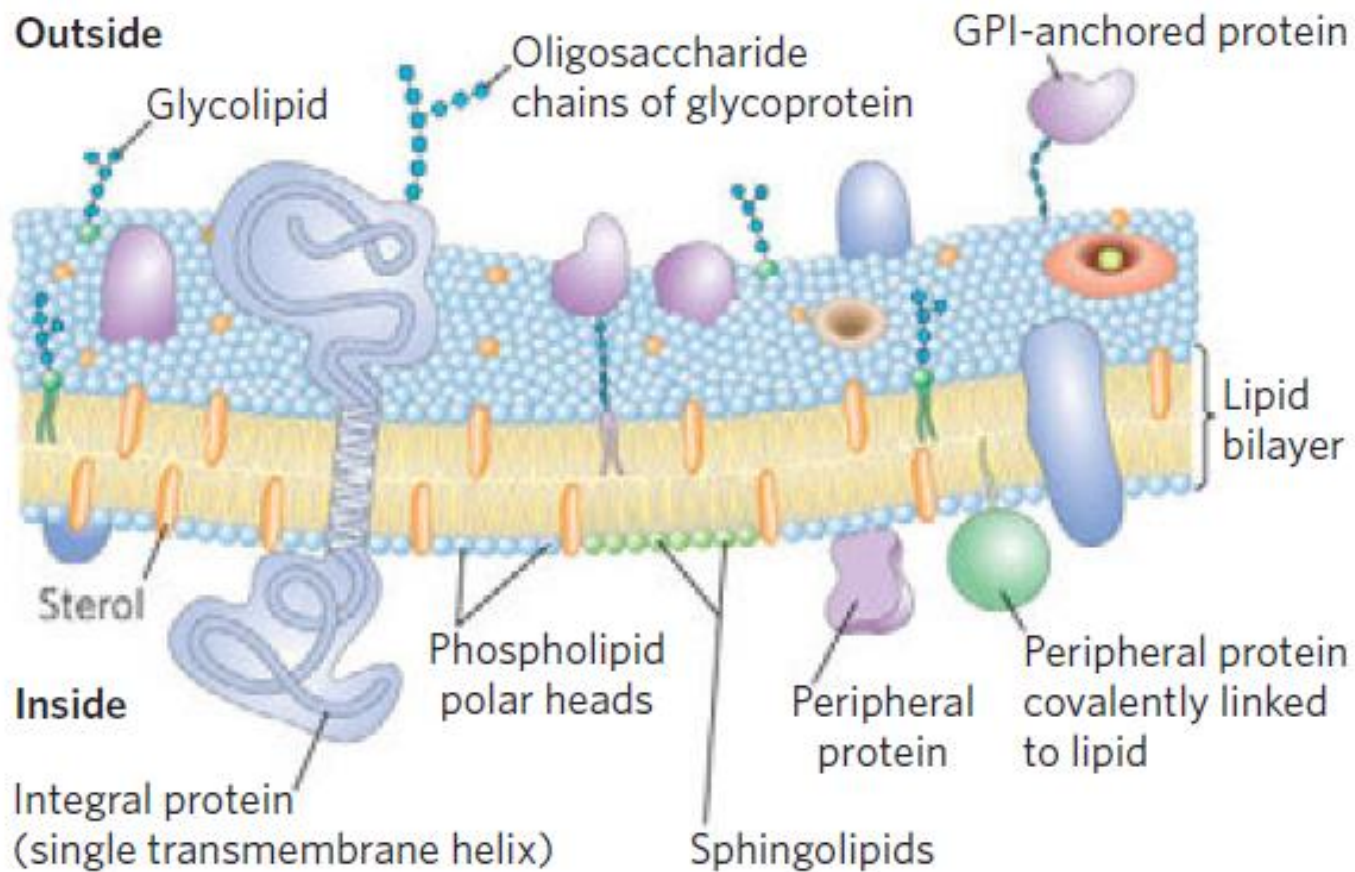


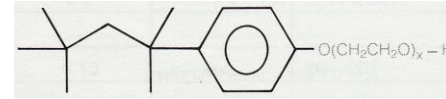
Struktura lipidnih mikrodomen

Singer-Nicholsonov model organizacije BM kot tekočega (fluidnega) mozaika (1972)

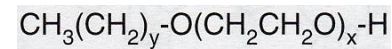


Le del BM je topen pri nizki T ($\leq 4^{\circ}\text{C}$) v detergentu:

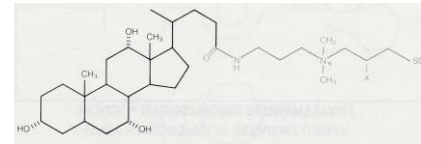
Triton X-100 (NP-40)



Brij-58

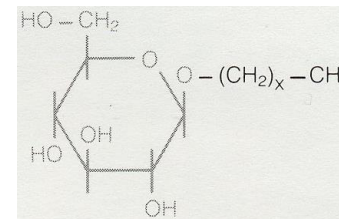


CHAPS



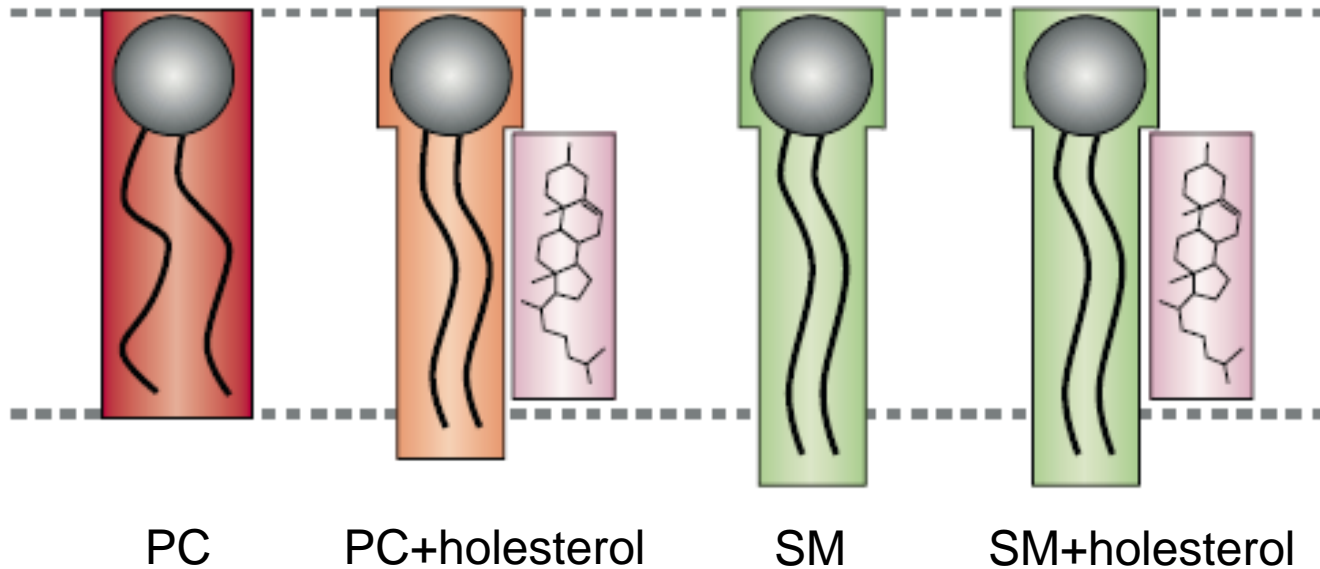
Preostanek membran je topen v detergentu:

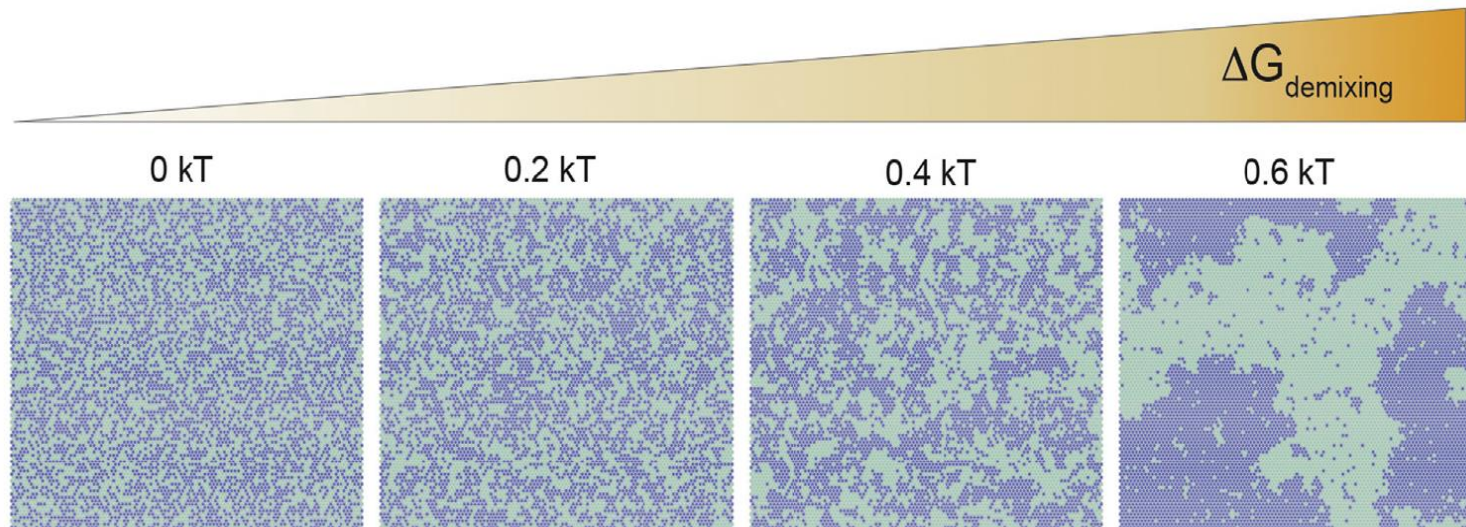
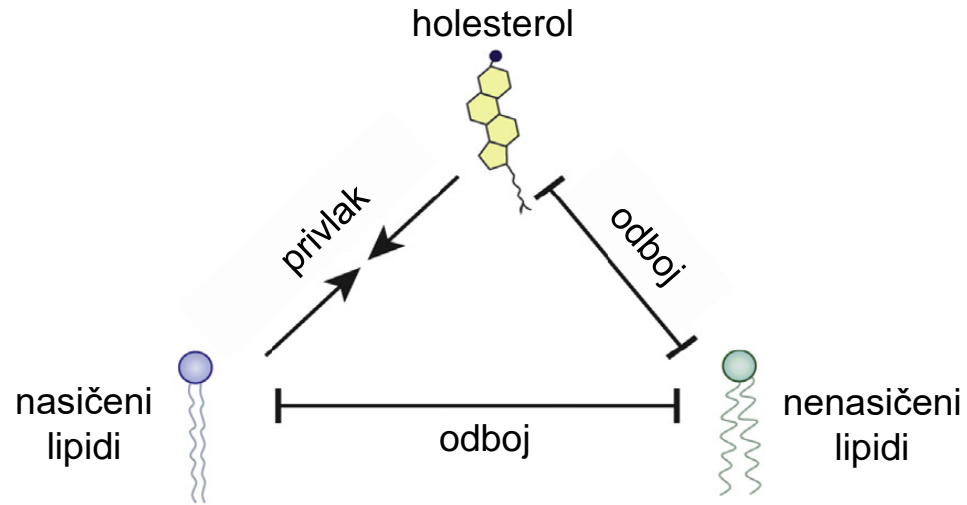
oktil glukozid



zgoraj naštetih detergentih pri višji T

Holesterol lahko izzove ločitev (segregacijo) tekočih faz





Spreminjanje vsebnosti sestavin lipidnih raftov

Sekvestracija holesterola

- Antibiotics:
Filipin | Nystatin | Amphotericin
- Pore-forming agents:
Saponin | Digitonin | Streptolysin O

Ekstrakcija holesterola

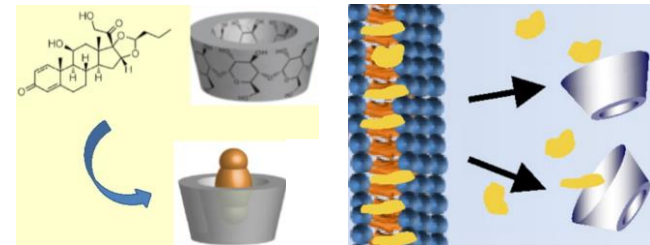
- Methyl- β -cyclodextrin

Inhibicija sinteze holesterola

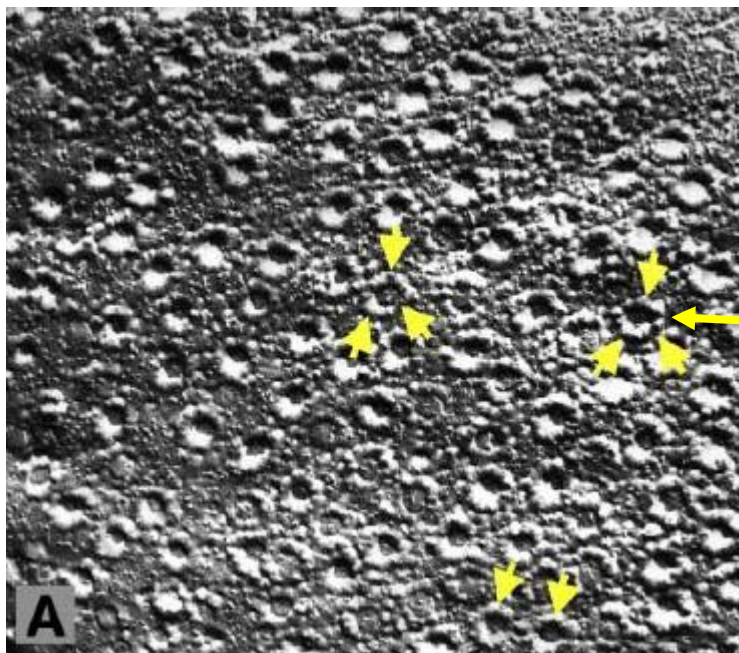
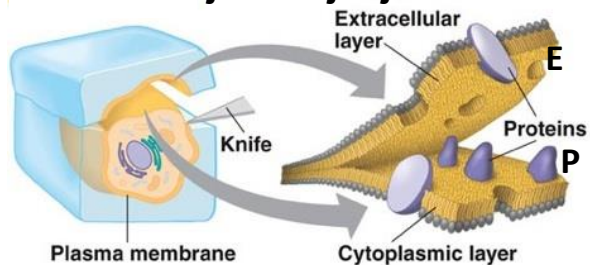
- Lovastatin

Perturbacija stabilnosti rafta

- Exogenous cholesterol
- Exogenous gangliosides
- Exogenous polyunsaturated fatty acids

