Proteins & Phospholipids on the move

Text books:

Biochemistry – Stryer	Chpt 12
The Cell – Alberts	-
Biomembranes – Gennis	Chpt 4, 5.4

Review articles:

Sprong, H. , van der Sluijs, P., van Meer, G. Nature Rev. Mol. Cell Biol. 2 (2001) 504-513

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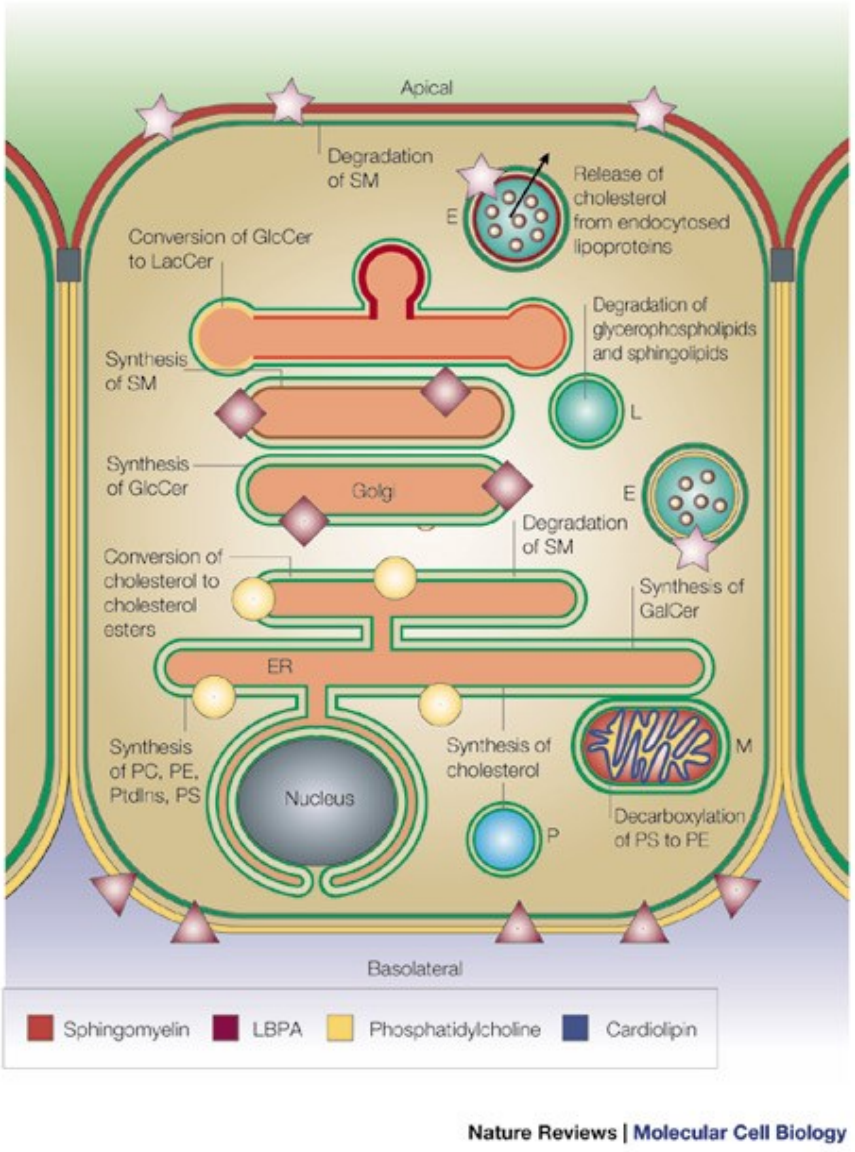
Phospholipids and proteins move within a cell



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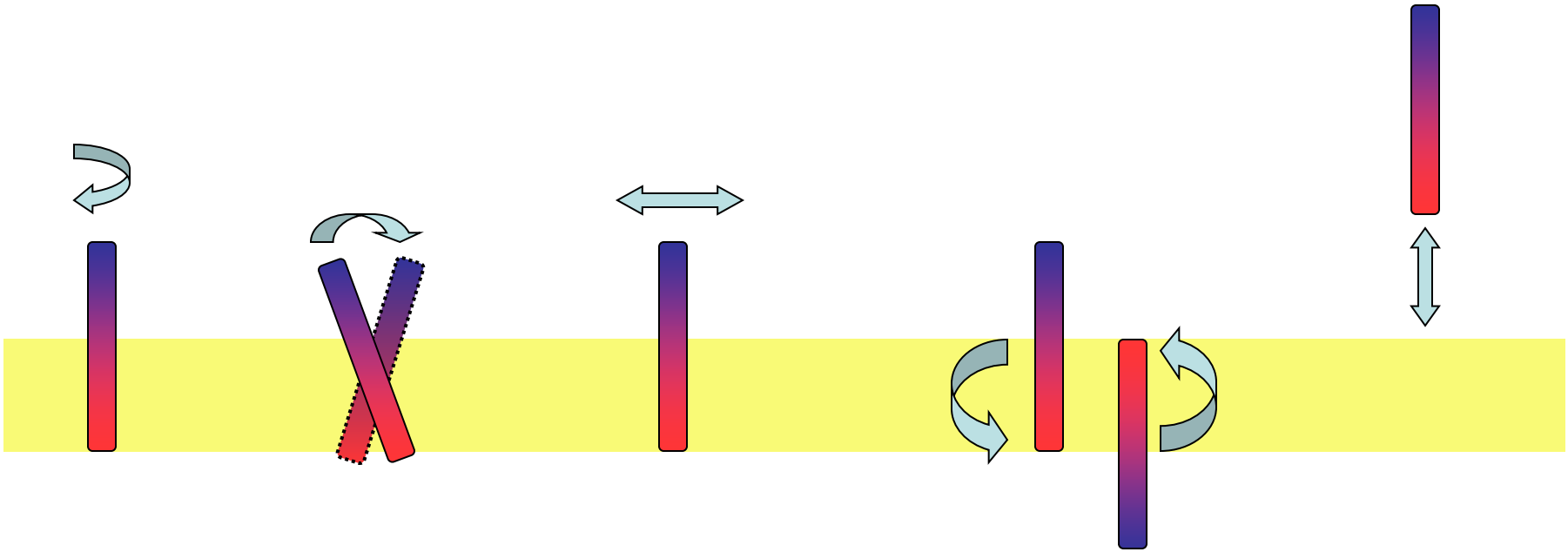
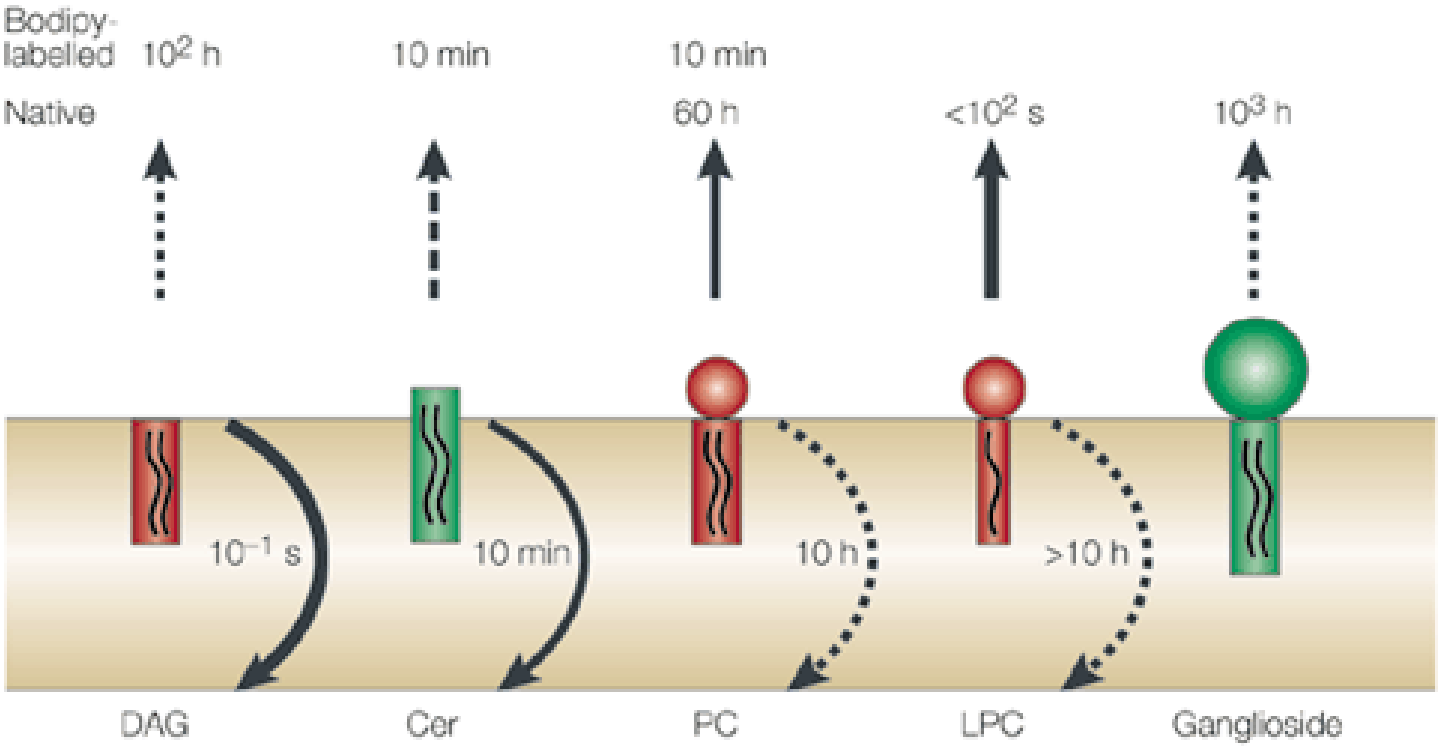
Movements in a membrane



