Take-home message

- don’t expend work if you can get it for free (Self-assembly)
- minimise energy by re-using whatever you can (Conservation)
- symmetry can minimise free energy (Symmetry)

be lazy

You want to move stuff around? *Use diffusion as it’s free, and only for longer distances where it doesn’t work use a motor that costs energy; and even then make the motor use diffusion too.*

You want stuff to react? *Let thermal motion randomly jiggle reactants around until they find each other*

You want to heal a hole in a membrane? *hydrophobic effect will do it*

You need to bring stuff in or out of the cell? *Create vesicles containing the material and pinch them off, then re-use the materials*

Membranes on different scales

Cell size / membrane thickness ~ 10 micron/4 nm = 2500

the PM is a very thin, flexible fluid sheet when viewed on the scale of the whole cell. We can ignore the molecules and treat the membrane as an infinitely thin elastic sheet.

How do we mathematically describe a membrane at this scale?

A 2-dimensional surface requires 2 in-plane coordinates (x_1, x_2).

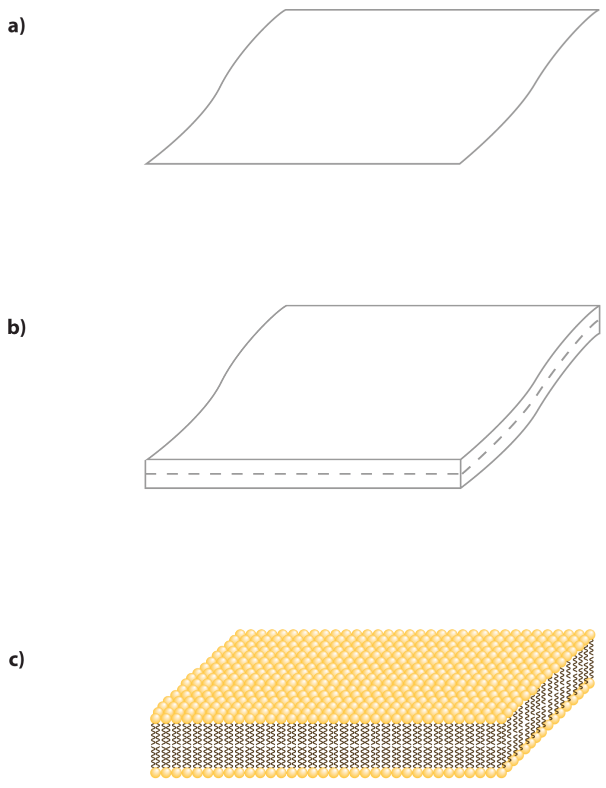
But in 3D, each point has 3 coordinates.

Nearly flat membranes can be described by Monge representation, where the height is specified at the projected (x,y) coordinates

$h(x_1, x_2)$ = function of (x, y) in the projected plane.

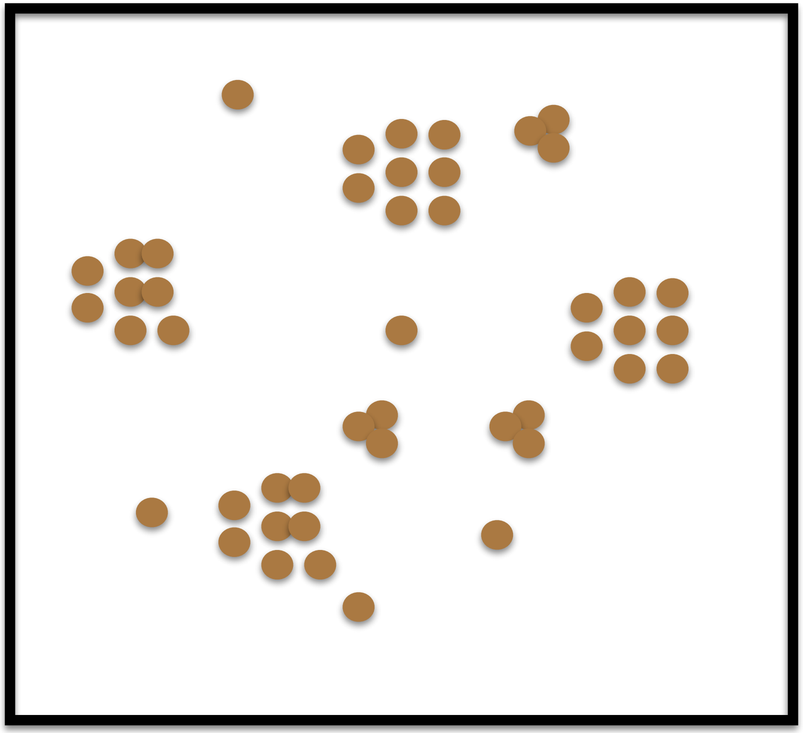
e..g, $h(x, y) = h0*\sin(k_x x)*\cos(k_y y)$

Now we have a coordinate system for the membrane surface, but what controls its dynamics?



