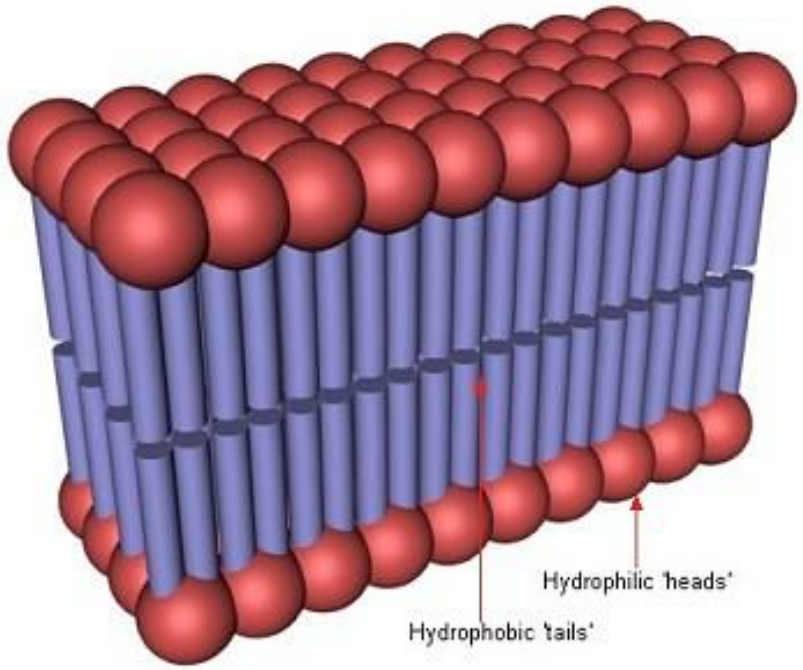
Struktura in dinamika bioloških membran

Stryer (5th), 12. poglavje
Voet (4th), 12. poglavje

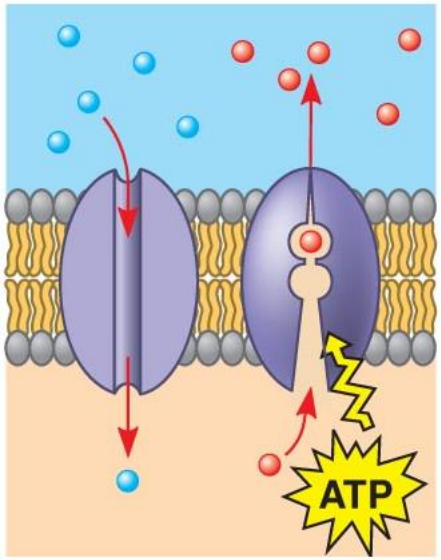


Pregled

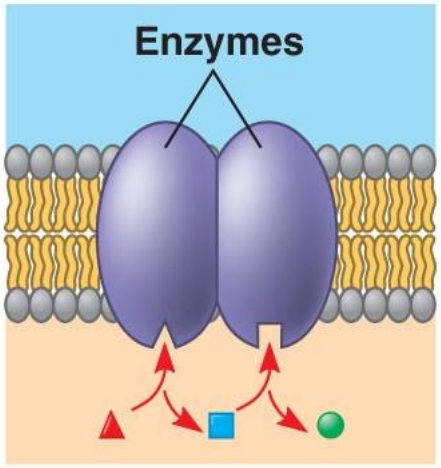
- Funkcije bioloških membran
- Lastnosti lipidnih membran
- Sestava membran
- Membranski lipidi
- Membranski proteini

Funkcije bioloških membran

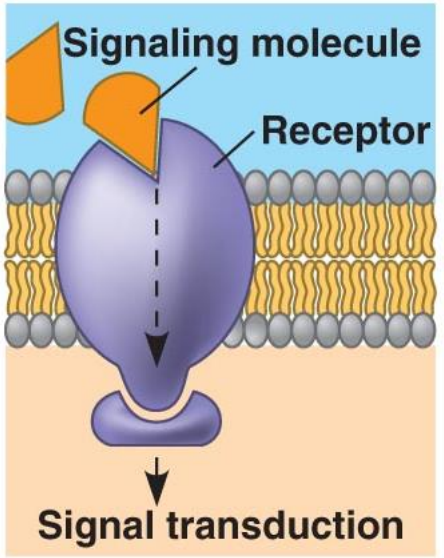
- (Selektivna) bariera za molekule.
- Energijska zaloga in pretvorbe.
- Prenos in modulacija signalov.
- Celični transport.
- Kompartimentizacija in akumulacija metabolitov.
- Posredovanje pri medceličnih interakcijah.
- Pomoč pri pomnoževanju celic (delitev).



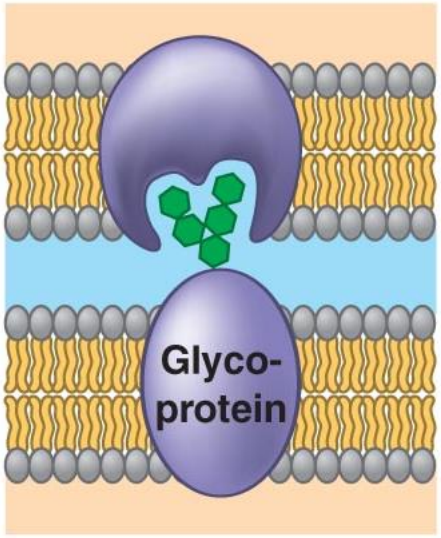
(a) Transport



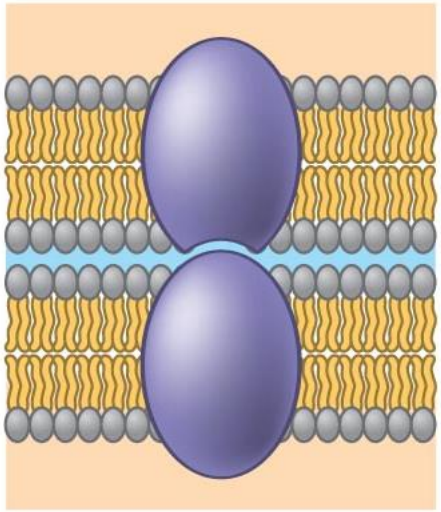
(b) Enzymatic activity



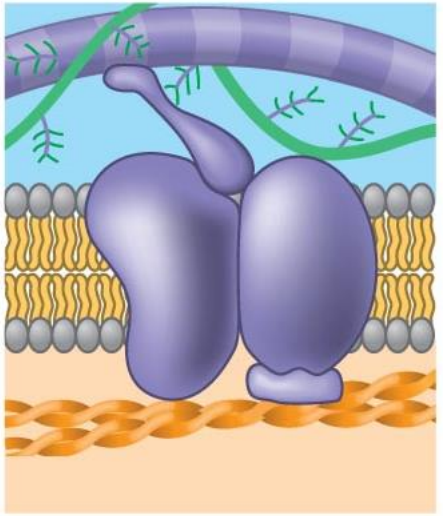
(c) Signal transduction



(d) Cell-cell recognition



(e) Intercellular joining



(f) Attachment to the cytoskeleton and extracellular matrix (ECM)

Lastnosti membran

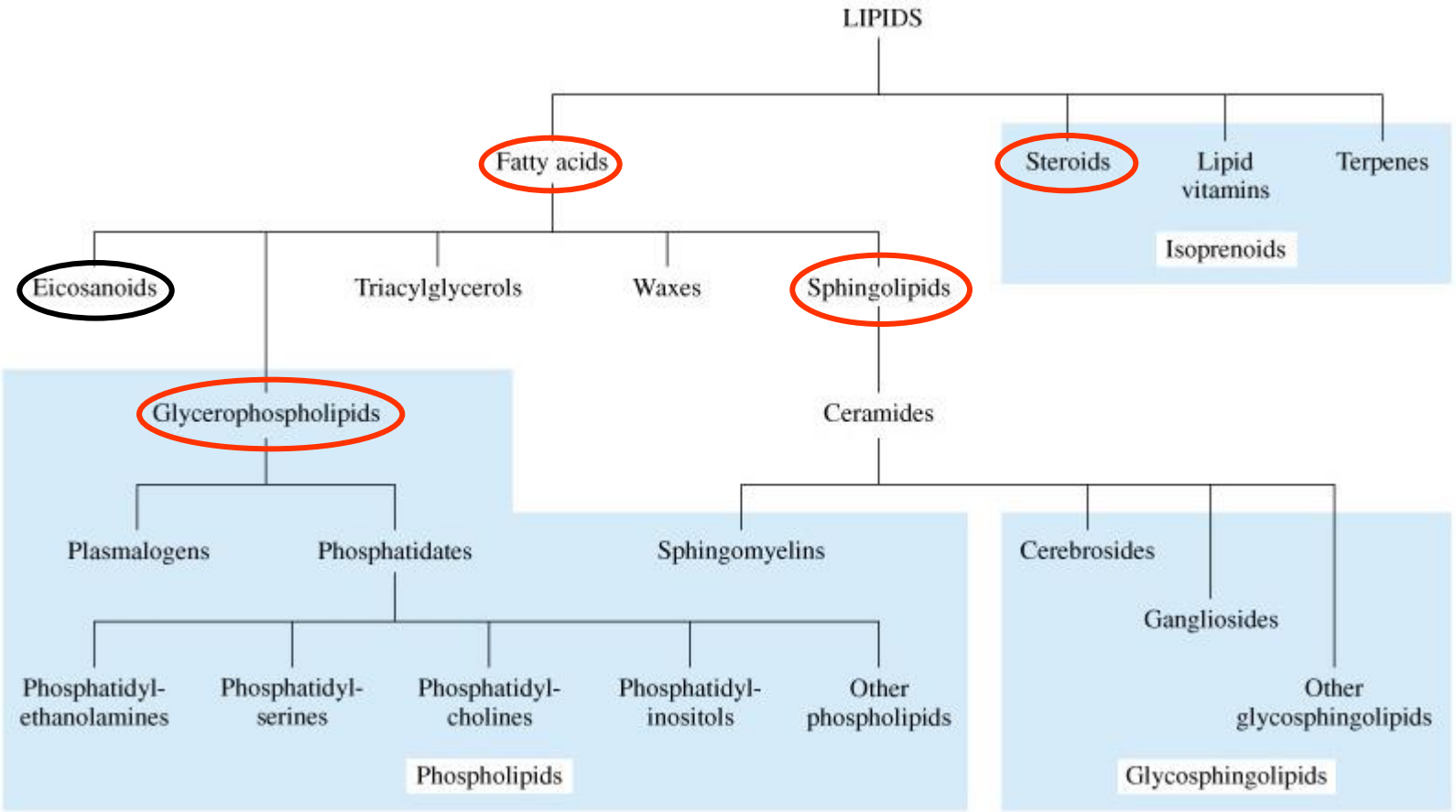
- Debelina 6-10 nm (2 molekuli).
- Sestavljene predvsem iz lipidov in proteinov.
- Lipidi predstavljajo hidrofobno bariero.
- Različnost funkcij predvsem zaradi proteinov.
- Asimetričnost.
- Tekoča (fluidna) struktura.
- Večina električno polarizirana.

Sestava bioloških membran (utež.%)

MEMBRANA	PROT.	LIP.	O.H.	P/L
Mielin	18	79	3.0	0.23
Trombocit (p.m.)	33-42	51-58	7.5	0.70
Miš. jetra (p.m.)	46	54	2-4	0.85
Človr. eritrocit	49	43	8	1.10
Ameba	54	42	4	1.30
Podg.jetra (p.m.)	58	42	5-10	1.40
Podg.jetra (j.m.)	59	35	2.9	1.60
Gov.retina (palč.)	51	49	4	1.00
Mitohondrij (z.m.)	52	48	2-4	1.10
SER	67	33	-	2.00
Kloroplast (lam.)	70	30	6	2.30
Mitohondrij (n.m.)	76	24	1-2	3.20
Halobacterium sp.	75	25	-	3.00
Mycoplasma sp.	58	37	1.6	1.60

LIPIDI

- **gradniki bioloških membran**
- **signalne molekule**
- **visoko-koncentrirana zaloga energije**



Membranski lipidi
(vsi so amfipatični)

- Maščobne kisline
- Sestavljeni lipidi (mašč. kisline + alkohol)
  - Glicerofosfatidi
  - Sfingolipidi
- Enostavni lipidi (izoprenski derivati)
  - Steroidi (holesterol)

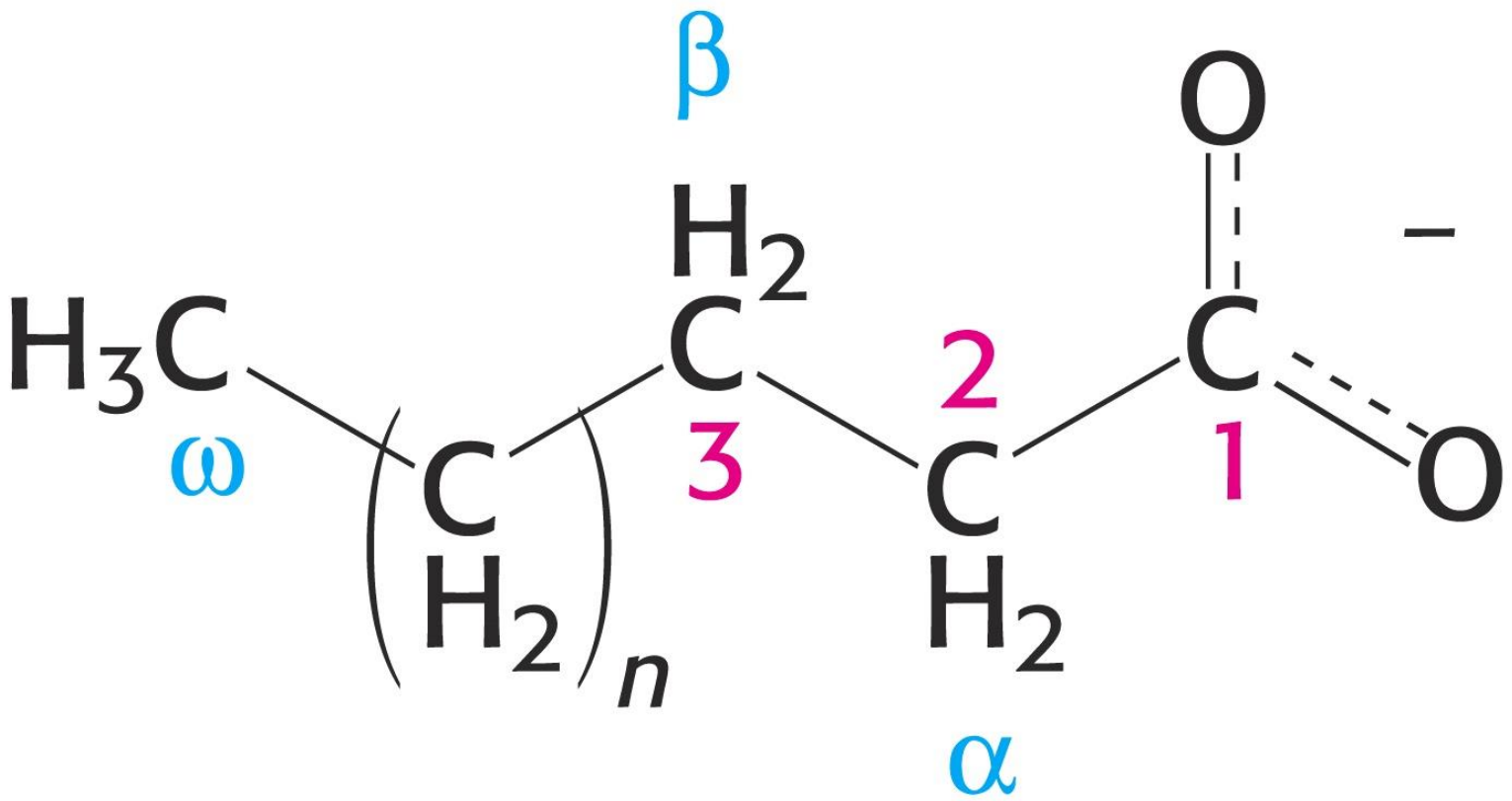
Maščobne kisline

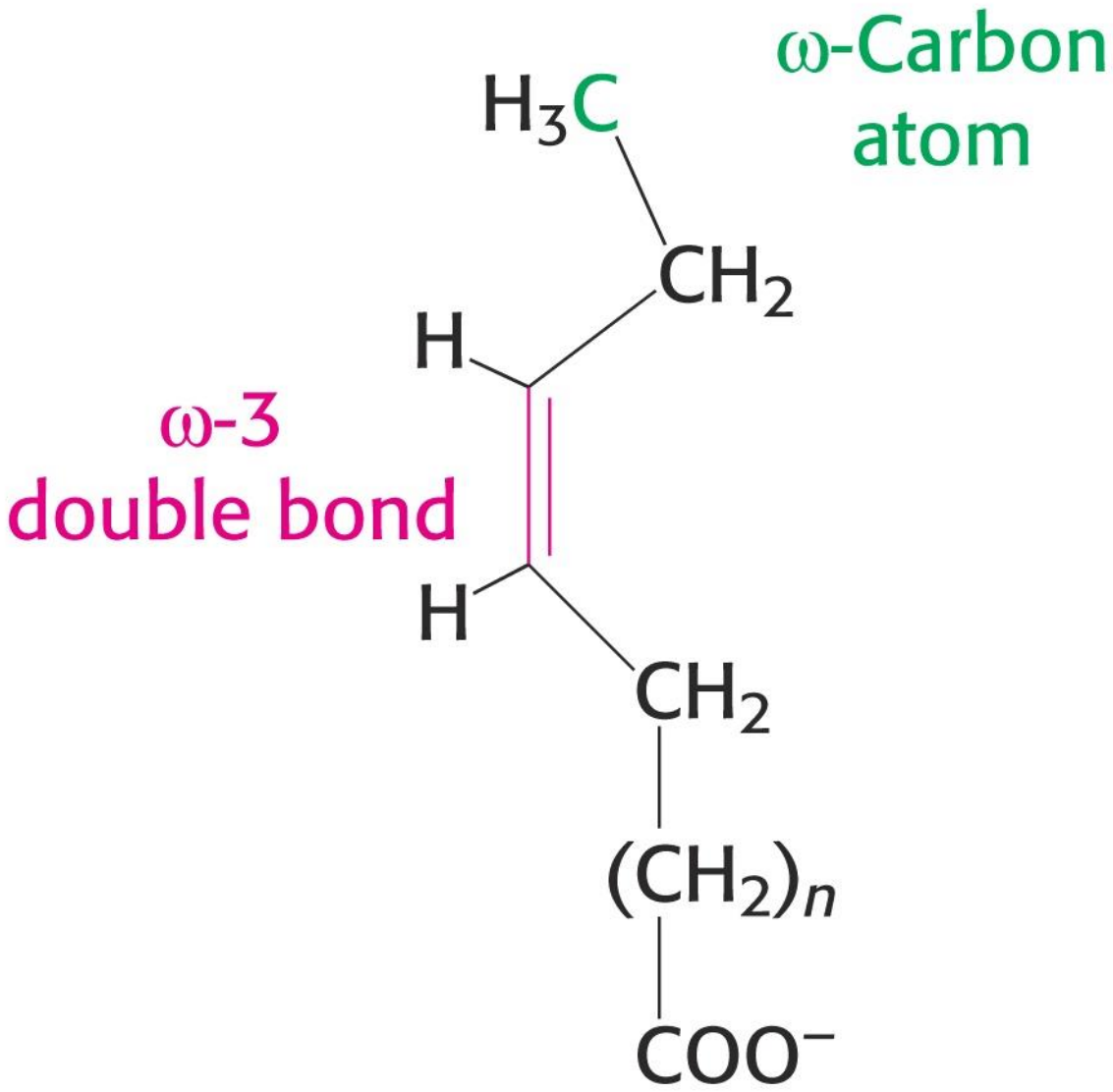
Maščobne kisline v bioloških membranah 14 – 24 C; najpogosteje 16 in 18 C atomov.

- Nasičene R-COOH
  - Laurinska kisl. $C_{12:0}$ – dodekanojska k.
  - Miristinska kisl. $C_{14:0}$ – tetradekanojska k.
  - Palmitinska kisl. $C_{16:0}$ – heksadekanojska k.
  - Stearinska kisl. $C_{18:0}$ – oktadekanojska k.
  - Arahidinska kisl. $C_{20:0}$ – eikozanojska k.

Maščobne kisline

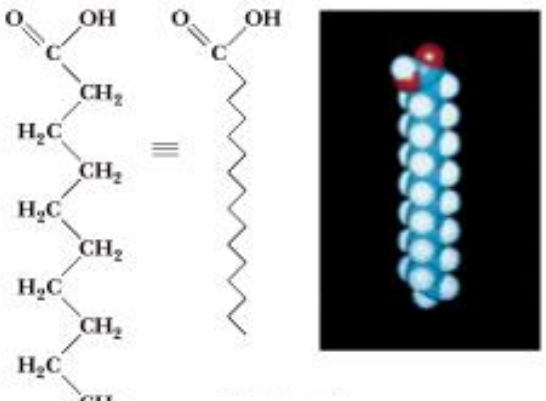
- Nenasičene — $R\text{-}CH{=}CH\text{-}(CH_2)_n\text{-}COOH$
  - Palmitoleinska kisl. — *cis*-Δ^9-$C_{16:1}$ (heksa<u>dekeno</u>jska k.)
  - Oleinska kisl. — *cis*-Δ^9-$C_{18:1}$ (okta<u>dekeno</u>sjka k.)
  - Linolna kisl. — *cis*-$\Delta^{9,12}$-$C_{18:2}$ (okta<u>dekadieno</u>jska k.)
  - α-Linolenska kisl. — *cis*-$\Delta^{9,12,15}$-$C_{18:3}$ (okta<u>dekatrieno</u>jska k.)
  - γ-Linolenska kisl. — *cis*-$\Delta^{6,9,12}$-$C_{18:3}$ (okta<u>dekatrieno</u>jska k.)
  - Arahidonska kisl. — *cis*-$\Delta^{5,8,11,14}$-$C_{20:4}$ (eikoza<u>tetraeno</u>jska k.)
- Vse dvojne vezi: *cis*-konfiguracija !


β
O
H2
–
H3C
C
2
C
ω
3
1
O
C
H2
H2
n
α

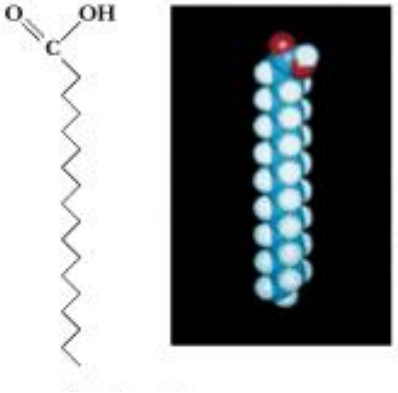


An ω-3 fatty acid

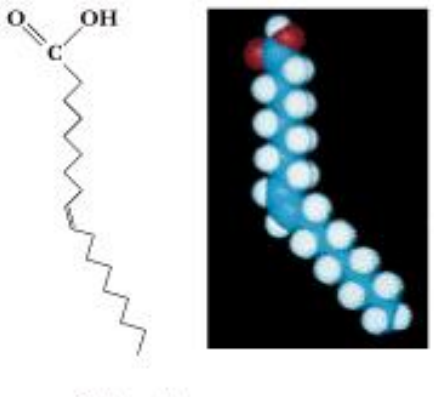
Zgradba maščobnih kislin



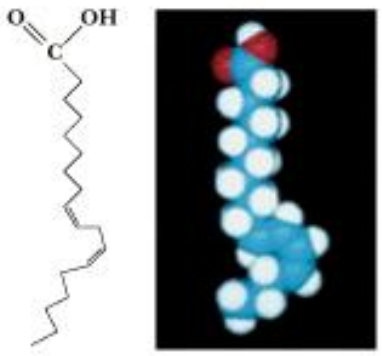
Palmitinska k.