Movements of lipids in a membrane



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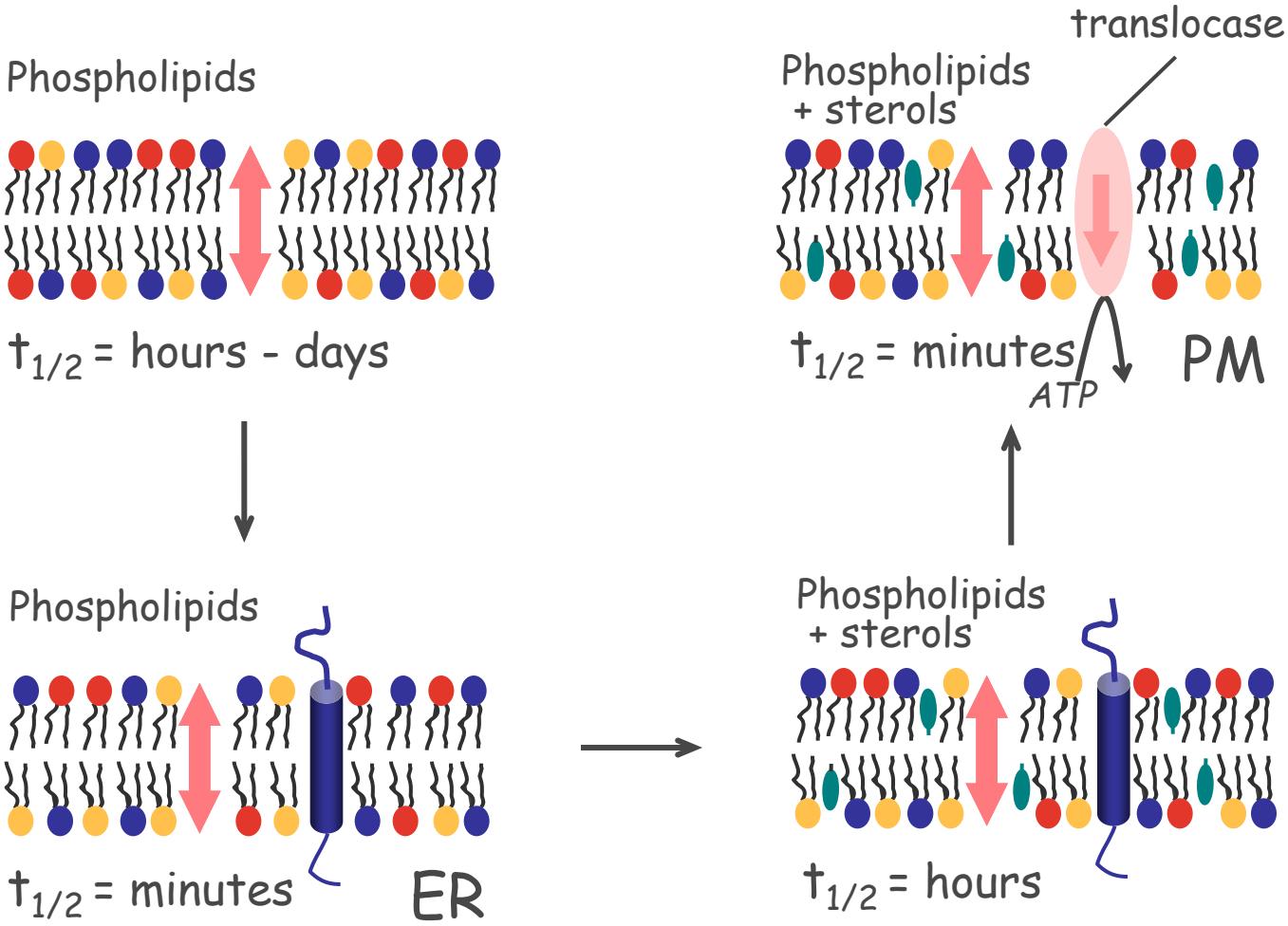
The larger the polar head group => slower flip-flop
=> faster exit

Membrane proteins accelerate flip-flop



Diffusion: thermal motion

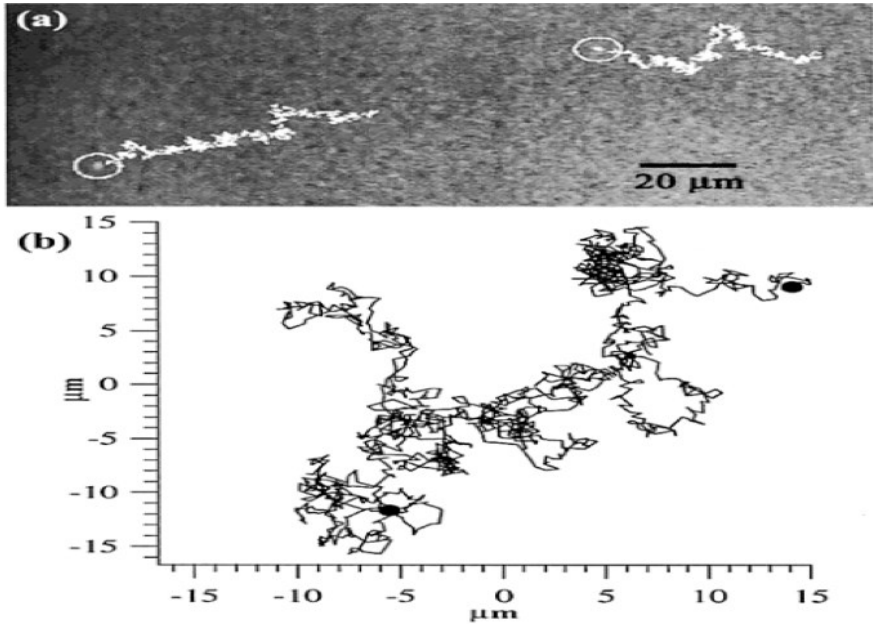
All particles under go Brownian motion.