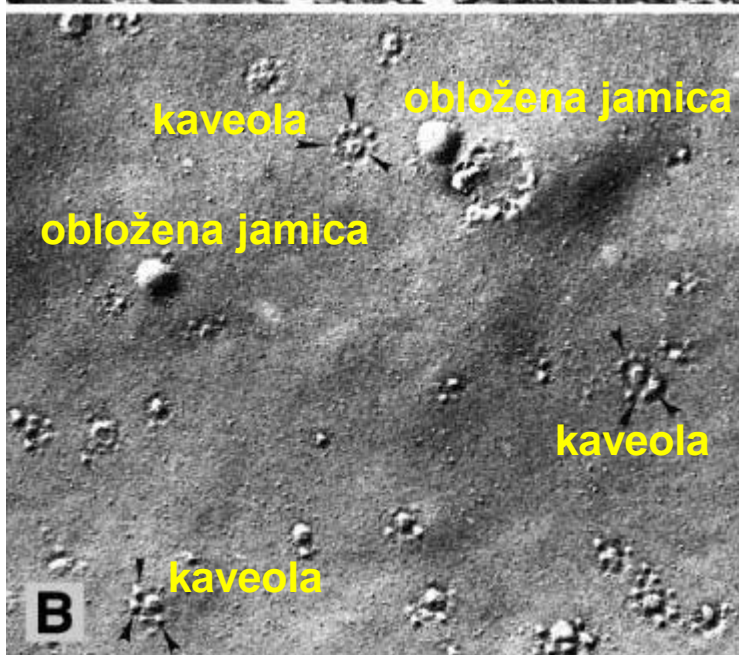


Zamrzovanje-lomljenje



PM endotelijske celice
Proto- ali P-stran

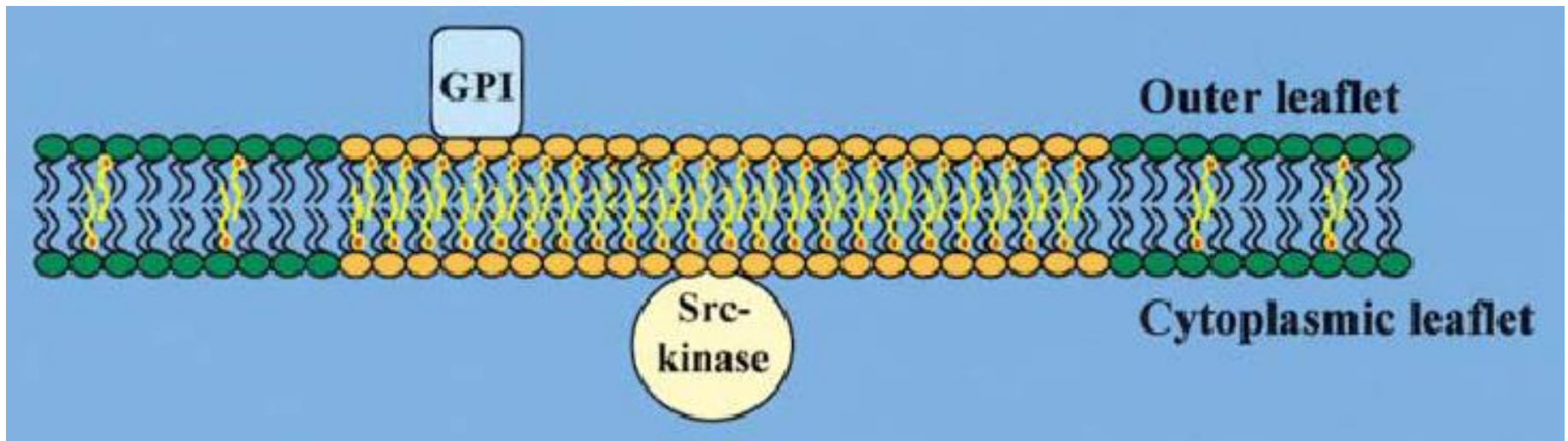
Oborina kompleksa
filipin-holesterol



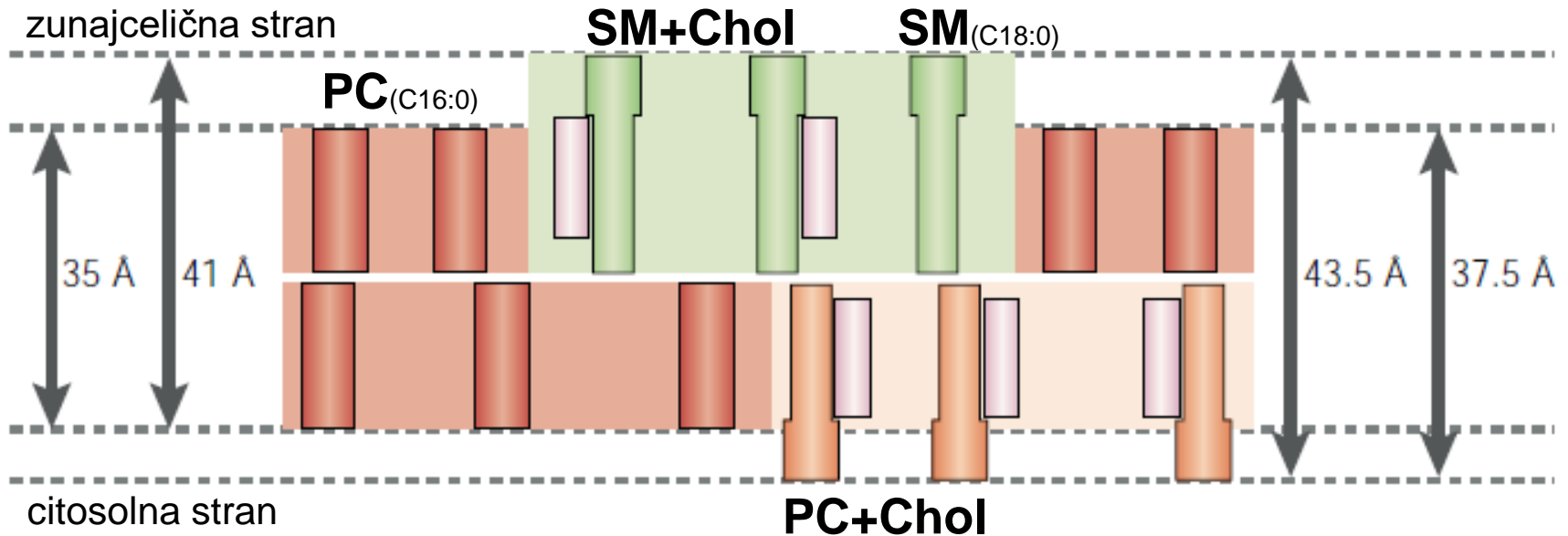
PM celice gladke mišice
Ecto- ali E-stran

**Holesterol v BM
ni enakomerno
razporejen!**

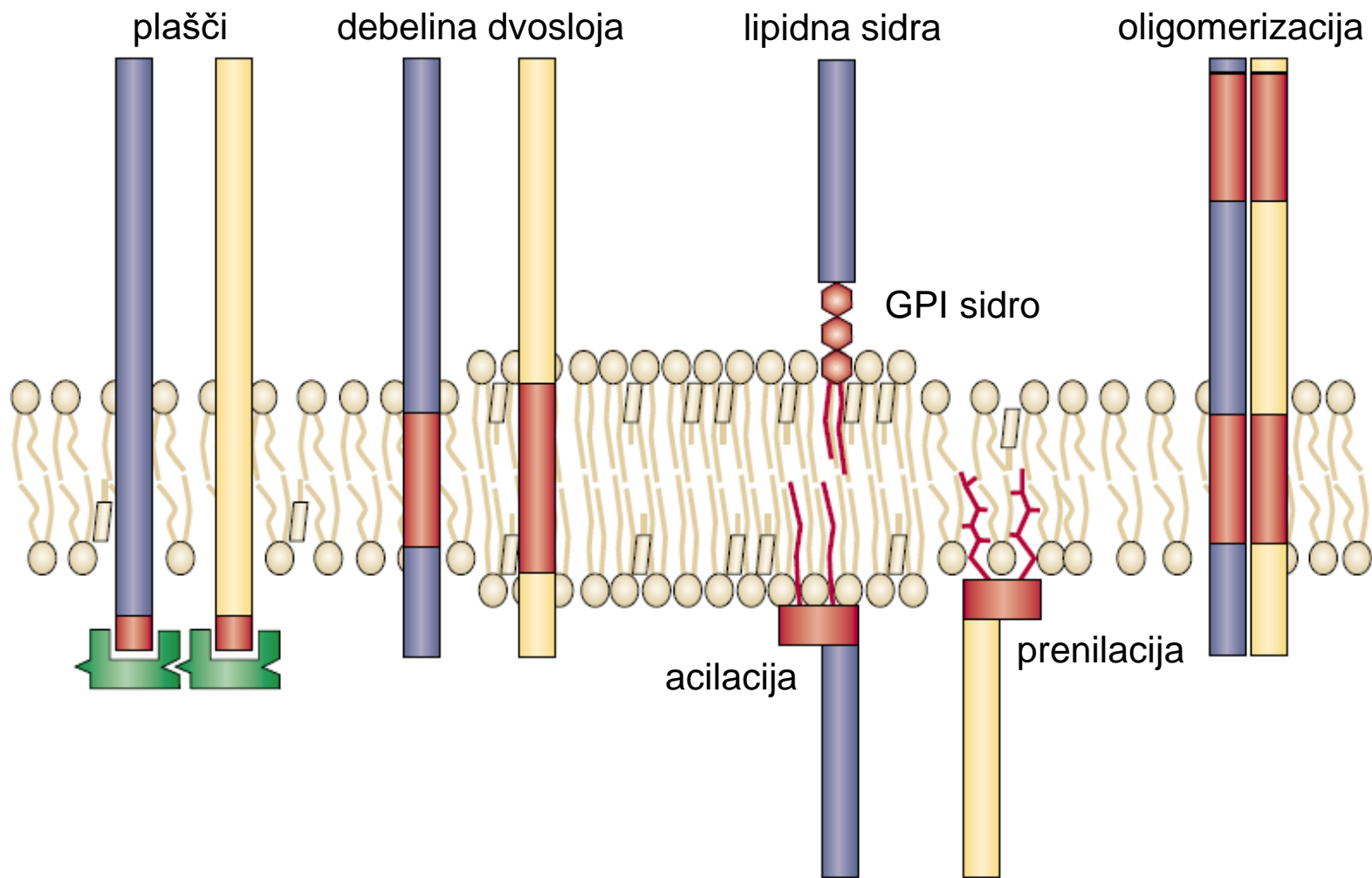
BM so notranje urejene: koncept lipidnega rafta



