. Vanden Eynde, K. Huygen , and J. De Coninck.

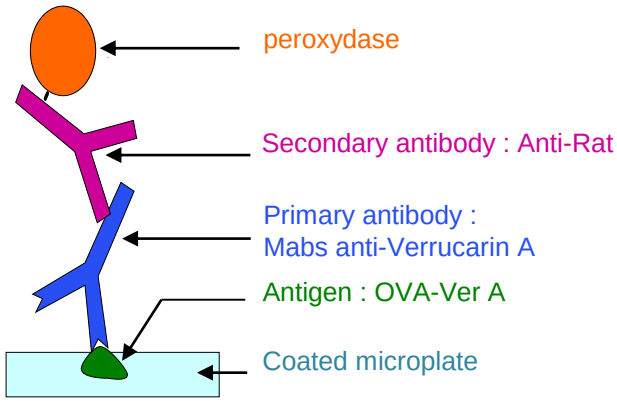


BSA Verrucarin-A injected

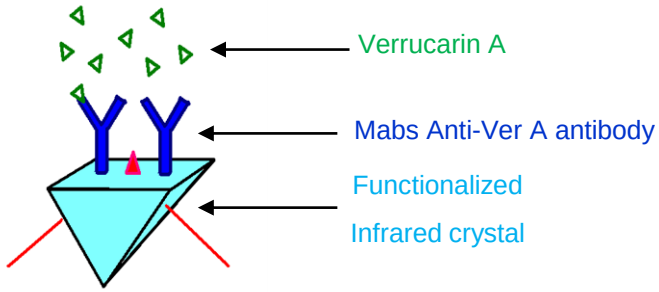


Dust

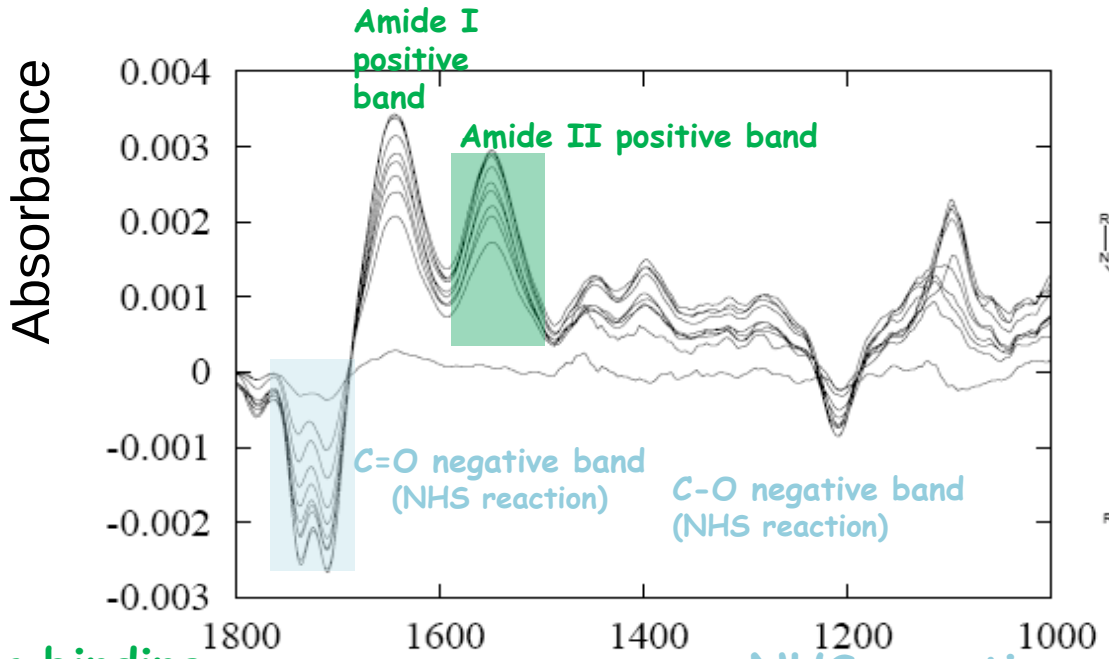
Indirect detection



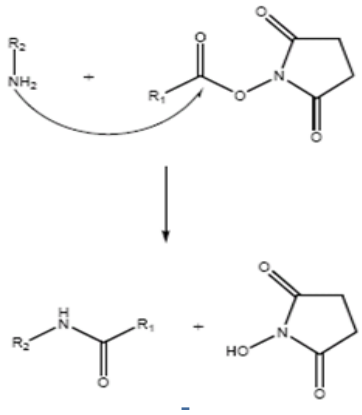
Direct detection



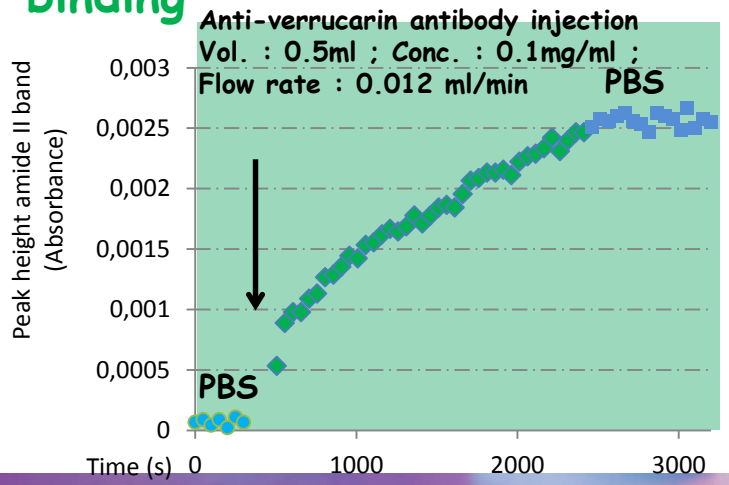
