EPFL

EDMI Microsystems and Microelectronics

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

Lecture #4

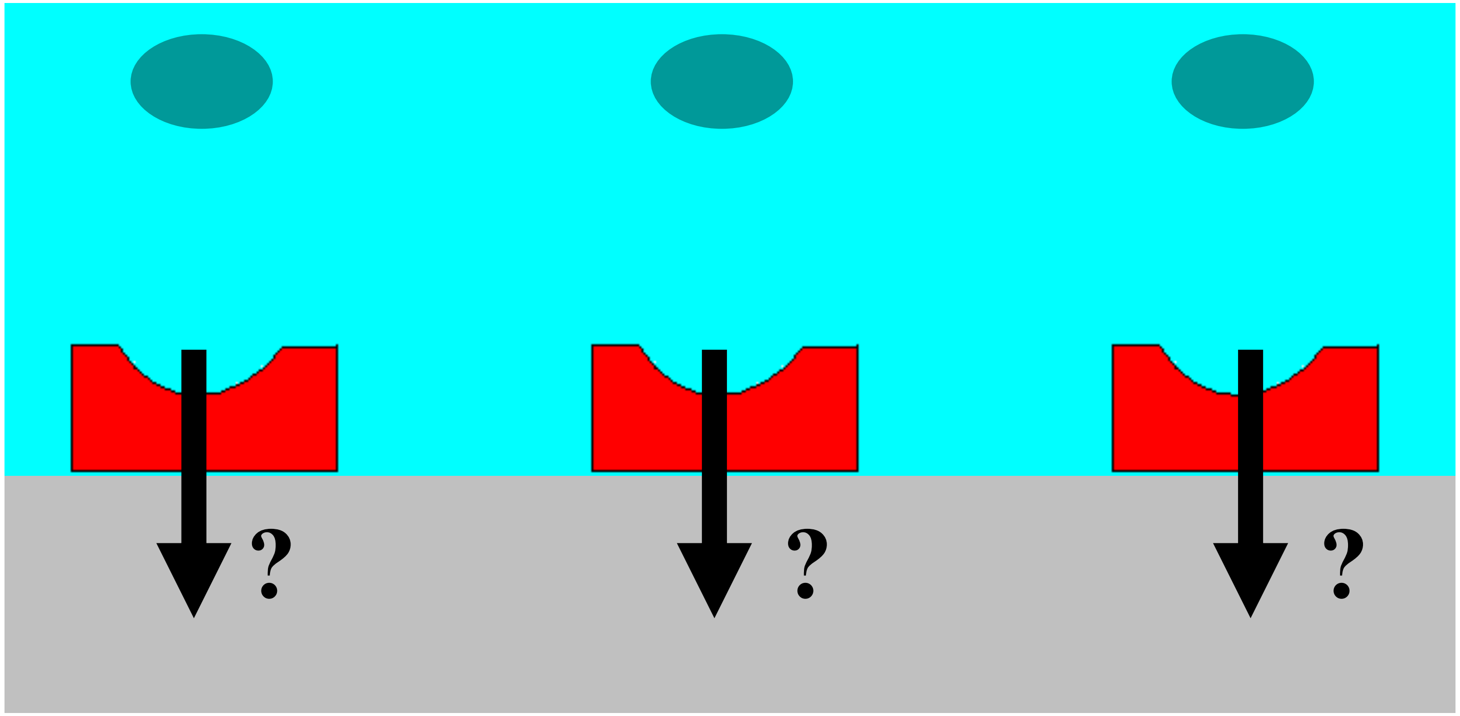
Probe Detection Principles (DNA, Antibodies & Oxidases)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs § 6.1-4 & 8.1)

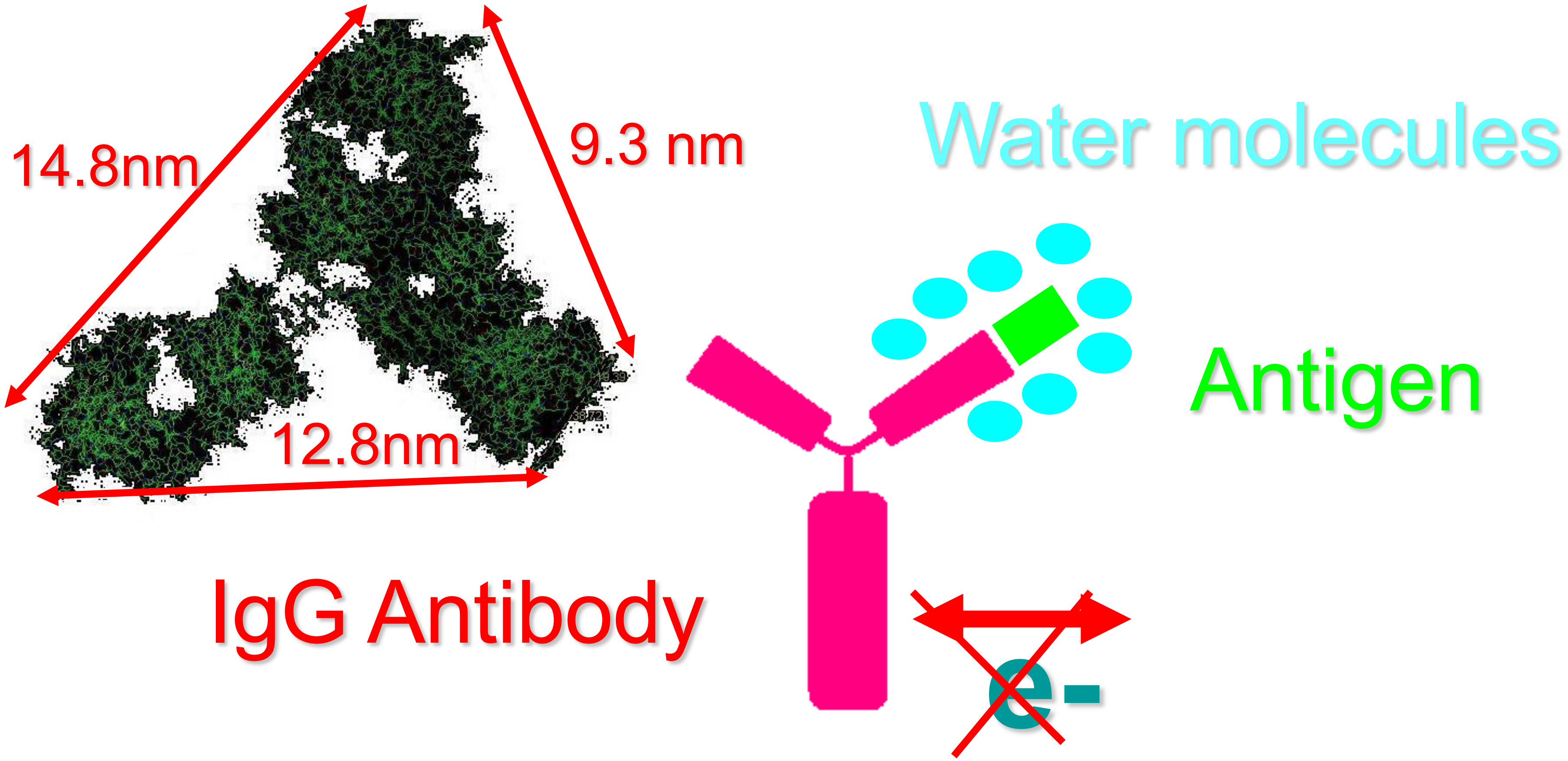
- DNA hybridization at Bio/CMOS interface
- Layering effects with DNA or Antibodies
- Helmholtz Planes & Debye Length
- Oxidases based principle of detection

CMOS/Sample interface



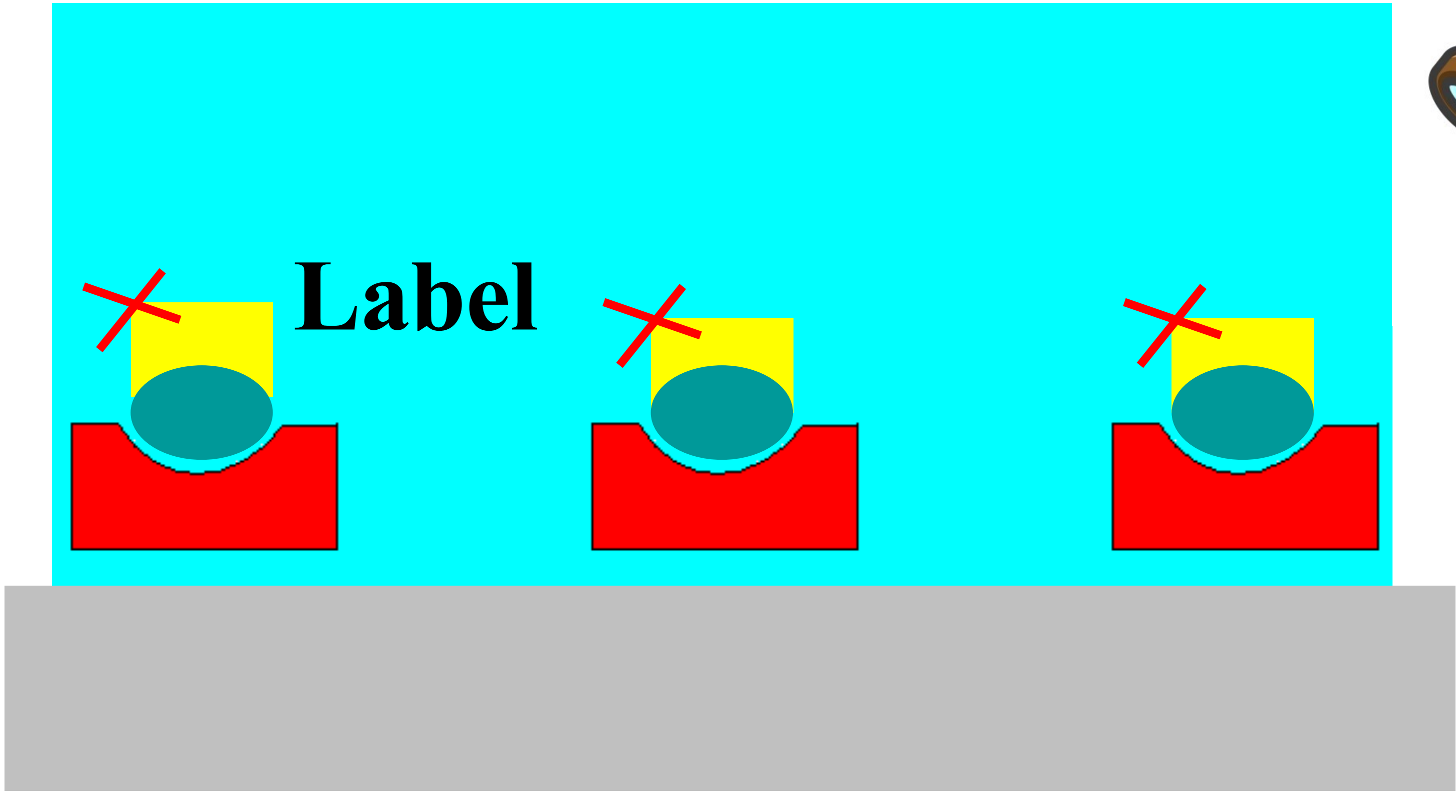
The interface between the CMOS circuit and the bio-sample needs to be deeply investigated and organized

The interaction IgG-Ag



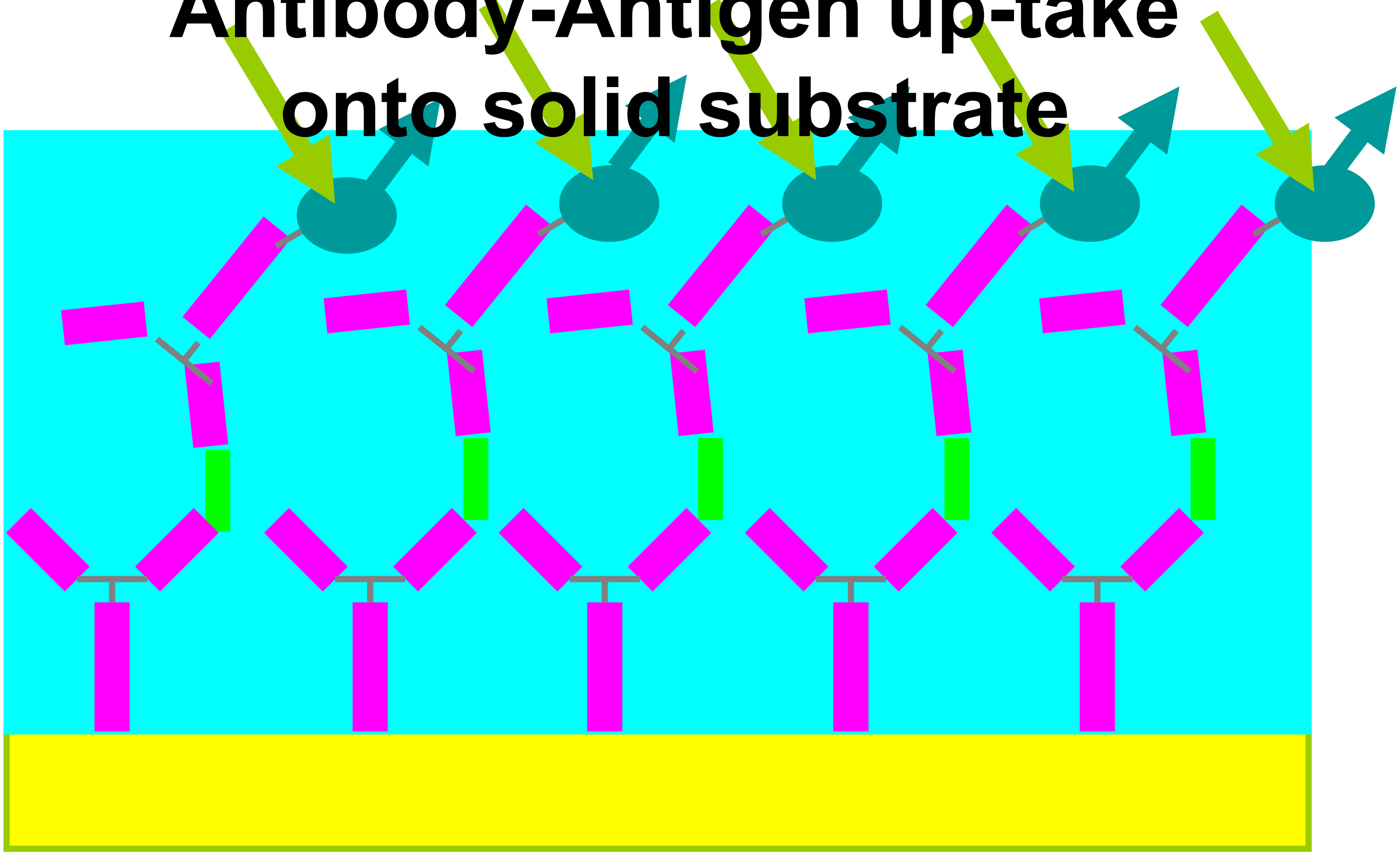
The antigen rests in a very tight binding pocket which is exactly the right size and shape to receive it. Other important factors include enthalpic contributions from van der Waals interactions and hydrogen bonds, and entropic contributions from the release of bound water upon antigen binding