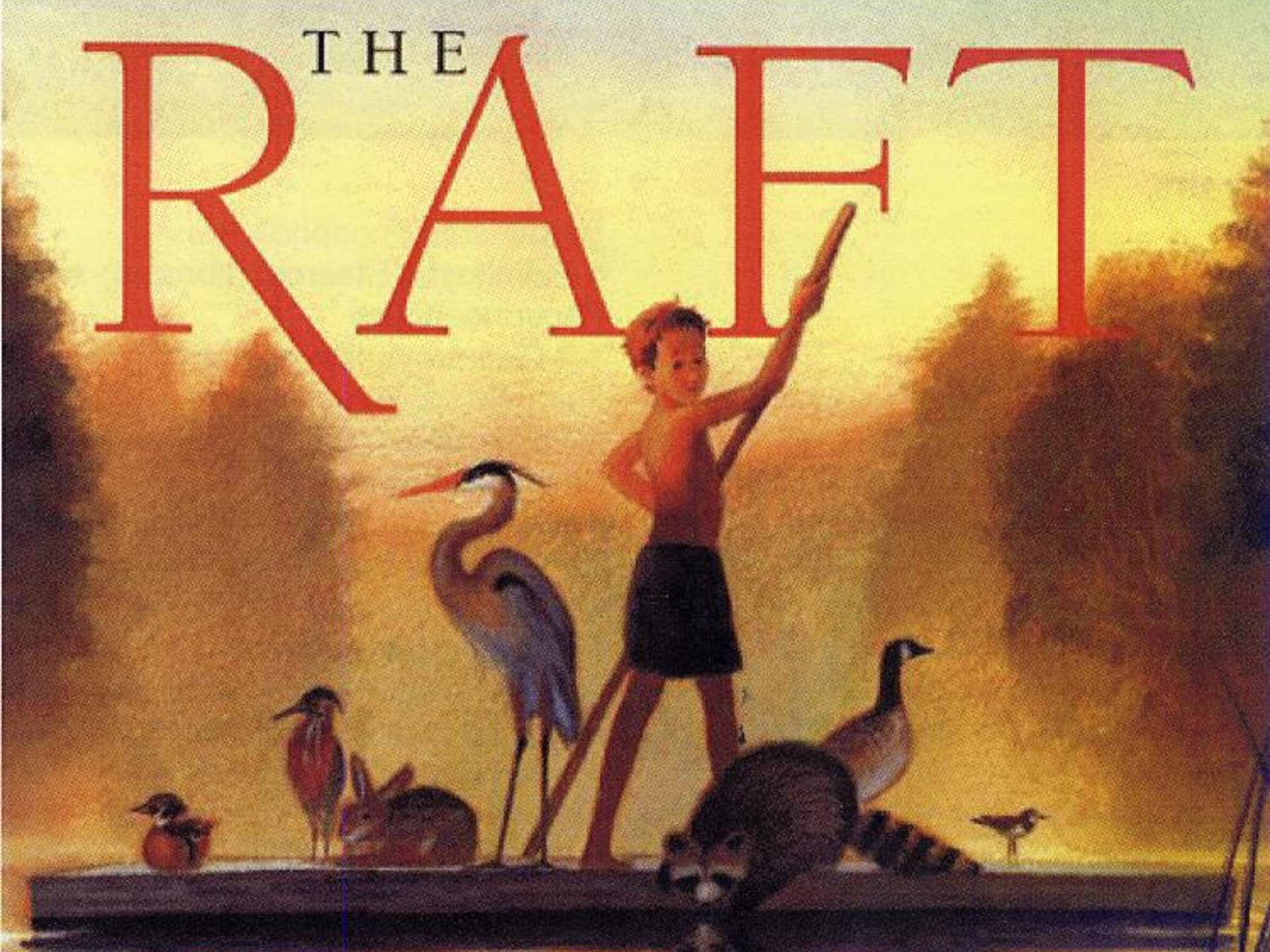
Debelina lipidne membrane je odvisna od njene sestave



Laterarno razvrščanje membranskih proteinov



THE
RAFT



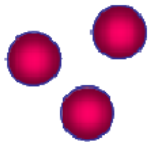
Eksperimentalne tehnike za študij lipidnih raftov

Approach*	Information available	Live cells	Comments
Flotation of detergent-resistant membranes (DRMs)	Identifies putative raft association Identifies possible raft proteins	No	<ul style="list-style-type: none"> • Easy to do • Most common approach for identifying putative proteins involved in signalling • Artefacts possible • Weak associations with rafts are difficult to detect
Antibody patching and immunofluorescence microscopy	Identifies putative raft association	No	<ul style="list-style-type: none"> • Easy to do • Common approach • Better than flotation for detecting weak raft associations • Cell-cell variability makes quantification difficult
Immunolectron microscopy	Determines location of raft components	No	<ul style="list-style-type: none"> • Promising results • Requires technical expertise
Chemical crosslinking	Identifies native raft protein complexes	Yes	<ul style="list-style-type: none"> • Straightforward • Choice of appropriate conditions and reagents is semi-empirical
Single fluorophore tracking microscopy	Monitors the diffusion and dynamics of individual raft proteins or lipids	Yes	<ul style="list-style-type: none"> • Requires highly specialized equipment and expertise
Photonic force microscopy	Determines the diffusion constant, size and dynamics of individual rafts	Yes	<ul style="list-style-type: none"> • Very informative technique • Requires highly specialized equipment and technical expertise • Time-consuming acquisition and analysis
Fluorescence resonance energy transfer (FRET)	Detects whether two raft components are spatially close (for example, <10 nm)	Yes	<ul style="list-style-type: none"> • Powerful approach • Choice of appropriate donor and acceptor probes is important

*The disruption of rafts by cholesterol depletion or sequestration is especially useful as a control for each of these approaches.

