EPFL

Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #6

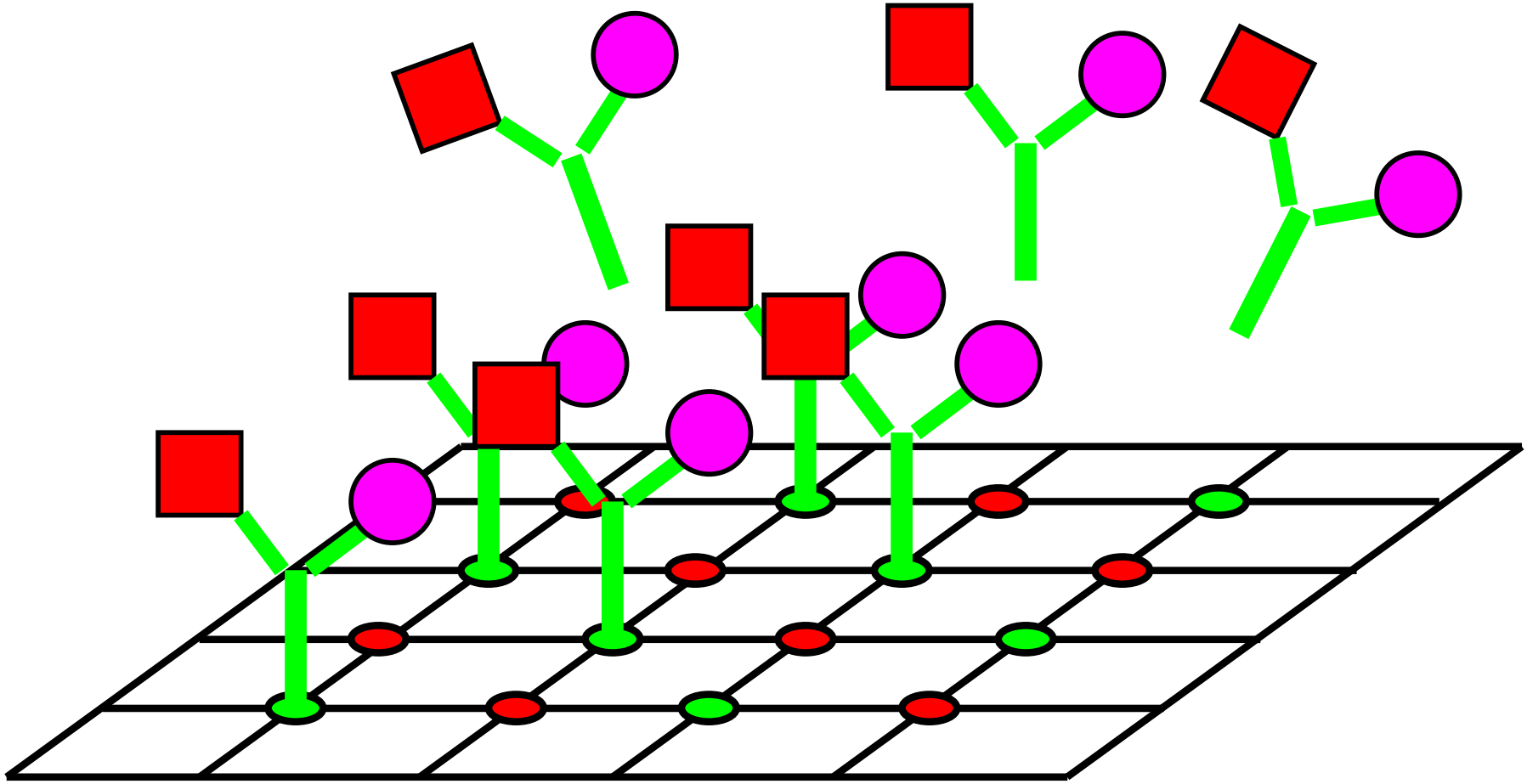
Probes immobilisation

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs § 5.1.1-5)

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

Self-assembly on a surface



Absorption methods and mechanisms

Q1

How is it possible molecular self-assembly on a surface?

A. By electrostatic forces

B. By hydrophobic forces

C. With chemical bonds

D. Never: each molecule needs to be forced on the surface

Different immobilization methods

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

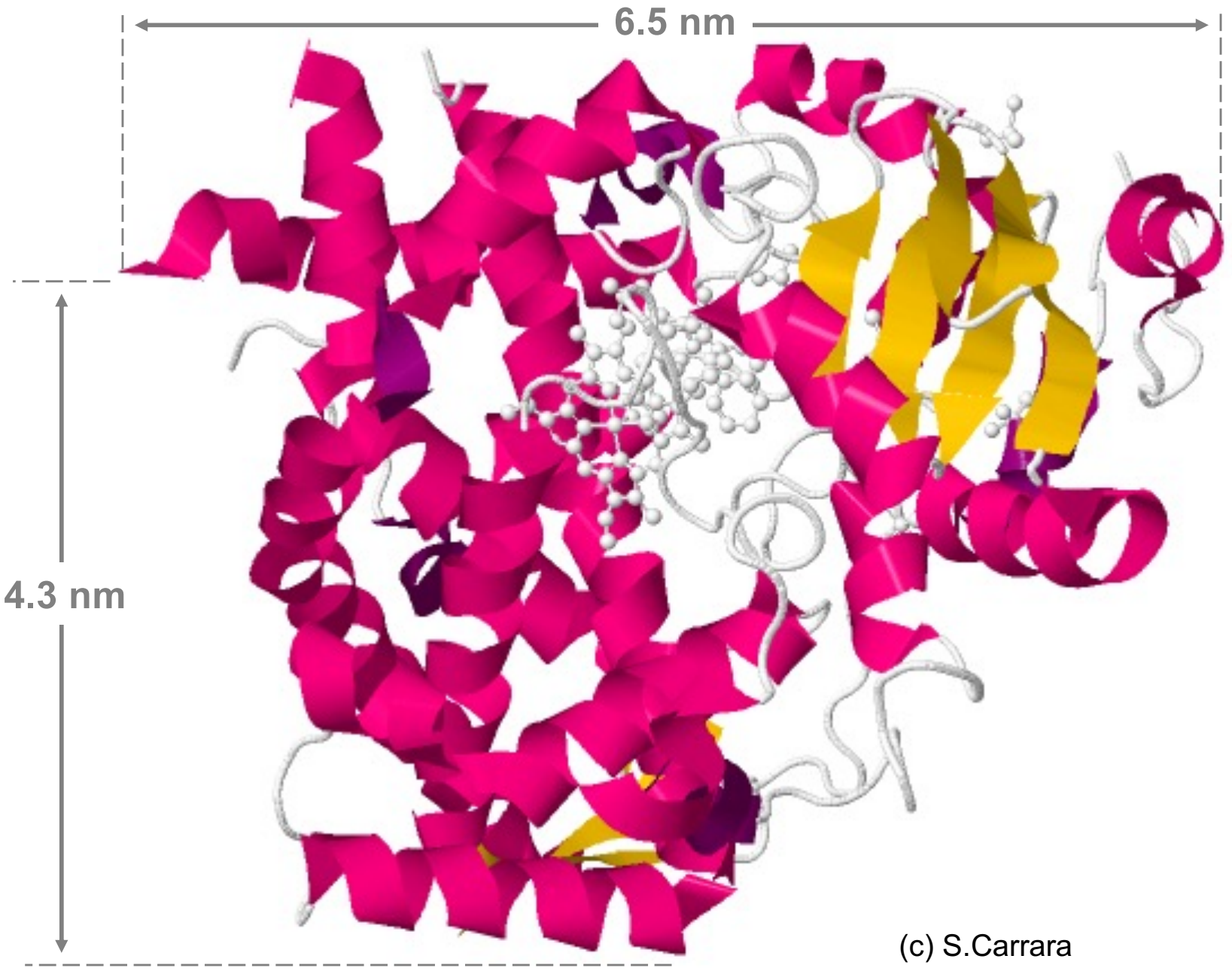
Proteins drop cast onto hydrophobic surfaces

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

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

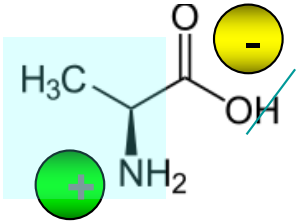
The hydrophobic forces are of the same intensity of the usual ones in Antigen/Antibody interactions

