

European Conference on Applications of Surface and Interface Analysis ECASIA 2026

September 13th-18th - Brussels, Belgium

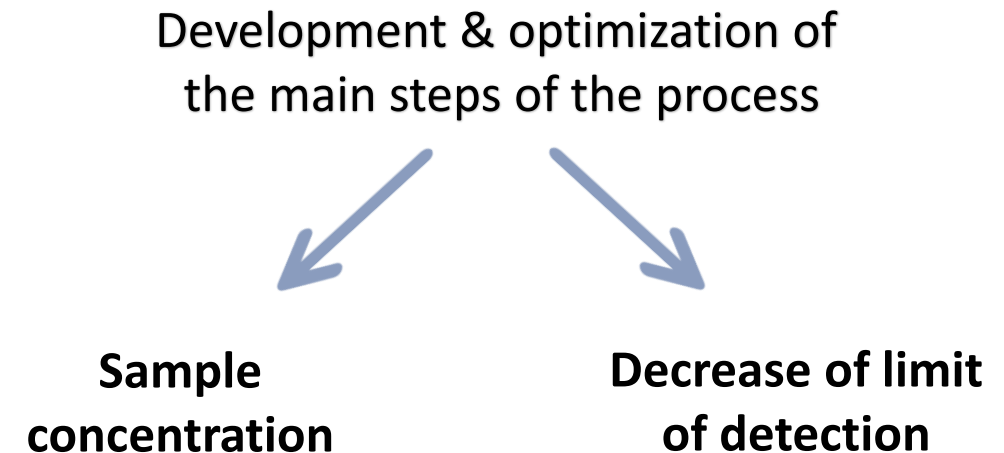
Surface-Functionalized Nanoparticles for Amplified QCM Detection of *Listeria monocytogenes*

Chloé RICHERT, Delphine LAROSE, Philippe TROSSAT, Thérèse LEBLOIS, Vincent HUMBLLOT

- Food industry affected by **pathogen contamination** : *Campylobacter*, *Salmonella* spp *E. coli*, *L. monocytogenes*, *Yersinia enterocolitica* & *pseudotuberculosis*
- European Standards : « 25 g of cheese (250 mL) or milk matrix must be free of pathogenic bacteria » ^[1]
- Existing methods (PCR or ELISA) :
 - **Very long waiting times** for results (up to 3-4 days)
 - **Analysis = not the entire 250 mL sample**



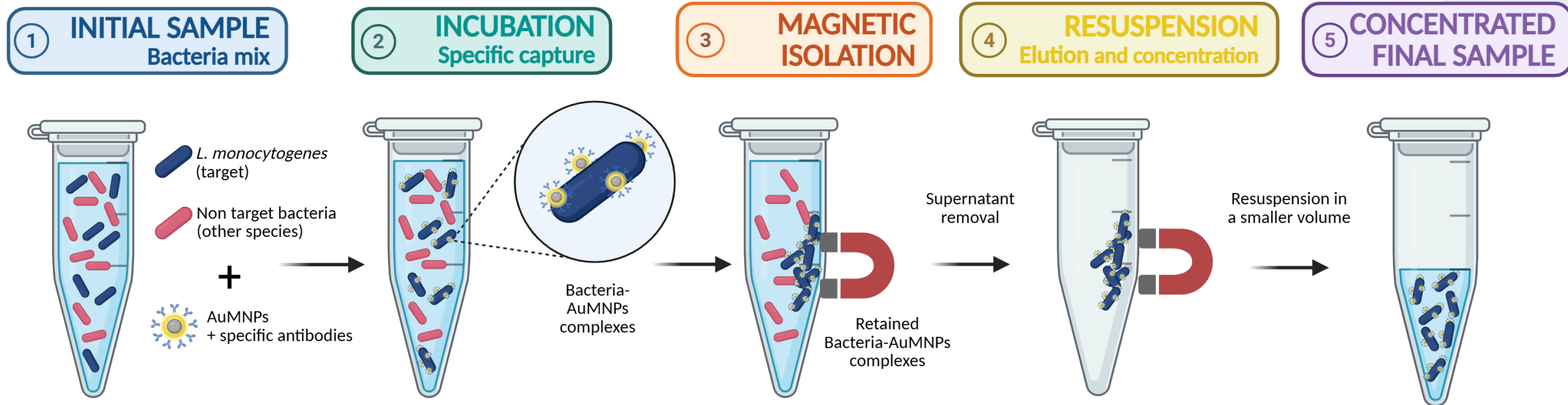
Objective : Significantly reduce the time to obtain the first negative result to 1.5 days



[1] COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, Official Journal of the European Union, Nov. 15, 2005.

THE PROJECT

Concentration step



INCREASE TARGETS CONCENTRATION IN FINAL SAMPLE

[2] Z. Li, J. Ruan, and X. Zhuang, "Effective capture of circulating tumor cells from an S180-bearing mouse model using electrically charged magnetic nanoparticles," J. Nanobiotechnology, vol. 17, no. 1, p. 59, Dec. 2019, doi: 10.1186/s12951-019-0491-1..

THE PROJECT

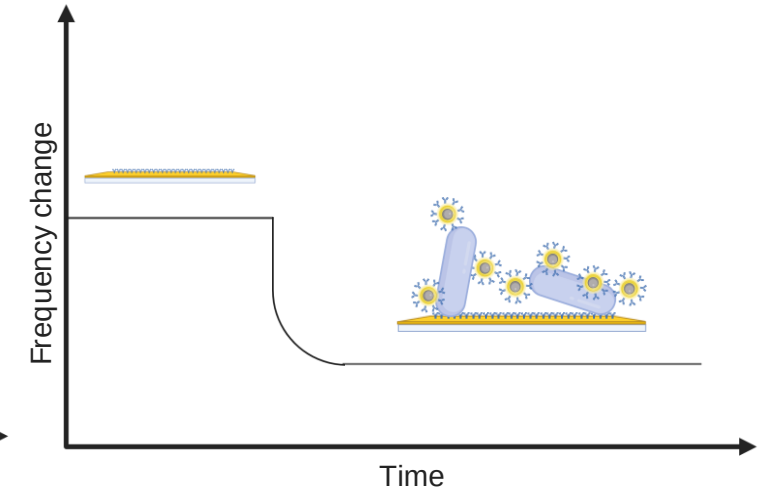
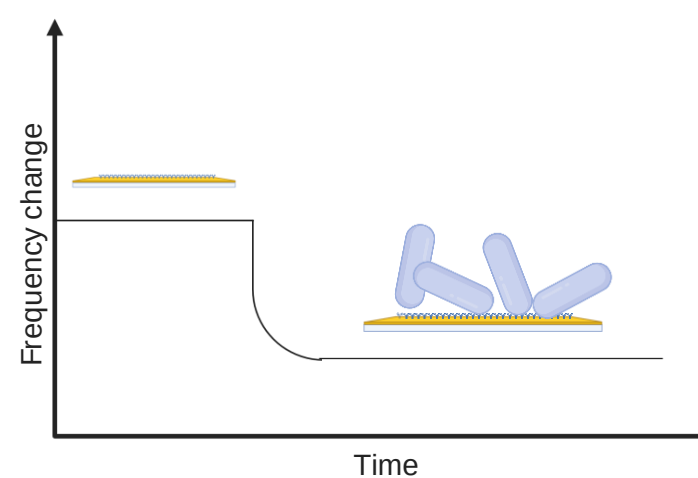
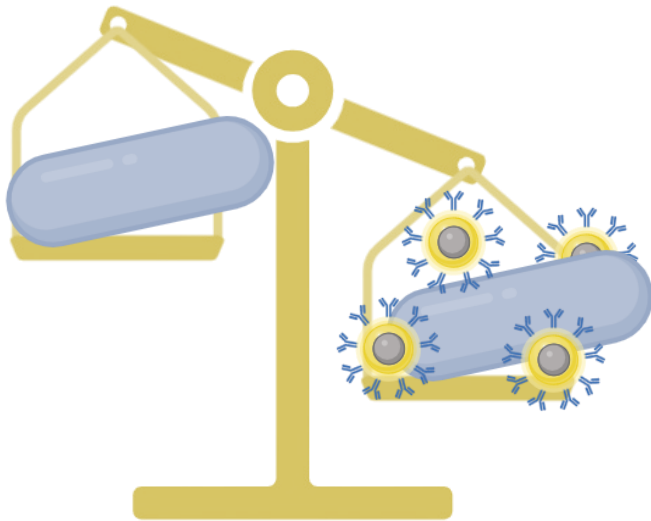
Detection step

Use of **Quartz Crystal Microbalance (QCM)** for detection : gravimetric analysis, measuring frequency shift in real time

Improving the detection method using gold magnetic nanoparticles to weigh down bacteria

$$\rho_{\text{bacteria}} = 1,4 \text{ g/cm}^3 < \rho_{\text{Fe}_3\text{O}_4} = 5,7 \text{ g/cm}^3 < \rho_{\text{Au}} = 19,7 \text{ g/cm}^3$$

$$m_{\text{bacteria}} < m_{\text{B-AuMNP}}$$



DECREASE OF LIMIT OF DETECTION FOR BACTERIA

[3] Liu et al., QCM immunosensor with nanoparticle amplification for detection of *Escherichia coli* O157:H7, Sens. & Instrumen. Food Qual. (2007) 1:161–168

