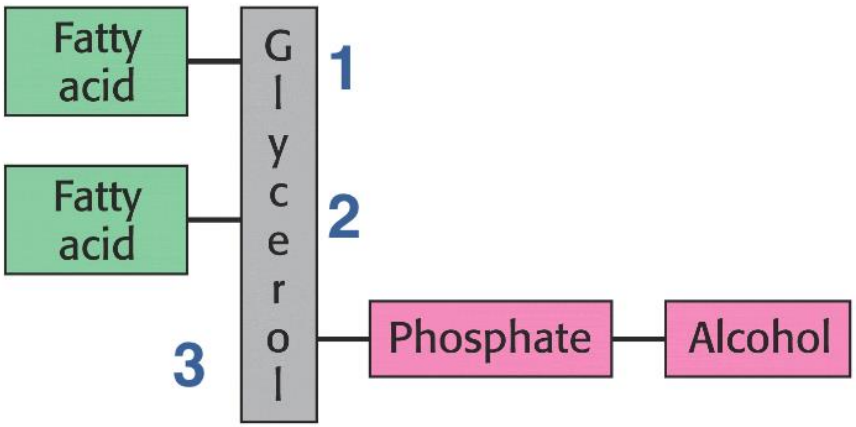
Localising proteins to and within membranes

Part II

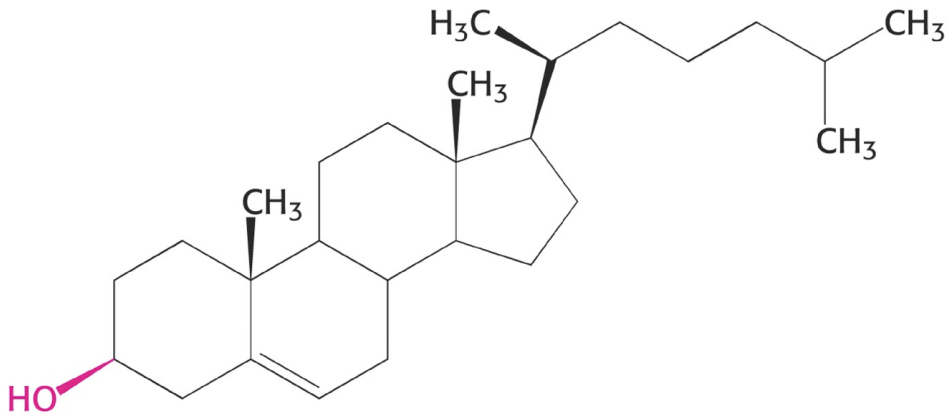
- Membranes
- Lipidation
  - GPI anchors
  - Prenylation
  - Myristoylation
  - Palmitoylation
- Modulation of signalling

Membrane lipids : Major classes

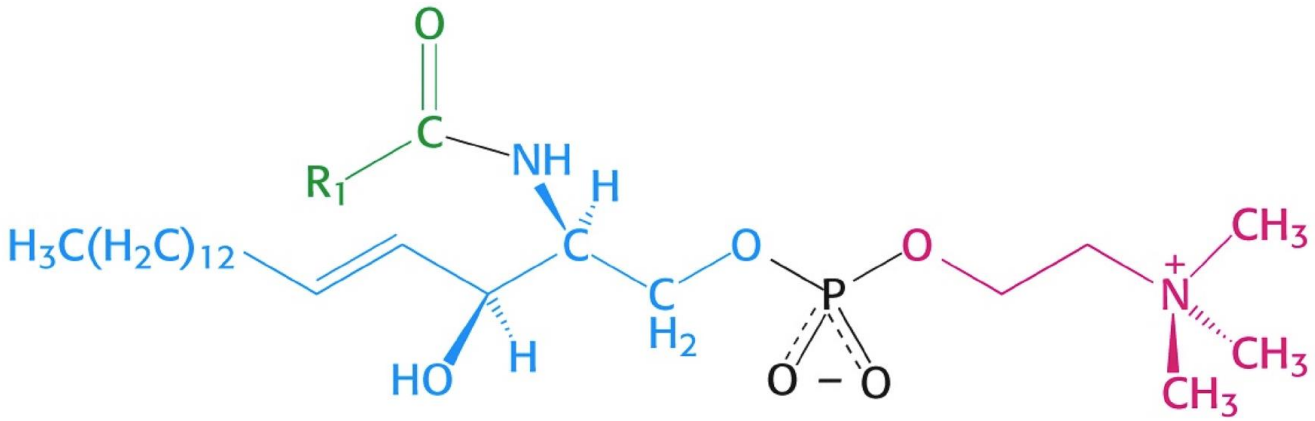
Phospho-glycerides



Sterols, e.g. cholesterol (Chol)

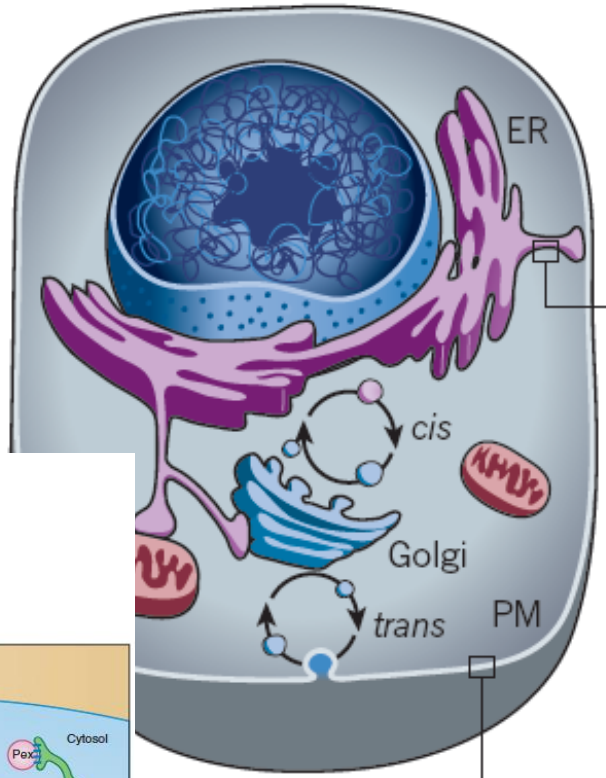


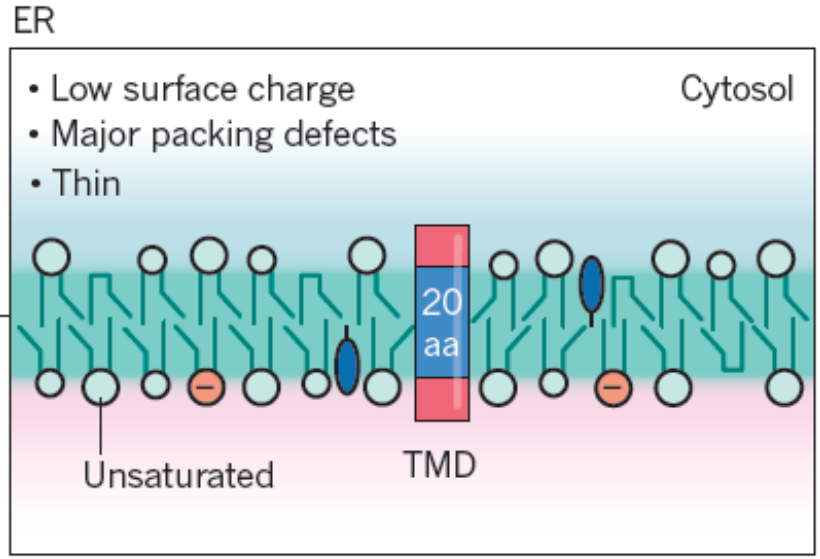
Phospho -sphingolipids, e.g. sphingomyelin (SM)

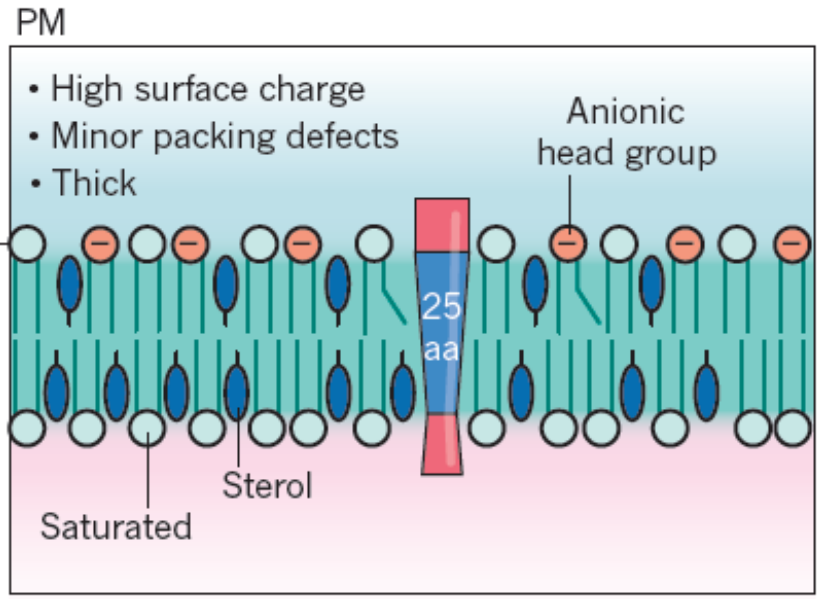


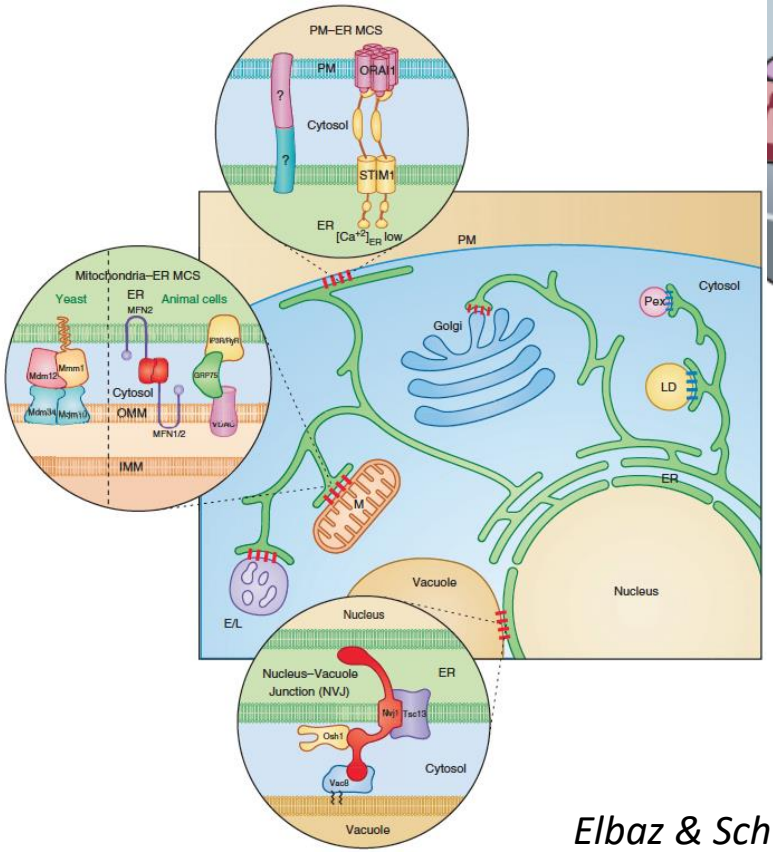
Transfersal lipid asymmetry in Eukaryotic membranes

