Institut des Nanotechnologies de Lyon UMR 5270

PMSE GDR 3430

ÉCOLE CENTRALE LYON

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Surface functionalization for plasmonic biosensors

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Outline

- From transduction to biosensing…

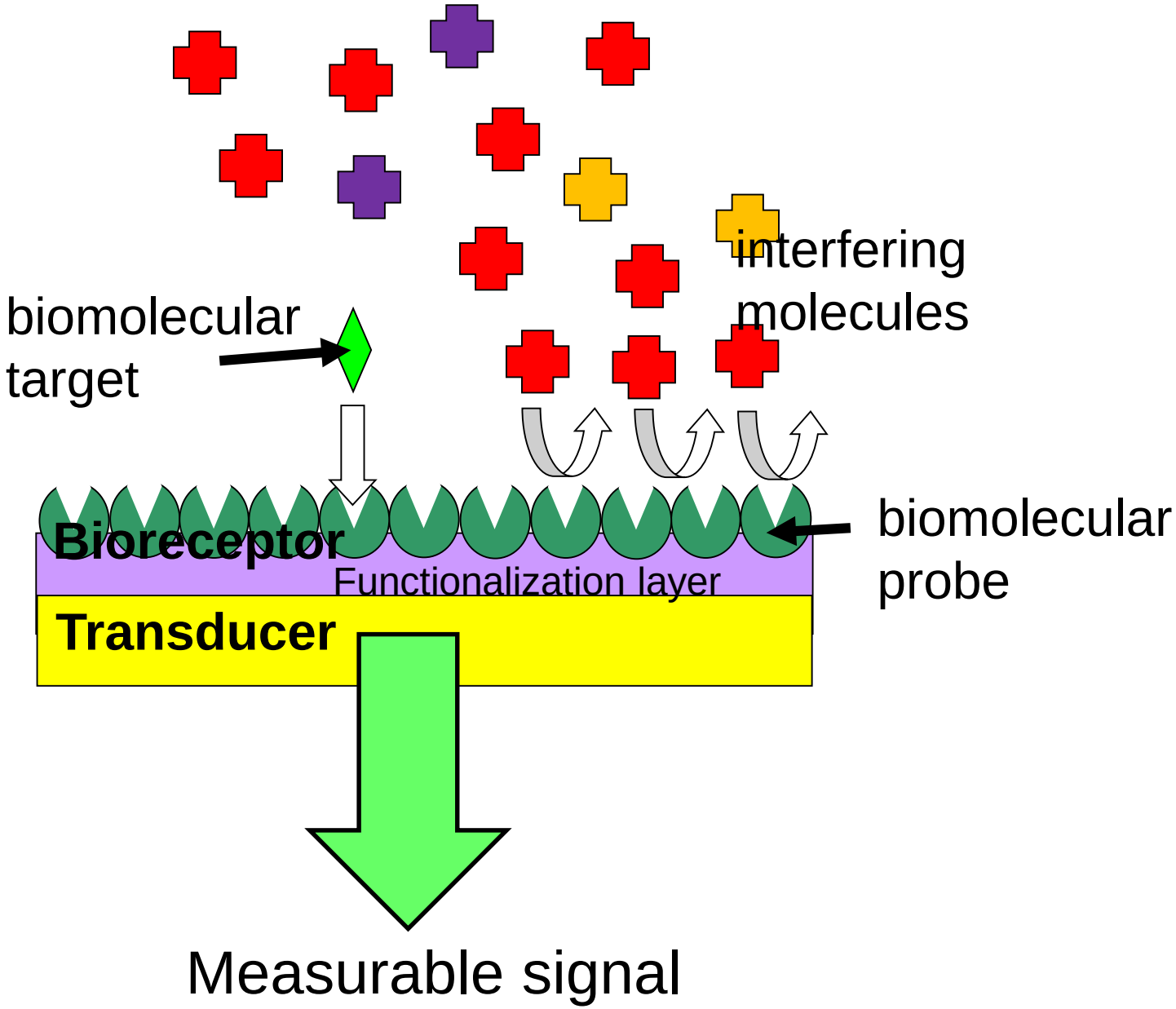
- How surface functionalization influences your biosensor measurements

- Classical gold functionalization
  - Homogeneous thiolate SAMs : how it can disfunction. Gold cleaning, gold oxydation, metal defects, …
  - Mixed SAMs

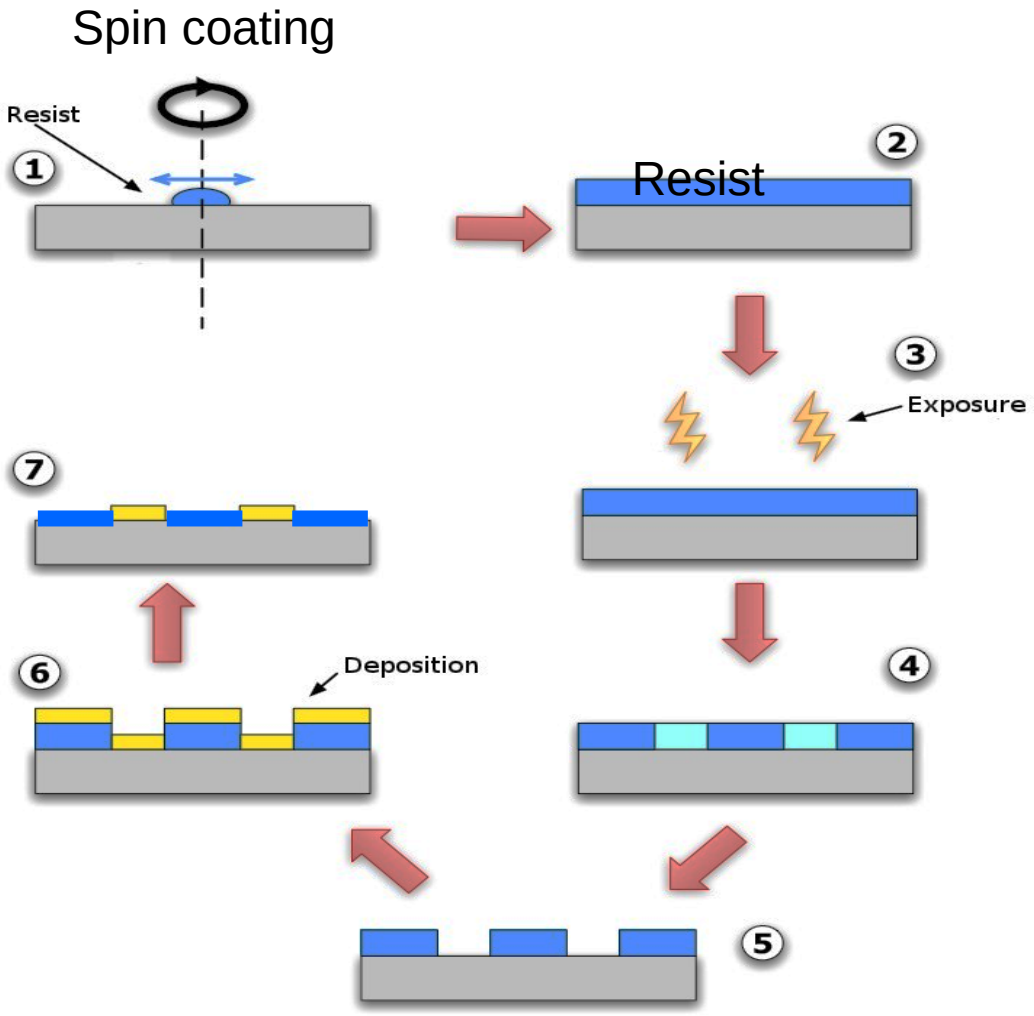
- Selective functionalization of multiple materials

- Conclusion

Principle of an affinity biosensor



Implementing patterned transducing zones



Example: lithography

Resist residues ?

Porosity of implemented layers ?

Other contaminations ?

Image adapted from A. Duval, PhD Thesis, 2009

Of surfaces and humans

- « God made the bulk,
  but surface was invented by the devil »
  Wolfgang Pauli

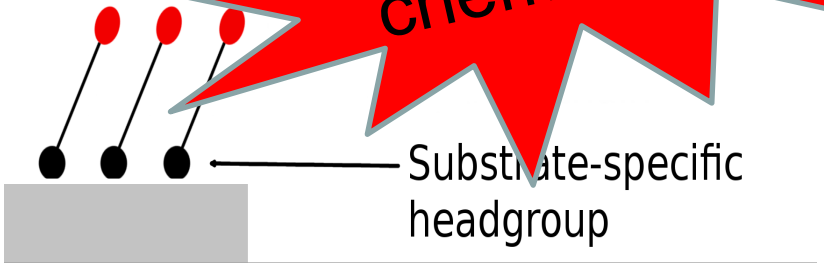
Surface chemistry 101

« powerpoint chemistry »

