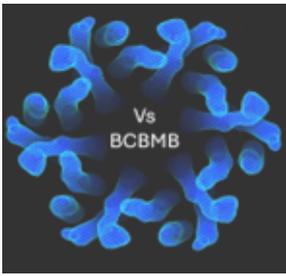




Biochemistry Fundamentals

LIPIDS

Goal Of this Unit



You will get the greatest gain from this short primer on lipid biochemistry if you looked over the following handouts (all free downloads):

- From Cell Biology to Social Media, the Internet and More (Page 17 most relevant)
- What Do You See?
- How Do Molecules See Part 1 & 2

Building on what is covered in the handouts mentioned above, the handouts about "Biochemistry – Fundamentals" will look at the chemical inventory of living systems from an “engineering“ point of view because that strategy will help you to understand **why** each class of biological macromolecules is uniquely suited for fulfilling certain roles.

Where to start?

From the first handout listed above, you learned that “life“ depends on partitioning of matter across boundaries → the most logical first “pit stop“ in exploring the chemical basis of "life" will be to explore “boundary formation“

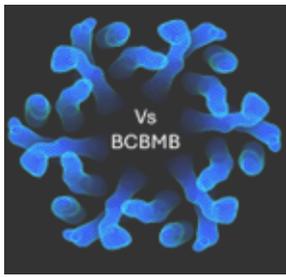
To do that, we want to find first levels answers to the following questions (***test your knowledge by trying to answer these questions before looking at the next slide!***):

What types of molecules can form boundaries?

Are all boundaries alike?

What causes these boundaries to form?

Goal Of this Unit



Did you get it right? Here are short answers

What types of molecules can form boundaries?

- Stereotypical answer: lipids ... but carbohydrates and nucleic acid:protein aggregates can form boundaries....and those boundaries are essential parts of life as a whole.

Are all boundaries alike?

- Absolutely not ... we will explore differences as we go, but a handwaiving big picture answer is "flexible/self-sealing" vs "rigid"

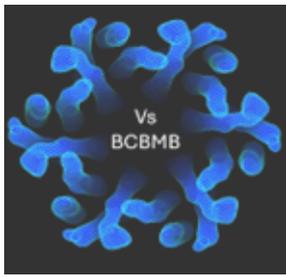
What causes these boundaries to form?

- Depends on the boundary lipids = hydrophobic effect causes spontaneous aggregation; carbohydrates – weak interactions between polymer chains; nucleic acid:protein aggregates create boundaries by weak interaction driven phase separation (more commonly known as "droplet formation")

For reasons you will understand later, this first handout will focus on water and lipids – because both play key roles in formation of some types of boundaries.

You will learn and should be able to explain

- **Why** unbiased observation is key for understanding molecular behaviors
- **Why** water is key for boundary formation by lipids (we'll find many other reasons for why water is the key for many things)
- **That** not all lipid-based boundaries are membranes
- **That** the double layered nature of biological membranes is a consequence of the structure of the lipids that form them



Water



Lets revisit this image (from the handout "What Do You See?" ...first 5 pages), but this time we want to dive into interpretations, instead of tackling "data blindness"

Blue: you know those are oceans

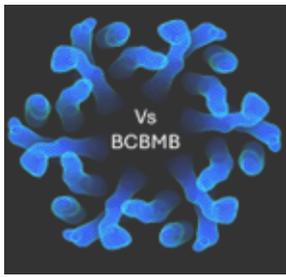
White: you know those are clouds + ice

What do oceans, clouds, and ice have in common?

ALL are based on the same chemical – water - but in different physical states: **liquid, aerosol, solid**

What about the color? How can the **same** thing have different colors?

Explaining the **colors** crosses over into physics: **scattering** (small water droplets = clouds scatter all wavelengths → see “white”) and **absorption** (larger volumes of liquid water absorb the longer wavelengths → blue is backscattered and dominates) lets not get too distracted by that....

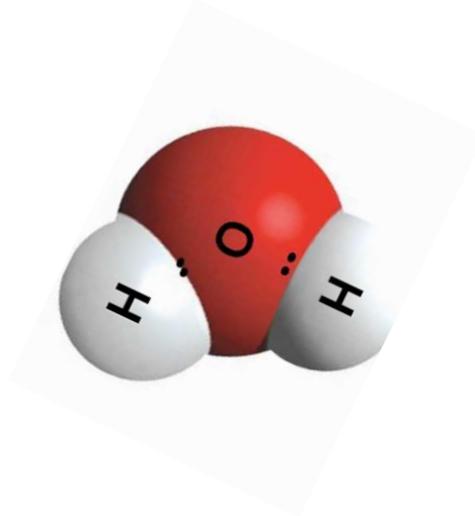


Water



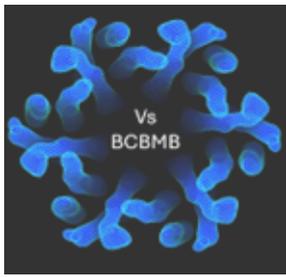
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We want to focus on the **structure of water**, how the structure affects water properties, and how the properties of water are the most **fundamental** aspect of chemistry that made **life** possible (as we know it on Earth).



What Do you See?

...try answering before looking at the answer on the next slide.....

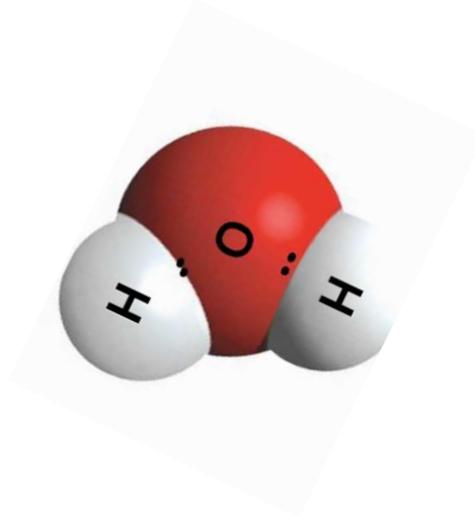


Water



Explaining the **colors** crosses over into physics: **scattering** (small water droplets = clouds scatter all wavelengths → see “white”) and **absorption** (larger volumes of liquid water absorb the longer wavelengths → blue is backscattered and dominates) ... lets not get too distracted by that....

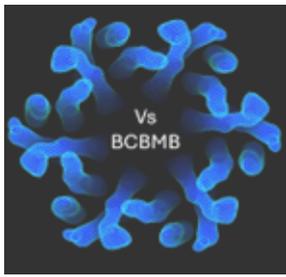
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What Do you See?

