Contributions to the design of biosensors for environmental and food contaminants monitoring

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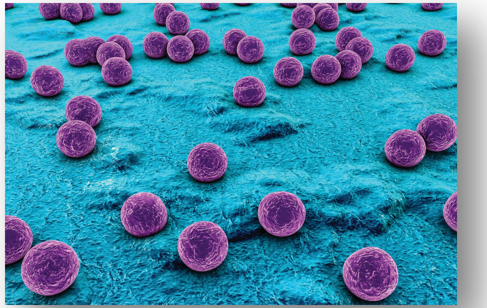
Biosensors development

The projects dealing with the design of biosensors were initiated thanks to a collaboration between Pr Souhir Boujday from the Laboratoire de Réactivité de Surface and I.

A first collaborative project was set up with teams from INRAE on the detection of *Staphylococcus Aureus*



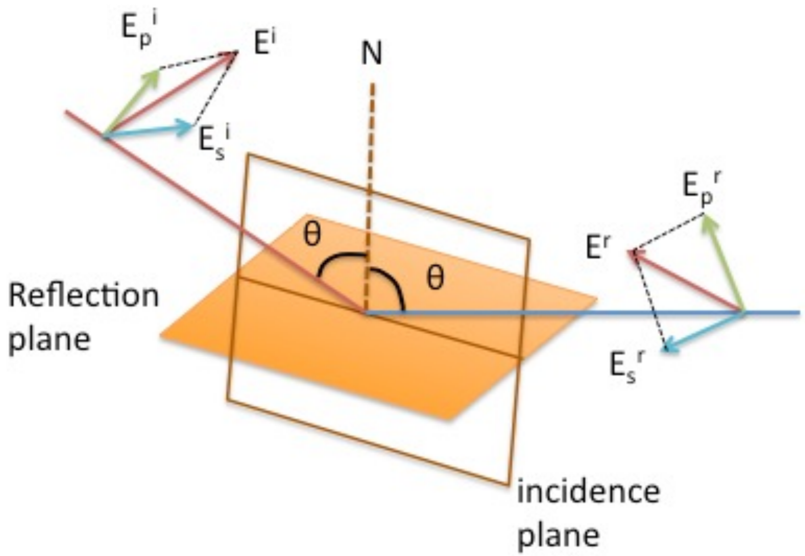
S. aureus is an anaerobic Gram-positive microorganism.
Some strains produce pathogenic toxins called enterotoxins (SE) that if ingested via consumption of contaminated food cause severe gastroenteritis

21 serotypes have been identified to date but the serotype A (SEA) is the most frequently encountered in foodborne disease outbreaks.

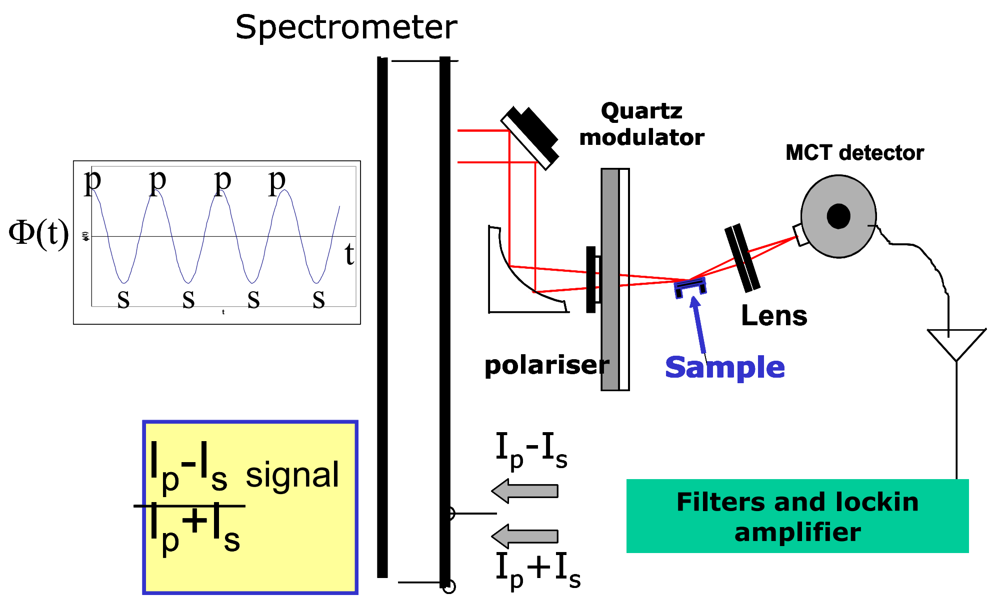
Dairy products (milk, cheese) and meat are the foods that are the most concerned by possible contaminations by *S. aureus*

Polarization Moldulation-IR Reflection-Absorption Spectroscopy (PM-IRRAS)

Principle of (PM)-IRRAS



Experimental setup



The incident IR beam is focused on the surface of the sample at grazing incidence and is subsequently reflected by the substrate. The standing wave generated near the surface is maximal for p-polarisation at this incidence and leads to the enhancement of the corresponding electric field component normal to the surface, E_p, and consequently, to an increase of the signal of the molecules perpendicular to the surface.
Gold surfaces are optimal for this sampling method

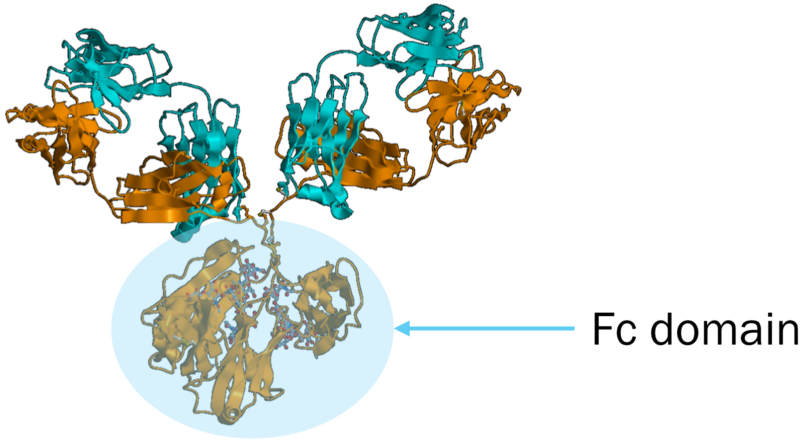
Toward an immunosensor for the detection of *S. aureus*

Principle : capture of S. aureus with specific antibody immobilized on transducer
Transducer: gold-coated planar substrate

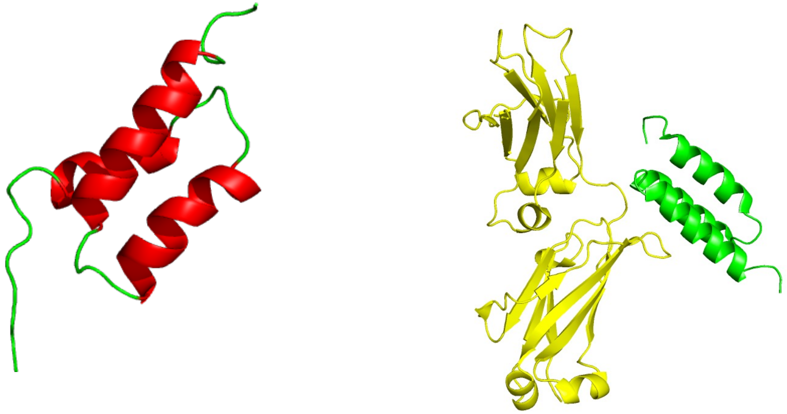
In a previous work, we had explored different strategies to immobilize an antibody on gold surface
The most effective strategy relied on the high affinity of rabbit antibodies for Protein A (Fc fragment)

Antibodies are immunoglobulins G produced by the immune system in response to antigens
Protein A is a bacterial protein (MW= 45 kDa) containing 4 IgG binding sites

X-ray structure of IgG



X-ray structures of Protein A (fragment) and IgG (Fc)-PrA complex



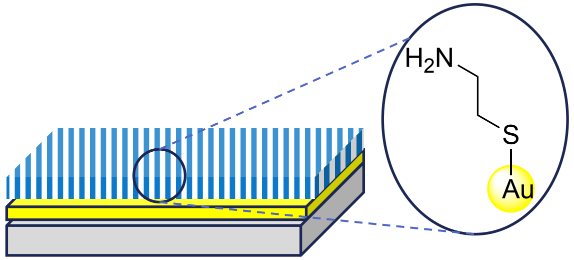
Toward an immunosensor for the detection of *S. aureus*

Functionalization of gold surfaces can be readily achieved by assembling a monolayer of thiolate (SAM) carrying a suitable functional group at its extremity

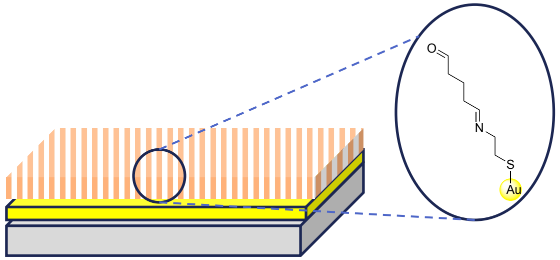
A SAM of **cysteamine** was first assembled on glass substrates coated with a thin layer of gold (200 nm)

The surface was next reacted with the cross-linking agent **glutaraldehyde**

1

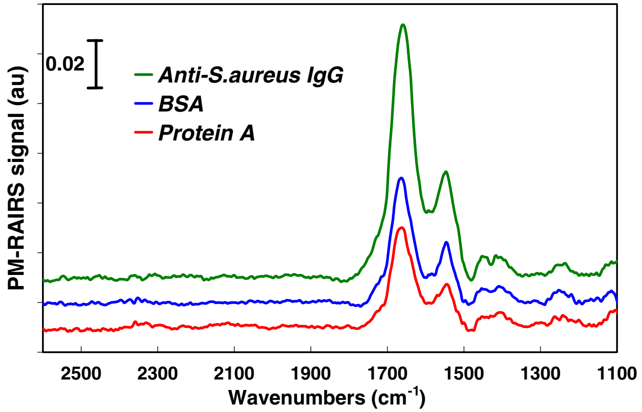


2



The surface was successively exposed to solutions of Protein A, BSA (as blocking agent) and *S. aureus* antibody