Measuring Bio-Markers



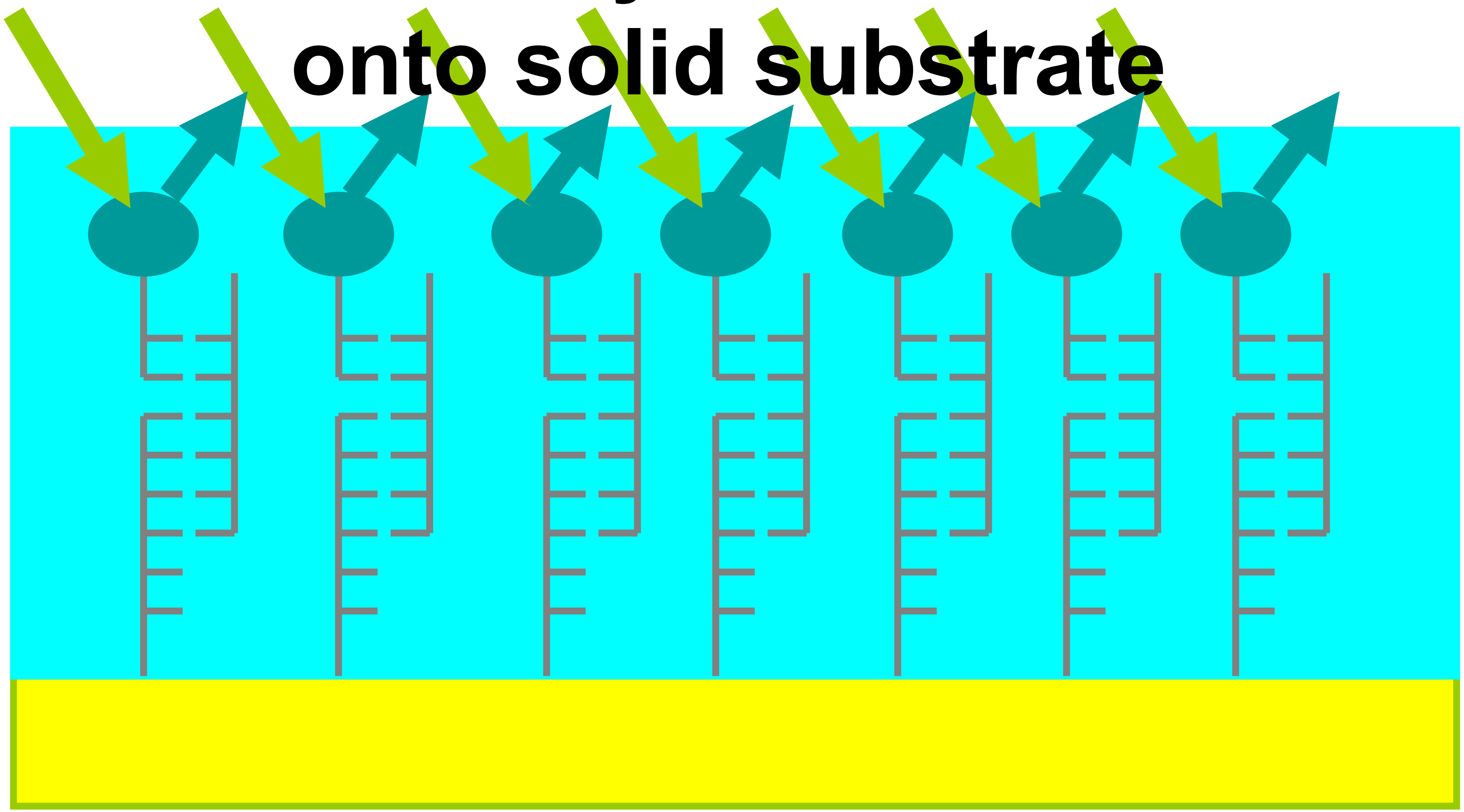
The Measure of Bio-markers may be performed in a labeled manner or in label-free mode

Antibody-Antigen up-take onto solid substrate



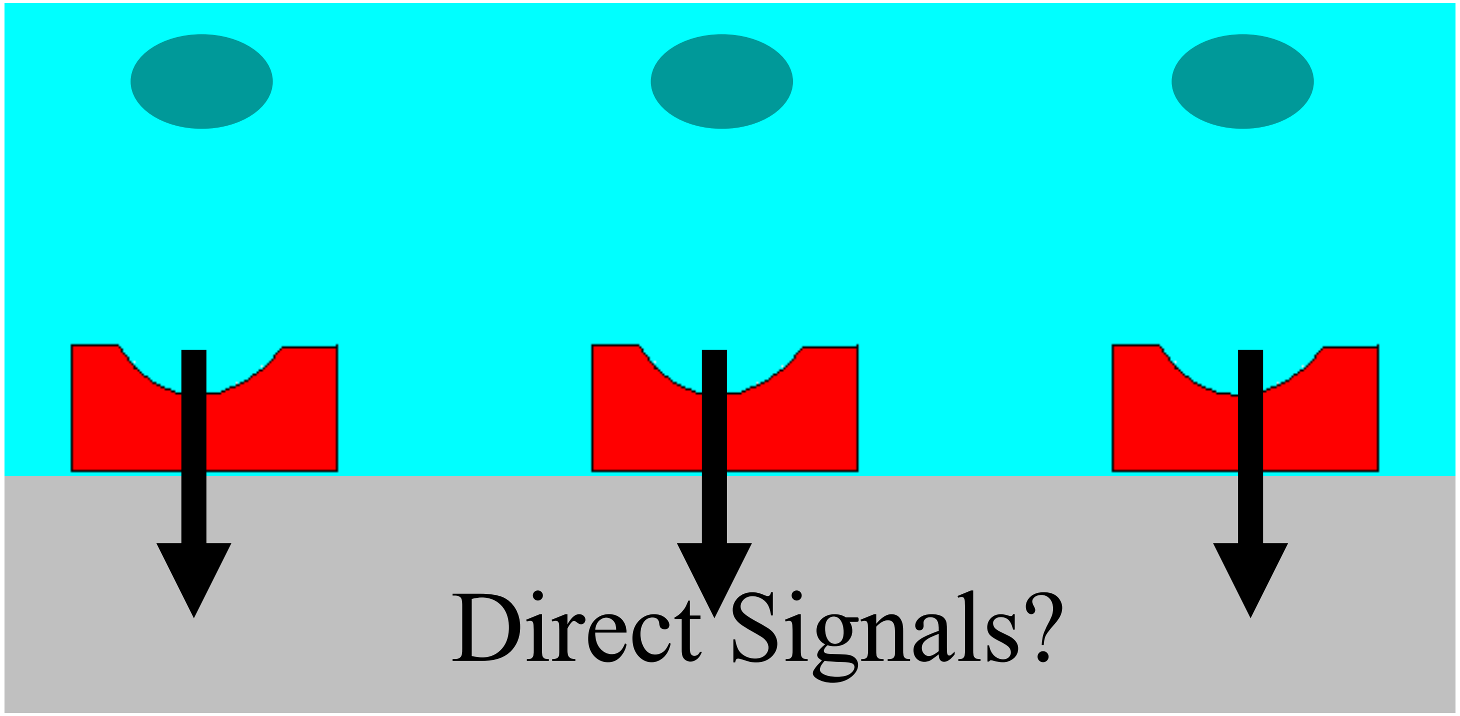
Antigens are specific detected by immobilizing the right antibodies

DNA hybridization onto solid substrate



DNA specific detection by immobilizing the right ssDNA sequence

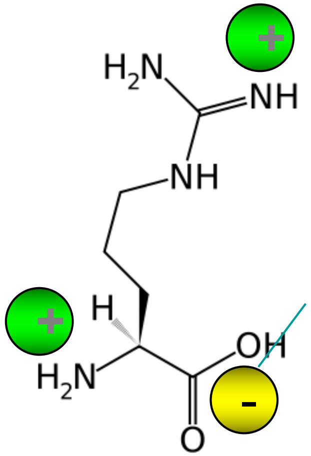
CMOS/Sample interface



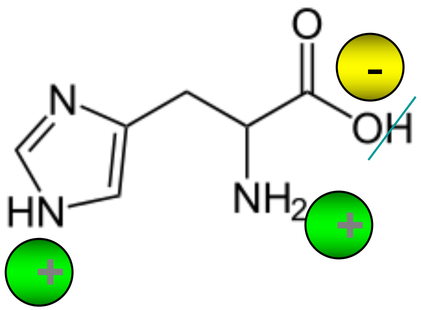
How to get direct signals of probe/target interactions in case of antibodies or ssDNA probes?

