“Rafts” as mixtures of lipids and cholesterol: Are we still at sea?

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The Basic Idea

1. That the plasma membrane is not homogeneous: These inhomogeneities permit proteins to aggregate and perform more efficiently.

2. That these inhomogeneities are due to phase separation.
THE PLAYERS IN MODEL MEMBRANES

Two representative constituent lipids and cholesterol.

DPPC  
\text{di}(16:0)pc

DOPC  
\text{di}(18:1c9)pc

Cholesterol
THEIR PHASES

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   two-dimensional solid
   well-ordered chains->high areal density
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b) Liquid disordered phase (Id)
   disordered chains -> low areal density
   rapid diffusion
   biologically useful

c) Liquid ordered phase: (Lo)
   chains of intermediate order -> intermediate areal density
   relatively rapid diffusion
   biologically useful
   cholesterol required
Liquid ordered and liquid disordered phases can coexist.

Domains of liquid ordered phase can float in a “sea” of liquid disordered phase like a RAFT.
Lateral phase separation is readily seen in these membranes with fluorescence microscopy.

1:1 DPPC/DOPC +30% Chol. @30C. (~303K)

2:1 DPPC/DOPC +20% Chol. @30C. (~303K)

-This is fluid/fluid coexistence.

Micrographs courtesy of Sarah Veatch.
RAFTS

lo and ld have different areal densities

Proteins attached by chains can distinguish the two

Sorting of proteins -> Efficiency
RAFTS

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Proteins attached by chains can distinguish the two—>

Sorting of proteins—> Efficiency

PHYSICAL ORGANIZATION LEADS TO FUNCTIONAL ORGANIZATION
Lateral phase separation is readily seen in these membranes with fluorescence microscopy.

-This is fluid/fluid coexistence.


Reminiscent of “raft” composition.
Typical Phase Diagram

Q: Where does liquid-liquid coexistence come from?

Usually, coexistence in the ternary comes from one of the binary systems.
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Usually, coexistence in the ternary comes from one of the binary systems.
SUPRISE!

Ternary system phase separates even though no binary system does!

Q: How do we understand this?
Theory

The physics:
Saturated and unsaturated lipids phase separate when saturated chains are sufficiently ordered, as in gel. This is a simple packing effect.

In liquid phase, the saturated chains are not, by themselves, sufficiently ordered to cause phase separation.
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Adding cholesterol to liquid orders the saturated chains.
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Adding cholesterol to liquid orders the saturated chains.

With addition of enough cholesterol, chains are sufficiently ordered to make separation favorable once again.
Results for $T$ above gel transition

Results for T below gel transition
Major Problem:

Ternary mixtures of inner leaf composition don’t show phase separation.

Fig. 1. Distribution of the main phospholipid classes between the two leaflets of the human erythrocyte membrane. Values are expressed in % of the total phospholipids. Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; PI, phosphoinositides.

Possible Solutions

1. Coupling to outer leaf which has strong tendency to phase separate.
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2. Cross-linking of a component.
Effect of dimerizing unsaturated lipids.
No dimers (dotted),
3% dimers (solid).

Crosslinking five saturated lipids of concentration $z$
Could rafts just be fluctuations?
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Of course; and they might be critical
Could rafts just be fluctuations?

Of course; and they might be critical and they might not.....
Q: What about charged PS and PI on inner leaf?
Possible effects of PS and PI

- Creates a charged layer on inner leaf only.
Possible effects of PS and PI

- Creates a charged layer on inner leaf only.
Possible effects of PS

- Creates a charged layer on inner leaf only.
- Charges introduce two new lengths:

Debye screening length

\[ L_D = \left( \frac{4\pi \kappa \epsilon_0 k_B T}{e^2 c} \right)^{1/2} \]

Gouy-Chapman length

\[ L_{GC} = \frac{4\pi \kappa \epsilon_0 k_B T}{e^2 \sigma} \]
Fluctuations of charged regions attract at large distances, repel at short distances;

Hence fluctuating regions of characteristic length, $L_D$ or $L_{GC}$. 
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We do now understand the behavior of the simple model ternary systems.

We are now adding to the theoretical models more biological details.

This is as it should be.
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SO WE MAY STILL BE AT SEA, BUT THE ORDEAL MAY SOON BE OVER!
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