

15. Self-Assembly¹

Cooperative self-assembly refers to the the spontaneous formation of sophisticated structures from many molecular units. Generally, we think of this as involving many molecules (cooperative units), although single- and bi-molecular problems can be wrapped into this description, as in the helix–coil transition. Examples include:

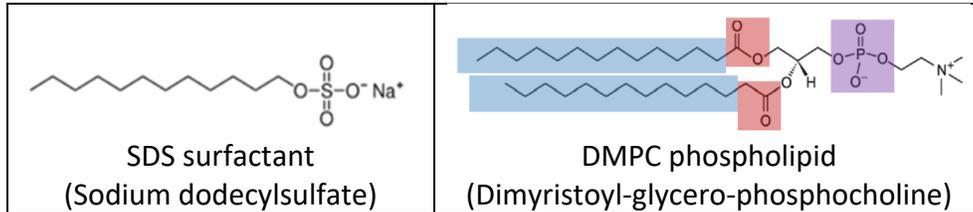
- Peptides and proteins
 - Protein folding, binding, and association
 - Amyloid fibrilization
 - Assembly of multi-protein complexes
 - Viral capsid self-assembly
- Nucleic acids
 - DNA hybridization, DNA origami
 - Folding and association of RNA structures: pseudoknots, ribozymes
- Lipids
 - Bilayer structures
 - Micelle formation

Although molecular structures also assemble with the input of energy, the emphasis here is on spontaneous self-assembly in the absence of external input.

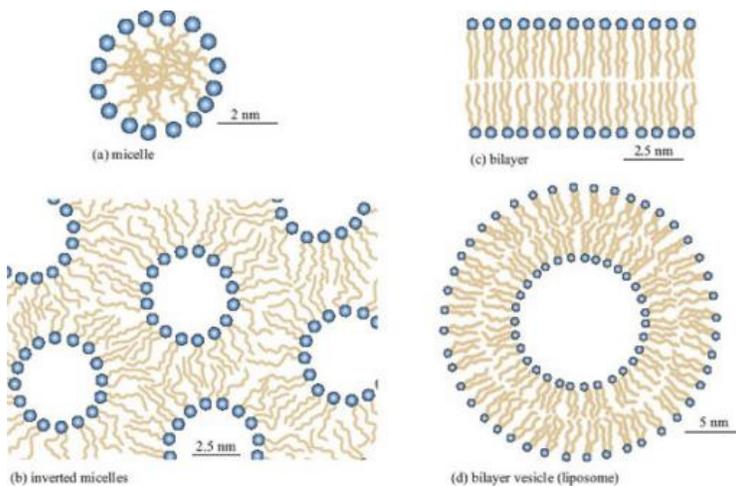
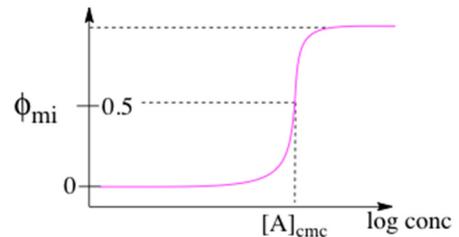
1. D. Philp and J. F. Stoddart, Self-Assembly in Natural and Unnatural Systems, *Angew. Chem. Int. Ed.* **35** (11), 1154-1196 (1996).

Micelle Formation

In particular, we will focus on micellar structures formed from a single species of amphiphilic molecule in aqueous solution.² These are typically lipids or surfactants that have a charged or polar head group linked to one or more long hydrocarbon chains.

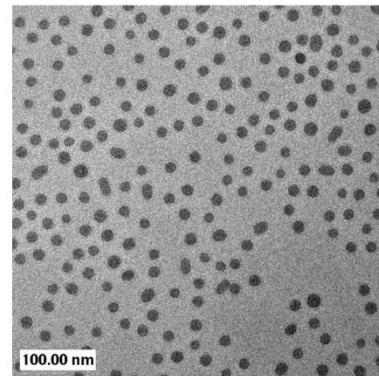


Such amphiphiles assemble into a variety of structures, the result of which depends critically on the concentration, composition, and temperature of the system. For SDS surfactant, micelles are favored. These condense hydrophobic chains into a fluid like core and present the charged head groups to the water. The formation of micelles is observed above a critical micelle concentration (CMC). As the surfactant is dissolved, the solution is primarily monomeric at low concentration, but micelles involving 30–100 molecules suddenly appear for concentrations greater than the CMC.



