Protein interactions with Nanostructures

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Outline

- Introduction
- Micro and Nano patterning: chemical contrasts
  - e-beam lithography
  - Colloidal lithography
- Proteins on nanopatterns

Structure of the JRC

7 Institutes in 5 Member States

IRMM – Geel, Belgium
- Institute for Reference Materials and Measurements

Staff: ≅ 250

IE – Petten, The Netherlands
- Institute for Energy

Staff: ≅ *180*

ITU – Karlsruhe, Germany
- Institute for Transuranium elements

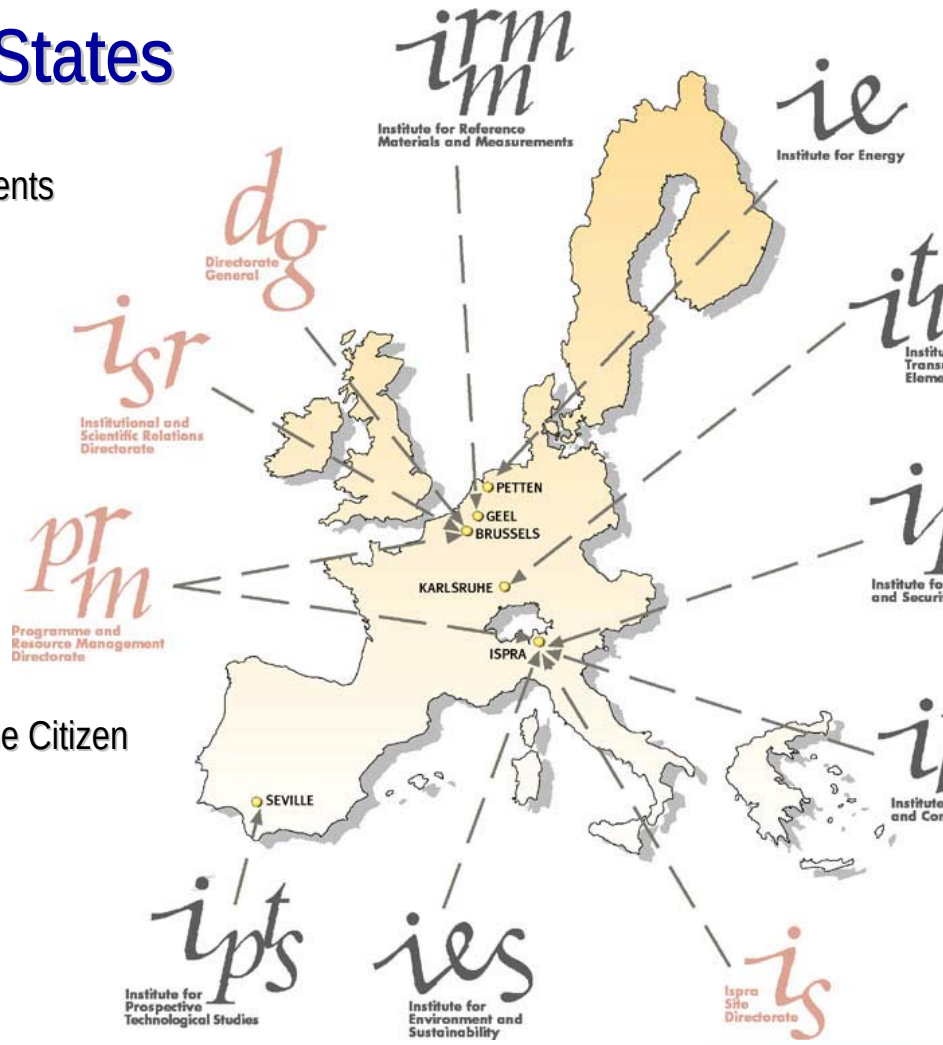
Staff: ≅ 250

IPSC - IHCP - IES – Ispra, Italy
- Institute for the Protection and the Security of the Citizen
- Institute for Health and Consumer Protection
- Institute for Environment and Sustainability

Staff: ≅ 350, 250, 370

IPTS – Seville, Spain
- Institute for Prospective Technological Studies

Staff: ≅ 100



Total staff: ~ 2200 people

IHCP 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
  - Cell sensing
  - Uptake and Cellular trafficking

(Bio)senso

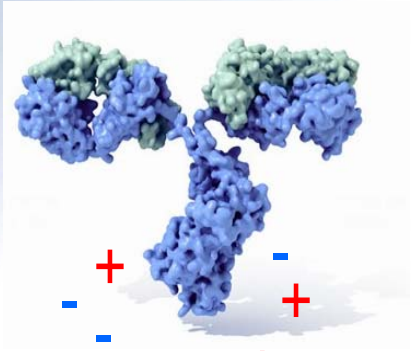
In vitro test

Cell on chi

NP/proteins complex

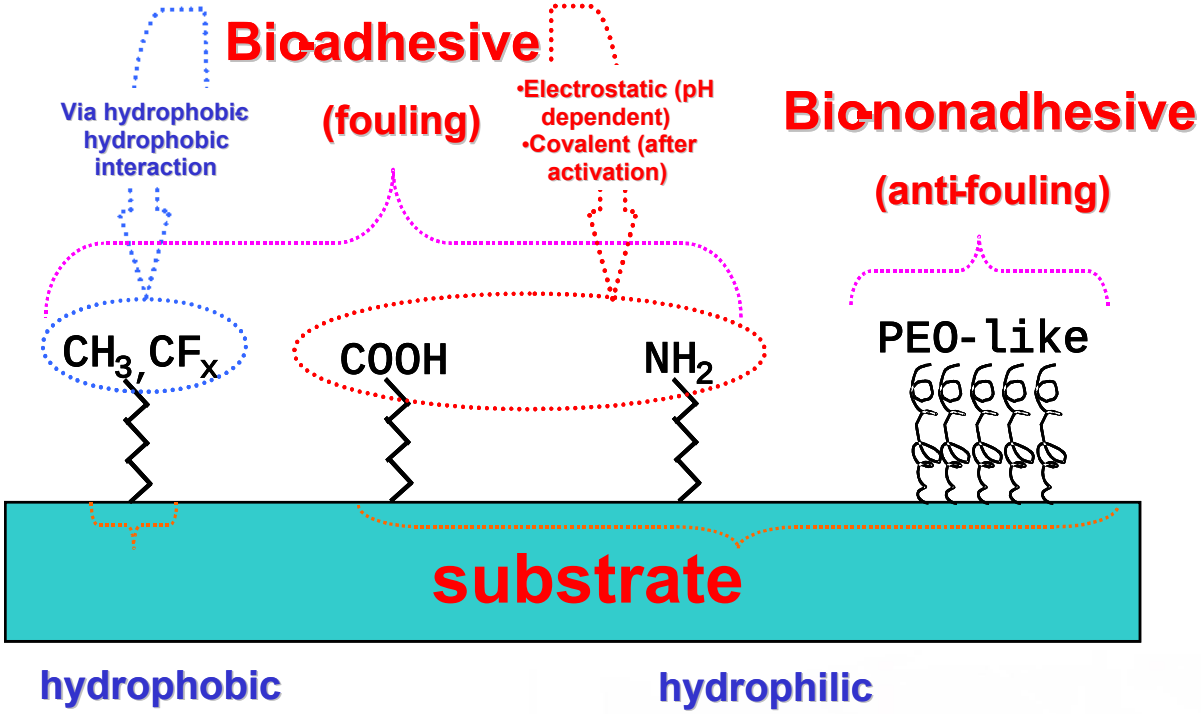
➢Protein-surface interactions

Immobilisation of Proteins on surfaces



Protein, Antibody

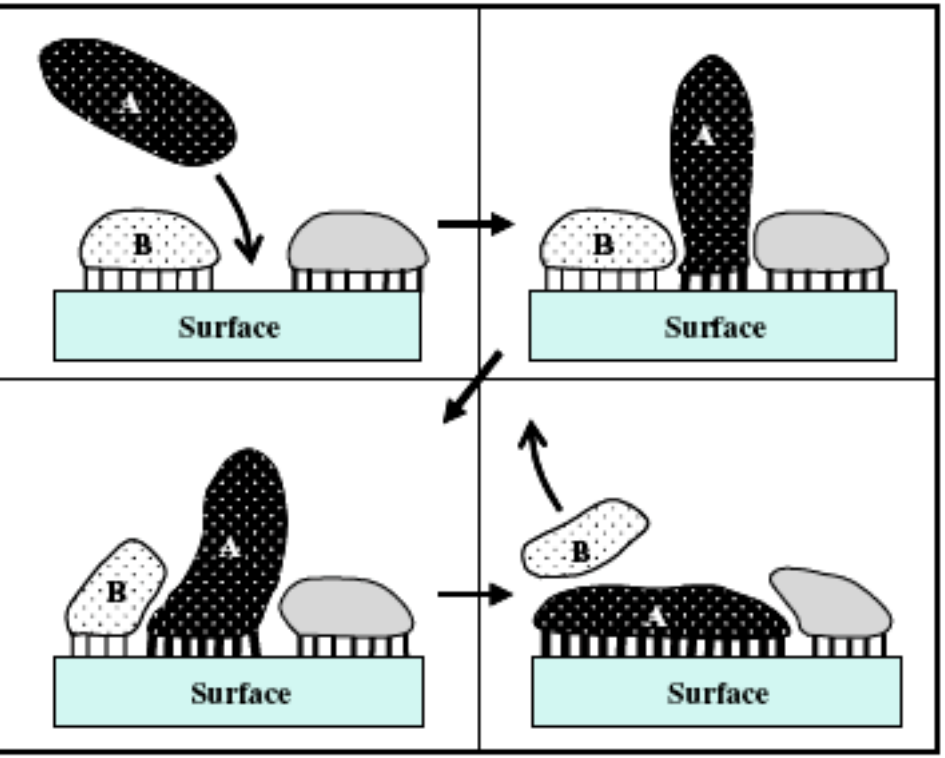
detector



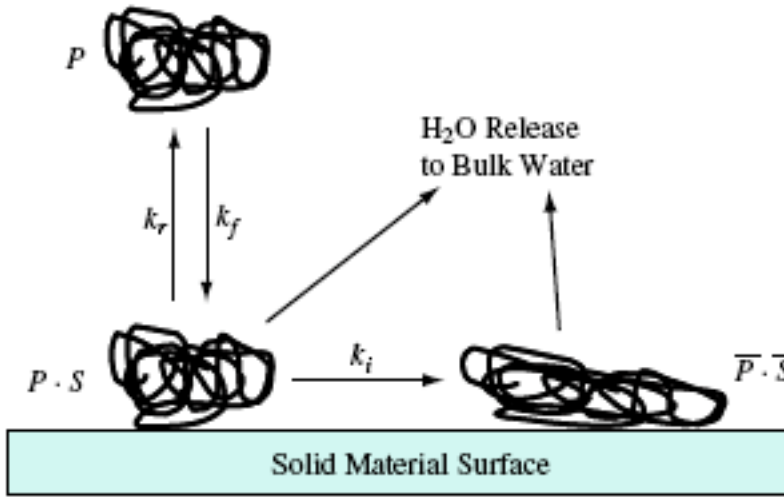
OBJECTIVE

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

Protein surface interactions



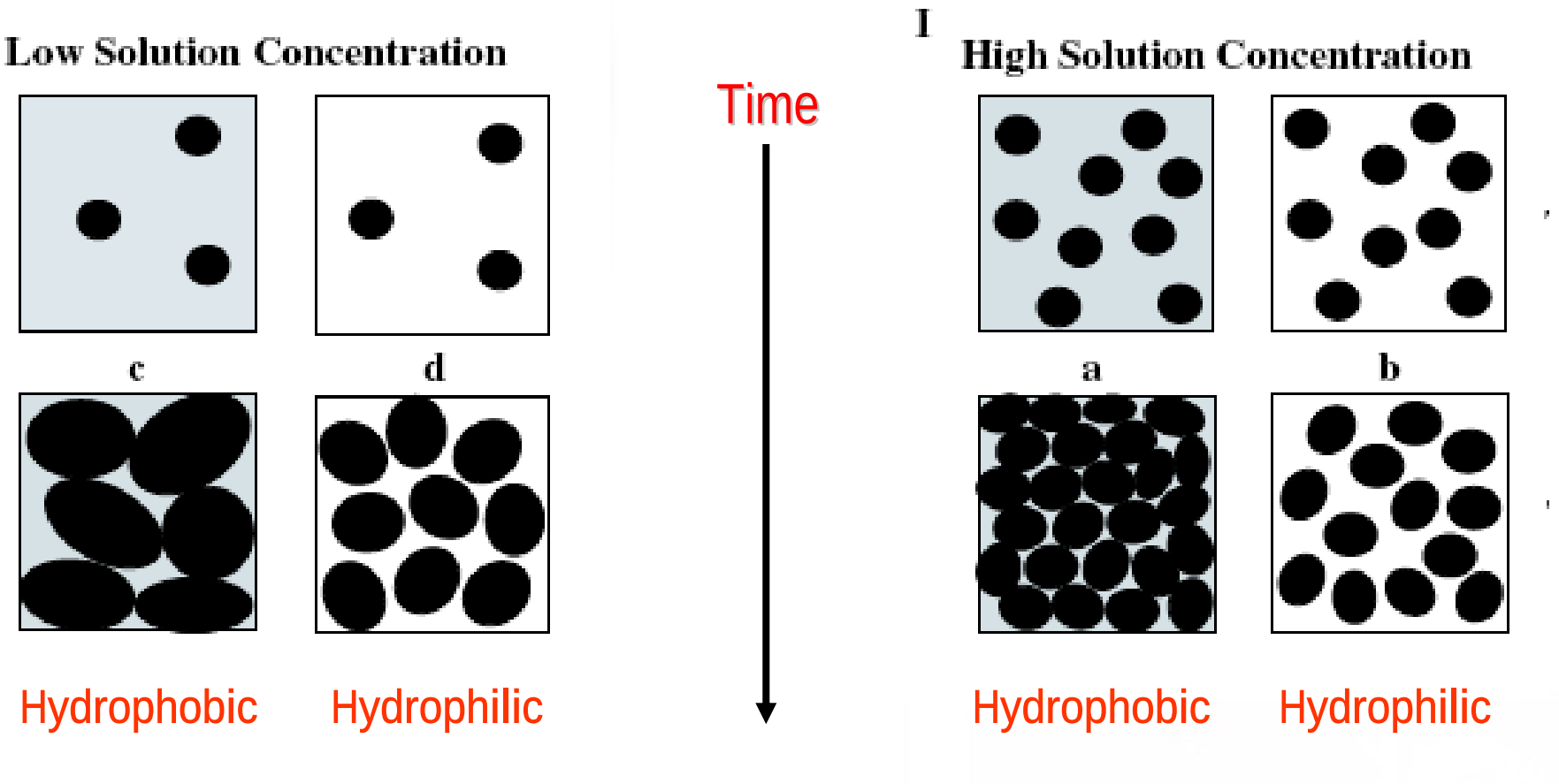
Vroman effect



Conformational changes

Latour, Encyclopedia of Biomaterials and Biomedical Engineering, 2005

Surface properties & adsorption kinetics



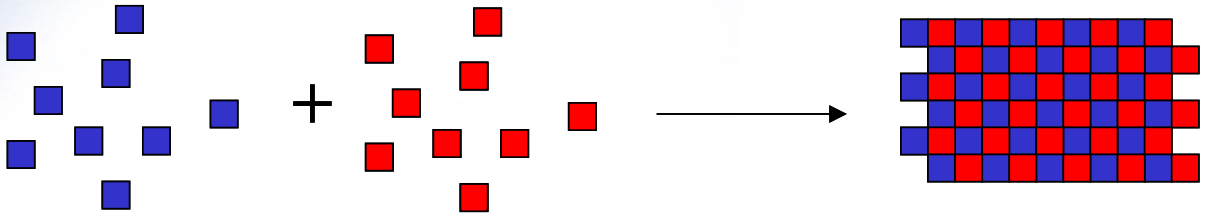
Surface concentration and activity of adsorbed proteins depend on:

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

Effect of confinement on nanostructures?

