Chapter 2

Which of the following has the lowest melting point?
   (a) oleic acid
   (b) linoleic acid
   (c) linolenic acid
   (d) stearic acid

A fatty acid with 3 carbon-carbon double bonds is
   (a) α-linolenic acid
   (b) linoleic acid
   (c) palmitoleic acid
   (d) arachidonic acid

Microorganisms maintain the lipid composition of their membranes to be
   (a) below T_m for $L\beta \rightarrow L\alpha$
   (b) at T_{LH} , the transition for $L\alpha \rightarrow H_{II}$
   (c) well above the T_m for $L\beta \rightarrow L\alpha$ [to maintain fluidity]
   (d) between T_{LH} and T_m

The fluidity of the lipid side chains in the interior of a bilayer is generally increased by
   (a) a decrease in temperature
   (b) an increase in fatty acyl chain length
   (c) an increase in the number of double bonds in fatty acids
   (d) an increase in the percentage of phosphatidyl ethanolamine

Which of the following fatty acids is most likely to be increased in membranes of organisms adapting to higher temperatures?
   (a) palmitic acid
   (b) palmitoleic acid
   (c) arachidic acid
   (d) arachidonic acid

Which of the following phospholipids has a net charge of zero?
   (a) PE
   (b) PS
   (c) PG
   (d) PI

A phospholipid with a net charge of -1 is
  (a) phosphatidyl ethanolamine
  (b) phosphatidyl choline
  (c) phosphatidyl serine
  (d) cardiolipin (diphosphatidyl glycerol)

A phospholipid head group which <u>by itself</u> carries a positive or negative net charge is
  (a) serine
  (b) choline
  (c) inositol
  (d) glycerol

Why don't membranes have more than 30% (by weight) cholesterol?
  (a) It is too expensive to for cells to synthesize that much cholesterol.
  (b) Cholesterol prefers raft lipids to bulk lipids.
  (c) Cholesterol precipitates out (crystallizes) at such high concentrations.
  (d) Cholesterol forms condensed complexes with lipids.

Cholesterol is an important component of
  (a) bacterial plasma membranes
  (b) animal cell plasma membranes
  (c) plant cell plasma membranes
  (d) all of the above

The difference between the rate of diffusion of phospholipids measured in reconstituted systems (fast) and the rate measured on the surface of cells (much slower) was finally explained by
  (a) single molecule tracking
  (b) hop diffusion
  (c) presence of cytoskeleton in cells
  (d) all of the above

Flippases
  (a) catalyze the transverse movement of lipids across the membrane
  (b) expend ATP
  (c) maintain lipid asymmetry in the two leaflets of many membranes
  (d) all of the above

Typical components of lipid rafts generally do **not** include
- (a) cholesterol
- **(b) farnesyl-linked proteins**
- (c) caveolin
- (d) GPI-linked proteins

The function of caveolin is to
- (a) bind lipid anchored proteins
- (b) form an ion channel
- **(c) cause inward curvature at lipid rafts**
- (d) all of the above

Curvature frustration results whenever
- (a) any different lipids are mixed in a bilayer.
- **(b) a lipid is pushed away from its intrinsic Ro value.**
- (c) cholesterol is present.
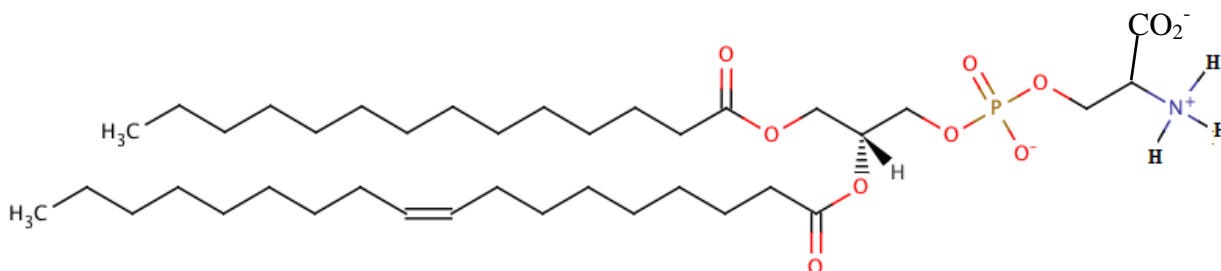- (d) any peptide is inserted into any lipid bilayer

What are the characteristics of naturally occurring fatty acids?