A substrate-binding headgroup

Sulfhydril (thiol)on gold : -SH

Silane on silica : -Si-$(X)_3$

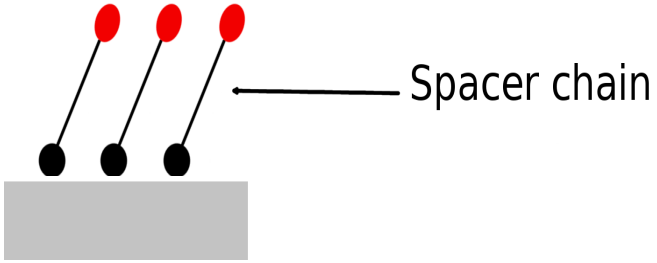


A spacer chain

Alkyl : -$(CH_2)_n$-

OEG : -$(CH_2$-CH_2-$O)_n$-

Perfluorinated : -$(CF_2)_n$–



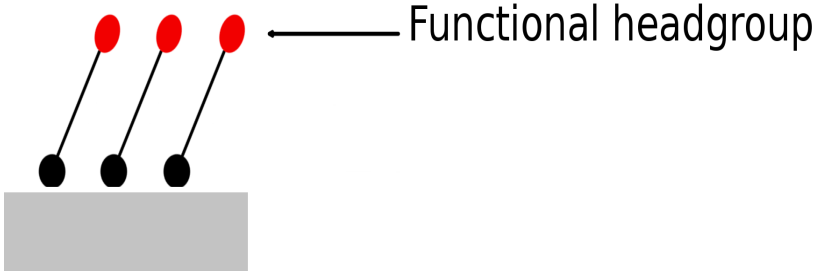
A functional headgroup

Target-binding on gold : -Biotin,

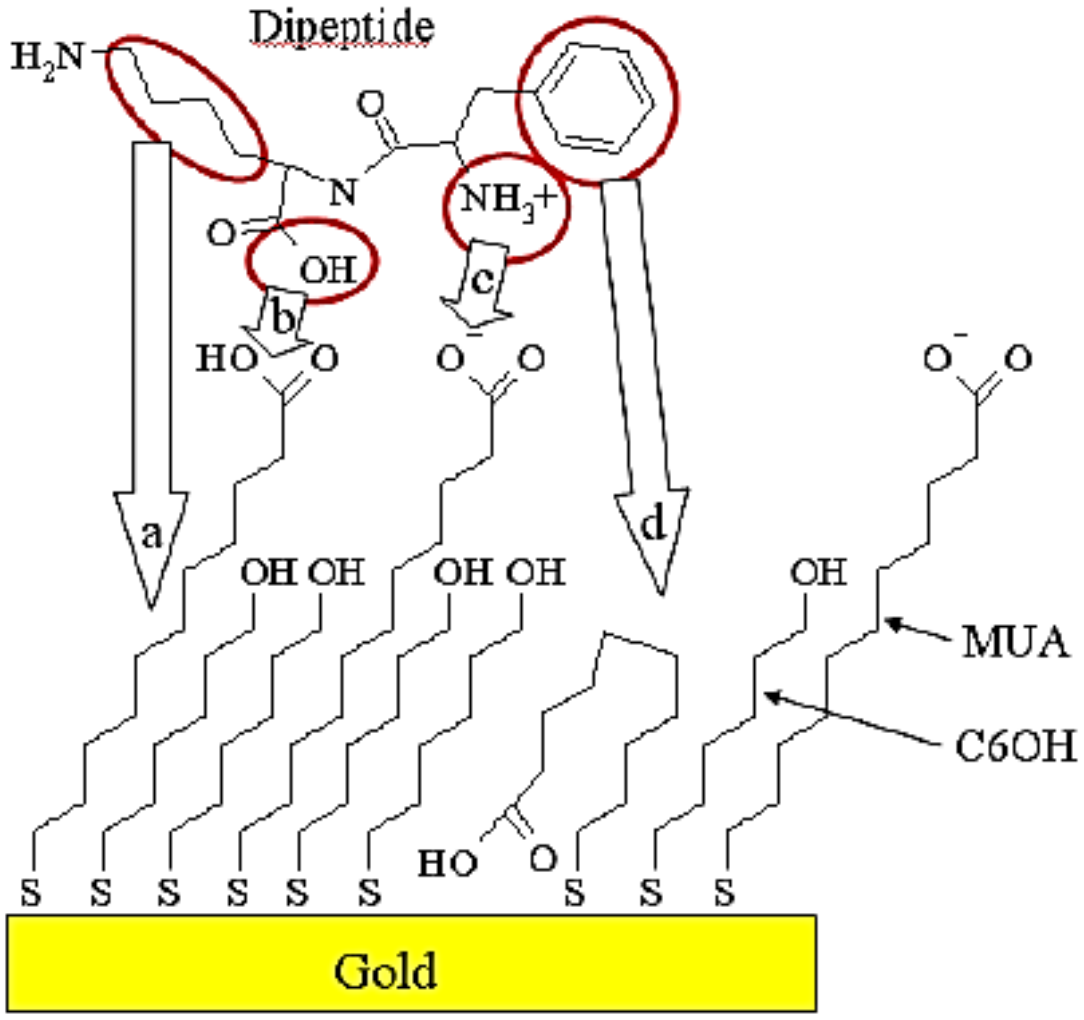
-COOH/NHS, -NH_2

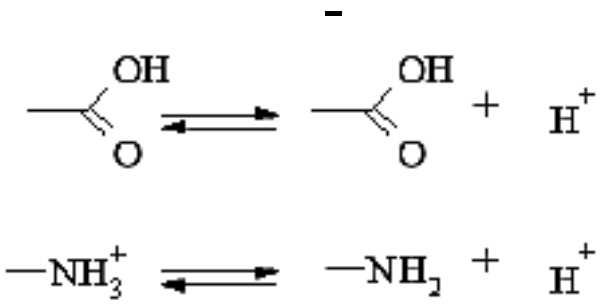
Anti-fouling on silica : -OCH_3

-CF_3



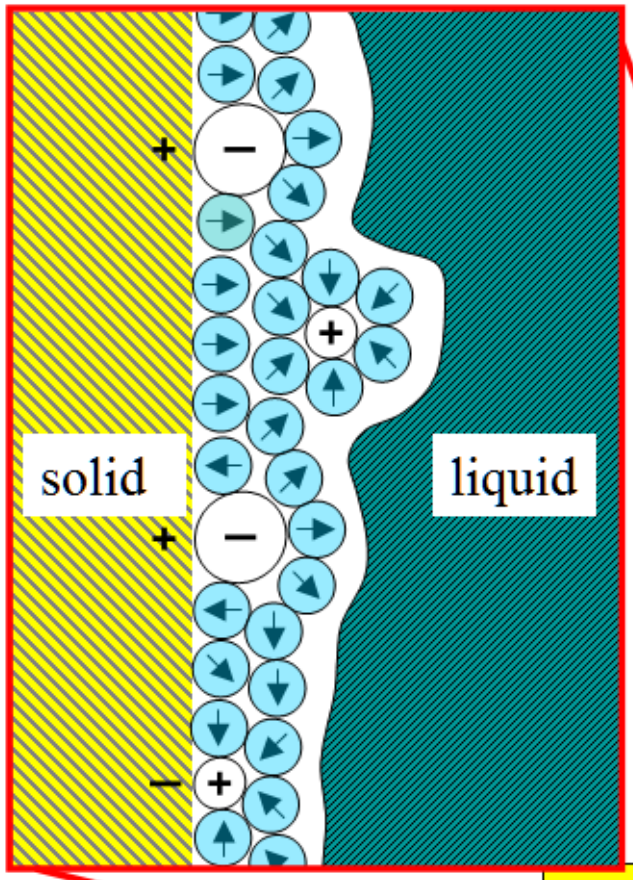
The trouble with surface chemistry : a diversity of bonds





Example of a simple dipeptide

Electrical / ionic organization of solid/liquid interface

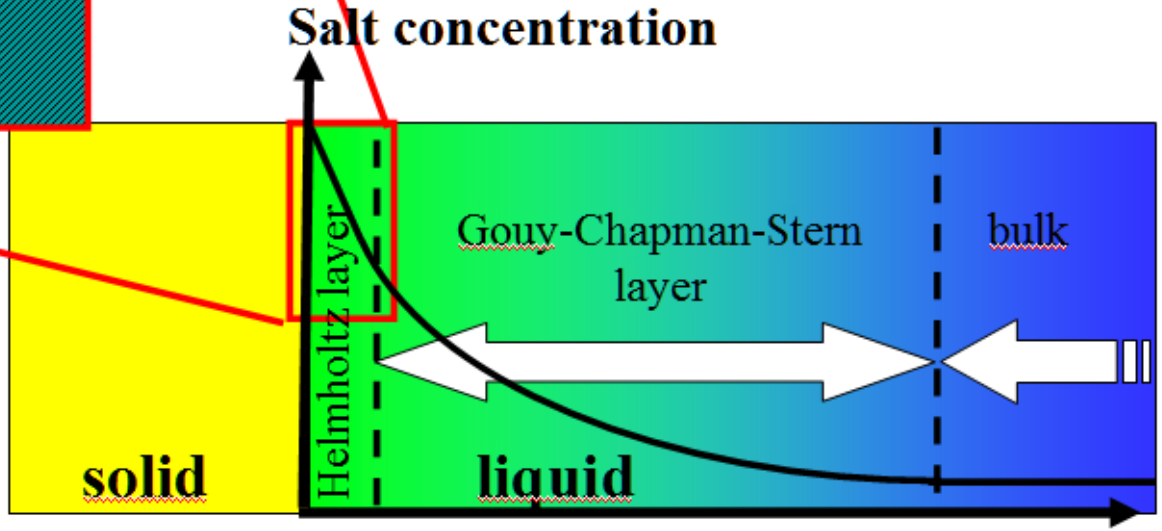


Water molecule (represented as a dipole)

Anion (typically Cl^-)

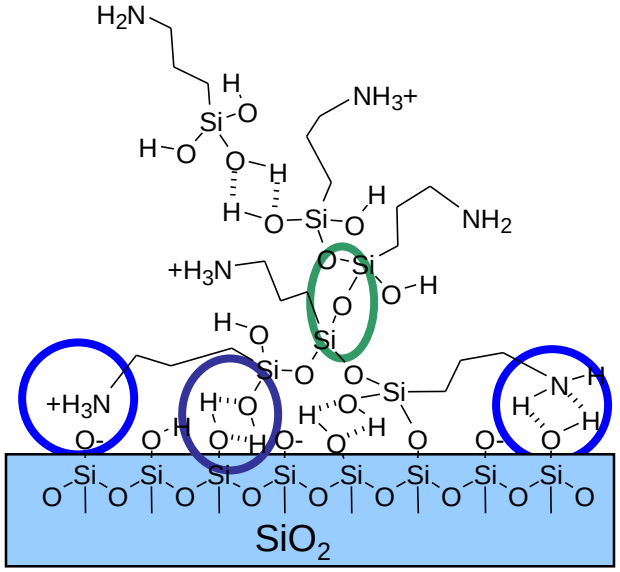
Cation (typically Na^+)

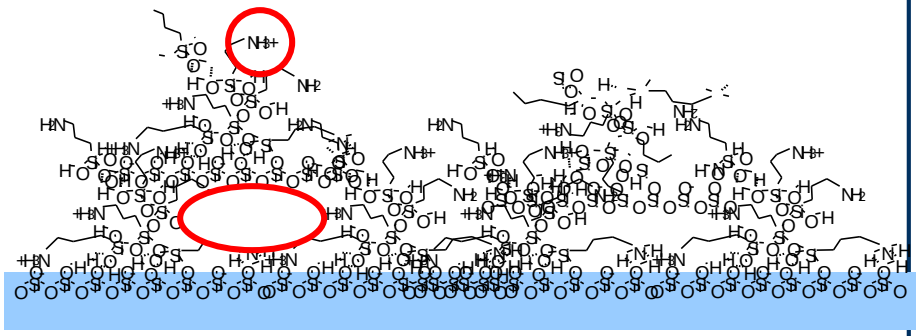
Electrical impurities or defects in solid



local pH on the surface may be different from the pH in bulk !

The trouble with surface chemistry…





- Molecular flip-flaps
- Physisorption
- Bulk polymerisation
- Heterogeneity
- Entrapments
- Non specific adsorption

G. Whitesides

A. Ulmann

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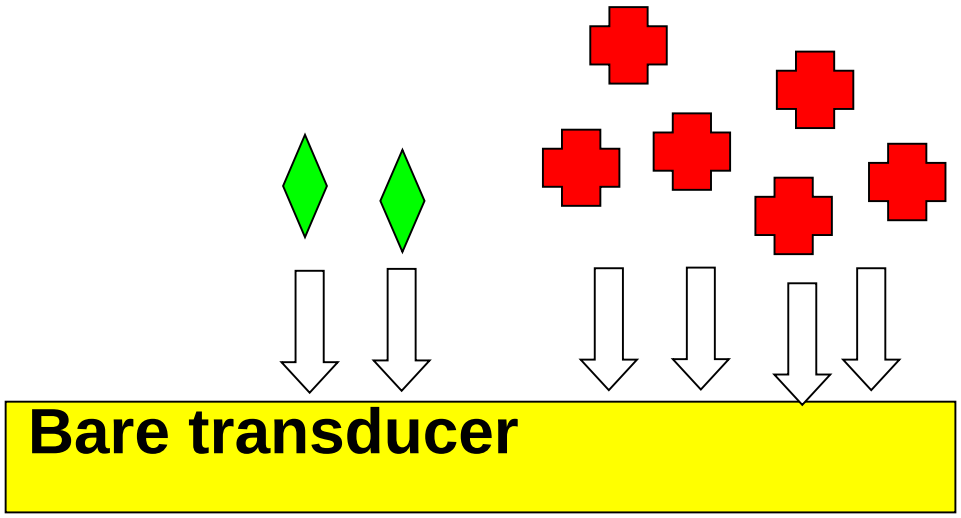
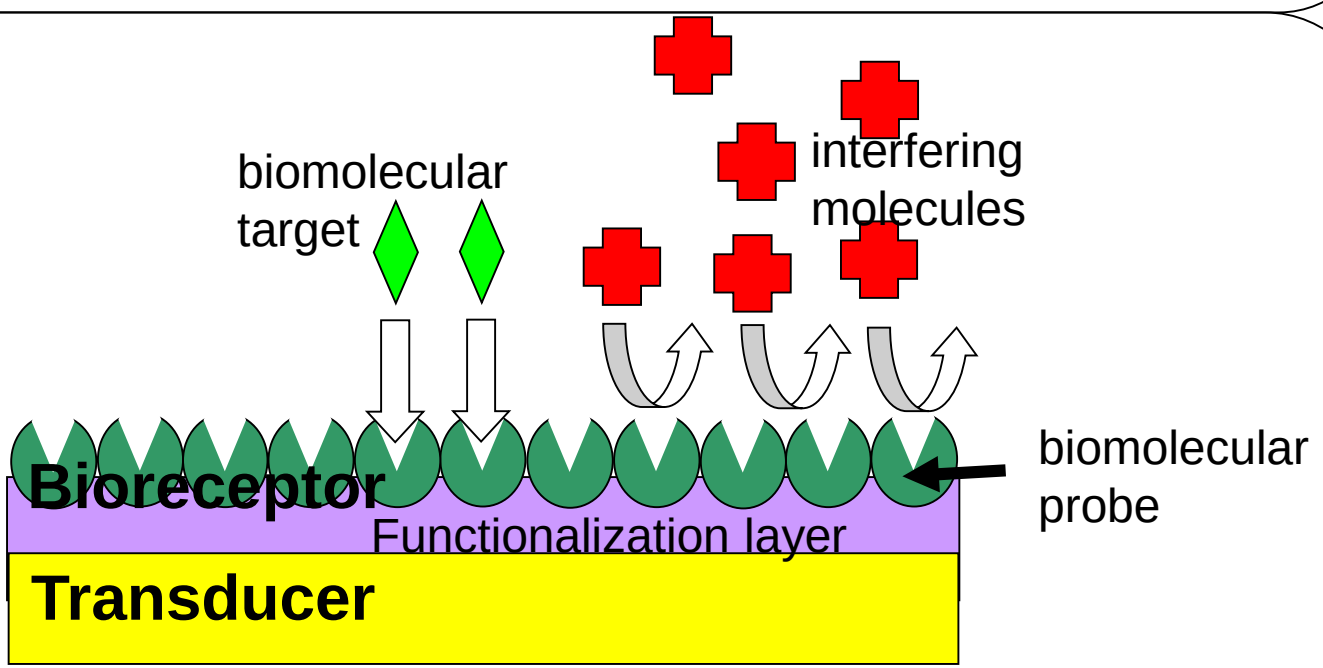
Influence of functionalization on biosensing efficiency

