Surface engineering for Biological interfaces

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EUROPEAN COM
DIRECTORATE-GENERAL

Outline

- Introduction
  - Protein surface interactions
- Production of surfaces with controlled properties
  - Plasma polymers, SAMs
- Micro and Nano patterning
  - e-beam lithography
  - Colloidal lithography
- Proteins on nanopatterns

JRC workprogramme

Support to the European Policy on:

- Exposure monitoring
  - Air, water, food monitoring
  - Indoor exposure measurements
- Chemicals policy
  - Toxicity evaluation of 30000 chemicals compounds
  - Reduction of animal testing
  - Validation of alternative methods
- Nanotoxicology
  - In vitro tests
  - Nanoparticles – cell interactions

(Bio)senso

In vitro test

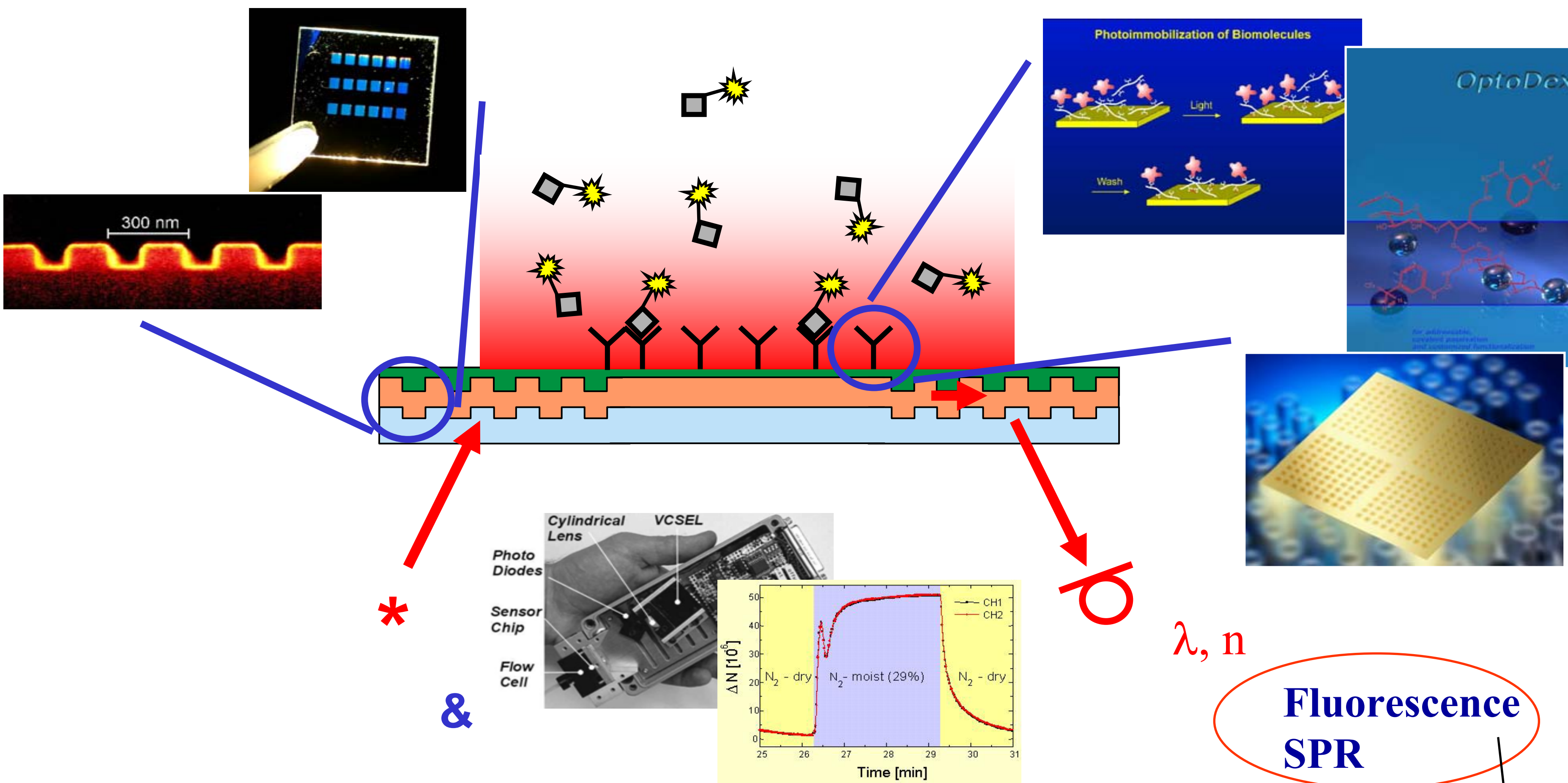
Cell on chi

Protein surface interac

➢ Need for development and characterisation of bio interfac

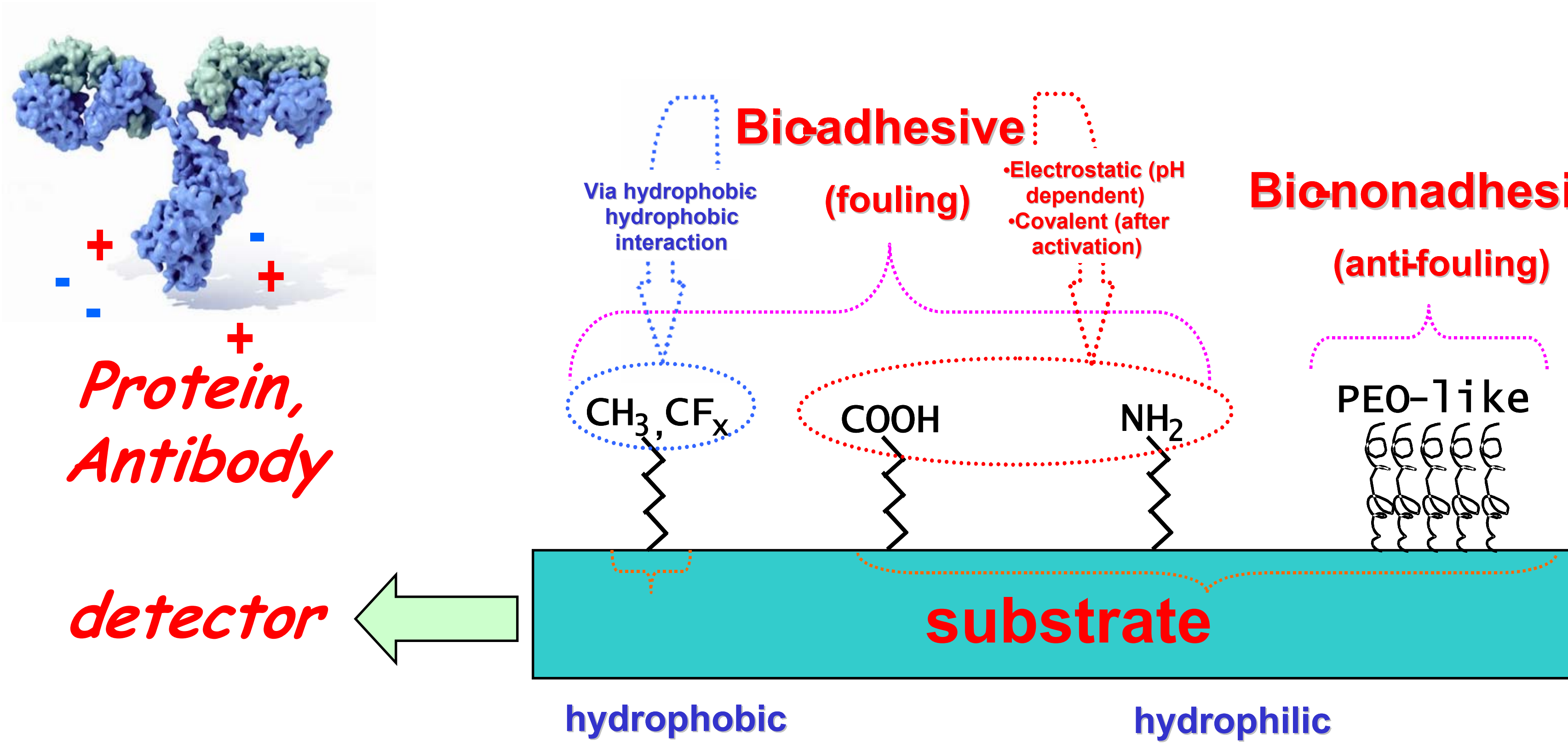
Bio interfaces studies

- **Micro / nanostructures**
- **Chemistry**



- **Detection / signal processing**

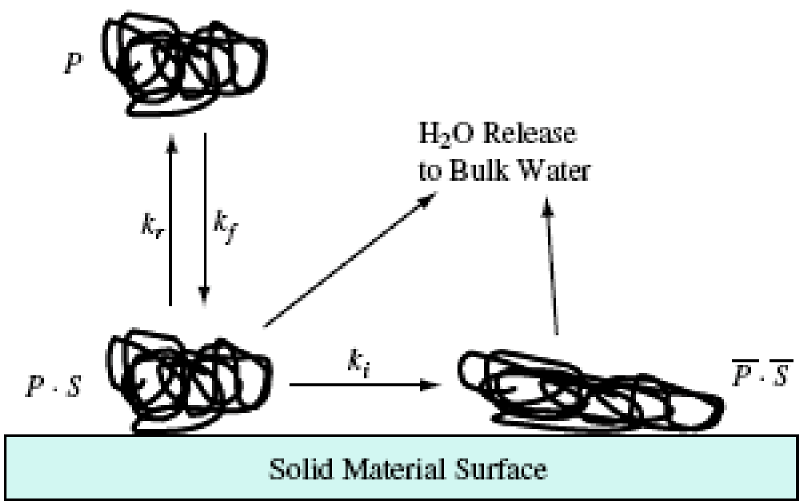
Protein surface interactions

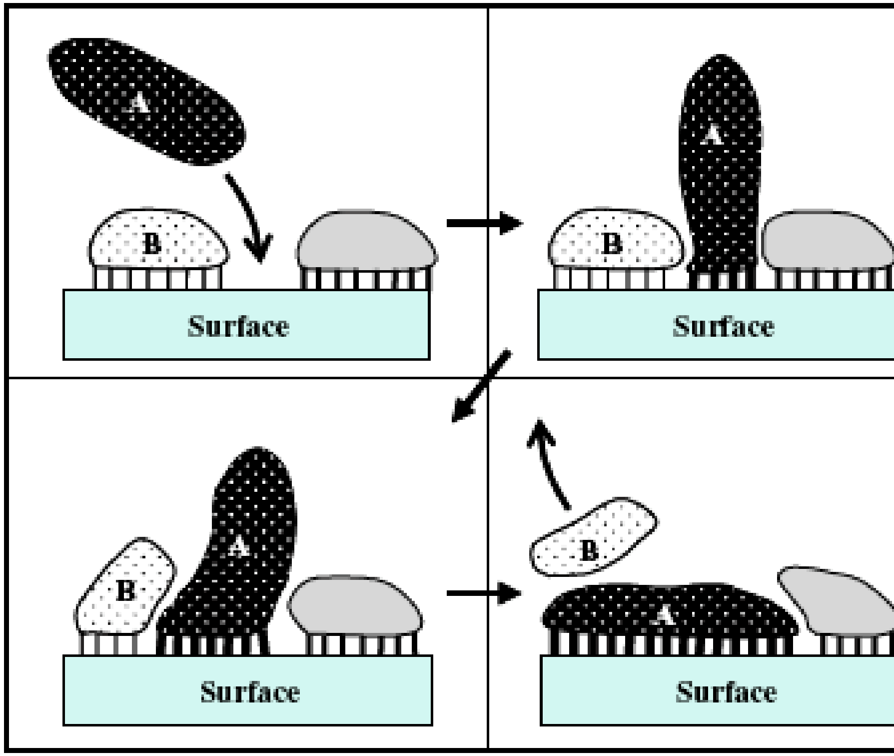


OBJECTIVE

Optimize the Recognition Element (biological) coupling with the Detector substrate (inorganic)