◊ Thermal energy equals kT/2 per direction

◊ Instantaneous velocity v_x : $\langle v_x^2 \rangle = kT/mass$

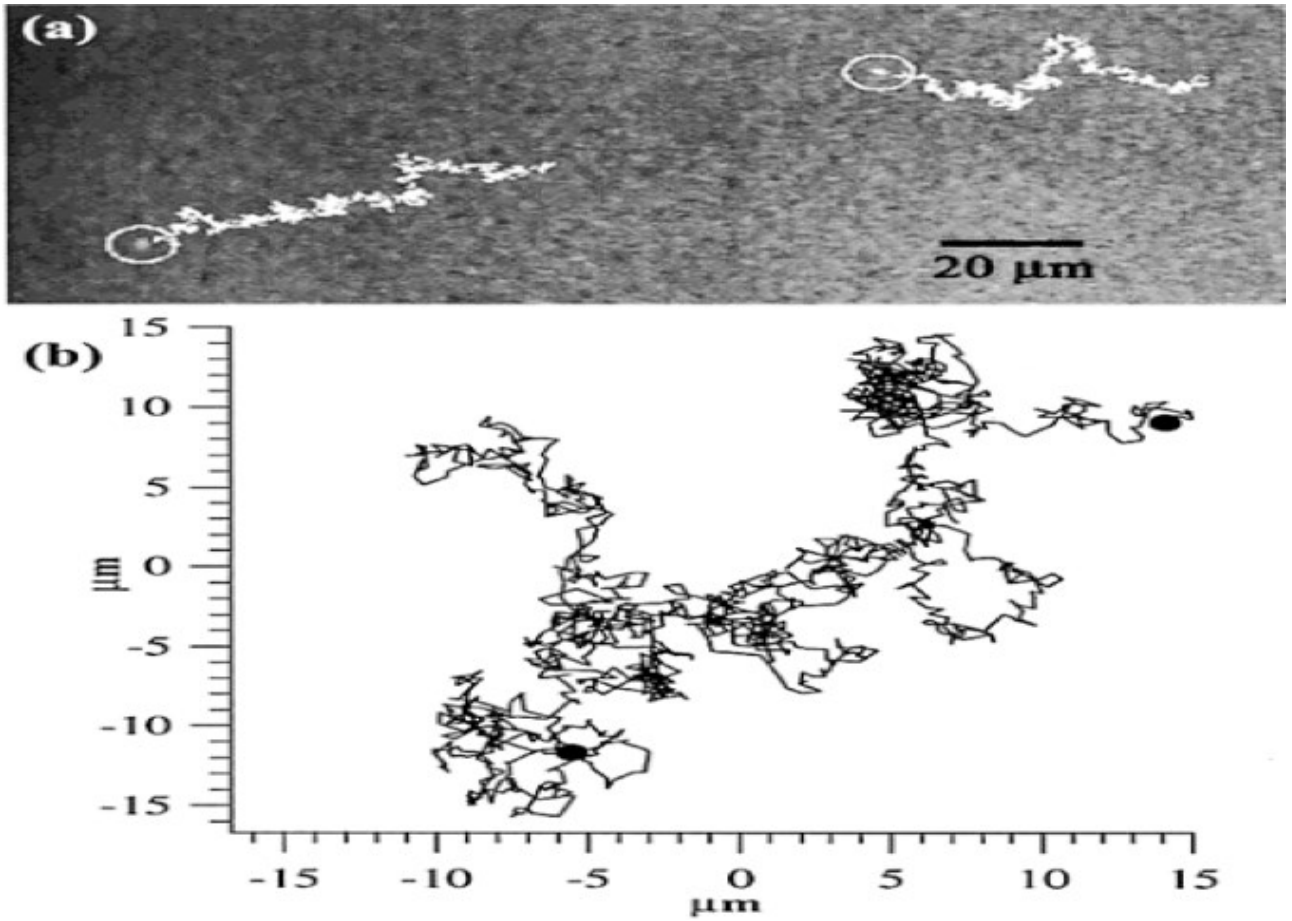
e.g. for a 14 kDa protein v_x = 13 m/s

◊ Random walk due to collisions



Diffusion of lipids

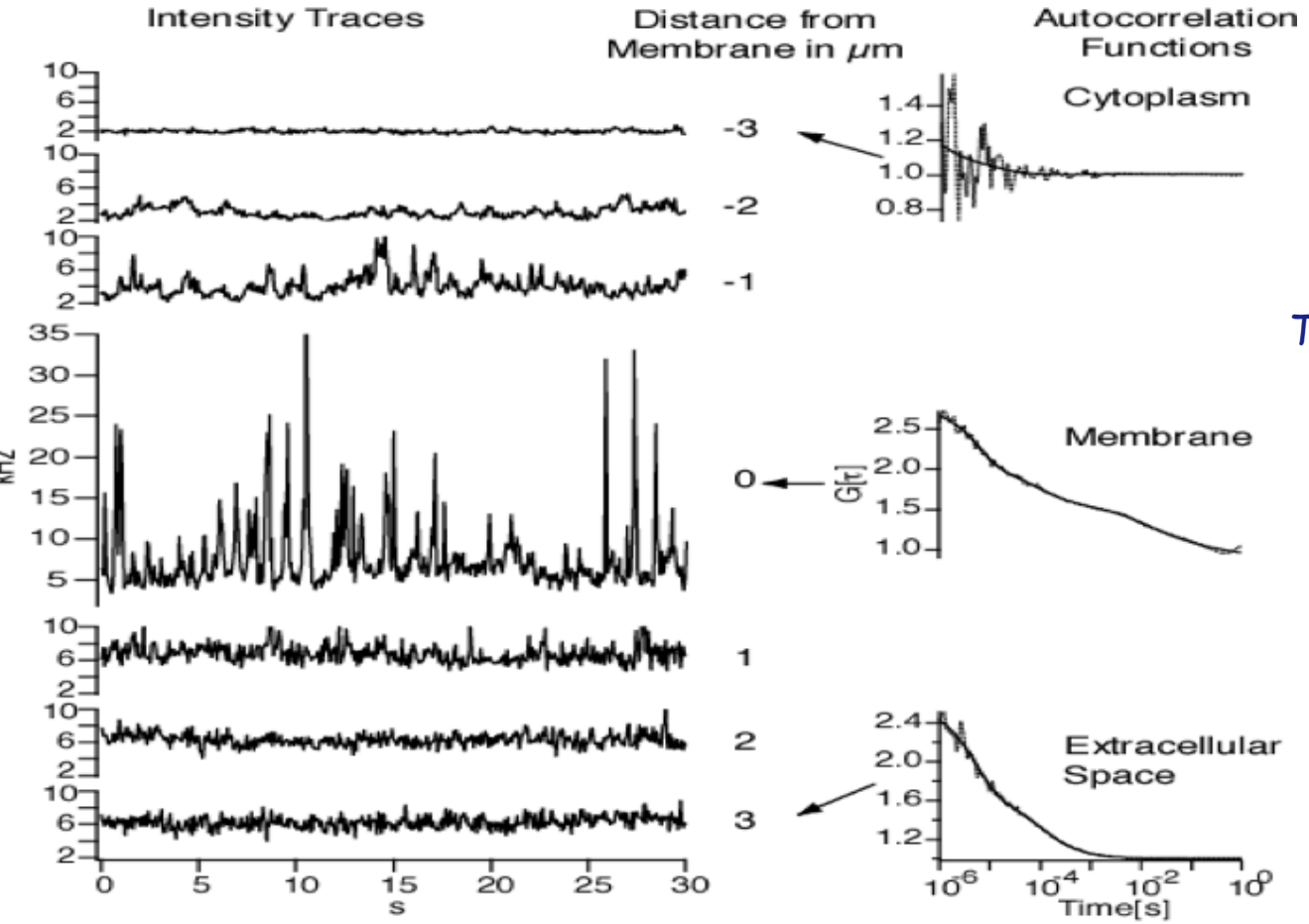
Fluorescent lipids in a Membrane.



T. Schmidt

Diffusion of lipids

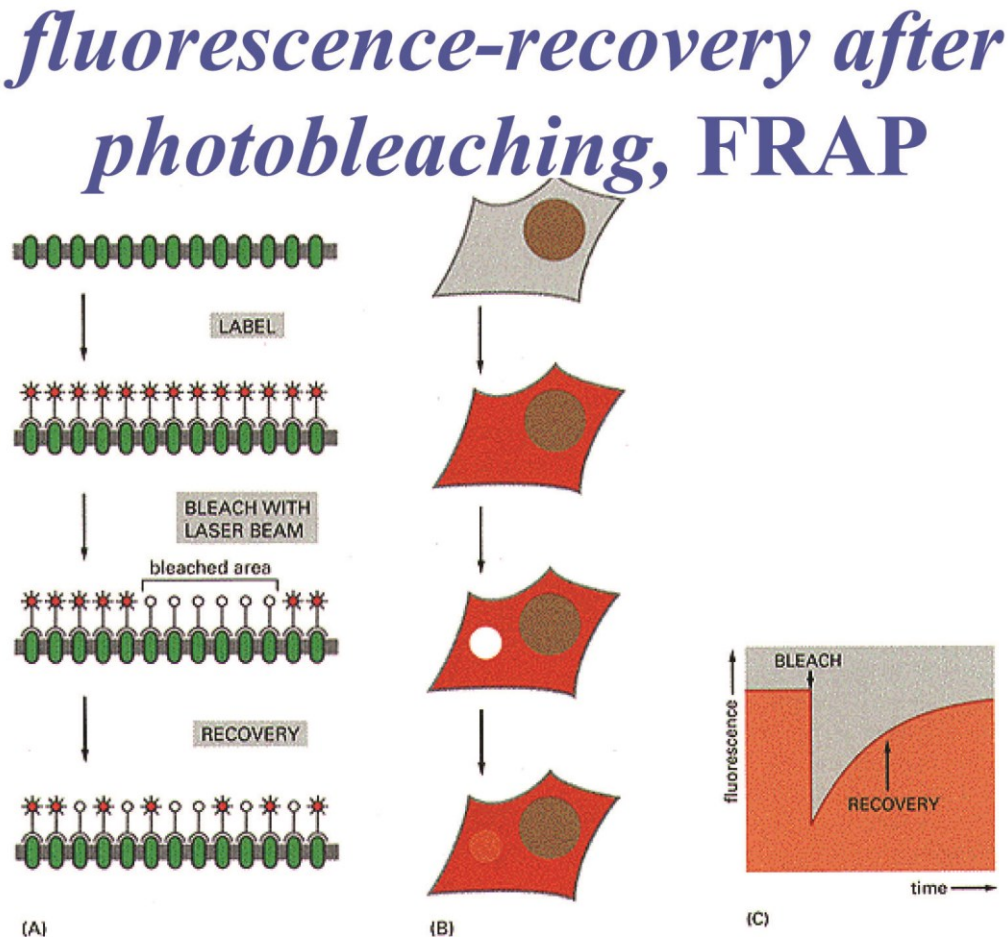
Fluorescent lipids in a membrane, detected at a single spot,quantified by auto-corrolation



T.Wholand

FRAP

Fluorescent lipids in a membrane,



Diffusion coefficient and friction

Einstein: $D = kT/f$ **f**: friction coefficient

Sphere in homogeneous medium: **f** $= 6\pi\eta R$ in case of 0 % slip

$= 4\pi\eta R$ in case of 100 % slip

$D \alpha R^{-1} \alpha M^{-1/3}$

$D \alpha \eta^{-1}$

R: radius of sphere

M: mass of sphere

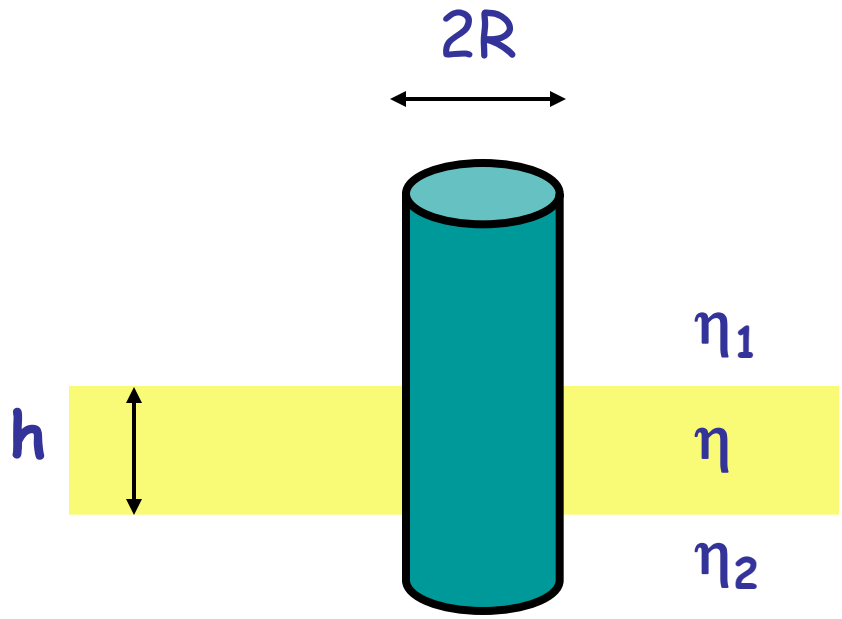
η: viscosity of medium

◊ Problem: membrane is a thin 2-D layer!

