Lipid Bilayer Membranes: Materials Science and Materials Engineering

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At Duke

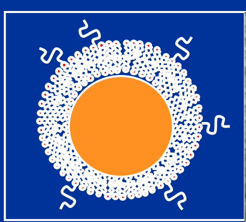
I am, and have been, a Professor in Mechanical Engineering and Materials Science, since 1987

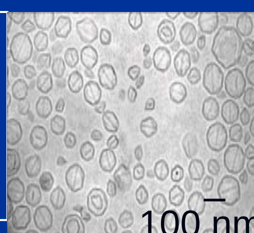
I teach classes in Biological and other Soft Wet Materials, Colloids and Surfaces, Biological Materials Science and Materials Engineering.

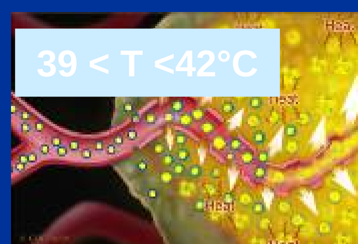
I work in my Lab with my students

I invented a cancer treatment which is now in clinical trials for prostate cancer

My new interests are in other 2-phase micro-systems, like micro-crystallization & Biomineralization in particular that has already inspired a new crystal nanocomposite

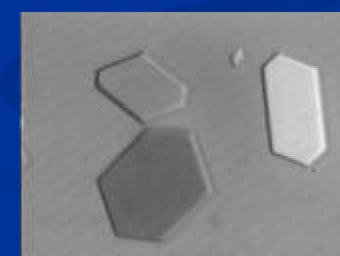


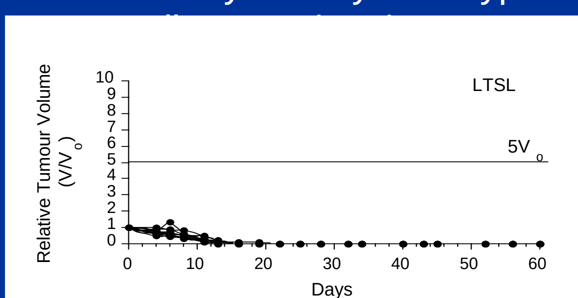




Temperature-sensitive liposome loaded with doxorubicin that releases drug in tumors when heated to only 41C by mild hyperthermia and

The abalone shell is a microlaminate composite of calcium carbonate crystals and proteins, (Morse UCSB)





Microcrystal (20 microns) of sodium chloride at 11M

Flat micro-crystals, inspired by the abalone, that flow with the turbulence and streamlines in this liquid filled bottle

Materials Matrix for Traditional Engineering

	Composition	Structure	Property	Performance + Processing
Metals				
Ceramics				
Polymers				
Semi Conductors				
Composites				

Please Learn This

- All materials used in products and processes engineered for society are made from four classes of materials and/or their combination as composites, --metals, ceramics, plastics, and semi conductors,
- (What are the equivalent materials that make up Biology?)
- characterize relationships between the material's
  - **composition** (atomic, chemically distinct entities)
  - **structure**, (atomic, defects, crystal or amorphous, microstructure),
  - **properties**, (the ratio of what you do, to how it responds, like stress/strain, giving the modulus of elasticity),
  - **processes** for shaping material into a product, (molding in plastics, or drawing and rolling in metals, or sintering in ceramics)
  - **performance-in-service**.
  - (Biology is also a series of products and processes).

How might we define Biology?

Can we define Biology via its materials?

biological materials science and materials engineering

	Composition	Structure	Property	Performance - Processing
lipids				
sugars				
amino acids				
nucleotides				
Salts and Water				

what you do
how it responds

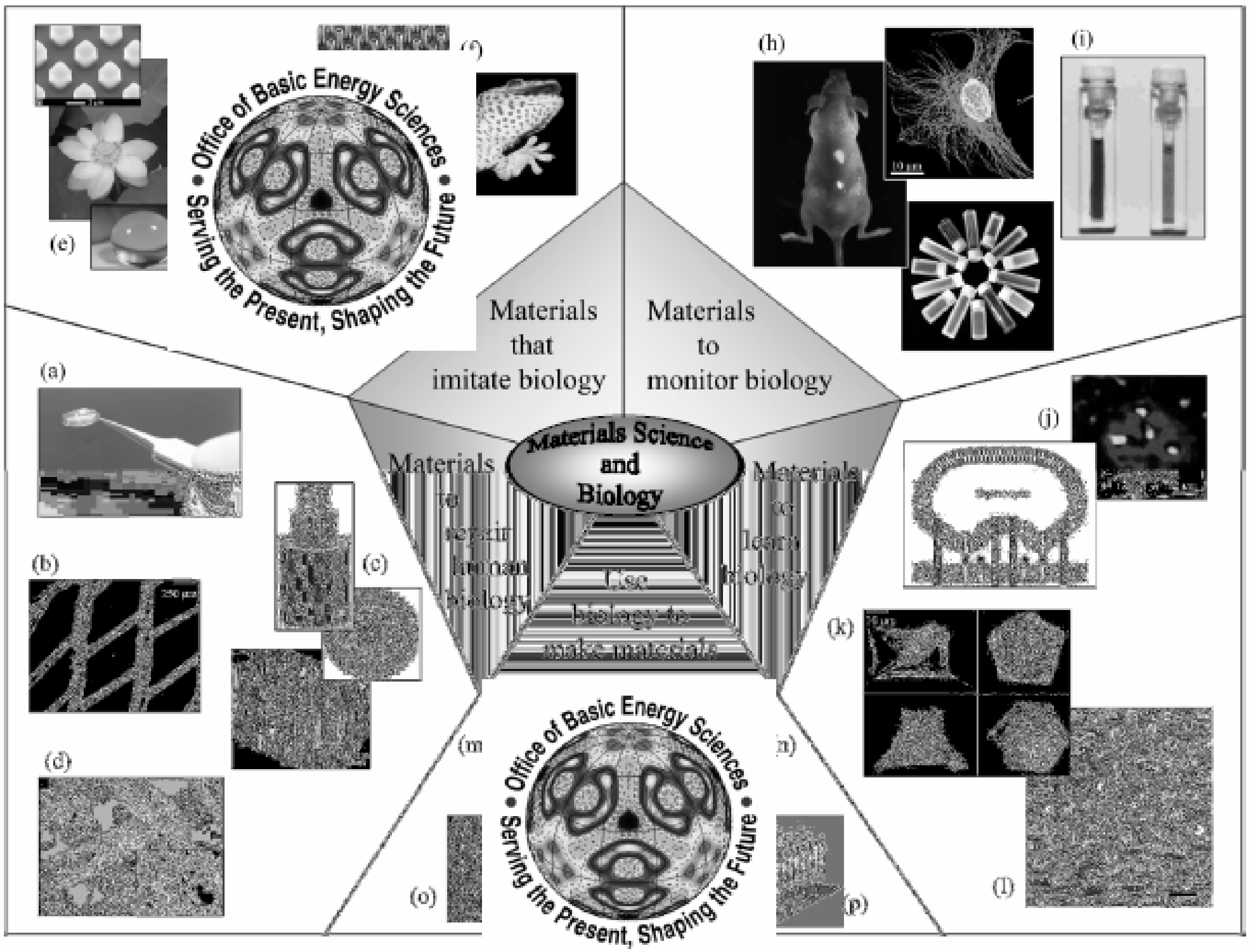
Please Learn This

Molecular Composites, Association Structures, Cells, Higher order system….
organs, tissues, organisms
Much of Biology is at the **Nano and Micro** scale

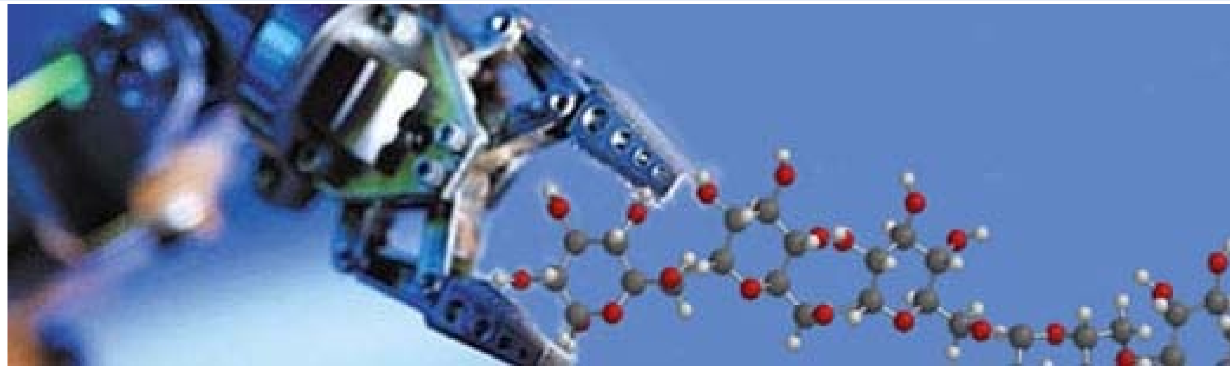
- Inherent in Biology's Products and Processes is DESIGN (Workshop I)

Materials Science and Biology Interface

Coutrtesy: *Sam Stupp, Northwestern University*



Duke University
CENTER FOR BIOLOGICALLY INSPIRED MATERIALS & MATERIAL SYSTEMS



Mapping Engineering onto Biology

--Reverse engineering nature's designs for molecules, membranes and cells: "what were the problems nature solved?", "How did nature solve the …x… problem?"

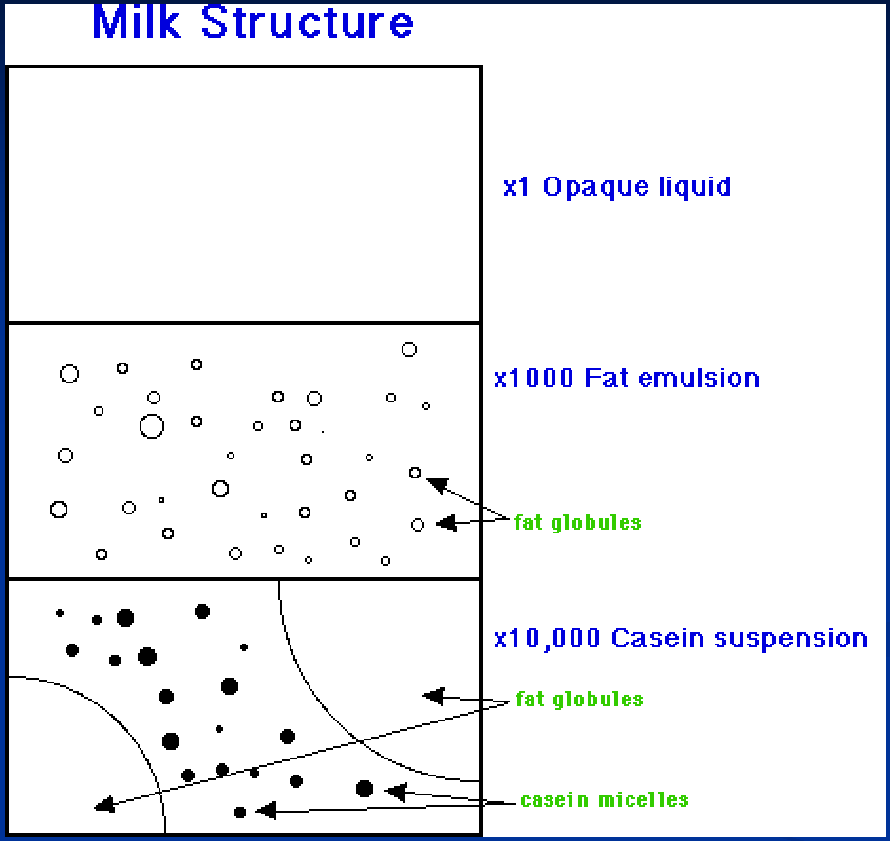
--Developing and using techniques to quantify composition structure, and properties at the nano and micro scale

--Forward engineering new products and processes inspired by nature's solutions

Outline

- 1 Its all about the Water: Oil and water don't mix
- 2 Cell Membranes
- 3 The Lipid Membrane as a Material: Composition
- 4 Lipid Membrane as a Material: Structure
- 5 Lipid Membrane as a Material: Properties
  - Mechanical, Electrical, Thermal Properties
  - Permeability, Molecular Exchange
  - Intersurface Interactions
  - Surface-Molecule Interactions
- 6 Lipid Membrane as a Material: Processing
- 7 Forward Engineering Bioinspired Membranes?
  - In Vivo Drug Delivery and Drug Release??

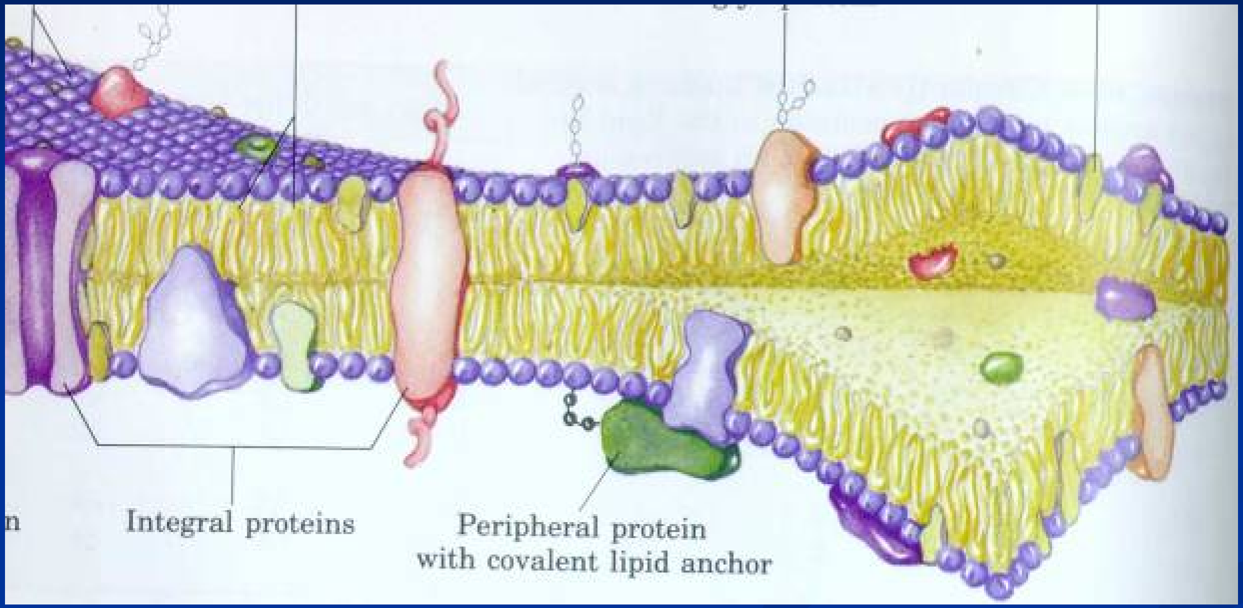
Let's make some milk??



- Requires homogenization, fluid forces……
- How did nature do it?
- Same physical and chemical compositions, structures, forces, properties and performance of these materials are present in every cell of every organism on the planet, --here the processing is different!

http://www.foodsci.uoguelph.ca/dairyedu/chem.html

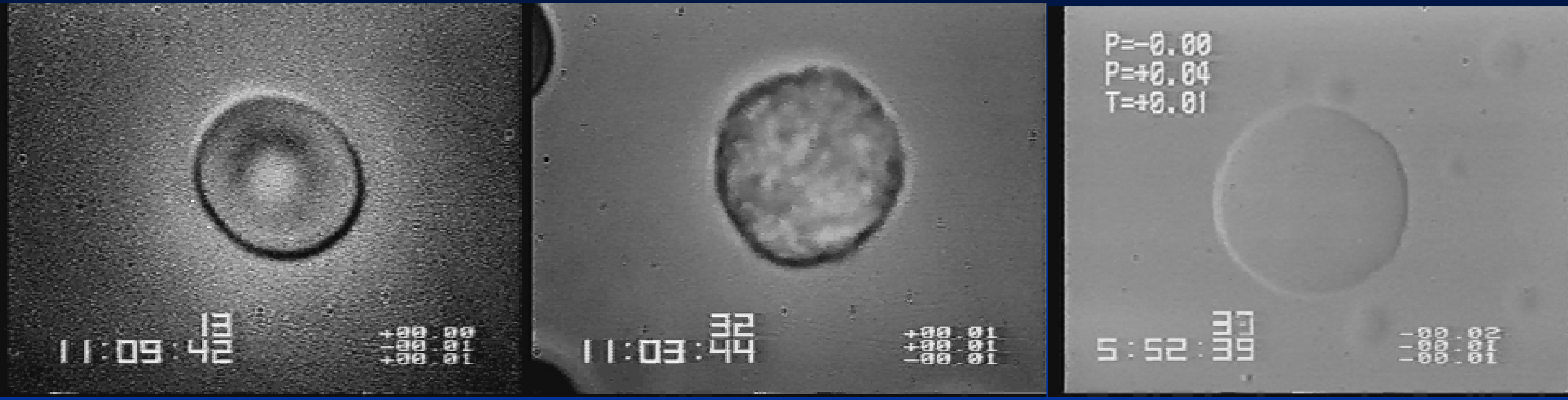
2 Cell Membranes: PERFORMANCE



http://www.bme.umich.edu/Research_lab_sites/BioMembrane/WebsitePics/pmembrane2.jpg

- multi-component cell membrane
- lipids, bilayer structure
- but what are its properties? --for pure lipid and lipid mixtures
- Understanding the Cell membrane is a motivation, but what about the lipid bilayer as an interesting material??

Some Lipid-Membrane-Bound Capsules



A *red blood cell (7 microns diameter) --delivers O_2, $t_{1/2}$ 60 days*

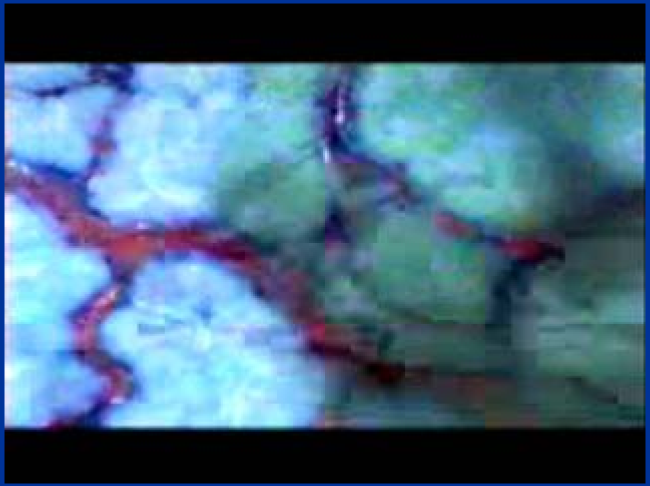
B *neutrophil (8 microns diameter) --"micron machine"! adhesion, motility, pharma*

C *flaccid giant lipid vesicle (12 microns diameter)*

What are the composition-structure-**<u>property</u>** relationships that allow them to perform their function?

Lipid-Membrane-Bound Capsules for transport in the blood stream

EM Picture by Ping Beal



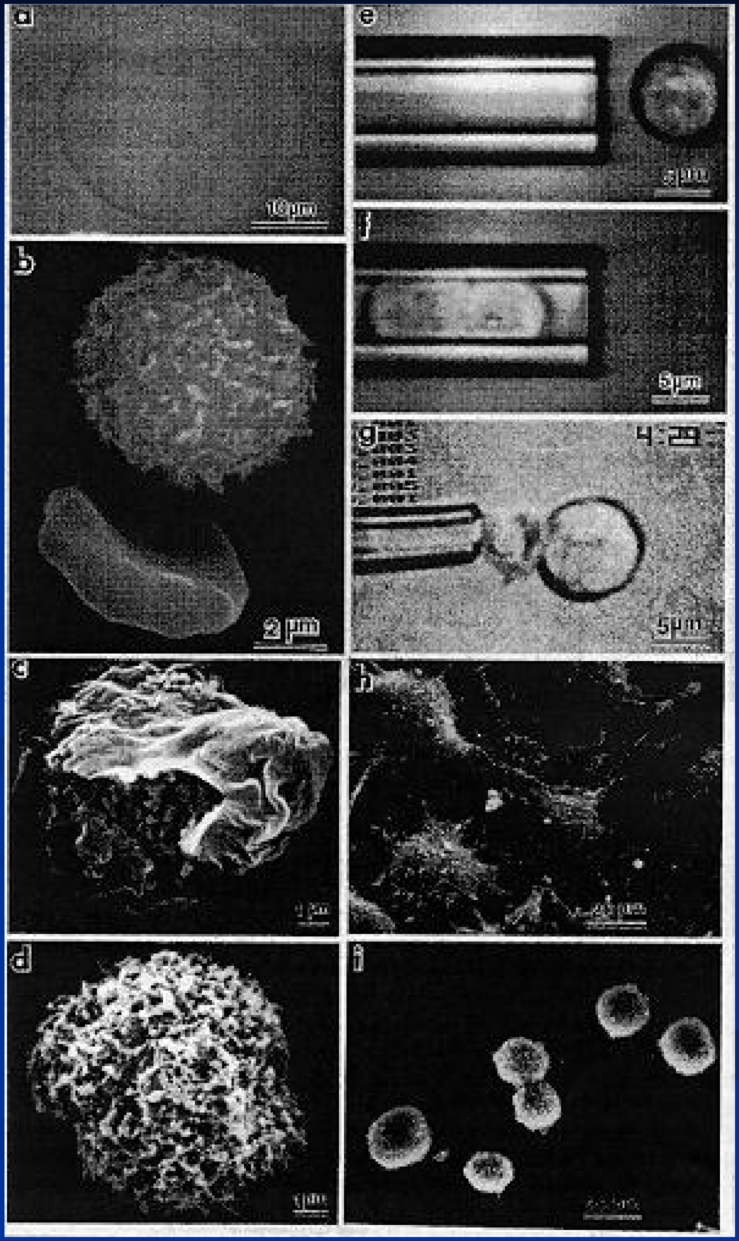
- both cells can deform and flow into vessels with diameters considerably less than their natural diameters.

neutrophil (8 microns diameter) --"micron machine"! adhesion, motility, pharma
excess surface area as microvilli is available to expand the projected area whilst staying in tact.

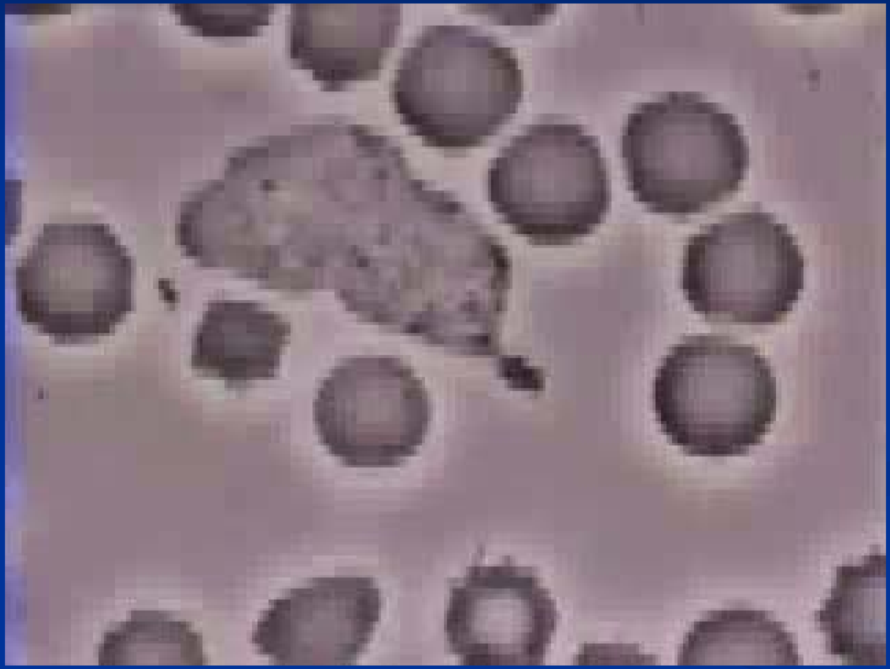
red blood cell (7 microns diameter) --delivers O_2 $t_{1/2}$ 60 days
smooth with excess area as flaccid membrane supported by spectrin network, --capable of being deformed into many different shapes at constant volume

Performance Requirements: Mechanical Properties of Lipids (bending)

- Lipid membrane (a) is a mechanical, chemical and electrical barrier that has to have flexibility and strength in order to perform its capsular function
- It has to bend around protein networks (b, c, d, h, i), and remain in tact during cellular extensions in deformation (e,f) and phagocytosis (g)



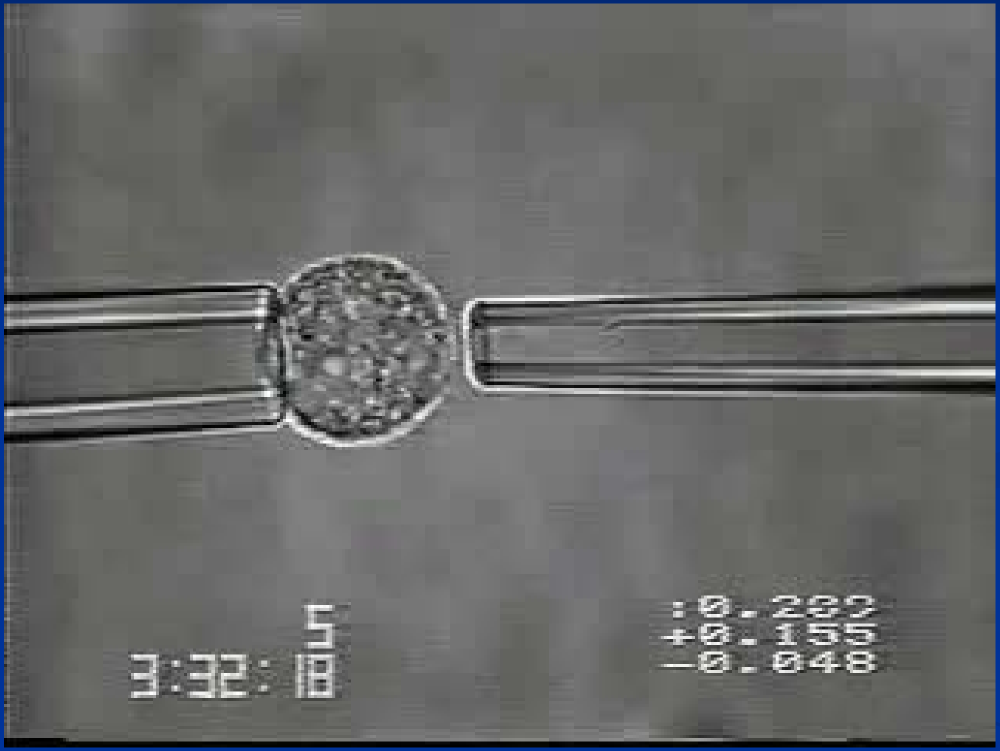
Neutrophil crawling after a bacterium



membrane stays in tact as cell adheres and crawls over surface chasing bacterium, and eventually engulfs it.
(David Rogers, Vanderbilt University, 1950s)
http://www.biochemweb.org/fenteany/research/cell_migration/neutrophil.html