Flotacija membran, netopnih v detergentu

celice



Liza celic v HLAD-
NEM ne-ionskem
detergentu



gostotni gradient
saharoze



ultracentrifugiranje



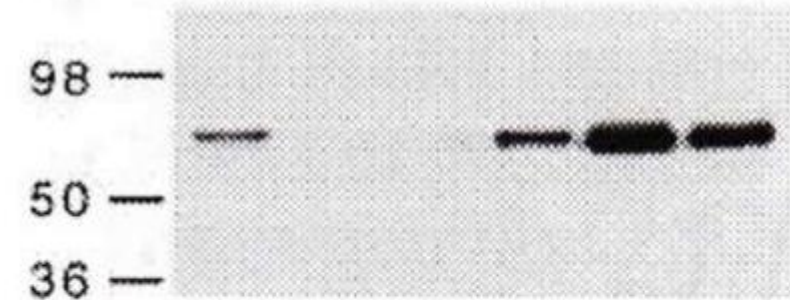
lipidni
rafti

topna
frakcija

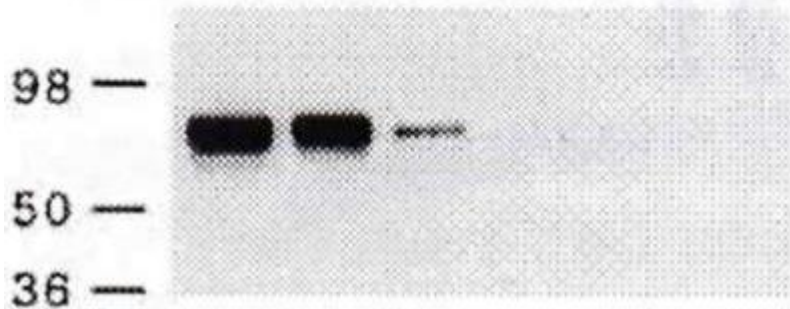
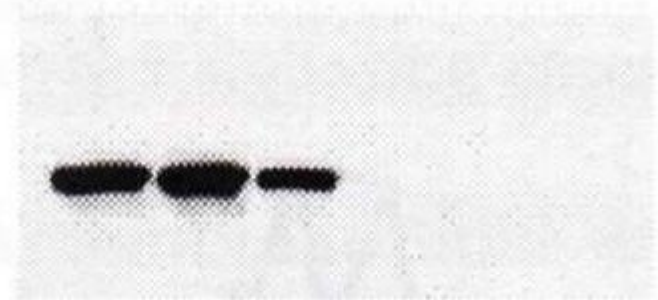
Raztapljanje BM v 2% (v/v) TR X-100 pri 4°C ali 30°C in analiza vzorca s centrifugiranjem v gradientu saharoze (flotacijska analiza).

PLAP (PLacental Alkaline Phosphatase)

VSV-G (Vesicular Stomatitis Virus Glycoprotein)



0 °C



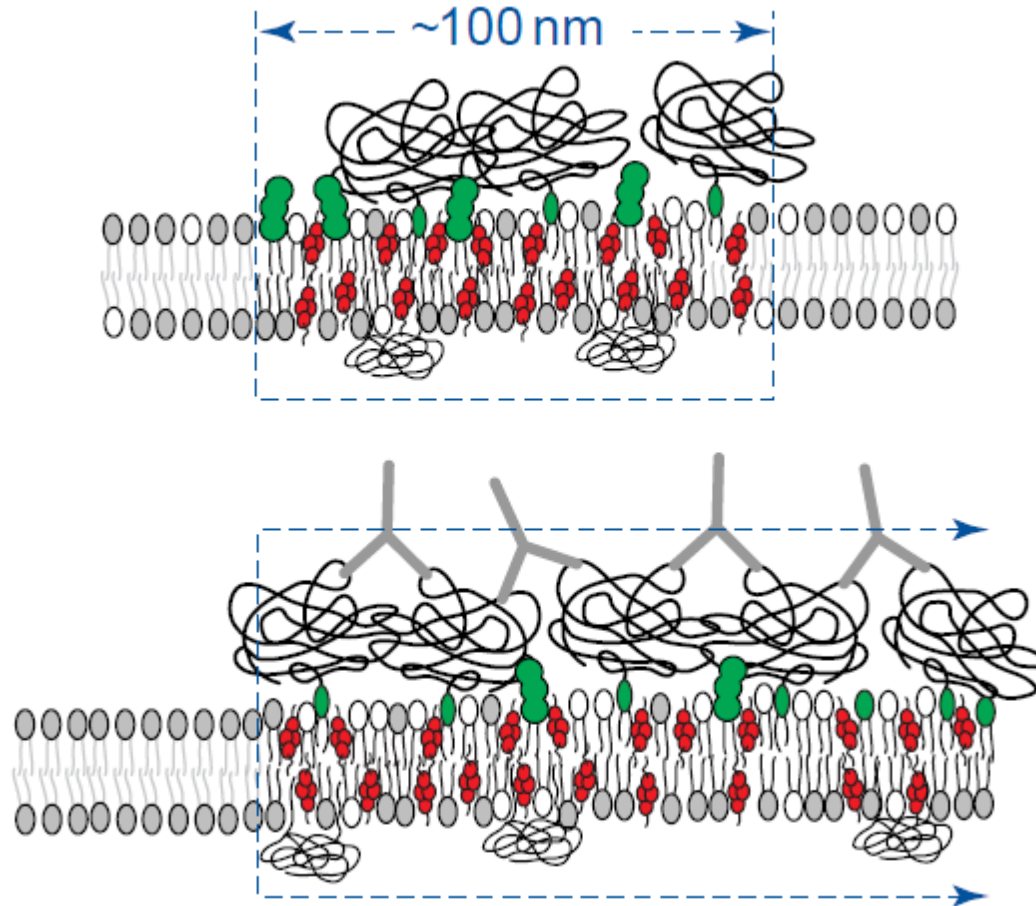
30 °C



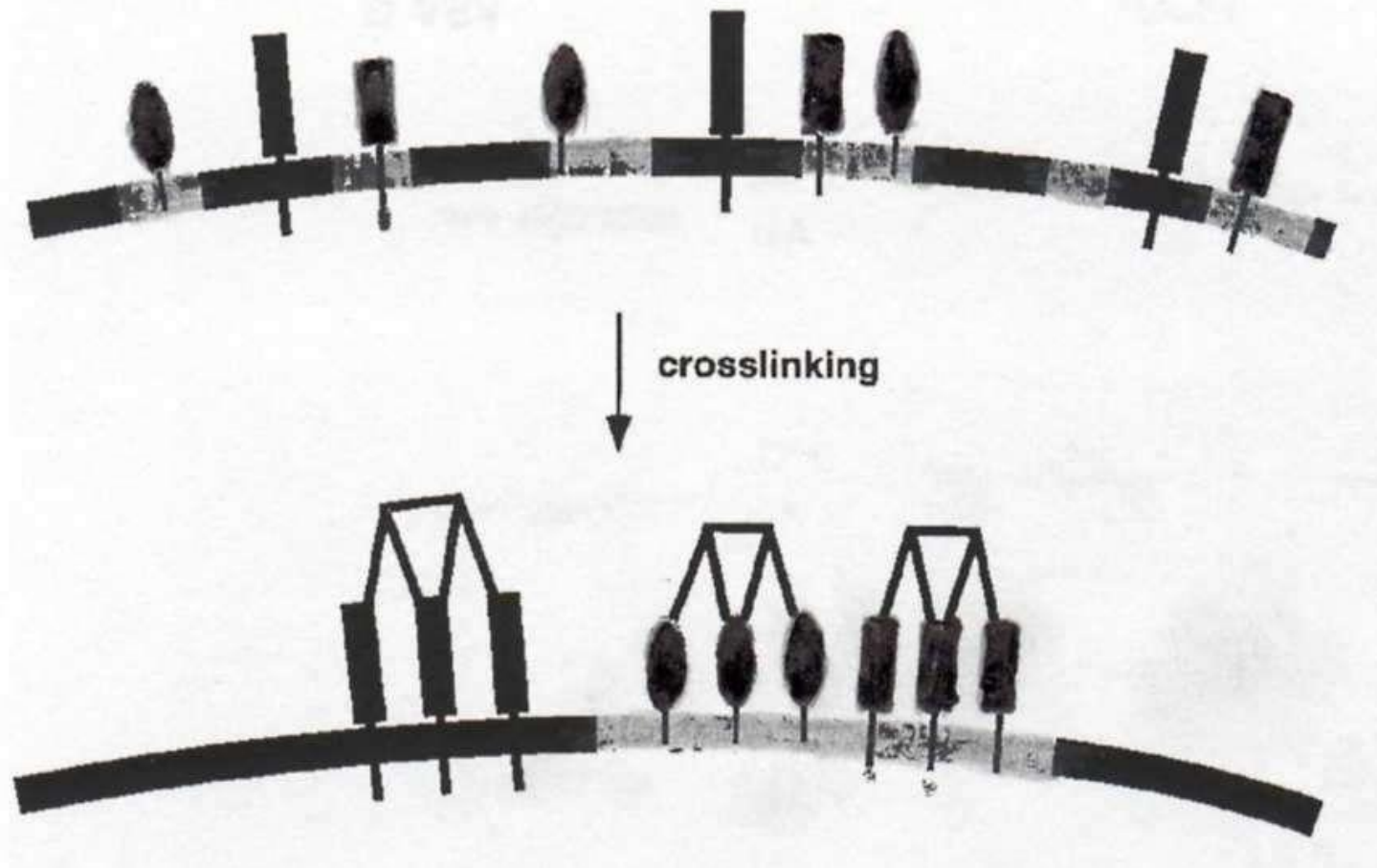
višja → nižja gotota

višja → nižja gotota

Skepljanje membranskih komponent (patching)

