

Biological Rafts and Biophysical Phases

Adam T. Hammond
ath28@cornell.edu

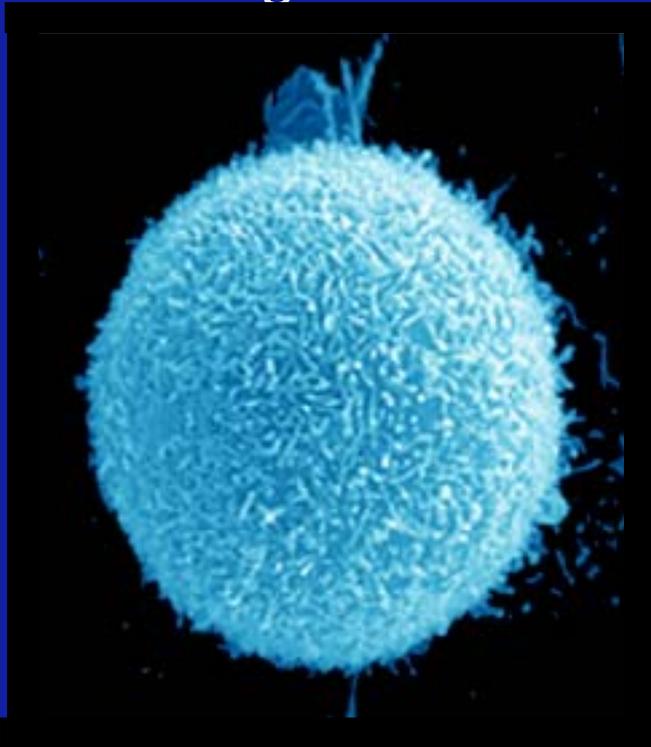
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What is the effect of crosslinking in biological membranes?

Adam T. Hammond
ath28@cornell.edu

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resting mast cell



activated mast cell

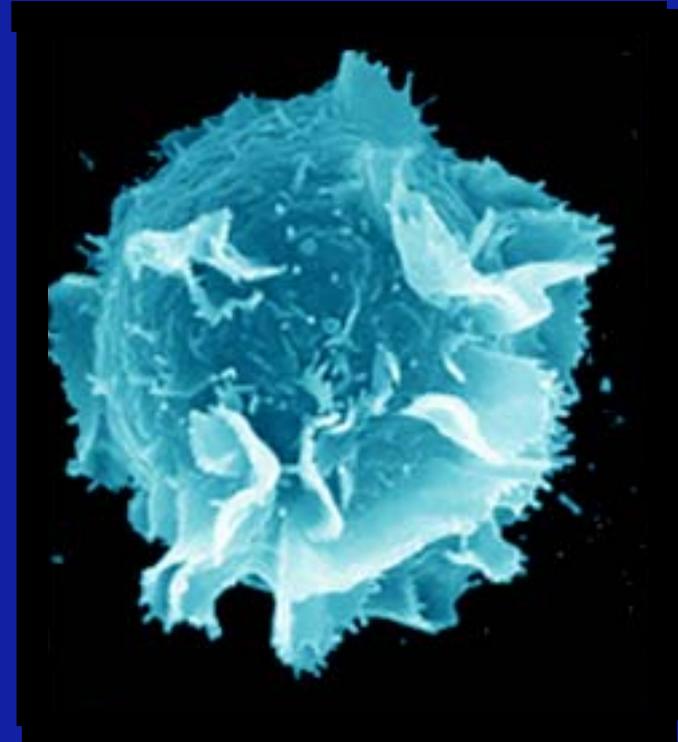
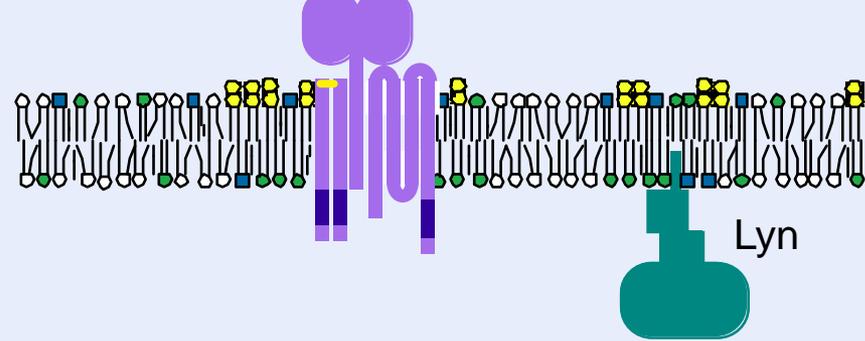


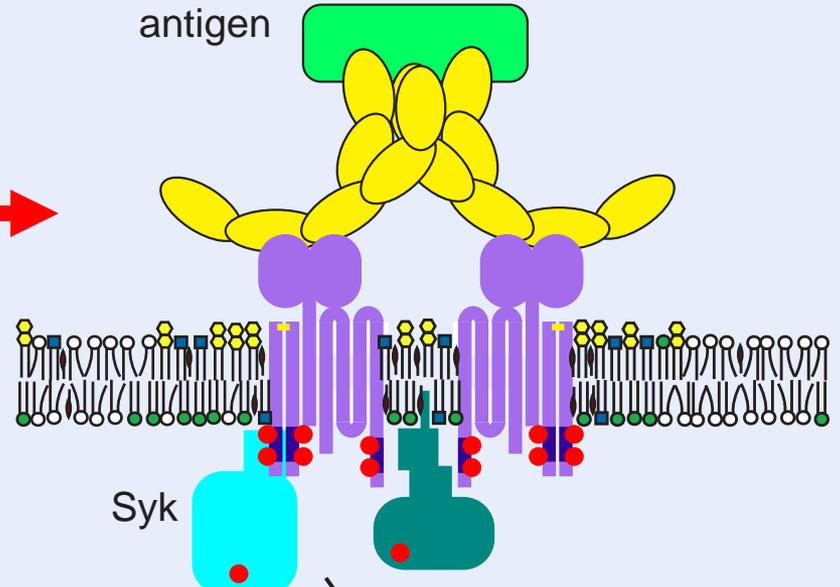
image source: Janet Oliver
University of New Mexico
School of Medicine

How does the RBL cell know?

IgE-Fc ϵ RI



antigen



Syk

Lyn

Cdc42 / Rac

PLC γ

IP $_3$

DAG

Ca $^{2+}$

PKC

Degradation

- cholesterol
- sphingomyelin
- glycosphingolipid
- glycerophospholipid

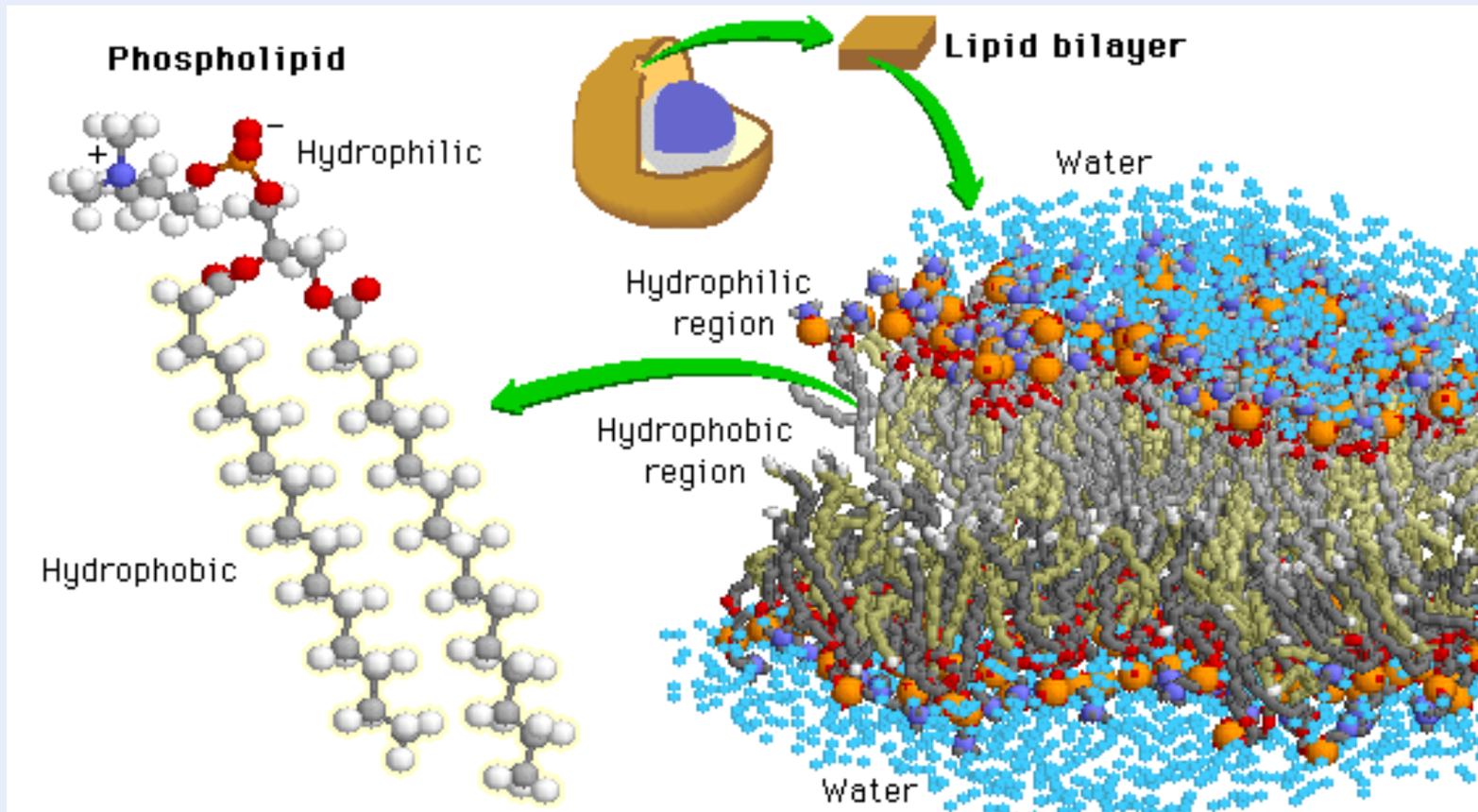
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ath28@cornell.edu