Protein surface interactions



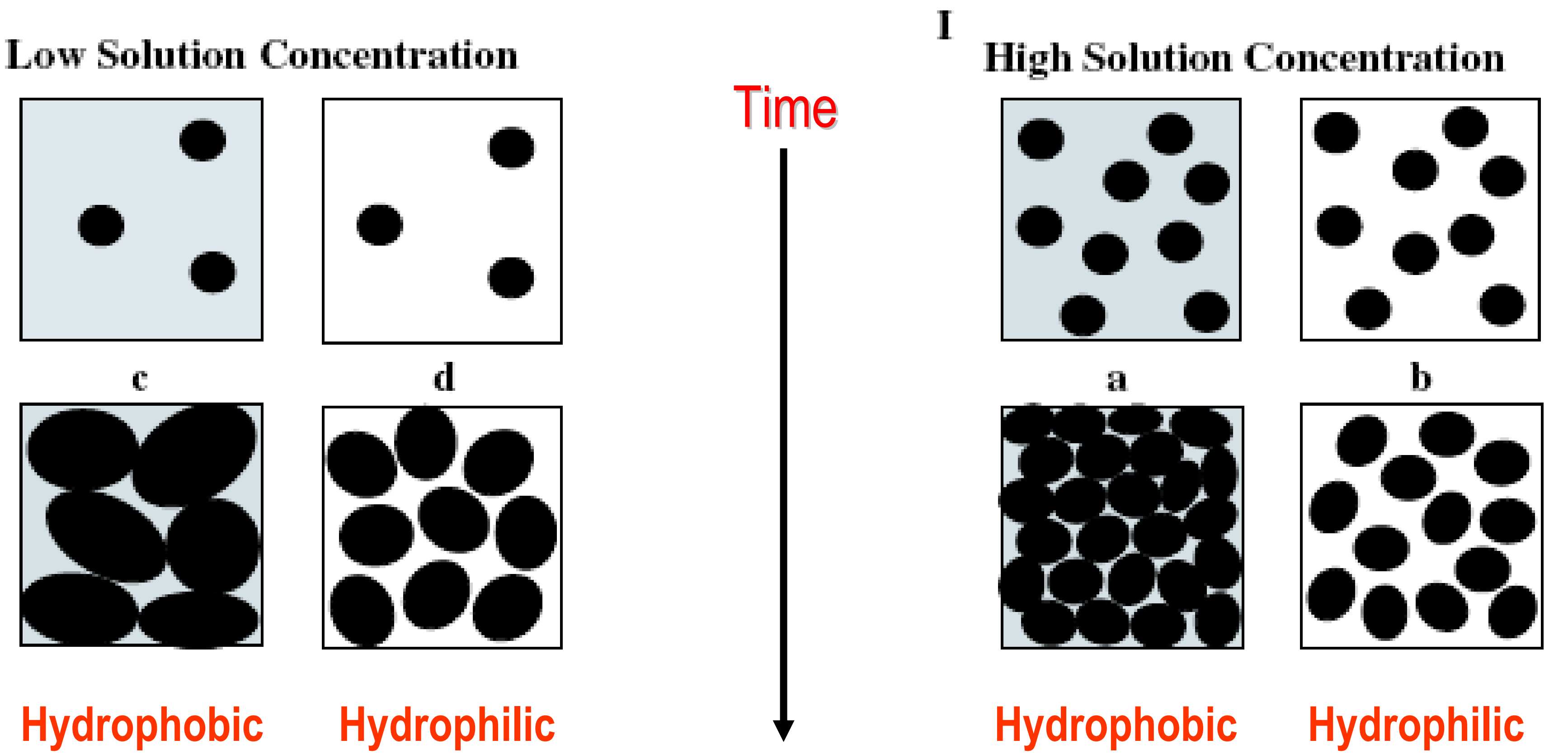


Conformational changes

Vroman effect

Latour, Encyclopedia of Biomaterials and Biomedical Engineering, 2005

Effects on adsorption kinetics



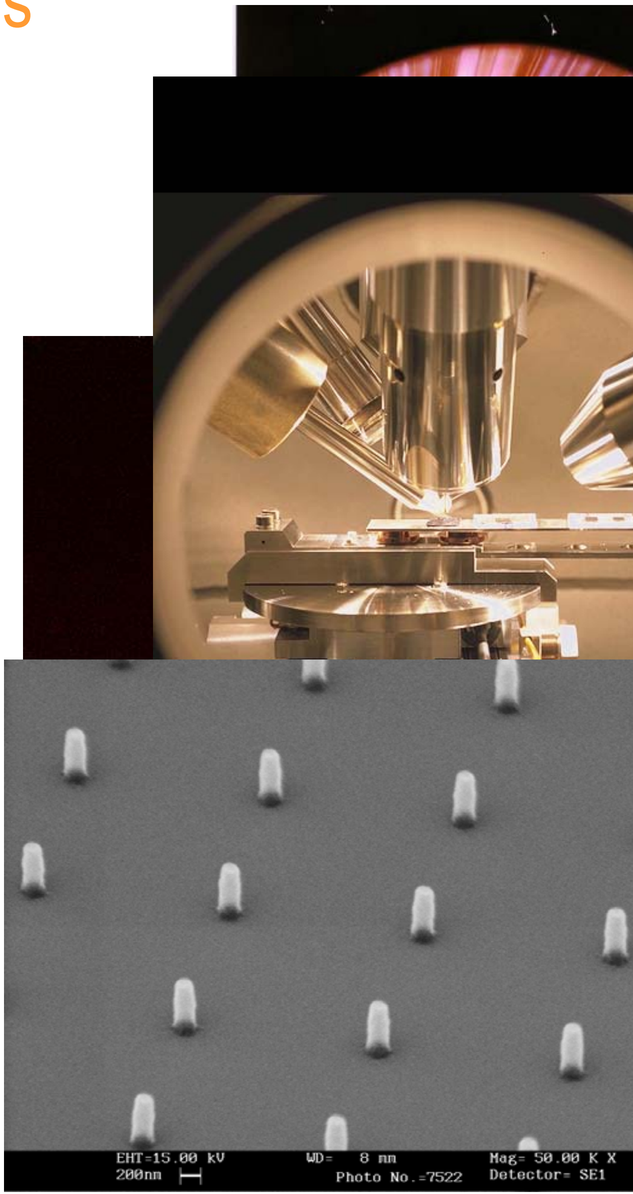
Surface concentration and activity of adsorbed proteins depend on:

- Initial concentration of the solution
- Phys. chem. properties of the surface

Effect of confinement (nanostructures)?

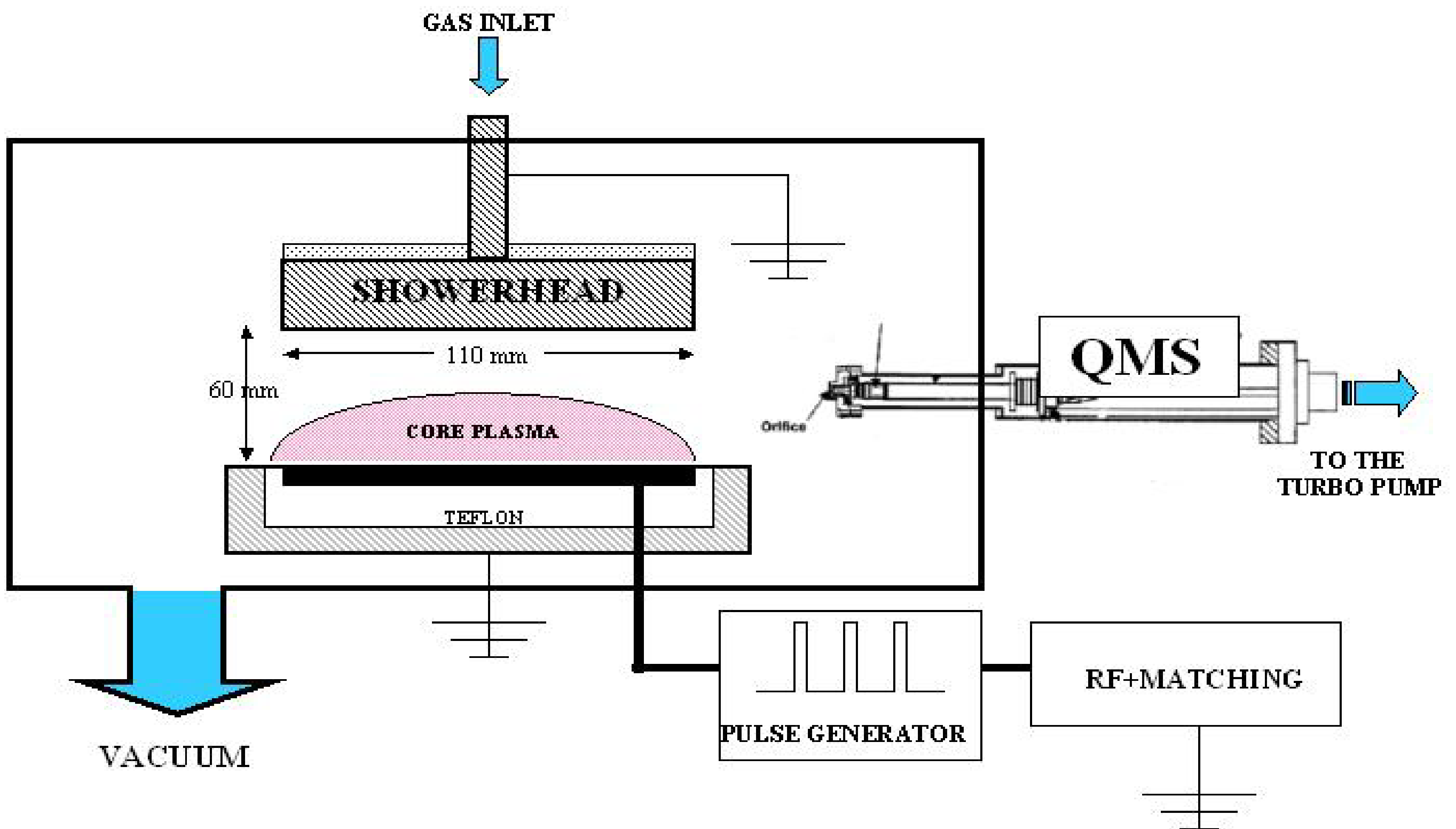
Functional surfaces production

- Production of films with controlled properties
  - Plasma deposition
  - Ion /electron beam modification
  - Self Assembled Monolayers (silanes, thiols)
  - Sol gel, spin coating…
- Characterisation/ Functional properties
  - Composition
  - Physico chemical properties (surface energy, hydrophobicity, surface charge)
  - Protein affinity
  - Cell adhesion
- Micro and nanopatterning
  - E-Beam and colloidal lithography
  - Micro contact printing, NIL (A. Ruiz)
  - Biosensors/ Cell culture



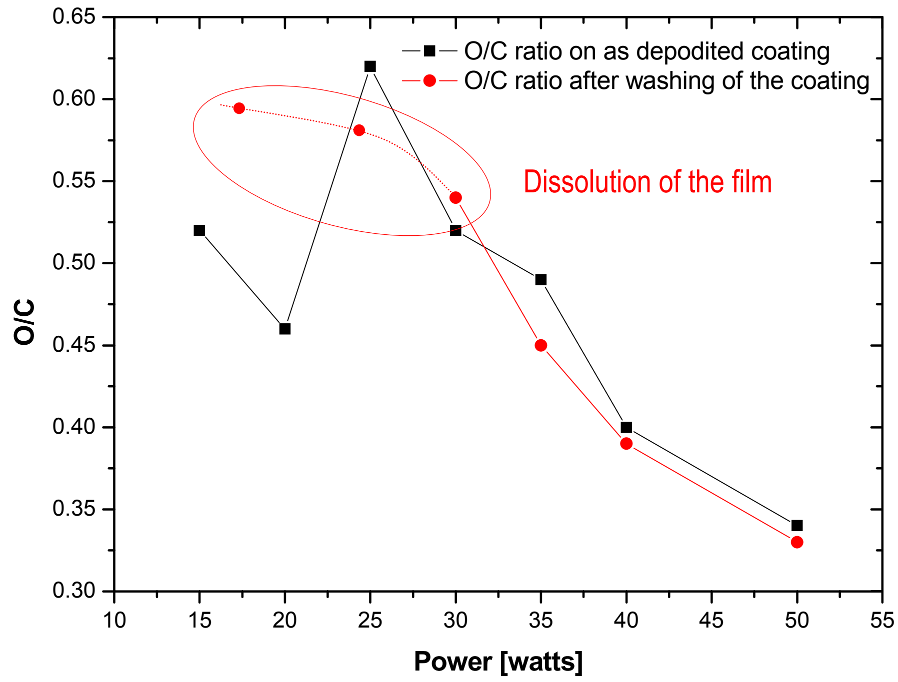
Plasma deposition of polymers

- **Precursors:**
  - **Acrylic Acid, Allylamine, Diethylen Glycol Dimethyl Ether…**
- **Working pressure 50 mTorr, monomer flow 10 sccm**
- **Power supply: RF unit (13.56 MHz) CW or pulsed mode (T_{on}=3ms Duty Cycle = 10**



Effect of power on Monomer Retention

Acrylic acid plasma polymer



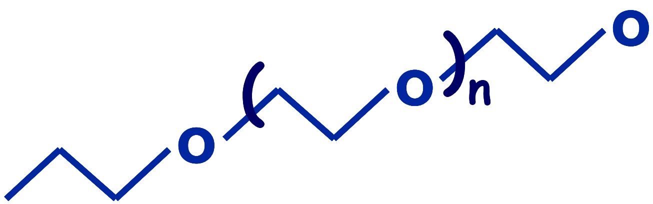
Trade-off between film stability and precursor retention

- Films deposited at low P are soluble in water.
- Stable films are obtained for P> 30W.

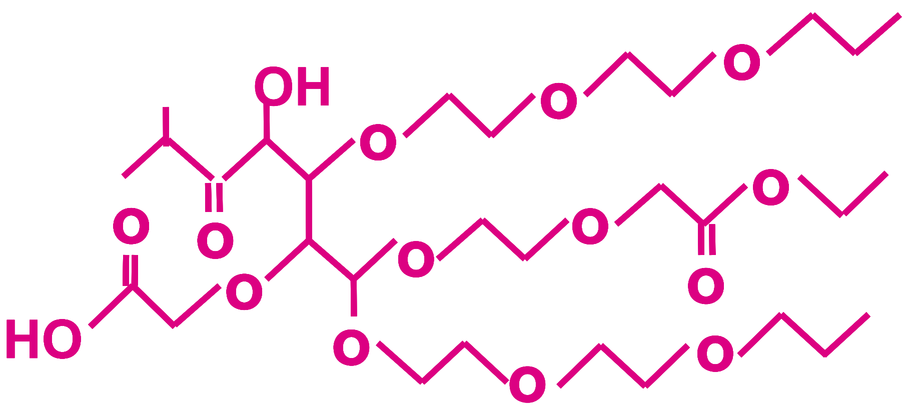
Plasma deposited PEO-like coatings

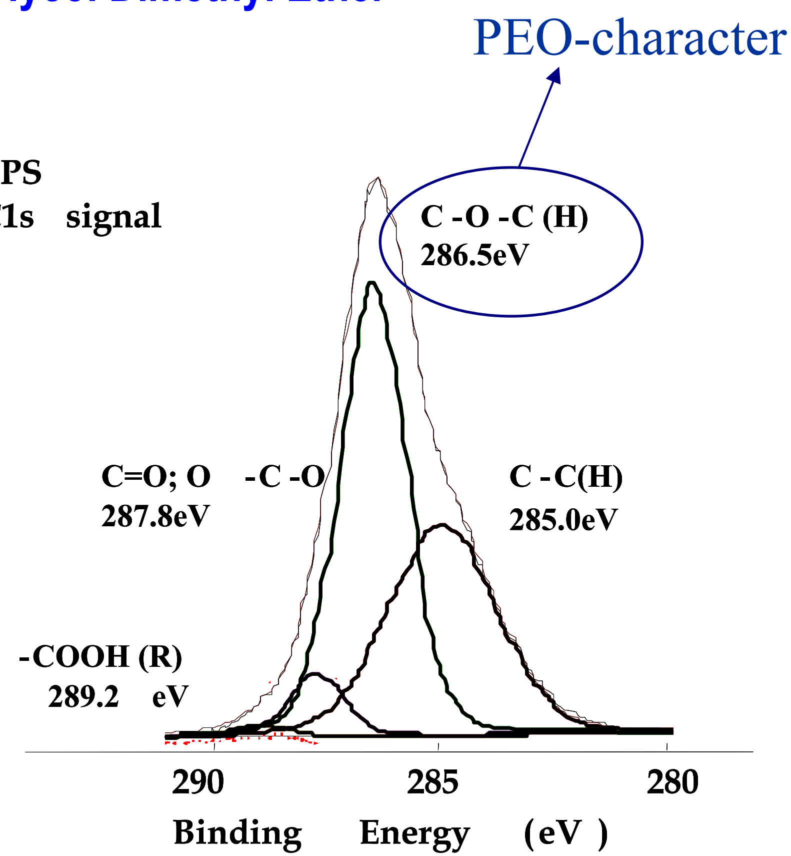
Precursor: Ditheylen Glycol Dimethyl Ether