Pseudopod extension due to blowing chemoattractant

(Doncho Zhelev)

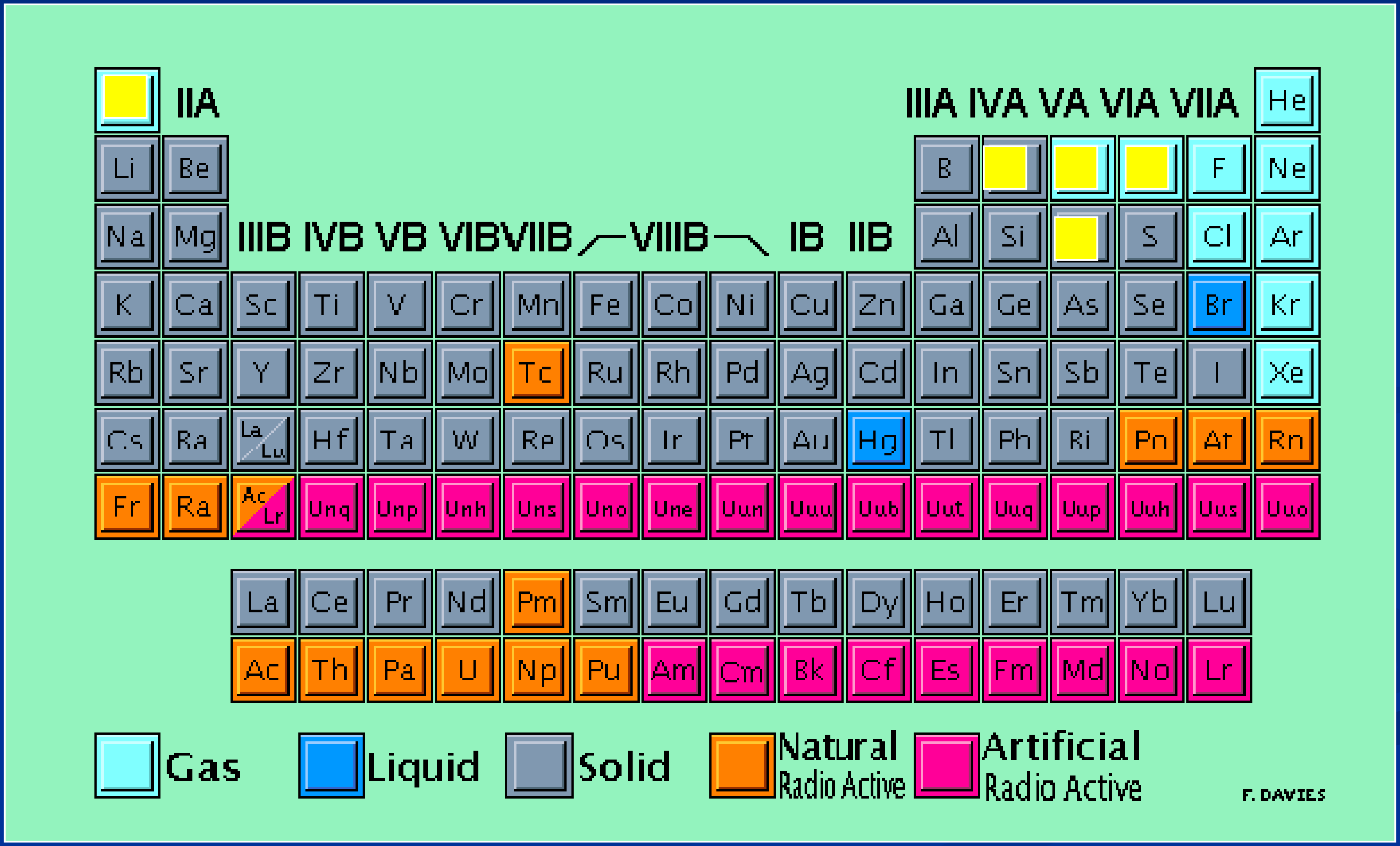


Again, even though cell creates new and extending stiff structures under membrane, Lipid membrane maintains integrity

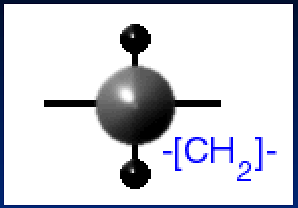
So Let's look at the LIPID BILAYER MEMBRANE as a Material

3 Lipid Membrane as a Material: Composition (and Molecular Structure)

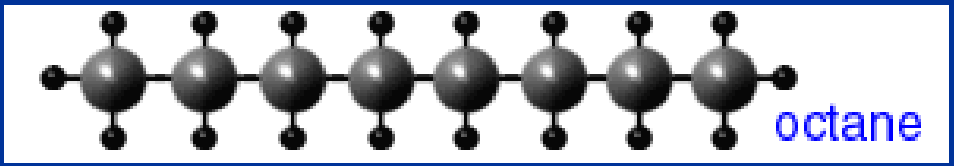
- H, C, O, P, N, ...



Hydrocarbons

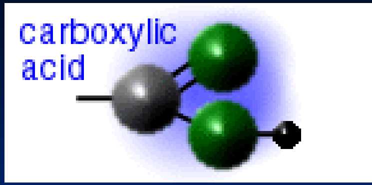


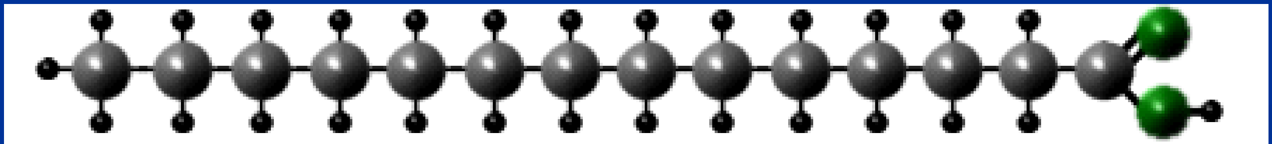
- The basic 'monomer' from which general hydrocarbons are constructed is a -[CH_2]- unit. These are joined together in long, straight chains to form molecules such as **octane**.



- Hydrocarbons contain and store a lot of energy in their bonds, and are thus good fuel molecules (gasoline, for example contains a lot of hydrocarbons). However, they are strongly hydrophobic (they 'hate' water), so it is very difficult for living cells and organisms to manipulate and use pure hydrocarbons.
- About the only use for nearly pure hydrocarbons is wax, e.g. candles, or because it is so strongly hydrophobic, it is used as a waterproofing material, e.g car wax.

Fatty Acids



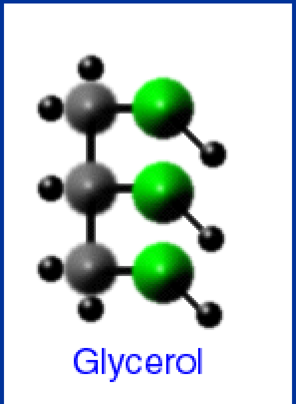
- Fatty acids consist of long, unbranched hydrocarbons with a carboxylic acid group at one end. The number of carbon atoms in a fatty acid molecule is usually even (6, 8, 12, 32, 36, etc.), although it is not impossible to find a fatty acid with an odd number of carbon atoms in its structure.
- 
- While the long, hydrocarbon chain of the fatty acid continues to be strongly hydrophobic, the presence of the carboxylic acid group at one end of the molecule adds some hydrophilic properties. Small fatty acids such as propionic acid (with 3 carbon atoms) mixes with water readily, caproic acid (with 6 carbon atoms) is only 0.4 percent soluble in water.

Saturated and Unsaturated

- Typical animal fatty acids: palmitic (C16), and stearic (C18),
- carbon atom is linked with two hydrogen atoms ($-CH_2-CH_2-CH_2-CH_2-CH_2-$).
- -- **saturated** fatty acids.
- Animals also contain fatty acids in which there are less hydrogen atoms joined to some of the carbon atoms, and a double bond between two carbon atoms takes their place. These are the **unsaturated** fatty acids, such as oleic acid ($CH_3-[CH_2]_7-CH{=}{=}CH-[CH_2]_7-COOH$), which is the most common fatty acid found in nature.
- Unsaturated fatty acids melt at lower temperatures than saturated fatty acids, -- liquids at room temperatures.
- some fatty acids in which there are more than one double bond, such as linolenic acid.

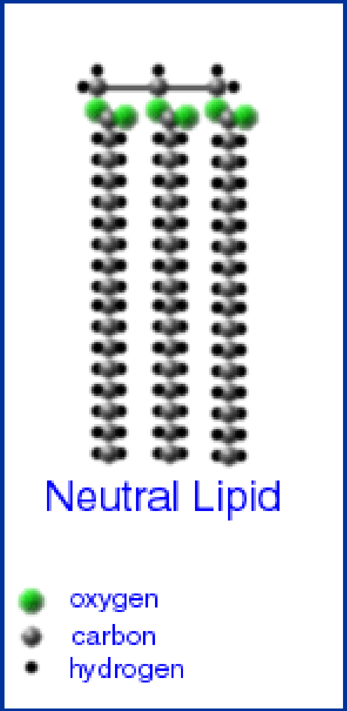
Glycerol

- Glycerol, -- a very thick, sticky, sweet tasting liquid that dissolves easily and readily in water.
- forms more complex molecules by reacting with e.g. fatty acids, or inorganic reactive groups, e.g., phosphate.
- -- **ethers**, -- general name **glycerides**.
- SO, one fatty acid linked to a glycerol molecule is called a **monoglyceride**.



Glycerides

- Neutral lipids are very abundant in nature.
- -- consist of one, two or three fatty acid molecules joined to one molecule of glycerol, -- mono-, di-, or triglycerides.
- Fats are insoluble in water, --most animal fats contain mainly palmitic, stearic, palmitoleic, oleic and linoleic fatty acids in their structure.



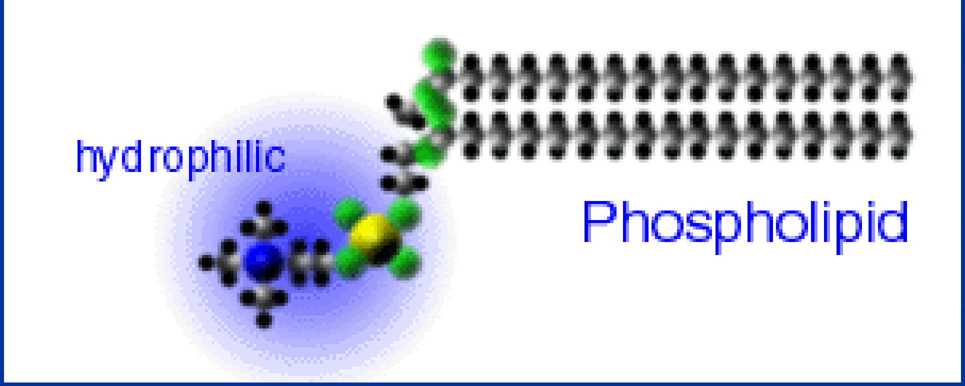
Fatty acids in Animals

Acid	Human	Cow	Pig
Palmitic	23	29	27
Stearicic	6	22	10
Palmitoleic	6	-	-
Oleic	50	40	59
Linoleic	10	2	4

approximate composition in molar percentage

Phospholipids

- -- a second class of glycerol based lipids in which (usually) two fatty acid molecules and one phosphate reactive group are all joined to one glycerol molecule.
- These phospholipids play many roles in cells, but one of their most important is in the cell membrane



Phospholipid Molecule

(different representations)

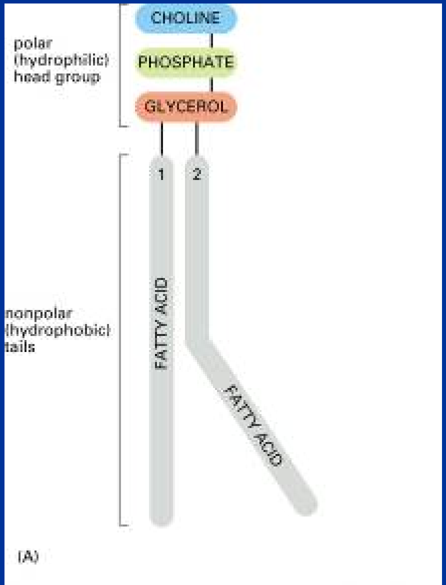


Figure 10–2 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

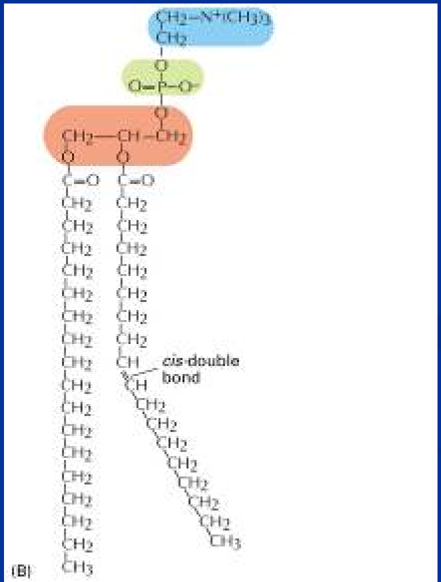


Figure 10–2 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

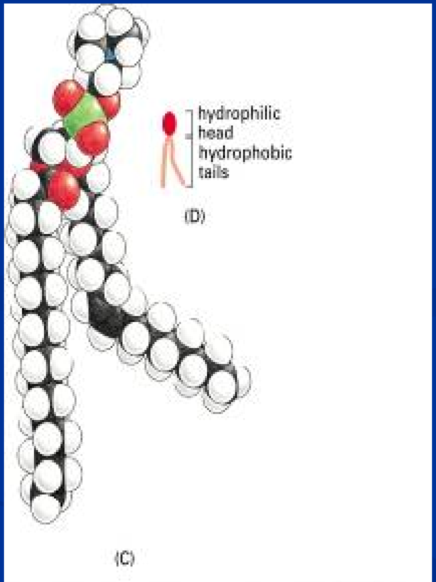


Figure 10–2 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

Phospholipids

4 major phospholipids in mammalian cell membranes

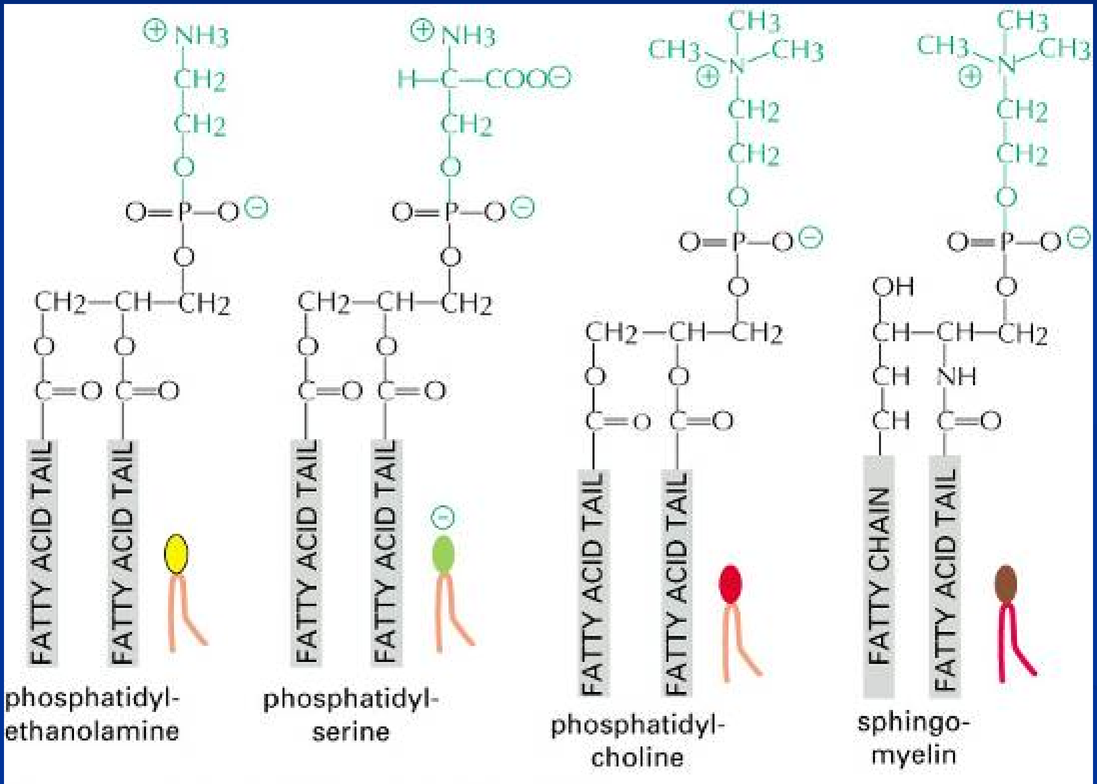


Figure 10–12. Molecular Biology of the Cell, 4th Edition.

inside outside

Cholesterol

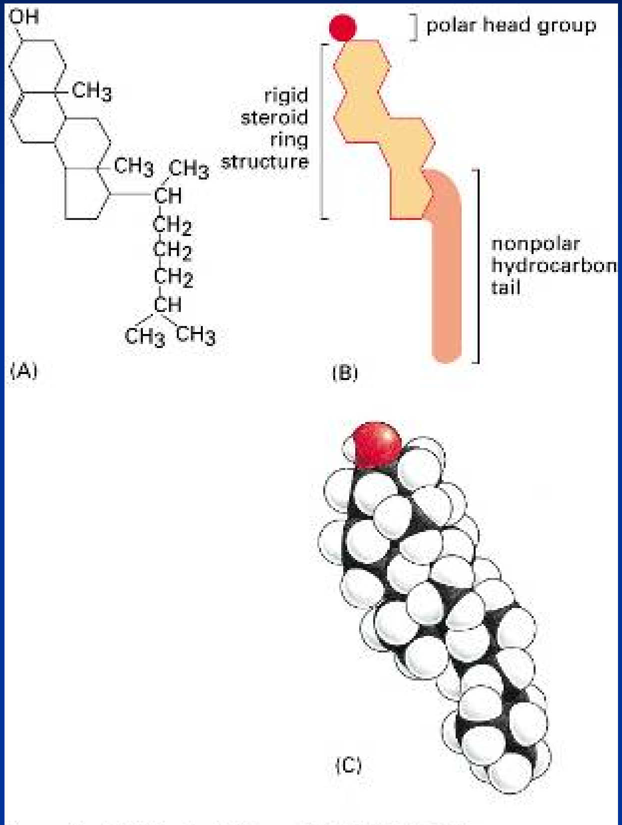


Figure 10–10. Molecular Biology of the Cell, 4th Edition.

Cholesterol influences material properties of bilayer via its molecular structure and interaction (packing) with lipid molecule

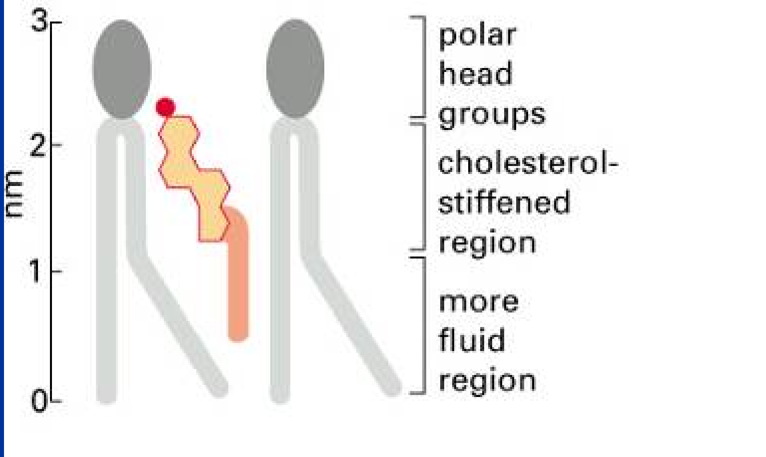


Figure 10–11. Molecular Biology of the Cell, 4th Edition.

4 Lipid Membrane as a Material: Bilayer Structure

- Biological and other soft wet materials:
- water is an organizing solvent
  - hydrophillic and hydrophobic effects help create molecular structure and self associating colloids
  - limits mass of solutes in solution by solubility
- influences energy of interactions
  - in electrodynamic and electrostatic intermolecular interactions it discounts the energy of interaction compared to vacuum by its dielectric constant of 80
- "Life began in the oceans, and conditions in that primordial environment put a permanent stamp on the chemistry of living things"
- Life hinges on the properties of water!! Because it is part of the composition and helps determines structure.....

Water Molecule

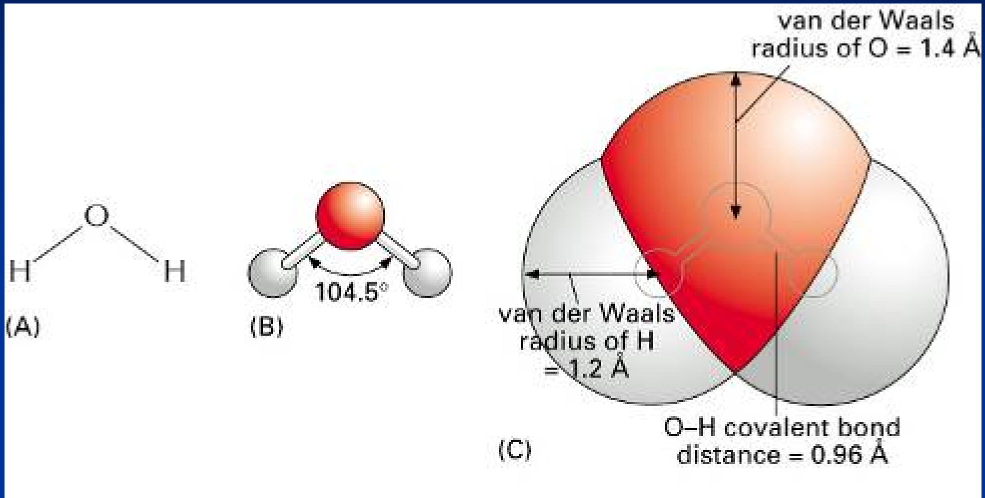


Figure 2–12. Molecular Biology of the Cell, 4th Edition.

70% of cell weight

very polar molecule forms hydrogen bonds with itself and other molecules that have polar or charge character

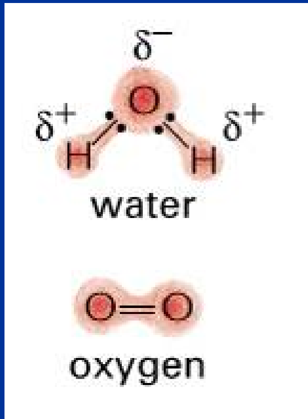


Figure 2–10. Mole

H_2O --polar covalent bonds

O_2 --non-polar covalent bond

some molecules form acids and bases with water

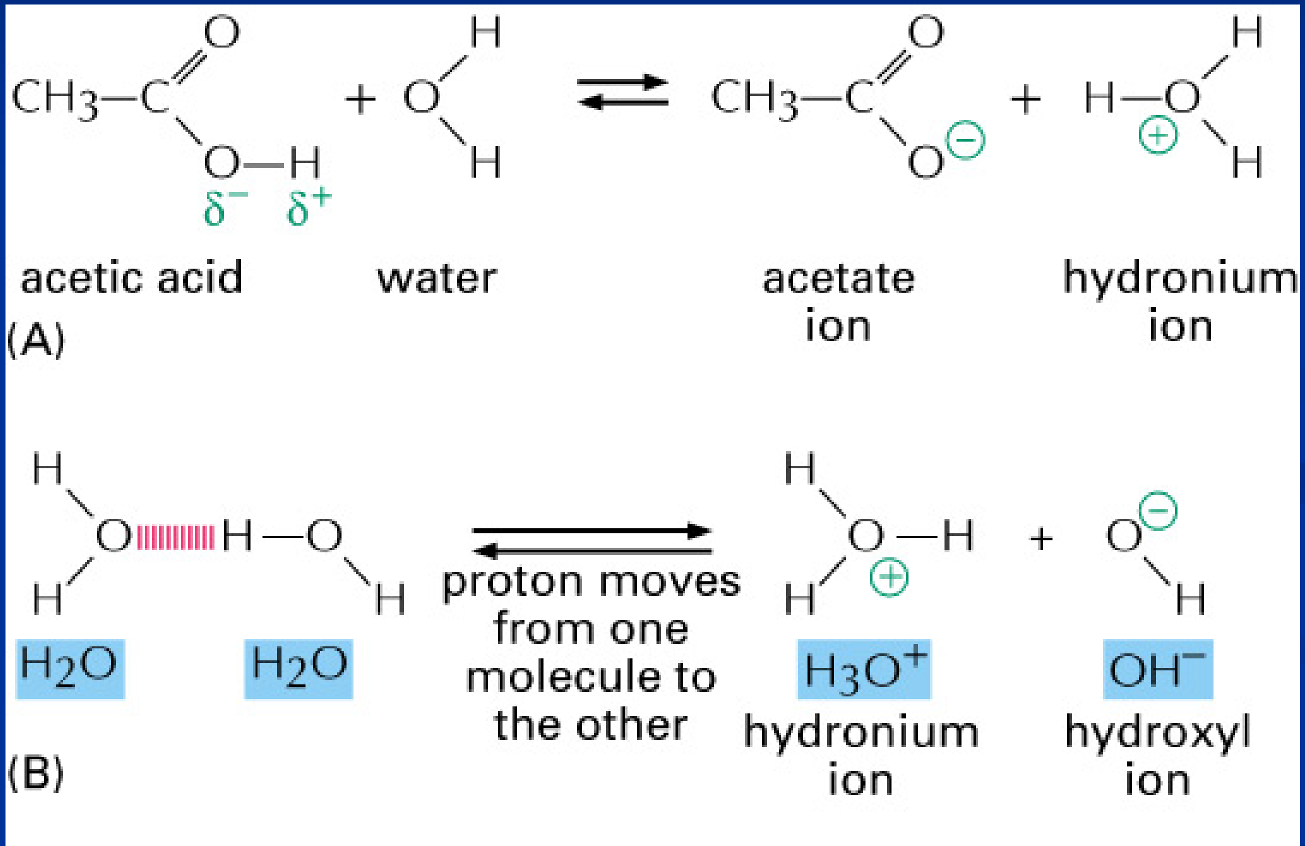
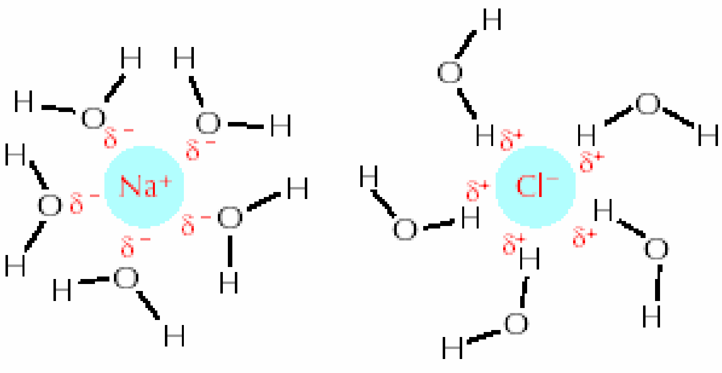


Figure 2–13. Molecular Biology of the Cell, 4th Edition.

