L. SPONTANEOUS ORGANIZATION OF LIPIDS

This title contains a seeming oxymoron: spontaneous organization. Spontaneity implies a negative change in free energy, while organization implies a loss of entropy, which means that the contribution of entropy to free energy ($-T\Delta S$) will be positive. So, how is it that organization may occur spontaneously among lipids…

The Structure of Lipids and Fellow Travelers

Basics

Lipids are generally defined as the solvent extractable compounds of the cell – the hydrophobic fraction. In general, they are not purely hydrophobic, but are rather **amphipathic** (dual feeling) or **amphiphilic** (dual loving) molecules, possessing the capacity to interact favorably with both non-polar and polar environments. These compounds in biochemistry are representatives of a larger class of compounds used as surfactants, which notably includes detergents. In the latter case, the function of detergents is to solubilize grease, which is achieved by using the non-polar part of the detergent to interact with the non-polar grease, and the polar part of the detergent to interact with the aqueous environment. In general, we can describe these amphipathic compounds as possessing a polar "head group" and a non-polar "tail", which is diagrammatically shown in Figure L.1. The simplest types of detergents contain an *n*-alkyl tail attached to a small, charged functional group. For example, sodium dodecyl sulfate (SDS; $Na^+C_{12}H_{25}OSO_3^-$) and *n*-dodecyltrimethylammonium bromide (DTAB; $C_{12}H_{25}N(CH_3)_3^+Br^-$) are also shown in Figure L.1.

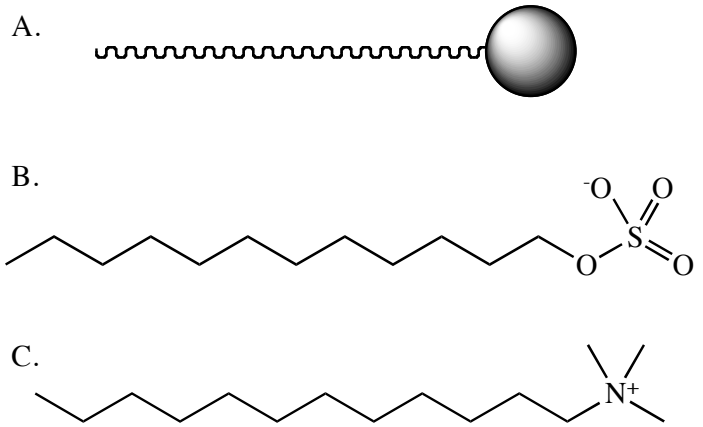

Figure L.1. (A) Diagram of an amphipathic surfactant. The polar head group is denoted with the circle and the non-polar tail with the squiggly line. (B) Dodecyl sulfate, and (C) dodecyltrimethylammonium.

Biological Amphiphiles

Most lipids are found in membranes – the molecular sheathes that enclose the cell. The simplest component is the fatty acid, typically a straight-chain carboxylic acid. Biologically, it's relatively rare to find a fatty acid playing a functional role with fewer than 14 carbons, or with an odd number of

carbons, but as a class we can include an alkanoic acid among the so-called "saturated" fatty acids – those carboxylic acids with a straight chain alkane substituent. A number of these acids have common names in addition to IUPAC names, but helpfully a shorthand exists to describe them as well. Any saturated fatty acid can be described simply as n:0, where "n" is the number of carbons in the compound (the "0" indicates the absence of additional functionality). For a sample listing of saturated fatty acids, see Table L.1.

Table L.1. Nomenclature of the saturated fatty acids.

Formula	Systematic Name	Common Name	Shorthand ID
$CH_3(CH_2)_{10}CO_2H$	Dodecanoic acid	Lauric acid	12:0
$CH_3(CH_2)_{12}CO_2H$	Tetradecanoic acid	Myristic acid	14:0
$CH_3(CH_2)_{14}CO_2H$	Hexadecanoic acid	Palmitic acid	16:0
$CH_3(CH_2)_{16}CO_2H$	Octadecanoic acid	Stearic acid	18:0
$CH_3(CH_2)_{18}CO_2H$	Arachidic acid	Eicosanoic acid	20:0
$CH_3(CH_2)_{20}CO_2H$	Behenic acid	Docosanoic acid	22:0

For every ying in chemistry, there is a yang, and for the saturated fatty acids, it is the unsaturated fatty acids – those that contain a double bond within the "tail" group. For biosynthetic reasons, these double bonds are almost universally *cis*- and are placed at intervals of three carbons distance from the carboxylic carbon atom (i. e. at carbon, 9, 12, 15, etc.). To denote the position of a double bond (a point of unsaturation), the nomenclature described above for saturated acids is modified to denote the number of double bonds "x", and the distance of the the double bond closest to the terminal methyl group from the methyl group. For example, linoleic acid, which is a C_{18} fatty acid, has a two points of unsaturation at carbons 9 and 12. Its shorthand name is 18:2*n*-6 (Figure L.2).

It should be noted that a variety of other modifications are possible in fatty acids. Most notoriously, a *trans*- double bond yields a "trans-fat". But also there are oxidation products that may incorporate epoxy rings or hydroxyl groups, and branched alkanes are present as well. For the moment, we will be content to focus on the major classes of straight chain saturated and unsaturated acids.

