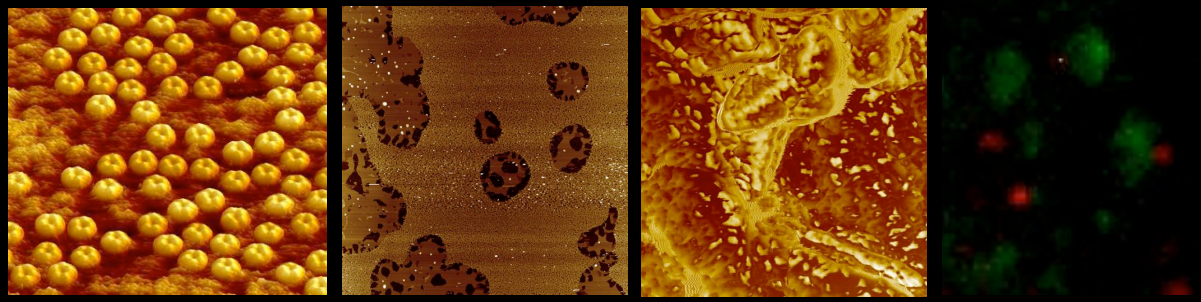
Summer School of AFM, Marcoule, 2011

AFM Imaging of Biological Membranes

Centre de Biochimie Structurale

U1054 INSERM, UMR5048 CNRS, Université Montpellier



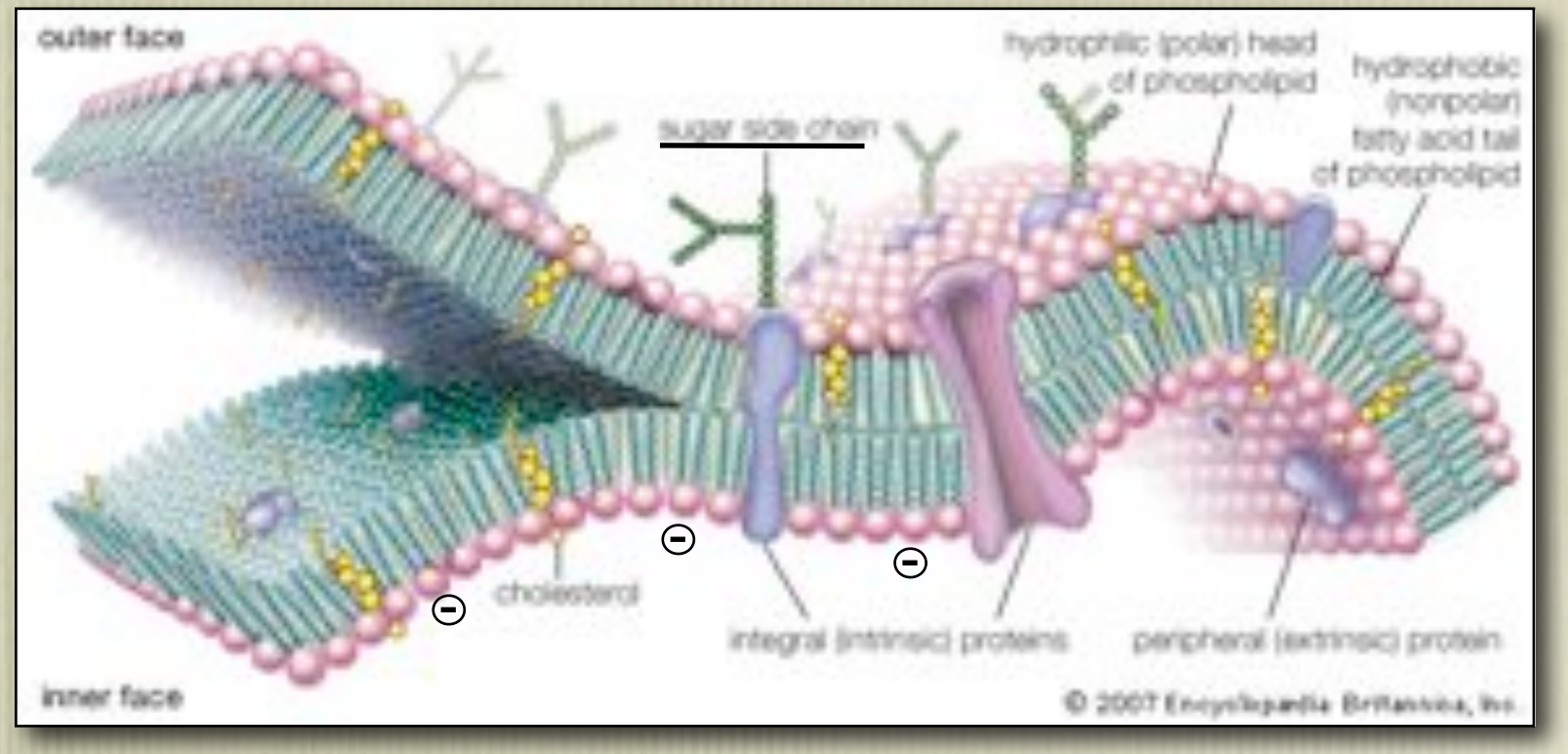
Single Molecule Biophysics Group

Structure and dynamics of membrane assemblies

pem@cbs.cnrs.fr

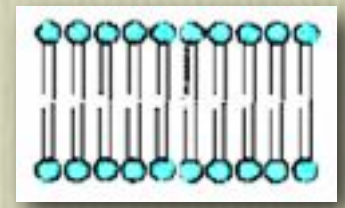
Outlines

- Introduction to biological membranes
- How to mimic biological membranes
- Imaging of artificial supported lipid bilayers
  - Force spectroscopy
- Imaging of biological membranes
- Main drawbacks and developments

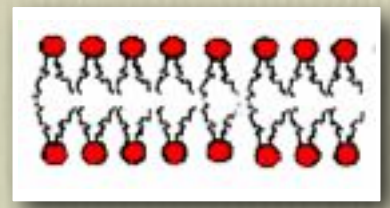


~ 1 protein per 50 lipids

Physical properties of phospholipids



Tm

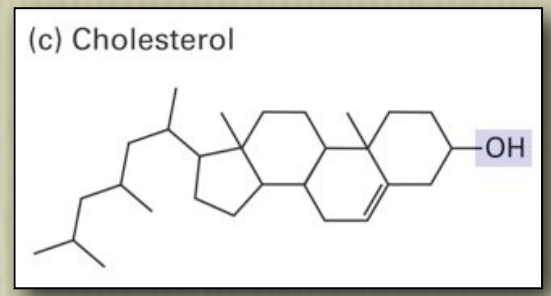


Gel phase

Fluid phase

Lipid	T_m (°C)	Surface area per lipid [a,c] (Å^2)	Molecular weight [f] (g/mol)
DOPC	-22[e]	72	786.15
DPPC	41.3[e]	44.5	734.05
egg PC [b]	-15[e]	62	760.09 [b]
DPPE	63[d]		691.97
egg PE [b]		42	744.05 [b]
DOPS	-11[b]	67[c]	810.04
PS [d]		50-100[c]	

Name	Structure	Number of carbon atoms	Number of double bonds
Myristoyl (M)	$CH_3(CH_2)_{12}COO$-	14	0
Palmitoyl (P)	$CH_3(CH_2)_{14}COO$-	16	0
Stearoyl (S)	$CH_3(CH_2)_{16}COO$-	18	0
Oleoyl (O)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COO$-	18	1
Linoleoyl (L)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COO$-	18	2



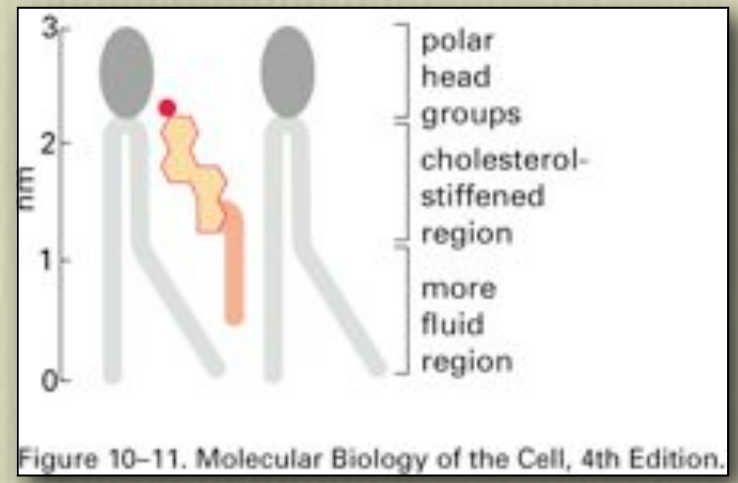


Figure 10–11. Molecular Biology of the Cell, 4th Edition.

Cholesterol molecules have several functions in the membrane

- They immobilize the first few hydrocarbon groups of the phospholipid molecules. This makes the lipid bilayer less deformable and decreases its permeability to small water-soluble molecules. Without cholesterol a membrane would be a cell wall (like in bacteria).

- Cholesterol prevents crystallization of hydrocarbons and phase shifts in the membrane.

Thickness

Curvature

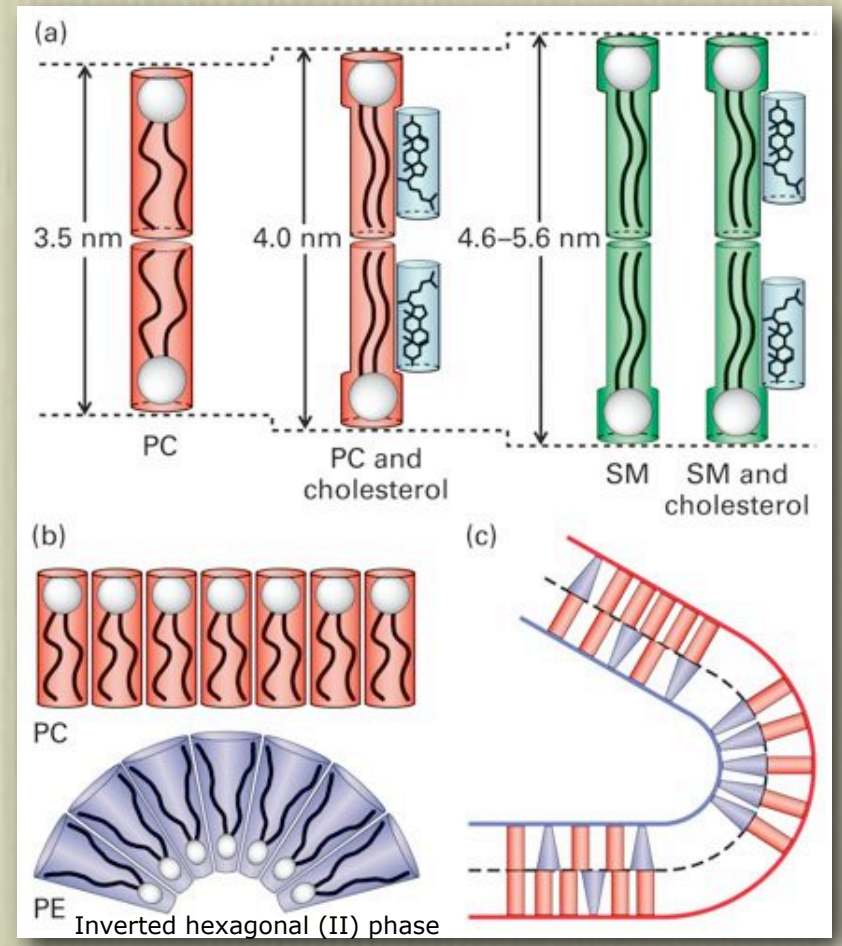
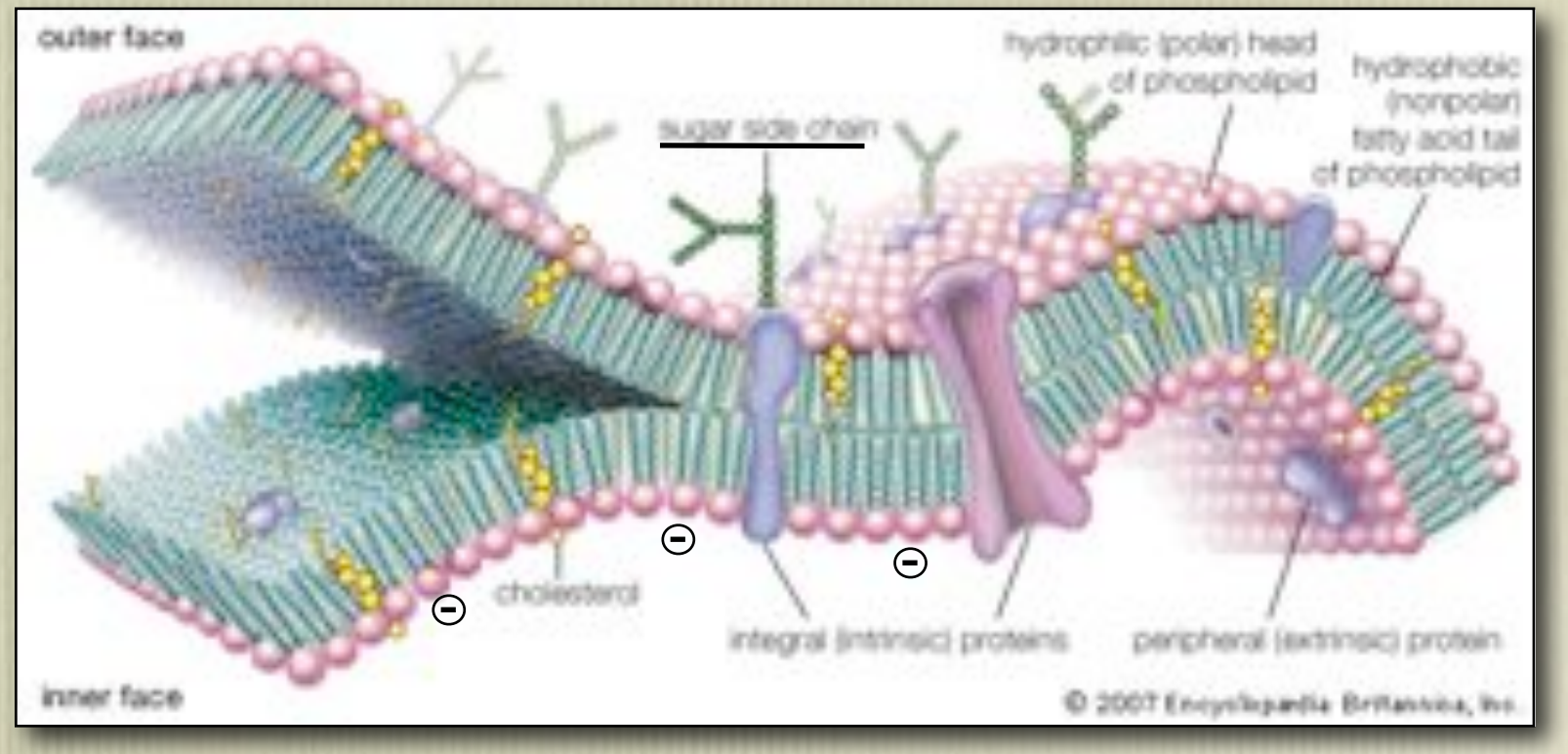


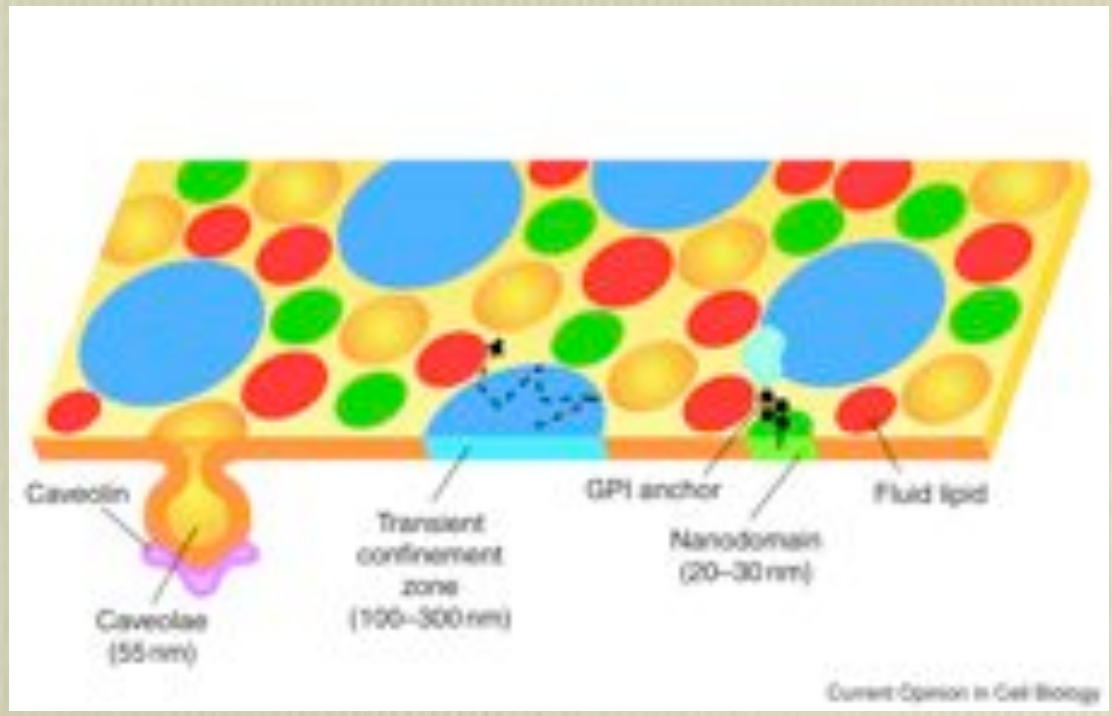
TABLE 5-1 Major Lipid Components of Selected Biomembranes

	Composition (mol %)			
Source/Location	PC	PE + PS	SM	Cholesterol
Plasma membrane (human erythrocytes)	21	29	21	26
Myelin membrane (human neurons)	16	37	13	34
Plasma membrane (*E. coli*)	0	85	0	0
Endoplasmic reticulum membrane (rat)	54	26	5	7
Golgi membrane (rat)	45	20	13	13
Inner mitochondrial membrane (rat)	45	45	2	7
Outer mitochondrial membrane (rat)	34	46	2	11
Primary leaflet location	Exoplasmic	Cytosolic	Exoplasmic	Both

PC = phosphatidylcholine; PE = phosphatidylethanolamine; PS = phosphatidylserine; SM = sphingomyelin.
SOURCE: W. Dowhan and M. Bogdanov, 2002, in D. E. Vance and J. E. Vance, eds., *Biochemistry of Lipids, Lipoproteins, and Membranes*, Elsevier.

