Structure of the Plasma Membrane Lecture-2

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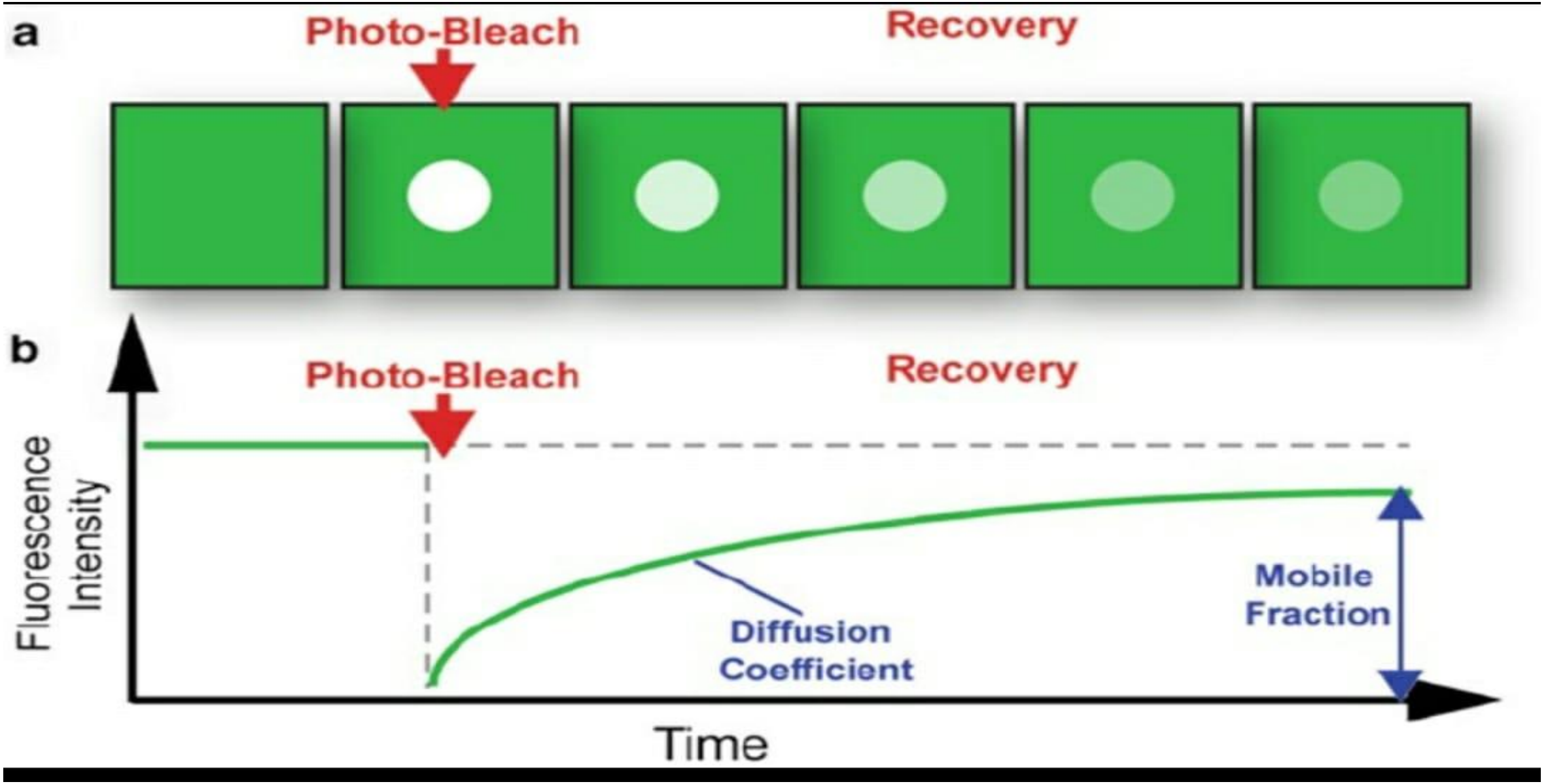