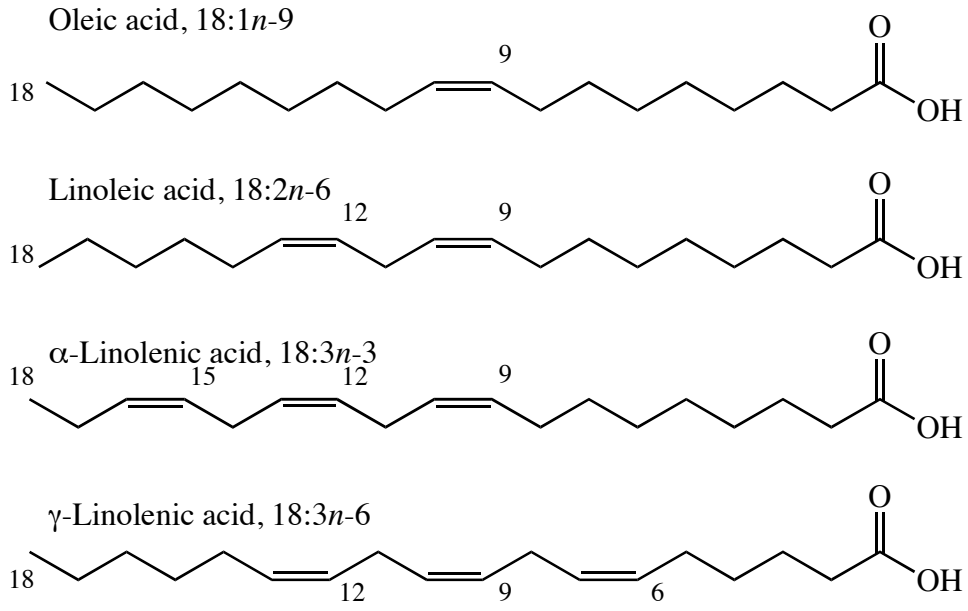

Figure L.2. Structure and nomenclature among some unsaturated fatty acids.

Another common class of lipids are the sterols, hydrophobic alcohols built off of a common four-ring structure. The most commonly known of these is cholesterol, but a number of different compounds exist. Examples are given in Figure L.3. Cholesterol plays a significant role in the structure of biological membranes of mammals.

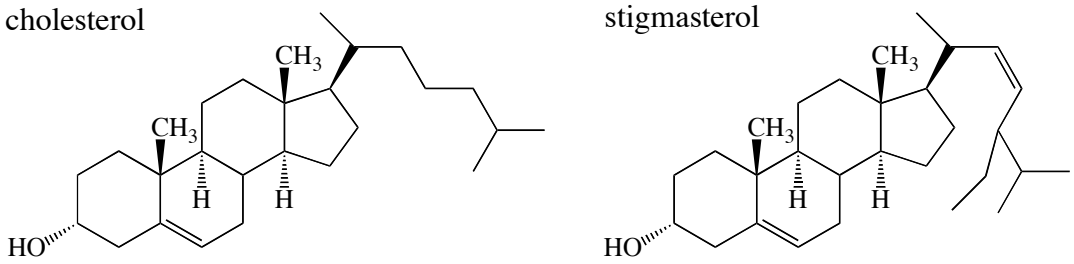

Figure L.3. Structures of a mammalian sterol (cholesterol), a plant sterol (stigmasterol).

Phospholipids

Although it will be instructive to study the properties of fatty acids, they are not generally found as isolated species in the cell. In their functional roles, fatty acids are generally modified by formation of esters or amides at the carboxyl carbon, creating covalent links to other molecules and creating new conjugates. Although many different esters and amides are found, we will concentrate on esters of fatty acids with glycerol (1,2,3-propanetriol). These compounds are the principal components of fats and oils (as triesters) and of phospholipids (as diesters of phosphoglycerol). Phospholipids play the key structural role in forming cell membranes. Note that, in a theme that will repeat several times this semester, a functional molecule is derived by the **condensation** of multiple subunits (Figure

L.4). A glycerol moiety is condensed with two fatty acids to create a dicarboxylic acid ester, and yet again with a phosphoric acid derivative to create a phosphate ester of glycerol. Although glycerol is itself an achiral molecule, it becomes chiral upon substitution of the terminal hydroxyl groups. To distinguish the two different hydroxyls, they are identified from 1-3 in the *sn* (stereochemical numbering system), which can be appreciated by holding the glycerol in a fixed position with respect to the page as in Figure L.4. (For details on the *sn* system, see the appendix to this chapter.) Most phospholipids in biology have *R*- absolute configuration, a byproduct of the biosynthetic process that makes them.

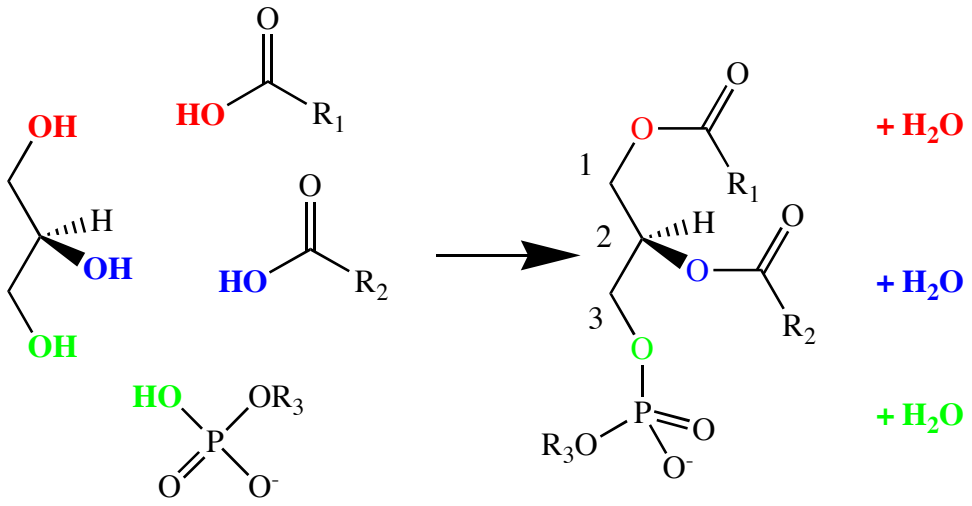

Figure L.4. Condensation of two fatty acids and a phosphoric ester with glycerol to form a phospholipid. Note that the product is labeled according to the *sn* numbering system (see Appendix).

Note that a third point of variation in phospholipid structure is related to the substituent on the phosphate moiety – often called the headgroup of the compound (see Figure L.1). If R_3 (Figure L.4) is hydrogen, a phosphate monoester is created, and the resulting phospholipid is referred to as a phosphatidic acid (PA). However, if R_3 is an alkyl group, the headgroup consists of a phosphodiester, reflecting the fact that a phosphoric acid equivalent has been modified by two alcohols, the diacyl glycerol and a second alcohol (the source of the undefined R_3 group). Although there are many possibilities, we can focus on three: ethanolamine, $HOCH_2CH_2NH_3^+$; choline, $HOCH_2CH_2N(CH_3)_3^+$; and serine ($HOCH_2CH(CO_2^-)NH_3^+$. These three create three additional headgroups: phosphatidylethanolamines (PE's), phosphatidylcholines (PC's) and phosphatidylserines (PS's; see Figure L.5). Note that PS's and PA's are anionic, PE's and PC's are charged, but neutral, at pH 7.

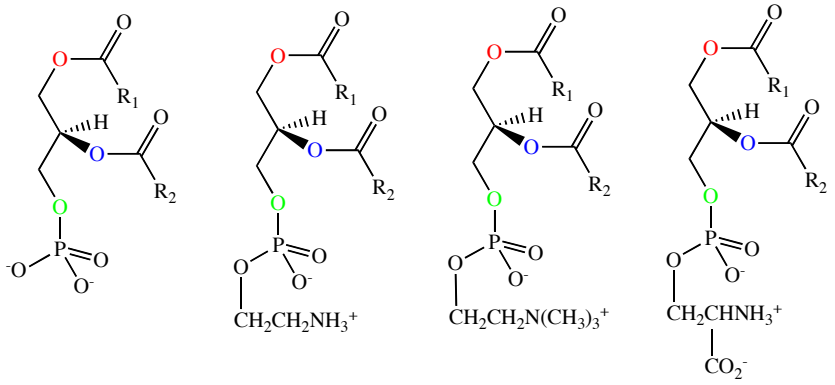

Figure L.5. Four classes of phospholipids, from left to right: a phosphatidic acid (PA), a phosphatidylethanolamine (PE), a phosphatidylcholine (PC) and a phosphatidylserine (PS).

In a given phospholipid, the two fatty acid moieties may be identical or different. They may both be saturated, unsaturated or a mix of the two. Typical nomenclature identifies the point of substitution of each substituent as well as giving the identity of the head group. For example, a phospholipids with two stearoyl chains (C_{18} saturated fatty acids) and a phosphatidylserine head group would be 1,2-distearoyl-*sn*-glycerol-3-phosphoserine. An example of a differentially substituted phospholipids is 1-palmitoyl-2-oleoyl-*sn*-glyerol-3-phosphocholine. Just to provide a wrinkle to the story, there is also a class of singly substituted phospholipids, in which one of the fatty acid chains has been cleaved off. These so-called lyso fatty acids may be labeled as 1-lyso or 2-lyso to indicate the position of loss. For example, 1-*lyso*-2-stearoyl-*sn*-glycerol-3-phosphoethanolamine lacks a fatty acid at the 1 position.

Aggregation of Lipids in Aqueous Solution[1]

As with all biochemical species, lipids function in an environment dominated by water, and the interaction of these compounds with water dictates much of their behavior – even if that behavior is to simply seek out hydrophobic environments. Ultimately, any lipid at a high enough concentration will aggregate, taking advantage of the hydrophobic effect to obtain a more entropically favorable state for the aqueous solvent. The mode of aggregation and the thermodynamics of that organizing process are determined both by the structure of the lipid and the solvent conditions.

The path towards aggregation can be viewed as following along an axis of increasing concentration of the lipid. At low concentrations, the lipid exists in equilibrium between two states: a disperse form in solution and an organized monolayer at the surface of the solution (Figure L.6). The monolayer at the surface places the hydrophobic regions of the lipid in contact with air (a non-interacting medium) and sequesters them from water. This is entropically unfavorable for the lipid, since its translational freedom is restricted, but is entropically favorable for the solvent, as unfavorable water-alkyl group interactions are minimized.

[1] A good chunk of the material for these notes was taken from two texts: (a) M. N. Jones & D. Chapman, *Micelles, Monolayers and Biomembranes*, Wiley-Liss, 1995, and (b) R. B. Gennis, *Biomembranes*, Springer-Verlag, 1989.

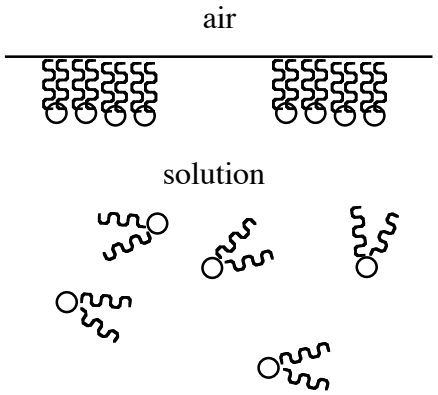

Figure L.6. Equilibrium between disperse lipids and a surface monolayer. When the surface monolayer is saturated, the disperse lipids aggregate in solution.

As the concentration of the lipid increases, the monolayer at the surface will eventually be completed, and there will be no additional space for any additional lipid units. That this point, termed the **critical micelle concentration**, any further aggregation will take place in disperse solution away from the surface. The exact nature of the aggregate depends on concentration, temperature, structure, and other factors. For simplicity, we will classify them as **micellar**, **bilayers** and **hexagonal cylinder** phases (see Figure L.7). Certain lipids and detergents strongly favor one form or another, for reasons that will be discussed below.

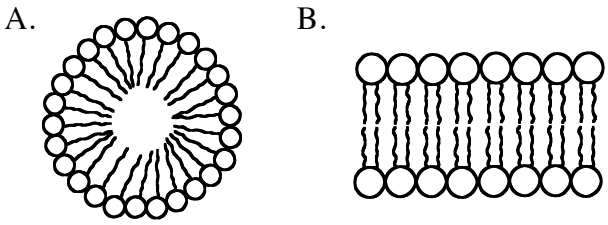

Figure L.7. Two possible aggregates of amphiphilic molecules. (A) a cross-section of a micelle, and a (B) a lipid bilayer.

There are relatively simple structural grounds that determine whether a given amphiphile will aggregate in a micelle or a bilayers. The question goes to a balance between the surface area required by the head group (S) and the length (l) and volume (v) of the tail groups. It is important to note that there is a huge enthalpic cost for a micelle to create a central void. Thus, the radius of a micelle must be less than, or equal to, the length of the tail group:

$$R \leq l$$

Note that we can calculate the volume and surface area of a micelle containing n molecules of amphiphile as follows:

$$\text{Volume} = {}^{4}/_{3}\pi R^3 = nv$$

$$\text{Area} = 4\pi R^2 = n\text{S}$$

By division and separation of variables, we find that:

$$R = 3v/S \leq l$$

So, we find that micelle formation requires that:

$$v/lS \leq 1/3$$

This will be true for amphiphiles with skinny tails (v/l, the cross-section of the tail, must be a small number) or a large head group (S is large). Conversely, for a lipid bilayers the cross-section of the tail should be about equal to the surface area of the head group ($v/lS \approx 1$) so that the horizontal stacking of the amphiphile is comparable between head and tail groups.

Generally speaking, simple amphiphiles such as fatty acids and detergents, which contain a single, saturated tail group will form micelles because the tail is reasonably narrow (the cross-section is estimated to be 19 $Å^2$), while the head group is charged and leads to a requirement for greater surface area to avoid charge repulsion. Conversely, phospholipids often form bilayers, because of the greater v/l ratio that accompanies two tail groups (38 $Å^2$) and the comparable surface area occupied by the head group – phosphatidylethanolamine groups require roughly 40 $Å^2$ as well. On the other hand, *lyso*-phospholipids, with a single tail group, or for diacyl phospholipids with short, unsaturated fatty acid tails retain the tendency to form micelles.

Micelles

Table L.2. Critical micelle concentrations for a series of sulfate-containing detergents.

Detergent	cmc (mM)
$CH_3(CH_2)_7OSO_3^-$	139.96
$CH_3(CH_2)_9OSO_3^-$	33.04
$CH_3(CH_2)_{11}OSO_3^-$	16.48
$CH_3(CH_2)_{13}OSO_3^-$	8.59
$CH_3(CH_2)_{14}OSO_3^-$	1.20
$CH_3(CH_2)_{11}O(CH_2)_2OSO_3^-$	4.02
$CH_3(CH_2)_{15}OSO_3^-$	1.20
$CH_3(CH_2)_{17}OSO_3^-$	0.16
$CH_3(CH_2)_{11}O(CH_2)_2O(CH_2)_2OSO_3^-$	2.80

The simplest aggregate structure formed by lipids are micelles. These are spherical aggregates that orient the polar head groups towards solution and bury the hydrophobic tail groups towards the inside (Figure L.7A). The classes of amphipathic molecules that favor this form are those with relatively large head groups relative to the volume of the hydrophobic tails: detergents, lysophospholipids and phospholipids with short fatty acid chains, or very large head groups. As the name suggests, the critical micelle concentration is a value that was originally used to describe the concentration at which detergents would saturate aqueous solution and start to form micelles. A brief glance at a table of cmc values for sulfate containing detergents reveals an obvious structural trend (Table L.2). As the number of methylene groups in the hydrophobic tail increases, the cmc becomes lower. This change can readily be tied to solubility issues and the hydrophobic effect. As the "tail" grows, the interactions of the compounds with water become significantly less favorable as the hydrophobic effect becomes more prominent.

The thermodynamics of micelle formation can be determined by treating association as a simple process in which a single type of micelle of a fixed number of subunits is present:

$$nS \leftrightarrows S_n$$

where n is the number of subunits, S, and S_n is a micelle formed from n subunits of S. The equilibrium constant is:

$$K = [S_n]/[S]^n$$

And the free energy of micelle formation can be calculated as:

$$\Delta G^\circ = -RT\ln K = -RT\ln[S_n] + nRT\ln[S]$$

At the critical micelle concentration, [S] = cmc, and if we calculate ΔG°_m as the free energy of micelle formation per mole of subunit, then we get:

$$\Delta G^\circ_m = \Delta G^\circ/n = -(RT/n)\ln[S_n] + RT\ln(cmc)$$

If n is large (usually between 50-100 as it turns out) we can discount the first term, and ΔG°_m is simply:

$$\Delta G^\circ_m = RT\ln(cmc)$$

Note that the cmc is generally considerably much less than 1.0 M, so the ΔG°_m will usually be negative, indicating spontaneous formation of micelles from the standard state.

By comparing the thermodynamics of micelle formation for a series of small phospholipids reveals that entropy is the significant and determining factor in the micelle formation – though not as one might generally expect. In going from n subunits of S to a single S_n micelle, there is a substantial loss of entropy for the subunits. However, overall the entropy of micelle formation is positive! Why? This is a clear example of the hydrophobic effect at work. As the hydrophobic tails of the amphipathic molecules aggregate, fixed water molecules engaged in solvating these groups are released from their cage-like structures, so solvent entropy is quite favorable. If many water molecules are fixed to a single tail, then the loss of entropy of a single subunit is nothing compared to the gain in entropy for the many solvent molecules. It is also worth noting that ΔH° for micelle formation is relatively small and does not contribute significantly to the stability of the micelle – at least not generally (Table L.3).

Table L.3. Thermodynamic properties of micelle formation for a few compounds at 25°C.

Amphiphile	cmc (μM)	ΔG_m° (kcal/mol)	ΔH_m° (kcal/mol)	$-T\Delta S_m^\circ$ (kcal/mol)
$CH_3(CH_2)_7OSO_3^-$	2300	-3.6	0.8	-4.4
$CH_3(CH_2)_9OSO_3^-$	590	-4.4	0.5	-4.9
$CH_3(CH_2)_{11}OSO_3^-$	150	-5.2	0.5	-5.7
$CH_3(CH_2)_{11}N(CH_3)_3^+$	0.58	-8.5	-0.3	-8.2
Dihexanoyl phosphatidylcholine	250	-4.9	1.6	-6.5

In general, the thermodynamic impetus for micelle formation closely follows the amount of hydrophobic surface are that becomes buried for a given class of compounds. However, there are significant effects that the polar head group can have on micelle formation, which become apparent when comparing differing classes of compounds. Note that in Table L.3, DTAB has a much lower cmc than does SDS, despite the fact that each possesses a dodecyl chain. Clearly the reason must lie in the difference between the sulfate and trimethyl ammonium groups. In a study of phospholipids with identical fatty acid substitution but differing head groups, it was found that the cmc could vary significantly, and showed a dianionic phosphitidate (PA^{2-}) has the highest cmc, due to the greater ionic repulsion felt relative to other groups, including a singly protonated PA group (PAH^-). On the other hand, neutral phospholipids head groups such as phosphatidylcholine and phosphatidylethanolamine have considerably lower cmc values (Table L.4). Crystal structures of phospholipids containing the PE group show that it is possible for intermolecular hydrogen bonds to form between the ammonium groups of the ethnolamine and the phosphate oxygens of an adjacent head group (Figure L.8). Ionic effects on micelle stability are very different between neutral and charged head groups. For PE, the addition of 2.0 M NaCl to solution decreases the cmc only four-fold, while for anionic PS, 2.0 M NaCl leads to a 100-fold decrease in cmc.

Table L.4 Effect of head group structure on cmc for a sample phospholipids.[2]

Head Group	cmc (mM)	ΔG° (kcal/mol)
PA^{2-} (pH 8)	0.77	-6.5
PAH^- (pH 5)	0.13	-7.6
PS^-	0.24	-7.2
PC	0.10	-7.7
PE	0.05	-8.1

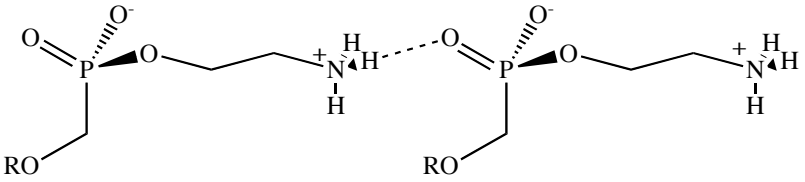

Figure L.8. Hydrogen bonding between phosphoethanolamine groups.

In discussing the effect of surfactant structure on the cmc, we are neglecting an important partner in micelle formation. Many synthetic surfactants (such as SDS and DTAB) are ionic, as are fatty acids at high pH (above 5) and several phospholipids. An interesting property of these compounds is that the conductivity of solution increases linearly with surfactant concentration below the cmc, but at the cmc it deflects to a lower slope as the conductivity increases more slowly with each added quantity of surfactant. This indicates that micelles block full dissociation of the ion pair that forms between an ionic surfactant and its counterion (say a Na^+ ion from dodecyl sulfate in SDS). Not surprisingly, the density of ionic charge at the surface of a micelle causes a condensation of counterions to the surface to balance that charge. It is not atypical for monovalent counterions to

[2] M. D. King & D. Marsh (1987) Head Group and Chain Length Dependence of Phospholipid Self-Assembly studied by Spin-Label Electron Spin Resonance. *Biochem.* **26**, 1224-1231.

balance about 80% of the charge at the surface of a micelle that contains between 50-100 individual surfactant molecules. Because of the importance of charge balancing in micelle formation, added electrolytes can have a significant effect on the cmc.

Table L.5. Variation in thermodynamics of micelle formation by DTAB with salt concentration.

[NaBr] (M)	cmc (μM)	ΔG_m° (kcal/mol)	ΔH_m° (kcal/mol)	$T\Delta S_m^\circ$ (kcal/mol)
0	302	-4.8	-0.3	4.5
0.0175	182	-5.1	-0.4	4.7
0.05	130	-5.3	-0.4	4.9
0.10	66	-5.7	-0.5	5.2

In Table L.5, the cmc for DTAB is measured at several different concentrations of NaBr. As the concentration of the bromide counterion increases, cmc decreases. There are two components to this decrease. The first is enthalpic. As the concentration of bromide increases, the electrostatic screening of charge-charge repulsion between ammonium groups in DTAB also increases, reducing an enthalpically unfavorable interaction. However, again, the entropic term changes more dramatically. As [NaBr] increases, the entropy of micelle formation becomes increasingly favorable. This is a common feature of many salts that act to increase the hydrophobic effect. We will return to this effect later in the class.

Lipid Bilayers

Generally, when water is added to dry phospholipids, bilayered structures form (Figure L.9). These structures can be discrete "liposomes" (literally "fatty bodies", which are defined by the enclosure of some volume of solution) that are either multi-layered, like an onion, or containing a single bilayer, a so-called **vesicle (**Figure L.9C). The simplest vesicles are called **small unilamellar vesicles** (SUVs). For dipalmitoyl-PC, the smallest possible vesicles are roughly 25 nm in diameter, but larger unilamellar vesicles (LUVs) can have diameters of up to 10^4 nm. In nature, the cell is essentially enveloped in a bilayer vesicle that is permeated by a variety of embedded proteins. Vesicles provide a simple model for understanding the more complex structural features of the cell membrane. Ultimately, what is of interest in the cellular context is the relationship of the properties of a bilayer to the structure of the component phospholipids.

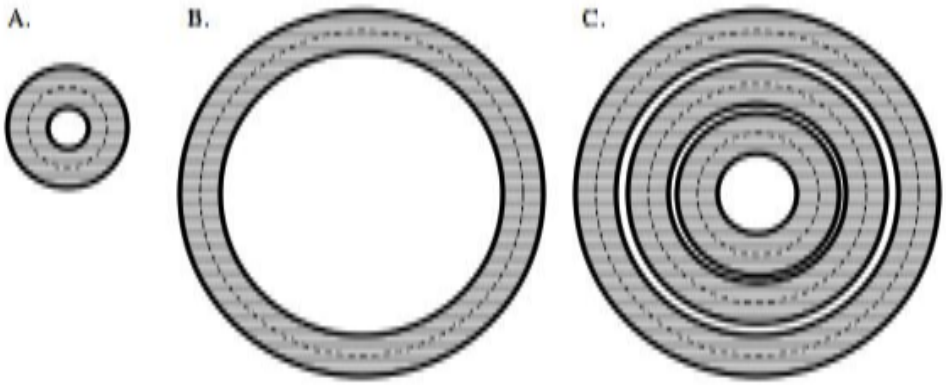

Figure L.9. Examples of liposomes. The bilayers is represented by bold lines for the positions of head groups, weak dotted lines for the interface between tail groups and horizontal lines for the volume filled by hydrophobic tail groups. Blank space indicates enclosed solvent. (A) Small unilamellar vesicle, (B) large unilamellar vesicle and (C) multi-layered liposome.

While one can speak of the cmc of a lipid that forms a bilayers (the cmc is therefore generically referring to the concentration at which aggregation takes place), of greater interest is the **transition temperature (T_m)** between two phases of the bilayers: the ordered, **gel**, state and the disordered, **liquid crystal***,* state. The transition temperature is analogous to a melting point. When the tightly packed and ordered tail groups of the gel obtain sufficient thermal energy, independent motion of each lipid is available in the plane of the bilayer. The term "liquid crystal" refers to the fluidity of the bilayers in two dimensions, but its strong ordering in the direction perpendicular to the bilayers is maintained by the thermodynamic drive to keep the hydrophobic tail groups buried away from solution. The structural change between the gel and liquid crystal states is best exemplified by the change in bilayer thickness. In the gel state, dipalmitoylphosphoethanolamine has a thickness of roughly 45 Å, but shrinks to 35 Å in the liquid crystal state. The phase transition is accompanied by adoption of *gauche* conformations within the alkyl chains that effectively shorten and widen the fatty acid tail, leading to weaker intermolecular contacts between lipids.

The technique used to measure the T_m of a bilayers is **differential scanning calorimetry** (**DSC**), which reports on the quantity of energy (heat) required to raise the temperature of a sample by a fixed amount. This ratio is the heat capacity of the bilayers. In the gel and liquid crystal phases, the heat capacity remains roughly constant, but near the transition temperature, the heat capacity of the sample spikes dramatically as a substantial quantity of the applied energy goes into "melting" the bilayer. By integrating the peak that appears in the experimental plot of heat capacity vs. temperature (Figure L.10) one obtains ΔH_m, the transition enthalpy, and because $\Delta G_m = 0$ at the transition temperature, one may calculate ΔS_m as well.

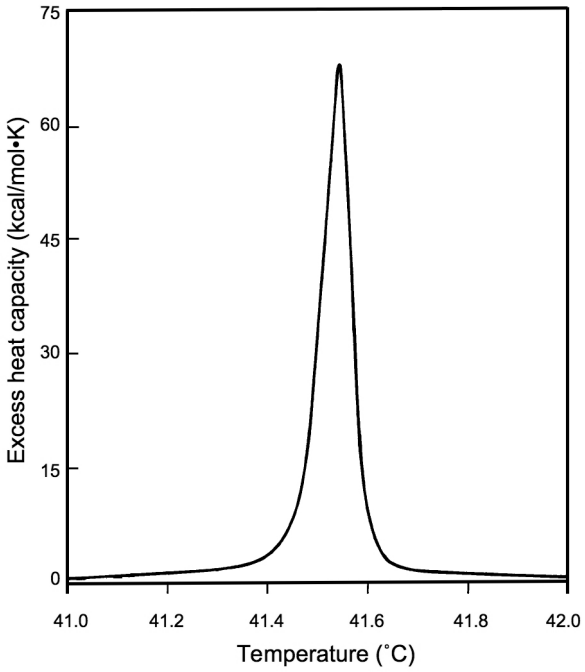

Figure L.10. Differential scanning calorimetry scan of dipalmitoylphosphocholine in water. The transition temperature is 41.5˚C. (Figure adapted from Albon & Sturtevant (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2258-2260).

Among phospholipids with saturated fatty acids, the T_m value correlates closely with the length of the alkyl substituents (Table L.6), with increasing T_m values accompanying longer tails. This observation is in accord with melting temperature data of pure, saturated fatty acids. The longer the hydrophobic tail, the greater the van der Waals attraction between chains and the greater the thermal energy required to disrupt those vdW forces. As in the micellar state, the identity of the head group can be important. For example, phosphatidylethanolamines generally melt 20˚C higher in temperature than phosphatidylcholines, due to the hydrogen bond that forms between the ammonium group and the unesterified phosphate oxygens of adjacent lipid molecules. Interestingly, the increase in melting temperature is not due to an increase in the enthalpic term associated with metling (Table L.6). Dimyristoyl PC and dimyristoyl PE have virtually identical heats of transition, yet melt 25˚C apart. Why? The difference in entropies of transition helps explain. PE phospholipids have a uniformly lower entropy of transition. Dynamics studies have indicated that this is because PE head groups remain relatively immobile in the liquid crystalline state to continue to take advantage of intermolecular hydrogen bonding, much as water does in the liquid state – thus PE phospholipids don't attain the same mobility in the liquid crystalline state as PC phospholipids.

