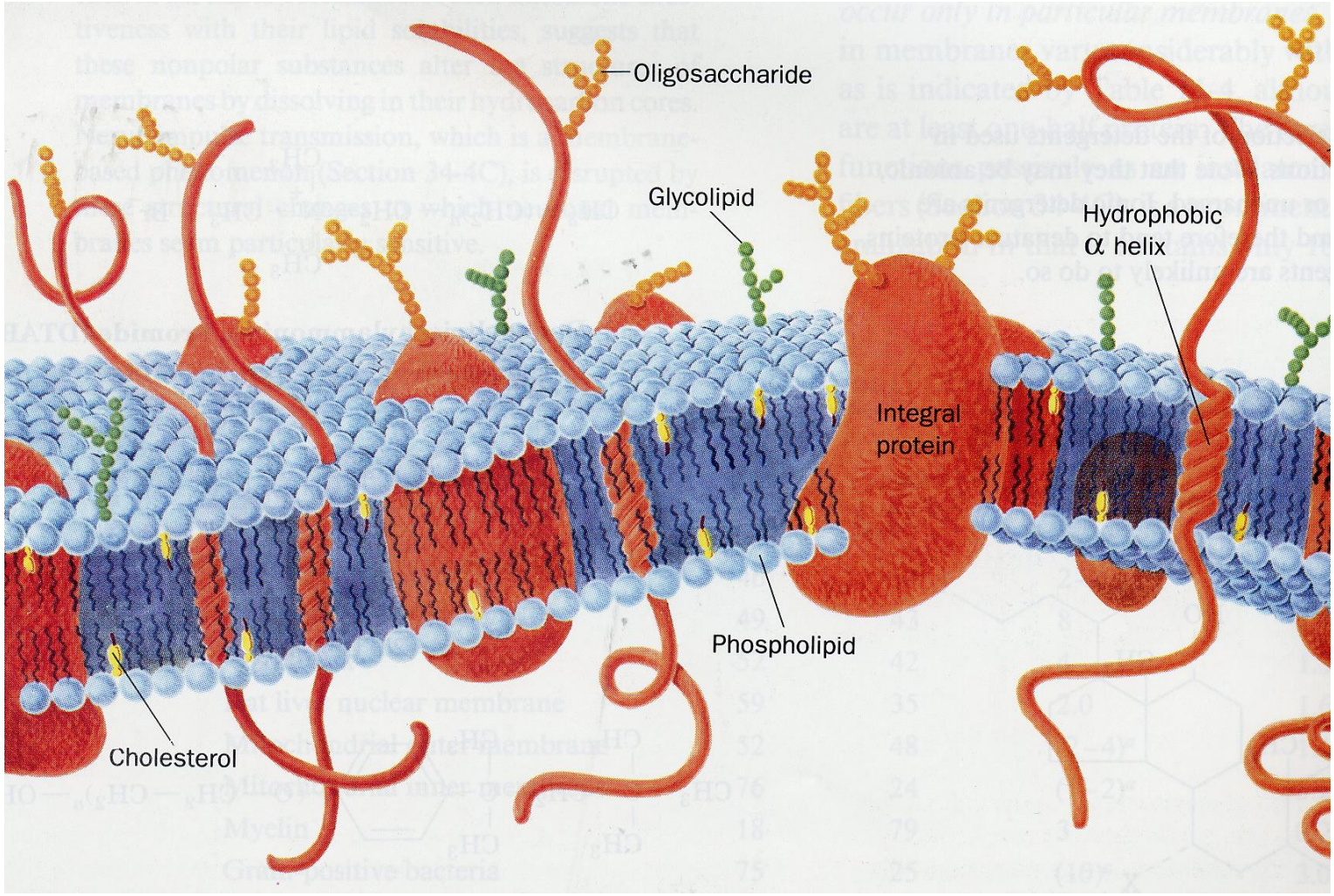
Lipidne mikrodomene

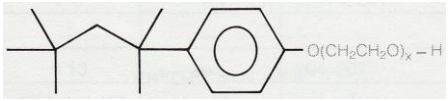
struktura in funkcija

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



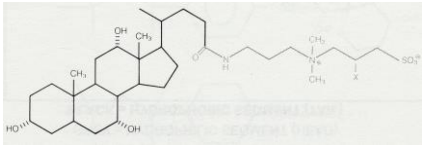
Only part of biological membranes is solubilized after treatment at low T (≤4°C) with:

Triton X-100 (NP-40)



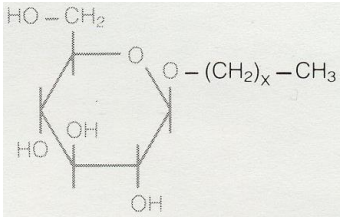
Brij-58

CHAPS



The remaining membranes are soluble in:

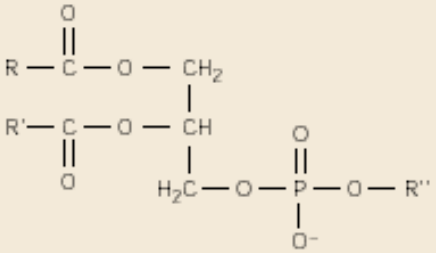
octyl glucoside



above mentioned detergents at higher T

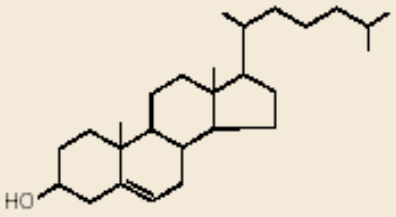
Basic lipid structures in Eukaryotic membranes

Phospholipids

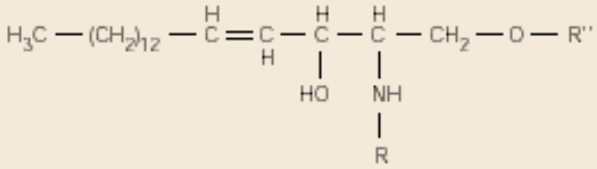


R, R', Hydrocarbon chains of fatty acids
R'', Head-group

Cholesterol



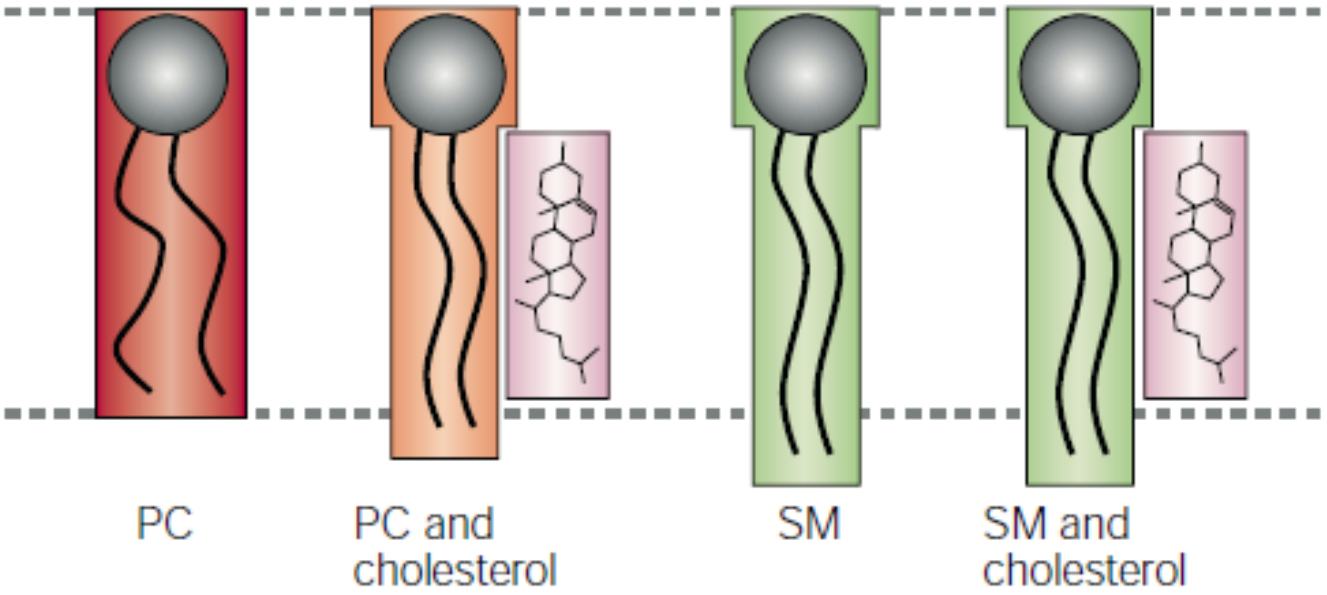
Sphingolipids



Lipids exist in:

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

Cholesterol can induce fluid-fluid immiscibility



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

Manipulation of raft lipid constituents

Cholesterol sequestration

- Antibiotics:

  Filipin | Nystatin | Amphotericin

- Pore-forming agents:

  Saponin | Digitonin | Streptolysin O

Cholesterol depletion

- Methyl-β-cyclodextrin

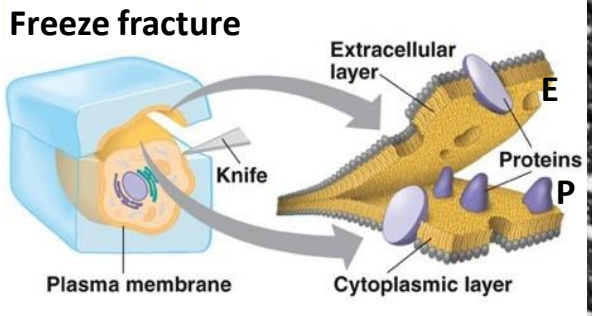
Inhibition of cholesterol biosynthesis

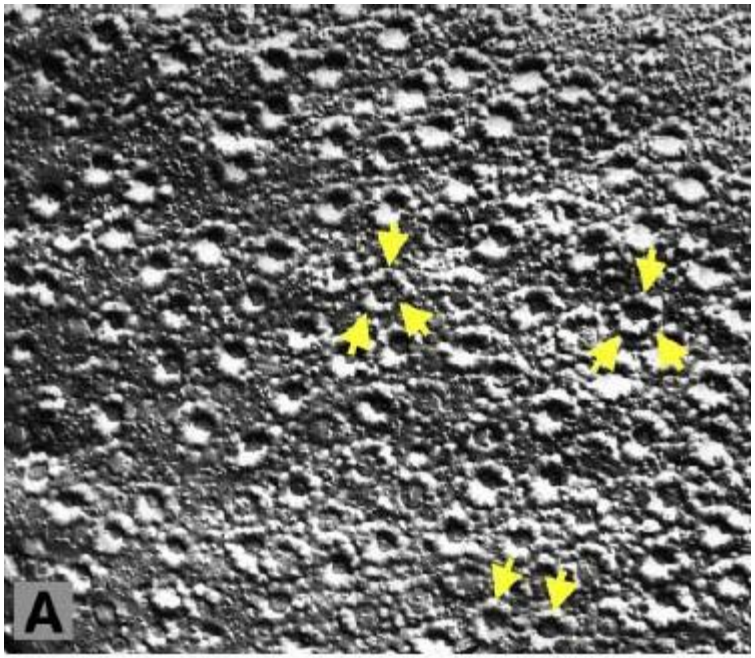
- Lovastatin

Perturbation of raft stability

- Exogenous cholesterol
- Exogenous gangliosides
- Exogenous polyunsaturated fatty acids

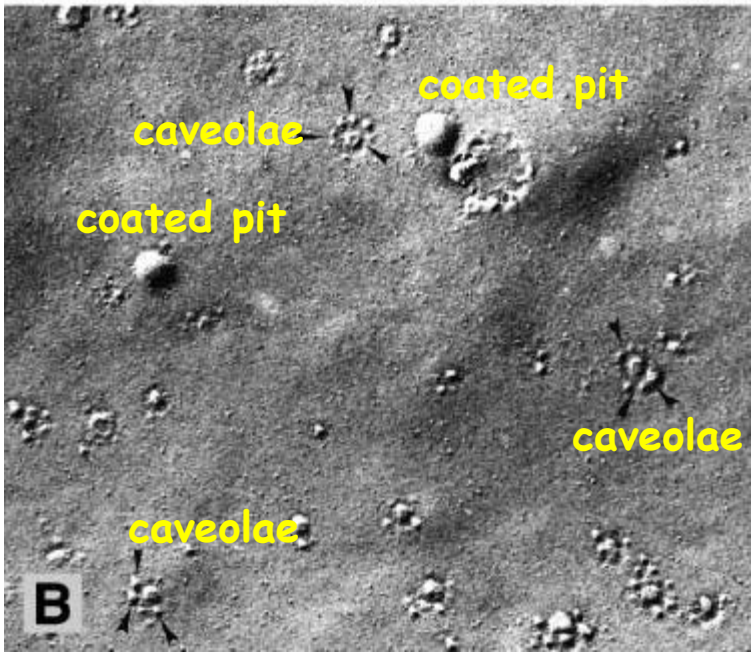
Simons & Toomre (2000) Nat. Rev. Mol. Cell Biol. 1, 31-40.





Endothelial cell PM
P face

filipin-cholesterol precipitate



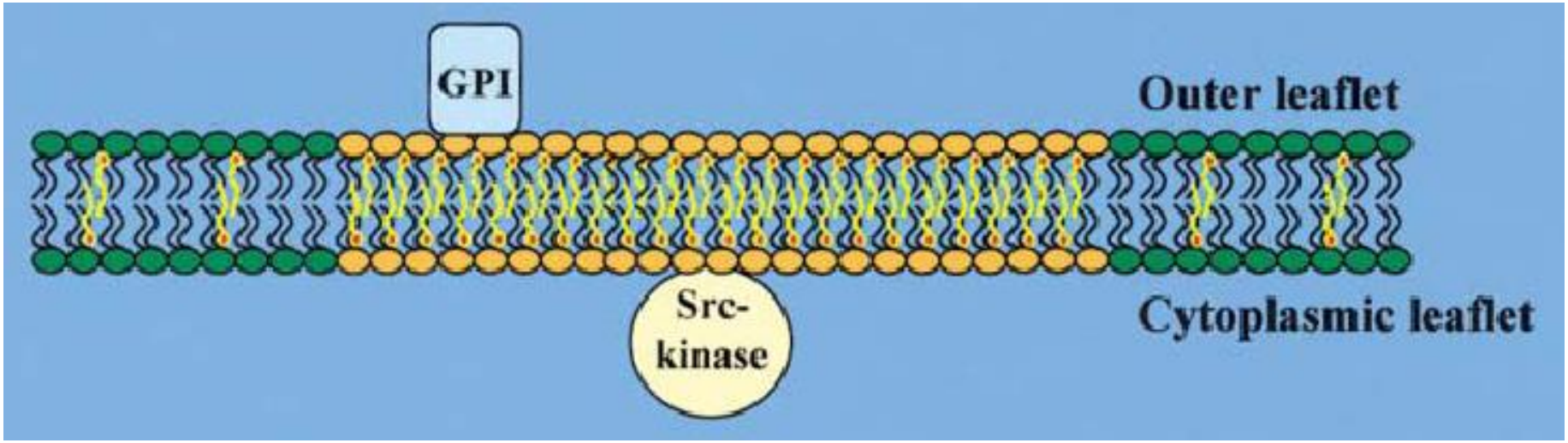
Smooth muscle cell PM
E face

Cholesterol is concentrated in BM!

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

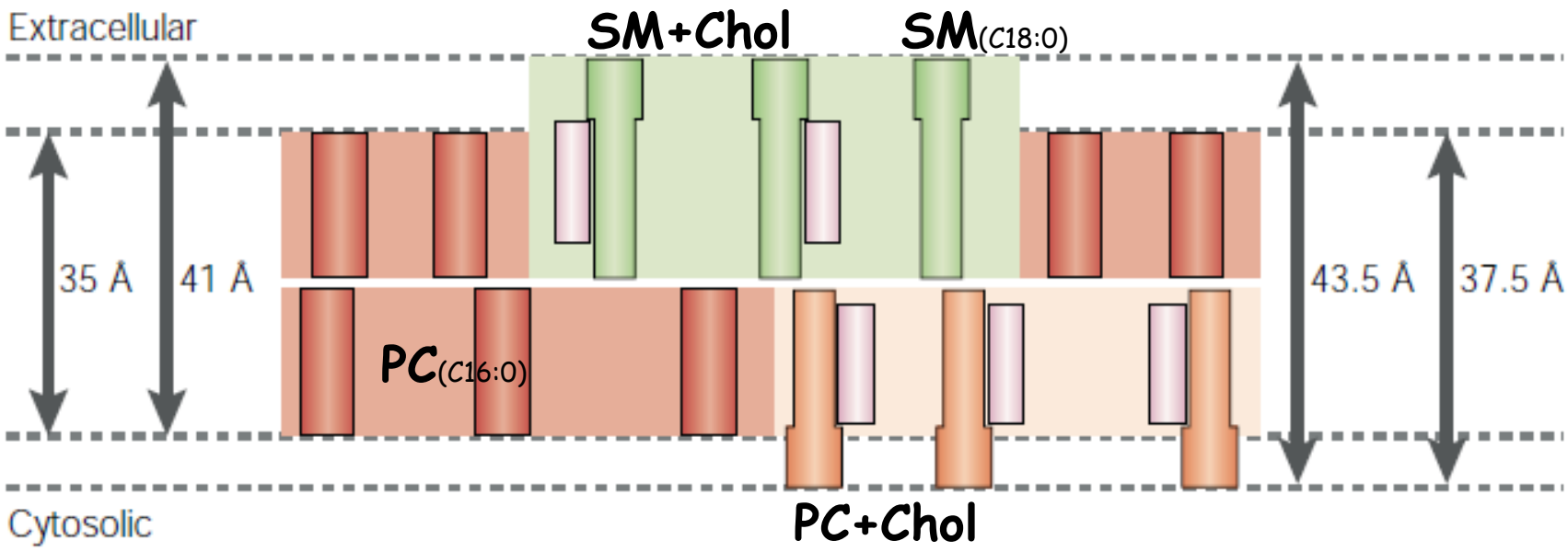
Biological membranes possess an intrinsic order:

raft concept



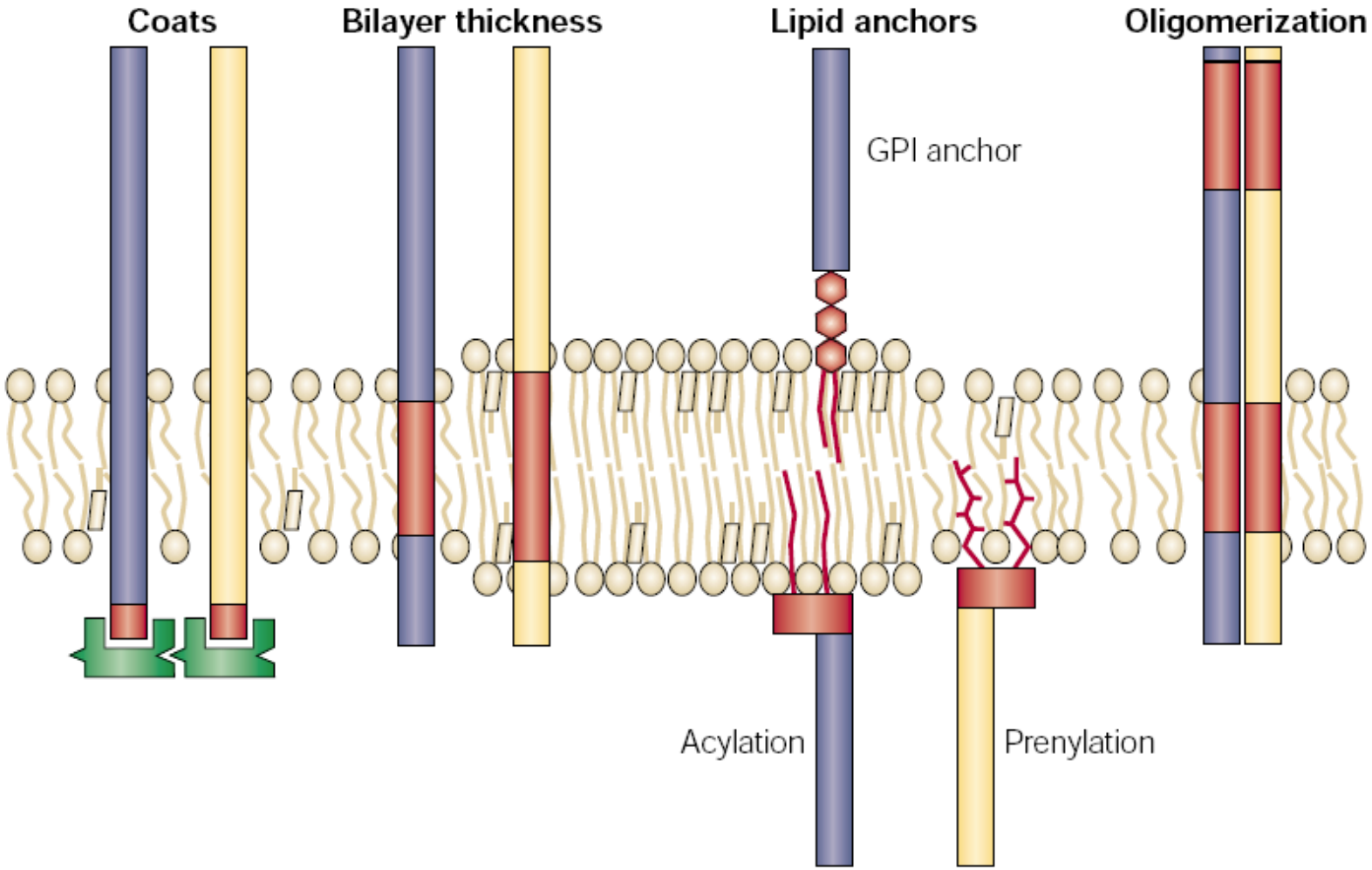
Galbiati et al. (2001) Cell 106, 403-411.

Membrane thickness depends on lipid composition



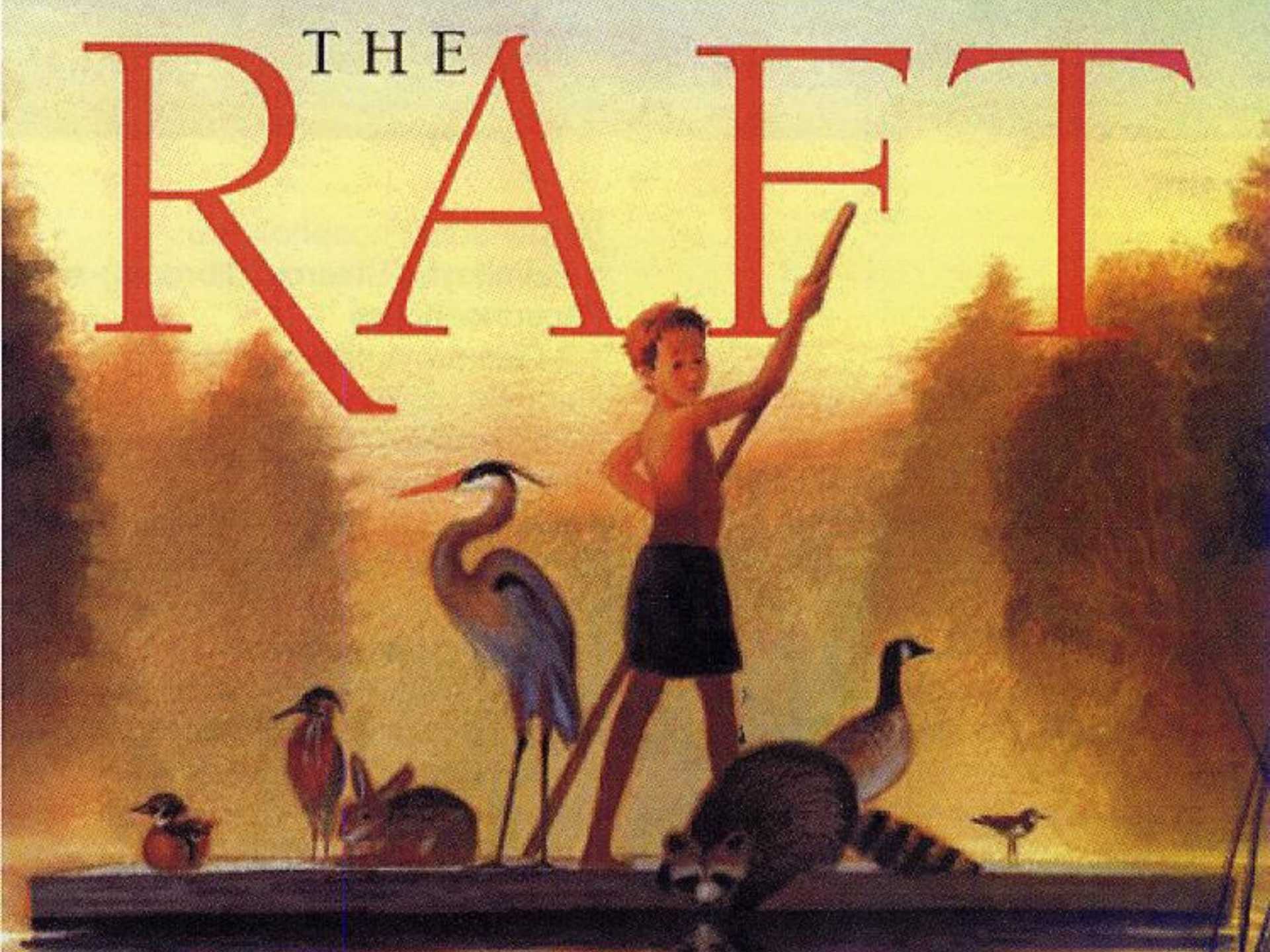
Sprong et al. (2001) Nat. Rev. Mol. Cell. Biol. 2, 504-513.

Lateral sorting of membrane proteins



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

THE RAFT



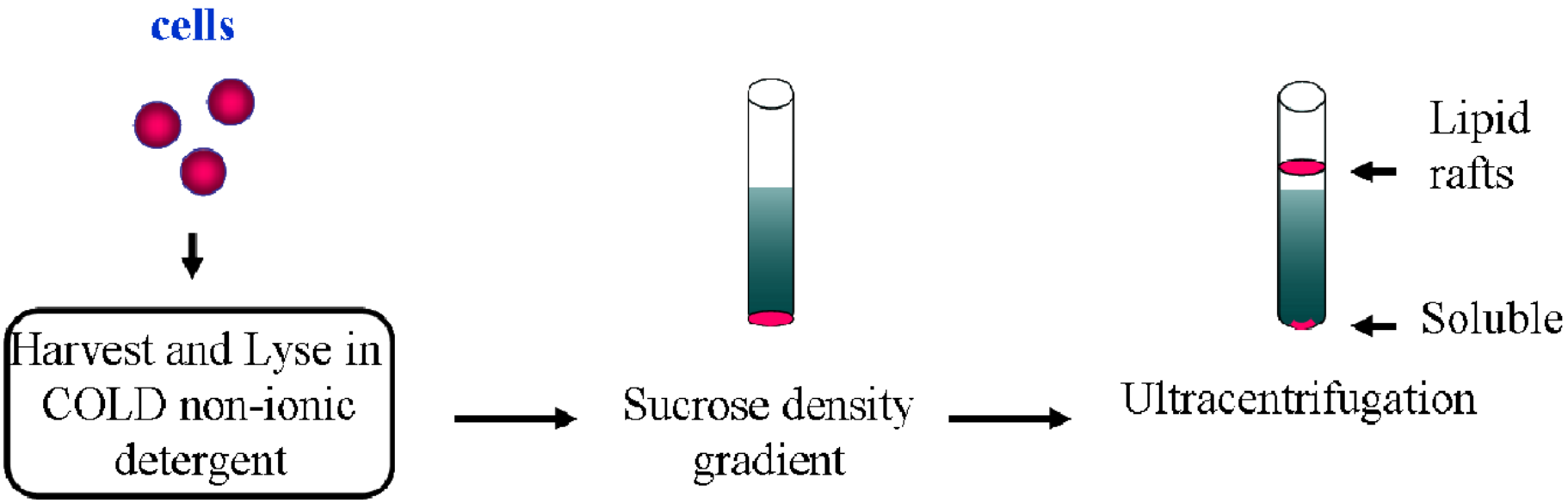
Techniques to study lipid rafts

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.

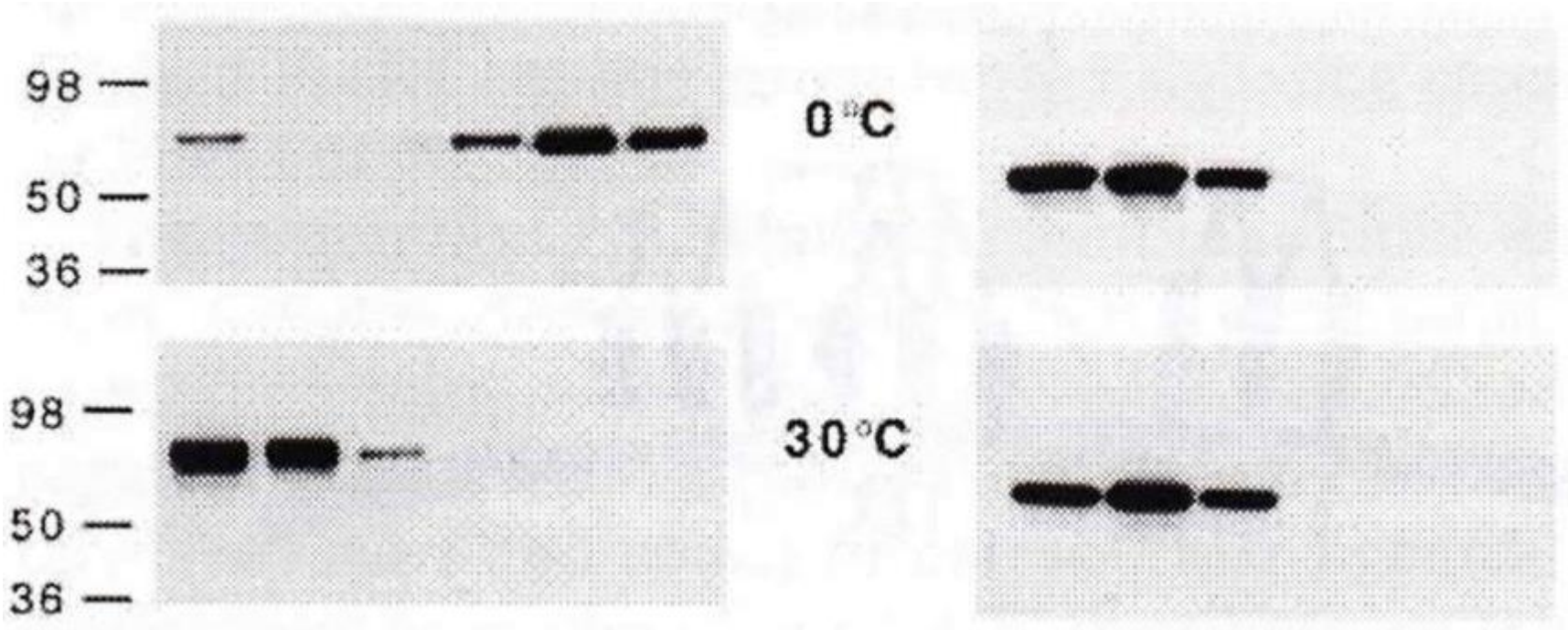
Flotation of detergent-resistant membranes



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

PLAP (PLacental Alkaline Phosphatase)

VSV-G (Vesicular Stomatitis Virus Glycoprotein)

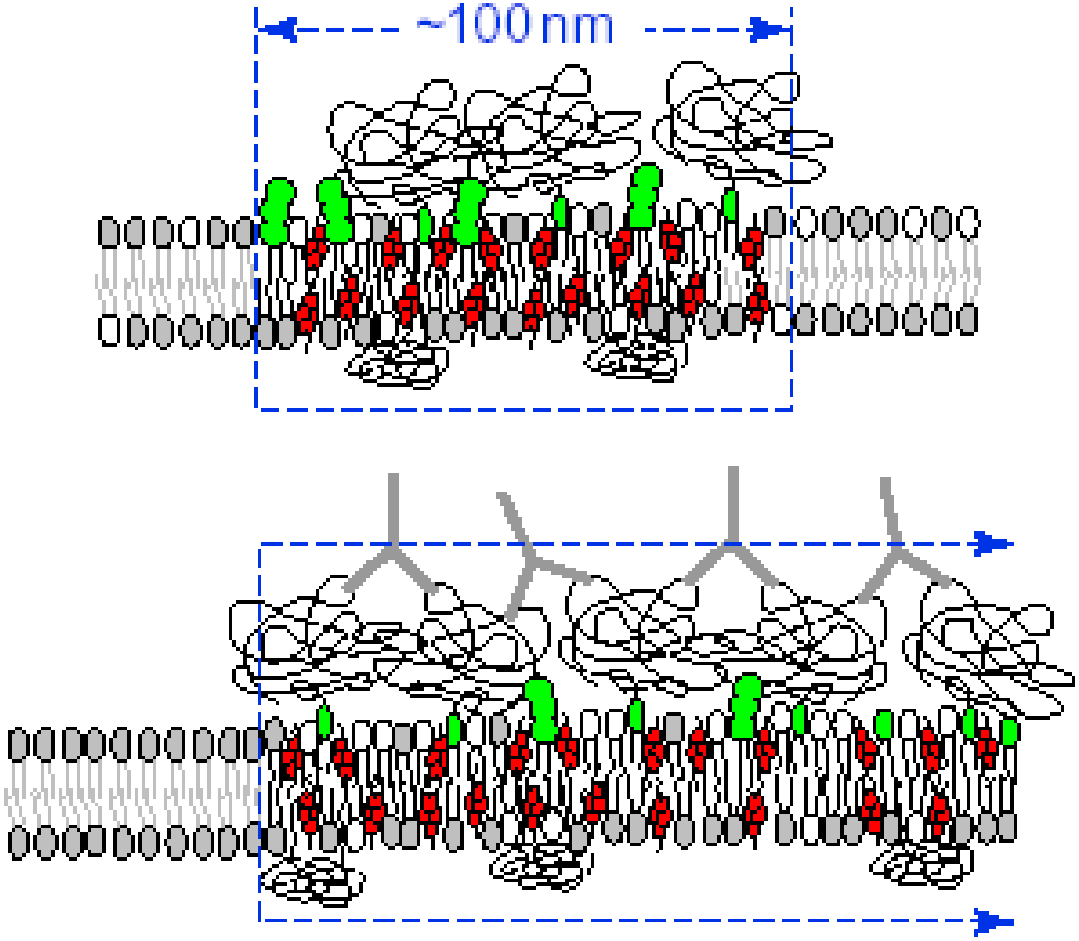


higher → lower density

higher → lower density

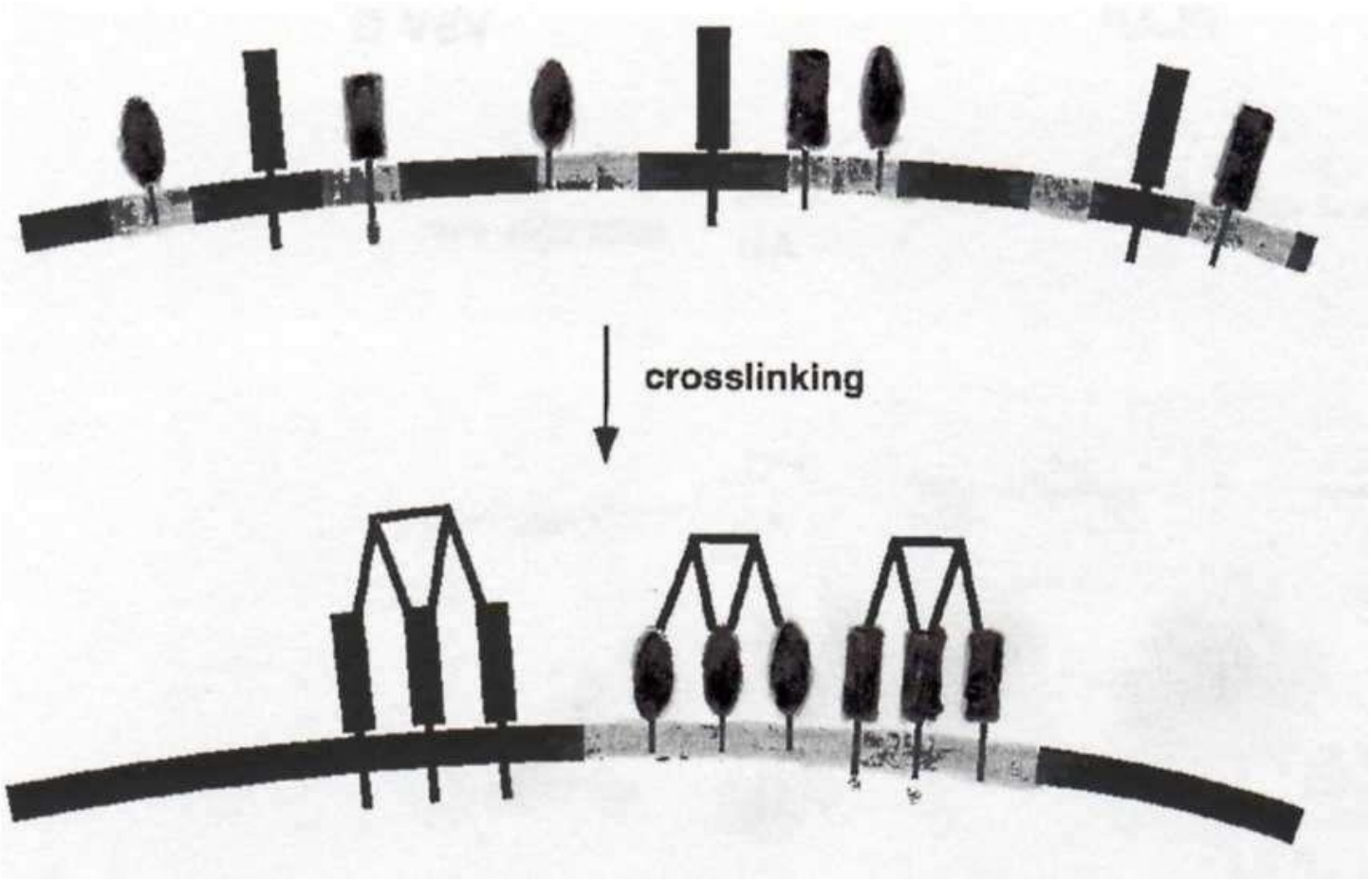
Harder et al. (1998) J. Cell Biol. 141, 929-942.