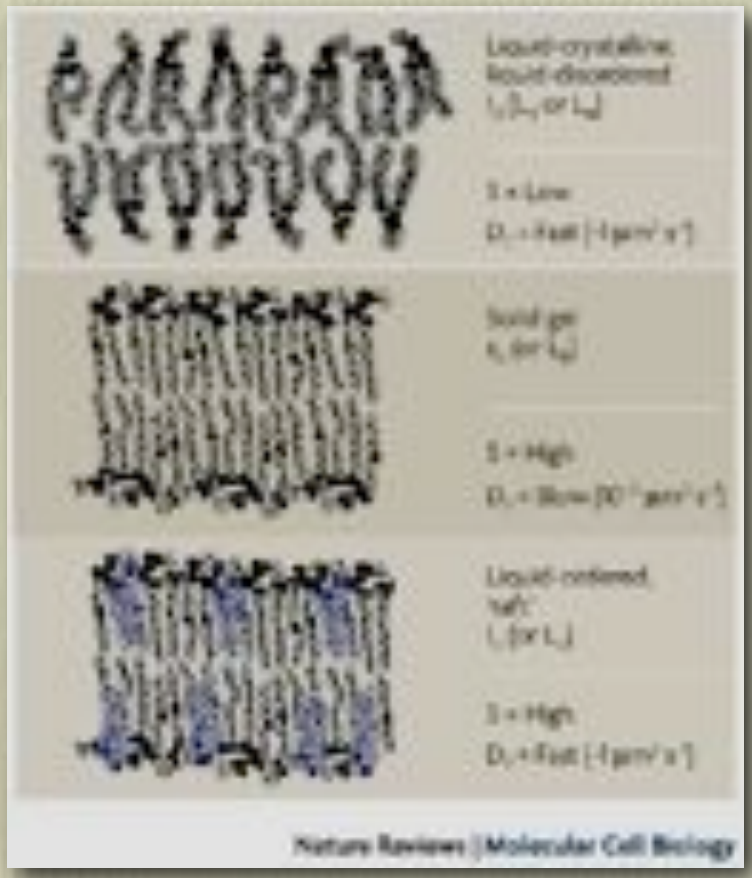


~ 1 protein per 50 lipids

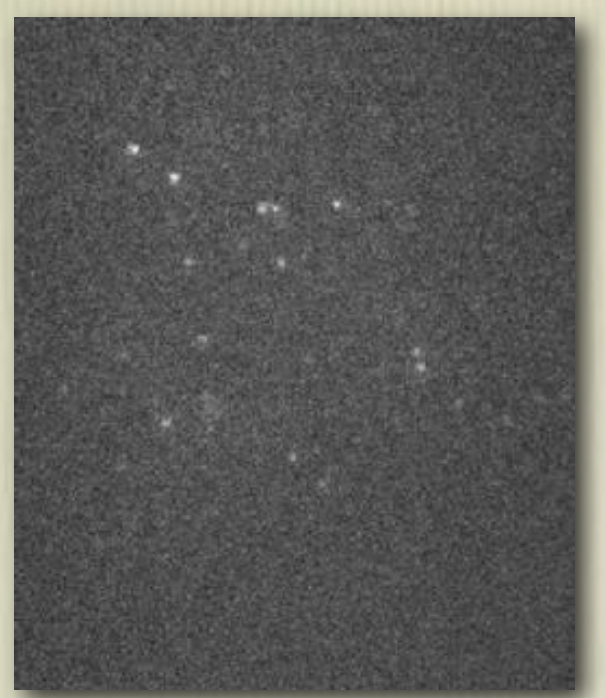

Caveolin
Caveolae
(55 nm)
Transient
confinement
zone
(100–300 nm)
GPI anchor
Nanodomain
(20–30 nm)
Fluid lipid
Current Opinion in Cell Biology

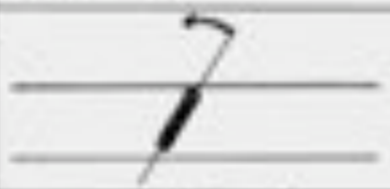
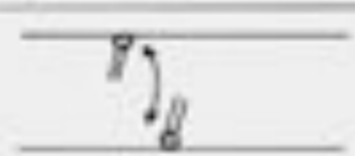
Lateral Diffusion of Lipids

D lipid model membrane
1 à 10 $\mu m^2/s$ (fluid phase)



D lipid plasma membrane
0.1 à 1 $\mu m^2/s$



NAME	PICTURE
LATERAL DIFFUSION 1-10 $\mu m^2/s$	
ROTATIONAL DIFFUSION WOBBLING 1-100 MHz	
ROTATIONAL DIFFUSION AXIAL ROTATION 10-1000 MHz	
DIRECTION FLUCTUATION	
CHAIN FLEXIBILITY	
FLIP-FLOP	

Outlines

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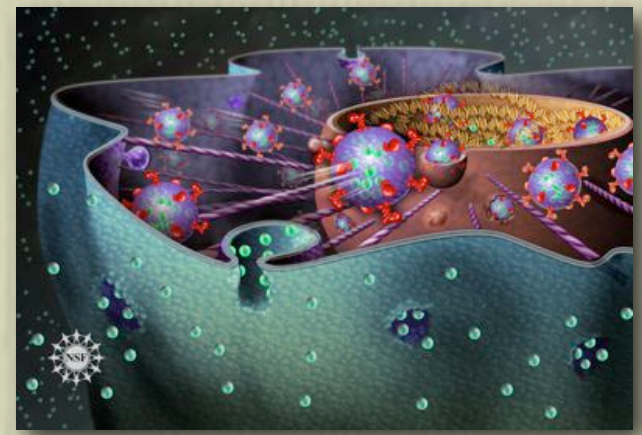
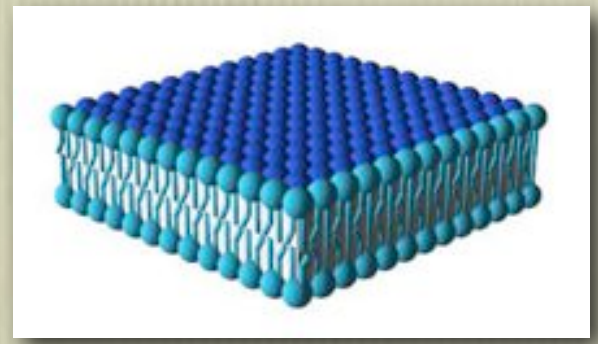
- **How to mimic biological membranes**
- Imaging of artificial supported lipid bilayers
  - Force spectroscopy
- Imaging of biological membranes
- Main drawbacks and developments

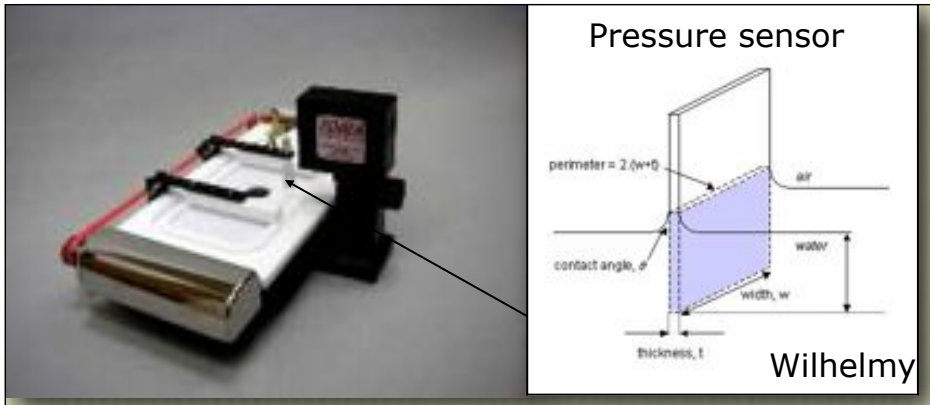
Membrane Reconstituted systems



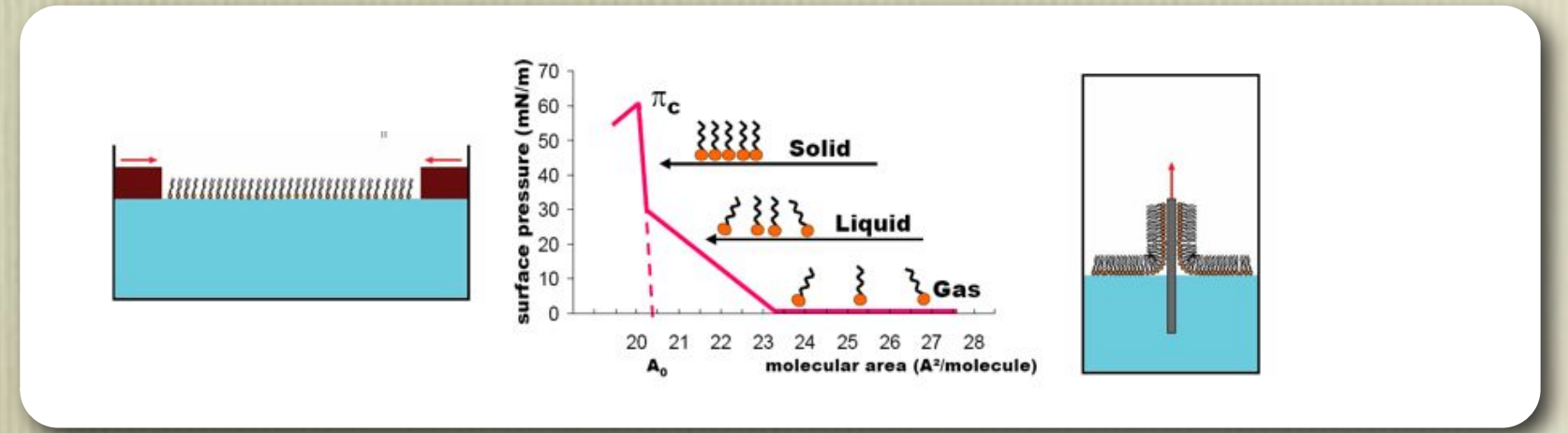
“Forming planar membranes suitable for AFM analysis”



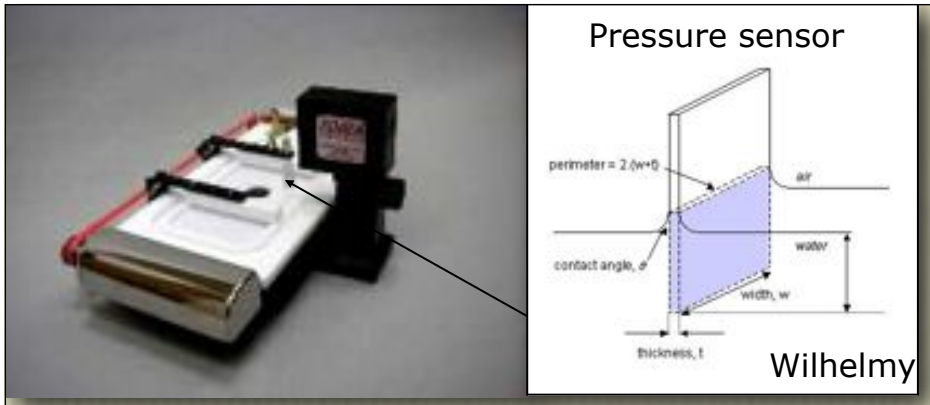
Lipids
Proteins



Supported lipid bilayer using Langmuir balance



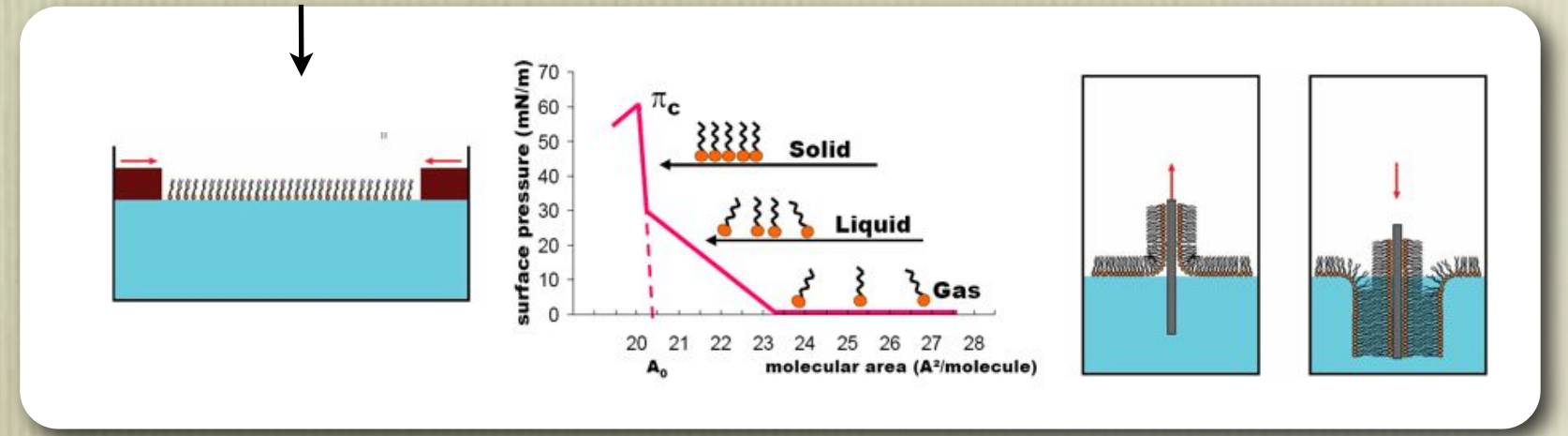
Lateral pressure in biological membranes = 30 mN/m



Supported lipid bilayer using Langmuir balance

Langmuir-Schaeffer

Langmuir-Blodgett



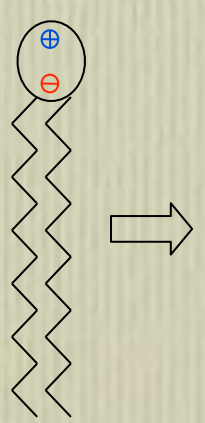
Lateral pressure in biological membranes = 30 mN/m

Supported lipid bilayer using Langmuir balance

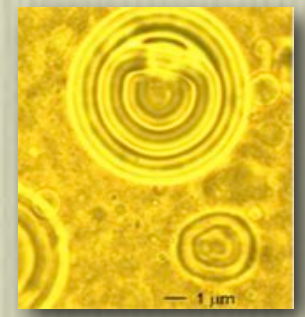
- **Advantages**
  - Asymetric bilayers
  - Poor sensitivity to the buffer
  - Monolayers

- **Drawbacks**
  - Dewetting problem (difficult to produce homogeneous films covering a large surface)
  - Freezing of the leaflet facing the substrate

Supported lipid bilayer by vesicle fusion



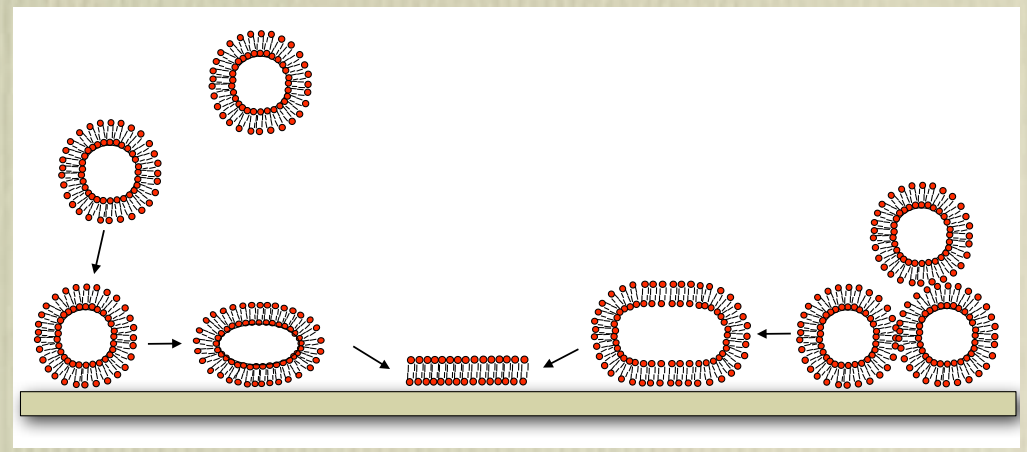
Multilamellar vesicle (MLV)



Aqueous environment

Extrusion

Sonication

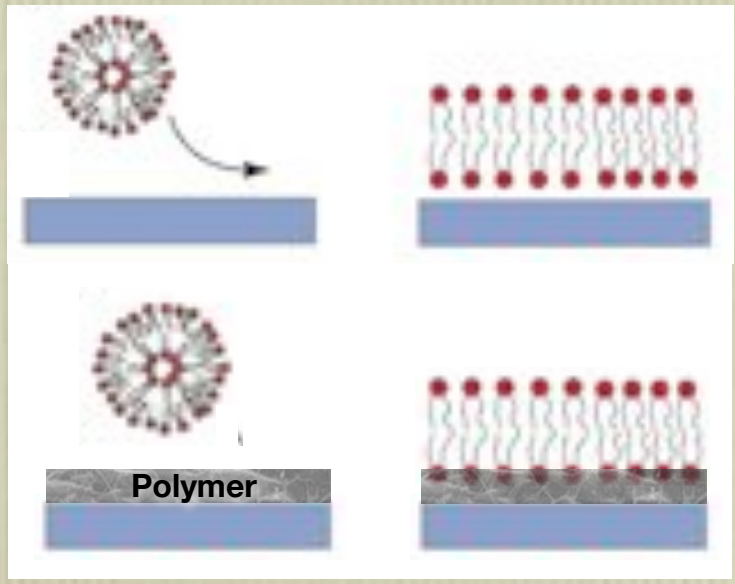


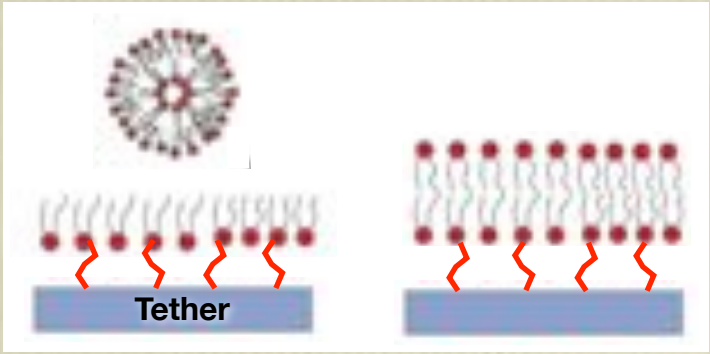
- Easy to prepare

- Liposome rupture
- Temperature control
- Sensitivity to the buffer
- Interaction of the leaflet with the substrate

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

Supported lipid bilayer by vesicle fusion

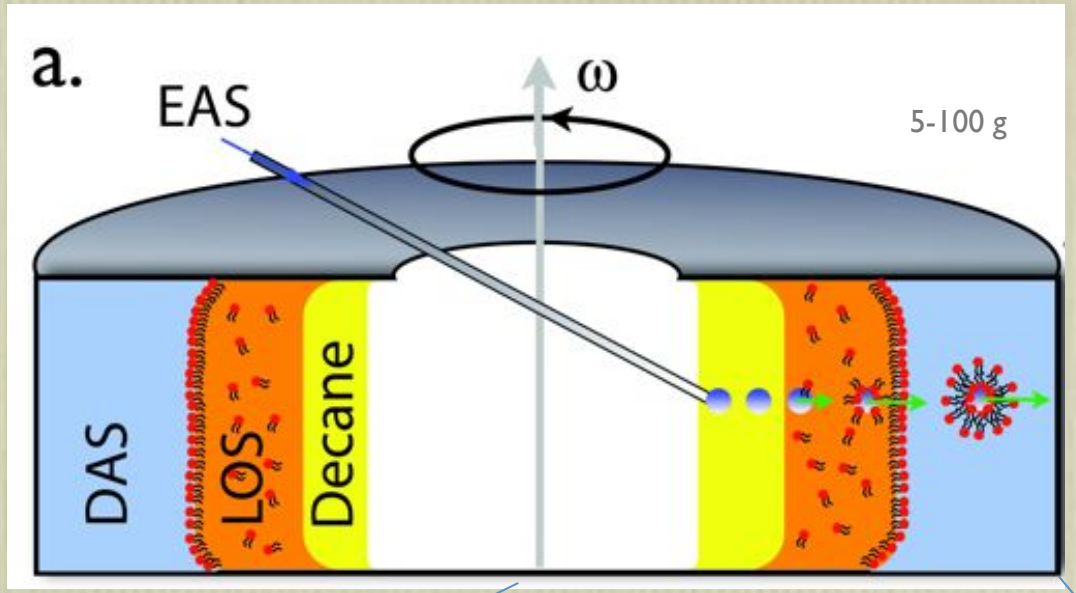




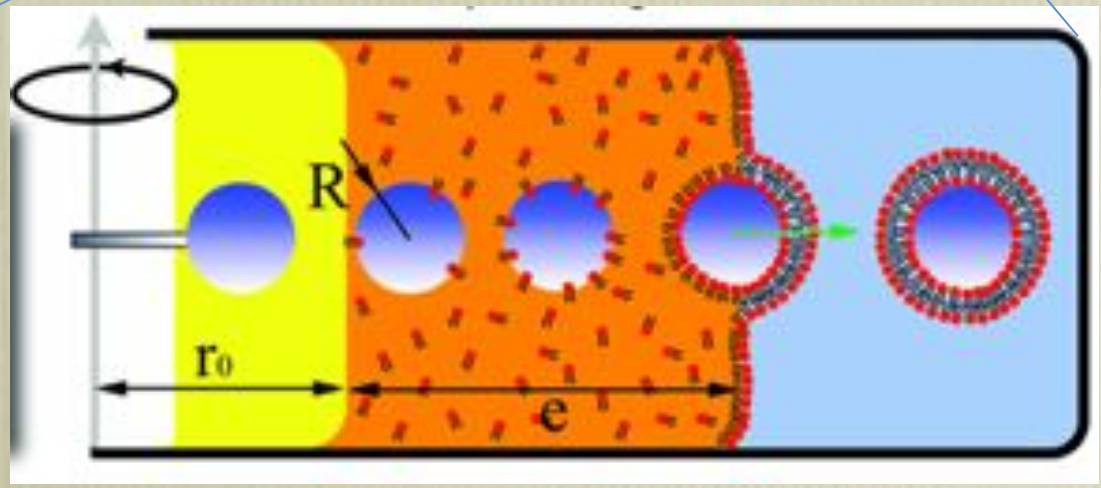
Tanaka et al (2005) Nature 437, 656-663

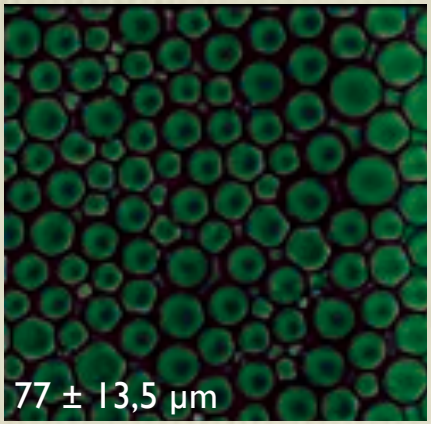
cDICE: continuous droplet interface crossing encapsulation

Abkarian et al (2011) Soft Matter, in press



LOS: Lipid in Oil Solution
DAS: Dispersing Aqueous Solution
EAS: Encapsulating Aqueous Solution





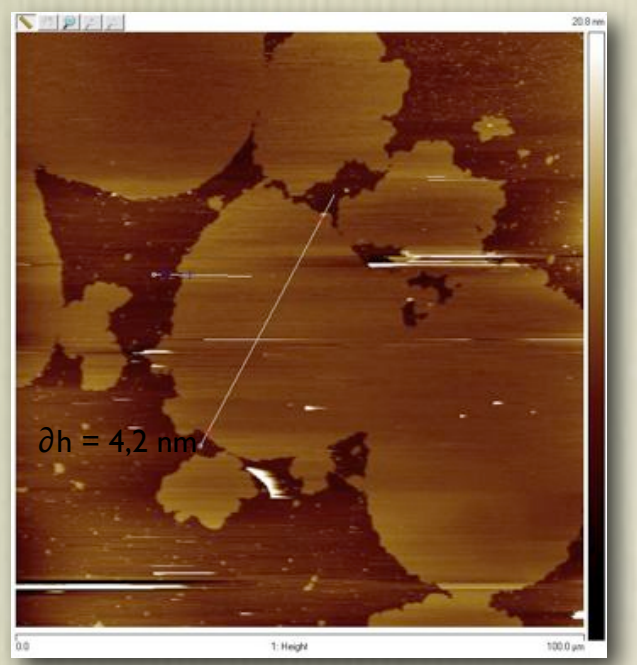
Protocol

Glucose buffer

Sucrose

4 mM $CaCl_2$ buffer

H_2O



AFM imaging in 10 mM Tris (pH 7.4), 150 mM KCl, 5 $MgCl_2$

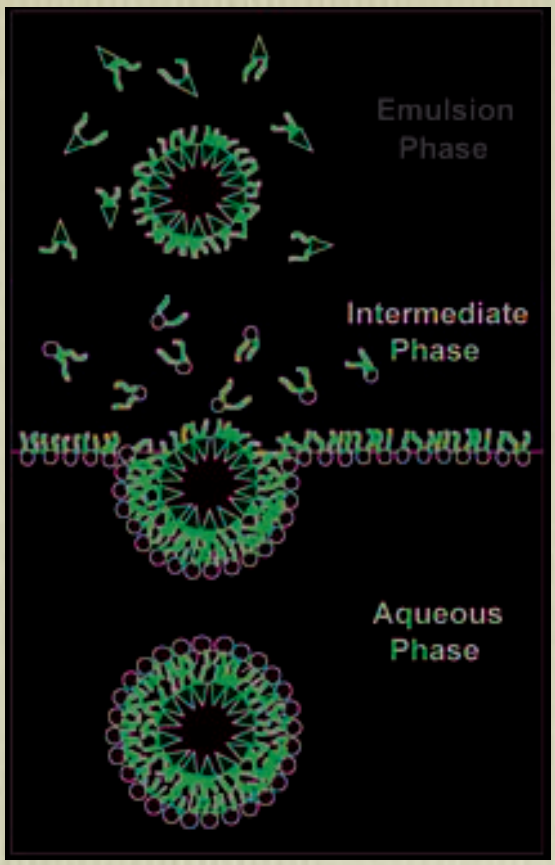
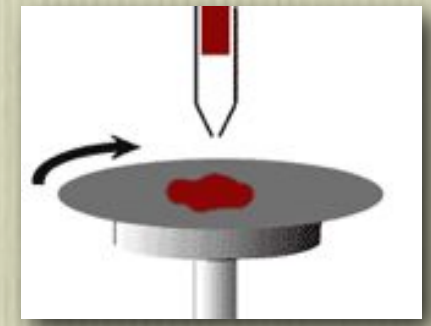


Fig. 1. Schematic illustration of the technique used to engineer asymmetric vesicles. The sample is composed of three parts: an inverted emulsion where water droplets in lipid-saturated oil are stabilized by lipid molecules destined for the inner leaflet; an intermediate phase of lipid-saturated oil heavier than the inverted emulsion phase, and whose lipids form a monolayer at the oil/water interface; and the bottom aqueous phase, which receives the final asymmetric vesicles. The lipids in the intermediate phase are completely different from those in the inverted emulsion and form the outer leaflet of the bilayer; the final structure is an asymmetric vesicle.

