Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #8

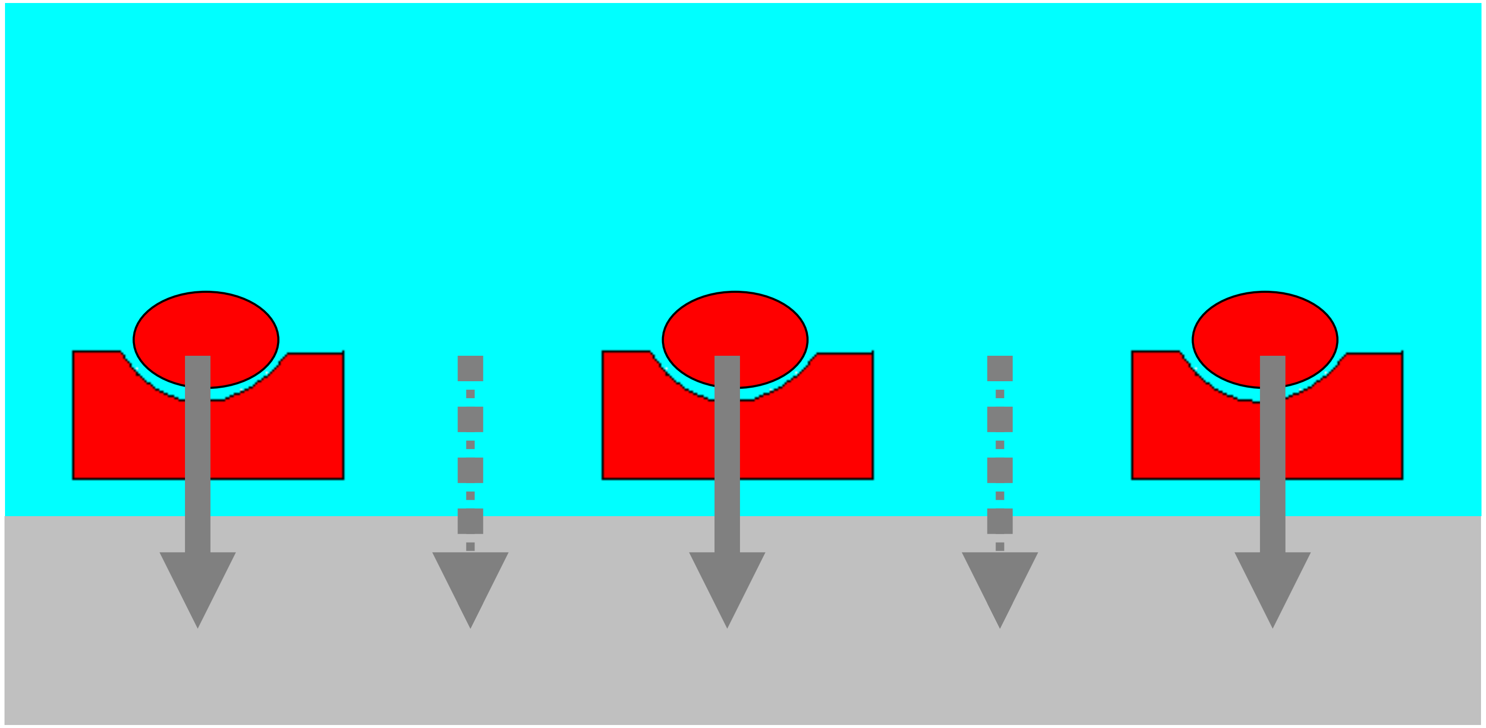
Nanotechnology to prevent Electron Transfer

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs § 6.3-8)

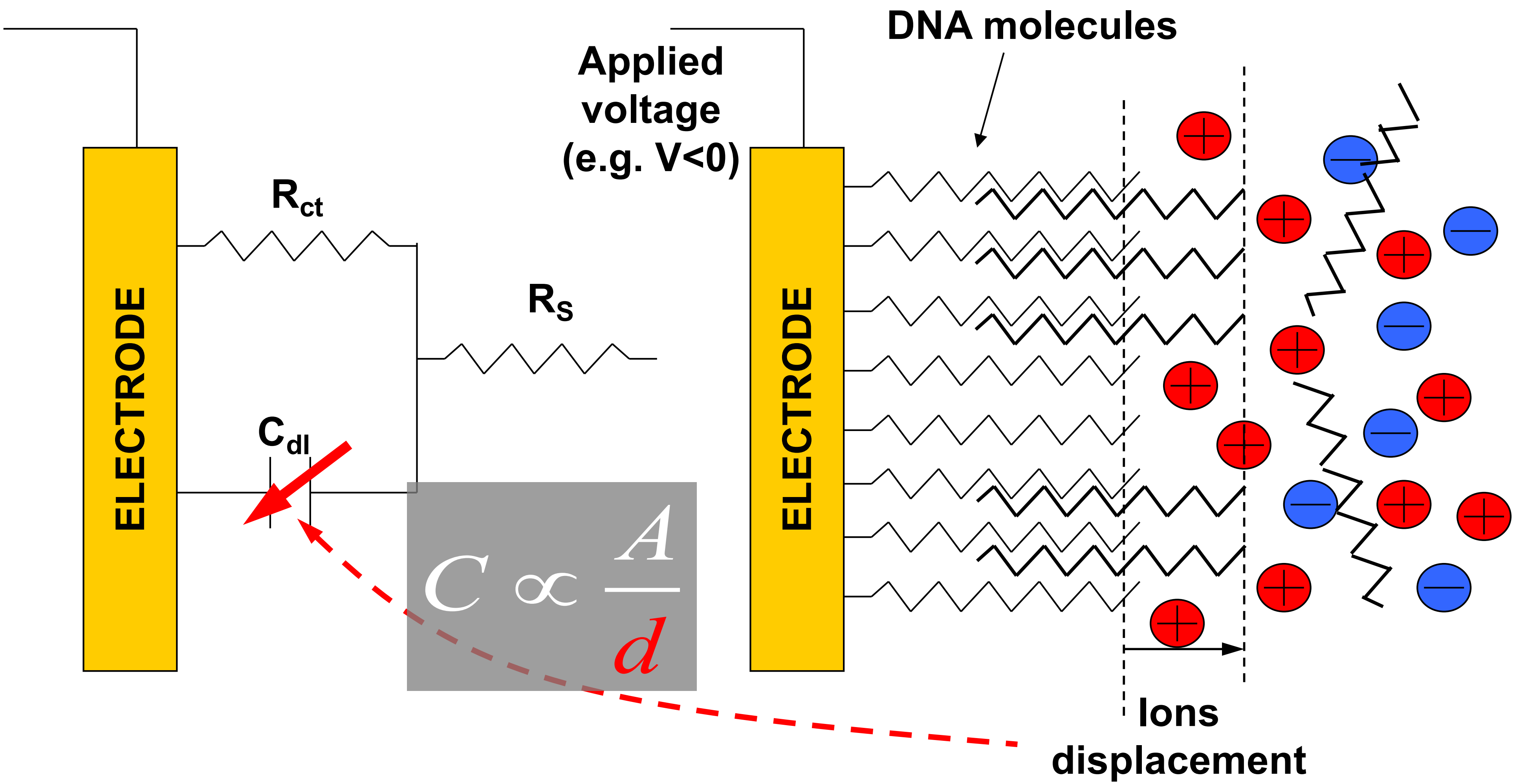
- Electrical behavior of DNA at the interface
- Nanoscale properties of DNA films
- Blocking agents
- Precursor films
- Electrical behavior of Antibodies

CMOS/Sample interface



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

The Capacitance DNA Detection



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

Q1

Does a ssDNA sensing layer behave as an insulator?

A. Yes: all the DNA molecules are insulators!
B. Mainly yes, but not exactly always
C. Mainly not, but in certain conditions
D. No: the DNA molecules are not insulators!

Q2

Does a dsDNA layer behave differently than ssDNA as an insulator?

A. Yes: the dsDNA layer is less insulating
B. Yes: the dsDNA layer is more insulating
C. Mainly not, but in certain conditions
D. No: all the DNA molecules behave similarly!

Q3

Does a DNA molecule behave as an insulator?

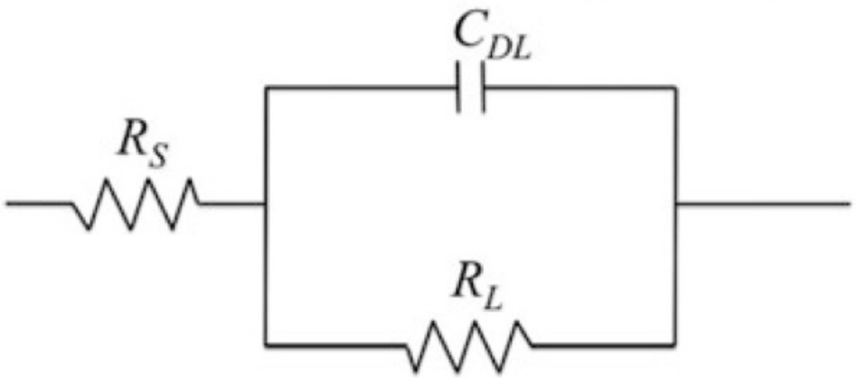
A. Yes: any organic molecules is an insulator

B. Mainly yes, but not exactly always

C. Mainly not, but in certain conditions

D. No: all organic molecules allow a certain conductivity

Randle Model



$$C_{DL} = \varepsilon_{DNA}\varepsilon_o \frac{A}{d}$$

$$Z_{//} = \frac{R_L}{j\omega C_{DL} R_L + 1}$$

$$I_{non-F} = \frac{V_{ref}}{Z} = \frac{1 + j\omega C_{DL} R_L}{R_L} V_{ref}$$

Equivalent circuits of DNA Bio/CMOS interface

Q4

Recall: do the layering phenomena always behave like an ideal C in the Randle model?

A. Yes, always!
B. Yes, but not in time-trends
C. Yes, but only on voltage-scan
D. No, but in frequency scan
E. No, especially in frequency scan
F. No, never!

Recall: Frequency behavior of a bare gold surface

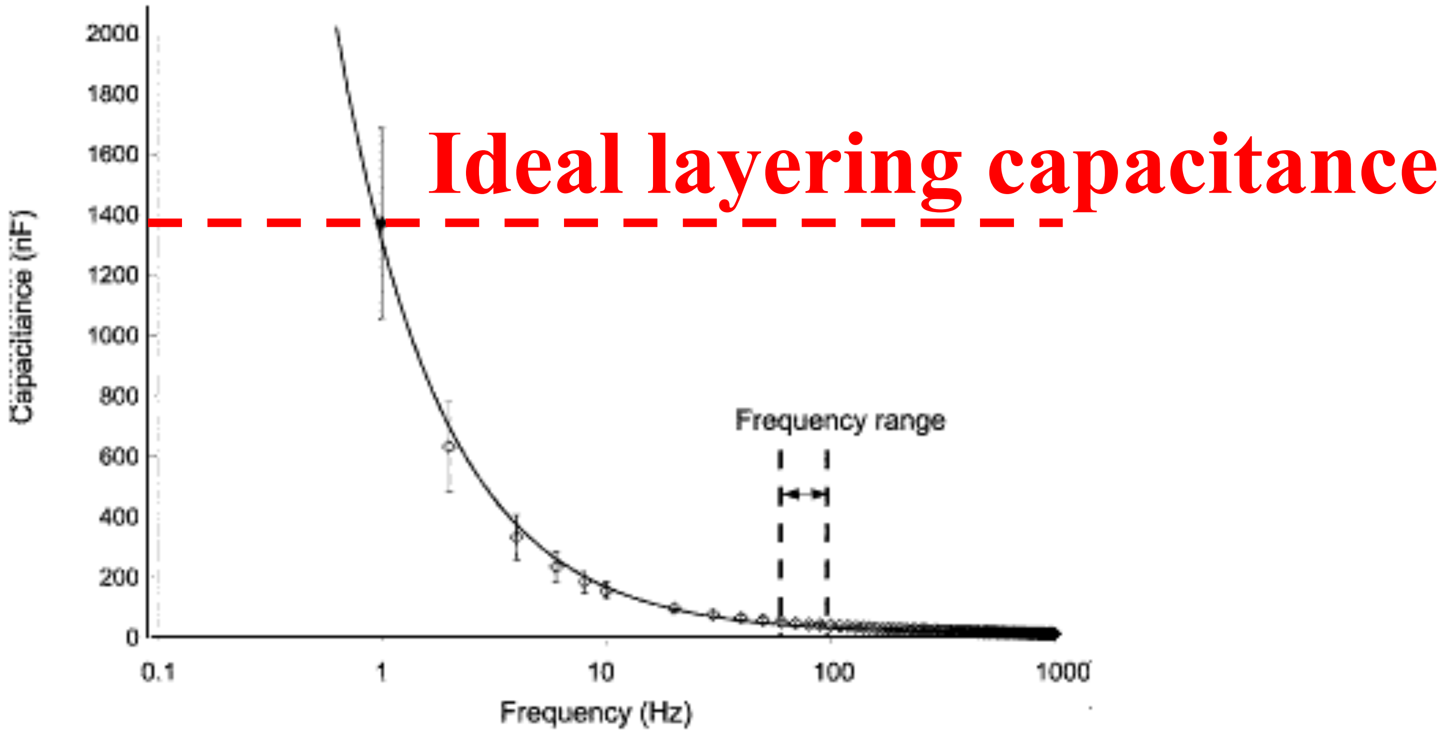
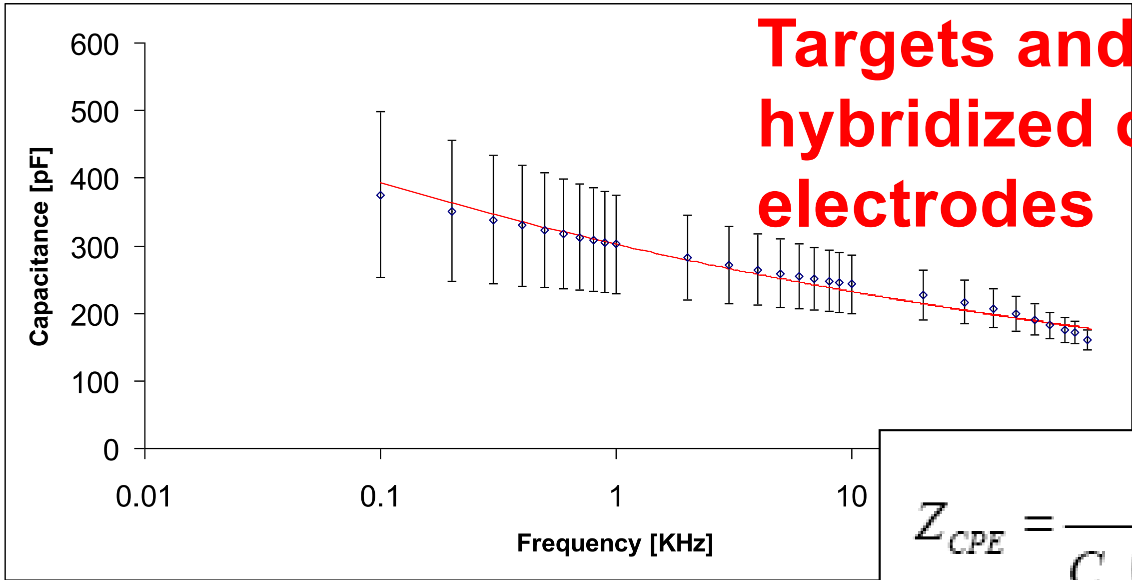


Fig. 9. Measured capacitance versus charge/discharge frequency on clean gold electrodes. The continuous line shows the fitting.

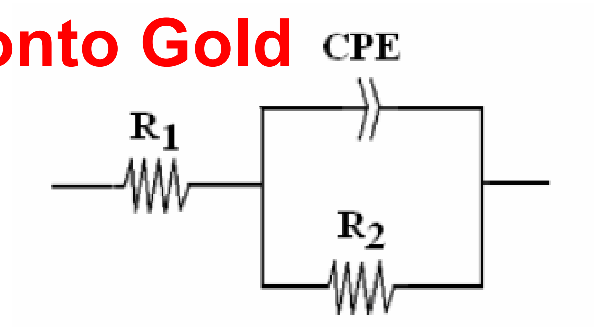
STAGNI *et al.*: FULLY ELECTRONIC LABEL-FREE DNA SENSOR CHIP IEEE SENSORS JOURNAL, VOL. 7, NO. 4, APRIL 2007

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

Recall: Frequency behavior of DNA surfaces



Targets and probes hybridized onto Gold electrodes



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

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

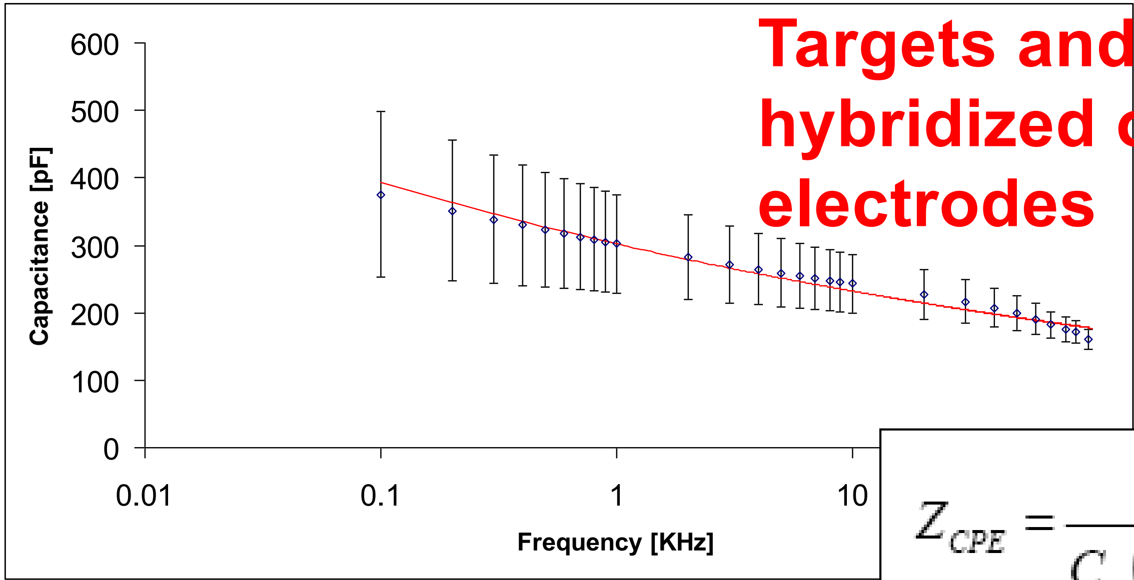
Q5

Does the idea of a C-measure of the target-DNA work then?