The gold surface was analyzed by Polarization-modulation IR Reflection-Absorption Spectroscopy (PM-IRRAS)



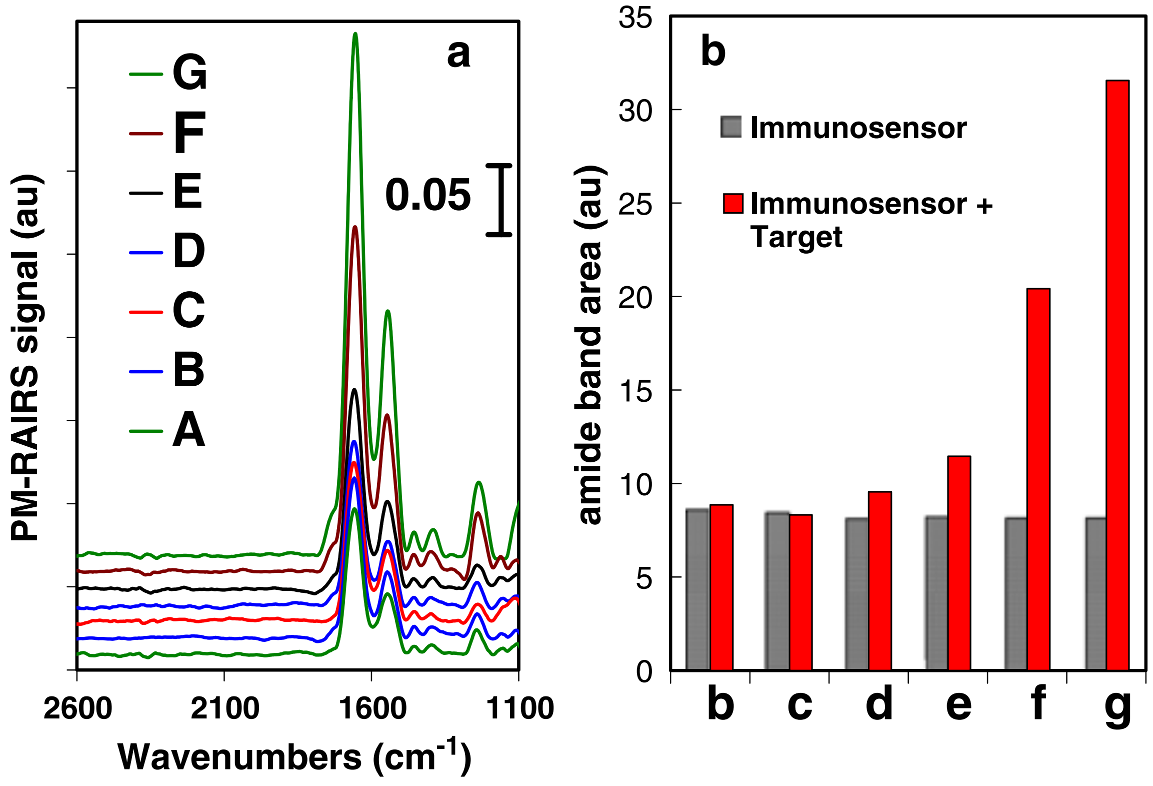
Two bands assigned to the peptide bonds (Amide I, 1660 cm^{-1}; Amide II, 1550 cm^{-1}) are observed which intensity is related to the surface concentration of proteins

IgG/PrA = 0.8

Toward an immunosensor for the detection of *S. aureus*

The immunosensing platform was exposed to suspensions of bacteria at different concentrations and the area of the peptide bands was measured on the IR spectra

A immunosensor
B reference sample
C non specific bacteria
D 10^5 CFU/ml
E 10^6 CFU/ml
F 10^7 CFU/ml
G 10^8 CFU/ml



Boujday, Microchim. Acta 2008, 163, 203

Staphylococcal enterotoxin A

Piezoelectric and optical biosensor configurations

Staphylococcus enterotoxin A

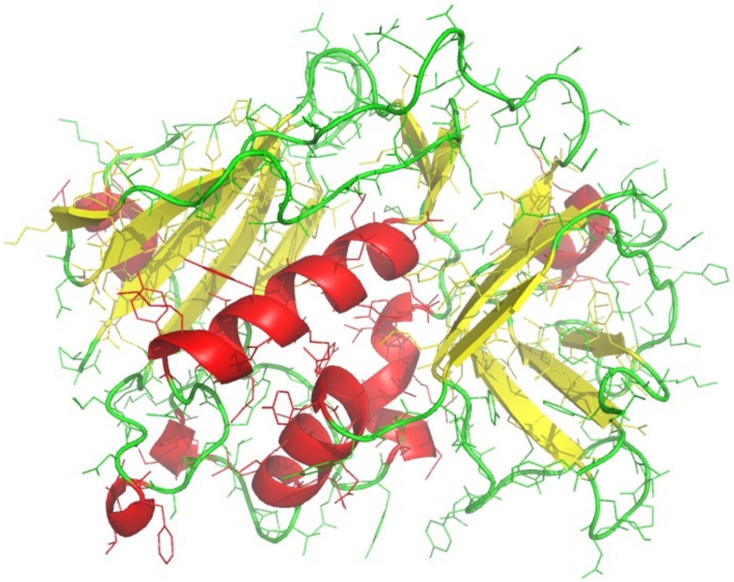
Main causative agent for staphylococcal food poisoning
Resistance to heat and proteolysis

Analysis during the manufacturing process when *S. aureus* > 10^5 cfu/g

Reference method = ELISA

Effective dose > 0.1 µg

X-ray structure of SEA

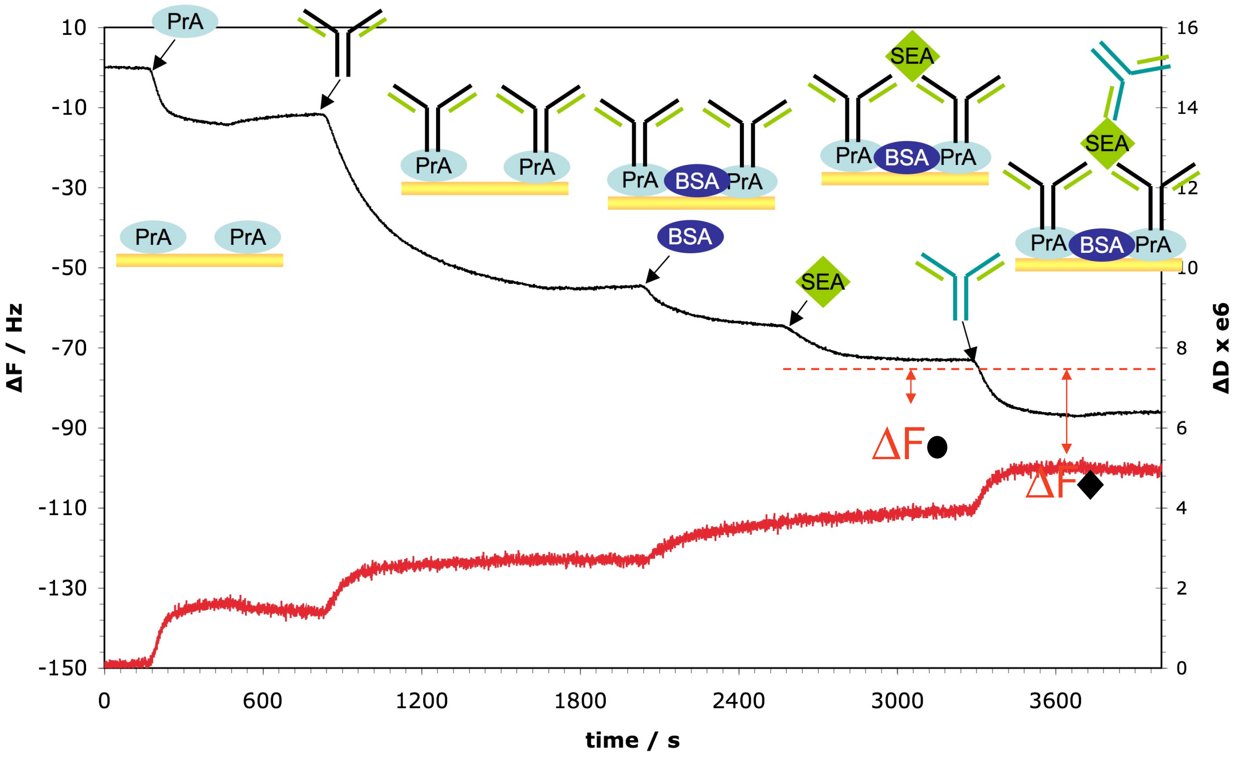


MW = 27.1 KDa
233 aminoacids

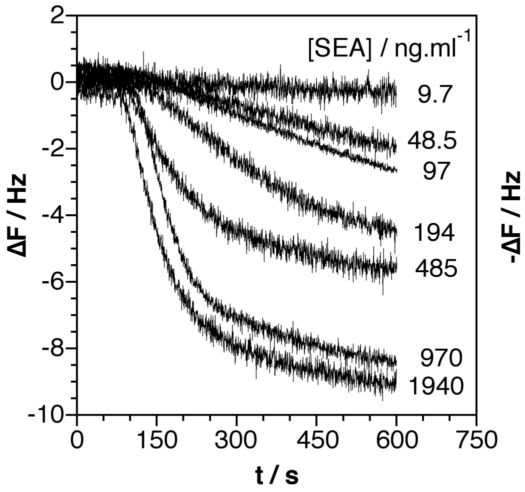
→ Necessity to check its absence to ensure safe food to consumers

