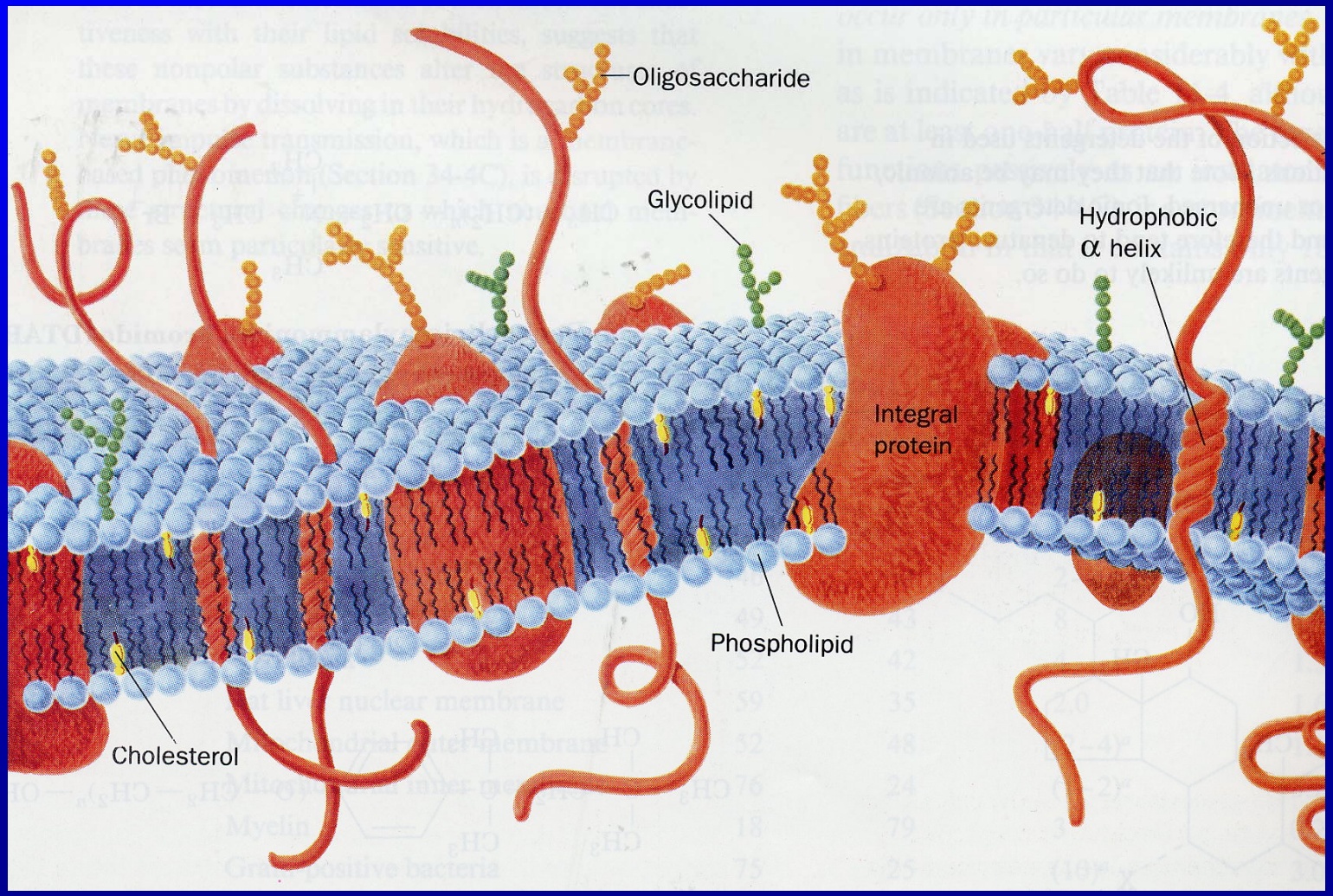
Lipidne mikrodomene

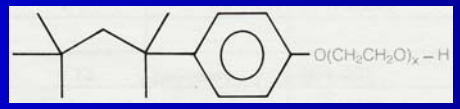
struktura

Singer-Nicholson fluid mosaic model of a biological membrane organization (1972)



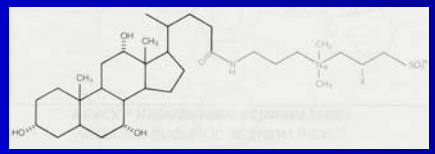
Only part of biological membranes is solubilized after treatment at low T (≤4°C) with:

Triton X-100 (NP-40)



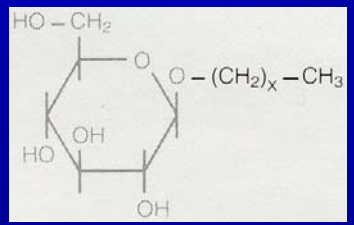
Brij-58

CHAPS



The remaining membranes are soluble in:

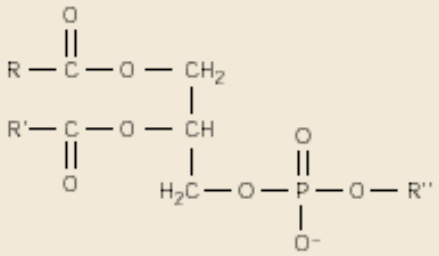
octyl glucoside



above mentioned detergents at higher T

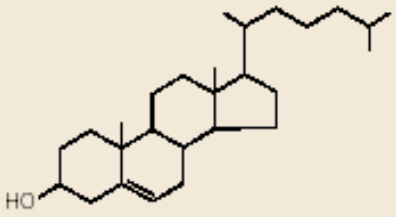
Basic lipid structures

Phospholipids

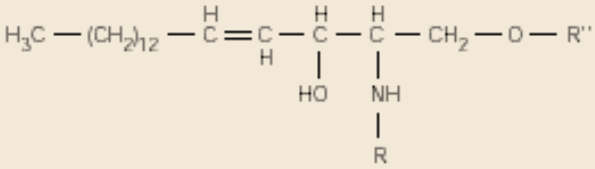


R, R', Hydrocarbon chains of fatty acids
R'', Head-group

Cholesterol



Sphingolipids

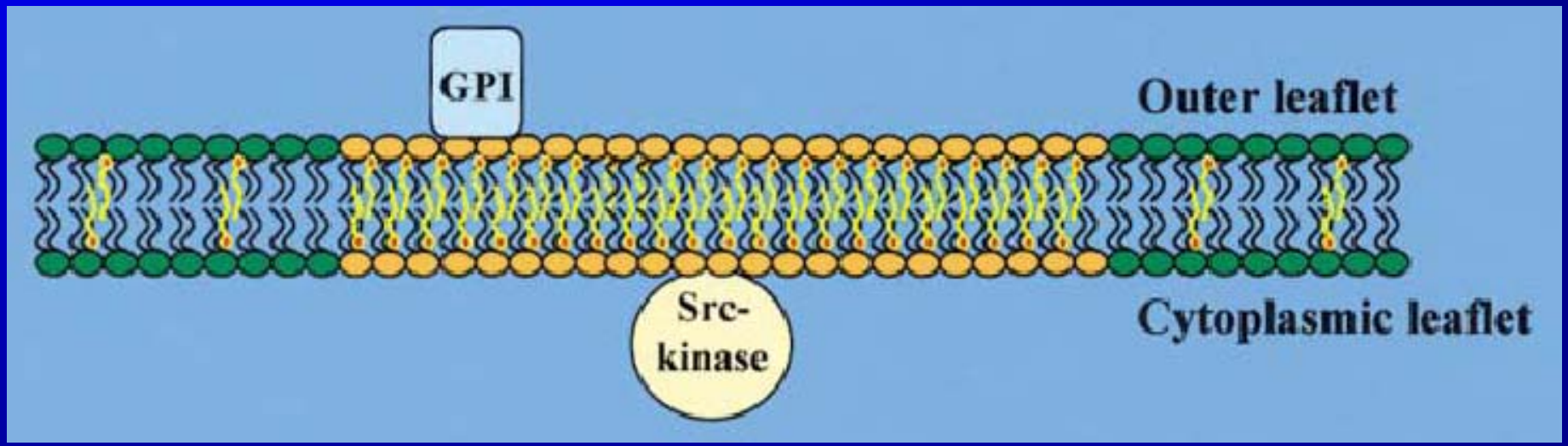


Lipids exist in:

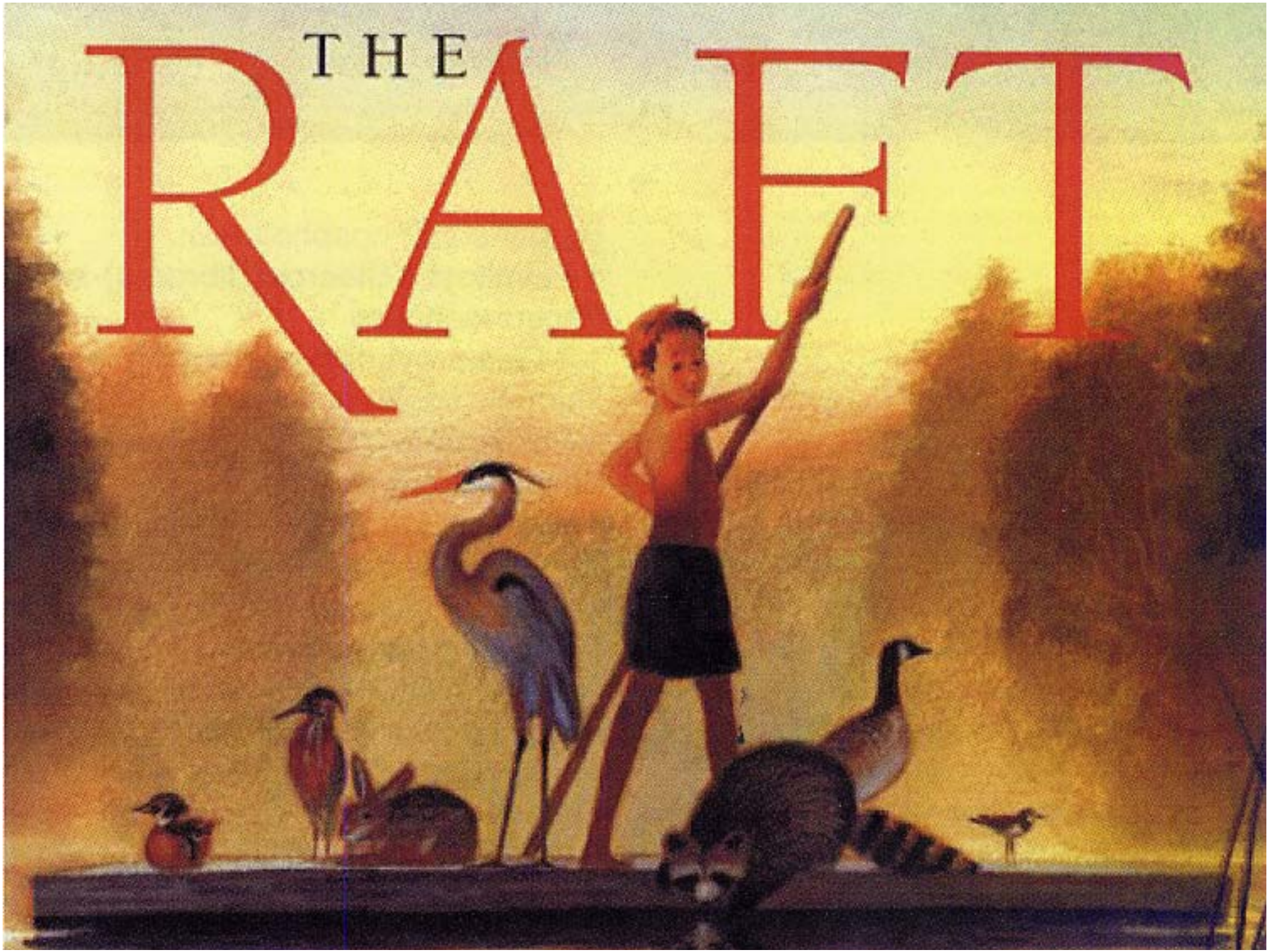
- gel state (semi-frozen)
- liquid-ordered state
- liquid-disordered state (fluid mosaic)