Cytochromes P450

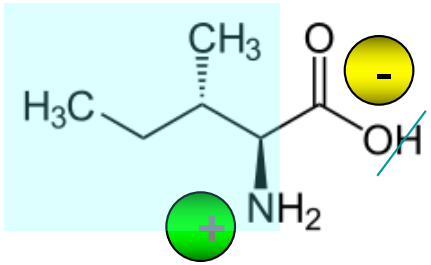


Hydrophobic AA

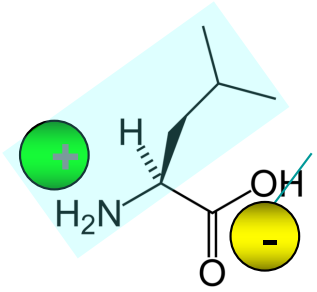
Hydrophobic Side Chains



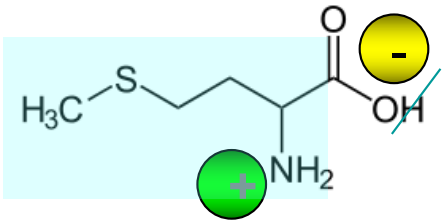
Alanine



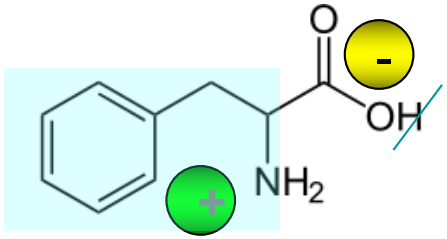
Isoleucine



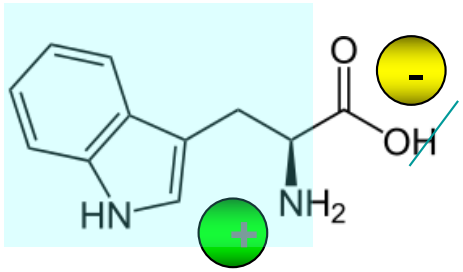
Leucine



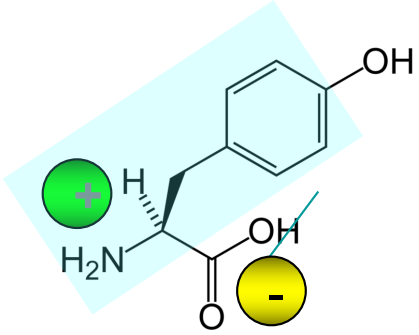
Methionine



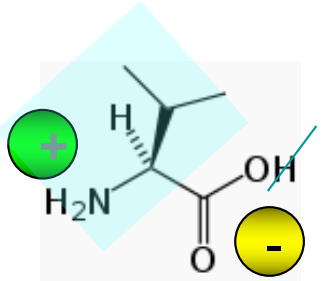
Phenylalanine



Tryptophan



Tyrosine



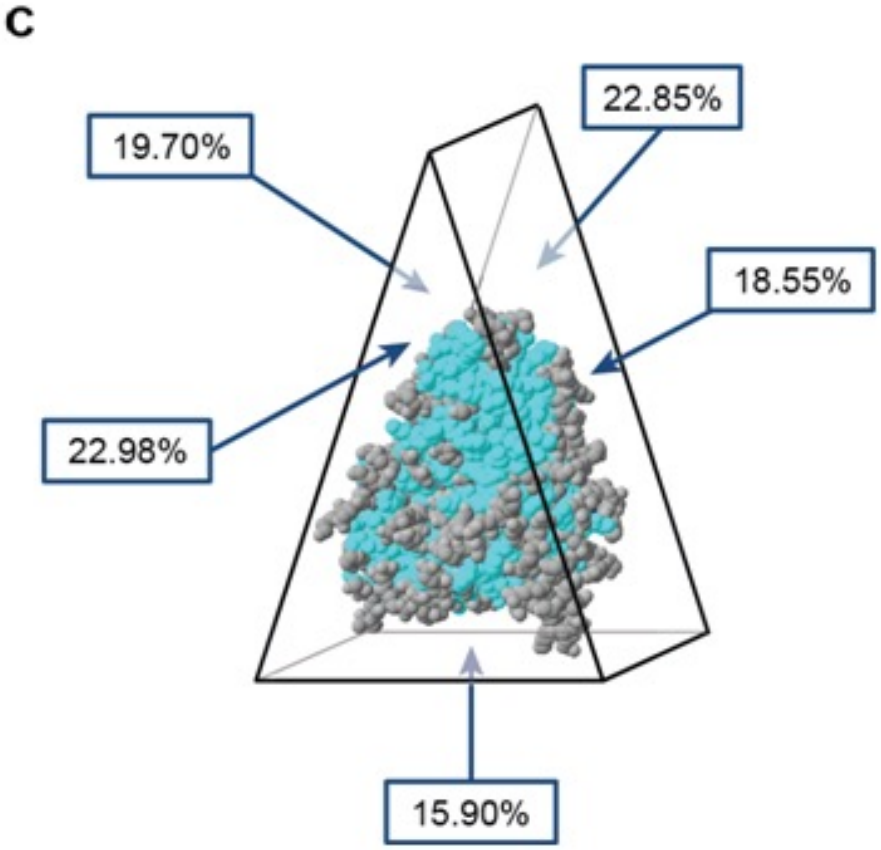
Valine

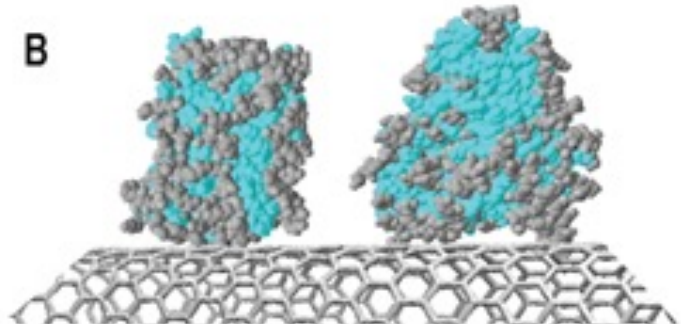
Q2

Are the hydrophobic residues located only inside in globular proteins?

A. Yes: the globular form is kept exactly to minimize their exposure to water
B. Mainly yes, but not exactly all
C. Only if the protein is an enzyme
D. No: the globular form is kept exactly to maximize their exposure to water

Hydrophobic Amino Acids anchor proteins onto carbon surfaces





C - percentage of hydrophobic residues in the different cytochrome' surfaces

B – The cytochromes anchored onto a carbon surface

The percentage of hydrophobic residues at the surface of a cytochrome P450

P450 onto carbon surfaces

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

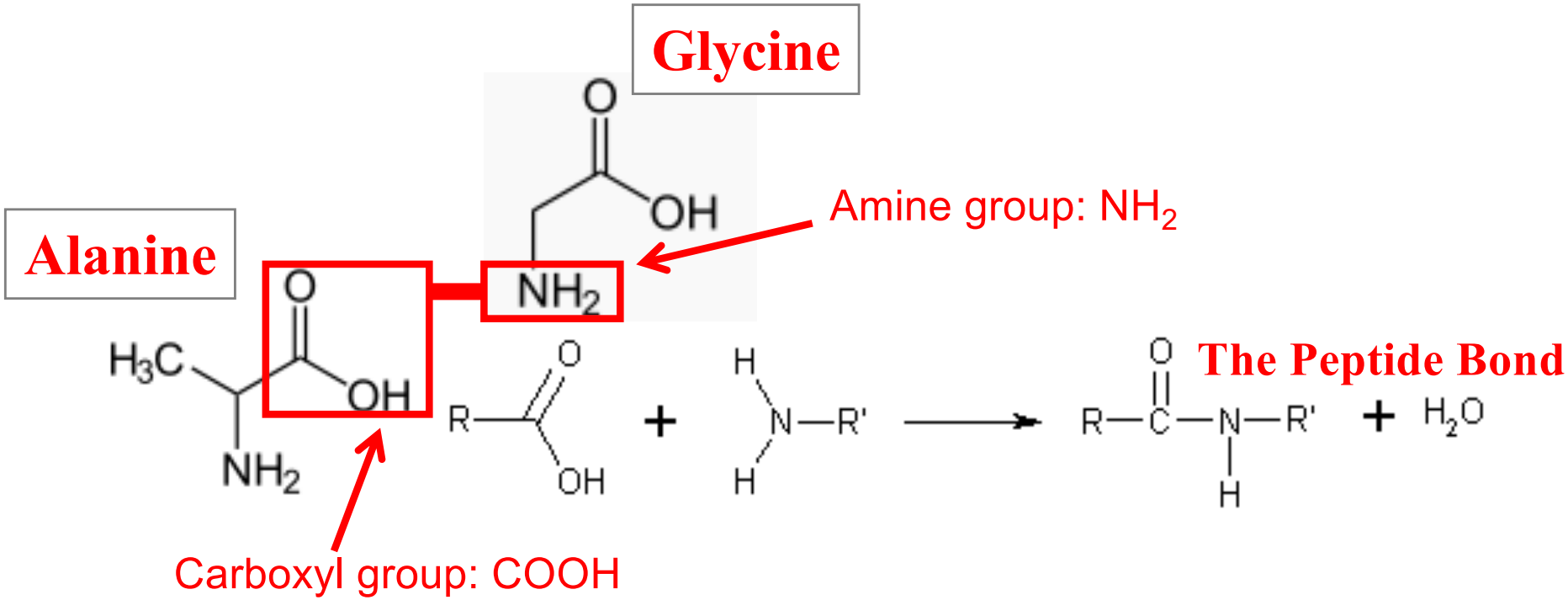
The hydrophobic forces are of the same intensity of the usual ones in Antigen/Antibody interactions

Q3

What is a peptide bond?

A. A bond between two peptides
B. A typical bond between two Amino Acids
C. A bond that could be established between COOH and NH_2 functional groups
D. Any bond between proteins

The Peptide Bond



The same mechanism used to form peptides may be used to covalently immobilize probes on surfaces

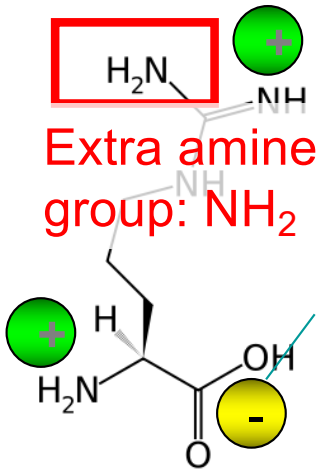
Q4

Is it possible to find amine groups exposed in proteins?