DEVELOPMENT OF BIOINTERFACES

- Flat surfaces
- Nanoparticles

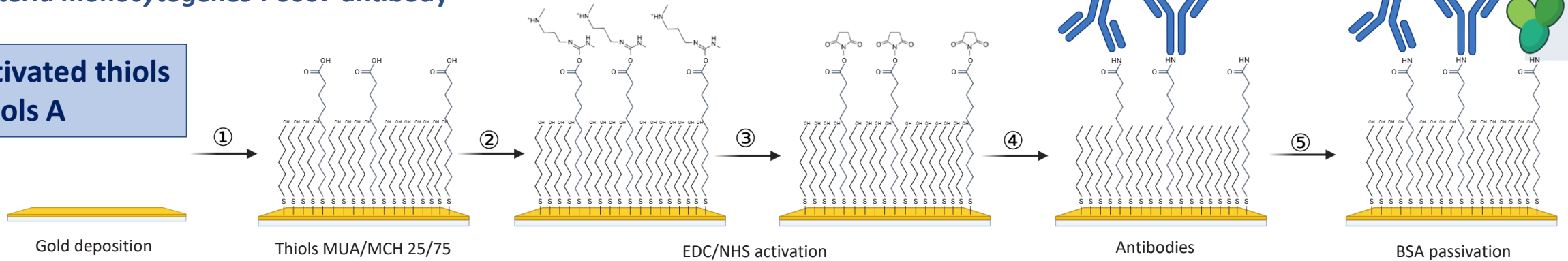


DEVELOPMENT OF BIONTERFACE

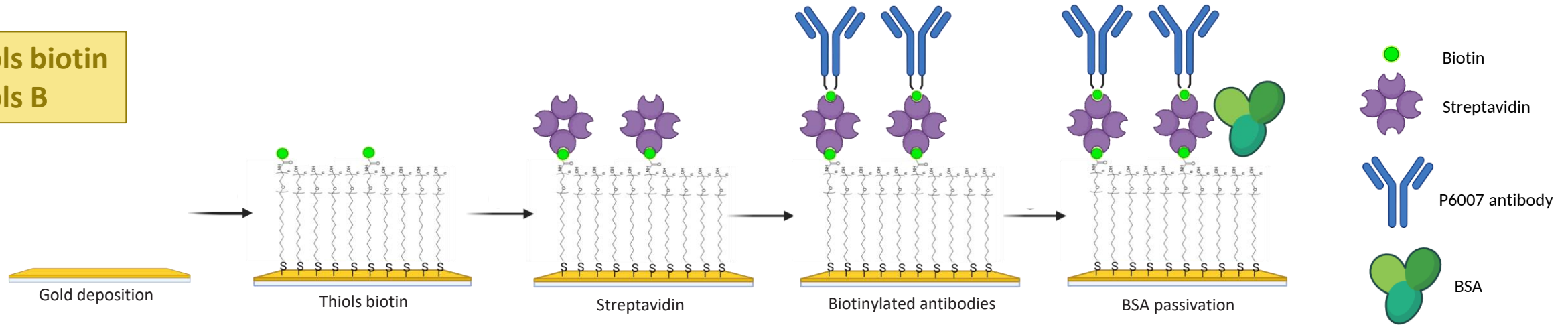
Surfaces functionalization – Flat surfaces

2 strategies of functionalization for flat surfaces: use of 2 different Self-Assembled Monolayers (SAM) to immobilize **monoclonal anti-*Listeria monocytogenes* P6007 antibody**

Activated thiols Thiols A

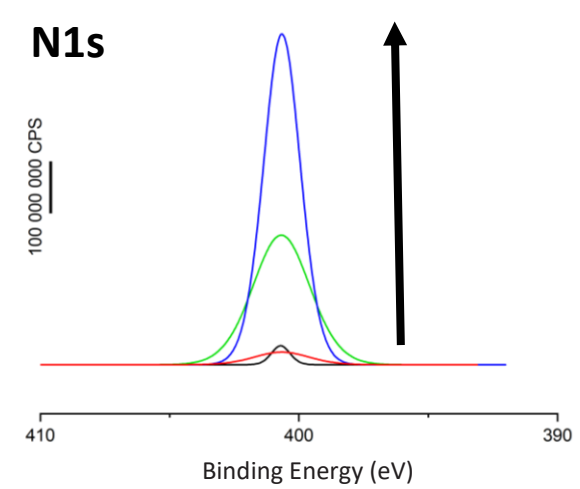
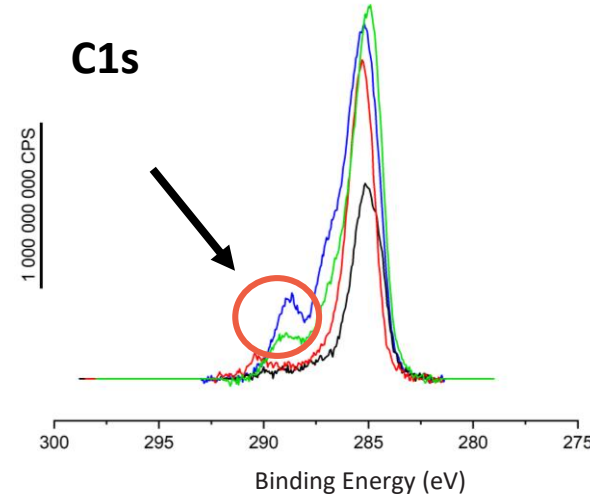
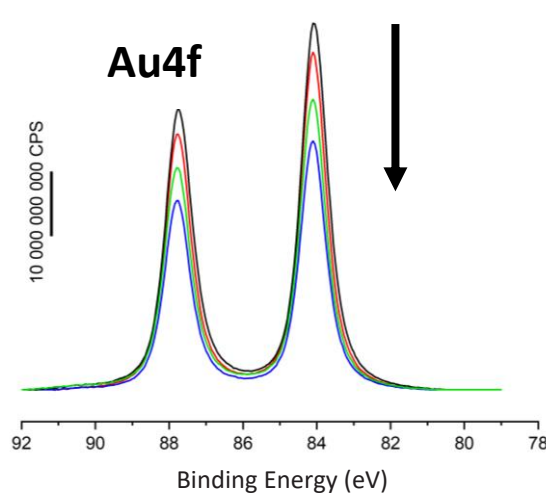
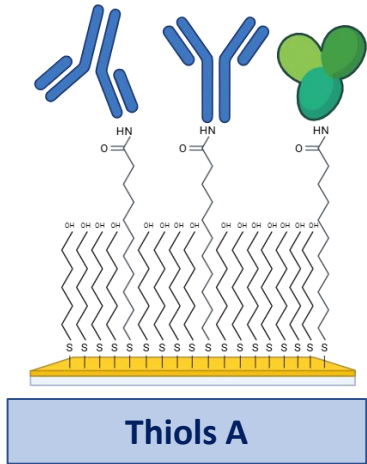


Thiols biotin Thiols B

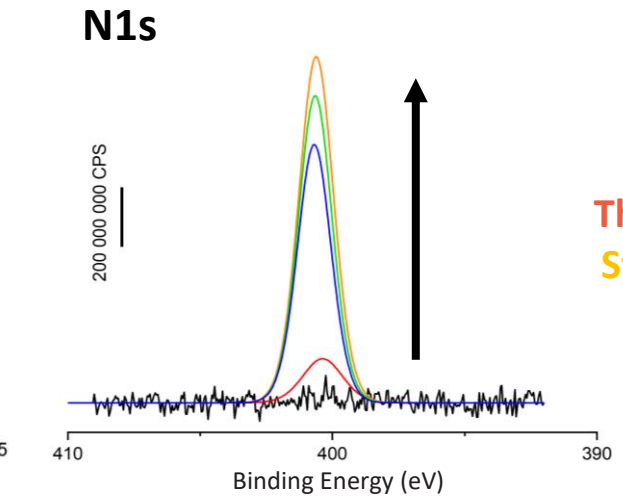
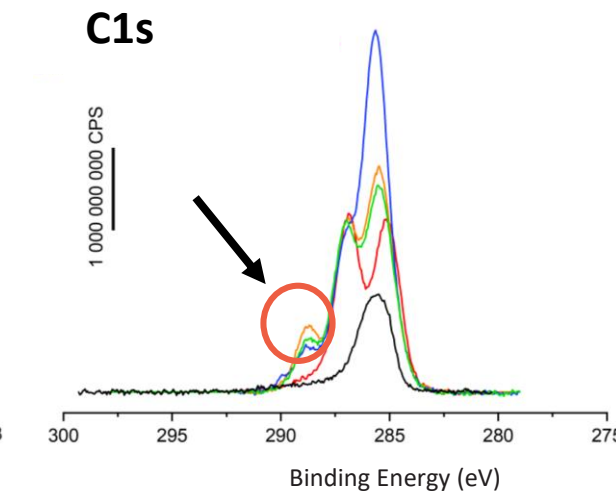
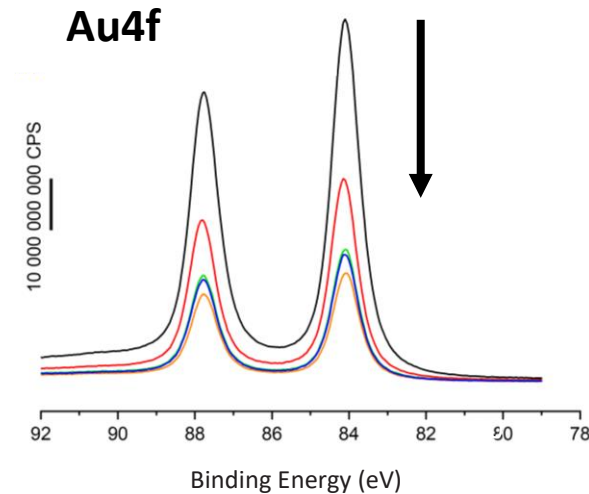
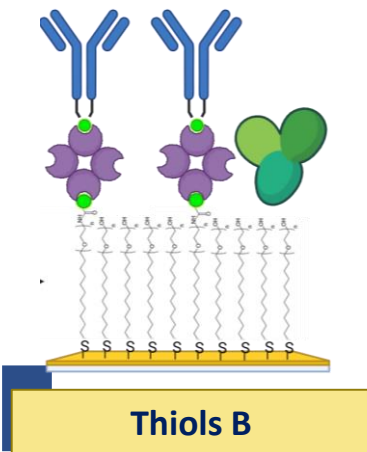