Biological membranes possess an intrinsic order:

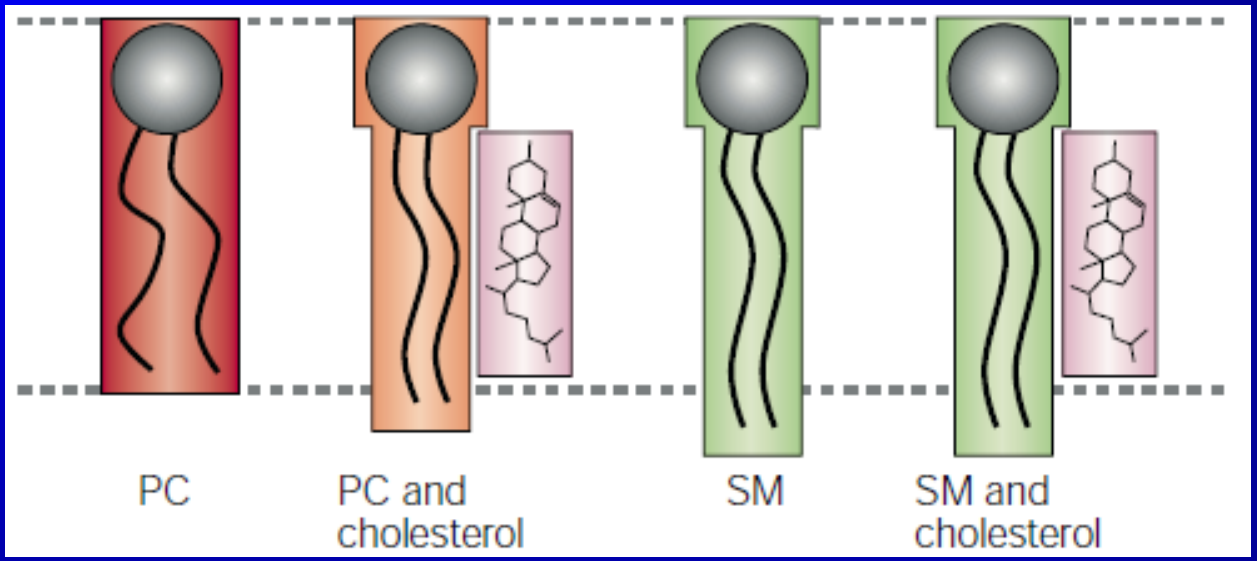
raft concept



Galbiati et al. (2001) Cell 106, 403-411.

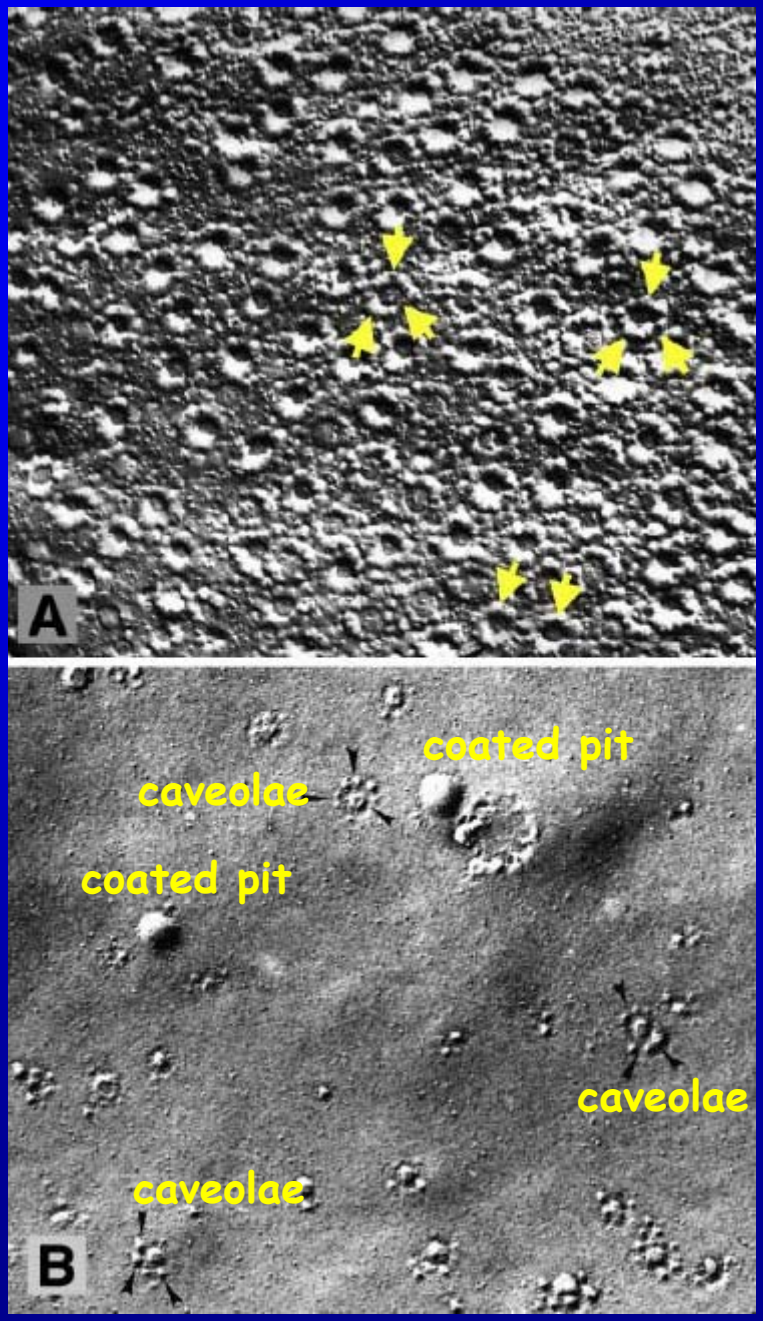

THE
RAFT

Cholesterol can induce fluid-fluid immiscibility



Sprong et al. (2001) Nat. Rev. Mol. Cell. Biol. 2, 504-513.

Cholesterol is concentrated in lipid rafts



Endothelial cell PM

filipin-cholesterol precipitate

Smooth muscle cell PM

Mineo & Anderson (2001) Histochem. Cell Biol. 116, 109-118.

Common tools to disrupt lipid rafts

Cholesterol sequestration

- Antibiotics:

  Filipin | Nystatin | Amphotericin

- Pore-forming agents:

  Saponin | Digitonin | Streptolysin O

Cholesterol depletion

- Methyl-β-cyclodextrin

Inhibition of cholesterol biosynthesis

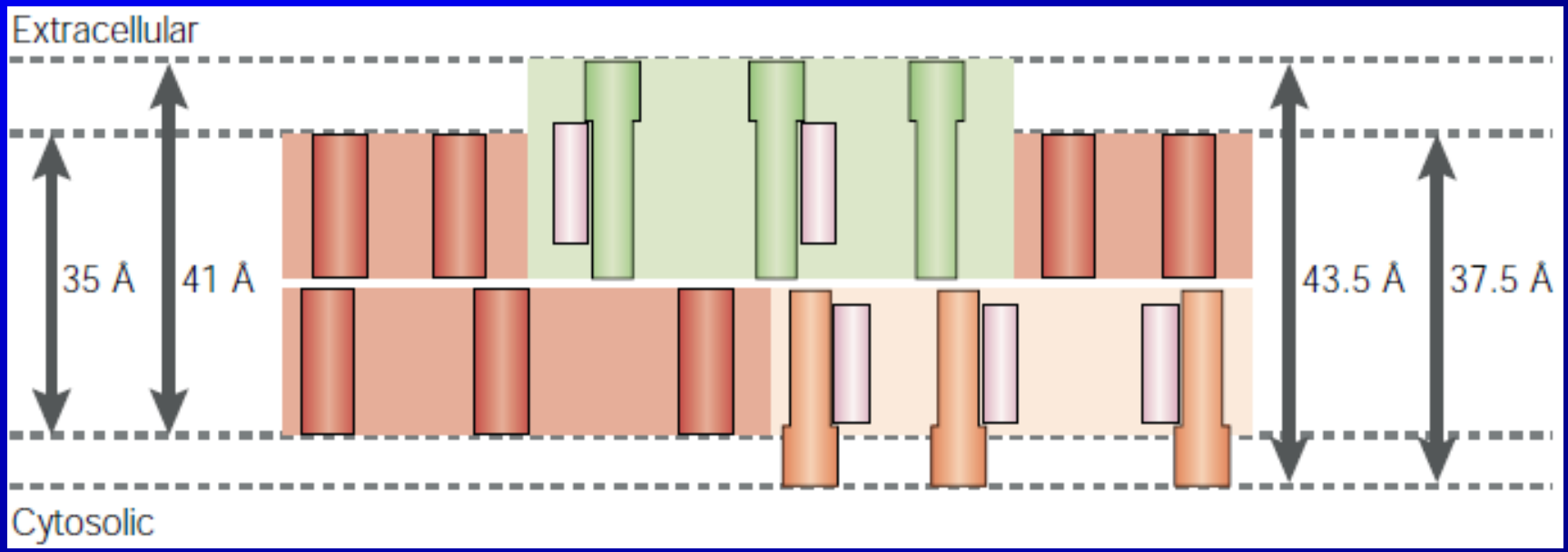
- Lovastatin

Perturbation of raft stability

- Exogenous cholesterol
- Exogenous gangliosides
- Exogenous polyunsaturated fatty acids