Anti-verrucarin mAb binding



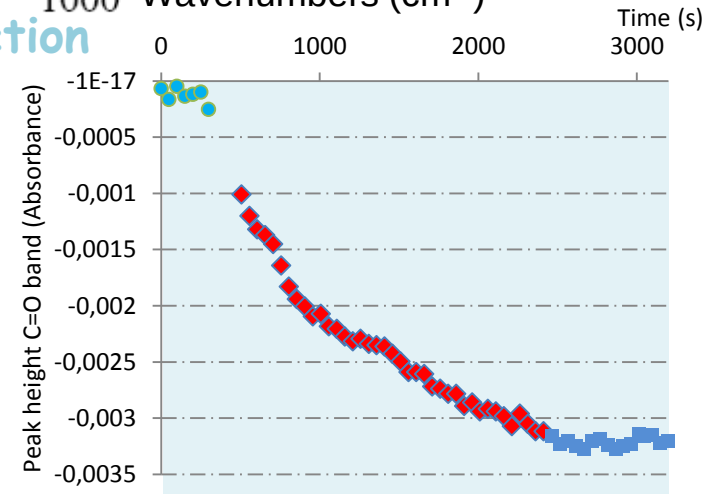
NHS reaction



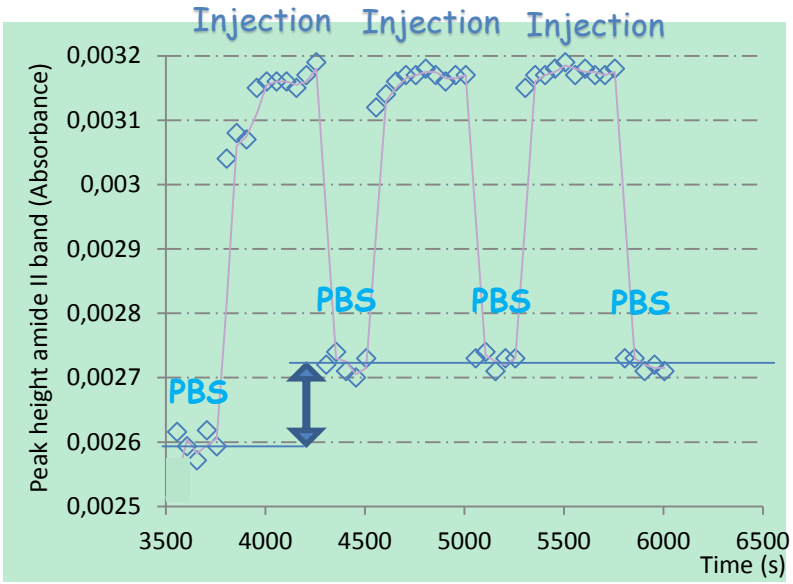
Receptor binding

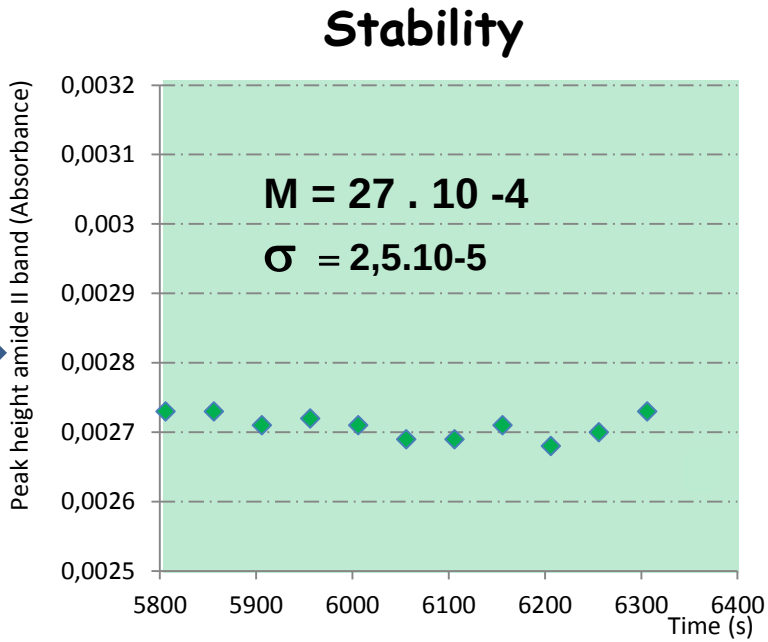


NHS reaction



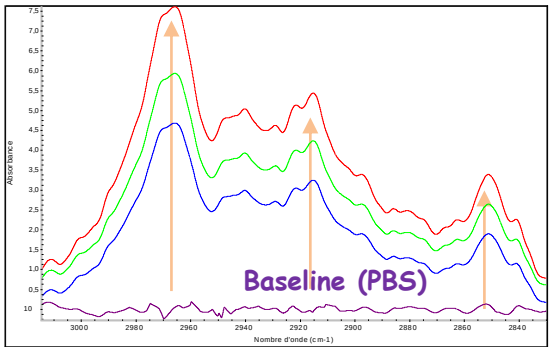
Saturation step
by primary amines or proteins injection





