EPFL

Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #5

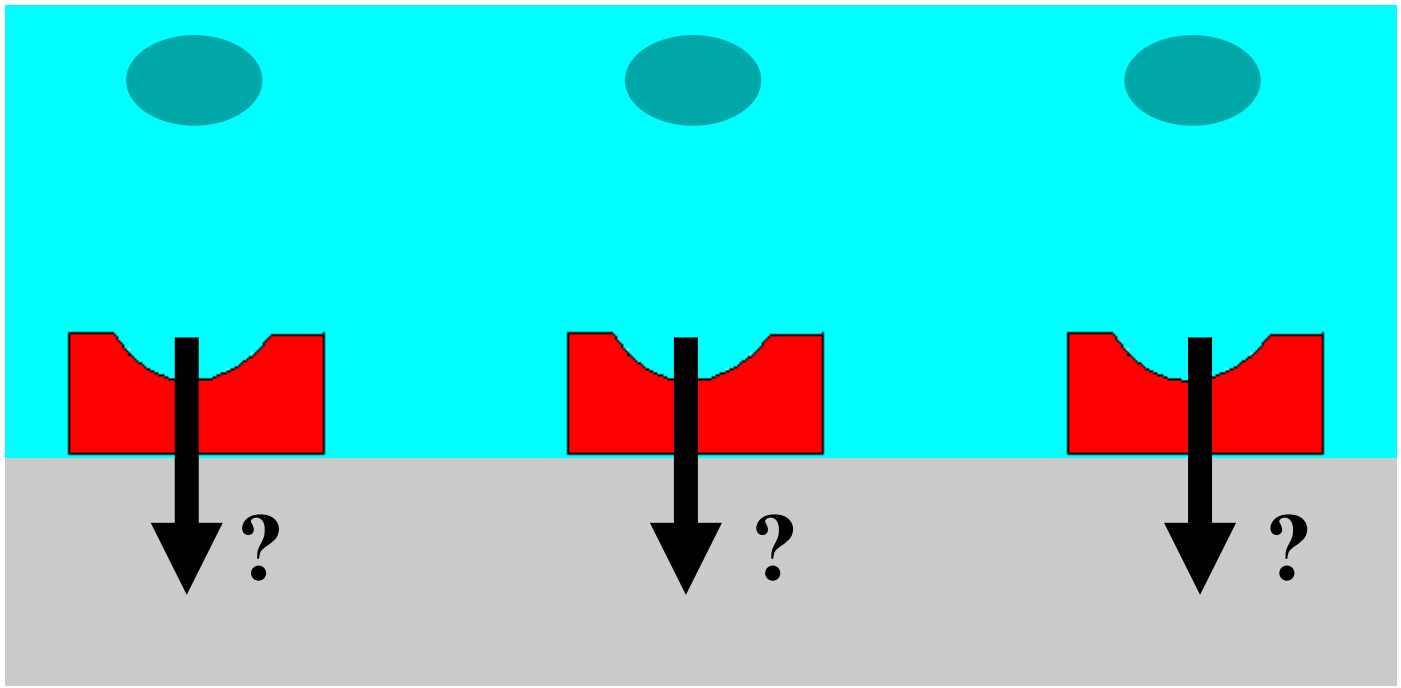
Probe Detection Principles (with Antibodies and DNA)

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs §6.1-4 & 6.8)

- Uptake Ab/Ag @ Bio/CMOS interface
- DNA hybridization @ Bio/CMOS
- Layering effects with DNA or Antibodies
- Helmholtz Planes & Debye Length

CMOS/Sample sensing interface



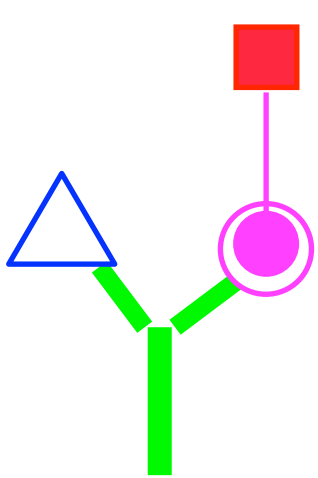
The interface between the CMOS circuit and the bio-sample needs to be deeply investigated and organized

Different Kinds of Antibody

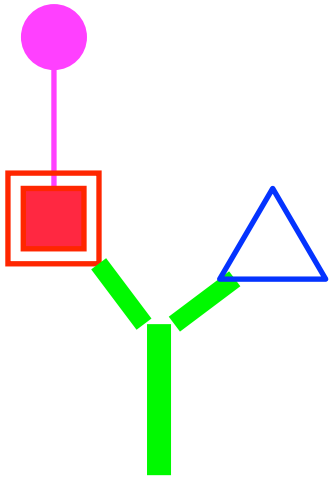
Dealing with real cases results in a bit more complex situation than just adding antigens to antibodies with a unique perfect match. **Monoclonal antibodies** are, then, all antibodies that have exactly the same specificity because they are from the same cloned single cell. However, antibodies are in general secreted in blood plasma by cells that are from different cell lines. Therefore, it is easy to obtain antibodies that are all against the same antigen but that do not have exactly the same specificity: these are **polyclonal antibodies**. Different kinds of antibodies means different kinetics on the same antigen.

We may also obtain different kinetics by involving the same antibody. It happens when the secreted antibody possesses two different paratopes to address two different epitopes of the same antigen

Polyclonal Antibodies

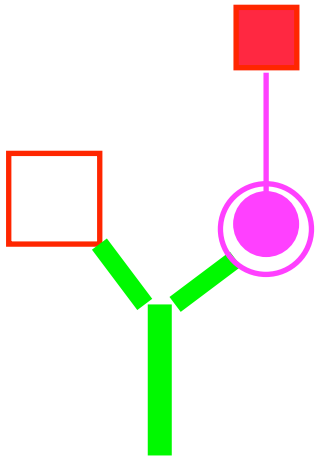


Antibody 1

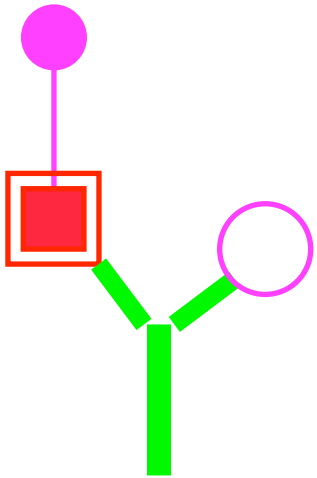


Antibody 2

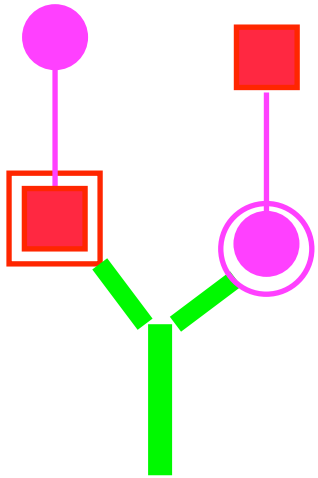
Different monomers with same antibody



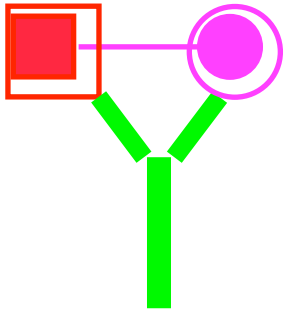
Simple Monomer



Simple Monomer

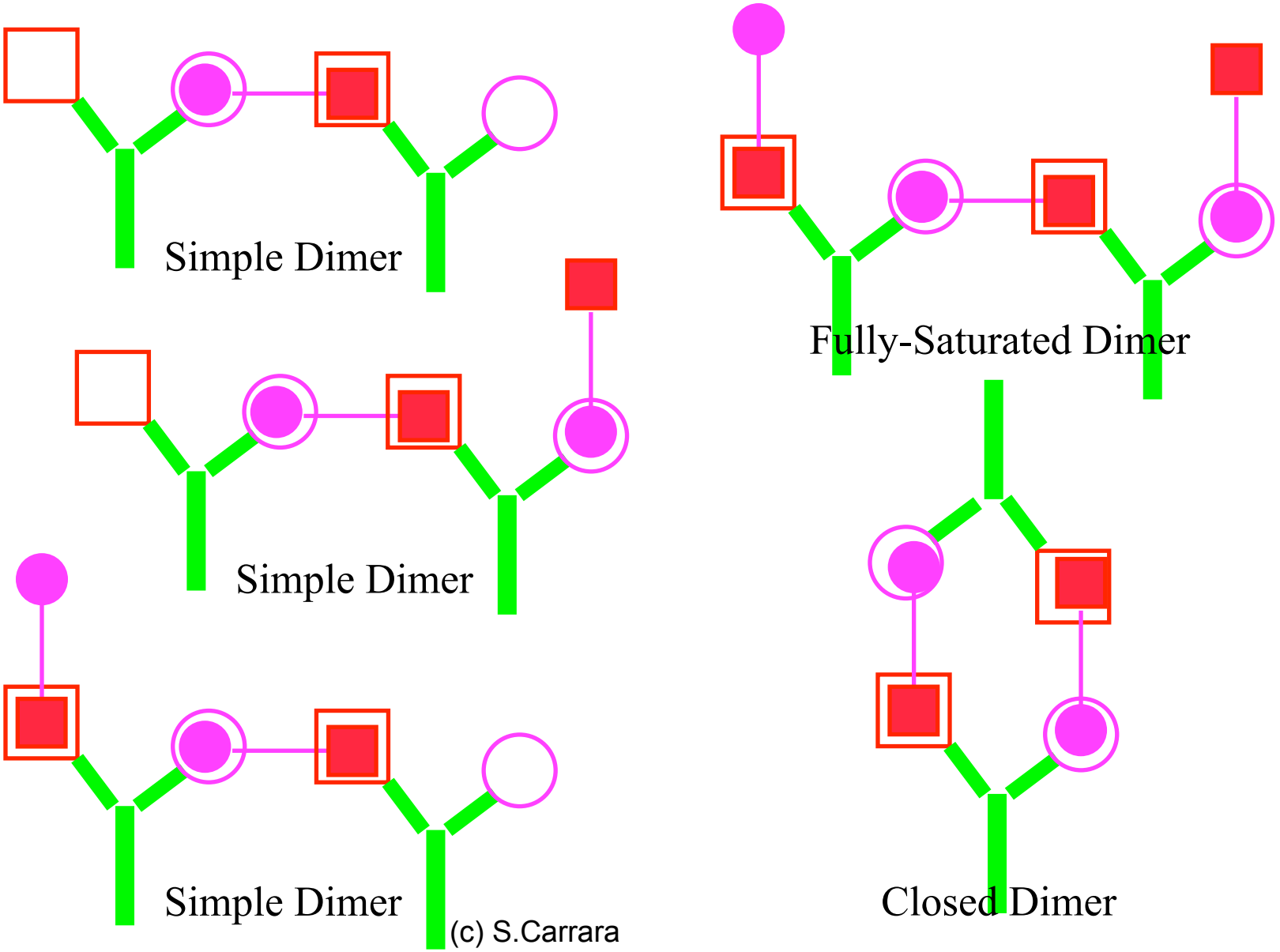


Fully-Saturated Monomer

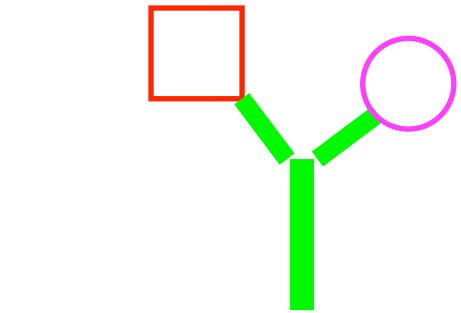


Closed Monomer

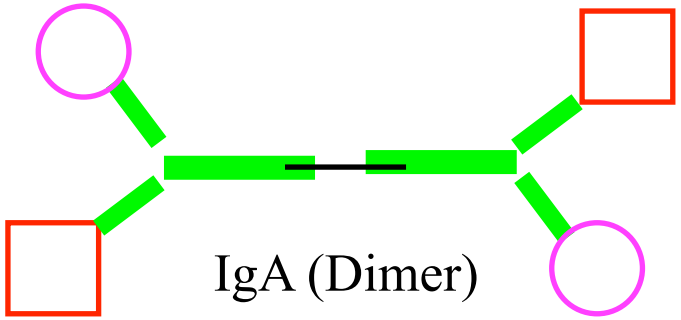
Different dimers with two antibodies



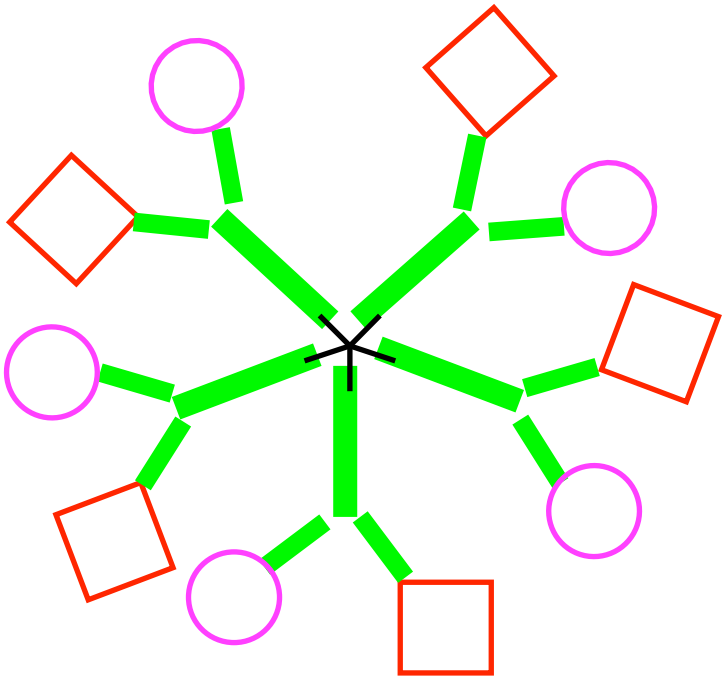
All possible classes of immunoglobulins



IgG, IgE, and IgD (Monomer)

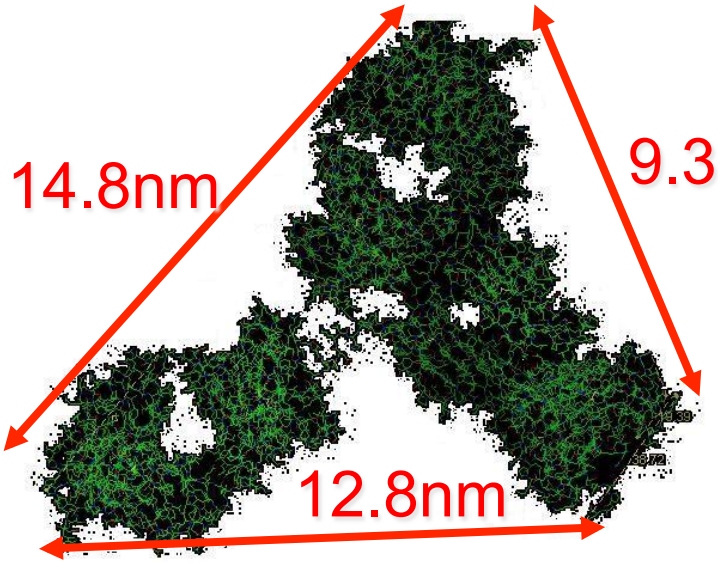


IgA (Dimer)