Piezoelectric immunosensor of SEA

Each binding event translates into a change of frequency and dissipation

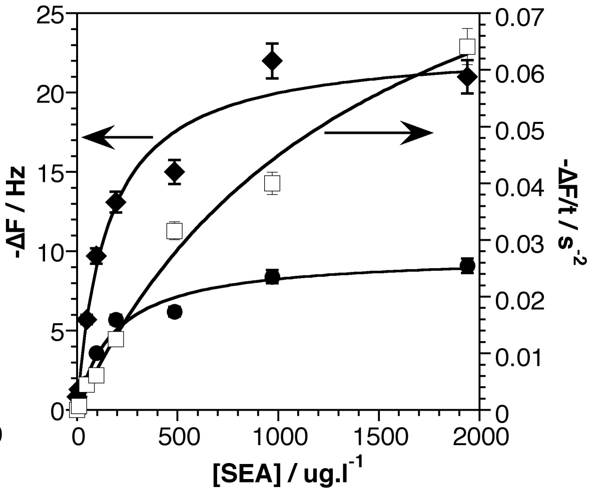


Successive immobilization of Protein A, anti-SEA, SEA (970 ng/mL) and anti-SEA (sandwich assay) monitored in real time by QCM-D



Direct format (●)

$$-\Delta F = 9.8 \times \frac{[SEA]}{[SEA] + 183}$$



Sandwich format (◆)

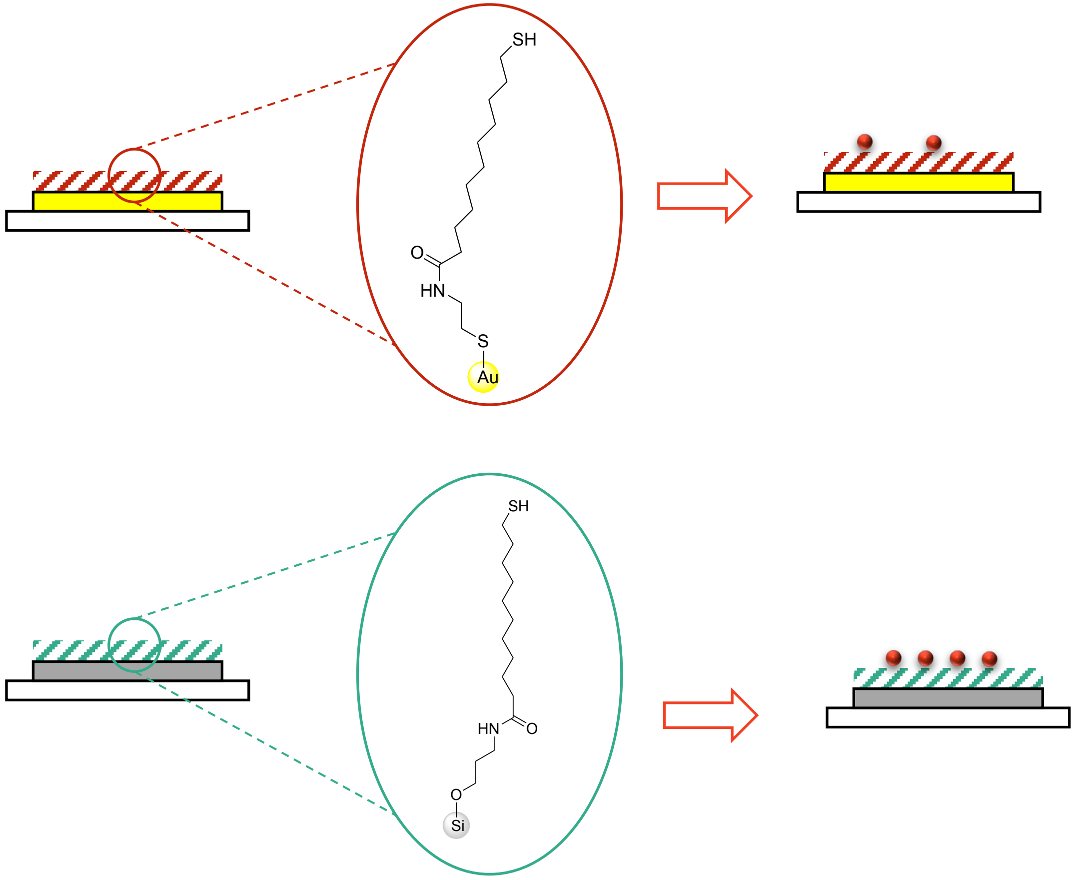
$$-\Delta F = 23 \times \frac{[SEA]}{[SEA] + 151}$$

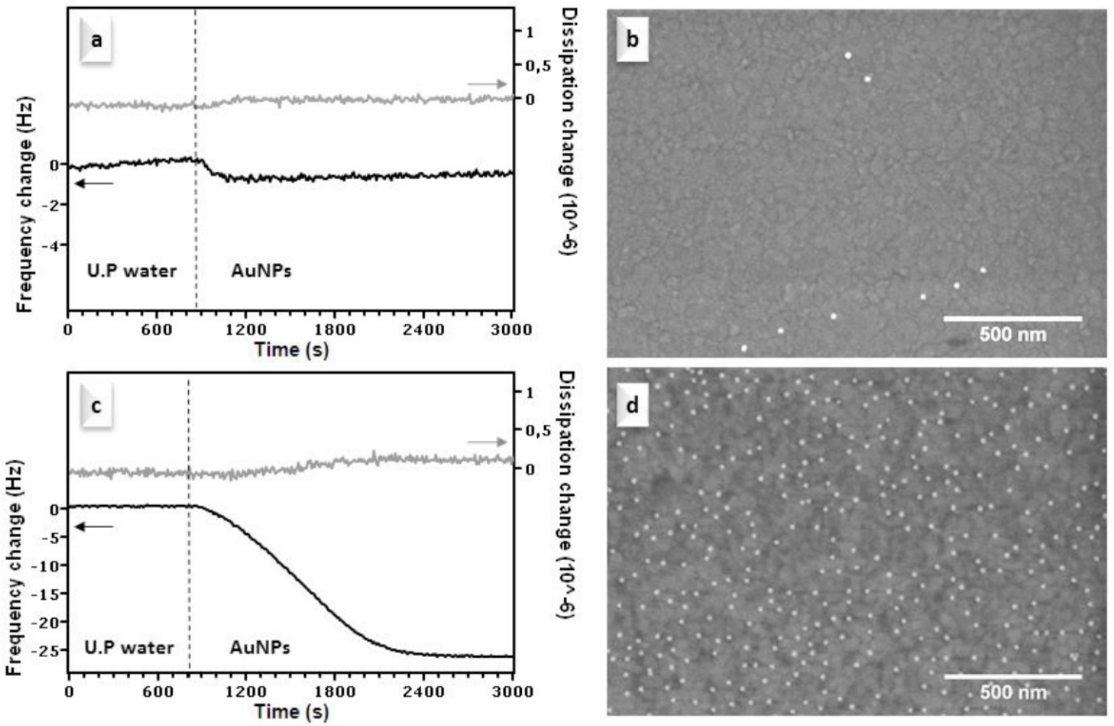
Limit of detection (LoD) = 20 ng/mL
Application to milk samples

Salmain, Biosens. Bioelectron. 2011, 29, 140 & Sens. Actuators B 2012, 173, 148

Piezoelectric immunosensor of SEA

Gold- or silicon-coated quartz crystals were nanostructured with gold nanoparticles to increase the surface area
Thiol groups were introduced by wet chemistry on both surfaces followed by AuNP attachment under flow
AuNP chemisorption was monitored in real time by QCM and surfaces were characterized by SEM





18x10^{9} AuNP/cm^{2}

Ben Haddada, Sens. Actuators 2018, 255, 1604

Piezoelectric immunosensor of SEA

Anti-SEA antibody was further immobilized on the QCM sensors via Protein A and SEA solution (485 ng/ml) was flown while resonance frequency was measured

Sensor → Step ↓	$AuNP_{13}$-coated Au	$AuNP_{40}$-coated Au	Planar Au	$AuNP_{13}$-coated Si	Planar Si
Protein A	-7.0	-7.9	-6.9	-9.4	-7.0
Anti-SEA	-33.9	-34.8	-33.0	-32.6	-33.7
SEA	-8.2	-7.9	-6.2	-8.3	-5.3
SEA/anti-SEA	1.3	1.2	1.0	1.4	0.8

On nanostructured surfaces:

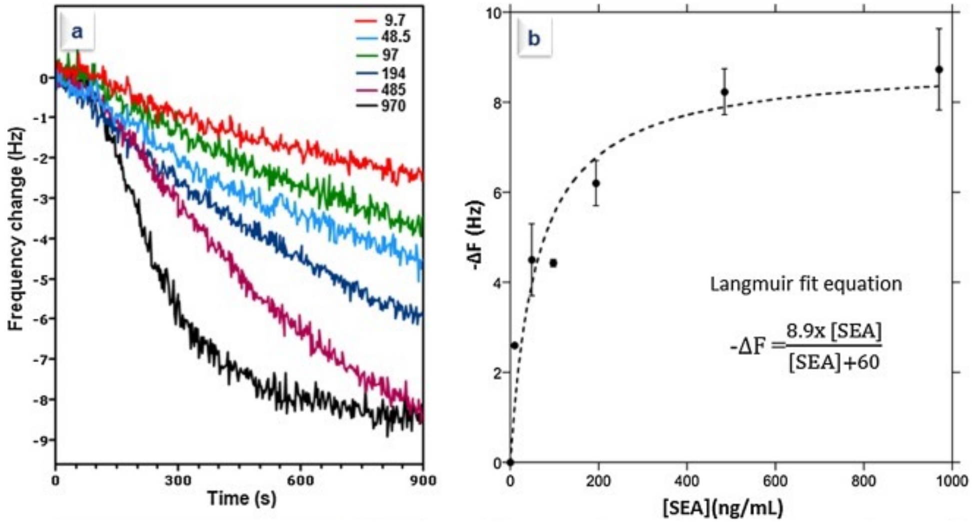
- ✓ Larger surface density in PrA
- ✓ Same surface density in anti-SEA
- ✓ More efficient capture of target