DEVELOPMENT OF BIONTERFACE

Surfaces functionalization – XPS characterization



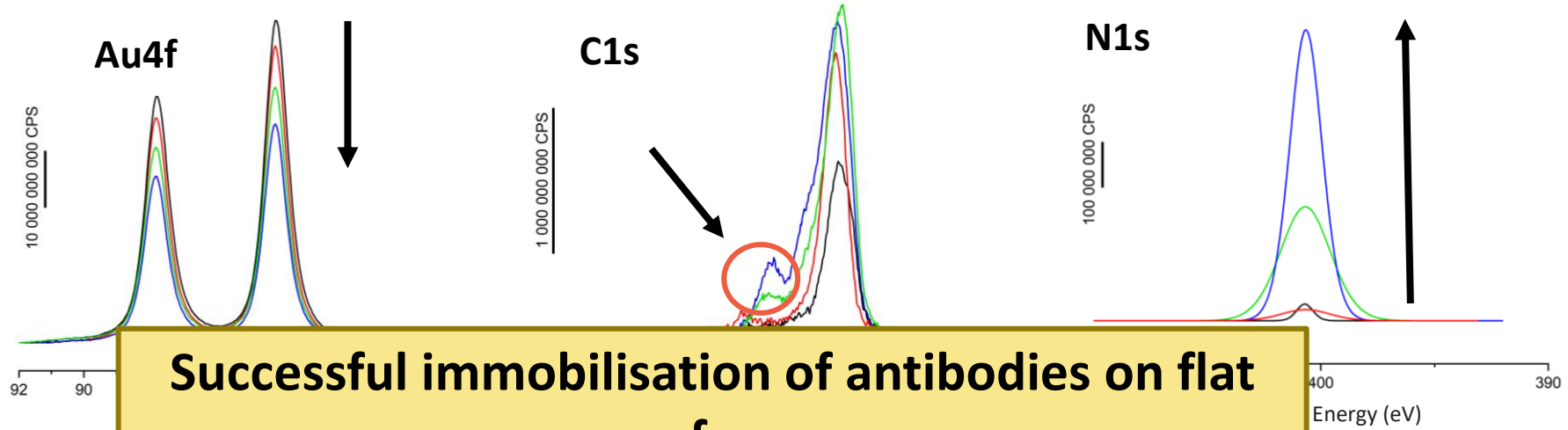
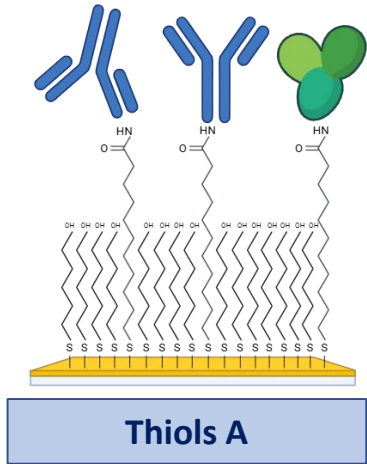
Gold
Thiols-COOH
Antibodies
BSA



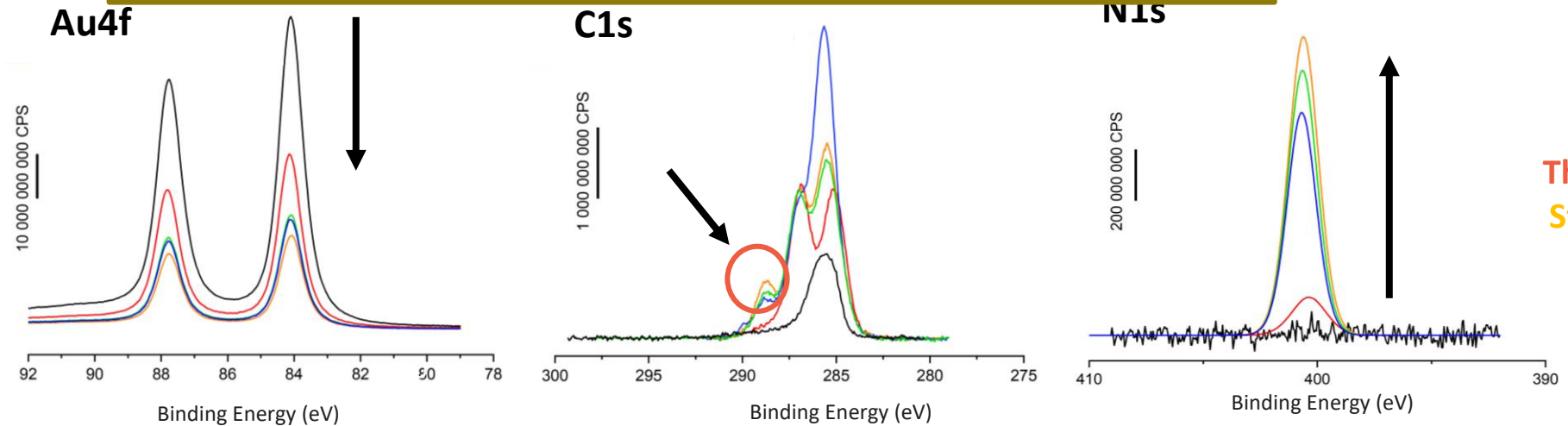
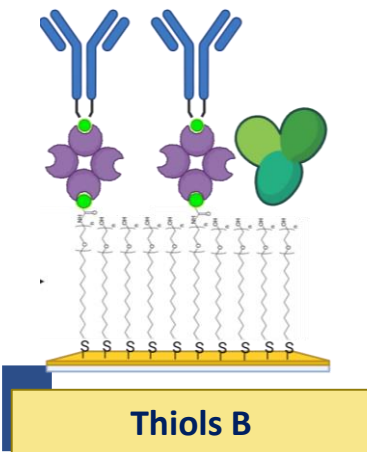
Gold
Thiols-biotin
Streptavidin
Antibodies
BSA

DEVELOPMENT OF BIONTERFACE

Surfaces functionalization – XPS characterization

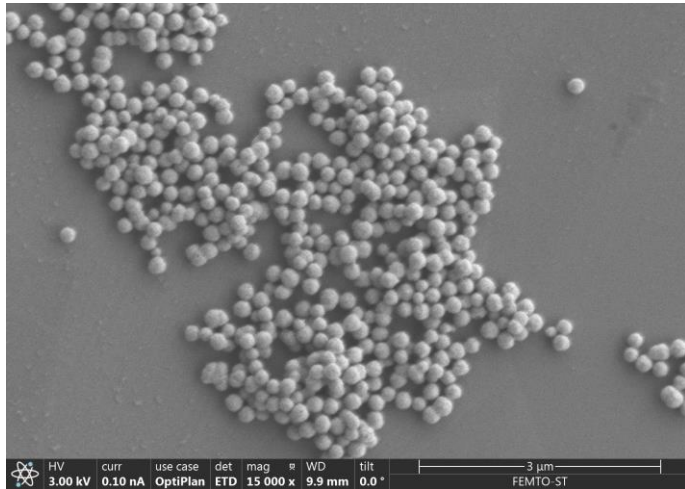


Successful immobilisation of antibodies on flat surfaces



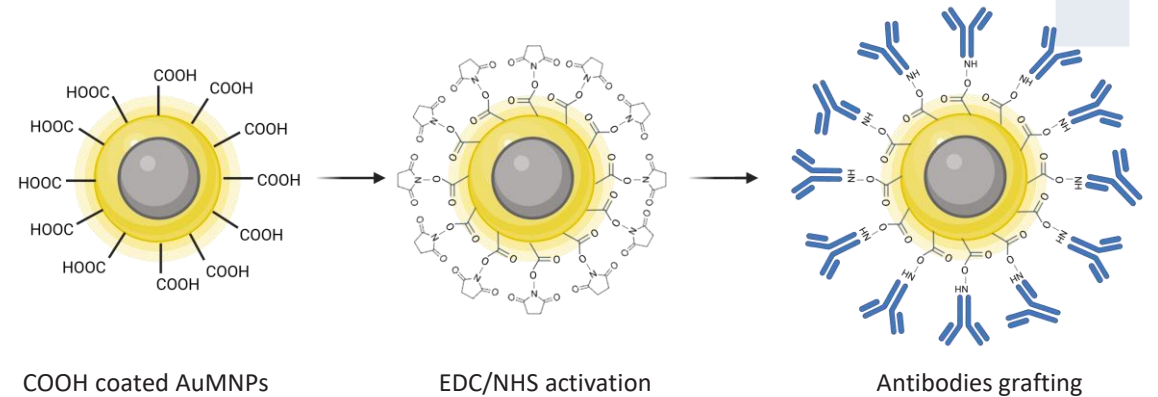
DEVELOPMENT OF BIONTERFACE

Surfaces functionalization – Nanoparticles



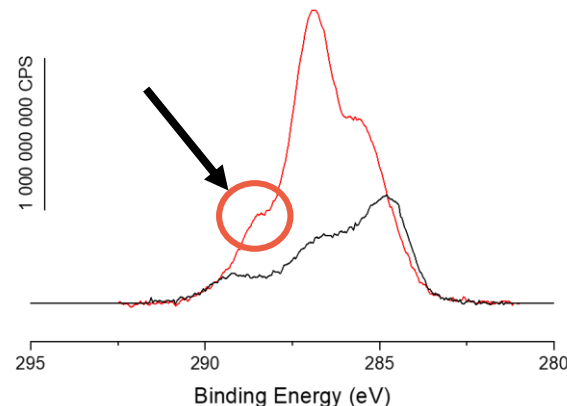
-COOH coated, spherical, homogeneous **gold magnetic 200nm nanoparticles** from Nanocomposix® were functionalized with monoclonal anti-*Listeria monocytogenes* P6007 antibody