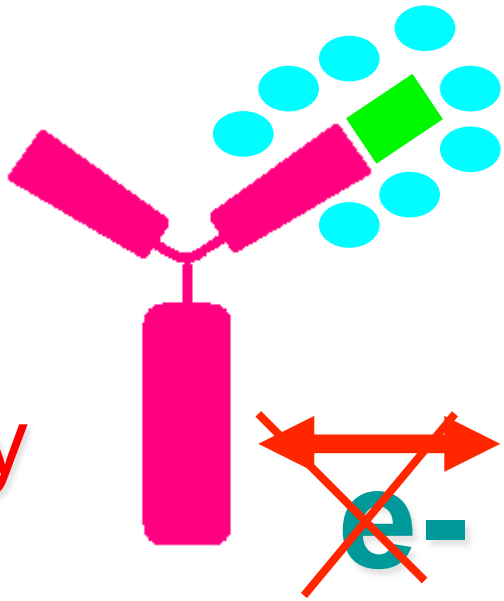
IgM (Pentamer)

The interaction Ab/Ag



IgG Antibody

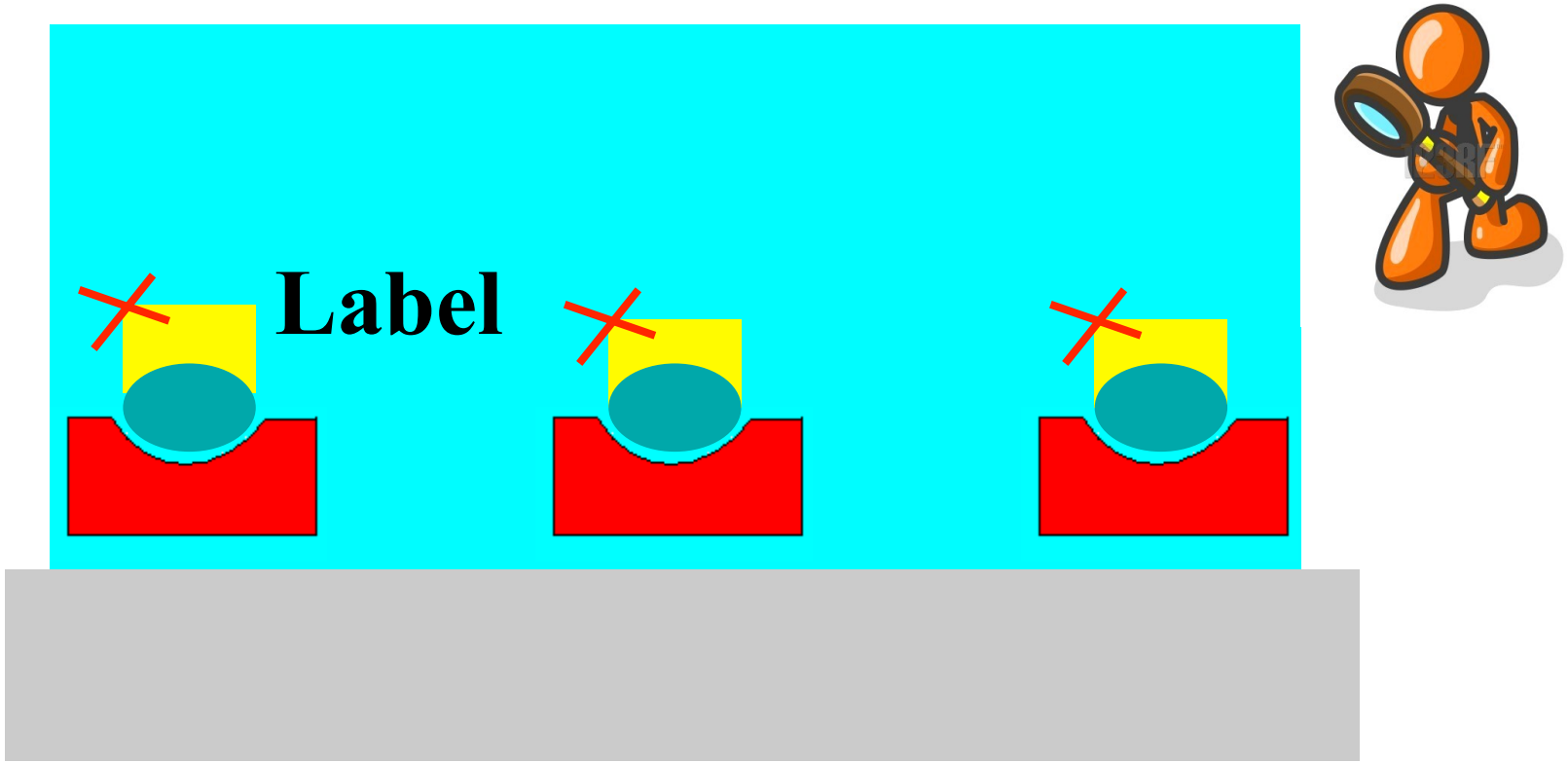
Water molecules



Antigen

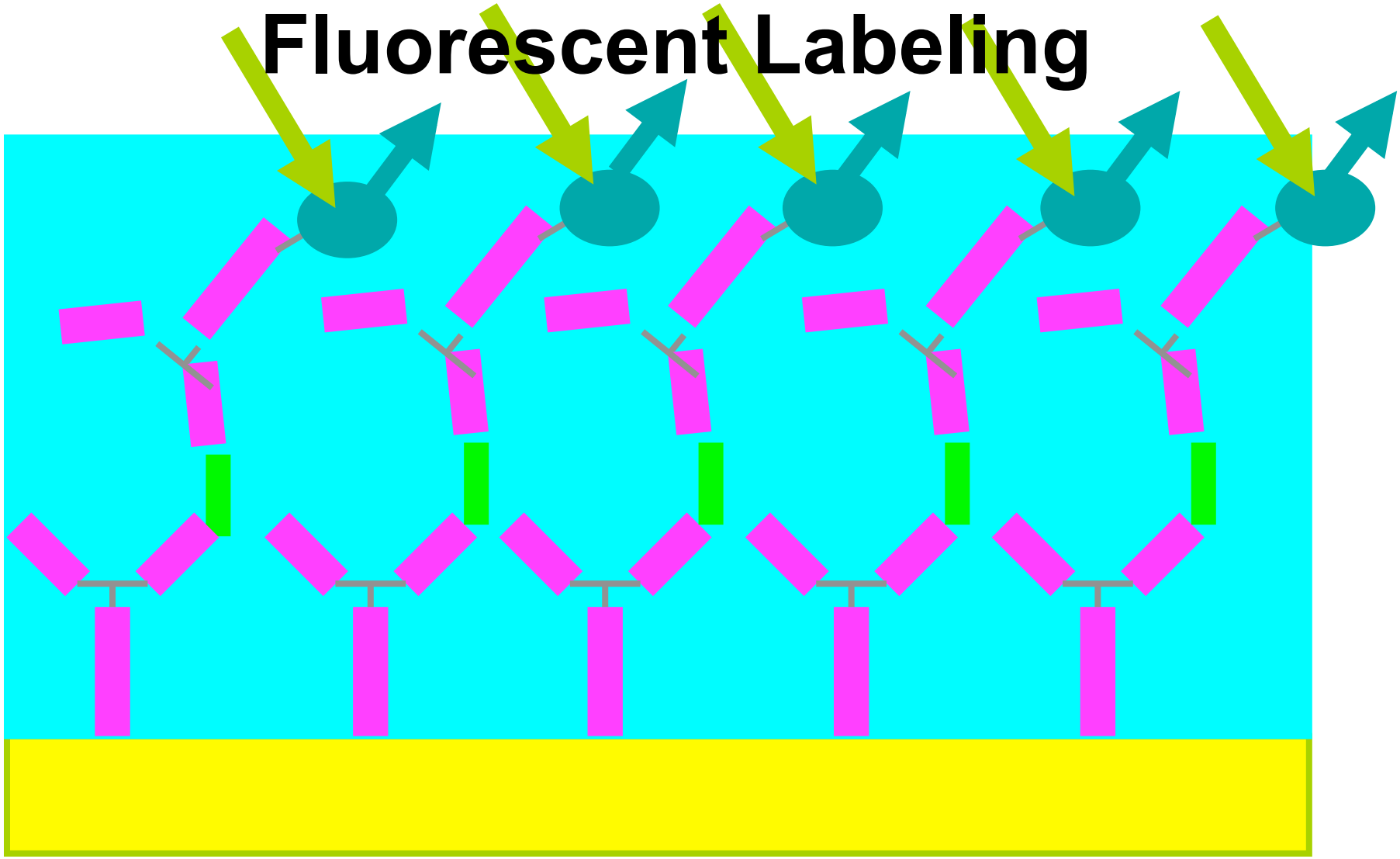
The antigen rests in a very tight binding pocket which is exactly the right size and shape to receive it. Other important factors include enthalpic contributions from van der Waals interactions and hydrogen bonds, and entropic contributions from the release of bound water upon antigen binding

Measuring Bio-Markers



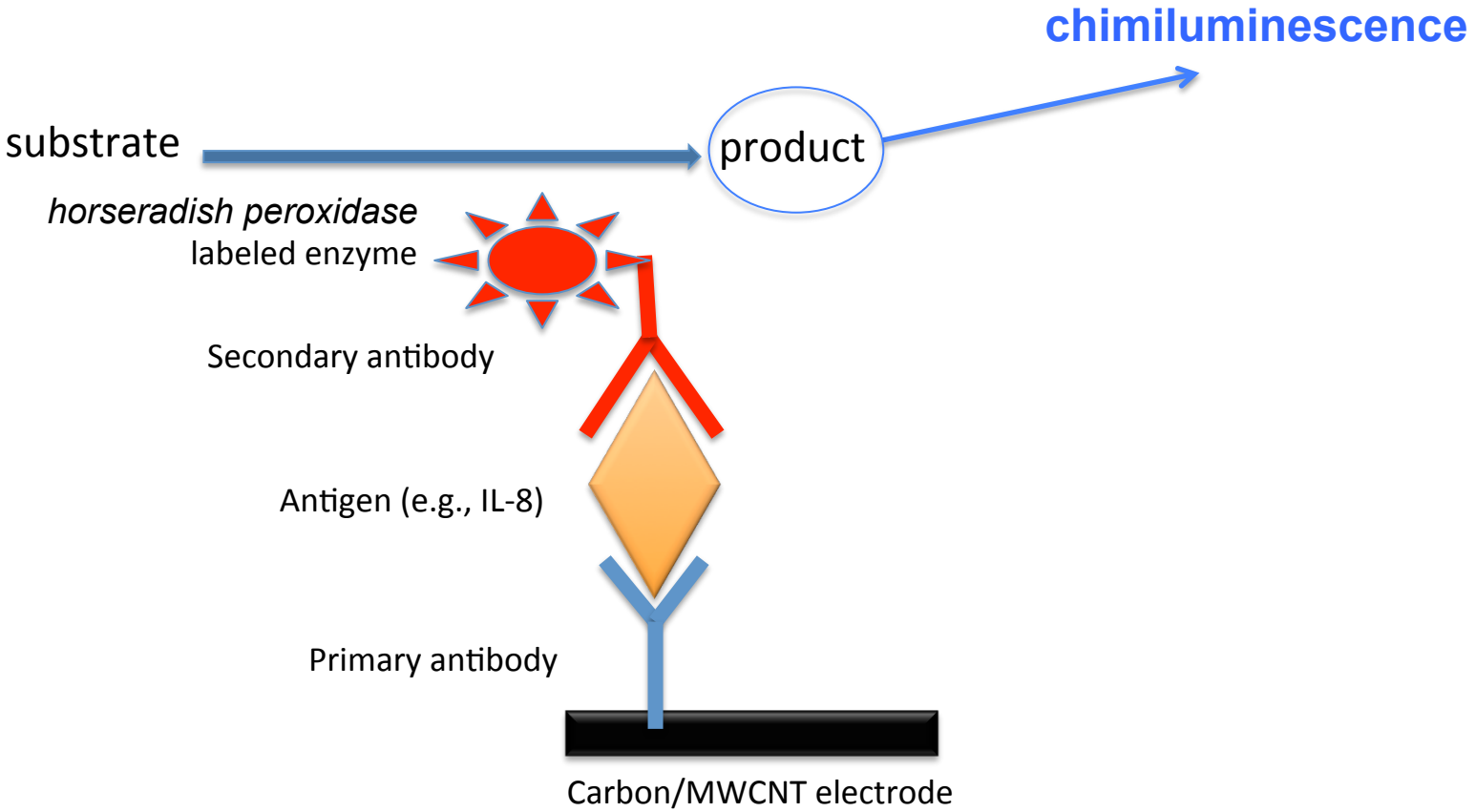
The Measure of Bio-markers may be performed in a labeled manner or in label-free mode

Fluorescent Labeling



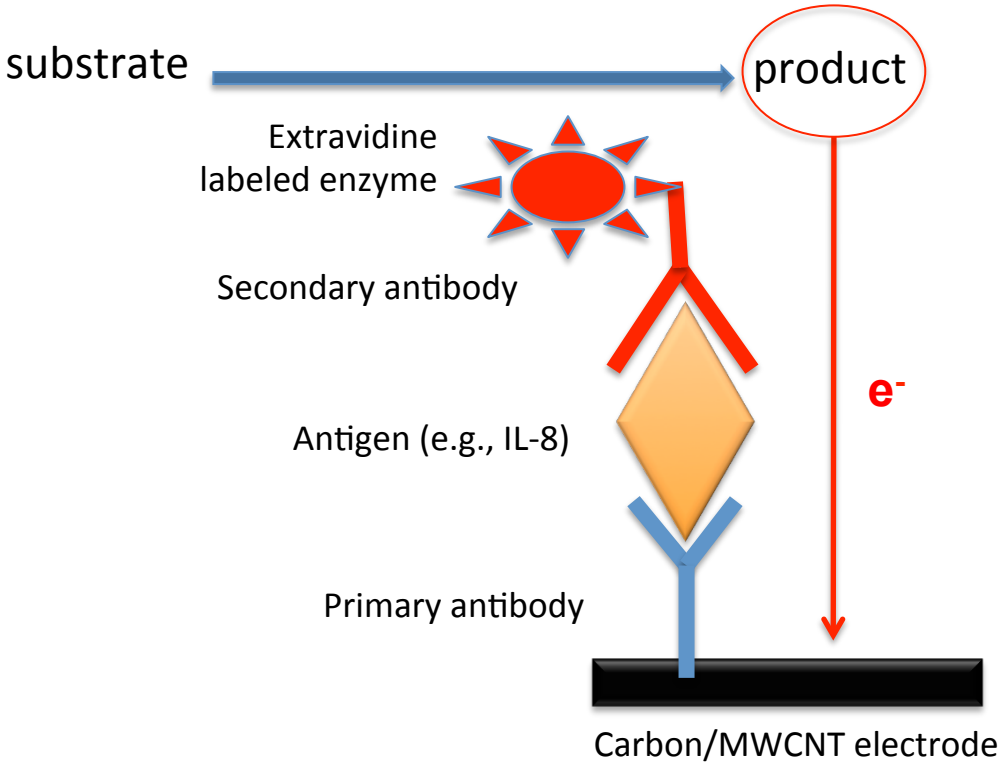
Antigens are specific detected by Secondary Antibodies with Fluorescent Labels

