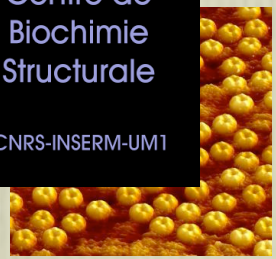
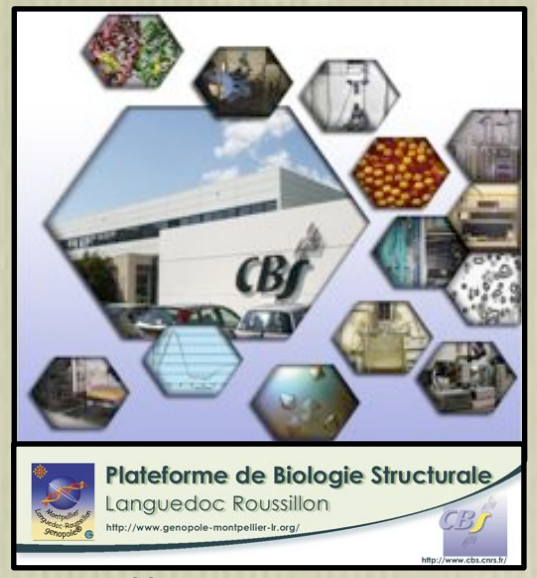
# Nanoscale Imaging of Artificial Membranes using Atomic Force Microscopy

Centre de Biochimie Structurale
CNRS-INSERM-UM1
pem@cbs.cnrs.fr



## Center of Structural Biology

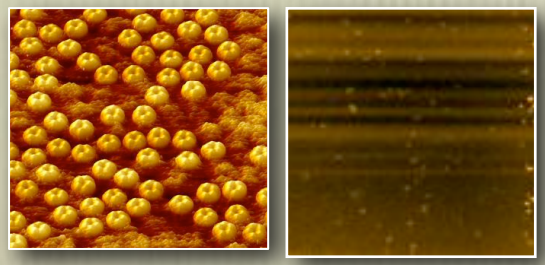
# CBS



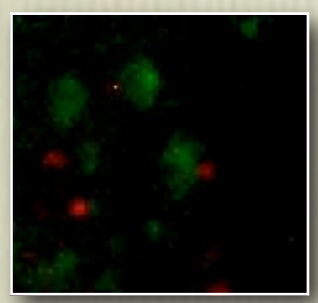

http://www.cbs.cnrs.fr

## Single Molecule Biophysics Group

Structure and dynamics of membrane assemblies



*Atomic Force Microscopy*



*Single Molecule Tracking*

# OUTLINES

- How to mimic biological membranes
  *Supported lipid bilayers (SLB) and Atomic Force Microscopy (AFM)*
- Development of a membrane biosensor based on porous silicon
- Direct incorporation of solubilized proteins into supported lipid bilayers

# Requirements for Membrane in Biosensor

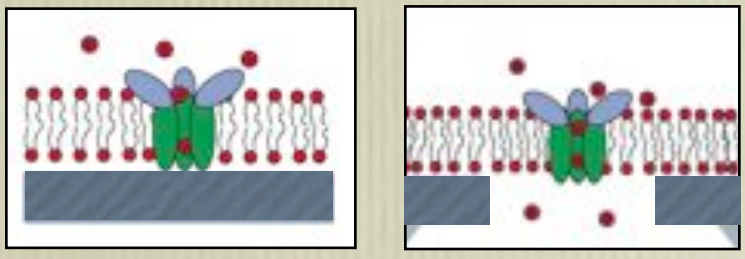
Mimicking the membrane composition

Continuous membrane

Separation of 2 compartments

Stability

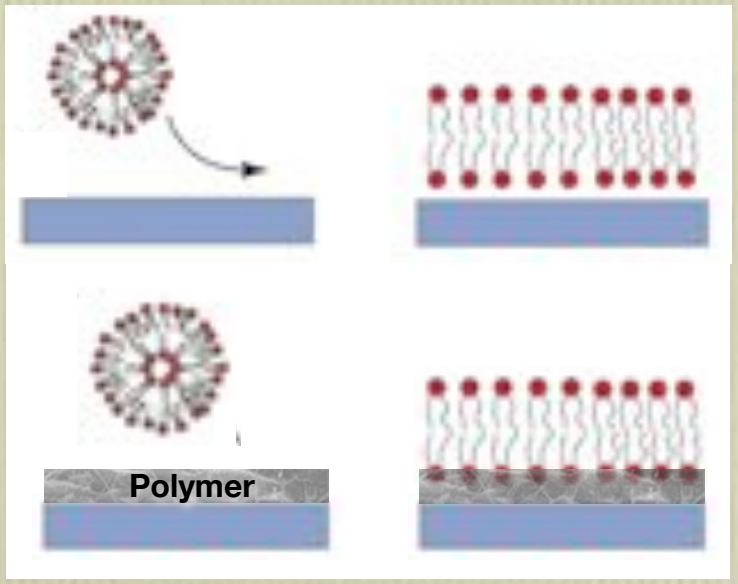
Membrane decoupling from the substrate

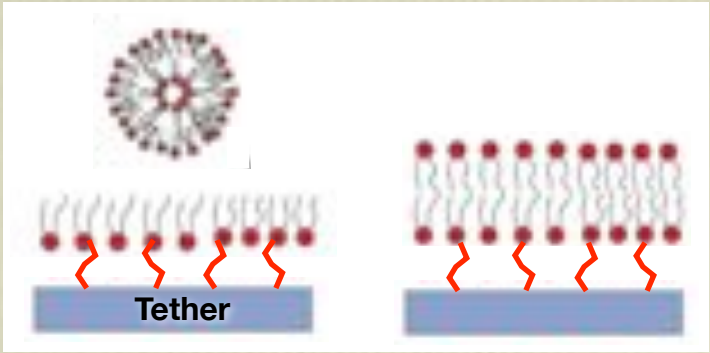


Adapted from Reimhult and Kumar (2007) TiBS

# Methods used to form lipid membrane on substrates

## Large Unilamellar Vesicle (LUV) Fusion



# Characterization of Membrane-inspired biosensor

**Quartz Crystal Microbalance with Dissipation (QCMD)**

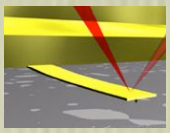
**Surface Plasmon Resonance *Spectroscopy***

**Fluorescence**

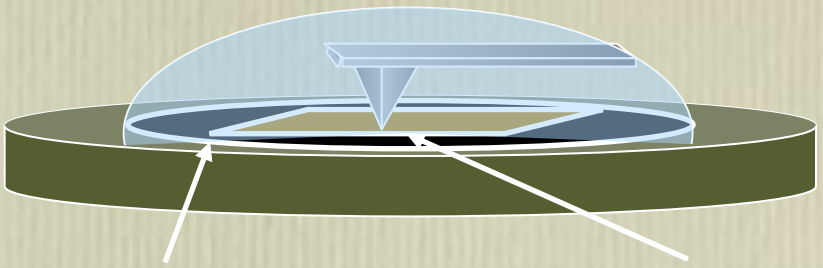
**Small angle X-rays scattering**

**Atomic Force Microscopy**

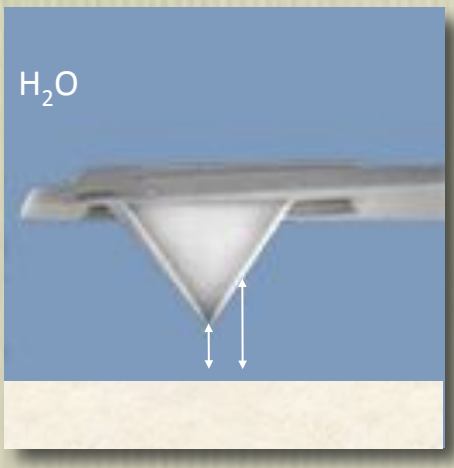
Current Opinion in Colloid & Interface Science 13 (2008) 326–337



# AFM Imaging in aqueous buffer




Electrostatic force
Repulsive
(0.1 to 1 µm)



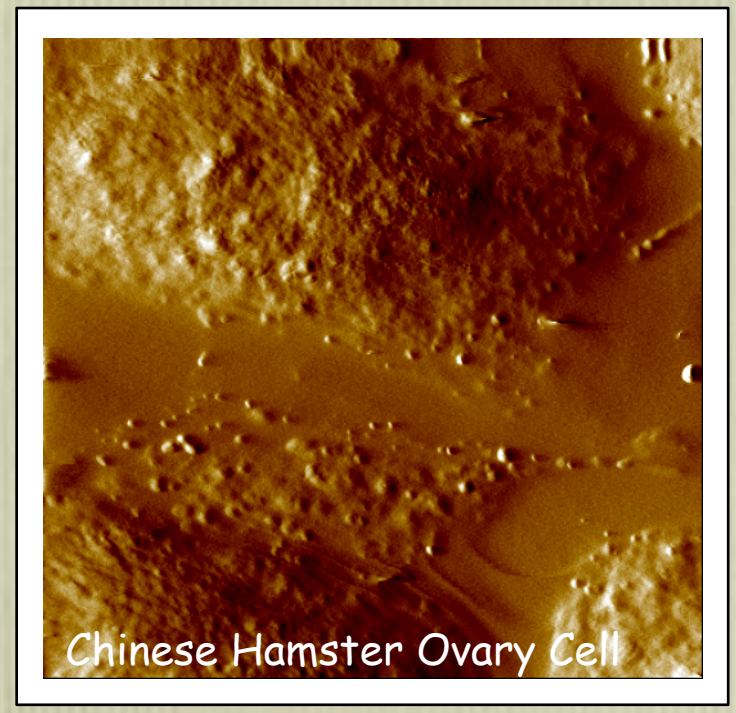

Van der Walls
Attractive Force (~Å)