How does **f** look like?

Protein diffusion: continuum model

A protein is large compared to a lipid molecule => the membrane is a "continuum"



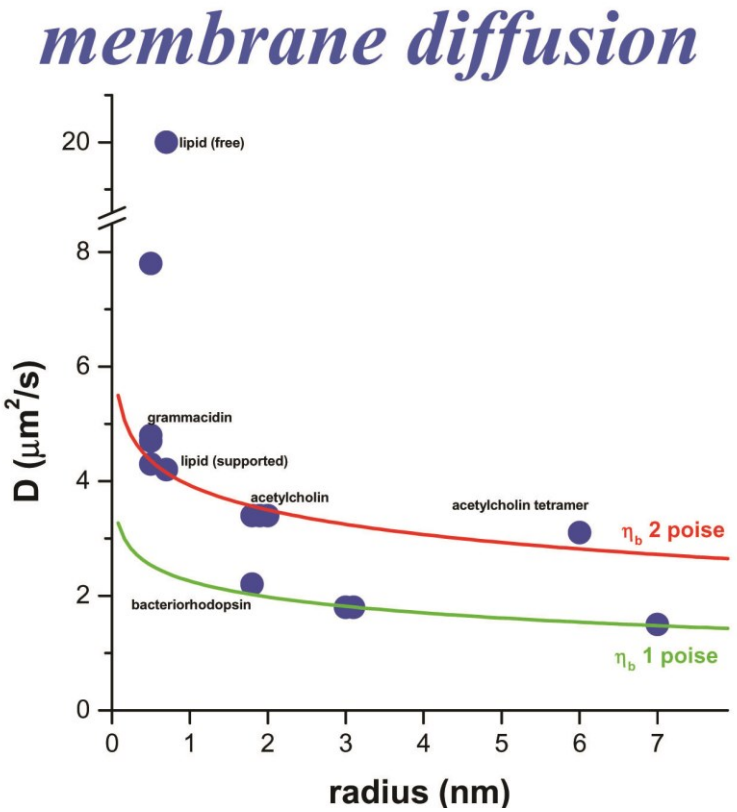
Assuming $\eta_1 = \eta_2$ and $\varepsilon = (R/h)\bullet[(\eta_1 + \eta_2)/\eta] \leq 0.1$

$f_{trans} = 4\pi\eta h\,[\ln(\eta h/\eta_1 R) - \gamma]^{-1}$ γ : Euler's constant = 0.5772

$f_{rot} = 4\pi\eta R^2 h$

Saffmann-Delbrück (1975)

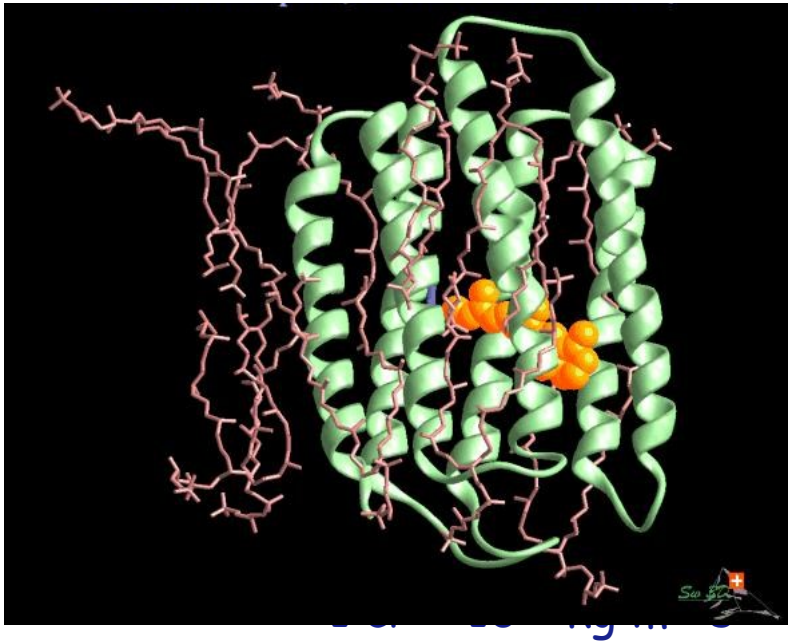
Protein diffusion: continuum model



=> f, and thus D, depend not so strong on R

Protein diffusion: continuum model

Example: rhodopsin



R: 2 nm

η_1: 0.01 P

η: 5 P

h: 4.5 nm

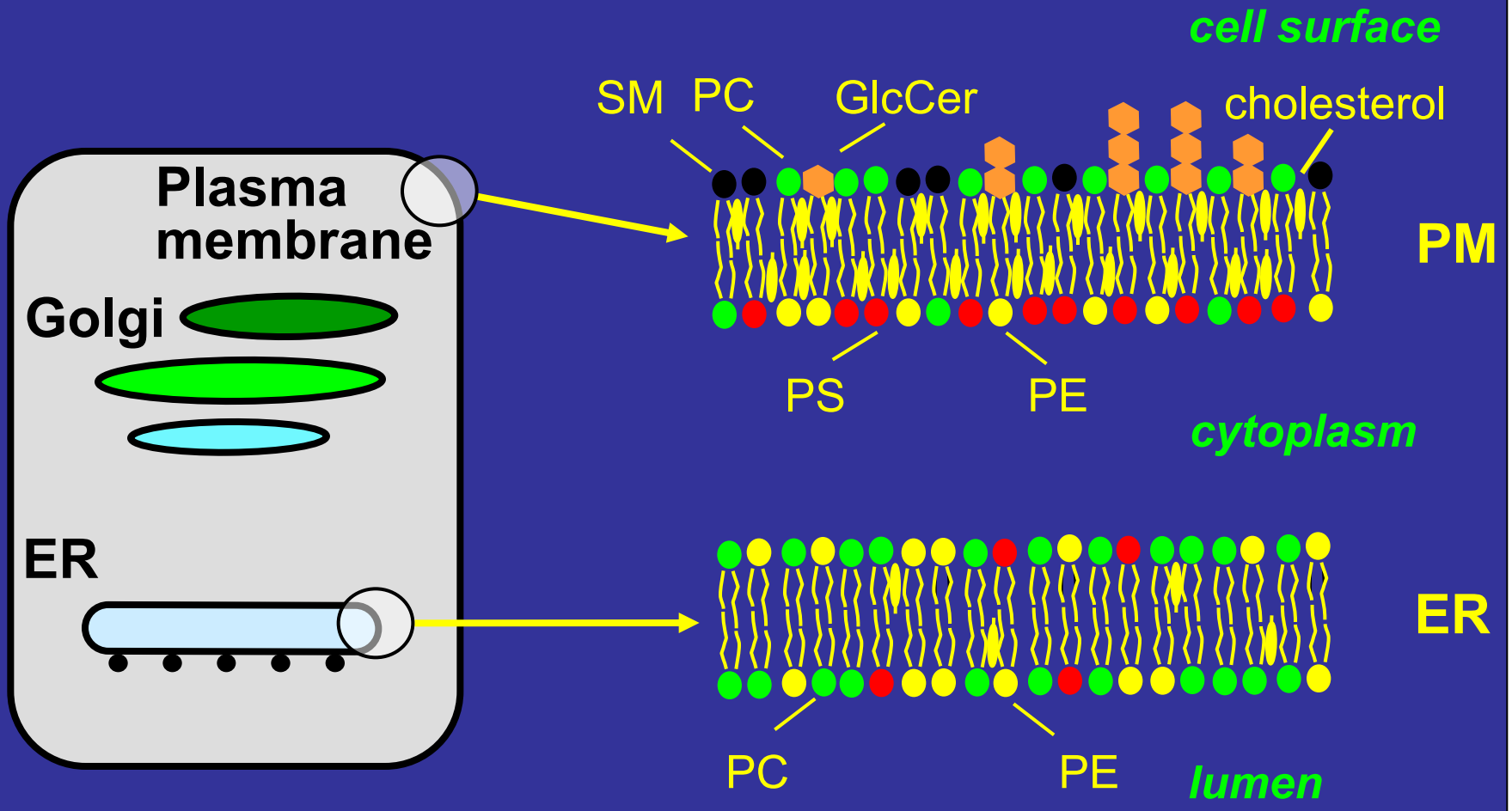
$f_{trans} = 4\pi\eta h\,[\ln(\eta h/\eta_1 R) - \gamma]^{-1}$ => $D_{trans} = 9.4 \bullet 10^{-9}$ cm^2 s^{-1}