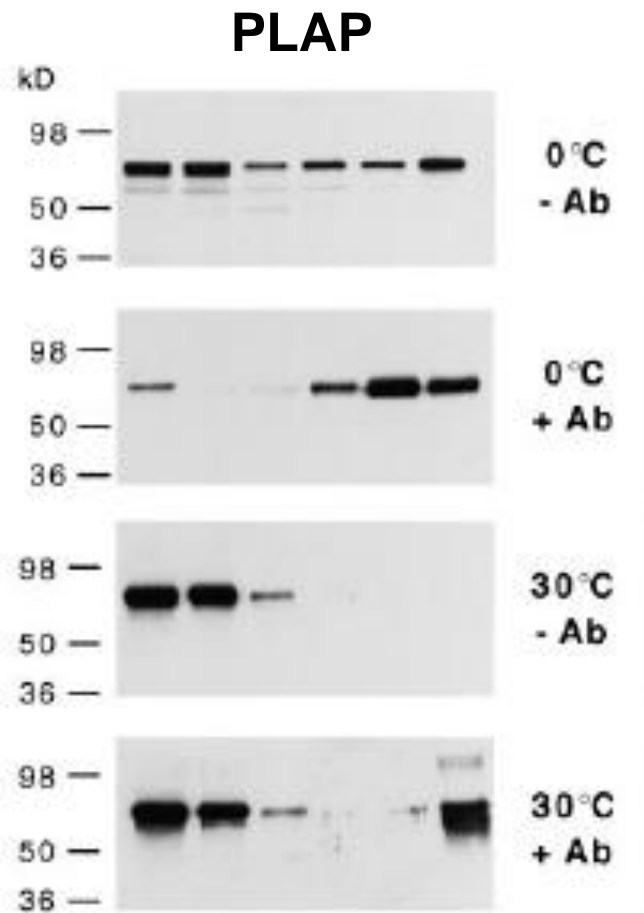


Ločevanje membranskih faz s skepljanjem membranskih komponent (patching)



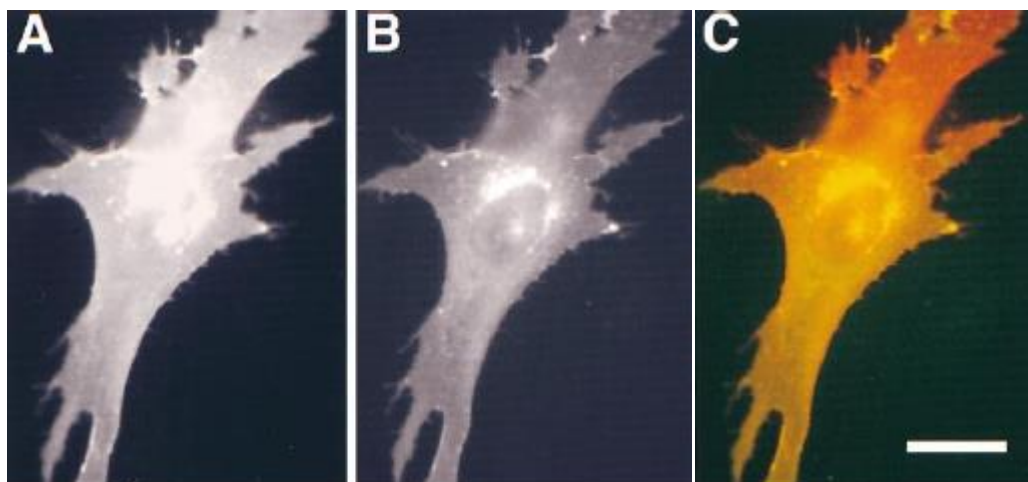
Stabilizacija membranskih domen s prečnim povezovanjem MP s protitelesi

GPI-protein PLAP izražen v nepolariziranih fibroblastoidnih BHK-21 celicah

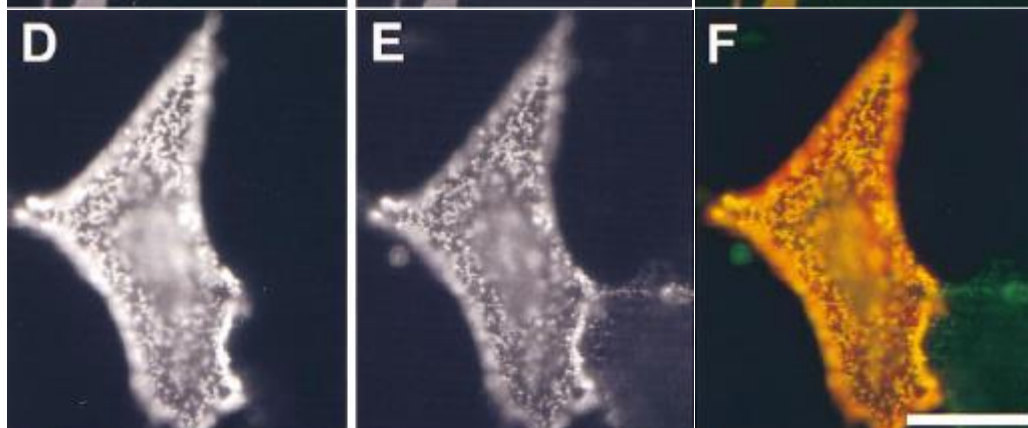


višja → nižja gostota

Skepljanje PLAP (rdeče) in HA (zeleno), proteina iz virusa gripe, so-izraženih v nepolariziranih BHK-21 celicah