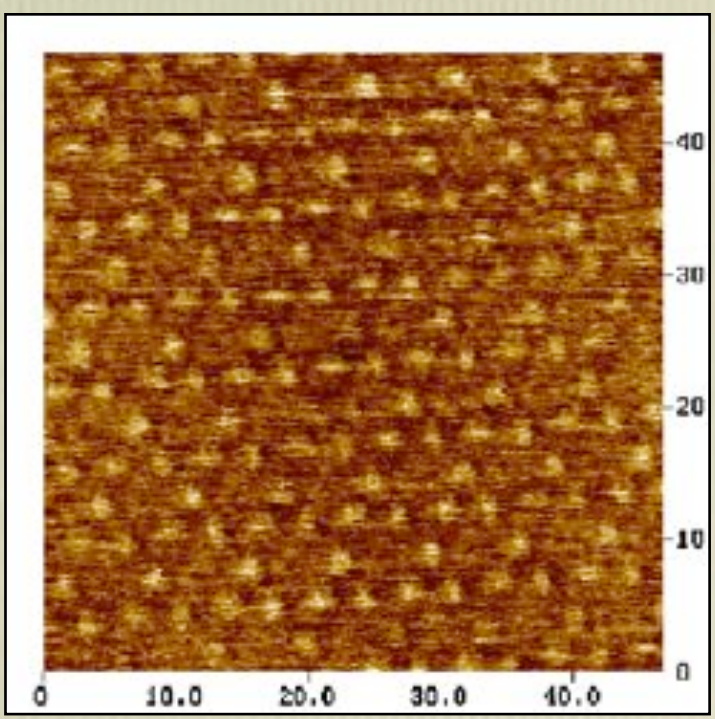
**Force applied during scanning < 100 pN**

Silicium Nitride k<100 mN/m

CBS

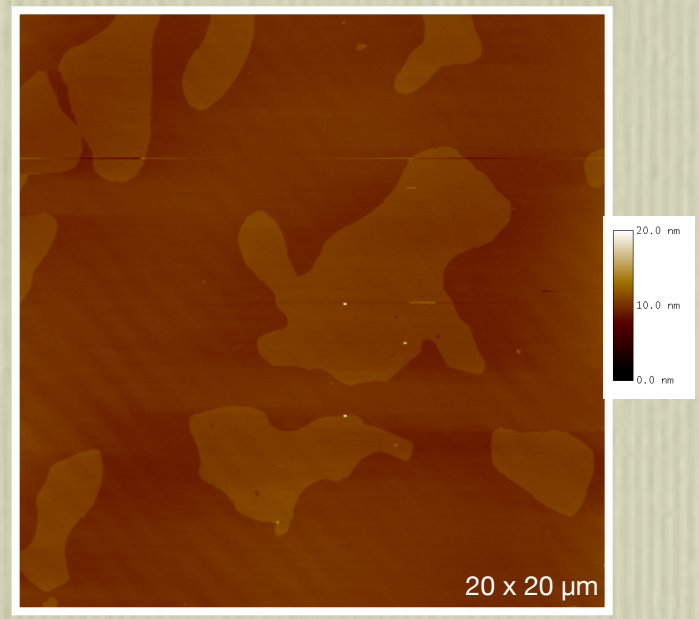
# From the intact cell to … the molecule

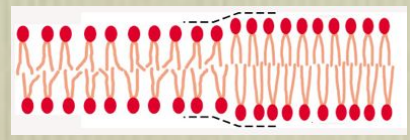


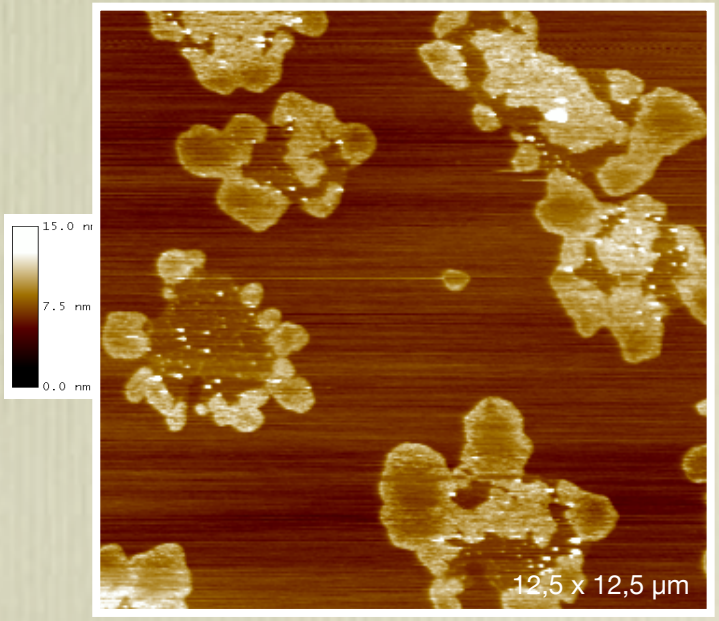




Vertical (0.1 nm) and lateral (0.5 nm) resolution

# Outstanding method for membrane characterization

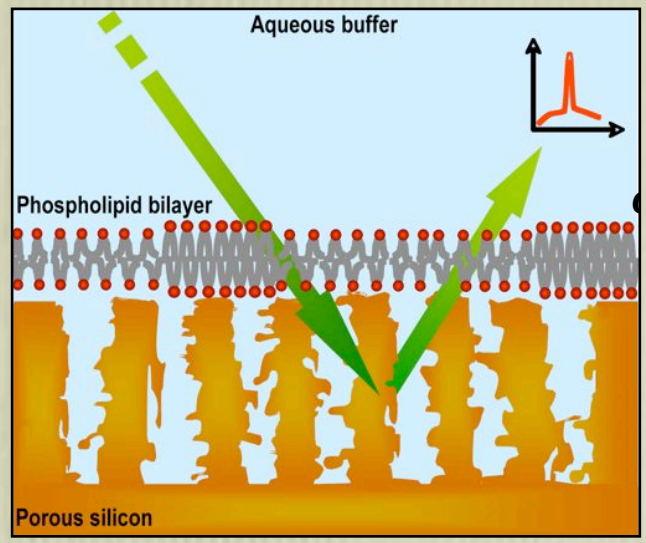


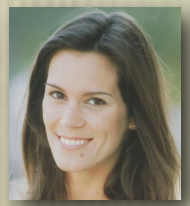






Pflugers Arch. – Eur J Physiol, (2008) 456(1):179-88.

# Development of a membrane-inspired biosensor on porous silicon



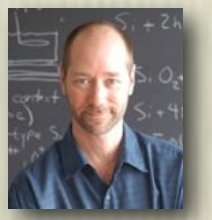



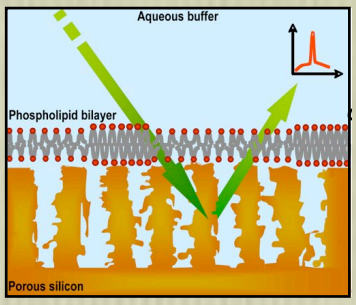
ICG Montpellier
UMR 5253 CNRS

Stéphanie Pace
**Frédérique Cunin**
Jean-Marie Devoisselle

UCSD

**Michael J. Sailor**
Emily J. Anglin






# Characterization of the ability of drugs to cross membranes

## Porous silicon

Optical properties (fluorescence, reflectivity)

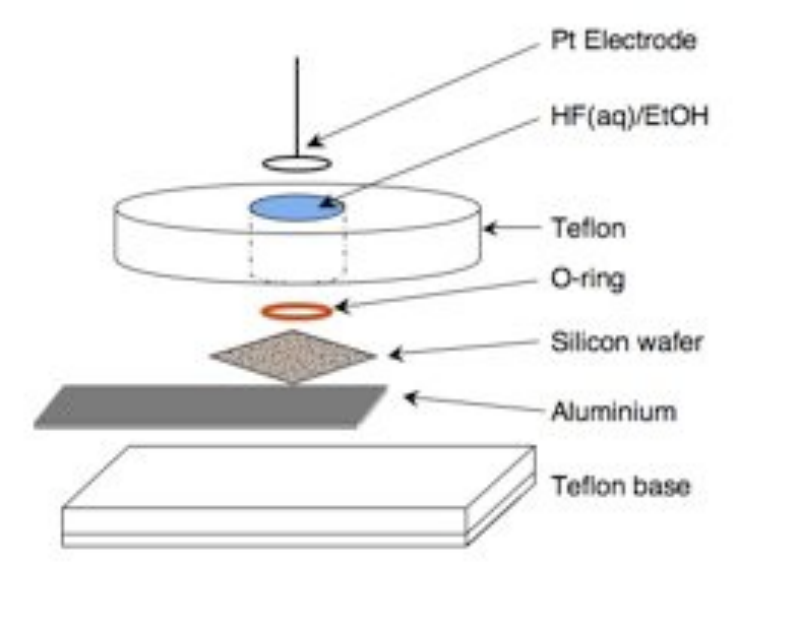
Tunable structural properties (surface, volume, size of the pores)

Silicon chemistry (surface modification)