Patching (clustering) of membrane components



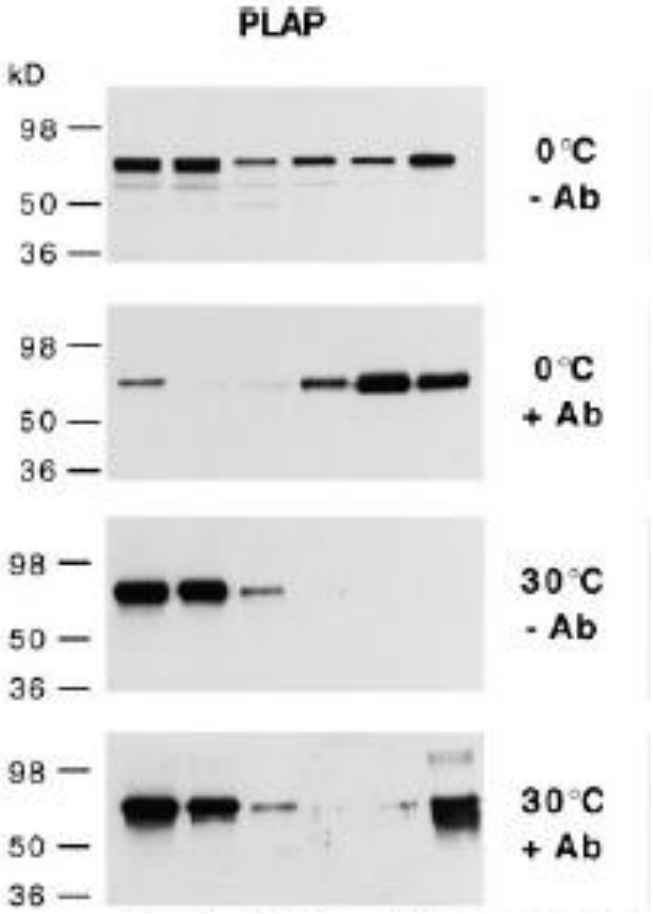
Jacobson & Dietrich (1999) Trends Cell Biol. 9, 87-91.

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



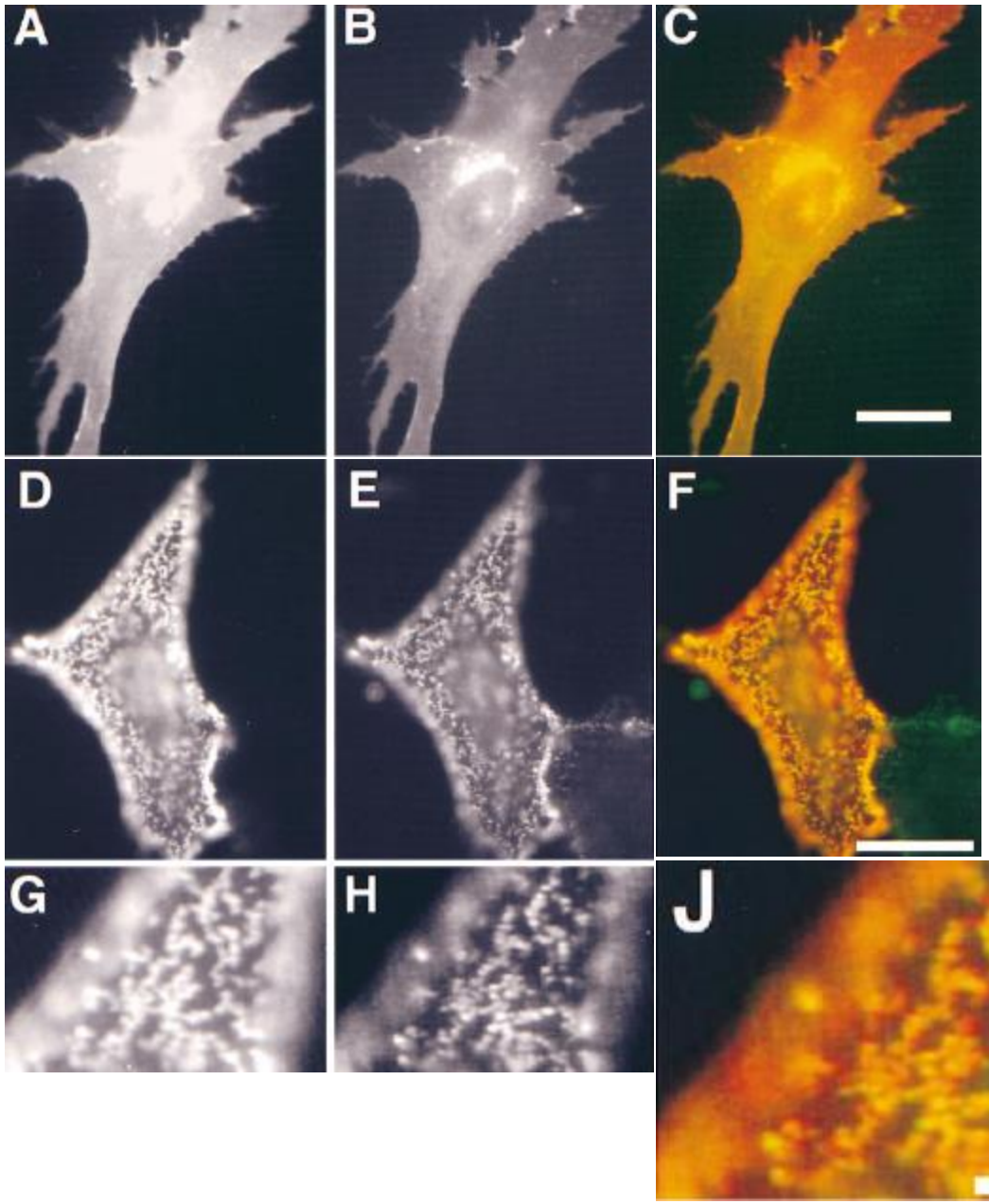
Harder et al. (1998) J. Cell Biol. 141, 929-942.

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



higher → lower density

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

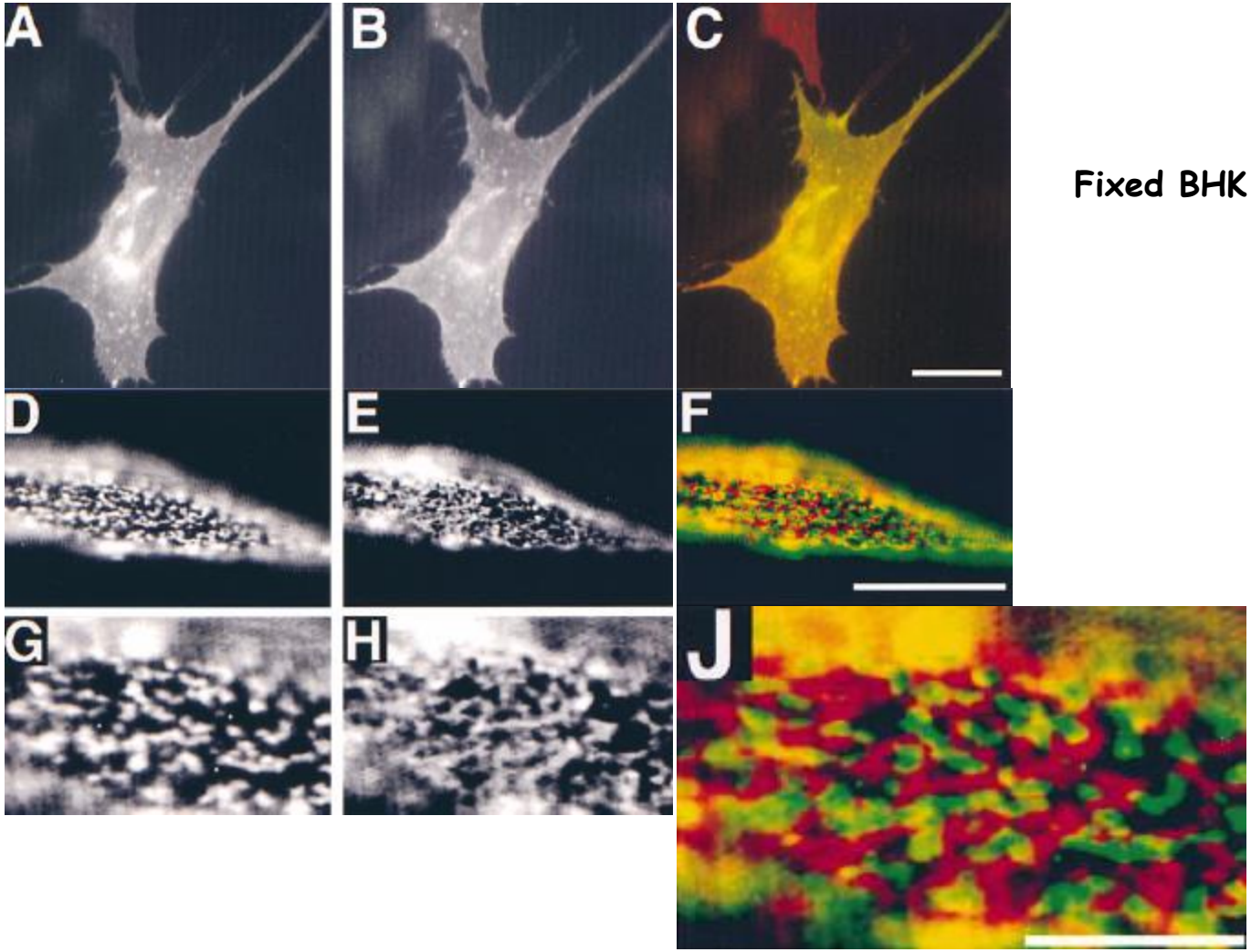


Fixed BHK cells

PLAP and HA

copatch

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

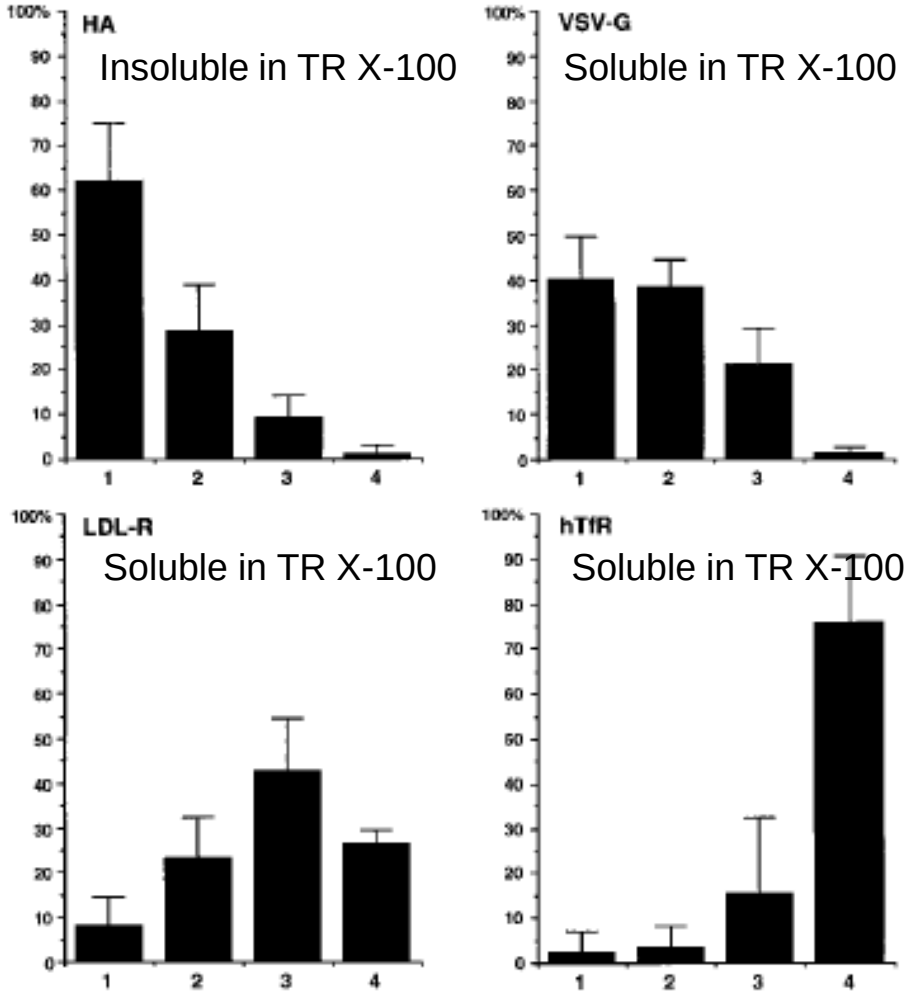


Fixed BHK cells

PLAP and hTfR segregate

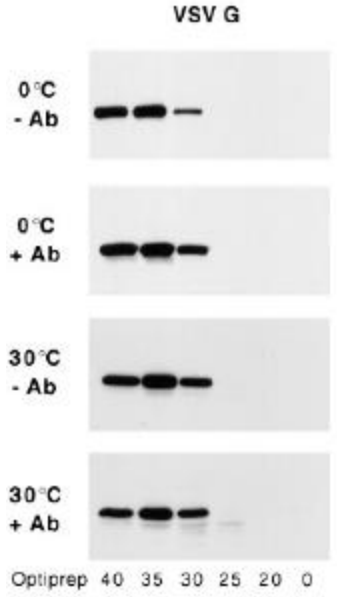
Certain proteins exhibit a weak but significant raft interaction which is not detectable by the TR X-100-solubility criterium

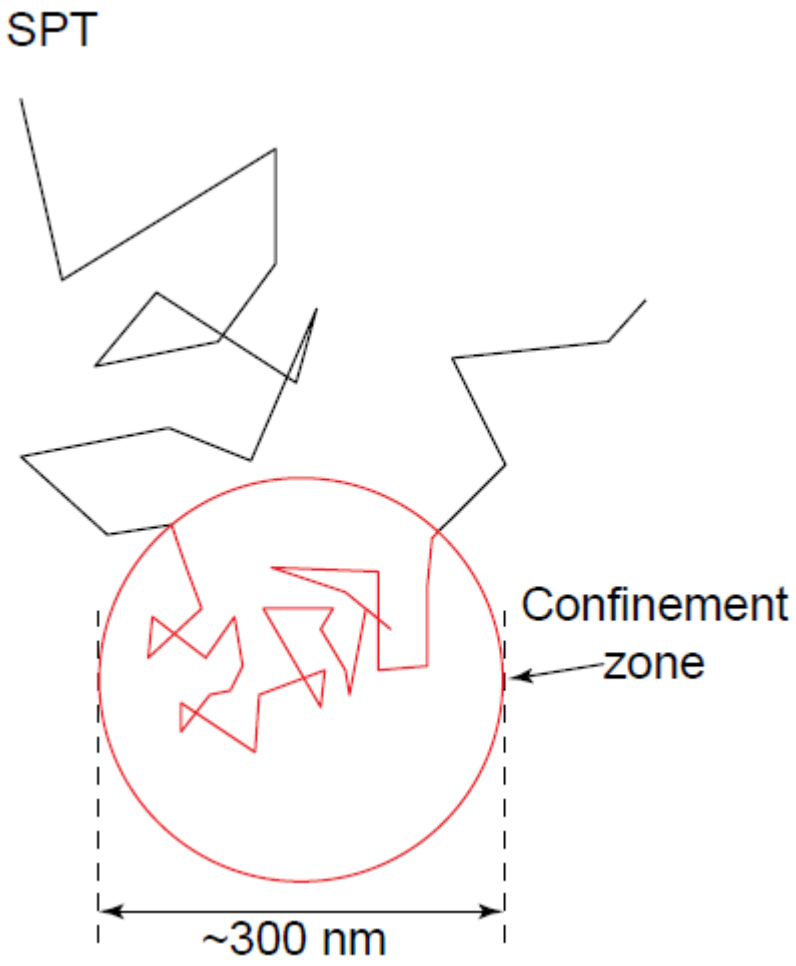
PLAP copatching



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

TR X-100 solubility



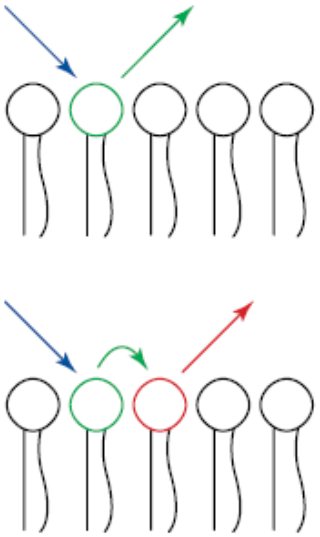


Jacobson & Dietrich (1999) Trends Cell Biol. 9, 87-91.