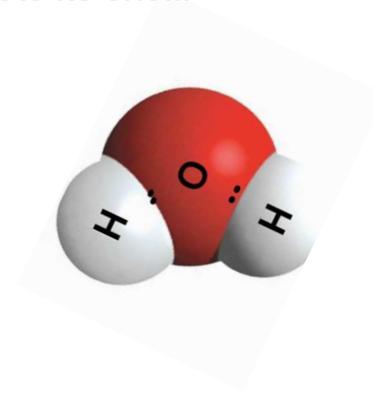
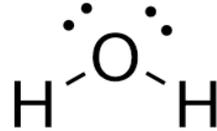
One doxygen, bonded to two hydrogen bonds through single σ -bonds that form an angle of $\sim 100\text{-}110^\circ$, leaving two lone pairs of electrons at the oxygen to complete its shell.

Water - Structure

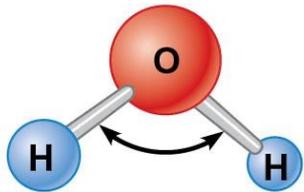


What are the **most relevant** pieces of information in that answer?

One oxygen, bonded to two hydrogen bonds through single σ -bonds that form an **angle of $\sim 100-110^\circ$** , leaving **two lone pairs** of electrons at the oxygen to complete its shell.



σ -bondsangle of $\sim 100-110^\circ$, leaving two lone pairs.....**what is the significance of these two pieces of information?**



σ -bonds: relevant to us are s+s, s+p orbital-combinations

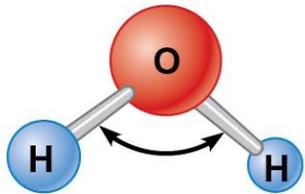
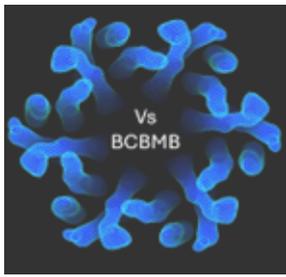
s orbitals are spherical, p-orbitals are dumbbell-shaped and at 90° angles

→ the bond angle of $\sim 100-110^\circ$ isn't compatible with basic orbital bonding geometry but suggests a tetrahedral configuration (ideal angle: 109.5°)

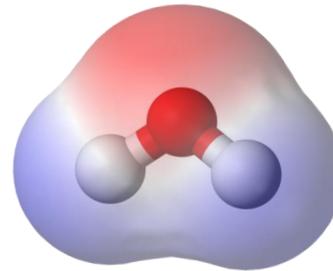
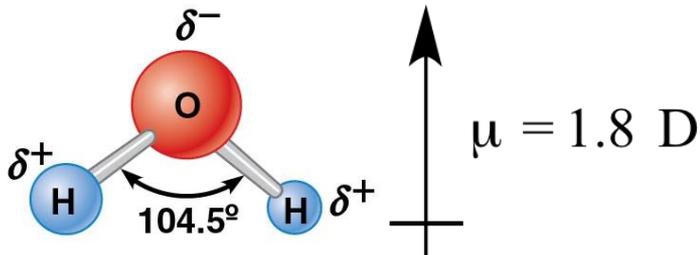
→ **oxygen (electron configuration: $2s^22p^4$ is sp^3 -hybridized when bonded to hydrogen. THIS IS A BIG DEAL! Why?**

→ **Water is not a linear molecule, it is bent**

Water - Structure



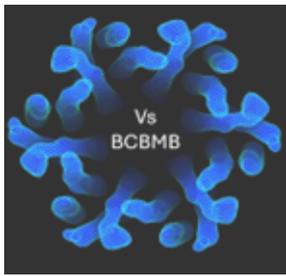
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- Water is not a linear molecule, it **is bent**



The tetrahedral geometry combined with the different electronegativities of hydrogen and oxygen means that the individual polarizations of the two σ -bonds add up to create a permanent charge asymmetry across the molecule = **water has a stable dipole.**

the angled structure
causes a permanent dipole moment

Note: the non-ideal angle of 104.5° is caused by electrostatic repulsion between the lone pairs

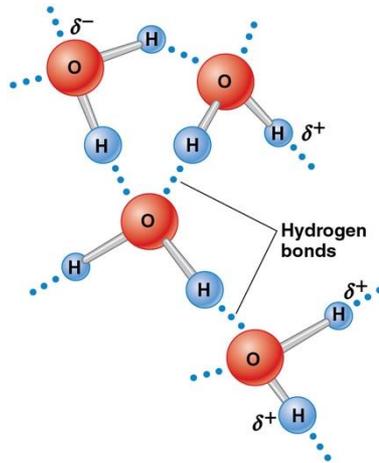


Water – Function 1



The permanent dipole and tetrahedral structure of water molecules give water an array of physicochemical properties that are **exceptionally** important for Earth chemistry and life:

1. Hydrogen bonds = weak interactions

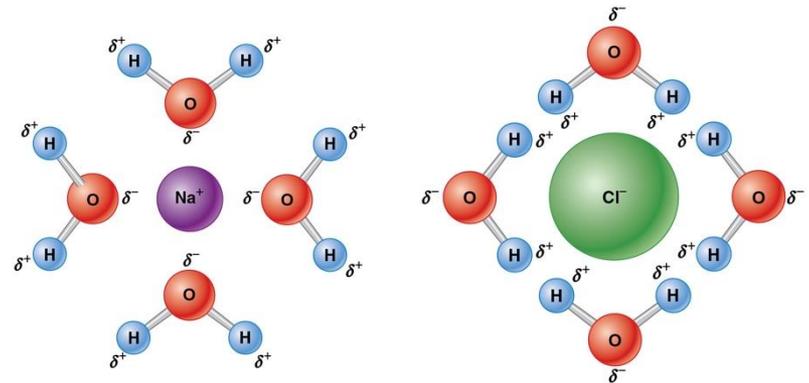


Water forms **extensive 3-dimensional H-bond networks with itself**

- **Very high melting and boiling points**
- **Large heat capacity and heat of vaporization** → water is an extremely efficient coolant for absorbing and dissipating heat (eg from metabolism)
- **High surface tension**

Water also can form **H-bonds with polar groups on other molecules** → such molecules are called hydrophilic [water loving] → water is excellent solvent for most (organic) biological macromolecules

2. Solvation of ions



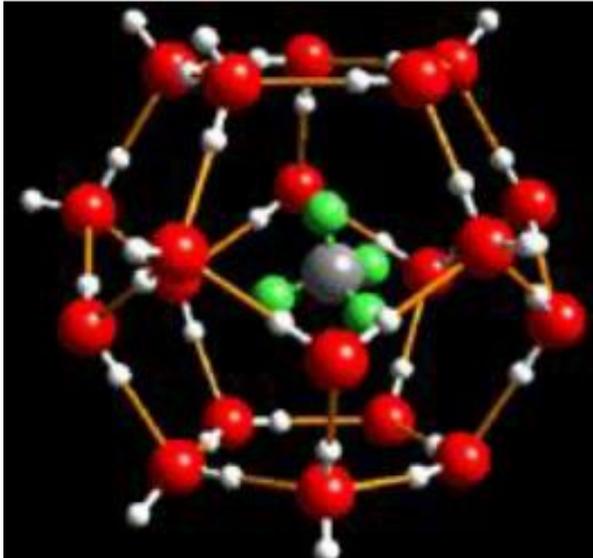
The **dipole** of the water molecule **allows it to form hydration shells around both positive and negative ions.**

- Water is an excellent solvent for many inorganic salts (eg NaCl, KCl, MgCl₂, CaCl₂).