Reservoir of buffer

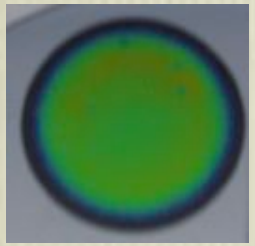
# Preparation of the porous silicon

*Anodization conditions*



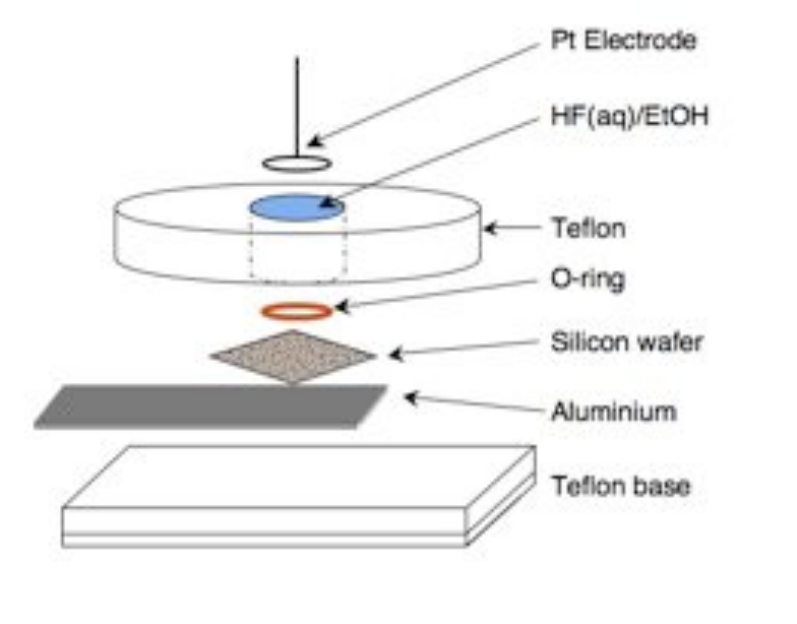

- P type silicon
- Solution of HF/Ethanol (3/1 in volume)
- Current density 22.5 mA/cm$^2$ for 5 min
- Thermal oxidation 450°C for 2 hours then treatment with NaOH (1M)

$$Si + 6F^- + 2h^+ + 2H^+ \longrightarrow SiF_6^{2-} + H_2$$

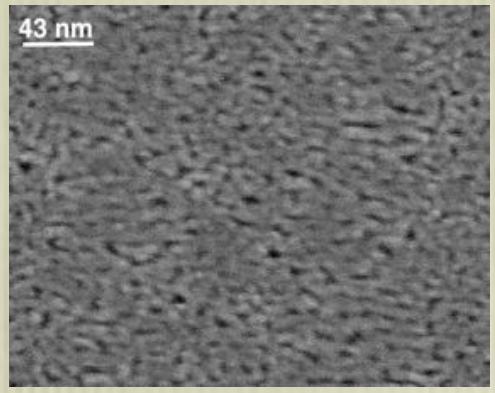


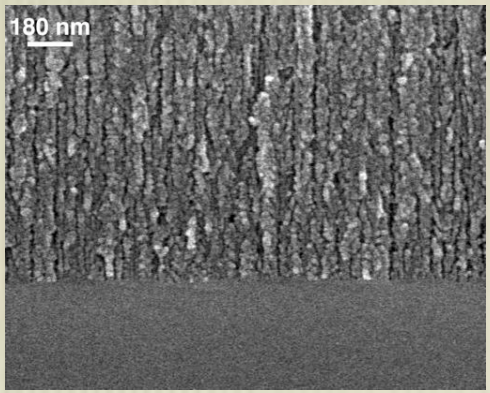
# Preparation of the porous silicon

*Anodization conditions*




- Pores diameter 5-9 nm
- Thickness 3.7 μm

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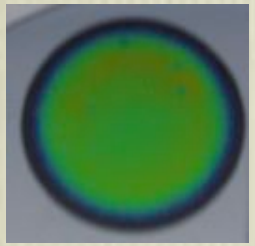
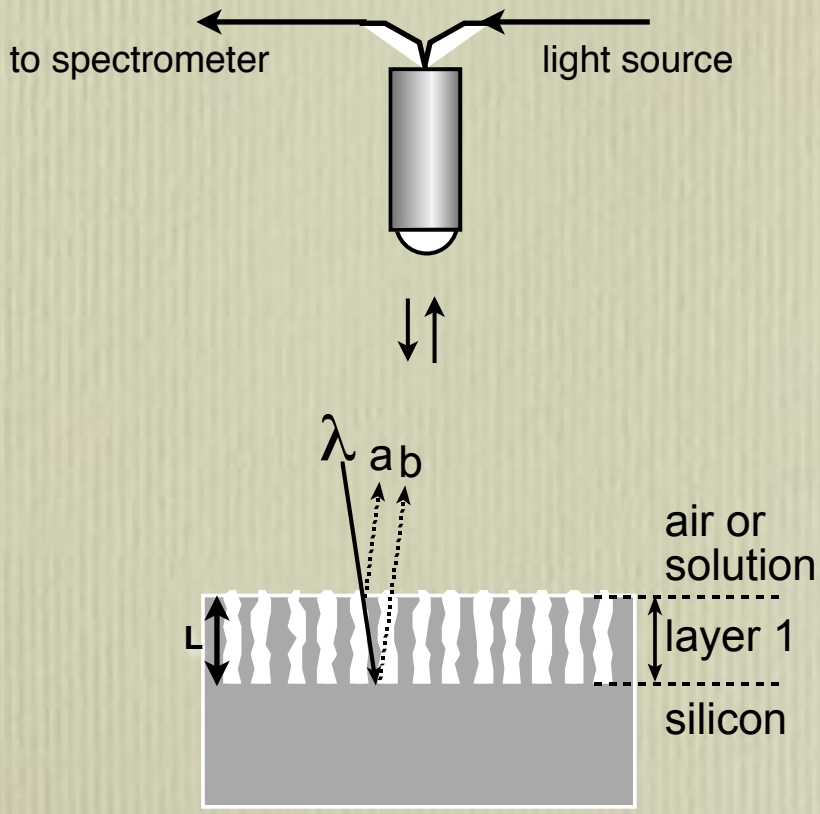


*22,5 mA/cm²*

# Optical properties of porous silicon

## Sensing using Optical Reflectivity from Fabry-Pérot Layers




**Fabry Perot interference pattern**

$m\lambda = 2nL$

*n* is the refractive index, *L* the thickness , λ the wavelength

# Optical properties of porous silicon

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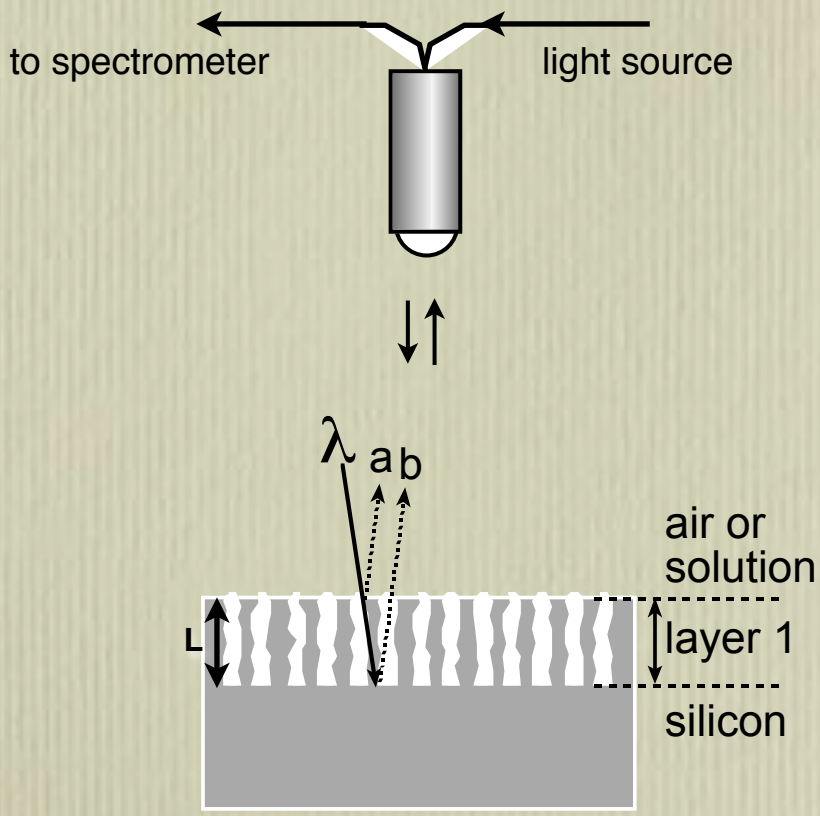