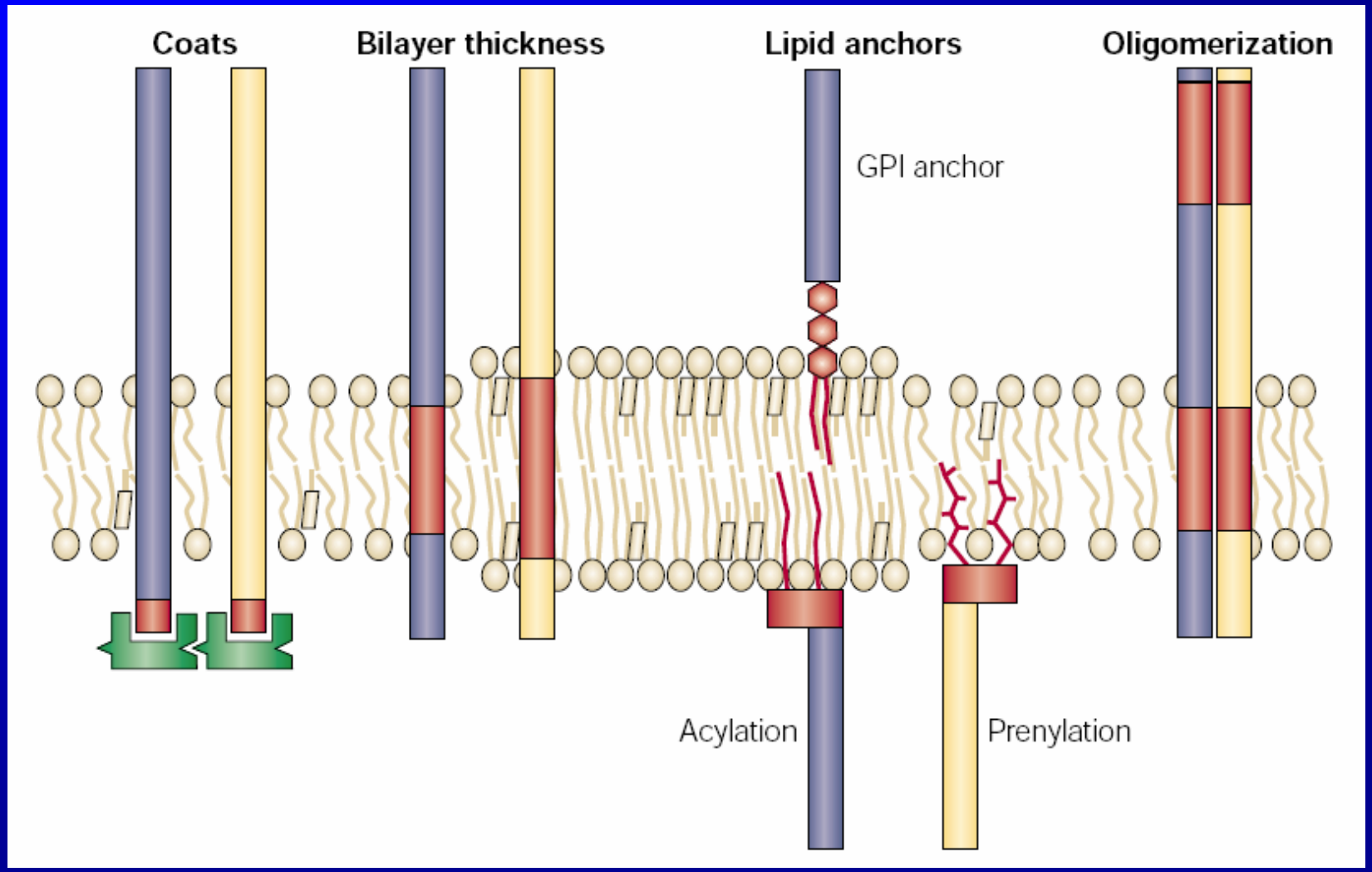
Simons & Toomre (2000) Nat. Rev. Mol. Cell Biol. 1, 31-40.

Membrane thickness depends on lipid composition



Sprong et al. (2001) Nat. Rev. Mol. Cell. Biol. 2, 504-513.

Lateral sorting of membrane proteins



Sprong et al. (2001) Nat. Rev. Mol. Cell. Biol. 2, 504-513.

Techniques to study lipid rafts

Approach*	Information available	Live cells	Comments
Flotation of detergent–resistant membranes (DRMs)	Identifies putative raft association Identifies possible raft proteins	No	• Easy to do • Most common approach for identifying putative proteins involved in signalling • Artefacts possible • Weak associations with rafts are difficult to detect
Antibody patching and immunofluorescence microscopy	Identifies putative raft association	No	• Easy to do • Common approach • Better than flotation for detecting weak raft associations • Cell–cell variability makes quantification difficult
Immunoelectron microscopy	Determines location of raft components	No	• Promising results • Requires technical expertise
Chemical crosslinking	Identifies native raft protein complexes	Yes	• Straightforward • Choice of appropriate conditions and reagents is semi-empirical
Single fluorophore tracking microscopy	Monitors the diffusion and dynamics of individual raft proteins or lipids	Yes	• Requires highly specialized equipment and expertise
Photonic force microscopy	Determines the diffusion constant, size and dynamics of individual rafts	Yes	• Very informative technique • Requires highly specialized equipment and technical expertise • Time-consuming acquisition and analysis
Fluorescence resonance energy transfer (FRET)	Detects whether two raft components are spatially close (for example, <10 nm)	Yes	• Powerful approach • Choice of appropriate donor and acceptor probes is important

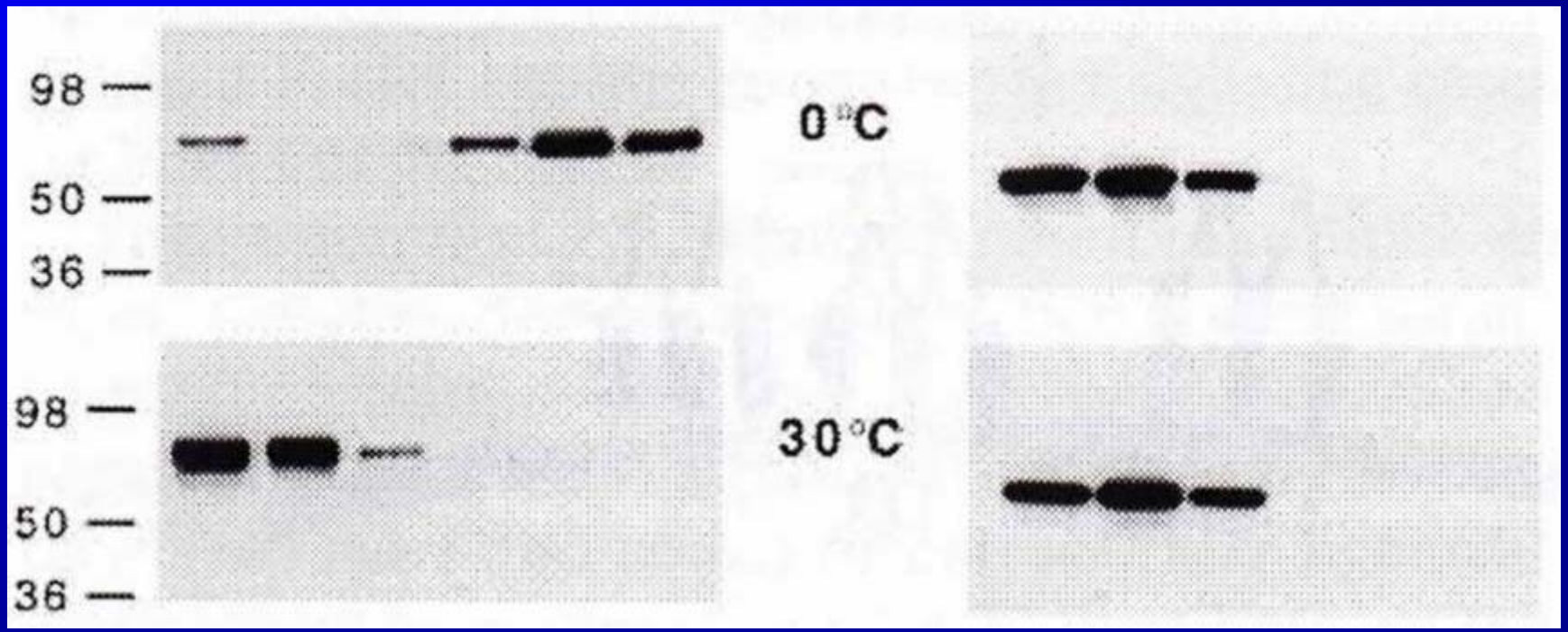
*The disruption of rafts by cholesterol depletion or sequestration is especially useful as a control for each of these approaches.

Simons & Toomre (2000) Nat. Rev. Mol. Cell Biol. 1, 31-40.

Solubilization of biological membranes in 2% (v/v) TR X-100 at 4 or 30°C followed by sucrose gradient centrifugation (flotation) analysis.

PLAP (PLacental Alkaline Phosphatase)

VSV-G (Vesicular Stomatitis Virus Glycoprotein)

