EPFL

EDMI Microsystems and Microelectronics

MICRO-614: Electrochemical Nano-Bio-Sensing and Bio/CMOS interfaces

Lecture #6

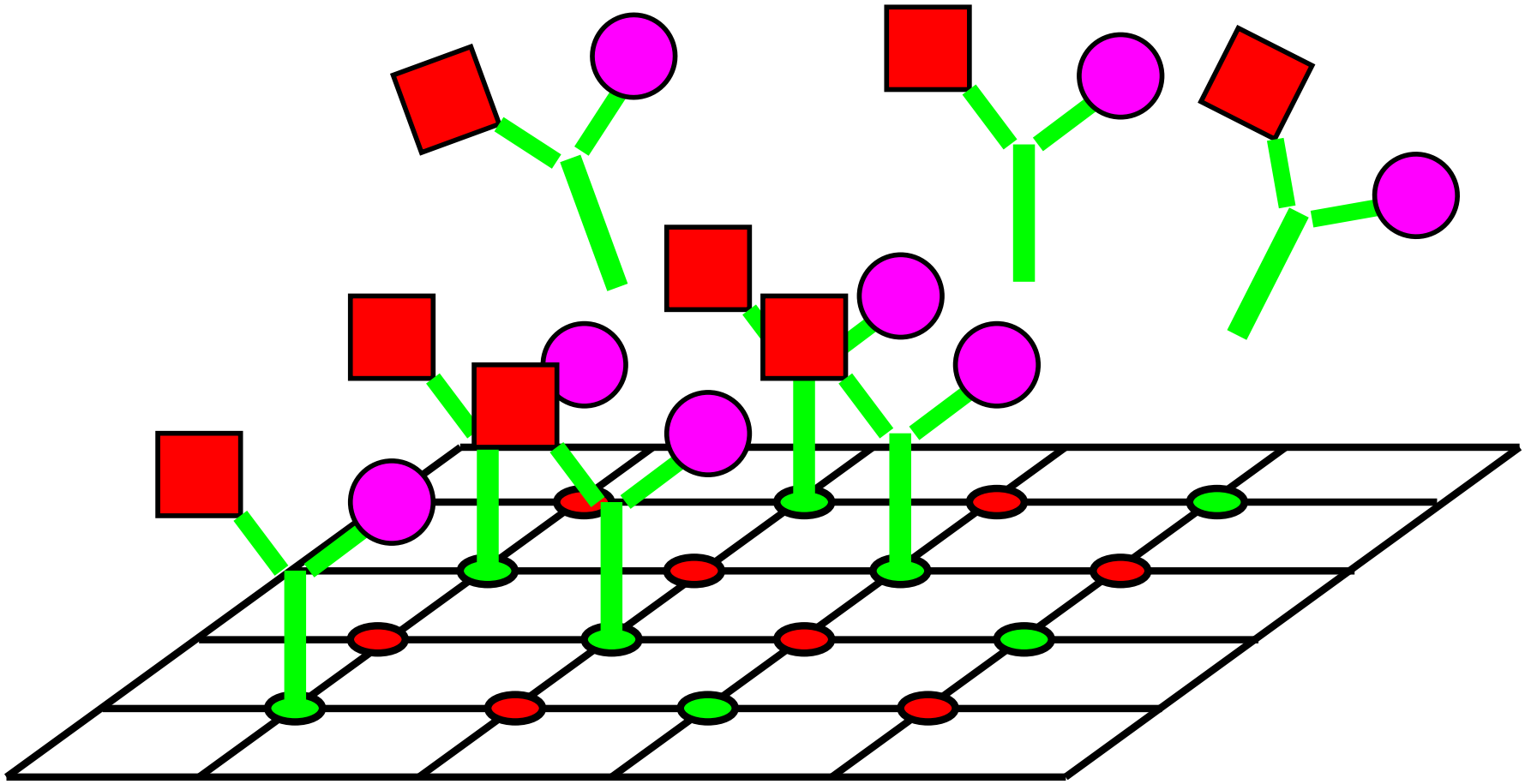
Probes immobilisation (methods & models)

Lecture Outline

(Book Bio/CMOS: Chapter' 6)

- Different immobilization methods
- Langmuir Model
- Kisliuk Model
- Steric Hindrance Model
- Spreading Model

Self-assembly on a surface



Adsorption models

Different immobilization methods

- Drop casting
- Covalent bonding:
  peptide bond
- Covalent bonding:
  thiol-groups
- Covalent bonding:
  silane-groups

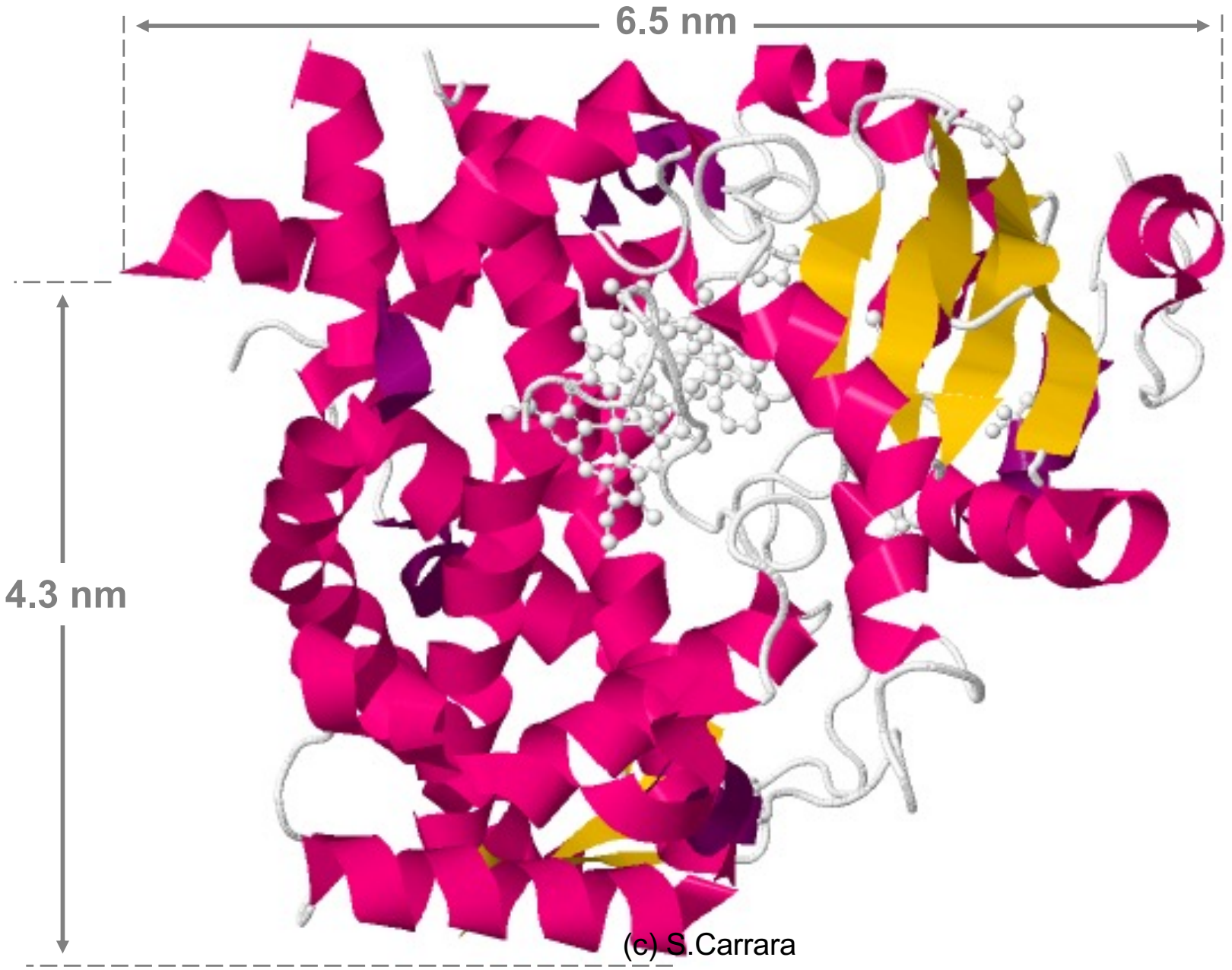
Proteins drop cast onto hydrophobic surfaces

$$\Delta H_{\Phi} = \alpha \, A_{contact}$$

$$\alpha = -104.5 \frac{kJ}{mol \, nm^2}$$

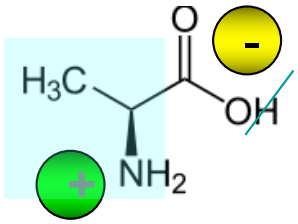
The hydrophobic forces are of the same intensity of the usual ones in Antigen/Antibody interactions