PEO $-(CH_2CH_2O)_n-$



PEO-like

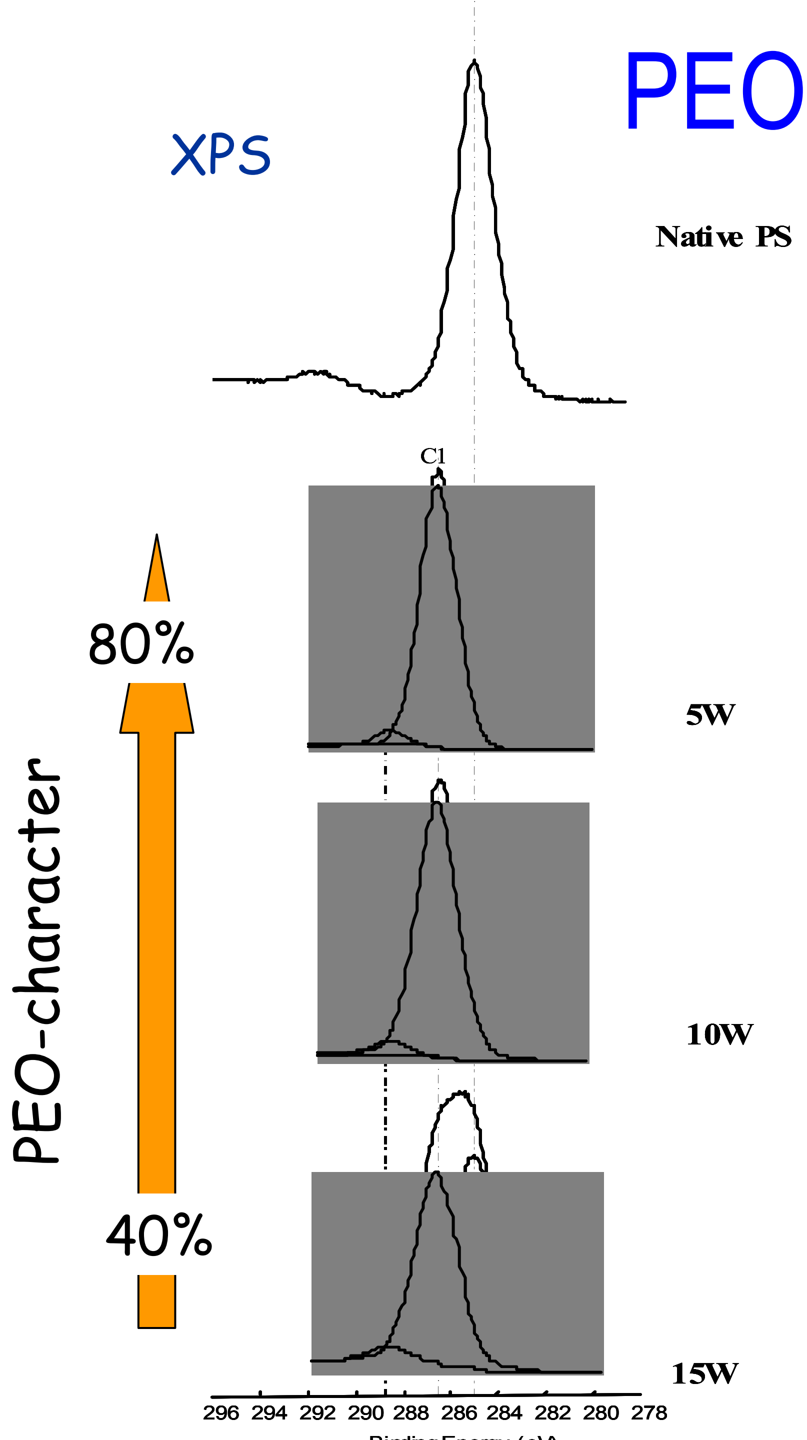




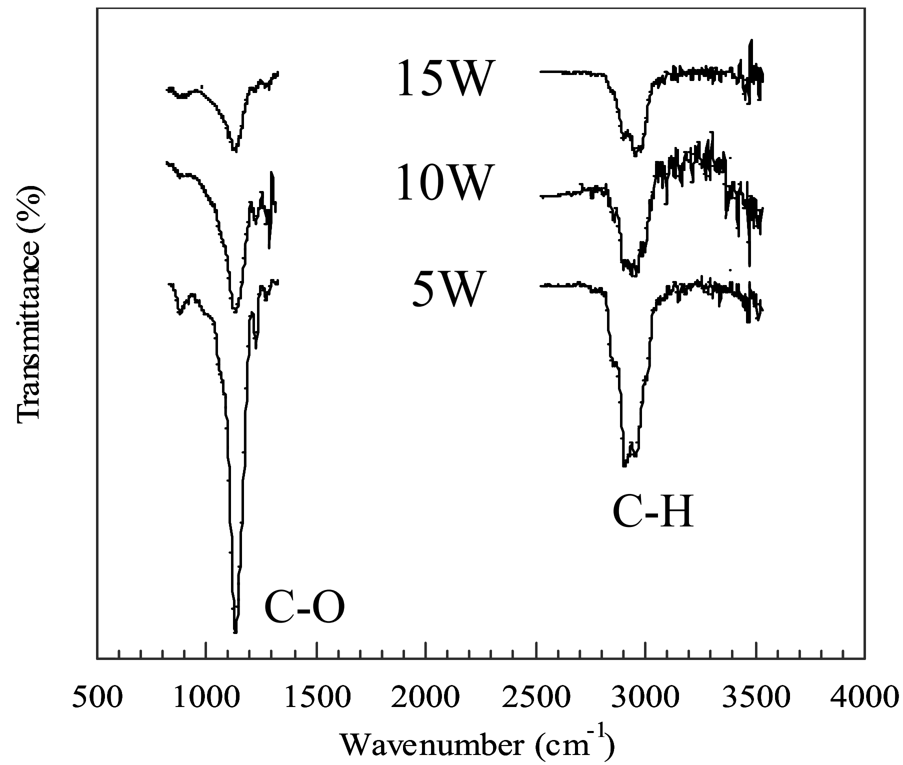
power ↑
%C-O-C↓

PEO character

XPS



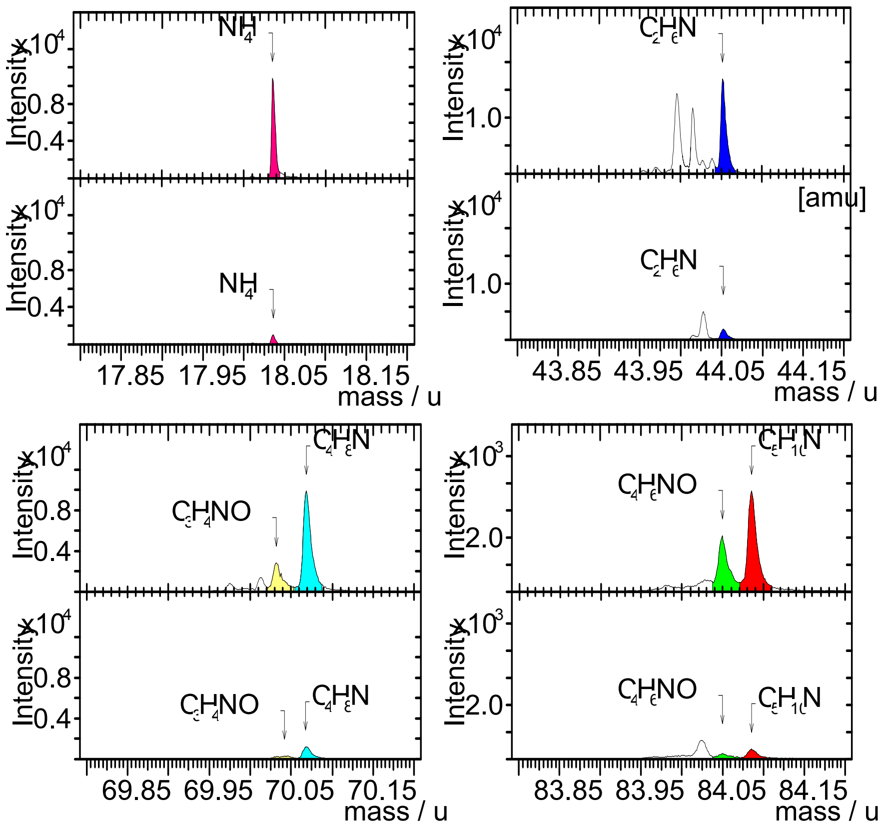
FT-IR spectroscopy

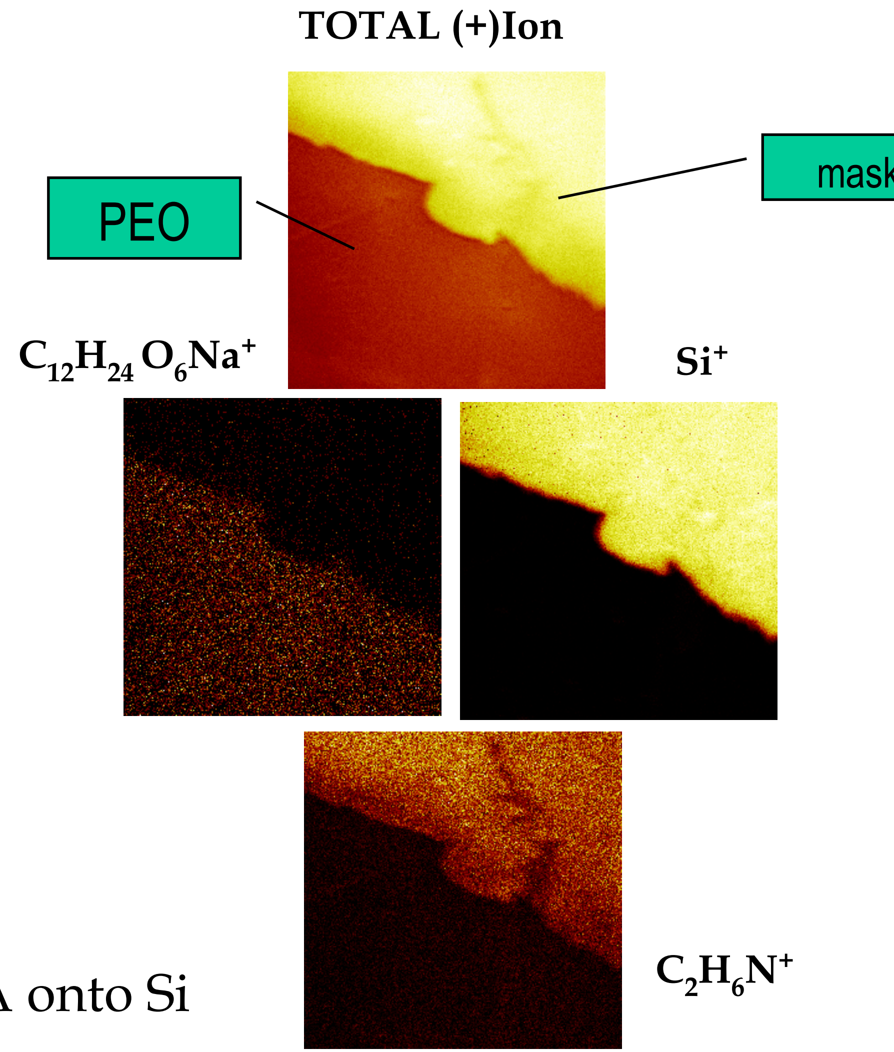


EO-like non fouling properties: BSA adsorptio

IBM-PEG 15 s micropatterned Si

IBM-PEG top- Si Bottom





Preferential adsorption of BSA onto Si

500µm x 500µm

Plasma polymers properties

Film	Dispersive (mJ/m^2)	Acid (mJ/m^2)	Base (mJ/m^2)	Zeta Pot mV@pH7	Prot. Ads. ng/cm^2
Acrylic - COOH	42-44	0.1-0.2	45-55	-55	200-700
Allylamine -NH_2	42-44	9-10	0.1-0.2	0 to 5	200-400
PeO	40-44	10^{-3}-0.1	24-28	-25	0-50
CFx	20-40	0.05-0.9	0.9-7	-20 -50	250-300

Different surfaces with large contrast of physico chemical properties

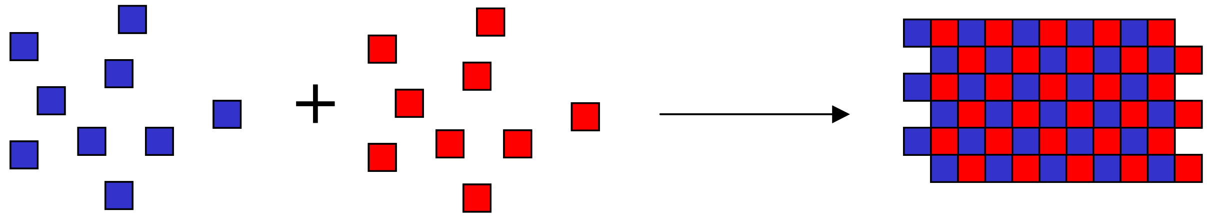
Physical and Chemical Patterning

E-beam lithography
Colloidal lithography

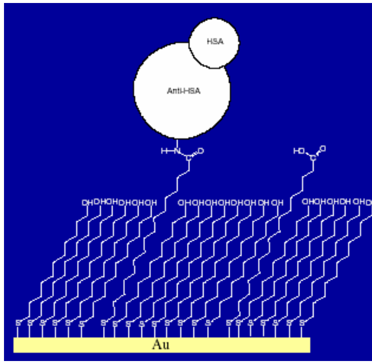
Chemical Nano-patterning

e.g. Mixed SAM (COOH+AF)

