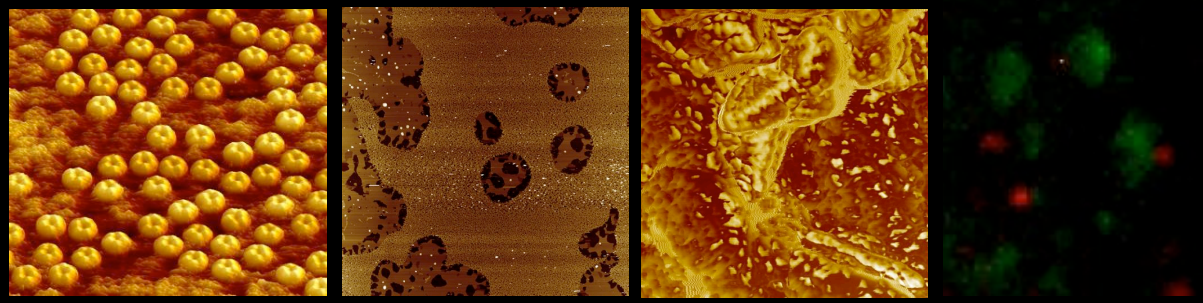
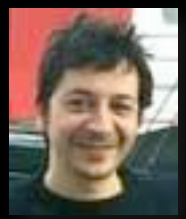
Centre de Biochimie Structurale
U1054 INSERM, UMR5048 CNRS, Université Montpellier



Summer School of AFM, Marcoule, 2011

Membrane Protein Reconstitution within Lipid Bilayer

Francesca Gubellini
Manuela Dezi
Daniel Levy



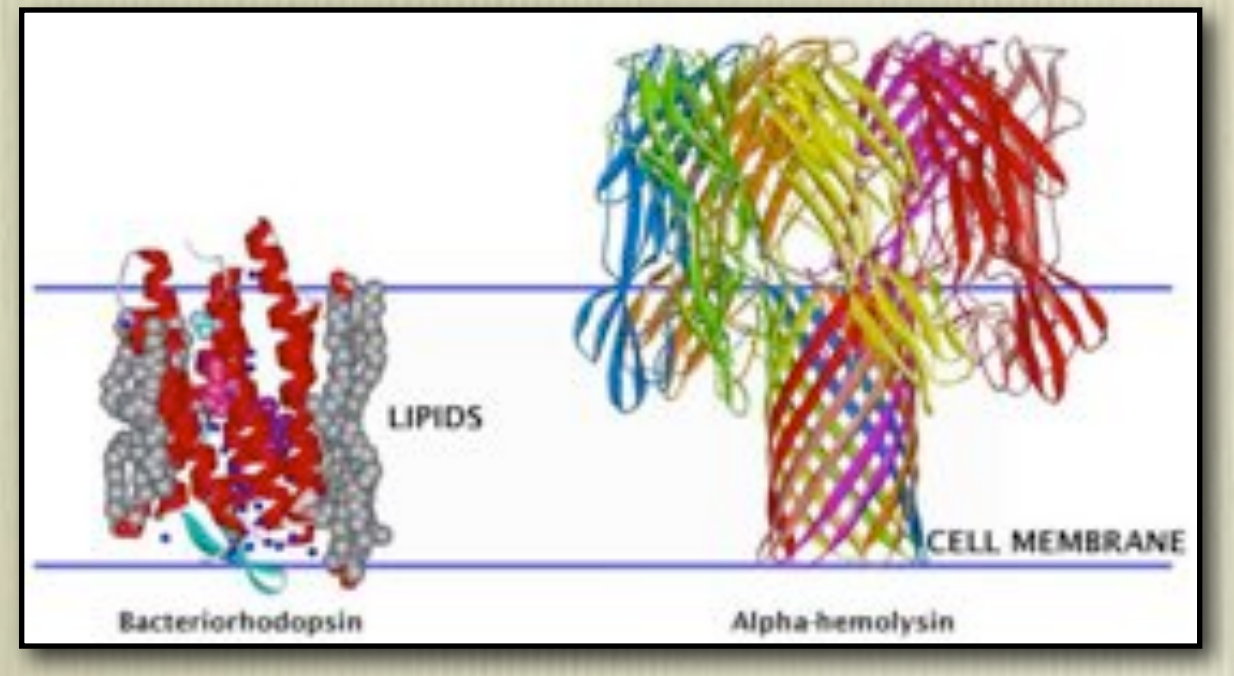
UMR168
CNRS
institutCurie
Ensemble, prenons le cancer de vitesse.

Outlines

- Membrane proteins and cell functions
- Reconstitution of membrane proteins
- High resolution AFM imaging of transmembrane proteins reconstituted into artificial membranes
  - Direct incorporation
  - Lipid layer technique
- Other methods

25% of the human genome encode transmembrane proteins

Target of 70% of the commercially available drugs



Alpha helix
(GPCR)

Beta barrels
(porin)

25% of the human genome encode transmembrane proteins

Target of 70% of the commercially available drugs

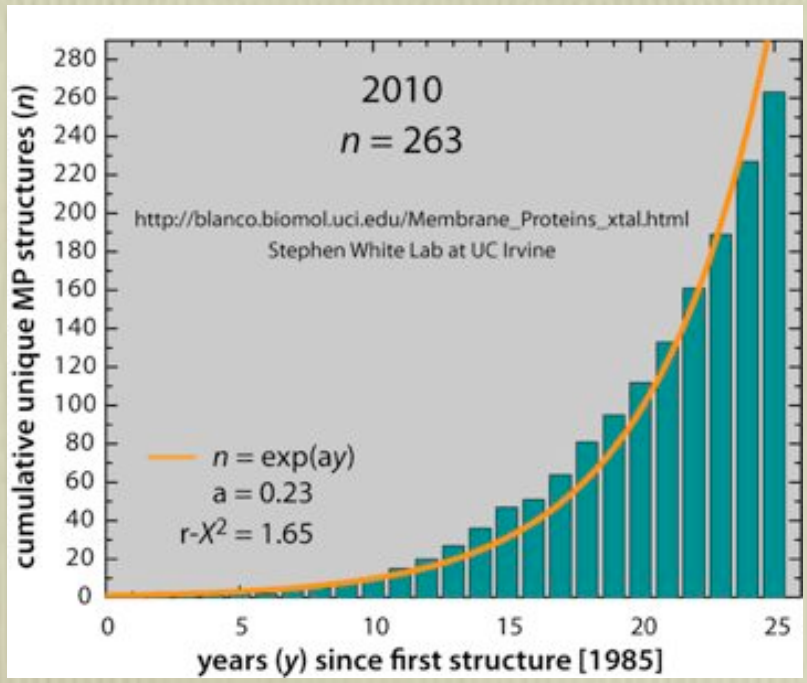
The Nobel Prize in Chemistry 1988 was awarded jointly to **Johann Deisenhofer, Robert Huber and Hartmut Michel** *"for the determination of the three-dimensional structure of a photosynthetic reaction centre"*.

The Nobel Prize in Chemistry 1997 was divided, one half jointly to **Paul D. Boyer and John E. Walker** *"for their elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)"* and the other half to Jens C. Skou *"for the first discovery of an ion-transporting enzyme, Na+, K+ -ATPase"*.

The Nobel Prize in Chemistry 2003 was awarded *"for discoveries concerning channels in cell membranes"* jointly with one half to **Peter Agre** *"for the discovery of water channels"* and with one half to **Roderick MacKinnon** *"for structural and mechanistic studies of ion channels"*.

Number of structures available in the pdb (protein data bank)

236 structures available in the PDB (~ 25 eukaryotic)



Monotopique
Prostaglandin H2 synthase-1
Cyclooxygenase-2
Monoamine Oxidase

Polytopique
GPCR (Rhodopsin, β2 adrenergic receptor)
Beta Barrel Membrane Proteins
Channels (ions, H_2O, NH_3)
Transporters (multidrug efflux, aa, ABC,…)
Proteins from photosynthetic complexes
ATPase
Respiratory proteins
Oxidases
Cytochrome

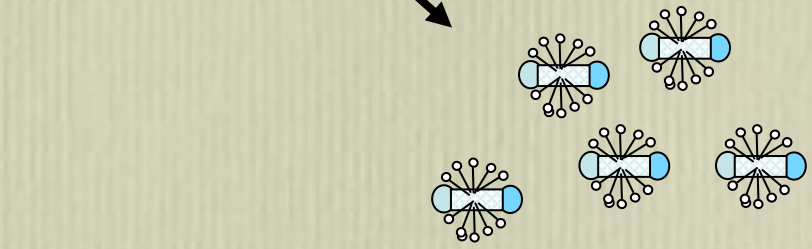
Mainly purified from biological membranes

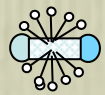
Classical strategies to solve the structure of transmembrane proteins

Native membranes

Ovexpression

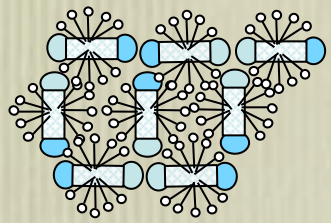
Detergent solubilization
Purification





Single particle analysis

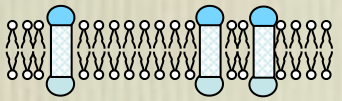
Electron Microscopy



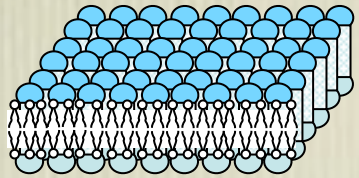
3D crystals

X rays

NMR, AFM

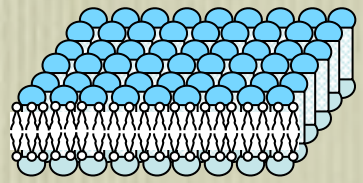


Membrane reconstitution



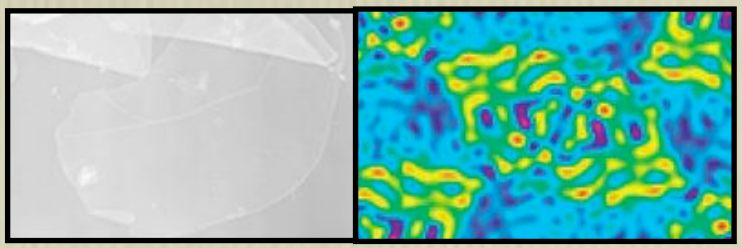
EM, AFM

Structural analysis of reconstituted transmembrane proteins



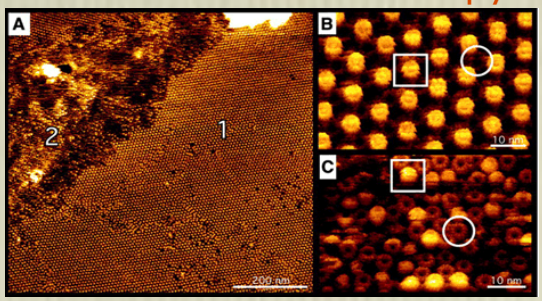
2D crystals

Electron Crystallography

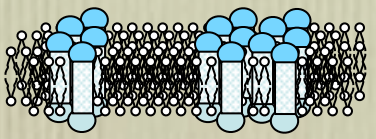


Chloride channel, J.A. Mindell et al, Nature (2001) 409, 219

Atomic Force Microscopy



Na^+-ATP synthase S. Scheuring, Curie

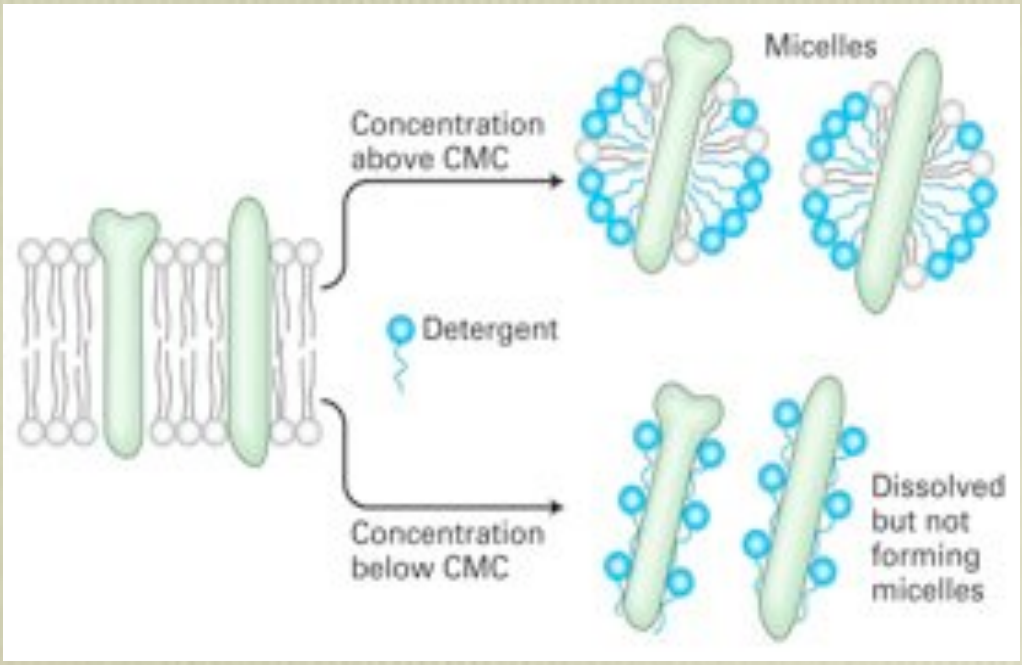


High density

Atomic Force Microscopy ?