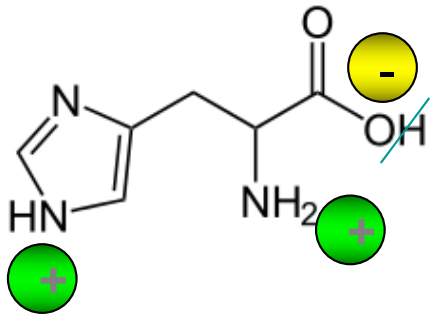
A. Of course: all amino acids have an NH_2 group
B. Yes, all protein' residues have an NH_2 group
C. No, all the NH_2 groups of AA are involved in peptide bonds
D. Yes, some protein' residues have an NH_2 group

Charged AA

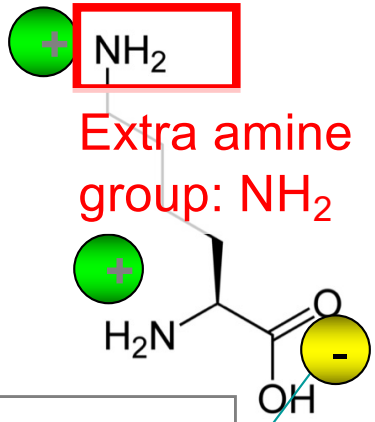
Positively Charged



Arginine

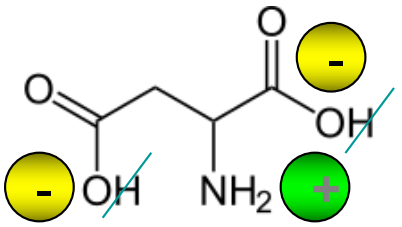


Histidine

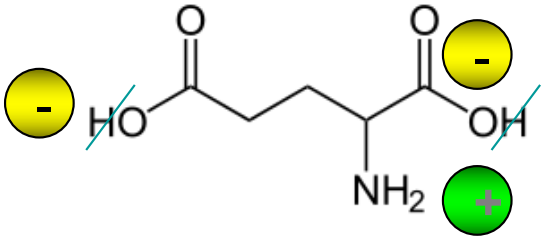


Lysine

Neg. Charged

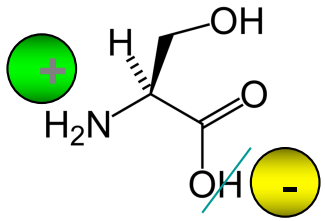


Aspartic Acid

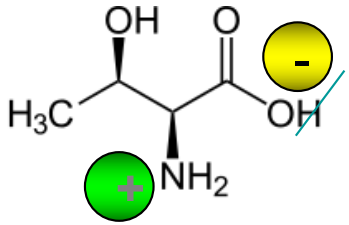


Glutamic Acid

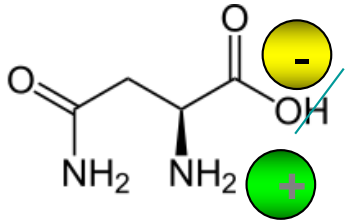
Polar Uncharged



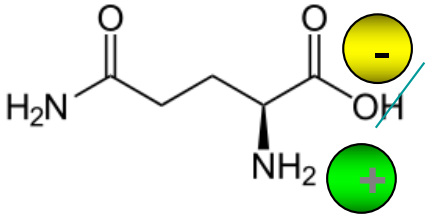
Serine



Threonine

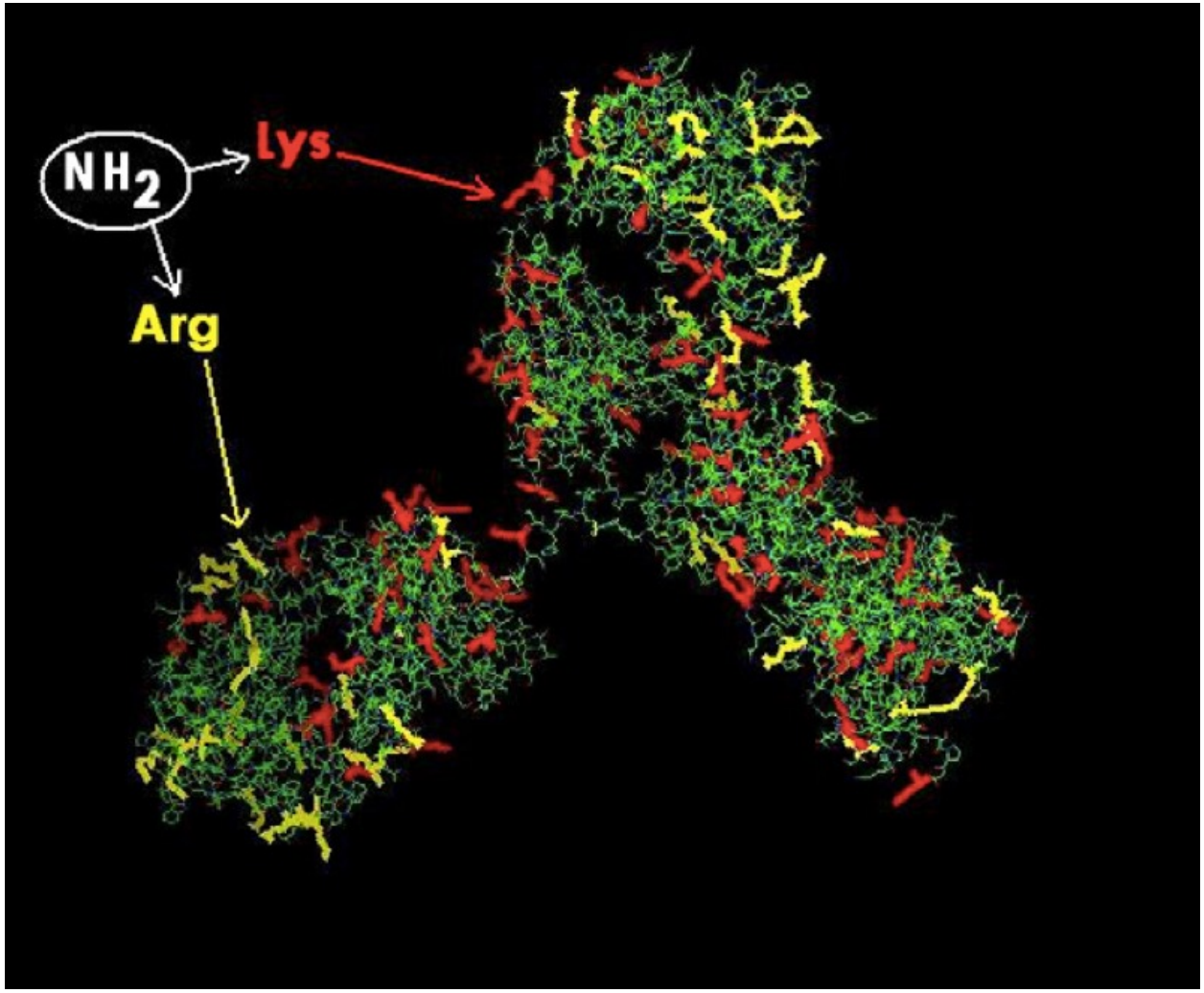


Aspargine



Glutamine

Residues with Amine group



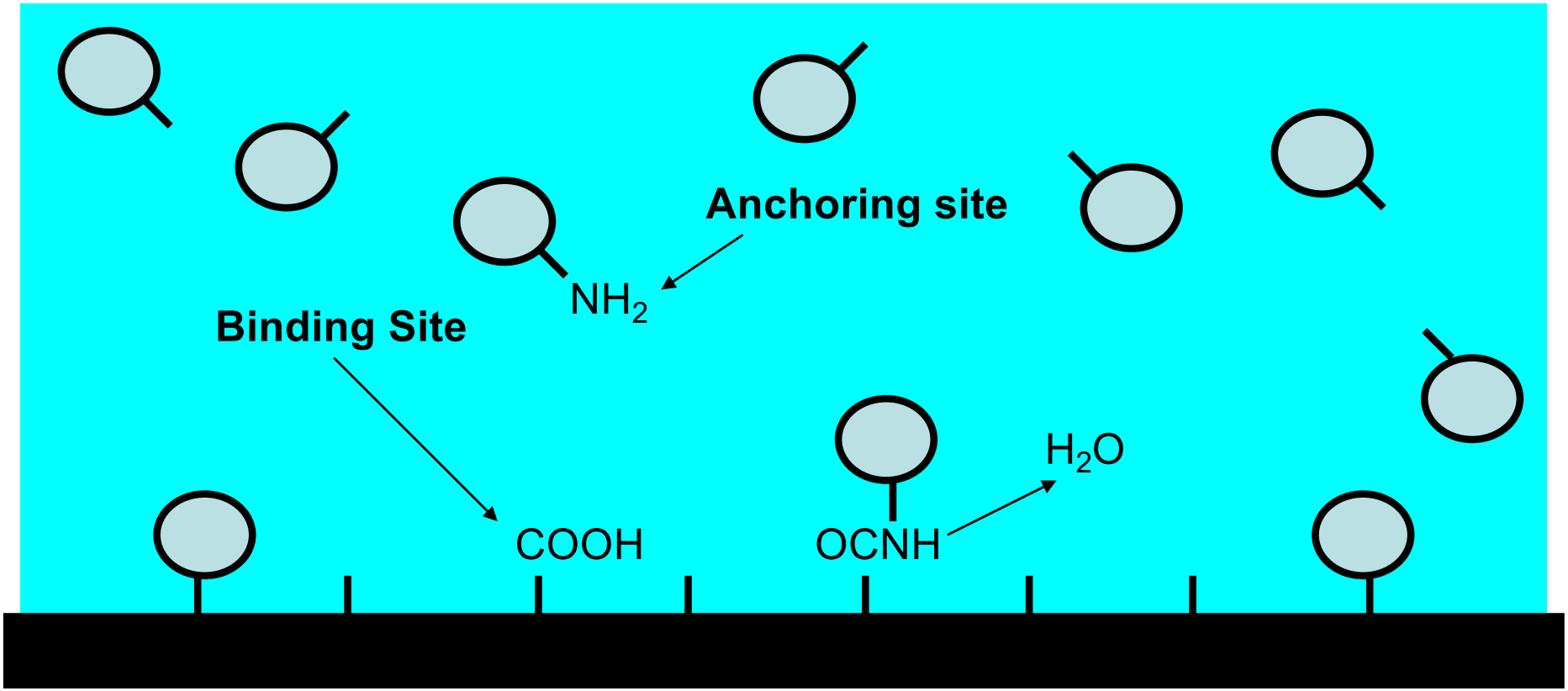
Location of Arginines and Lysines in an antibody

Q5

What I need more to obtain a Peptide bond on the surface?