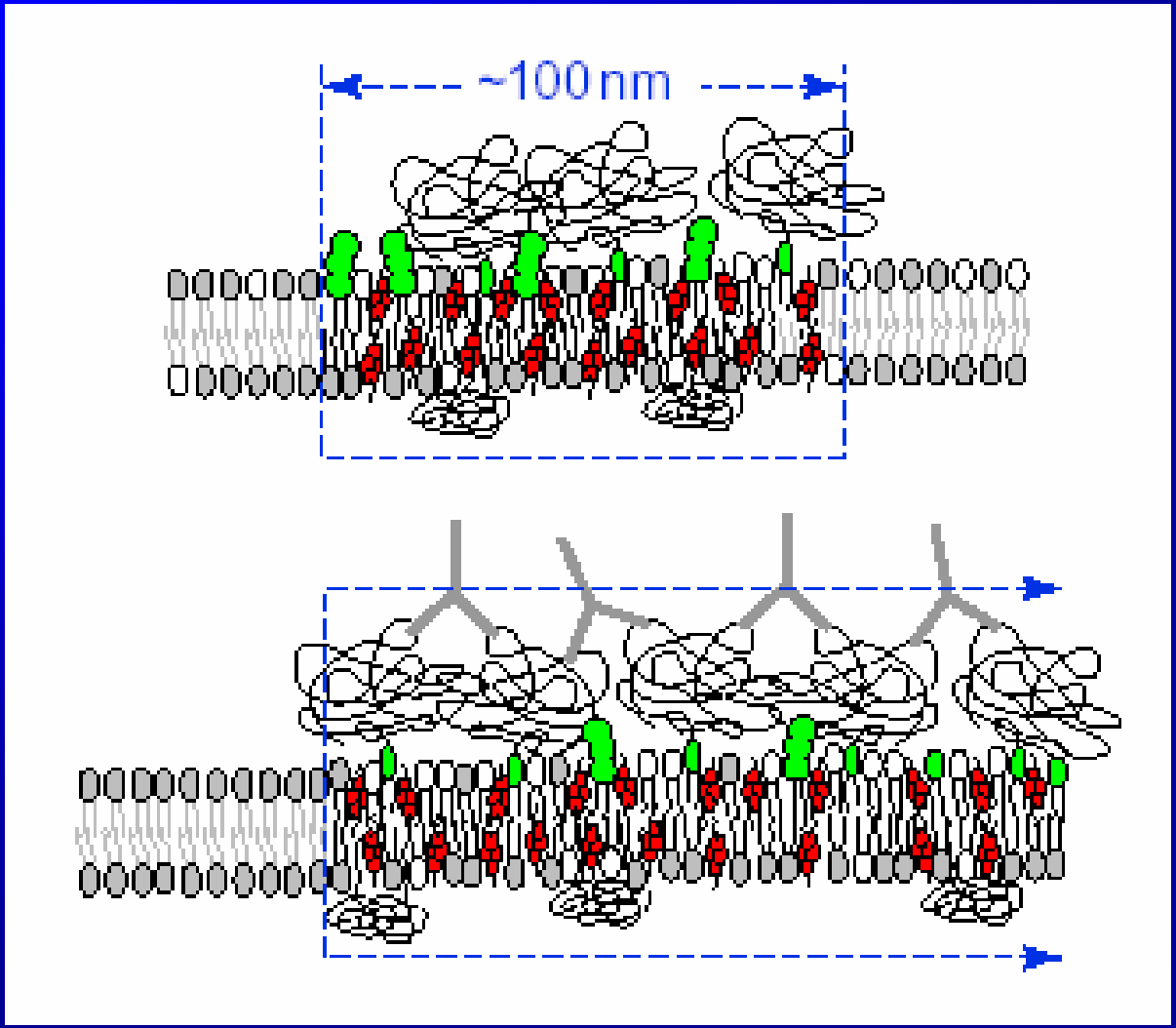


higher → lower density

higher → lower density

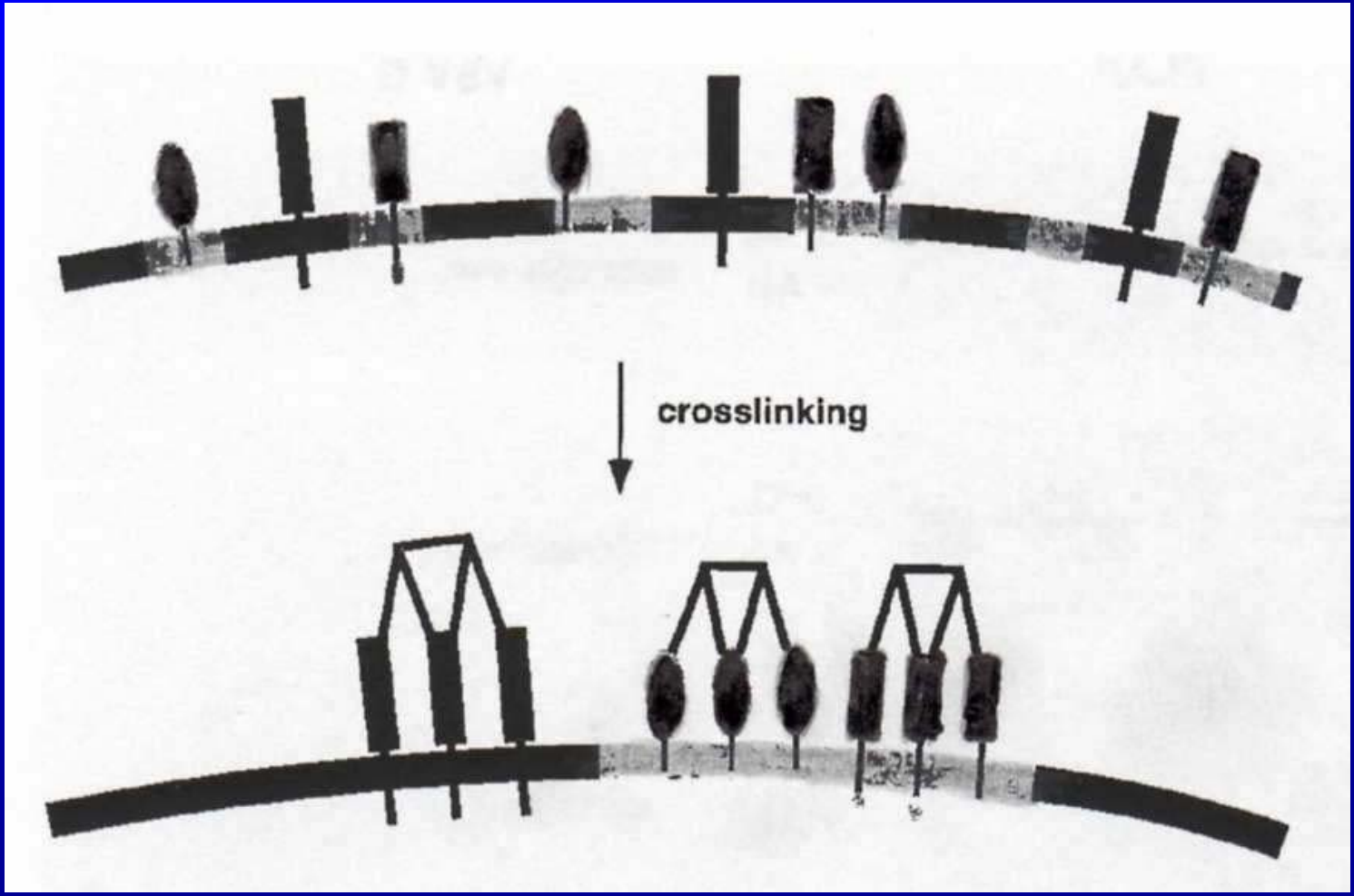
Harder et al. (1998) J. Cell Biol. 141, 929-942.

Patching (clustering) of membrane components



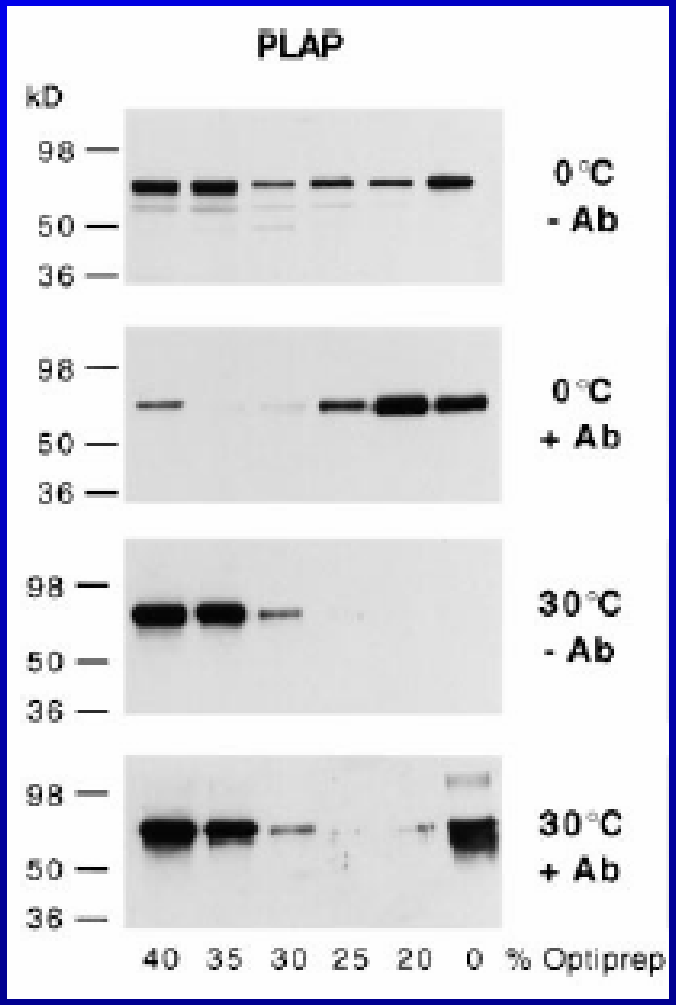
Jacobson & Dietrich (1999) Trends Cell Biol. 9, 87-91.

Bulk separation of membrane phases caused by clustering (patching) of membrane components



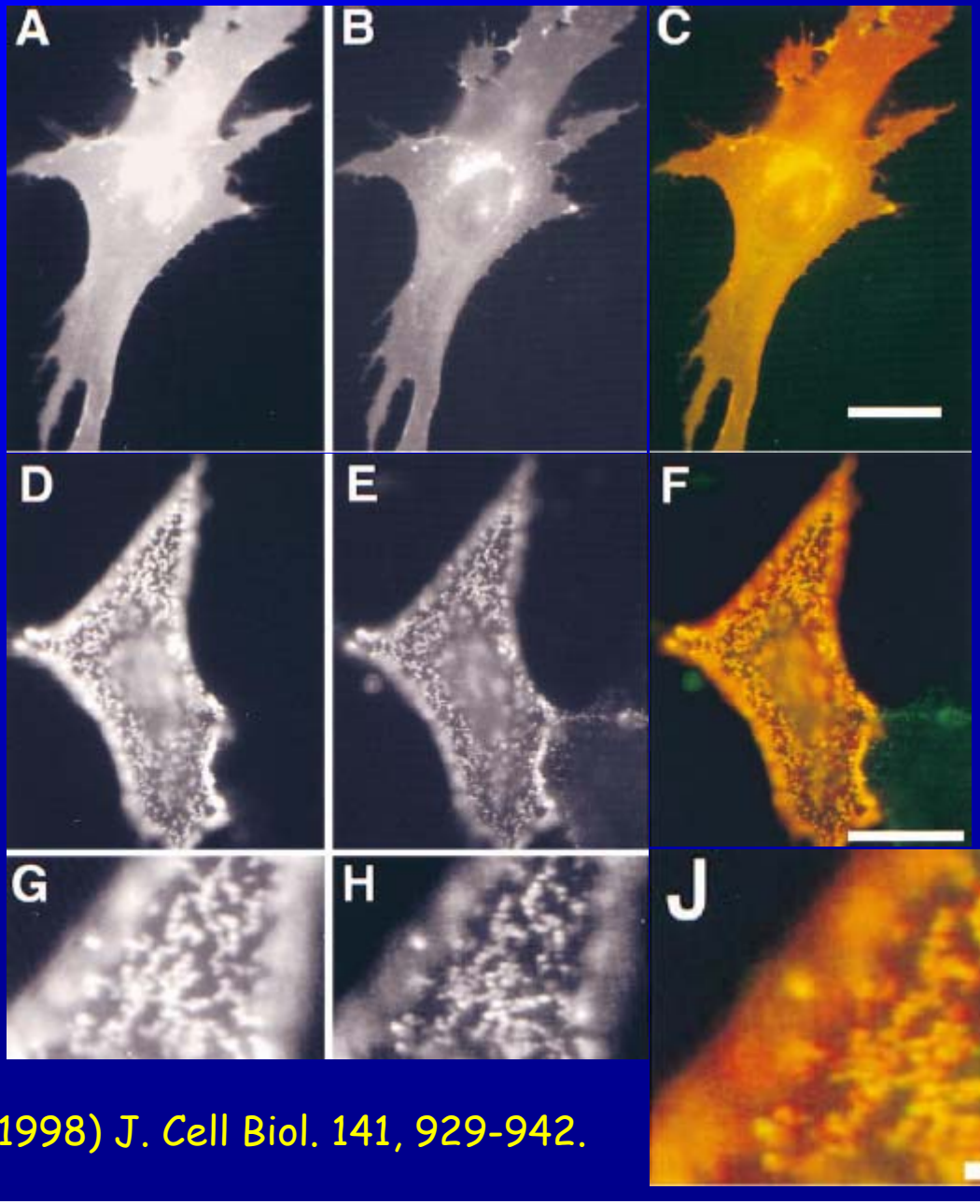
Harder et al. (1998) J. Cell Biol. 141, 929-942.

Stabilization of membrane domains by Ab crosslinking of a GPI-protein PLAP, transiently expressed in nonpolarized fibroblastoid BHK 21 cells



Harder et al. (1998) J. Cell Biol. 141, 929-942.

Patching of GPI-anchored PLAP (red) and influenza HA (green) transiently coexpressed in nonpolarized BHK-21 cells