Experiment: $3.5 \bullet 10^{-9}$ cm^2 s^{-1}

$f_{rot} = 4\pi\eta R^2 h$ => $D_{rot} = 3.6 \bullet 10^{4}$ s^{-1}

Experiment: $5 \bullet 10^{4}$ s^{-1}

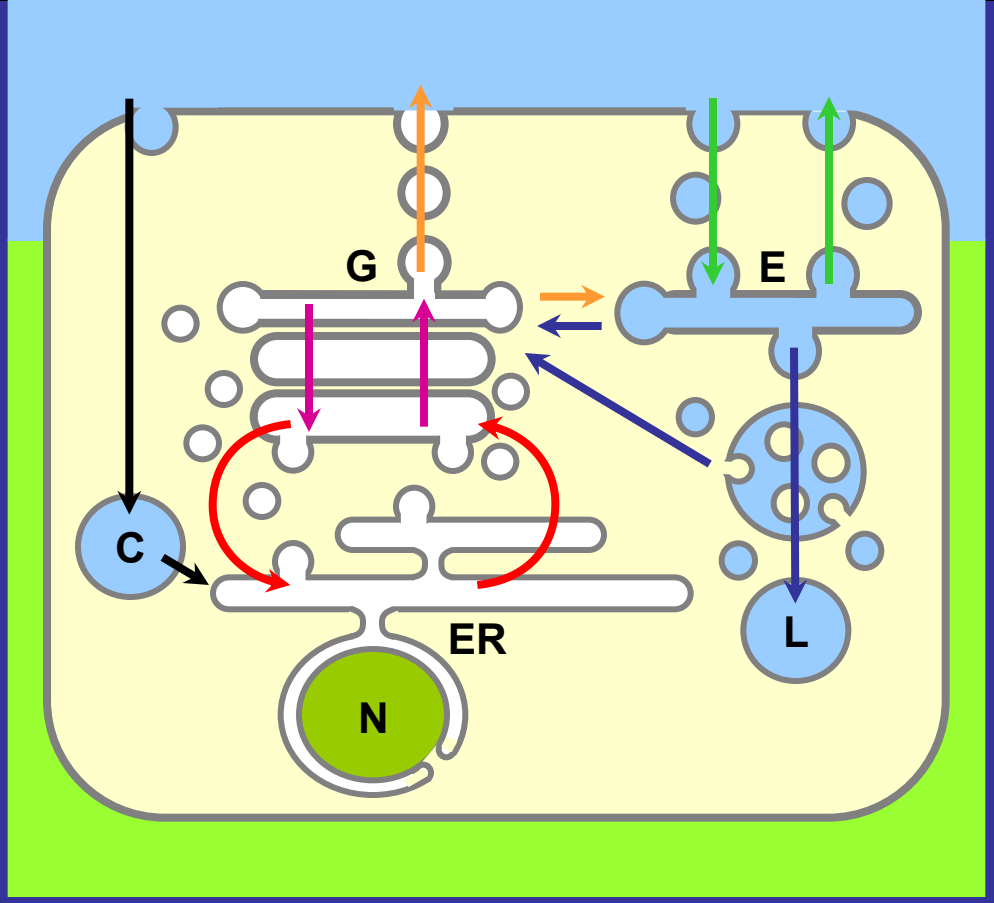
Phospholipids on the move



1. Cells have some 500 different membrane lipids

Cellular membranes differ in

2. lipid composition
3. transbilayer distribution

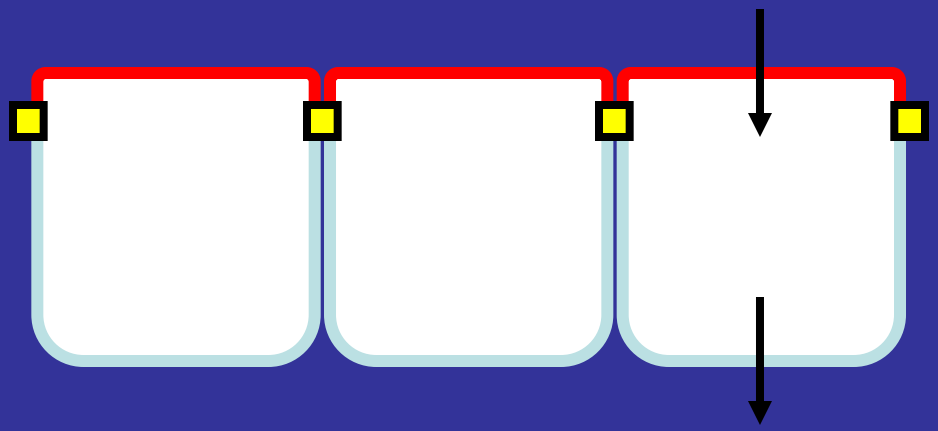


Most organelles are connected by vesicular traffic that is bidirectional

So:

How are differences between membranes generated and maintaine

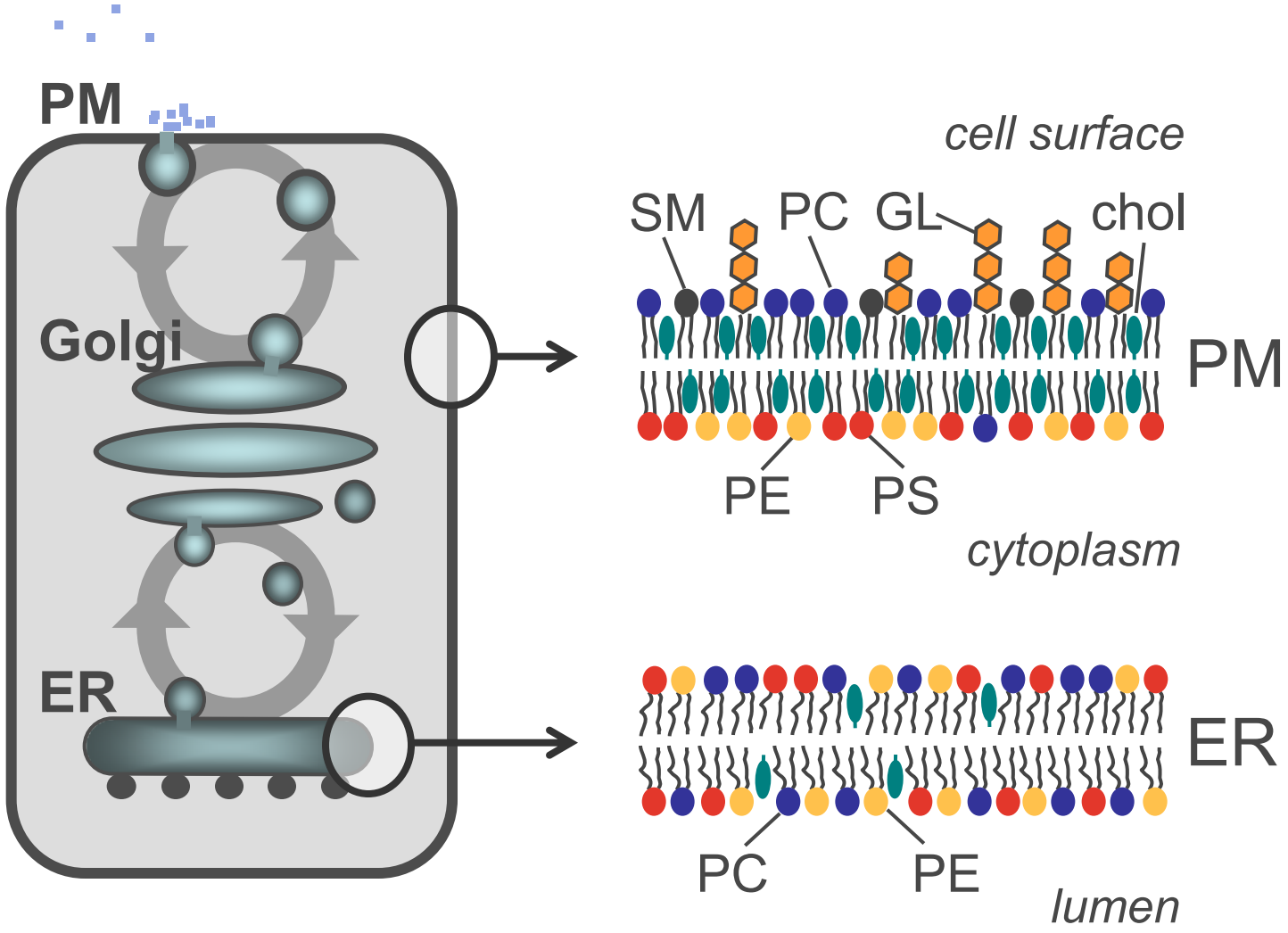
Surface polarity of membrane lipids in epithelial cells



GSL	PL	Chol
33	33	33
8	66	26

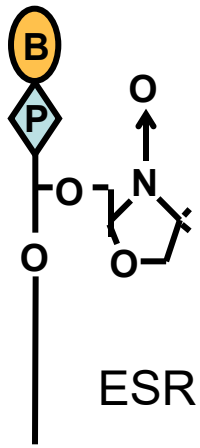
How are these differences maintained in view of the fact that the membrane is a continuous layer of oily consistency?

Lipids are heterogeneously distributed among and within cellular membranes



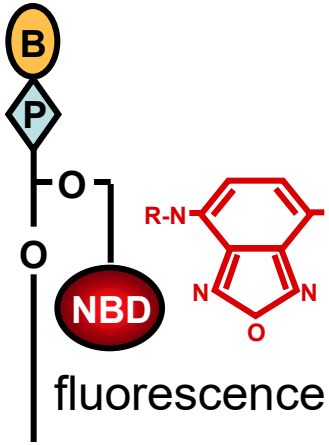
Measuring phospholipid transport across the plasma membrane