BOTTOM – UP: self-assembling process

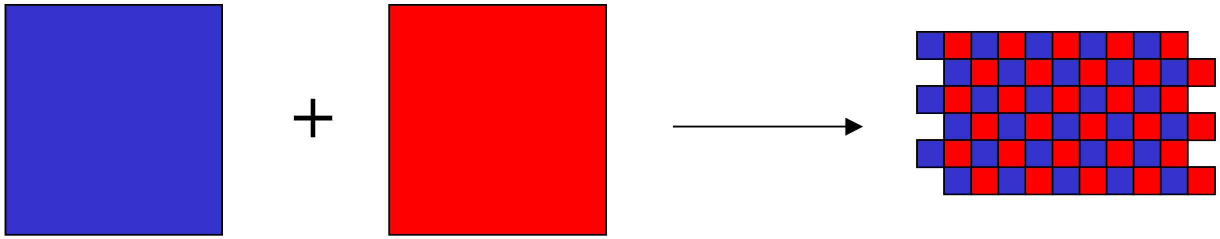


Component A   Component B   Nanostructured material



Ostuni, *Langmuir*, 17(
Frederix, *JBBM*, 58 (2

TOP – DOWN: lithographic techniques



Component A   Component B   Nanostructured material

SERIAL (dip-pen, e
beam, nano-founta

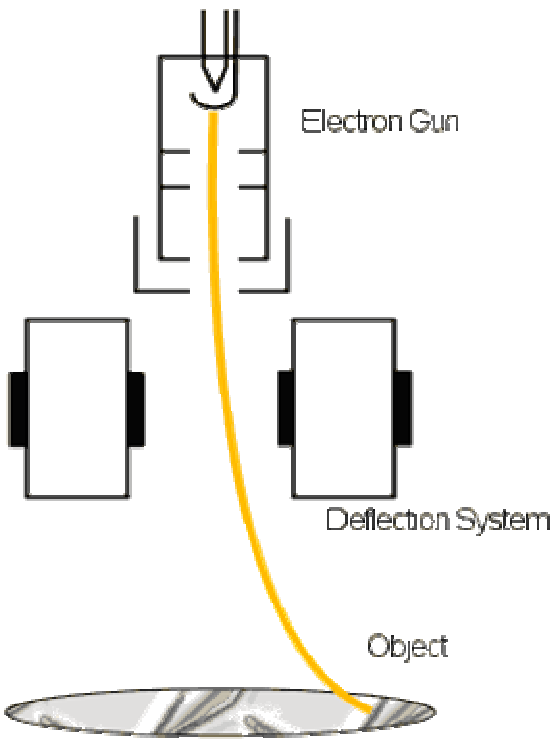
Lee, *Science*, 295 (
Taha, *APL*, (2003)

PARALLEL (nano
imprint, colloidal

Falconnet, *Nano Lett.*, 4
Valsesia, *Nano Lett.*, 4 (

Interest of Electron Beam Lithography

<u>General principle:</u> Similar to classical lithography (used of electron sensitiv resist but with a higher definition since it is no limited by diffractio phenomenon)



- High resolution definition (around 20 nm)
- Possibility to fabricate pattern with a free geometry
- Ideal for miniaturization of arrays

Electron beam lithography: resolution

More sensitive to the electron backscattering
if not baked → **Decrease of lateral resolution**

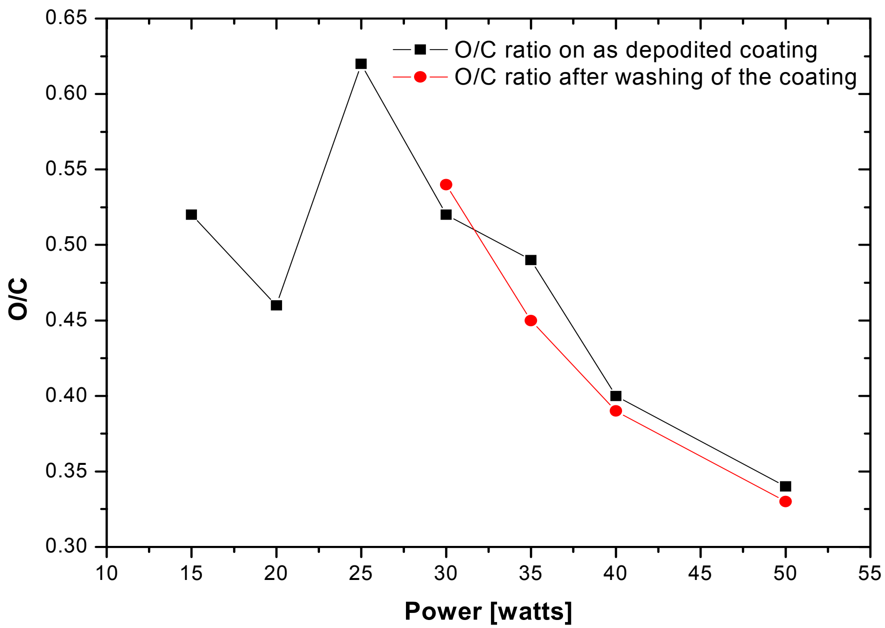
Figure 1: Simulation of electron scatter for 2 keV and 10 keV

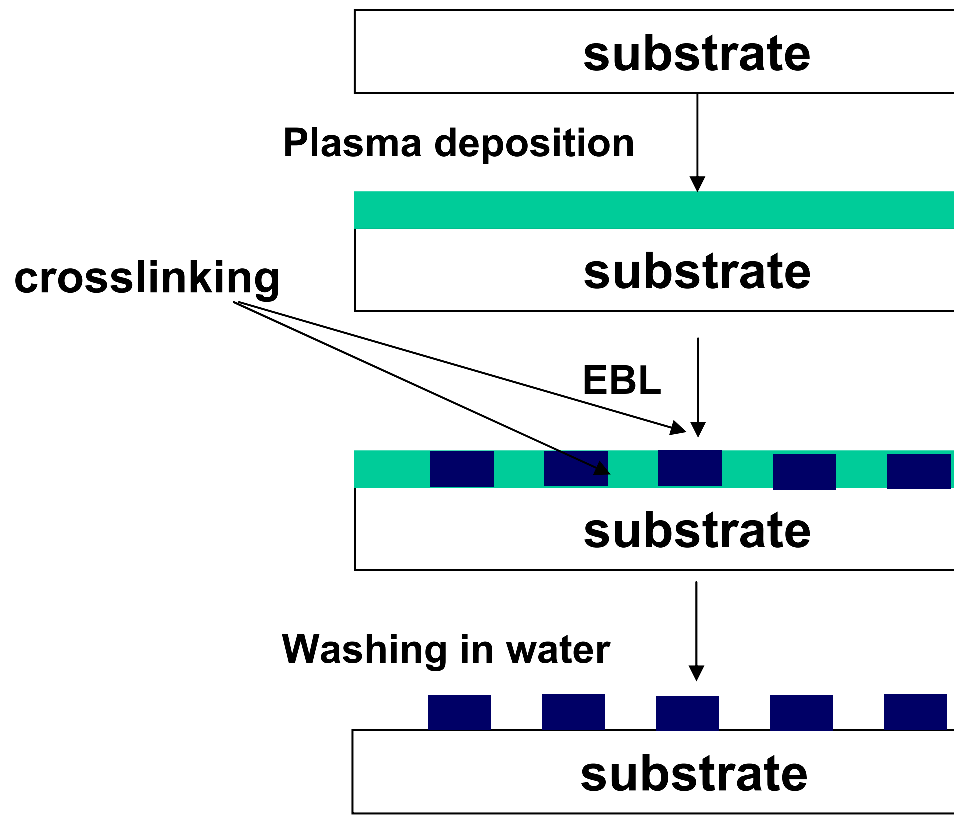
Limit of achievable resolution with SEM:

200 nm with baked PMMA and 400 nm with non baked
(if dedicated lithography tool is used 20nm can be reached)

Direct patterning of an unstable COOH plasma polymer

rinciple: When a plasma polymer is deposited is the rich monomer re (typically low power, high pressure), the degree of reticulation o created polymer obtained is weak= soluble

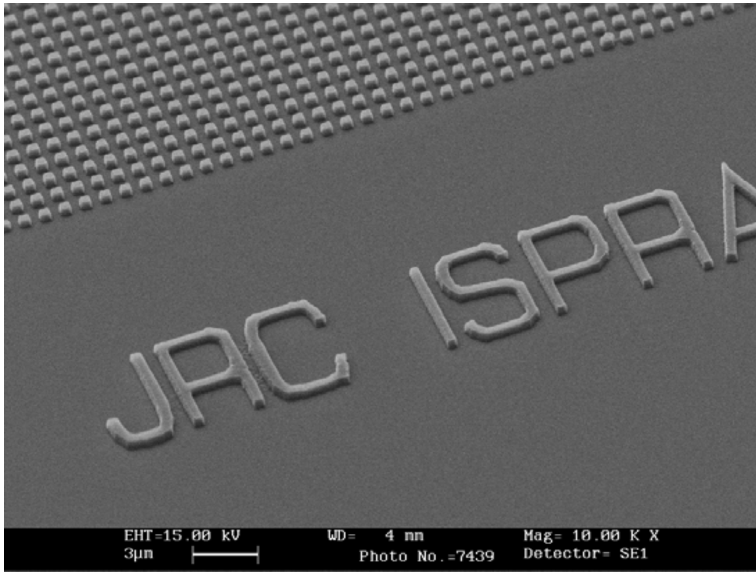


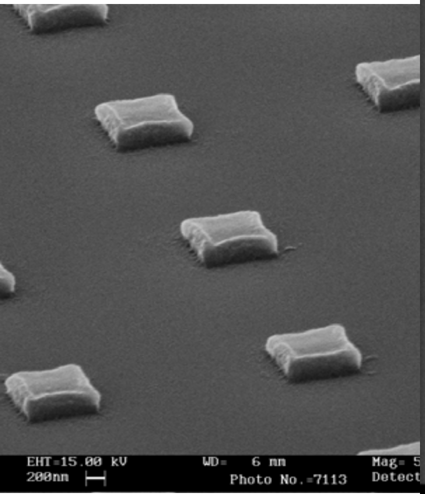


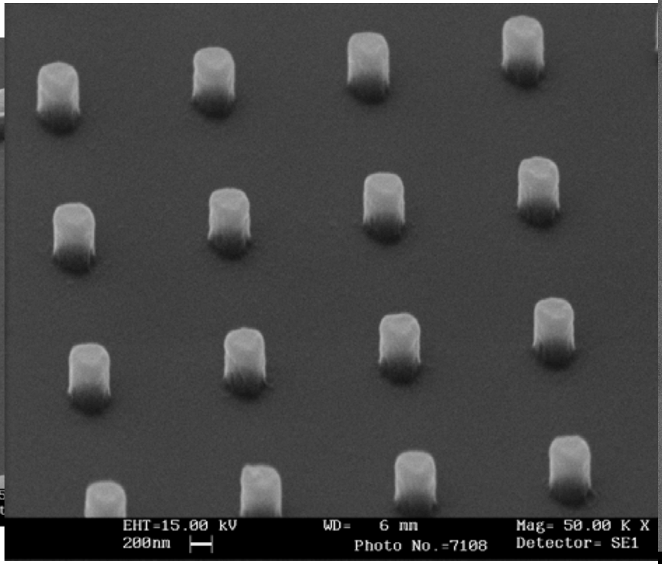
But the degree of reticulation could be improved directly on the surface by the help of the electron beam energy (cross-linking)

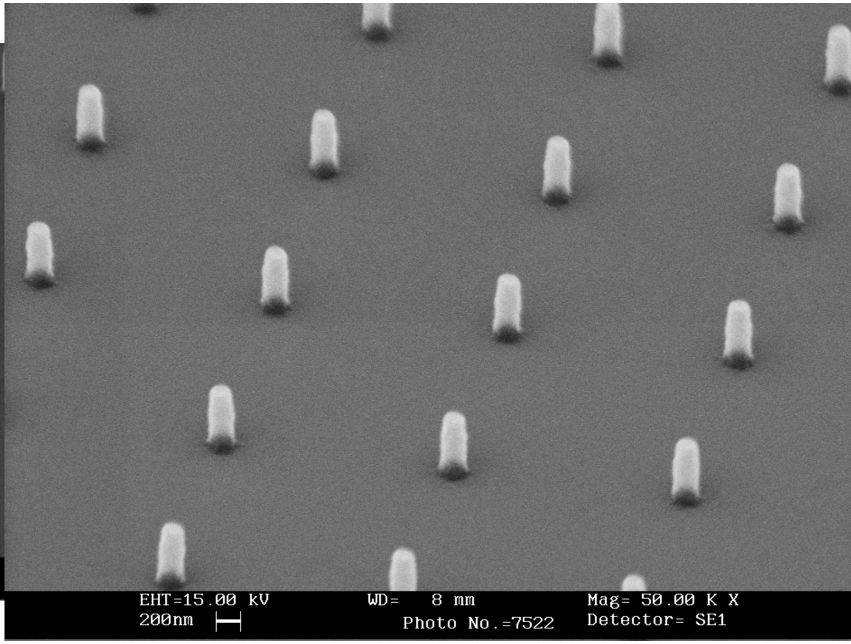
E-Beam lithography

Direct nano-structures of functional polymers
No baking
No resist
Lift off in water (in few minutes)
3-Dimensional structures to be used in *in vitro* tests



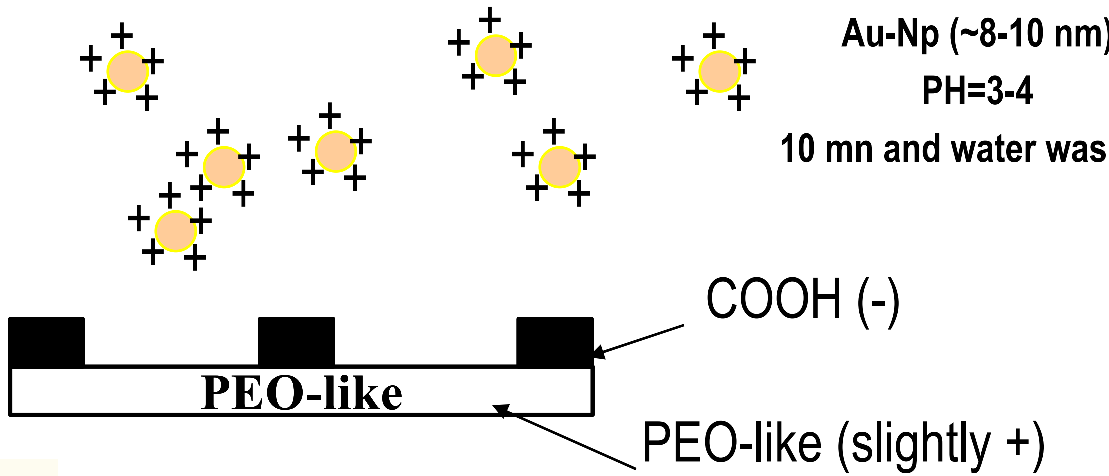






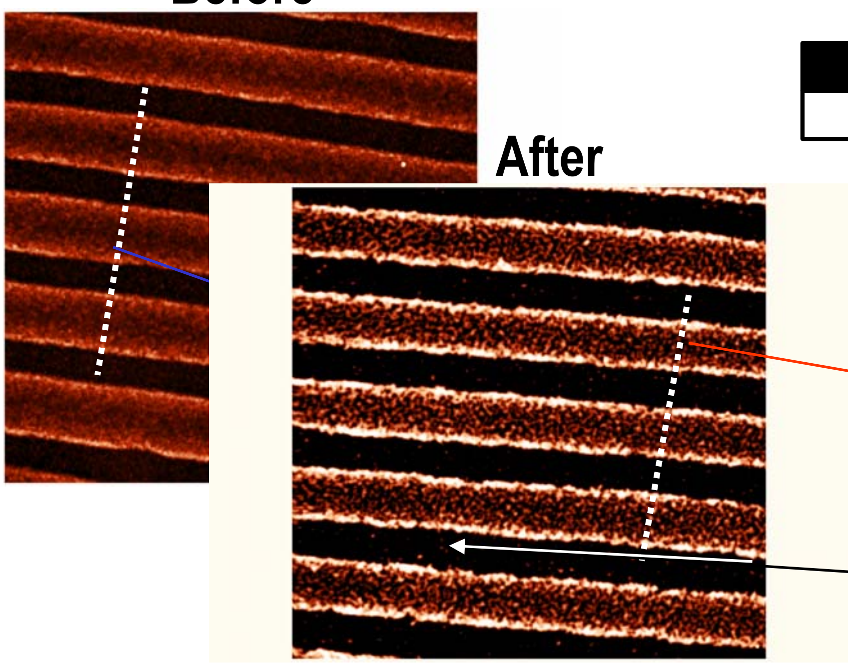
Evidence of the chemical contrast

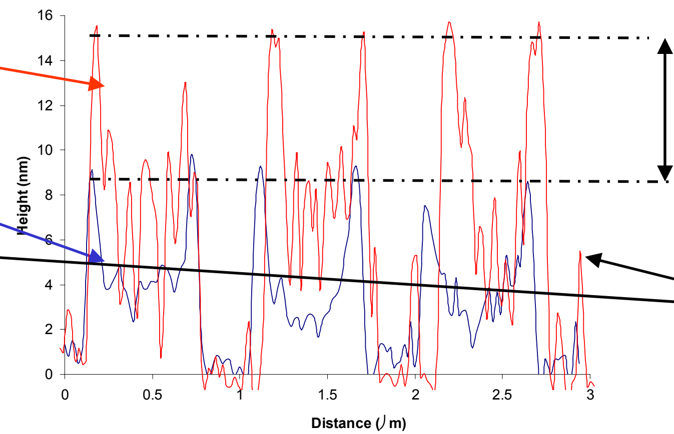
Incubation of Au NP positively charged on the patterned surface