Worst case

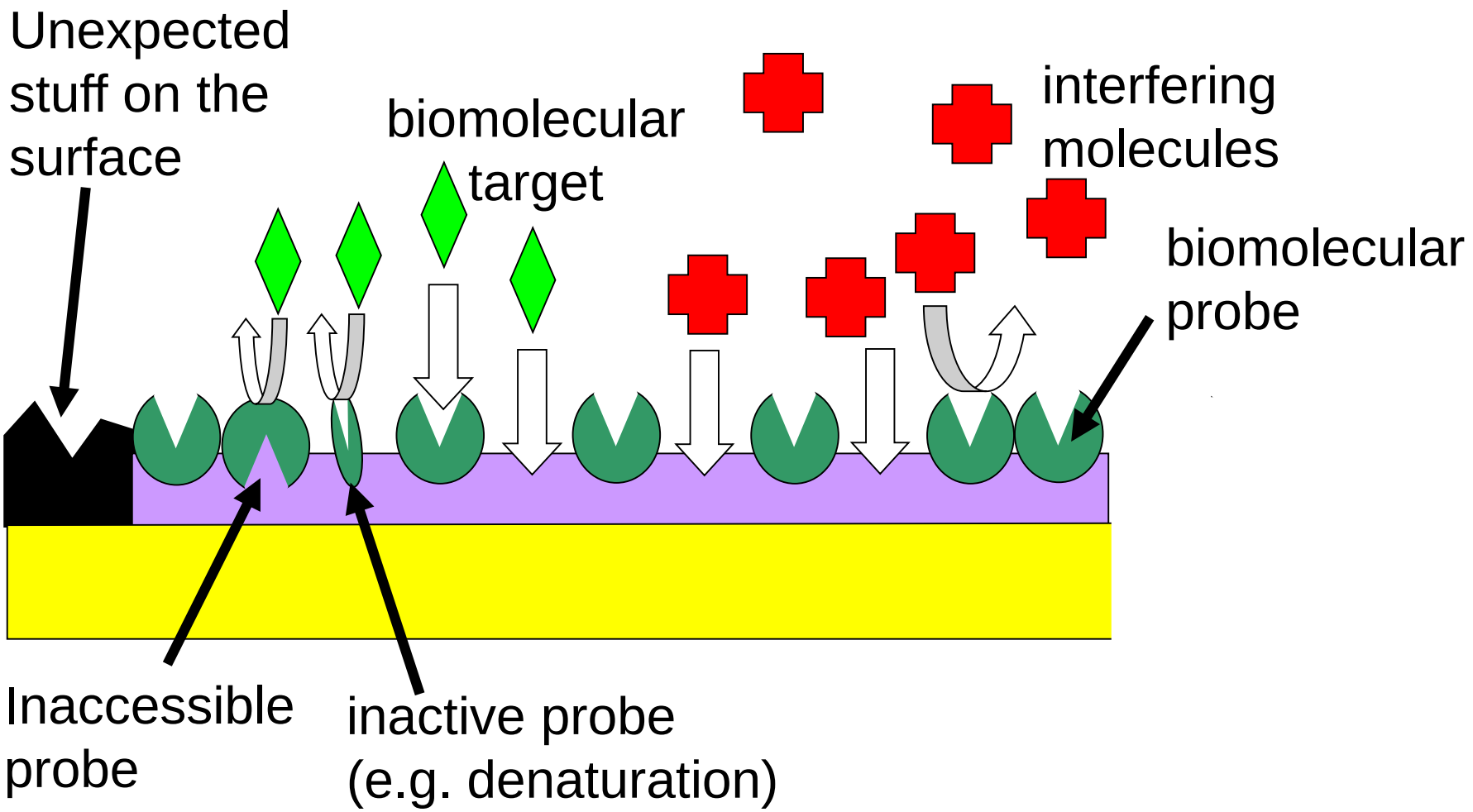


Best case



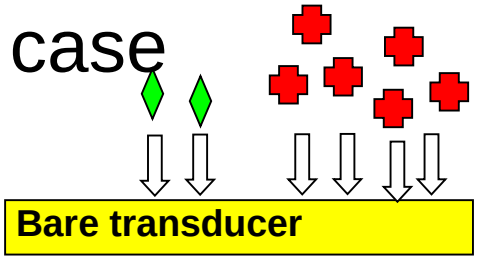
Influence of functionalisation on biosensing efficiency