A. A series of COOH groups on the surface
B. A further peptide differently anchored onto the surface
C. A series of COOH groups on the protein
D. A series of NH_2 group on the surface

Peptide bond on carbon surface



Q6

How to get COOH groups on the surface?

A. By further peptide differently anchored onto the surface

B. By self-assembly of OOH molecules

C. By exposing the surface to OH^- ions

D. By breaking C-C bonds on the 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

Q7

May we obtain Peptide bonds with COOH groups on Proteins?

A. Yes, with series of NH_2 groups directly on the surface

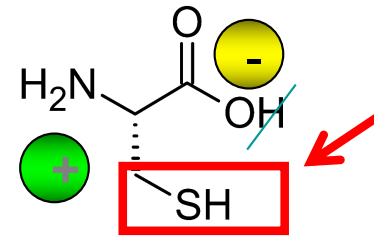
B. Yes, but with another self assembly on the surface

C. No, there are not proteins' residues with exposed COOH

D. No, but in case of antibodies

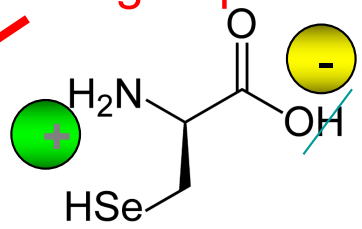
Neutral AA

Special Cases

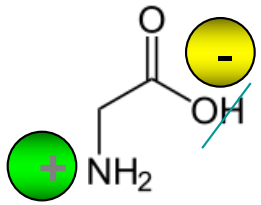


Cysteine

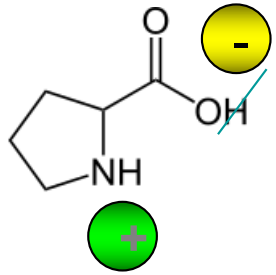
Sulfur group: SH



Selenocysteine

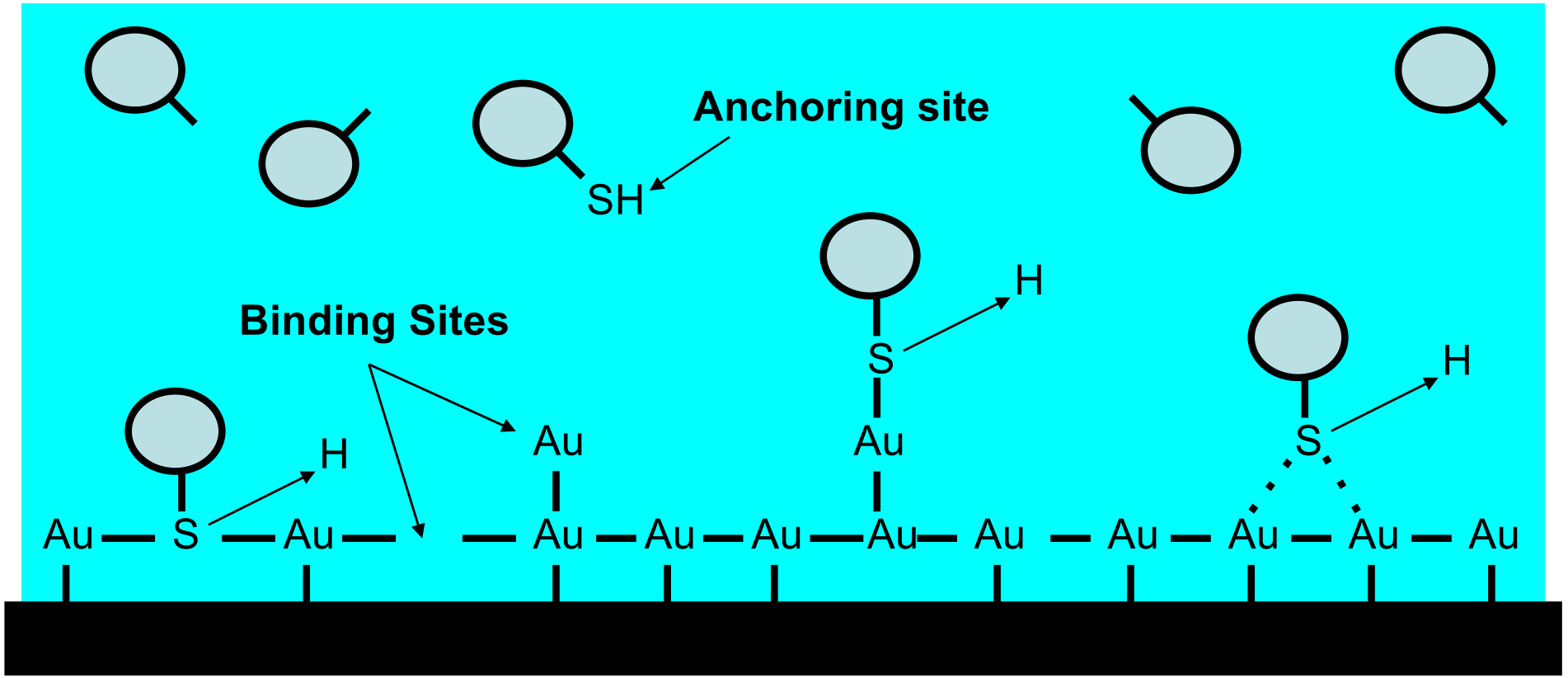


Glycine



Proline

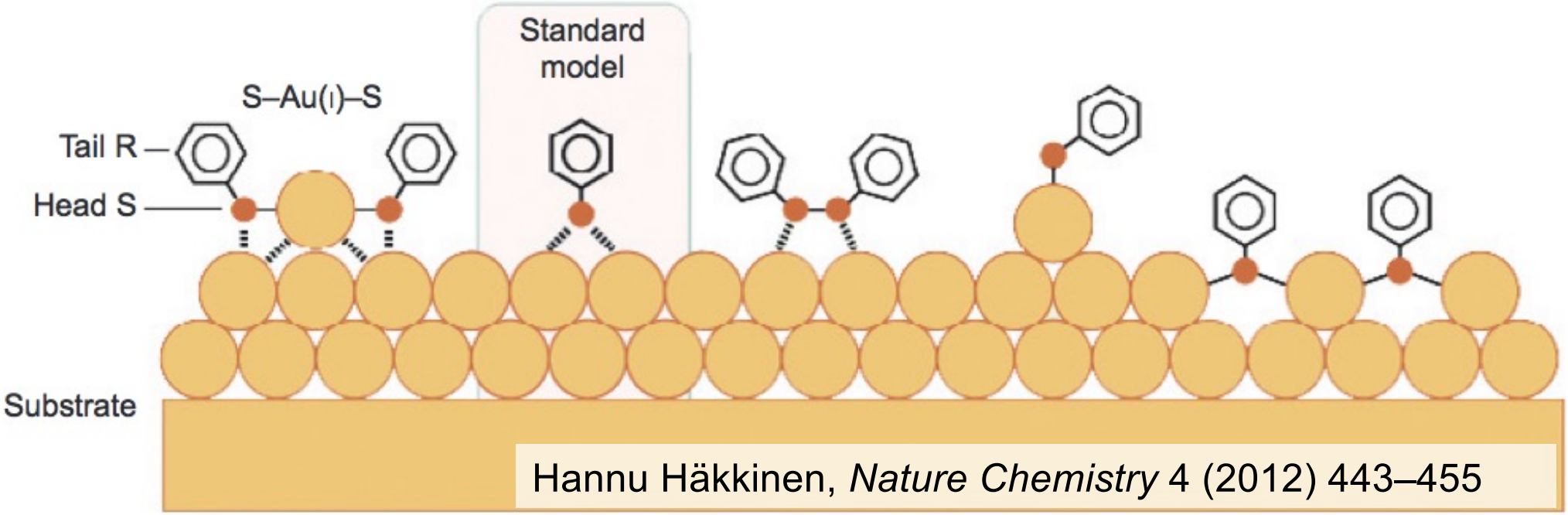
Thiol bond on gold surface



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)