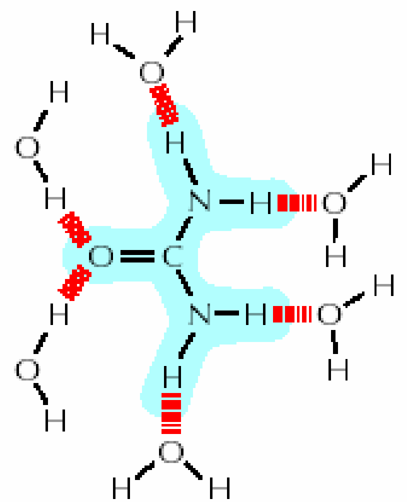
Salts: ions dissociate and become hydrated

HYDROPHILIC MOLECULES

Substances that dissolve readily in water are termed hydrophilic. They are composed of ions or polar molecules that attract water molecules through electrical charge effects. Water molecules surround each ion or polar molecule on the surface of a solid substance and carry it into solution.



Ionic substances such as sodium chloride dissolve because water molecules are attracted to the positive (Na^+) or negative (Cl^-) charge of each ion.



Polar substances such as urea dissolve because their molecules form hydrogen bonds with the surrounding water molecules.

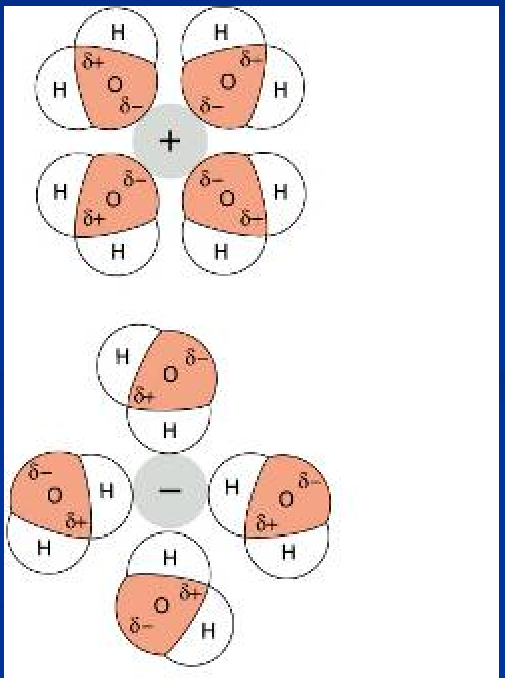


Figure 2–14. Molecular Biology of the Cell, 4th Edition.

6M

10M

Hydrophilic and hydrophobic molecules interact differently with water

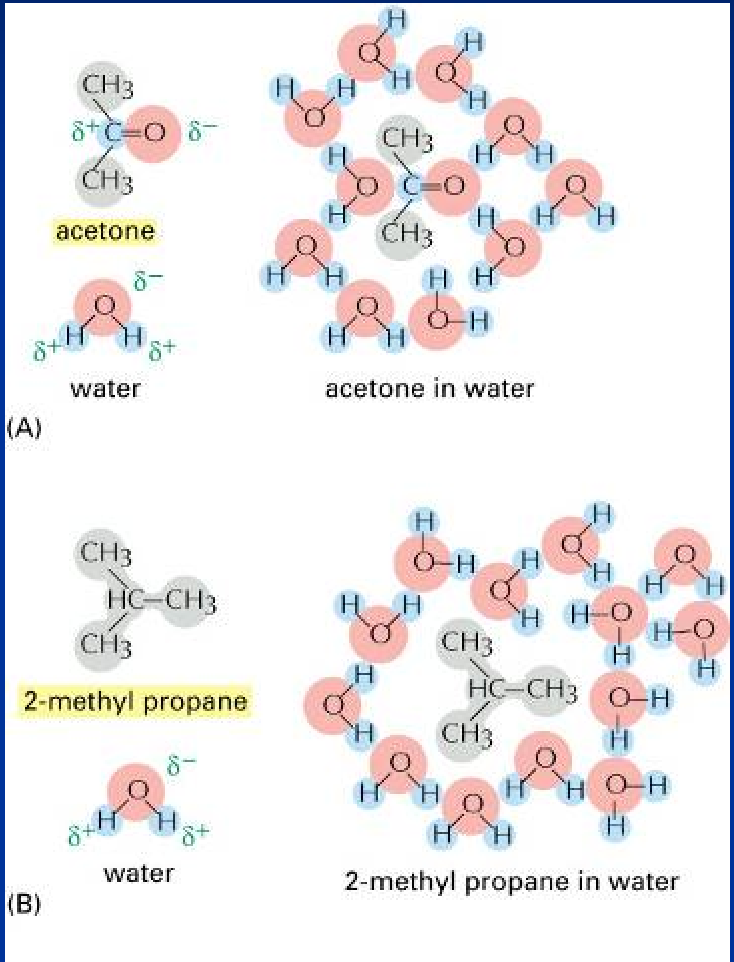


Figure 10-3. Molecular Biology of the Cell, 4th Edition.

Acetone is polar and forms favorable interactions with water and so readily dissolves

2-methyl-propane is non-polar, can't form favorable interactions and in solution forces water to form ice-like structure, so virtually insoluble.

Lipids Self-Associate to form Bilayers

e.g., SDS

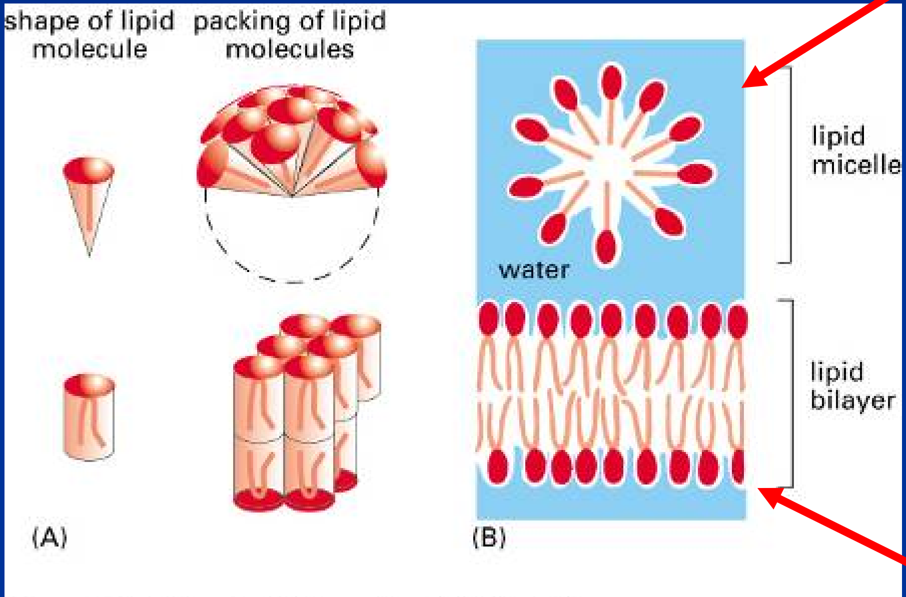


Figure 10–4. Molecular Biology of the Cell, 4th Edition.

Lipid molecules spontaneously form aggregates that bury hydrophobic (insoluble) HC tails in the aggregate interior and expose hydrophilic heads to water.

Molecular shape dictates micelles or bilayer (or inverted micelle)

e.g., Phospholipids

Lipid Bilayer is a 2 D fluid

Lipid molecule mobility

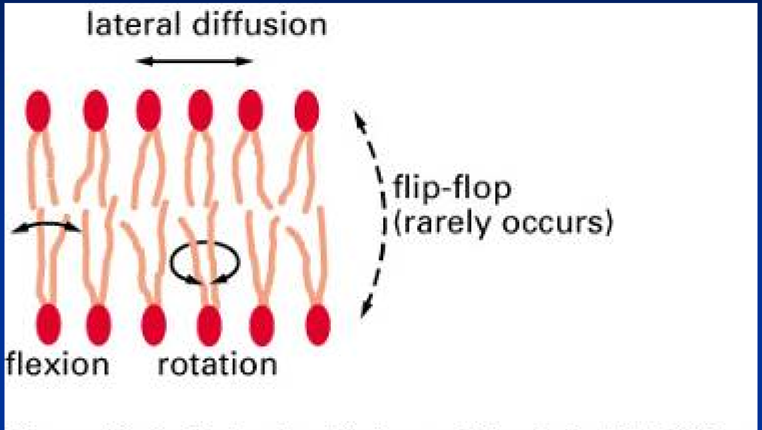


Figure 10–8. Molecular Biology of the Cell, 4th Edition.

effect of cis double bond on packing

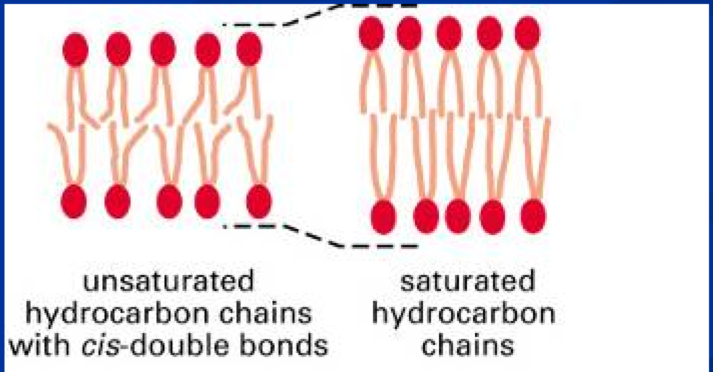
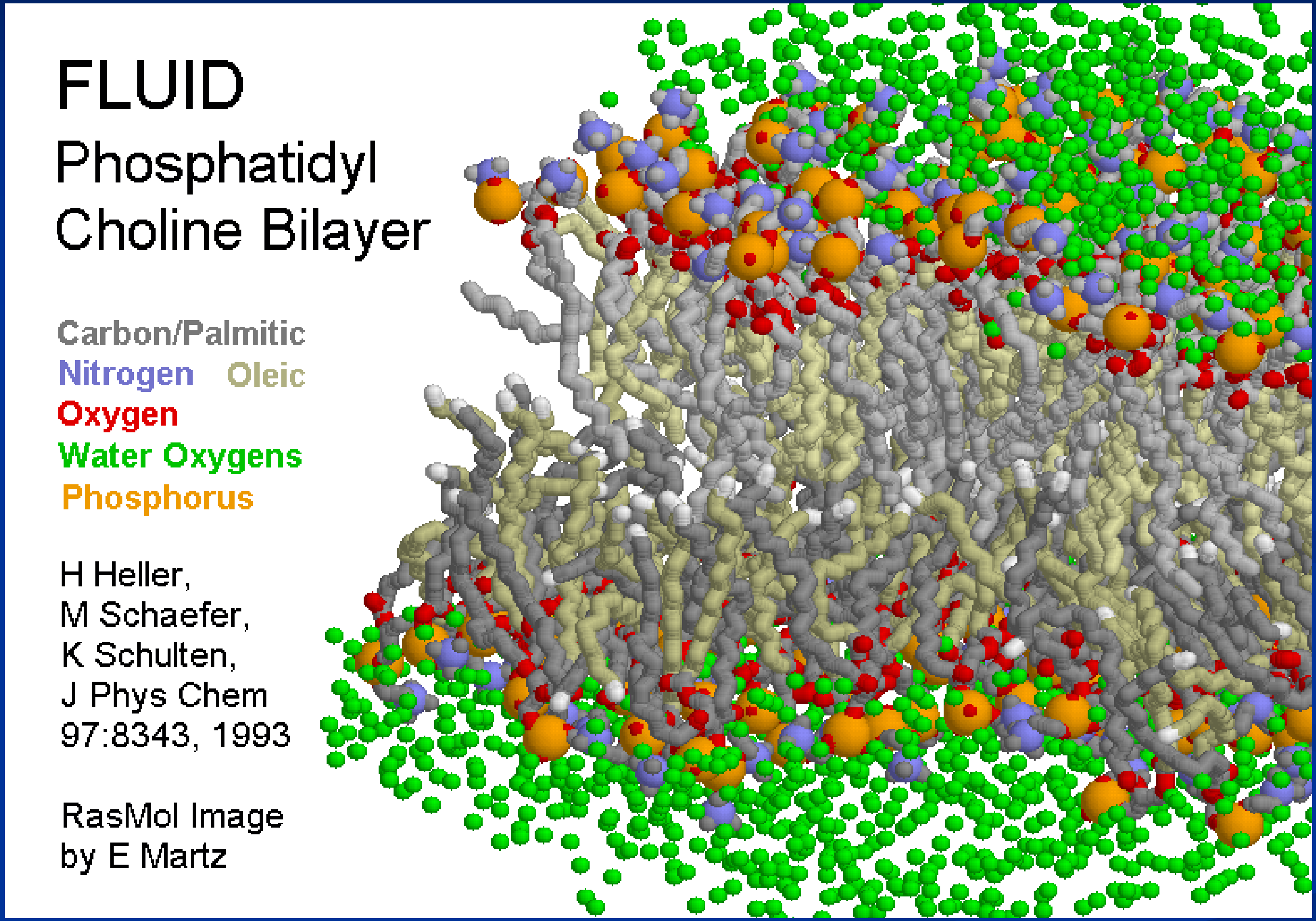
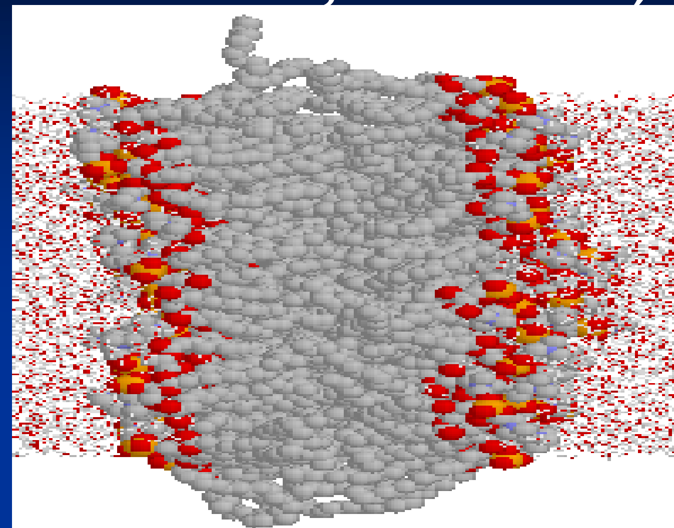


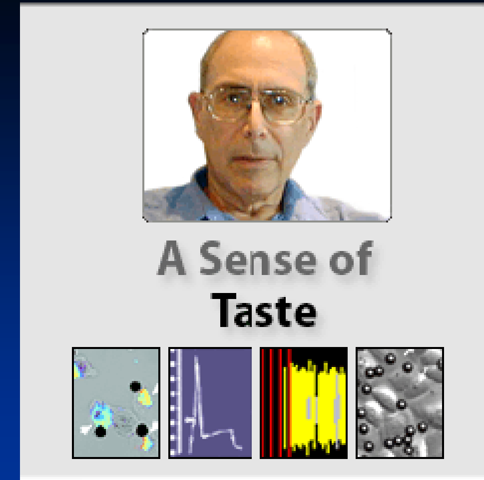
Figure 10–9. Molecular Biology of the Cell, 4th Edition.

Bilayers are Hydrated!!



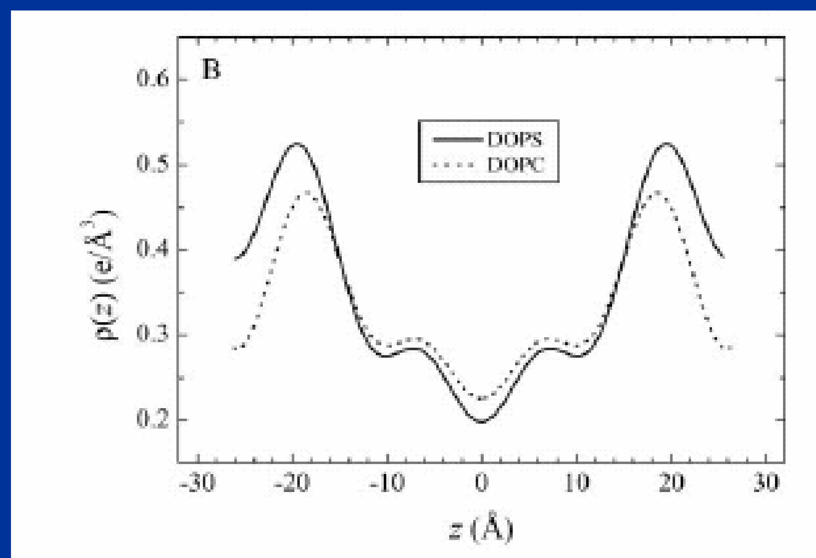
X-ray Diffraction
(Tom McIntosh, Sid Simon)





- 128 Phospholipiden (POPC)
- ~ 3000 Wassermoleküle
- Boxgröße 6x6x7nm

Liquid Phase – fluid



http://www.ciw.uni-karlsruhe.de/mvm/bio/projekte/adsorption_liposomen.html

STRUCTURE!! Nuclear Pore Complex Structure and Dynamics Revealed by Cryoelectron Tomography

Ju¨rgen M. Plitzko

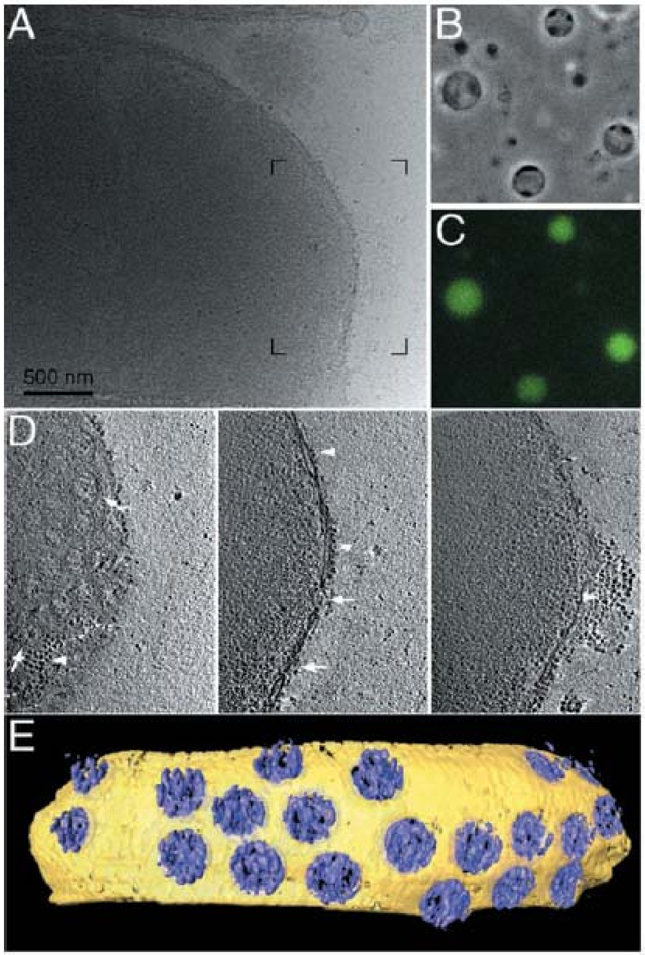


Fig. 1. Cryo-ET of transport-competent nuclei. (A) Transmission electron micrograph of a vitrified *Dictyostelium* nucleus. The image was recorded after acquisition of a complete tilt series; the frame marks the area representative for the reconstruction shown in (D). (B) Phase-contrast image and (C) the corresponding fluorescence image showing uptake of the transport substrate (FITC-BSA-NLS) into isolated, enriched nuclei. (D) Three-dimensional reconstruction of an intact nucleus. Three sequential *x-y* slices of 10 nm thickness along the *z* axis through a typical tomogram are indicated. Different orientations of NPCs are shown: top-views (left) and side-views (right, arrows). Ribosomes connected to the outer nuclear membrane are visible, as is a patch of rough ER (right, arrowheads). (E) Surface-rendered representation of a segment of nuclear envelope (NPCs in blue, membranes in yellow). The dimensions of the rendered volume are 1680 nm × 984 nm × 558 nm. The number of NPCs was ~45/μm².

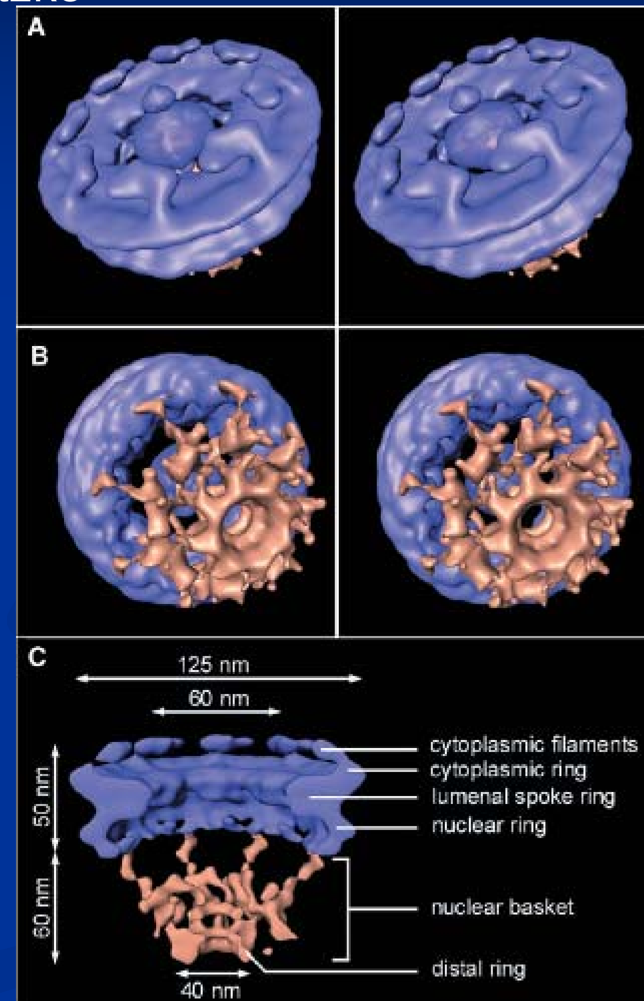


Fig. 2. Structure of the *Dictyostelium* NPC. (A). Cytoplasmic face of the NPC in stereo view. The cytoplasmic filaments are arranged around the central channel, they are kinked and point toward the CP/T. (B) Nuclear face of the NPC in stereo view. The distal ring of the basket is connected to the nuclear ring by the nuclear filaments. (C) Cutaway view of the NPC with the CP/T removed. The dimensions of the main features are indicated. All views are surface-rendered (nuclear basket in brown).

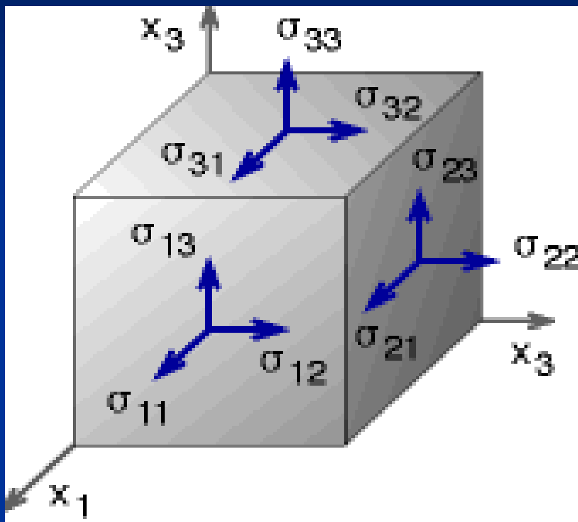
SCIENCE VOL 306 19 NOVEMBER 2004

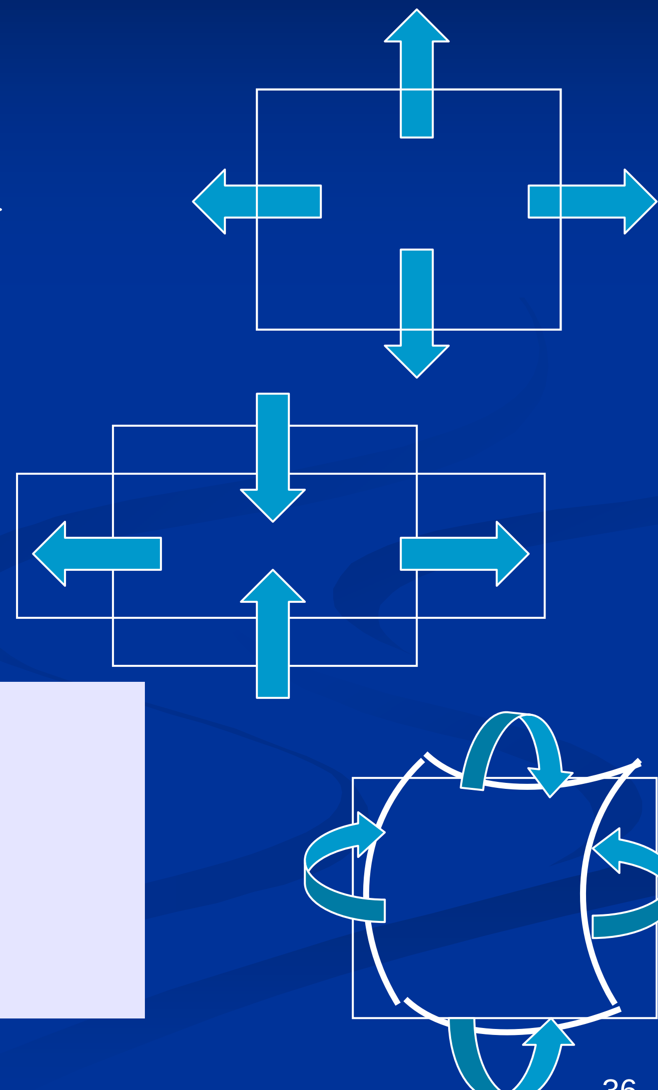
5 Membrane Mechanical Properties

- modes of membrane deformation
- membrane bending, elastic expansion, and failure
  - elastic modulus and drug partitioning
  - elastic modulus and liposome circulation time
- electroporation
- thermal transitions
- solid bilayers and solid monolayers, microcrystalline defects

The stress acting on the three faces perpendicular to the axes of the cube can be resolved into their component parts

Collapse the cube to an infinitely thin sheet…..