Menon (2014) Nature





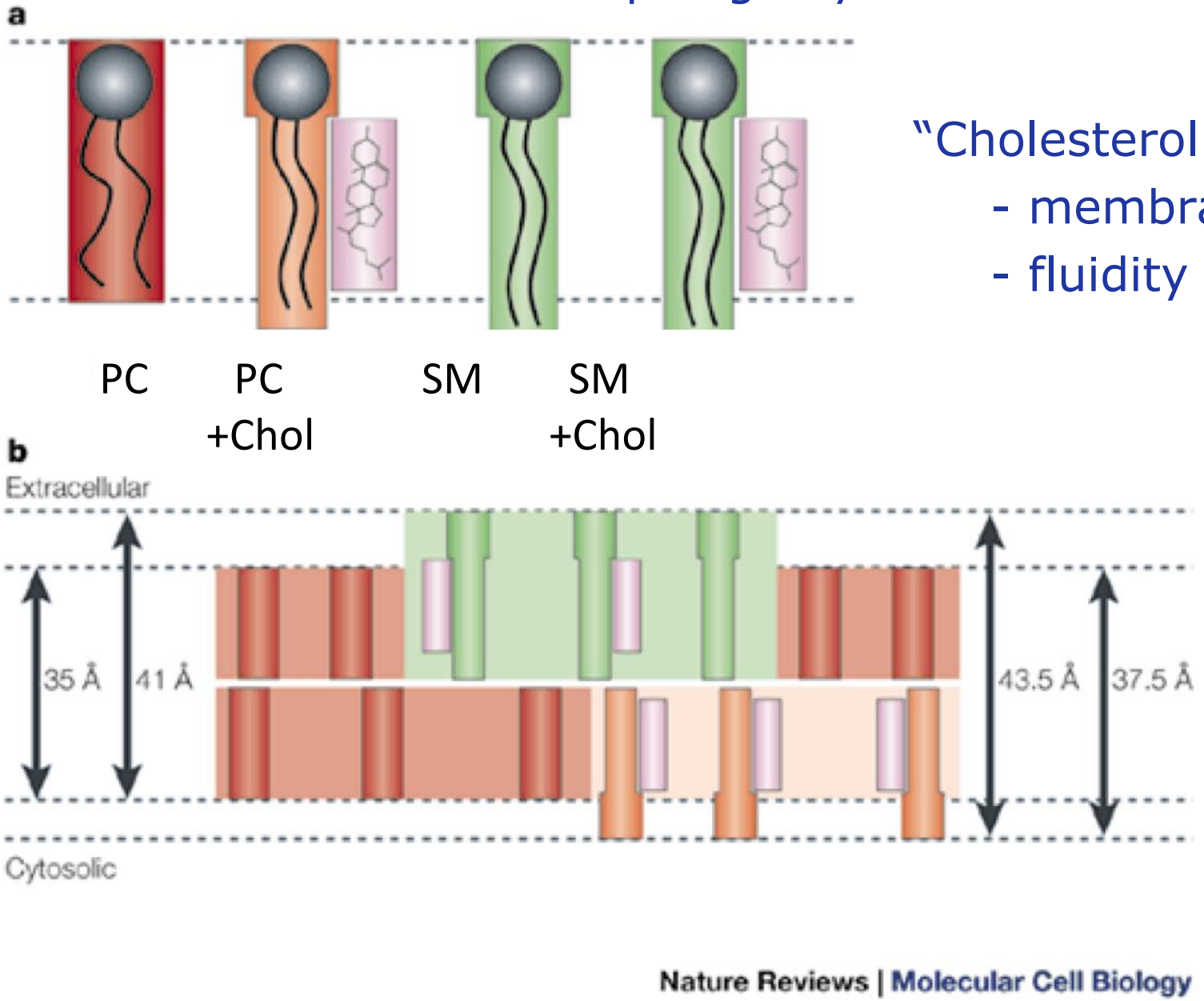




Elbaz & Schuldiner (2011) TiBS 36, 616

Lateral lipid asymmetry and phase-coexistence : Plasma membrane

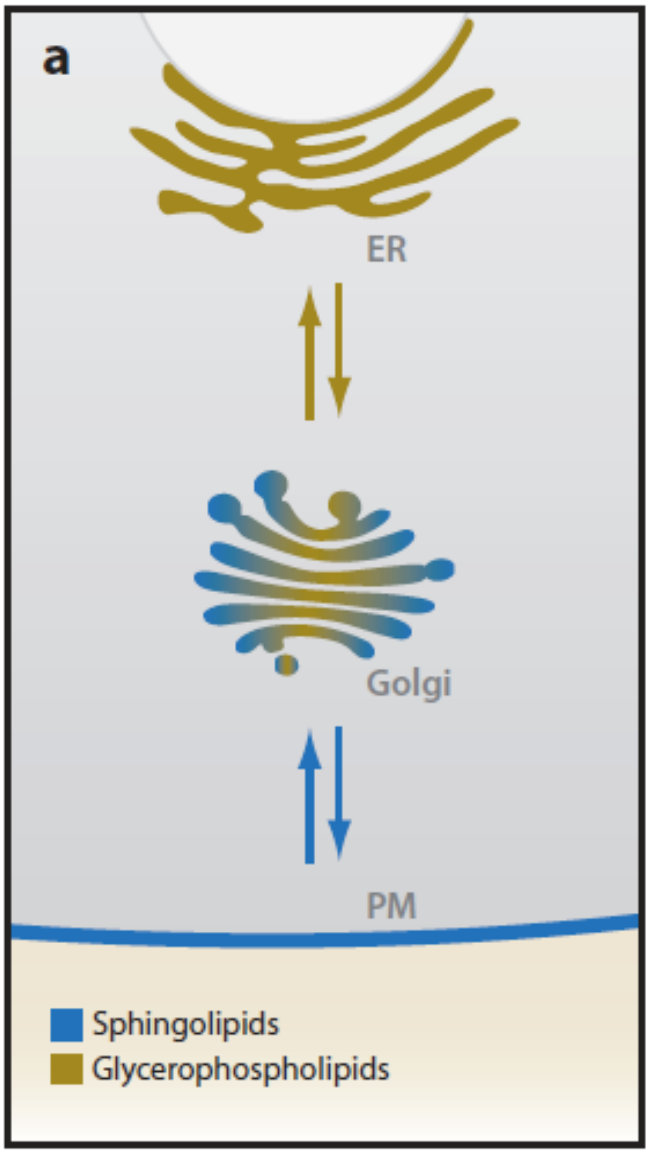
- Cholesterol: Preference for sphingomyelin and not so "fluid" lipids