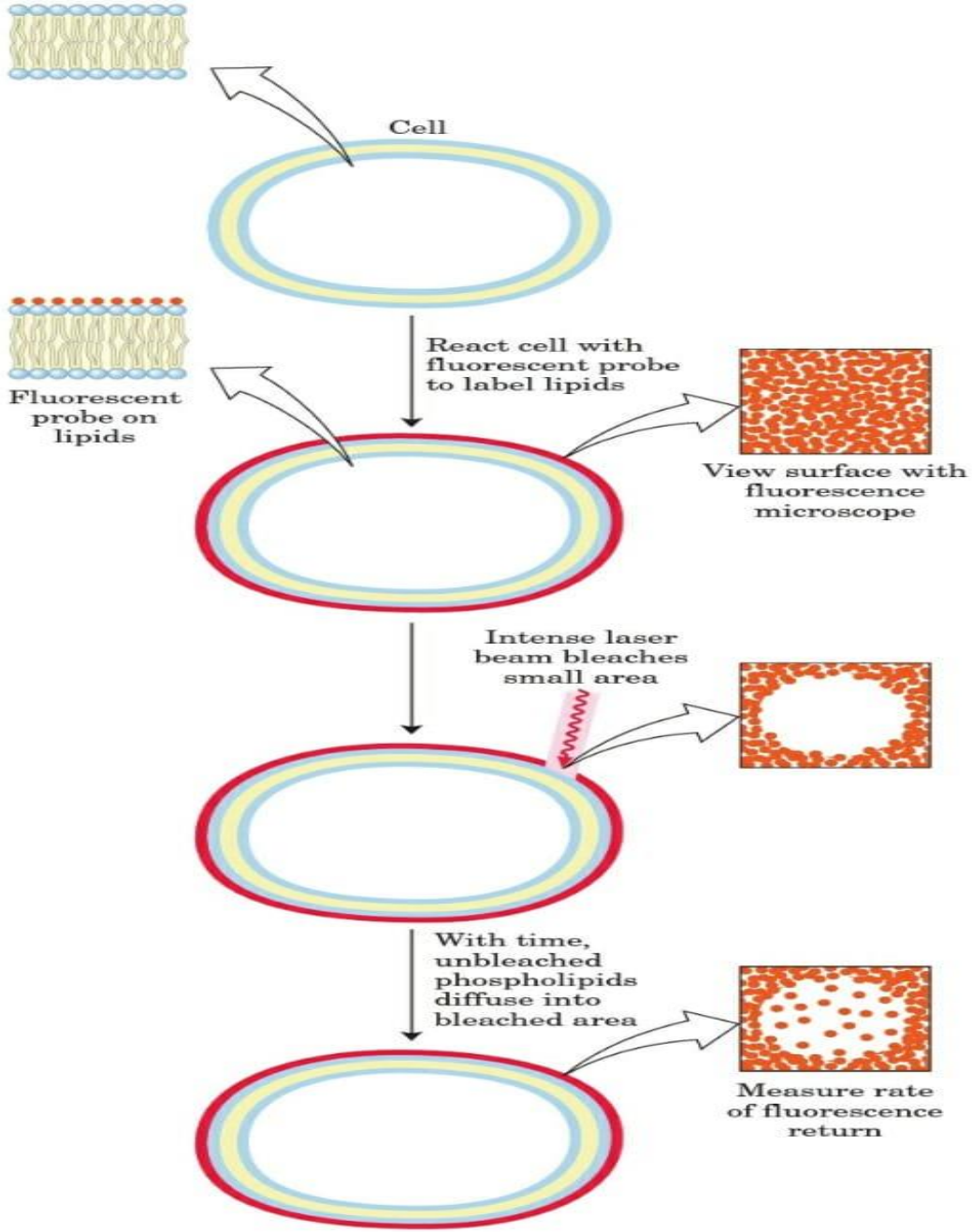
How to study membrane fluidity? FRAP is the method of choice.