Before

After

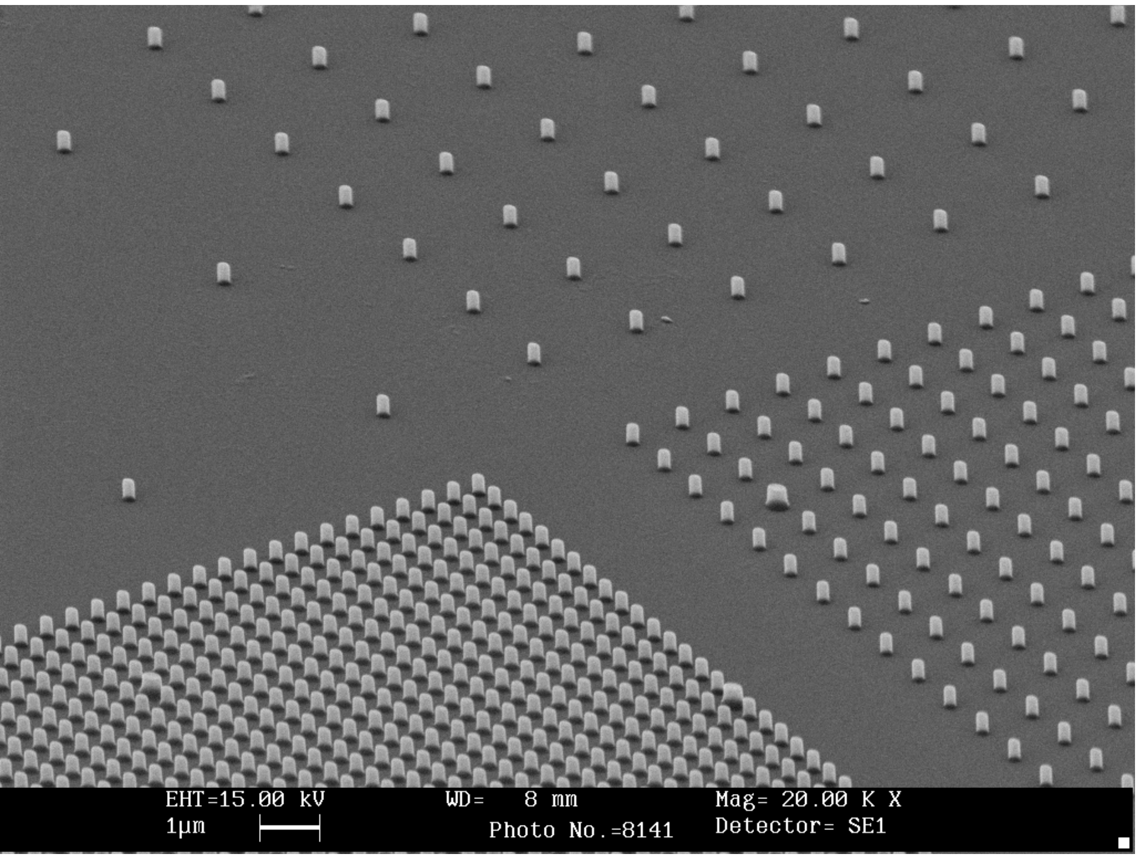


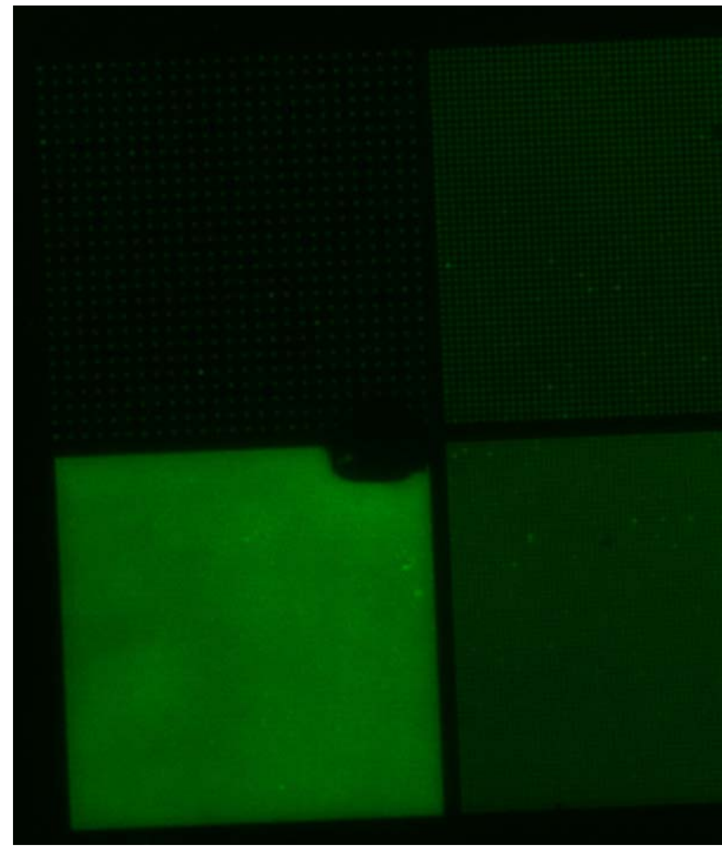


Positively charged particles are mainly adsorbed on the PAA

Chemical contrast

Incubation of the surface with FITC-PLL ((+) polypeptides)

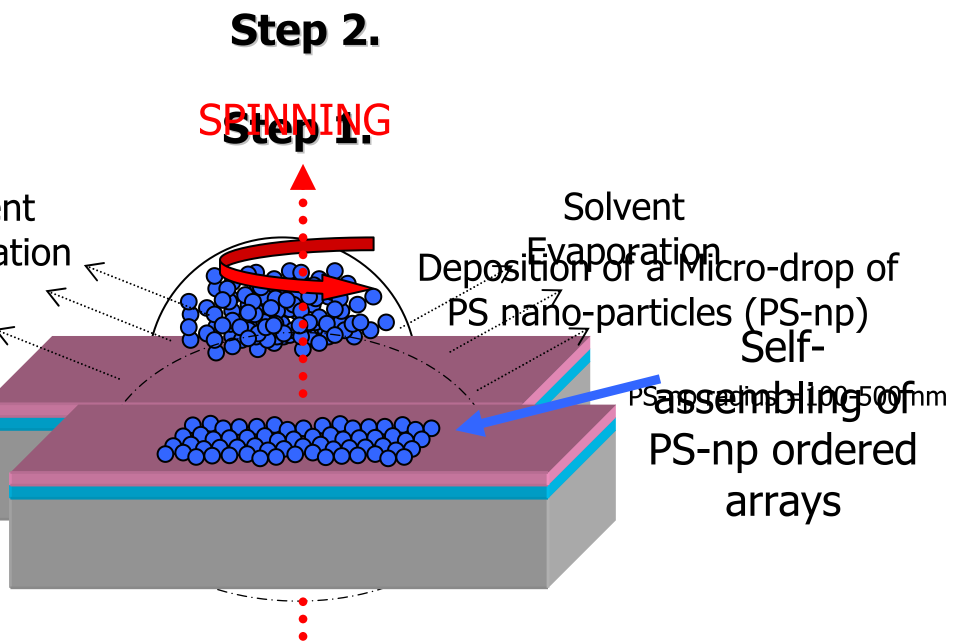


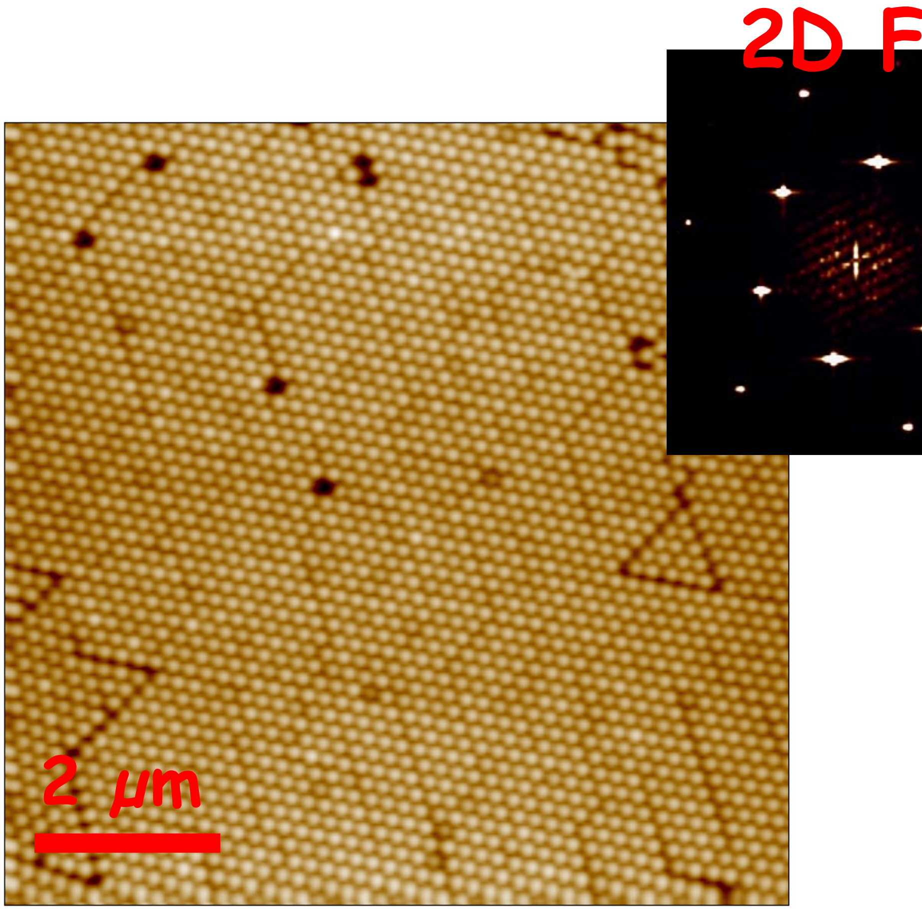


Nanopillars (h= 100nm, d= 100 nm) of stabilized PAA on PEO-like

Colloidal lithography

* Dipersion in MetOH + Triton
* 800 rpm



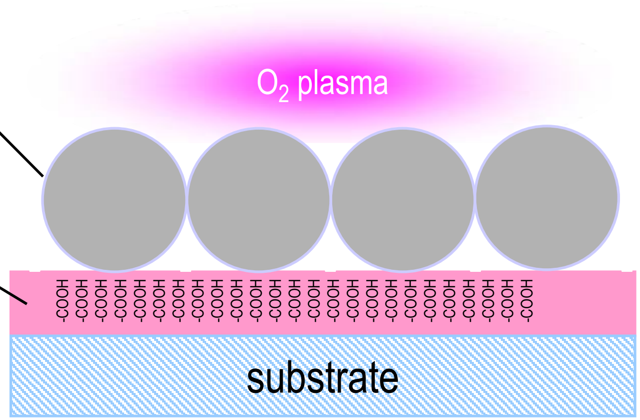


Fast and cheap. Nano-sphere Surface coverage up to 95 % over mm^2. Polycrystalline hexagonal monolayer of nanospheres

Colloidal Lithography + Plasma Polymers
(self-assembly+top-down)

PS beads

Plasma deposited Poly Acrylic acid

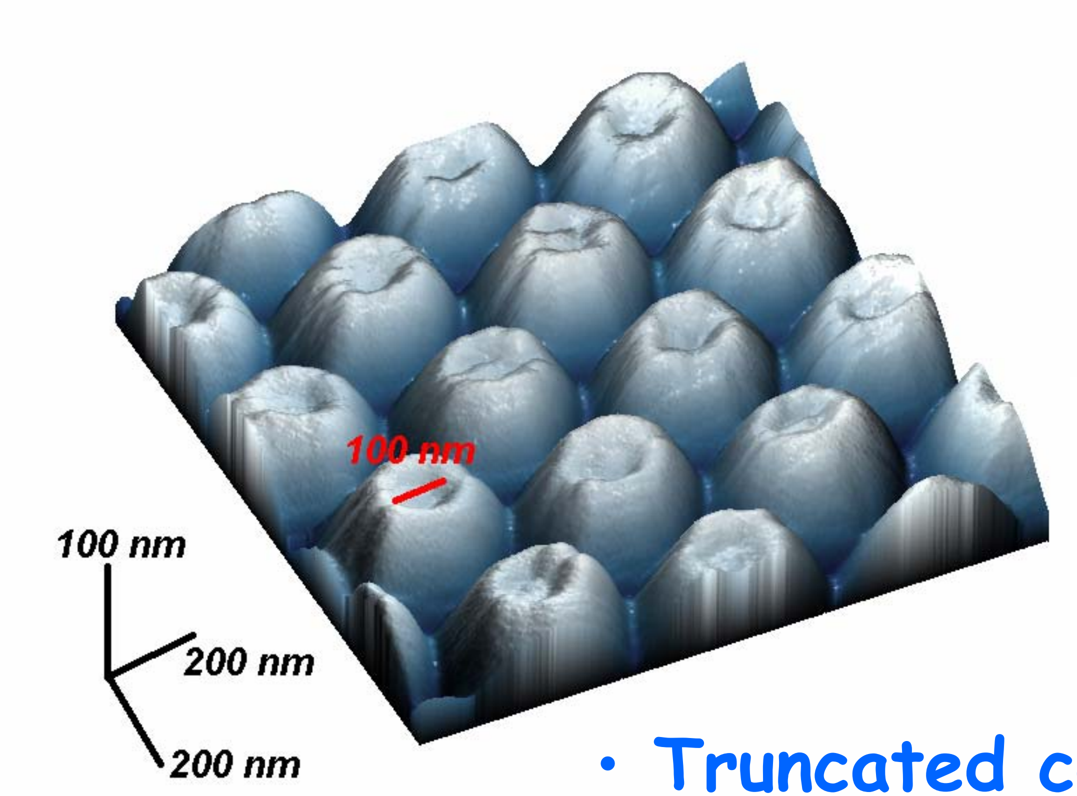


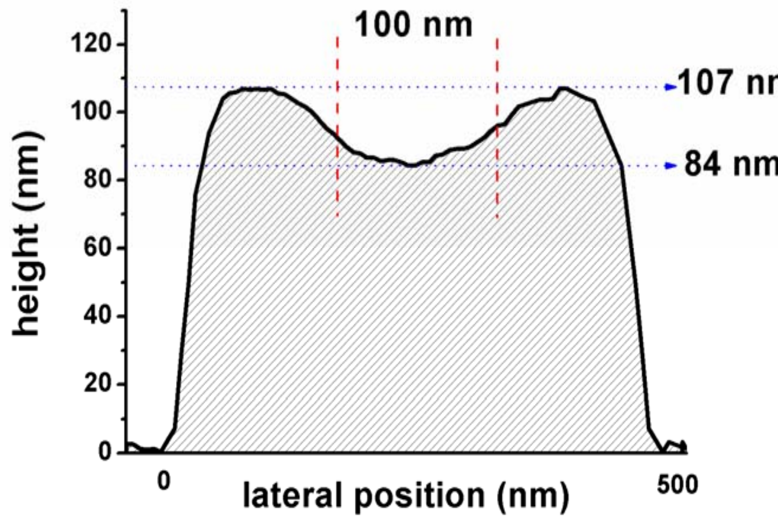
Ultrasound bath

Valsesia,Colpo,Meziani,Manso, Ceccone,Rossi *Nano Lett.*, 4 (2004), 1047-1050

Valsesia,Colpo,Meziani,Bretagnol,Garcia,Bouma,Rossi,, *Adv. Func. Mat. ,16* (2006),1242-1246

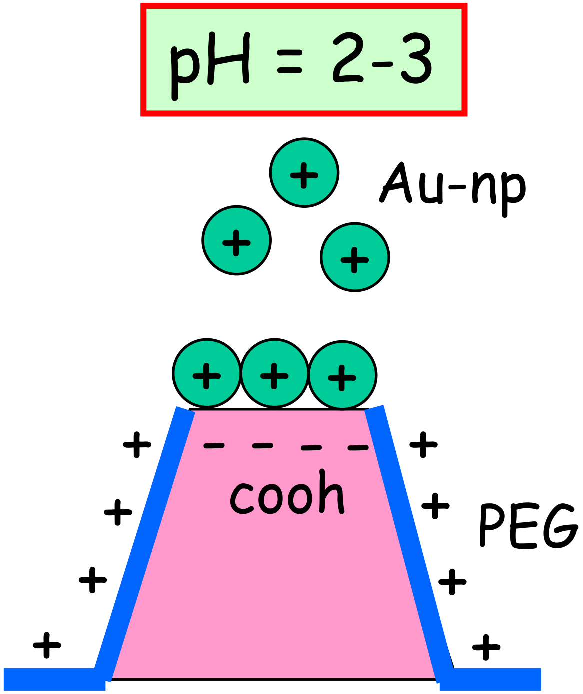
Morphological characterization (AFM)



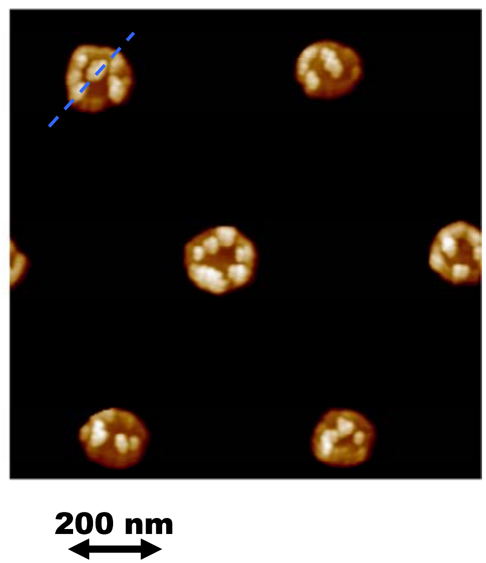


- Truncated conical shape
- 100 nm – wide -COOH plateau
- 500 nm – lattice constant
- 200 nm - high

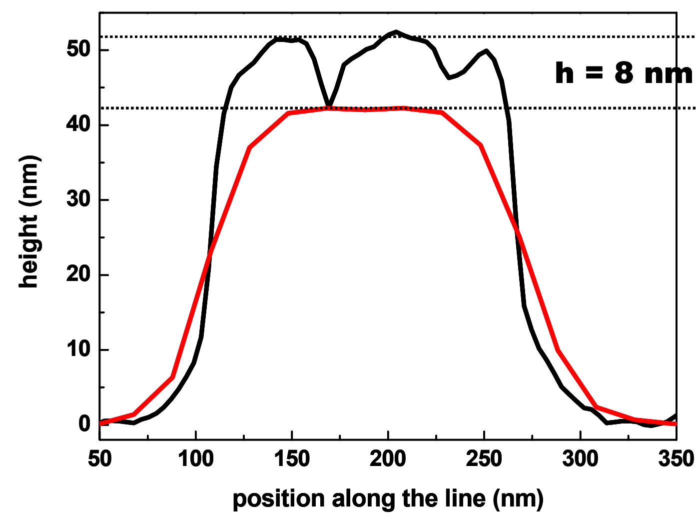
COOH-functionalized area of 1ND and measured chemical contrast



(a)



(b)



Gold nanoparticles are selectively absorbed on the COOH areas

$$A^{cooh} = 9500 \pm 200\ nm^2$$

(approx. 100 nm in diame

Application

Surfaces for Protein Surface interaction studies

Application of Nanostructures to detection systems: Enzyme Linked Immuno Sorbent Assay (ELISA)

1-Antibody immobilization

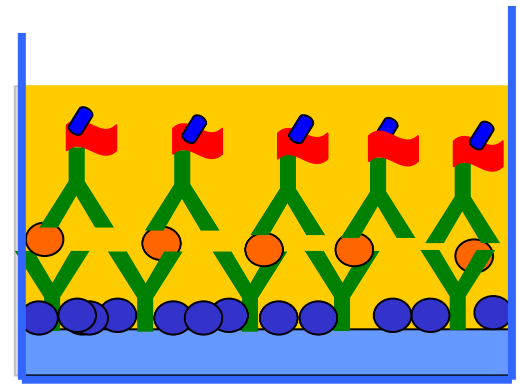
2- Blocking Step (prevent unspecific binding)

3- Antigen Recognition (Analyte)

4- Labeled Antibody (Enzyme linked)

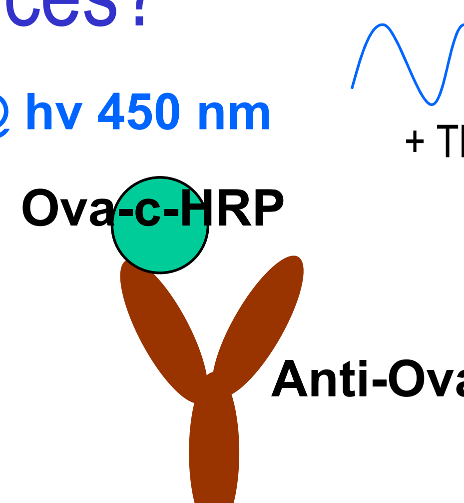
5- TMB (changes the colour of the solution)

6- Measure colour change ⇨ Proportional to Recognised Antigen concent



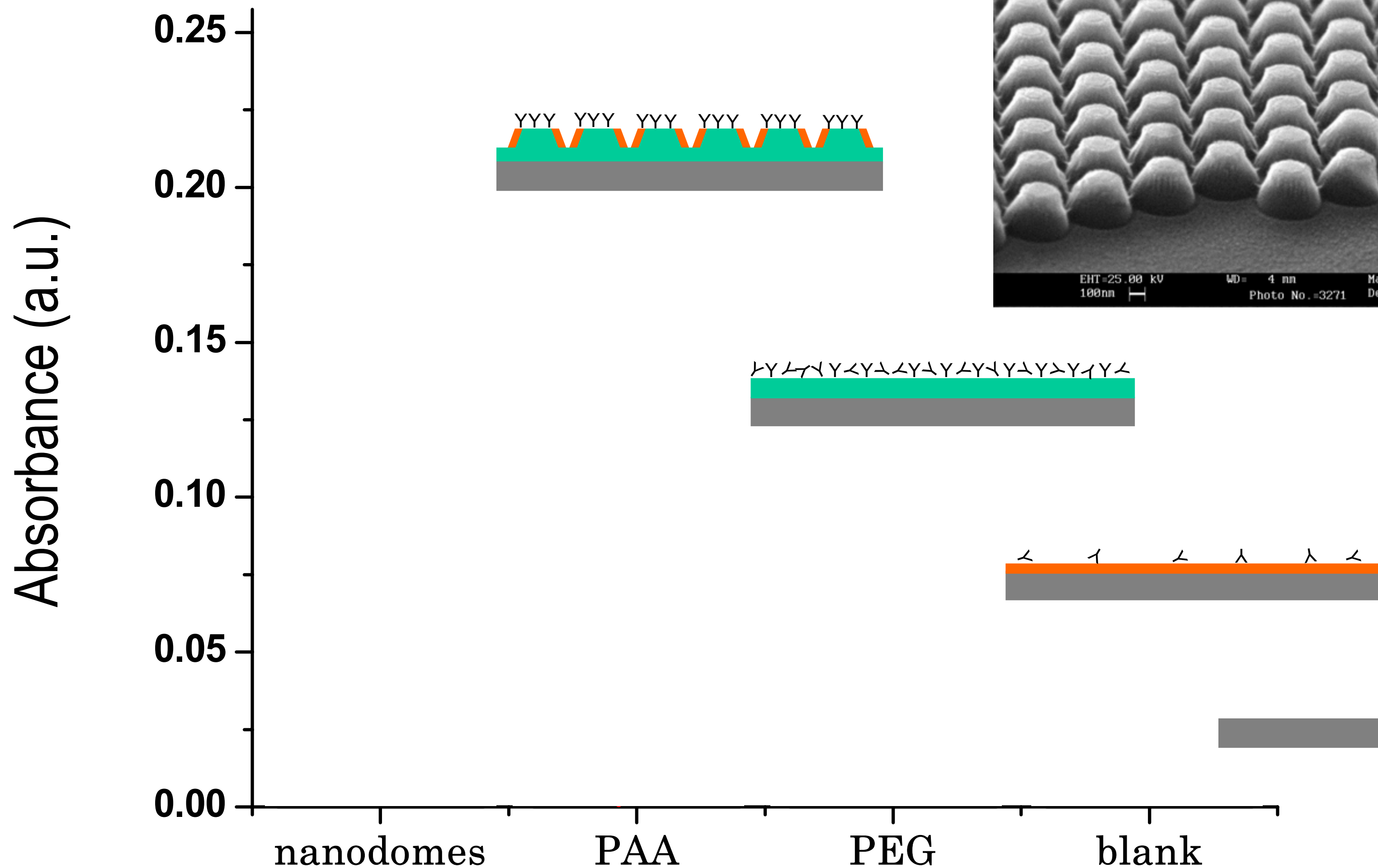
Application of nanostructured surfaces?

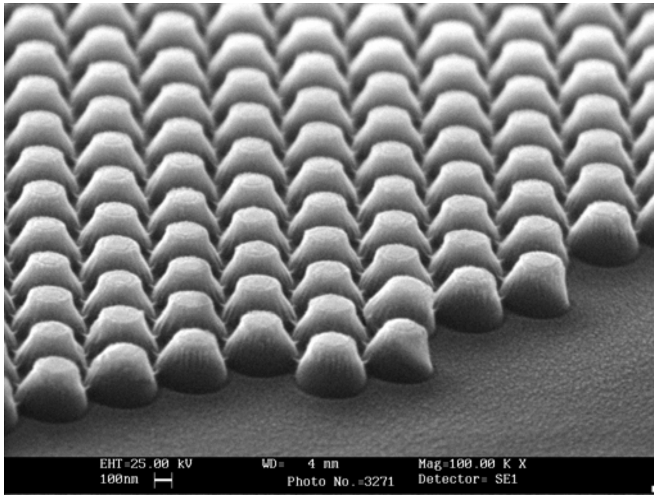
Absorbance @ hv 450 nm



- Ovalbumin conjugated with HRP for chemiluminescence, Reaction with TMB and absorbance measured @ 450 nm

Immunosensing performance: ELISA assay



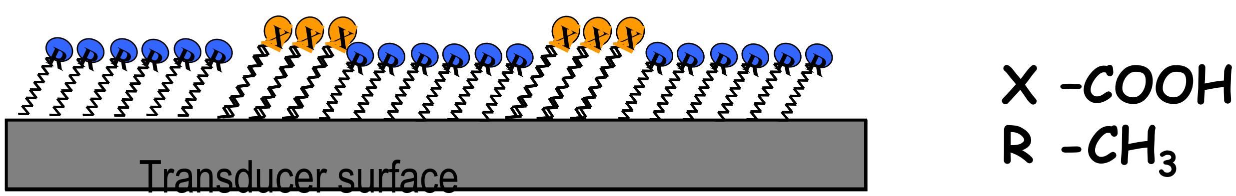


3-times larger signal from the nanostructures

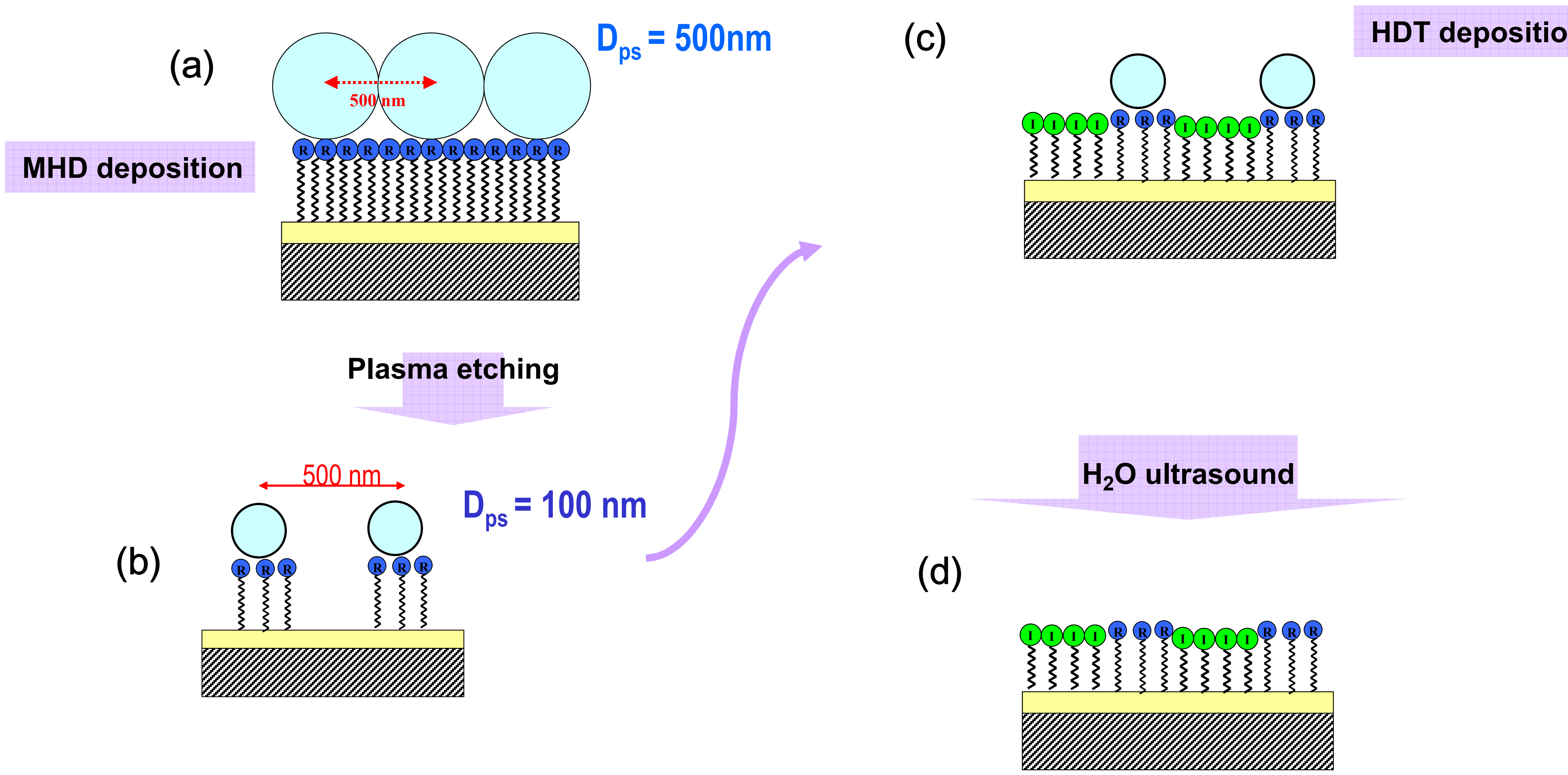
Mixed approach

Top-down/bottom-up

- Use of Self Assembled Monolayers (SAM) for chemical contrast $CH_3/COOH$
- Colloidal lithography for chemical nanopattern production
- Test of chemical nanopatterns with an ELISA assay



Proposed Method: SAM + Colloidal Lithography

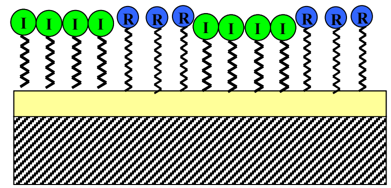


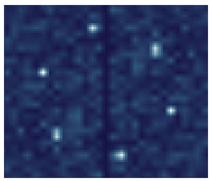
	MHD	MHD/Gold
Contact angle	35	26

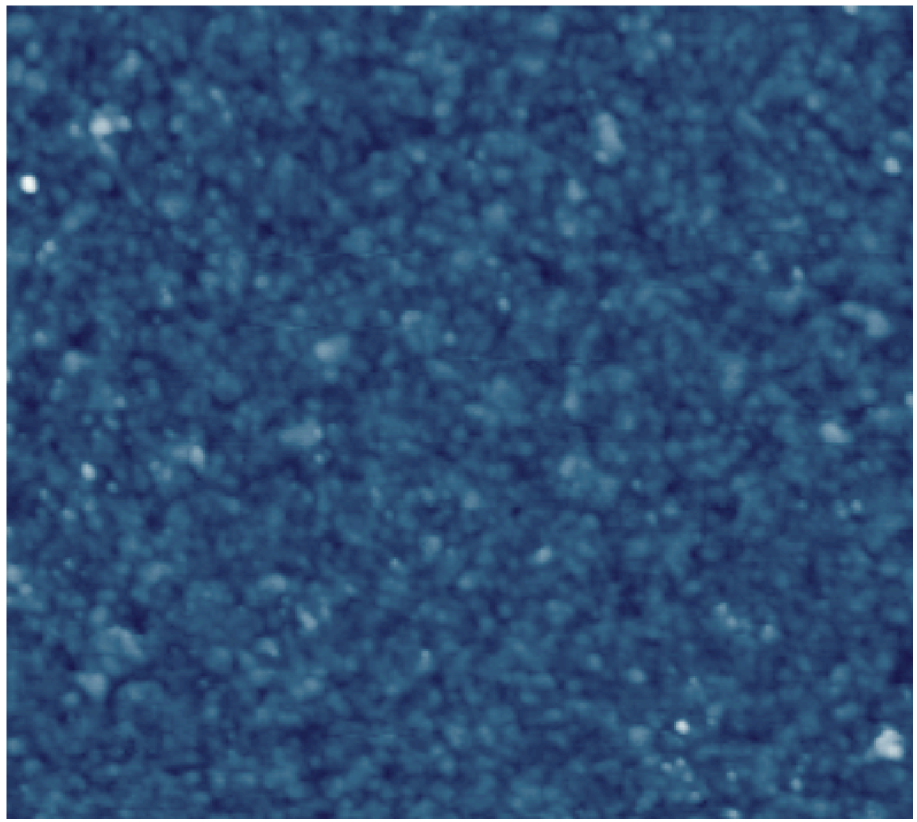
HDT/MHD	HDT
96	103

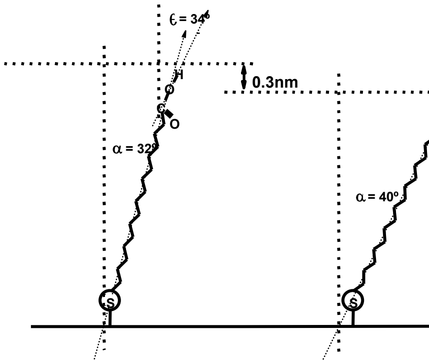
AFM measurements

(d)



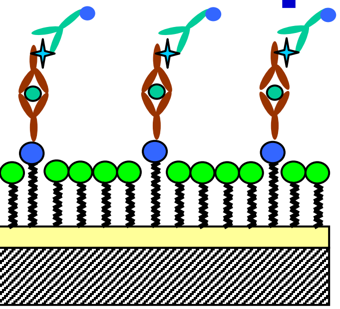


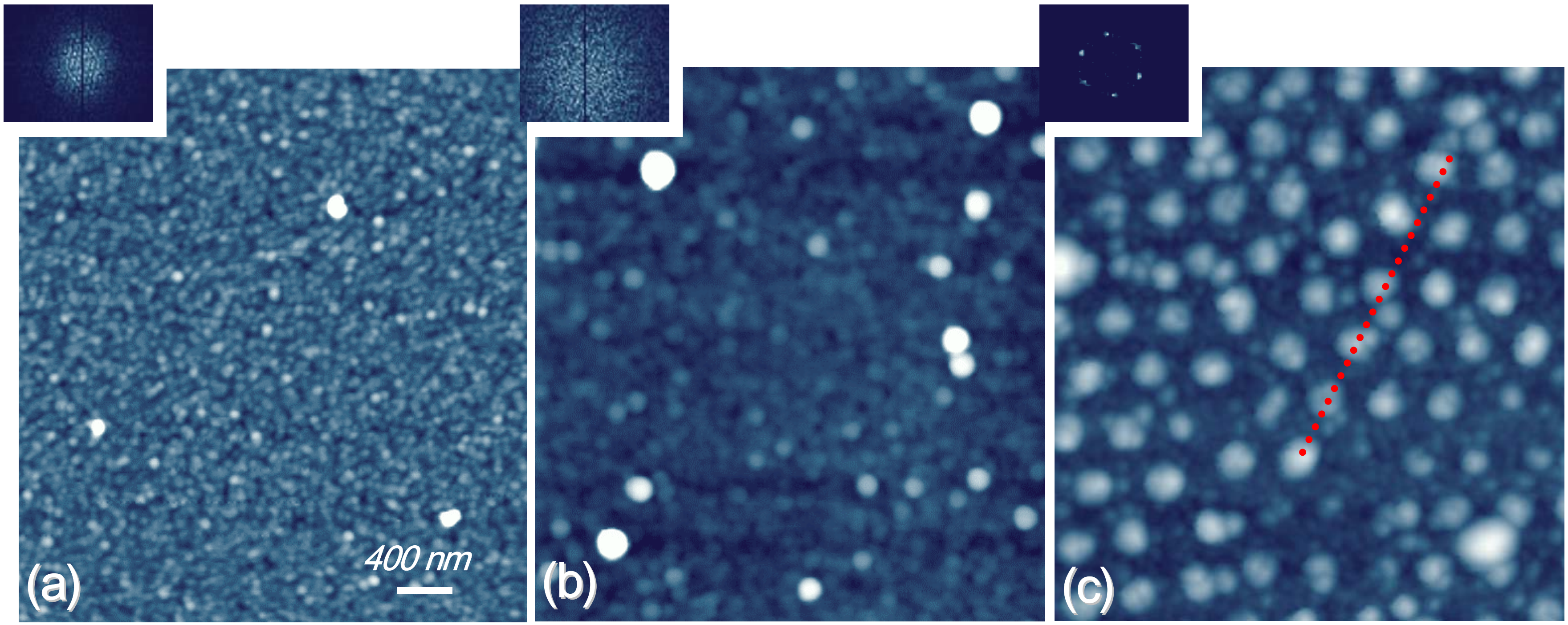




2D crystalline organization is conserved

Protein adsorption: AFM analysis

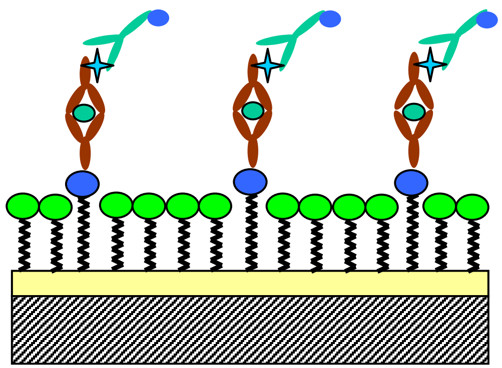




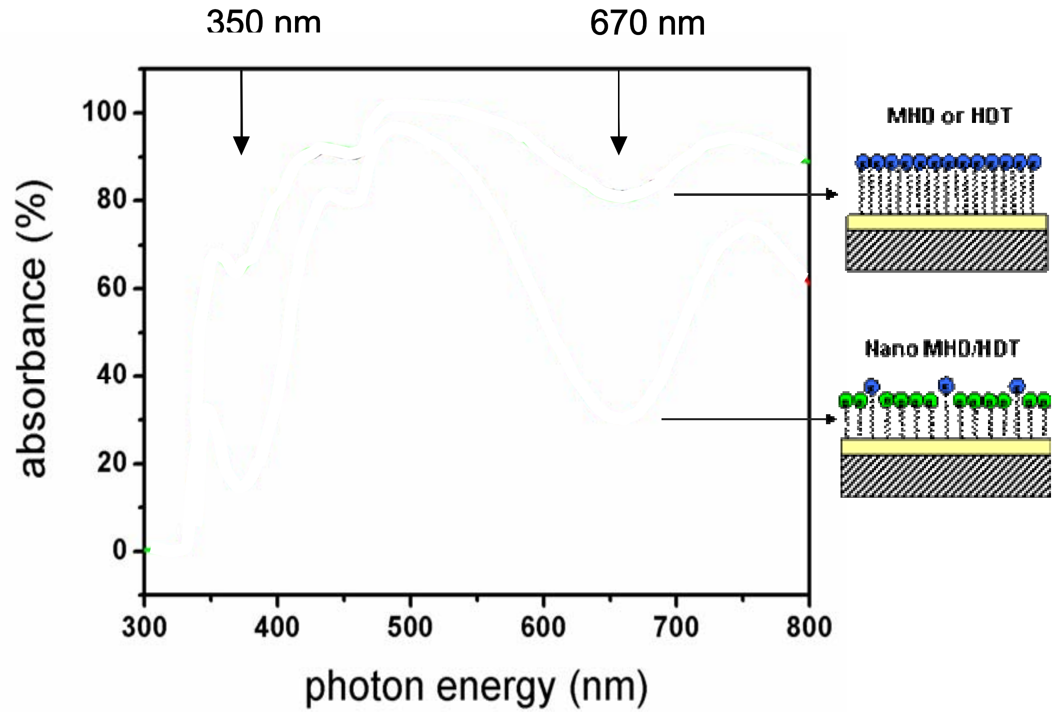
HDT (CH3) | MHD (COOH) | MHD/HDT (COOH/CH_3)

This chemical contrast at the nano-scale enables the absorption of antibodies in active state

Enzyme Linked Immunosorbent Assay (ELISA)