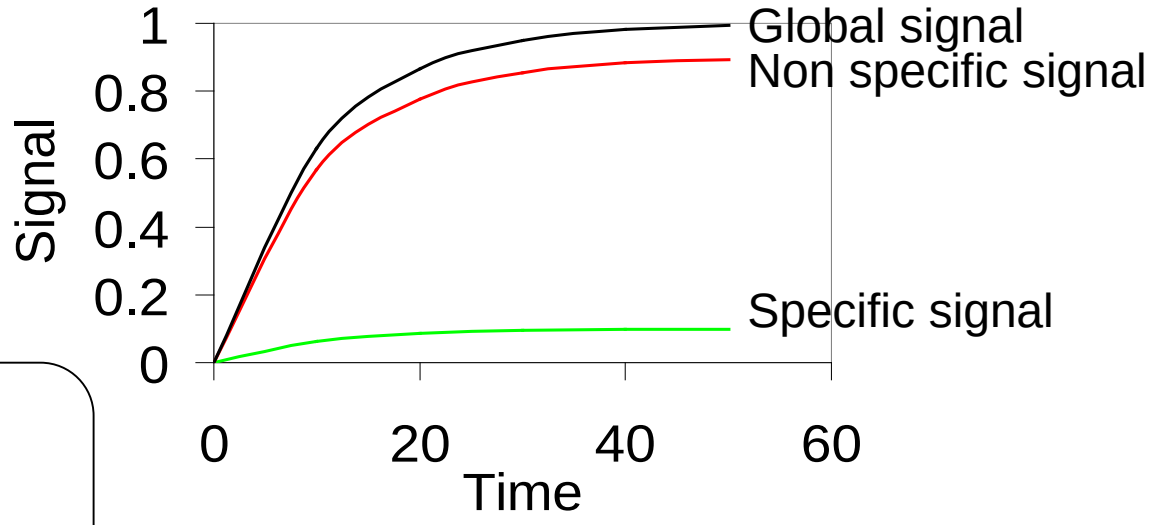
realistic case



Influence of functionalisation on biosensing efficiency

Worst case





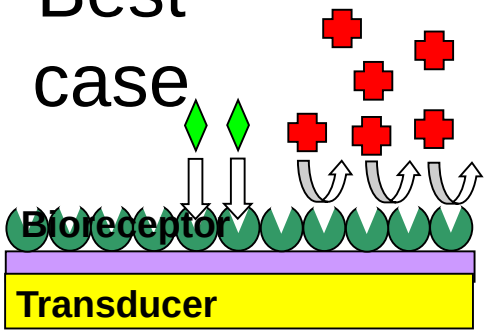
probe

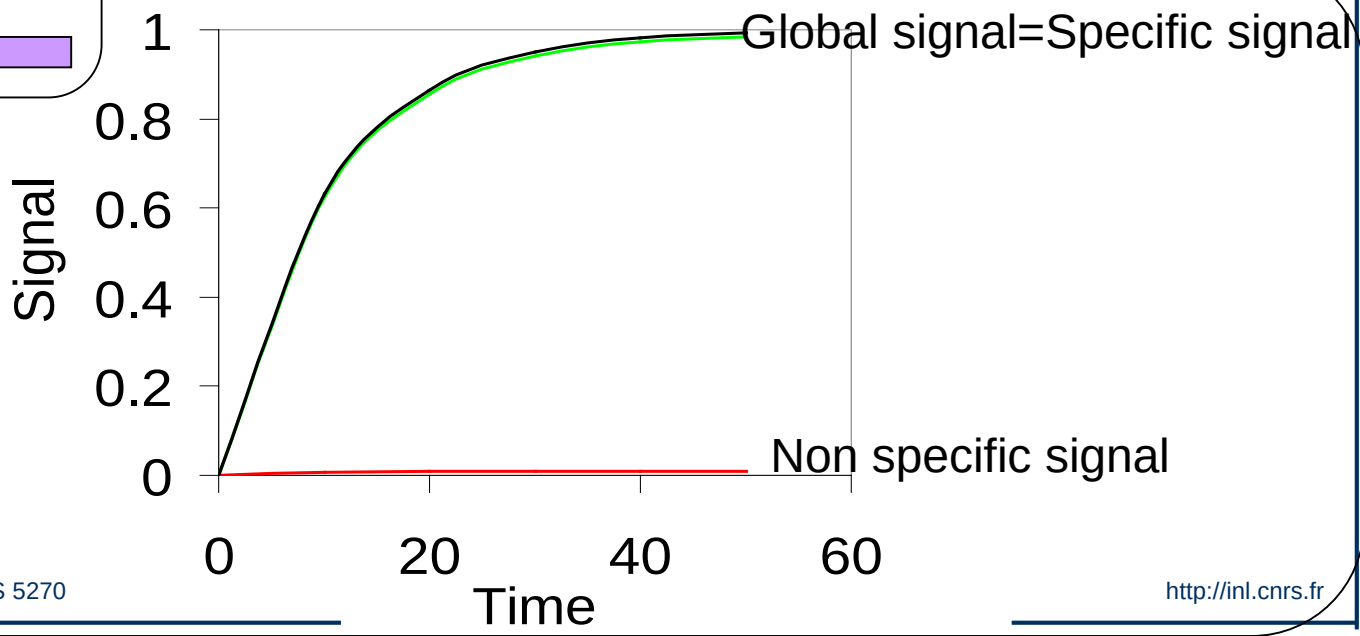
target

Interfering molecule

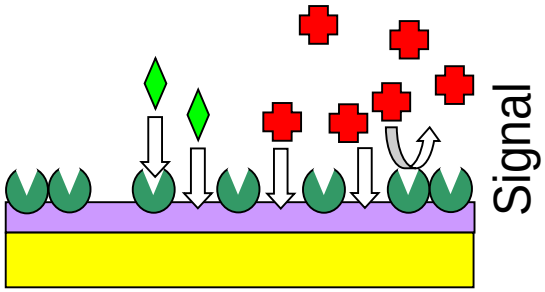
Functionalization layer

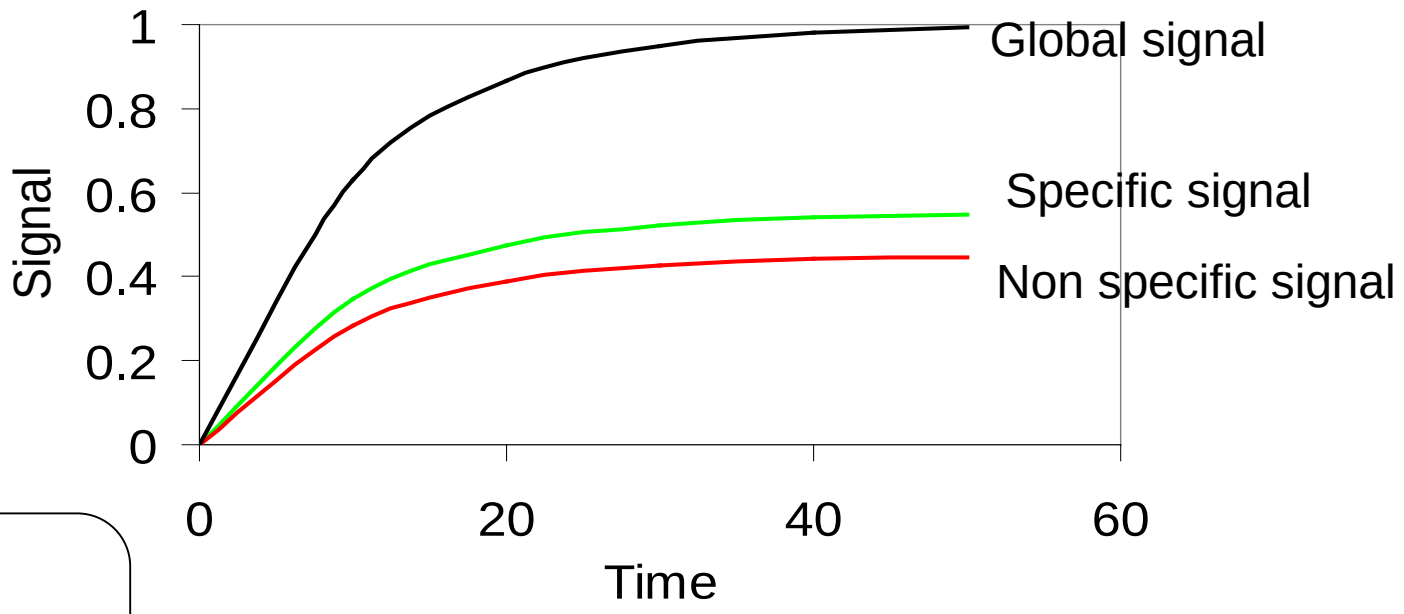
Best case





Realistic case





probe

target

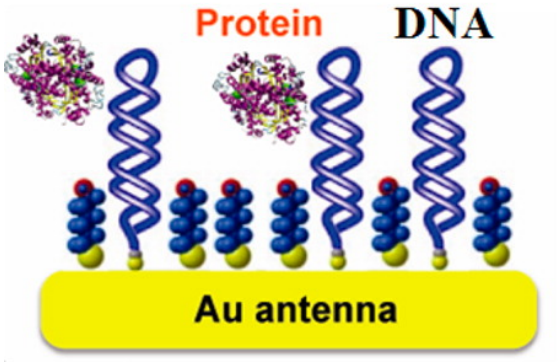
Interfering molecule

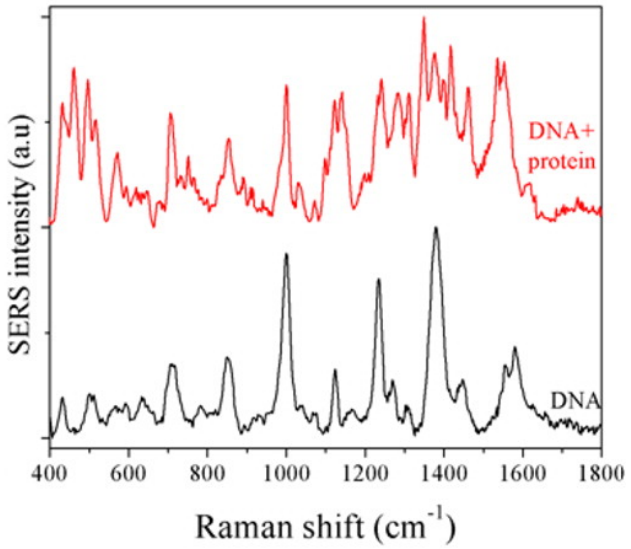
Functionalization layer

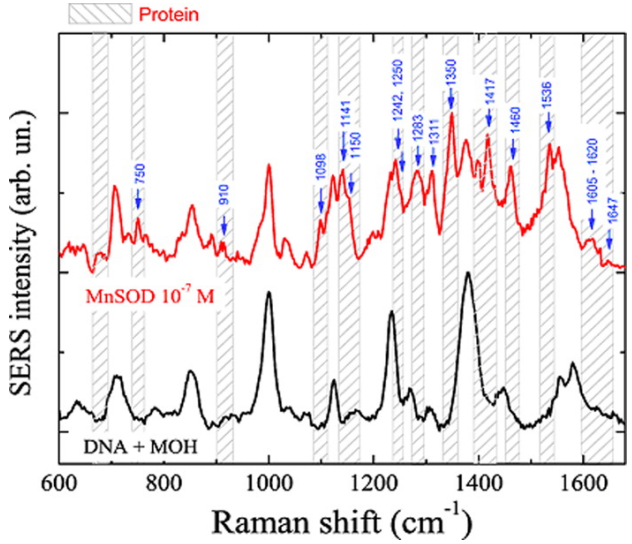
Targets are usually less concentrated than interfering species

Targets may have different properties compared to interfering species (eg : mass is important for SPR analysis)

SERS as a transduction method : how to get the targets close to the nano-antennas ?







Minimize the immobilization layer
Use « small » biomolecular probes

Examples :
thiolated DNA aptamers
ScFv antibody fragments

Cottat et al., *J. Phys. Chem. C* 2015, 119, 15532−15540

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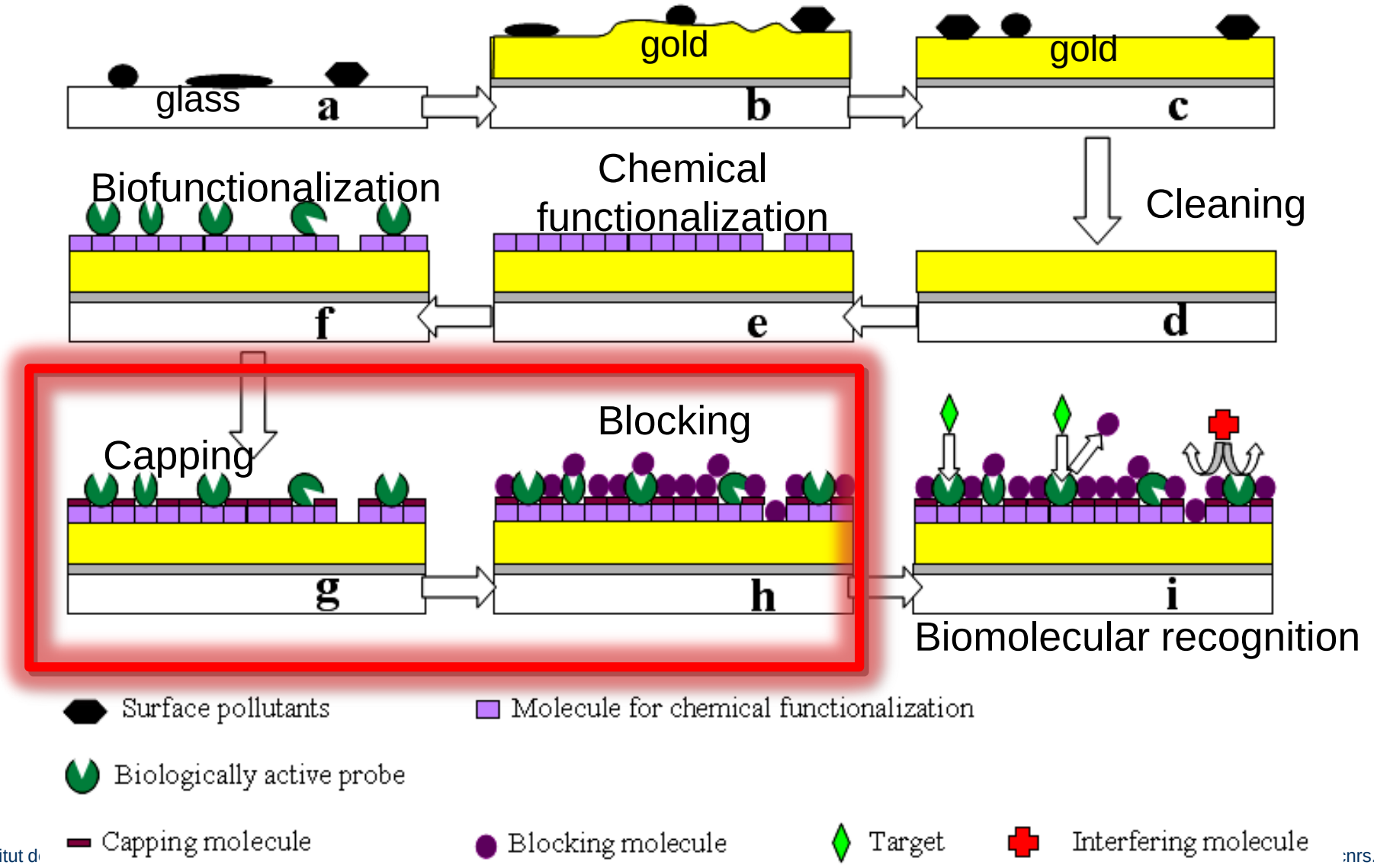
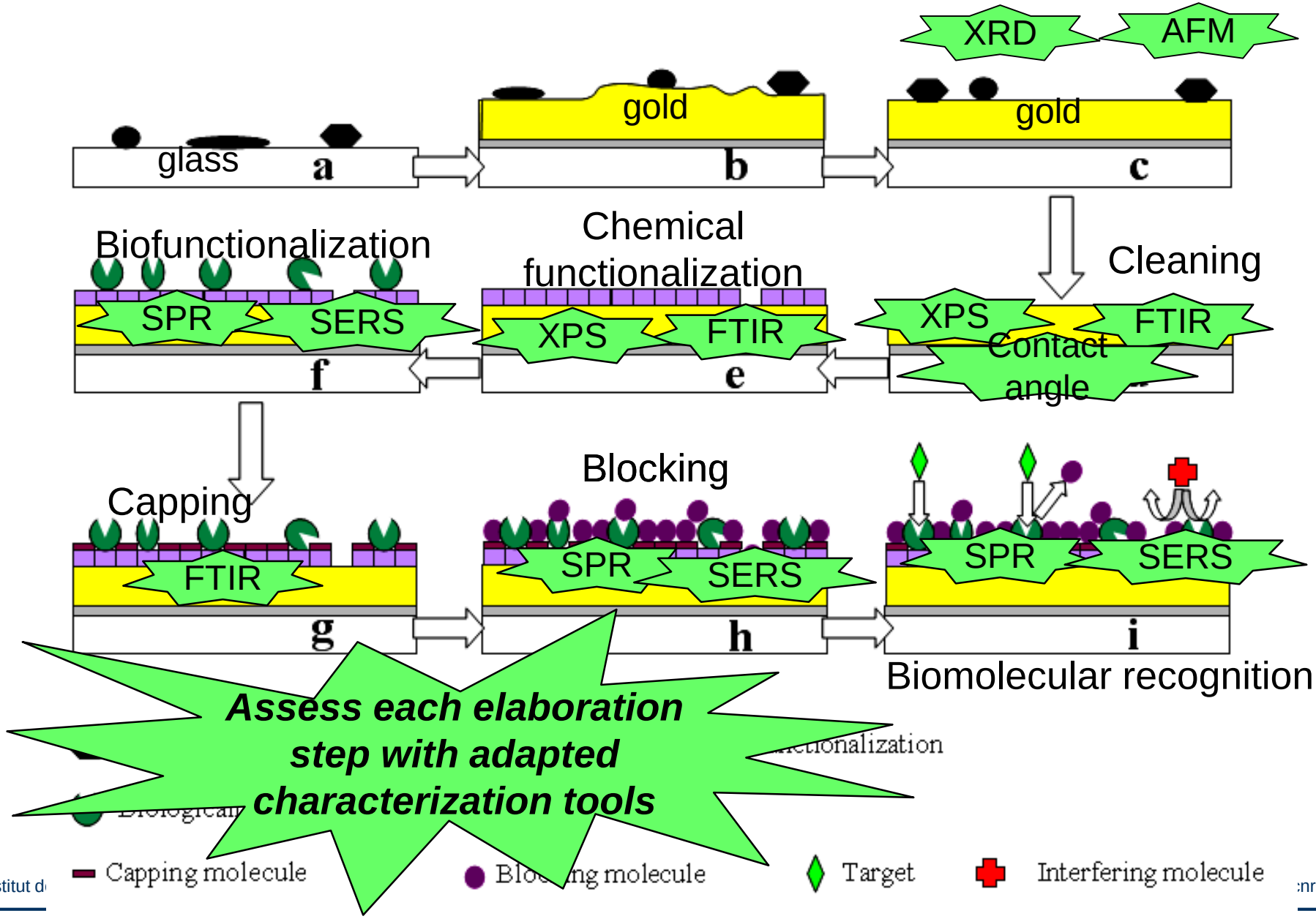
An example of classical steps for functionalization : the case for a glass/gold/thiols SPR biosensor



Elaboration has to be characterized



Surface characterization tools : example @ INL

Technique	**Chemical information**	**Lateral resolution**	**Other limitations**
PM-IRRAS	YES	mm	Only on metal
XPS	YES	μm	Availability
ToF-SIMS	YES	μm	Availability
Fluorescence scan	NO	μm	Only on dielectric
SEM	NO	nm	-
AFM	NO	nm	Scan area $\leq$ 50 $\times$ 50μm

