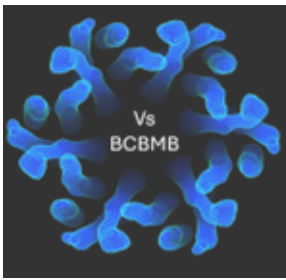


# Advanced Biochemistry

## LIPIDS & MEMBRANES



# This is a Tutorial/Silent Lecture



## What is a Tutorial/Silent Lecture?

a sequence of "slides" formatted to guide you through the exploration/study of the topic

**you are the main actor in this active learning experience**

think of it as working with a tutor without having to pay for it

as the slide sequence unfolds, you will get opportunities to engage with the material

- **by thinking about/answering questions,**

(my answer is always provided on the next slide).

- **by completing a "short assignment"**

(it never will take more than a few minutes, if at all that long),

- **by watching a short video/clip**

(the embedded links will take you to my YouTube@VsBCBMB channel;

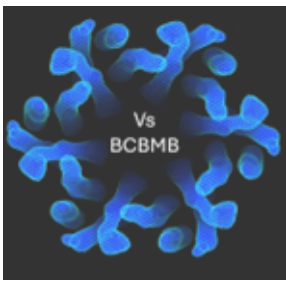
key moments are shown in the slide-deck as still images, in case you don't want to watch the videos)

of course, you can skip the active learning aspect and look at the answers right away.

## Why Give This a Go?

- **benefits: you set the pace** taking as much or as little time as you need.
- you **can turn tutorials/silent lectures into fully immersive experiences** (eg playing your favourite music while working through the content),
- **or invite friends to over the Q&A structured/guided materials together**, discussing the questions before looking at answers.

each of these features help you to hold on to the material.



# Advanced Biochemistry

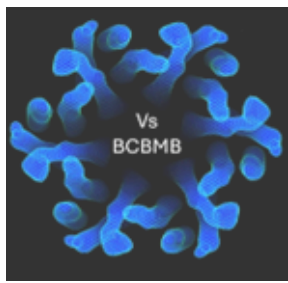


this collection of handouts builds on the "Biochemistry Fundamentals" collection = the chapters assume that you know the basics covered in the "Fundamentals" handouts (free downloads), and have some basic knowledge of molecular/cell biology

while this tutorial can be used stand alone, you will benefit more from the "silent lectures" by

- working through the chapters in the order that they are posted in the "download gallery"
- **reviewing the associated "Biochemistry Fundamentals" chapter** to refresh your memory
- spending 5-10 minutes to **summarize for yourself what you already know/remember** about the topics of the handout you are about to look at.
  - **take advantage of the "interactive" elements**

**I welcome your thoughts and ideas for further improvements of the chapters. You can submit your comments by contacting me at [pdf-comments@vsbcbmbstudy.com](mailto:pdf-comments@vsbcbmbstudy.com)**



## Goals

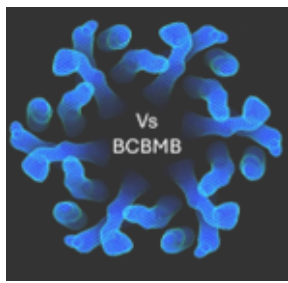


the exploration of chemical evolution has given us the “big picture” frame for all that is to come.  
from here on out, we want to focus in more detail on the various aspects of life’s design,

taking an engineering point of view – the first pit stop is to revisit the “system” aspect of our definition for life: >>a .....system<< implies boundaries

in the "Biochemistry Fundamentals" collection, we took a cursory look at the molecules that can form boundaries ...what were they?

*.....what does your memory/knowledge tell you .... ?*



## Goals



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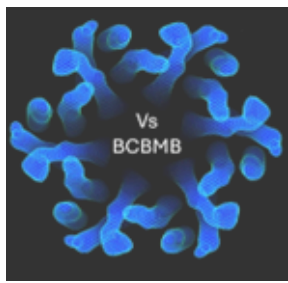
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in the "Biochemistry Fundamentals" collection, we took a cursory look at the molecules that can form boundaries ...what were they?

**Answer: Lipids and Carbohydrates**

in this more advanced chapter, we will significantly expand knowledge about lipids and membranes

- by introducing a more complete survey of biologically relevant lipids, and their large spread of biological functions that go beyond membrane formation and energy storage.
- by reviewing how the structure of certain lipids relates to the ability to form biological membranes that are compatible with life
  - by learning about and understanding the complexity of membranes
    - by learning about membrane dynamics



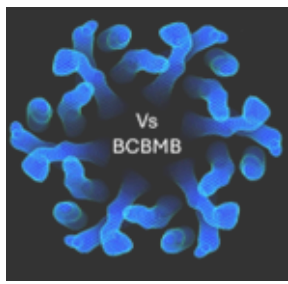
## We Will Start This Tutorial With a SUMMARY of Some Basic Facts

(to prime your mind ..just go over it and take note... it will start making sense as we go)



- lipids are relatively small - a few hundred dalton = The term “lipid” refers to the monomeric unit of this class of biologically active molecules.

I CANNOT emphasize this point enough .... yes, the aggregates of certain types of lipids form bilayers ... but there are MANY OTHER functions that are associated with individual lipid molecules



## SUMMARY of Some Basic Facts - Continued

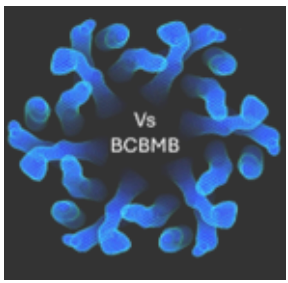


- *lipids are relatively small - a few hundred Da = The term “lipid” refers to the monomeric unit of this class of biologically active molecules.*
- lipids are **structurally diverse** ranging from simple sterols and fatty acids, to complex, carbohydrate-conjugated gangliosides
- acknowledging that lipids lack distinctive chemical hallmarks, lipids are **collectively defined by the insolubility / poor solubility in water.**
- this definition does not preclude the existence of polar groups within a lipid → many lipids are amphiphiles = contain hydrophobic **and** hydrophilic regions
- the hydrophobic/amphiphilic nature of complex lipids allows them to **assemble non-covalent polymers/aggregates.** The most important are **emulsions** used for energy storage, and **biological membranes.**
- formation of **polymers (membranes, emulsions)** is driven by the **hydrophobic effect**
- stereotypically, lipids are associated with biological membranes.

...remember: the "pink" words are **true concepts** that help tying materials together.  
If you want to learn more about how these concepts help unifying your knowledge, consider

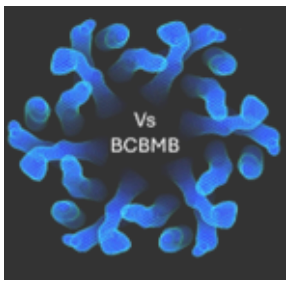
[\*\*registering for the related workshop\*\*](#)  
[\*\*"Conceptual Frameworks"\*\*](#)

## SUMMARY of Some Basic Facts - Continued



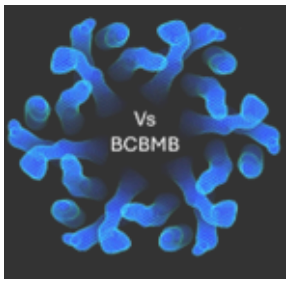
- while key for formation of bilayers, **lipids play numerous other roles in biology**. Examples are: energy storage, hormones, (precursors for) signaling molecules, electron transfer, hydrophobic anchors for proteins, light-absorbing pigments, and emulsifiers.

## SUMMARY of Some Basic Facts - Continued



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- **formation of polymers (membranes, emulsions)** is driven by the hydrophobic effect
- Stereotypically, lipids are associated with biological membranes.
- **while key for formation of bilayers, lipids play numerous other roles in biology.** Examples are: energy storage, hormones, (precursors for) signaling molecules, electron transfer, hydrophobic anchors for proteins, light-absorbing pigments, and emulsifiers.
- imbalances in lipid homeostasis result in devastating, mostly lethal diseases
- not all lipids/lipid components can be synthesized by higher organisms
- **except for** isoprenoids (&derivatives) and archeal lipids, the hydrophobic component of lipids is unbranched
- **except for** isoprenoids (&derivatives) + most eicosanoids + fatty acids: lipids are complex compounds that contain two or more chemically distinct components

you don't need to memorize this, but it does present a compilation of important, basic facts. Students in my classes were expected to know these facts because they help with understanding more complicated things



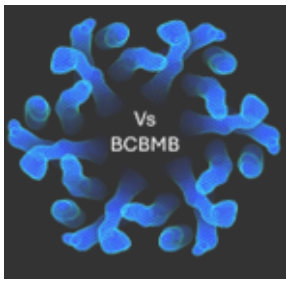
**over the next few slides, you will be introduced to different classes of lipids**

**this is dense** - there is no expectation that you retain all of it

just look at these examples carefully as you go ....

.....try to describe in your own words what you think is characteristic of each class

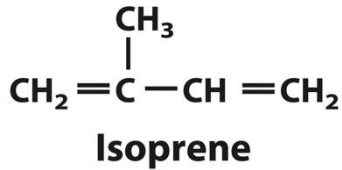
.... and .... if you want to go beyond the call of duty .. take a pen and paper and draw some of them



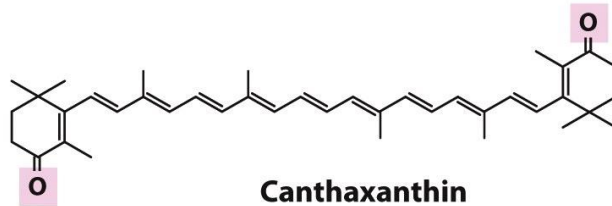
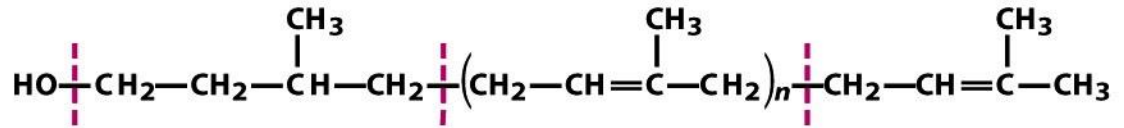
# Isoprene - A Building Block of Isoprenoids



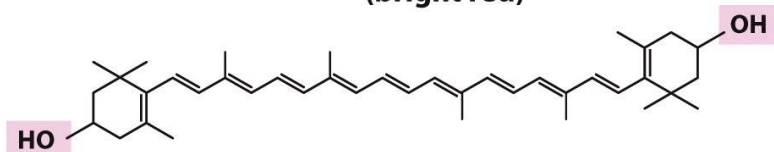
- isoprene serves as building block for
- membrane anchors for some proteins,
  - scaffold for assembly of oligosaccharides (dolichol)
  - form the basis of many (plant) secondary metabolites (such as pigments, and the typical scents of pine trees, limes, menthol, geraniums → recall: Fundamentals Collection - Lipids)



**(f)**  
**Dolichol: a sugar carrier**  
 ( $n = 9$  to  $22$ )



**Canthaxanthin**  
 (bright red)

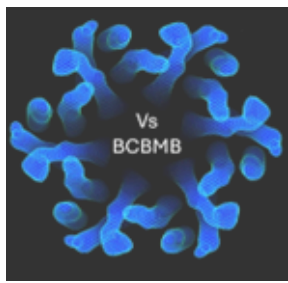


**Zeaxanthin**  
 (bright yellow)

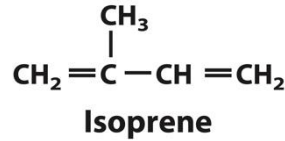


**chemistry trivia:** why does change from ketone to hydroxyl cause color shift? ..... *try to explain*

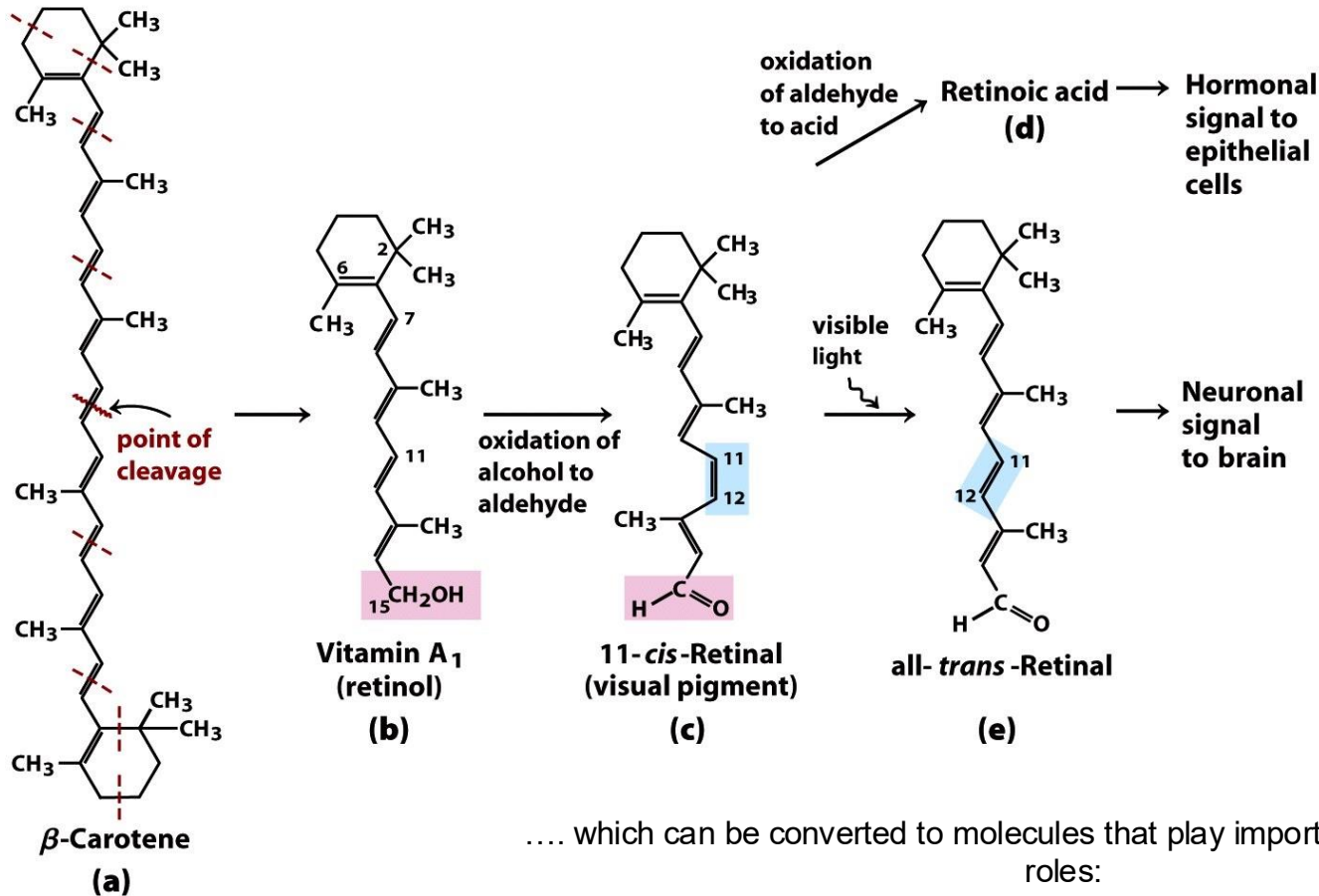




# Isoprene - A Building Block of Isoprenoids

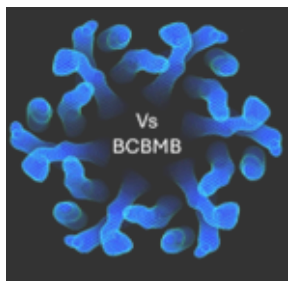


.... also serves as building block for intermediates in the biosynthesis of pigments such as carotene

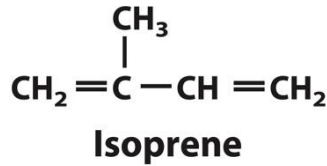


.... which can be converted to molecules that play important physiological roles:

- **retinal** - chromophore in photoreceptor rhodopsin (mediates vision; interesting trivia: fully dark adapted, a single isomerization event in a single rhodopsin molecule allows you to detect a **single photon** )
- **retinoic acid** – regulates cell growth and gene expression

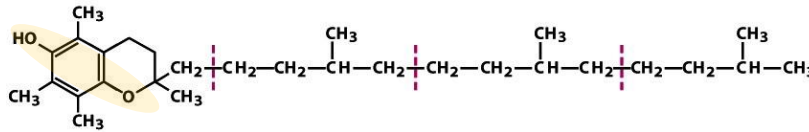


# Isoprene - A Building Block of Isoprenoids

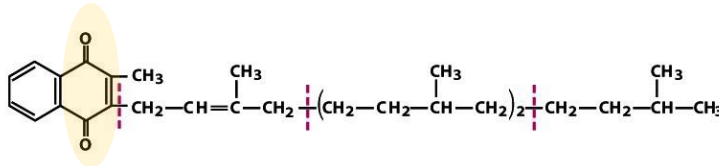


- redundant derivatives of isoprene act as
- antioxidants (Vitamin E)
  - blood clotting co-factor (Vitamin K)

(a)  
Vitamin E: an antioxidant

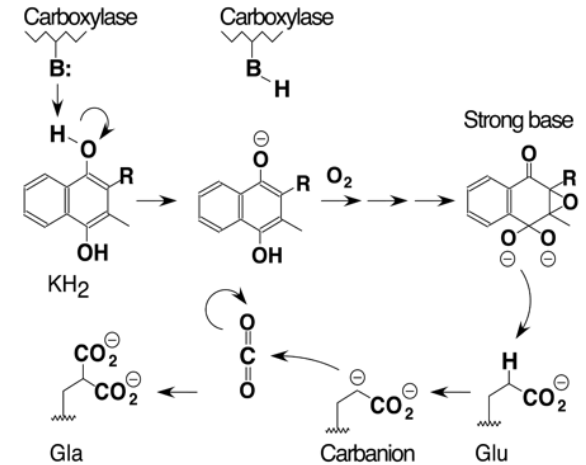
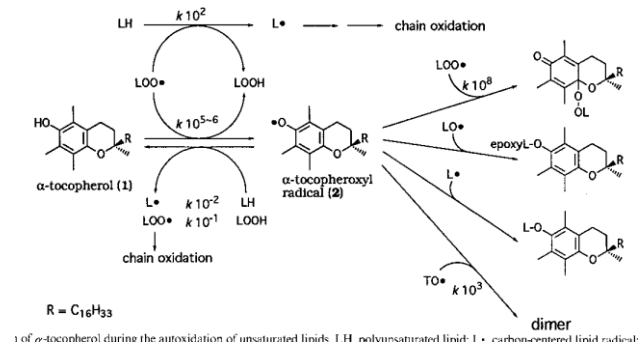


(b)  
Vitamin K<sub>1</sub>: a blood-clotting cofactor (phylloquinone)

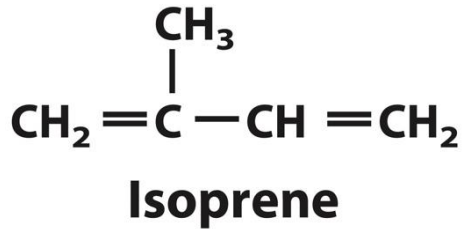
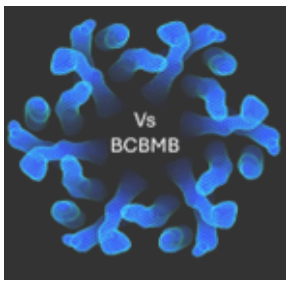


- the aromatic ring in Vitamin E scavenges reactive oxygen radicals, which prevents oxidative damage to biological membranes
- vitamin K undergoes an oxidation-reduction cycle during the posttranslational carboxylation of glutamate to  $\gamma$ -carboxyglutamate, which is an essential modification in the maturation of some blood clotting factors.

## Vit E mechanism

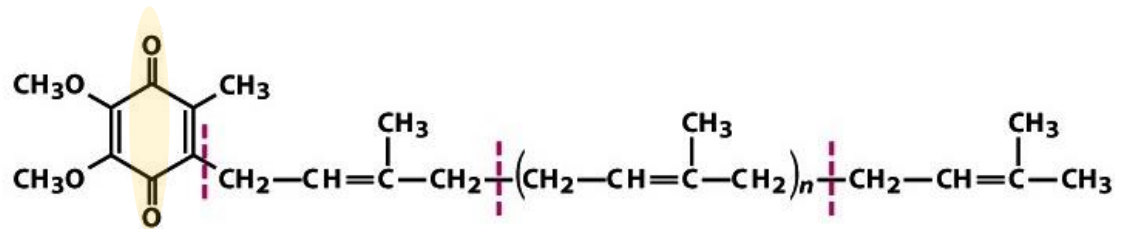


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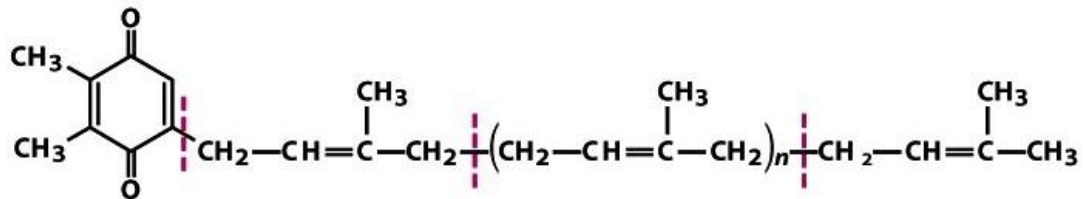


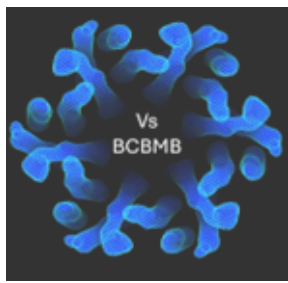
derivatives of isoprene also act as electron carriers in the respiratory chain and photosynthesis

**(d)**  
**Ubiquinone: a mitochondrial electron carrier (coenzyme Q)**  
( $n = 4$  to  $8$ )

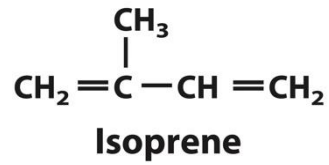


**(e)**  
**Plastoquinone: a chloroplast electron carrier** ( $n = 4$  to  $8$ )

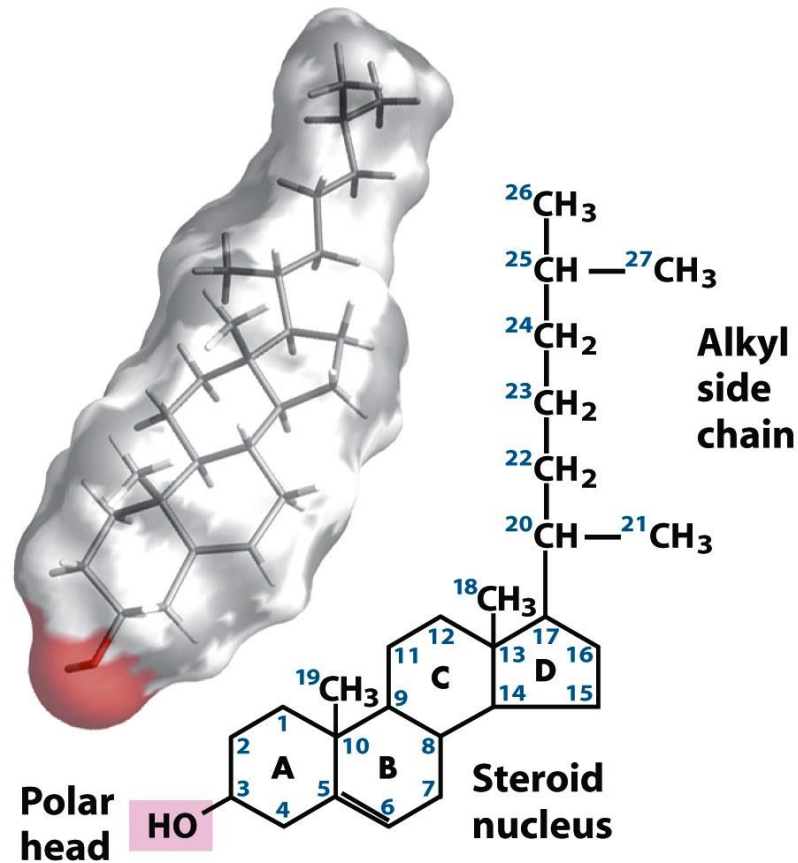


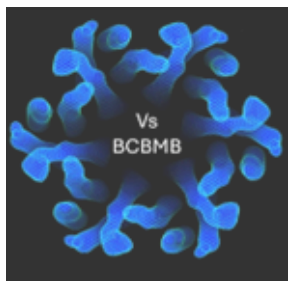


# Isoprene - A Building Block of Isoprenoids

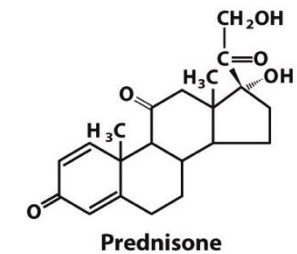
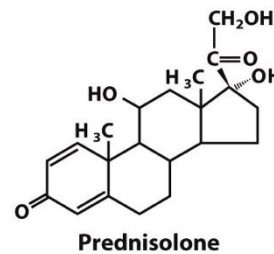
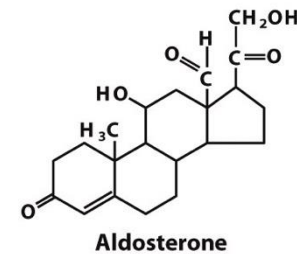
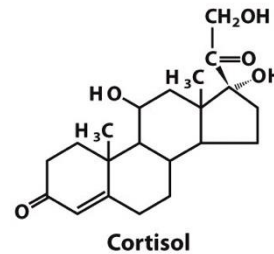
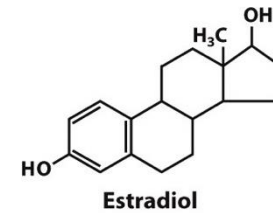
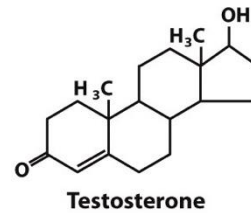
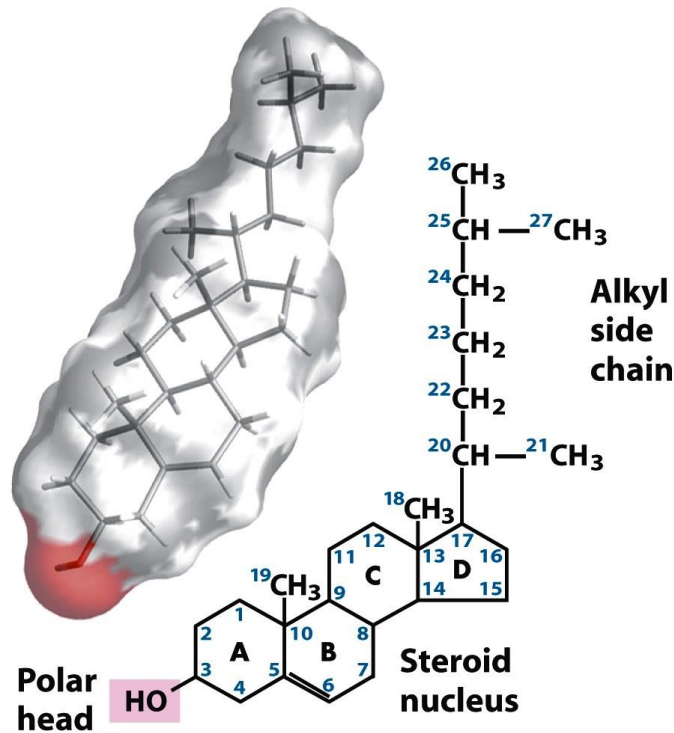


isoprene units also serve as intermediates in the biosynthesis of another important and notorious lipid - **cholesterol**

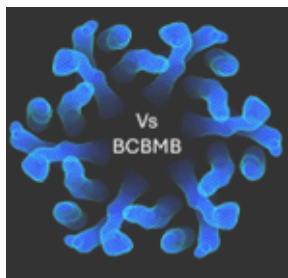




## Which in Turn is an Important Building Block For Redundant Group of Lipid Hormones (aka Steroids)



- **testosterone, estradiol:** sex hormones - regulate transcription; **cortisol:** regulates glucose metabolism, **aldosterone:** regulates salt excretion
  - synthesized in specific locations (eg testis, ovary, placenta, adrenal gland); travel through bloodstream in protein bound form.
- **synthetic steroids** like prednisolone/prednisone serve as anti-inflammatory drugs in part by inhibiting synthesis of arachidonic acid derivatives (prostaglandins and thromboxans, see later in this Chapter)



moving on from isoprenoids .....