Charged Residues

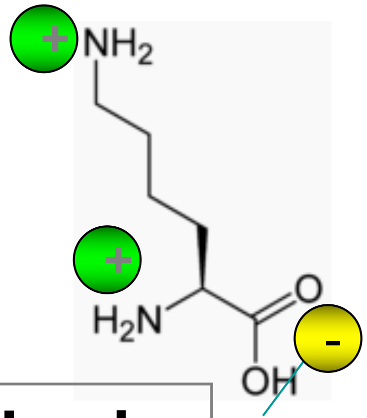
Positively Charged



Arginine

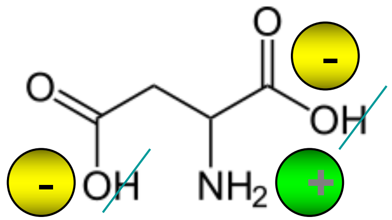


Histidine

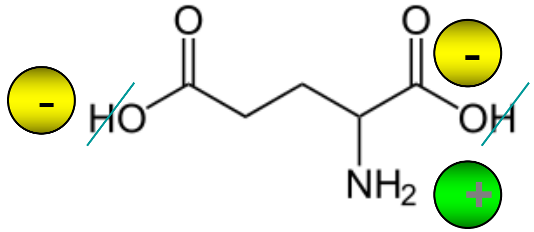


Lysine

Neg. Charged

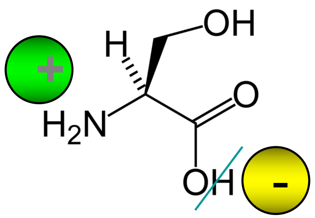


Aspartic Acid

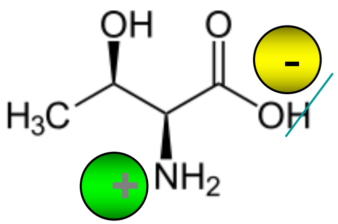


Glutamic Acid

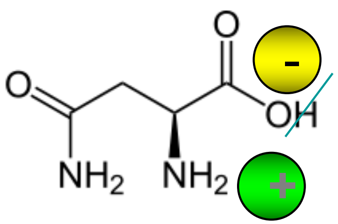
Polar Uncharged



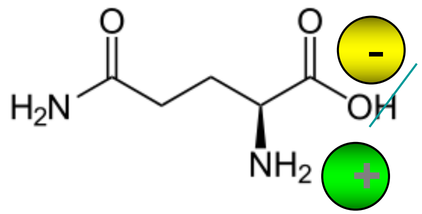
Serine



Threonine



Aspargine

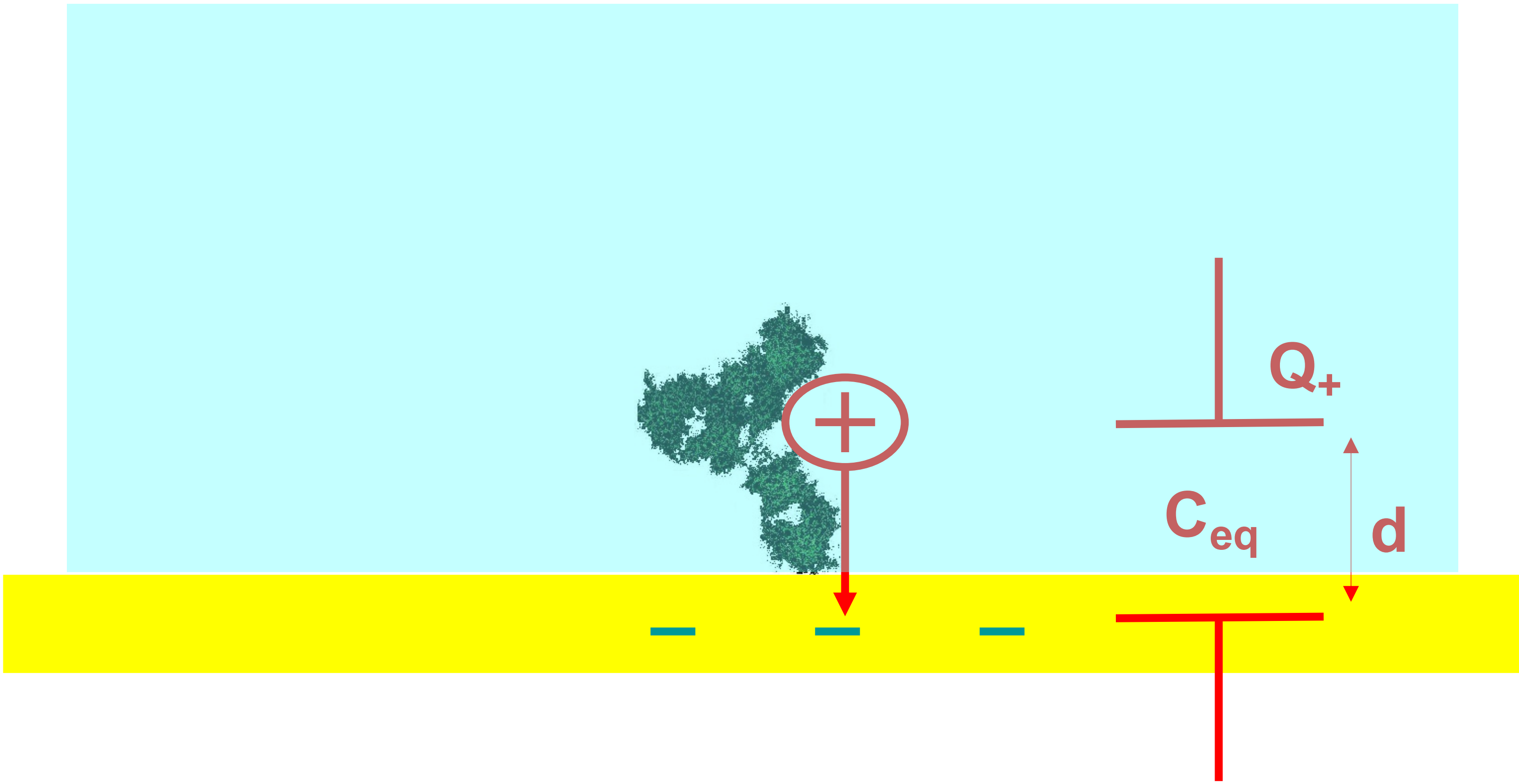


Glutamine

The charges of an Antibody

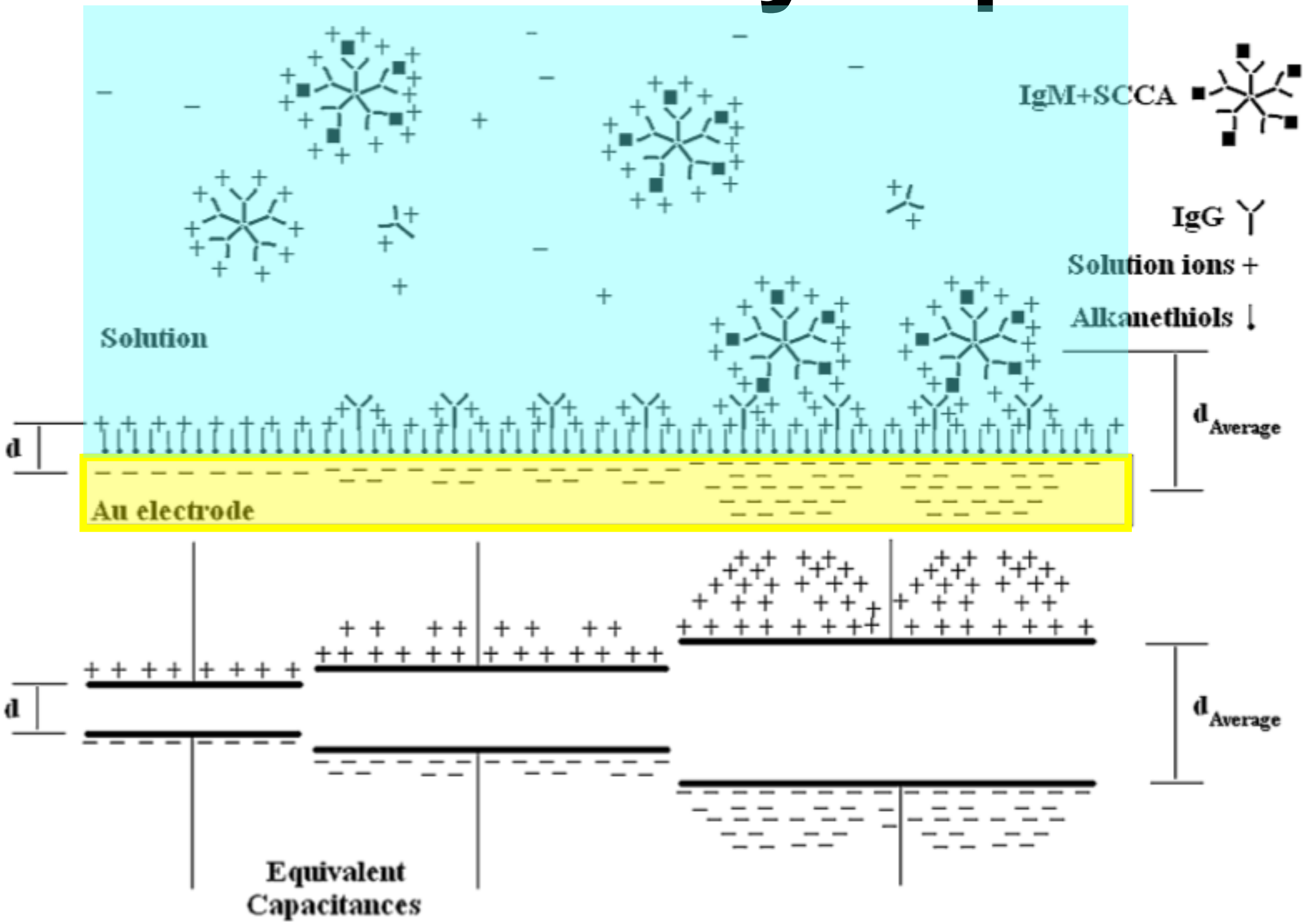
The crystallographic structure of an antibody

Capacitive detection



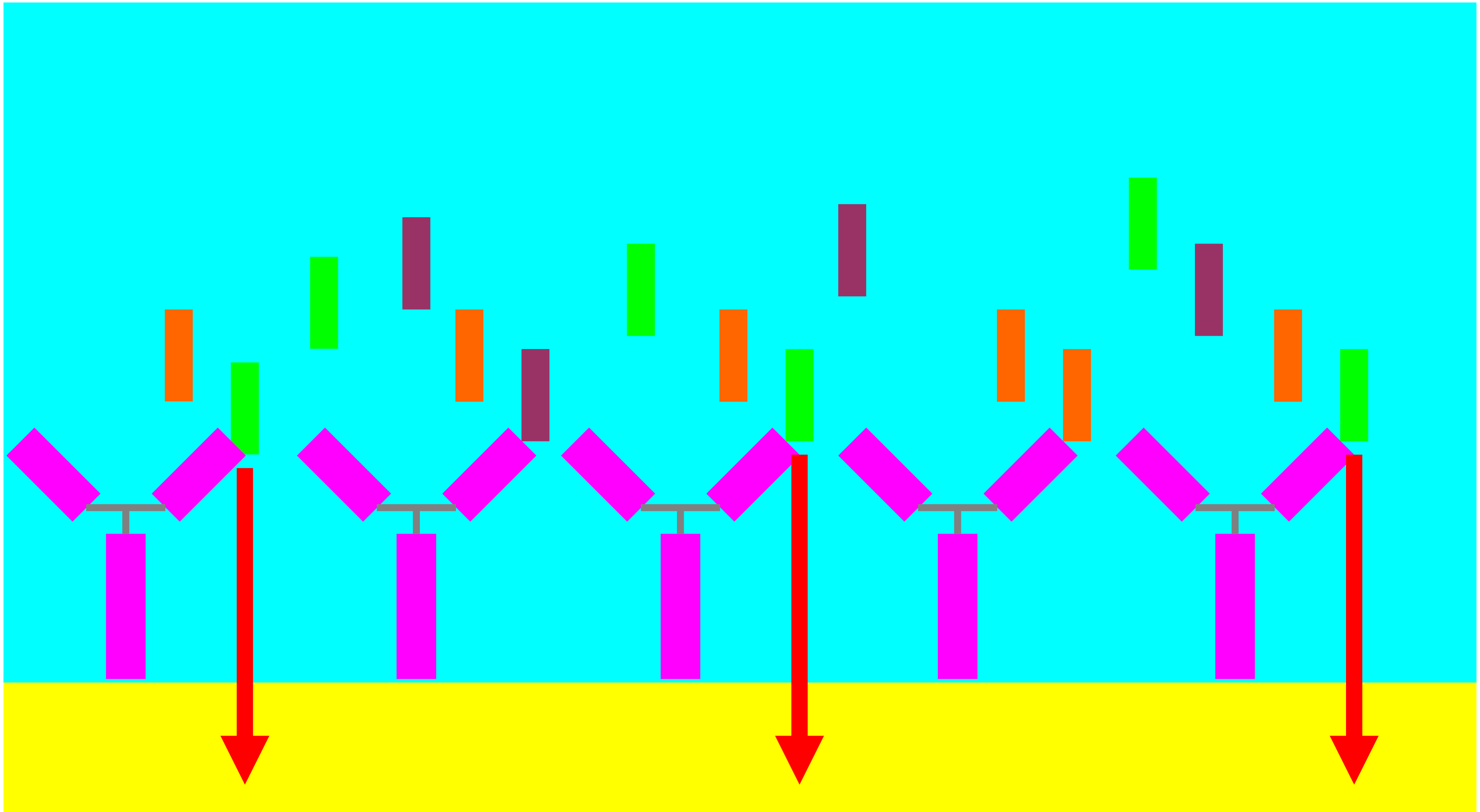
Charged residues of the antibody may affect charge carriers in the electrode

Cancer Detection by capacitance



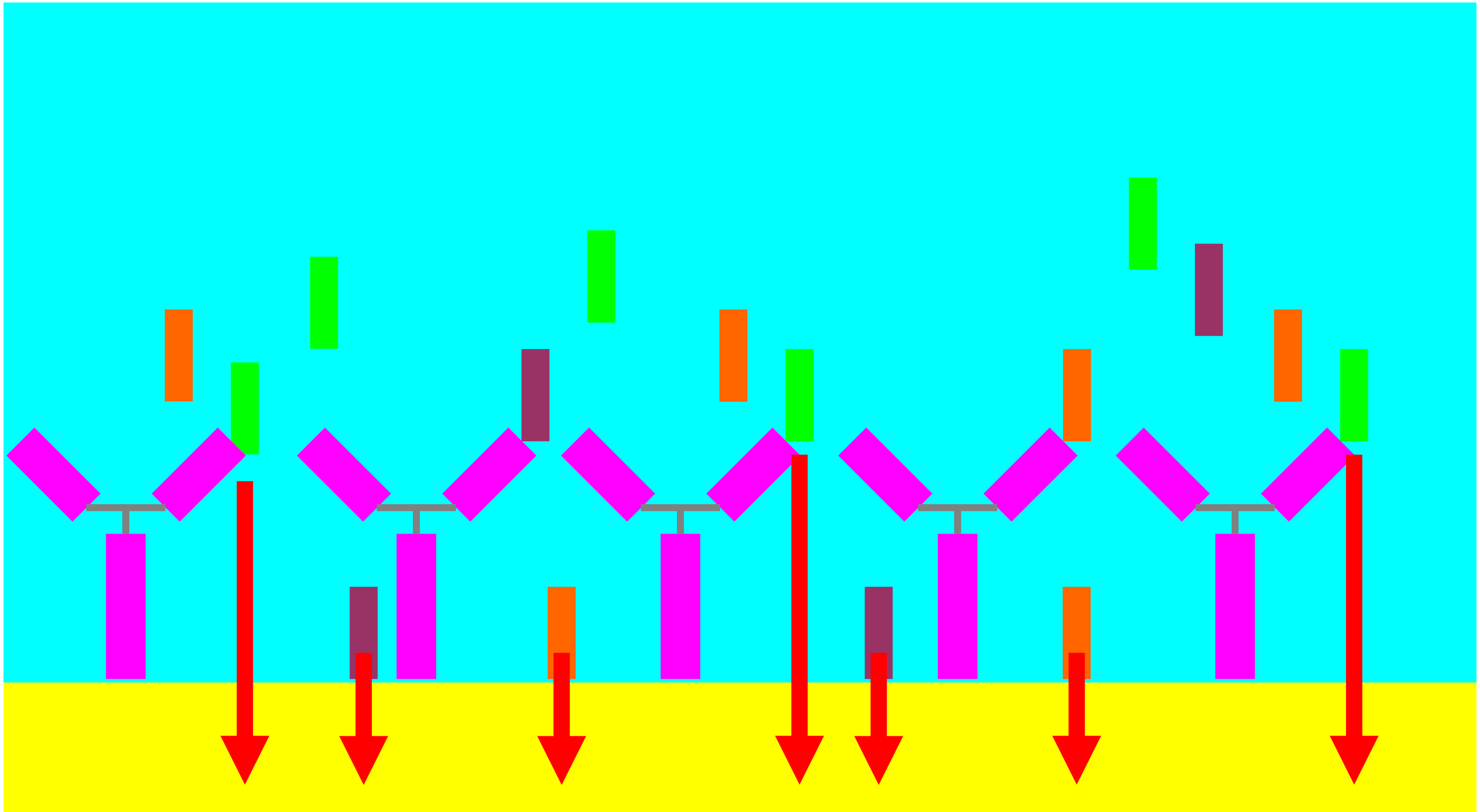
Schematic of the capacitive detection principle