Ben Haddada, Sens. Actuators 2018, 255, 1604

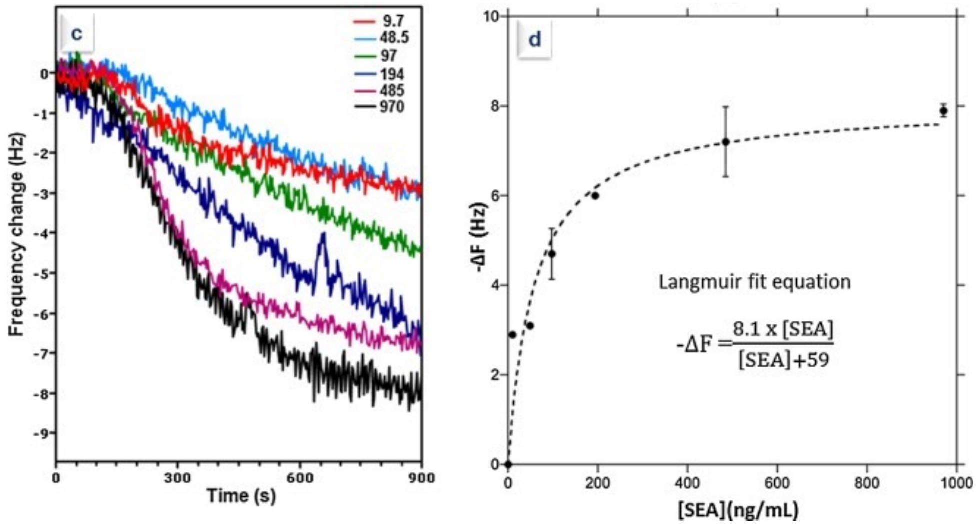
Piezoelectric immunosensor of SEA

Immunosensors’ responses to SEA and calibration curves (direct format)

$AuNP_{13}$-Au



$AuNP_{13}$-Si

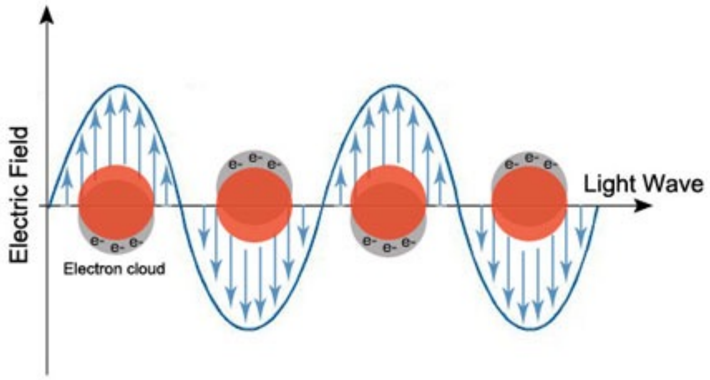


LoD = 8 ng/ml in the direct format
LoD = 1 ng/mL in the sandwich format

Ben Haddada, Sens. Actuators 2018, 255, 1604

Localized Surface Plasmon Resonance (LSPR)

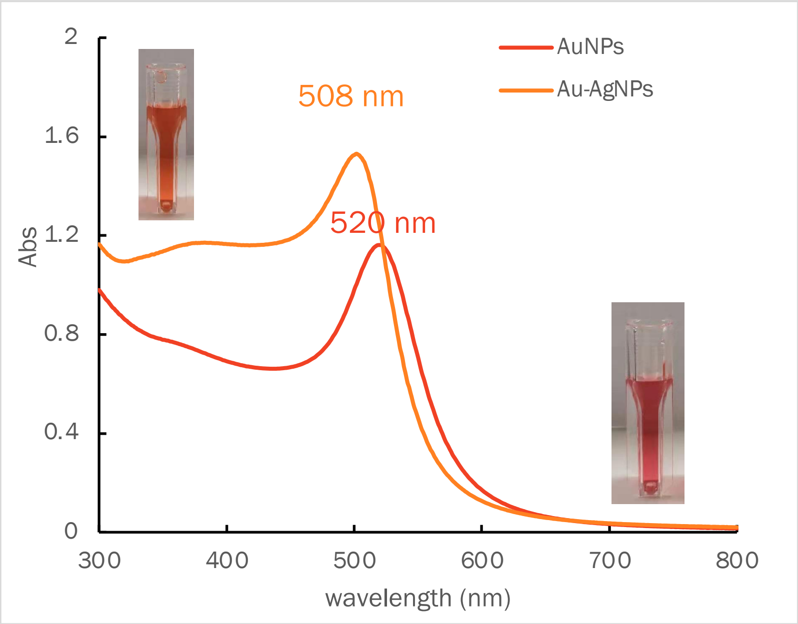
When a small spherical metallic nanoparticle is irradiated by light, the oscillating electric field causes the conduction electrons to oscillate coherently. The oscillation frequency depends on the density of electrons and the size and shape of the charge distribution



- The electric fields near the particle's surface are greatly enhanced
- The particle's optical absorption has a maximum at the plasmon resonant frequency (λ_{max})
- Extinction coefficients are extremely high (compared to organic molecules)
- **The resonant frequency is in the visible range for noble metals**
- **It is highly sensitive to the local refractive index**

LSPR

Uv-vis spectra of colloidal solutions of spherical AuNP and Au@AgNP



The plasmon band shift $\Delta\lambda$ is governed by the equation:

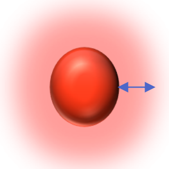
$$\Delta\lambda = m(n_{adsorbate} - n_{medium})\times(1 - e^{\left(-\frac{2d}{l_d}\right)})$$

n = refractive index
l_d = decay length of the electric field
d = thickness of the adsorbate layer
m = intrinsic RI sensitivity factor

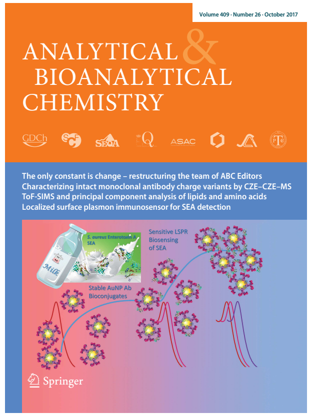
m and l_d can be determined experimentally

✓ **l_d is typically short (in the tens nm range)**

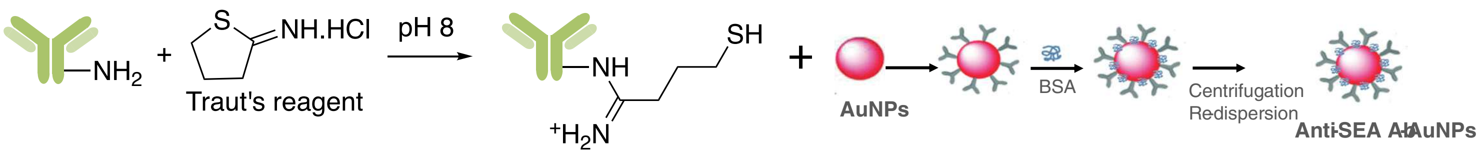
✓ **m strongly depends on size, shape, composition**

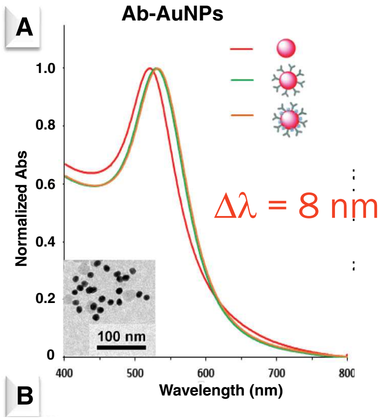


LSPR immunosensor of SEA with gold NP

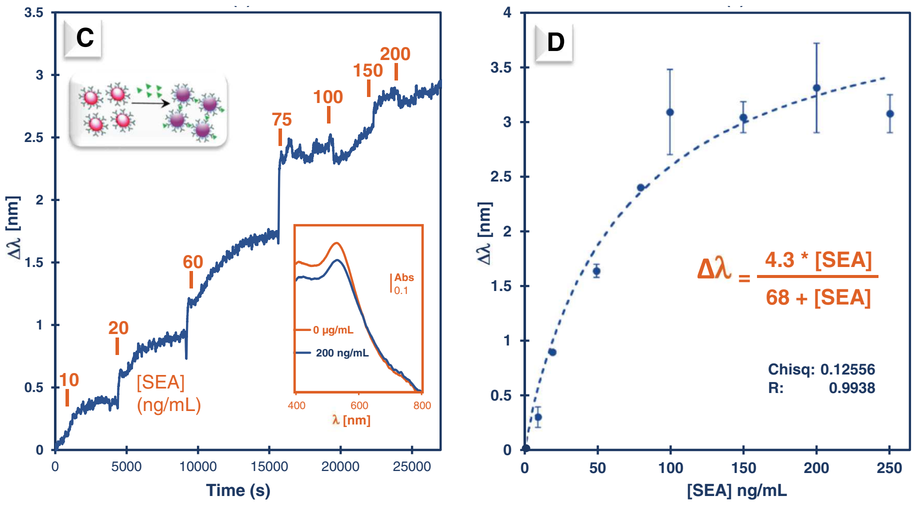


Homogeneous immunosensor engineering

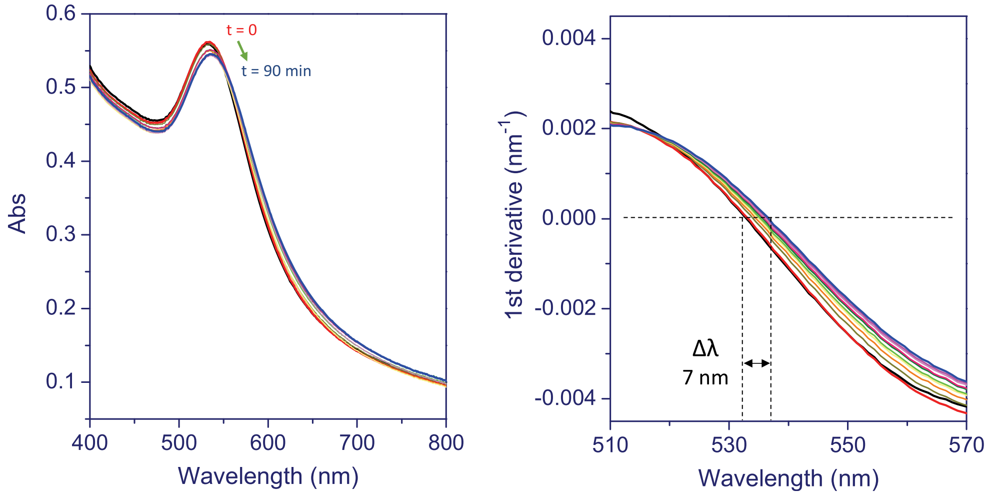




Immunosensor response to SEA (real time measurement with Insplorion Xnano II)



