Protein-lipid interactions and the lipid raft hypothesis

Fivaz & Meyer 2003 *Neuron* 40, 319-30
Munro 2003 *Cell* 115, 377-88

How to target proteins to membranes?
Covalent lipid modification of proteins

• Palmitoylation (16 C acyl chain)
  – (Cys) thioester bonds
  – The most abundant
  – The only reversible (can be cleaved by thioesterases)

• N-myristoylation (14 C acyl chain)
  – In most cases not enough to anchor a protein to membrane

• Prenylation (farnesyl or geranylgeranyl)
  – Thioether linkage to a C-terminal Cys

Protein binding domains

• Polybasic domains
  – Short cluster of basic amino acids (<30)
  – non-specific binding to acidic lipids
  – Act synergistically with lipid modifications

• Phospho inositides binding modules
  – Low affinity lipid interaction modules that regulate transient association w/cellular membranes
  – Examples:
    • PH, FERM, ENTH [PI(4,5)P₂]
    • FYVE, PX [PI(3)P]

• C1 and C2 domains
How fast are proteins localized to their targets in the cell?

Table 1. Occurrence of Membrane Targeting Motifs in the Human Genome

<table>
<thead>
<tr>
<th>Membrane Targeting Motif</th>
<th>Number of Motifs in the Human Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid-based:</td>
<td></td>
</tr>
<tr>
<td>Palmitoylation</td>
<td>?</td>
</tr>
<tr>
<td>Myristoylation</td>
<td>270</td>
</tr>
<tr>
<td>Prenylation (CaaX)</td>
<td>214</td>
</tr>
<tr>
<td>Protein-based:</td>
<td></td>
</tr>
<tr>
<td>Polybasic</td>
<td>?</td>
</tr>
<tr>
<td>PH domains</td>
<td>448</td>
</tr>
<tr>
<td>ENTH domains</td>
<td>16</td>
</tr>
<tr>
<td>FYVE domain</td>
<td>66</td>
</tr>
<tr>
<td>PX domains</td>
<td>65</td>
</tr>
<tr>
<td>C2 domain</td>
<td>225</td>
</tr>
<tr>
<td>C1 domain</td>
<td>97</td>
</tr>
</tbody>
</table>
Suggested roles for Rafts

- Signal transduction pathways (Leah, Paejonette, Daniel)
- Apoptosis (Sait)
- Cell adhesion and migration (Casey)
- Synaptic transmission (Kathryn, Andrew, Khursheed)
- Organization of cytoskeleton (Ban-Yoon, Kristina)
- Protein sorting during exo- and endocytosis (Nat)
- Point of entry of viruses, bacteria, and toxins (William, Fabian)
- Site of viral assembly and formation of prion and Alzheimer amyloid (Dorota, Adriana)

Alicja, David, Krovi, Paul
(?) Kevin, Navin

However…

- Current knowledge does not unequivocally support the existence of rafts

- Alternative models for the structure of the plasma membrane are equally plausible
Mixtures of lipids form lipid phases

A Lipid structures
- cholesterol
- phospholipids
- sphingolipids

B Membrane phases
- gel
- liquid disordered (Lo)
- liquid ordered (Ld)

'Rafts' in the Plasma Membrane

- Liquid-Ordered (Ld) Domain (Raft)
- Liquid-Disordered (Lo) Bilayer
- Transmembrane Proteins
- Multiply Acylated Proteins
- Glycosphingolipids
- (Cytoplasm)
Detergent/Low Temperature Method to Isolate and Characterize ‘Rafts’

1. Triton X-100 0 - 4°C
2. Apply under Density Gradient
3. 100,000 x g (0 - 4°C) Raft Fraction

A sphingolipid/cholesterol rich raft in the outer leaflet connects to a cholesterol rich domain in the inner leaflet, to allow co-clustering of GPI-anchored proteins and signalling components.

A sphingolipid/cholesterol rich outer leaflet forms a permeability barrier but allows free lateral diffusion of components, and signalling via protein-protein interactions.

Figure 2. Models for the Organization of the Plasma Membrane
Detergent-resistant membranes

• There are a number of reasons why it cannot be assumed that detergent resistance reflects a protein being present in a microdomain that was present before triton was added.

  – A number of nonphysiological rearrangements of the bilayer could occur during detergent solubilization
    ▪ Reduction in T can alter lipid organization
    ▪ The highly asymmetric nature will lead to anomalous processes
    ▪ Triton actually promotes the formation of ordered domains in model bilayers (Heerklotz 2002, Biophys. J. 83, 2693)

Lipid phase behavior is highly temperature dependent

![Image of lipid phase behavior](image_url)

FIGURE 5 Two-photon excitation fluorescence intensity images (false color representation) of LAURDAN-labeled GUV as a function of temperature formed from DOPC:cholesterol/sphingomyelin (1:1:1) with 1 mol % GM1 added. The images have been taken at the top part of the GUV. The lower right panel shows an equatorial section demonstrating the absence of internal vesicles. Bar, 50 μm.

(Dietrich et al., Biophys. J. 2001)
Relative Affinities of Different Bimane-labeled Diacyl PE Probes for Lc vs. Ld Domains in 1:1:1 Spin-labeled PC/Sphingomyelin/Cholesterol Bilayers

**Relative Affinities of Bimane-labeled Diacyl PEs for Liquid-ordered Domains**

(Sphingomyelin/Spin-labeled PC Cholesterol Bilayers, 37°C)

Acyl Chains of Bimane-labeled PE
Sensitivity to cholesterol depletion

- Cholesterol affects a number of physical properties of the membranes
- Cellular processes are sensitive to changes in any one physical property
- Any consequences would not necessarily be due to the loss of rafts
Conclusion

• The existence of lipid rafts cannot be taken as an established fact
• We should proceed with caution and open mind to alternative mechanisms for phenomena currently attributed to rafts