- Normal, and in-plane stresses become:
- Isotropic dilational
- Shear
- And cutting or bending stresses

Define the function component to carry oxygen

Material Selection | **Component Design**

Analysis of Materials Performance

Detailed Specifications and Design

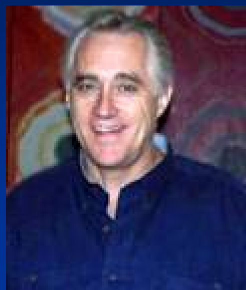
Choice of Production Methods

Prototype Testing

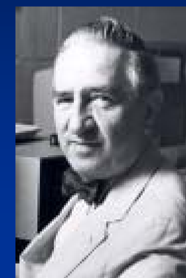
Establish Production

Further Development

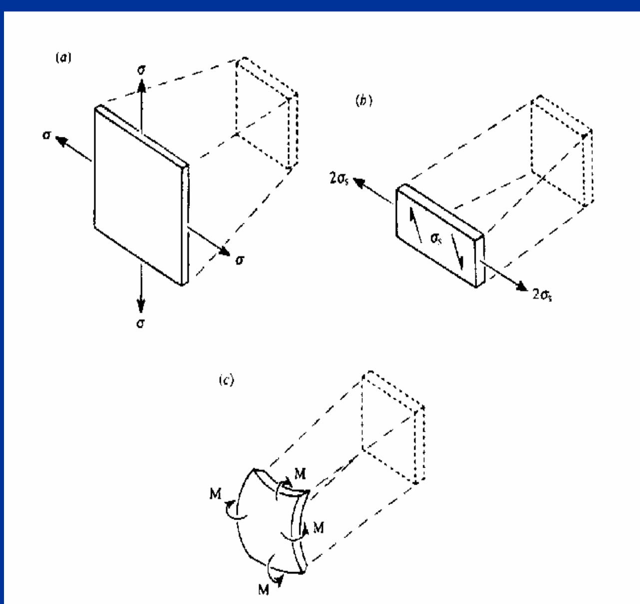
Analysis of Materials Performance Modes of Deformation



Evans and Skalak
Mechanics and Thermodynamics of Biomembranes
(1980)



3 modes describe deformation of thin shells



- **Area dilation** characterized by isothermal area expansion modulus K_a

  $$\tau = K_a \alpha$$

- **Membrane bending** characterized by the bending rigidity k_c,

  $$k_c = M/\Delta c$$

- **Surface shear rigidity**, characterized by shear modulus

  $$\tau_s = 2\mu e_s$$

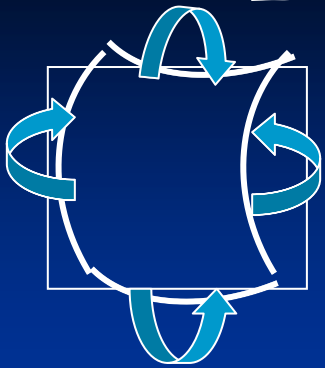
  For liquid membranes shear rigidity is zero
- **Viscous coefficients** characterize liquid behavior (viscosity) --for dilational and bending deformation are on a "molecular time scale" (10^{-5} s to 10^{-10} s)

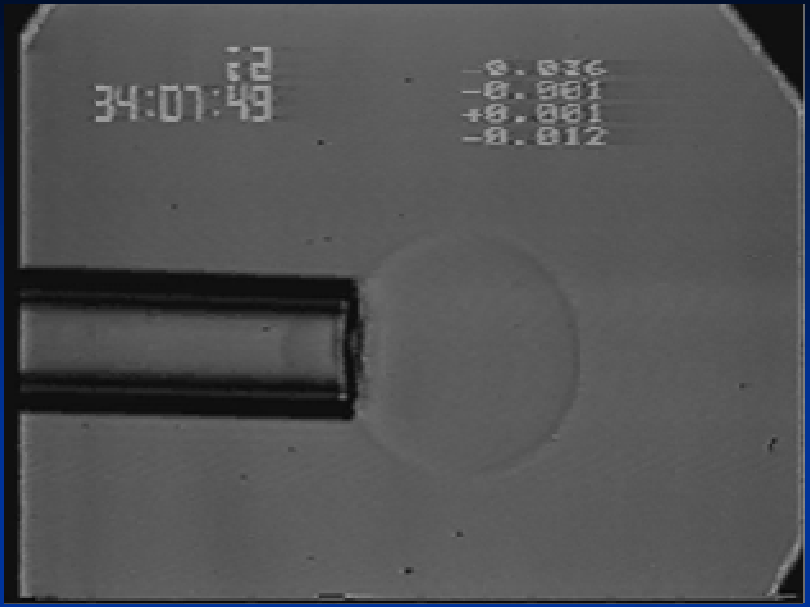
Measurement Technique:
Micropipet manipulation of single microparticles

D. Needham and D. V. Zhelev, (1999). In “Giant Vesicles”, P. Walde and L. Luisi, Eds, J. Wiley

- Pressure control: microatmospheres to milliatmospheres
- Temperature control: 5^{o}C to 50^{o}C
- Manipulator has fine positional control of pipet
- Record on video tape for analysis
- Ability to expand, stretch and bend micronsized membrane capsules

Bending Modulus



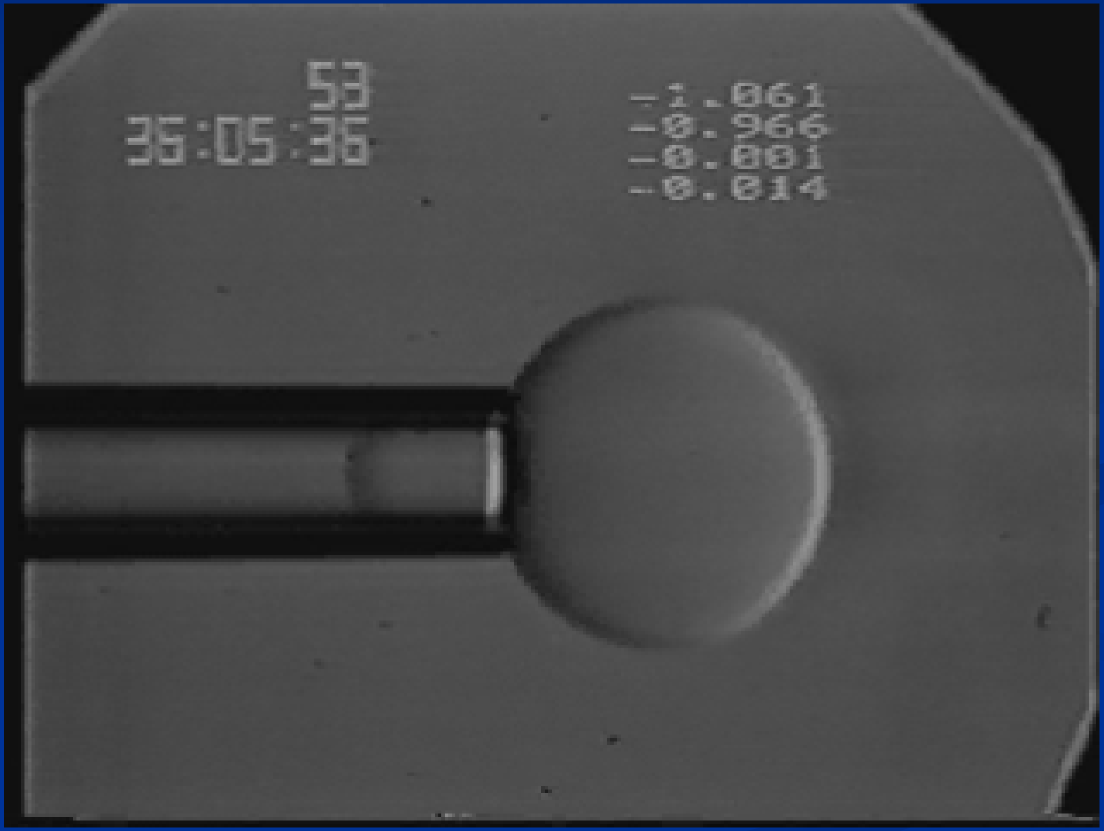


Threshold pressure for entry into micropipet

- Equate PdV work with bending energy
- Threshold ΔP for entry for 4 micron radius pipet ~ 2 dyn/cm^2 (limit of resolution)
- $k_c \sim \Delta P\, Rp^3/8 = 16 \times 10^{-12}$ dyn.cm (over estimate)
- More sensitive measurements (Evans, Sackmann, Waugh, Zhelev) give $k_c \sim 1 - 5 \times 10^{-12}$ dyn.cm (1 kT is 4×10^{-13} ergs)

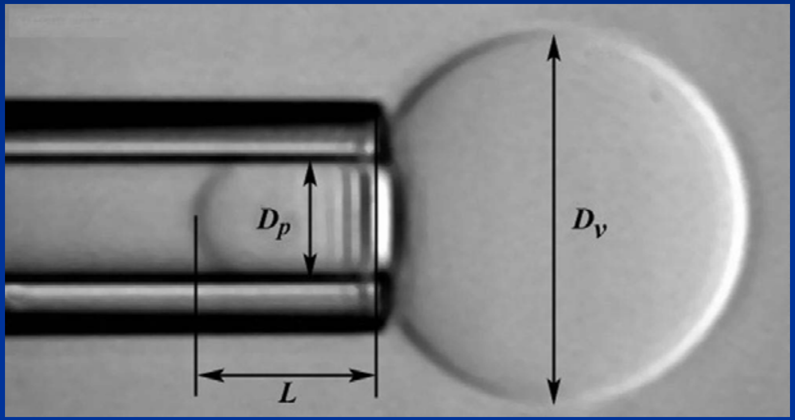
Elastic Expansion, and Failure

- Expansion and Failure



Mechanical Properties of Lipids
(expansion and failure)

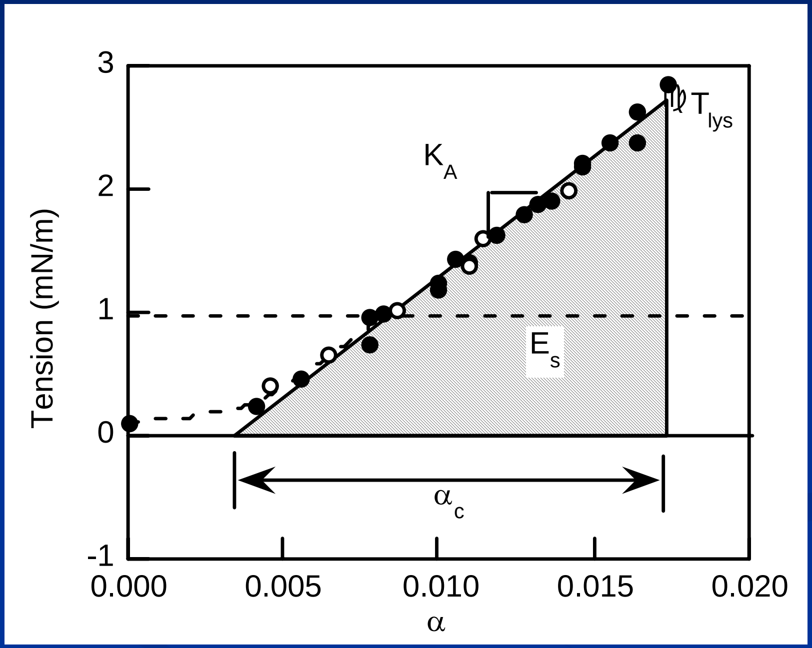
Needham, D. and D. V. Zhelev. 1996. In Vesicles, Marcel Dekker



Lipid vesicle held in a micropipet. pipet ~8 μm, vesicle diameter ~25 μm, projection length ~12 μm.

$$\tau = \Delta P R_p / 2(1 - R_p/R_v)$$

$$\Delta A = 2\pi R_p (1 - R_p/R_v)\Delta L$$



Tension vs area strain

⇒ area expansion modulus K_A

lysis tension T_{lys}

critical strain at failure α_c,

strain energy E_s

Area dilation Modulus is ~200mN/m

SIDEBAR Core Tutorial:
So, what's a Newton?

Things That Weigh a Newton

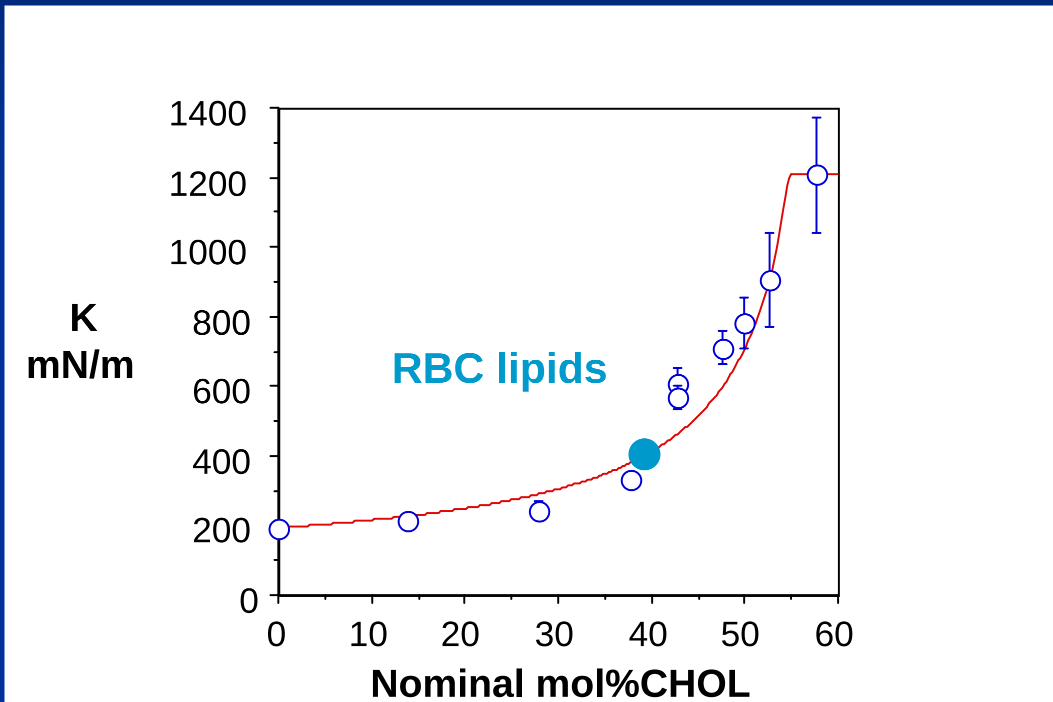
http://hypertextbook.com/facts/2004/WaiWingLeung.shtml

Maybe Not!

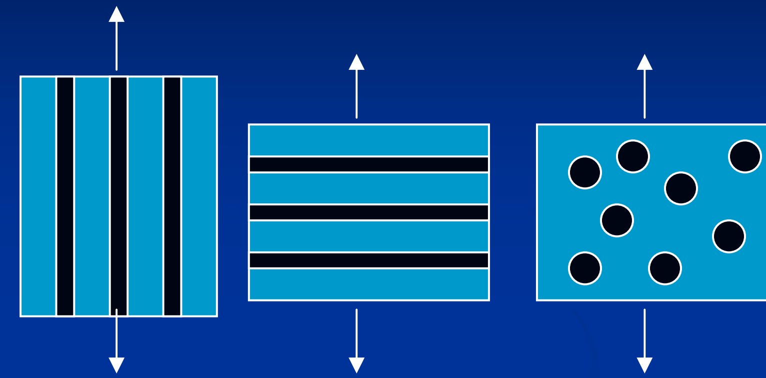
- Weight is the downward force of gravity exerted on an object by the planet it is located on. The weight, *W*, of an object can be determined by the formula...
- $$W = mg = G\frac{Mm}{r^2}$$
- where...
- *m* is the mass of the object
  g is the acceleration due to gravity
  G is the universal gravitational constant
  M is the mass of the planet
  r is the distance
- Therefore, the formula for the acceleration of gravity is...
- $$g = G\frac{M}{r^2}$$
- ~ 9.81 m/s^2.
- The SI unit of weight is the Newton [$N = kg \cdot m/s^2$],
- One Newton on the surface of the Earth is equal to 101.972 grams, 0.224809 lb, or 3.59694 oz. Objects that weigh one Newton on the Earth's surface include
- a quarter-pound burger,
- a stick of margarine, and
- a medium size apple given the alleged story of how Newton discovered gravity.

Effect of Cholesterol

Needham, D. and R. S. Nunn. 1990. Biophys. J. 58: 997-1009.



Elastic area expansion modulus versus cholesterol concentration for SOPC/CHOL mixtures

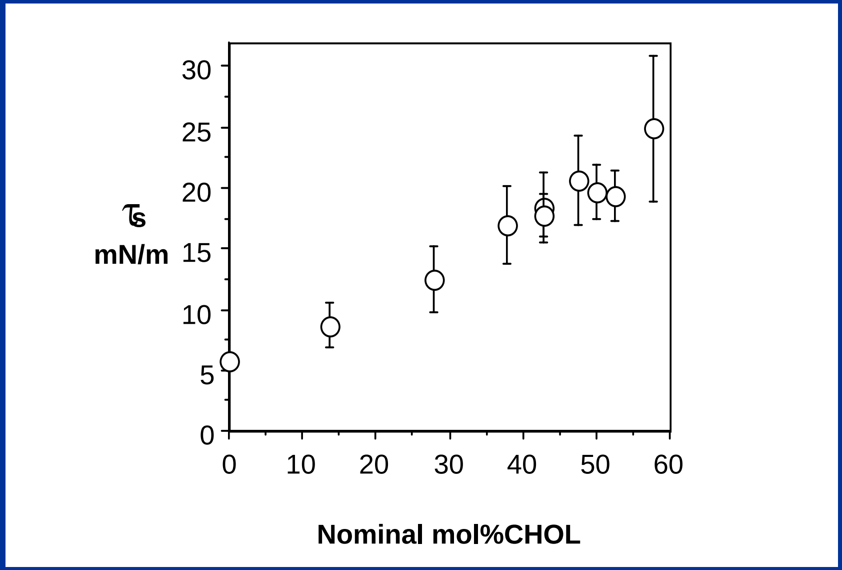


- simple isostress “molecular composite” theory for lipid and lipid/cholesterol components
- linear combination of free lipid K_L and lipid/cholesterol molecular complex $K_{L/C}$, scaled by their area fractions a_L and $a_{L/C}$

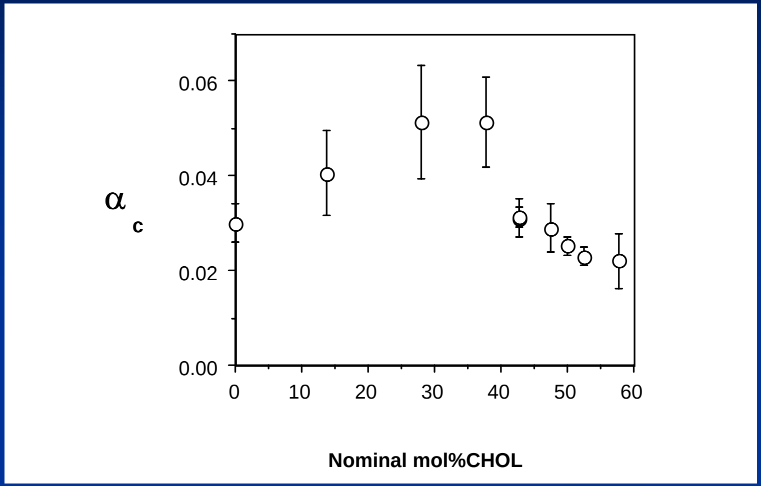
$$K_m = \left(\frac{a_L}{K_L} + \frac{a_{L/C}}{K_{L/C}} \right)^{-1}$$

Failure Parameters for SOPC/Chol

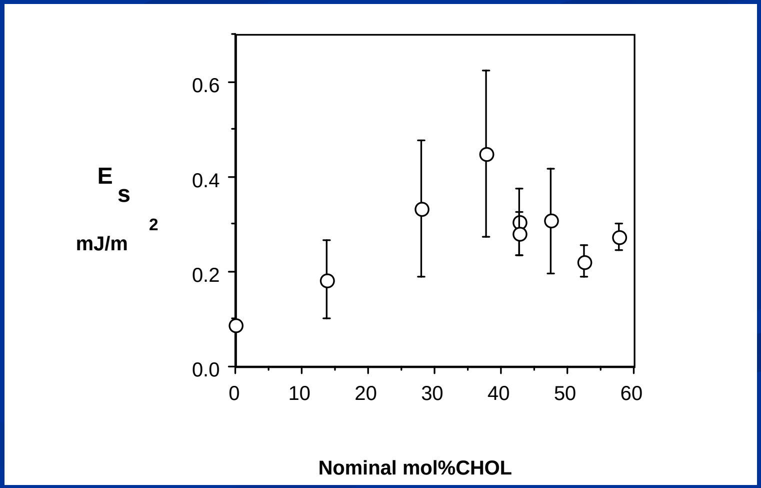
- lysis tension increases linearly,
- critical strain shows a maximum
- strain energy shows maximum at ~40 mol% (stability due to mixing?)



tensile strength

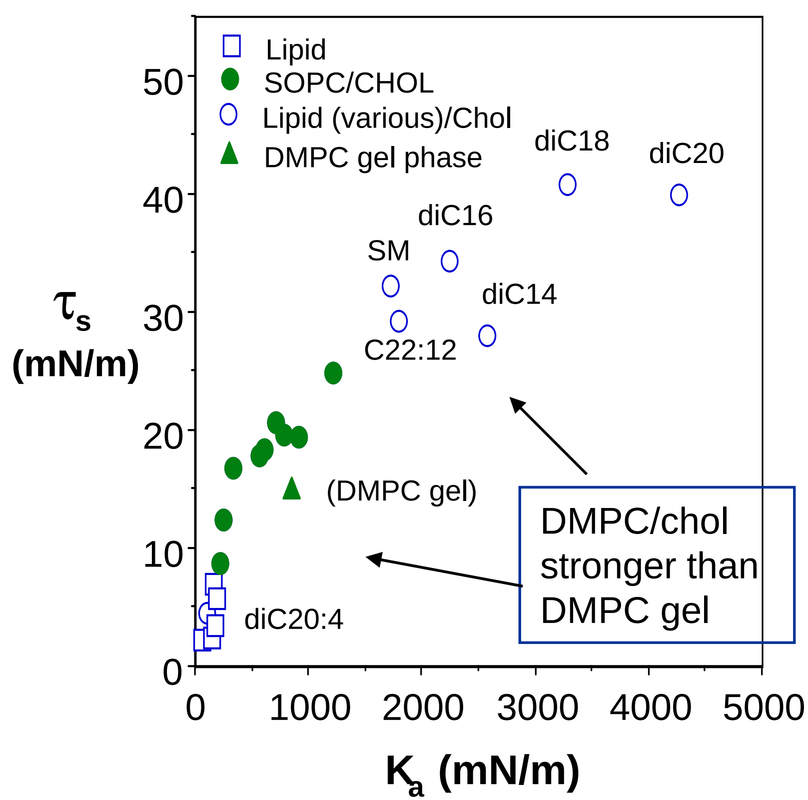


critical strain



strain energy

Elasticity and Failure for Lipids and Lipid Cholesterol mixtures



- Add cholesterol --most effective way to increase strength and modulus of lipid bilayers
- t_s limit is 42 mN/m; "equivalent bulk strength" of ~10^7 N/m^2 equal to tensile strength of polyethylene
- modulus of ~4,000 mN/m converts to "equivalent bulk modulus" of 10^9 N/m^2, ~Young's modulus of polyethylene
- for cholesterol-rich bilayers, strength is from van der Waals bonding between largely all-trans hydrocarbon chains

Micropores: Electroporation

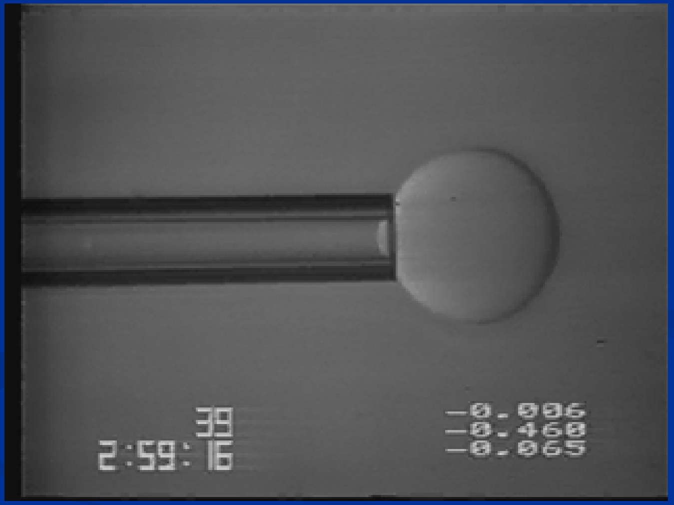
D. Zhelev and D. Needham. (1993).Biochim. Biophys. Acta, 1147, 89-104.