Micelles have a surprisingly uniform size distribution.

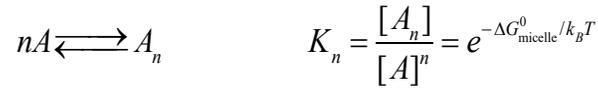
TEM of PEG-PPS micelles



Reprinted from <http://swartz-lab.epfl.ch/page-20594-en.html>.

2. D. H. Boal, *Mechanics of the Cell*, 2nd ed. (Cambridge University Press, Cambridge, UK, 2012), p. 250.

To begin investigating this phenomenon, we can start by simplifying the equilibrium to a two-state form:



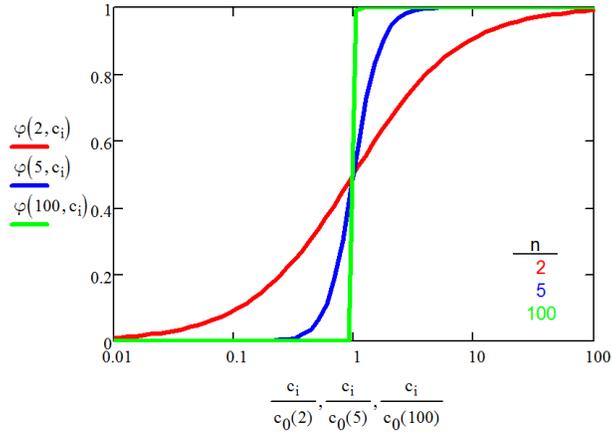
K_n is the equilibrium constant for assembling a micelle with n amphiphiles from solution. n is called the aggregation number. The total number of A molecules present is the sum of the free monomers and those monomers present in micelles: $C_{TOT} = [A] + n[A_n]$. The fraction of monomers present in micelles:

$$\phi_{mi} = \frac{n[A_n]}{C_{TOT}} = \frac{n[A_n]}{[A] + n[A_n]} = \frac{nK_n[A]^{n-1}}{1 + nK_n[A]^{n-1}} \quad (1)$$

This function has an inflection point at the CMC, for which the steepness of the transition increases with n . Setting $\phi_{mi} = 0.5$, we obtain the CMC (c_0) as

$$c_0 = [A]_{cmc} = (nK_n)^{-\frac{1}{n-1}}$$

Function steepens with aggregation number n :



Thus for large n , and cooperative micelle formation:

$$\Delta G_{\text{micelle}}^0 = -RT \ln c_0$$

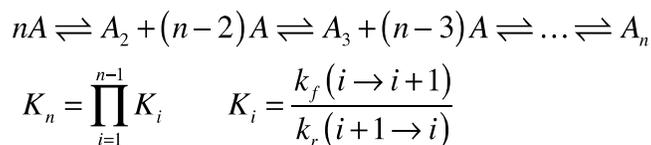
Note the similarity of eq. (1) to the results for fractional helicity in the helix-coil transition:

$$\frac{s^n}{1 + s^n}$$

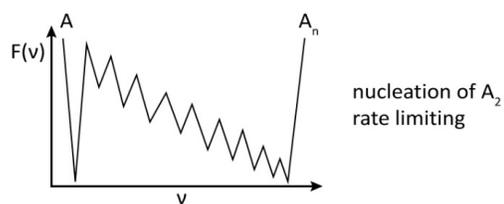
This similarity indicates that a cooperative model exists for micelle formation in which the aggregation number reflects the number of cooperative units in the process. Cooperativity can be

obtained from models that require surmounting a high nucleation barrier before rapidly adding many more molecules to reach the micelle composition.

The simplest description of such a process would proceed in a step-wise growth form (a zipper model) for n copies of monomer A assembling into a single micelle A_n .



