

iBio Seminars Part I: What are Membrane Rafts?

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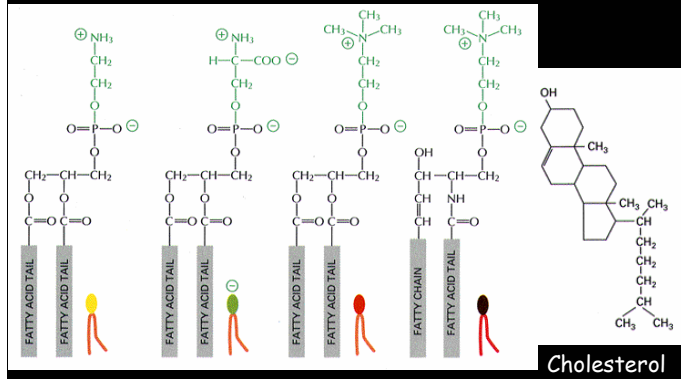


The cell membrane:



The Art of MBOC, Garland Publishing

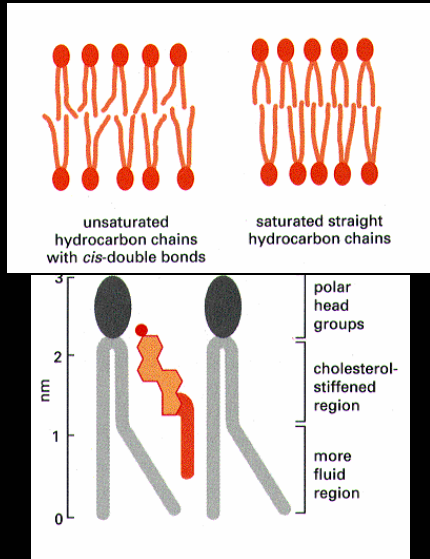
The cell membrane:



phosphatidylethanolamine
phosphatidylserine
phosphatidylcholine
sphingomyelin

The Art of MBOC, Garland Publishing

The cell membrane



The Art of MBOC, Garland Publishing

History of cell membrane structure

Time-line by Michael Edidin

(Nature Reviews MCB, 2003).

From Agnes Pockels (1880s) to Langmuir (1917) for lipid bilayer organization, to

The debate between trilamellar and bilayer models where proteins and lipids are incorporated.

Reveals an inter-twined relationship between concepts borrowed from studies on artificial membranes and with experiments on cell membranes

History of cell membrane structure

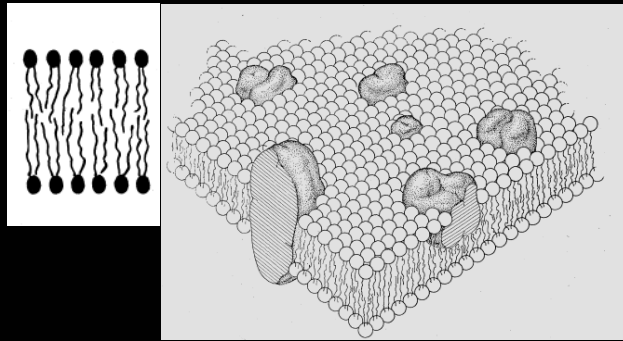
Singer and Nicholson Picture: the fluid-mosaic model

(*Science*, 1972)

- derived from observations of EM analysis of protein arrangement and protein diffusion on cell membranes
- coupled with an understanding of how membrane proteins are associated with bilayer- peripheral and integral membrane proteins

History of cell membrane structure

- Singer and Nicholson Picture:
the fluid-mosaic model (*Science*, 1972)



Proteins embedded in a sea of lipids
bilayer arrangement of lipids

History of cell membrane structure

'The membrane is an oriented, two-dimensional, viscous solution of amphiphatic proteins (or lipoproteins) and lipids *in instantaneous thermodynamic equilibrium*'