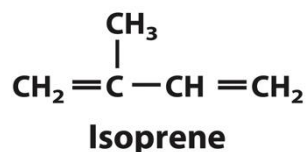


one of the points in the initial Summary of Basic Facts stated that,

collectively

lipids are defined as organic compounds that are either insoluble or only marginally/poorly soluble in water

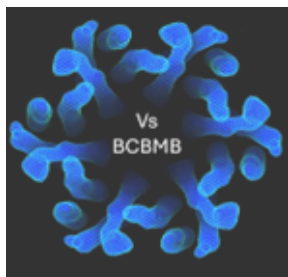
(= lipids are not defined by a characteristic chemical feature, but a shared physicochemical behavior)



certainly meets that definition, and so do all the isoprenoids we looked at, even those that have a few oxygen containing functional groups (hydroxyl-, aldehyde, ketone, carboxylic acid)

**if some polarity is OK, can you use other hydrocarbons to make a lipid?**

*...your thoughts?...*



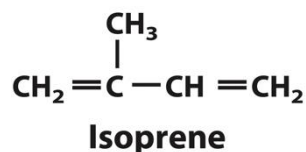
## Moving on From Isoprenoids .....



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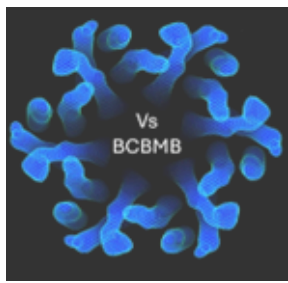
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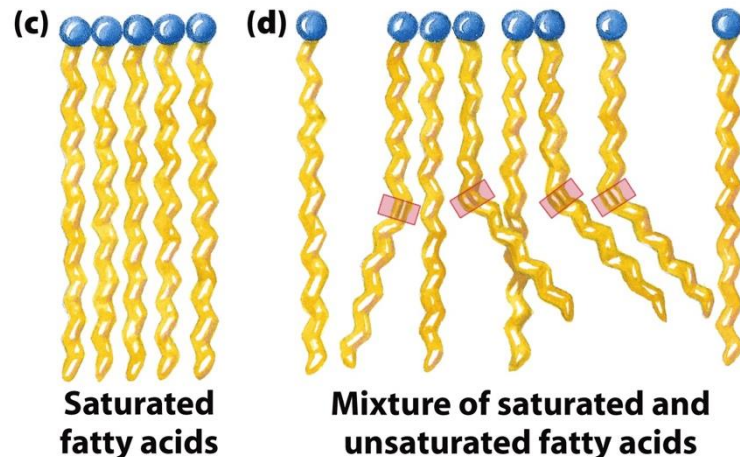
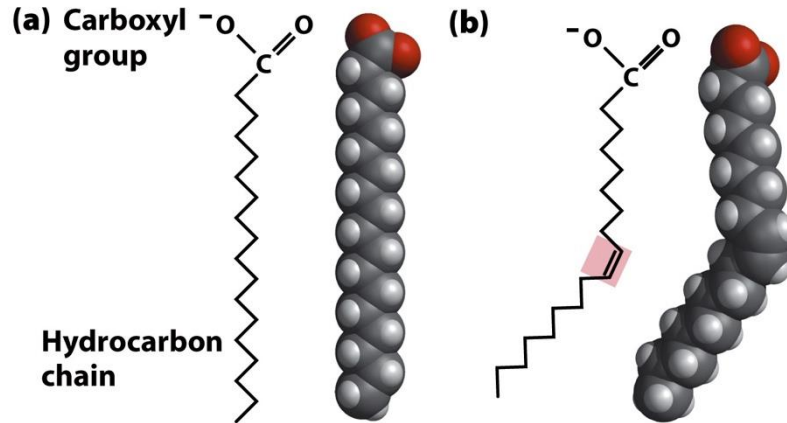
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if some polarity is OK, can you use other hydrocarbons to make a lipid?

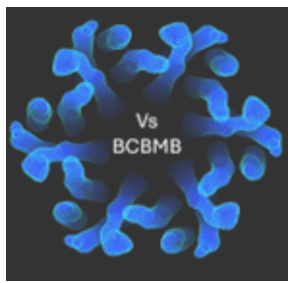
**Answer:** sure thing .... in fact, the easiest is to take a simple, unbranched alkane that has at least 4 carbon atoms and add a carboxyl group ... doing so brings you to a class of lipids known as .....



## Fatty Acids Are Hydrocarbon Derivatives



- **Fatty acids are a building block of most lipids.**
- Fatty acids are derived from hydrocarbons (**4-36 carbon atoms**) and feature a **single terminal carboxyl group**. With exception of short chain fatty acids, these molecules are water insoluble
- Fatty acids **can be fully saturated, or unsaturated.**
- Aggregates of fully saturated fatty acids have very high melting points.
- **Aggregates of cis-unsaturated fatty acids have low melting points** because the kink introduced by the cis-double bond causes packing defects in the aggregate. This feature is exploited in the construction of biological membranes.
- **Many biologically important fatty acids have multiple double bonds.** These bonds **always have “cis”** configuration, and – in their **unmodified forms** - are **never conjugated** (= have a methylene group separating the double bonds)

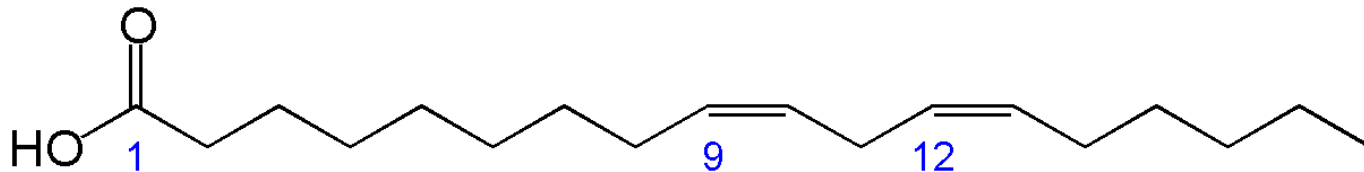


.... so many facts ... is there ever any logic to any of it?



**YES. ....let's, for instance, digest the very last statement....**

*>>Many biologically important fatty acids have multiple double bonds. These bonds always have "cis" configuration, and – in their unmodified forms - are never conjugated (= have a methylene group separating the double bonds)<<*



**the fatty acid shown here is "linoleic acid" – a component of many relevant lipids**  
it has two cis double bonds that are separated by a methylene group.

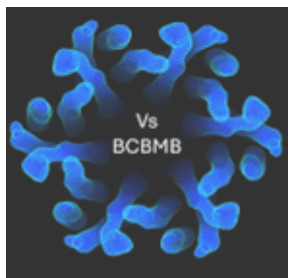
**why "cis"?**

that will become obvious once we take a closer look at membranes ... the "cis" conformation introduces kinks and packing defects in the hydrophobic region of the bilayer, lowering its **melting point** to something that **supports life at ambient temperatures**.

**Why separated by –CH<sub>2</sub>-?**

that needs a bit more careful analysis ... starting with a necessary "evil" .... oxygen....

oxygen needs to pass through cell membranes to get to the mitochondria where it is used for generating energy... this uptake of oxygen happens by diffusion. What is so bad about that? Well, here you will discover that knowing some basic chemistry is really helpful at times ....

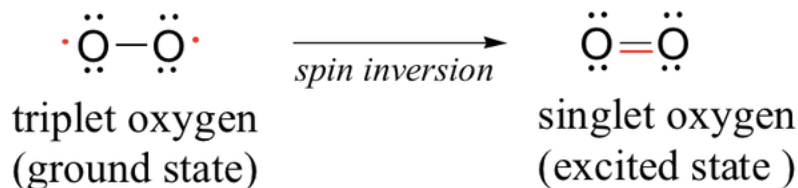


## ...The Thing We Easily Forget About Oxygen....

### why separated by $-\text{CH}_2-$ ?

That needs a bit more careful analysis ... starting with a necessary "evil" .... oxygen....

Oxygen needs to pass through cell membranes to get to the mitochondria where it is used for generating energy... this "uptake" of oxygen happens by diffusion. What is so bad about that? Well, here you will discover that knowing some basic chemistry is really helpful at times ....



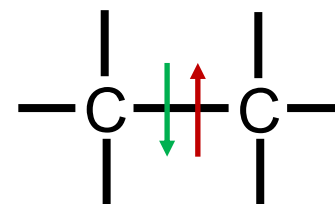
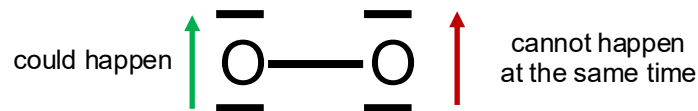
**meaning: molecular oxygen ( $\text{O}_2$ ) is a diradical in its ground state**

(= how it exists most of the time (eg when you breathe it in))

you may remember from your Orgo classes .... radicals are very reactive....and that is mostly true here too, except that the two unpaired electrons have the same spin.....

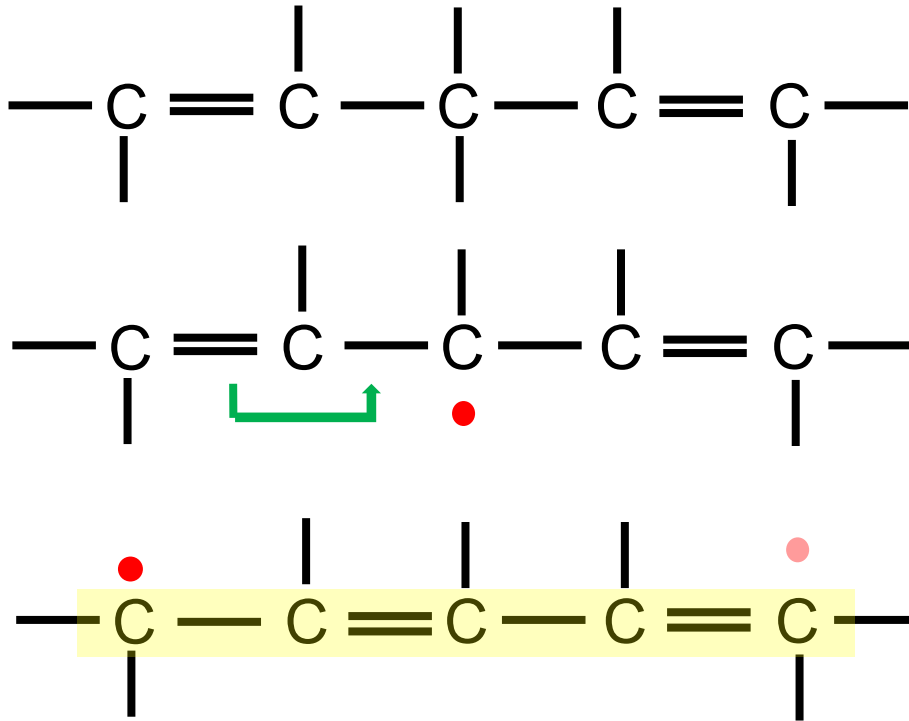
being a **triplet state** (as it is called) is **LIFESAVING** – it **prevents molecular oxygen to spontaneously insert itself into bonds** (eg. carbon-carbon) because the two electrons in a bond have opposite spins = simultaneous insertion of both oxygen atoms would violate quantum mechanics (Pauli Principle)

....but this does not mean that the triplet oxygen could not snatch the H-atom from a  $-\text{CH}_2-$  bond .. creating a carbon radical .....and that brings us back to why there is a  $-\text{CH}_2-$  group.



# Isolated vs Conjugated Dienes .... When Stability Turns Sour....

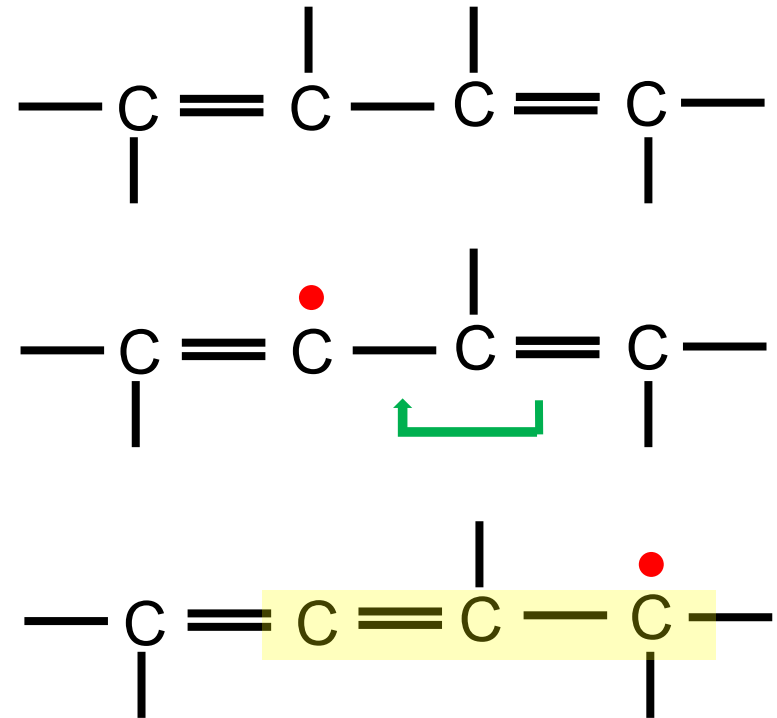
## Isolated Diene



the ability to delocalize the radical over five carbon atoms renders H-atoms on the  $-\text{CH}_2-$  group quite reactive

→ radical formation is fairly easy, **BUT** the resulting radical is not very aggressive

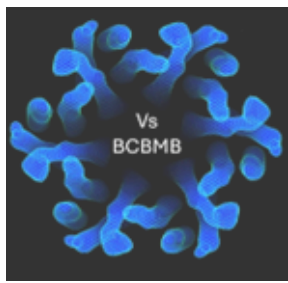
## Conjugated Diene



the H-atoms on the CH-groups of conjugated dienes are less reactive than H-atoms on the methylene group in the isolated diene to the left.

however, if a radical is formed delocalization only involves 3 C-atoms → more reactive than in case of the isolated diene. In addition, the allene that remains after the radical is satisfied is also very reactive.

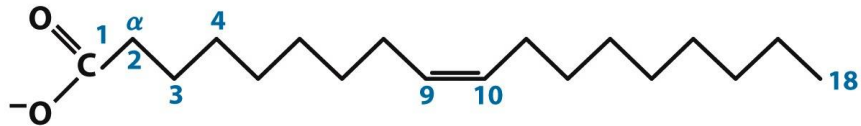
→ maintaining isolated double bonds in fatty acids with >1 double bond is **LESS** prone to lead to chemical degradation of the lipids in the membrane, thus preventing oxidative membrane damage!



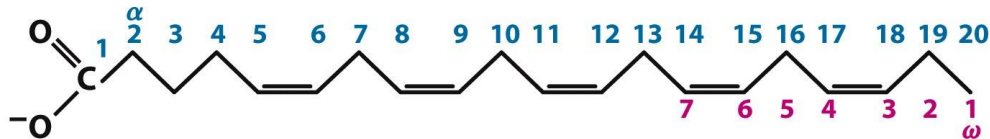
# Unsaturated Fatty Acids - Nomenclature



two alternate nomenclature systems for unsaturated fatty acids (systematic and  $\omega$ )



**(a) 18:1( $\Delta^9$ ) *cis*-9-Octadecenoic acid**



**(b) 20:5( $\Delta^{5,8,11,14,17}$ ) Eicosapentaenoic acid (EPA), an omega-3 fatty acid**

## systematic Name

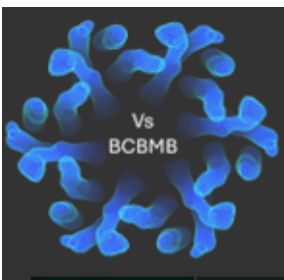
- First number - chain length
- Number of double bonds indicated after colon
- Position of double bond(s) indicated as superscript to  $\Delta$

## $\omega$ -System

- Last carbon in chain (=farthest away from carboxyl) is designated  $\omega$
- Position of first double bond is indicated counting backwards from  $\omega$

polyunsaturated fatty acids (PUFAs) are essential. However, two of them - linoleic acid [ $18:2(\Delta^{9,12})$ ], and  $\alpha$ -linolenic acid [ $18:3(\Delta^{9,12,15})$ ] cannot be synthesized by humans and thus must be taken up through diet.

the ratio of  $\omega$ -3 and  $\omega$ -6 is physiologically important. Too much  $\omega$ -6 is associated with cardiovascular disease .. and the reason why you can buy  $\omega$ -3 supplements at grocery stores.



# Fatty Acids - The Most Important Players



TABLE 10-1 Some Naturally Occurring Fatty Acids: Structure, Properties, and Nomenclature

Carbon skeleton	Structure*	Systematic name <sup>†</sup>	Common name (derivation)	Melting point (°C)	Solubility at 30 °C (mg/g solvent)	
					Water	Benzene
12:0	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	<i>n</i> -Dodecanoic acid	Lauric acid (Latin <i>laurus</i> , "laurel plant")	44.2	0.063	2,600
14:0	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	<i>n</i> -Tetradecanoic acid	Myristic acid (Latin <i>Myristica</i> , nutmeg genus)	53.9	0.024	874
16:0	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	<i>n</i> -Hexadecanoic acid	Palmitic acid (Latin <i>palma</i> , "palm tree")	63.1	0.0083	348
18:0	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	<i>n</i> -Octadecanoic acid	Stearic acid (Greek <i>stear</i> , "hard fat")	69.6	0.0034	124
20:0	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	<i>n</i> -Eicosanoic acid	Arachidic acid (Latin <i>Arachis</i> , legume genus)	76.5		
24:0	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$	<i>n</i> -Tetracosanoic acid	Lignoceric acid (Latin <i>lignum</i> , "wood" + <i>cera</i> , "wax")	86.0		
16:1( $\Delta^9$ )	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -9-Hexadecenoic acid	Palmitoleic acid	1 to -0.5		
18:1( $\Delta^9$ )	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -9-Octadecenoic acid	Oleic acid (Latin <i>oleum</i> , "oil")	13.4		
18:2( $\Delta^{9,12}$ )	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -, <i>cis</i> -9,12-Octadecadienoic acid	Linoleic acid (Greek <i>linon</i> , "flax")	1-5		
18:3( $\Delta^{9,12,15}$ )	$\text{CH}_3\text{CH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	<i>cis</i> -, <i>cis</i> -, <i>cis</i> -9,12,15-Octadecatrienoic acid	$\alpha$ -Linolenic acid	-11		
20:4( $\Delta^{5,8,11,14}$ )	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_3\text{COOH}$	<i>cis</i> -, <i>cis</i> -, <i>cis</i> -, <i>cis</i> -5, 8,11, 14-Icosatetraenoic acid	Arachidonic acid	-49.5		

common names are frequently used for the most abundant fatty acids (those in the table)

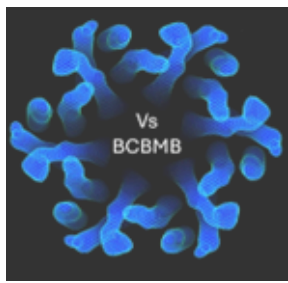
while extensive memorization of compounds is not desirable,

knowing a select few is helpful for understanding anything related to membranes.

For fatty acids – knowing the content highlighted by red boxes is advantageous

\*All acids are shown in their nonionized form. At pH 7, all free fatty acids have an ionized carboxylate. Note that numbering of carbon atoms begins at the carboxyl carbon.

†The prefix *n*- indicates the "normal" unbranched structure. For instance, "dodecanoic" simply indicates 12 carbon atoms, which could be arranged in a variety of branched forms; "*n*-dodecanoic" specifies the linear, unbranched form. For unsaturated fatty acids, the configuration of each double bond is indicated; in biological fatty acids the configuration is almost always *cis*.

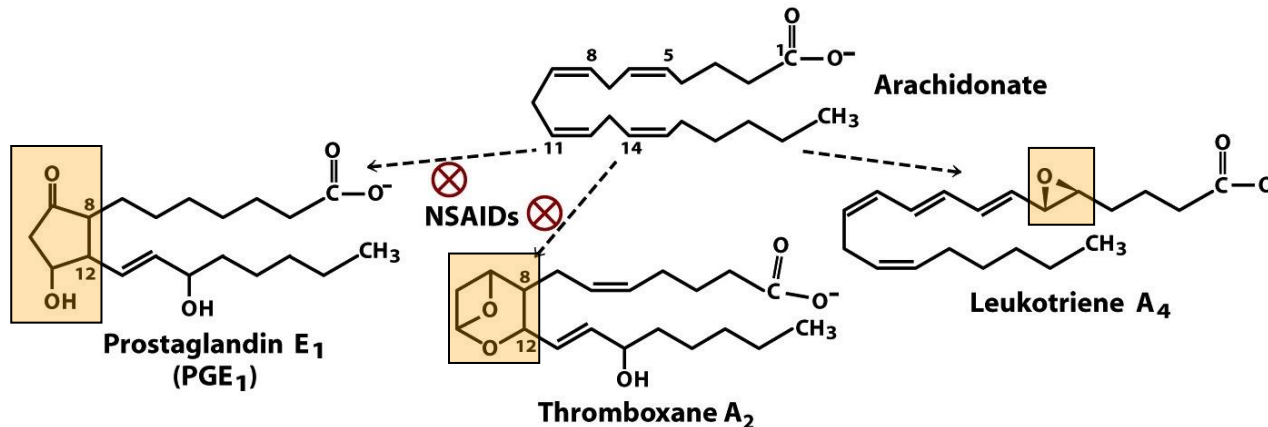


## Arachidonic Acid - Precursor For Paracrine Modulators (aka Eicosanoids)

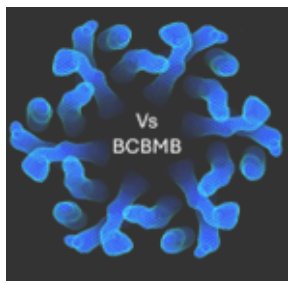


as stated earlier (slide 8), lipids play many functional roles in biology. **arachidonic Acid is one really important example for how fatty acids contribute beyond being part of membrane forming lipids:**

**paracrine modulators:** these substances act in the immediate environment in which they are formed. this sets them apart from “classical” hormones, which are secreted in locations different from the place of action (eg insulin - made in pancreatic  $\beta$ -cells, but active in muscle, liver, fat tissue, etc)



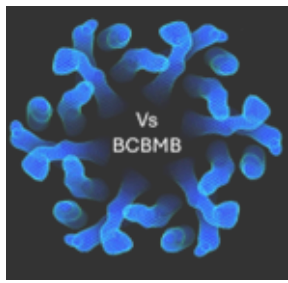
- only one example is shown for each of the **three major groups of eicosanoids: prostaglandins, thromboxanes, leukotrienes**
- eicosanoids can cause smooth muscle contraction (important in menstruation&labor, constricting blood vessels at site of injury, constricting airways), aid blood clotting, play roles in inflammation, rise of body temperature (fever), wake-sleep cycle, and tuning of tissue sensitivity to other hormonal signals
- eicosanoids are chemically labile and have very short half-lives (only a few seconds in some cases)
- NSAID: non-steroidal anti-inflammatory drugs (eg aspirin, ibuprofen) block synthesis of prostaglandins and thromboxans; interesting trivia fact: use of NSAIDs is preferable over the use of corticosteroids (synthetic derivatives of cholesterol) because there are fewer unwanted side-effects.



Now that you have familiarized yourself with fatty acids ...  
what can you do with them??