Cytochromes P450

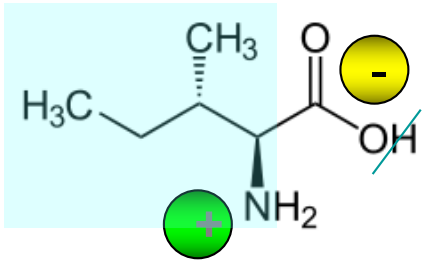


Hydrophobic AA

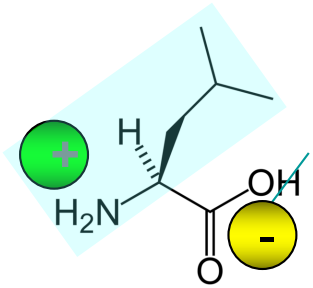
Hydrophobic Side Chains



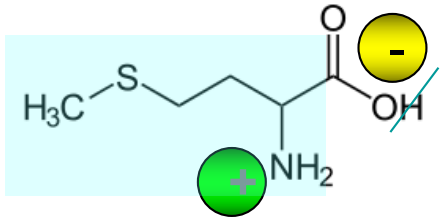
Alanine



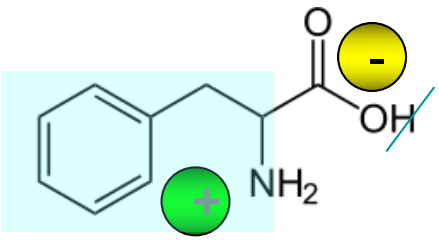
Isoleucine



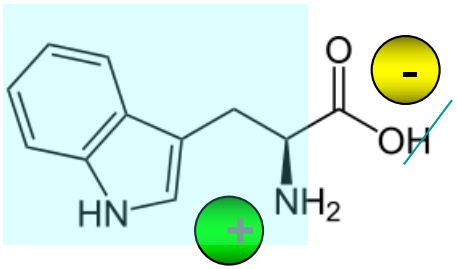
Leucine



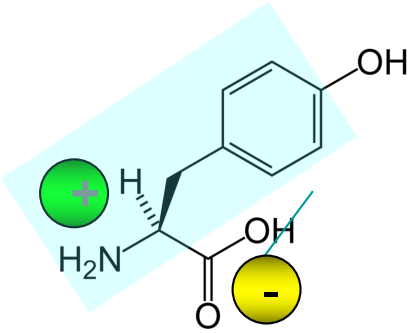
Methionine



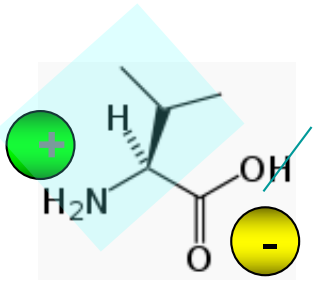
Phenylalanine



Tryptophan

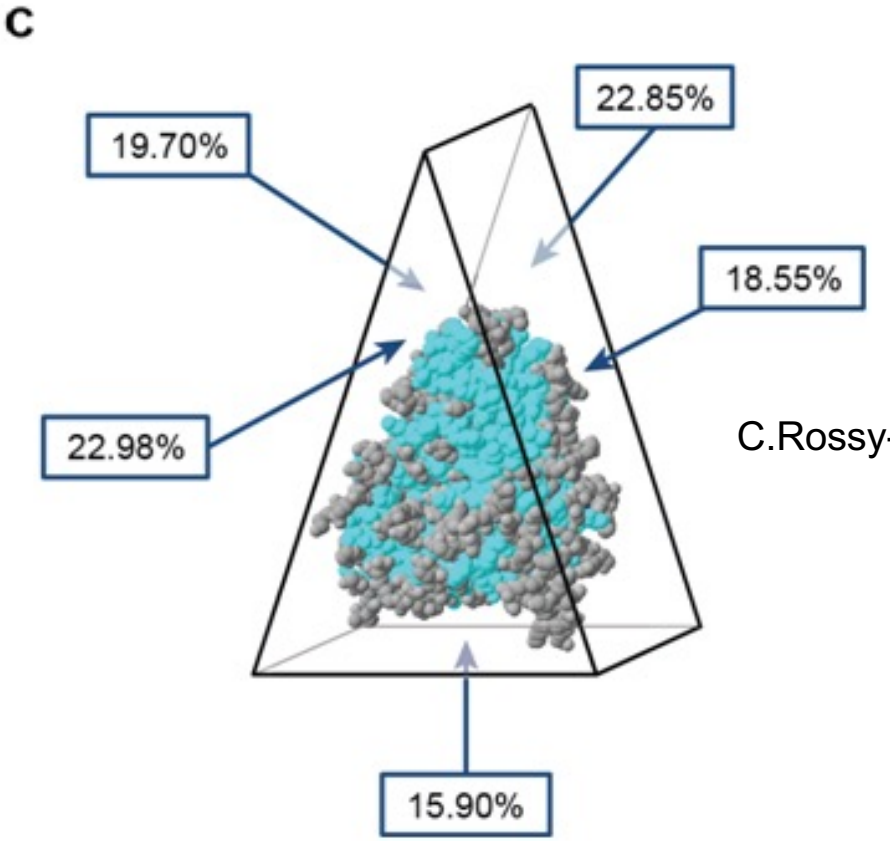


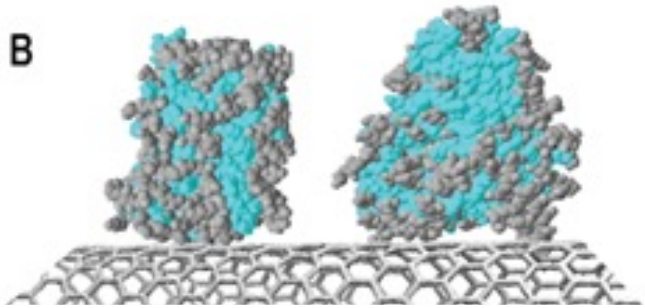
Tyrosine



Valine

P450 onto CNT surface





C.Rossy-Bai, S. Carrara, et al., Sensors 2013

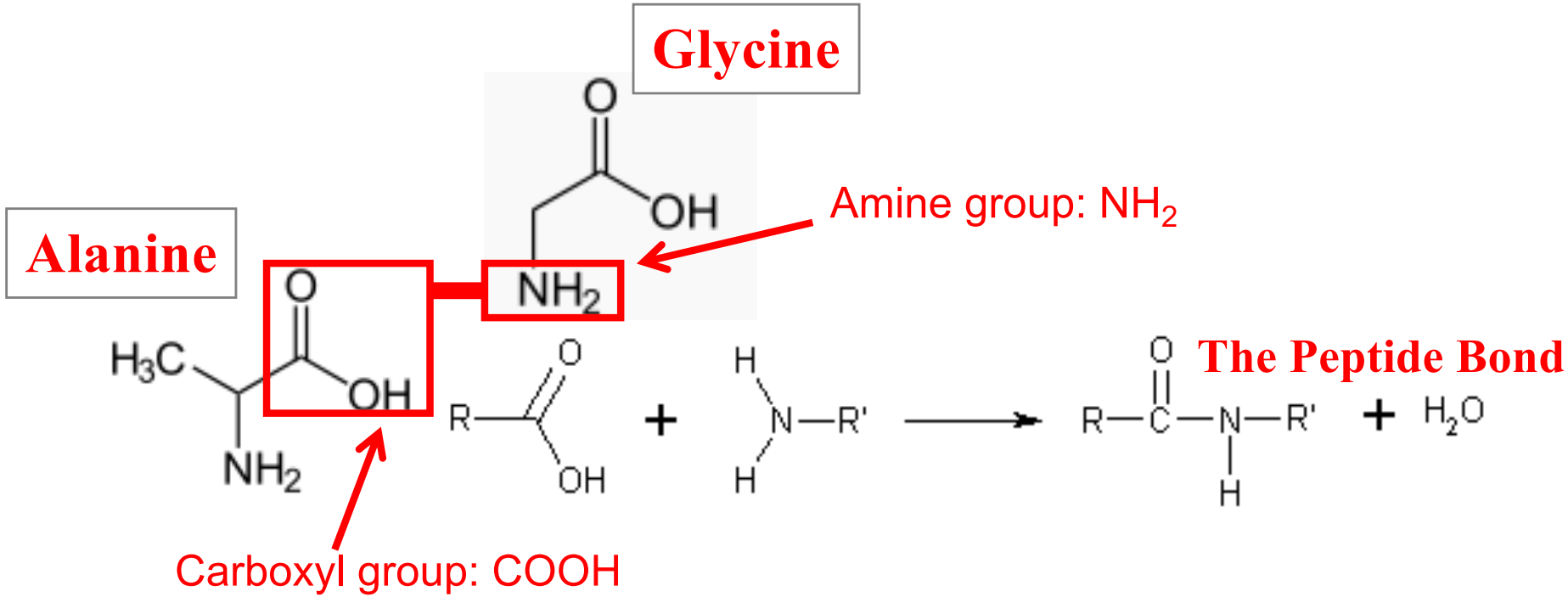
The hydrophobic Amino Acids anchor the proteins P450 onto the Carbon Nanotubes' surface

P450 onto CNT surface

Complex	Area [Å^2]	Enthalpy [-kJ/mol]
Ab/Ag	150-690	16-74
P450/Carbon	272-378	28-40

The hydrophobic forces are of the same intensity of the usual ones in Antigen/Antibody interactions

The Peptide Bond

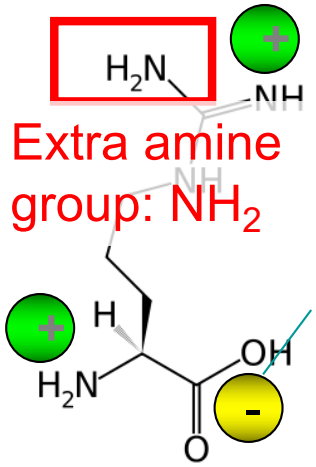


The same mechanism used by Peptides may be used to covalently immobilize probes on a sensing surface