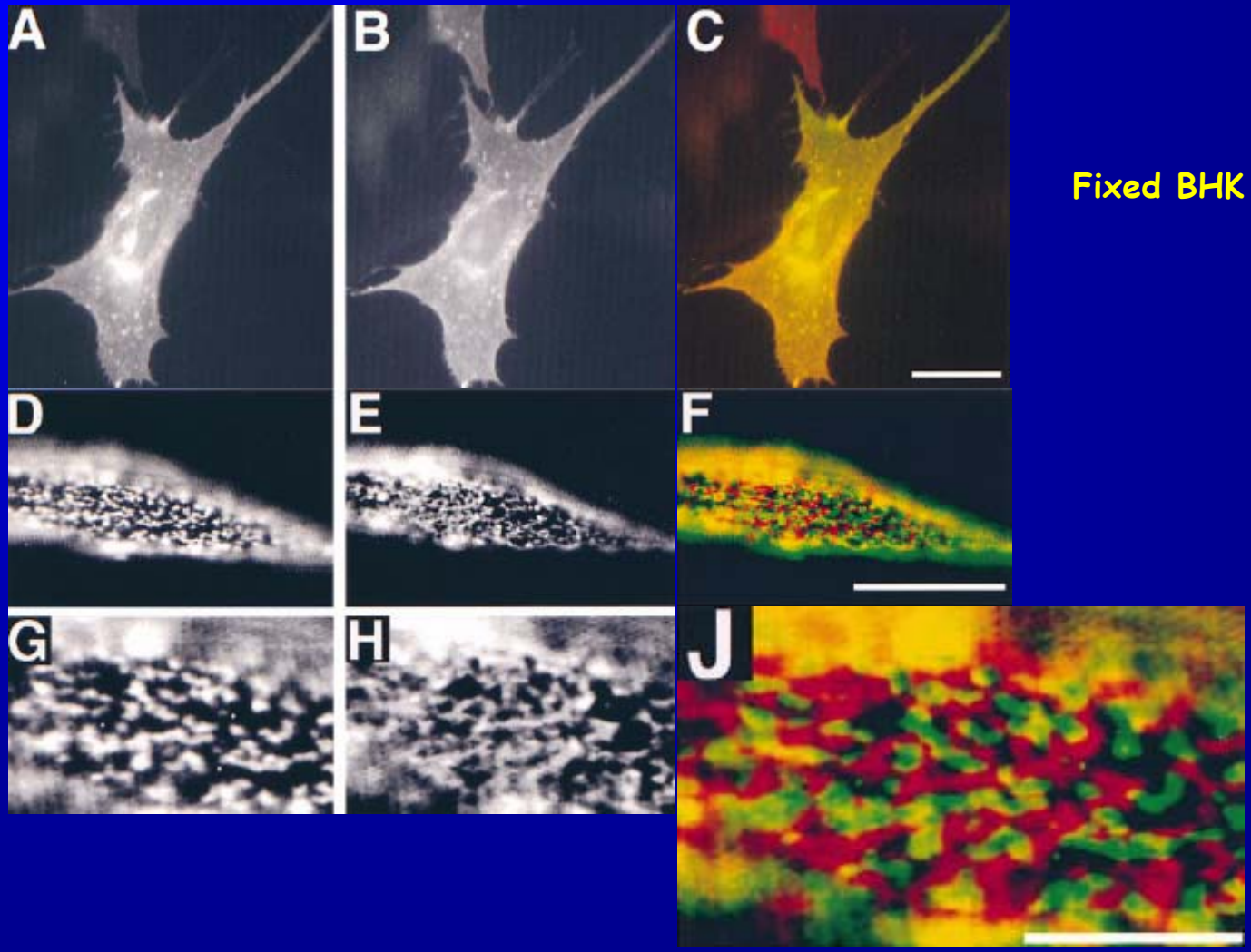


Fixed BHK cells

PLAP and HA copatch

Harder et al. (1998) J. Cell Biol. 141, 929-942.

Patching of GPI-anchored PLAP (green) and hTfR (red) transiently coexpressed in nonpolarized BHK-21 cells



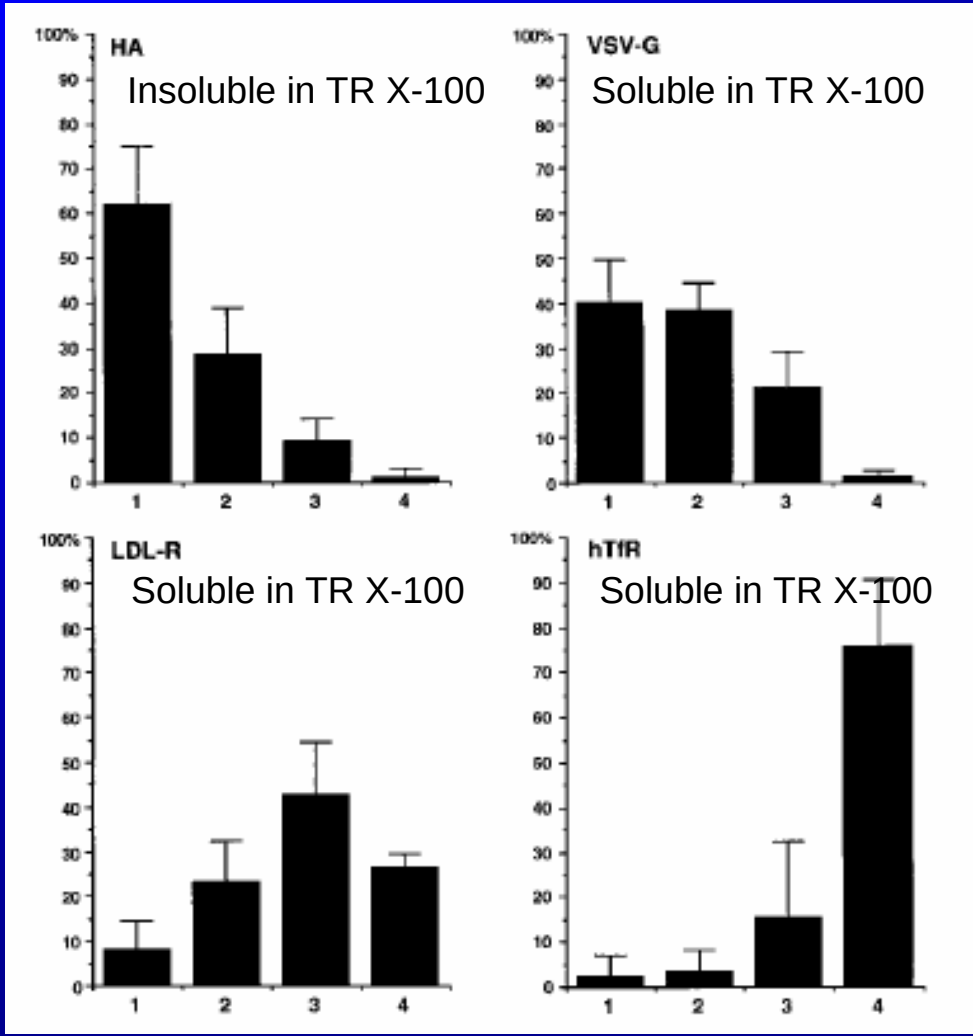
Fixed BHK cells

Harder et al. (1998) J. Cell Biol. 141, 929-942.

PLAP and hTfR segregate

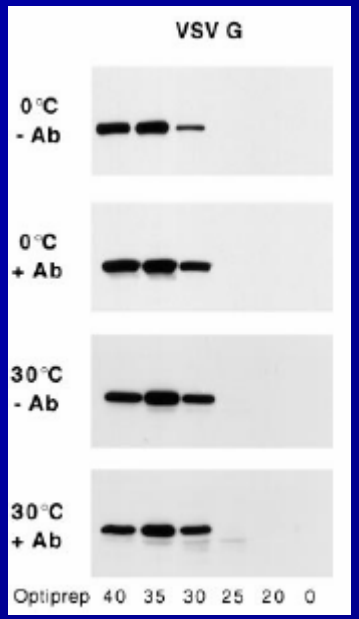
Certain proteins exhibit a weak but significant raft interaction which is not detectable by the TR X-100-solubility criterium

PLAP copatching



(*1*) copatching (80% overlap)
(*2*) partial copatching
(*3*) random distribution
(*4*) segregation

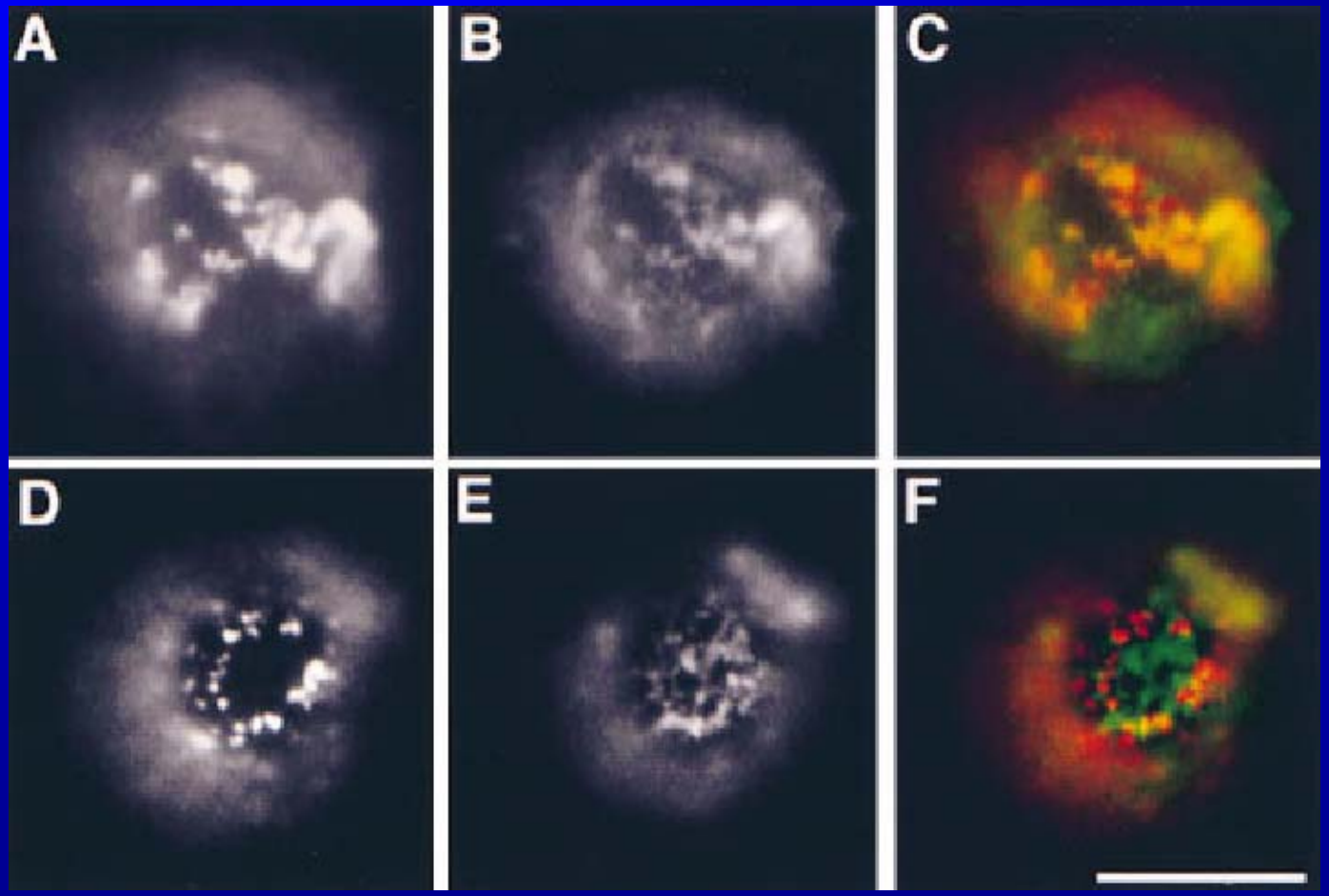
TR X-100 solubility



Harder et al. (1998) J. Cell Biol. 141, 929-942.

Specific involvement of lipid interactions in the copatching phenomenon

PLAP (A-C) and hTfR (D-F) were transiently coexpressed in Jurkat T-lymphoma cells.