History of cell membrane structure

The fluid-mosaic model : consequences

'There is generally no long-range
order in a mosaic membrane with a
lipid matrix'

'The lipids of a functional cell
membrane are in a fluid-state rather
than a crystalline state'

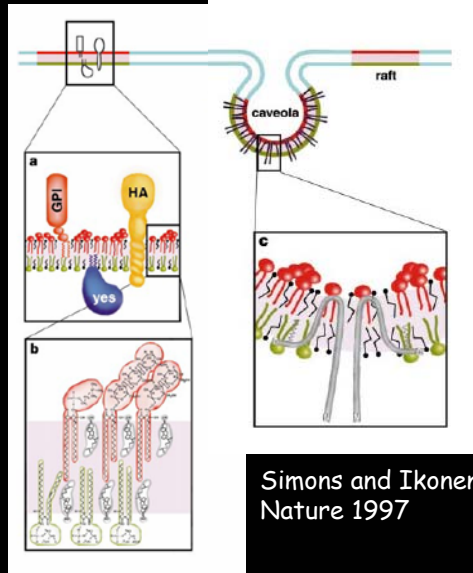
What are Membrane Rafts?

- Pub Med survey ~ 2007
~ 2500 hits
- Involved in diverse cellular processes
 - Membrane trafficking
 - Membrane sorting
 - Signaling
 - Cell Migration

Membrane Rafts are hypothesized to be involved in diverse biological phenomena

- Nervous system
- Immune function
- Nutrient uptake
- Cell cycle
- Virus budding and entry
- Pathogen biology
- Cell Motility

What is understood by the term 'Membrane Rafts'?



Simons and Ikonen,
Nature 1997

What is understood by the term 'Membrane Rafts'?

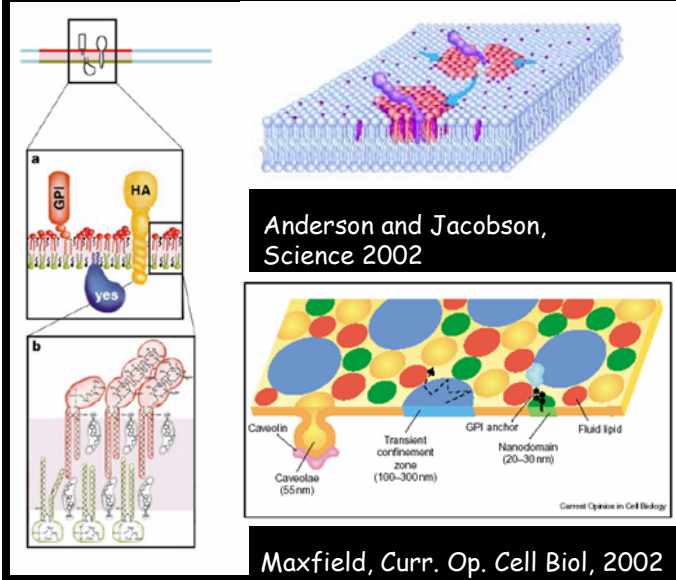
- Cholesterol and sphingolipid-enriched domains
- Detergent resistant membranes (DRMs)
- Cholesterol-sensitive functional membrane complexes

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- Cholesterol and sphingolipid-enriched domains
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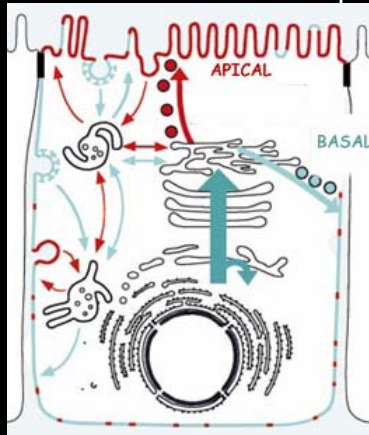
- Illusive or elusive!
- Controversial

What is understood by the term Membrane Rafts?