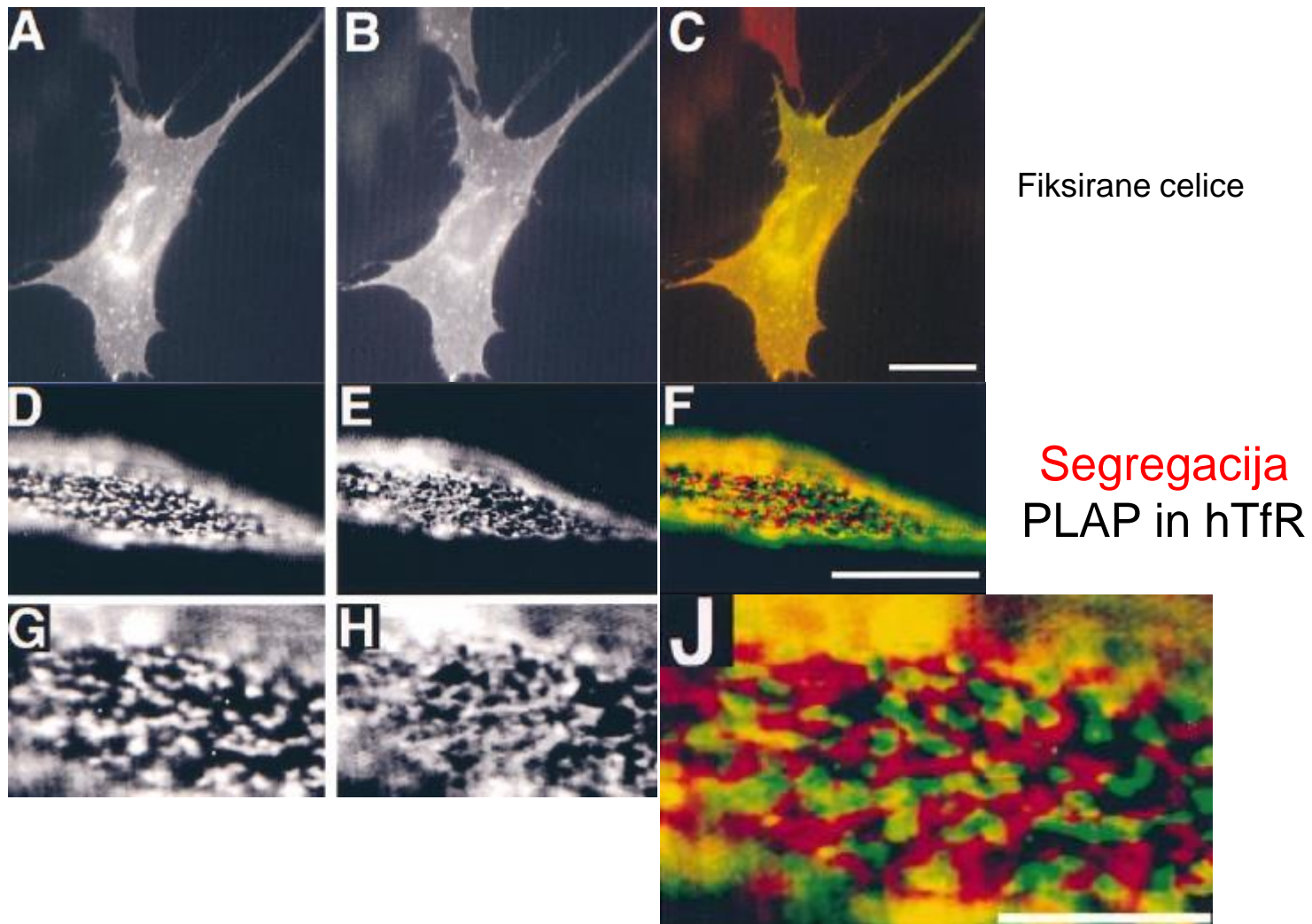
Fiksirane celice



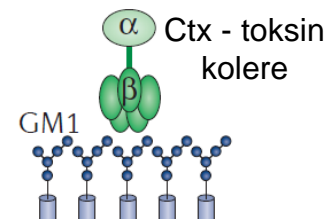
Kolokalizacija
PLAP in HA



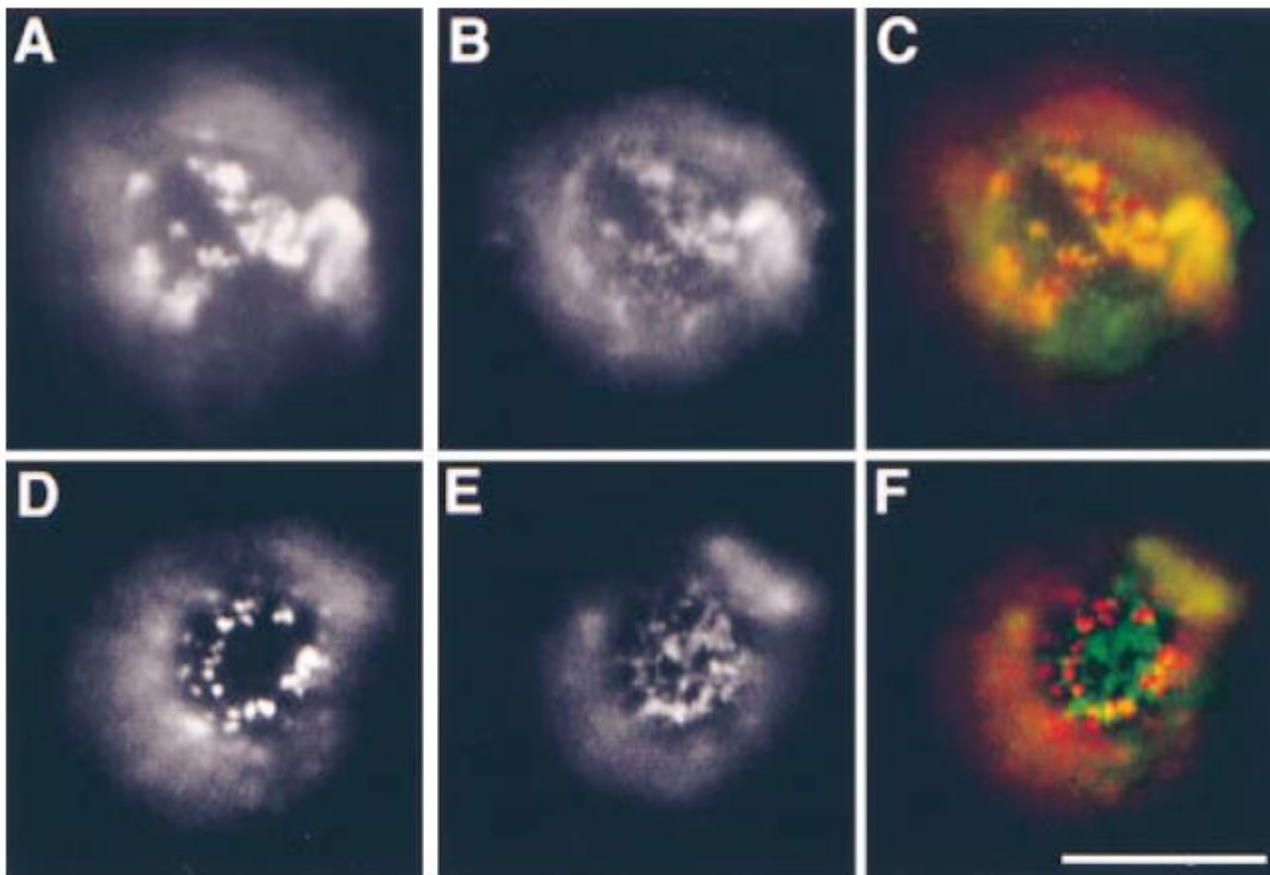
Skepljanje PLAP (zeleno) in hTfR (rdeče), človeškega receptorja za transferin, so-izraženih v nepolariziranih BHK-21 celicah



Skepljanje PLAP ali hTfR (rdeče), prehodno izraženih v nepolariziranih Jurkat celicah, in gangliozida GM1 (zeleno)

