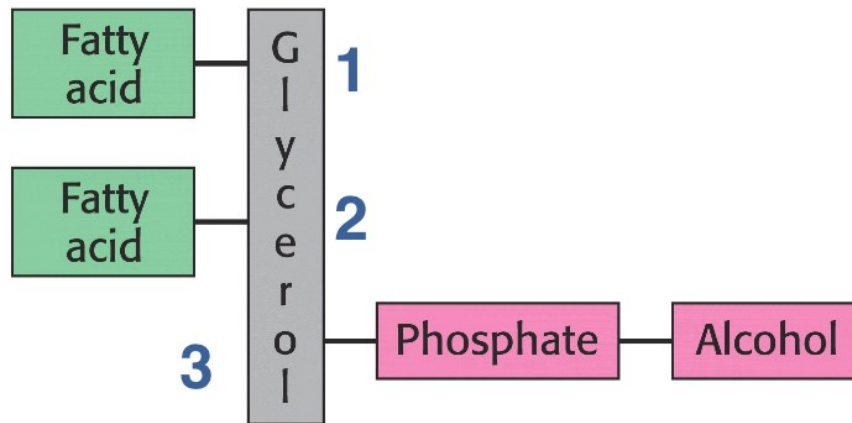


Localising proteins to and within membranes

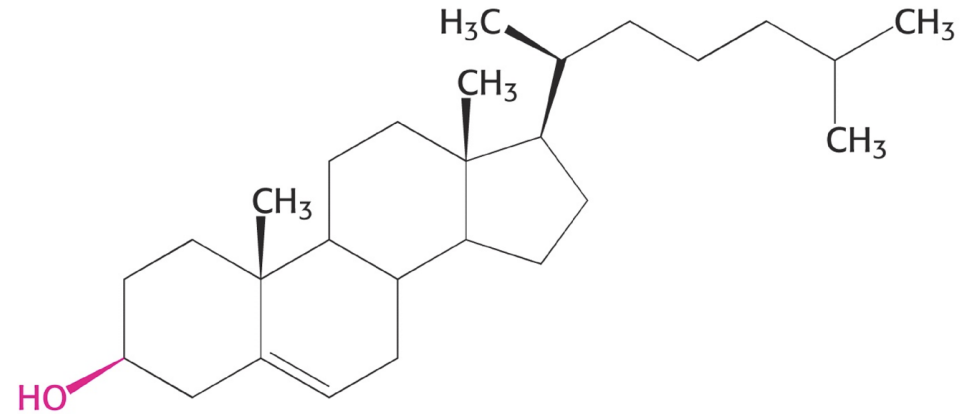
- Membranes
- Lipidation
 - GPI anchors
 - Prenylation
 - Myristoylation
 - Palmitoylation
- Modulation of biological outcome..

Membrane lipids : Major classes

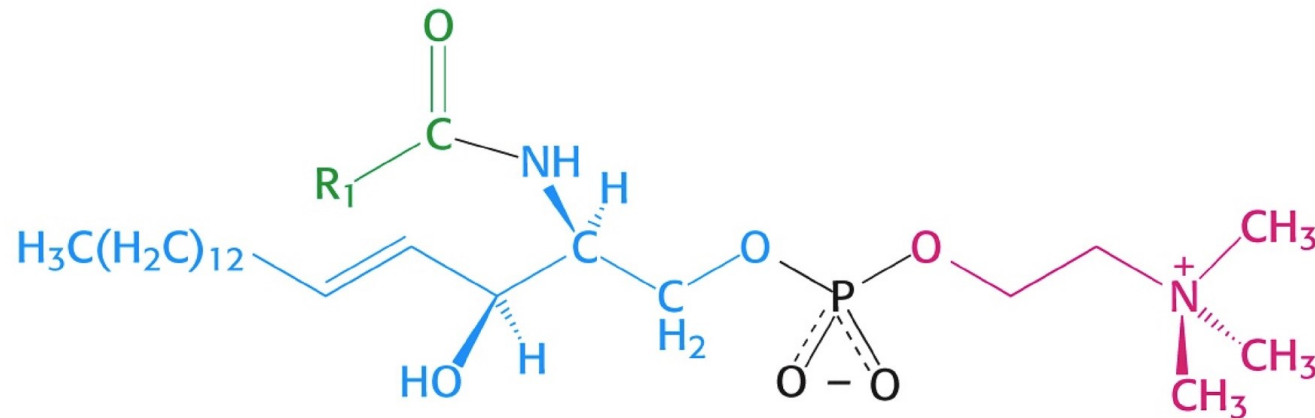
Phospho-glycerides



Sterols, e.g. cholesterol (Chol)

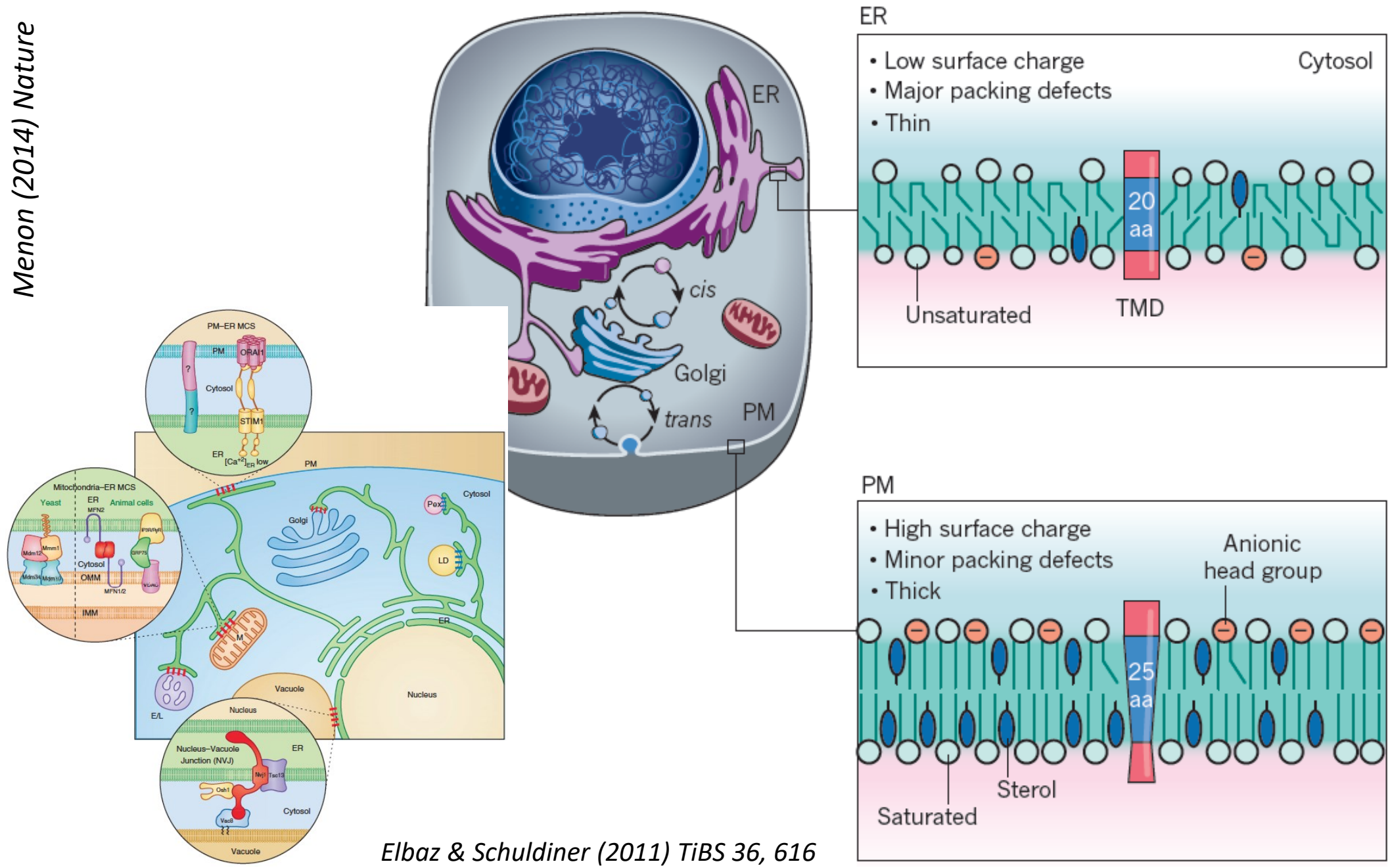


Phospho -sphingolipids, e.g. sphingomyelin (SM)