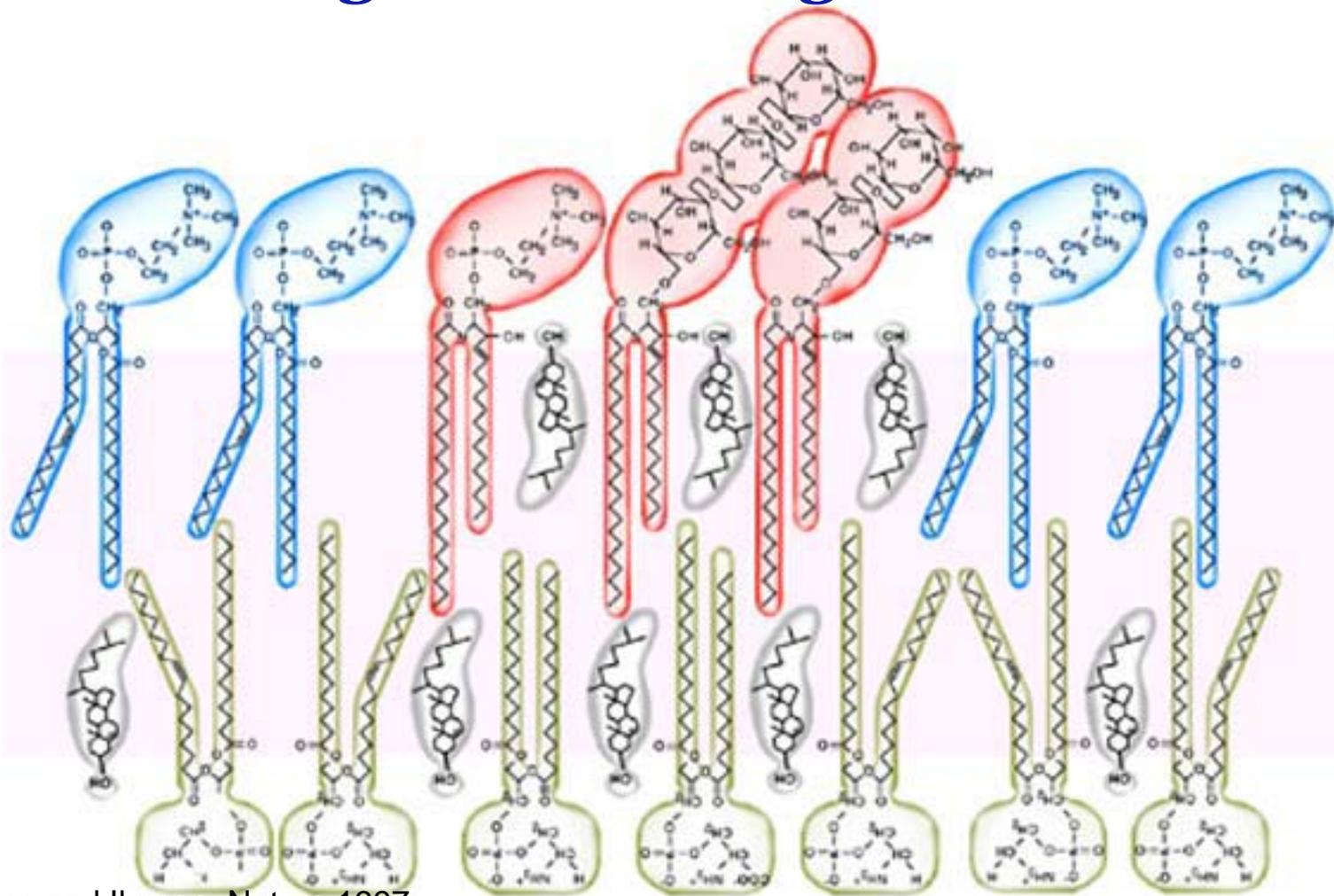
Membrane Domains in Cells - a biochemically distinct region with in a continuous lipid bilayer

- They absolutely exist: e.g. coated pits; caveolae; rough, smooth, and transitional ER, desmosomes; TGN; and various endosomes.
- Sorting of lipids occurs: e.g. cholesterol and sphingomyelin gradients.
- Protein aggregation will alter lipid content to some degree.
- Lipid aggregation will alter protein content as well.
- But, higher entropy drives membrane components towards random distributions.

Not so simple



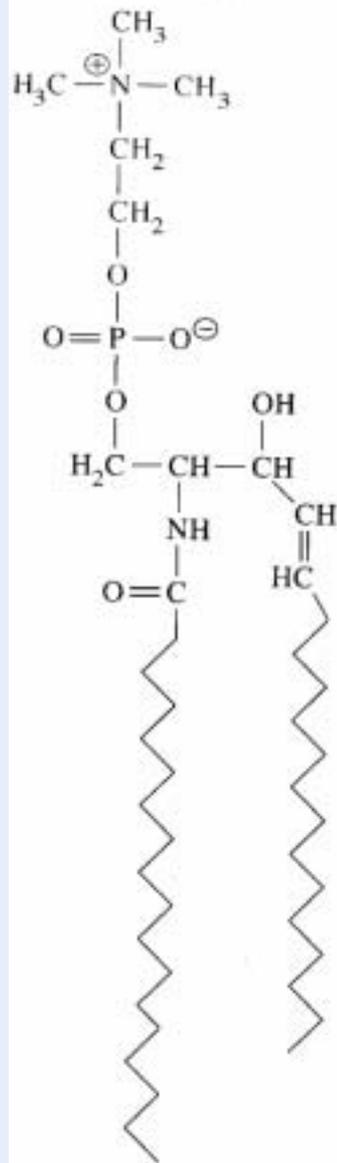
Lipid rafts are cholesterol-dependent ordered regions of biological membranes



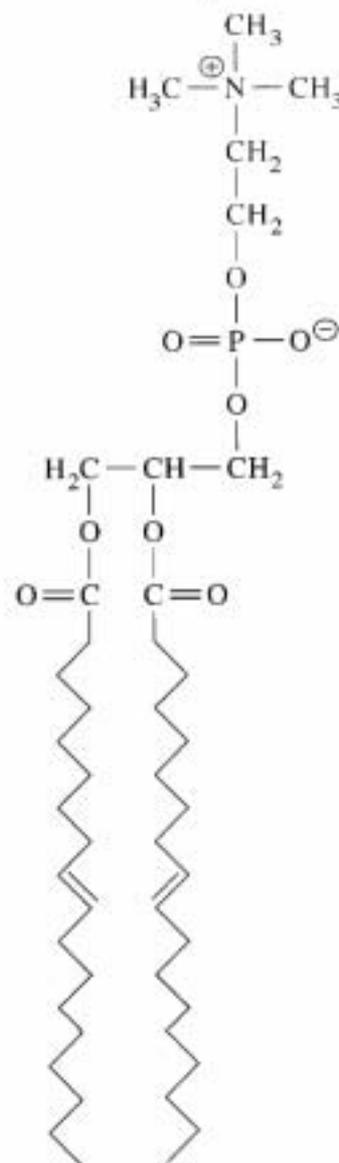
Simons and Ikonen, Nature 1997

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3-Component Mixtures: Sph/DOPC/cholesterol



Sphingomyelin
(bovine brain)

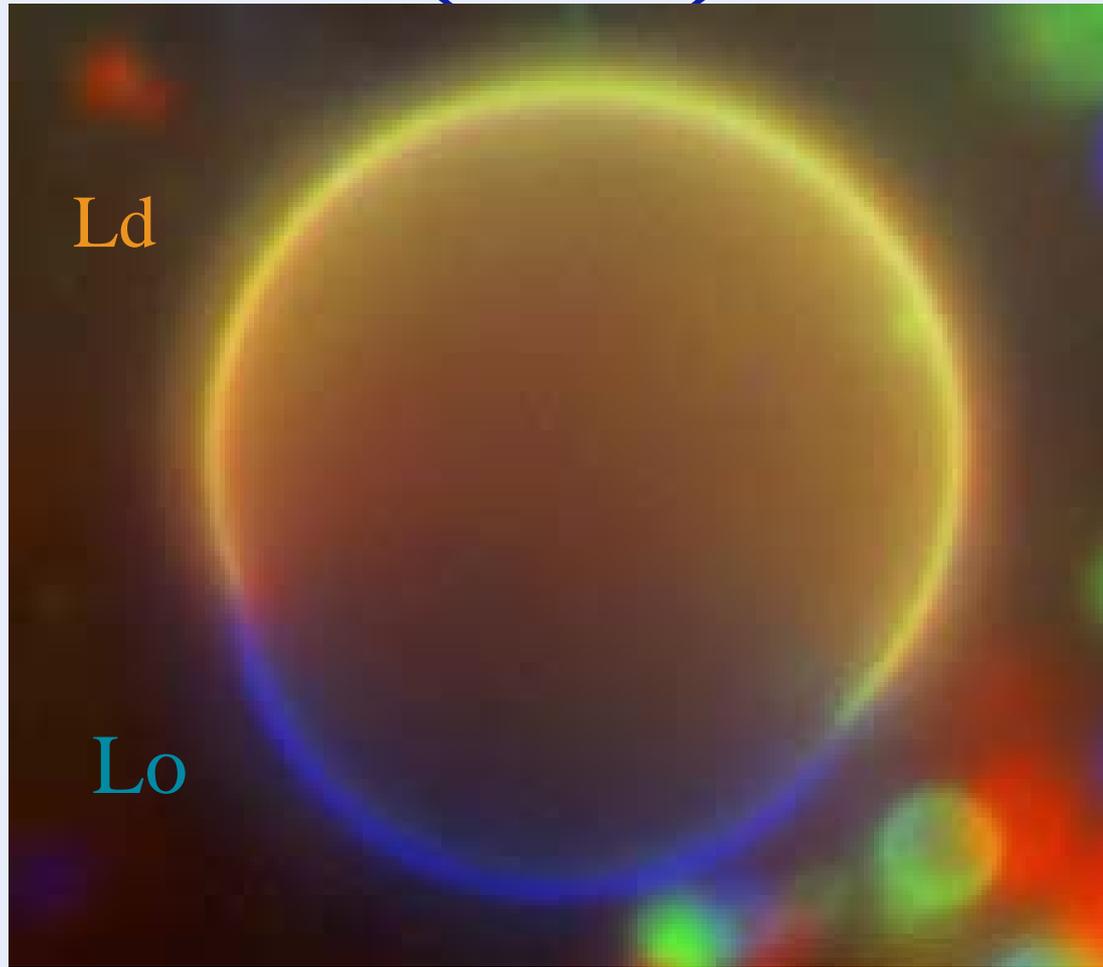


DOPC
(18:1,18:1-PC)



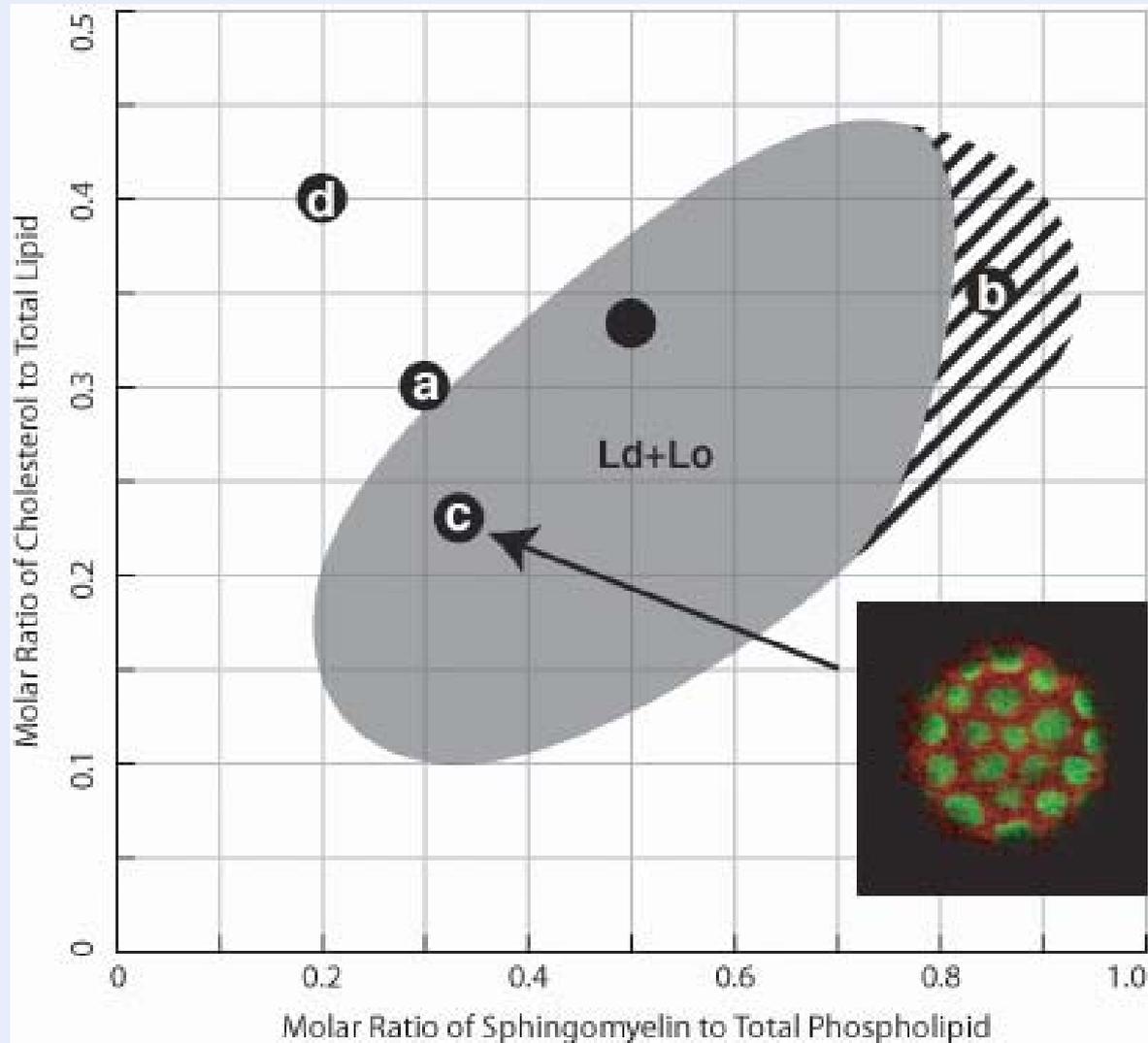
cholesterol

Giant Unilamellar Vesicle (GUV)