Origin of the Raft Hypothesis-1

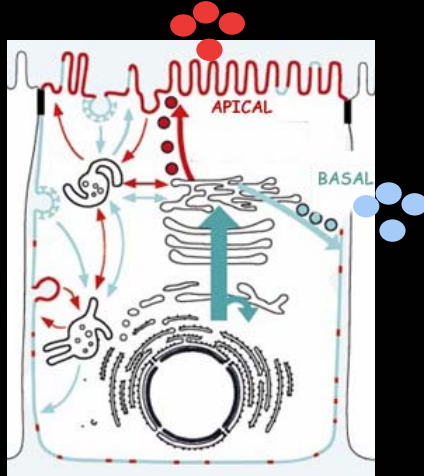
Epithelial Cells have distinct protein and lipid compositions at their apical and basolateral surfaces: *GSLs* and Sphingomyelin are concentrated at the apical surface



From Simons and Ikonen, 1997

Origin of the Raft Hypothesis-1

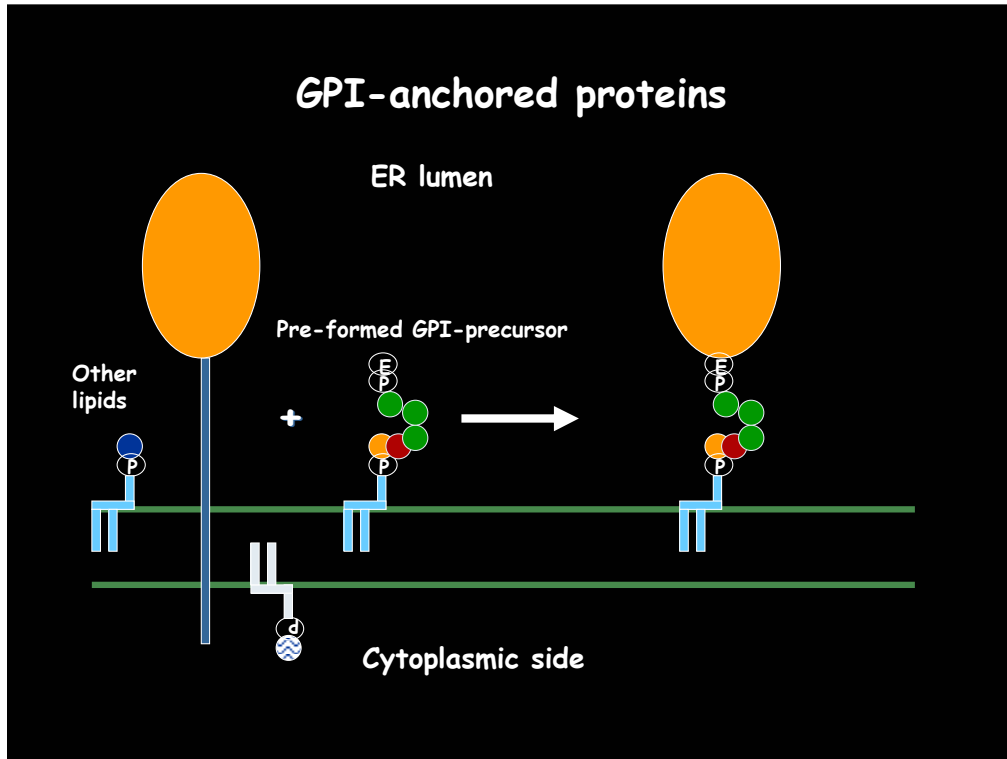
Viruses that bud from apical and basolateral surfaces sample distinct protein and lipid compositions