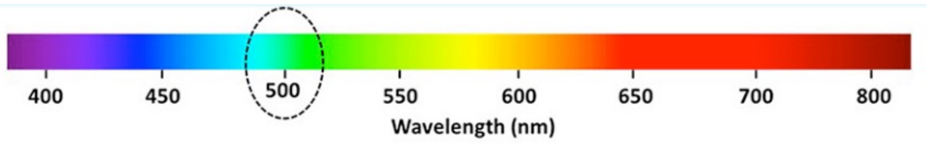
Response to 1 ug/mL SEA spiked in milk



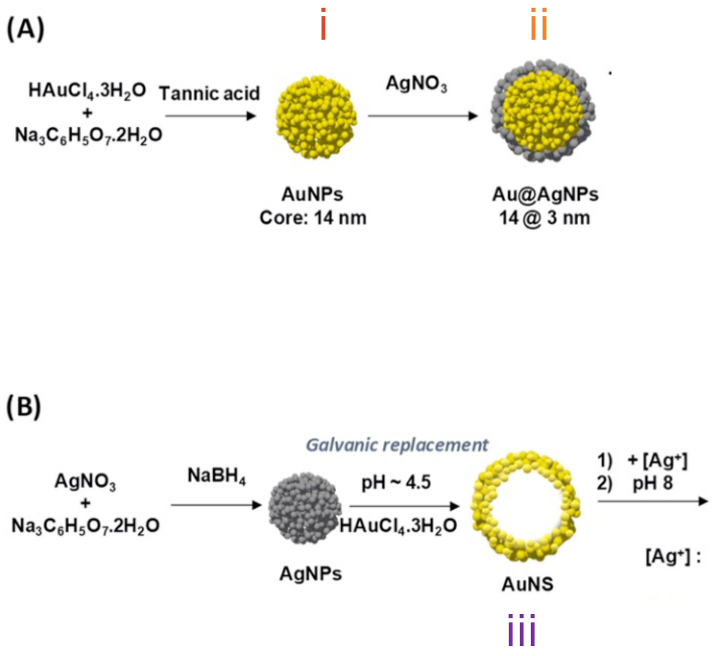
Ben Haddada, Anal. Bioanal. Chem. 2017, 409, 6227

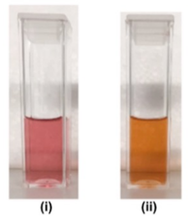
LSPR immunosensor of SEA with core-shell NP

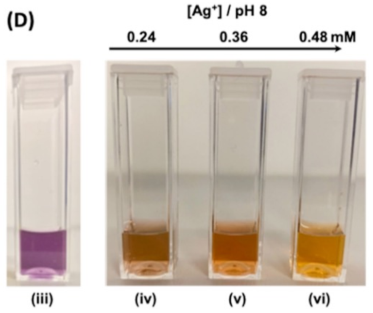
Choose noble metal nanoparticles for which very small $\Delta\lambda$ are visually detectable