Specificity of the Surface



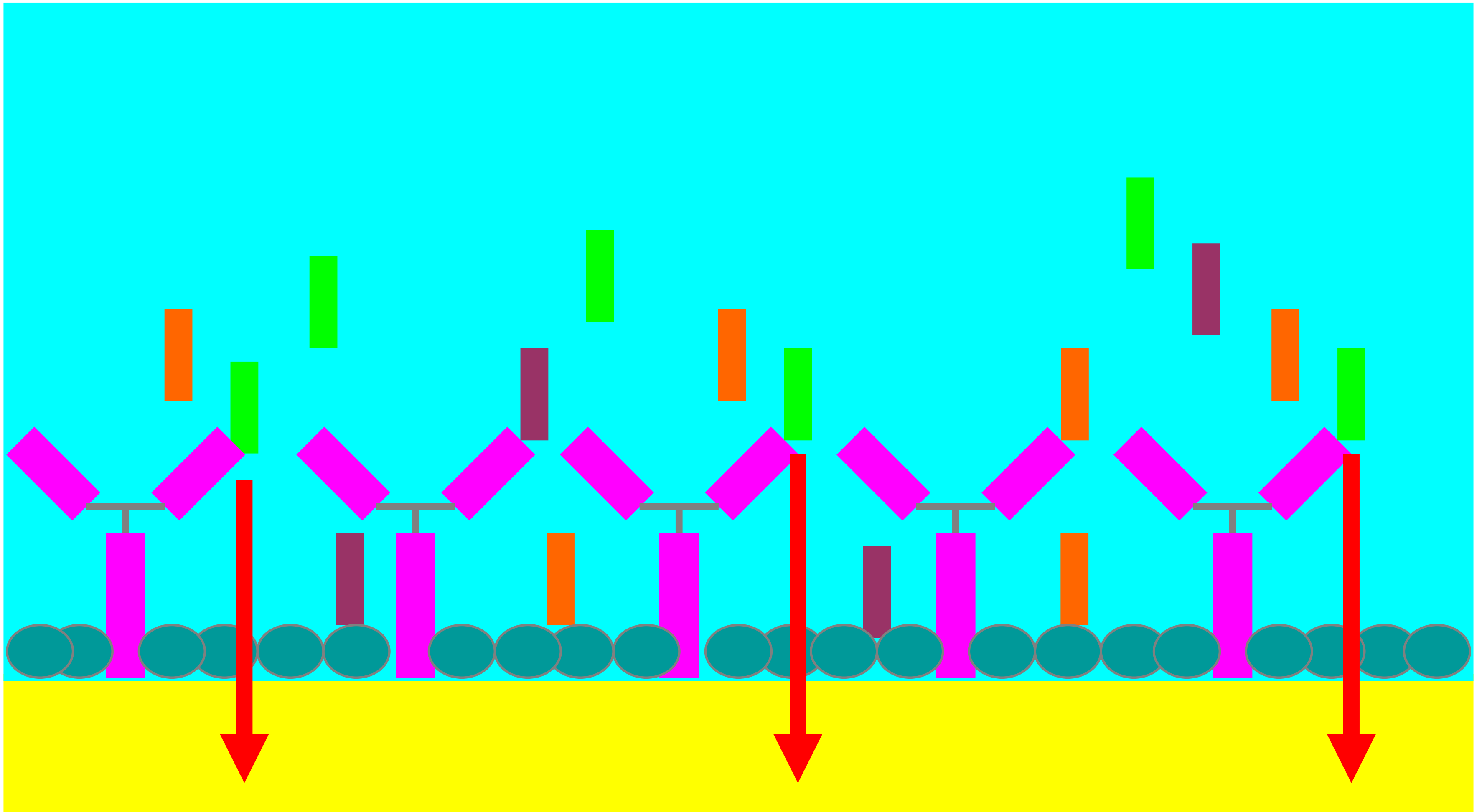
Antigens are specific detected by immobilizing the right antibodies

Specificity of the Surface



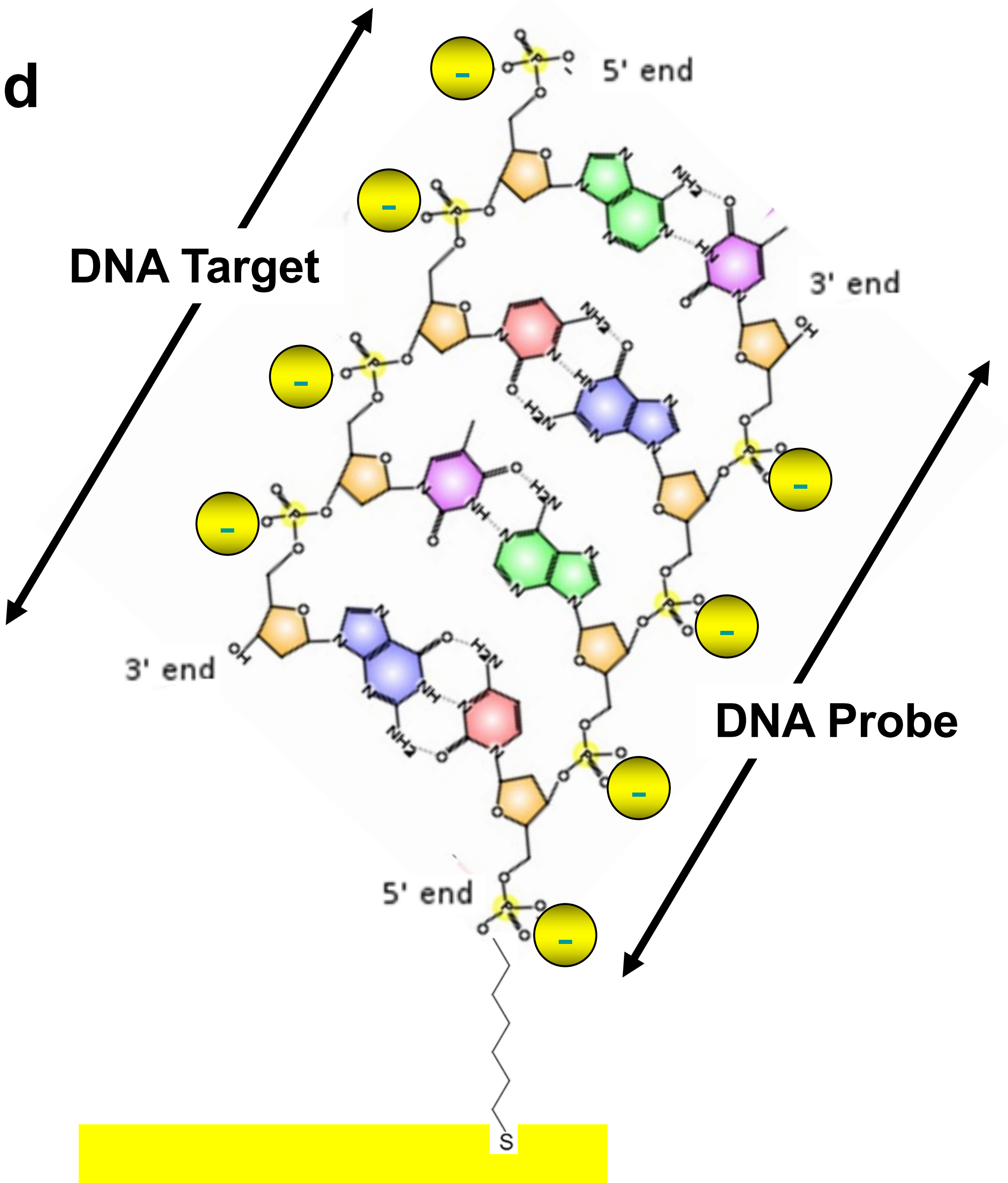
Antibody are specific but the resulting surface might not be specific enough

Specificity of the Surface



Blocking agents are used
to improve surface specificity

DNA probe and target hybridized on a solid substrate

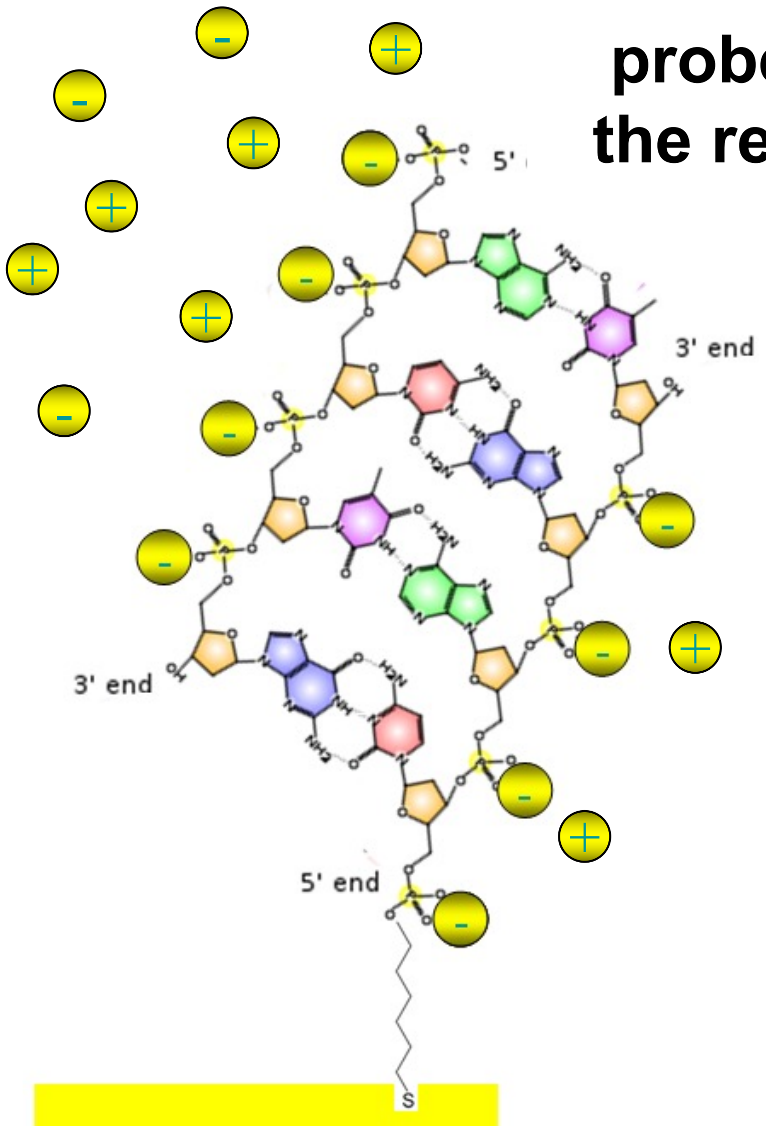


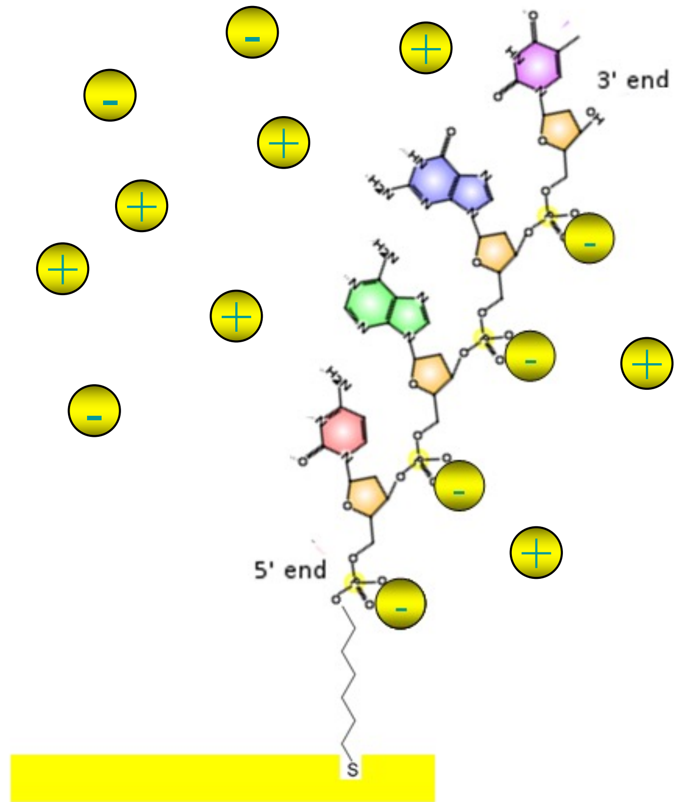
Hybridization degree of DNA

Duplex	ΔG [kJ/mol]
GGTTATTGG CCAATAACC	-26.8
GGTTATTGG CCAAAAACC	-12.0
GGTTCTTGG CCAATAACC	-12.4

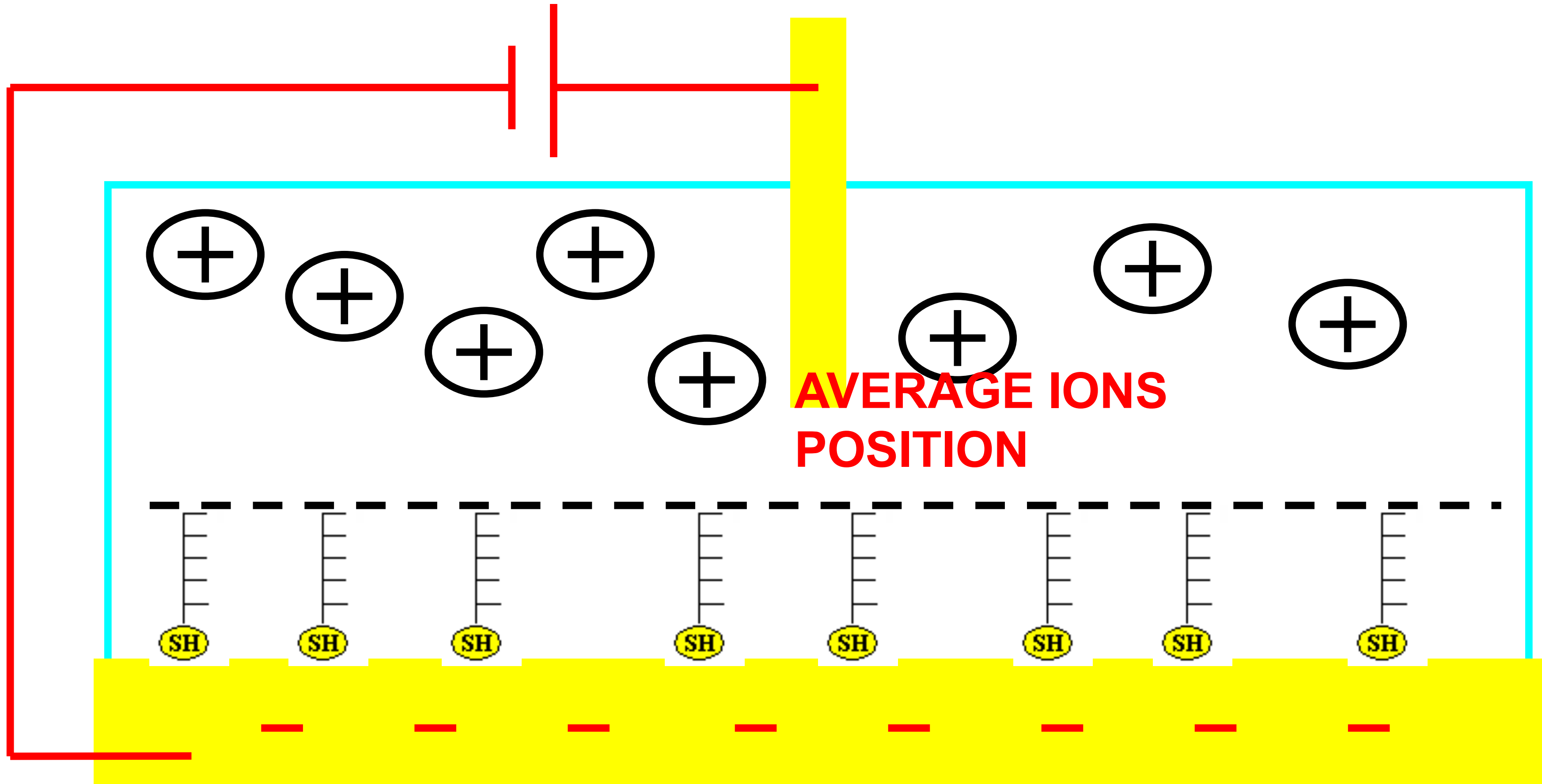
Gibbs free energies of different matching/nonmatching duplexes

DNA probe and hybridized probe/target on a solid substrate and the related solution ions distributions



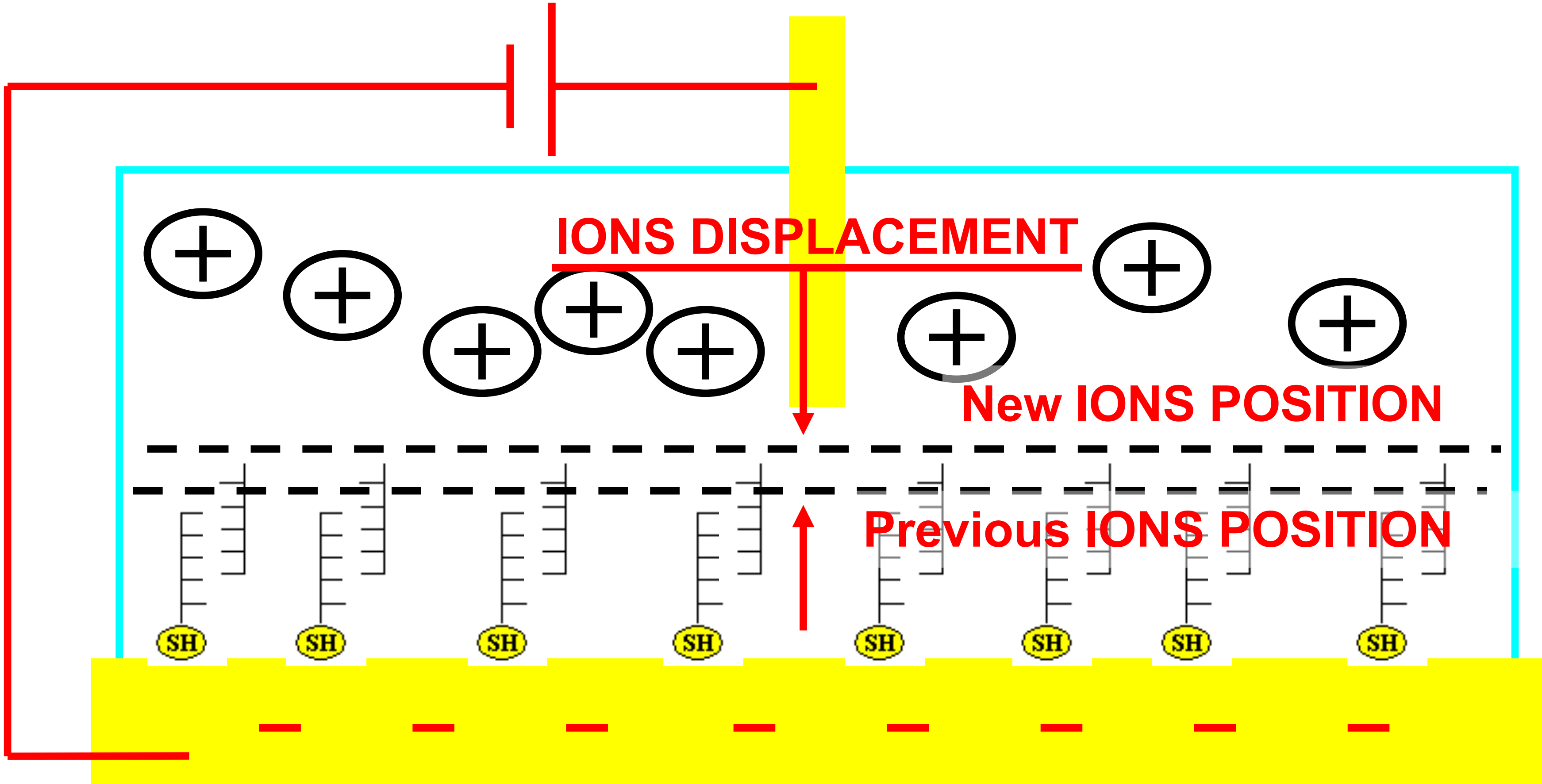


Electrochemical Interface



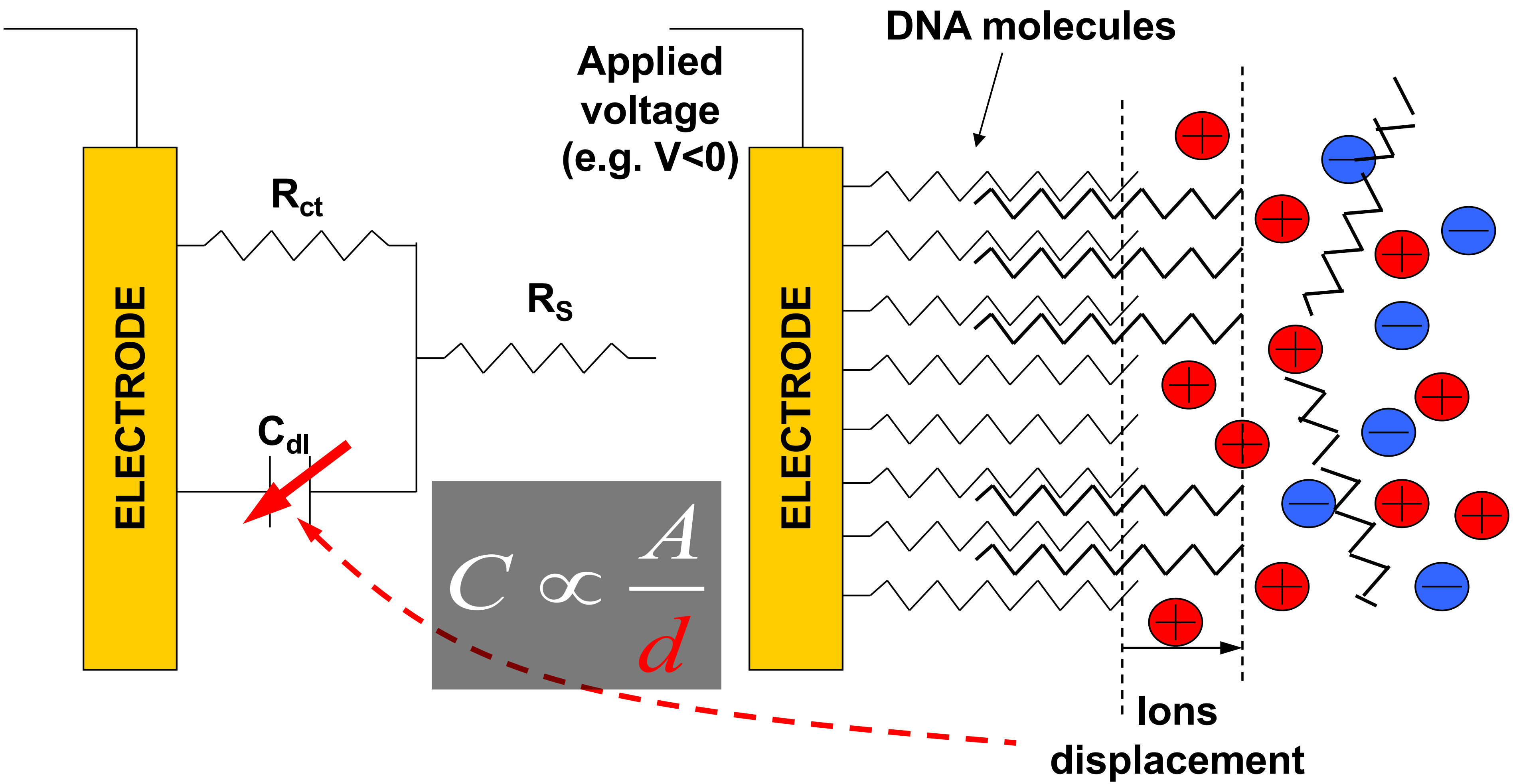
Ion planes are formed at the interface when electrodes immersed in solution are polarized

Electrochemical Interface



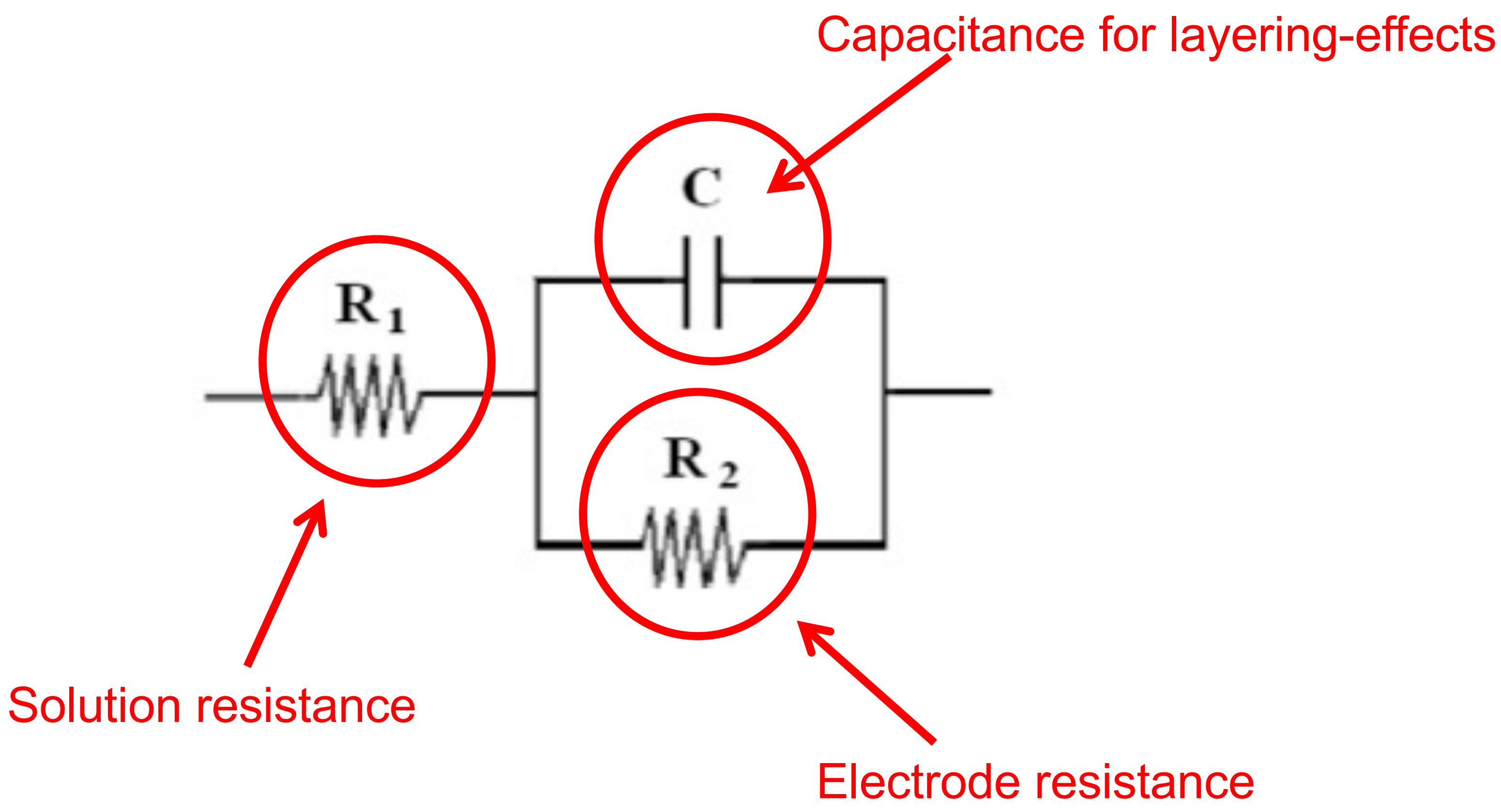
Ion planes are formed at the interface when electrodes immersed in solution are polarized

The Capacitance DNA Detection



Unlabeled ssDNA may be detected with capacitance measurements as due to charge displacement

Equivalent Circuit with Layering effects



Equivalent C of sensing electrodes

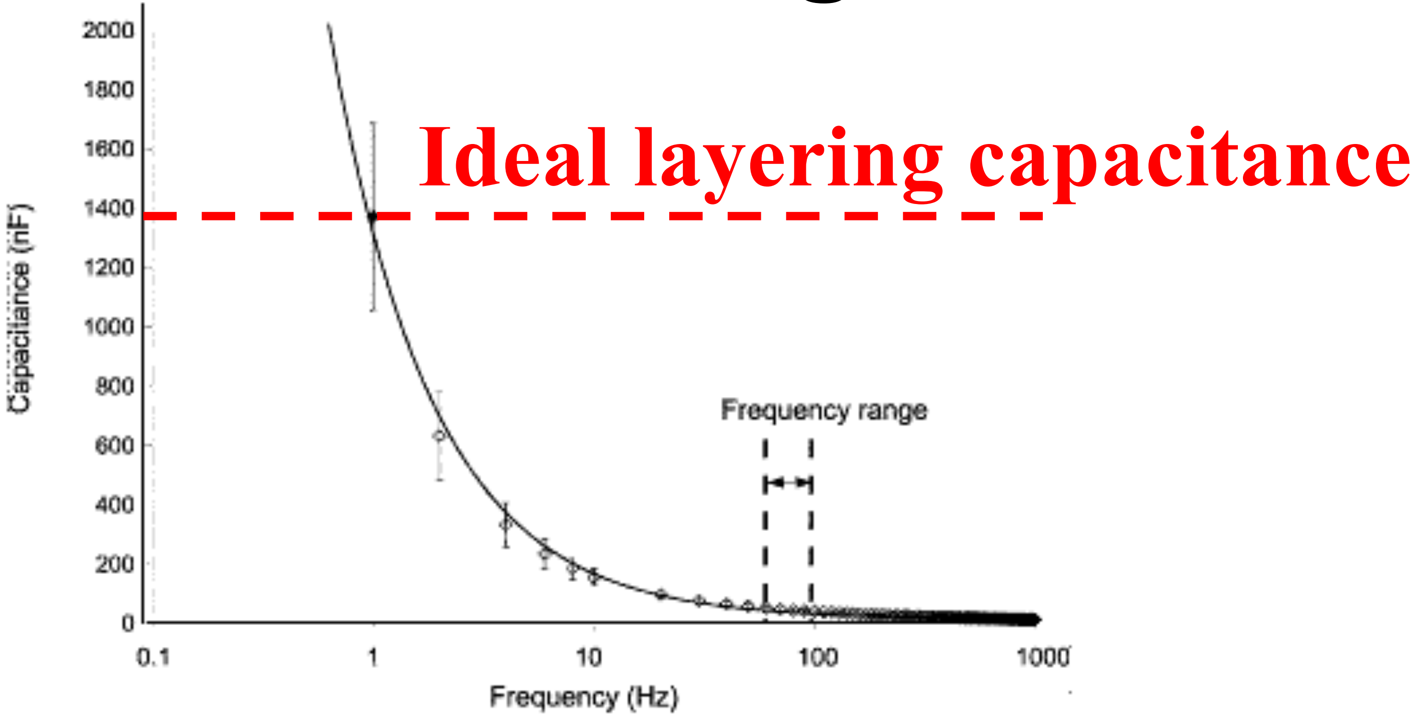
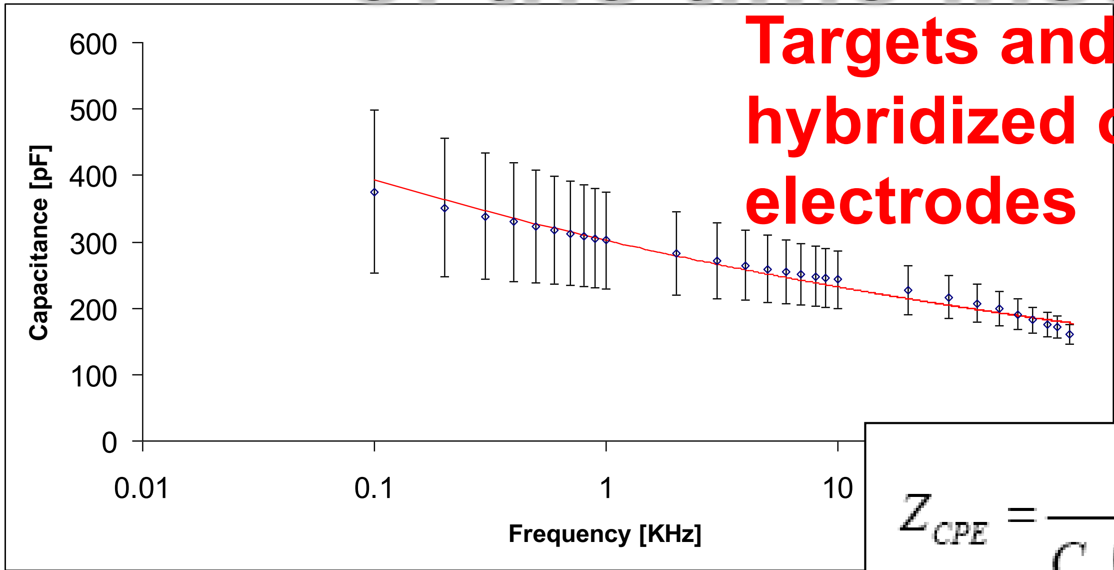


Fig. 9. Measured capacitance versus charge/discharge frequency on clean gold electrodes. The continuous line shows the fitting.

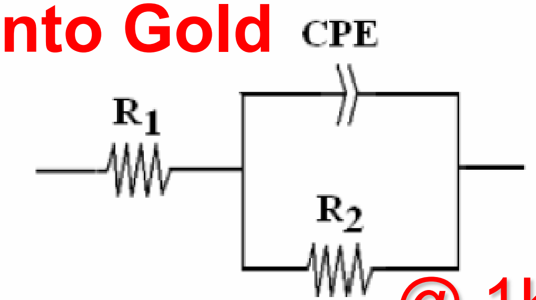
STAGNI *et al.*: FULLY ELECTRONIC LABEL-FREE DNA SENSOR CHIP IEEE SENSORS JOURNAL, VOL. 7, NO. 4, APRIL 2007

The equivalent capacitance of Helmholtz ion planes on bare electrodes is frequency-dependent

How to understand the reason of the time instability?



Targets and probes hybridized onto Gold electrodes



@ 1KHz

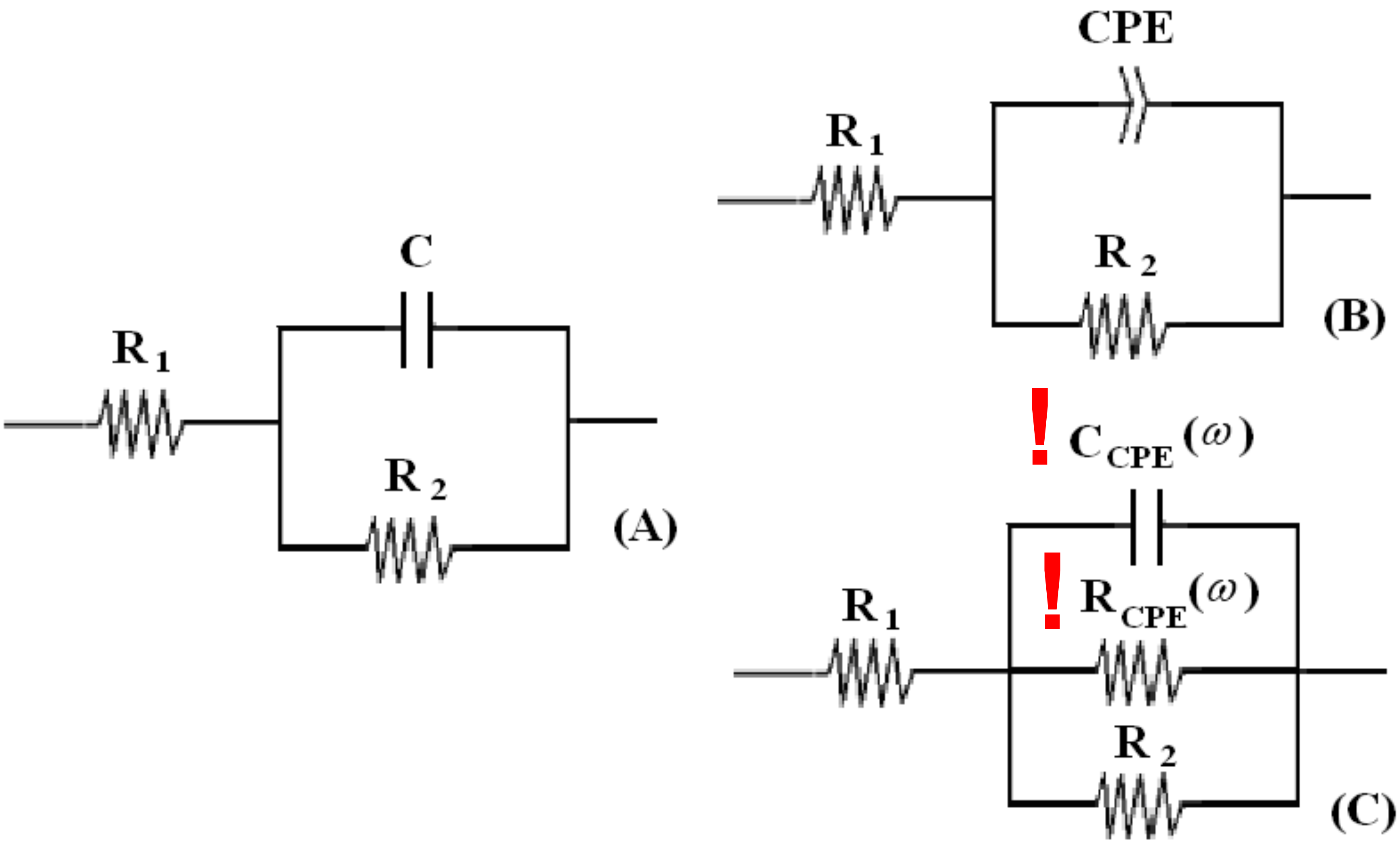
$$Z_{CPE} = \frac{1}{C_p (j\omega)^\alpha} \cong \frac{1}{\omega^\alpha C_p}\sqrt{1-\alpha^2} - j\frac{1}{\omega^\alpha C_p}\alpha$$

CPE parameters	Bare electrode	DNA Probes	DNA Target
R(GΩ)	45	177	177
X(GΩ)	123	837	970

S.Carrara et al., Sensors and Transducer Journal 76 (2007) 969-977

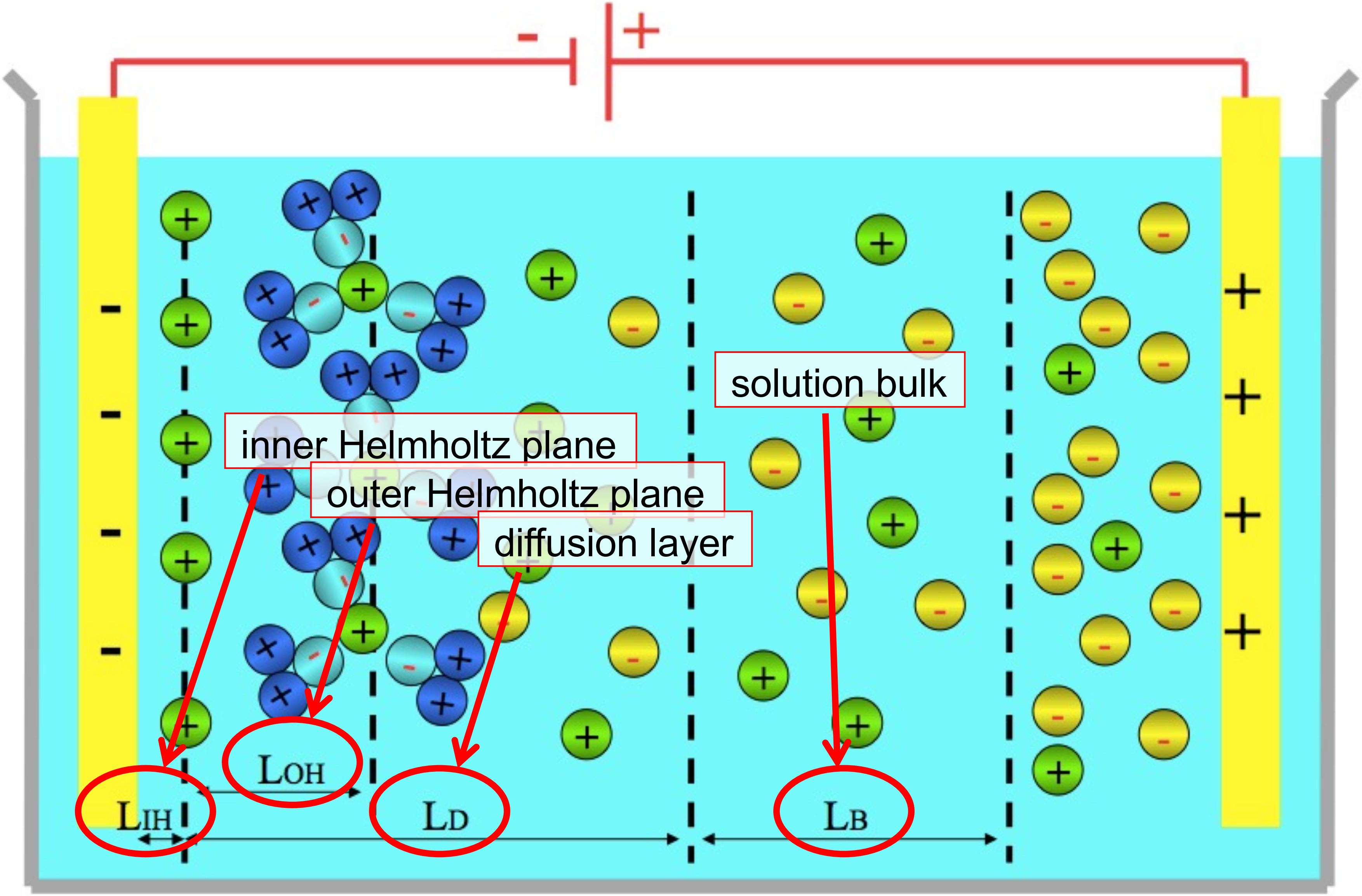
Charge transfer pathways through the DNA layer affect the ideal Capacitance behavior of the interface with the solution sample

Equivalent circuits



Equivalent circuits of DNA Bio/CMOS interface

Helmholtz Planes



Debye Length

Charge density: $$\rho_e = \sum_i z_i e n_i$$

z_i = charge of species i (*e.g.* +2, -1, etc.)

n_i = concentration of species i (number per volume)

$$\nabla^2 \phi = 0$$ In the bulk

$$\nabla^2 \phi = -\frac{\rho_e}{\kappa \varepsilon_0}$$ Close to electrodes

For perturbation away from equilibrium at finite temperature

$$\hat{\phi} \equiv \phi - \phi_0 \qquad \rho_e = \sum_i z_i e n_{i0} \exp\left(-\frac{z_i e \hat{\phi}}{k_B T}\right)$$

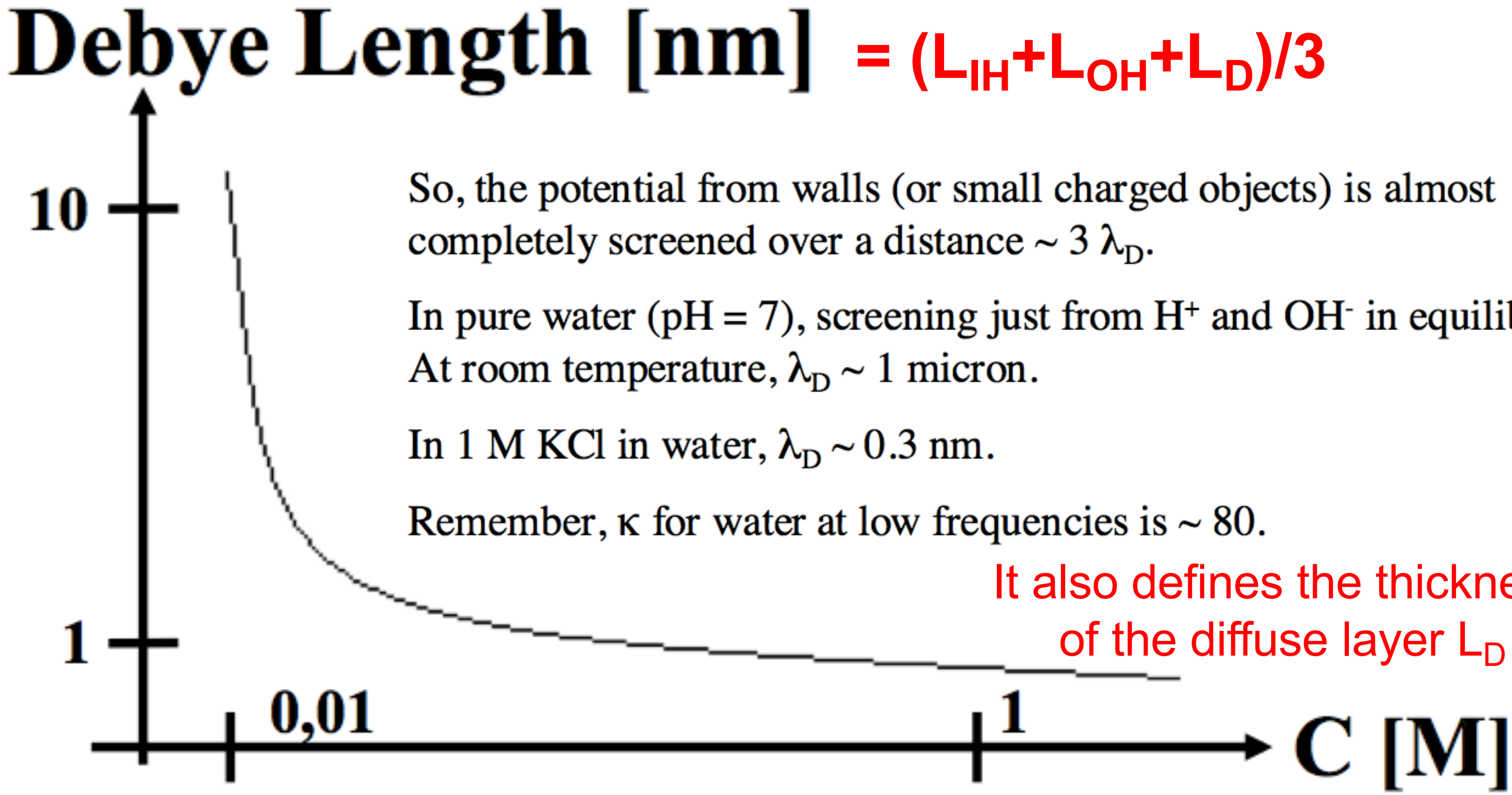
Debye Length

$$\nabla^2 \hat{\phi} = -\frac{1}{\kappa\varepsilon_0}\sum_i z_i e n_{i0} \exp\left(-\frac{z_i e\hat{\phi}}{k_B T}\right) \approx -\frac{1}{\kappa\varepsilon_0}\sum_i z_i e n_{i0} + \frac{e^2}{\kappa\varepsilon_0 k_B T}\sum_i z_i^2 n_{i0}\hat{\phi} \equiv \frac{1}{\lambda_D^2}\hat{\phi}$$

~ 0 for equilibrium neutrality

$$\lambda_D \equiv \left(\frac{e^2}{\kappa\varepsilon_0 k_B T}\sum_i z_i^2 n_{i0}\right)^{-1/2}$$

Debye Length



The Bebye Length is defined as the region of charge carrier's net electrostatic effect in solution

Enzymes' based detection

Some enzymes provide redox reactions in catalysing their substrates. In the case of these enzymes, we can exploit their catalysis for the aim of an electrochemical direct detection. That's the case of both oxidases and cytochromes.

Redox with oxidases

The typical redox involving an oxidase is as follows:

$$XOD/FAD + X \rightarrow XOD/FADH_2 + X_p$$

The FAD (Flavin Adenine Dinucleotide) is a functional part of the protein that gains a hydrogen molecule after the reaction. Therefore, the oxidase is not yet ready for another transformation because the FAD has gained the H_2. To return to its initial state, the enzyme needs to release that hydrogen molecule:

$$XOD/FADH_2 + O_2 \longrightarrow XOD/FAD + H_2O_2$$

Redox with oxidases

The hydrogen peroxide provide two possible redox reactions. An oxidation:

$$H_2O_2 \xrightarrow{+650mV} 2H^+ + O_2 + 2e^-$$

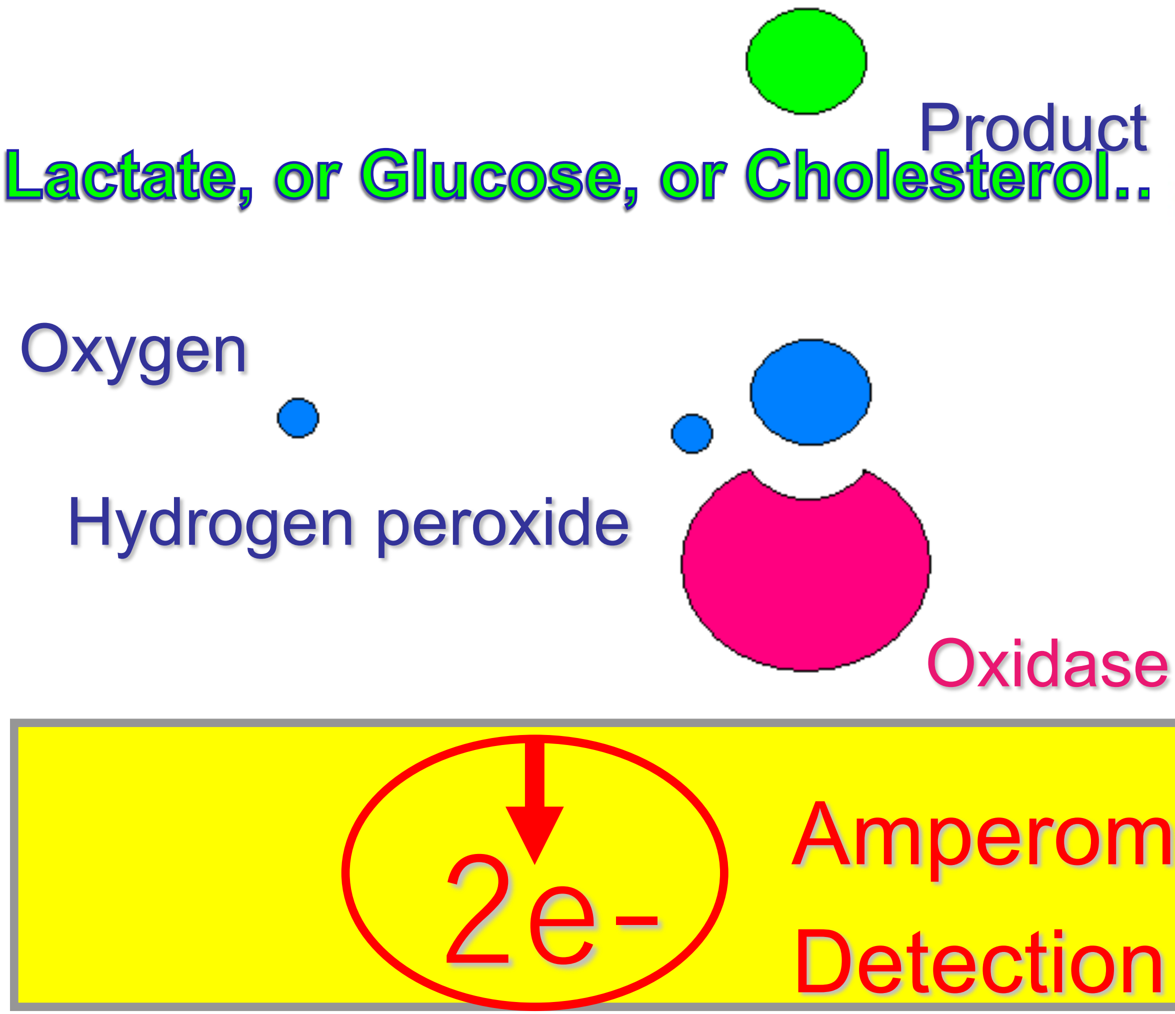
And a reduction:

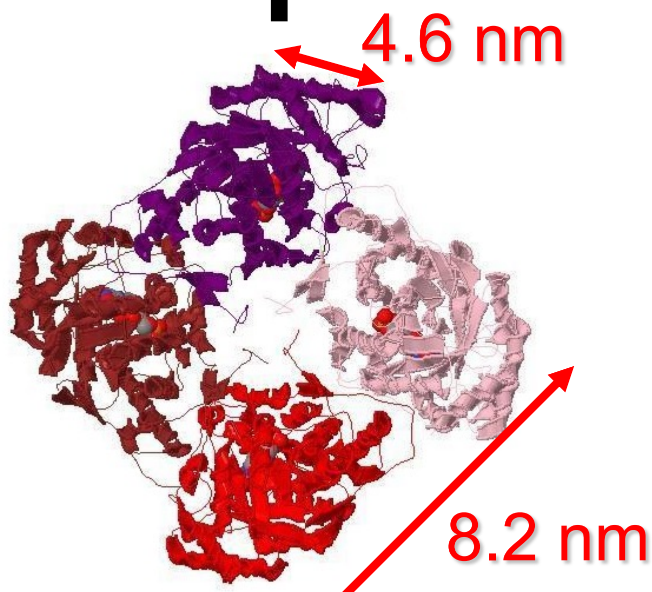
$$H_2O_2 + 2H^+ + 2e^- \xrightarrow{+1540mV} 2H_2O.$$

A third redox is provided by the oxygen reduction:

$$O_2 + 4H^+ + 4\,e^- \xrightarrow{-700\ mV} 2H_2O$$

Oxidases working principle



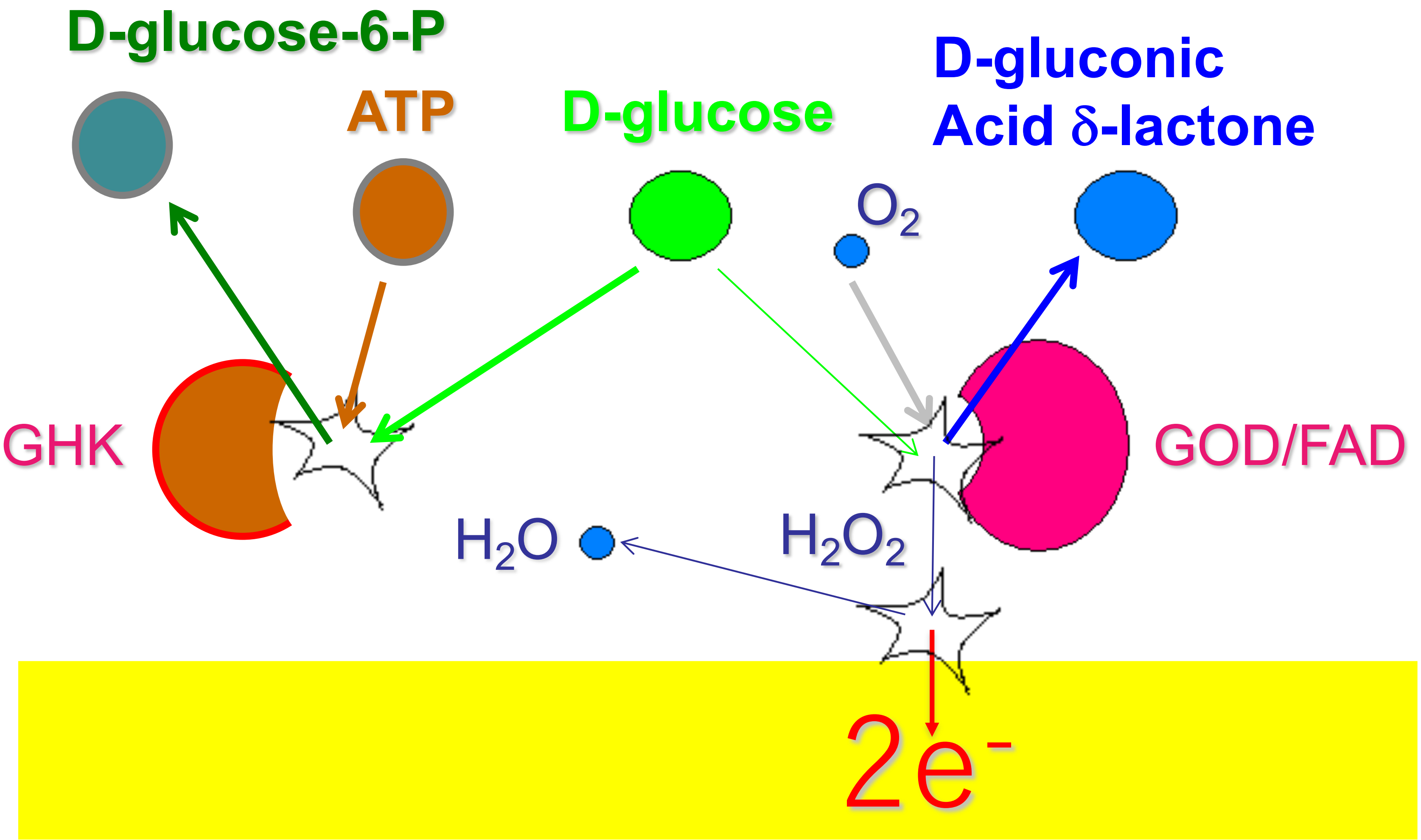


An Oxidase

Enzymes’ based detection

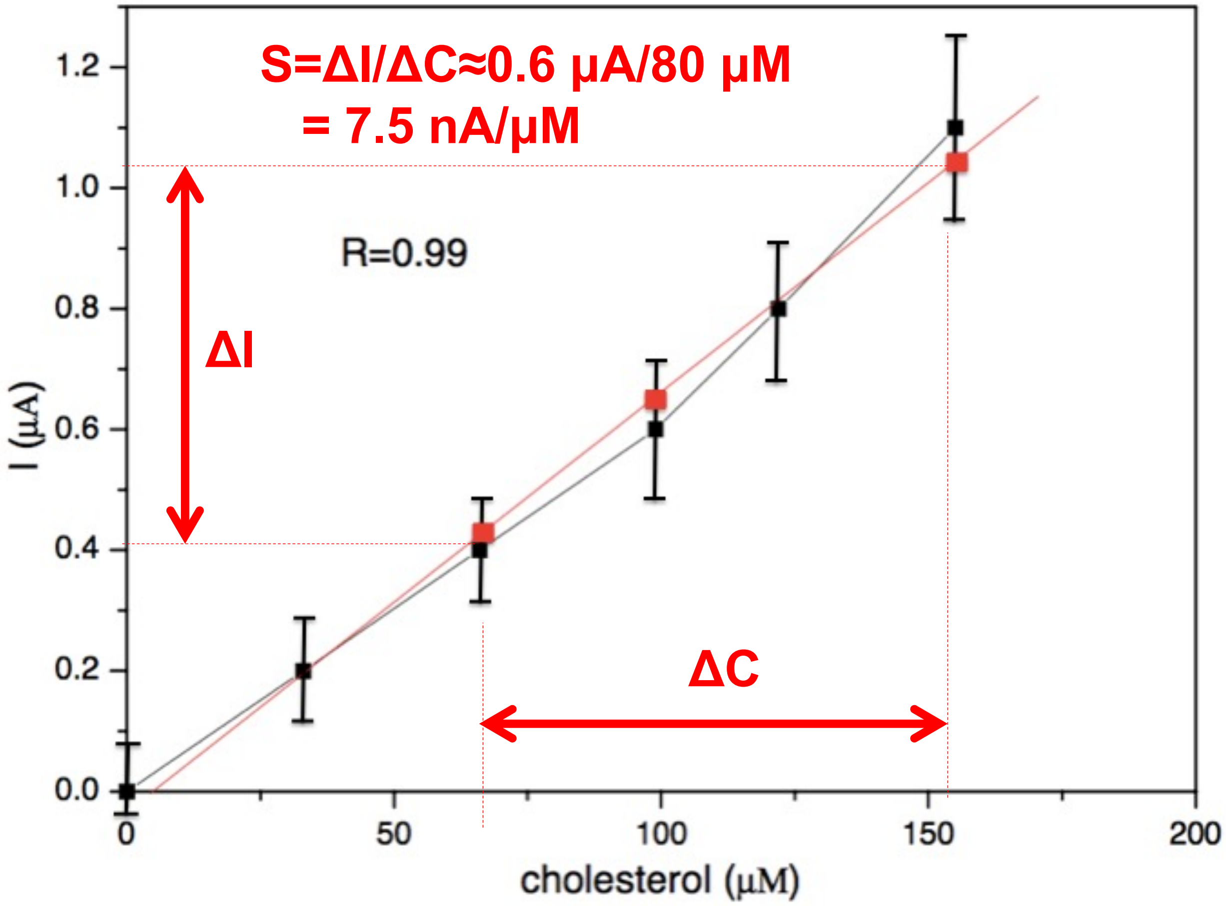
Other enzymes do not provide any redox reaction in catalysing their substrates However, some of them may be used together with enzyme that do it. In the case, we can exploit their catalysis for the aim of an electrochemical direct detection by combining two different kind of enzymes on the same Bio/CMOS interface. That is the case of the detection of ATP

ATP detection



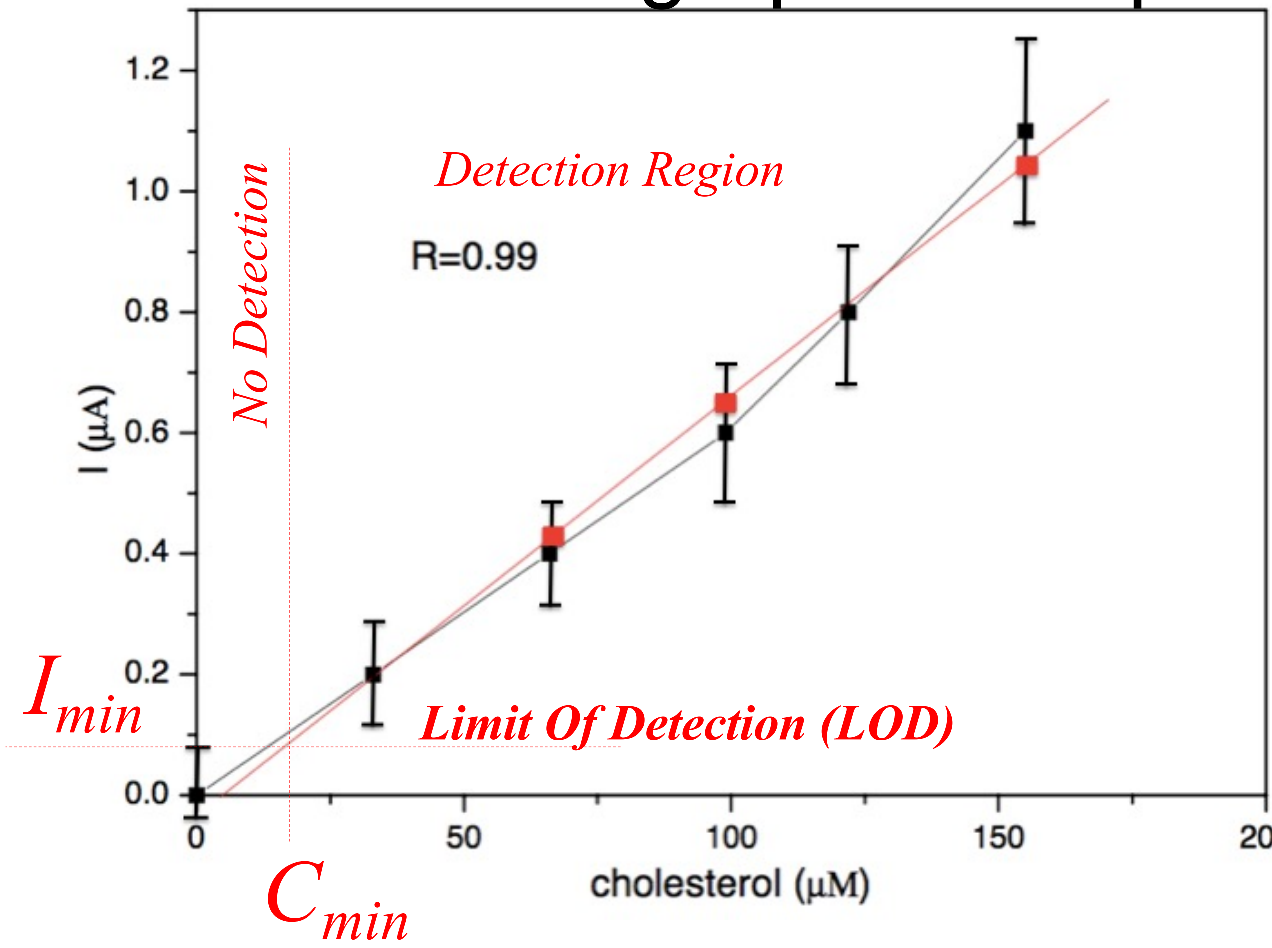
Detection Principle

- Sensitivity: example – A linear sensor



Detection Principle

- Detection Limit: a graphic interpretation



$$LOD = \frac{\delta I_{zero}}{S}$$