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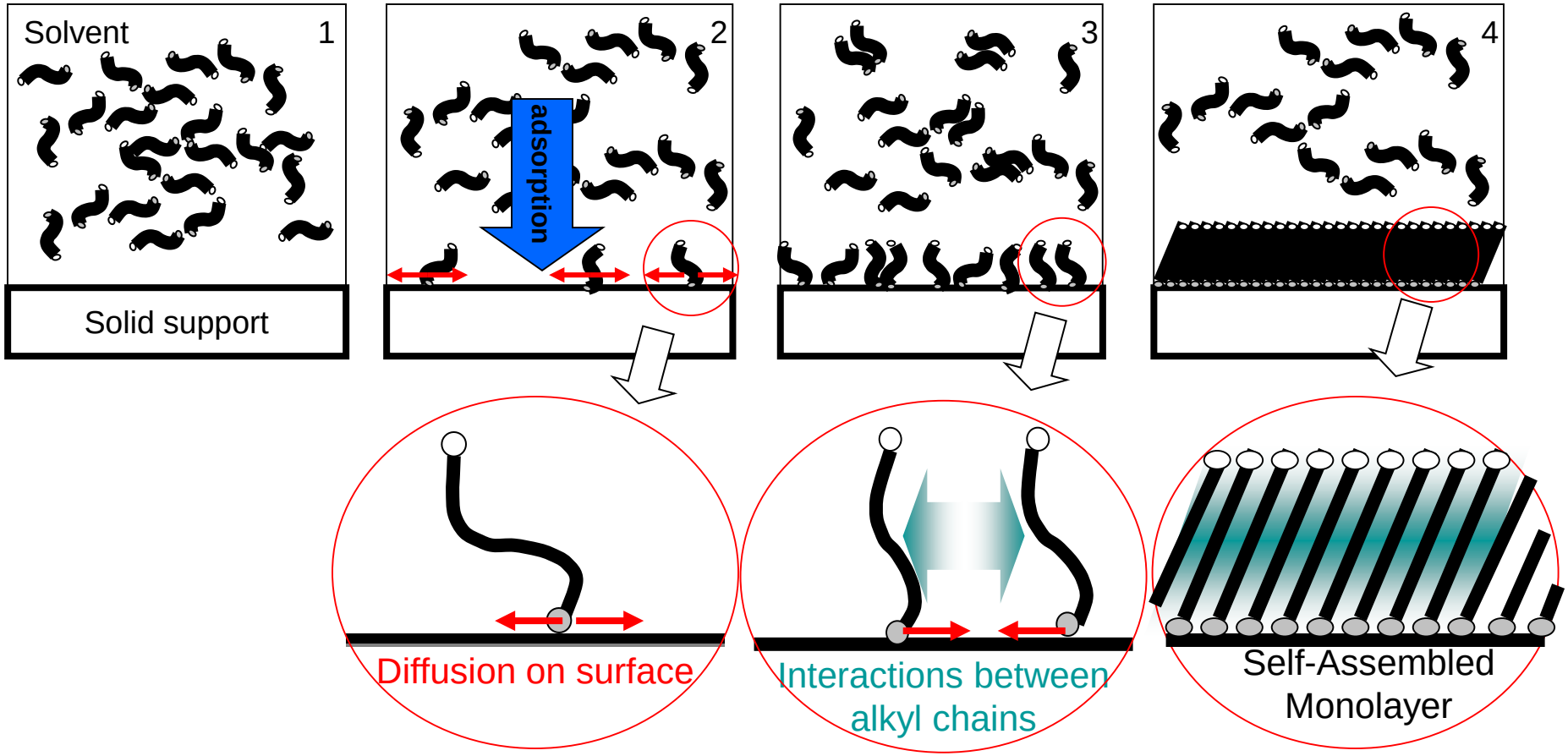
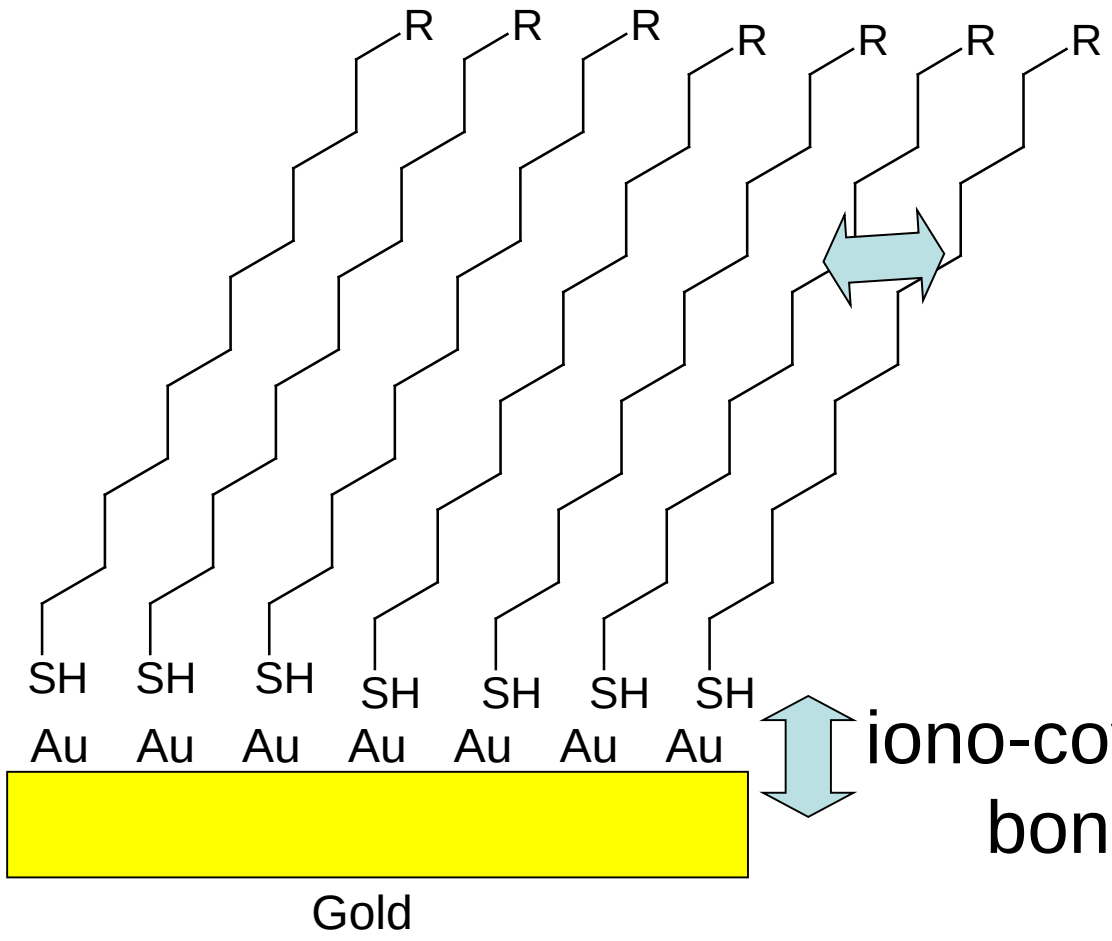
Self-Assembled Monolayers (SAM) : general mechanism of formation



Self-Assembled Monolayers

Alkylthiols

Self-assembled monolayer (SAM)



Van der Waals interactions

iono-covalent bonds

SAMS defects : example of alkylthiols on gold supports

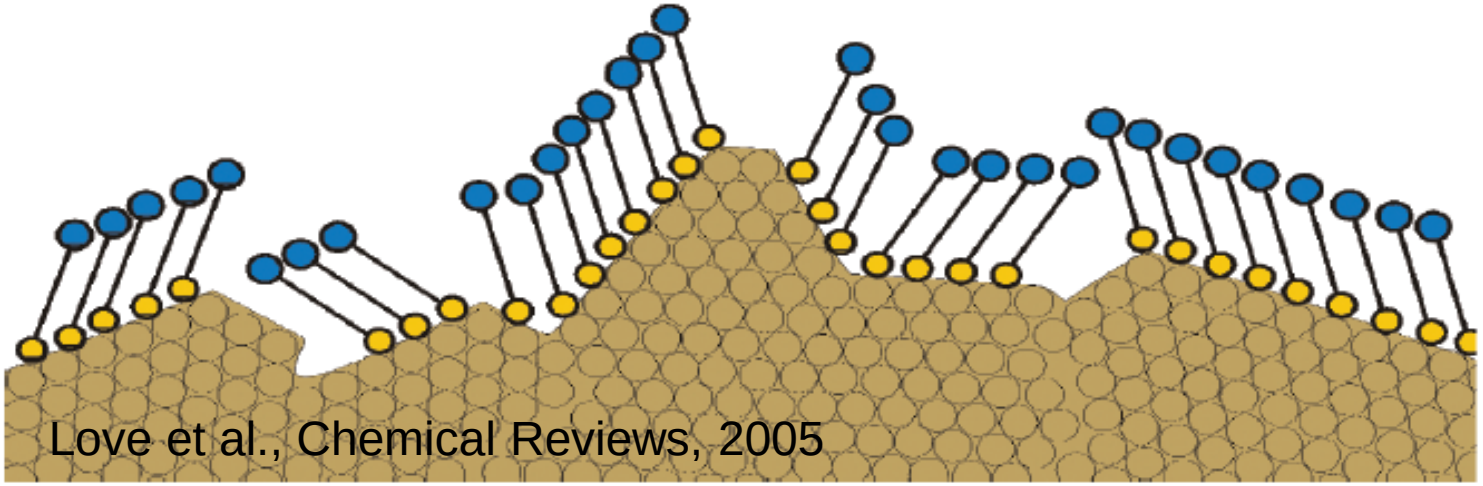
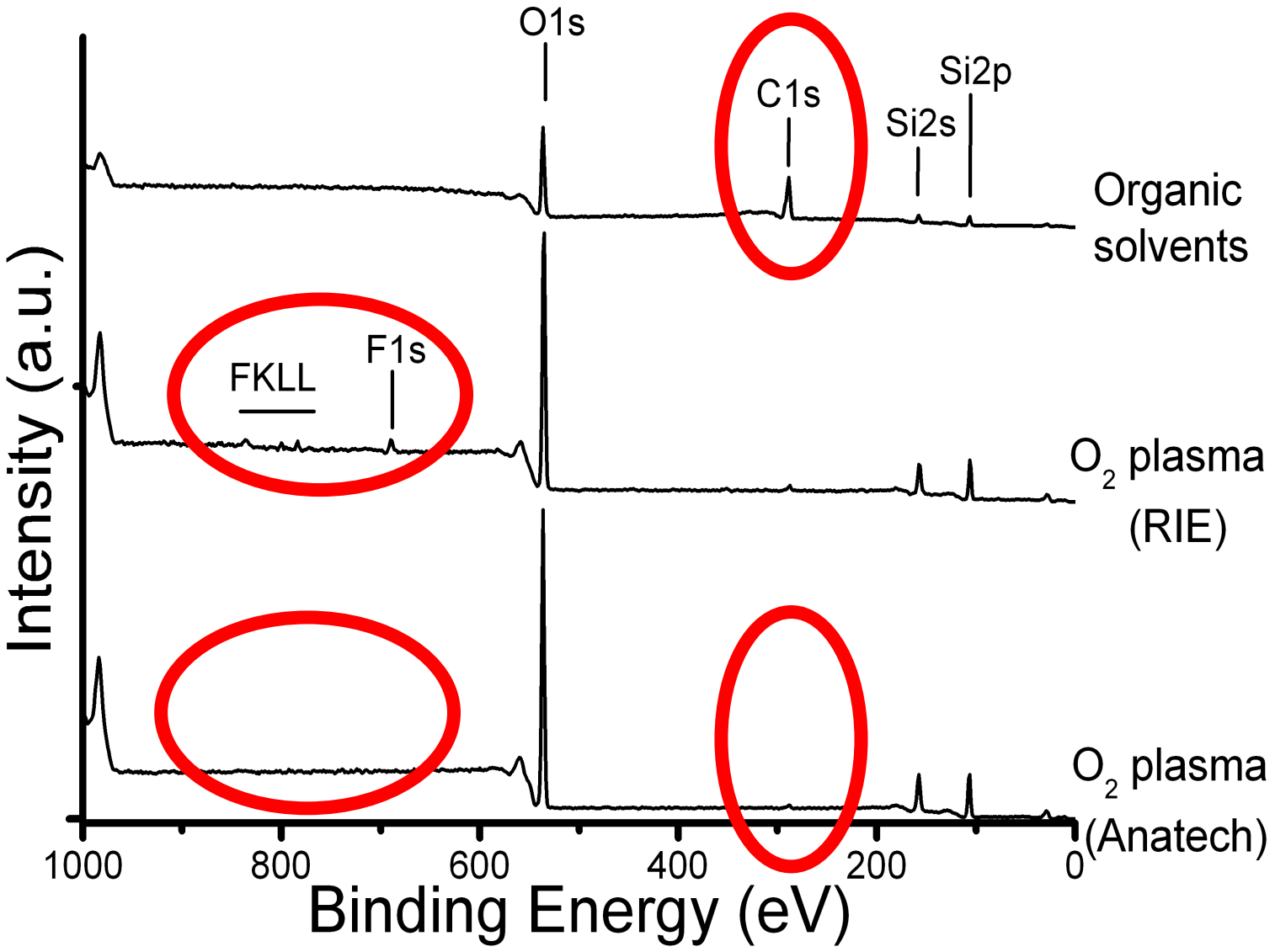


Figure 6.11 Schematic illustration of possible defects of SAMs adsorbed on a molecular scale "nanorough" gold surface. Such defects can cause local non-specific interactions.