Examples of how the energy landscape looks as a function of oligomerization number v are shown on the right. However, if you remove the short-range correlation, overall we expect the shape of the energy landscape to still be two-state depending on the nucleation mechanism.



This picture is overly simple though, since it is not a one-dimensional chain problem. Rather, we expect that there are equilibria connecting all possible aggregation number clusters to form larger aggregates. A more appropriate description of the free energy barrier for nucleating a micelle is similar to classical nucleation theory for forming a liquid droplet from vapor.

Classical Nucleation Theory³

Let's summarize the thermodynamic theory for the nucleation of a liquid droplet by the association of molecules from the vapor. The free energy for forming a droplet out of n molecules (which we refer to as monomers) has two contributions: a surface energy term that describes the energy needed to make droplet interface and a volume term that describes the cohesive energy of the monomers.

$$\Delta G_n = \gamma a - \Delta \epsilon V$$

Note the similarity to our discussion of the hydrophobic effect, where γ was just the surface tension of water. $\Delta \epsilon$ is the bulk cohesive energy—a positive number. Since this is a homogeneous cluster, we expect the cluster volume V to be proportional to n and, for a spherical droplet, the surface area a to be proportional to $V^{2/3}$ and thus $n^{2/3}$ (remember our discussion of hydrophobic collapse). To write this in terms of monomer units, we can express the total area in terms of the average surface area per molecule in the droplet:

$$\alpha = a/n$$

and as the monomer volume V_0 . Then the free energy is

$$\Delta G_n = \gamma \alpha n^{2/3} - \Delta \epsilon V_0 n \quad (2)$$

and the chemical potential of the droplet as

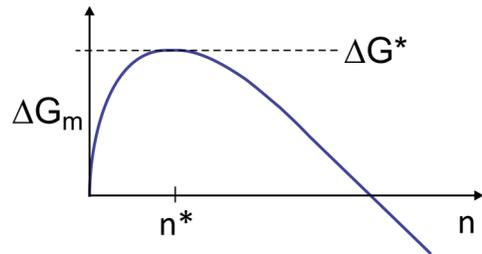
$$\Delta \mu_n = \frac{\partial \Delta G_m}{\partial n} = \frac{2}{3} \gamma_0 \alpha n^{-1/3} + \Delta \epsilon V_0 \quad (3)$$

These competing effects result in a maximum in ΔG versus n , which is known as the critical nucleation cluster size n^* . The free energy at n^* is positive and called the nucleation barrier ΔG^* . We find n^* by setting eq. (3) equal to zero:

$$n^* = \left(\frac{2\gamma_0 \alpha}{3\Delta \epsilon V_0} \right)^3$$

and substituting into eq. (2)

$$G^* = \frac{4}{27} \frac{(\gamma_0 \alpha)^3}{(\Delta \epsilon V_0)^2}$$



3. P. S. Richard, Nucleation: theory and applications to protein solutions and colloidal suspensions, J. Phys.: Condens. Matter **19** (3), 033101 (2007).

For nucleation of a liquid droplet from vapor, if fewer than n^* monomers associate, there is not enough cohesive energy to allow the growth of a droplet and the nucleus will dissociate. If more than n^* monomers associate, the droplet is still unstable, but the direction of spontaneous change will increase the size of the droplet and a liquid phase will grow from the nucleus.

The process of micelle formation requires a balance of attractive and repulsive forces that stabilize an aggregate, which can depend on surface and volume terms. Thus the $\Delta G_{\text{micelle}}$ has a similar form, but the signs of different factors may be positive or negative.

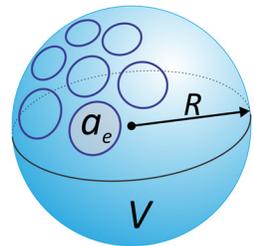
Why Are Micelles Uniform in Size?⁴

Micelles are formed by amphiphiles that want to bury hydrophobic chains and expose charged head groups to water. Since a cavity must be formed for the micelle, the resulting surface tension of the cavity (the hydrophobic effect) results in the system trying to minimize its surface area, and thereby the number of molecules in the micelle. At the same time, the electrostatic repulsion between headgroups results in driving force to increase the surface area per headgroup. These competing effects result in an optimal micelle size.