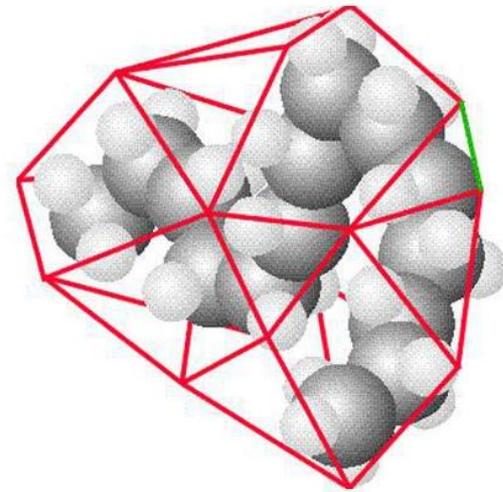
However, many inorganic compounds such as Mg₃(PO₄)₂ or CaSO₄ are largely insoluble in water [eg. Mg₃(PO₄)₂ solubility is ~1μM/liter] → can form solids in presence of water.

Water – Function 2Clathrates

The ability of water to form complex 3D networks of H-bonds, also allows water to cope with molecules, such as hydrocarbons, that are incapable of supporting polar interactions. However, the encounter with such molecules – which are called hydrophobic [water fearing] – causes water to adopt highly ordered, cage-like structures, known as **clathrates**, around the **hydrophobic molecule**



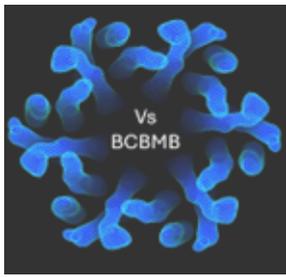
Methane (CH₄) in water



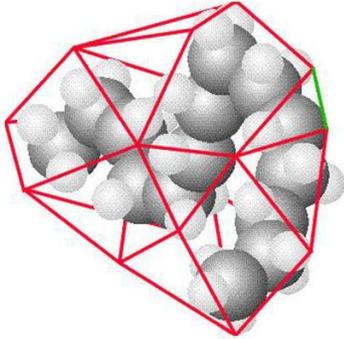
Clathrate water cage around a long chain hydrocarbon

Red lines sketch the overall structure of the cage, but does not explicitly show individual water molecules.

Water and the Hydrophobic Effect



Looking at the structure of clathrates from the perspective of how molecules “see”, recognize and select interaction partners – what does your intuition tell you about the interaction between water and hydrophobic substances? (suggestion: if you struggle to come up with an idea here, review "*How Do Molecules See Pts 1&2*")



Clathrate water cage around a long chain hydrocarbon

Answer: they tolerate each other, but will not interact if they don't have to. **Why?**

Answer: can be found in the Gibbs Free Energy Equation

$$\Delta G = \Delta H - T\Delta S$$

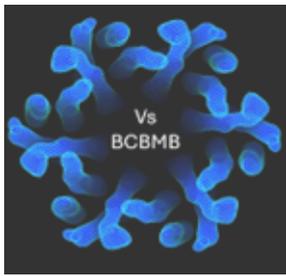
Recall: for interactions to be spontaneous $\Delta G_{\text{overall}} < 0$

Evaluating Gibbs Free Energy Equation tells you that **formation of clathrates carries a very significant entropic penalty** (= very unfavorable)

Consequence: if a large enough number of hydrophobic molecules is forced into water, these molecules will spontaneously aggregate if they encounter each other through random collision of their clathrate cages. **This tendency of hydrophobic molecules to aggregate in aqueous solution is called the hydrophobic effect and has fundamental implications for life. Why?**

Answer: aggregation means spontaneous **self-assembly into non-covalent polymeric structures** simply by “being there”. At a molecular size scale – these structures are “macroscopic” = many times the size of their constituent building blocks.

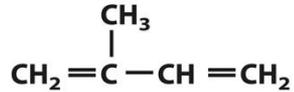
→ In other words: the hydrophobic effect/properties of water drove formation of macroscopic structures **without** a need for chemical bond formation (or an instruction manual).



Water and the **Hydrophobic Effect**



Lets explore some more what happens when you put hydrophobic molecules into water, starting with hydrocarbons that collectively are called "terpenes" (chemically derived from a small hydrocarbon called isoprene:



3-methyl-1,3-butadiene (isoprene)

Terpenoid	Structure	Commonly encountered in
Limonene		 Lemon
α-Pinene		 Pine
β-Myrcene		 Hops
Linalool		 Lavender



Step 1
Terpene gently added without perturbing water → "large "oil droplets" on water surface

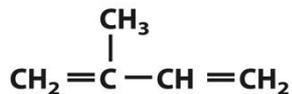
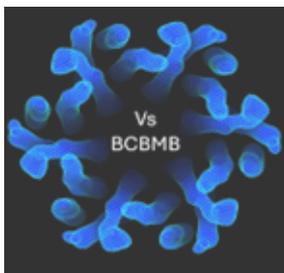


Step2: shake vigorously
"milky" appearance after shaking

Water and the **Hydrophobic Effect**

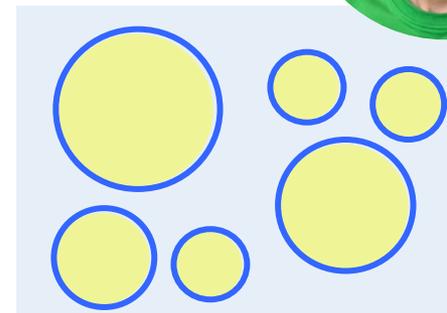
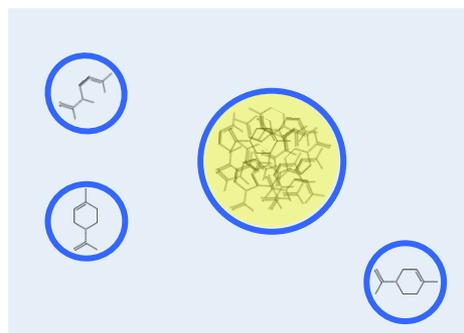
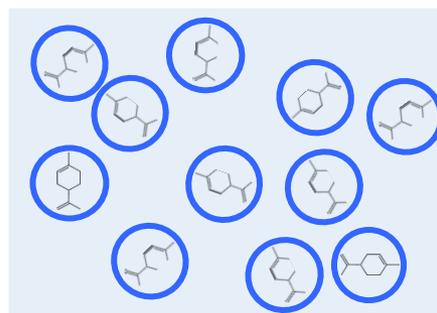


How can you explain the "milky" appearance of the solution after shaking?



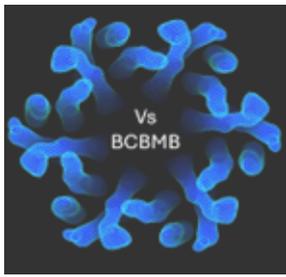
3-methyl-1,3-butadiene (isoprene)

Terpenoid	Structure	Commonly encountered in
Limonene		Lemon
α -Pinene		Pine
β -Myrcene		Hops
Linalool		Lavender



Top left: Solvated monomer
 → increase [concentration]
 → phase separation by fusion of clathrate cages [blue circles]
 → fully formed emulsion with droplets of different sizes (light scattering by the droplets makes it look milky/opaque)

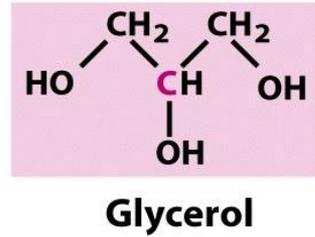
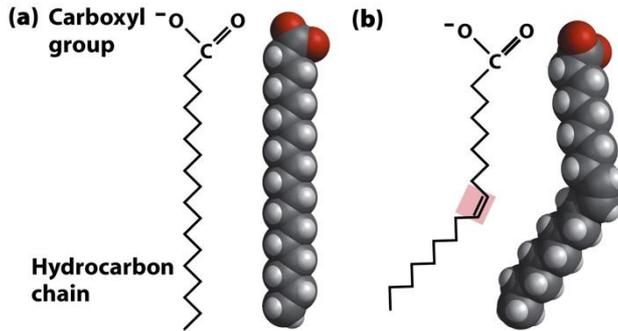
Emulsion: a mixture of two liquids that are mutually immiscible



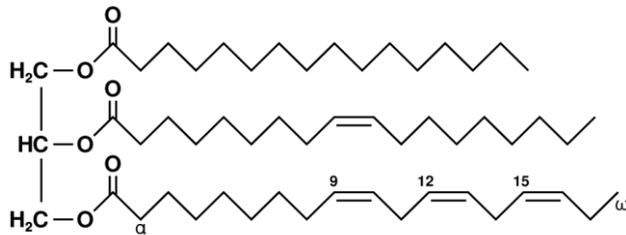
Water and the Hydrophobic Effect



Next: lets consider a different type of hydrophobic molecule that is made from three long chain fatty acids and glycerol



Recall some basic Orgo:
 Alcohol + Acid \rightleftharpoons Ester + H_2O
 (condensation that, in this case, eliminates water)

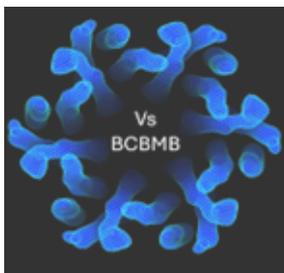


What do you see? Any difference compared to terpenes? If so – what do you expect to happen when this meets water?Lets find out

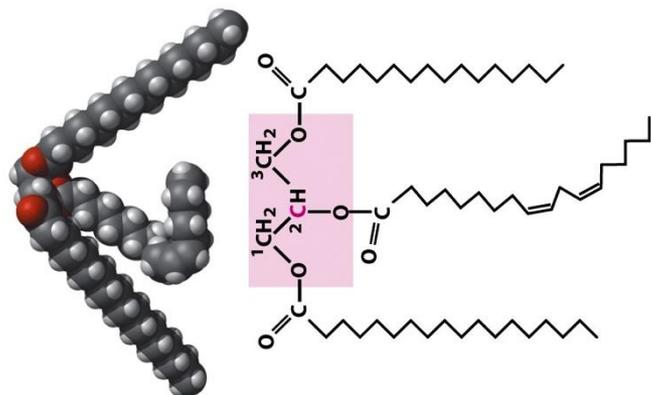
By Wolfgang Schaefer - author, Public Domain,
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triacylglycerol

Water and the **Hydrophobic Effect**



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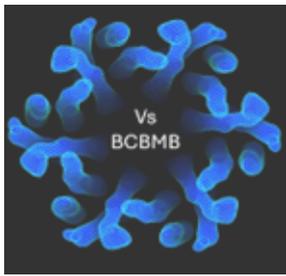
triacylglycerol
(olive oil in this case)



before



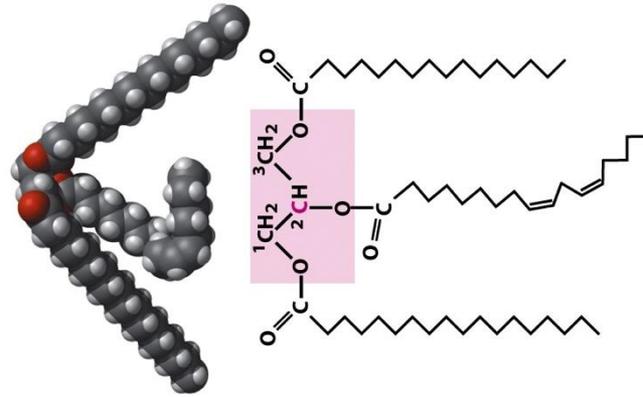
after shaking



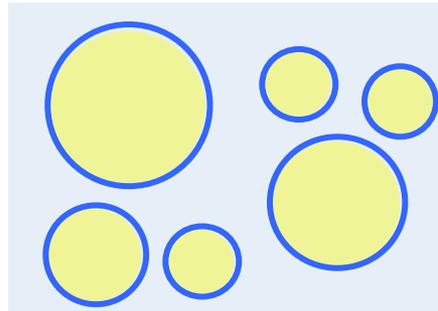
Water and the **Hydrophobic Effect**



Conclusion – Triacylglycerol in Water



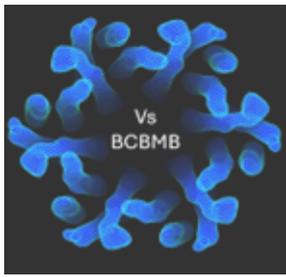
triacylglycerol



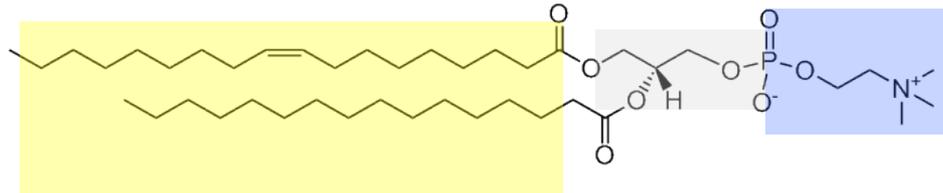
Triacylglycerols also form emulsions because the two carbonyl O-atoms are not sufficient to stably engage water
→ Forces clathrate formation → will phase separate if the concentration of triacylglycerol gets above a critical threshold