- FRAP is abbreviation for Fluorescent recovery after photobleaching.
- It uses fluorescent probes to study the fluidity of a particular component in the PM.
- The mobility rates are determined using the time taken for fluorescence to recover at the spot where it was bleached using high intensity light beam.


a
Photo-Bleach
Recovery
b
Photo-Bleach
Recovery
Fluorescence Intensity
Diffusion Coefficient
Mobile Fraction
Time


Cell
Fluorescent probe on lipids
React cell with fluorescent probe to label lipids
View surface with fluorescence microscope
Intense laser beam bleaches small area
With time, unbleached phospholipids diffuse into bleached area
Measure rate of fluorescence return

Micelle or liposome?

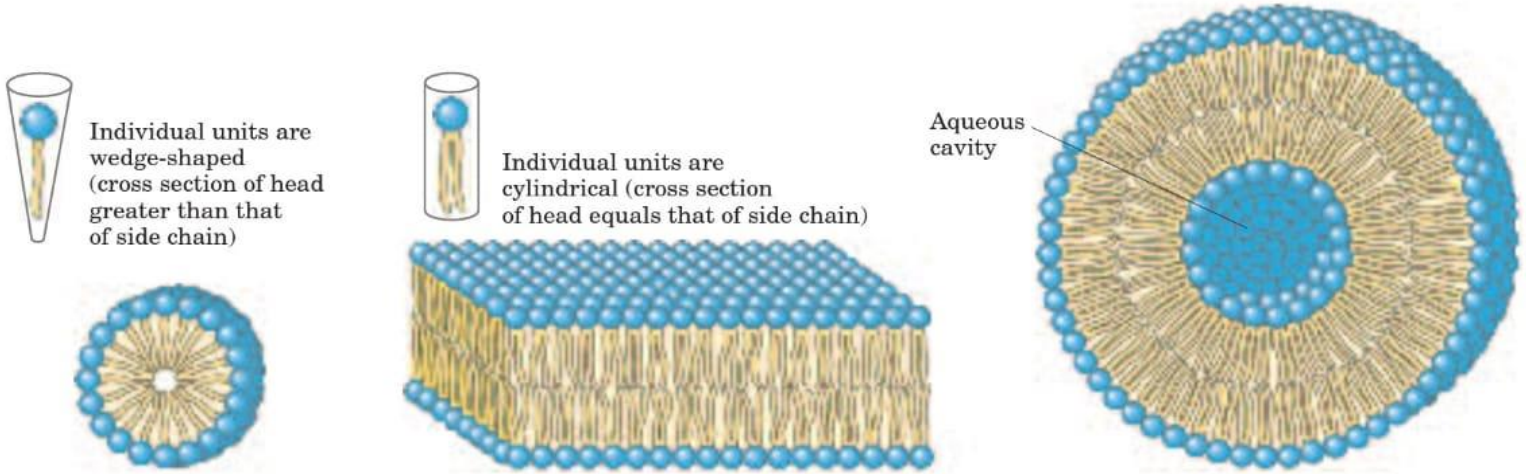


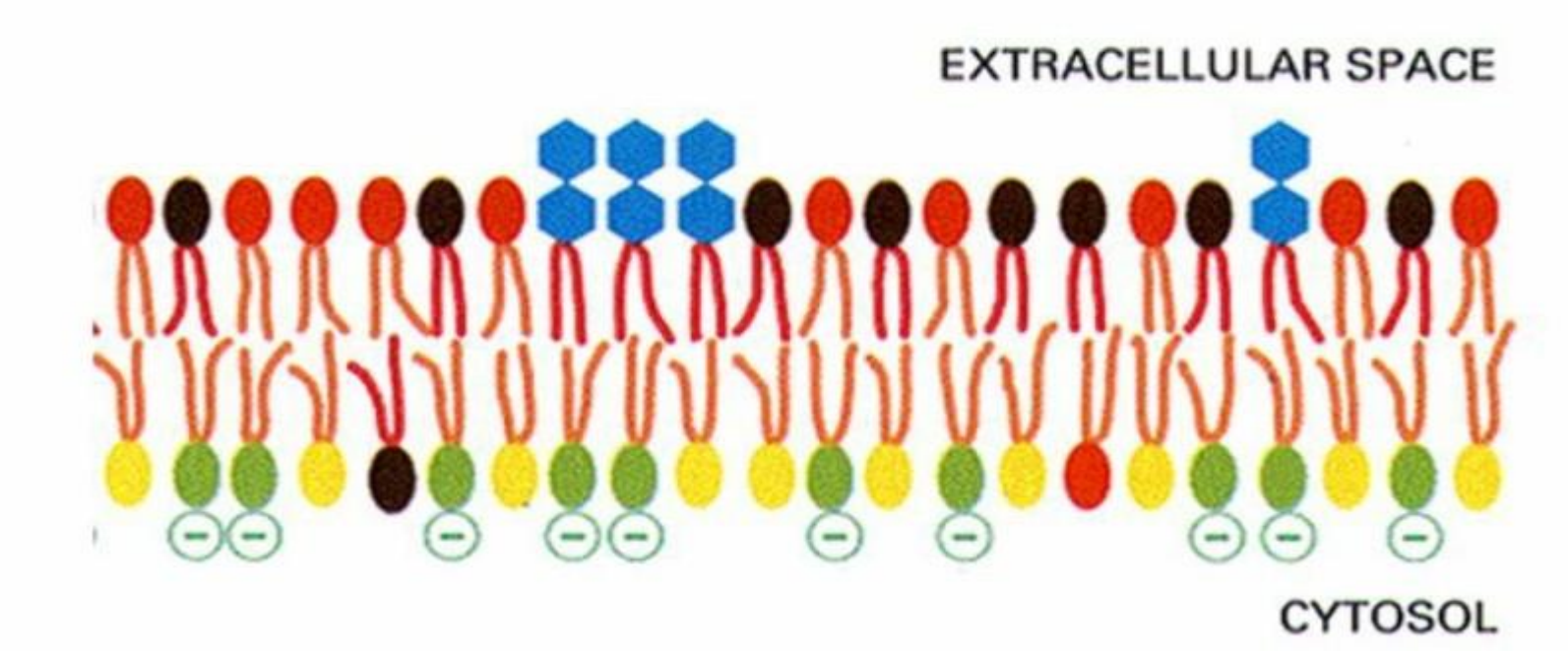
FIGURE 11–4 Amphipathic lipid aggregates that form in water. **(a)** In micelles, the hydrophobic chains of the fatty acids are sequestered at the core of the sphere. There is virtually no water in the hydrophobic interior. **(b)** In an open bilayer, all acyl side chains except those at the edges of the sheet are protected from interaction with water. **(c)** When a two-dimensional bilayer folds on itself, it forms a closed bilayer, a three-dimensional hollow vesicle (liposome) enclosing an aqueous cavity.