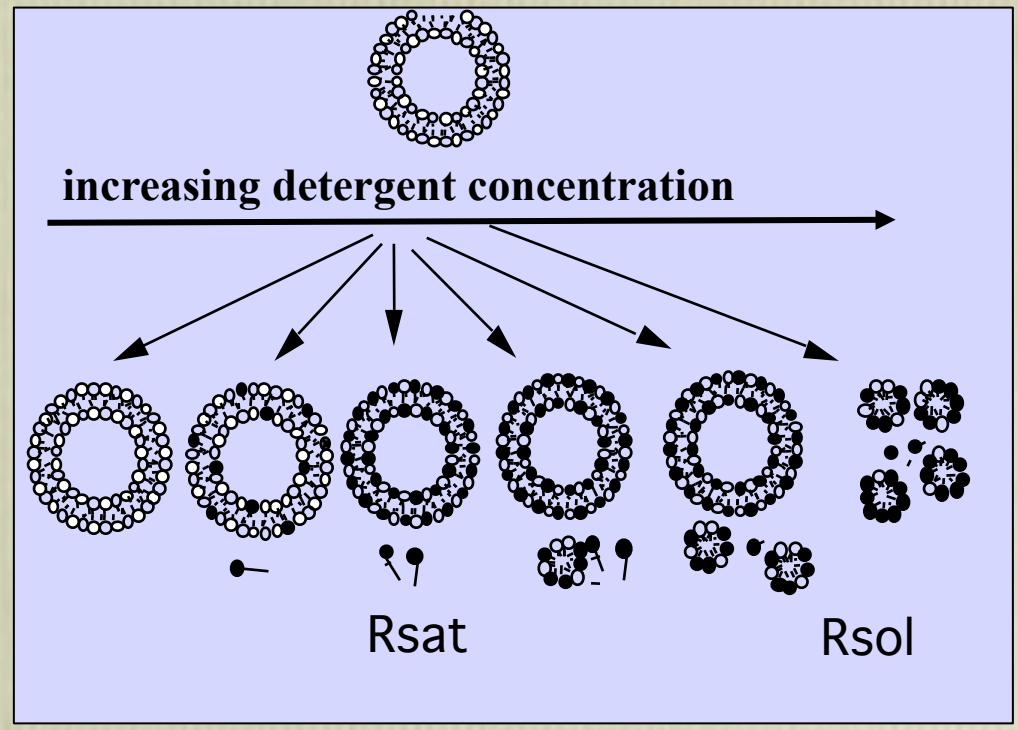
→ **Developing new strategies to escape the main critical steps**
→ **Imaging under physiological conditions**

Effect of detergents



Effect of detergents on lipid assemblies

(Lichtenberg, 1983; Almog, 1986; Ollivon,1988; Paternostre, 1988;
Da Graça Miguel, 1989; Vinson, 1989...)



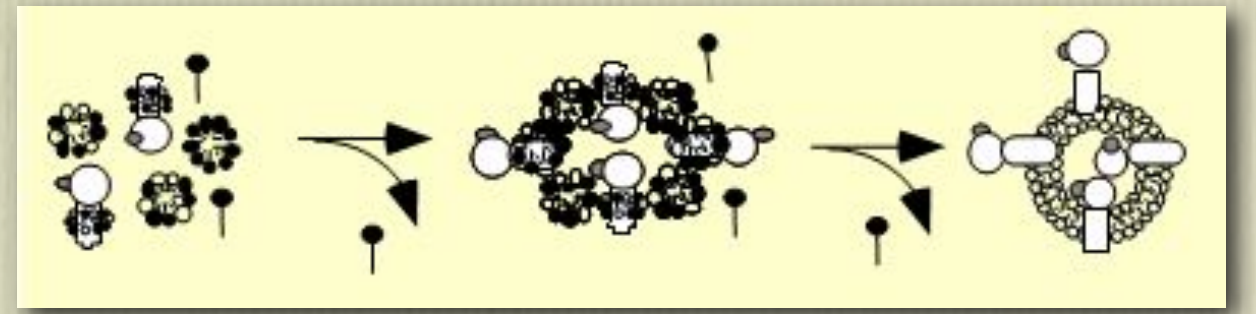
Dtotal = Dwater + Reff[Lip]

Reff = [det]/[Lip] in mixed aggregates

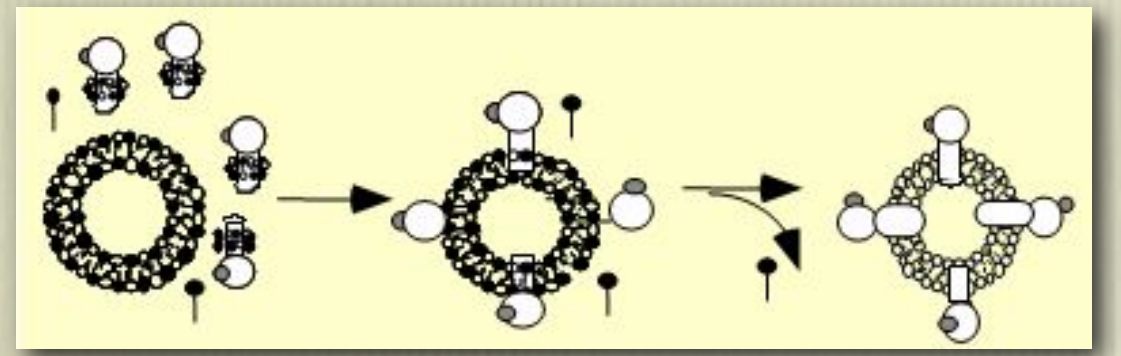
$$D_{sat} = cmc + R_{sat}.[Lipide]$$

$$D_{sol} = cmc + R_{sol}.[Lipide]$$

Direct incorporation of protein into detergent-saturated liposomes



Reconstitution from fully solubilized samples



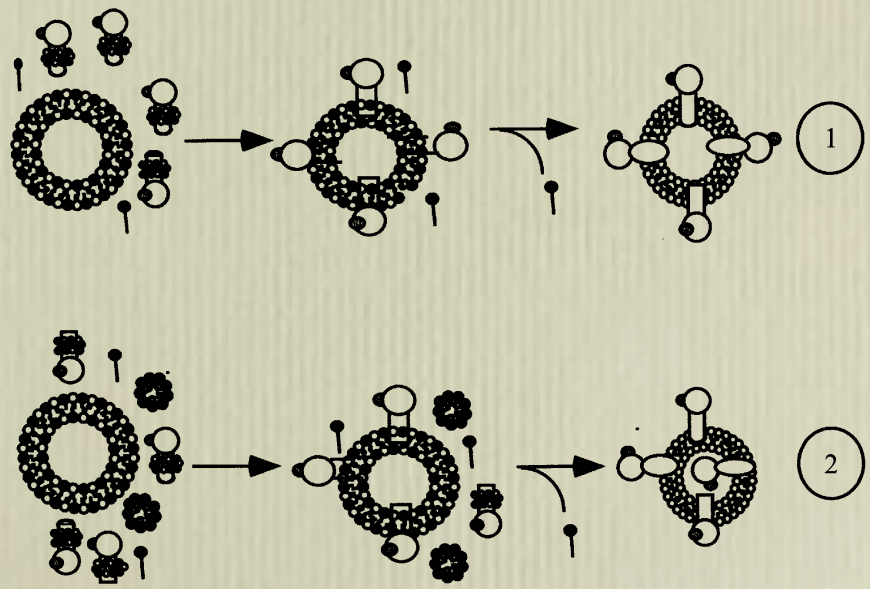
Direct incorporation in glycosylated detergent-destabilized liposomes

Unique orientation

Rigaud et Levy, Meth in Enzymol. (2003)

Direct Incorporation of transmembrane proteins within supported lipid bilayers

The detergent induces the mechanism of incorporation

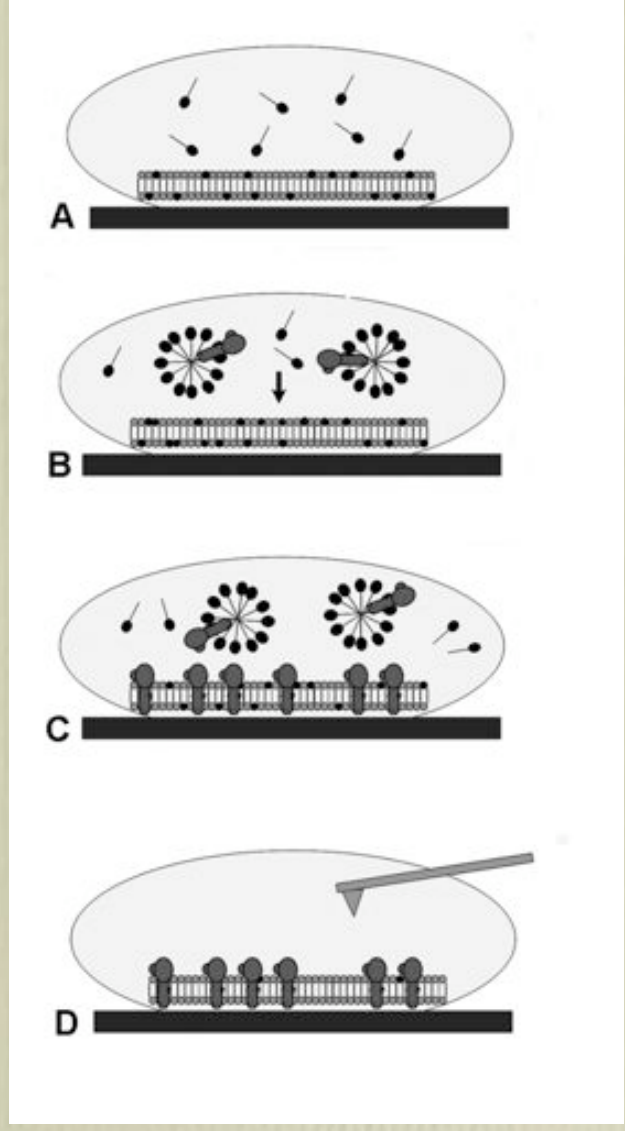


OG, DDM

Triton X100

Rigaud et Levy, Meth in Enzymol. (2003) 372, 65-86

Direct Incorporation of transmembrane proteins within supported lipid bilayers



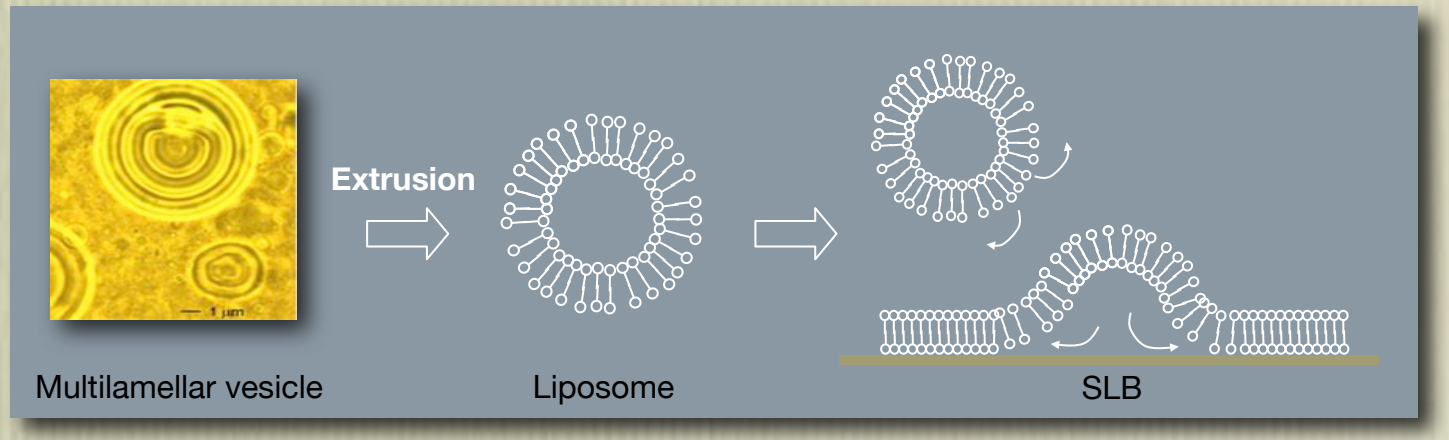
SLB destabilization

[detergent] ~ cmc

Incorporation

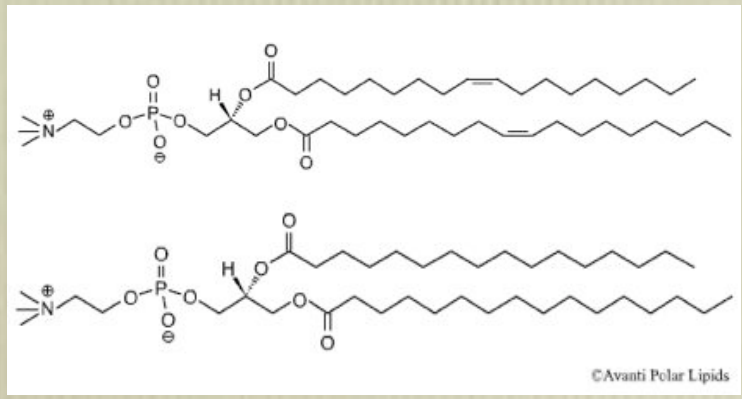
AFM Imaging
of non crystalline proteins

Direct Incorporation of transmembrane proteins within supported lipid bilayers



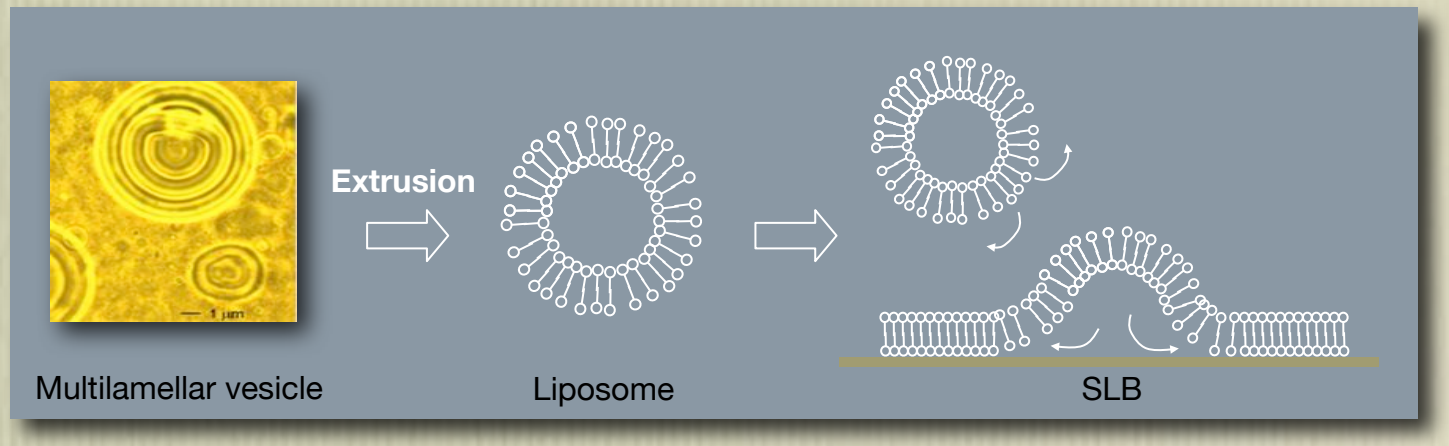
DOPC
Tm = - 20°C

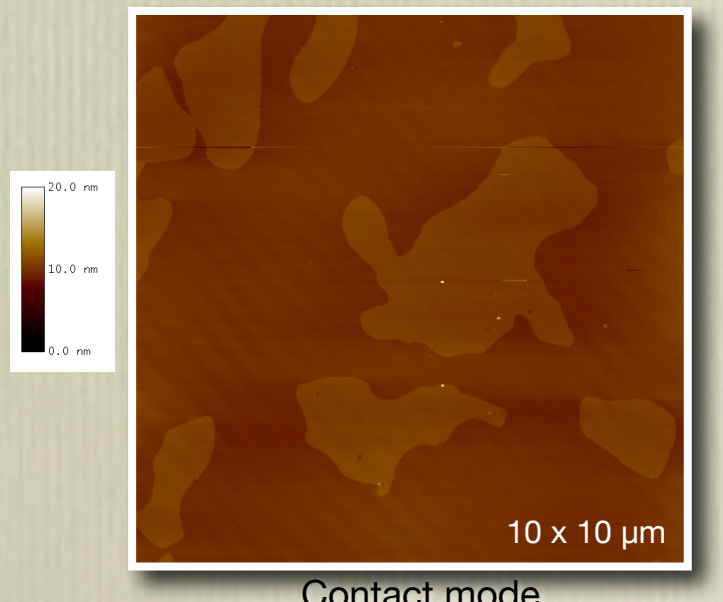
DPPC
Tm = 41°C



Fusion in PBS buffer
(20 mM phosphate, NaCl 150 mM, pH 7.4)
2 h at 65°C

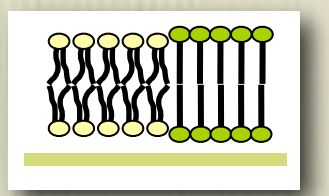
Direct Incorporation of transmembrane proteins within supported lipid bilayers





Contact mode

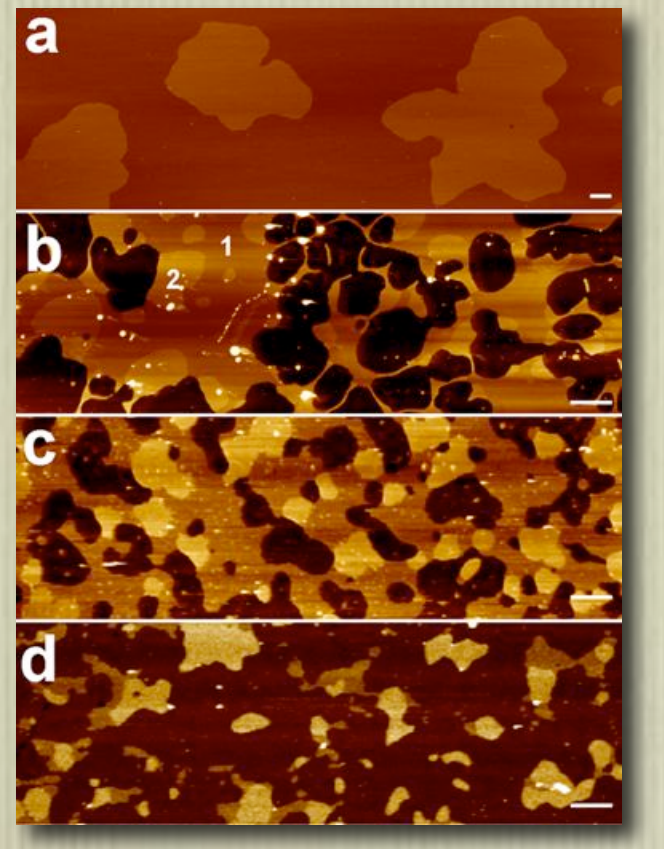
DOPC/DPPC



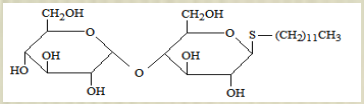
No leaflet decoupling
$D = 2\text{-}5\ \mu m^2/s$

Imaging setup in liquid
Silicon nitride tip 100 mN/m
Applied force < 100 pN

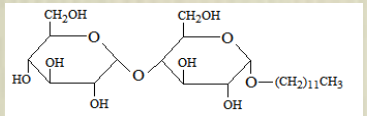
Membrane destabilization by detergents



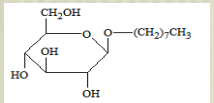
n-Dodecyl-β-D-Thiomaltopyranoside (DOTM)
cmc = 0.05 mM



n-Dodecyl-β-D-Maltopyranoside (DDM)
cmc = 0.17 mM



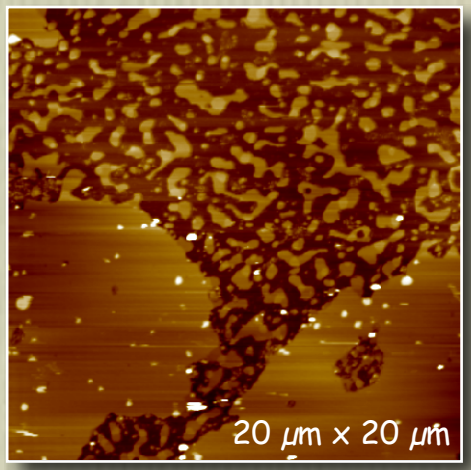
Octyl-β-D-glucopyranoside (OG)
cmc = 17 mM



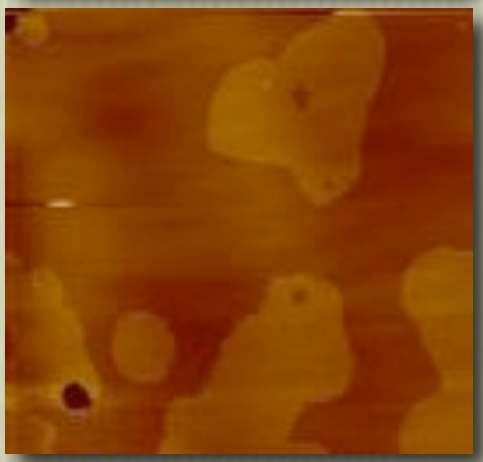
- SLB are stable above the cmc with low but not with high cmc detergent
- Planar lipid membrane are more resistant than liposomes
- Both gel and fluid phases are preserved

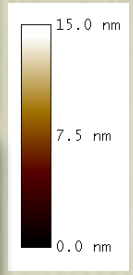
Membrane destabilization by detergents

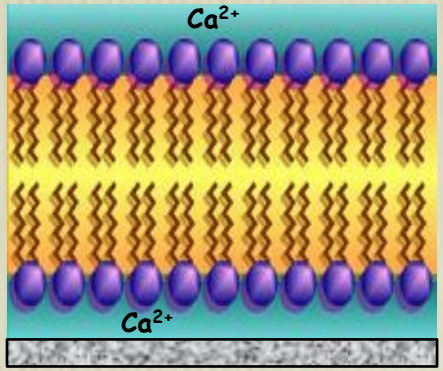
0.2 mM DDM



5 mM $CaCl_2$





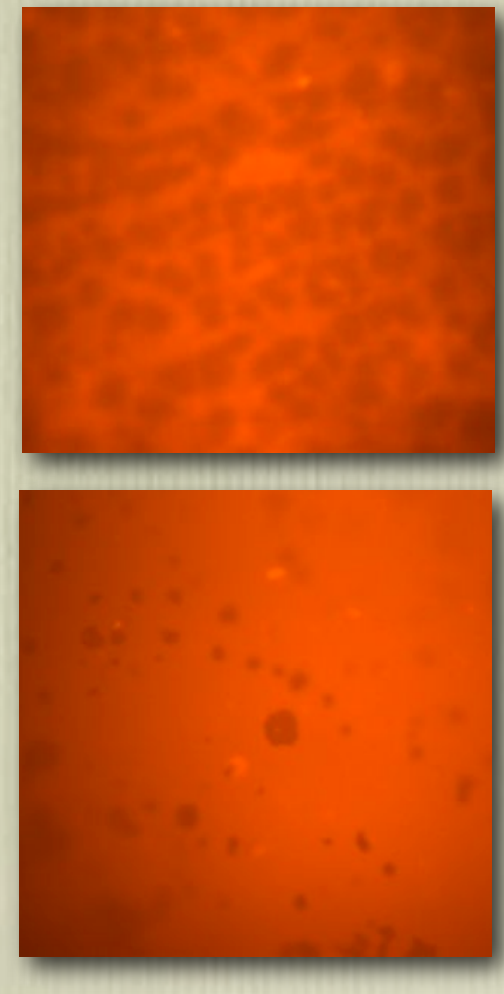


	- Ca^{2+}	+ Ca^{2+}
δh DOPC-mica	5.67 ± 0.56	4.36 ± 0.25
δh DPPC -mica	6.69 ± 0.35	5.37 ± 0.14

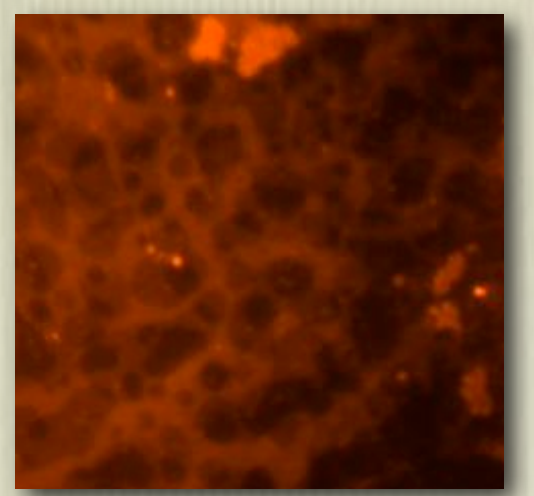
Membrane destabilization by detergents

Fluorescence

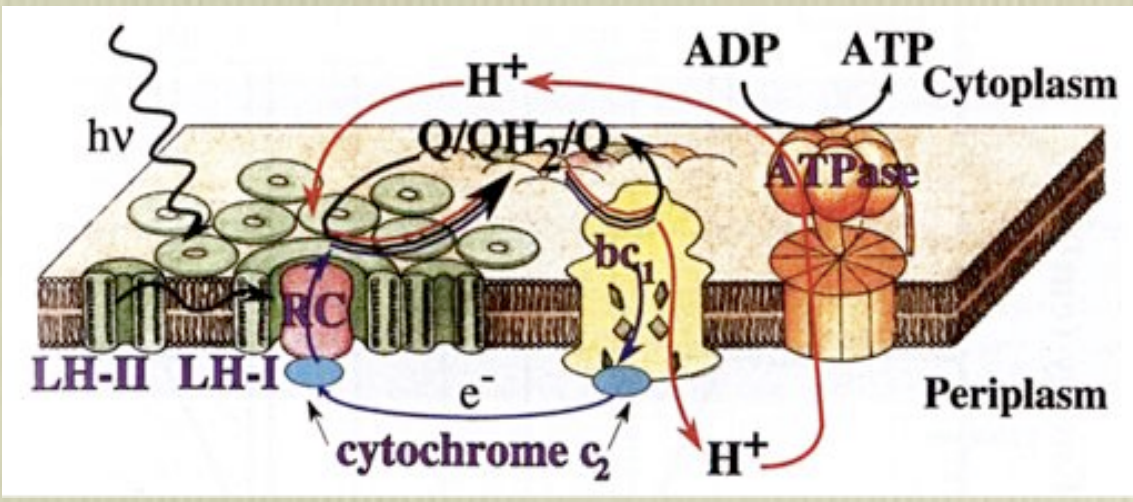
Upright microscope
LD objectives
HBO lamp



150 µm

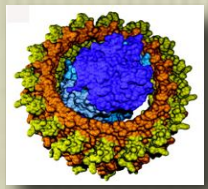


Direct Incorporation of transmembrane proteins within supported lipid bilayers

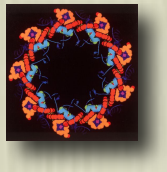


Photosynthetic apparatus of bacteria

Rhodobacter spheroides and Rhodopseudomonas acidophila



RC-LH1



LH2

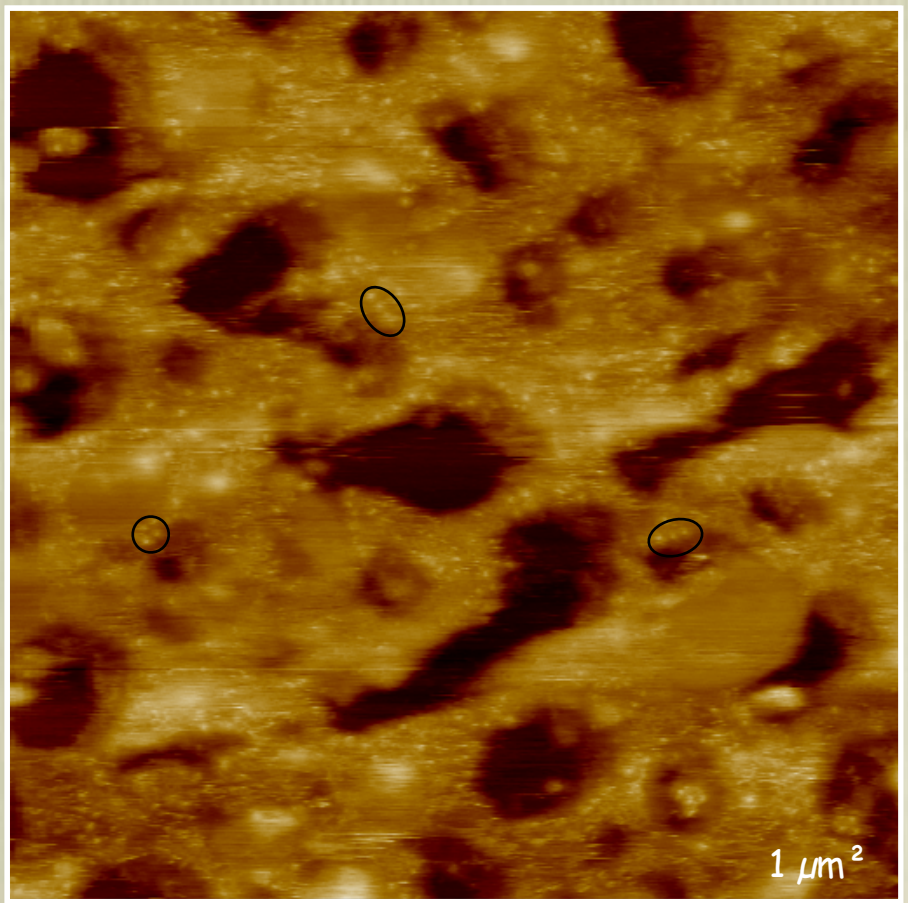
Incorporation of RC-LH1 from *Rhodobacter spheroides*

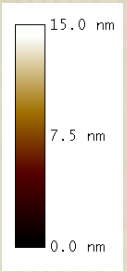
15 min incubation with RC-LH1

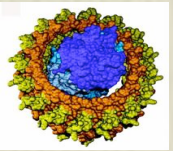
500 ng (1.5 picomole) in 0.075 mM DOTM, 150 mM KCl, 10 mM Tris pH 7.4

Contact mode

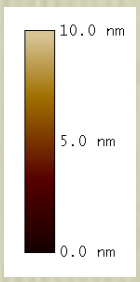
in 150 mM KCl, 10 mM Tris pH 7.4

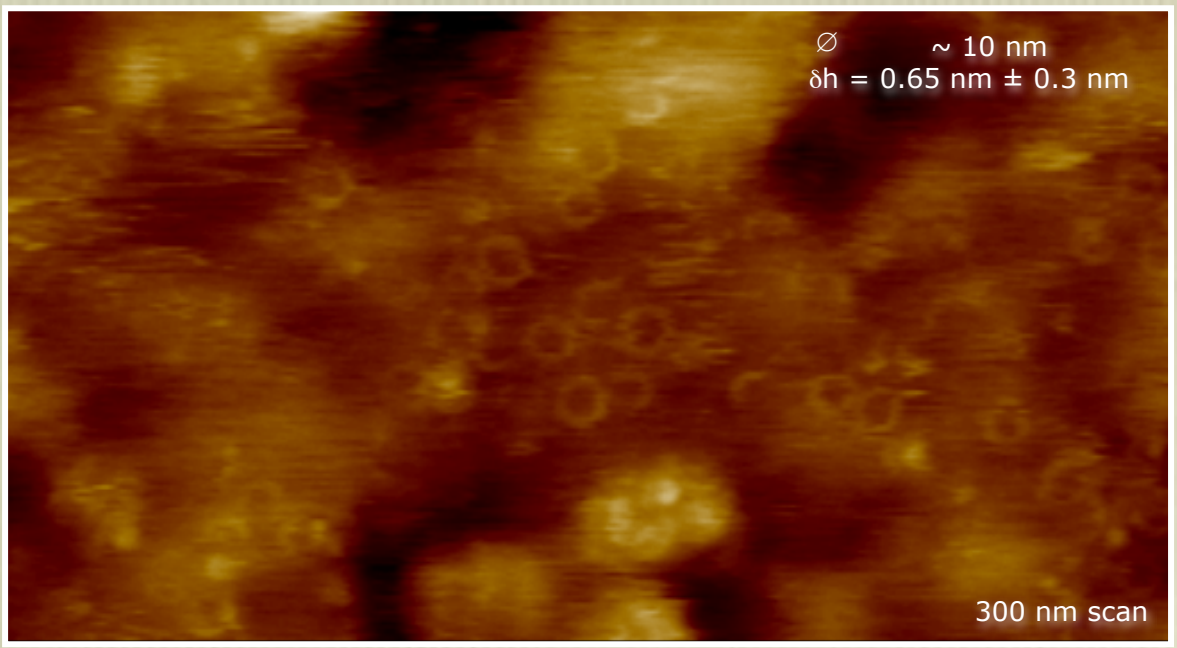


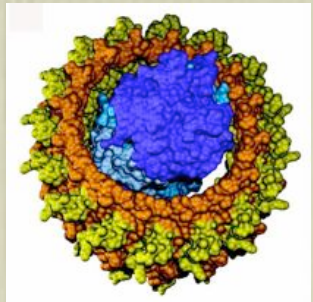




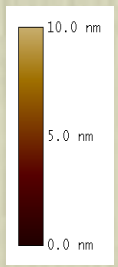
Incorporation of RC-LH1 from *Rhodobacter spheroides*

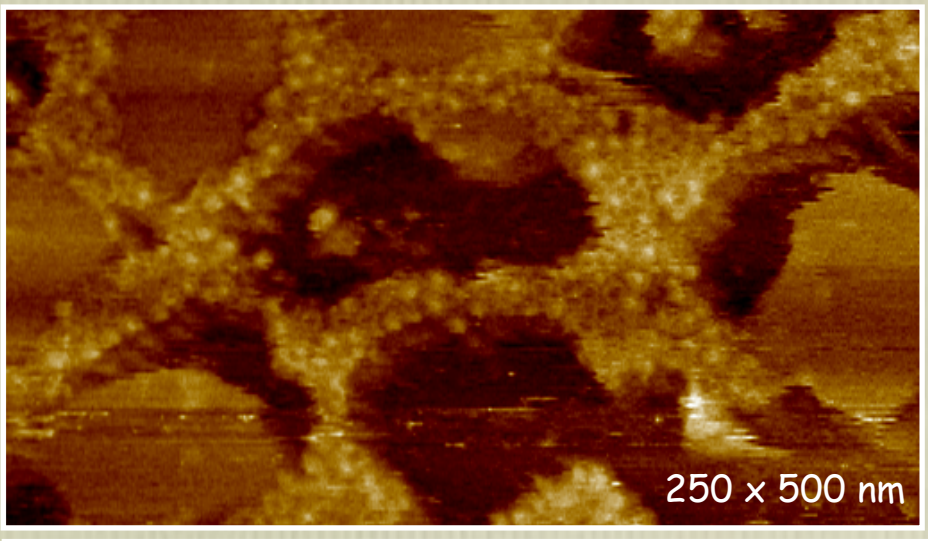


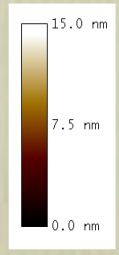


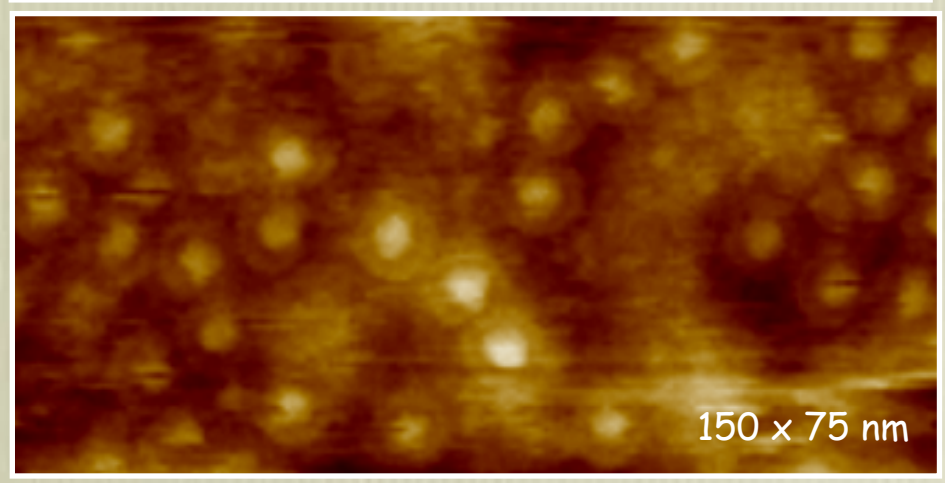


Incorporation of RC-LH1 from *Rhodobacter spheroides*









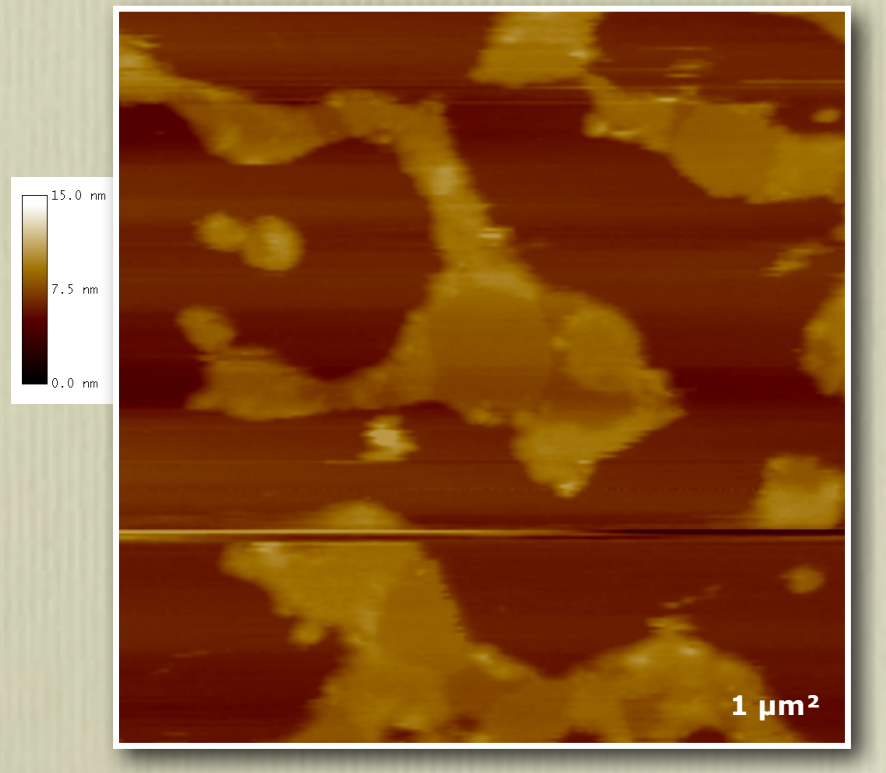
Unidirectional incorporation

Proteins diffuse in the fluid phase and segregate in the lipid bilayer

Ultramicroscopy (2007) 107(10-11):928-33.

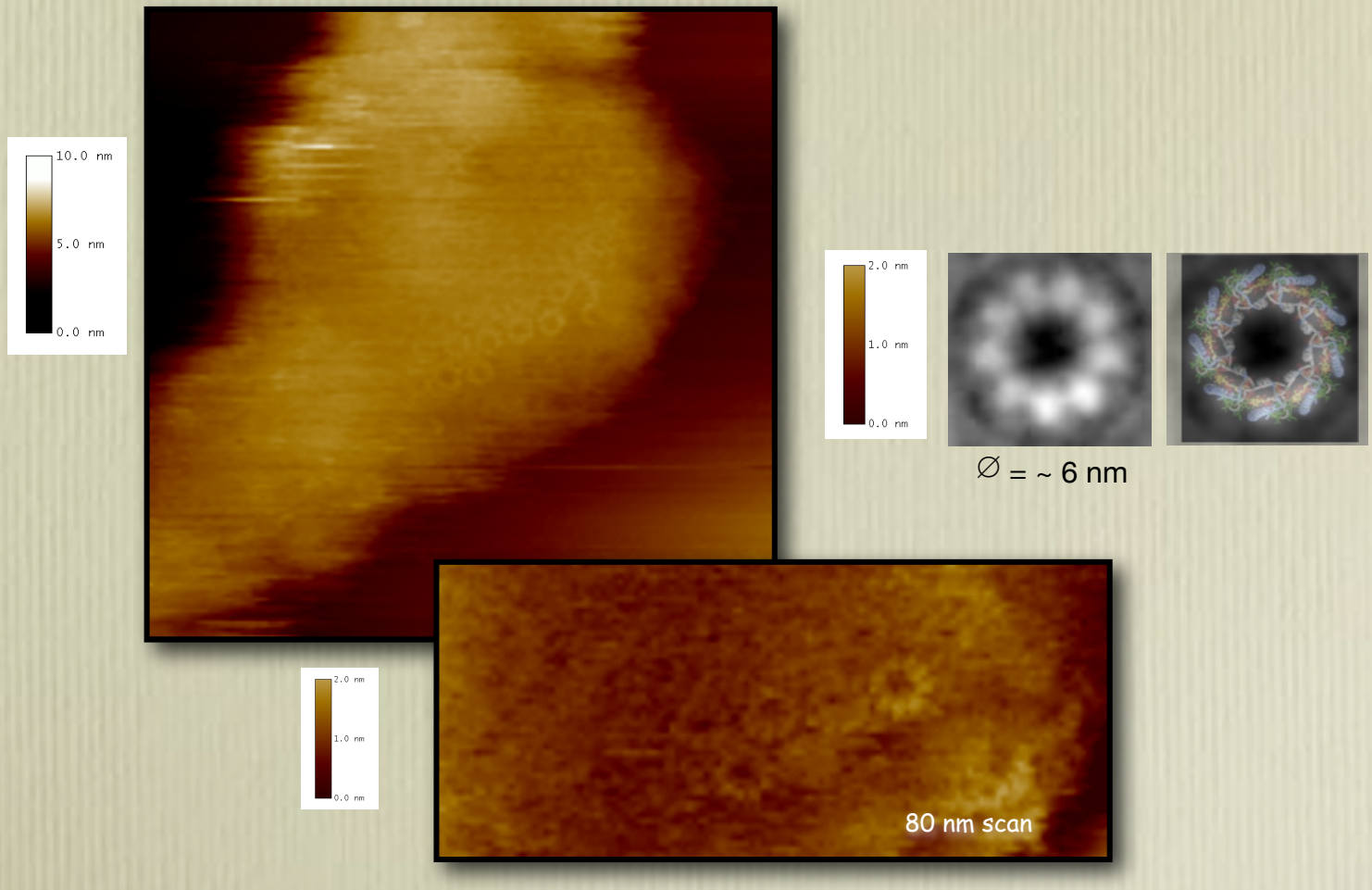
Incorporation of LH2 from *Rhodopseudomonas acidophila*

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



Biophysical J (2006) 91, 3268-3275

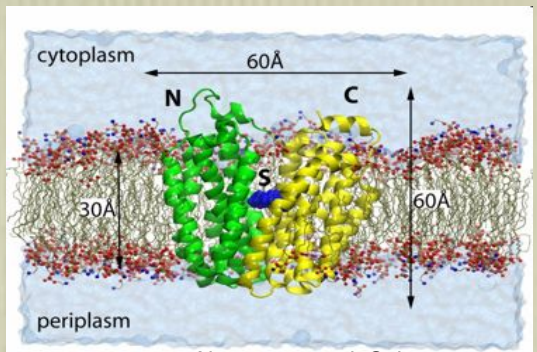
Incorporation of LH2 from *Rhodopseudomonas acidophila*



∅ = ~ 6 nm

Biophysical J (2006) 91, 3268-3275

Incorporation of the lactose permease Lac Y



Abramson et al. Science, 2003

UNIVERSITAT DE BARCELONA

INSTITUT DE NANOCIÈNCIA I NANOTECNOLOGIA

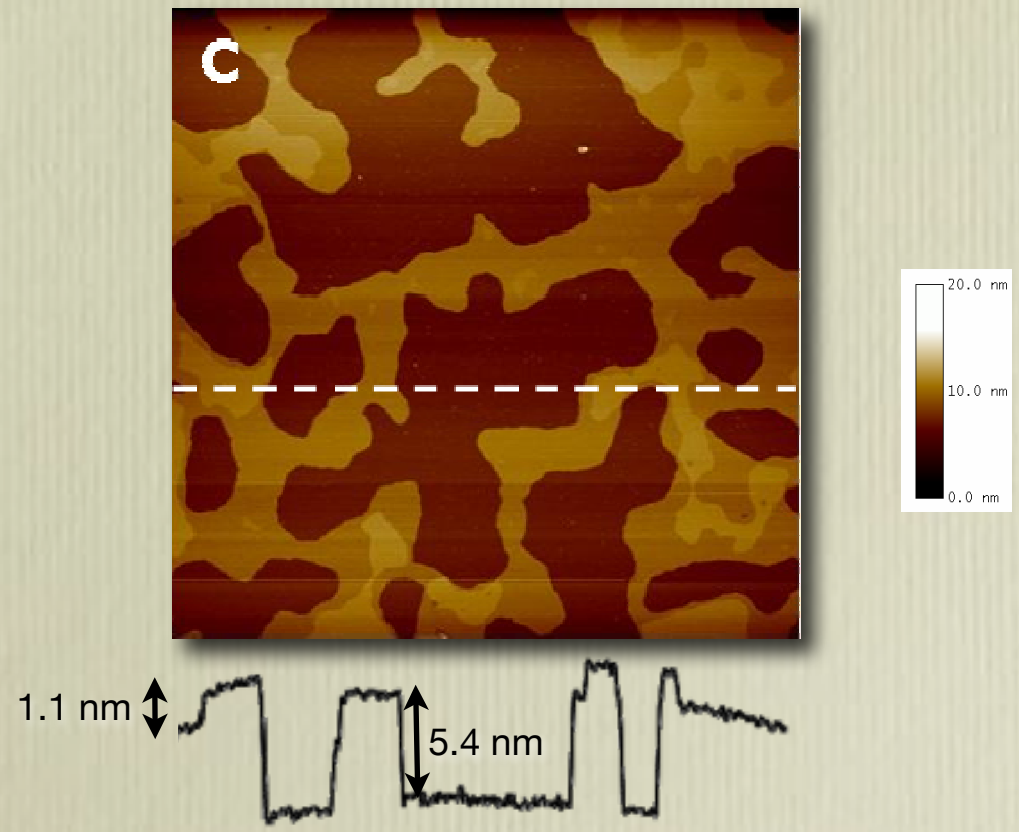
Laura Picas Escoffier
M. Teresa Montero
Jordi Hernández-Borrell

CBS

Incorporation of the lactose permease Lac Y

Detergent effect on POPE/POPG bilayer

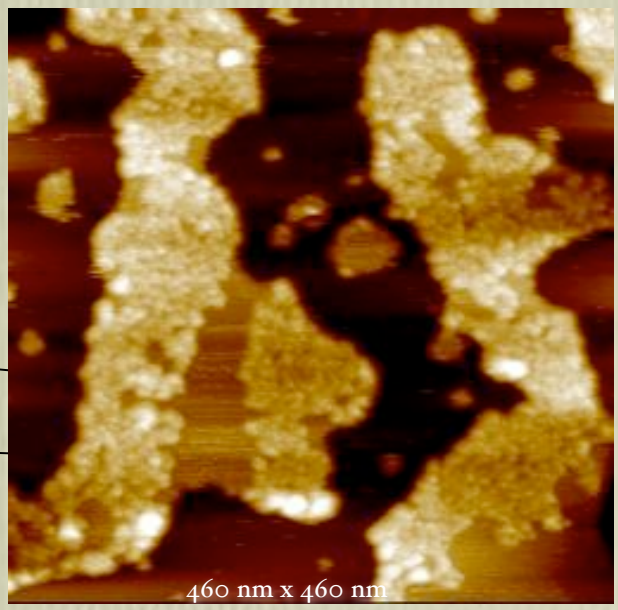
n-Dodecyl-β-D-Maltopyranoside (DDM)(2 cmc)

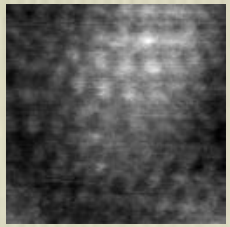


Incorporation of the lactose permease Lac Y

lipid bilayer

protein incorporation

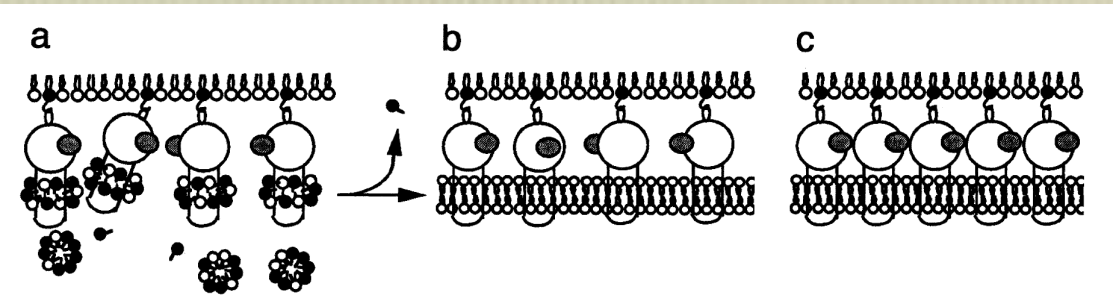




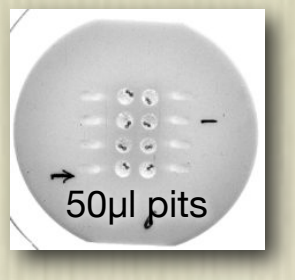
Images acquired in TM-AFM

Picas et al (2010) BBA Biomembranes

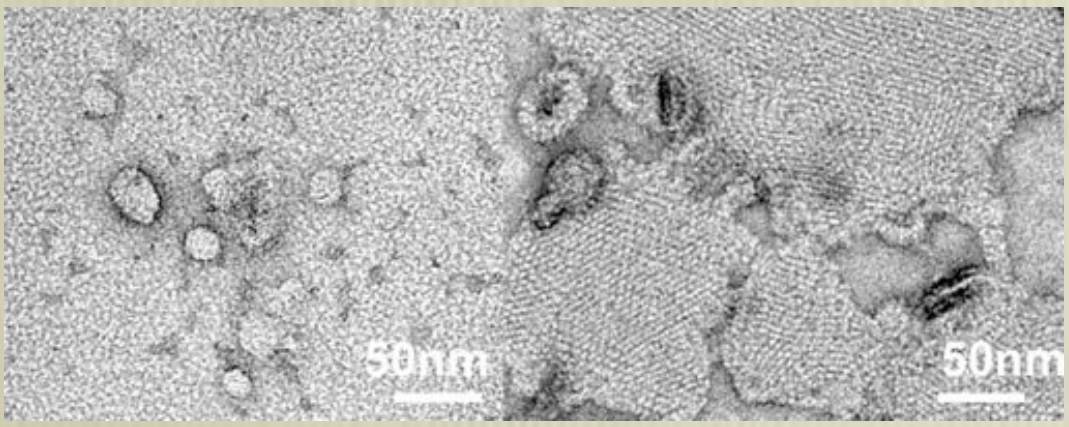
AFM imaging of proteins reconstituted using the lipid-layer 2D technique



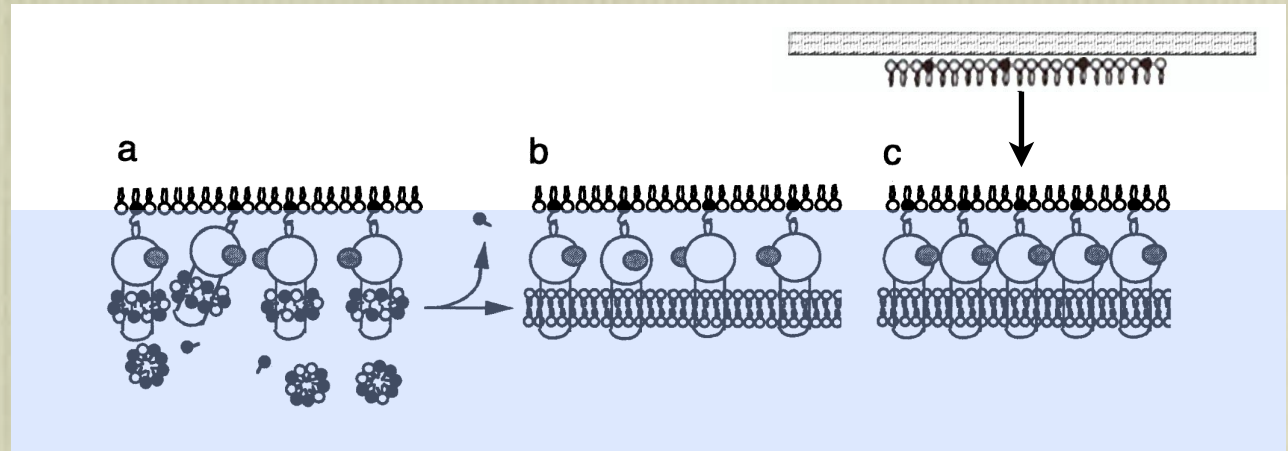
Levy et al, J Struct Biol (1999) 127:44-52

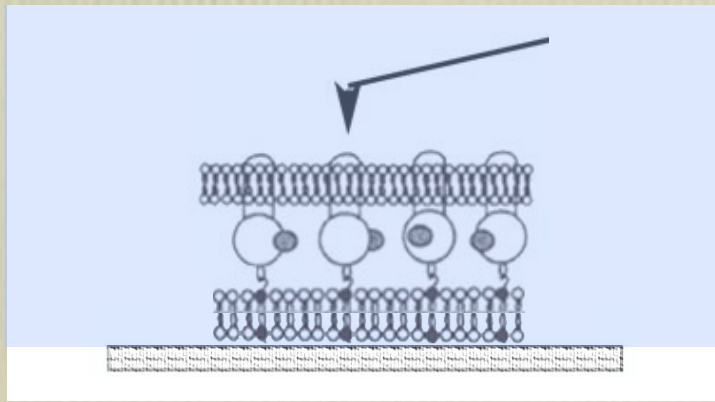


Proteins in a native environment
1 µg per assay



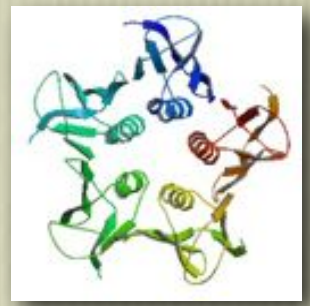
AFM imaging of proteins reconstituted using the lipid-layer 2D technique

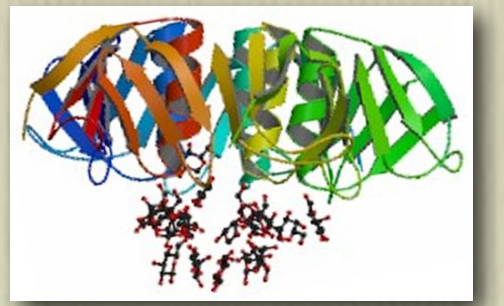




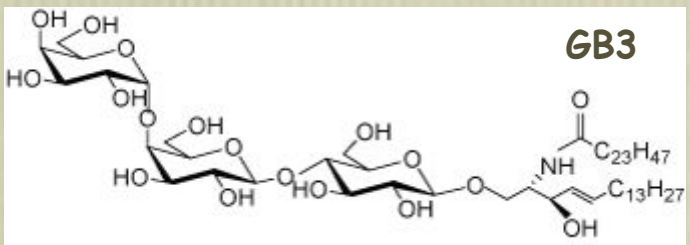
Transfer of shiga toxin B-subunit on lipid monolayer

Ø = 6 nm



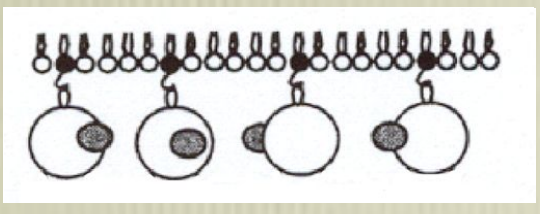


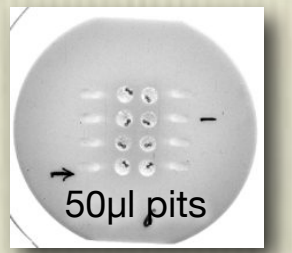
∂h = 2.5 nm



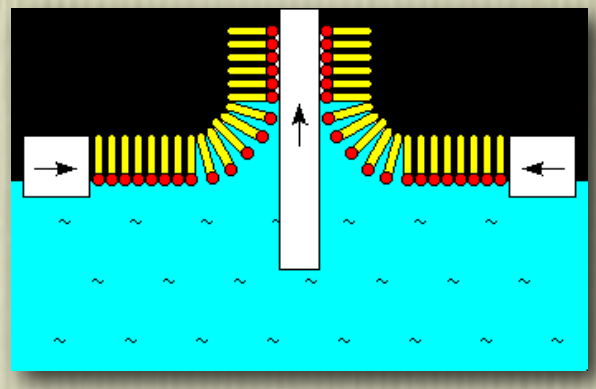
DOPC/ GB3

Shiga toxin
B-subunit



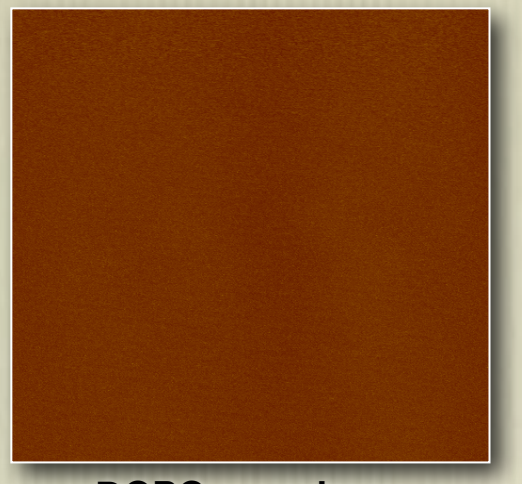


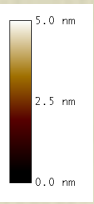
Transfer of shiga toxin B-subunit on lipid monolayer



Teflon barrier

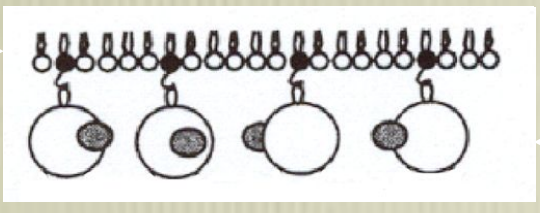
Langmuir-Blodget transfer
30 mN/m





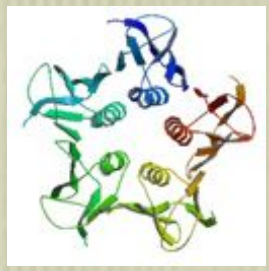
DOPC monolayer
Rms roughness = 0.8 Å

DOPC/ GB3
(Globotriaosylceramide)
(6:4)

