The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

**2268-19**

# Conference on Nanotechnology for Biological and Biomedical Applications (Nano-Bio-Med)

*10 - 14 October 2011*

## From Bio- to Nano-Interfaces

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**Supramolecular NanoMaterials and Interfaces Laboratory**

INSTITUTE OF MATERIALS - INSTITUT DE MATÉRIAUX

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सानमिल

ΣουΝΜιΛ

سنمل

# From Bio- to Nano-Interfaces

**Kislon Voitchovsky**
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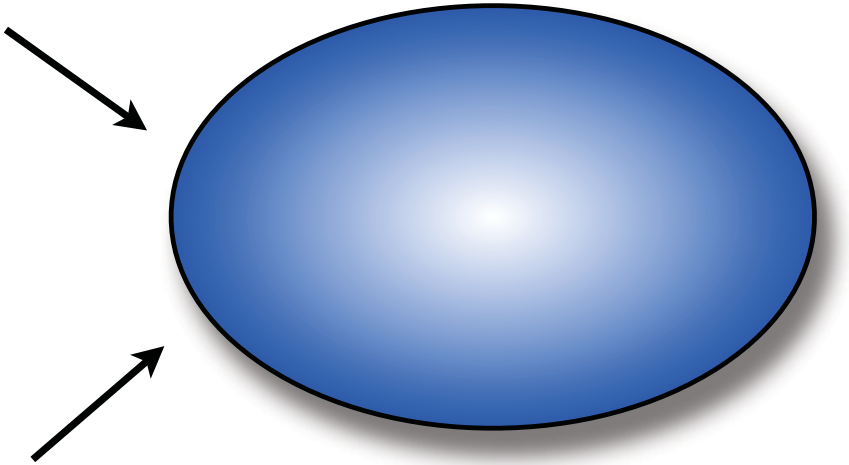
ICTP-KFAS *NanoBioMed* Conference, Trieste, 11 October 2011

***In nature***
molecules
protein
ions
lipid vesicles
...



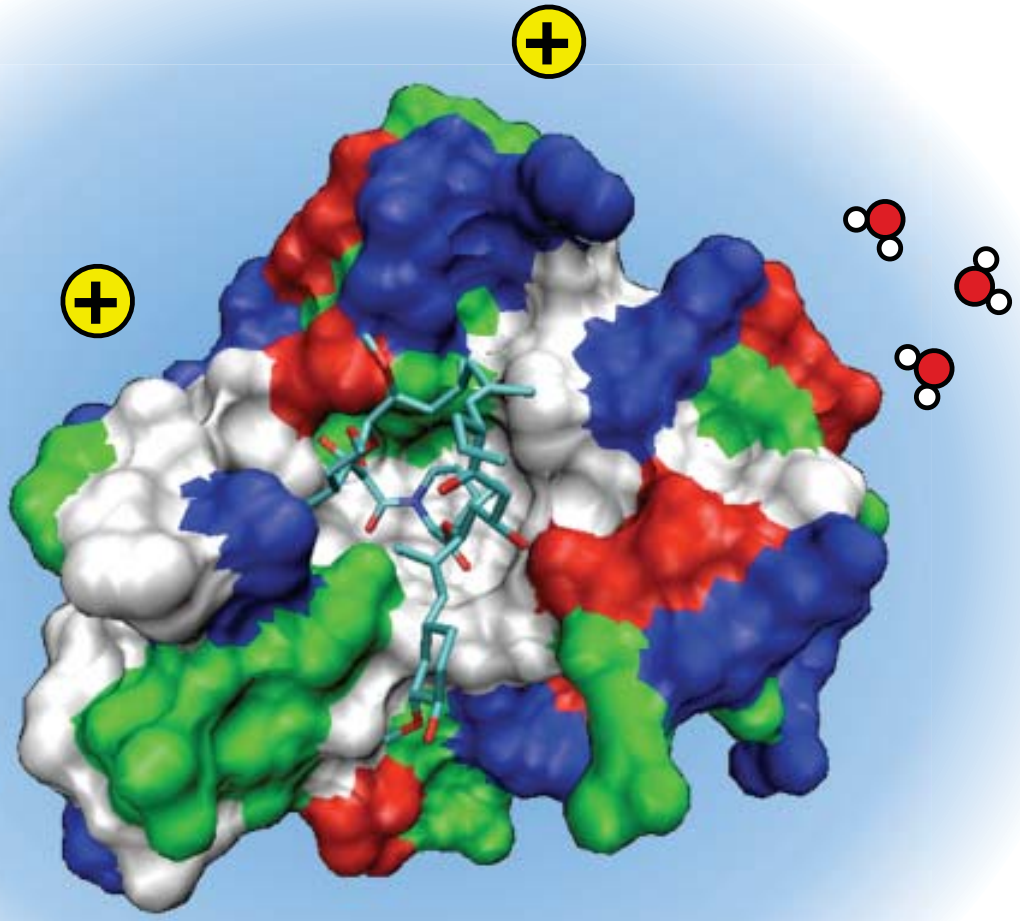
**biological process**

**biological material (cell)**

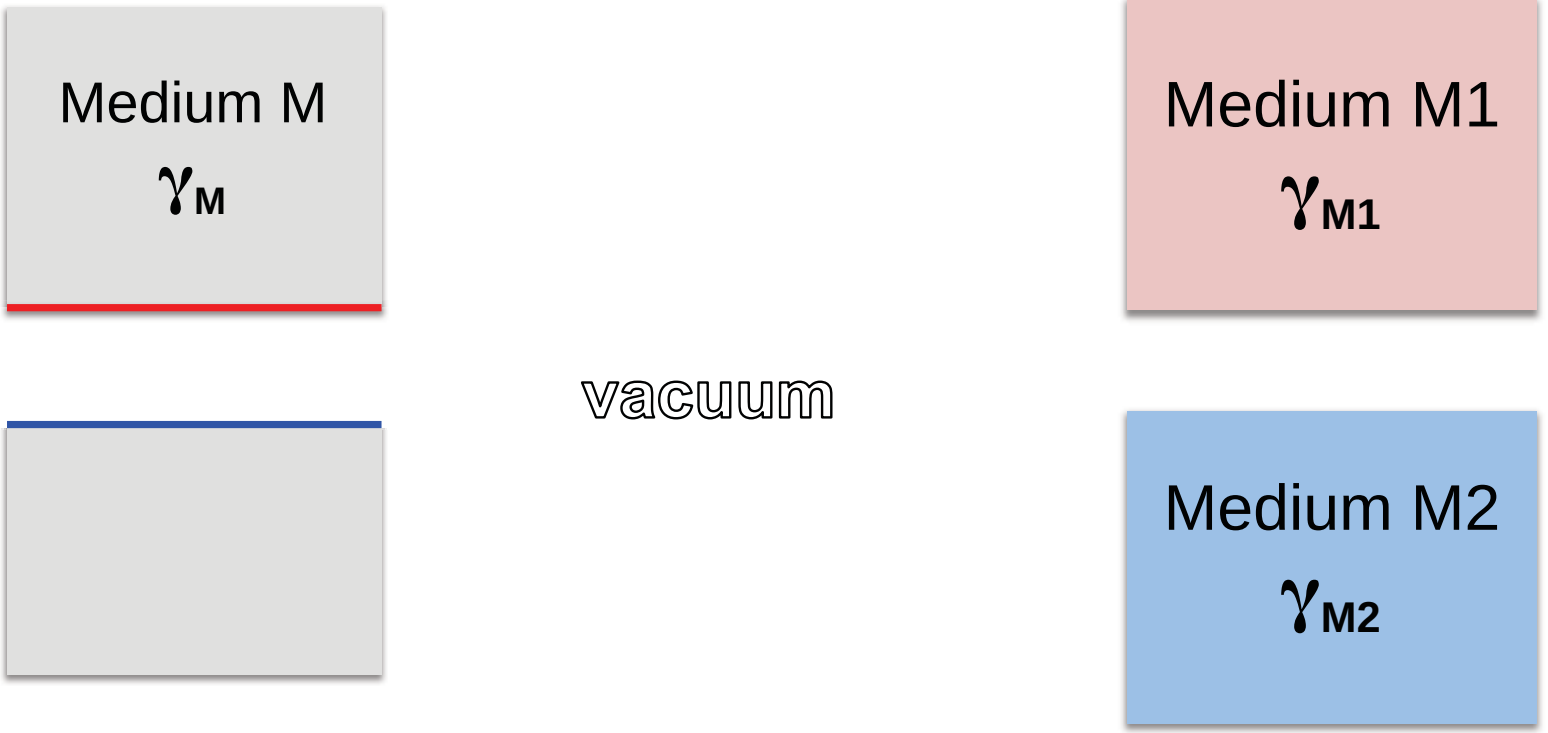
***In nanomedicine***
drug molecules
nanoparticles
polymer aggregates
...

**How do synthetic nano-objects interact with their surrounding?**

# Trypsin



**How are interfaces at the nanoscale?**
**Does the coexistence of hydrophobic and hydrophilic nanoscale domains (e.g. on proteins) provide surfaces with special properties?**



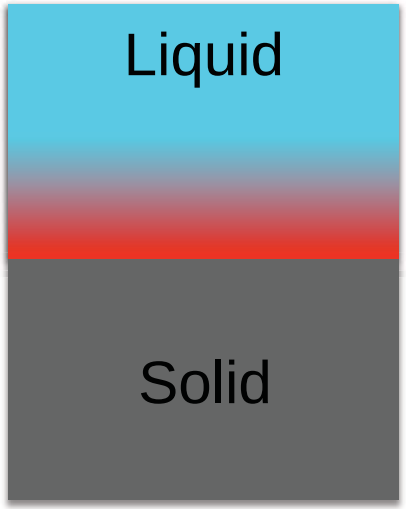

Energy cost:

$\gamma_{MM} = \gamma_M + \gamma_M = 2\gamma_M$

Interfacial energy:

$\gamma_{M1M2} = \gamma_{M1} + \gamma_{M2} - W_{M1M2}$

## Thermodynamics at the interface: the work of adhesion

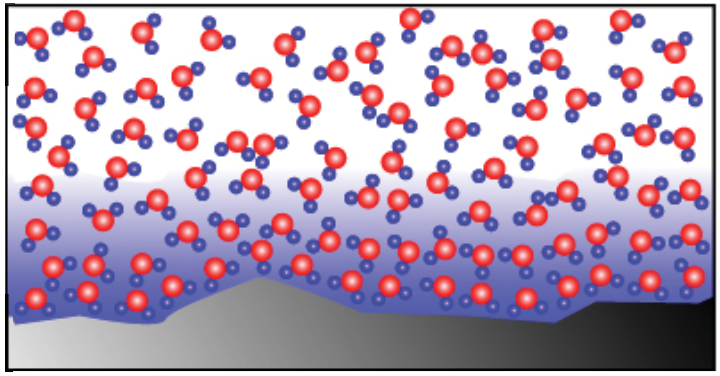



**Interfacial energy $\gamma_{SL}$ :**

$\gamma_{SL} = \gamma_S + \gamma_L - W_{SL}$ (Dupré equation)

**interfacial energy ~ work of adhesion**

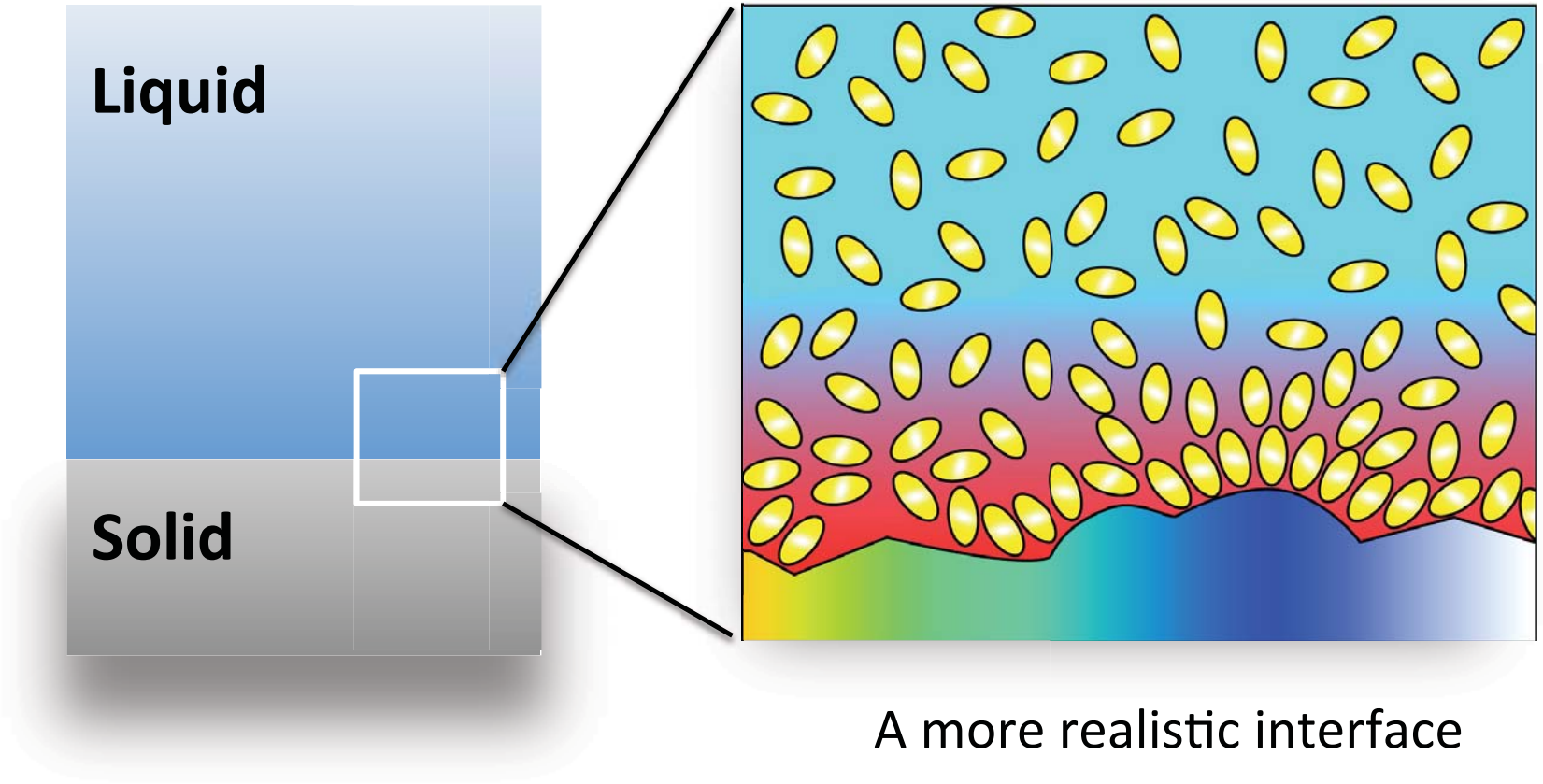
**Bulk liquid**



**Solid**

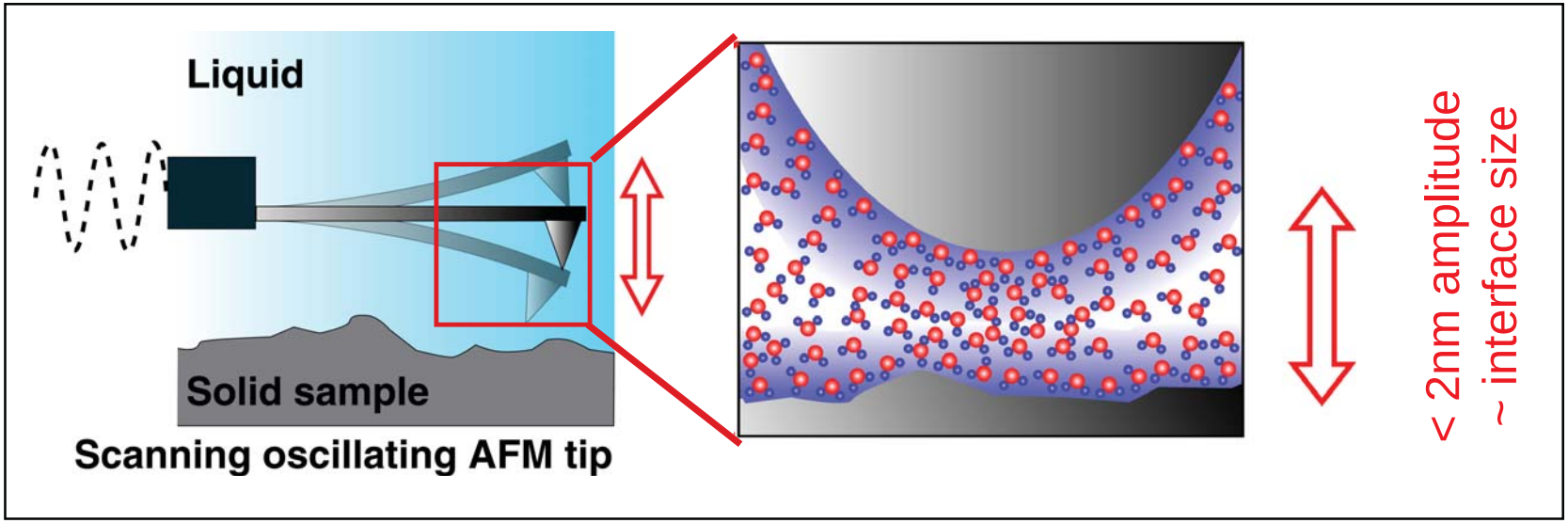
**Interfacial liquid**

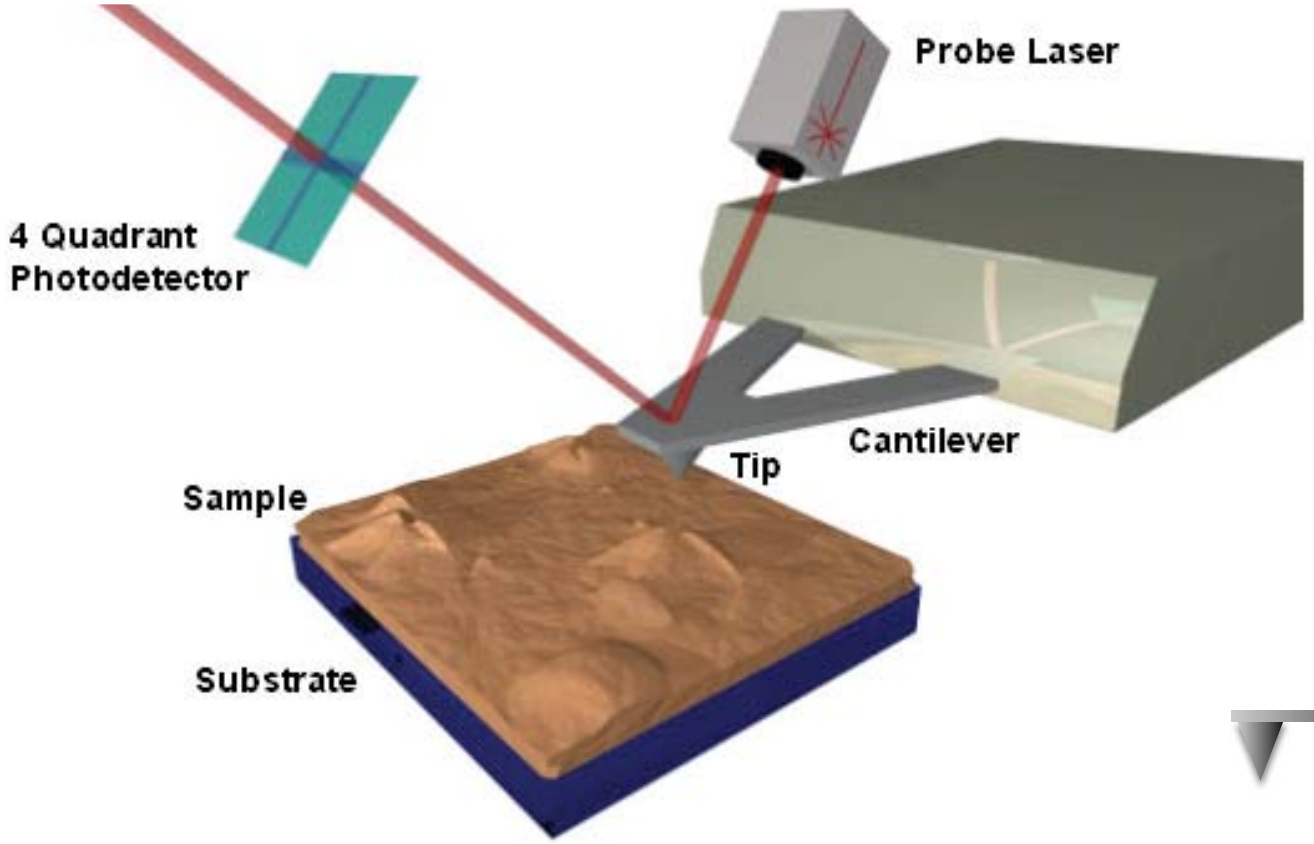
# Solid-liquid interfaces at the nanoscale

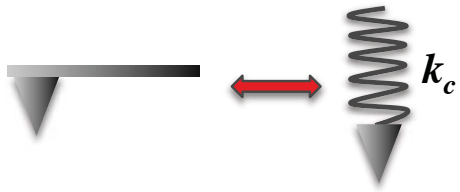



A more realistic interface

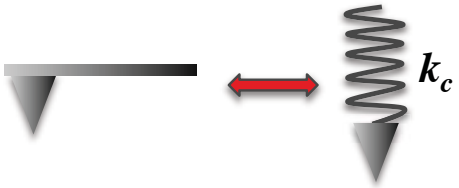
# Probing the solid-liquid interface with AFM



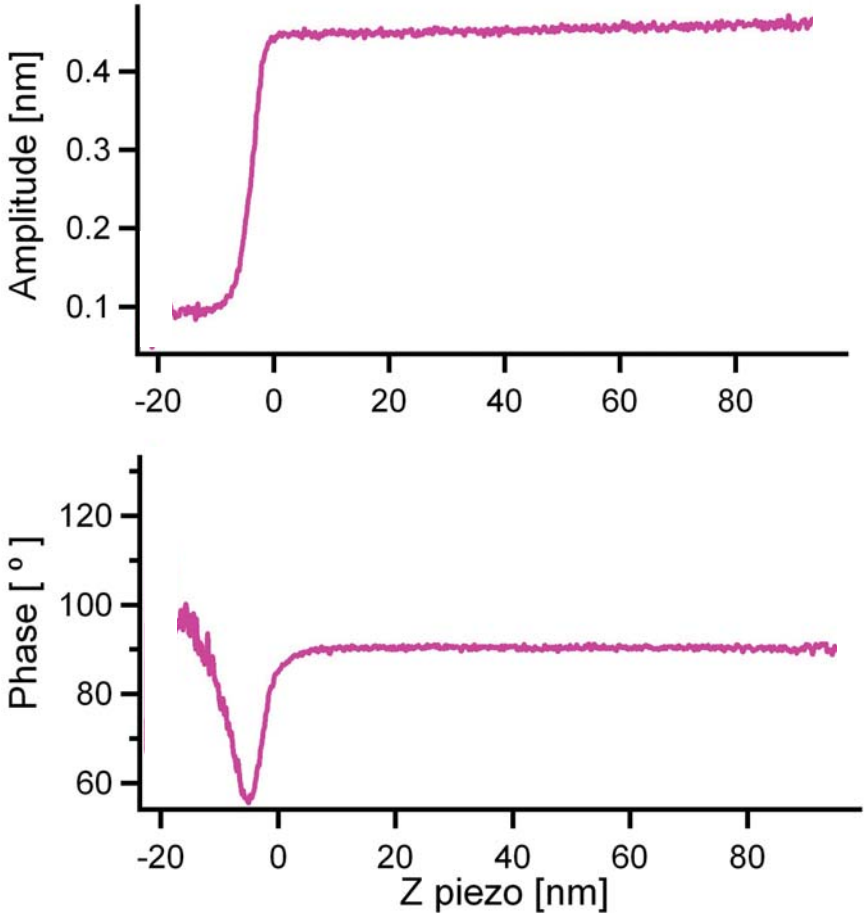



**A nanoscale tip mounted on a flexible cantilever is used to probe a sample locally**

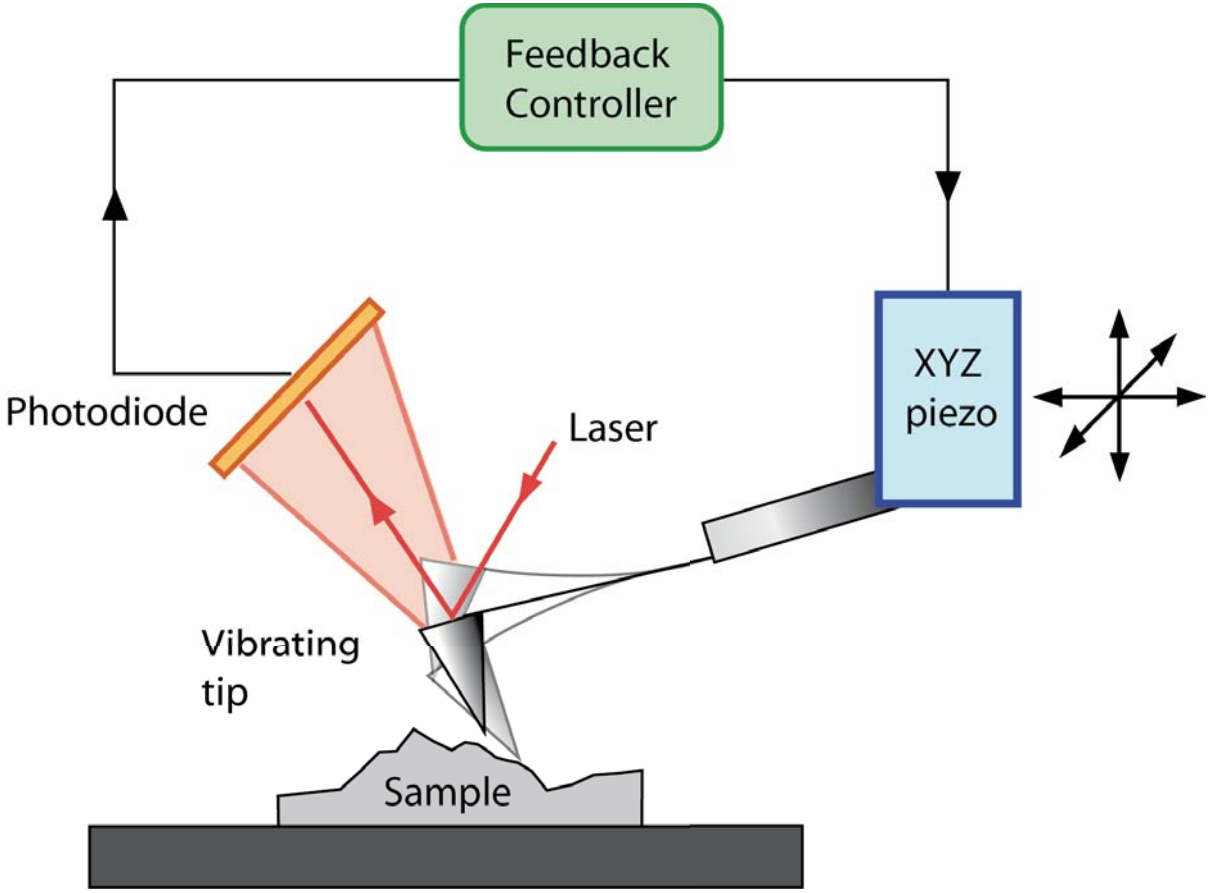



## A vibrating tip ⇨ Amplitude A and phase ϕ



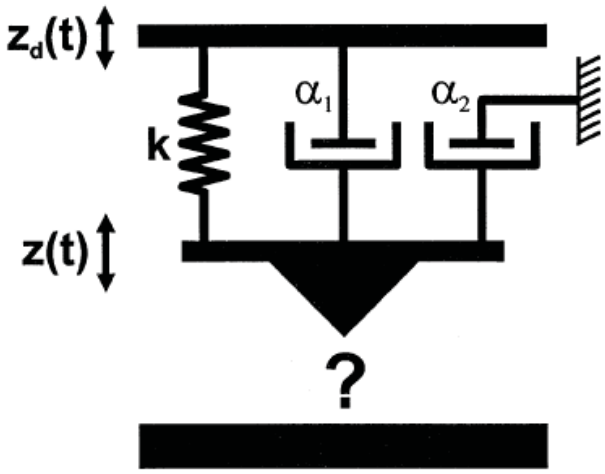

The tip vibration is damped by as the cantilever approaches the surface (here in liquid)

A feedback loop keeps the cantilever vibration amplitude ***A*** constant




Detection: amplitude ***A*** and phase **ϕ**

# The harmonic oscillator formalism




Flow of energy: $\overline{P}_{in} = \overline{P}_{o} + \overline{P}_{tip}$

Equation of motion of the tip:

$$m\ddot{z} - \gamma_{TS}\dot{z} + (k_c + k_{TS})z = A_0 k_c \cos(\omega t)$$

Solution of the type: $z(t) = A\cos(\omega t + \varphi)$

Amplitude **A**
Phase **φ**

Linear damping $\Upsilon_{TS}$

Interaction stiffness $k_{TS}$

Energy dissipation $E_{TS}$

B. Anczykowski et al. Applied Surface Science 140 (1999) 376-382

**In AM-FM, the amplitude is kept constant**

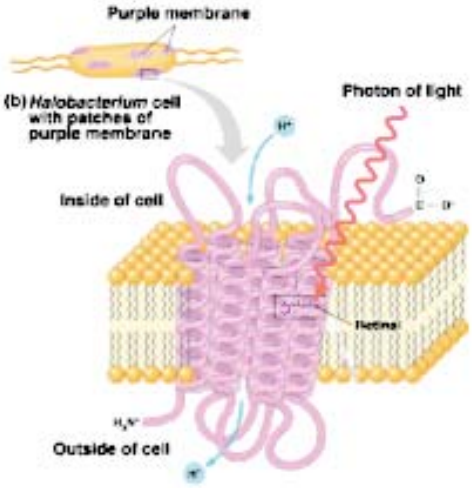
→ **only the phase ϕ can vary freely**

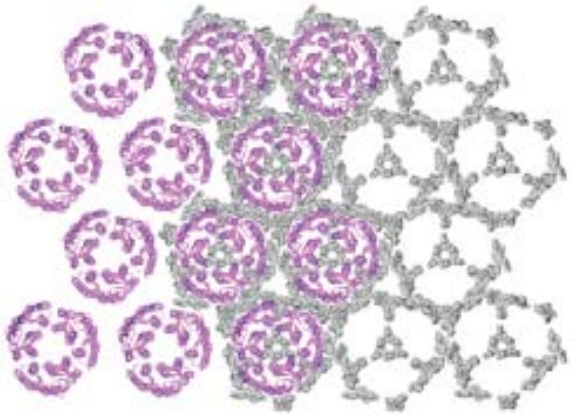
→ **the phase ϕ is directly related to the energy dissipation**

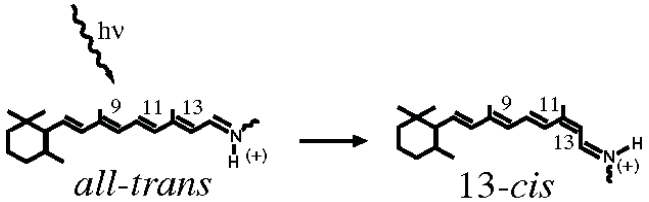
**Local energy dissipation by the tip ~ local phase contrast**

**No indication on the origin of the dissipation! (and hence on the origin of the local phase contrast)**

# A model bio-interface:
## *Purple membranes* from *H. salinarium in solution*

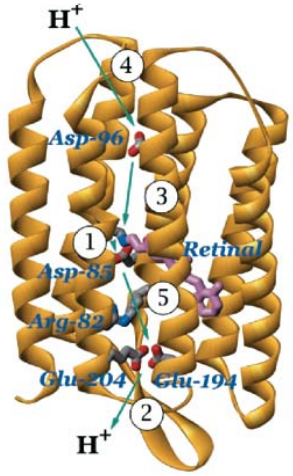




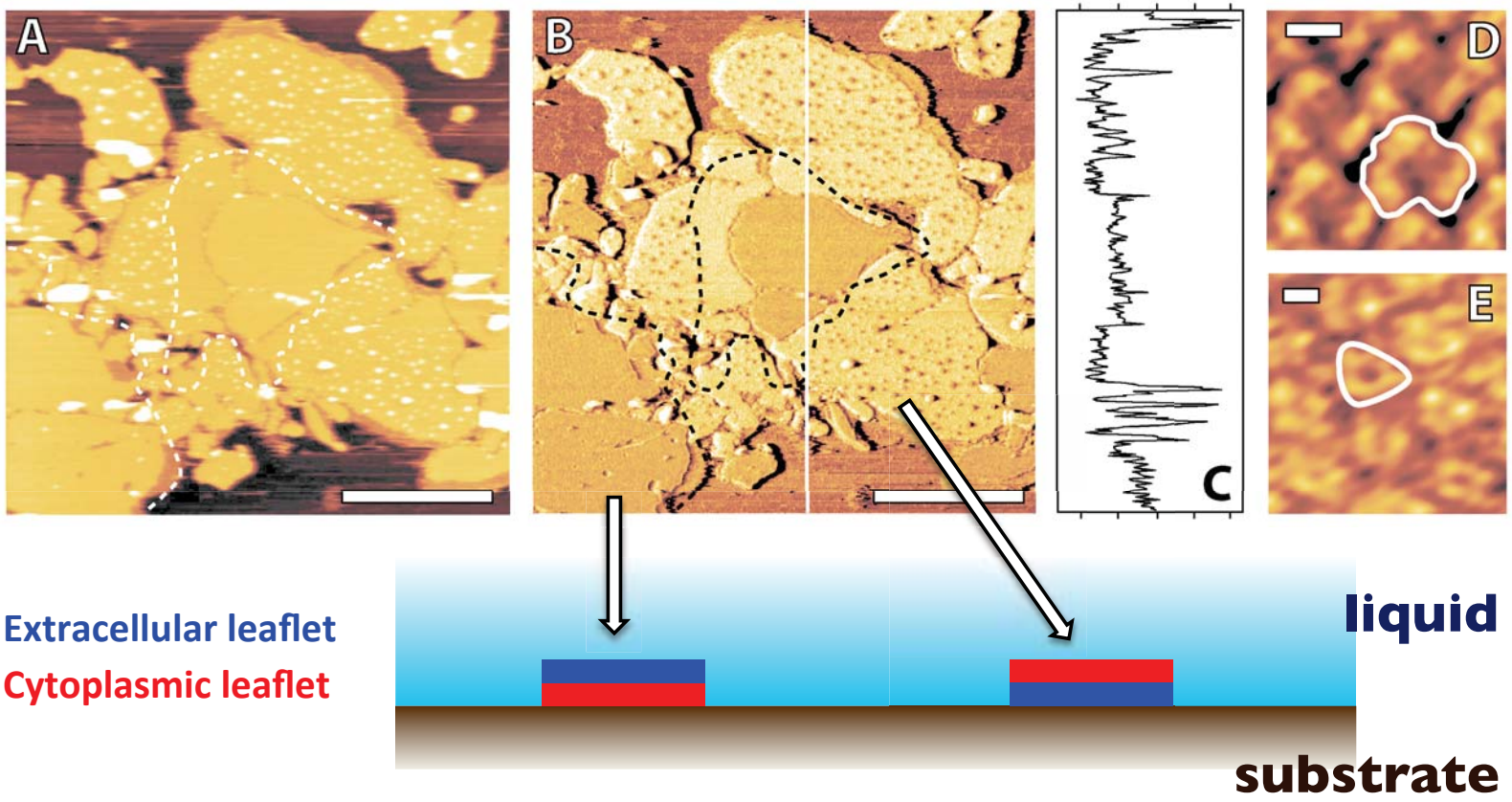

$H^+$

10 – 20 ms
QE ~ 0.6




Luecke *et al*. Science, 280 (1998), 1934–193

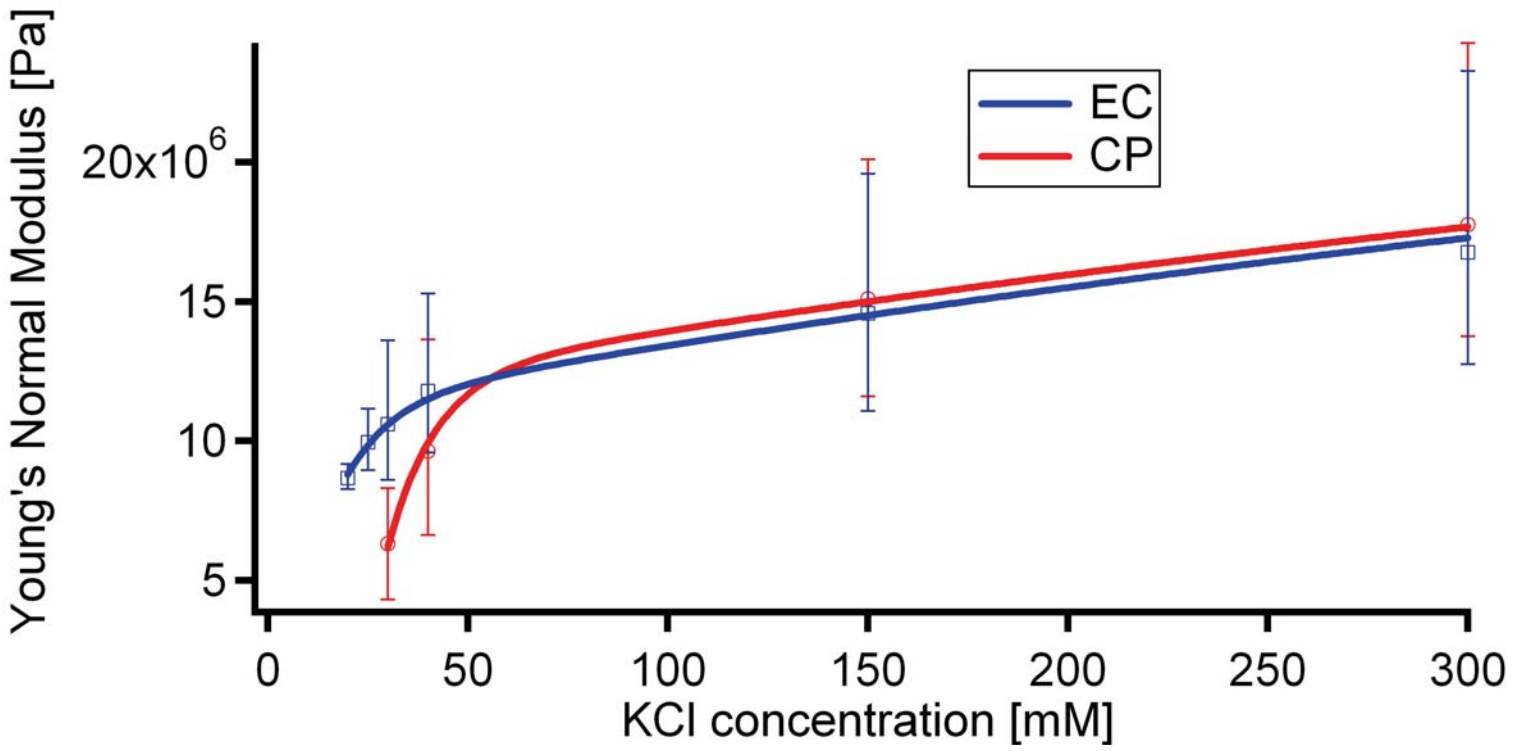
# AM-AFM of purple membrane in liquid



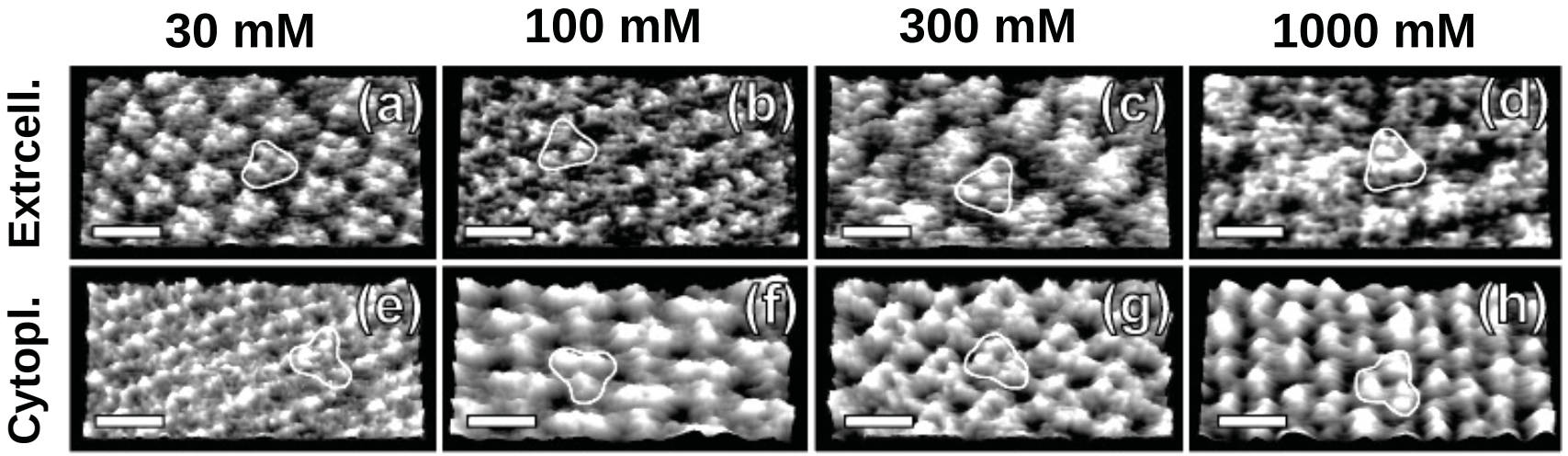


*Biophysical Journal* (2006) 90, 2075-2085

# Nano-indentation at different ionic concentrations show *leaflet-specific effects*

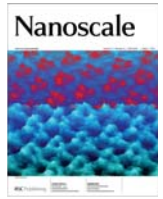


# Ionic effects on the membrane/interface with KCl




5 nm bar

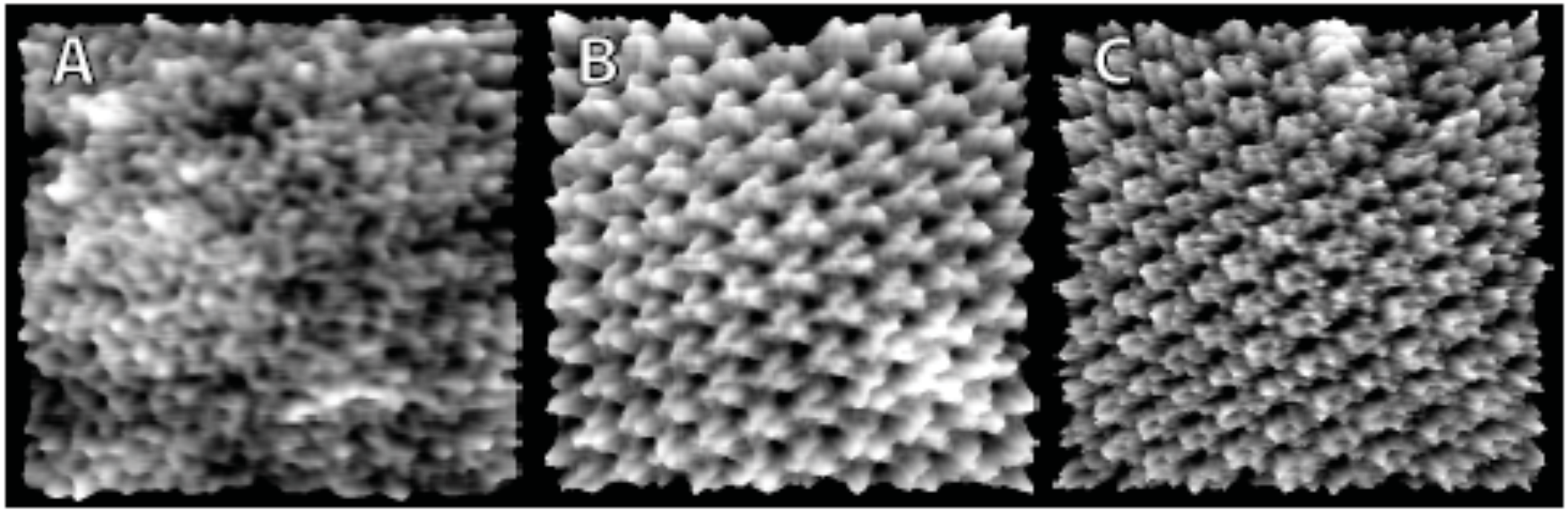
RMS extracell.: 0.15 nm / 0.15 nm / 0.15 nm / 0.13 nm
RMS cytopl.: 0.15 nm / 0.25 nm / 0.25 nm / 0.26 nm




*Nanoscale* (2010) 2, 222-29

## Looking at specific ionic effects: $Li^+$, $K^+$ and $Cs^+$

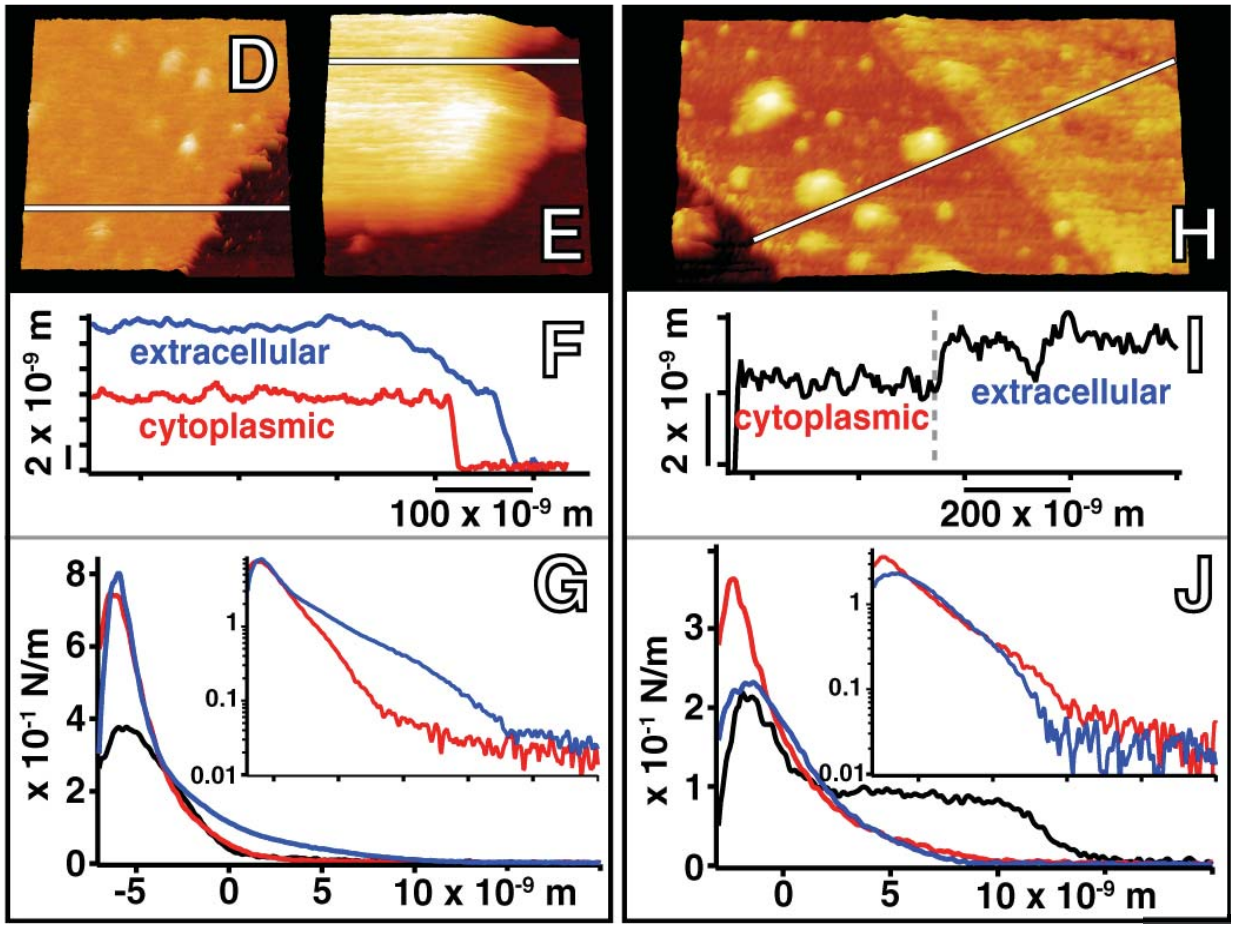
Purple Membrane cytoplasmic surface at 50mM salt concentration




50nm x 50nm

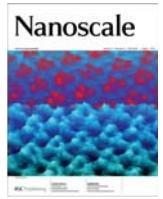
**Voitchovsky *et* al. 2007**

# Modification of the membrane/interface with specific ions



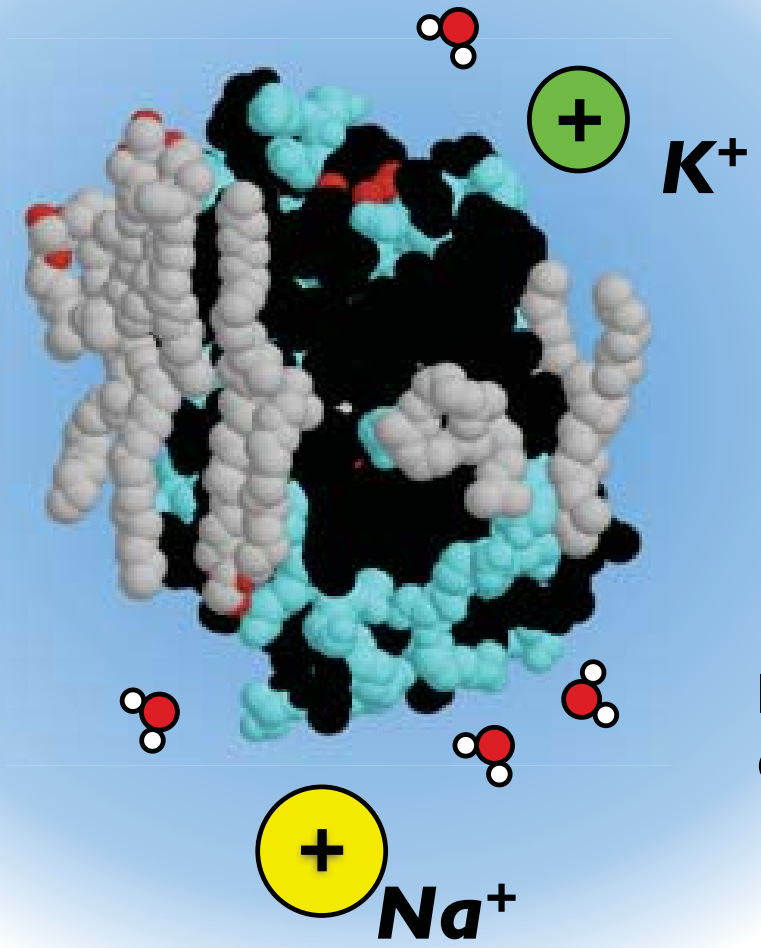

1M NaCl, 20mM $MgCl_2$, 10mM KCl

50 mM $MgCl_2$



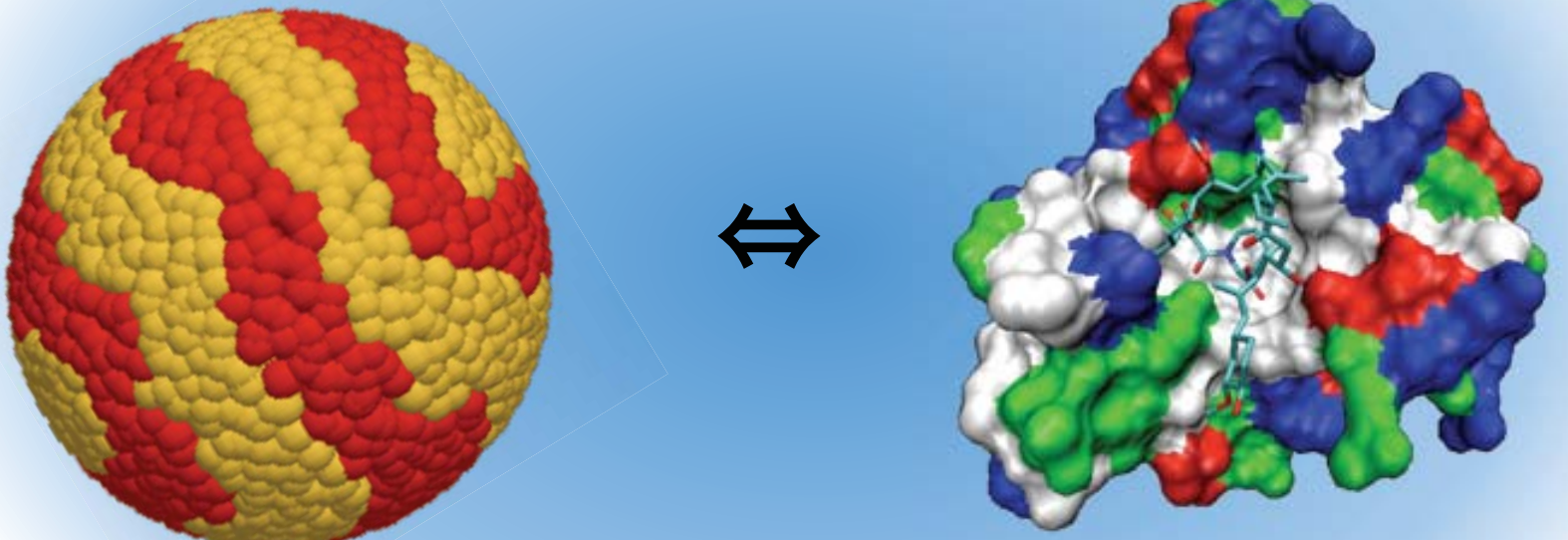

*Nanoscale* (2010) 2, 222-29

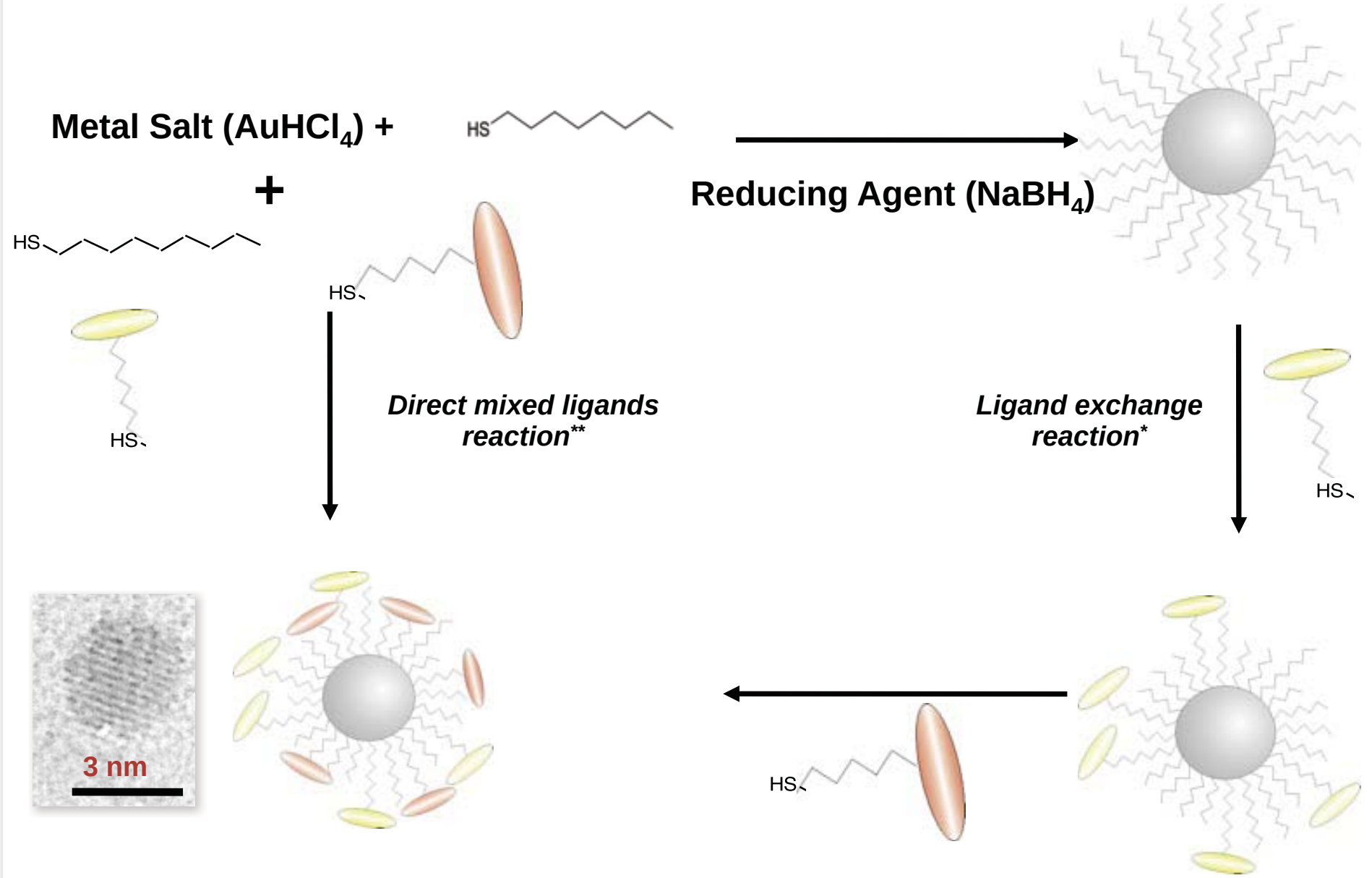
⇨Importance of the protein surface structure in determining the membrane unique interfacial properties




- Alternation of hydrophobic/philic domains
- Specific ionic effects
- Controlled local flexibility
- …

# Striped nanoparticles can mimic the interface of proteins with the surrounding liquid

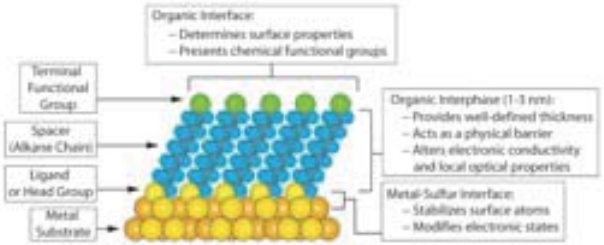


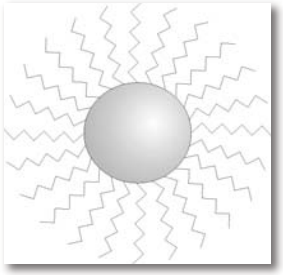


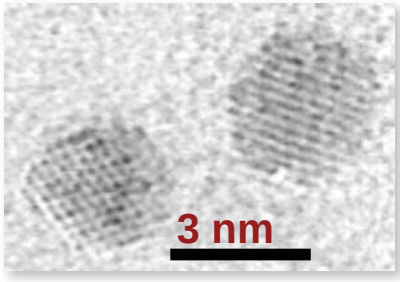

F. Stellacci, et al. *Adv. Mat.* **2002**, 14, 194

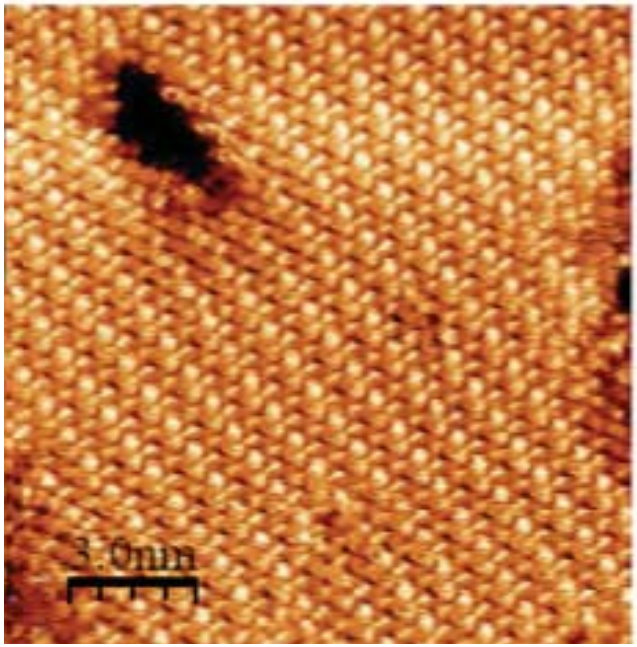
A. C. Templeton, M. P. Wuelfing and R. W. Murray, *Accounts Chem. Res.* **2000**, 33, 27

# Scanning Tunneling Microscopy

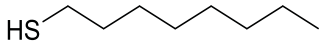


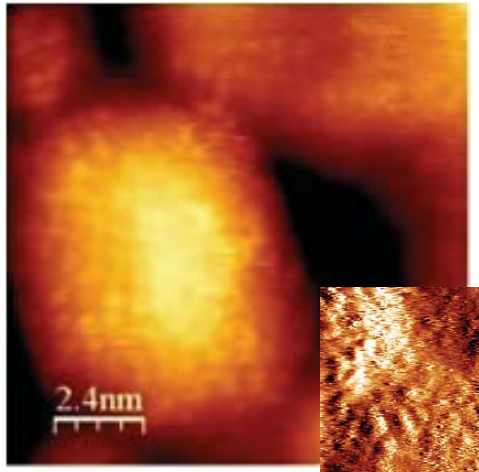



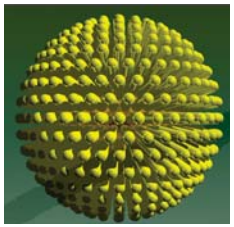


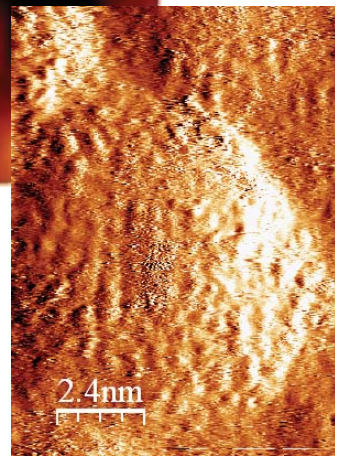




OT

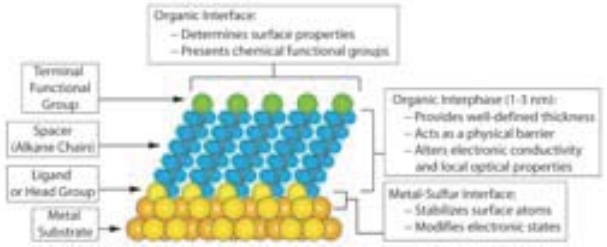


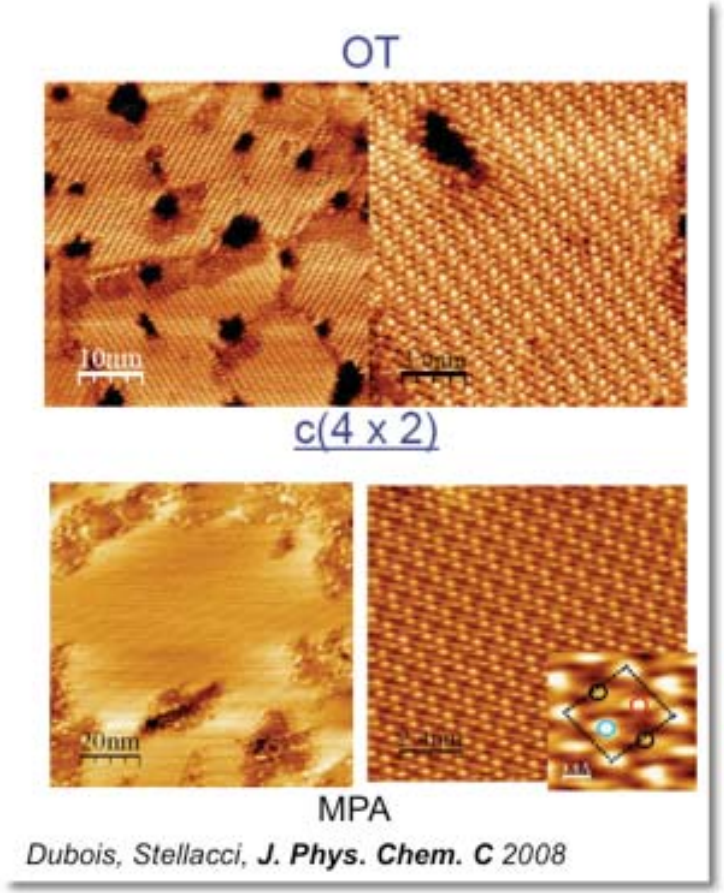



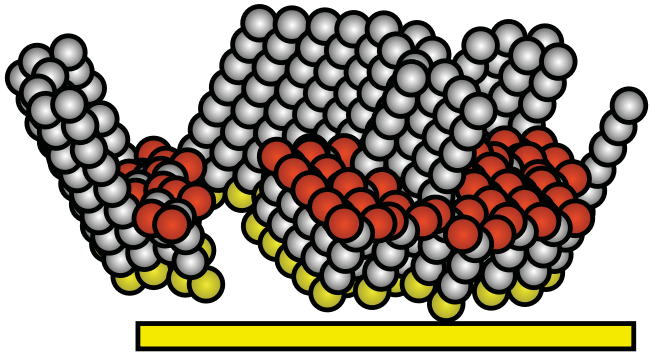





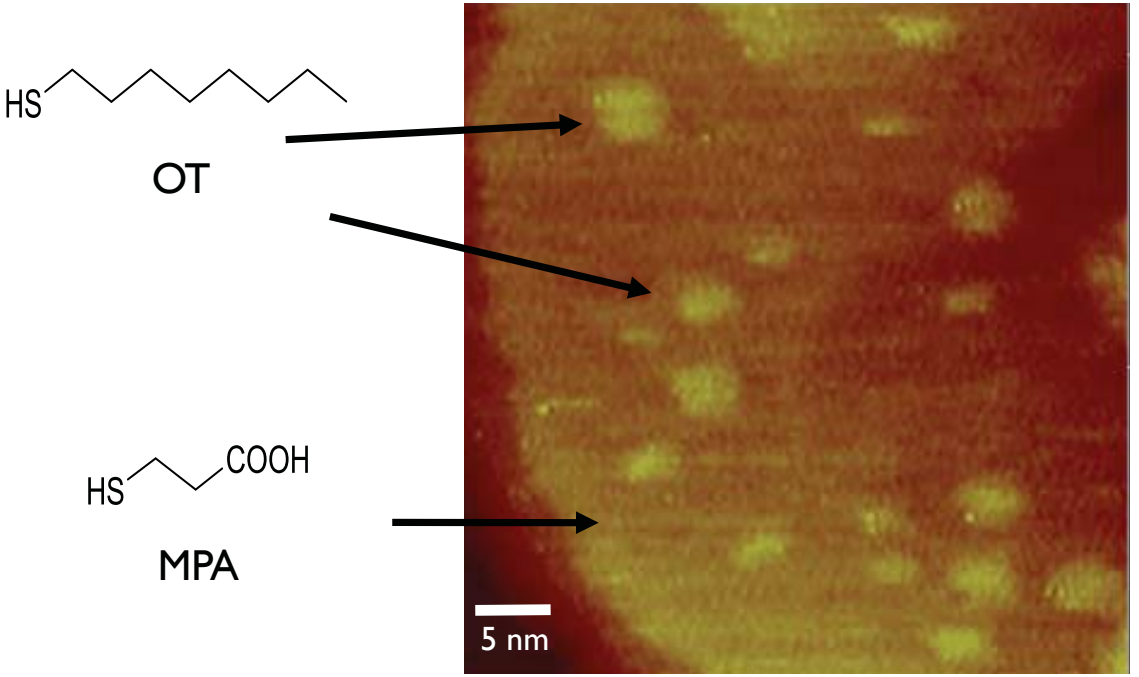
# Mixed Self-Assembled Monolayers



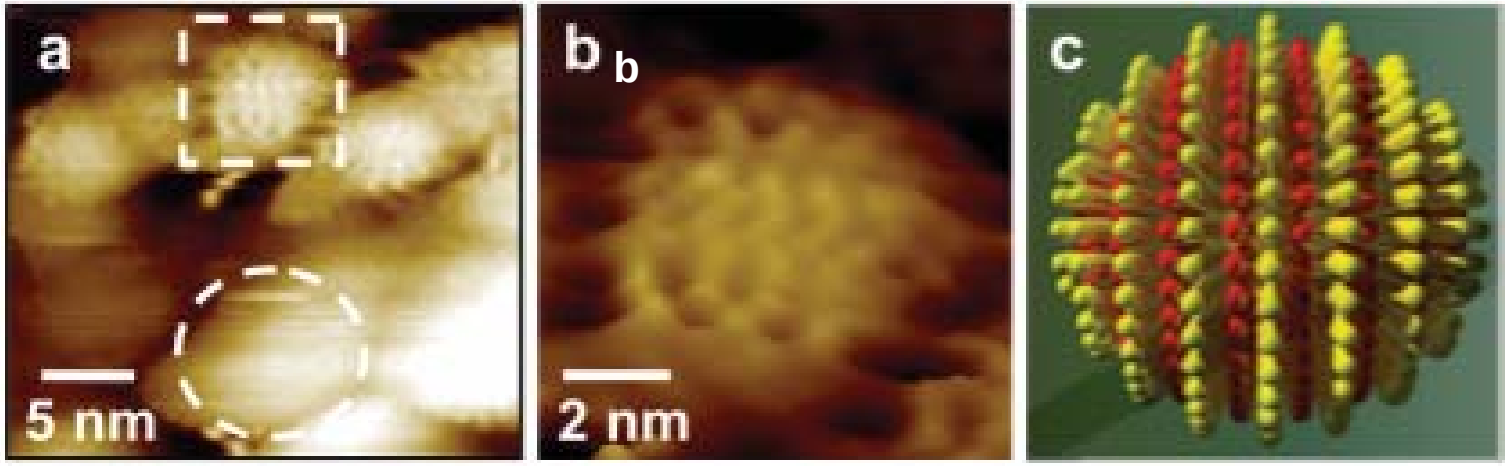
Au (111)

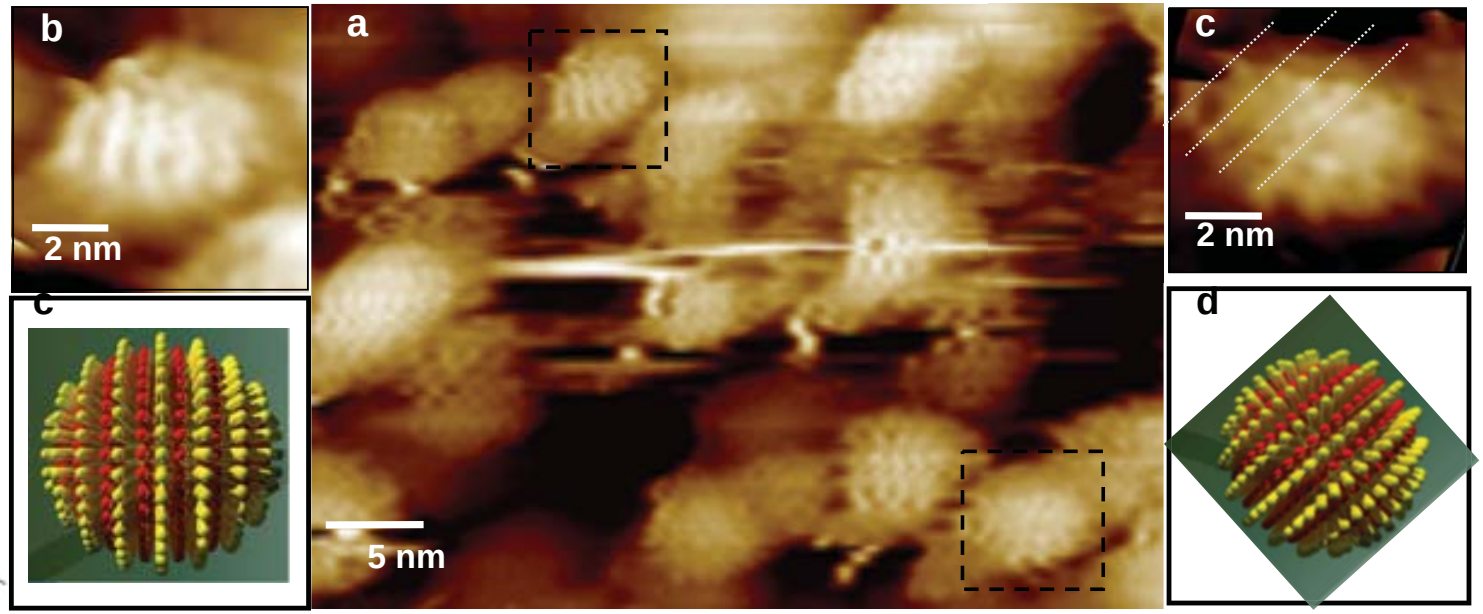


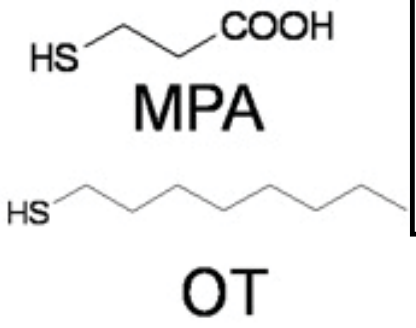



R. Smith, P. Weiss. *J. Phys. Chem. B* **2001**, 105, 1119-1122.

# STM Images of 'Striped' Nanoparticles



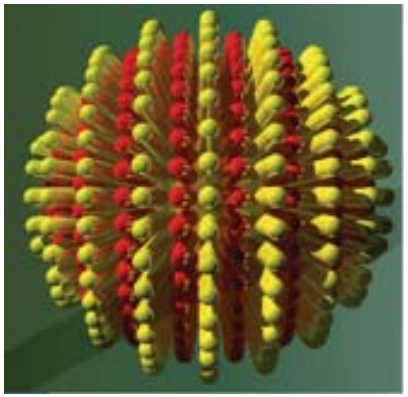



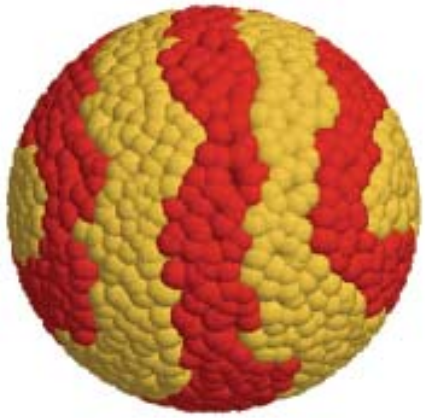



Jackson, Myerson, Stellacci, **Nat. Mat.**, 2004; Jackson, Silva, Hu, Stellacci, **JACS.**, 2006

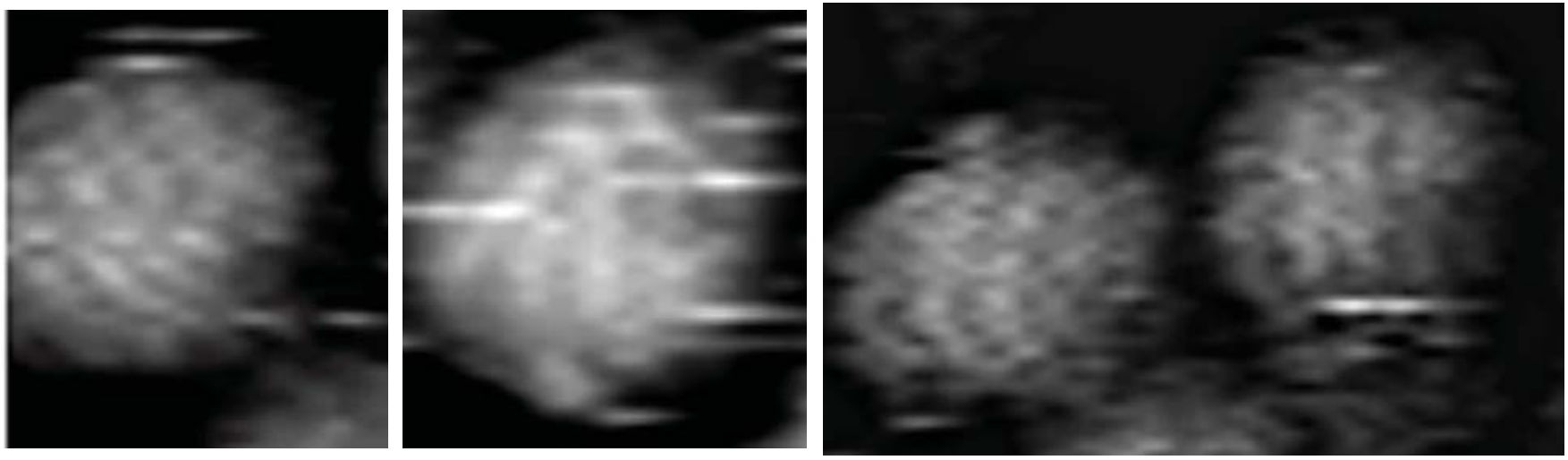
## Cartoon



## Simulations



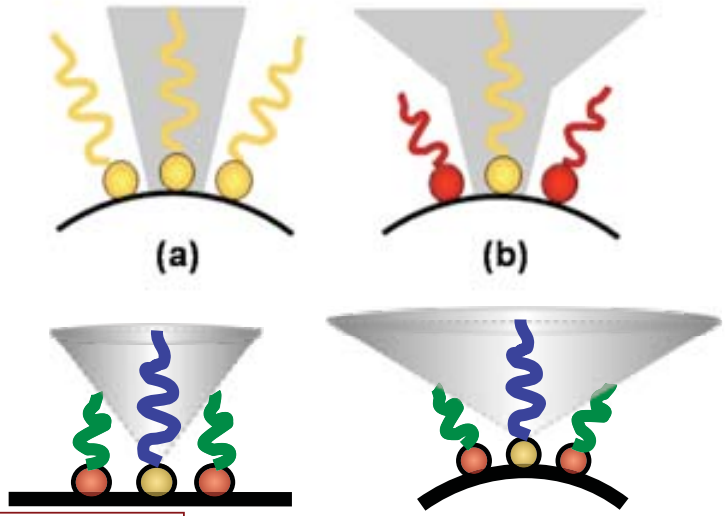
## Microscopy Images

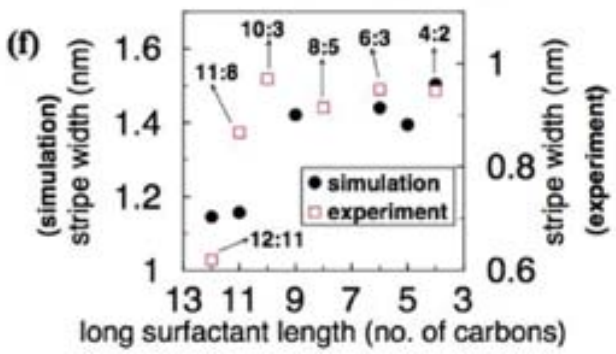


# Stripe Formation in Mixed Monolayers

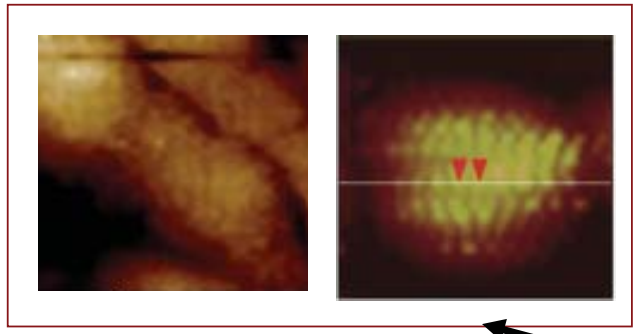
Entropy gain
in stripe formation

*with Sharon Glotzer, U. Mich;* ***PRL*** *2007*

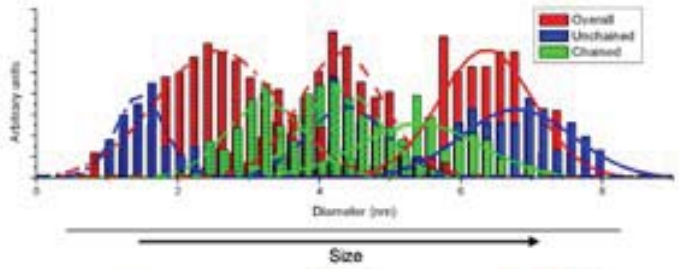


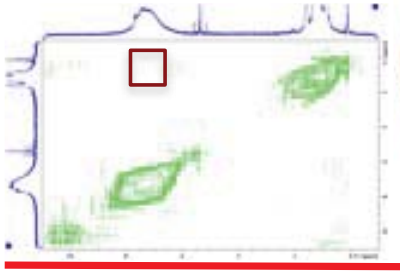

*Singh,* ***PRL*** *2007*

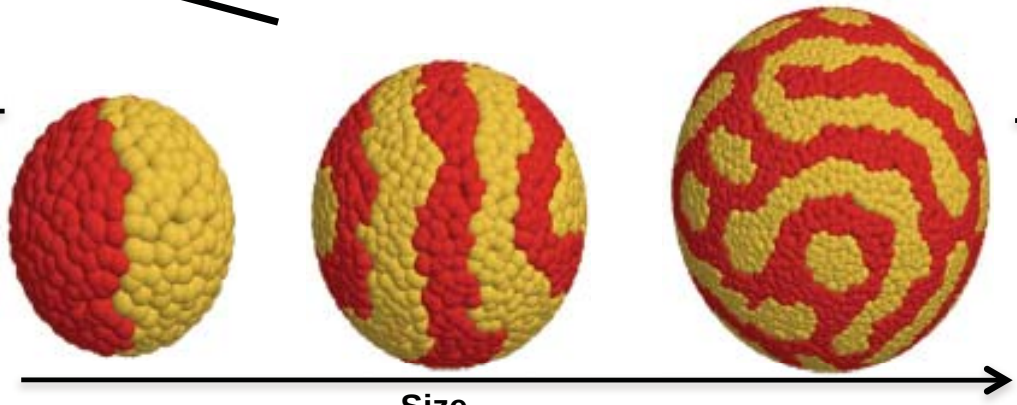


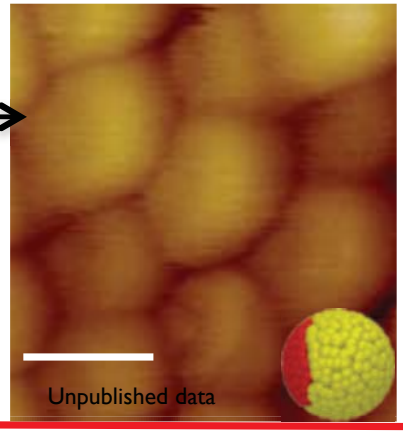
Unpublished data
TEM,
NMR, Noesy
Gel Electrophor.
AUC



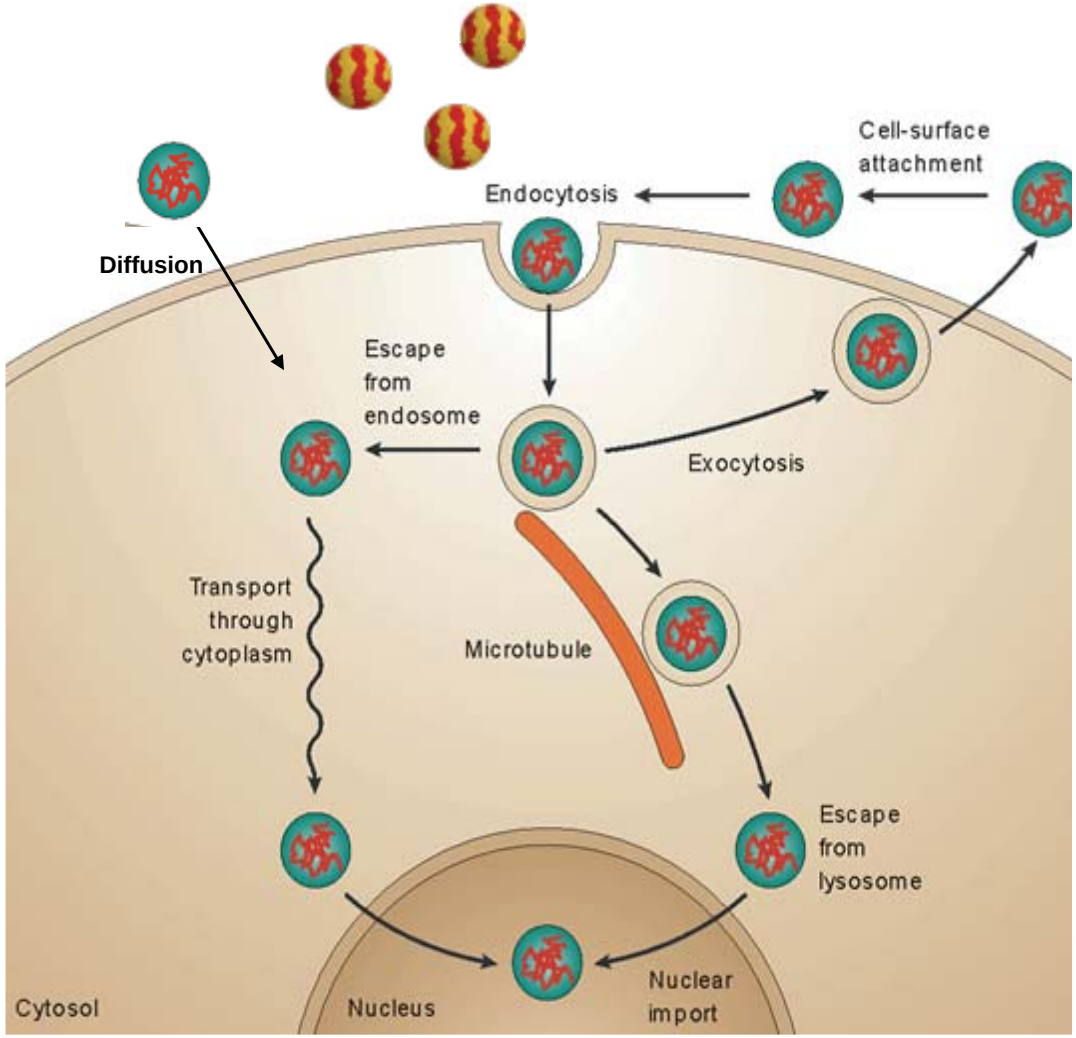

*DeVries, Stellacci,* ***Science*** *315, 358, 2007Carney,* ***JACS*** *2008*





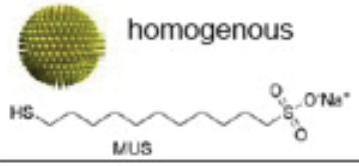
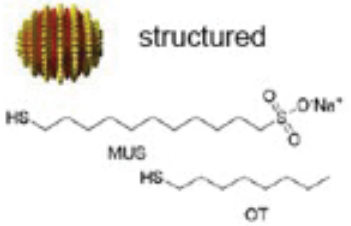
# How do these 'synthetic proteins' interact with biological cells?

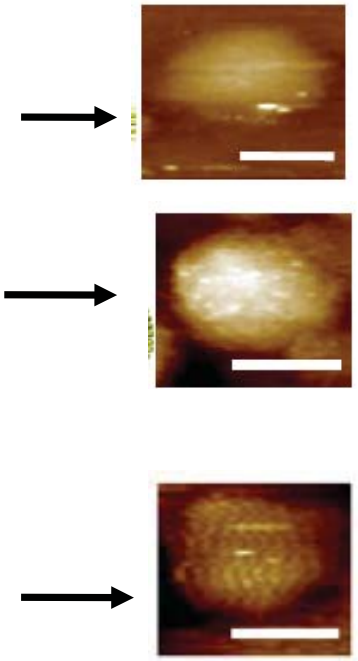



Challenge of cell-membrane penetration

Adapted from:
Nature Reviews Drug Discovery (2005) **4** 581-593

# Effect of Nanoparticle Coating

| Nano-particles | Ligand Shell Composition[a] | Core Size[b] (nm) | TGA[c] | ζ Potential[d] (mV) | Ligand shell morphology/ chemical structures |
|---|---|---|---|---|---|
| **MUS** | 100% MUS | 4.3±1.3 | 15 | -38±5.3 | homogenous   |
| **66-34 br-OT** | 67% MUS | 4.3±1.2 | 13 | -31.1± 0.73 | unstructured   |
| **66-34 OT** | 66% MUS | 4.5±1.0 | 15 | -33.1± 0.64 | structured   |



| | TEM total diameter[a] before incubation (nm) | TEM total diameter[a] after incubation (nm) | DLS diameter before incubation (nm) | DLS diameter after incubation (nm) |
|---|---|---|---|---|
| MUS | 7.4±1.3 | 7.5±0.9 | 6.8±0.2 | 12.0±0.4 |
| 66-34 br-OT | 7.4±1.2 | 7.4±0.9 | 8.0±0.2 | 10.0±0.4 |
| 66-34 OT | 7.6±1.0 | 7.4±0.8 | 7.2±0.2 | 7.8±0.2 |

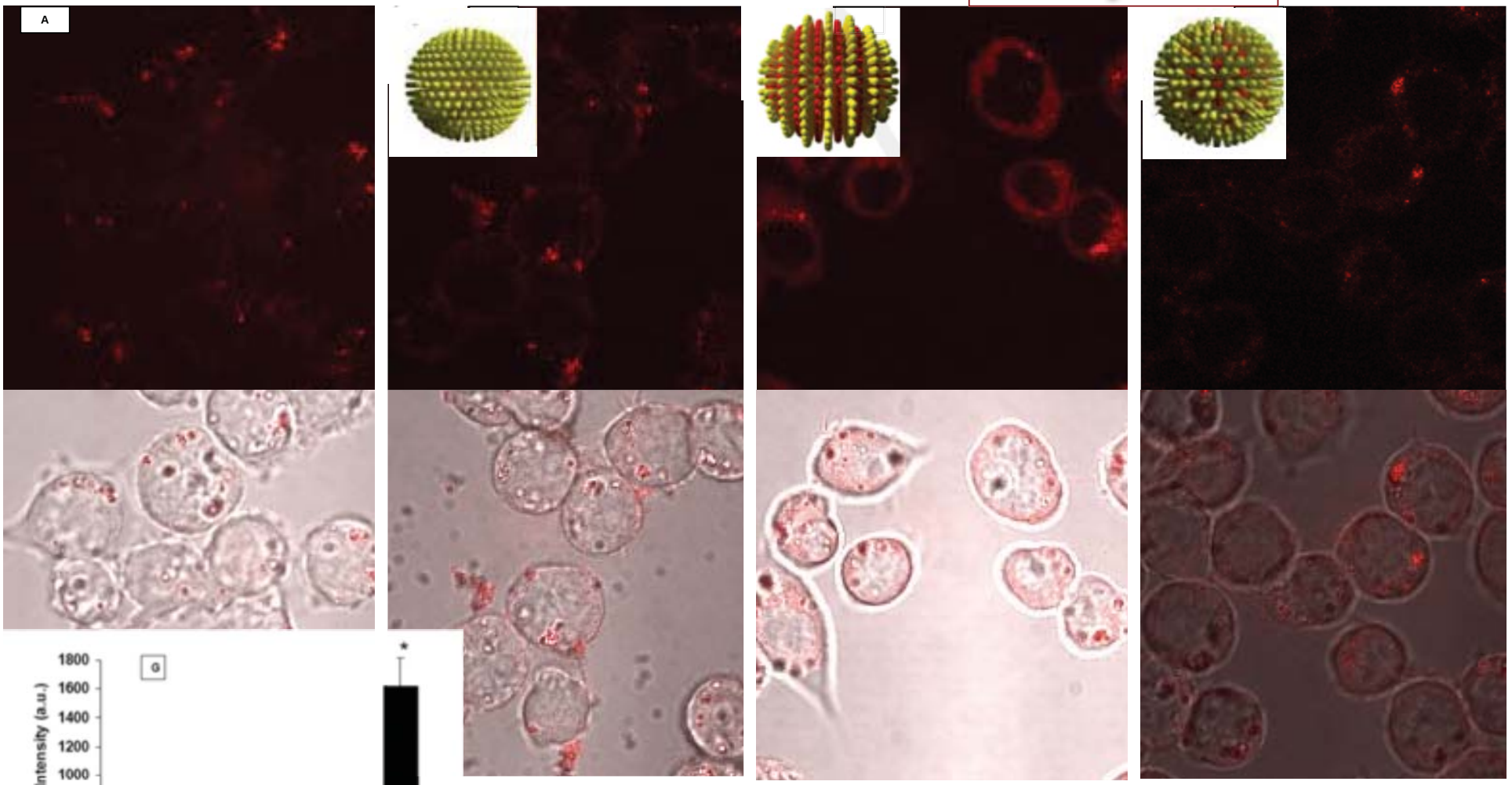
Protein Interactions

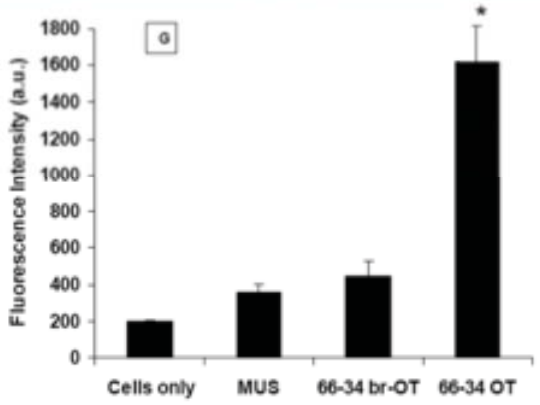


Verma, Uzun, Irvine, Stellacci, **Nature Mat.** *2008*

# Cell Membrane Penetration

## 4 $^0$C Experiment



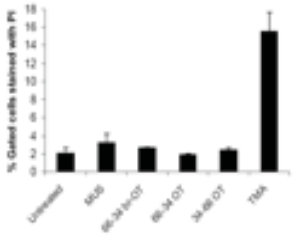

The absence of endocytosis has been independently confirmed via TEM studies



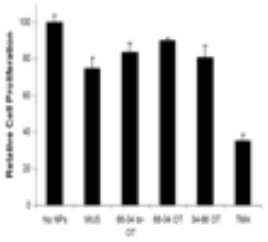
Verma, Uzun, Irvine, Stellacci, **Nature Mat.** *2008*

# Endocytosis Inhibitors

Calcein Only



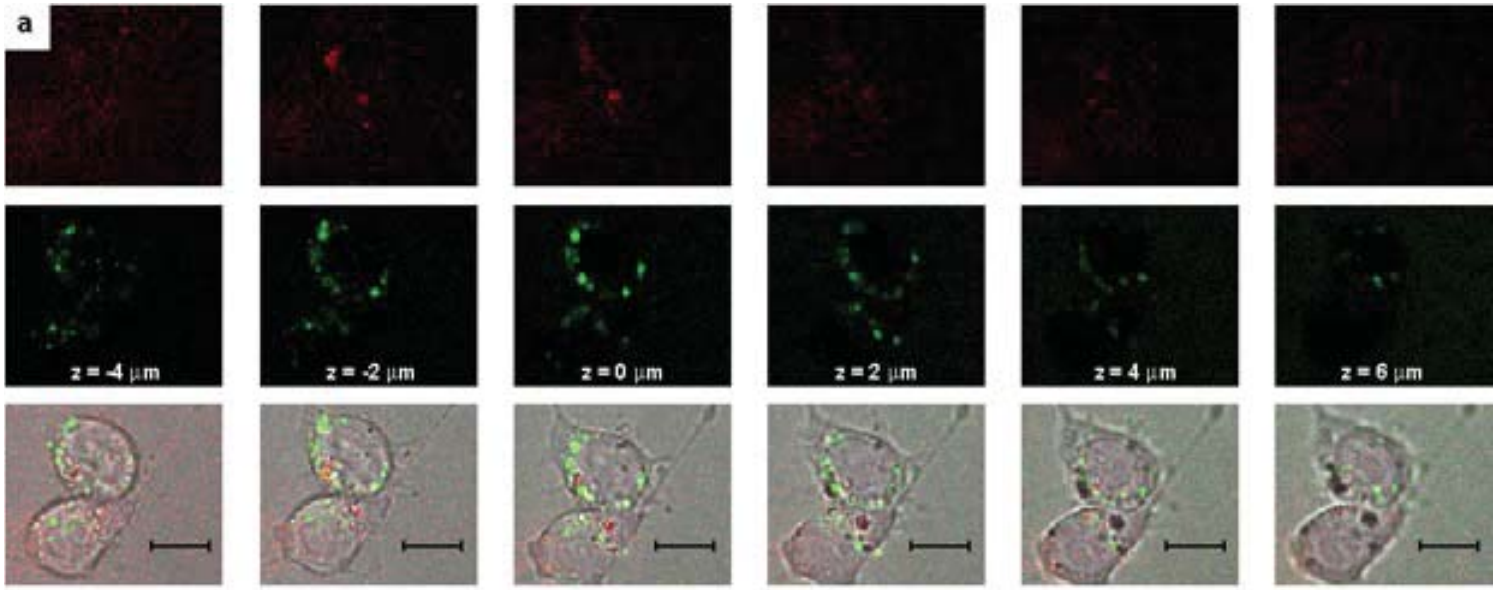
PI Staining



CyQuant

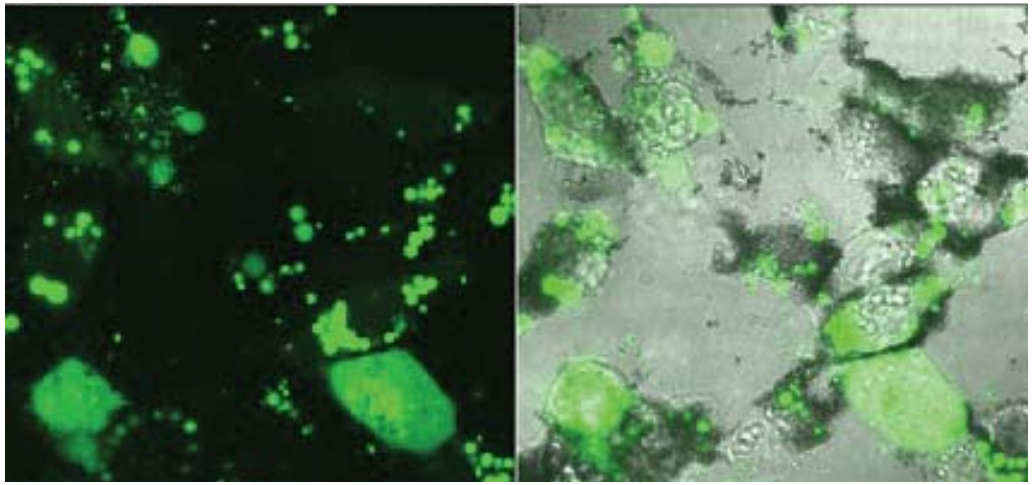
Calcein and 'Striped' NPs

No sodium azide and 2-deoxyglucose




Z-Stack Images

sodium azide and 2-deoxyglucose

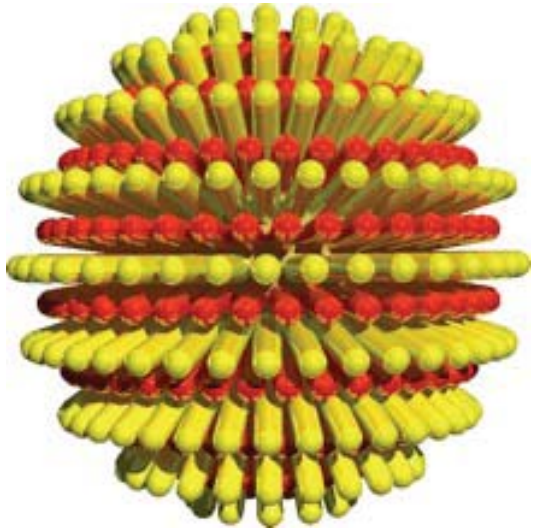


Calcein and TriMethyl Ammonium NPs

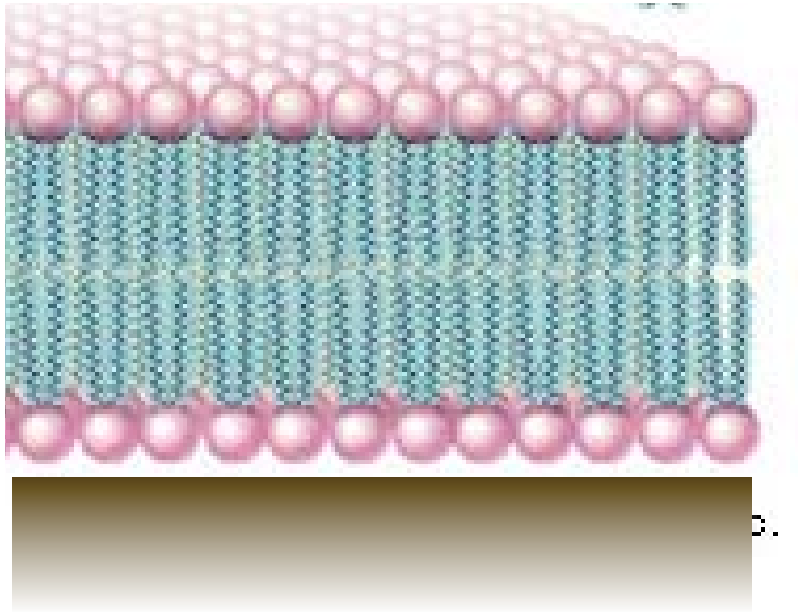


Verma, Uzun, Irvine, Stellacci, **Nature Mat.** *2008*

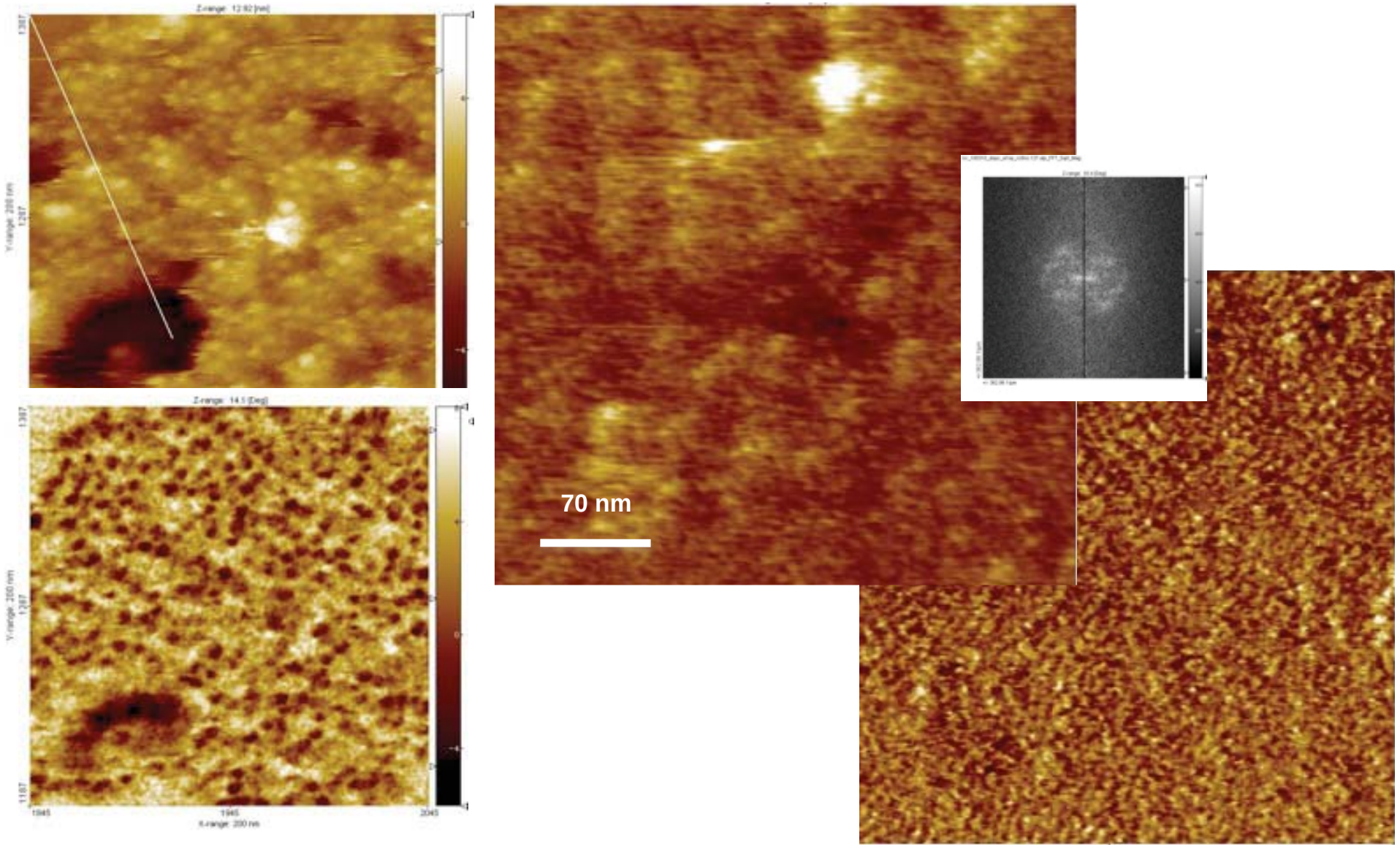
## Striped Nanoparticles



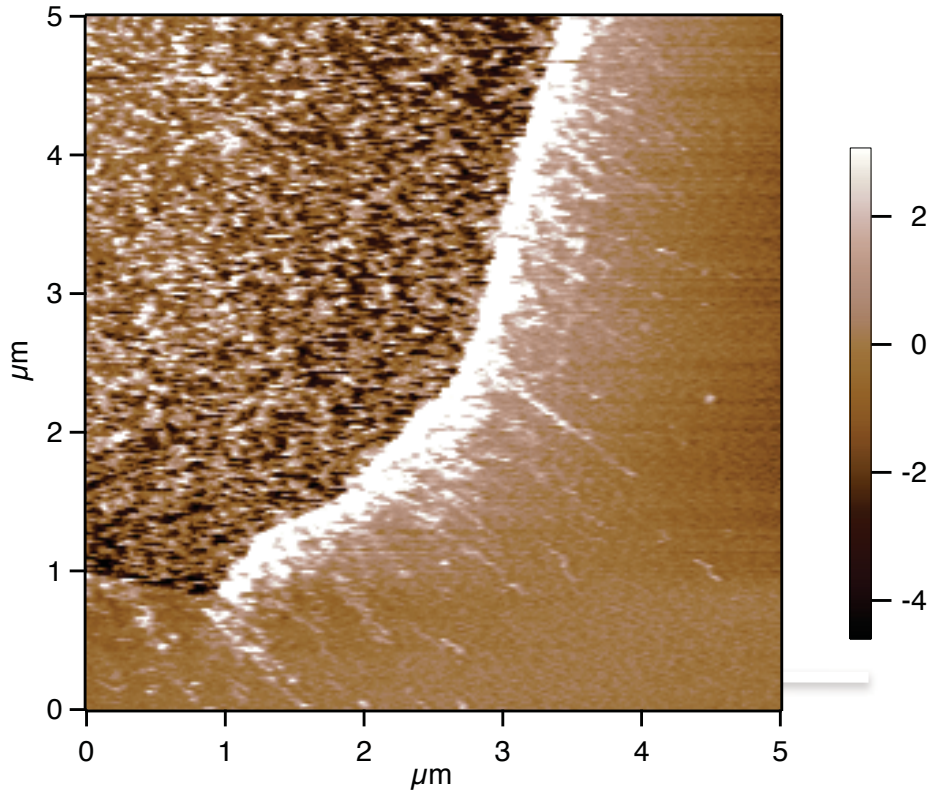
## Lipid Bilayers

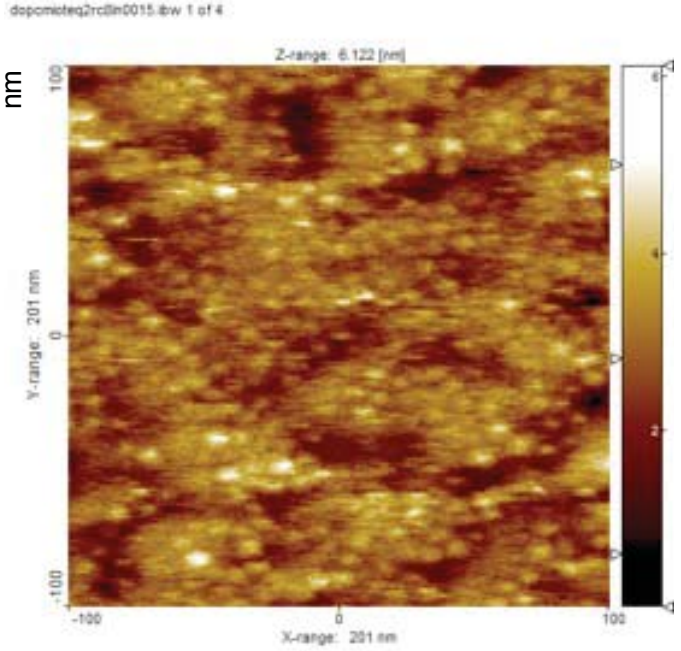


## AFM images of supported bilayers and striped particles



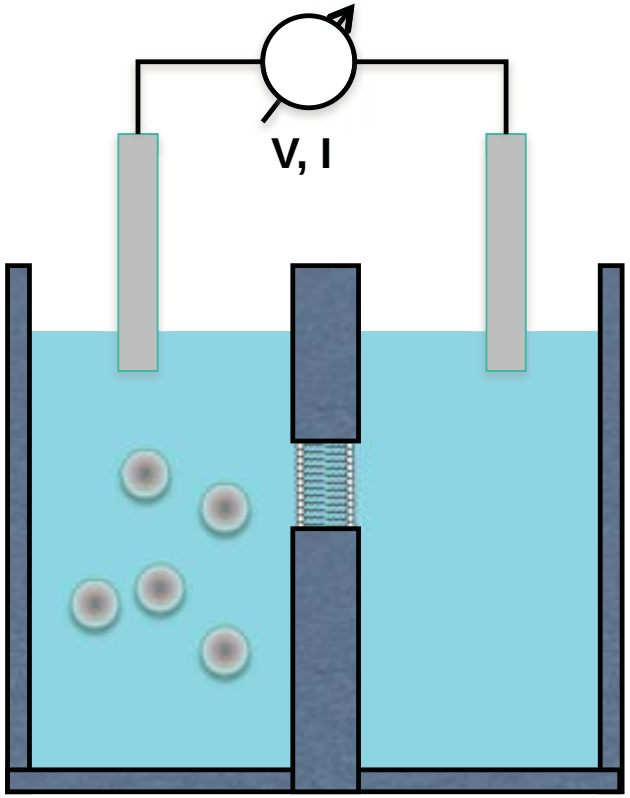
# AFM study of Nanoparticles-bilayer interactions

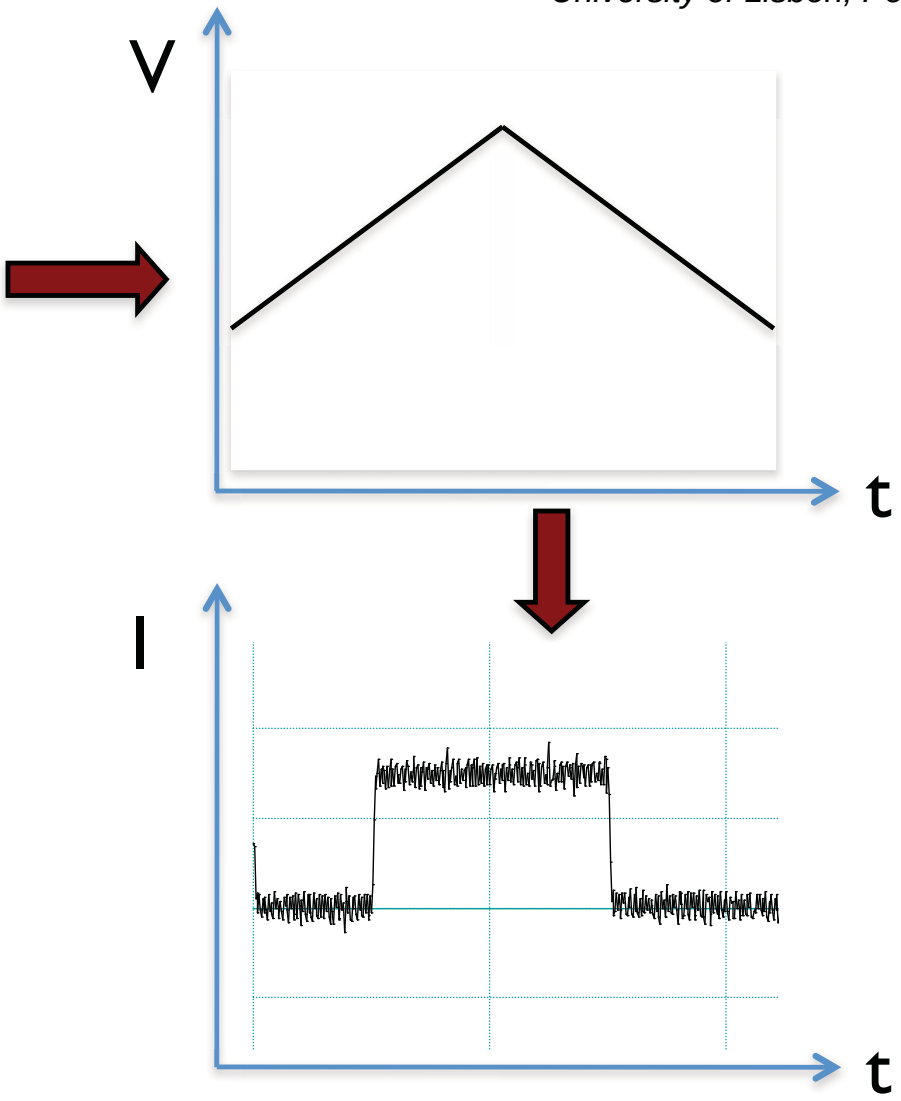


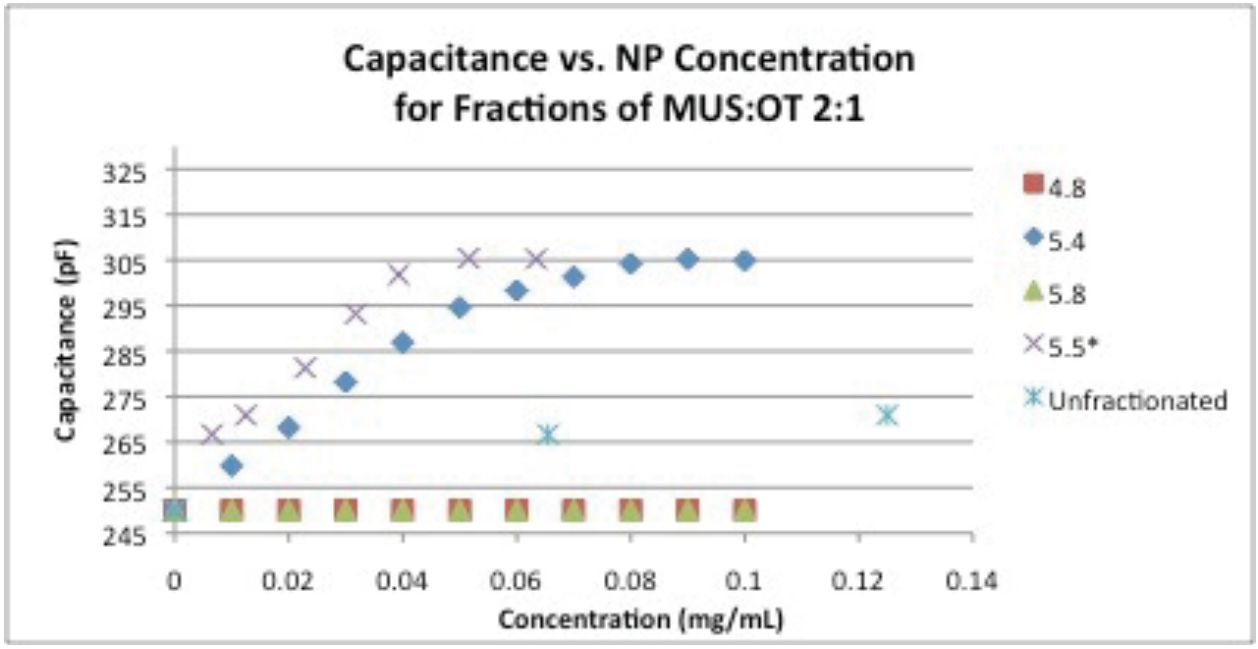

## Poster: Maria RICCI

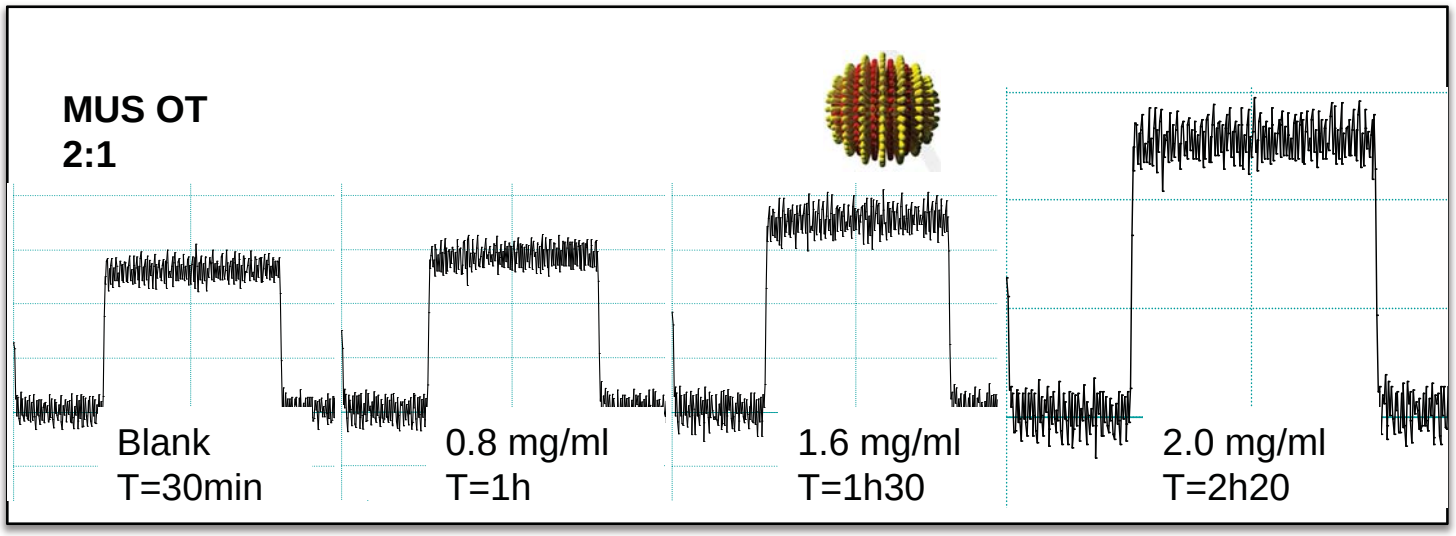
*In collab. with Prof. Yann Astier, University of Lisbon, Portugal*




- **1,2 diphytanoyl-sn-glycero phosphocholine lipid bilayer**
- pH 8.1 Tris-HCl 0.1 M, KCl 2 M
- Reference Ag/AgCl electrodes

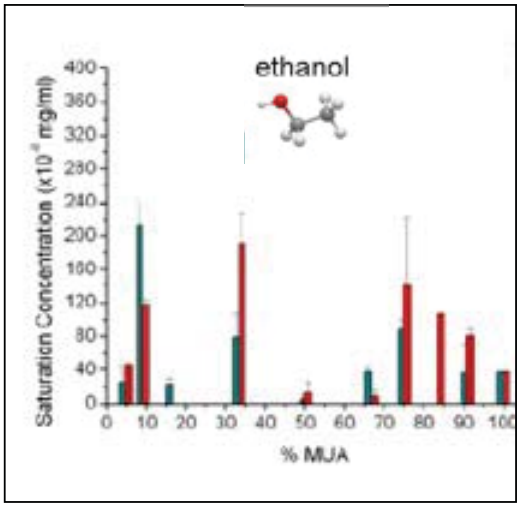





Carney, Stellacci, ***in preparation***

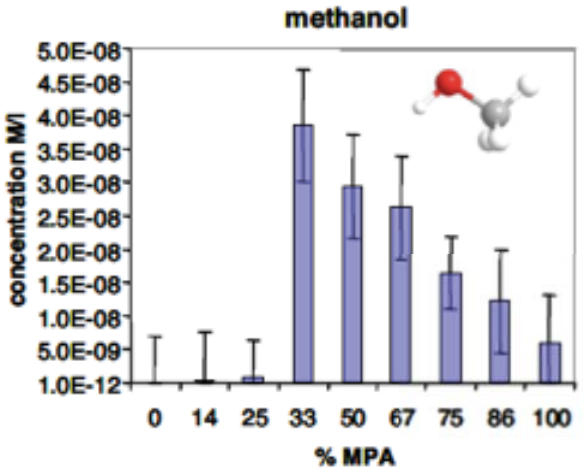
# Solubility of Rippled Nanoparticles

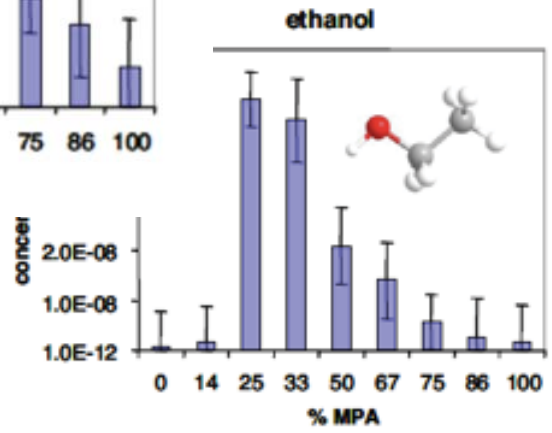



OT

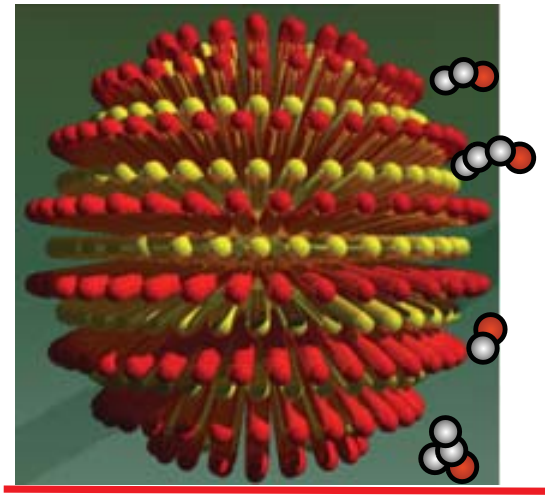
MUA

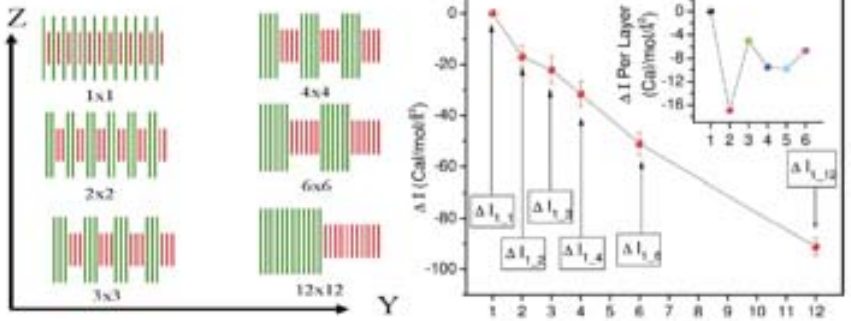
HT

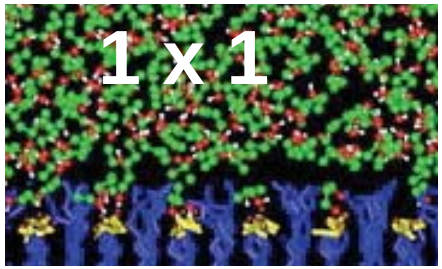


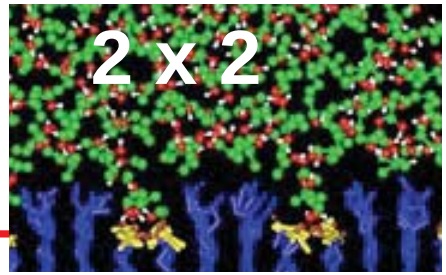




*with N. Marzari, MIT;*
***PNAS** 9886, 2008*

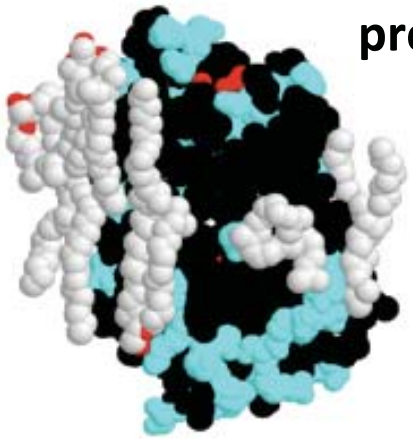




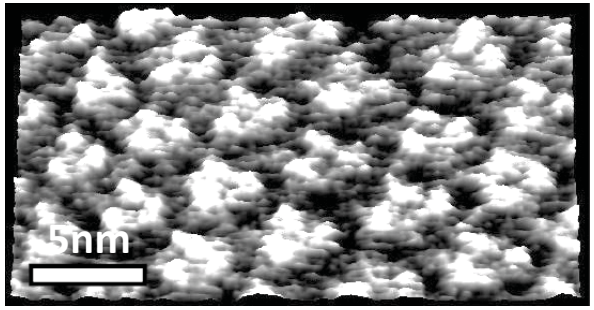



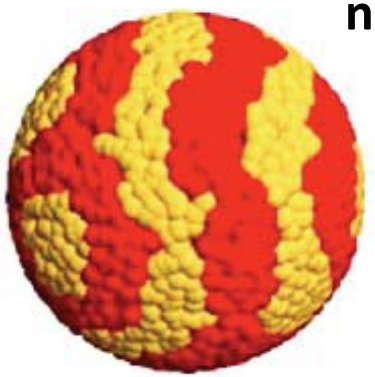
**What is so special about theses nanoparticles?**
**Is it possible to quantify their interface with the surrounding?**

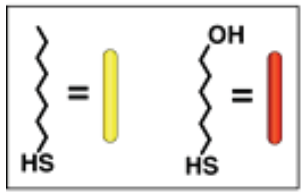


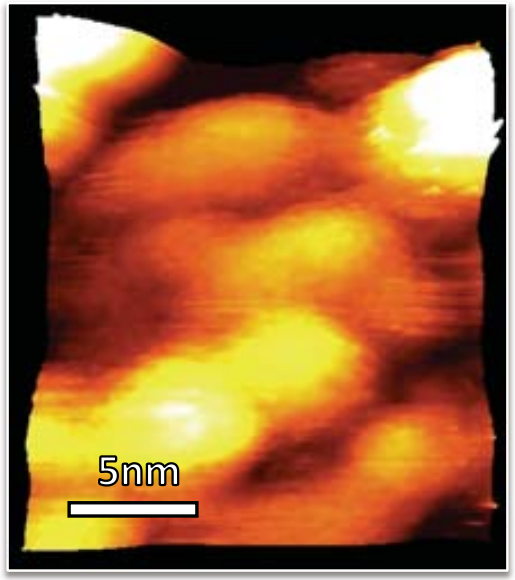
**protein**



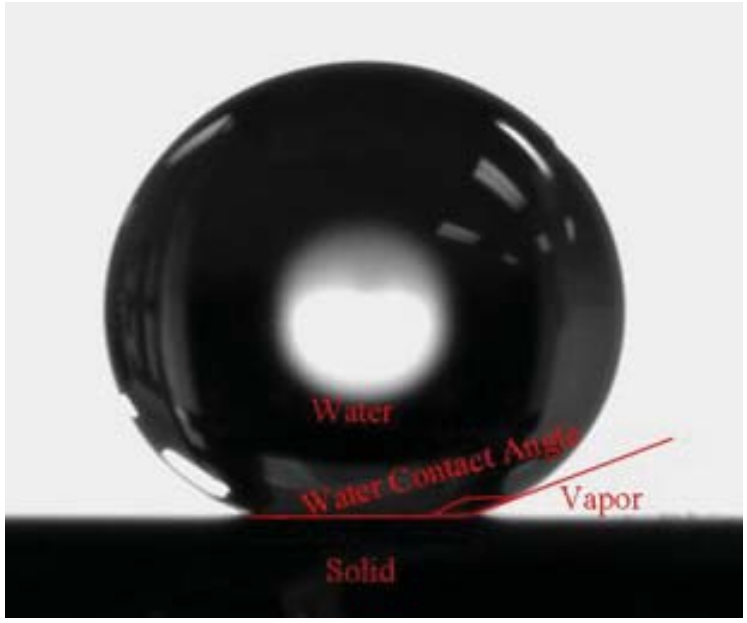

**ligand-coated nanoparticule**





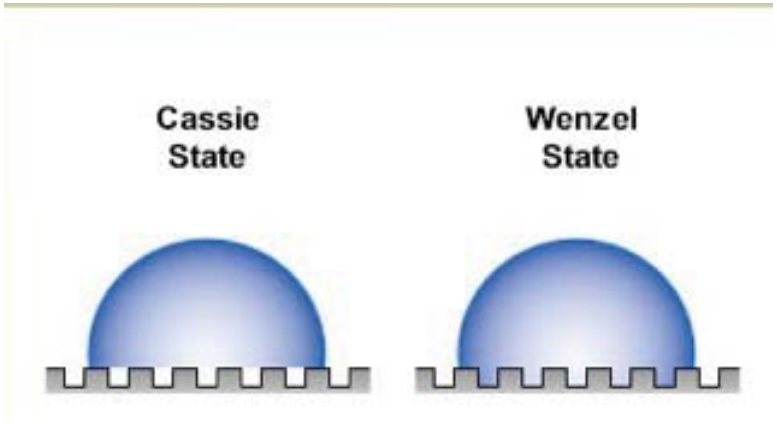



$$I.E. = \frac{1}{2}W_{11} + \frac{1}{2}W_{22} - W_{12} = \gamma_S + \gamma_L - W_{SL}$$




$$W_{SL} = \gamma_{LV}(1 + \cos\theta_{CA}) \approx$$
$$\approx \gamma_L (1 + \cos\theta_{CA})$$

## At the micron/nano scale




*Cassie State:*

$(\cos\theta_{CA})^{obs} =$

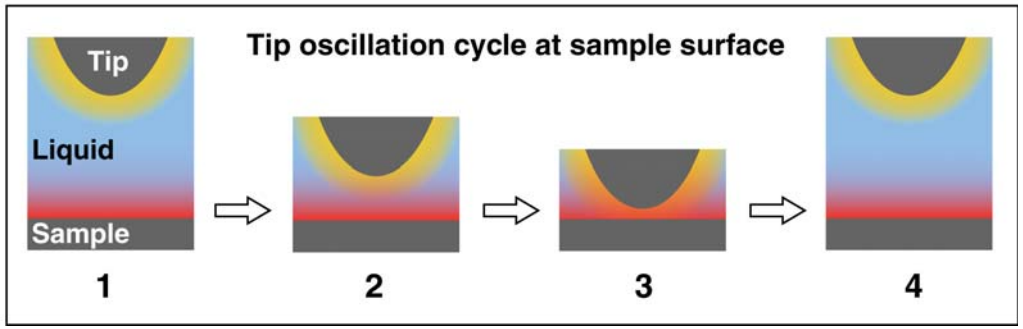
$= f_1 (\cos\theta_{CA})_1 + f_2 (\cos\theta_{CA})_2 =$

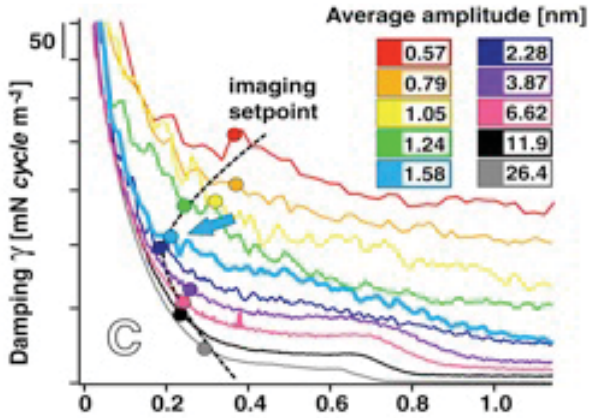
$= f_1 + f_2 (\cos\theta_{CA})_2$

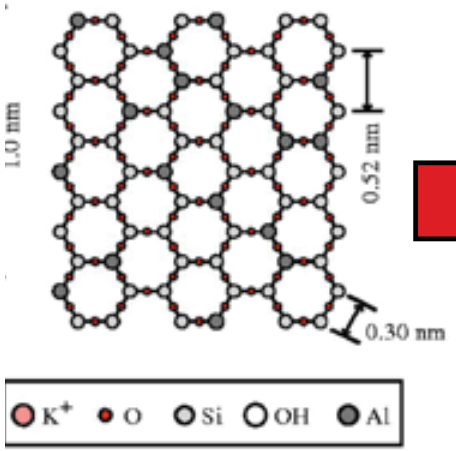
*Wenzel State:*

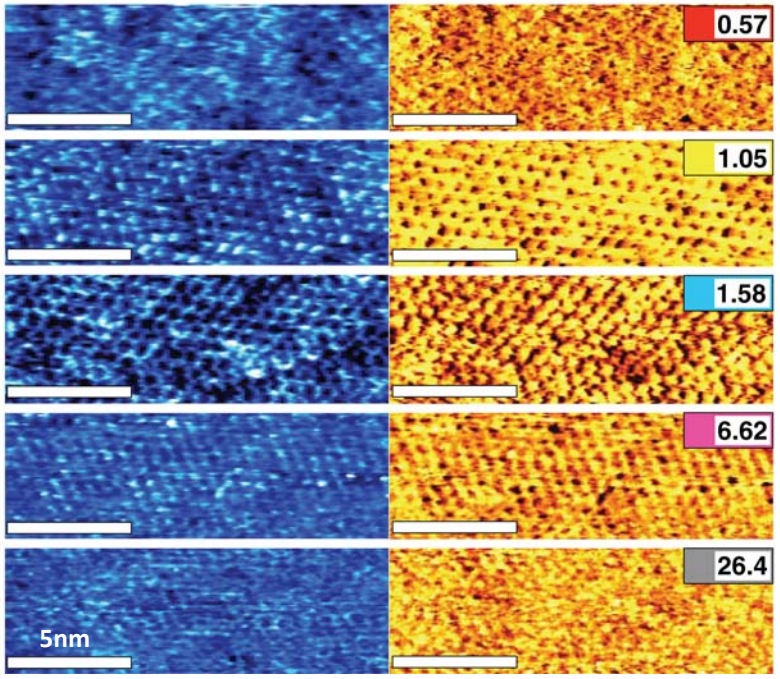
$(\cos\theta_{CA})^{obs} = r (\cos\theta_{CA})$

# Interfacial Energy Microscopy



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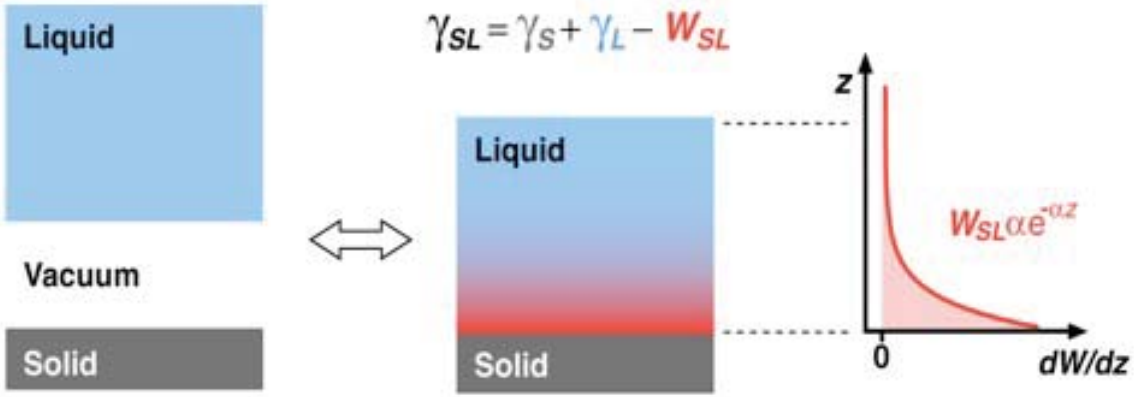


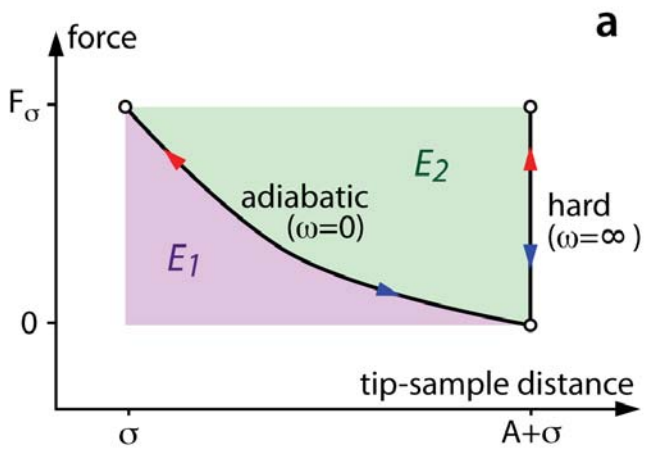


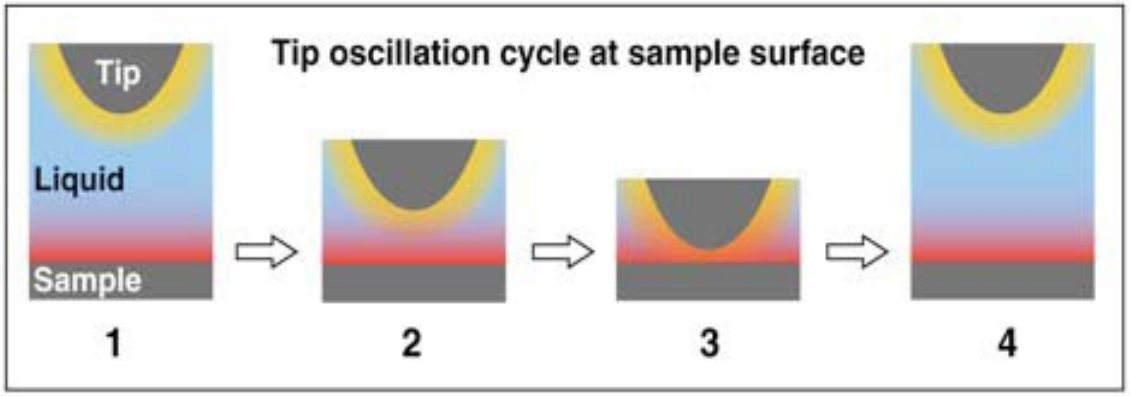


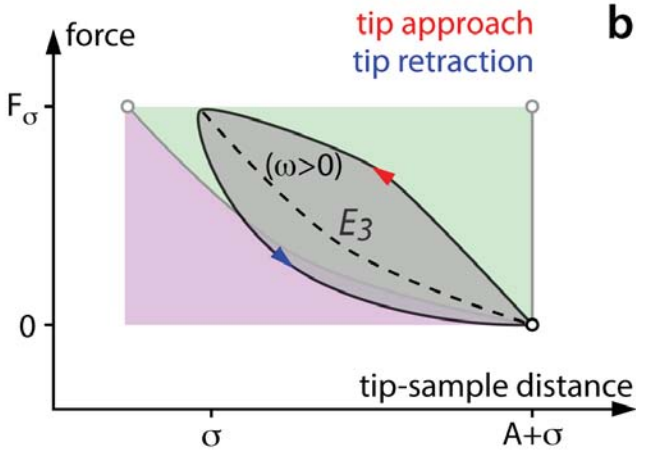


Pressure to remove liquid near the sample and the tip surfaces:

$$P_S(z) \approx W_S \alpha e^{-\alpha z}$$
$$P_T(z, D) \approx W_T \beta e^{\beta (z-D)}$$

$\alpha \sim \beta$

$$E_{cycl,ext} \propto \frac{R}{\alpha}\sqrt{W_S W_T}\left(1 - e^{\frac{-\alpha}{2}A}\right)$$



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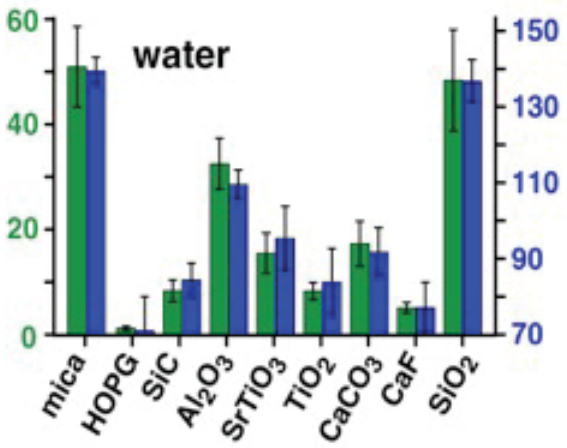


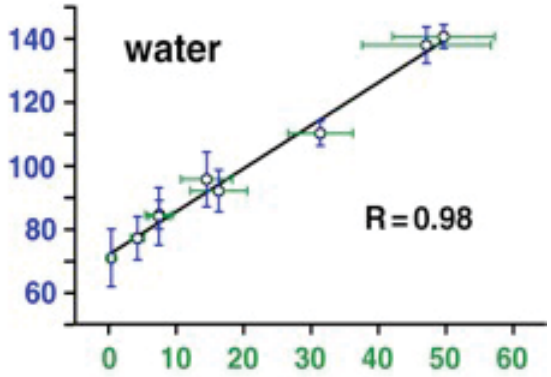


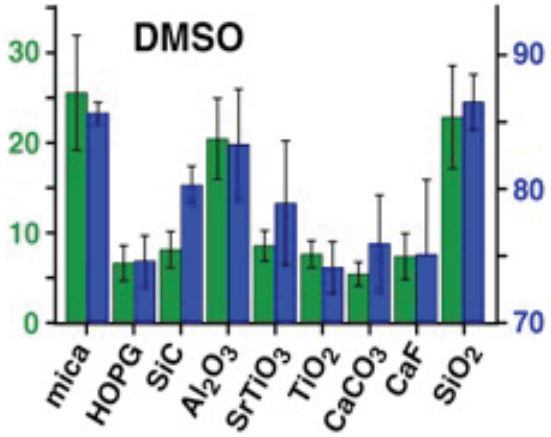


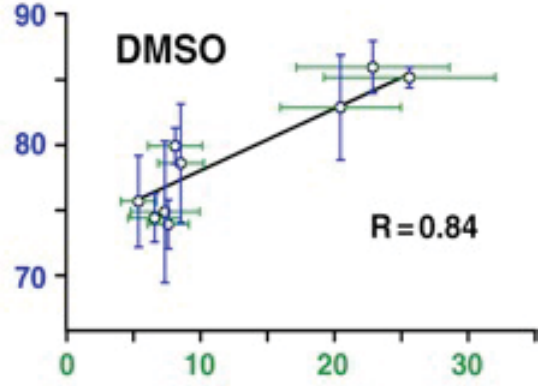


$$E_{cycl,ext} \propto \frac{R}{\alpha}\sqrt{W_S W_T}\left(1 - e^{\frac{-\alpha}{2}A}\right)$$

⇒ high-resolution

phase $\phi \approx E_{cycl,\,ext}$

⇒phase $\phi \approx$ *local wetting* (work of adhesion)



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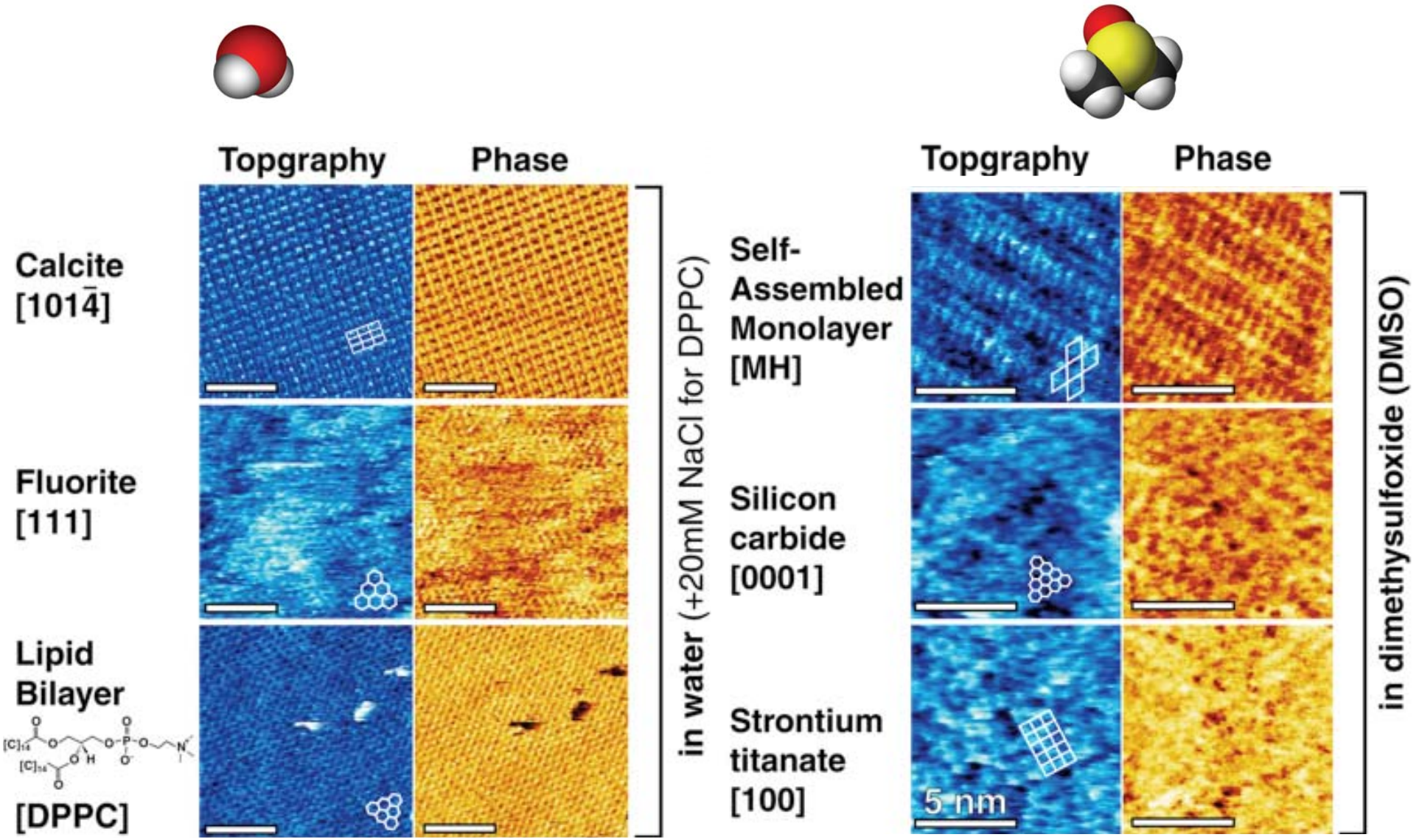
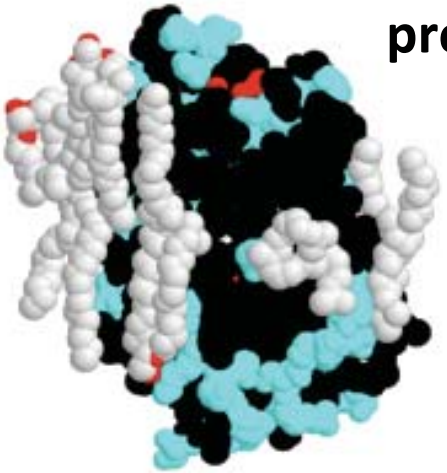
# High Resolution Images

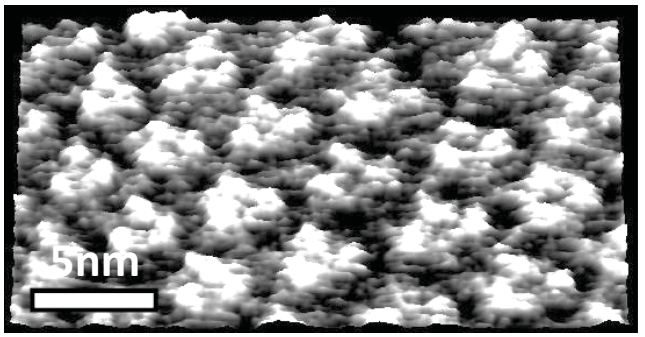




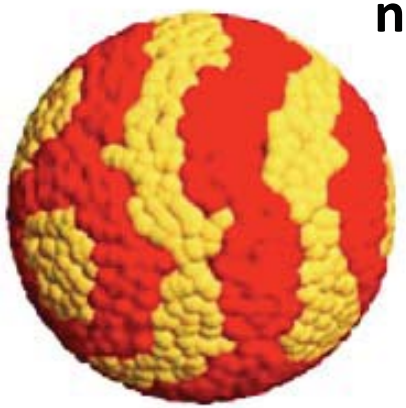

Voitchkovsky et al. **Nature Nanotechnology** 2010

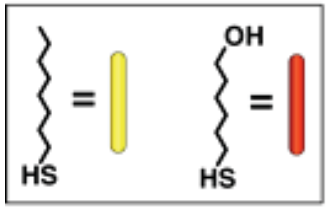


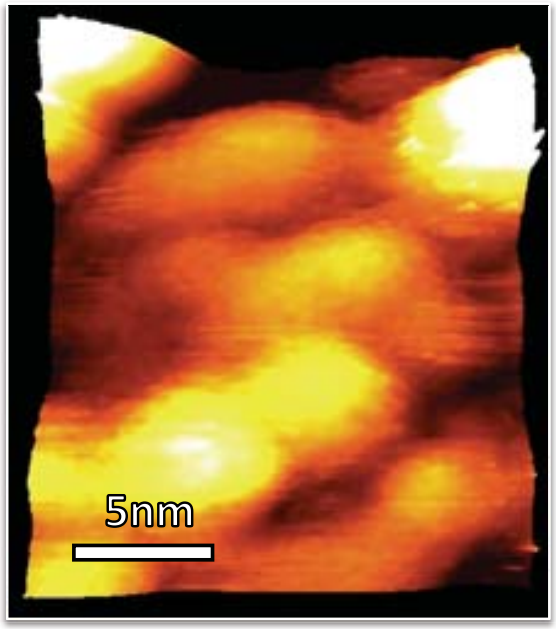
protein

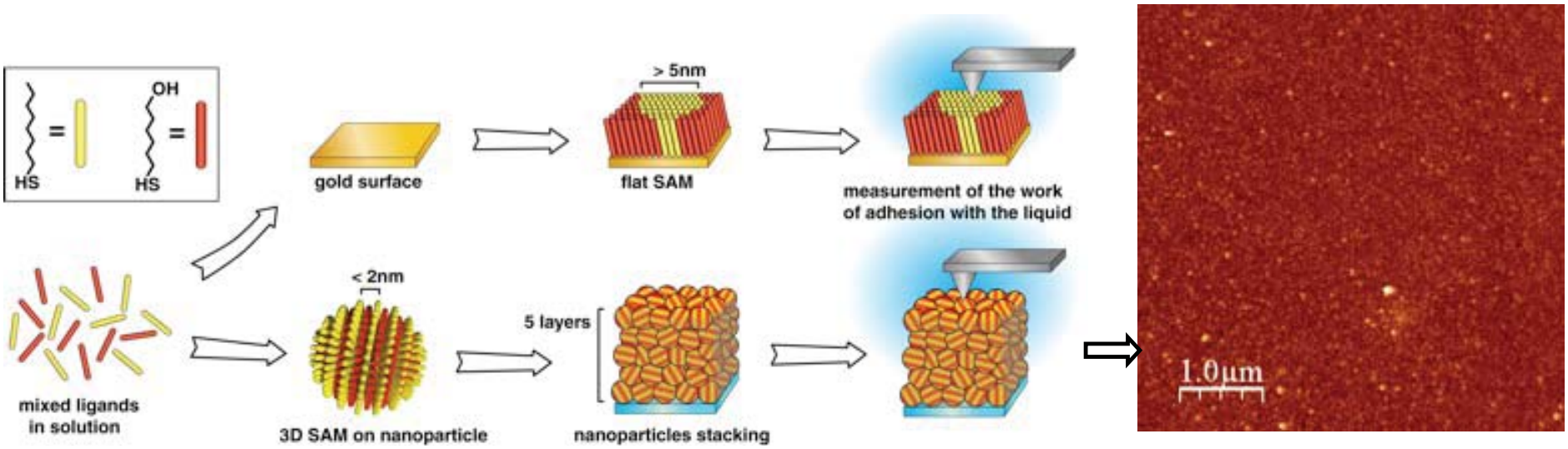



ligand-coated nanoparticule





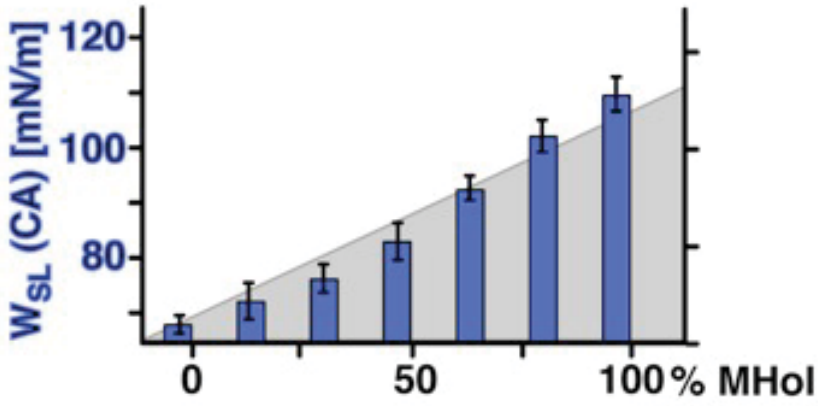






$$W_{sl} = x_A W_{sl}^A + x_B W_{sl}^B$$

$x_A$ = component A surface fraction

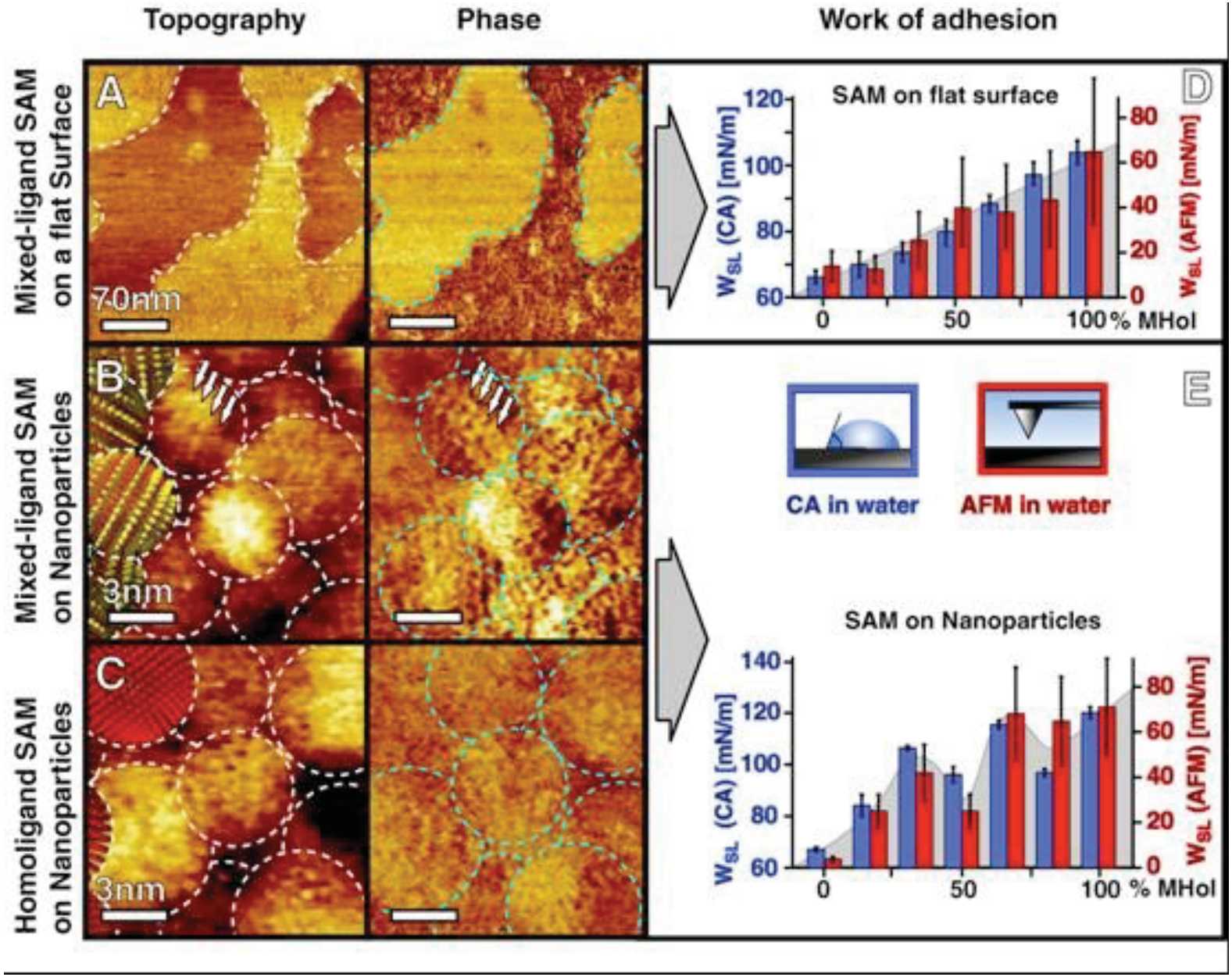
$x_B = 1 - x_A$






Kuna, Voitchovsky,, Stellacci, **Nature Mat.** *2009*

# Work of Adhesion Measurements



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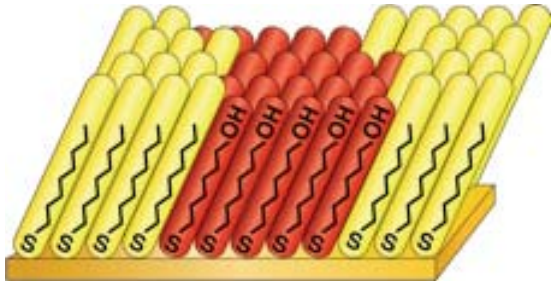
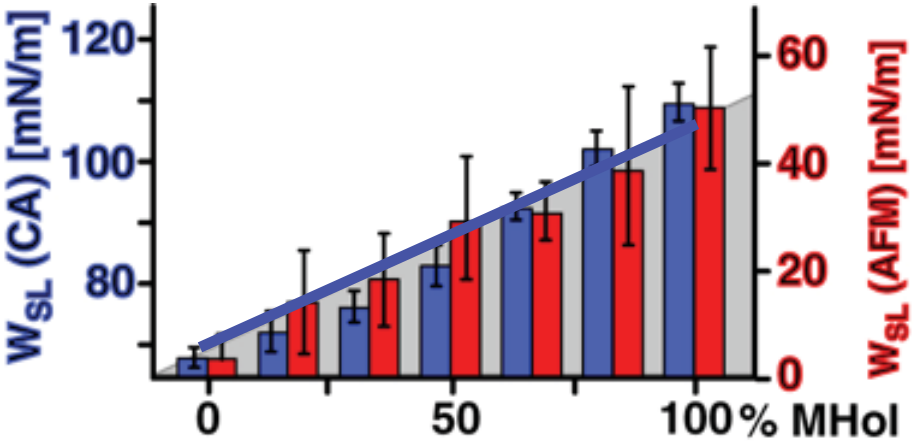
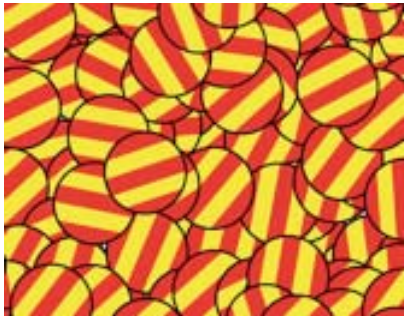
# Structure Component to I.E.

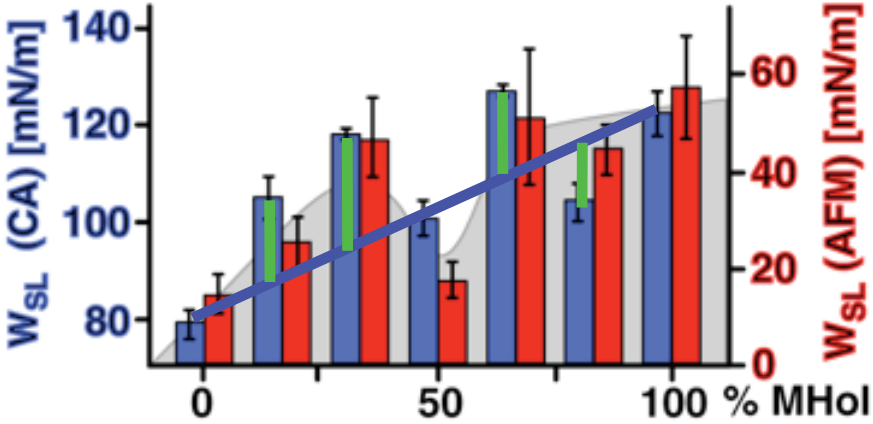



$$W_{SL}^{AB} = x_A W_{SL}^{A} + x_B W_{SL}^{B}$$



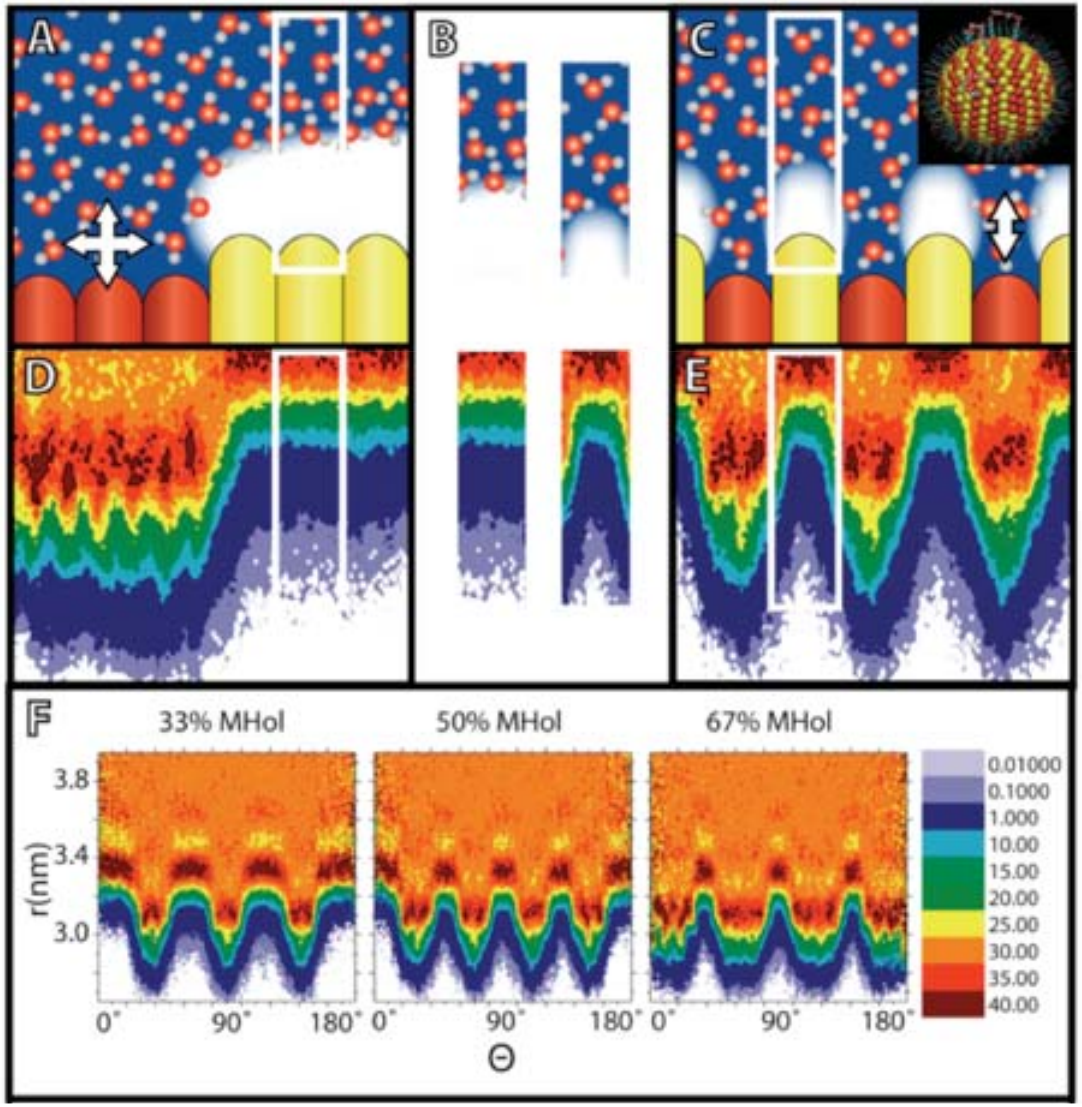



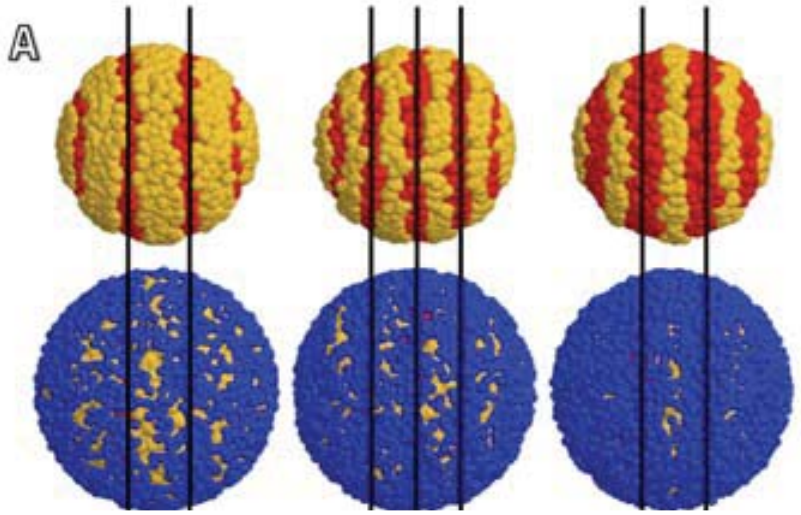
$$Wsl = x_A W_{sl}^{A} + x_B W_{sl}^{B} + f(structure)$$

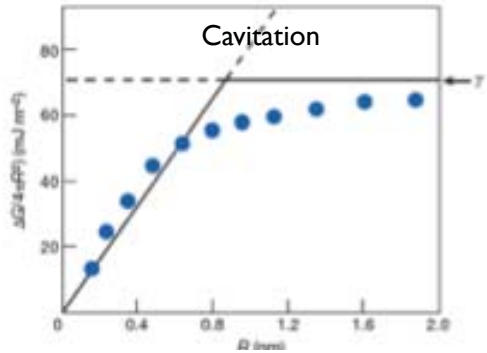





Kuna et al. ***Nature Materials***, 2009



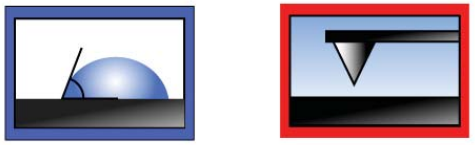




From D. Chandler, **Nature**, 2005



*Kuna et al.* ***Nature Materials,*** 2009

# Adding salt should decrease cavitation…

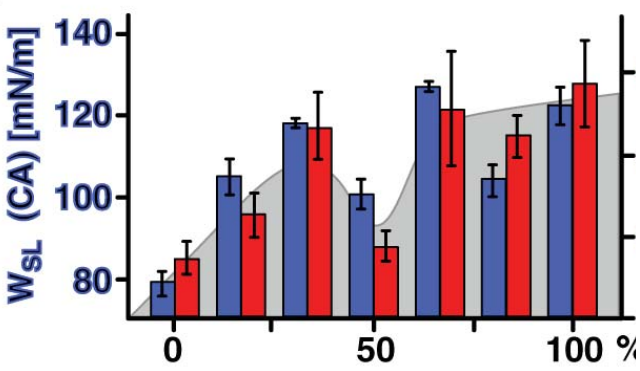


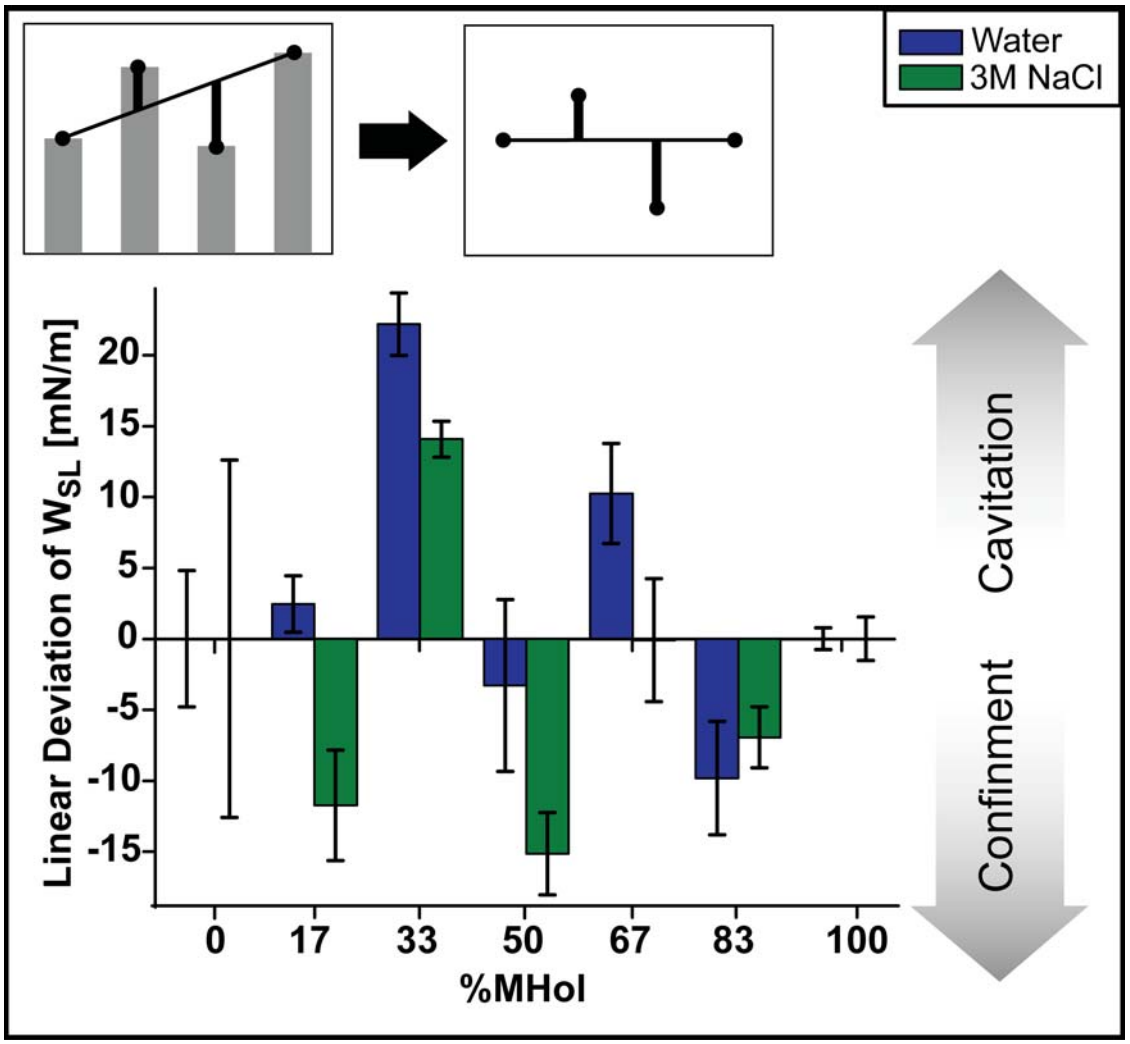
CA in water



AFM in water

SAM on Nanoparticles



- Atomic Force Microscopy can be used to locally probe and map solid-liquid interfaces

- At the nanoscale, interfacial properties (interfacial energy) strongly depend on structure.

- This becomes particularly important when the size of the different interaction sites (hydrophilic/phobic) becomes commensurate with that of the solvent molecules, as illustrated here with nanoparticles

- This is the norm in biology!

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