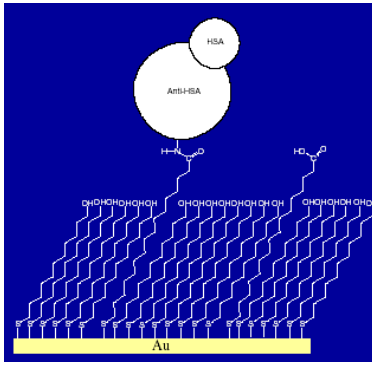
Chemical nano-patterns

BOTTOM – UP: self-assembling process



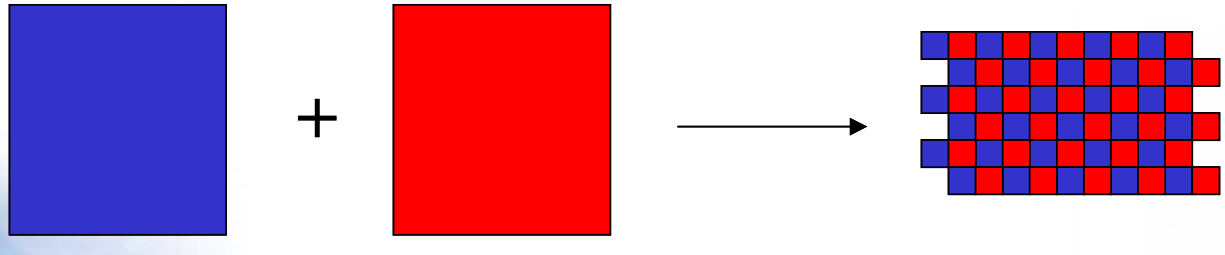
Component A Component B Nanostructured material

e.g. Mixed SAM (COOH+AF)



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

TOP – DOWN: lithographic techniques



Component A Component B Nanostructured material

SERIAL (dip-pen, beam, nano-founta

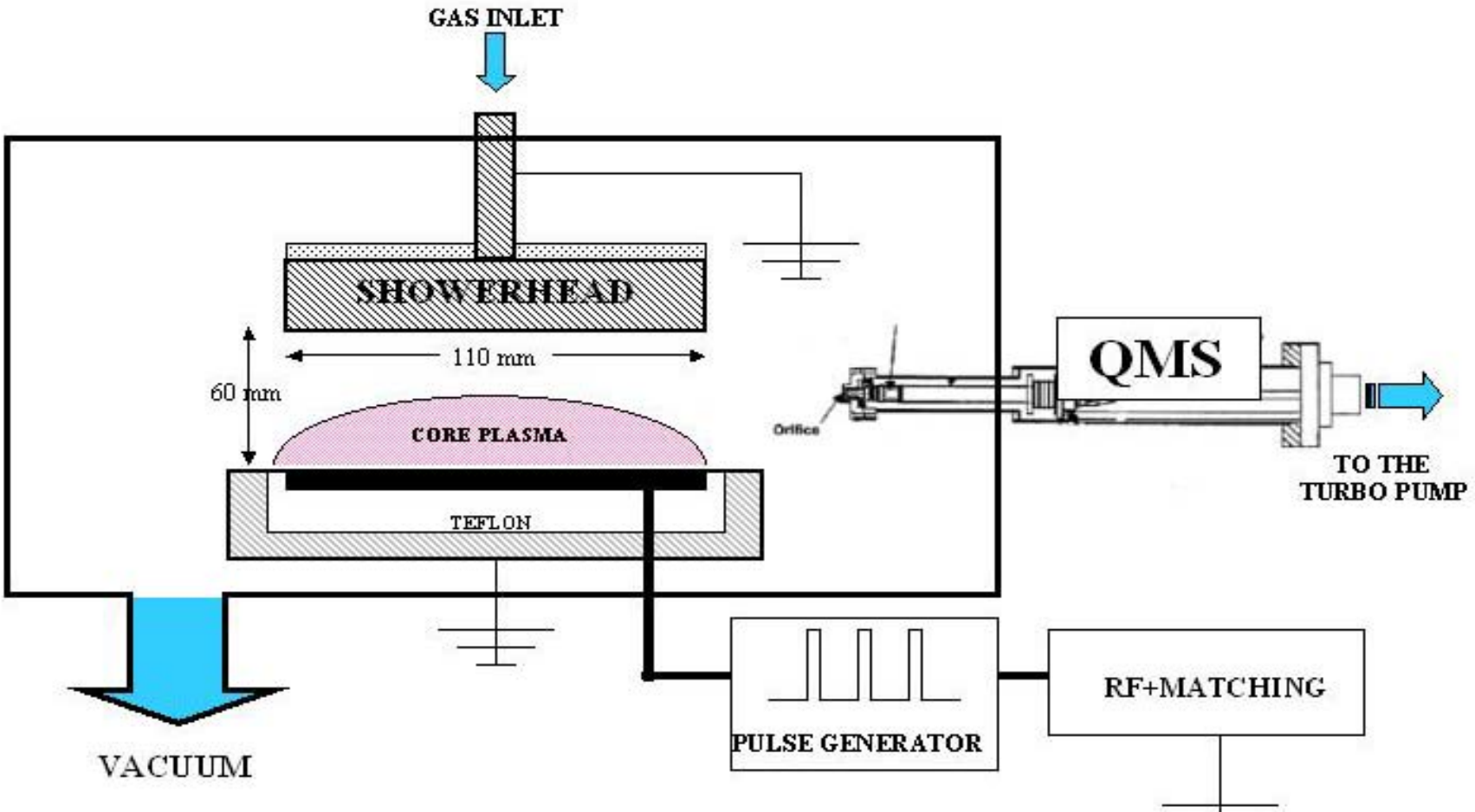
Lee, *Science*, 295 (
Taha, *APL*, (2003)

PARALLEL (nano imprint, colloidal

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

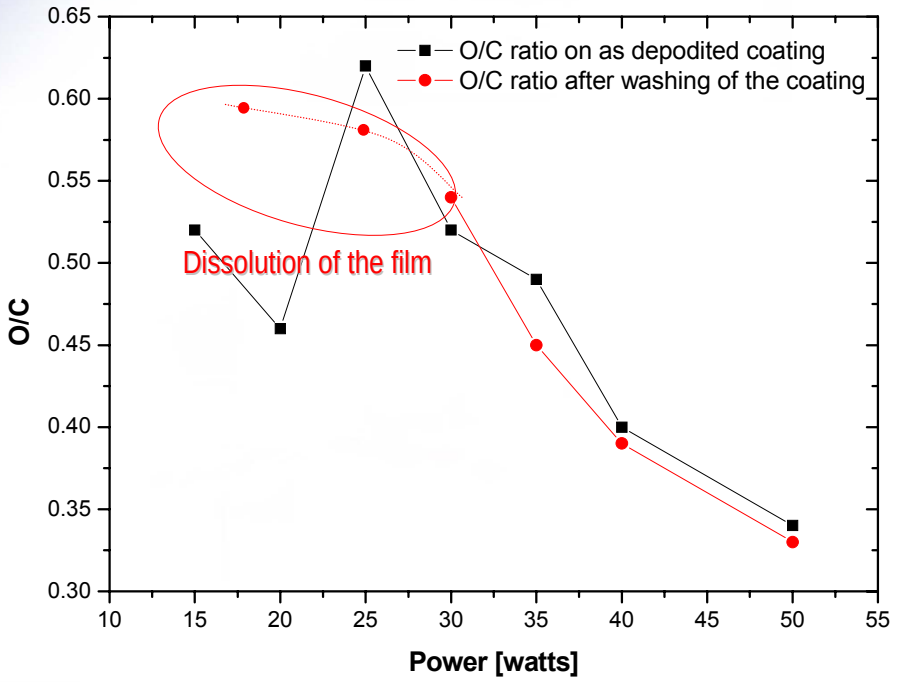
Plasma deposition of polymers

- **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**

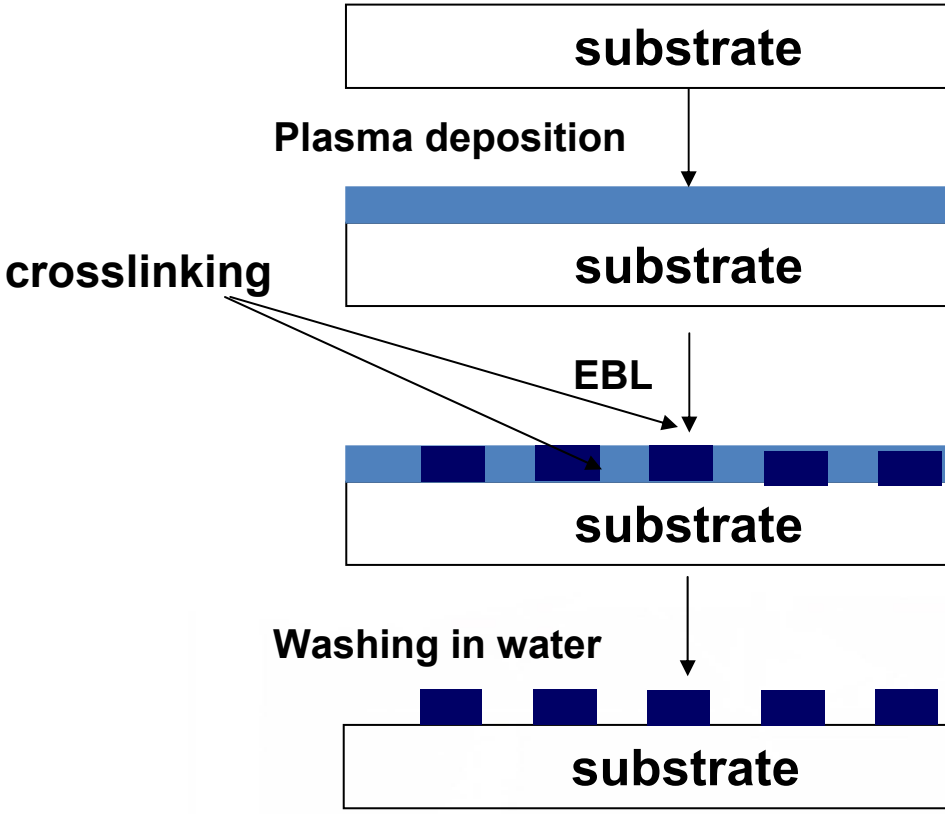


Direct patterning of unstable PPAA

Principle: Change of plasma polymer c
linking and solubility by e-beam irradiation

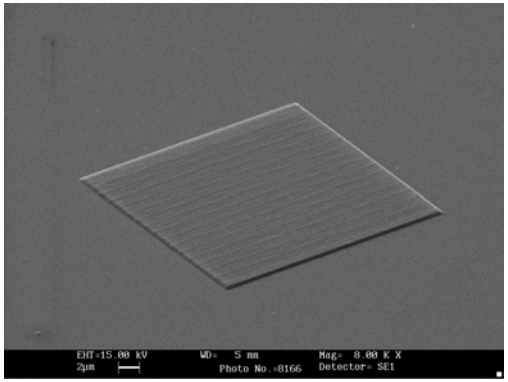


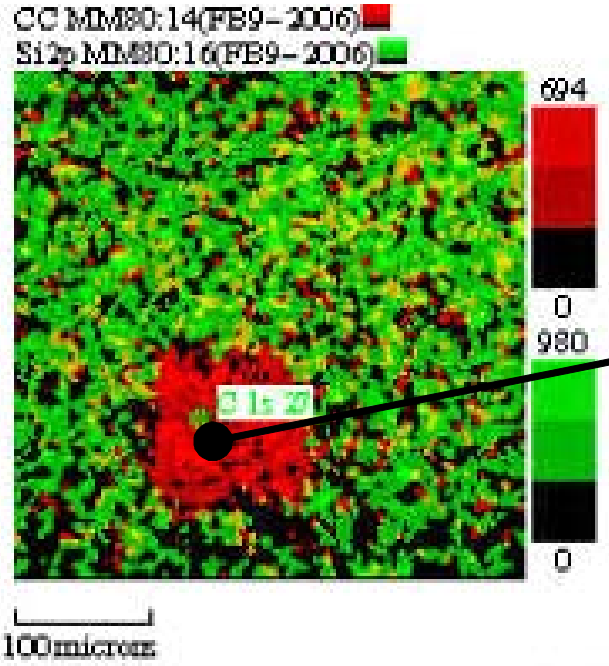
Acrylic acid plasma polymer

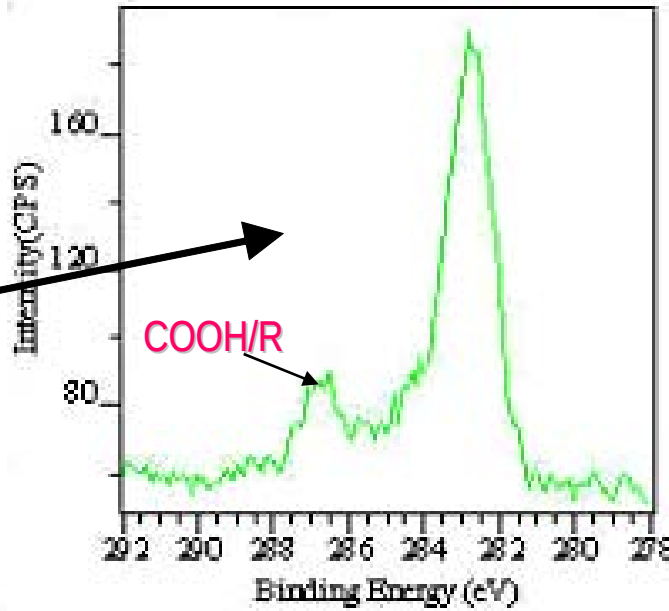


Chemistry of the stabilized PAA

μ-XPS analysis





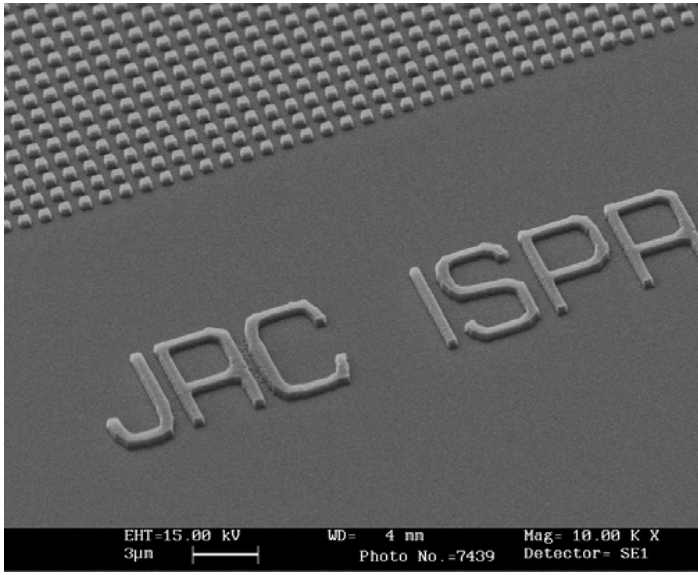


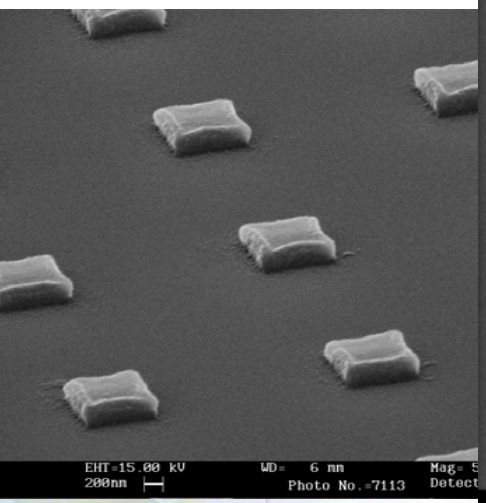
COOH/R retention of around 10 % suitable for bio applications[1]