Thiols A-like strategy



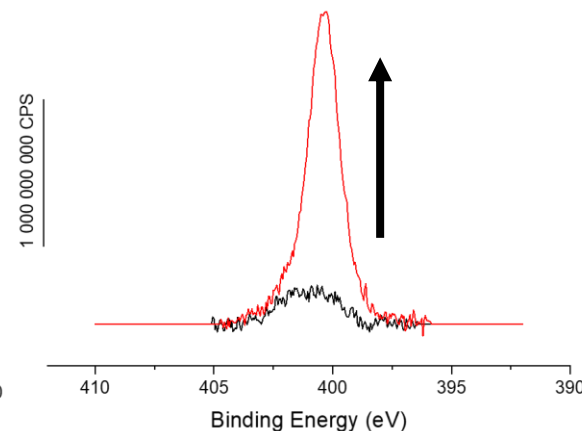
C1s

AuMNPs - COOH
Anti-*L. monocytogenes* monoclonal P6007 antibodies



N1s

EDC-NHS activation
Anti-*L. monocytogenes* monoclonal P6007 antibodies



Successful immobilisation of antibodies on nanoparticles

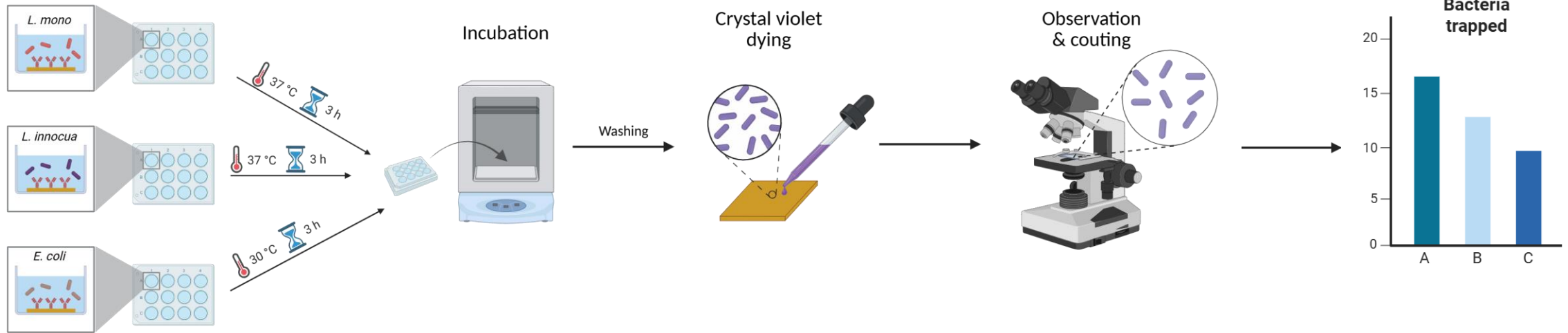
BACTERIA CAPTURE

- Static conditions
- Dynamic conditions



BACTERIA CAPTURE

Flat gold surfaces – Static conditions (proof of concept)



Choice of bacteria :

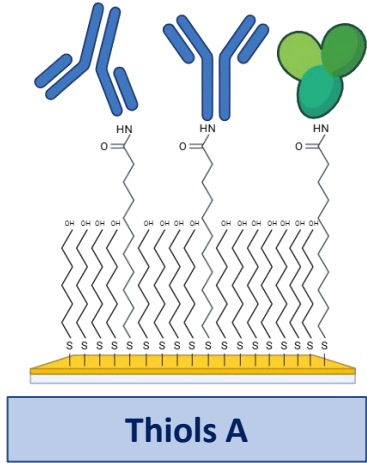
- *L. monocytogenes* : target bacterium
- *L. innocua* : non-target, species control bacterium
- *E. coli* : non-target, other species control

Inoculum : 10^7 CFU/mL suspension, in PBS

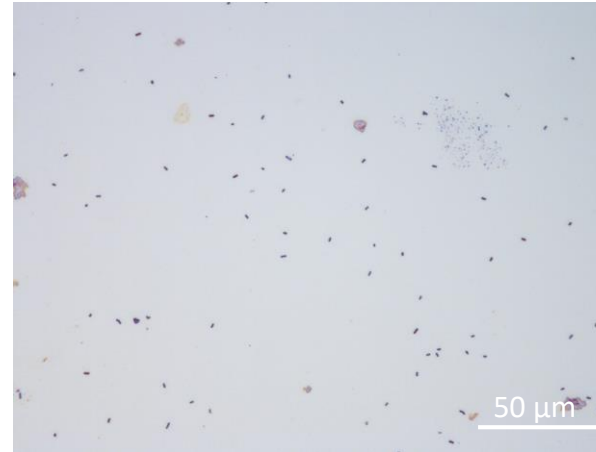
Number of samples : 2 surfaces, 2 independant tests

BACTERIA CAPTURE

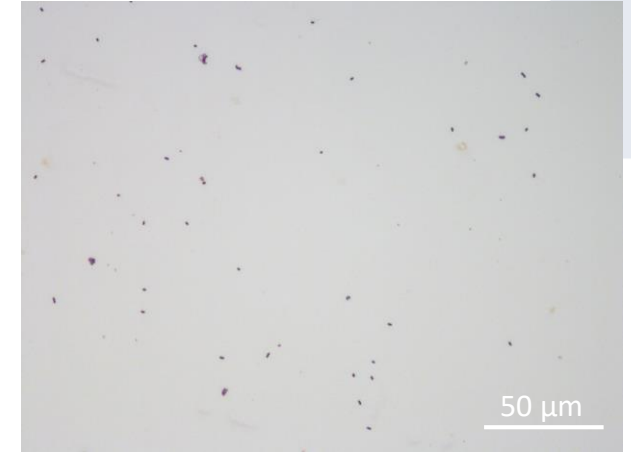
Flat gold surfaces – Static conditions (proof of concept)



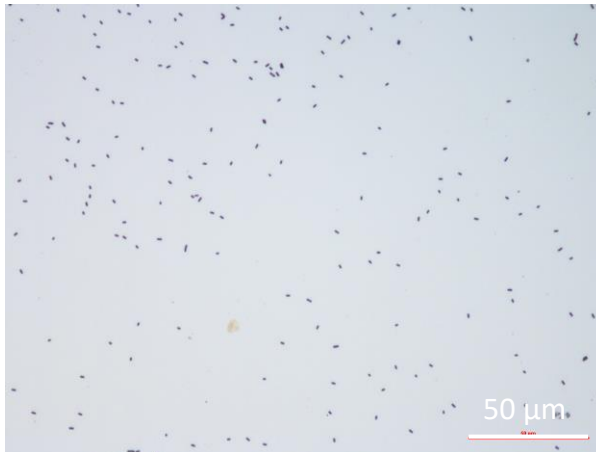
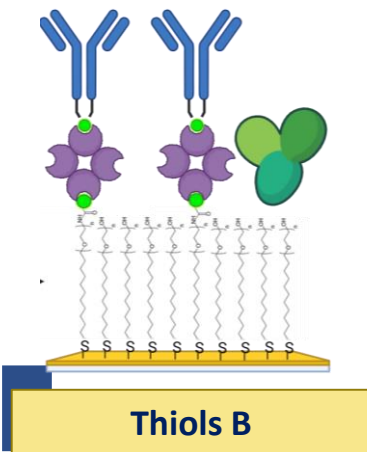
L. monocytogenes