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ath28@cornell.edu

Not a Phase Diagram



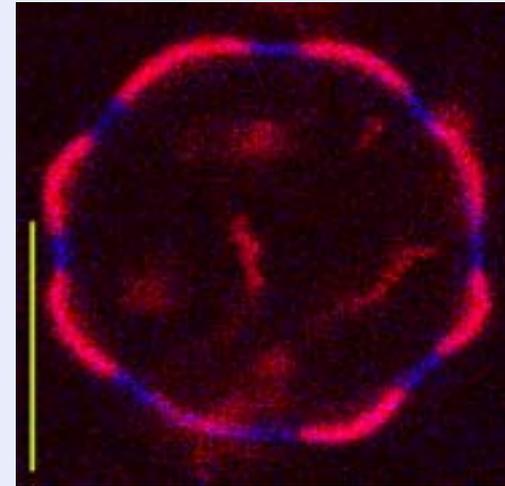
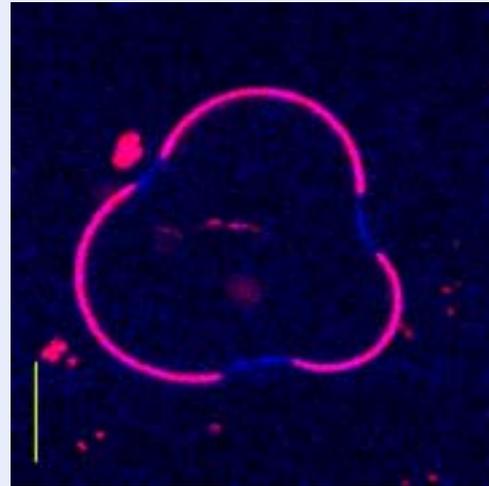
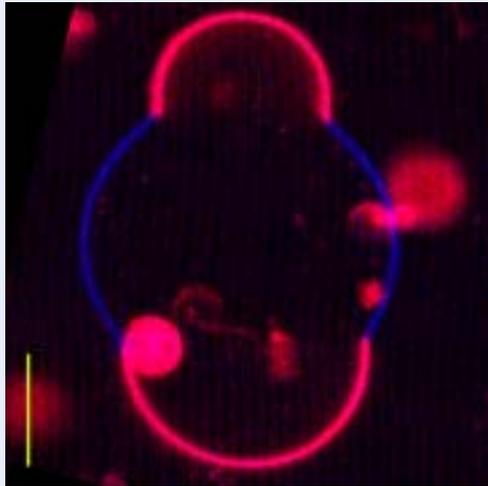
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Answers we crave from the model membranes:

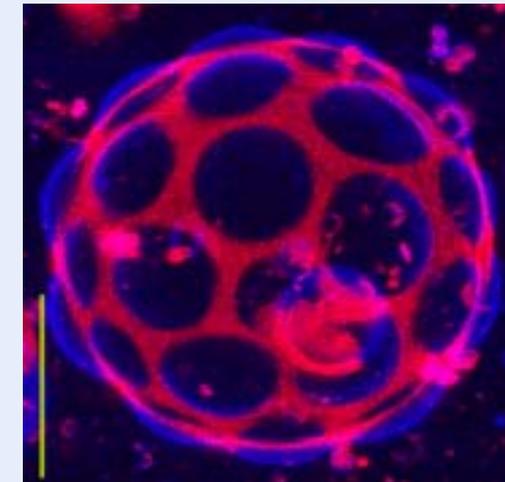
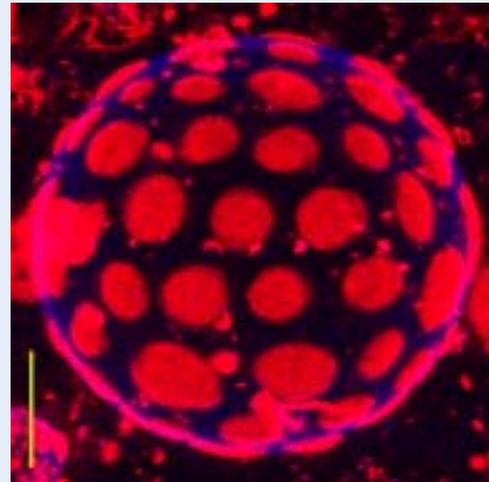
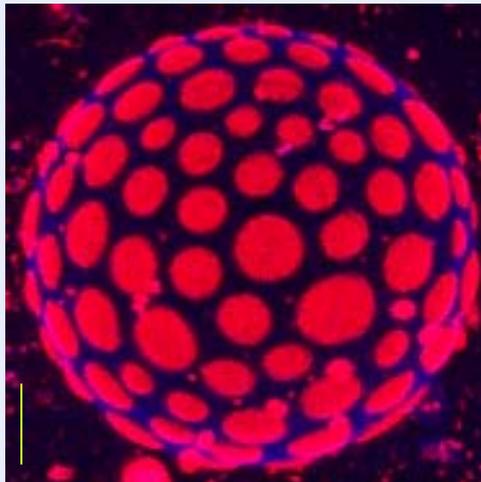
- When a membrane component is crosslinked, is there a change in its partitioning?
- Are other components affected?

GUVs with more than one domain: superstructures

Symmetric domain arrangement minimizes mechanical energy

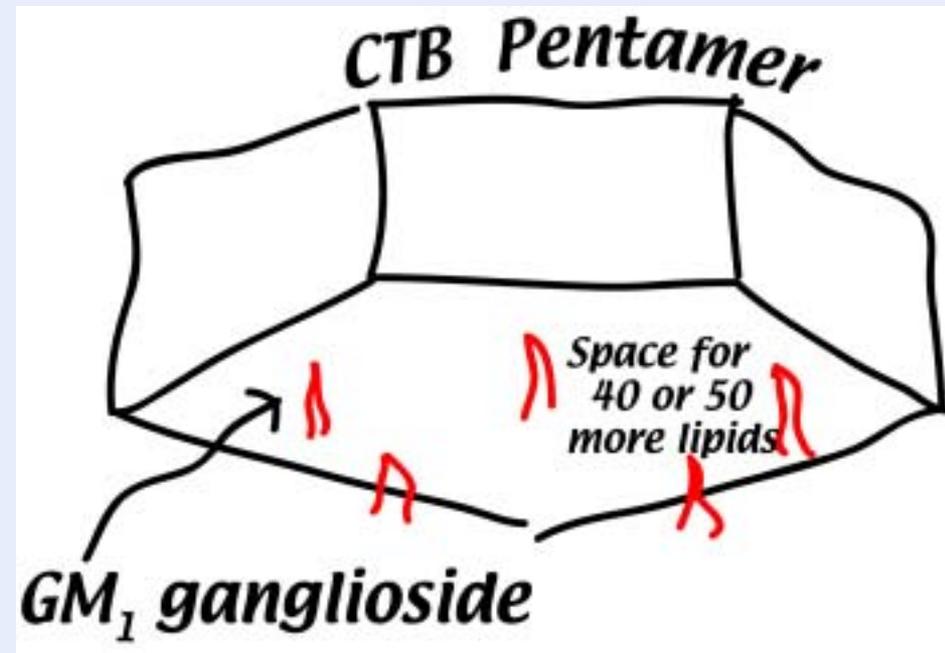


Energy minimization leads to hexagonal membrane superstructures

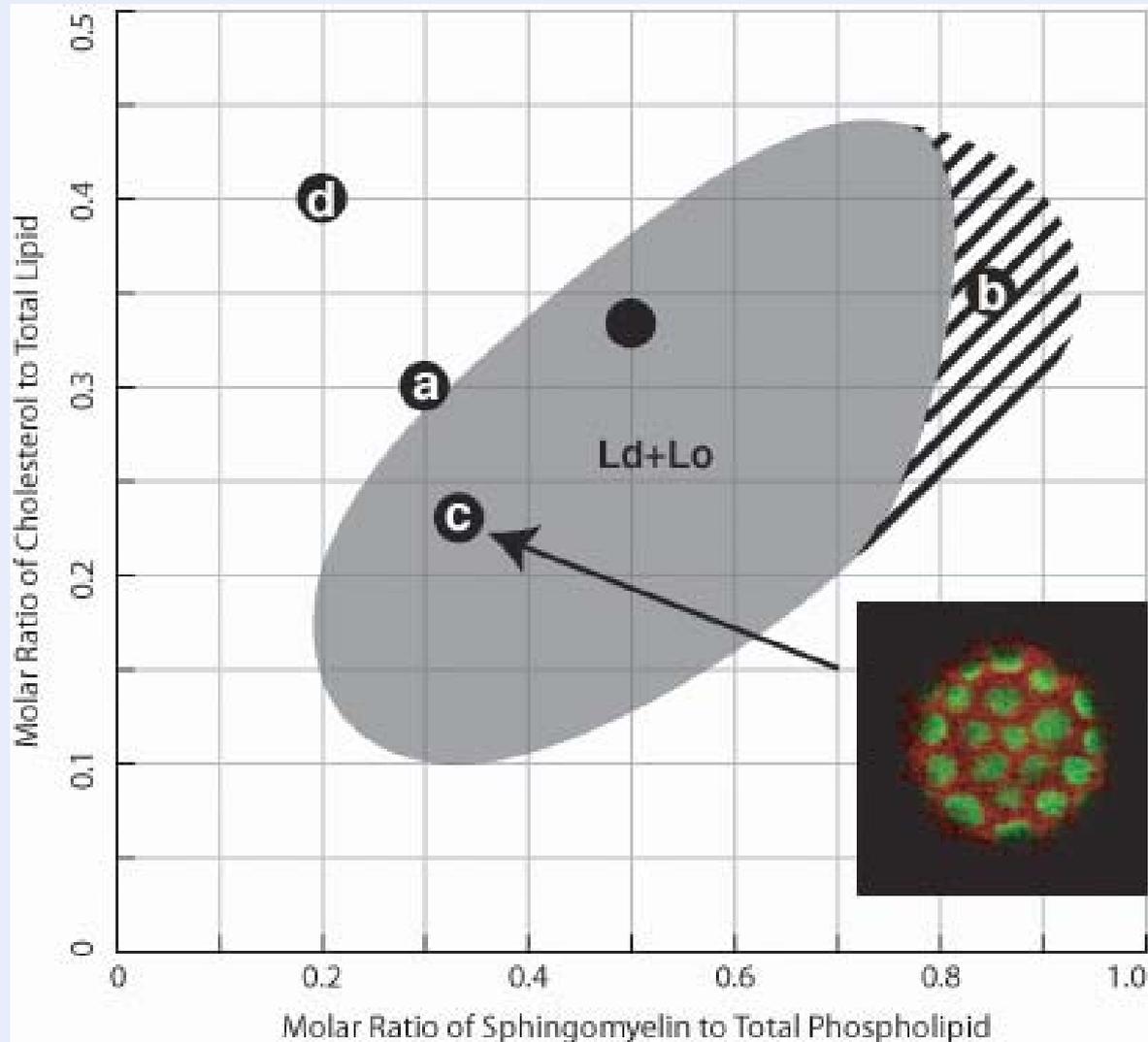


The ganglioside GM₁ is the cellular receptor for cholera toxin.