*...test your knowledge ... what do you know already ...  
other than what was just said about one particular fatty acid  
....arachidonic acid....*

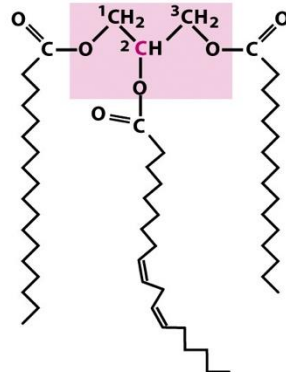
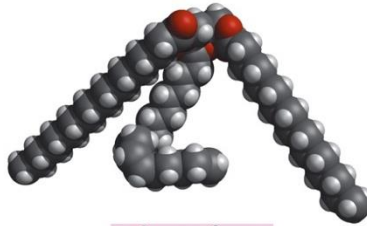
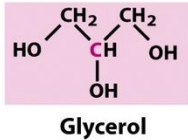


## Triacylglycerols = Storage Fats

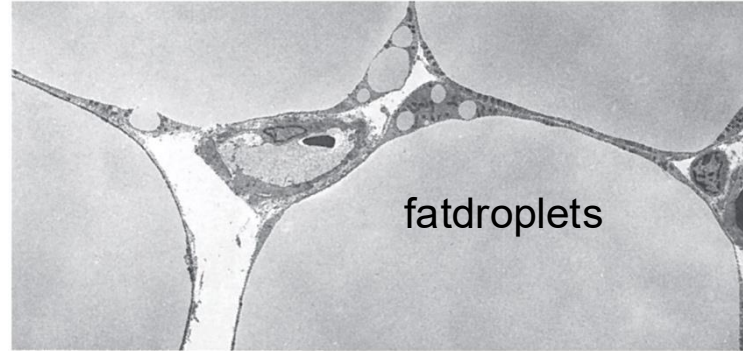
this, perhaps, is one of the most notorious aspects other than contributing to the structure of biological membranes

...in fact ...

this biological role supports multibillion \$\$ fitness, weight loss and fashion industries



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol



8 μm

triacylglycerols are stored in specialized cells, called adipocytes (see above).

the fat forms an emulsion (droplets) inside the cell, comprising ~80% of its mass (if you don't remember, review "Fundamentals – Lipids" chapter)

Specific enzymes - **lipases** - release free fatty acids from triacylglycerols

free fatty acids are exported from the adipocyte and transported to the site of utilization. While in transit, free fatty acids are bound to albumin.

**triacylglycerols are much better for energy storage form than the polysaccharide glycogen - why?**

*...what are your thoughts? ....*

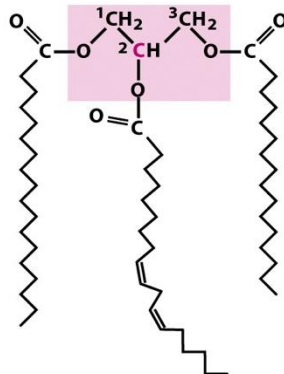
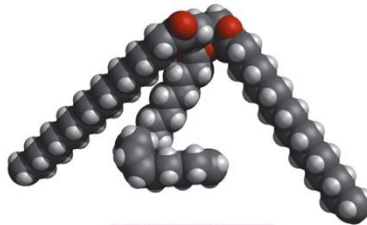
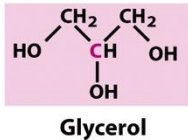
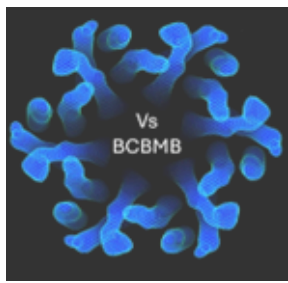
# Triacylglycerols = Storage Fats



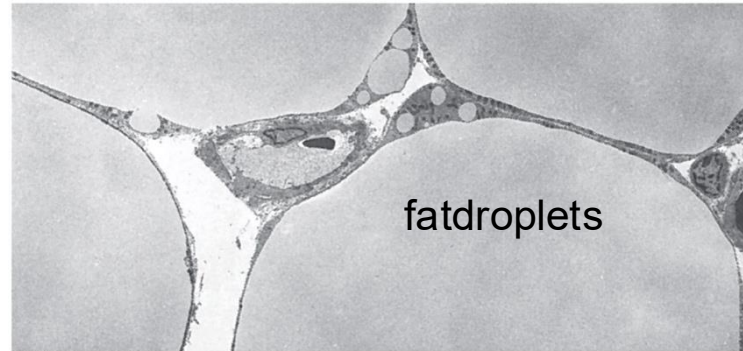
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1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol



fat droplets

8  $\mu\text{m}$

Triacylglycerols are stored in specialized cells, called adipocytes (see above).

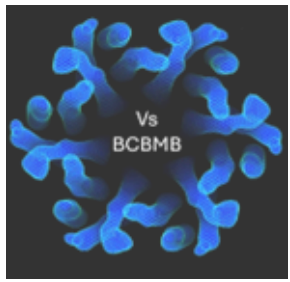
The fat forms an emulsion (droplets) inside the cell, comprising ~80% of its mass (if you don't remember, review "Fundamentals – Lipids" chapter)

Specific enzymes - **lipases** - release free fatty acids from triacylglycerols

Free fatty acids are exported from the adipocyte and transported to the site of utilization. While in transit, free fatty acids are bound to albumin.

Triacylglycerols are much better for energy storage form than the polysaccharide glycogen - why?

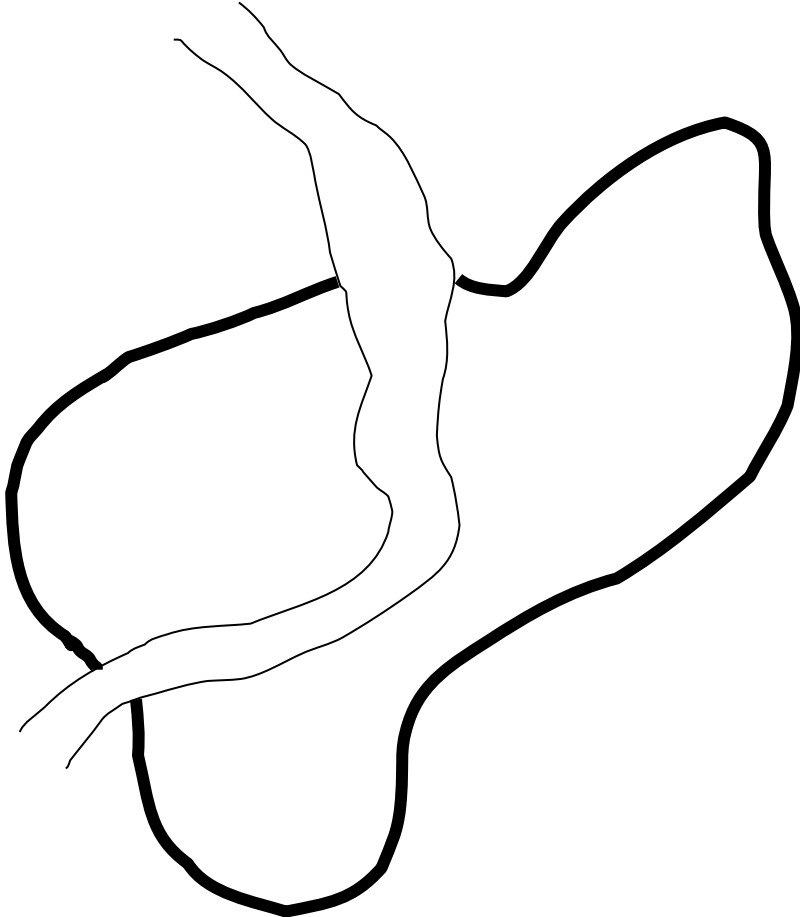
- oxidation of fatty acids to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  yields much more ATP than oxidation of glucose (body fat stored = several weeks/months of energy supply; glycogen stores only last for few hours)
- the aggregation/emulsion is not hydrated = need no water to solvate = less weight



# Switching Gears - Lipids and Membranes = Life and the **Hydrophobic Effect**



## •Historic City Wall

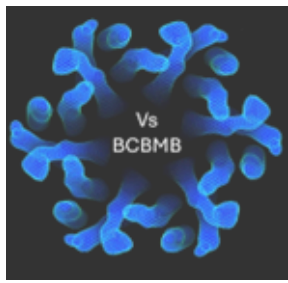


### properties of city wall

- divided inside from outside with an uncrossable barrier
- used controlled gates (and river) to let in selected sources
- used gates (and river) to get rid of waste
- watchmen in towers informed the inside about the outside
- toll houses captured “energy” from influx
- but city walls were rigid, un-adaptable, locally un-responsive, and hard to fix

this is one example where humans "borrowed" ideas from biology ... but as it turns out, cells do all of this SO MUCH better .....

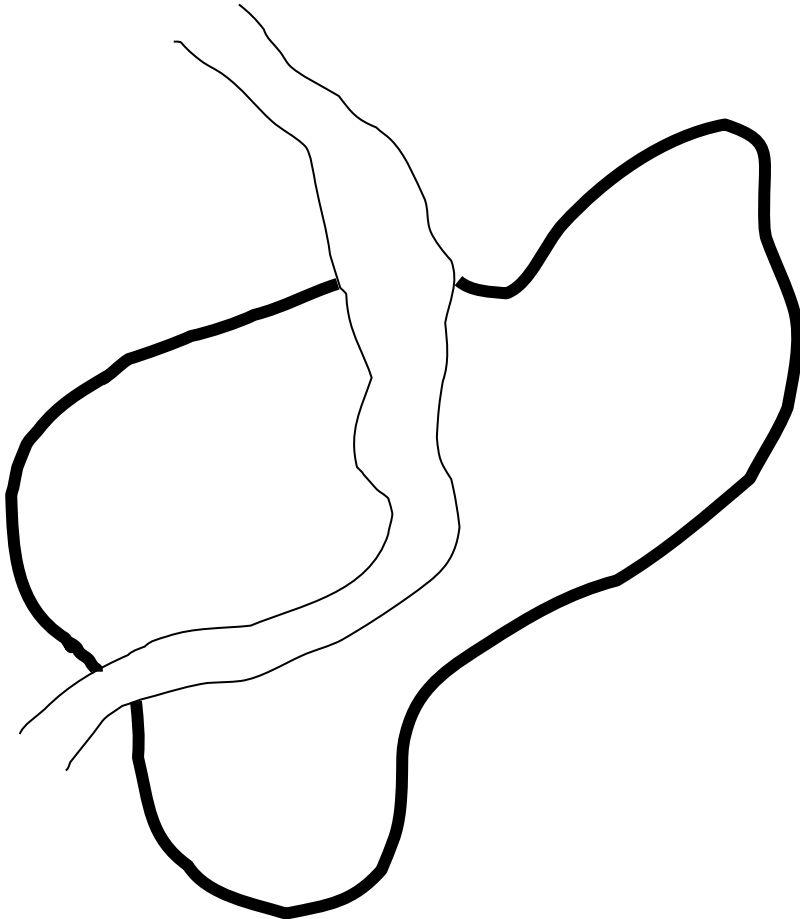
yes, I know this looks like a stomach with indigestion .... :)



# Switching Gears - Lipids and Membranes = Life and the **Hydrophobic Effect**



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- properties of city wall
- divided inside from outside with an uncrossable barrier
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- used gates (and river) to get rid of waste
- watchmen in towers informed the inside about the outside
- toll houses captured “energy” from influx
- but city walls were rigid, un-adaptable, locally un-responsive, and hard to fix
  
- cells do it better!
- cells use lipid membranes as boundaries and to compartmentalize special functions within
- membrane proteins serve as shuttles for food and waste, to inform and to transduce energy
- membranes are flexible, self-sealing, laterally responsive
- boundedness is as basic as heredity or metabolism, but has been less understood

yes, I know this looks like a stomach with indigestion .... :)

# Membranes are Dynamic Organelles and Their Self-Sealing Properties Can Literally be Life-Saving

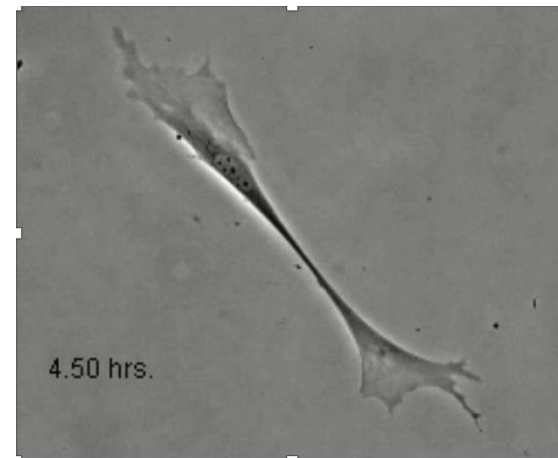
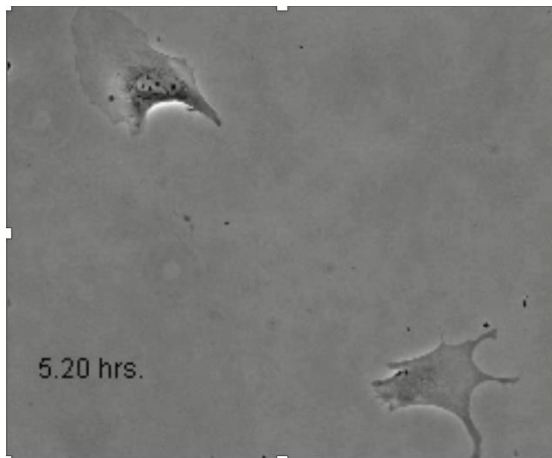
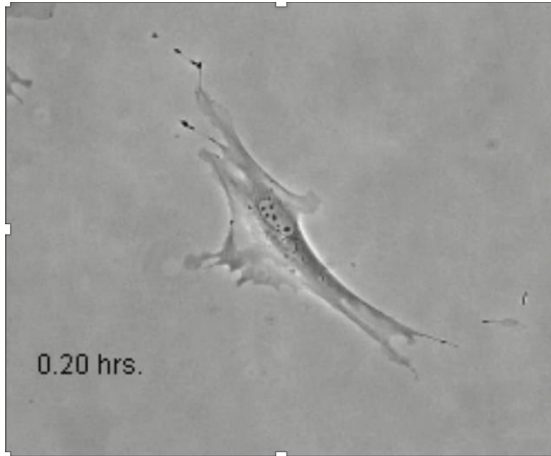
[Check Out This Short Video](#)



arg<sup>-/-</sup> cell migrating on fibronectin  
for ~7 hrs @ 3min/frame.  
Courtesy Dr Ann Miller (U Michigan).

# Membranes are Dynamic Organelles and Their Self-Sealing Properties Can Literally be Life-Saving

video stills (sequence: clockwise)



# Membranes are Dynamic Organelles and Their Self-Sealing Properties Can Literally be Life-Saving



arg<sup>-/-</sup> cell migrating on fibronectin  
for ~7 hrs @ 3min/frame.  
courtesy Dr Ann Miller (U Michigan).

**integration:** ... if you worked through the Molecular and Cell Biology Collection ... test how much you remember..... how are the extended structures called that form during "ruffling" of the cell?

what cytoskeletal component is a key player in the process you see?

what – if anything – is “wrong” with this cell’s behavior?

# Membranes are Dynamic Organelles and Their Self-Sealing Properties Can Literally be Life-Saving



arg-/- cell migrating on fibronectin for ~7 hrs @ 3min/frame.  
Courtesy Dr Ann Miller (U Michigan).

**Integration:** ... if you worked through the Molecular and Cell Biology Collection ... test how much you remember.....

How are the extended structures called that form during "ruffling" of the cell? (Cytokinesis – Actin and Myosin Chapter)

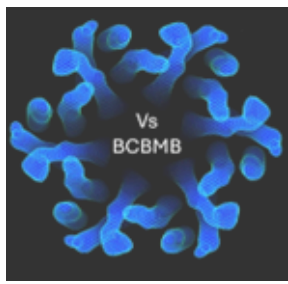
## FILOPODIA

What cytoskeletal component is a key player in the process you see?

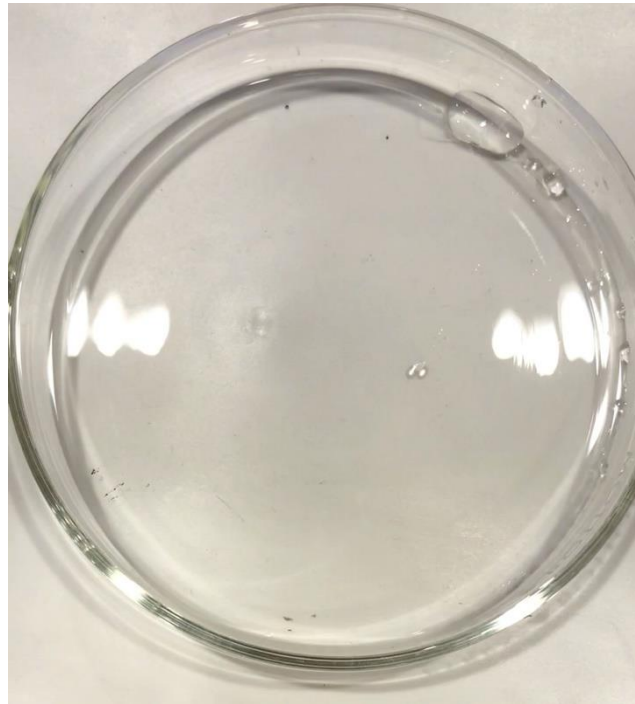
## ACTIN

What – if anything – is "wrong" with this cell's behavior?

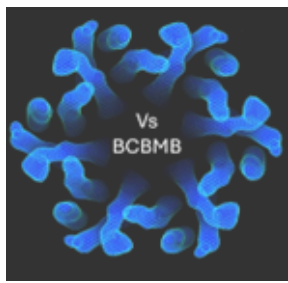
**Actin dynamics are uncoordinated. Instead of migrating in one direction – uncoordinated actin dynamics lead the cell to literally rip itself into two parts**



To Understand How Lipids Can Form Such Amazingly Flexible Boundaries ... Let's Watch a Short "V-Productions" Video....



[Watch Clip](#)



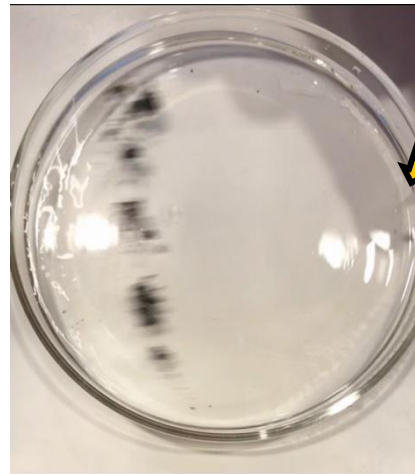
# Select Stills from “V-Productions” Clip...to start explaining why lipids can form amazingly flexible, self-sealing boundaries



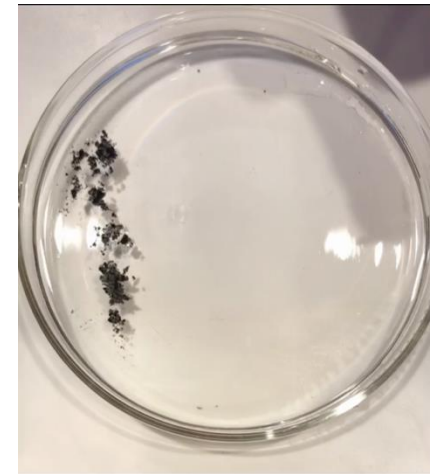
dip a "yellow tip"  
(like you use in biolab)  
into a detergent similar to dish  
washing detergent



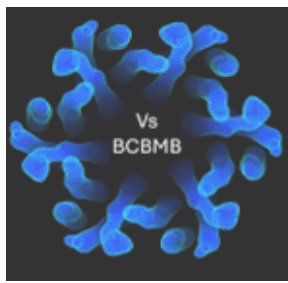
sprinkle graphite powder  
on water surface to have a "probe"



touch yellow tip to water  
surface at right edge  
(position marked by arrow)  
and watch behavior of  
graphite powder



less than 1 second after  
touching yellow tip to water  
surface  
magic??

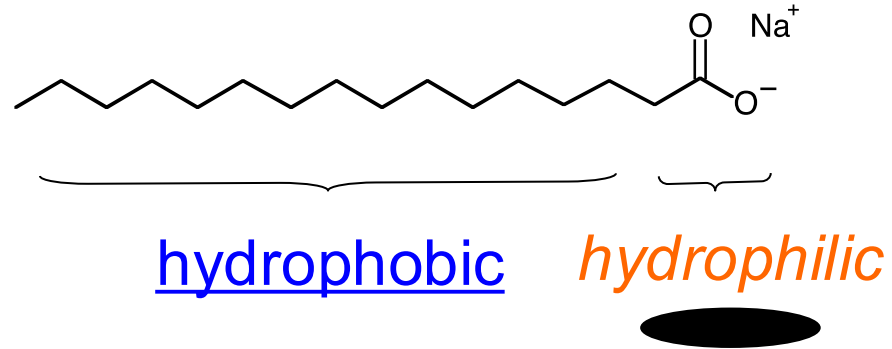


to Understand What You Saw in the Short Clip (or Stills) we need to deal with



## amphiphiles (and their properties)

spatial separation of *water soluble* and *water insoluble* regions in a molecule creates an amphiphile

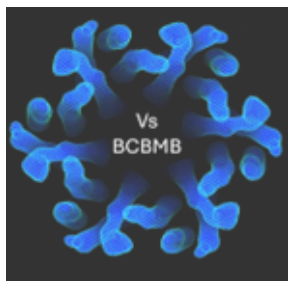


amphiphilic = amphipathic

amphi = of both kinds  
philos = loving  
phobos = fear  
pathos = suffering

in the example above, I chose the sodium salt of a fatty acid

**what will happen if this molecule comes into contact with water?**  
start thinking about just one, then many

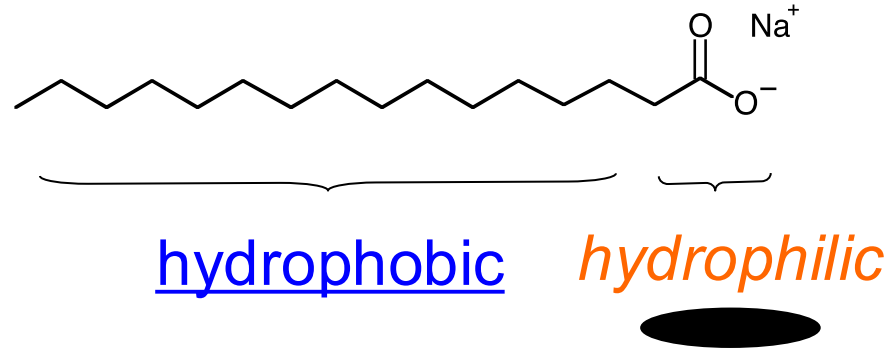


to Understand What You Saw in the Short Clip (or Stills) we need to deal with



## amphiphiles (and their properties)

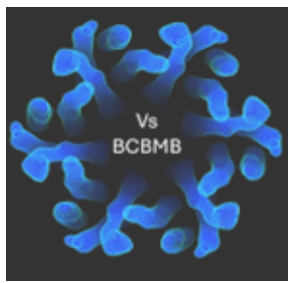
Separation of *water soluble* and water insoluble regions in a molecule creates an amphiphile



what will happen if this molecule comes into contact with water?  
start thinking about just one, then many

➤ the hydrophilic part WANTS to be in water  
➤ the hydrophobic part WILL AVOID water at all cost .....  
...but from life you know that you "*can't have your cake and eat it too*" ...

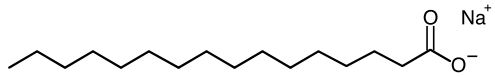
➔ is there any "truce/deal" that can be reached that would make both parts happy? ... and the answer is .....????



Yes ... It Will Do The Same As The Swan  
In This Clip ....



.....and that is ....?



Hydrophobic

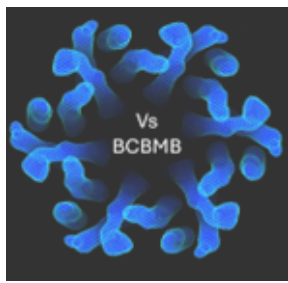
*hydrophilic*

"body/tail"

*"head"*



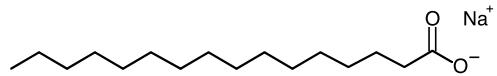
[Watch the Clip](#)



Yes ... It Will Do The Same As The Swan  
In This Clip ....



.....and that is ....?



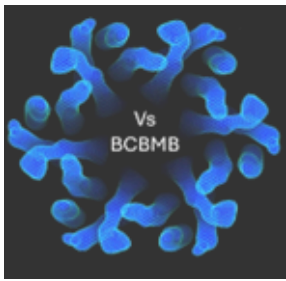
Hydrophobic

*hydrophilic*

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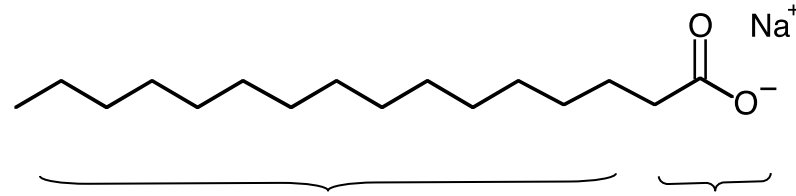


To Understand What You Saw in the Short Clip (or Stills) we need to deal with



## amphiphiles (and their properties)

separation of  
*water soluble* and water  
insoluble regions in a  
molecule creates an  
amphiphile



hydrophobic

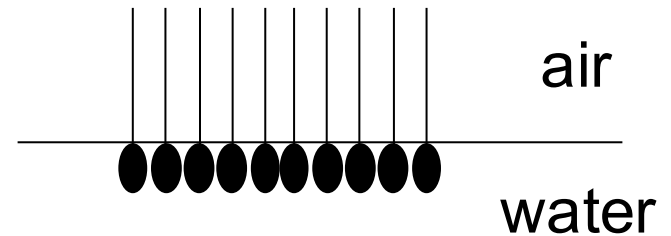
hydrophilic



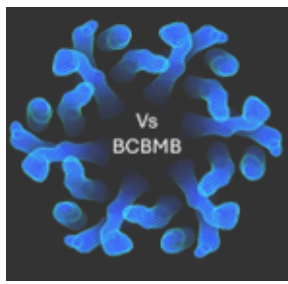
called "headgroup"

just like the swan sticks its head into the water....