Ready to detect the analyte of interest.

Verrucarin A detection

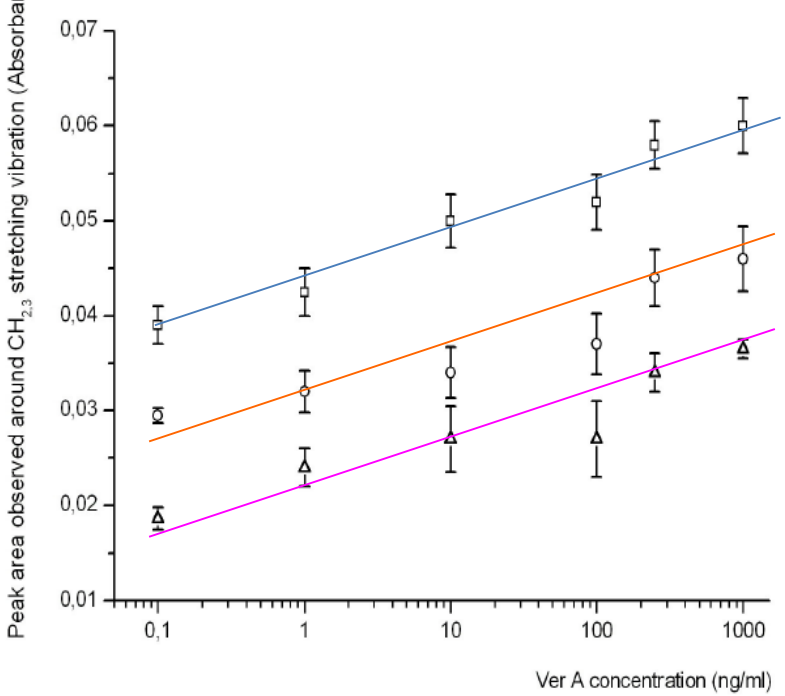


Ver A concentration

1000 ng/ml

10 ng/ml

0.1 ng/ml



Lane 1

Lane 2

Lane 3

Low reproducibility ?

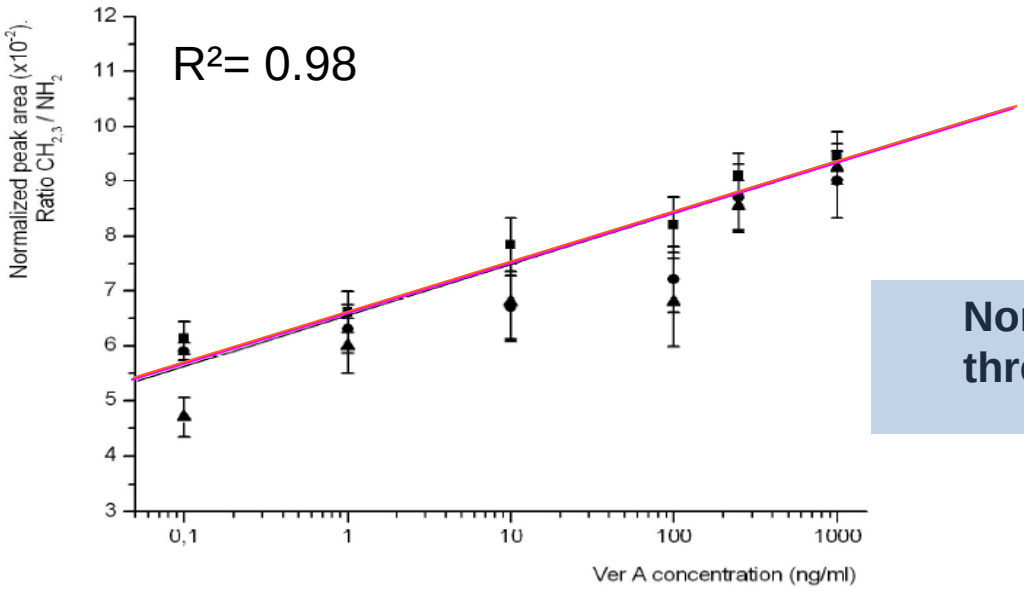
The binding of the Verrucarin A was dependent upon…