NP synthesis

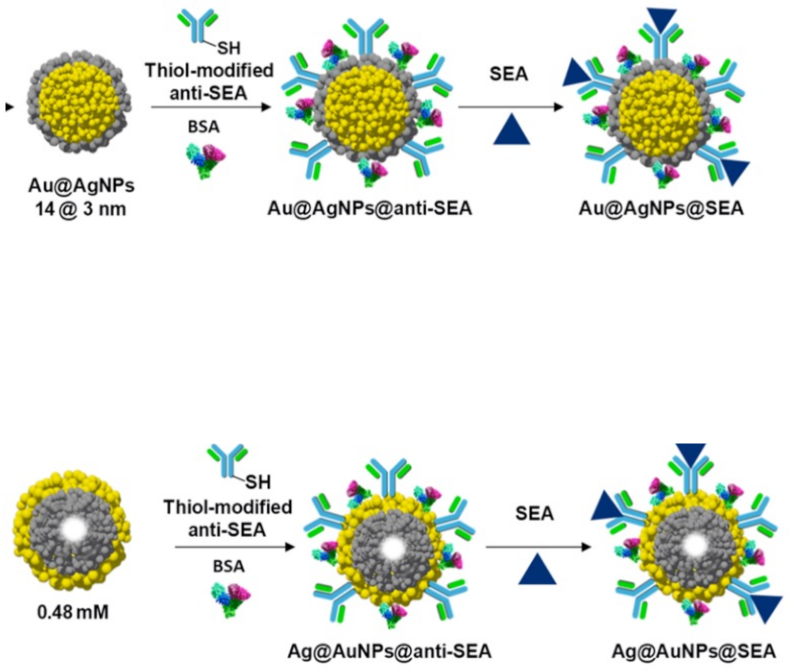


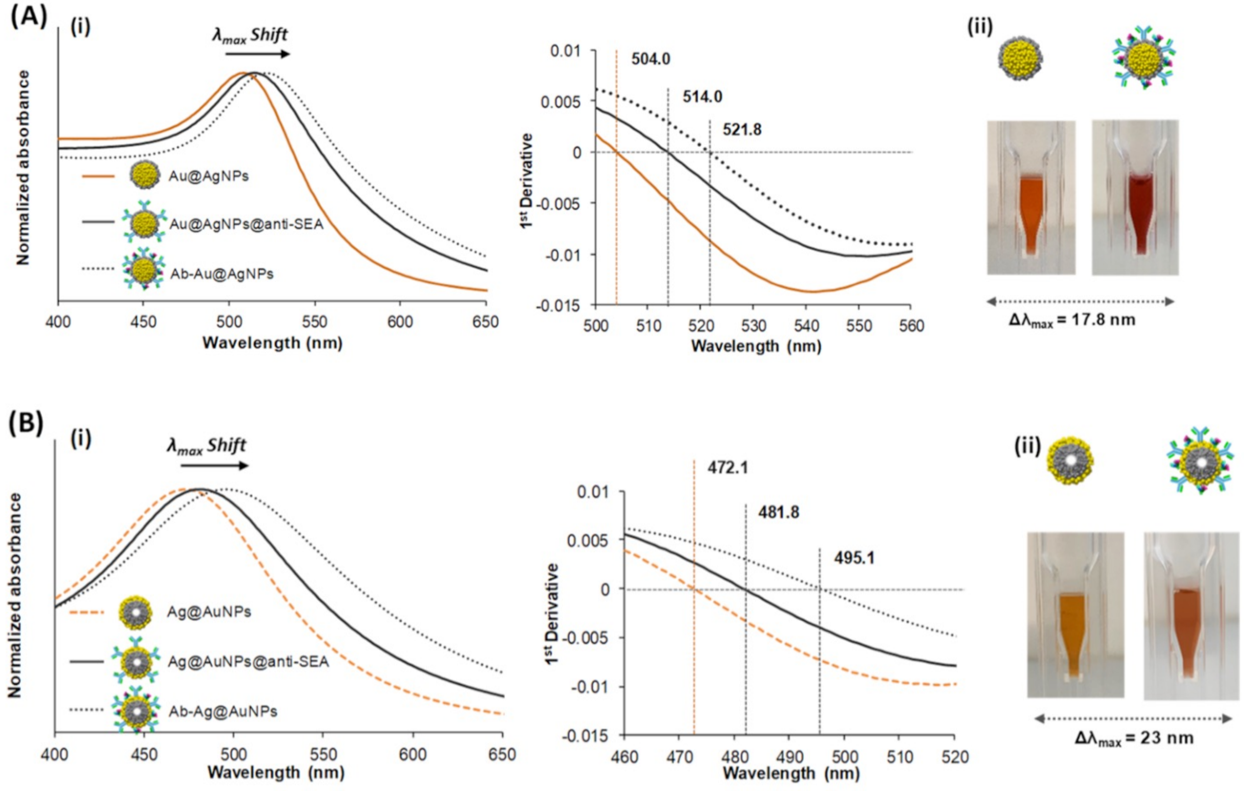




LSPR immunosensor of SEA with core-shell NP

Immunoprobes' engineering

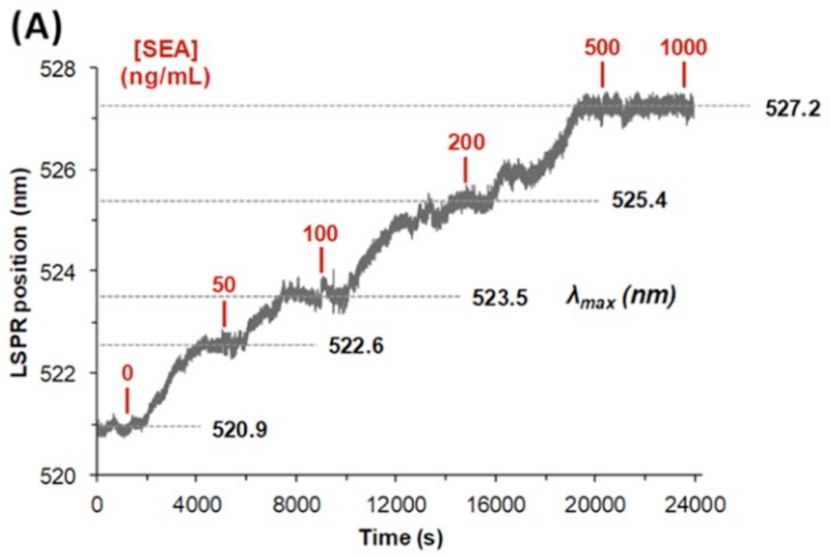




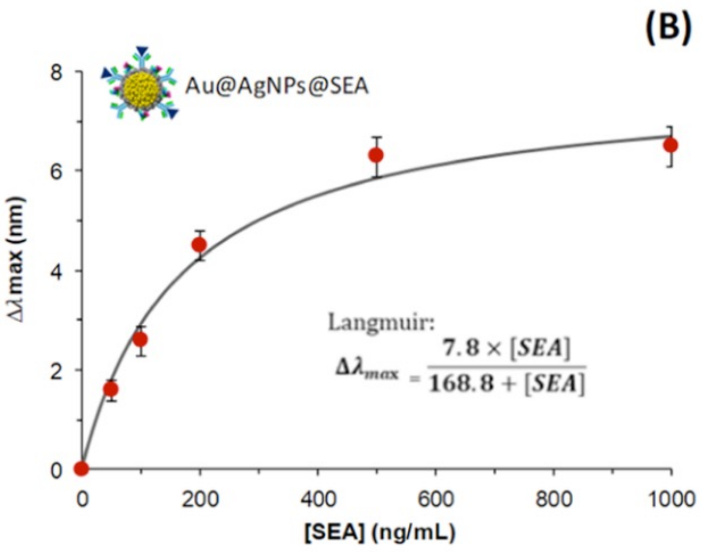
Loiseau, ACS Appl. Mater. Interfaces 2019, 11, 46462

LSPR immunosensor of SEA with core-shell NP

Au@AgNP immunosensor response to SEA
(real time measurement with Xnano II)

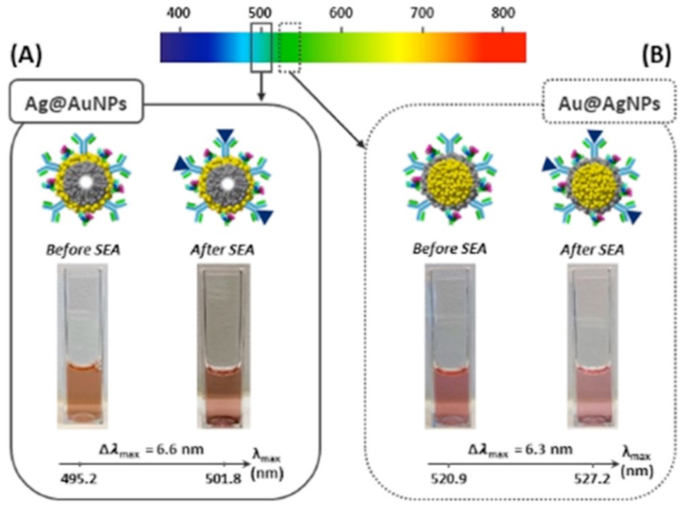


Calibration curve



LoD ~ 5 ng/mL

Visual detection



orange -> red red -> pinkish

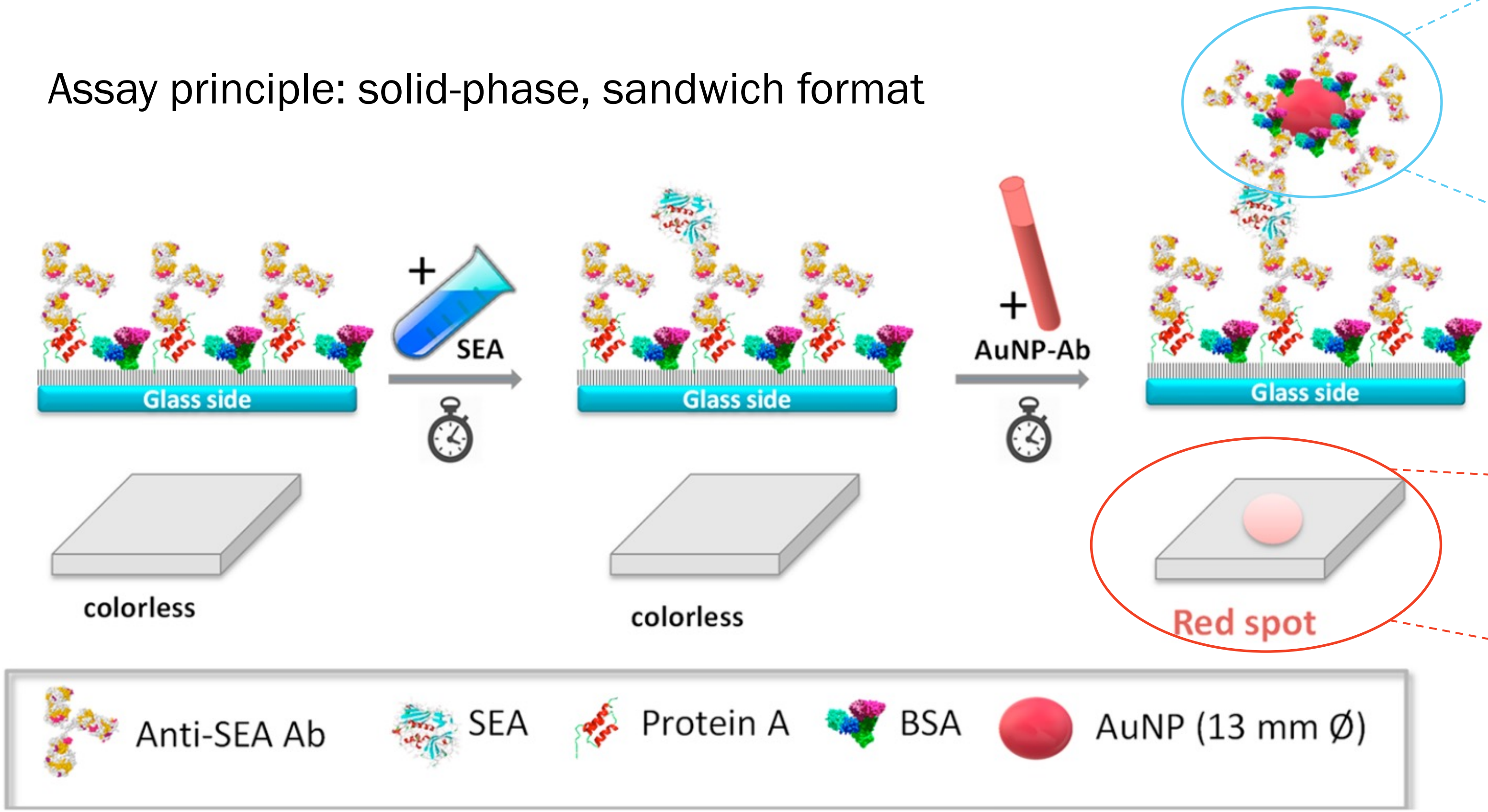
([SEA] = 500 ng/mL)

Loiseau, ACS Appl. Mater. Interfaces 2019, 11, 46462

Colorimetric, gold-nanoparticle based immunosensor of SEA

AuNP as colorimetric tag

Assay principle: solid-phase, sandwich format



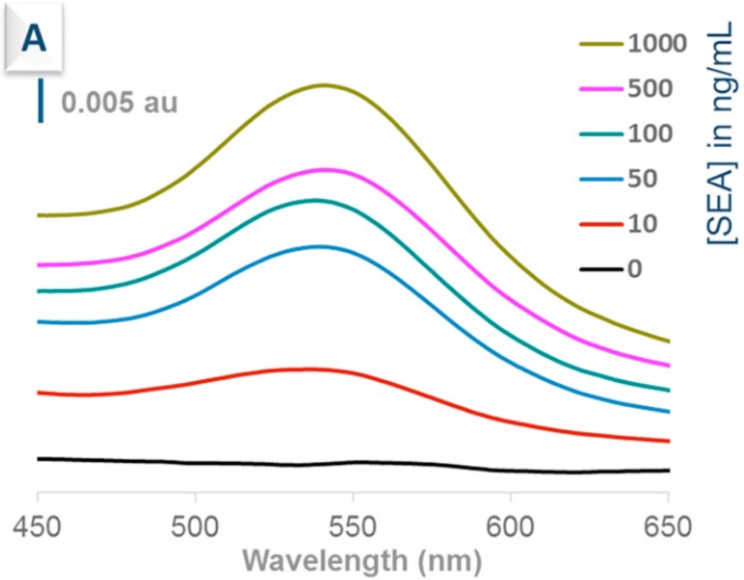
Revelation reagent (= AuNP-Ab bioconjugate) was synthesized by direct physisorption of anti-SEA

Detection by the naked eye or with benchtop uv-vis spectrophotometer

Zhang, ACS Appl. Nano Mater. 2019, 2, 4150

Colorimetric, gold-nanoparticle based immunosensor of SEA

Absorption spectra of glass slides



Calibration curve (SEA spiked in skimmed milk)