spin-labeled phospholipid

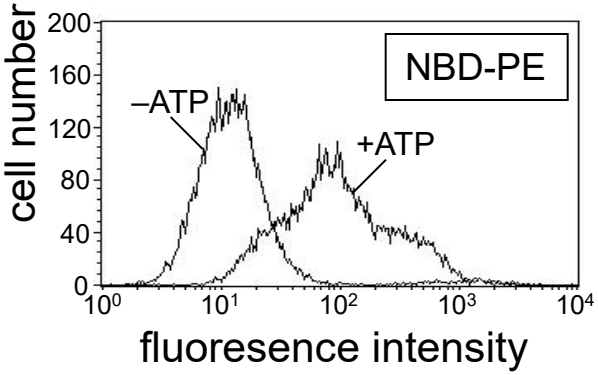


ESR

NBD-labeled phospholipid

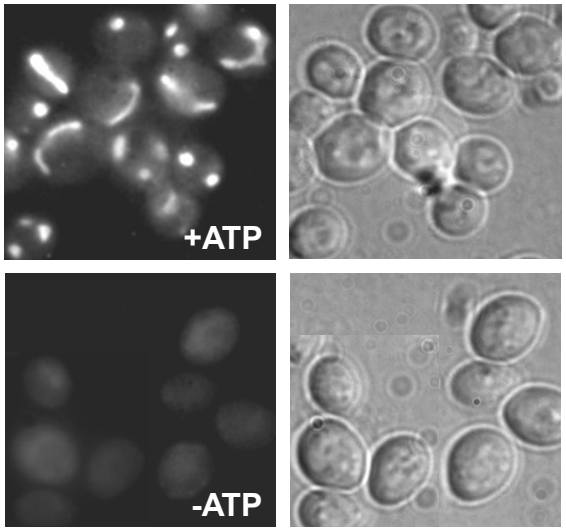


fluorescence

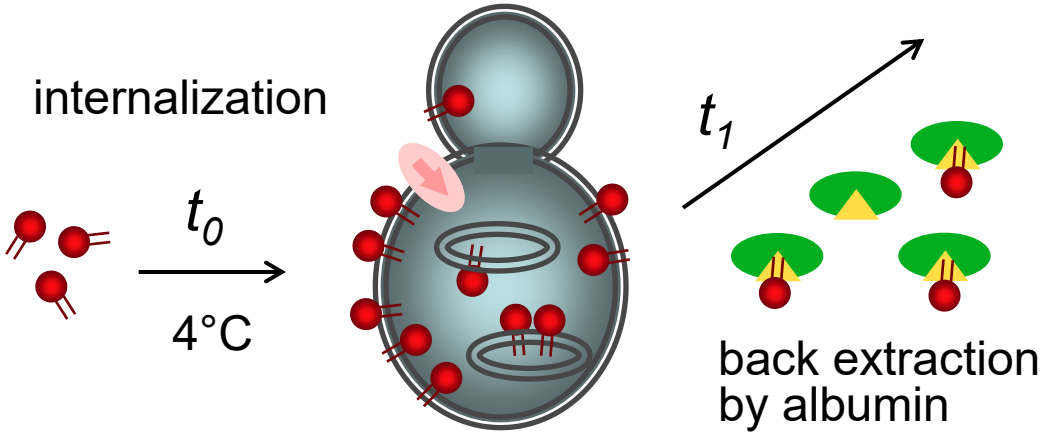


flow cytometry

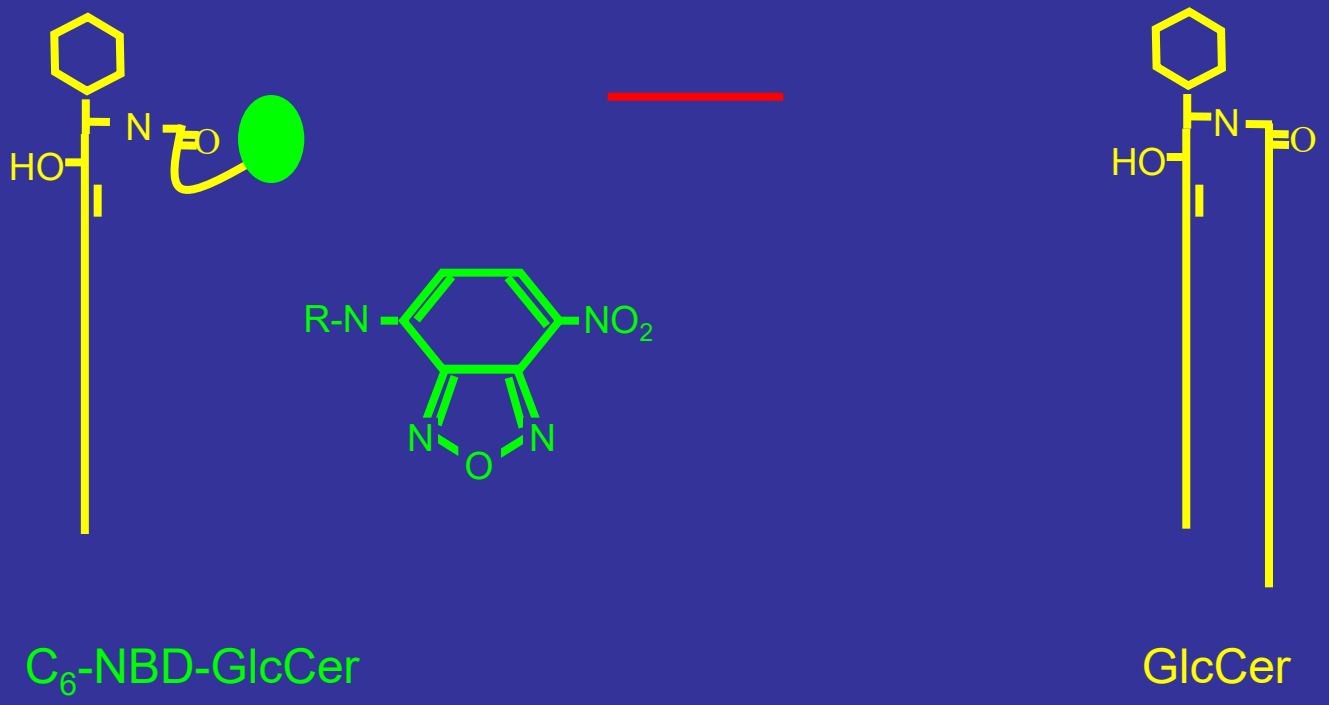
fluoresc. microsc.