L. innocua

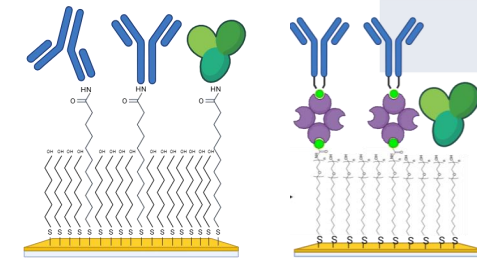


E. coli

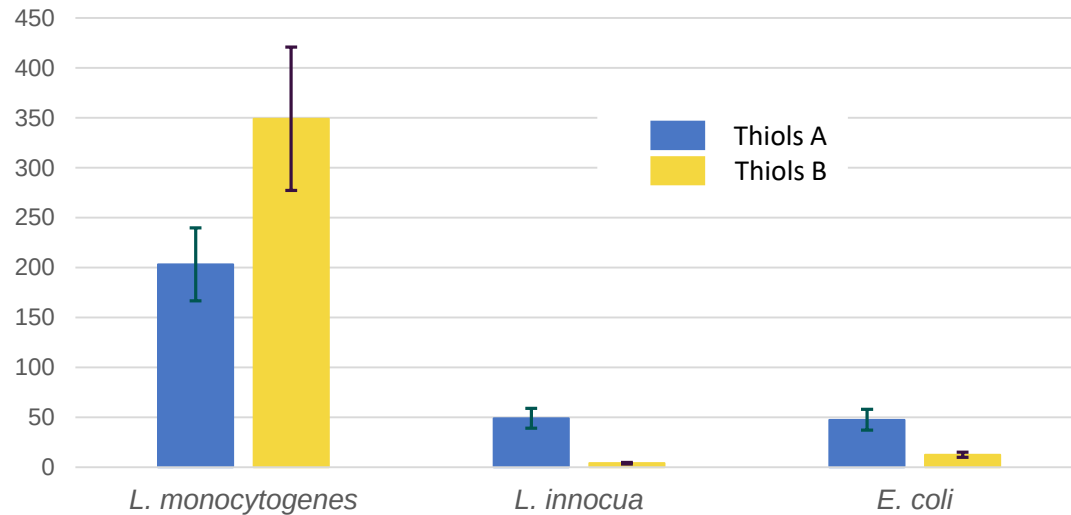


BACTERIA CAPTURE

Flat gold surfaces – Static conditions (proof of concept)



Number of bacteria captured on surface per unit of surface (a.u.)



Relative specificity of <i>L. monocytogenes</i>	Thiols MUA/MCH	Thiols biotin
<i>L. innocua</i>	76.0%	98.9%
<i>E. coli</i>	76.7%	96.5%

$$\text{Relative specificity (\%)} = \left(1 - \frac{N_{\text{control}}}{N_{\text{target}}}\right) \times 100$$

Significant differences of trapping between strains and depending on the SAM used

→ Specific capture of the target for both biointerface

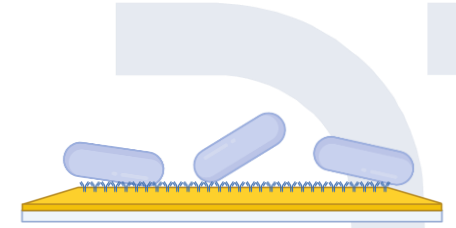
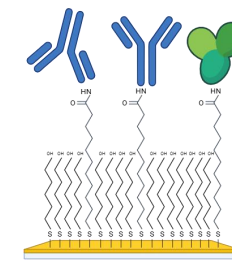
→ Thiols biotin SAM allows specific capture of target, decreasing non-specific capture of other bacteria

Specific capture of the target by both biointerface : thiols biotin SAM is the best for static capture

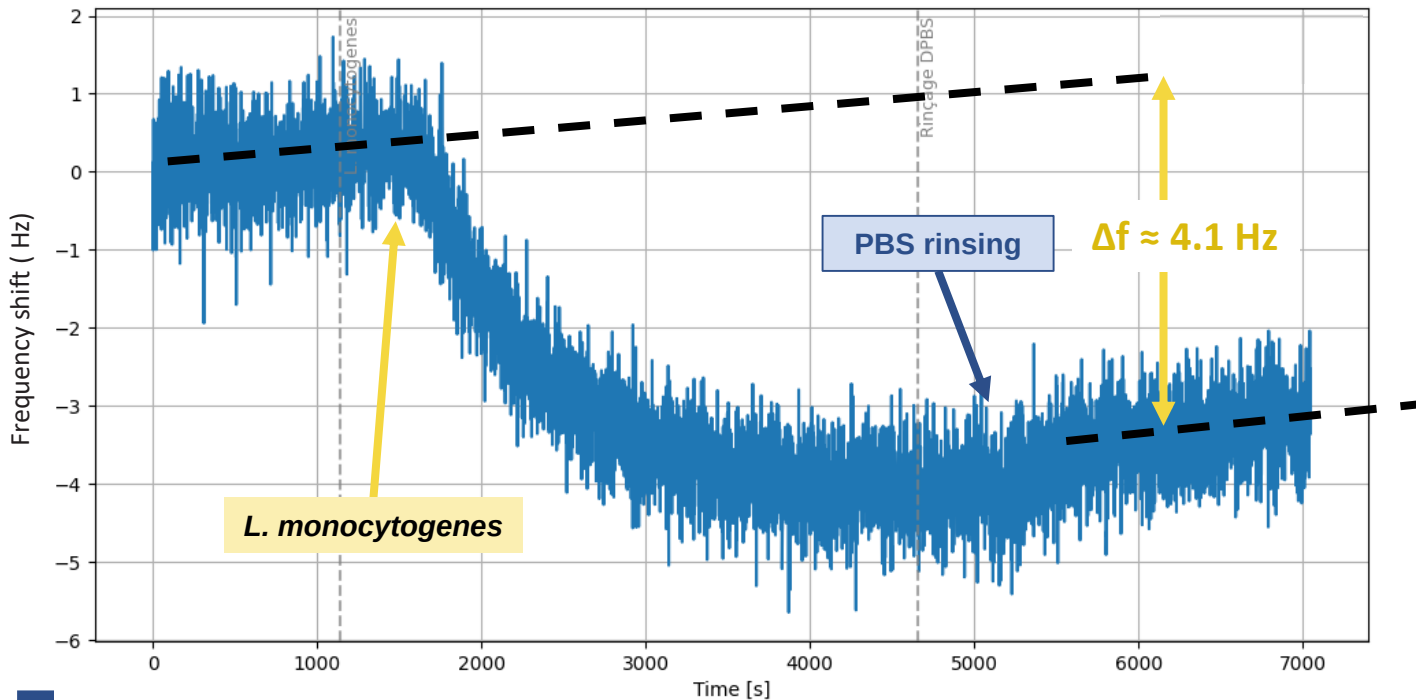
BACTERIA CAPTURE

Flat gold surfaces – Dynamic conditions, target bacterium

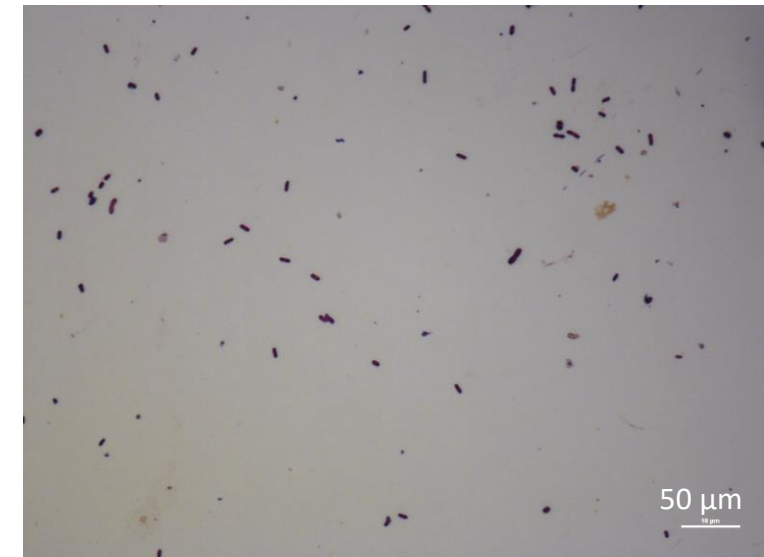
Dynamic capture of *L. monocytogenes* (10^7 CFU/mL) on 10 MHz functionalized quartz with thiols MUA/MCH SAM, measure of frequency variation with QCM device.



Thiols A



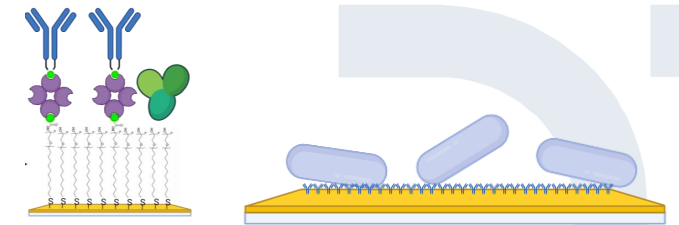
$$\Delta f_{\text{mean (3 captures)}} \approx 4.1 \pm 0.12 \text{ Hz}$$