- Micelle is formed when we add free fatty acid or detergent in aqueous medium.
- Liposome is formed when phospholipids and sphingolipids are added in aqueous solution.

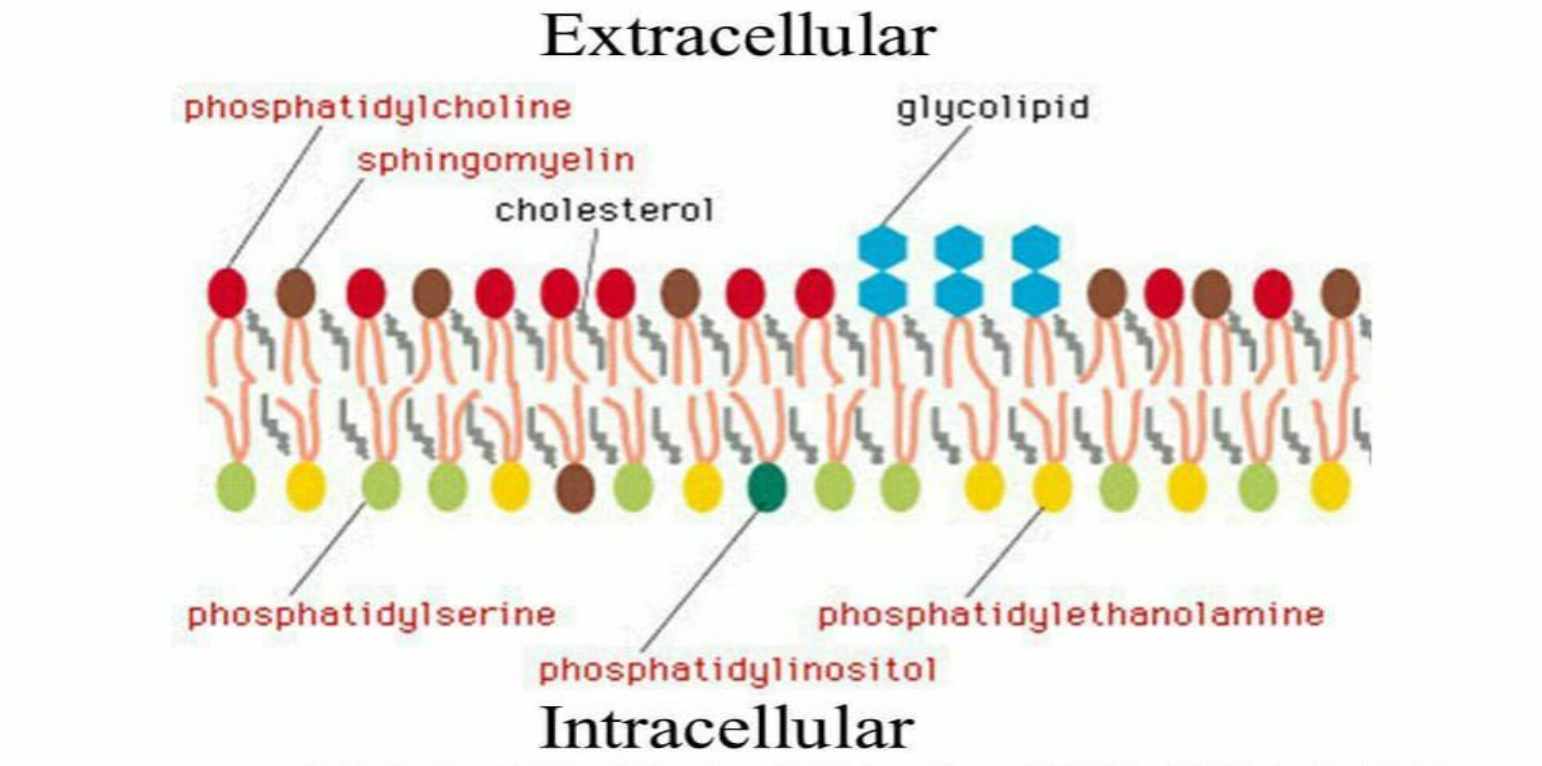
Membrane asymmetry

- Plasma membrane has two faces viz cytosolic face because it faces cytosol and exoplasmic face because it faces extracellular side.