- single lipid vesicles manipulated to measure tension - voltage relationships for breakdown and line tension for pore ($T \sim V^2$)
- line tension Γ calculated from applied far field membrane tension τ and measured pore radius R_p

$$\Gamma = \tau_c R_p$$

- for SOPC and SOPC/Chol (1:1) membranes line tensions are 0.9×10^{-11} N and 3.1×10^{-11} N
- Pore in membrane maintained open for ~1.5s

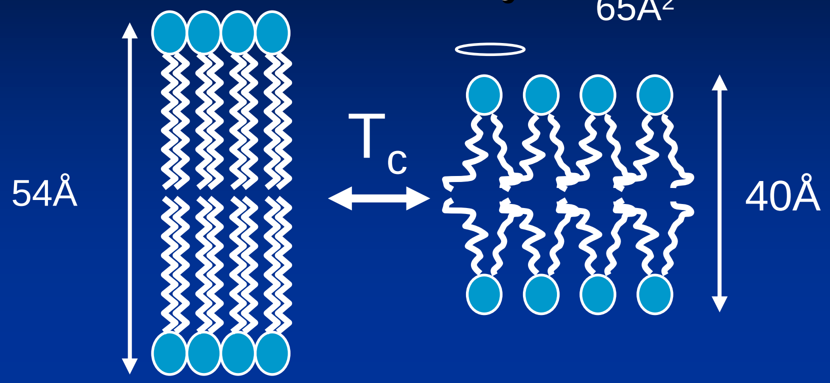
ELECTRODE



ELECTRODE

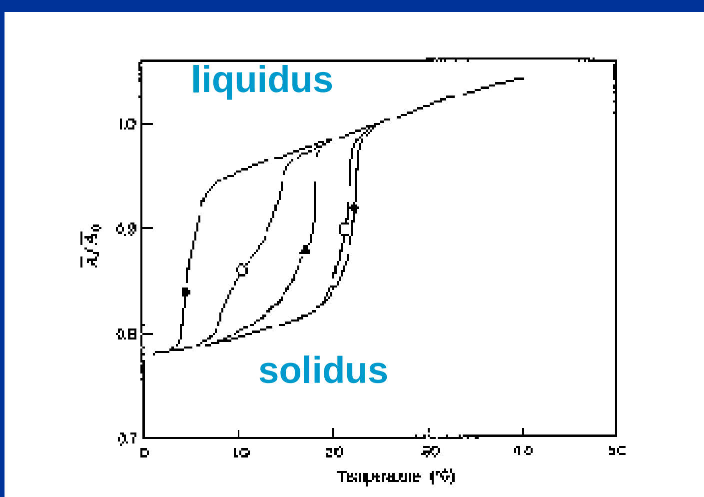
Lipid Membrane Thermal Properties

Acyl chain freezing and remelting

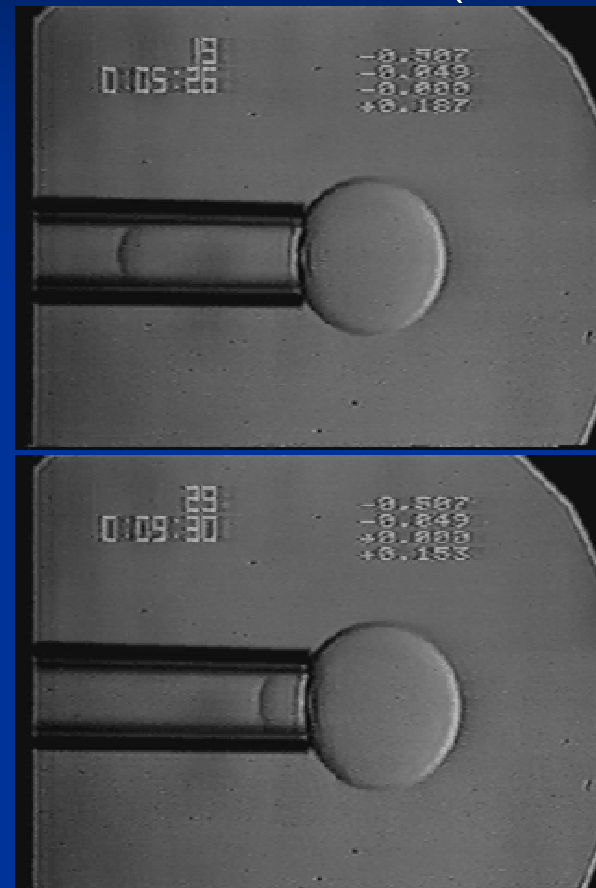


Solid
(gel)

Liquid
(liquid crystalline)



SOPC/POPE 40/60 (ideal mixture)



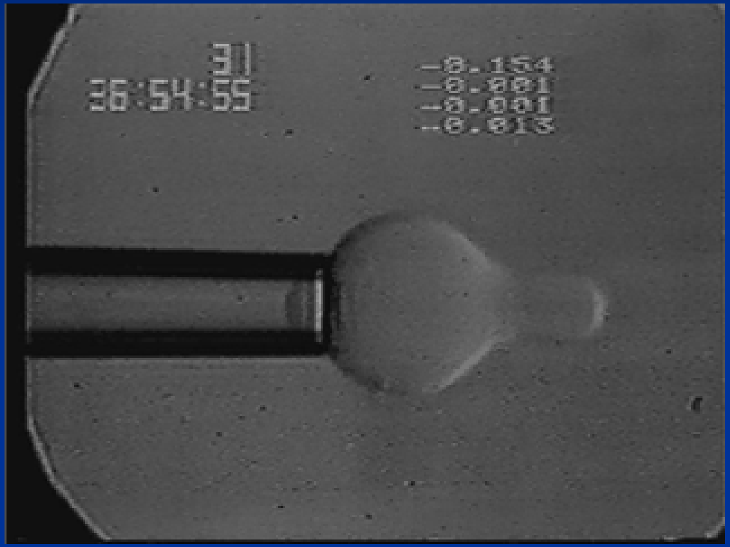
Liquid Lα
freezing

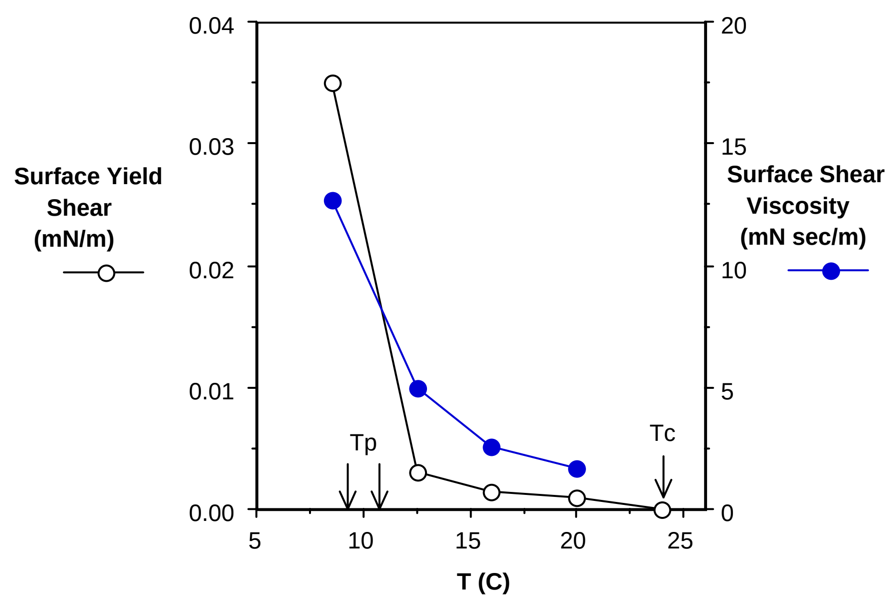
Solid Lβ
remelting

at solid/liquid boundaries there will be a mismatch in molecular area and thickness

Solid Bilayers:
Yield Shear and Shear Viscosity

Evans, E. and D. Needham. 1987. J. Phys. Chem. 91: 4219-4228.



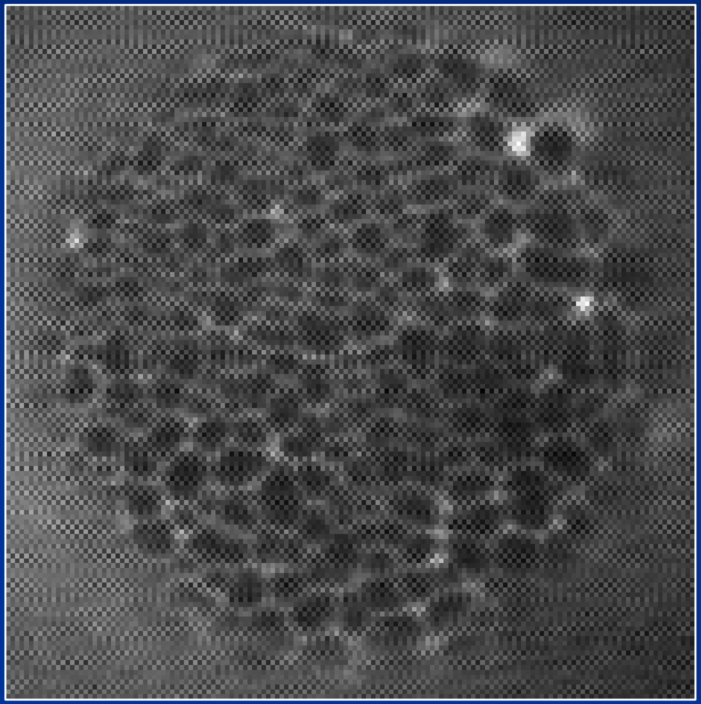


“yield shear and shear viscosity reflect density and mobility of crystal defects, (grain boundaries and/or intragrain dislocations), in the solid bilayer membranes”.

Surface shear viscosity and surface yield shear vs temperature for frozen DMPC vesicles below the main crystallization (Tc) and pretransition (Tp) temperatures

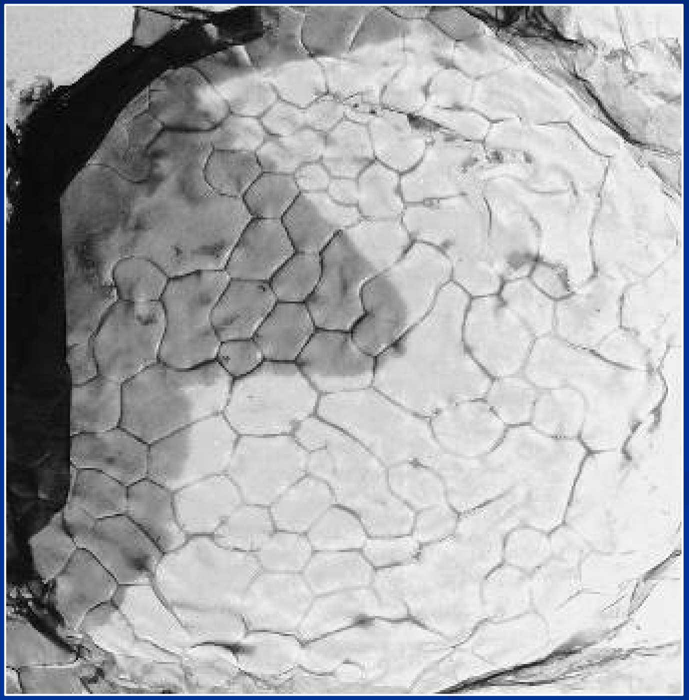
Waxy Lipids (DSPC) Coating Gas Bubbles

Fluorescence Microscopy



40 μm

Freeze Fracture EM (Joe Costello, UNC)



5 μm

D. H. Kim, et al, (2003). *Langmuir*, **19, 845**5-8466

What do they "feel" like?

Micro-Indentation test

Plastic Micro-Shopping Bag! -- One-molecule thick

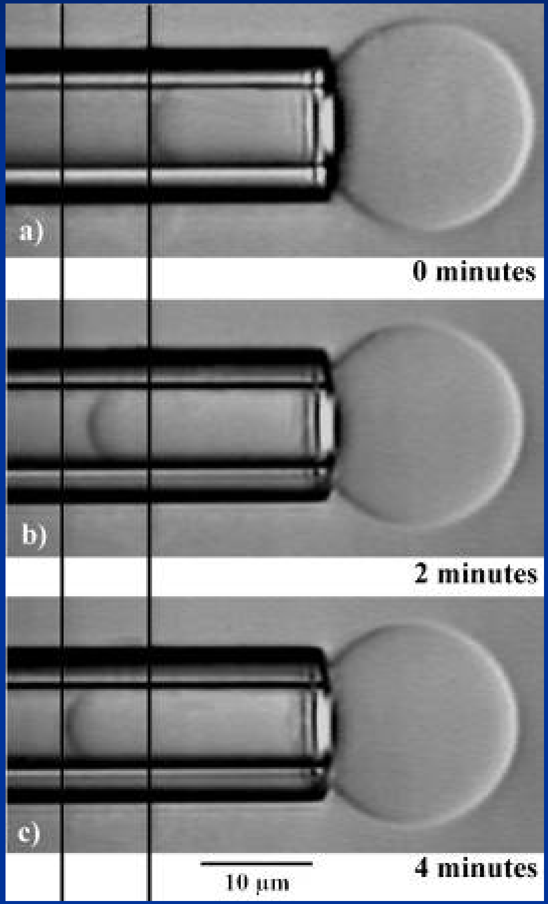
Membrane Permeability and Molecular Exchange

Transport properties and Drug Release

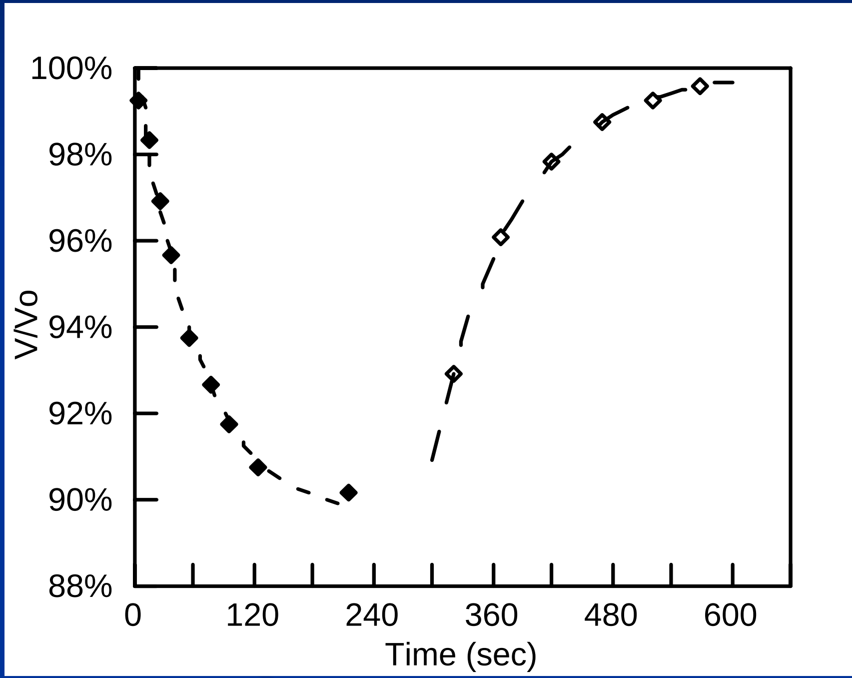
- water
- non-electrolytes (urea)
- permeability and compressibility
- Membrane soluble molecules
- Molecular Exchange at Bilayer Surface and repulsion barriers (influence of PEG)

Membrane permeability to water

with Kevin Olbrich, Wieslawa Rawicz and Evan Evans



$$P_f = \frac{kV_\infty}{Ac_\infty \bar{v}_w}$$

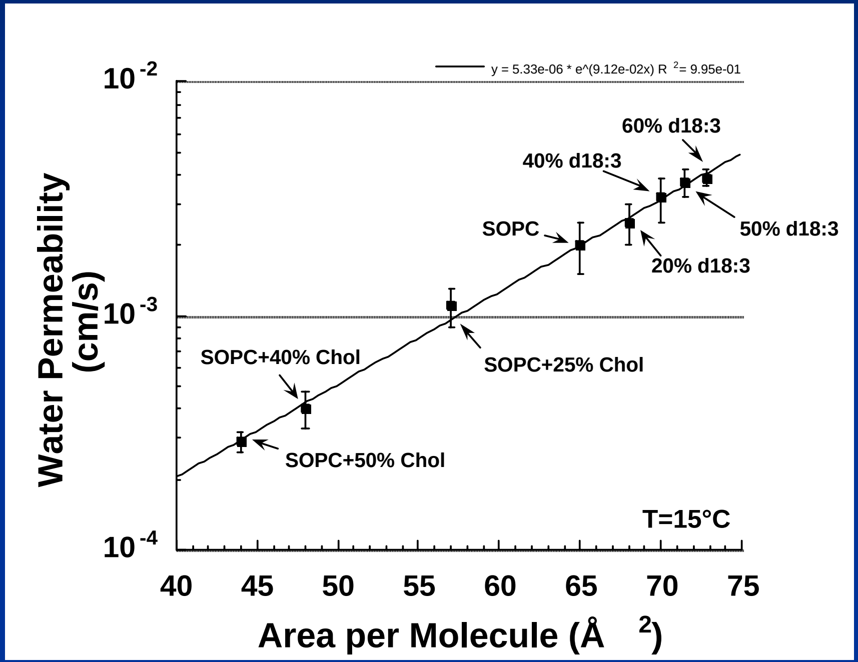


Relative vesicle volume vs. time; transfer from a 200 mOsm glucose into 220 mOsm glucose solution. Vesicle returned to original solution at 300s.

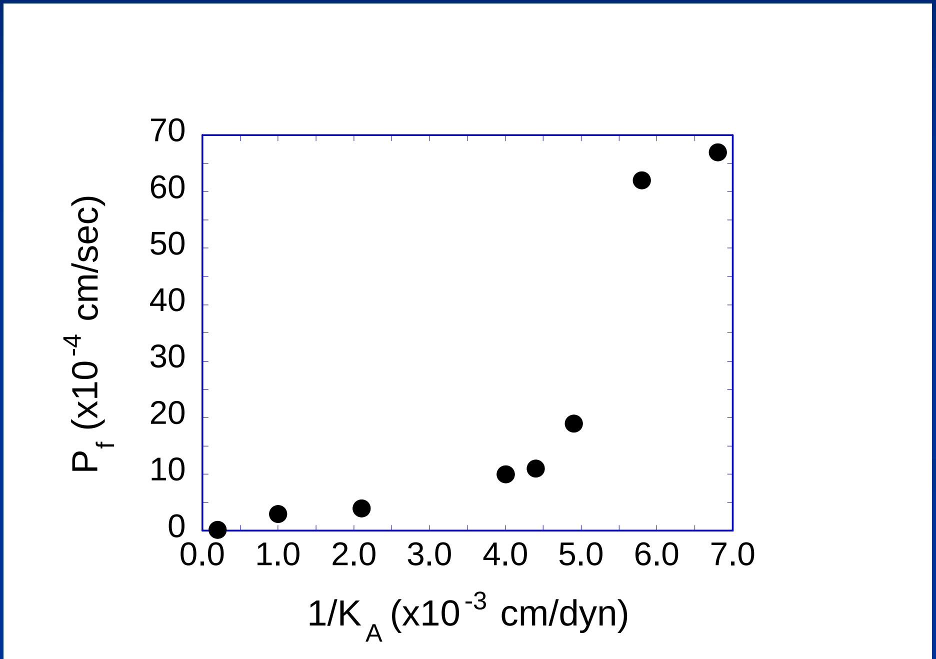
Vesicle volume change $(V/V_{inf} - 1) \exp(V/V_{inf}) = (V_o/V_{inf} - 1) \exp(kt + V_o/Vi_{nf})$

Property-Property Correlations: Membrane Permeability and Compressibility

Bloom, M., E. Evans and O. G. Mouritsen. 1991. Q. Rev. Biophys.24:293-397.



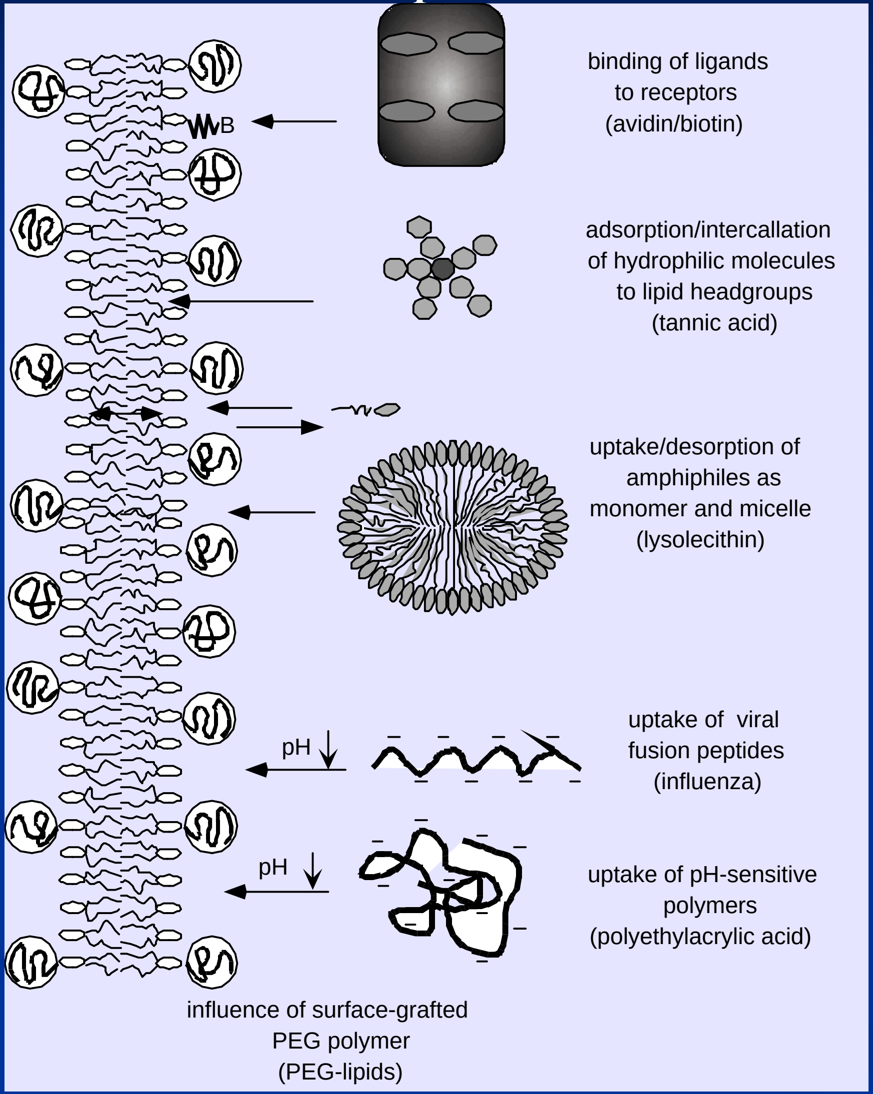
Water Permeability vs. Area per Molecule. Mixtures of unsaturated lipids and SOPC/Cholesterol mixtures.



Water Permeability vs. membrane compliance.

