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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 electrolyt 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

- 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
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 $\rightarrow$ 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 ($\mu_N$°)
  - attractive interfacial free energy contribution: $\gamma a$ ($\gamma \approx 20\sim 50\text{mJ/m}^2$)
  - 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 $\propto 1$/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,
  \[ \mu^o_N = \gamma a + K / a \]  

- Minimum energy
  \[ \mu^o_N (\text{min}) = 2\gamma a_o, \quad a_o = \sqrt{k / \gamma} \]  

( \( a_o \) : optimal surface area per molecule )

\[ \mu^o_N = 2\gamma a_o + \frac{\gamma}{a}(a-a_o)^2 \]  

( Unknown constant \( K \) : eliminated )
( \( \gamma \) 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 $\mu_N$°
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)

  $l_c \leq l_{\text{max}} \approx (0.154 + 0.1265n) \text{ nm}$
  $v \approx (27.4 + 26.9n) \times 10^{-3} \text{ m}^3$

  $\Rightarrow$ Larger $n$, $v/ l_c \approx 0.21 \text{ m}^2$ constant $\Rightarrow$ 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 $\mu_N$ 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) - $v/a_0l_c$

- $(v/a_0l_c < 1/3)$: spherical micelles
- $(1/3 < v/a_0l_c < 1/2)$: non-spherical micelles
- $(1/2 < v/a_0l_c < 1)$: vesicles or bilayers
- $(1 < v/a_0l_c)$: ‘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 $M$

$$M = 4\pi \frac{R^2}{a_0} = 4\pi \frac{R^3}{3v}$$

$$R = 3v / a_0, \quad \frac{v}{a_0 l_c} < \frac{1}{3}$$
Spherical micelles

- Example) SDS(12-carbon chain sodium dodecyl sulphate surfactant)
  - \( M \approx 74 \)
  - \( n=12 \rightarrow v \approx 0.3502 \text{ nm}^3 \), \( a_0 \approx 0.57 \text{ nm}^2 \), \( l_c \approx 1.67 \text{ nm} \), \( R \approx 1.84 \text{ nm} \)
  - \( v / a_0 l_c \approx 0.37 > 1/3 \)
  - ⇒ Just cannot pack into spheres, slightly non-spherical

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

\[
\mu_N^0 = \mu_M^0 + \frac{\gamma}{a} (a-a_0)^2
\]

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

\[
\mu_N^0 - \mu_M^0 = \Lambda \left( N-M \right)^2, \quad \Lambda = \gamma a_0 / 9 M^2
\]

\[
\sigma = \sqrt{\left(9kT / 2\gamma a_0\right)M}
\]

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

\[
\sigma \approx \sqrt{M}
\]
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
  - $\rightarrow$ partially screens the electrostatic inter-headgroup repulsion
  - $\rightarrow$ reduce headgroup area: $1/3 < \nu/a_0 l_c < 1/2$
  - $\rightarrow$ cannot pack into spherical micelles
  - $\rightarrow$ 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
     $\Rightarrow$ 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 $\sqrt{v/a_0 l_c} = 1/3$, so that $a > a_0$ since $\sqrt{v/a_0 l_c} > 1/3$.
  - The unfavourable energy of these end lipids determines the magnitude of the interaction parameter $a$ in $<N> = 2\sqrt{C e^a}$

- **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
  - $\frac{1}{2} < v/a_0 l_c < 1$
  - 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}~10^{-5}M)
     - bilayer forming lipids CMC (10^{-6}~10^{-10}M)
  2. increasing ‘lifetimes‘ (or residence times) $\tau_r$
     - $\tau_r$ (micelles) $\sim 55 \times 10^{-9}/10^{-3} \sim 10^{-4}$ s
     - $\tau_r$ (bilayer) $\sim 55 \times 10^{-7}/10^{-10} \sim 10^{+4}$ 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 \( k_a \)
    \[ k_a \approx 2 \gamma \text{ per monolayer, } \approx 4 \gamma \text{ per bilayer} \]

- 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
**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 $T_c$**

<table>
<thead>
<tr>
<th>Lipid (giving number of carbons per chain)</th>
<th>Headgroup type and chain melting temperature, $T_c(°C)$</th>
<th>Melting point of $n$-alkane with same number of carbon atoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC</td>
<td>PG$^-$</td>
</tr>
<tr>
<td>Saturated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilauroyl (12)</td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>Dimyristoyl (14)</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Dipalmitoyl (16)</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Distearoyl (18)</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Unsaturated (cis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dioleoyl (18)</td>
<td>-22</td>
<td>-18</td>
</tr>
</tbody>
</table>

* PC: phosphatidylcholine (zwitterionic); PG$^-$: phosphatidylglycerol (negatively charged); PS$^-$: phosphatidylinositol (negatively charged); PE: phosphatidylethanolamine (zwitterionic).