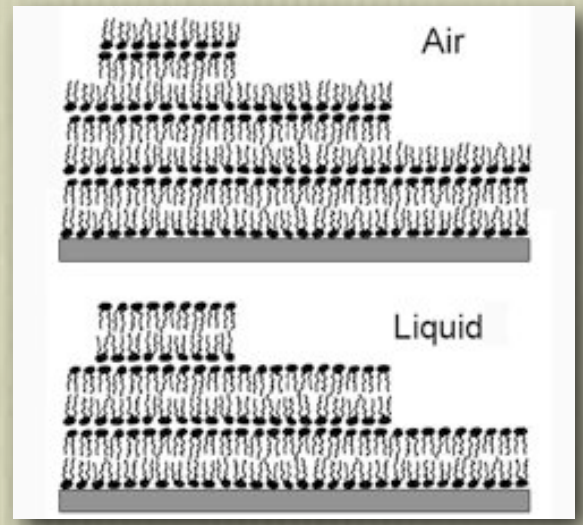
Pautot et al, PNAS (2003)

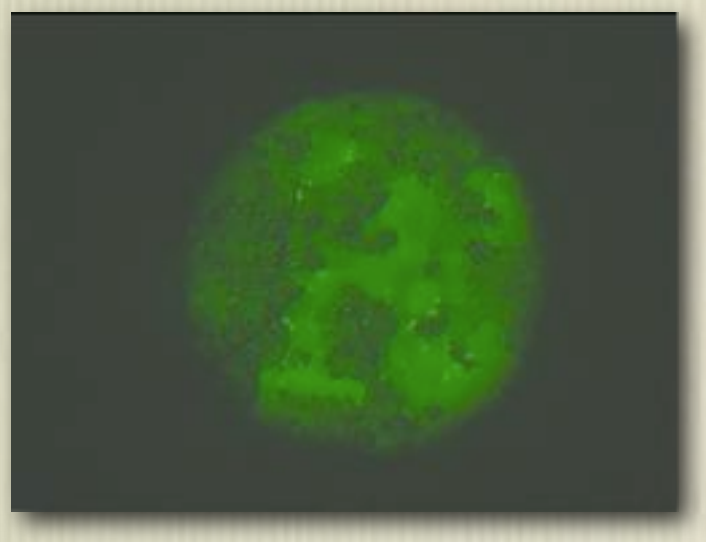
Problem: presence of organic solvent or oil in the membrane?

Supported lipid bilayer by spin coating



Lipids in organic solvant





http://www.brewerscience.com/products/cee/technical/spintheory/
Cohen-Simonsen, Langmuir 2004

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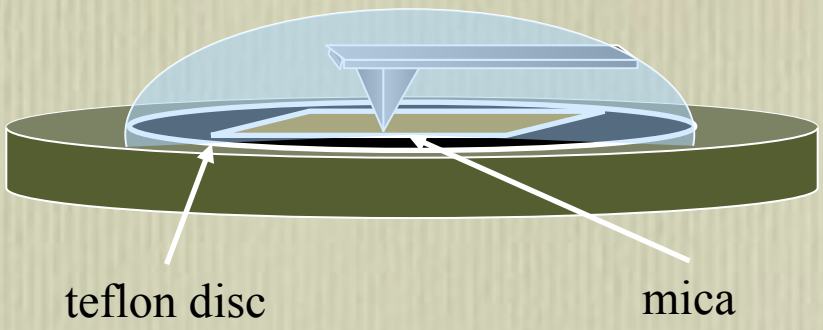
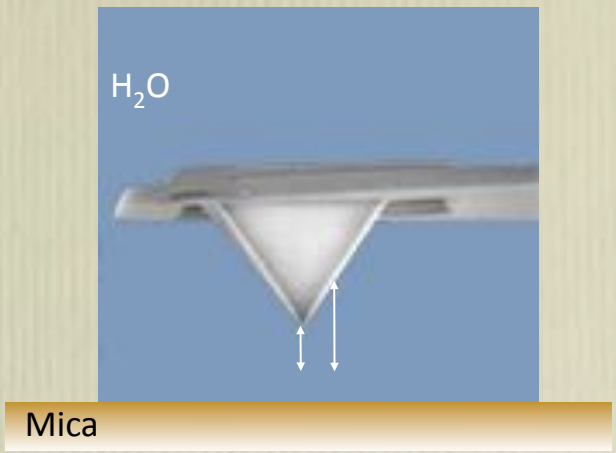
AFM imaging





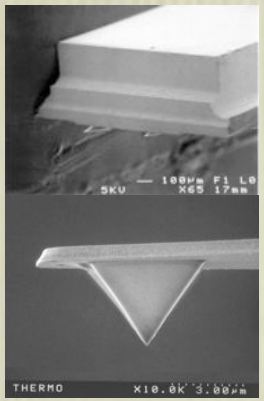
Electrostatic Repulsive Force (0.1 to 1 µm)

Van der Walls (attractive Force, ~Å)

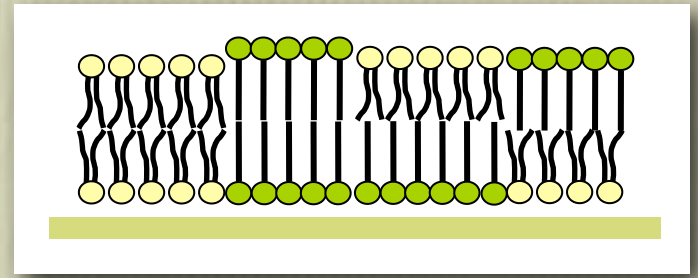
Force applied during scanning < 100 pN

Soft cantilevers

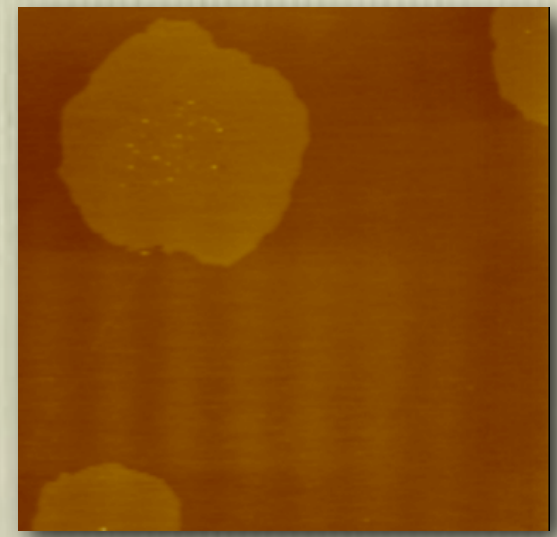
Tapping or contact mode



Substrate - lipid polar heads interaction



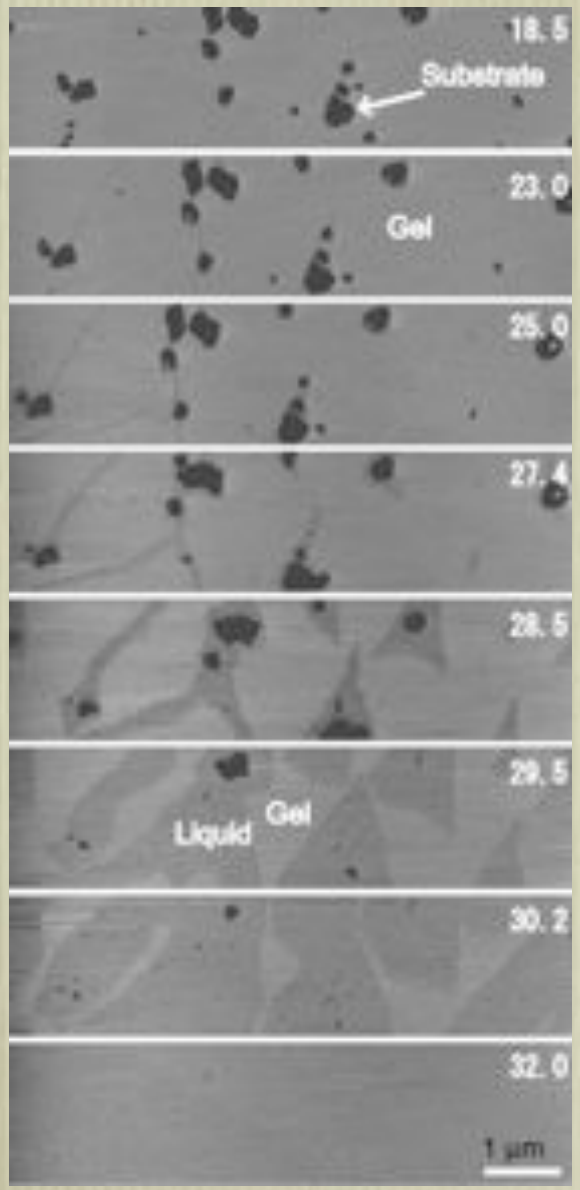
Bilayer with two components
Lateral segregation



Substrate-lipid polar head interaction

E.I. Goksu et al. Biochimica et Biophysica Acta (2008)

Substrate - lipid polar heads interaction

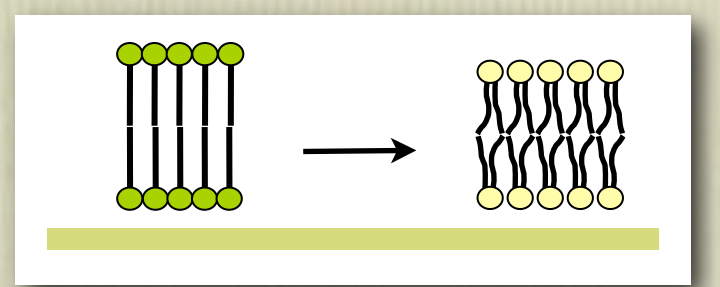


Simultaneous melting of the two leaflets

Temperature-controlled AFM

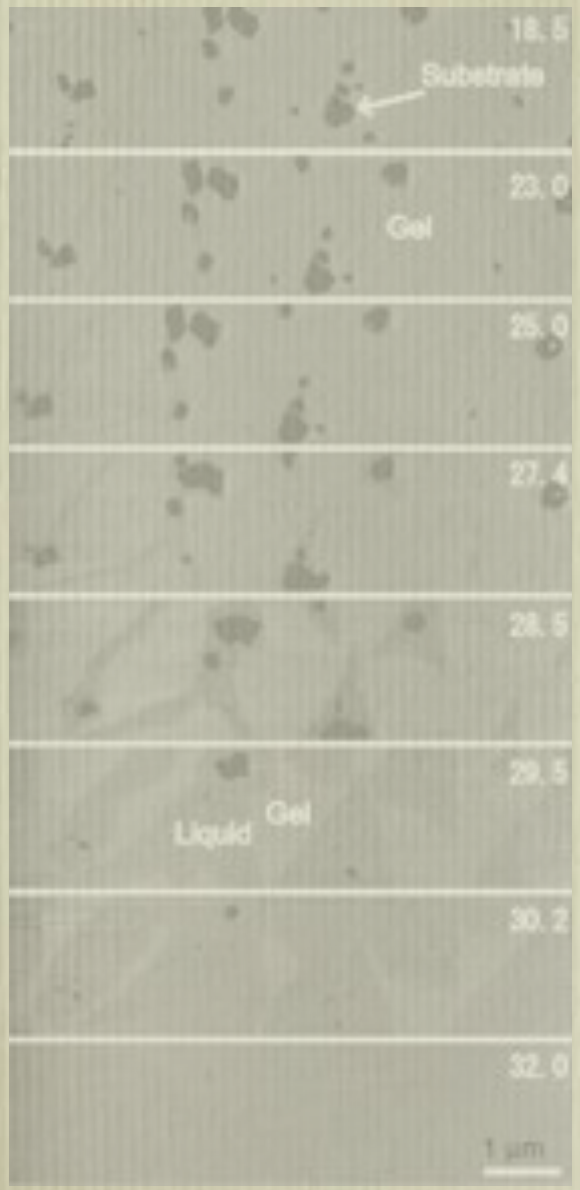
DMPC

Tm = 23°C



Tokumasu et al., Ultramicroscopy 97 (2003) 217-227

Substrate - lipid polar heads interaction



Temperature-controlled AFM

DMPC

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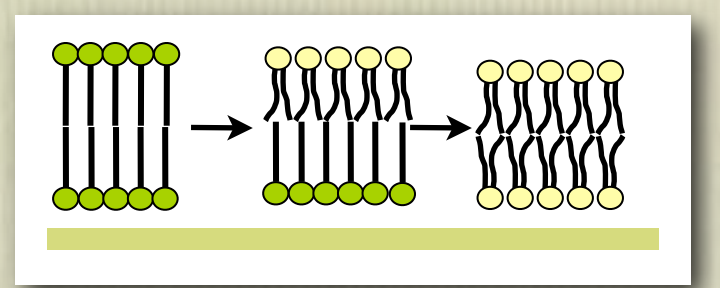
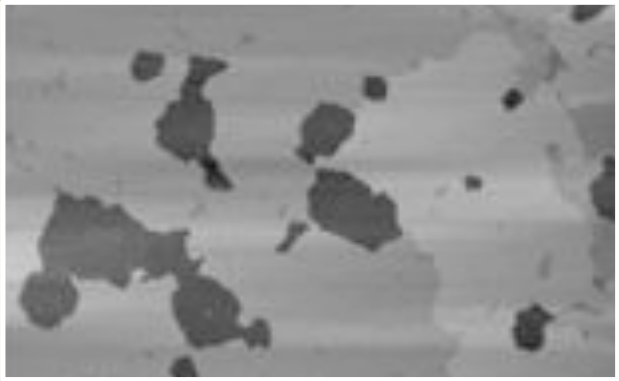
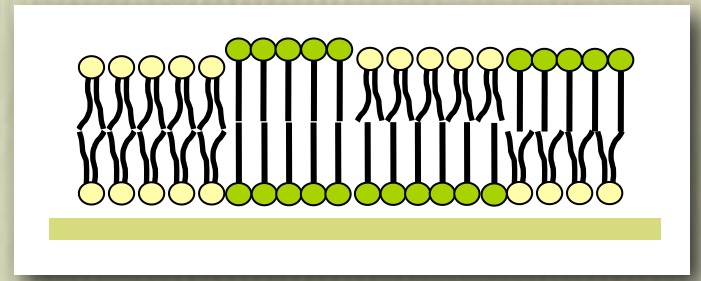




Differential melting of the two leaflets

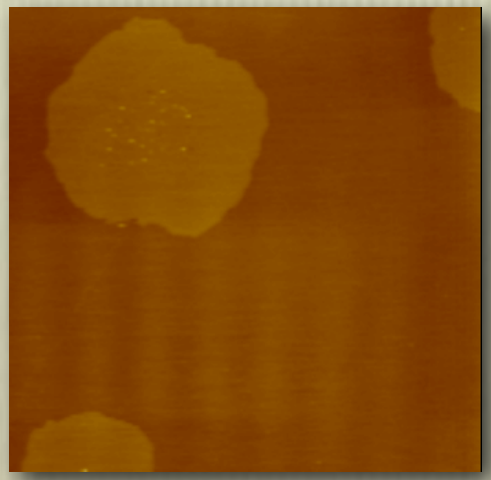
Seeger et al., Biophys. J. (2009) 97, 1067-1076

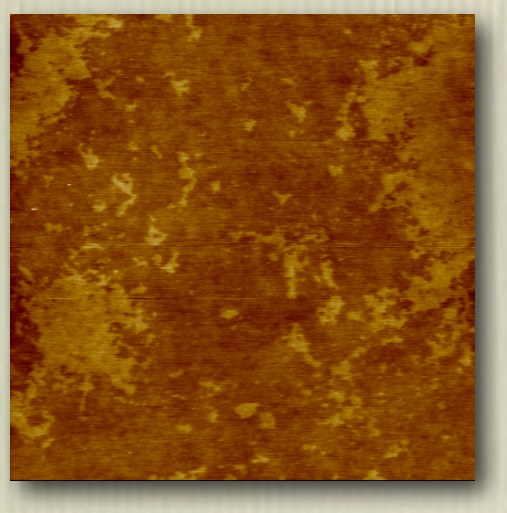
Substrate - lipid polar heads interaction



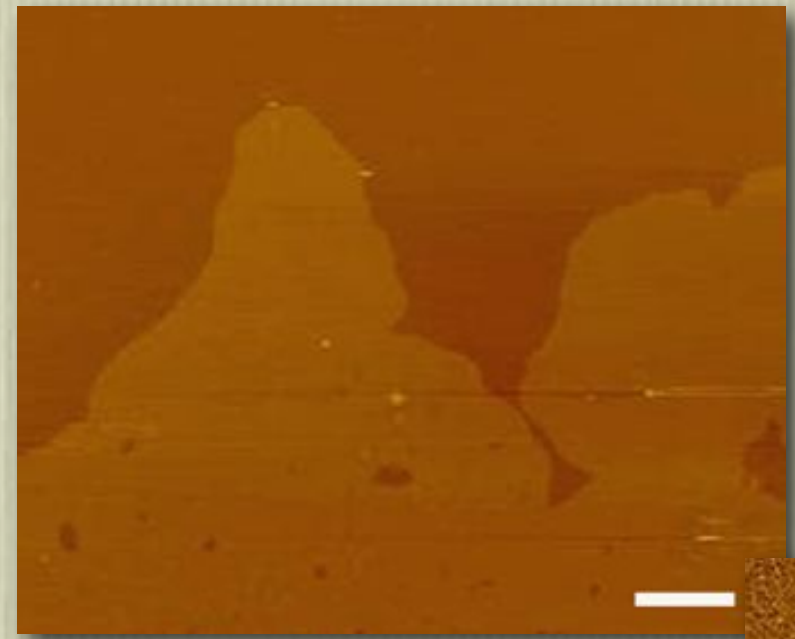
Bilayer with two components
Lateral segregation