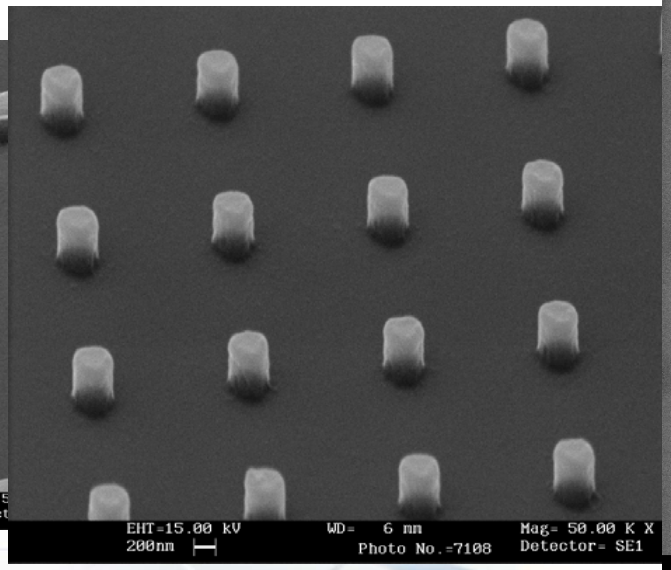
1: Loredana Detomaso, Roberto Gristina, Giorgio S. Senesi, Riccardo d'Agostino and Pietro Favia *Biomaterials*, *Volume 26, Issue 18, June 2005, Pages 3831-3841*

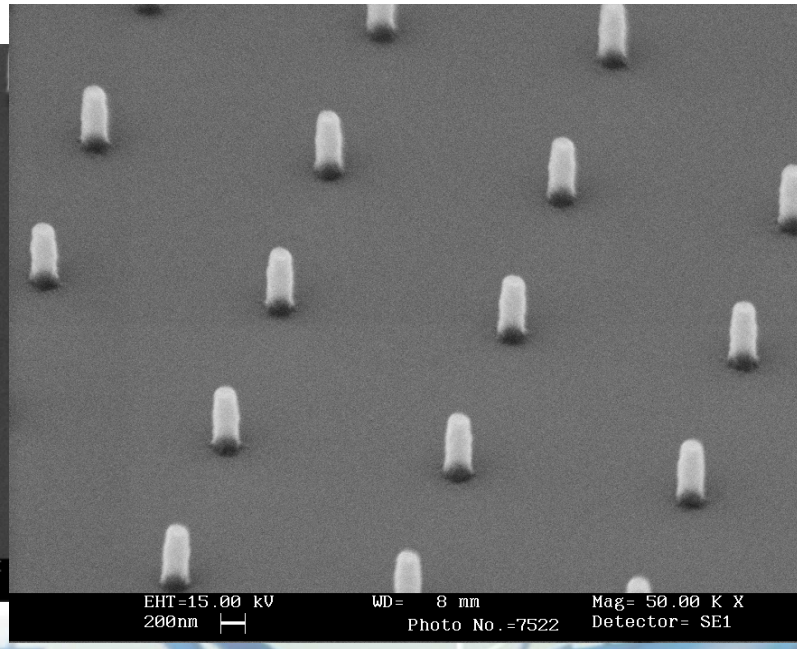
E-Beam lithography

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





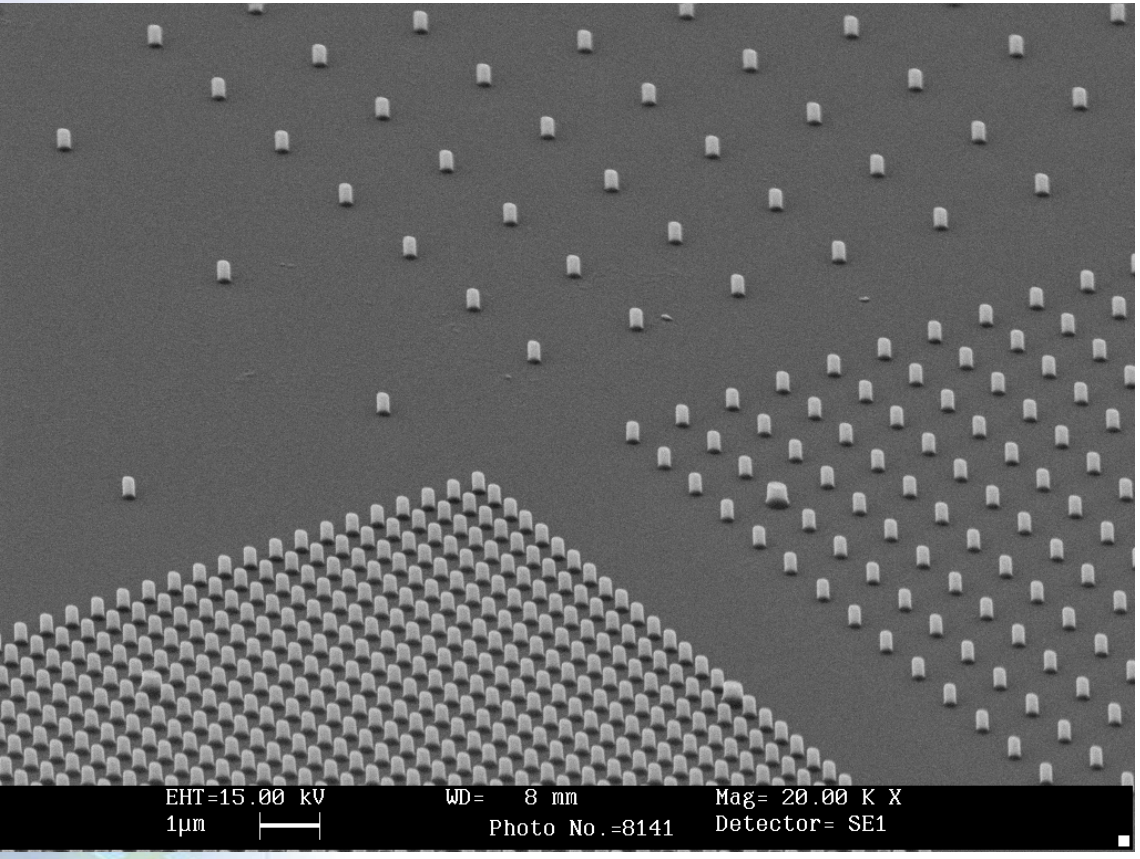


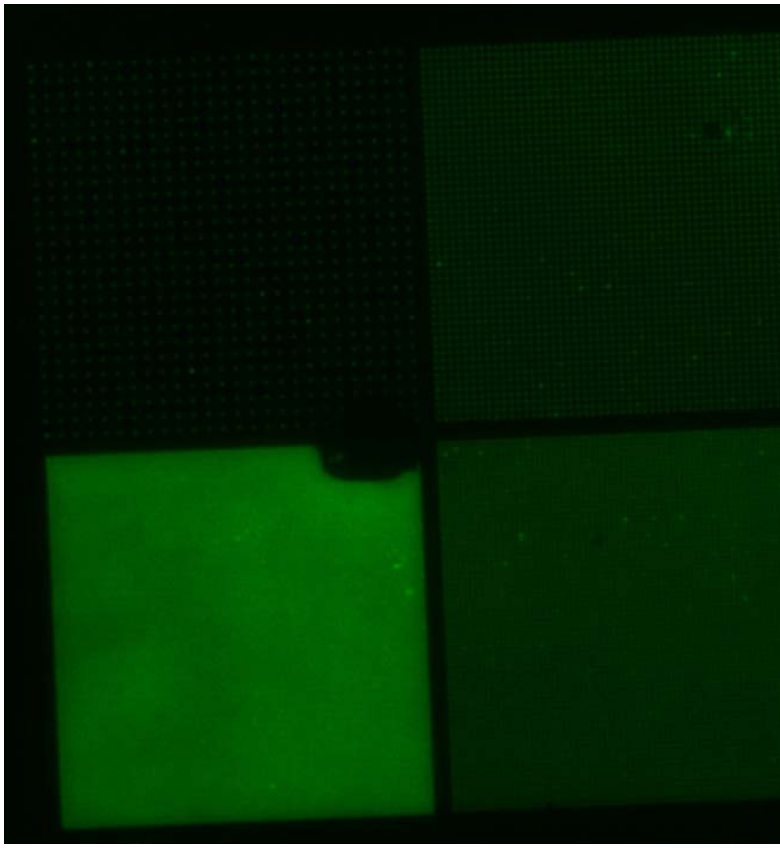


Protegnol et al. Adv. Materials 2007 (19) 1247-1250

Chemical contrast

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

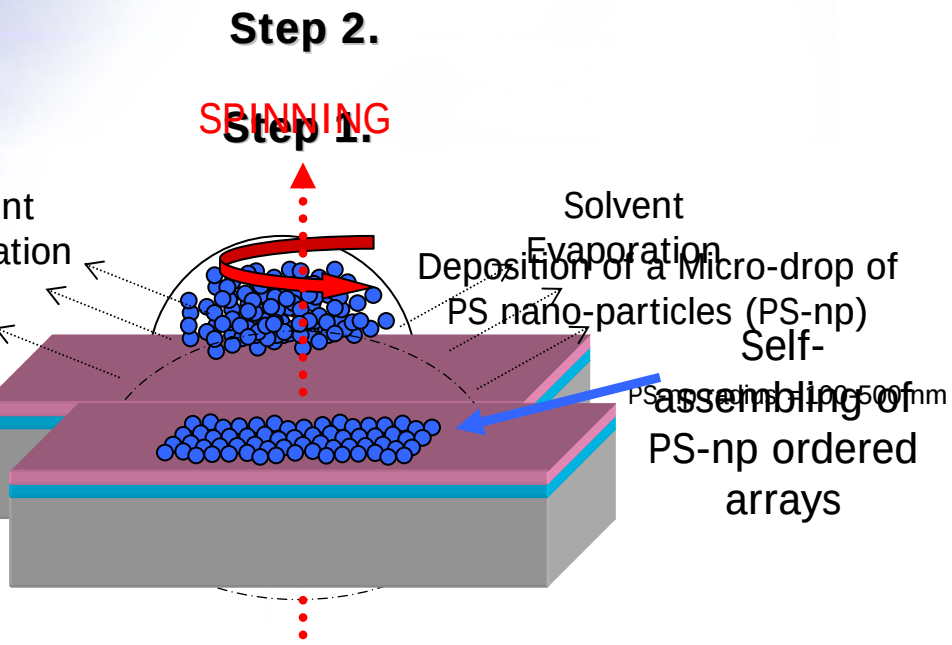


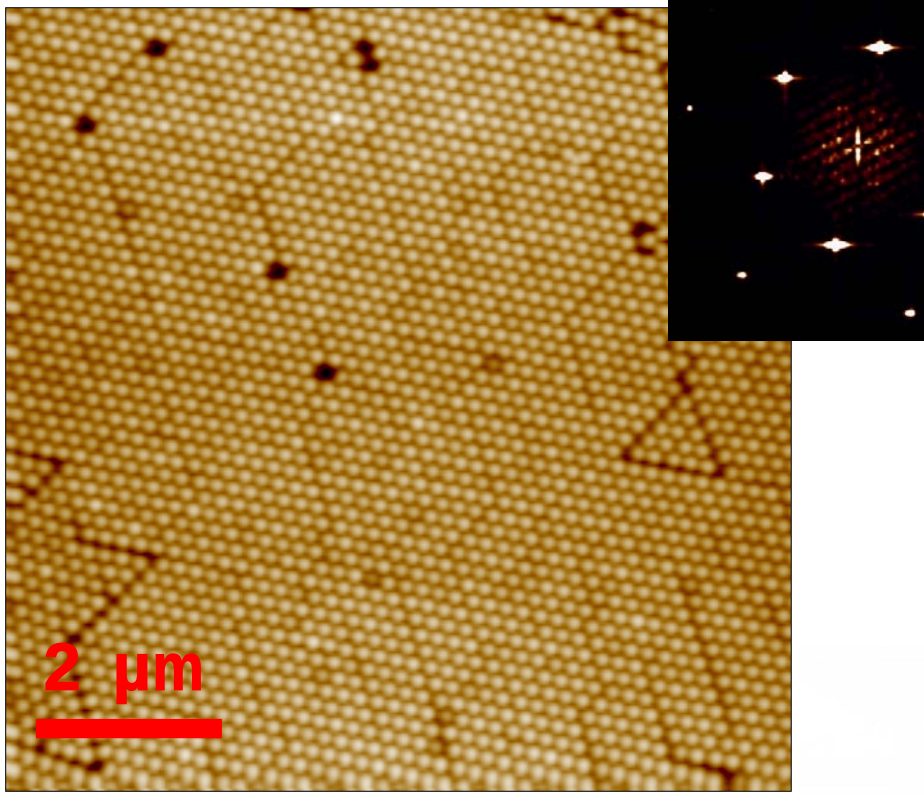


Nanopillars (h= 100nm, d= 100 nm) of stabilized PAA on PEO-like

Colloidal lithography

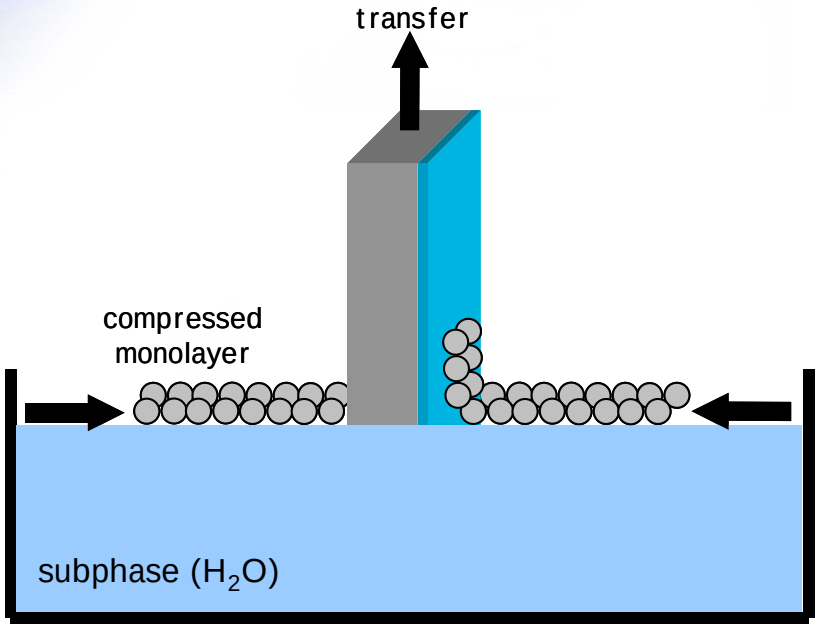
* Dispersion in MetOH + Triton
* 800 rpm