…the quantity of receptors present at the sensor surface !

$$\text{Normalized peak area} = \frac{\text{Observed peak area in the CH2,3 stretching vibration}\ ^{i}}{\text{Peak area of the amide bands}\ ^{ii}}$$

i : Measured quantity of ligands during washing step
ii : Measured quantity of receptors during washing step

Normalized quantification of Verrucarin A

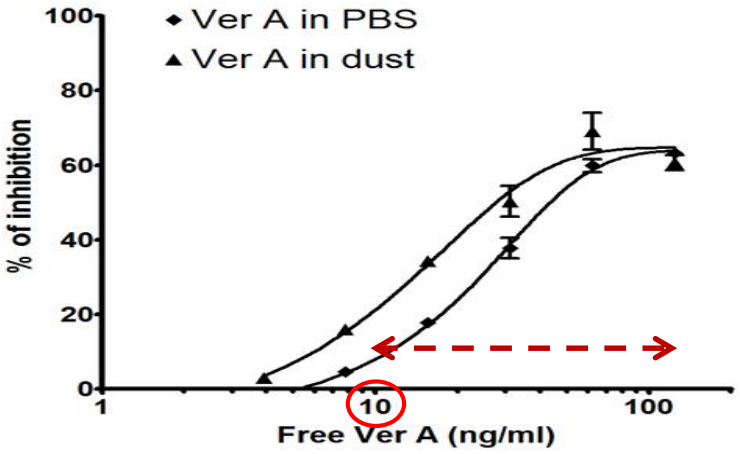


Lane 1
Lane 2
Lane 3

$$\text{Normalized threshold} = \frac{3 \times \text{SD max value}}{\text{Peak area of the amide bands}}$$

mAb based competitive ELISA

ELISA curves



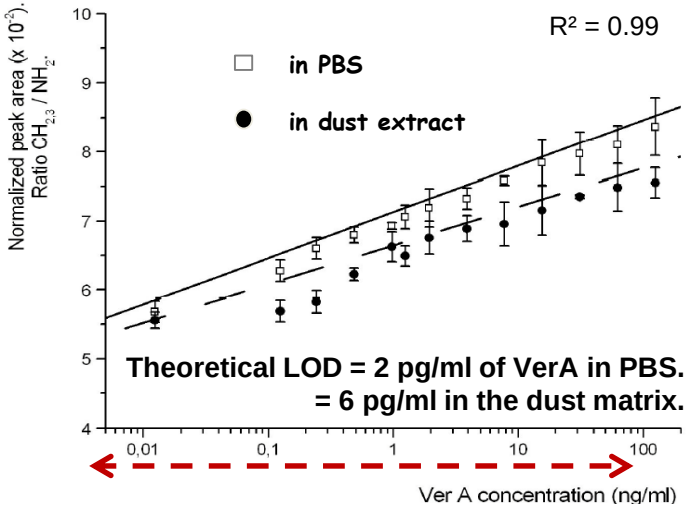
Sensitivity around ng/ml of free verrucarin-A in complex media

Dynamic range ~ 1 log

mAb based FTIR-ATR biosensors

B

IR dilution curves



Theoretical LOD = 2 pg/ml of VerA in PBS.
= 6 pg/ml in the dust matrix.

A very high sensitivity in complex media

(reaching pg/ml for Trichothecene Verrucarin-A quantified in environmental samples)

Dynamic range ~ 5 log

Robustness :

- High recovery values
- Reproducibility (run to run recovery)

Offers chemical signature

Precision

(replicates at the same conc. within run thanks to normalization)

Conclusions

1 New functionalization method of ATR elements based on organic layers only.

2 Generic devices for (bio)detection.

3 Spectroscopic sensor response (multivariate analysis, multi analyte detection, conformational transitions)

4 Efficient antifouling layer

5 Detection of low-molecular and high molecular weight ligands

6 Detection in complex fluids

7 Adapted for standard immunochemistry protocols (ELISA in competition, …)

Acknowledgments

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pharmaceuticals

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