internalization



back extraction by albumin



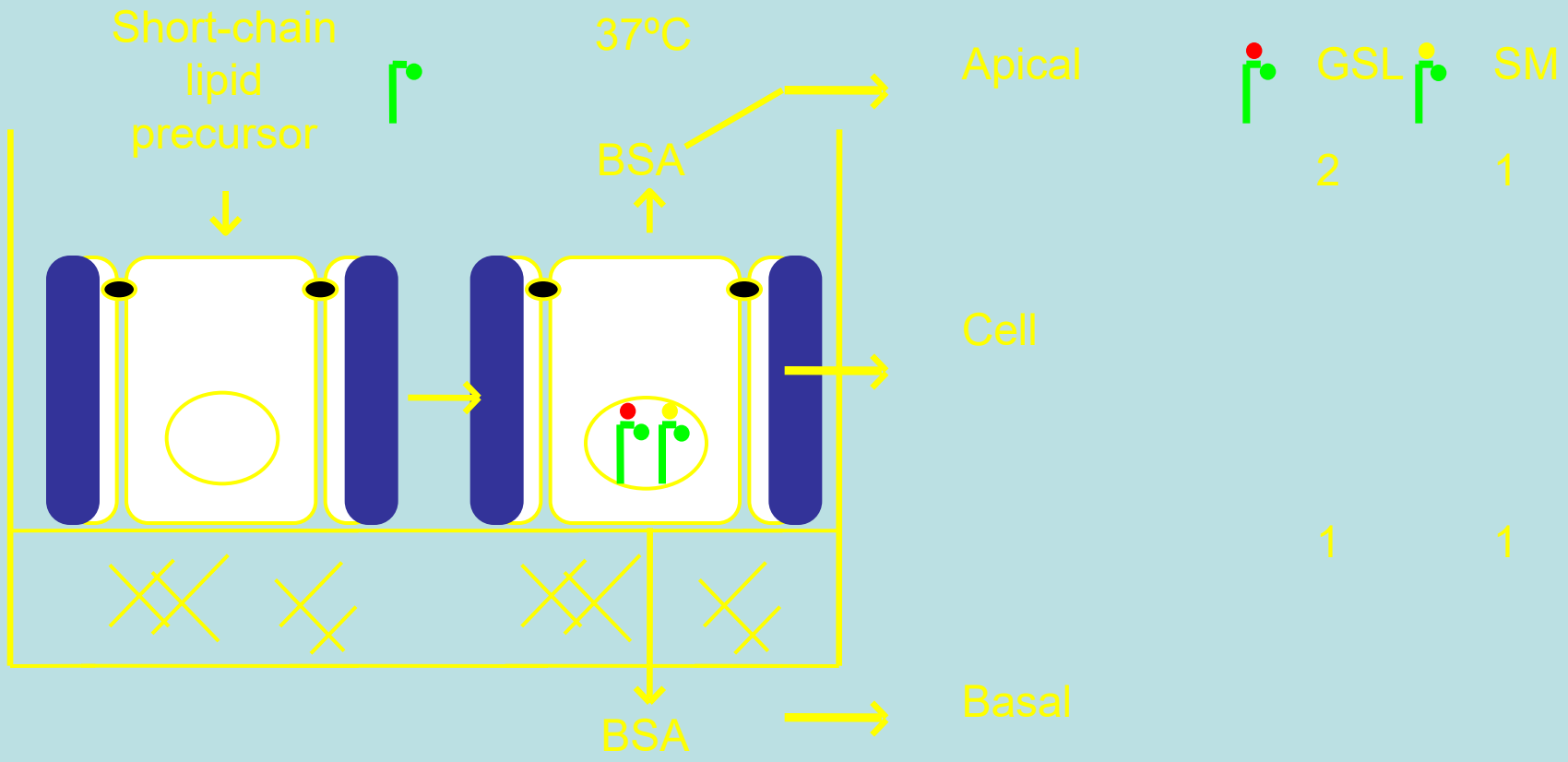
C_6-NBD-GlcCer

GlcCer

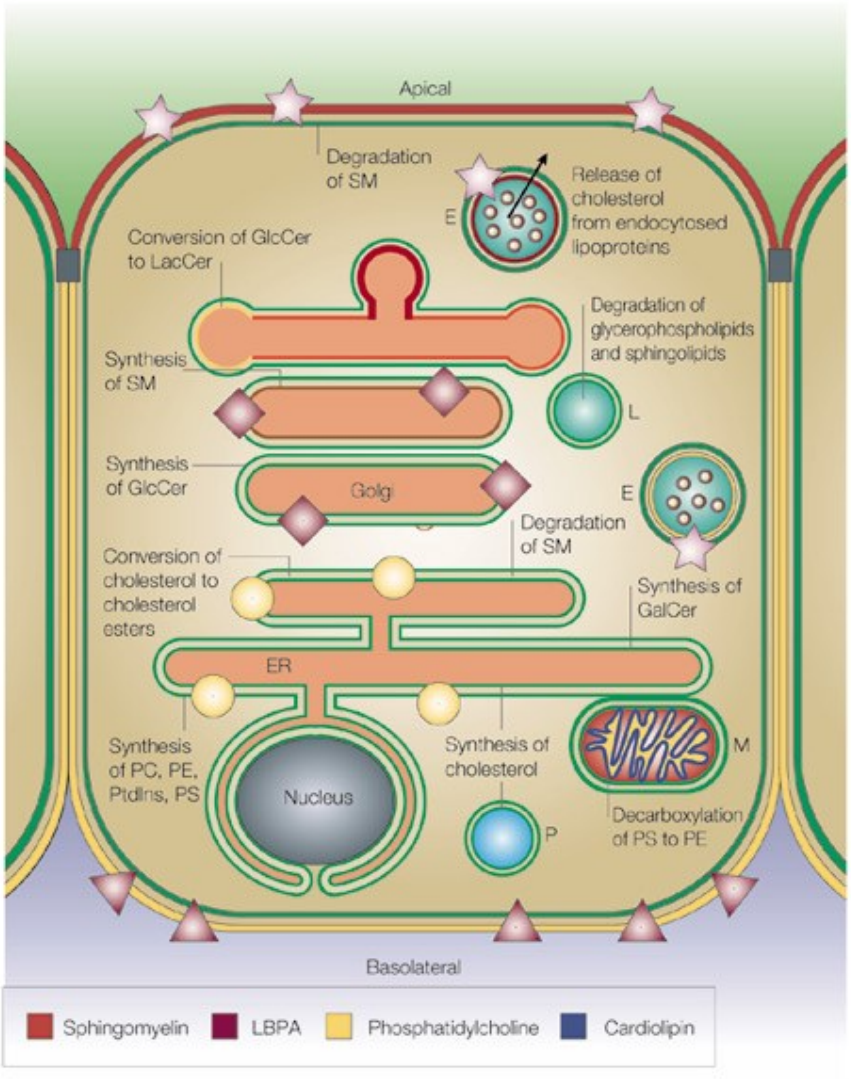
C_6-NBD-lipids are fluorescent and more water-soluble
They can be depleted from membrane surfaces by BSA (Pagano et al., 1983)

(of course, any results obtained with these lipids must be confirmed for real lipids)

Quantitative assay of epithelial lipid transport



Cells use the short-chain precursor for the synthesis of short-chain GlcCer, an apical lipid, and short-chain sphingomyelin, a basolateral lipid. Transport is studied by depleting specifically these lipids from the apical and basal surface by using the l,ipid binding protein BSA.



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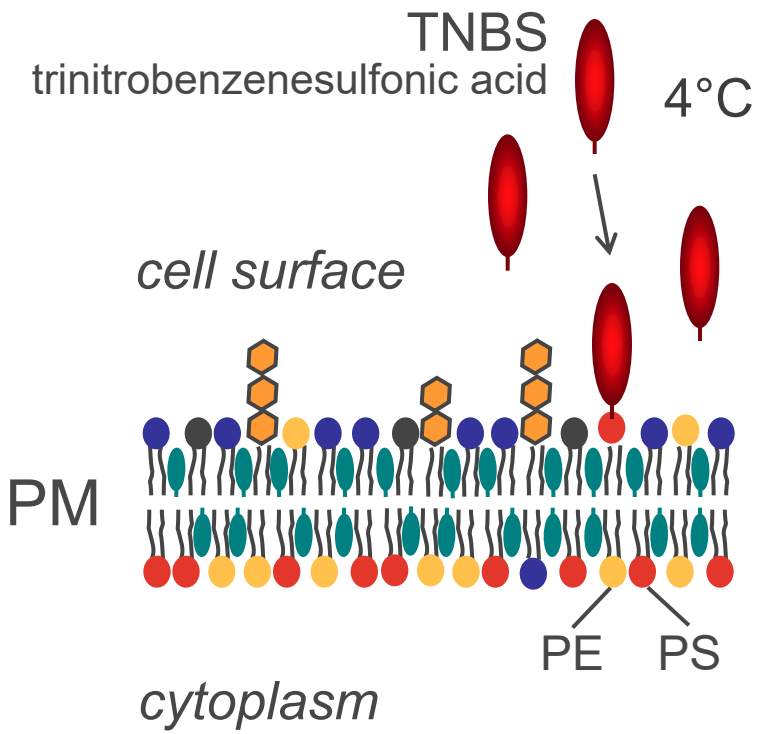
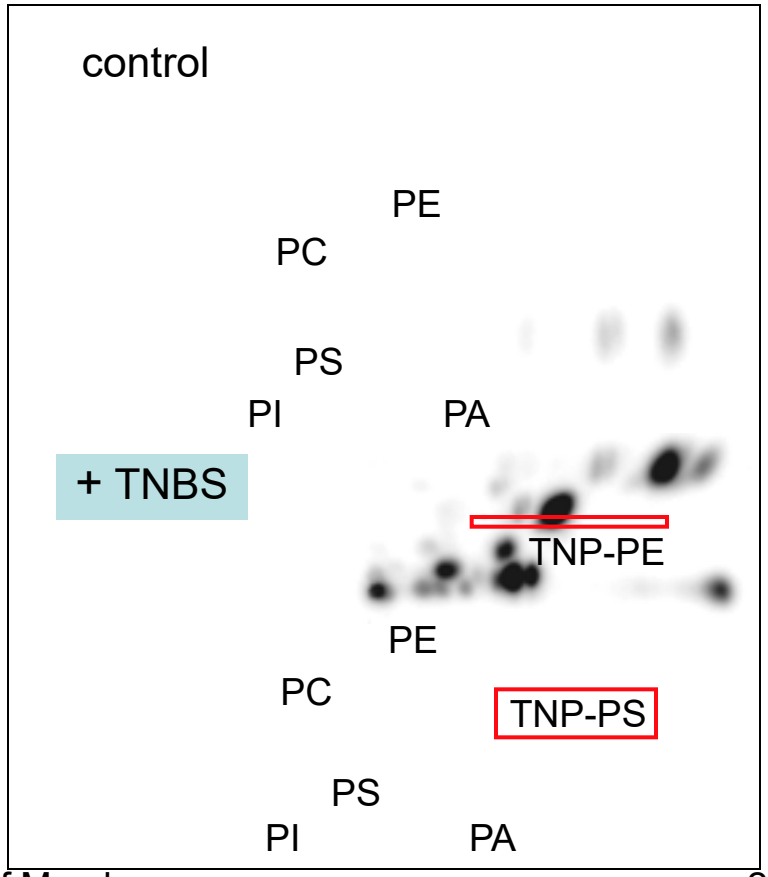
Measuring the aminophospholipid distribution across the plasma membrane

TNBS labeling



Lipid extraction & 2D-TLC analysis



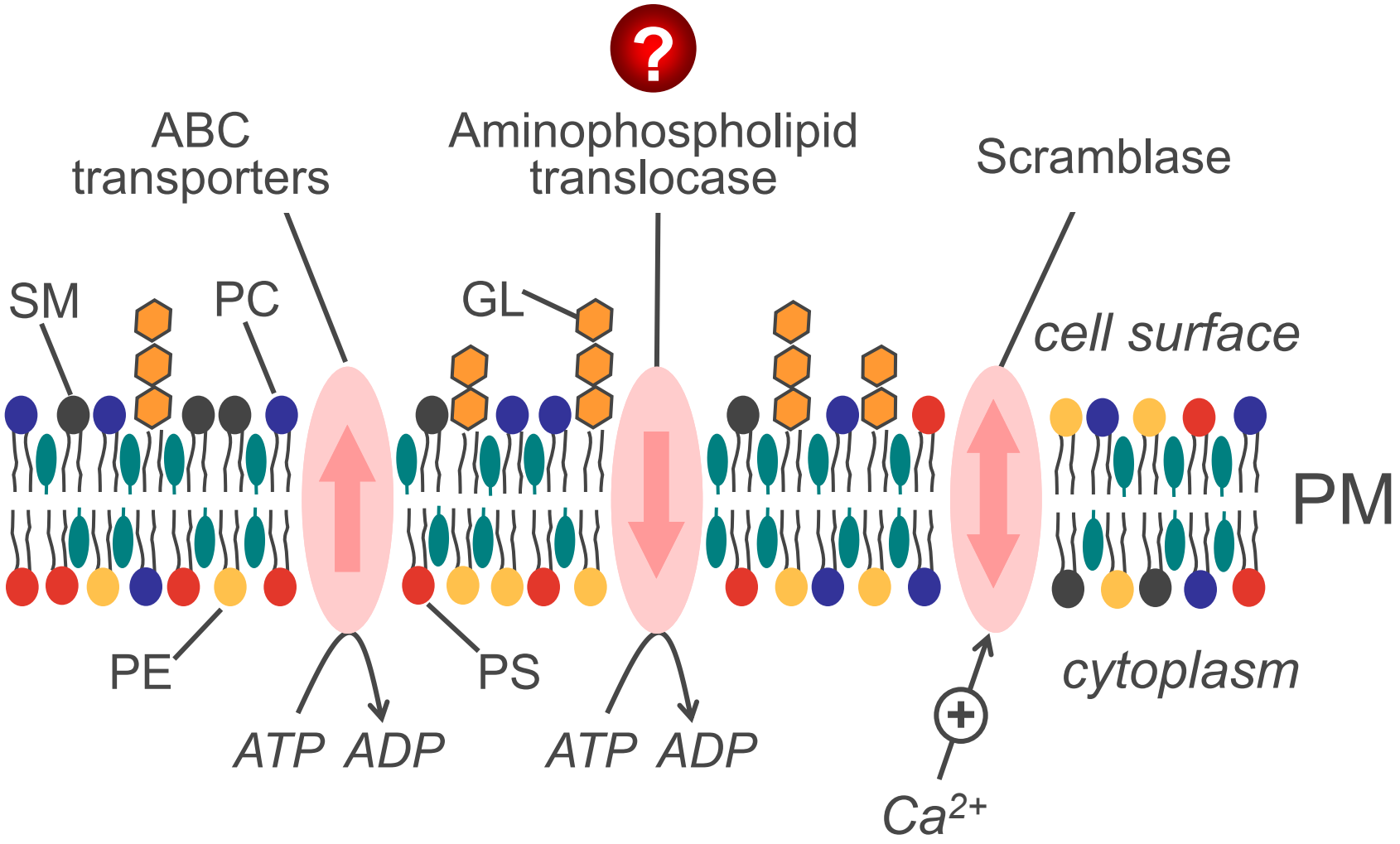
Significance of phospholipid asymmetry at the PM