Transfersal lipid asymmetry in Eukaryotic membranes

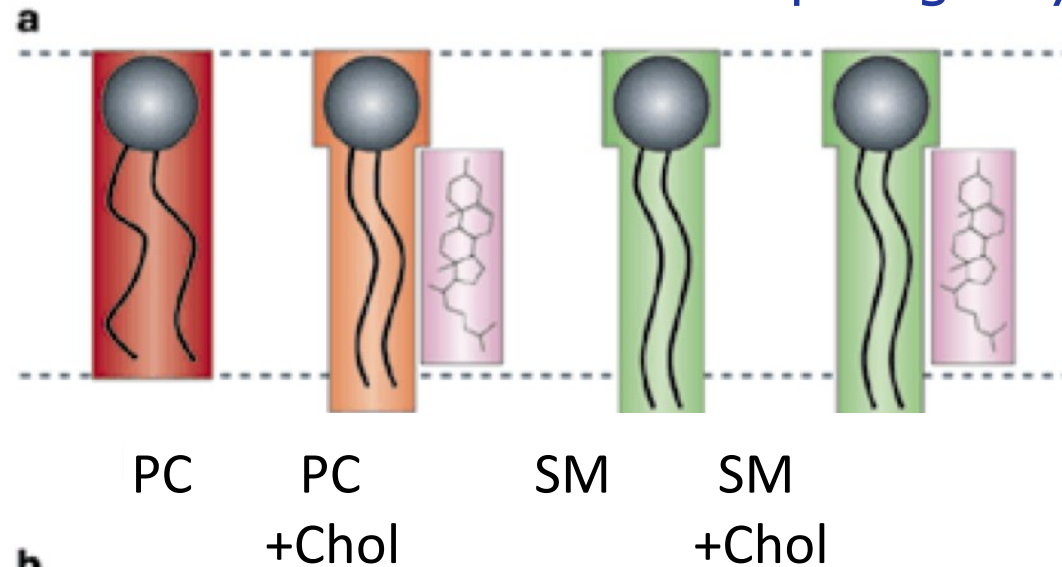
Menon (2014) Nature



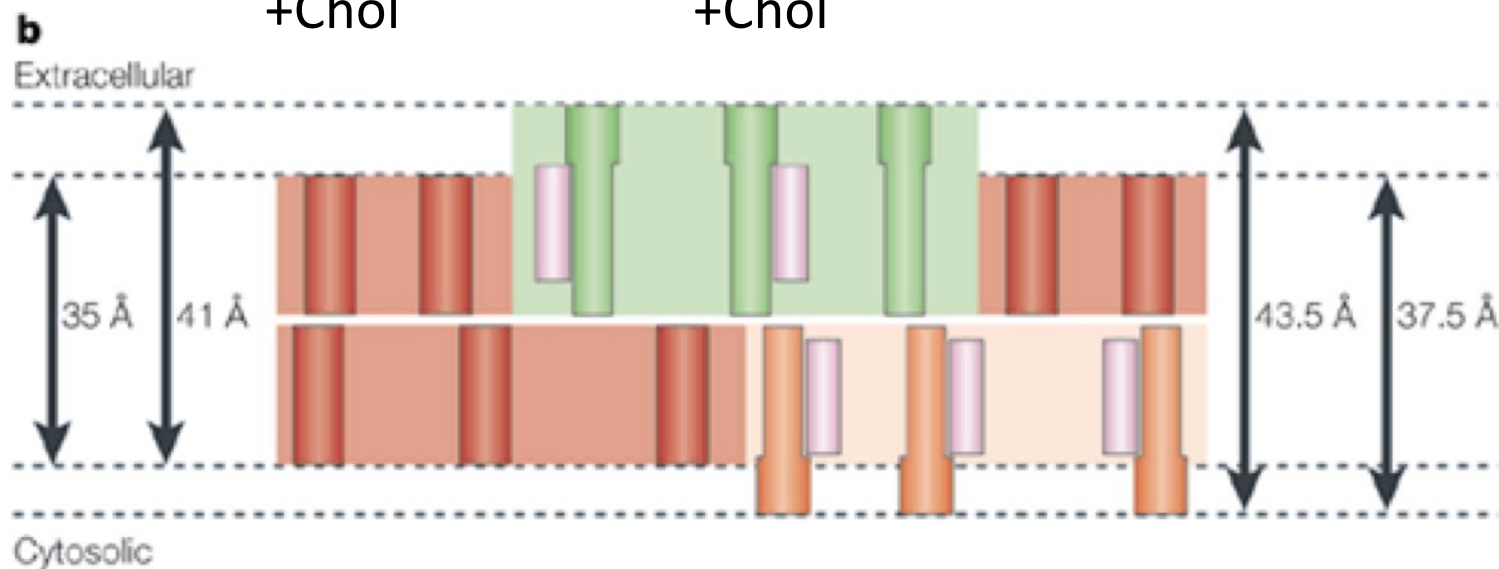
Elbaz & Schuldiner (2011) TiBS 36, 616

Lateral lipid asymmetry and phase-coexistence : Plasma membrane

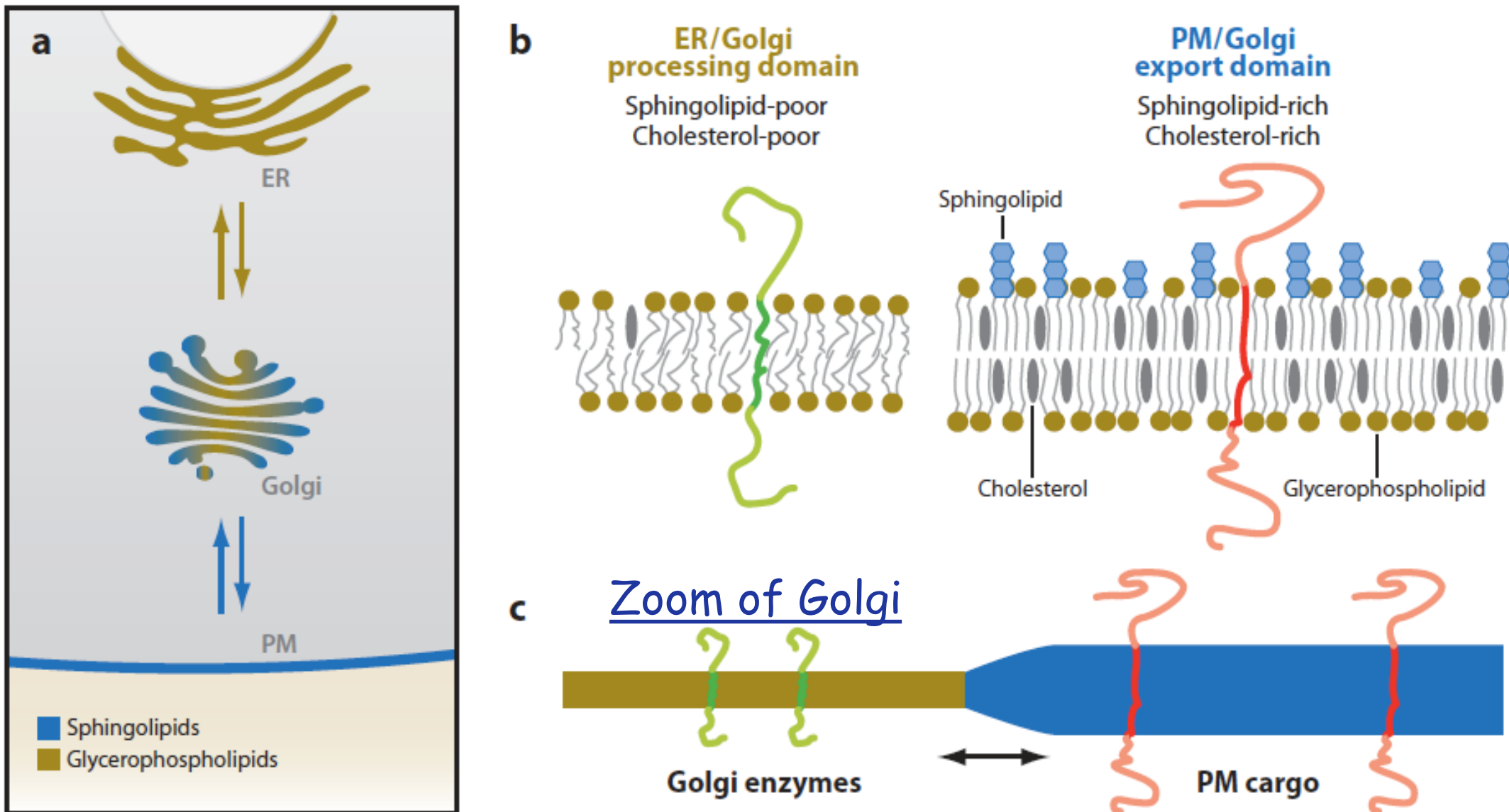
- Cholesterol: Preference for sphingomyelin and not so “fluid” lipids



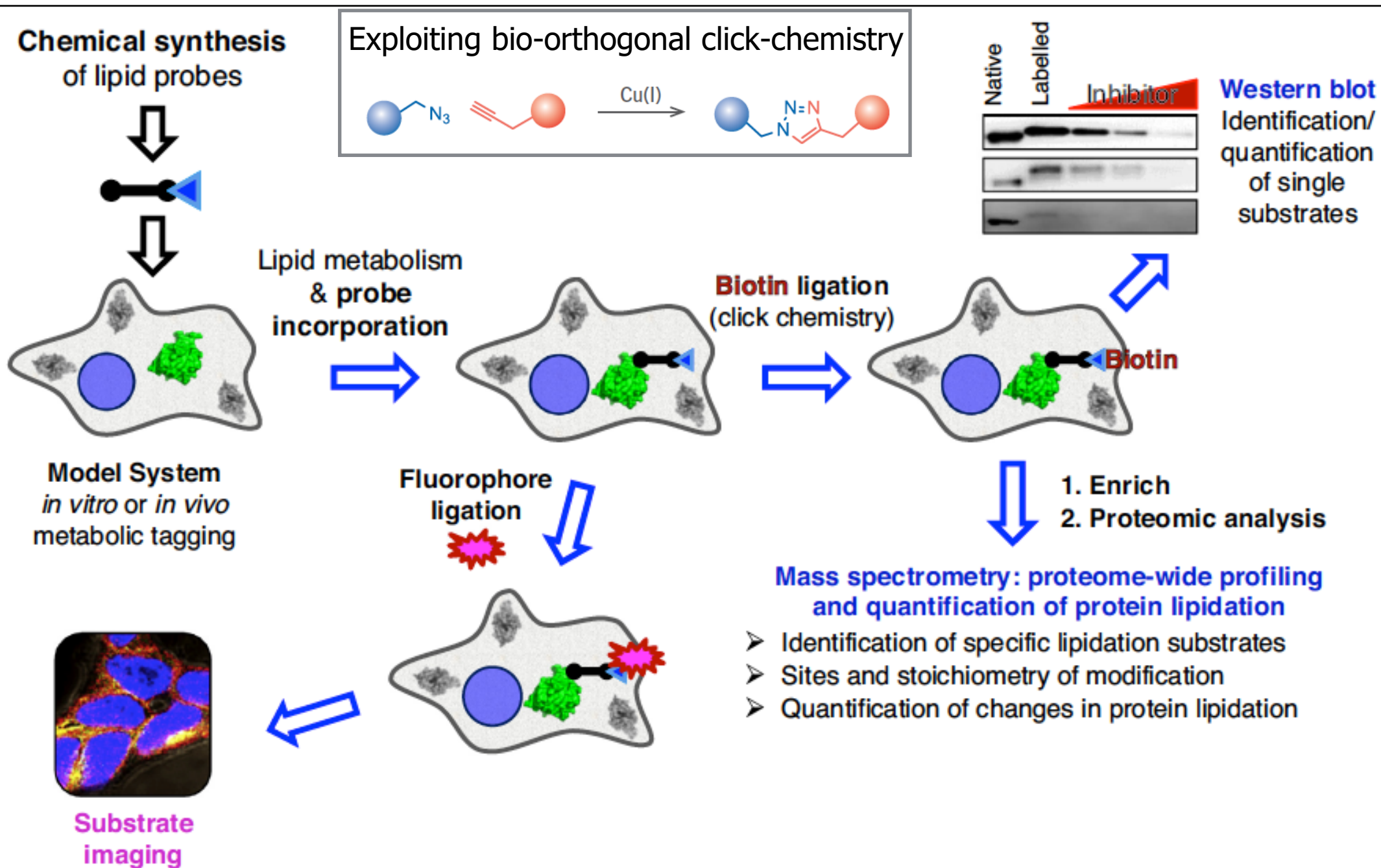
“Cholesterol domains”:
- membrane thickening
- fluidity decrease