- Influence of the substrate on bilayer topology and domain shape

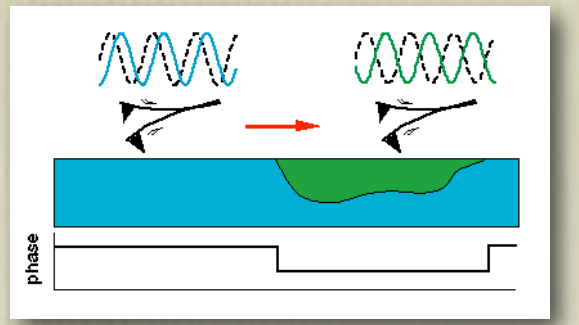




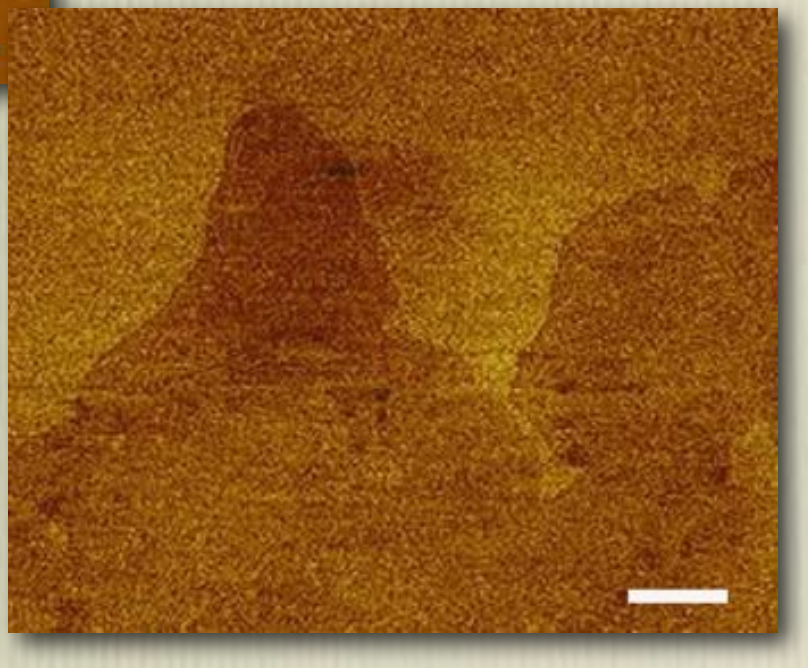
Probing visco-elastic properties using tapping mode



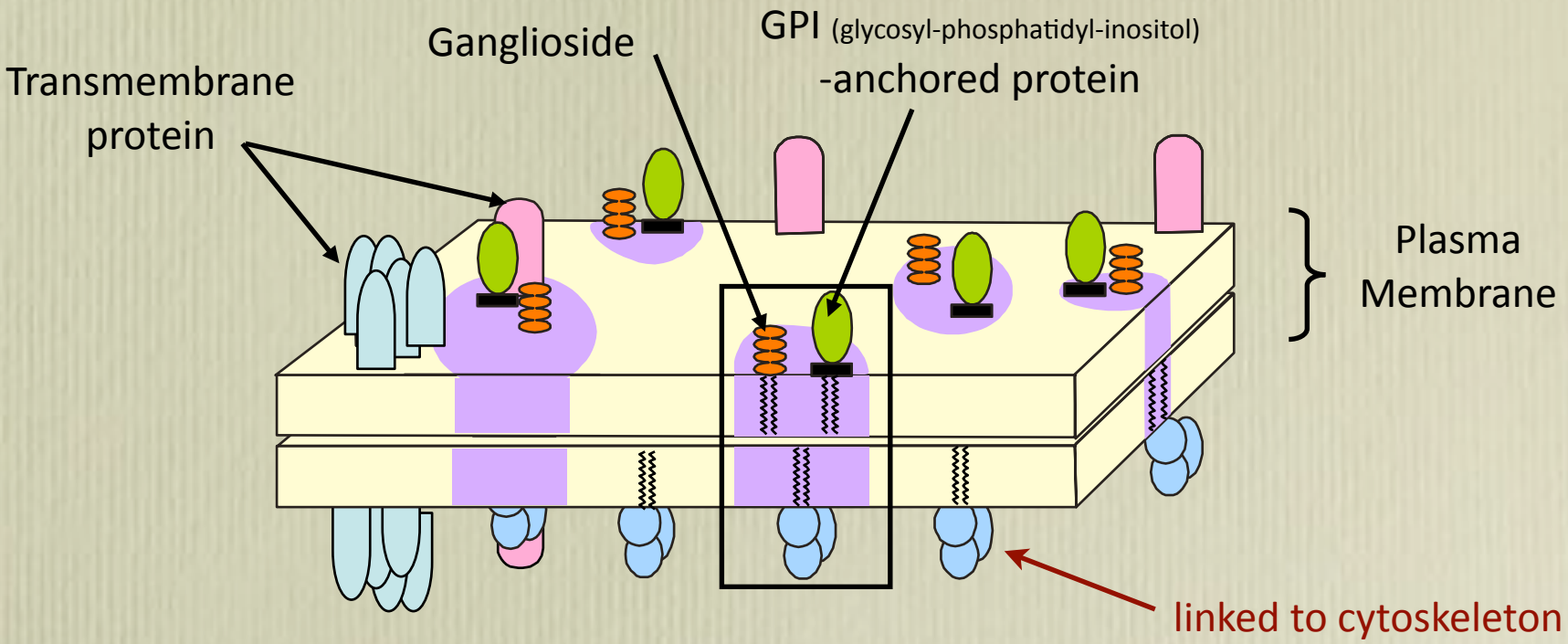
Tapping



Peak Force Tapping



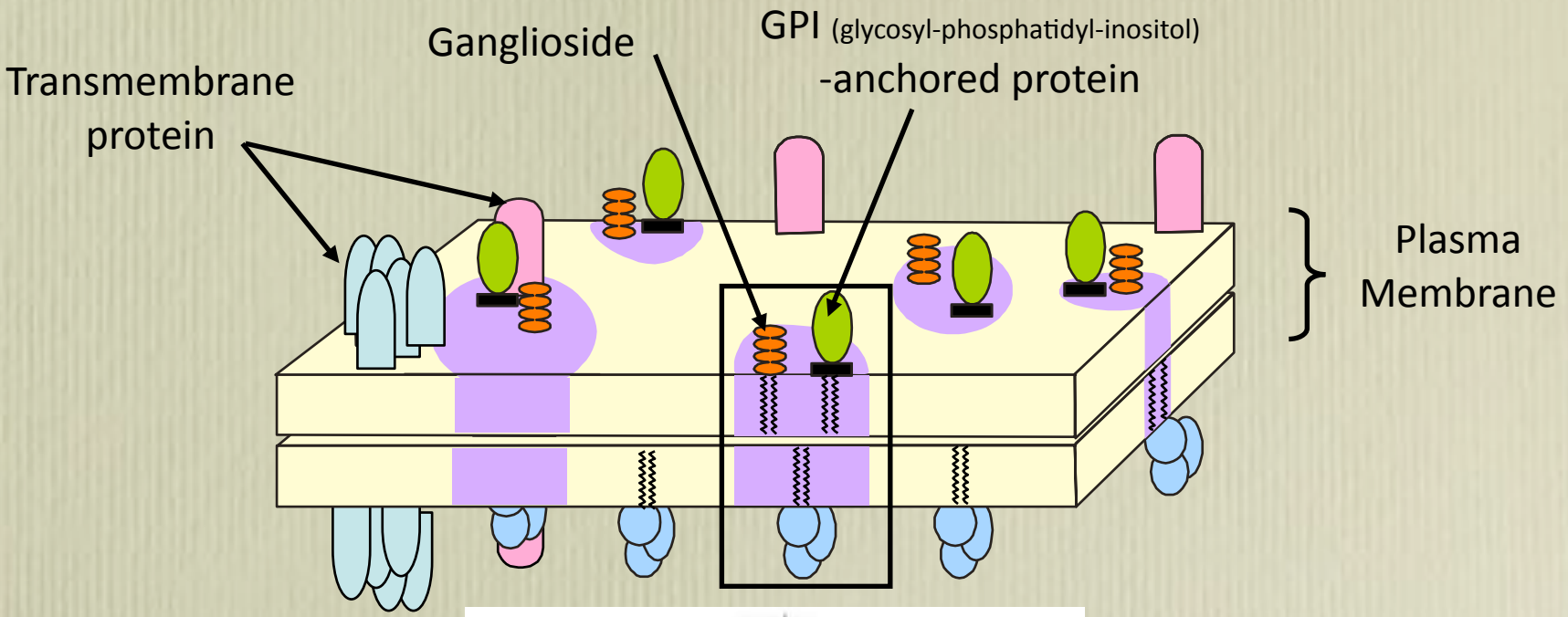
Lateral heterogeneity of membrane components

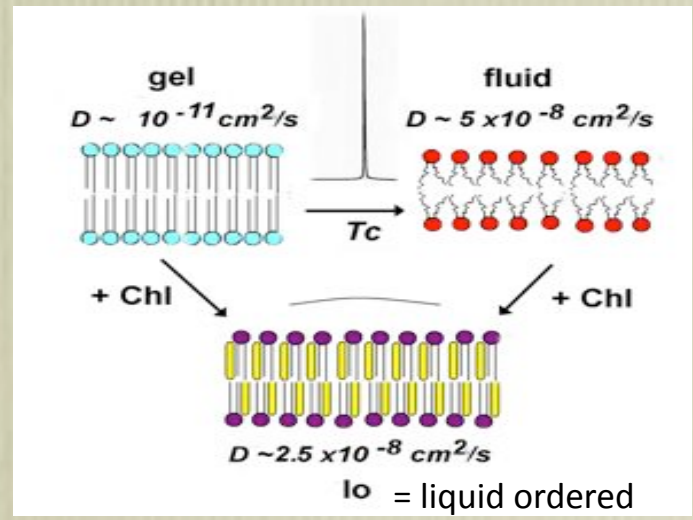


Raft: "Small, heterogeneous, highly dynamic, sterol- and sphingolipids-enriched domains that compartmentalize cellular processes"

Molecular mechanism of microdomain formation ?

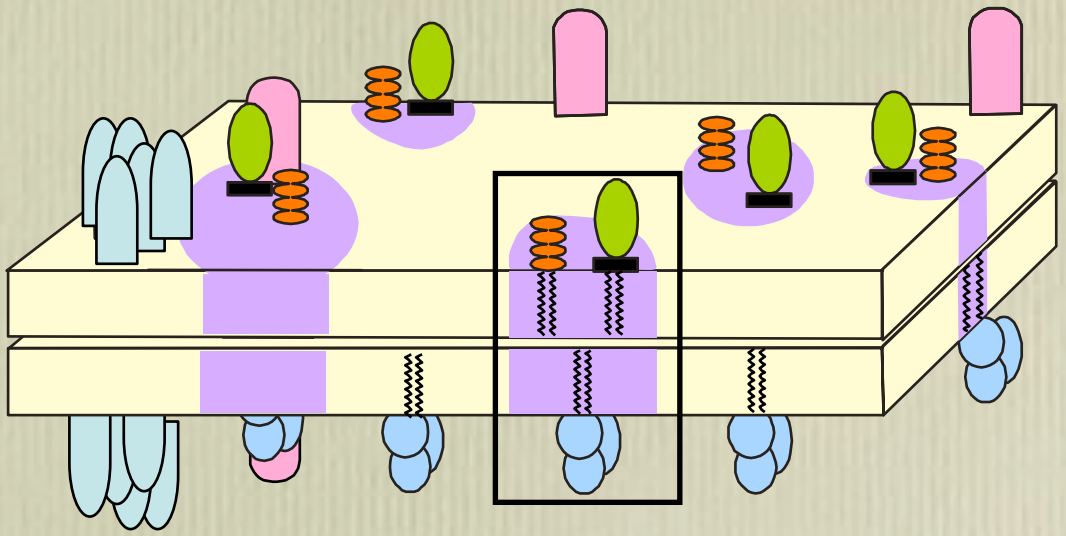
Lateral heterogeneity of membrane components



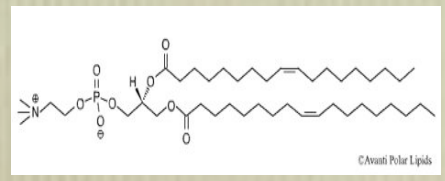


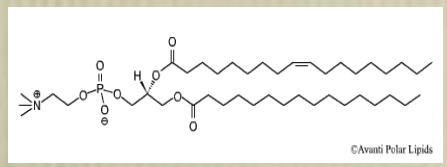
Morrisett, J.D. 1976; Simons, K. 1988

AFM imaging of model raft microdomains

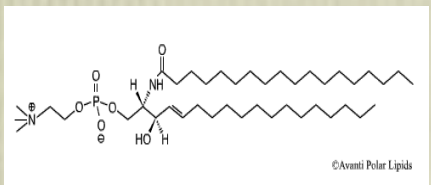


DOPC *Tc=- 20°C*

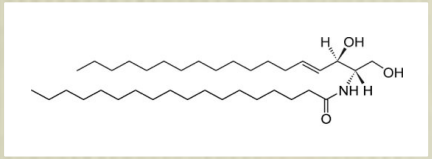




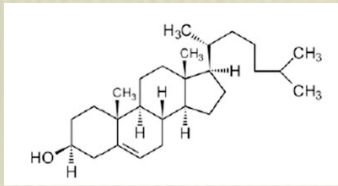
POPC *Tc=-2°C*



SM *Tc=20°-45°C*



Ceramide



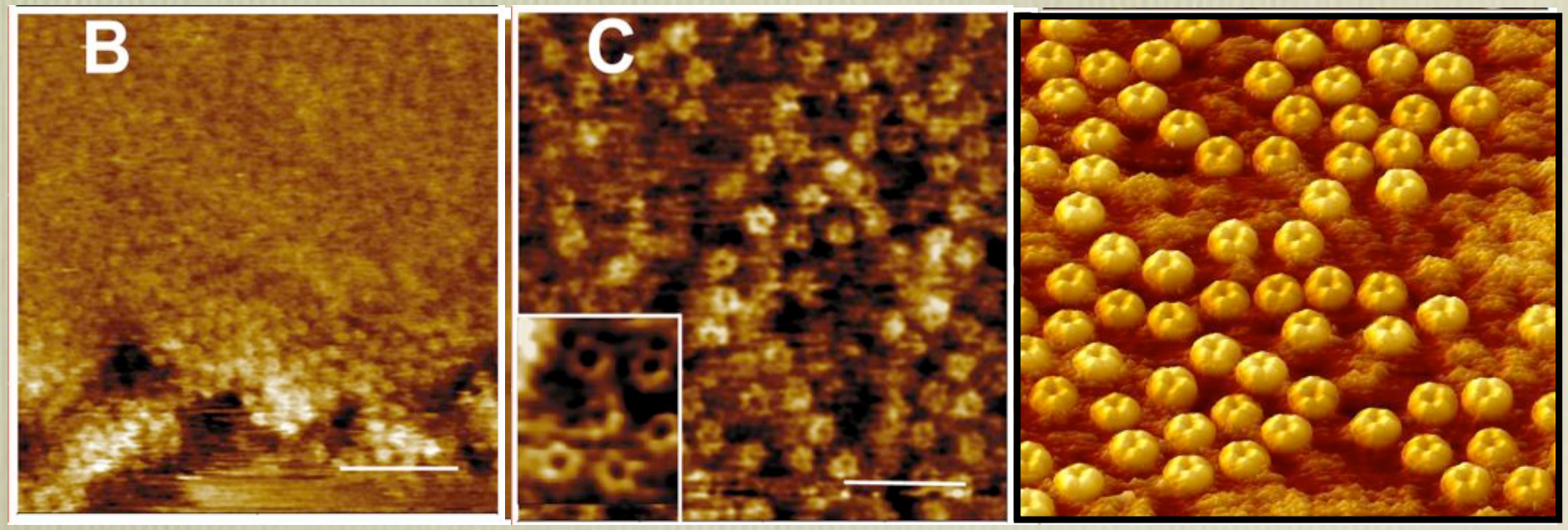
Cholesterol

Ganglioside

AFM imaging of model raft microdomains

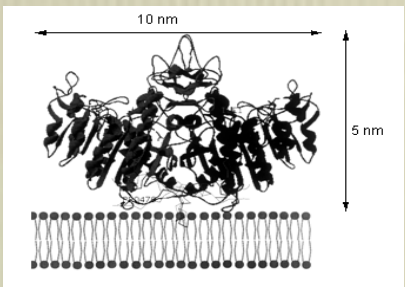
GM1 partitioning in DOPC/DPPC bilayers

dioleoylPC(C18:1, DOPC)/dipalmitoylPC (C16:0, DPPC)



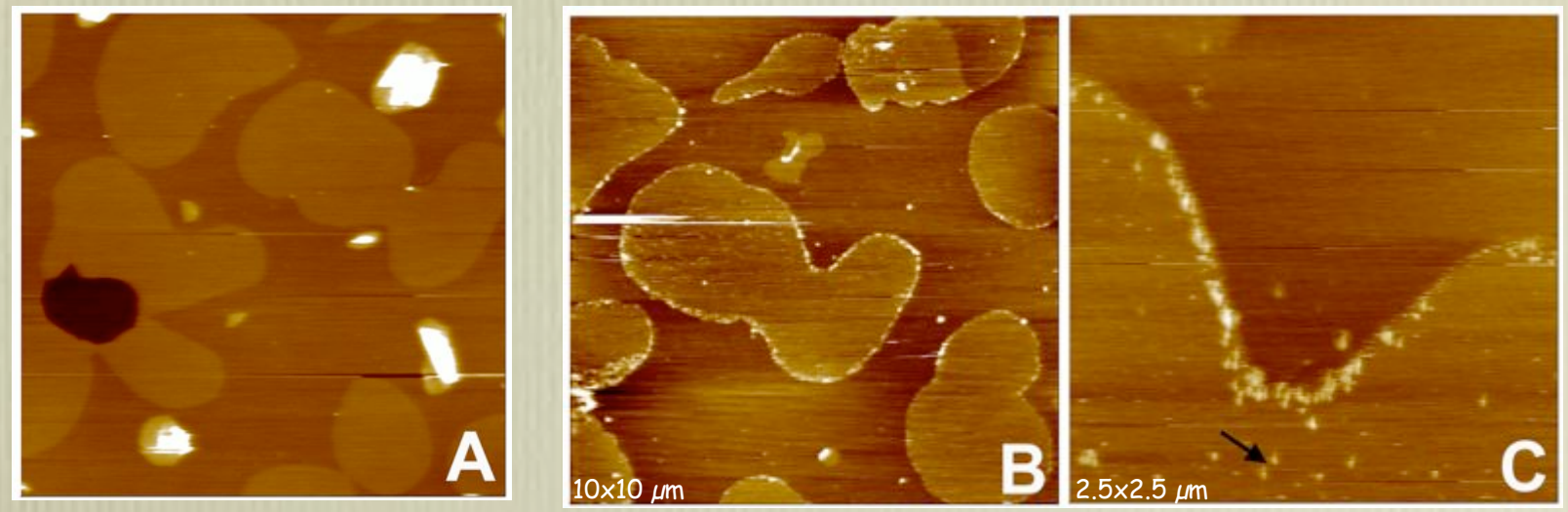
AFM imaging of model raft microdomains

Partition of the GPI-anchored Alkaline Phosphatase



Lheto, 2002

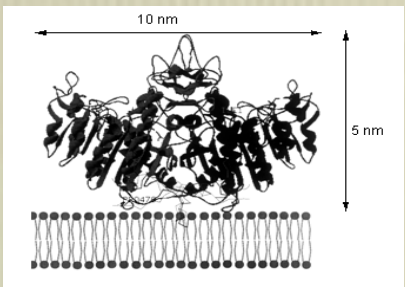
DOPC/SM



δh, 2 à 5 nm
Ø, 15 à 50 nm

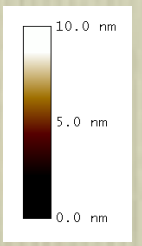
AFM imaging of model raft microdomains

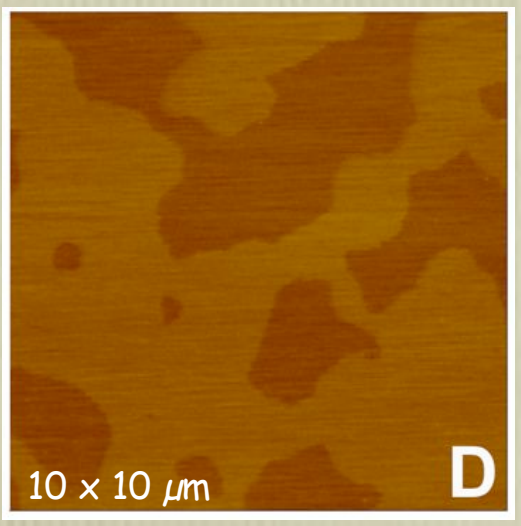
Partition of the GPI-anchored Alkaline Phosphatase

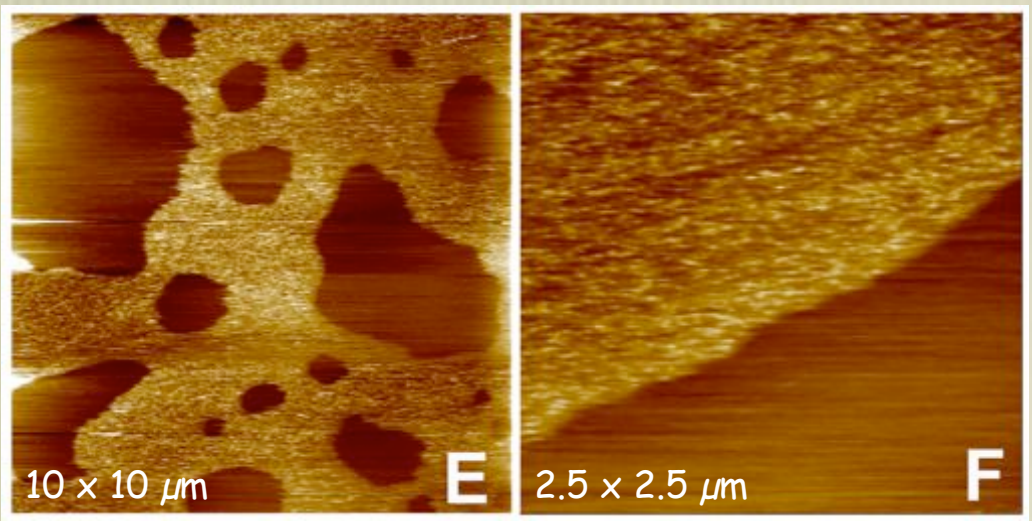


Lheto, 2002

DOPC/SM/Chl (1:1:0.35)