...**amphiphiles** such as the salts  
of fatty acids can satisfy the needs of both the  
hydrophobic and hydrophilic regions **by aligning  
themselves at the water-air interface.**



in this alignment, the **hydrophilic part will go in the water,**  
**while the hydrophobic part will stick out in the air.**

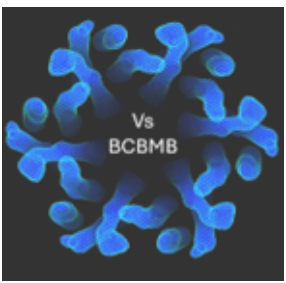


# Micelle Formation 1



as more and more detergent molecules moved from the "yellow tip" to the water, spontaneous alignment of the detergent molecules at the air water interface provided the "force" to push the graphite particles away, keeping them ahead of the expanding frontline of the spreading monolayer

**this is easy to appreciate but raises the question ... what happens if you add more detergent molecules once the surface is completely occupied?**



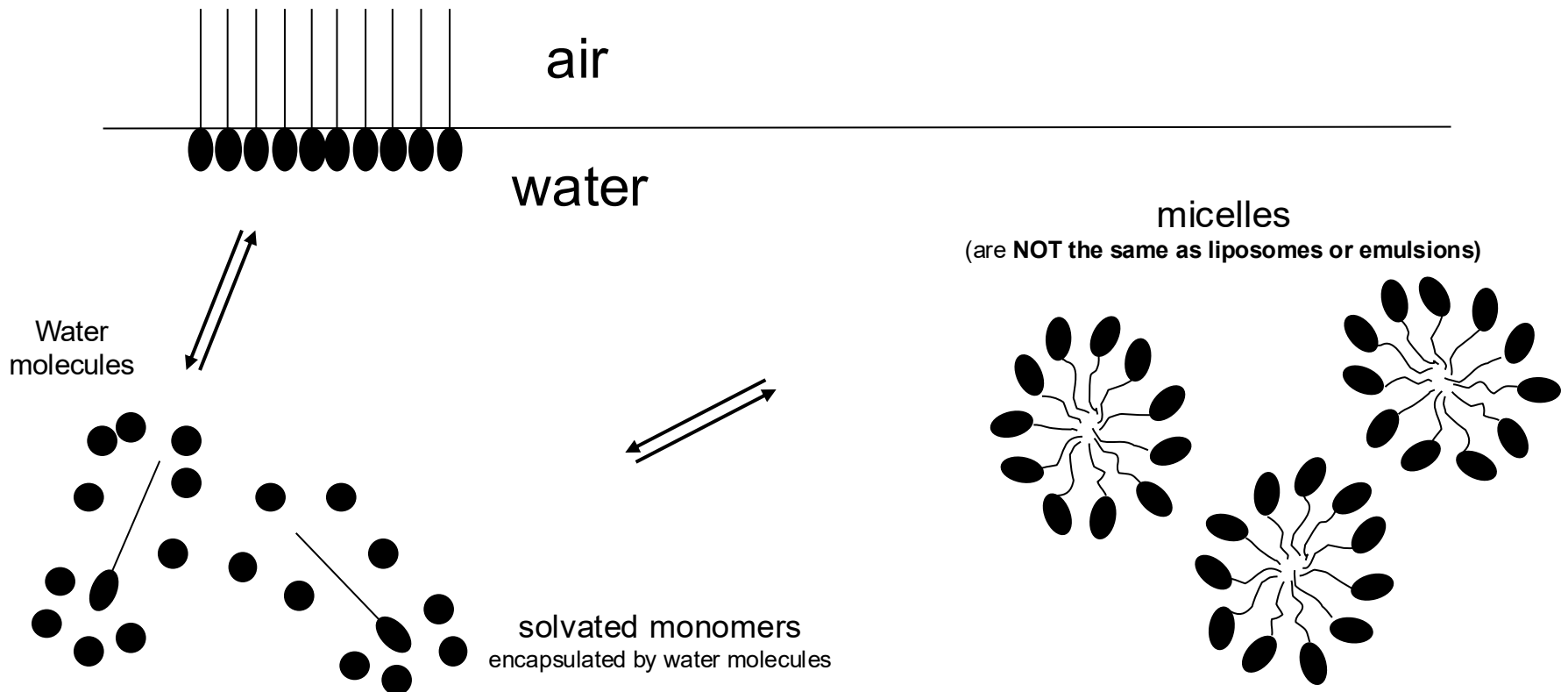
# Micelle Formation 1

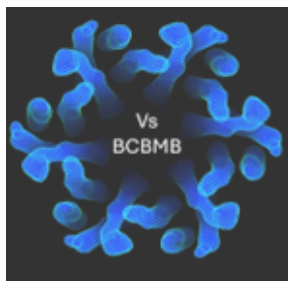


this is easy to appreciate but raises the question ... what happens if you add more detergent molecules once the surface is completely occupied?

amphiphilic molecules in solution

- **First: monomer covers surfaces**
- **second: monomer will be solvated** (clathrate cage – see "Fundamentals – LIPIDS" chapter)
- **third: aggregates form** above the “critical” micelle concentration (next slide)



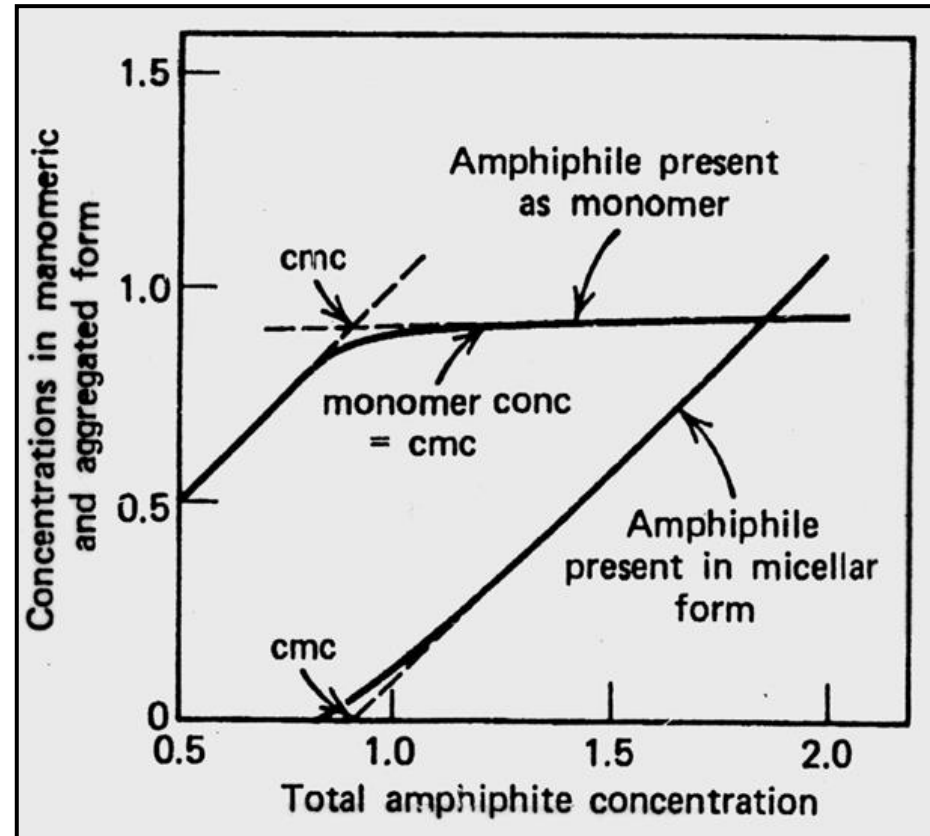


# Micelle Formation 2



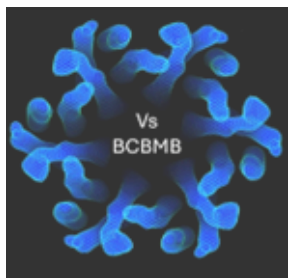
upon partitioning of hydrophobic and hydrophilic parts of amphiphile in water

- **hydrophobic effect** tends to increase size (=wants to push more of the monomers into the same micelle)
  - > increase volume/surface ratio (volume increases as function of  $r^3$  vs surface  $r^2$ )
- **steric, solvation and electrostatic effects** tend to increase surface (the tails and headgroups getting too close and repel each other)
  - > reduce volume/surface ratio
- packing of hydrocarbon chains is ordered near center, disordered towards outside
- shape of micelles is not necessarily spherical, can be elongated

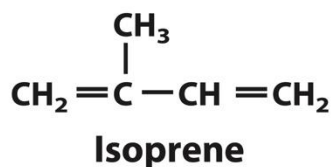


cmc: critical micelle concentration

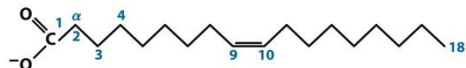
(each detergent has its own, characteristic cmc. The cmc depends on the size and character of the headgroup, how it relates to the size and character of the hydrophobic part, the solvent and temperature)



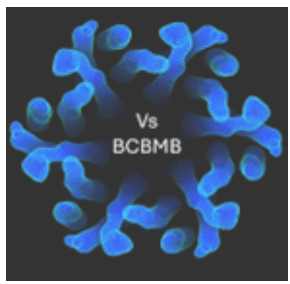
## QUICK RECAP Before Moving On



Uncoloured 10/2009  
Copyright © 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025  
© 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025



- Learned that lipids are a chemically diverse group of biologically active macromolecules
- Learned that many lipids are derived from a small hydrocarbon called isoprene, including steroid hormones that are formed from cholesterol
- Learned that "fatty acids" are class of lipids formed by simple linear hydrocarbons that carry a terminal  $-\text{COOH}$  group (carboxyl group) if the chain is 4 or more carbon atoms long.
- Learned that unsaturated fatty acids have cis-stereochemistry.
- Learned that paracrine modulators known as eicosanoids are derived from the highly unsaturated fatty acid - arachidonic acid ( $20:4\Delta^{5,8,11,14}$ )
- Learned that fatty acids are stored as triacylglycerols
- Learned that fatty acids are amphiphiles, and that such "single chain" amphiphiles form micellar structures in water/aqueous solutions if the concentration of the amphiphile exceeds a threshold that is called "critical micelle concentration"

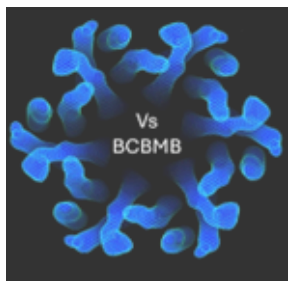


## Bilayer Forming Amphiphiles - Phospholipids, Glycolipids and Ether Lipids



in discussing **triacylglycerols** - you saw that they form emulsions ... but **not micelles or membranes. Why is that?**

*....try to remember/answer .... (or go back to refresh your memory....\_*

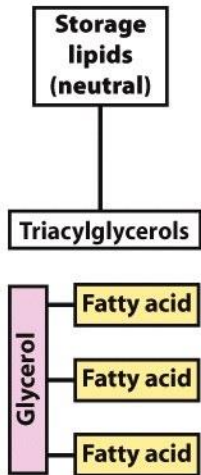


## Bilayer Forming Amphiphiles - Phospholipids, Glycolipids and Ether Lipids

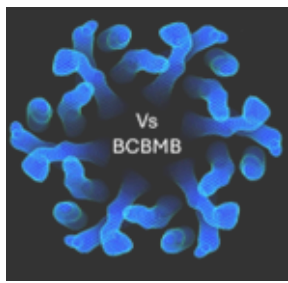


In discussing **triacylglycerols** - you saw that they form emulsions ... but **not micelles or membranes**. **Why is that?**

**Answer:** triacylglycerols are not amphiphilic! ....they can and do form emulsions (rather than micelles) because the steric bulk of these molecules does not allow for the micellar geometry



**what will change/happen, if you were to replace one of the fatty acids in a triacylglycerol with a hydrophilic group?**

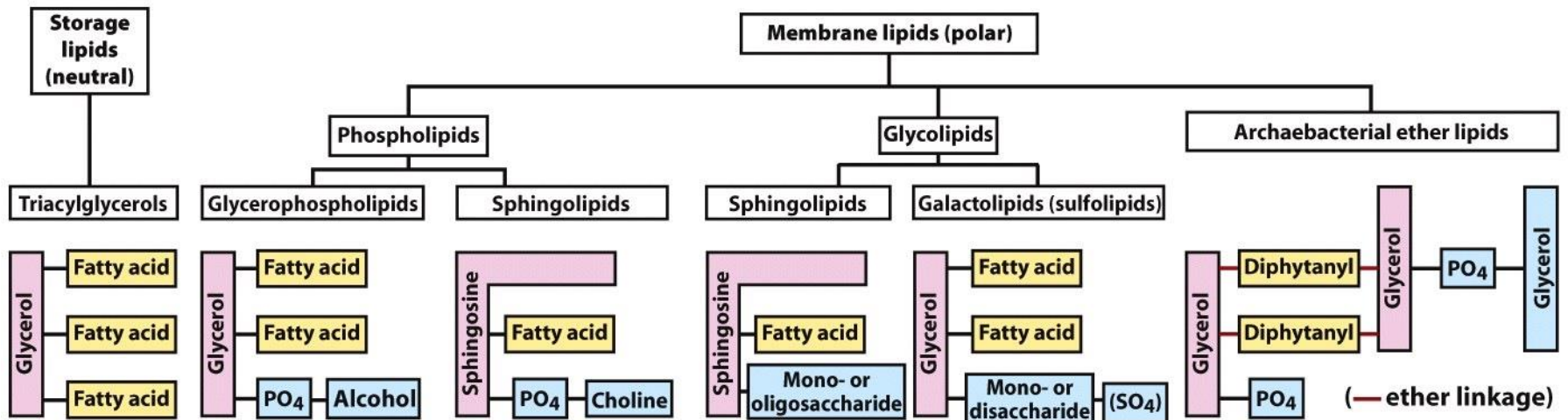


## Bilayer Forming Amphiphiles - Phospholipids, Glycolipids and Ether Lipids



In discussing **triacylglycerols** - we saw that they form emulsions ... but **not micelles or membranes**. Why is that?

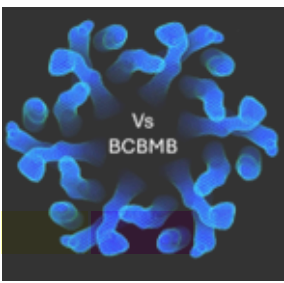
**Answer:** triacylglycerols are not amphiphilic! ... they can and do form emulsions (rather than micelles) because the steric bulk of these molecules does not allow for the micellar geometry



what will change/happen, if you were to replace one of the fatty acids in a triacylglycerol with a hydrophilic group?

replacement of one fatty acid with a hydrophilic component **creates very powerful amphiphiles with very low CMCs ( $\mu\text{M}$  to sub-nM range)**

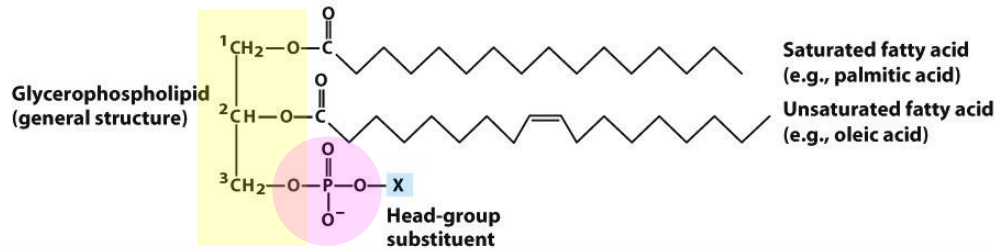
**nomenclature:** the hydrophilic part of a membrane forming lipid is called “headgroup”



# Phospholipids

## Are a Diverse Group of Lipids

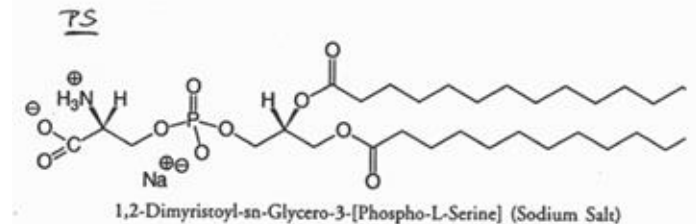
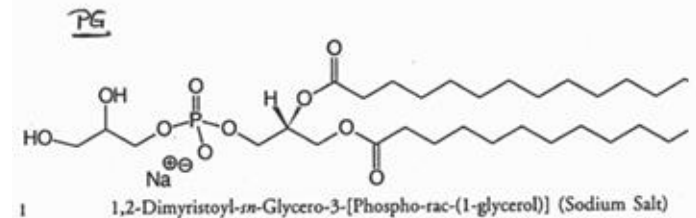
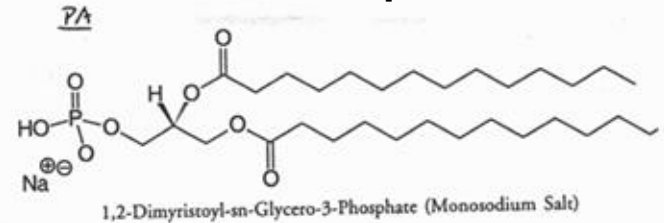
### That are Key to Formation of Membranes



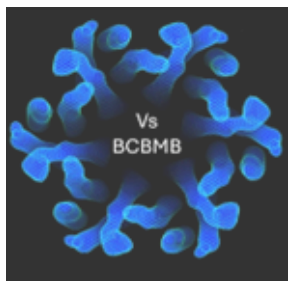
knowing what type of headgroups exist, and what their properties are is quite useful for understanding issues related to membranes

Name of glycerophospholipid	Name of X	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	— H	PA - 1
Phosphatidylethanolamine	Ethanolamine	— CH <sub>2</sub> —CH <sub>2</sub> —NH <sub>3</sub> <sup>+</sup>	PE 0
Phosphatidylcholine	Choline	— CH <sub>2</sub> —CH <sub>2</sub> —N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>	PC 0
Phosphatidylserine	Serine	— CH <sub>2</sub> —CH—NH <sub>3</sub> <sup>+</sup>   COO <sup>-</sup>	PS - 1
Phosphatidylglycerol	Glycerol	— CH <sub>2</sub> —CH—CH <sub>2</sub> —OH   OH	PG - 1
Phosphatidylinositol 4,5-bisphosphate	myo-Inositol 4,5-bisphosphate		PI - 4
Cardiolipin	Phosphatidylglycerol		- 2

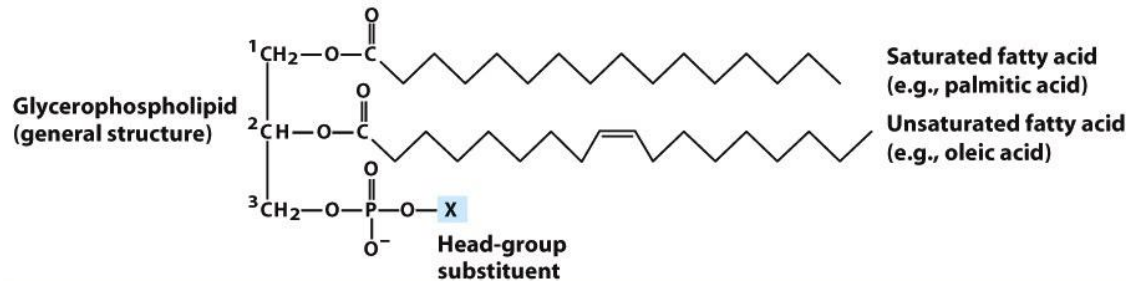
### examples







# Phospholipids



naturally occurring phospholipids usually have a saturated (C<sub>16</sub> or C<sub>18</sub>) fatty acid on C1, an unsaturated acyl chain (C<sub>18</sub> or C<sub>20</sub>) on C2.

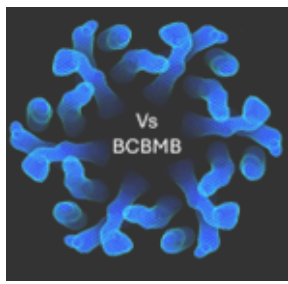
textbooks sometimes say that apart from few exceptions the variations in chain length/saturation and headgroup are not understood .... that is not entirely true .... you can understand some if it .....

Comment	Lipid	Melting Temp [°C]	if membranes were made of only these ...what would be the consequences?
	12:0 PC	-1	
not in mammals	13:0 PC	14	
	<b>14:0 PC</b>	23	➤ only could use short fatty acid chains to avoid "freezing the bilayer" = turns it rigid (which happens below the melting temperature)
not in mammals	15:0 PC	33	
	<b>16:0 PC</b>	41	
not in mammals	17:0 PC	48	
	<b>18:0 PC</b>	55	
not in mammals	19:0 PC	60	
	<b>20:0 PC</b>	66	<b>rigid bilayer = BAD</b> <b>even if you choose short chains to keep the membrane liquid, these membranes would be quite "thin" = MORE leaky = BAD</b>

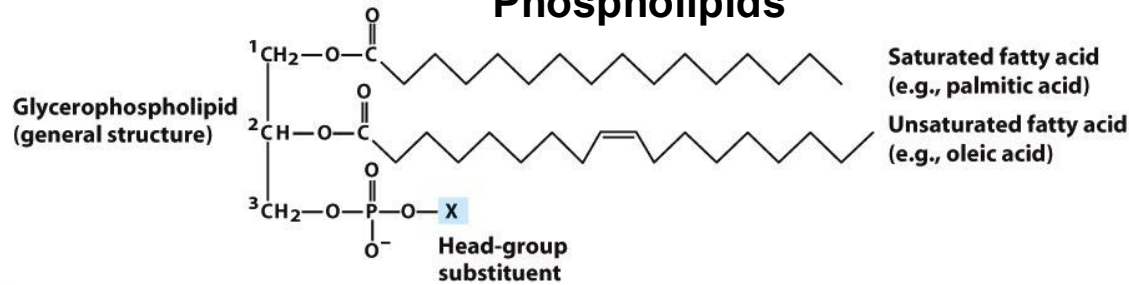
12:0 = both acids with 12 carbons, no double bond ...

even numbered because of biosynthesis from acetic acid (H<sub>3</sub>C-COOH)

**What Can you Do to Save Life??**



## Phospholipids



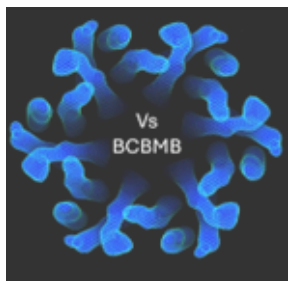
Comment	Lipid	Melting Temp [°C]	Lipid	Melting Temp [°C]
	12:0 PC	-1	14:0-16:0 PC	35
not in mammals	13:0 PC	14	14:0-18:0 PC	40
	<b>14:0 PC</b>	23	16:0-14:0 PC	27
not in mammals	15:0 PC	33		
	<b>16:0 PC</b>	41	<b>BUT</b>	
not in mammals	17:0 PC	48	16:0-18:0 PC	49
	<b>18:0 PC</b>	55	<b>16:0-18:1 PC</b>	-2
not in mammals	19:0 PC	60		
	<b>20:0 PC</b>	66	Similar	
			16:0-18:1 PG	-2
			16:0-18:1 PS	14
			16:0-18:1 PA	28
			16:0-18:1 PE	25

12:0 = both acids with 12 carbons, no double bond ...

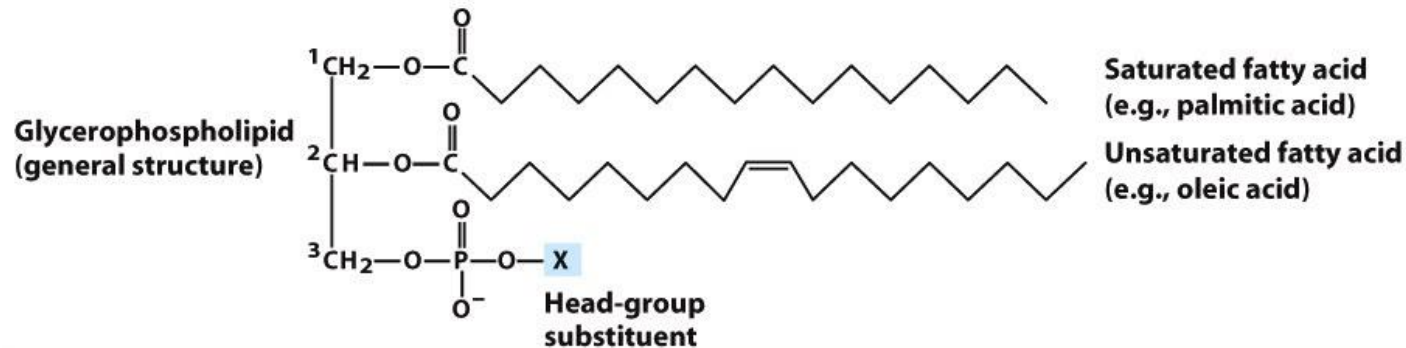
**even numbered because** of biosynthesis from acetic acid (H<sub>3</sub>C-COOH)

16:0 -18:1 = one saturated acid with 16 carbons, 1 singly unsaturated acid with 18 carbons (oleic acid, slide 24)

**you add unsaturated fatty acids ...**even substituting one of the two fatty acids with a singly unsaturated fatty acid decreases the melting temperature to well below body temperature! You will understand in a little bit why that is ...