- Sphingomyelin plus a large pentasaccharide head group.
- The most widely accepted raft marker
- A pentamer of Cholera Toxin B (CTB) binds to five GM1 molecules

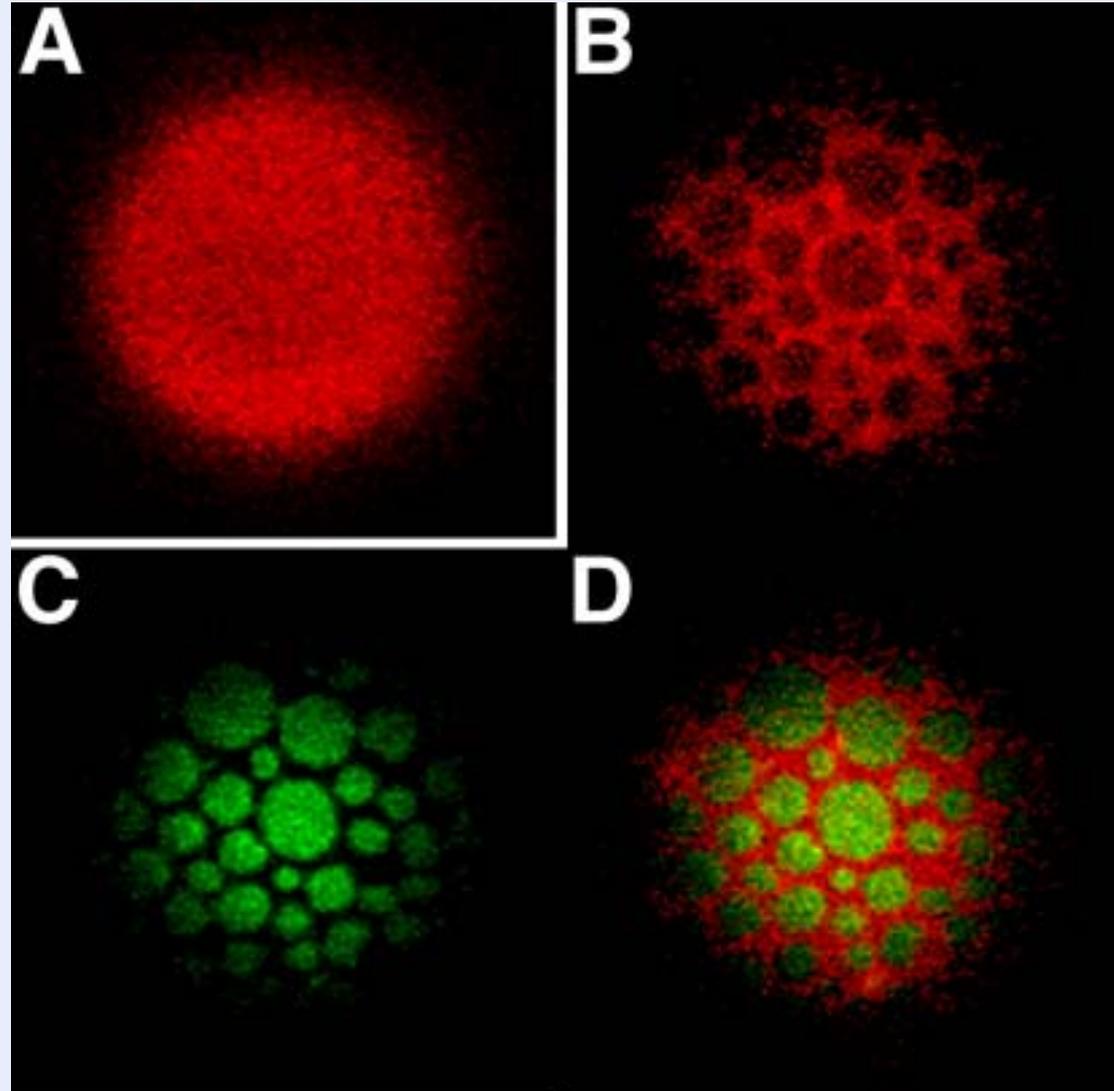
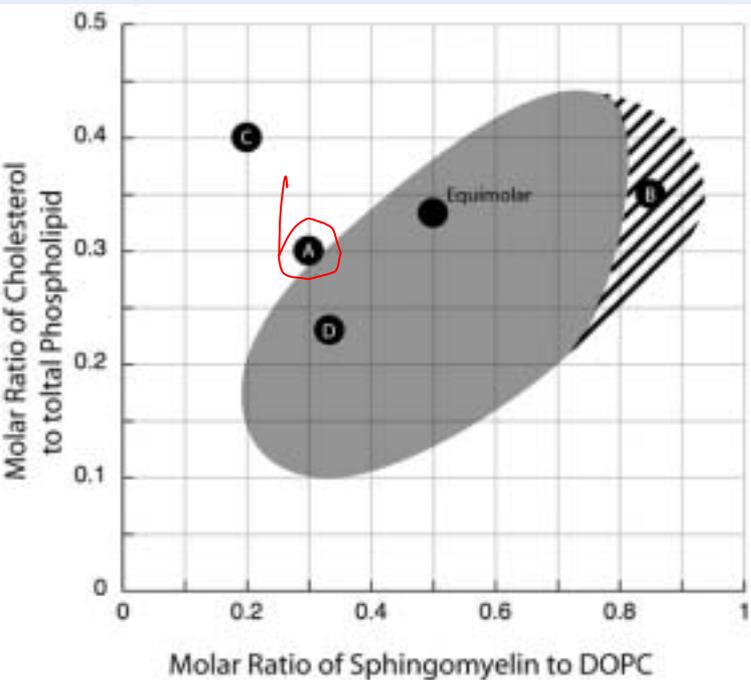


Not a Phase Diagram

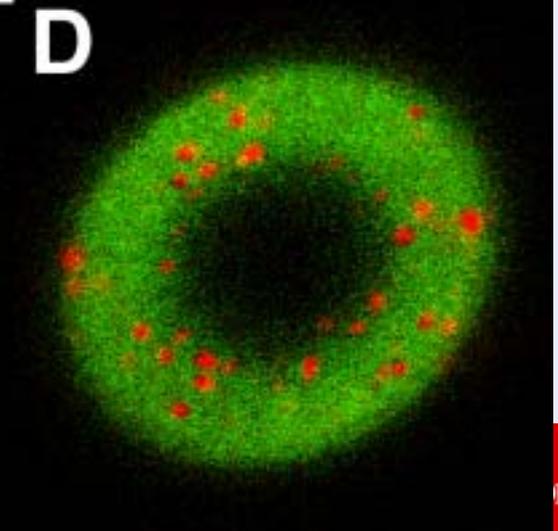
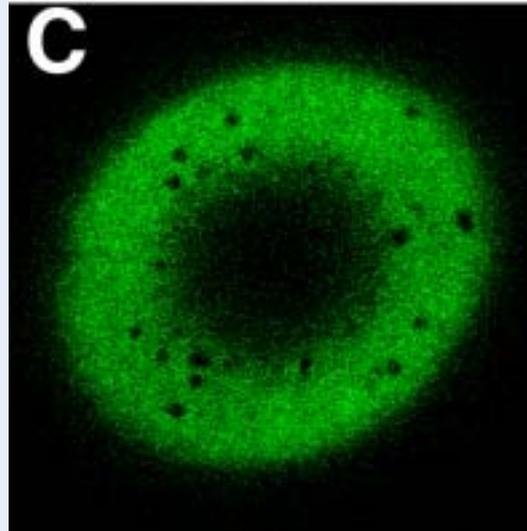
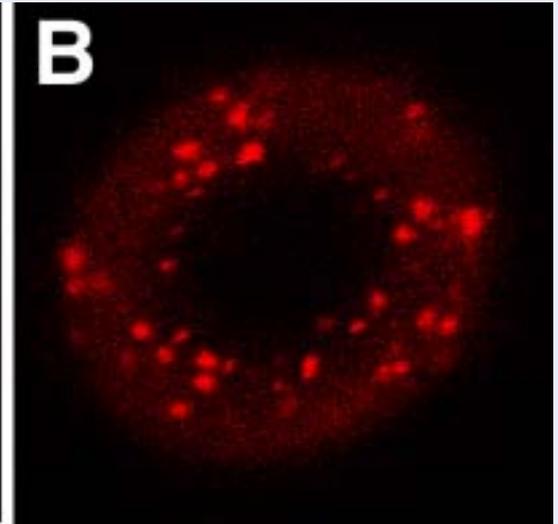
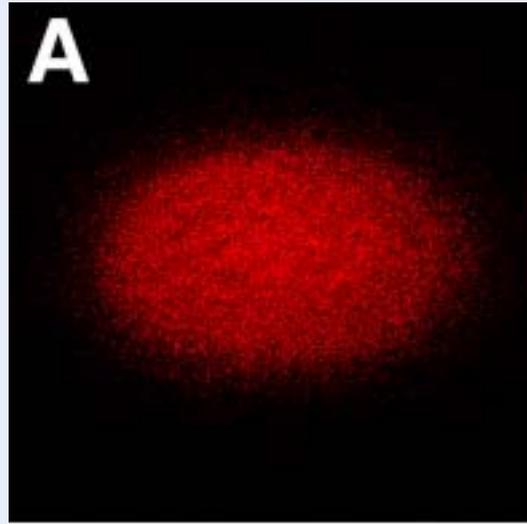
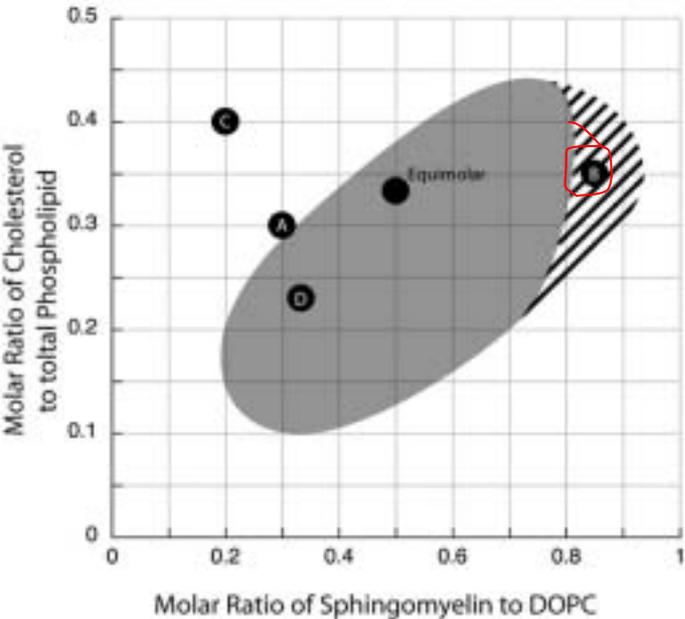