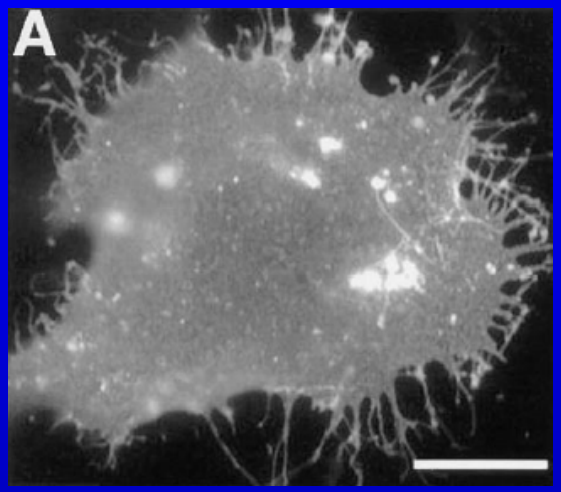


PLAP and GM1 copatch

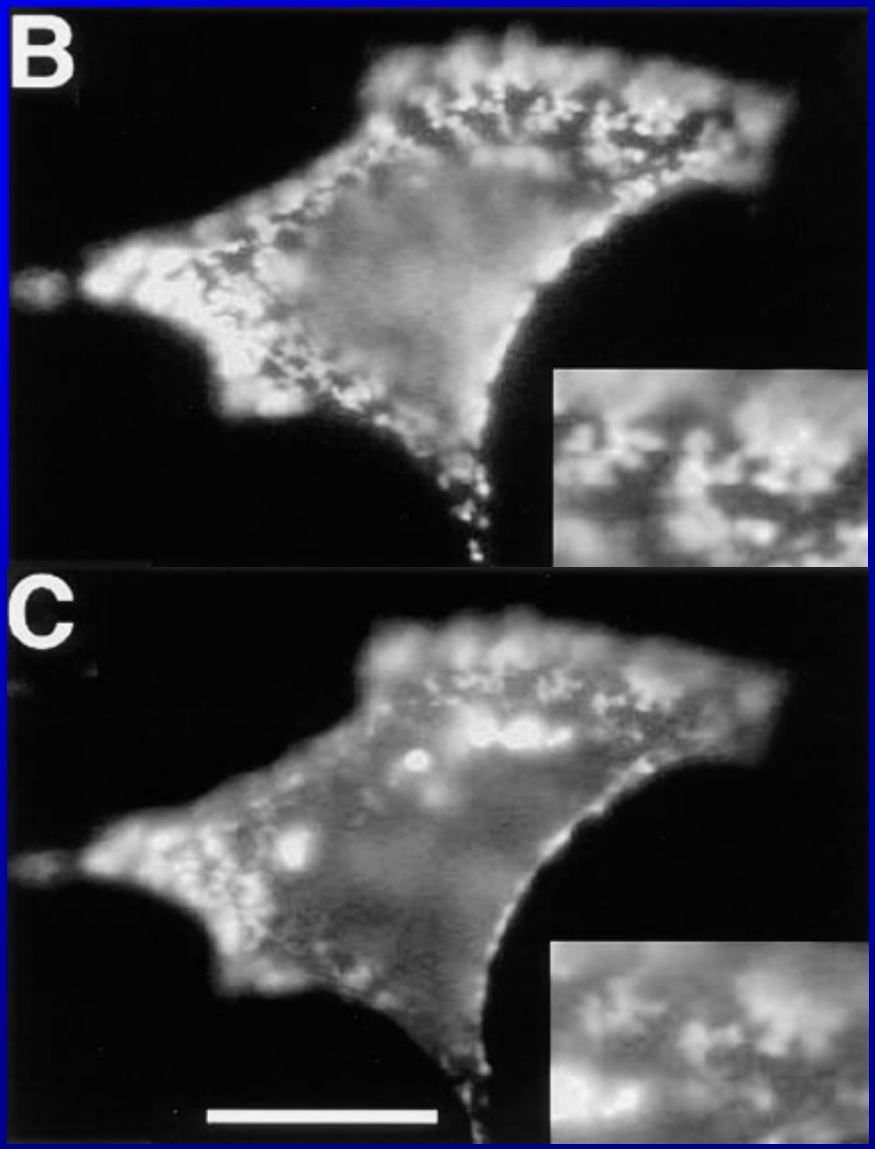
hTfR and GM1 segregate

Harder et al. (1998) J. Cell Biol. 141, 929-942.

Accumulation of src-like Tyr kinase fyn in membrane domains formed by patched PLAP transiently expressed in nonpolarized BHK-21 cells.



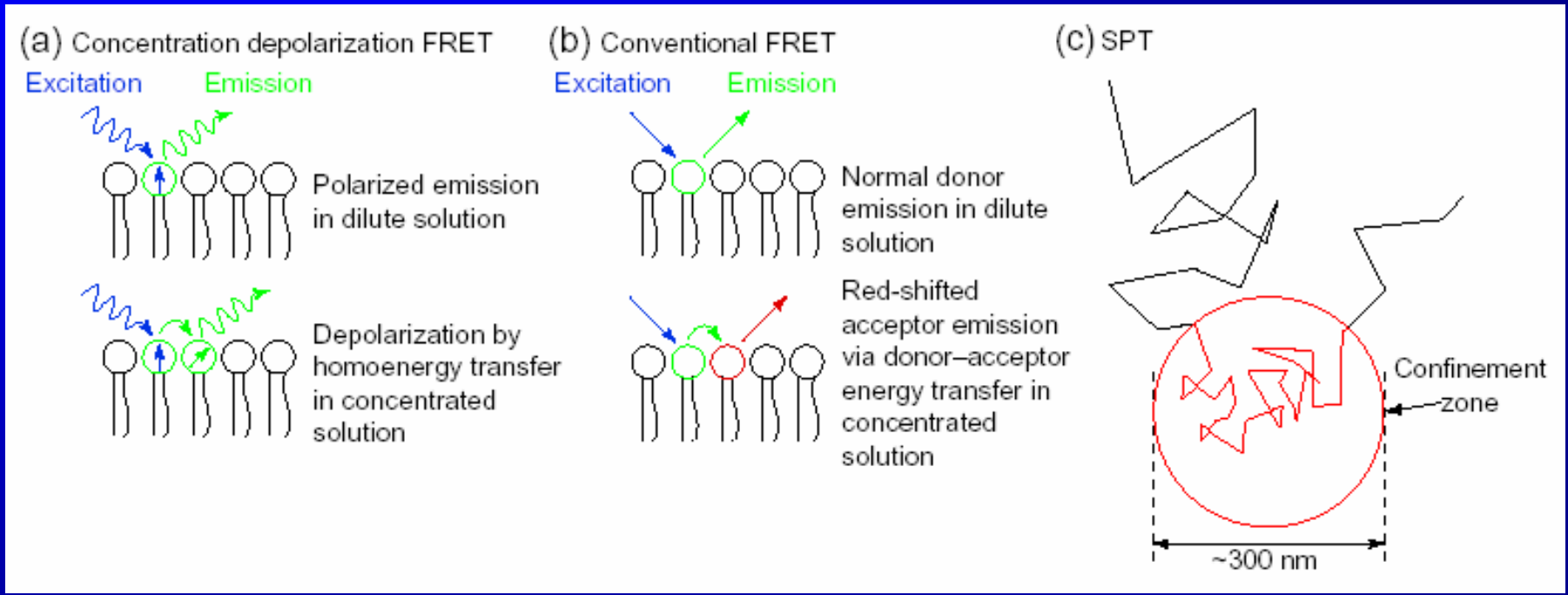
Distribution of overexpressed fyn in fixed BHK cells is even.



Patches of PLAP

Distribution of fyn in PLAP patched cells

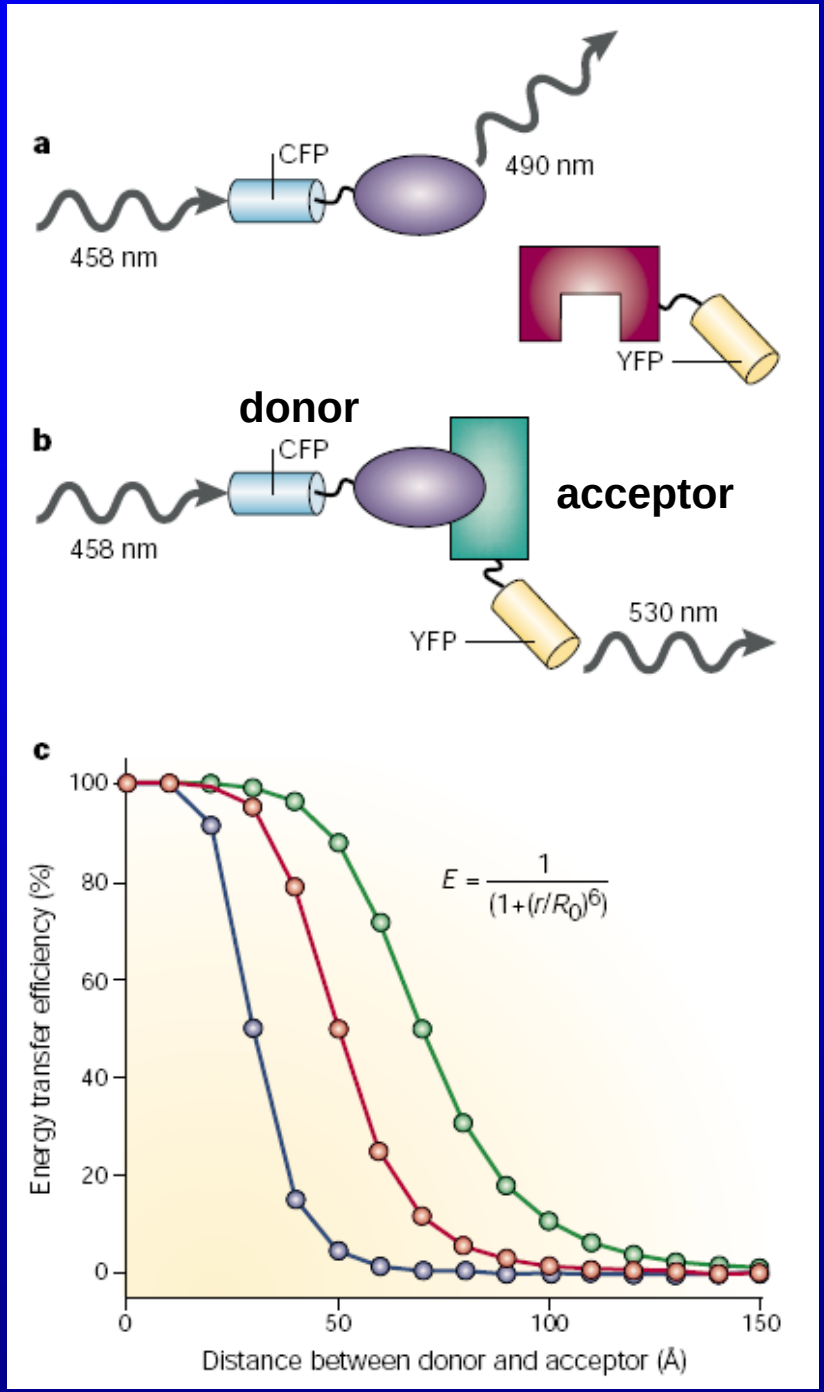
Harder et al. (1998) J. Cell Biol. 141, 929-942.



Jacobson & Dietrich (1999) Trends Cell Biol. 9, 87-91.