- PS exposed on surface of activated platelets promotes blood coagulation
  (Rosing *et al.,* 1980)

- PS exposed on surface of apoptotic cells forms recognition signal for engulfment by macrophages
  (Fadok *et al.,* 2000)

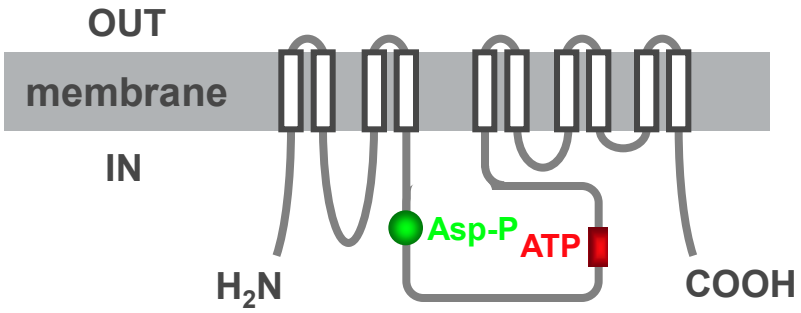
- Dynamic regulation of the transbilayer lipid arrangement is required for the biogenesis of endocytic vesicles
  (postulated by Devaux *et al.,* 1991)

Maintenance of phospholipid asymmetry



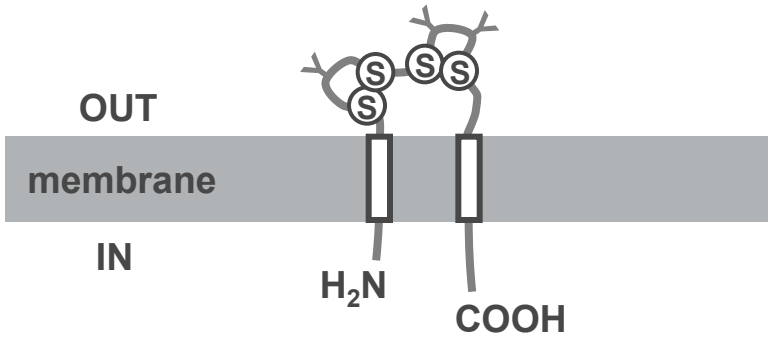
Inward translocation of aminophospholipids across the PM involves members of distinct protein families

1 P-type ATPases



Devaux et al., 1994
Williamson et al., 1996

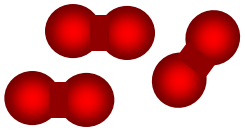
2 Ros3p-related proteins



Umeda et al., 2002

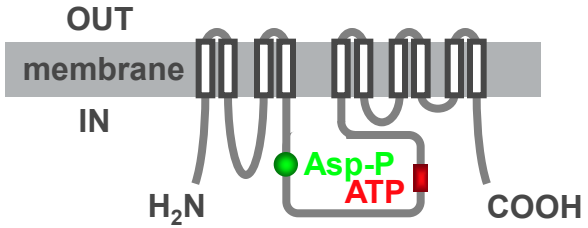
Inward translocation of aminophospholipids across the PM involves P-type ATPases

- Devaux *et al.* (1984) discover a vanadate-sensitive, Mg^{2+}-dependent ATPase mediating a fast ($t_{1/2}$ min) inward-directed transport of NBD-labeled aminophospholipids in human erythrocytes



- Devaux *et al.* (1994) purify and reconstitute a 112 kDa Mg^{2+}ATPase, named ATPase II, that stimulates translocation of NBD-labeled aminophospholipids in proteoliposomes

- Williamson *et al.* (1996) show that ATPase II purified from chromaffin granules is a member of a conserved subfamily of P-type ATPases, and that removal of the yeast homolog - Drs2p - abolishes NBD-PS transport across the PM



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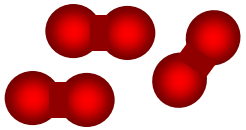

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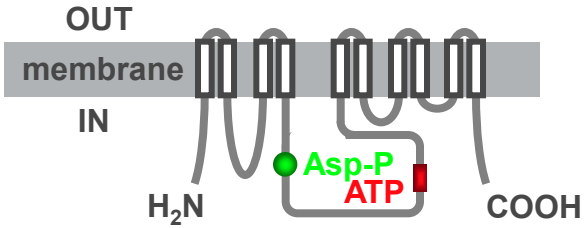
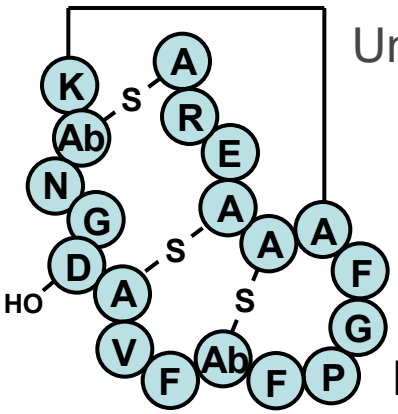


- However, two other groups show that NBD-PS transport in the *drs2* mutant is fine and that Drs2p is localized to the Golgi, not the PM!!

Inward translocation of aminophospholipids across the PM involves members of the Ros3p protein family

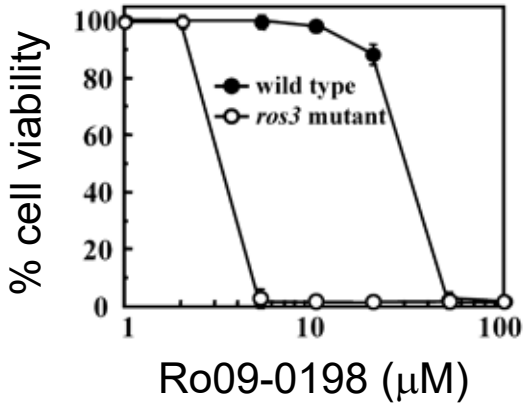
Genetic screen in yeast

- Chemical mutagenesis of yeast
  ethyl methanesulfonate
- Select mutants hypersensitive to cytolytic PE-binding peptide, Ro09-0198

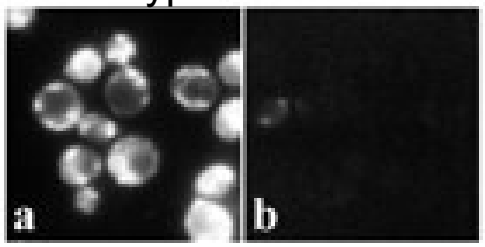


Umeda et al., 2002

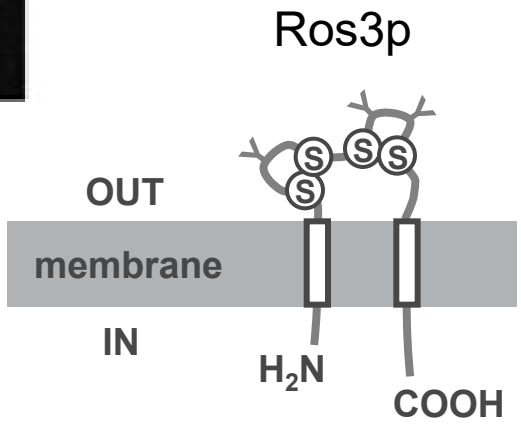
Ro09-0198



NBD-PE



- Transfect *ros3* mutant with gDNA library and isolate gene restoring resistance to Ro-0198



Conclusions

- Flip-flop of phospholipids in liposomes is extremely slow
- Flip-flop in biological membranes is facilitated by membrane proteins and modulated by lipid composition
- Distribution and transport of phospholipids across the PM is subject to a dynamic, ATP-dependent regulation and involves members of distinct protein families
- Regulation of the phospholipid arrangement across the PM has been functionally linked to blood coagulation, clearence of apoptotic cells and endocytsis
- The identity of the lipid translocases operating at the PM remains to be established
- Short-chain phospholipid analogues are extremely usefull to analyse lipid transport across biological membranes, but only partially reflect the behaviour of natural phospholipids

Phospholipids on the move

Text books:

Biochemistry – Stryer	Chpt 12
The Cell – Alberts	-
Biomembranes – Gennis	Chpt 4, 5.4

Review articles:

Sprong, H. , van der Sluijs, P., van Meer, G. Nature Rev. Mol. Cell Biol. 2 (2001) 504-513