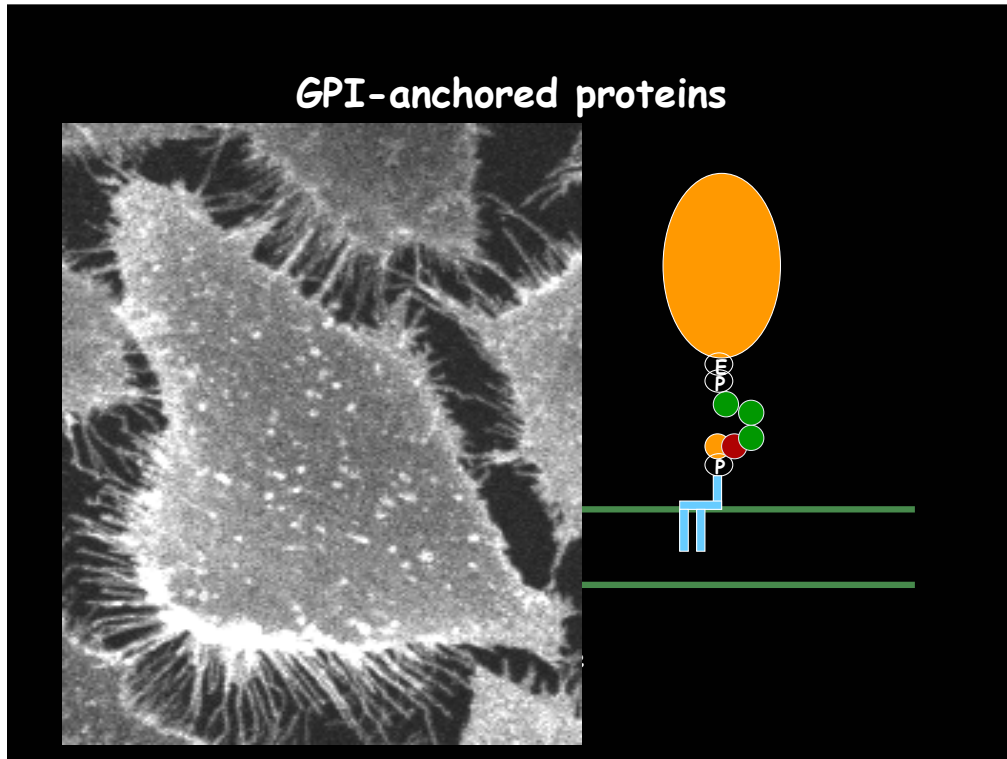


Origin of the Raft Hypothesis-2

- Lipid and GPI-anchored protein behavior
- Most GPI-anchored proteins are apical
 - GPI-anchored protein traffic is polarized
 - GPI-anchor acts as a sorting signal for secretory and endocytic cargo

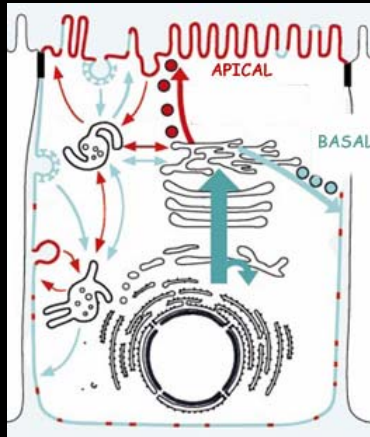






Origin of the Raft Hypothesis-2

GPI-anchoring serves as an apical sorting signal in many contexts



Origin of the Raft Hypothesis-3

DRM-association and phase segregation

- Detergent insolubility of *GSLs* and *GPI*-anchored proteins ~ 60- 90% insoluble in cold Triton X-100 (N. Hooper and colleagues)
- DRM-association during apical transport: (Brown and Rose; *Cell*, 1992)
- Artificial membrane studies
 - Cholesterol-induced phase segregation
 - Neutral *GSLs* are clustered

Origin of the Raft Hypothesis-4

(Linking up with transbilayer-signaling)

- Crosslinking GPI-anchored proteins activates intracellular signalling
- Cold detergent isolation of src-family tyrosine complexes and GPI-anchored proteins
- IgE Receptor signaling and DRMs
- B Cell Receptor and lipid-domains

