

Gold surface bio-engineering: a chemical perspective

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Goutorbe, A-L. Deman, E. Laurenceau, J-P. Cloarec and Y. Chevolot

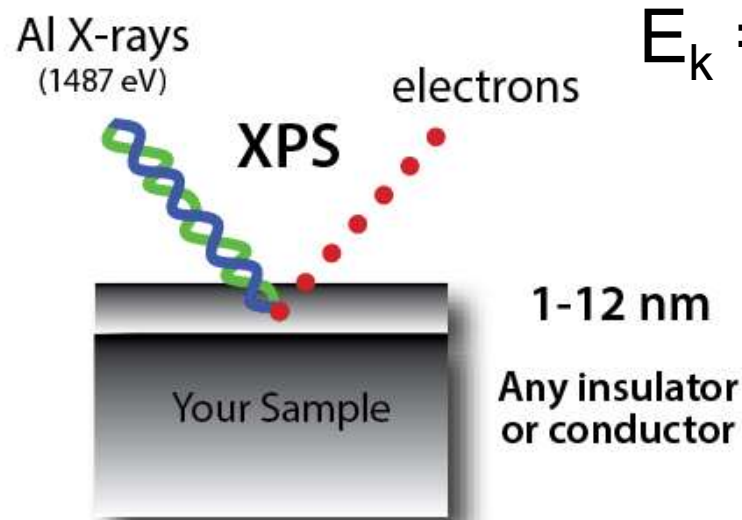


Outline

- Gold pre-conditioning and its impacts,
- Gold-thiol reaction and the stability of Au-S,
- Grafting biomolecules,
- Strategies for reducing non specific interactions,
- Multimaterial substrates.

Characterisation tools: XPS

- X-ray Photoelectron Spectroscopy (XPS or ESCA)



<https://www.nanolabtechnologies.com/xps-esca/>

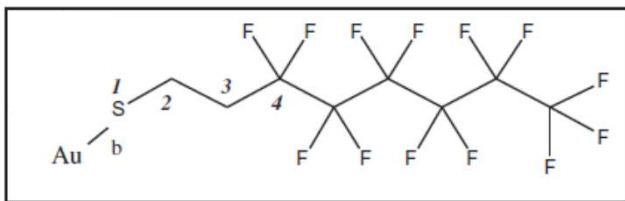
$$E_k = h\nu - E_b - \phi$$

→ E_b

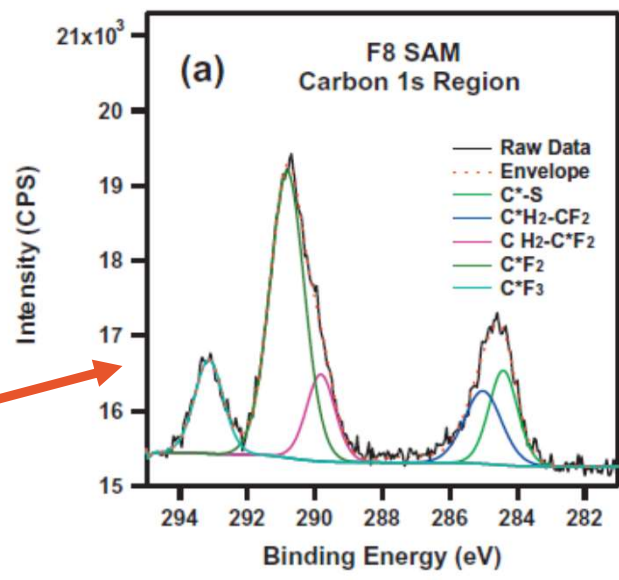
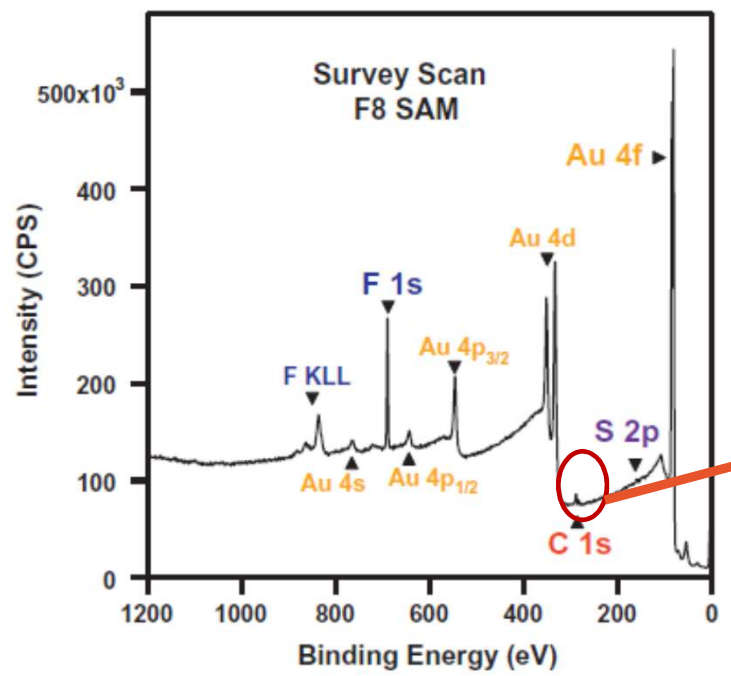
- What element? (Excepted H and He)
- How much (semi-quantitative)?
- What is its chemical state?

$$\%I = \frac{I_i/S_i}{\sum I_i/S_i}$$

XPS: Chemical shift



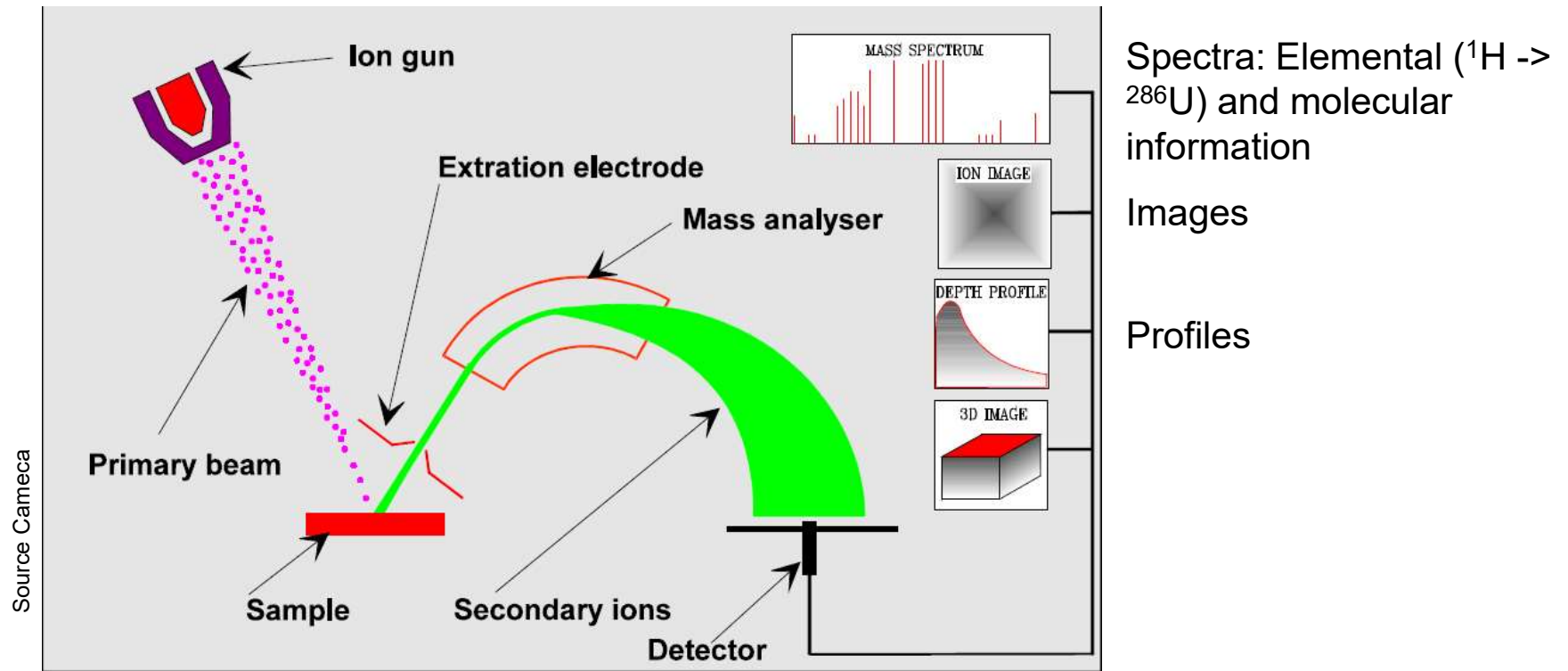
C1s



K. Ford et al, J. Colloid Interfac. Sci., 2012, 162–169

Characterisation tools: SIMS

Dynamic vs **Static** Secondary Ion Mass Spectrometry



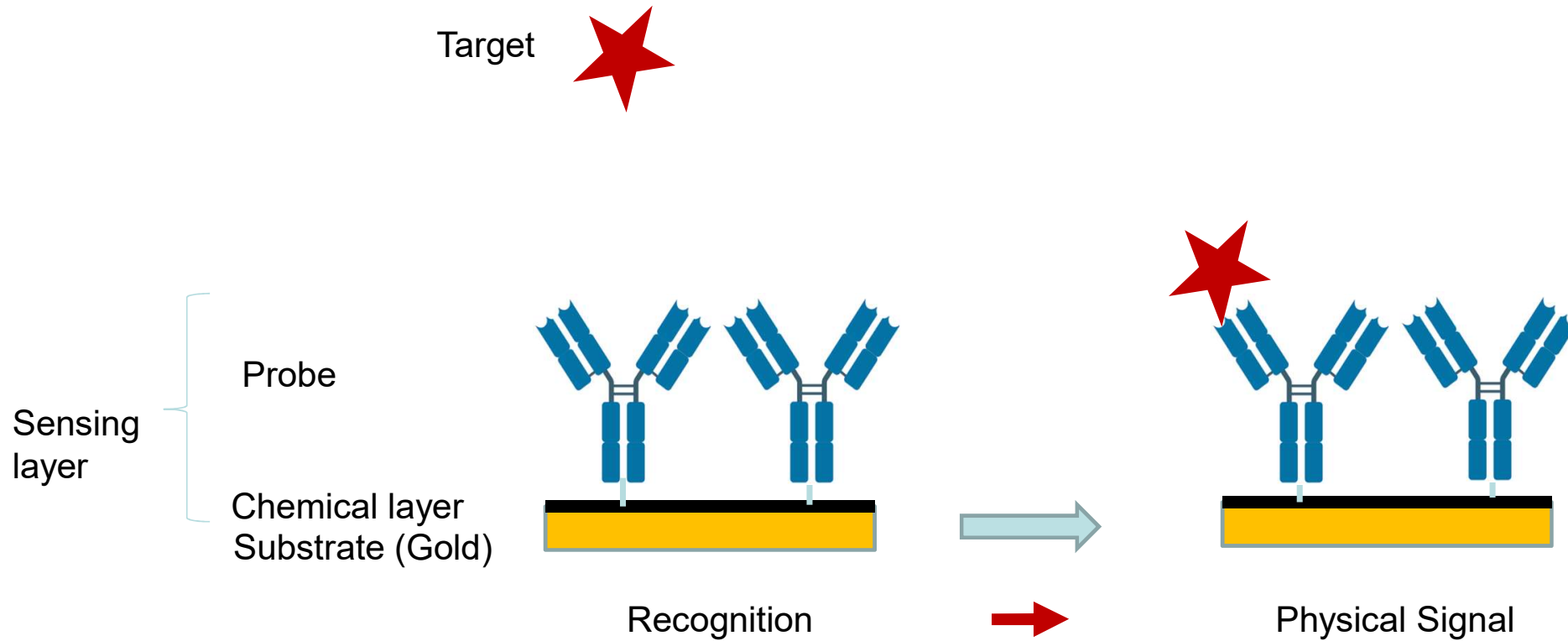
Spectra: Elemental (^1H -> ^{286}U) and molecular information

Images

Profiles

But quantification difficult => standard

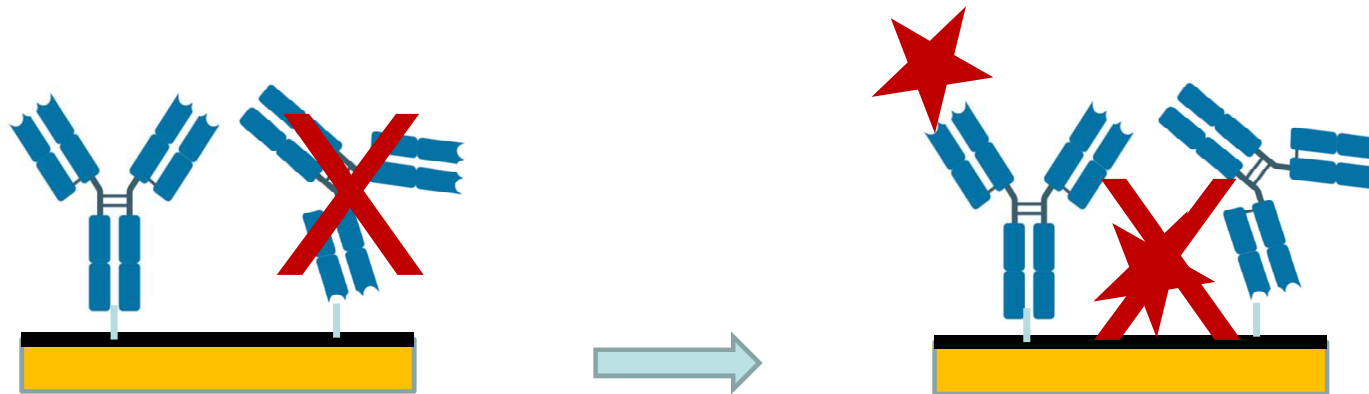
Biosensors



Gold surface chemical functionalisation for biosensing

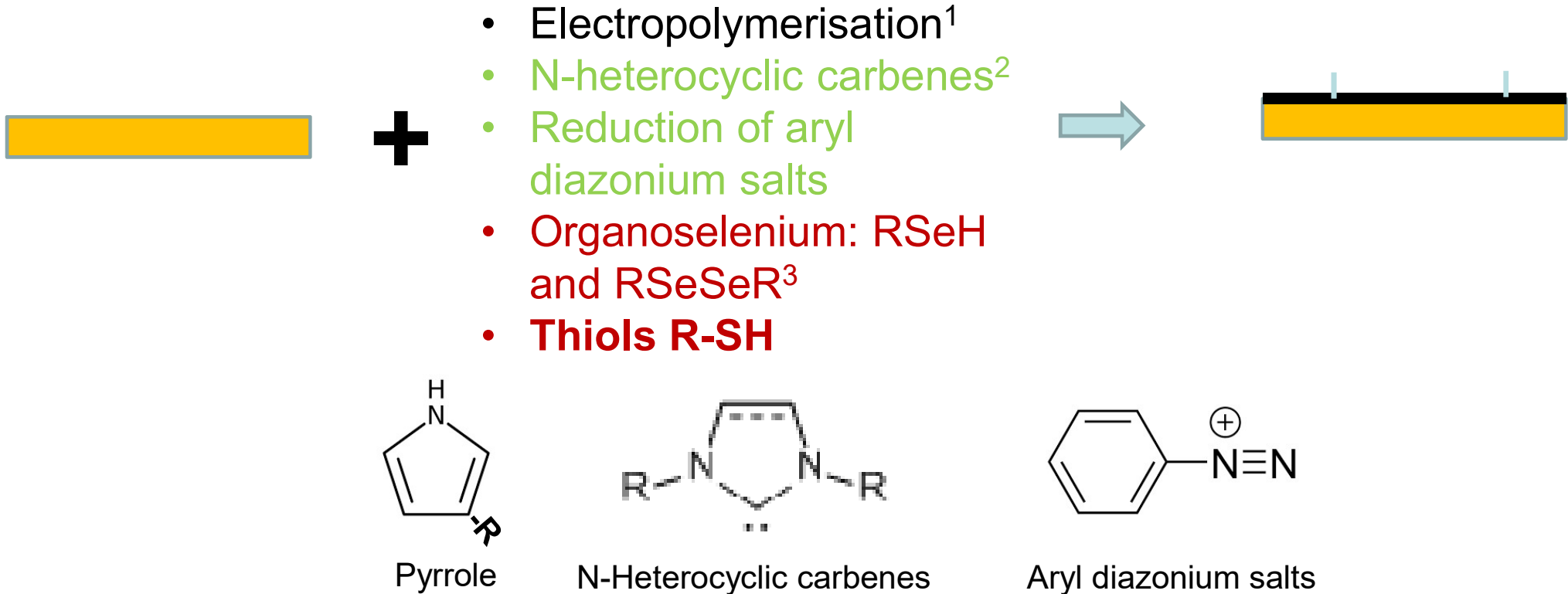
Surface chemistry :

- Stability
- Reproducible
- To favor signal vs noise



Cruz et al Langmuir 2016, 8660–8667
Ha et al Proteomics 2005, 416–419

Gold surface chemical functionalisation for biosensing



¹ Morlay *et al*, Measurement, 2017, 305-310

² Engel *et al*, Chem. Soc. Rev., 2017, 2057-2075

³ Romashov *et al*, Chem. Eur. J. 2013, 19, 17640 – 17660

Gold surface chemical functionalisation for biosensing



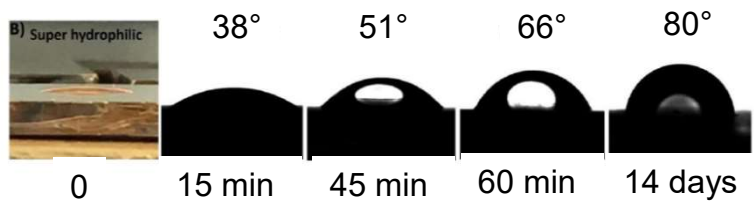
Know what you start from:
Clean, purity¹

¹ Bain, et al, J. Am. Chem. Soc. 1989, 321–33

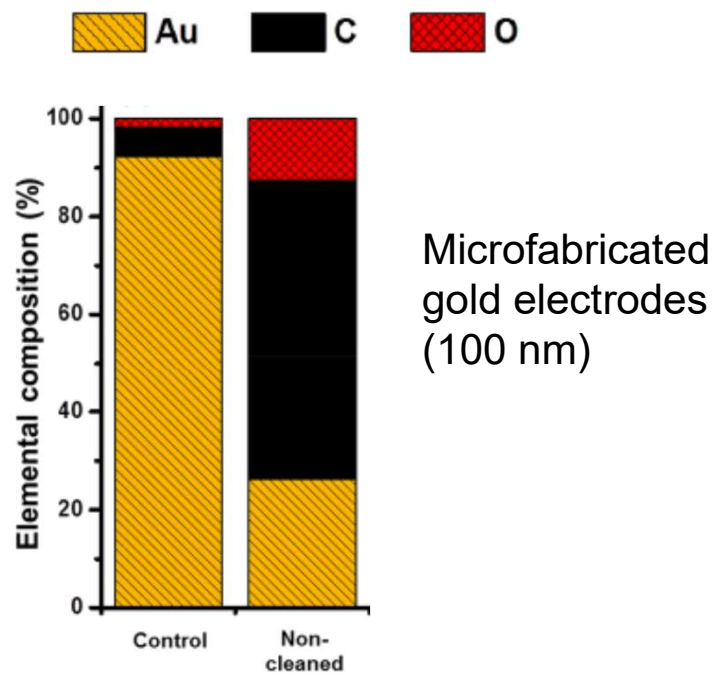
Limit undesired
reactions
Reproducibility

Why cleaning gold?

T= 0s Freshly evaporated gold



M. Li PhD thesis, Ecole Centrale de Lyon, 2018



Makaraviciute et al, ACS Appl. Mater. Interfaces 2017, 26610–26621
Palazon PhD thesis, Ecole Centrale de Lyon, 2014

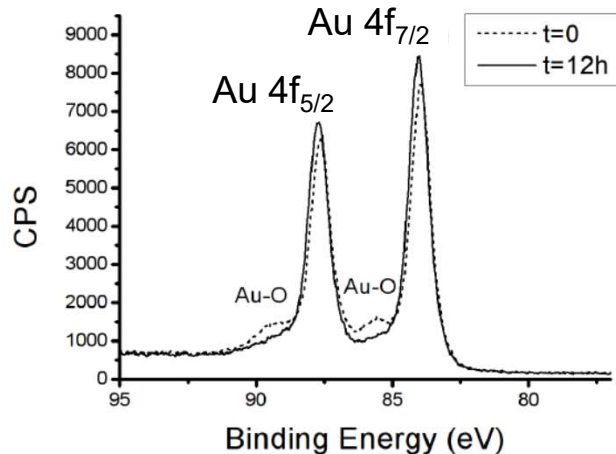


How to clean?
What are the consequences?

Cleaning procedures

- Most of the contamination is due to adventitious hydrocarbons which is remove thanks to oxidative treatments
 - Electrochemical cleaning.
 - Piranha ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, Caro Acid H_2SO_5),
 - UV/ozone,
 - Oxygen plasma,
- However:
 - Au_2O_3
 - Contamination with sulfonate
 - Surface roughening

Au₂O₃ and possible impact



Au: 84 eV and 87,7 eV
 Au₂O₃: 85.5 and 89.2 eV

F. Palazon PhD thesis Ecole Centrale de Lyon

- Au₂O₃ thickness is treatment dependent=> stability
 - RF O₂ plasma²⁻³ 300 W => 5-7 nm, 600 W => 6-10 nm
 - UV/ozone : 1-2 nm¹
 - Piranha: controversy
- Effect on SAM organisation⁴
- How to get rid of it?
 - Wait, EtOH⁵

1 King *et al*, J. Vac. Sci. Technol. Vac. Surf. Films, 1995, 1247-1253

2 Stadnichenko *et al*, Surf. Eng., 2018, 1-5

3 Stadnichenko *et al*, J. Struc. Chem., 2015, 557-565

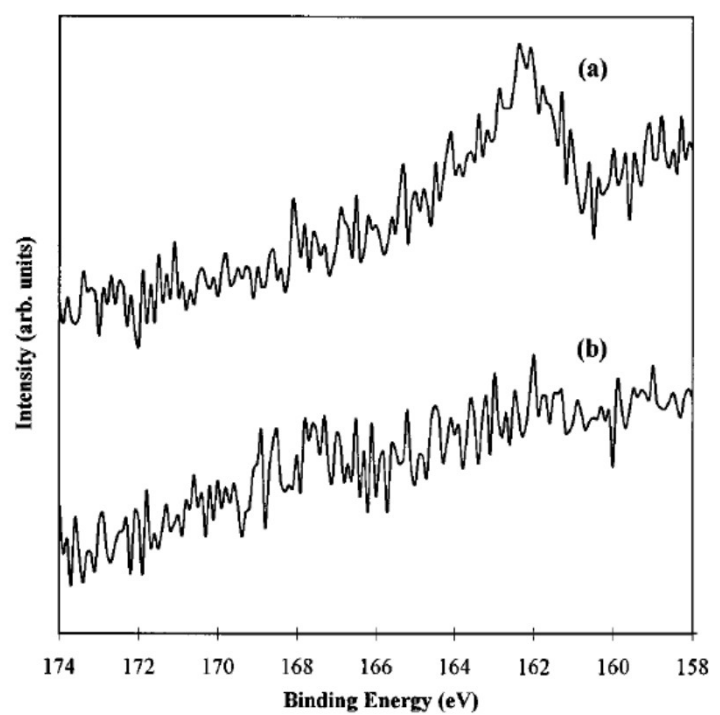
4 Woodward *et al* Langmuir 2000, 5347-5353

5 Ron *et al*, Langmuir, 1994, 4566-457, Makaraviciute *et al*, ACS Appl.

Mater. Interfaces 2017, 26610-26621

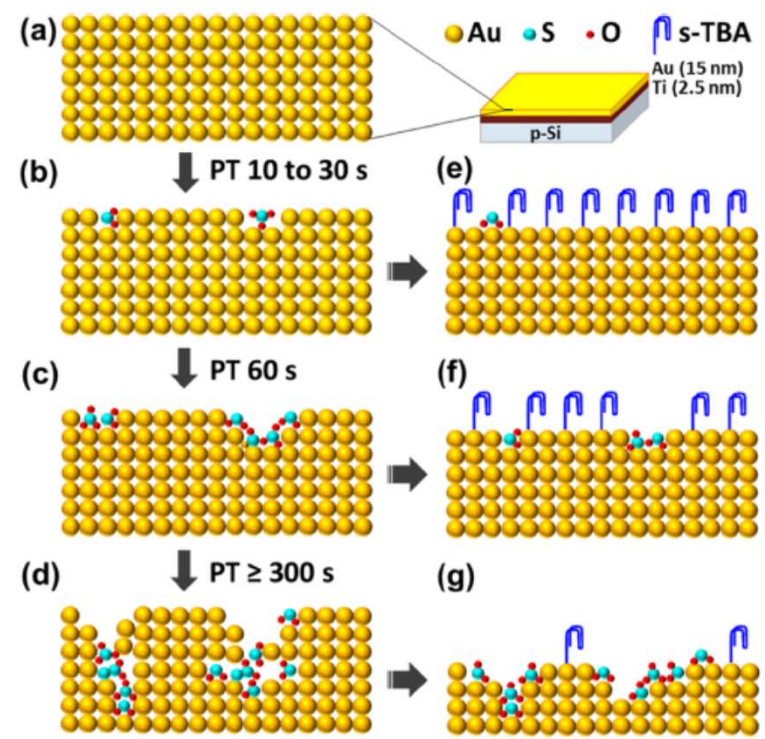
Sulfonate contamination

Oxidation of sulphur containing compounds



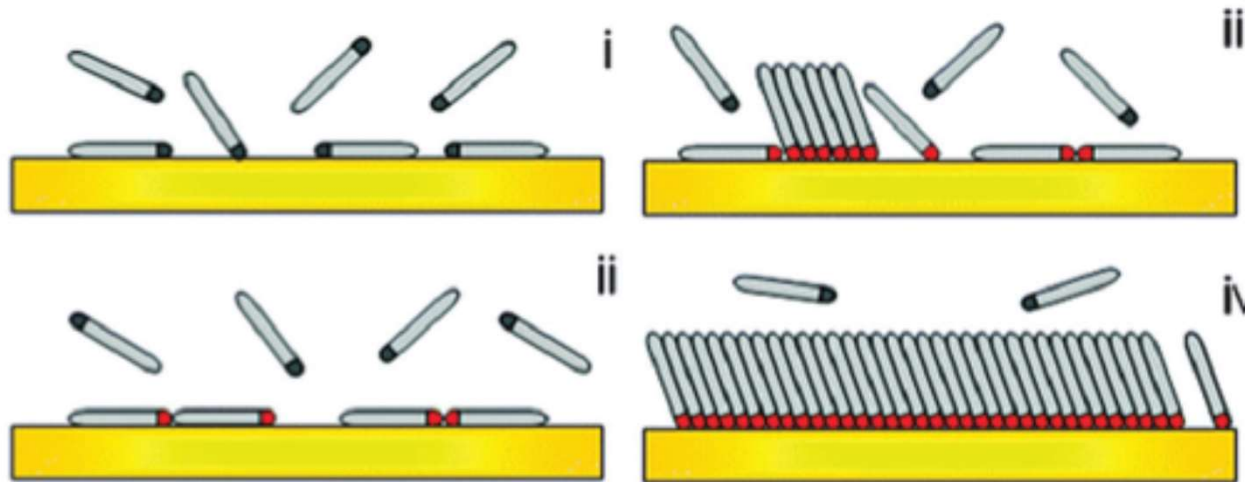
Worley *et al*, J. Vac. Sci. Technol. A, 1995, 2281-2284

Piranha treatment time



Thomas *et al*, ACS Appl. Mater. Interfaces 2012, 4, 5945-5948

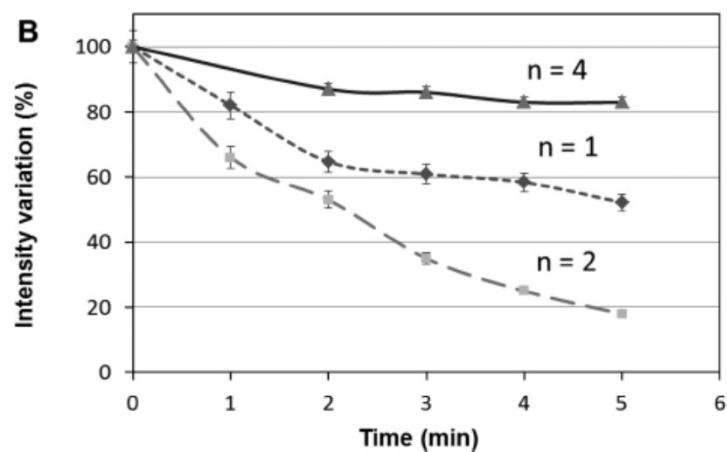
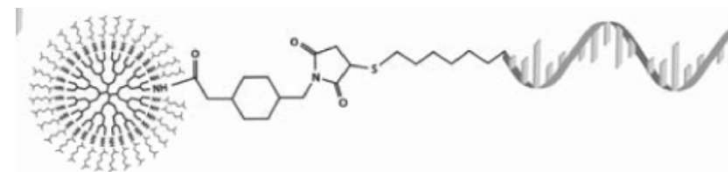
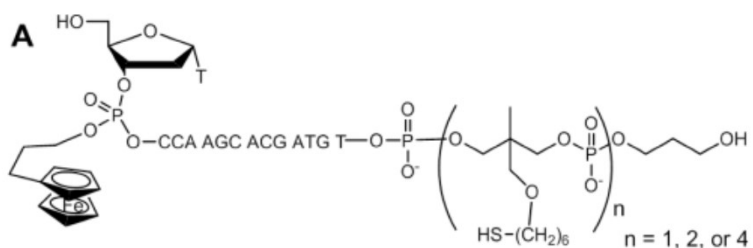
Formation of Au-S



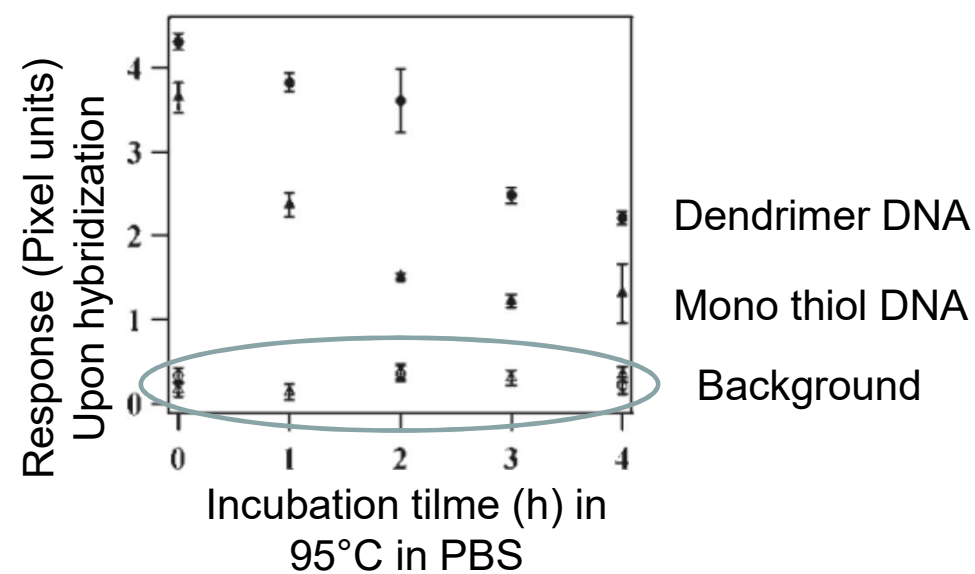
Engel *et al*, Chem. Soc. Rev., 2017,2057-2075

- Physisorption followed by H-S cleavage => Reaction with adatoms: -161 kJ/mole
- XPS: Free thiol S2p **163 eV**, Thiolate S2p at **162 eV**
- IR: **2925 cm⁻¹** or above for more disordered monolayers -> **2916-2917 cm⁻¹** for highly crystalline, long-chain alkanethiols.
- 1- 10mM in EtOH 12-18h at RT

Stability of Au-S bond as a function of temperature

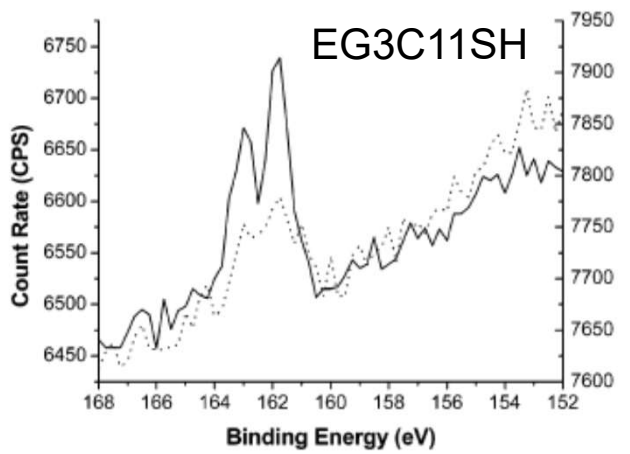
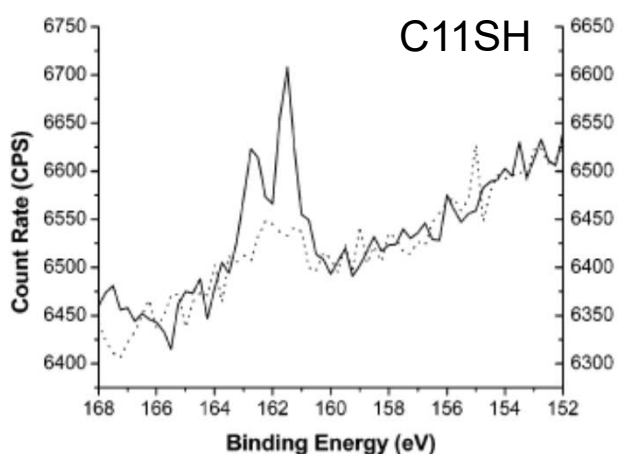


Lerau et al, Anal. Chem. 2013, 85, 9204–9212

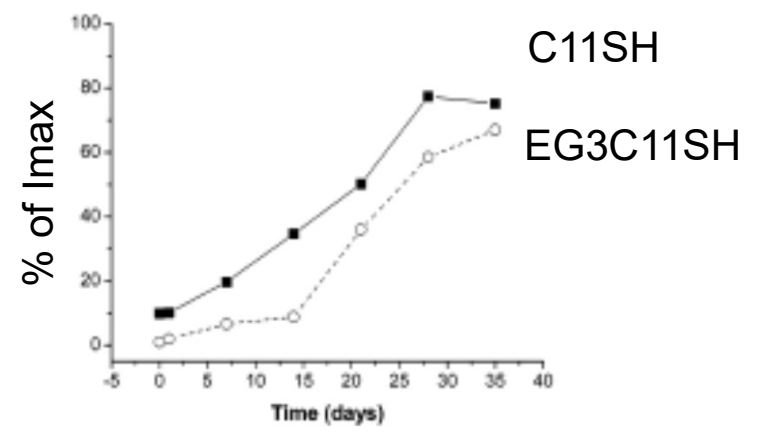


Scott Day et al, Langmuir 2011, 27, 12434–12442

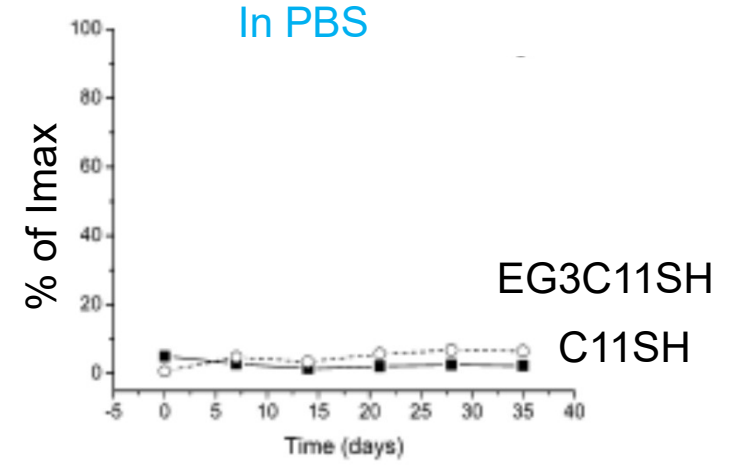
Stability of Au-S bond upon storage



In PBS



In PBS

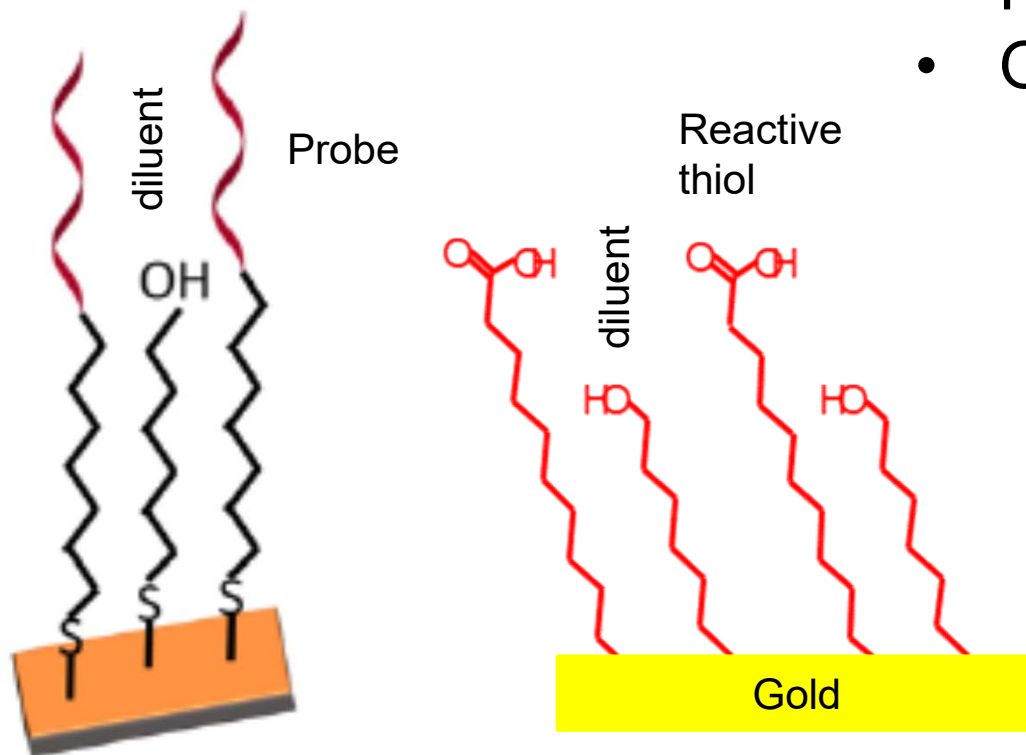


In degassed PBS + Uric acid

Flynn *et al*, Langmuir, 2003, 10909-10915



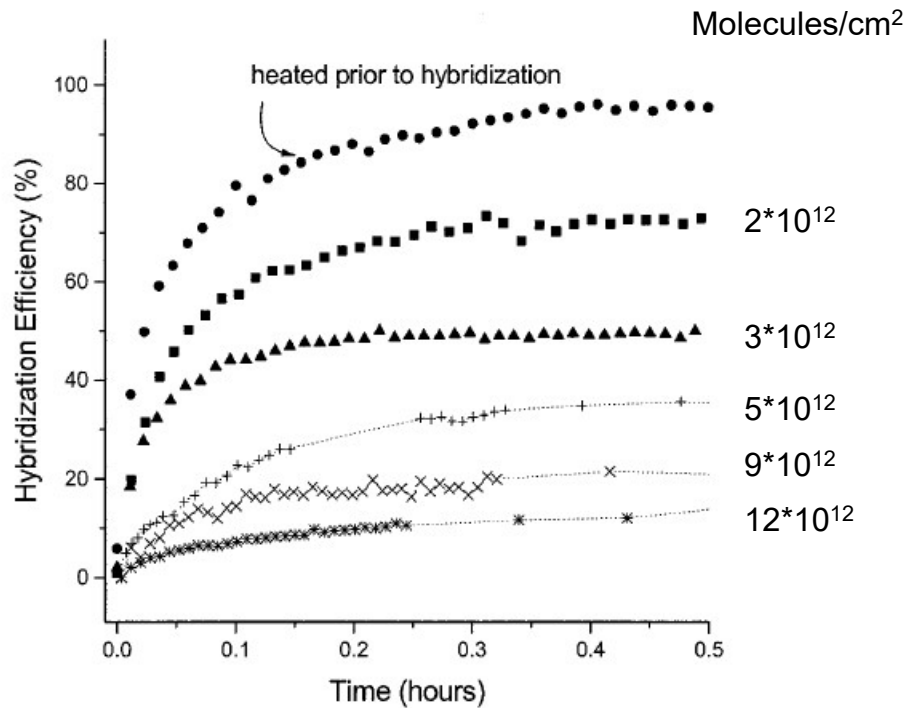
mixed Self Assembled Monolayer



- Improve reactivity
- Reduce steric hindrance
- Orientation of immobilized DNA

Why mixed SAMs

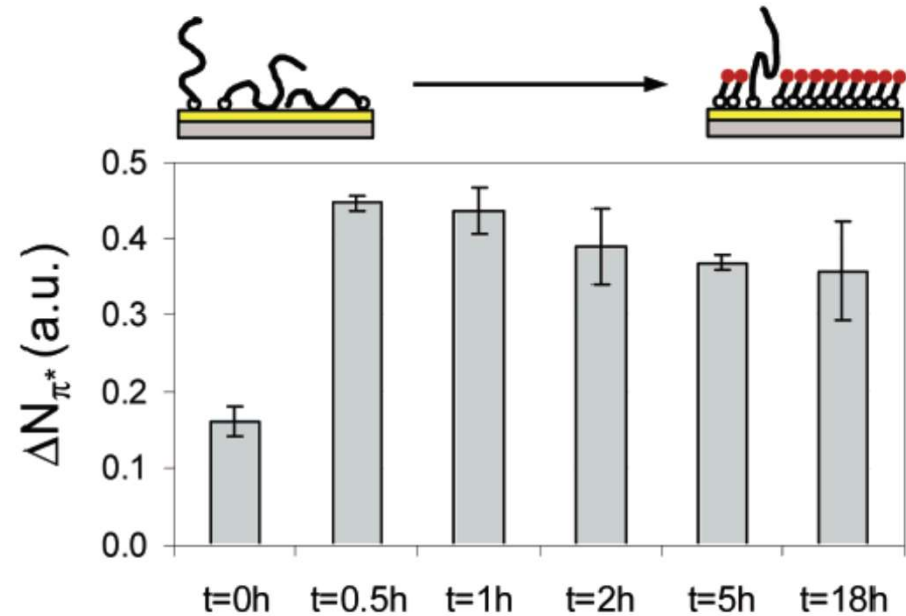
- Steric hinderance



Peterson et al, NAR, 2001, 29, 5163-5166

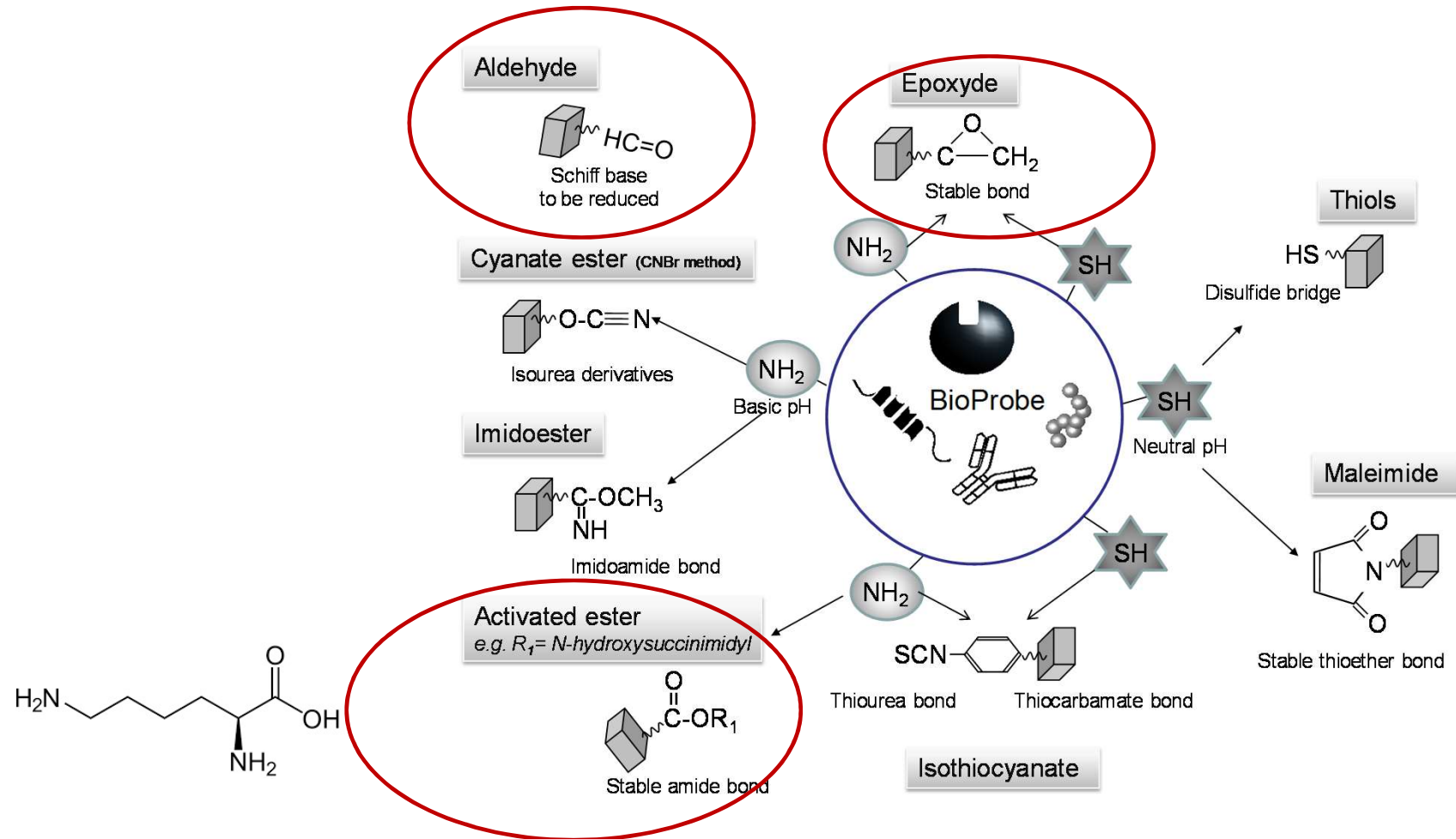
- Orientation of the probe
- A > C > G > T

Kimura-Suda *et al*, J. Am. Chem. Soc. 2003, 125, 9014-9015
Also Liu, Phys. Chem. Chem. Phys., 2012, 14, 10485-10496

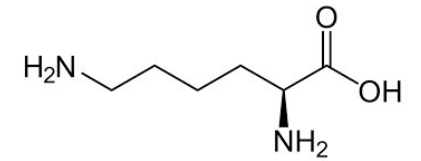
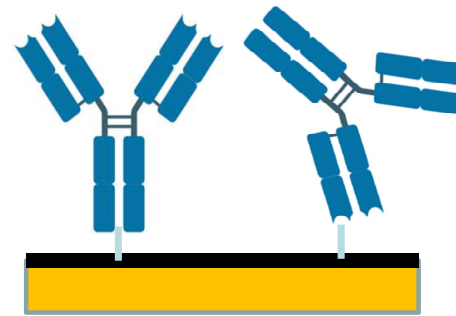
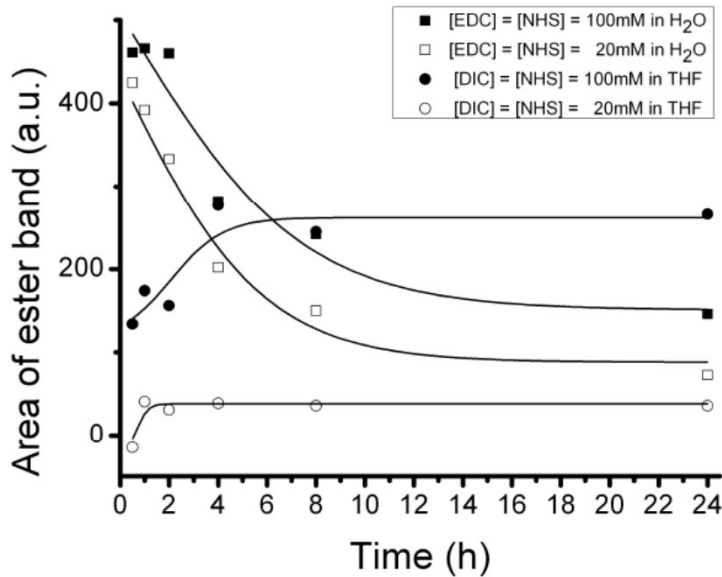
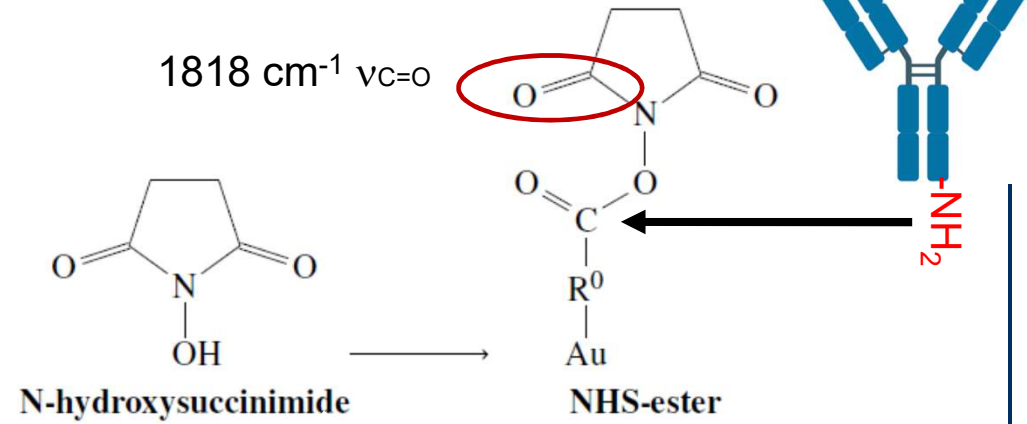
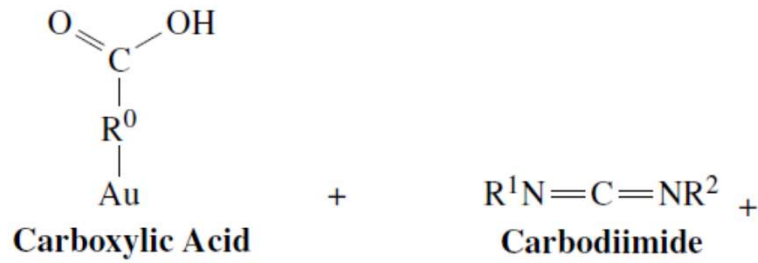


Lee *et al*, Analytical Chemistry, 2006, 3321-3325

How to graft biomolecules via chemical functionalisation

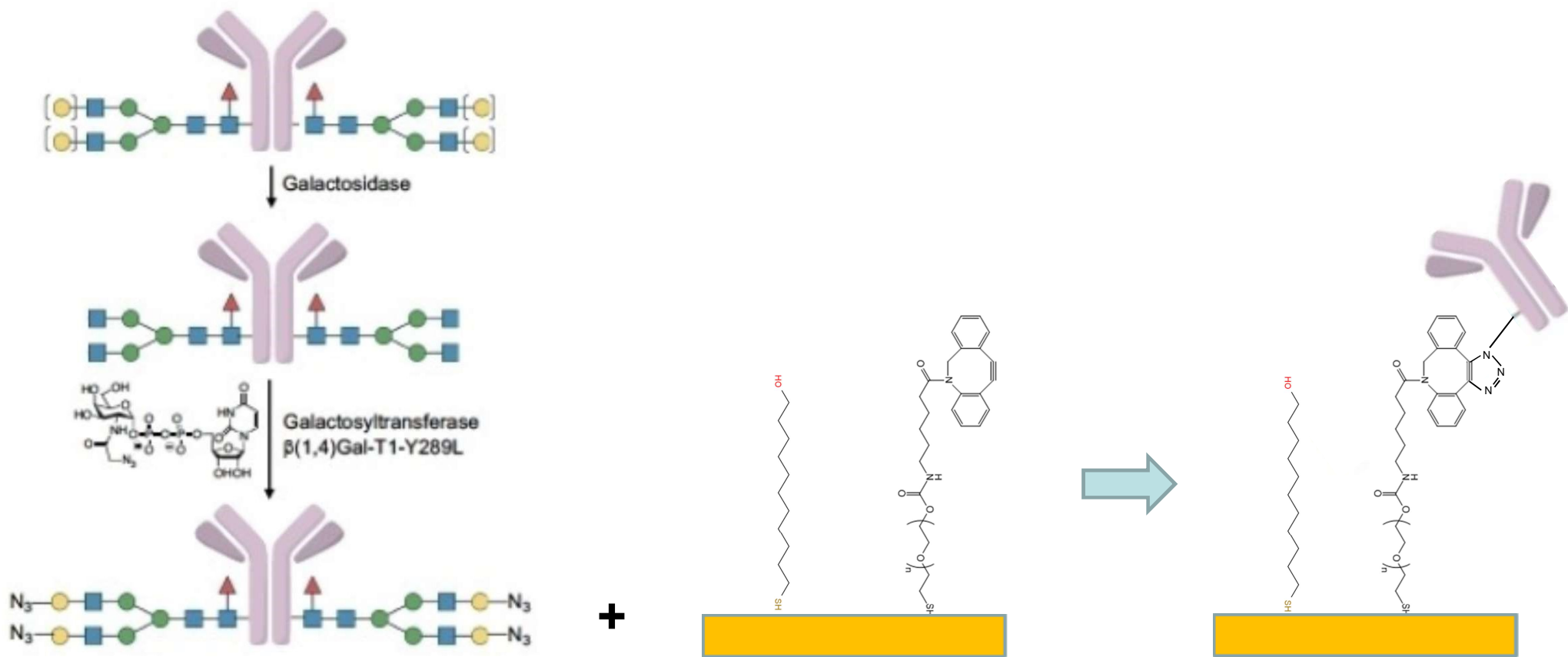


NHS ester activation



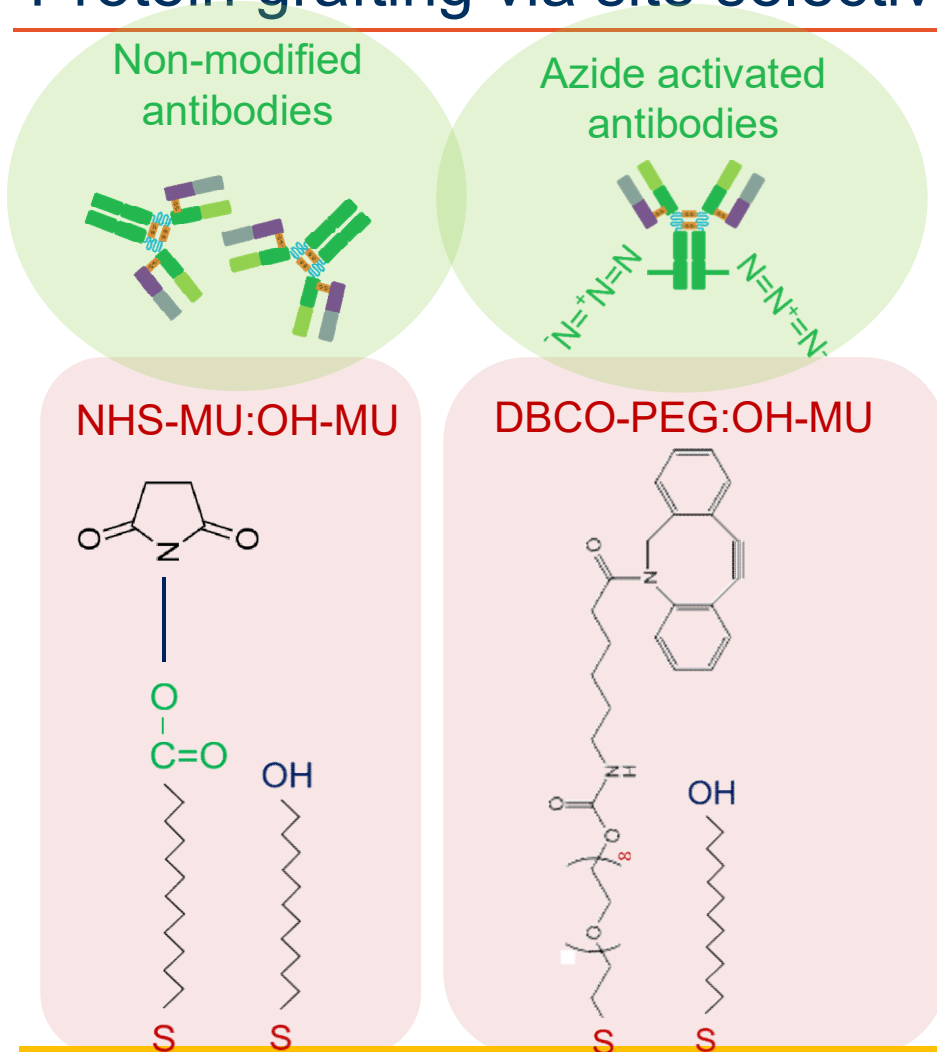
=> 85 lysine per IgG

Protein grafting via site selective modification of antibodies

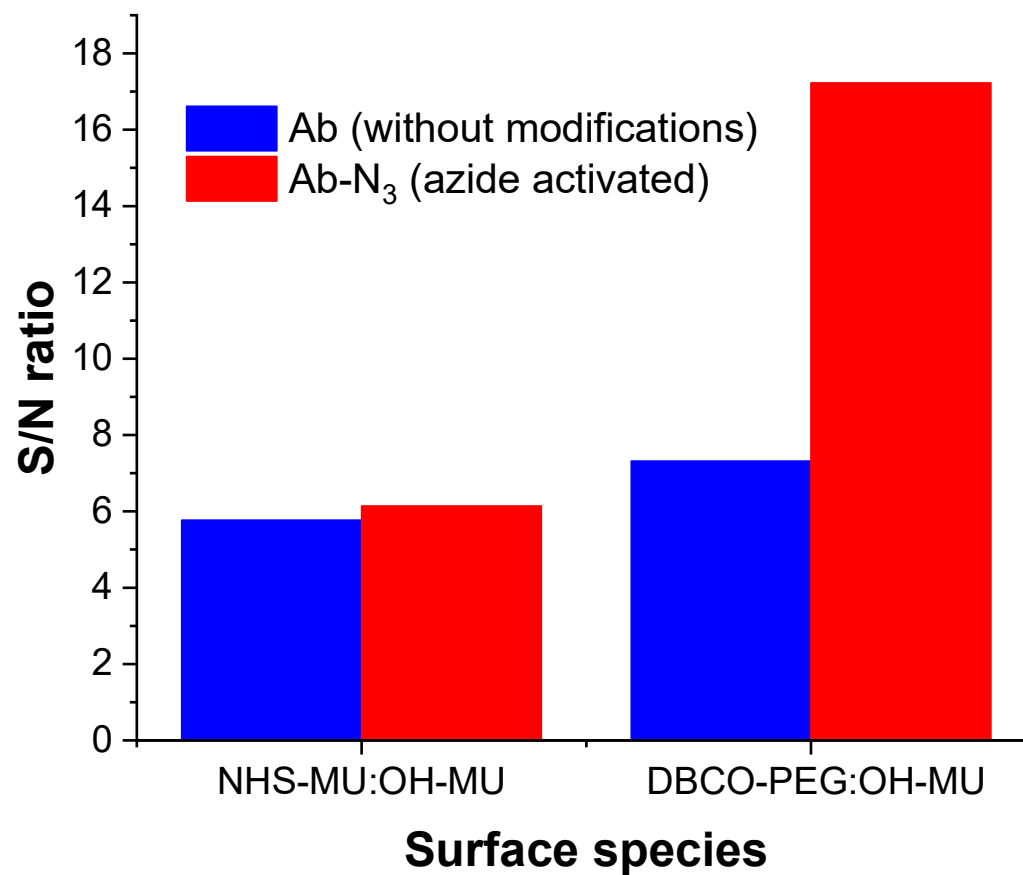


Chem. Rec. 2021, 21, 3005– 3014

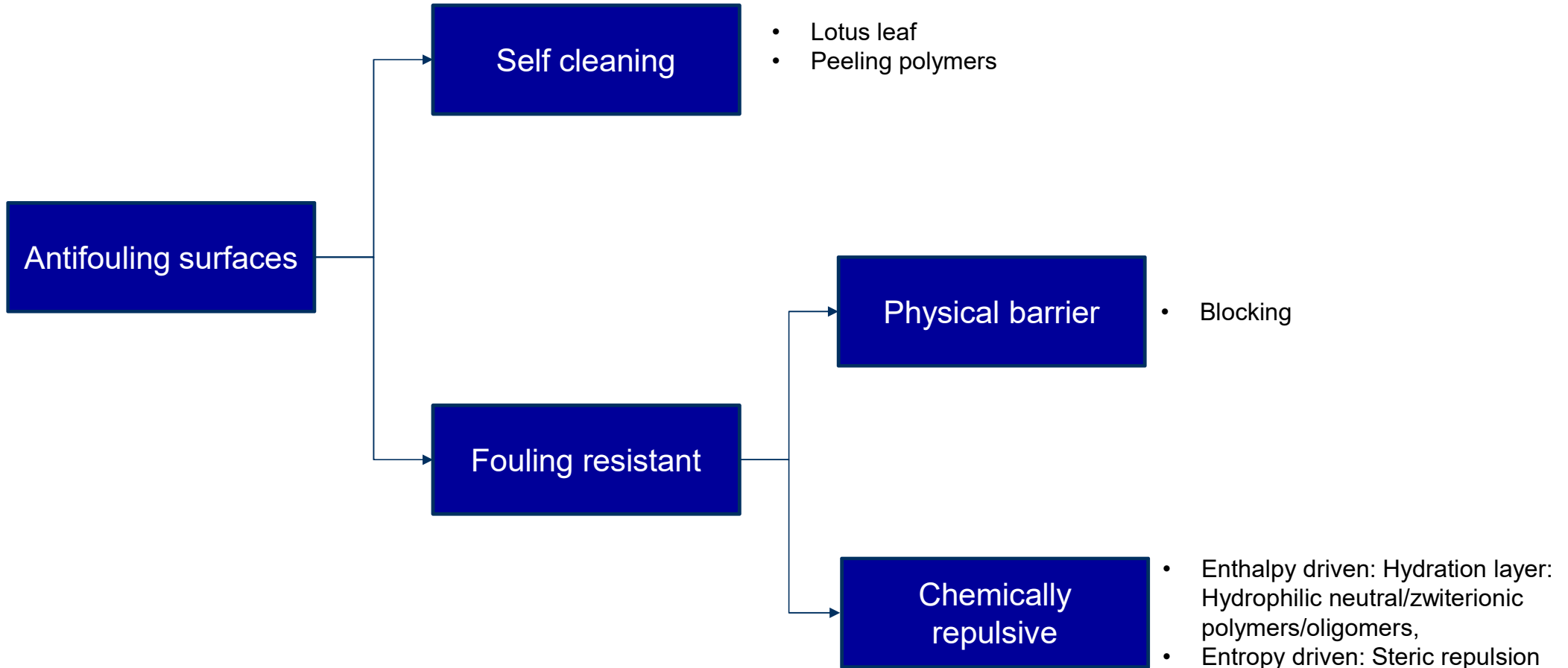
Protein grafting via site selective modification of antibodies



Signal/Noise (S/N) ratio for Immobilized Species

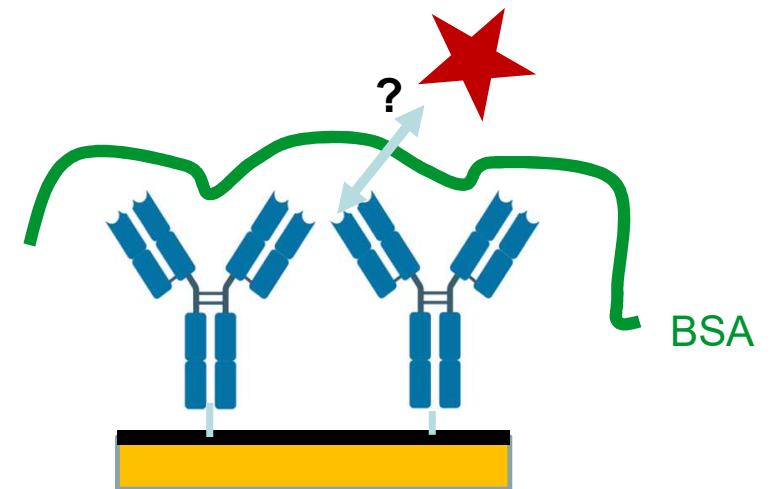


Anti-fouling strategies



Fouling resistant surfaces

- Chemically repulsive
 - Hydrophilic neutral molecules
 - **PEG₃₋₈**, Polyglycerol, Dextran, Hyaluronic acid, agarose, Poly(2-hydroxyethyl methacrylate)...
 - Zwitterion: carboxybetaine, sulfobetaine phosphorylcholine (PC), zwitterionic peptides...
- Blocking,
 - **BSA** (1%, rinsing with PBS or PBS-T 0,02)
 - Casein or dry milk (contains biotin),
 - Mucin
 - Salmon Sperm DNA,
 - PolyA....
 - Commercial blocking solution



Review

Chen *et al*, Acta Biomaterialia, 2021, 45–62

Lin *et al*, Analyst, 2020, 1110–1120

Maan *et al*, Adv. Funct. Mater. 2020, 2000936

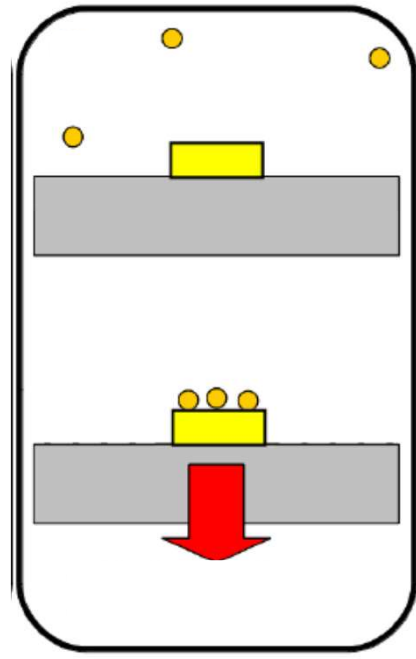
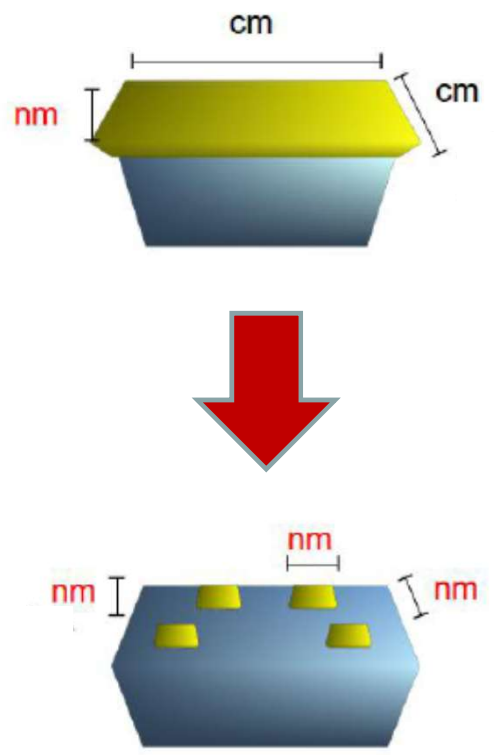
Ruiz *et al*, J Biomater Sci Polym Ed, 1999, 931–55

Tan *et al*, Int. J. Electrochem. Sci., 2020, 9446 – 9458

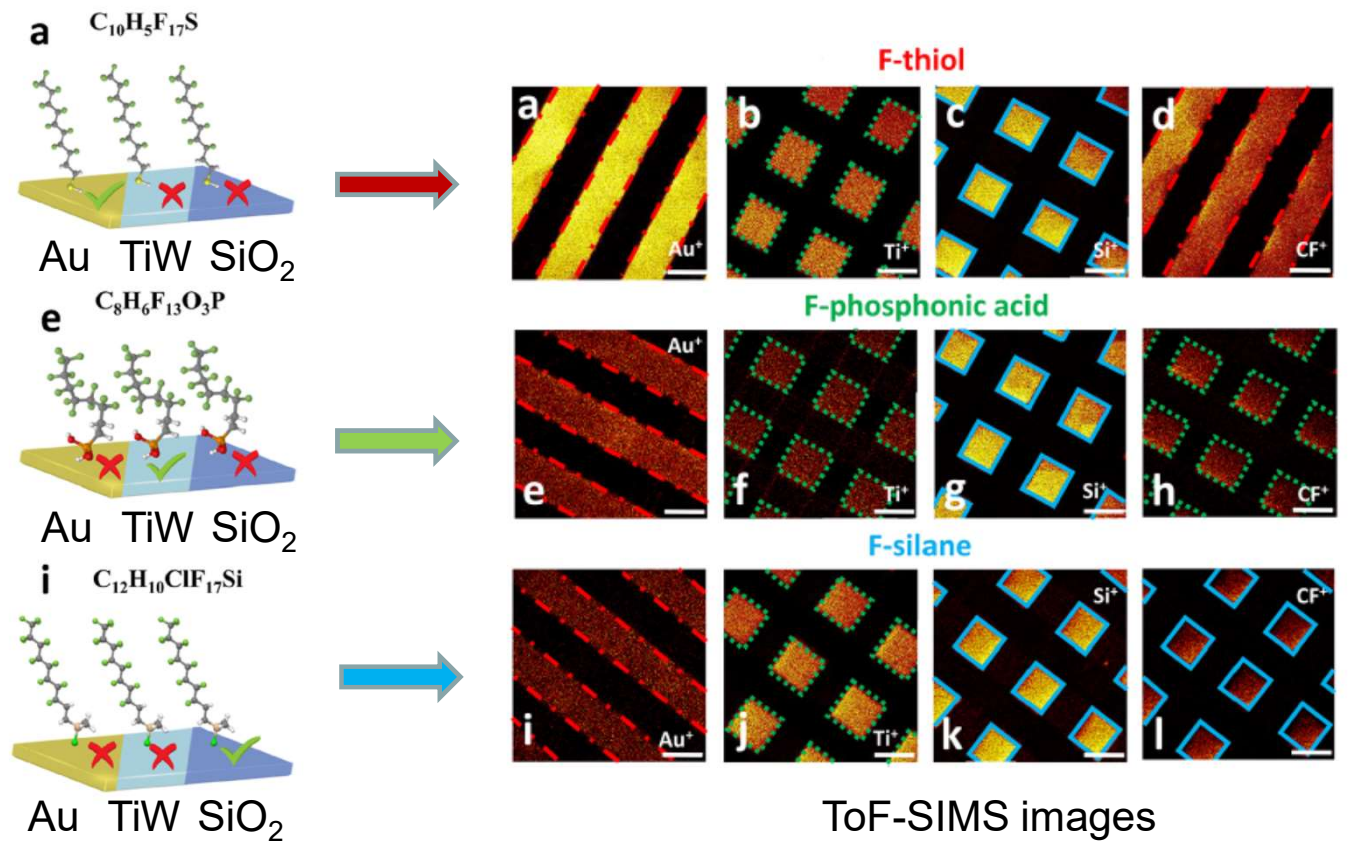
A. Morlay *et al*, Measurement, 2017, 305–310

B. Petrou *et al*, Biomater. Sci., 2018, 2282–2297

Multimaterial substrate



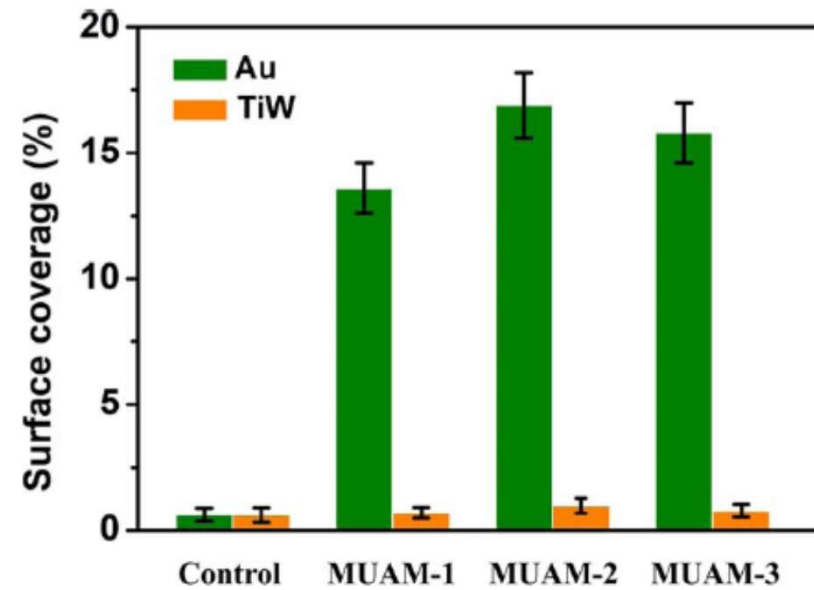
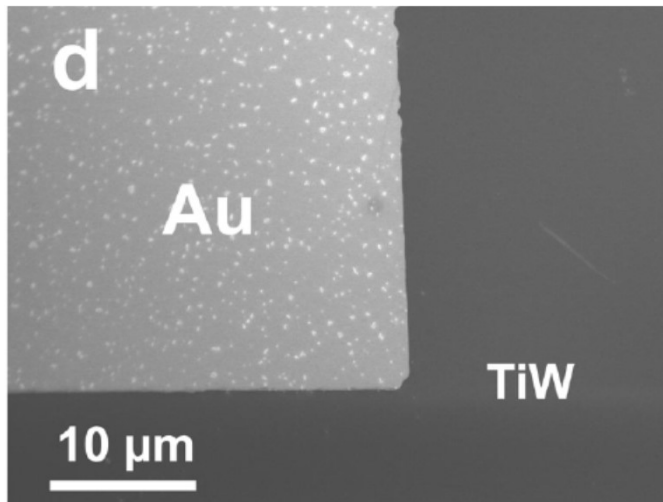
Orthogonal chemical functionalisation of multimaterial substrate



Zhang *et al*, *Langmuir*, **2020**, 14960-14966
Palazon *et al*, *Beilstein J. Nanotechnol.*, **2015**, 2272-2277



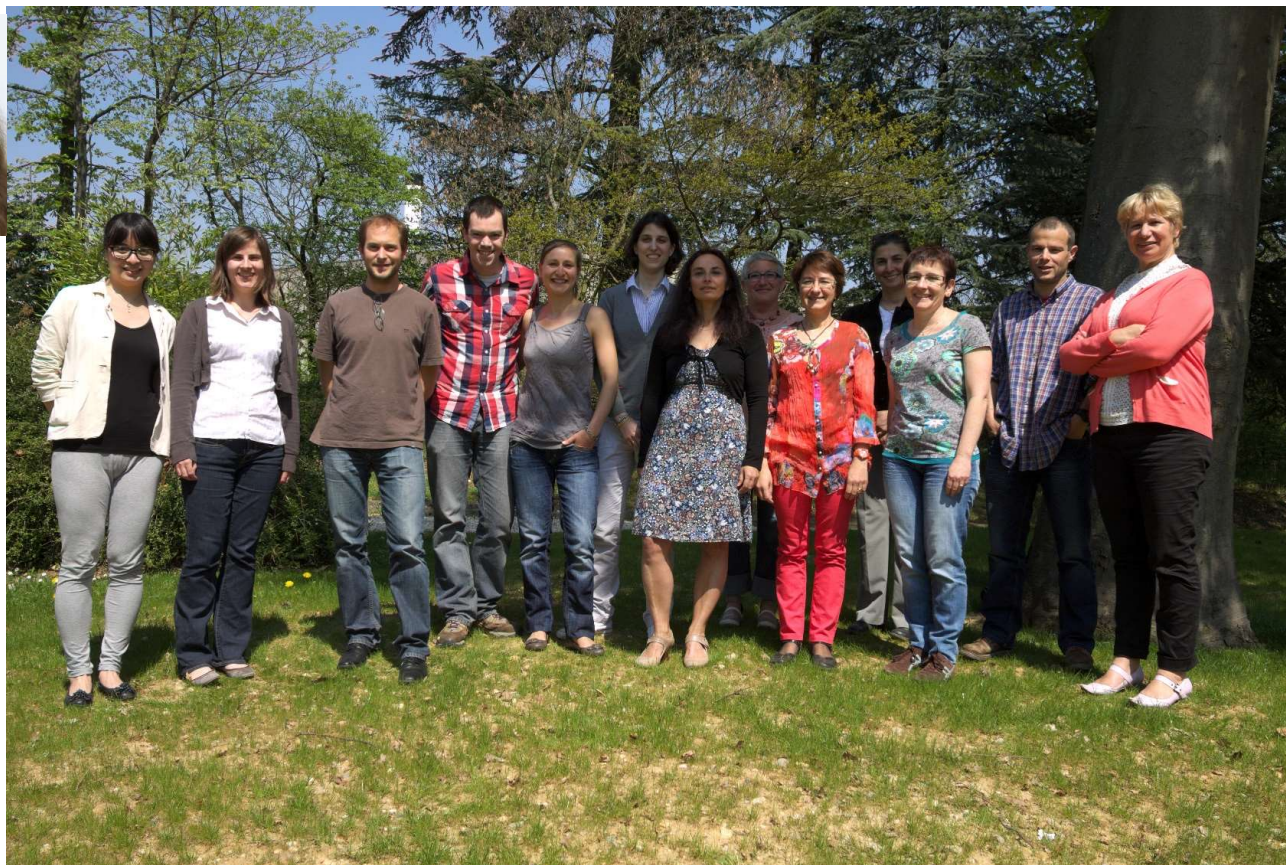
Orthogonal chemical functionalisation of multimaterial substrate



Conclusions

- Cleaning
 - Oxygen Plasma Cleaning
 - $\text{Au}_2\text{O}_3 \Rightarrow$ Thickness dependence
 - Time or EtOH
- Au-S is reversible
- Oriented immobilization
 - DNA: A rich sequences, mixed SAMS
 - Protein
- In complex media, blocking remains to be tackle on a case to case \Rightarrow no universal anti-foulant.
 - BSA blocking and PEG a good starting point

Acknowledgement



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Thank you for your attention