Self-assembly - Oil

Consider N_0 oil molecules initially, uniformly dispersed in a closed box of bulk water with volume $V = L^3$.



We intuitively expect the oil to form droplets that coalesce and grow in time, but how precisely do the radius and mass change with time?

Assumptions

1) energy cost of the interface drives droplets spherical
2) at any time, all droplets are spheres with radius $R(t)$
3) droplets of radius R diffuse with a coefficient that is given by the Stokes-Einstein relation: $D = k_B T / 6\pi\eta R$ according to $\langle X^2 \rangle \sim 6\,D\,t$

Let $N(t)$ = mean number of droplets at time t
$R(t)$ = mean radius of droplets "

$$\langle vol/droplet \rangle \sim L^3 / N(t)$$

$$\langle separation \rangle \sim L / N(t)^{1/3}$$

Droplets diffuse around and grow by coalescing when they touch, and we assume that the merging time is short compared to the diffusion time.

$$(L / N^{1/3}(t))^2 \sim 6\ (k_B T / 6\pi\eta R(t))\ .\ t$$

But the number of oil molecules is constant, so:

$N(t).4\pi R(t)^3/3$ = constant or $N(t) \sim 1 / R(t)^3$

$\pi\eta R(t) L^2 / k_B T = N(t)^{2/3}.t \sim t / R(t)^2$

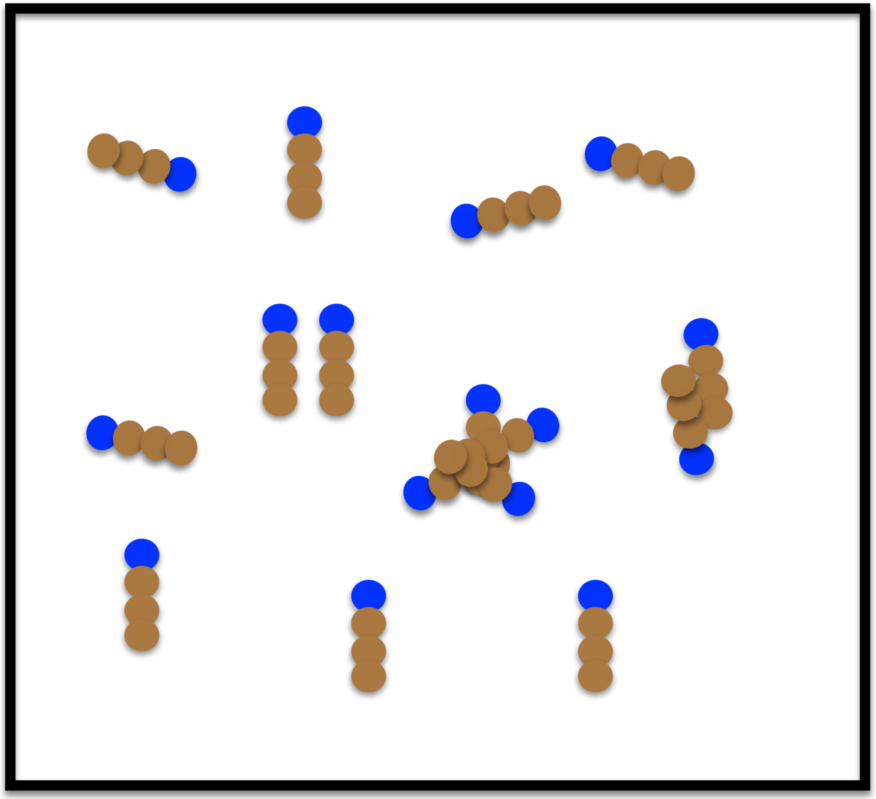
$$R(t) \sim (k_B T / \pi\eta L^2)\ t^{1/3}$$

$$M(t) \sim R(t)^3 \sim t$$

Self-assembly - lipids

How does this change if we have N *lipid* molecules in bulk water?