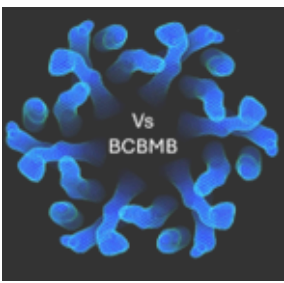


# Phospholipids



## ➤ Roles of Phospholipids

- phospholipids are the primary components of biological membranes
- PI (phosphatidylinositol) plays important roles in cellular signal transduction (we will talk about it later in a later chapter; example for **modularity = chemically identical molecule with different biological functions**; we came across this already in the case of ATP, "Chemical Evolution" Chapter)
- PS (phosphatidylserine) can serve as an apoptotic signal and to recruit/stabilize/retain peripheral membrane proteins at the membrane surface (also an example for **modularity**)
- phospholipids often modulate the function of membrane embedded proteins



# Adding to the Repertoire of Lipids Involved in Membrane Formation: Sphingolipids

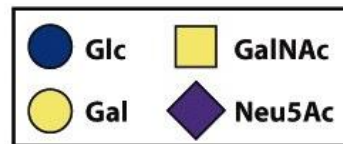


Sphingosine		
Sphingolipid (general structure)	$\begin{array}{c} \text{HO}-\text{CH}^3-\text{CH}=\text{CH}-(\text{CH}_2)_{12}-\text{CH}_3 \\   \\ \text{CH}^2-\text{N}-\text{C} \\   \quad   \quad    \\ \text{H} \quad \text{H} \quad \text{O} \\   \\ \text{CH}_2^1-\text{O}-\text{X} \end{array}$	Fatty acid
Name of sphingolipid	Name of X—O	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	$\begin{array}{c} \text{O} \\    \\ \text{P}-\text{O}-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3 \\   \\ \text{O}^- \end{array}$
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

## important differences to glycerophospholipids

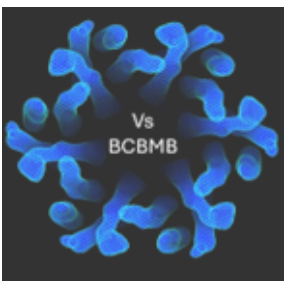
➤ **sphingolipids use the amino alcohol sphingosine (instead of glycerol) as scaffold.**

- ✓ note: sphingosine has a long, unsaturated hydrocarbon chain (16 C-atoms) = no need to add an unsaturated fatty acid at C2
- ✓ the fatty acid at C2 is bonded through a peptide bond (not an ester)...hugely important
- ✓ sphingolipids often have complex sugar based headgroups
- ✓ sphingomyelin has a phosphocholine headgroup = can also be classified as "phospholipid"



Glc: glucose; Gal: galactose;  
GalNAc: N-acetyl-galactosamine  
Neu5Ac: N-acetylneuraminic acid  
→ All carbohydrates ... we will cover this in more detail in the "Carbohydrates" chapter

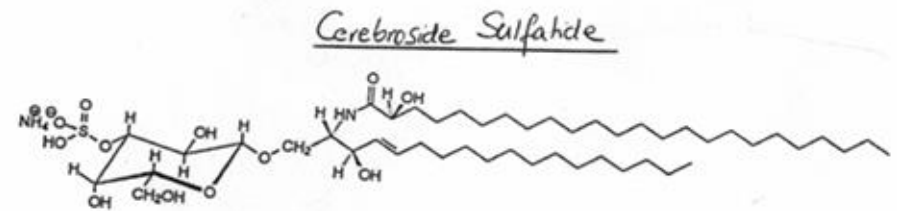
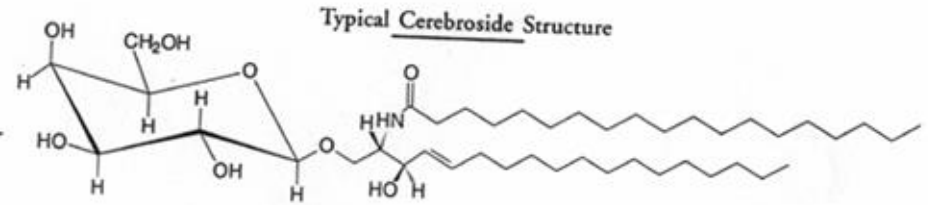
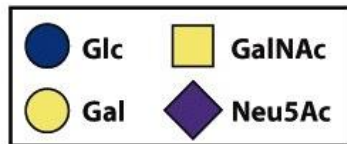
**knowing common sphingolipids and how they differ from phospholipids is useful to understand membrane biochemistry**



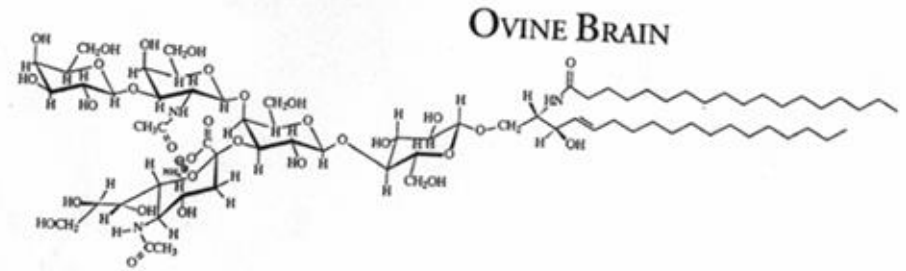
# Here Are Three Physiologically Relevant Examples



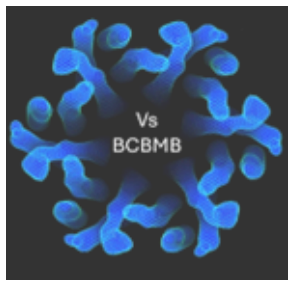
Sphingolipid (general structure)		
Name of sphingolipid	Name of X—O	Formula of X
Ceramide	—	— H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	



## GANGLIOSIDE G<sub>M1</sub>



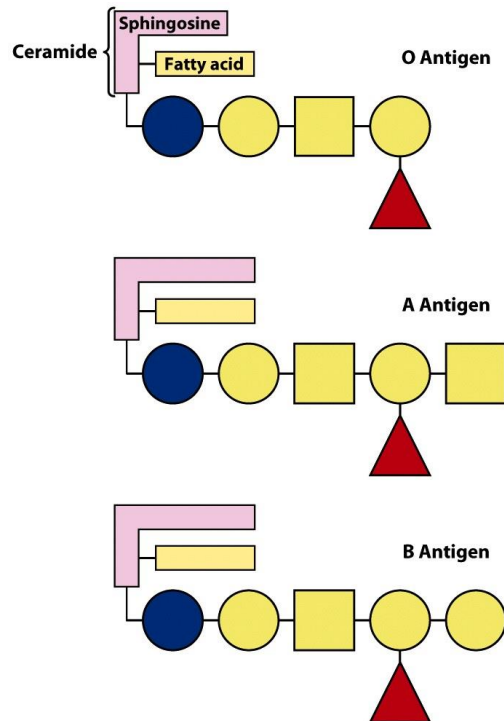
curiously: the "sulfatide" carries a negatively charge sulfate modification



## Some Biological Roles of Sphingolipids

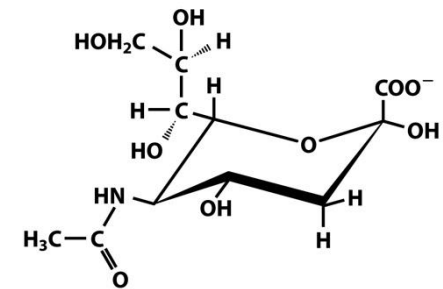


sphingomyelin (a phospholipid) plays important roles in the myelination of nerve fibers

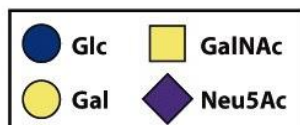


glycolipids are predominantly found on the cell surface where:

- glycosphingolipids **determine blood group** (left) and
- gangliosides play **important but poorly understood roles in molecular recognition**. They are characterized by the presence of “sialic acid” (N-acetylneuraminic acid)

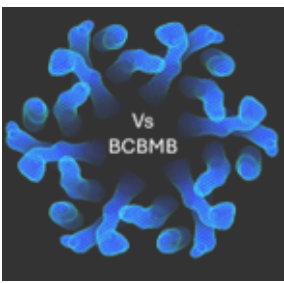


*N*-Acetylneuraminic acid (a sialic acid)  
(Neu5Ac)

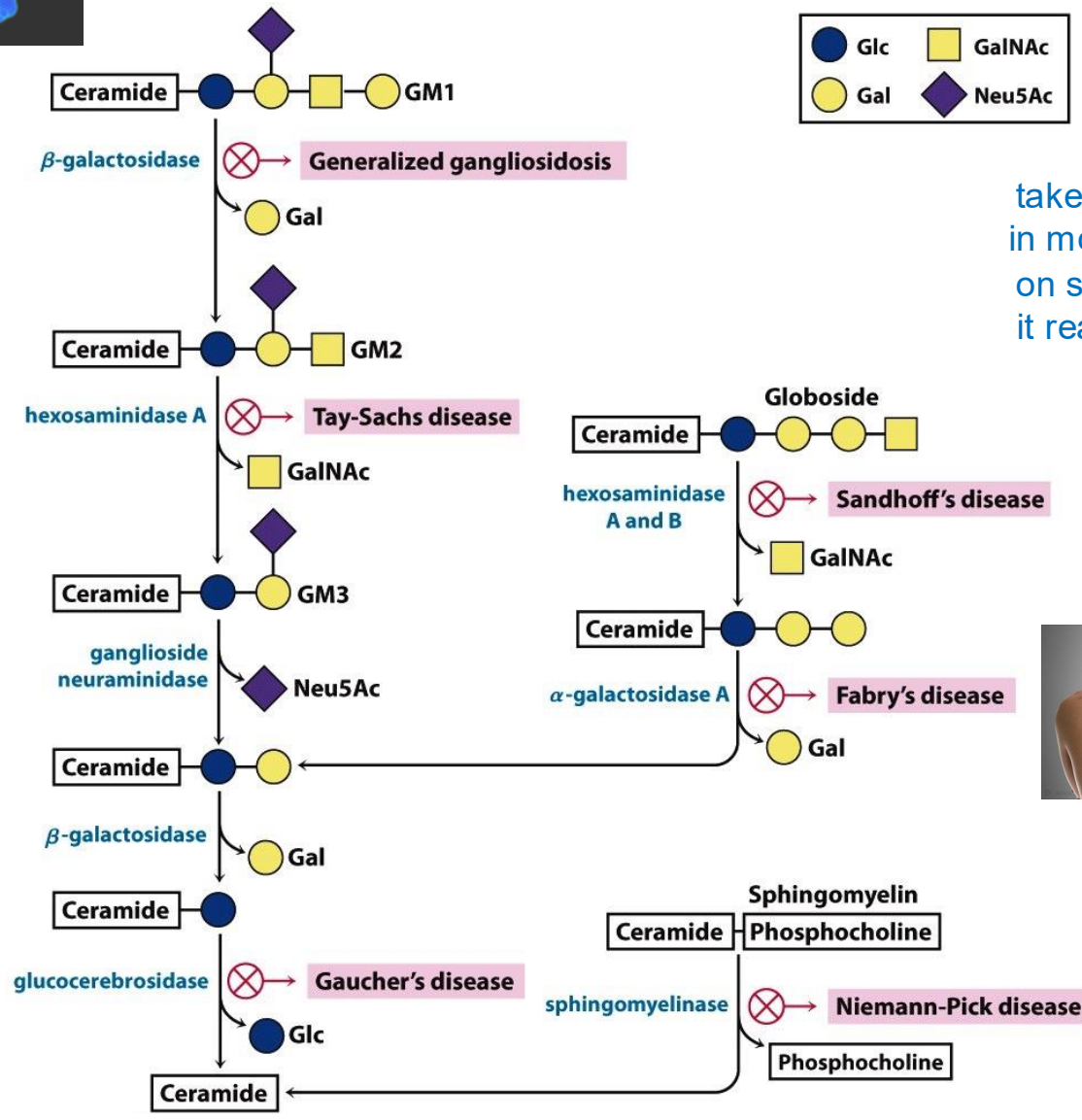


Fucose

(sugar with 6 carbons, but lacking the –OH group at C6)



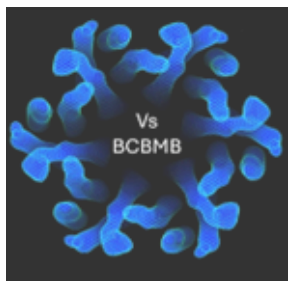
If you are "Premed" ... Here is the Angle for You:  
**Diseases Associated with Sphingolipid Metabolism**



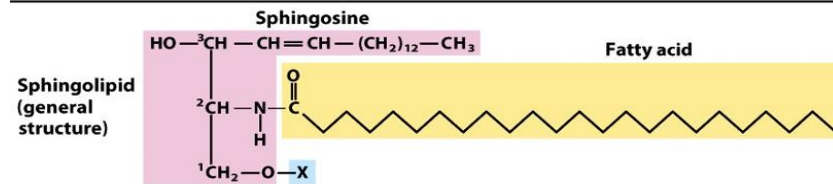
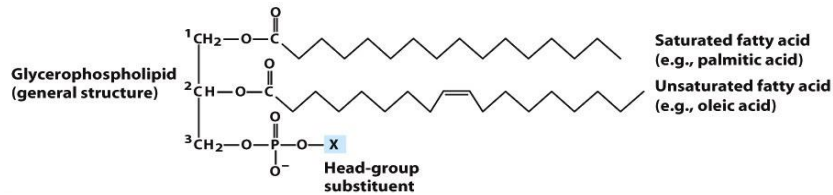
take Note how almost every defect in modifying the sugar modifications on sphingolipids leads to disease – it really drives home how important this class of lipids is



**Fabry's Disease**  
 Early on presents with skin lesions, but in 3<sup>rd</sup>/4<sup>th</sup> decade progresses to affect internal organs  
 → kidney damage/failure and heart dysfunction are common



# Recap

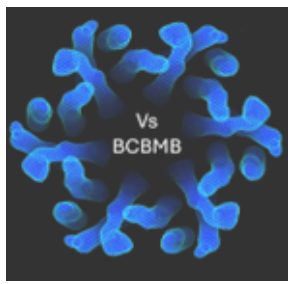


- learned that fatty acids are a major component of more complex lipids known as phospholipids and sphingolipids
- learned that just like free fatty acids and cholesterol, phospholipids and sphingolipids are amphiphiles

	Scaffold	Fatty Acid	Linkage	Headgroup Linkage
<b>Glycerophospholipid</b>	Glycerol	2	Ester	Phosphodiester
<b>Sphingolipid</b>	Sphingosine	1	Amide	Glycosidic (if carb) Phosphodiester (sphingomyelin)

## Goals From Here On Out

- get an appreciation for the molecular complexity of biological membranes
  - get an appreciation for dynamic behavior of biological membranes

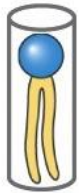


## From Single Lipids to Biological Bilayers

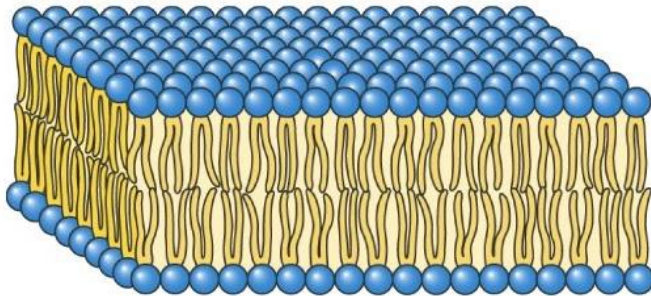


just as it drives the formation of micelles (or initial states of protein folding), the **hydrophobic effect** dictates that **diacylglycerols, and sphingolipids (in most cases) form bilayered structures (aka biological membranes) - why?**

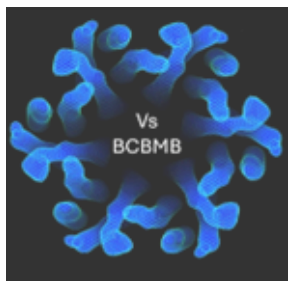
*....try to find your own words to answer this question...  
if you worked through the Fundamentals Lipids Tutorial, you may recall  
how we derived the answer to this question....*



**Individual units are cylindrical (cross section of head equals that of side chain)**



**(b) Bilayer**

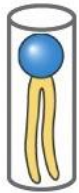


## From Single Lipids to Biological Bilayers

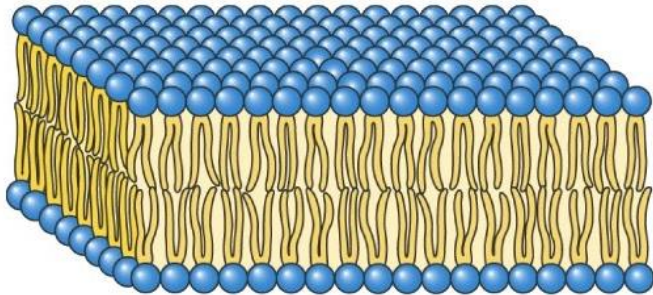


just as it drives the formation of micelles (or initial states of protein folding), the hydrophobic effect **dictates that diacylglycerols, and sphingolipids (in most cases) form bilayered structures (aka biological membranes) - why?**

**Answer:** hydrophobic part of membrane forming lipids is too large to allow micelle formation



**Individual units are cylindrical (cross section of head equals that of side chain)**

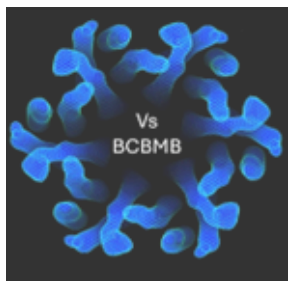


**(b) Bilayer**

while cartoons of bilayers - like the one shown here - are very common and familiar, there is something fundamentally wrong with this picture  
(and that "wrong" makes it really difficult to understand how membranes function)

**what is the big shortfall in this cartoon?**

*....try to answer ....*



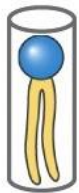
## From Single Lipids to Biological Bilayers



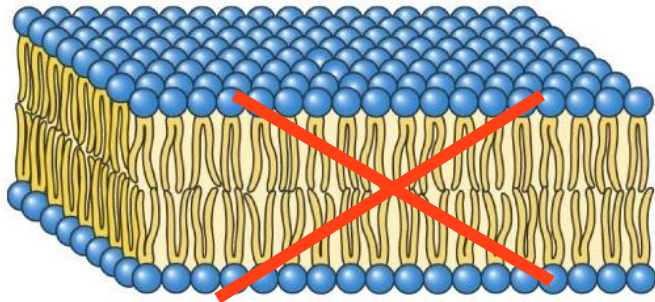
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**Answer:** hydrophobic part of membrane forming lipids is too large to allow micelle formation

while cartoons of bilayers - like the one shown here - are very common and familiar, there is something fundamentally wrong with this picture  
(and that "wrong" makes it really difficult to understand how membranes function)



**Individual units are cylindrical (cross section of head equals that of side chain)**

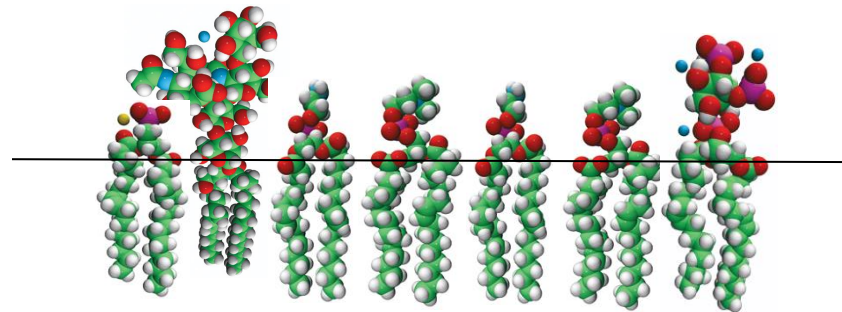


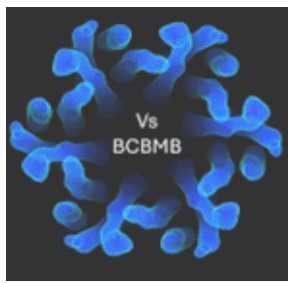
**(b) Bilayer**

**what is the big shortfall in this cartoon?**

**Answer:** it ignores molecular structure!

**more correctly:** bilayer is mix of different (>100) types of lipids





## Structural Diversity of Membrane Lipids Causes Complex Phase Diagrams



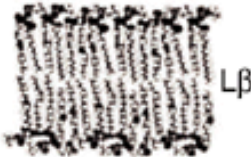
outsch....yes – this looks complicated and difficult ... though you may remember some from general chemistry....

the take home message here: the physical state and structure of the bilayer depend on the mixing ratio of different lipids in the mix ....

liquid crystalline



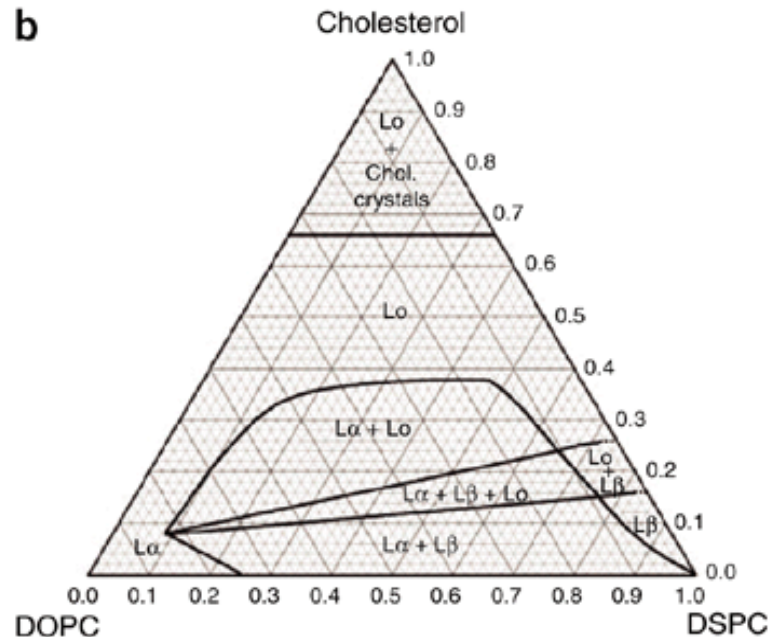
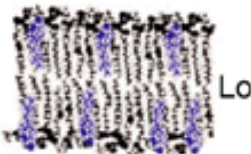
gel phase



tilted gel phase

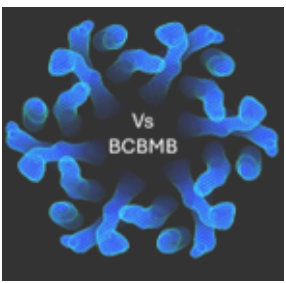


liquid ordered



Feigenson, GW (2006), *Nat Chem Biol*, 2:560-563

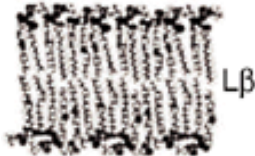
you can find more detailed, easy to read and short description of these different phases here: [lipid phases](#)  
the ones that have a blue underlay are the ones relevant in biology



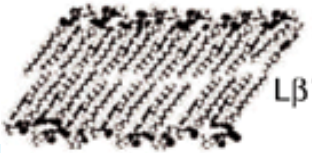
liquid crystalline



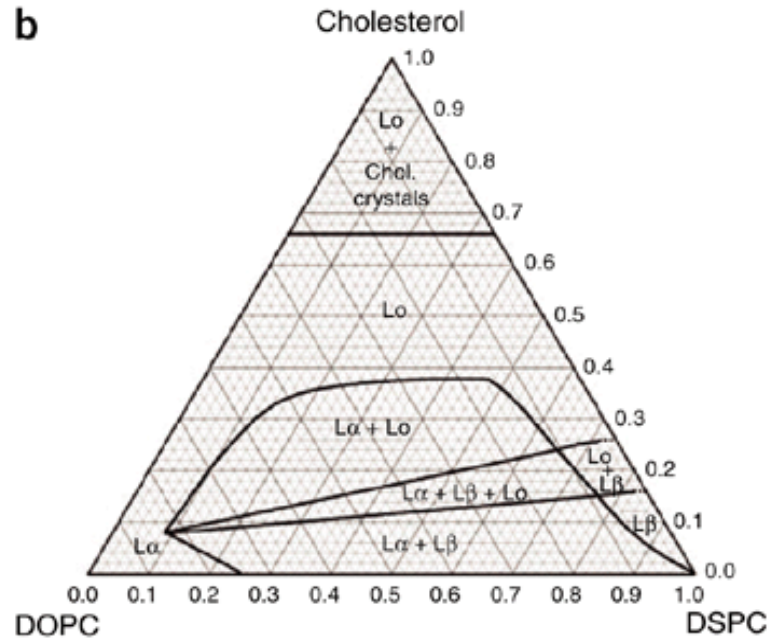
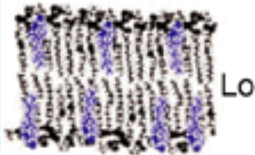
gel phase



tilted gel phase



liquid ordered



Feigenson, GW (2006), *Nat Chem Biol*, 2:560-563

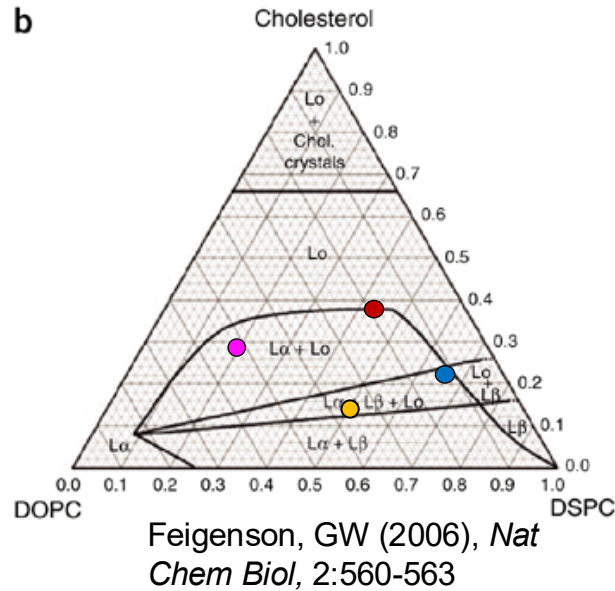
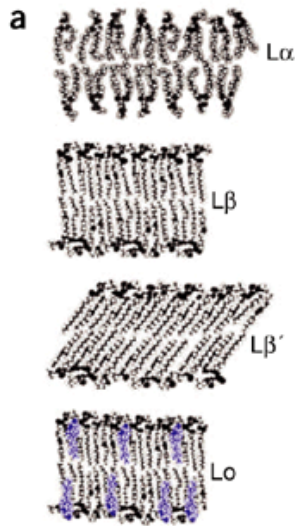
### What Does Any of This Mean.....?

let's look at an example.

in this example (next slide), researchers made very large liposomes from the three lipids shown in the phase diagram above, using different mixing ratios. They added a fluorescent dye whose color depends on the physical state of the lipid bilayer (eg whether it is liquid or rigid)

# Phase Diagrams – What Do They Mean in Real Life?

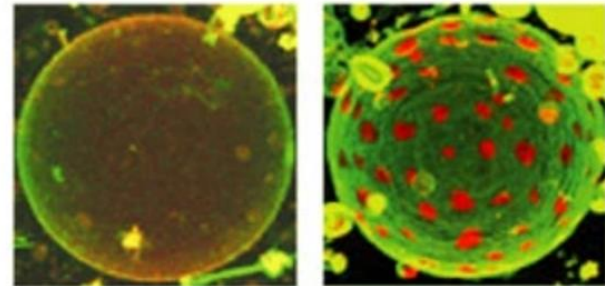
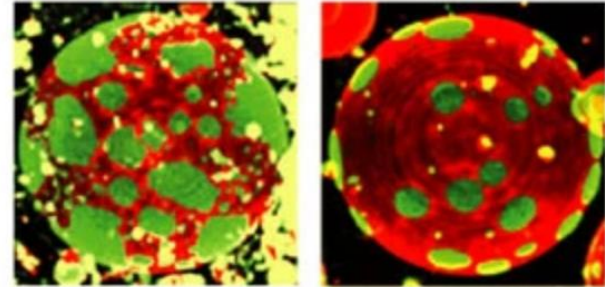
liquid crystalline



DSPC(18:0,18:0):DOPC (18:1,18:1): Cholesterol

50:36:14

66:12:22



20:52:28

42:19:39

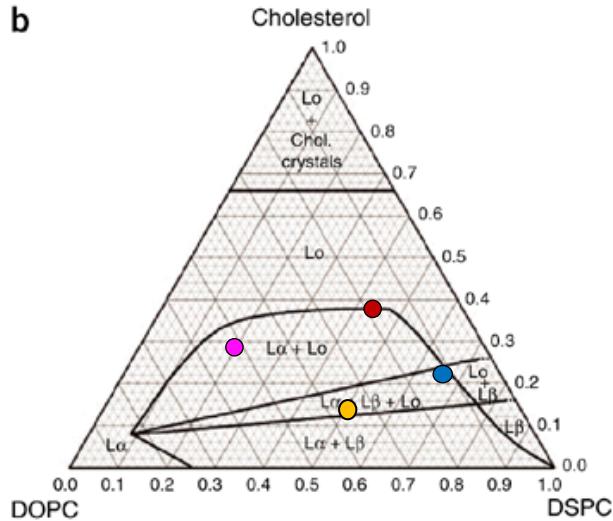
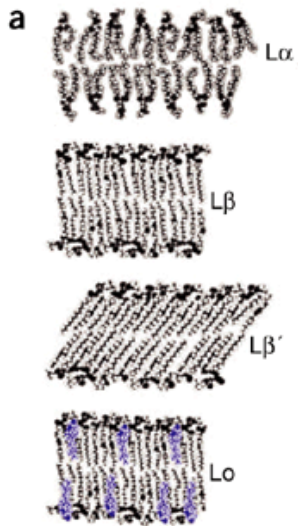
**Note:** Colors in images of Giant Unilamellar Vesicles to the right mark different phases (not lipids): green marks  $L_{\alpha}$ ; red marks  $L_{\beta}$

this looks wild ... but it tells you that at the 20:52:28 ratio, all three components co-exist in the liquid crystalline state (most common in biological bilayers), while at any of the other mixing ratios you get spontaneous "phase separations" = formation of regions where one of the components gets locally enriched, changing the physical state (liquid or solid) of that region.

curious Question: how will the complexity of the phase diagram change as you go from 3 to 100 of different lipid types?