They are straight chain carboxylic acids, ~C14 to C22 long. They can be saturated or unsaturated.

Name and draw five types of glycerophospholipids, including the three main classes in eukaryotes.

PC, PE, PI are the three main classes in eukaryotes, plus PS, PG and DPG=cardiolipin full names and structures (see Figure 2.5)

Draw the structure of 1-myristoyl-2-oleoyl phosphatidylserine in the protonation state it will have at pH 7.

Briefly define each of the following abbreviations in the context of membrane biochemistry. You do not have to describe the items.

a) SpM
b) DSPE
c) L_α
d) H_I
e) H_{II}
f) PG
g) $18:1^{\Delta9}$
h) DRMs

a) SpM - sphingomyelin
b) DSPE - distearoyl phosphatidylethanolamine
c) L_α - lamellar liquid-crystalline phase
d) H_I - normal hexagonal phase with nonpolar inside and polar outside
e) H_{II} - inverted hexagonal phase with polar inside and nonpolar outside
f) PG – phosphatidylglycerol or proteoglycan
g) $18:1^{\Delta9}$ - Oleic acid or oleoyl
h) DRMs - detergent-resistant membranes

The following table gives the T_m value for the gel-liquid crystalline phase transition of the indicated bilayer. Predict whether the T_m values will be higher or lower than **the reference value of 55°** by writing either ↑ for higher T_m or ↓ for lower T_m.

Fatty Acids	Head Group	T_m
18:0/18:0	**PC**	**55**
16:0/16:0	PC	
18:0/18:1$^{\Delta 9}$	PC	
18:0/18:0	PE	
18:0/18:0	PS	

Answers:

16:0/16:0	PC	↓
18:0/18:1$^{\Delta 9}$	PC	↓
18:0/18:0	PE	↑
18:0/18:0	PS	↑

Name and draw five different types of lipid aggregates (that is, morphologies) that have been observed.
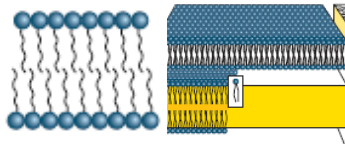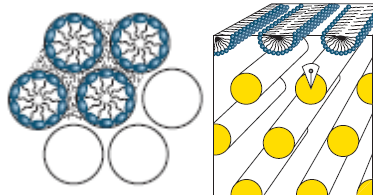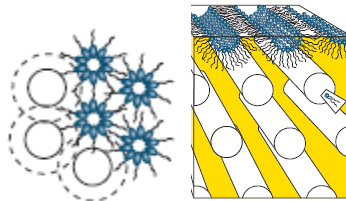
Answers:

Micelle

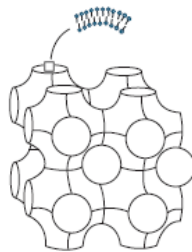*Inverted micelles (spherical)*

Lamellar bilayers

Long rods H$_I$ phase

Long rods H$_{II}$ phase

Bicontinuous cubic phase

The conformation and motion of the acyl chains of phospholipids are determined by the properties of the linkages between carbon atoms. These linkages can be described for C=C bonds as cis or trans, while for C-C bonds they are gauche or anti (also referred to as trans).

   a.   Describe each of these four kinds of bonds and how they affect acyl chain conformation and order.
   b.   For a specific phospholipid structure, how will each of these four kinds of bonds change with increasing temperature?
   c.   How will this affect the motional order and conformation of the acyl chain?

   a.   C=C cis bonds cause the formation of a kink in the direction of the acyl chain. Increase acyl chain splay and disorder, particularly in the centre of the bilayer.

   C=C trans bonds cause the formation of only a mild kink in the acyl chain. They cause similar but much smaller effects to the acyl chain conformation and order compared with C=C cis bonds.

   C-C gauche bonds cause the acyl chain to move away from the bilayer normal and not to extend as far into the centre of the bilayer. Increases disorder, especially for the part of the acyl chain between the gauche bond and the methyl terminus.

   C-C anti (trans) found as only rotomer in gel state lipid. High degree of order. Acyl chain conformation like stiff rod, extending to a large degree toward the centre of the membrane making the bilayer thicker.

   b.   C=C cis and trans will not interconvert with increasing temperature. C-C anti will convert to C-C gauche.

   c.   The conversion of anti to gauche will decrease the order and induce a conformation with more acyl chain splay (or more negative curvature).

Trans-fatty acids have detrimental health effects. These health effects may be related to the difference in the physical properties been cis and trans acyl chains. Describe the differences in physical properties one would expect in replacing the natural cis fatty acids with trans acids in foods and why this might be detrimental to health.

   Unlike a cis double bond, a trans C=C does not introduce a kink in the acyl chain, so it is more similar to a saturated fatty acid. Saturated fatty acyl chains and chains with trans double bonds are extended, while chains with a kink have increased splay that makes the curvature of the bilayer more negative. Replacing cis-FAs with trans-FAs is like increasing saturated FAs, so it affects membrane fluidity, permeability, and protein activity.   In humans, increased consumption of trans fatty acids is correlated with increased heart disease, which results from diets high in saturated fats.

Why does the ratio of unsaturated fatty acids to saturated fatty acids decrease when the temperature of a culture of bacteria is raised?

Since the membrane should be fluid but not too fluid (close to Tm), bacterial cells maintain a mixture of acyl chains that are more unsaturated (lower Tms) at low temperatures and a mixture that are more saturated (higher Tms) at higher temperatures.

What structural features are shared by phosphatidyl choline and sphingomyelin? What are two/three major differences in their structure?

They share the phosphocholine headgroup, and they both have two chains coming from the three C "body". The differences are:
1. *PC has a glycerol backbone; SM has sphingosine*
2. *PC has ester bonds to two acyl chains; SM has one amide bond and one C-C bond (as the second chain is part of sphingosine)*
3. *The acyl chain on the middle C tends to be unsaturated in PC and saturated in SM*

Sphingomyelin is enriched in Detergent Resistant Membranes (DRM).
A. What is different about the physical properties of sphingomyelin compared with that of the majority of glycerol-based phospholipids found in mammalian membranes?
B. What other lipid is enriched in DRMs?
C. How are the physical properties of a membrane that is enriched in these two lipids described (i.e. what phase do these two lipids form)?

A. *Sphingomyelin has a high melting temperature compared to other bilayer lipids.*
B. *Cholesterol*
C. *Liquid ordered*

What are three major reasons the liquid crystalline phase of a bilayer made from natural sphingolipids is less leaky than when made from natural glycerophospholipids?

   a)   *Hydrophobic tails are more saturated. This promotes tighter packing of the lipid monomers with each other.*
   b)   *The sphingosine carbon-carbon double bond is trans, which disrupts close packing less than the cis double bond of unsaturated fatty acids.*
   c)   *Sphingolipids are more H-bonded with donors and acceptors in the interfacial region close to the tails than glycerophospholipids are. This promotes tighter packing of the lipid monomers with each other.*
   d)   *Sphingolipids are more H-bonded with donors and acceptors in the very polar X head group than glycerophospholipids are. This promotes tighter packing of the lipid monomers with each other.*

Although the rate of flip-flop (transbilayer diffusion) of phospholipids in biological membranes is of the order of minutes to hours, these membranes are still able to maintain a high degree of transbilayer asymmetry (i.e. each monolayer of the bilayer has a different lipd compositon). How can this lipid asymmetry be maintained over a long period of time despite the occurrence of flip-flop?

   Lipid asymmetry is maintained because of the presence of an ATP-dependent phospholipid translocase that can use the energy of ATP hydrolysis to drive PS and PE from the outer to the inner membrane.

The lateral diffusion of lipids in bilayers has been measured by EPR, fluorescence, and NMR techniques. In artificial and biological membranes, lipids usually have a diffusion coefficient (D) within an order of magnitude of 10^{-8} cm^2/sec. The mean-squared distance, x^2, traveled by a lipid molecule in the membrane is related to the diffusion coefficient by the following equation:

$$x^2 = 4\,Dt \quad \text{(t in sec)}$$

How many times in one minute could a lipid travel around the outer monolayer of a phospholipid vesicle having a diameter of 400 Å?

Answer:

Calculate x = square root of 4 Dt = 1.55 x 10^{-3} cm/min

Calculate circumference as π x diameter = 1.26 x 10^{-5} cm

Ratio is 12.3 times in 1 min.

The lipid distearoylphosphatidylcholine (DSPC) (18:0/18/0) has two 18:0 acyl chains and has a gel to liquid crystalline phase transition temperature at around 60°C. Adding one double bond to the acyl chain at the C-2 position of glycerol to give 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC) (18:0/18:1) reduces the phase transition temperature to about 0°C.

    a.   Indicate how the order parameter profiles measured by ^{2}H NMR would compare for DSPC vs. SOPC. (You can draw a diagram of the two order parameter profiles but you don't have to show the NMR spectra from which they were obtained.)

    b.   Suggest why the phase transition temperature of SOPC is lower than that of DSPC.

    c.   Which of these two lipids would function better as a major lipid component of biological membranes? Why?

    d.   A different, but structurally related lipid, dioleoylphosphatidylethanolamine (DOPE) (18:1/18:1) has a gel to liquid crystalline phase transition below zero degrees, but is not compatible as a major lipid component of biological membranes. Suggest why.

[Hint: Choline = $HOCH_2CH_2N(CH_3)_3^+$; Ethanolamine = $HOCH_2CH_2NH_3^+$]

a.   The order parameters are lower for SOPC than for DSPC, i.e. DSPC has a higher order. In addition, the order parameter becomes lower near the center of the membrane.

b.   The gel state of SOPC is less stable because of the lower density of hydrocarbon packing in the gel state as a result of the kink in the acyl chain caused by the presence of the double bond.

c.   SOPC would function better because DSPC would be in the solid (gel) phase and now allow sufficient motion to sustain certain biological functions.

d.   DOPE, because of a small headgroup and C=C kinks in the acly chains, would form inverted phases because of the high negative curvature and therefore destroy the permeability barrier. If the lipid is not gel state and it is not compatible with biological membranes, a good likelihood is that it forms inverted phases- hexagonal or cubic.

Polymorphic phase behavior of membrane lipids is critical to function. Answer each question below about polymorphic properties.

   a)   A lipid molecule that has an S value of 0.5 will aggregate to form what phase in excess buffer at pH 7?

   b)   PC forms what phase in water?

   c)   An agitated mixture of glycerophospholipid, sphingolipid, and cholesterol in the ratio 6:1:1 suspended in excess buffer at pH 7 and 37°C is likely to do what?

   d)   High temperature is a membrane-membrane fusogen. Why?

   e) Ca^{2+} is a membrane-membrane fusogen. Why?

a) H_{II}, or inverted micelle

b)   lamellar or bilayer

c)   It will form lipid rafts having close to 1:1:1 composition of glycerophospholipid, sphingolipid, and cholesterol floating in a sea of ordinary L_a phase composed mostly of glycerophospholipid.

d)   The hydrocarbon tails will have more spreading between their ends due increased thermal motions. This promotes formation of H_{II} phase during encounters of two bilayers. H_{II} phase is the hemifusion intermediate in membrane-membrane fusion.

e) Ca^{2+} is chelated by negatively charged lipids on the surfaces of different bilayer membranes (for example, CL), screens the electrostatic repulsion and brings the two surfaces into close apposition. Also, Ca^{2+} promotes the H_{II} phase that forms during membrane-membrane fusion.

Name two characteristics of Lo phase that are shared with two other lamellar phases (and name the phase that shares them)

   1.   extended acyl chains – like Lβ

   2.   fast lateral diffusion – like La

Give two specific experiments that indicate the mobility of the acyl chains increases with the distance from the glycerol backbone.

   1.   NMR, putting 2H on different Cs of the acyl chains

   2.   EPR, putting nitroxide group on different Cs of the acyl chains

In the amphiphile shape hypothesis, what are the three types of lipids and what phases do they produce? Give an example of each.
Define the S factor and give approximate values of S for each type.

S is the ratio of the volume to the area of the polar headgroup times the length.

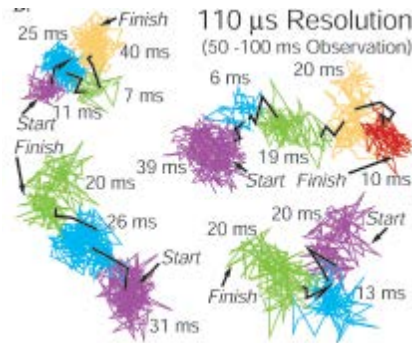| 1	Cylinder	lamellar or bilayer	DPPC	S=1
2	Cone	micelles/H$_I$	lysophospholipid	S>1
3	Wedge	inverted micelles/H$_{II}$	POPE	S<1

Name and briefly describe three of the four components of free energy that determine shape/phase in lipid polymorphism.
– *Hydrocarbon-packing energies along acyl chains*
  • *Due to the hydrophobic effect*
– *Hydration (which affects the hydrocarbon packing)*
– *Electrostatic potentials*
  • *Attraction or repulsion of headgroups*
– *Elastic bending of monolayers*

The fatty acid composition in the microorganism *Acholeplasma laidlawii* can be experimentally manipulated. As the acyl chain unsaturation is increased, the ratio of diglucosyldiglyceride (DGluDG) to monoglucosyldiglyceride (MGluDG) increases dramatically. DGluDG is a bilayer lipid, whereas MGluDG is an H$_{II}$ phase lipid. Indicate how the change in ratio of DGluDG to MGluDG can be rationalized in terms of lipid shape properties.

   The unsaturated acyl chains have increased splay, so the lipids are more wedge shaped (wider area for acyl chains than for head group) and are more likely to form H$_{II}$ phase. In order to maintain membrane integrity there needs to be an increase in the size of the lipid head group that will compensate for the increased area of the acyl chains and promote lamellar phase.

Do the tenets of the Fluid Mosaic Model have to change significantly to accommodate the data from single particle tracking shown below? Explain fully.

Single particle tracking revealed hop diffusion of a molecule of DOPE in a fluid bilayer. The irregular path observed indicates there are barriers to the lateral diffusion of a lipid. The FMM emphasized fast lateral diffusion of lipids in bilayers, based on measurements of overall rates of lipid diffusion in the plane of the membrane. With the techniques available at the time, there was no prediction of hop diffusion. However, Singer and Nicholson do allow for specialized regions of membranes. It was known that attachment of the cytoskeleton could define regions within the plasma membrane. Theoretical physical chemists have long studied molecular movements within "corrals". One could argue either way, that the basic tenets of the FMM do allow for regions, which imply some kinds of barriers to lateral diffusion or that the lack of single particle data prevented them from really understanding the nature of lateral diffusion of bilayers. (Not one correct answer, asking students to think about the issues.)

Recent experiments have revealed unexpected patterns of lipid movements in membranes.
   a. What is meant by "hop-diffusion" of components of biological membranes?
   b. What is the molecular basis of this phenomenon?
   c. How can this property be measured?
   a. Non-random diffusion. Diffusion confined to zones with barrier to go from one zone to another. This is diffusion along the plane of the membrane.
   b. Presence of a cytoskeleton on the cytoplasmic surface of the plasma membrane presenting a barrier.
   c. By single particle tracking using fluorescence microscopy. FRAP does measure lateral diffusion, but it measures the property of an ensemble of molecules over longer times. (FRAP is not the correct answer.)

Give a simple definition of Lipid Rafts. Then discuss three characteristics of rafts that make the definition less simple.
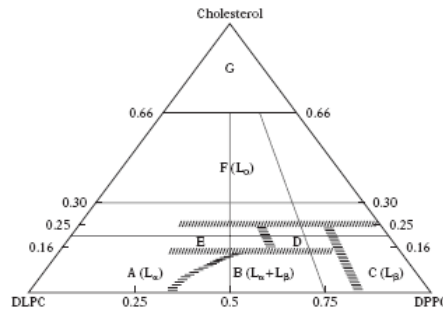
Lipid rafts are lateral regions of biomembranes that are distinguished from the rest of the membrane by protein and lipid composition and that play important roles in lipid trafficking, protein targeting, and signal transduction.

1) Rafts are dynamic. The size and number of rafts observed depend on the time-scale (as well as the length-scale) of the technique for observation. Fusion of smaller rafts into larger rafts is likely important biologically, but can also be stimulated by methods of observation, e.g. addition of antibodies.

2) There are variations to the defined composition of rafts. A typical lipid composition for rafts is phospholipid, sphingomyelin and cholesterol in 1:1:1 molar ratio. However, in membranes the composition can vary. It turns out that the sterol is not even required, depending on the mixture of phospholipids present. Since lipid-protein interactions play an important role in raft formation, the presence of particular proteins will affect the lipid composition as well.

3) Do rafts encompass both leaflets of the bilayer? Rafts were initially observed on the membrane surface, thus involving the outer leaflet. Mixtures of lipids corresponding to the composition of the inner leaflet do not form rafts in reconstituted systems. However, interdigitation of long acyl chains can be expected to couple the two leaflets. New methods of reconstituting supported bilayers have given evidence for raft formation in both leaflets.