We expect the lipids also form aggregates that coalesce and grow in time, but how do the radius and mass change with time now?

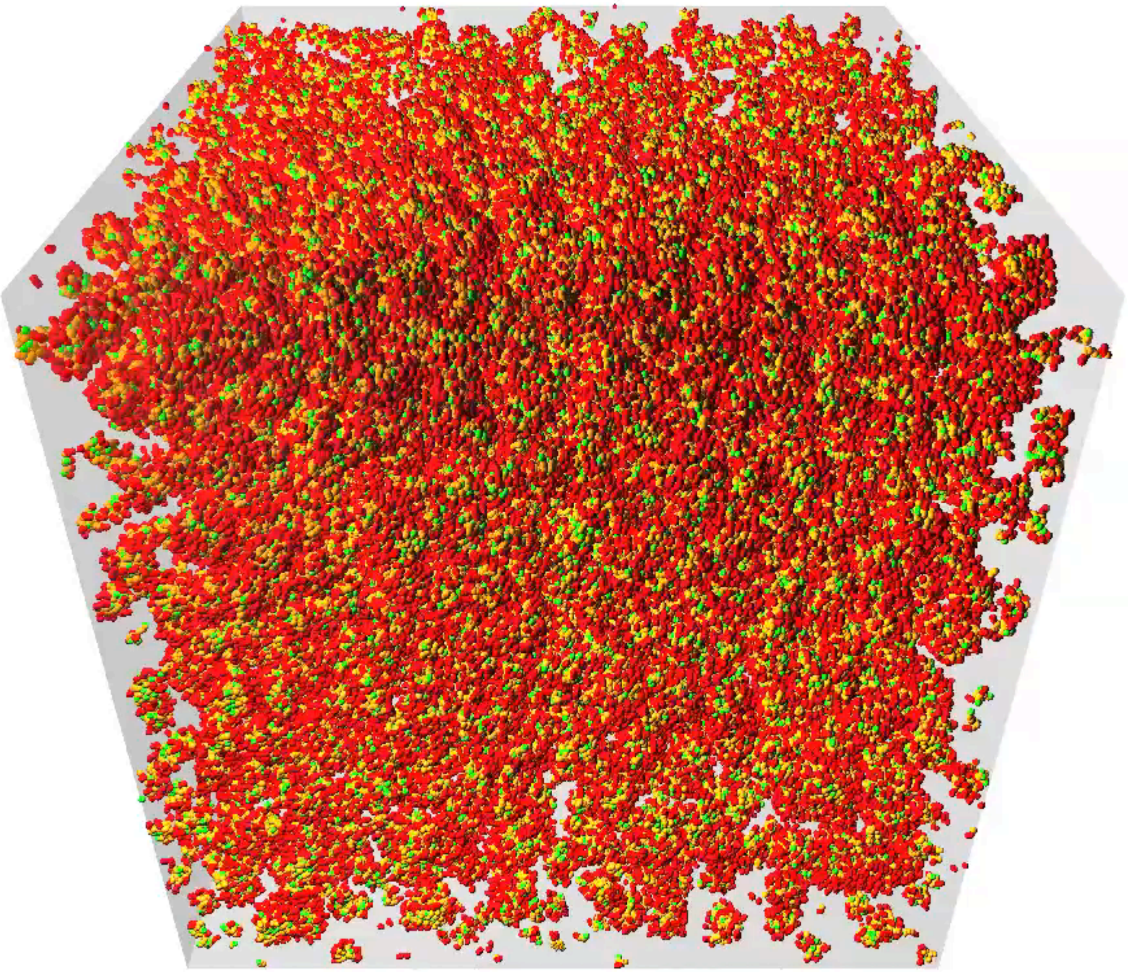


Assumptions

1) at any time, all aggregates have same mean size
2) aggregates of radius R diffuse with a coefficient that is given by the Stokes-Einstein relation: $D = k_B T/6\pi\eta R$ according to $\langle X^2 \rangle \sim 6\,D\,t$

The difference now is that lipids don't form solid droplets but hollow spheres, or vesicles, and the number of lipids in a vesicle is proportional to its area not its volume.