Principle of

F luorescence
R esonance
E nergy
T ransfer

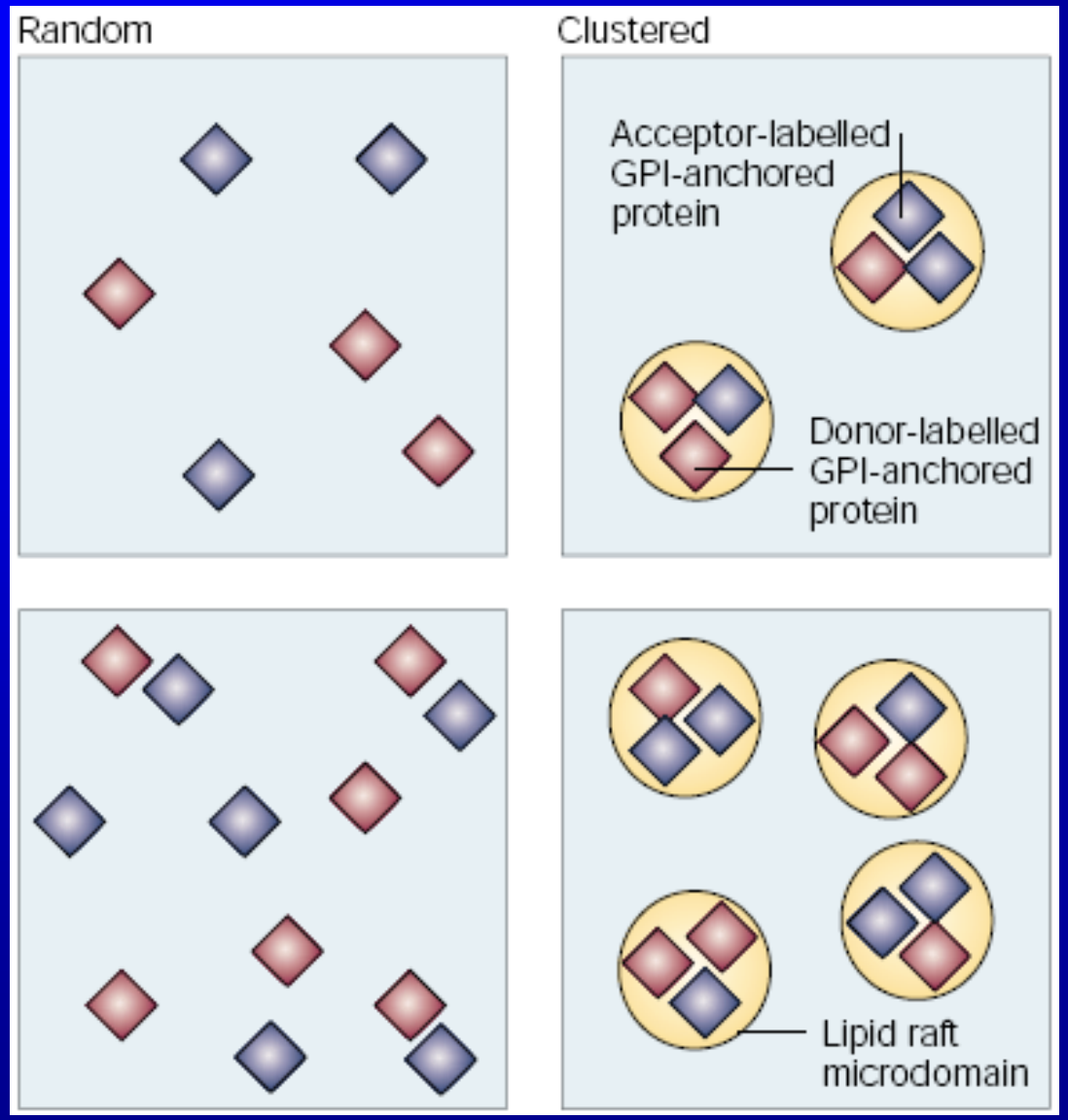


FRET pairs:
CFP-GFP
FITC-rhodamine
Cy3-Cy5
GFP-Cy3
GFP-Cy5

Lippincott-Schwartz et al. (2001) NRMCB 2, 444-456.

FRET assay for detecting lipid rafts

FRET as a function of donor and acceptor surface density



Lippincott-Schwartz et al. (2001) NRMCB 2, 444-456.

Table 1. Lipid and Protein Components of Lipid Rafts/Caveolae

- Lipids
  - Cholesterol
  - Sphingo-myelin
  - Glyco-sphingolipids (e.g., GM_1)
  - PIP_2
- Proteins
  - Integral/Structural
    - Caveolins (Cav-1, -2, and -3)
    - Flotillins (FLO-1 and -2; aka, Reggies or Cavatellins)
    - LAT/PAG
    - MAL/BENE
    - Stomatins
    - VIP36
  - Acylated
    - Exoplasmic
      - GPI-linked proteins (e.g., Thy-1, alkaline phosphatase, folate receptor)
    - Cytoplasmic
      - Src-family tyrosine kinases (NRTKs)
      - G proteins
      - eNOS
      - H-Ras
  - Scavenger Receptors
    - CD 36
    - SRBI
    - RAGE
  - Other receptors
    - Receptors Tyrosine kinases (RTKs; e.g., EGF-R, PDGF-R, Insulin-R)
    - Hepta-helical Receptors (e.g., Endothelin receptor)

Galbiati et al. (2001) Cell 106, 403-411.

Raft distribution and trafficking is cell type-dependent

Polarized epithelial cells (tight junctions)
- accumulated in apical PM

Neurons (cytoskeleton, extracellular matrix)
- accumulated in axonal PM

Osteoclasts (cytoskeleton, extracellular matrix)
- asymetric distribution in PM

Lymphocytes and fibroblasts
- uniform distribution

Raft nomenclature

Present raft nomenclature*

Rafts | DRMs | DIGs | DICs | GPI domains | Glycosphingolipid signalling domains | Caveolae-like domains | Microdomains | LDM | Liquid-ordered domains | DIM | GEMs | TIFF

Suggested raft nomenclature

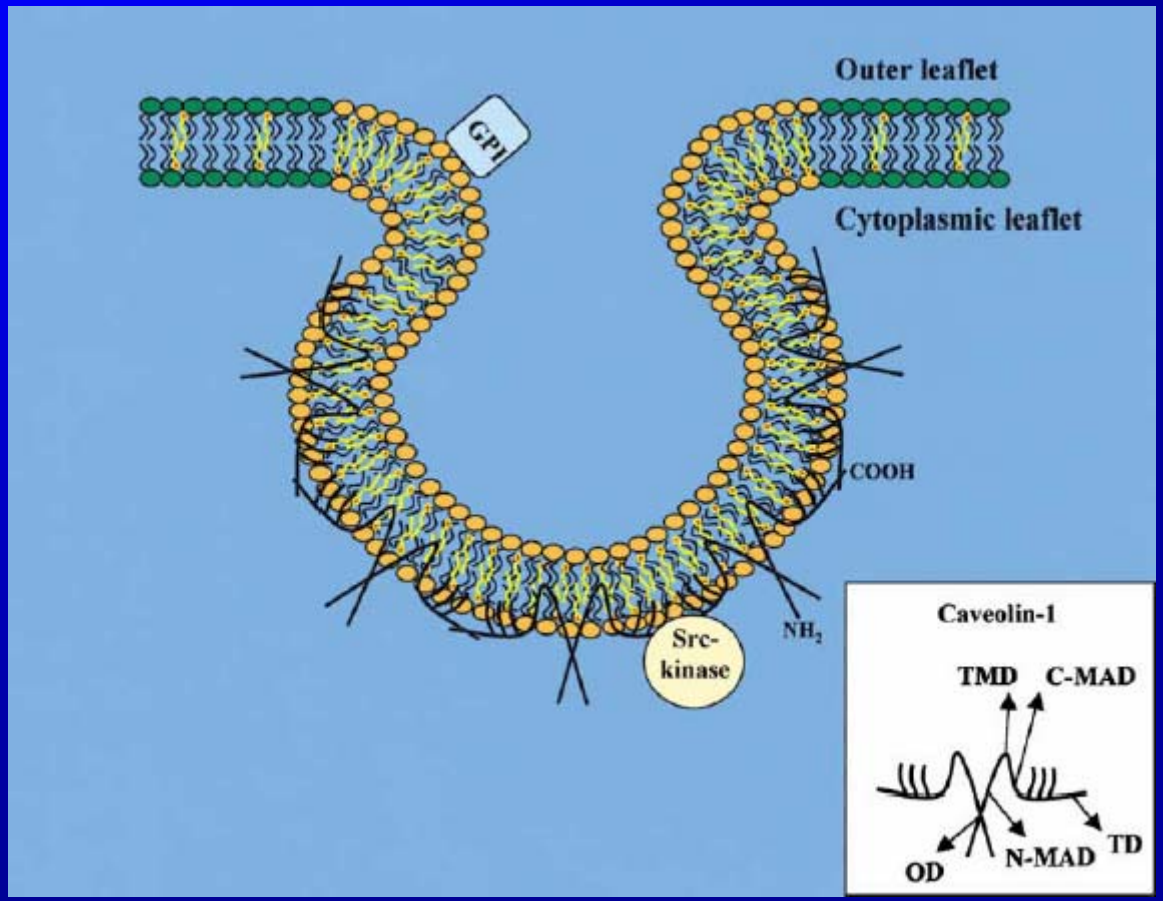
	I. Rafts	II. Clustered rafts	III. DRMs	IV. Caveolae
Components	• Glycosphingolipids • Cholesterol • Lipid-modified proteins containing saturated acyl chains: – GPI-anchored proteins – Doubly acylated Src-type kinases • Transmembrane proteins	• Rafts clustered by: – Antibody – Lectin – Adjacent cell proteins – Physiological crosslinking proteins	• Rafts remaining insoluble after treatment on ice with detergent‡§: Triton X-100 (most popular), Brij-58, CHAPS, NP-40	• Raft proteins and lipids • Caveolins
Properties	• 50 nanometres in diameter • Mobile (~10^{-8} cm^{-2} sec^{-1}) • Liquid-ordered phase	• Large, often hundreds of nanometres to micrometres in size • Often bound to cytoskeleton	• Float to low density in sucrose or Optiprep™ density gradients	• Morphological 'cave-like' invaginations on the cell surface
Comments	• Native rafts are only detected in living cells	• Clustering is used both artificially and physiologically to trigger signalling cascades	• Non-native (aggregated) raft • Variable effects depending on: – Detergent type – Detergent:lipid ratio – Cell type	• Raft subcategory • Highly specialized

* DRM, detergent-resistant membrane; DIG, detergent-insoluble glycolipid-rich domain; DIC, detergent-insoluble complex; LDM, low-density membrane; DIM, detergent-insoluble material; GEM, glycolipid-enriched membrane; TIFF, Triton X-100 insoluble floating fraction.
‡Care should be taken when choosing solubilization conditions for co-immunoprecipitation experiments, as these popular detergents do not solubilize rafts on ice. Co-localization of proteins in rafts or DRMs could be mistaken for direct protein–protein interactions if rafts are not completely solubilized.
§Rafts can be solubilized in octyl glucoside or in the detergents listed above at raised temperatures.

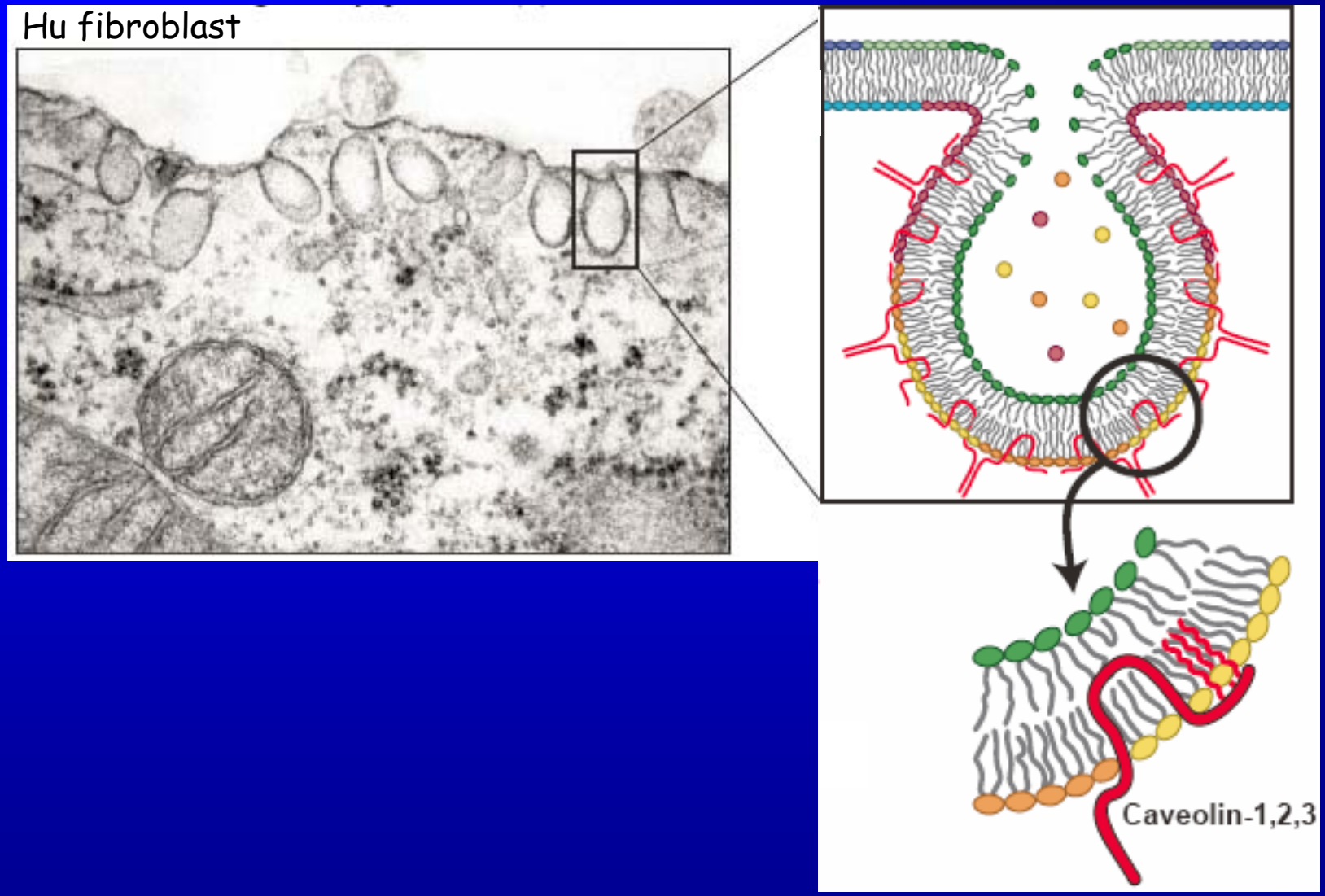
Simons & Toomre (2000) Nat. Rev. Mol. Cell Biol. 1, 31-40.