**Fabry Perot interference pattern**

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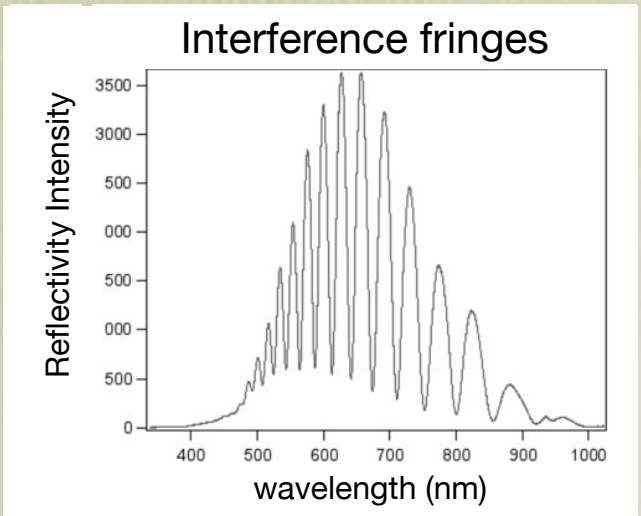
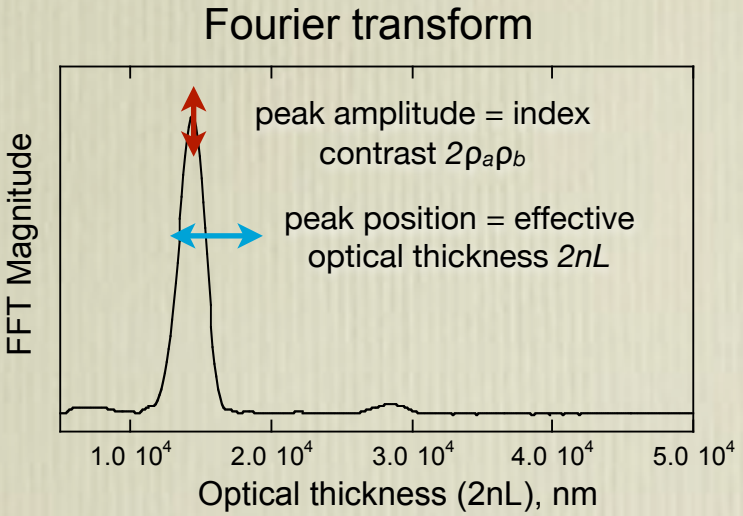
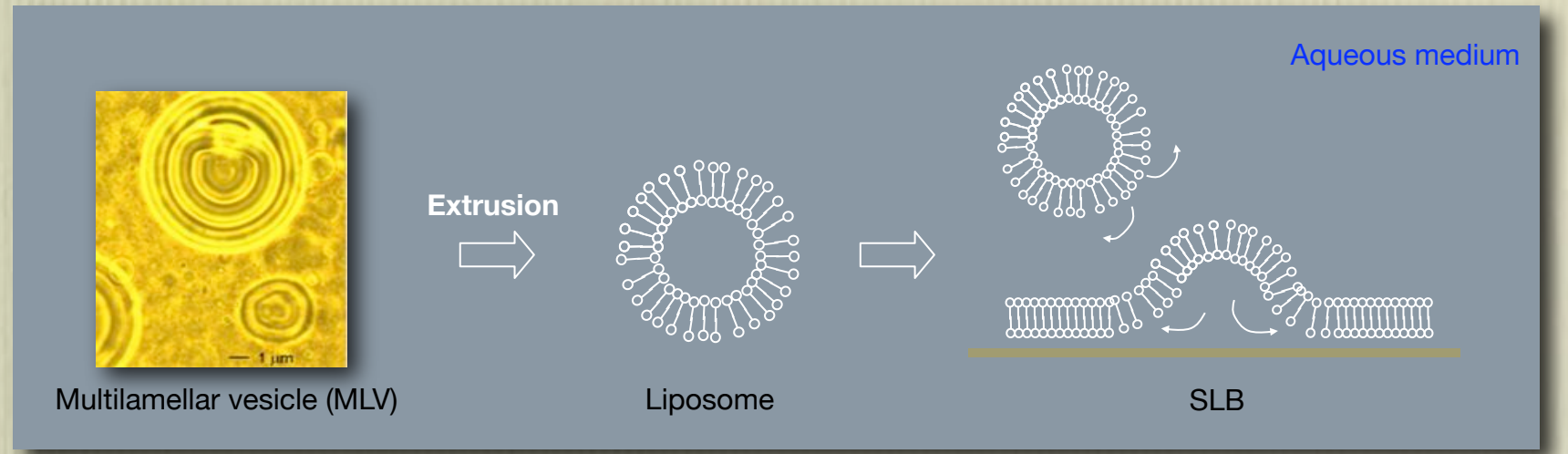
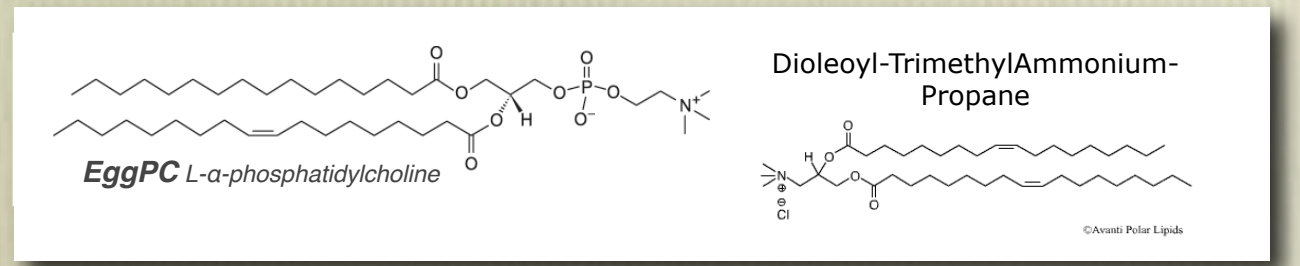


# Supported lipid bilayer by vesicle fusion

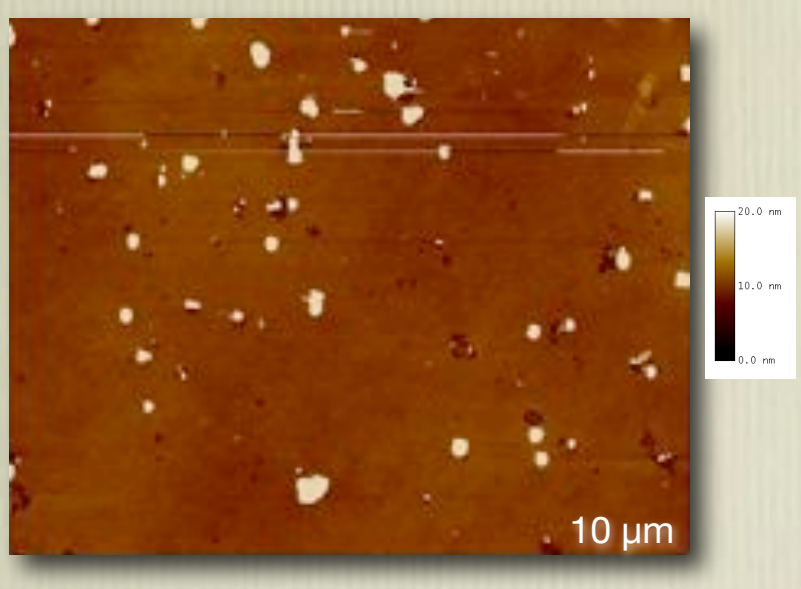



EggPC + 10% DOTAP/DPTAP + 0.1% Rhodamine-DHPE

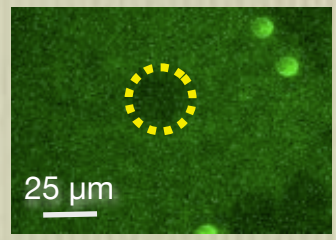
# EggPC/DOTAP
# Supported Lipid Bilayer

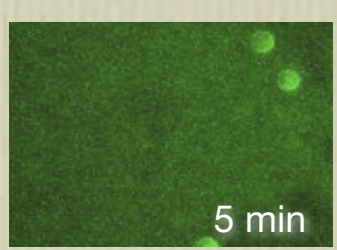

99,9% coverage



# EggPC/DOTAP
# Supported Lipid Bilayer

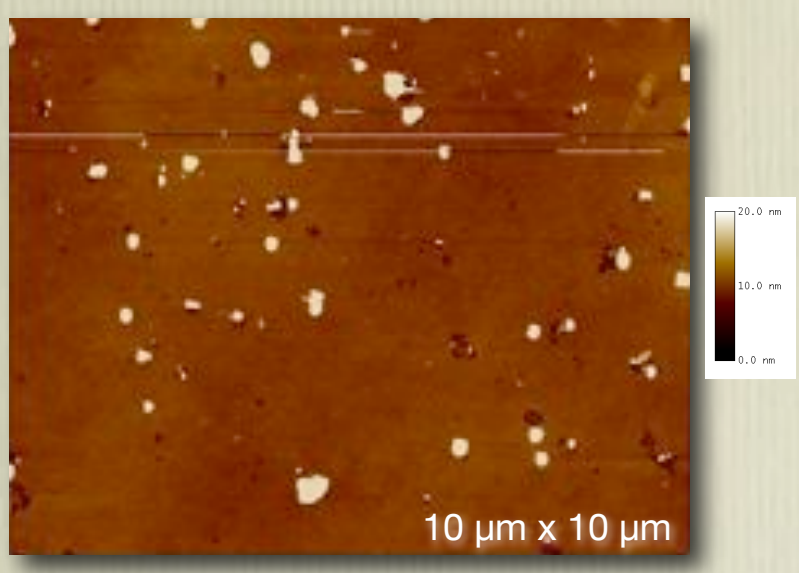
FRAP




D =1.58 µm$^2$/s
Mobile fraction = 78%


99,9% coverage



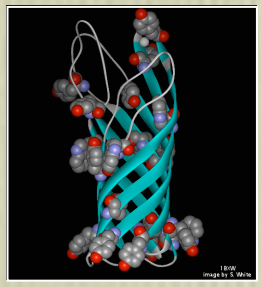
# Direct Incorporation of transmembrane proteins within artificial bilayers

25% of the human genome encode transmembrane proteins.

Target of 70% of the commercially available drugs.

## In a Structural point of view

~ 200 structures available in the PDB (~ 20 eukaryotic).