Aggregates diffuse around and grow by coalescing when they touch, and we assume that the merging time is short compared to the diffusion time. Aggregates are now bilayer fragments that form closed vesicles when they are large enough.



Let $N(t)$ = mean number of aggregates at time t
$R(t)$ = mean radius of aggregates “

Lipid aggregates also diffuse around and grow by coalescing when they touch but they are now bilayer fragments that can form closed vesicles when they are large enough. Again, the mean separation grows as $\sim L / N(t)^{1/3}$

$$L^2 / N^{2/3}(t) \sim (k_B T/6\pi\eta R(t)) \,.\, t$$

Number of lipid molecules is constant, so:

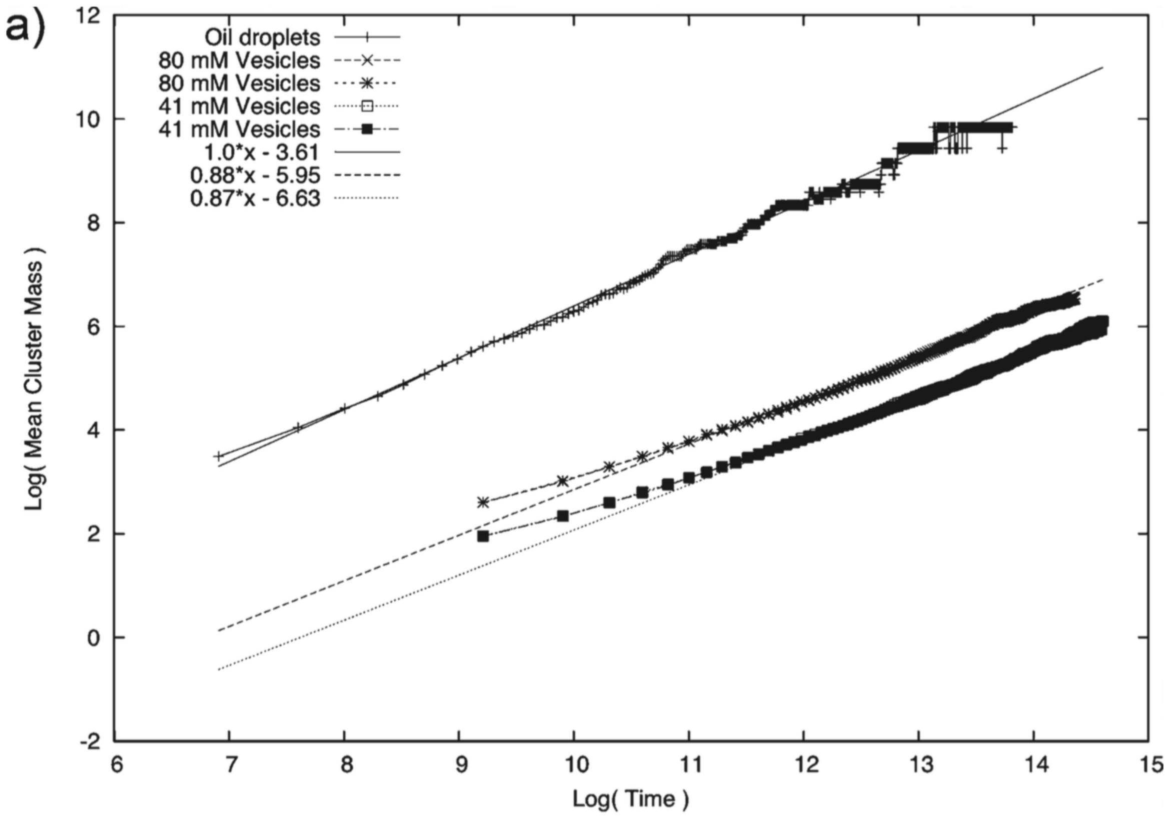
$$N(t).4\pi R(t)^2 = \text{constant}$$

$$N(t) \sim 1 / R(t)^2$$

$$\pi\eta R(t)L^2/k_B T = N(t)^{2/3}.t \sim t / R(t)^{4/3}$$

$$R(t) \sim (k_B T/6\pi\eta L^2)\, t^{3/7}$$

$$M(t) \sim R(t)^2 \sim t^{6/7} \sim t^{0.86}$$



JC Shillcock, Spontaneous vesicle self-assembly: a mesoscopic view of membrane dynamics, Langmuir 28:541-547 (2012)

Thermodynamics of self-assembly

Amphiphiles self-assemble because of the hydrophobic effect, but what determines the size and shape of the resulting aggregates?