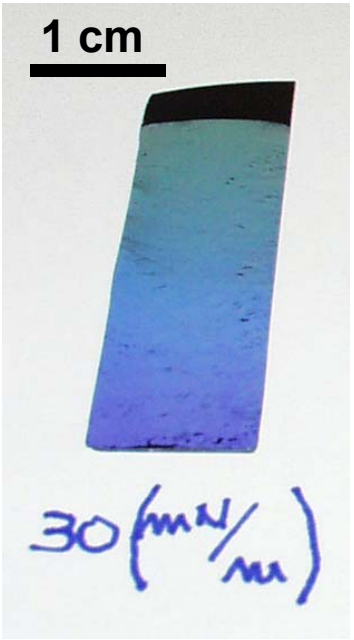


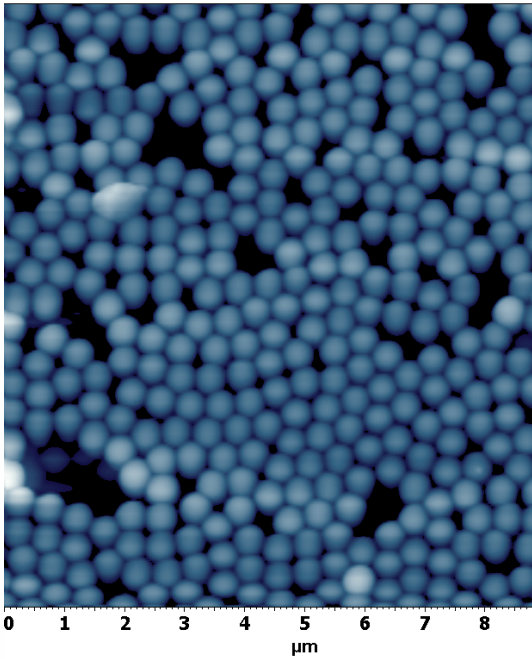


Fast and cheap. Surface coverage up to 95 % over mm

Langmuir-Blodgett

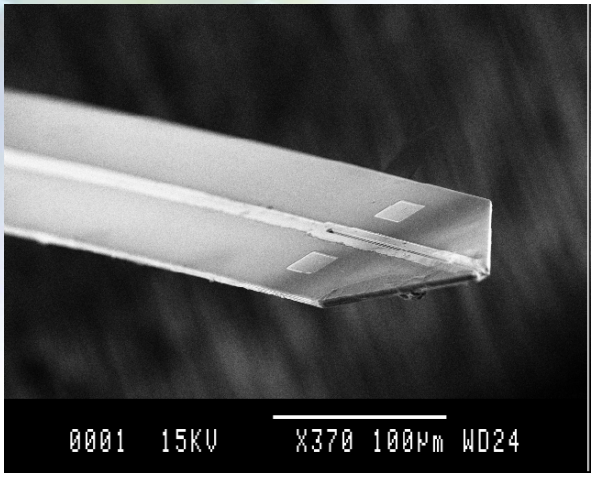


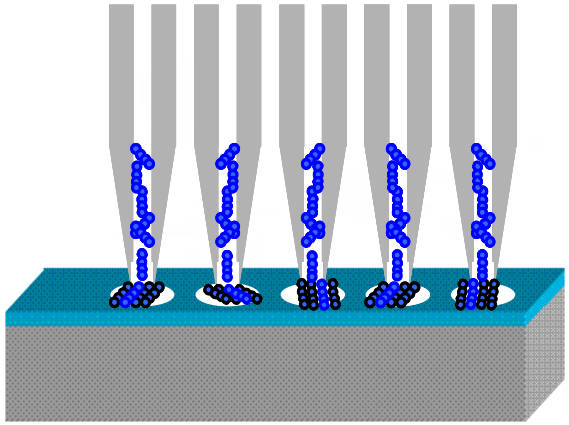




Surface coverage = 100% over cm^2.
2D monolayers. Many defects

Micro-Spotting

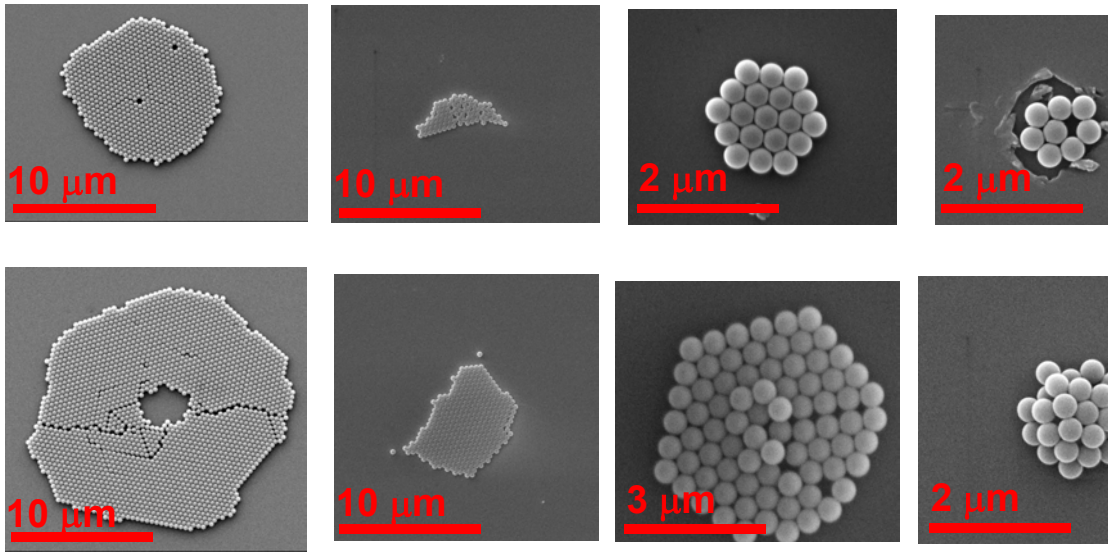




0.1 s

5 s

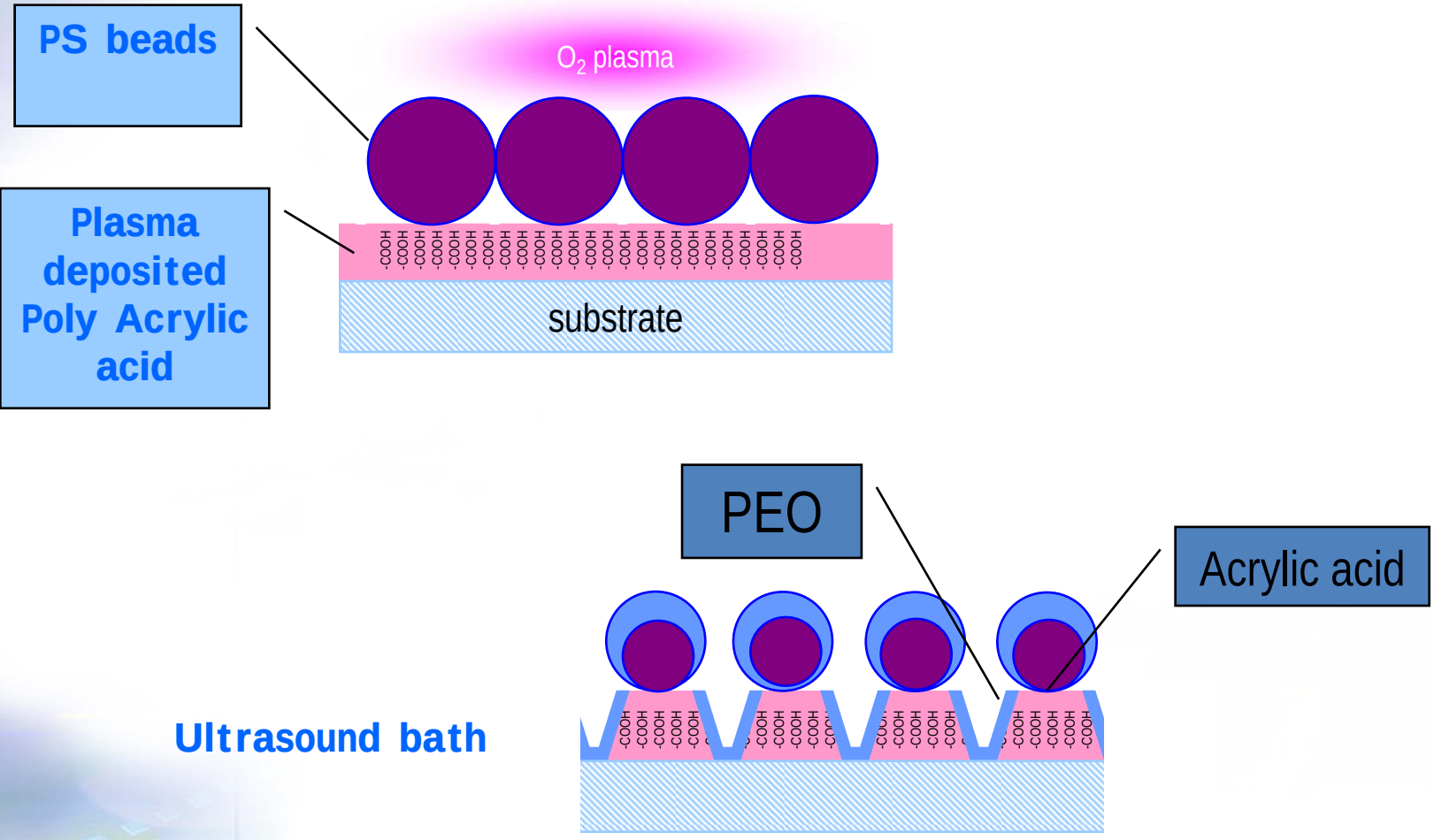
PAA (CA = 50°) MHD (CA = 60°) PAL (CA = 70°) CF_x (CA =



Spot size and nanostructure depend on surface wettability.
Possibility to create "nanostructured micropatterns"

Valsesia et al. ***Small,*** 4 (2006) 1444-1447

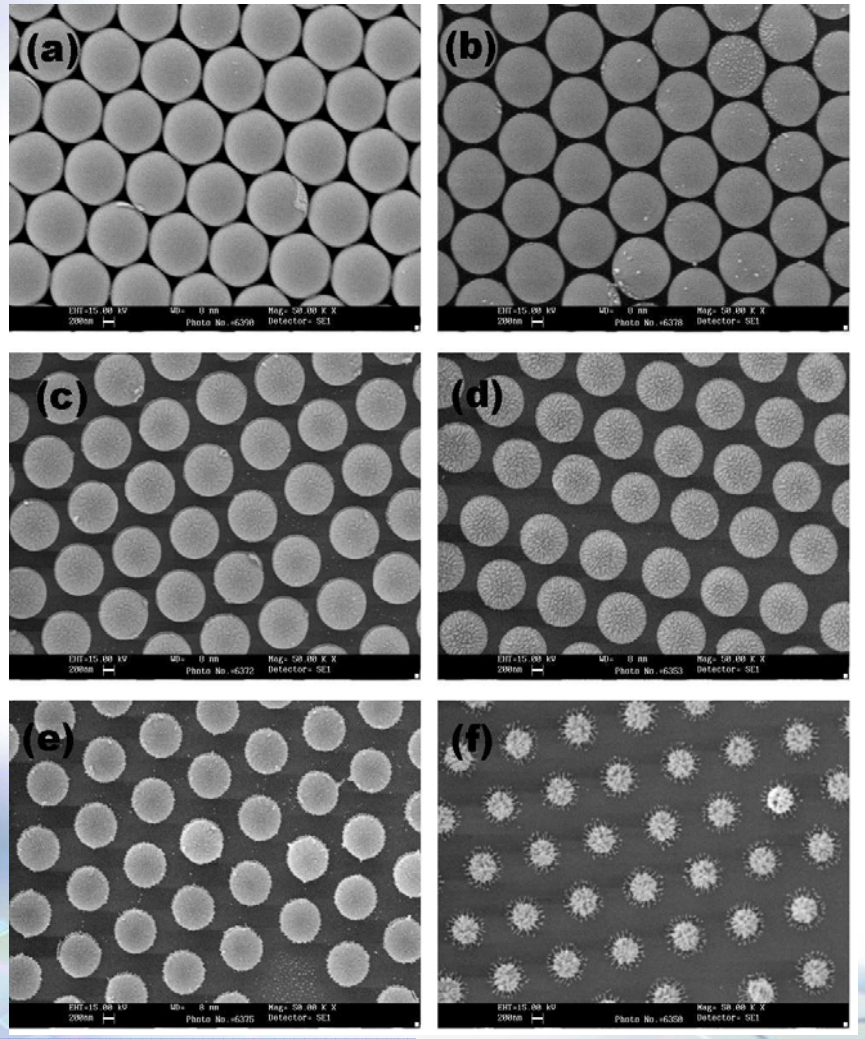
Colloidal Lithography + Plasma Polymers (self-assembly+top-down)

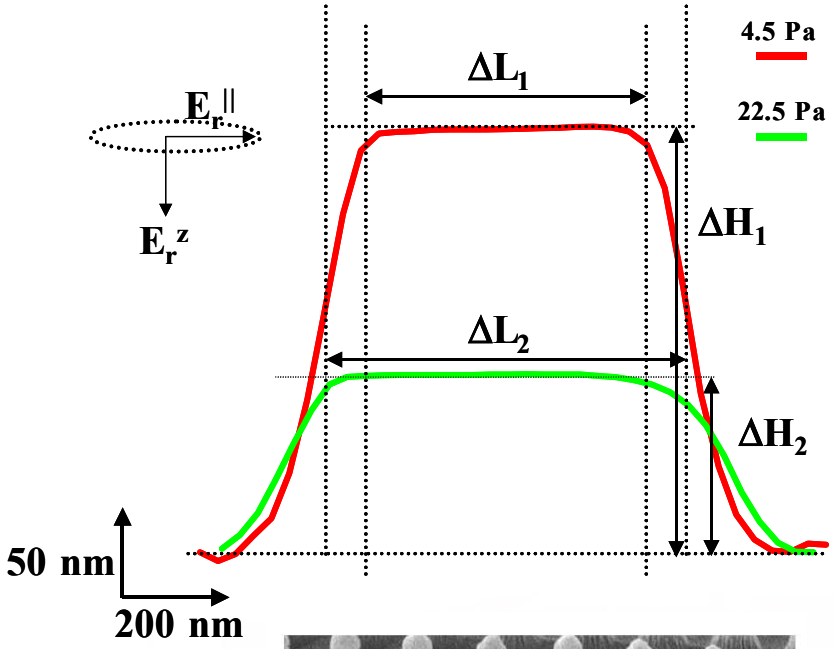


Valsesia,Colpo,Meziani,Manso, Ceccone,Rossi *Nano Lett.*, 4 (2004), 1047-1050
Valsesia,Colpo,Meziani,Bretagnol,Garcia,Bouma,Rossi., *Adv. Func. Mat. ,16* (2006),1242-1246

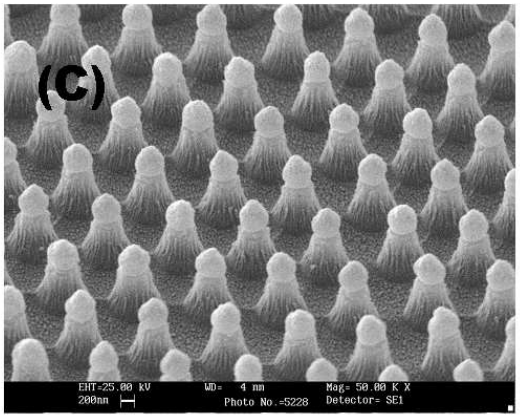
Colloidal Lithography: plasma etching

* Low Pressure Process (< 30 mTorr) (for anisotropic etchir
* Inductive-capacitive coupling (fast chemical etching for reducing processing
* Constant etching-rate