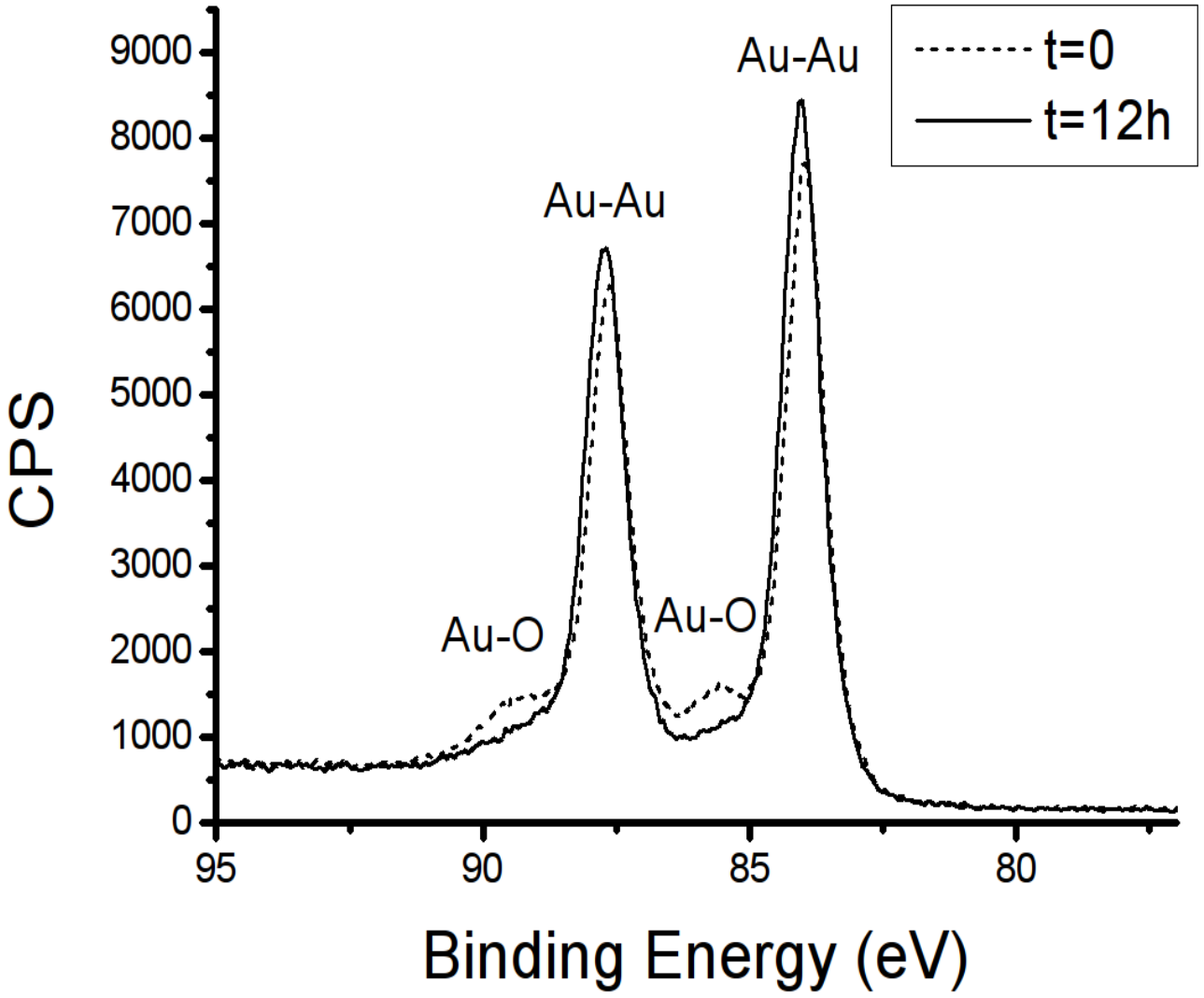
The quality of metal surface just before SAM formation is of primary importance

Importance of surface cleaning



∗. G. Grenet, C. Botella, D. Ferrah

Cleaning can have side effects



† G. Grenet, C. Botella, D. Ferrah

Results : Wait for gold de-oxidation after plasma cleaning †

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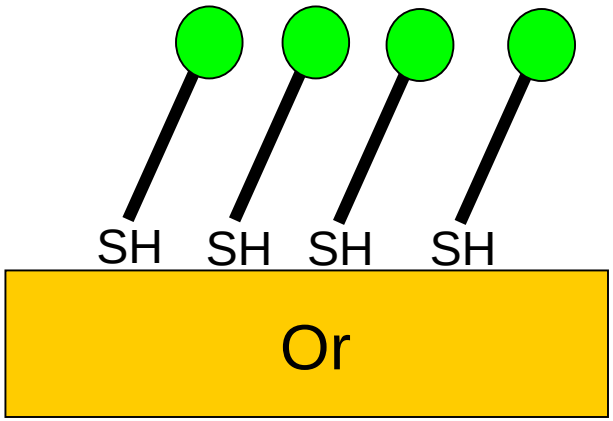
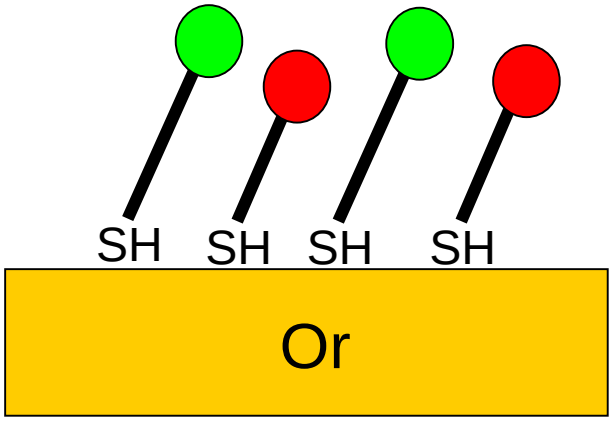
Fonctionnalisation chimique de surfaces

Fonctionnalisation de surfaces homogènes avec **SAM homogènes**

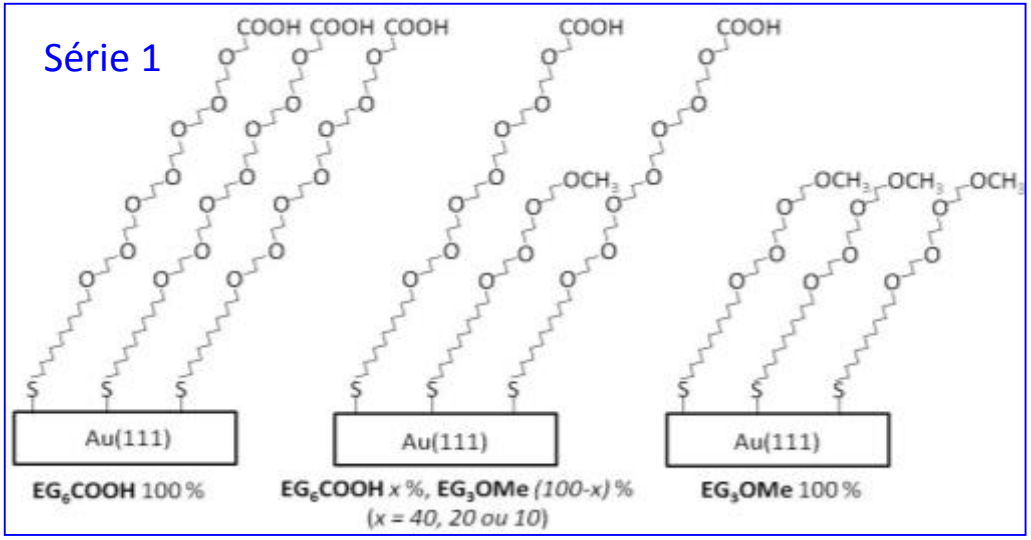


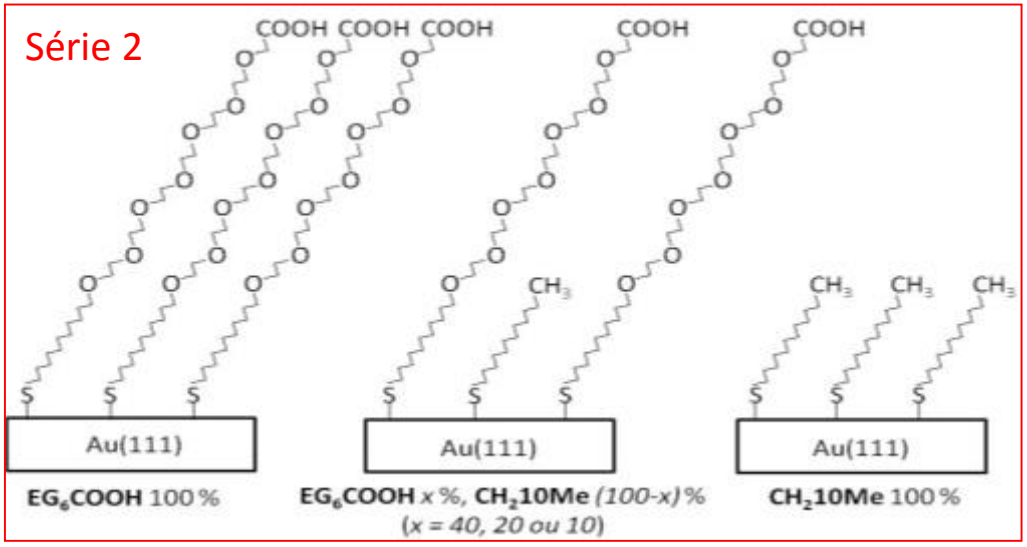
Fonctionnalisation de surfaces homogènes avec **SAM mixtes**

2 thiols différents mélangés sur une même surface d'or



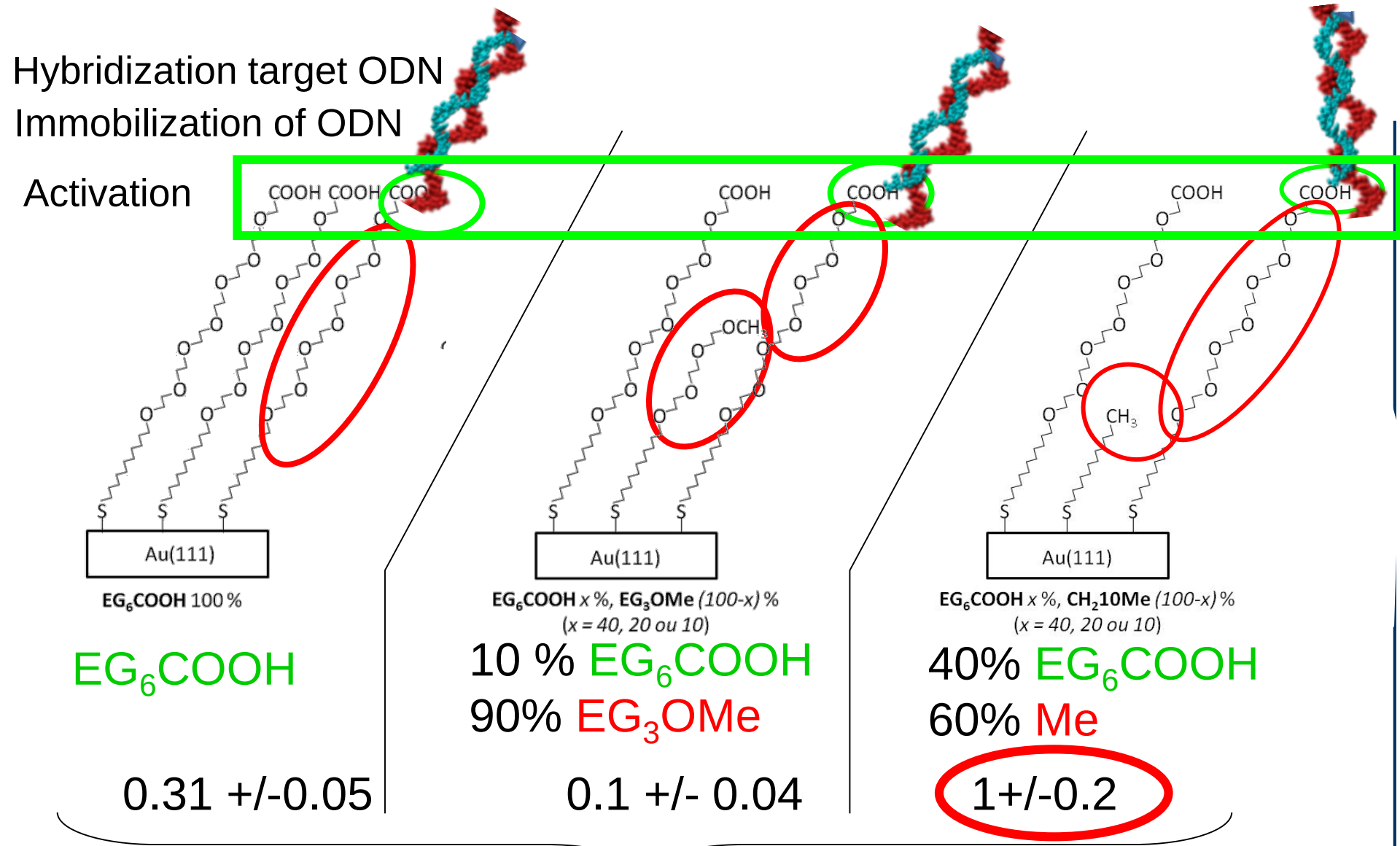
Mixed SAMs chemical functionalization





Refer to work of Claire-Marie Pradier's group, Laboratoire de Réactivité des Surfaces, Paris