Israelachvili produced a thermodynamic argument that the *molecular shape* of the constituents (the packing parameter) controls the equilibrium *shape* (rod, disk, sphere, etc) and *size distribution* of the aggregates.

This is a *thermodynamic result*: it does not depend on details of the interactions (ionic, hydrophobic, steric, charge), duration, history of the sample, etc. - only on free energy differences, P, T, composition. It will be valid for lipids, polymers, actin monomers, colloids, etc.

But note that we ignore inter-aggregate interactions; and the mean lifetime of a molecule in a micelle is very short, around 10^{-5} - 10^{-3} sec. In TD terms, we have a dilute solution of aggregates that maintain their equilibrium state by fast exchange of molecules: they form an homogeneous *phase*.

It's a powerful argument of the form:

Molecular shape → Free energy difference in solution/aggregate → Thermodynamic force driving aggregation → Aggregate shape and size distribution

J. Israelachvili,, Intermolecular and Surface Forces, Ch. 16, Academic Press 1992

Consider aggregate formation as a “chemical reaction”: N monomers combine into an N-mer.

In equilibrium, the rate of association and disassociation are equal:

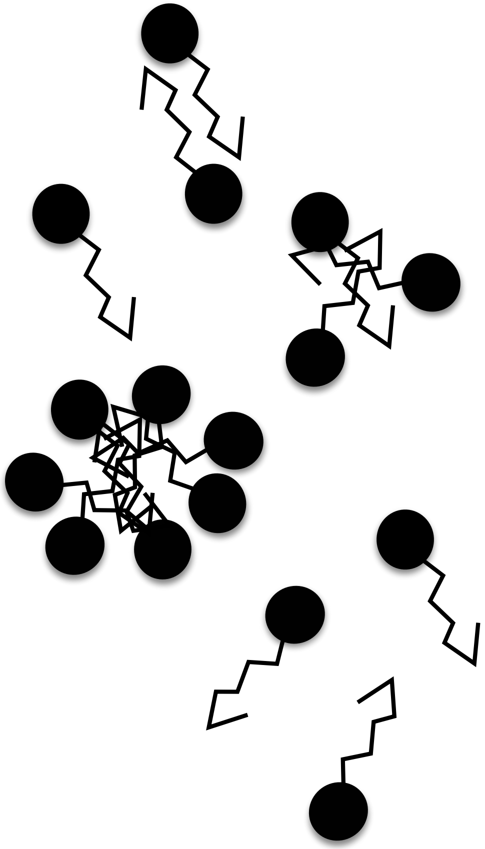
$$k_1 X_1^N = k_N (X_N / N)$$

where X_1 = volume fraction of monomers in solution

X_N = volume fraction of monomers in N-mers

k_1 = association constant (forward reaction)

k_N = dissociation constant (backward reaction)



Now $k_1/k_N = K$ = equilibrium constant = $\exp(-\beta N(\mu_N^0 - \mu_1^0))$

μ_N^0 is the mean interaction free energy per molecule in aggregates of size N.

Let C be the total concentration of molecules in all aggregates:

$$C = \sum X_N = X_1 + X_2 + \ldots.$$

All X_N and $C \leq 1$.

Combining the reaction equations leads to the equilibrium conditions:

$$\mu_1^0 + k_BT \ln X_1 = \mu_N^0 + (k_BT/N) \ln (X_N/N) \text{ for all } N$$

or, equivalently:

$$X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N$$

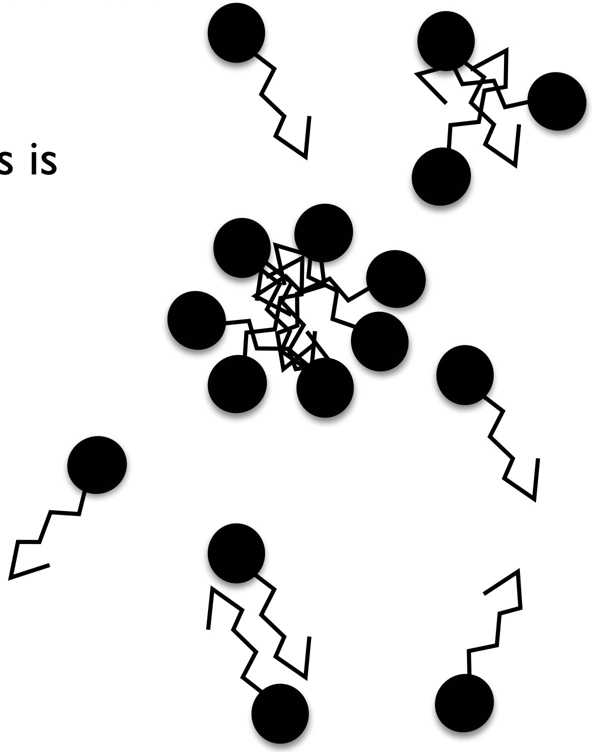
The inter-molecular forces are wrapped up in the Chemical Potential - μ - a measure of the free energy of a substance in a given phase. In Lecture 11 we saw how opposing forces between lipids in a membrane gave rise to a quadratic minimum of $\mu(a)$ on area/molecule.

Whether aggregates form and what fraction of monomers form N-mers is determined by how the function μ_N^0 varies with N:

$$\mu_N = \mu_N^0 + (k_BT/N) \ln(X_N/N)$$

We can imagine that μ_N^0 :

1. increases with N
2. is constant
3. decreases with N or has a more complicated relation



$X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N$ $C = \sum X_N$

Case 1. μ_N^0 increases monotonically with N

$X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N \rightarrow$ zero as N increases because $X_1 < 1$ and exponent is < 0

⇒ No aggregates or very few

Case 2. μ_N^0 independent of N

$X_N = \text{const.}\, N X_1^N \rightarrow$ zero as N increases because $X_1 < 1$

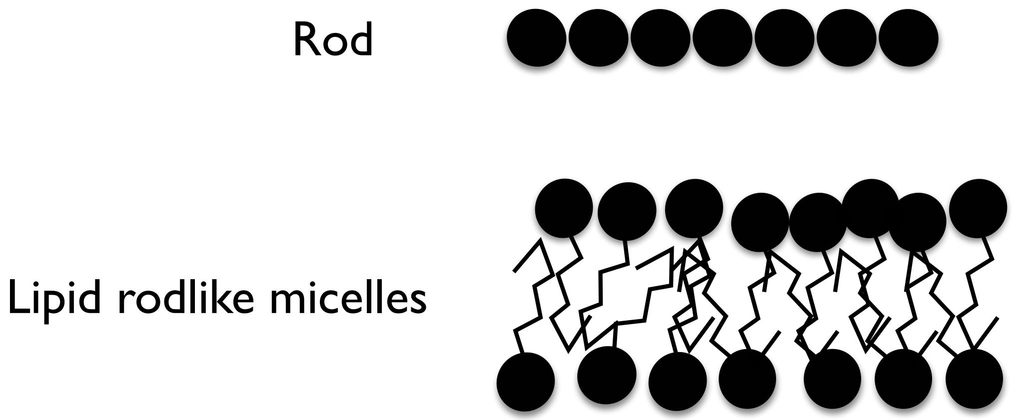
⇒ No aggregates or very few

Case 3. μ_N^0 decreases with or has complex dependence on N.

$X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N \rightarrow$ large as N increases because the exponent is > 0.

X_N depends on *how* μ_N^0 varies with N which depends on the *geometry* of the aggregates.

Rods

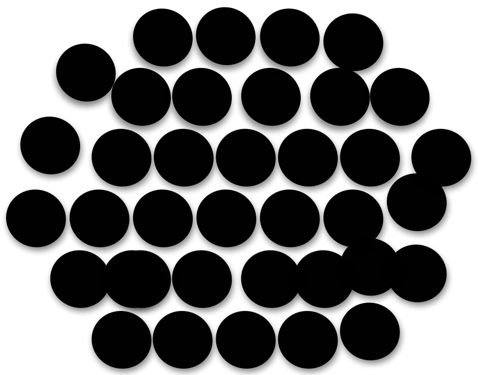


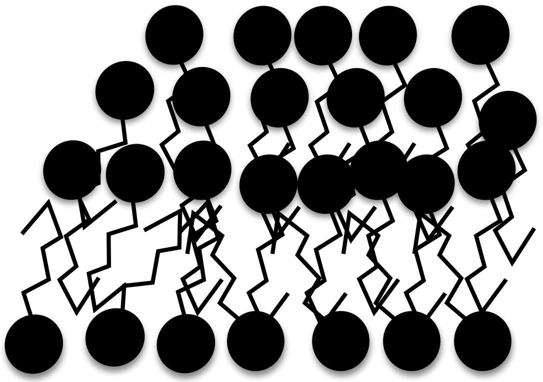
Case 3A. $N \mu_N^0 = -(N - 1)\, \alpha\, k_B T$ where $\alpha\, k_B T$ is the "bond energy" of a monomer in the infinite aggregate.

