Three lectures on membranes

Composition, properties, function - *why are there so many types of lipid?*

Self-assembly of lipid membranes - *thermodynamics and amphiphile molecular shape drive membrane formation*

Membrane models on different scales - *fluid surfaces, pores and neuronal synapses*

Membranes and cellular structure

Dimension dominates behaviour

Thermodynamics and shape drives self-assembly of lipid membranes

Membranes are active cellular materials

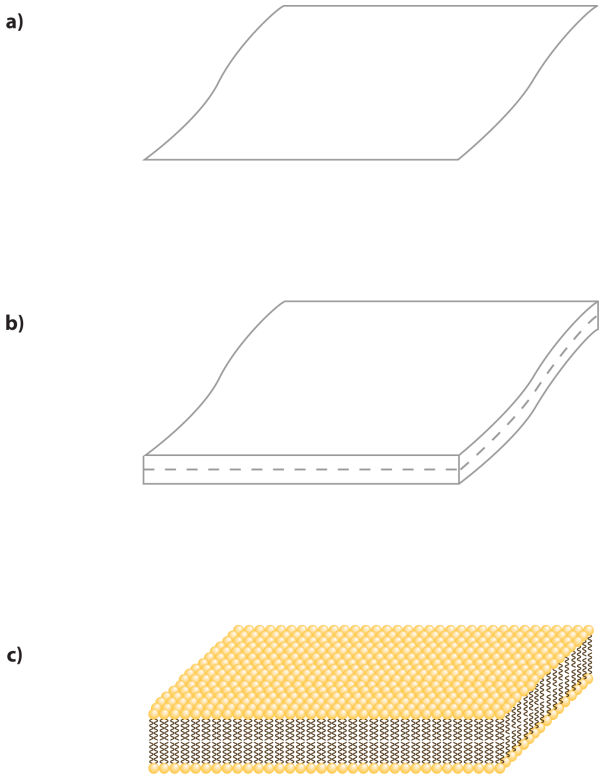
Membranes on different scales

How we describe a membrane mathematically depends on the scale of the question of interest:

Macroscopic - 2d surface, elasticity theory, shape equations, (sufficient for red blood cells), triangulated network "fish net", attach a field (e.g., lipid tilt or different lipid types) to points in the membrane, pore formation

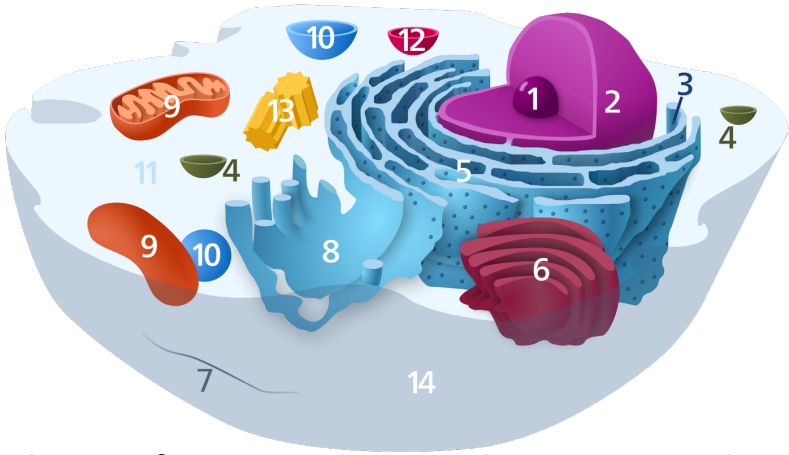
Mesoscopic - adds physical properties involving thickness (e.g. lipid "shape" lateral stress profile/protein conformational changes), geometric asymmetry but no molecular details, pore formation

Molecular - lipids, proteins, protein channel dynamics, permeation, molecular rearrangements, pore formation, fusion



Membrane properties

Lipid membranes provide the cell with:



Structure - elastic, fluid, flexible 2d surface, forms smooth spheres as well as long, thin tubules in the ER, and flattened disks in the Golgi

Stability - self-heal small pores, relieve local tension by surface tension gradients, provide excess area

Controlled permeation - prevent ions and large molecules crossing, allow water and small molecules to pass

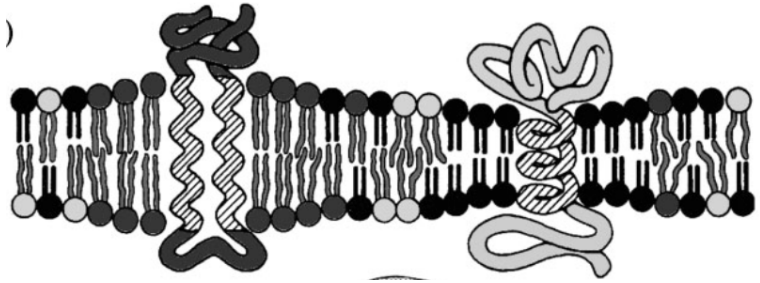
Functional protein environment - proteins need a specific mechanical and chemical environment to function

Membranes are MORE than a barrier

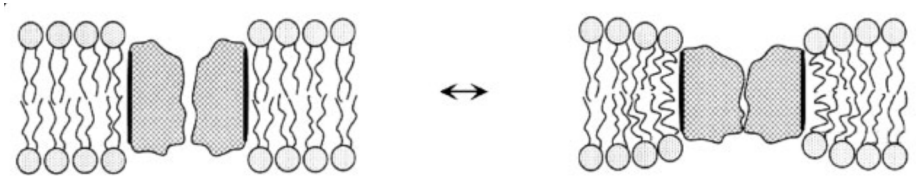
Membrane material properties

Thickness, bending modulus, stretch modulus, fluidity

Protein hydrophobic region must match the membrane **thickness** **(or pay high energy cost)**, hence they prefer to be surrounded by lipids with matching thickness