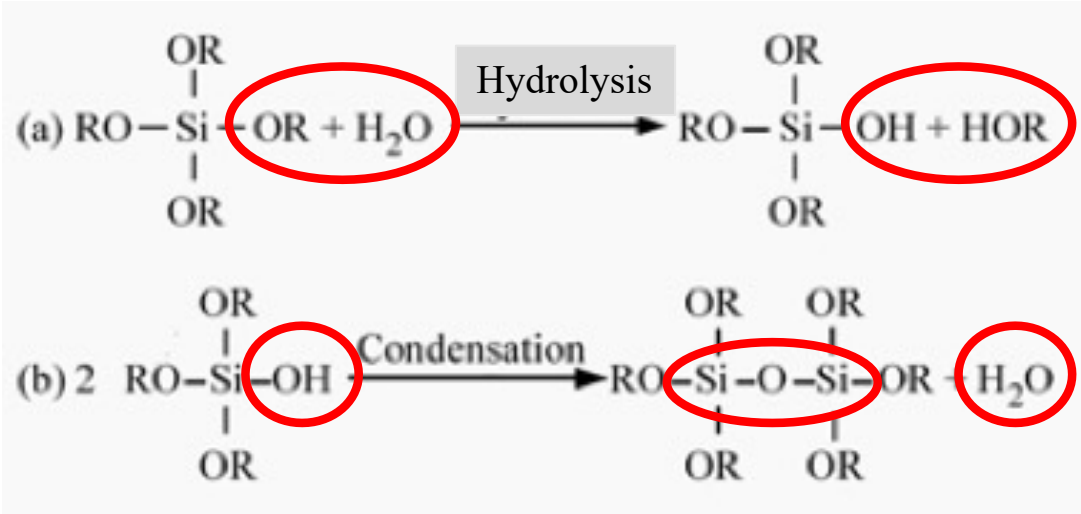
Thiol bond models



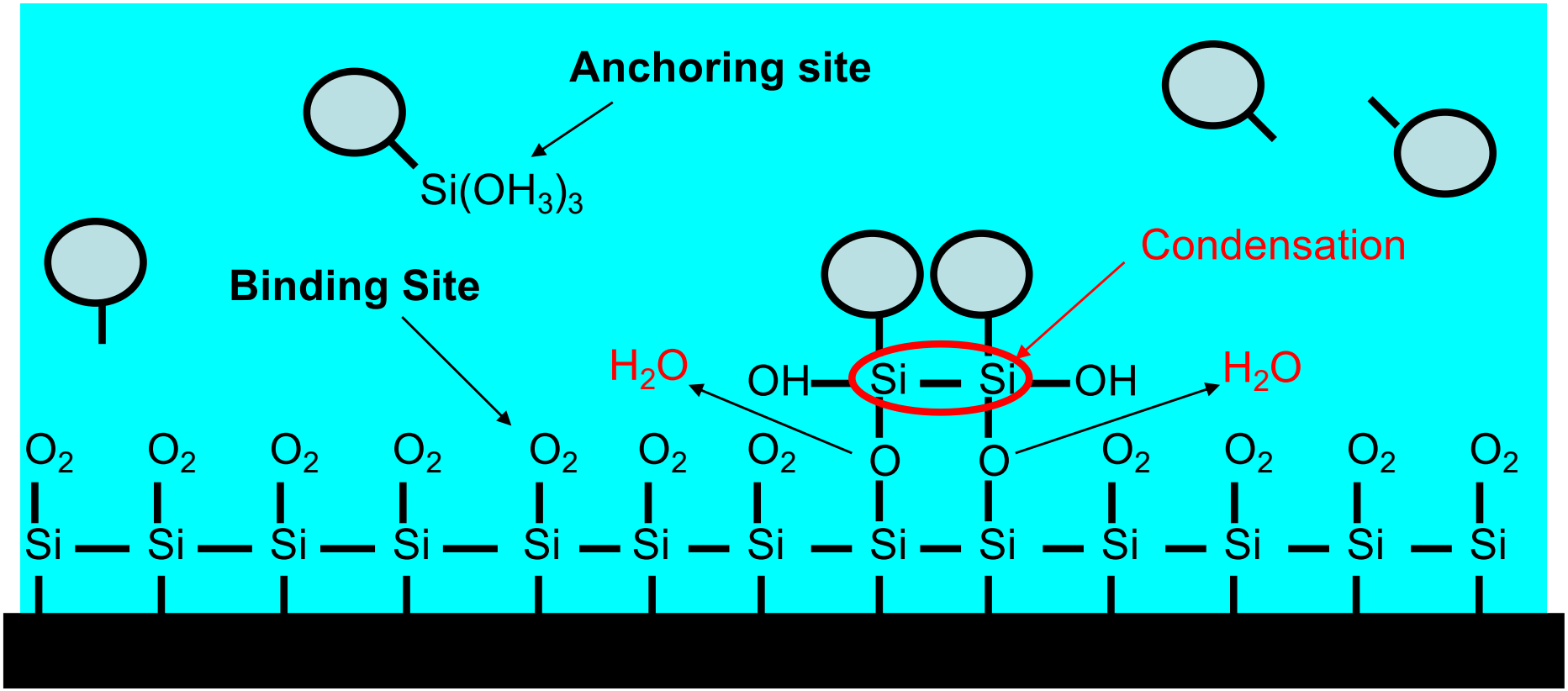
The ‘standard model’ foresees a monothiolate binding at atop, while new experimental evidence shows other key structural with complexes where the bridging gold atom is in a formal oxidation state of +1

Silanes bond

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



Silanes bond on silicon surface



Silanes bond

Figure 3. Hydrolysis of alkoxysilanes.

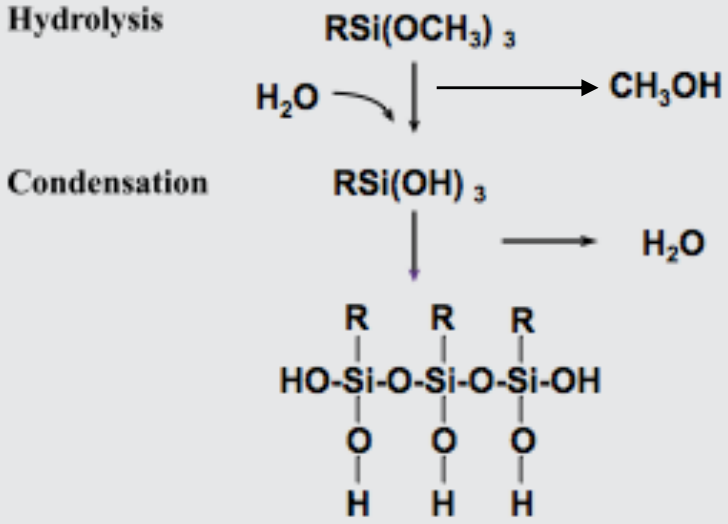
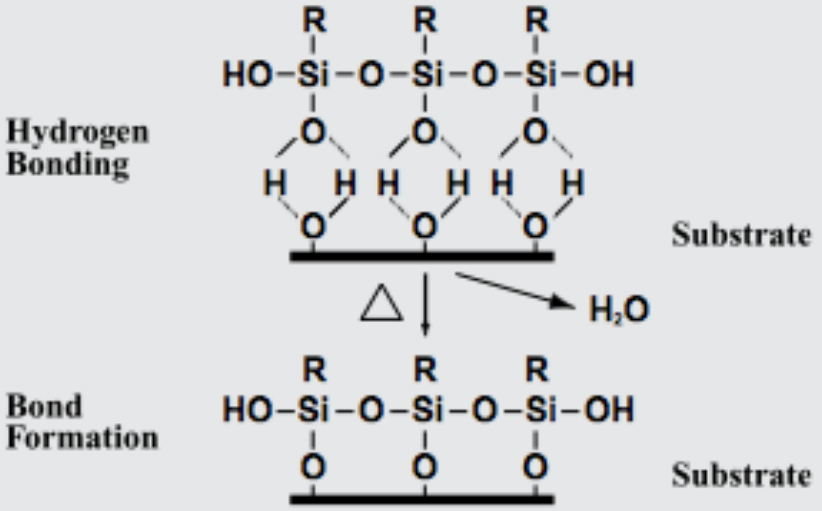
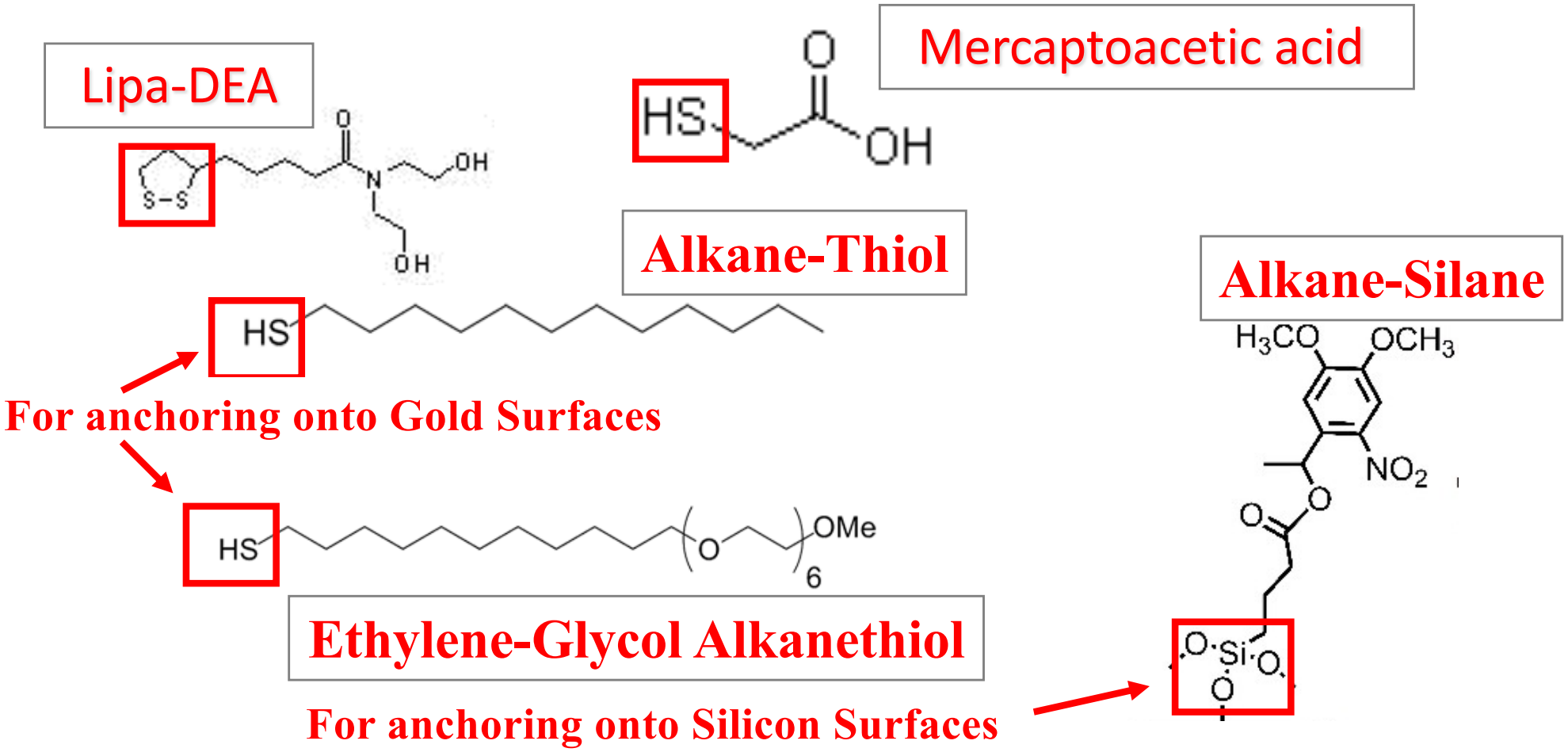


Figure 4. Bonding to an inorganic surface.



