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Phase Transition in Lipid Bilayers

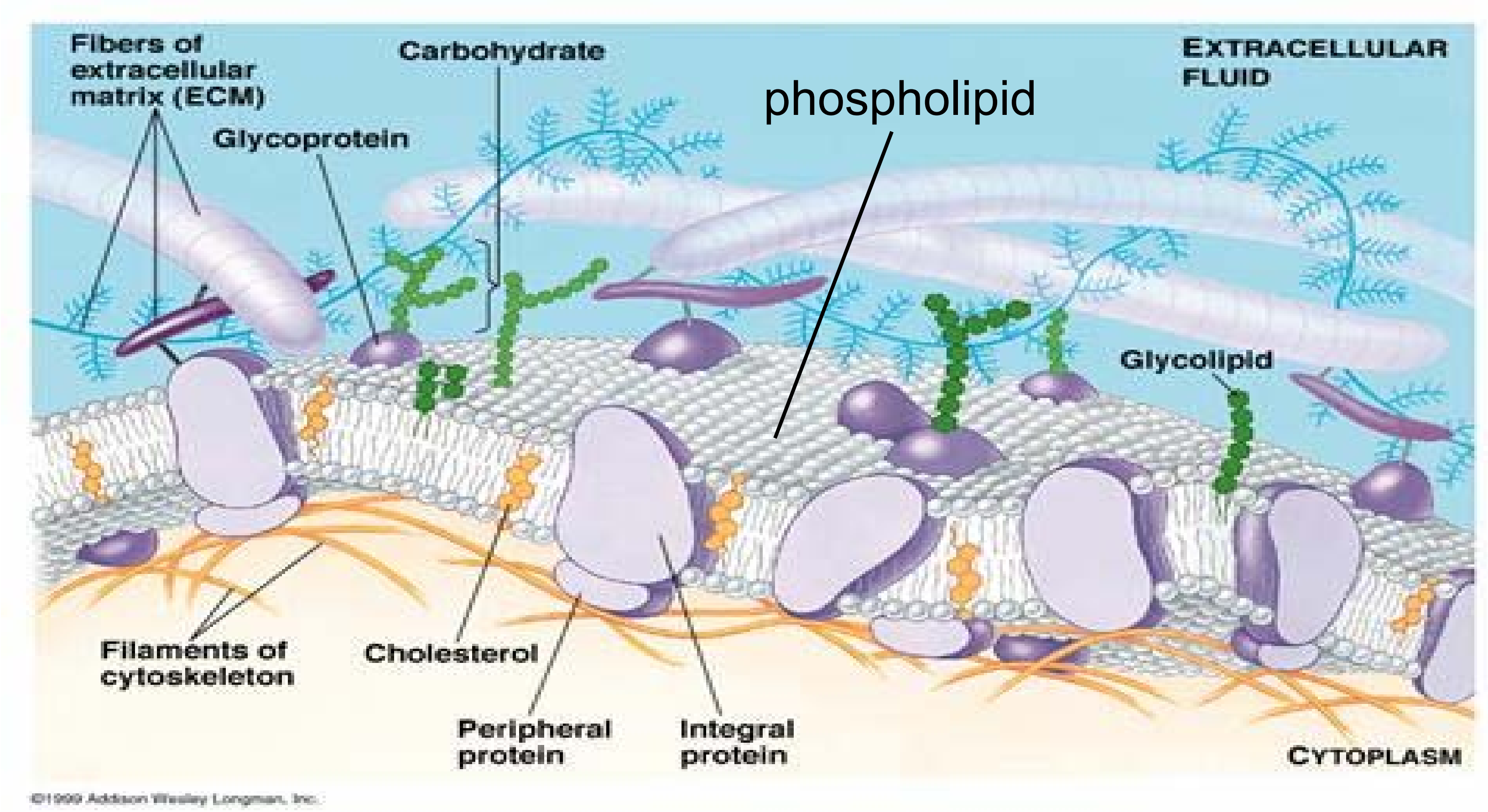
Ethanol's Effect on Miscibility Transition Temperature in Ternary Vesicles

Biology and Physics

- Biology: Why do we make vesicles?
- Physics: Phase Transition
- Our Experiment

- Why do we make vesicles?

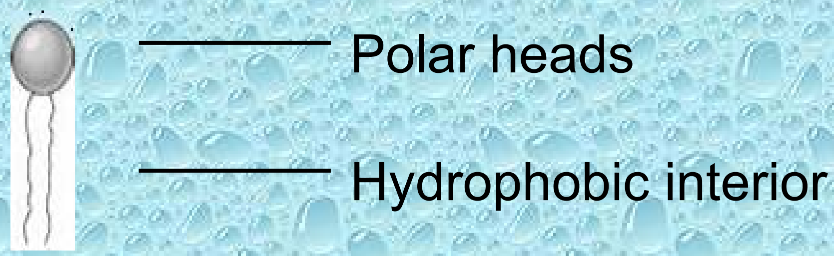
Fluid-Mosaic Model



- first proposed by S.J. Singer (1971) as a **lipid protein** model
- and extended to include the **fluid** character in a publication with G.L. Nicolson in "Science" (1972)

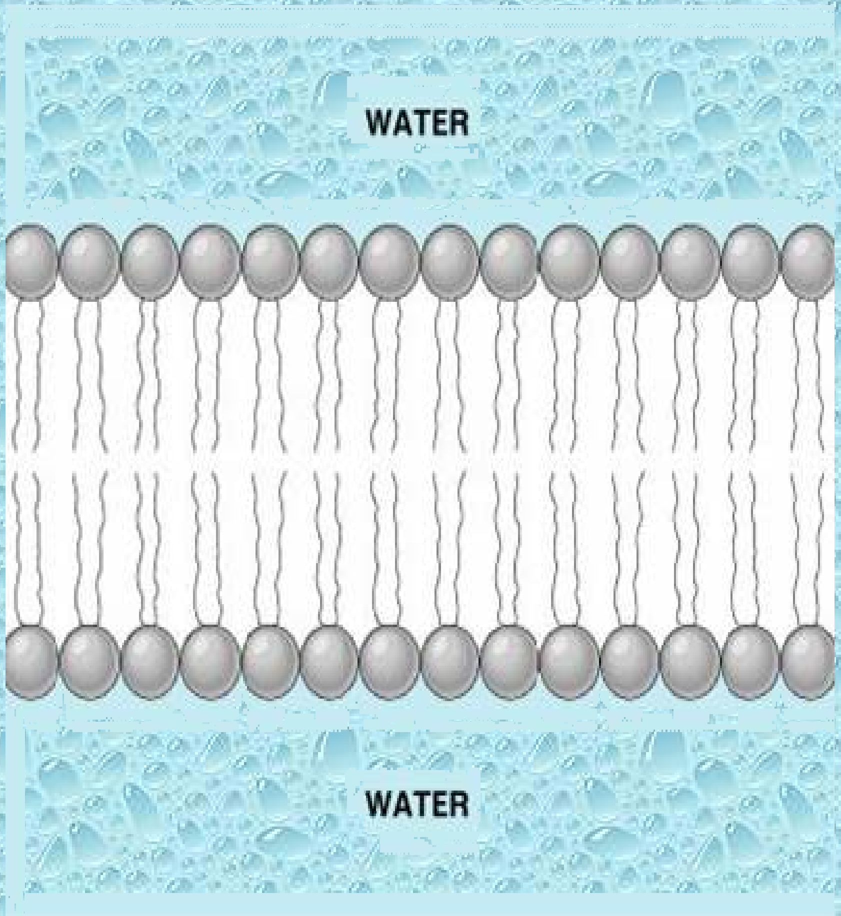
from en.wikipedia.org

Phospholipid



From fajerpc.magnet.fsu.edu

Phospholipid bilayer

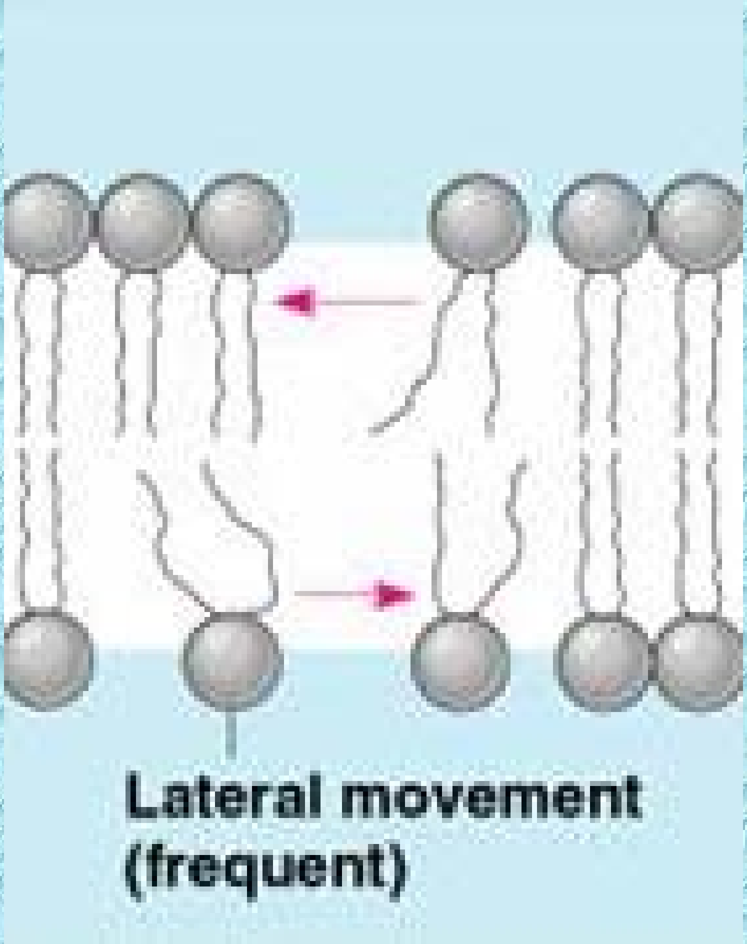


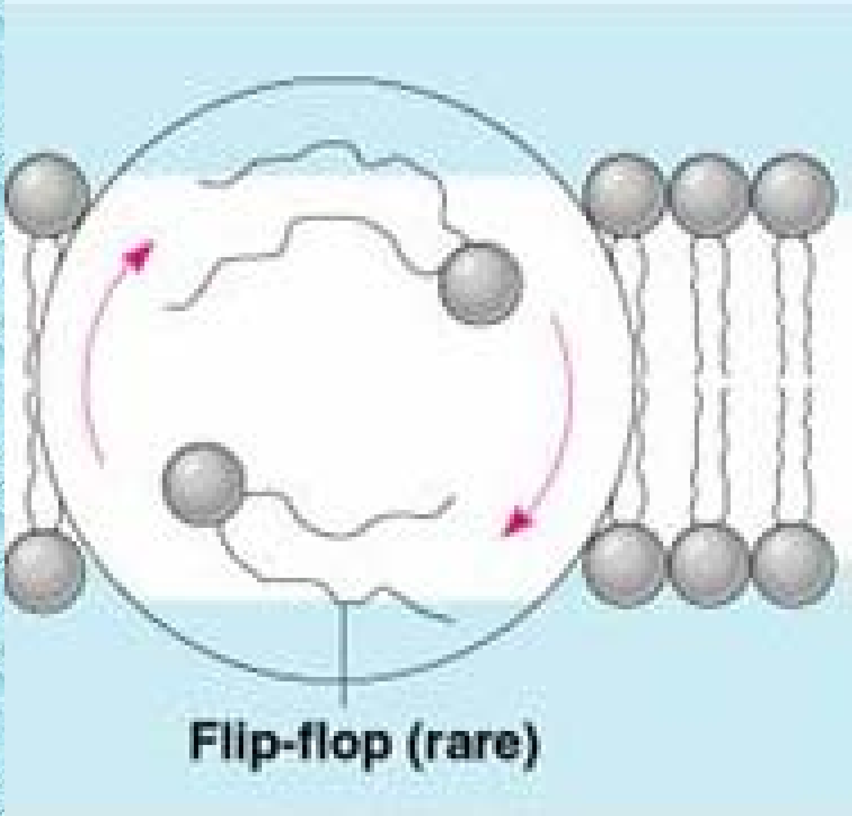
Polar heads

Hydrophobic interior

From fajerpc.magnet.fsu.edu

Lipid movement





From fajerpc.magnet.fsu.edu

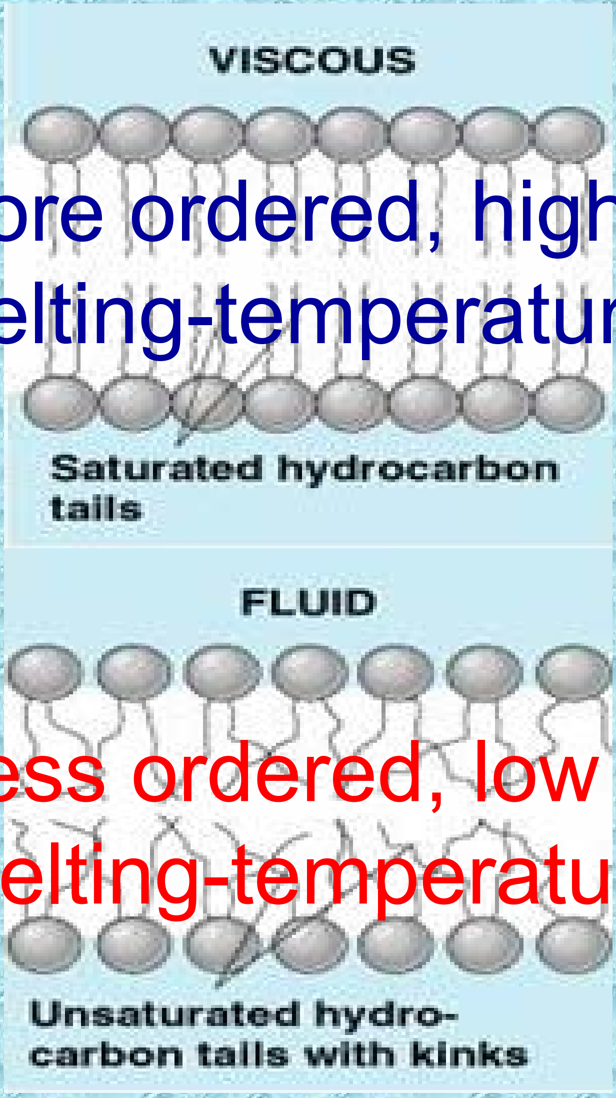
Viscosity depends on…

- The saturation of lipids

- Other molecules (e.g. Cholesterol)

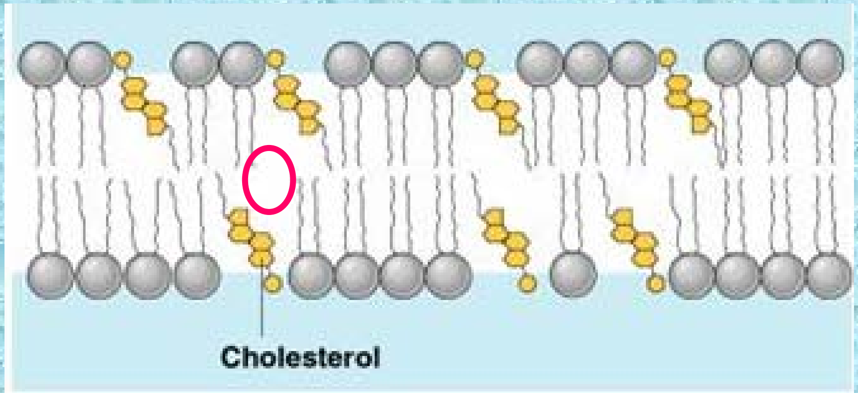
More ordered, high melting-temperature

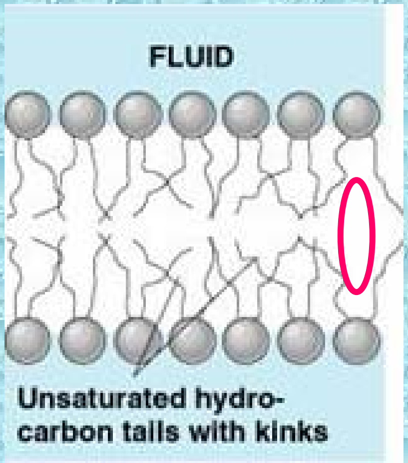
Less ordered, low melting-temperature

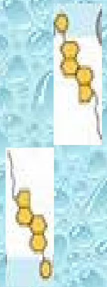


From fajerpc.magnet.fsu.edu

Cholesterol has a bad reputation… moderates membrane viscosity…







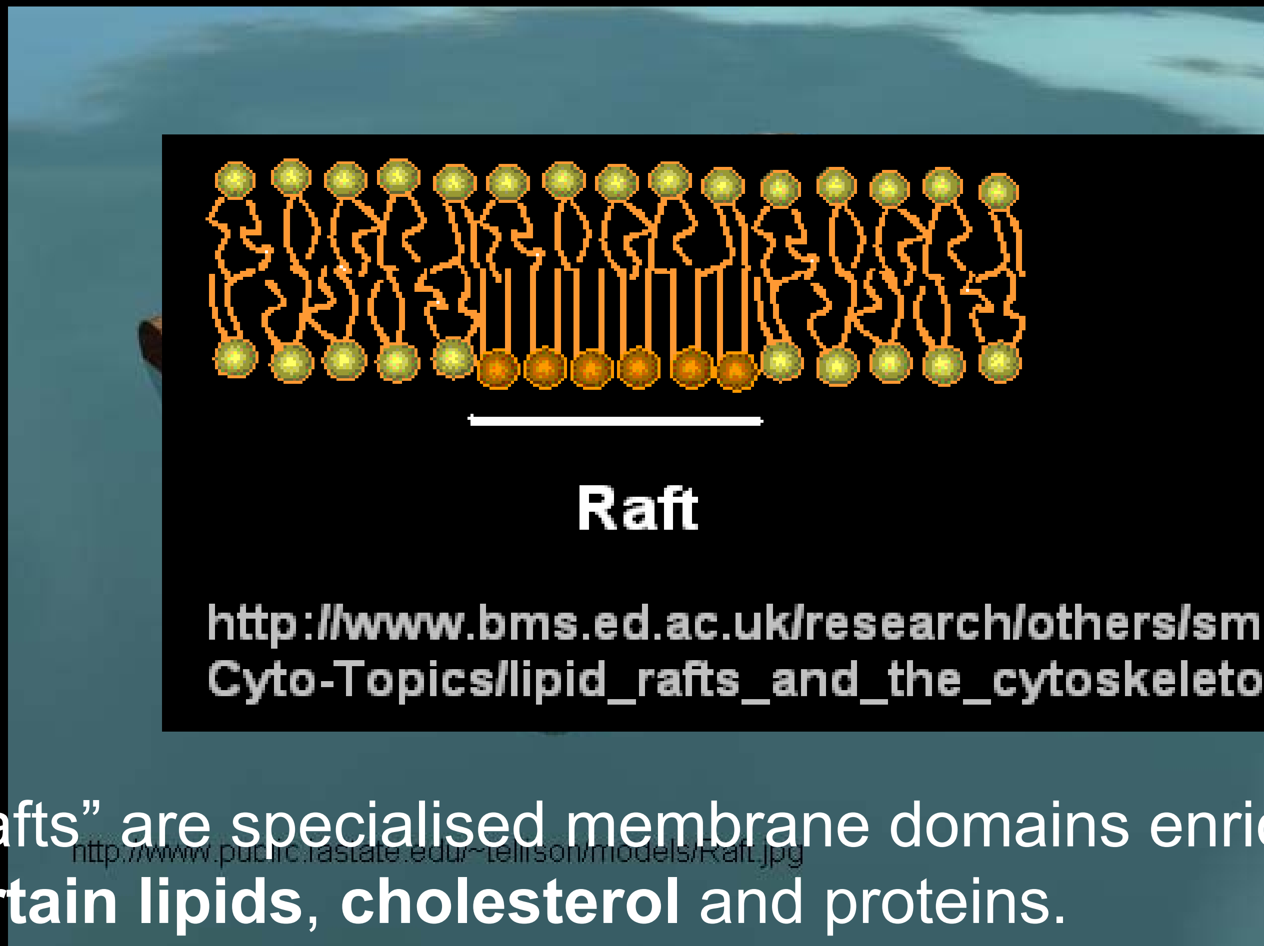
- disrupts long range order (prevents solid phase);
- enhances short range order (increases acyl chain order in fluid phase)

From fajerpc.magnet.fsu.edu

Remember this fluid mosaic model was proposed in the early 1970's…

- Simons & van Meer (Simons, K. & van Meer, G. (1988). *Biochemistry 27*, 6197-6202.) first hypothesized the existence of lipid rafts in 1988…

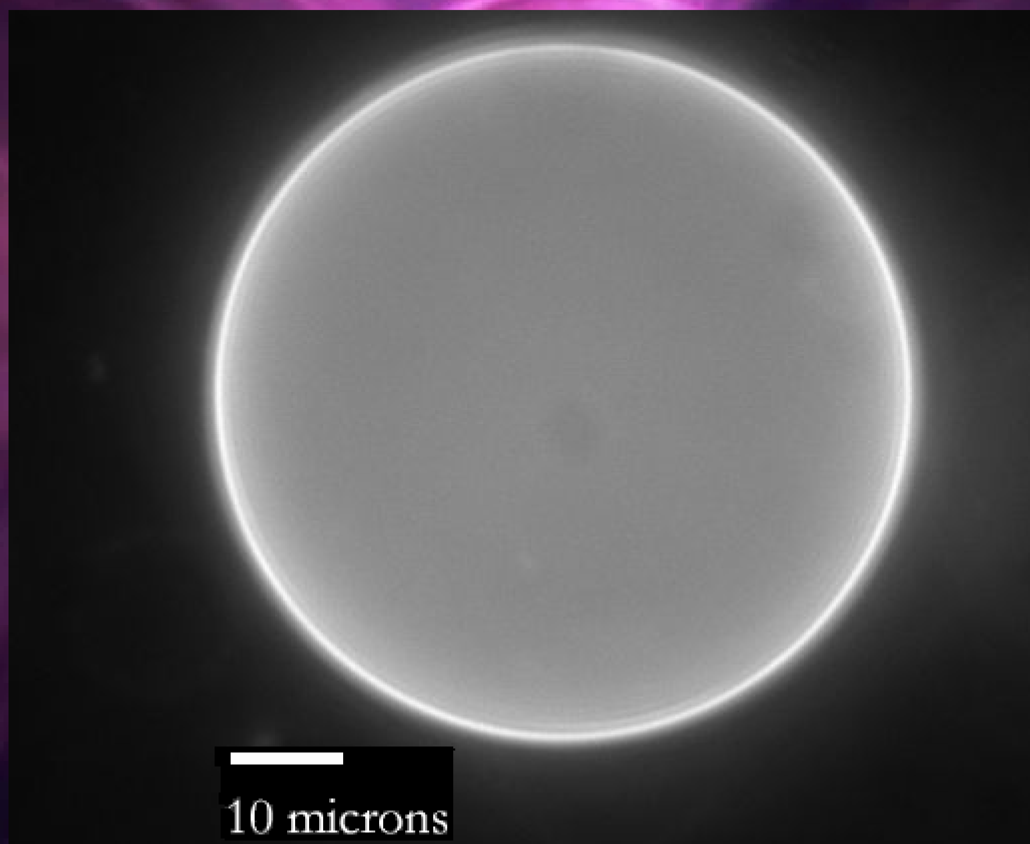
“Rafts”???



“Rafts” are specialised membrane domains enriched in **certain lipids**, **cholesterol** and proteins.

- Rafts are important, but they are in cell membrane.

- Giant Unilamellar Vesicles (GUVs)
- Why do we make vesicles?



Order

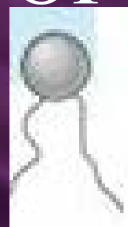
Solid > Liquid ordered (e.g. rafts), L_o > Liquid disordered, L_d

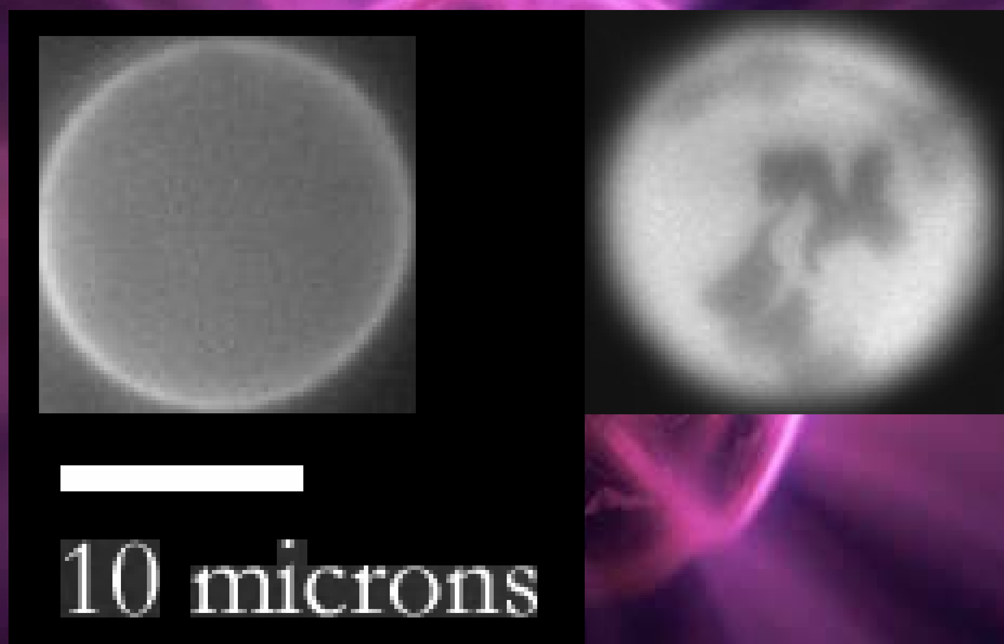
Gel > Liquid > Gas

Q: Is it possible for our vesicles to have L_o and L_d coexisting?

Q: If so, under what conditions?

Binary System

DPPC  + DOPC  = gel – liquid coexistence



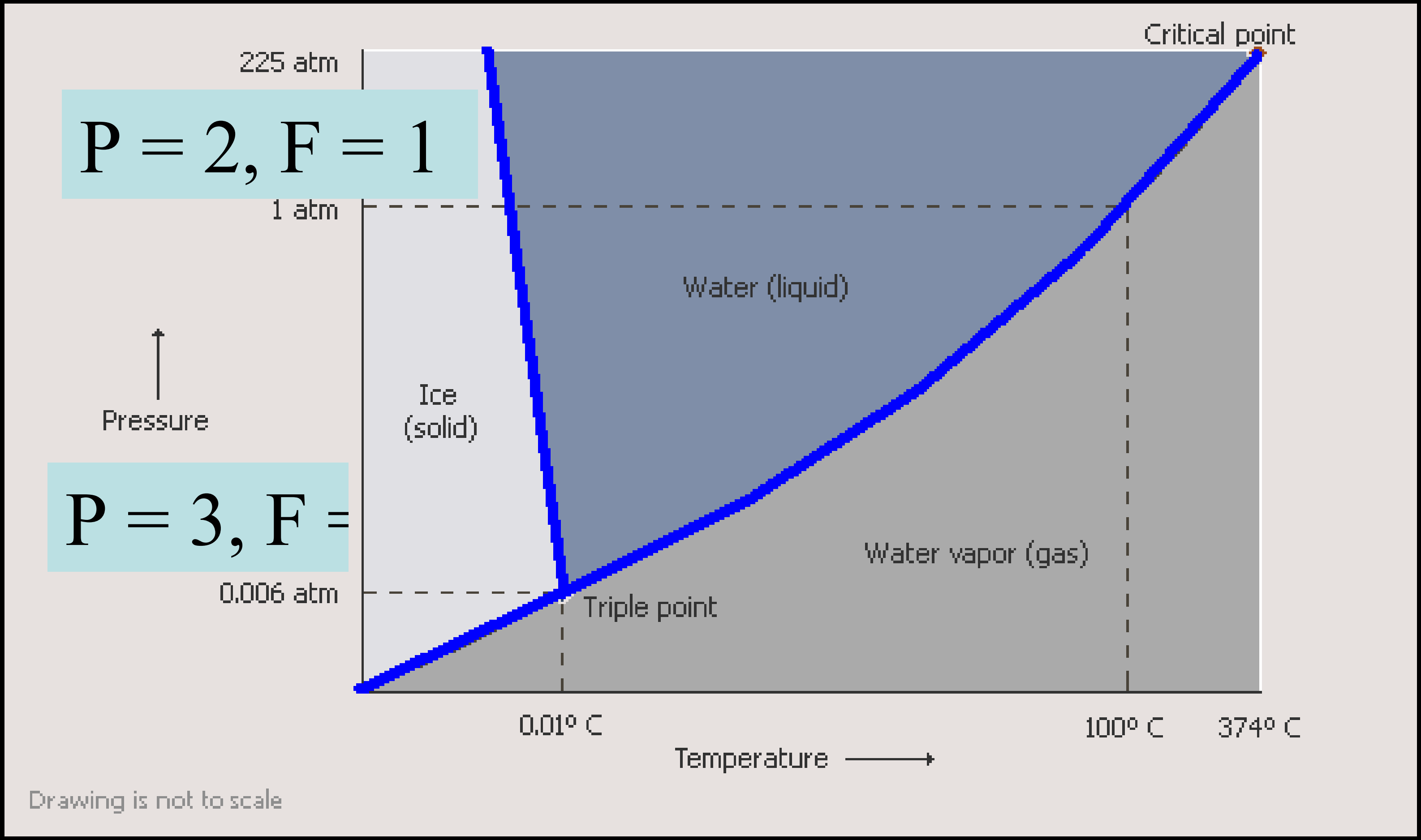
Gibb's Phase Rule

- At equilibrium, $F = C + 2 - P$
- ➔ $P = C + 2 - F$
- For $C = 1$, $P = 1 + 2 – F$

  $= 3 – F$

Triple point occurs at a fixed temperature and pressure.

Phase diagram of water



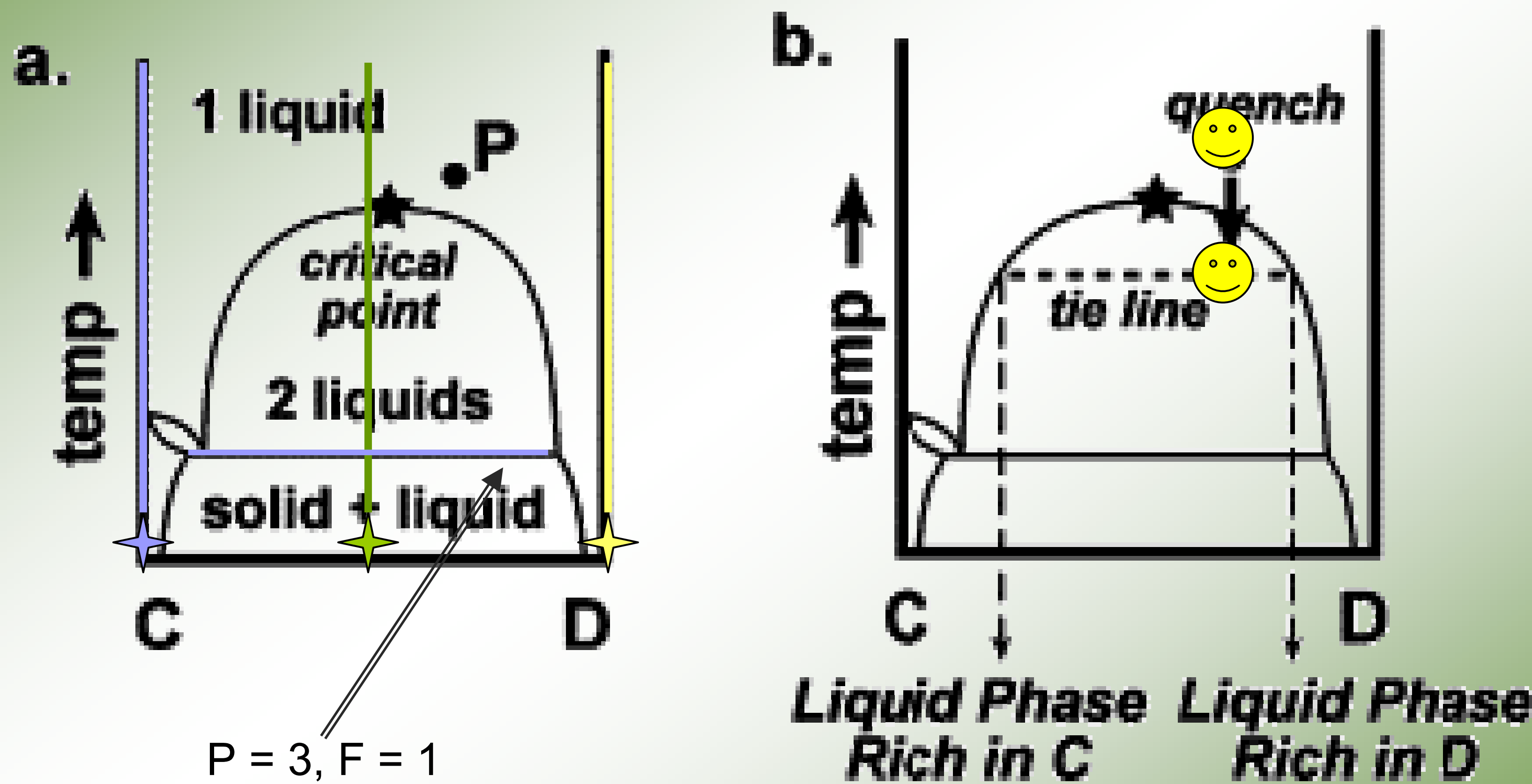
http://encarta.msn.com/media_461541579/Phase_Diag

Gibb's Phase Rule

- At equilibrium, F = C + 2 - P
- ➔ P = C + 2 - F
- For C = 2, P = 2 + 2 – F

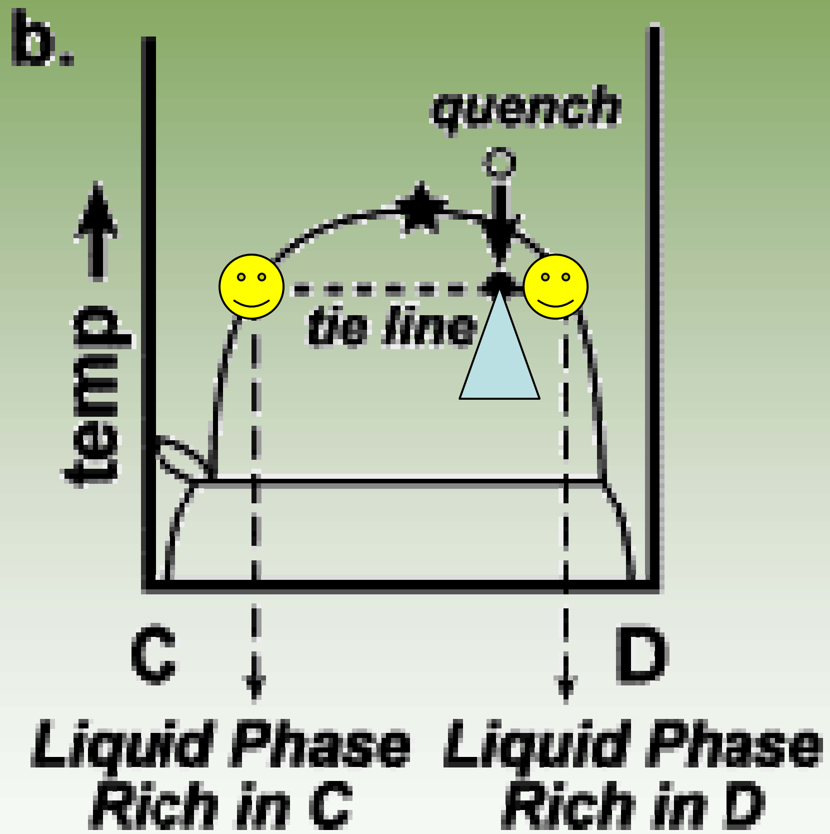
  = 4 – F

Binary System and Tie-line



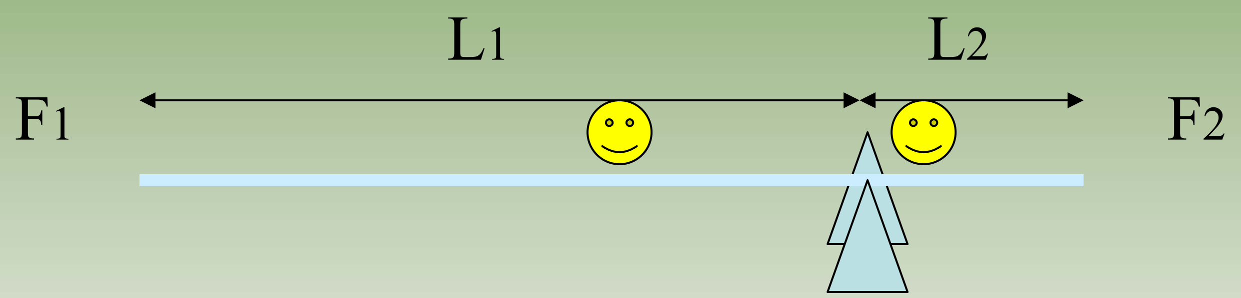
Sarah L. Veatch and Sarah L. Keller, (2005) BBA Review, In Press, (http://www.sciencedirect.com/science/article/B6T20-4GJV954-1/2/2ce878c214514e584c1545a8d520edcc)

Lever Rule



b.
quench
temp
tie line
C
D
Liquid Phase Rich in C
Liquid Phase Rich in D

Lever Rule



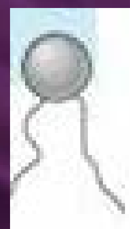
$$F_1 L_1 = F_2 L_2$$

$$\Rightarrow \quad F_1 / (F_1 + F_2) = L_2 / (L_1 + L_2)$$

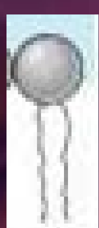
$$F_2 / (F_1 + F_2) = L_1 / (L_1 + L_2)$$

Q: Is it possible for the two liquid phases L_o and L_d to coexist in a <u>binary</u> system experimentally?

Binary system

 +  = gel – liquid coexistence

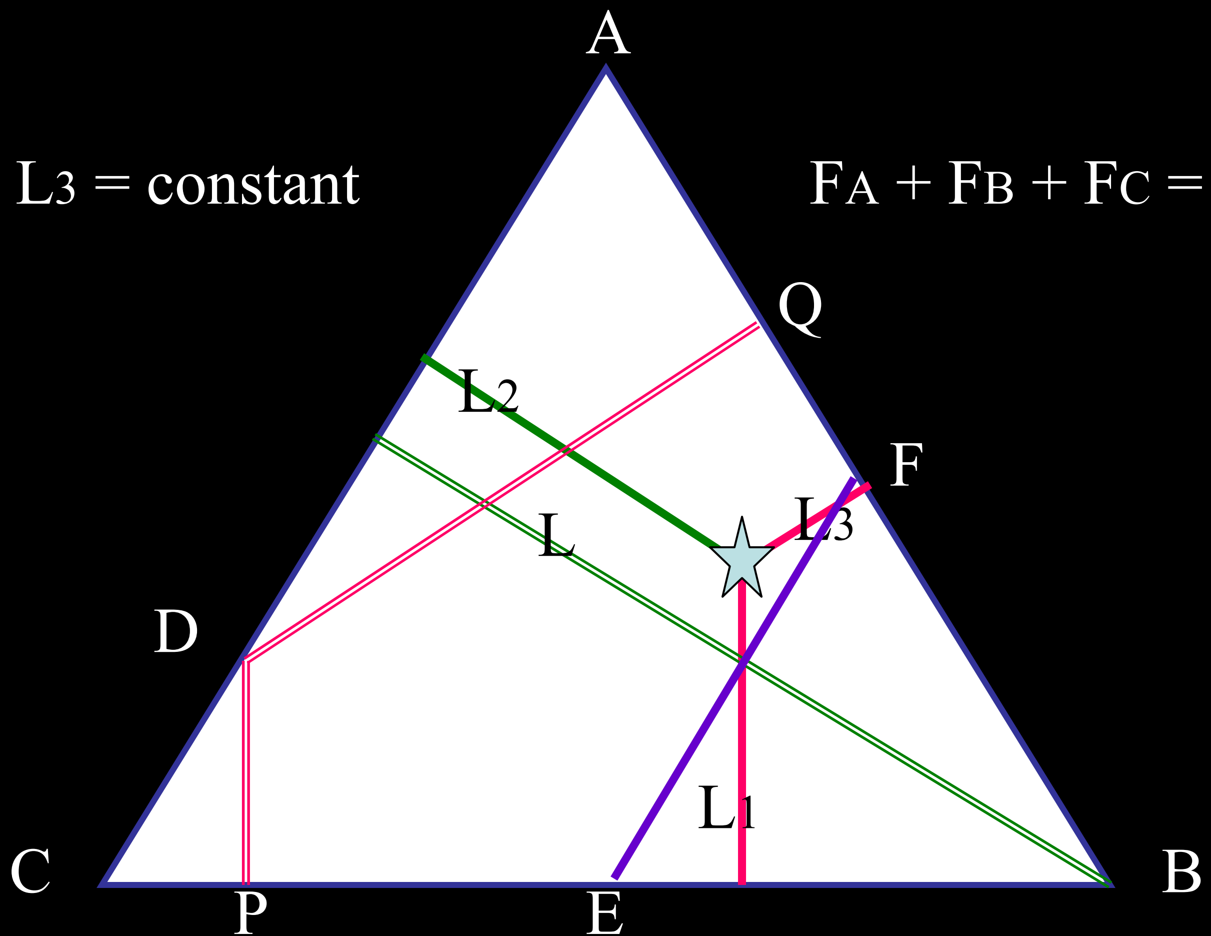
 +  = one uniform liquid phase

 +  = nothing detectable with fluorescence microscopy

Gibb's Triangle and Ternary System

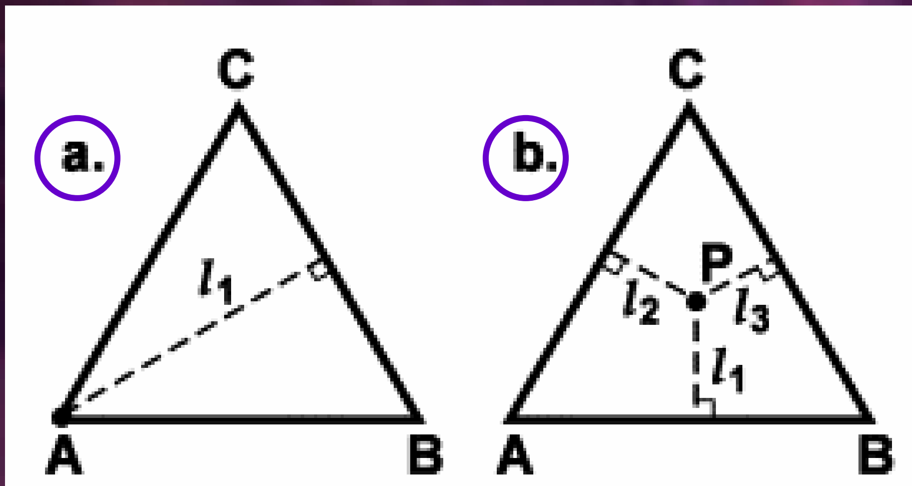
$L_1 + L_2 + L_3 = \text{constant}$

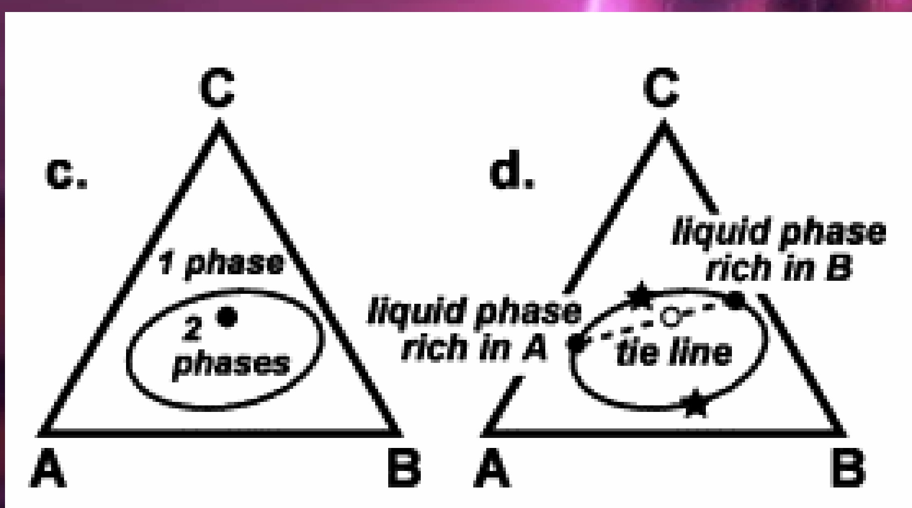
$F_A + F_B + F_C = 1$



$$\frac{\text{Amount of A}}{\text{Amount of C}} = \frac{DP}{DQ} = \frac{DC}{DA}$$

Gibb's Triangle and Ternary System

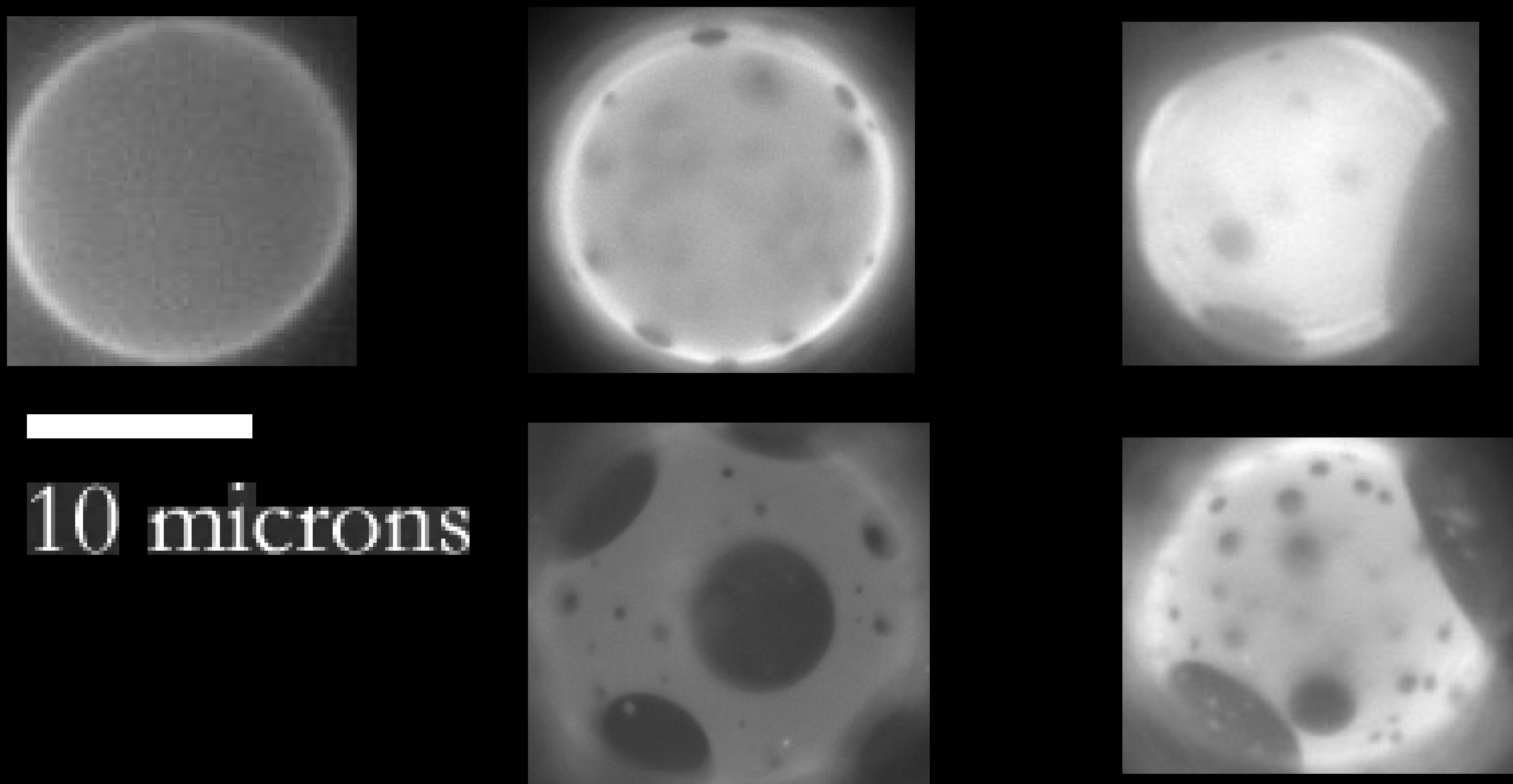




Sarah L. Veatch and Sarah L. Keller, (2005) BBA Review, In Press, (http://www.sciencedirect.com/science/article/B6T20-4GJV954-1/2/2ce878c214514e584c1545a8d520edcc)

Q: Is it possible for the two liquid phases L_o and L_d to coexist in a ternary system experimentally?

This is easy to observe…



Giant Unilamellar Vesicles (GUVs) of
1:1 DPPC:DOPC 20% cholesterol

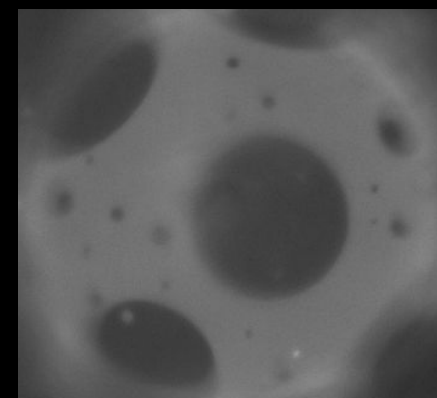
This is easy to observe…

High Temp

10 microns

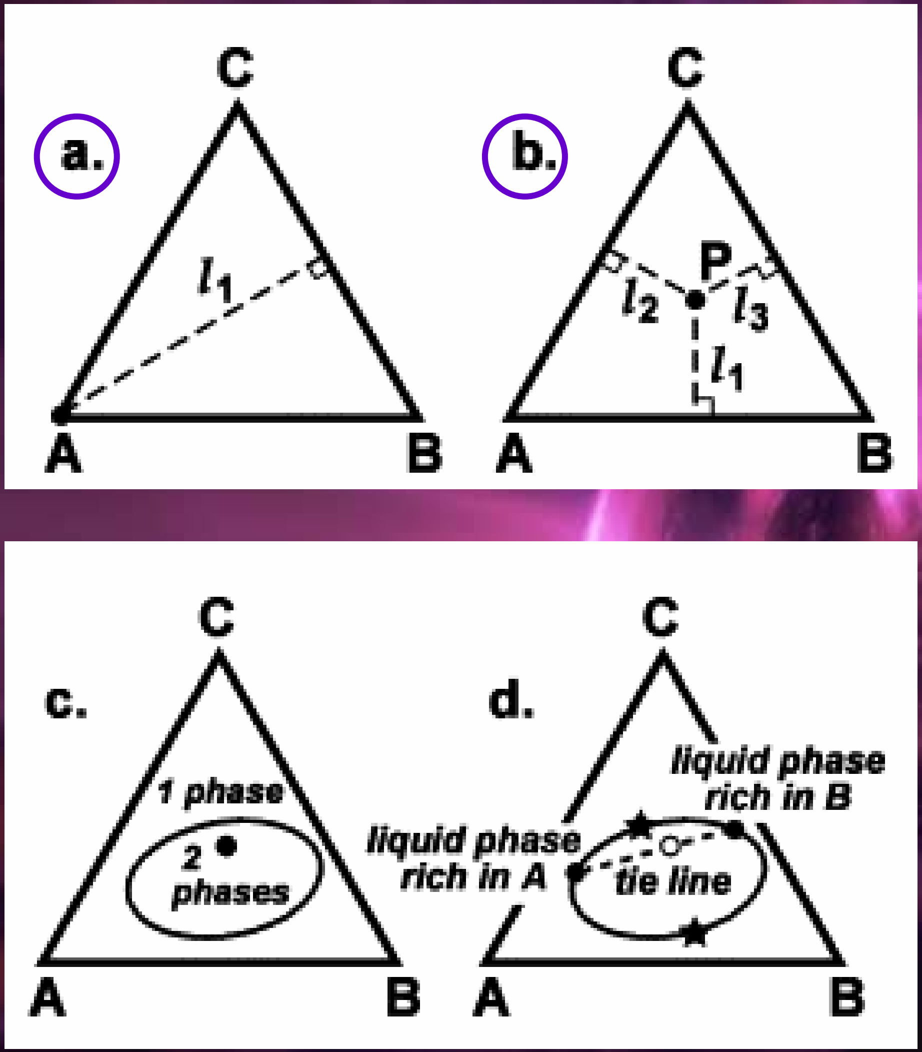
Phase Transition

Low Temp



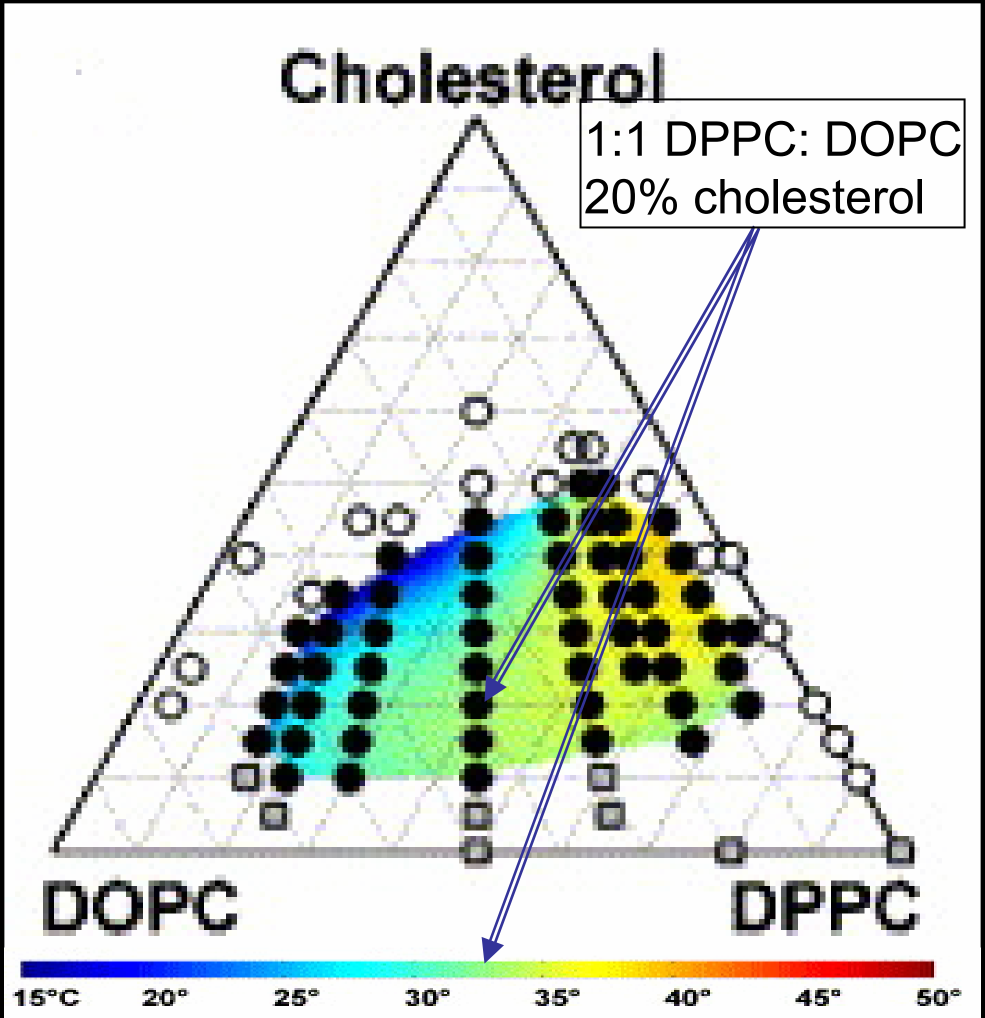
Giant Unilamellar Vesicles (GUVs) of 1:1 DPPC:DOPC 20% cholesterol

Gibb's Triangle and Ternary System



Sarah L. Veatch and Sarah L. Keller, (2005) BBA Review, In Press, (http://www.sciencedirect.com/science/article/B6T20-4GJV954-1/2/2ce878c214514e584c1545a8d520edcc)

Phase Transition Temperature



Sarah L. Veatch and Sarah L. Keller, (2005) BBA Review, In Press, (http://www.sciencedirect.com/science/article/B6T20-4GJV954-1/2/2ce878c214514e584c1545a8d520edcc)

Why ethanol?

www.strw.leidenuniv.nl/astrochem/Ethanol.gif

Ethanol as general anesthesia

- "It is generally known that the primary action of ethanol is on the central nervous system, resulting in depression of neurotransmission functions in the intoxicated state and development of tolerance and physical dependence after chronic exposure."

"Like most general anesthetics, ethanol was thought to cause depression of neuronal functions by interfering with the cationic translocation of ions across the neuronal membrane."

(GY Sun, AY Sun - Alcohol Clin Exp Res, 1985)

How much ethanol should we add?

http://medicine.ucsd.edu/HFP/images/needle%20syringe.jpg http://www.intox.com/wheel/drinkwheelresults.asp

BAC (g/100 ml of blood)	Stage	Clinical symptoms
0.01 - 0.05	Subclinical	Behavior nearly normal by ordinary observation
0.03 - 0.12	Euphoria	Mild euphoria, sociability, talkitiveness Increased self-confidence; decreased inhibitions Diminution of attention, judgment and control Beginning of sensory-motor impairment Loss of efficiency in finer performance tests
0.09 - 0.25	Excitement	Emotional instability; loss of critical judgment Impairment of perception, memory and comprehension Decreased sensitory response; increased reaction time Reduced visual acuity; peripheral vision and glare recovery Sensory-motor incoordination; impaired balance Drowsiness
0.18 - 0.30	Confusion	Disorientation, mental confusion; dizziness Exaggerated emotional states Disturbances of vision and of perception of color, form, motion and dimensions Increased pain threshold Increased muscular incoordination; staggering gait; slurred speech Apathy, lethargy
0.25 - 0.40	Stupor	General inertia; approaching loss of motor functions Markedly decreased response to stimuli Marked muscular incoordination; inability to stand or walk Vomiting; incontinence Impaired consciousness; sleep or stupor
0.35 - 0.50	Coma	Complete unconsciousness Depressed or abolished reflexes Subnormal body temperature Incontinence Impairment of circulation and respiration Possible death
0.45 +	Death	Death from respiratory arrest

How much ethanol should we add?

- Membrane concentration:

0.70 moles EtOH / kg dry membrane

(Philip Seeman – Pharmacological Reviews, 1972 Vol. 24, No. 4)

- Partition coefficient:

K = 0.2 (Molarity in membrane/Molarity in aqueous phase)

$$\frac{0.070 \text{ mol EtOH}}{1 \text{ kg of membrane}} * \frac{1 \text{ kg}}{10^{6} \text{ mg}} * 0.25 \text{ mg membrane}$$

$= 1.75 * 10^{-8}$ mol of EtOH in membrane;

$$\frac{3.5895 * 10^{-7} \text{ mol of lipids in membrane}}{} * \frac{6.022 * 10^{23} \text{ molecules}}{1 \text{ mol}}$$

$$* \frac{1 \text{ molecule}}{1145 \text{ Å}^3} * \frac{10^{-27} \text{ L}}{1 \text{ Å}} = 2.48 * 10^{-7} \text{ L of membrane lipids;}$$

0.2 = partition coefficient

$$= \frac{1.75 * 10^{-8} \text{ mol of EtOH} \;/\; 2.48 * 10^{-7} \text{ L of membrane lipids}}{?? \text{ mol of EtOH} \;/\; 1 \text{ L of aqueous solution}}$$

?? = 0.35 mol EtOH/L H_2O

= 16 g EtOH/L H_2O

INTRODUCTION

The interaction of short-chain alcohols with biological membranes forms an important area of exploration because of the role of alcohols in metabolism, membrane fusion, drug delivery, alcohol toxicity, alcohol tolerance, and general anesthesia. Of particular interest is fundamental understanding of the molecular mechanism of action of short-chain alcohols on the lipid portions of biomembranes. To this end, model systems of known lipid composition such as unilamellar vesicles or supported bilayers have served as ideal platforms to elucidate the details of the effect of short-chain alcohols on the physical and thermodynamic properties of lipid membranes.

The emerging paradigm is that short-chain alcohols (given their amphiphilic nature) primarily confine themselves to the hydrophilic headgroup region instead of the hydrocarbon core as demonstrated by nuclear magnetic resonance (NMR; Barry and Gawrisch, 1994), nuclear Overhauser effect spectroscopy (Feller et al., 2002; Holte and Gawrisch, 1997), fluorescence spectroscopy (Rottenberg, 1992), and Fourier-transform infrared studies (Chiou et a [illegible] Their location in the headgroup region disturbs the natural microstructure of the lipid membrane and is apparently responsible for observed increases in membrane fluidity or disorder (Chin and Goldstein, 1977), increases in the membrane lipid lateral mobility (Chen et al., 1996), decreases in the main phase transition temperature (Rowe, 1985), and the formation of an interdigitated gel phase (Slater and Huang, 1988). Past works have also shown that alcohols increase membrane permeability (Komatsu and Okada, 1995, 1997), induce shape transformations of vesicles (Angelova et al., 1999), and influence membrane thermodynamic parameters (Rowe et al., 1998; Trandum et al., 2000; Westh and Trandum, 1999; Westh et al., 2001). Other important physical properties that short-chain alcohols may affect, but which need exploring, are mechanical properties and thickness, which are closely tied to integral membrane protein activities and cell shape. A close relationship between mechanical stress and activities in membrane-embedded proteins has been established with mechanical sensitive ion channels (Sukharev et al., 1997), gramicidin (Goulian et al., 1998), and the meta-I to meta-II transition in rhodopsin (Mitchell and Litman, 1999, 2000). Theoretical works exist relating the structure, function, and surface arrangement of membrane-bounded proteins and enzymes to membrane mechanics and thickness (Cantor 1997a,b; Dan et al., 1994; Dan and Safran, 1998). Shape and stability of cells and liposomes are regulated by mechanics as demonstrated by observed shape deformations of erythrocytes (Evans, 1989) and neutrophils (Tsai et al., 1993) and vesicle budding-fission-fusion events involved in vesicle-mediated material transport (Sackmann, 1994). Certainly, a quantification of

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HV Ly and ML Longo (2004): Biophysical Journal Vol. 87

Amazing…

Rowe, 1985: B.B.A. 813 : 321-330

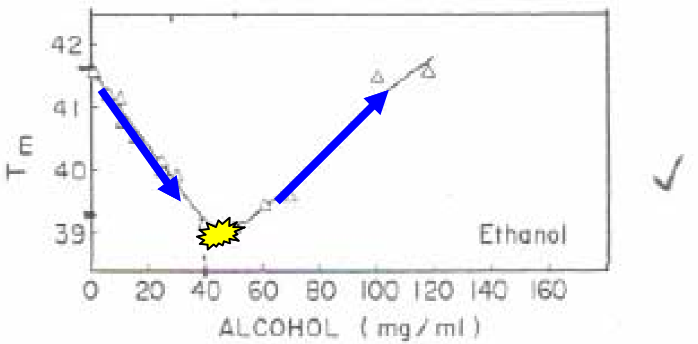


Fig. 1. Dependence of the transition midpoint temperature of PC(16 : 0) on alcohol concentration for ethanol (Δ) as determined by absorbance at 400 nm.

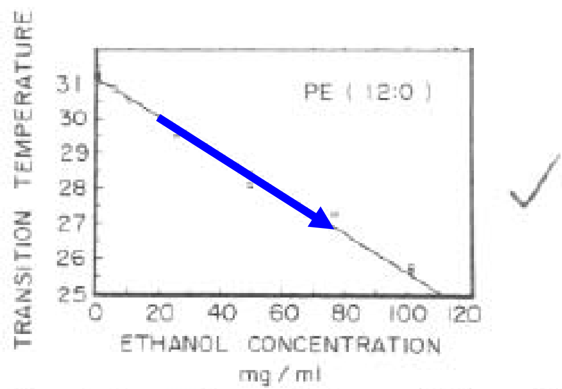
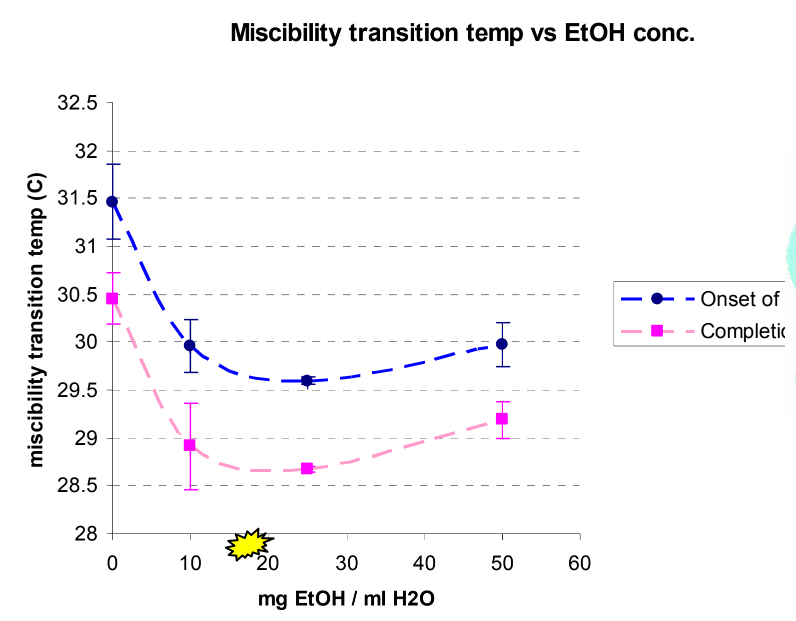
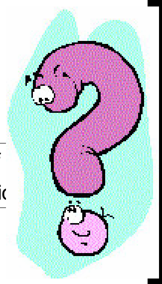


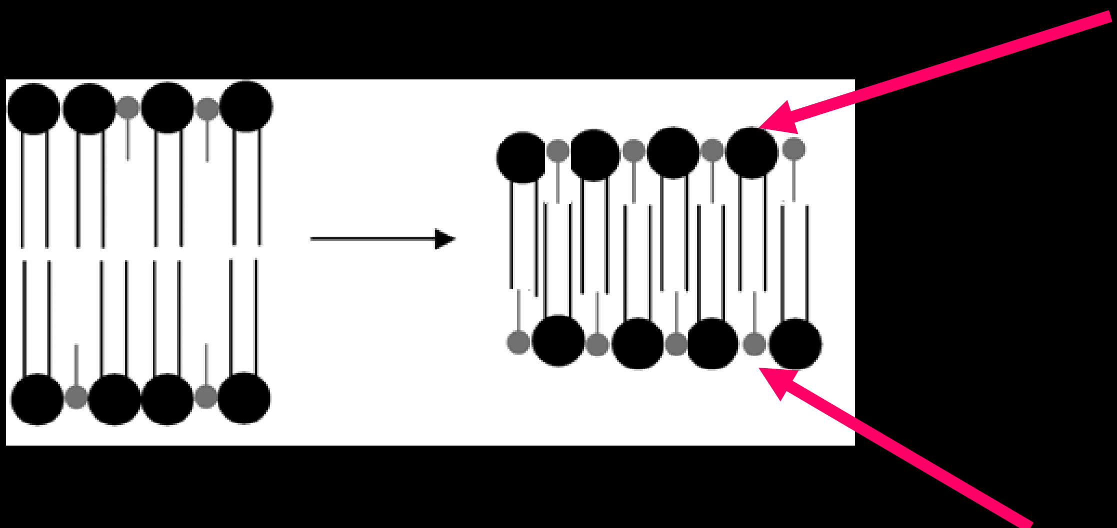
Fig. 2. Dependence of the transition midpoint temperatures of PE(12 : 0) as a function of ethanol concentration, as followed by absorbance at 400 nm.





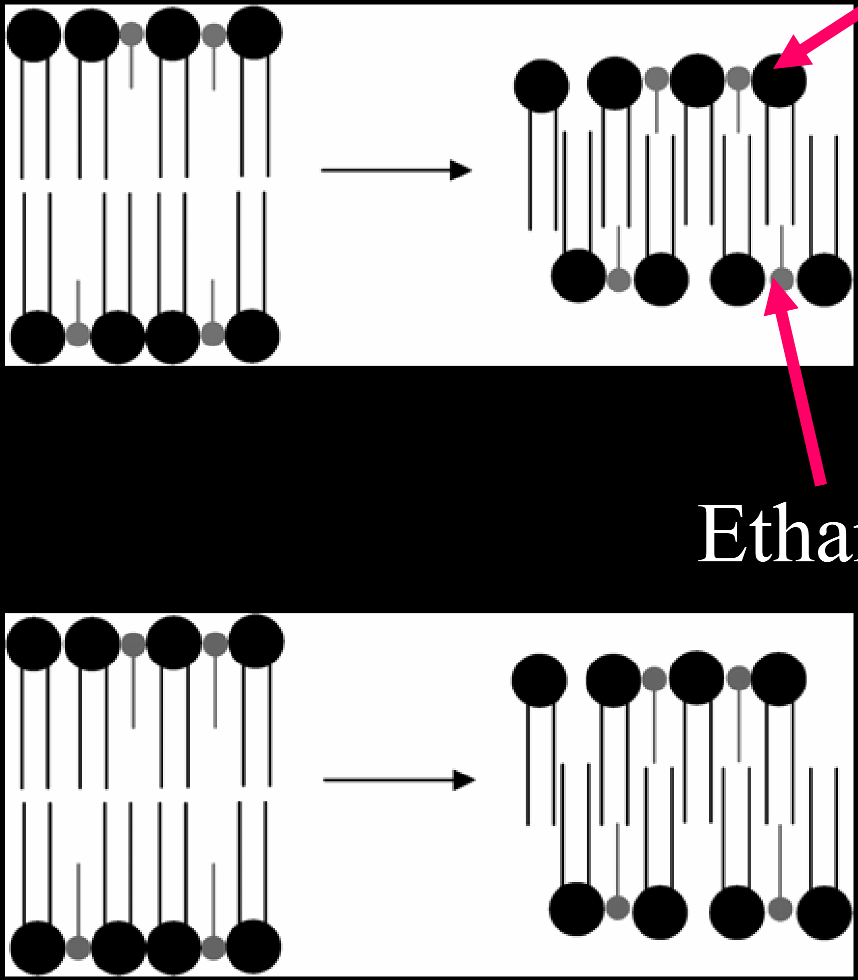
PE's don't make good vesicles.

Interdigitation



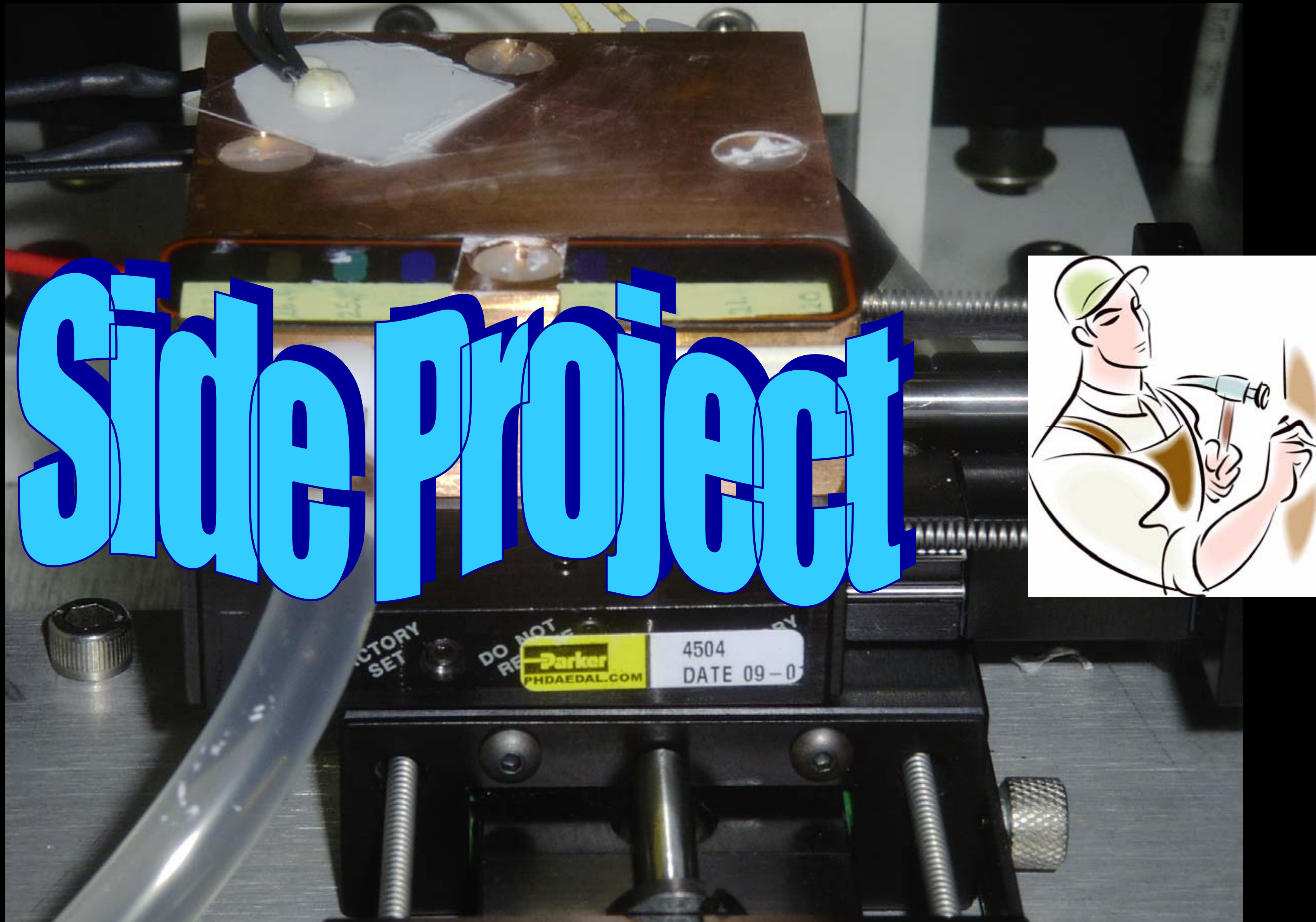
http://www.biophysj.org/cgi/content/full/87/3/1596

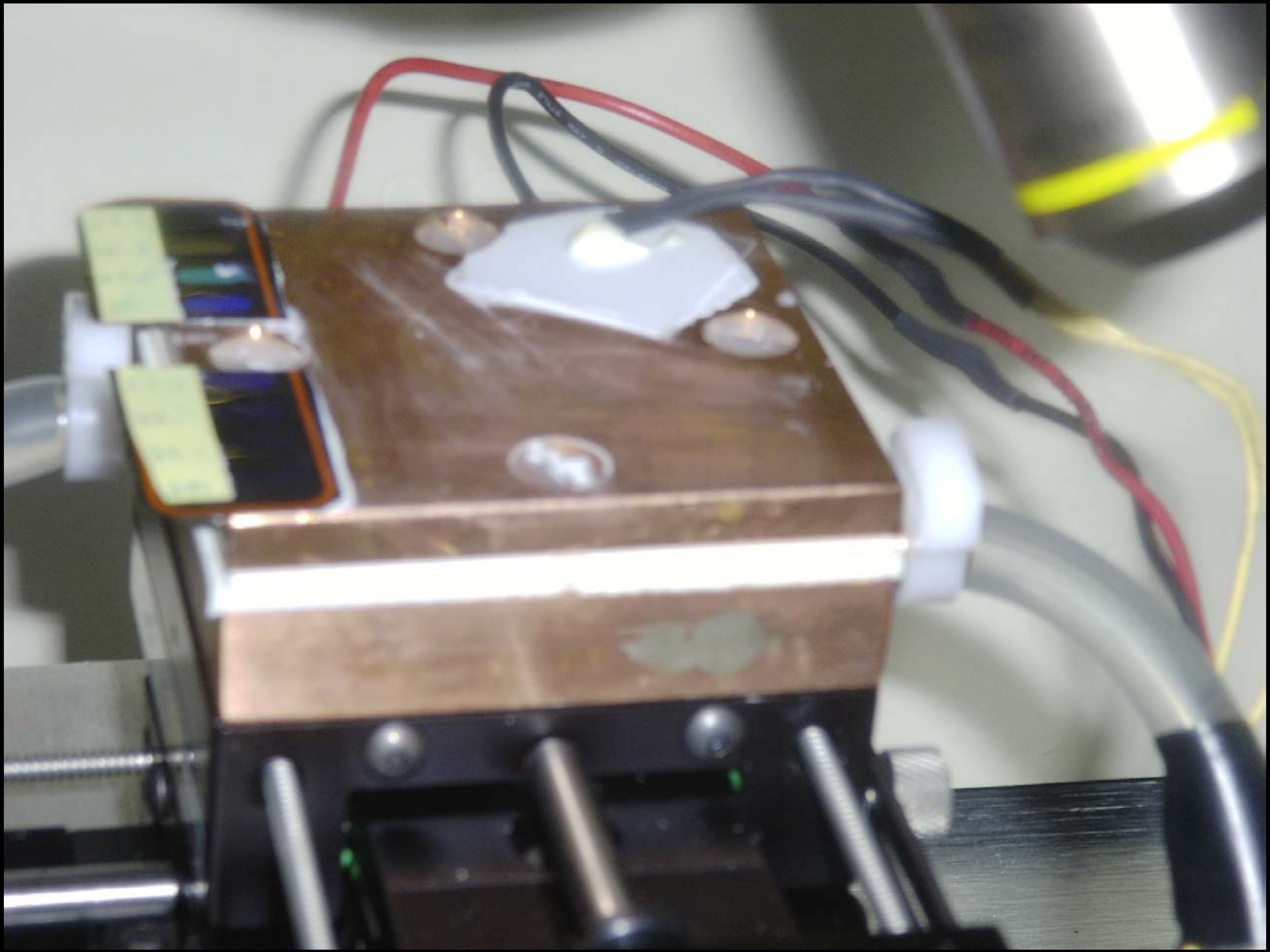
Interdigitation

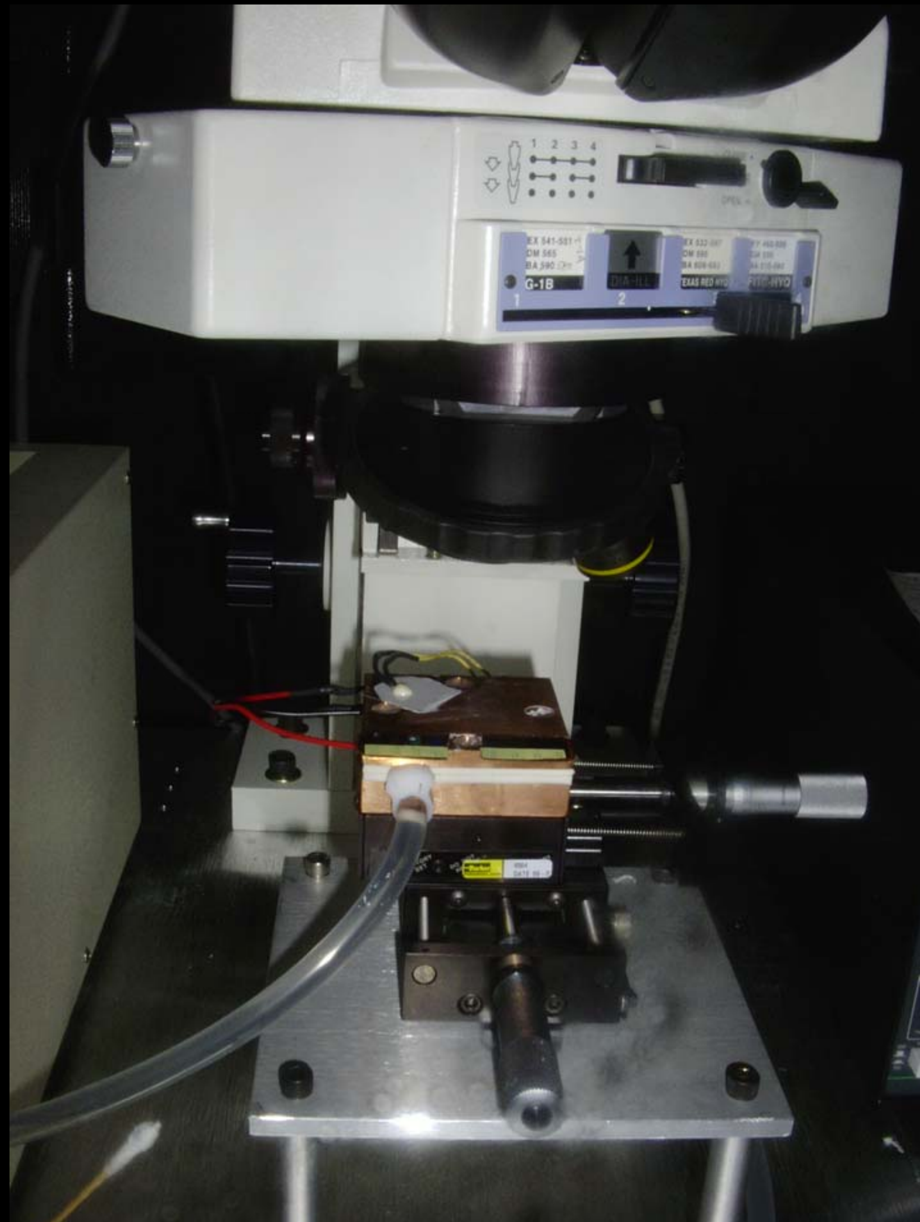


http://www.biophysj.org/cgi/content/full/87/3/1596

Side Project






1 2 3 4
DM 565
BA 590
G-1B
1
2

Funky Vesicles