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ath28@cornell.edu

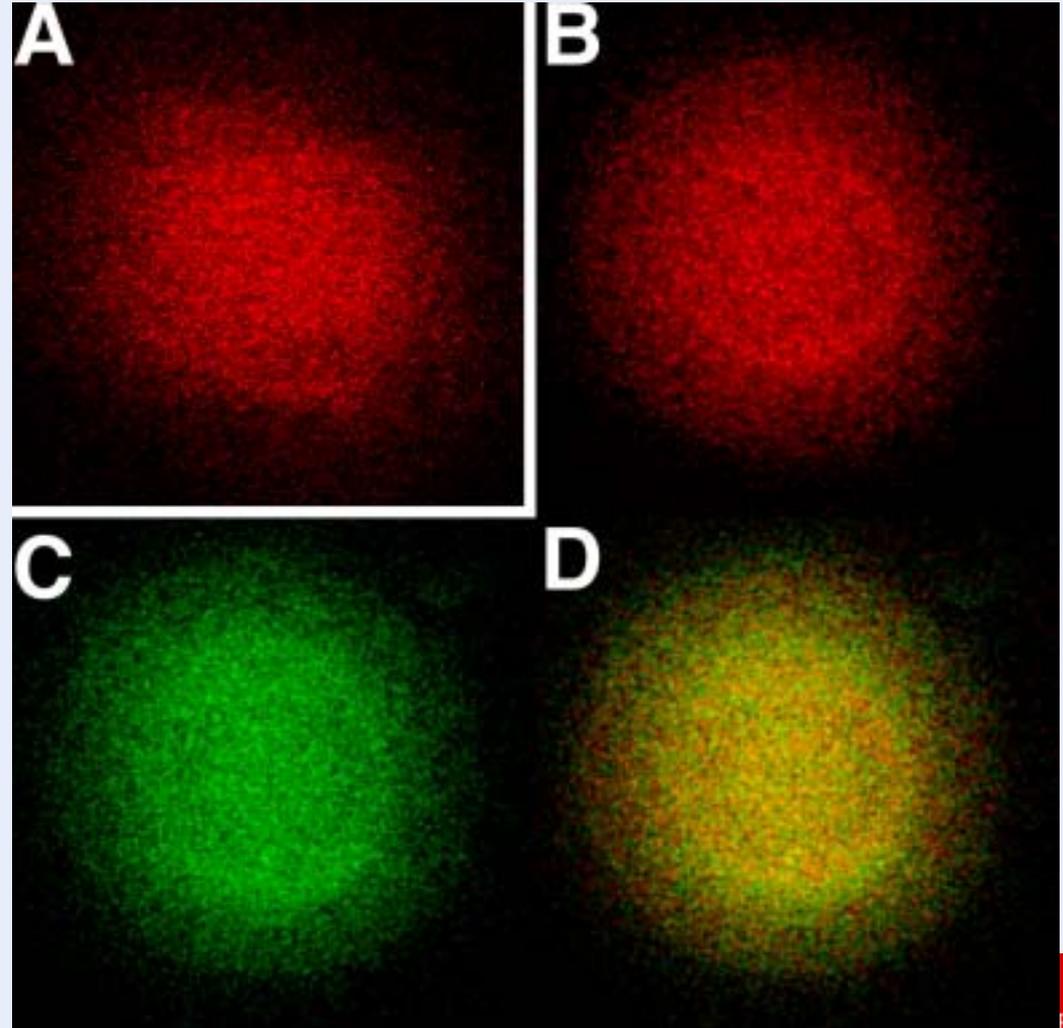
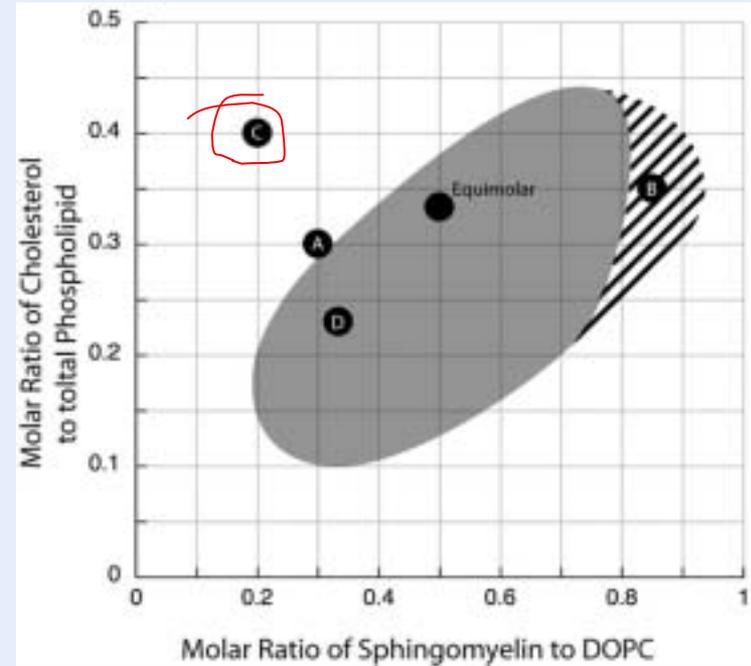
Crosslinking can induce a large change in the organization of the membrane



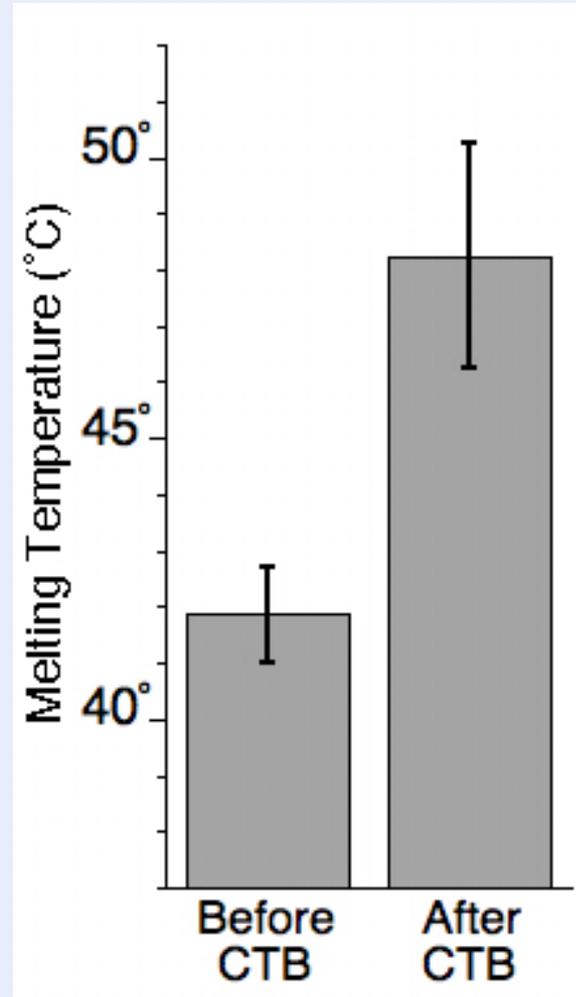
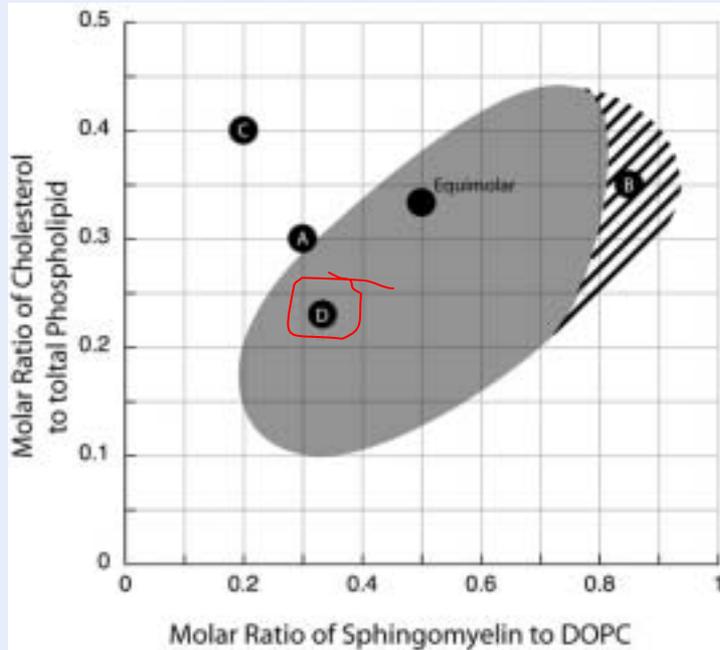
This reorganization also occurs in membranes that are highly ordered to begin with.



Cholera Toxin binding doesn't always cause new domains.



Miscibility transition temperature



Adam T. Hammond
ath28@cornell.edu

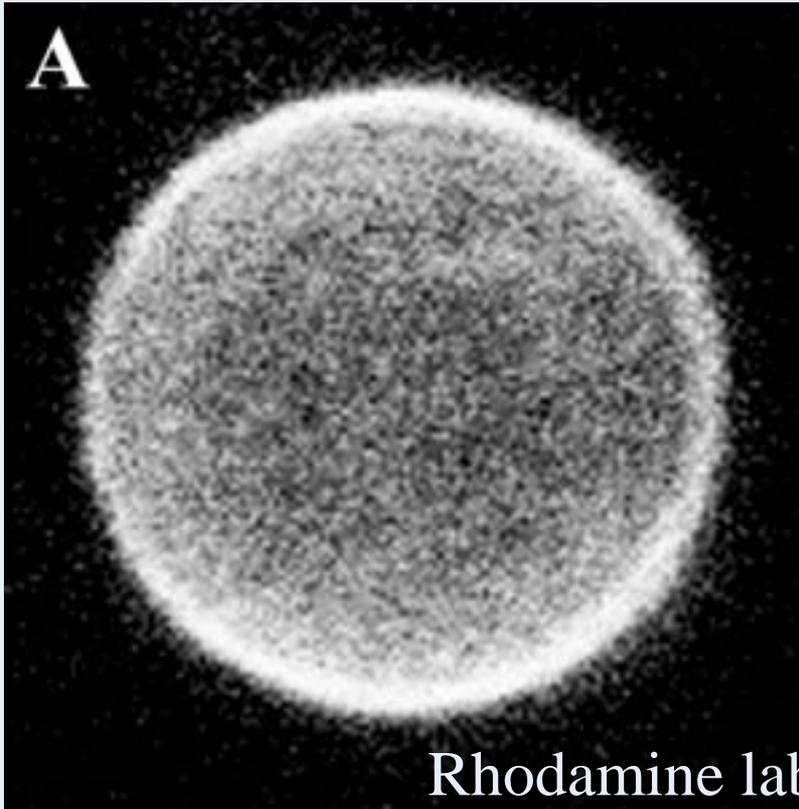
Can this crosslinking sort proteins?