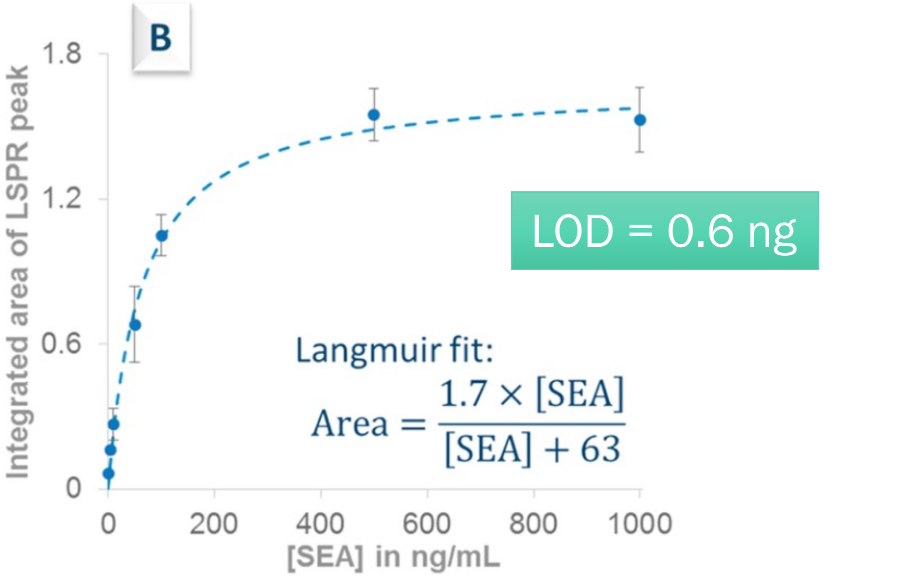
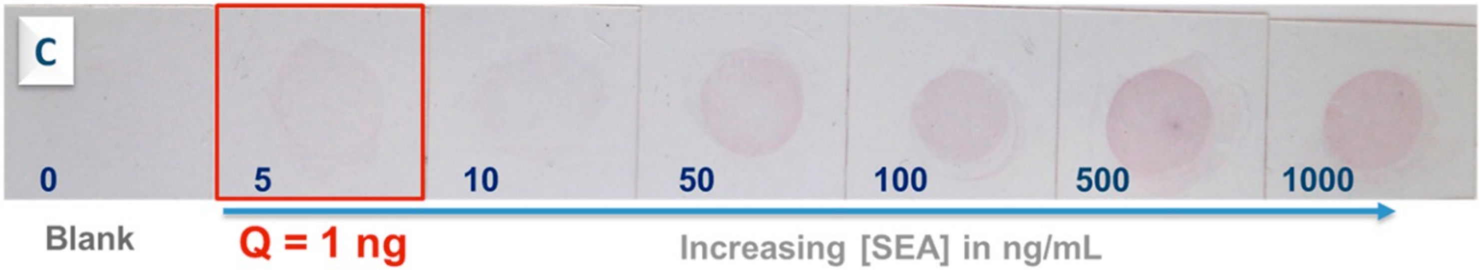


Photo of glass slides



L. Zhang, ACS Appl. Nano Mater. 2019, 2, 4150;
W. Ait Mammar, Talanta 2025, 291, 127864

Small molecule targets

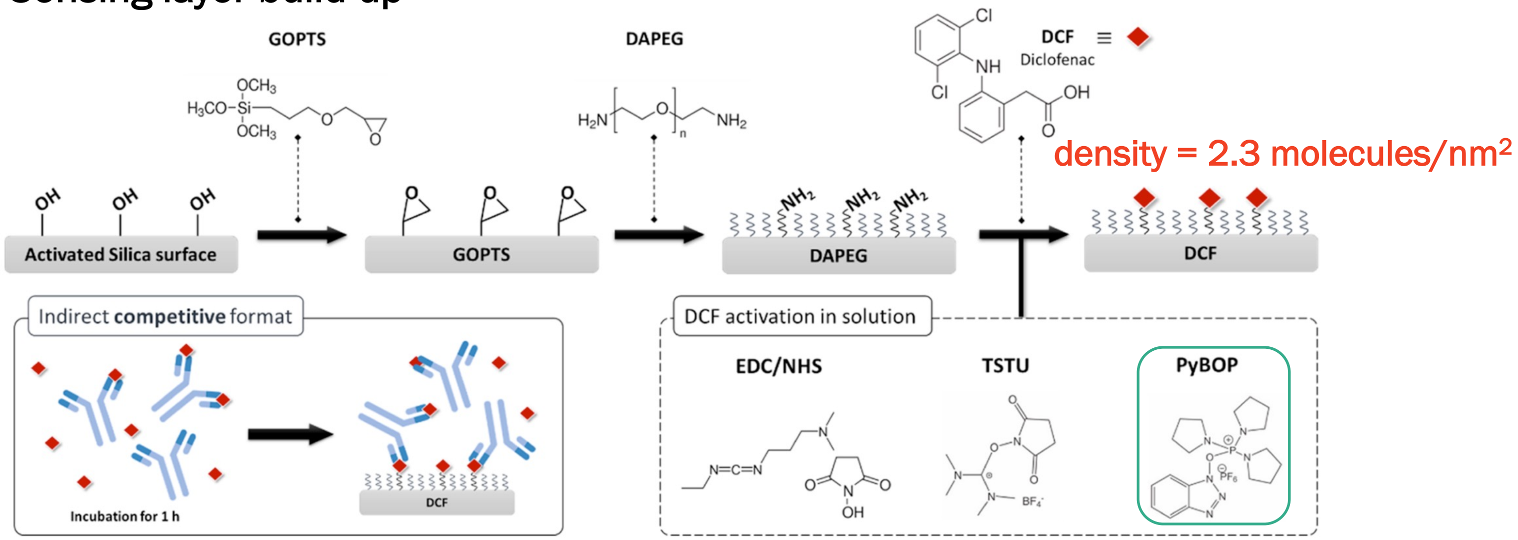
diclofenac; aflatoxin B

Piezoelectric immunosensor of drug residue in river water

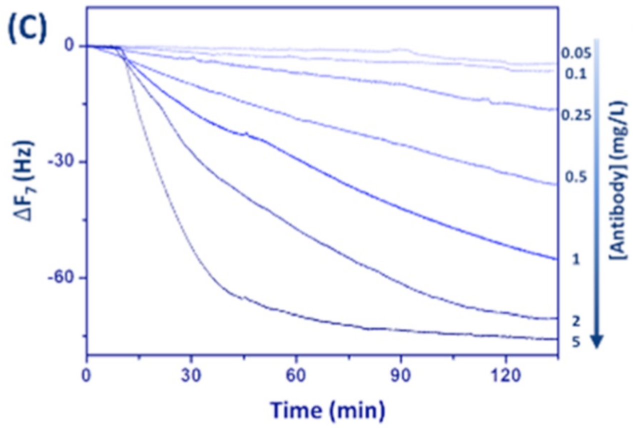
Diclofenac is the active component of Voltaren™. It is a non steroidal anti-inflammatory drug (NSAID) broadly employed (40-60 tons per year in France) to treat mild pains.
Because of inefficient waste water treatments plants, diclofenac is released in surface waters and is considered as an emerging pollutant. Guide value = 0.4 µg/L (ANSES)

An piezoelectric immunosensor operating in competitive format was designed to assay diclofenac first in model matrix then in surface water taken from 3 different rivers

Sensing layer build up



Capture of anti-DCF monitored by QCM

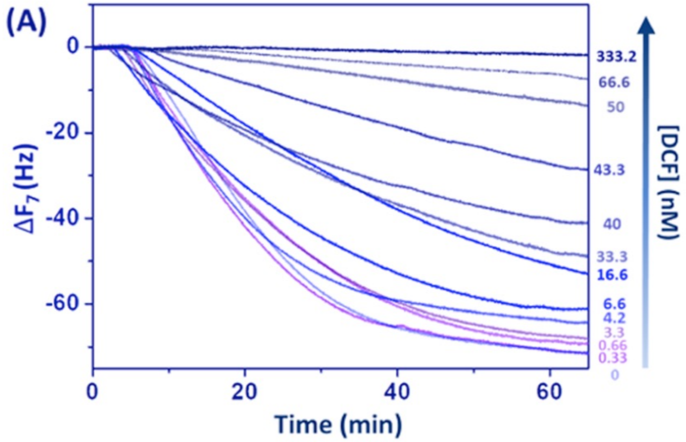


K_D = 0.23 nM

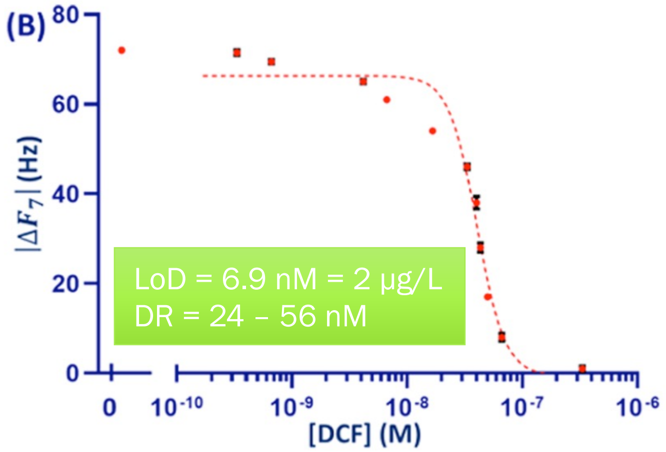
Mazouzi, ACS Sens. 2012, 6, 3485

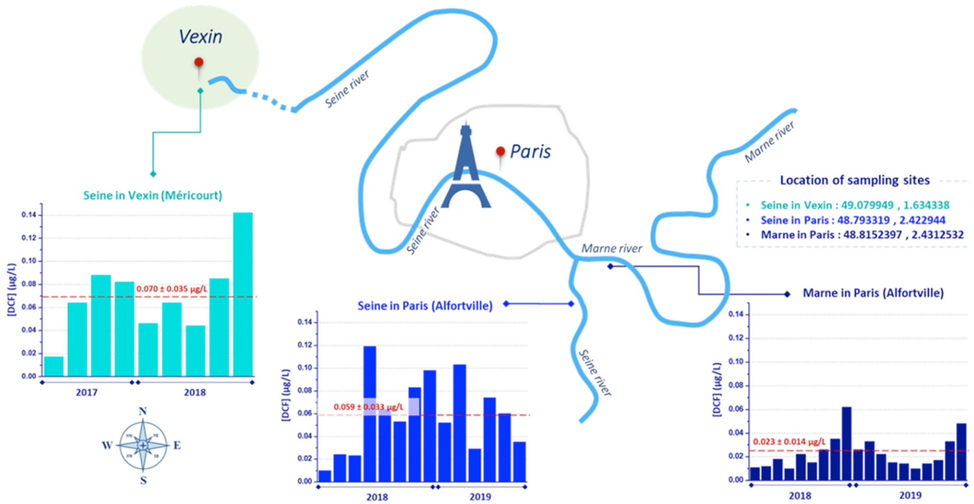
Piezoelectric immunosensor of drug residue in river water