An operational definition of Rafts

- DRM-association
- Perturbation of lipid constituents-
cholesterol and/or sphingolipids

by the use of a cholesterol chelating agent
'cyclodextrin' or metabolic perturbation of lipid
levels-

Proliferation of Raft-related studies (~ 90's to 2000)

- **Artificial Membranes:**
Investigation of the phase-behavior of 'DRM-composition' lipid-mixtures
- **Cellular context:** Application of the operational definition of 'Rafts' to numerous cellular processes

Phase behavior of Raft- composition artificial membranes

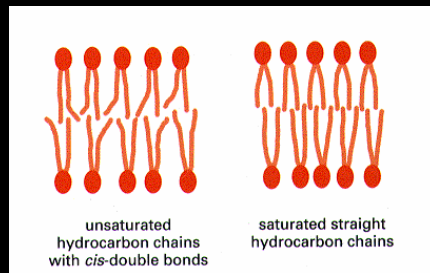
- DRM-composition - Chol:Sph:PC
(~1:1:1)

Supported membranes and Giant
Unilamellar Vesicles (GUVs)

Fluorescently-labeled lipid-probes
/optical imaging/spectroscopy

Phase behavior of Raft-composition artificial membranes

- Fluid membranes where two types of immiscible liquid phases co-exist
 - Liquid-ordered (lo) phase (promoted by cholesterol and long, saturated acyl chain containing phospholipids)
 - Liquid-disordered (ld) phase



Connection of Lo domains, rafts and DRMs

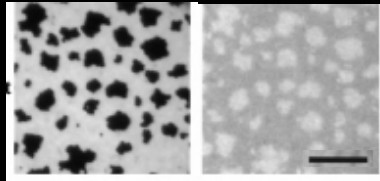
- DRM-association and
Lo domains

Brown and London, 1990s

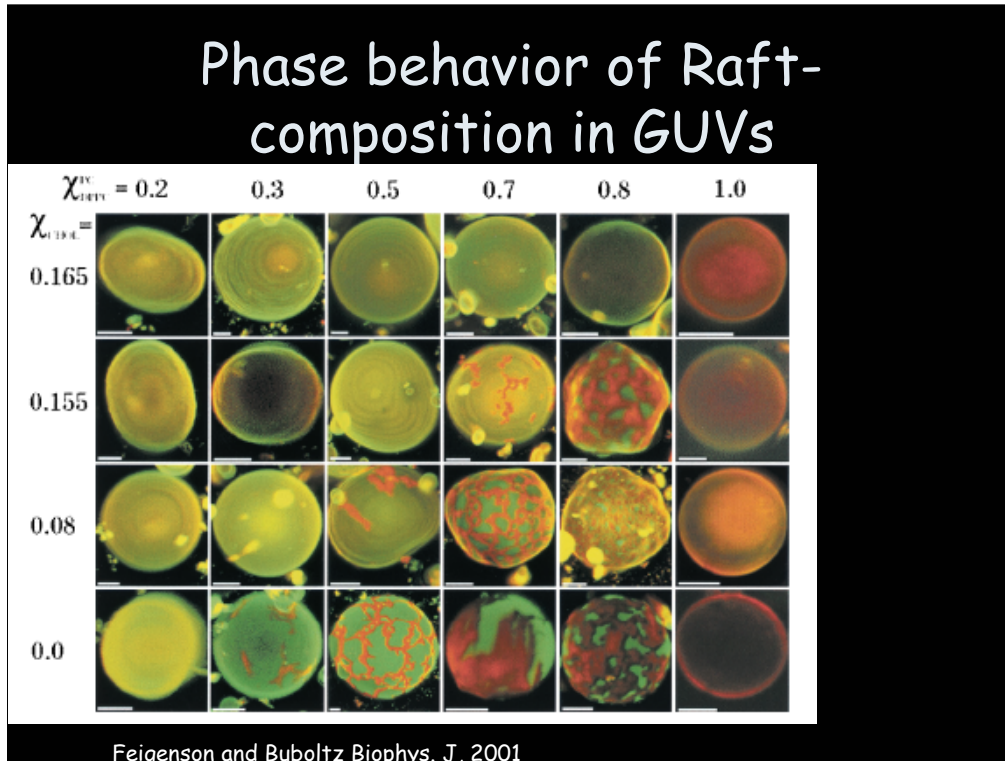
Phase behavior of Raft- composition artificial membranes