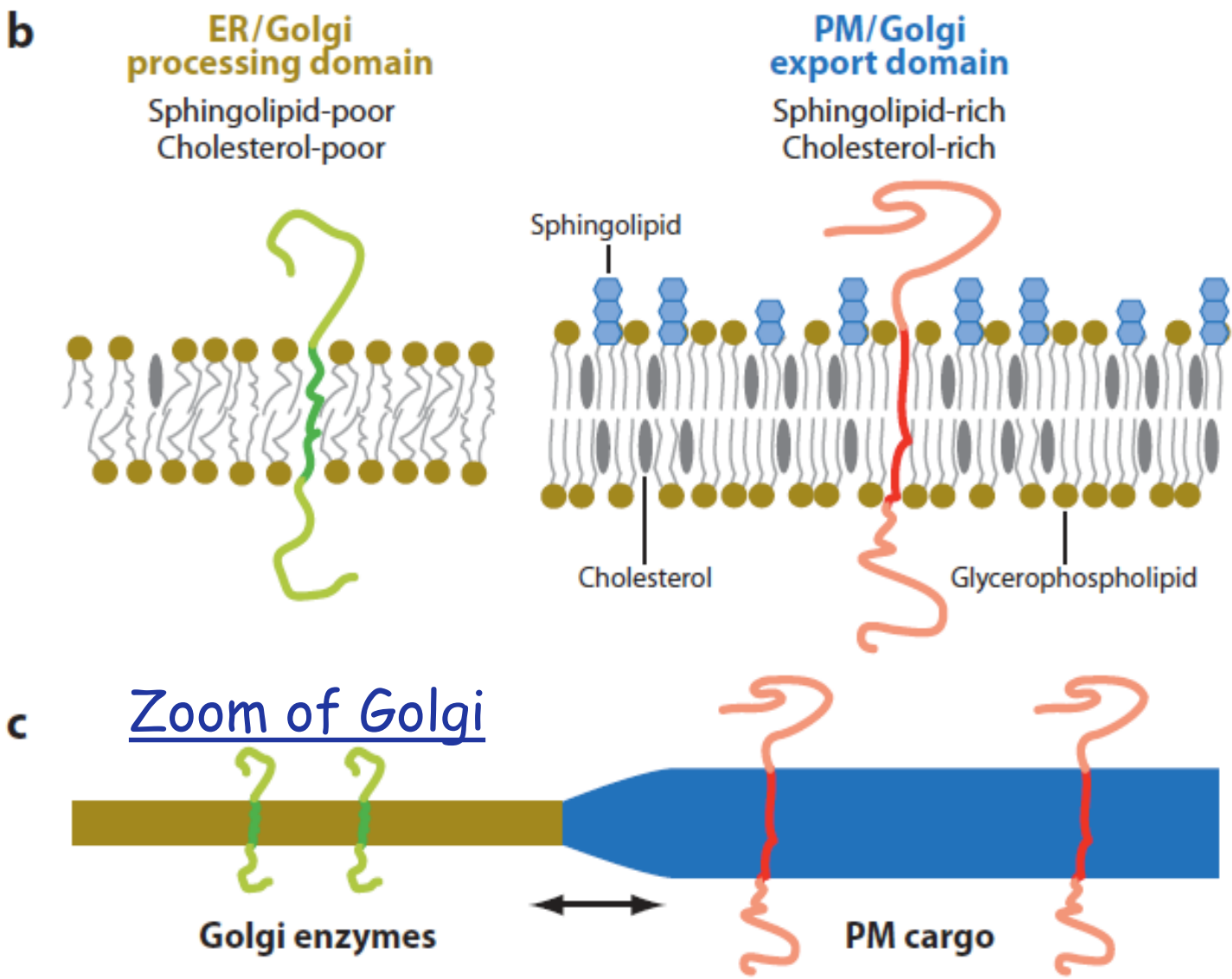


"Cholesterol domains":
- membrane thickening
- fluidity decrease

Nature Reviews | Molecular Cell Biology

Phase separations facilitate sorting and transport



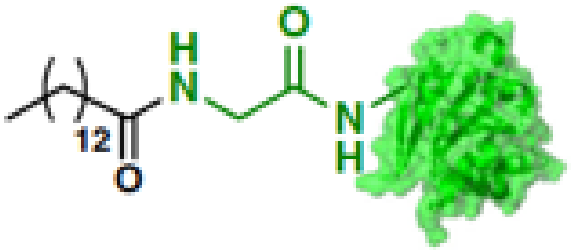


Lippincott-Schwartz, Ann Rev Biophys 2010

Lipidation of proteins

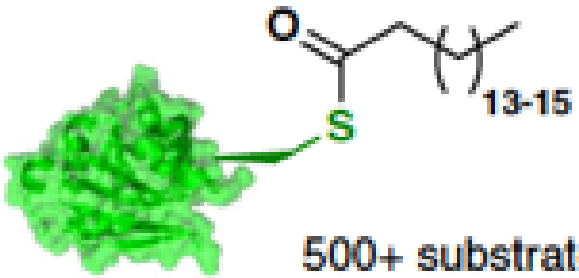
Current Opinion in Chemical Biology 2015, 24:48–57

N-terminal *N*-myristoylation

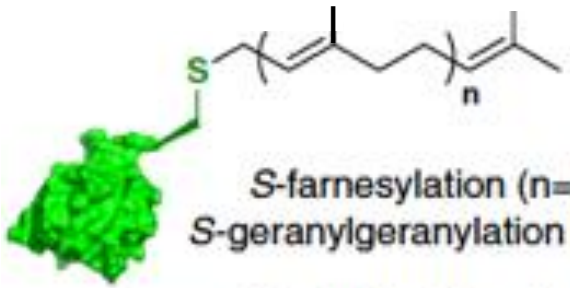


100+ substrates
in humans

Cysteine *S*-acylation

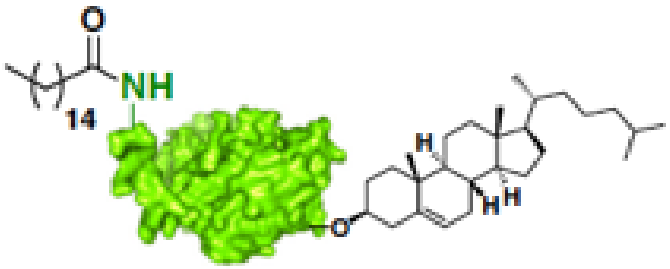


500+ substrates
in humans



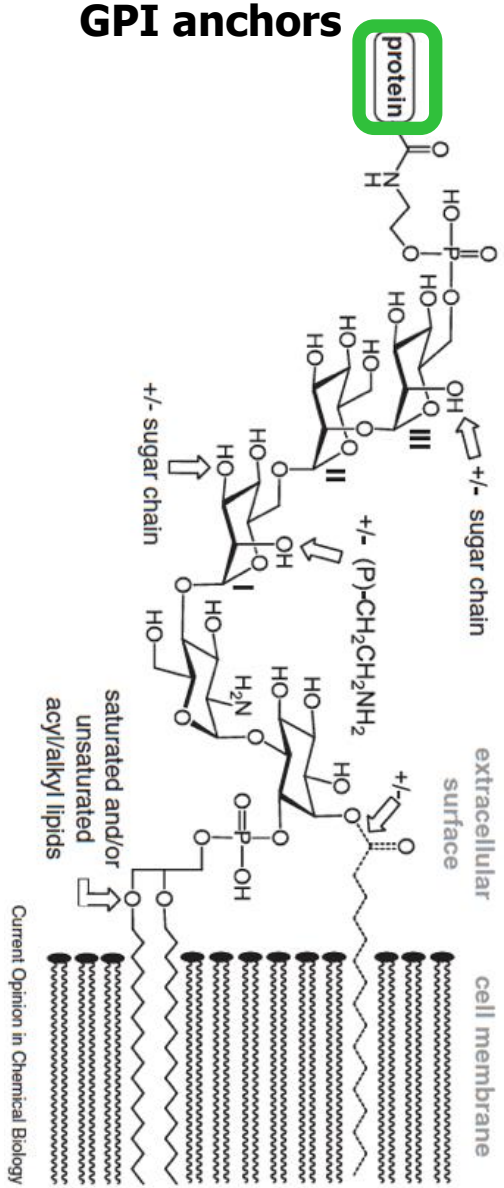
S-farnesylation (n=2)
S-geranylgeranylation (n=3)

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



Hedgehog *O*-cholesterylation

GPI anchors



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.

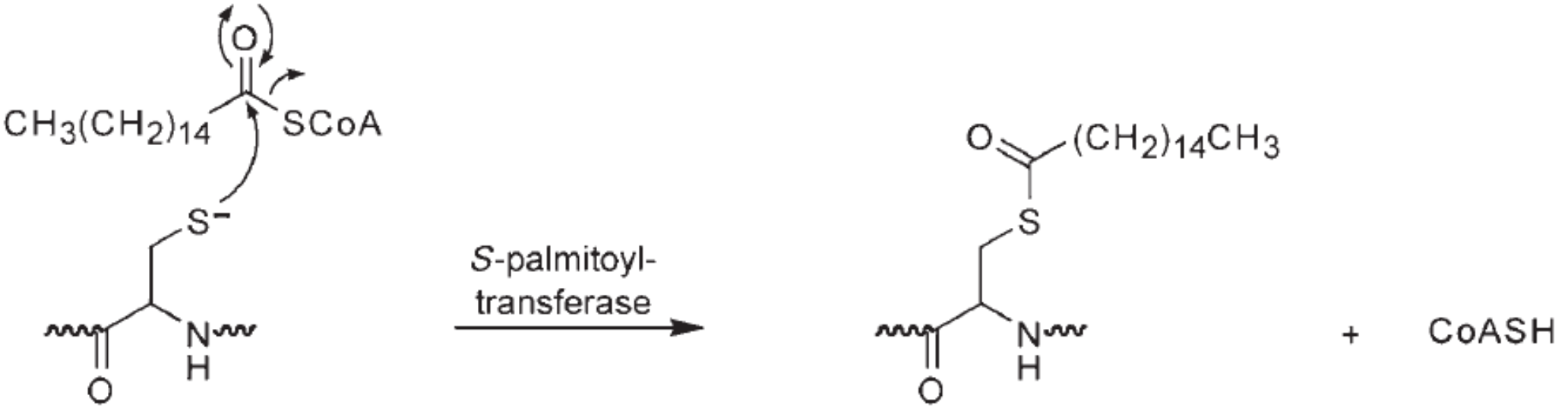
-C-•-C, **-•-•-C-C**, **-•-C-•-C**, **-C-C-•-•**

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

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* ***due to labile thioester***

=> regulation of location, sorting and activity

- *Acylation*:

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

- *De-acylation*:

  => S-acylprotein thioesterase (APT) in cytosol

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

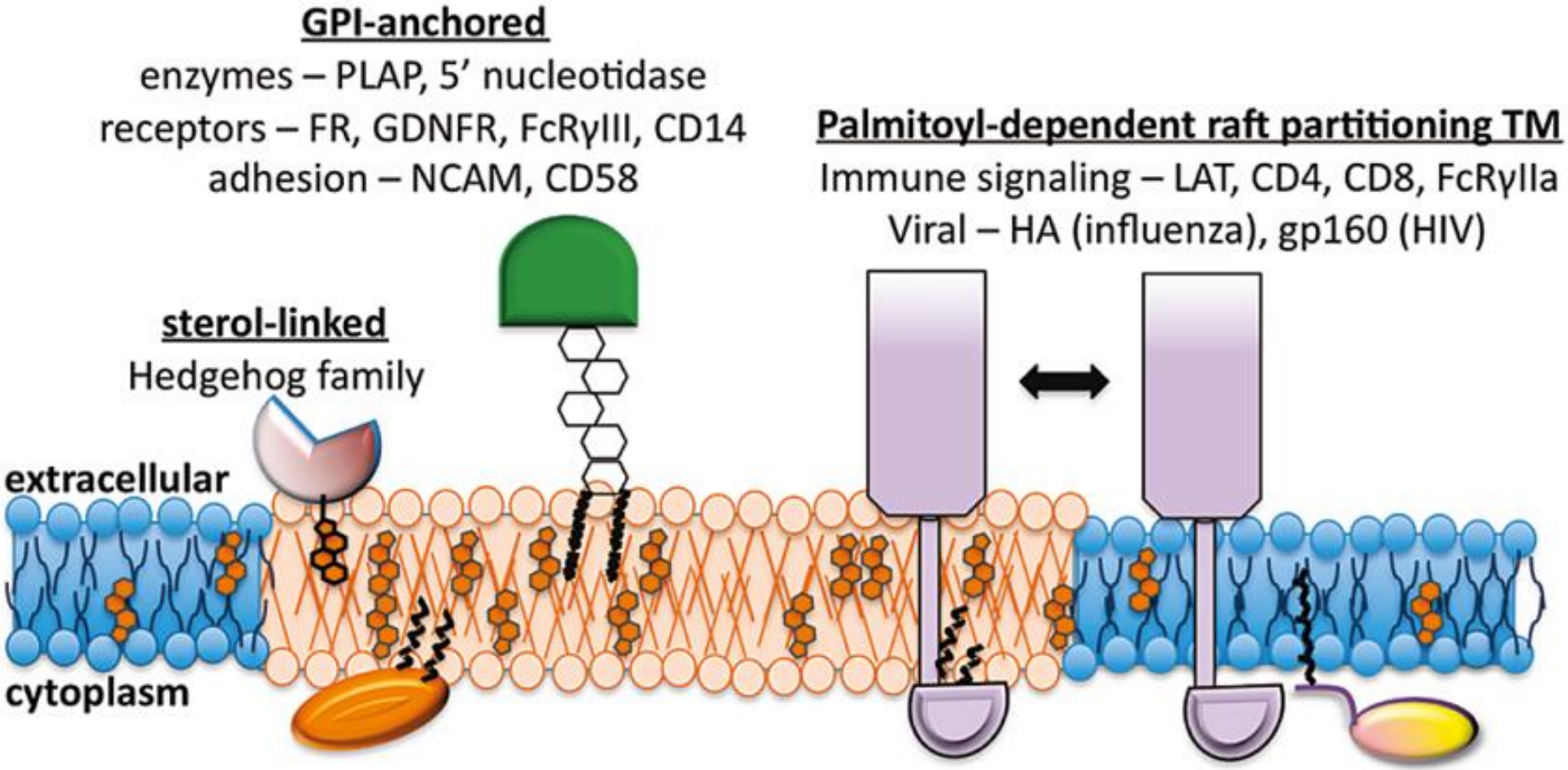
  Modification close to C-terminus

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

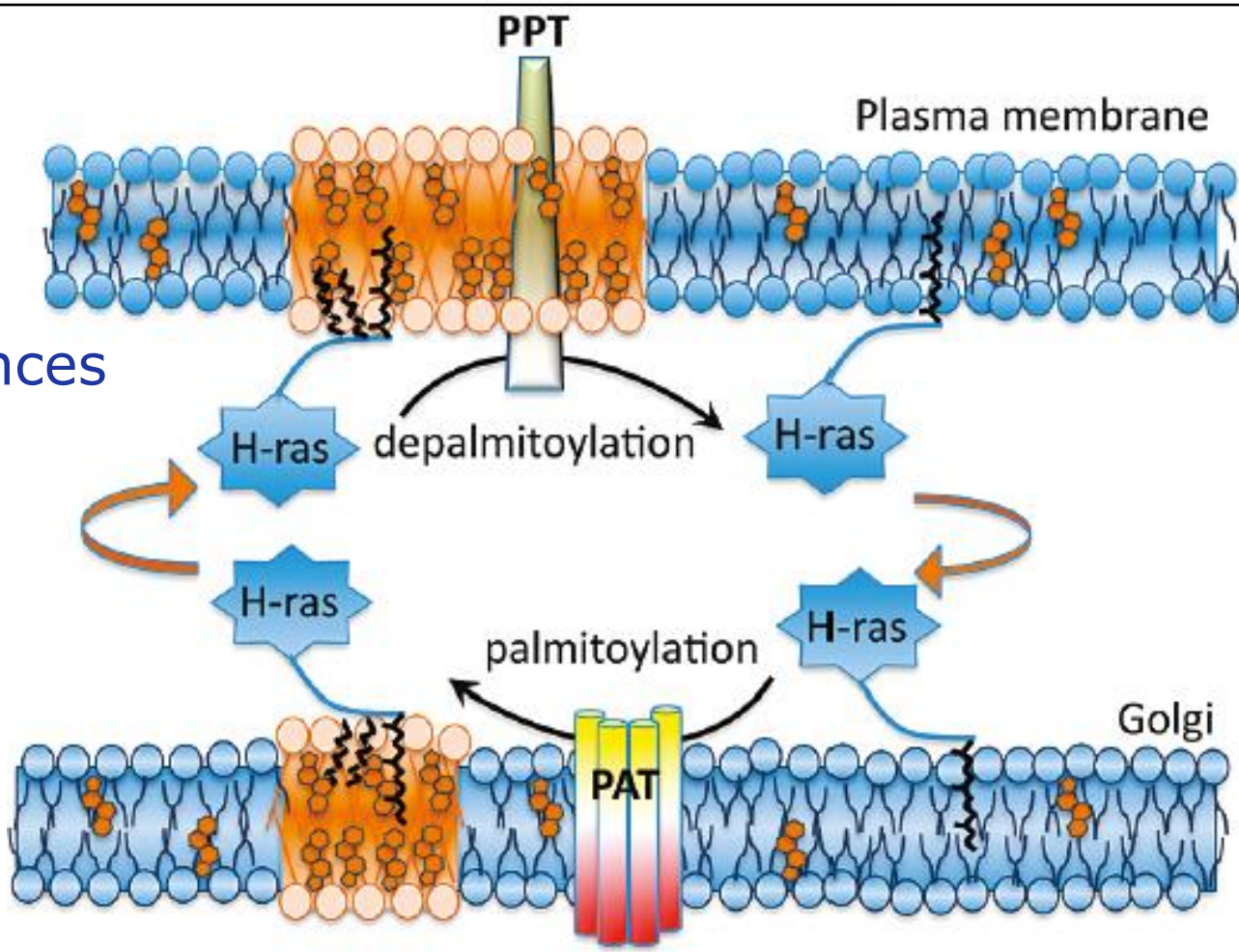


Levental 2010

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

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****.*

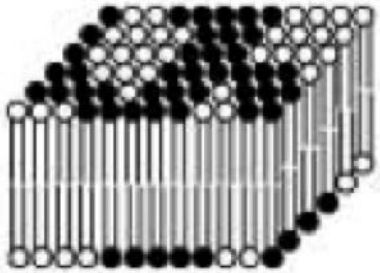
Modulation of signalling
by
lipids and membrane domains

Biological lateral phase separations : Rafts

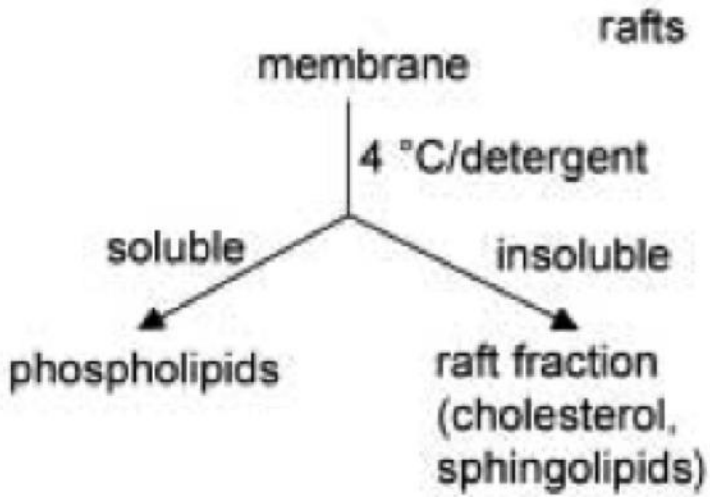
Historically, lipid rafts have been defined as :

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

- low buoyant density



domains

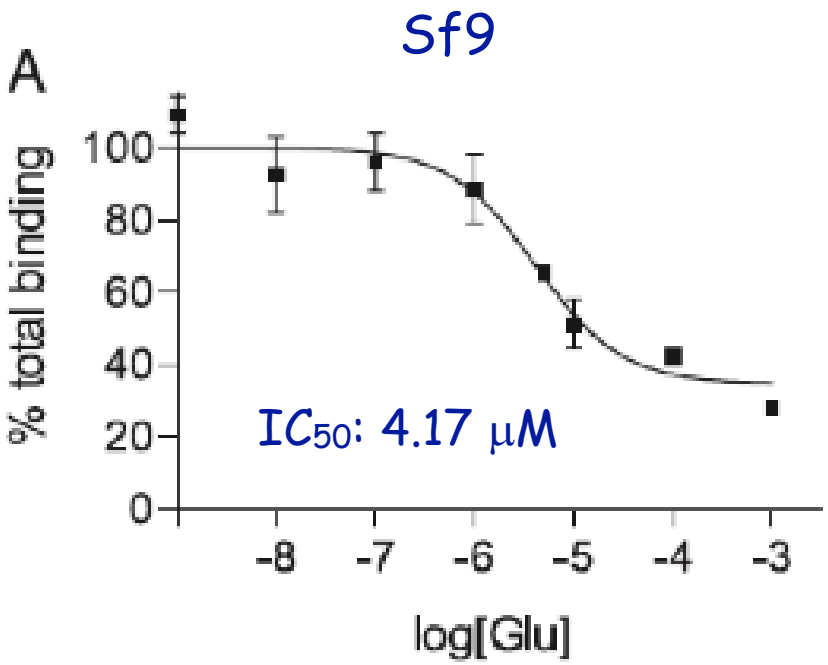


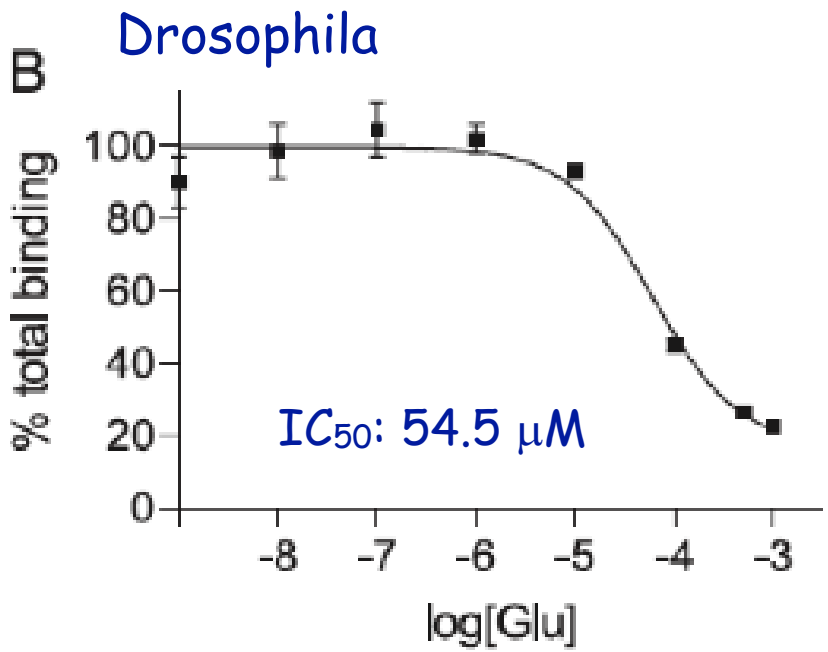
Binder, Angew Chem Intl Ed 2003.