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

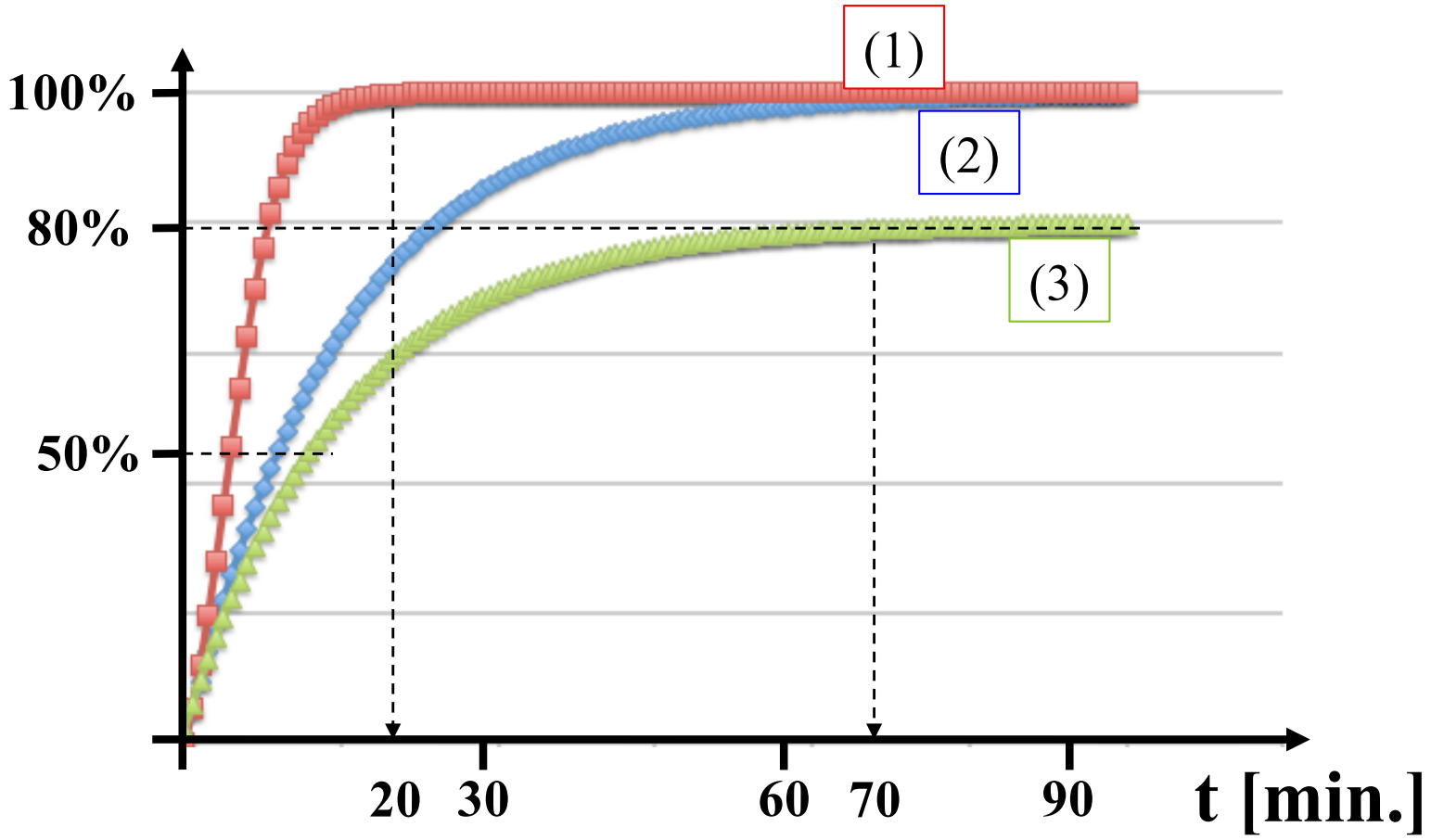
Molecules with alkyl chains



Aliphatic chains may provide thiols or silanes for several aims, including linkers to proteins

Different molecules result in different Yields onto the same surface

Yield = Percentage of covered surface



Q8

Why different molecules result in different Yields ?

A. Since different molecules have different isoelectric point
B. Since different molecules have different shape
C. Since different molecules have different steric hindrance
D. Since different molecules have different characteristics

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

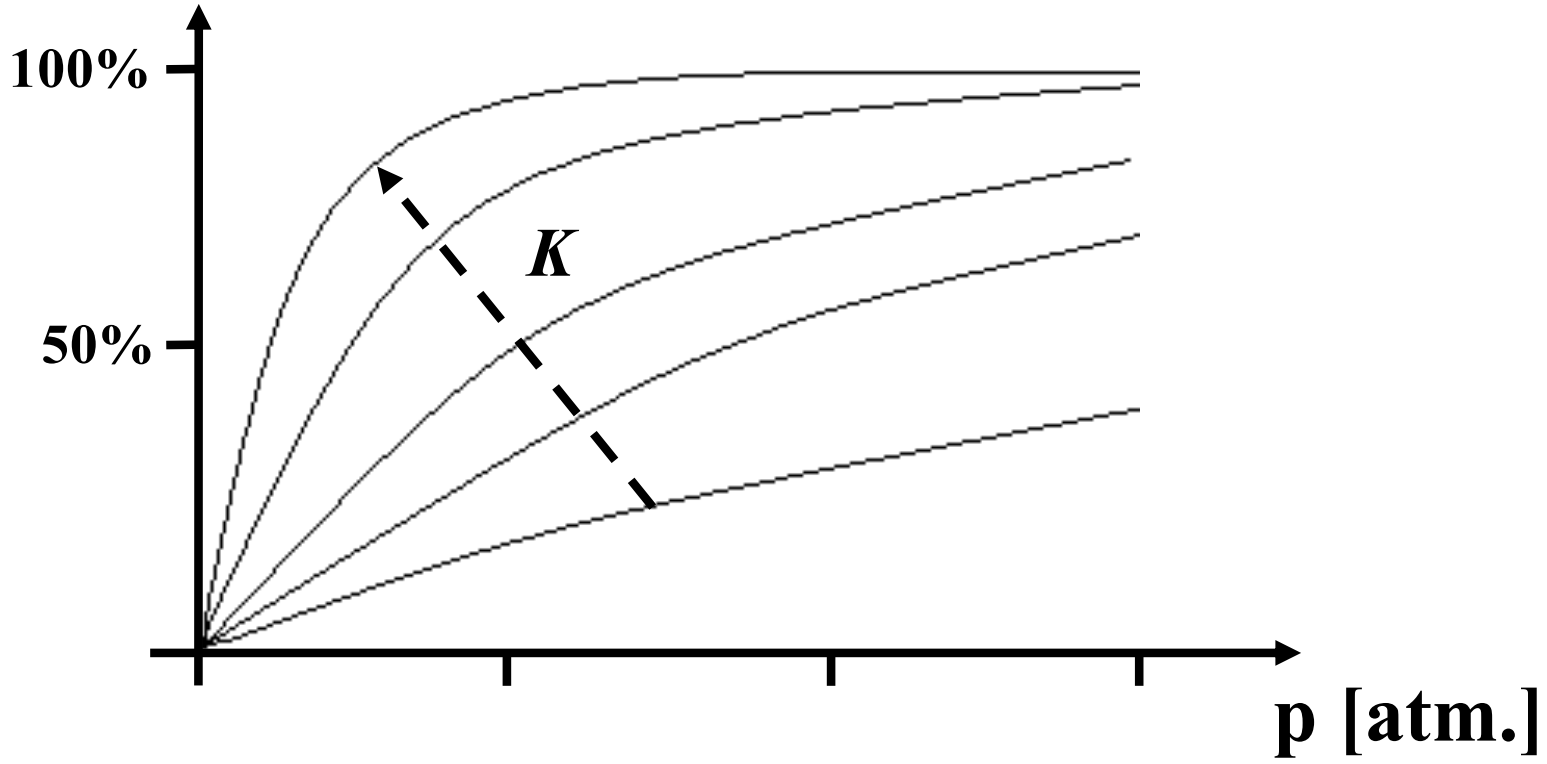
$$[BS_0] = [BS_{free}] + [A] = \frac{[A]}{pK} + [A] = \frac{1 + pK}{pK}[A]$$

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

The Yield

The Yield in Langmuir Model

Yield = Percentage of covered surface



Q9

Does an equation upon the pressure help for Yielding in liquid ?