- These two layers form each layer of the bilayer structure.



- Composition of these two layers is different in terms of lipids.
- Thus, lipids are said to have asymmetric distribution in the plasma membrane.
- It gives rise to membrane asymmetry.

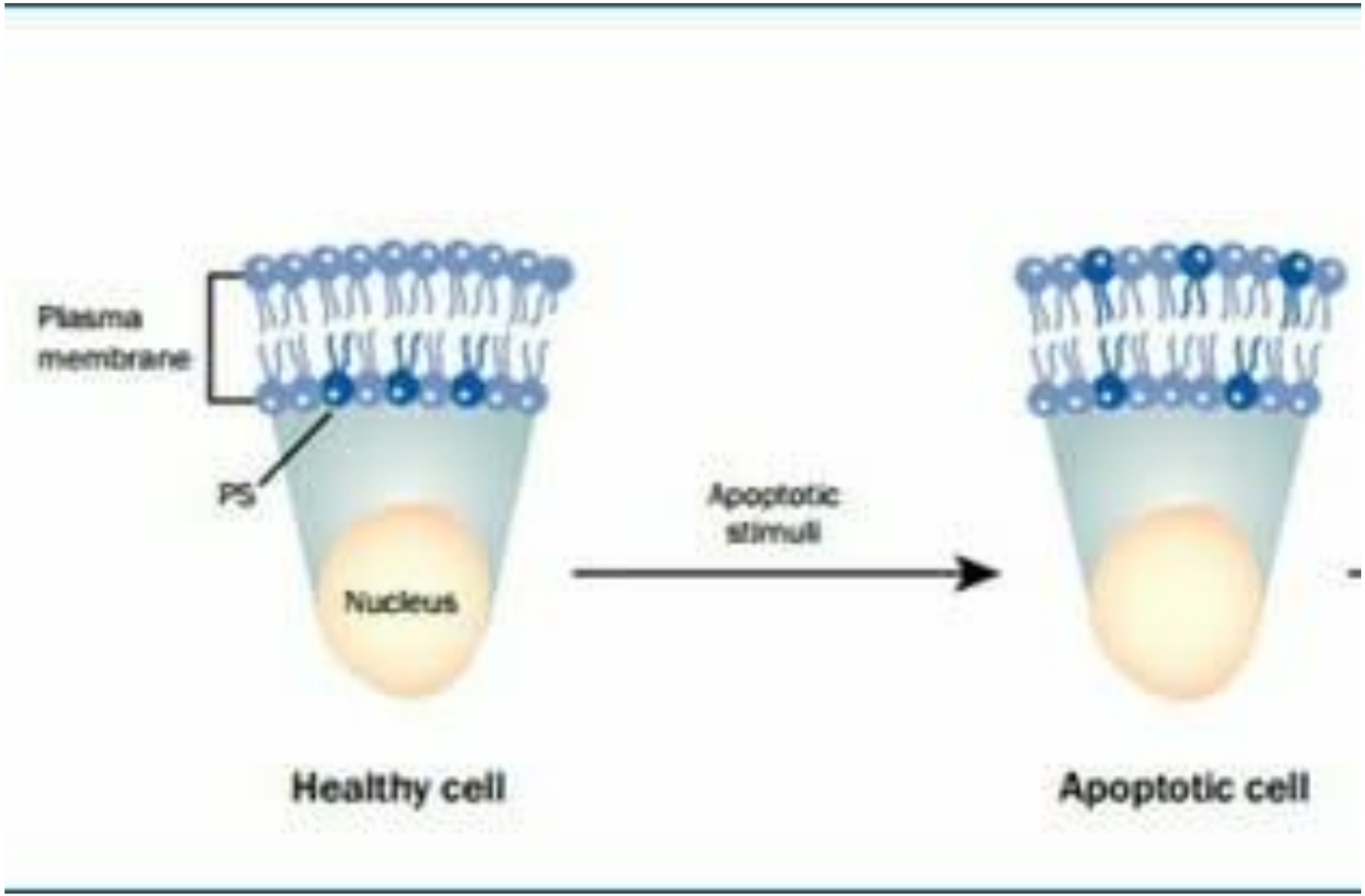


- This asymmetry has functional importance.

- Movement of phosphatidylserine to the outer leaflet in plasma membrane helps in blood clotting.

- In nucleated cells, exposure of phosphatidylserine to the outer leaflet marks the cell for destruction by apoptosis.

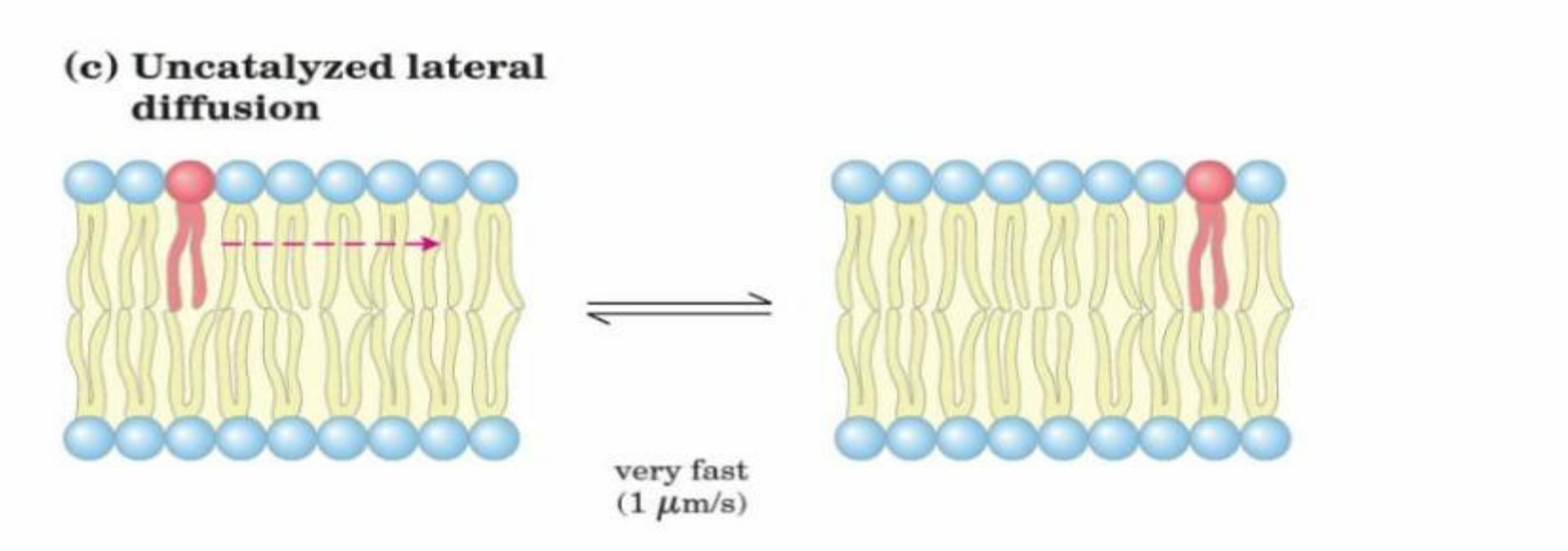
- Thus, cells must be able to maintain this asymmetry for their proper functioning.


Plasma membrane
PS
Nucleus
Apoptotic stimuli
Healthy cell
Apoptotic cell

Maintaining membrane asymmetry.