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 :





Eroglu PNAS 2003

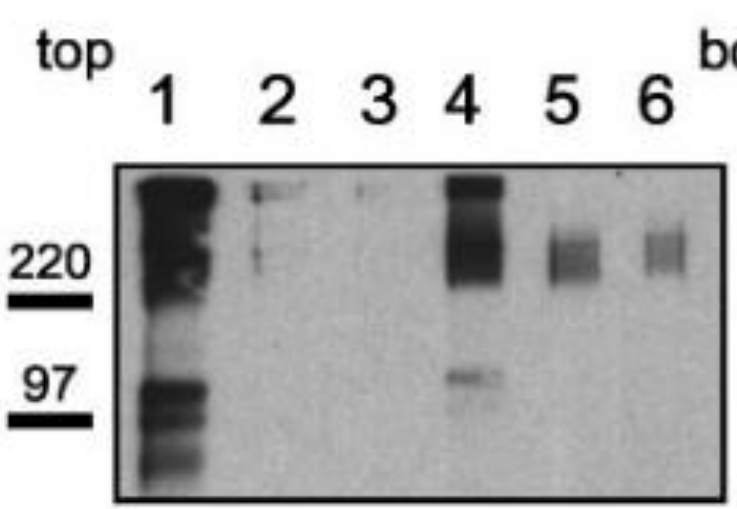
Modulation of mGlu-R signalling by lipid domains

Are rafts involved ?

Sf9 insect cells or Drosophila eyes

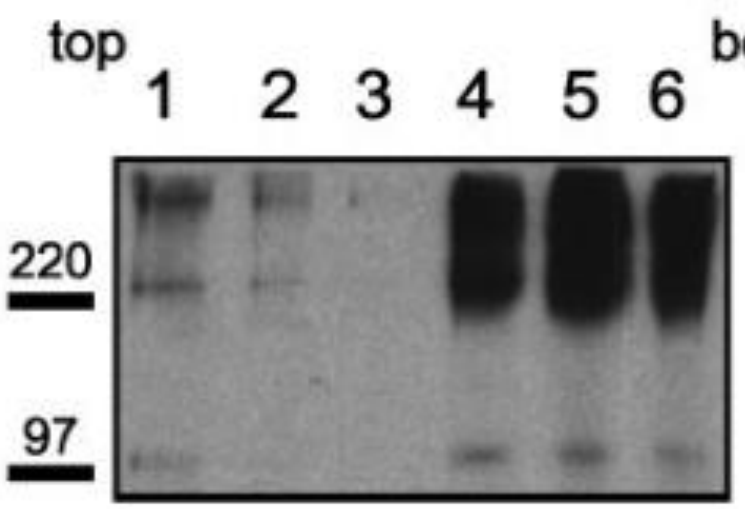
- Membrane density fractionation followed by SDS-PAGE analysis :

Sf9



B

Drosophila



Modulation of mGlu-R “fitness” by lipid domains

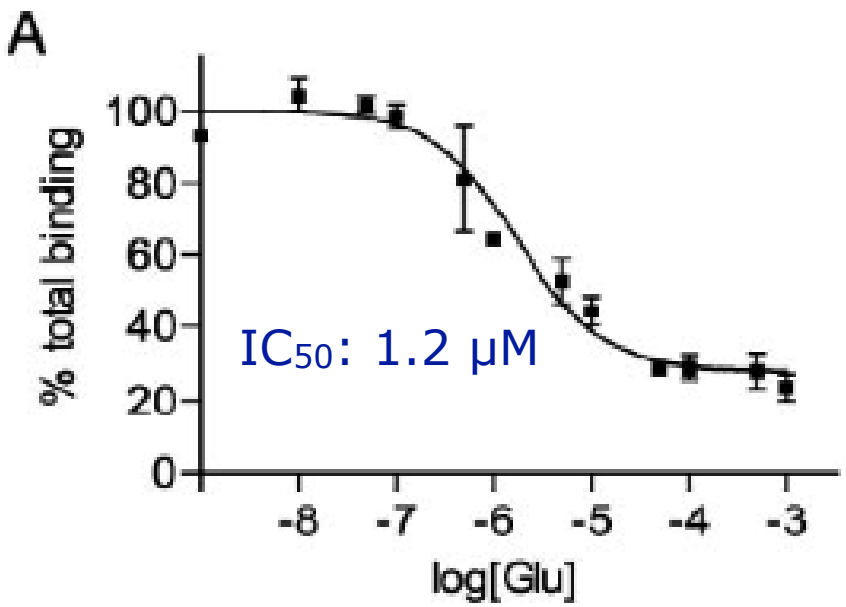
- Observation: Drosophila membranes contain very little cholesterol
  Q: Is cholesterol needed for high affinity?

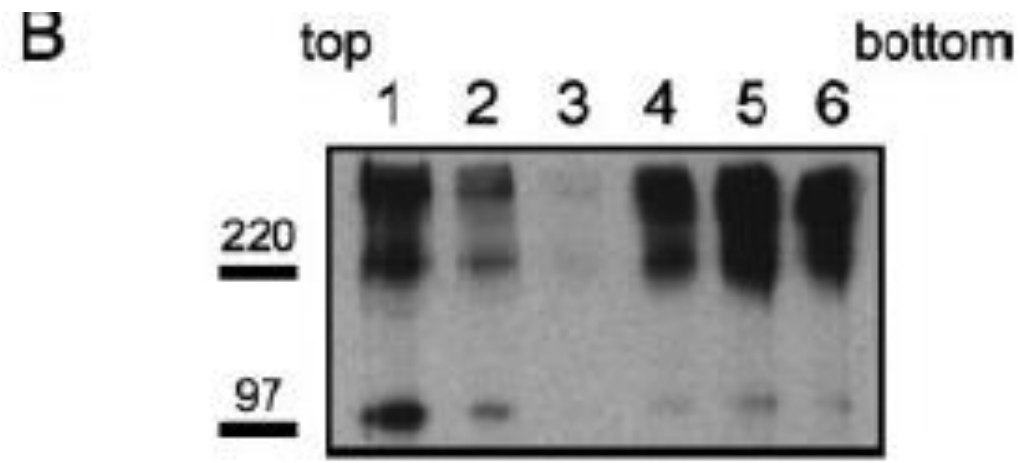
Experiment 1:

◇ Cholesterol **addition** to Drosophila membranes

=> receptor gains affinity
IC_{50} 54.5 => 1.2 µM

=> receptor goes to rafts
“light” fractions gradient



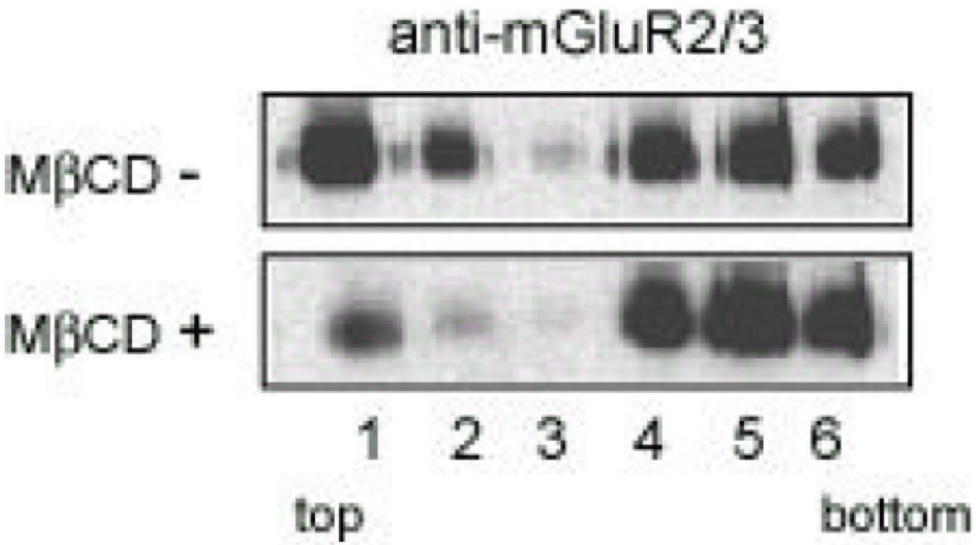


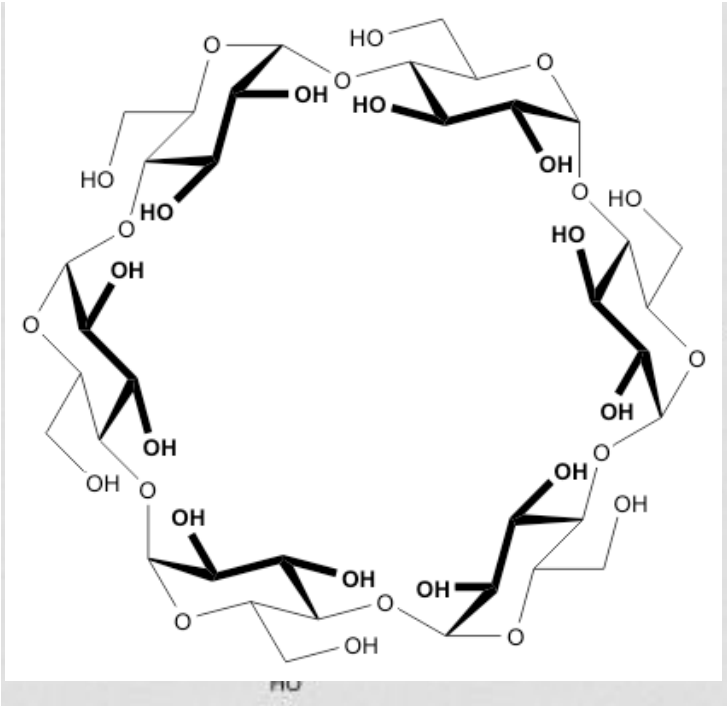
Eroglu PNAS 2003

Modulation of mGlu-R “fitness” by lipid domains

Experiment 2:

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





=> receptor goes to heavy membranes upon mβCD treatment

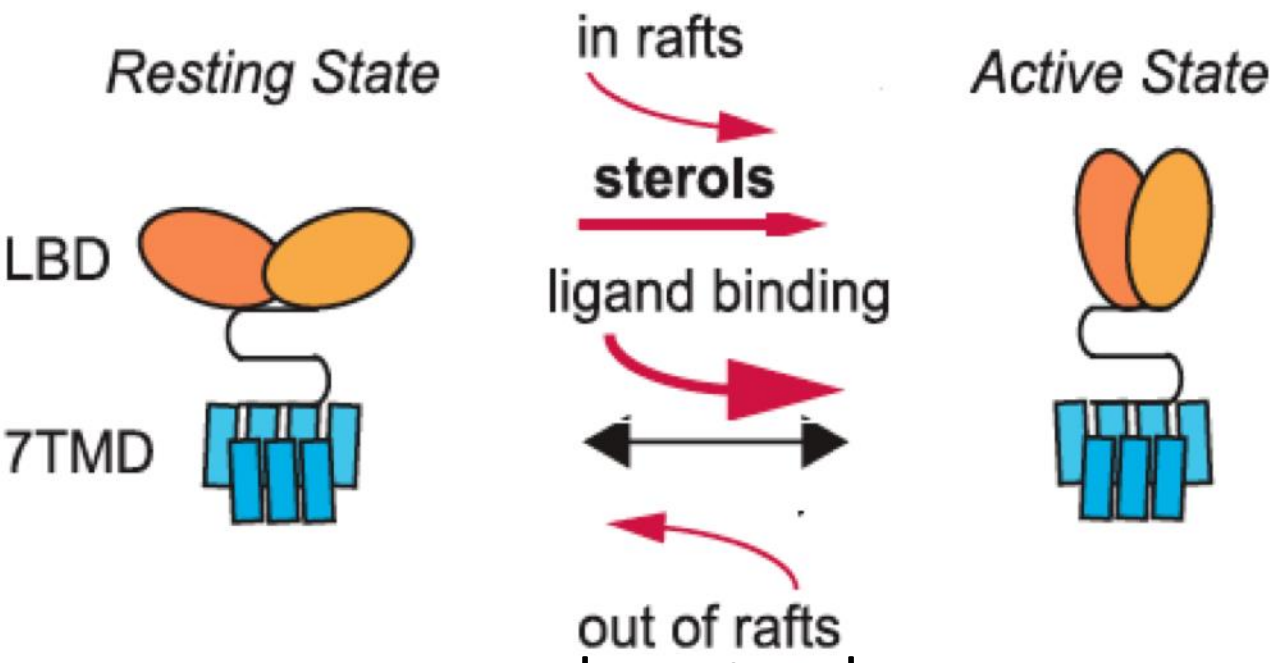
=> Yes, cholesterol is needed for both high affinity & raft localisation !

Eroglu PNAS 2003

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.



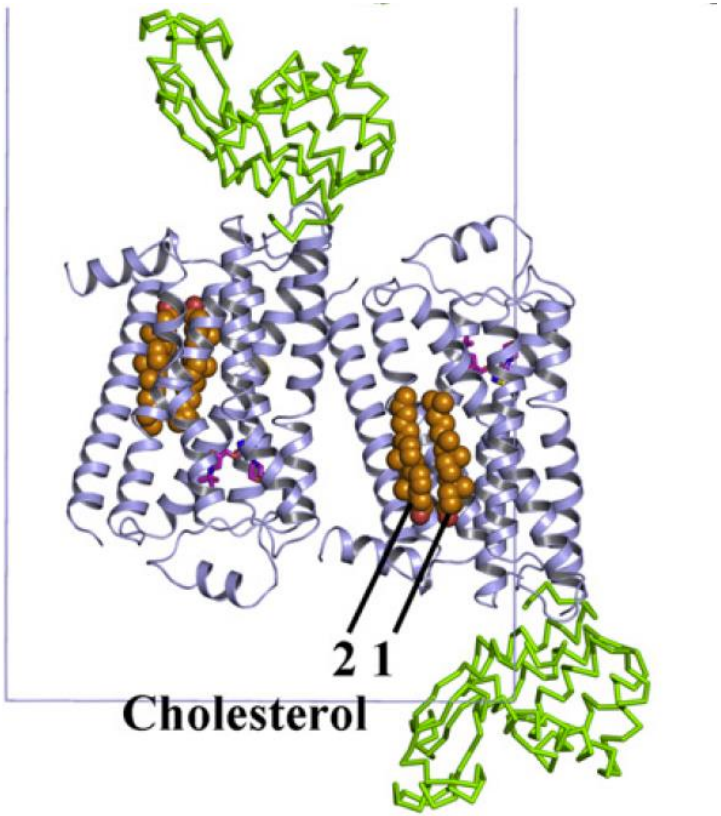
less sterols
ligand dissociation

Eroglu PNAS 2003

Cholesterol binding site on GPCRs

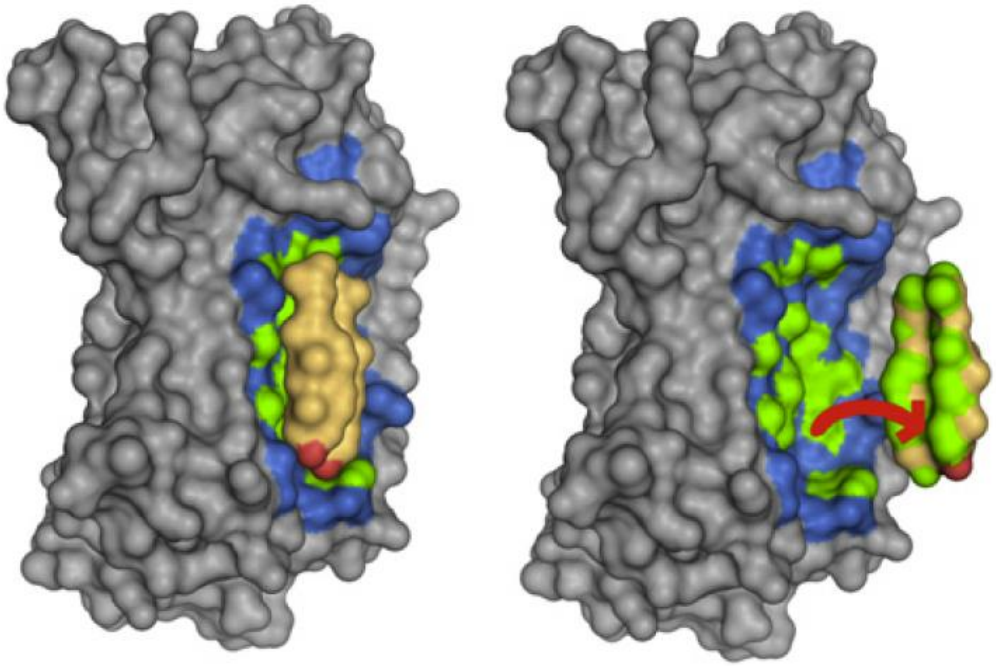
Crystal structure of the 2β-adrenergic receptor
=> 2 cholesterol molecules / receptor within the protein structure

Unit cell



Green : T4 lysozyme

Cholesterol 1 docks on receptor

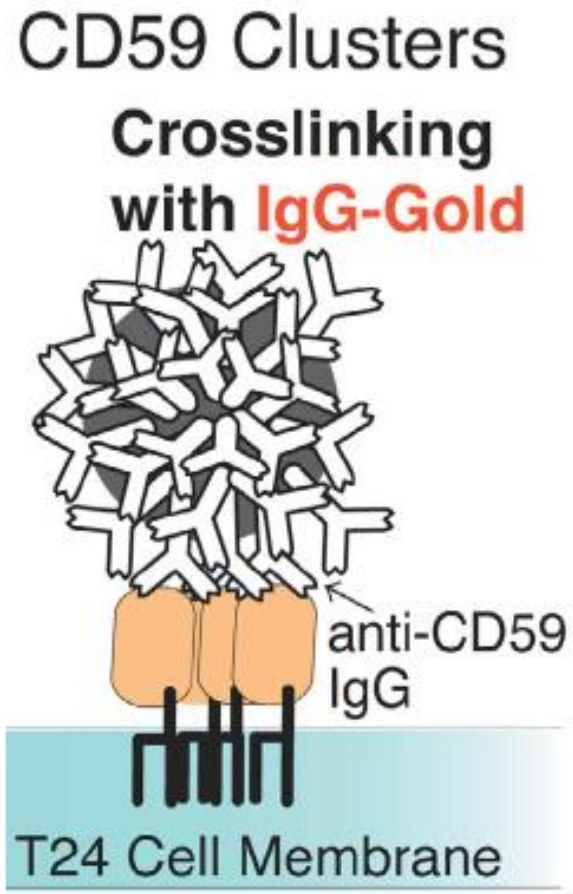


Brown — Cholesterol 1
green — d < 4Å
blue — 4 Å < d < 5 Å

Hanson Nature 2008

Case 2 : Signaling cascade of CD59

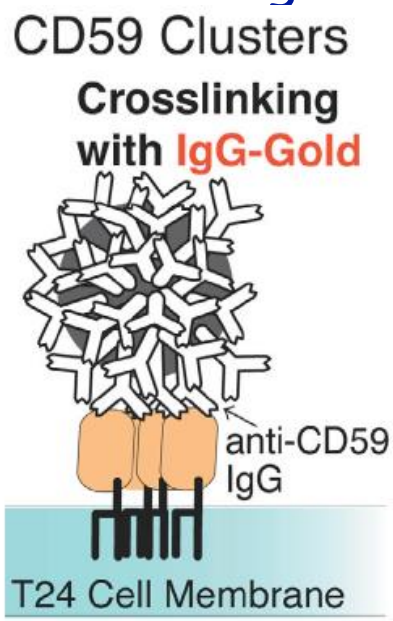
- CD59 is a GPI anchored receptor



Suzuki, J Cell Biol May 21, 2007

Single molecule observation of CD59 and Lyn

Observations of signalling pathway :