Capture of the target in dynamic conditions on thiols A

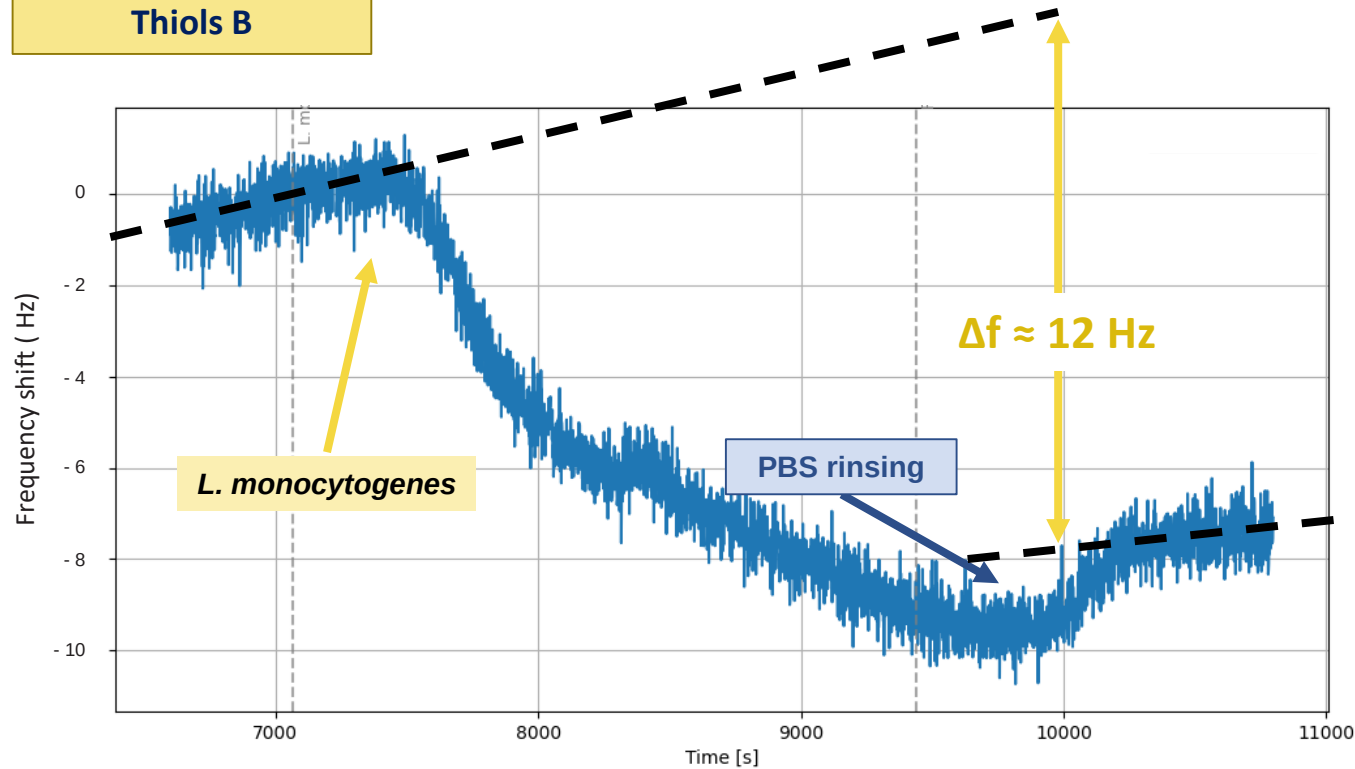
BACTERIA CAPTURE

Flat gold surfaces – Dynamic conditions, target bacterium



Dynamic capture of *L. monocytogenes* (10^7 CFU/mL) on 10 MHz functionalized quartz with thiols biotin SAM, measure of frequency variation with QCM device.

Thiols B



$$\Delta f_{\text{mean (3 captures)}} \approx 7.6 \pm 1.63 \text{ Hz}$$

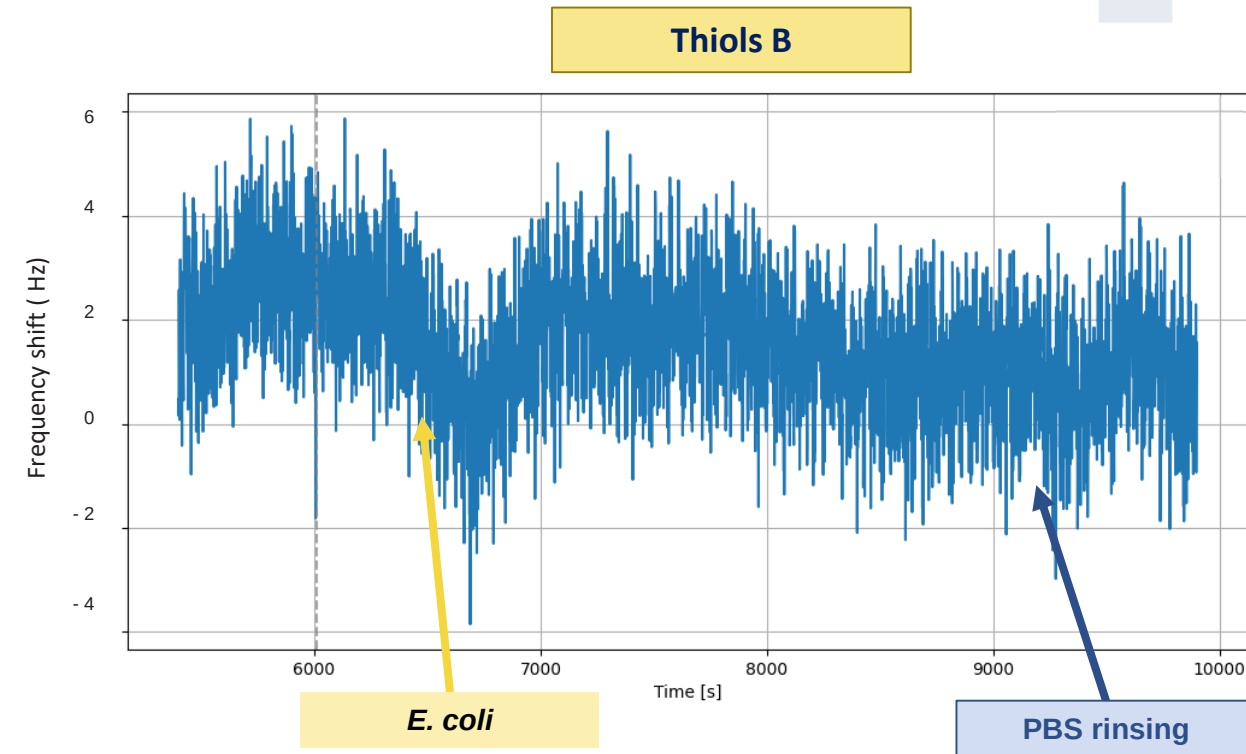
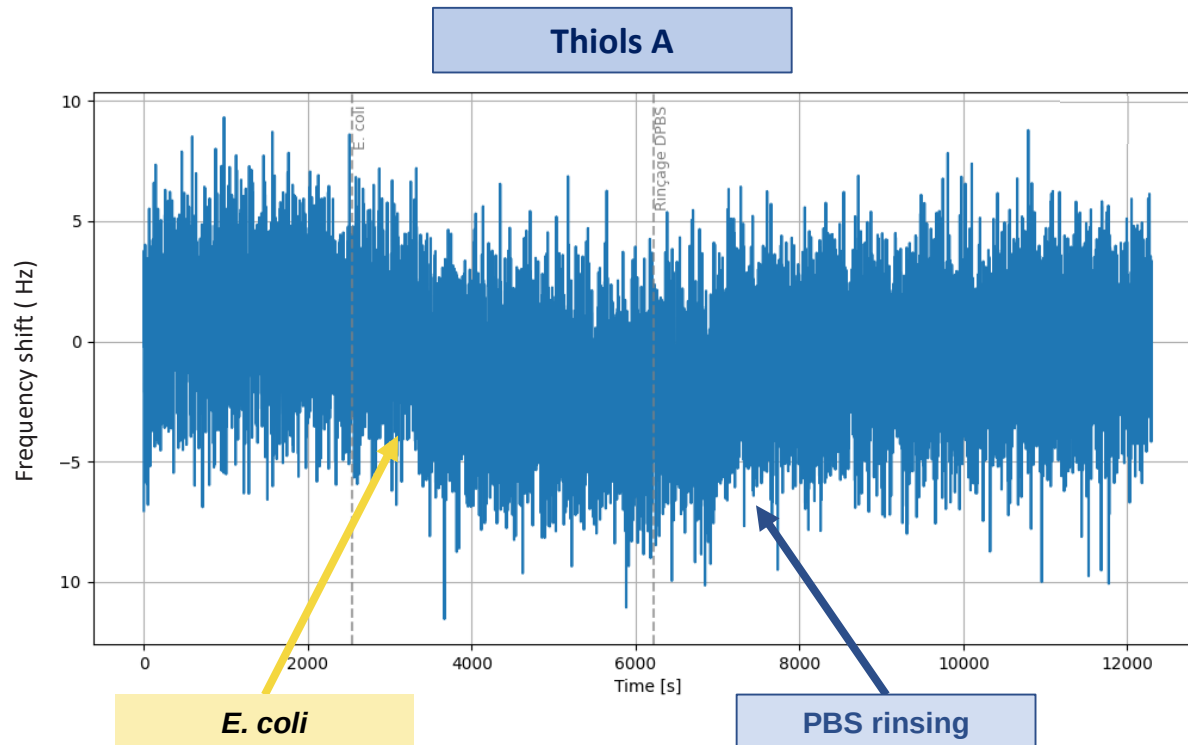
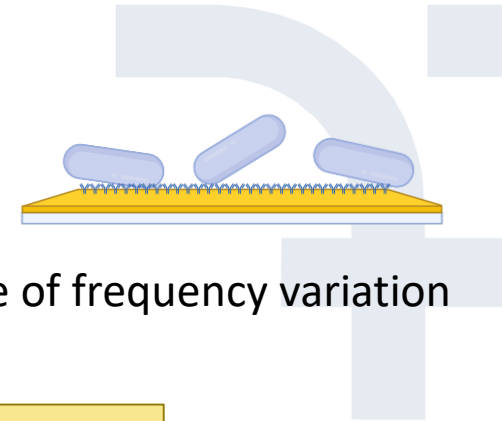


Capture of the target in dynamic conditions on thiols B

BACTERIA CAPTURE

Flat gold surfaces – Dynamic conditions, negative control (specificity)

Dynamic capture of *E. coli* (10^7 CFU/mL) on 10 MHz functionalized quartz with both SAM, measure of frequency variation with QSENSE device.



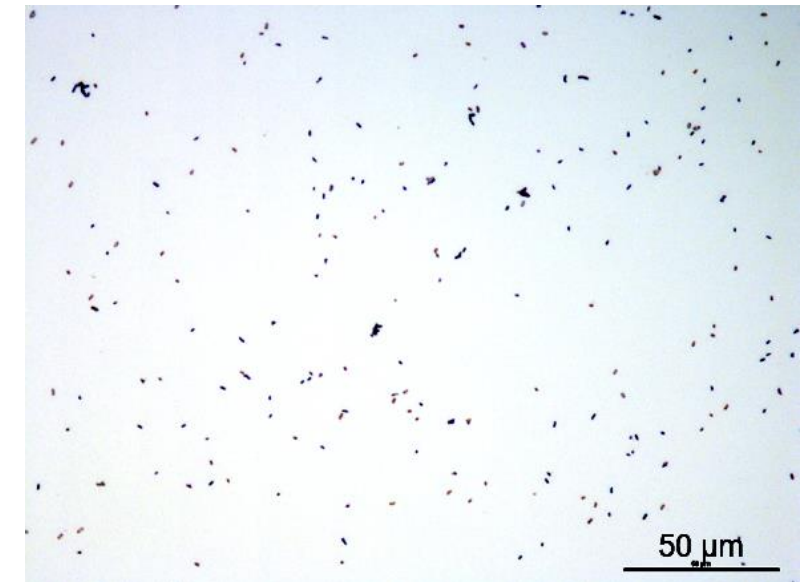
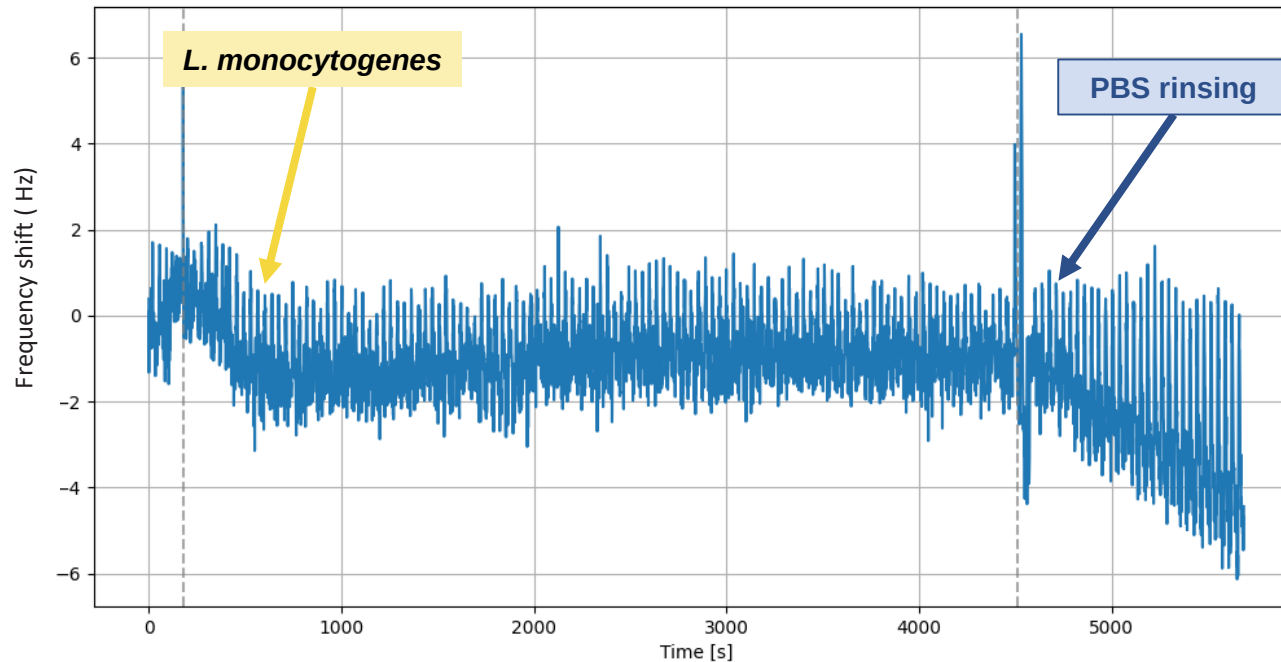
Specificity of biointerfaces in dynamic conditions : no measured signal for negative control

BACTERIA CAPTURE

Flat gold surfaces – Dynamic conditions

Dynamic capture on 10 MHz functionalized quartz for *L. monocytogenes* shows a negative shift of frequency but for a **concentrated suspension of bacteria**.

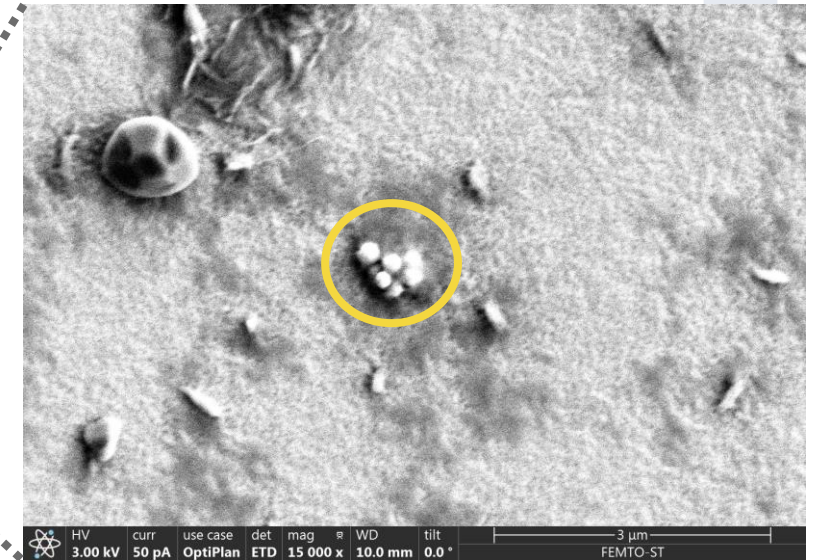
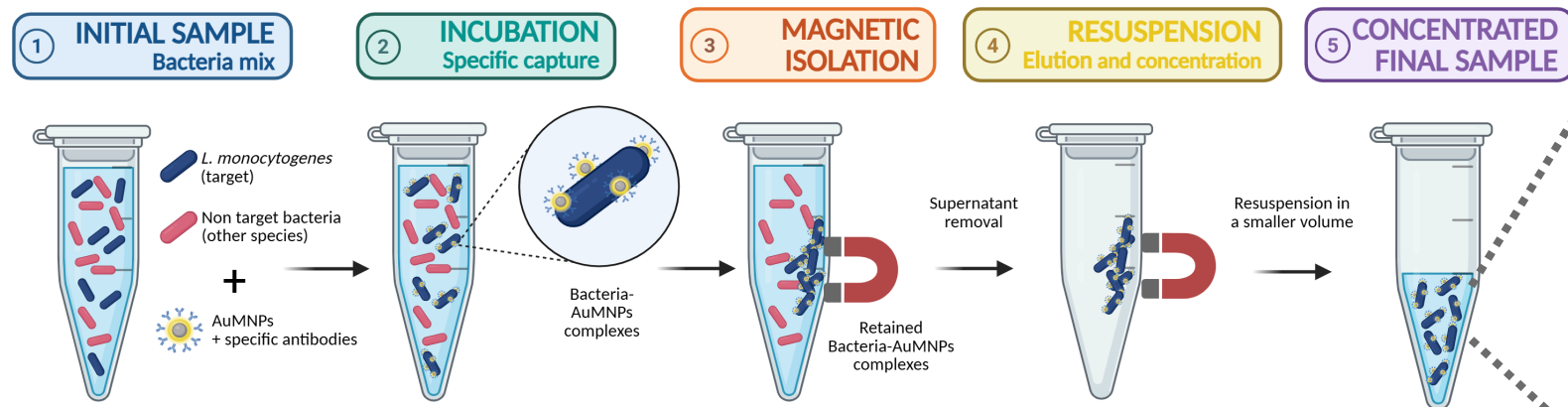
Real conditions : 10^4 CFU/mL bacteria suspension (after microbiological enrichment step)



Bacteria capture at low concentration is not detected by QCM.

COMPLEXES FORMATION

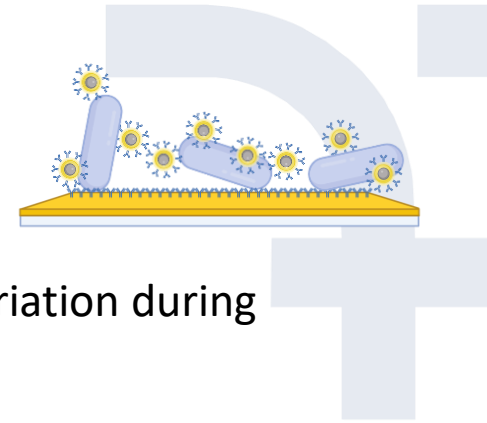
Bacteria capture by nanoparticles



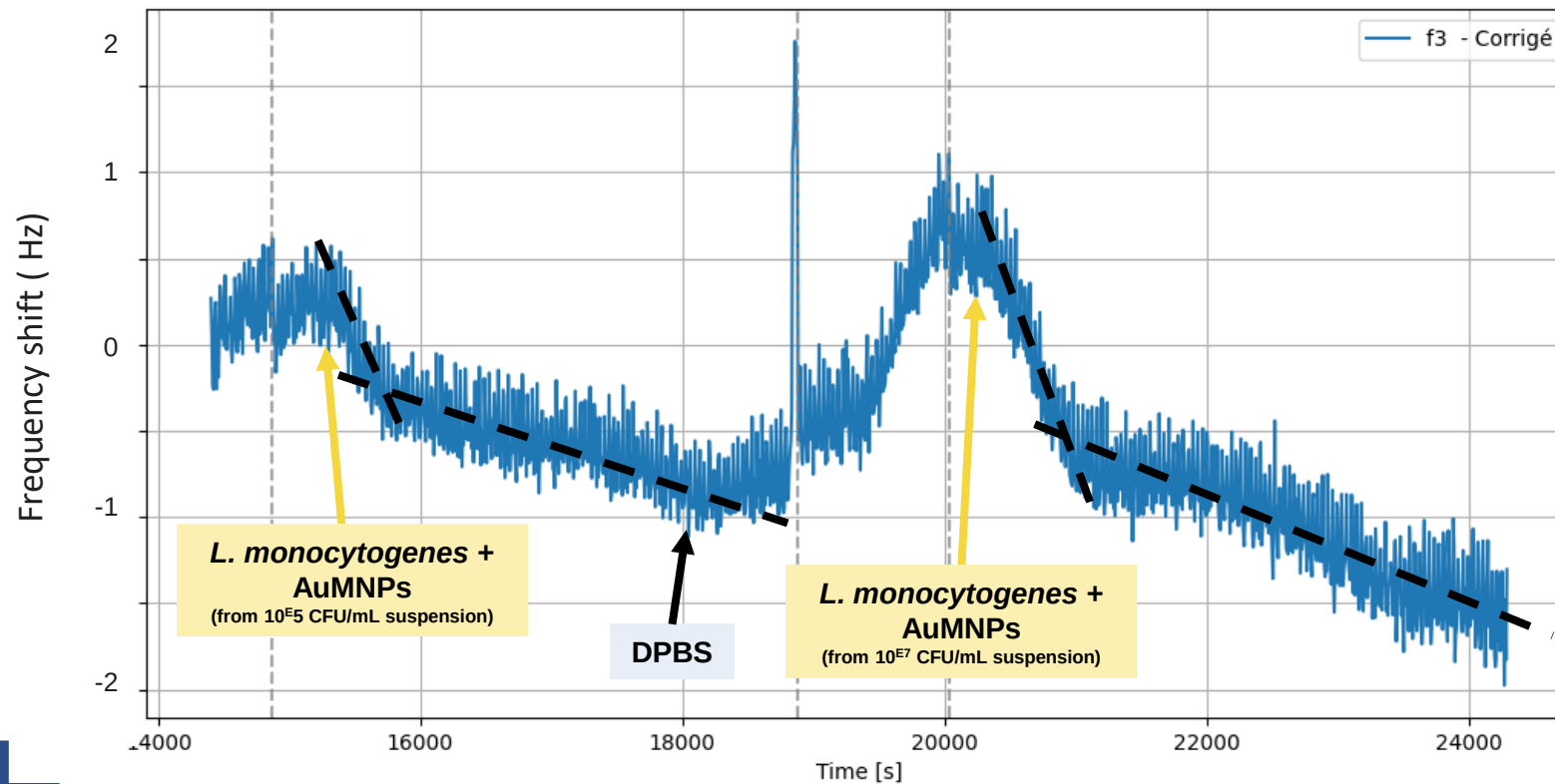
SEM observation of complexes

COMPLEXES CAPTURE

Bacteria capture by nanoparticles and detection with QCM



Dynamic capture on 10 MHz functionalized quartz with thiol-biotin SAM, measure of frequency variation during complexes sample injection.



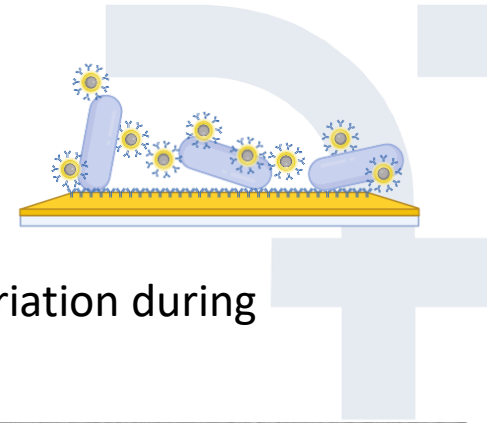
Change in slope at the entrance of the complexes in the chamber
→ **Effect of complexes in signal**

Rise in the signal at the moment of rinsing

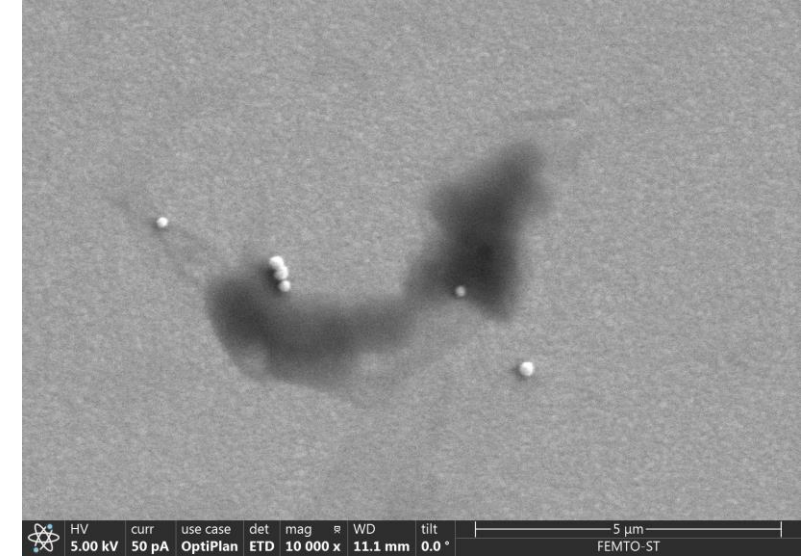
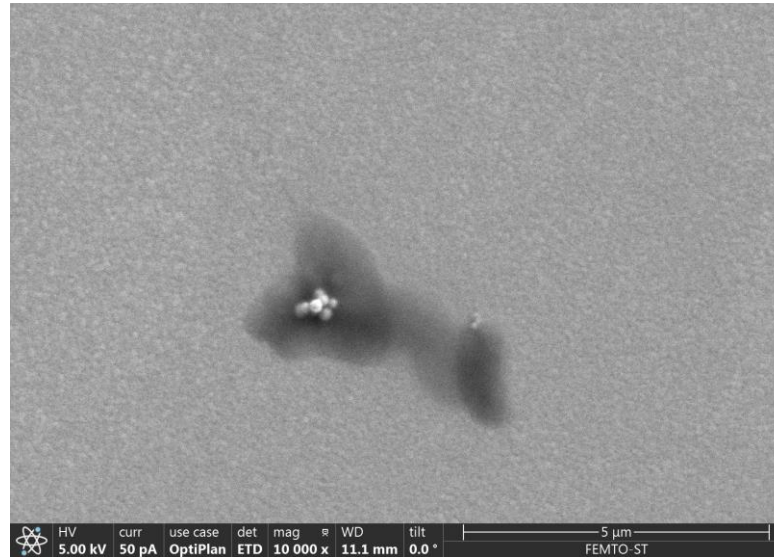
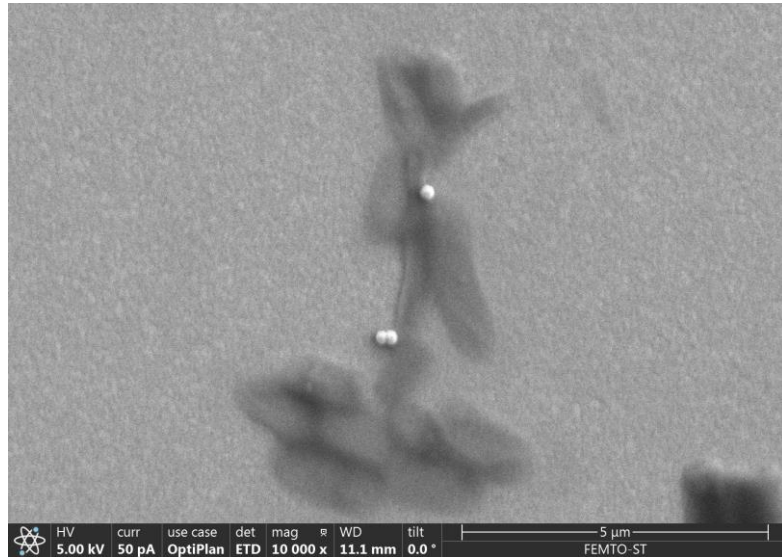
Big frequency shift expected with bigger mass captured

COMPLEXES CAPTURE

Bacteria capture by nanoparticles and detection with QCM



Dynamic capture on 10 MHz functionalized quartz with thiol-biotin SAM, measure of frequency variation during complexes sample injection.



SEM observations

Presence of captured complexes on the quartz
Groups of bacteria + AuMNPs

Thank you for your attention.

AKNOWLEDGEMENTS:

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- French RENATECH Network and FEMTO-ST technological facilities (Mimento Cleanroom)

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