$$\mu_N^0 = -\alpha\, k_B T + \alpha\, k_B T/N \rightarrow \mu_\infty \text{ for } N \rightarrow \infty$$

so it decreases towards an asymptotic value $\mu_\infty = -\alpha\, k_B T$ when adding a single monomer to the aggregate has the same free energy change as adding it in the bulk.

Disks





Cohesive energy ~ Area = πR^2
Unbonded energy ~ circumference = $2\pi R$

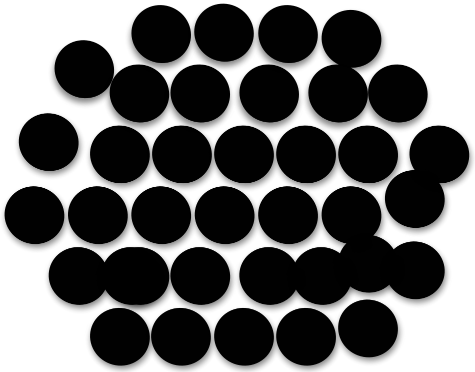
Lipid disklike micelles
(imagine this is circular!)

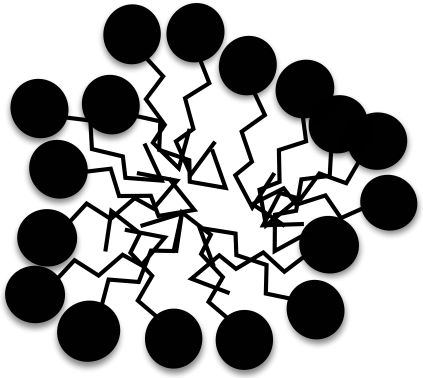
Case 3B. $N \mu_N^0 = -(N - \sqrt{N})\, \alpha\, k_B T$

$$\mu_N^0 = \mu_\infty + \alpha\, k_B T / \sqrt{N}$$

and again decreases towards an asymptotic value.

Spheres or micelles





Cohesive energy ~ Vol = $4/3\pi R^3$
Unbonded energy ~ Area = πR^2

Lipid micelles (imagine this is spherical!)

Case 3C. $N\,\mu_N^0 = -(N - N^{2/3})\,\alpha\,k_BT$

$$\mu_N^0 = \mu_\infty + \alpha\,k_BT / N^{1/3}$$

and again decreases towards an asymptotic value.

General case

All three cases can be collected together if we define a parameter **p** that depends on their geometry and dimension. The free energy per molecule in an aggregate of size N varies as:

$$\mu_N^0 = \mu_\infty + \alpha k_B T / N^p$$

A necessary condition for aggregates to form is that μ_N must decrease with N (at least for some range of N > 1).

Now the questions are:

1) At what concentration of monomers do aggregates start to form?

2) What distribution of sizes will we get?

We have rearranged

$$\mu_1^0 + k_B T \ln X_1 = \mu_N^0 + (k_B T/N) \ln (X_N/N) = \text{constant}$$

to give the fraction of N-mers:

$$X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N$$

Low monomer concentration

Given $\mu_N^0 = \mu_\infty + \alpha k_B T / N^p$

$$\mu_1^0 - \mu_N^0 = \alpha k_B T(1 - 1 / N^p)$$

and inserting this into $X_N = N X_1^N (\exp(-\beta(\mu_N^0 - \mu_1^0))^N$ gives:

$$X_N = N(X_1 \exp(\alpha(1 - 1 / N^p))^N \sim N(X_1 \exp(\alpha))^N$$

as $N^{-p} \sim 0$.