Supplemental video 1

G40-crosslinked CD59 on T24 Cells

Real time replay
Scale = 1 µm

CD59 and Lyn

Supplemental video 2

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

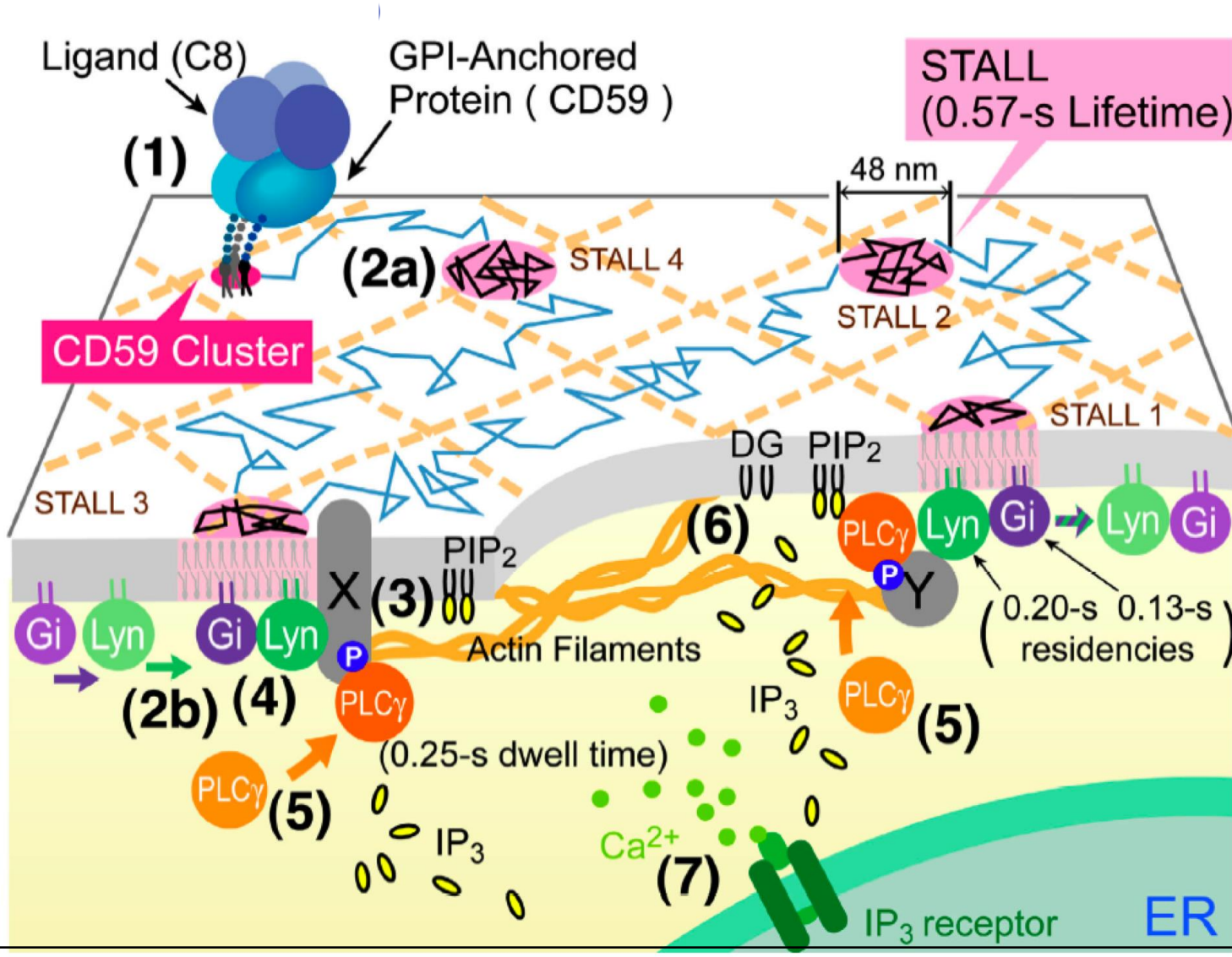
Real time replay, 10 sec
Scale = 500 nm

Suzuki, J Cell Biol May 21, 2007

Single-molecule imaging reveals transient interactions

1) Clustering of the GPI-anchored receptor CD59

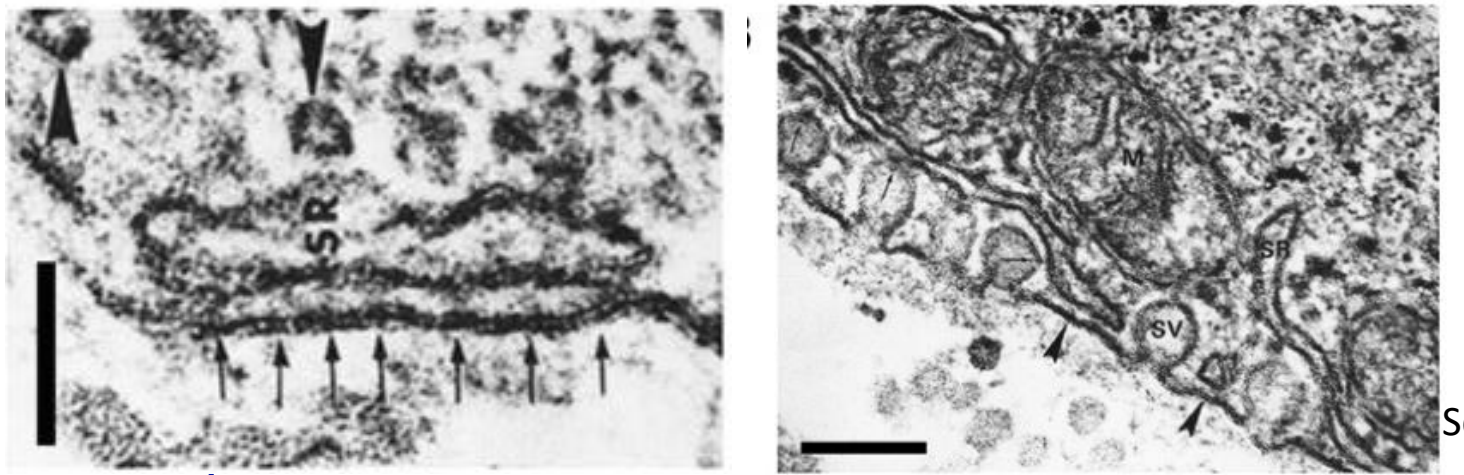
STALL = Stimulation-activated ***transient*** arrest of lateral diffusion



Suzuki, J Cell Biol May 21, 2007

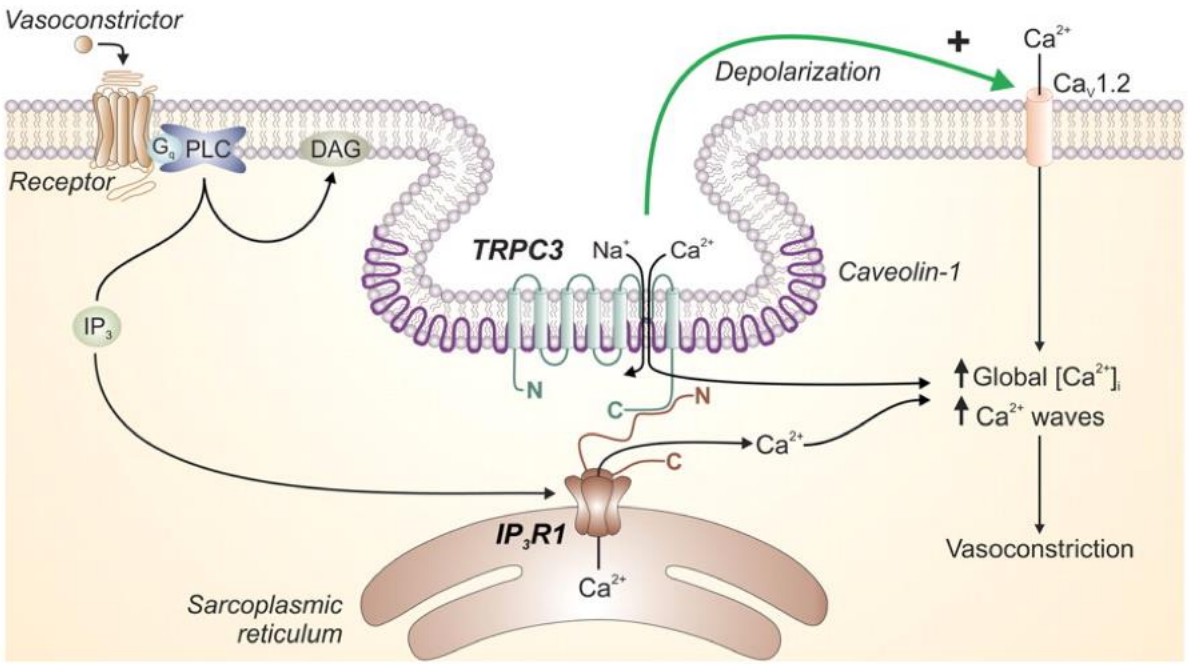
Close proximity between plasma membrane and ER

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



Scale bar : 100 nm

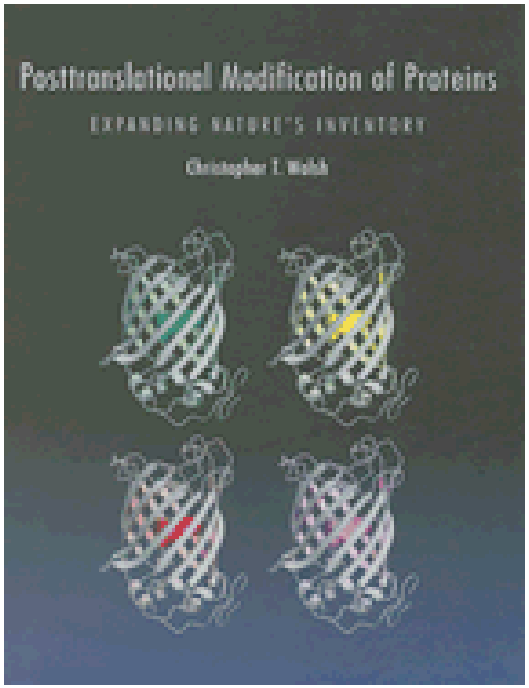
Schematic representation



Narayanan et al. Heart & Circul Physiol 302(2012) H2190

Further reading:

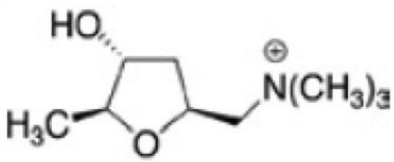
Walsh	Post-translational modifications of proteins Chpt. 7
Van Meer	Membranes & lipids (2008)
Yu	GPI (2013)
Levental	Membrane association (2010)
Tate	Lipidation profiling (2014)



Next week

Amanita muscaria

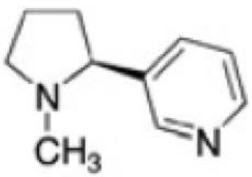
Muscarinic



(+)-Muscarine*

Isolation 1954
Structure 1957
Pharmacology 1911

Nicotinic



S*-(–)-Nicotine

Isolation 1828
Structure 1893
Pharmacology 1889

Nicotiana tabacum

Does membrane localisation enhance diffusion-limited (inter/re)actions ?