# Phase Diagrams – What Do They Mean in Real Life?

liquid crystalline

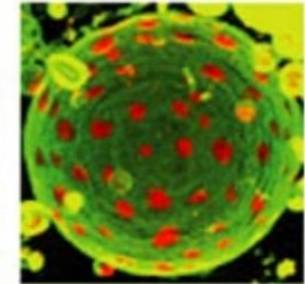
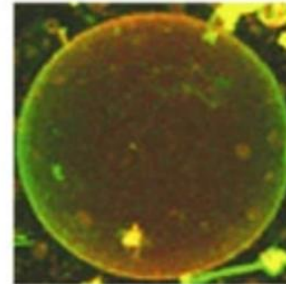
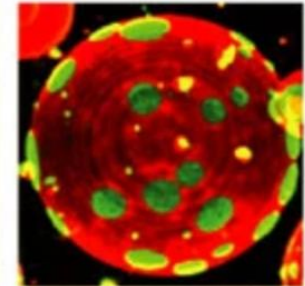
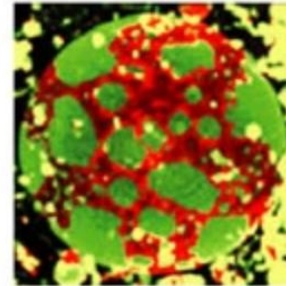


Feigenson, GW (2006), *Nat Chem Biol*, 2:560-563

DSPC(18:0,18:0):DOPC (18:1,18:1): Cholesterol

50:36:14

66:12:22



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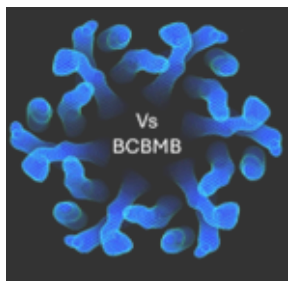
42:19:39

**Note: Colors** in images of Giant Unilamellar Vesicles to the right **mark different phases** (not lipids): green marks  $L_{\alpha}$ ; red marks  $L_0$

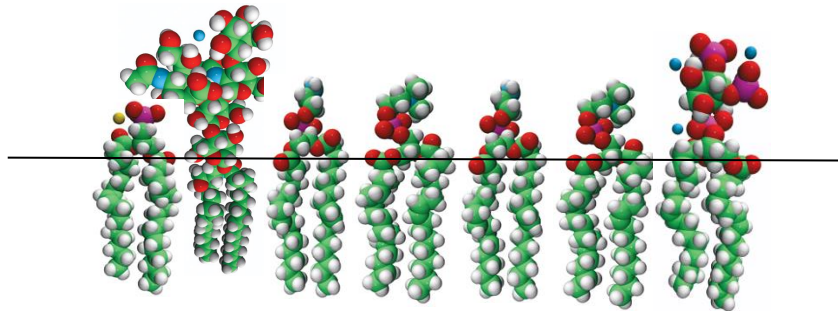
Curious Question: how will the complexity of the phase diagram change as you go from 3 to 100 of different lipid types?

**Answer: immensely**

for instance, if you assume 100 different lipids, you – theoretically – have up to 101 coexisting phases  
 → why on Earth would you want such a “messy” system??

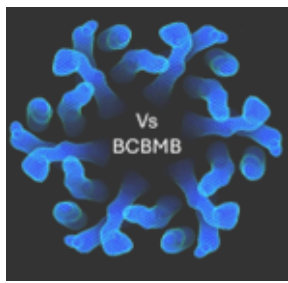


## Structural Complexity is Necessary For Bilayer Function

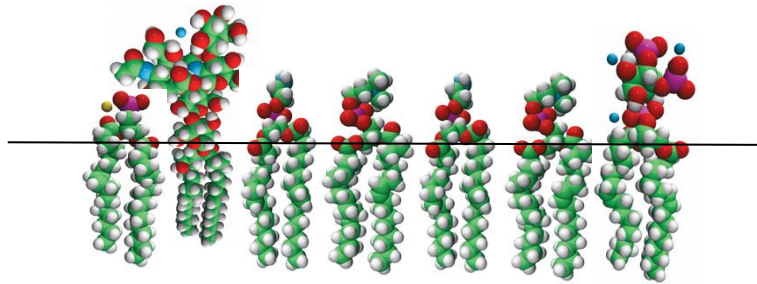


the complexity of the phase diagrams of biological membranes has two, causally related tangible consequences:

1. **(partial) demixing** = formation of lipid domains with lipid composition being different from bulk. This process is driven by differences in headgroup interactions (more/less favorable) and incompatibility in acylchain (eg lipids with shorter chains do not like to mix with lipids that have long acylchains)
2. the large number of different lipids + thermal energy in the system prevents the "demixing" from EVER finding a stable endpoint  
→ bilayers are forever "frustrated" and metastable



## Structural Complexity is Necessary For Bilayer Function



the complexity of the phase diagrams of biological membranes has two, causally related tangible consequences:

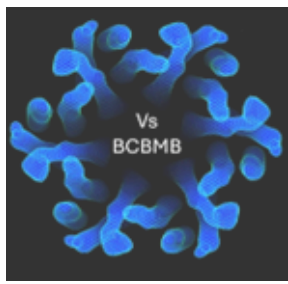
(partial) demixing & forever “frustrated” and metastable

why would the cell “want” a bilayer that is thermodynamically frustrated and constantly tries to demix?

**Answer:** membranes serve many functions (envelope, scaffolding, host to membrane embedded proteins, signaling organelle, capacitor, ...)

→ functional constraints **require** differences in lipid shape & dynamics

- (partial) demixing **provides the means to create spatially and temporally dynamic domains** (next slide) with specialized functions (eg accommodate different types of proteins) **by changing the physical properties of the bilayer (eg thickness, stiffness)**. The most notorious example for this are “rafts”, which form through interactions between cholesterol and sphingolipids (more on that two slides on)
- partial demixing also facilitates/induces membrane curvature (which is key for things like vesicle transport membrane fusion)



## Bilayers are Dynamic Entities



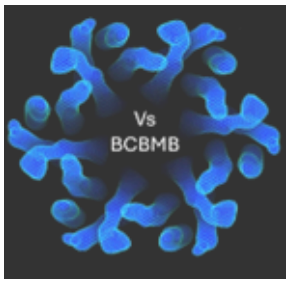
now that you hopefully gained some “respect” for the chemical complexity of bilayer - let's dispel the myth of them being “boring and passive” sheets

first stop: **compartmentalization** ... bilayers do that at a macroscopic level (= form membrane bound organelles) ..but they also have complex local substructures.

the thermodynamics of simple lipid mixes predict that substructures should form, but is there any experimental evidence for this happening in **actual** biological membranes?

**Answer: Yes – there is ....**and it came from studying the diffusion behavior of single, fluorescently labeled lipid molecules.

**to understand the evidence, let's first ask what you would expect to observe if you were to insert a labeled lipid into a bilayer and watch it over some time.....**



# Bilayers are Dynamic Entities



now that you have gained some “respect” for the chemical complexity of bilayer - let's dispel the myth of them being “boring and passive” sheets

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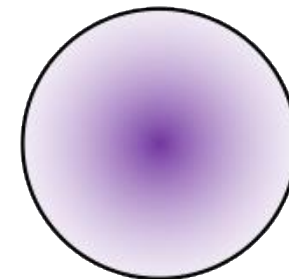
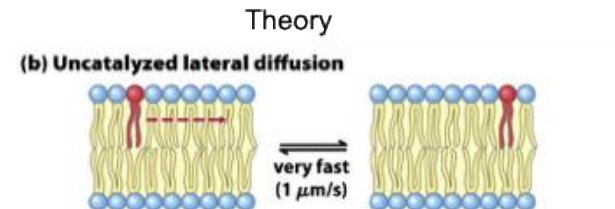
**to understand the evidence, let's first ask what you would expect to observe if you were to insert a labeled lipid into a bilayer and watch it over some time.....**

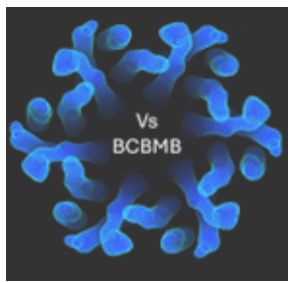
here is what you expect to see if diffusion was unimpeded and following the regular laws of diffusion:

If you follow the lipid over time you will find that it covers an area that has circular symmetry

(more specifically, the molecule samples the area close to where it started quite extensively, but occasionally explores regions farther away and in doing so, shows no bias where to go)

**does this hold up in practice??**



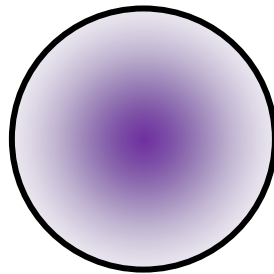
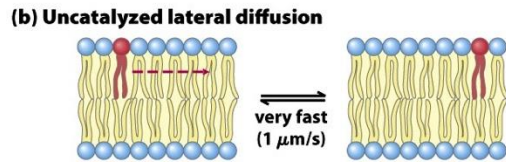


# Bilayers are Dynamic Entities

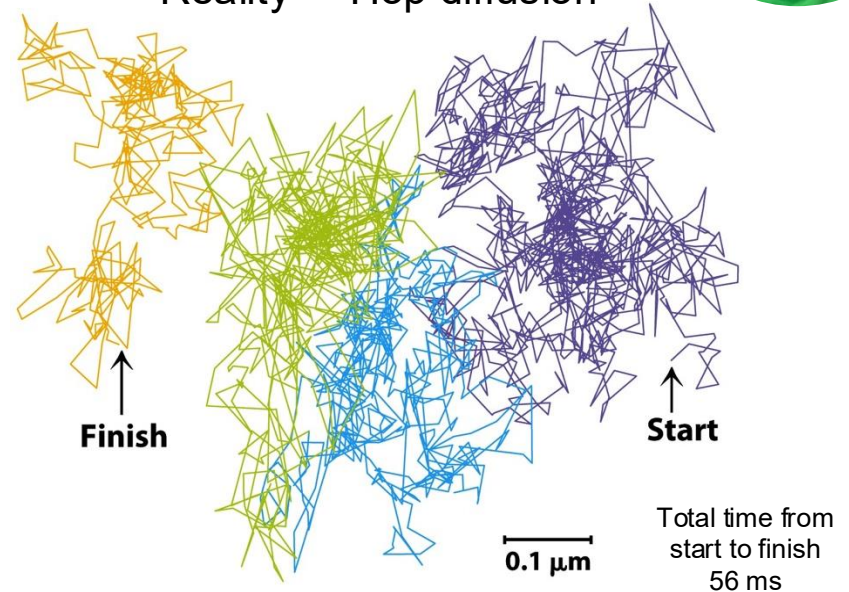


No ..... It Does Not

Theory



Reality - "Hop diffusion"



in **reality**, you initially see the molecule exploring a region in ways very similar to what theory predicts...but then something "weird" happens....

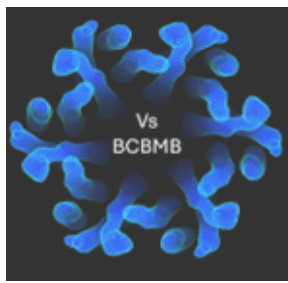
the molecule strays to an adjacent, but distinctively different "neighborhood"...explores (never returning to the original neighborhood) before getting bumped into yet another region, and on and on .....

There are also regions where the molecule never seems to go ....

**Only two ways to explain this behavior:**

➤ there are **physical obstacles** (like a membrane protein that takes up space the lipid cannot explore), and/or

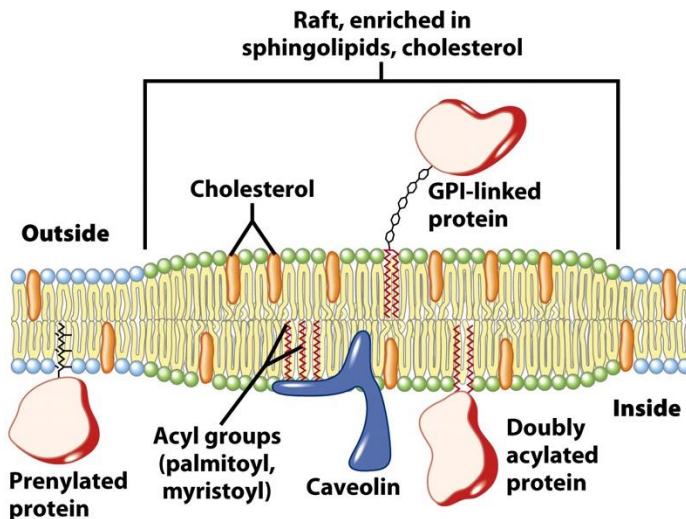
➤ there are distinct **microdomains in the bilayer that differ from each other in their lipid composition** ...the boundaries around these domains act like fences that make entering or leaving these region "unfavorable" ...but ...occasionally, the lipid "jumps", or is pushed into such a different domain .... and once there, it starts exploring (looking for a way out ... )



## The **Best “Understood” Example** for Membrane “Domain” Formation Are So-Called **“Lipid Rafts”**



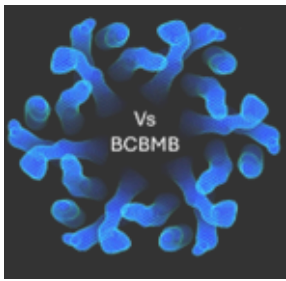
- rafts are regions that are rich in sphingolipids and cholesterol. These two lipids like each other because the hydroxyl group on cholesterol can hydrogen bond with the amide nitrogen in the sphingolipid scaffold (amino alcohol, sphingosine)
  - ➔ this interaction gives an energetic advantage to interactions between these two types of lipids, resulting in recruitment of more molecules to the raft, increasing its size (local depletion of the components, thermal motion, and actively regulated modifications of lipid/protein components counteract the recruitment to prevent the raft from becoming too large or too stable)
- biological function of rafts is still under investigation - but there is good evidence that some membrane proteins like to be in these environments
  - ➔ rafts become “hotspots” for some biological processes.



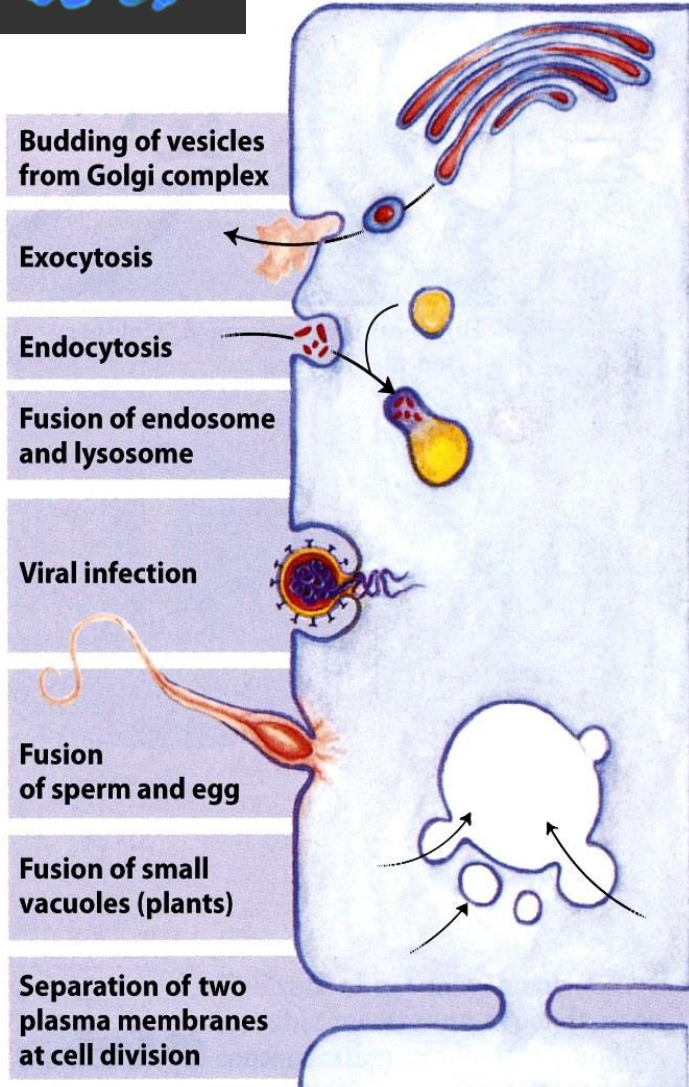
the existence of other types of “membrane domains” can be observed in *in vitro* systems, and is supported by “hop-diffusion” observed in real membranes

however, the biological functions and dynamics of non-raft domains are largely unknown, and difficult to study (very small, very dynamic, and short lived)

what is becoming abundantly clear though is that – aided by the metastability - membranes are constantly being “remodeled”



# Membrane Remodeling - A Crucial Process in Cell Biology

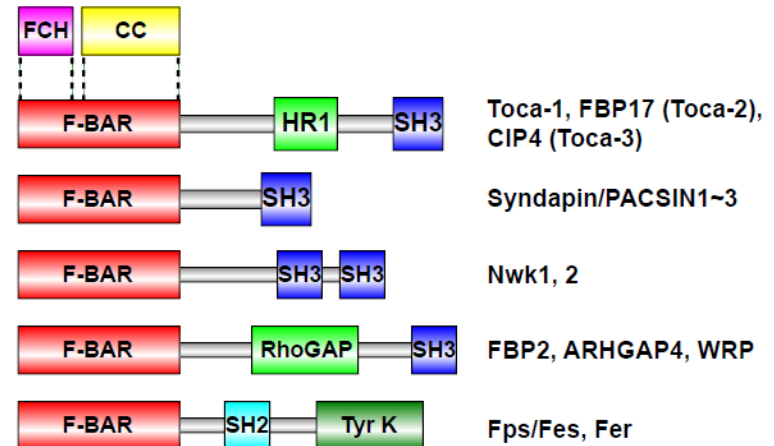


## examples for processes requiring membrane remodeling :

- vesicle transport
  - viral entry and exit
  - synaptic signal transmission
  - cell migration
  - cell division
  - exocytosis
  - endocytosis
- } separate chapter

## common to all these processes:

membranes need to change their degree of curvature (=need to be bent), which - at the rate required to support cellular processes - is accomplished by proteins from the BAR-domain superfamily



# Lipids - The Shape Matters

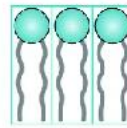
on slides 60-62, you learned how simplified cartoons of bilayers greatly misrepresent their actual structure. In fact – if the simplified structure was true, none of the processes listed on the previous slide could happen because they all require a "flat bilayer" to be bent. This "change in curvature" – as it is called - is aided by the different geometric and charge properties of different lipids

- Phosphatidylserine (PS)
- Phosphatidylcholine (PC)
- Sphingomyelin (SM)
- Phosphatidylinositol (PI)

Cylinder



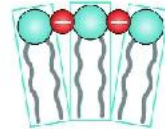
Flat



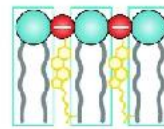
Scheme of differently-shaped lipids and their associated membrane curvature impact. Because their headgroup size is similar to that of their hydrophobic tails, lipids such as PS, PC, and SM exhibit roughly cylindrical shape and tend to form flat membranes. However, the repulsive forces of the negatively charged PS headgroup can induce membrane bending. This curvature can be buffered by cholesterol intercalation, which reduces the local charge density. In contrast, PI and lysophospholipids (LysoPLs) have larger headgroups relative to their tails, giving them an inverted cone shape that favors positive (convex) membrane curvature. Conversely, PA, PE, and ceramide (Ce) possess small headgroups, resulting in a conical shape that promotes negative (concave) membrane curvature.

Phosphatidylserine (PS)

Convex



Flat



+ Cholesterol

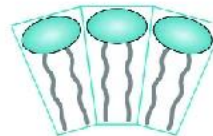


- Phosphatidylinositol phosphates (PIPs)
- Lysophospholipids (LysoPLs)
- Gangliosides

Inverted conical



Convex

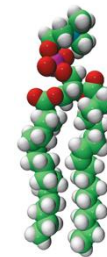
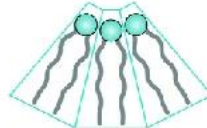


- Phosphatidylethanolamine (PE)
- Phosphatidic acid (PA)
- Ceramide (Ce)

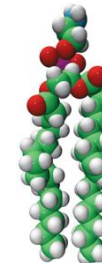
Conical



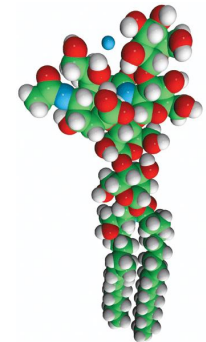
Concave



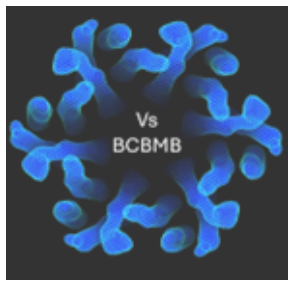
PC



PE



gangliosides

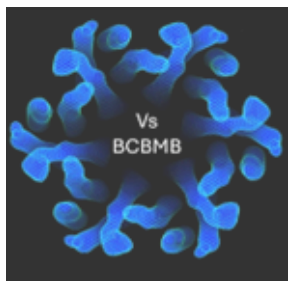


## Biological Bilayers - Another Paradigm:

**The Hydrophobic Effect Also Dictates That “Planar”  
Bilayers Fold Back On Themselves To Form Closed  
Vesicular Structures - Why?**



*.....try to answer before moving to next slide*



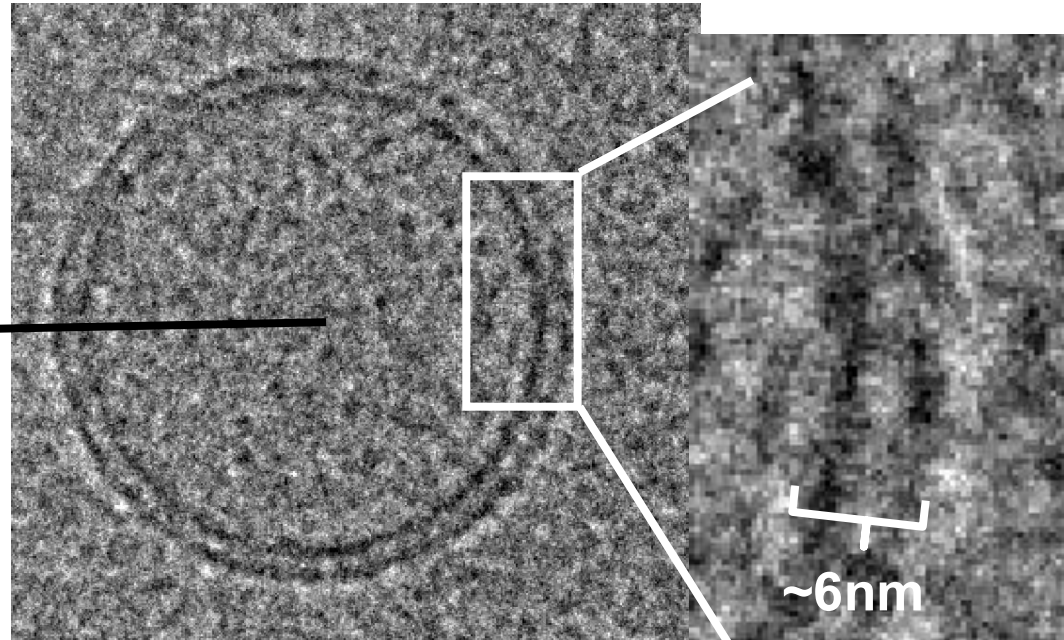
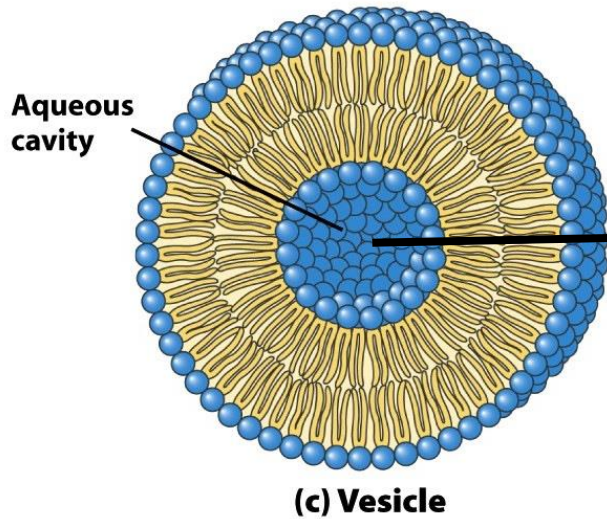
## Biological Bilayers - Another Paradigm:



The Hydrophobic Effect Also Dictates That “Planar” Bilayers Fold Back On Themselves To Form Closed Vesicular Structures - Why?