A. No, since all liquid depositions are done at room atmosphere
B. No, since different depositions might be done at the same pressure
C. Yes, since different depositions might be done at different pressures
D. Of course: the pressure always matters

The Langmuir Model

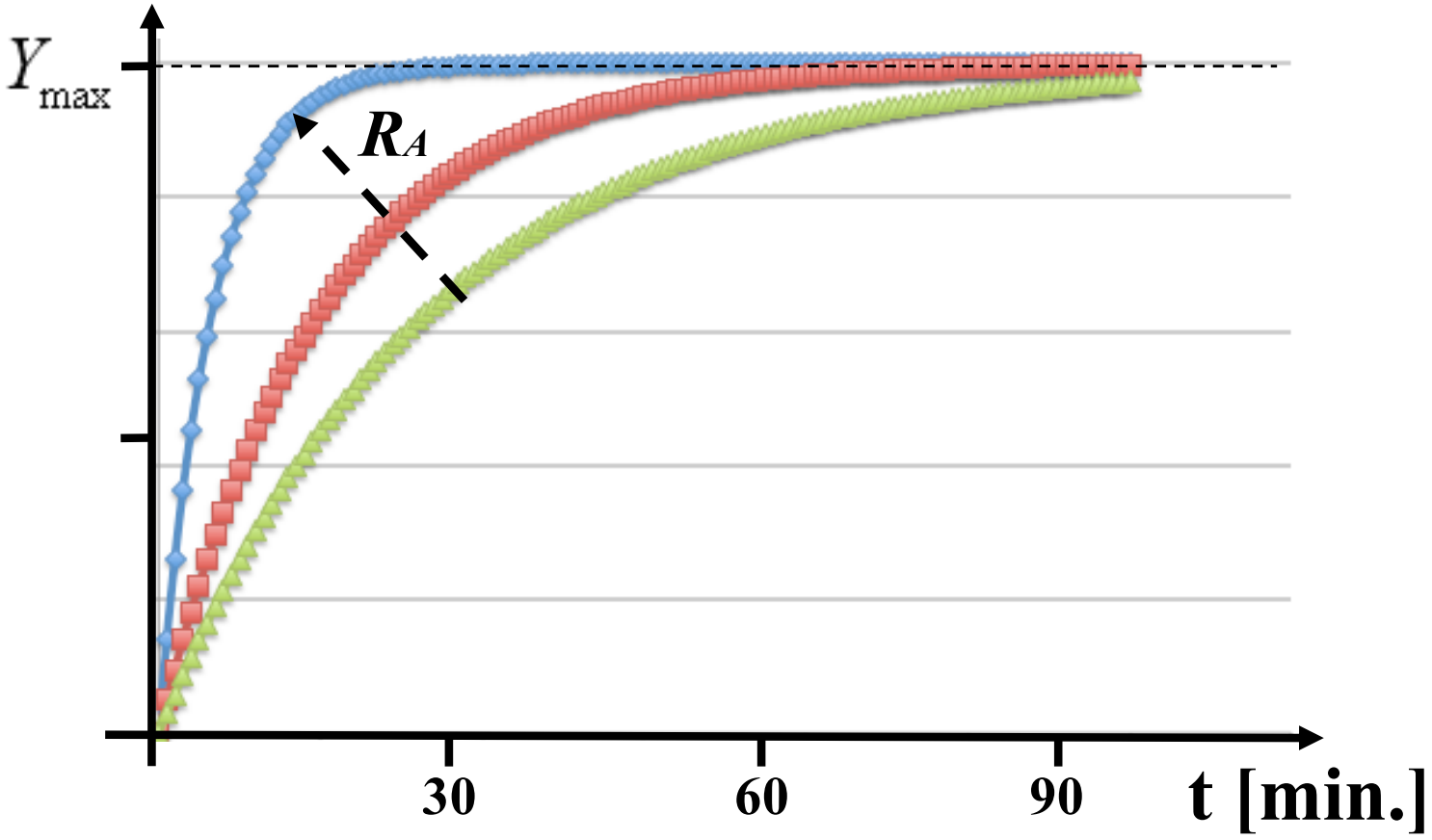
$$\frac{dY}{dt} = R_A - R_D Y \xrightarrow{equilibrium} R_A(1-Y)$$