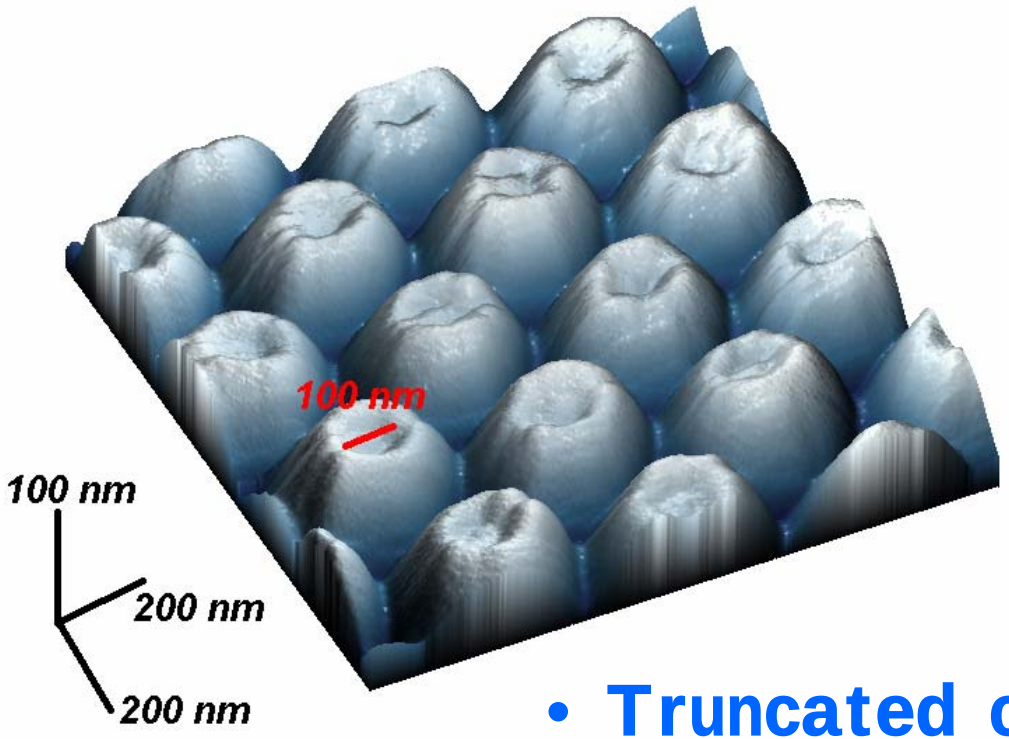


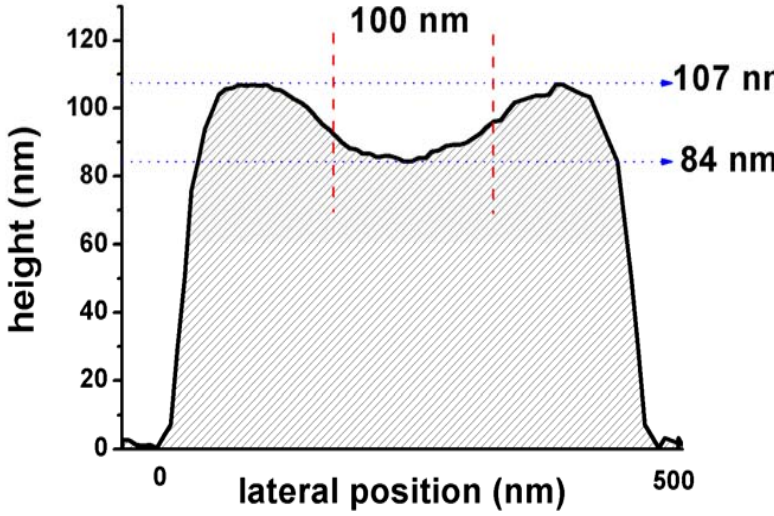


High aspect-ratio polymeric structures. Geometric Flexibility



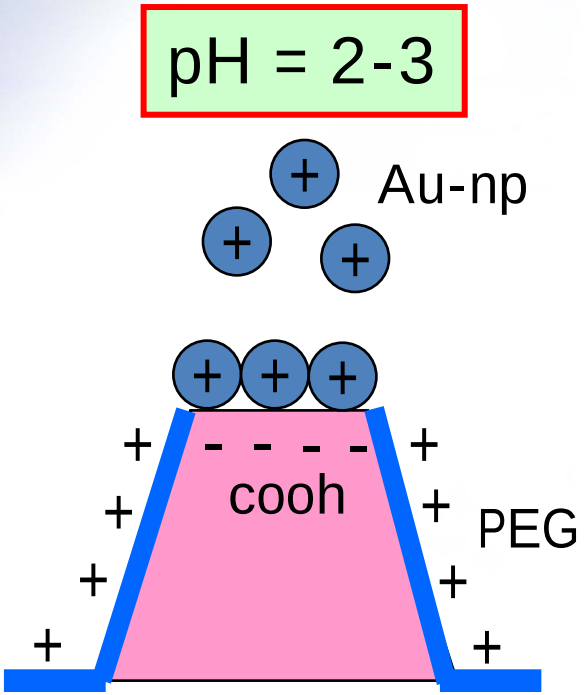
Morphological characterization (AFM)



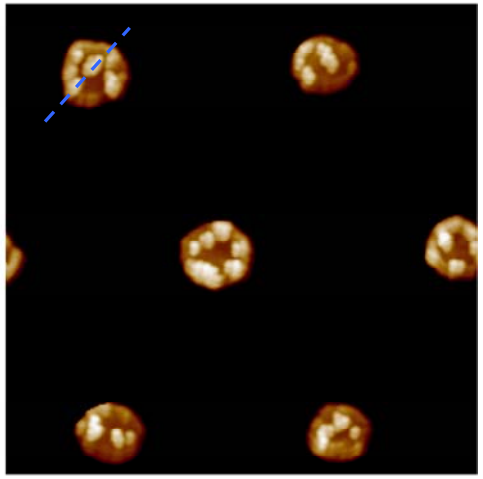


- **Truncated conical shape**
- **100 nm – wide -COOH plateau**
- **500 nm – lattice constant**
- **200 nm - high**

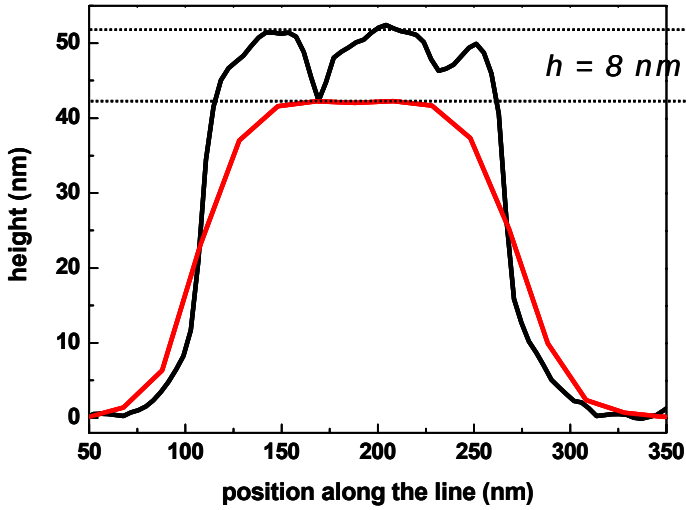
COOH-functionalized area of 1ND and measured chemical contrast



(a)



(b)



Gold nanoparticles are selectively absorbed on the COOH areas

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

(approx. 100 nm in diame

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

1- Antibody immobilization

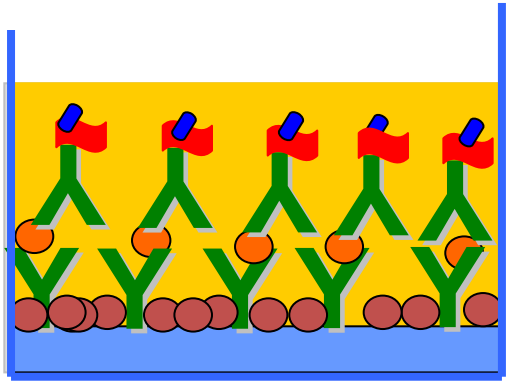
2- Blocking Step (prevent unspecific binding)

3- Antigen Recognition (Analyte)

4- Labeled Antibody (Enzyme linked)

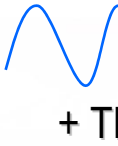
5- TMB (changes the colour of the solution)

6- Measure colour change ⇨ Proportional to Recognised Antigen concentr

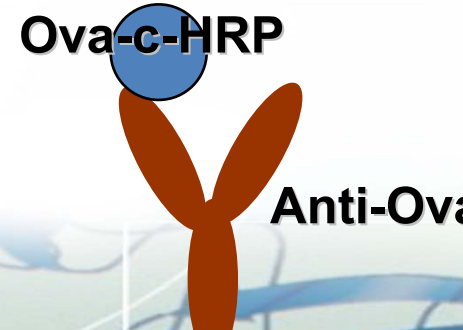


Application of nanostructured surfaces?

Absorbance @ hv 450 nm

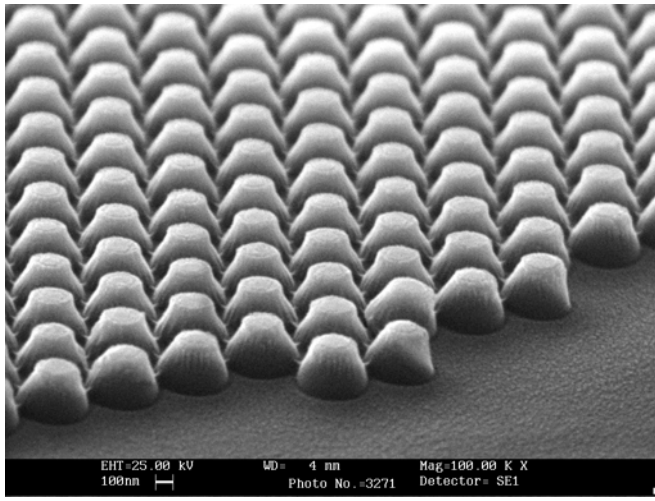


+ T



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

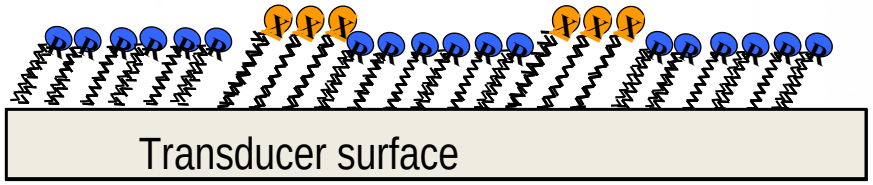
Immunosensing performance: ELISA assay



3-times larger signal from the nanostructures

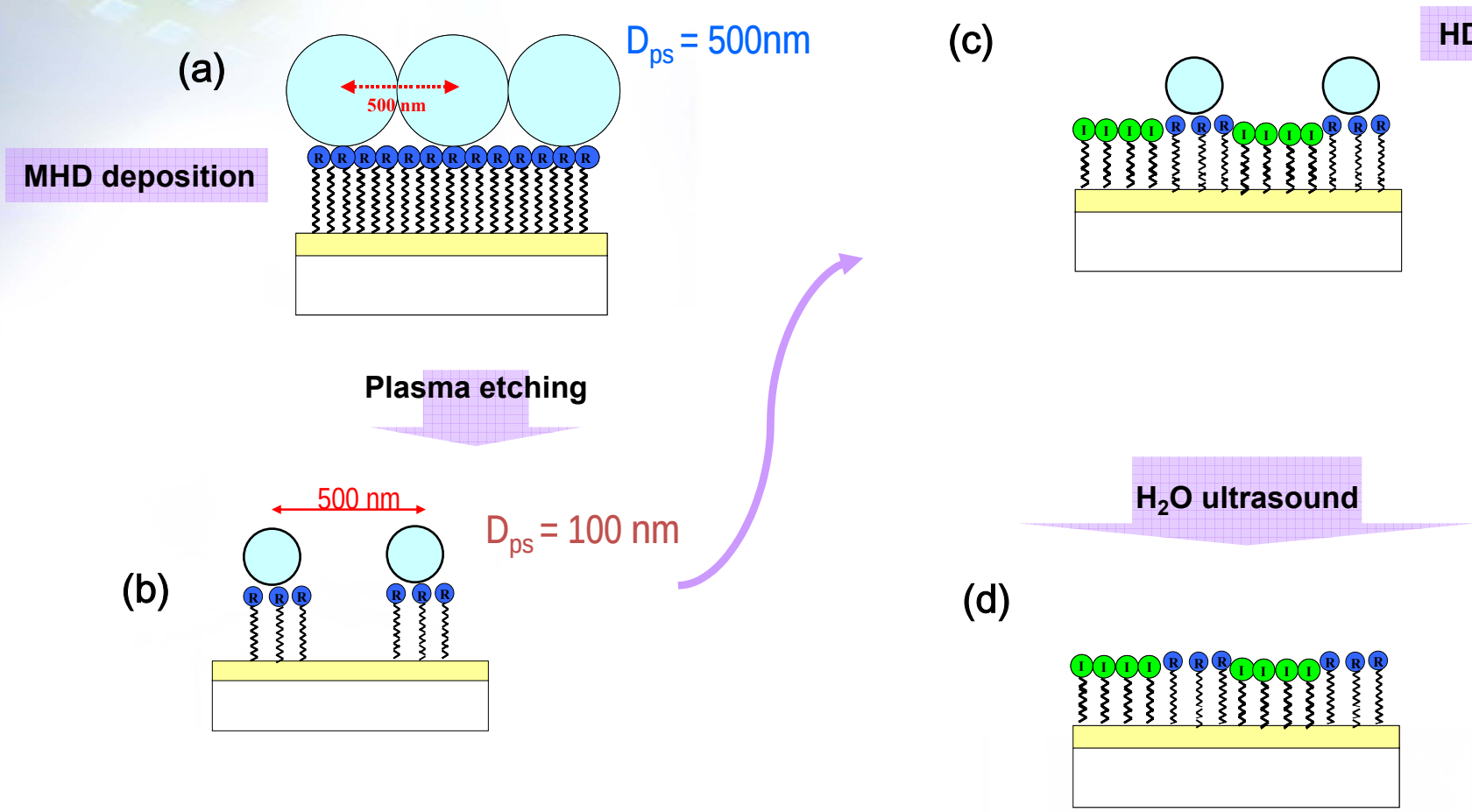
SAM's and colloidal lithography

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



X –COOH
R -CH_3

Proposed Method: SAM + Colloidal Lithography

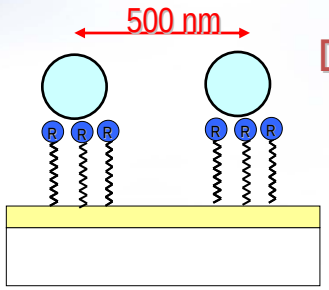


	MHD	MHD/Gold
Contact angle	35	26

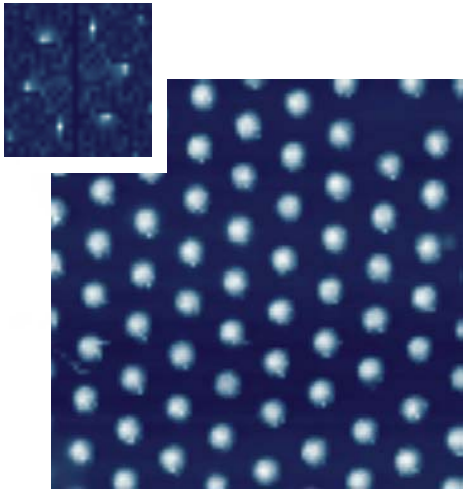
HDT/MHD	HDT
96	103

AFM measurements

(b)

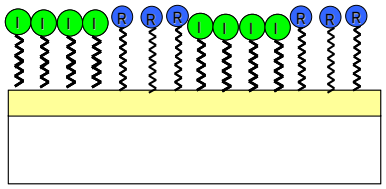


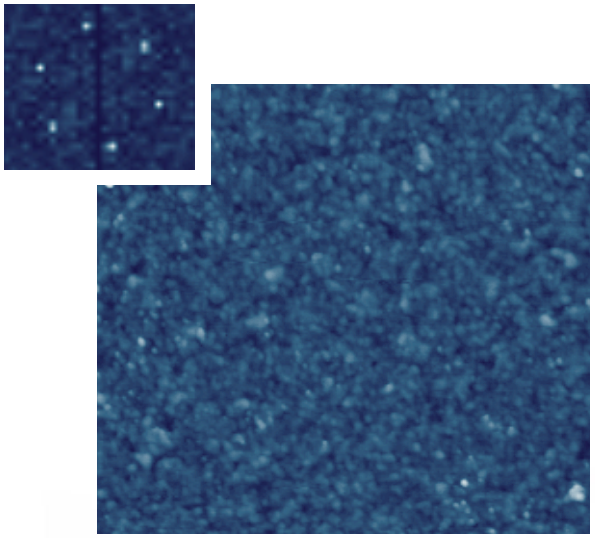
D_{ps} = 100 nm

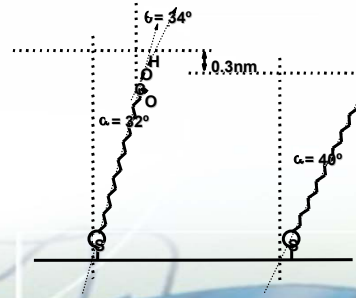


400 nm

(d)