Functionalization of gold with mixed SAMs : example



Outline

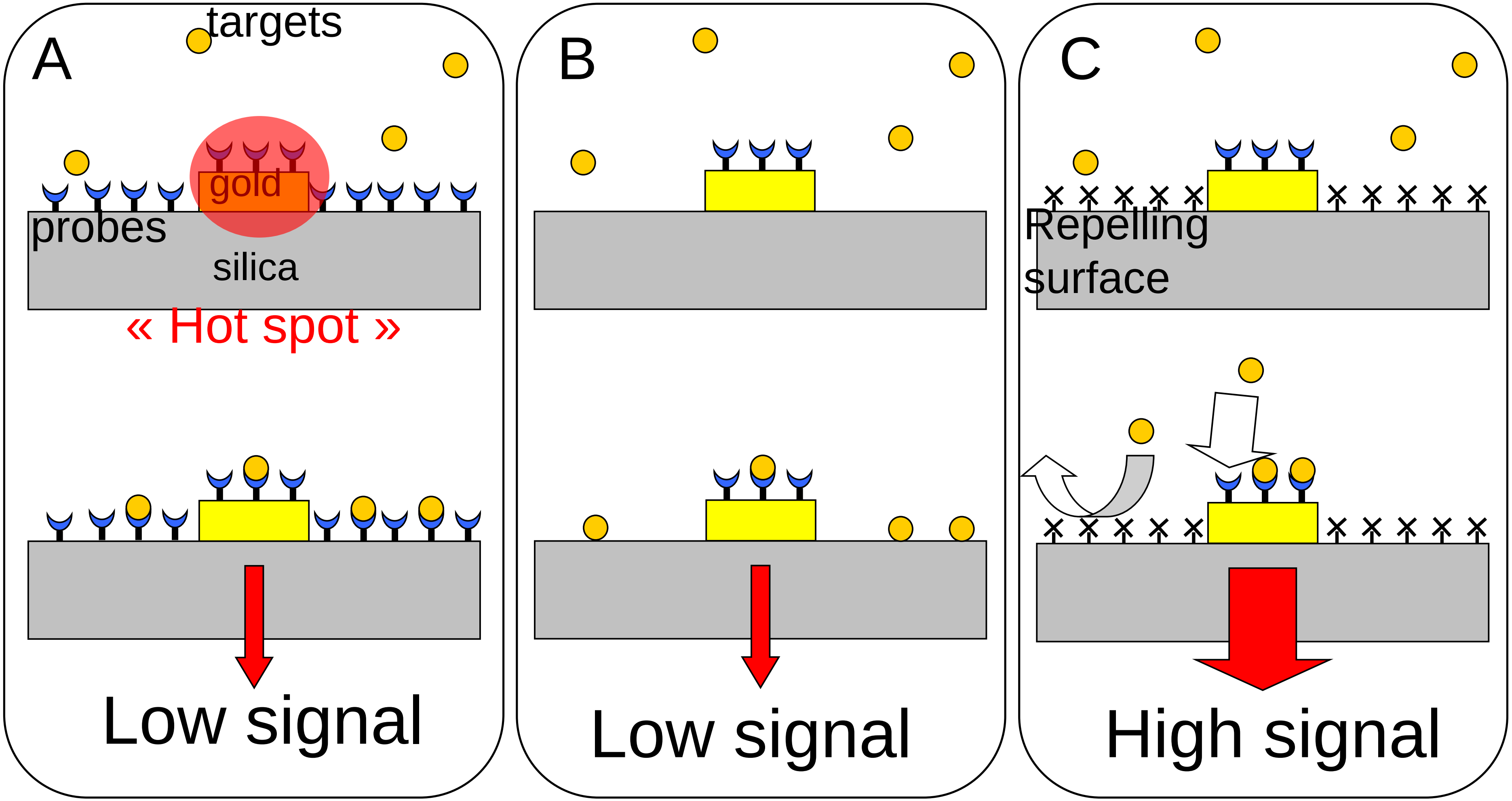
- How surface functionalization influences your biosensor measurements ?
  - Sensors analytical performances…
  - …and how bioreceptor elaboration can influence it
  - From molecular interaction to non specific interactions

- Classical approaches
  - Homogeneous thiolate SAMs : how it can disfunction. Gold cleaning, gold oxydation, metal defects, …
  - Capping, blocking
  - Mixed SAMs

- Selective functionalization of multiple materials

Selective surface functionalization

Example of Localized Surface Plasmon Resonance sensor



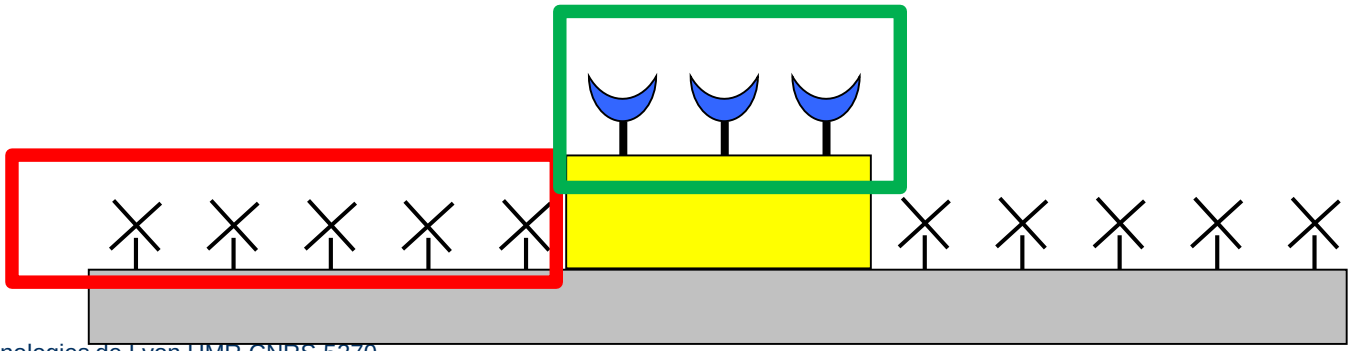
Palazon et al., *Beilstein Journal of Nanotechnology* **2015,** *6,* 2272–2277.

Managing complexity…

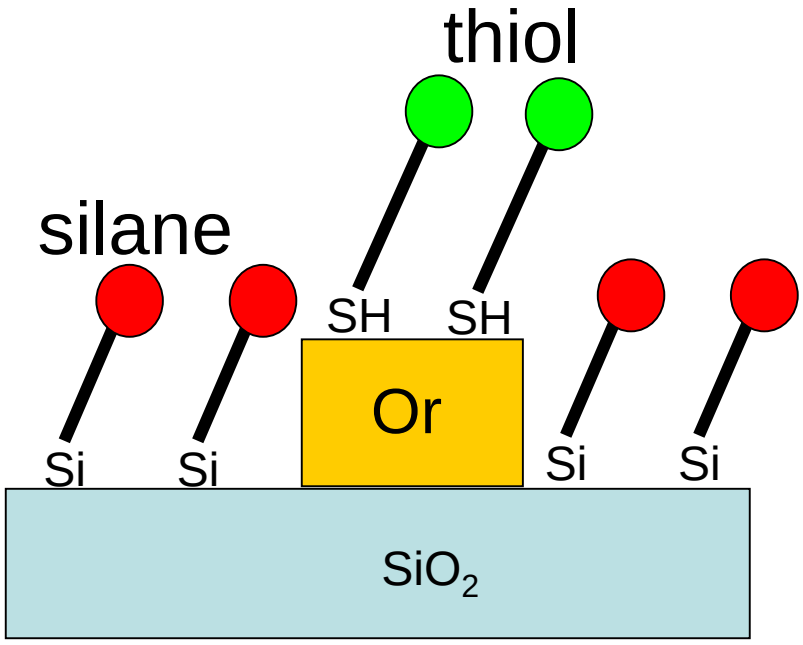
When possible, use materials with intrinsinc adapted surface properties (e.g. electric charge)

If necessary, use surface functionalization steps

Goal : use simultaneous, surface specific functionalization reactions

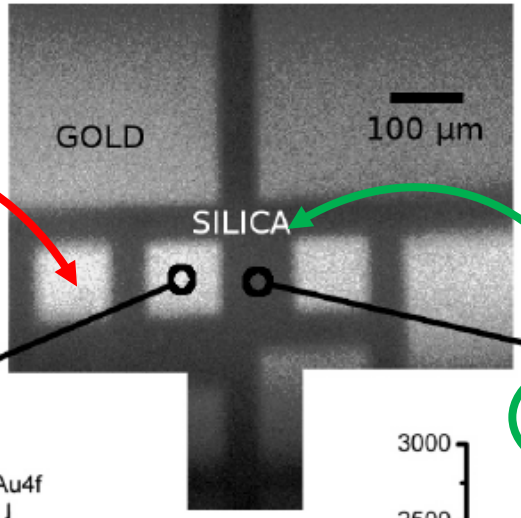


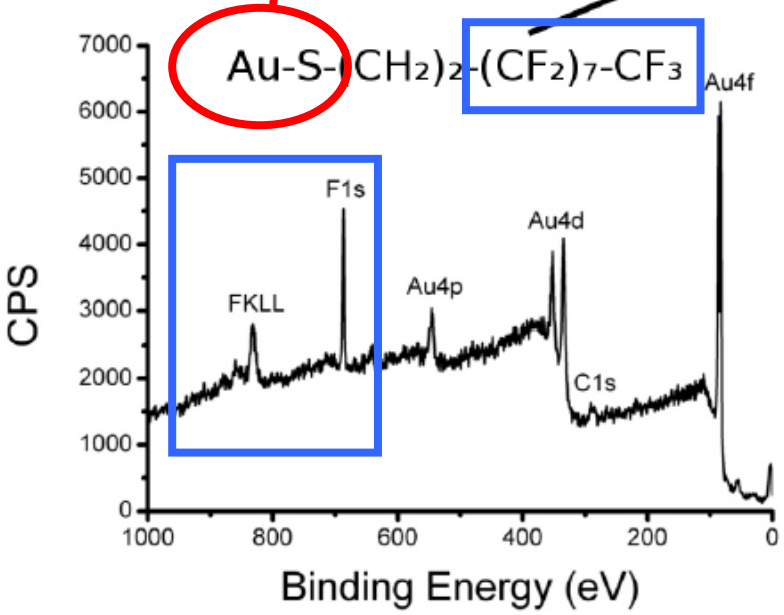
Selective functionalization of mixed surfaces