Water permeability decreases with condensation of lipid interface

Molecular Exchange with Membrane (Barrier Properties of PEG for adsorption interactions)

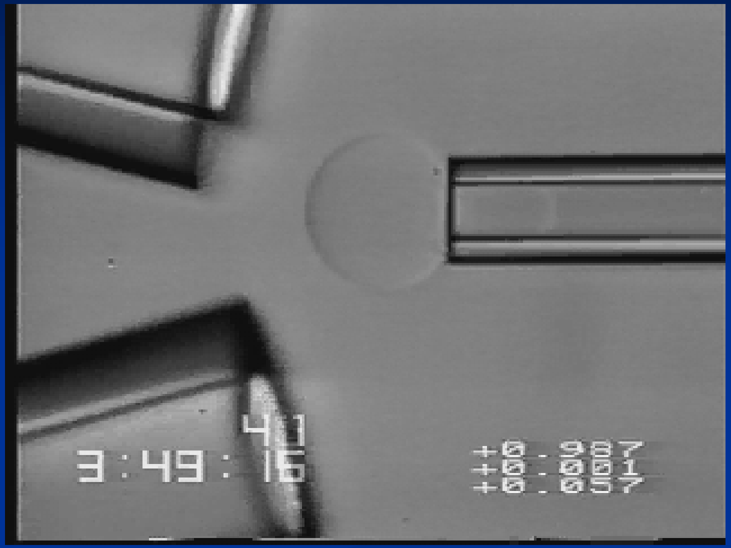


- Avidin binding to lipid-grafted Biotin
- Tannic acid
- Micelle fusion with Lipid bilayer
- pH-Sensitive peptide
- pH-sensitive polymer
- PEG as a molecular barrier to protein macromolecules and surfactant micelles

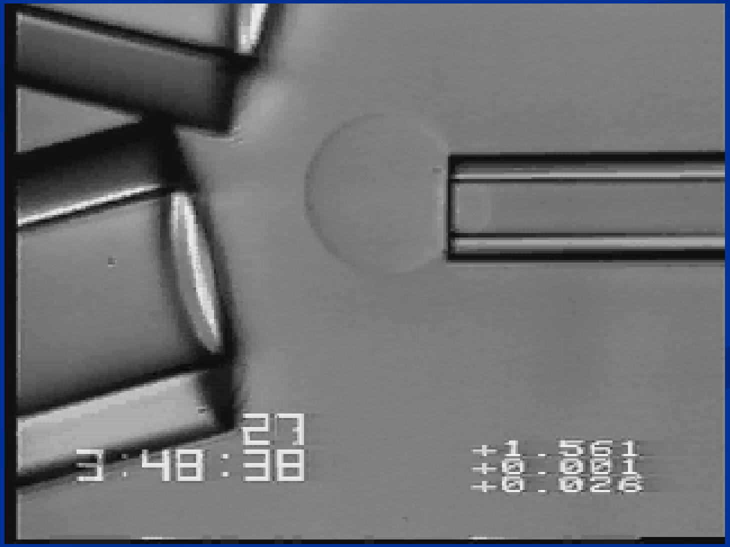
Lysolipid Uptake and Desorption

Needham, D., N. Stoicheva, et al. (1997). Biophys. J. **73**: 2615-2629.

holding pipette and two flow pipettes that deliver MOPC and MOPC-free solution at controlled flow rate to test vesicle



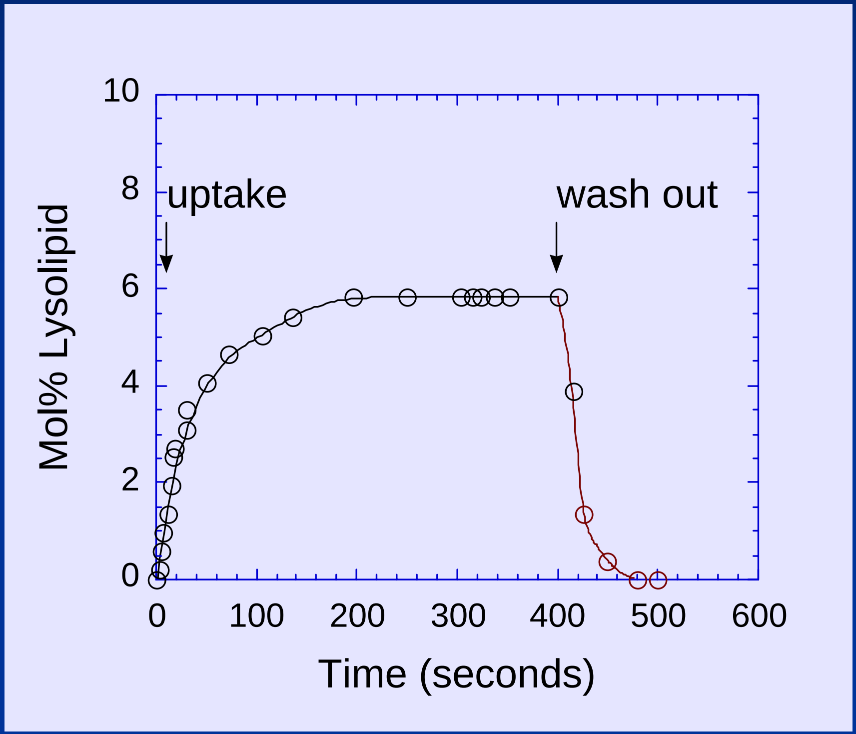
Lysolipid solution, produces area change as lysolipid enters lipid bilayer membrane



Control solution returns vesicle area back to original

Lysolipid Uptake and Desorption

(non-pegylated bilayers)



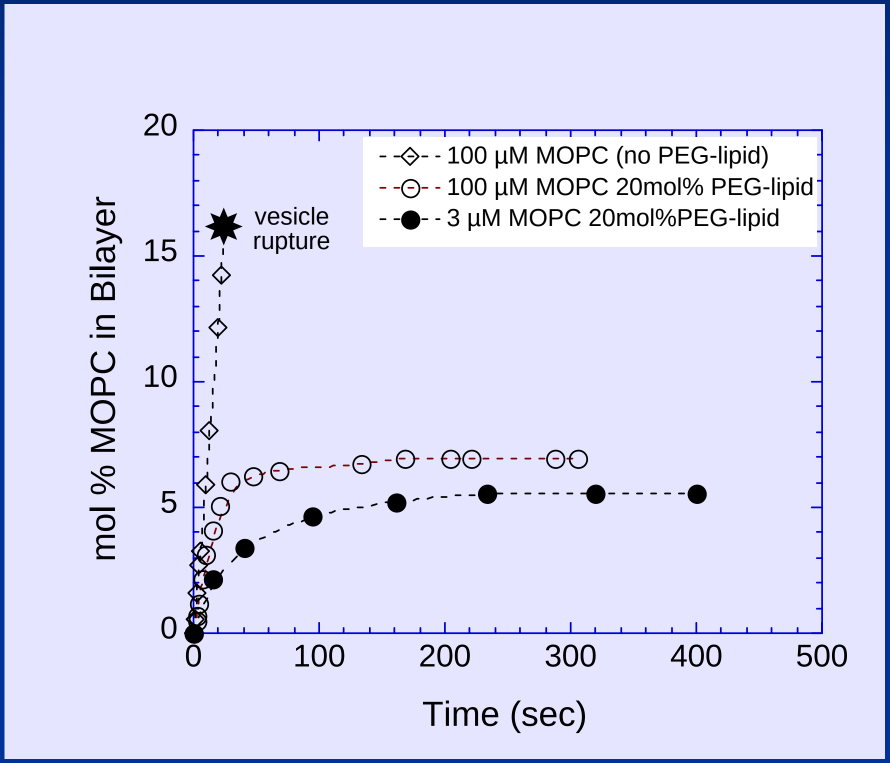
Uptake and desorption of Lysolipid by single SOPC vesicle after exposure to Lysolipid solution

Membrane area changes give mol% in bilayer

$$mol\%MOPC_{bilayer} = \frac{\Delta A}{A_o}\frac{A_{SOPC}}{A_{MOPC}} x100$$

- Lysolipid leaves bilayer rapidly in seconds upon wash out

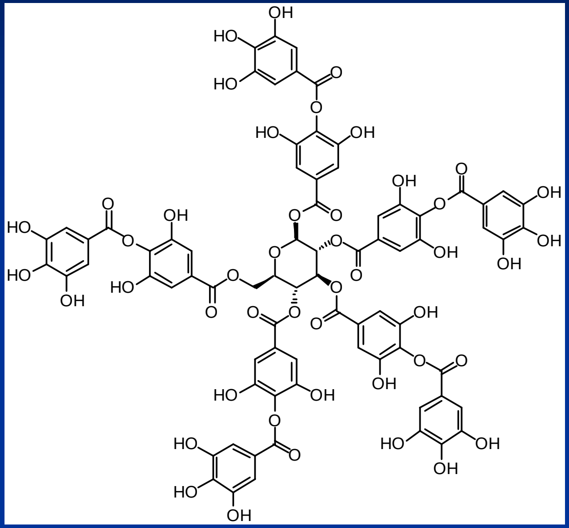
Micelle fusion with lipid vesicle



- Exposure of single vesicle to flow of MOPC solution
- No PEG -rapid uptake and vesicle breaks in seconds for 100 uM MOPC
- 20 mol% PEG750 -limited uptake, vesicle stable in 100 uM MOPC, equivalent to uptake in non-micellar solution (3 uM)
- PEG750 prevents micelle fusion with membrane but does let lysolipid monomer through

Tannic Acid

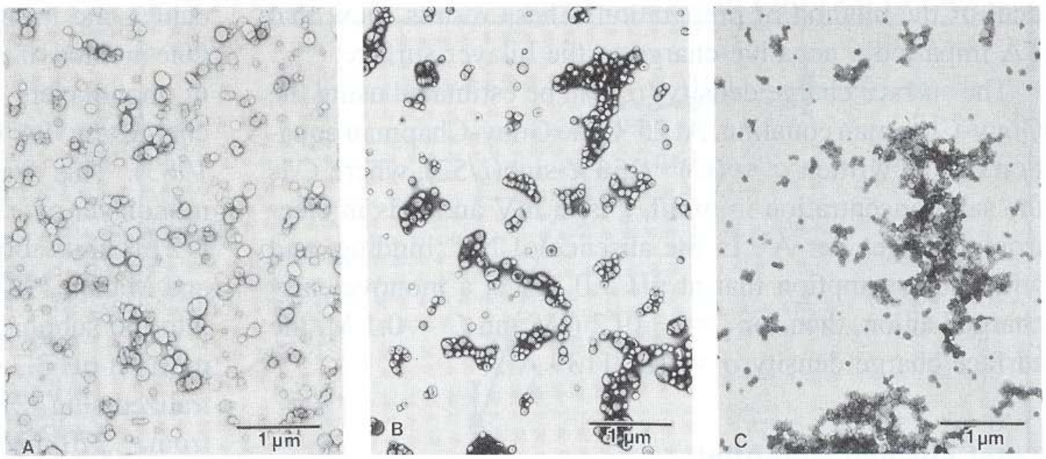
S. A. Simon, et al. (1994). Biophys. J. 66, 1943-1958



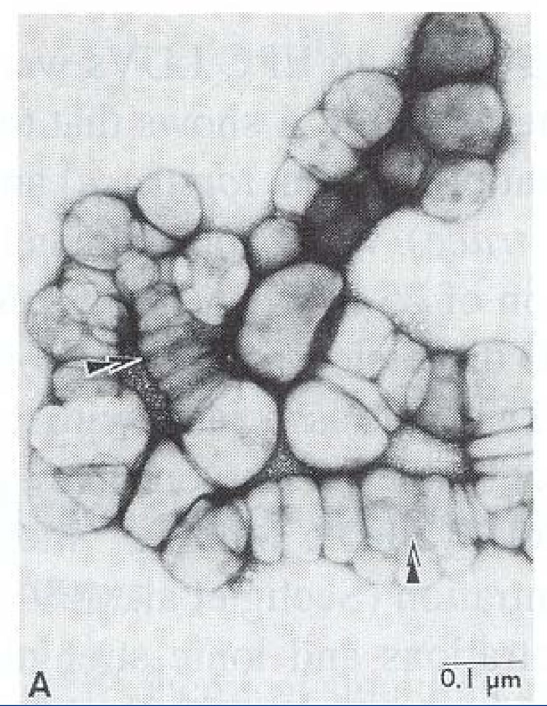
tannic acid is a polyphenolic compound capable of strong hydrogen bonding

It causes lipid membranes to adhere at Angstrom levels, (no water!)

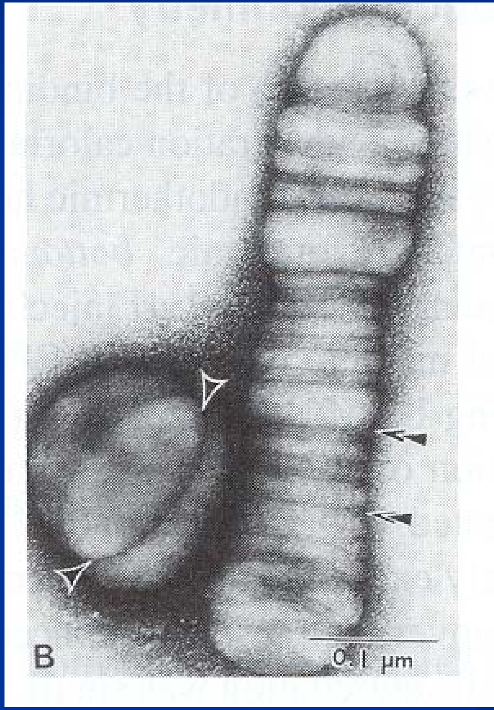
FIGURE 3 Micrographs of negatively stained large unilamellar vesicles of EPC in the absence (A) and in the presence (B and C) of 0.1 mM tannic acid. In the absence of TA, individual vesicles and small clusters of vesicles are observed. The addition of TA causes the formation of larger aggregates. Scale bar = 1 μm.



Like this…..



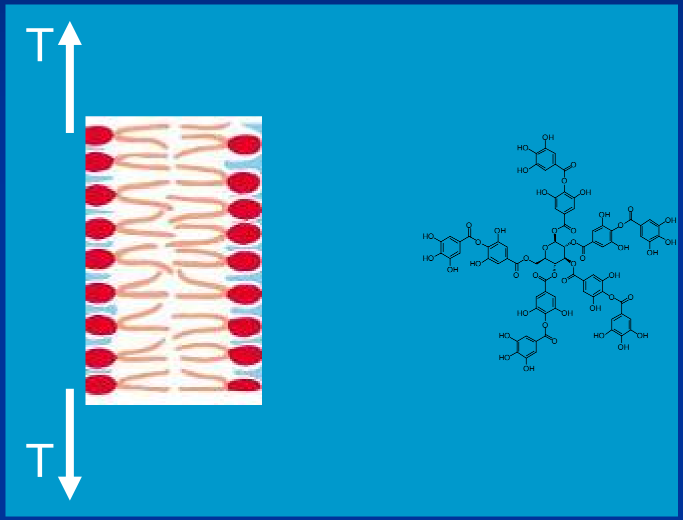
And sometimes….. Like this



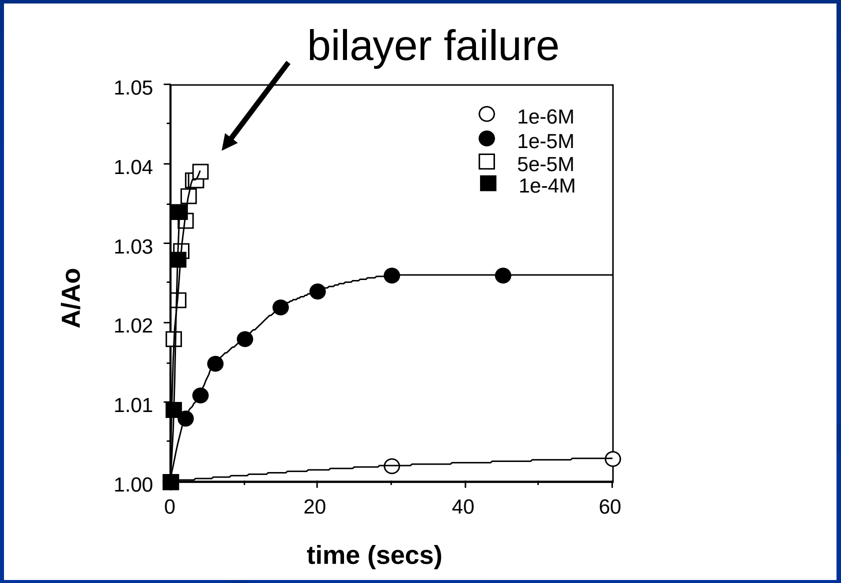
- This is what I have to put up with!!!

….And it causes lipid membranes to fail

Compressive tension



Compressive tension



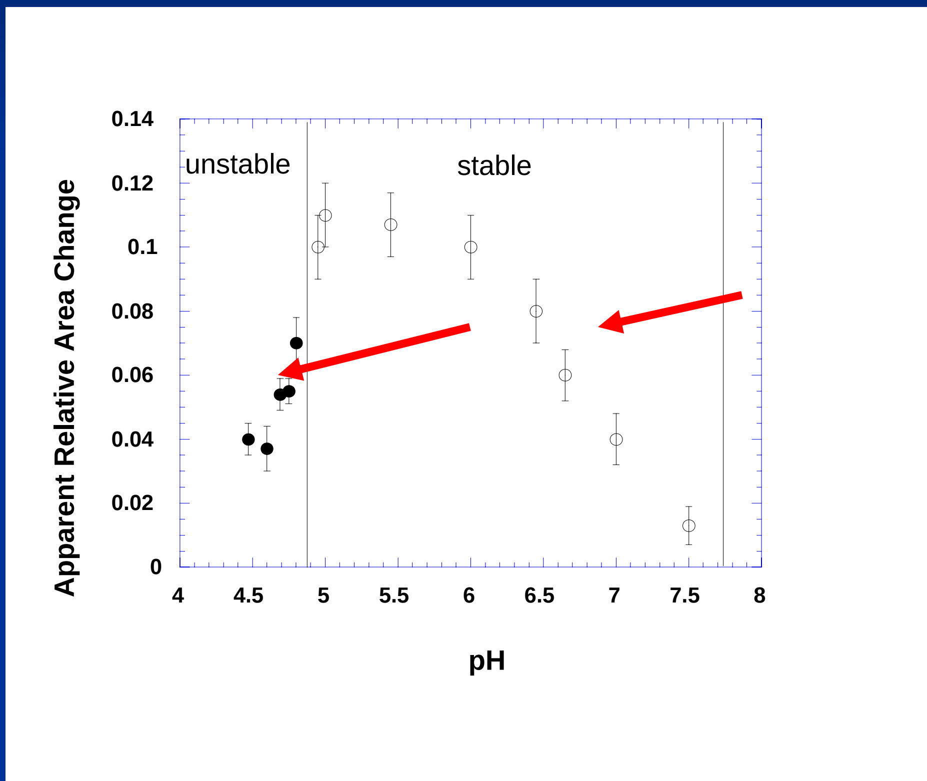
Relative change in vesicle area upon transfer into equiosmolar TA solutions

- tannic acid binds strongly to outer monolayer of bilayer, expands the interface, creating tension in the inner monolayer which eventually fails at only 3% strain because neither lipid nor TA can cross the bilayer fast enough to relax the tension

Fusion Peptides

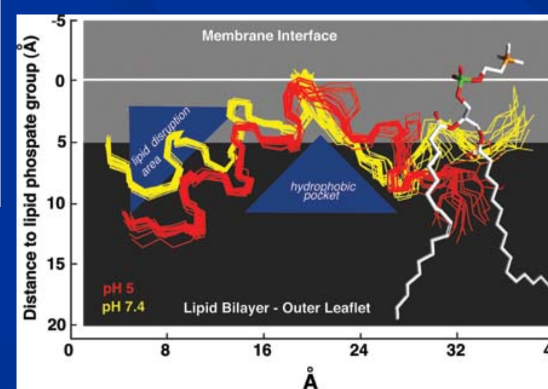
(intracellular delivery, viral fusion)

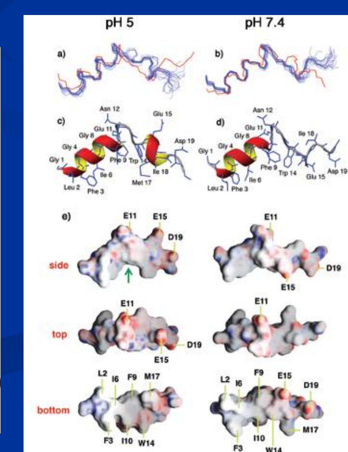
Doncho Zhelev and Natalia Stoicheva



Dependence of vesicle area due to peptide insertion on pH of buffer solution

- Exposure of vesicle to peptide solution gives increase in vesicle area
- above pH 4.8 the membrane is stable,
- below pH 4.8 the membrane breaks down.
- tendency of peptide to insert into membrane when pH decreases correlates with the peptide's own tendency for self-aggregation.





Intersurface Interactions

("stealth" and targeting by binding)

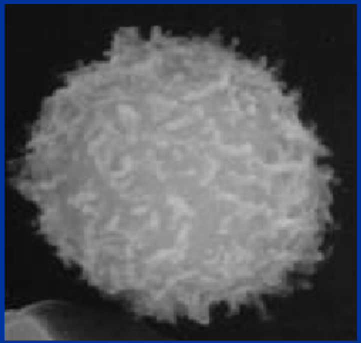
- Non-Specific Adhesion Between Vesicles
  - colloidal interactions and membrane fusion
- Specific Adhesion
  - mobile avidin biotin crosslinks (strengthen binding)
  - immobile avidin-biotin crosslinks
  - hierarchical polymer brush
- Biointerface Probe
  - single bond attachment and detachment
  - cell interface compliance

UBIQUITOUS REPULSION--SPECIFIC ATTRACTION

What did Evan Say??

Evan Evans, Mechanics of Cell Deformation and Cell Surface Adhesion, in Physical Basis of Cell-Cell Adhesion, Pierre Bongrand Ed.

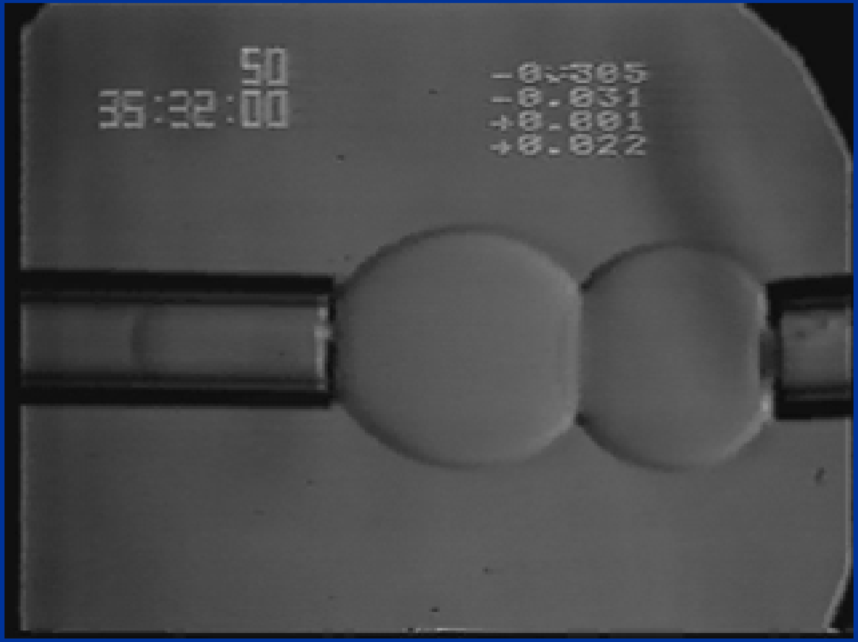
- "Formation of (specific or non-specific) adhesive contact induces stresses in the cells that eventually limit the extent of contact"
- "Subsequent separation of adherent cells by physical force also creates stresses that are transmitted through the cell cortex to the contact zone"
- "Implicitly significant, although less obvious (than receptors/ligands), is the mechanical rigidity of cells, because cell stiffness directly opposes adhesion of cells"



Non-specific adhesion between vesicles

E. A. Evans and D. Needham (1988). Macromolecules, 21, 1822-1831.

- lipid bilayer vesicles provide unique and versatile systems for study of a range of colloid and surface phenomena at and between surfaces
- extent of adhesion is controlled via tension in left hand adherent vesicle membrane



spread contact

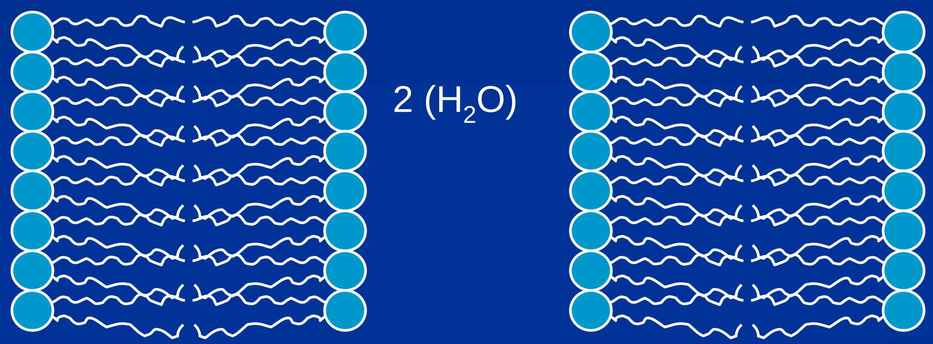
$w_a = \tau(1-\cos\theta)$

Interaction Potentials involved in binding and stability

Needham, D. and D. V. Zhelev. 1996. In Vesicles, Marcel Dekker

System	Attraction	Repulsion
Neutral Bilayers	van der Waals	hydration (+ thermal)
Charged Bilayers	van der Waals	electrostatic
Neutral Bilayers & Polymer in Solution	depletion flocc'n	hydration
Charged Bilayers & Polymer in Solution	depletion flocc'n	electrostatic
Grafted Polymer	van der Waals	steric
Grafted Polymer & Polymer in Solution	depletion flocc'n	steric, electrostatic
Neutral Bilayer Fusion (PE)	van der Waals	hydration

Cumulate Interaction Potentials starting with Neutral Bilayers

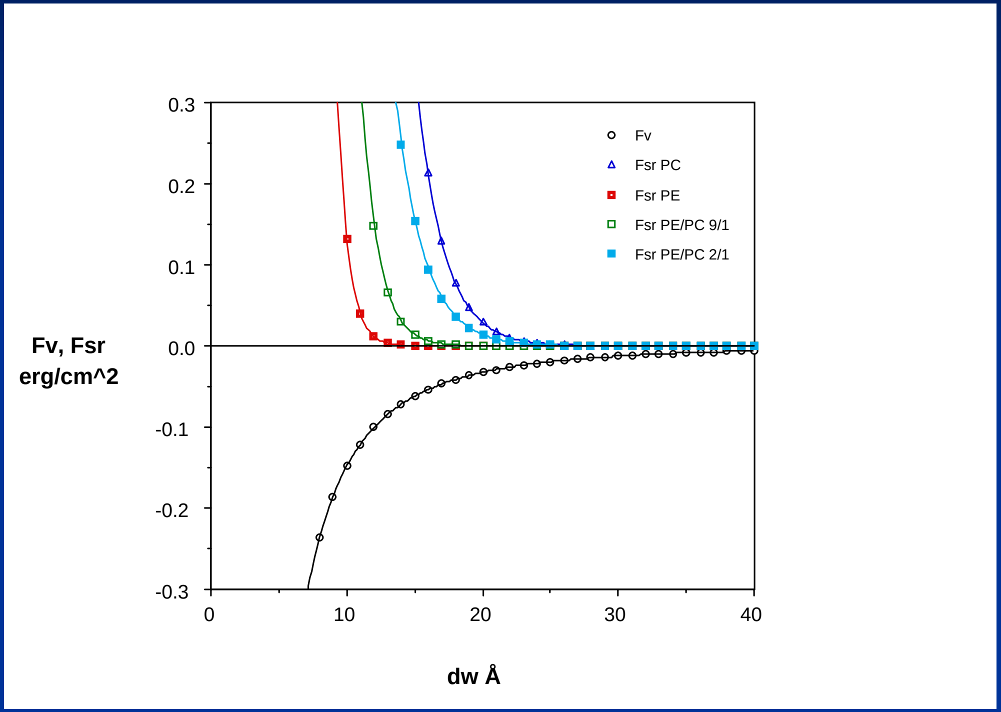


- Add in:
- - ve charge
- polymer in solution
- polymer grafted to surface
- additional lipids with low hydration, Phosphatidyl Ethanolamine

$-CH_2-CH_2-NH_3^+$

Neutral Bilayers:

van der Waals, hydration (+ thermal undulation)