Phase separations facilitate sorting and transport

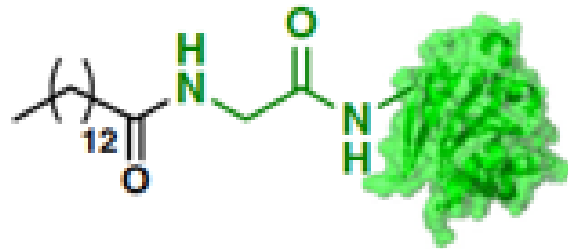


Identifying & localising lipidated proteins



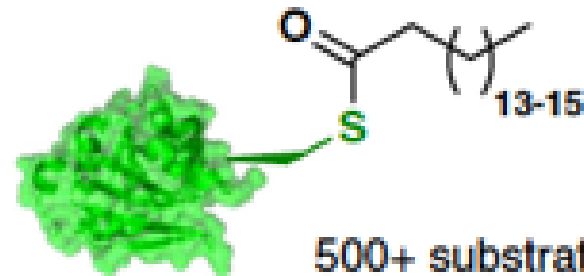
Lipidation of proteins

N-terminal N-myristoylation

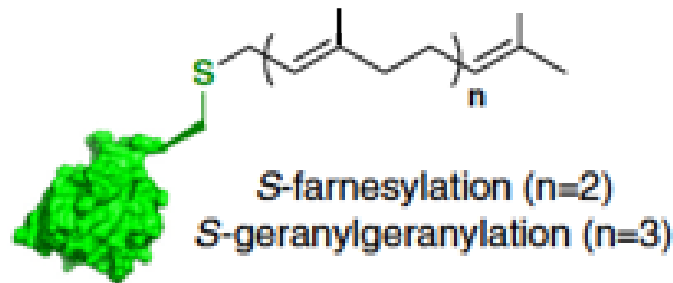


100+ substrates
in humans

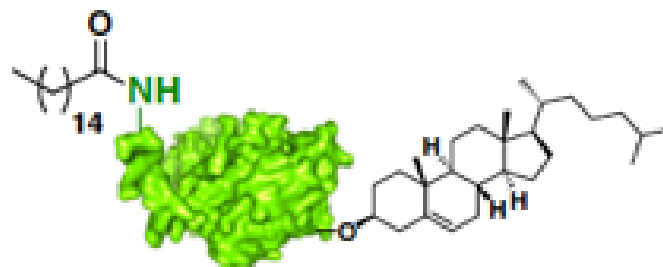
Cysteine S-acylation



500+ substrates
in humans

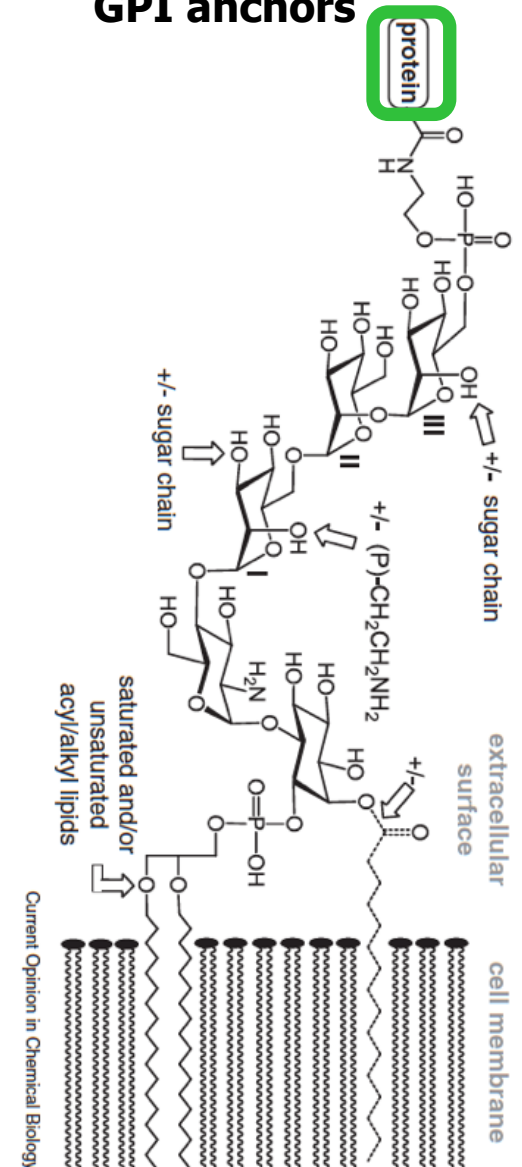


60+ Rabs, 70+ other
substrates (Ras, Rho, etc.)



Hedgehog O-cholesterylation

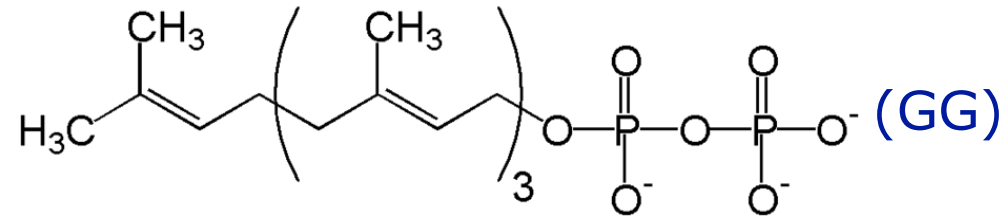
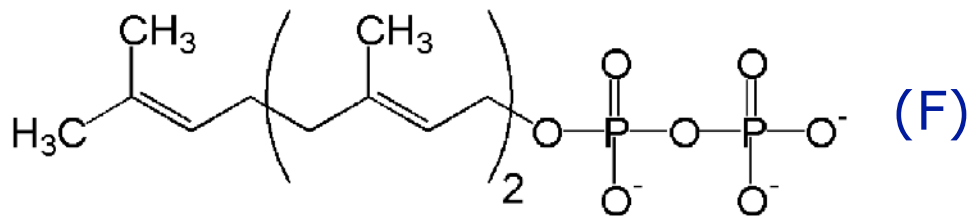
GPI anchors



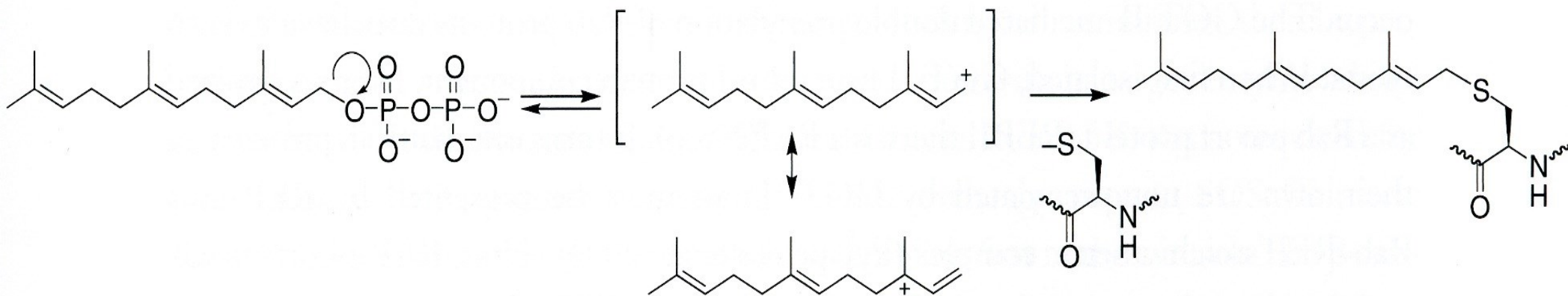
Current Opinion in Chemical Biology

S-Prenylation

Conjugation of farnasyl-(F) or geranylgeranyl- (GG)pyrophosphate precursors by the enzymes protein F-transferase or protein GG-transferase



Allyl cation reacts with thiolate to form a stable thioether



÷ About 1% of the proteome is prenylated

÷ These proteins are involved in cell growth, differentiation, transport and motion

S-Prenylation close to the C-terminus

Post-translational addition to Cys residues at the C-terminus:

÷ Motif 1: -**C**-a-a-X a = aliphatic

=> *Geranylgeranylation* if X = Leu

=> *Farnesylation* if X = Ala, Ser

NB: upon farnesylation a-a-X are proteolytically removed

÷ Motif 2: 2 Cys in a short amino acid motifs, e.g.

-**C**-•-**C**, -•-•-**C**-**C**, -•-**C**-•-**C**, -**C**-**C**-•-•

=> *Geranylgeranylation* of one or both Cys-residues

N-Myristoylation at the N-terminus

Co-translational addition of a myristate (C14:0) through formation of a stable **amide bond** to amino group of **N-terminal glycine**

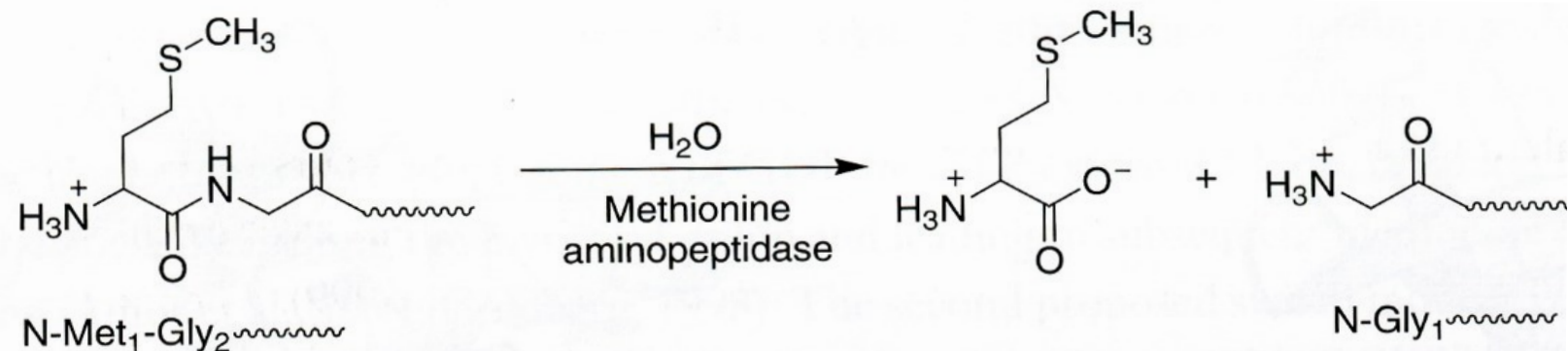
if N-terminus is : $\text{H}_2\text{N-Met-Gly-x-x-x-[Ser/Thr]-y-y}$ x: small & uncharged

y: basic

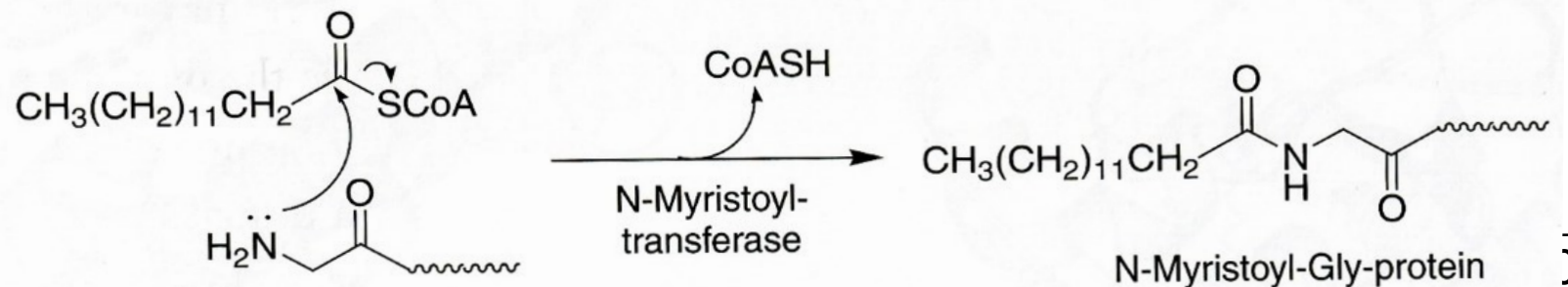
Enzyme: myristoyl-CoA:protein N-myristoyl transferase