Lipid rafts do not occur in bacterial and many internal membranes in eukaryotes. Why?

Such membranes contain little or no cholesterol and sphingolipids.

Describe the effects of cholesterol on an aqueous mixture of DLPC and DPPC [or sphingomyelin and a PC] based on the ternary phase diagram of the system.

[provide the figure]

In contrast to binary mixtures of lipids that contain Lα and Lβ phases, ternary mixtures containing cholesterol have a new phase called Lo, a liquid ordered phase with extended, tightly-packed acyl chains in lipids that are still fluid. The Lo state requires cholesterol, as it results from close interactions of certain acyl chains with the sterol molecule. It can coexist with either Lα or Lβ, depending on the lipid composition. However, when there is too much cholesterol (above ~0.66 mole percent), the cholesterol crystallizes out.

How does addition of cholesterol affect the thickness of membrane bilayers? Suggest the molecular basis of this effect.

It increases the thickness by inhibiting trans to gauche rotomer conversion of single bonds in the acyl chain.

Do detergent–resistant membranes correspond to Lipid Rafts? Support your answer with experimental data. [OR Give the pros and cons based on experimental data.]

A strong argument that DRMs are rafts is that they both have a lipid mixture (usually including sterols and sphingomyelins) that produces Lo phase. They are similar in thickness (width across the plane of the bilayer), both about 9Å wider than normal membranes. Also they are both enriched in fatty acid- or GPI-linked proteins. DRMs contain many important signaling proteins that are thought to be in the raft regions of biomembranes. However, the composition of DRMs is a function of detergent and conditions used for the membrane extraction. Indeed, since DRMs are isolated at 4°C, the lower temperature is a critical factor for the phase of membrane lipids. Since the protein composition of rafts is understood to vary in dynamic membrane processes (such as signaling), comparison of protein composition between DRMs and rafts is not clear cut.

Assuming that "raft" domains exist in biological membranes, indicate the <u>differences</u> between these "raft" domains and:

a.   Liquid-ordered domains in giant unilamellar vesicles (GUVs) composed of an equimolar mixture of dioleoylphosphatidylcholine (DOPC) (18:1/18:1); sphingomyelin and cholesterol. (assume the raft domains of biological membranes are also in the liquid ordered phase.)

b.   Detergent-resistant membrane fractions.

c.   Caveolae.

a.   Size. Model domains are much larger.

b.   Detergent resistant fraction is assumed to be a preexisting "raft" but the components may segregate as a result of the addition of the detergent.

c.   Caveolae are distinct morphological features in the plasma membrane and are enriched in the protein caveolin.

Mutant *E. coli* lacking a gene required for the synthesis of phosphatidylethanolamine (PE) can only be grown in the presence of high concentrations of divalent cations, while wild-type *E. coli* do not require these cations. Suggest why the mutant bacteria may need these divalent cations to survive.

Divalent cations are needed to maintain monolayer curvature homeostasis. Divalent cations binding to anionic lipids give them more negative curvature tendency to compensate for loss of PE.

What evidence directly supports the need for diversity of lipids in biomembranes?

Genetic manipulation of the PL content of E. coli (and more recently other microorganisms) illustrates that anionic lipids are essential for cell growth: pgsA⁻ mutants unable to synthesize PG and CL do not grow. E. coli membranes are normally 70 to 80% PE, and pssA⁻ mutants that have less than 0.1% PE grow poorly on minimal media and regulate their CL content.

Define Ro.   What factors affect Ro? What does Ro indicate about a lipid bilayer?

R is the radius of curvature at the lipid-water interface of a monolayer or bilayer leaflet. Ro is the intrinsic value of R for a particular lipid species. Each pure lipid species has a particular Ro it will reach at equilibrium (in the absence of other forces.) In terms of lipid shape, factors that widen the splay of the lipid tails (e.g. temperature or unsaturation) make Ro more negative, while factors that increase the effective headgroup area (e.g. size and charge of the headgroup) make Ro less negative. Other components of the membrane can push the curvature away from the Ro value, resulting in curvature frustration.   In this case the lateral pressure pushing apart the lipids is countered by the favorable lipid-lipid and lipid-protein interactions (hydrophobic effect). The extent of curvature frustration is related to the elasticity of the bilayer, which is an important factor for such processes as protein insertion—but this is described in later chapters.