Individual contributions to total interaction potential (F_T)from van der Waals attraction and short range (hydration) repulsion for PC and PE bilayers

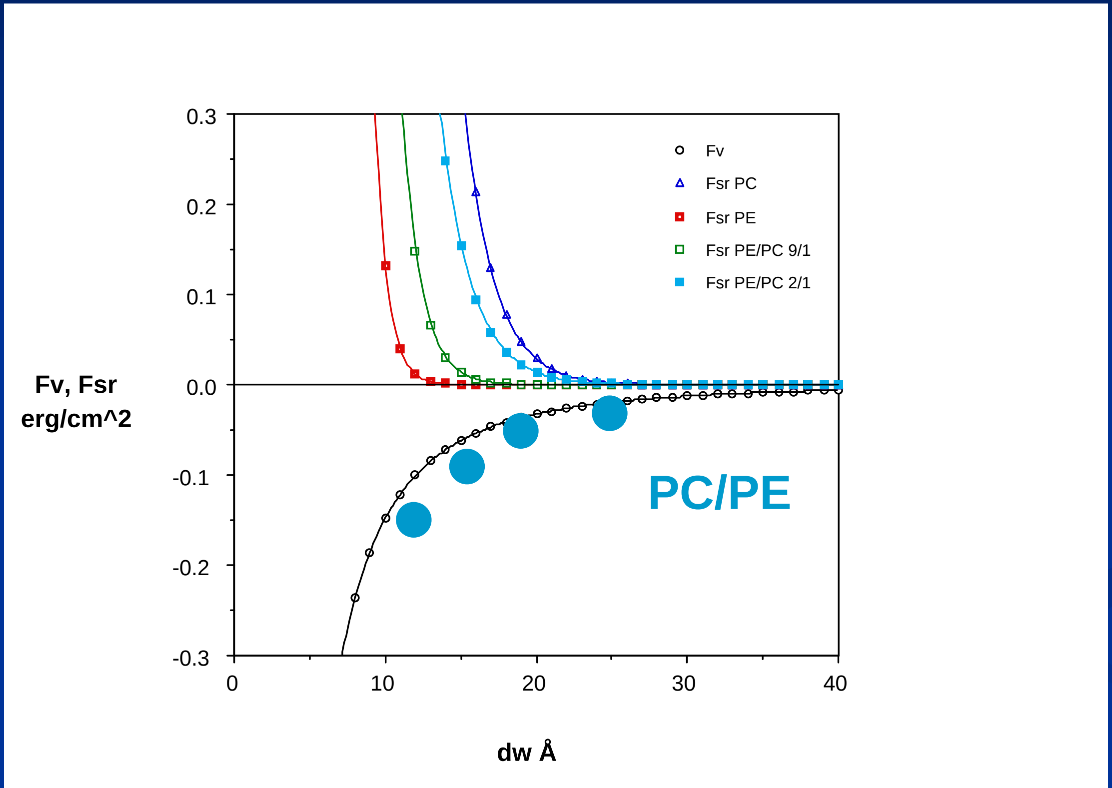
$$F_T = \lambda_{sr} P_{sr} \exp(-d_w/\lambda_{sr}) - \frac{A_H f\left(\frac{d_w}{d_b}\right)}{12\Pi d_w^2}$$

(λ_{sr} ~ 1 - 2Å)

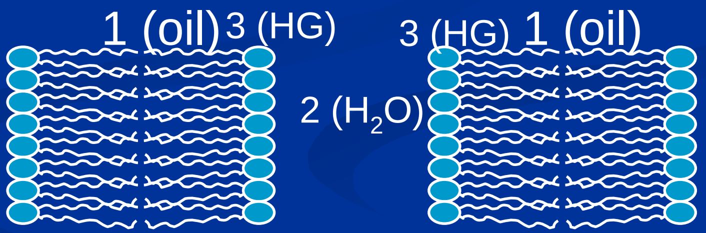
- Short range hydration repulsion limits van der Waals attraction at distances of ~25Å for PCs and ~11Å for PEs (from x-ray difraction, McIntosh)
- At large separations ~100 Å, thermal undulation stress is insignificant for most phospholipid membranes, --this weak steric repulsion does not prevent vesicle-vesicle adhesion.
- undulation effects do however renormalize all the other forces involved (Ipsen and Evans)

Experiment and Theory

neutral bilayers

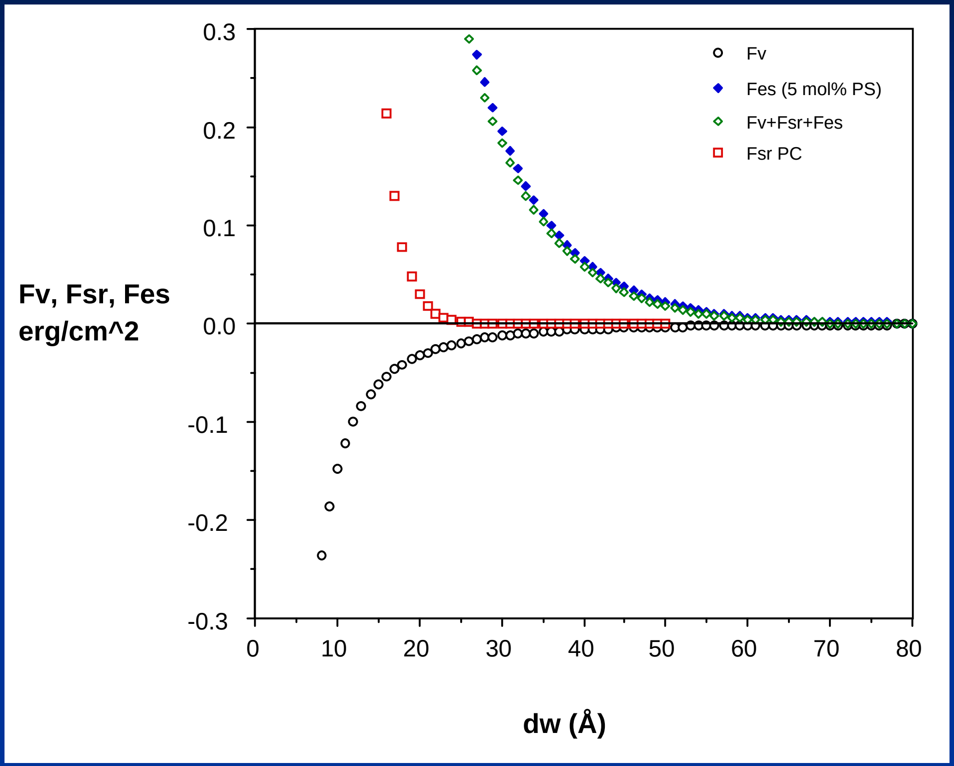


Total interaction Potential F_T for neutral PC, PE Bilayers: experimental adhesion energy w_a FOLLOWS VAN DER wAALS

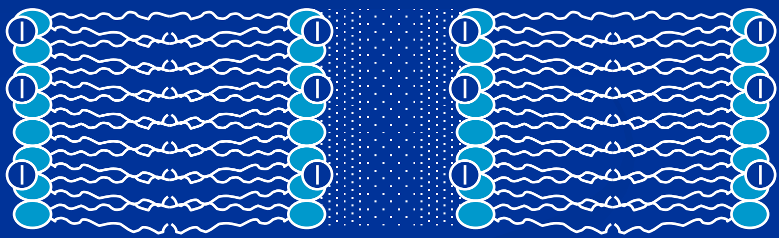


- Van der Waals potential represents ~90% of total interaction potential for PC and PE for two oil layers across water

Add Electrostatics



Individual and cumulated interaction potentials for PC bilayers containing 5 mol% negatively charged lipid PS

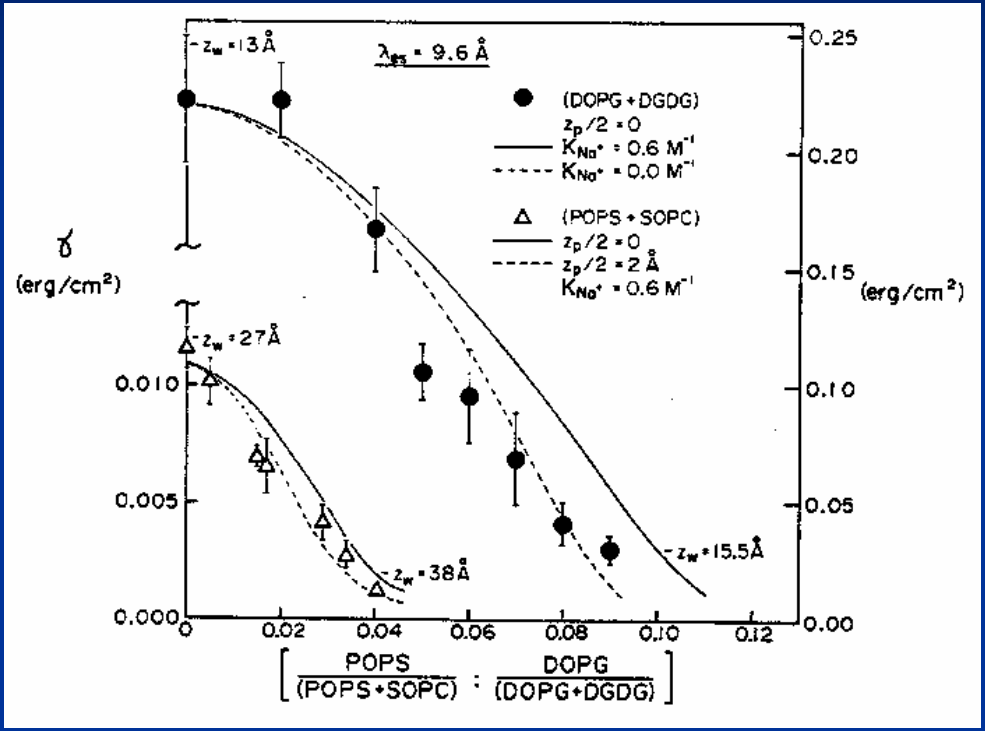


$$F_{es} = \kappa^{-1}\, 64\, n_o\, k\, T\, \psi_0^2 \exp\left(\frac{-d_w}{\kappa^{-1}}\right)$$

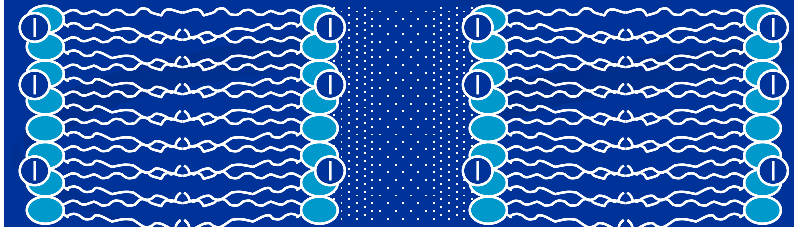
Repulsion due to ionic double layer

- Electrostatic decay (~9Å in 100mM salt solution), opposes van der Waals, but power law should always win
- Vesicles become non adherent when F_{vdW}-F_{es} ~kT and thermal undulations drive separation

Attenuation of Attraction by Double Layer Repulsion



Adhesion energies for charged bilayers in 0.1M NaCl.
Curves are predictions of Gouy Chapman theory

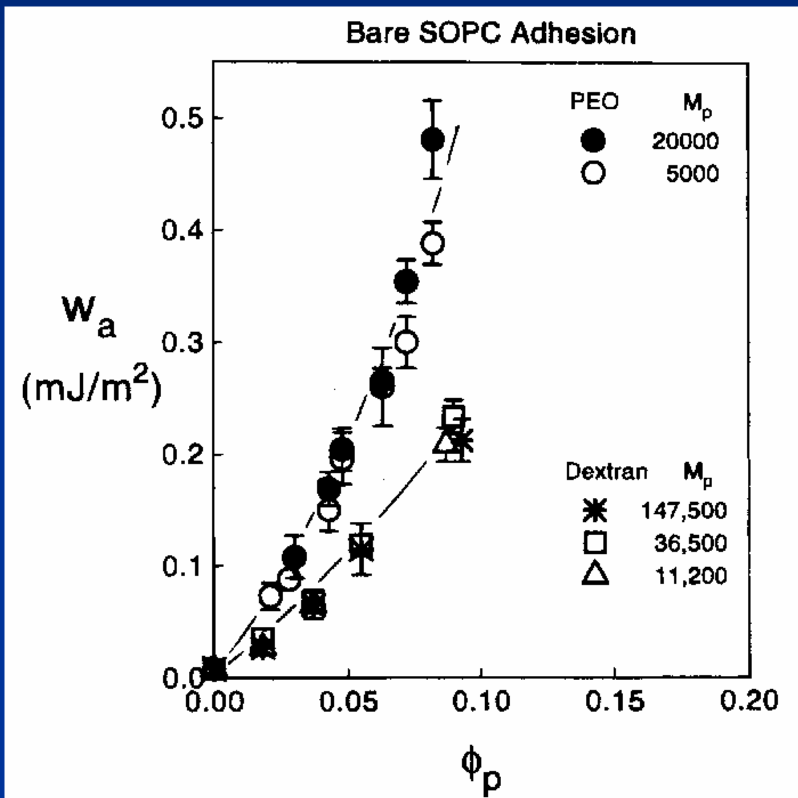


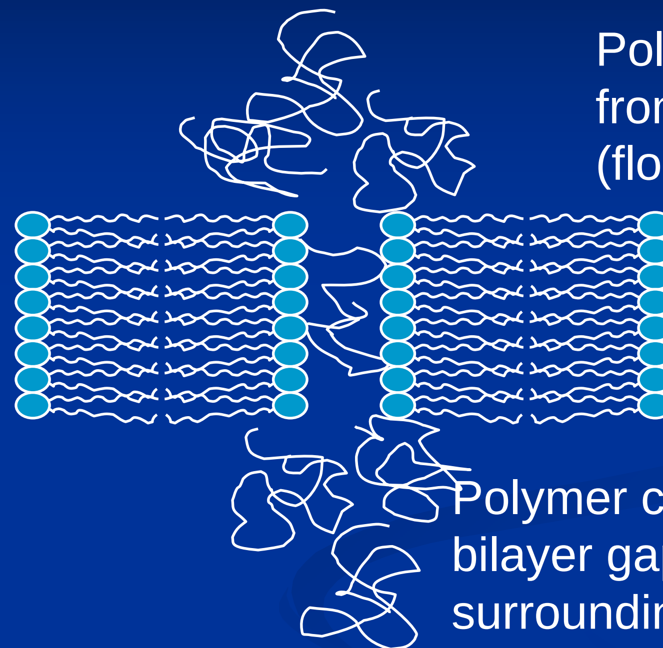
Repulsion due to ionic double layer

- A few mol% of charge overcomes weak van der Waals attraction at 5 mol% charge for PC and 10 mol% charge for DGDG

Neutral Bilayers and Non-Adsorbing polymer
(Dextran or PEG)

E. A. Evans and D. Needham (1988). Macromolecules, 21, 1822





Polymer excluded from inter bilayer gap (flocculation depletion)

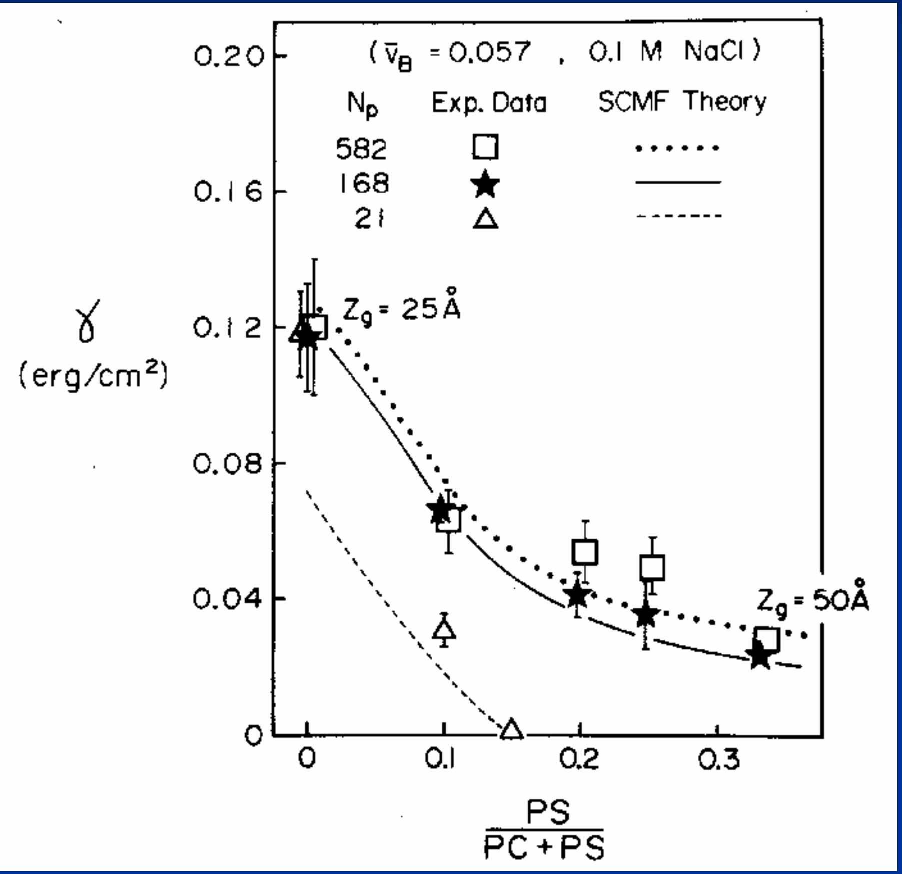
Polymer conc^n (Π) in inter bilayer gap is lower than in surrounding solution leads to normal attractive stress

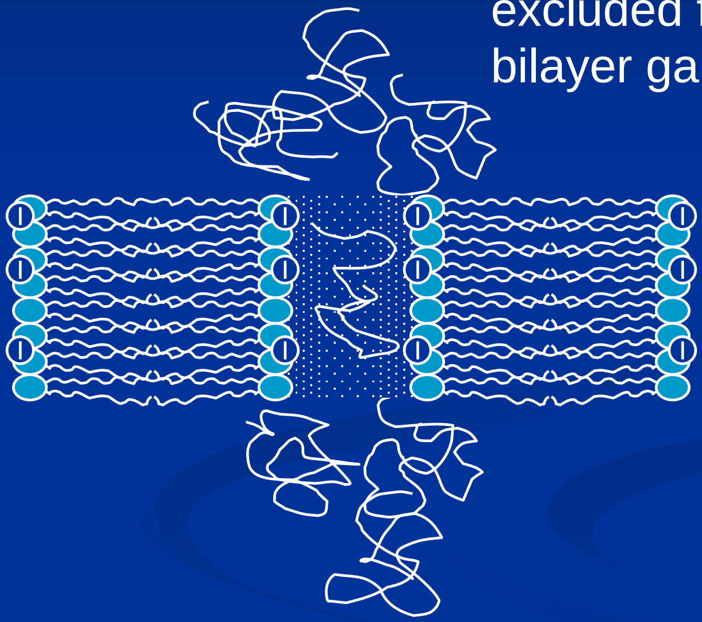
Cumulated stress is additional adhesion energy

$$\omega_a^p = \int_{\infty}^{d_{w/2}} \sigma_n^p \partial d_w$$

- Attraction in PEG is 2-3 fold higher than in dextrans at similar volume fractions

Non-Adsorbing polymer and electrostatic repulsion





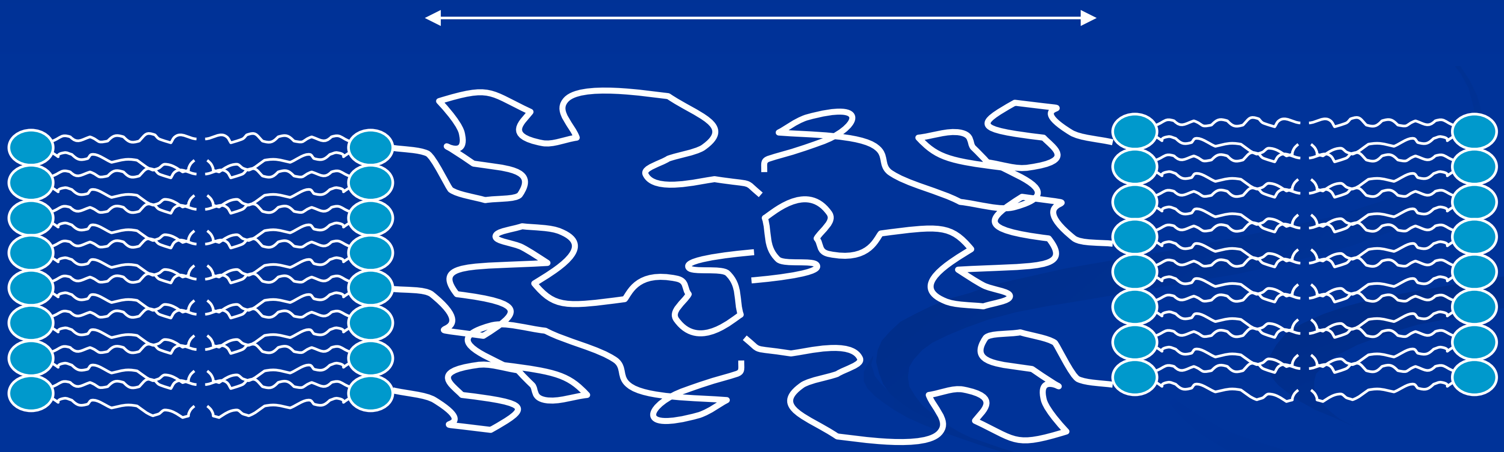
Again, Polymer is excluded from inter bilayer gap

Total potential is now electrostatic double layer plus flocculation depletion (plus van der Waals)

- High Mwt dextran (30K, 100K) can overcome even 33 mol% negative charge, still promote significant levels of attraction

Grafted Polymer

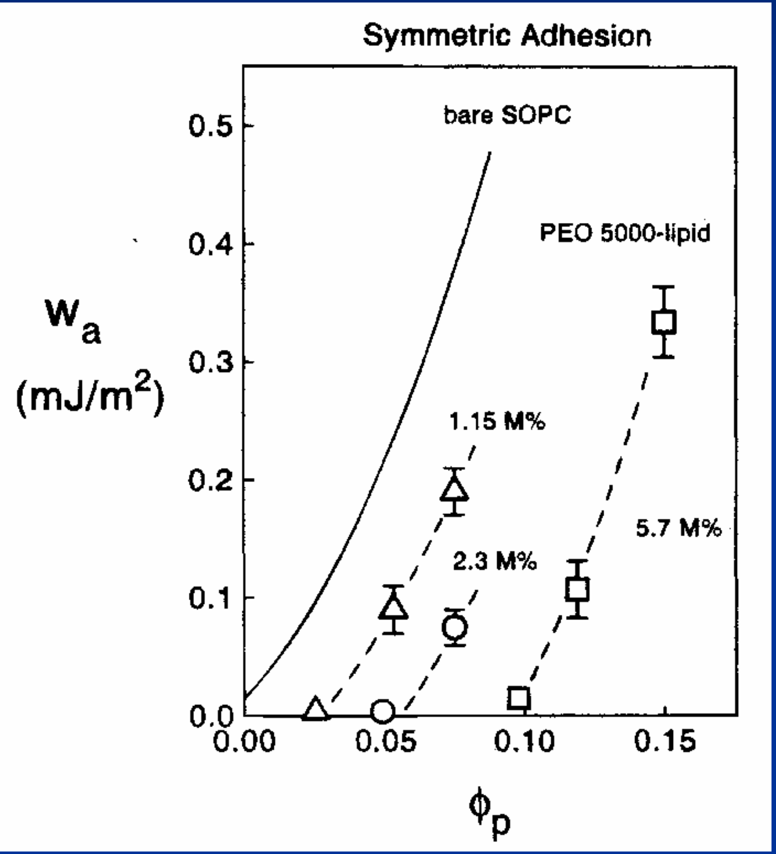
Repulsive steric pressure opposes inter bilayer attraction



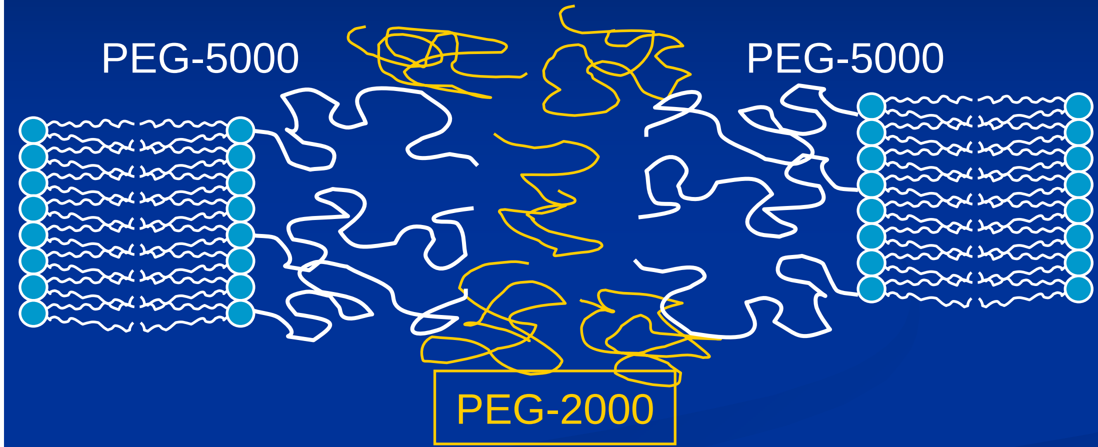
- Presence of 5 mol% PEG2000-lipid completely inhibits vesicle-vesicle adhesion due to van der Waals: inter bilayer gap is ~120Å (see x-ray diffraction data)

Grafted polymer in Non-Adsorbing Polymer Solution

Evans et al, Langmuir, 12, 3031-3037, 1996



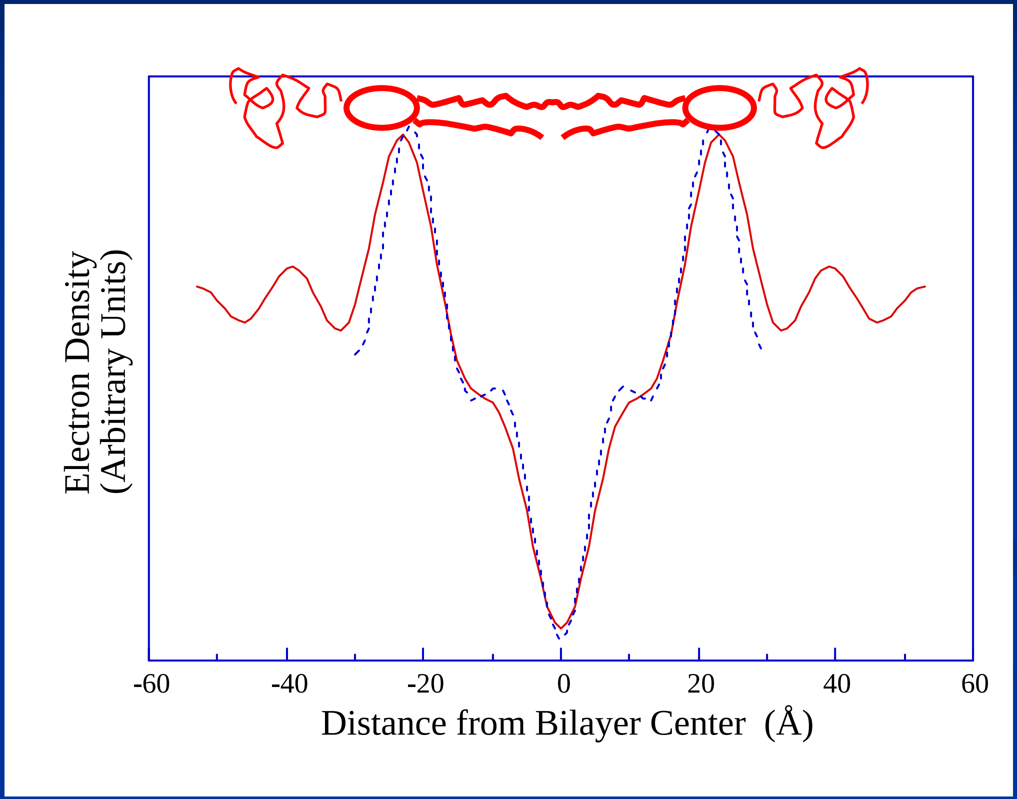
Adhesion energies between symmetric PEG-5000 -grafted surfaces in PEG-2000



- Surfaces are stabilized with no detectable attraction below a threshold free polymer concentration
- Above threshold polymer concentration, adhesion strength progressively increases

Steric Barrier by Grafted Polymer

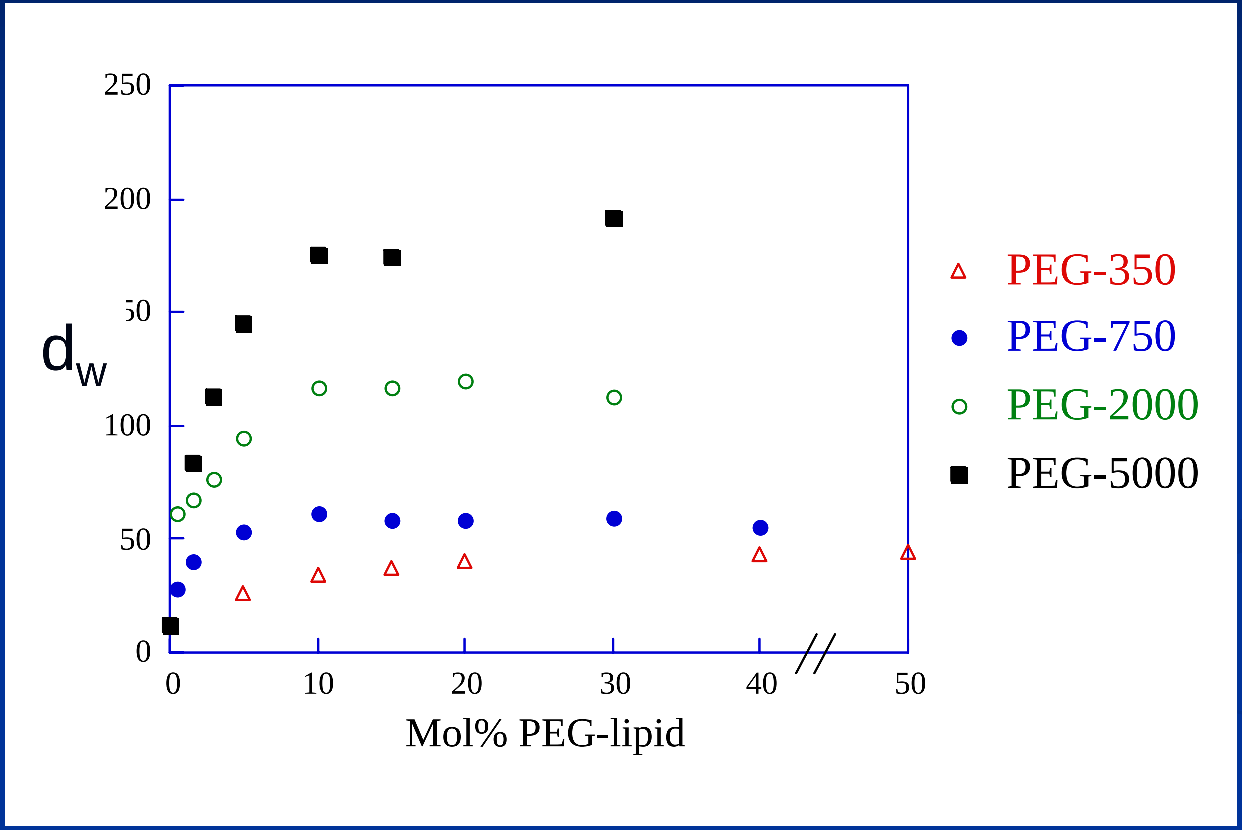
D. Needham, T. J. McIntosh and D. D. Lasic. (1992). Biochim. Biophys. Acta, 1180, 40-48



- Electron density profiles of bilayers in absence (dotted line) and presence (solid line) of 4 mol% PEG-2000. (applied osmotic pressure P = 2.8×10^7 dyn/cm^2. Each profile contains one unit cell, with the origin at the center of the bilayer

Distance between DSPC:PEG-lipid bilayers vs mol% PEG-lipid

A. K. Kenworthy, K. Hristova, T. J. McIntosh, and D. Needham. (1995). Biophys. J., 68, 1921-1936

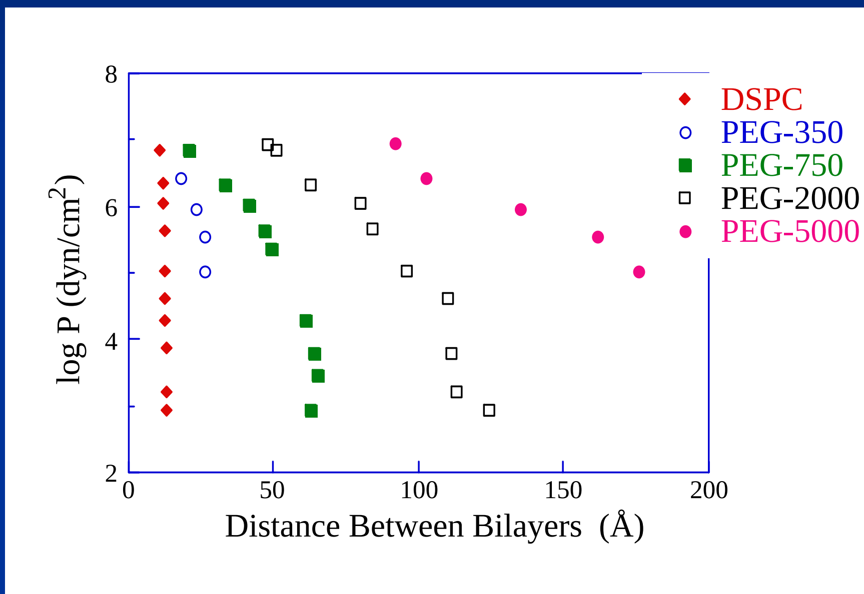


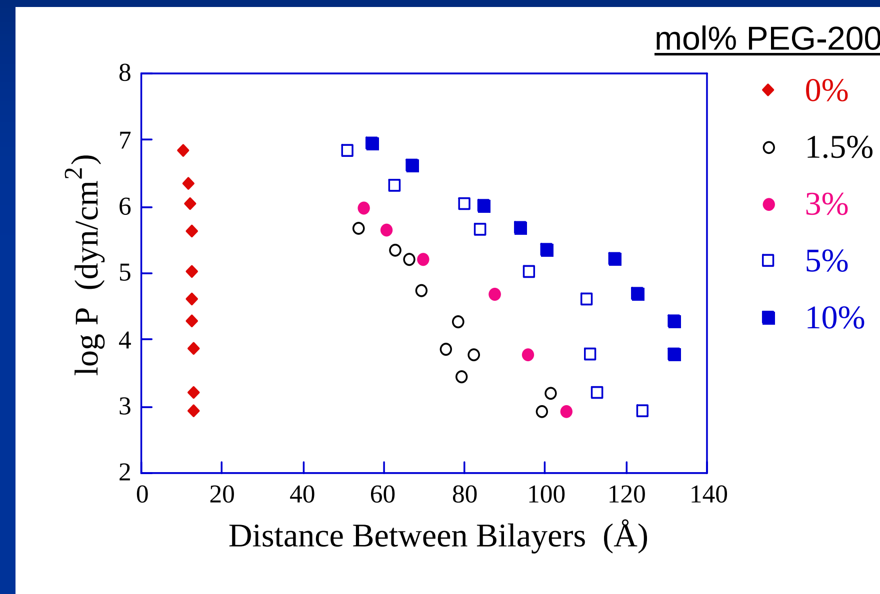
applied pressure =1.0×10^5 dyn/cm^2

- distance between opposing DSPC:PEG-lipid bilayers is a function of both concentration of PEG-lipid in bilayer and size of the grafted PEG chain
- extension of PEG chain from bilayer surface is 20 Å, 28 Å, 60 Å, and 100 Å for PEG-350, PEG-750, PEG-2000, and PEG-5000, respectively

Steric repulsive pressure
pressure vs interbilayer distance

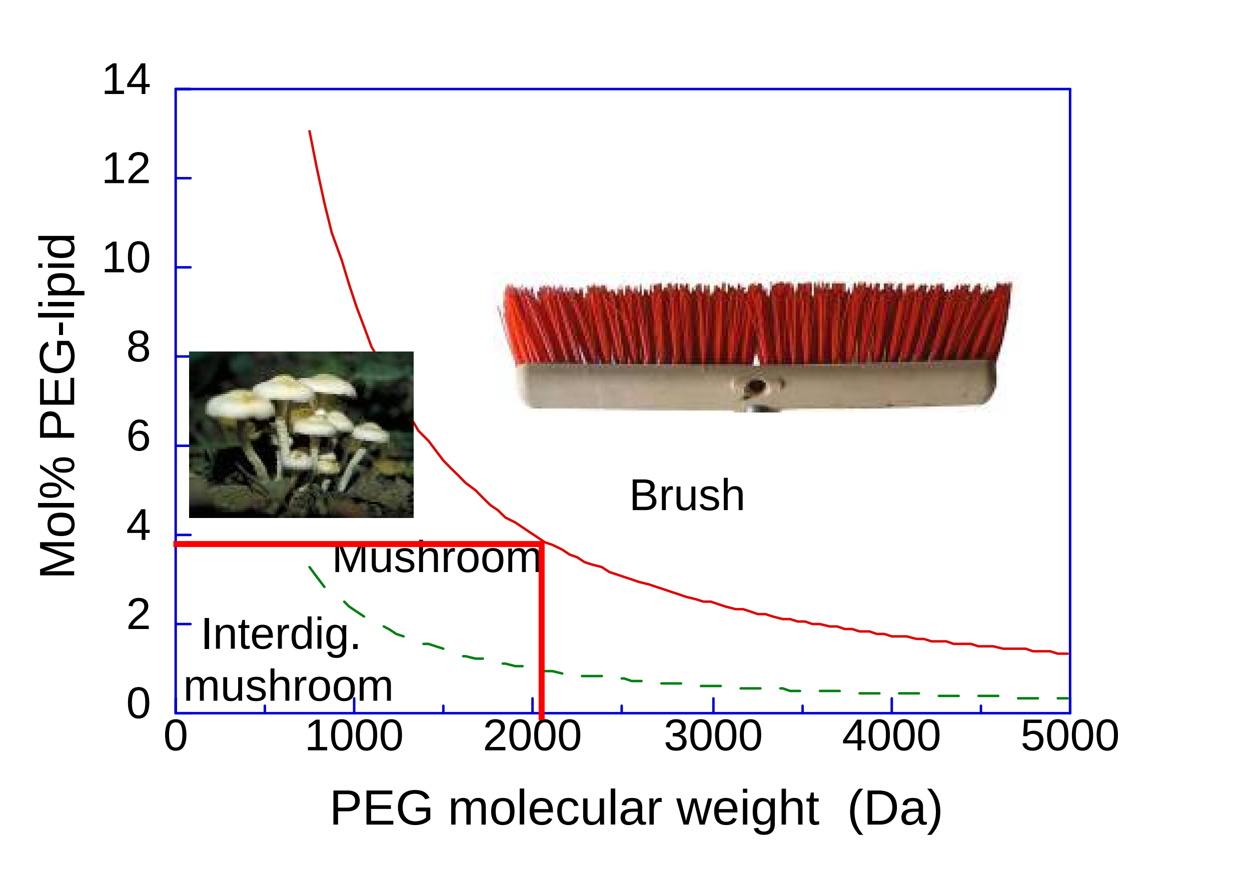
5 mol% PEG lipid





- for each PEG-lipid, total repulsive pressure increased monotonically with decreasing distance between bilayers.
- the range of the total repulsive pressure, (maximum fluid separation at low applied pressure), systematically increased with increasing polymer length
- both the range and magnitude of the total repulsive pressure increased with increasing concentration of PEG-2000

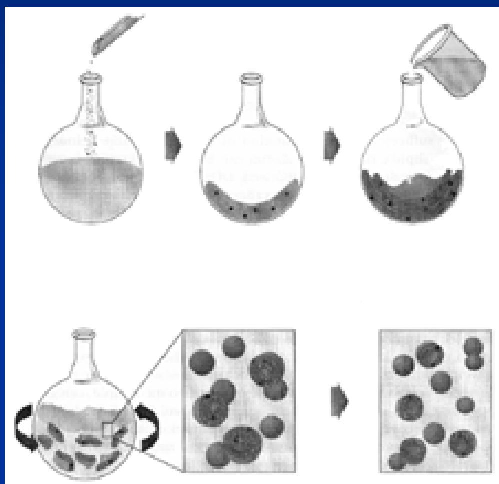
Grafted PEG regimes and Bilayer Surface Coverage



- This data allows selection of PEG-lipids to provide desired coverage of bilayer surface
- 4-5 mol% PEG200 gives complete "mushroom" coverage

6 Lipid Membrane as a Material: Processing

Solvent evaporation, rehydration



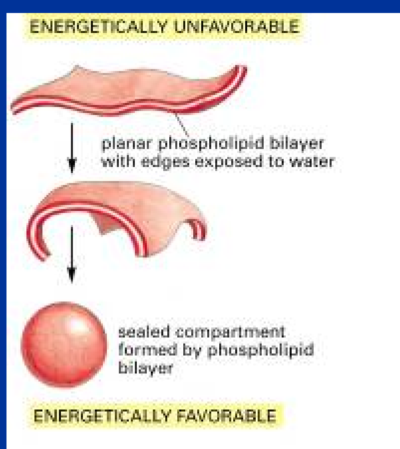
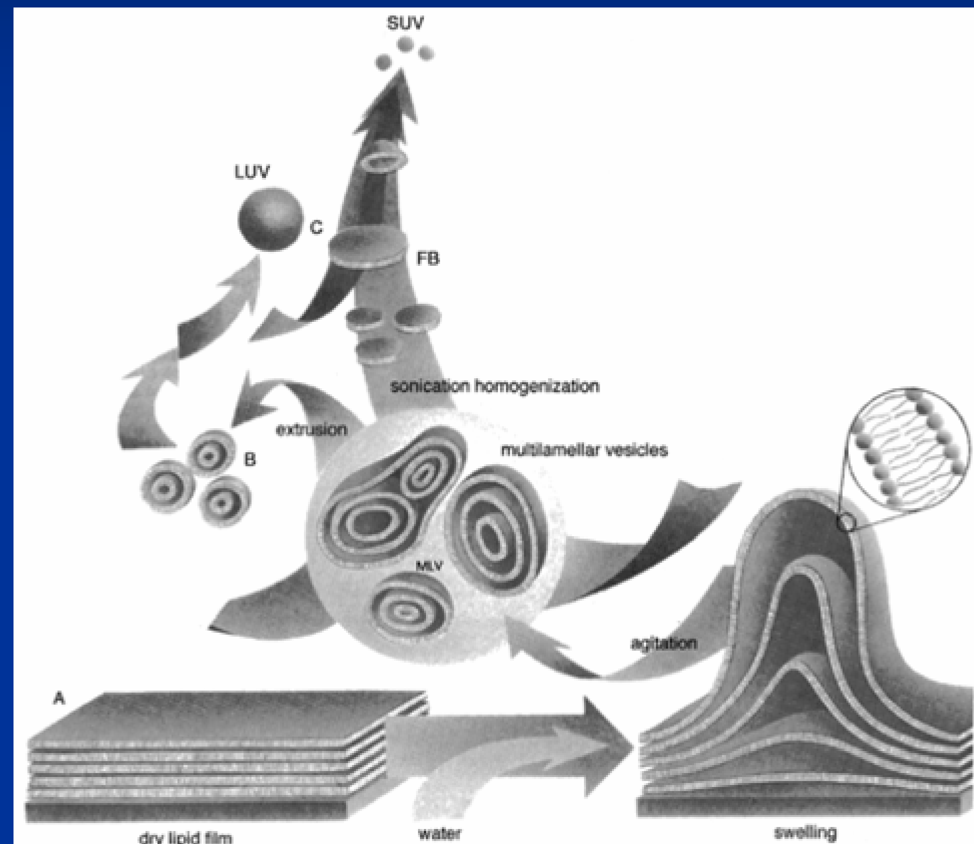


Figure 10-5. Molecular Biology of the Cell, 4th Edition.

Schematic rehydration process



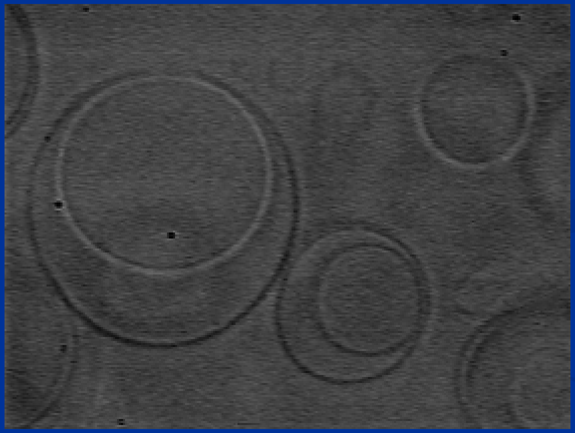
Avanti Polar Lipids, Inc
www.avantilipids.com

6 Lipid Membrane as a Material: Processing

Vesicle Formation

- Lipid lamellae hydrate and form vesicles when hydrated with non-electrolyte solutions,
- in electrolyte solutions, lamellae are remain adherent, and less hydrated, unless highly charged lipid is used

Dried lipid film on glass cover slip showing lamellar structure



Same lipid film after hydration with water (or non-electrolyte solution - 200 mM sucrose)

Lipid film after hydration with 90 mM NaCl solution gives partially rehydrated “myelin”-like structures

Self-Forming, Self-Healing Membranes

liposomes

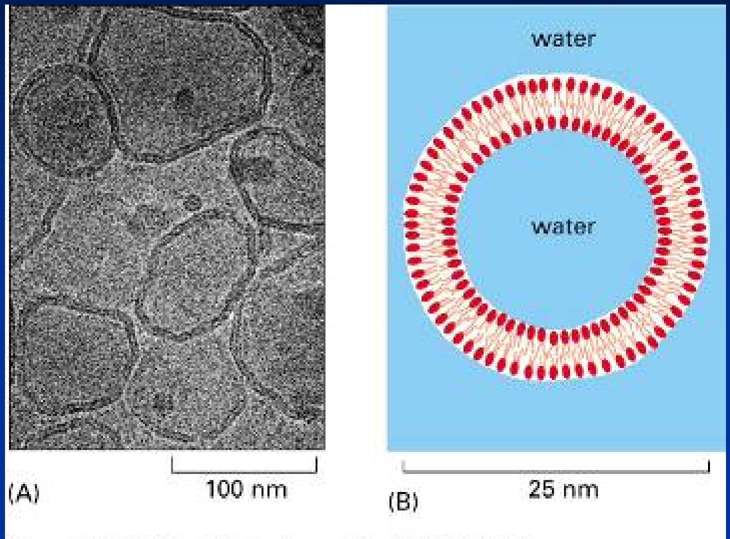


Figure 10–6. Molecular Biology of the Cell, 4th Edition.

and BLMs
(soap film underwater
D A Haydon)

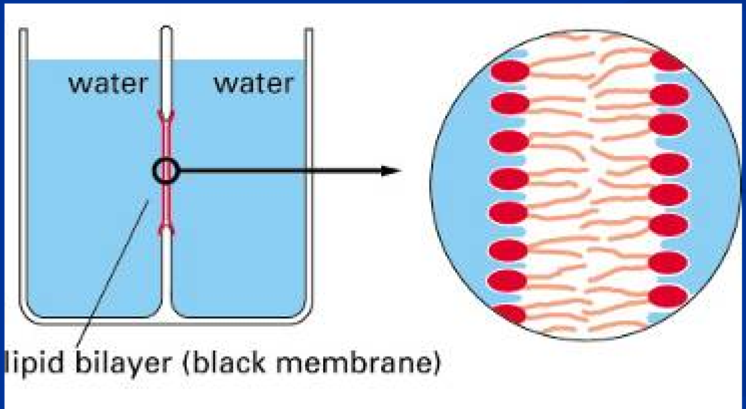
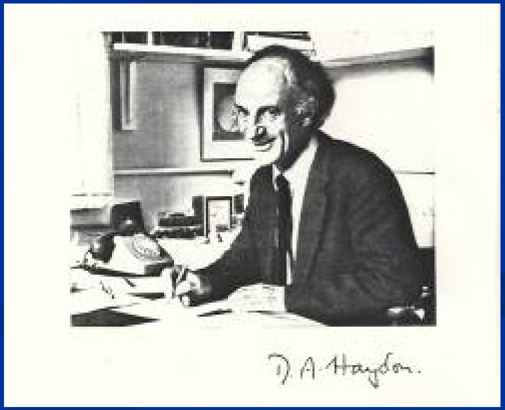
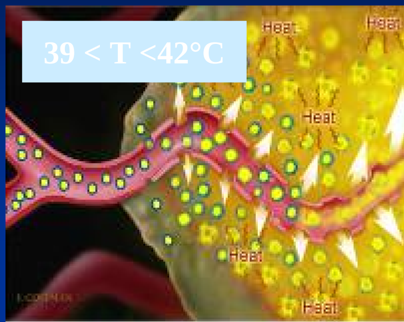


Figure 10–7. Molecular Biology of the Cell, 4th Edition.



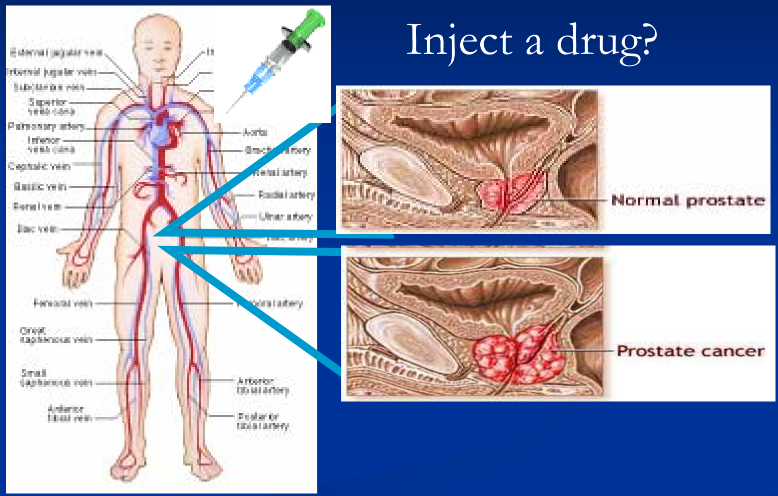
7 Forward Engineering Bioinspired Membranes?
In Vivo Drug Delivery and Drug Release??



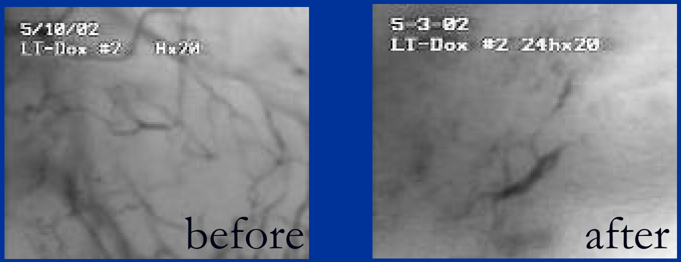
Mechanism: drug release in blood vessels

Tentative Component Liposome Design: TSL

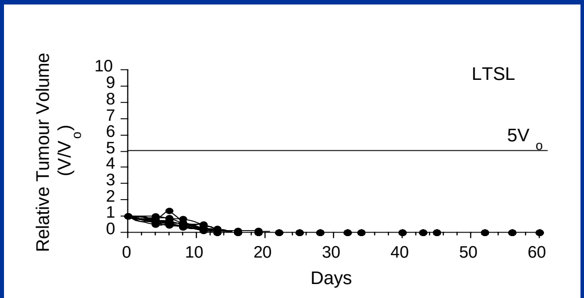
Inject a drug?



System/Problem to be Solved: Prostate cancer



Effect: vascular shut down



Complete regression of tumor growth

- physical parameters/limitations:
  - solubility
  - diffusion
  - convection
- Engineering
  - Design Methodology
  - Materials (CSP)
  - Analyses
  - Processing, Performance

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The end