We start by defining the chemical potential $\Delta\mu_n$, which is the free energy per mole of amphiphilic molecule A to assemble a micelle with n molecules. Instead of using n , we will try to express the size of the micelle in terms of its surface area a and assume that it is spherical. Then, the free energy for forming a cavity for the micelle grows as γa , where γ is the surface tension. The surface area is expressed as an average surface area spanned by the charged headgroup of a monomer unit:

$$a_e = a/n$$

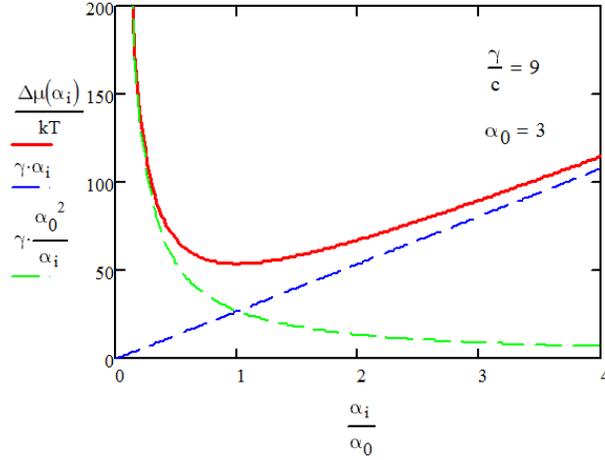
The repulsion term is hard to predict and depends on many variables. There are the electrostatic repulsions between head groups, but there is also the entropic penalty for forming the micelle that depends on size. As an approximation, we anticipate that the free energy should be inversely proportional to surface area. Then the free energy for forming a micelle with n molecules is



$$\begin{aligned}\Delta G_n &= \gamma a + \frac{x}{a} \\ &= \gamma n a_e + \frac{x}{n a_e}\end{aligned}\tag{4}$$

where x is a constant.

4. K. Dill and S. Bromberg, *Molecular Driving Forces: Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience*. (Taylor & Francis Group, New York, 2010); J. N. Israelachvili, *Intermolecular and Surface Forces*, 3rd ed. (Academic Press, Burlington, MA, 2011), Ch. 20.



Solving for $\Delta\mu=\partial\Delta G/\partial n$, differentiating it with respect to a_e , and setting to zero, we find the optimal micelle size, a_0 , is

$$a_0 = \sqrt{\frac{x}{\gamma n^2}} \quad (5)$$

Solving for x and substituting in eq. (4), we obtain the chemical potential as:

$$\Delta\mu = \frac{\gamma}{a_e} (a_e^2 + a_0^2) = 2\gamma a_0 + \frac{\gamma}{a_e} (a_e - a_0)^2$$

It has a parabolic shape with a minimum at a_0 .

Next, we can obtain the probability distribution for the micelle size as a function of head group surface area and aggregation number

$$P_n = \exp(-n\Delta\mu / k_B T)$$

$$P_n(a_e) \sim \exp\left(-\frac{n\gamma(a_e - a_0)^2}{a_e k_B T}\right)$$

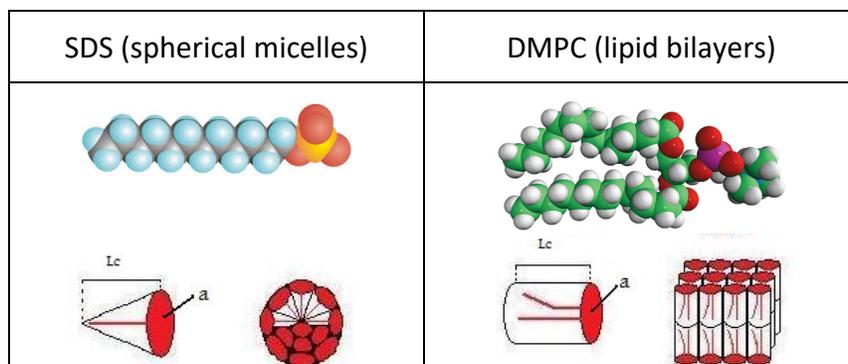
The relative populations of micelles are distributed in a Gaussian distribution about a_0 . The distribution of sizes has a standard deviation (or polydispersity) given by

$$\sigma = \sqrt{\frac{na_e k_B T}{2\gamma}}$$

From $a = 4\pi r^2 = na_e$, we predict that the breadth of the micelle size distribution will scale linearly in the micelle radius, and as the square root of temperature and molecule number.