Chemilunescence Labeling



Antigens are specific detected by Secondary Antibodies with Chemiluninescence Labels

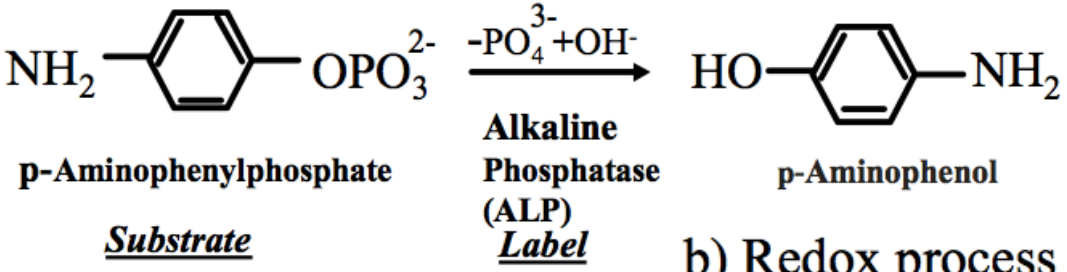
Electrochemical Labeling



Antigens are specific detected by Secondary Antibodies with Electrochemical Labels

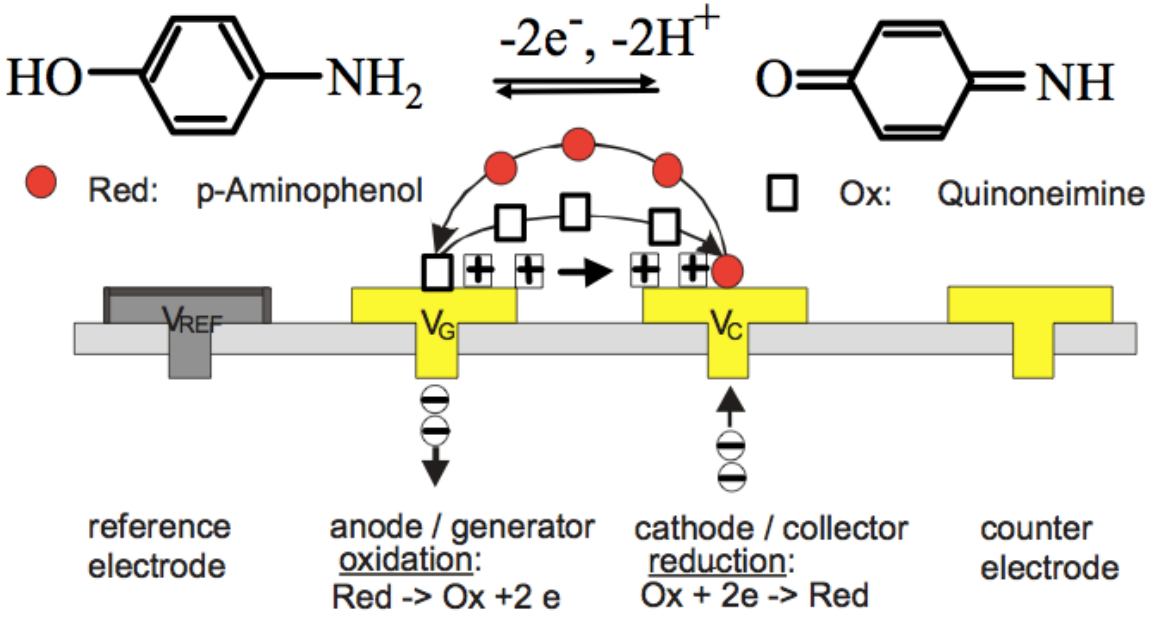
Electrochemical Labels

a) Process at the label



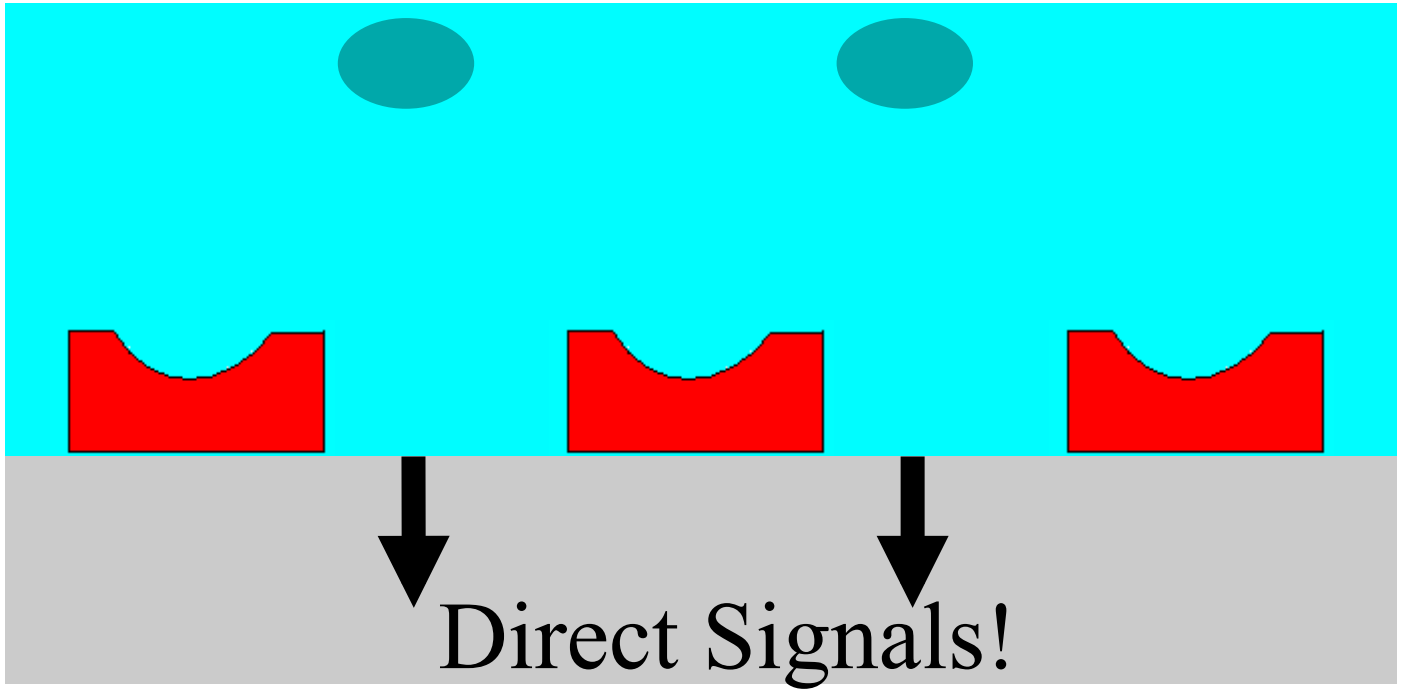
- ✓ The label cleaves the secondary probe

b) Redox process at the electrodes



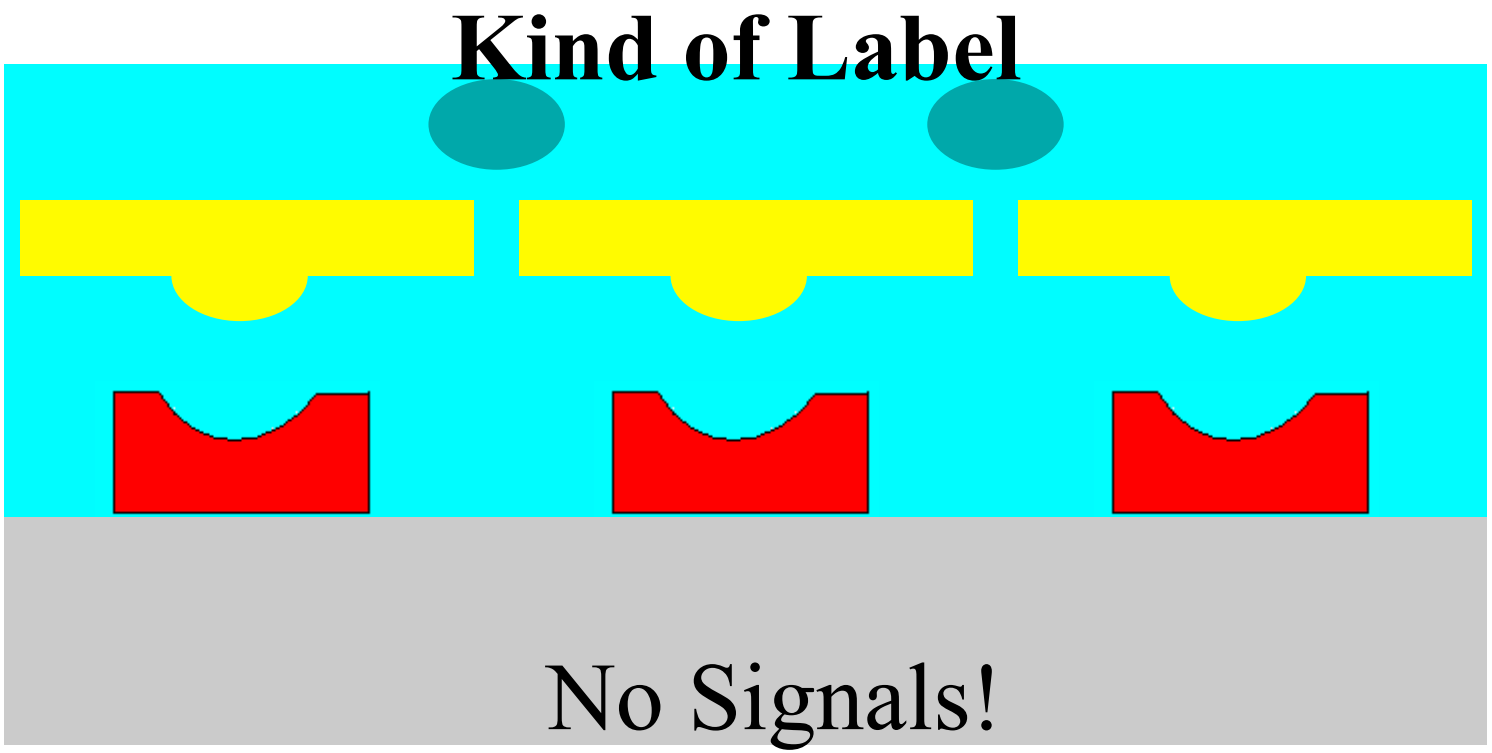
- ✓ The product of the cleavage is generating an oxidation process at the anode and, once oxidized, a reduction at the cathode

CMOS/Sample interface



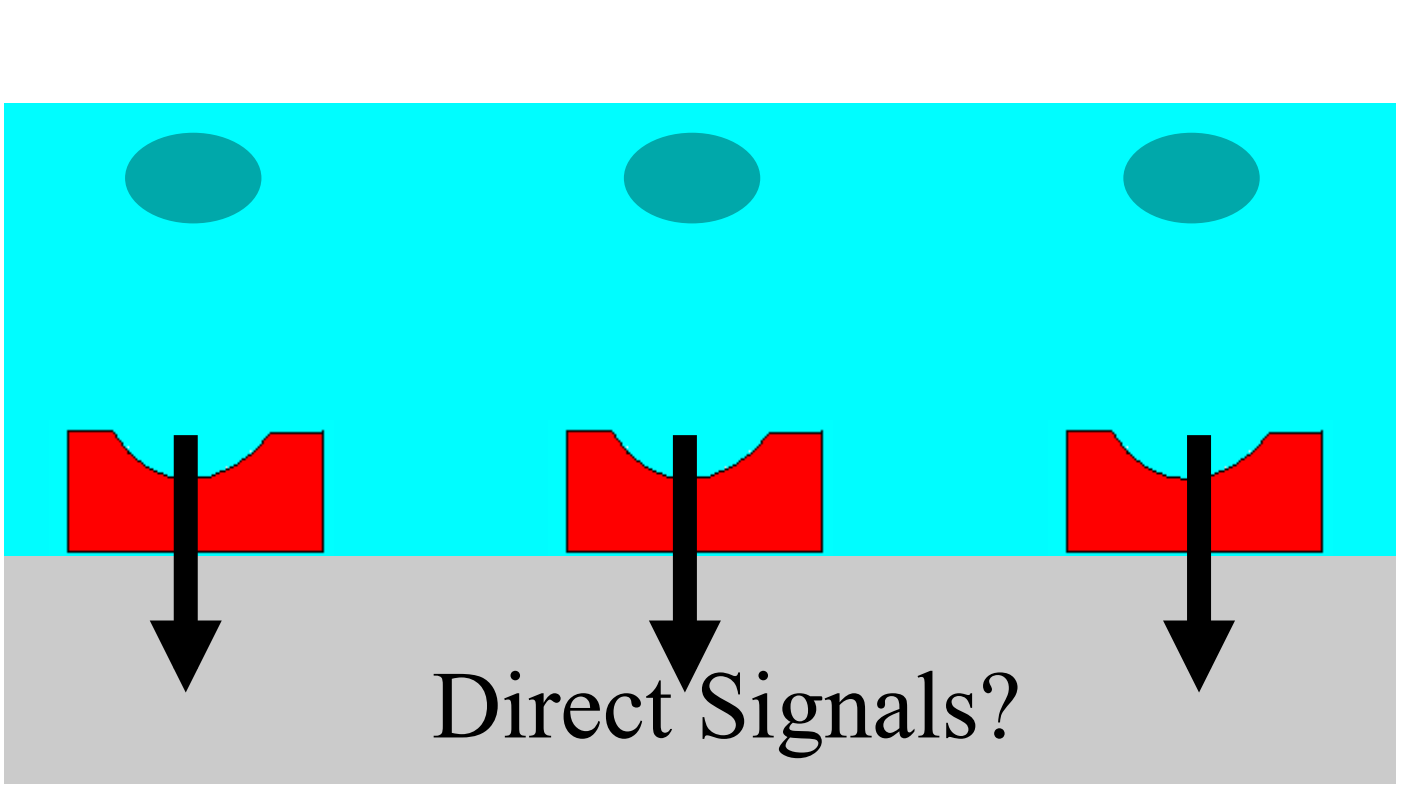
How to get direct signals of probe/target interactions?

CMOS/Sample interface



How to get direct signals of probe/target interactions?