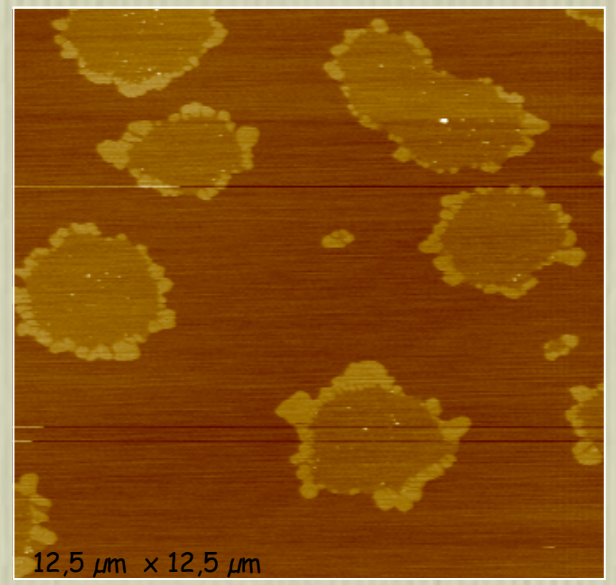


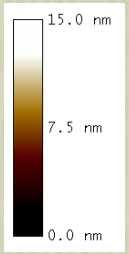


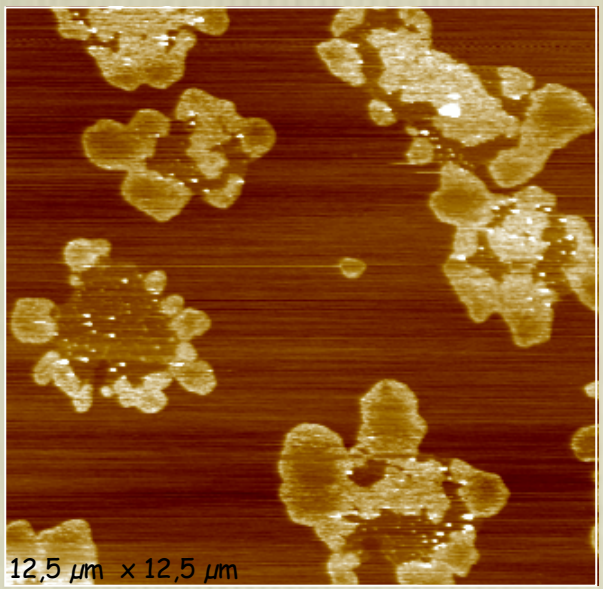
EMBO Reports (2002) 3, 485-490

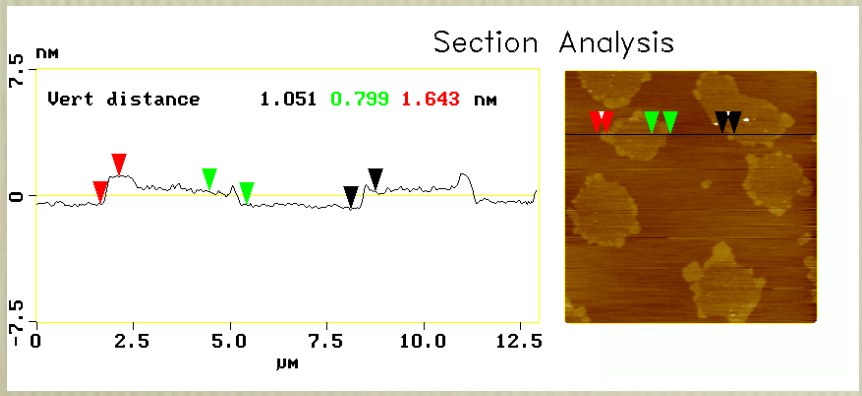
AFM imaging of model raft microdomains

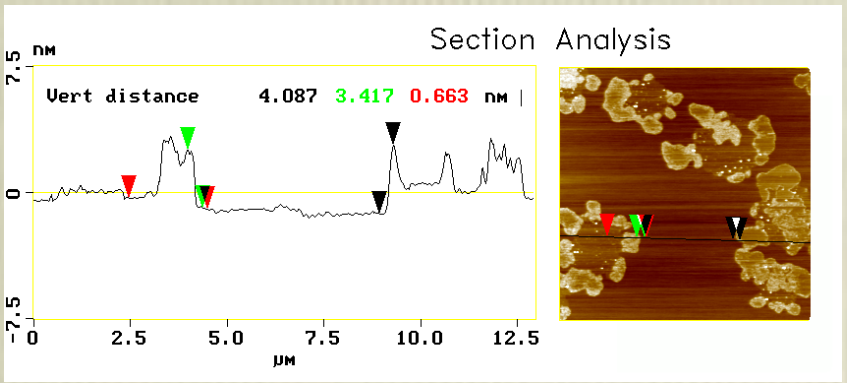
POPC/SM/Chl (1:1:0.35)





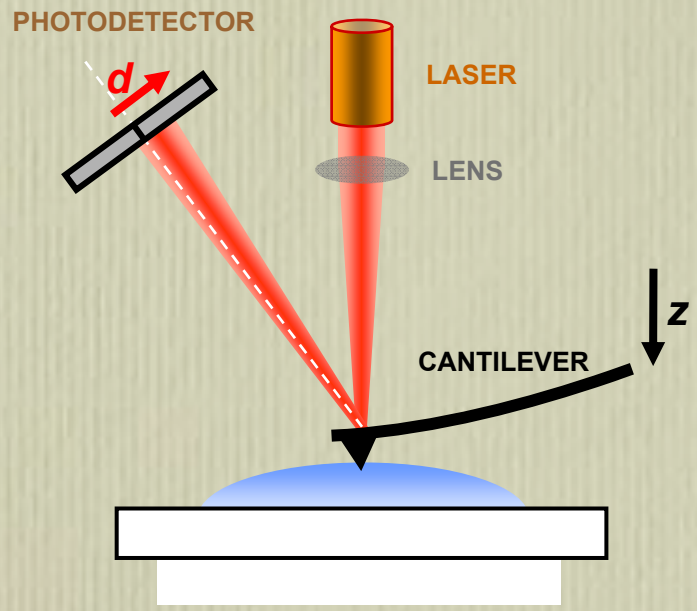


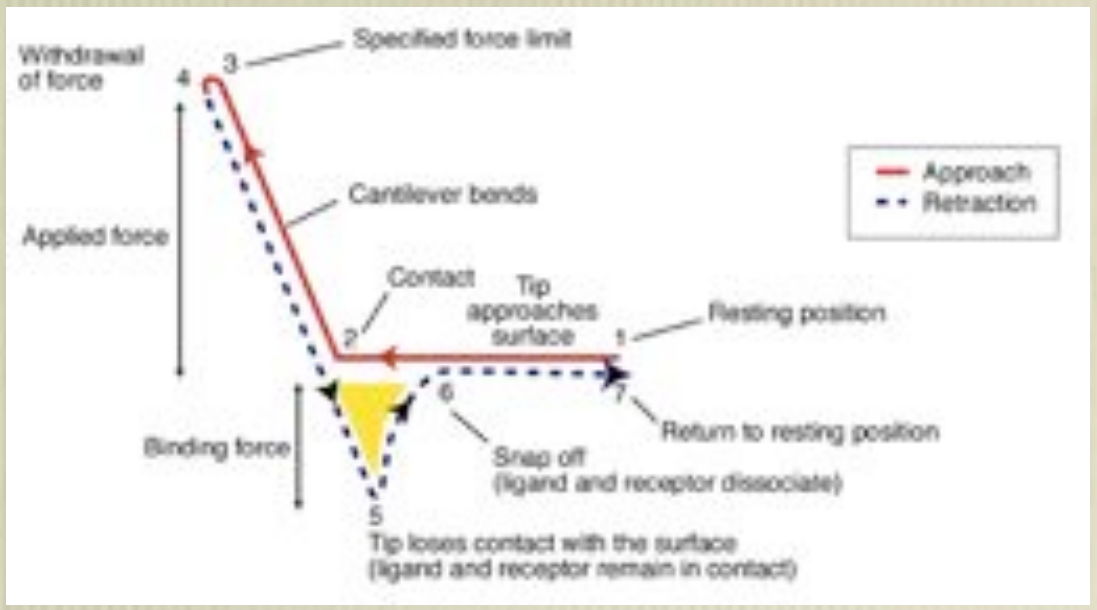


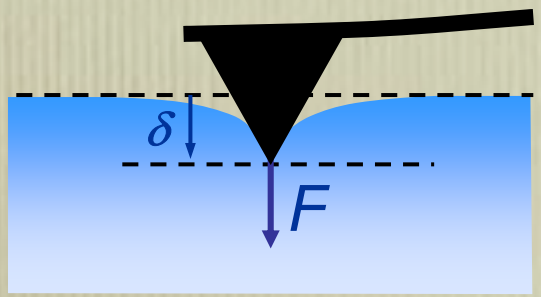


Pflugers Arch. – Eur J Physiol, (2008) 456(1):179-88.

Force-displacement curves on membranes



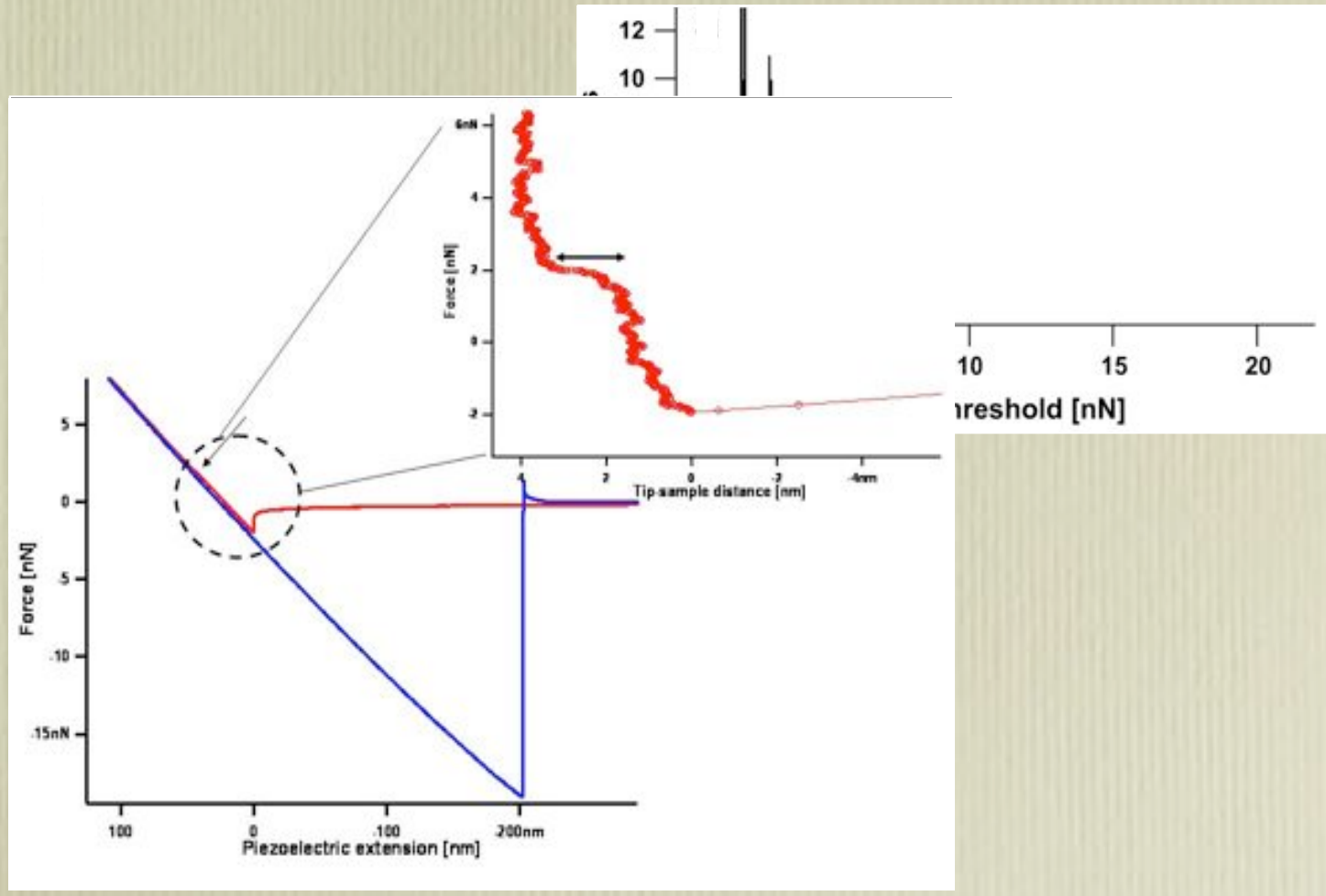




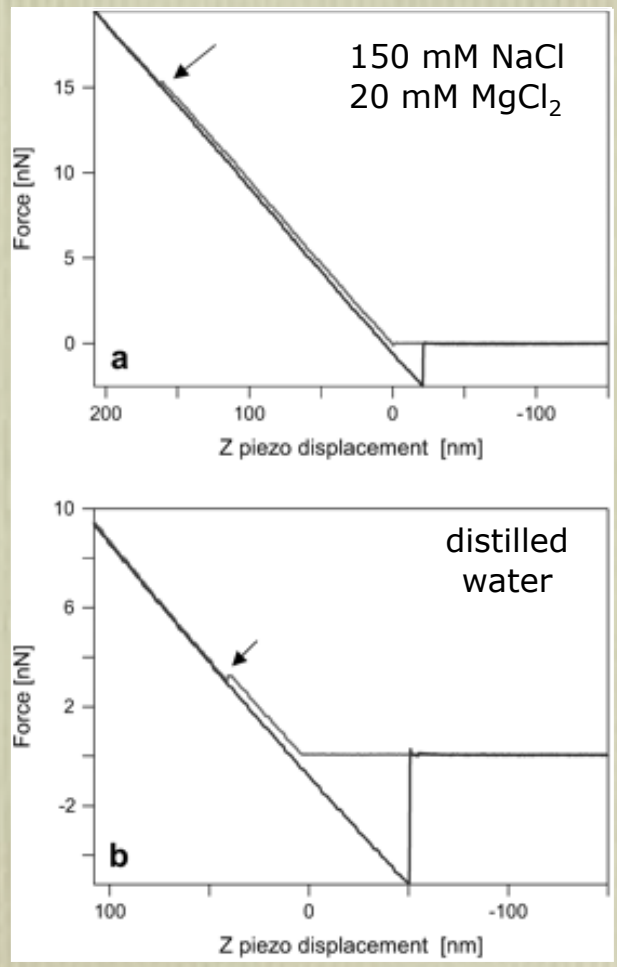
Young modulus

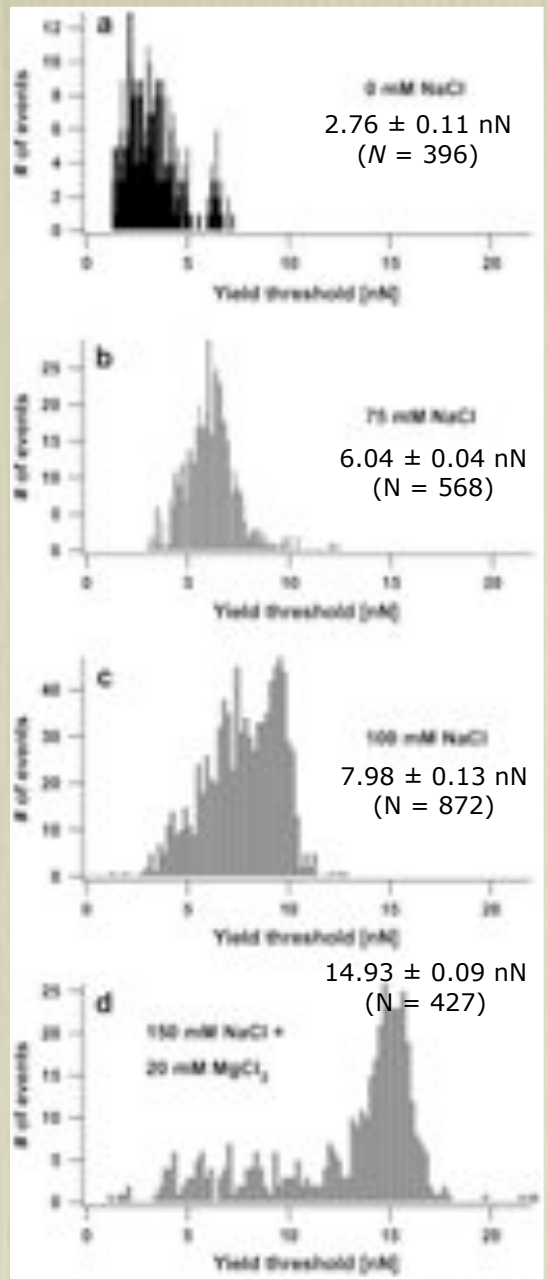
Depend on:
- the shape of the tip
- the model used to fit the data
- the velocity of the approach

Force curves on planar bilayers
Towards nanomechanics of biomembranes



Force versus *z*-piezo displacement plot for a DMPC bilayer

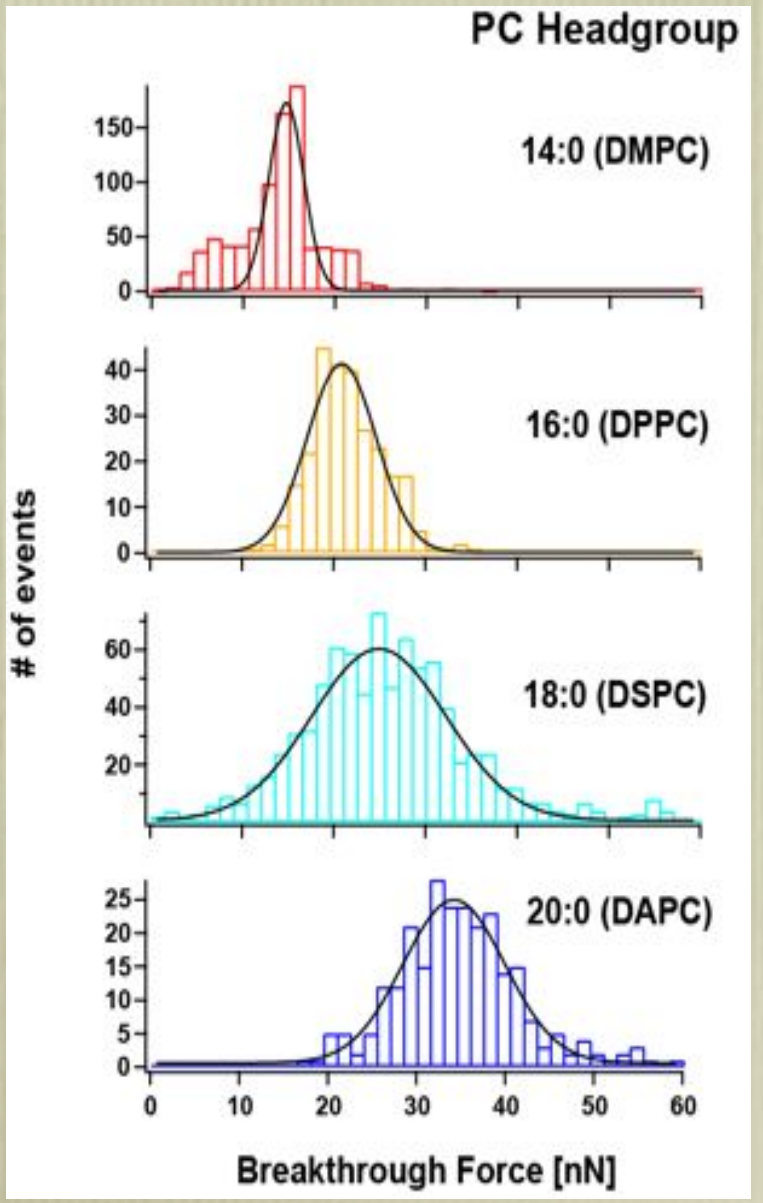


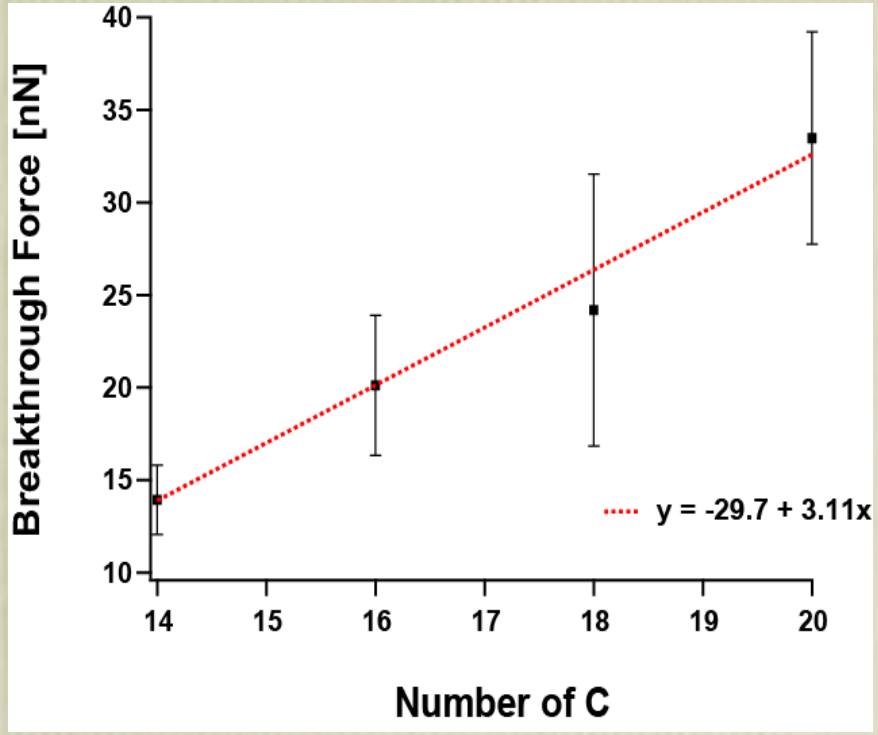


Yield threshold is denoted by black arrows at 15 nN (*a*) and 2.1 nN (*b*). The width of the jump, 4.5 nm, corresponds well with the bilayer height measured using contact mode AFM.