Channel proteins can have their open/closed equilibrium changed by **local membrane constituents**



Membrane **bending stiffness** (or degree of saturation of lipids) controls their shape; flexible lipids can form curved membranes, vesicles, tubes, rigid lipids form flat bilayers;

Stretch modulus is high which maintains surface area constant

Fluidity of membrane components allows cell to create/remove lipids that associate by diffusion; transient domains can form to aid signalling, tension can be relieved by lipid flow

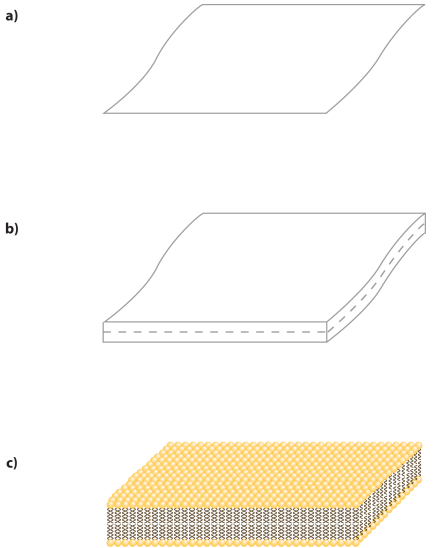
Membrane composition

Why are there so many different types of lipid in the body?

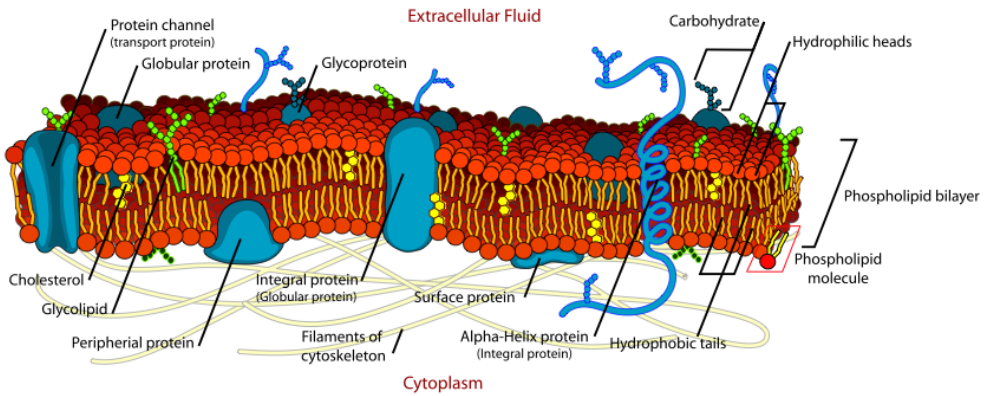
- If all nature needed was a barrier, a few lipid types would be sufficient - but there are 1000s of different types of lipid in cell membranes (cp. 4 bases in DNA and 20 amino acids in proteins)

- Membrane is a fluid so all components might diffuse freely - but different proteins need distinct environments due to thickness, tension, and their function depends on local lipid composition

- Different organs have different compositions: > 50% of neural membrane lipids are poly-unsaturated



Life as a Matter of Fat, O. Mouritsen, Frontiers Collection, (Springer 2007)

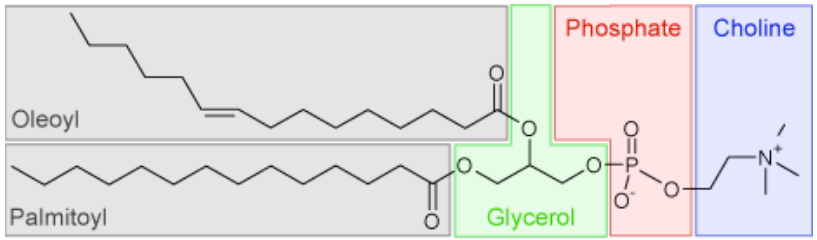


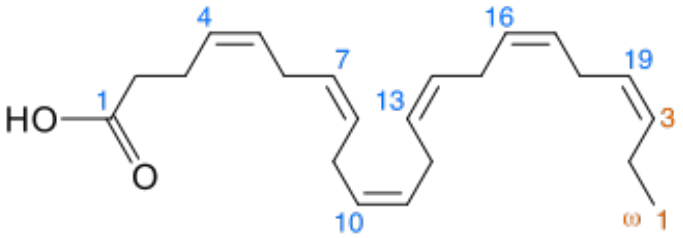
This diversity is expensive to maintain so it must have a reason

Membrane composition

How do the many types of lipid in cells differ?

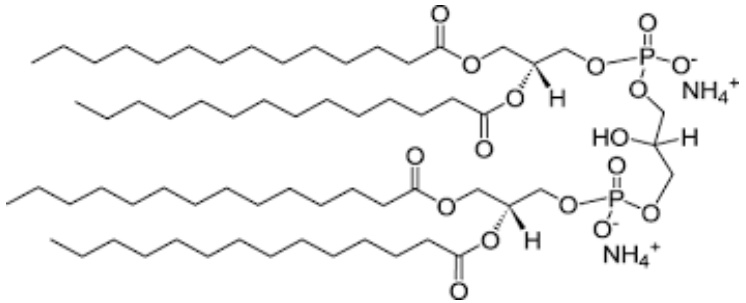
Many lipids are like DOPC below (Di-oleoyl palmitoyl phosphatidylcholine), that make bilayers and vesicles and are in the fluid phase at body temperature. Others like PE cannot form bilayers along.

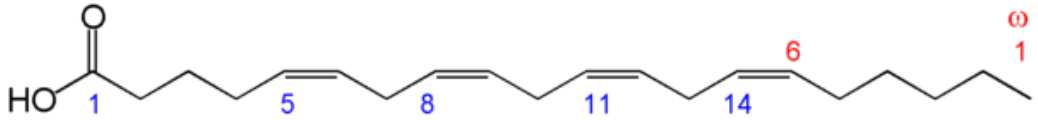




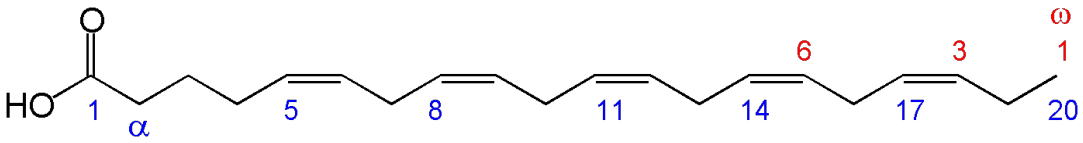
Docosahexaenoic acid in the brain - 6 double bonds, 22 C

But our bodies have more exotic lipids:





Arachidonic acid in the brain - 4 double bonds, 30 C



Cardiolipin in the heart - 4 tails

Eicosapentaenoic acid in the brain - 4 double bonds, 20 C

Most cell membranes contain substantial amounts of unsaturated lipids - especially the brain as more than half its lipids are DHA, AA, and EA which are poly-unsaturated (PUFAs)

Cholesterol

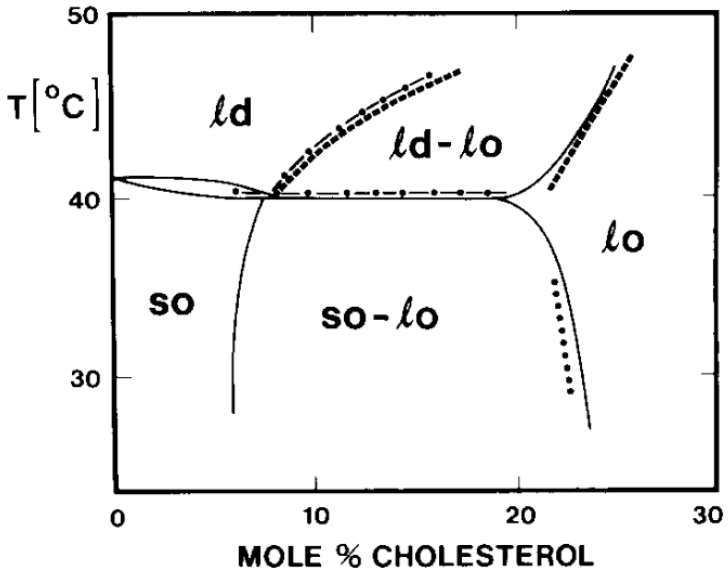
Cholesterol makes up 30-50% of typical mammalian membrane constituents. Paradoxically, adding cholesterol has two apparently contradictory effects:

makes fluid phase membranes less fluid

makes solid phase membranes more fluid

Recall lipid bilayer phases as T increases:

Gel = solid ordered (so)
Liquid ordered (lo)
Liquid disordered (ld)



J. H. Ipsen et al. Phase equilibria in the phosphatidylcholine cholesterol system, Biochim. Biophys. Acta. 905:162-172 (1987)

ld - Liquid disordered phase has high lateral mobility (large diffusion of lipids), large disorder in chains (rotations and bends), headgroups bounce up and down. The membrane thickness is decreased because of increased chain disorder - typical fluid

so - Solid ordered phase has crystalline structure, no translation, lipid tails are all trans - typical solid

What is the **lo** phase? A thermodynamic model and Monte Carlo simulations discovered it in 1987 …

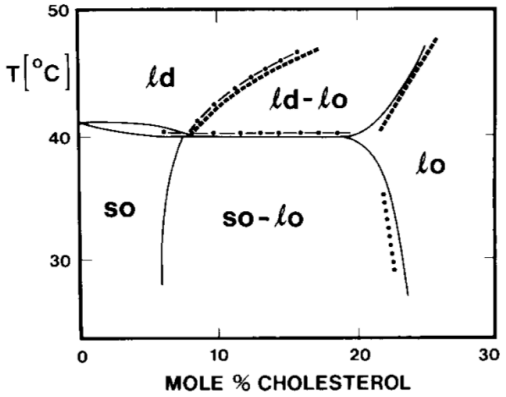


Fig. 1. Experimental phase diagram for the DPPC-cholesterol system as determined by NMR spectroscopy and differential scanning calorimetry (———) [16,17], EPR spectroscopy (- - - - - -) [10], freeze-fracture (· · · · · ·) [29], and micromechanics (· – · – ·) (Needham, D. and Evans, E., unpublished data). Note that the NMR and calorimetry studies were carried out on d_{62}-DPPC for which T_m is about 38° C. The experimental data have been scaled accordingly to facilitate the comparison within a single figure.

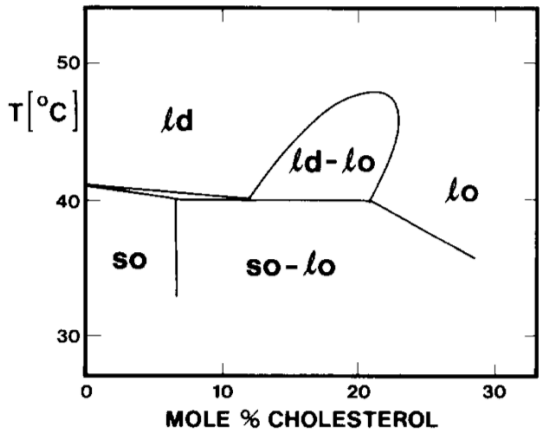


Fig. 3. Phase diagram for the thermodynamic model of the phosphatidylcholine-cholesterol system allowing for solid and liquid phases. The model parameters used are the same as in Fig. 2. $\mu_L^\theta = -1.3307$ kJ/mol and $\mu_C^\theta = 3.9322$ kJ/mol.

J. H. Ipsen et al. Phase equilibria in the phosphatidylcholine cholesterol system, Biochim. Biophys. Acta. 905:162-172 (1987)

- impurities usually lower freezing point of solids; cholesterol doesn't
- cholesterol greatly stabilises LO phase
- thermodynamic model reproduced phase diagram with assumption that translational dof *decouple* from tail conformational dof
- cholesterol separates these two type of disorder; although lipid + chol is a binary system, it behaves like a ternary one because of tail order/disorder

SO = 2d solid + ordered tails phase (solid)
LD = 2d fluid + disordered tails phase (fluid)
LO = 2d fluid but ordered tails (all trans conformations), fast diffusion but low area compressibility (part fluid part ordered)

The LO phase is fundamental to the "lipid raft" concept

K. Simons and E. Ikonen, Nature 387:569-572 (1997)

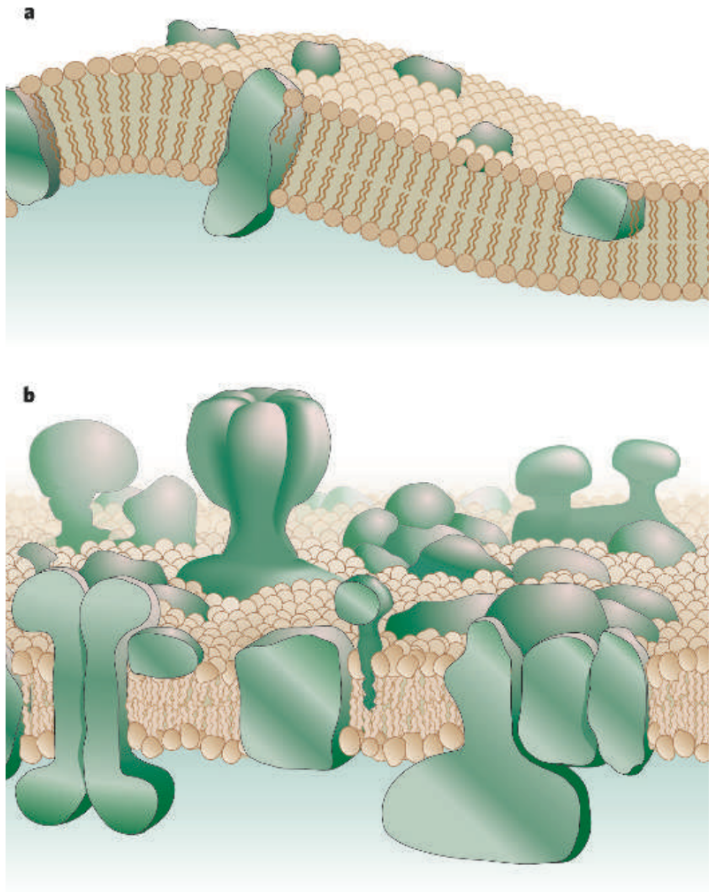
Proteins

Proteins are a large fraction of membrane constituents.

In E. Coli, for example, there are > 1000 distinct trans-membrane helical proteins.

In general, 20-30% of all genes code for trans-membrane proteins and the fraction increases with genome size (bacteria, C. Elegans, humans,)

Genome-wide analysis of integral membrane proteins from eubacterial, archaean, and eukaryotic organisms, Protein Science 7:1029-1038 (1998)



Engelman DM Membranes are more mosaic than fluid Nature 438:578-580 (2005)

Membrane function

Membrane provides a stable regulatory environment for:

Channels and pores - selective permeability, gating open/closed, blockers

Receptors - self-heals small pores, relieves local tension by surface tension gradients

G- Protein - coupled to channels and receptors to transduce signals

Transmembrane proteins are affected by:

membrane thickness

fluidity or diffusion rates

rigidity or order/disorder of the lipids

trans-membrane lateral tension or pressure profile

Diversity implies complex *organization* at the molecular scale: **structural** (material properties, phase stability, formation/dissolution, e.g., cell division) and **dynamic** (transient domains around proteins, binding/unbinding of ligand/receptors, pore formation and vesicle fusion, etc.)

Specific lipid interactions in membranes

In Lecture 12, we will look at the thermodynamics of amphiphile self-assembly and how it is driven by the free energy difference between the molecules in solution and in aggregates of different size.

But what is the origin of this free energy difference for phospholipids?

It depends on molecular details (headgroup charge, H-bonds, tail saturation, …) the environment (pH, ion concentrations), and the shape of aggregate (flat bilayer, spherical vesicle, micelle, rod, …), but can be simplified to:

- Headgroup properties
- Tail properties

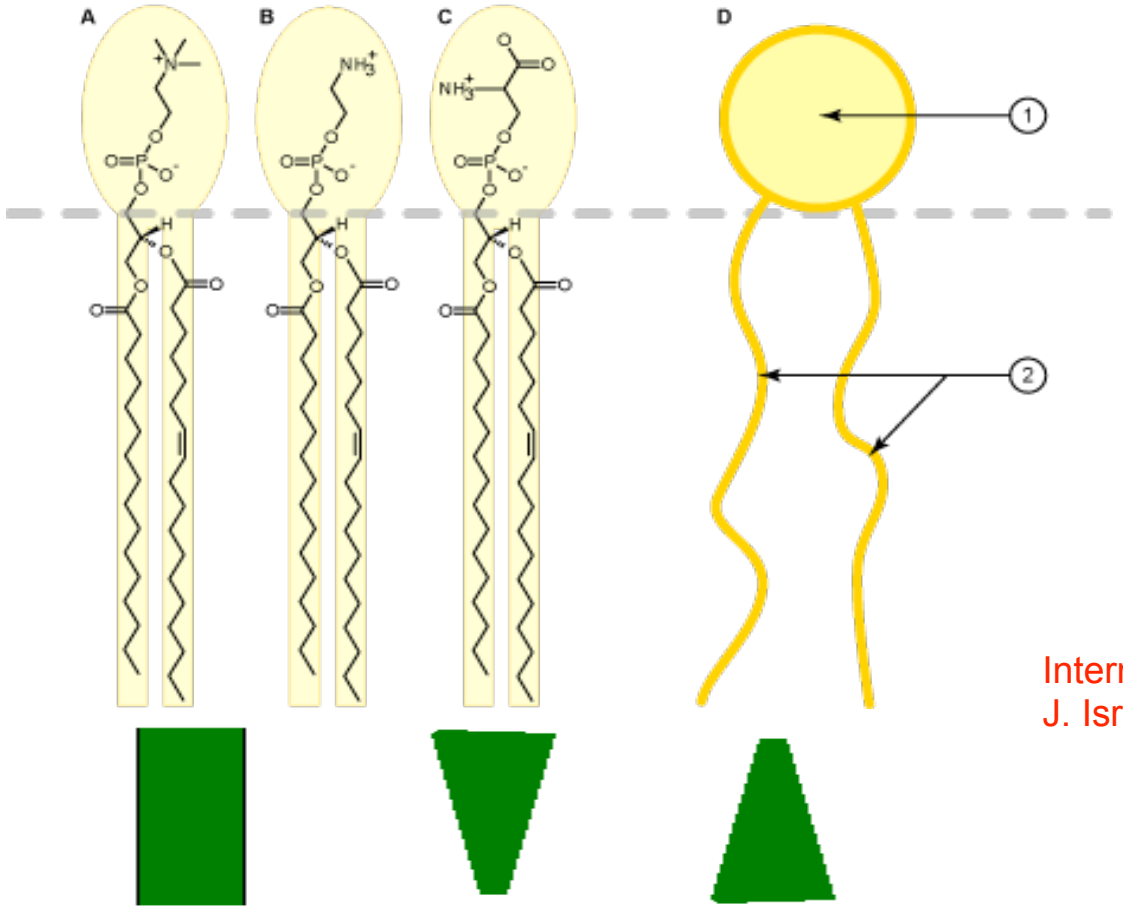
If we find a relation between the lipids' chemical potential and their aggregate size, i.e., μ_N , we can lump all the specific molecular details into a few parameters, and then use thermodynamics to tell us what any molecule with these properties will do (using results from next week's lecture).

Analogy with Newton's 2nd law: F = ma

Once you know F, you can integrate to get the trajectory and this is the same for all masses m; but F itself arises from details of the interacting objects.

Lipids have a shape

If the lipid headgroup has the same "size" as the tails the molecule is like a cylinder; if the head or tail has a smaller volume, the molecule is like a cone. This shape strongly influences their behaviour.



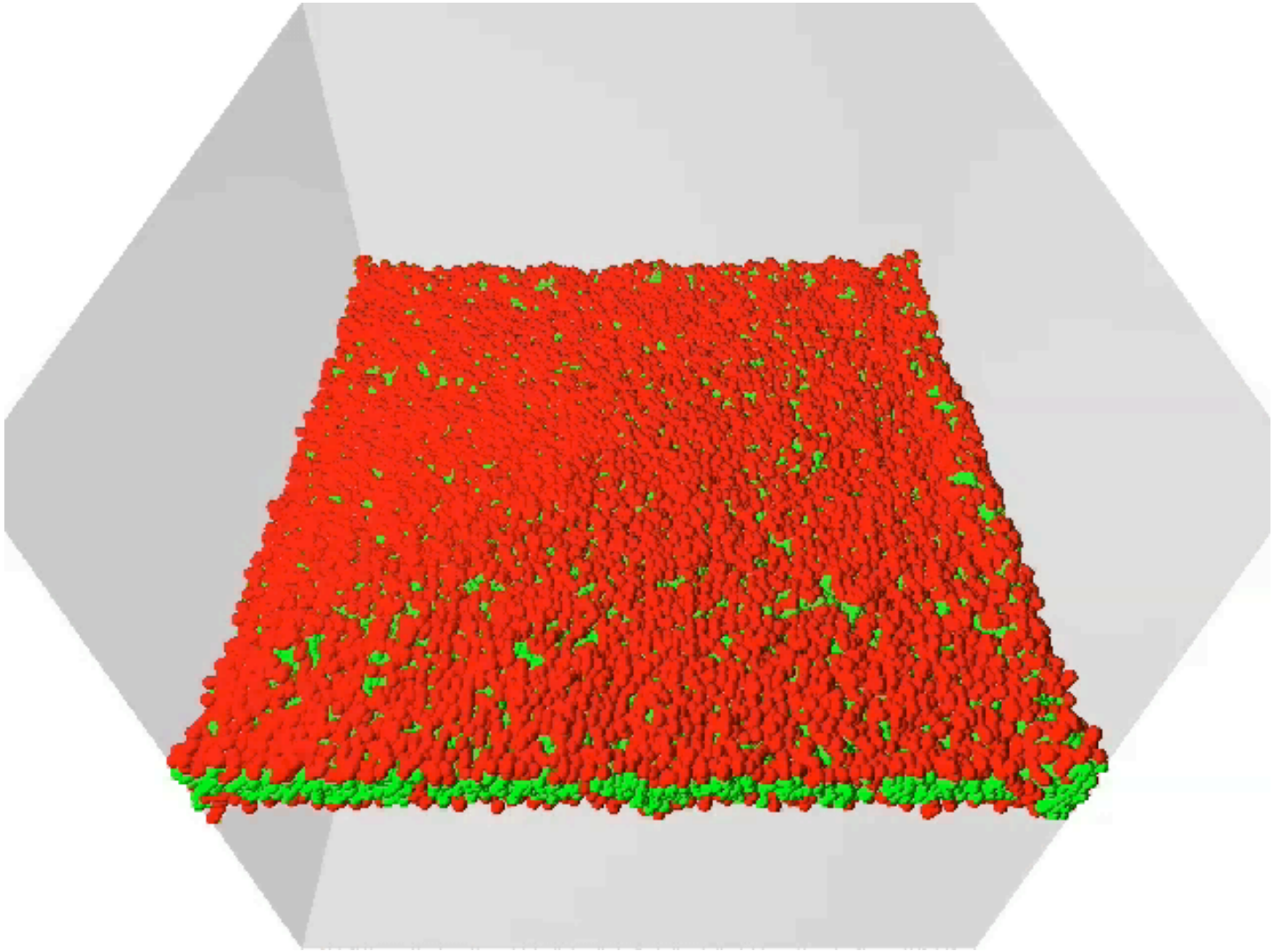
Packing parameter ~ v/a_0L_c

v = lipid volume
a_0 = headgroup area
L_c = tail length

Intermolecular and Surface Forces, 2nd edn.
J. Israelachvili, Academic Press, London, 1992.

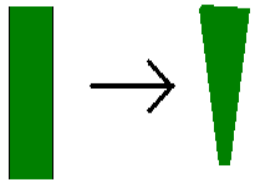
If v/a_0L_c ~ 1 planar bilayers; < 1 curved; < 1/3 micelles; > 1 inverted micelles

DPD is useful for lipid shape effects …



Initially-tensionless membrane
5538 lipids
40 nm x 40 nm

$C_0 = 0 \rightarrow > 0$

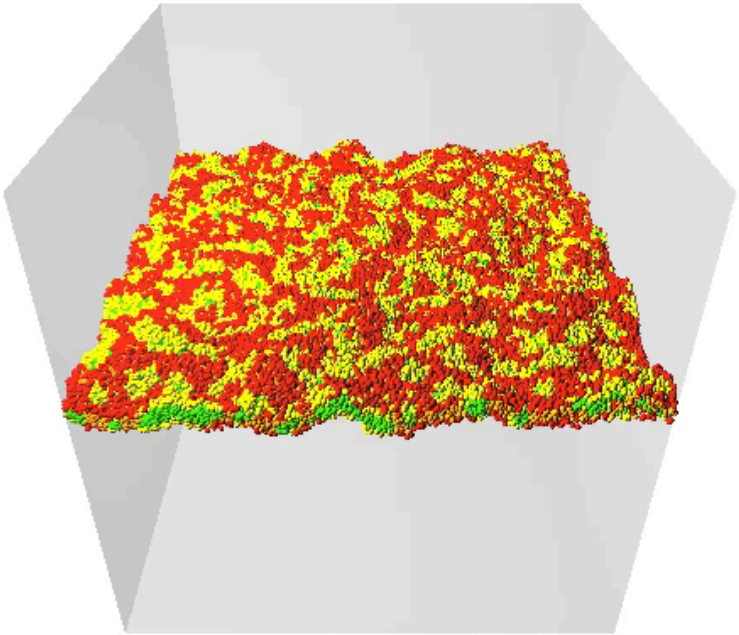


NB Water is invisible

Illya, G, Lipowsky, R, Shillcock JC, J. Chem. Phys. 122:244901 (2005)

… and multicomponent membranes

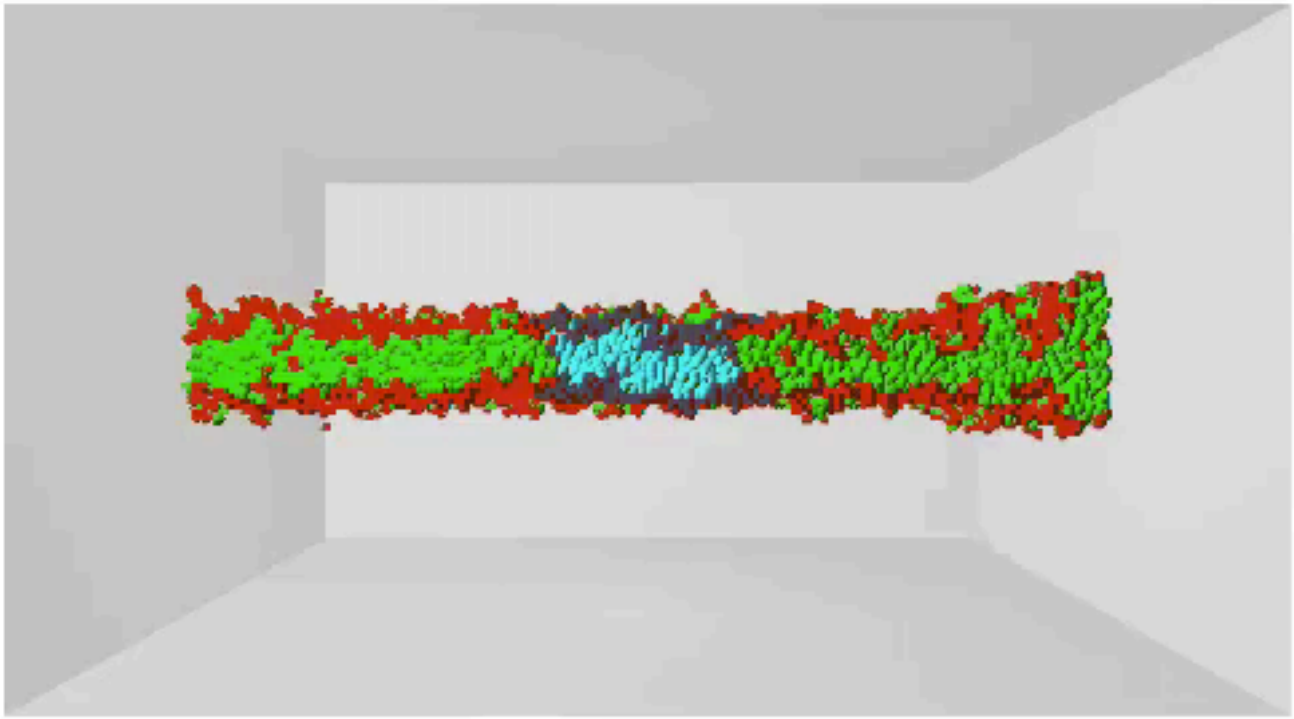
Vesicles self-assemble when lipids are immersed in water, but the vesicle membrane is itself a fluid - a two-dimensional fluid. It too can support self-assembly within it.



Domains form when the two lipid species don't want to pack together, (i.e., there is a line tension around the domain the makes the interface cost energy proportional to its length) but now more complex processes can occur - budding - in which the domain forms a small vesicle and pinches off.

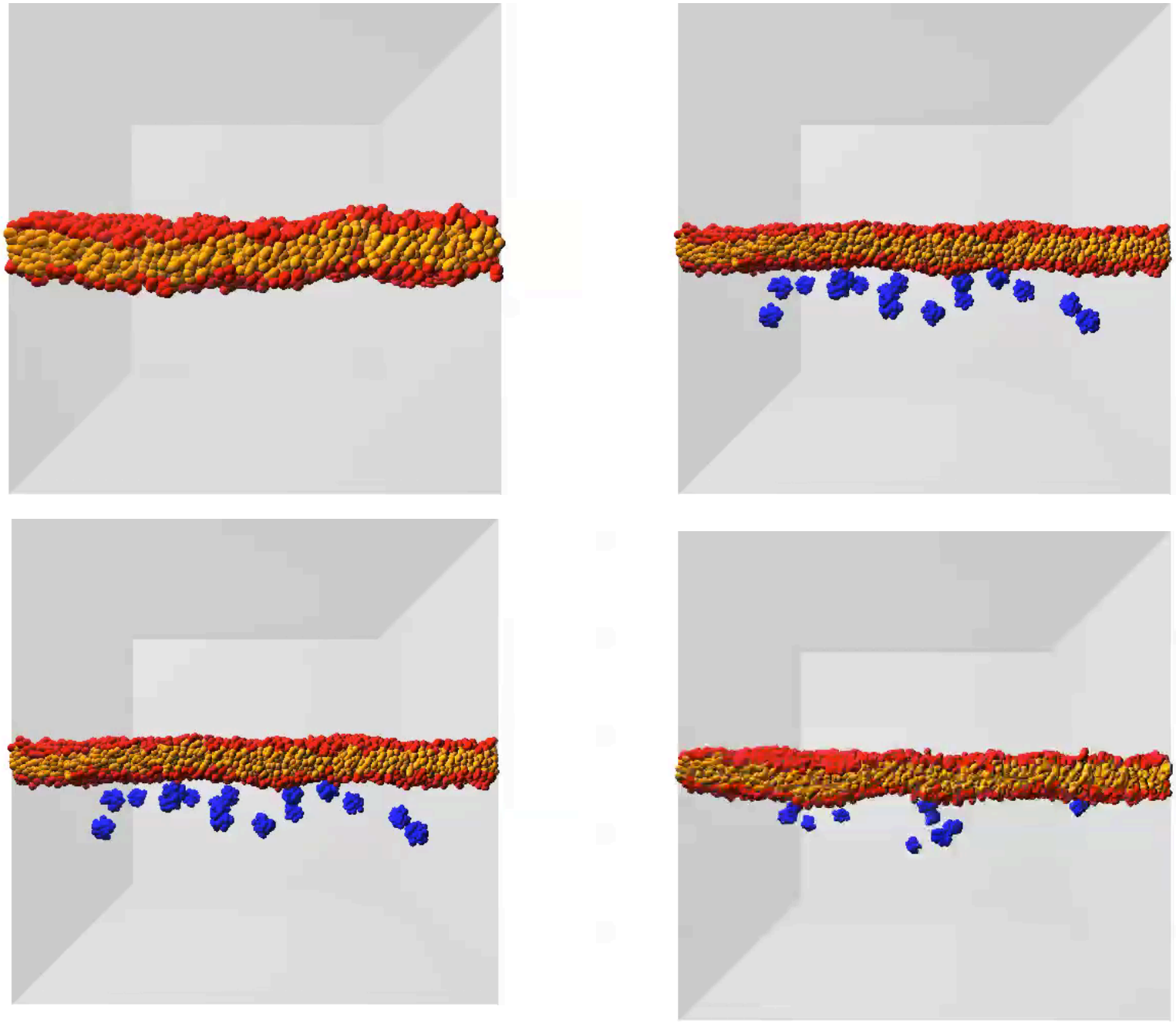
Illya, G, Lipowsky, R, Shillcock JC, J. Chem. Phys. 125:114710 (2006)

… and nanoparticle interactions with membranes



Line tension + curvature energy of membrane/NP drives translocation

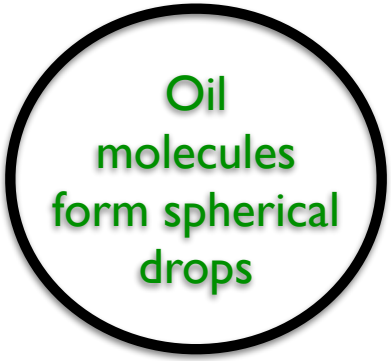
Size-dependent entropic aggregation



Opposing forces between lipids in membranes

Oil molecules separate into an infinite phase because their chemical potential in an aggregate of size N decreases monotonically with N.

Oil/hydrocarbon ~ tiny spheres ⇒ spherical droplets (what else could they do?)

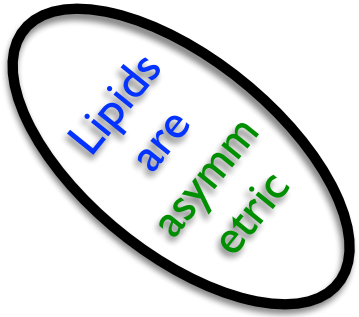


Lipids are asymmetric (hence - *amphiphiles*) ~ cannot form spheres

We try to lump all the complexity of the molecular interactions into two broad categories:

Headgroup properties - typically repulsive due to:

steric repulsion
charge or dipole interactions
mobility (protrusion from membrane)



Hydrocarbon tail properties - typically attractive due to:

hydrophobic repulsion from water
high flexibility/many conformational states within membrane interior

Opposing forces in lipid aggregates

From the assumption that lipid-lipid interactions result from *opposing forces* we can predict the direct interaction part of their chemical potential, μ_N^0:

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

To a first approximation we assume the lipid molecular volume is constant and their direct interactions scale with the area per lipid as:

Headgroup steric repulsion ~ 1 / a

Tail hydrophobic repulsion from water ~ a

This ignores some small effects (direct binding between headgroups, ions/H-bonds, non-ideal tails, curvature of aggregate).

Then

$$\mu_N^0(a) \sim \gamma a + K / a$$

where γ , K are constants with γ related to the surface tension but K has no simple interpretation.

This leads to a minimum preferred area for lipids in an aggregate.

Opposing forces in lipid aggregates

Minimising wrt headgroup area gives the equilibrium value:

$$a_0 = \sqrt{(K / \gamma)}$$

Substituting back into the expression for μ(a) gives a quadratic expression for deviations of the headgroup area around its minimum value:

$$\mu^0_N(a) = 2 \gamma a_0 + (\gamma / a)(a - a_0)^2$$

and this means that aggregates will grow until they reach a size such that the lipids have an area per molecule near this value as this minimizes their free energy.

Smaller micelles/vesicles will be highly curved (exposing the lipid tails to water) while bigger ones will force the headgroups closer together to make the tails fit into the fixed volume (that depends mainly on tail length). Both raise the free energy.

What is this preferred size?

Come back next week …

Fatty acids and lipids

Lipids are made from precursor fatty acids that are also converted into hormones.

Animals get essential polyunsaturated fatty acids (PUFAs) in their diet as they cannot synthesize them all, e.g., animals can add a double bond at C_9 but only plants can add them at C_{12} and C_{15}. For humans, the essential PUFAs are:

Linoleic acid - C18:2n-6, 18 carbon chain with 2 double bonds starting at C_6 from the end

α-linolenic acid - C18:3n-3 18 carbon chain with 3 double bonds starting at C_3 from the end

Linoleic acid (n-6 family) ⇍⇏ **α-linolenic acid (n-3 family)**

Arachidonic acid (AA)

Eicosapenteanoic acid (EPA)

Docosahexaenoic acid (DHA)

These families cannot efficiently be interconverted, but can be (expensively) made in the liver from the precursors, but it is less costly to eat them.

AA, Linoleic acid is found in seeds, corn, soybean; linolenic acid only in green plants and algae.

DHA is found in algae, eggs, meat, cold-water fish and shell fish that eat algae.

Polyunsaturated lipids in the brain

60% of dry weight of brain is lipids, and 50% of this fraction is polyunsaturated lipids with roughly equal amounts of AA and DHA.

Mammals, reptiles and fish have different lipids in their muscles, livers, other organs but their brain lipids are similar - only long-chain, super-unsaturated fatty acids.

Where do these exotic lipids come from? Fish - 50% of all fatty acids in salmon are DHA, while it is only 0.2% in cows.

Michael Crawford's hypothesis:

Human brain mass and brain/ body ratio are high

PUFA composition of human brain is high

This can only happen where DHA and AA are plentiful, i.e., near the sea or eating brain/tissue of other animals

Why aren't fish smart? They have only the DHA present in egg, but mammals have a continuous supply from mother during gestation and breast milk.

Animal	Brain/Body ratio (%)
Mouse	2
Chimp	0.5
Gorilla	0.25
Cow	0.1
Human	2.1
Dolphin	1.5

Why polyunsaturated lipids in the brain?

1) DHA may provide membrane conditions optimal for trans-membrane G-proteins and the membrane fragility needed for plasticity in neuron membranes, as well as electrical properties. DHA is a non-lamellar forming lipid (Meyer Bloom)

2) Connectivity is the key factor in human brain growth - how easy is to for synapses to grow/ shrink? (David Horobin's hypothesis)

In the embryo, tight control of the growth/shrinkage of new synapses is crucial, which requires tight regulation of the production of AA and DHA in the growth zones. This requires enzymes (acyl-transferases, phospholipase A2 and C, etc), lipoproteins that transport/cleave fatty acids at synapses.

If AA, DHA increase due to excess phospholipase activity ⇒ manic depression

AA, DHA decrease due to reduced acyl-transferases ⇒ schizophrenia

Schizophrenia arises with the appearance of the biochemical machinery that produces the connectivity and plasticity of our brains: schizophrenia is the illness that makes us human.

Only then (50 - 200, 000 years ago) could H. Sapiens evolve.

Myer Bloom, Evolution of membranes from a physics perspective, in In Search of a New Biomembrane Model, OG. Mouritsen and O. S. Andersen eds.) Biol. Skr. Dan. Vid. Selsk. 49:13-17 (1998)

Summary

Lipid membranes are more than a barrier

Many aspects of membranes are still unknown:
- why so many lipid types?
- how do lipids affect membrane proteins?
- role of PUFAs?

Membrane composition is actively tuned for function