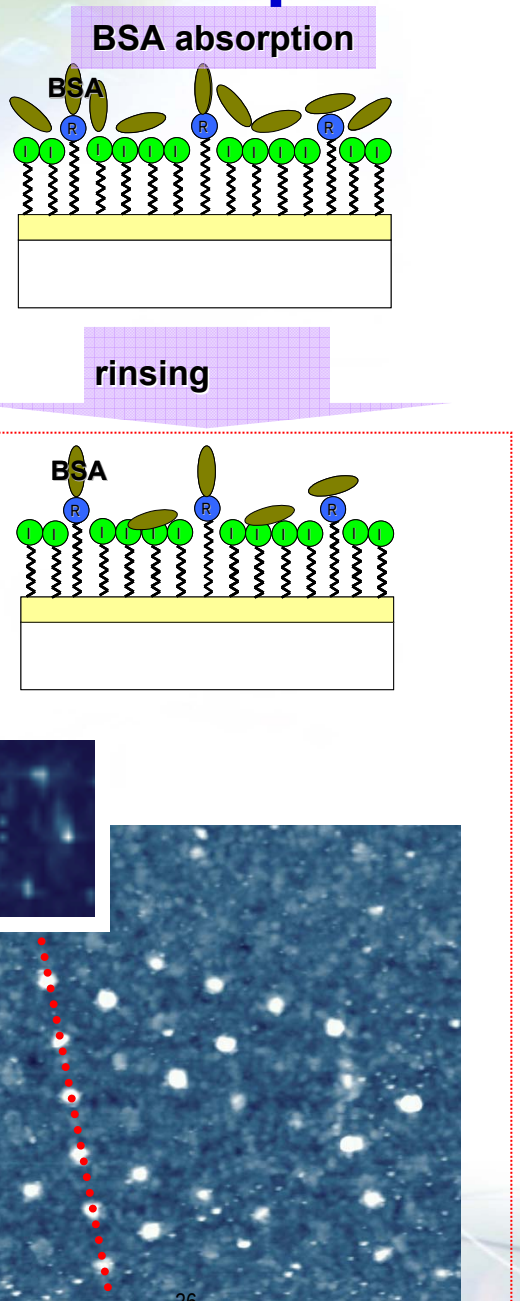




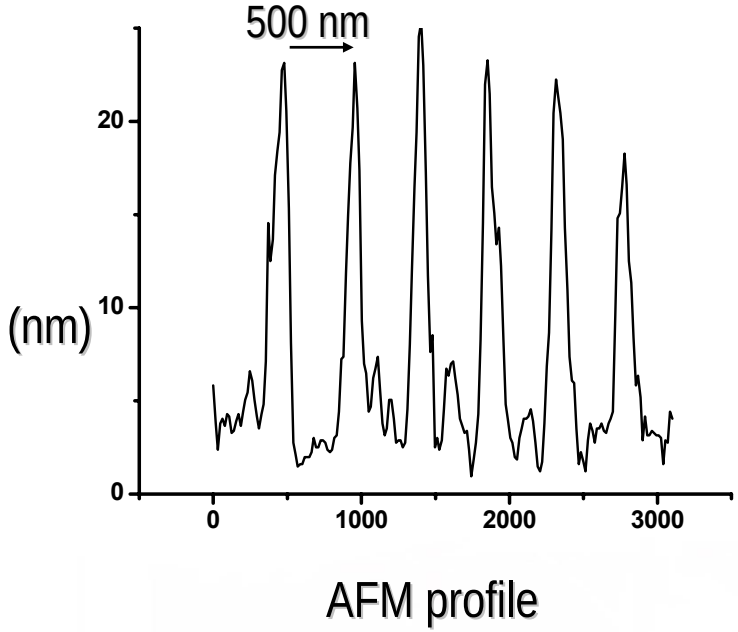


2D crystalline organization is maintained

BSA absorption on MHD/HDT nano-patterr



Preferential absorption on the MHD spots

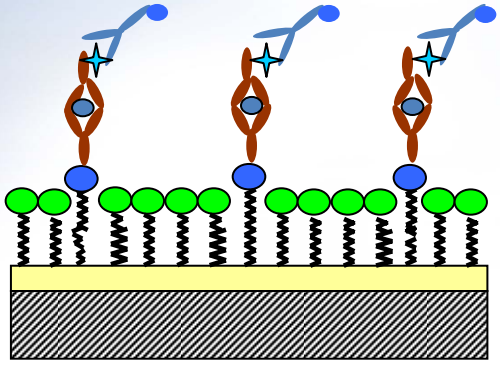


AFM profile

Two height distributions : 17nm ±2
5 nm ±2

~ BSA dimension (14 x 4 nm)

Enzyme Linked Immunosorbent Assay (ELISA)



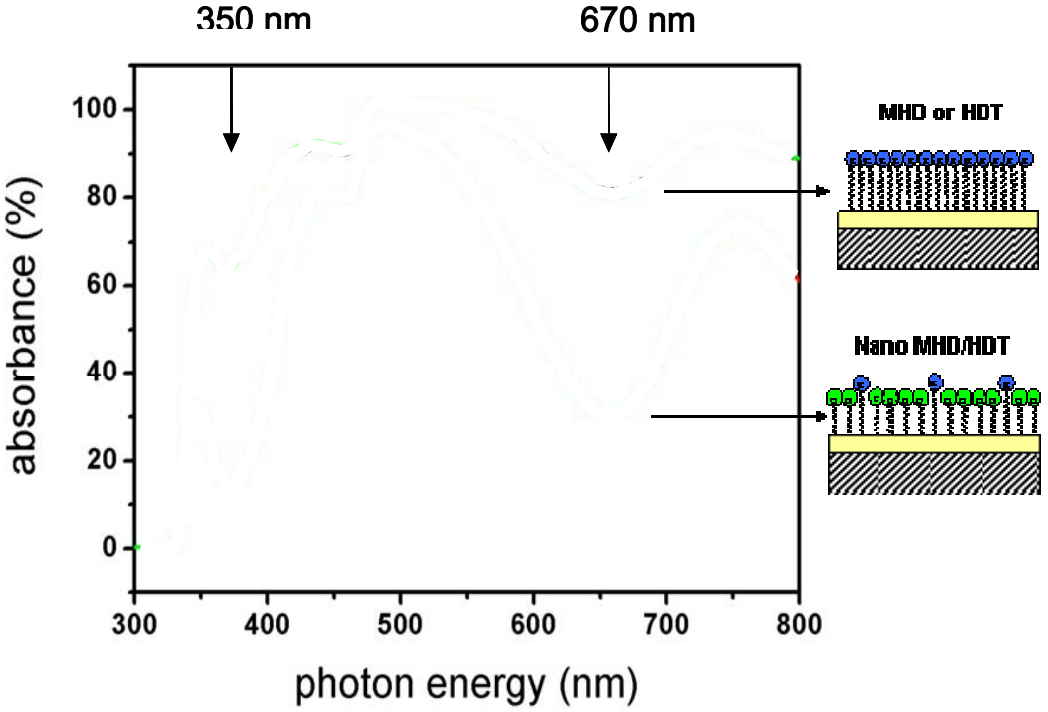
Il-1β Elisa tests

Antibody

Antigen

biotinylated Ab

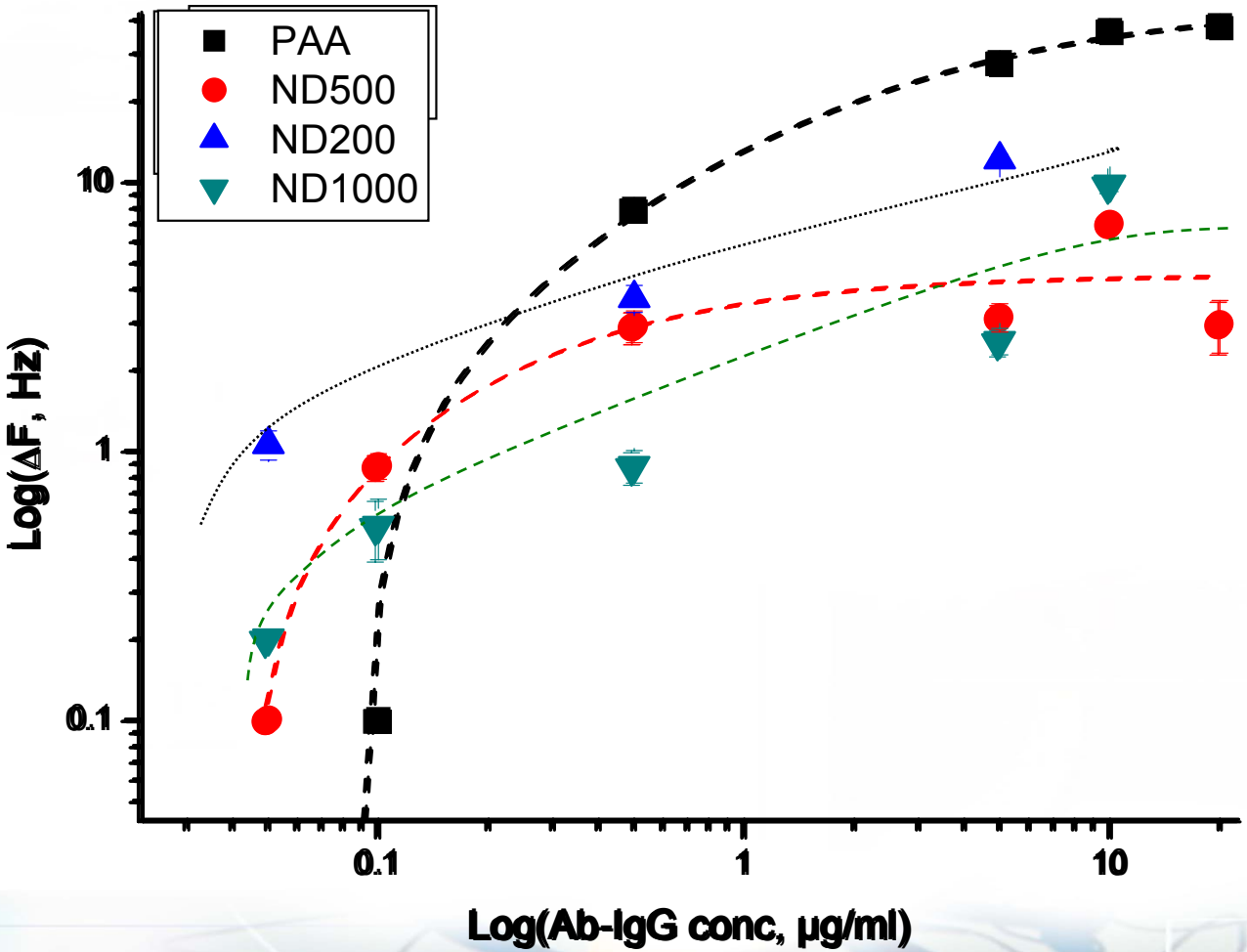
Steptavidin labelled Enzyme (Horse radish peroxidase)
For chemiluminescent reaction



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

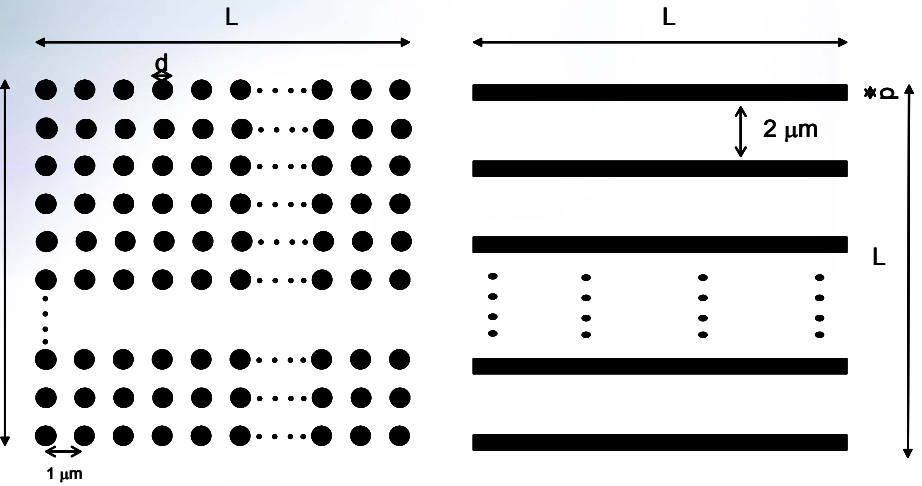
Calibration curves: QCM studies

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



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

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

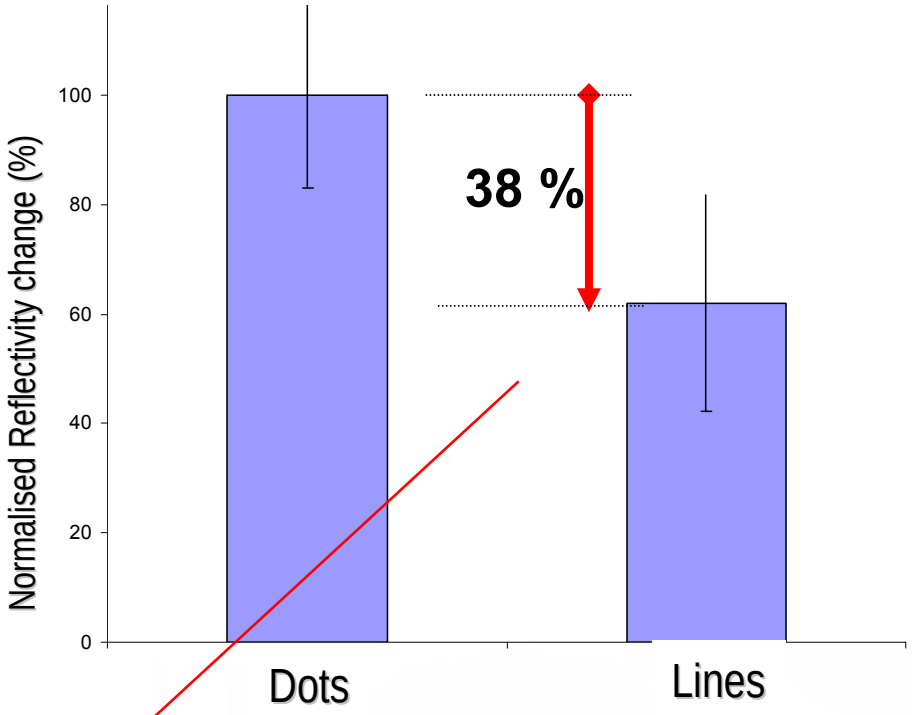
$$S_{line} = dL \qquad \sum_L S_{line} = d \times L \times \frac{L}{2} = d \times \frac{L^2}{2}$$