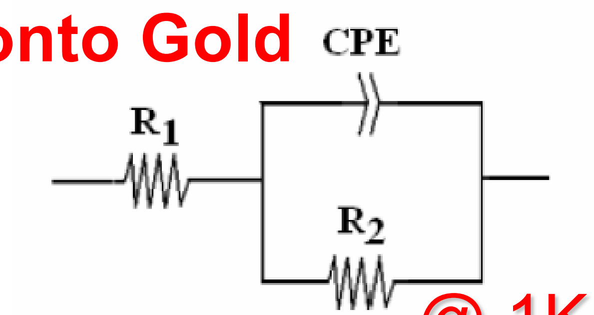
A. Yes: always!
B. Yes, but not in time-trends
C. Yes, but only on voltage-scan
D. No, but in frequency scan
E. No, especially in frequency scan
F. No, never!

(c) S.Carrara

Recall: Frequency behavior of DNA surfaces



Targets and probes hybridized onto Gold electrodes



@ 1KHz

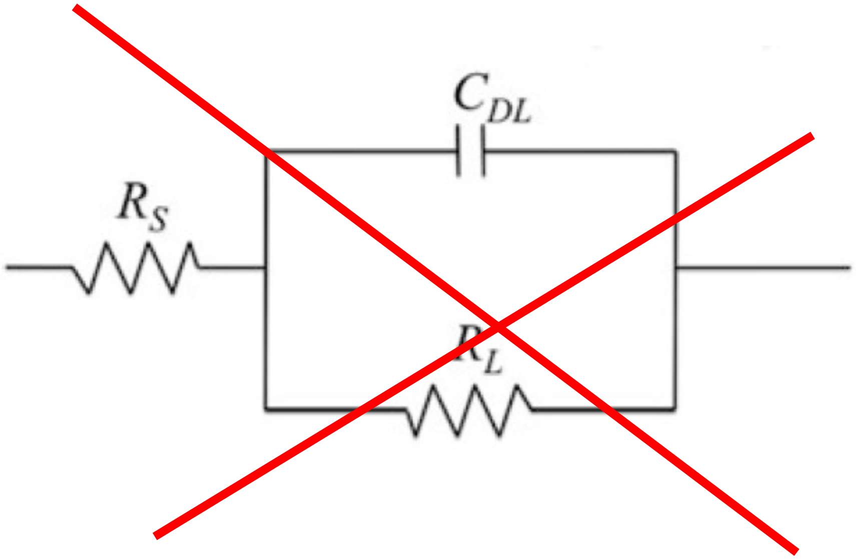
$$Z_{CPE} = \frac{1}{C_p (j\omega)^{\alpha}} \cong \frac{1}{\omega^{\alpha} C_p}\sqrt{1-\alpha^2} - j\frac{1}{\omega^{\alpha} C_p}\alpha$$

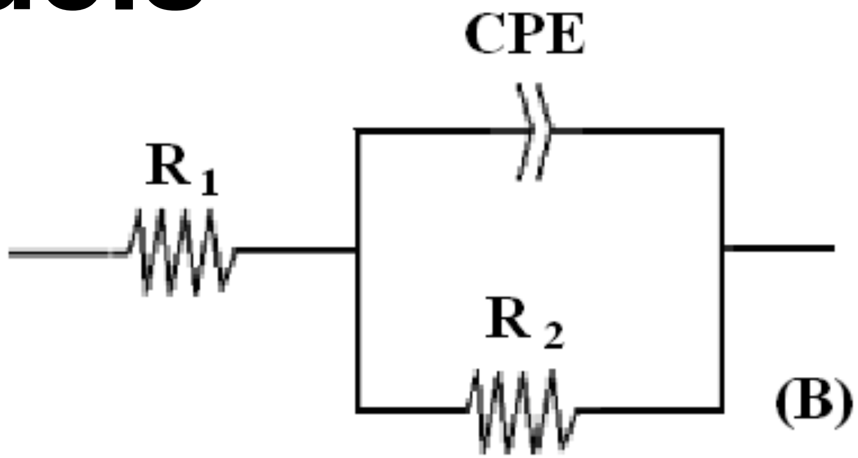
CPE parameters	Bare electrode	DNA Probes	DNA Target
R(GΩ)	45	177	177
X(GΩ)	123	837	970

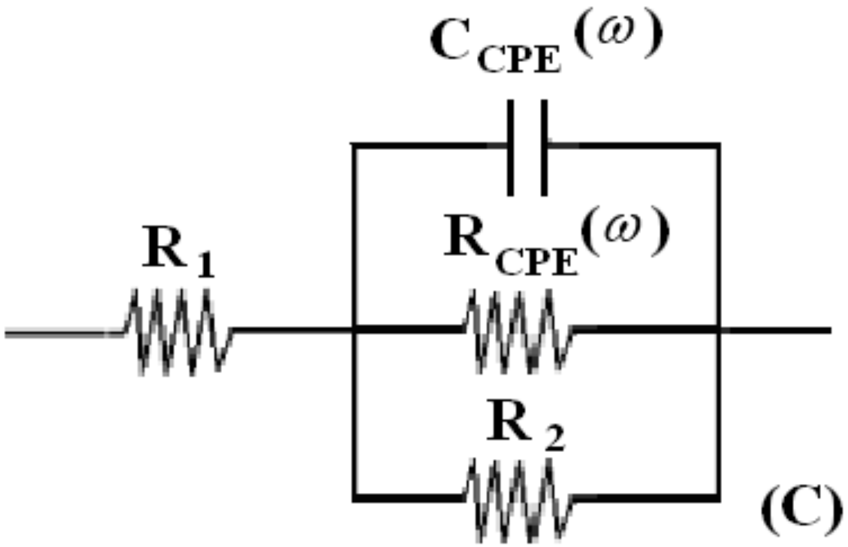
S.Carrara et al., Sensors and Transducer Journal 76 (2007) 969-977

From ssDNA to dsDNA, R_2 does not change, nor the Re(CPE), while the Im(CPE) does

Interface models







Equivalent circuits of DNA Bio/CMOS interface

CPE element

$$Z_{CPE} = \frac{1}{C_p(j\omega)^\alpha} = \frac{\cos\left(\frac{\pi}{2}\alpha\right)}{C_p\omega^\alpha} - j\frac{\sin\left(\frac{\pi}{2}\alpha\right)}{C_p\omega^\alpha}$$

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

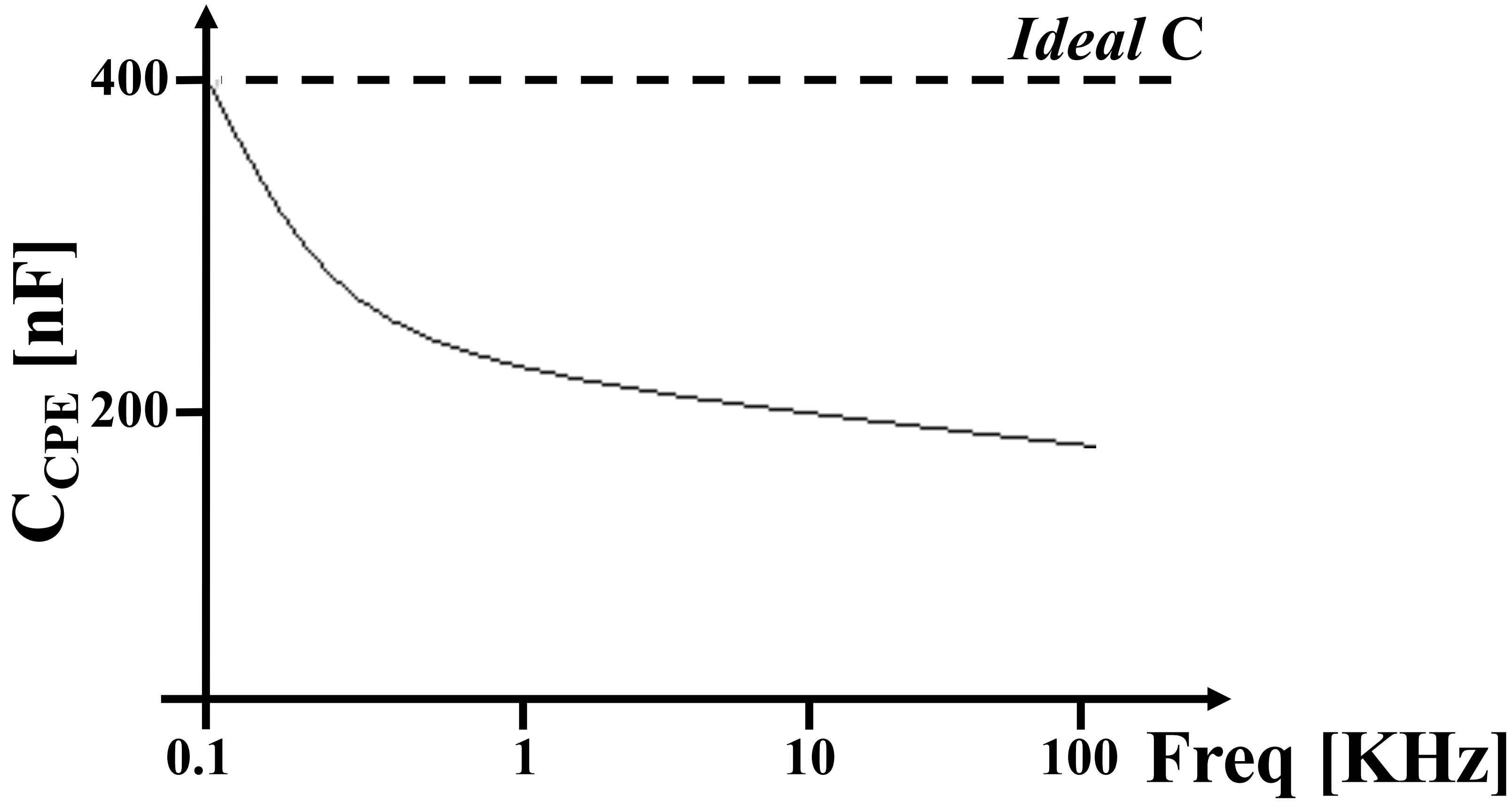
CPE element

$$Z_{CPE}\begin{cases} R_{CPE} \cong \frac{1}{\omega^{\alpha} C_p}\sqrt{1-\alpha^2} \\ X_{CPE} \cong \frac{-1}{\omega^{\alpha} C_p}\alpha \end{cases}$$

$$|X_{CPE}| \cong \frac{1}{\omega^{\alpha} C_p}\alpha = \frac{1}{\omega^{\alpha-1}\omega\, C_p}\alpha = \frac{1}{\omega\left(\frac{C_p}{\alpha\,\omega^{1-\alpha}}\right)}$$

$$C_{CPE} \cong \frac{C_p}{\alpha\,\omega^{1-\alpha}}$$

Equivalent Capacitance vs frequency



Q6

Does conductivity through the DNA layer affect the quality of the sensing?

A. Yes: always!
B. Yes, but not in time-trends
C. Yes, but only in a negligible way
D. No, if not done not in V-scan
E. No, if done in frequency scan
F. No, never!

Q7

Does the conductivity through the DNA layer affect the sensing quality in terms of stability in time?

A. Yes: always!
B. Yes, but not in time-trends
C. Yes, but only in a negligible way
D. Negligible, if done with proper coatings
E. No, if done in frequency scan
F. No, never!

The problem of time instability

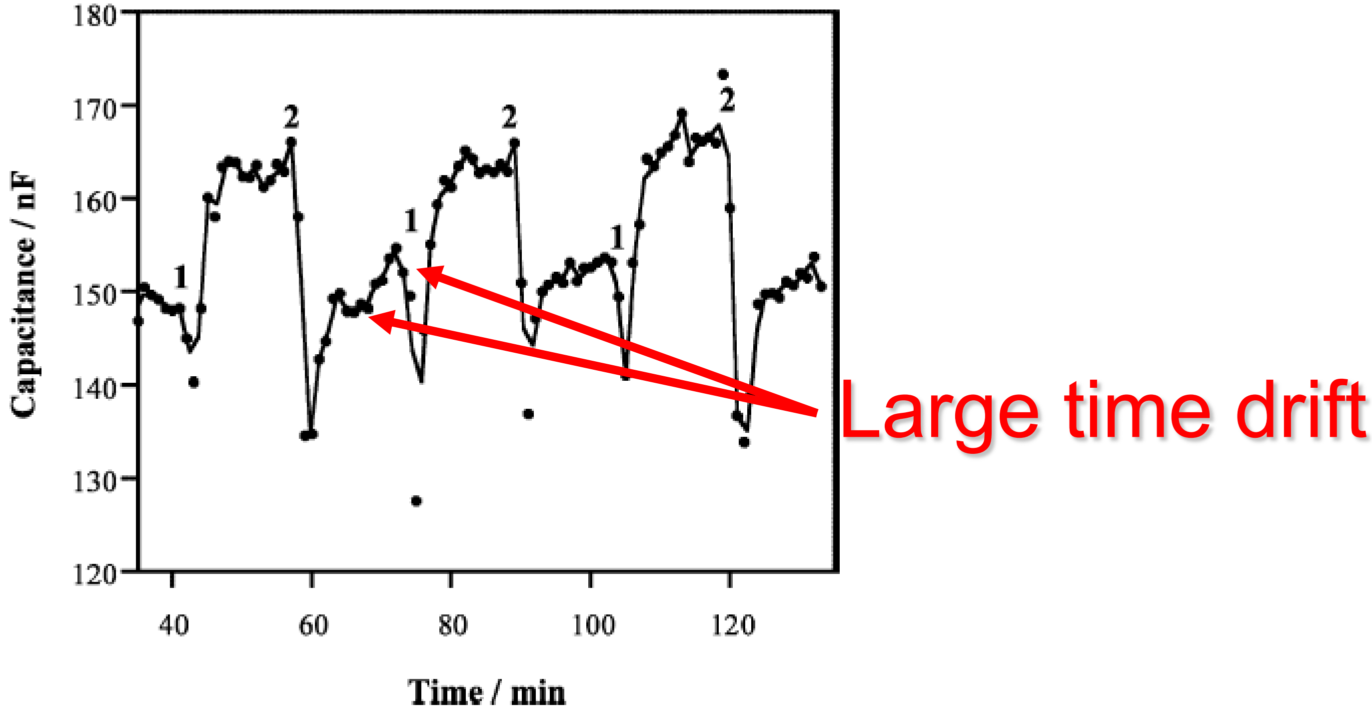


Fig. 7. Regeneration of the EC20 modified electrode with (**1**) 1 mM EDTA after injections of (**2**) 100 μM Zn^{2+}. Other experimental conditions are as in Fig. 5.

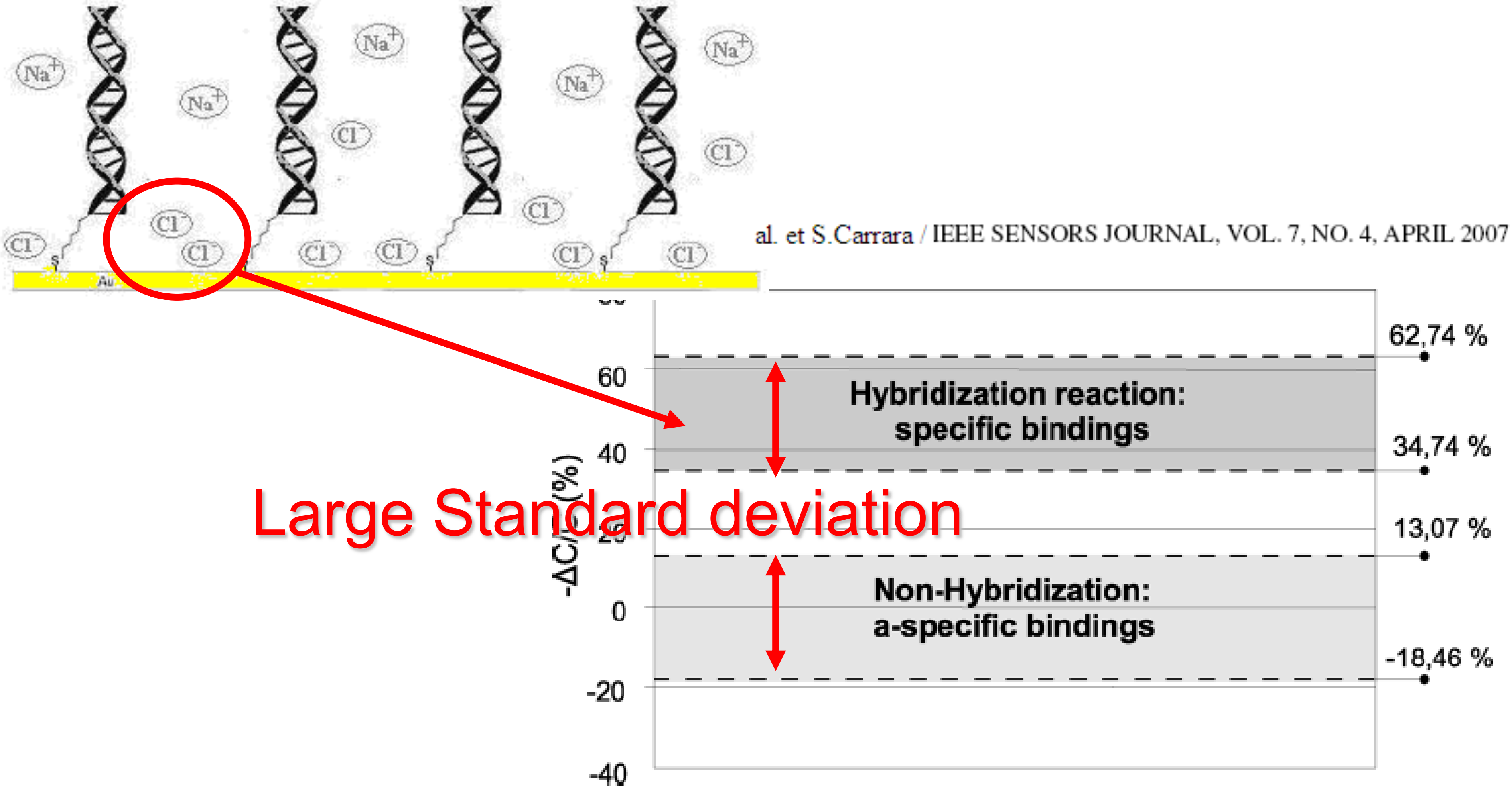
Heavy metal ion capacitance detection by using phytochelatine as probe molecule

Q8

Does the time instability affect the quality in terms of measurements errors?

A. Yes: always!
B. Yes, but not in time-trends
C. Yes, but only in a negligible way
D. Negligible, if done with proper coatings
E. No, if done in frequency scan
F. No, never!

Nano-aperture in the probes film



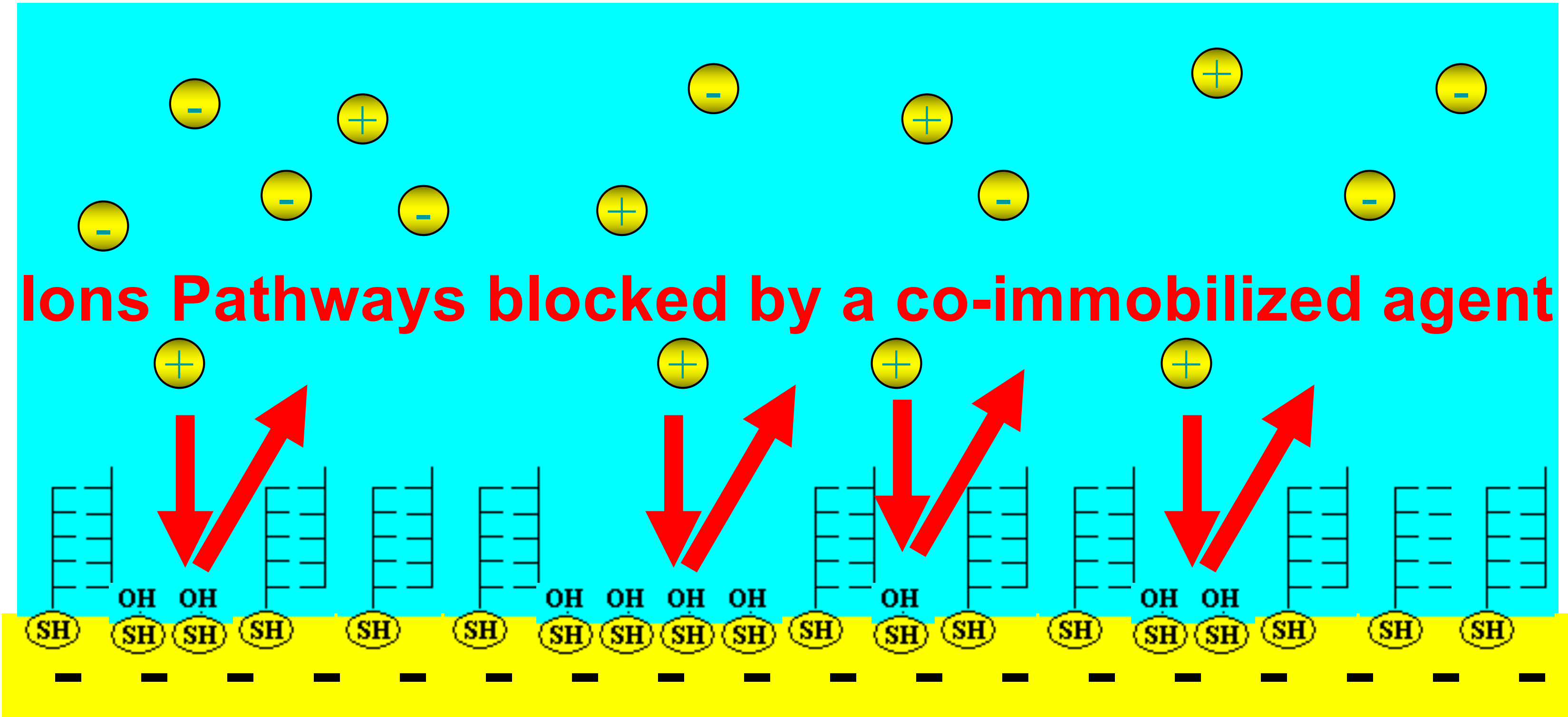
Solution ions in free contact with electrodes surface results in very large standard deviations

Q9

How to close the nano-apertures in the DNA layers?

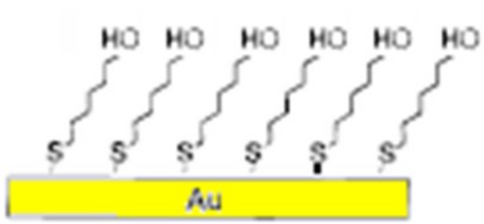
A. Impossible!
B. With a more packed DNA probes-layer
C. With synthetic DNA probes
D. With longer DNA targets
E. With some other organic molecules as blocking caps
F. With underneath fully insulating layers

Nano-aperture blocked in the probes film

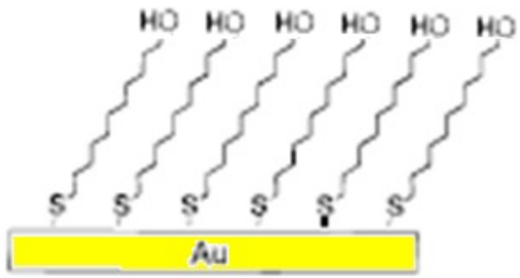


Ion Pathways blocked at the nanoscale

Blocking Agents



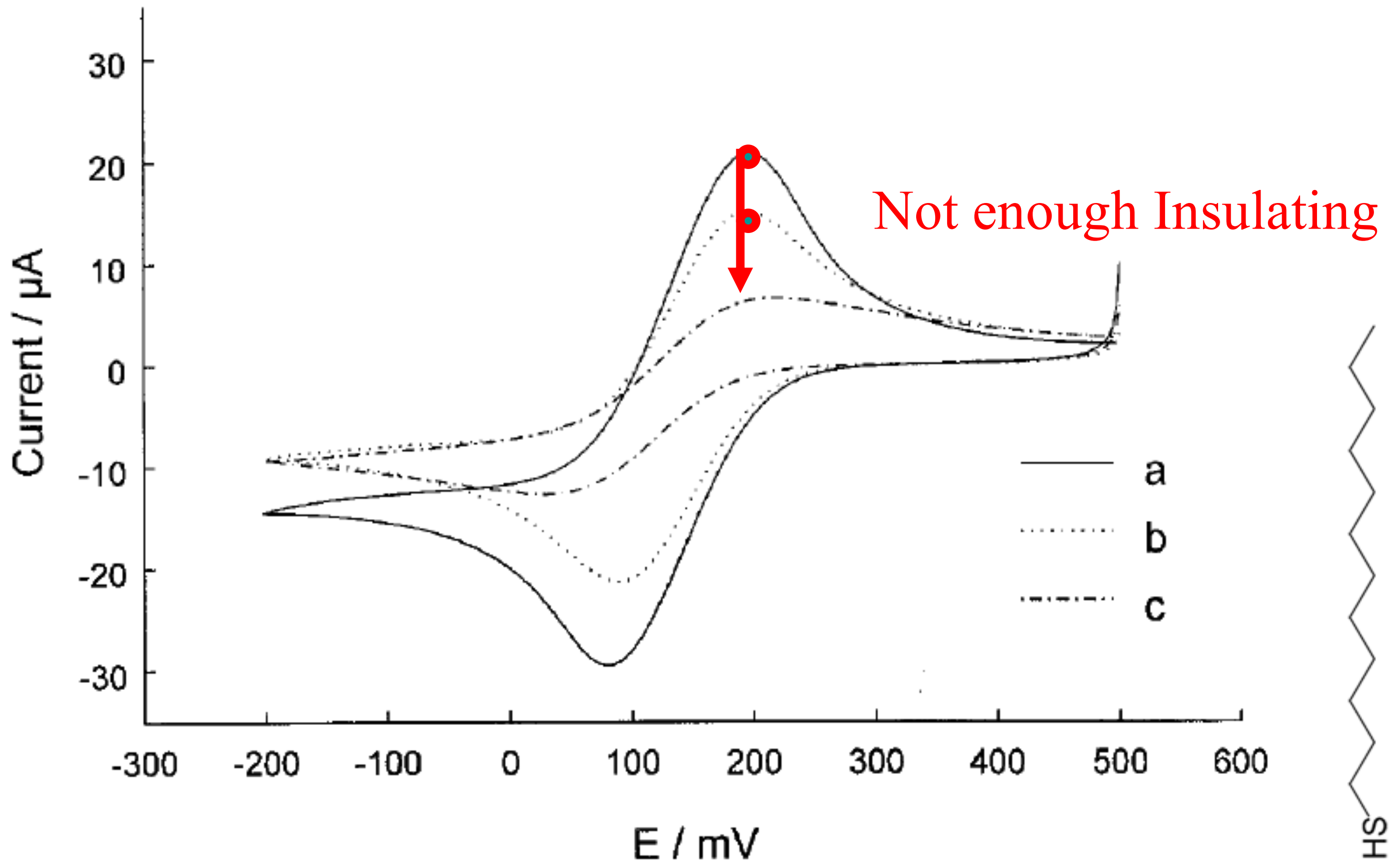
Mercapto-Hexanol



Mercapto-Undecanoic Acid

Increased stability by using mercapto-hexanol or longer thiols

Improved insulating behavior



C. Berggren et al. Electroanalysis 1999, 11, No. 3

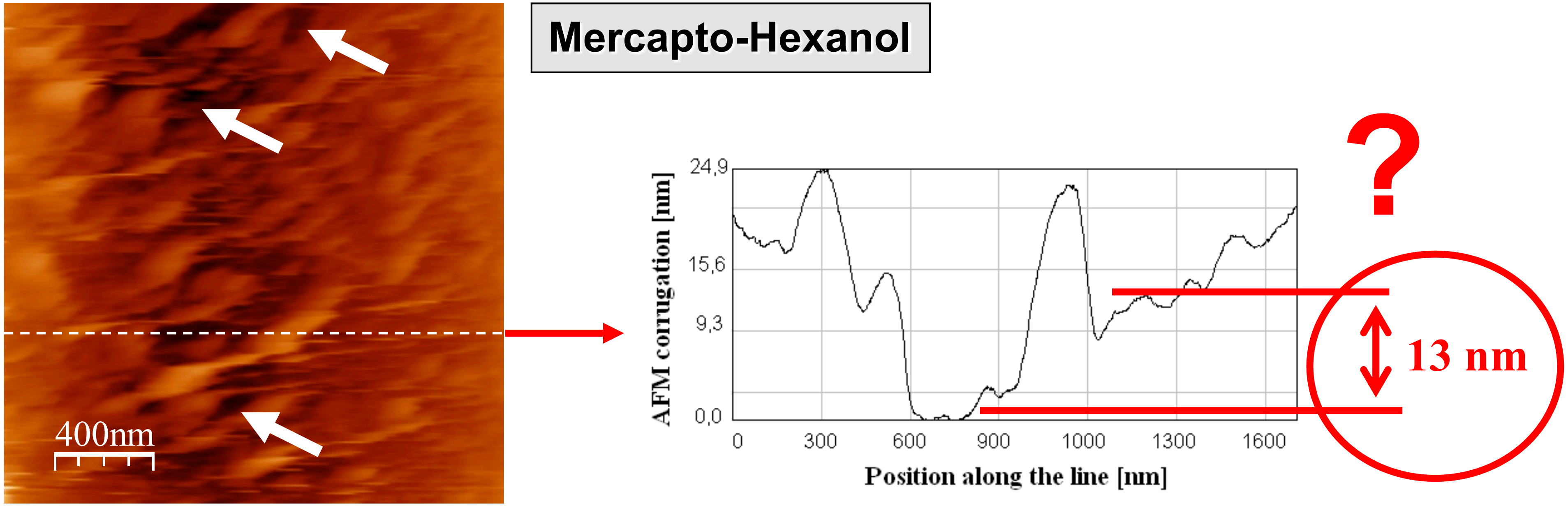
Redox reaction of K3Fe(CN)6 on gold electrode (a), ss-DNA onto gold (b) and ss-DNA + 1-dodecanethiol onto gold (c)

Q10

With which nano-technology may we directly investigate the nano-apertures of DNA on gold?

A. By acquiring time-trends with Resonant Mirrors
B. By acquiring time-trends with Surface Plasmon Resonance
C. By using the Transmission Electron Microscopy
D. By using the Atomic Force Microscopy

Nano-scale Apertures



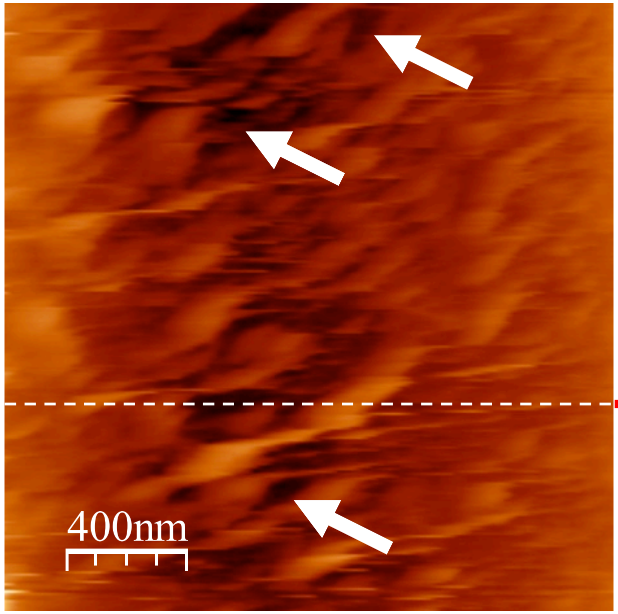
Monolayers of ssDNA with blocking agents present groves with a certain depth

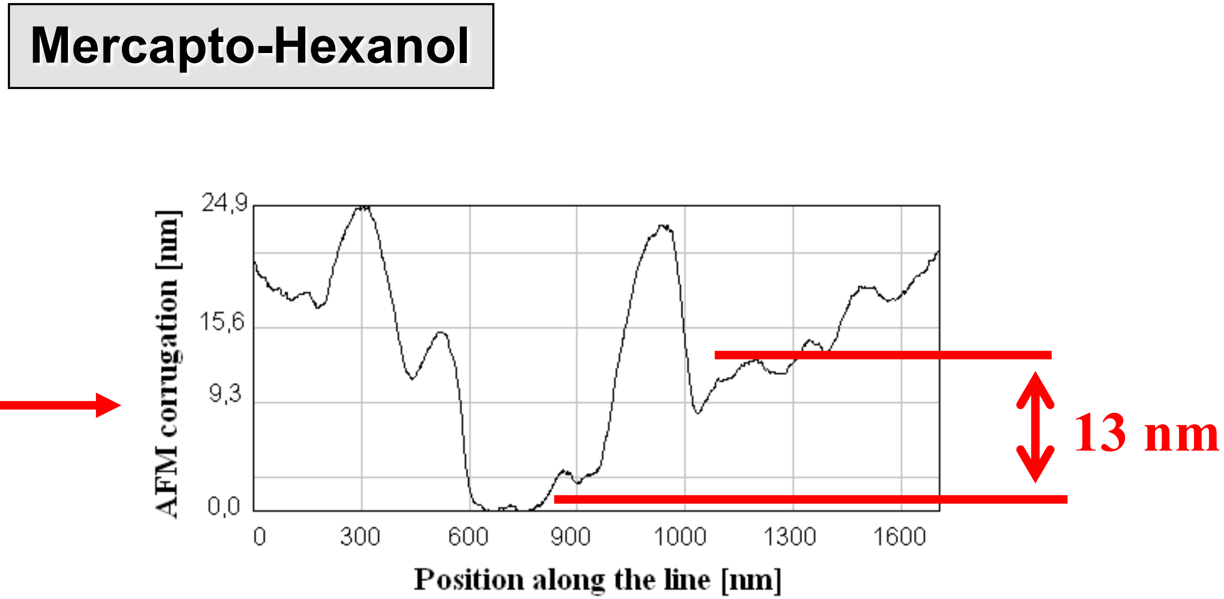
Q11

May we trust the groves' depth as estimated by AFM?

A. Yes: of course!
B. It depends on the material of the tip
C. It depends on the material of organic layer
D. No, but in case of insulating substrates
E. Definitely not!

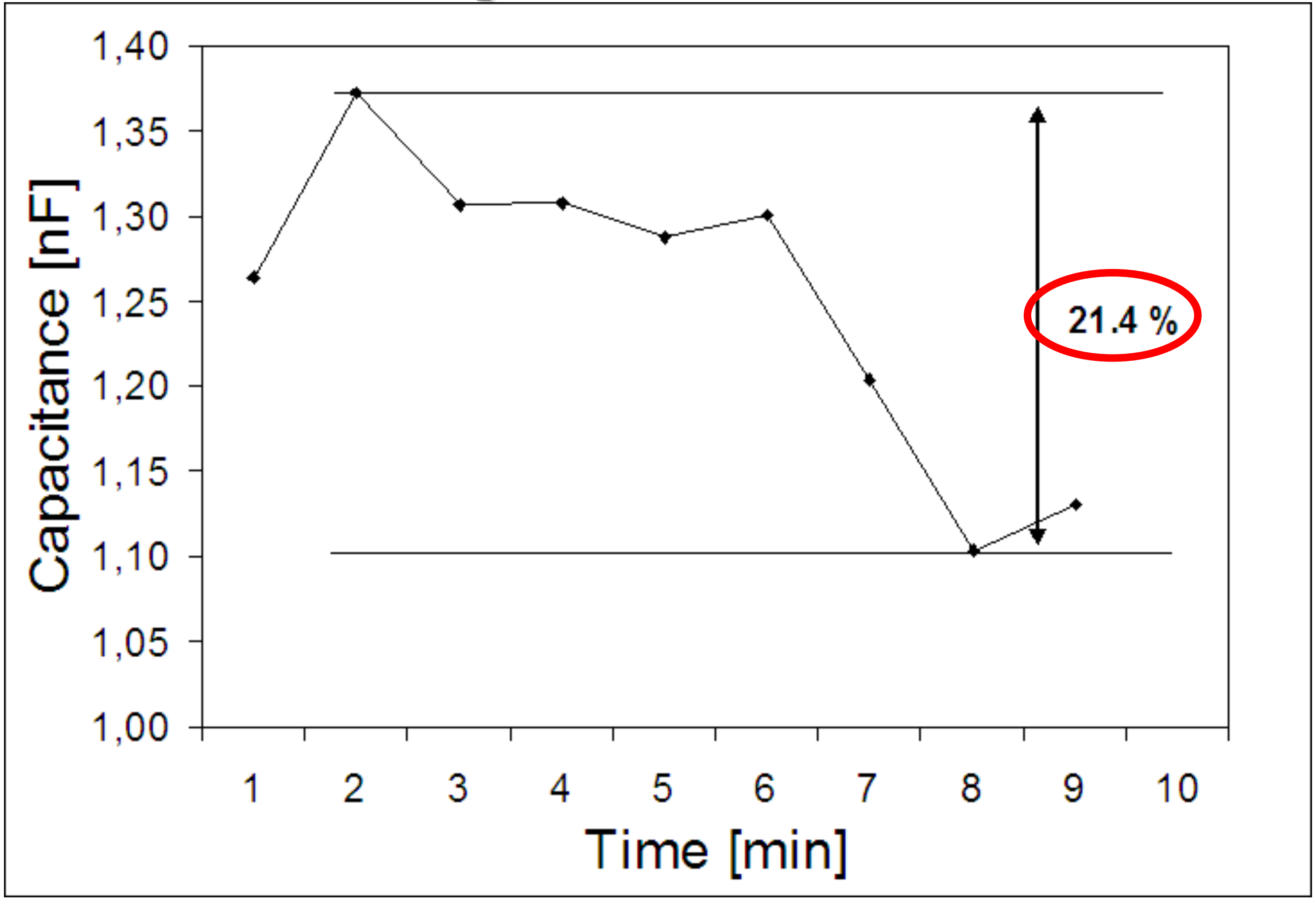
Nano-scale Apertures

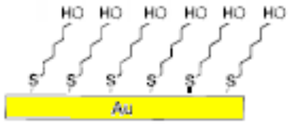




Monolayers of ssDNA with blocking agents still present deep groves crossing the film

Capacitance Instability

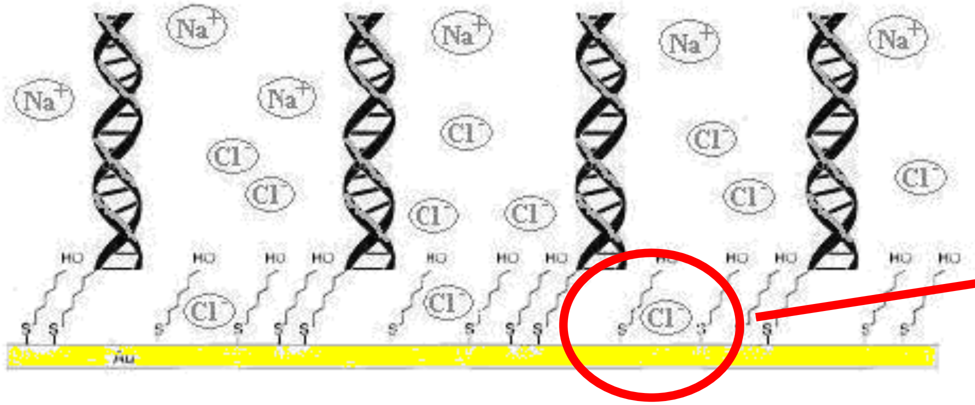


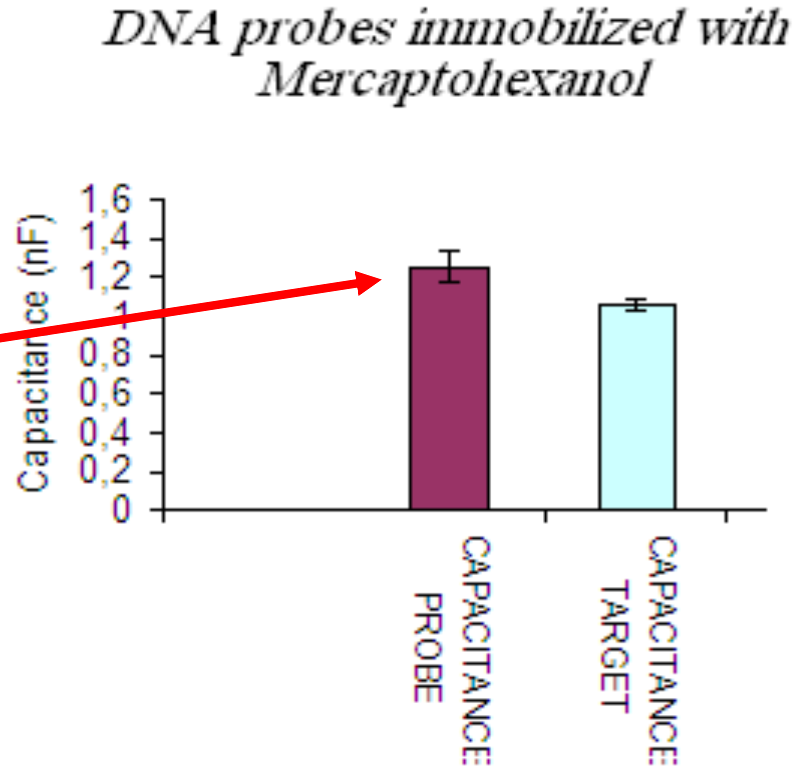


Mercapto-Hexanol

Mercapto-Hexanol is not enough as blocking agent

Mercapto-Hexanol partially block

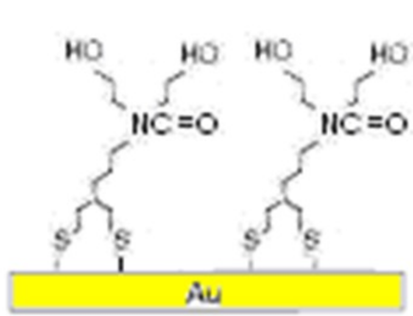




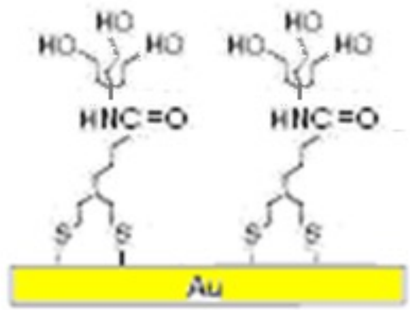
The presence of mercapto-hexanol partially prevent the solution ions in free contact with electrodes and results in a reduction of the standard deviations

La pause

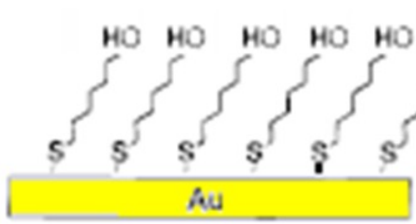
More Effective Blocking Agents



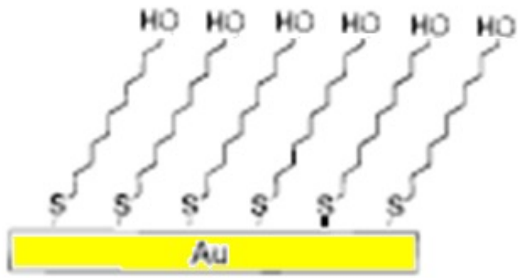
Lipa-DEA



Lipa-Tris



Mercapto-Hexanol

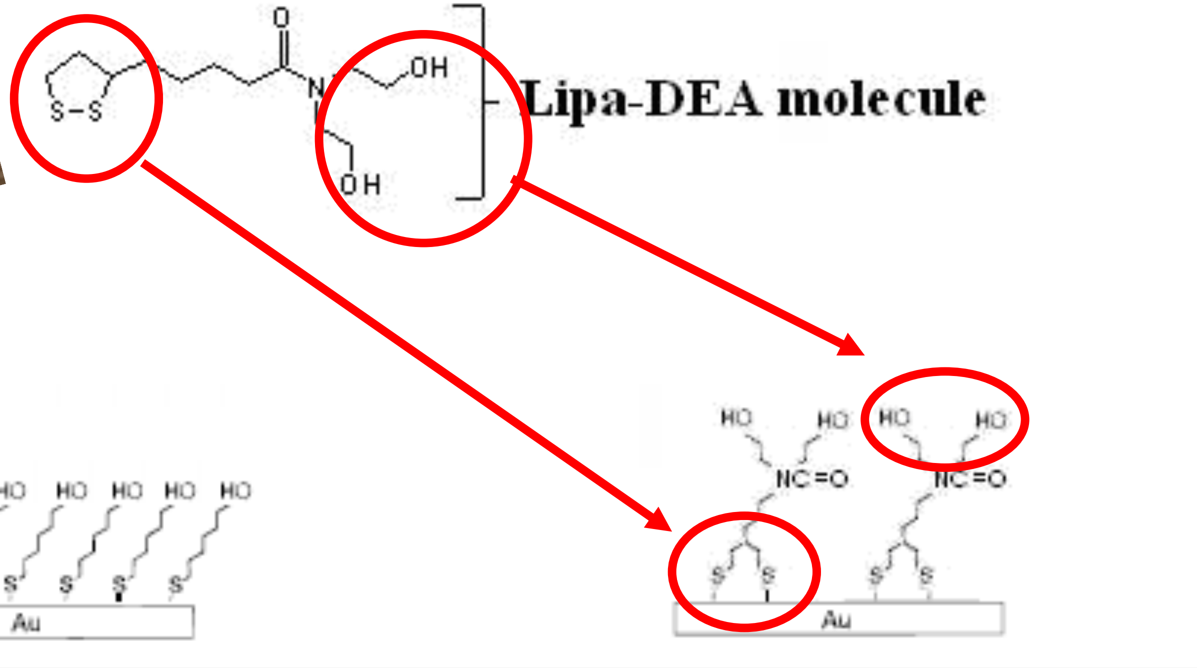


Mercapto-Undecanoic Acid

Increased stability by using Lipa-DEA as blocking agent

Lipa-DEA blockers

Different kind of thiols developed by Inger Vikholm



N,N-bis(2-hydroxyethyl)-α-lipoamide (Lipa-DEA) may be used as more efficient blocking agents

Q12

With which nano-technology may we investigate the deposition of blocking agents on gold?

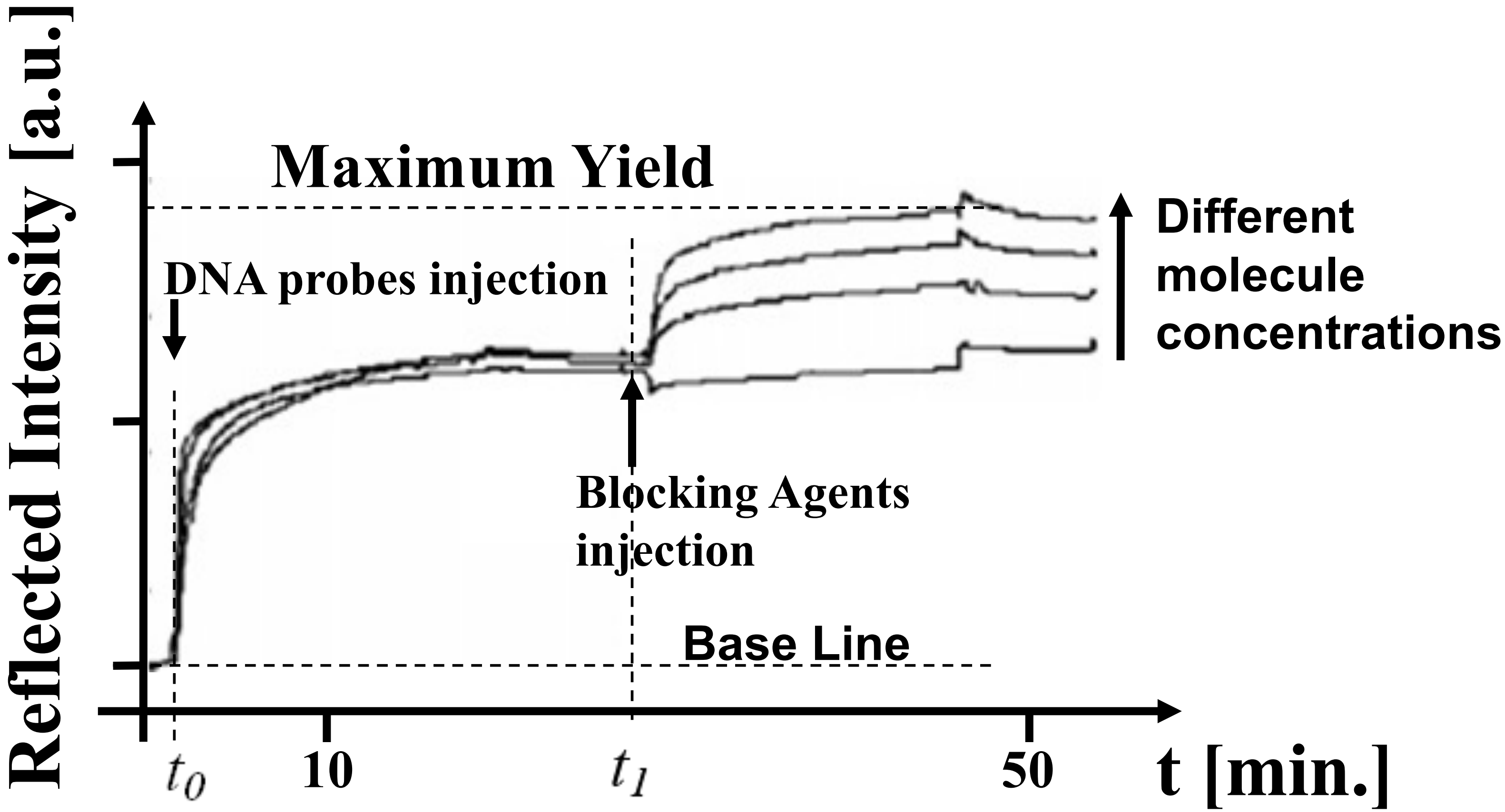
A. By acquiring time-trends with Resonant Mirrors

B. By acquiring time-trends with Surface Plasmon Resonance

C. By using the Transmission Electron Microscopy

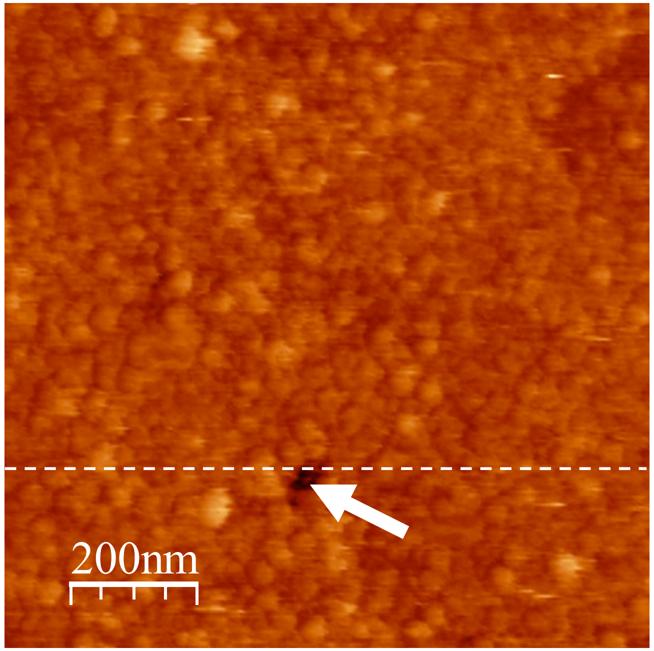
D. By using the Atomic Force Microscopy

SPR on successive deposition of Blocking Agents

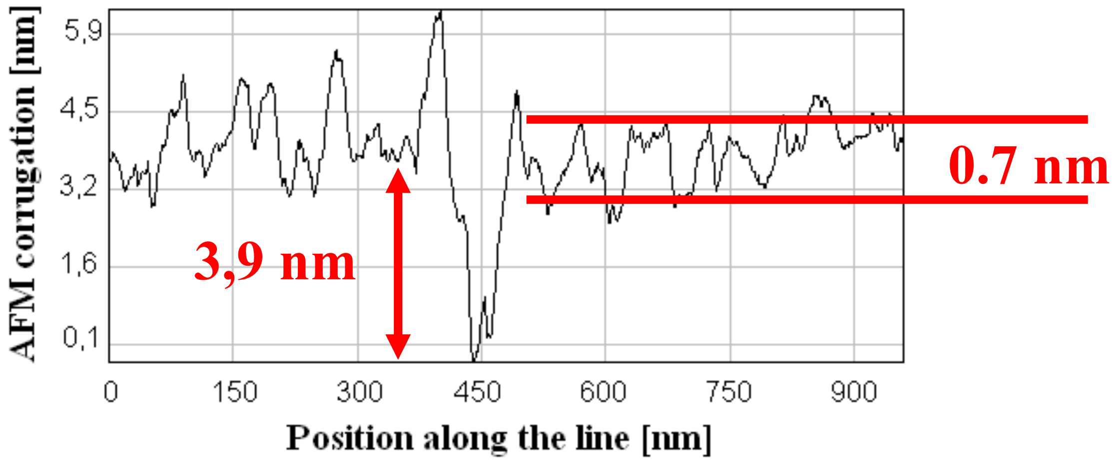


I. Vikholm-Lundin et al. / Biosensors and Bioelectronics 22 (2007) 1323–1329

Nano-scale Apertures

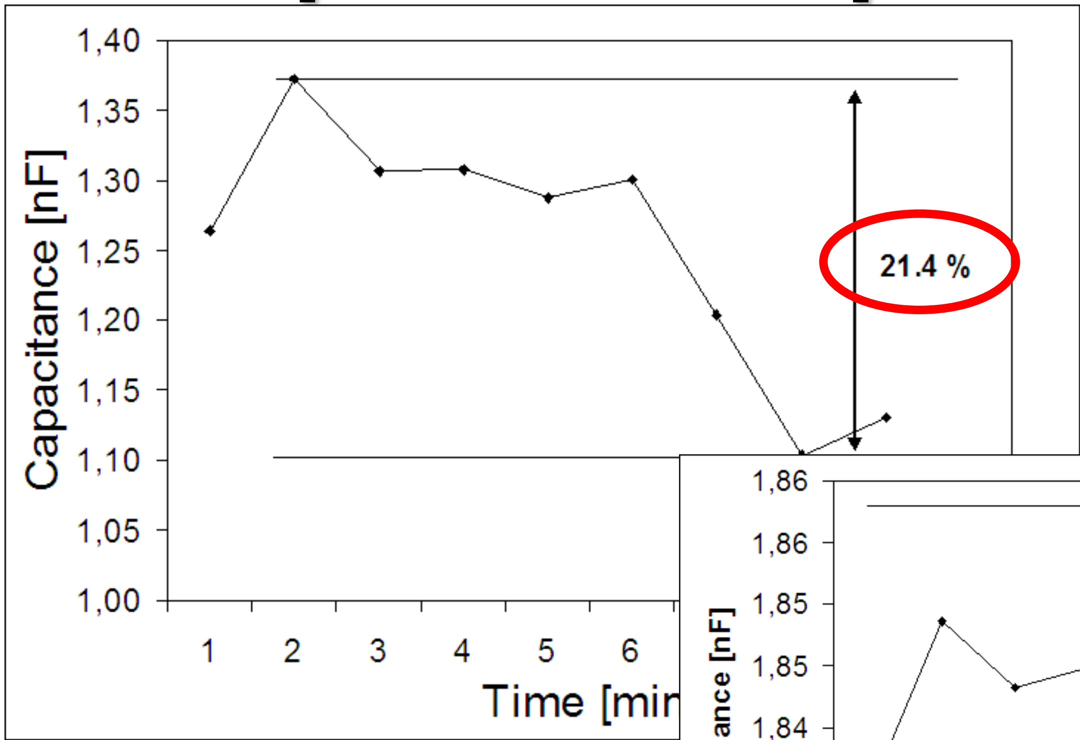


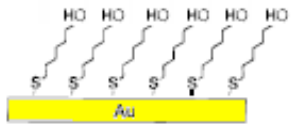
Lipa-DEA



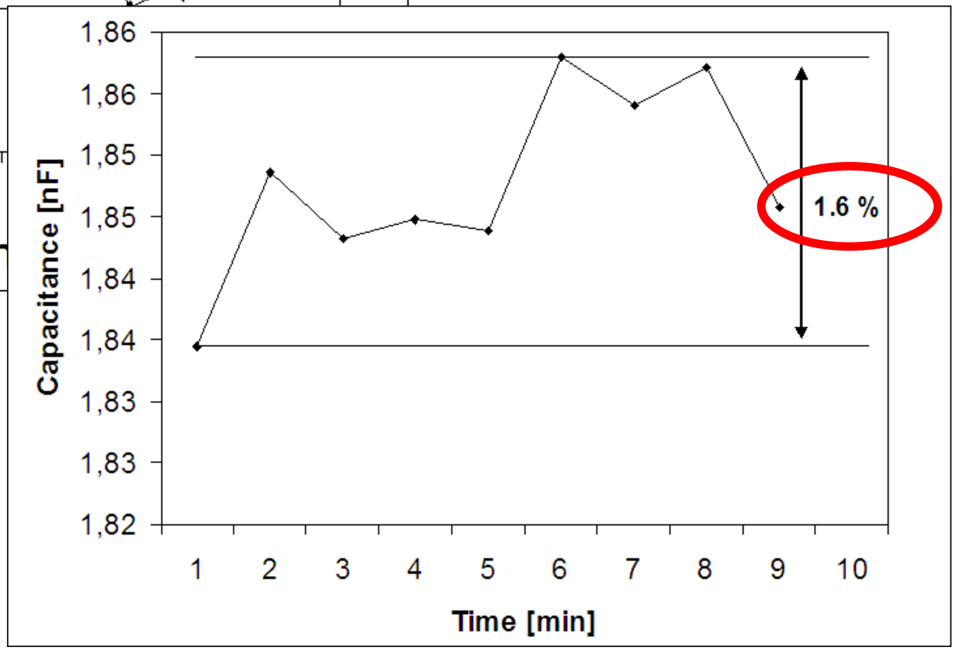
Monolayers of ssDNA with blocking agents still present deep groves crossing the film

Improved Capacitance Stability

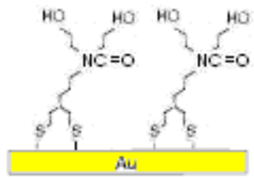




Mercapto-Hexanol

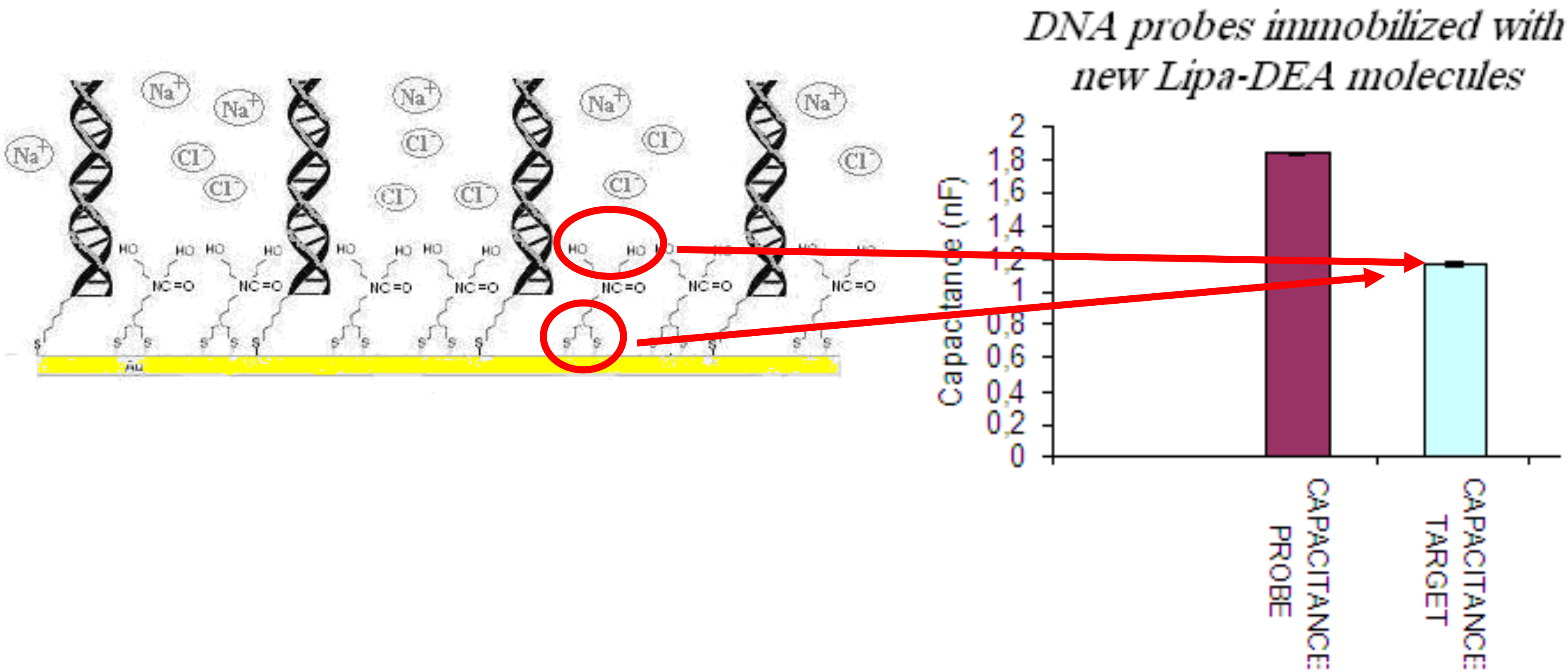


Lipa-DEA



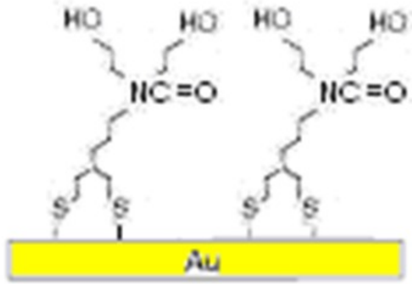
Increased stability by using Lipa-DEA as blocking agent

Lipa-DEA further improves

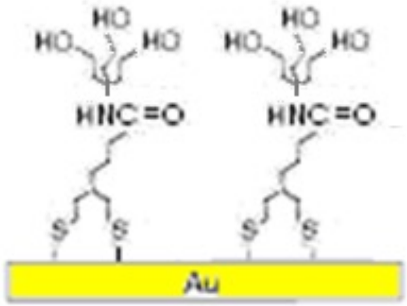


The presence of Lipa-DEA further prevent the solution ions in free contact with electrodes and results in a strong reduction of the standard deviations

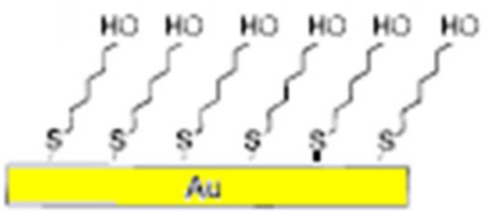
Precursors Monolayers



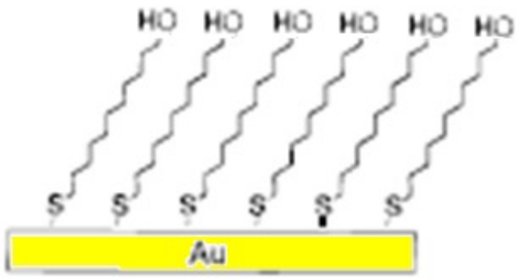
Lipa-DEA



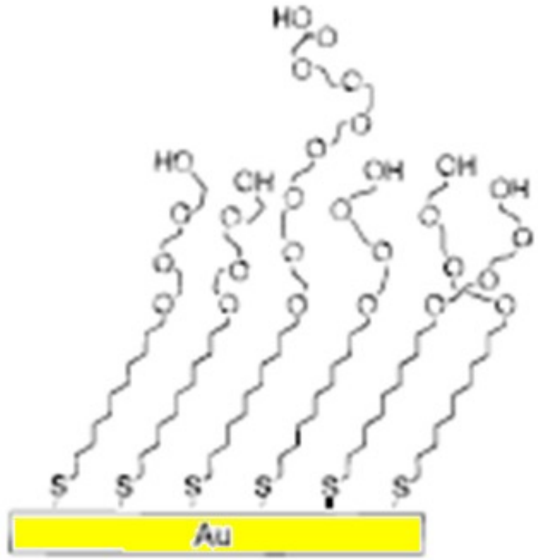
Lipa-Tris



Mercapto-Hexanol



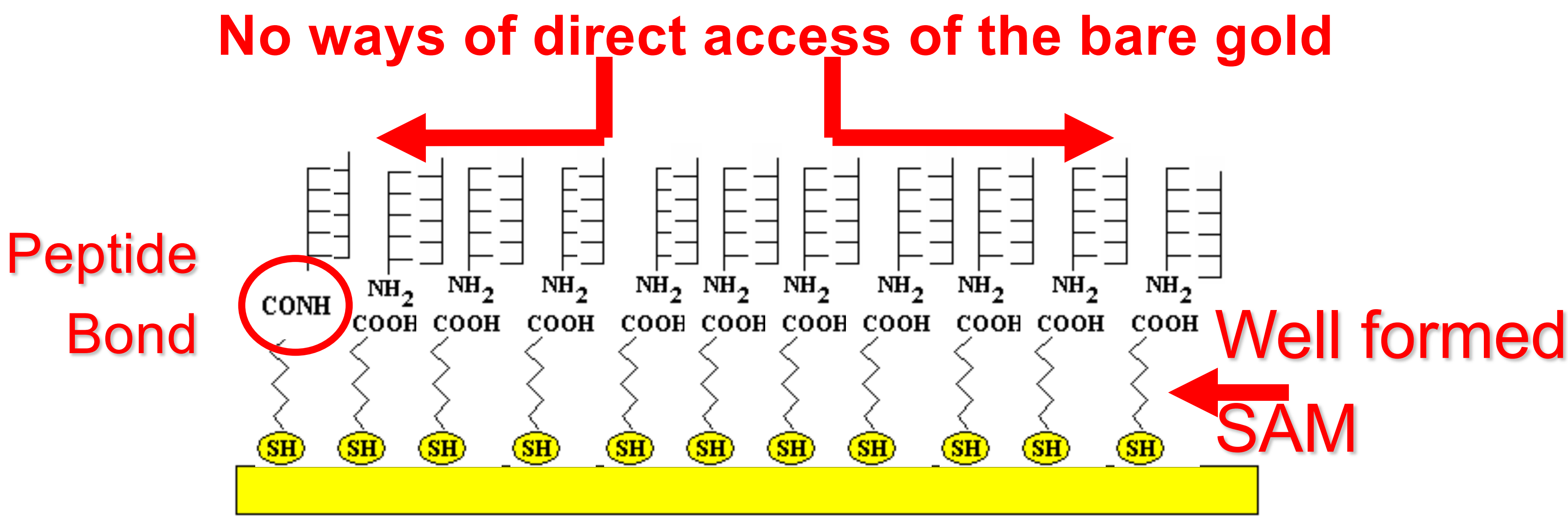
Mercapto-Undecanoic Acid



Ethylene-Glycol Thiols

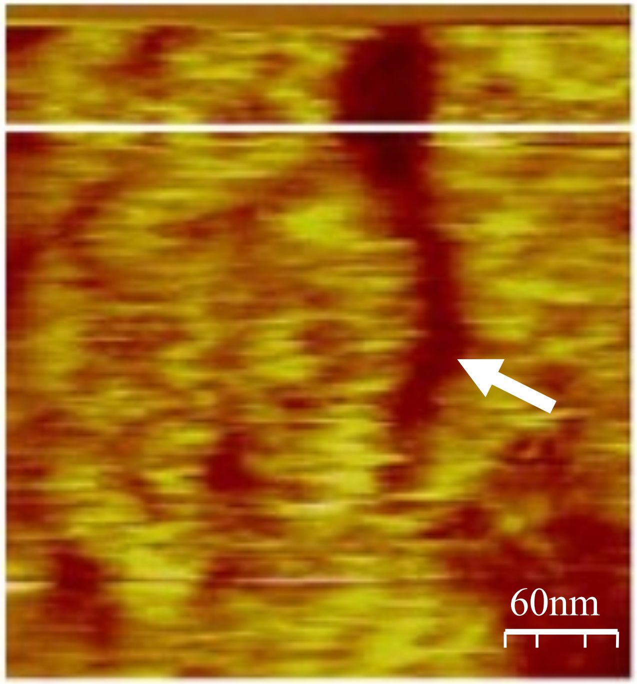
Increased stability by introducing precursors monolayers with Ethylene-Glycol function

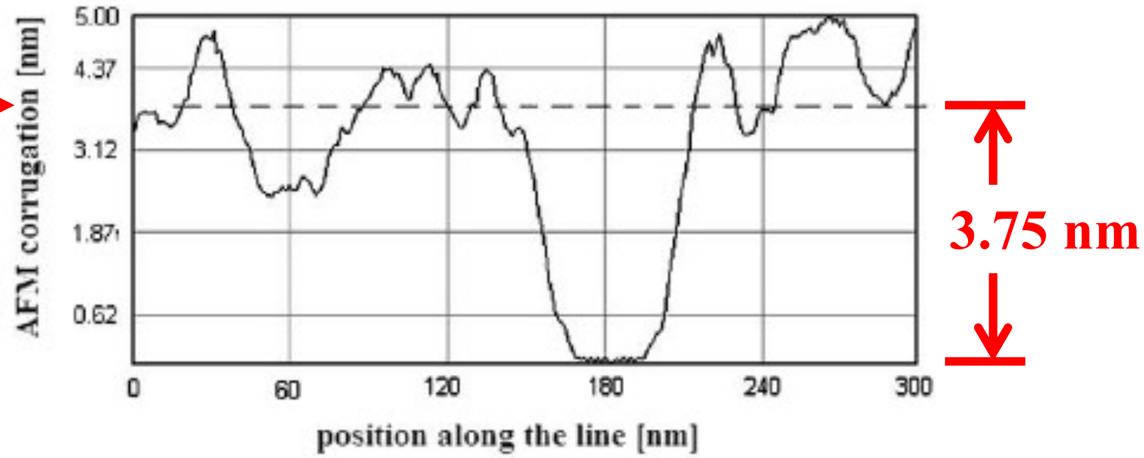
Film precursors below probes



Highly packed thiols monolayer may be used to improve the DNA detection capability

Nano-scale Apertures

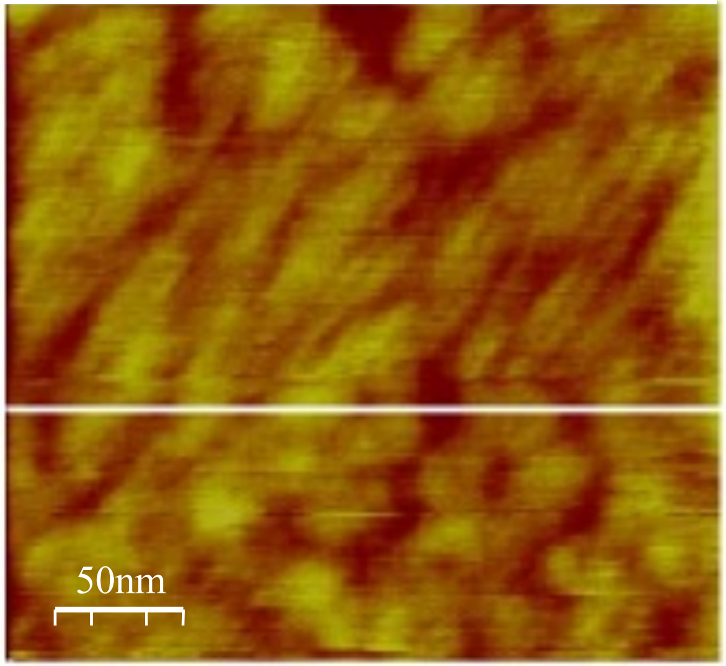




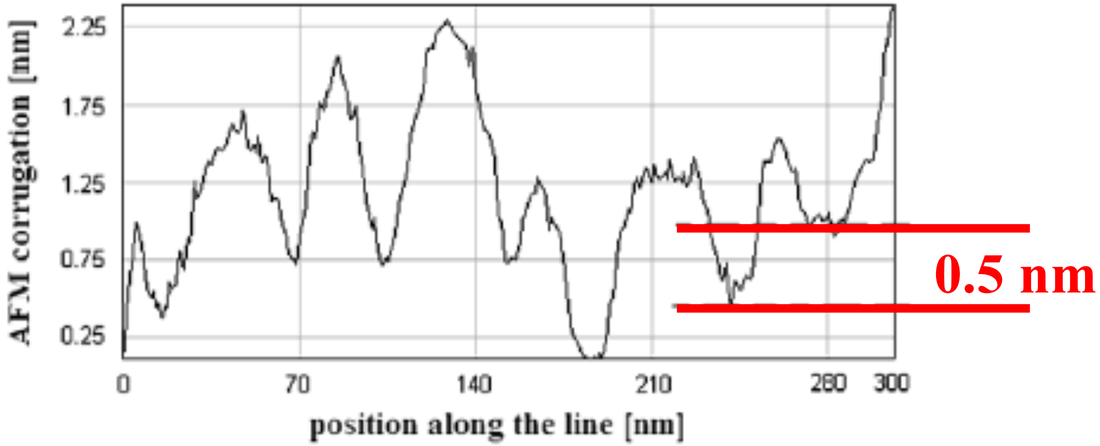
Mercapto-Undecanoic Acid

Monolayers of alkanethiols without ethylene-glycol chains still do present deep groves crossing the film

Absence of Nano-scale Apertures



Ethylene-Glycol Film



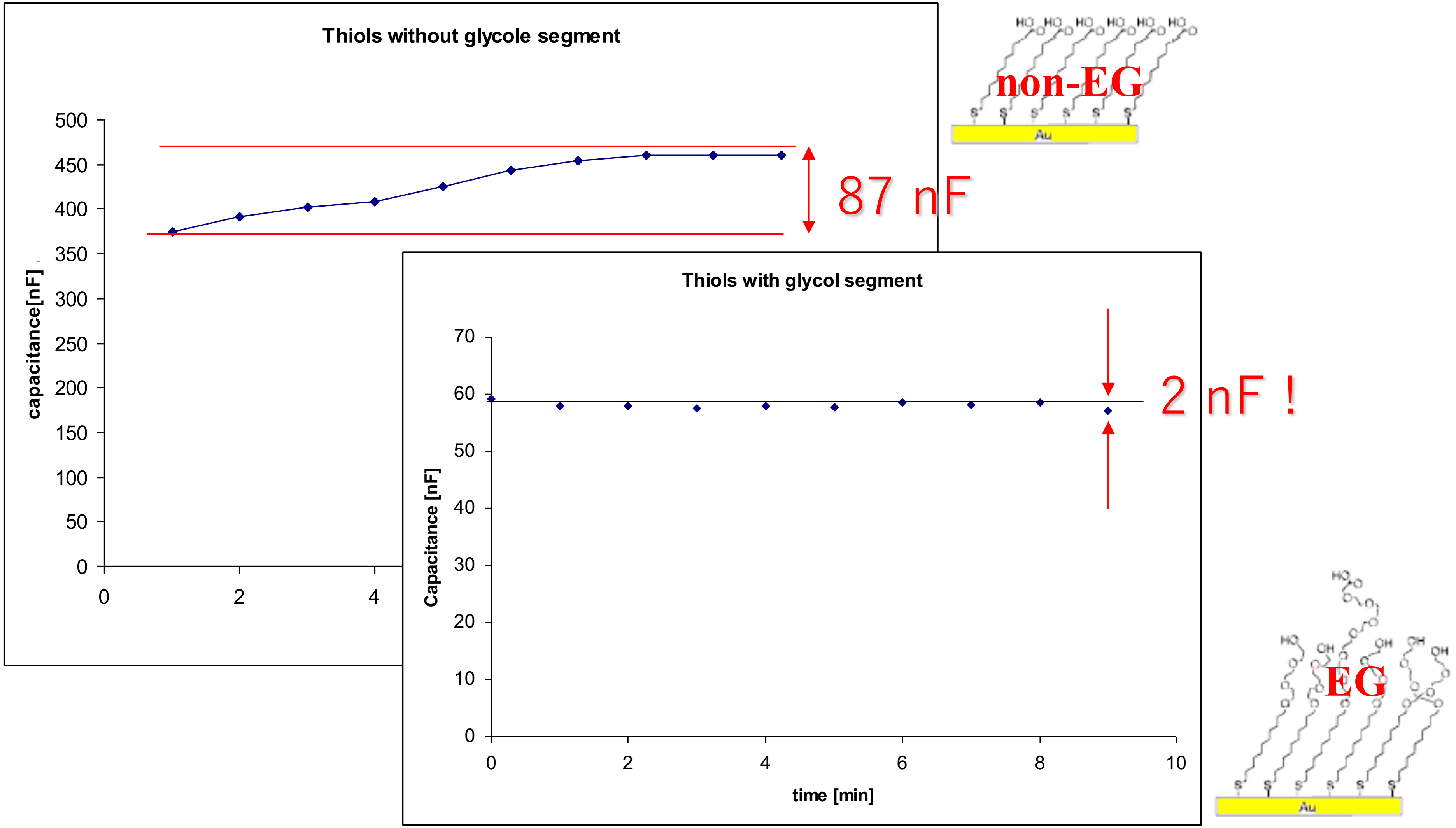
Monolayers of alkanethiols with ethylene-glycol chains do not present deep groves crossing the film

Q13

Why AFM imaging on ethylene-glycol appears more blurred than on undecanoic acid?

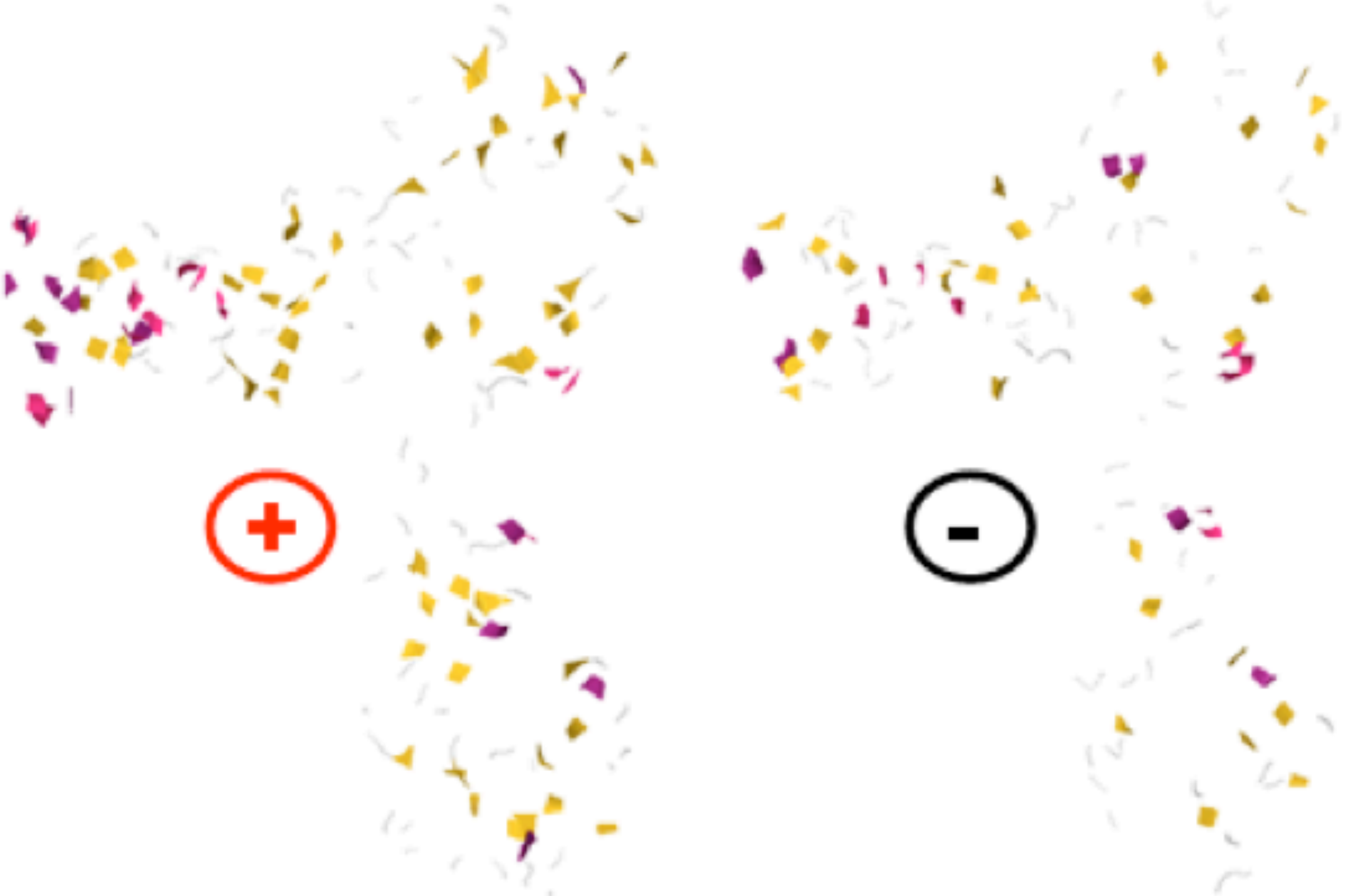
A. Since the size of images are different
B. Since different AFM tips have been used
C. Since the second is more in-focus than the first
D. Since the different interactions of the two organics

Improved Capacitance Stability



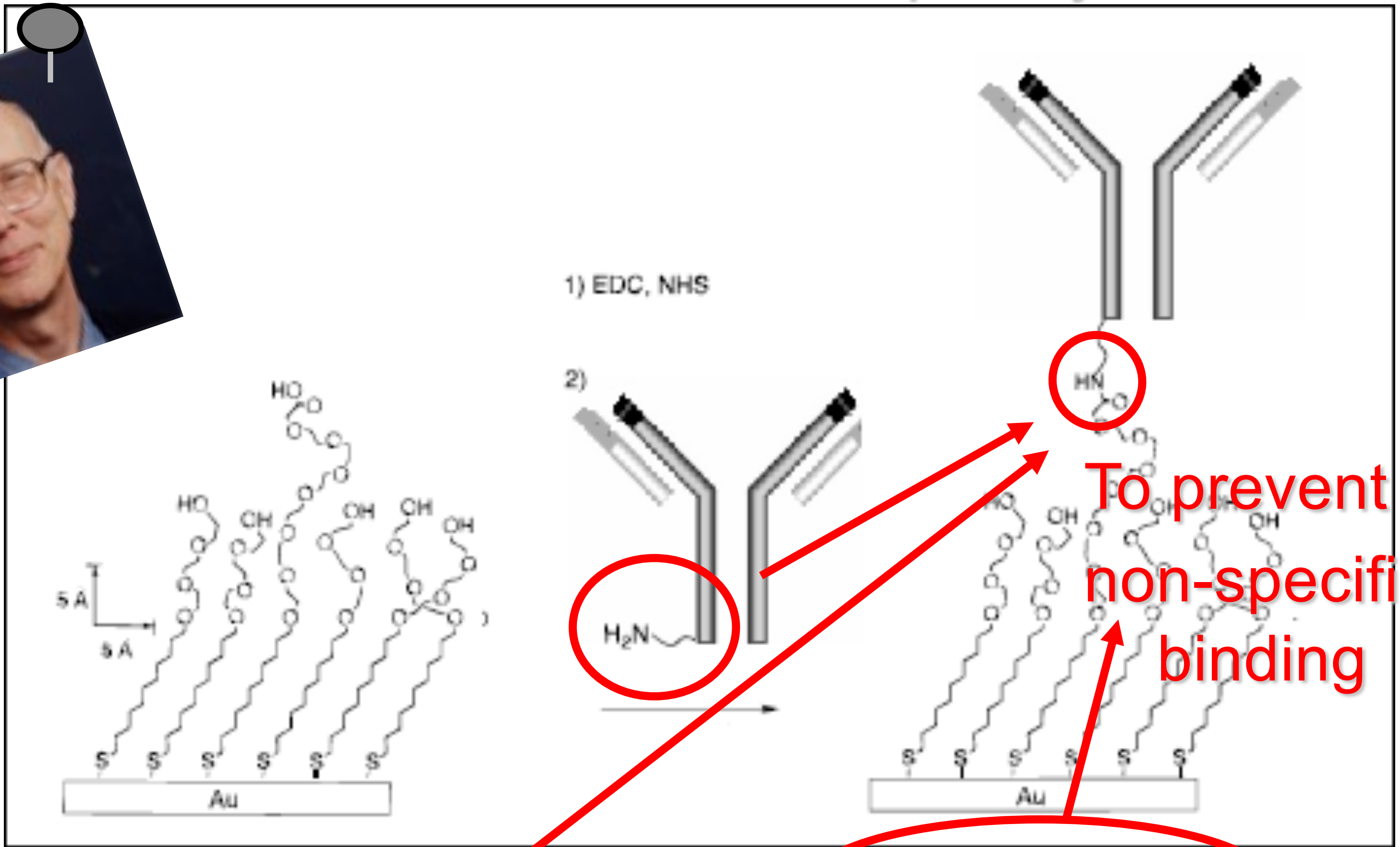
Increased stability by using Ethylene-Glycol thiols

Charges on an Antibody



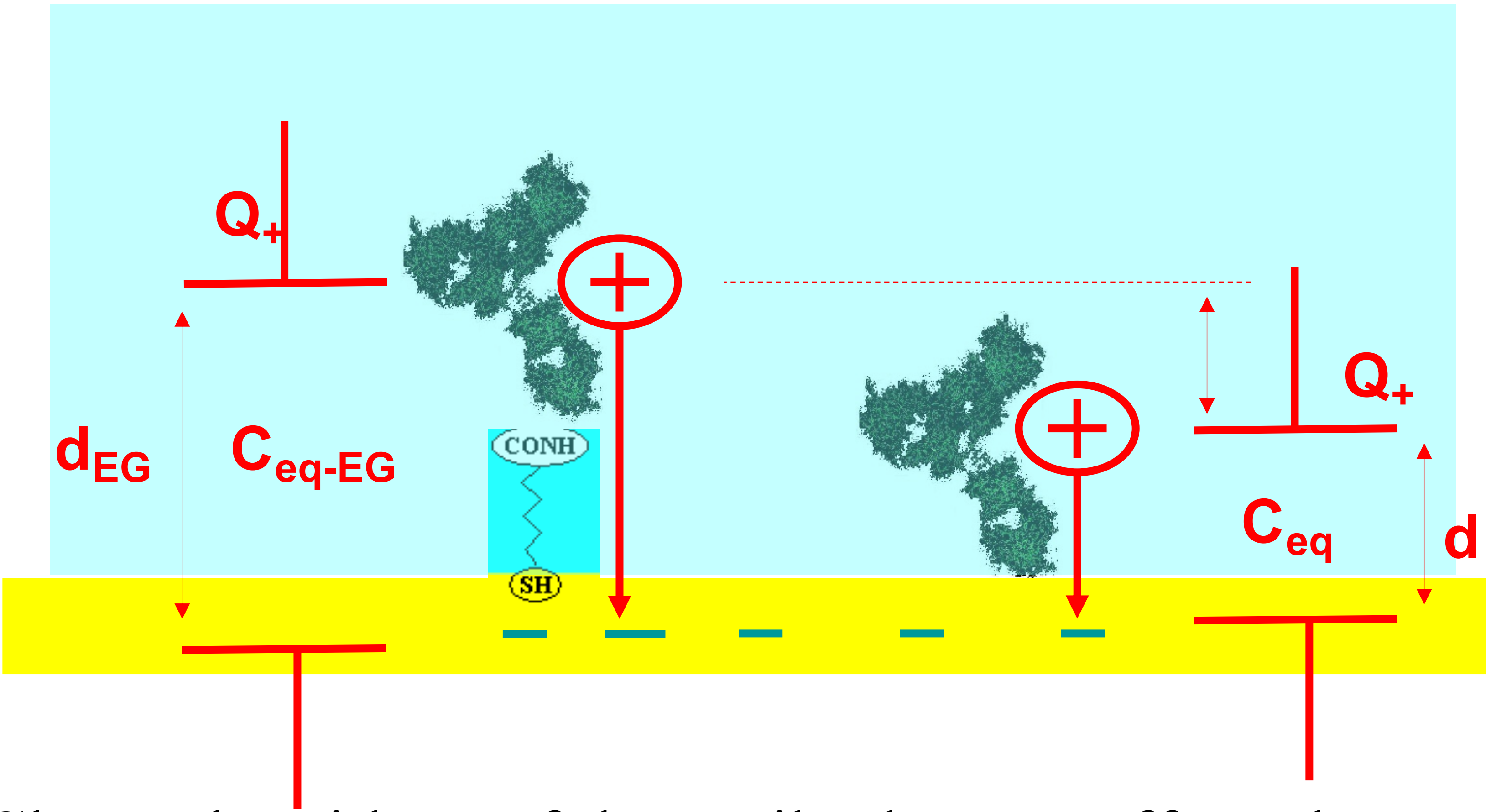
Ethylene-Glycol Blockers

Different kind of thiols developed by G.M.Whitesides



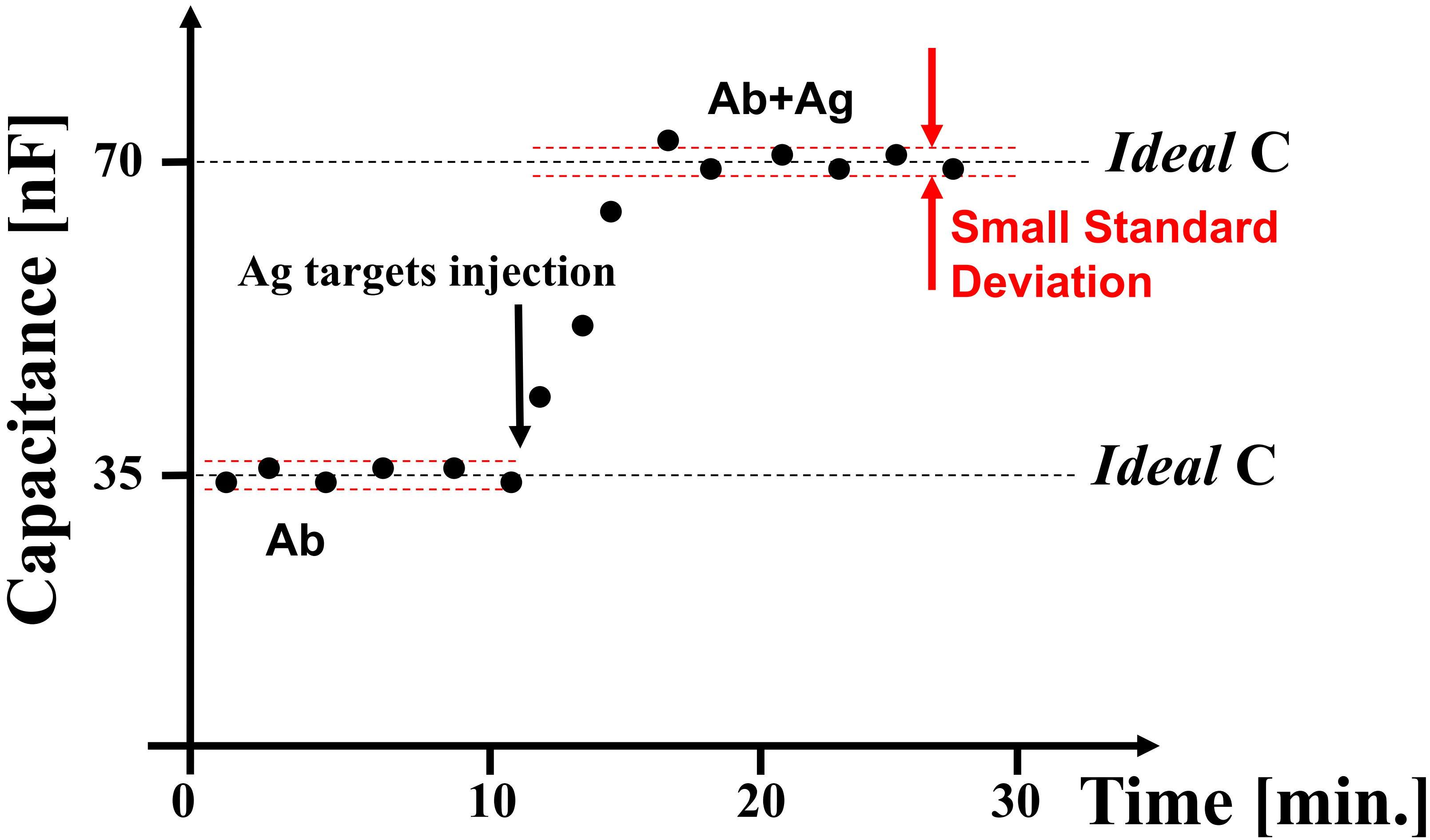
Covalently linked by using Ethylene-Glycol carboxyl terminated alkyl Thiols

Capacitive detection

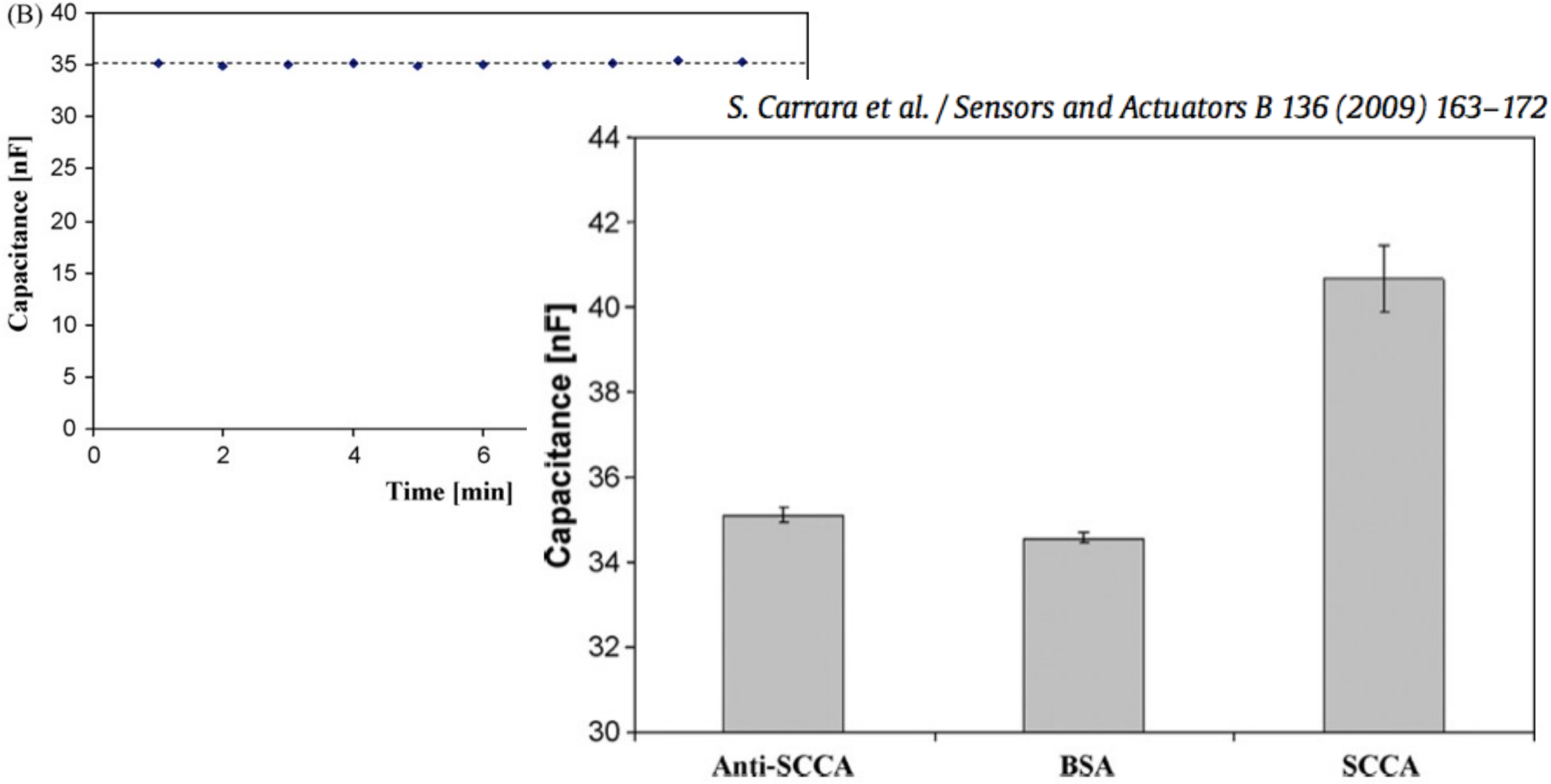


Charged residues of the antibody may affect charge carriers in the electrode

Reduced scattered-data



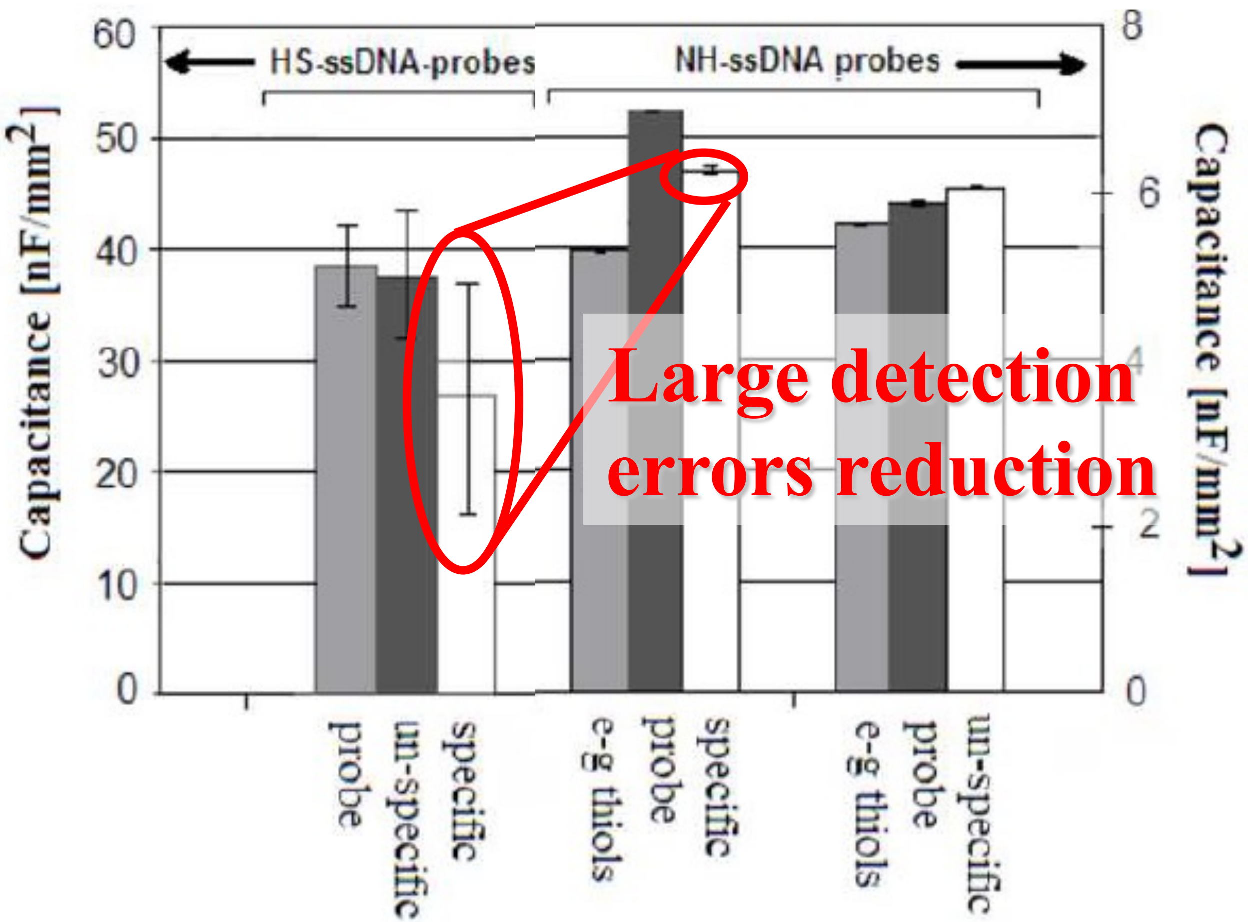
Cancer Marker Capacitance Detection



Highly reproducible Capacitance detection of the cancer-marker protein SCCA by immobilizing the antibodies onto EG-Thiols precursors

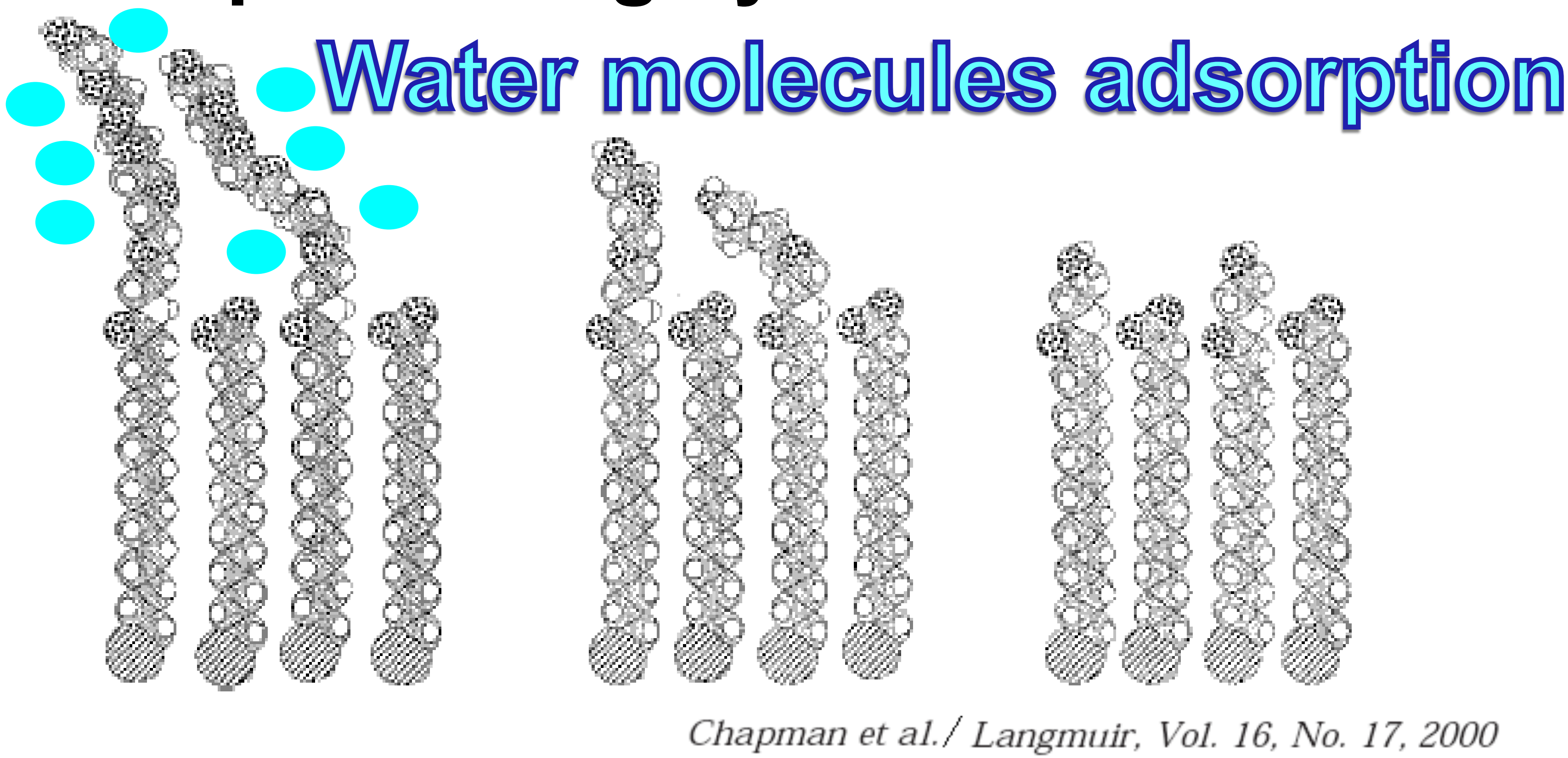
Capacitance detection of DNA

S. Carrara et al. / Biosensors and Bioelectronics 24 (2009) 3425–3429



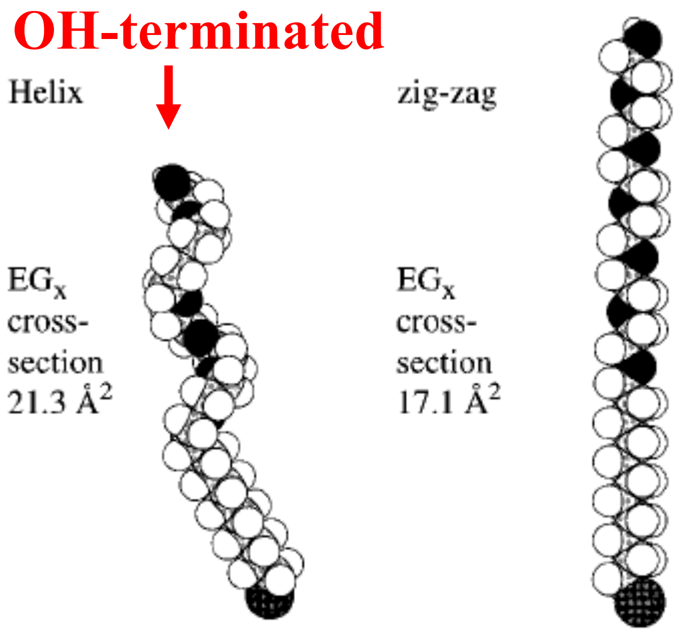
Highly –reproducible DNA detection based on ss-DNA-SH terminated directly immobilized onto gold and ss-DNA-NH2 terminated immobilized onto EG-Thiols

Why it works so well to close nano-apertures? Space filling by EG thiols



Space filling molecular model illustration of EG-thiols film presenting a 1:1 mixture of -CON(CH2CH2O)*n*H (*n*) 1, 3, or 6) and O2H/CO2- groups.

Water Adsorption in thin films

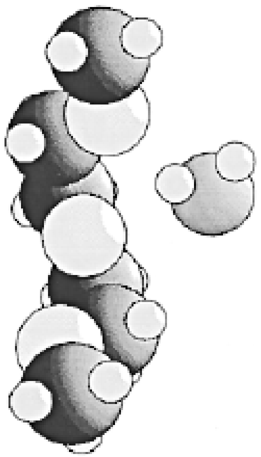


Harder et al. / *J. Phys. Chem. B, Vol. 102, No. 2, 1998*

Infrared spectroscopy demonstrates that ethylene-glycol chains in the methoxy-terminated monolayer are mainly in great disorder, while the hydroxyl-terminated monolayer chains are in a crystalline helical phase (Harder et al 1998)

Molecular conformations driven by terminal groups in EG-thiols

Water Adsorption in thin films



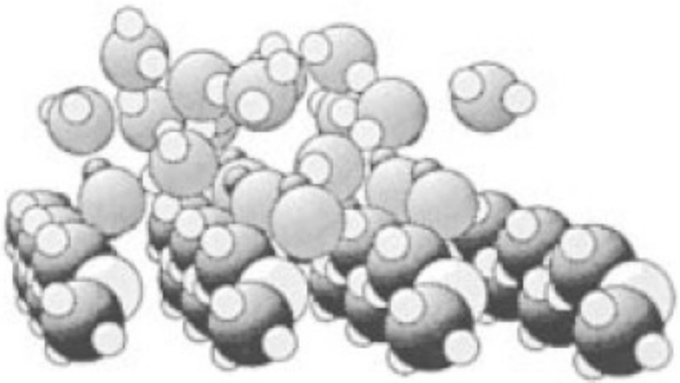
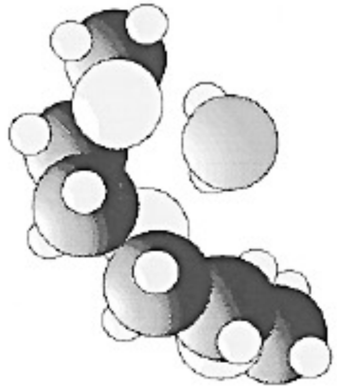


Figure 7. 20 water molecules form three adsorption layers on a hexagonal cluster of a helical OEG film with lattice constant 5 Å consisting of 3 × 4 helical OEG strands.

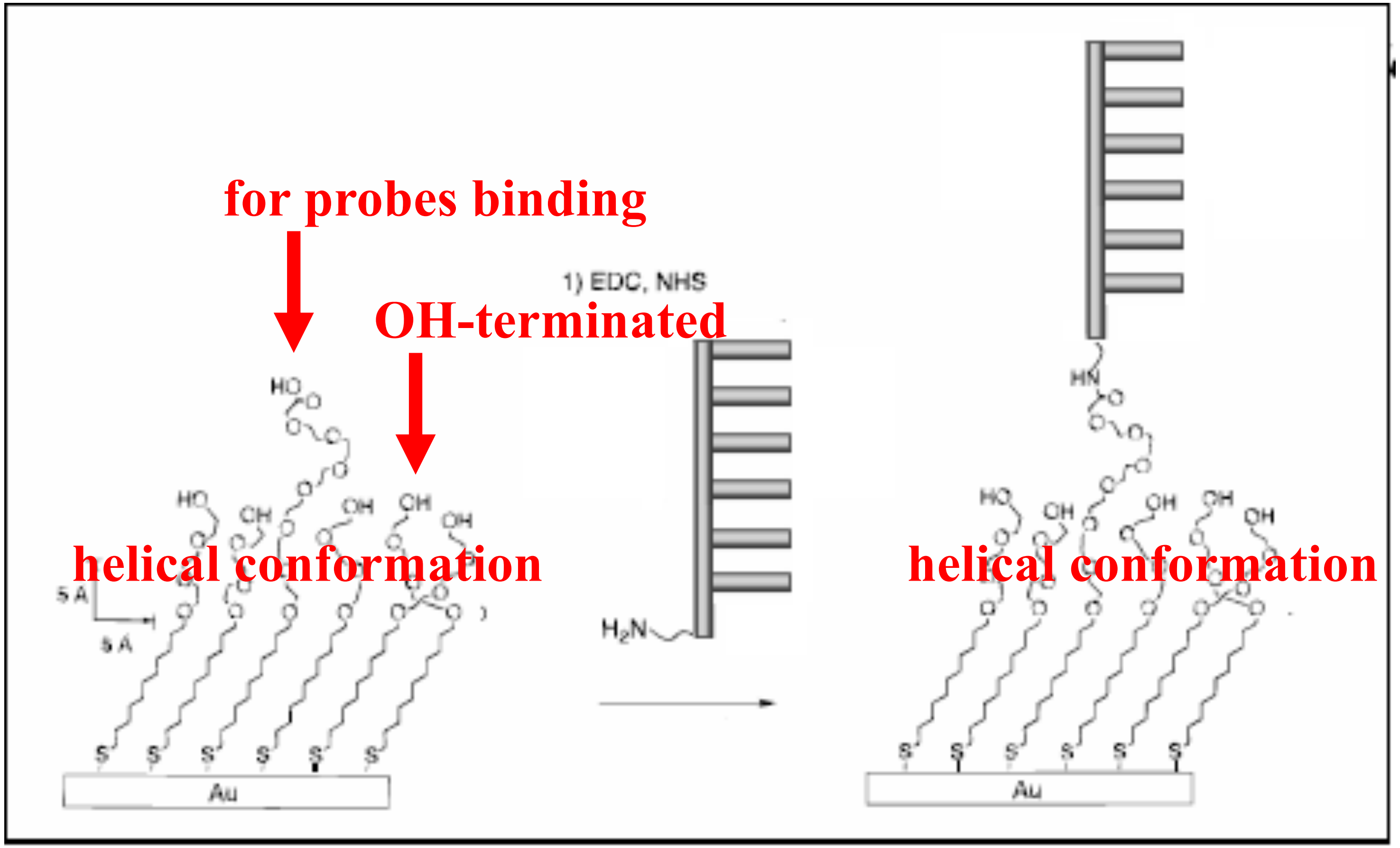


Wang et al. / *J. Phys. Chem. B, Vol. 101, No. 47, 1997*

We conclude the following: OEG in its helical conformation, but not in its "all-trans" form, is amphiphilic with respect to water. The stability of the water interface with helical OEG prevents proteins and other molecules from adsorbing irreversibly on the OEG surface.

Water adsorption and Molecular conformation
in EG-thiols Film

Water Adsorption in thin films



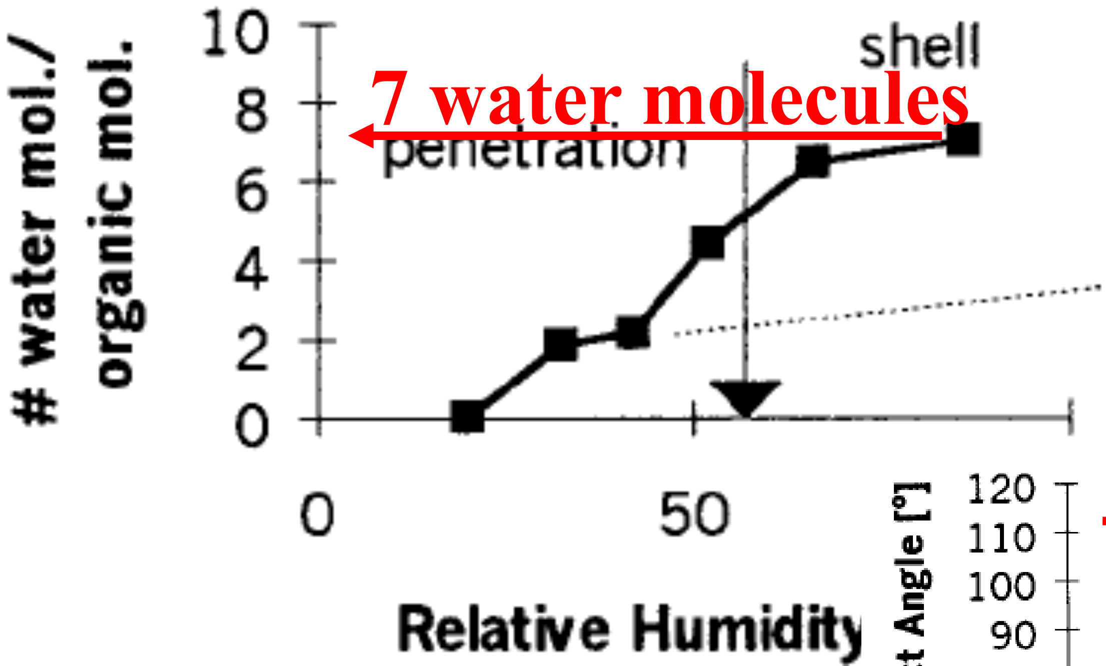
Covalently linked by using three-glycol carboxyl terminated alkyl Thiols

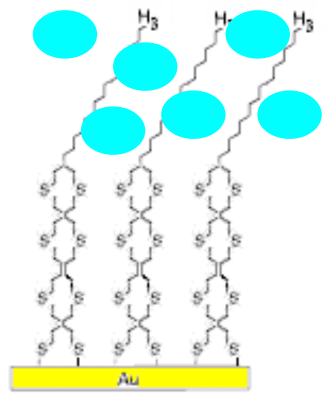
Q14

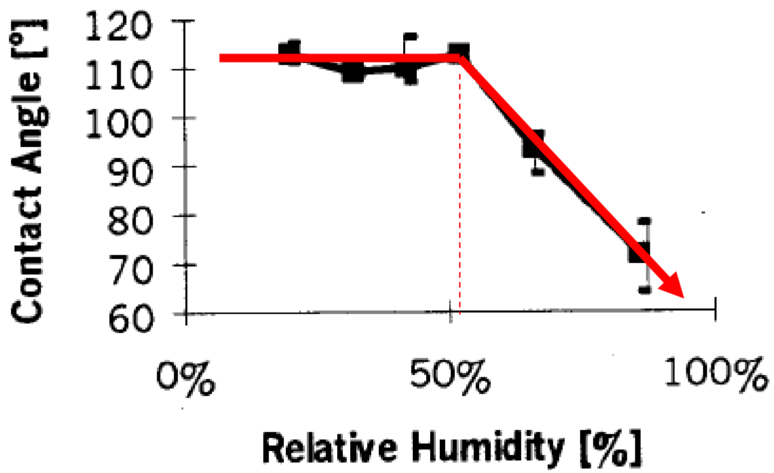
Are there any water molecule inside the hydrophobic alkyl chain?

A. Yes, of course!
B. Yes, even thought the hydrophobic character is right there
C. No, since the hydrophobic character is right there
D. Not a all!

Water Adsorption in thin films



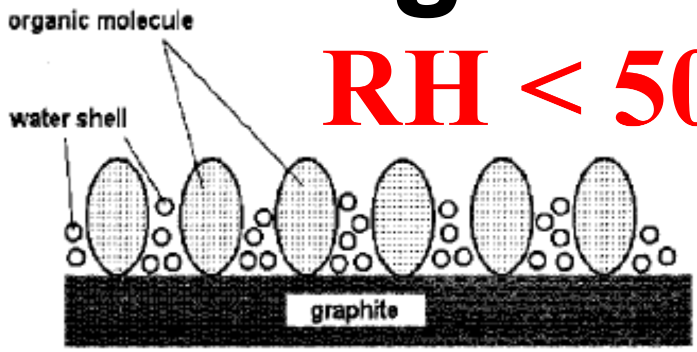




Film Water adsorption on alkyl thiol film chains

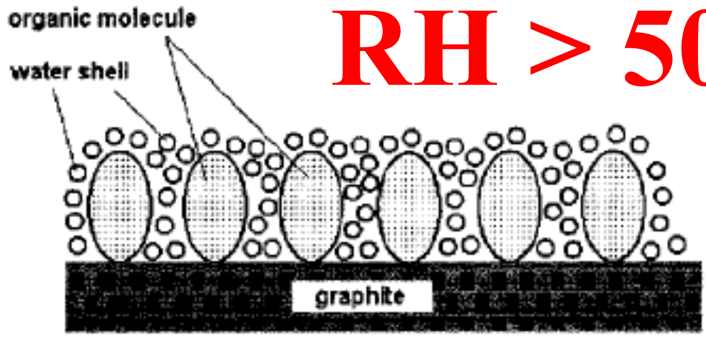
Water in Organic Films

RH < 50%



(A)

RH > 50%

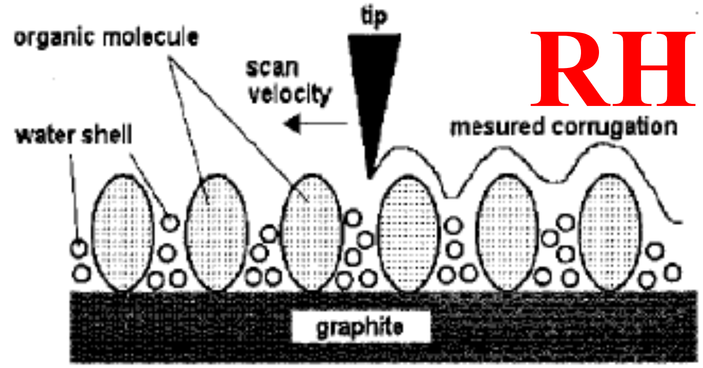


(B)

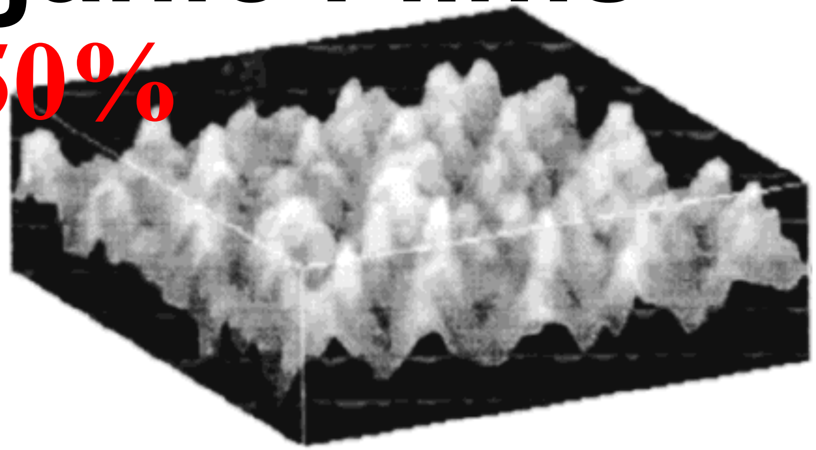
Water molecule are stored in the intra-molecular space in the thin film

Water in Organic Films

RH < 50%

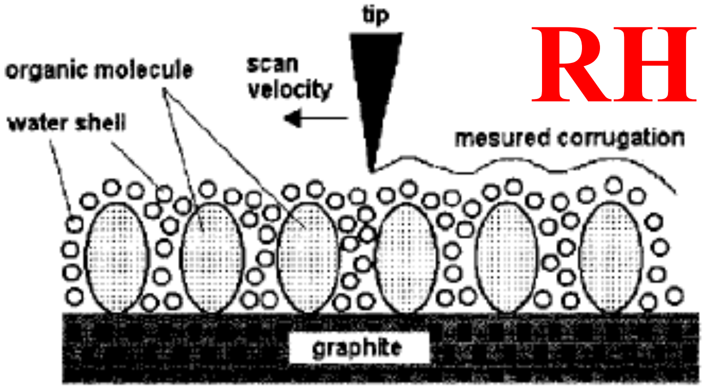


(A)

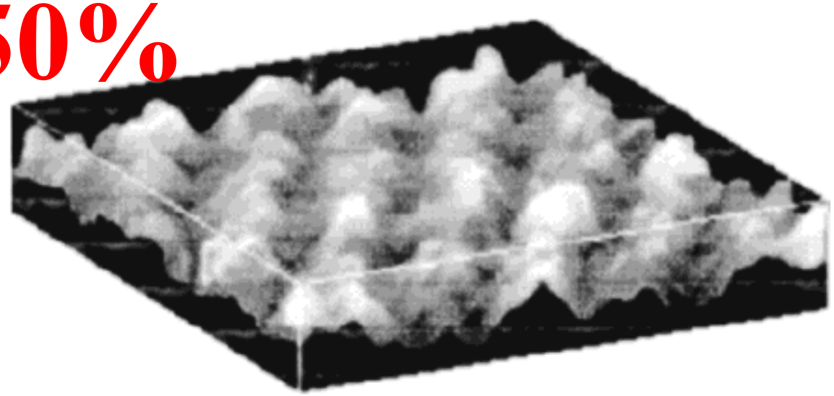


(A)

RH > 50%



(B)



(B)

STM imaging showed water molecule stored in the intra-molecular space in the thin film

Water Adsorption in thin films

Mass change	# of water molecules/EG-molecules
0,14 ng/mm^2	16

The estimated mass changing correspond to 16 water molecules each ethylene-glycol alkanethiol. Up to 7 water molecules for each organic molecules may be fund in dried alkyl chains film (Carrara et al. 2000). The other water molecules are strongly coordinated by ethylene-glycol chains in our film

S. Carrara et al. / Biosensors and Bioelectronics xxx (2008) xxx–xxx

QCM measurements on dried EG-thiols film conditioned with water buffer