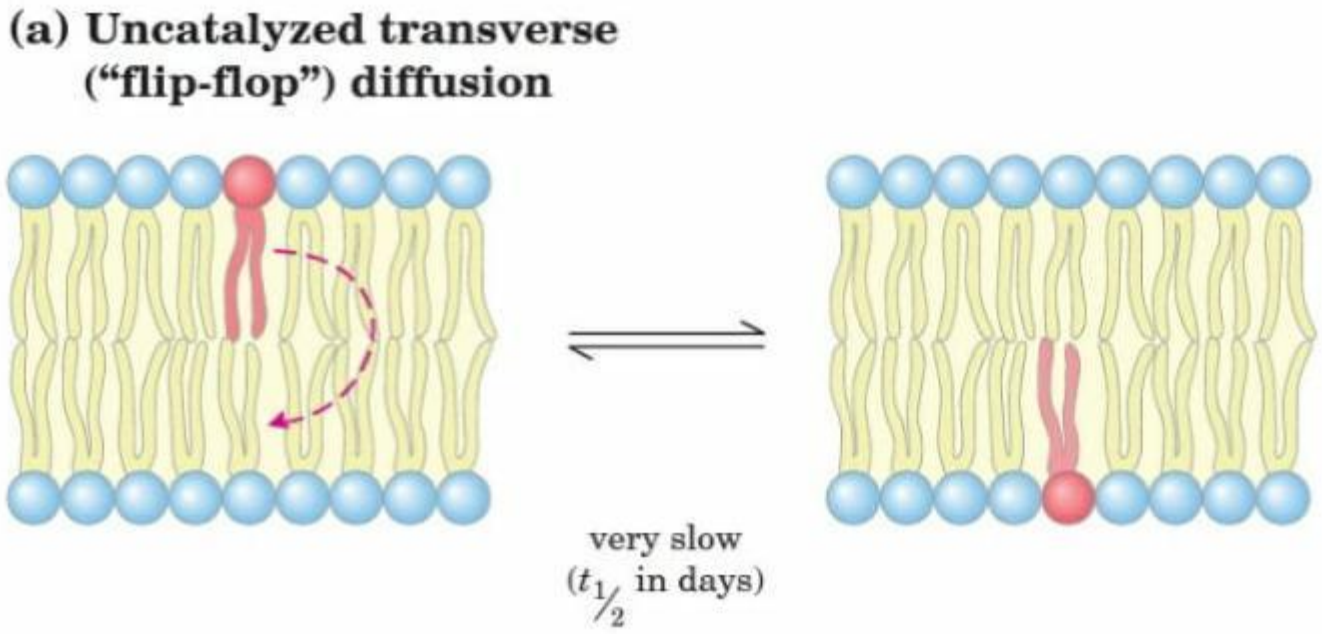
- Lipids in plasma membrane are capable of two types of diffusion.

- 1. Lateral diffusion in given monolayer i.e. Either cytosolic or exoplasmic.
- This movement is efficient without any catalysis but has no role in membrane asymmetry.