Adam T. Hammond
ath28@cornell.edu

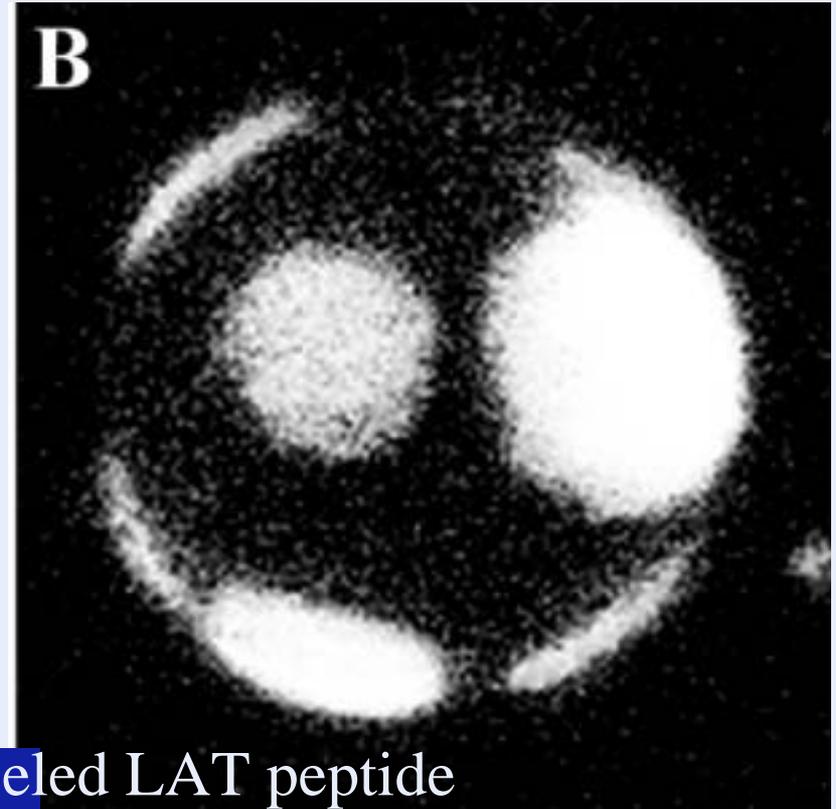
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Transmembrane alpha-helices are redistributed when the membrane changes.

Before CTB



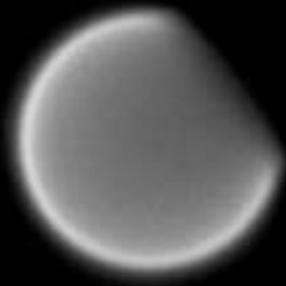
After CTB



Rhodamine labeled LAT peptide

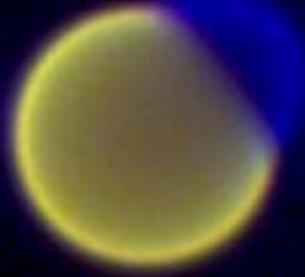
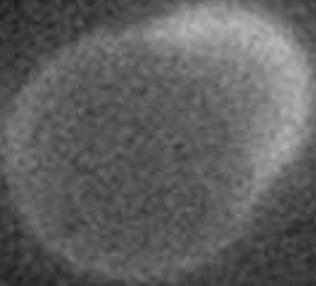
Palmitoylated LAT peptide

Ld
C12:0 DiI



peptide

Lo
perylene



Answers we crave from the model membranes:

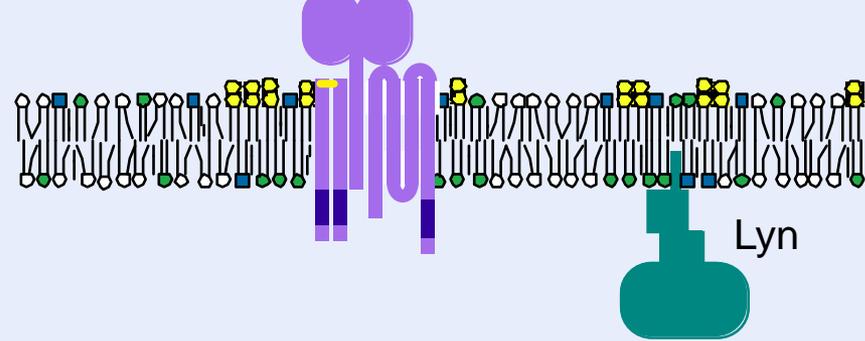
- When a membrane component is crosslinked, is there change in partitioning?
 - Are other components affected?
-

Answers we got:

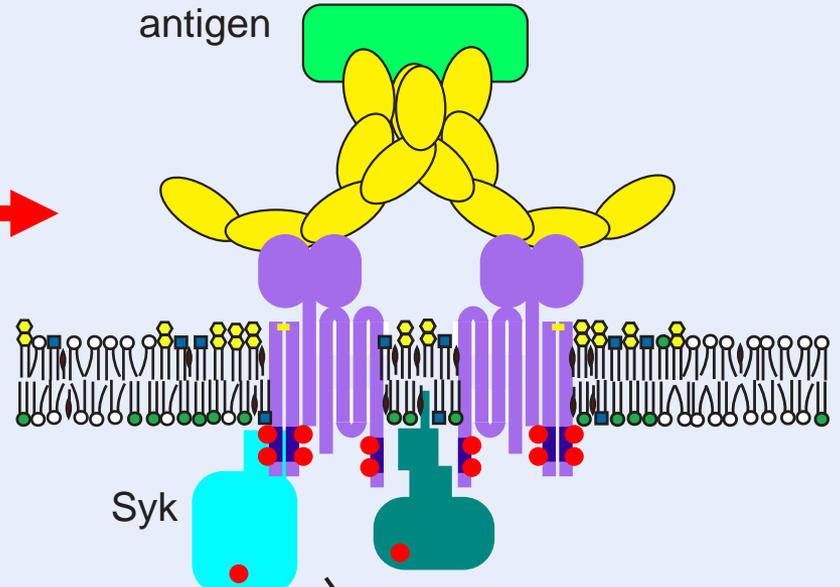
- Crosslinking changes the nature of the underlying membrane.
- This change is a thermodynamic stabilization and ordering of the membrane.
- One protein can change the distribution of others by causing such a change.

Are we any closer?

IgE-Fc ϵ RI



antigen



Syk

Lyn

Cdc42 / Rac

PLC γ

IP $_3$

DAG

Ca $^{2+}$

PKC

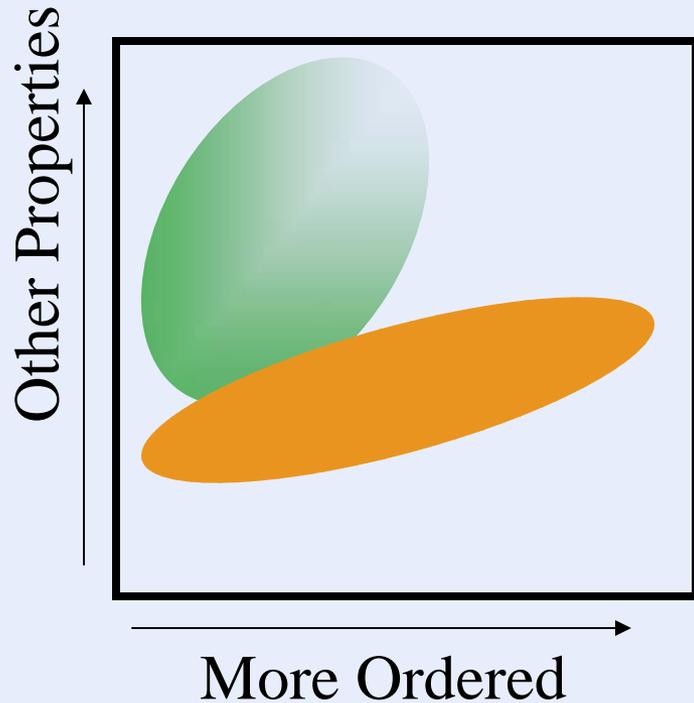
Degranulation

- cholesterol
- sphingomyelin
- glycosphingolipid
- glycerophospholipid

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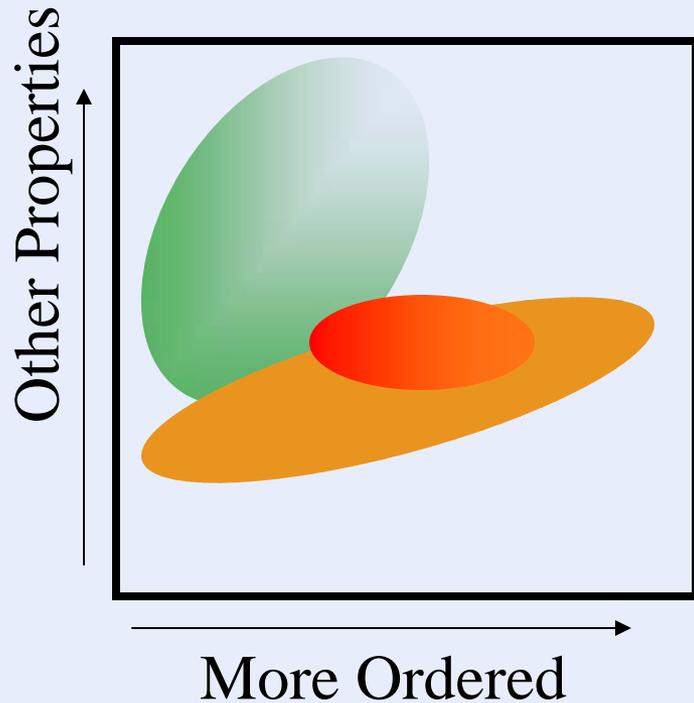
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RBL plasma membrane before stimulation



- Not at equilibrium
- A wide range of ordering, sizes, compositions, and durations
- ● On average, IgE receptor is in less ordered environments
- ● Lyn kinase is more evenly distributed (but down regulated in less ordered environments)

RBL plasma membrane at the initiation of stimulation



- Still just as diverse
- ● Lyn kinase is relatively unchanged
- ● Monomeric receptor is the same
- ● Crosslinked receptor creates a small transient environment where phosphorylation occurs

A potential mechanism for how a cell can respond to a crosslinking event

Cells could use the increase in membrane order caused by a crosslinking event to reorganize key proteins.