*b* Compiled from Cevc and Marsh (1987) and Marsh (1990).
* Structure of Vesicle

Bilayer Vesicle (Closed Uni-Lamellar)

Interior Aqueous Solution

Phospholipid Bilayer

Hydrophobic Tail

Hydrophilic Head Group

Phospholipid

R1 Chain

R2 Chain

Characterizing group
Vesicles

- Driving force for vesicle formation: elimination of the energetically unfavorable edges
- What determines the radii of vesicles?
  - Packing parameter: \( \frac{v}{a_0 l_c} \)
    - \( 1 \rightarrow \) bilayer
    - \( <1 \rightarrow \) vesicles
    - \( >1 \rightarrow \) inverted micellar structure/precipitation

- Critical radius by geometric consideration for \( \frac{1}{2} < \frac{v}{a_0 l_c} < 1 \)
  \[
  R_c \approx l_c \left[ \frac{3 + \sqrt{3(4v/a_0 l_c - 1)}}{6(1 - v/a_0 l_c)} \right] \approx \frac{l_c}{(1 - v/a_0 l_c)}
  \]

- Aggregation number
  \[
  N \approx 4\pi \left[ R_c^2 + \left( R_c - t \right)^2 \right] / a_o \\
  \text{here, } t \approx 2v/a_o
  \]
  Bilayer hydrocarbon thickness
**Vesicles**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Critical packing parameter $\nu/a_0/a_c$</th>
<th>Critical packing shape</th>
<th>Structures formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-chained lipids (surfactants) with large head-group areas:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS in low salt</td>
<td>$&lt; 1/3$</td>
<td>Cone</td>
<td>Spherical micelles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-chained lipids with small head group areas:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS and CTAB in high salt, nonionic lipids</td>
<td></td>
<td>Truncated cone</td>
<td>Cylindrical micelles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-chained lipids with large head-group areas, fluid chains:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphatidyl choline (lecithin), phosphatidyl serine,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phosphatidyl glycerol, phosphatidyl inositol, phosphatidic acid,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sphingomyelin, DGDG*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dihexadecyl phosphate, dialkyl dimethyl ammonium salts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Truncated cone</td>
<td>Flexible bilayers, vesicles</td>
</tr>
<tr>
<td></td>
<td>$1/2 - 1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-chained lipids with small head-group areas, anionic lipids in high salt, saturated frozen chains:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phosphatidyl ethanolamine, phosphatidyl serine + Ca$^{2+}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cylinder</td>
<td>Planar bilayers</td>
</tr>
<tr>
<td></td>
<td>$\sim 1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Double-chained lipids with small head-group areas, nonionic lipids, poly (cis) unsaturated chains, high T: unsat. phosphatidyl ethanolamine, cardiolipin + Ca$^{2+}$ phosphatidic acid + Ca$^{2+}$ cholesterol, MGDG*</td>
<td></td>
<td>Inverted truncated cone or wedge</td>
<td>Inverted micelles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$&gt; 1$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DGDG, digalactosyl diglyceride, diglucosyl diglyceride
* 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 $\mu_N^0$ on $R$

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

- 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 \(R (R > R_c)\)

\[
N \mu^o_N = N \mu^o_\infty + \left(\frac{k_b}{R^2}\right)4\pi R^2 = N \mu^o_\infty + 2\pi k_b
\]

\[
\mu^o_N = \mu^o_\infty + 2\pi k_b / N, \quad \alpha = 2\pi k_b / kT
\]

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

**vesicle distribution** \(X_N / N = \text{Const.} e^{-N/M}\)

- In case of dilute lipid concentration \(C = 10^{-4} \text{ moldm}^{-3}\)
  
  \(\checkmark k_b > 2 \times 10^{-20} \text{ J} \rightarrow \text{vesicles will be large (}M > 10,000\text{), polydisperse, and } R \propto C^{1/4}.\)

  \(\checkmark k_b < 2 \times 10^{-20} \text{ J} \rightarrow \text{small and monodisperse (No significant effect due to low bending modulus)}\)
Negative Curvature Modulus \((k_b < 0)\)

- Bending of a bilayer right from the start
- Smaller vesicles
- For large repulsive headgroups and shorter hydrocarbon chains \(\rightarrow\) smaller vesicles
- Below a certain chain length \(\rightarrow\) 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: $\alpha$-helical or $\beta$-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.