Table L.6. Thermodynamic properties for the phase transition of some lipid bilayers[3]

Phospholipid	T_m (°C)	ΔH_m° (kcal/mol)	ΔS_m° (cal/mol•K)
Dimyristoyl PC	24.0	6.5	21.9
Dipalmitoyl PC	41.5	8.7	27.7
Distearoyl PC	54.3	10.4	33.3
Diarachidoyl PC	64.1	12.3	37.6
Dimyristoyl PE	49.9	6.6	20.4
Dipalmitoyl PE	63.9	8.6	25.5
Distearoyl PE	70.4	10.5	30.6
Diarachidoyl PE	81.1	12.2	34.5
1-stearoyl-2-oleoyl PC	3.8	6.5	23.3

Unsaturated fatty acids are a second key variable in determining the transition temperature for a bilayers. In essence, the *cis*- double bonds introduced in the alkyl tails mimic the *gauche* conformations found in the liquid crystal phase. Even in the gel phase, bilayers containing unsaturated fatty acids experience reduced levels of attractive dispersion forces, and the enthalpic favorability of the gel state is less able to compensate for the entropic costs of ordering the alkyl tail groups, and individual lipid molecules with respect to each other. Thus, the transition temperature for unsaturated phospholipids bilayers is lowered. Membrane function is dependent upon fluidity, which is achieved at physiological temperatures by incorporating unsaturated fatty acids in the membrane. *E. coli* cells lacking the capacity to synthesize their own unsaturated fatty acids are non-viable in the absence of those compounds provided in the medium.

Sterols likewise contribute to the properties of the bilayers. Cholesterol, and other sterols, are embedded in the bilayers so that the hydroxyl group is adjacent to the glycerol oxygens and the rest of the molecule extends to the interface of the bilayers (Figure L.11).

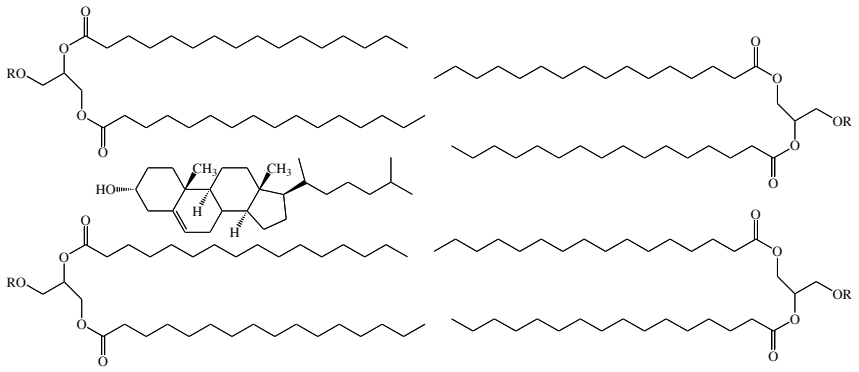

Figure L.11. Comparison of the dimensions of cholesterol to the lipid bilayer.

[3] A. Blume (1983) Apparent Molar Heat Capacities of Phospholipids in Aqueous Dispersion. Effect of Chain Length and Head Group Structure. *Biochemistry* **22**, 5436-5442.

Appendix: Stereochemical numbering

Many achiral molecules would become chiral upon substitution of a single hydrogen atom with deuterium at select positions within the molecule. This "cryptic" chirality speaks to a level of asymmetry that is not apparent in comparing the molecule to its mirror image, but rather the inequivalence of two substituents from a common **prochiral** center in a chiral environment. Since you are a chiral environment yourself (you distinguish your left hand from your right), you are capable of noting this inequivalence by inspection. Consider glycerol – place the molecule on the page such that the 2-hydroxyl group is pointing forward and to the right. With that set of instructions, one hydroxymethyl group will consistently be placed above C2 and one hydroxymethyl group will consistently be placed below. We are thus distinguishing between them.

An alternate approach would be to label one hydroxymethyl group with a deuteron on the hydroxyl. That substitution breaks the plane of symmetry in the molecule and it becomes chiral. Depending upon which hydroxymethyl (1 or 3) is substituted, the molecule takes on either *R*- or *S*- absolute configuration. According to standard nomenclature, if substituting on one group creates *R*-stereochemistry, that group is the *pro*-*R* group. By extension, the other group is *pro*-*S*. Absent a deuteron, the *pro*-*R* and *pro*-*S* groups are chemically equivalent in an achiral environment. But – in a chiral environment (such as your hand, or in an enzyme) they are chemically distinct.

The stereochemical numbering (*sn*) system defines an ordering of atoms in a linear molecule by starting from the end of the molecule that belongs to the *pro*-*S* group and numbering sequentially from 1 at that terminus. Thus the *pro*-*S* carbon in glycerol is 1 and the *pro*-*R* carbon is 3. A similar scheme can be given for xylitol, a five-carbon polyol, and citric acid, a tri-carboxylic acid (left as exercises for those who'd like one).

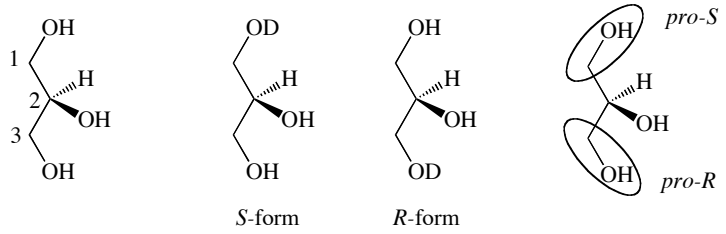

Figure L.App. Stereochemical numbering of glycerol, showing prochiral assignments.

An Exercise – Assign numbers to the following