Thanks

- Barbara Baird and David Holowka
- Gerald Feigenson
- Tobias Baumgart (W.W.Webb)
- Andrew Smith
- Hidehiko Shogomori
- Deborah Brown
- Erwin London
- Cornell University
- Keck Foundation

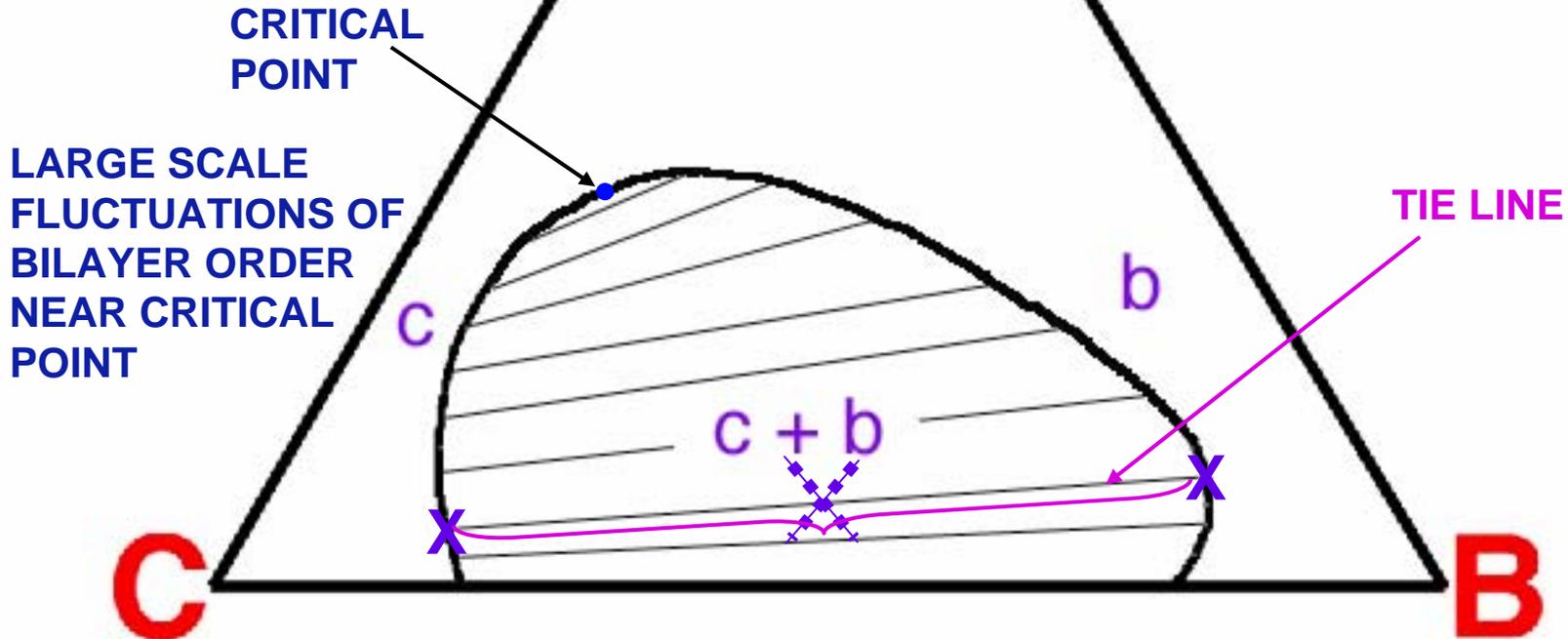
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A GENERIC 3-COMPONENT PHASE DIAGRAM

ALL MIXTURES OF
COMPONENTS A, B, AND C
AT CONSTANT
TEMPERATURE

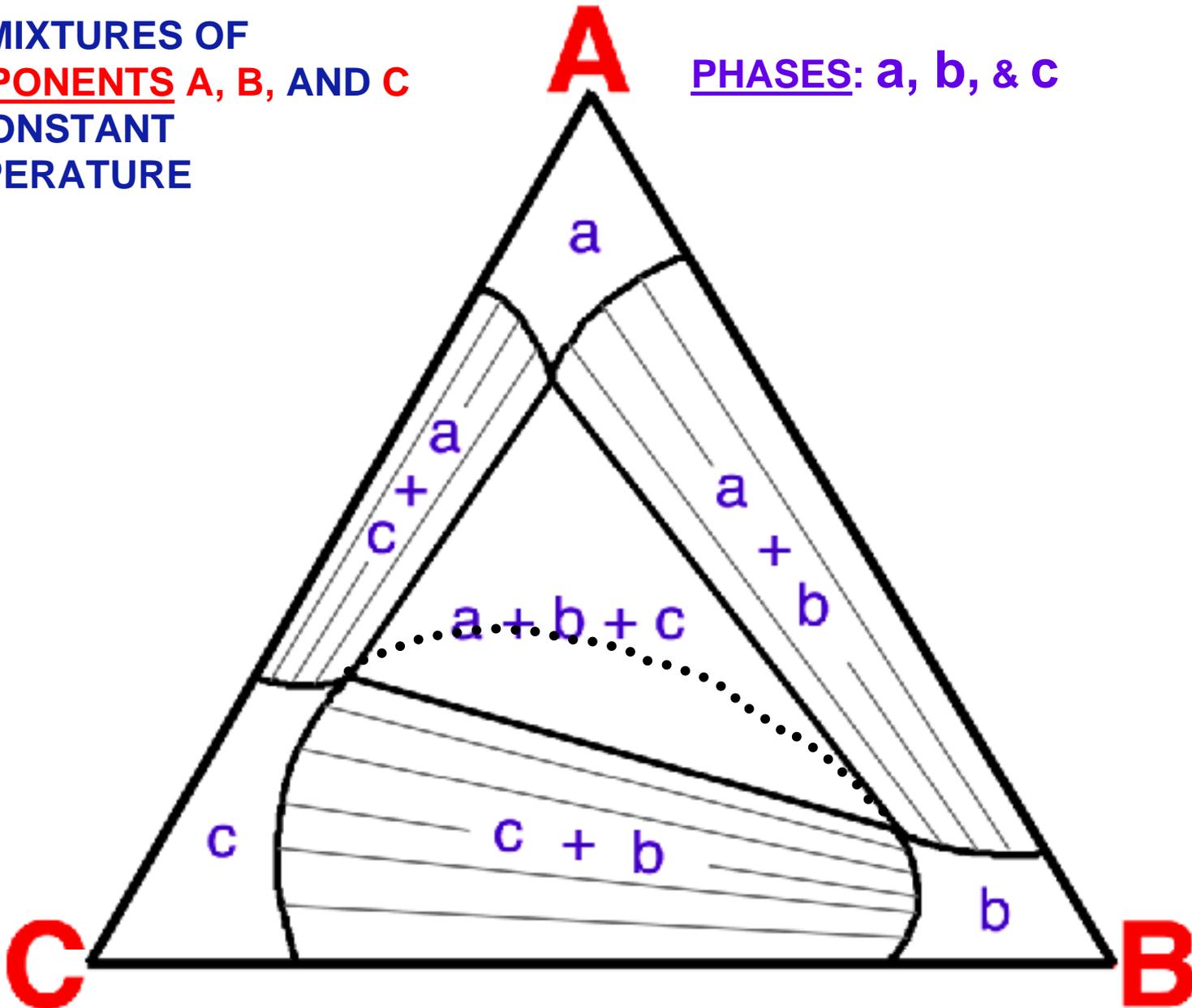
PHASES: b & c
COULD BE NO 2-PHASE OR 3-PHASE
COEXISTENCE REGIONS, OR SEVERAL



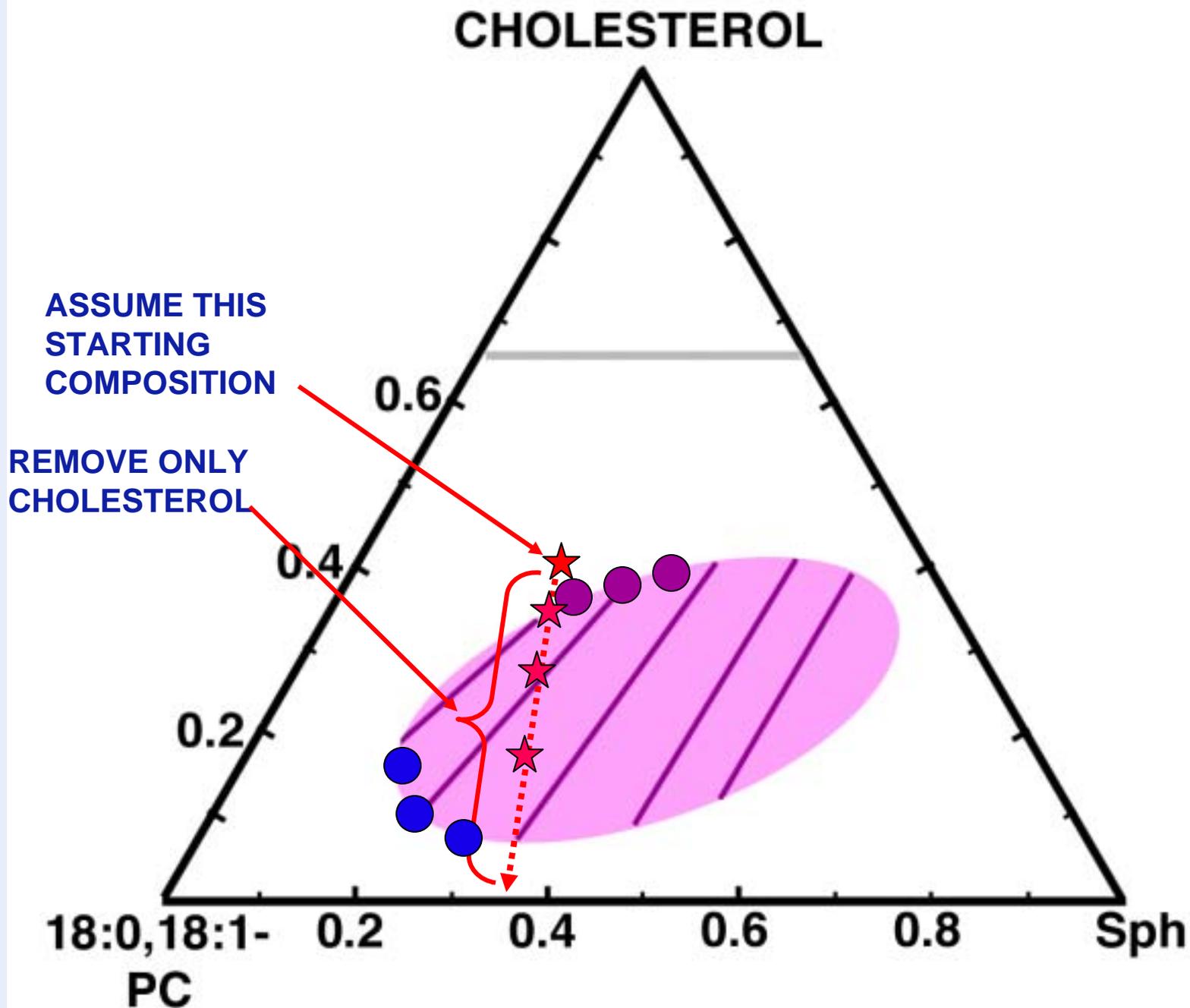
A DIFFERENT GENERIC 3-COMPONENT PHASE DIAGRAM