F luorescence
R esonance
E nergy
T ransfer

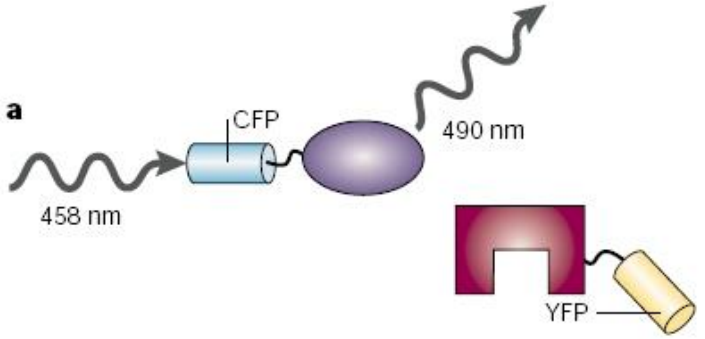
Conventional FRET

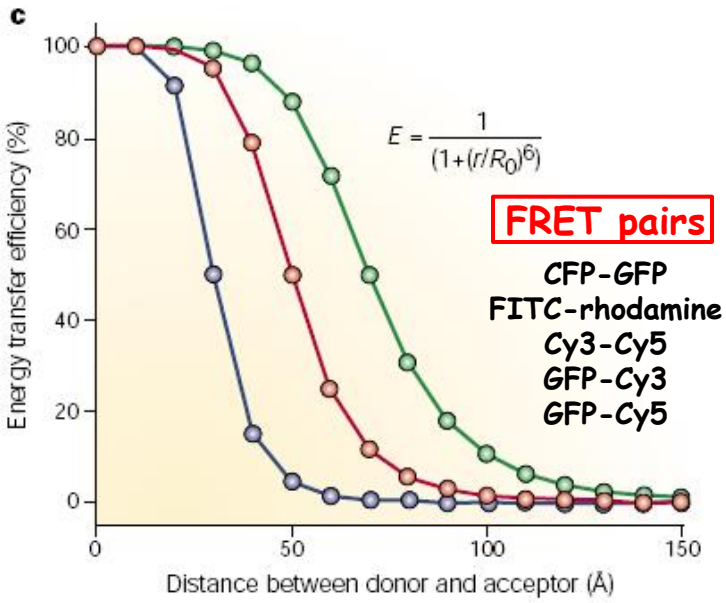
Excitation Emission



Normal donor emission in dilute solution

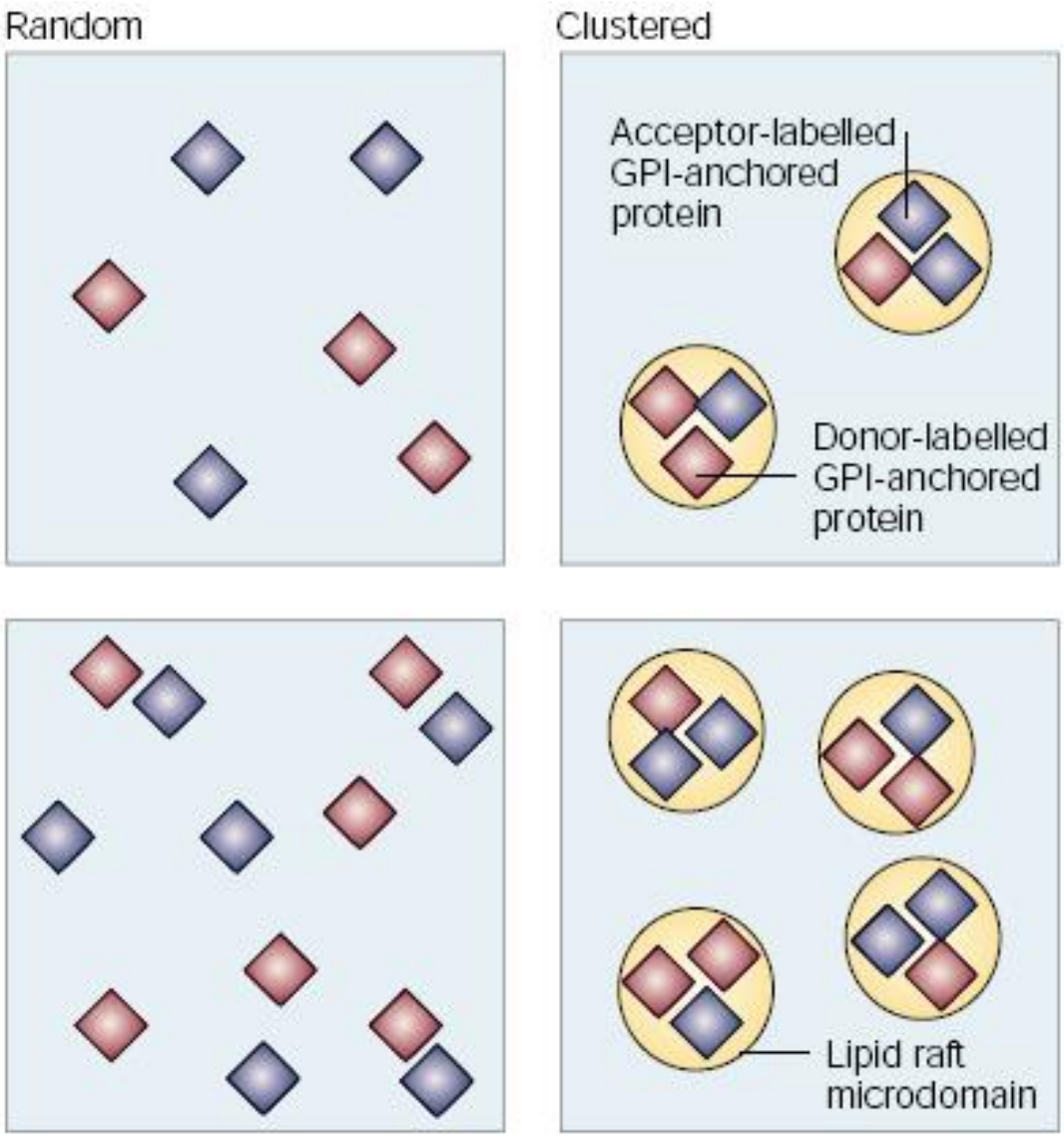
Red-shifted acceptor emission via donor–acceptor energy transfer in concentrated solution





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.

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

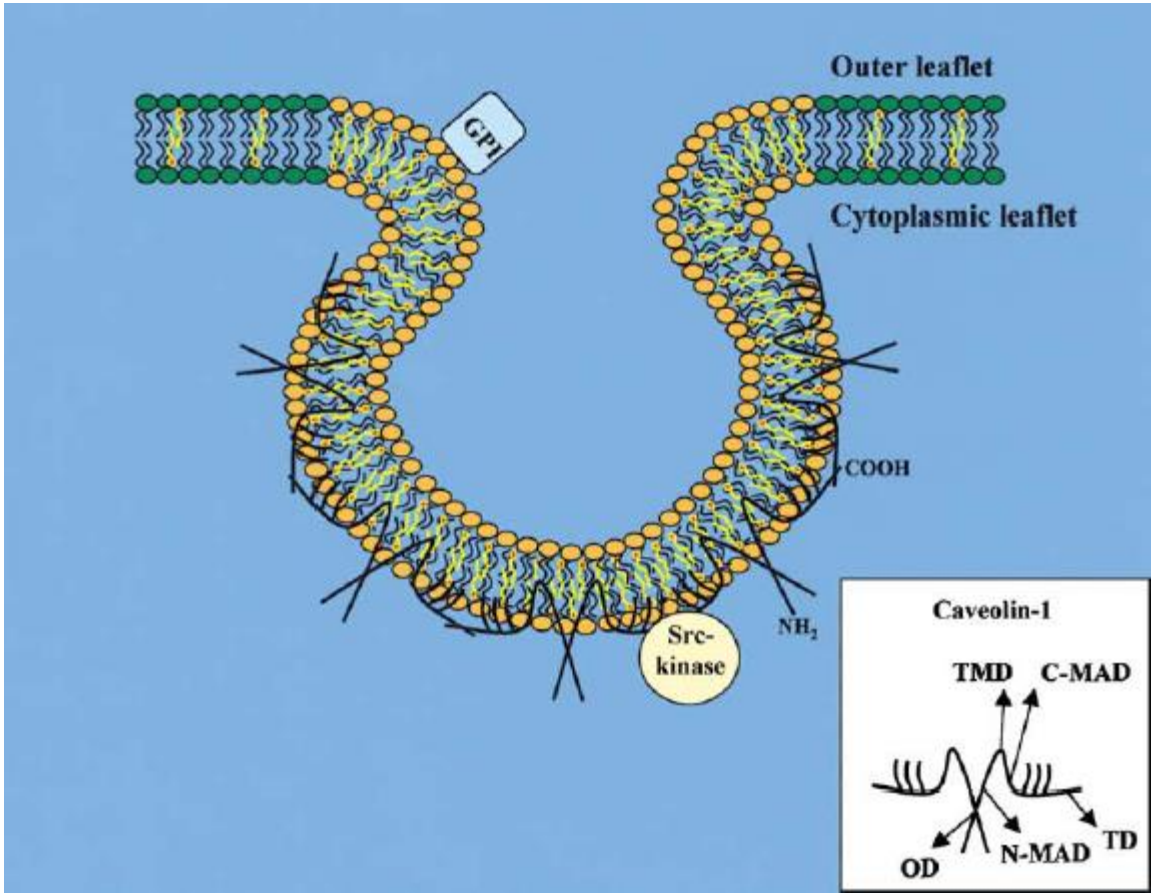
Neurons (cytoskeleton, extracellular matrix)
- accumulated in axonal PM

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

Lymphocytes and fibroblasts
- uniform distribution

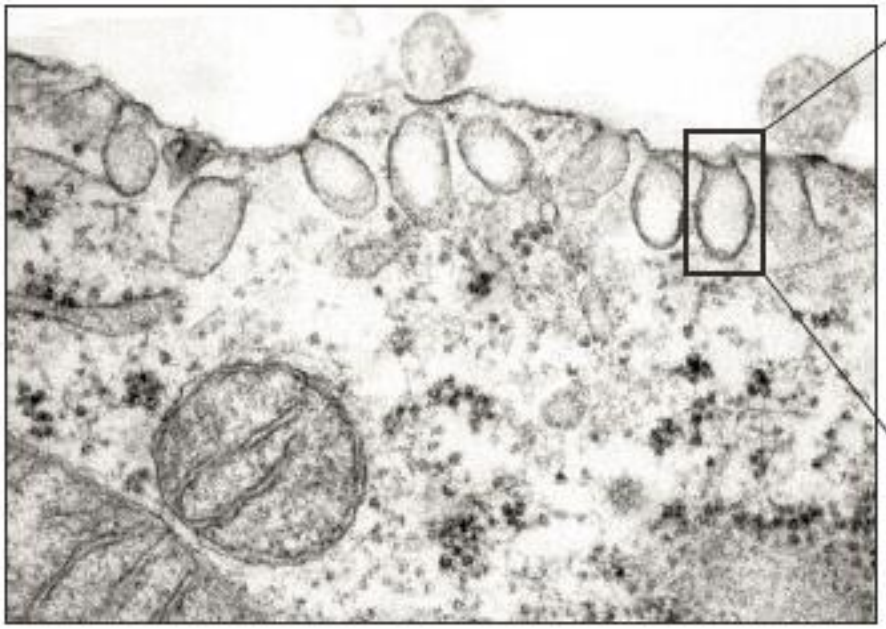
Caveolae

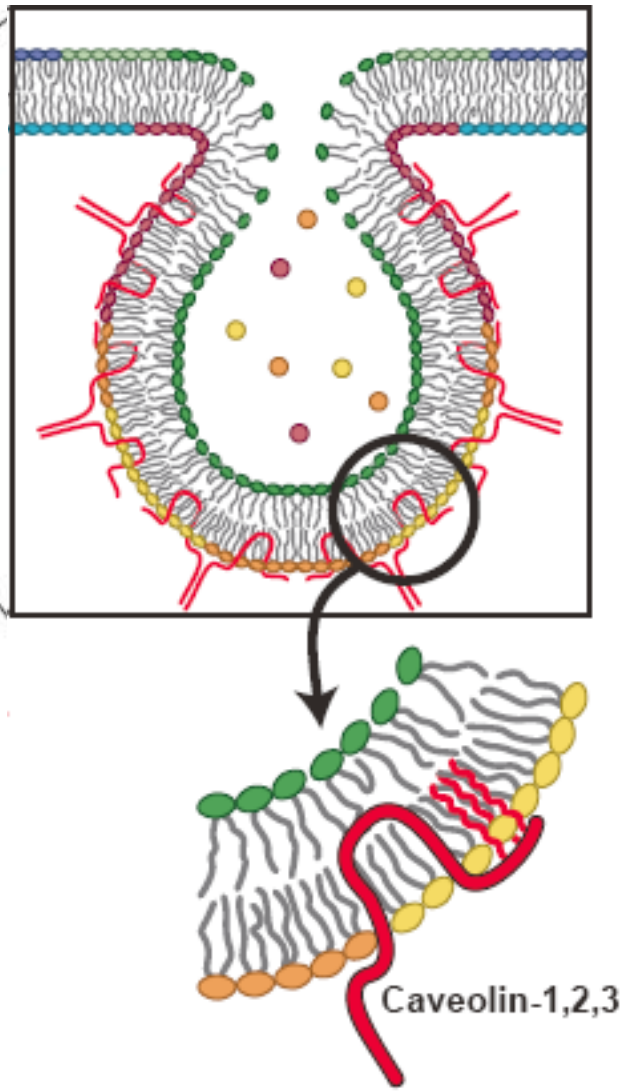
highly specialized raft subcategory



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

Hu fibroblast



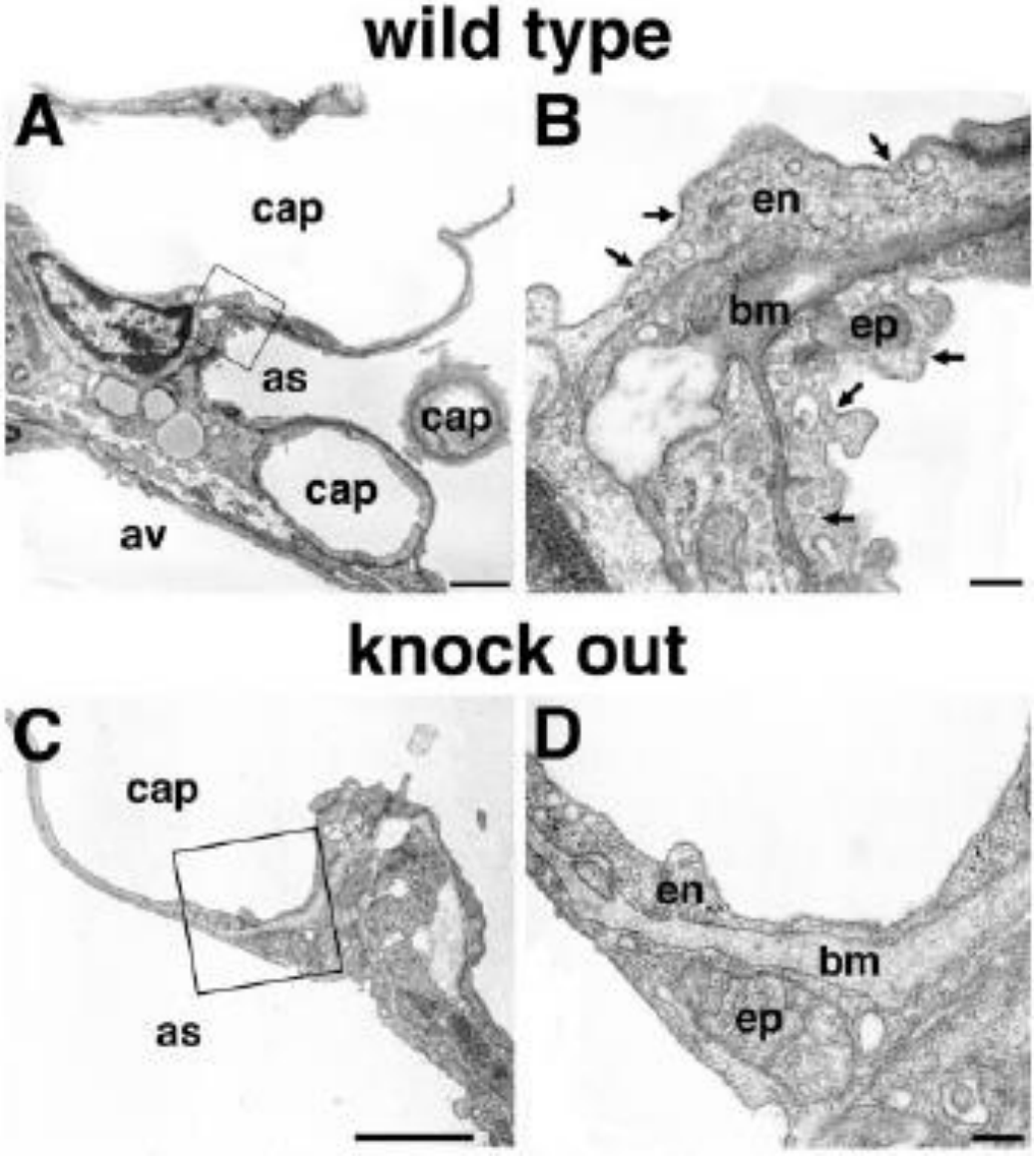


Parton (2001) Science 293, 2404-2405.