A cell with a radius of 10 uM contains 10'000 molecules of each A and B.

How long will it take for a molecule A to meet for the first time a molecule B ?

Make an educated guess using the following diffusion constants D:

2D diffusion in the membrane : D = 10^{-9} to 10^{-10} cm^2/s

3D diffusion in the cytosol : D = 10^{-8} cm^2/s

A hint: what is the average distance between the molecules of the same species either in the membrane or in the cytosol ?

Do submit your answer or questions to the Moodle site .

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**

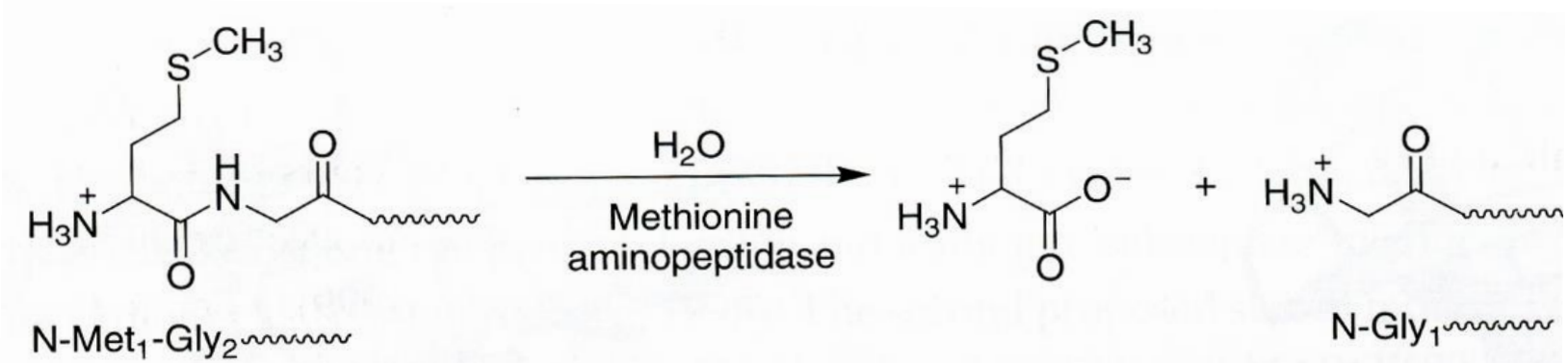
if N-terminus is : H_2N-Met-**Gly**-x-x-x-[Ser/Thr]-y-y

x: small & uncharged

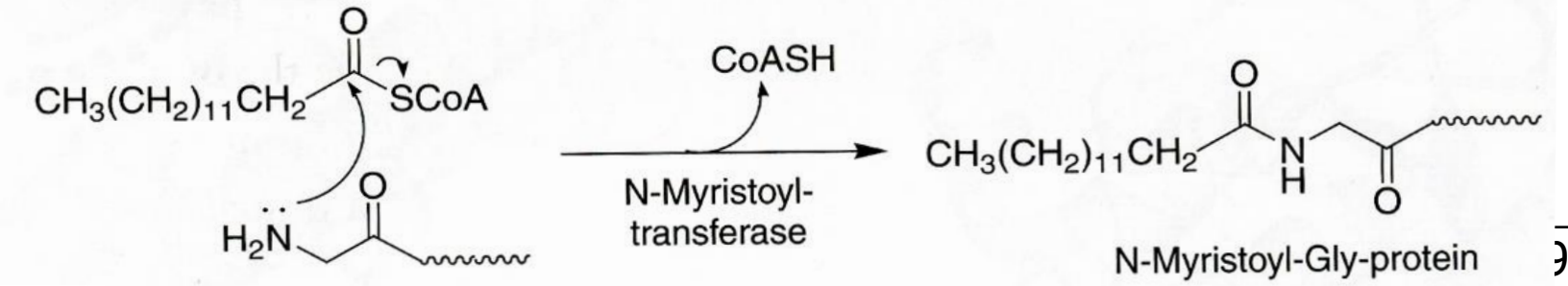
y: basic

Enzyme: myristoyl-CoA:protein *N*-myristoyl transferase
Substrate: myristoyl-CoA

Step 1:
removal of Met



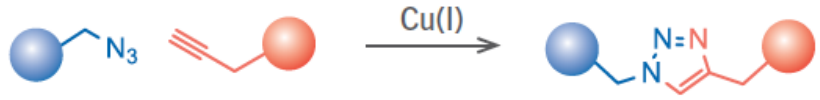
Step 2:
addition C14:0

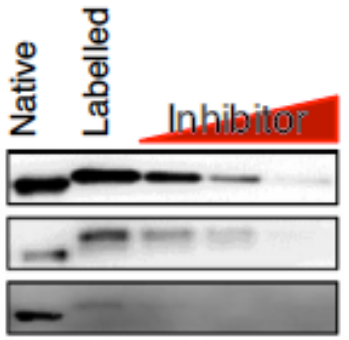


Identifying & localising lipidated proteins

Chemical synthesis
of lipid probes

Exploiting bio-orthogonal click-chemistry





Western blot
Identification/ quantification of single substrates

Model System
in vitro or *in vivo* metabolic tagging

Lipid metabolism & probe incorporation

Biotin ligation
(click chemistry)

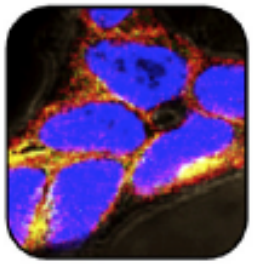
Biotin

Fluorophore ligation

1. Enrich
2. Proteomic analysis

Mass spectrometry: proteome-wide profiling and quantification of protein lipidation

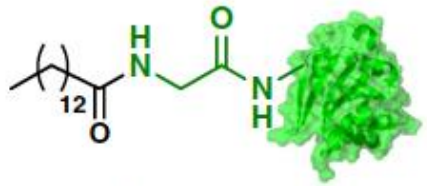
- Identification of specific lipidation substrates
- Sites and stoichiometry of modification
- Quantification of changes in protein lipidation



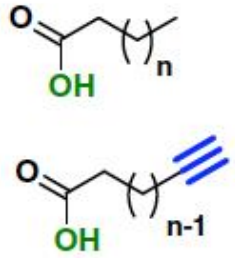
Substrate imaging

Current Opinion in Chemical Biology 2015, 24:48–57

N-terminal *N*-myristoylation
Transferases: **NMT1** and **NMT2**
Mediates switchable membrane binding

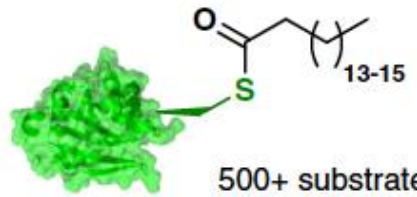


100+ substrates
in humans



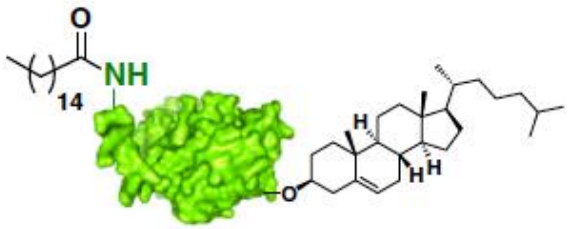
Acylation: myristate
and palmitate probes

Cysteine *S*-acylation
Transferases: **DHHC** class
Hydrolases: **APT1**, **APT2…?**



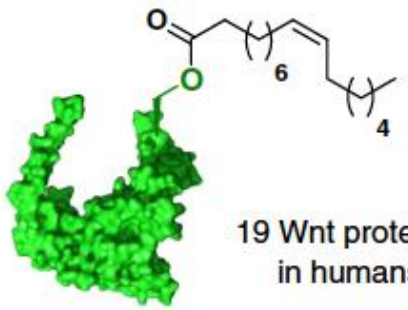
500+ substrates
in humans

Hedgehog *N*-acylation
Transferase: Hedgehog
acyltransferase (Hhat)



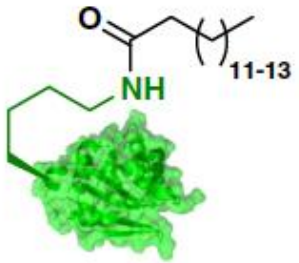
Hedgehog *O*-cholesterylation
Transferase: autocatalytic

Wnt *O*-acylation
Transferase: Porcupine



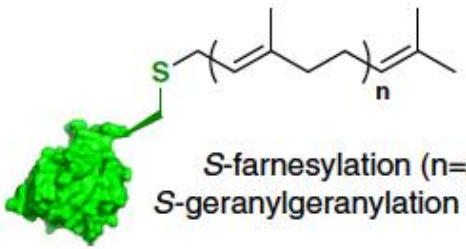
19 Wnt proteins
in humans

Lysine *N*-acylation
Modulated by: **sirtuins**
(Sirt6; Sirt2?; Sirt3?)



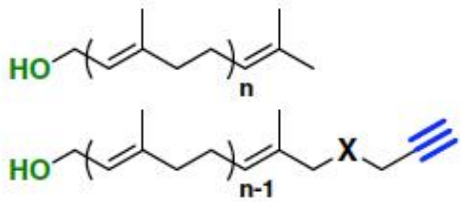
TNF-α; other
substrates?

Cysteine *S*-farnesylation and *S*-geranylgeranylation
Transferases: **FTase** & **GGTase** at CAAX box, and by **RabGGTase** on Rab proteins

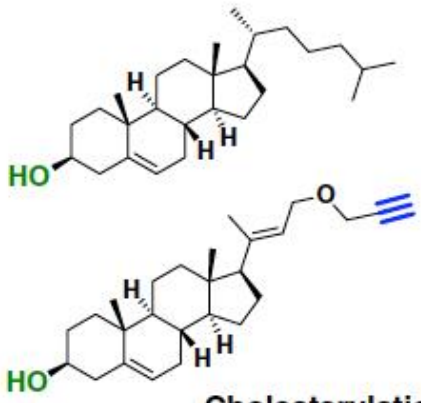


S-farnesylation (n=2)
S-geranylgeranylation (n=3)

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



Prenylation: farnesyl and
geranylgeranyl alcohol probes



Cholesterylation: