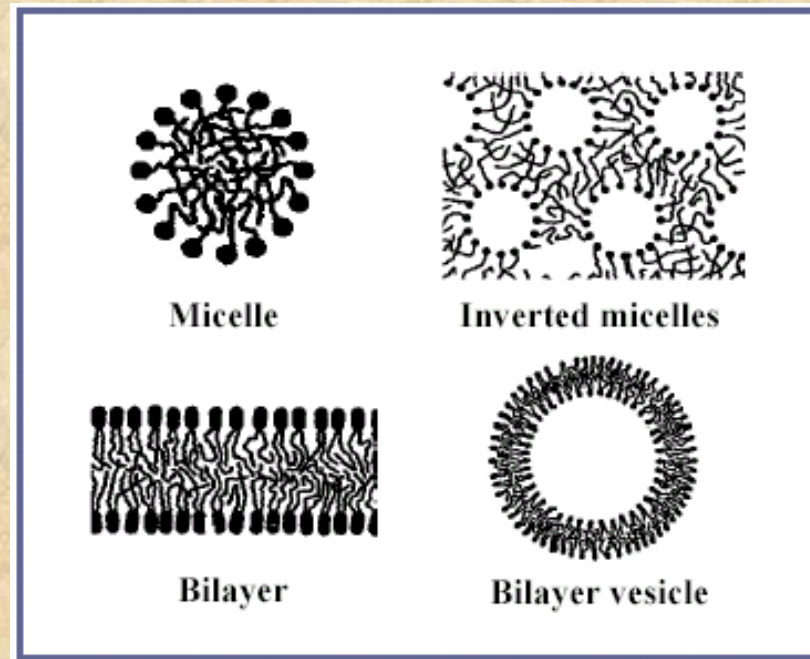


Aggregation of Amphiphilic Molecules Into Micelles, Bilayers, Vesicles And Biological Membranes



Colloids, Chapter 17

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- Equilibrium considerations of amphiphilic structures
- Optimal head-group area
- Geometric packing considerations
- Spherical micelles
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- Vesicles
- Factors affecting changes from one structure to another
- Curvature elasticity of bilayers and membranes
- Biological membranes
- Membrane lipids
- Membrane proteins and membrane structure

Equilibrium considerations of amphiphilic structures

➤ Amphiphilic molecules?

- Can associate into a variety of structures in aqueous solutions
- Can change from one form to the other according to the exterior conditions, e.g. pH or electrolyte concentration
- e.g. surfactants, lipids, copolymers, proteins

➤ Amphiphilic molecules

- thermodynamics of self-assembly
- intra-aggregate forces
- inter-aggregate forces

=> Equilibrium structures of the system

Equilibrium considerations of amphiphilic structures

➤ Equilibrium structures?

- reference to Gibbs phase rule

Amphiphilic structures can be:

hard and solid-like

soft or fluid like

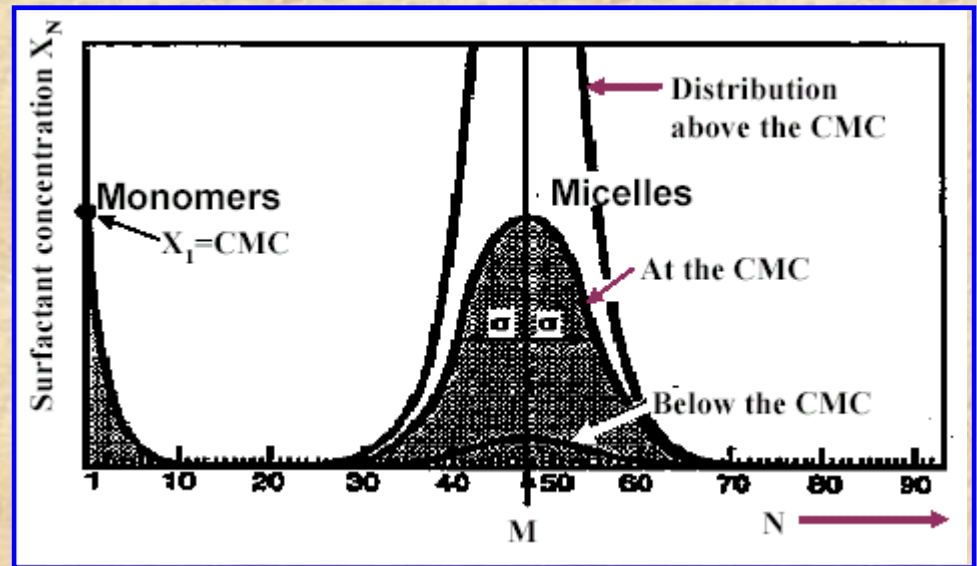
molecules in thermal motion

1. No definite size or shape

1. No definite size

2. Only distribution about some mean value

3. Equilibrium distribution to peak at more than one value of N
(aggregation number)



Equilibrium considerations of Amphiphilic Structures

- Equilibrium distribution can peak at more than one N value
- Small aggregates → micelles
- Thermodynamic equilibrium with larger structures
- Vesicles or liposomes
- Not a separate phase
- Structure size does not play a role in the thermodynamic definition of a separate phase
- Two and three phase systems can occur when
- Monomers, micelles, and vesicles separate out in equilibrium
- This can take a very long time

Optimal headgroup area

- The major force : hydrophobic attraction
(at the hydrocarbon-water interface)

Two 'opposing forces'

- attraction : between hydrocarbon
- repulsion : between head-group

Acting region : interfacial region

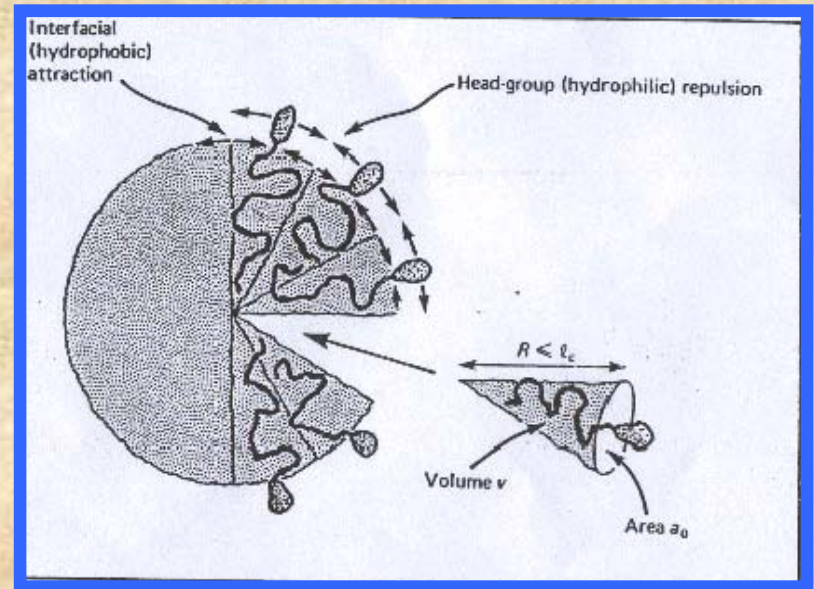
- Interfacial area A /molecule

- attractive force

; decrease Interfacial area A /molecule

- repulsive force

; increase Interfacial area A /molecule



Optimal head-group area

➤ Attractive interaction

- hydrophobic or interfacial tension
- positive interfacial free energy per unit area
 - (hydrocarbon – water interface $\gamma \approx 50\text{mJ/m}^2$)
 - (headgroup – water interface $\gamma \approx 20\text{mJ/m}^2$)
 - (C=C bonds – water interface $\gamma \approx 50\text{mJ/m}^2$)

➤ Total interfacial free energy(μ_N°)

- attractive interfacial free energy contribution : γa ($\gamma \approx 20\sim 50\text{mJ/m}^2$)
- repulsive interfacial free energy contribution : K/a

- Too difficult to formulate explicitly

steric contribution, hydration force contribution (mobile headgroups),
electrostatic double-layer contribution charged headgroups

- two-dimensional van der Waals equation of state

=> the first term in any energy expansion $\propto 1/\text{surface area}$
occupied per headgroup a (cf. pressure $\propto 1/a^2$)

Optimal head-group area

- Total interfacial free energy per molecule in an aggregate can be written to first order as,

$$\mu_N^o = \gamma a + K / a \quad (17.1)$$

- Minimum energy

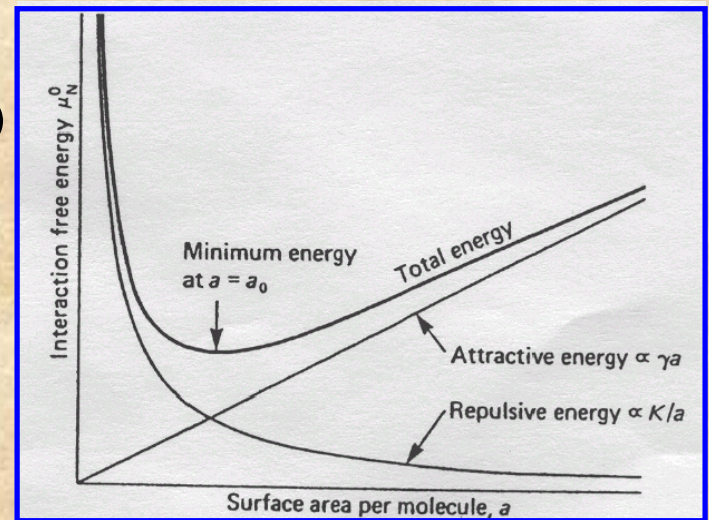
$$\mu_N^o(\text{min}) = 2\gamma a_o, \quad a_o = \sqrt{K / \gamma} \quad (17.2)$$

(a_o : optimal surface area per molecule)

$$\mu_N^o = 2\gamma a_o + \frac{\gamma}{a} (a - a_o)^2 \quad (17.3)$$

(Unknown constant K : eliminated)

(γ and a_o : measurable parameters)



Optimal head-group area

- a_0 : optimal surface area per molecule
→ total interfacial energy per lipid molecule is minimum
- optimal area should not strongly depend on the chain length or chain number (in fluid hydrocarbon)
- lipid interaction energy between lipids
 - minimum at optimal surface area
 - parabolically vary
- (Eq17.3) ignore three second-order effects
 1. Specific head-group interactions such as ionic bridging
 2. Specific chain-chain interactions (never perfectly fluid hydrocarbon)
 3. The effect of surface curvature on μ_N°

Geometric packing considerations

➤ Geometry or Packing properties

1. Optimal area : a_0

2. Volume of hydrocarbon chain or chains : v

3. Critical chain length : l_c

➤ Critical chain length l_c

- How far the chains can extend; smaller extensions are allow?

- Semi-empirical parameter

- Saturated hydrocarbon chain (carbon number = n) (by Tanford)

$$l_c \leq l_{\max} \approx (0.154 + 0.1265n) \text{ nm}$$

$$v \approx (27.4 + 26.9n) \times 10^{-3} \text{ nm}^3$$

=> Larger n , $v / l_c \approx 0.21 \text{ nm}^2 \approx \text{constant} \rightarrow \text{minimum cross-sectional area}$

Geometric packing considerations

- Optimal surface area a_0 , hydrocarbon chain volume v , critical length l_c are determine their packing structures
 - ✓ These parameters can be satisfied by a variety of different structures
 - ✓ For all these structures μ_N° same. (since a_0 is the same)
 - ✓ Entropy favors structure with the smallest aggregation number $N=M$ this structure is unique!
 - ✓ Larger structure will be entropically unfavored
 - ✓ Smaller structures , packing constraints force a above a_0 energetically unfavoured
- Packing parameter (or shape factor) - $v/ a_0 l_c$
 - ✓ ($v/ a_0 l_c < 1/3$) : spherical micelles
 - ✓ ($1/3 < v/ a_0 l_c < 1/2$) : non-spherical micelles
 - ✓ ($1/2 < v/ a_0 l_c < 1$) : vesicles or bilayers
 - ✓ ($1 < v/ a_0 l_c$) : 'inverted' structure
- All structures have minimum sized aggregation

Spherical micelles

- Spherical micelles
 - ✓ Optimal surface area (a_0) sufficiently large
 - ✓ Hydrocarbon volume v sufficiently small
 - ✓ Radius of micelle, $R < l_c$

➤ From simple geometry we have

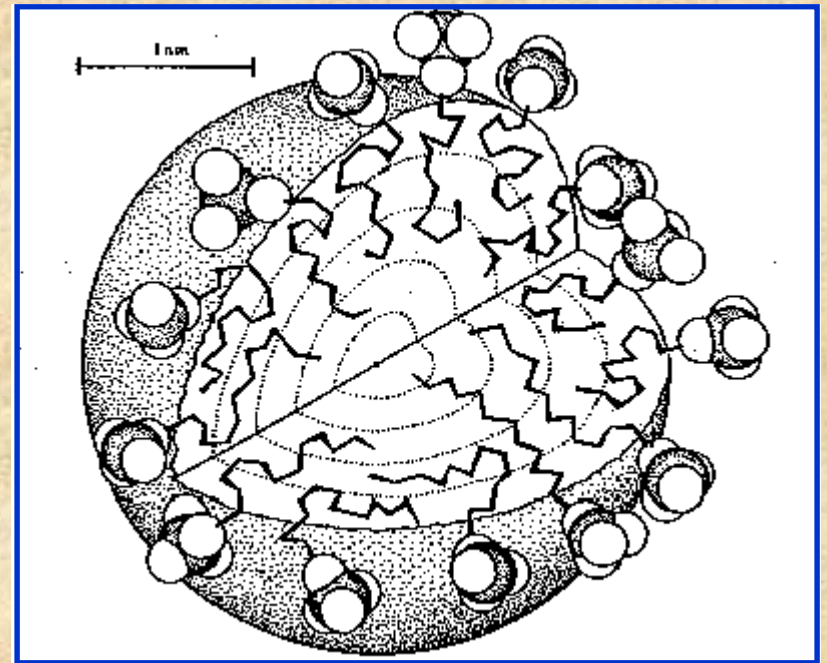
➤ For a spherical micelle

➤ With Radius R and a

➤ Mean aggregation number M

$$M = 4\pi R^2 / a_o = 4\pi R^3 / 3v$$

$$R = 3v / a_o, \quad \frac{v}{a_o l_c} < \frac{1}{3}$$



Spherical micelles

➤ Example) SDS(12-carbon chain sodium dodecyl sulphate surfactant)

✓ $M \approx 74$

✓ $n=12 \rightarrow v \approx 0.3502 \text{ nm}^3$, $a_0 \approx 0.57 \text{ nm}^2$, $l_c \approx 1.67 \text{ nm}$ $R \approx 1.84 \text{ nm}$

✓ $v / a_0 l_c \approx 0.37 > 1/3$

\Rightarrow Just cannot pack into spheres , slightly non-spherical

Standard deviation can be obtained at $N \approx M$, where $a = a_0$

$$\mu_N^o = \mu_M^o + \frac{\gamma}{a} (a - a_o)^2$$

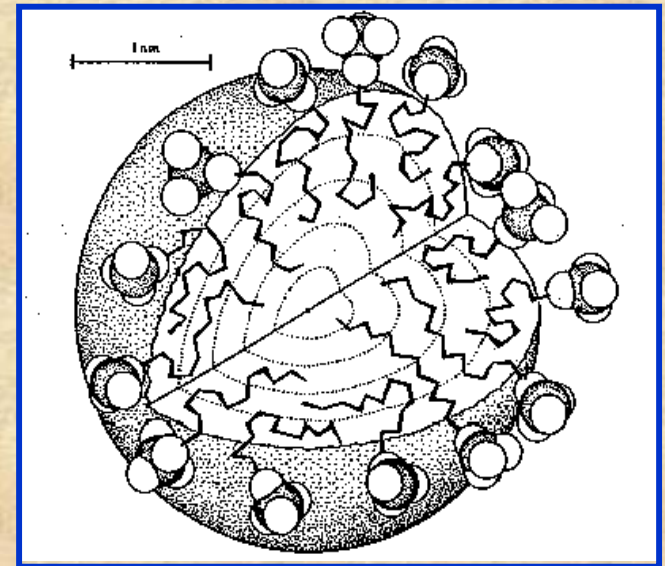
$$N = 4\pi R^2 / a = 4\pi R^3 / 3v = 36\pi v^2 / a^3$$

$$\mu_N^o - \mu_M^o = \Lambda (N - M)^2, \quad \Lambda = \gamma a_o / 9 M^2$$

$$\sigma = \sqrt{(9kT / 2\gamma a_o) M}$$

($\gamma \approx 20 \sim 50 \text{ mJ/m}^2$) , $a_0 \approx 0.06 \text{ nm}^2$

$$\sigma \approx \sqrt{M}$$



Non-spherical and cylindrical micelles

- Most spherical micelles forming lipid – **charged head-group** (since large head-group area a_0)
- Add salt to Charged head-group lipid
 - > partially screens the electrostatic inter-headgroup repulsion
 - > reduce headgroup area : $1/3 < v/a_0 l_c < 1/2$
 - > cannot pack into spherical micelles
 - > can form cylindrical (rod –like) micelles
- Rod-like aggregate – unusual properties
 1. Large and polydisperse
 2. $\langle N \rangle = 2\sqrt{C} e^\alpha$ above CMC

=> due entirely to **‘end effects’**

Non-spherical and cylindrical micelles

➤ End effects

- ✓ At each end the lipids are forced to pack into hemispherical caps with a headgroup area a determined by $v/a_0 l_c = 1/3$, so that $a > a_0$ since $v/a_0 l_c > 1/3$.
- ✓ The unfavourable energy of these end lipids determines the magnitude of the interaction parameter a in $\langle N \rangle = 2\sqrt{C} e^a$

➤ Cylindrical micelles

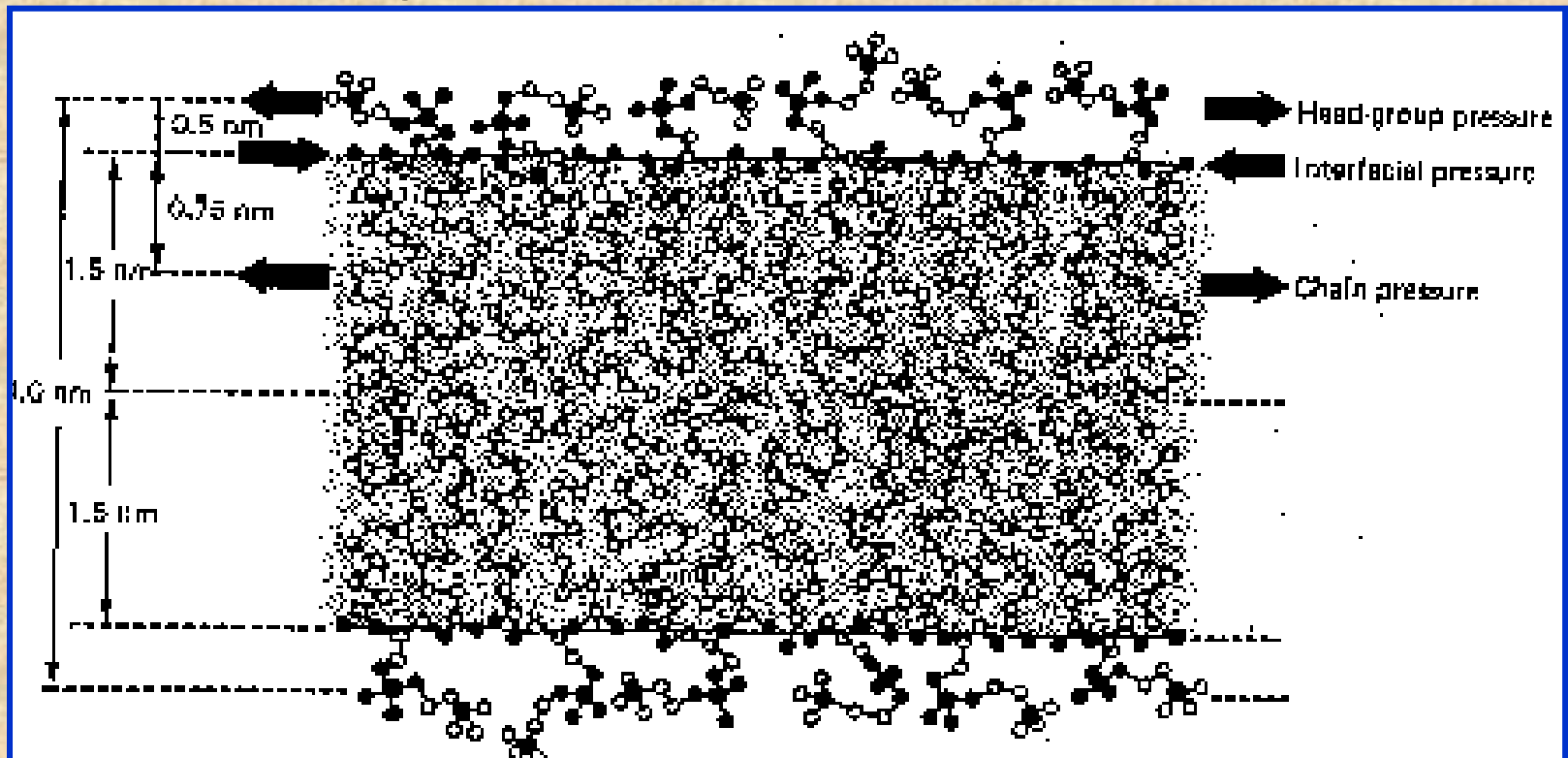
- ✓ Sensitive to
 - temperature changing
 - chain length
 - ionic strength (for ionic lipids)

- Toroidal micelles (experimentally not observed yet)
 - 2D vesicle, end effect minimum

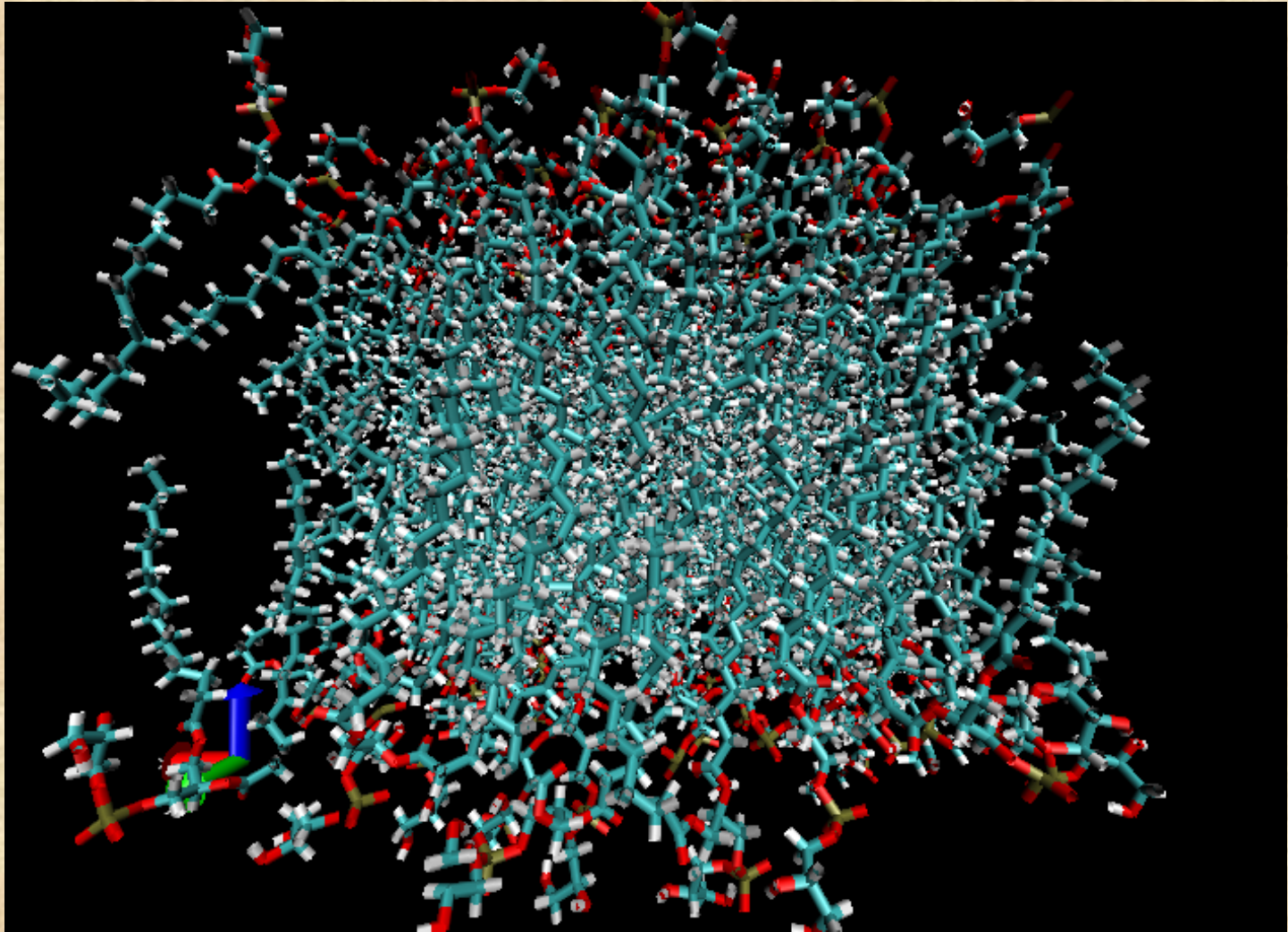
Bilayers

➤ Bilayer forming lipids

- ✓ $1/2 < v/a_0 l_c < 1$
- ✓ small a_0
- ✓ too bulky hydrocarbon chain
- ✓ tow chain lipids – almost same a_0 , l_c but v is twice
- ✓ Twice chain lengths



Membrane



Bilayers

➤ Doubling of the chains affects other aggregate properties

1. increase hydrophobicity -> decrease CMC

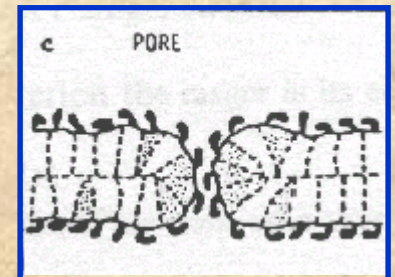
- (biologically very important)
- common micelle forming lipids CMC ($10^{-2} \sim 10^{-5} \text{M}$)
- bilayer forming lipids CMC ($10^{-6} \sim 10^{-10} \text{M}$)

2. increasing 'lifetimes' (or residence times) τ_r

- τ_r (micelles) $\sim 55 \times 10^{-9} / 10^{-3} \sim 10^{-4} \text{ s}$
- τ_r (bilayer) $\sim 55 \times 10^{-7} / 10^{-10} \sim 10^{+4} \text{ s}$
- suggests exchange rates should fall a factor of about 4-10 per two CH_2 groups added to the chain
- residence times depend on the individual molecules and not on the structures

3. 'transbilayer lipid exchange'

- 'flip-flop' of molecules from one side to the another
- diffusive exchange process
- diffusion of lipids around the walls transient pores



Bilayers

➤ Bilayer is elastically stretched

- ✓ elastic energy

$$\text{elastic energy} = \frac{1}{2} k_a (a - a_o)^2 / a$$

- ✓ Compressibility modulus k_a

$$k_a \approx 2\gamma \quad \text{per monolayer, } \approx 4\gamma \quad \text{per bilayer}$$

➤ Elastic bending or curvature modulus, k_b

- ✓ K_b Curved bilayer vesicle

➤ Fluid-like properties

- ✓ Assume that hydrocarbon chains in micelles and bilayers are in the fluid state
- ✓ Fluid at Room temperature
 - almost micelle forming single-chain surfactant
 - bilayer forming double-chain lipid
- ✓ Fluid at lower temperature : unsaturated or branched chained lipid

* Chain Melting Temperatures T_c

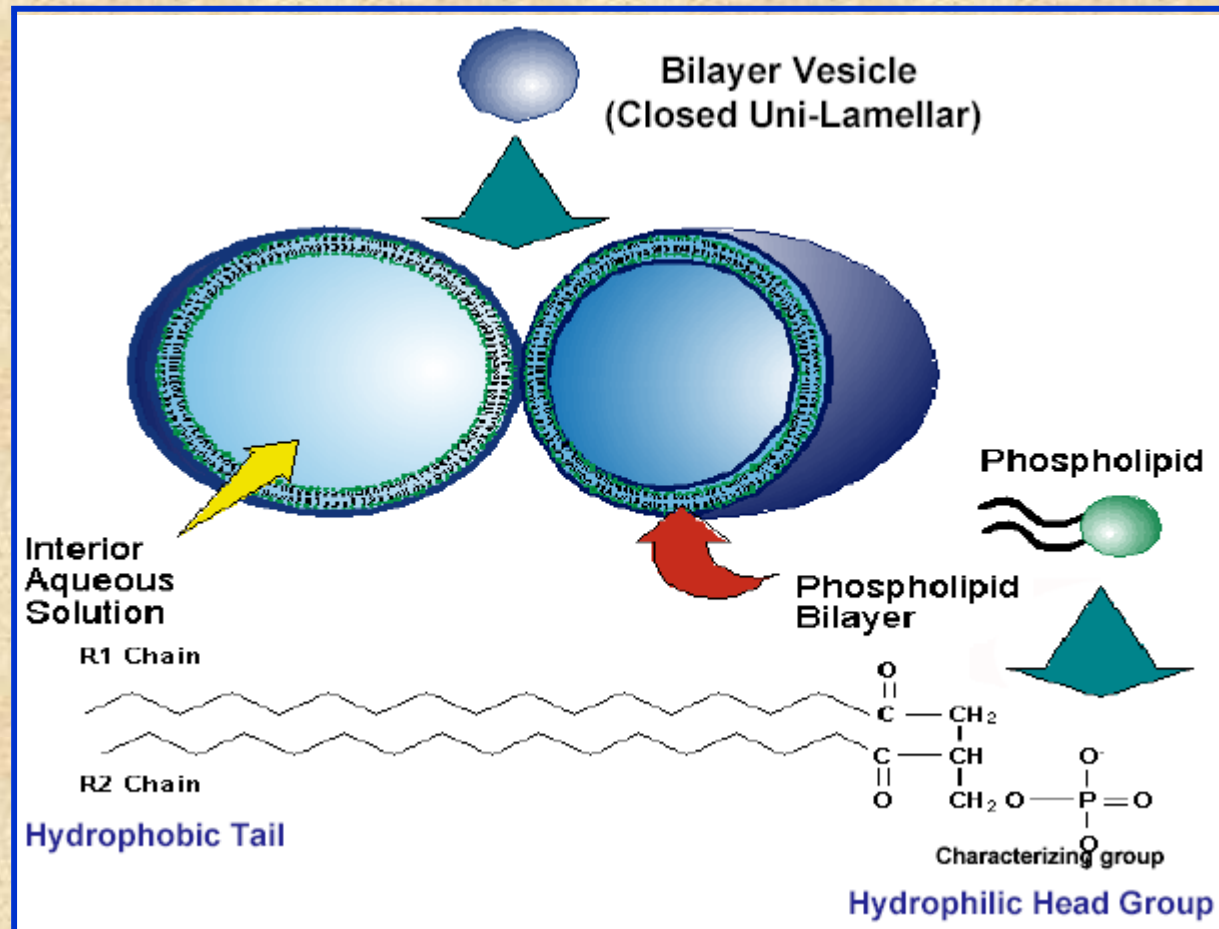
TABLE 17.1 Chain melting (phase transition) temperatures, T_c , of some common double-chained lipid bilayers in water (at pH 7) in order of increasing T_c

Lipid (giving number of carbons per chain)	Headgroup type ^a and chain melting temperature, ^b T_c (°C)				Melting point of <i>n</i> -alkane with same number of carbon atoms
	PC	PG ⁻	PS ⁻	PE	
<i>Saturated</i>					
Dilauroyl (12)	-2	0	13	30	-9.6
Dimyristoyl (14)	23	24	36	49	5.9
Dipalmitoyl (16)	41	41	52	64	18.2
Distearoyl (18)	55	55	68	74	28.2
<i>Unsaturated (cis)</i>					
Dioleoyl (18)	-22	-18	-7	-16	-30

^a PC: phosphatidylcholine (zwitterionic); PG⁻: phosphatidylglycerol (negatively charged); PS⁻: phosphatidylserine (negatively charged); PE: phosphatidylethanolamine (zwitterionic).

^b Compiled from Cevc and Marsh (1987) and Marsh (1990).

* Structure of Vesicle

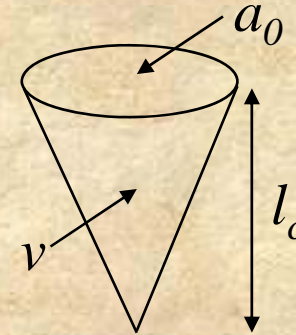


Vesicles

- Driving force for vesicle formation: elimination of the energetically unfavorable edges
- What determines the radii of vesicles?

✓ **Packing parameter:** $v/a_0 l_c$

- $1 \rightarrow$ bilayer
- $<1 \rightarrow$ vesicles
- $>1 \rightarrow$ inverted micellar structure/precipitation



✓ **Critical radius** by geometric consideration for $1/2 < v/a_0 l_c < 1$

$$R_c \approx l_c \left[\frac{3 + \sqrt{3(4v/a_0 l_c - 1)}}{6(1 - v/a_0 l_c)} \right] \approx \frac{l_c}{(1 - v/a_0 l_c)}$$

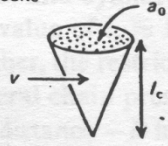
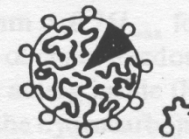

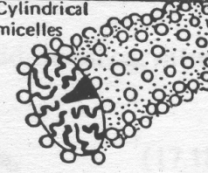

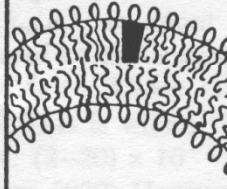
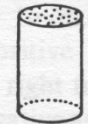
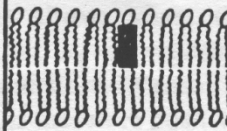

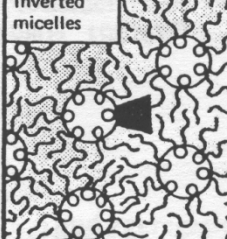
✓ **Aggregation number**

$$N \approx 4\pi \left[R_c^2 + (R_c - t)^2 \right] / a_o \quad \text{here, } t \approx 2v/a_o$$

Bilayer hydrocarbon thickness

Vesicles

TABLE 17.2 Mean (dynamic) packing shapes of lipids and the structures they form

Lipid	Critical packing parameter $v/a_0 l_c$	Critical packing shape	Structures formed
Single-chained lipids (surfactants) with large head-group areas: <i>SDS in low salt</i>	$< 1/3$	Cone 	Spherical micelles 
Single-chained lipids with small head-group areas: <i>SDS and CTAB in high salt, nonionic lipids</i>	$1/3-1/2$	Truncated cone 	Cylindrical micelles 
Double-chained lipids with large head-group areas, fluid chains: <i>Phosphatidyl choline (lecithin), phosphatidyl serine, phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid, sphingomyelin, DGDG^a, dihexadecyl phosphate, dialkyl dimethyl ammonium salts</i>	$1/2-1$	Truncated cone 	Flexible bilayers, vesicles 
Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains: <i>phosphatidyl ethanolamine, phosphatidyl serine + Ca²⁺</i>	~ 1	Cylinder 	Planar bilayers 
Double-chained lipids with small head-group areas, nonionic lipids, poly (<i>cis</i>) unsaturated chains, high <i>T</i> : <i>unsat. phosphatidyl ethanolamine, cardiolipin + Ca²⁺, phosphatidic acid + Ca²⁺, cholesterol, MGDG^b</i>	> 1	Inverted truncated cone or wedge 	Inverted micelles 

^a DGDG, digalactosyl diglyceride, diglucosyl diglyceride.

^b MGDG, monogalactosyl diglyceride, monoglucosyl diglyceride.

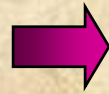
Factors Affecting Changes from One Structure to Another

- I. **Headgroup area**: small headgroup areas (high v/a_0l_c) \rightarrow large vesicles, less-curved bilayers, or inverted micellar phases
- II. **Chain packing**: chain branching and unsaturation \rightarrow reduction of $l_c \rightarrow$ increase of v/a_0l_c
- III. **Temperature**: $T \rightarrow$ affecting both a_0 and l_c
 - ✓ increase of $T \rightarrow$ reduction of l_c by the hydrocarbon chain motion
 - ✓ increase of $T \rightarrow$ hydrophobic group: decrease of a_0 , hydrophilic group: increase of a_0
- IV. **Lipid mixture**: The size of vesicles can be conveniently modulated by adding another component (cosurfactant).

Curvature Elasticity of Bilayers & Membranes

➤ More delicate treatment

- ✓ Headgroup repulsion
- ✓ Chain repulsion



Additional curvature dependence
of μ_N^0 on R

$$\Delta E = -2\gamma t D / R^2 = k_b / 2 R^2 \quad \text{per unit area}$$

■ Bending modulus k_b

headgroup repulsion: $k_b < 0$

chain repulsion: $k_b > 0$

Positive Curvature Modulus ($k_b > 0$)

- Interaction energy per vesicle of large radius R ($R > R_c$)

$$N \mu_N^o = N \mu_\infty^o + (k_b / R^2) 4\pi R^2 = N \mu_\infty^o + 2\pi k_b$$

$$\mu_N^o = \mu_\infty^o + 2\pi k_b / N, \quad \alpha = 2\pi k_b / kT$$

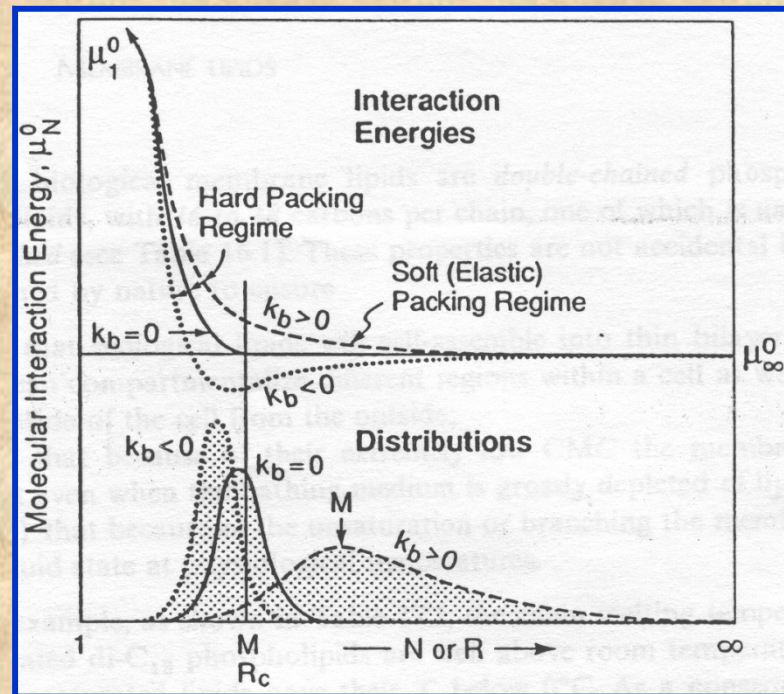
$$\text{Mean aggregation number } M = \sqrt{C e^\alpha} = e^{\pi k_b / kT} \sqrt{C}$$

$$\text{vesicle distribution} \quad X_N / N = \text{Const.} e^{-N/M}$$

- In case of dilute lipid concentration $C = 10^{-4} \text{ mol dm}^{-3}$
- ✓ $k_b > 2 * 10^{-20} \text{ J} \rightarrow$ vesicles will be large ($M > 10,000$), polydisperse, and $R \propto C^{1/4}$.
 - ✓ $k_b < 2 * 10^{-20} \text{ J} \rightarrow$ small and monodisperse (No significant effect due to low bending modulus)

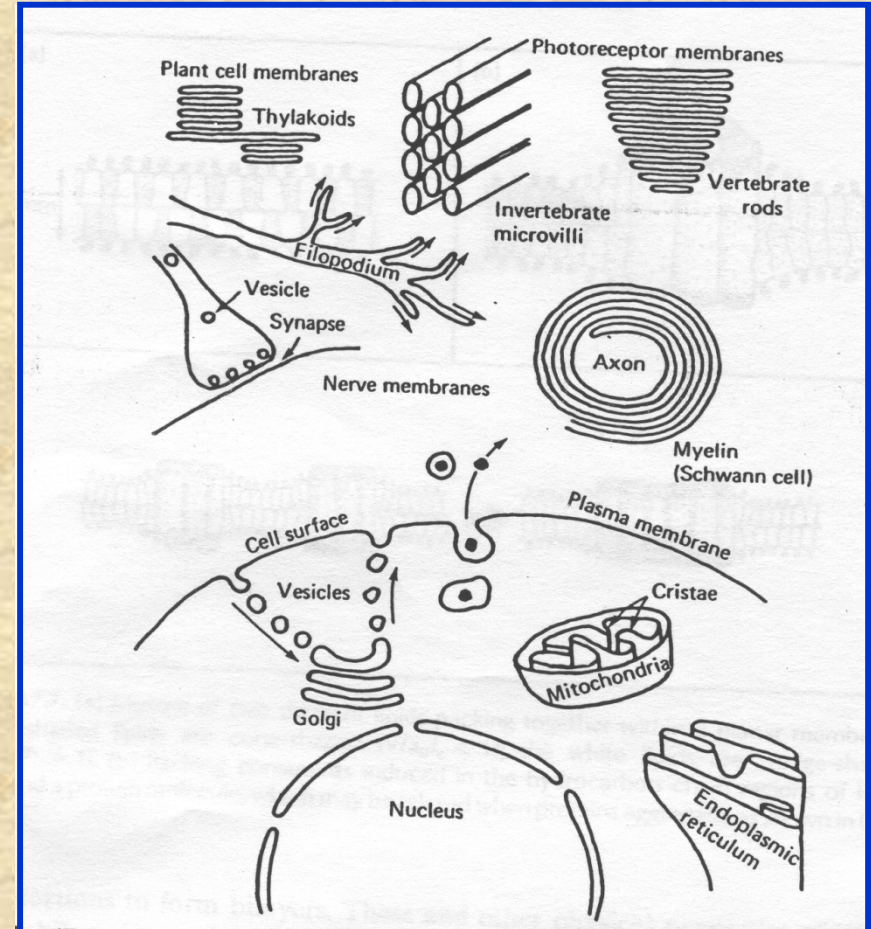
Negative Curvature Modulus ($k_b < 0$)

- Bending of a bilayer right from the start
- Smaller vesicles
- For large repulsive headgroups and shorter hydrocarbon chains → smaller vesicles
- Below a certain chain length → cylindrical or spherical micelles



Biological Membranes

- The most common cellular structure in both animals and plants
- Various function such as mobility, food entrapment, energy transduction, immunological recognition, nerve conduction, and biosynthesis



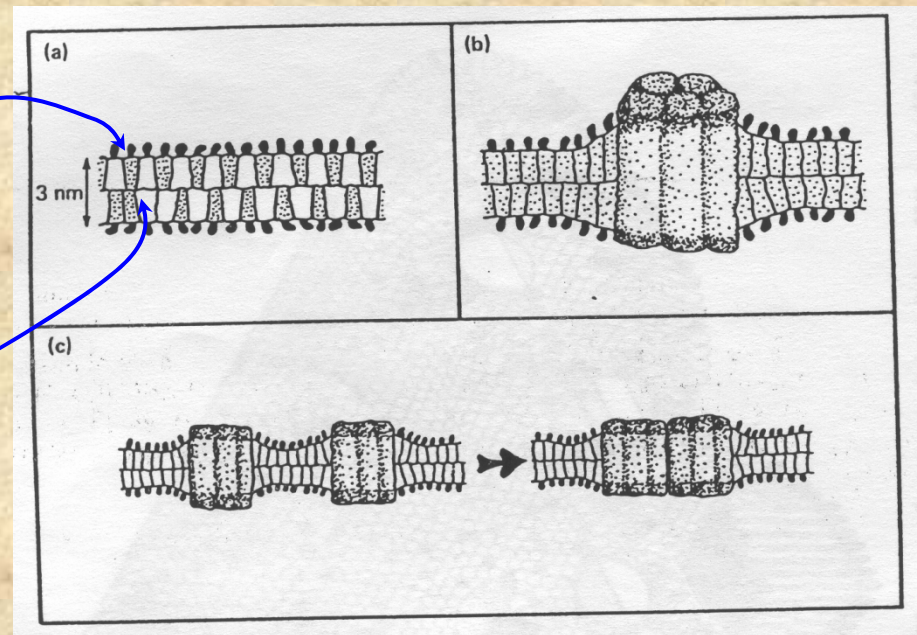
Membrane Lipids

- **Double-chained** phospholipids or glycolipids with 16 to 18 carbons per chain
- Properties
 - ✓ **Self-assembly** of biological lipids into thin bilayer membranes
 - ✓ Extremely **low CMC**
 - ✓ **Fluid state** at physiological temperatures by unsaturation or branching

- Mixture of two different lipids

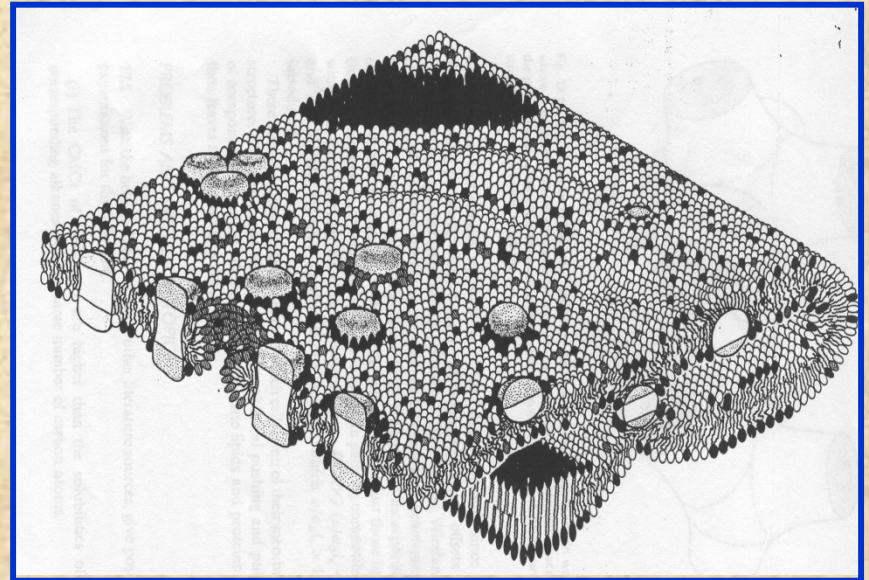
Phosphatidylcholine (PC):
cone-shaped ($v/a_0 l_c < 1$)

Phosphatidylethanolamine
(PE): wedg-shaped ($v/a_0 l_c > 1$)



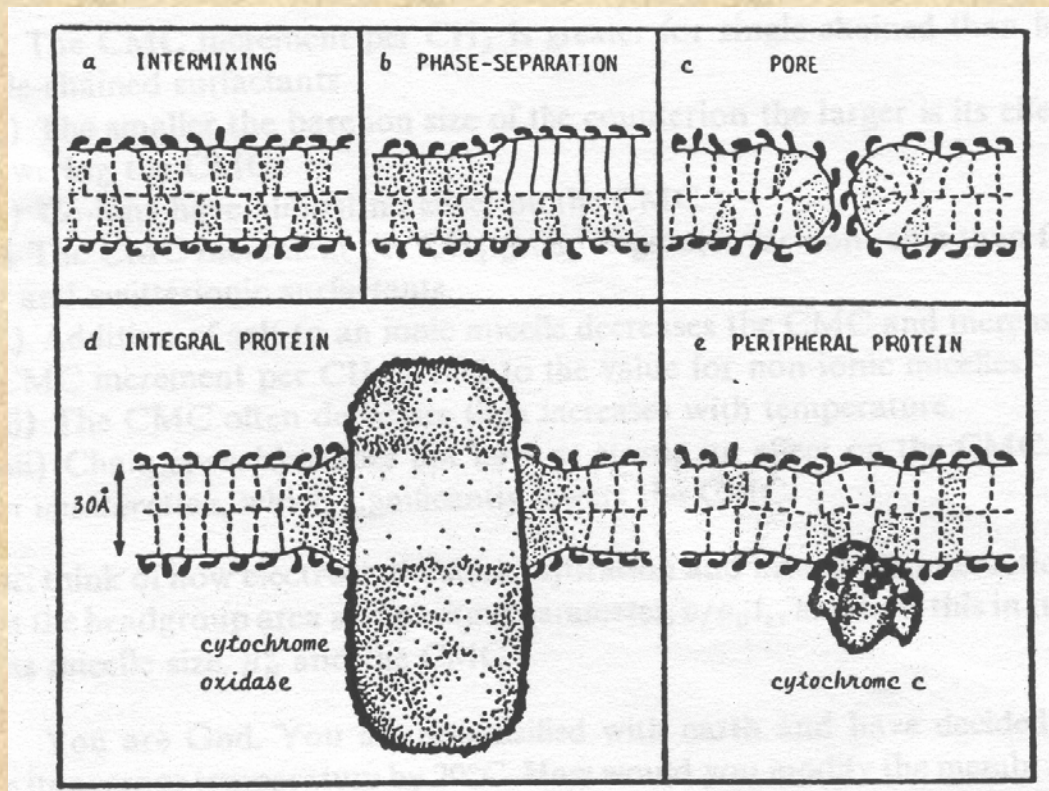
Membrane Proteins and Membrane Structure

- Membrane proteins are **long-chained polypeptide polymers** consisting of a long string of amino acid residues.
- Protein structures
 - ✓ Primary: residue sequence
 - ✓ Secondary: α -helical or β -pleated sheets
 - ✓ Tertiary: globule
- Incorporation of proteins into a lipid bilayer → **stress induction** (Boundary lipids)
- Consideration of proteins within a fluid membrane: **coexistence of attractive and repulsive forces**



Membrane proteins and Membrane Structures

- Both the lipids and the proteins move about rapidly in the plane of the membrane.
- Heterogeneous domains and local clustering of lipids and proteins



Mean packing conformations of mixed lipid and lipid-protein membranes, showing how local packing stresses may cause clustering of specific lipids and/or non-bilayer shapes.