QCM responses to different concentrations of DCF



Calibration curve





DCF was extracted from water samples (0.5 l) by SPE
Samples (0.5 ml) were analysed in triplicate by QCM
ΔF were measured over 60 min

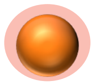
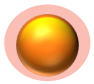
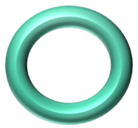
Seine in Vexin	Seine in Paris	Marne in Paris
0.069 ± 0.005 µg/l	0.047 ± 0.006 µg/l	< LoD
0.07 ± 0.035	*0.059 ± 0.033*	*0.023 ± 0.014*

Mean values (2 years) provided by eaufrance agency

Mazouzi, ACS Sens. 2012, 6, 3485

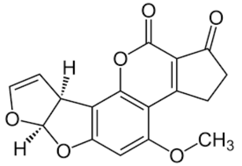
Refractive index sensitivity of plasmonic particles

The plasmon band position of metal nanoparticles is known to be sensitive to the local refractive index. This sensitivity can be characterized by the refractive index sensitivity factor (RIS) in nm/RIU.
The RIS value of NP in colloidal solution strongly depends on the size, shape and composition of the material.

Nanoparticle	Size	RIS
Gold nanospheres 	15 nm diameter	44 nm/RIU
Gold nanorods 	102x40 nm (AR=2.6)	274 nm/RIU
Core-shell Au@AgNP 	14 nm core 3 nm shell	Similar to pure AuNP
Core-shell Ag@AuNP 	22 nm core 5 nm shell	2.2x amplification / AgNP
Hollow shell AuNP 	96 nm diameter 11 nm shell (AR = 9.6)	360 nm/RIU

LSPR aptasensor of aflatoxin B1

Aflatoxin B1 is a highly toxic mycotoxin mainly produced by Aspergillus flavus
Group I carcinogen (hepatotoxicity)
Fungi can proliferate on various foodstuffs including corn, rice, spices, dried fruits, nuts, and figs
Maximum tolerated level in the EU = 2 – 4 ppb (=μg/g)

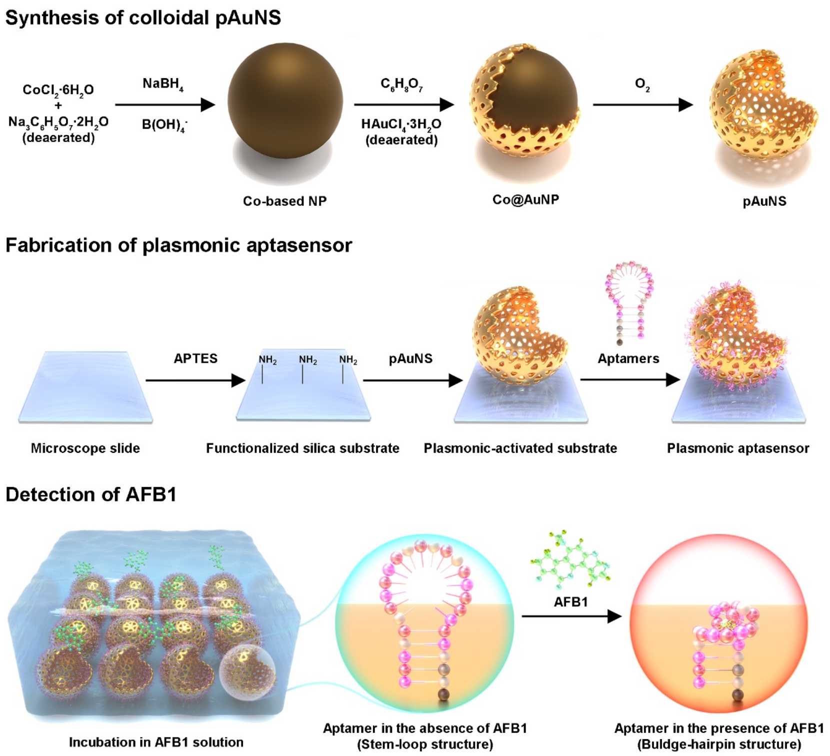


26-mer aptamer was preferred to antibody because of its much lower size (MW = 8.3 vs. 150 kDa)

It is more compatible with LSPR refractometric detection because of very short sensing depth

Porous hollow shell gold nanoparticles were selected as transducer elements

Sensors were exposed to solutions of AFB at different concentrations and analyzed with a benchtop spectrophotometer

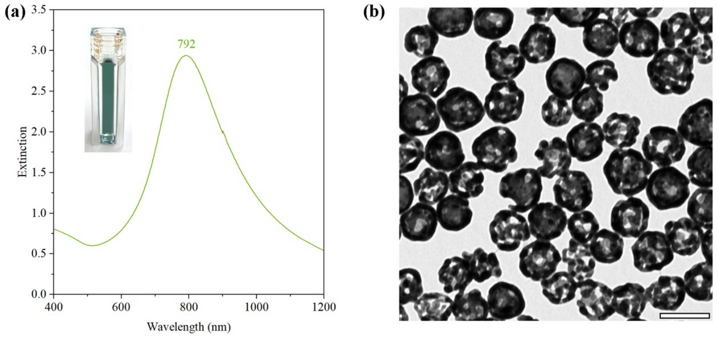


D. Sun, unpublished results

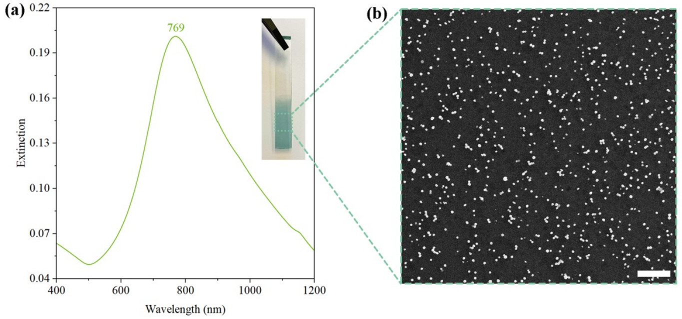
LSPR aptasensor of aflatoxin B1

Hollow gold nanoshells characterization

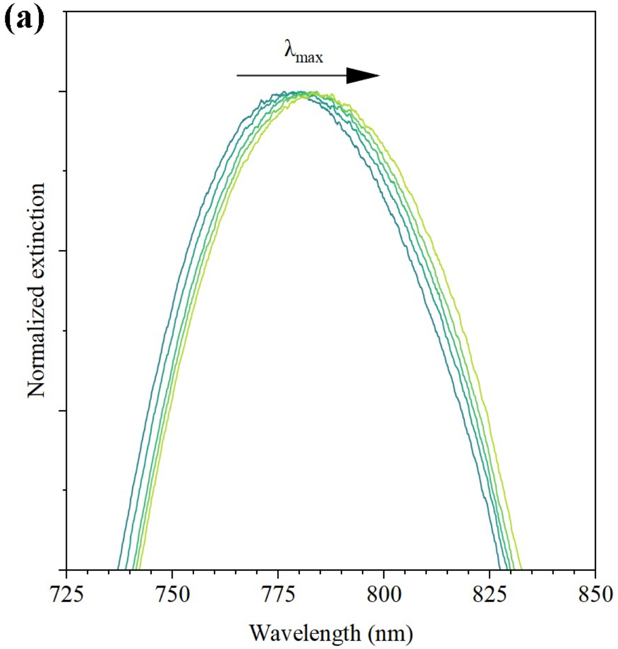
In solution



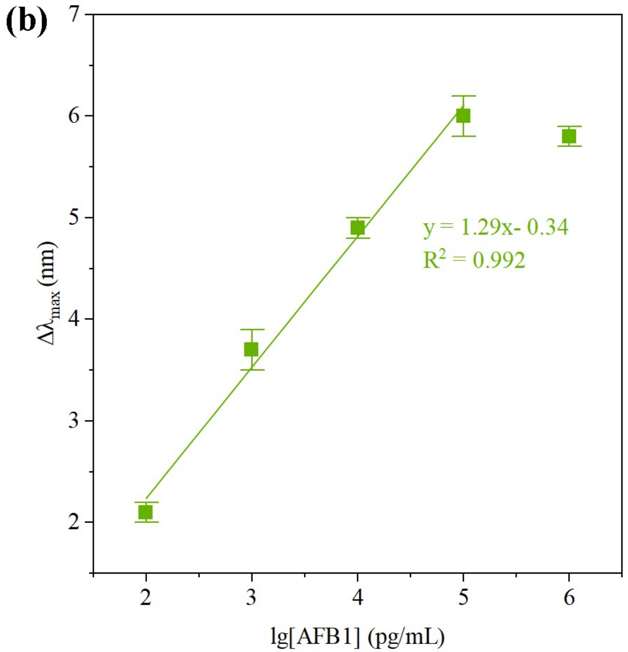
Immobilized on glass slide



Normalized spectra



Calibration curve



LoD = 5 pg/mL
DR = 100 pg/ml – 100 ng/ml

Conclusion and perspectives

We have developed a large set of biosensors using antibodies or more recently aptamers as bioreceptors

Targets ranged from small molecules to whole cells (bacteria)
We selected gravimetric and optical transduction methods (both label-free)

We are currently working on new targets of civil and military interests, food allergens and disease biomarkers

We also aim at transposing the systems to portable ones combining microfluidics and smartphone-based readout for on-field measurements

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