Beta barrels
(porin)

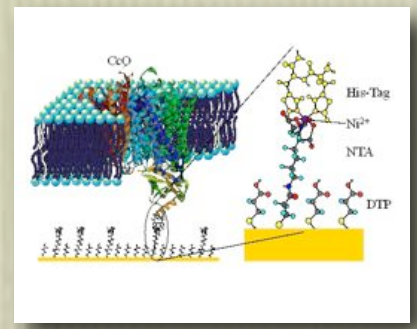
Alpha helix
(GPCR)

## In a nano-biotechnological point of view

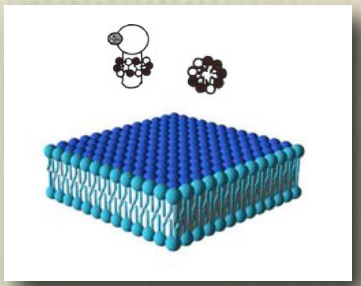
# How to incorporate transmembrane proteins within artificial bilayers ?

**Proteoliposome fusion**

**Tethered proteins reconstitution**




**Incorporation**

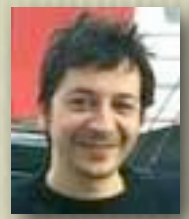


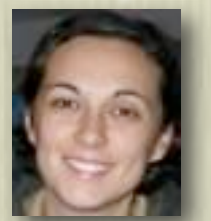
CBS

# Direct Incorporation of transmembrane proteins within artificial bilayers

UMR168
CNRS
institutCurie
Ensemble, prenons le cancer de vitesse.

Francesca Gubellini
**Manuela Dezi**
**Daniel Levy**

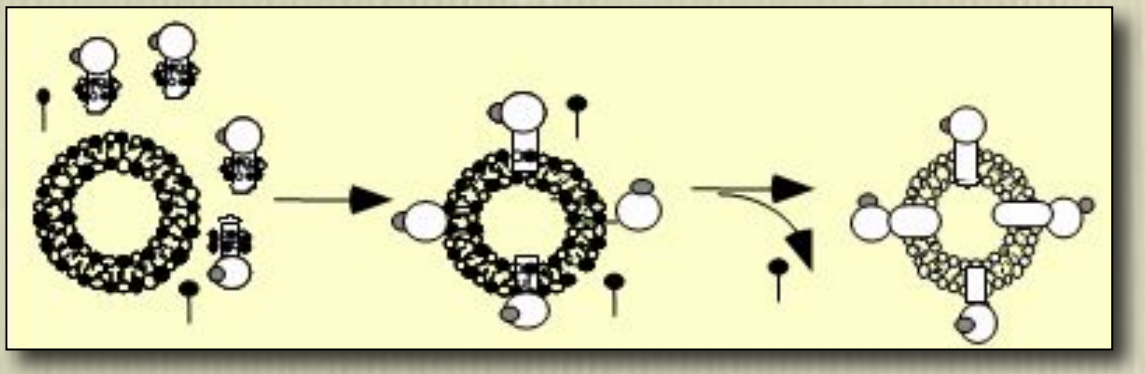




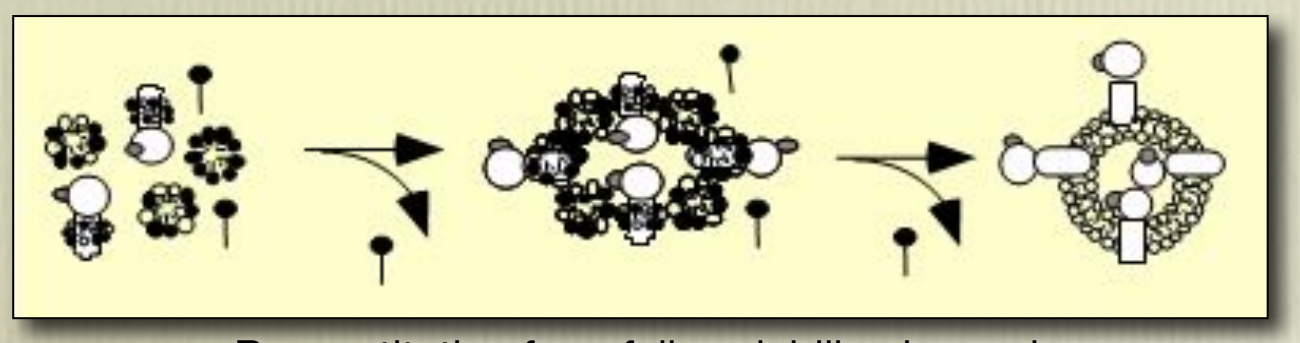
CBS

# Direct incorporation of protein into SLB

Direct incorporation of protein into liposomes
destabilized with glycosylated detergent



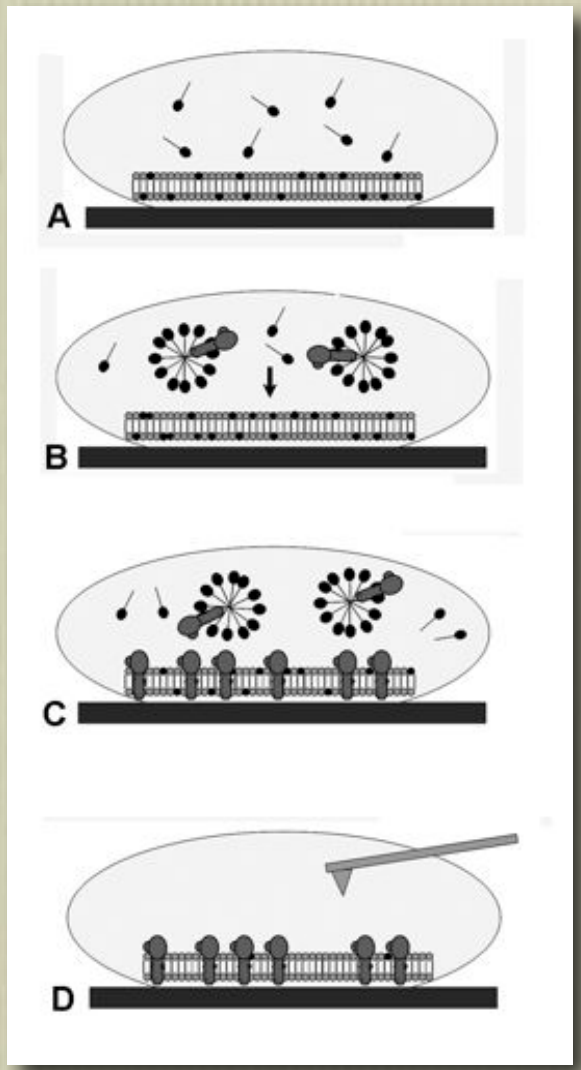
Unique
orientation



Reconstitution from fully solubilized samples

Rigaud et Levy, Meth in Enzymol. (2003)

# Direct incorporation of protein into SLB




1

SLB destabilization

[detergent] ~ cmc

2

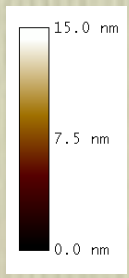
Incorporation

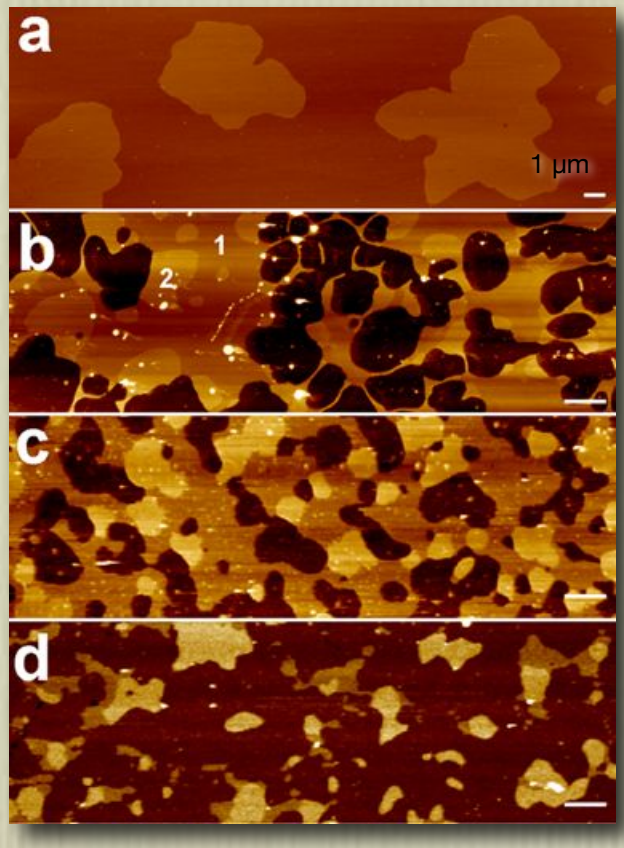
3

AFM Imaging
of non crystalline proteins

# Control SLB treated with detergent

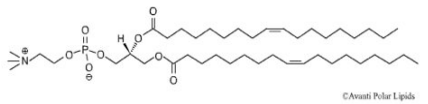
15 min incubation with detergent (1.5 x cmc à 20°C)



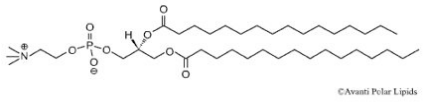




DOPC/DPPC (1:1)