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

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

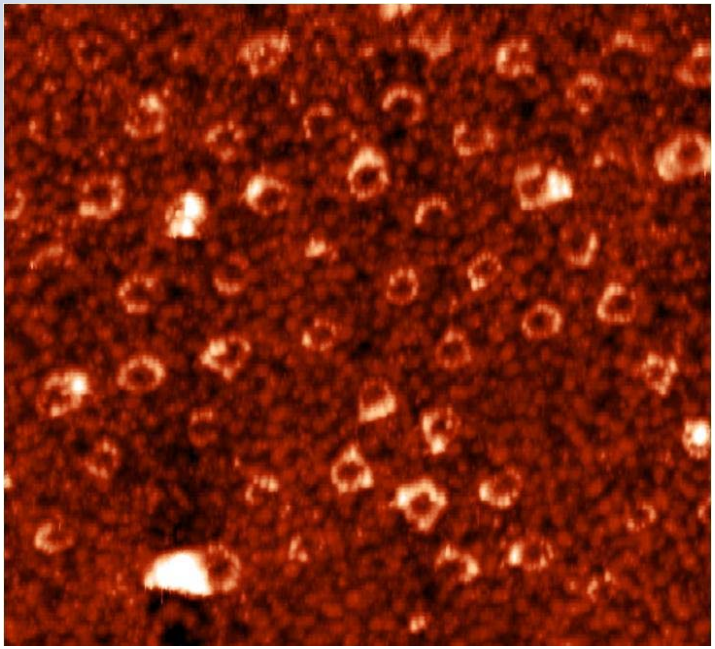
S/A=25% **S/A=33%**

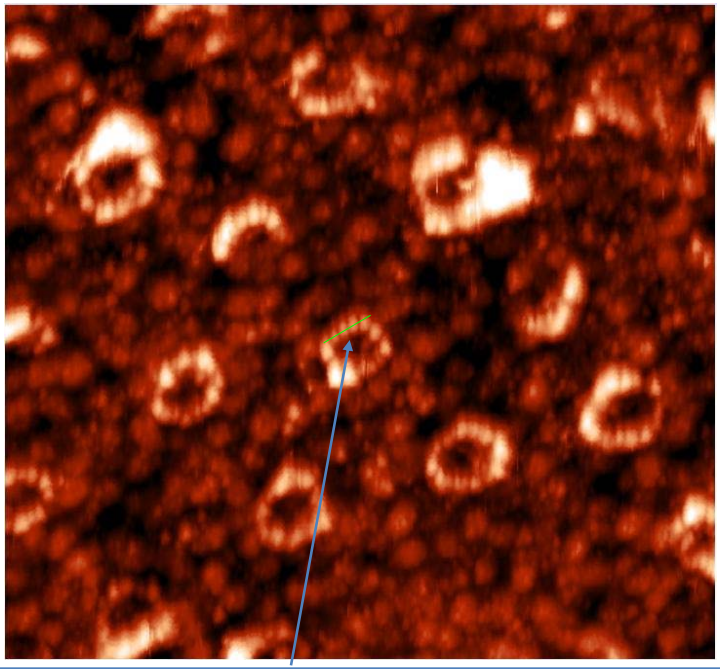
L/A = 1µm^{-1} **L/A = 0.66 µm^{-1}**

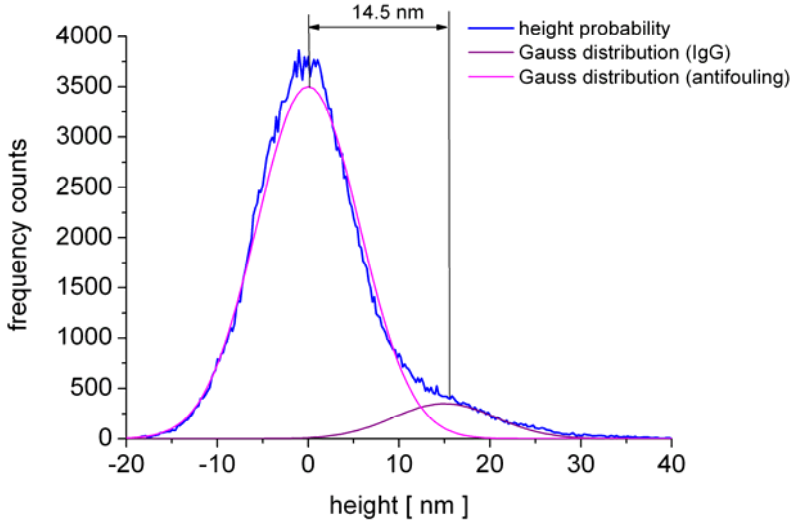


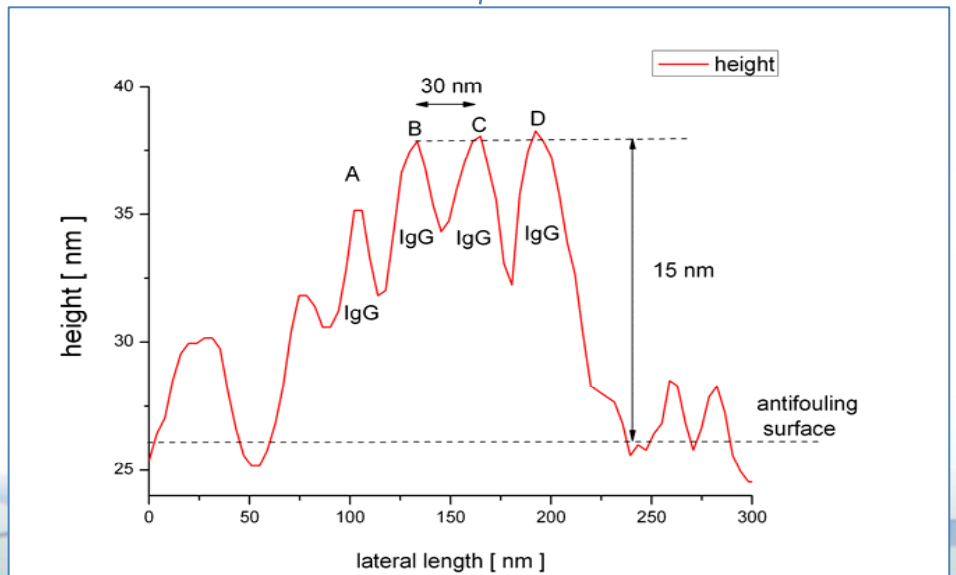
The signal intensity scales with the boundary length!