Caveolae

highly specialized raft subcategory



Galbiati et al. (2001) Cell 106, 403-411.

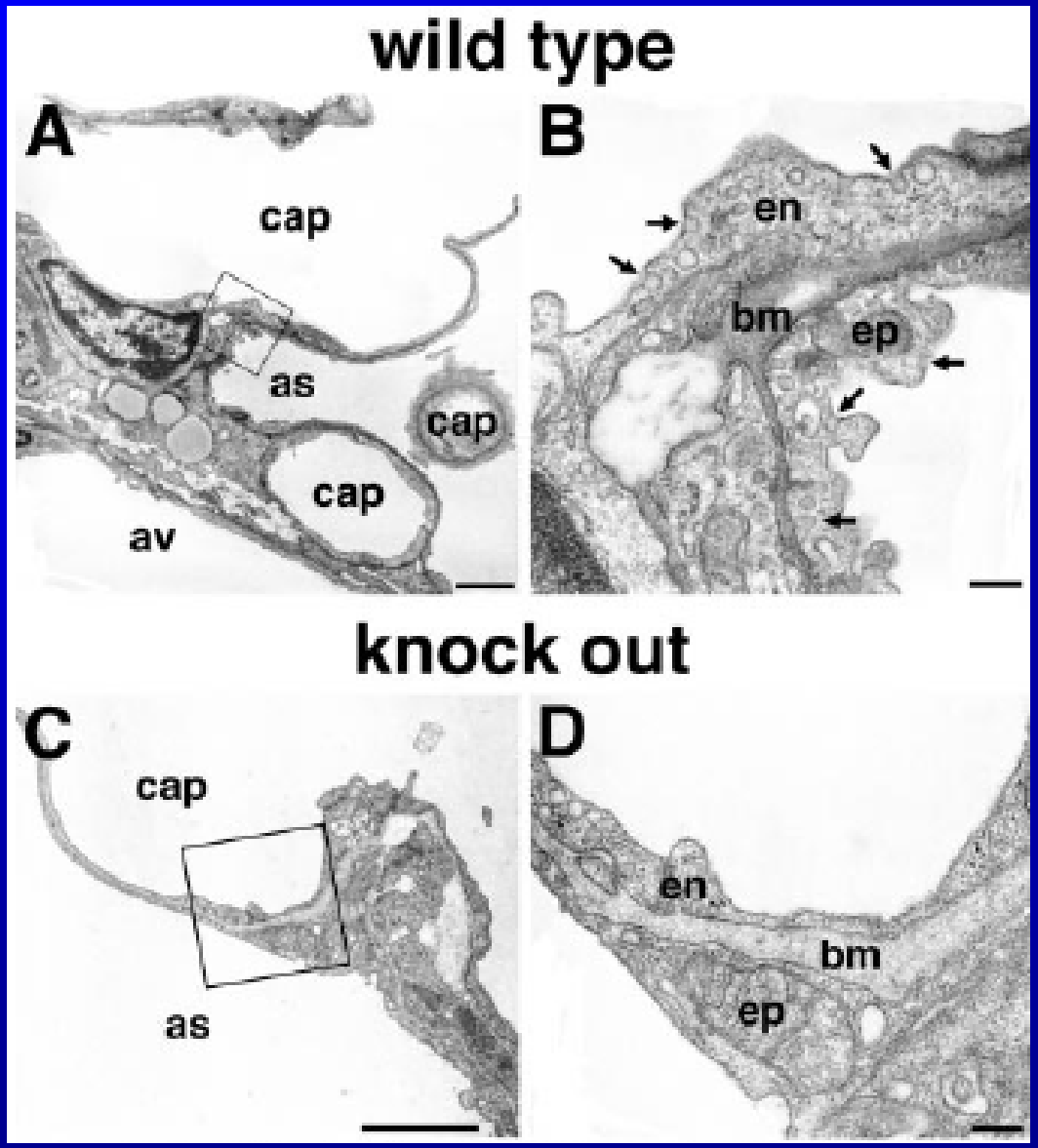


Parton (2001) Science 293, 2404-2405.

Caveolins (Cav)

- Essential for the formation of caveolae.
- Cav gene family structurally and functionally conserved from worms (*C. elegans*) to humans.
- Cav-1(α and β), -2 and -3 in mammals (21- to 25-kDa).
- Integral membrane proteins (tri-palmitoylated).
- Cav-1 and -2 are coexpressed, Cav-3 is muscle-specific.
- Polymerize (14-16) and shape up caveolae.
- Bind cholesterol, fatty acids and interact with the broad range of signal transducing molecules (*e.g.* Tyr kinase R, eNOS, heterotrimeric G proteins).
- Not present in lymphocytes and neurons.

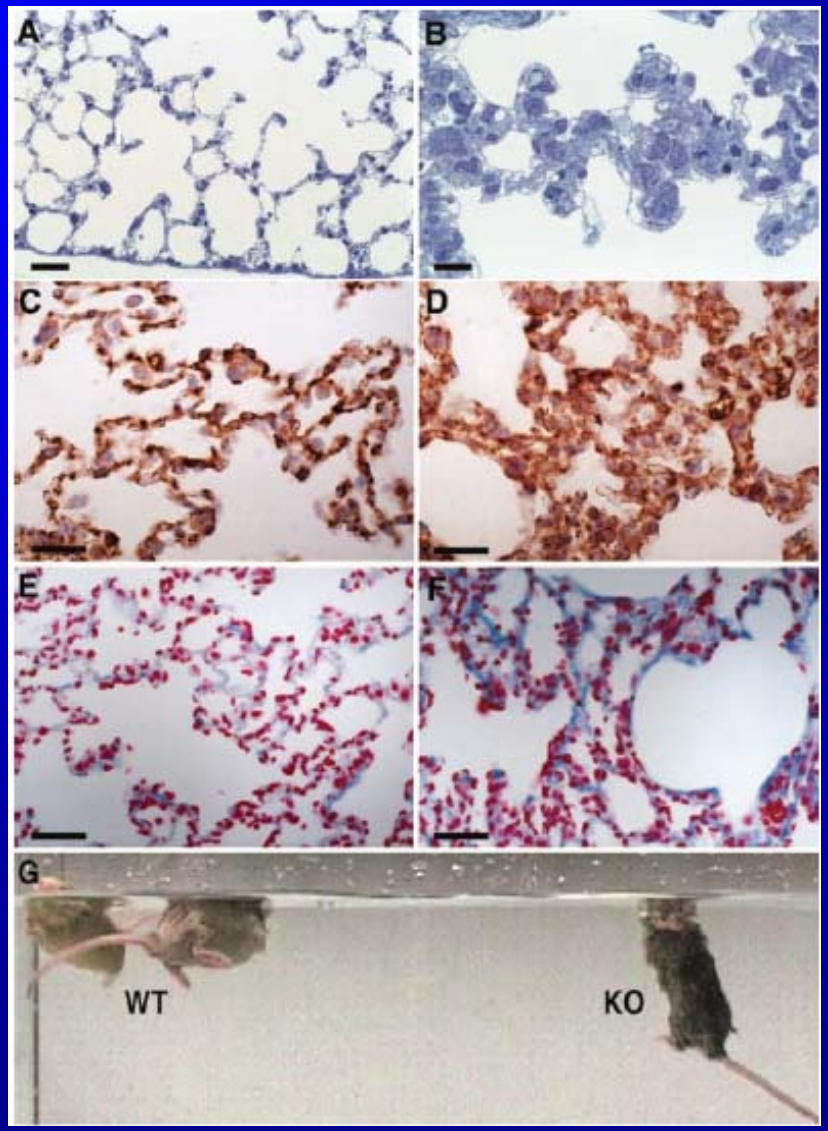
Disappearance of caveolae from cells in cav-1 (-/-) mice



as – alveolar space
cap – capillary
av – arterial vessel
en – endothelium
ep – epithelium
bm – basal membr.

Drab et al. (2001) Science 293, 2449-2452.

Patomorphological defects in lungs and physical disability of cav-1 (-/-) mice



Thickening of alveolar walls

caused by:

- uncontrolled endothelial cell proliferation

- increased content of extracellular fibrillar matrix (fibrosis)

results in:

physical weakness.

Drab et al. (2001) Science 293, 2449-2452.

Features of caveolin-defficient mice

Gene knockout	Features
Caveolin-1	Loss of caveolae in non-(striated) muscle cells, loss of caveolin-2 protein, pulmonary defects — exercise intolerance*, vasoconstriction or dilation abnormalities, resistance to diet-induced obesity — high serum triglyceride levels, defects in albumin uptake or transendothelial transport, defects in glycosylphosphatidylinositol-anchored protein transport
Caveolin-2	Pulmonary defects — exercise intolerance
Caveolin-3	Loss of caveolae in striated muscle, dystrophic muscle, disorganization of T-tubule network

*Proposed to be due to the loss of caveolin-2 protein in *caveolin-1*-null mice.

Parton (2003) Nature Rev. MCB 4, 162-167.

Recommended reading:

Jacobson, K. & Dietrich, C. (1999): Looking at Lipid Rafts? Trends Cell Biol. 9, 87-91.

Drab, M. et al. (2001): Loss of Caveolae, Vascular Dysfunction, and Pulmonary Defects in Caveolin-1 Gene-Disrupted Mice. Science 293, 2449-2452.

Galbiati, F. et al. (2001): Emerging themes in lipid rafts and caveolae. Cell 106, 403-411.

Lippincott-Schwartz, J., et al. (2001): Studying protein dynamics in living cells. Nat. Rev. Mol. Cell Biol. 2, 444-456

Parton, R.G. (2003): Caveolae - from ultrastructure to molecular mechanisms. Nat. Rev. Mol. Cell Biol. 4, 162-167.

Riethmuller, J., et al. (2006): Membrane rafts in host-pathogen interactions. Biochim. Biophys. Acta1758, 2139-2147.

Jacobson, K., et al. (2007): Lipid rafts: at a crossroad between cell biology and physics. Nat. Cell Biol. 9, 7-14.

Coskun, Ü. & Simons, K. (2010): Membrane rafting: From apical sorting to phase segregation. FEBS Lett. 584, 1685-1693.

Levental, I., et al. (2010): Greasing their way: Lipid modifications determine protein association with membrane rafts. Biochemistry 49, 6305-6316.