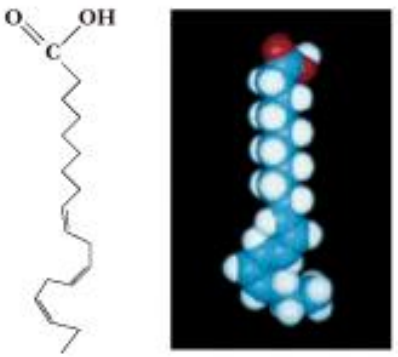
Stearinska k.



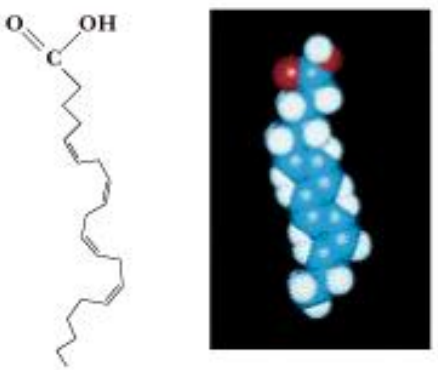
Oleinska k.



Linolna k.



α-linolenska k.



γ-linolenska k.

Lastnosti maščobnih kislin

Funkcija velikosti, stopnje nasičenosti in naboja.

- Nasičene verige: tesno zlaganje, višje tališče, urejene in rigidne v membranah.
- Nenasičene verige (*cis*): ukrivljene, manj tesno zlaganje, nižje tališče, bolj gibljive v membranah.
- Pri fiziološkem pH vse v obliki $R\text{-}COO^-$.
- Lastnosti detergentov (tvorijo kroglaste micele).

Maščobne kisline

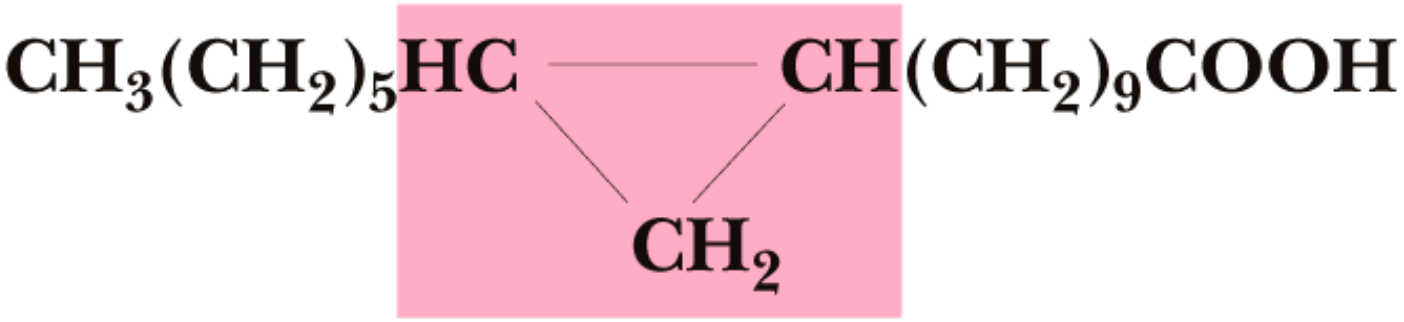
Oznaka	Ime	Sistematsko ime	Tališče (^{o}C)
1.nasičene:			
12:0	**Lavrinska**	**Dodekanojska**	**44.2**
14:0	**Miristinska**	**Tetradekanojska**	**52**
16:0	**Palmitinska**	**Heksadekanojska**	**63.1**
18:0	**Stearinska**	**Oktadekanojska**	**69.6**
20:0	**Arahidinska**	**Eikozanojska**	**75.4**
22:0	**Behenska**	**Dokozanojska**	**81**
24:0	**Lignocerinska**	**Tetrakozanojska**	**84.2**

Maščobne kisline

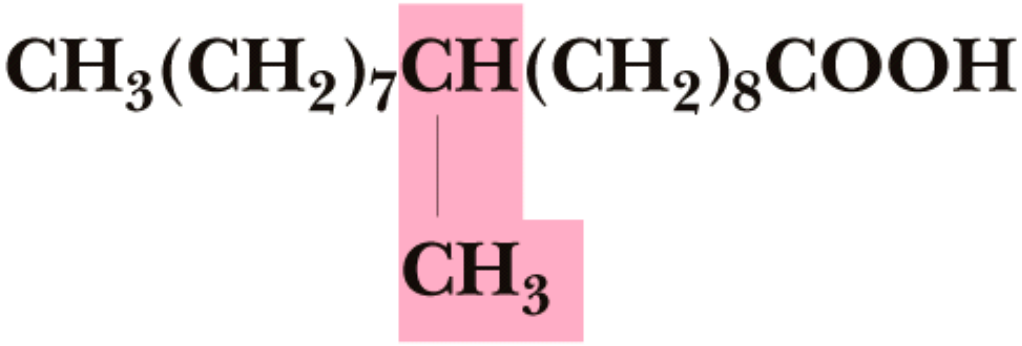
Oznaka	Ime	Sistematsko ime	Tališče (^{o}C)
2. nenasičene:			
16:1	**Palmitoleinska**	**9-heksadekenojska**	**-0.5**
18:1	**Oleinska**	**9-oktadekenojska**	**13.4**
18:2	**Linolna**	**9,12-oktadekendienojska**	**-9**
18:3	**α-Linolenska**	**9,12,15-oktadekentrienojska**	**-17**
18:3	**γ-Linolenska**	**6,9,12-oktadekentrienojska**	**-11**
20:4	**Arahidonska**	**5,8,11,14-eikozatetraenojska**	**-49.5**
20:5	**EPA**	**5,8,11,14,17-eikozapentaenojska**	**-54**
24:1	**Nervonska**	**15-tetrakozaenojska**	**39**

Neobičajne maščobne kisline

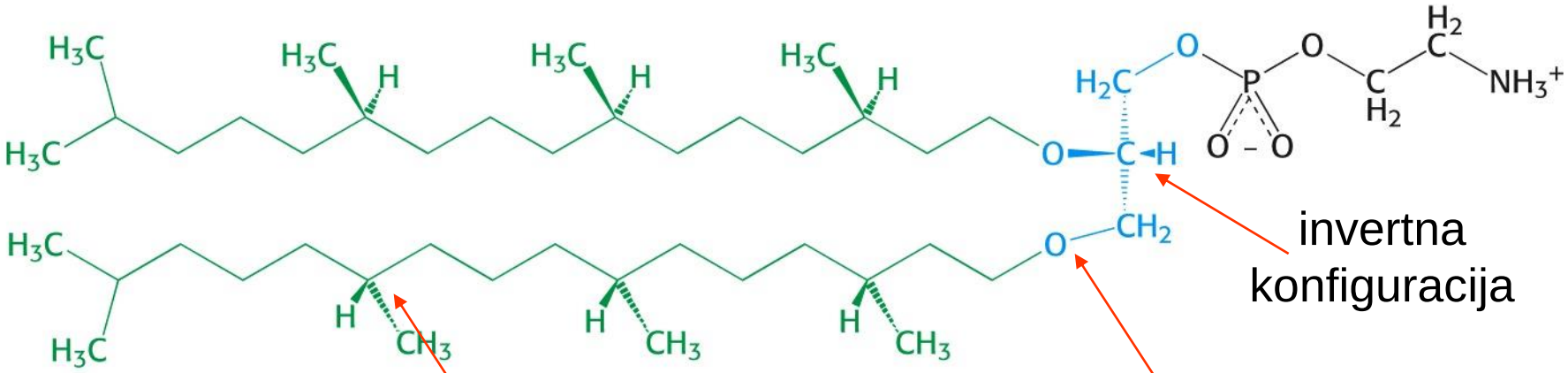
Laktobacilna kislina
(alicíklična k.)



Tuberkulostearinska kislina



Fosfolipidi pri arheah so drugačni kot tisti pri evkariontih in bakterijah



invertna konfiguracija

razvejane, nasičene verige odporne na oksidacijo

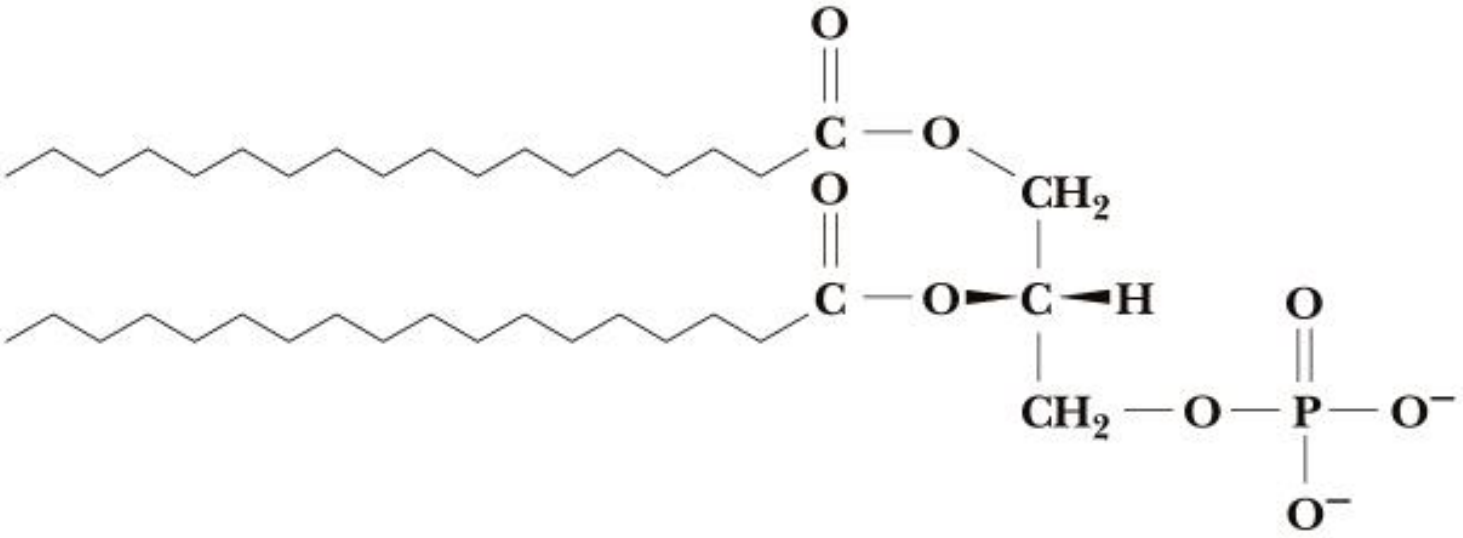
eterske vezi odporne na hidrolizo

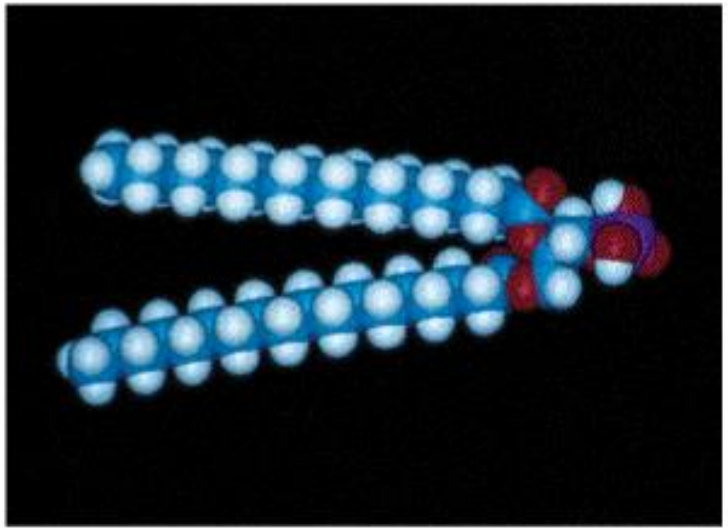
Glicerofosfatidi

Glicerofosfatidi so fosfolipidi.

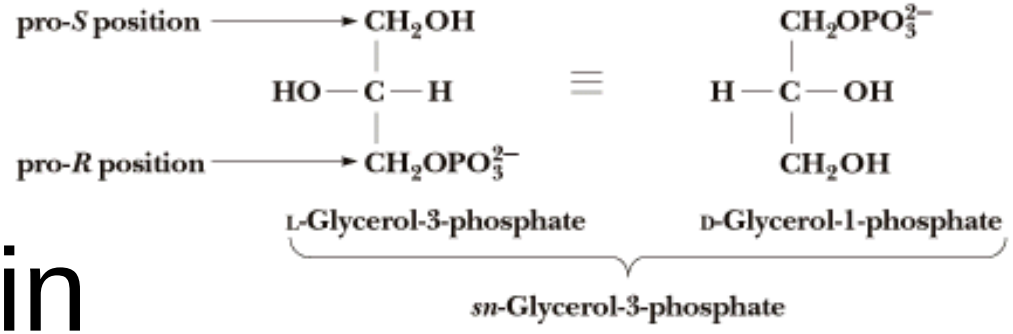
- Osnova glicerol (alkohol).
- Glicerol zaestren na mestih 1, 2 z maščobno kislino (če nenasičena, je običajno na mestu 2).
- Mesto 3 zaestreno s fosforno kislino.
- Fosfatidna kisl. + alkoholna komponenta = fosfatid:
  - Fosfatidilholin
  - Fosfatidiletanolamin
  - Fosfatidilserin
  - Fosfatidilinozitol
  - Fosfatidilglicerol in kardiolipin itd.

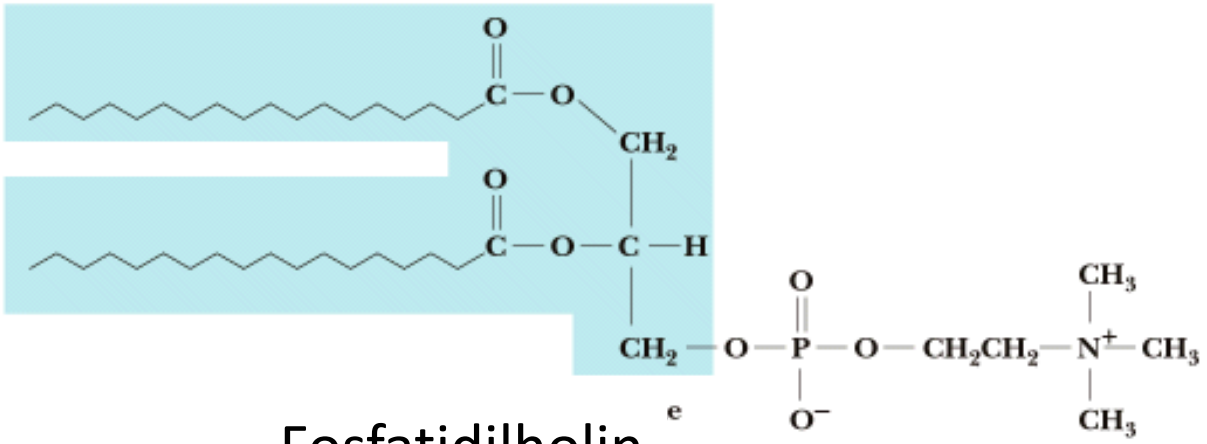
Fosfatidna kislina



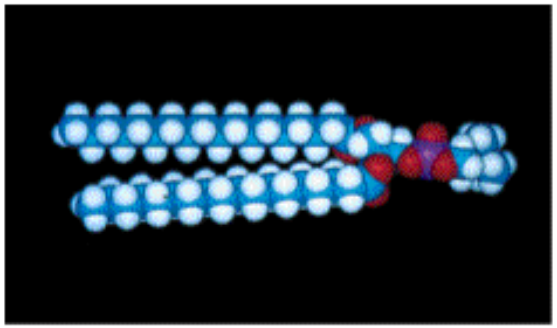


Fosfatidil-holin



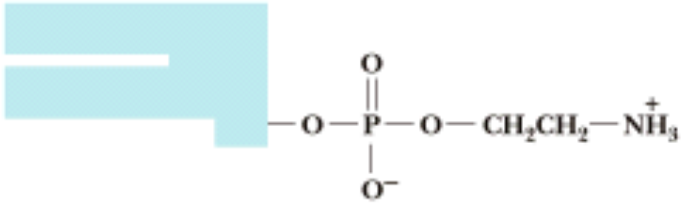


Fosfatidilholin

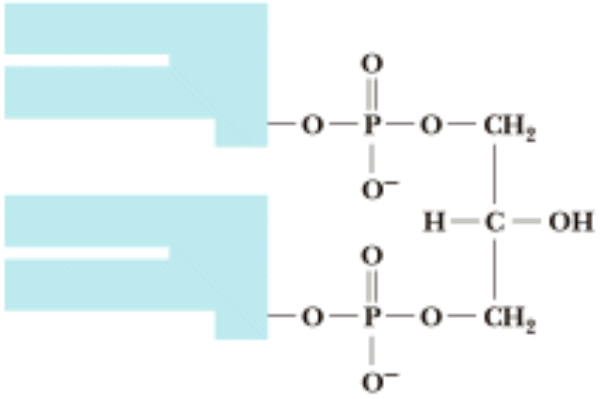


Fosfatidi

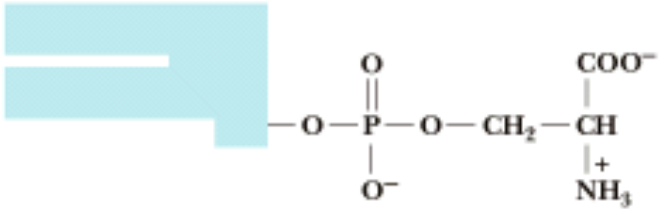
GLYCEROLIPIDS WITH OTHER HEAD GROUPS:



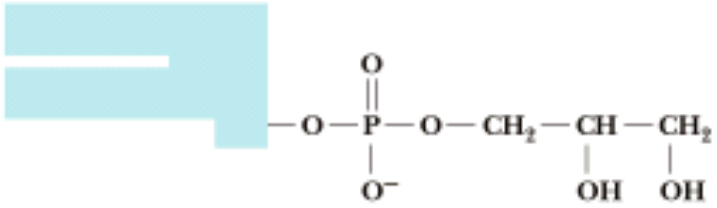
Fosfatidiletanolamin



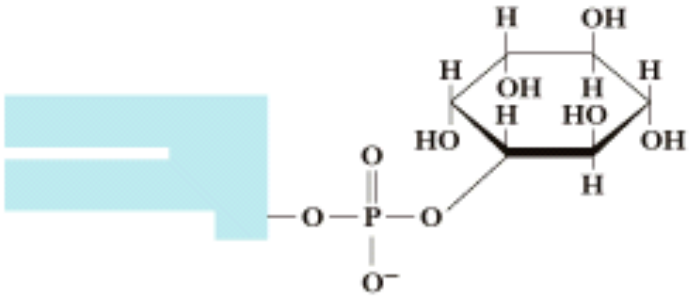
Kardiolipin



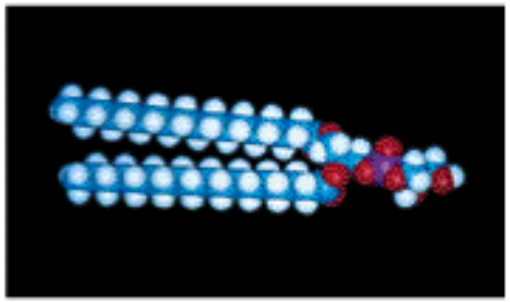
Fosfatidilserin

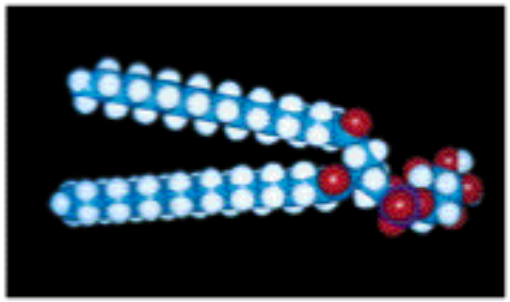


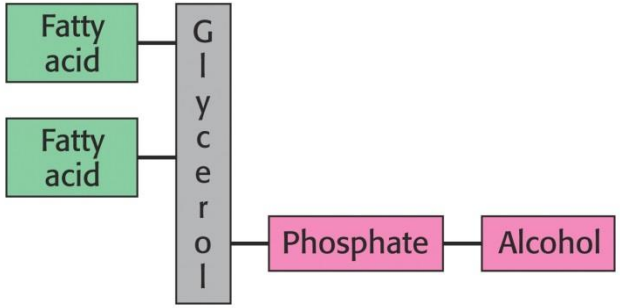
Fosfatidilglicerol

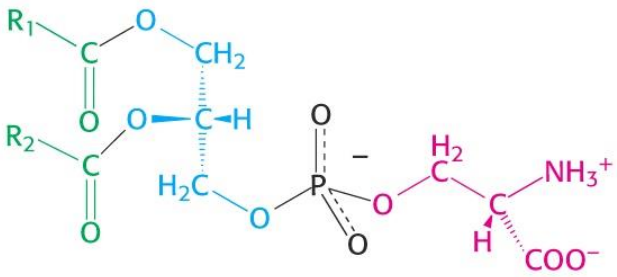


Fosfatidilinozitol

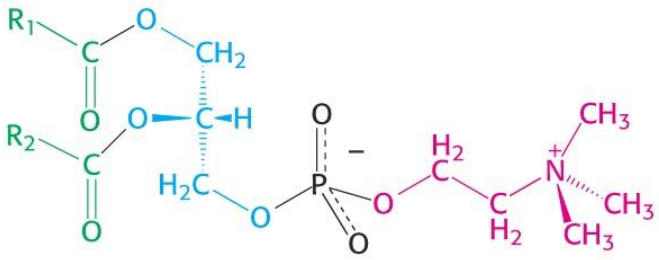




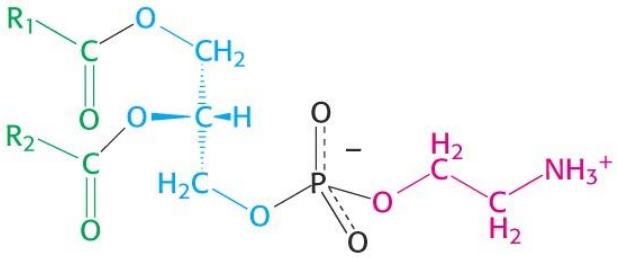




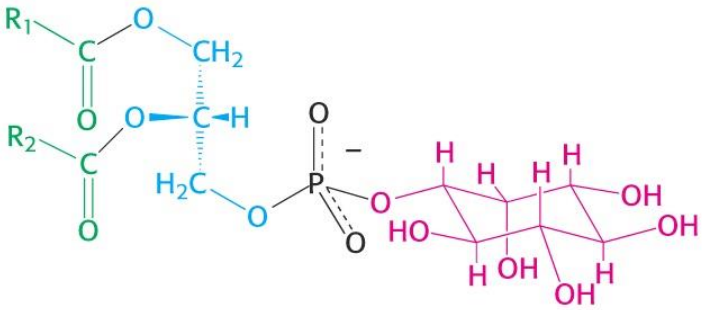
Phosphatidyl serine



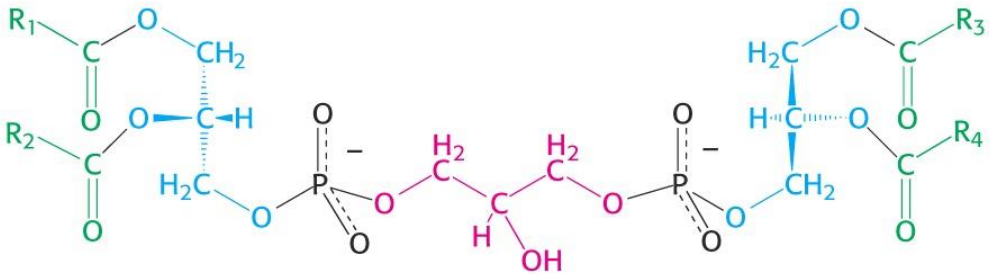
Phosphatidyl choline



Phosphatidyl ethanolamine

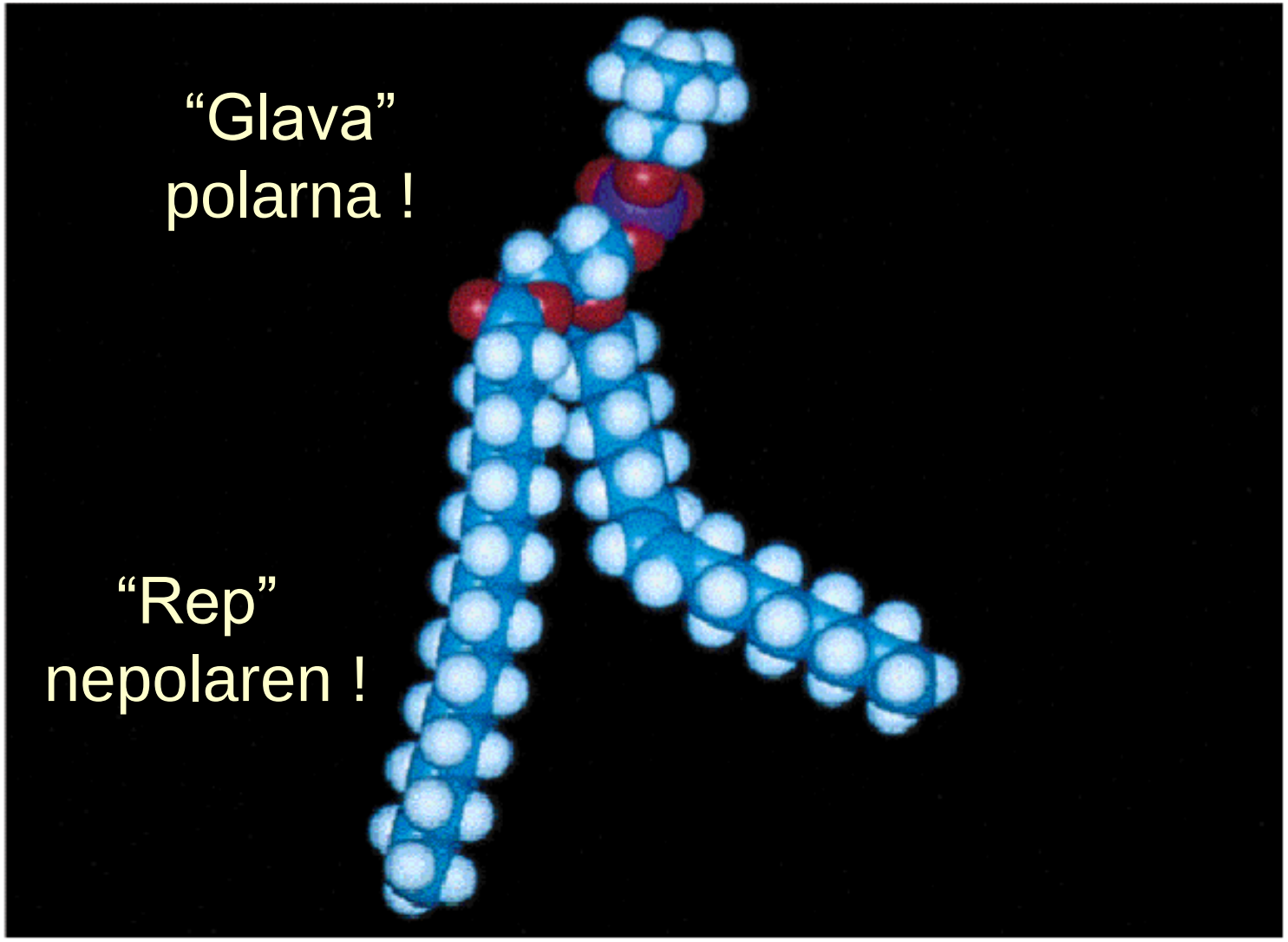


Phosphatidyl inositol



Diphosphatidyl glycerol (cardiolipin)

Glicerofosfatid – model



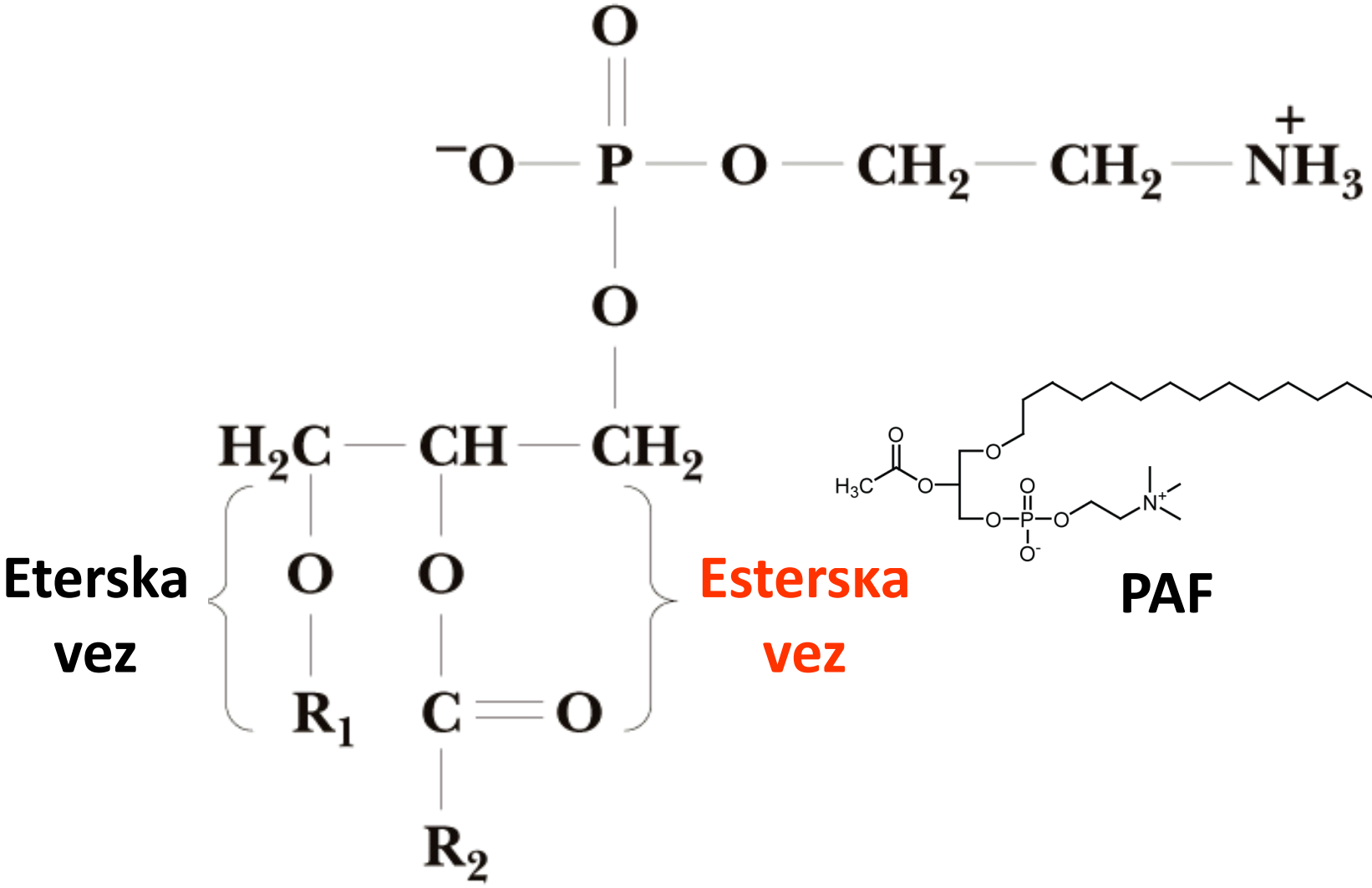
Eterski glicerofosfatidi

Na C-1 eterska vez namesto esterske!

- Plasmalogeni:

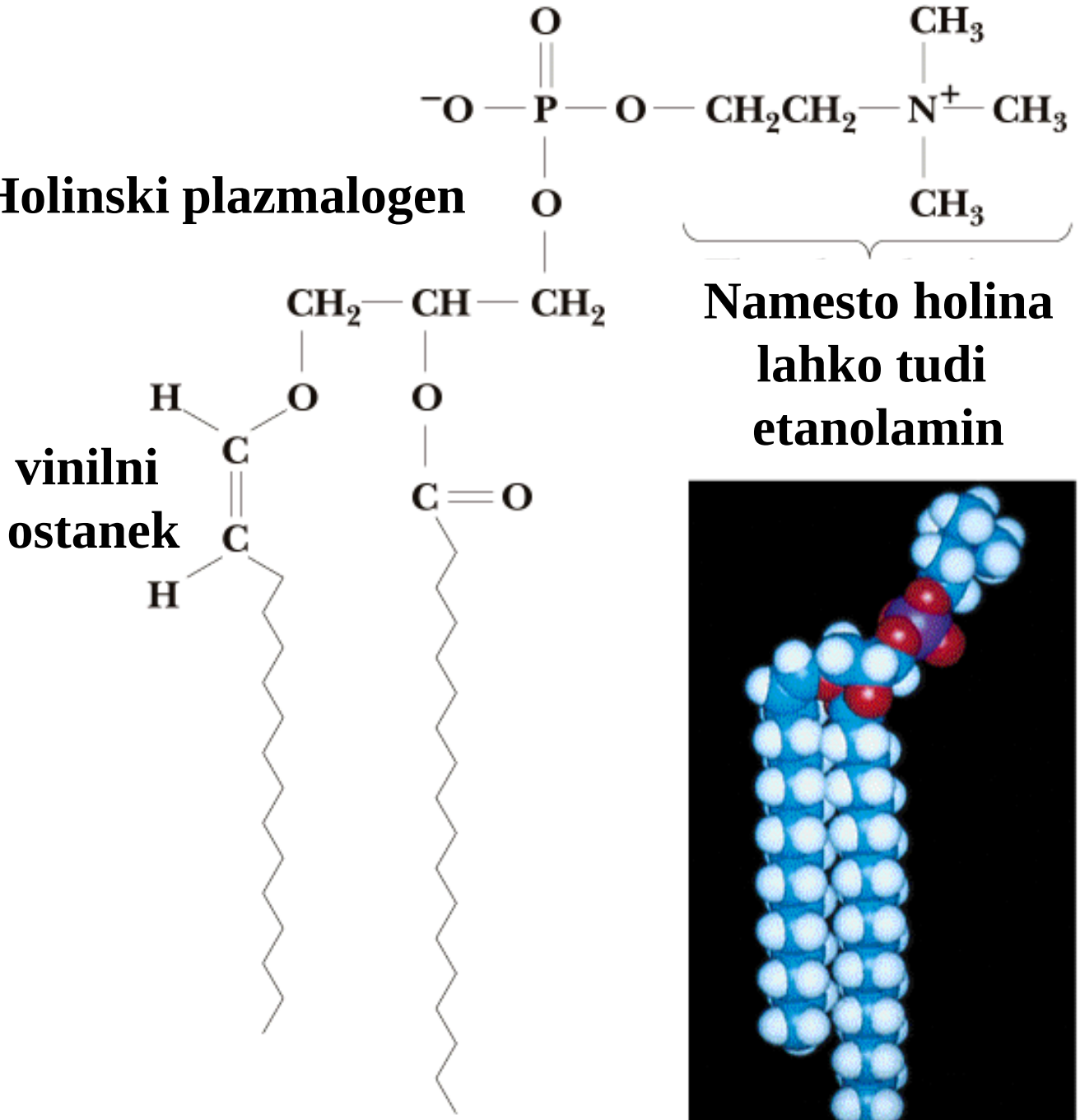
  eterski glicerofosfolipidi z nenasičeno alkilno verigo (vinilni ostanek na *sn*-1= dvojna vez takoj ob eterski vezi)
- Zalsti v membr. srca, živcev, ledvic in testisov
- PAF (platelet activating factor) – signalna molekula

Eterska vez na C-1 glicerola



Membranski plazmalogen
1-alkil-2-acil-glicerofosfoholin

Holinski plazmalogen



Namesto holina lahko tudi etanolamin

Sfingolipidi

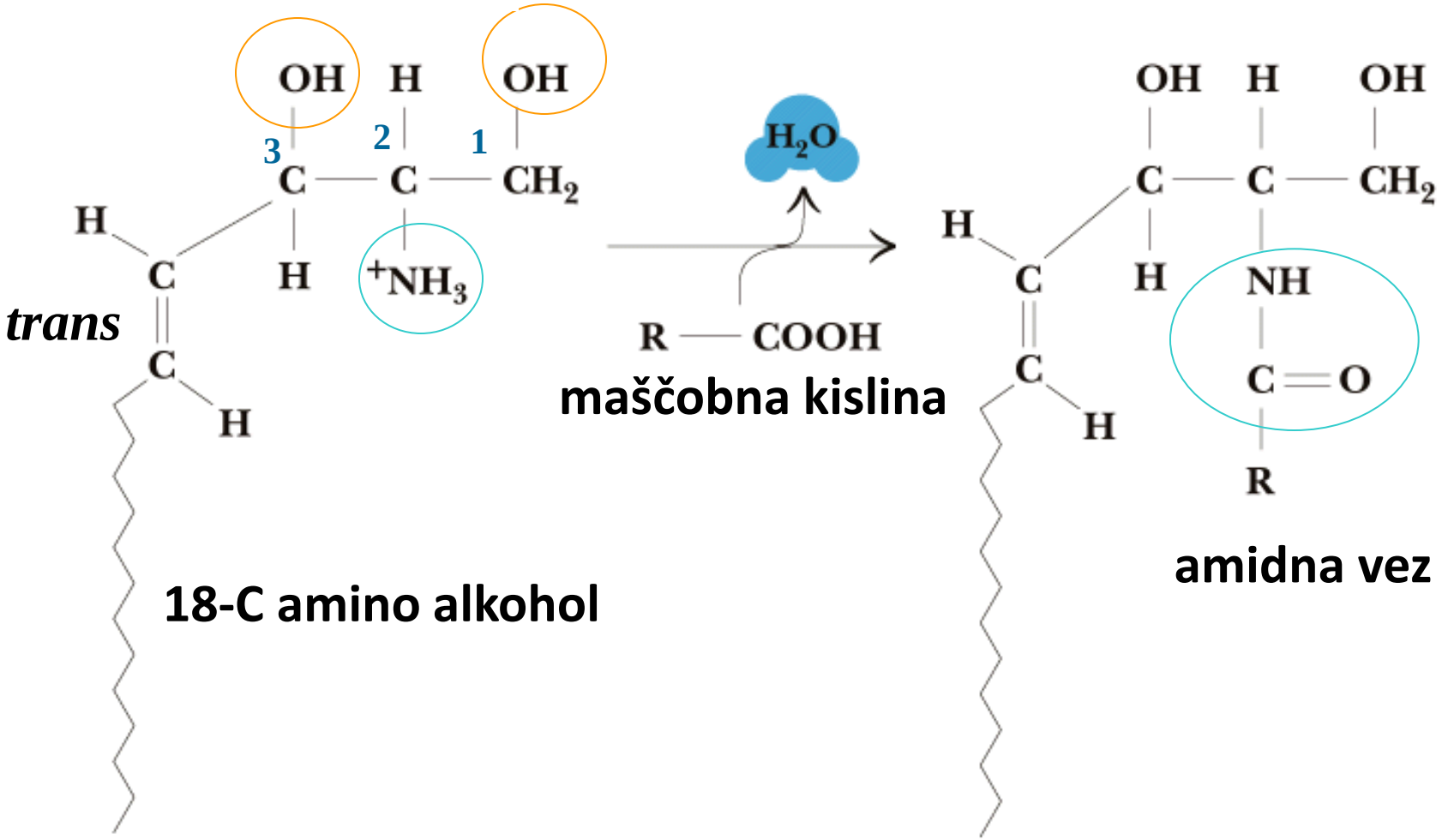
Osnova je sfingozin.

- **Sfingozin** je 18-C amino alkohol (2-OH).
- **Ceramidi**: sfingozin + amidno vezana maščobna kislina.
- **Fosfosfingolipidi** : ceramidi + fosfoholin = SM.
- **Glikosfingolipidi**: ceramidi + β-glikozidno vezan monosaharid (**cerebrozidi**) ali oligosaharid (**gangliozidi**) na 1-OH skupino sfingozina.

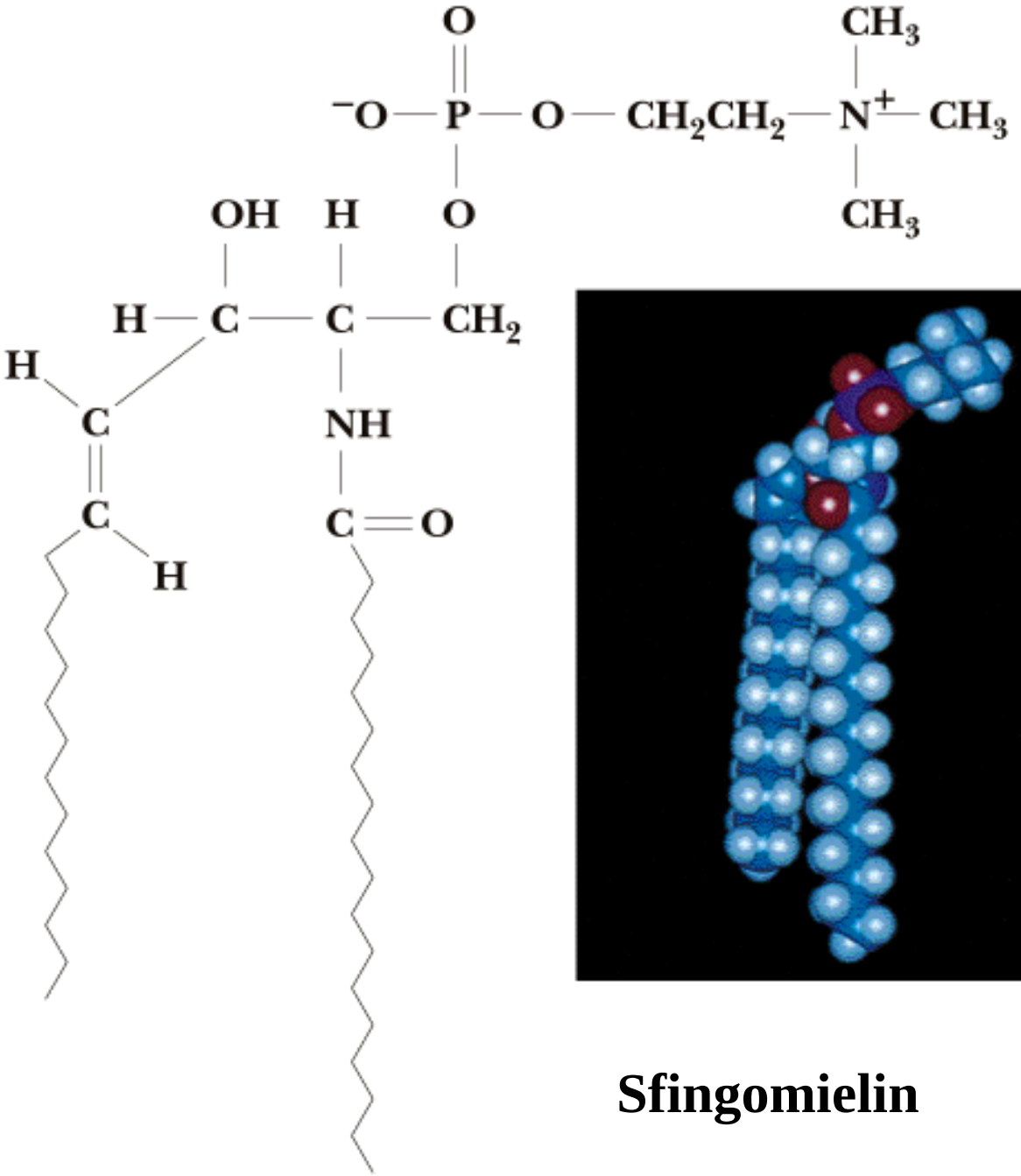
  Vedno na ekstracelularni strani p.m.

  Gangliozidi vsebujejo vsaj en ostanek sialične kisline (npr. NANA).

Zgradbi sfingozina in ceramida



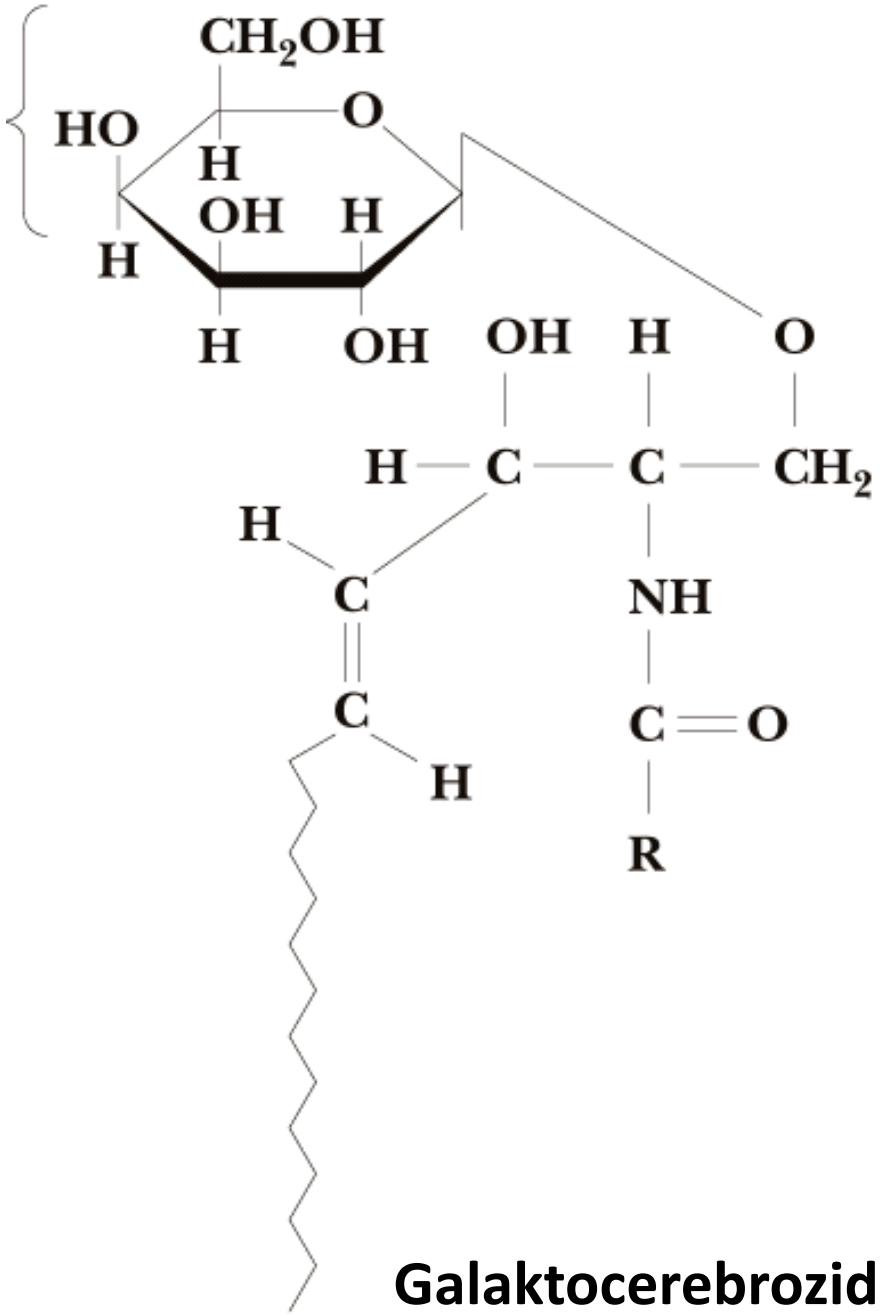
Zgradba sfingomielina (SM)



Sfingomielin

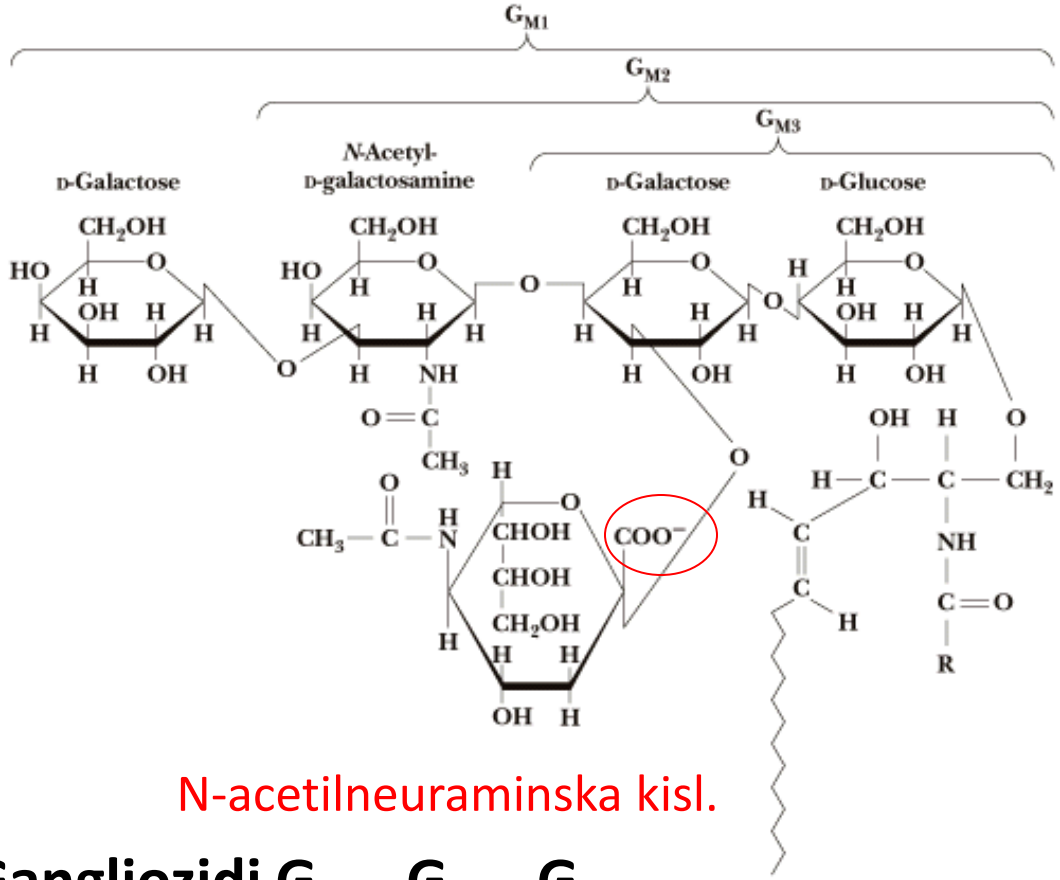
Zgradba cerebrozida

β-D-galaktoza



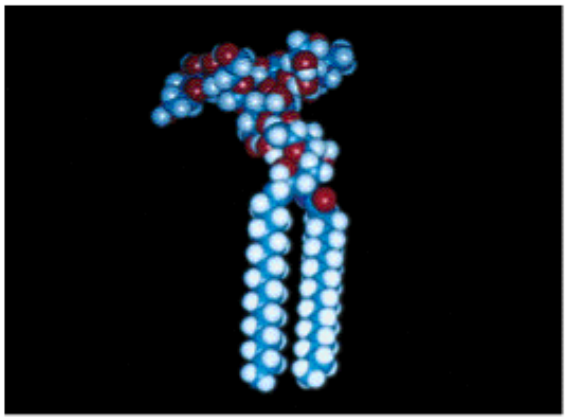
Galaktocerebrozid

Gangliozidi

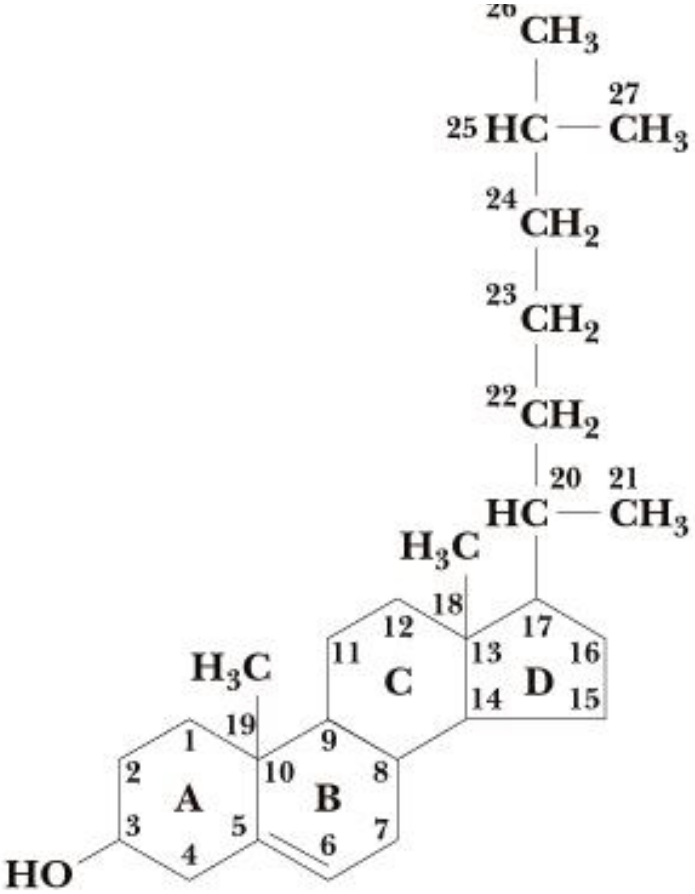


N-acetilneuraminska kisl.

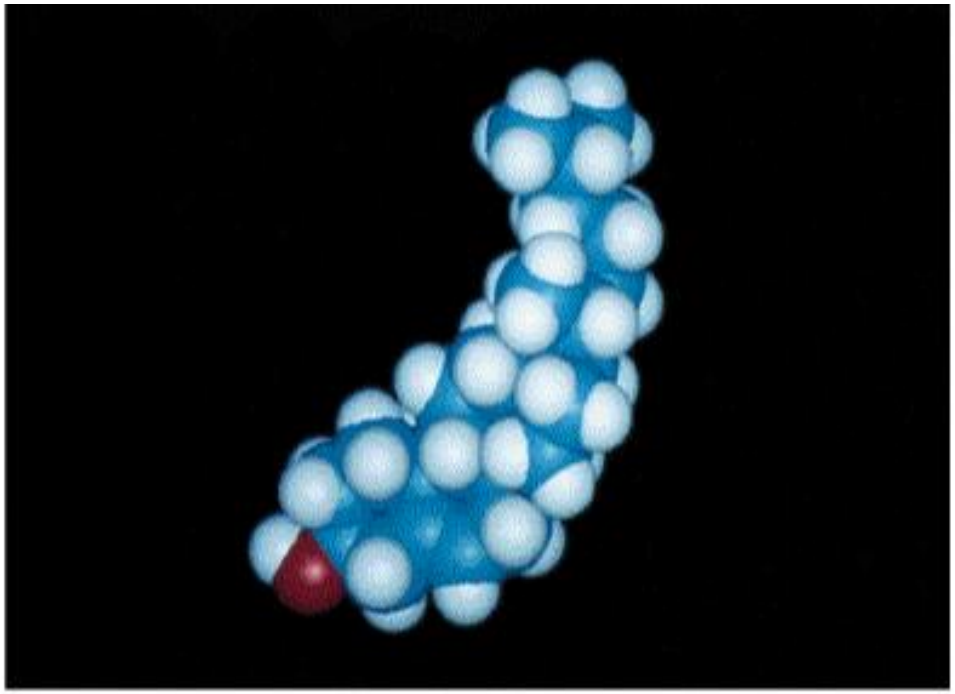
Gangliozidi G_{M1}, G_{M2}, G_{M3} …



Enostavni membranski lipid : holesterol



Holesterol (27-C)
(steroid, triterpenski derivat)



Holesterola ni v prokariontskih m.!

Lipidna sestava b.m. (utež.%)

LIPID	ERITROCIT (Človek)	MIELIN (Človek)	MITOH. (Gov. srce)	*E. coli*
Fosfatidna k.	1.5	0.5	0	0
Fosfatidilholin	19	10	39	0
Fosfatidiletanolamin	18	20	27	65
Fosfatidilglicerol	0	0	0	18
Fosfatidilinozitol	1	1	7	0
Fosfatidilserin	8.5	8.5	0.5	0
Kardiolipin	0	0	22.5	12
Sfingomielin	17.5	8.5	0	0
Glikolipidi	10	26	0	0
Holesterol	25	26	3	0

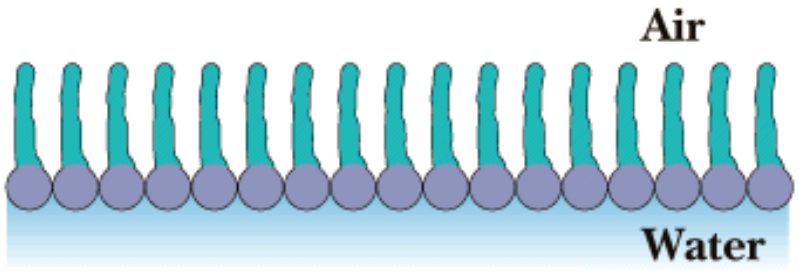
Lipidne strukture se spontano tvorijo

Hidrofobne interakcije, van der Waalsove sile!

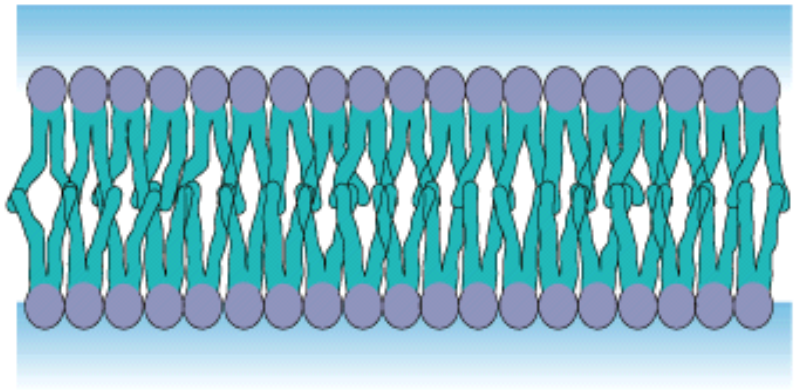
- Malo lipidov monomernih v vodi.
- Lipidni monosloji - nepolarni lipidni repi v zraku.
- Lipidni dvosloji:
  - unilamelarni vezikli (liposomi)
  - multilamelarni vezikli.
- Miceli - lipidni repki zaprti v kroglaste ali cilindrične strukture v polarnih topilih.
- Obrnjeni (reverzni) miceli v nepolarnih topilih.

Lipidne tvorbe v vodi

Sloji

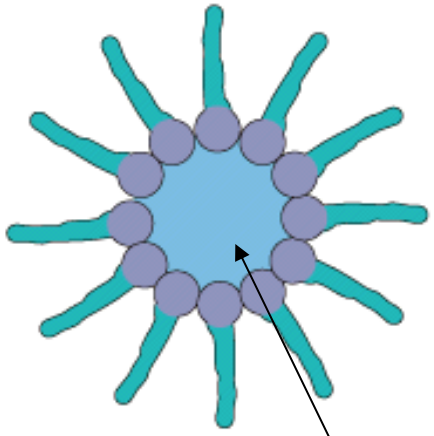


Monomolekulski sloj

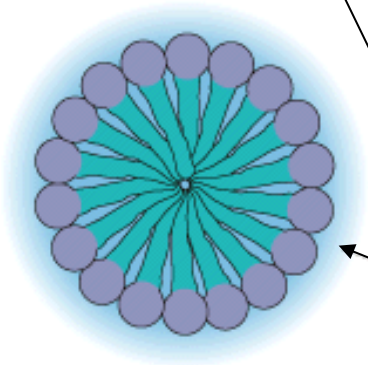


Bimolekulski sloj
= lipidni dvosloj

Miceli



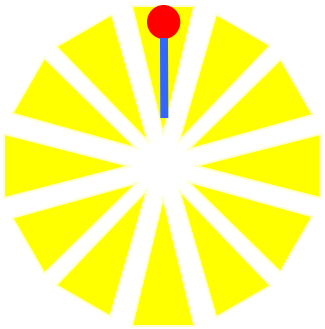
Obrnjen

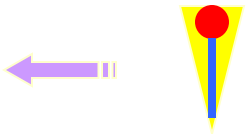


Običajen

Voda

Kritični Parameter Pakiranja - KPP





⅓ – ½

inverzni konus

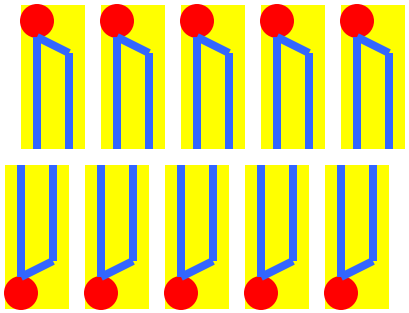
Lizofosfatidi, detergenti

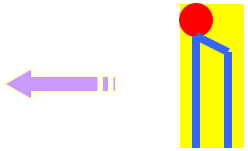
KPP = V/L×S

V = volumen monomera

L = dolžina verige

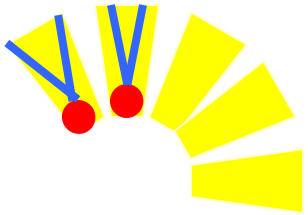
S = površina polarne glave monomera

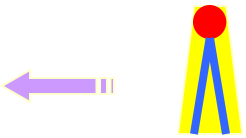




½ – 1

PC, SM, PS, PI, itd.





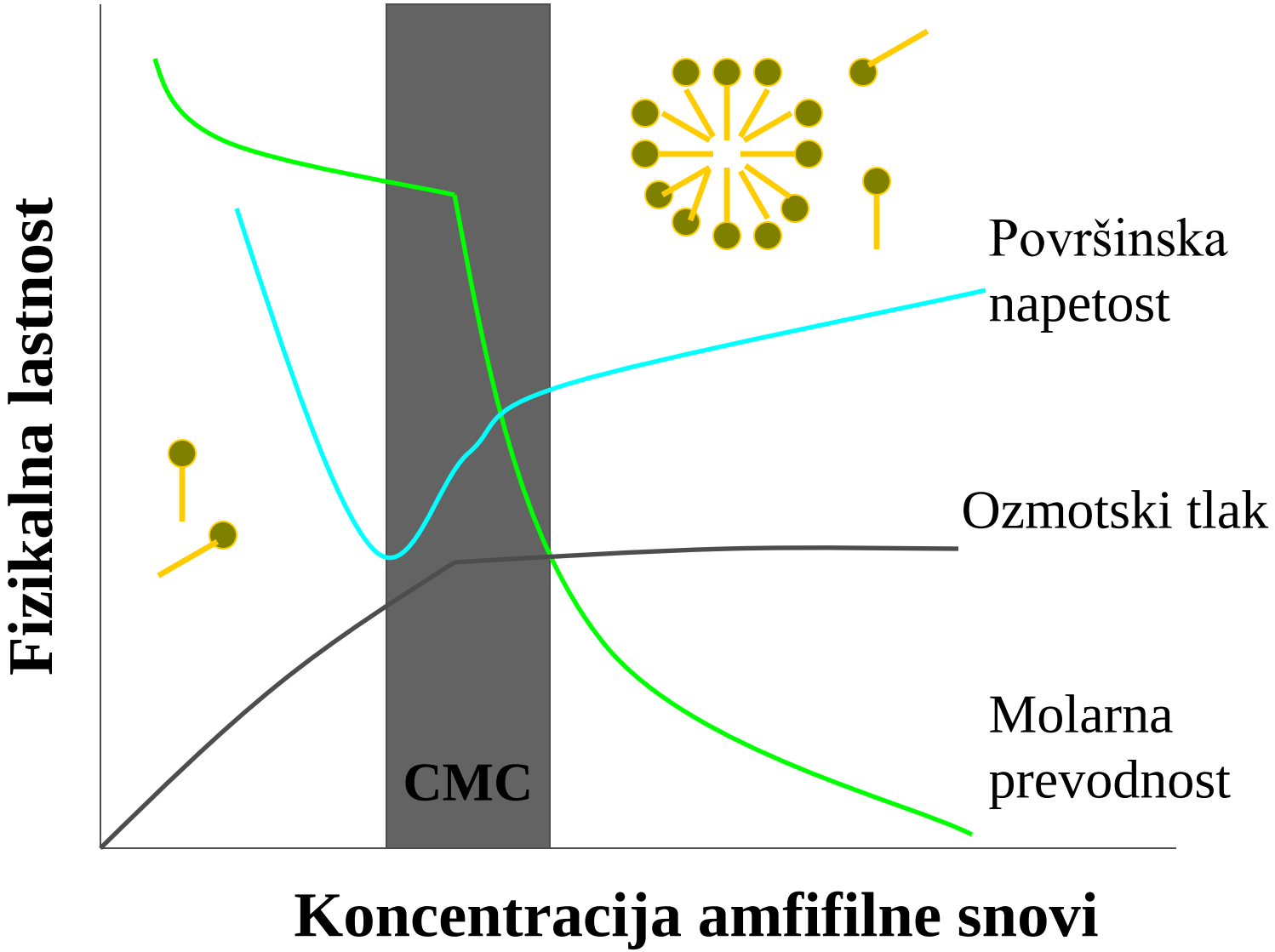
> 1

konus

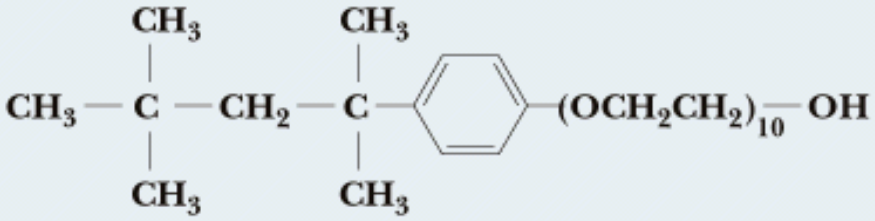
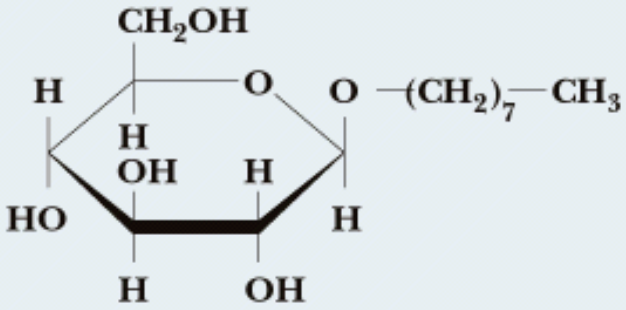
Heksagonalna faza (H_{II})

PE, kardiolipin-Ca^{2+}, PA- Ca^{2+}

Kritična micelna koncentracija (CMC)

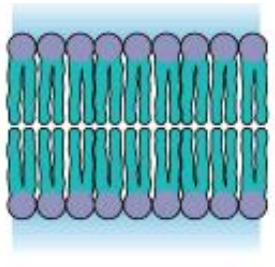


Detergenti - CMC

Structure	M_r	CMC	Micelle M_r
Triton X-100 	625	0.24 mM	90–95,000
Octyl glucoside 	292	25 mM	
$C_{12}E_8$ (Dodecyl octaoxyethylene ether) 	538	0.071 mM	

Lipidni dvosloji

Planarni dvosloj

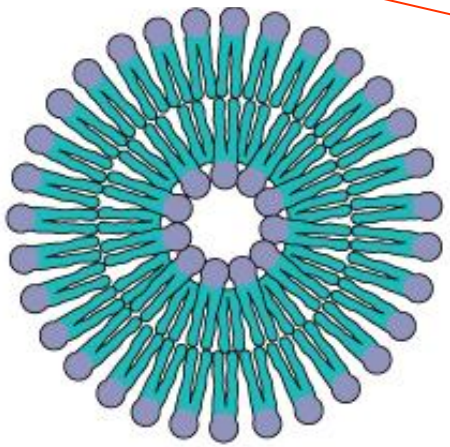


(a)

Unilamelarni vezikli → **SUV**

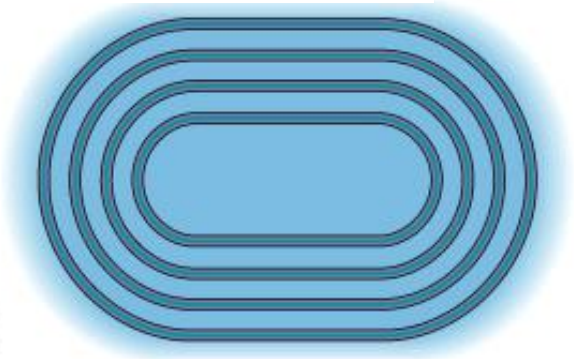
Unilamelarni vezikli → **LUV**

SUV



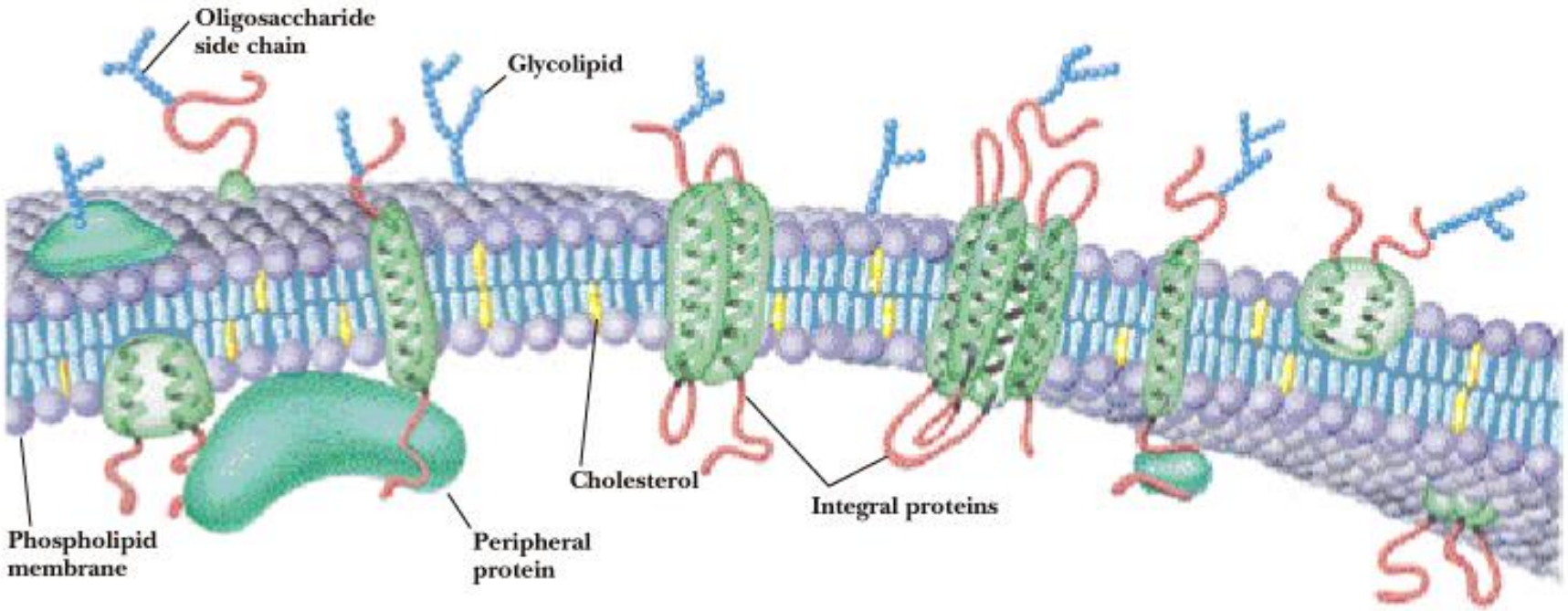
(b)

Multilamelarni vezikel



(c)

Modela biološke membrane



Fluidni mozaični model b.m.

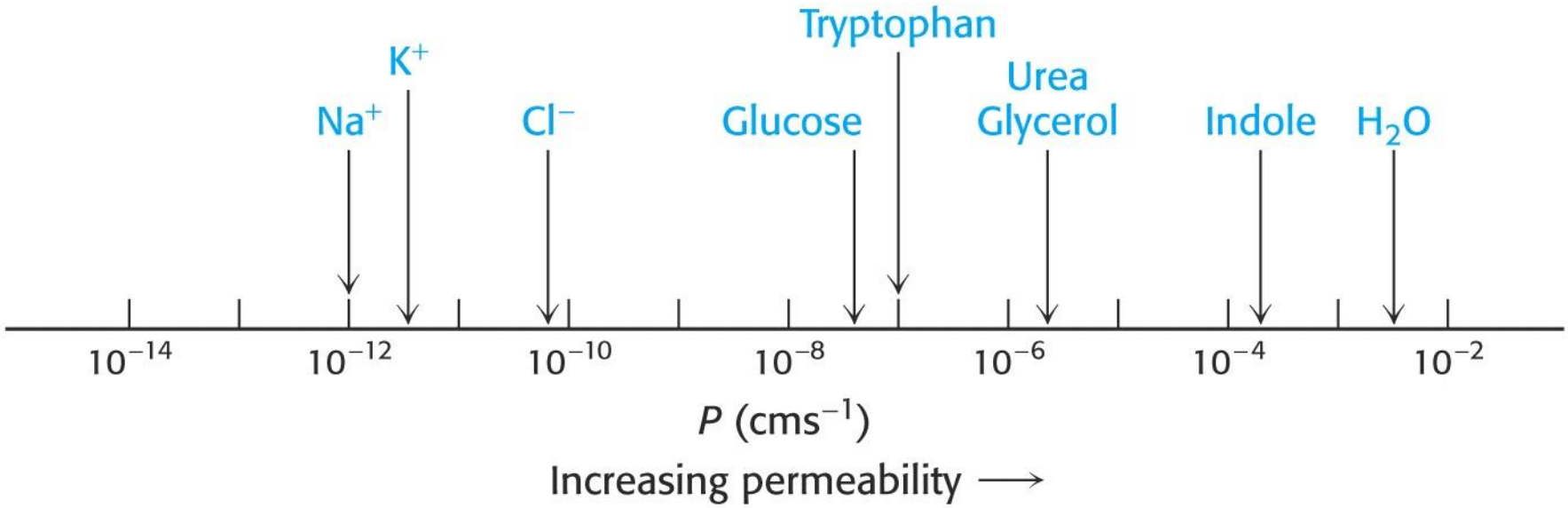
Singer in Nicolson, 1972

- Fosfolipidni dvosloj - *fluidni matriks.*
- Lipidni dvosloj je dvo - dimenzionalno topilo.
- Lipidi in proteini - gibljivost
  - lateralna difuzija
  - rotacijska difuzija
  - difuzija “flip-flop”.
- Dva razreda proteinov:
  - periferni (zunanji) membranski proteini
  - integralni (notranji) membranski proteini.

Gibanja v lipidnem dvosloju

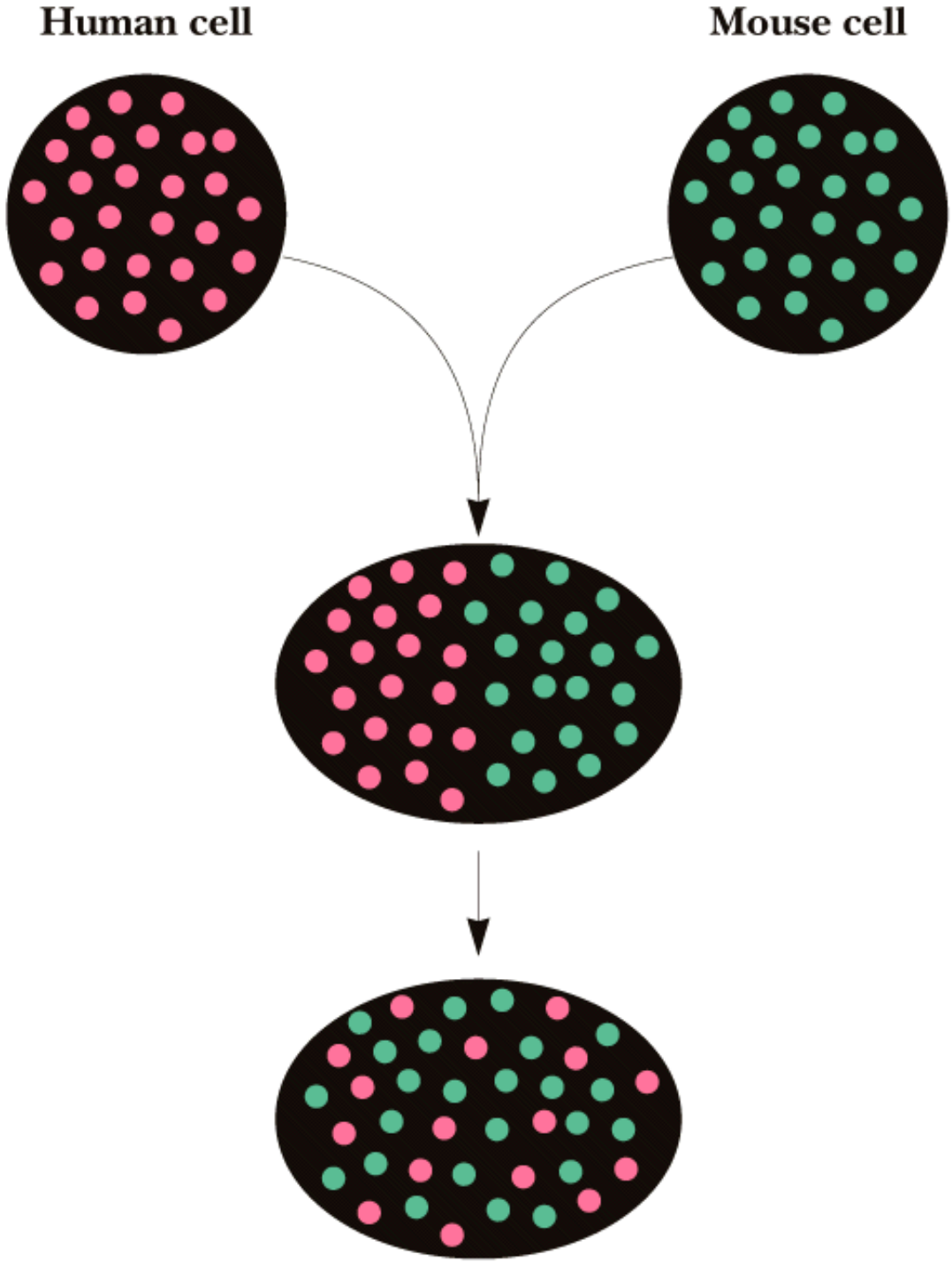
- Lipidne verige: upogibanje, nagib glede na normalo in rotacija.
- Lipidi in proteini - lateralna difuzija v dvosloju.
- Metode za meritve lipidne difuzije:
  - NMR,
  - EPR,
  - FRET, FRAP.

Prepustnostni koeficienti nekaterih ionov in molekul čez lipidni dvosloj

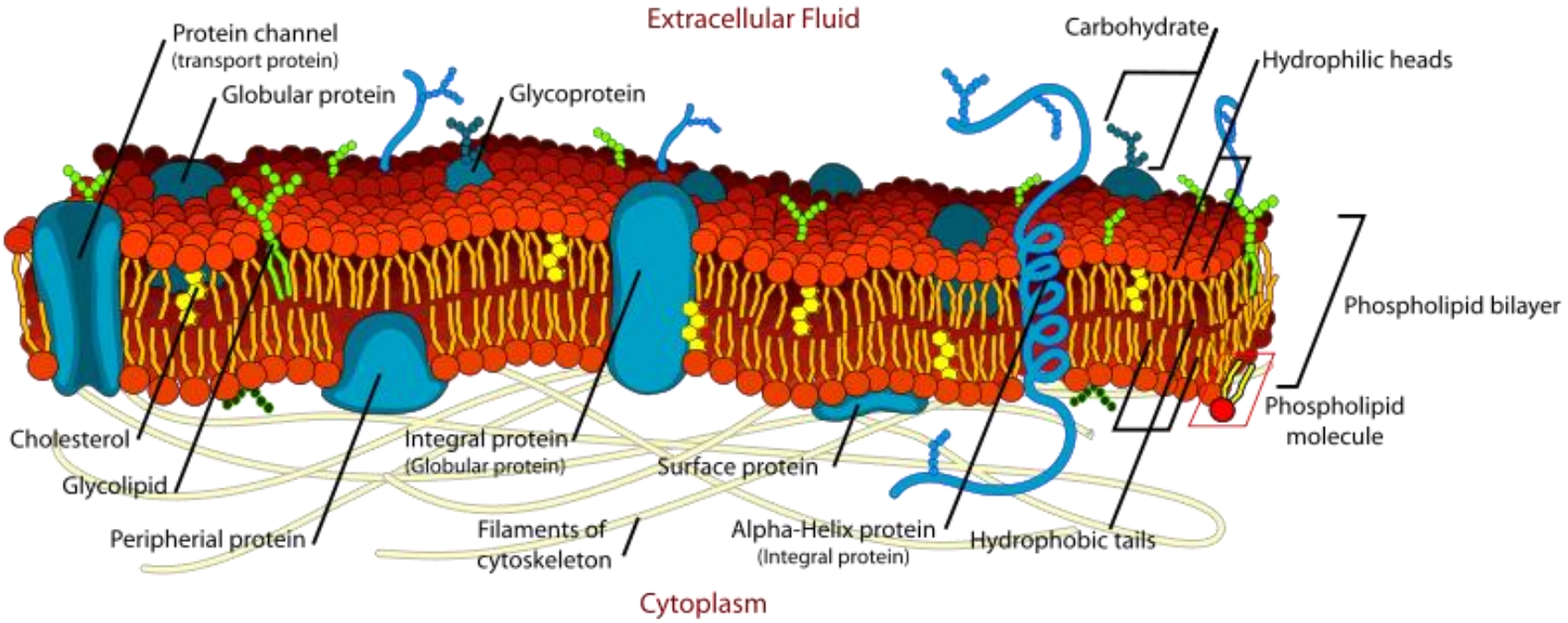


Lateralna difuzija lipidov in proteinov v membrani

FRET:
Fluorescence
Resonance
Energy
Transfer



Biološke membrane so asimetrične

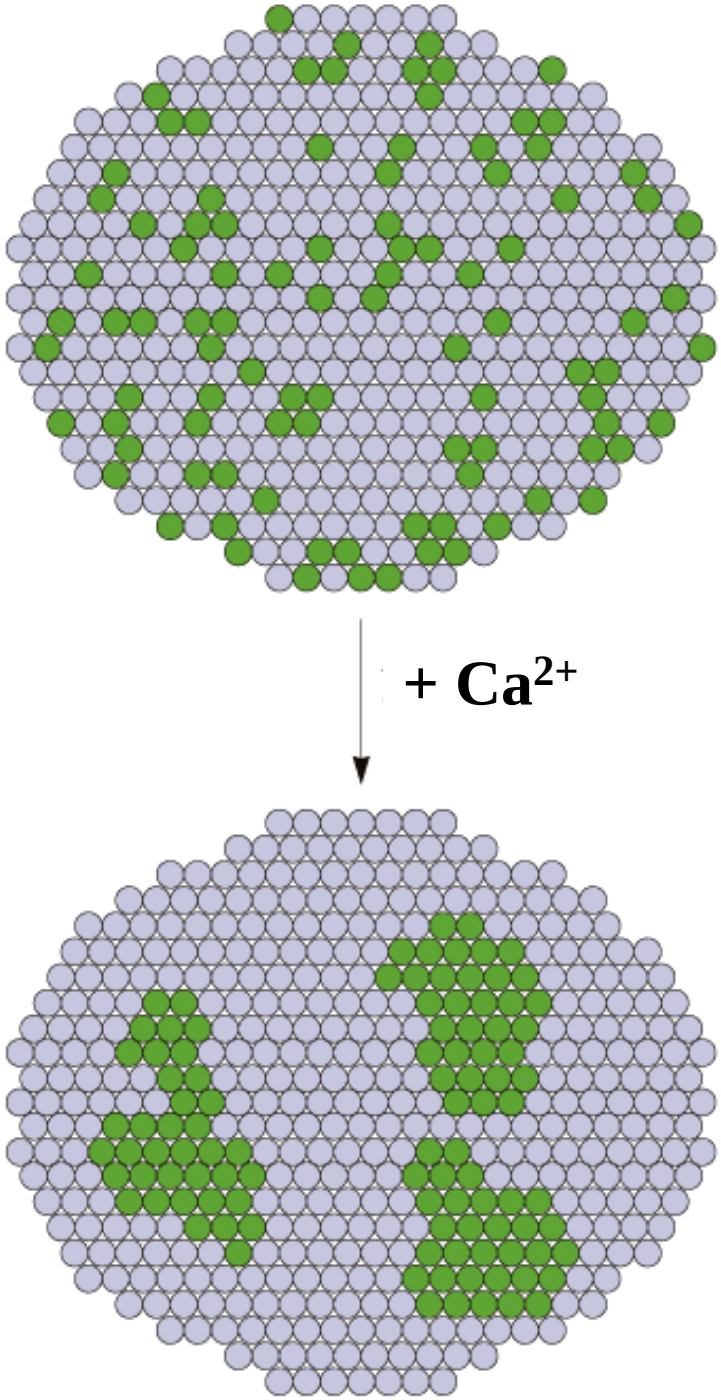


Lateralna asimetričnost membran

- Lateralna asimetrija proteinov:
  - Proteini se lahko oligomerizirajo v ravnini membrane - večinoma ni enakomerne porazdelitve.
- Lateralna asimetrija lipidov:
  - Lipidi se grupirajo v ravnini membrane - fazna separacija - “**lipidni rafti**”.

Fazna separacija lipidov

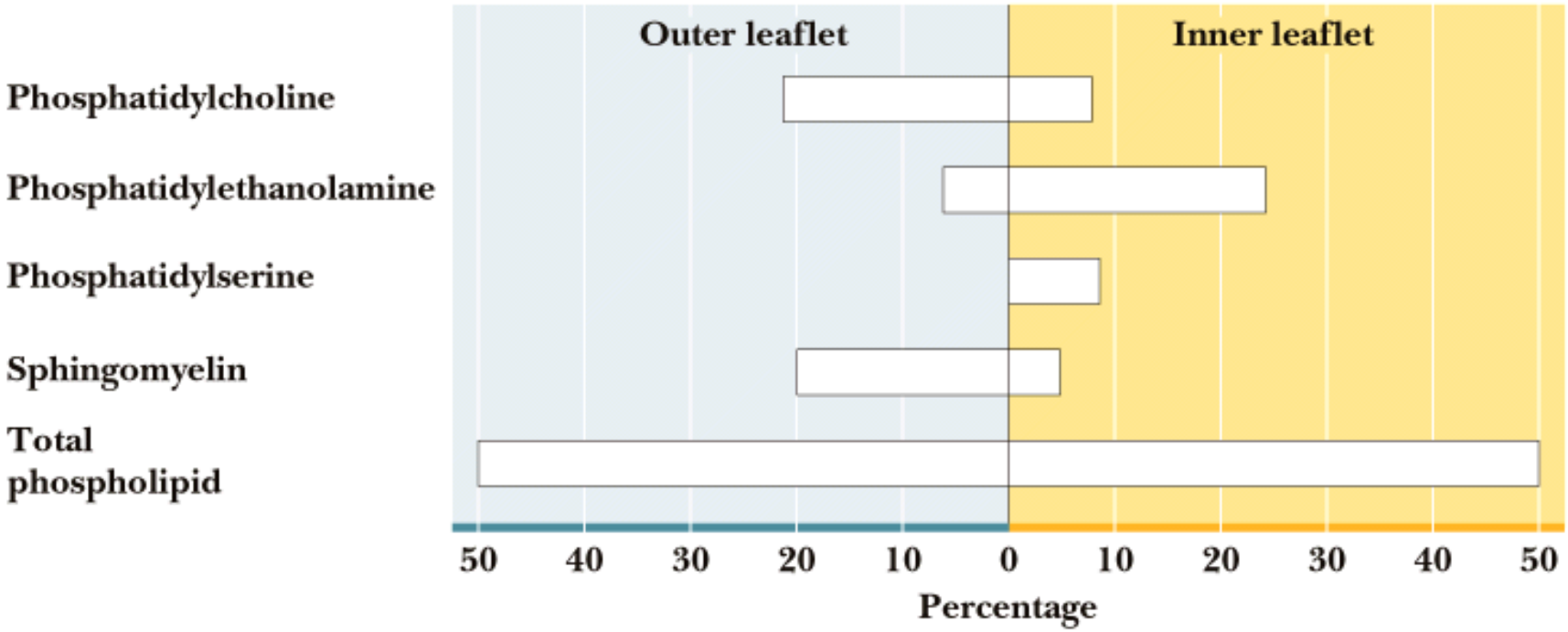
Raftni model b.m.



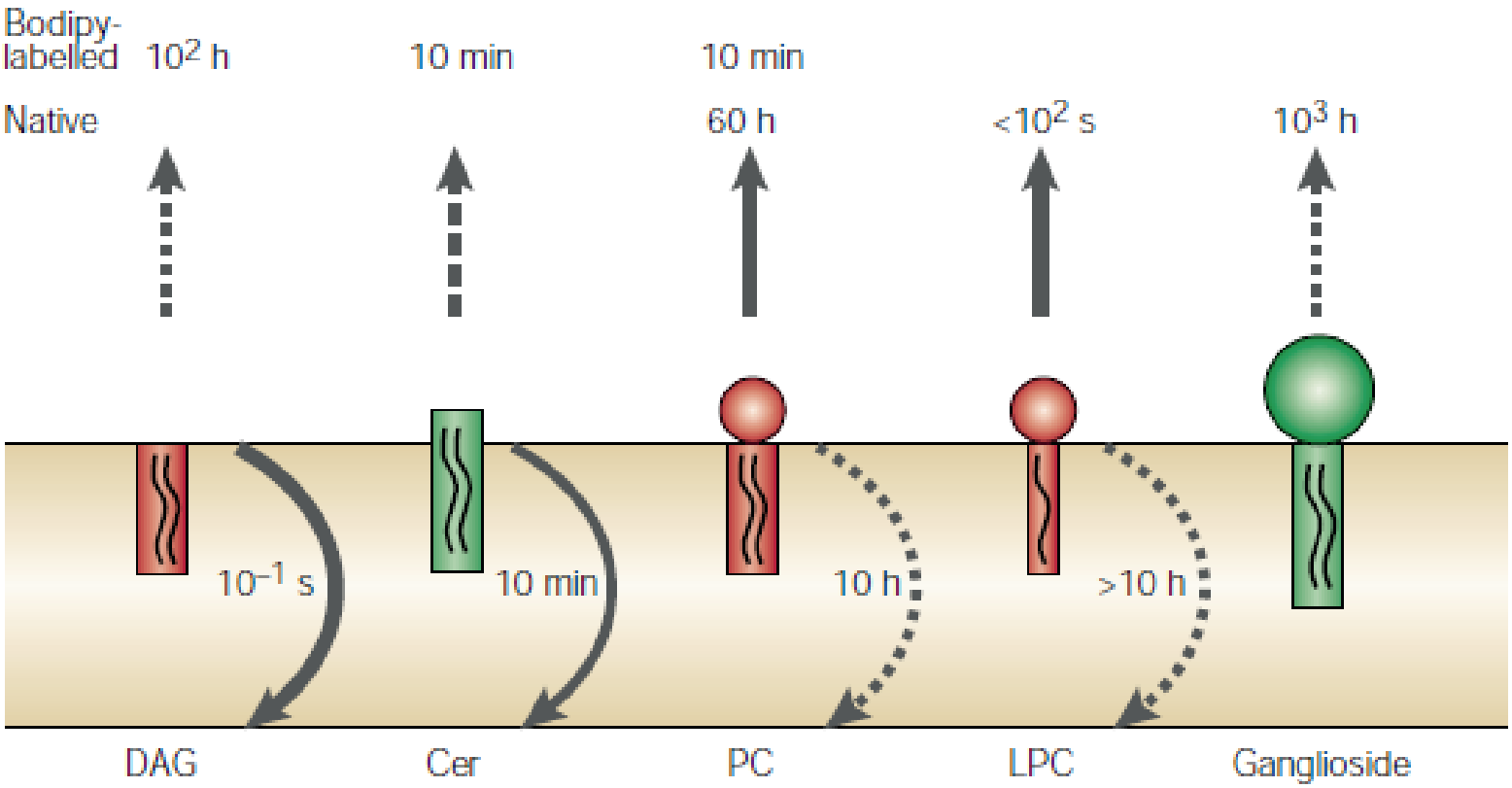
Transverzalna asimetričnost membran

- Transverzalna asimetrija proteinov
  - Primer: N-terminus glikoforina je ekstracelularen, C-terminus intracelularen.
- Transverzalna asimetrija lipidov
  - Sestava zunanjega monosloja je drugačna od notranjega.

Eritrocitna membrana: razlike v lipidni sestavi obeh slojev



Časa potrebna za spontan prehod lipida čez membranski dvosloj in za difuzijo v vodno fazo

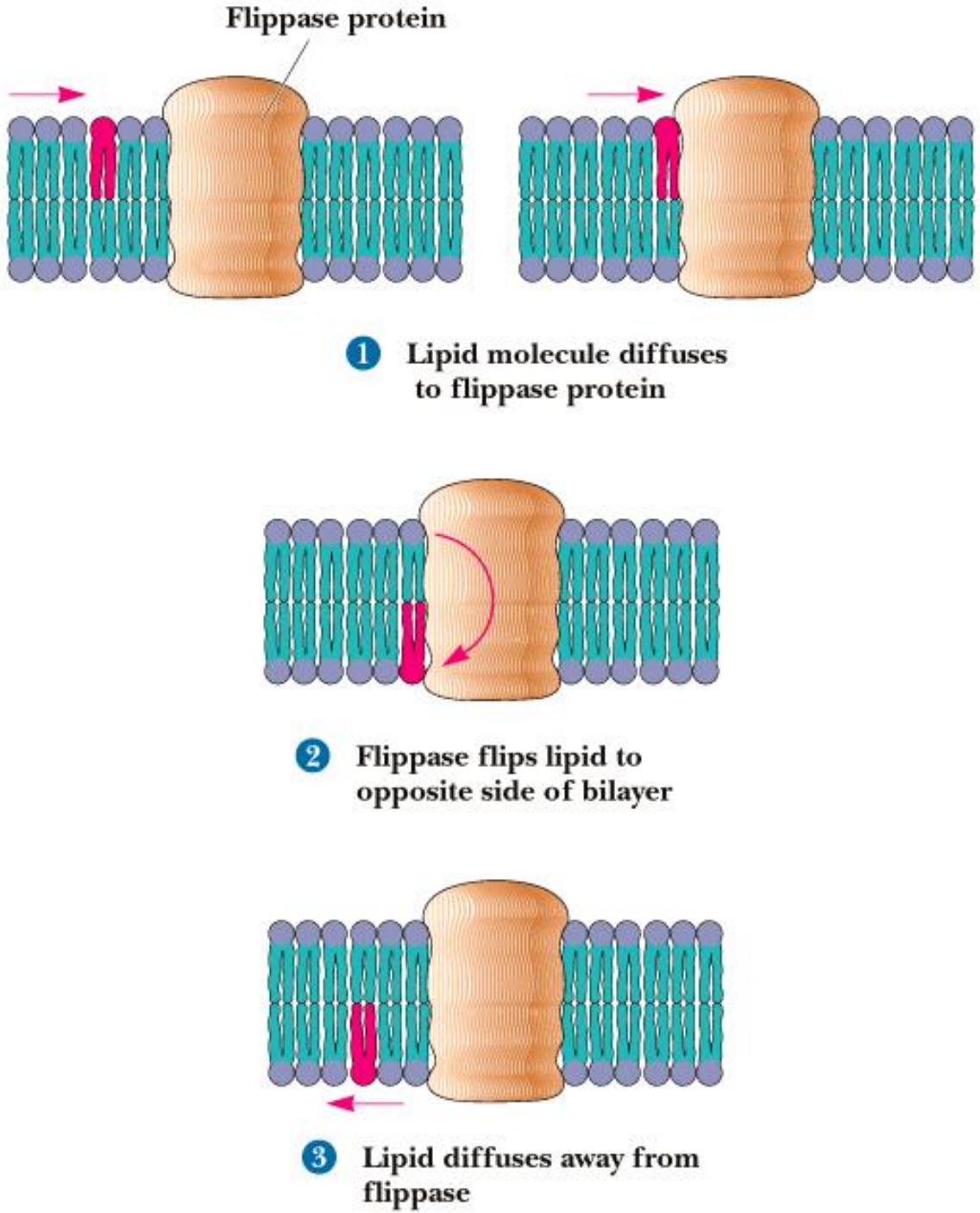


Flipaze

Kataliza difuzije “flip-flop”!

- Flipaze, proteini, katalizirajo “flip-flop” gibanje lipidov.
- Flipaze
  - pasivni transport
  - aktivni transport (poraba energije, hidroliza ATP).
- Aktivne flipaze generirajo asimetrijo lipidov v membranah.

Flipaze


Flippase protein
1 Lipid molecule diffuses to flippase protein
2 Flippase flips lipid to opposite side of bilayer
3 Lipid diffuses away from flippase

Fazni prehodi v membranah

”Taljenje" membranskih lipidov -
temperatura prehoda oz. “melting T” (T_m)

- Pod T_m - membranski lipidi togi, tesno zloženi.
- Nad T_m - lipidi bolj fleksibilni, lateralna difuzija.
- T_m - je posledica lastnosti lipidov v membrani.
- Samo čisti lipidni sistemi - ostri, določeni fazni prehodi v ozkem temperaturnem intervalu.

Fazni prehod

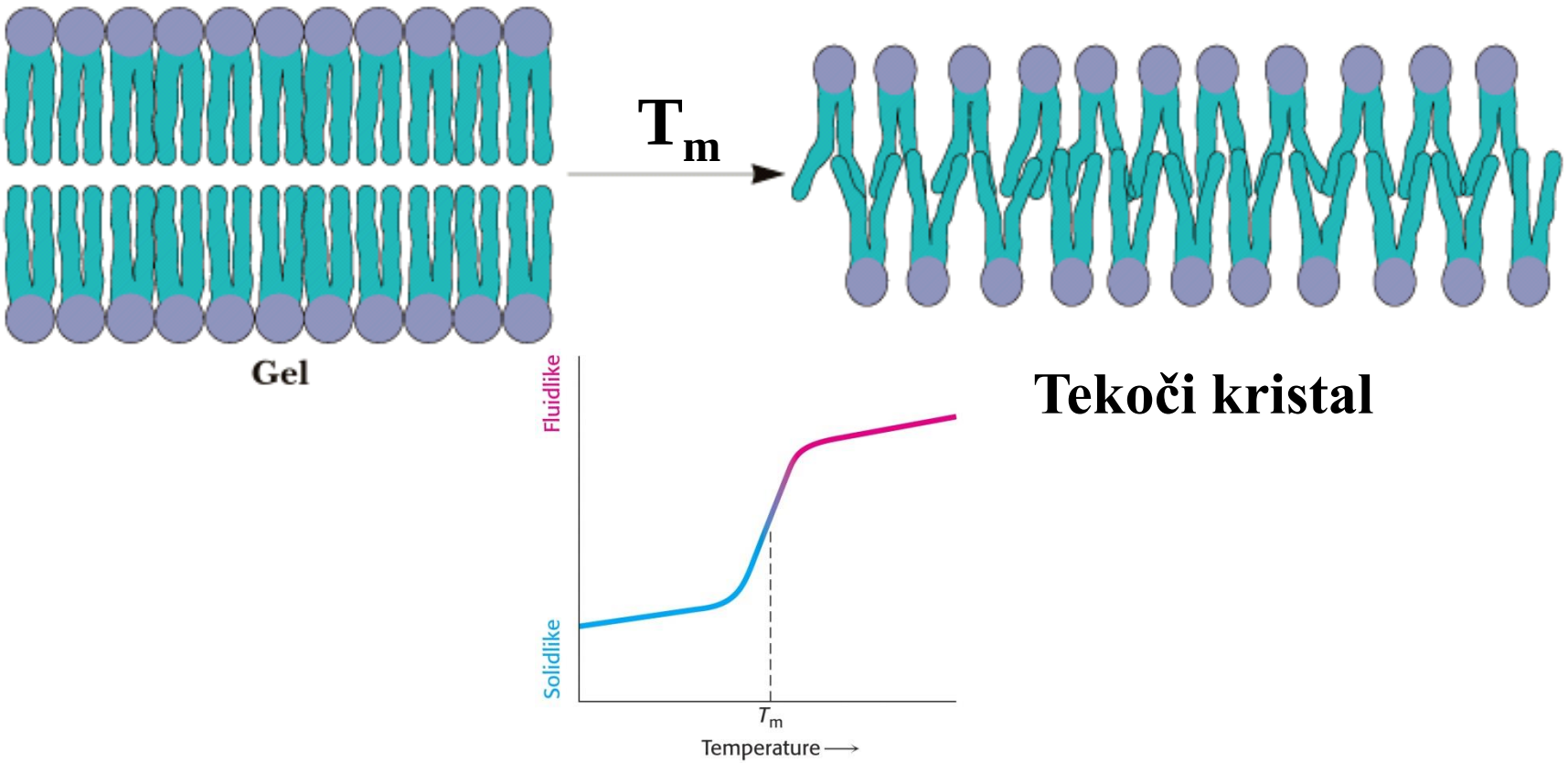
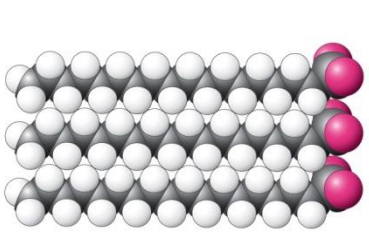
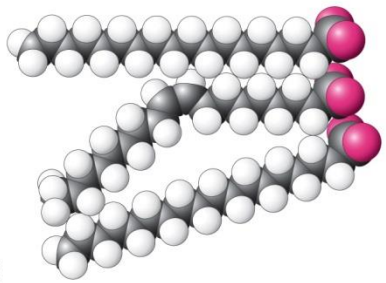


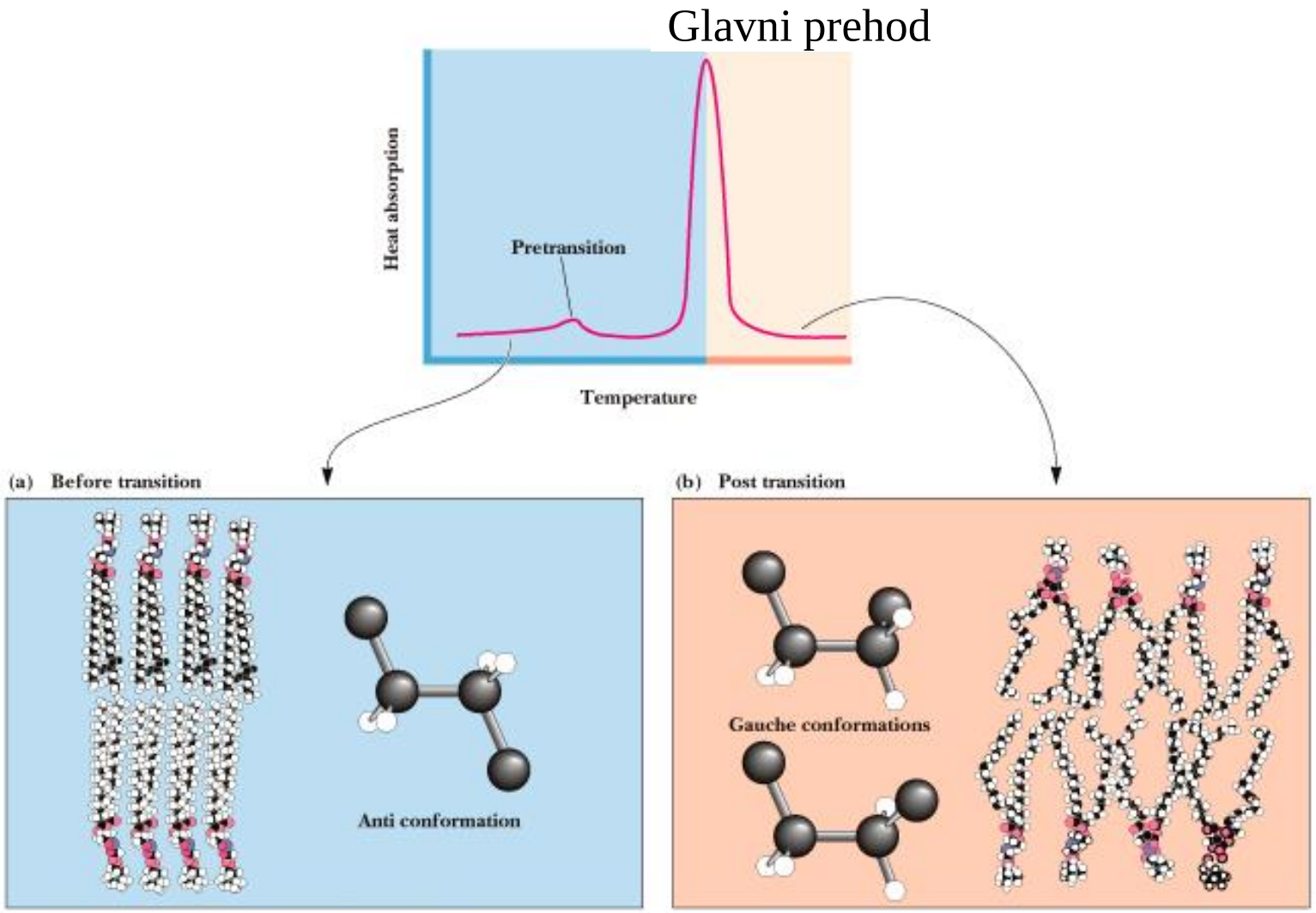
TABLE 12.3 **The melting temperature of phosphatidyl choline containing different pairs of identical fatty acid chains**

Number of carbons	Number of double bonds	Fatty acid		T_m (°C)
		Common name	Systematic name	
22	0	Behenate	*n*-Docosanote	75
18	0	Stearate	*n*-Octadecanoate	58
16	0	Palmitate	*n*-Hexadecanoate	41
14	0	Myristate	*n*-Tetradecanoate	24
18	1	Oleate	*cis*-Δ^9-Octadecenoate	−22





Fazni prehod (kalorimetrija)

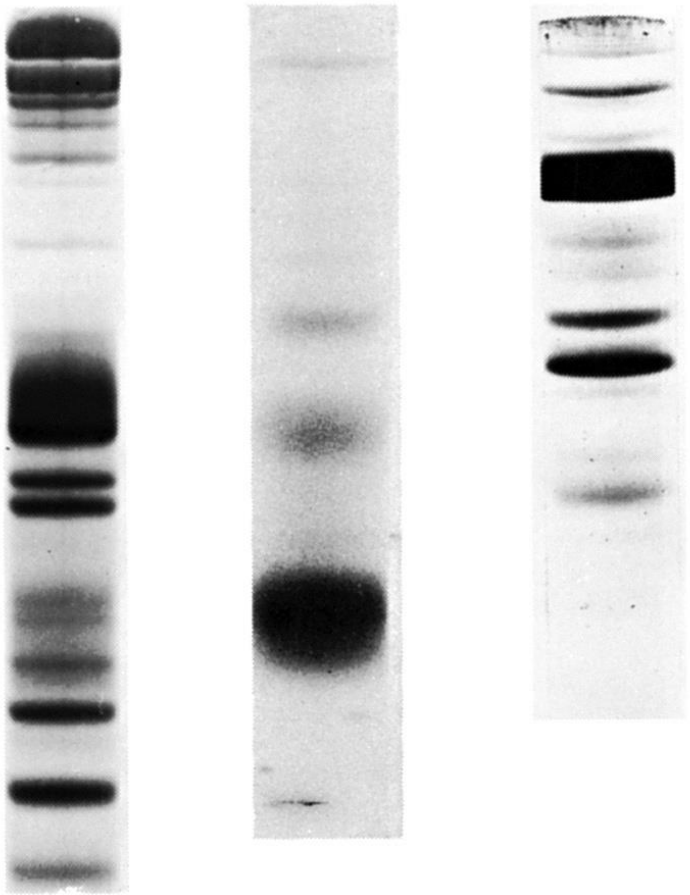


Membranski proteini

- Periferni membranski proteini.
- Integralni membranski proteini.
- Proteini, zasidrani v membrani z lipidnimi “repki”.

Vsebnost proteina v b.m. je lahko zelo različna

- mielin (oligodendrociti) 18%
- notranja m. MT 75%
- povprečno v PM 50%



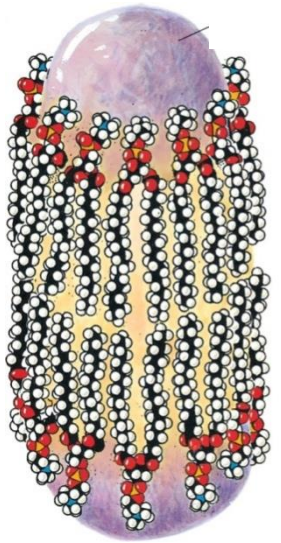
eritrocit fotoreceptor sarkopl. ret.

Periferni proteini

- Periferni proteini šibko vezani na površino membran.
- Se odtopijo (oddisociirajo od membrane) z detergenti ali povečano ionsko jakostjo (npr. 1M KCl, 1M NaCl).

Integralni membranski proteini

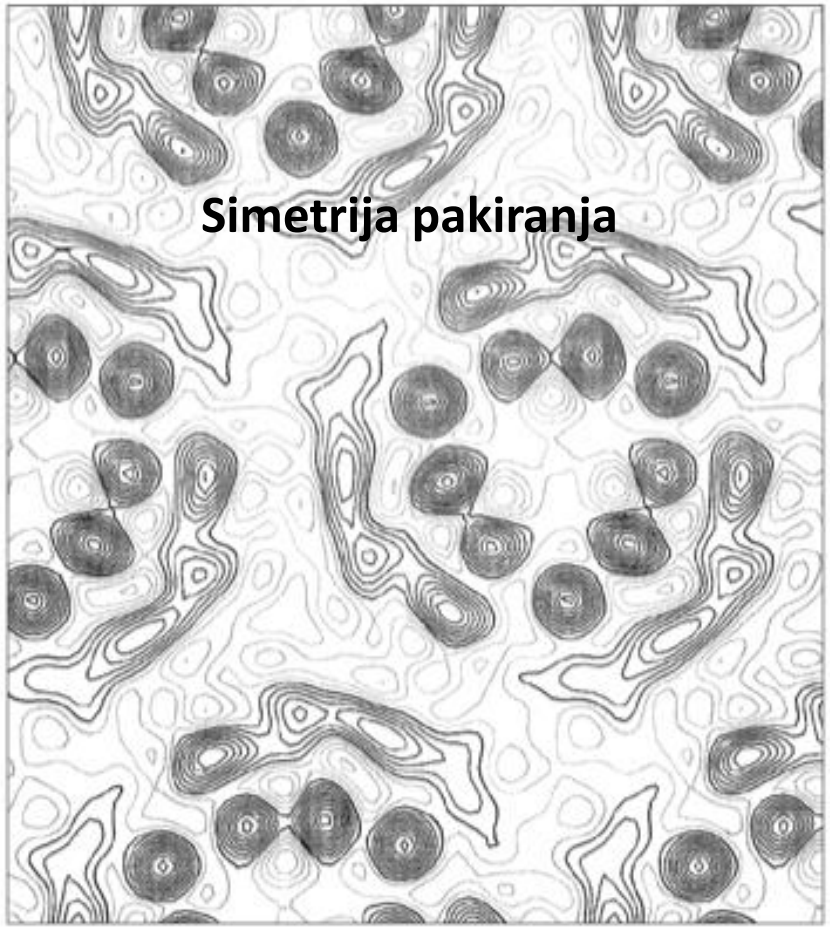
- Močno zasidrani v lipidni dvosloj.
- Ekstrakcija le z “raztapljanjem” lipidnega dvosloja:
  - organska topila,
  - Detergenti.
- Pogosto transmembranski, ni pa nujno.
- Primeri: bakteriorodopsin, glikoforin, porini.

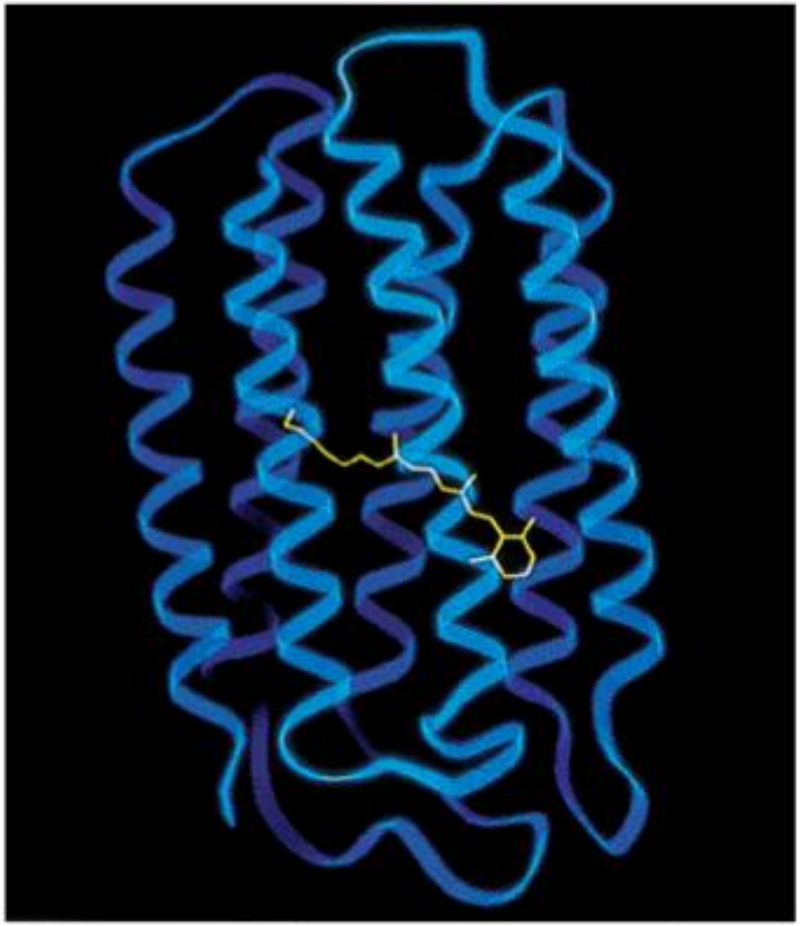


Bakteriorodopsin

- Ima 7 transmembranskih α-helikalnih segmentov s kratkimi vmesnimi zankami.
- Vir: *Halobacterium halobium* (škrlatna bakterija - arhea).
- Funkcija: sodeluje pri pretvorbi svetlobne v kemijsko energijo (ATP) - je svetlobno-gnana protonska črpalka! Nastali pH gradient poganja sintezo ATP.

Bakteriorodopsin





α-vijačnica (α-helix) iz pretežno nepolarnih ak ostankov.
Je najbolj pogost strukturni element za prehajanje membrane pri m. proteinih.

Glikoforin

- Transmembranski protein v PM eritrocitov.
- En transmembranski segment + globularni domeni na N- in C-koncu.
- Transmembranski segment je α-helikalen - 19 hidrofobnih aminokislin.
- Ekstracelularni del: močno glikoziliran (60%): sladkorji tvorijo hidrofilen plašč eritrocitov in določajo krvno skupino.

Glikoforin (eritrocit)

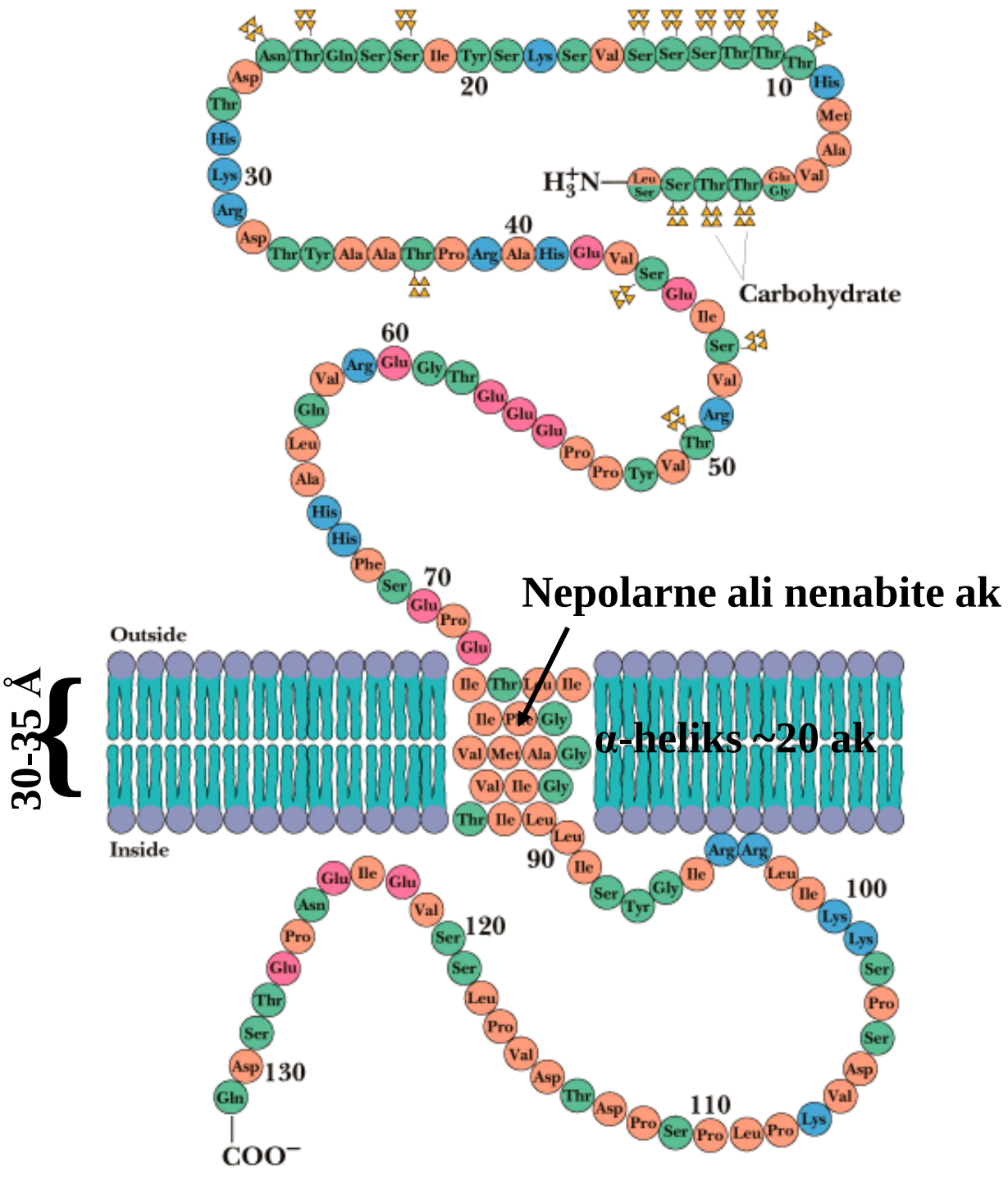
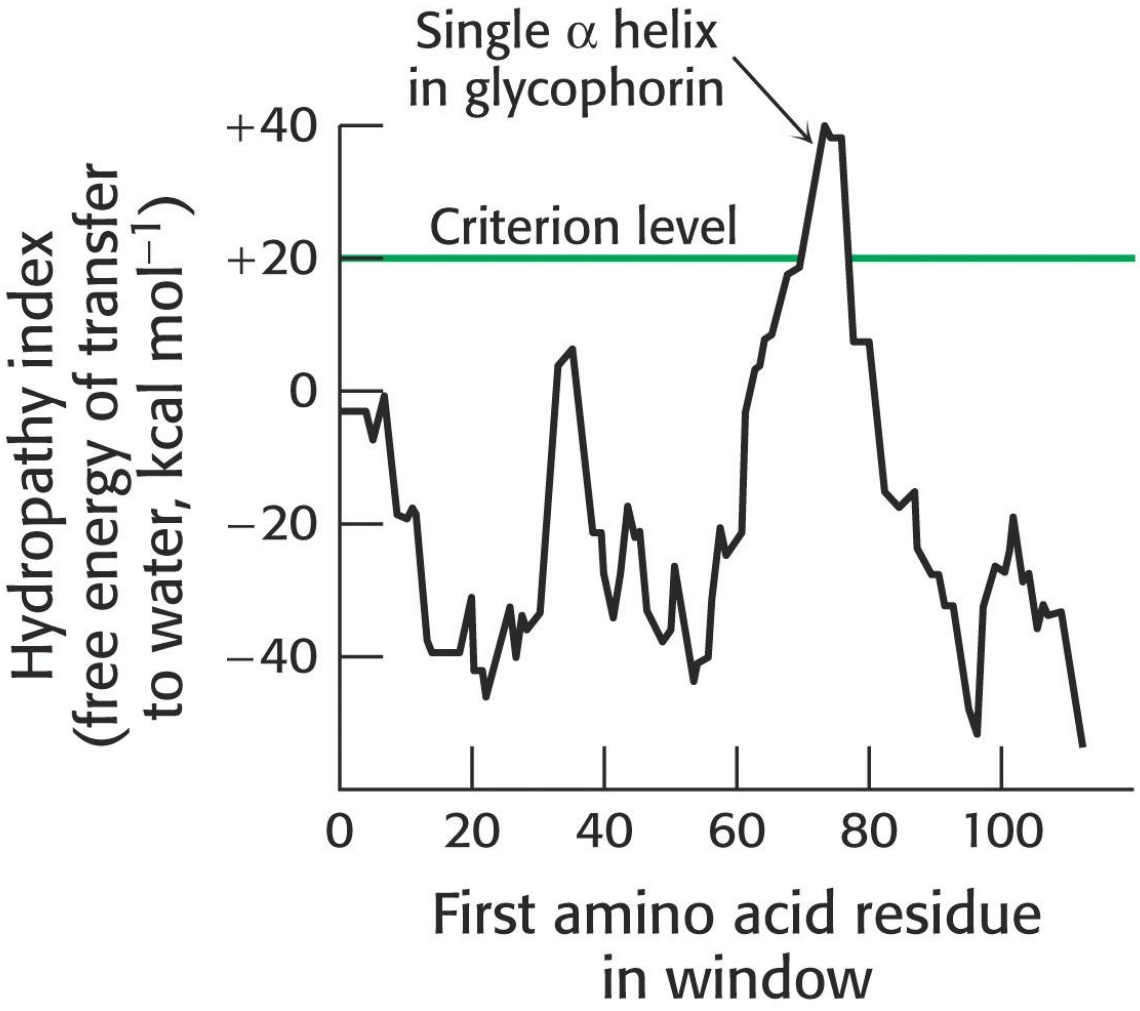


TABLE 12.2 Polarity scale for identifying transmembrane helices

Amino acid residue	Transfer free energy kcal mol^{-1} (kJ mol^{-1})
Phe	3.7 (15.5)
Met	3.4 (14.3)
Ile	3.1 (13.0)
Leu	2.8 (11.8)
Val	2.6 (10.9)
Cys	2.0 (8.4)
Trp	1.9 (8.0)
Ala	1.6 (6.7)
Thr	1.2 (5.0)
Gly	1.0 (4.2)
Ser	0.6 (2.5)
Pro	−0.2 (−0.8)
Tyr	−0.7 (−2.9)
His	−3.0 (−12.6)
Gln	−4.1 (−17.2)
Asn	−4.8 (−20.2)
Glu	−8.2 (−34.4)
Lys	−8.8 (−37.0)
Asp	−9.2 (−38.6)
Arg	−12.3 (−51.7)

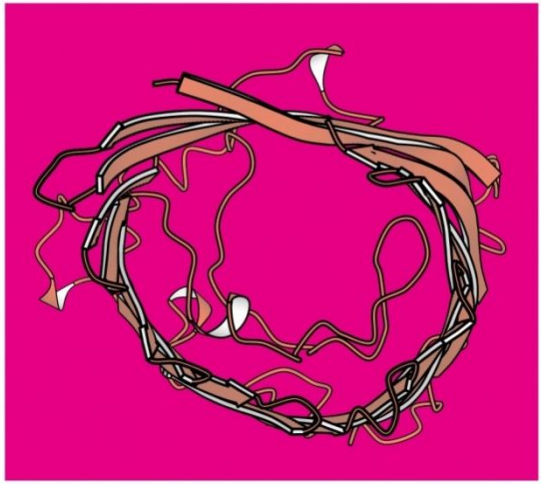
Source: After D. M. Engelman, T. A. Steitz, and A. Goldman. *Annu. Rev. Biophys. Biophys. Chem.* 15(1986):330.
Note: The free energies are for the transfer of an amino acid residue in an α helix from the membrane interior (assumed to have a dielectric constant of 2) to water.

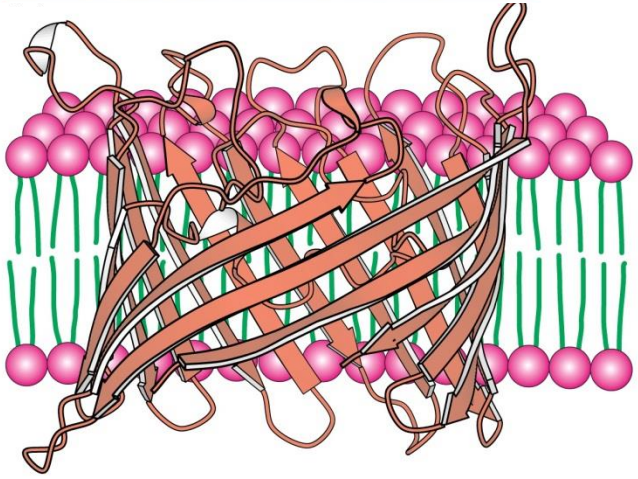

Single α helix
in glycophorin
Criterion level
+40
+20
0
−20
−40
Hydropathy index
(free energy of transfer
to water, kcal mol−1)
0
20
40
60
80
100
First amino acid residue
in window

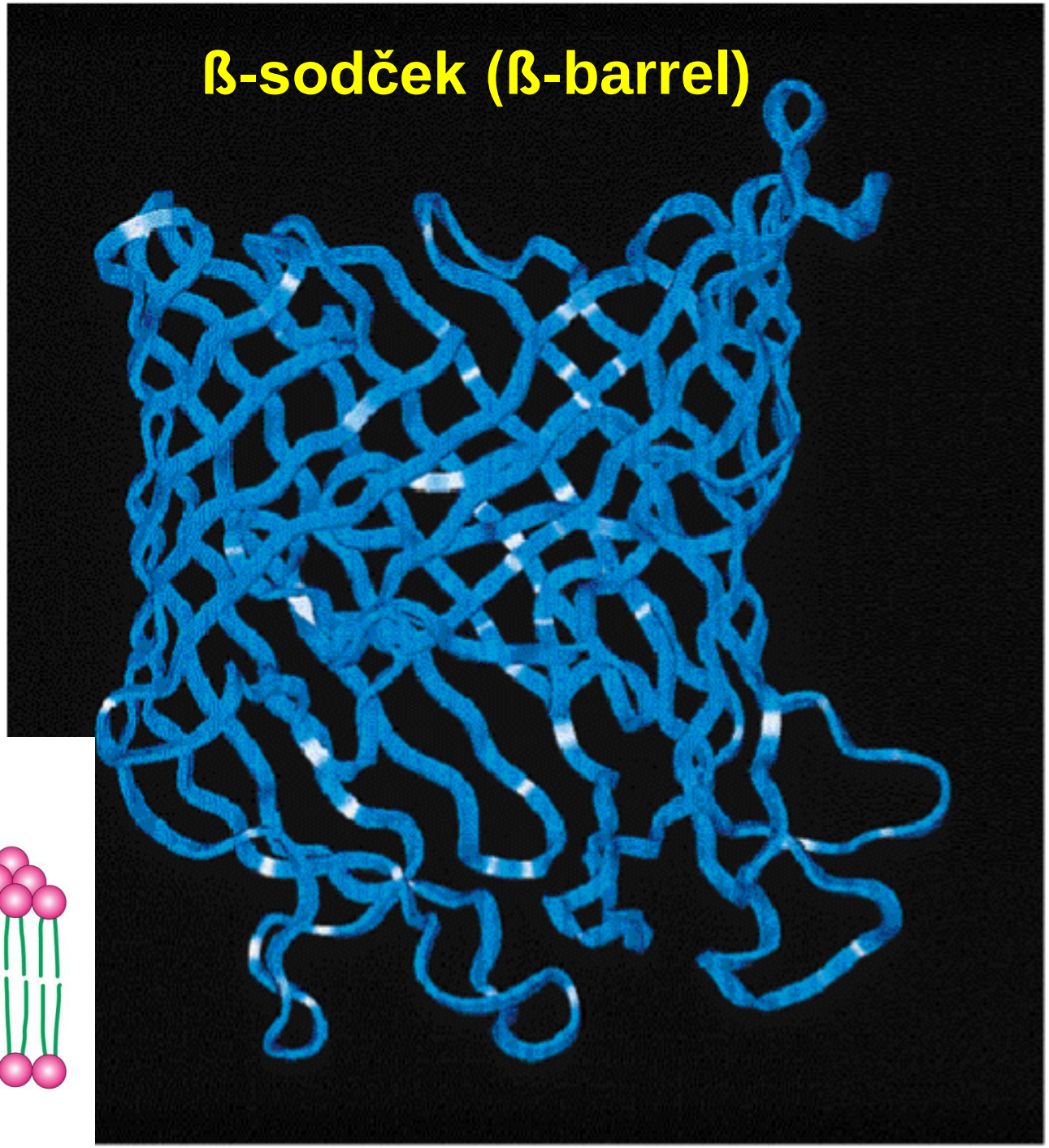
Porini

- Transmembranski protein v zunanji m. Gram-negativnih in nekaterih Gram-poz. bakterij, MT in kloroplastih.
- Transmembranski proteini s strukturo ß-sodčka.
- Tvorijo kanalčke, ki omogočajo pasivno difuzijo različnih vrst molekul.

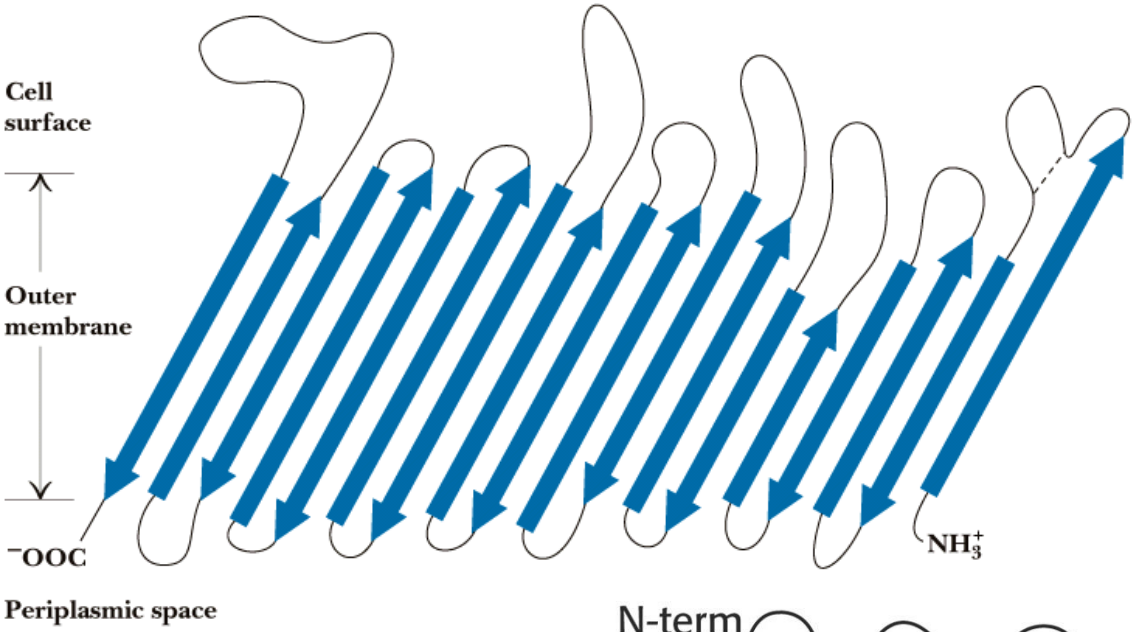
Bakterijski porin

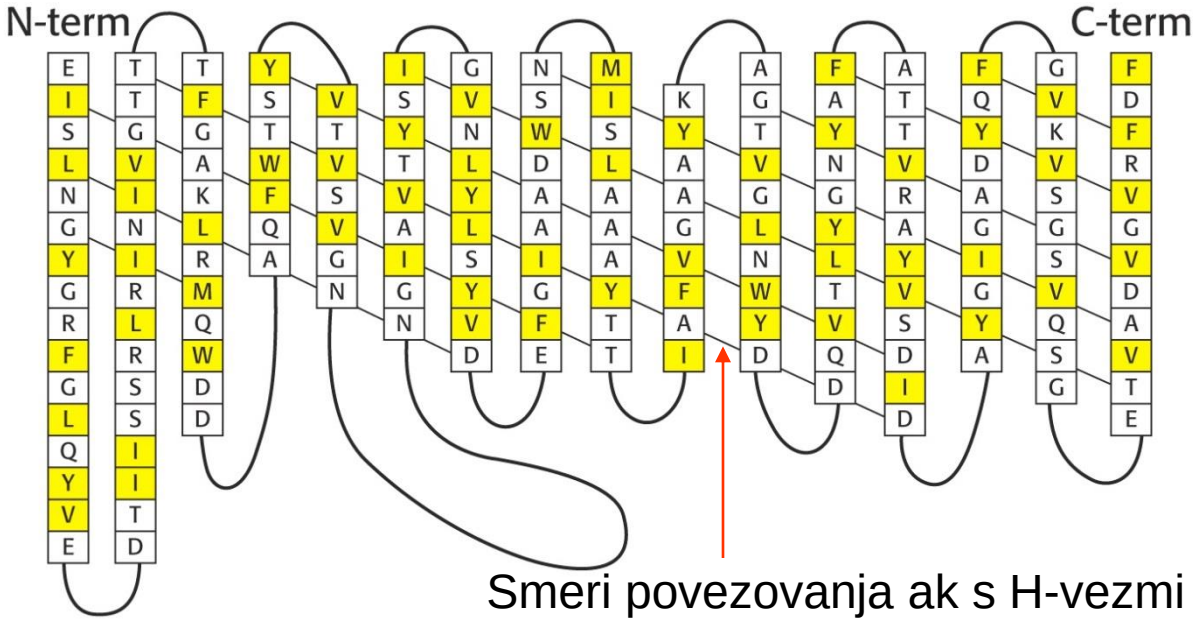




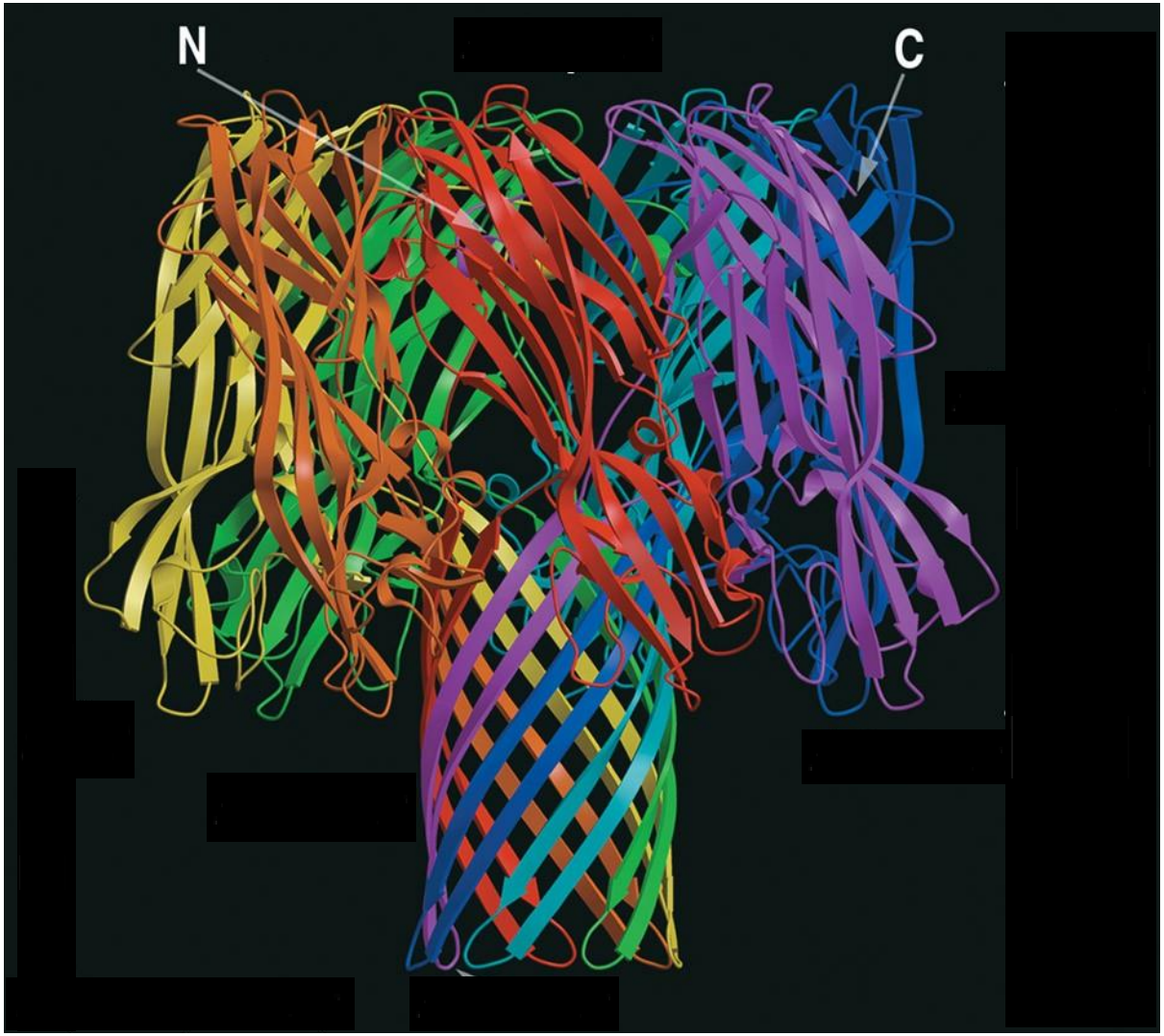


Bakterijski porin

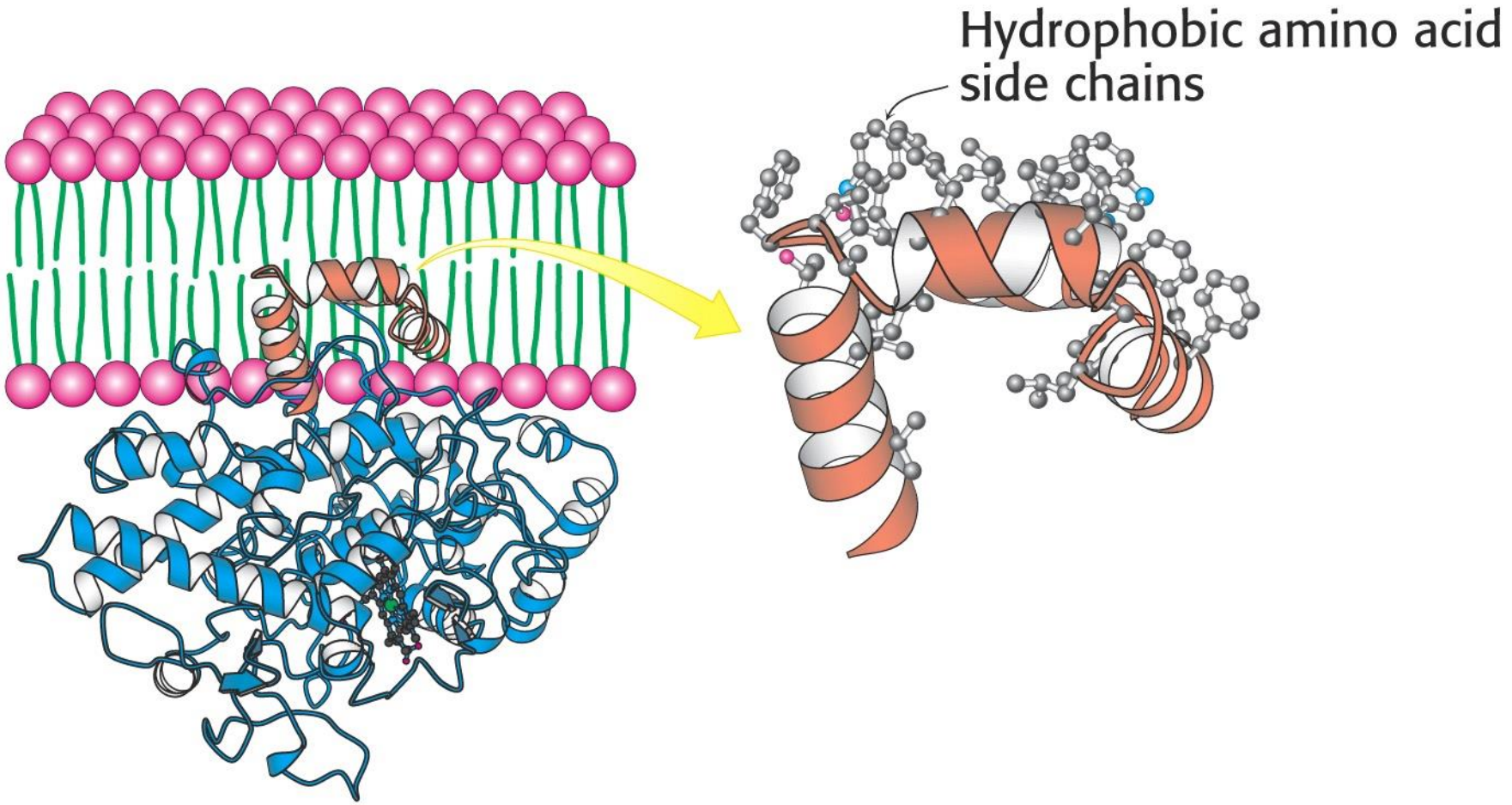


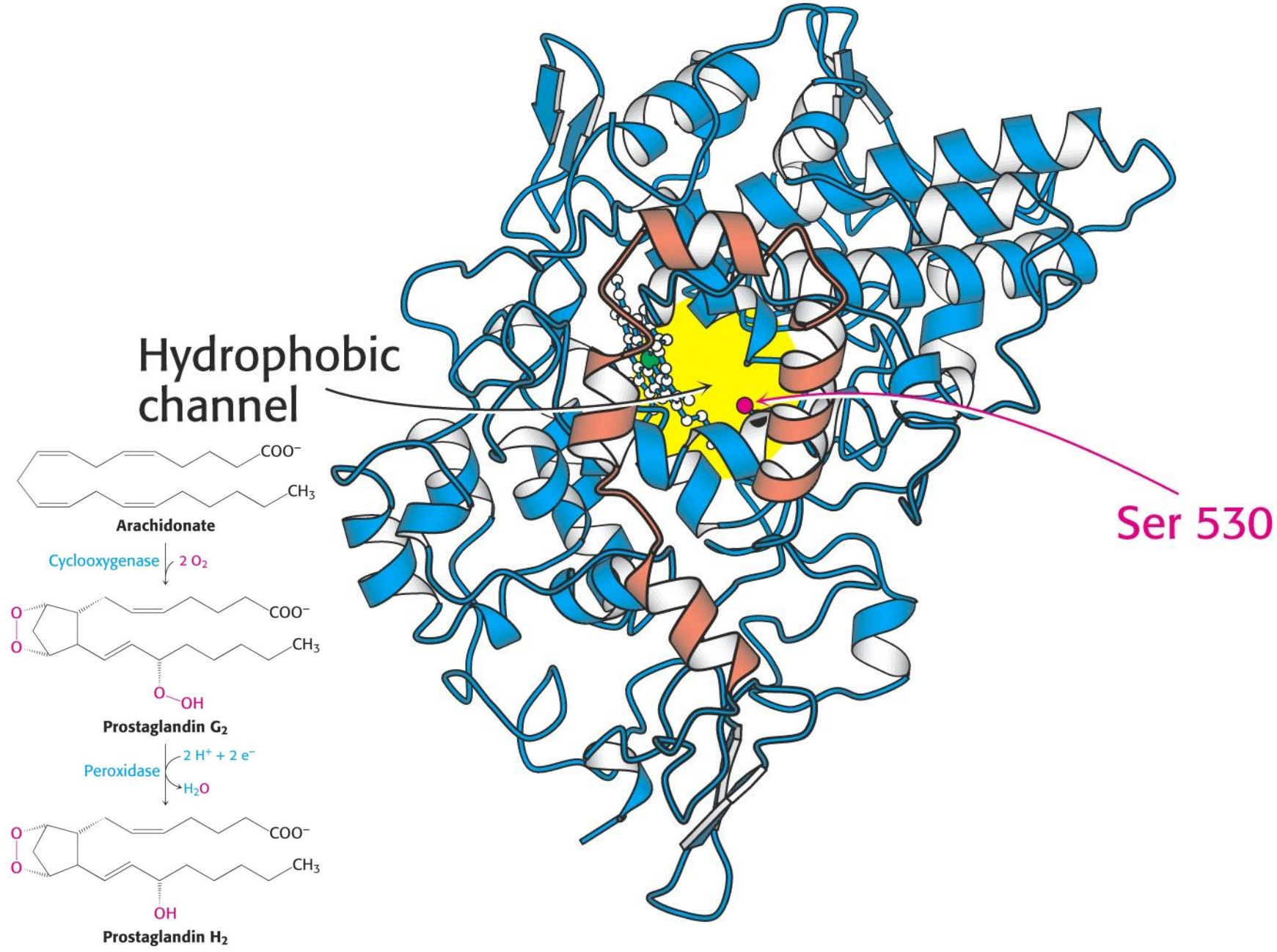


Kristalna struktura α-hemolizina (*Staphylococcus aureus*)



Prostaglandin-sintaza: ne-transmembranski integralni m.p.




Hydrophobic channel
Ser 530
COO⁻
CH3
Arachidonate
Cyclooxygenase
2 O2
O
O
OH
Prostaglandin G2
Peroxidase
2 H+ + 2 e⁻
H2O
Prostaglandin H2

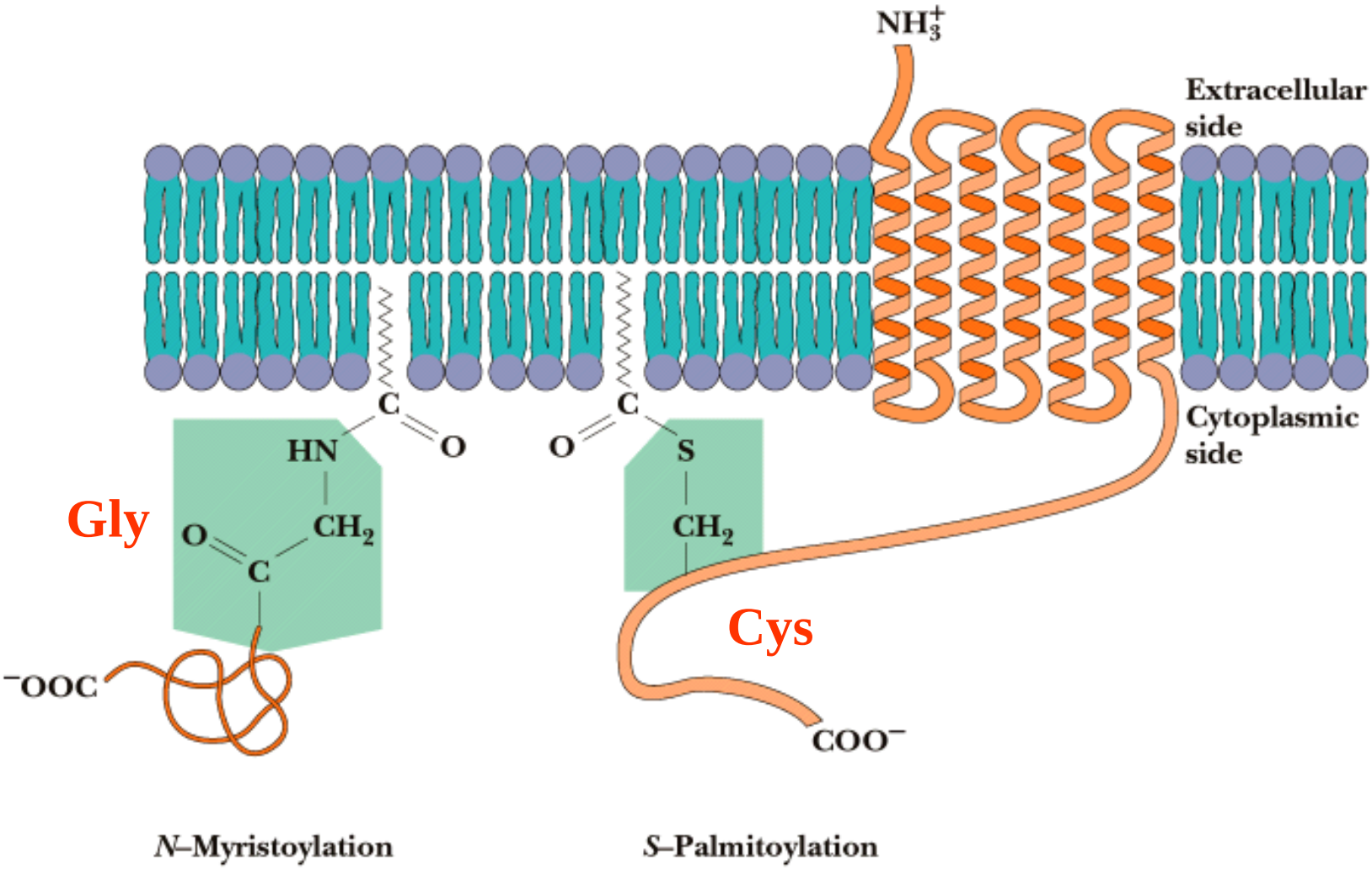
Membranski proteini, sidrani v p.m. s kovalentno vezanimi lipidi

- 4 tipi vezav v lipidno plast:
  - Miristoilno sidro - amidna vezava na protein.
  - Acilno sidro - tioesterska vez na Cys.
  - Prenilno sidro - tioeterska vez.
  - Glikozil-fosfatidilinozitolno sidro.

Miristoilno sidro - amidna vez

- Vedno miristinska kislina ($C_{14:0}$).
- Vedno vezana na N-terminus.
- Vedno povezava na Gly ostanek.
- Primeri: cAMP-odvisna proteinska kinaza, $pp60^{src}$ tirozinska kinaza, kalcineurin B, α-podenota G-proteina, gag-protein HIV-1.

Miristoilno in acilno sidro



Acilno sidro – (tio)esterska vez

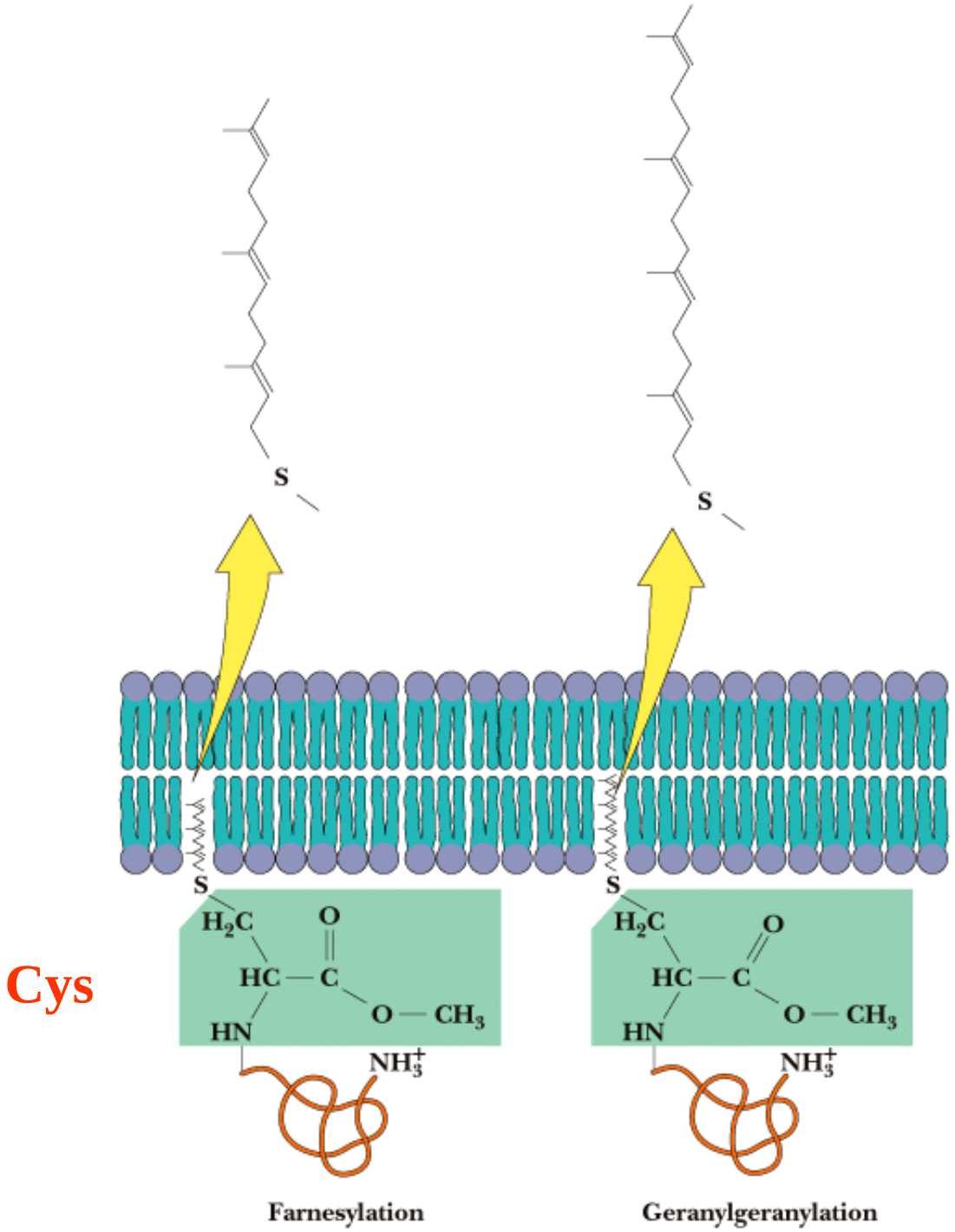
- Širša specifičnost za mašč. kisline: miristat, palmitat, stearat, oleat.
- Širša specifičnost za aminokisline: Cys, Ser, Thr.
- Primeri: receptorji odvisni od G-proteina, površinski glikoproteini nekaterih virusov, transferinski receptor, SNAP-25, kaveolini.

Prenilno sidro - tioeterska vez

- Prenilacija: vezava “izoprenskih derivatov”
- Vedno Cys iz CAAX zaporedja na C-koncu proteina (C=Cys, A=alifatski, X=poljubni ostanek).
- Izoprenske skupine (C_5):
  - farnezilni ostanek (C_{15}, 3 dvojne vezi) - seskviterpen
  - geranilgeranilni ostanek (C_{20}, 4 dvojne vezi) - diterpen
- Primeri: Ras in Rab proteini, jedrni lamini.

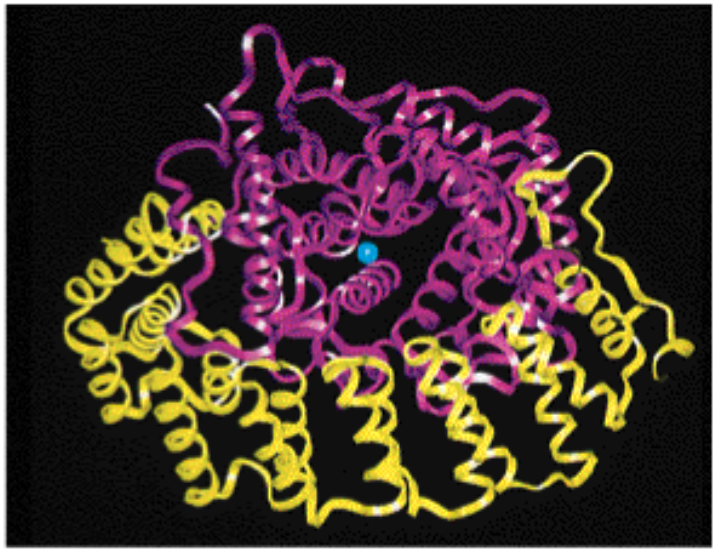
Prenilni sidri:

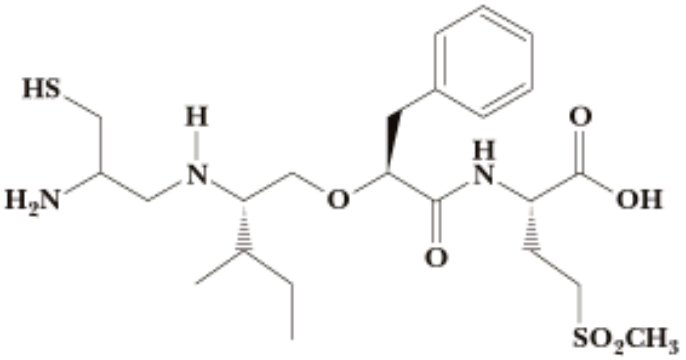
- farnezilno
- geranil-geranilno



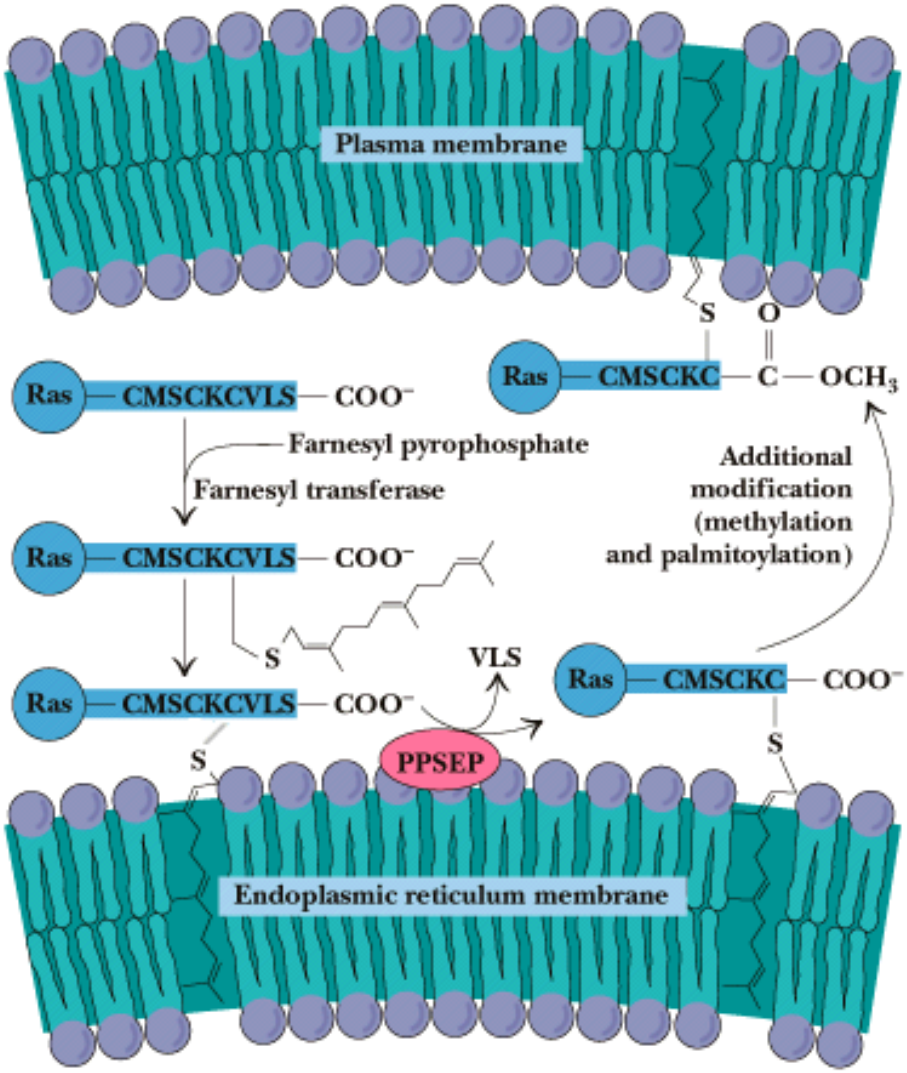
Farnezilacija Ras-proteina

Farnezil transferaza





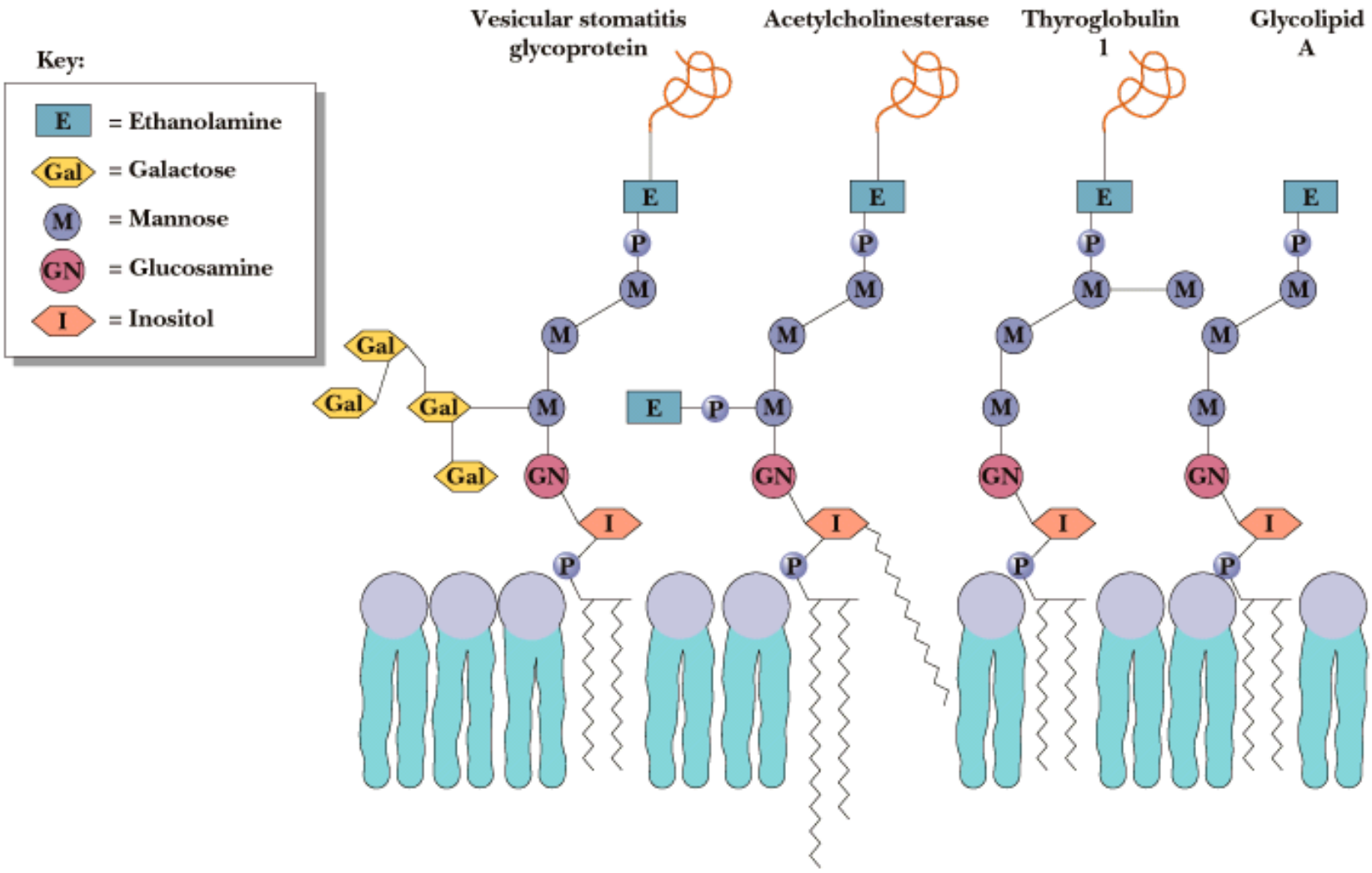
Inhibitor farnezil transferaze



Glikozil-fosfatidilinozitolno sidro
GPI sidro

- Vedno vezava na C-terminalni ostanek.
- Fosfoetanolamin povezuje C-terminal proteina preko oligosaharidne enote na fosfatidilinozitol (PI).
- Primeri: adhezijske molekule, hidrolaze celične površine, PrPC, PLAP, Thy-1.
- Pogosto v lipidnih “raftih”.

Različna GPI-sidra



Lipidna sidra - pomoč pri signaliziranju

- Lipidna sidra niso stalna - vezava je prehodna.
- Reverzibilna vezava sidra - kontrolna funkcija - modulacija v signalnih poteh.
- Podobnost s fosforilacijo/defosforilacijo, vezavo/disociacijo substrata, proženjem s proteolitičnim razcepom, itd …