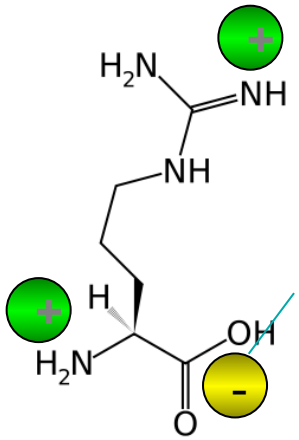
CMOS/Sample interface



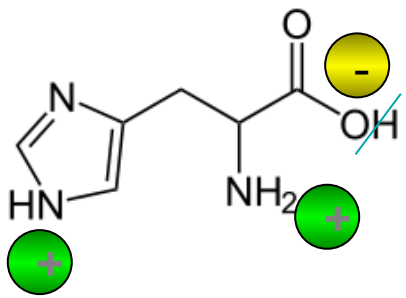
How to get direct signals of probe/target interactions?

Charged Residues

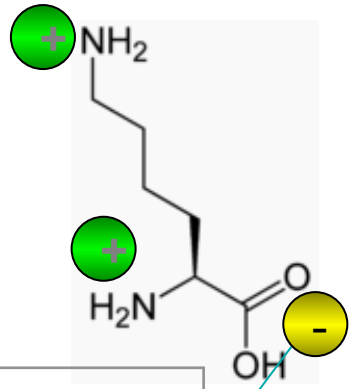
Positively Charged



Arginine

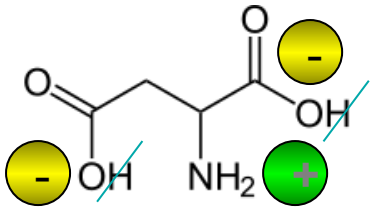


Histidine

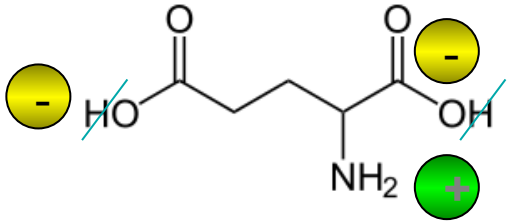


Lysine

Neg. Charged

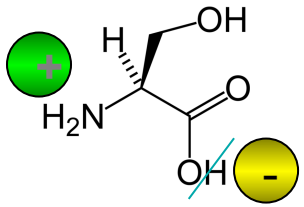


Aspartic Acid

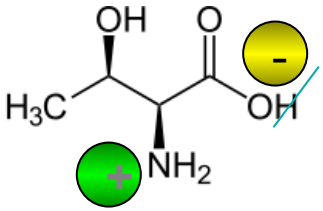


Glutamic Acid

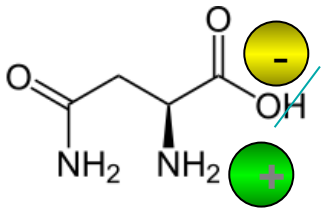
Polar Uncharged



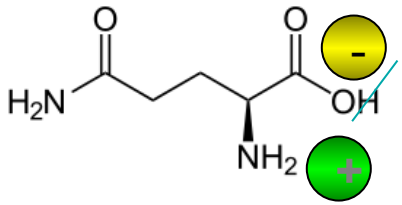
Serine



Threonine



Aspargine

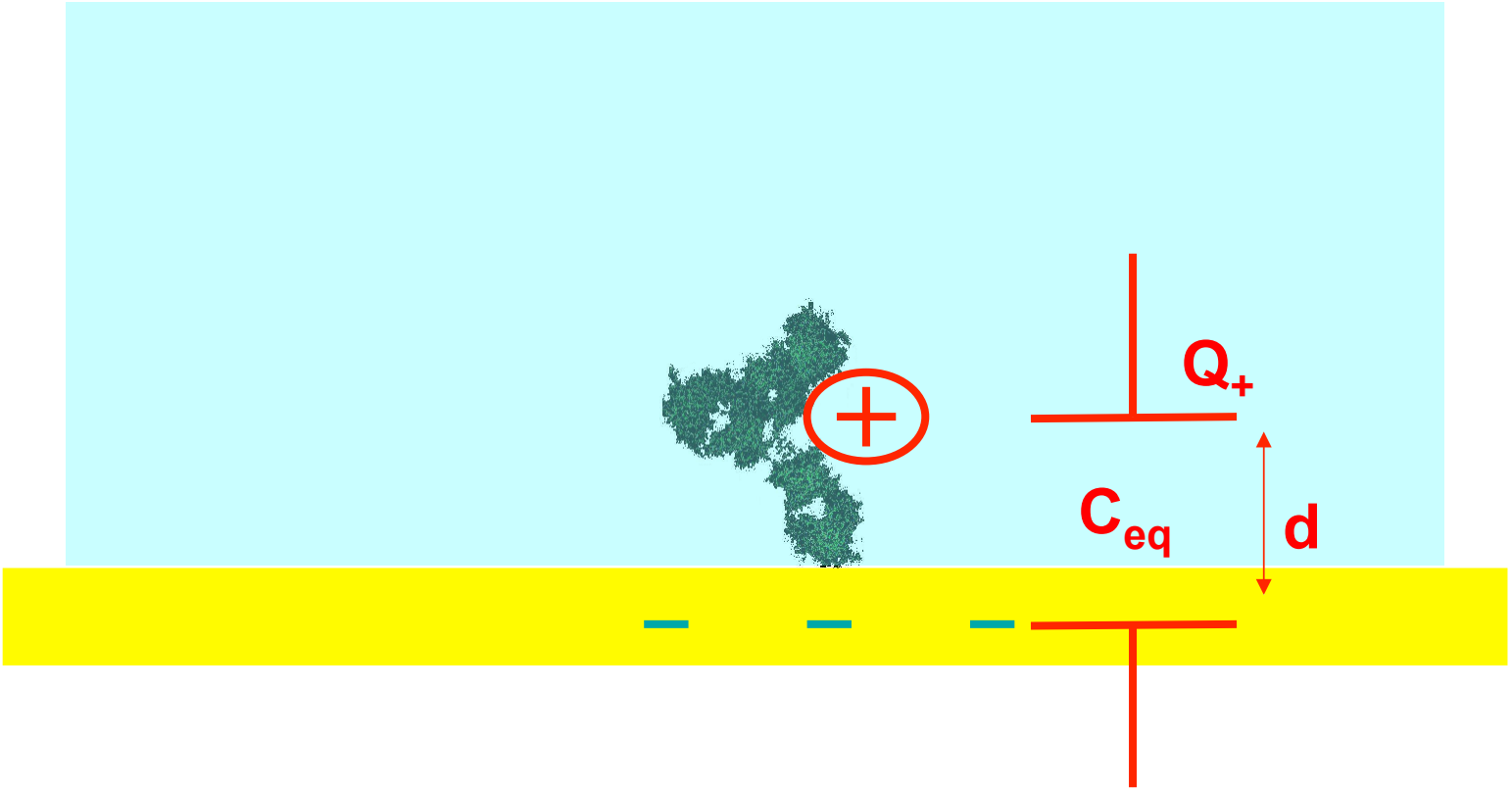


Glutamine

The charges of an Antibody

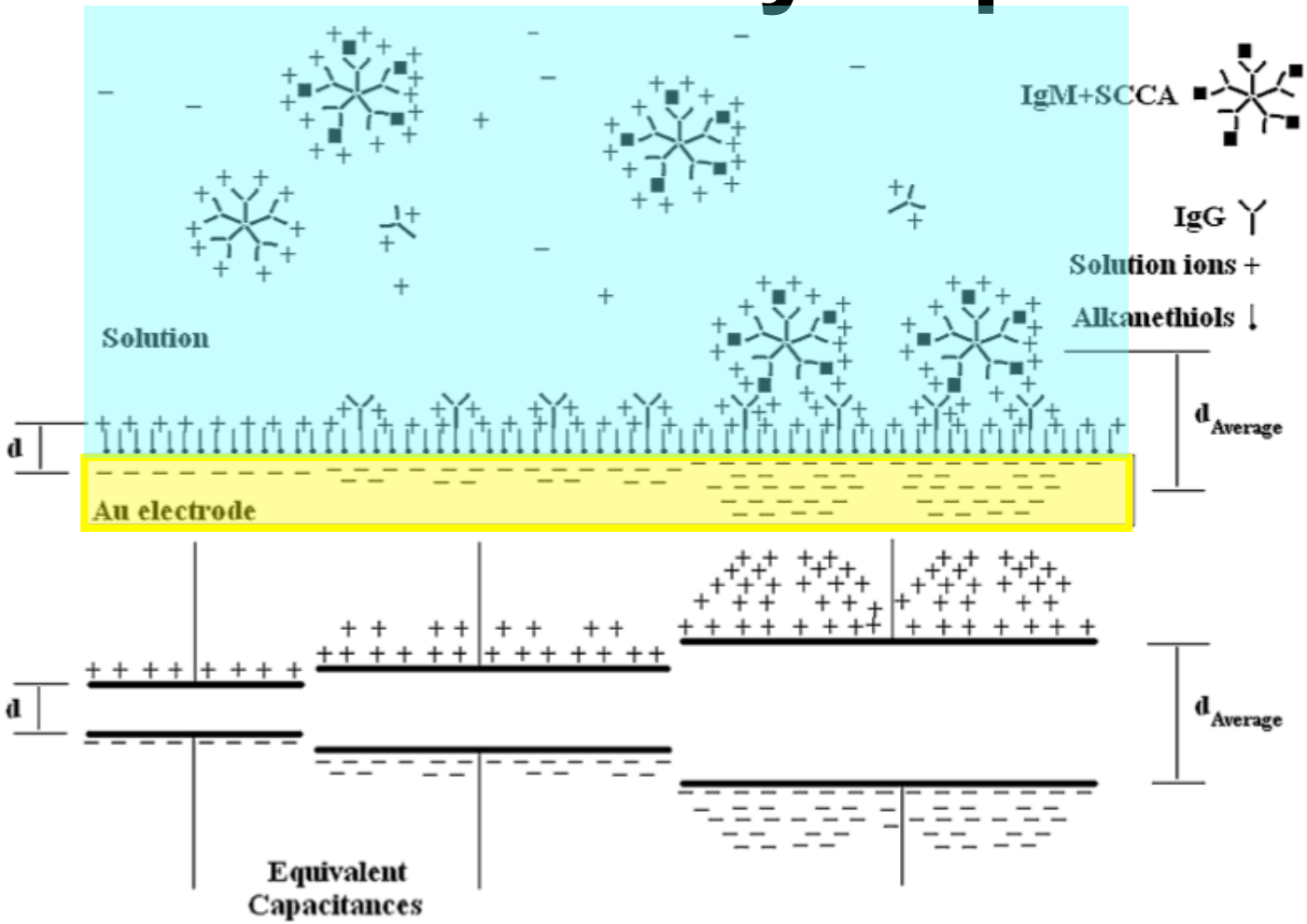
The crystallographic structure of an antibody

Capacitive detection



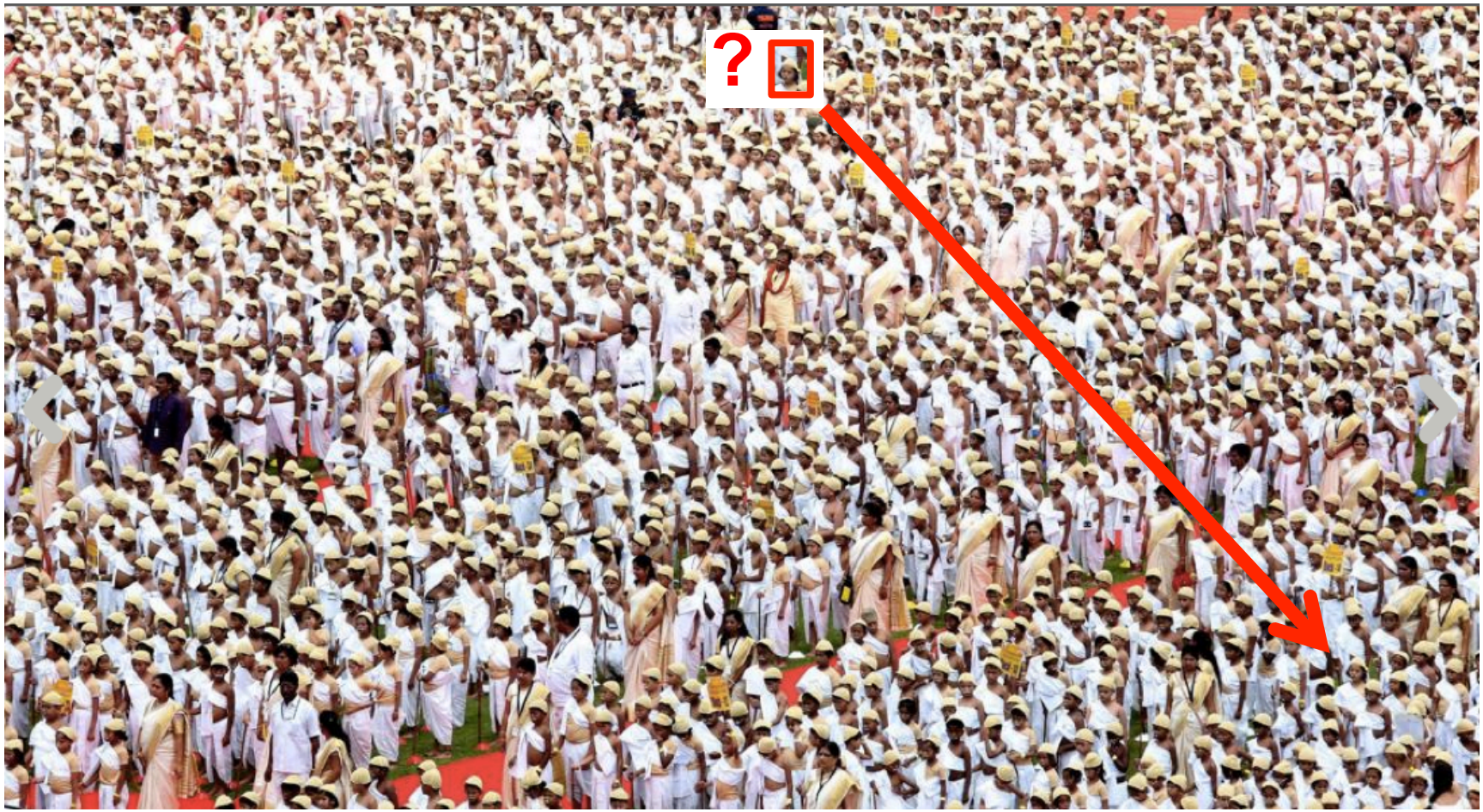
Charged residues of the antibody may affect charge carriers in the electrode