Shape of Self-Assembled Amphiphiles

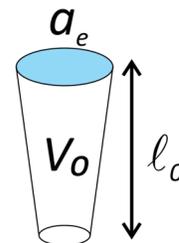
Empirically it is observed that certain features of the molecular structure of amphiphilic molecules and surfactants are correlated with the shape of the larger structures that they self-assemble into. For instance, single long hydrocarbon tails with a sulfo- group (like SDS) tend to aggregate into spherical micelles, whereas phospholipids with two hydrocarbon chains (like DMPC) prefer to form bilayers. Since structure formation is largely governed by the hydrophobic effect, condensing the hydrophobic tails and driving the charged groups to a water interfaces, this leads to the conclusion that the volume and packing of the hydrophobic tail plays a key role in shape. While the molecular volume and the head group size and charge are fixed, the fluid nature of the hydrocarbon chain allows the molecule to pack into different configurations.

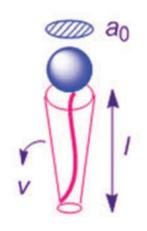
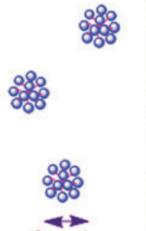
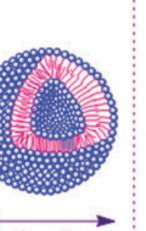


This structural variability is captured by the packing parameter:

$$p = \frac{V_0}{a_e \ell_0}$$

where V_0 and ℓ_0 are the volume and length of the hydrocarbon chain, and a_e is the average surface area per charged head group. V_0/ℓ_0 is relatively constant at $\sim 0.2 \text{ nm}^2$, but the shape of the chain may vary from extended (cylindrical) to compact (conical), which will favor a particular packing.



				
$p = v/a_0l$	$p < 1/3$	$1/3 < p < 1/2$	$1/2 < p < 1$	$p \sim 1$
aggregates	sphere	cylinder	vesicle	bilayer

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Empirically it is found that systems with $p < 1/3$ typically form micelles, for cylindrical structures for $1/3 < p < 1/2$, and for bilayer structures for $1/2 < p < 1$. Simple geometric arguments can be made to rationalize this observation. Taking a spherical aggregate with radius R and aggregation number n as an example, we expect the ratio of the volume to the surface area to be

$$\frac{V}{A} = \frac{nV_0}{na_e} = \frac{R}{3} \rightarrow V_0 = \frac{a_e R}{3}$$

Substituting into the packing parameter:

$$p = \frac{V_0}{a_e \ell_0} = \frac{R}{3\ell_0}$$

Now, even though the exact conformation of the hydrocarbon chain is not known, the length of the hydrocarbon tail will not be longer than the radius of the micelle, i.e., $\ell_0 \geq R$. Therefore

$$\therefore p \leq \frac{1}{3} \quad (\text{spheres})$$

Similar arguments can be used to explain why extended lipid bilayers have $p \approx 1$ and cylinders for $p \approx 1/2$. In a more general sense, we note that the packing parameter is related to the curvature of the aggregate surface. As p decreases below one, the aggregate forms an increasingly curved surface. (Thus vesicles are expected to have $1/2 < p < 1$). It is also possible to have $p > 1$. In this case, the curvature also increases with increasing p , although the sign of the curvature inverts (from convex to concave). Such conditions result in inverted structures, such as reverse micelles in which water is confined in a spherical pool in contact with the charged headgroups, and the hydrocarbon tails are project outward into a hydrophobic solvent.

Readings

1. J. N. Israelachvili, *Intermolecular and Surface Forces*, 3rd ed. (Academic Press, Burlington, MA, 2011).