In biology, emulsions of triacylglycerols are exploited for energy storage in adipose tissue (fat cells)

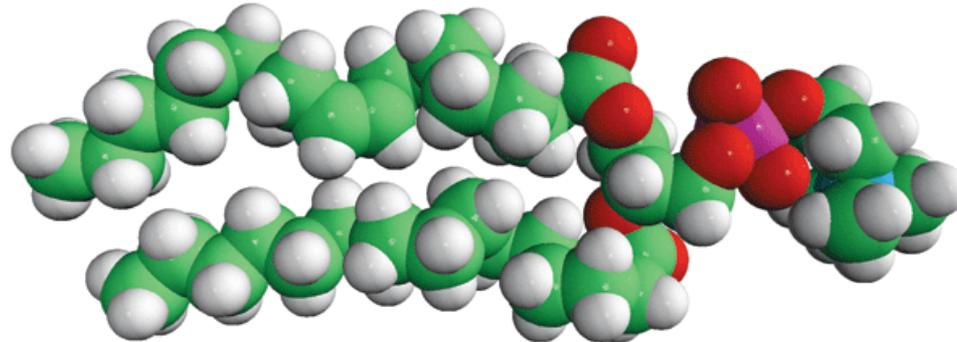
Water and the **Hydrophobic Effect**



Lets try a third one that belongs to the family of phosphatidylcholines



source:
avantilipids.com

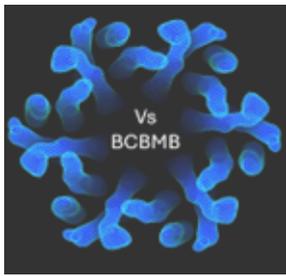


What do you see?

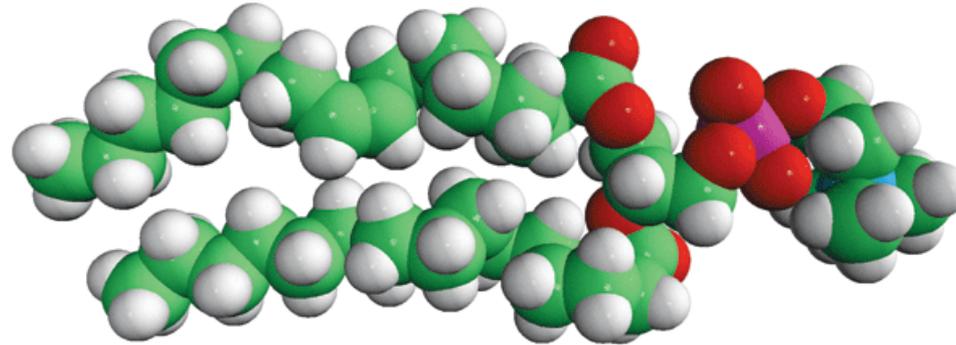
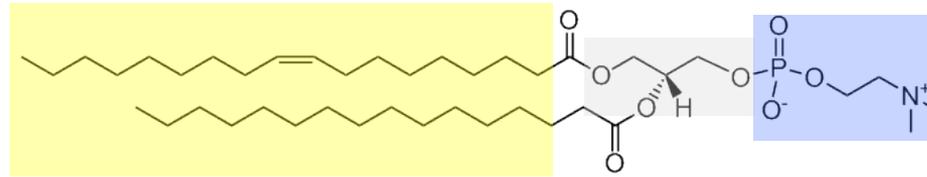
....try to answer yourself first before looking at next slide

How will this molecule behave if you put it into water?

....try to answer yourself first before looking at next slide



Water and the **Hydrophobic Effect**



source:
avantilipids.com

What do you see?

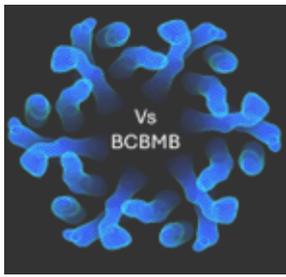
Answer: molecule that is similar to triacylglycerol, but with one of the fatty acid chains replaced by a small substituent that has both negative and positive charge (phosphate and quaternary amine) = no net charge

How will this molecule behave if you put it into water?

Answer: this likely is going to be more complicated because this molecule has conflicting chemical properties

- Fatty acid acyl chains = hydrophobic = clathrate → wants to phase separate in water
- Exposed ester bonds, phosphate, quaternary amine = hydrophilic = this loves water! It doesn't want to phase separate and certainly doesn't want to go into a purely hydrophobic phase

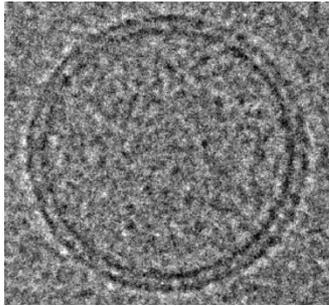
Molecules that combine **spatially separated** hydrophobic and hydrophilic components are called amphiphiles [=love both ...water and grease]



Water and the **Hydrophobic Effect**



With the coarse data collected ...lets now move on and interpret them:



Interpretation:

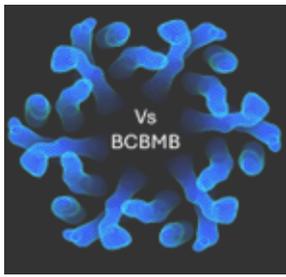
- *grayscale, speckled*: hard to interpret without any context
- *two-dimensional projection of a 3D-object*: means this is like an “X-ray”
- *in water*: you don't obscure anything by an “electron dense cover” (like you would if you were to take a chest X-ray wearing a lead vest).

→ **Most prominent features are two dark concentric circles**: here it starts to get more interesting because there really is only one 3D shape that will appear like a circle when seen/projected from any direction → **has to be some a sphere.**

→ If it is the projection of a sphere –

- will it be **two “thin walled spheres”** that are inside of each other?
- **or** will it be a **single sphere whose wall thickness corresponds with the spacing between the lines** and whose surface regions appear more dense on either side of the wall?

→ **How can you distinguish between these two possibilities?**



Water and the **Hydrophobic Effect**

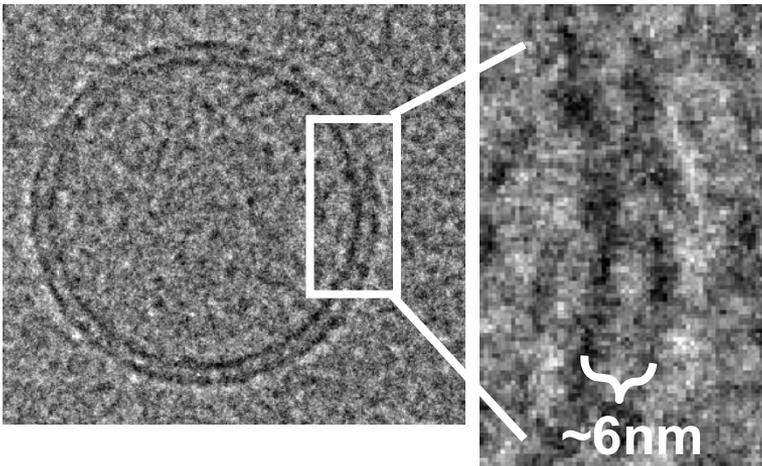


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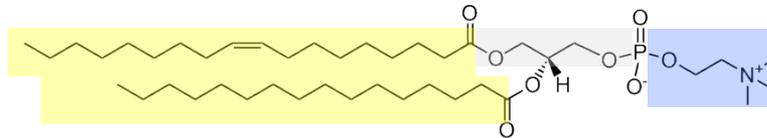
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→ **How can you distinguish between these two possibilities?**

Answer: this requires you to look closer and to extract some quantitative data + circle back to the molecules that make these structures + put some basic knowledge/reasoning to work.....

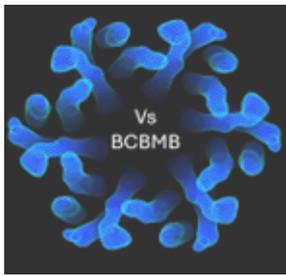


18 C-C bonds * 0.15nm/bond + C-H bond = ~2.9nm
 16 C-C bonds * 0.15 = ~2.5nm (short chain)

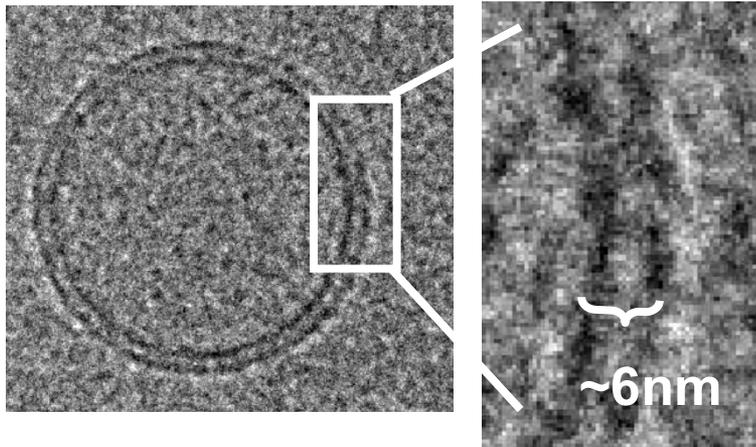


6 bonds (CO to P): ~0.9nm

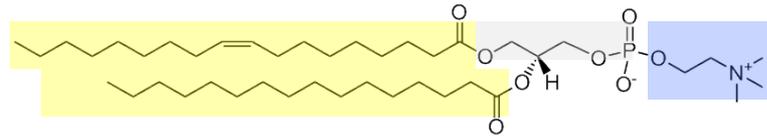
5 bonds (P to terminal -H) = ~0.8nm



Amphiphiles vs Water



18 C-C bonds * 0.15nm/bond = 2.9nm
 16 C-C bonds * 0.15 = 2.5nm (short chain)



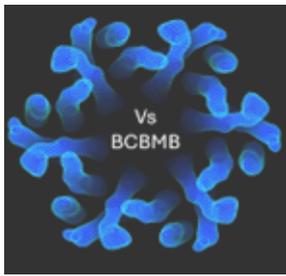
6 bonds (CO to P): ~0.9nm

5 bonds (P to terminal -CH₃) = ~0.8nm

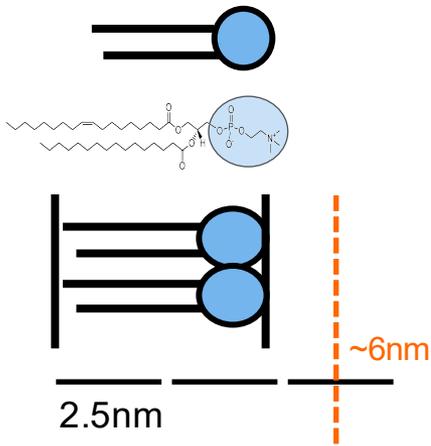
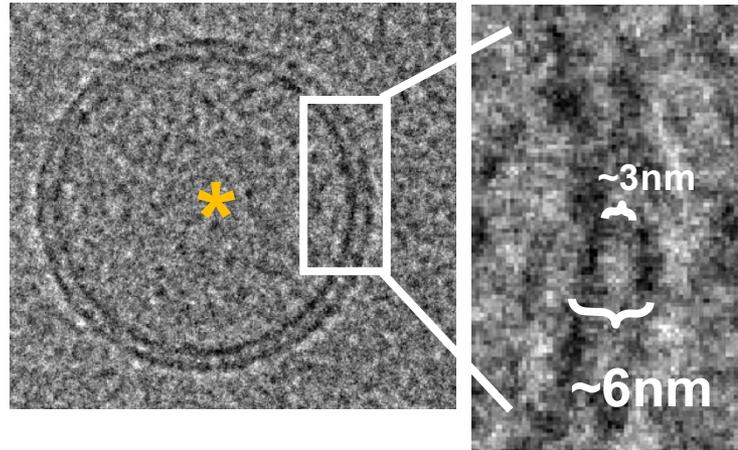
→ looking at these dimensions: one **phospholipid is ~4.6nm long** (2.9+0.9+0.8)
 → **rules out** “two independent” spheres because in that case, **each** dark line (~1.5nm) would have to fit an entire amphiphile molecule (~4.6nm) ... while – with a lot of imagination – you can fit the smaller into the larger..... you absolutely CANNOT fit it the other way round = larger into the smaller (violates everything we know about matter)

→ ...nope...“two independent spheres” is not going to happen.

→ has to be one thick-walled sphere it

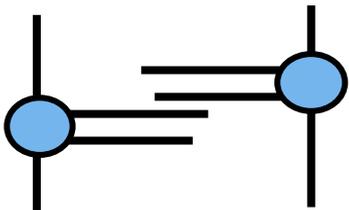


Amphiphiles vs Water

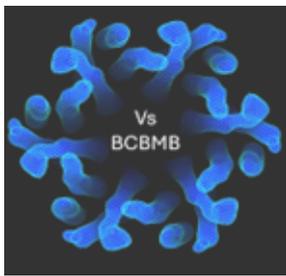


→ one thick-walled spherethat will leave you with two options

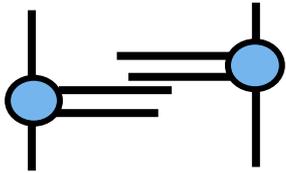
Upper sketched hypothesis: Cannot be because this **model** is inconsistent with the observation that there are **two** concentric dark circles = how would this represent chemically (very) different ends of the molecule? Furthermore, this model would not explain what is in the center region of the liposome (*) [can't be more copies of the molecule like in the case of the emulsions we looked at because that would force the hydrophilic and hydrophobic parts of the molecule to directly interact with each other. → This model is unlikely.....



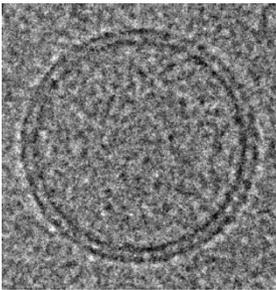
What if instead we use a double-layered wall structure? → Intuitively that makes a lot more sense...but intuition is hardly proof....so what makes this model promising – given the experimental data?



Amphiphiles of the Phospholipid Family form Bilayers



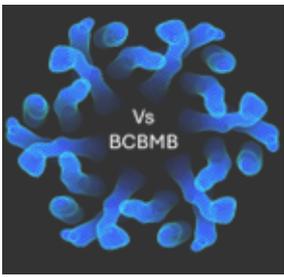
.....what makes this model promising – given the experimental data?



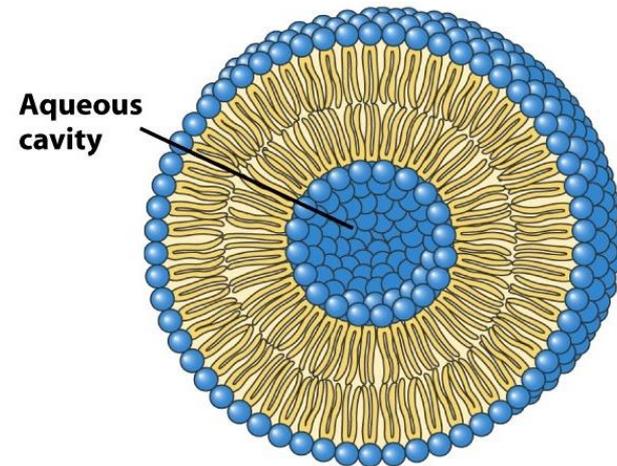
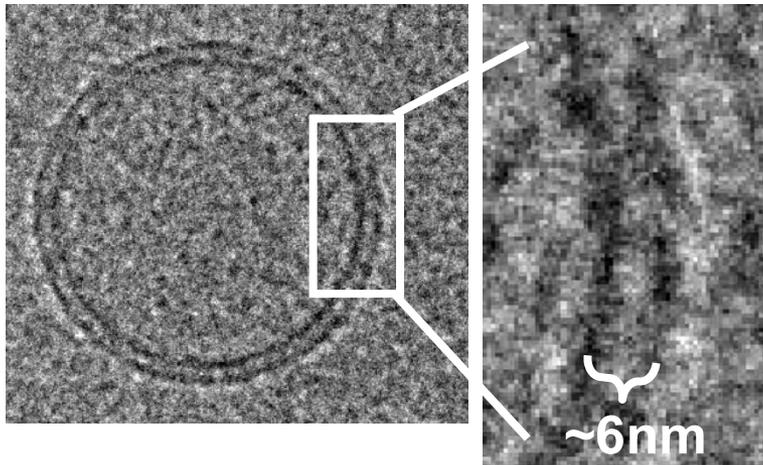
- it's "symmetric" (explains both dark circles),
- Within acceptable error/uncertainty, the observed dimensions are consistent with the known molecular structures of the component molecules
- it completely explains how the amphiphile can stabilize itself in water without any conflicts, by utilizing the full spectrum of weak interactions: H-bonds/ionic (in the regions that face the water) and van der Waals interactions (in the center between the hydrophilic boundaries on either side),
- sphere is closed on itself = no exposed hydrophobic rims

→ the structural model that best explains the experimental observations holds that what macroscopically looked like an emulsion is actually a suspension of spherical particles (**called liposomes**) that are **bounded by a double-layered membrane enclosing an aqueous cavity....**

Amphiphiles of the Phospholipid Family form Bilayers

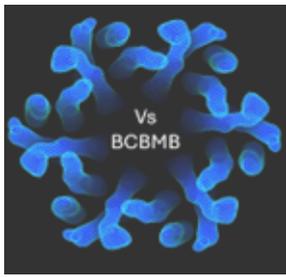


→ the structural model that best explains the experimental observations holds that what macroscopically looked like an emulsion is actually a suspension of spherical particles (**called liposomes**) that are **bounded by a double-layered membrane enclosing an aqueous cavity**....



→ We just “discovered” how the **chemical properties of phospholipds, water, and the hydrohobic effect cause spontaneous selfassembly of “units” that compartmentalize space, are separate/independent from each other, and stably co-exist in aqueous solutions** (= different from emulsions where droplets keep merging).

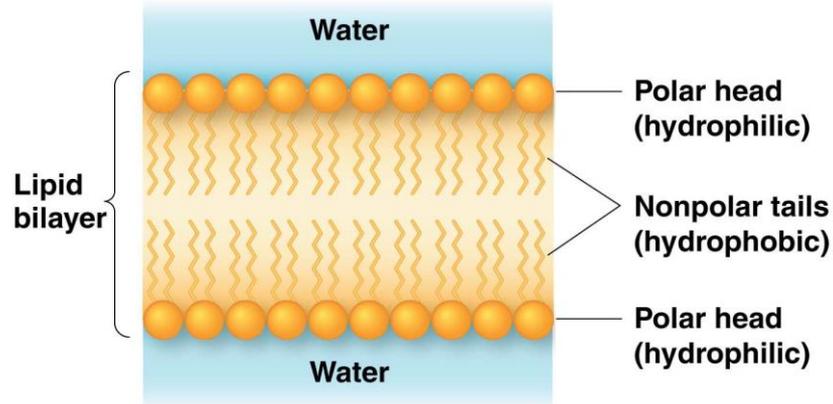
→ **In other words: we “discovered” the basis for how biological cells could emerge.**



Bilayers are a Key to Life



Looking at how a phospholipid behaves in water, you also can start to understand why microscopies (in many forms and flavors) have been and remain an essential tool/catalyst for the advancement of molecular and cell biology.



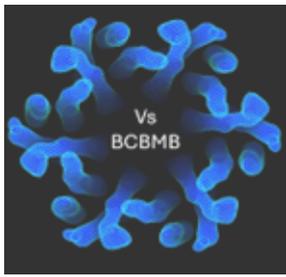
Now that we discovered how nature could exploit chemistry to begin constructing the physical boundaries of what will eventually be “cells” (as we know them).....

.....do all amphiphiles make bilayer structures?

Answer: no...there are other types of amphiphiles that form different structures, and we will look into that in the "Advanced Biochemistry" units. Here, we want to focus our attention on bilayer forming amphiphiles.

Getting carried away with the selfassembly processes that happen when hydrophobic and amphiphilic molecules encounter water...**isn't bilayer formation also a complete disaster?**

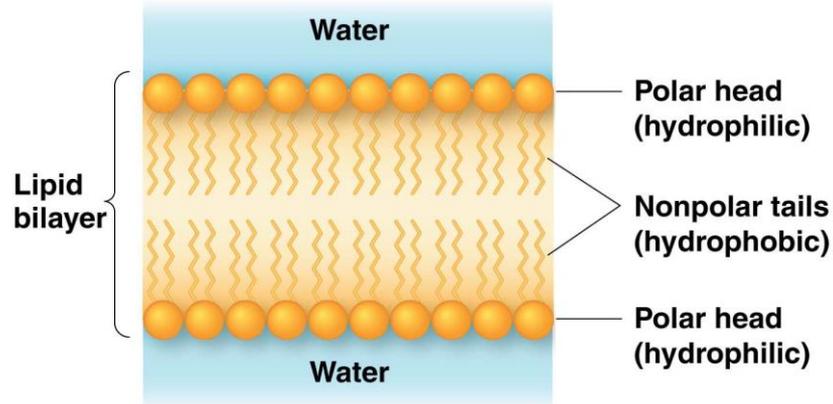
Answer: *.....try to answer yourself before looking at the next slide*



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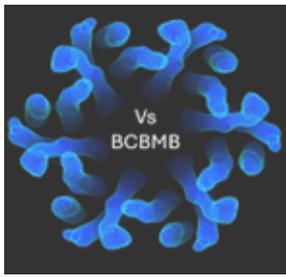
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Getting carried away with the selfassembly processes that happen when hydrophobic and amphiphilic molecules encounter water...**isn't bilayer formation also a complete disaster?**

Answer: you could think so, and you would be correct if bilayers were “pure”meaning if they only consisted of phospholipids. In that case: bilayers would create impermeable barriers for everything that has significant amount of polarity =all ions, most nutrients and cellular components.

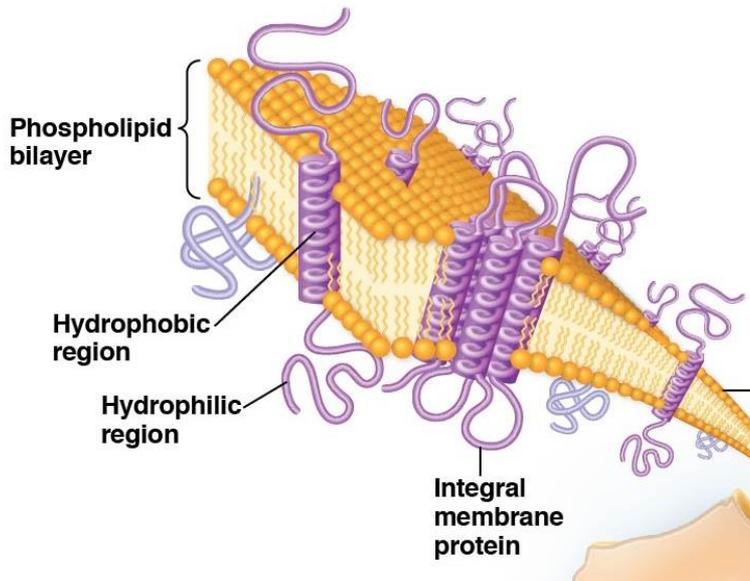
How can that be reconciled with the fact that all biological cells have bilayer membranes?



Bilayers are a Key to Life



Answer: bilayers are NOT pure. In fact – what we “rediscovered” today is equivalent to what was thought to be true in the 1920-ties. Some 50 years later, **Singer and Nicolson**, proposed their “**fluid mosaic model**”, which poses that biological membranes are complex mixtures of phospholipids, other membrane forming amphiphiles and another type of biological polymer: proteins.

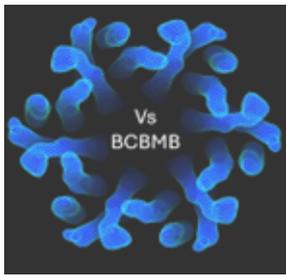


In this model, the bilayer itself is a viscous fluid that serves as a scaffold for proteins that function in:

- Transport of polar molecules across bilayers
- Cellular signaling
- Cell adhesion
- (Cellular structure)

Some of these functions will feature in other parts of the "Fundamental Biochemistry" set of handouts + in much more detail in the "Advanced Biochemistry" Units. For now we just want to retain that

- that biological membranes are semipermeable = allow & control the flux of polar/charged molecules
- biological membranes are capable of transmitting signals from one side to the other
- biological membrane are very flexible and dynamic

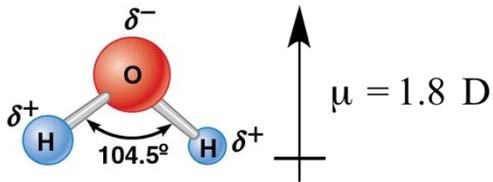


SUMMARY and ONE LOOSE END



Lipids

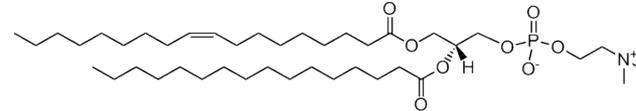
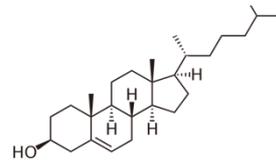
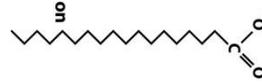
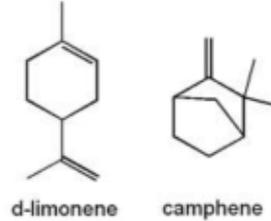
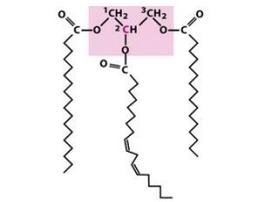
Water



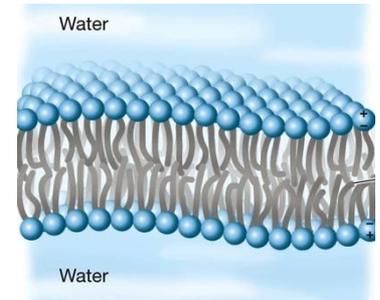
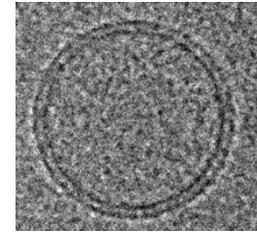
Water is a polar molecule that can act as H-bond donor **and** acceptor; it can also engage in weak ionic interactions.

The bent structure causes water to have a stable dipole

The asymmetry in charge distribution is key for water's unique chemical and physical properties



Bilayers



Lipids are collectively defined by the poor solubility/insolubility of their monomers in water

At concentrations above a characteristic threshold, the hydrophobic effect will drive solvated lipids to spontaneously self-assemble into of non-covalent polymers that are called emulsions, (micelles), and bilayers

The hydrophobic effect drives amphiphilic phospholipids to self-assemble into bilayers.

Bilayers are permeability barriers that enabled the emergence of biological cells