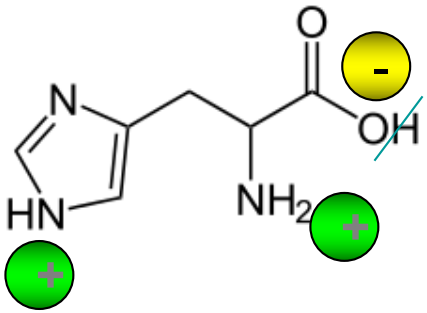
Charged AA

Neg. Charged

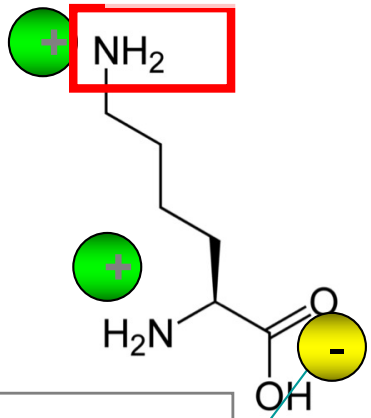
Positively Charged



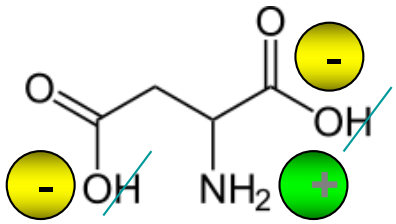
Arginine



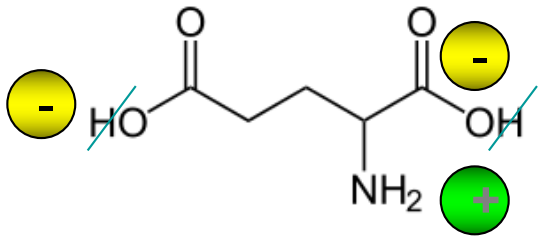
Histidine



Lysine

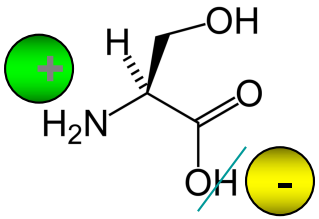


Aspartic Acid

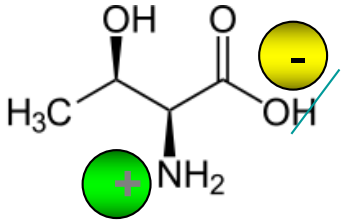


Glutamic Acid

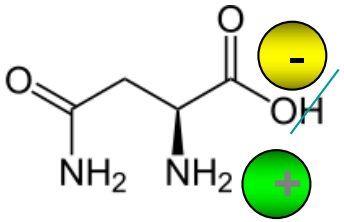
Polar Uncharged



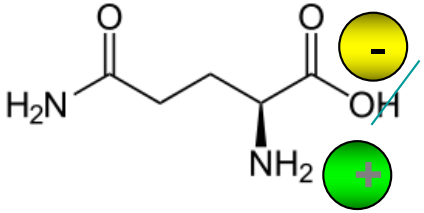
Serine



Threonine

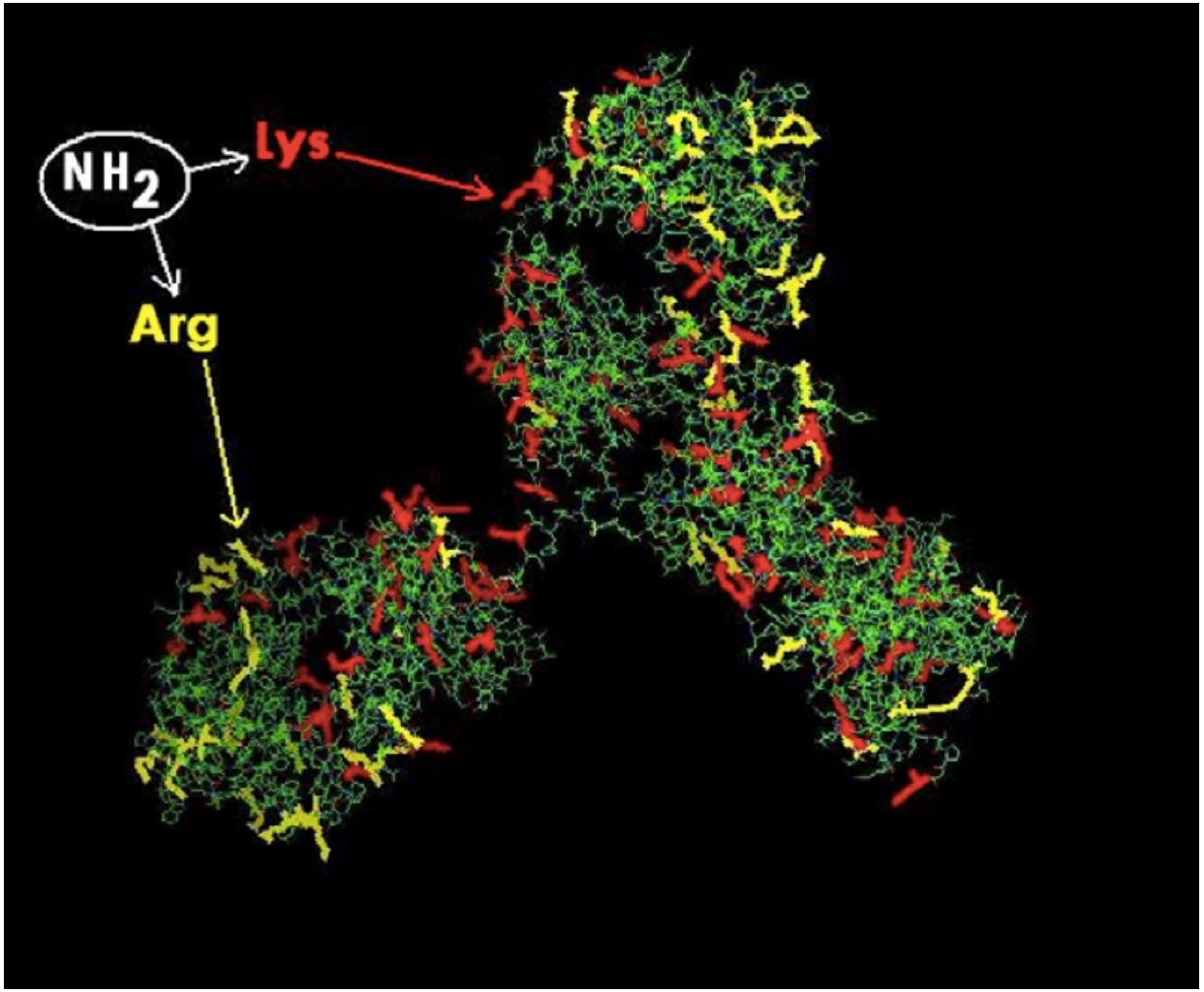


Aspargine



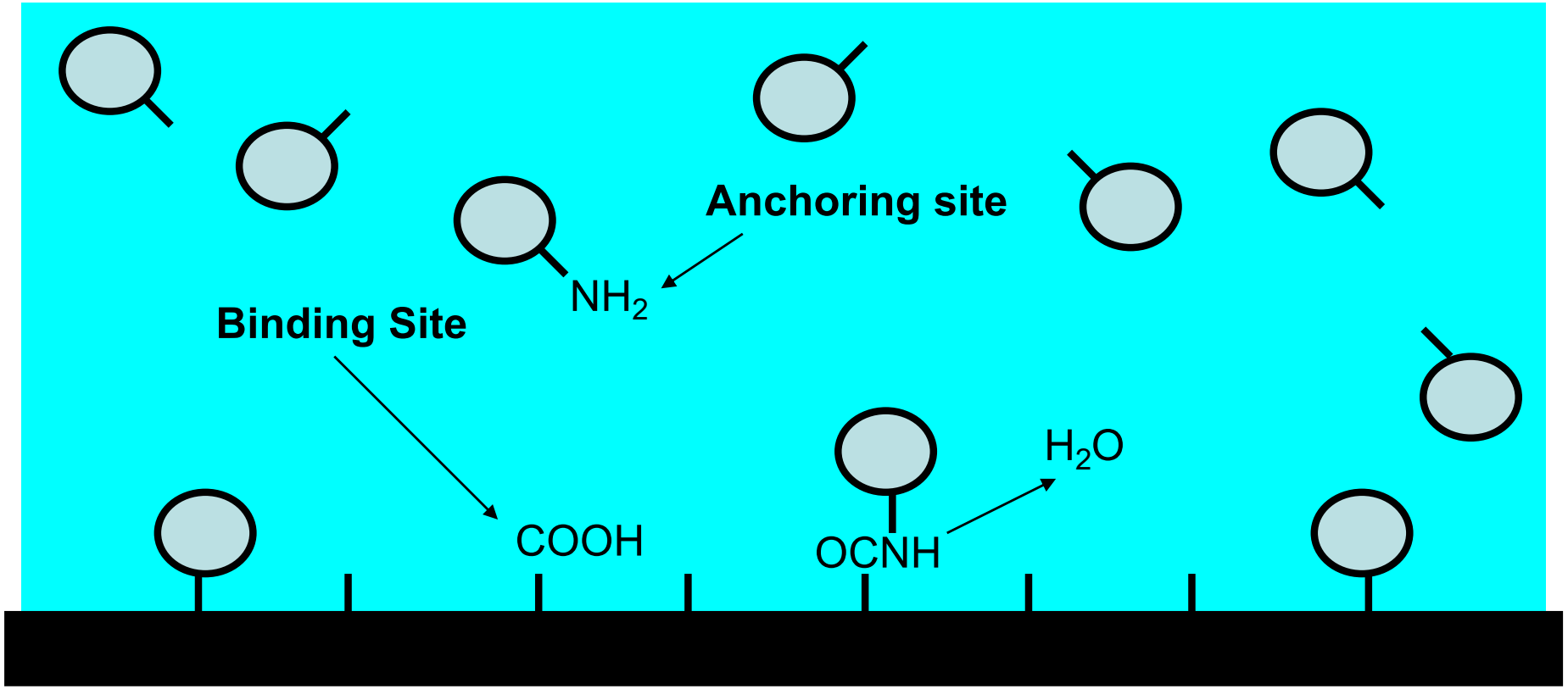
Glutamine

Hydrophobic Residues



Arginines and Lysines in an antibody

Peptide bond on carbon surface



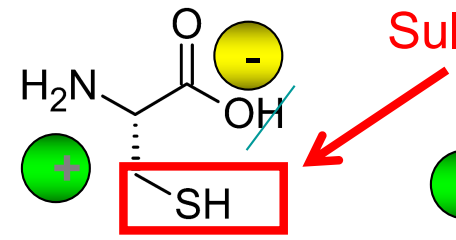
How to obtain COOH groups?