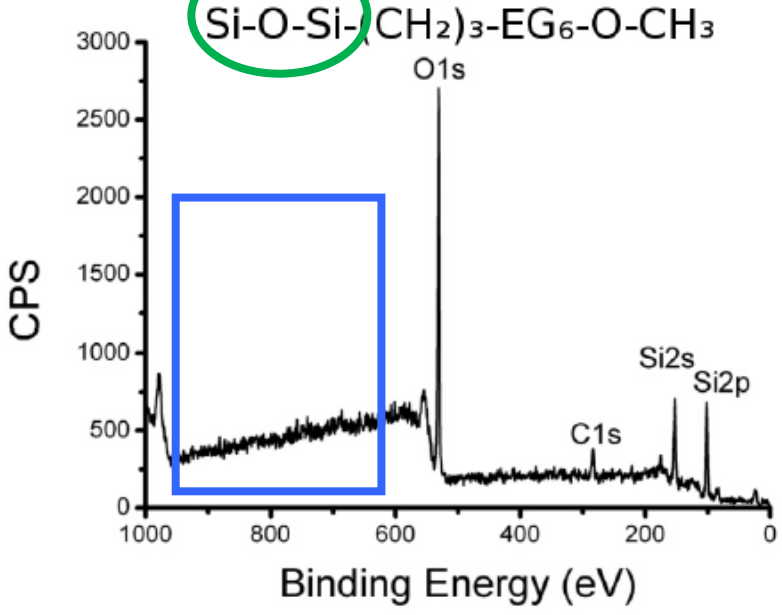


Orthogonal functionalization of bi-structured substrates

White zones : gold
Dark zones : SiO_2







Palazon et al., *Beilstein Journal of Nanotechnology* **2015,** *6,* 2272–2277

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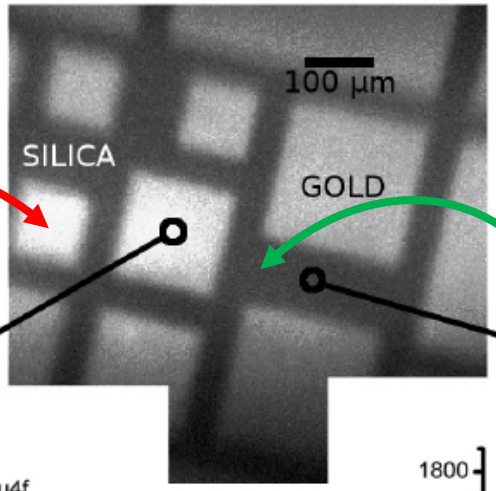
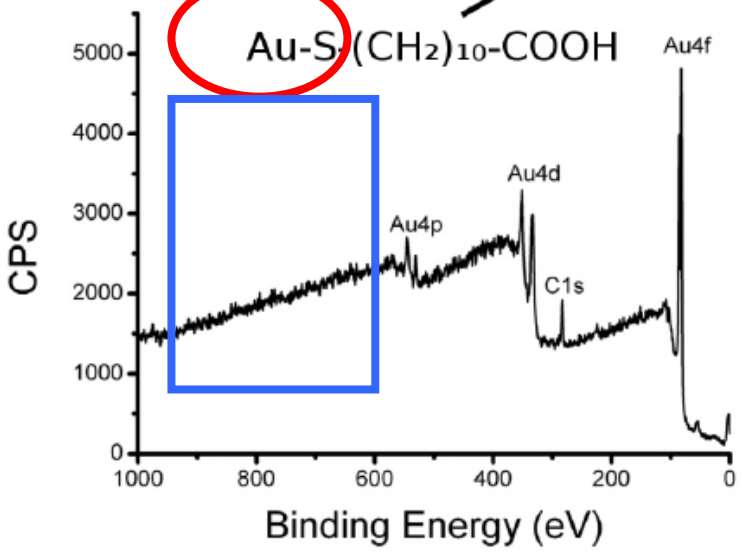
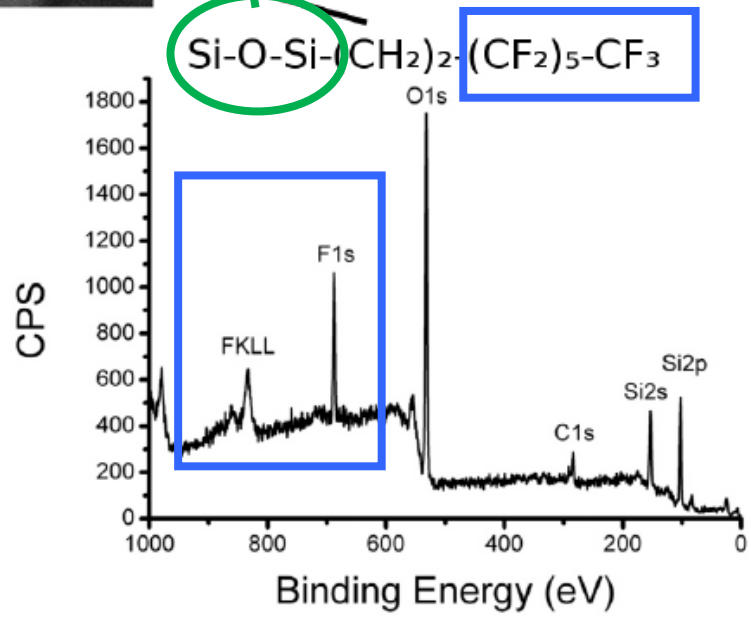
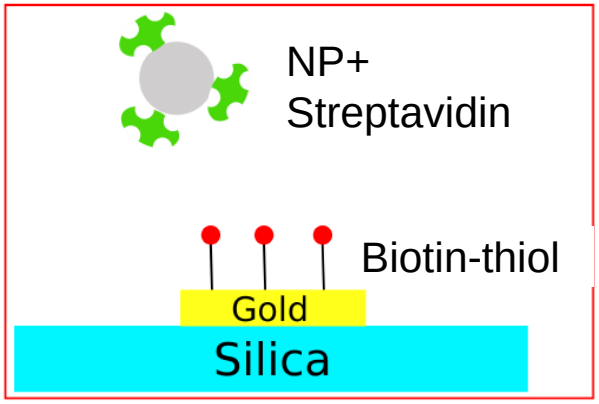




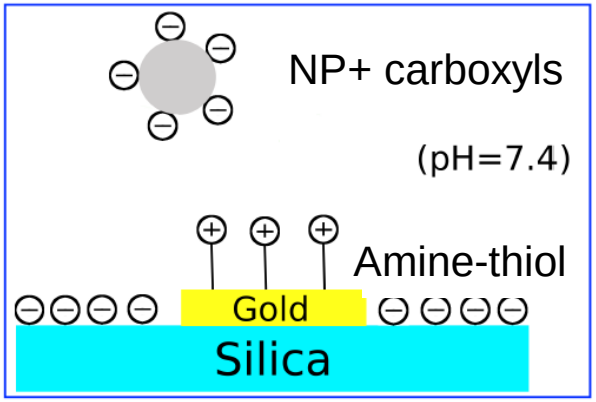


Palazon et al., *Beilstein Journal of Nanotechnology* **2015,** *6,* 2272–2277

Specific capture of microparticles on bi-structured substrate

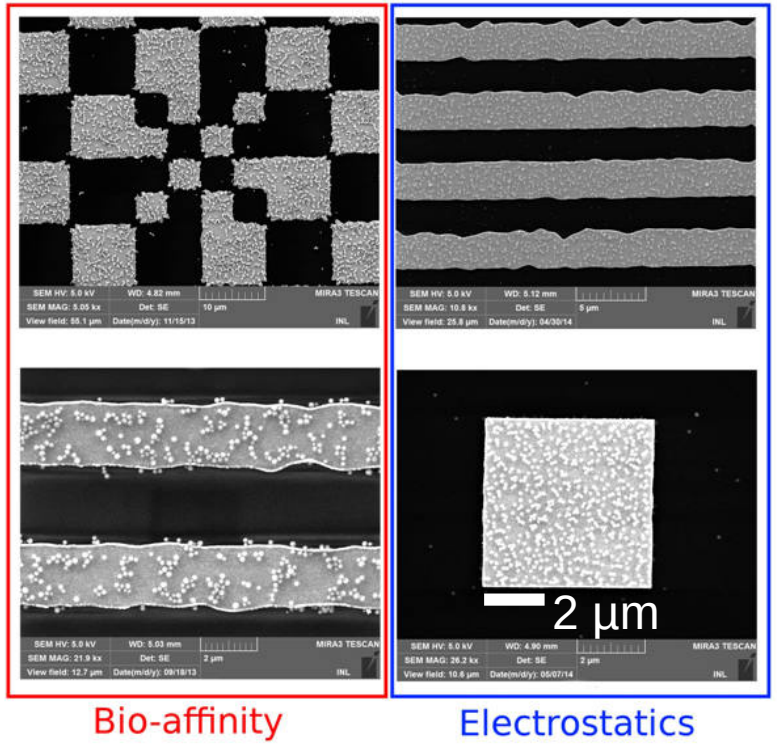


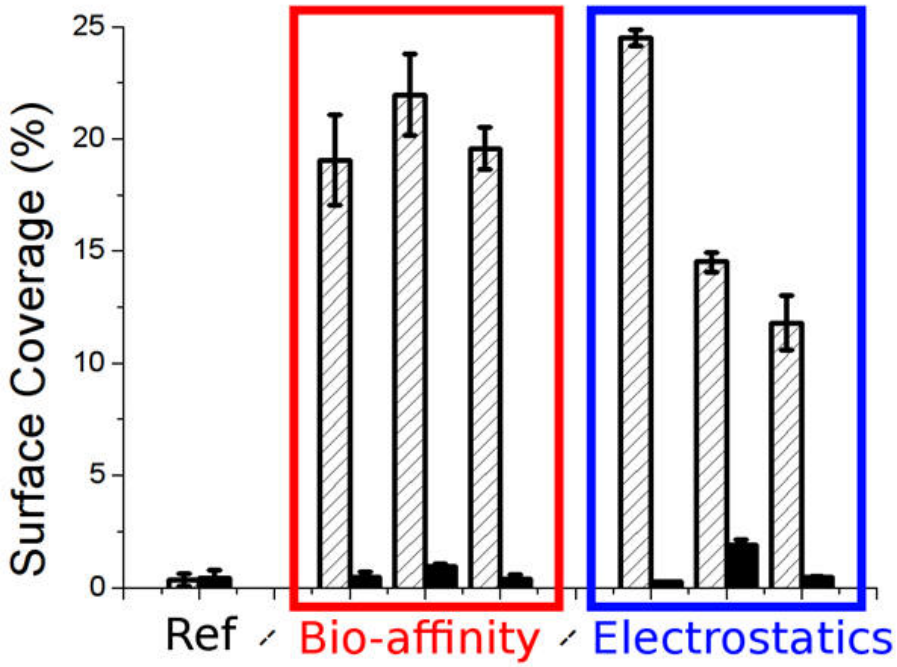
Biotin-streptavidin affinity



Electrostatic interactions

Nanoparticles captured on micronic size zones





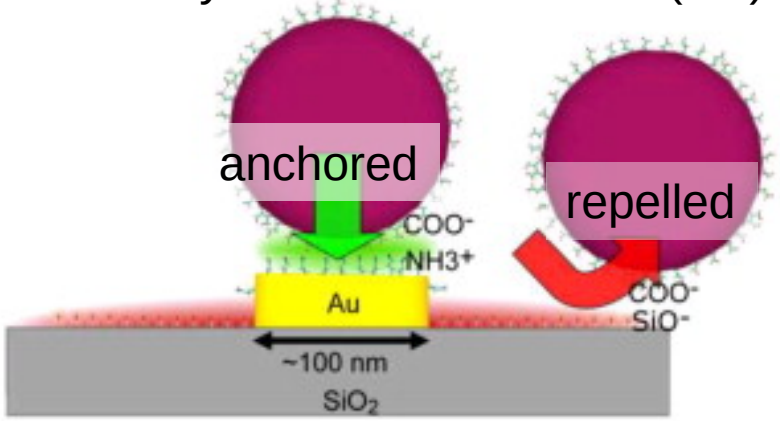
Palazon et al. JCIS (2014)

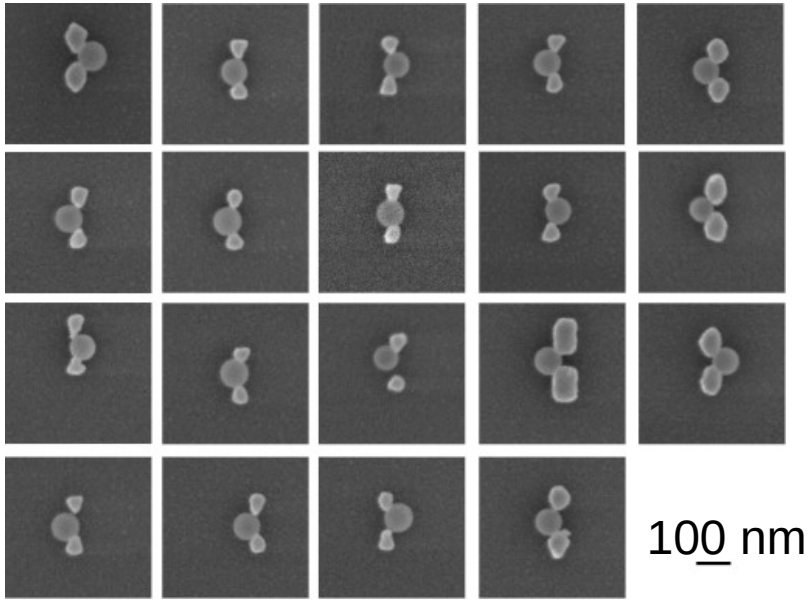
Interaction of bi-structured support with nanoparticles

Matrix of nano-antenna (NA) on SiO_2 support

1000 fabricated nano-antennas
109 inspected by SEM

Carboxy-latex NanoParticles (NP)

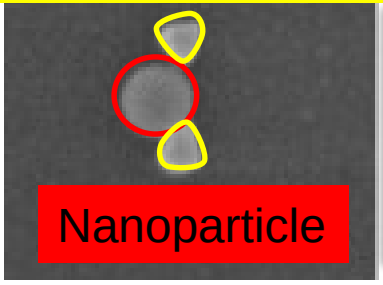




Capture of nanoparticles on nanoantennas

Very low non specific adsorption

Gold dimer nanoantenna



~70 % of NA have captures a NP
Only 0.2 NP per µm² of SiO_2

Palazon et al., *Journal of Colloid and Interface Science* 447 (2015) 152–158

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Modelling & SPR imaging

Merci de votre attention