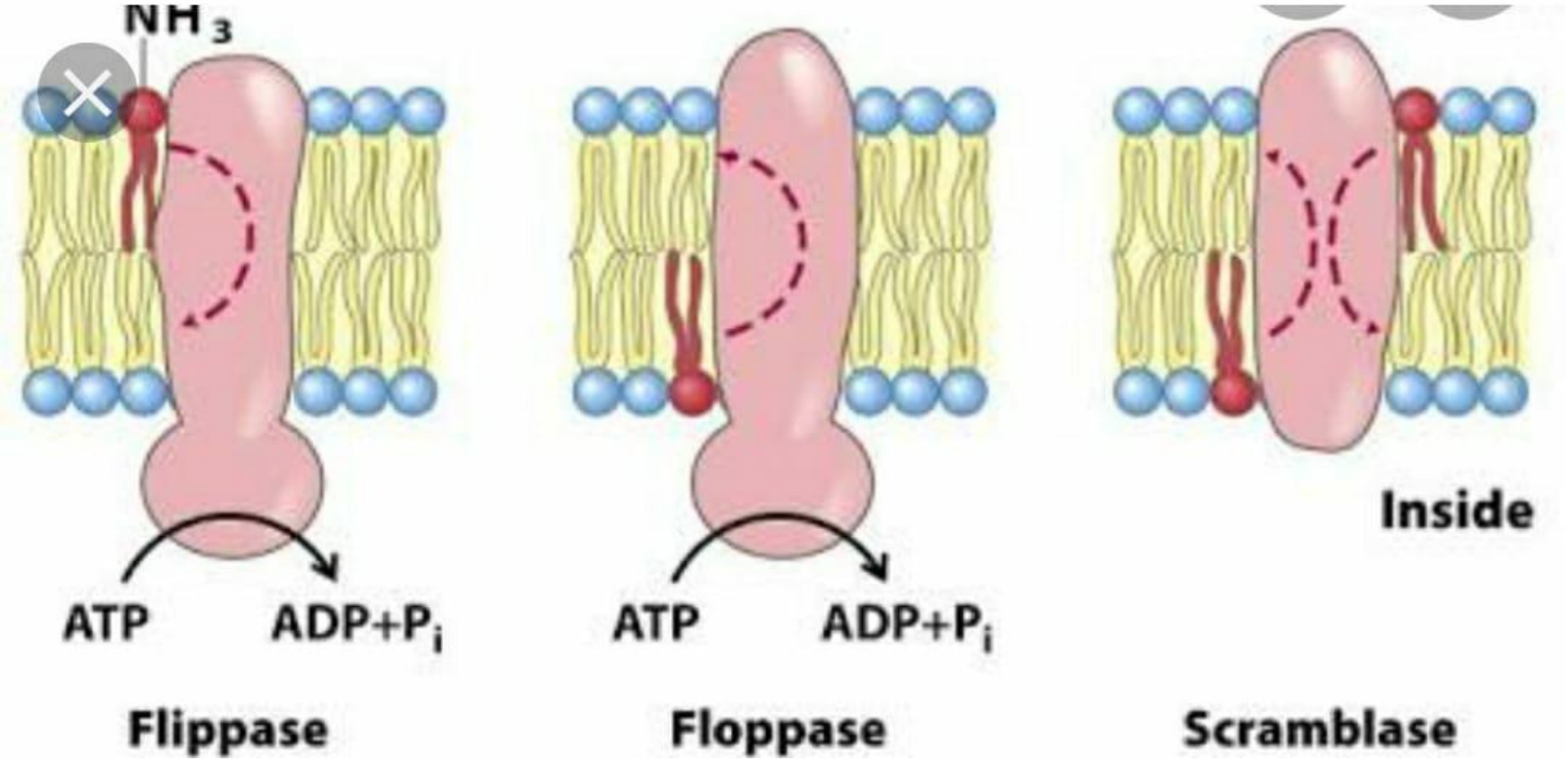


- The other possible movement type is transverse diffusion from one monolayer to the other.

- This is inefficient without any catalysis but has effect on membrane asymmetry.



- This transverse diffusion is called as flip flop and is catalysed by enzymes so that membrane asymmetry can respond to needs of the cell. These enzymes are



Flippase: *Outer to cytosolic leaflet.*

Floppase: *Cytosolic to outer leaflet.*

Scramblase: *To either leaflet.*

Brainstorming for concepts.

Question 1.

Phosphotidyl serine, an important component of biological membrane. is located in

(a) the outer leaflet but flip flop to inner

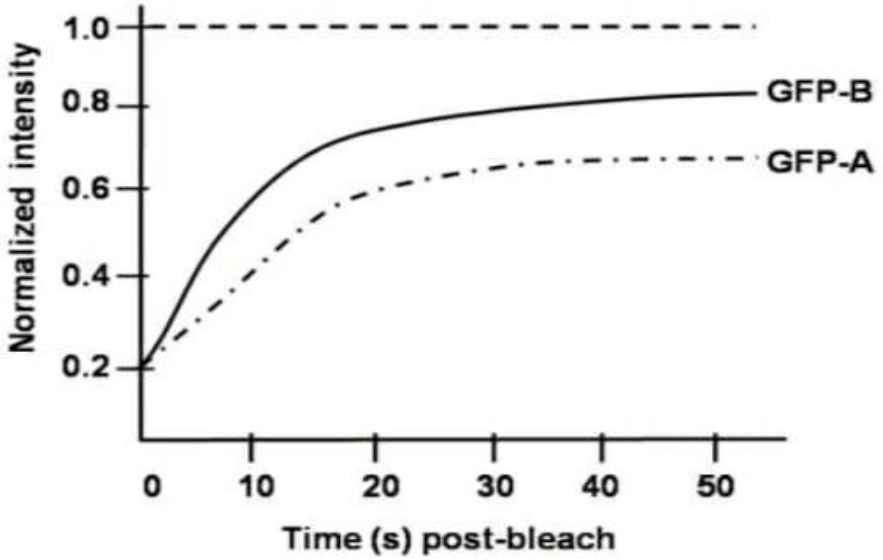
(b) both leaflets of plasma membrane

(c) middle of the bilayer

(d) the inner leaflet but flip flops to outer leaflets under specific conditions

Question 2

To investigate the dynamic nature of two unrelated centrosome-localized GFP-tagged proteins [GFP-A; GFP-B], a team of scientists conducted fluorescence recovery after photo bleaching (FRAP) experiment. The FRAP profile of these two proteins is given below:



The following statements for this FRAP analysis were made

A. GFP-B shows faster exchange rate than GFP-A

B. GFP-A shows faster exchange rate than GFP-B

C. GFP-A has more immobile fraction than GFP-B

D. GFP-B has more immobile fraction than GFP-A

Which of the above mentioned statements for GFP-A and GFP-B are correct?

(1) A and C

(2) A and D

(3) B and C

(4) B and D

Question 3

Column A		Column B	
A	Lipases	(i)	Catalysis of ATP-dependent translocation of the aminophospholipids, phosphatids, phosphatidylethanolamine and phosphatidylserine and phosphatidylserine from the extracellular to the cytosolic leaflet of the plasma membrane.
B	Flippases	(ii)	Catalysis of ATP-dependent translocation of the plasma membrane phospolipids from the cytosolic to the extracellular leaflet.
C	Floppases	(iii)	Catalyze hydrolysis of triacyglycerols.
D	Scramblases	(iv)	Catalyze the movement of any membrane phospholipd across the bilayer down its concentration gradient

Thank you