



qbio
quantitative
biology

QBIO MASTER PROGRAM

quantitative biology in practice

$$\frac{du}{dt} = \frac{\alpha_1}{1 + v^\beta} - u$$

MEMBRANE BIOPHYSICS

Pierre-Emmanuel Milhiet, Luca Costa, Manouk Abkarian

OCTOBER 7, 2022

INTRODUCTION TO MEMBRANE BIOPHYSICS



I - Introduction (PE Milhiet)

Self-assembling

Principles of Membrane Organization

II - Physics of Membranes - A theoretical point of view (M Abkarian)

- Membrane deformations
- Bending energy
- Equilibrium shapes
- Membrane fluctuation

III - Experimental characterization of biological membranes (L Costa & PE Milhiet)

A - Model membranes versus native membranes

B - How to manipulate membrane components (detergents, SMALP, nanodiscs...)

C - Mimicking biological membranes

D - Imaging and spectroscopy

- Spectroscopy and Microscopy (SMLM, FFS, NSNOM, EPR, SAXS/SANS, Raman, FTIR,
Cryo-EM, NMR, X-ray crystallography)
- Computational characterization

E - Probing membrane mechanics (L Costa)

- Methodologies
- AFM and cell mechanics

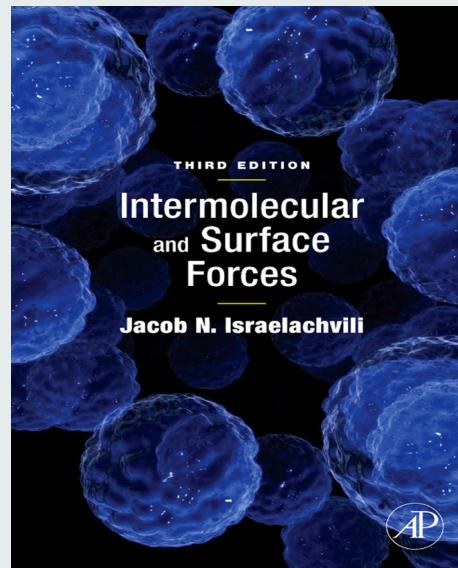
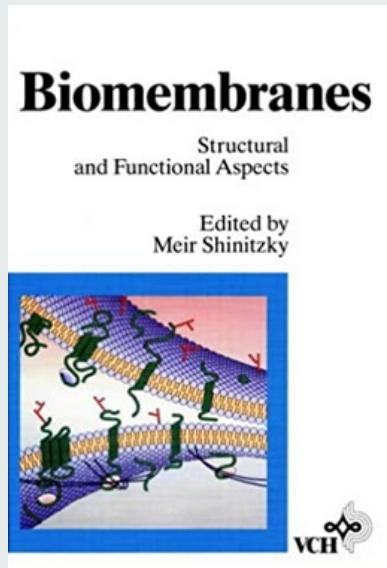
INTRODUCTION TO MEMBRANE BIOPHYSICS



Membrane biophysics is the study of the physical principles governing biological membranes, including microdomain formation and protein–lipid coupling, as well as their mechanical characteristics, and the effect they have on paracellular transport and phenomena relating to cell shape.

Membrane biophysics is the study of biological membrane structure and function using physical, computational, mathematical, and biophysical methods. A combination of these methods can be used to create phase diagrams of different types of membranes. As opposed to membrane biology, membrane biophysics focuses on quantitative information and modeling of various membrane phenomena, such as microdomain formation, rates of lipid and cholesterol flip-flop, protein-lipid coupling, and the effect of bending and elasticity functions of membranes on inter-cell connections. *Wikipedia*

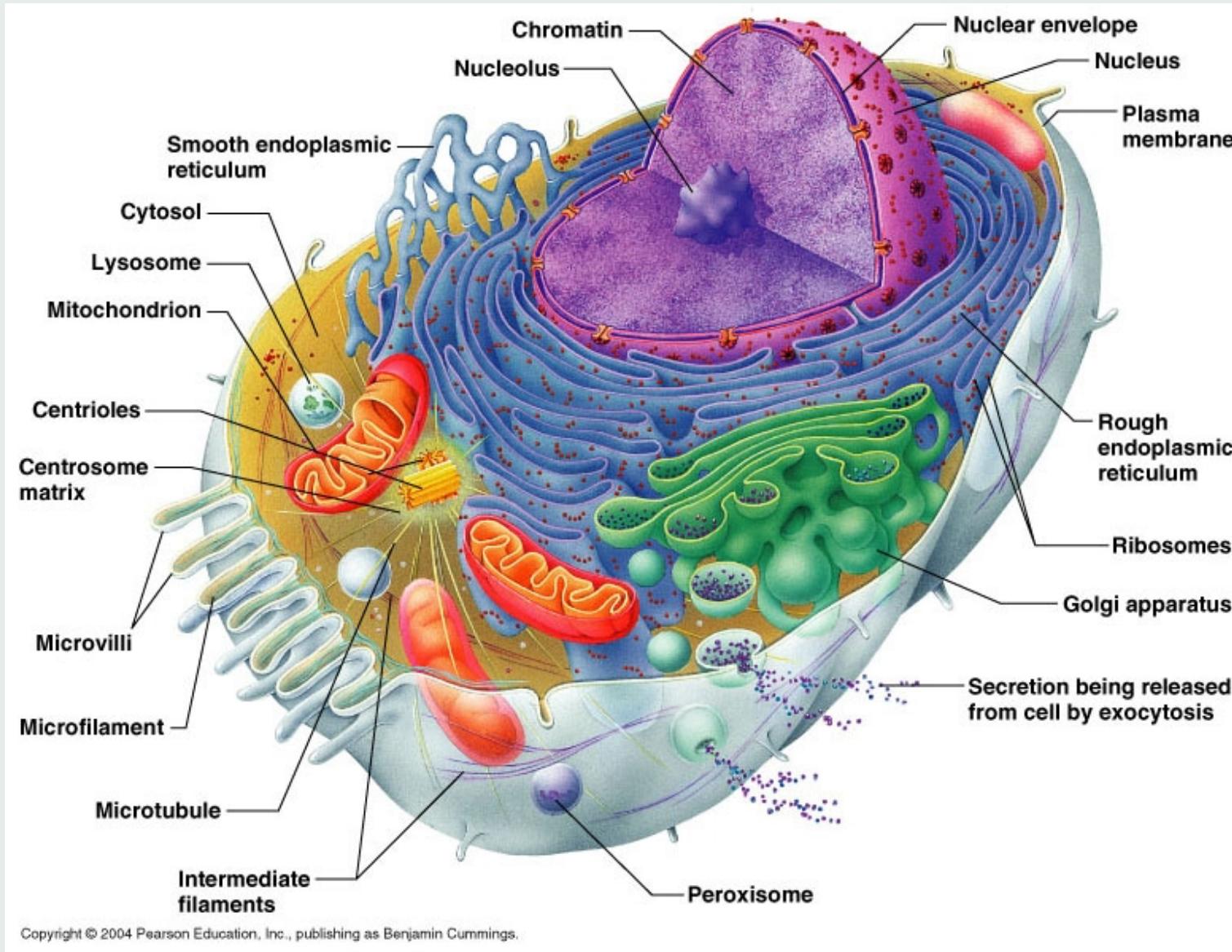
Bibliography



Richard P.
Feynman
(1918 – 1988)

“... If, in some cataclysm, all of scientific knowledge were to be destroyed, and only one sentence passed on to the next generations of creatures, what statement would contain the most information in the fewest words? I believe it is the atomic hypothesis (or the atomic fact, or whatever you wish to call it) that all things are made of atoms—little particles that move around in perpetual motion, attracting each other when they are a little distance apart, but repelling upon being squeezed into one another...”

INTRODUCTION TO MEMBRANE BIOPHYSICS



INTRODUCTION TO MEMBRANE BIOPHYSICS



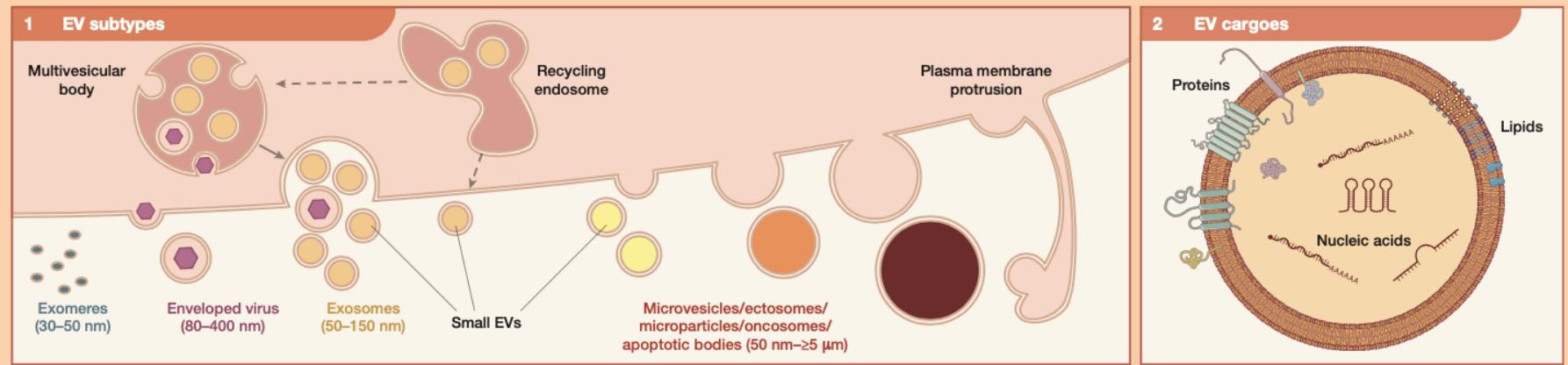
SnapShot: Extracellular Vesicles

Federico Cocozza,^{1,2,3} Eleonora Grisard,^{1,3} Lorena Martin-Jaular,^{1,3} Mathilde Mathieu,^{1,2,3} and Clotilde Théry^{1,3}

¹Institut Curie, INSERM U932, PSL Université, 26 rue d'Ulm, Paris 75005, France

²Université de Paris, 85 Bd St germain, Paris 75006, France

³These authors contributed equally

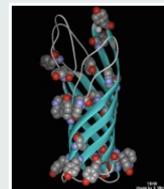
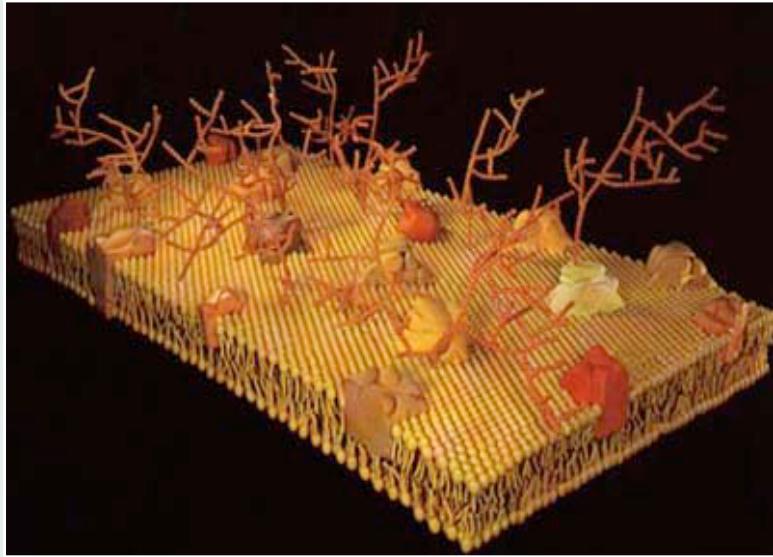


Functions of Biological Membranes



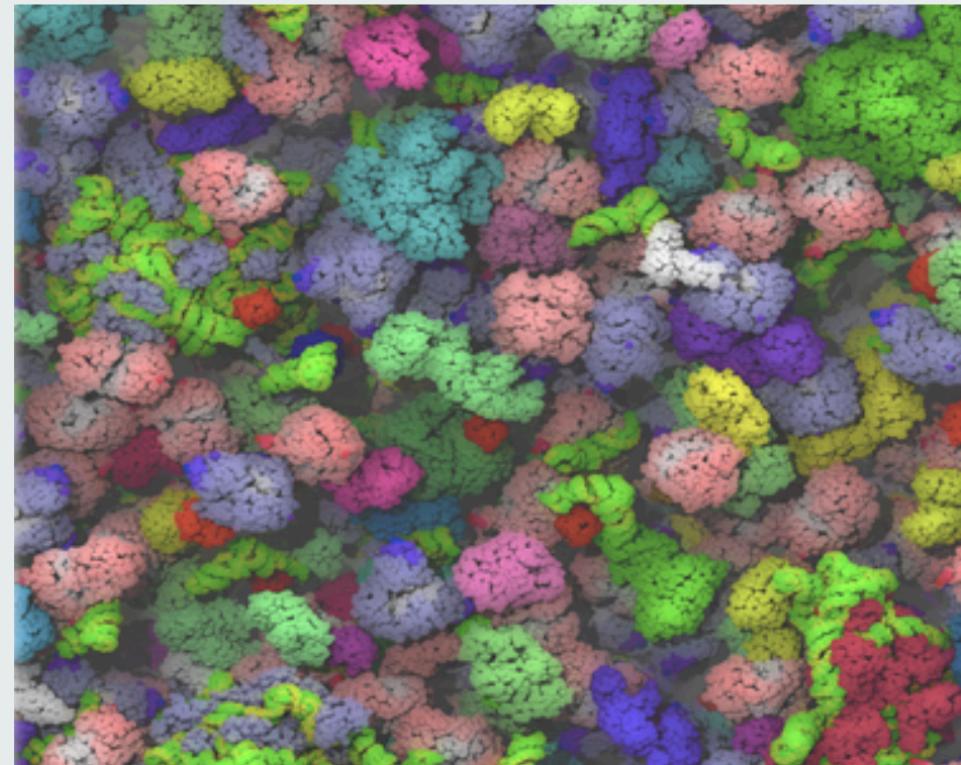
- fences, « gatekeepers » (ex: the nucleus protects chromatin from cell metabolites)
 - => restrict diffusion of large molecules (nutrients, proteins) charges (ions)
 - => specific transport, regulate sublocalization of components within the cell.
 - => restrict certain functions in specific locations (ex: degradation) => regulatory function
 - => create gradients (protons, RanGTP), used as energy source - signal transduction (through transmembrane proteins)

Composition & Organization of Biological Membranes



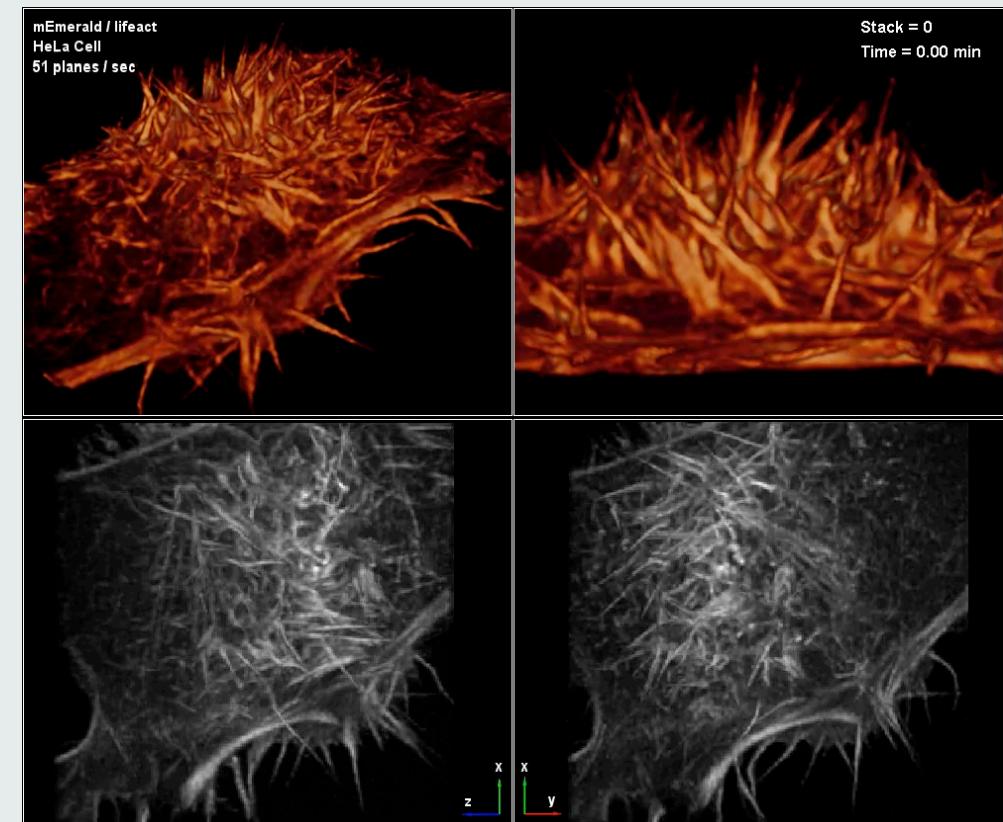
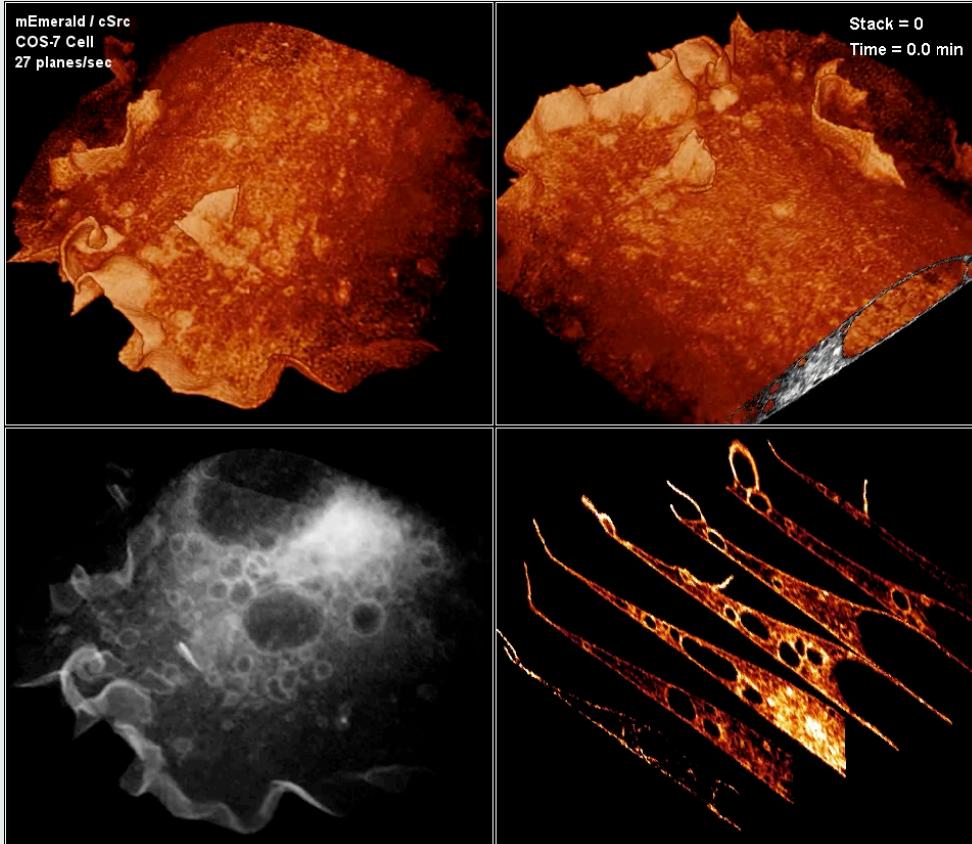
~ 1 protein per 50-100 lipids

Lipid/protein ratio (in mass) ~ 1



Iippo Vattulainen, Helsinki & Tempere Univ of Technology

Biological membranes are dynamic



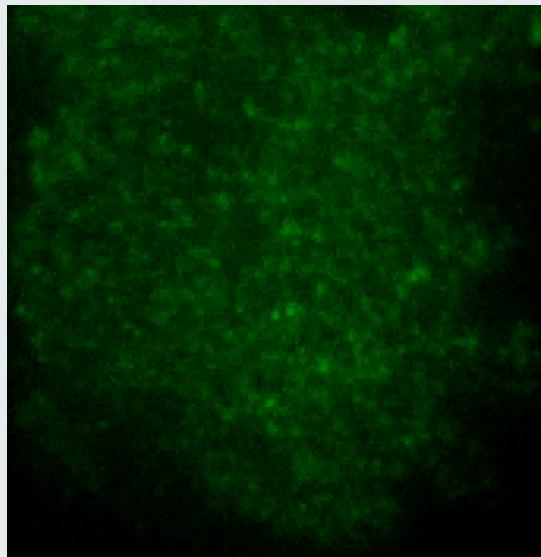
Biological membranes are dynamic



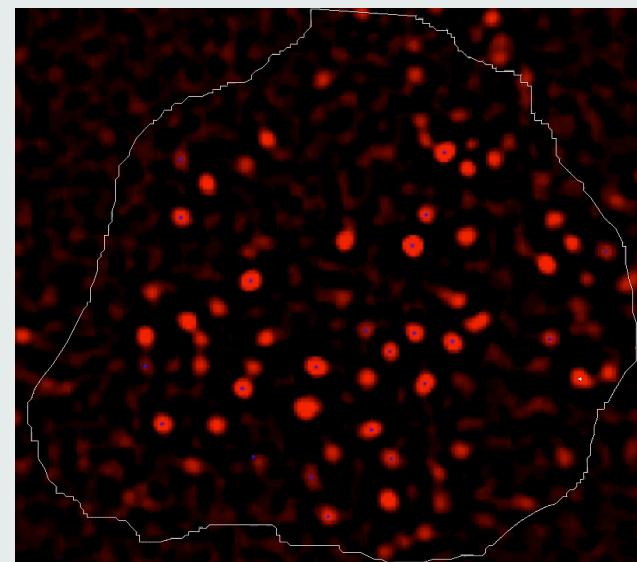
Lateral Diffusion

D lipid in model membrane: $D \sim 1 \text{ à } 10 \mu\text{m}^2/\text{s}$

D lipid in plasma membrane: $D \sim 0.1 \text{ à } 1 \mu\text{m}^2/\text{s}$

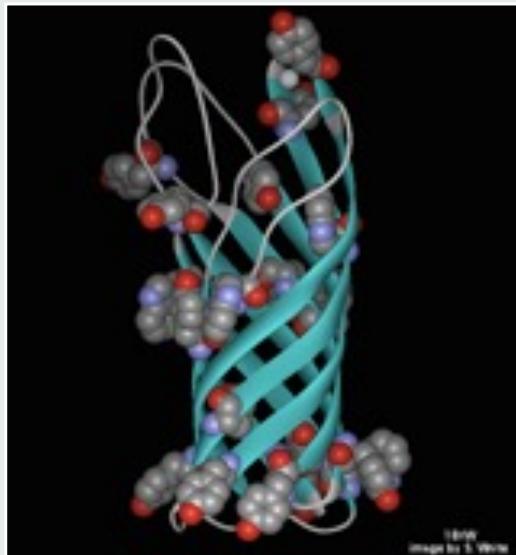


Ganglioside GM1

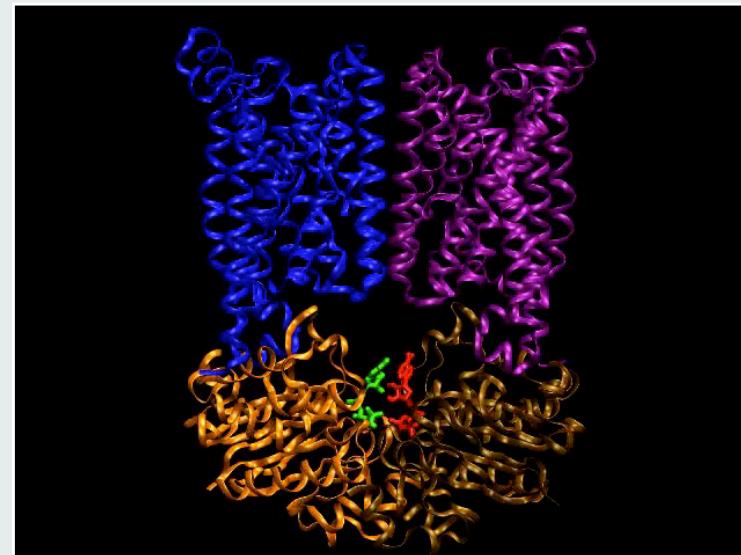


Tetraspanin CD9

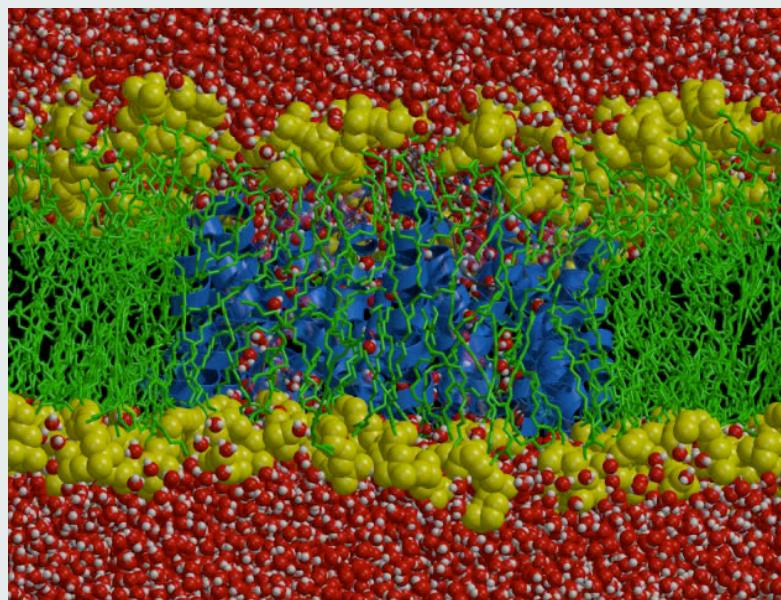
Biological membranes are dynamic



Porin



ABC Transporter



INTRODUCTION TO MEMBRANE BIOPHYSICS

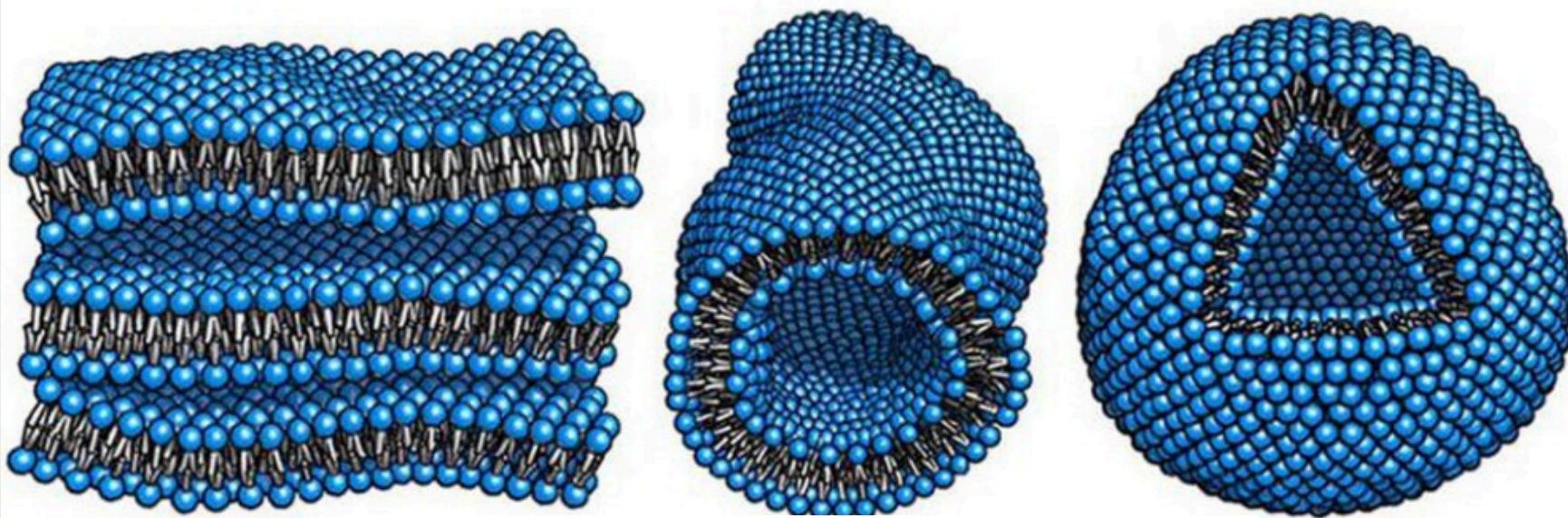
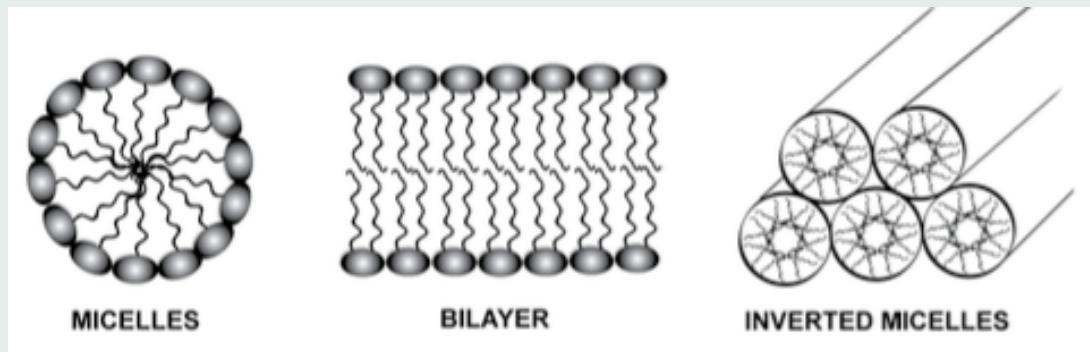


MECHANISMS OF MEMBRANE ORGANIZATION



Theory of self-assembly of lipid bilayers and vesicles

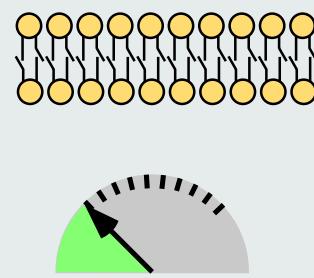
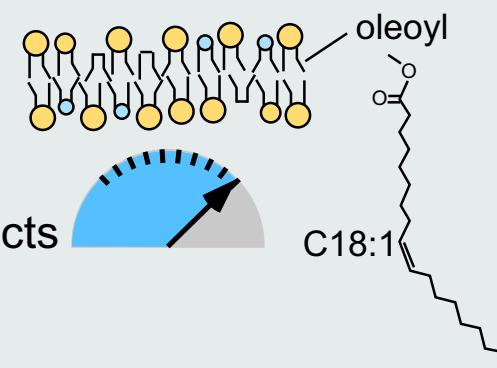
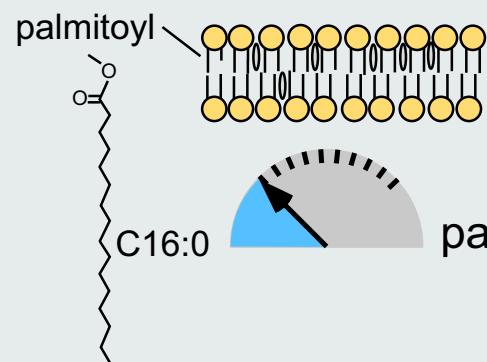
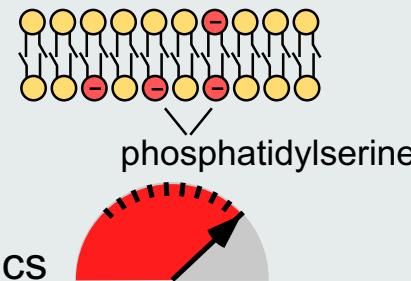
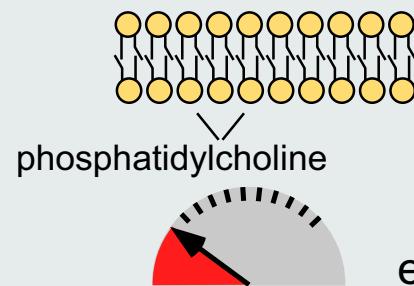
Biochimica et Biophysica Acta, 470 (1977) 185–201



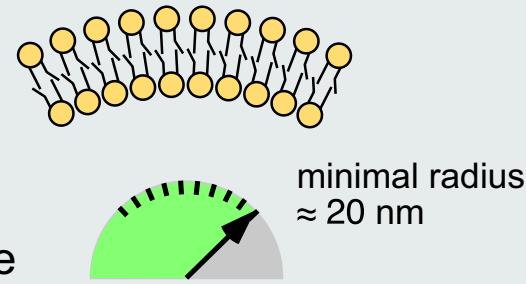
NATURE COMMUNICATIONS | 8:15856 | DOI: 10.1038/ncomms15856 | www.nature.com/naturecommunications

Composition/courbure

MECHANISMS OF MEMBRANE ORGANIZATION

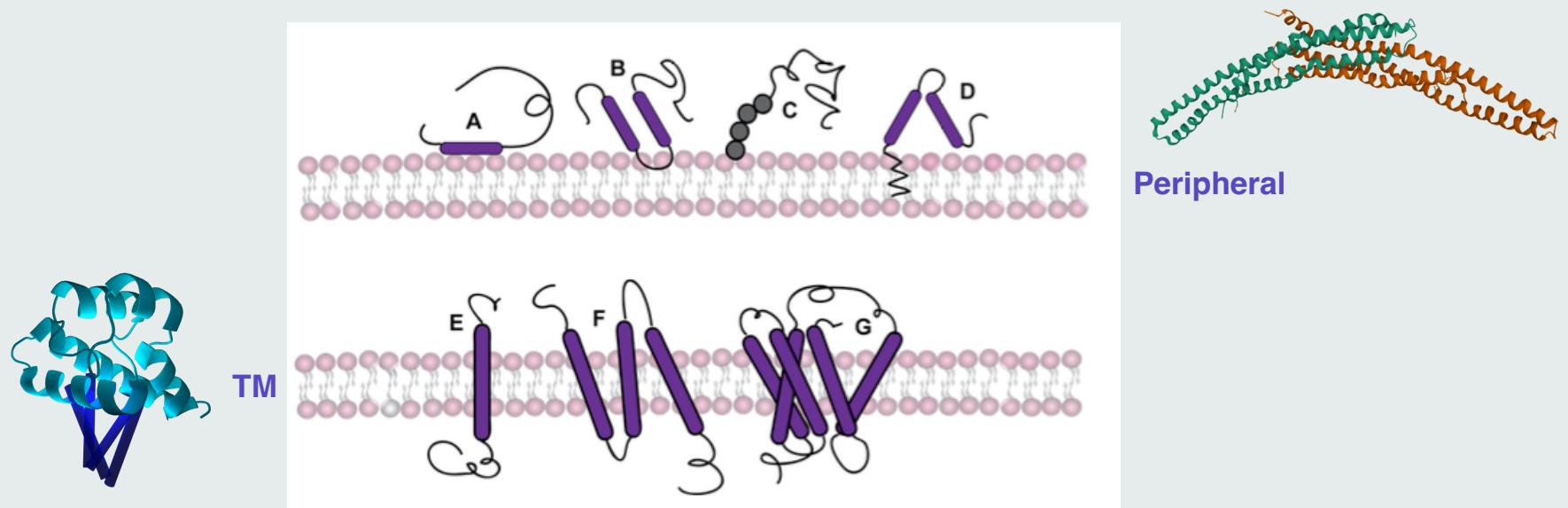


curvature

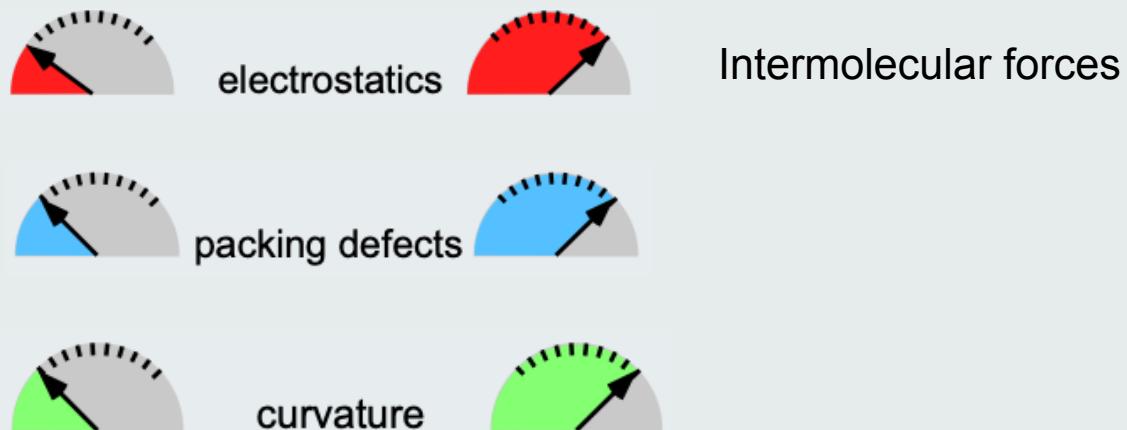


- PC 14:0/16:0
- PC 16:1/16:1
- PC 16:0/16:1
- PC 14:0/18:1
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- PC 14:0/20:4
- PC 16:1/18:2
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- PC 16:1/20:4
- PC 18:2/18:3
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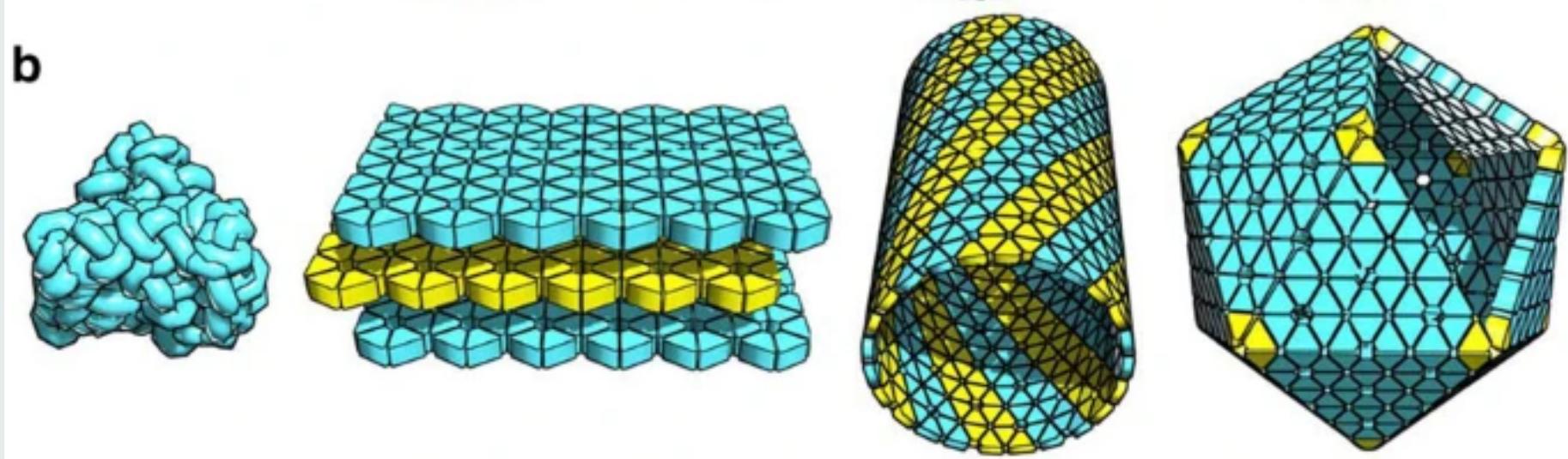
MECHANISMS OF MEMBRANE ORGANIZATION



25% of the coding sequences
of the human genome



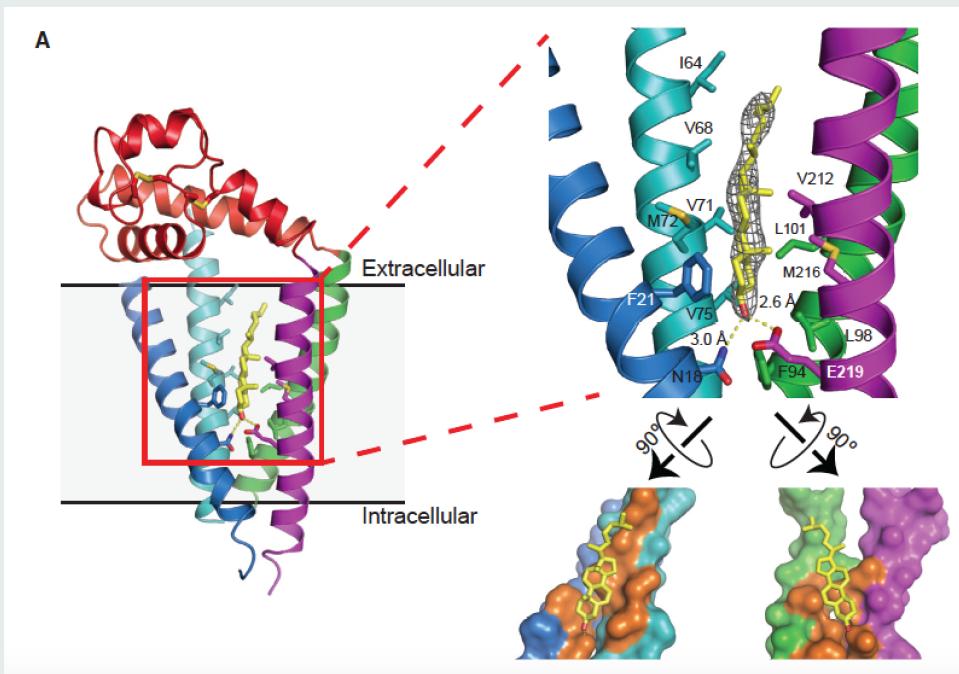
MECHANISMS OF MEMBRANE ORGANIZATION



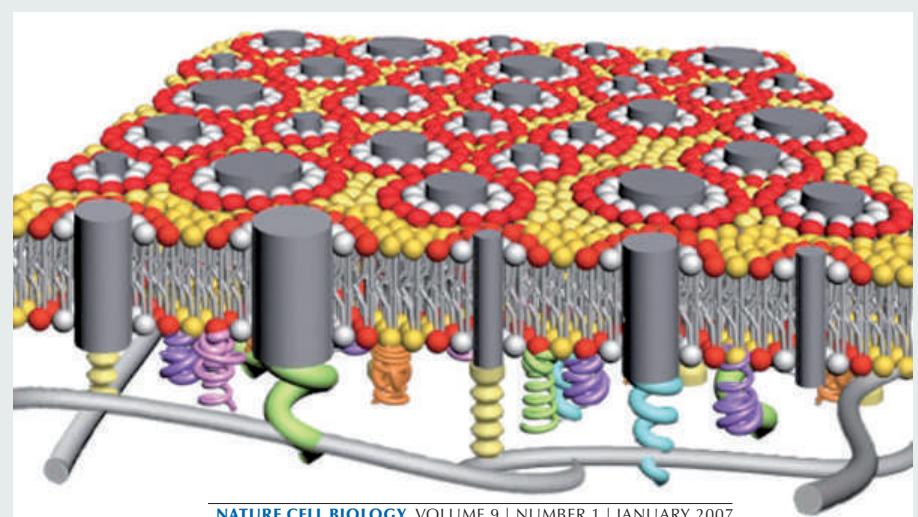
MECHANISMS OF MEMBRANE ORGANIZATION



Protein-protein-lipid interactions

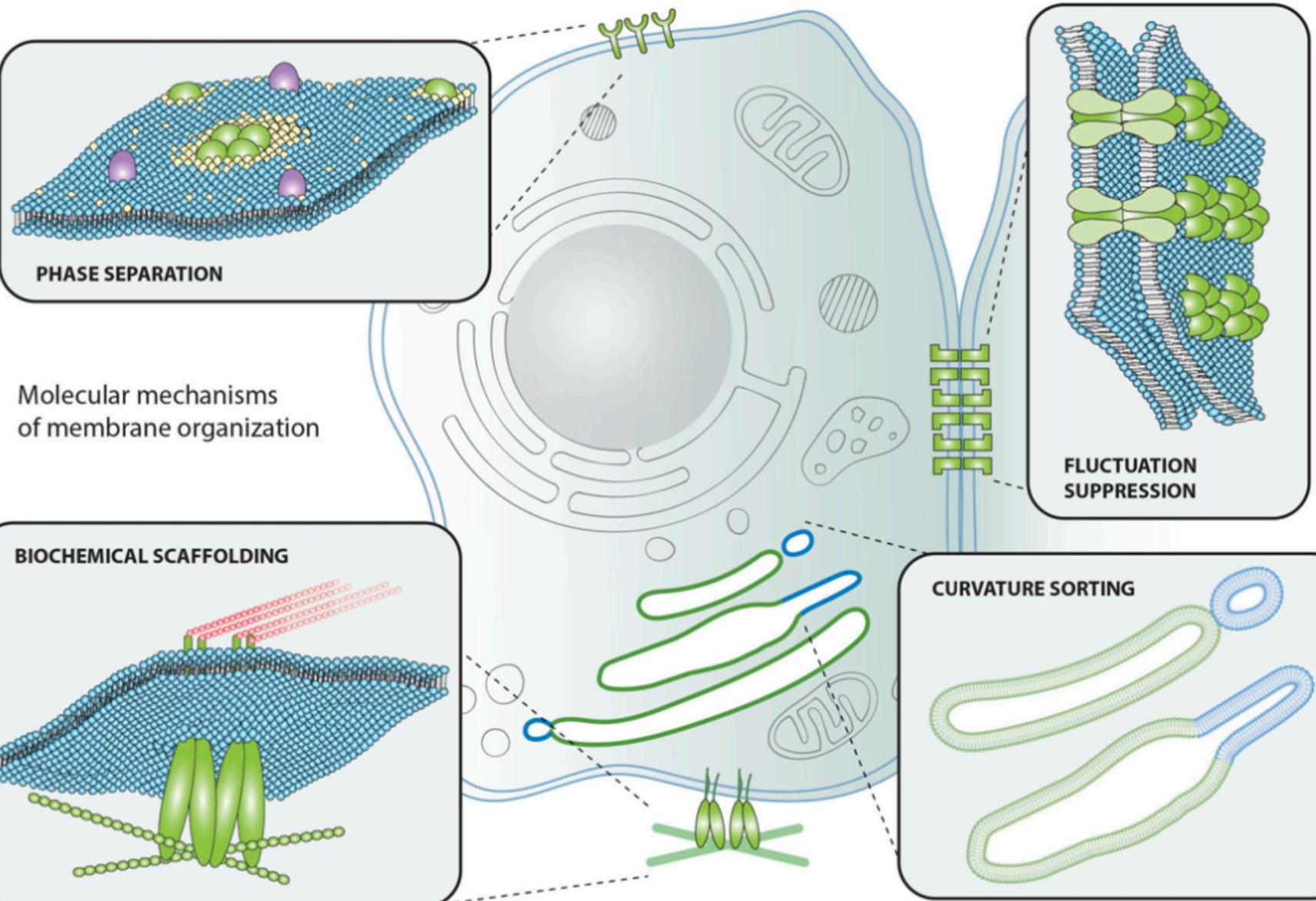


Protein-cytoskeleton interactions



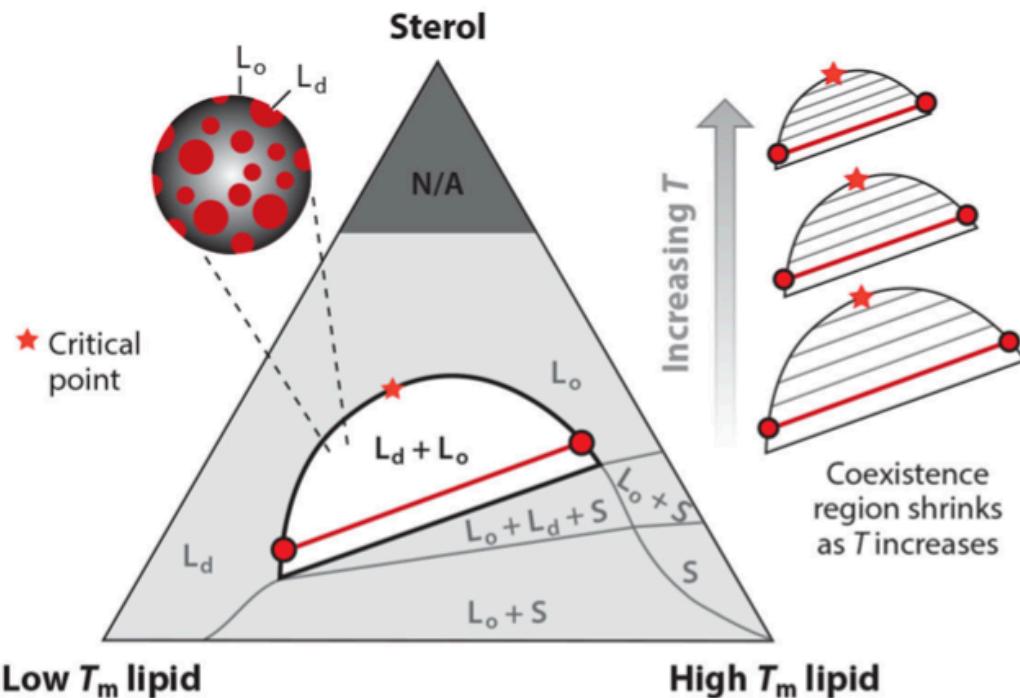
NATURE CELL BIOLOGY VOLUME 9 | NUMBER 1 | JANUARY 2007

MECHANISMS OF MEMBRANE ORGANIZATION

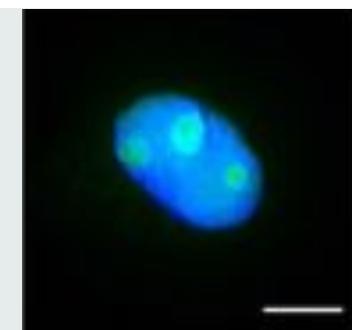
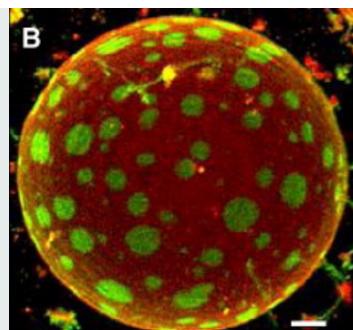
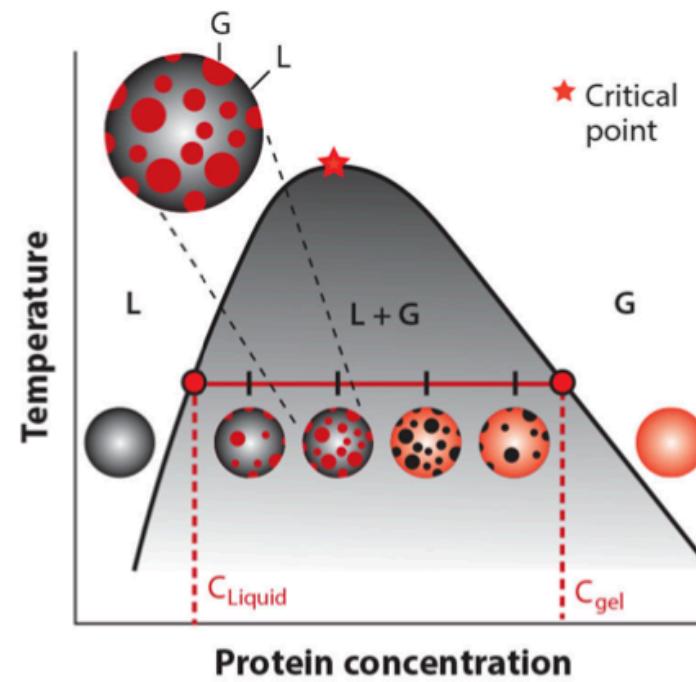


PHASE SEPARATION

a Lipid phase separation

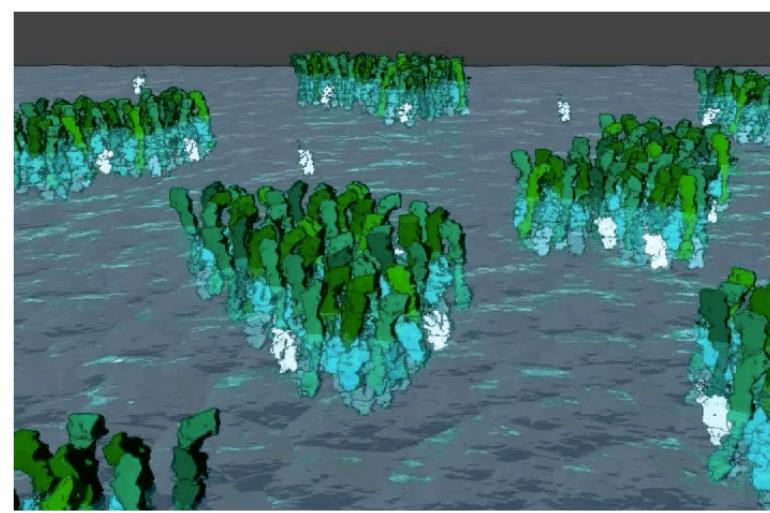


b Protein phase separation



LATERAL SEGREGATION

« Protein-protein-lipid networks »



Douglass et al *Cell* 121 (2005), pp 937-950

CD2-LAT
complex

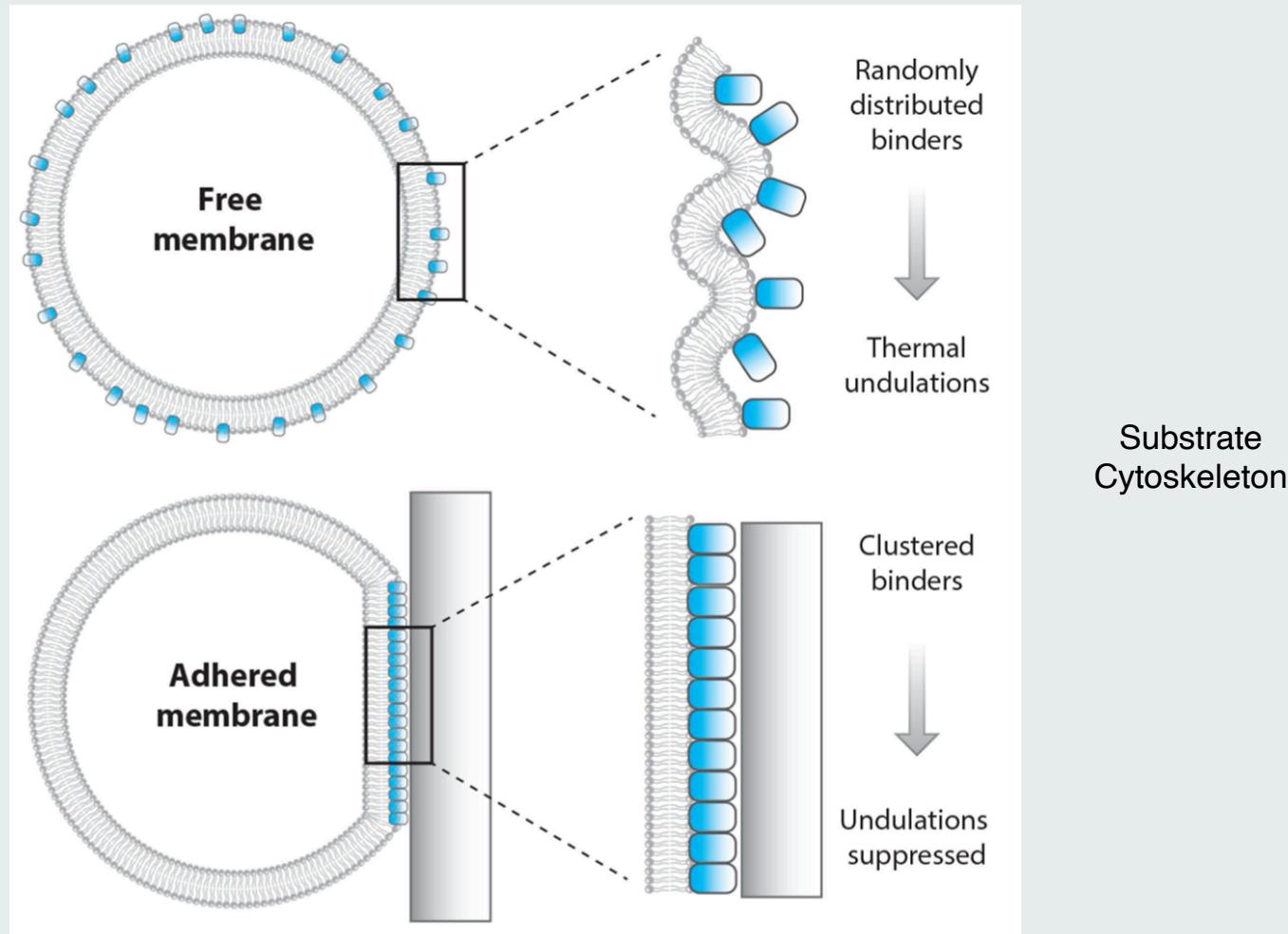
Notion of
Membrane Micro or nanodomains

« Fence picket »



Suzuki A. et al. *Biophysical J.*
Vol. 88 (May, 2005), pp
3659-80

FLUCTUATION SUPPRESSION

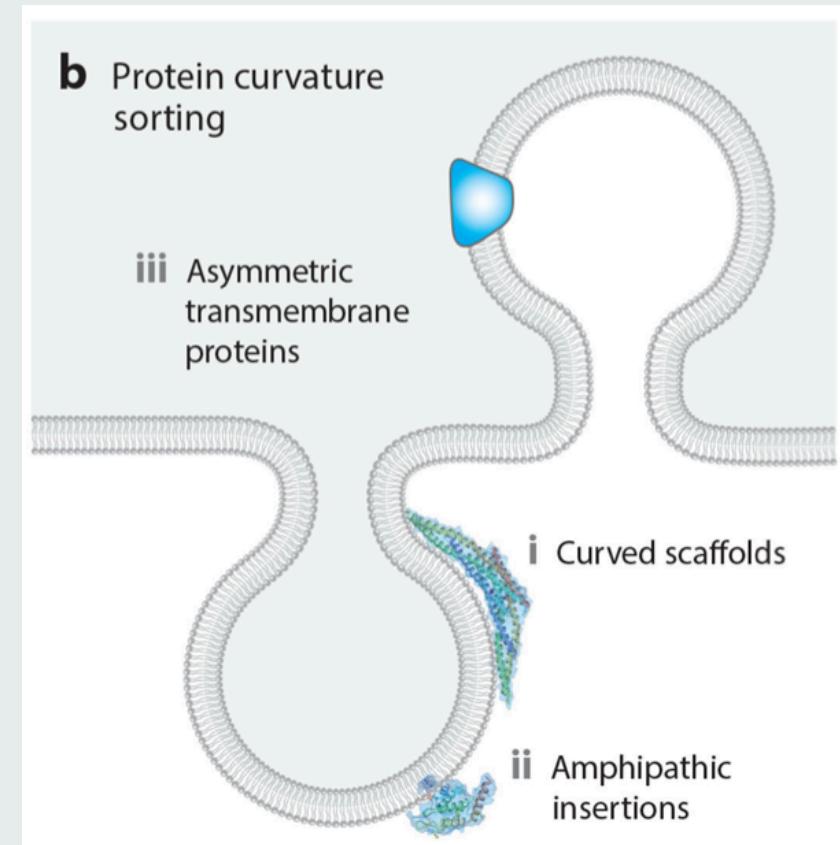
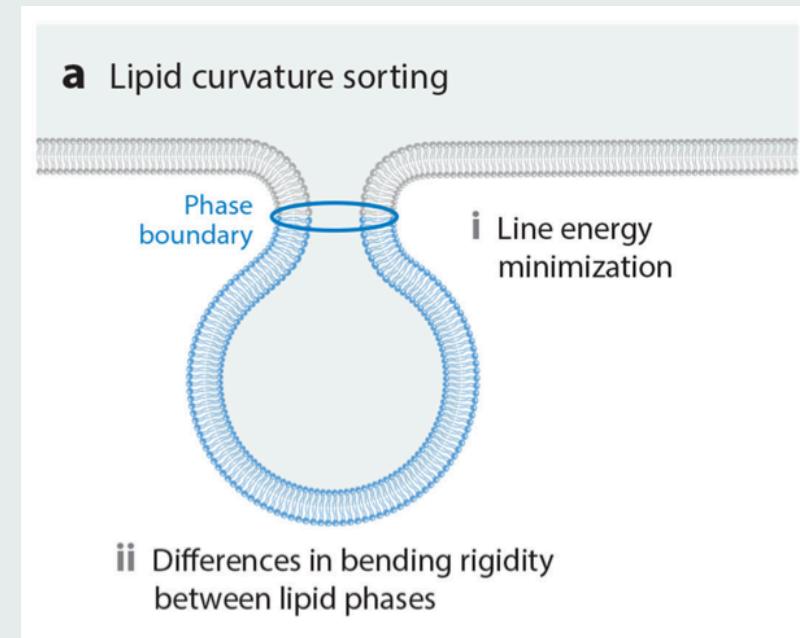


MECHANISMS OF MEMBRANE ORGANIZATION



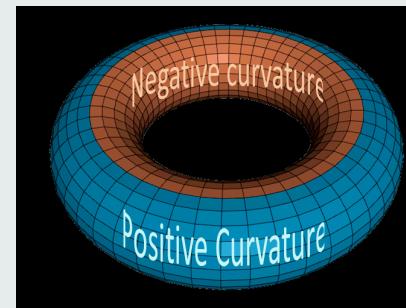
By Gregors - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=16945128>

MEMBRANE CURVATURE

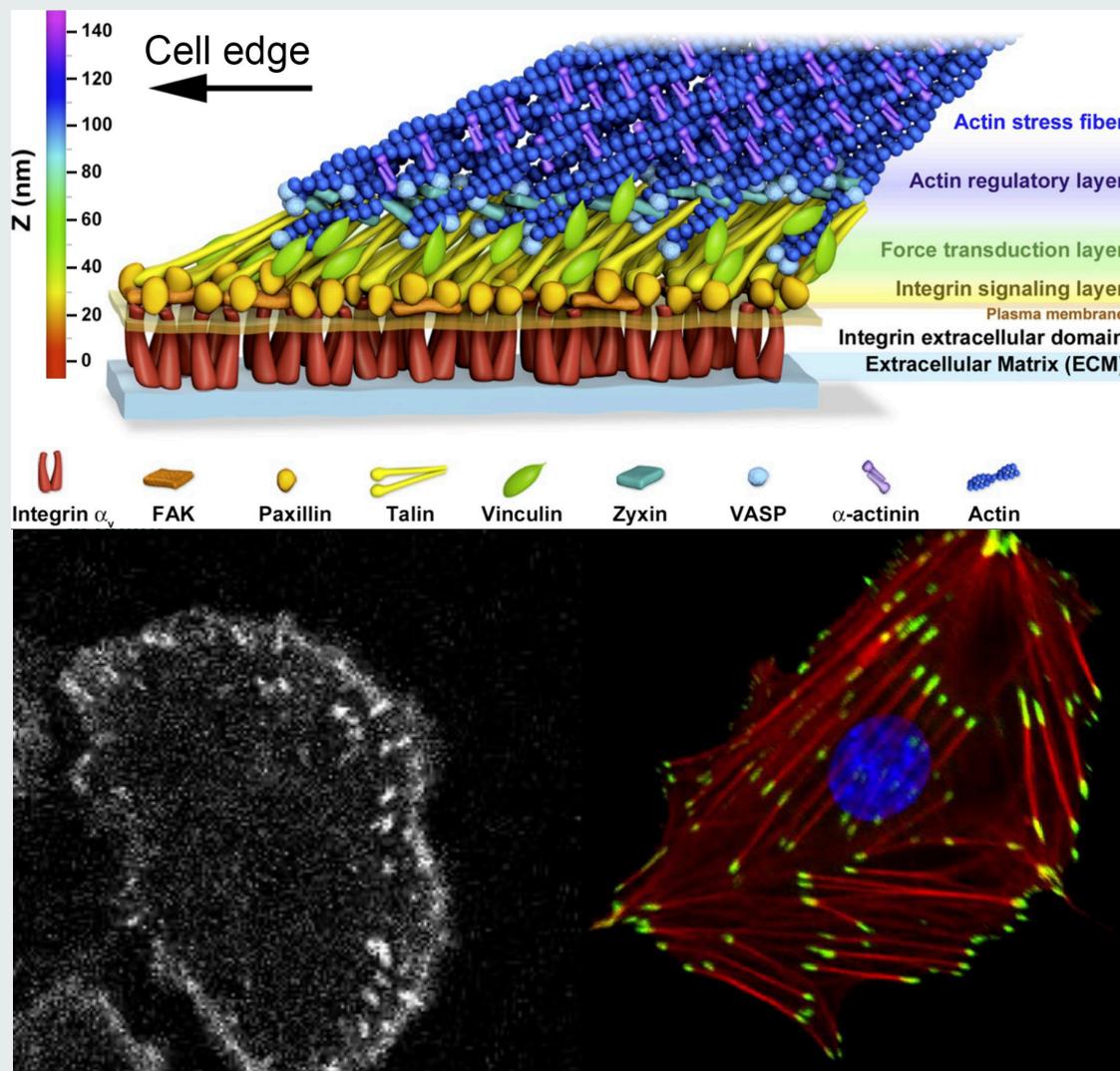


$$g = \frac{1}{2}K_B (c_1 + c_2 - c_0)^2 + K_G c_1 c_2$$

Helfrich 1973



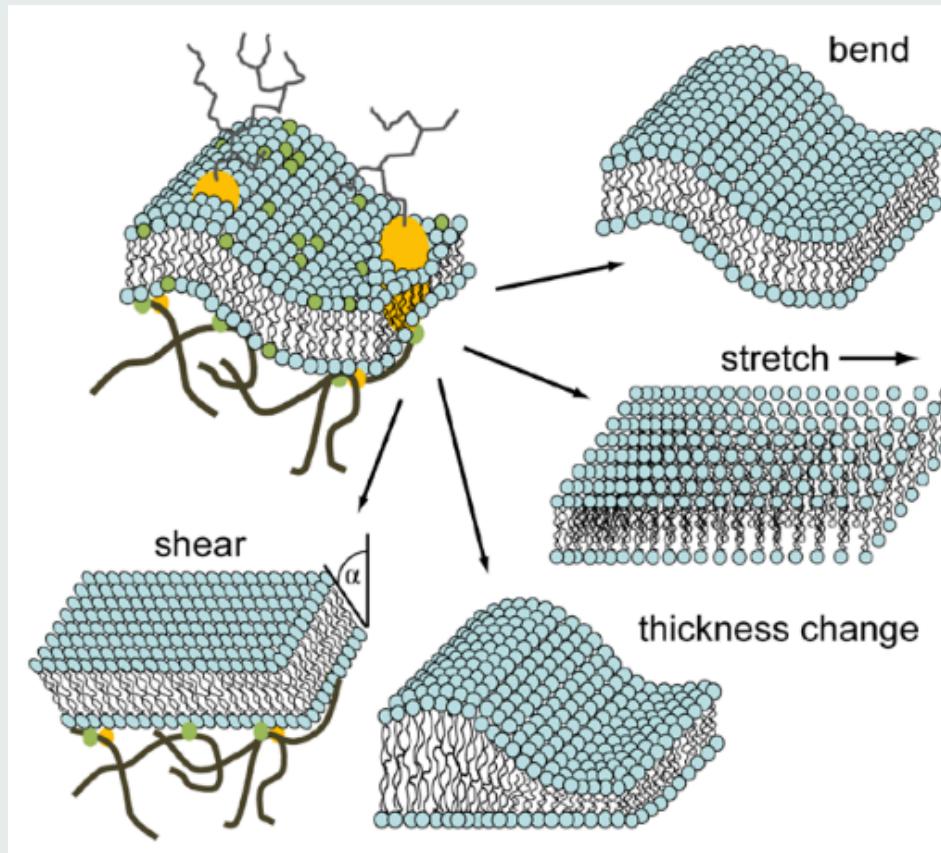
BIOCHEMICAL SCAFFOLDING



Cell focal adhesion

Physics of Membranes - A theoretical point of view (M Abkarian)

- Membrane deformations
- Bending energy
- Equilibrium shapes
- Membrane fluctuation



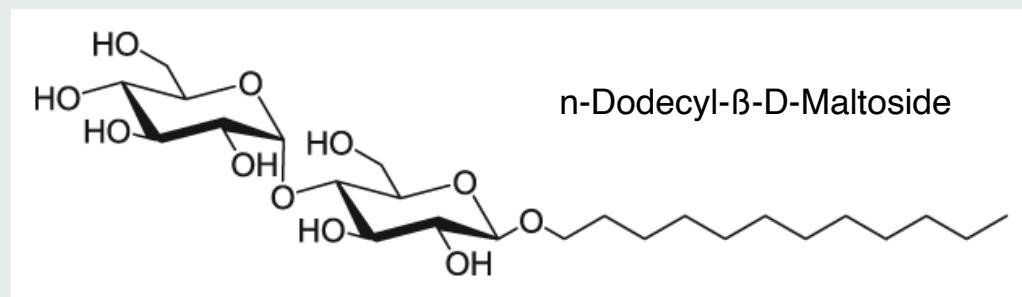
- Thickness
- Tension
- Rigidity
- Elastic modulus
- Curvature radius

HOW TO MANIPULATE MEMBRANE COMPONENTS

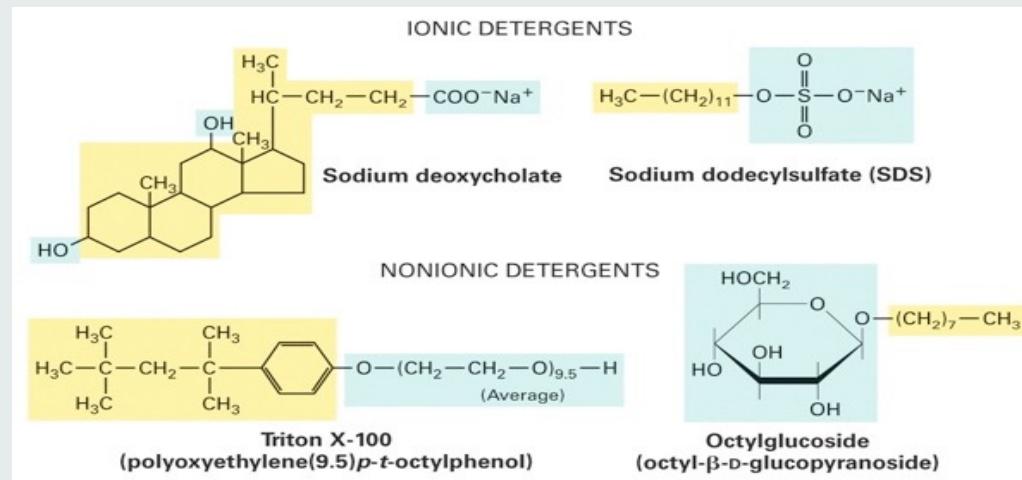


DETERGENTS

Detergents are a group of compounds with an amphiphilic structure, where each molecule has a hydrophilic (polar) head and a long hydrophobic (non-polar) tail.



Detergent molecules aggregate to form micelles, which makes them soluble in water. The hydrophobic group of the detergent is the main driving force of micelle formation, its aggregation forms the hydrophobic core of the micelles.



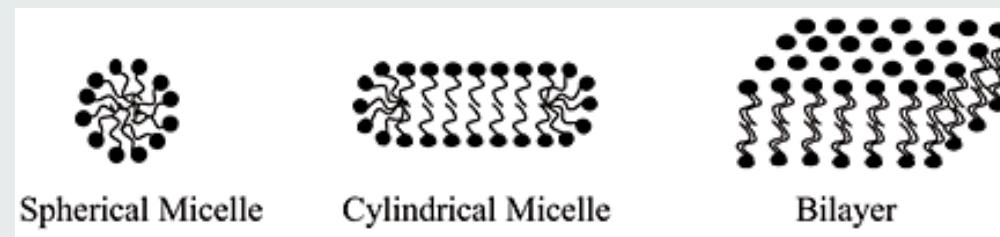
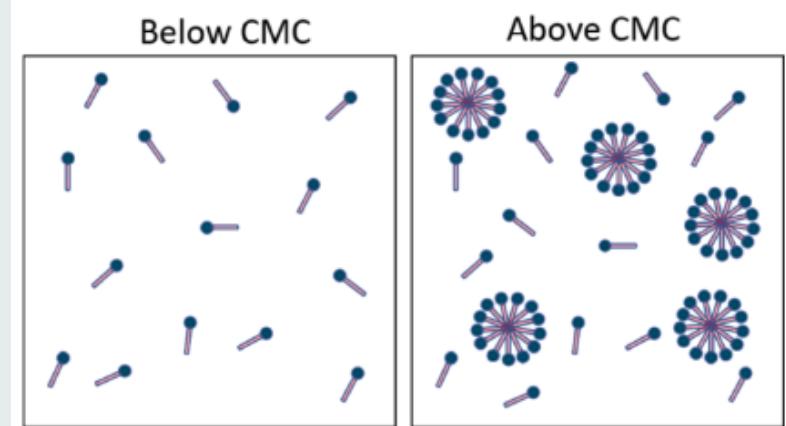
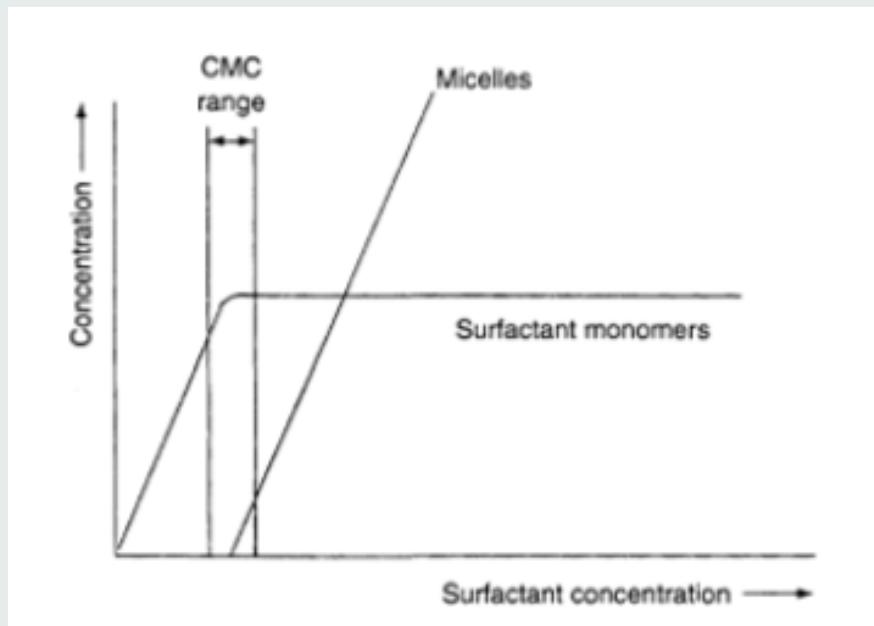
Huge diversity of detergents (C Bechara)

HOW TO MANIPULATE MEMBRANE COMPONENTS



DETERGENTS

CMC: Critical Micellar Concentration

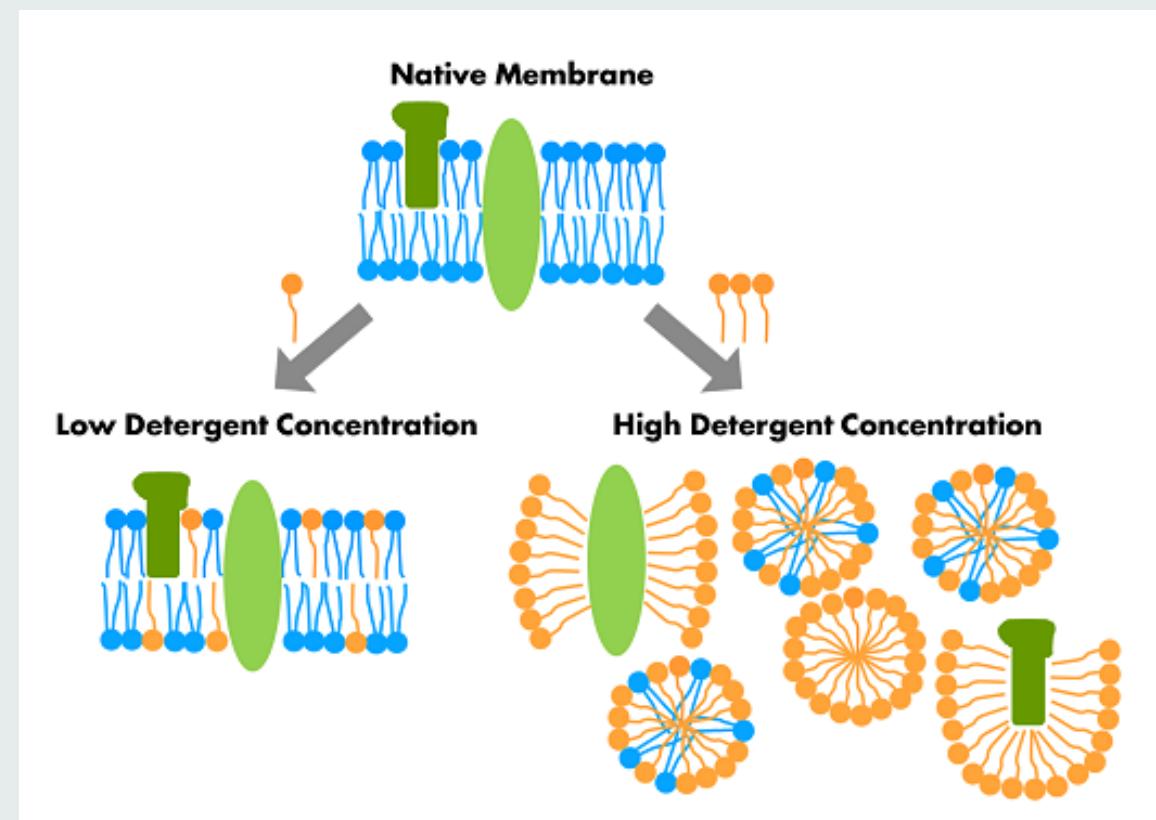
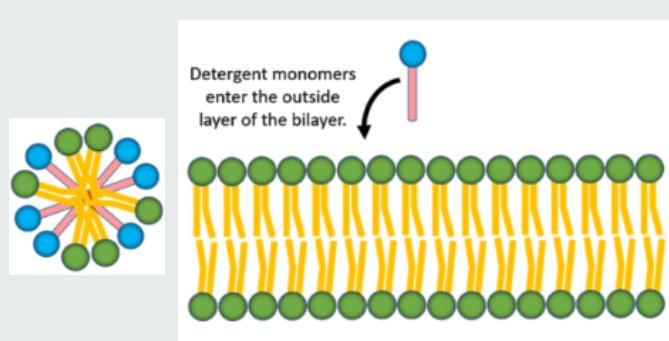


HOW TO MANIPULATE MEMBRANE COMPONENTS



DETERGENTS

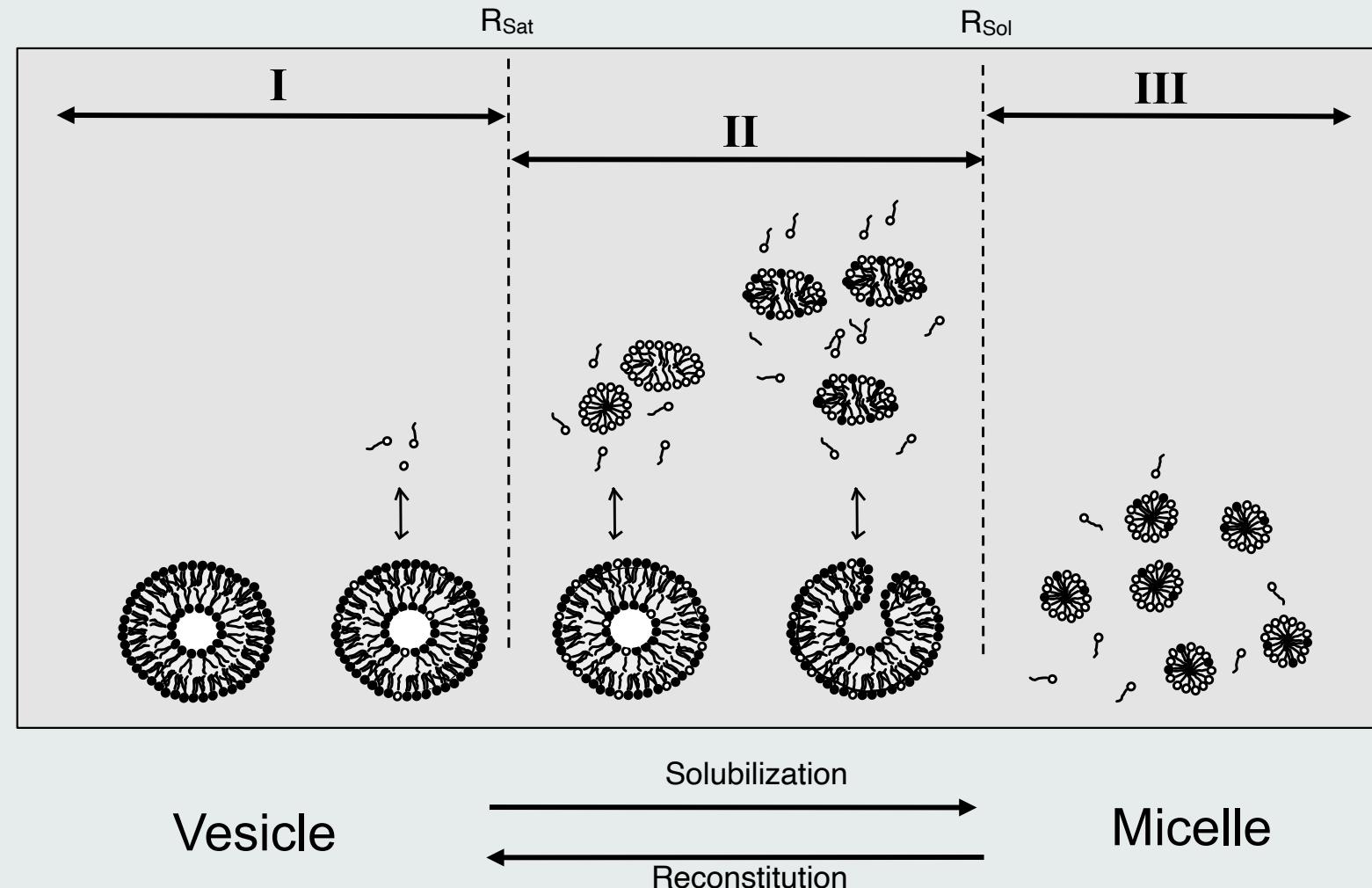
A tool to solubilize biological membranes



HOW TO MANIPULATE MEMBRANE COMPONENTS



DETERGENTS



Native membranes:

- + Composition
- Difficult to purify w/o perturbing the organization
- Difficult to analyze

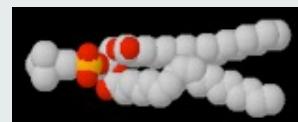
Membrane models must meet the following criteria:

- Be stable and robust with reproducible preparation
- Must separate two compartments "cis" and "trans"
- Must have minimum defects and especially good electrical properties
- The membrane must be fluid
- Must be able to incorporate functional membrane proteins

HOW TO MANIPULATE MEMBRANE COMPONENTS



LIPIDS



Soluble in organic solvents

Lipid vesicles

SUV: Small Unilamellar Vesicle

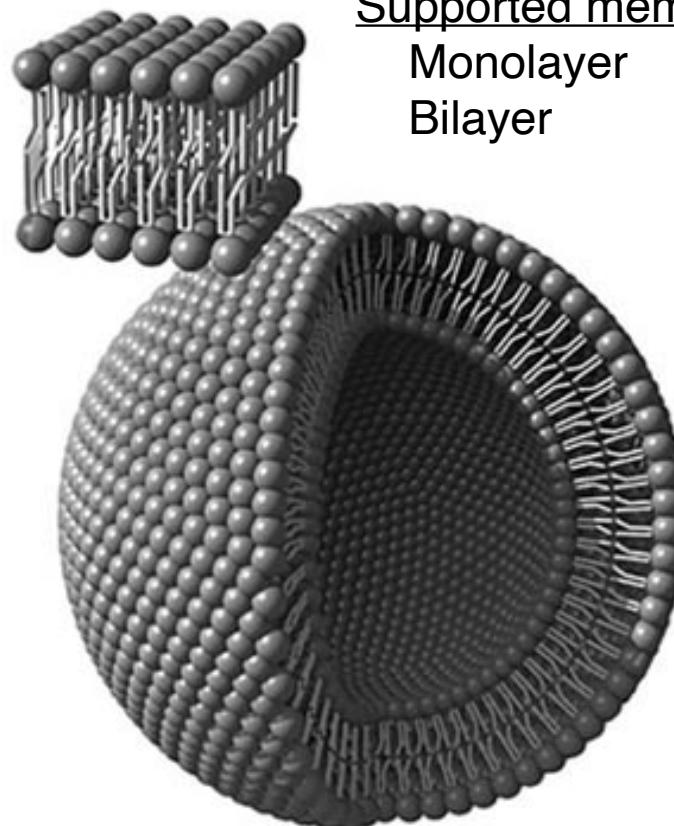
20-100 nm Ø

LUV: Large Unilamellar Vesicle

100-250 nm

Giant Unilamellar Vesicle

µm range

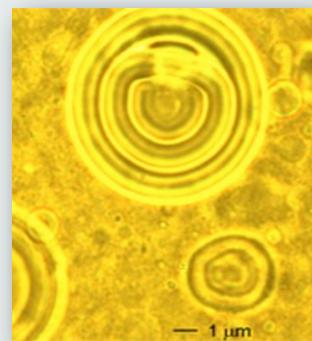


Supported membranes
Monolayer
Bilayer

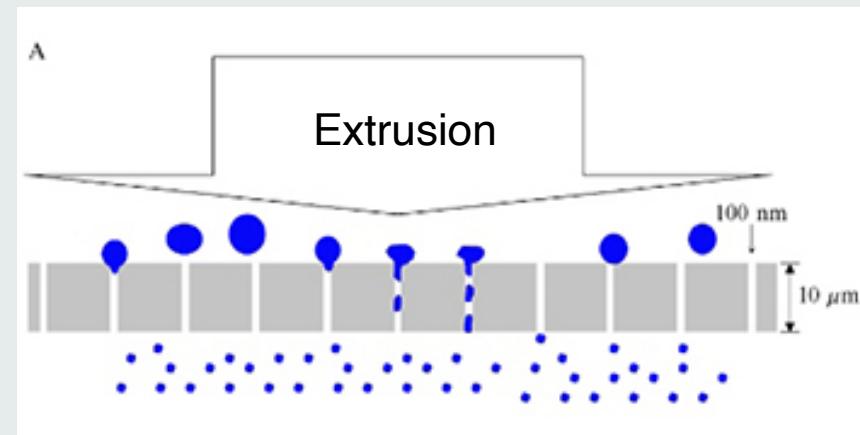
Lipid vesicles

- Extrusion
- Detergent dialysis
- Reverse evaporation

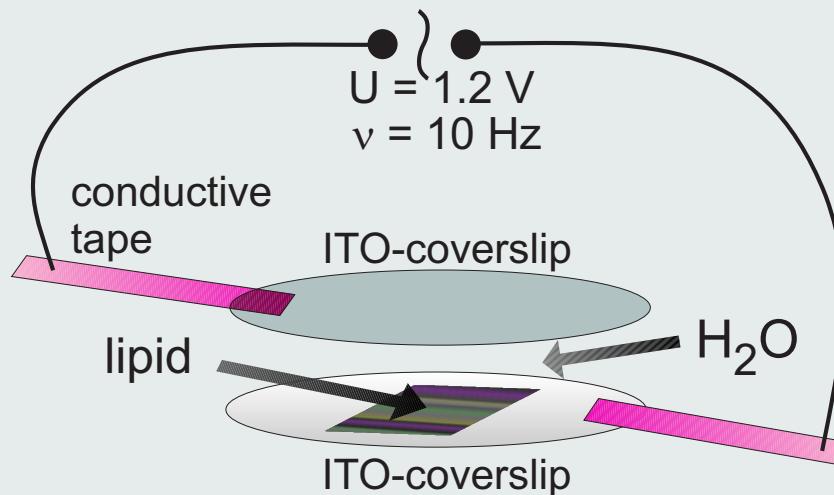
Multilamellar vesicle (MLV)



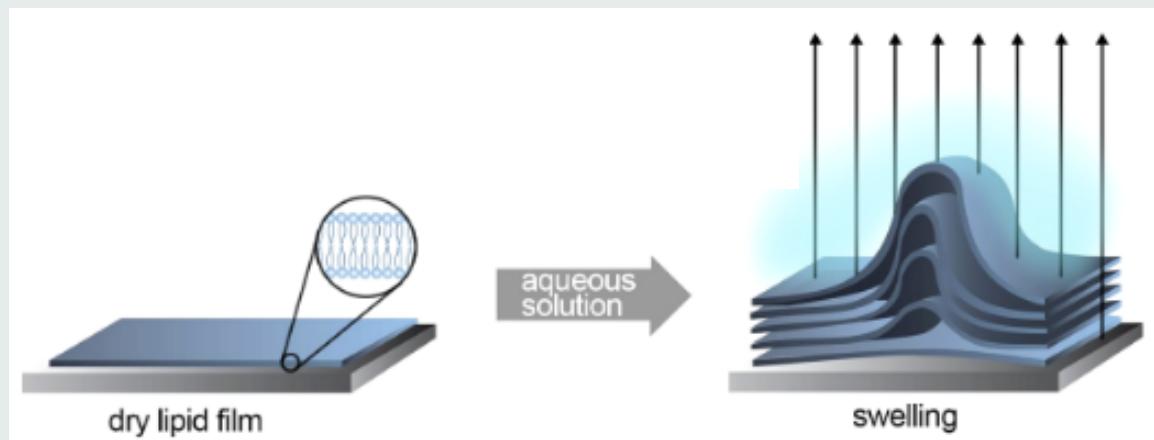
Aqueous environment



Giant Unilamellar Vesicle



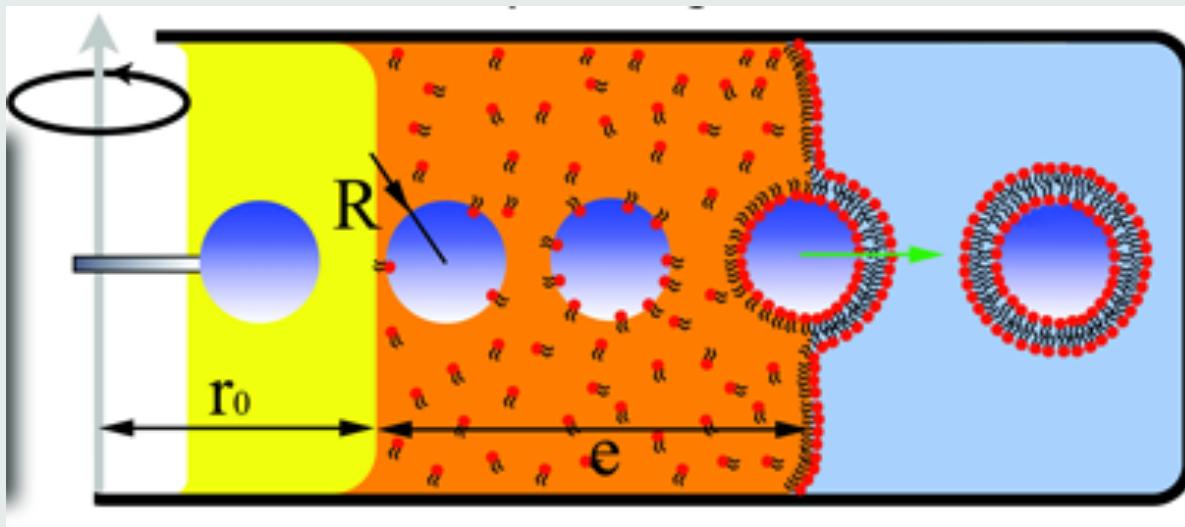
(a) Capacitor-type configuration for electroformation.



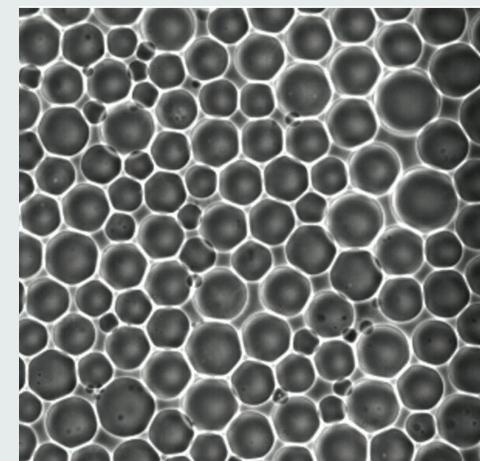
HOW TO MANIPULATE MEMBRANE COMPONENTS



Giant Unilamellar Vesicle

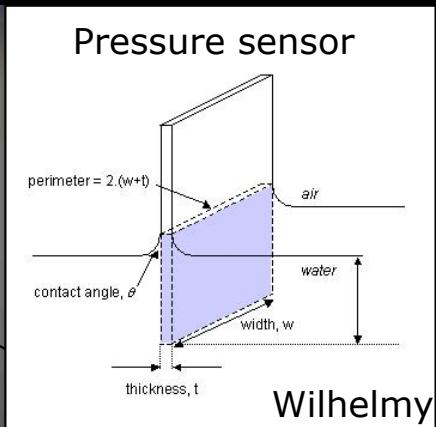
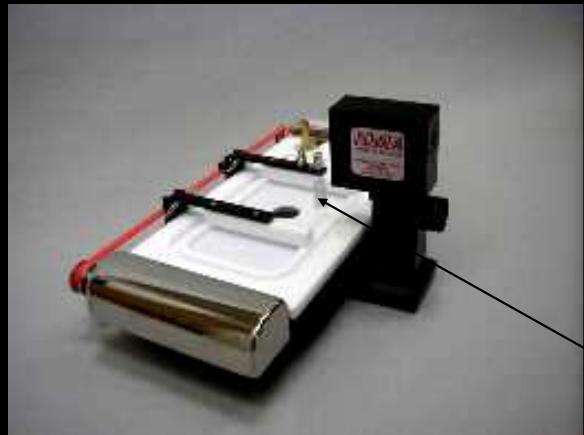


DAS: Dispersing Aqueous Solution
LOS: Lipid in Oil Solution

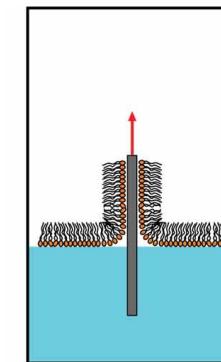
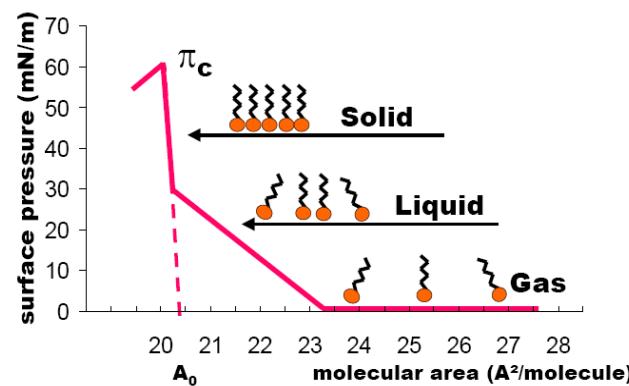


Loiseau et al (2011) Soft matter

HOW TO MANIPULATE MEMBRANE COMPONENTS



Supported lipid membrane using Langmuir balance

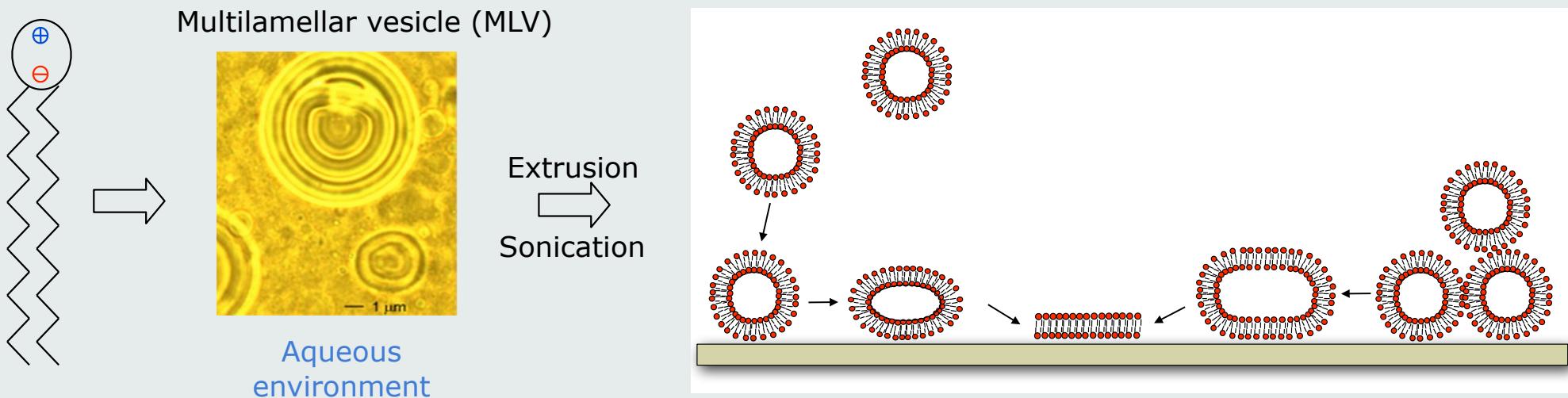


Lateral pressure in biological membranes = 30 mN/m

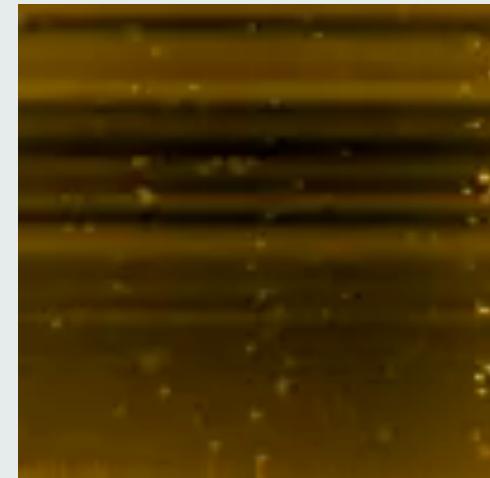
HOW TO MANIPULATE MEMBRANE COMPONENTS



Supported Lipid Bilayer



- Easy to prepare
 - Liposome rupture
 - Temperature control
 - Interaction of the leaflet with the substrate

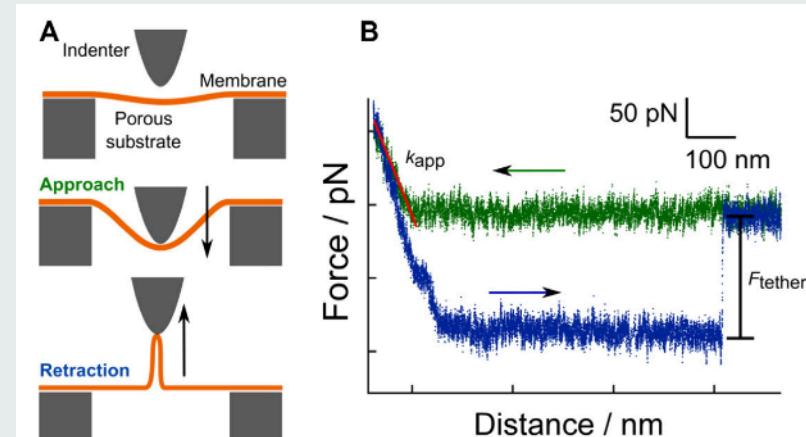
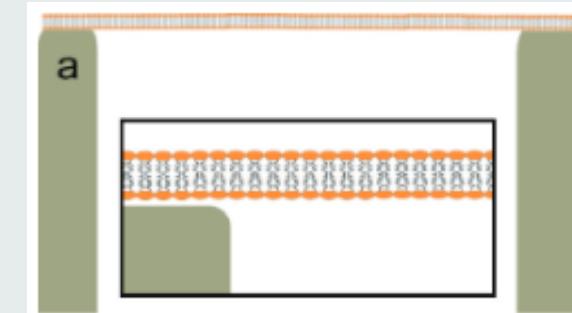
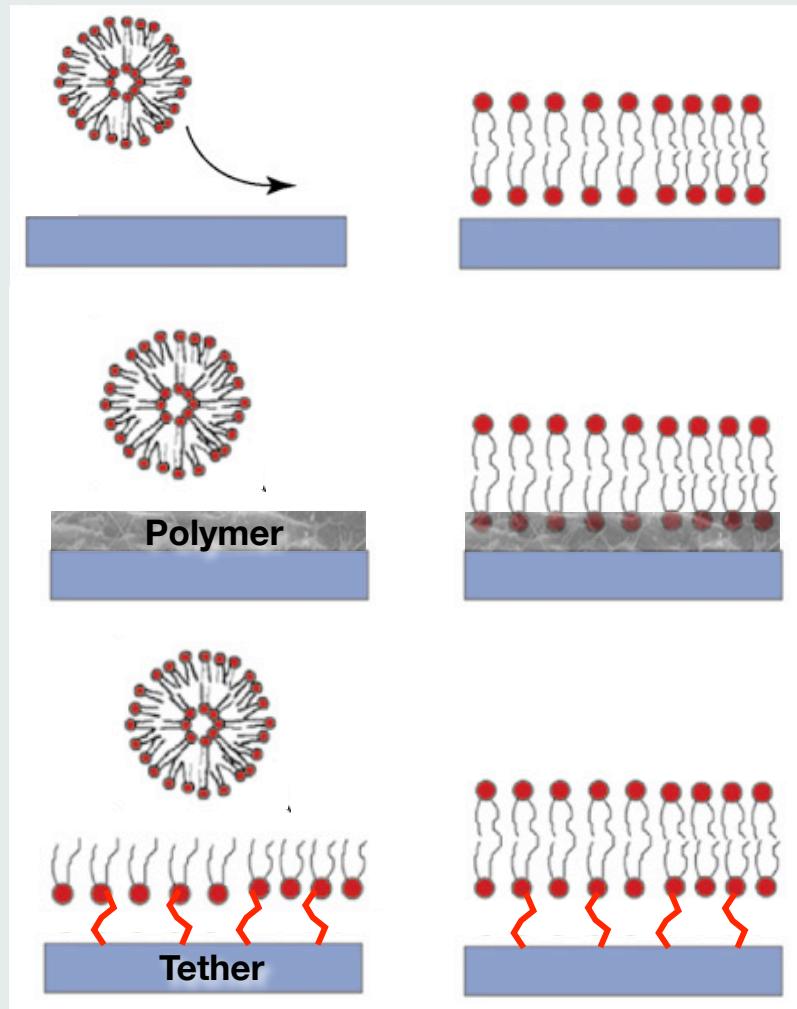


DOPE-containing membrane

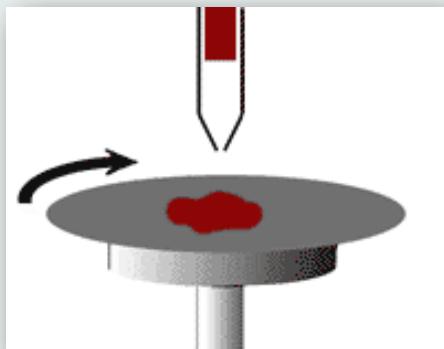
HOW TO MANIPULATE MEMBRANE COMPONENTS



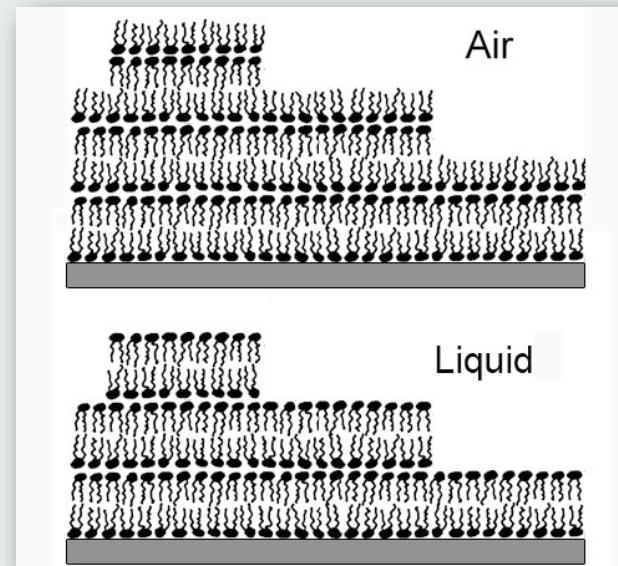
Pore-Spanning Membranes



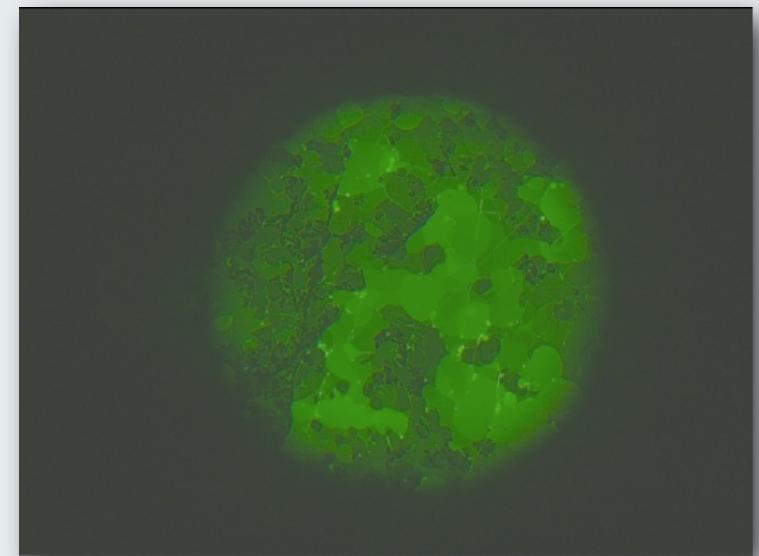
HOW TO MANIPULATE MEMBRANE COMPONENTS



Lipids in organic solvent



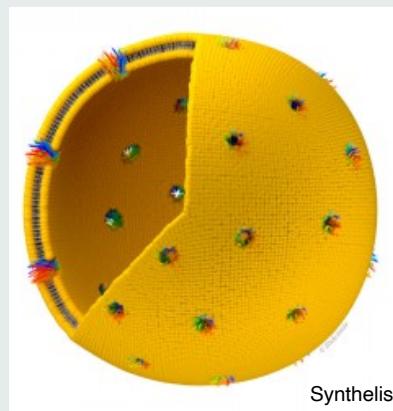
SLB using spin coating



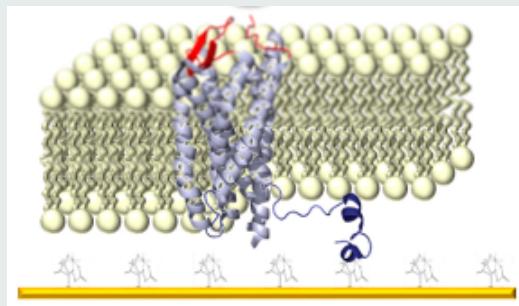
HOW TO MANIPULATE MEMBRANE COMPONENTS



LIPIDS + PROTEINS



**Proteoliposome
fusion**



**Tethered proteins
reconstitution**



Direct incorporation

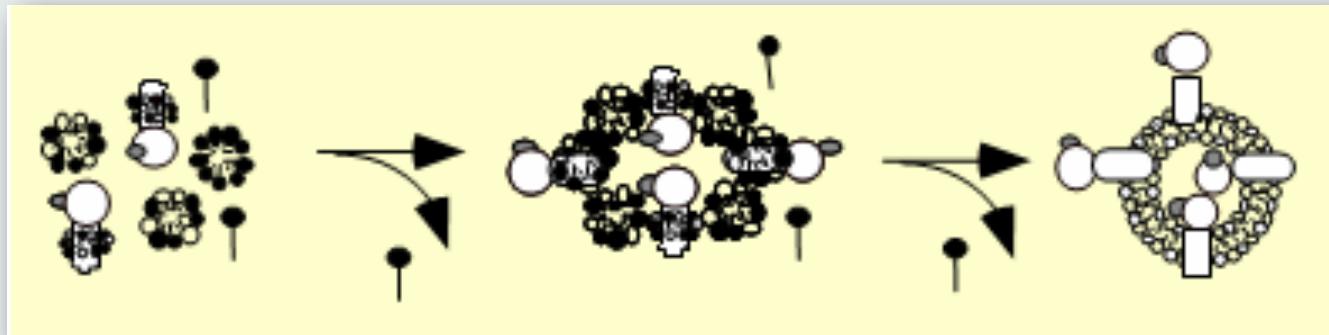
HOW TO MANIPULATE MEMBRANE COMPONENTS



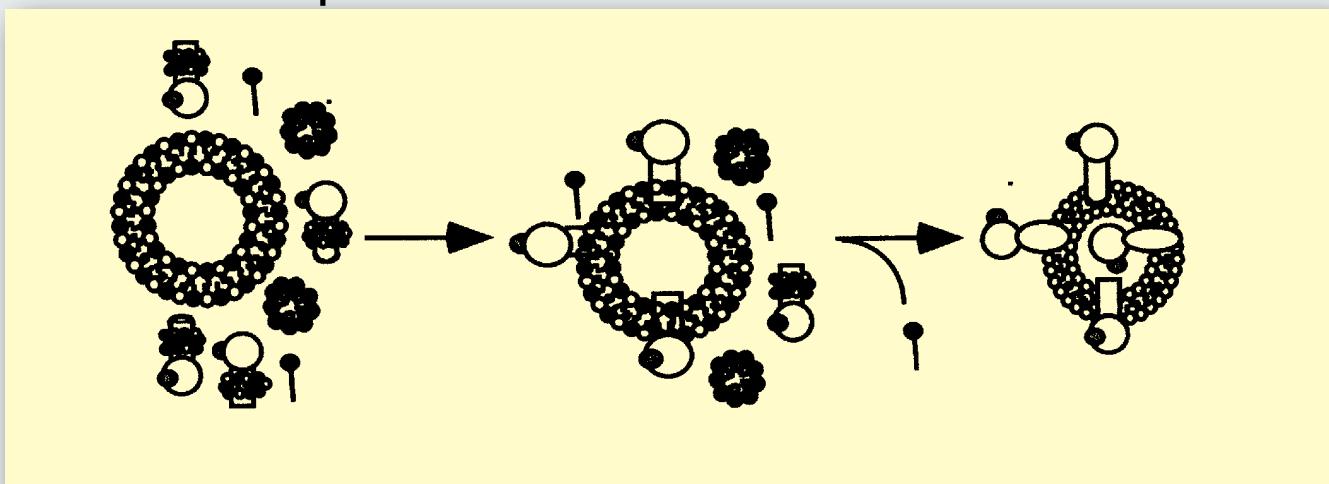
PROTEOLIPOSOMES



Reconstitution



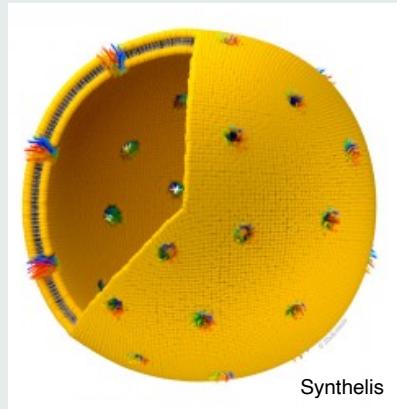
Direct incorporation



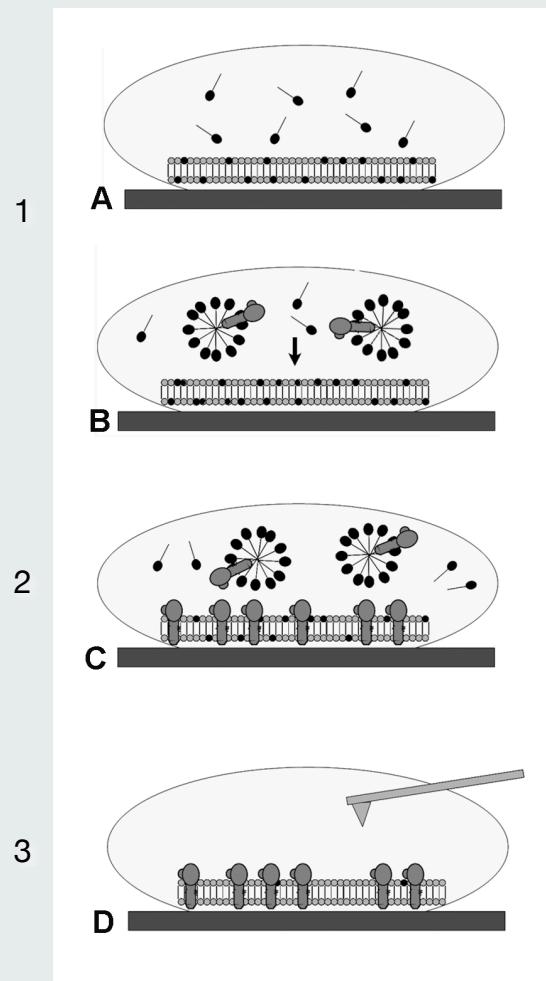
HOW TO MANIPULATE MEMBRANE COMPONENTS



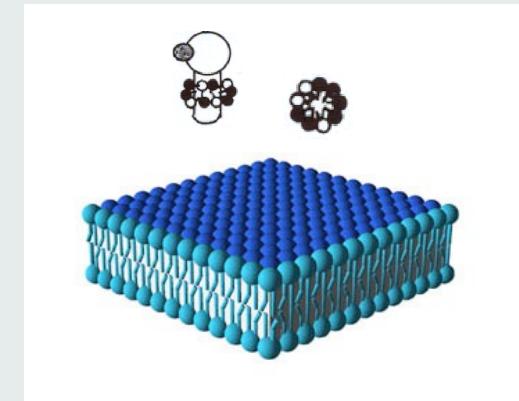
SUPPORTED MEMBRANES



Fusion on a solid substrate



Levy and Milhiet (2013) Methods Mol Biol



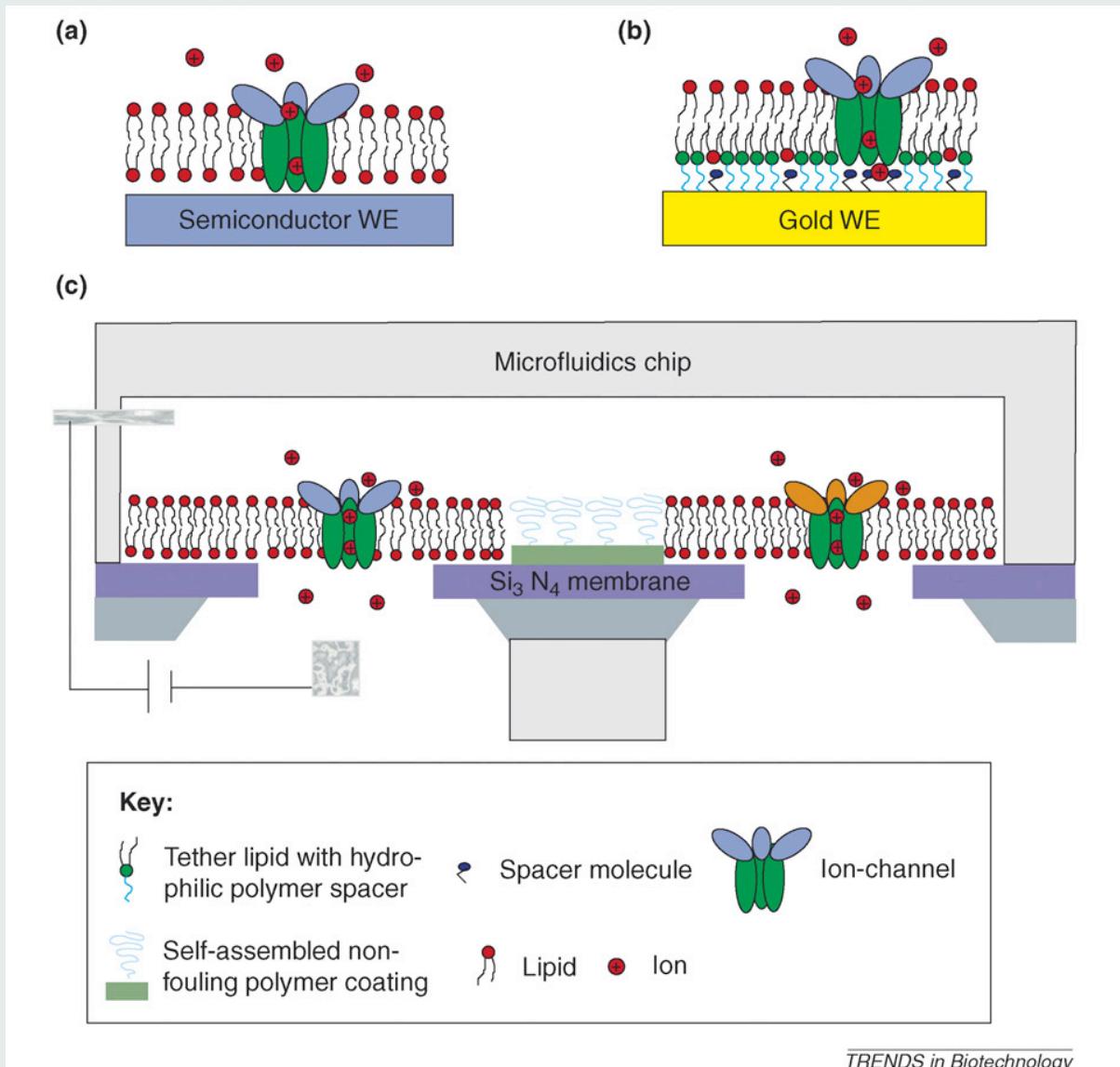
Incorporation

AFM Imaging
of non crystalline proteins

HOW TO MANIPULATE MEMBRANE COMPONENTS



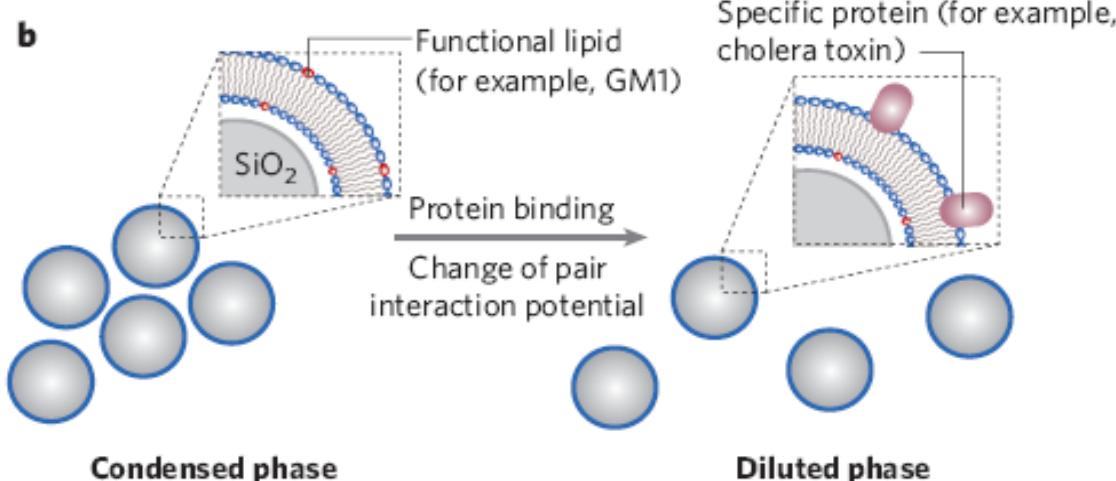
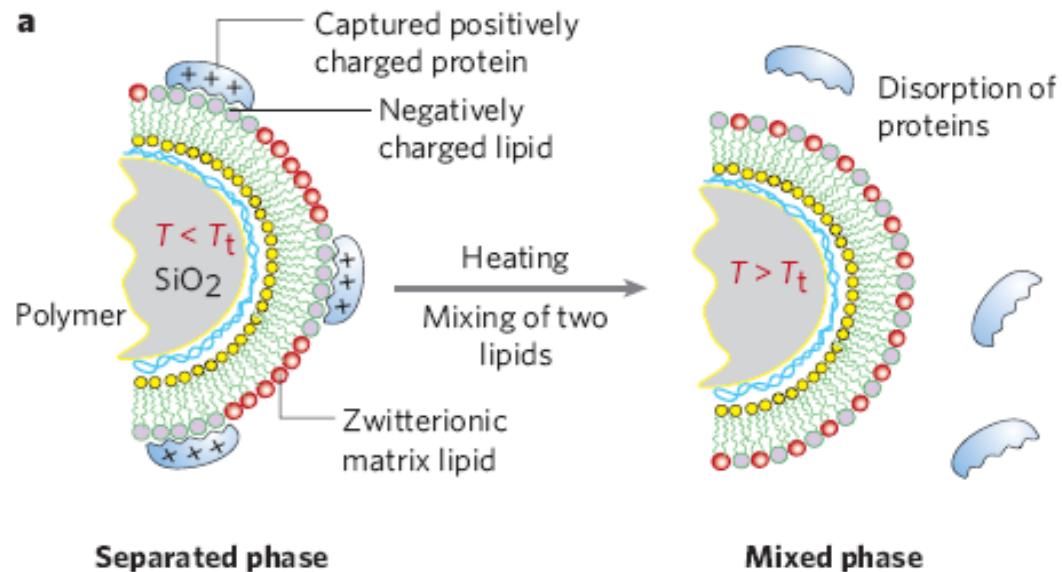
SUPPORTED MEMBRANES



HOW TO MANIPULATE MEMBRANE COMPONENTS



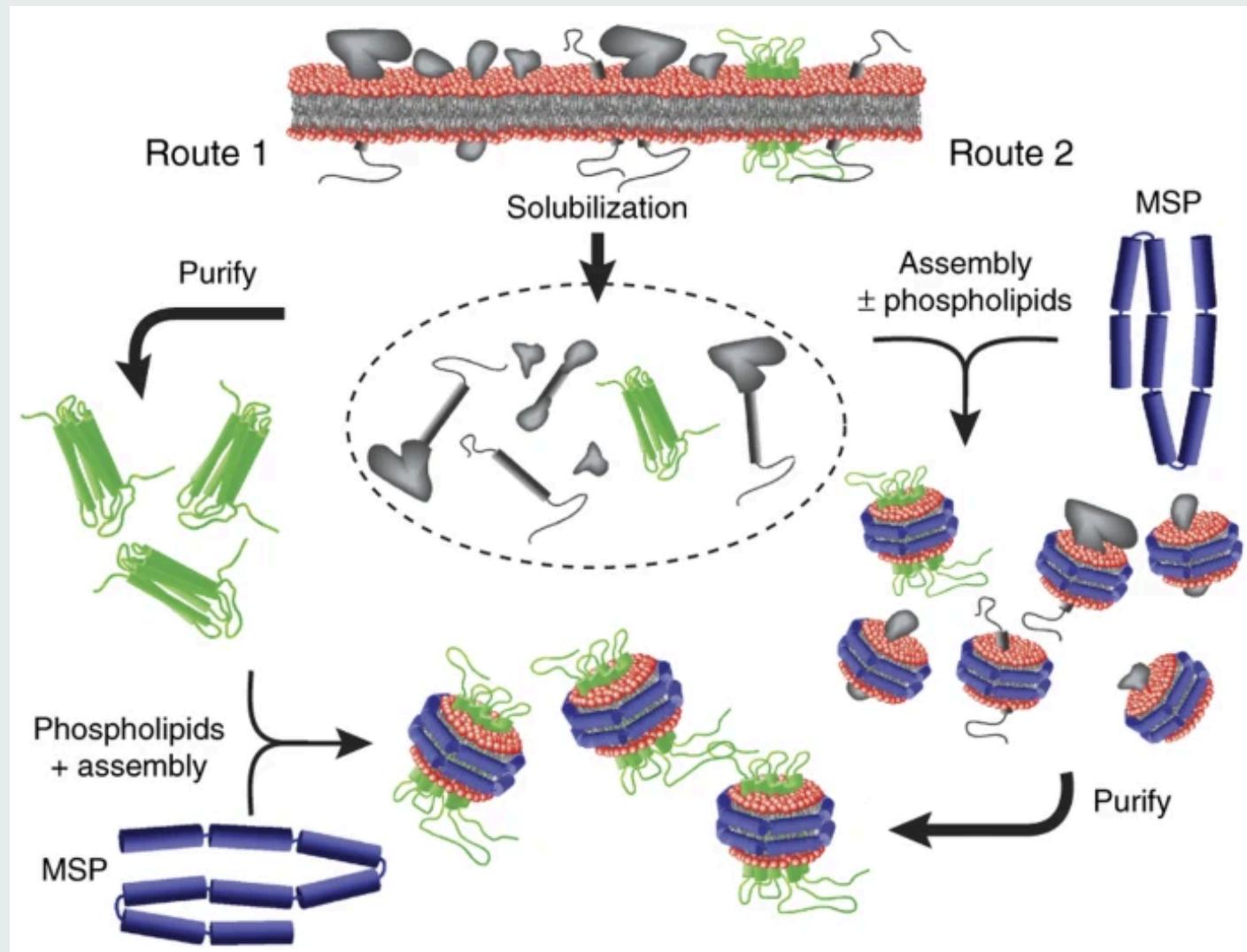
SUPPORTED MEMBRANES



HOW TO MANIPULATE MEMBRANE COMPONENTS



NANODISC



Bayburt, T.H. and Sligar, S.G. (2010) Membrane protein assembly into nanodiscs. FEBS Lett. 584, 1721–1727

HOW TO MANIPULATE MEMBRANE COMPONENTS



NANODISC

THE NANODISC SOLUTION

GPCRs: β_2 AR, RHO
BioTechniques (2006) 40, 601
JBC (2007) 282, 14875

TAR Receptor (1, 2, 3 - dimers)
PNAS (2006), 103, 11509

Bacteriorhodopsin (mono/tri-mer)
ABB (2006) 450, 215

P450 +/- Reductase
ABB (2005) 430, 218
JBC (2007) 282, 7066

Coagulation Factors
JBC (2007) 282, 6556

SecYEG
EMBO J. (2007) 26, 1995
JBC (2009) 284, 7897

Cholera Toxin
Anal. Chem. (2008), 80, 6245

Aromatase
BBRC (2008) 372, 379

Methods Enzymol. (2009)
464, 211-231
FEBS Lett. (2010)
584, 1721-1727

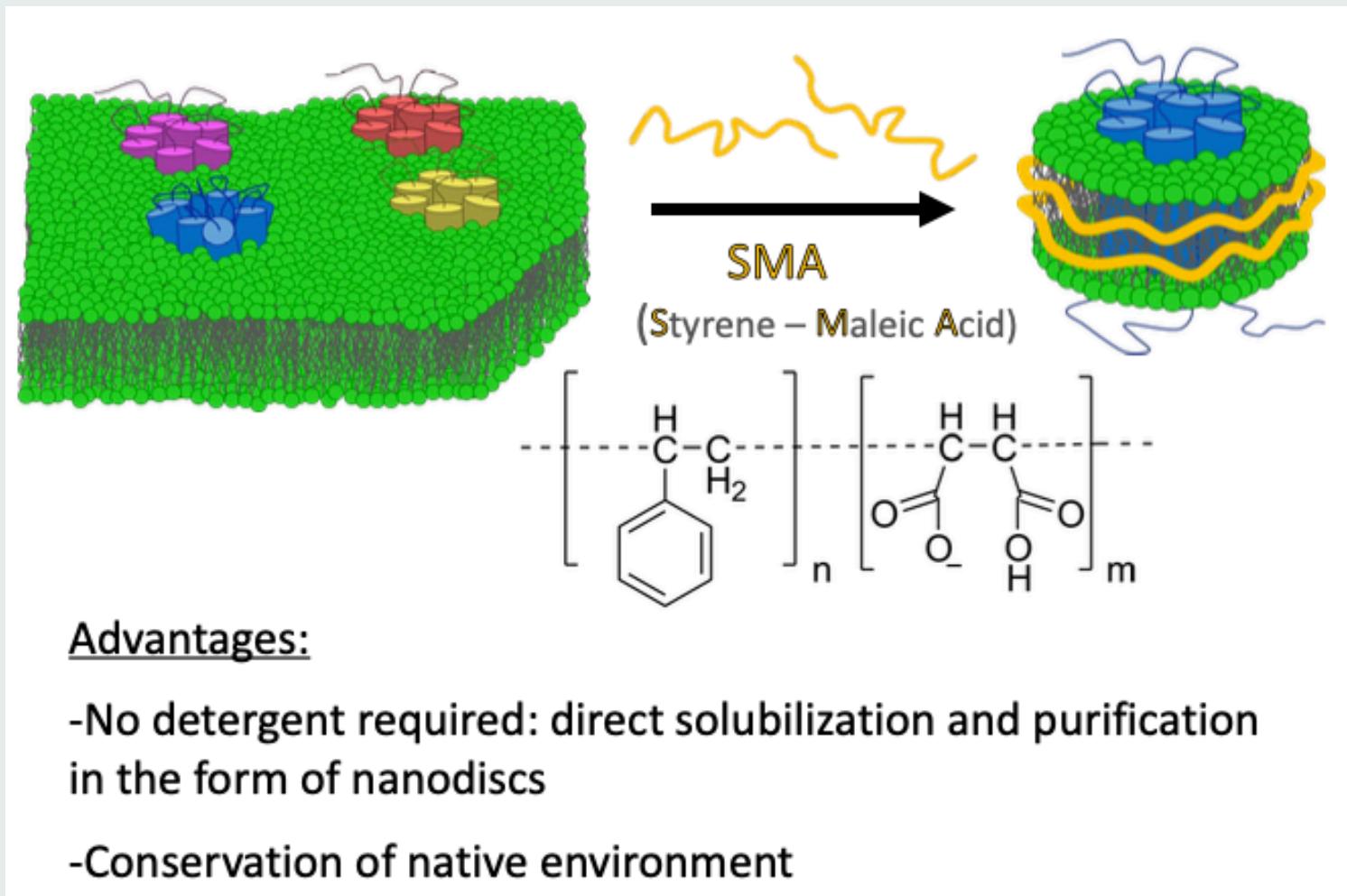
Biochemistry (2007)
46, 2059

MSP: Membrane Scaffolding Protein

HOW TO MANIPULATE MEMBRANE COMPONENTS



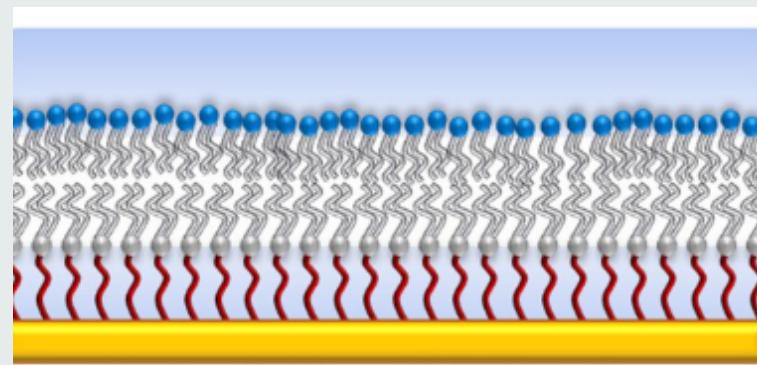
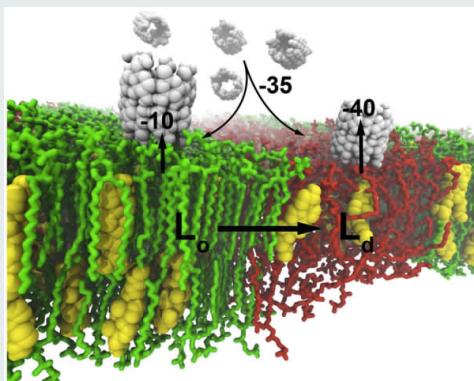
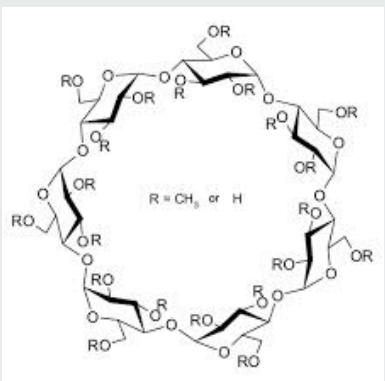
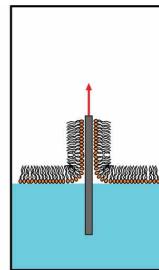
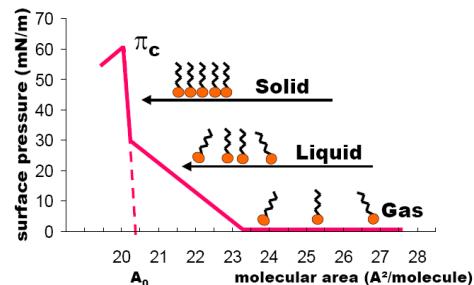
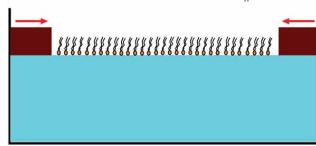
Styrene-maleic acid lipid particles (SMALP) ~ native nanodiscs



HOW TO MANIPULATE MEMBRANE COMPONENTS



HOW TO CREATE MEMBRANE ASYMMETRY

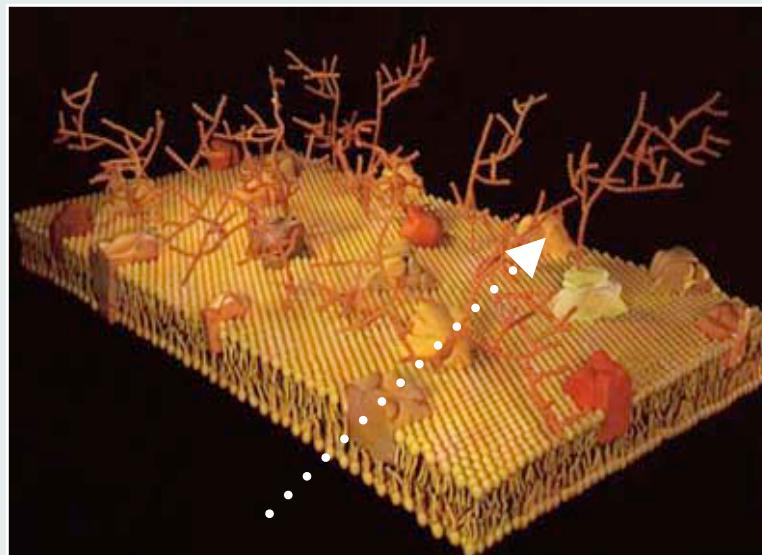


<https://doi.org/10.3389/fmats.2018.00055>

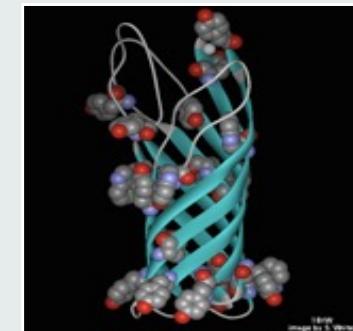
EXPERIMENTAL CHARACTERIZATION OF MEMBRANES



Prostate Cell



Artificial Membrane



Individual Component



Membrane biophysics is the study of biological membrane structure and function using physical, computational, mathematical, and biophysical methods. A combination of these methods can be used to create phase diagrams of different types of membranes. As opposed to membrane biology, membrane biophysics focuses on quantitative information and modeling of various membrane phenomena, such as microdomain formation, rates of lipid and cholesterol flip-flop, protein-lipid coupling, and the effect of bending and elasticity functions of membranes on inter-cell connections. *Wikipedia*

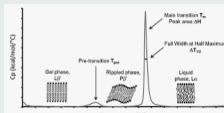
Biophysical techniques – methods used for gaining information about biological systems on an atomic or molecular level.

Microscopies and Spectroscopies for Characterizing Membranes and their Components

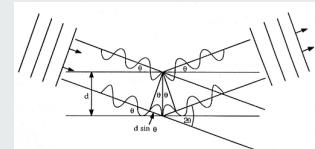


DSC

Differential Scanning Calorimetry

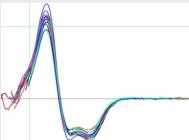


Scattering techniques



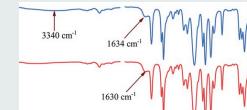
CD

Circular Dichroism



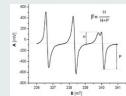
FTIR

Fourier Transform Infrared Spectroscopy

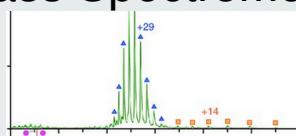


EPR

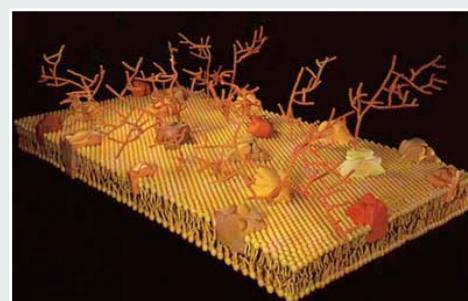
Electron Paramagnetic Resonance



Native Mass Spectrometry



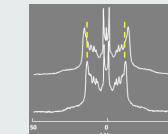
Integrative



Membrane Biophysics

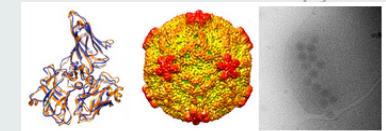
NMR

Nuclear Magnetic Resonance



EM

Electron Microscopy



Electrophysiology



AFM

Atomic Force Microscopy



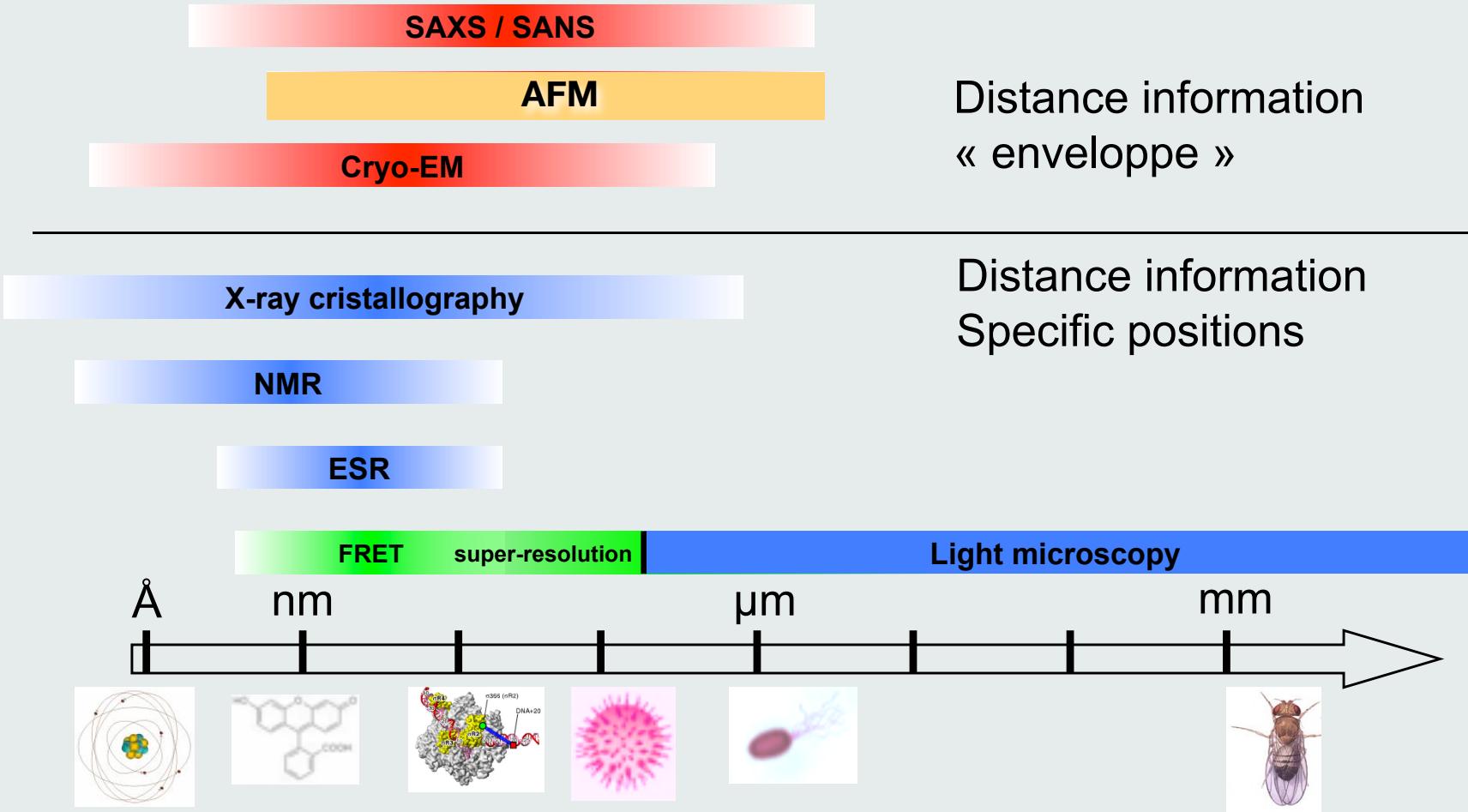
Fluorescence Spectro & μcopy



Molecular Modeling & Dynamics



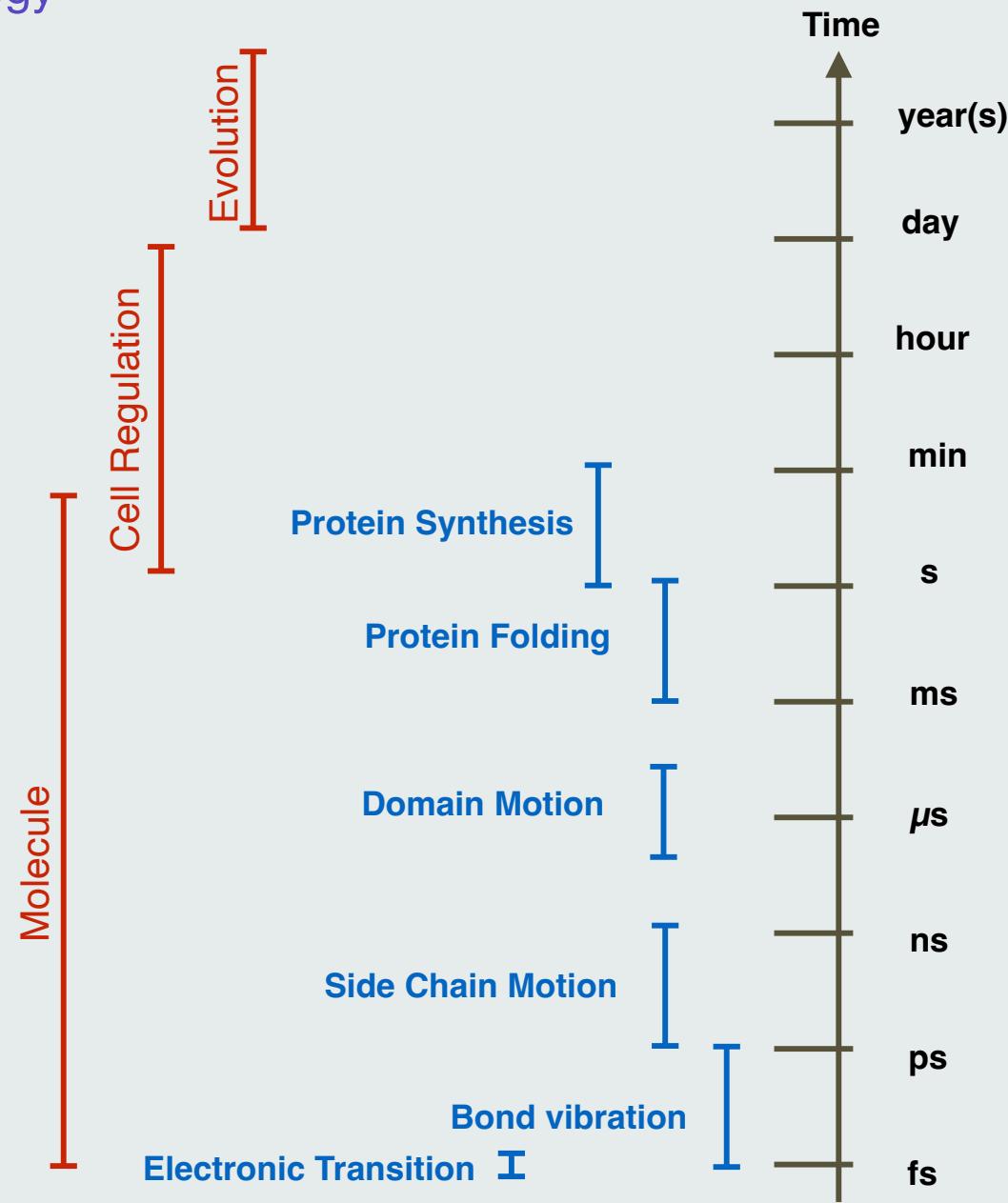
EXPERIMENTAL CHARACTERIZATION OF MEMBRANES



EXPERIMENTAL CHARACTERIZATION OF MEMBRANES



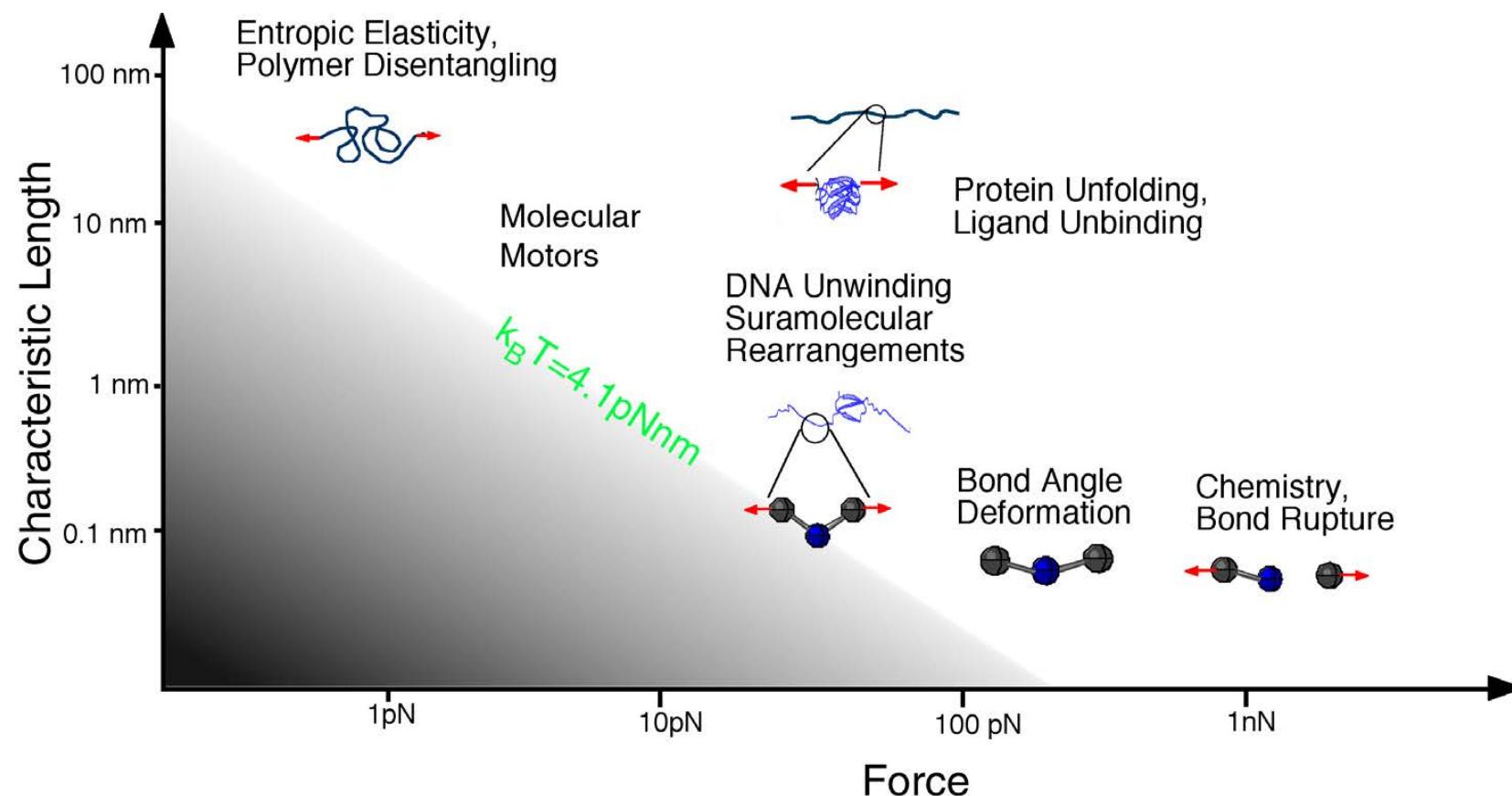
Time Scale in Biology



EXPERIMENTAL CHARACTERIZATION OF MEMBRANES



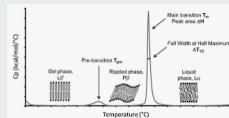
Force Scale in Biology (Costa's lecture)



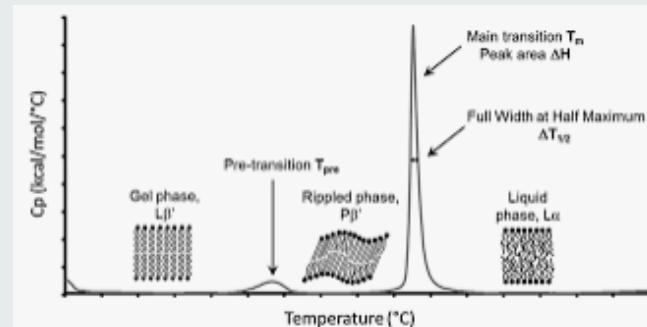
H. Clausen-Schauman, M. Seitz, R. Krautbauer & H.E. Gaub, Curr. Opp. Cem. Biol. 2000, **4**, 524-530

DSC

Differential Scanning Calorimetry

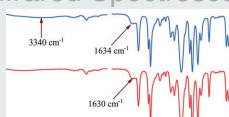


DSC works by measuring the amount of heat energy required to raise the temperature of the sample. By slowly ramping the temperature of the sample and accurately measuring temperature change, DSC can determine phase transition phases.

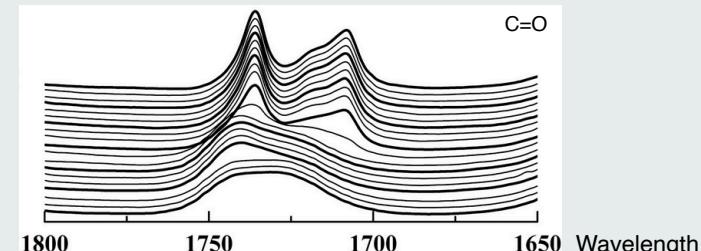
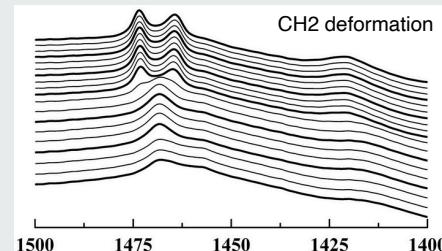
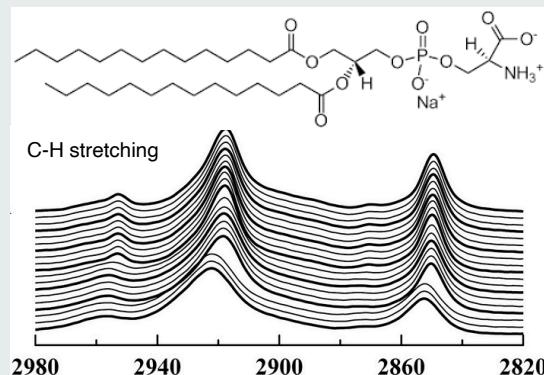


FTIR

Fourier Transform Infrared Spectroscopy



FTIR detects the absorbance of IR type light emission, which at certain wavelengths, is absorbed by different carbon-carbon bonds (chemical bond vibrations). From FTIR absorbance spectra, information about lipid conformation can be extrapolated.

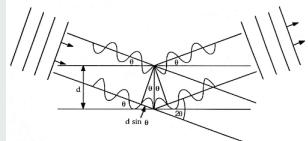


<http://dx.doi.org/10.1016/j.bbamem.2012.10.018>

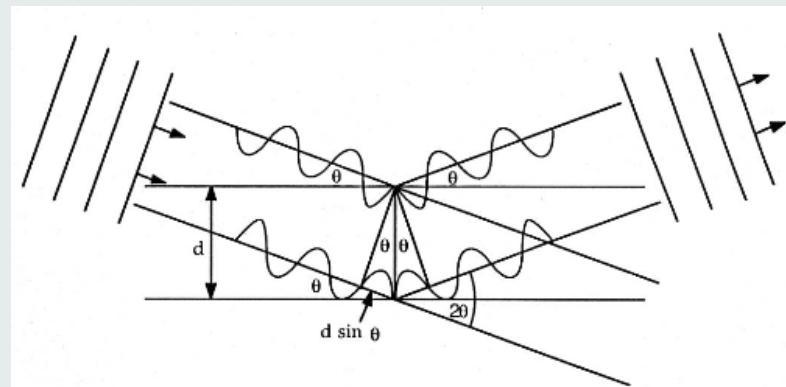
Lewis, R.N.A.H., McElhaney, R.N., 1996. FTIR spectroscopy in the study of hydrated lipids and lipid bilayer membranes. In: Mantsch, H.H., Chapman, D. (Eds.), *Infrared Spectroscopy of Biomolecules*. Wiley, New York, pp. 159– 202.

A table of IR absorbance's can also be found at www2.chemistry.msu.edu/faculty/reusch/virttxtjml/Spectrpy/InfraRed/infrared.html

Scattering techniques



- The sample is bathed in a monochromatic beam of radiation of wavelength λ , the incident beam direction being defined by the wave vector k_i , of magnitude $|k_i| = 2\pi/\lambda$.
- Each atom in the sample scatters a tiny fraction (wavelet) of the incident intensity in all directions.
- For elastic scattering the scattered wave has the same wavelength as the incident wave.

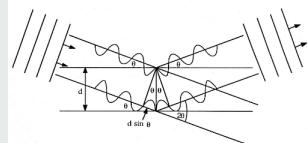


Bragg's Law

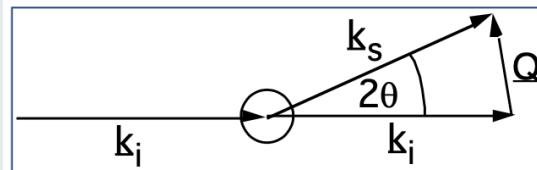
$$2d \sin \theta = n\lambda$$

$$s \text{ (scattering vector)} = n/d$$

Scattering techniques

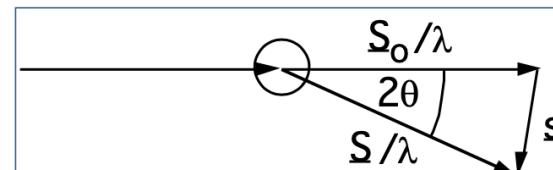


Neutron scattering



SANS

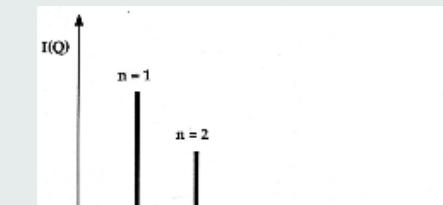
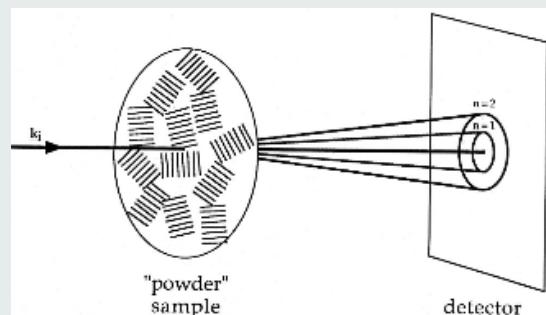
X-ray scattering



SAXS or WAXS

X-ray or neutron reflectivity and diffraction, Brewster angle microscopy, ellipsometry, X-ray interferometry, infrared reflection-adsorption spectroscopy, vibrational sum frequency generation spectroscopy, and any electron scattering techniques such as SEM or TEM

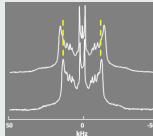
Characterizing the structure of the lipid membrane such as the membrane thickness, unit cell organization, or where in the membrane proteins prefers to reside.



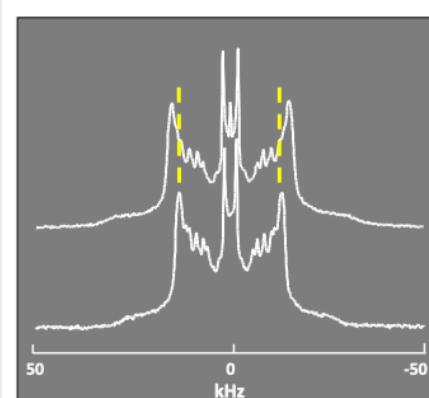
The observed Bragg peaks correspond to the different values of n.

NMR

Nuclear Magnetic Resonance



Nuclear magnetic resonance (NMR) refers to a property of certain atomic nuclei with a nuclear spin (e.g. ^1H , ^{13}C , ^{19}F , ^{31}P , ^{129}Xe ...), placed in a magnetic field. When subjected to electromagnetic radiation (radio frequency), most often applied in the form of pulses, the atomic nuclei can absorb the energy of the radiation and then release it during relaxation.



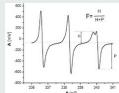
Required: lipids with perdeuterated acyl chains



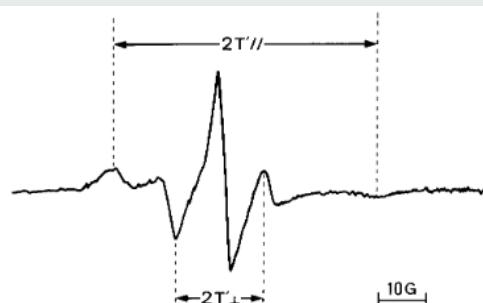
Lipids more ordered: increased quadrupolar splittings

EPR

Electron Paramagnetic Resonance



The basic concepts of EPR are known to be analogous with Solid-state NMR, except it is electron spins that are excited as opposed to spins of atomic nuclei.



Typical electron paramagnetic resonance spectra of erythrocytes for the fatty acid spin-label agents (5-nitroxide stearate, 5-NS). S: order parameter The greater the values of the order parameter (S) and the peak height ratio ($\text{I}_{\parallel}/\text{I}_{\perp}$), the lower the membrane fluidity of erythrocytes.

Biophys. Res. Commun., 275 (3), 2000, p. 946-954.

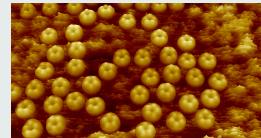
$$\text{Order Parameter (S)} = \frac{\text{T}'_{\parallel} - \text{T}'_{\perp}}{\text{T}_{zz} - \text{T}_{xx}} \cdot \frac{\alpha_n}{\alpha' n}$$

TECHNIQUES FOR DETECTING AND MEASURING LIPID PHASE TRANSITION

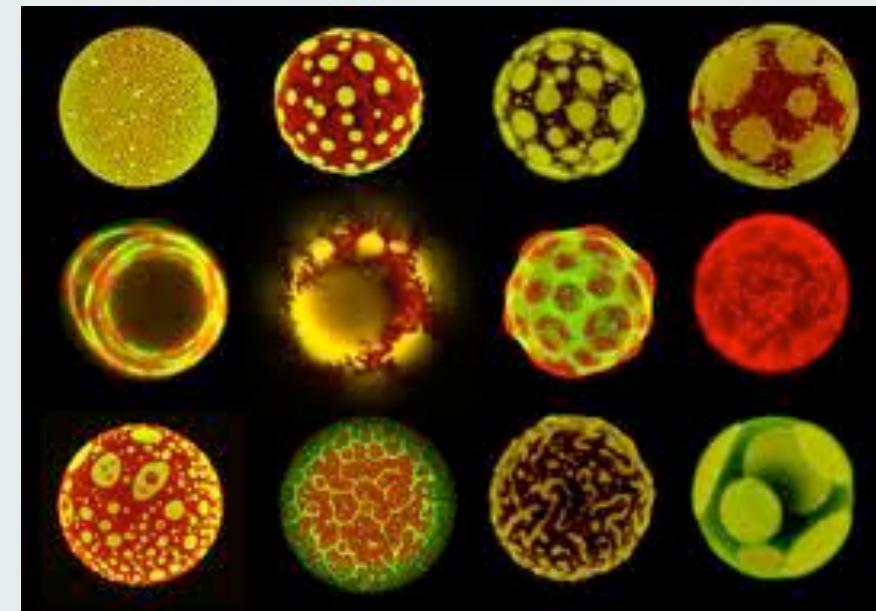
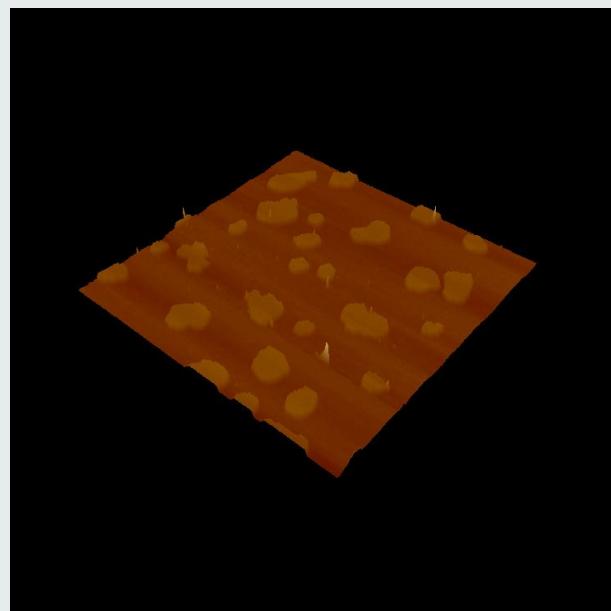
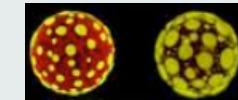


AFM

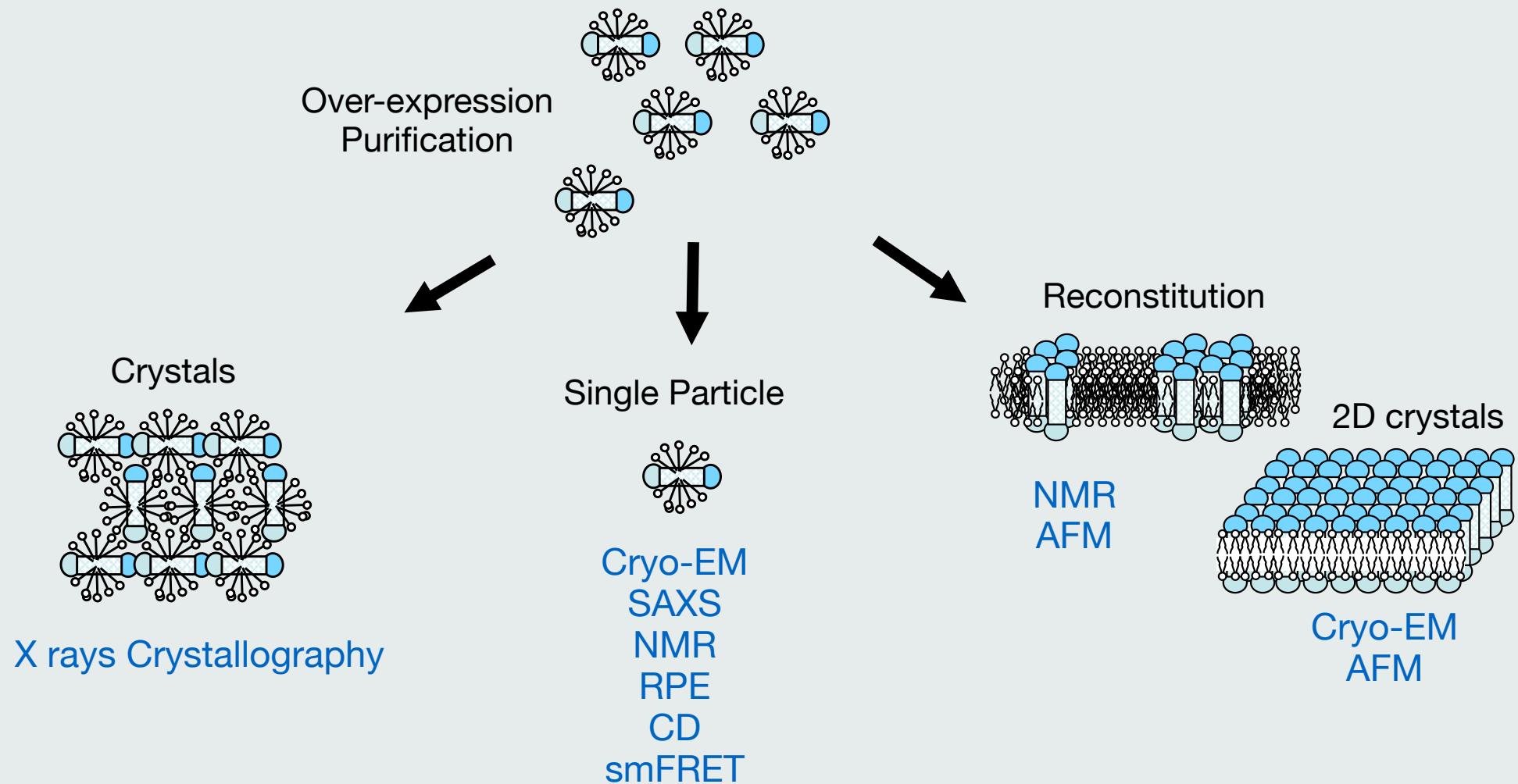
Atomic Force Microscopy



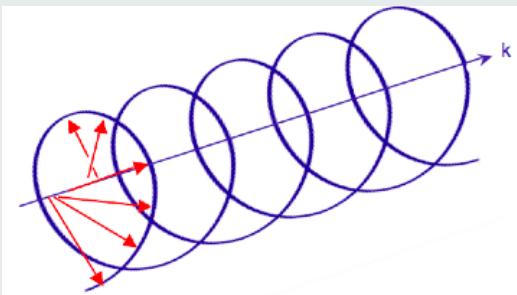
**Fluorescence
Spectro & μ copy**



STRUCTURAL ANALYSIS MEMBRANE PROTEINS



Circular dichroism (CD) is dichroism involving circularly polarized light, i.e., the differential absorption of left- and right-handed light.



- Absorption spectroscopy using circularly polarized light
- For biological molecules, UV and VUV (Vacuum UV, 100-200 nm) are the most important wavelength ranges
- Chiral or asymmetric structures produce characteristic signals
- Each type of secondary structure gives rise to a unique spectrum
- A dynamic technique for molecules in solution

CIRCULAR DICHROISM SPECTROSCOPY

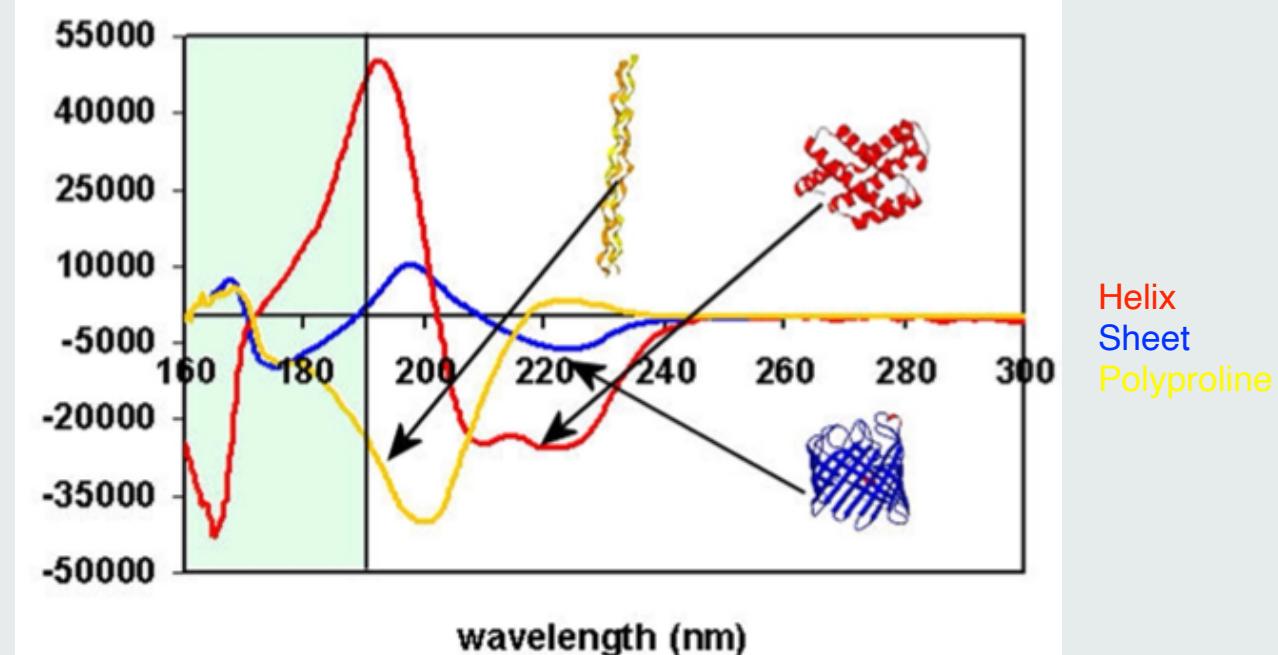


Spectral regions of interest:

Far UV: peptide bond

Near UV: W, Y, P

VUV: charge transfer



• Mean Residue Ellipticity ($\text{deg}\cdot\text{cm}^2\text{dmol}^{-1}\text{residue}^{-1}$)

$$[\theta] = \theta * 0.1 * \text{MRW}/cl$$

where θ is measured ellipticity
c is concentration in mg/ml
l is pathlength in cm

Advantages

- Small amount of material needed
- Small changes in conformation detectable
- Easy quantitation
- No probes needed

Information

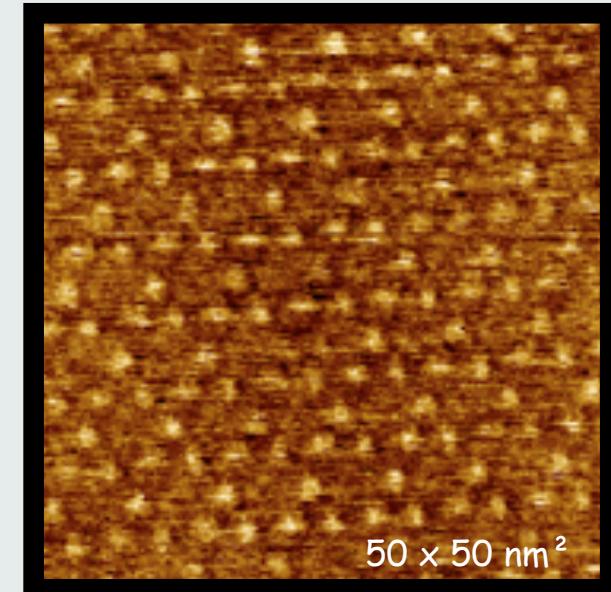
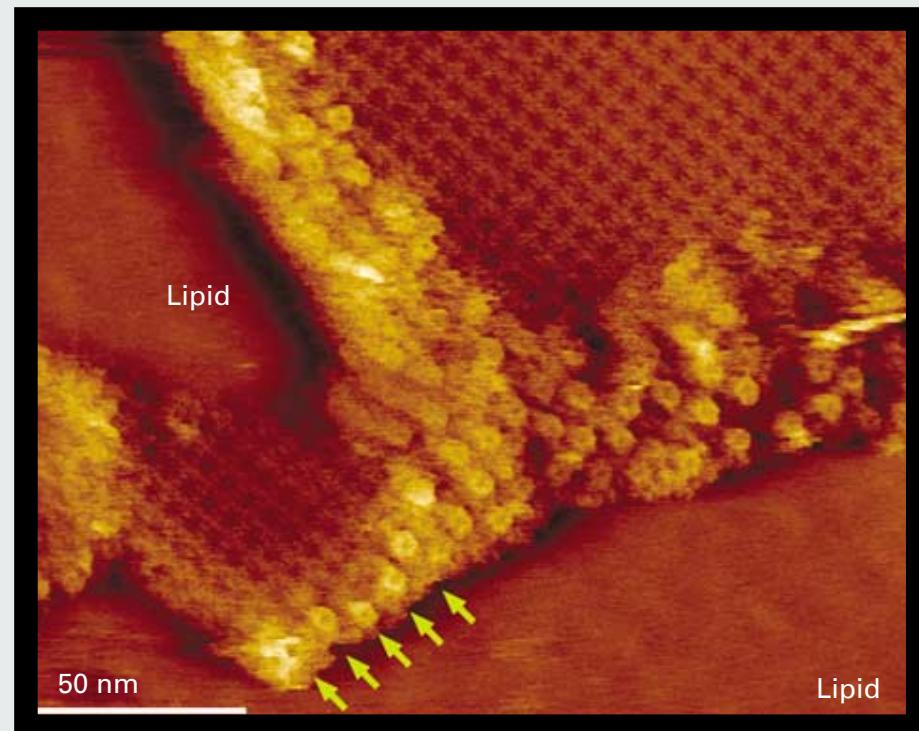
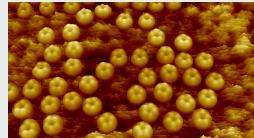
- Secondary structures, conformational changes, folding, thermal stability, molecular interactions...

TECHNIQUES FOR ANALYZING MEMBRANE PROTEINS



AFM

Atomic Force Microscopy



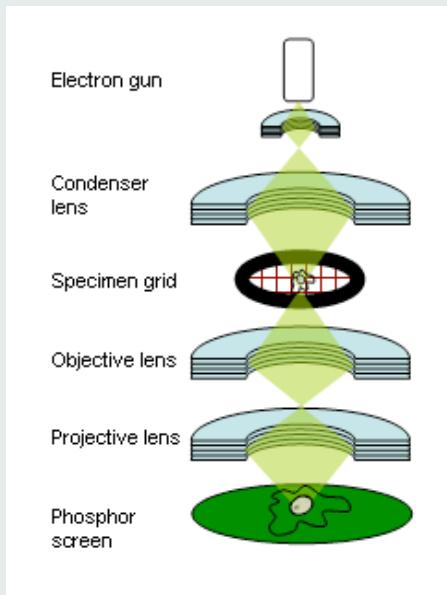
Bacteriorhodopsin

Supramolecular architecture of gap junctions in native lens membranes

CRYO-ELECTRON MICROSCOPY



The Nobel Prize in Chemistry 2017



© Nobel Media AB. Photo: A.Mahmoud

Jacques Dubochet

Prize share: 1/3



© Nobel Media AB. Photo: A.Mahmoud

Joachim Frank

Prize share: 1/3



© Nobel Media AB. Photo: A.Mahmoud

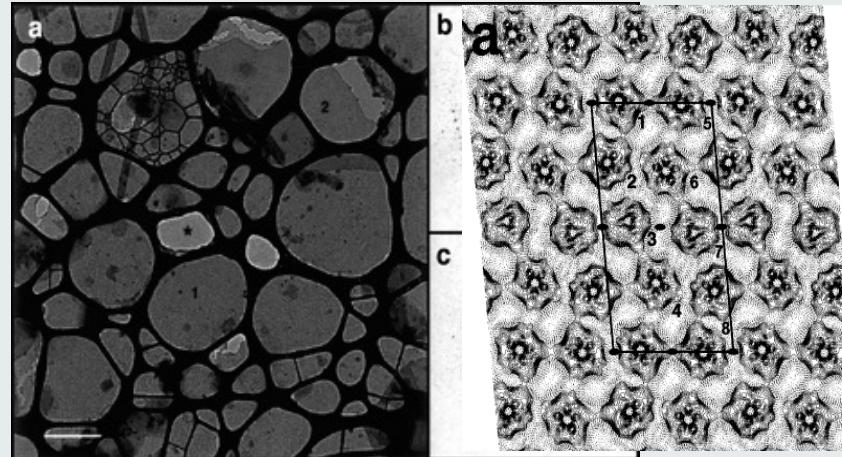
Richard Henderson

Prize share: 1/3

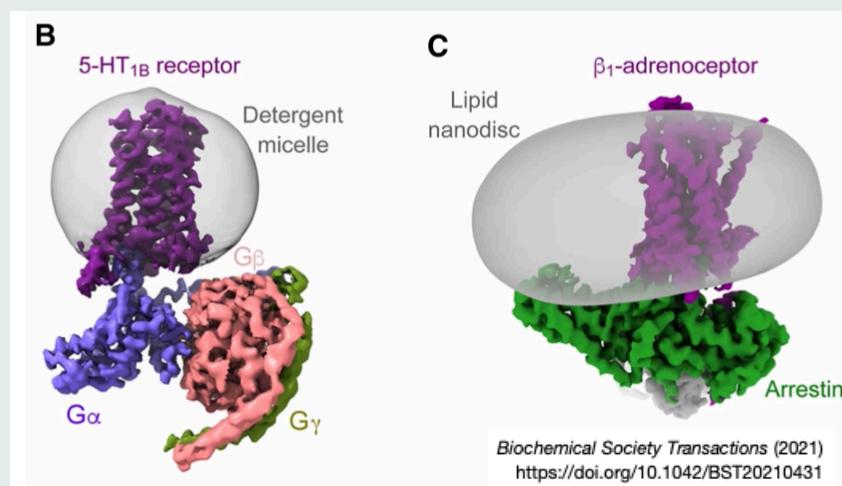
The Nobel Prize in Chemistry 2017 was awarded jointly to Jacques Dubochet, Joachim Frank and Richard Henderson "for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution."



In the membrane field



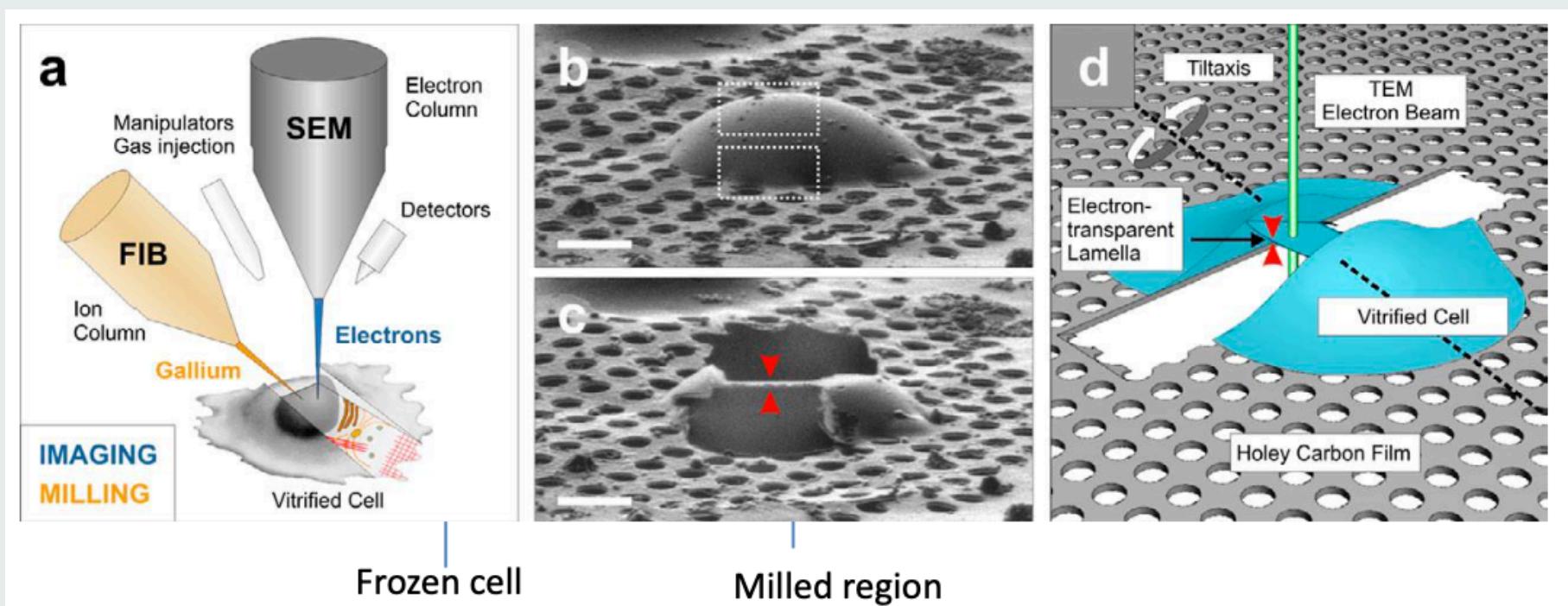
Electron crystallography
Shiga toxin B-subunit, D. Levy, Curie - 8.5 Å)



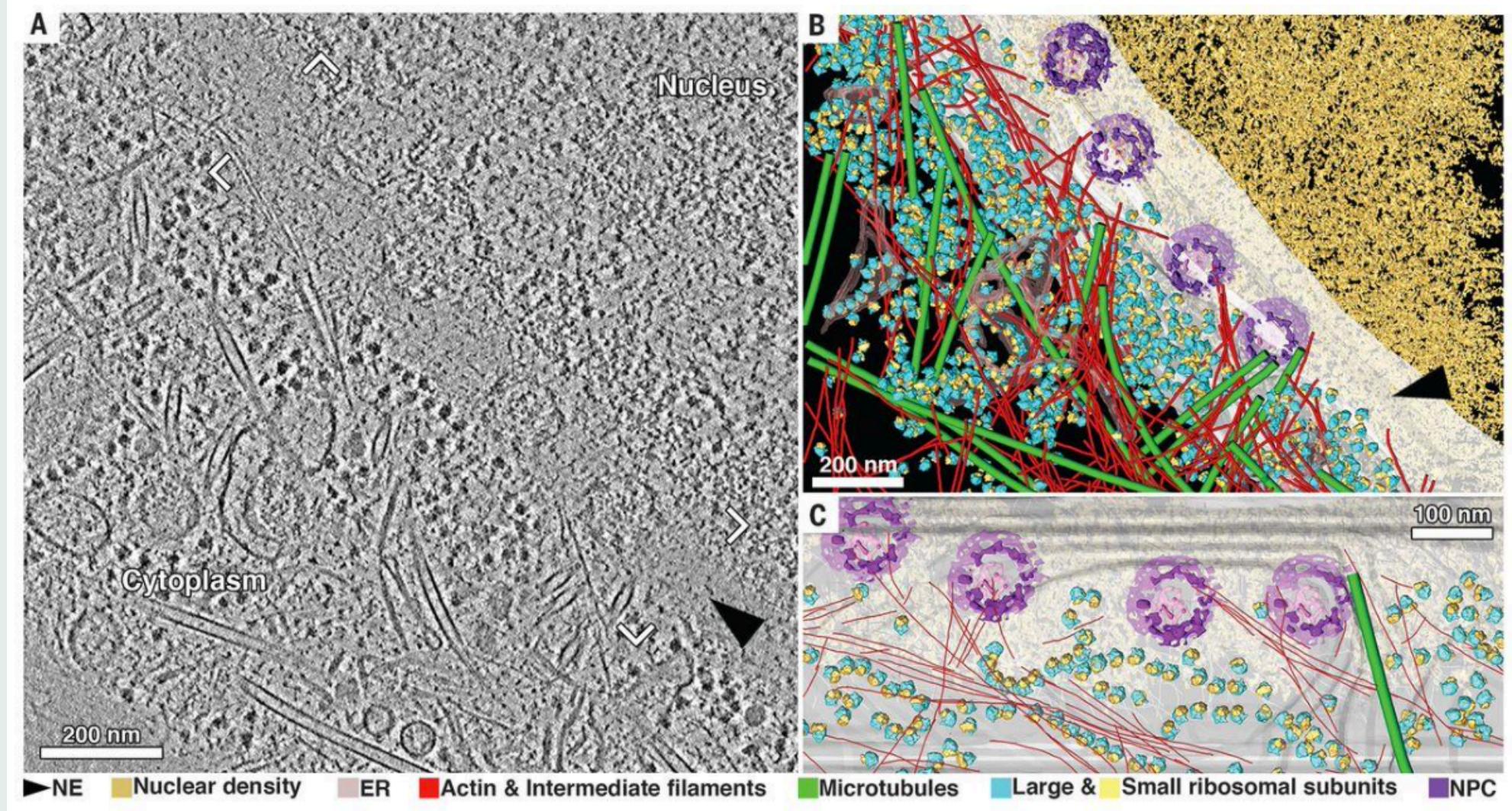
Single Particle Analysis

FIB (focused ion beam) et Cryo-FIB to see the interior of cells

Milling with an ion beam frozen eucaryotic cells to decrease the thickness of areas of interest before cryo-ET



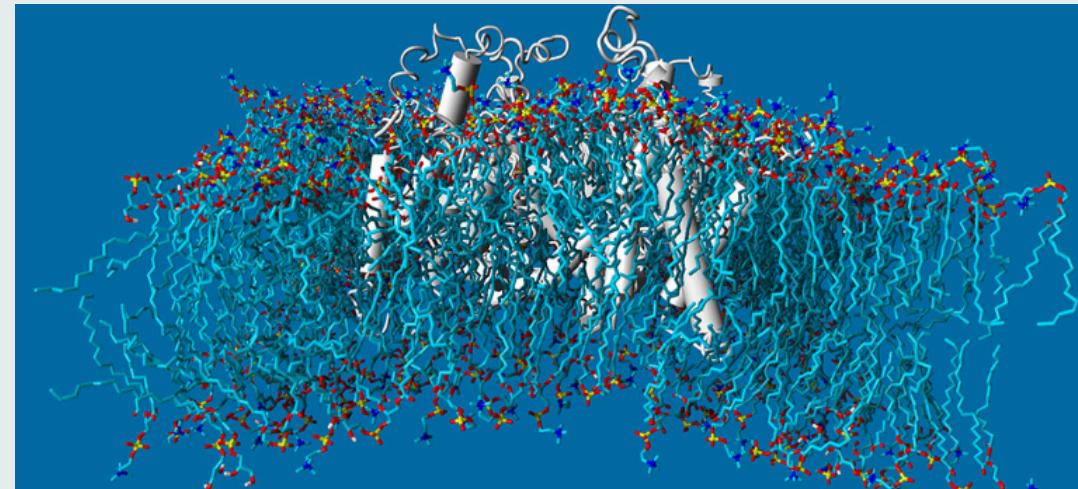
The nuclear periphery of a HeLa cell revealed by cryo-ET



MOLECULAR MODELING



Atomistic simulation



Coarse-Grained Simulation