Protein distribution on nanopatterned surfaces



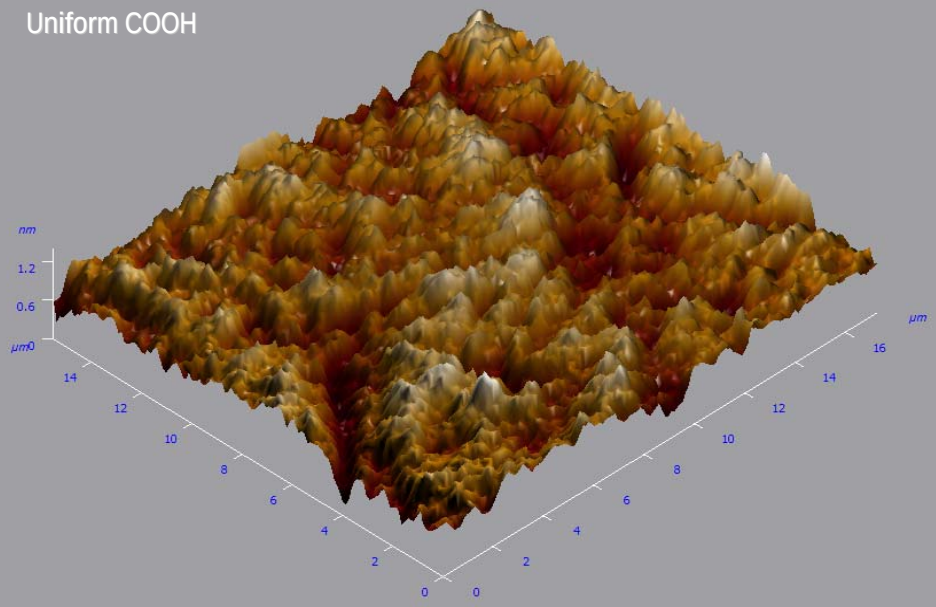




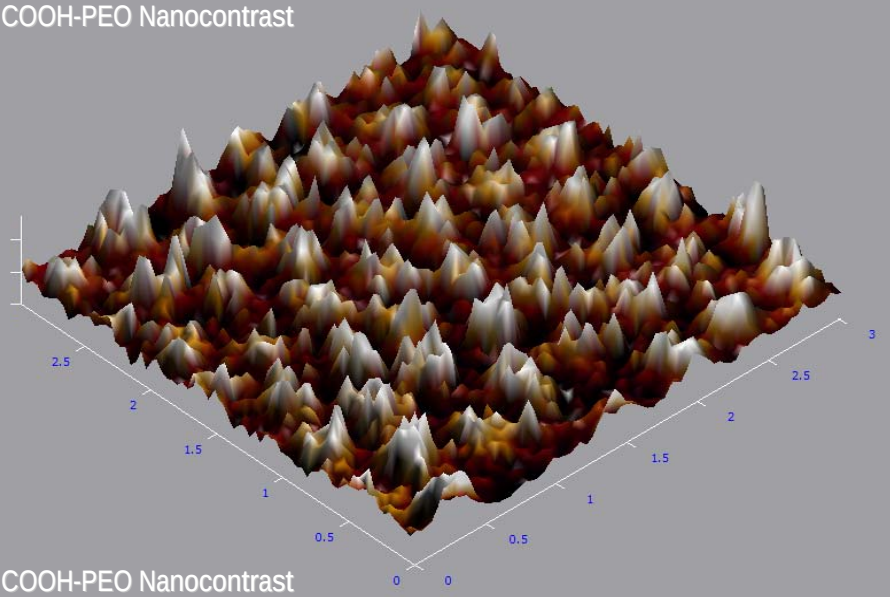


COOH-PEO contrast with covalent binding

Uniform COOH

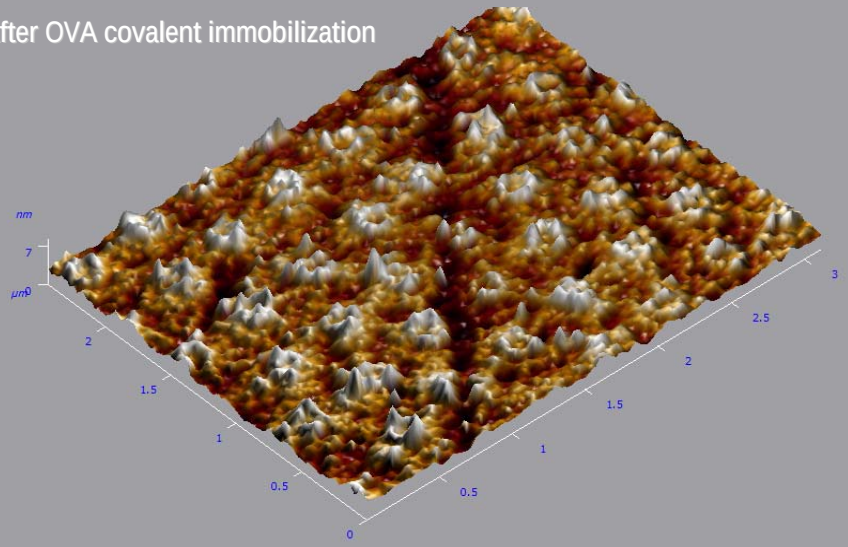


COOH-PEO Nanocontrast



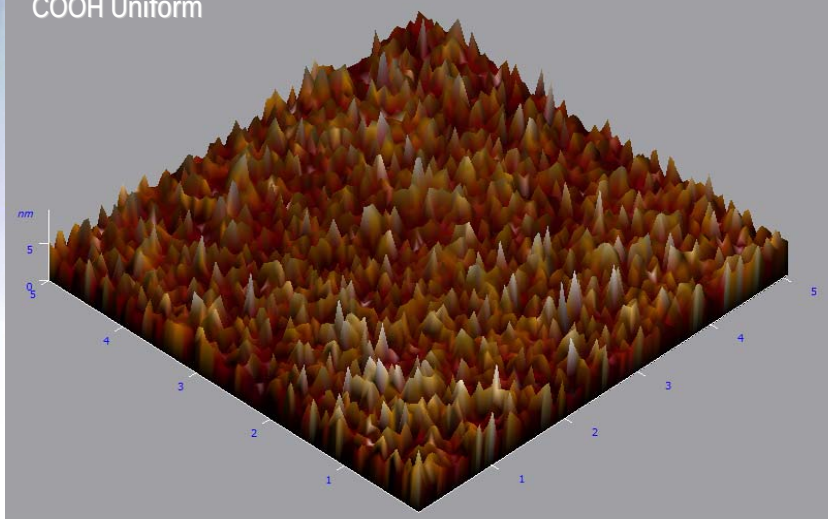
COOH-PEO Nanocontrast

After OVA covalent immobilization

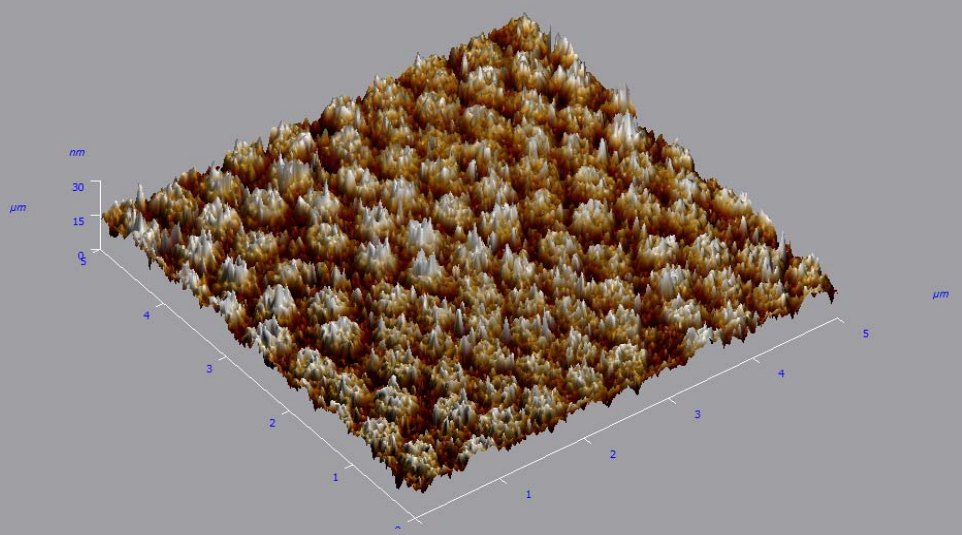


COOH-CH_3 contrast

COOH Uniform

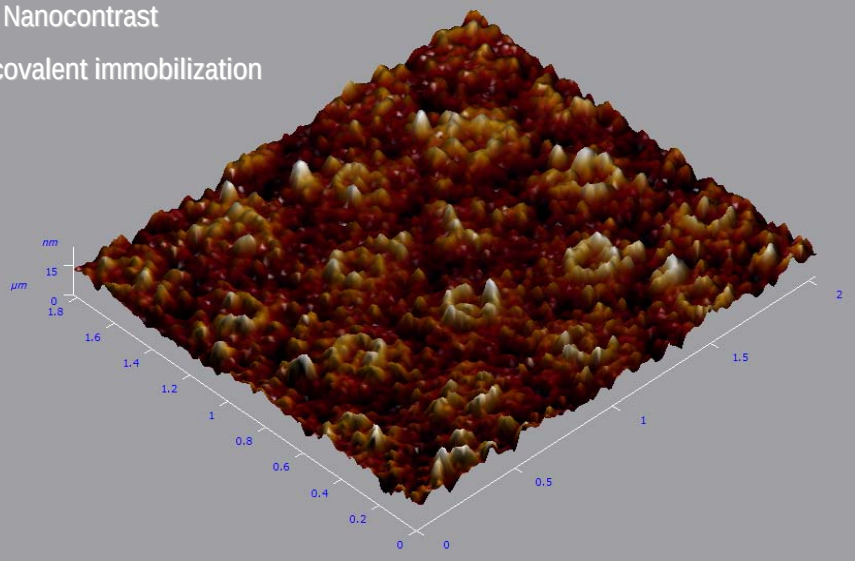


COOH-CH3 Nanocontrast

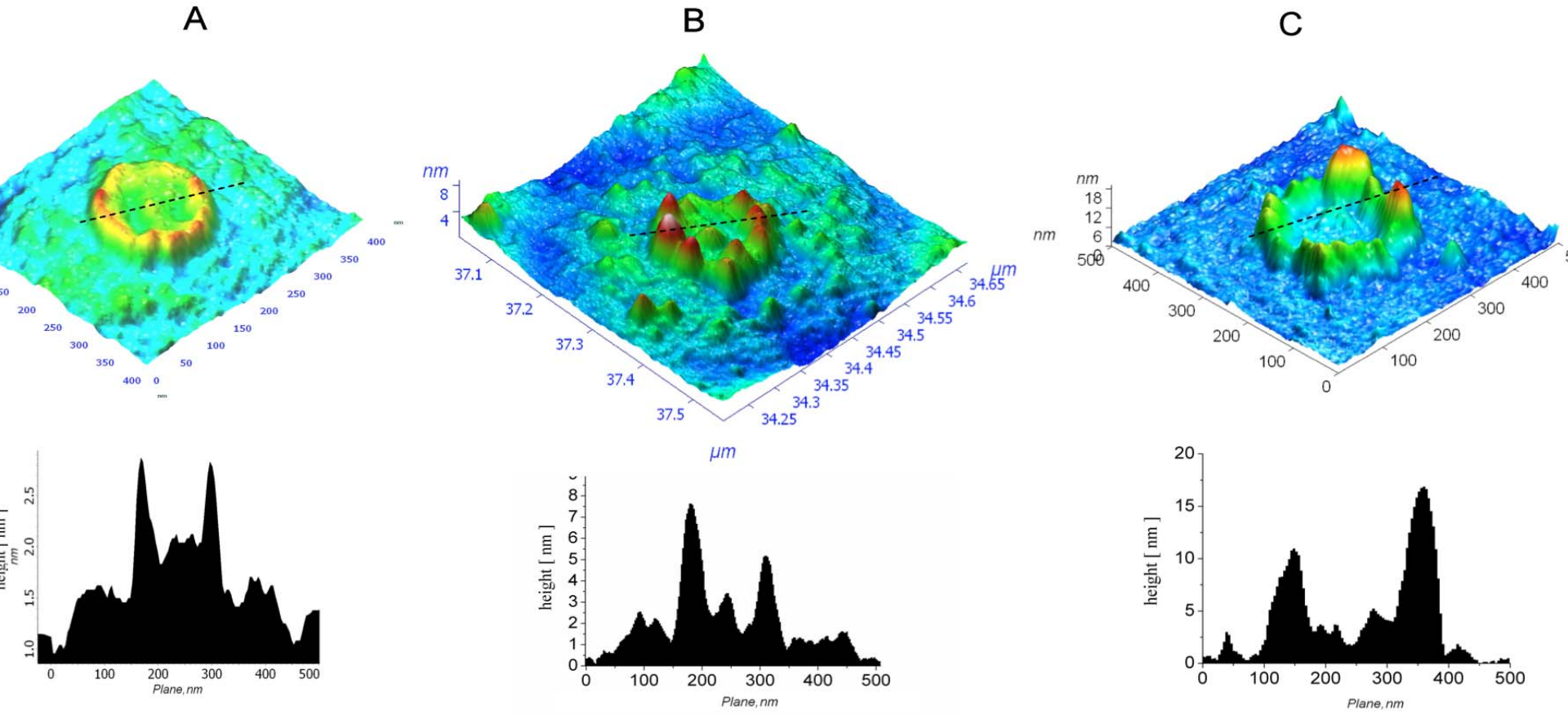


COOH-CH3 Nanocontrast

After OVA covalent immobilization



AFM analysis of an immunoreaction



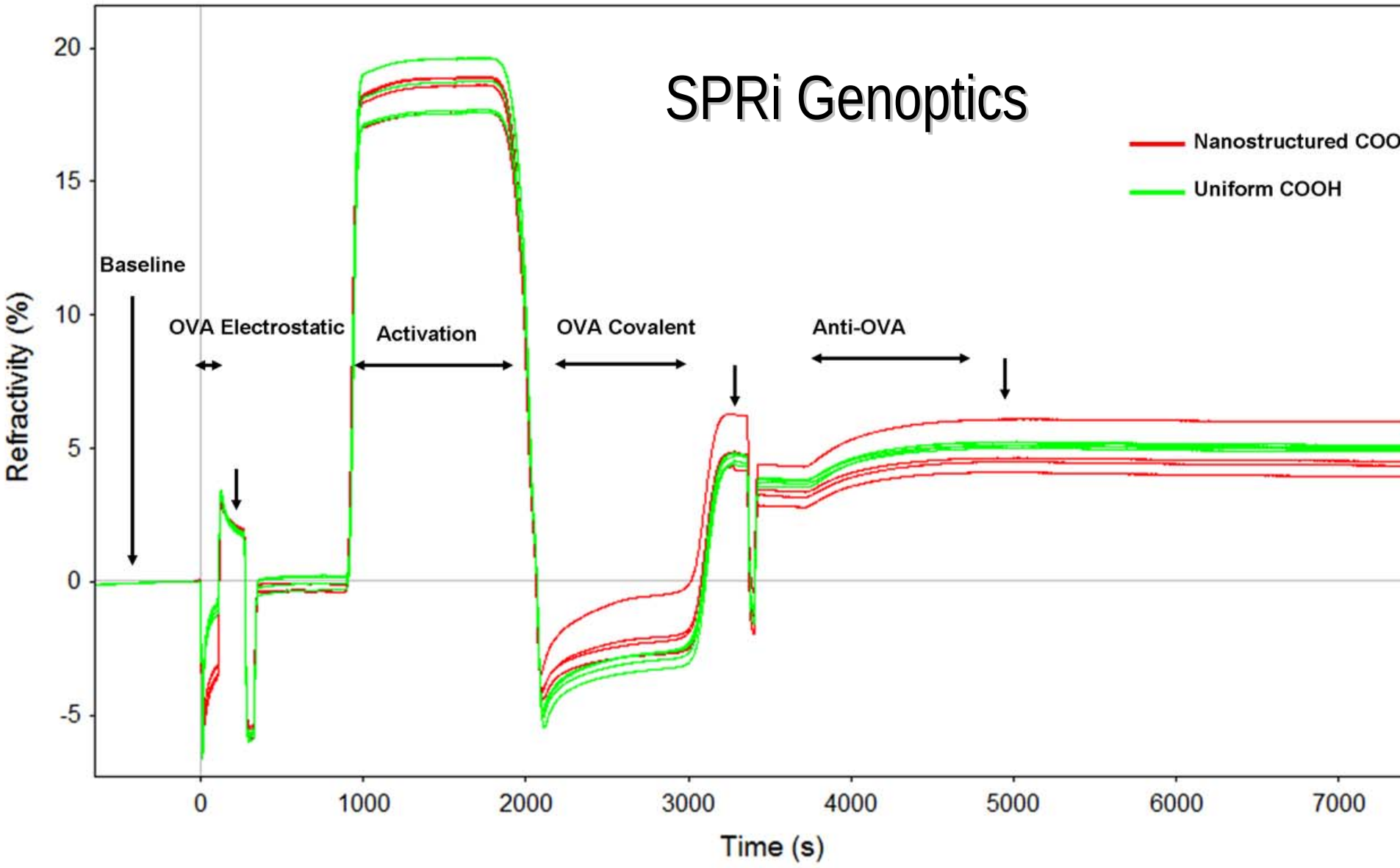
MHD/PEO nanospot

After IgG incubation

After Ab-IgG incubation

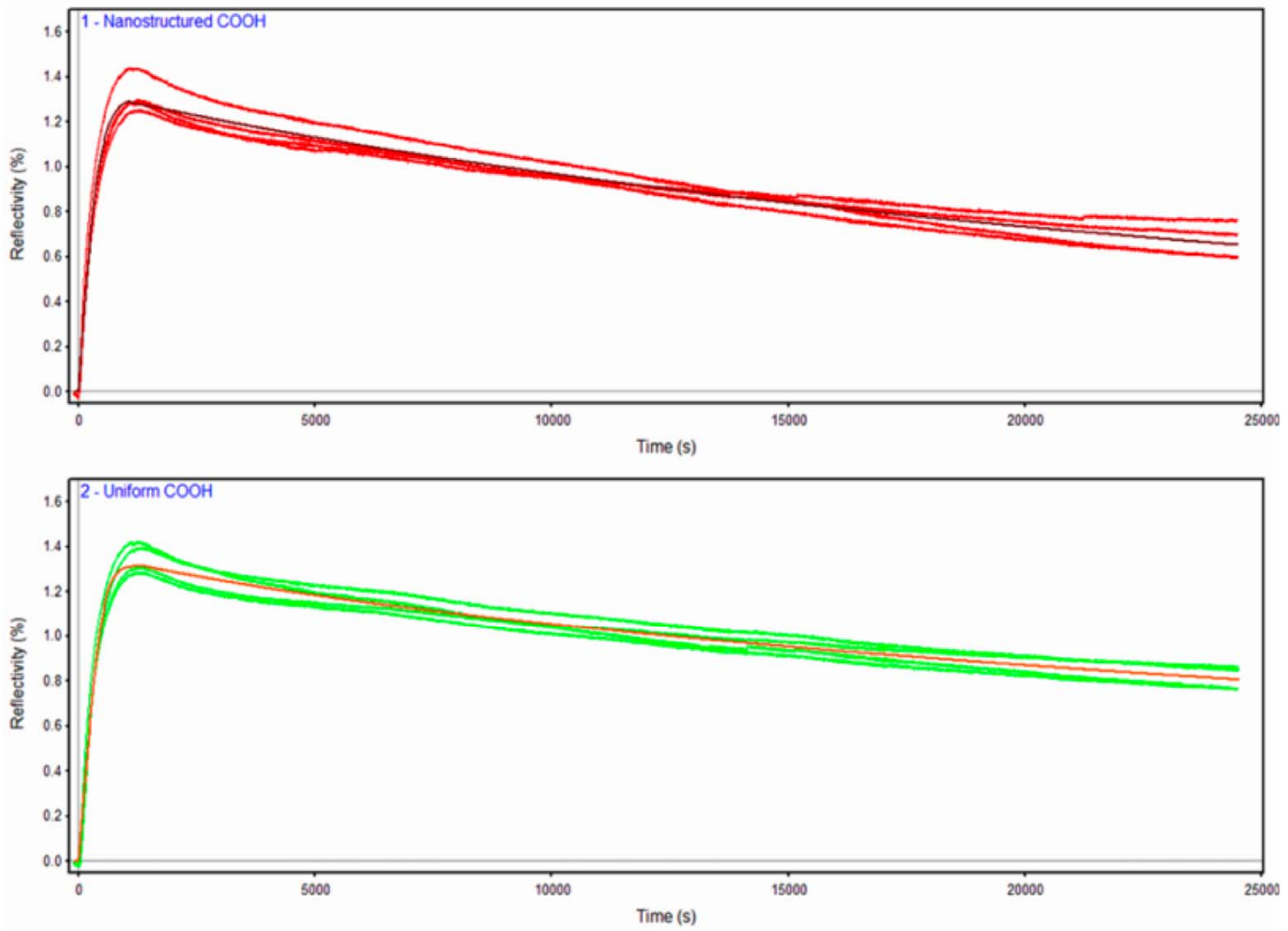
IgG-Ab IgG observed in dry state (AFM tapping mode)

Kinetics studies by SPR



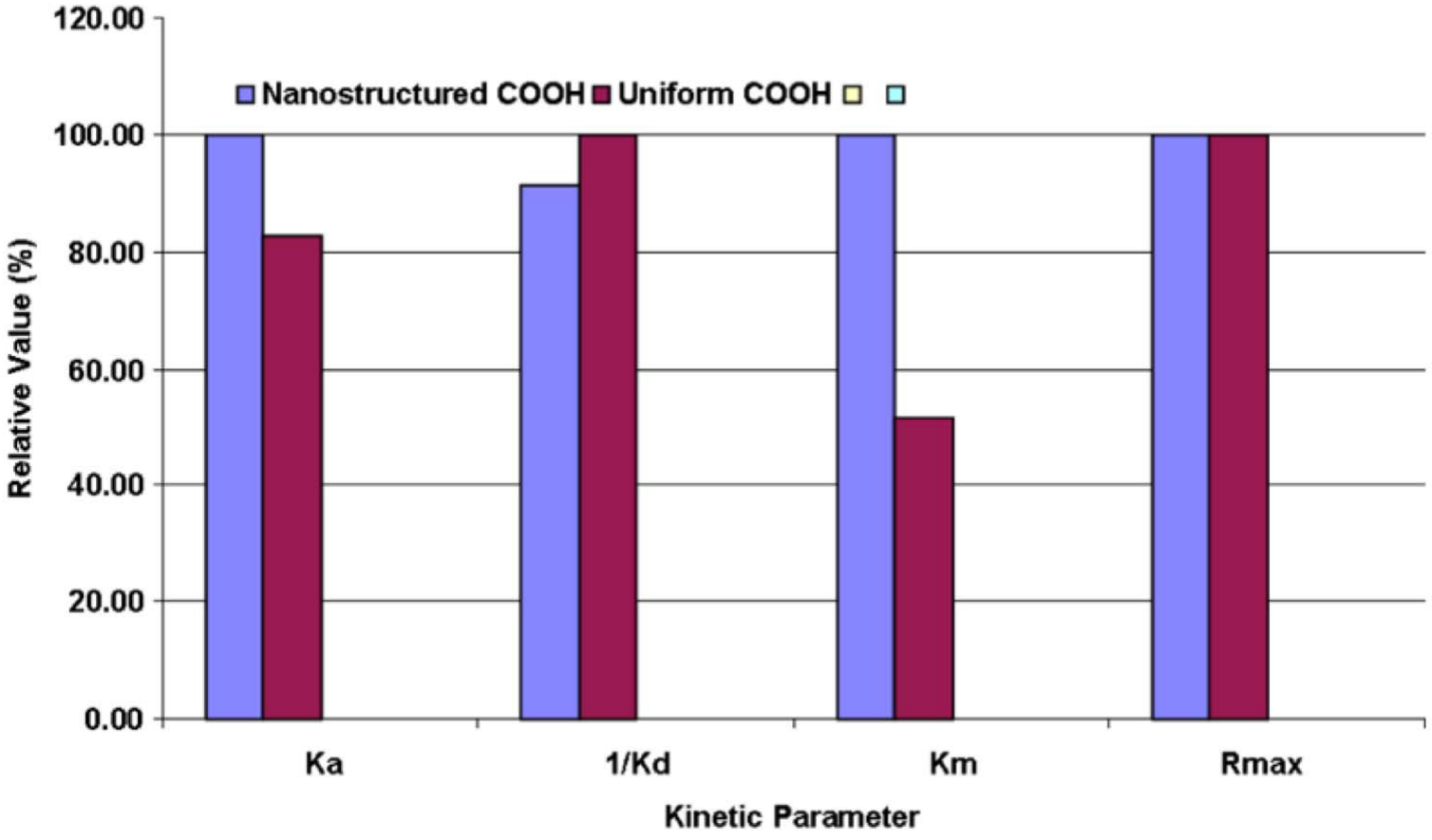
Adsorption kinetics modeling

- Model:
  - $A \overset{k_m}{\rightleftharpoons} A^* + B \overset{k_A}{\underset{k_D}{\rightleftharpoons}} A^*B$



Adsorption Kinetics analysis

$$A \overset{k_m}{\rightleftharpoons} A^* + B \overset{k_A}{\underset{k_D}{\rightleftharpoons}} A^*B$$



Conclusions

Amplification:

- Regular distribution of proteins at domain boundaries (low C)
- Reduction of steric hindrance
  - Better access of reactive sites
  - Works at low concentration
- Better orientation of proteins on surfaces?
  - Effect of boundary, local curvature? Largely unexplored
- Kinetics, thermodynamics: seem to present large advantage as compared to homogenous surfaces

Future work:

- Other contrasts to be tested
  - Hydrophobic/hydrophilic
  - Positive/negative
- Size /geometry effects
- Effect of nanostructure on reaction kinetics and conformational changes

Thanks

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