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.

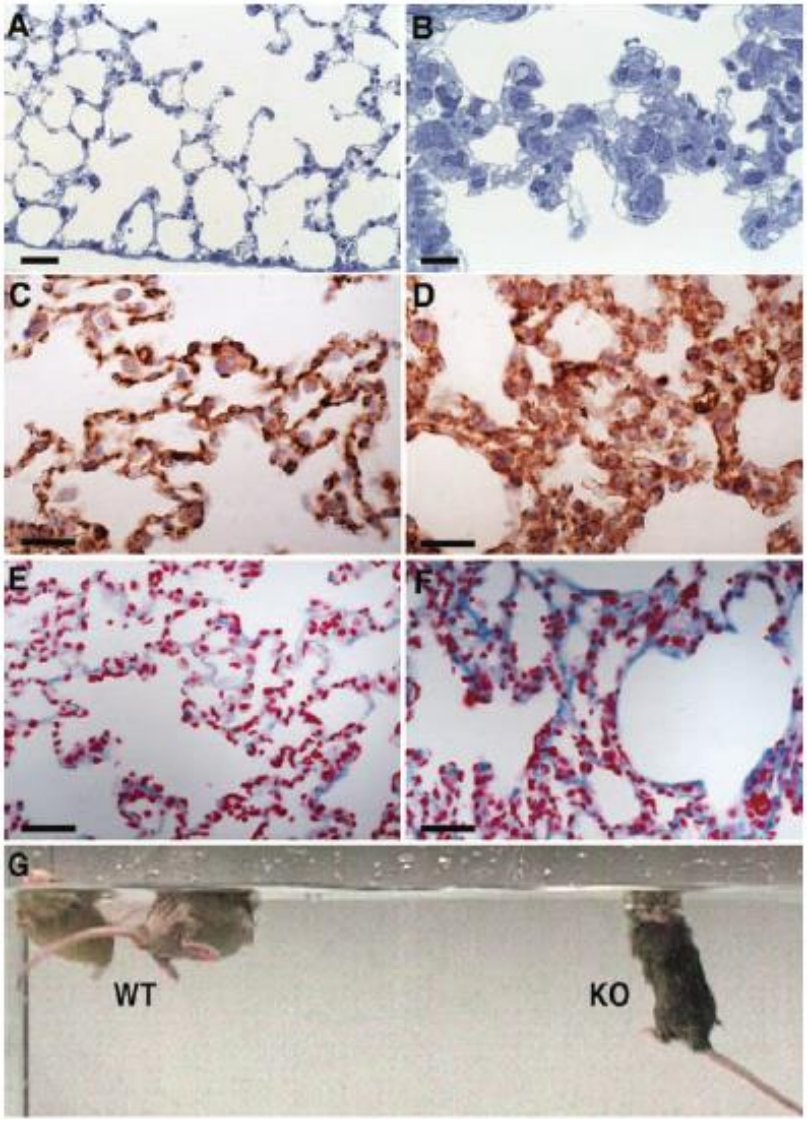
Disappearance of caveolae from cells in cav-1 (-/-) mice



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.

Cellular processes involving lipid rafts

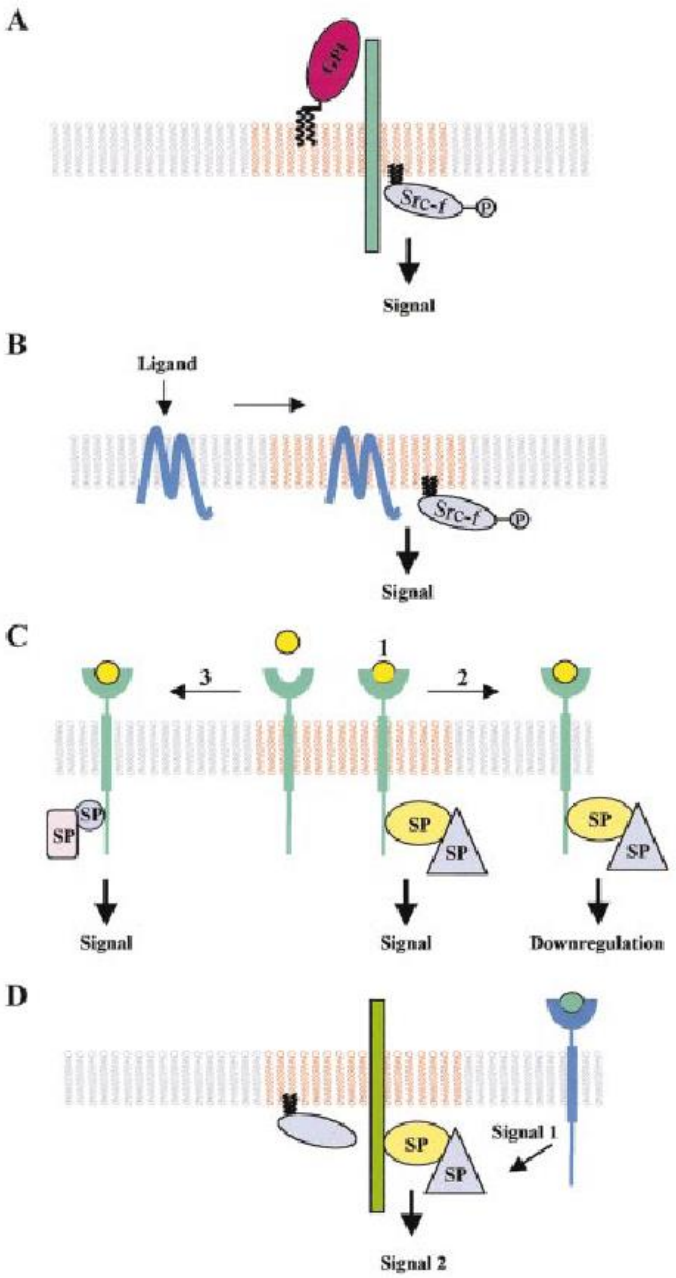
- Signal transduction
- Protein and lipid trafficking and sorting
- Clathrin-independent endocytosis:
    - ceveolin-dependent (potocytosis)
    - ceveolin-independent endocytosis
- Ca^{2+} homeostasis

Protein and lipid signalling molecules identified in lipid rafts

Protein/lipid
Transmembrane receptors
EGF receptor
Bradykinin B2 receptor
Eph family receptors
TCR
BCR
FcεRI
β1 integrins
Lipid signalling molecules
Sphingomyelin
Ceramide
Phosphoinositides
Diacylglycerol
GPI-linked proteins
CD59
uPAR
EphrinA5
Signalling effectors
$G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$
Src-family kinases
Ras
PKC α
Shc
Adenylate cyclase
eNOS
PLCγ
PI3K
SHIP
Cbp/PAG

Zajchowski & Robbins (2002) Eur. J. Biochem. 269, 737-752.

Proposed modes of signal transduction via lipid rafts



CD59, ephrin

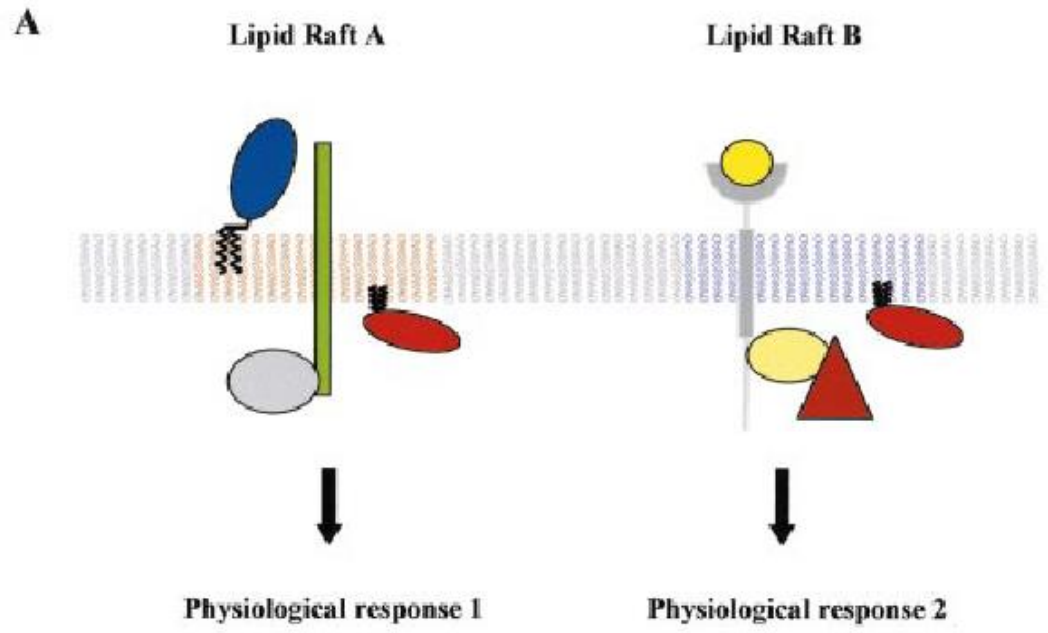
CD20, FcεRI

(1,2) EGFR, PDGFR

(3) IL-2R

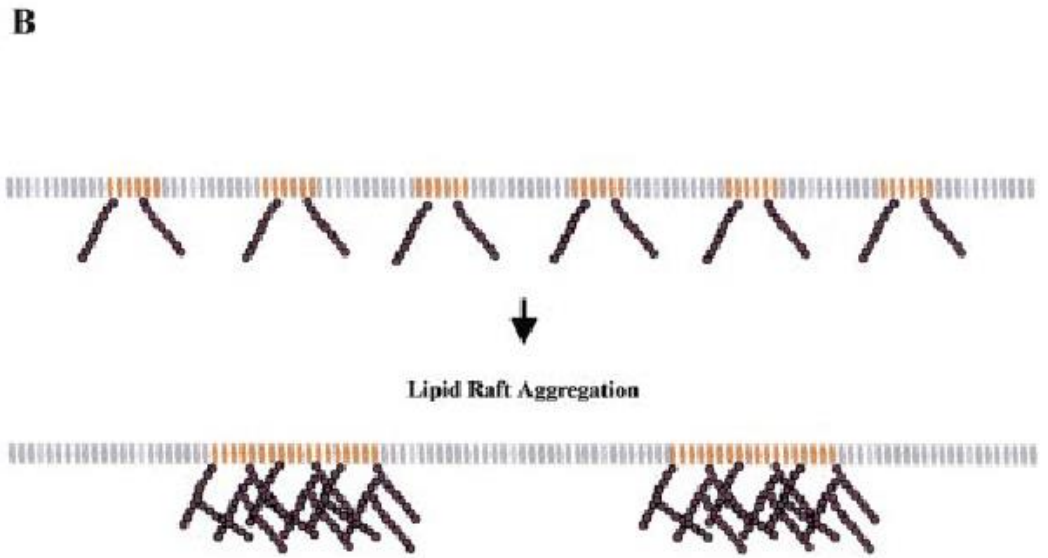
Zajchowski & Robbins (2002) Eur. J. Biochem. 269, 737-752.

Signalling specificity by distinct subpopulations of lipid rafts.

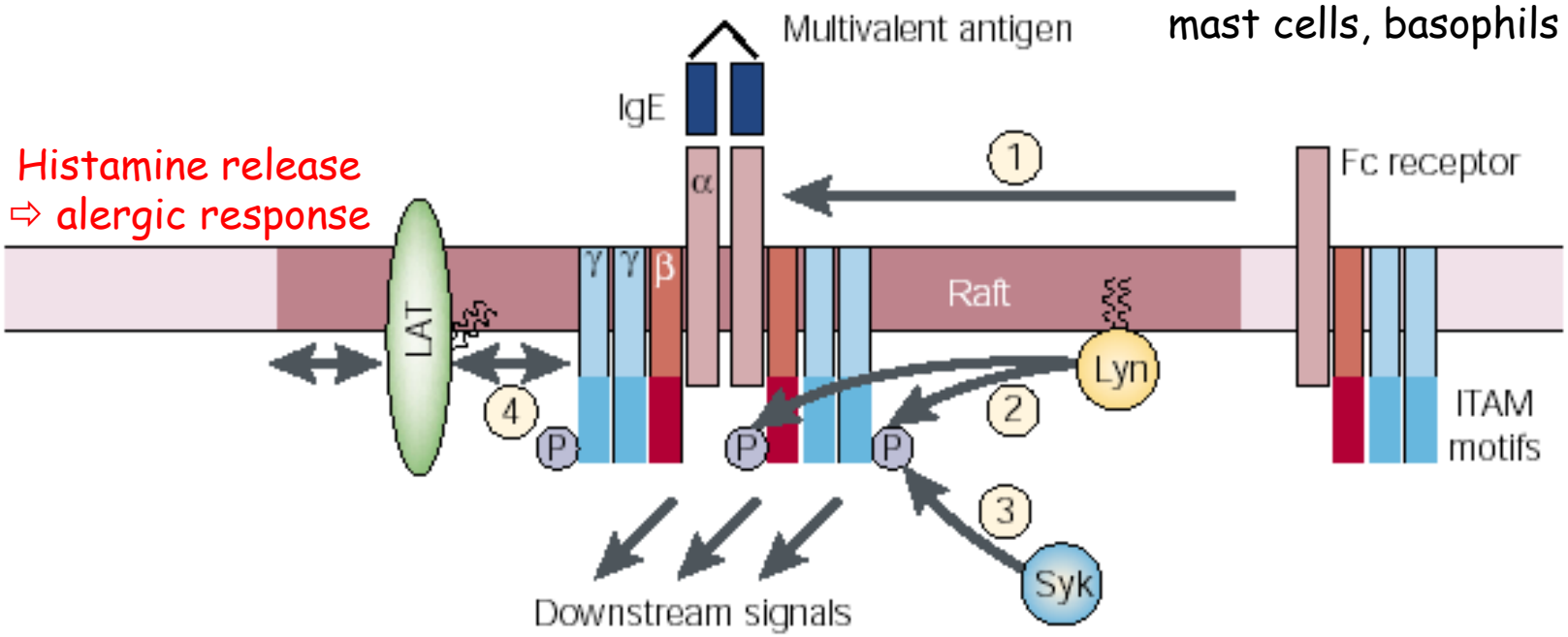


Formation of higher-order signalling complexes by clustering of lipid rafts:

- signal amplification
- cross-talk
- spatial regulation



IgE receptor (FcεRI)-mediated signalling in allergic immune response

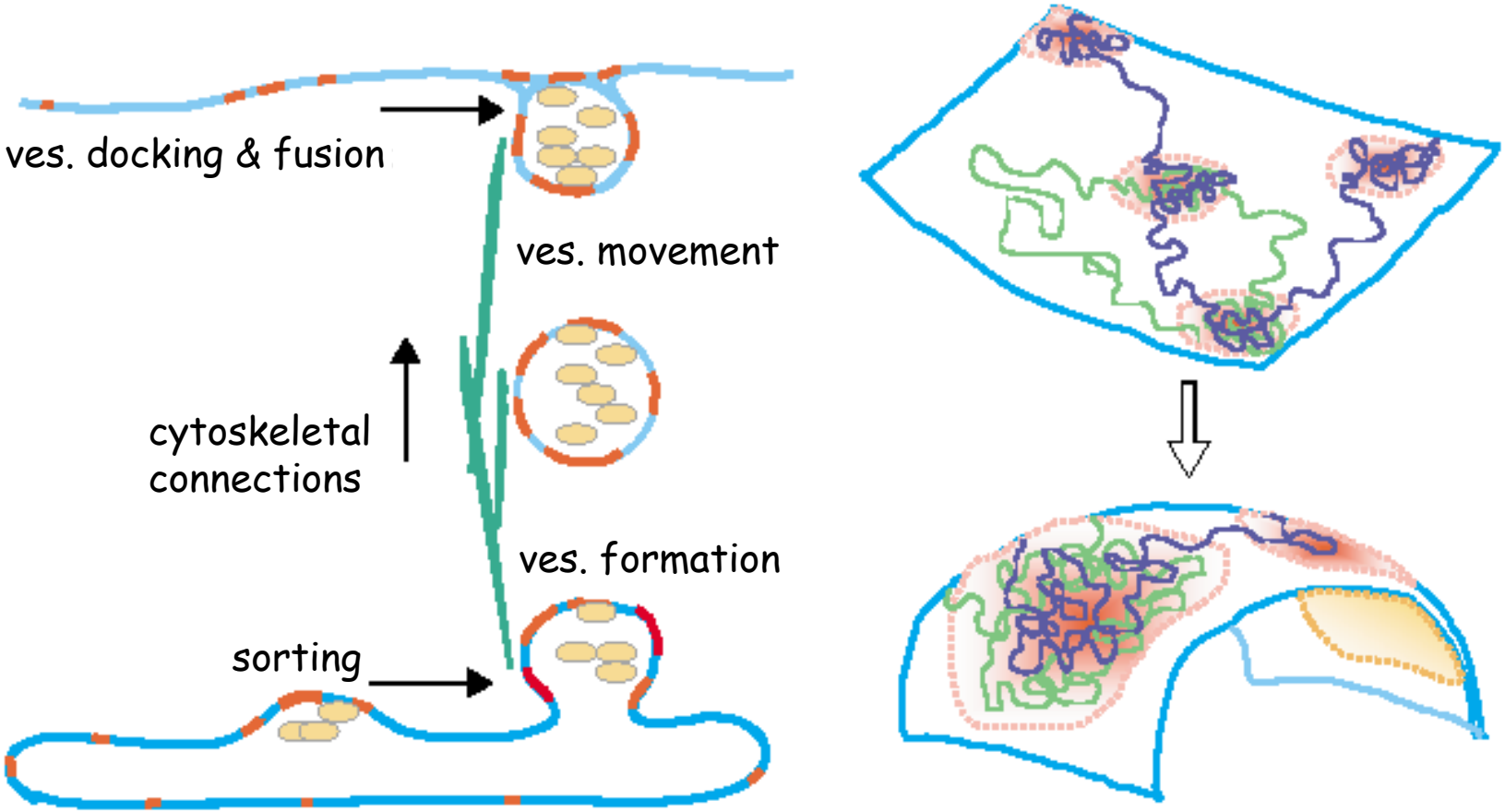


Simons & Toomre (2000) Nat. Rev. Molec. Cell Biol. 1, 31-40.

Cellular processes involving lipid rafts

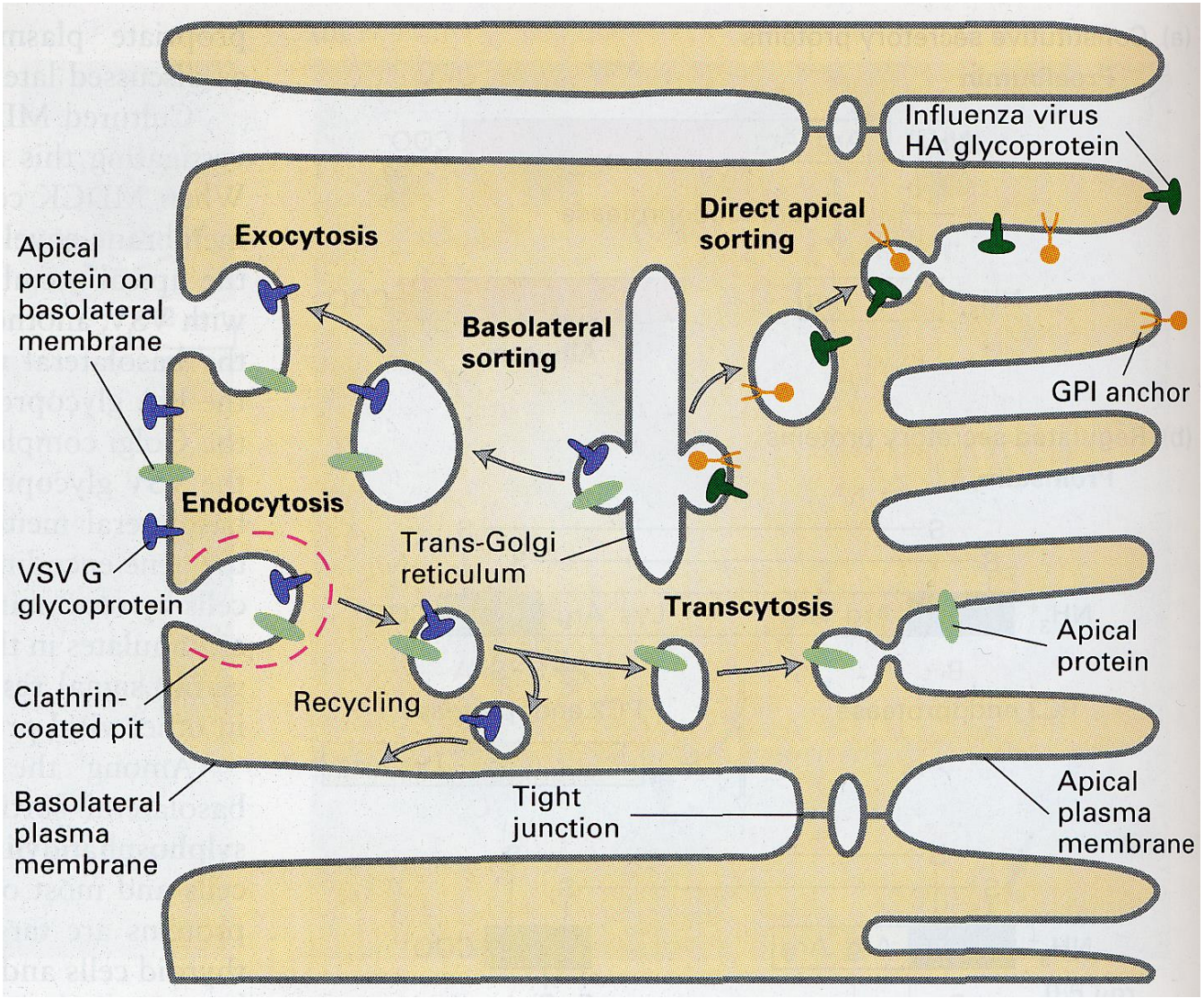
- Signal transduction
- Protein and lipid trafficking and sorting
- Clathrin-independent endocytosis:
    - ceveolin-dependent (potocytosis)
    - ceveolin-independent
- Ca^{2+} homeostasis

Potential roles of lipid rafts in vesicular transport

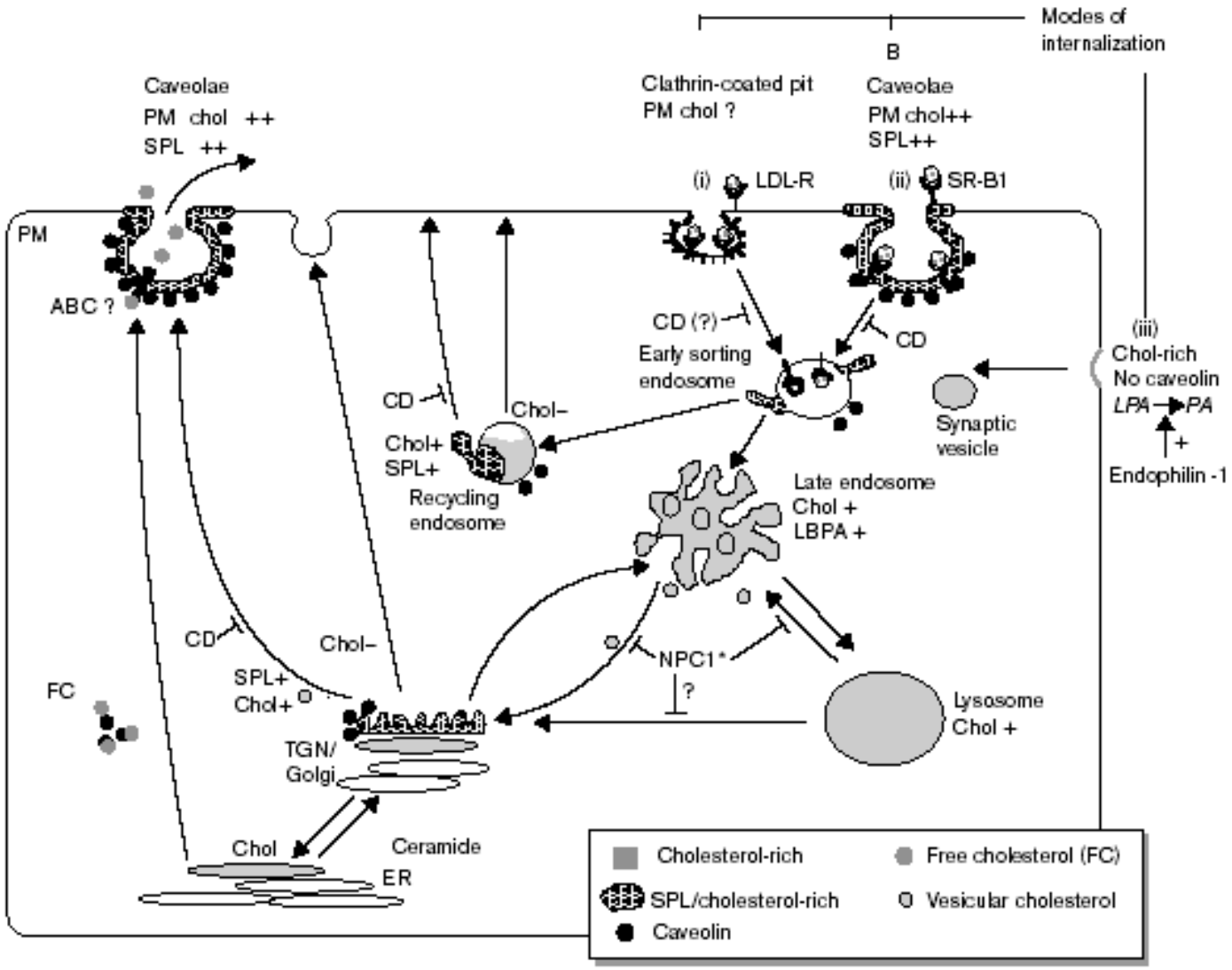


Ikonen (2001) Curr. Opin. Cell Biol. 13, 470-477.

The sorting of proteins in polarized cells
(*e.g.* MDCK epithelial cell)



Lipid rafts are involved in cholesterol and sphingolipid traffic



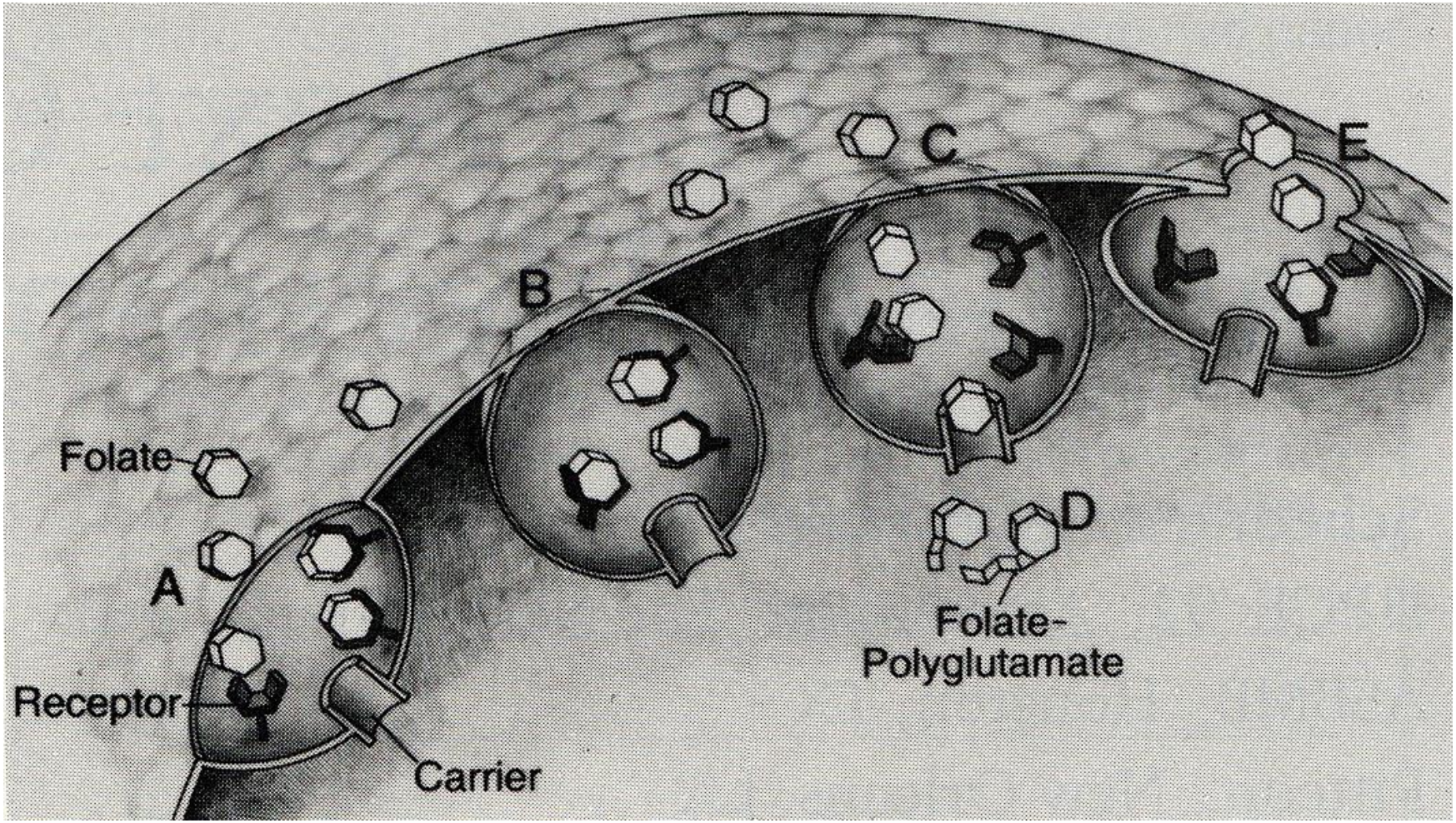
Hoekstra et al. (2001) Curr. Opin. Cell Biol. 12, 496-502.

Cellular processes involving lipid rafts

- Signal transduction
- Protein and lipid trafficking and sorting
- Clathrin-independent endocytosis:
    - ceveolin-dependent (potocytosis)
    - ceveolin-independent
- Ca^{2+} homeostasis

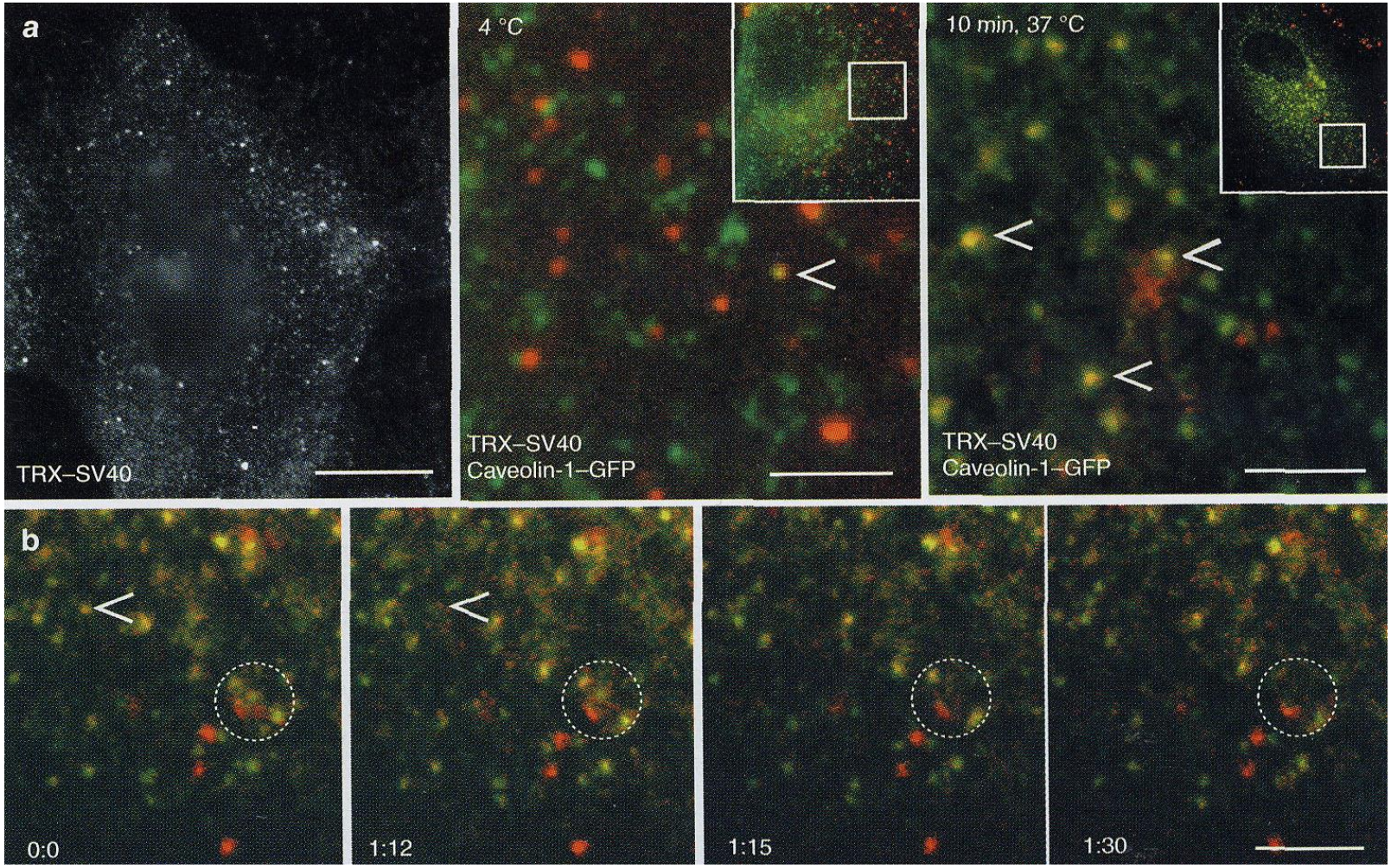
Potocytosis:

sequestration and internalization of molecules and ions by caveolae



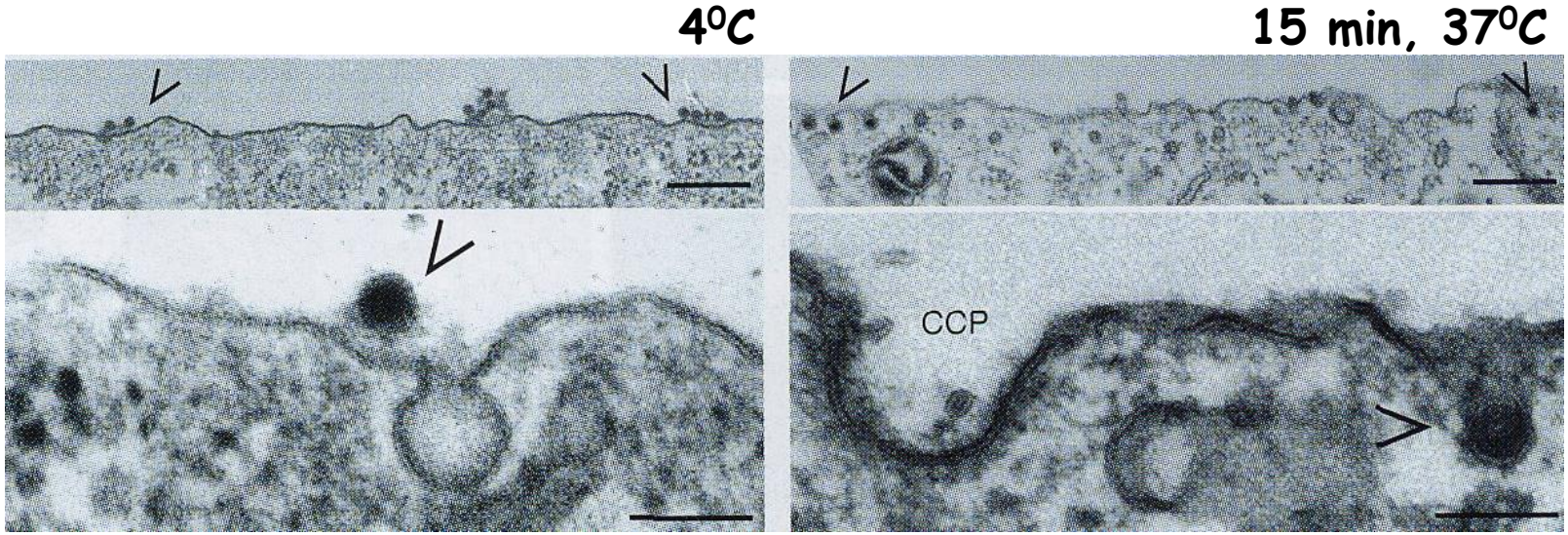
Anderson et al. (1992) Science 255, 410-411.

Caveolar endocytosis of simian virus 40 (SV40) by CV-1 cells



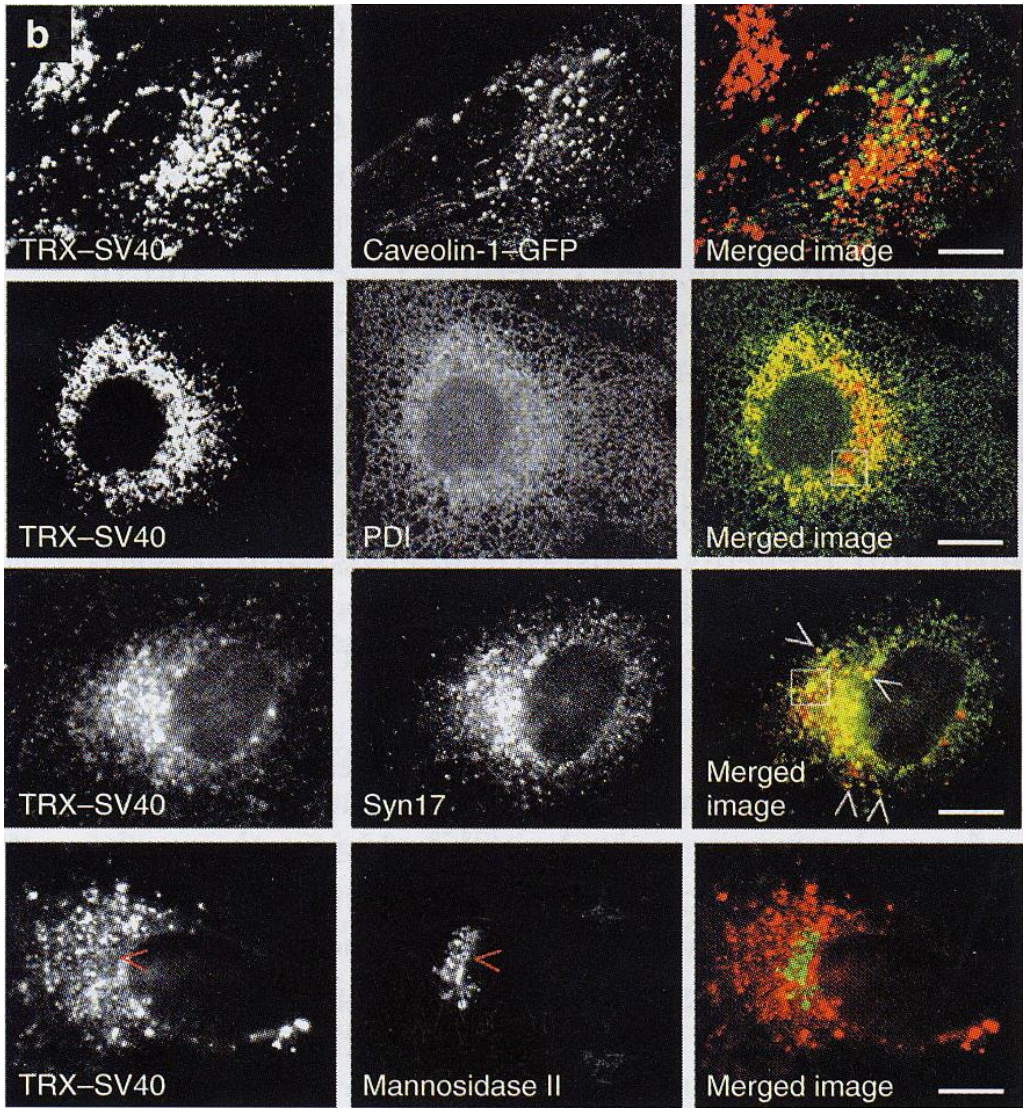
Pelkmans et al. (2001) Nat. Cell Biol. 3, 473-483.

Caveolar endocytosis of SV40 by CV-1 cells



Intracellular localization of SV40 in CV-1 cells

(16h at 37°C after virus binding)



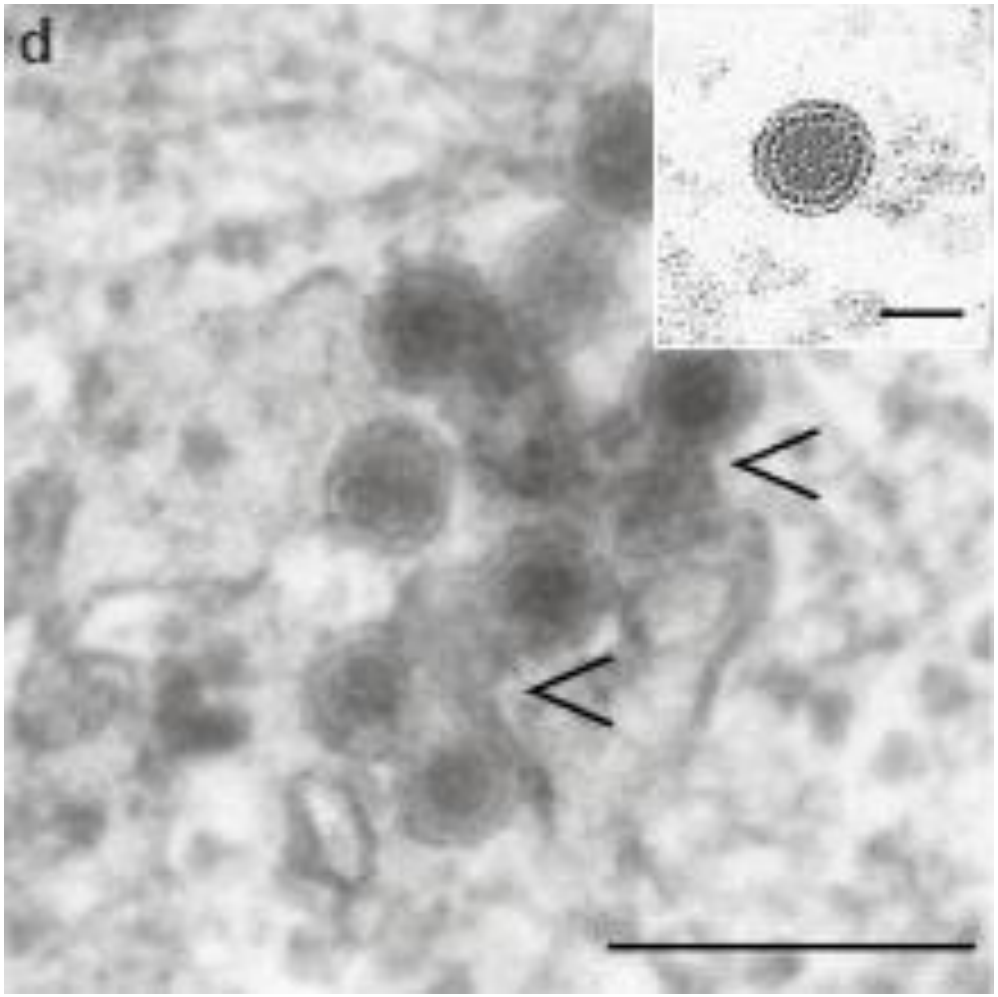
Cav-1 organelles
9 % overlap

reticular ER
70 % overlap

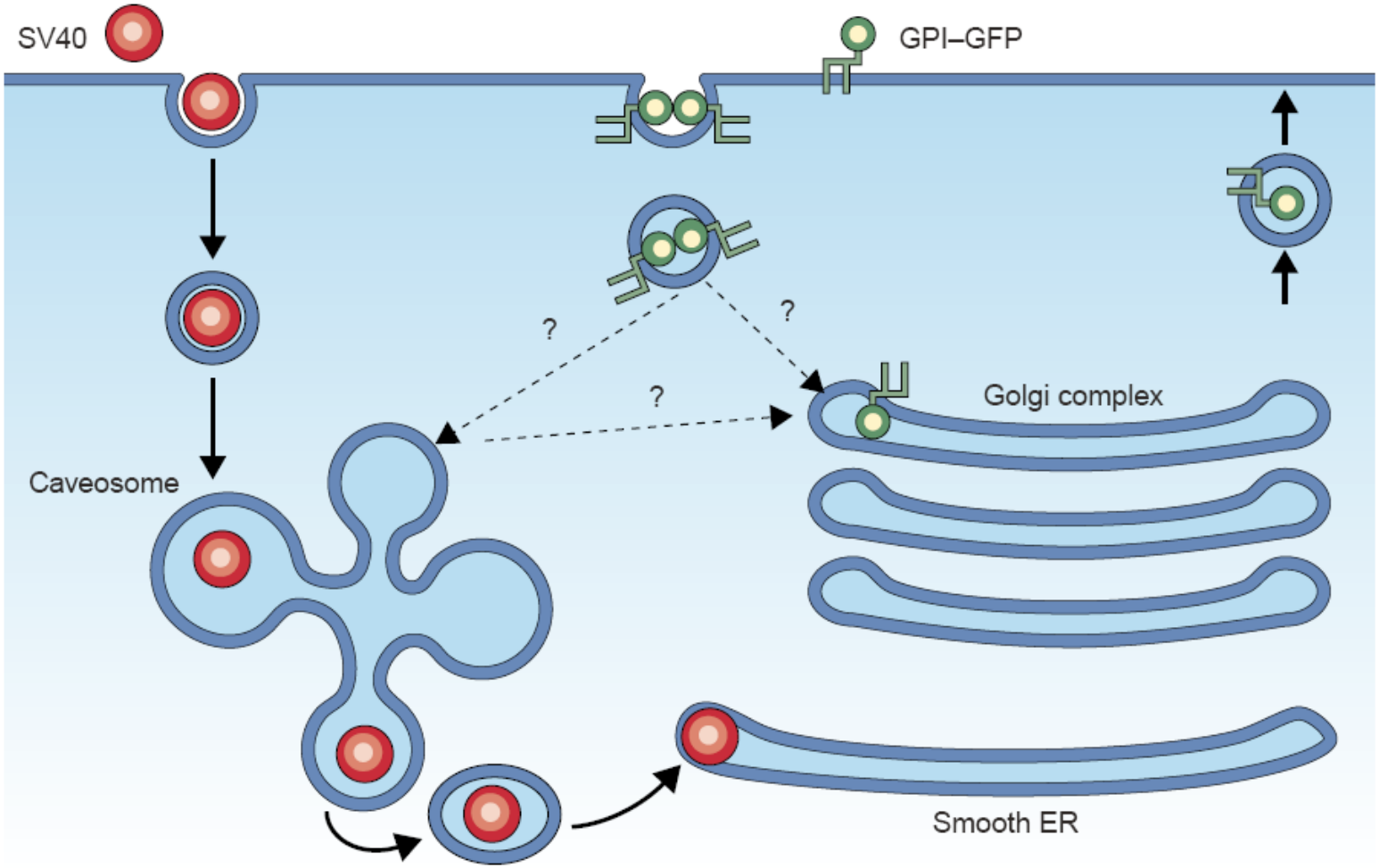
smooth ER
80 % overlap

Golgi
5 % overlap

A two-step transport from PM caveolae to ER, through an intermediate organelle - caveosome



Endosome-independent routs for endocytic transport to the ER and Golgi



Pfeffer (2001) Nat. Cell Biol. 3, E108-E110.

Caveosome

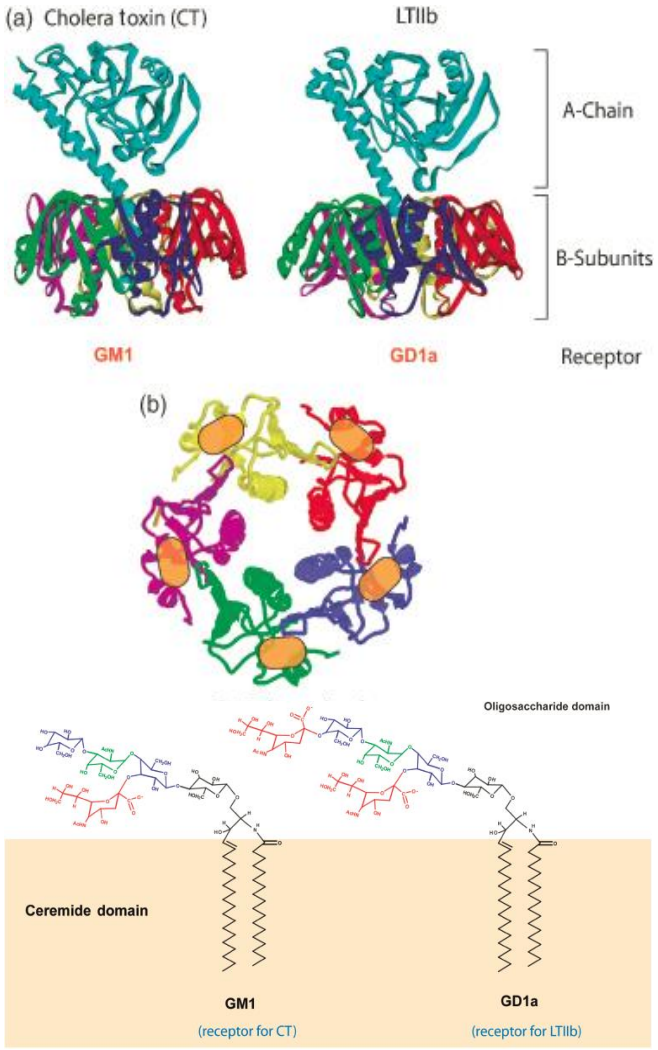
- does not acidify (neutral pH),

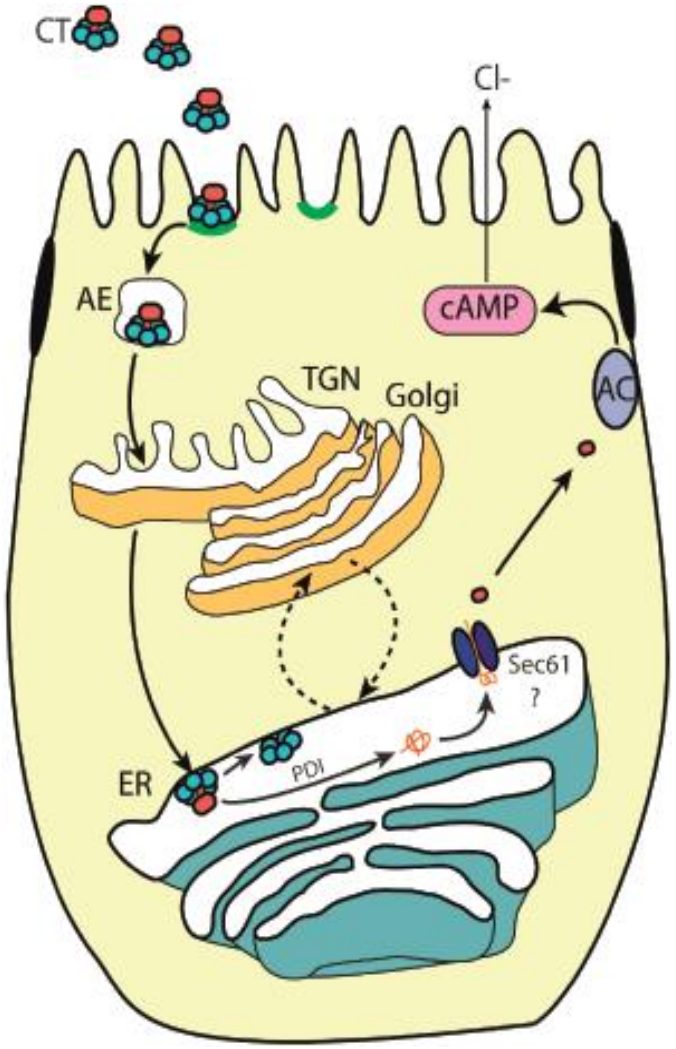
- caveolin-containing compartment,

- lacks coated pit-pathway markers (endosomal, lysosomal, ER or Golgi),

- does not acquire ligands of clathrin-coated vesicle endocytosis.

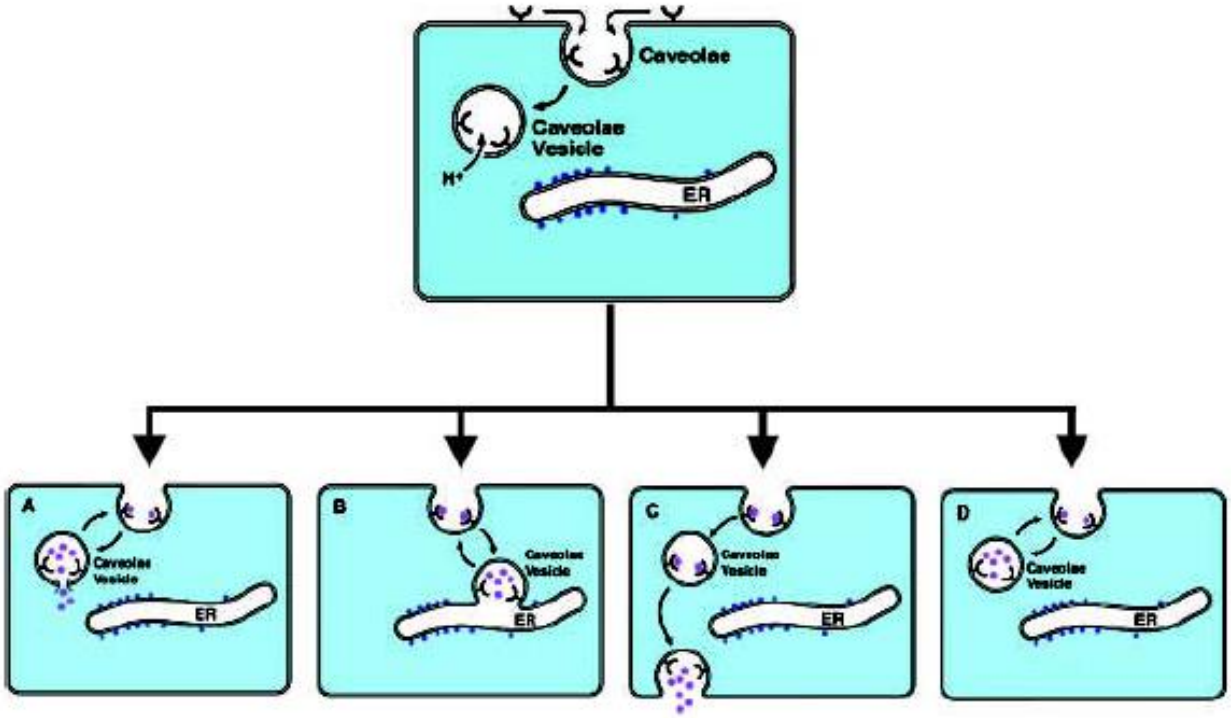
Retrograde transport via caveolae





Chinnapen et al. (2007) FEMS Microbiol. Lett. 266, 129–137

Four possible fates for molecules internalized by potocytosis



- Folate
- Iron
- Calcium
- Water
- Retinol

- Cholesterol
- SV40 Virus
- AGE
- CD59
- bFGF
- AMF
- GH
- CT

- Albumin
- Transthyretin
- Thrombomodulin
- Plasma Proteins
- Water

- AP
- Bradykinin
- Acetylcholine
- Cholecystokinin
- Endothelin
- gp114

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

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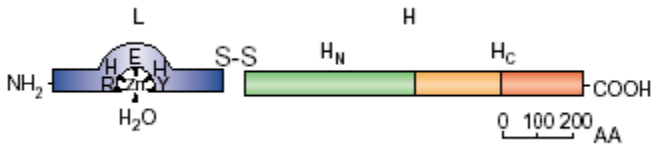
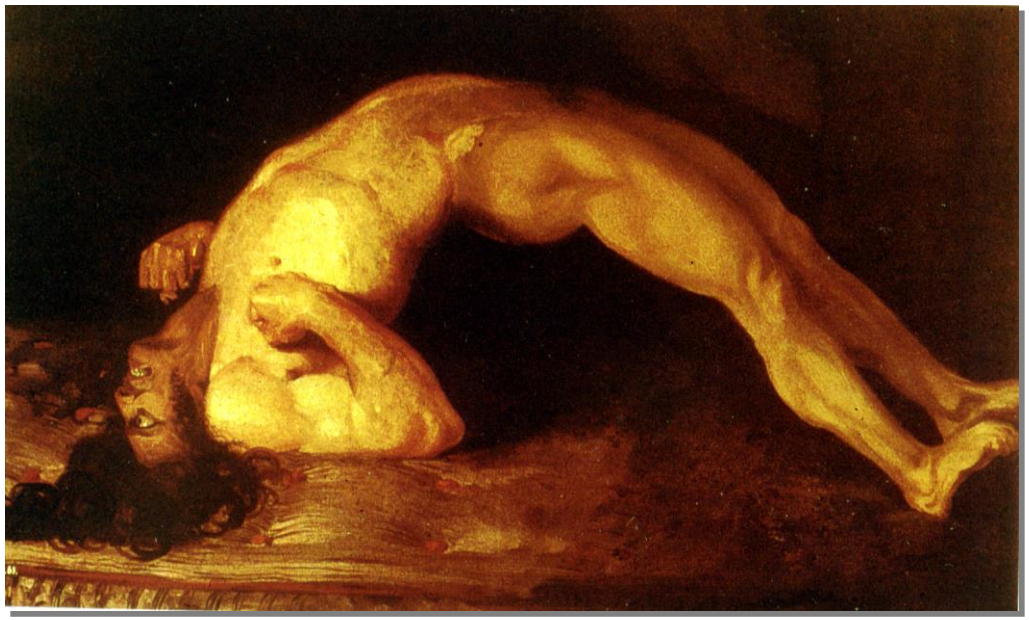
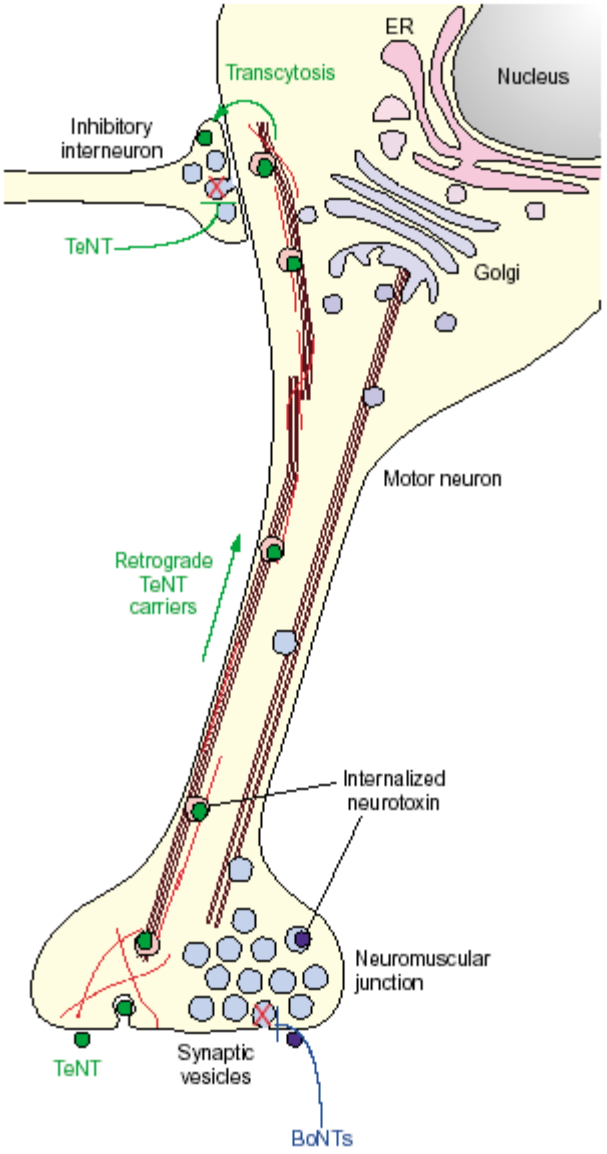
Clathrin-independent receptor-mediated endocytosis of TeNT

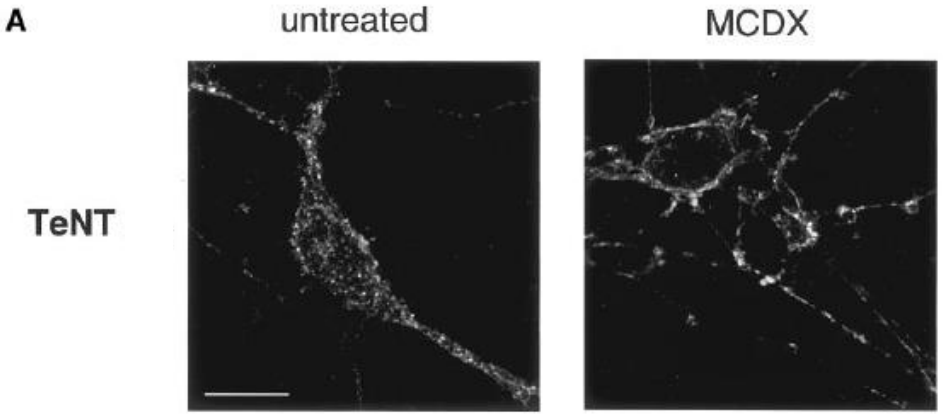




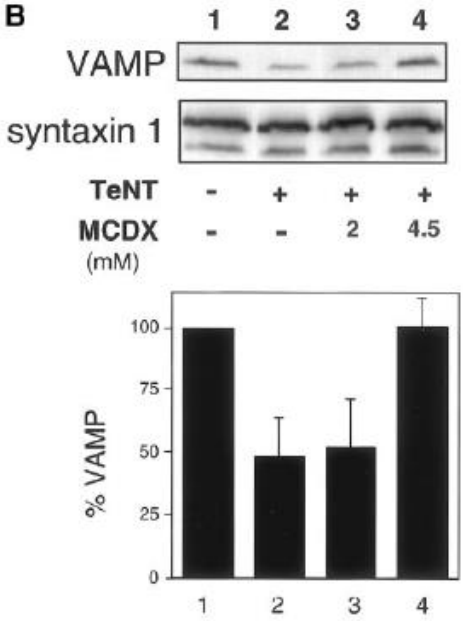


Lalli et al. (2003) TRENDS in Microbiol. 11, 431-437.

Cholesterol depletion (raft disruption) blocks the internalization and intracellular activity of TeNT

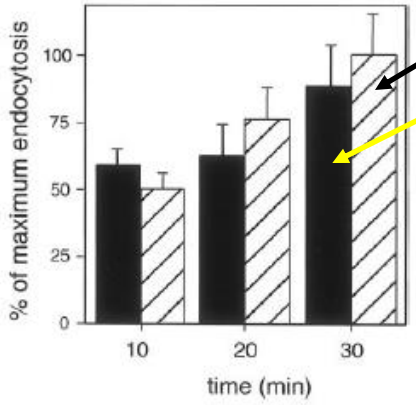


Spinal cord cells
(neurons lack caveolae)



C

Endocytosis of ^{125}I-transferrin
(a clathrin-mediated process)



MCDX-treated
MCDX-untreated

TeNT receptor is
a GPI-protein Thy-1

Herreros et al. (2001) Mol. Biol. Cell 12, 2947–2960.

Cellular processes involving lipid rafts

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    - ceveolin-independent
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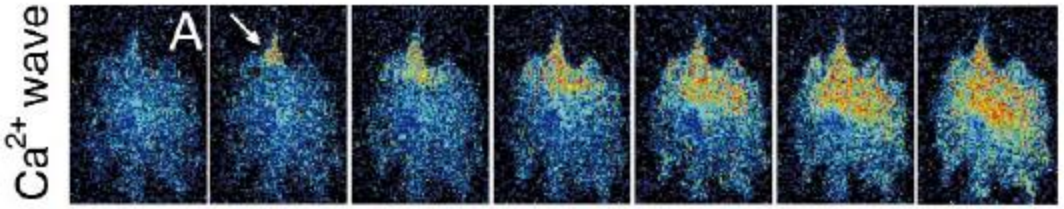
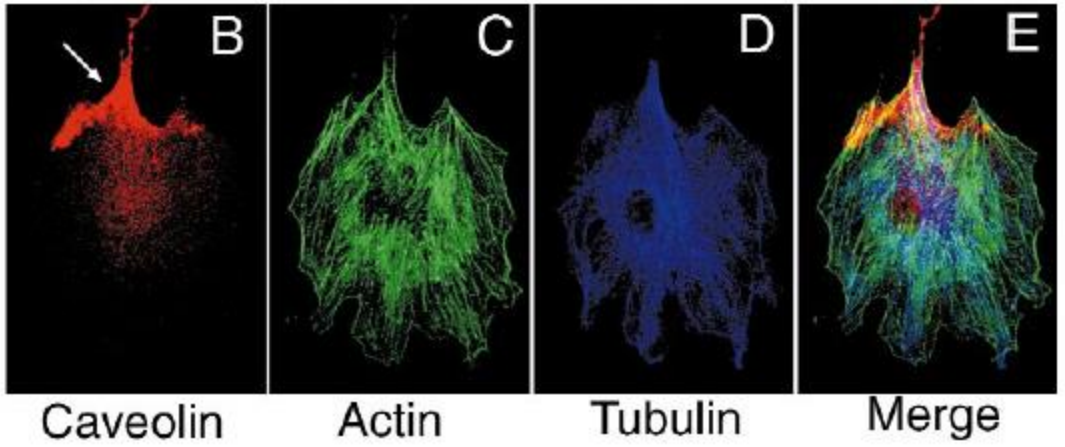
Ca^{2+} wave originates at the caveolin-rich cell edge

ATP stimulation ⇨ IP_3 mobilization

Indo-1 loaded endothelial cell (bovine aortic)



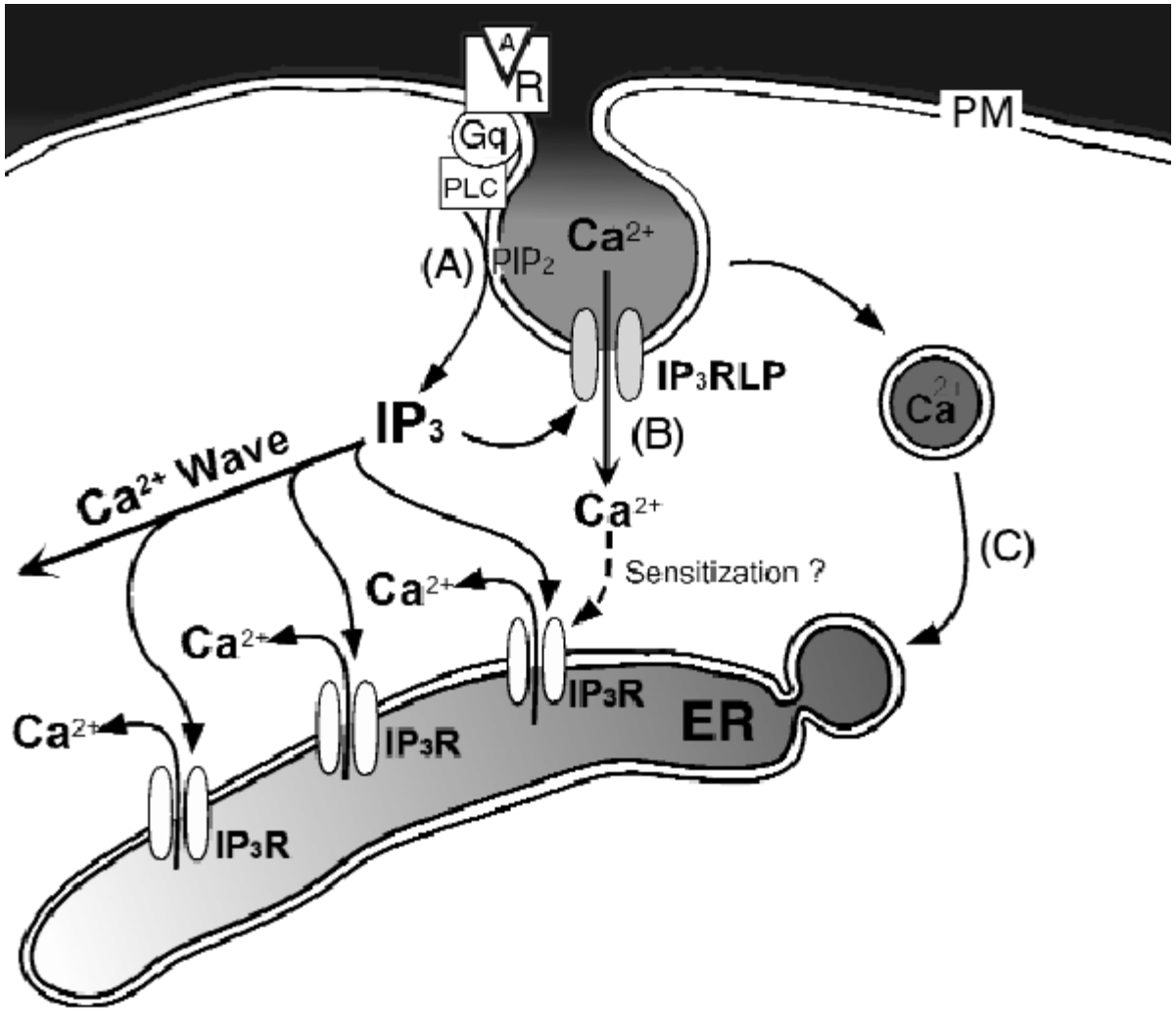
0.34 s intervals



Isshiki & Anderson (1999) Cell Calcium 26, 201-208.

Caveolae are enriched in molecules involved in Ca^{2+} regulation: IP_3R-like protein and Ca^{2+}-ATPase.

Three ways for how caveolae might regulate Ca^{2+} wave initiation



Key functions for caveolae in Ca^{2+} homeostasis

- regulation of the spatial organization of Ca^{2+} entry sites,
- control of the amount of Ca^{2+} that is delivered at these sites,
- initiation of Ca^{2+} wave formation,
- modulation of Ca^{2+}-dependent signalling cascades in caveolae (*e.g.* eNOS/CaM^{+}/$caveolin^{-}$).

Lipid rafts and human disease

- Muscular distrophy (cav-3 mutation)
- Alzheimer`s disease (generation of β-amyloid)
- Encephalopathies (a conversion of Pr^{C} to Pr^{Sc} in caveolae)
- Cancer (loss of caveolin-1, *i.e.* caveolae)
- Pathogens (cellular entrance point)
- Cardiovascular diseases

Further reading:

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.

Simons, K. & Gerl, M.J. (2010): Revitalizing membrane rafts: new tools and insights. Nat. Rew. Mol. Cell Biol. 11, 688-699.

Lingwood, D. and Simons, K. (2010): Lipid Rafts As a Membrane-Organizing Principle. Science 237, 46-50.

Simons, K. & Sampaio, J.L. (2011): Membrane organization and lipid rafts. Cold Spring Harb. Perspect. Biol. 3, a004697.