**Answer:** cannot have hydrophobic core exposed to water around the edges of a flat sheet

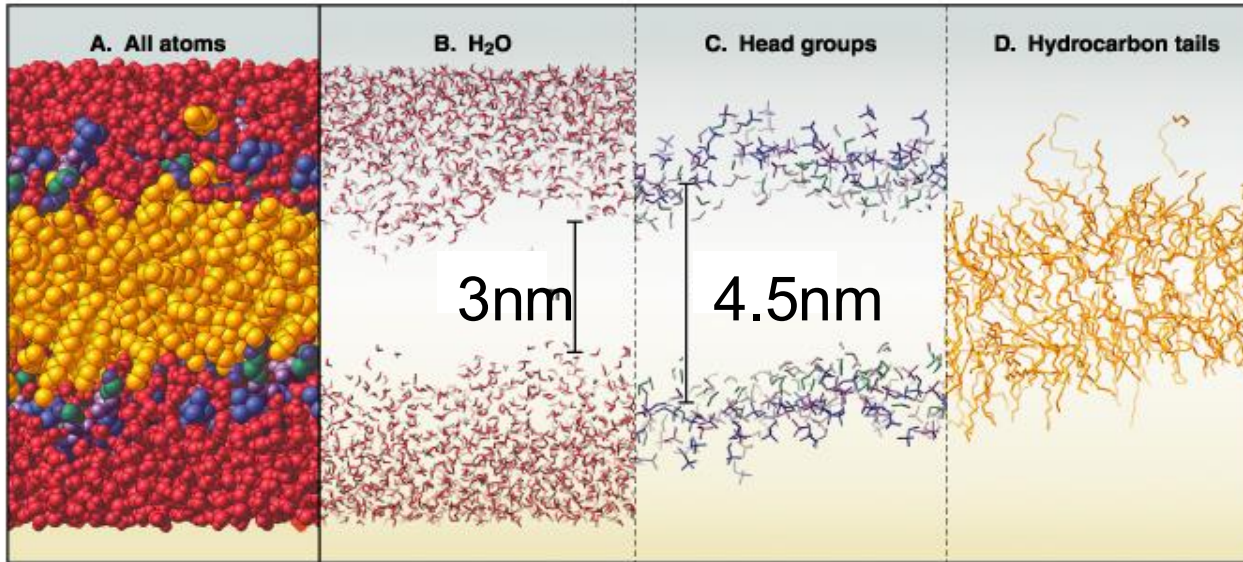
electron micrograph of a liposome in aqueous solution



Remember this from "Fundamentals – LIPIDS" Chapter?

# Lipid Bilayer as seen by Molecular Dynamic Calculations

a computational approach to simulate/calculate molecular models and dynamics



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Figure taken from Pollard & Earnshaw (2002, "Cell Biology", Ch6)

Ô modeling of bilayers makes insightful contributions to our understanding of membrane dynamics. The example here is from some of the pioneering efforts in this area.

Ô current state-of-the-art-all atom simulations can be done for ~1000 fully hydrated lipid molecules (including mixes of different types of lipids) for up to a few microseconds. For larger, more "life like" mixes (or time scales), simplifications are necessary to make computation manageable.

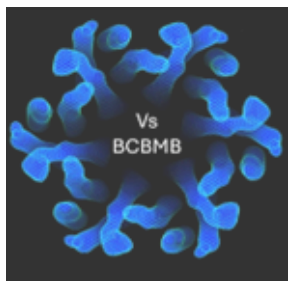
## TAKE NOTE:

Simulations predict two things that are counterintuitive

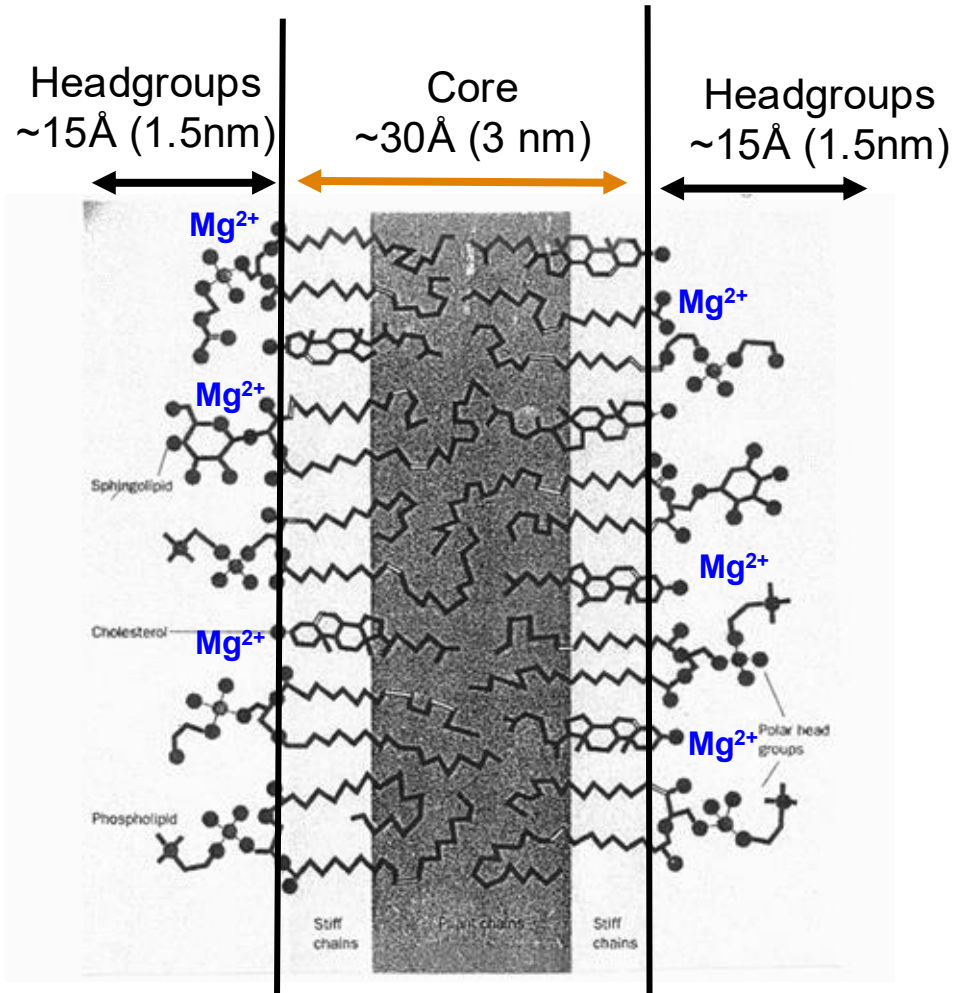
- At any time, a few water molecules can be found inside the hydrophobic core!
- At any time, hydrophobic acylchains can be "slung" out into the aqueous phase!

you would not guess this to be the case, but it actually is essential for processes like membrane fusion to work.

If you are unclear how the acylchain could escape from the core ... look at the structures in slide 50 and remember that the C-C single bonds in the glycerol backbone have free rotation= the molecule can adopt conformations where acyl-chains point in the opposite direction.



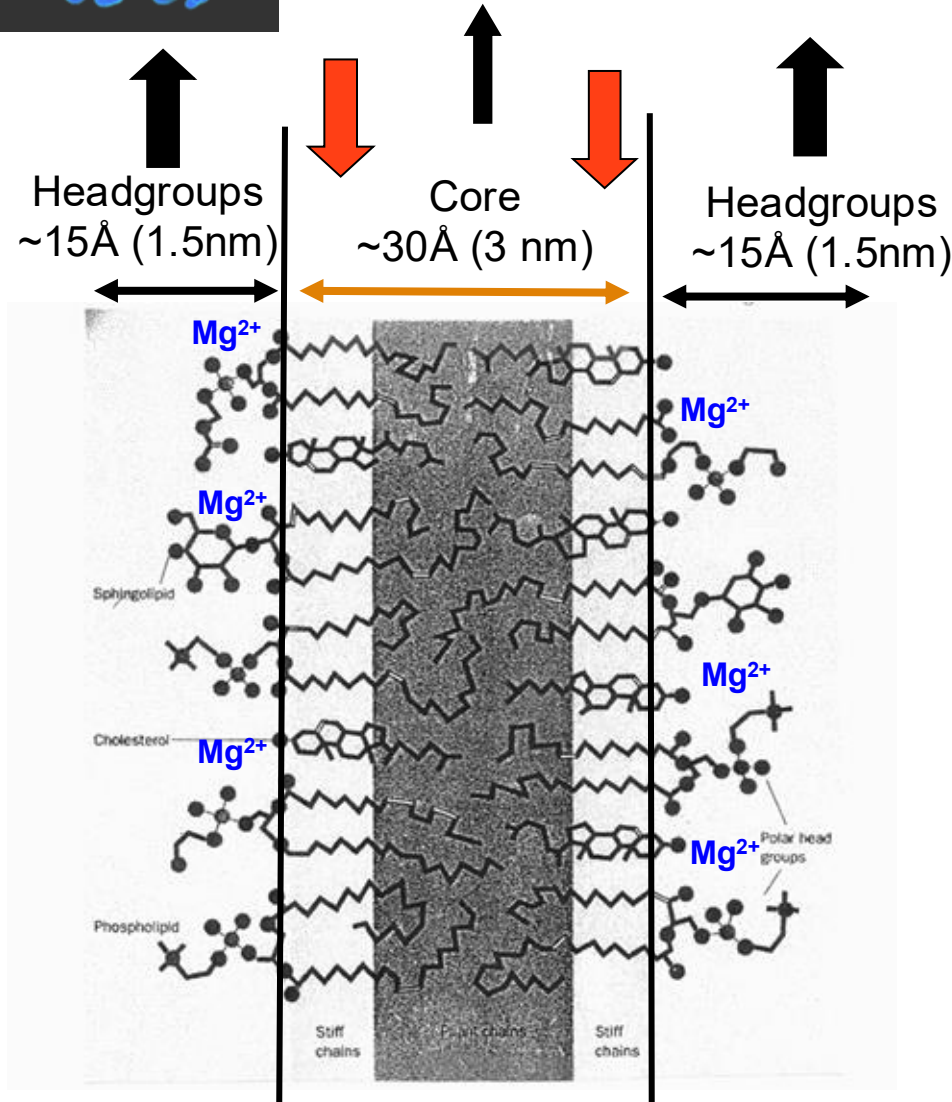
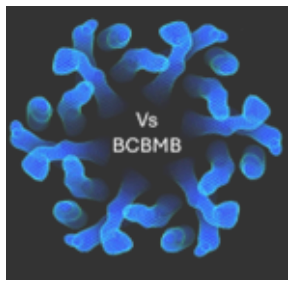
## Bilayers are Violent Environments!



Thermodynamics has quite interesting consequences for bilayers .... Why?



# Bilayers are Violent Environments!



Thermodynamics has quite interesting consequences for bilayers .... Why?

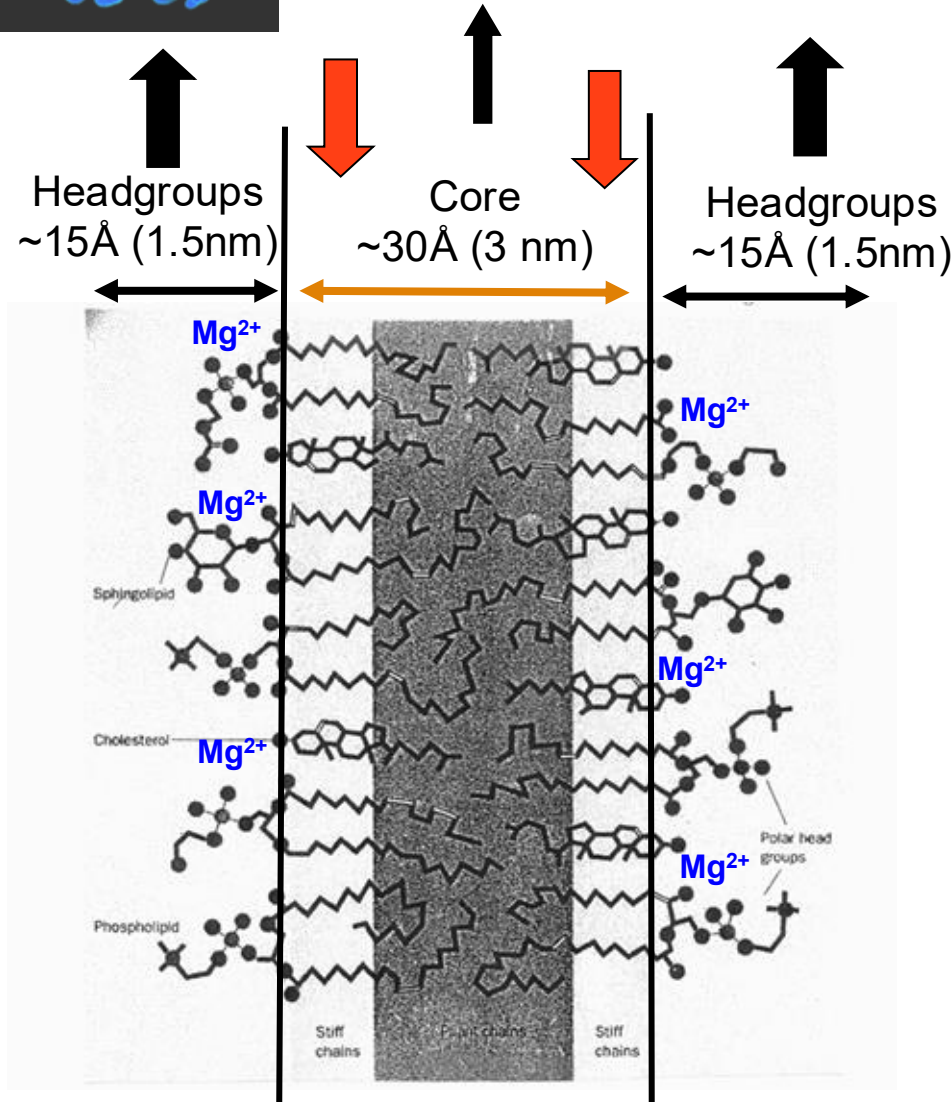
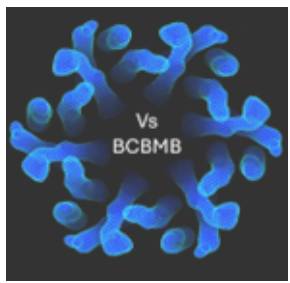
the **headgroups** on the surface **as well as** the disordered, more **loosely packed acylchains** in the center of the bilayer **want to move away** from each other to **maximize surface/space occupancy** ....(black arrows)

**trying to do this opens gaps along the interface** with the aqueous environment, leading acylchains to become exposed to water ....

→ **this is not stable** → the **atoms right at the interface between the head groups and acylchains try to pull back together** (orange arrows), aided by **Mg<sup>2+</sup>-ions** that reduce electrostatic repulsion between phosphate linkage of headgroups (phospholipids!) + ordering of acylchains along the first few atoms.



## Bilayers are Violent Environments!

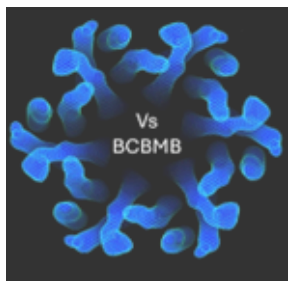


thermodynamics has quite interesting consequences for bilayers .... Why?

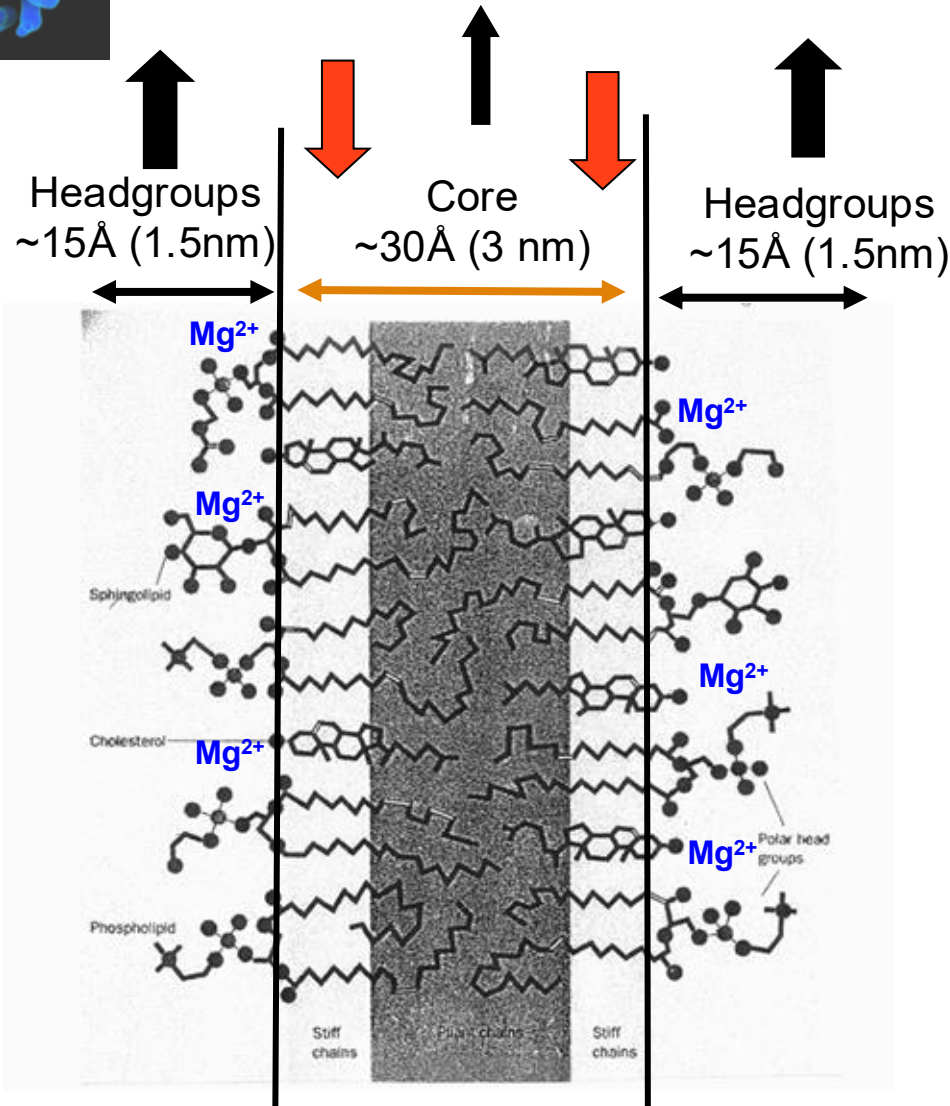
the conflicting tendencies = spreading out vs contracting create pressures that in this case are called:

**lateral pressures.** In the bilayer, these pressures are fighting each other (arrows) and, at the interfacial regions, reach well over 100 atm - this is a scary number ... why?

*...do you know? ... where on Earth would you find a pressure like this...?*



# Bilayers are Violent Environments!



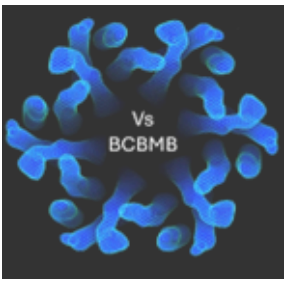
lateral pressures inside the bilayer are fighting each other (arrows) and, at the interfacial regions, reach well over 100 atm - this is a scary number ... why?

**Answer:** a lateral pressure of 100 atm is equivalent to the hydrostatic pressure at ~1000m depth.

curious trivia: some membrane transport proteins exploit these pressures to help them change shape during their conformational transport cycles.

in addition: cholesterol molecules rotate about their own long axis ... at what speed do you think these molecular blades go?

**Answer:** ~100,000 rpm  
(almost top speed of ultracentrifuges!)

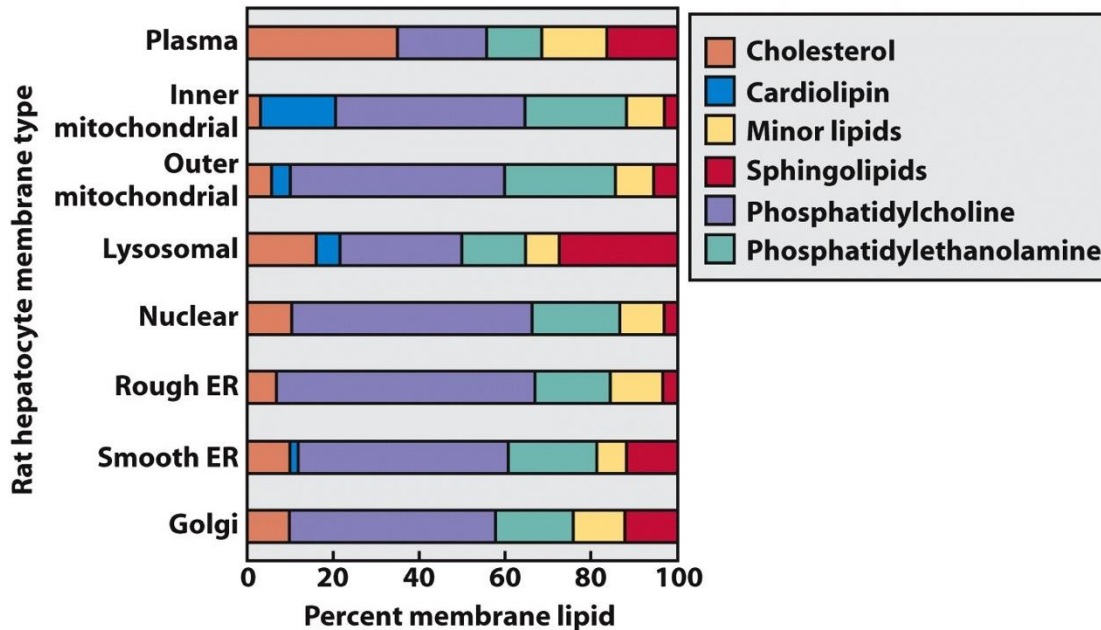


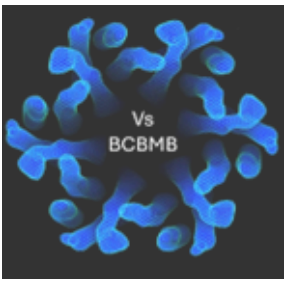
## Bilayers - Marvels and Mysteries



now that you know the basic makeup of bilayers - we are done ...aren't we? what else is there to know - other than that bilayers are “greasy”, “liquidish” envelopes whose function is to separate whatever is on one side from whatever is on the other side?

turns out, we only scratched the surface - bilayers are probably the most mind-boggling organelle of all ... here is why....look at the figure and verbalize what you see....



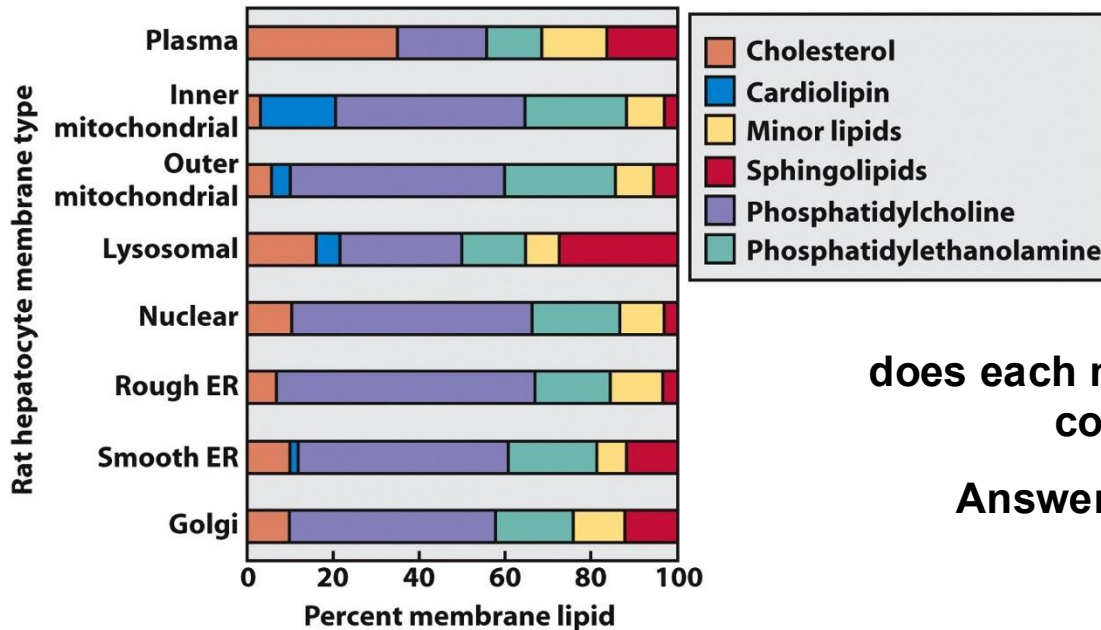


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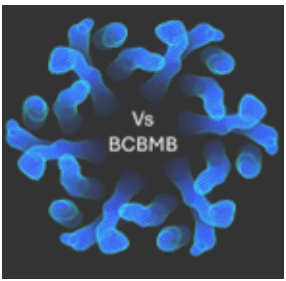


within a cell, each membrane has its own compositional "fingerprint" = each membrane is specific.

What questions does this trigger?

**does each membrane synthesize its own complement of lipids?**

**Answer:** *...what do you think?....*

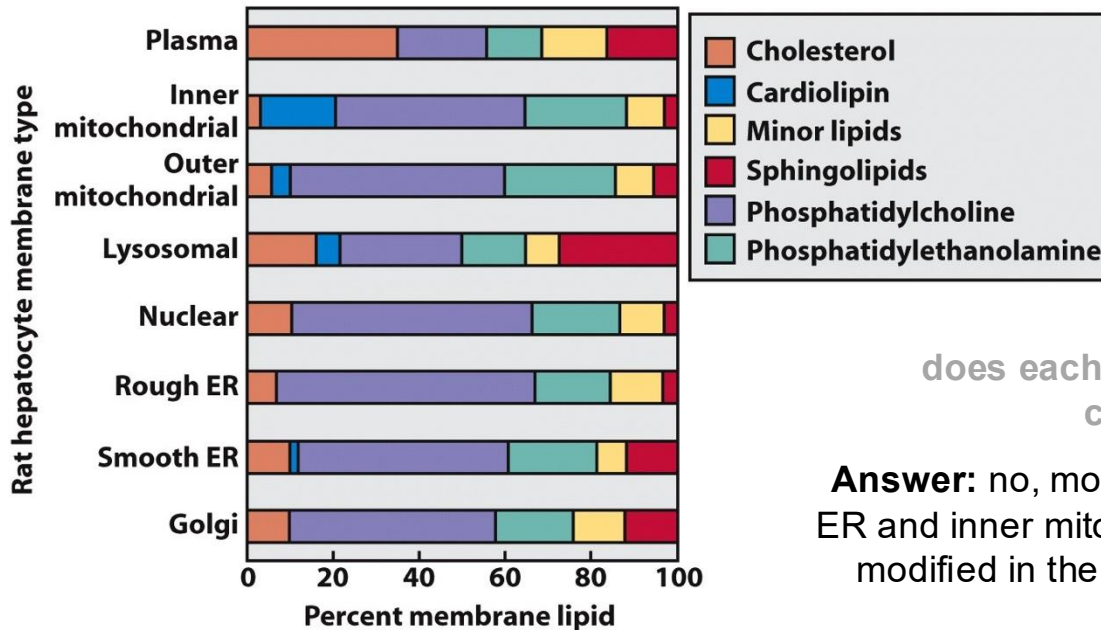


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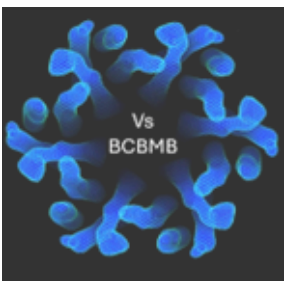


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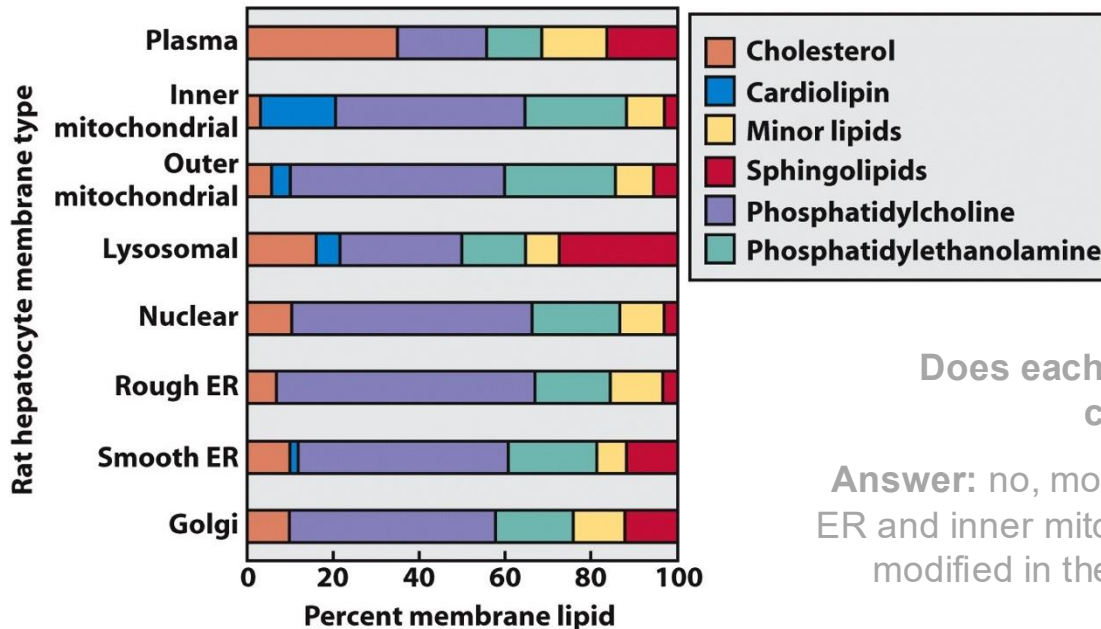


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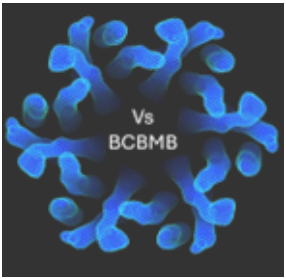
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gives more control over how much of what lipid is synthesized by making regulating synthesis easier + minimizes synthetic machinery + reduces overhead of distributing proteins necessary for synthesis to various cellular locations

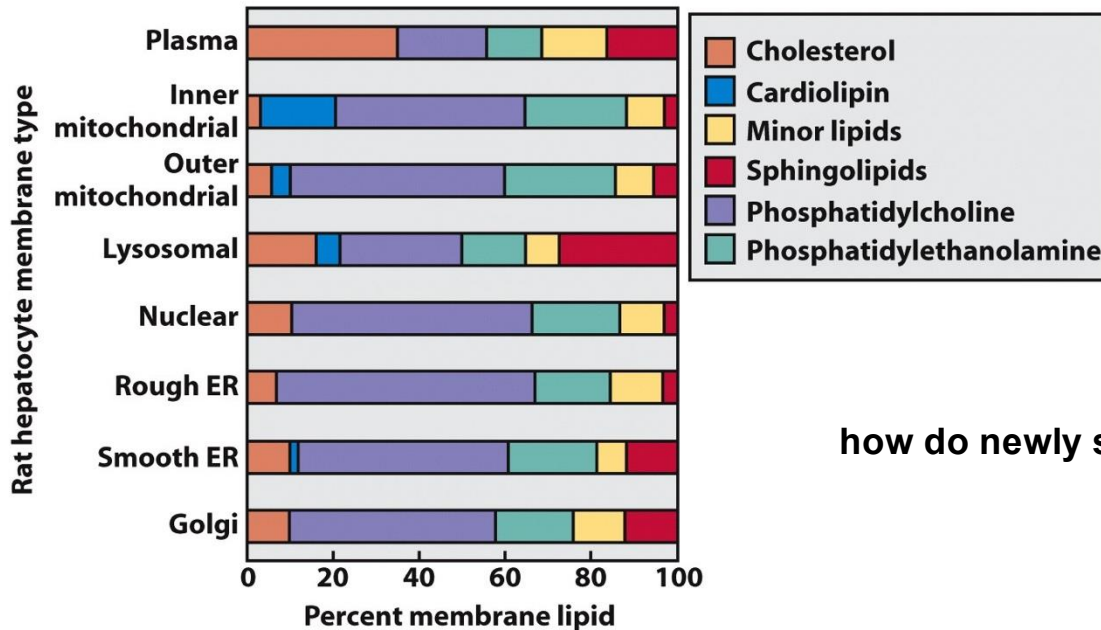


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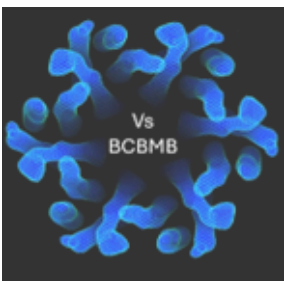
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having found the answer for why restricting biosynthesis of lipids to a small number of cellular locations raises another question ...

**how do newly synthesized lipid get to their final destinations??**

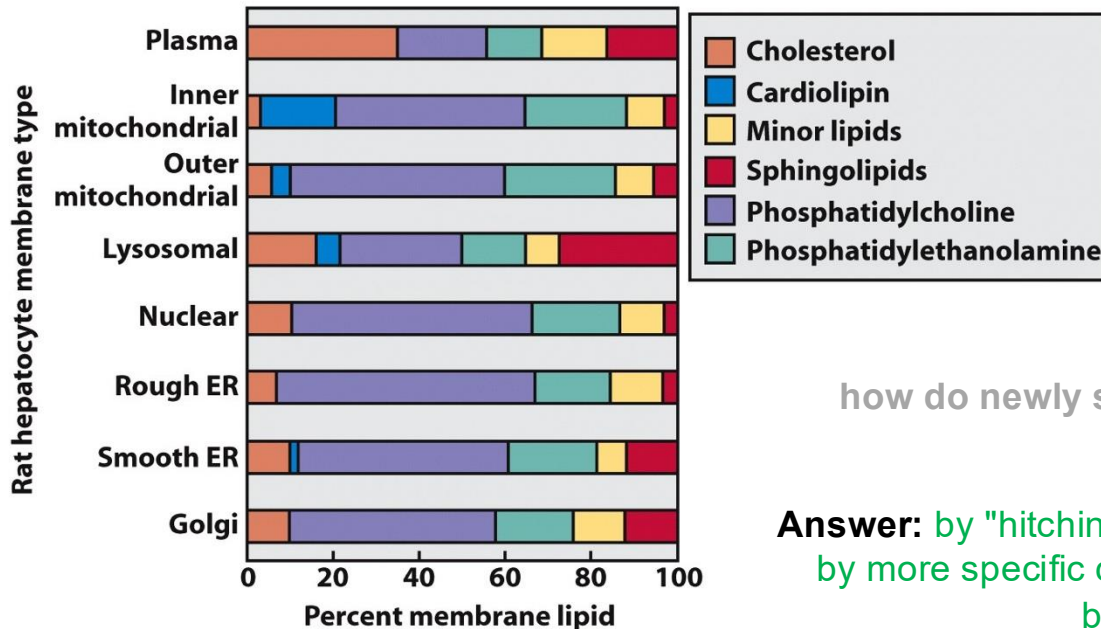


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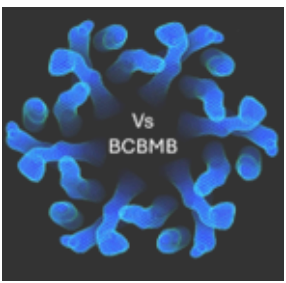
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how do newly synthesized lipid get to their final destinations??

**Answer:** by "hitching a ride" in transport vesicles or delivery by more specific carrier proteins that act at contact sites between cell organelles

this leaves yet other question .... **how does each organelle "measure" the content of each type of lipid? ....and: how does it "know" what mix of acyl-chains/headgroups is correct for its identity?**

Answers for these questions are only beginning to emerge, pointing to mechanisms that sense the packing density, packing mismatches and chemical markers on the lipid headgroups.



# If You Think All This Is Complicated .... It Gets Even More Complex Once You Consider The Following

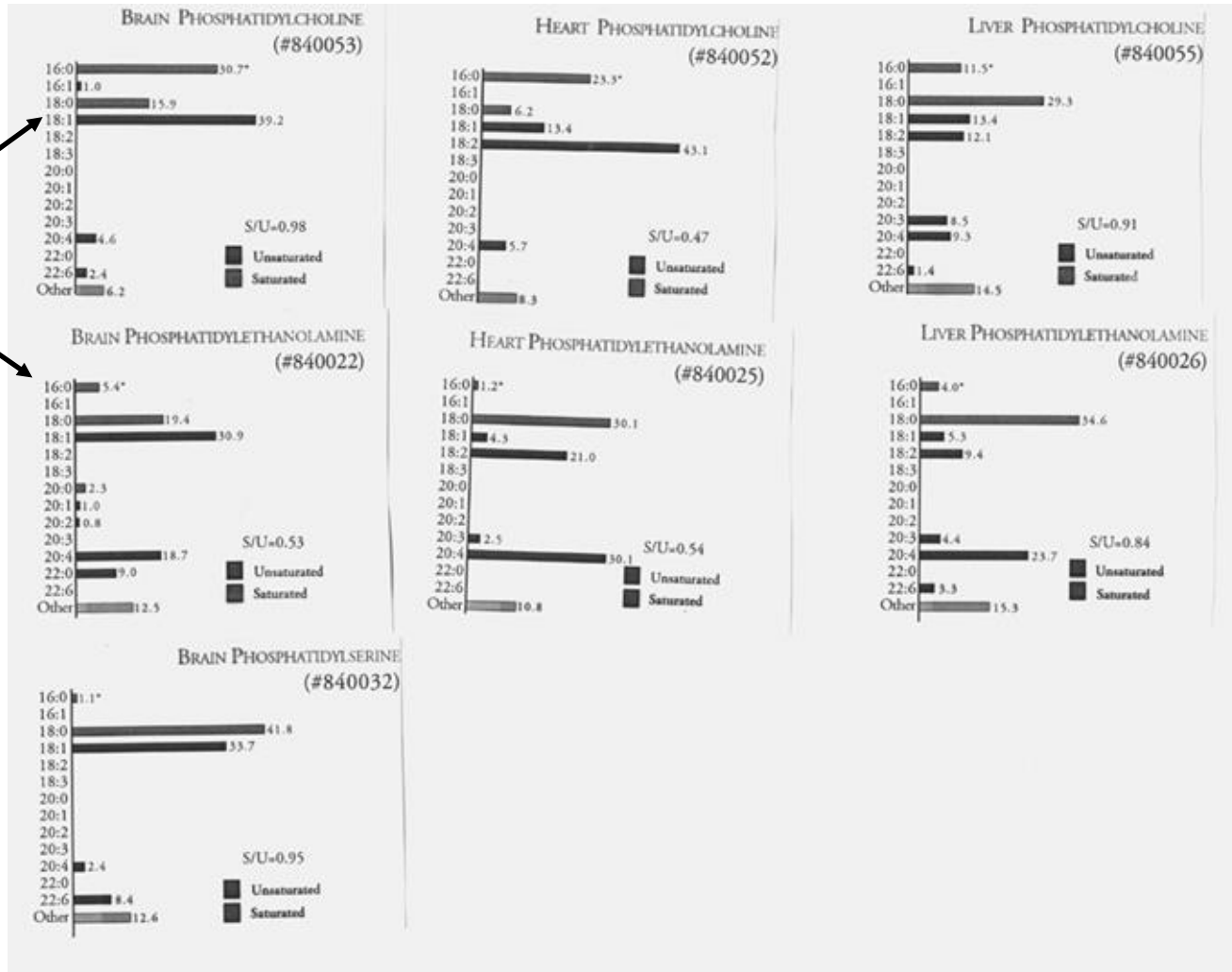


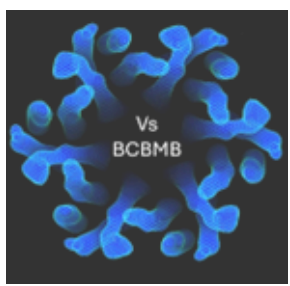
....

...look at the panels below and try to verbalize what you see...

Unsaturated  
X:2,3,4,6  
(eg 18:1)

Saturated  
X:0 (eg 16:0)



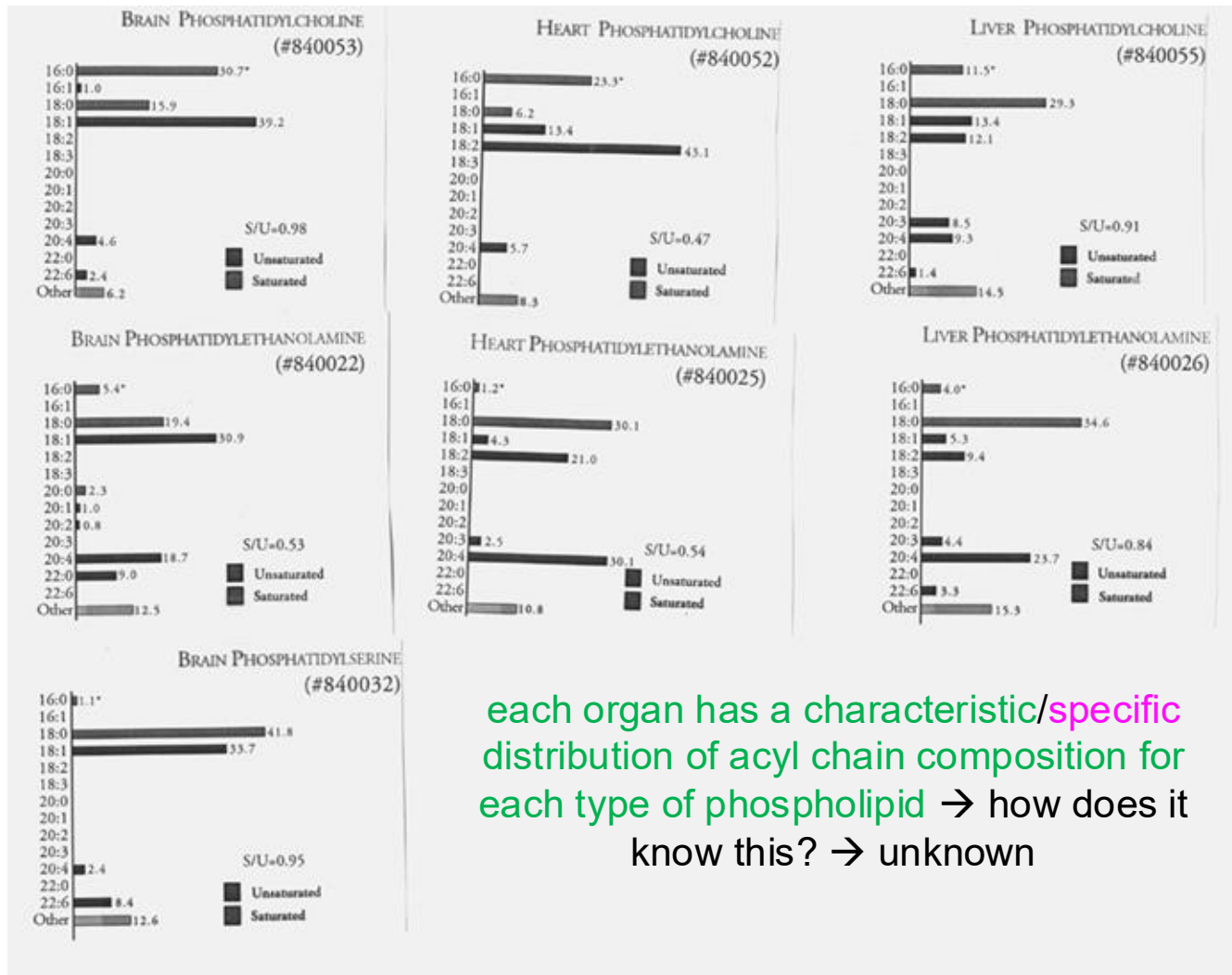


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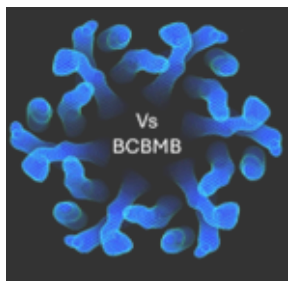


....

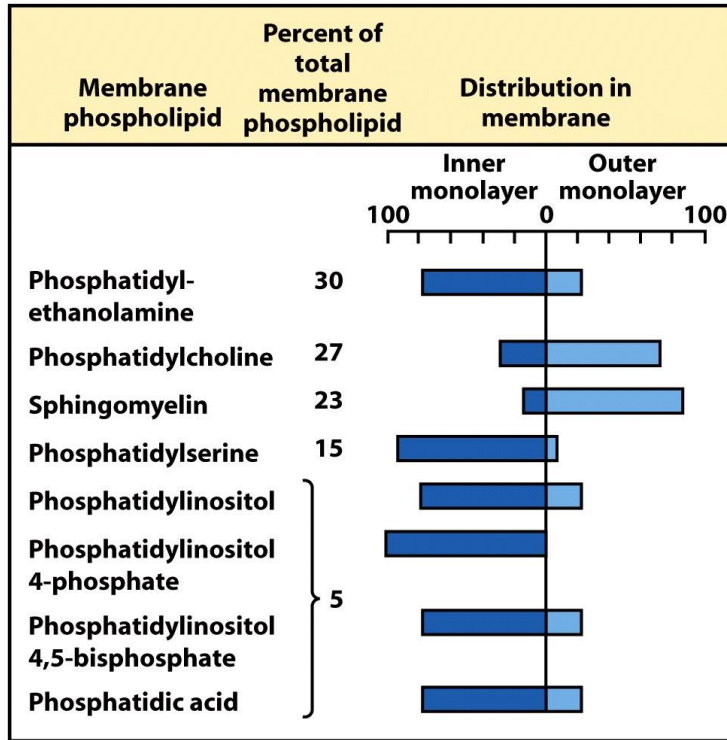
...look at the panels below and try to verbalize what you see...



each organ has a characteristic/specific distribution of acyl chain composition for each type of phospholipid → how does it know this? → unknown



## And Adding More to the Complexity ....



bilayers are **asymmetric (yet another important concept)** - that is, lipids with different headgroups are preferentially found in the inner or outer leaflet of the bilayer!

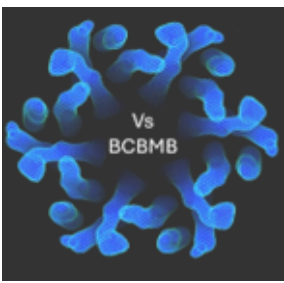
**can one make any sense of their preferences?**

**Answer:** some

- inositols are precursors for important intracellular signaling molecules = makes sense to find them inside.
- sphingomyelin has a neutral headgroup → less electrostatic charge on the surface → makes the bilayer “less sticky” (unless specifically designed, electrostatic interactions are generic and non-specific)

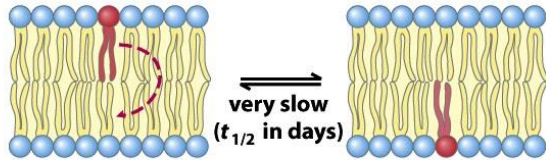
On the inner surface: many intracellular proteins that are needed at the membrane interface have specifically designed charged surfaces that allow them to exploit electrostatic interactions --> makes some sense to have negatively charged headgroups there.

Apart from those notions - **the asymmetry raises an yet another question - what is it?**

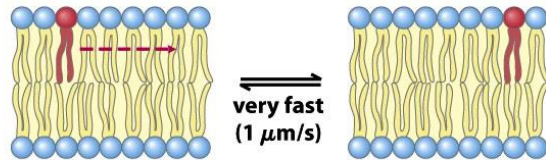


# How is Membrane **Asymmetry** Achieved/Maintained?

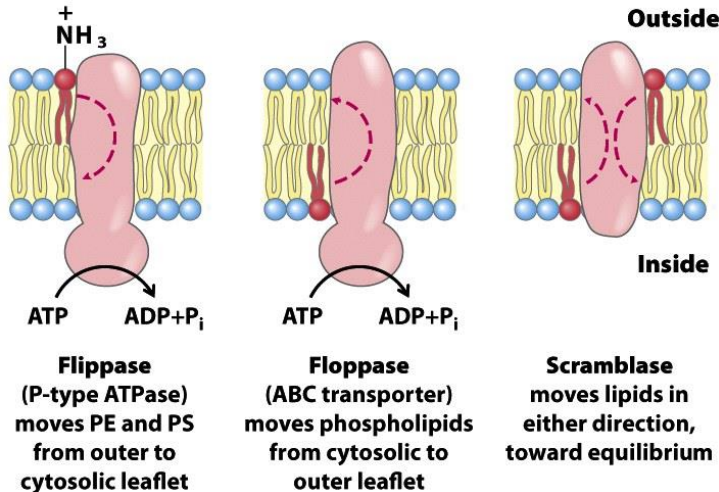
**(a) Uncatalyzed transbilayer (“flip-flop”) diffusion**



**(b) Uncatalyzed lateral diffusion**



**(c) Catalyzed transbilayer translocations**



- except for cholesterol, lipids cannot readily exchange between the leaflets (slow spontaneous “flip-flop”)
  - Contrasts with lateral diffusion, which is fast

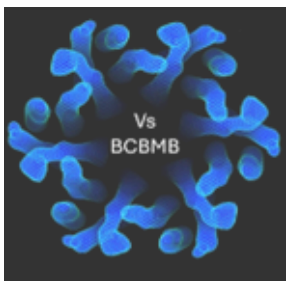
need **catalysis** to “drag” lipids from one leaflet to the other: three enzymes do this **flippase, floppase, and scramblase**.

function of these enzymes is essential because accumulation of PS on the outer leaflet is “read” as cellular malfunction, and triggers apoptosis.

but there is a **second reason why these enzymes are important** - can you see what it is?

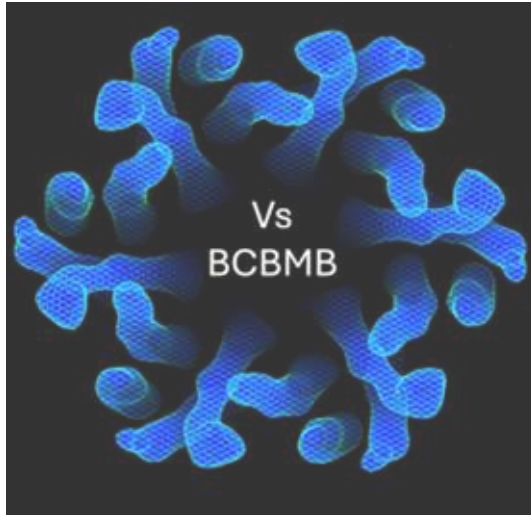
**Answer:**

new lipids are synthesized in either the inner or outer leaflet in the ER --> if there were no enzymes to “move” them between leaflets ... their size would become imbalanced!



## SUMMARY MEMBRANES

- membranes are bilayer structures composed of >100 different species of phospholipids, sphingolipids, and sterols (mostly cholesterol)
- at a molecular level, bilayers are asymmetric = different lipid preferences for each of the two leaflets
- lipid compositions of membrane compositions are specific for each type of cellular membrane, and furthermore vary depending on cell/tissue type
- the chemical diversity is matched by the complexity of phase behaviors that overall characterize biological membranes as thermodynamically metastable non-covalent aggregates.
- the metastability causes a constant drive towards demixing of lipids, which is an essential aspect of membrane physics and allows membrane to serve multiple biological functions
- one consequence of “demixing” is the formation of lipid territories (“rafts” are the most commonly known example)
- membranes show distinct lateral pressure profiles that are exploited to power certain structural transition in membrane resident proteins and membrane dynamics that are related to changes in curvature.



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