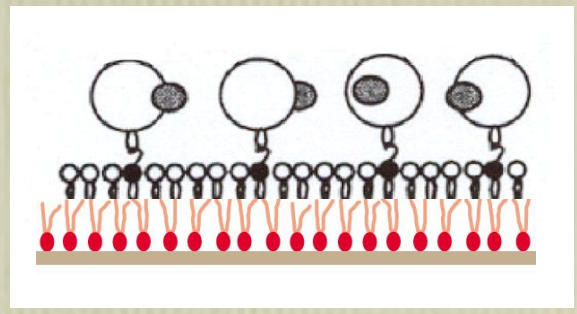


Shiga toxin
B-subunit

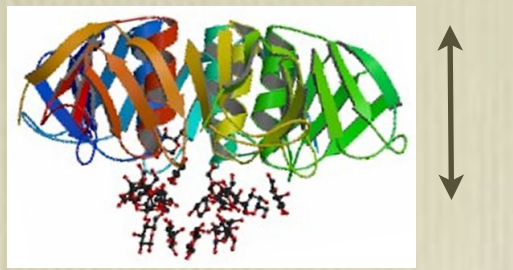
Transfer of shiga toxin B-subunit on lipid monolayer

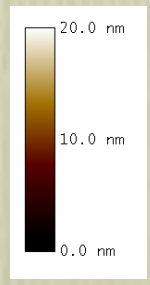


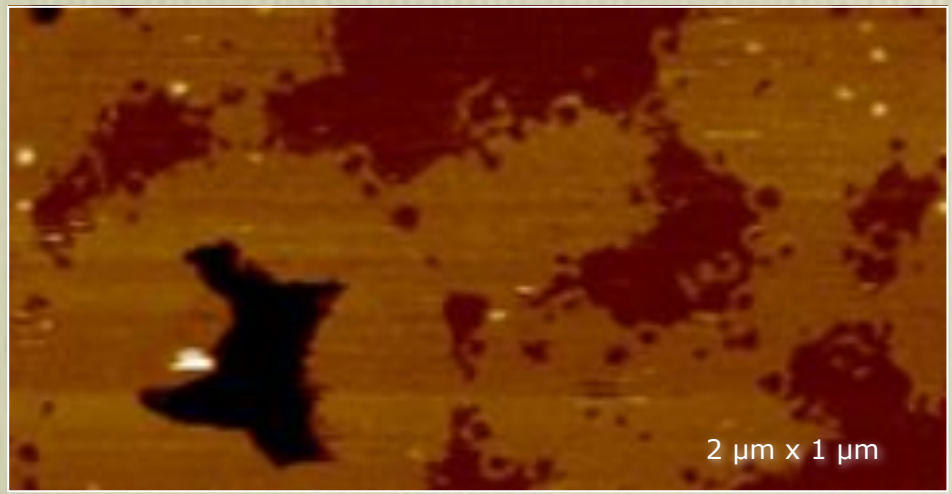
Ø = 6 nm



2.5 nm

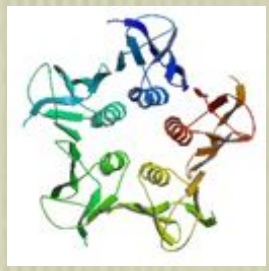


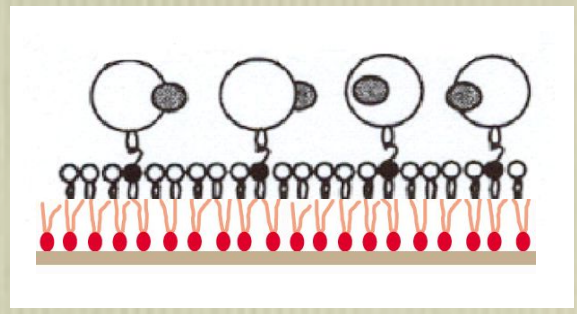


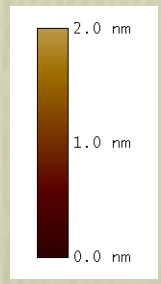


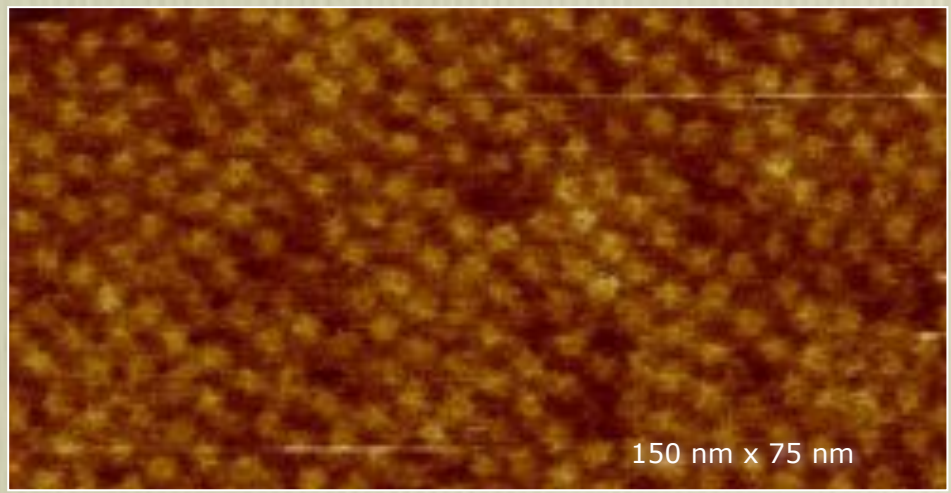
Contact mode

Transfer of shiga toxin B-subunit on a lipid monolayer

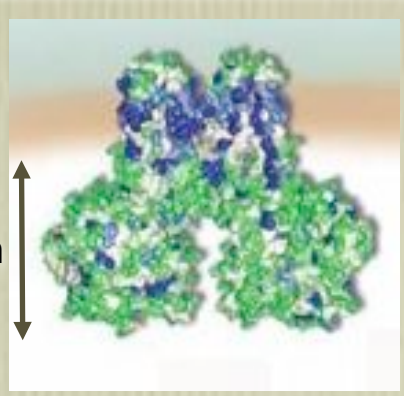




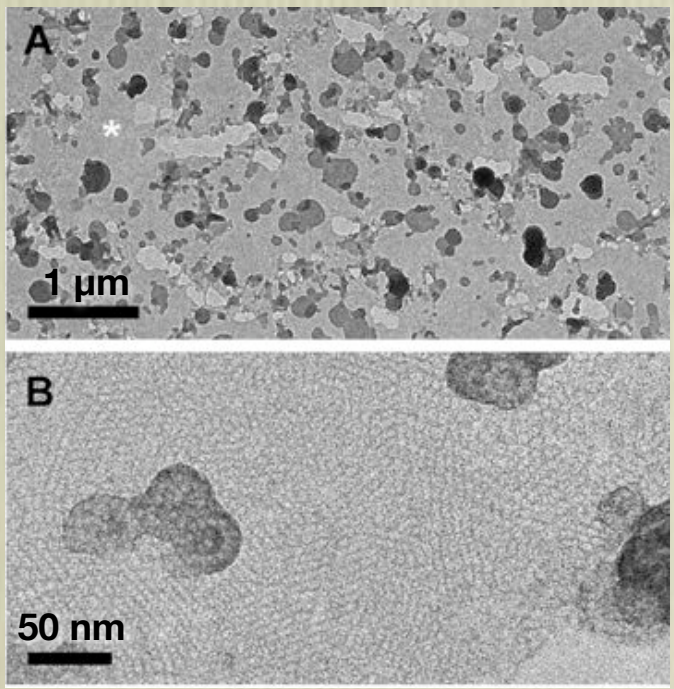


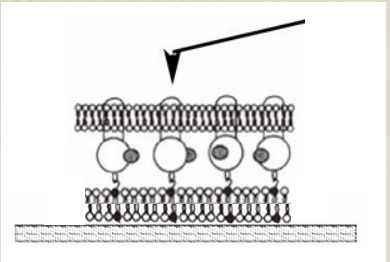


Transfer of reconstituted BmrA on a lipid monolayer

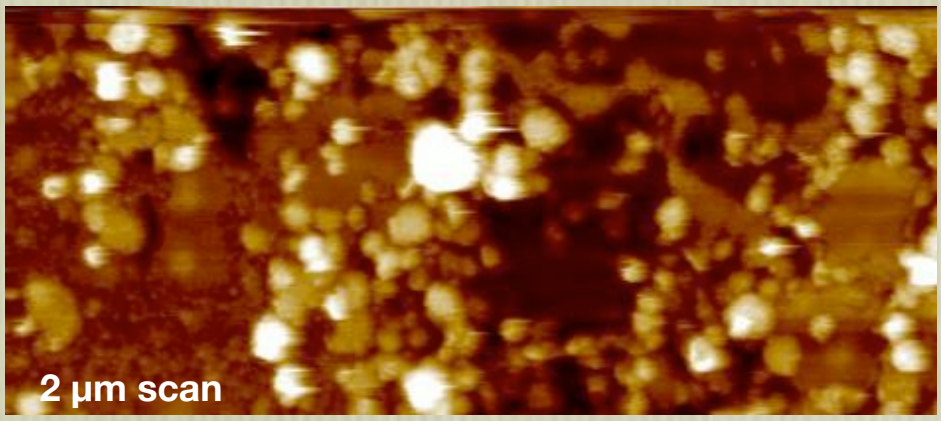


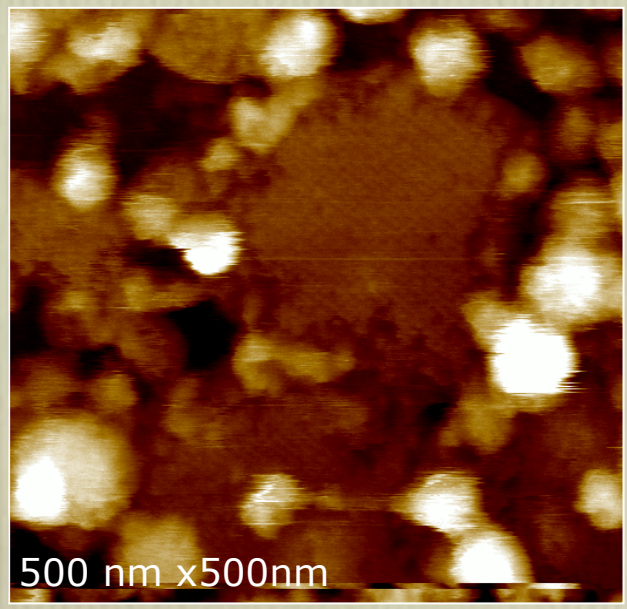
ATP-binding cassette transporter (ABC transporter)