Il-1β Elisa tests



Antibody

Antigen

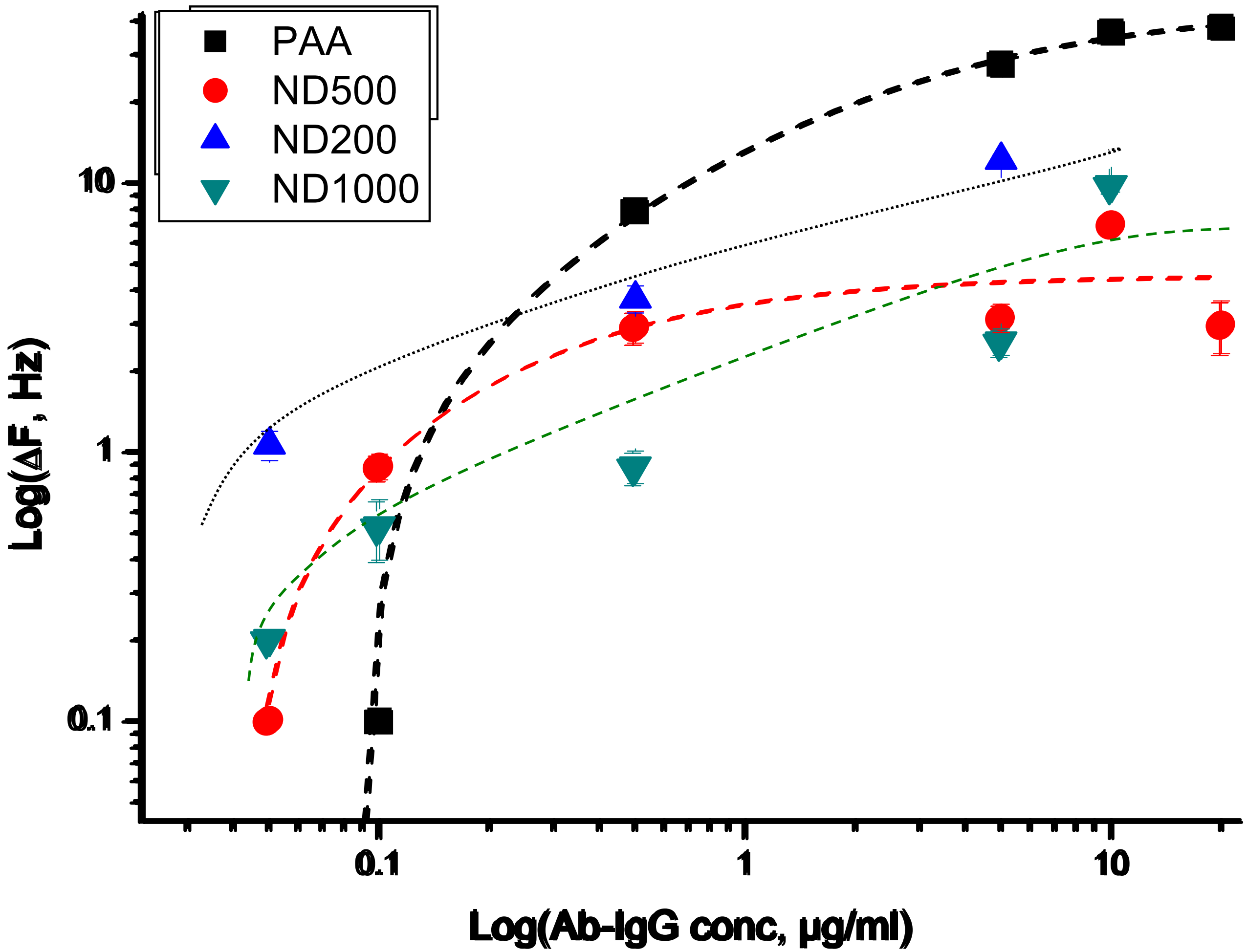
biotinylated Ab

Steptavidin labelled
Enzyme (Horse radish peroxidase)
For chemiluminescent reaction

Increase by a factor 3-4 of the ELISA signal with the nano-structured sample as compared to homogenous surfaces

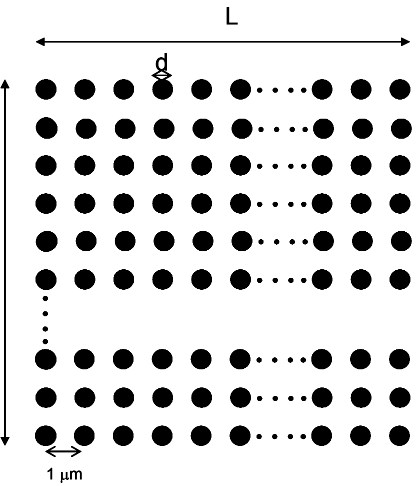
Calibration curves: QCM studies

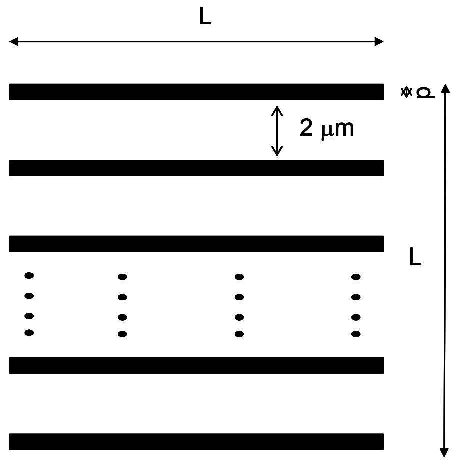
Nanostructures done with beads of 200, 500, 1000 nm diameter



Major interest of nanostructures at low analyte concentration

E-Beam Nano-Patterned surfaces response SPRi





$${}_{dot} = \pi\left(\frac{d}{2}\right)^2 \qquad \sum_L S_{dot} = L^2\pi\left(\frac{d}{2}\right)^2$$

$$P_{dot} = \pi d \qquad \sum_L P_{dot} = L^2\pi d$$

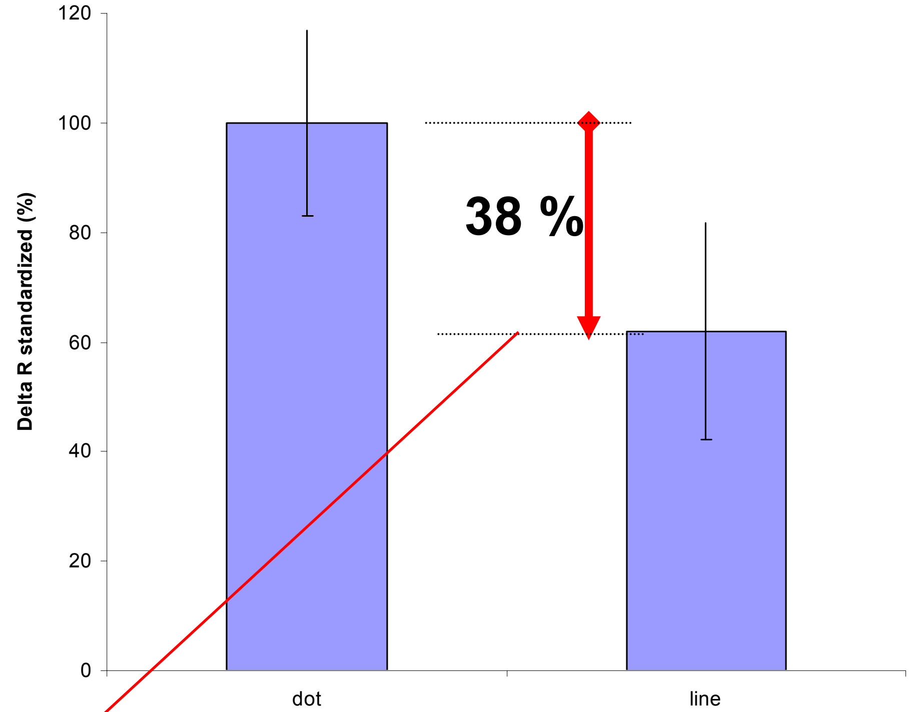
$$S_{line} = dL \qquad \sum_L S_{line} = d\times L\times\frac{L}{2} = d\times\frac{L^2}{2}$$

$$P_{Line} = 2L \qquad \sum_L P_{Line} = 2L\times\frac{L}{2} = L^2$$

Dot 1 x0.75 | **Line 1 x2**

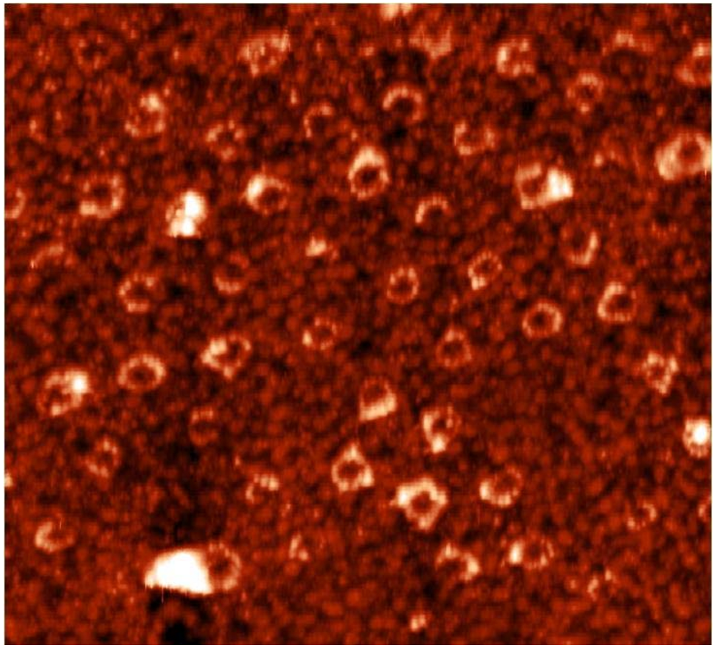
S/A=25% | **S/A=33%**

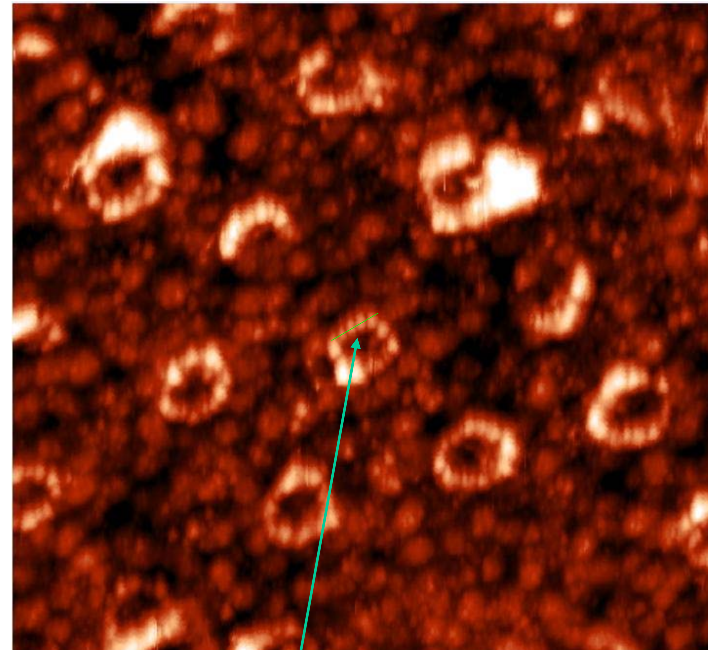
L/A = 1µm^{-1} | **L/A = 0.66 µm^{-1}**

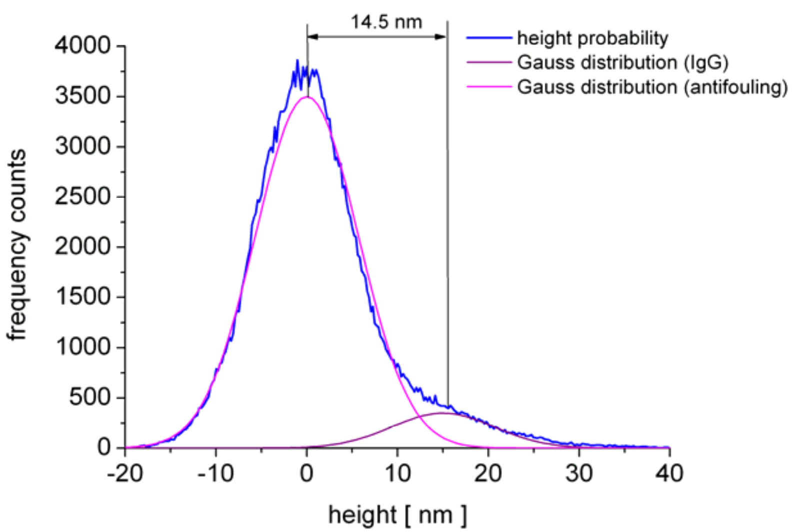


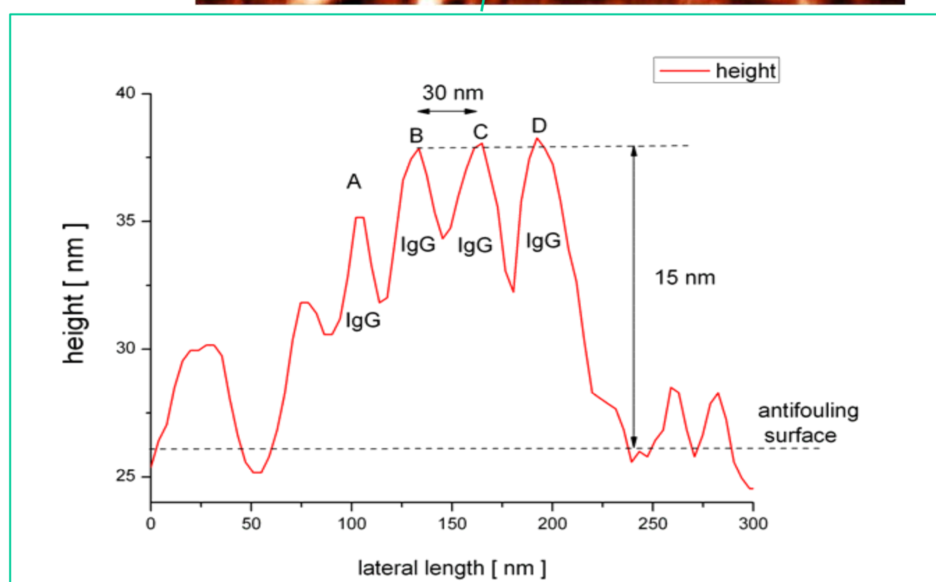
The amplification linked to nanostructures surfaces seems related to a boundary effect

Protein distribution on nanopatterned surfaces









Protein adsorption on nanostructures

Amplification:

- Reduction of steric hindrance
  - Better access of reactive sites
  - Works at low concentration
- Better orientation of proteins on surfaces?
  - Effect of electrostatic interaction
    - questionable with IgG on –COOH
  - Effect of boundary: largely unexplored

Future work:

- Other interfaces and boundaries to be tested
  - Hydrophobic/hydrophilic
  - Positive/negative
- Effect of nanostructure on adsorption kinetics (on going) and protein conformational changes

Conclusions

- Family of functional surfaces with high contrast properties produced by plasma deposition
- Nanopatterns have important effects on protein adsorption on surfaces
  - Produced by e-beam lithography: large flexibility
  - Colloidal lithography: Ordered hexagonal 2D structures
  - Effect on control of adsorption have been demonstrated
- Direct nano-structuring of the biosensors supports (QCM, SPR, ELISA)
  - Control of protein adsorption on surfaces
  - Large amplification of signal at low concentrations
  - Effect of boundaries still to be studied

Thank you for your attention!

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