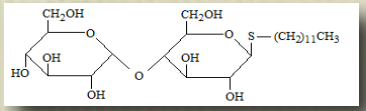
Dioleoyl-phosphatidylcholine



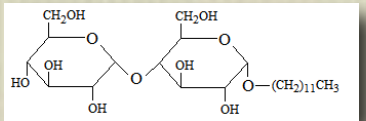
Dipalmitoyl-phosphatidylcholine



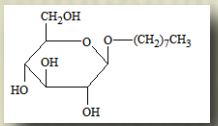
n-Dodecyl-β-D-Thiomaltopyranoside (DOTM)
cmc = 0.05 mM



n-Dodecyl-β-D-Maltopyranoside (DDM)
cmc = 0.2 mM



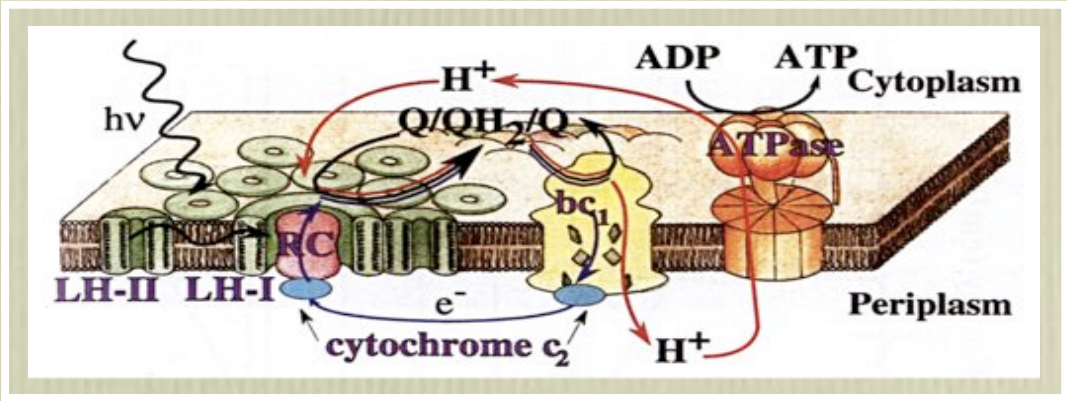
Octyl-β-D-glucopyranoside (OG)
cmc = 17 mM



**- SLB are stable above the cmc with low cmc detergent and more resistant than liposomes.**

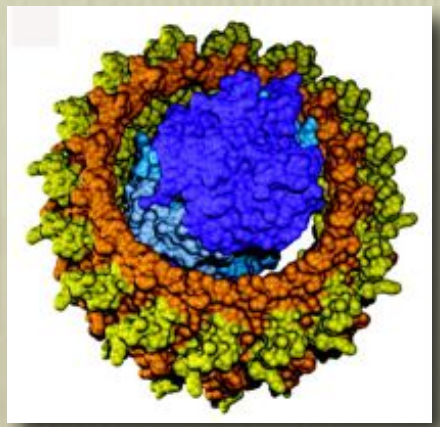
**- Both gel and fluid phases are preserved.**

# Incorporation of proteins from the photosynthetic apparatus of bacteria



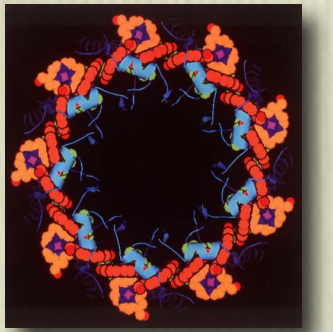

LH, Light-Harvesting

RC-LH1
*Rhodobacter spheroides*



MW 300 kDa

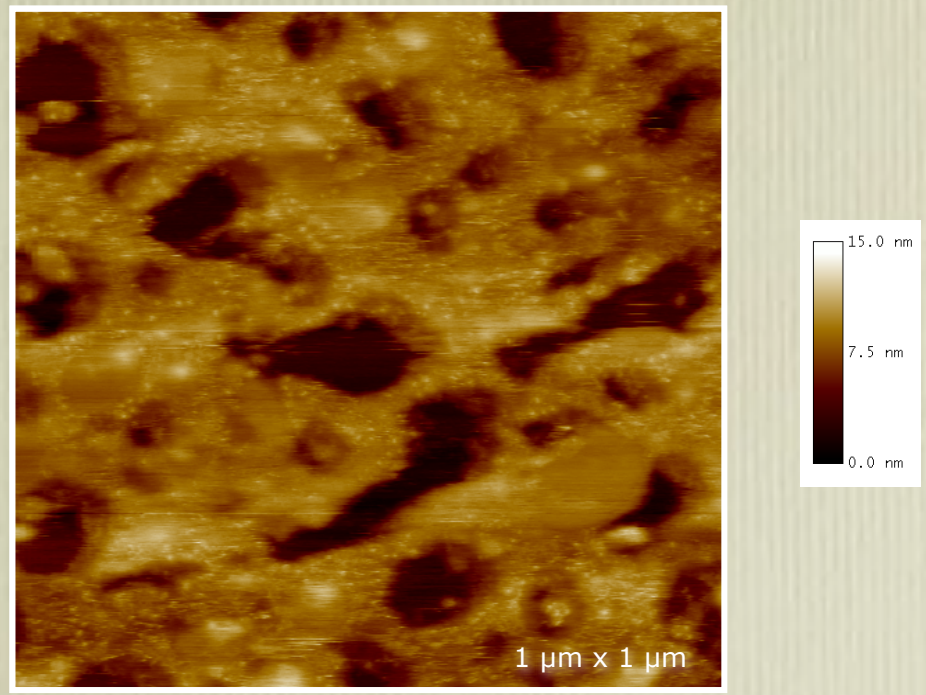
LH2
*Rhodopseudomonas acidophila*



MW 110 kDa

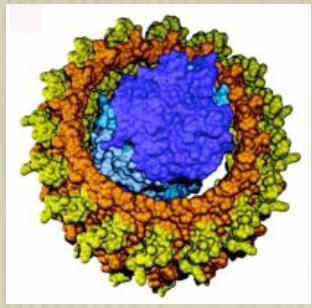
# Incorporation of RC-LH1 from *Rhodobacter spheroides*

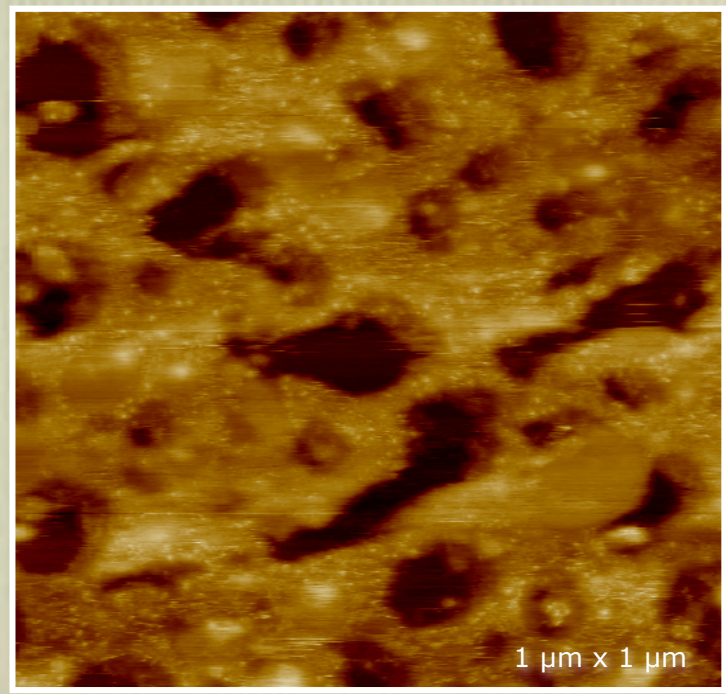
Experimental procedure
500 ng (1.5 picomole) in 0.075 mM DOTM, 150 mM KCl, 10 mM Tris pH 7.4
15 min incubation with RC-LH1

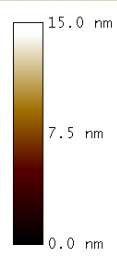



Contact mode

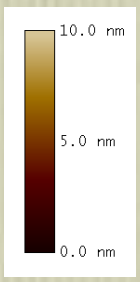
# Incorporation of RC-LH1 from *Rhodobacter spheroides*

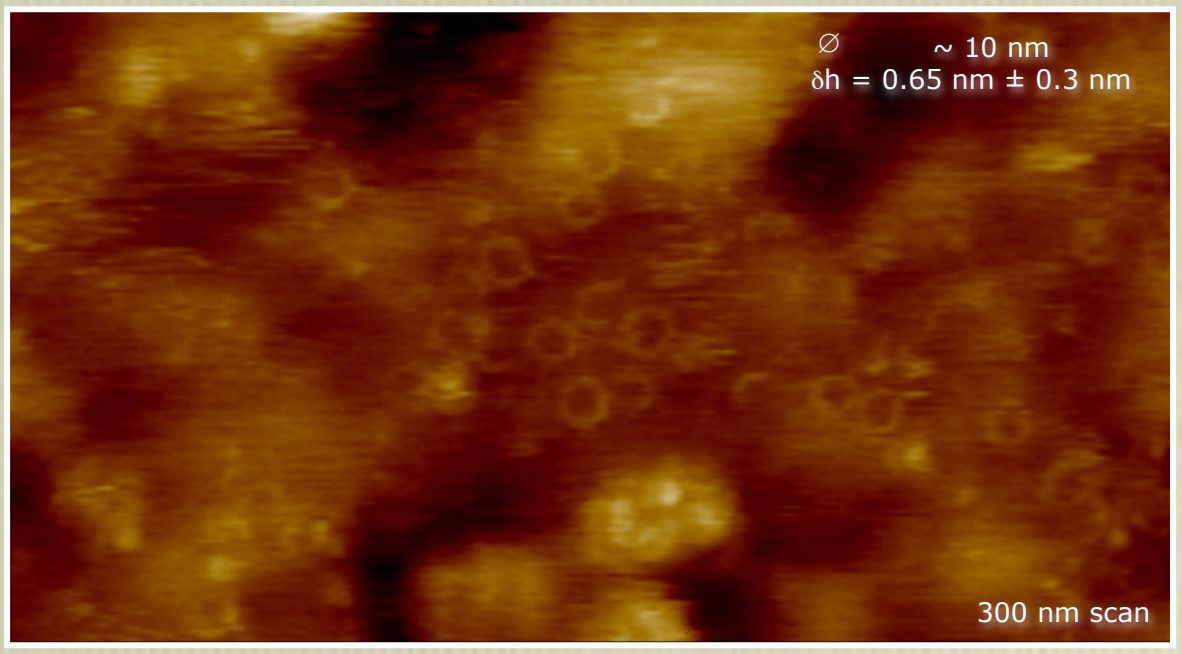


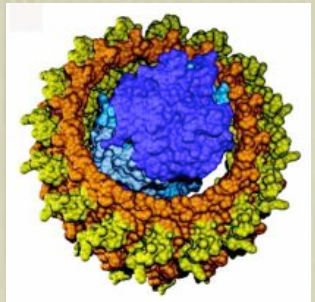


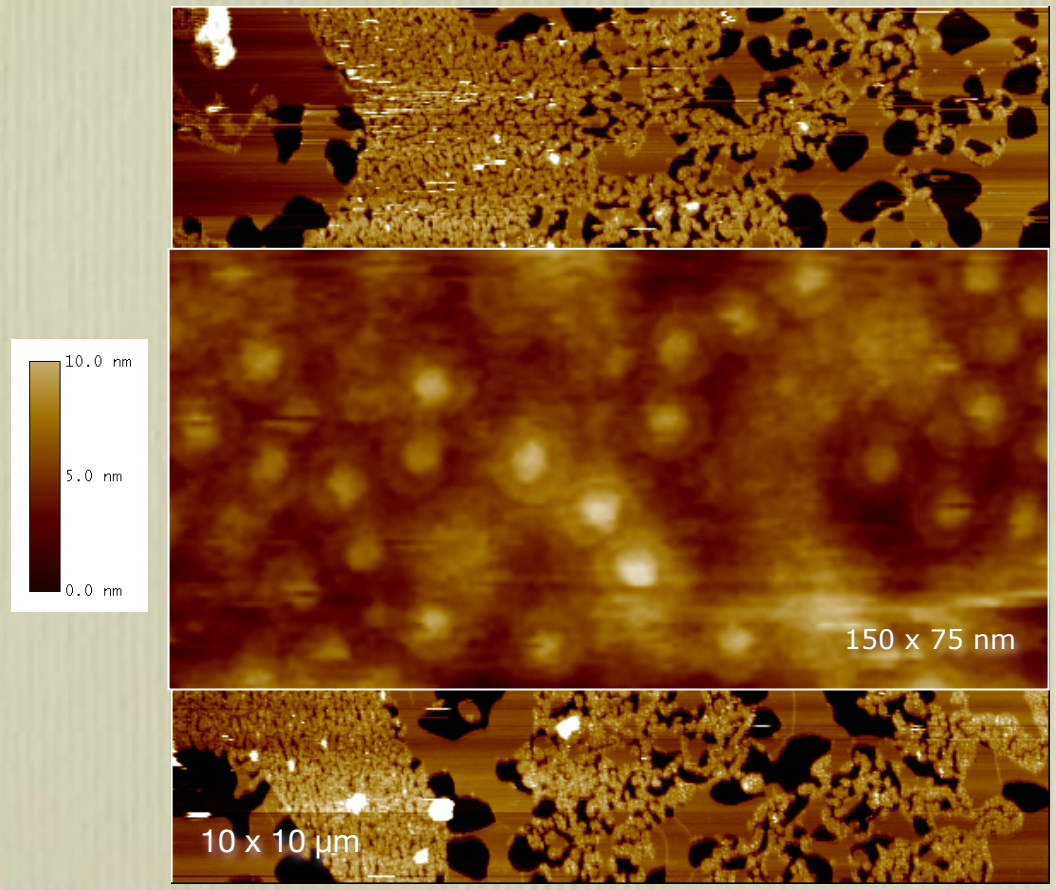
# Incorporation of RC-LH1 from *Rhodobacter spheroides*



# Incorporation of RC-LH1 from *Rhodobacter spheroides*




Contact mode

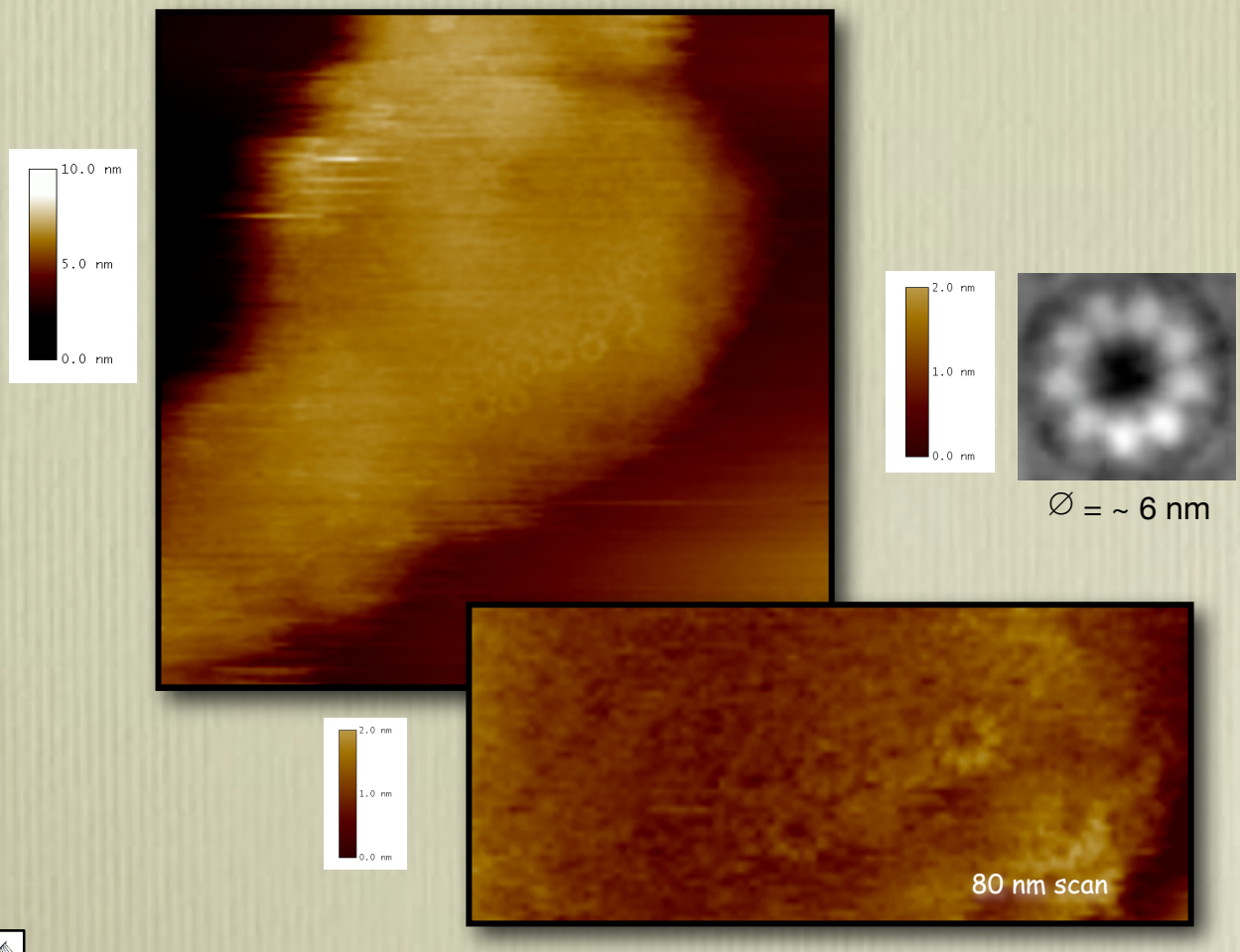
Membrane remodelling

Unidirectional incorporation

**Proteins diffuse in the fluid phase and segregate in the lipid bilayer**
**Formation of quasi-crystalline areas**

# Incorporation of LH2 from *Rhodopseudomonas acidophila*

Experimental procedure
100 ng (1 picomole) in 0.075 mM DOTM, 150 mM KCl, 10 mM Tris pH 7.4
15 min incubation with LH2







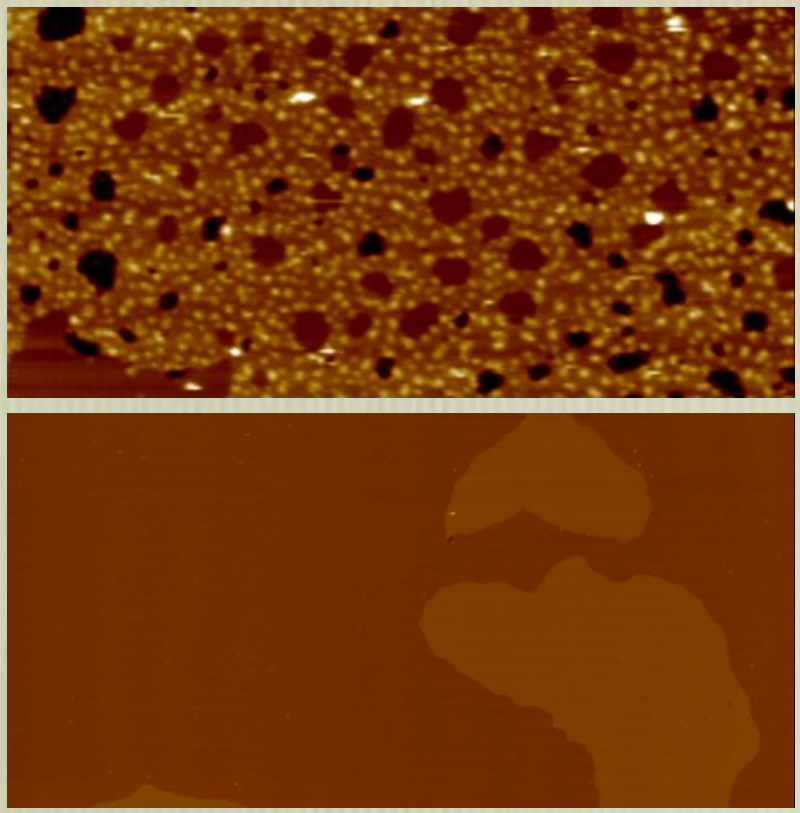
∅ = ~ 6 nm

Biophysical J (2006) 91, 3268-3275
Ultramicroscopy (2007) 107(10-11):928-33.