Now, if X_1 is small so that $X_1 \exp(\alpha) << 1$, we have

$$X_1 > X_2 > X_3 \ldots . > X_N$$

and almost all the molecules occur as monomers, with a few dimers, trimers maybe, so that

$$X_1 \sim C = \text{total concentration}$$

High monomer concentration

But if X_1 increases beyond $X_1 \exp(\alpha) \sim 1$, then it has to stay constant because $X_N < 1$.

Where do the extra molecules go?

Given $X_1 \exp(\alpha) \sim 1$,

$$X_N = N\,(\,X_1 \exp(\alpha\,(1 - 1 / N^p\,)\,)^N$$

$$\sim N\,(\,X_1 \exp(\alpha) \exp(-\,\alpha / N^p\,)\,)^N$$

$$\sim N\,(\,X_1 \exp(\alpha)\,)^N \exp(\,-\alpha.N^{1-p}\,)$$

$$\sim N \exp(\,-\alpha\,N^{1/2}\,) \text{ for disks} \quad \text{and } N \exp(\,-\alpha\,N^{2/3}\,) \text{ for spheres.}$$

For any reasonable value of α, there are very few aggregates with $N > 1$, and all the molecules go into an infinite phase similar to oil separating from water.

The concentration $X_1 \sim \exp(-\alpha)$ is the *Critical Micelle Concentration,* it is the concentration at which monomers start forming aggregates (or the infinite phase) instead of staying isolated in solution.

CMC calculation

Consider adding oil or alkane molecules to water.

Treating each molecule as a small sphere with $r \sim 0.2$ nm, we have the interfacial energy from the hydrophobic effect:

$\alpha = 4\pi r^2 \gamma / k_B T$

where $\gamma \sim 50$ mJ/m^2 is the surface tension for oil in water. So the CMC is

$$\exp(-\alpha) = \exp(-4\pi r^2 \gamma / k_B T)$$

The free energy of transferring one molecule from bulk hydrocarbon to bulk water is then:

$4.\pi.r^2.\gamma = 2.5.10^{-20}$ J ~ 15 kJ/mol ~ $6.1\ k_B T$

so, $\alpha = 6.1$ and the CMC ~ $e^{-6.1}$ ~ 0.002 M or 2 mM.

cp. DOPC with two tails for which CMC ~ 10^{-10} M (Q. Can one simulate the CMC with DPD?)

Phospholipids typically have 2 tails to ensure that membranes stay intact!

Critical micelle concentration

What happens when p = 1, i.e., rods? We have $\mu_N^0 = \mu_\infty + \alpha k_B T / N$

$X_N = N (X_1 \exp(\alpha (1 - 1 / N))^N$

$\sim N (X_1 \exp(\alpha))^N \exp(-\alpha)$

And now when $X_1 \exp(\alpha) \sim 1$, this gives $X_N \sim N \exp(-\alpha)$ and larger aggregates are possible.

Recall the total concentration:

$C = \sum X_N = \exp(-\alpha) \sum N(X_1.\exp(\alpha))^N$

$= \exp(-\alpha)(X_1 \exp(\alpha) + 2 (X_1 \exp(\alpha))^2 + 3 (X_1 \exp(\alpha))^3 + \dots)$

$$C = X_1 / (1 - X_1 \exp(\alpha))^2$$

Invert this equation to find how X_1 varies with C, and plot it.

(Required derivation in the limits C << CMC and C > CMC

Micelle size distribution

We can go further and find the mean aggregate size using

$$\langle N \rangle = \sum N X_N / \sum X_N = \sum N X_N / C$$

and given that the total concentration $C = \sum X_N$, and $X_N = N (X_1 e^{\alpha})^N e^{-\alpha}$

$$\langle N \rangle = e^{-\alpha} / C \sum N^2 (X_1 e^{\alpha})^N$$

To solve this we need the following results (note that the sums start at N = 1 not 0):

$$\sum_{N=1}^{\infty} x^N = x / (1 - x)$$

$$\sum_{N=1}^{\infty} N x^N = x / (1 - x)^2$$

$$\sum_{N=1}^{\infty} N^2 x^N = x (1 + x) / (1 - x)^3$$

(Required derivation - find <N> in the two limits where C << CMC and C > CMC)

Summary

Molecular shape propagates up to aggregate type/shape

Chemical potential of amphiphiles in aggregates determines aggregate size distribution

Cell membranes are stable against spontaneous pores unless embedded inclusions/peptides weaken their line tension

Derivation 3 due next week.

Project presentations on 29th May.