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

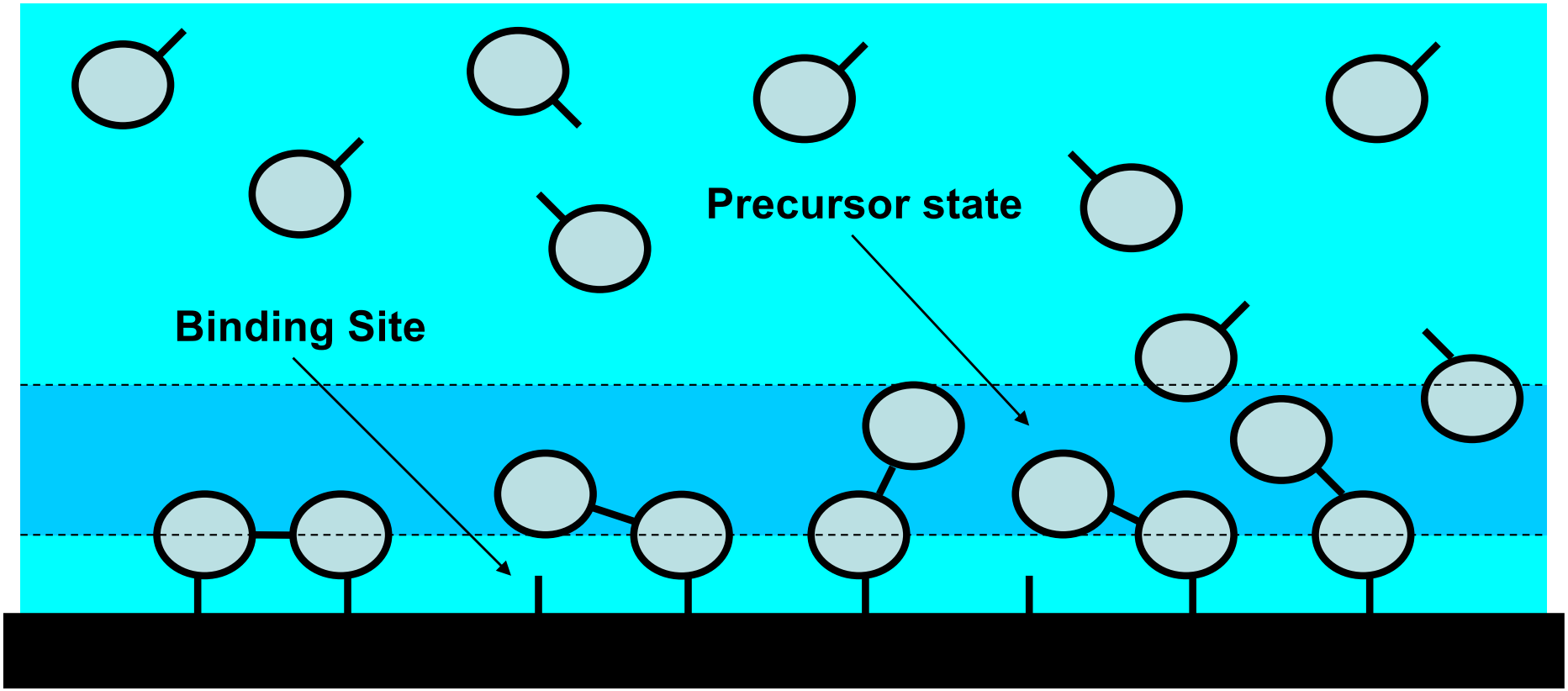
$$Y(t) = 1 - e^{-R_A t}$$

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

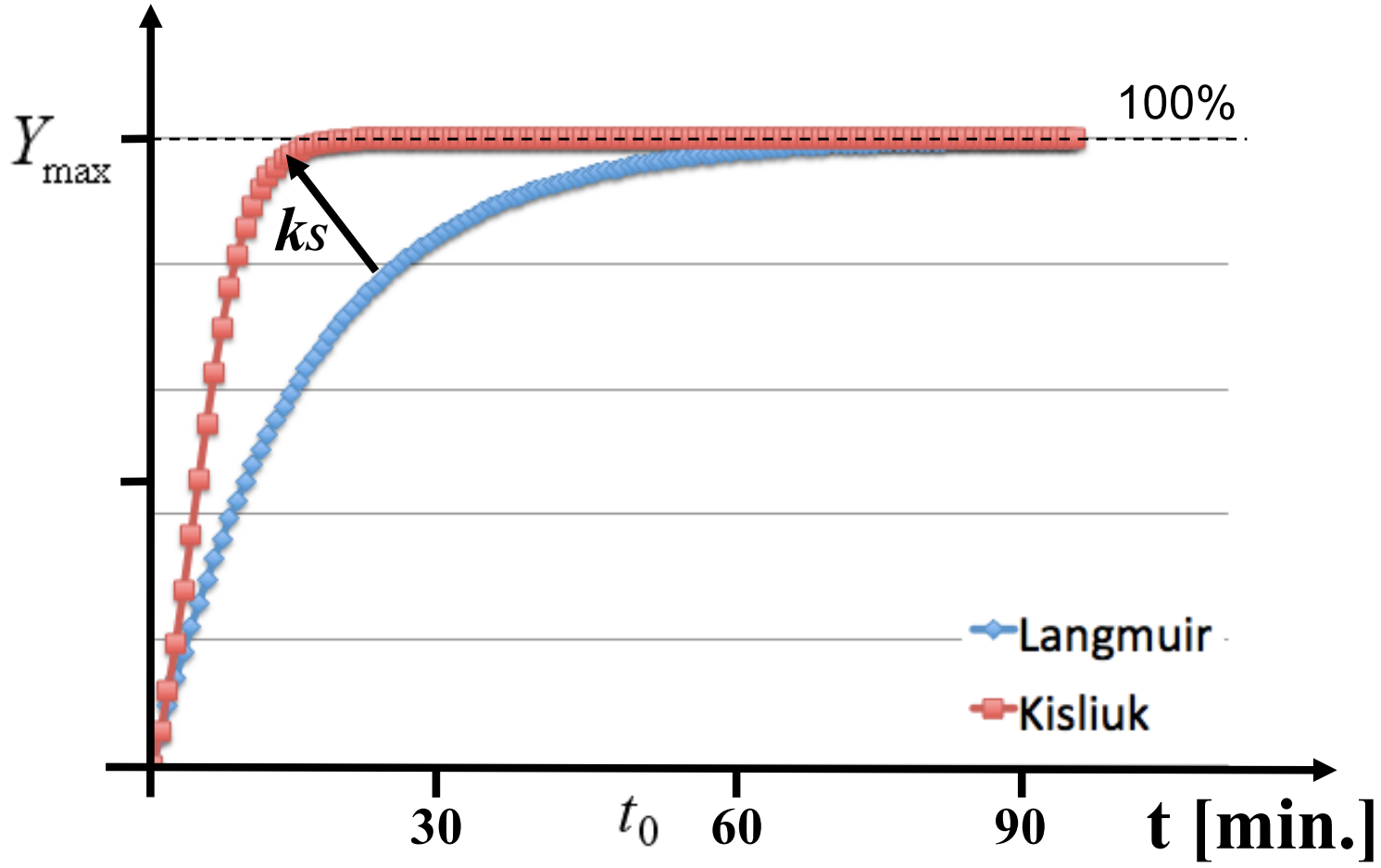
$$\frac{dY}{dt} = R_A(1-Y)(1+k_S Y)$$

The Sticking coefficient

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

The Yield in Kisliuk Model

Yield = Percentage of covered surface



The Langmuir Model

Four Assumptions

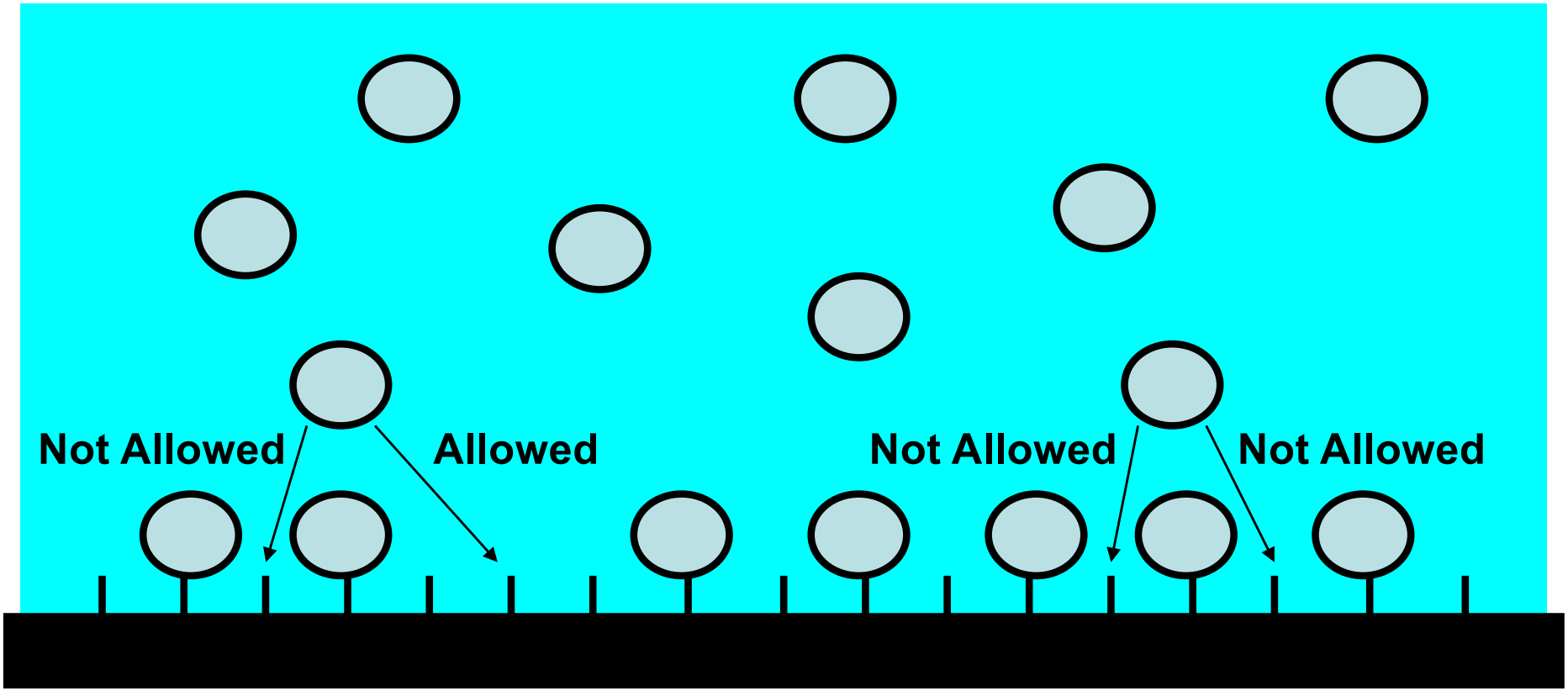
The Surface of adsorbent is uniform

~~**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

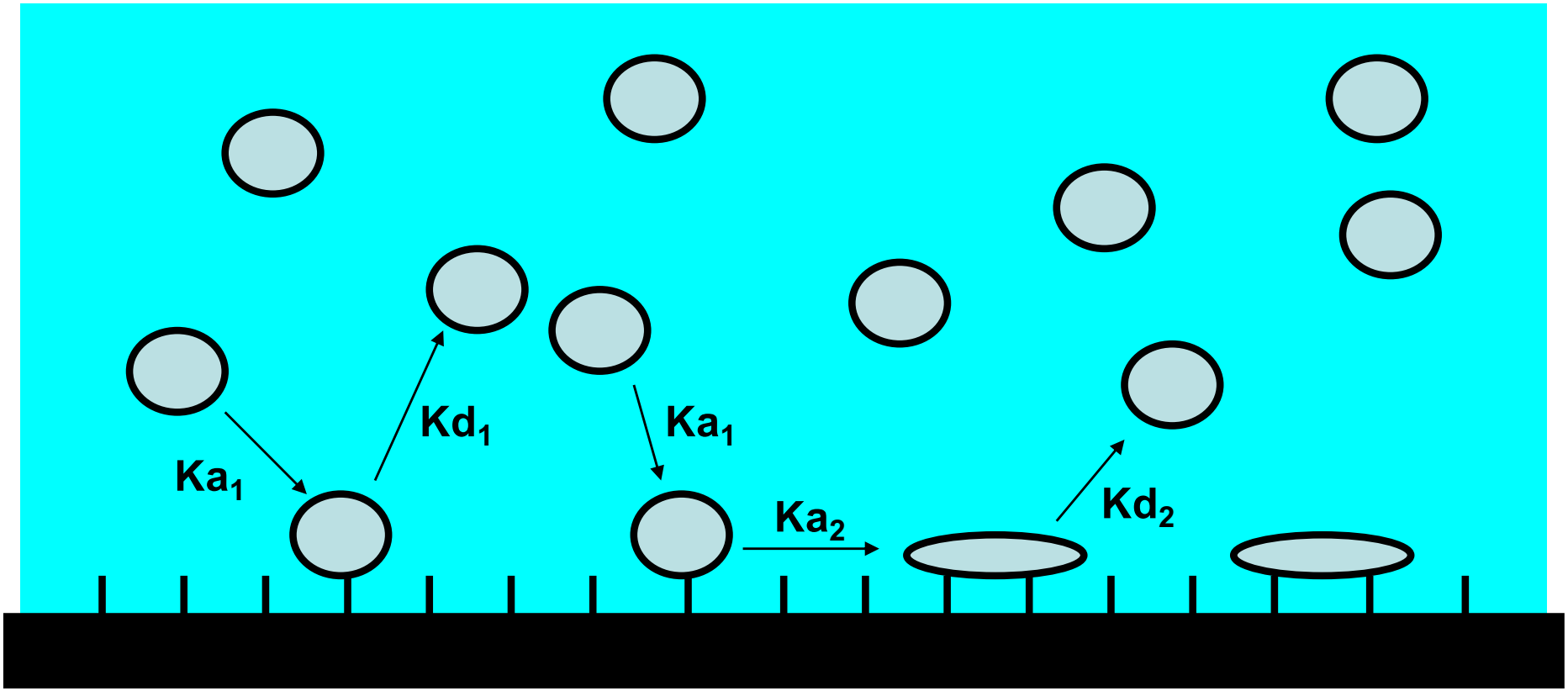
The Surface of adsorbent is uniform

Adsorbed molecules do not interact

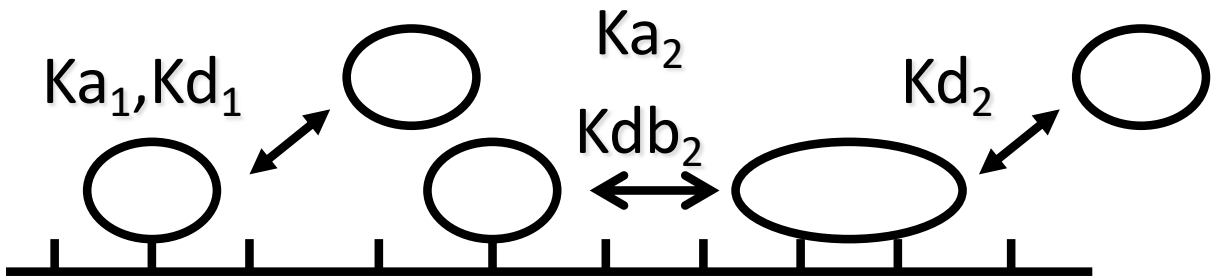
~~**All adsorptions occur with simple mechanism**~~

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

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

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

Direct Comparison for several Yields by different models