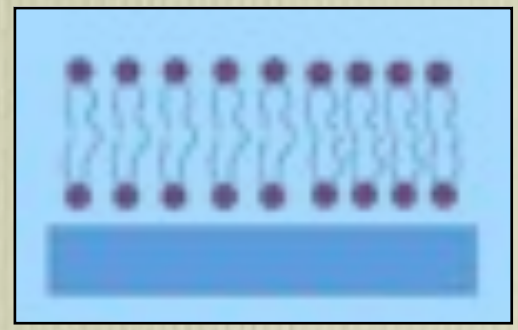
# Calcium effect on protein incorporation

FosCholine-16

5 mM $CaCl_2$

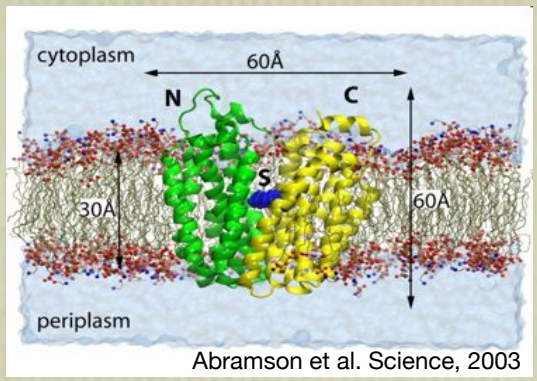


# Calcium effect on protein incorporation



| | - $Ca^{2+}$ | + $Ca^{2+}$ |
|---|---|---|
| δh DOPC-mica | 5.67 ± 0.56 | 4.36 ± 0.25 |
| δh DPPC -mica | 6.69 ± 0.35 | 5.37 ± 0.14 |

# Incorporation of the lactose permease Lac Y



Abramson et al. Science, 2003

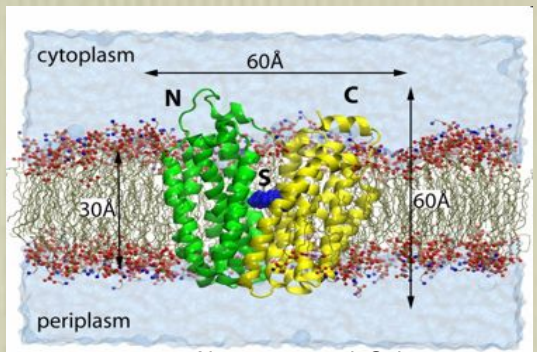
UNIVERSITAT DE BARCELONA

INSTITUT DE NANOCIÈNCIA I NANOTECNOLOGIA

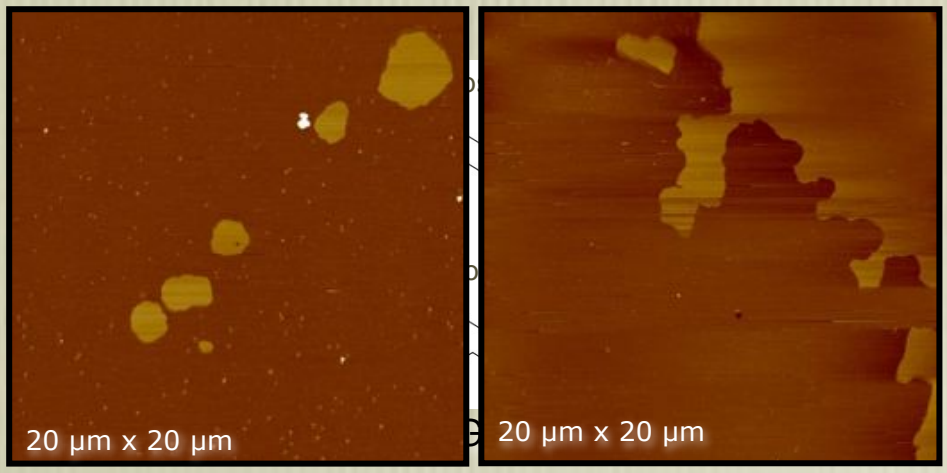
Laura Picas Escoffier
M. Teresa Montero
Jordi Hernández-Borrell

CBS

# Direct incorporation of the permease Lac Y



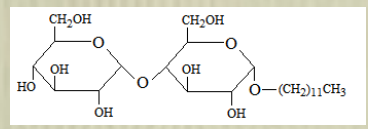
Abramson et al. Science, 2003

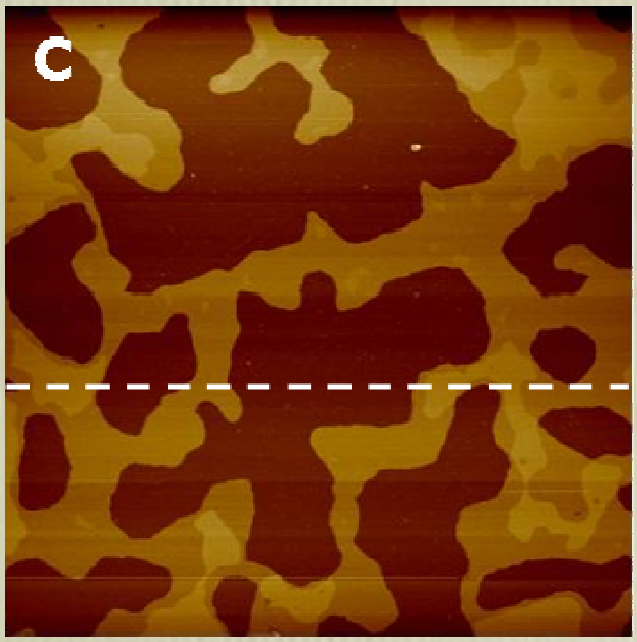


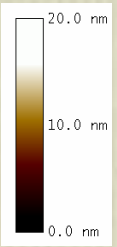

Calcium buffer
TM-AFM

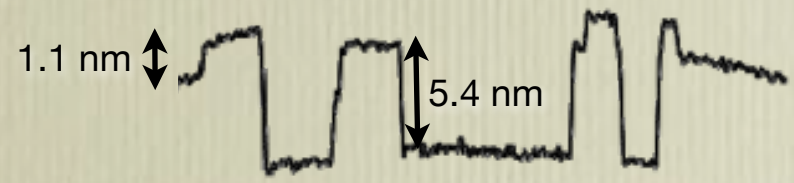
# Detergent effect on POPE/POPG bilayer

n-Dodecyl-β-D-Maltopyranoside (DDM)(2 cmc)



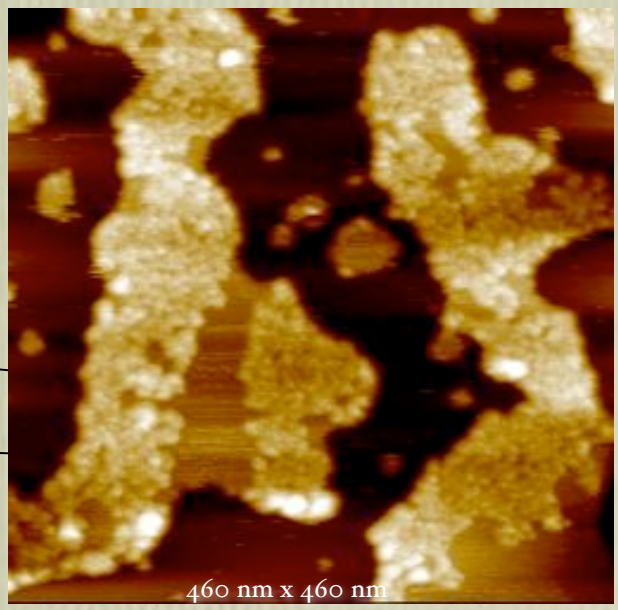



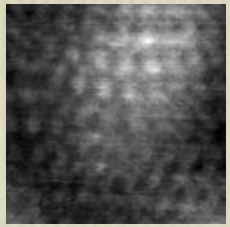



# Direct incorporation of the permease Lac Y

lipid bilayer

protein incorporation

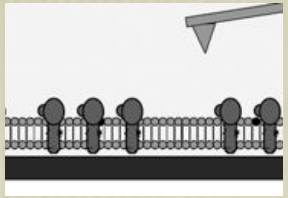


Images acquired in TM-AFM in calcium buffer.

**Picas et al (2010) BBA Biomembranes, in press**

# Summary



Low amount of protein (picomole range)

Unique orientation

Incorporation in the fluid phase and diffusion (weak interaction with the substrate)

Lateral resolution below the nanometer range (subunit of oligomers can be delineated).

Suitable for functional and nano-biotechnological applications

⟶ Fill-in (continuous bilayer)

CBS

Current Opinion Colloid Interface Science 13 (2008) 326–337

# Acknowledgements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cnrs advancing the frontiers

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Inserm