ALL MIXTURES OF
COMPONENTS A, B, AND C
AT CONSTANT
TEMPERATURE

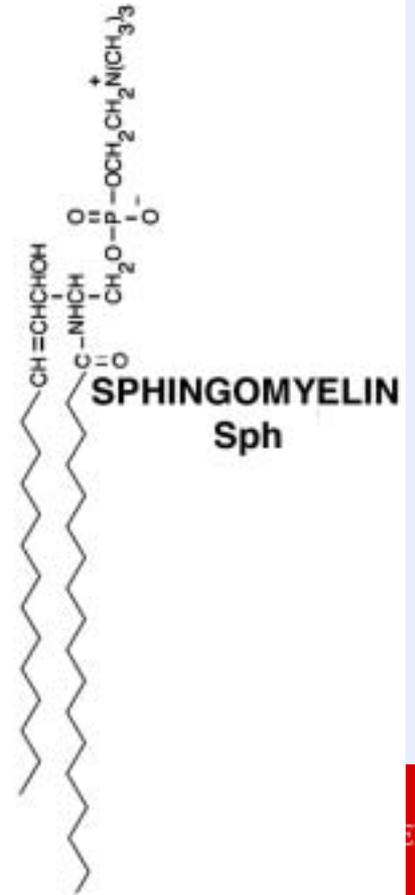
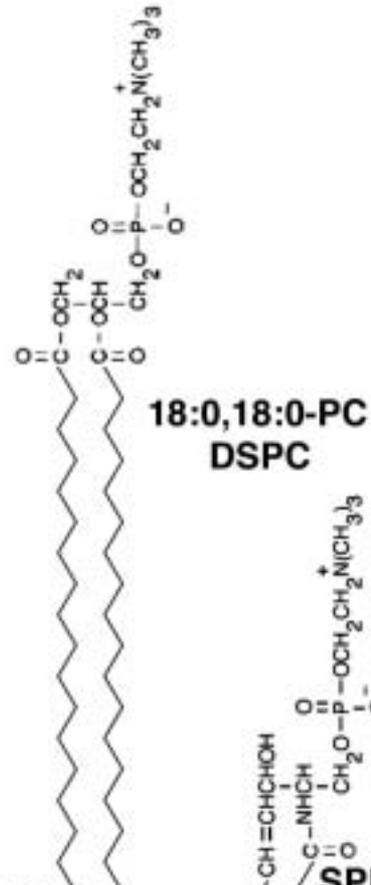
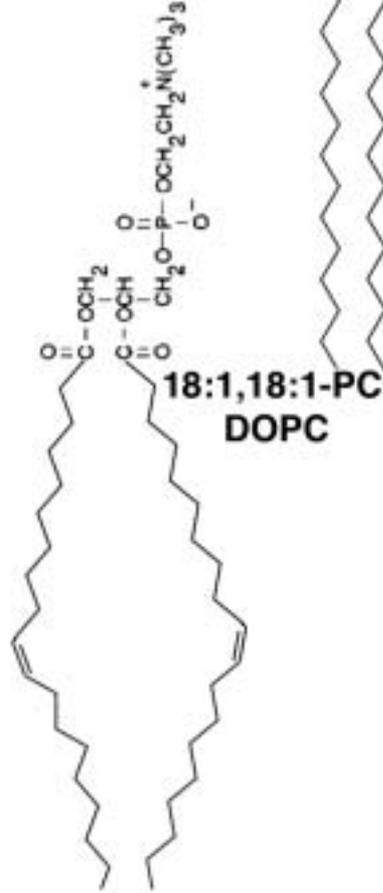
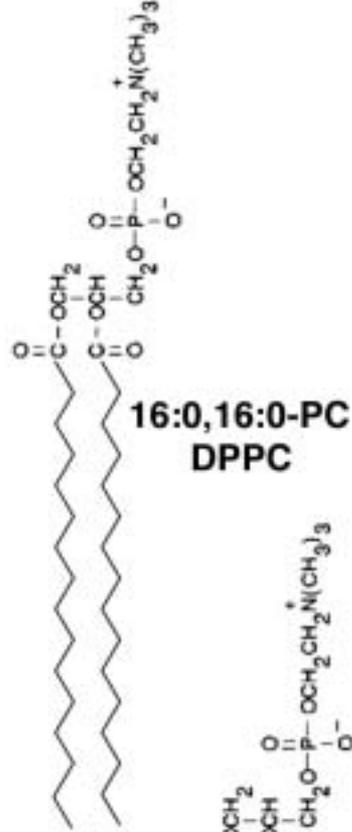
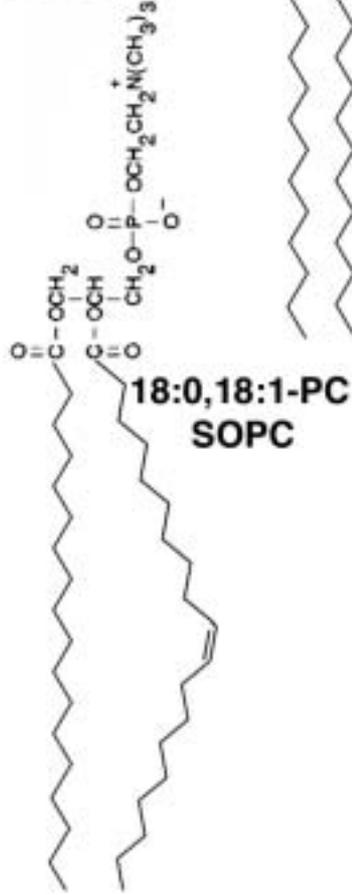
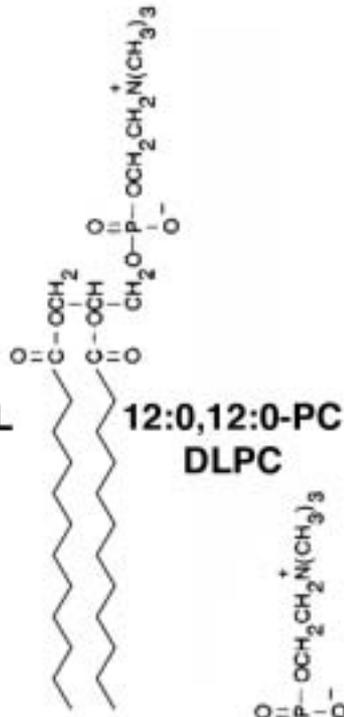
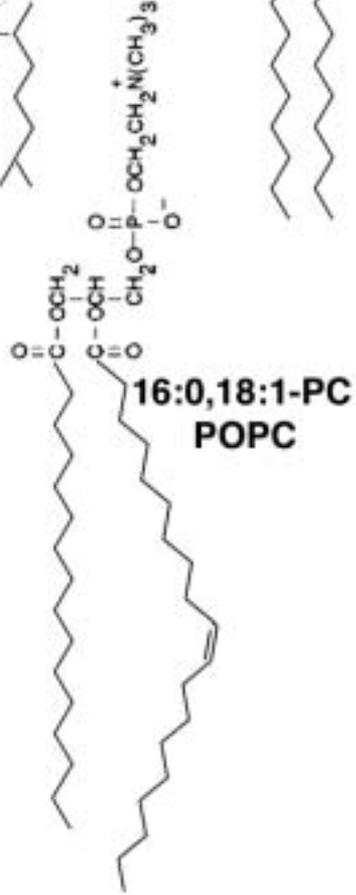
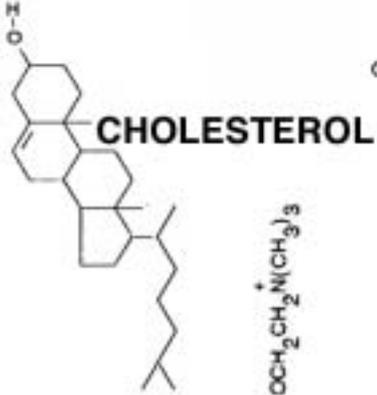
PHASES: a, b, & c



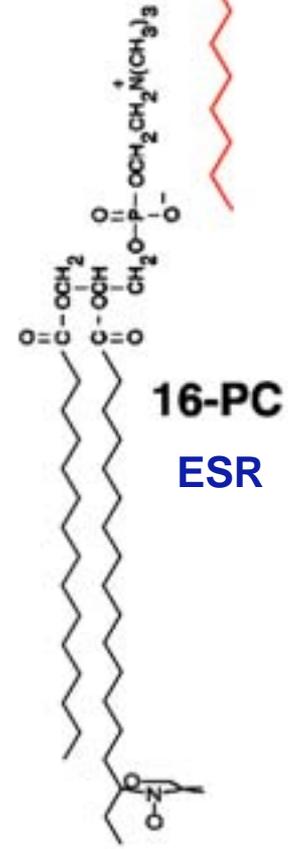
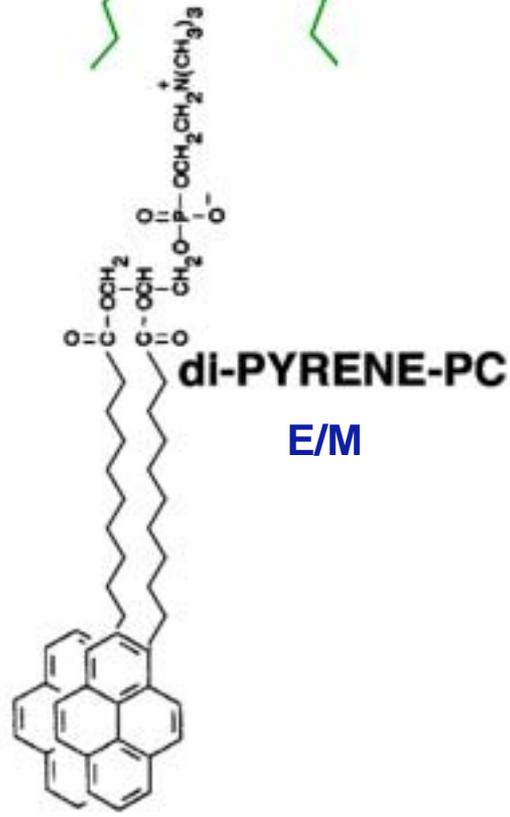
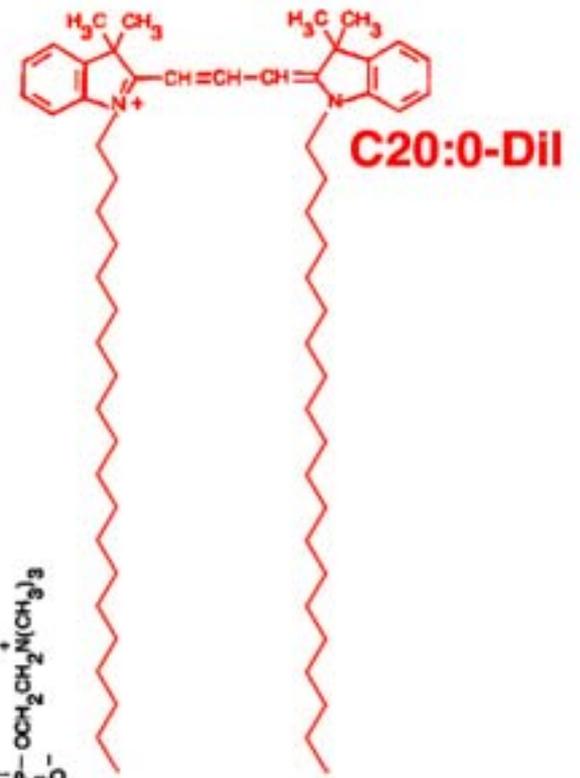
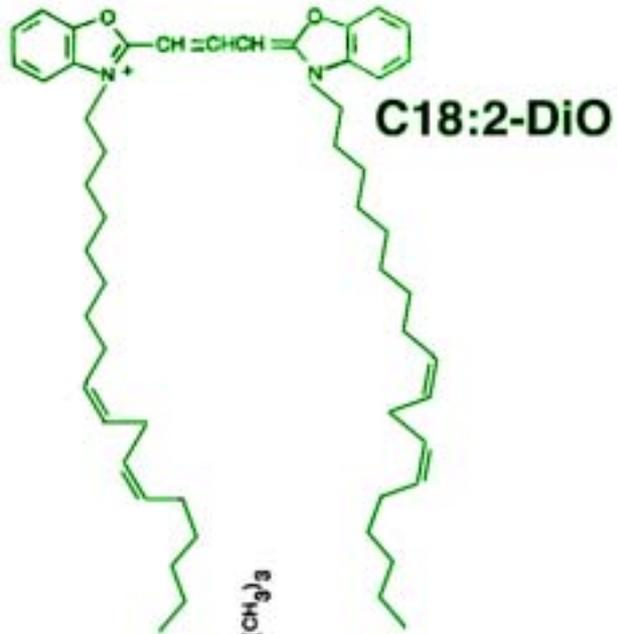
CHOLESTEROL DEPLETION EXPERIMENT: WHAT REALLY HAPPENS?!



LIPIDS STUDIED



SOME PROBES OF
LIPID BILAYER
PHASE BEHAVIOR



Move toward biology

- Create coexistent phases in more complex mixtures.
- Develop an assay to screen the partitioning of full-length transmembrane proteins.
- Examine the effect of other membrane binding proteins.
- Test the partitioning of transmembrane peptides with different properties.