Cancer Detection by capacitance



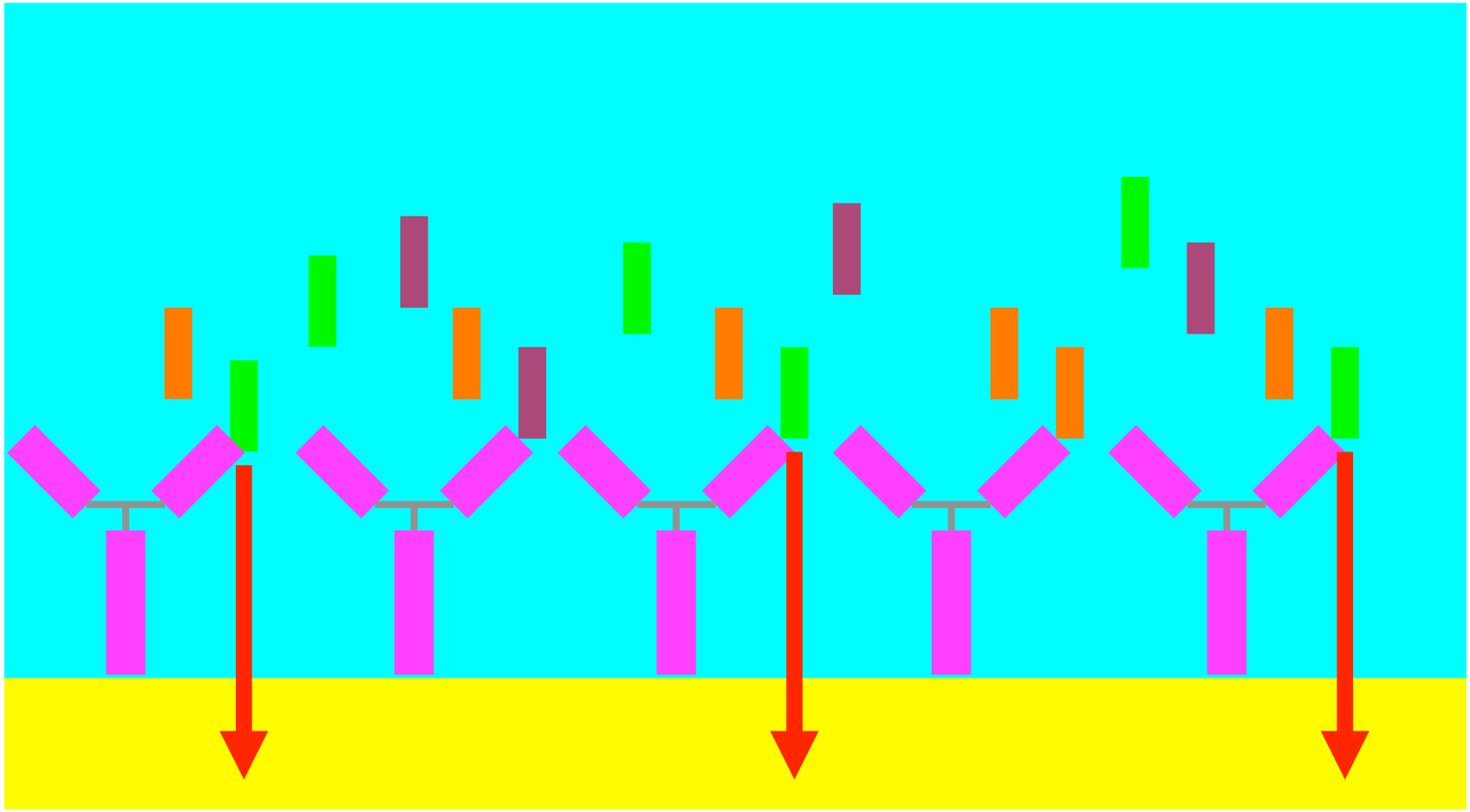
Schematic of the capacitive detection principle

Problem of Specificity



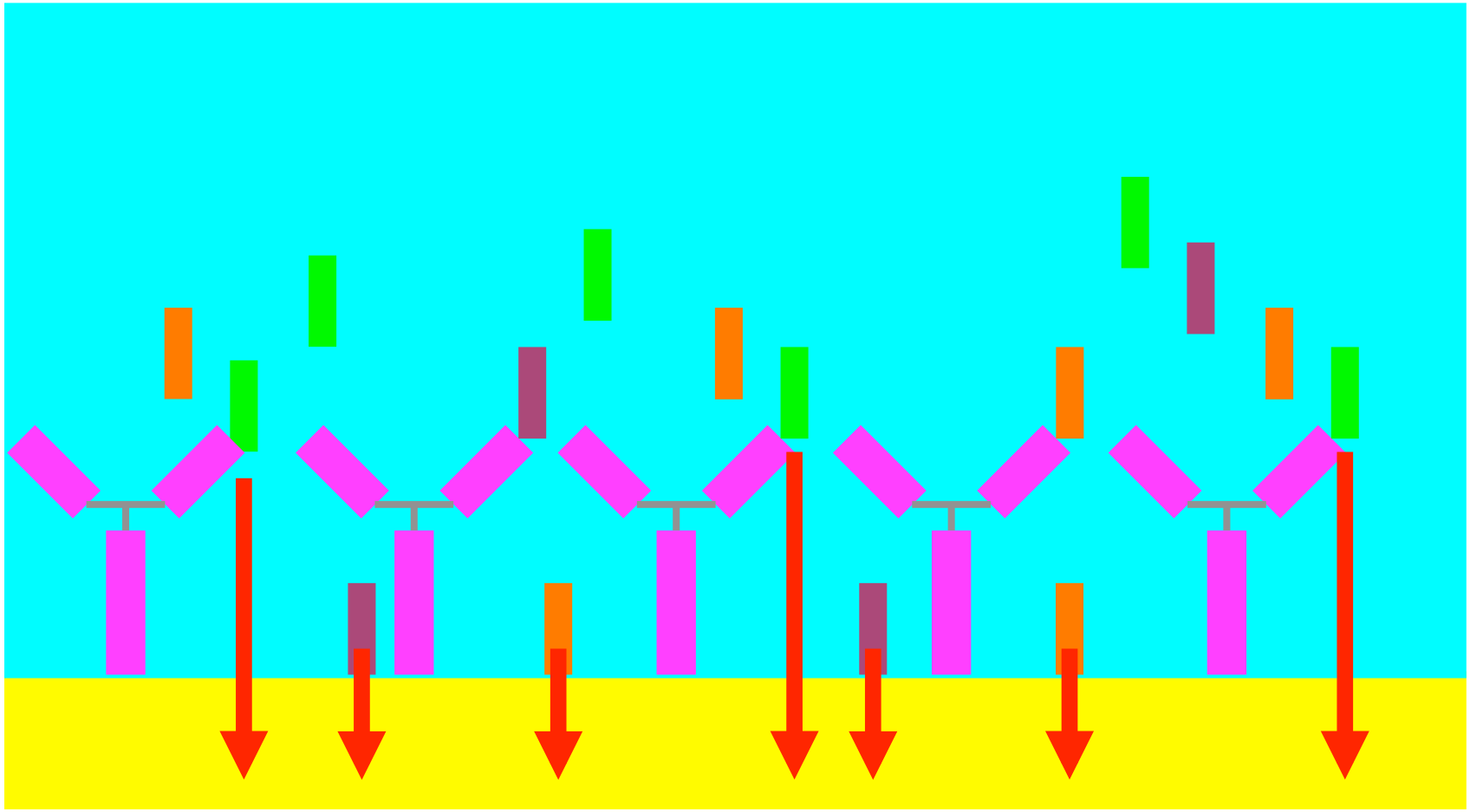
Find your friend in a very crowded square!

Specificity of the Probe



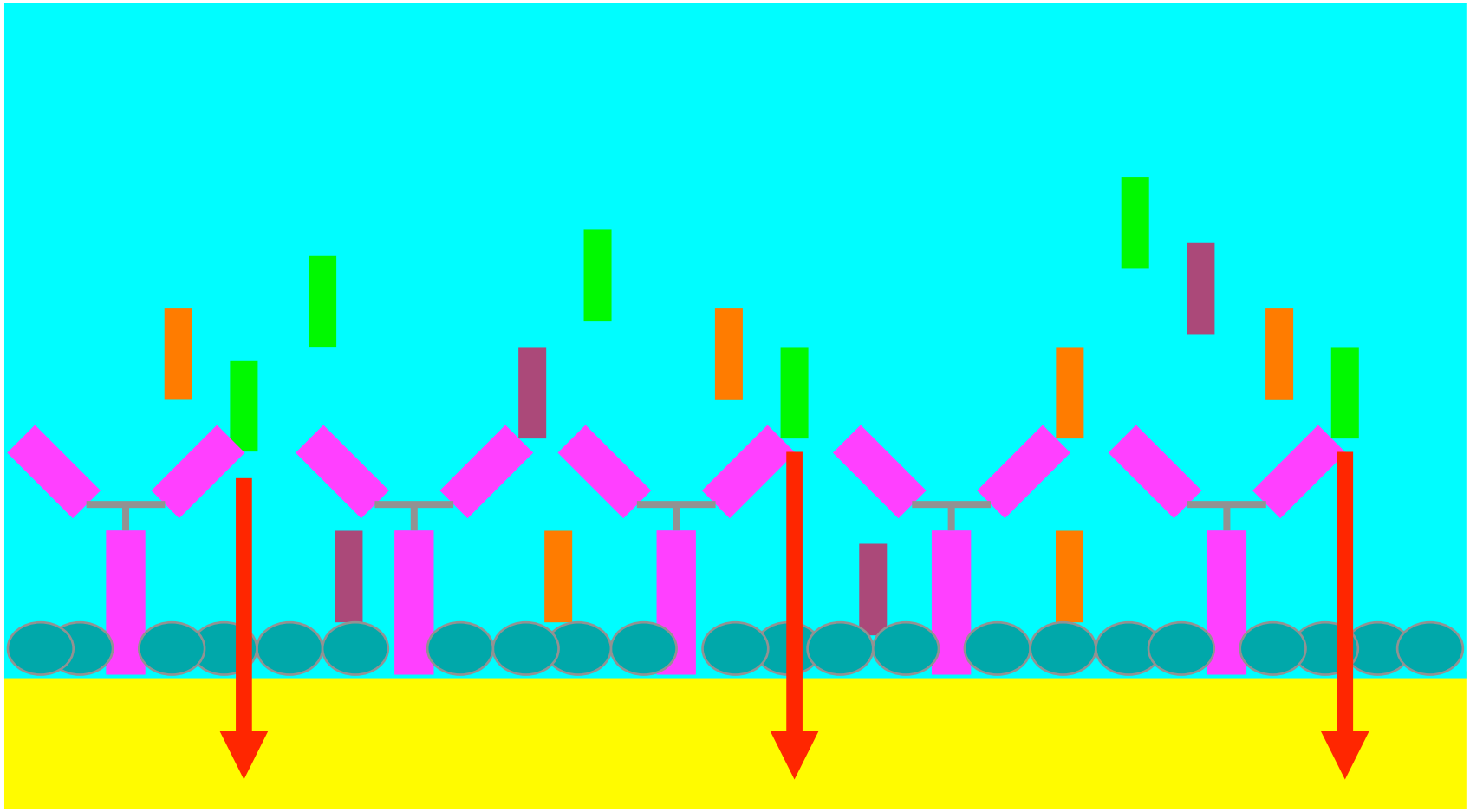
Antigens are specific detected by immobilizing the right antibodies

Specificity of the Surface



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

Specificity of the Surface



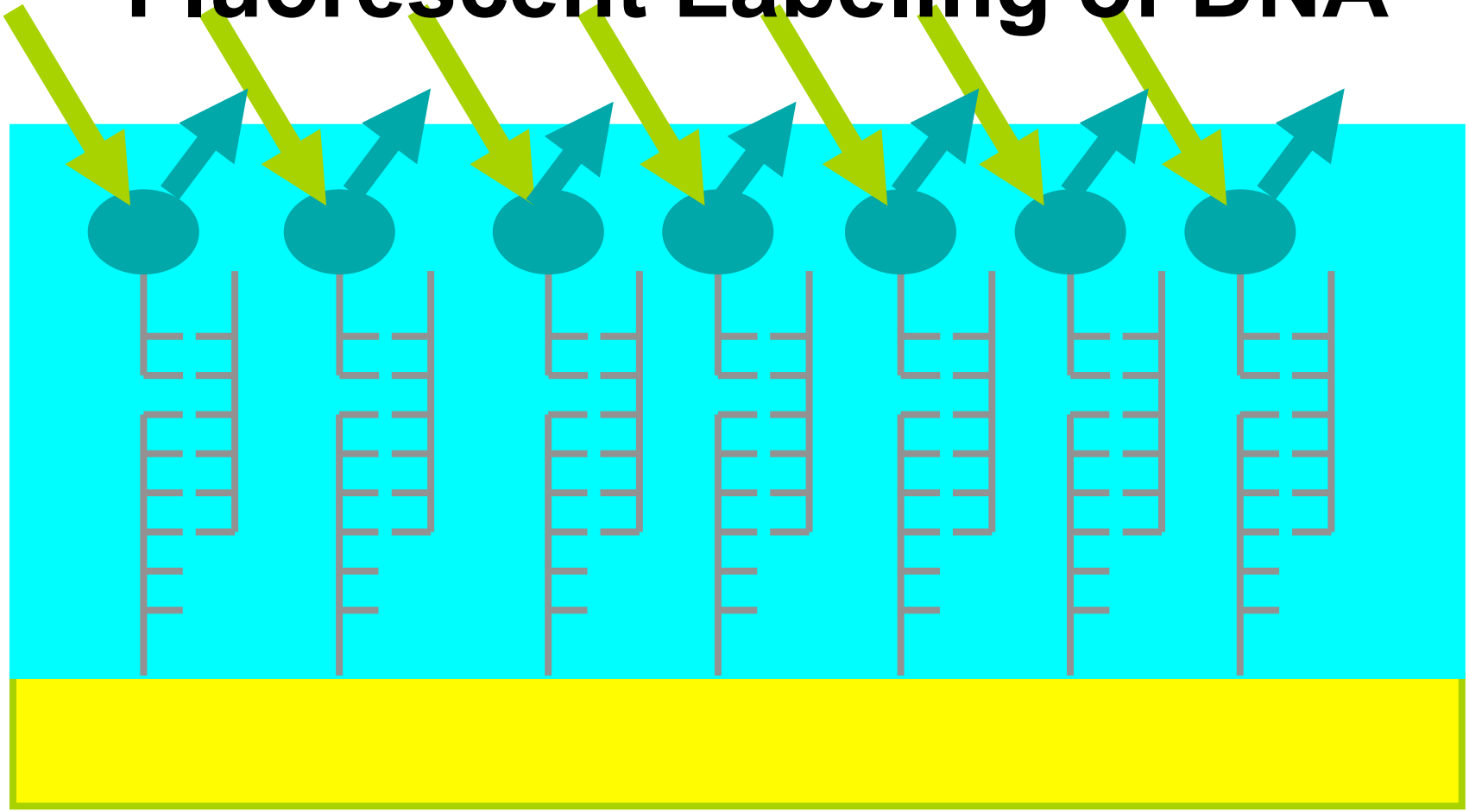
Blocking agents are used
to improve surface specificity

Specificity of the Probe

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

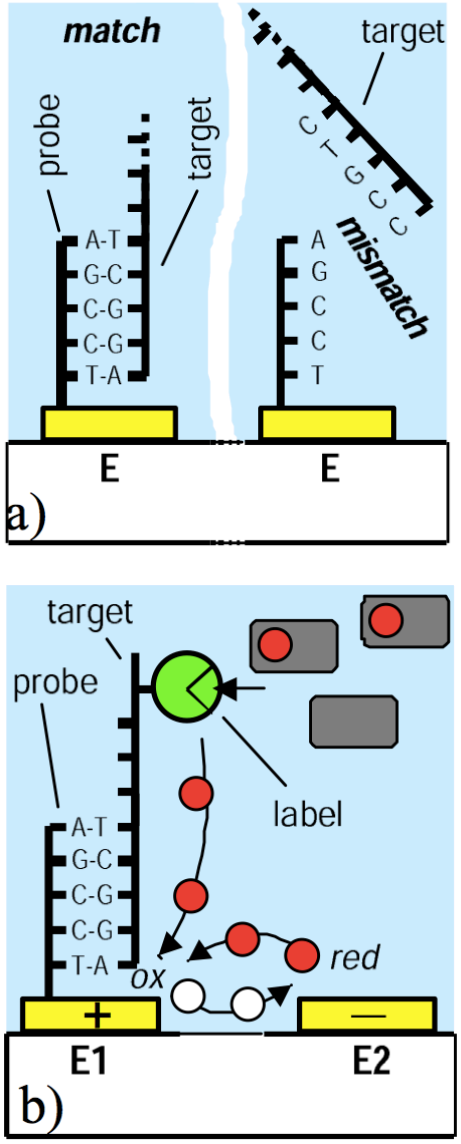
Specificity of the DNA probe: the Gibbs free energy of non-matching duplexes is not zero!

Fluorescent Labeling of DNA



DNA targets are specific detected by Secondary DNA-probes with Fluorescent Labels

Amperometric Labeling of DNA



How it works:

✓ First, single stranded DNA molecules (about 20 bases) are immobilized by using a spotting machine on top of the gold electrodes due to gold-thiol coupling.

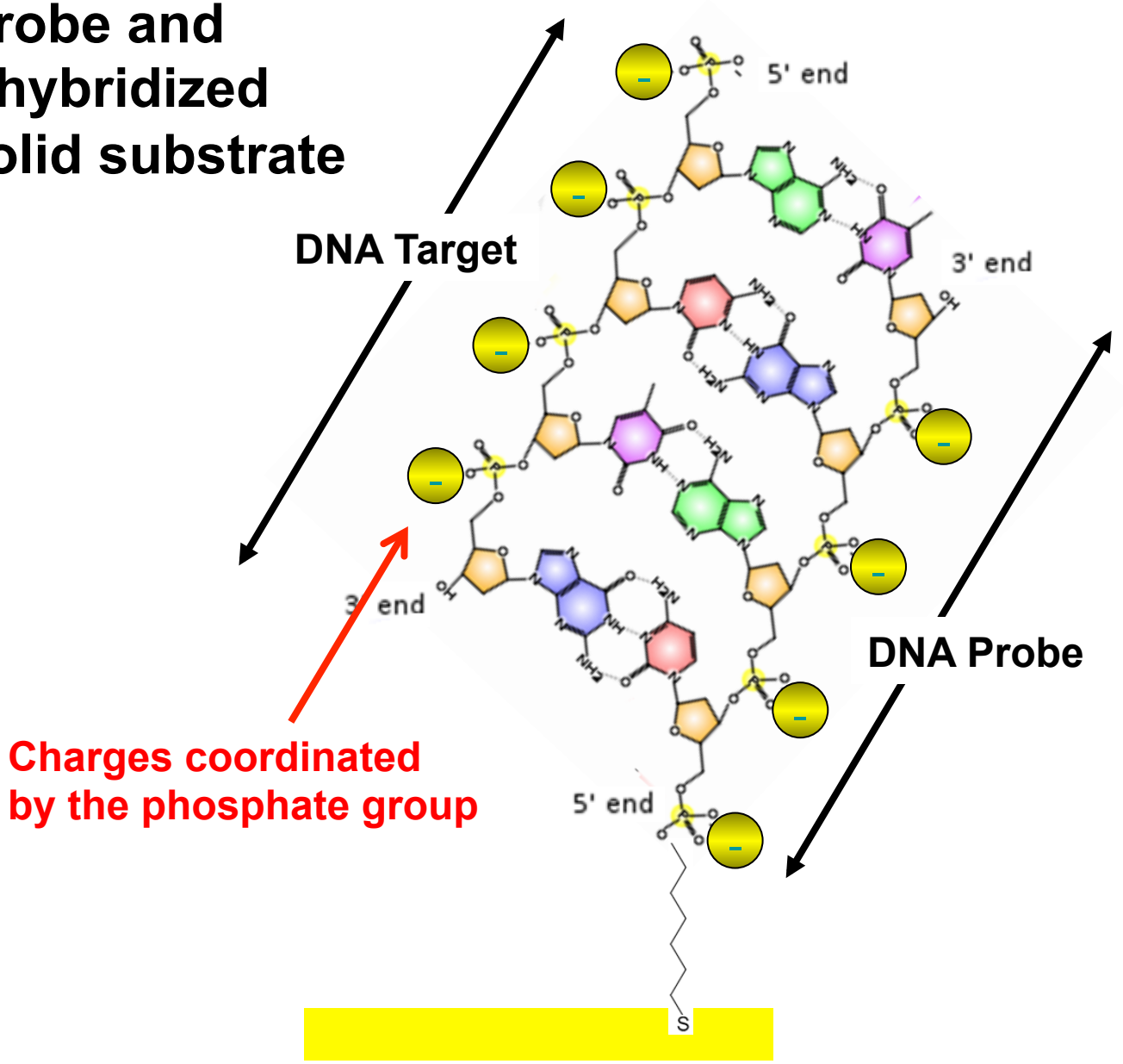
✓ Then, the chip is flooded with an analyte containing labeled target DNA ss: hybridization takes place in case of matching.

✓ A suitable substrate is applied to the buffer solution and it is enzymatically cleaved by the label.

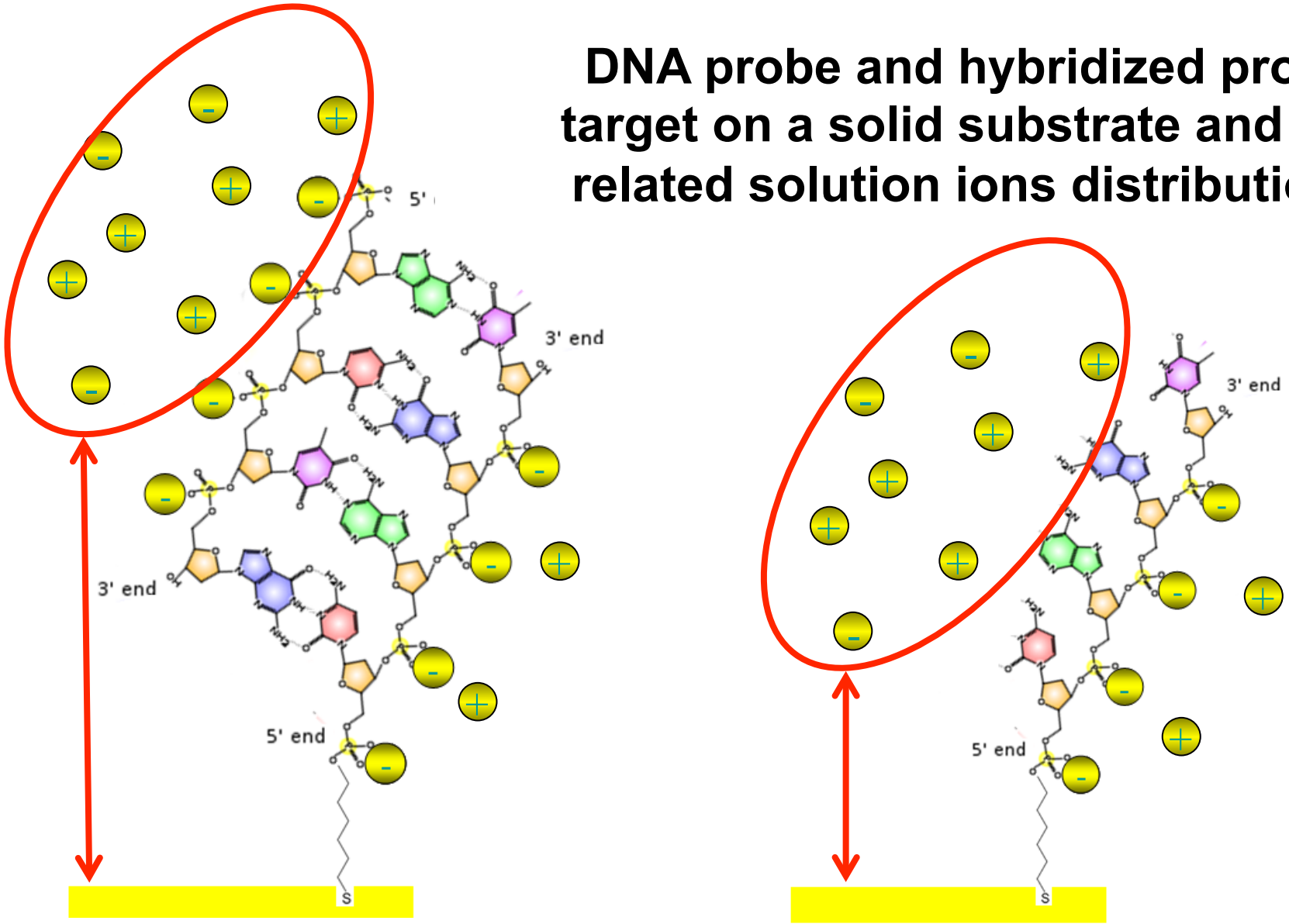
✓Resulting species starts an electrochemical redox process at the electrodes.

✓Faradaic currents generated by the related redox process is detected and transduces DNA hybridization

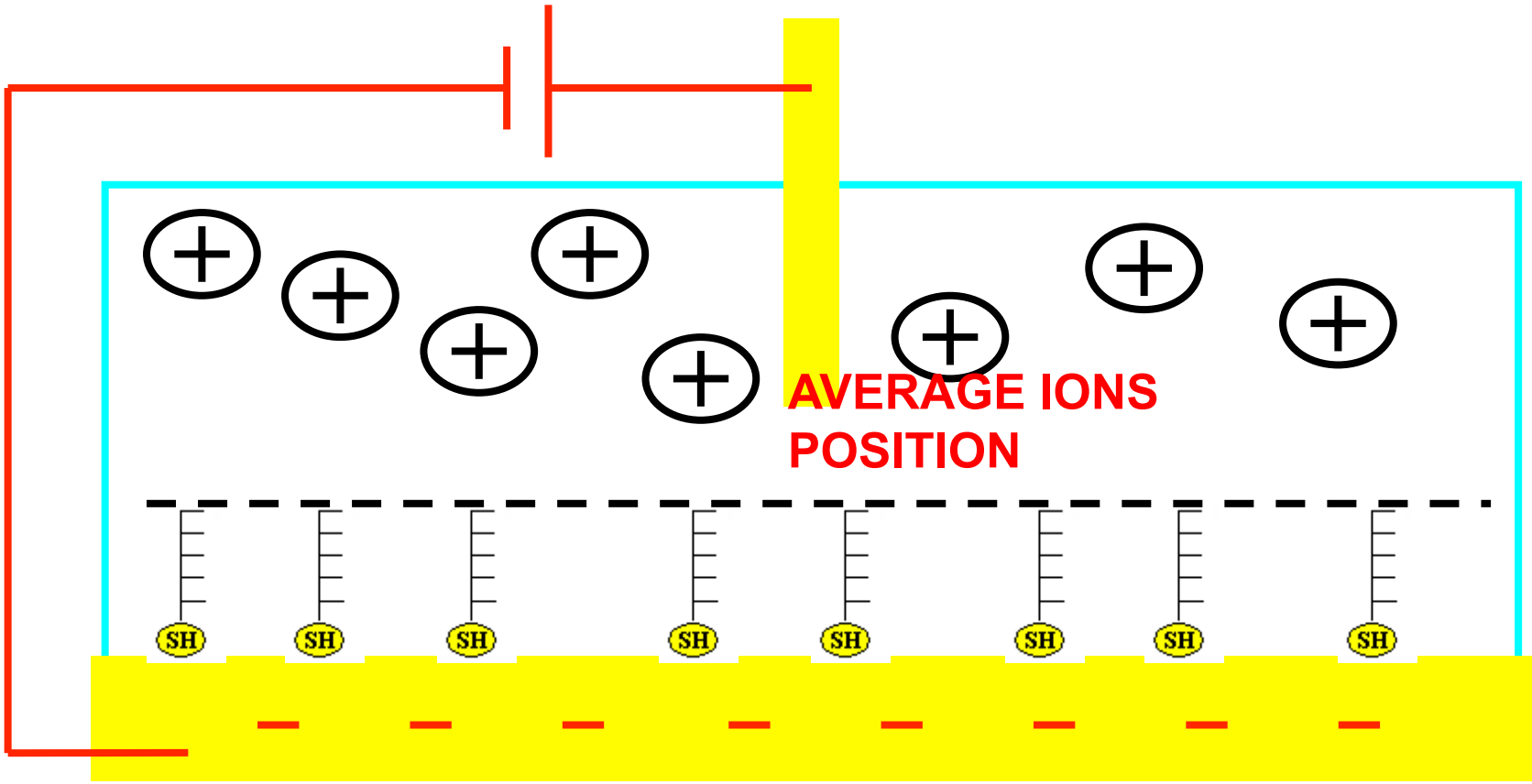
DNA probe and target hybridized on a solid substrate



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

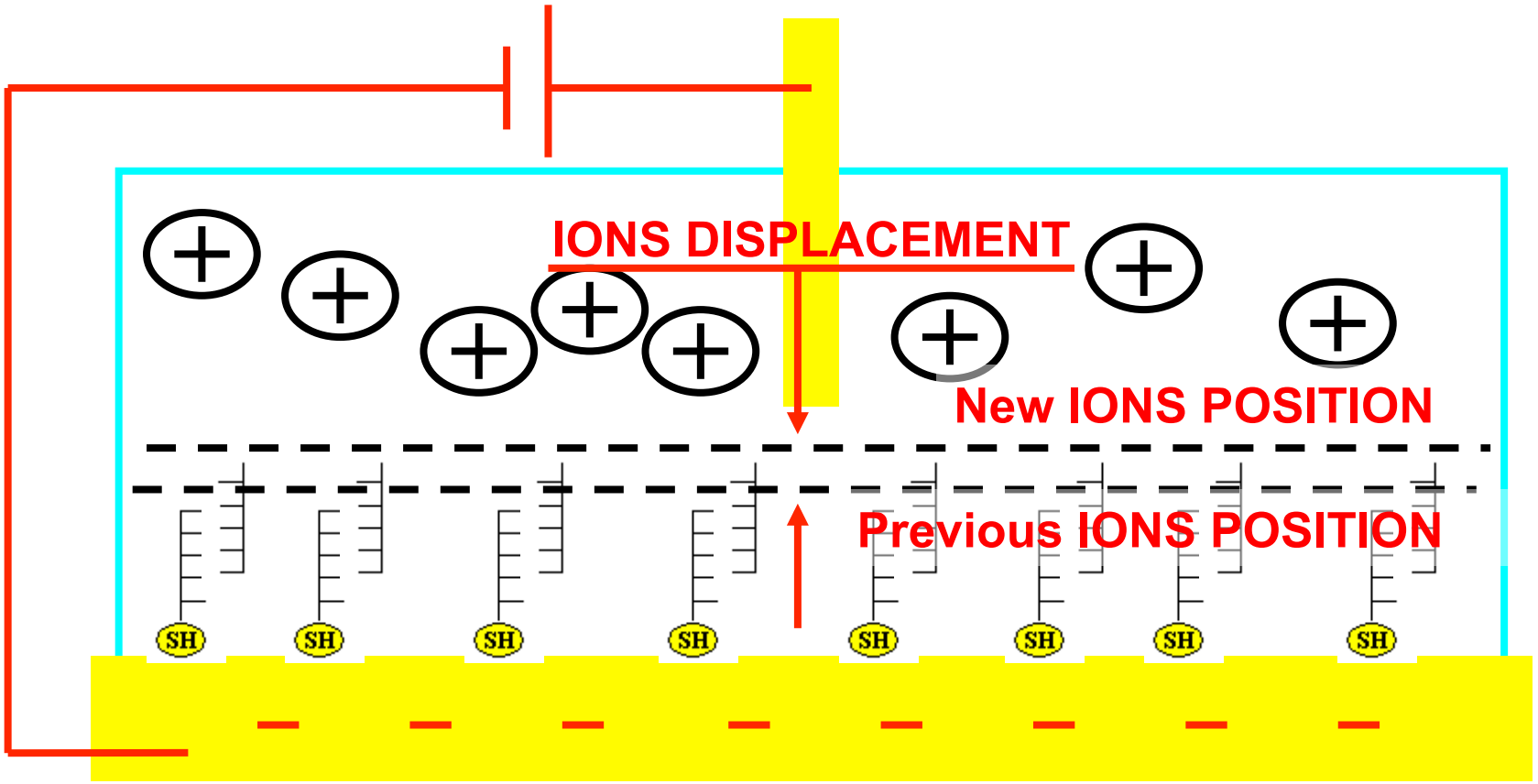


Electrochemical Interface



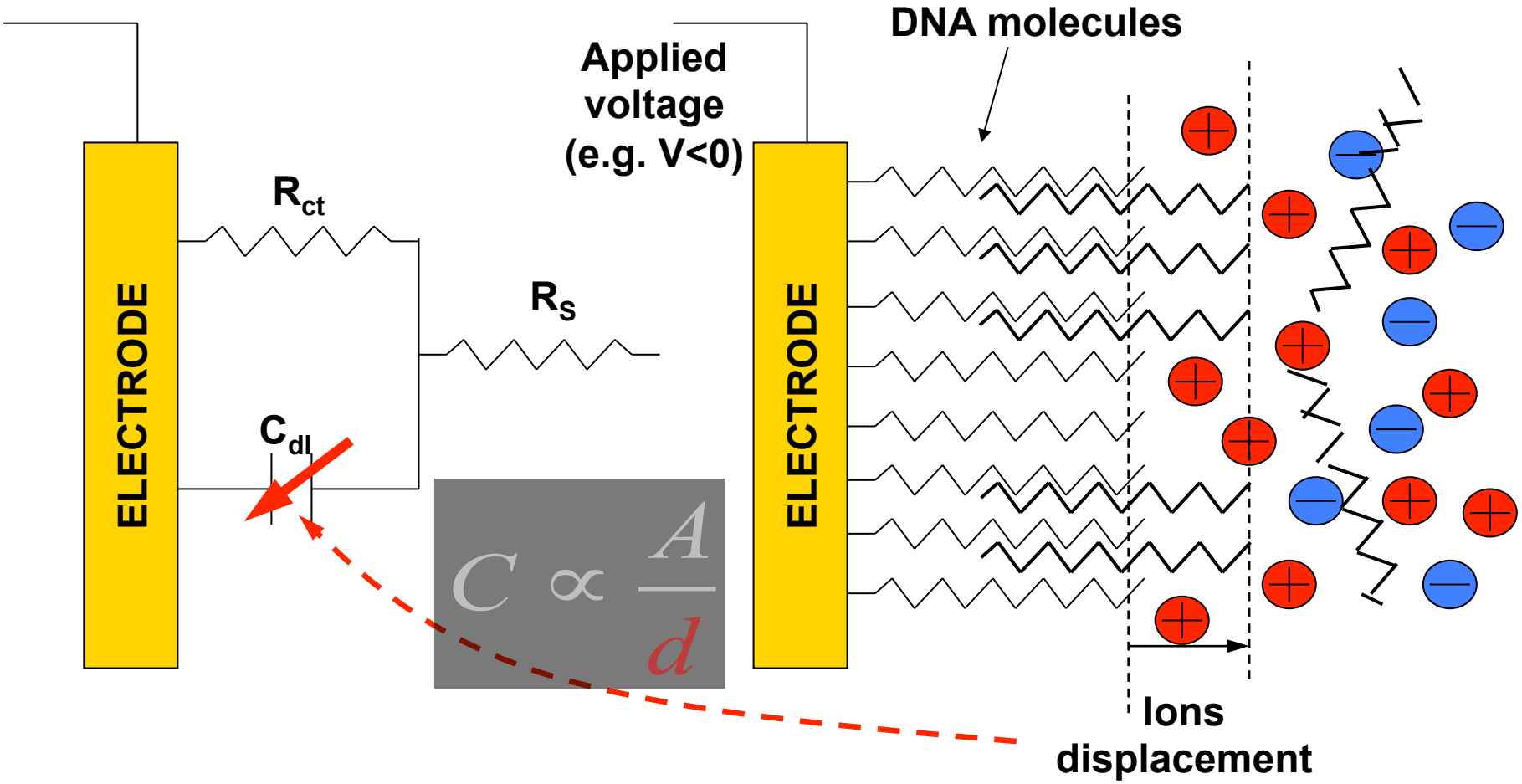
Ion planes are formed at the interface when electrodes immersed in solution are polarized

Electrochemical Interface



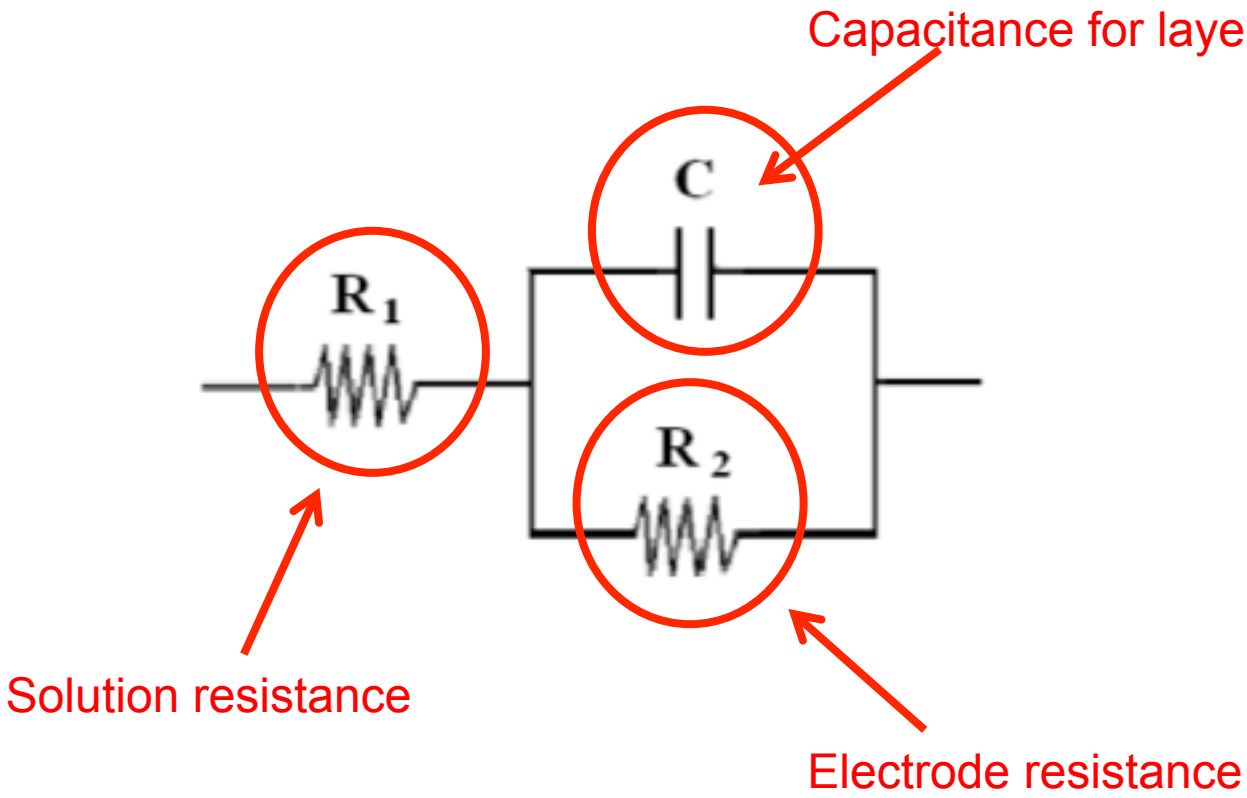
Ion planes are formed at the interface when electrodes immersed in solution are polarized

The Capacitance DNA Detection



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

Equivalent Circuit with Layering effects (Randle Model)



Equivalent C of sensing electrodes

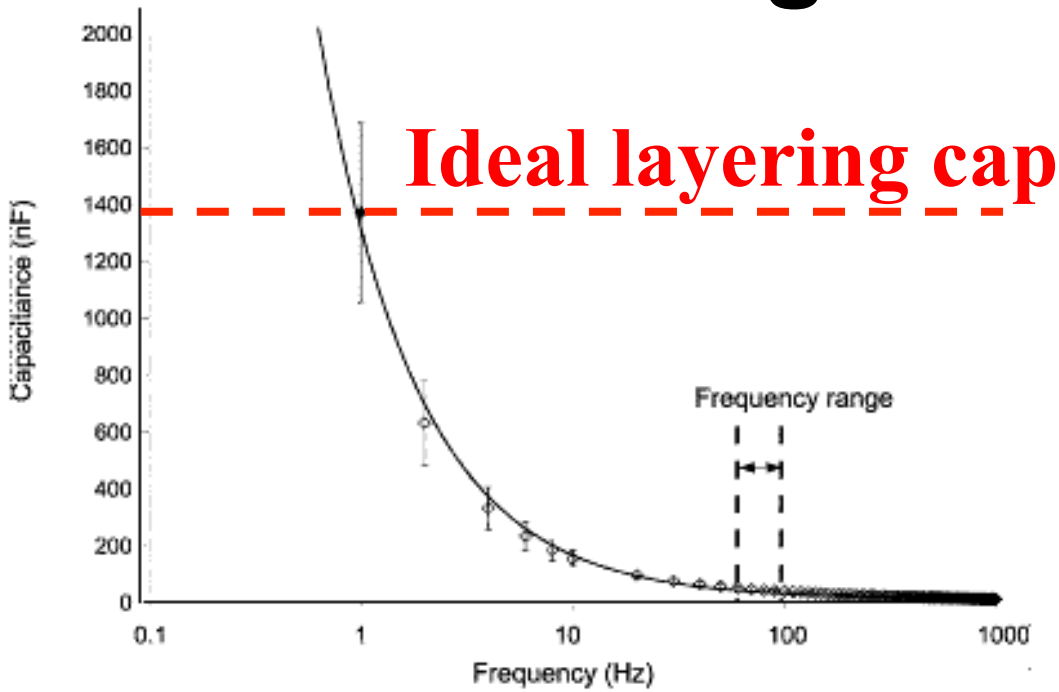
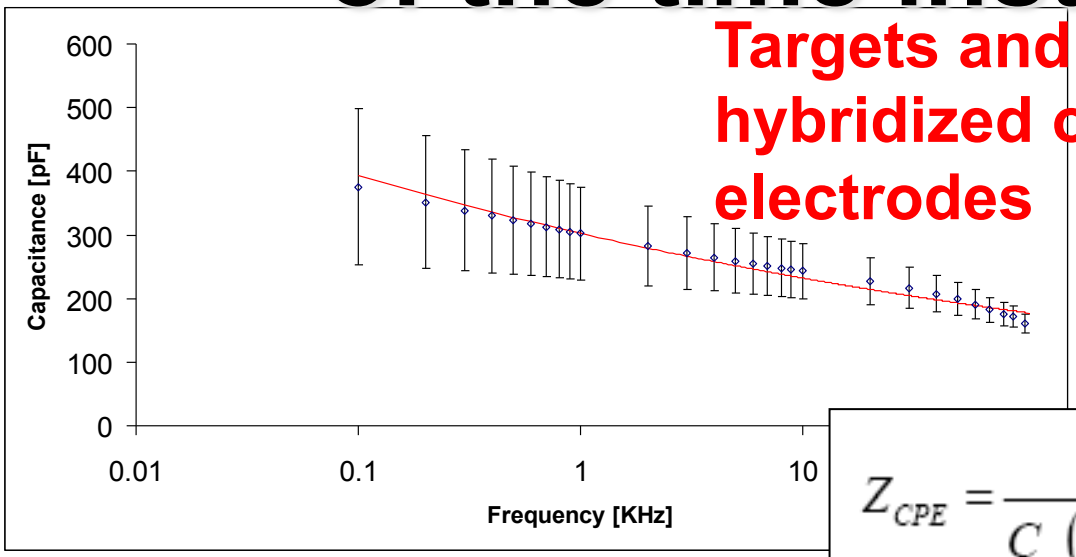


Fig. 9. Measured capacitance versus charge/discharge frequency on clean gold electrodes. The continuous line shows the fitting.

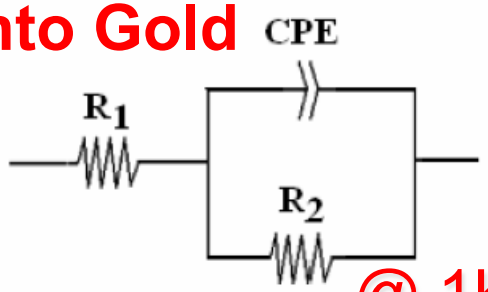
STAGNI *et al.*: FULLY ELECTRONIC LABEL-FREE DNA SENSOR CHIP IEEE SENSORS JOURNAL, VOL. 7, NO. 4, APRIL 2007

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

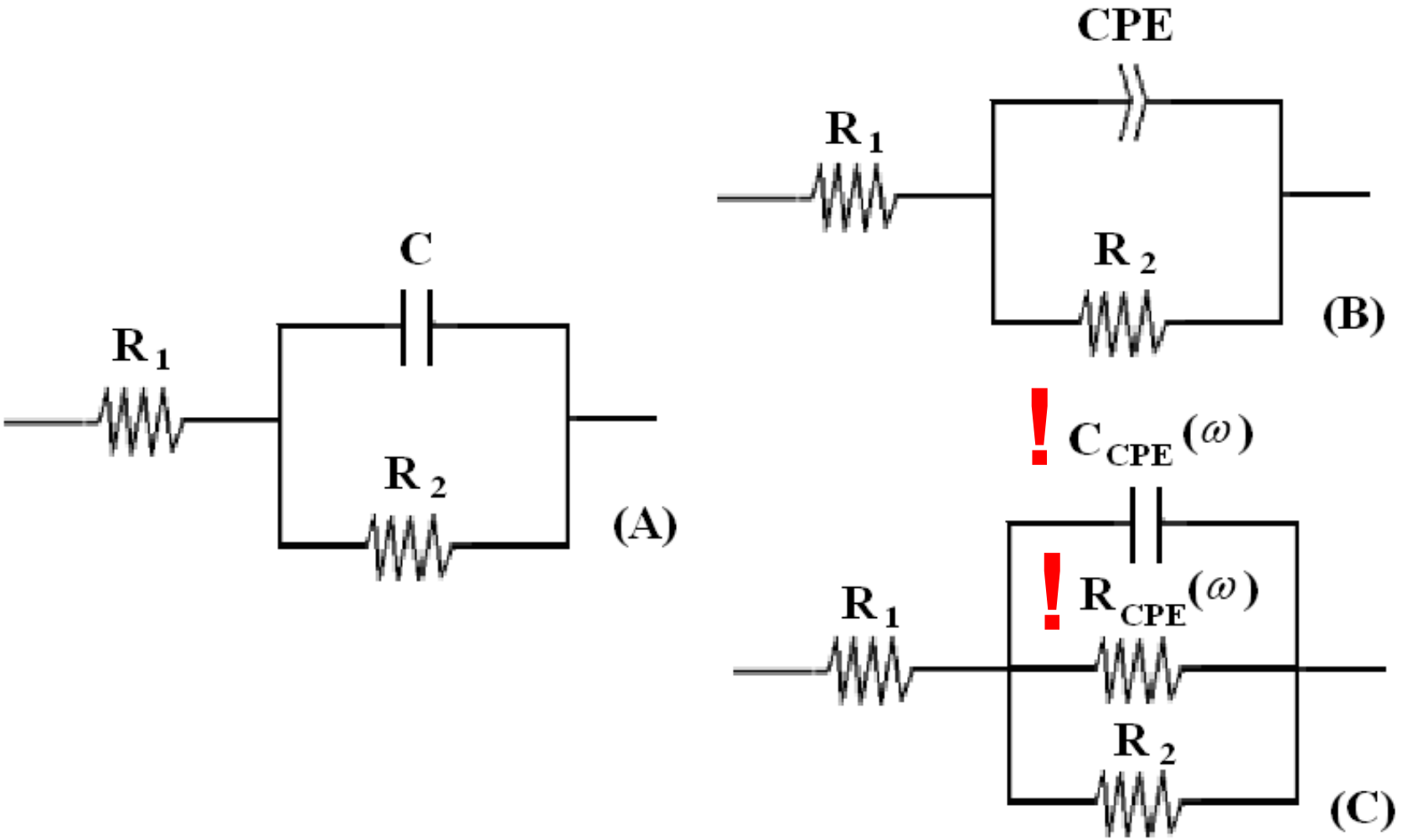
$$Z_{CPE} = \frac{1}{C_p (j\omega)^{\alpha}} \cong \frac{1}{\omega^{\alpha} C_p}\sqrt{1-\alpha^2} + j\frac{1}{\omega^{\alpha} C_p}\alpha$$

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

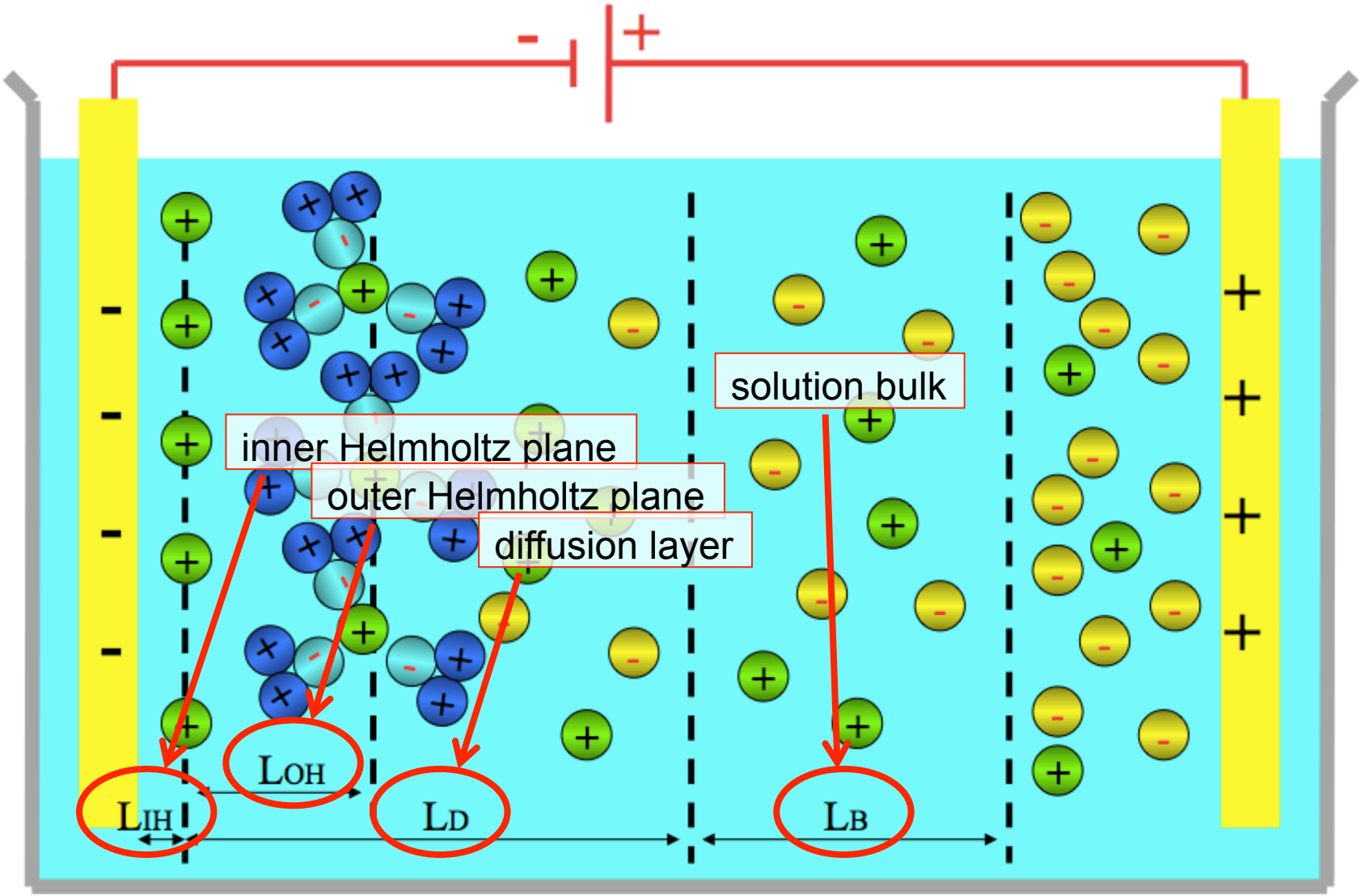
Charge transfer pathways through the DNA layer affect the ideal Capacitance behavior of the interface with the solution sample

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)

$$\nabla f = \frac{\partial f}{\partial x}\hat{\mathbf{x}} + \frac{\partial f}{\partial y}\hat{\mathbf{y}} + \frac{\partial f}{\partial z}\hat{\mathbf{z}}$$

$$\nabla \cdot \nabla f = \nabla^2 f$$

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

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

For perturbation away from equilibrium at finite temperature

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

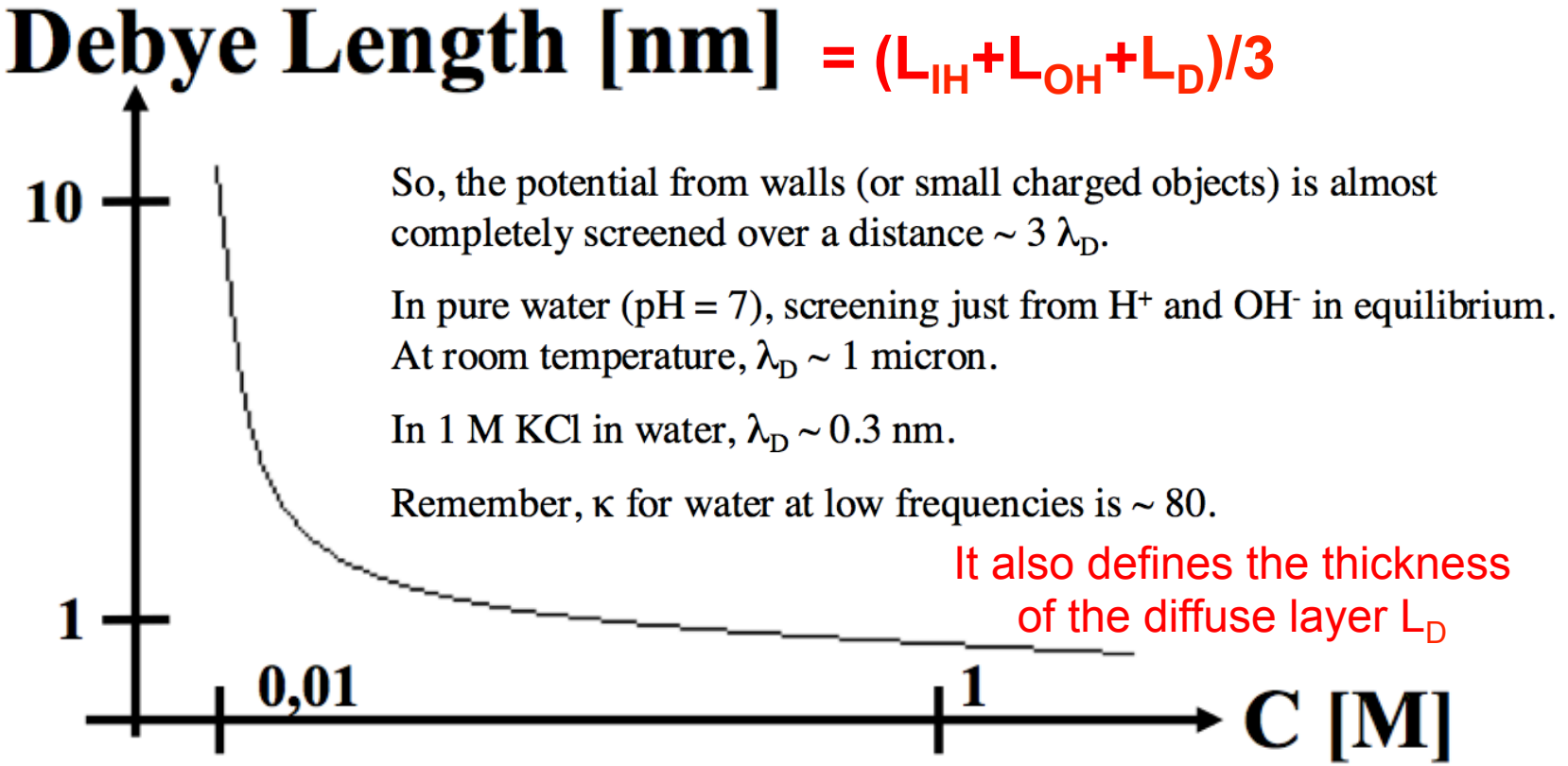
Debye Length

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

~ 0 for equilibrium neutrality

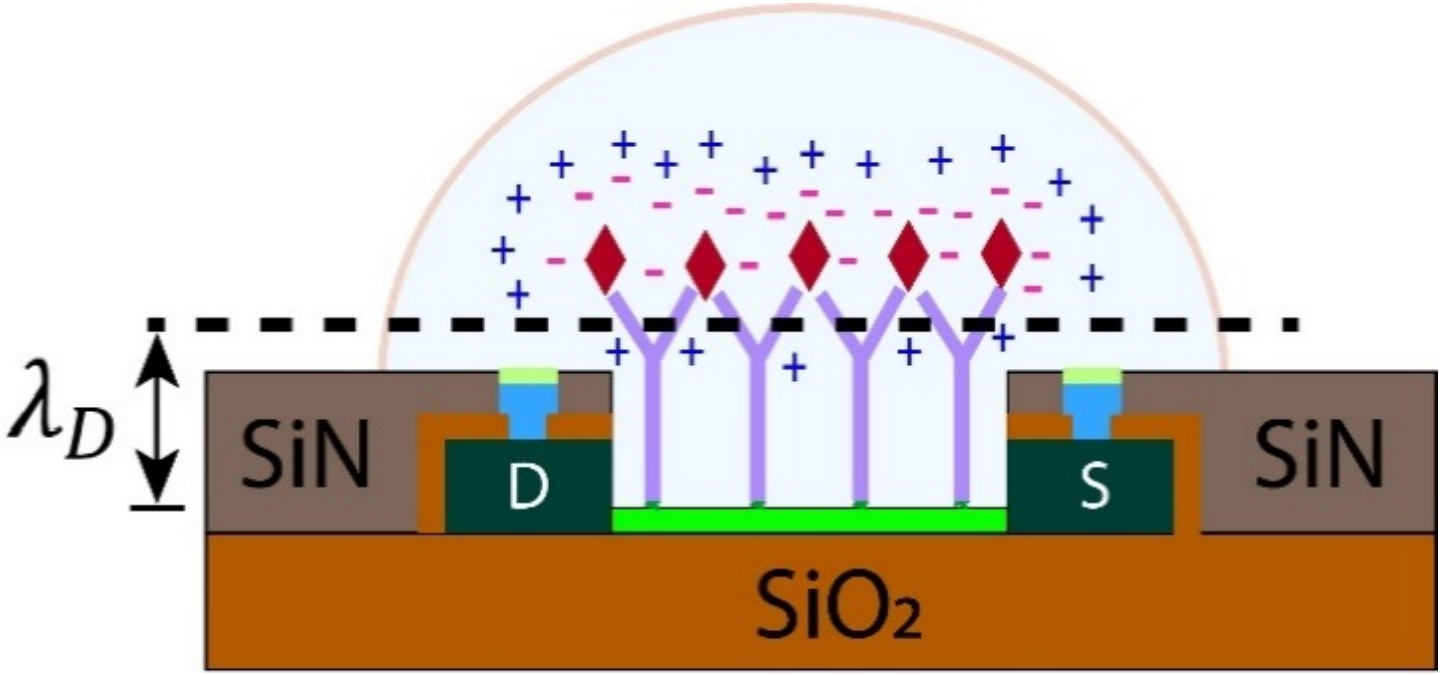
$$\lambda_D \equiv \left(\frac{e^2}{\kappa\varepsilon_0 k_B T}\sum_i z_i^2 n_{i0}\right)^{-1/2}$$

Debye Length



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

Capacitance Detection & Debye Length



In liquid electrical detection

The Debye Length is top important to establish the limits of charge-based detections in solution