Supported monolayers consisting of
Cholesterol and lipid mixtures

Fl-Phospholipid GPI-anchored protein



Dietrich et al., *PNAS*, (2001)



Lessons from artificial membranes

- Role of Cholesterol in making domains
 - umbrella effect
 - condensed complexes

Lessons from artificial membranes

- Trans-bilayer coupling
- Charge of lipid-headgroup affects partitioning

Do DRMs represent pre-existing structures or 'Rafts' in cell membranes ?

Quantitative physico-chemical studies with addition of Triton in artificial membranes suggest that they do not.

Heerklotz and Seelig, *Biophys J.* 2000

Heerklotz, *Biophys J.* 2001

Lichtenberg et al., *TiBS* (2005)

Effects of cholesterol removal

- Dramatic effects on actin cytoskeleton- Kwick et al, PNAS, (2003)
- Depletes intracellular Ca^{+2} and depolarizes the plasma membrane - Pizzo et al. Eur. J. Immunol (2002)

What about lipid rafts ?

Are 'rafts' creations of the operational definition?

What about lipid rafts ?

Are 'rafts' creations of the operational definition?

Yes!

What about the 'Raft' hypothesis?

Regardless, cellular
phenomenon provide the
reason to expect specialized
membrane domains endowed
with function.

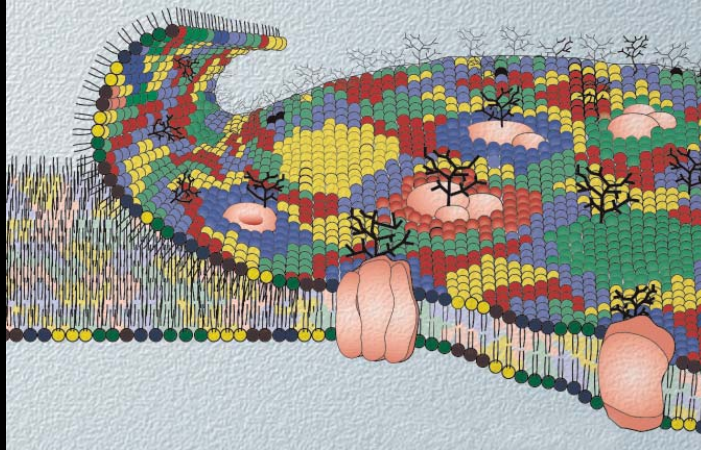
Functional membrane domains must exist

- Lipid and *GPI*-anchored protein sorting- apical polarity, and endocytosis
- Co-crosslinking of lipid-anchored species in membranes
- Lipid-dependence of H-Ras signalling
- IgE, B cell receptor signaling

Revisiting the 'Rafts' hypothesis?

Can we visualize specific regions of the cell membrane where specific lipid and protein components are concentrated in a functional context?

Is the membrane a patchwork-quilt where lipids are organized with the help of proteins?



Created by P. Kinnunen (University of Helsinki, Finland); source Edidin, Nature Reviews MCB, 2003

Parts II and III

- In Part II, I describe how studying endocytosis of GPI-anchored proteins has compelled me to examine lipid-anchored protein organization in living cell membranes
- In Part III, we explore new ideas about how the plasma membrane may be organized