Substrate: myristoyl-CoA

Step 1:
removal of Met



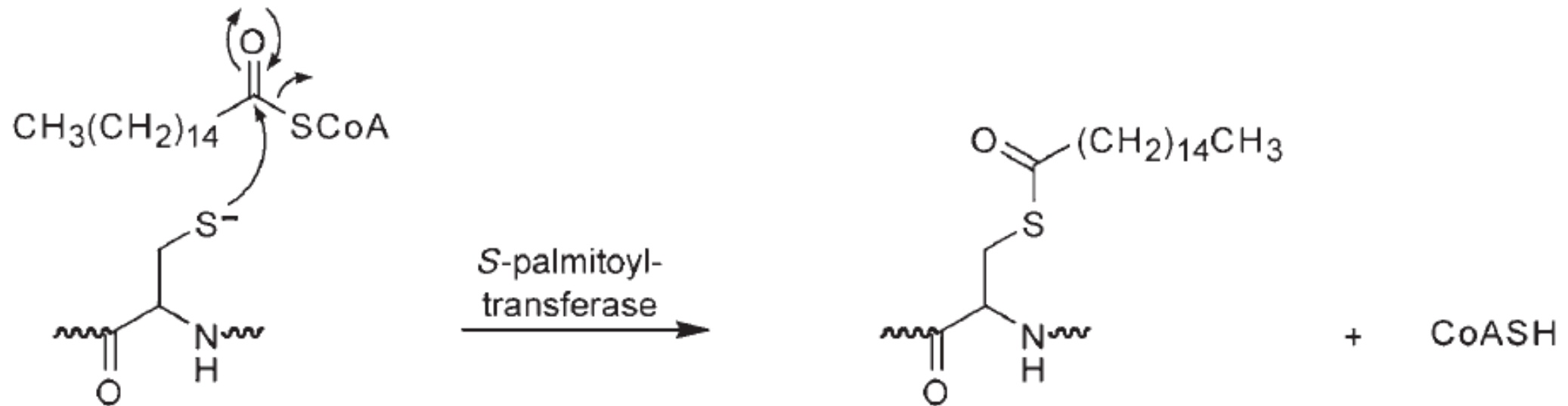
Step 2:
addition C14:0



S-Palmitoylation

Addition of a palmitate (C16:0) via of a labile thioester bond

Post-translational attachment of a C16:0 to thiol group of a Cys within the endoplasmic reticulum or Golgi system.



- Targets:
- Intrinsic membrane proteins
Cys a few (3-6) residues next to a TM domain on cytosolic side
 - Soluble proteins

=> No clear consensus sequence,
but often close to myristoylation or prenylation site

S-Palmitoylation : To be or not anymore to be

Palmitoylation is *dynamic and reversible*

=> regulation of location, sorting and activity

- *Acylation:*

=> protein-S-acyltransferase (PAT) in ER and Golgi

- *De-acylation:*

=> S-acylprotein thioesterase (APT) in cytosol

=> Palmitoyl protein thioesterase (PPT) in lysosomes,
 plasma membrane

Lipidation of proteins

Summary

GPI anchor	C-term	co-trans	“perm”	raft & cell surface
S-prenylation	C-term	post-trans	perm	non-raft
N-myristoylation	N-term	co-trans	perm	raft
S-palmitoylation		post-trans	reversible	raft

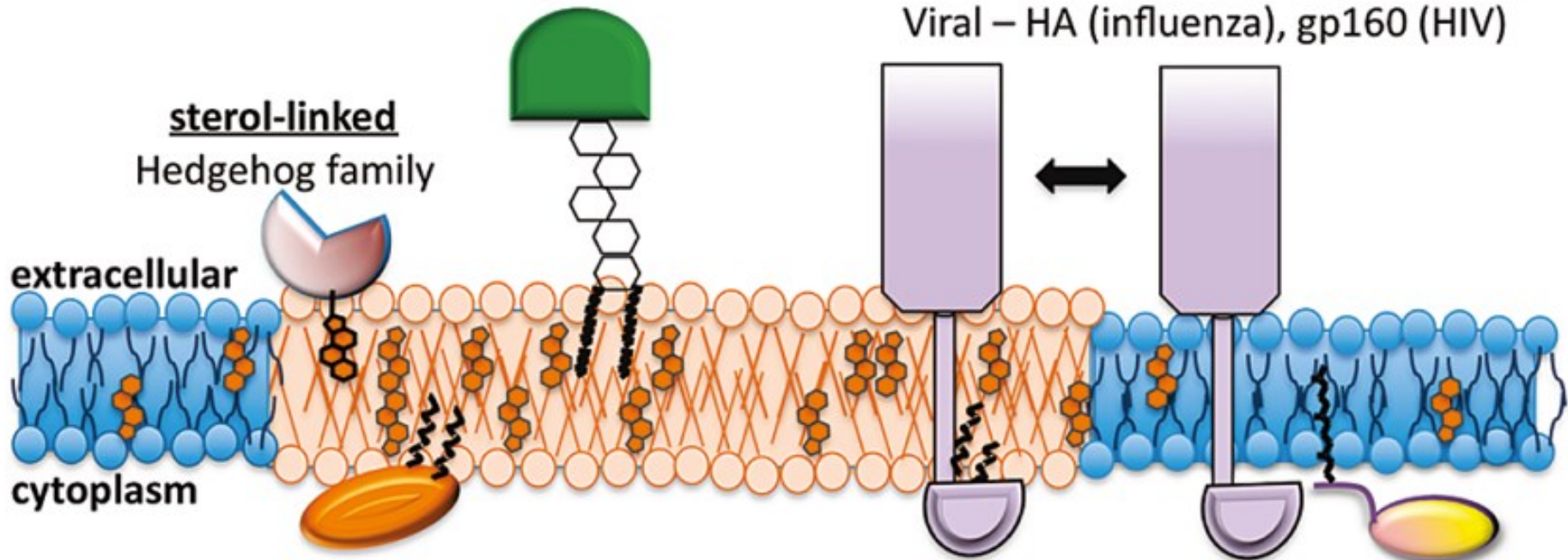
Lipidation rules location of proteins in membrane

GPI-anchored

enzymes – PLAP, 5' nucleotidase
receptors – FR, GDNFR, FcR γ III, CD14
adhesion – NCAM, CD58

sterol-linked

Hedgehog family



Palmitoyl-dependent raft partitioning TM

Immune signaling – LAT, CD4, CD8, FcR γ IIa
Viral – HA (influenza), gp160 (HIV)

Palmitoylated intracellular

Src-family kinases
H-ras
G protein α subunits

Prenylated

G protein $\beta\gamma$
K-ras4B
Rab family

RAFT

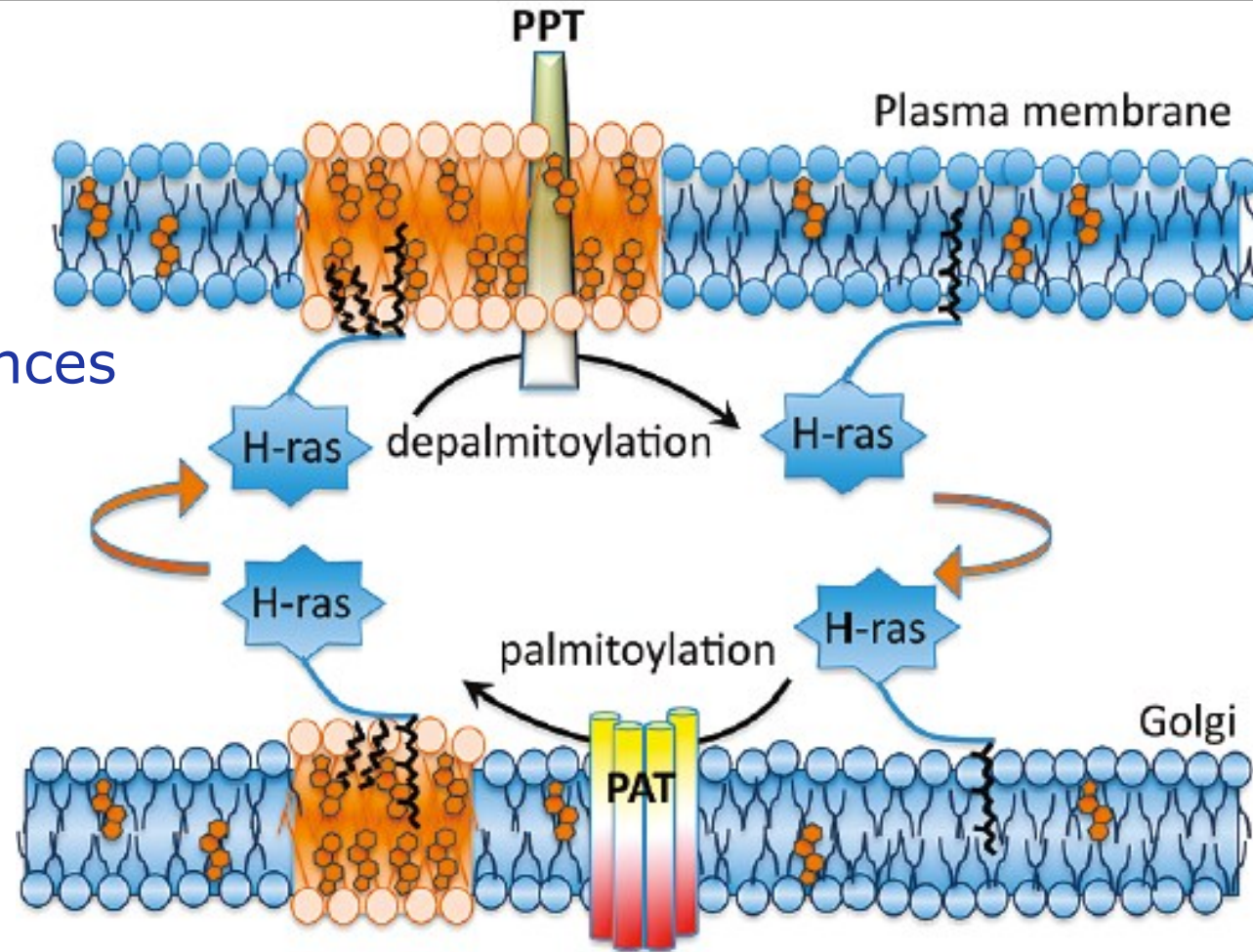
NON-RAFT

An example : H-*ras*

H-*ras*:

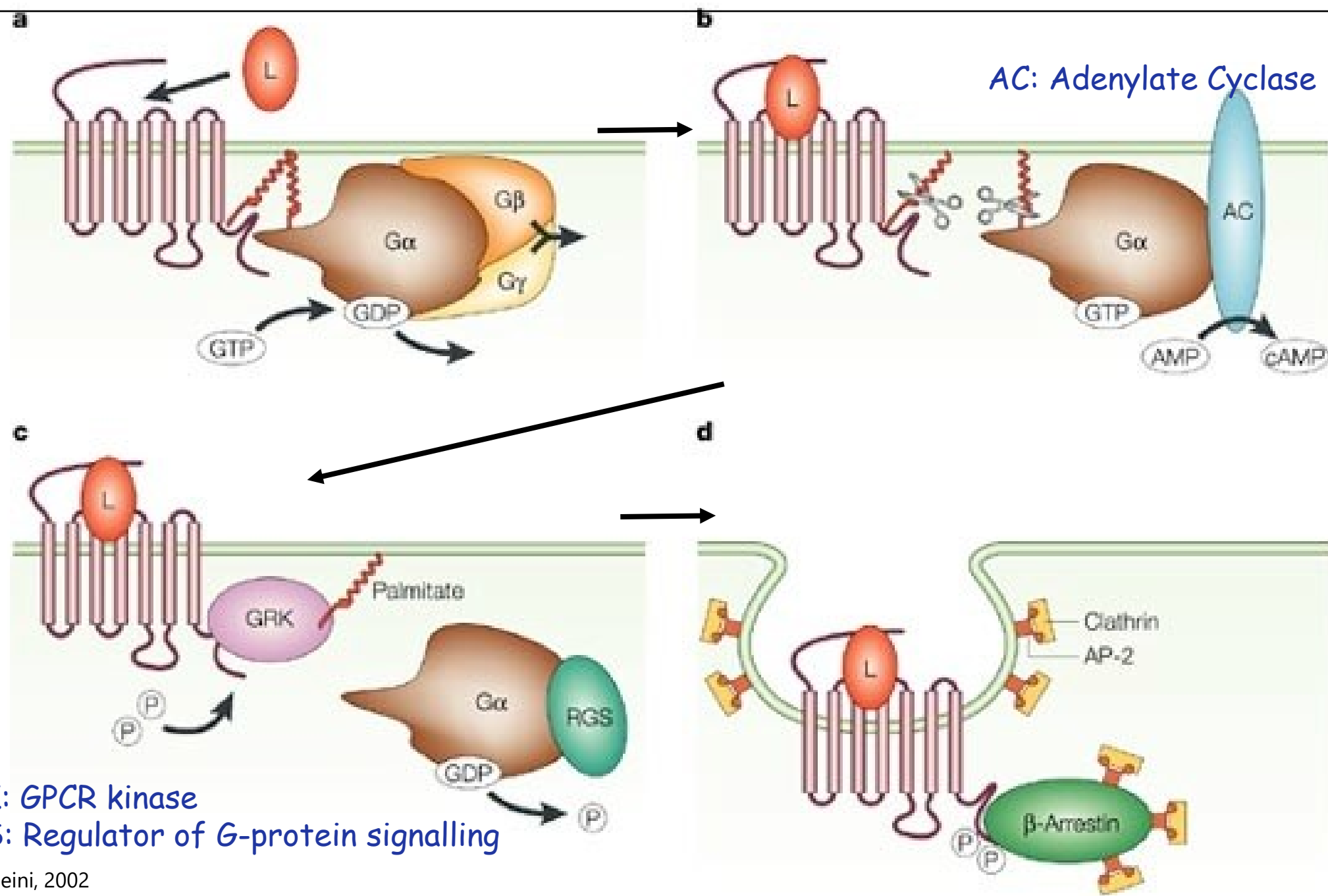
- farnesylated
- 2 palmitoylation sites

=> H-*ras* activation enhances de-palmitoylation



- T-lymphocytes reaction upon antigen presence:
 - i - strong antigens => negative selection => DEATH :: ras in PM
 - ii - weak antigens => positive selection => proliferation :: ras in ER

Dynamic palmitoylation : Modulating GPCR signalling

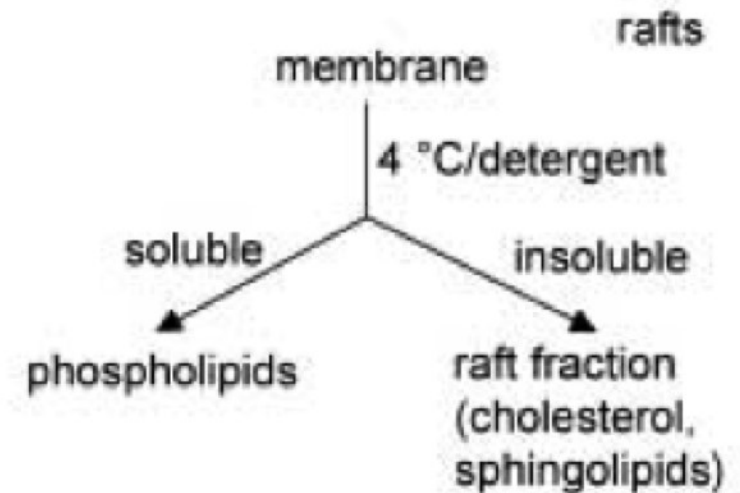
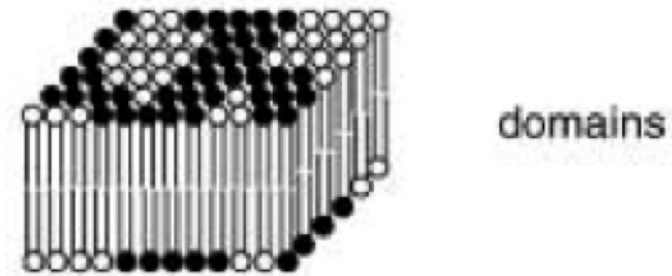


El-Husseini, 2002

Biological lateral phase separations : Rafts

Historically, lipid rafts have been defined as :

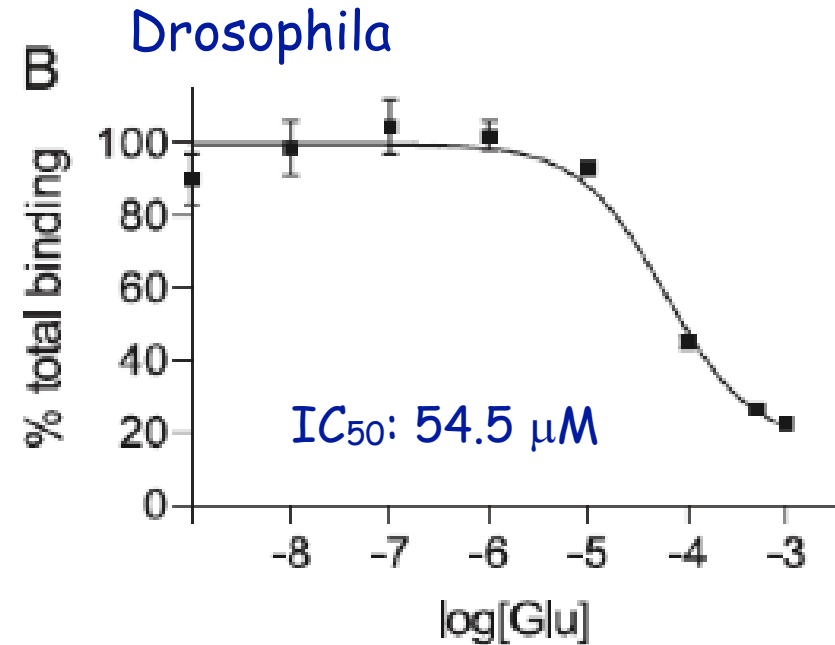
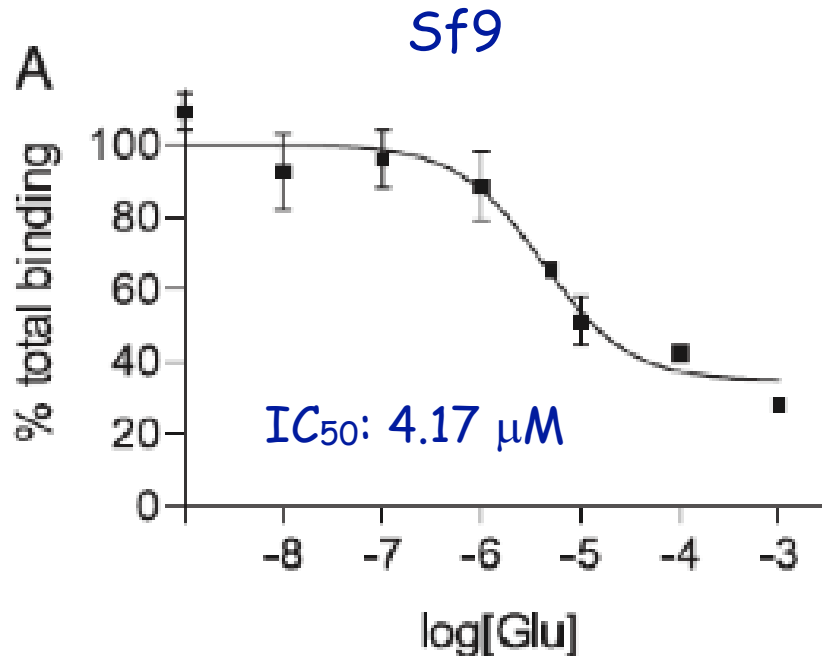
- insoluble in cold 1%-solution of Triton X-100, a detergent.
=> thus named: **D**etergent-**R**esistant **M**embrane (DRM)
- low buoyant density



Case 1: Modulation of GPCR signalling by lipids domains

E.g., a metabotropic glutamate receptor (mGlu-R) expressed in Sf9 insect cells or Drosophila eyes

- Radioligand competition binding assays :



=> higher affinity in Sf9

Modulation of mGlu-R “fitness” by lipid domains

- Observation: Drosophila membranes contain very little cholesterol
Q: Is cholesterol needed for high affinity?

Experiment 1:

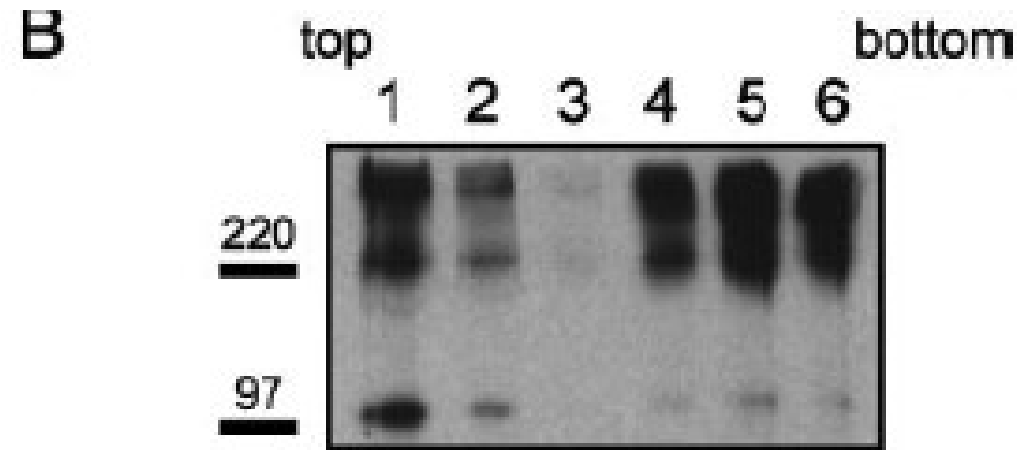
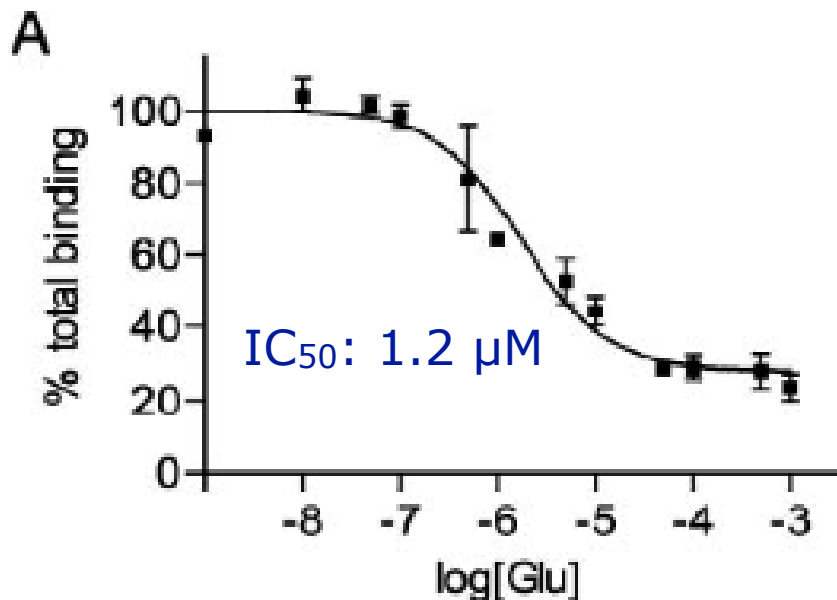
- ◇ Cholesterol **addition** to Drosophila membranes

=> receptor gains affinity

IC₅₀ 54.5 => 1.2 μ M

=> receptor goes to rafts

“light” fractions gradient



Modulation of mGlu-R signalling by lipid domains

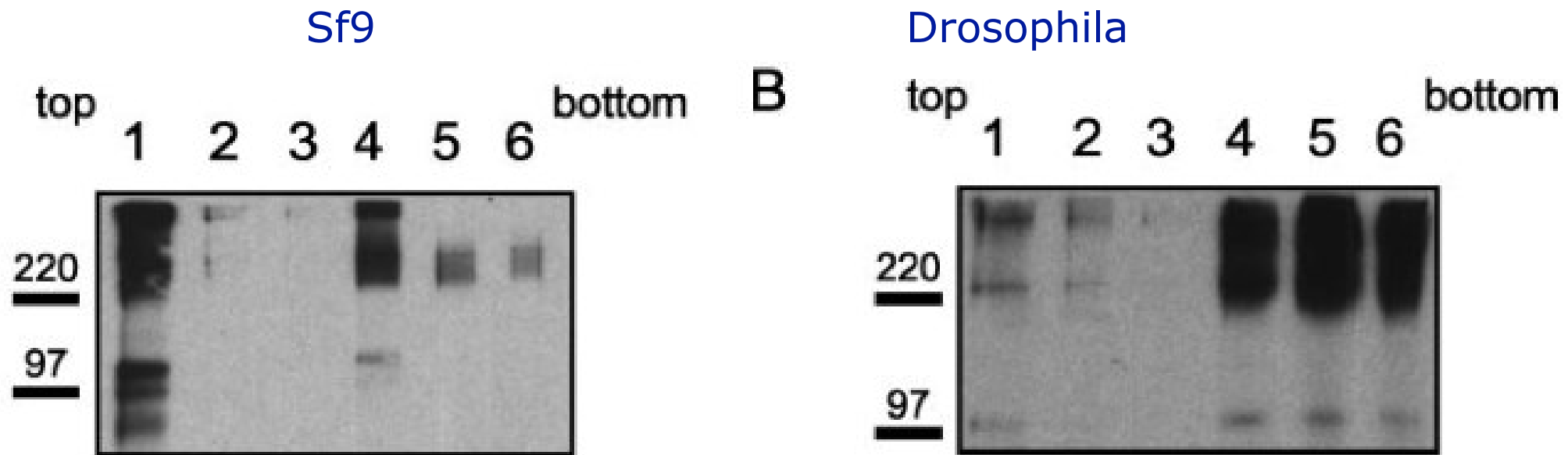
Are rafts involved ?

Sf9 insect cells

or

Drosophila eyes

- Membrane density fractionation followed by SDS-PAGE analysis :



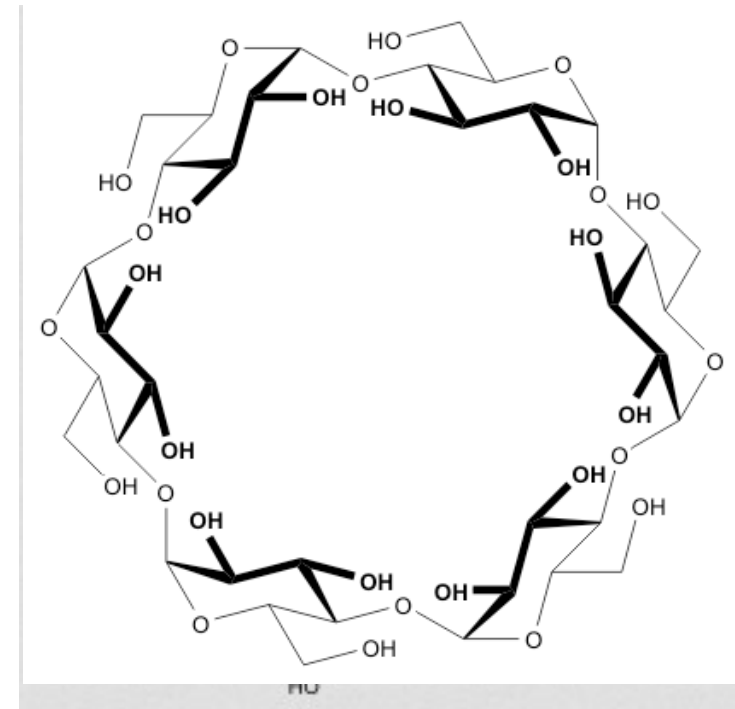
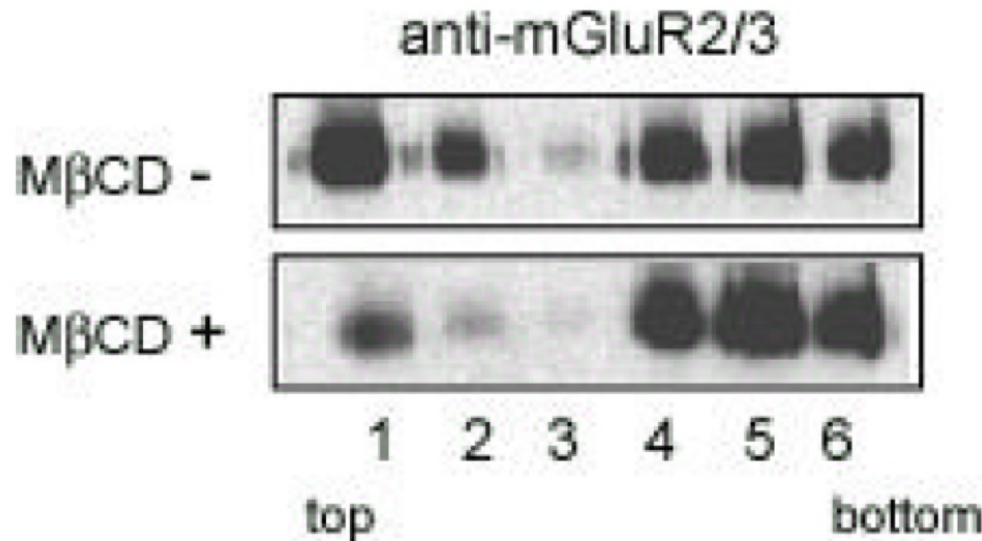
=> In Drosophila, the receptor is present only in the heavy fractions

=> In Sf9 the receptor is present mainly in the light raft fraction, rich in cholesterol and sphingomyelin

Modulation of mGlu-R “fitness” by lipid domains

Experiment 2:

◇ Cholesterol **depletion** in Sf9 by methyl- β -cyclodextrin (m β CD)



=> receptor goes to heavy membranes upon m β CD treatment

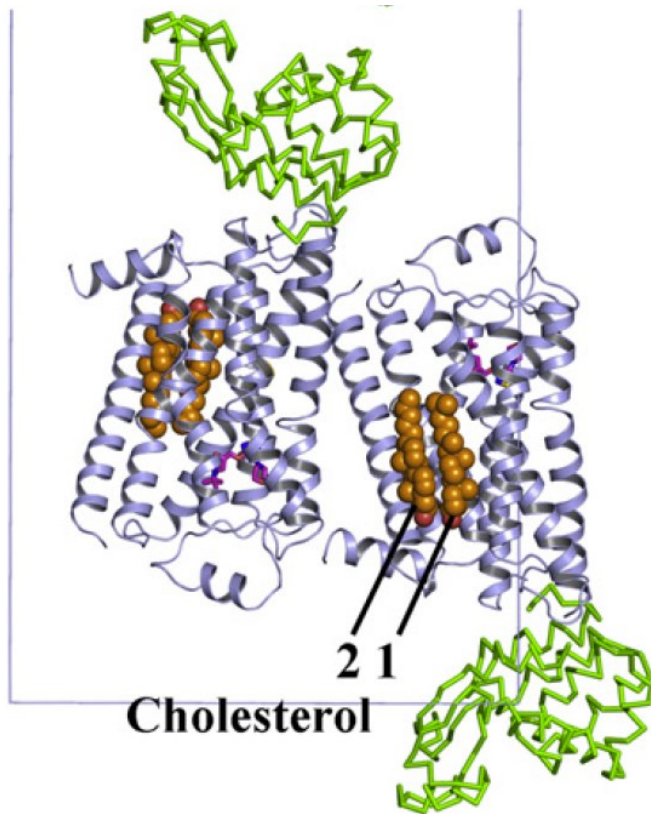
=> Yes, cholesterol is needed for both high affinity & raft localisation !

Cholesterol binding site on GPCRs

Crystal structure of the 2 β -adrenergic receptor

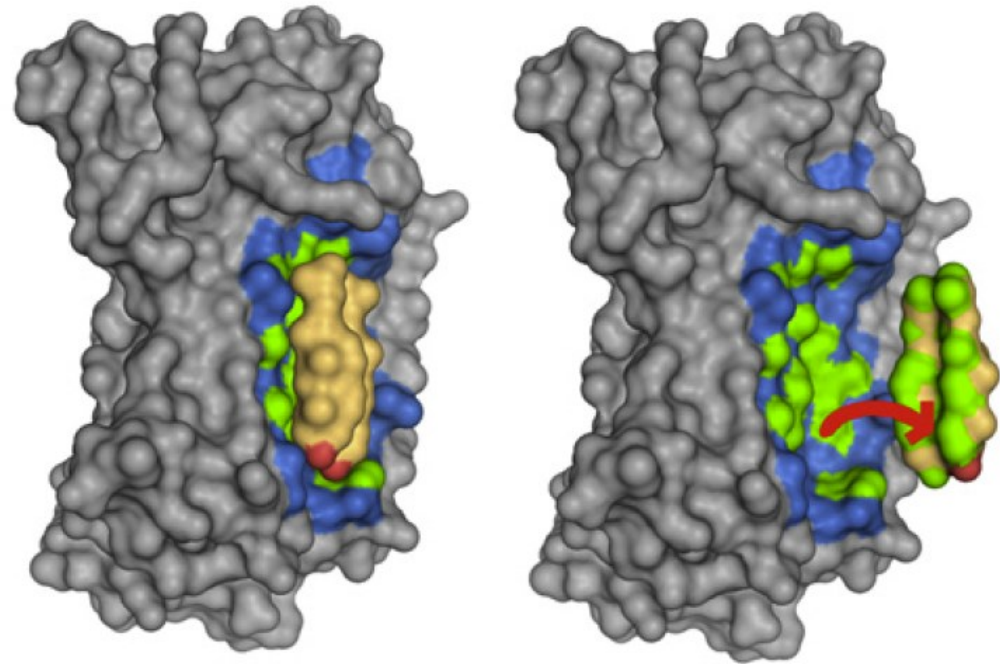
=> 2 cholesterol molecules / receptor within the protein structure

Unit cell



Green : T4 lysozyme

Cholesterol 1 docks on receptor



Brown
green
blue

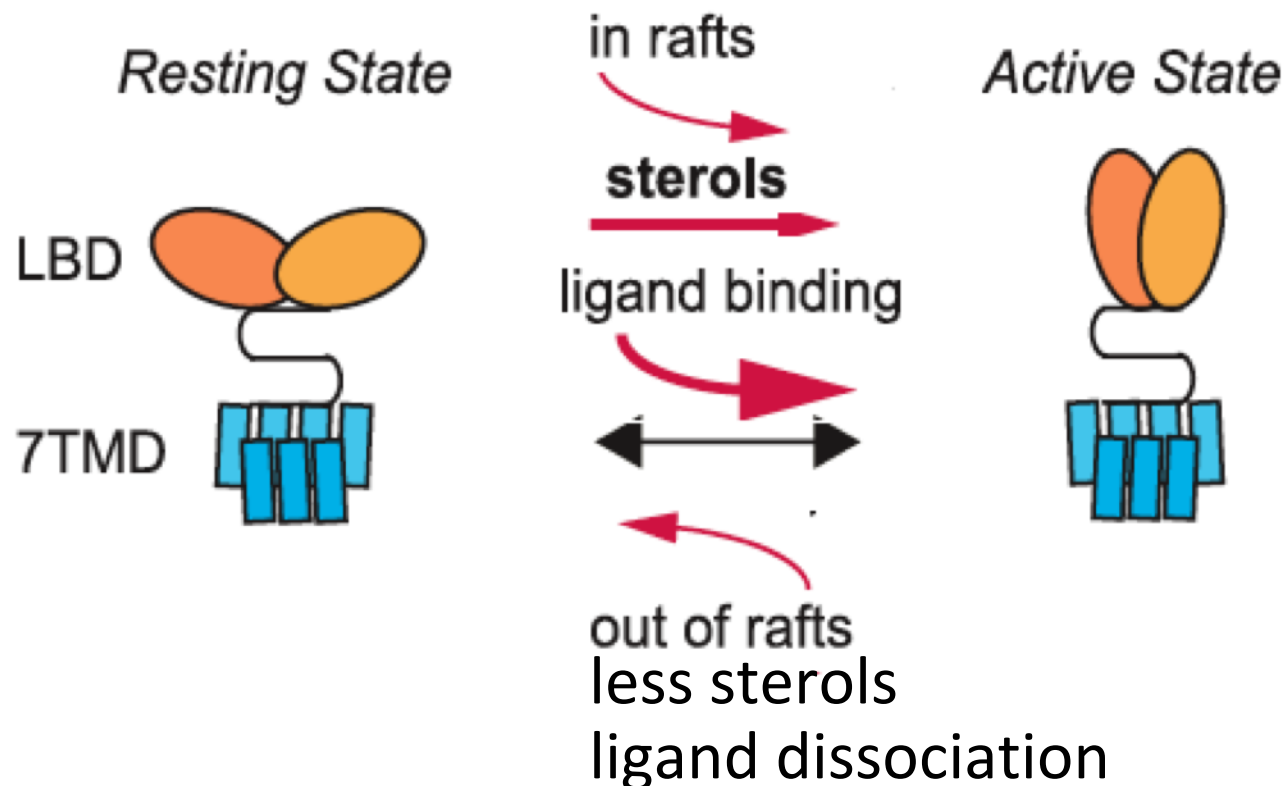
Cholesterol 1
 $d < 4\text{\AA}$
 $4\text{\AA} < d < 5\text{\AA}$

Hanson Nature 2008

Modulation of mGlu-R “fitness” by lipid domains

Model to explain effect cholesterol:

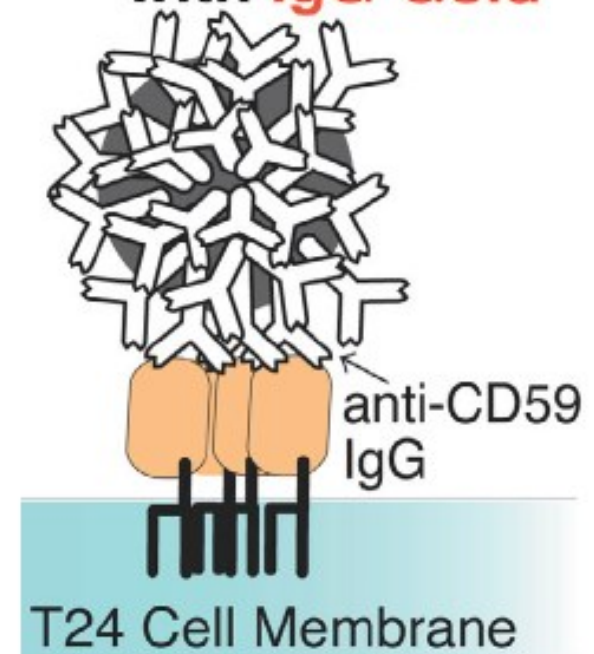
Cholesterol rich domains act as positive allosteric modulator, favouring active state that has a higher affinity.



Case 2 : Signaling cascade of CD59

- CD59 is a GPI anchored receptor

CD59 Clusters
Crosslinking
with **IgG-Gold**

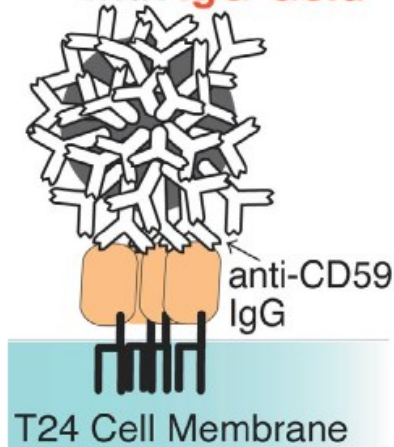


Single molecule observation of CD59 and Lyn

Observations of signalling pathway :

CD59 Clusters

Crosslinking
with IgG-Gold



Supplemental video 1

G40-crosslinked CD59 on T24 Cells

Real time replay
Scale = 1 μ m

CD59 and Lyn

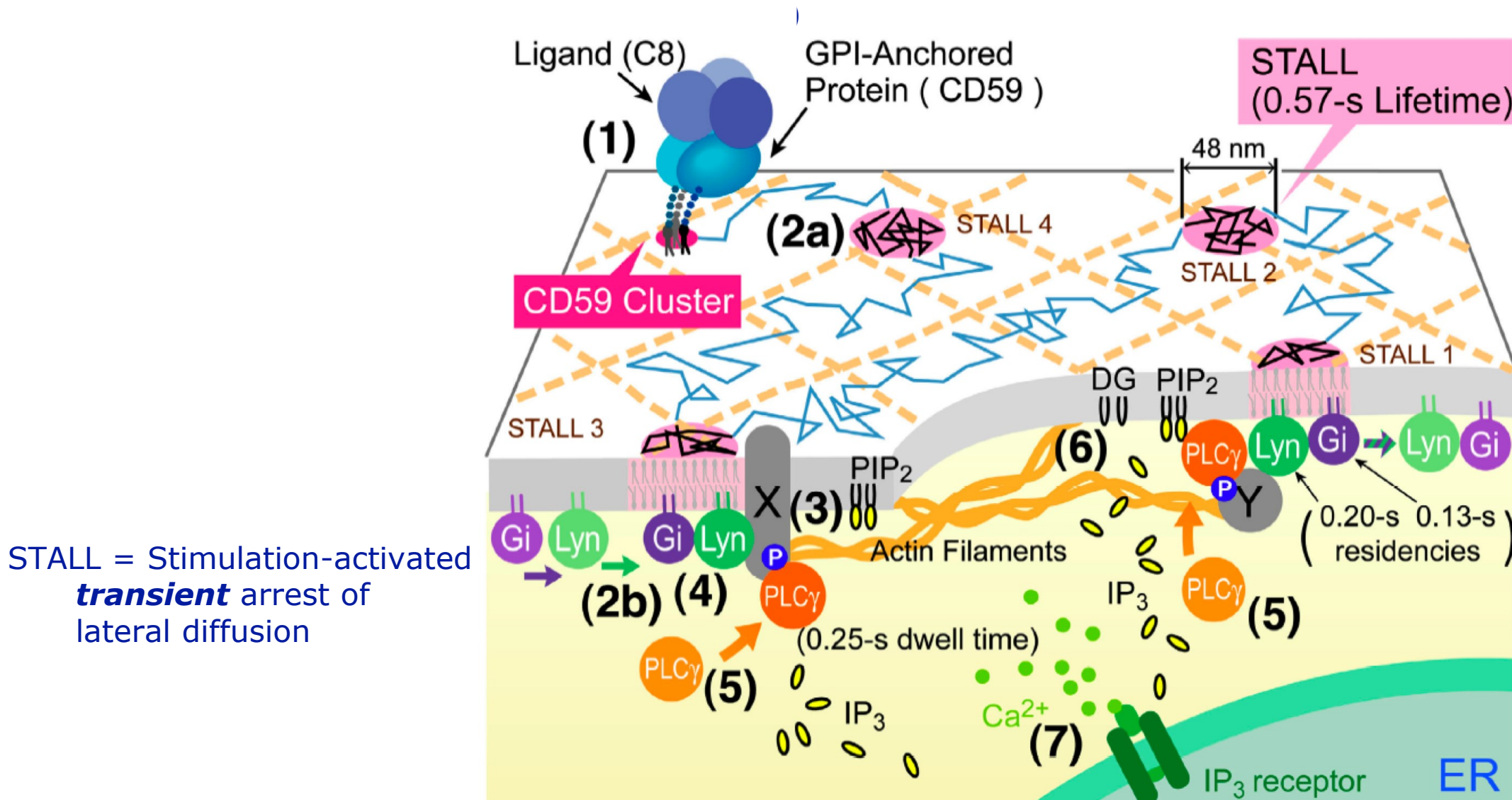
Supplemental video 2

Simultaneous observation
of a CD59 cluster and
a single Lyn-GFP molecule

Real time replay, 10 sec
Scale = 500 nm

Single-molecule imaging reveals transient interactions

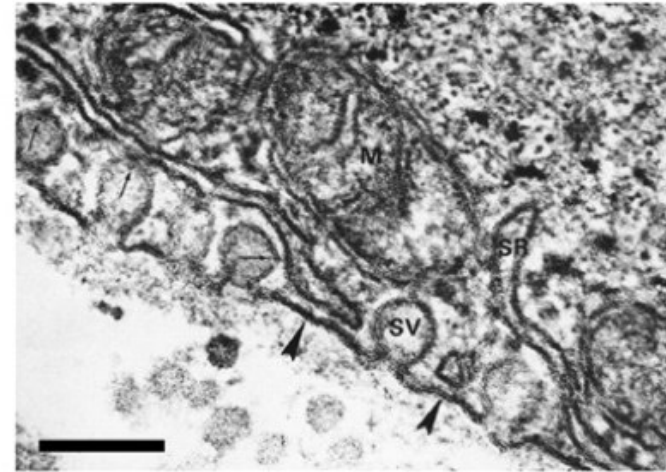
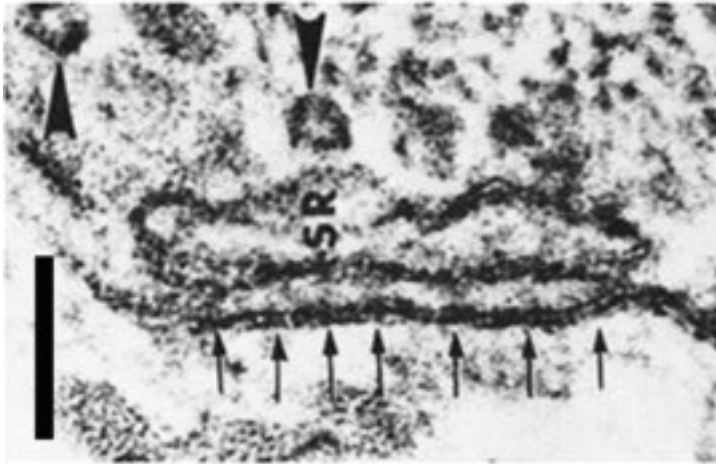
1) Clustering of the GPI-anchored receptor CD59



STALL = Stimulation-activated
transient arrest of
lateral diffusion

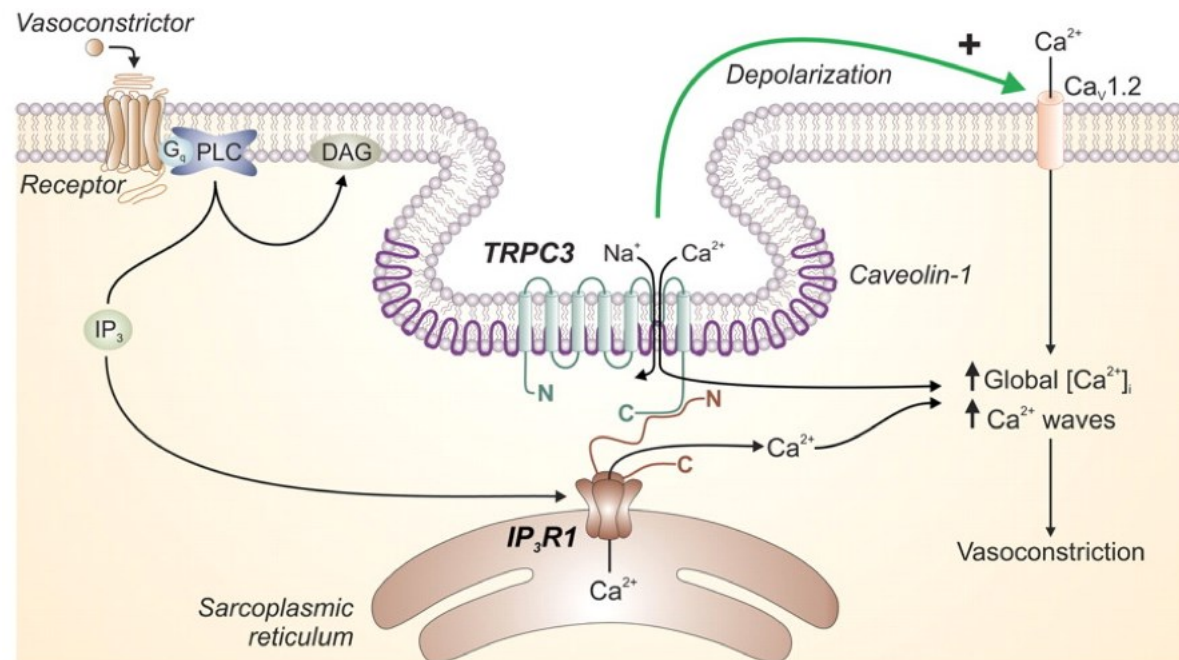
Close proximity between plasma membrane and ER

Electron microscopic image showing apposition of sarcolemma & plasma membrane (arrow head)



Scale bar : 100 nm

Schematic representation



Further reading:

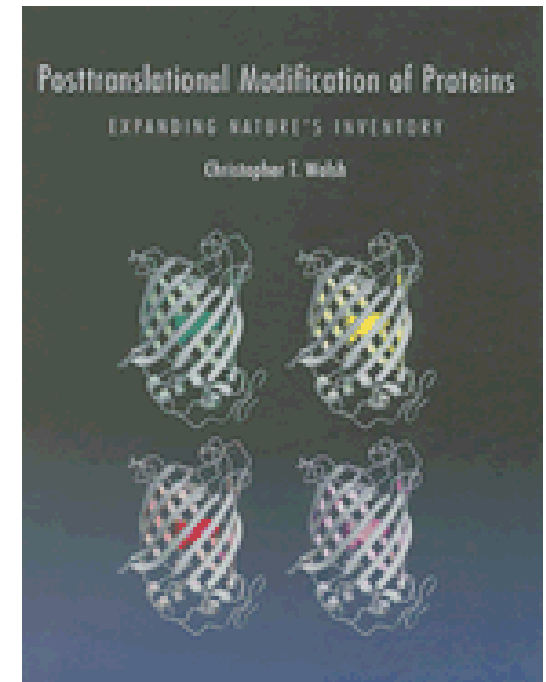
Walsh Post-translational modifications of proteins Chpt. 7

Van Meer Membranes & lipids (2008)

Yu GPI (2013)

Levental Membrane association (2010)

Tate Lipidation profiling (2014)



Protein lipidation

- *Attachment of one or several acyl chain or lipids*
 - *reversible or irreversible modifications*

=> *translocation from cytosol to membrane*

=> *location to a specific membrane or membrane domain*

=> *change in protein structure/function*

=> *change in accessibility*

◇ *Manipulation of location in space & time*

- *In general, **location rimes with activity.***

Modulation of signalling
by
lipids and membrane domains

Further reading:

Walsh Post-translational modifications of proteins Chpt. 7

Van Meer Membranes & lipids (2008)

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Levental Membrane association (2010)

Tate Lipidation profiling (2014)