- Strong bases (e.g., NaOH) or acids (e.g., H_2SO_4)
- Energy to break the C-C structure (e.g., electrochemical potentials, plasma irradiation, heating, etc…)

Several treatments are possible to create the right functional groups on the sensing surface

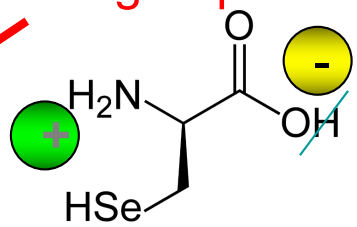
Neutral AA

Special Cases

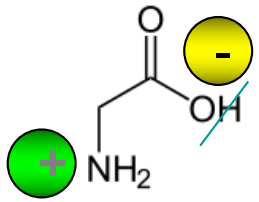


Cysteine

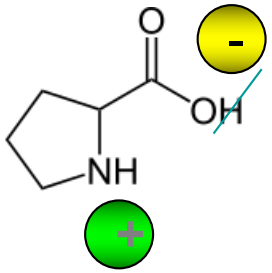
Sulfur group: SH



Selenocysteine

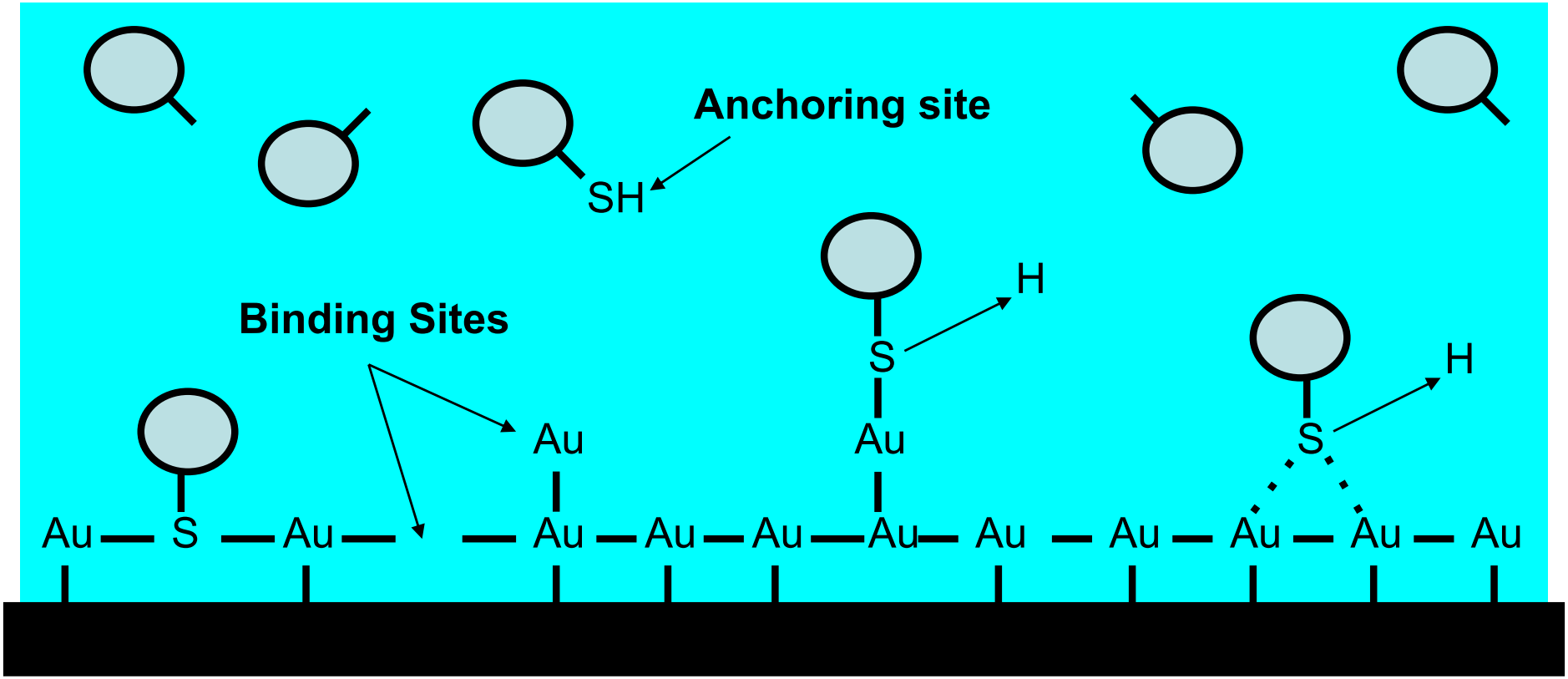


Glycine



Proline

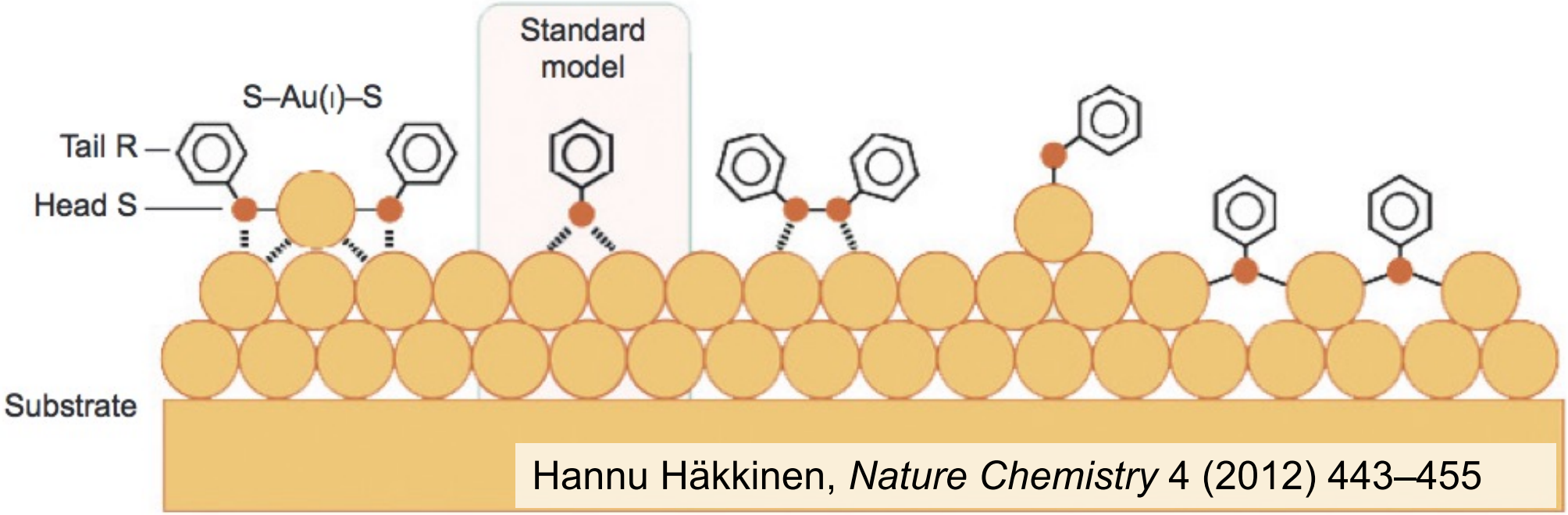
Thiol bond on gold surface



Thiols bond

- Thiols are frequently used on metallic substrates because of the strong affinity of sulfur for noble metals (e.g., for our aim, platinum, gold, silver, copper, …)
- The sulfur gold interaction provides a semi-covalent bond with a strength in the order of **100kJ/mol** (covalent O-H bond in water molecules is about 460 kJ/mol)

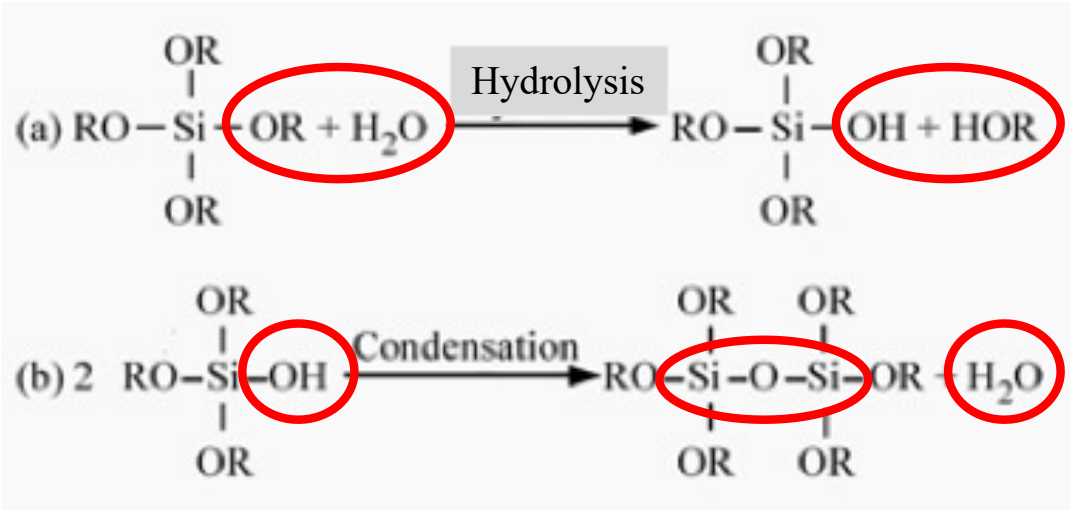
Thiol bond models



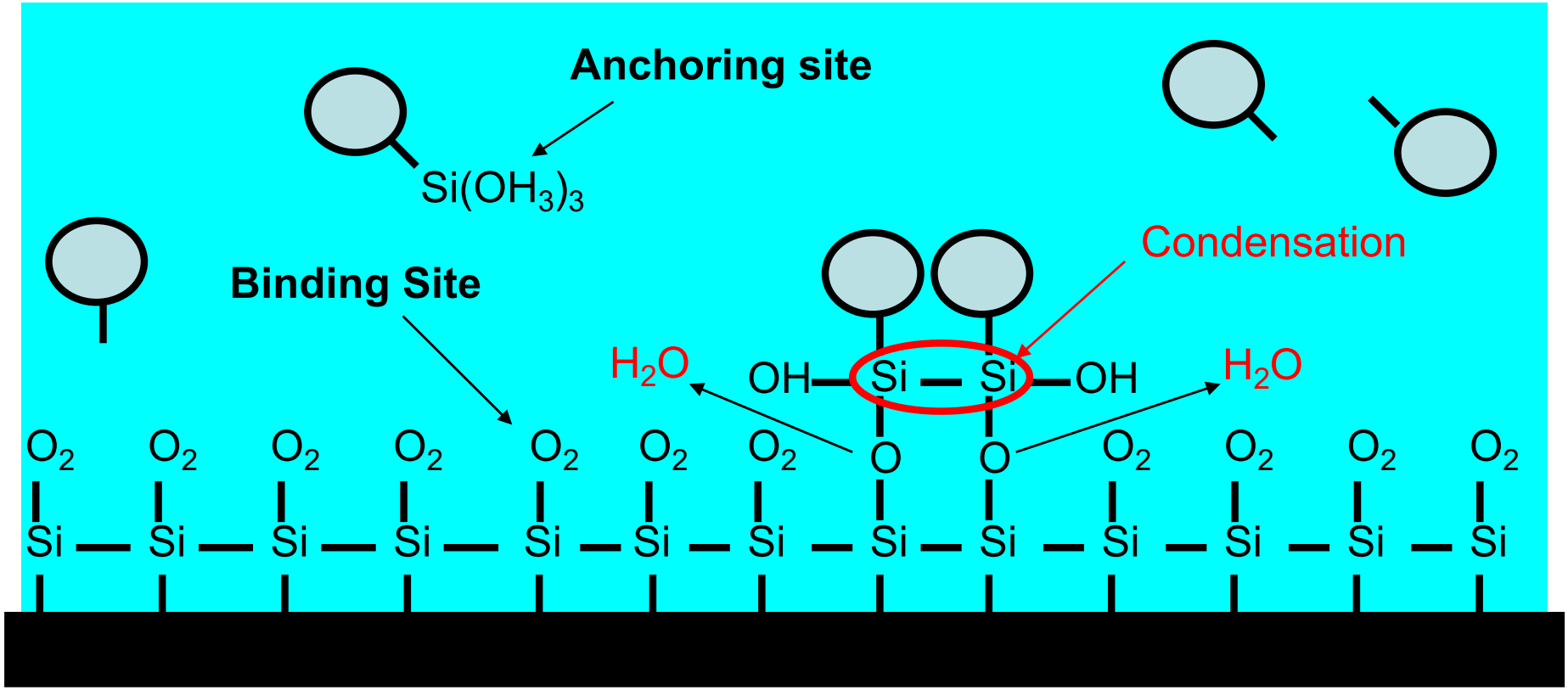
The ‘standard model’ foresees a monothiolate binding at atop, while new experimental evidence shows other key structural with complexes where the bridging gold atom is in a formal oxidation state of +1

Silanes bond

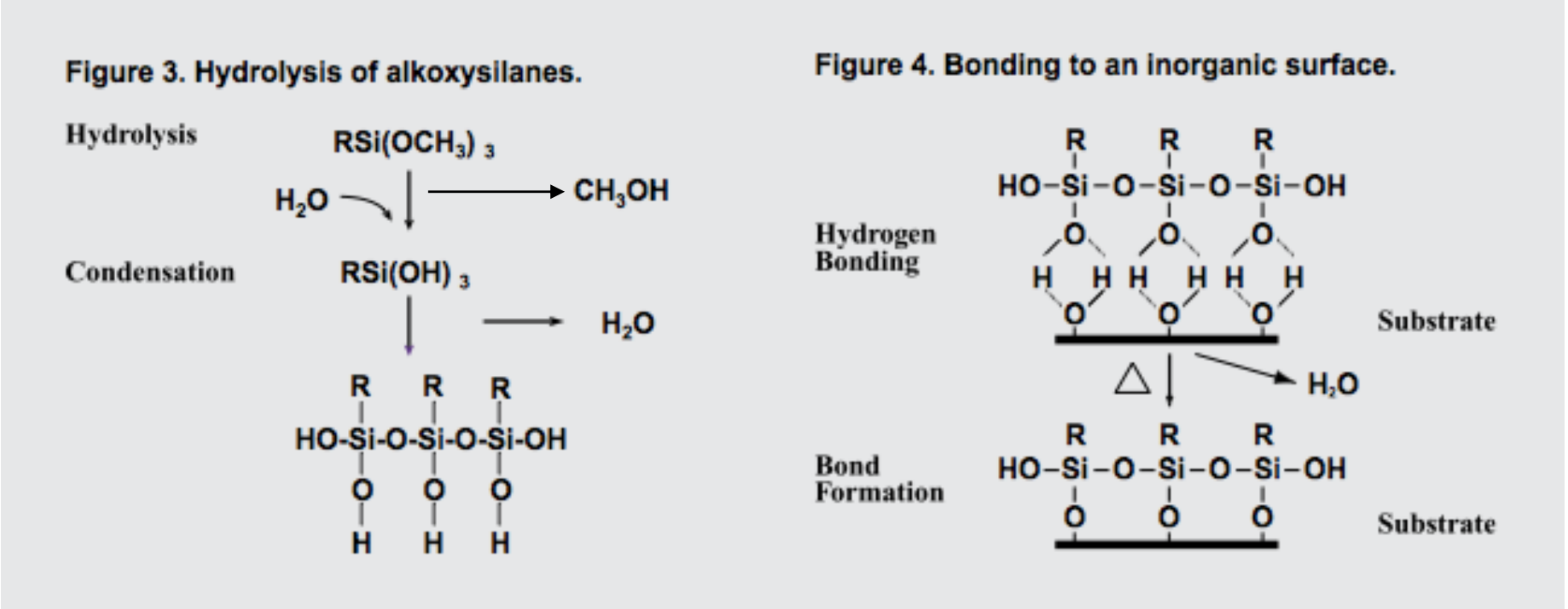
Silanes usually bond well to most inorganic silicon substrates. Typically, the alkoxy groups on silicon hydrolyze to silanols, either through the addition of water or from residual water on the inorganic surface



Silane bond on silicon surface



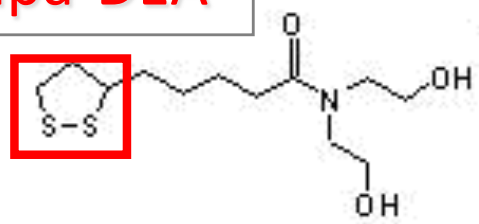
Silanes bond



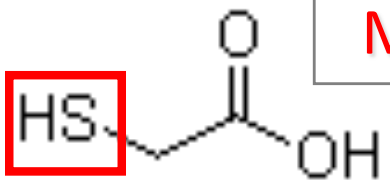
The formation of silanes film is due to different mechanisms: condensation and hydrolysis

Molecules with alkyl chains

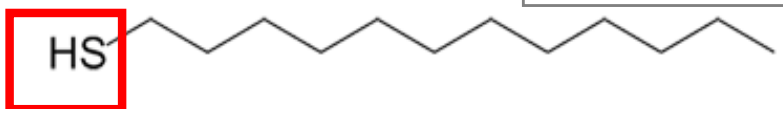
Lipa-DEA



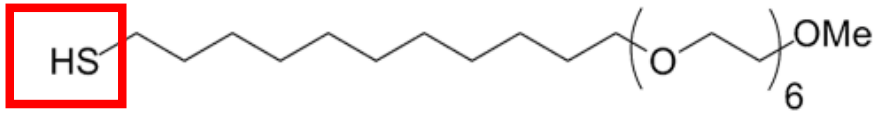
Mercaptoacetic acid



Alkane-Thiol

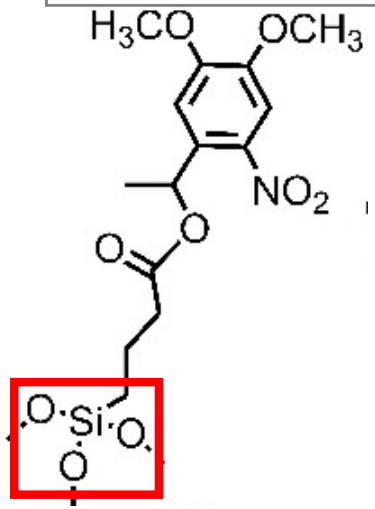


For anchoring onto Gold Surfaces



Ethylene-Glycol Alkanethiol

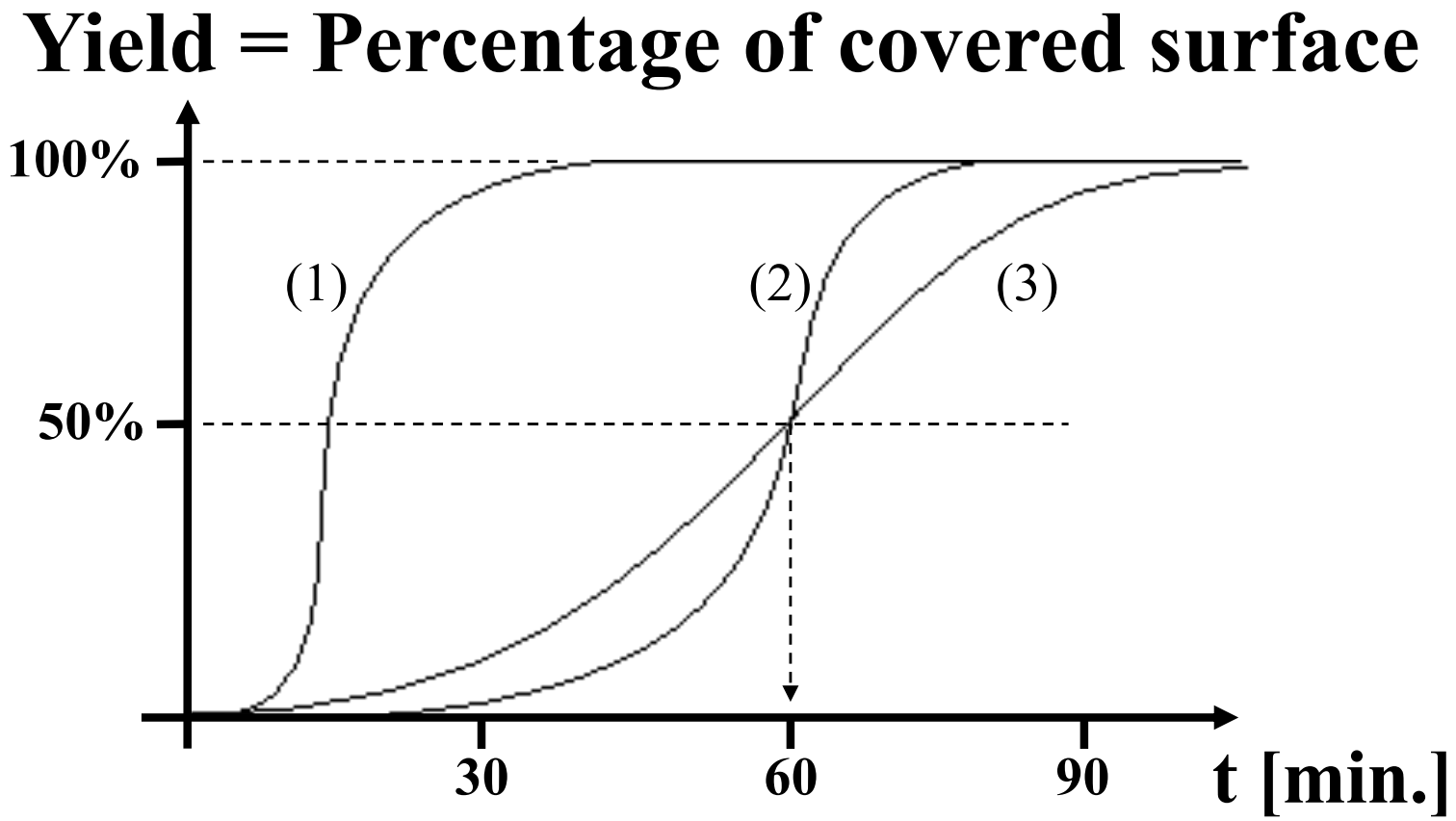
Alkane-Silane



For anchoring onto Silicon Surfaces

Aliphatic chains may provide Fatty Acids as well as Thiols or Silanes

Different molecules result in different Yields onto the same surface



How to characterize the Probes Immobilization?

What are the mechanisms of self-assembly?

How to monitor the self-assembly process?

How to check the film quality?

Adsorption kinetic models

1. Langmuir Model
2. Kisliuk Model
3. Steric Hindrance Model
4. Spreading Model

The Langmuir Model

Ideal Gas Adsorption.

Four Assumptions:

- The Surface of adsorbent is uniform
- Adsorbed molecules do not interact
- All adsorption occur with the same mechanism
- Only a monolayer is obtained at the maximum adsorption

The Langmuir Model

A molecule L is adsorbed in quantity A onto a surface with an amount BS of free binding sites

$$L + BS_S \Leftrightarrow A$$

$$R_A = k_A p[BS_{free}]$$

$$R_D = k_D[A]$$

$$Equilibrium \rightarrow R_A = R_D \rightarrow K = \frac{k_A}{k_D} = \frac{[A]}{p[BS_{free}]}$$

The Langmuir Model

A molecule L is adsorbed in quantity A onto a surface with an amount BS of free binding sites

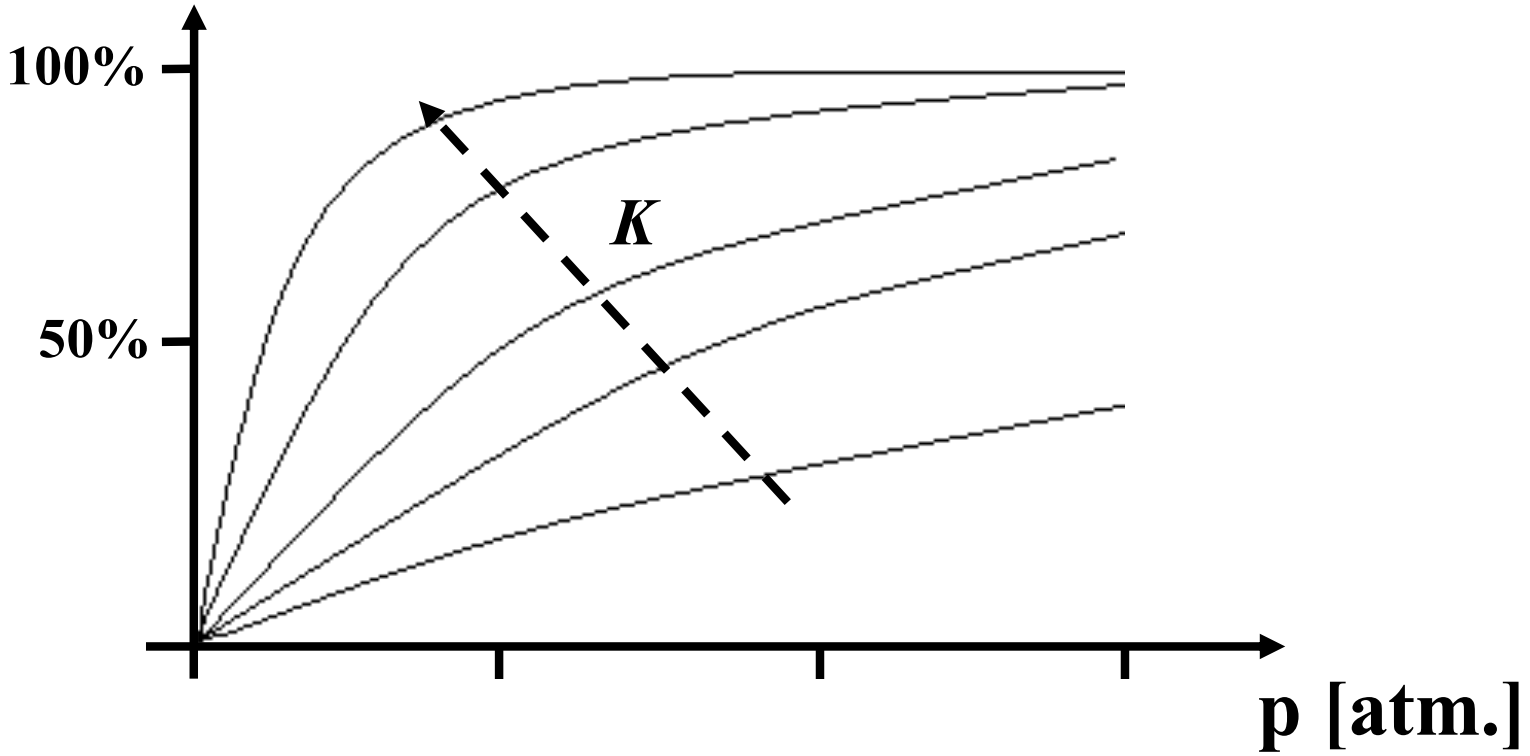
$$[BS_0] = [BS_{free}] + [A] = \frac{[A]}{pK} + [A] = \frac{1 + pK}{pK}[A]$$

$$Y = \frac{[A]}{[BS_0]} = \frac{pK}{1 + pK}$$

The Yield

The Yield in Langmuir Model

Yield = Percentage of covered surface



The Langmuir Model

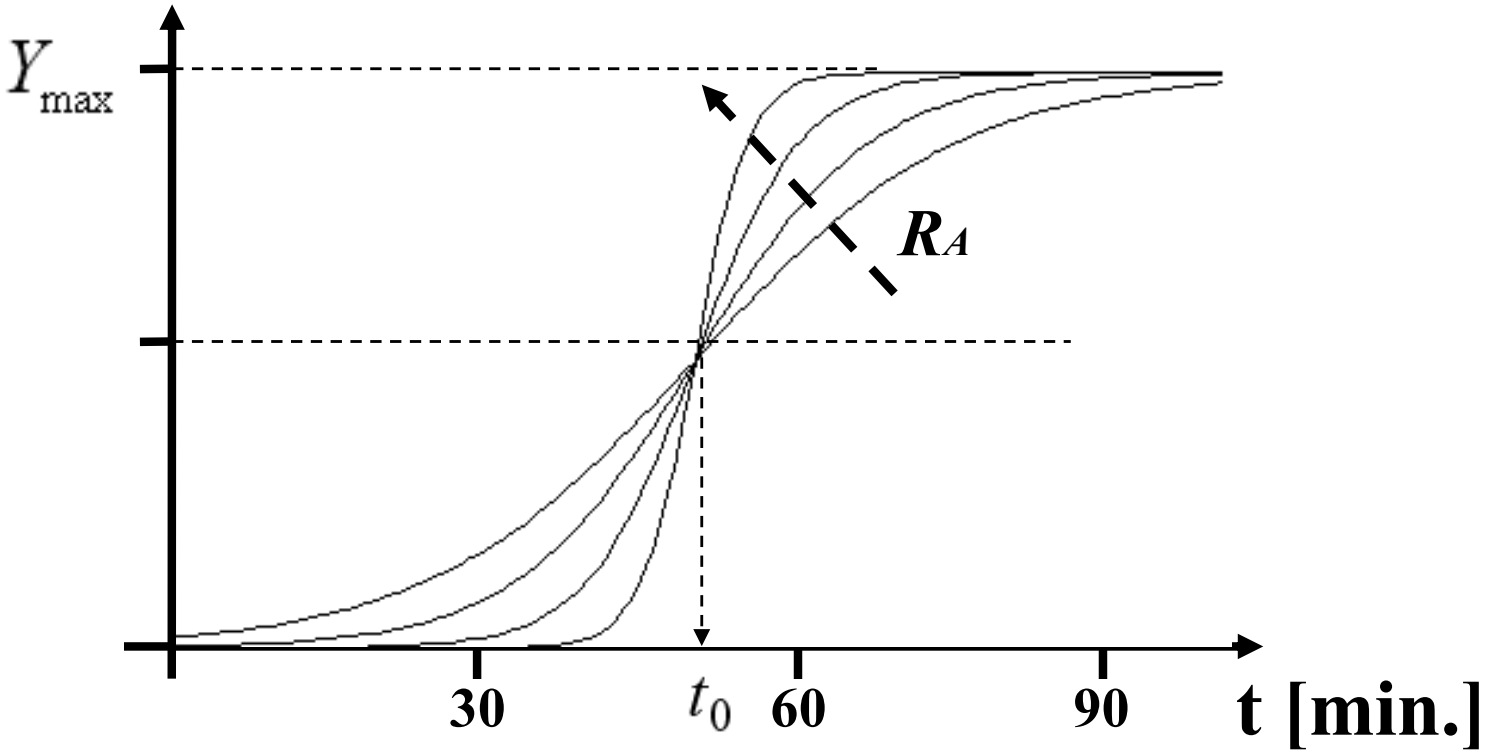
$$\frac{dY}{dt} = R_A - R_D Y \xrightarrow{equilibrium} R_A(1-Y)$$

$$\frac{dY}{dt} = R_A(1-Y)$$

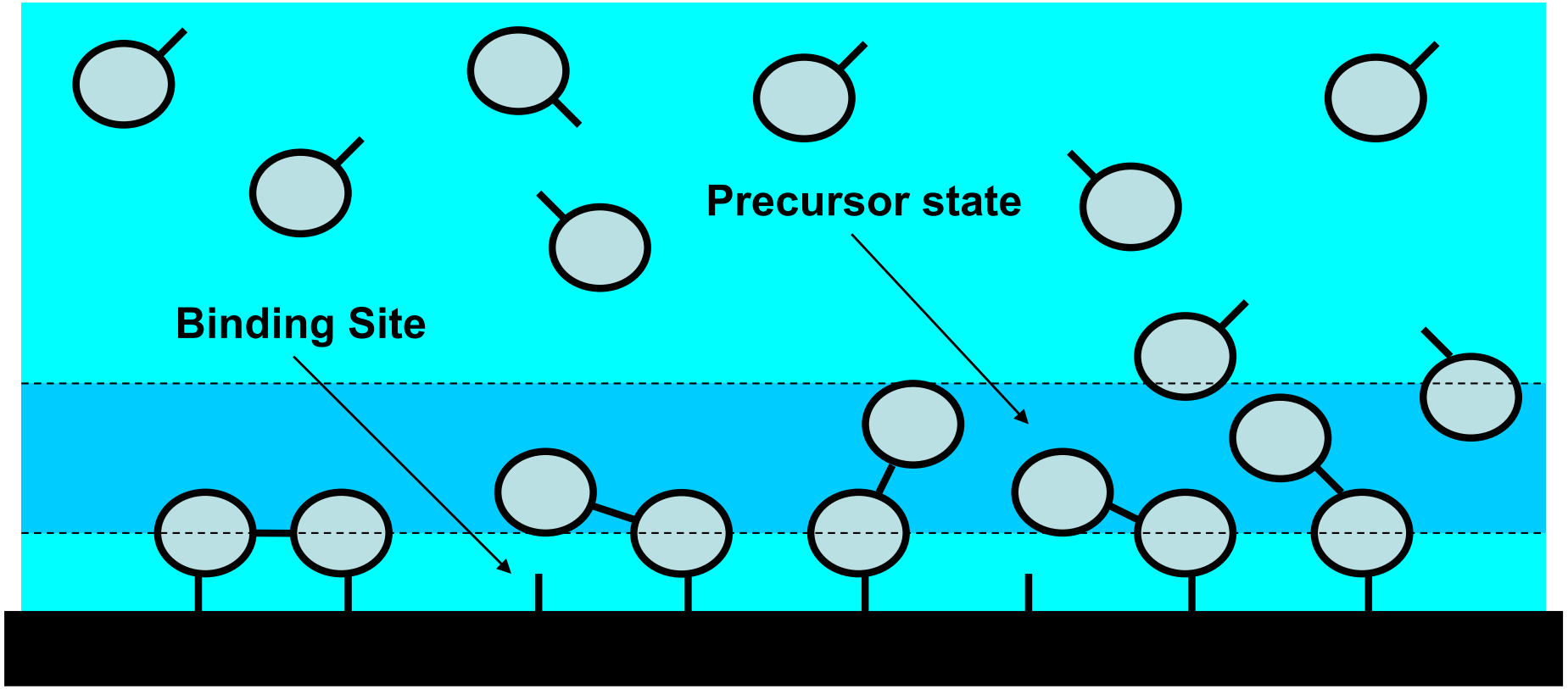
$$Y(t) = \frac{1-e^{-R_A t}}{1+e^{-R_A t}}$$

The Yield in Langmuir Model

Yield = Percentage of covered surface



The precursor state



The Kisliuk Model

Langmuir

$$\frac{dY}{dt} = R_A - R_D Y \xrightarrow{equilibrium} R_A(1-Y)$$

Precursor states at equilibrium interacting with gas

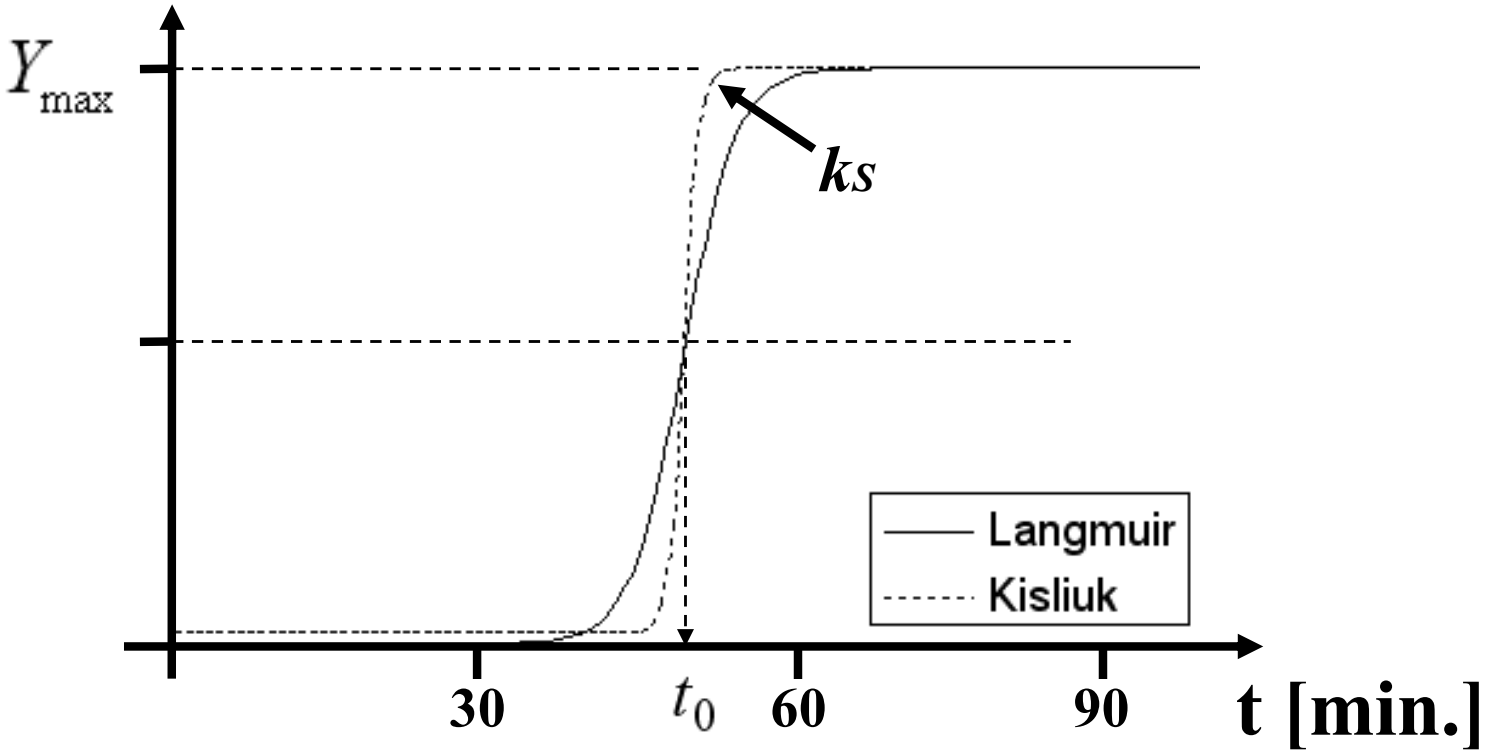
The Sticking coefficient

$$\frac{dY}{dt} = R_A(1-Y)(1+k_S Y)$$

$$Y(t) = \frac{1-e^{-R_A(1+k_S)t}}{1+k_S e^{-R_A(1+k_S)t}}$$

The Yield in Kisliuk Model

Yield = Percentage of covered surface



The Langmuir Model

Four Assumptions

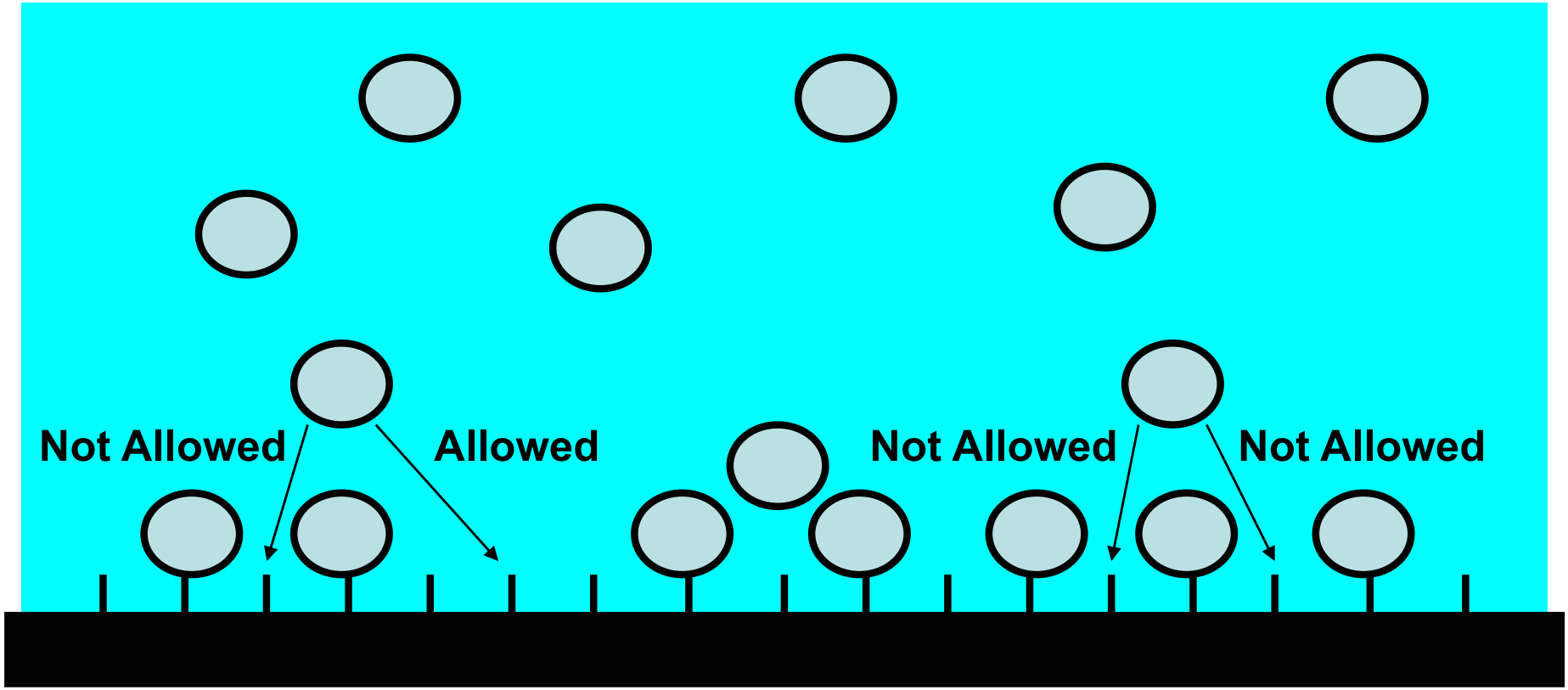
The Surface of adsorbent is uniform

~~**Adsorbed molecules do not interact**~~

All adsorptions occur with simple mechanism

Only a monolayer is obtained at the maximum adsorption

The Steric Hindrance Model



The Steric Hindrance Model

In the steric hindrance model, the previously adsorbed molecules prevent the adsorption of the next incoming ones:

$$\frac{dY}{dt} = R_A(1-Y) - \alpha Y[R_A(1-Y)].$$

A polynomial expansion of the molecular interaction term:

$$\frac{dY}{dt} = R_A(1-Y) - \sum_{\forall n>0} \alpha_n Y^n[R_A(1-Y)].$$

The Steric Hindrance Model

It is easy to show that the first approximation returns the Kisliuk model, while the second returns:

$$\frac{dY}{dt} = R_A(1-Y)[1-\alpha_1 Y - \alpha_2 Y^2].$$

As often written in literature. The fourth approximation gives us instead:

$$\frac{dY}{dt} = R_A(1-Y)[1-\alpha_1 Y - \alpha_2 Y^2 - \alpha_3 Y^3 - \alpha_4 Y^4]$$

Which corresponds to: $$\frac{dY}{dt} = R_A(1-Y)[1-AY-BY^2]^2$$

Wei-Dong Chen, Han-Hua Hu, Yan-Dong Wang (2006) Chem Eng Sci 61:7068–7076

The Langmuir Model

Four Assumptions

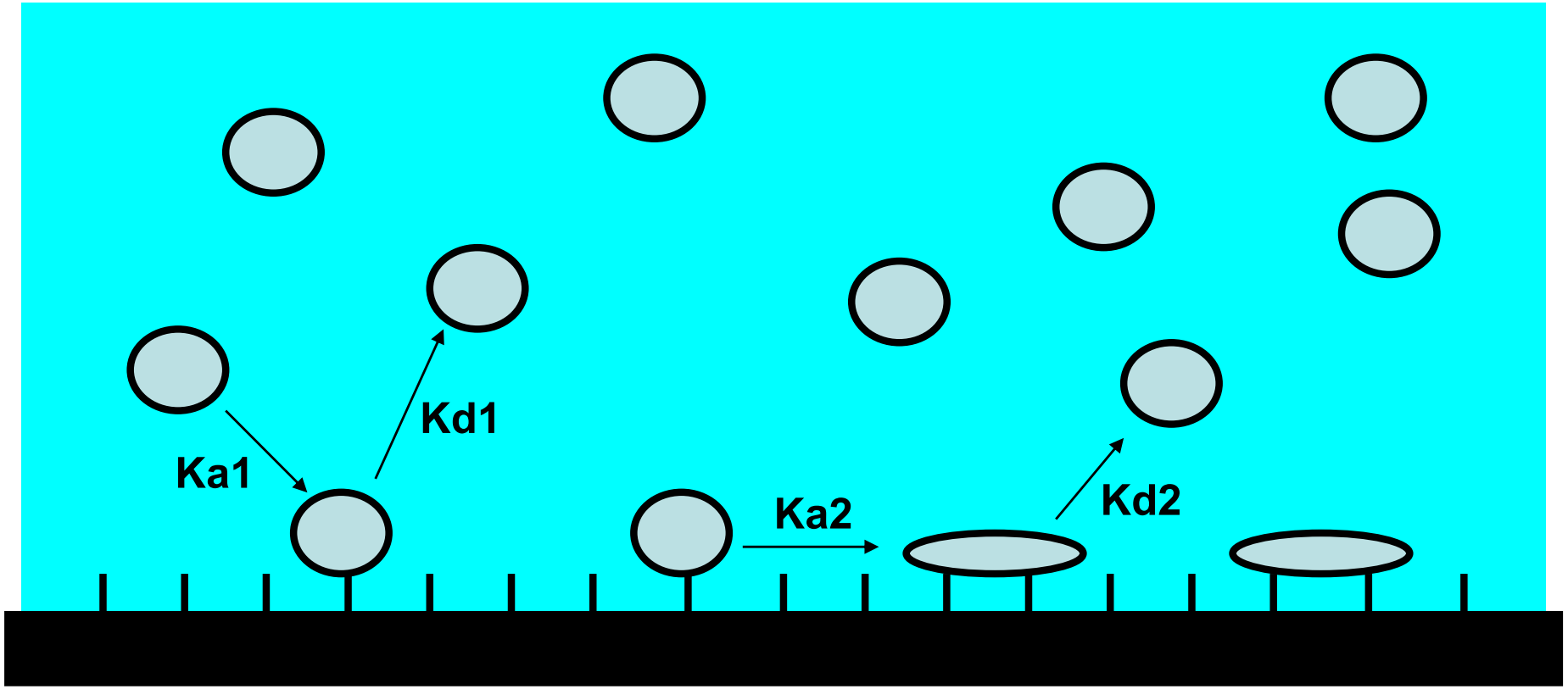
The Surface of adsorbent is uniform

Adsorbed molecules do not interact

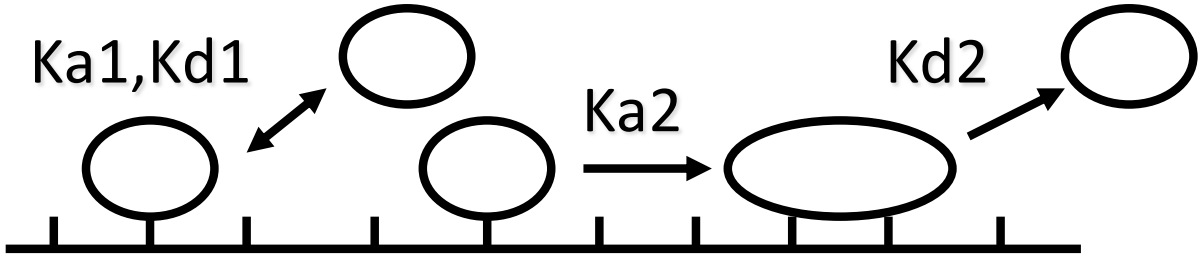
~~**All adsorptions occur with simple mechanism**~~

Only a monolayer is obtained at the maximum adsorption

The Spreading Model



The Spreading Model



If adsorbed molecules anchoring is by thought the Langmuir mechanism

$$\frac{dY_1}{dt} = R_{A1}(1 - Y_1 - \alpha Y_2)$$

$$\frac{dY_2}{dt} = R_{A2}(1 - Y_1 - \alpha Y_2)$$

Direct Comparison for several Yields by different models

Yield = Percentage of covered surface