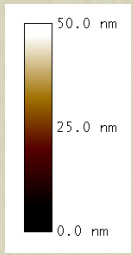


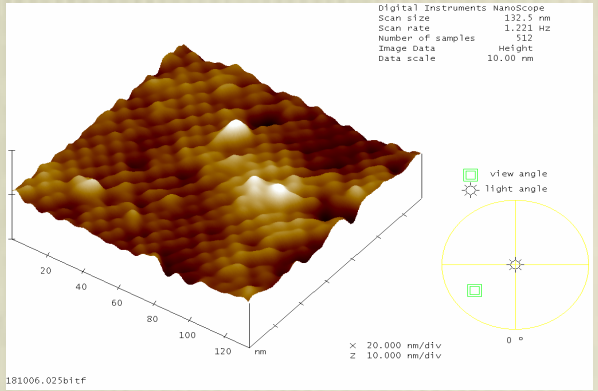


Transfer of reconstituted BmrA on HOPG

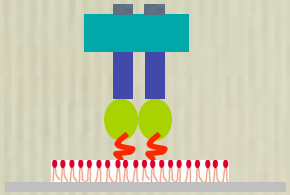




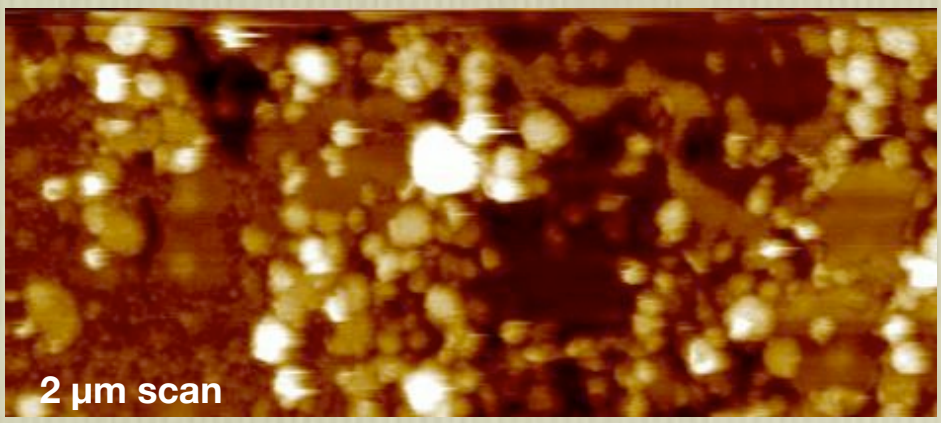


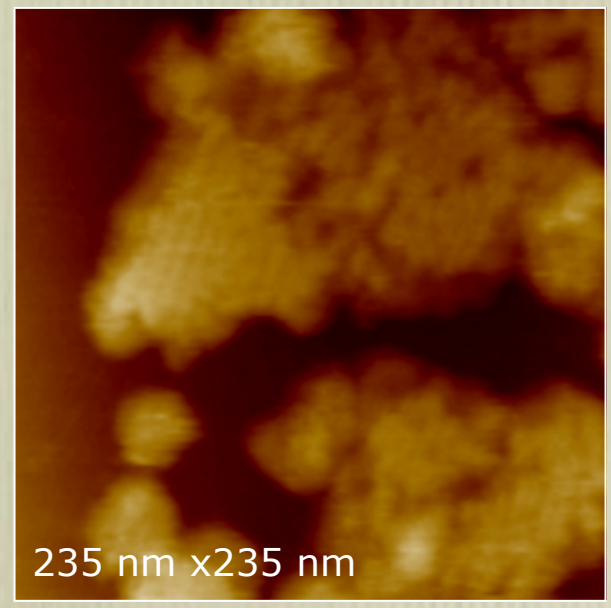


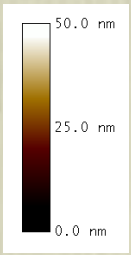
7 nm lattice, δh 1 nm

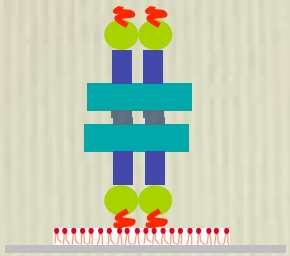


Transfer of reconstituted BmrA on HOPG

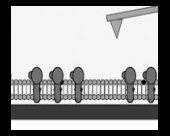




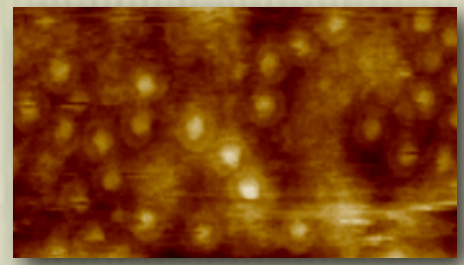




Summary



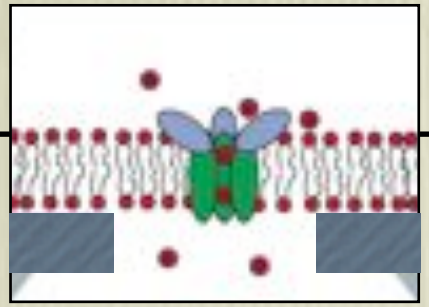
- Low amount of protein
- Single/control of the orientation
- High resolution imaging



- Proteins can segregate in the bilayer

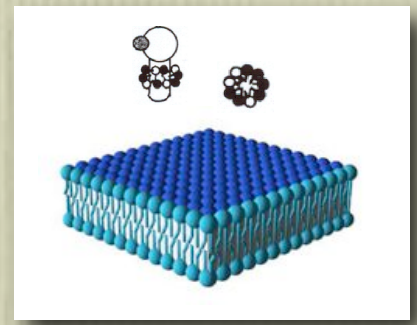
- Suitable for functional and nano-biotechnological applications (transfer of large membrane patches)

- Improvement of AFM resolution

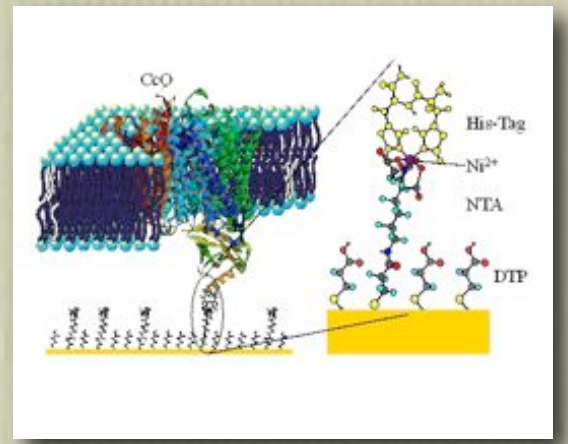


Membrane Biosensor

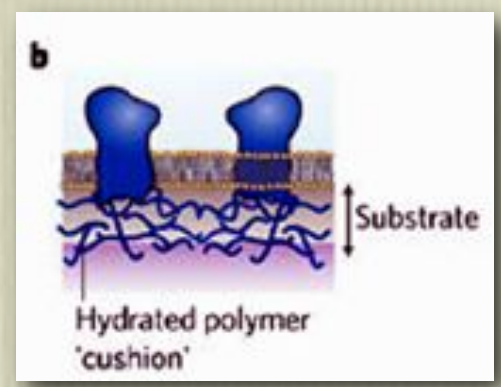
Comparison with other techniques



Incorporation



Tethered protein reconstitution



Polymer cushion

Tollin et al. 2003. *Trends Pharmacol Sci*. 24:655-9
Sinner et al. Angew. Chem. Int. Ed. 2006, 45, 1 – 5
Giess et al 2004. *Biophys J*. 87:3213-20.
Tanaka & Sackmann *Nature* 2005, 437, 656

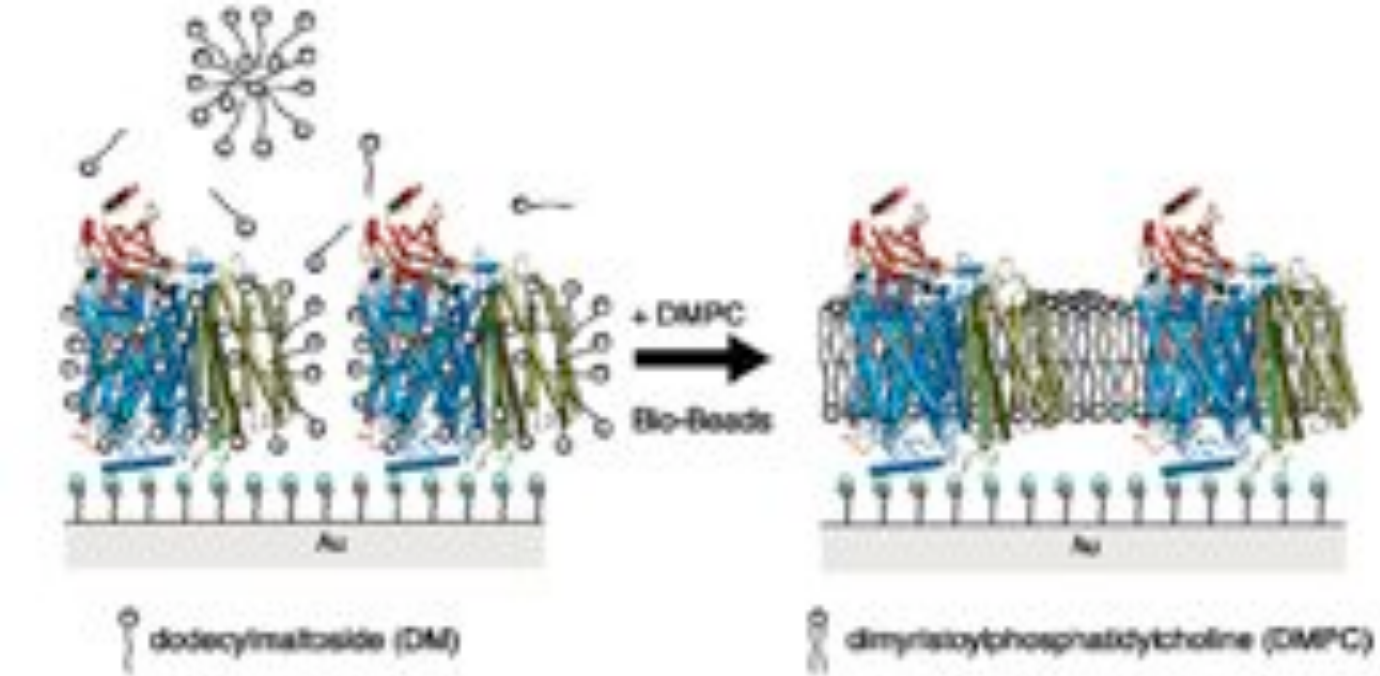


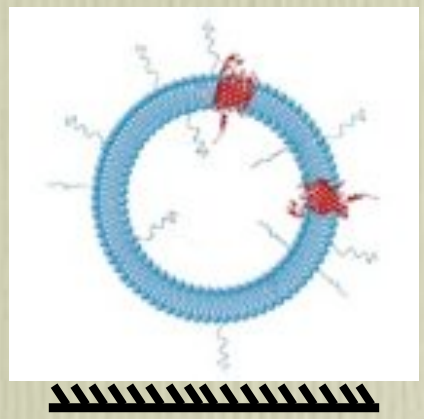
Figure 2. Schematic representation of the reconstitution of surface-tethered cytochrome *c* oxidase into a lipid bilayer. The surface-adsorbed cytochrome *c* oxidase via the affinity of the His-tag to the Ni−NTA surface (see Figure 1) is surrounded by the detergent dodecylmaltoside (left). After reaching maximum surface coverage, the protein is exposed to detergent-destabilized lipid vesicles (DMPC), and microporous Bio-Beads are added. The retreat of the detergent molecules by the beads drives the lipids to form a layer around the membrane protein (right).

Giess, *Biophys. J.* 2004, 87, 3213

Proteoliposome fusion

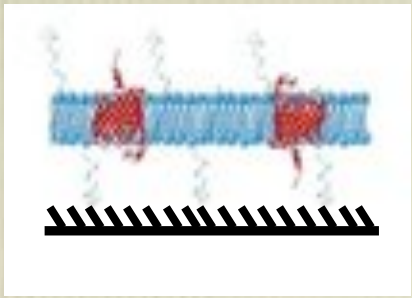
Triton removal

by BioBeads



Vesicles anchorage

and disruption



DSPE-PEG-NHS

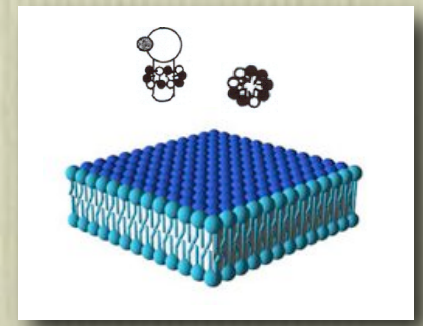
VDAC

POPC

Triton-X100

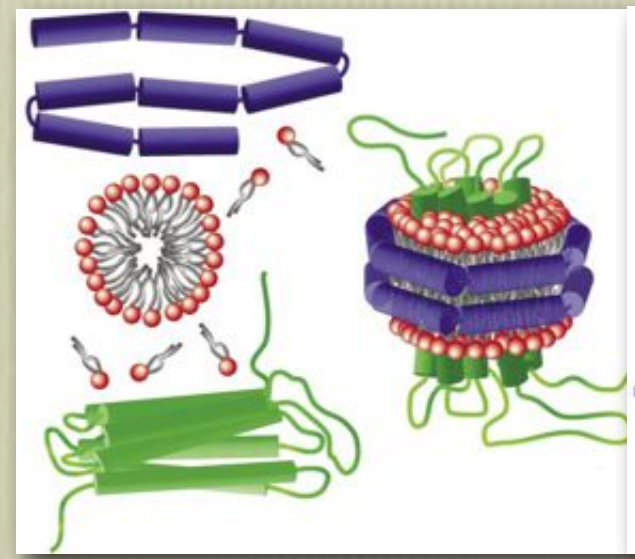
Amine-grafted surface (gold-cysteamine or glass-ADMS)

Comparison with other techniques



Incorporation

Nanodisc?



Tollin et al. 2003. *Trends Pharmacol Sci*. 24:655-9
Sinner et al. Angew. Chem. Int. Ed. 2006, 45, 1 – 5
Giess et al 2004. *Biophys J*. 87:3213-20.
Tanaka & Sackmann *Nature* 2005, 437, 656