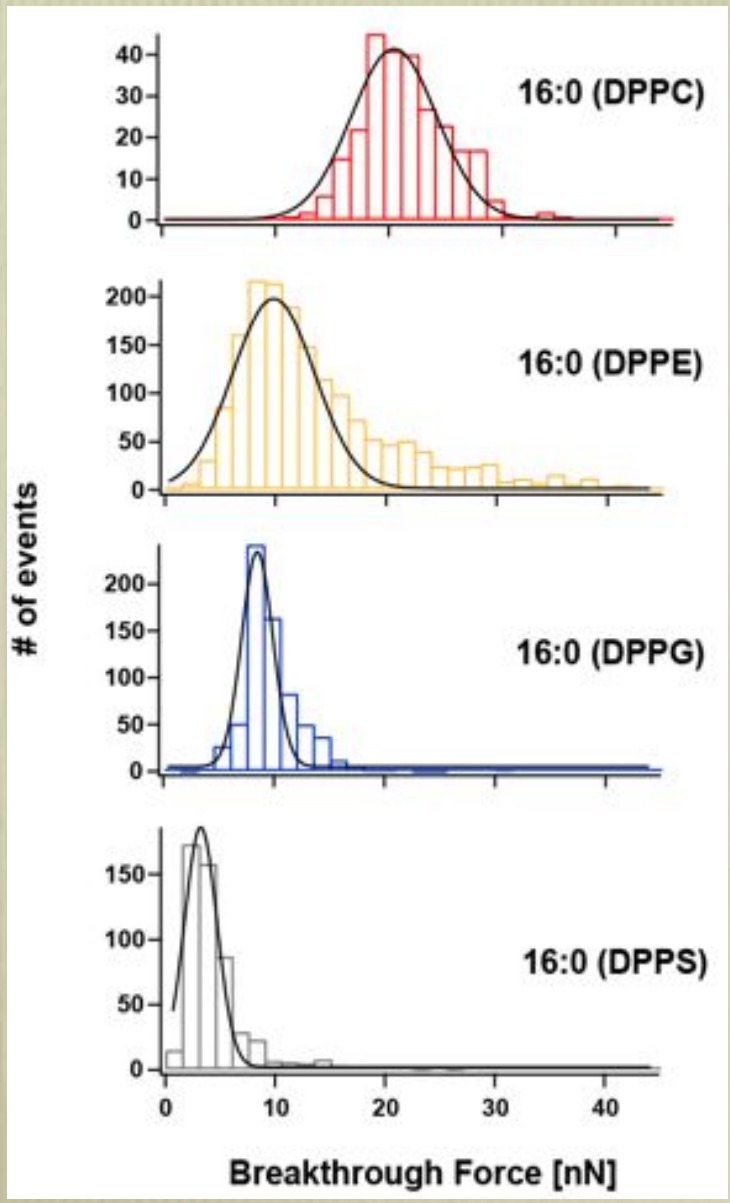
Garcia-Manyes, Sergi; Oncins, Gerard; Sanz, Fausto. *Biophysical J.* 89, (2005), 4261-4274.

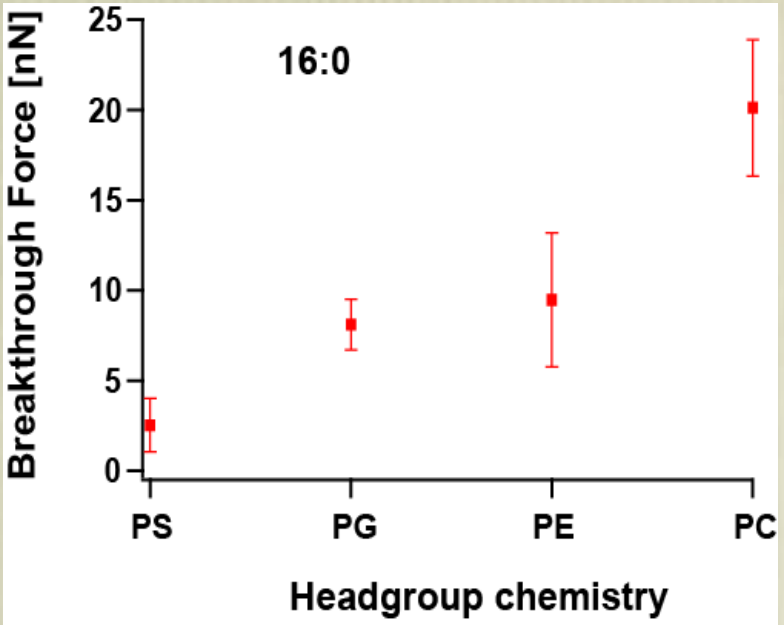
Effect of the tail length





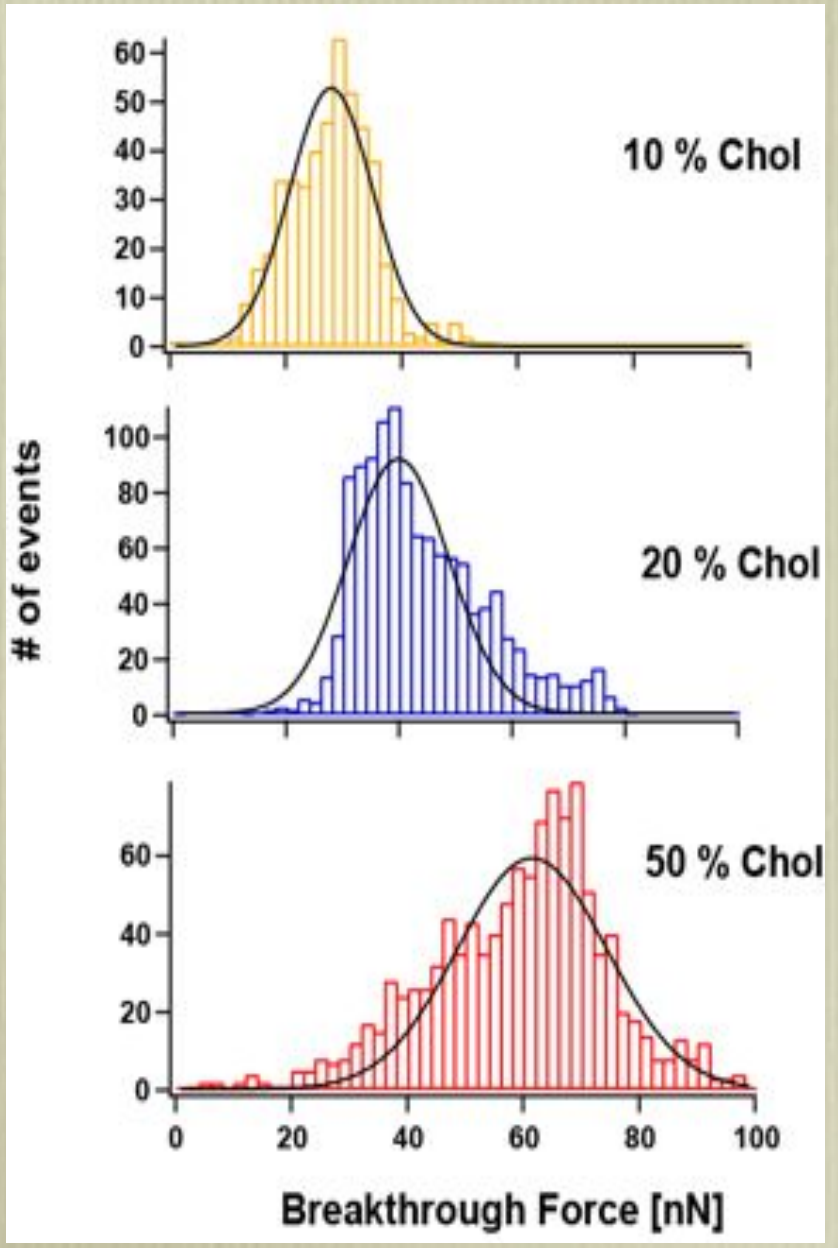
Effect of the headgroup

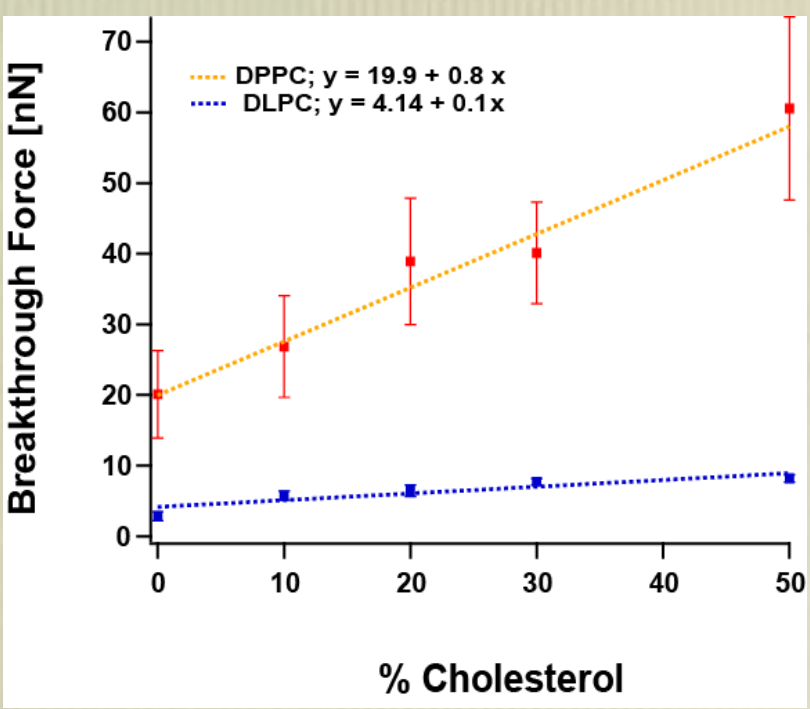




Different headgroups can give rise up to ~ 15 nN increase in force!!!

Effect of cholesterol





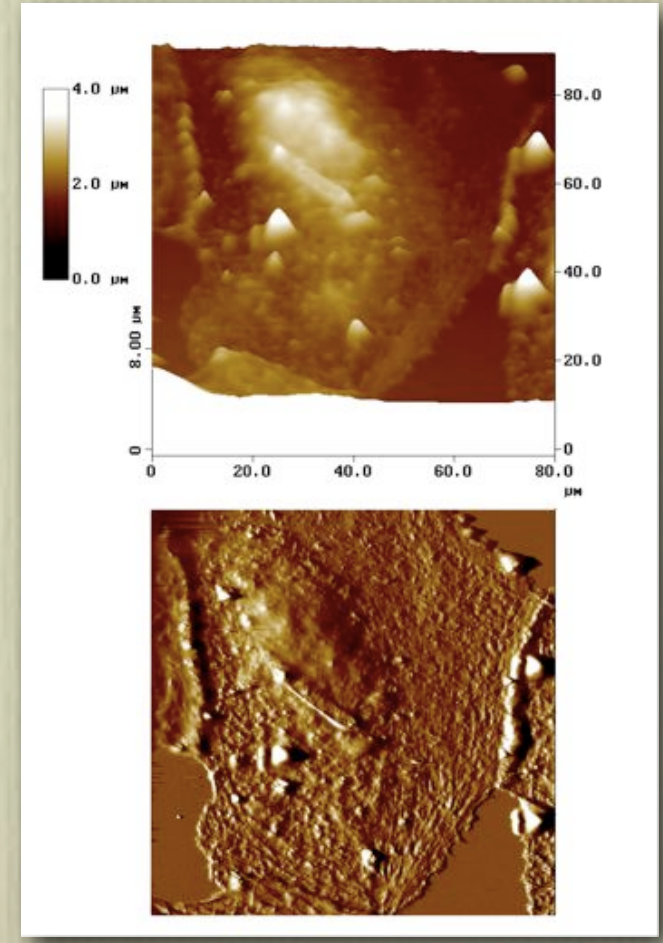
Different phases have different nanomechanical properties

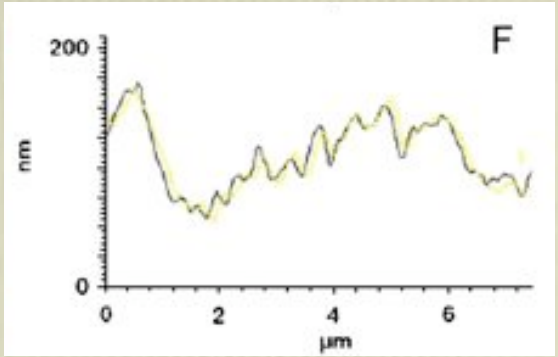
Outlines

- Introduction to biological membranes
- How to mimic biological membranes
- Imaging of artificial supported lipid bilayers
  - Force spectroscopy
- **Imaging of biological membranes**
- Main drawbacks and developments

AFM imaging of biological membranes

Living cells





Membrane stiffness ≤ 1 mN/m

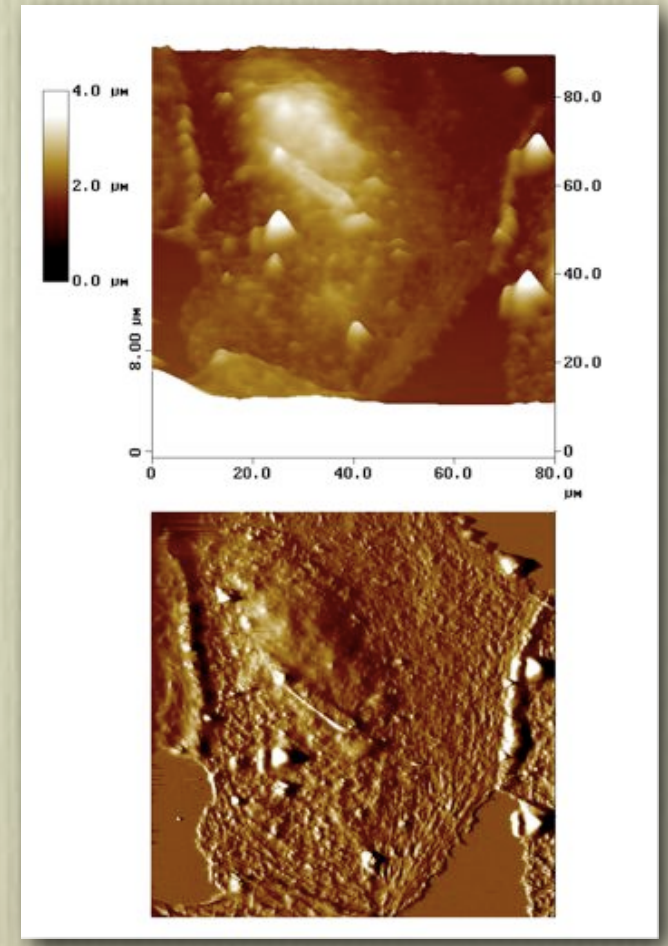
Superimposed Trace and Retrace

CV-1 cells
80 µm scan
contact mode

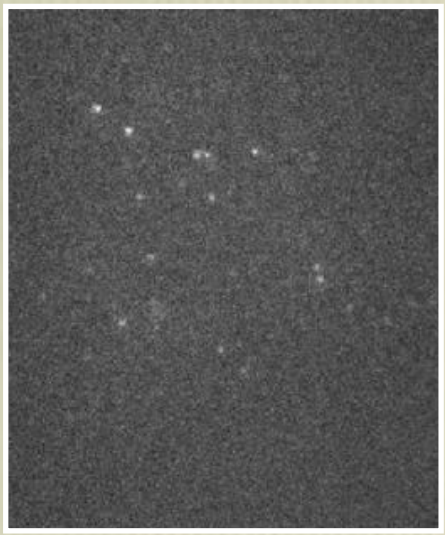
Giocondi et al (2003) Med/Sci

AFM imaging of biological membranes

Living cells



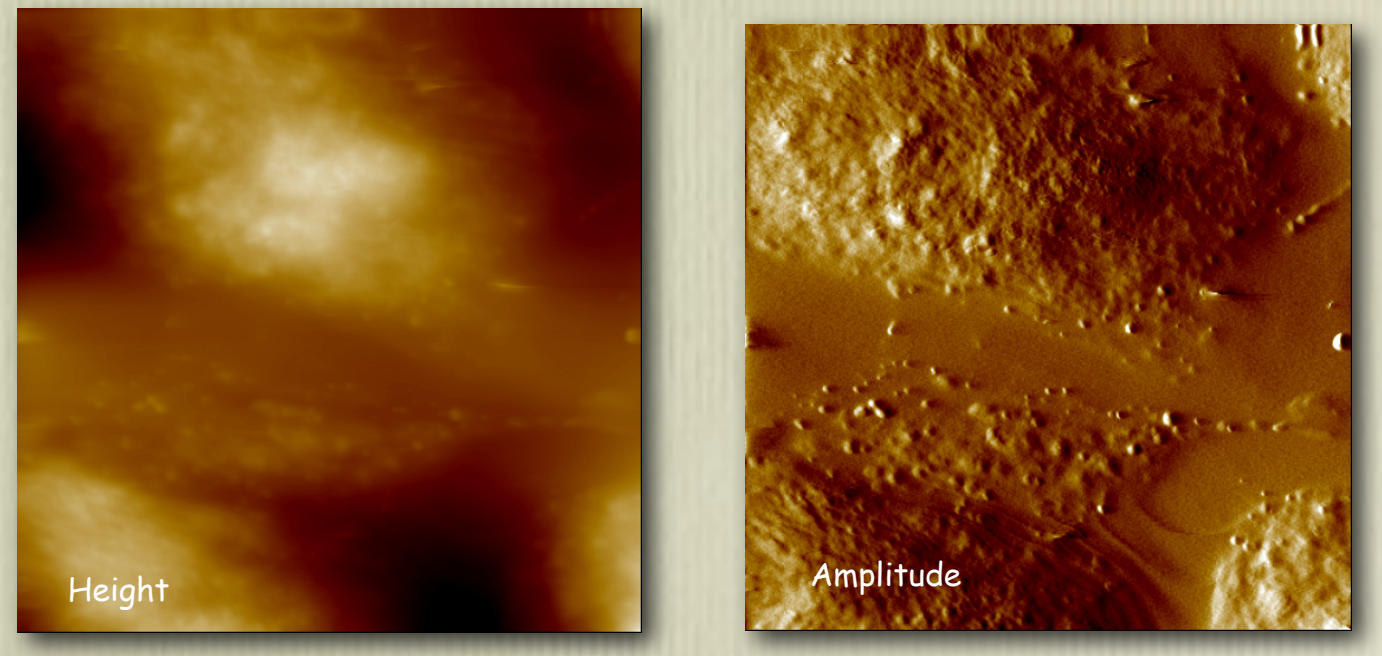
CV-1 cells
80 µm scan
contact mode



Lateral diffusion of membrane
Components (~0.01-10 µm2/sec)

AFM imaging of biological membranes

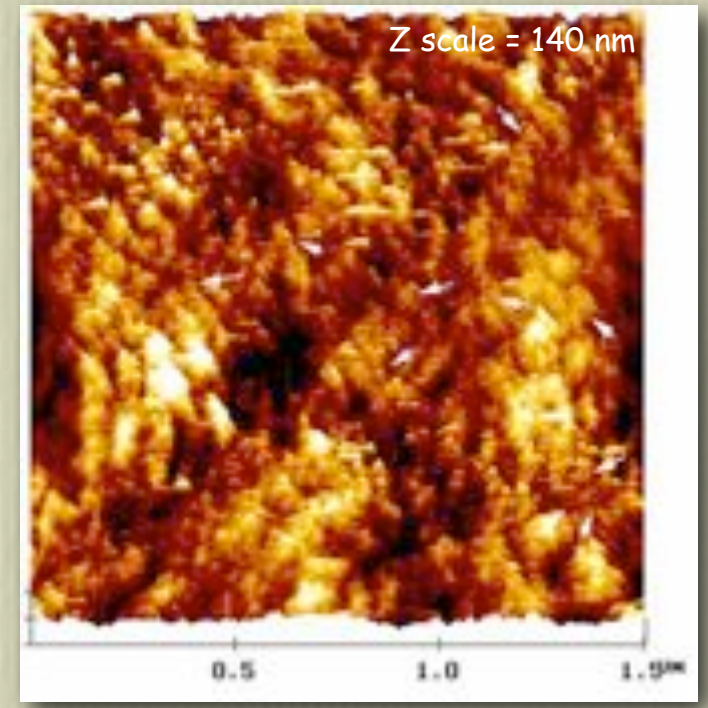
Fixed intact cells



CHO cells
40x40 μm scan
Tapping

AFM imaging of biological membranes

Cochlear outer hair cells



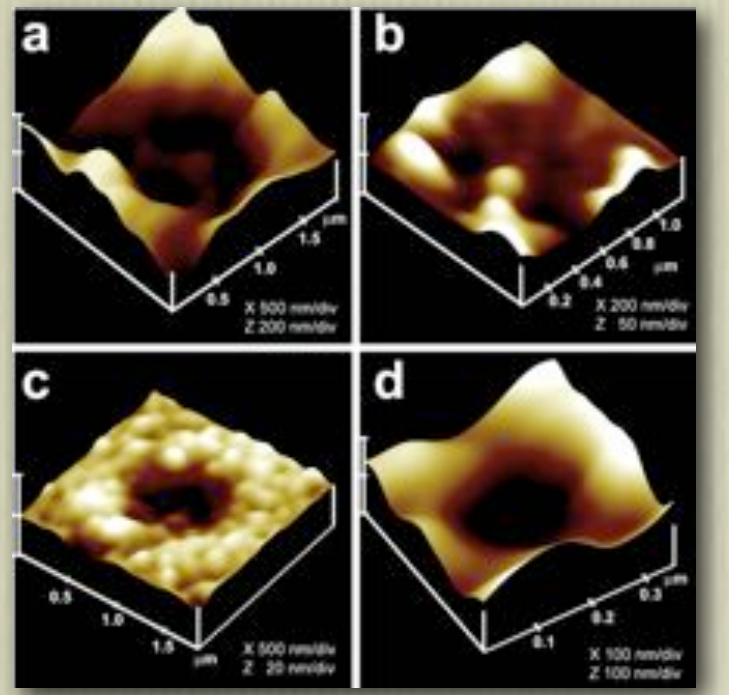
Le Grimellec et al., 2002 J. Comp. Neurol.

Outlines

- Introduction to biological membranes
- How to mimic biological membranes
- Imaging of artificial supported lipid bilayers
  - Force spectroscopy
- Imaging of biological membranes
- **Main drawbacks and developments**

Recognition of Membrane Components

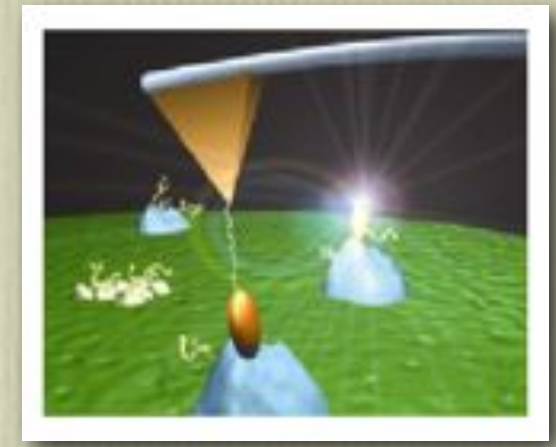
Pancreatic cells



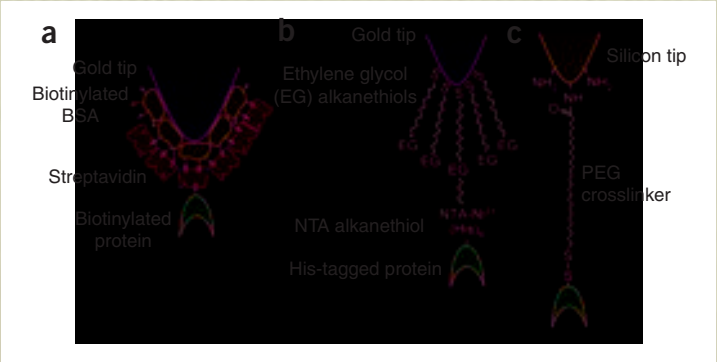
AFM and immunoAFM micrographs of the fusion pore
Biophysical J. 2003, 84: 1337-1343].

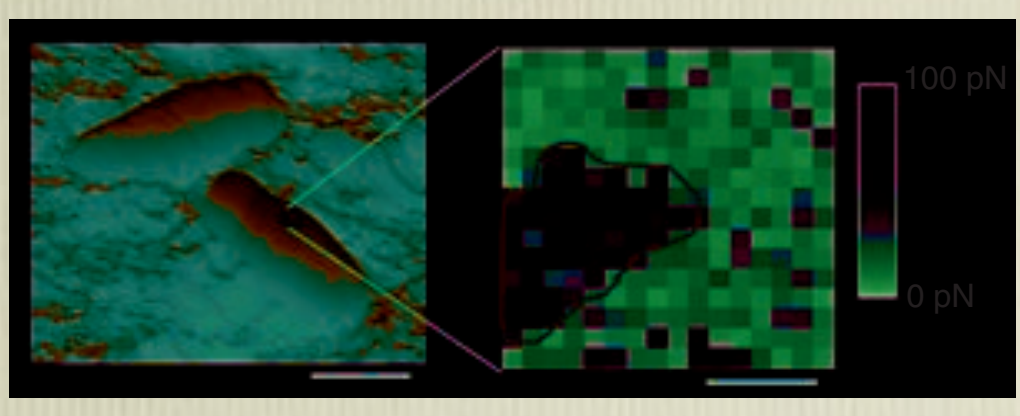
Beads

Recognition of Membrane Components



Recognition imaging

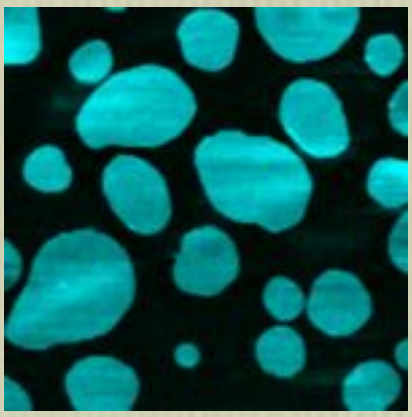


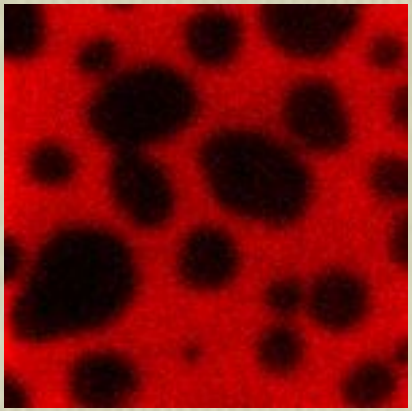


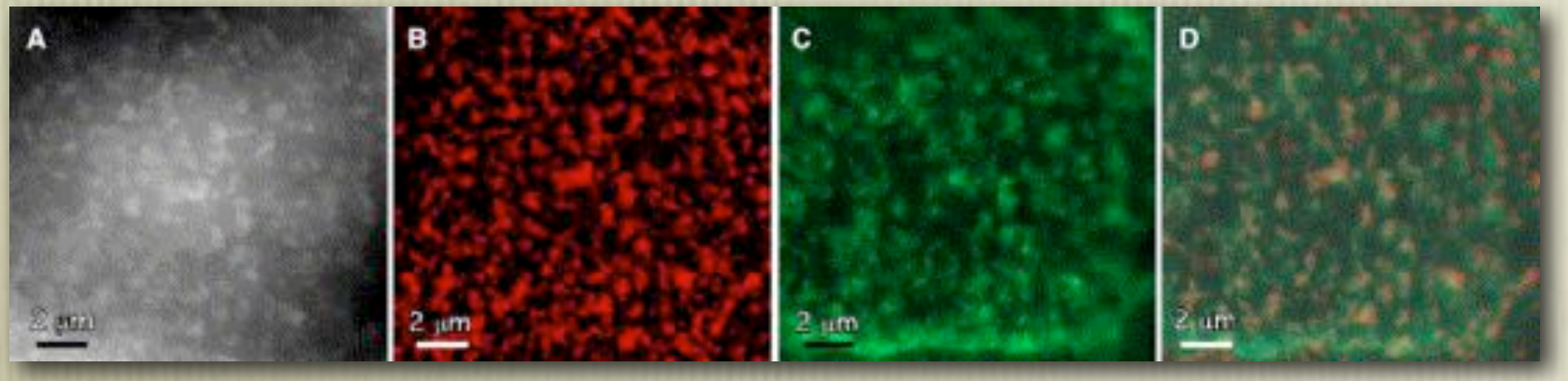
Hinterdorfer and Dufrene, Nature Methods (2006), 3, 347-355

Recognition of Membrane Components

Coupling Fluorescence and AFM





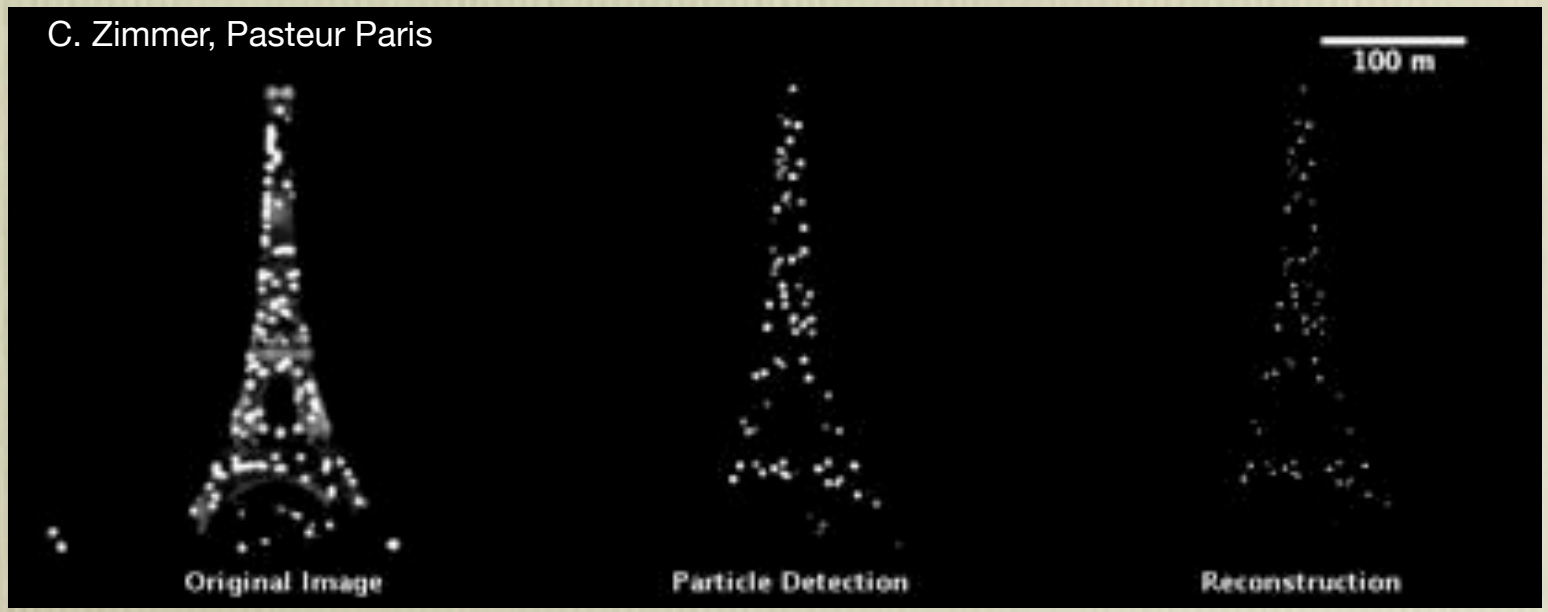


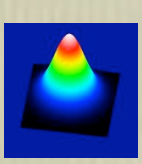
ChemPhysChem 7: 2409-2418 (2006); FEBS Lett. 2004 May 7;565(1-3):53-8

Recognition of Membrane Components

Coupling Fluorescence and AFM

Super resolution microscopy



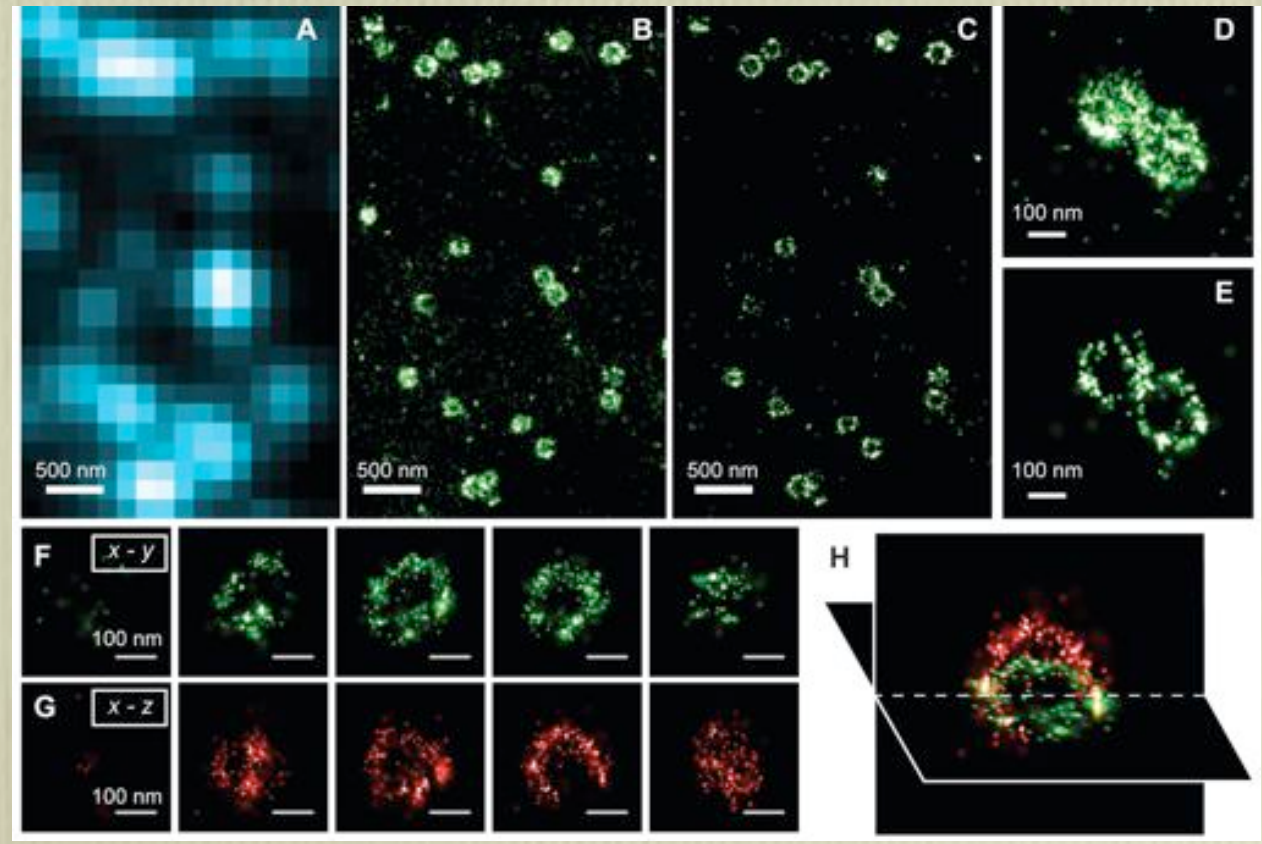


Super-resolution fluorescence technique
(PALM, STORM, STED, 3D structured illumination,...)

Science. 2008 February 8; 319(5864): 810–813.

Recognition of Membrane Components

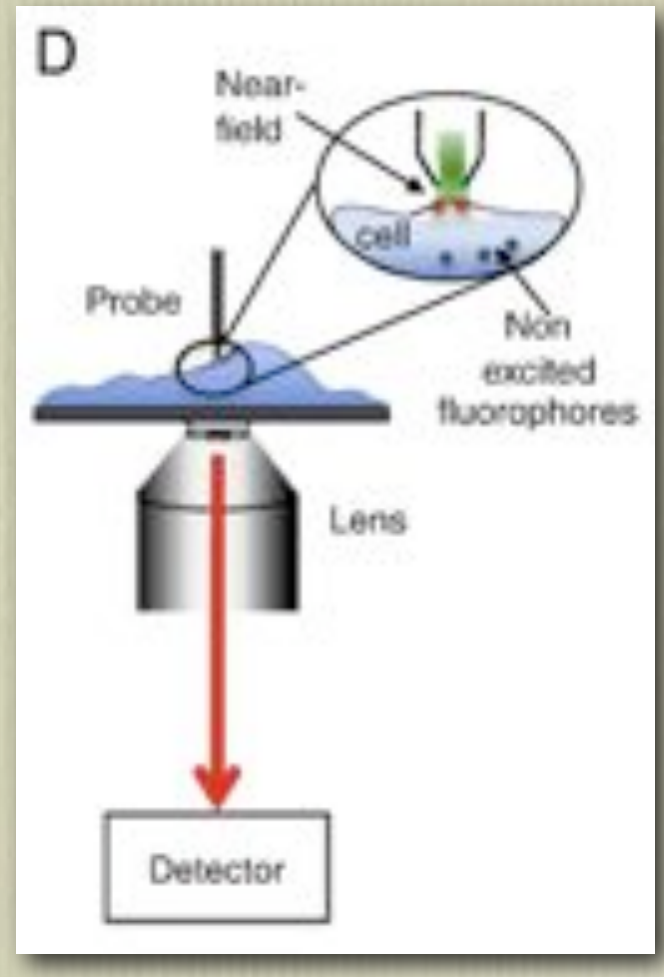
3D-STORM, PALM, STED,
Structured illumination...



AFM and Fluorescence Microscopies resolution in the same range

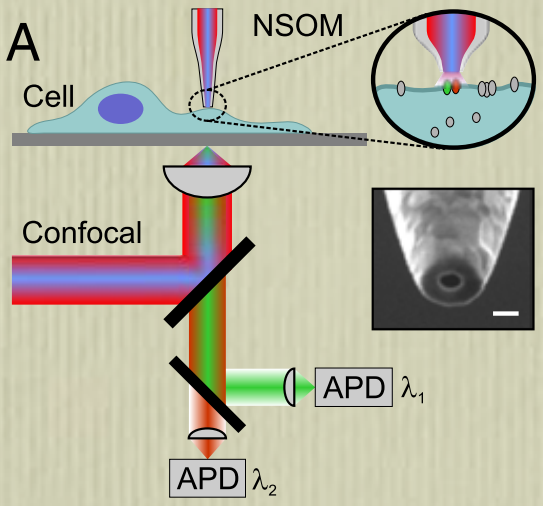
Science. 2008 February 8; 319(5864): 810–813.

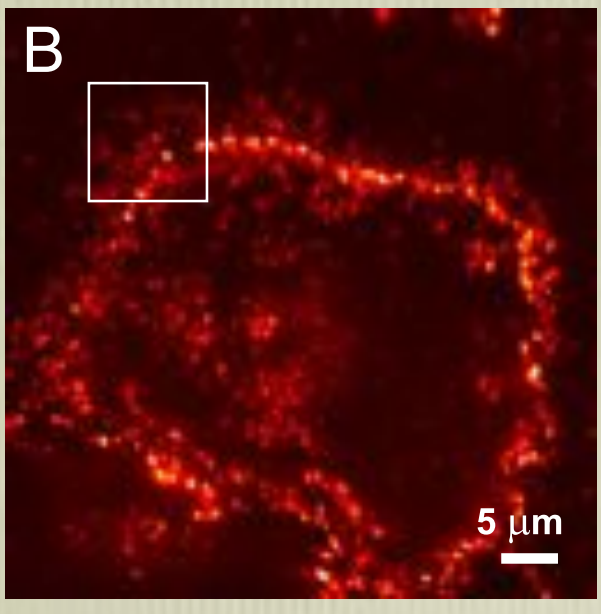
Scanning Near-Field Optical Microscopy
SNOM

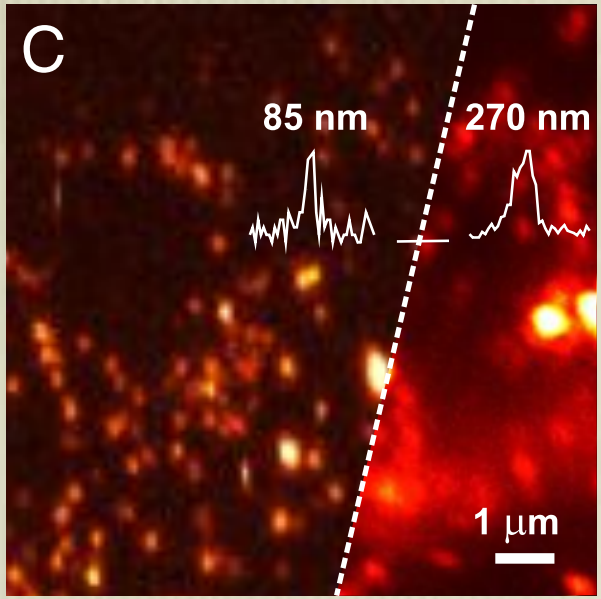


Biochimica et Biophysica Acta (BBA) - Biomembranes
Volume 1798, Issue 4, April 2010, Pages 777-787

Scanning Near-Field Optical Microscopy
SNOM

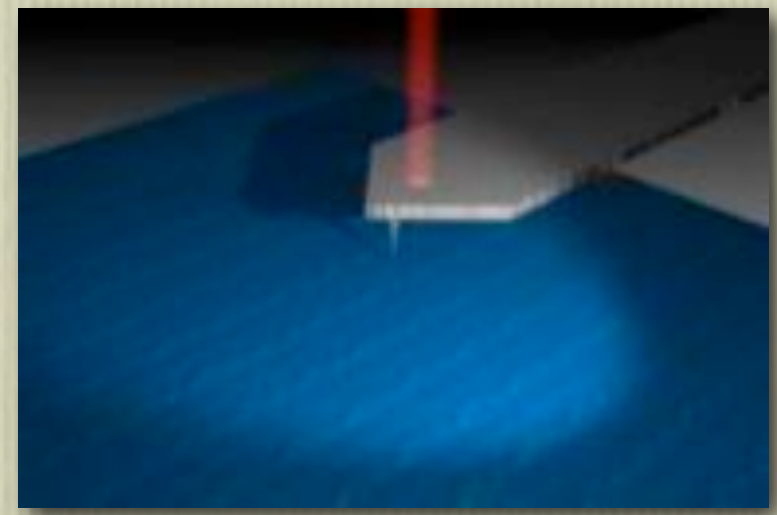


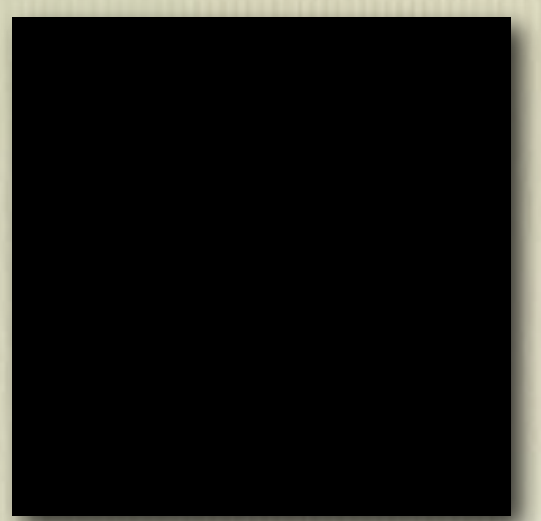




Zanten et al, PNAS (2009) 106, 18557–18562

Atomic Force Microscopy

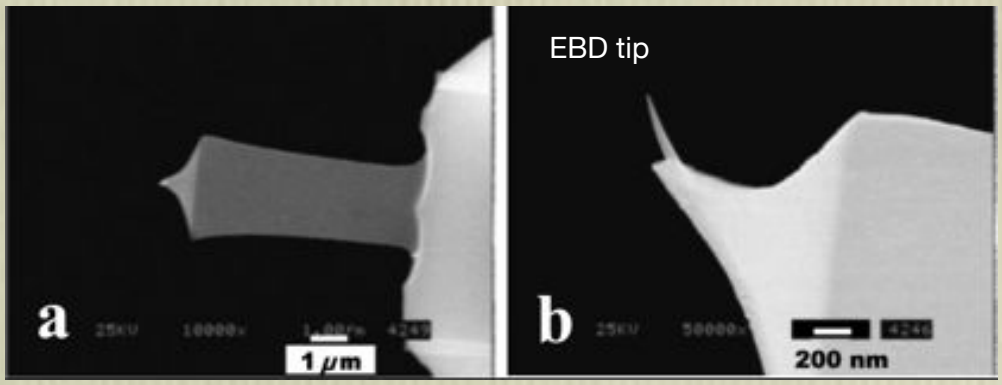




Scan rate < 6Hz (contact), 85 s/image
" < 2 Hz (tapping), 4.2 min/image

High-speed AFM

Cantilevers with high resonant frequency and low spring constant

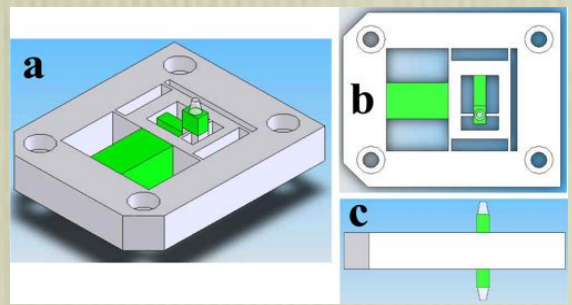


Resonance Frequency
Water: 1.2 MHz

Spring Constant: 200 pN/nm

L=7 μm, w=2 μm, thickness=90 nm

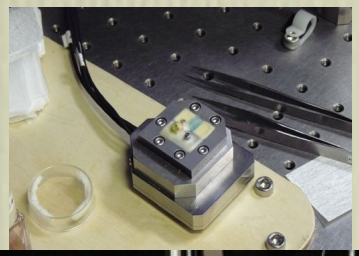
High scanning rate scanner

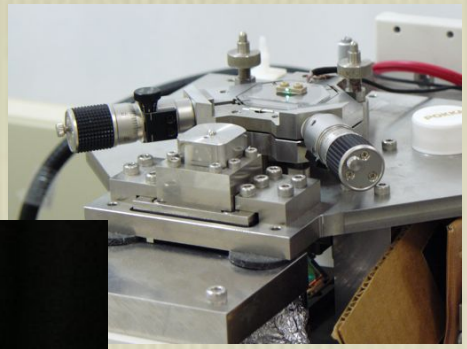


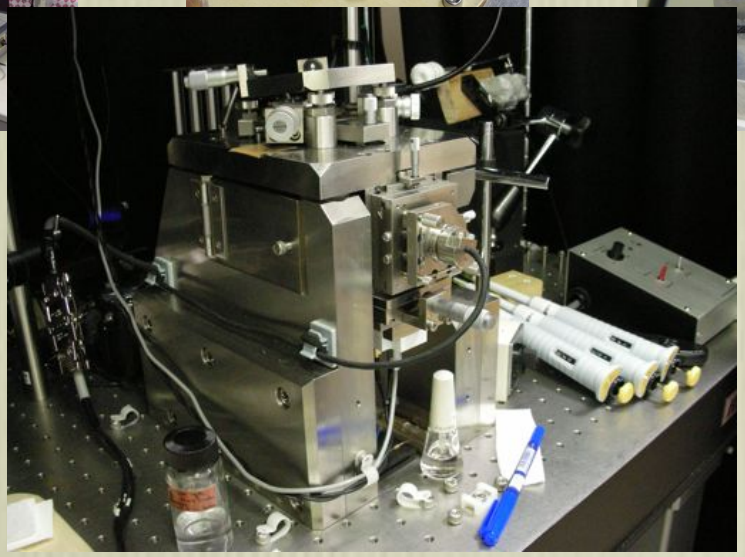
120 kHz in Z, 4 kHz in X&Y

Highly efficient feedback to minimize the tip-sample interaction force

High-speed AFM prototype







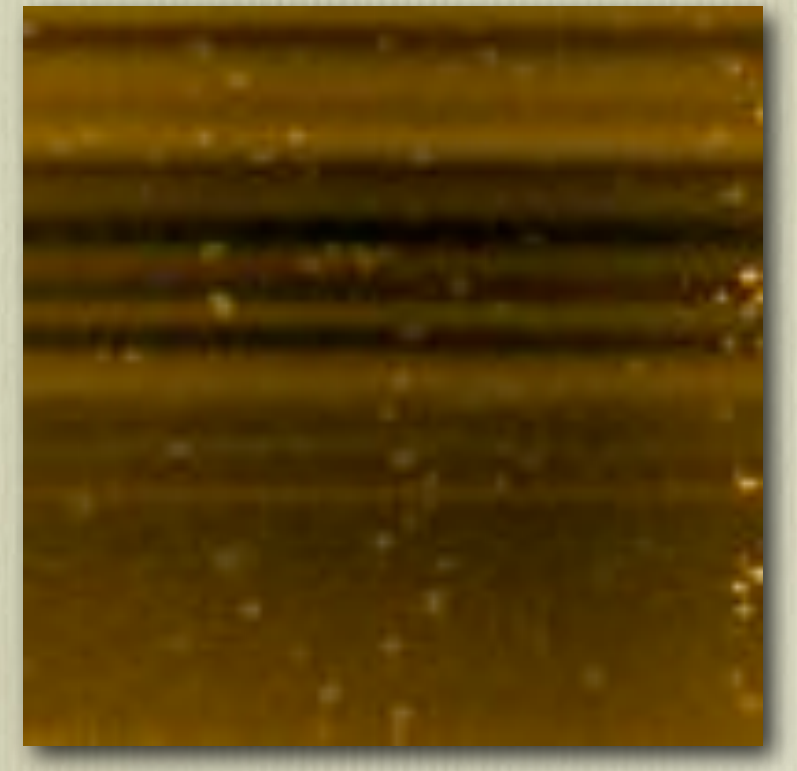
1 µm scan at video rate
Sample stage: 1.5 mm

High-speed AFM with SLBs

DOPC/DOPE/DPPE SLB

800 nm x 800 nm

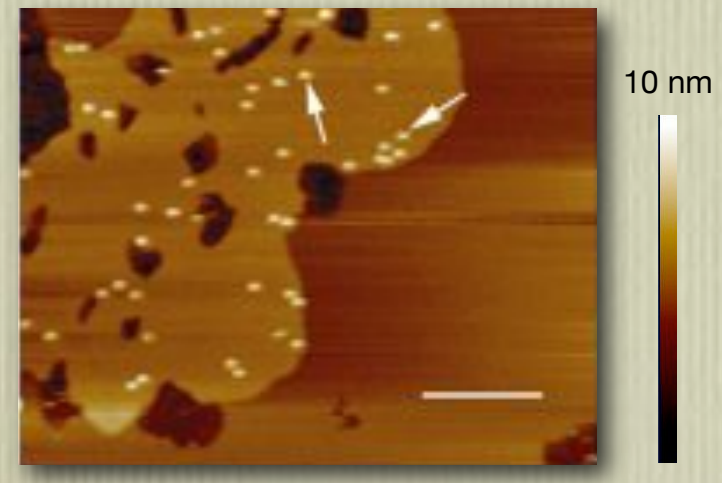
1 image/s - z color scale:75 nm

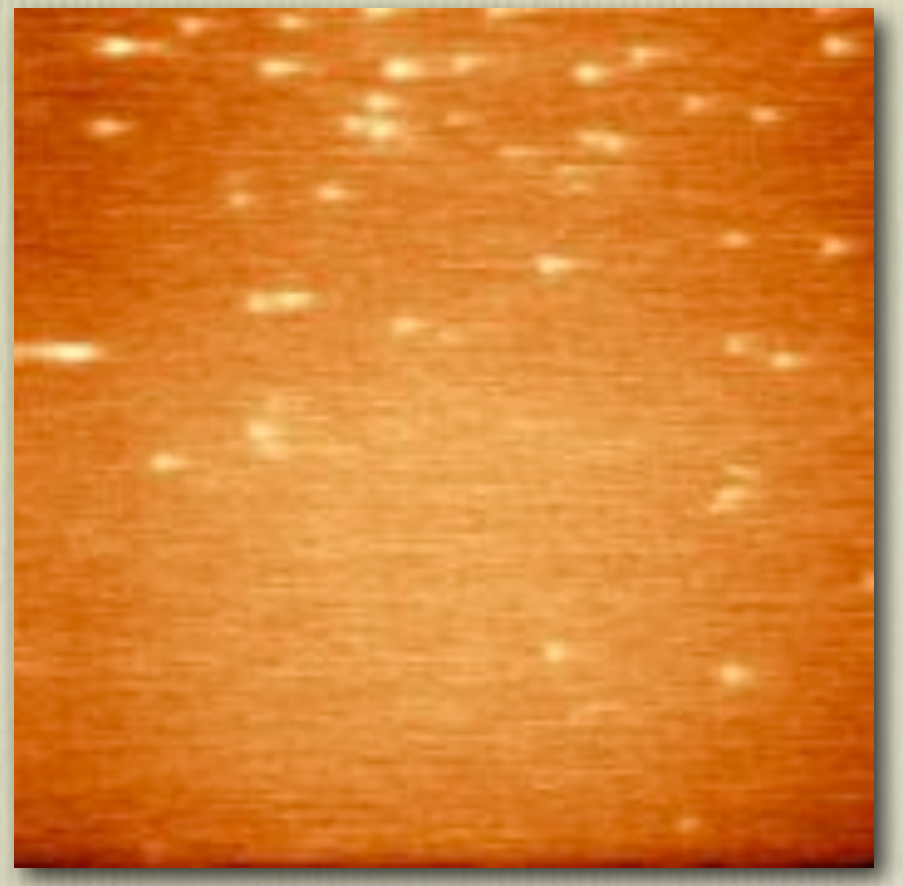


BBA Biomembr (2009) 1798(4):703-18

Membrane Partition of Lipids within Biological Membranes

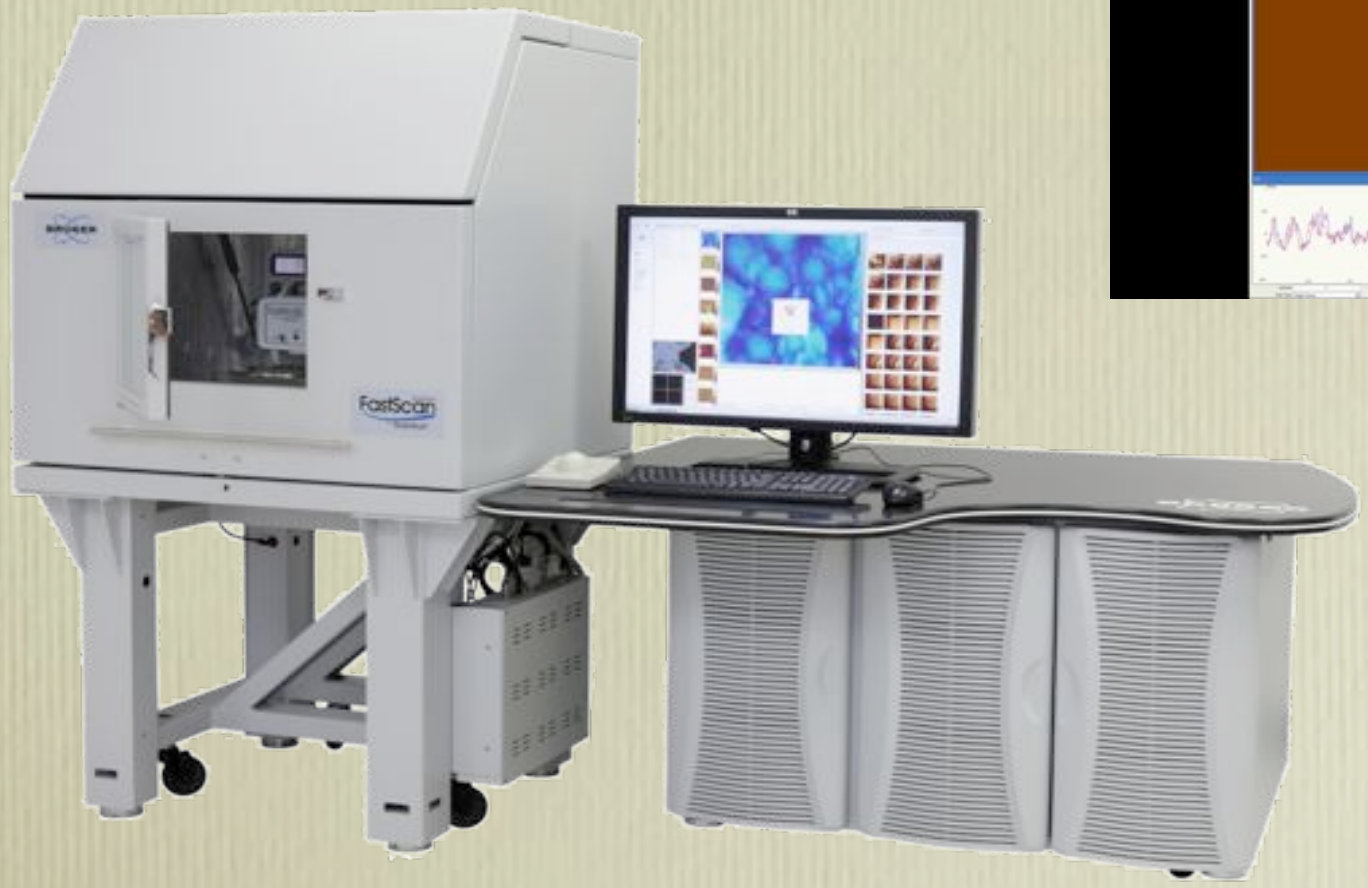
DOPC/DPPC/GM1

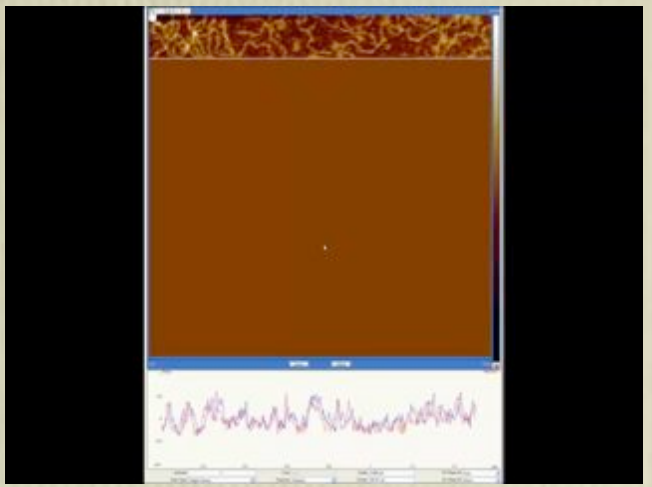




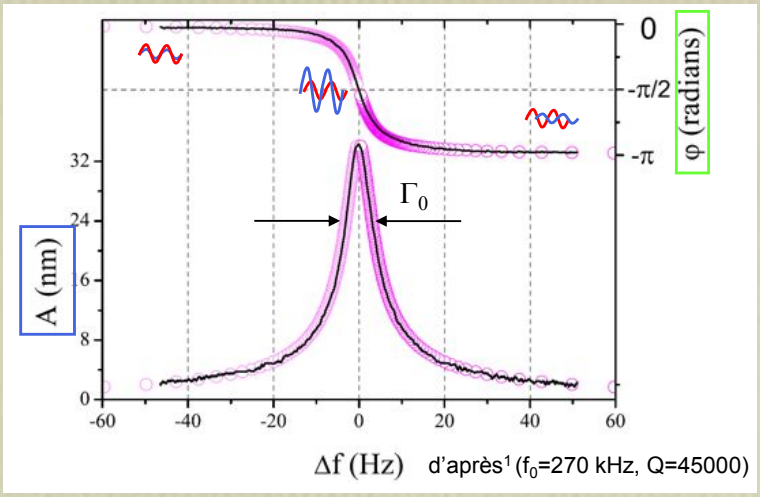
DPPC/GM1

Dimension FastScan™ Atomic Force Microscope





Non contact AFM - Frequency Modulation AFM

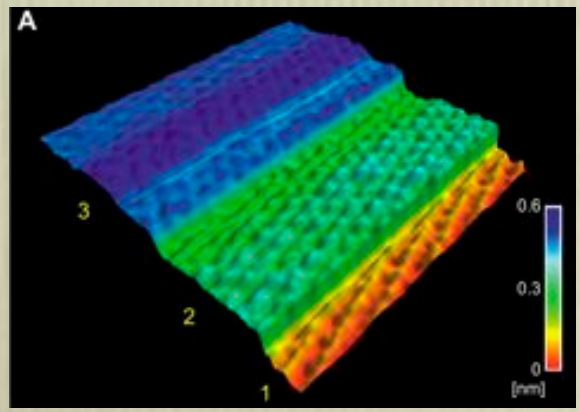


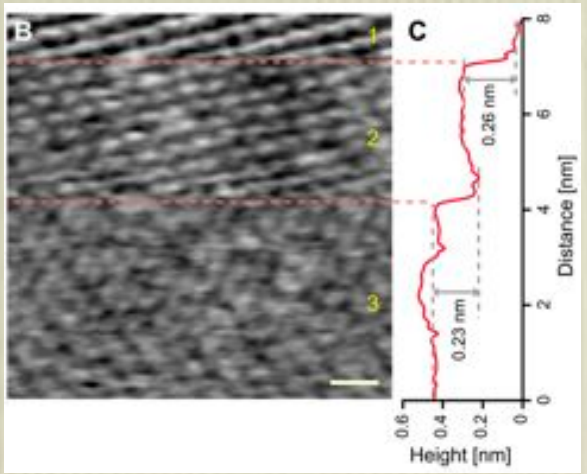
d'après[1] (f_0=270 kHz, Q=45000)

Biophysical Journal Volume 92 May 2007 3603–3609

Direct Imaging of Individual Intrinsic Hydration Layers on Lipid Bilayers at Ångstrom Resolution

Takeshi Fukuma, Michael J. Higgins, and Suzanne P. Jarvis
Centre for Research on Adaptive Nanostructures and Nanodevices, Trinity College Dublin, Dublin 2, Ireland





Postdoc Position in AFM Imaging of Membrane-Nanoparticle Interaction

A 24 month postdoctoral position is available in Montpellier to study the interaction between biological membranes and peptide-based nanoparticles used as drug or biosensor carriers. The project aims to characterize the shape and mechanical properties of the nanoparticles as well as their interaction with mica-supported artificial membranes. Dynamics of the interaction will be probed with high-speed AFM available in the Single Molecule Biophysics group. Applicants are expected to have a training and research experience in physical chemistry, artificial membrane fabrication and AFM. Some expertise in electron microscopy is also welcome.

The position is funded by the ANR (French Science Fundation) and the project will be conducted in tight collaboration with biologists.

Please contact Pierre-Emmanuel Milhiet (pem@cbs.cnrs.fr) and visit our website for further details about the “single Molecule Biophysics” group: http://www.cbs.cnrs.fr/rubrique.php3?id_rubrique=127.