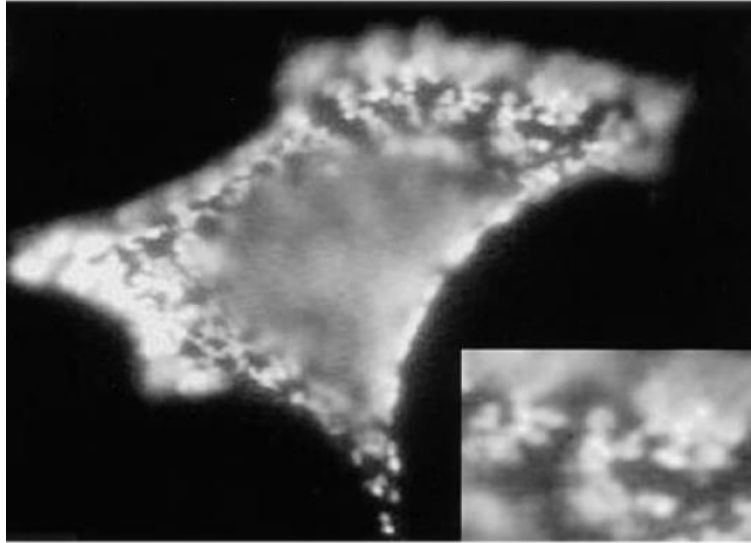


Kolokalizacija
PLAP in GM1

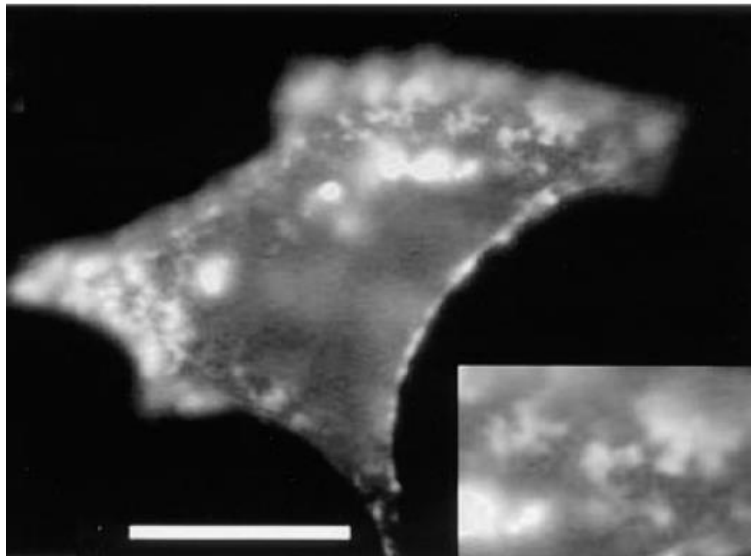


Segregacija
hTfR in GM1

**Skepljanje PLAP v BHK-21 celicah izzove enak vzorec
akumulacije src-podobne Tyr kinaze Fyn na citosolni strani PM**



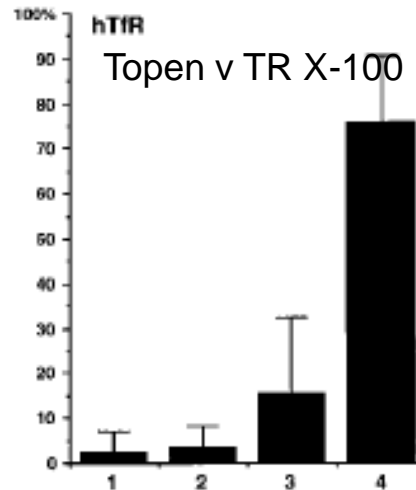
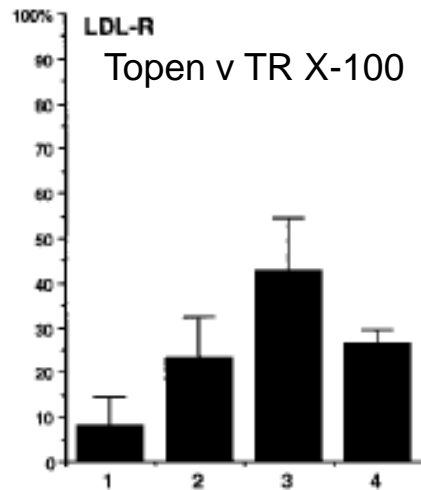
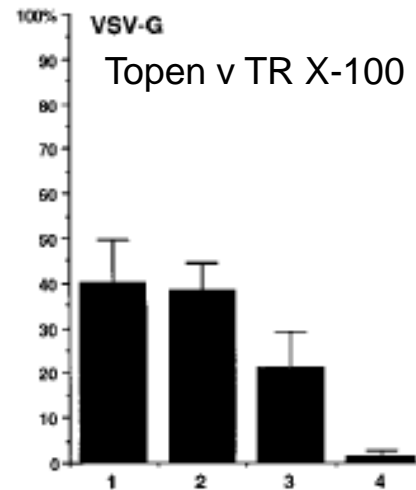
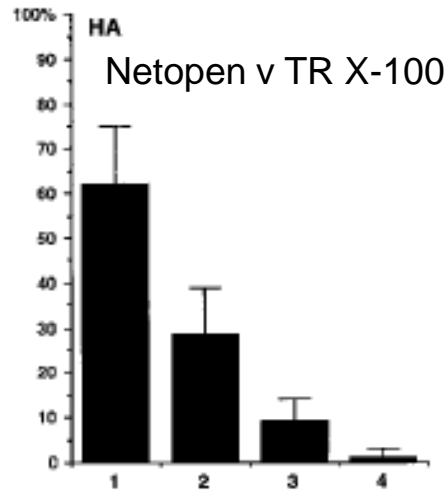
Razporeditev PLAP
po **skepljanju z Ab**



Razporeditev Fyn
po **skepljanju PLAP**

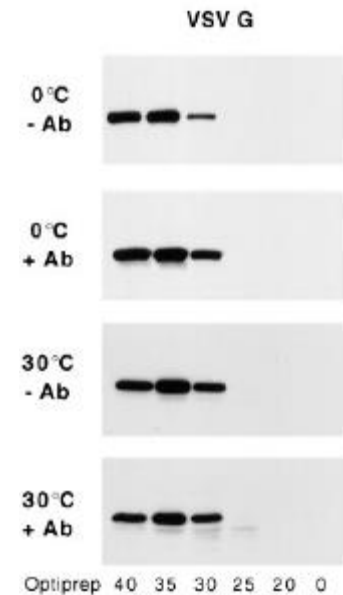
Nekateri proteini izražajo šibko, a občutno interakcijo z rafti, ki je ne moremo zaznati s kriterijem topnosti v TR X-100

Stopnja kolokalizacije s skepljenim PLAP



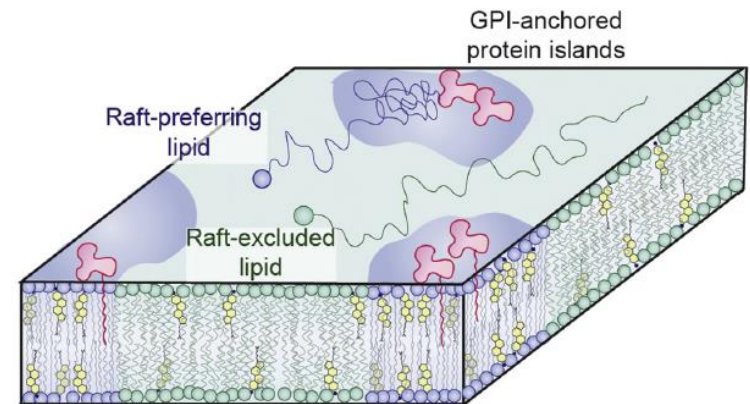
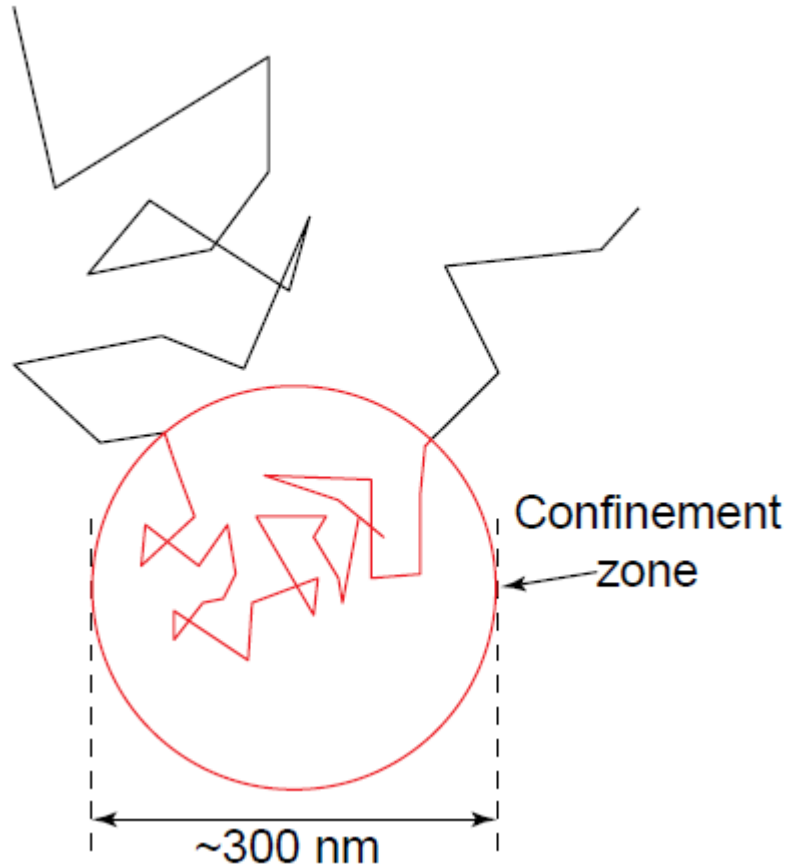
- (1) >80% prekrivanje
- (2) Delno prekrivanje
- (3) Delna segregacija
- (4) Popolna segregacija

Topnost v TR X-100



Sledenje posameznemu delčku (angl. „Single Particle Tracking“ – SPT)

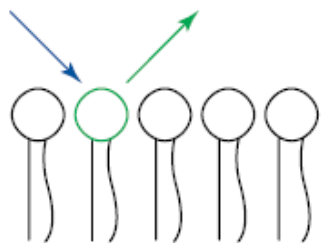
SPT



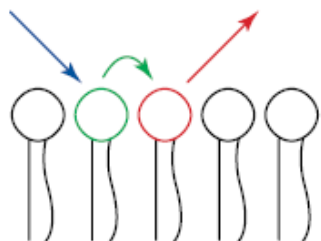
Prenos energije z resonanco fluorescence – FRET

F luorescence
R esonance
E nergy
T ransfer

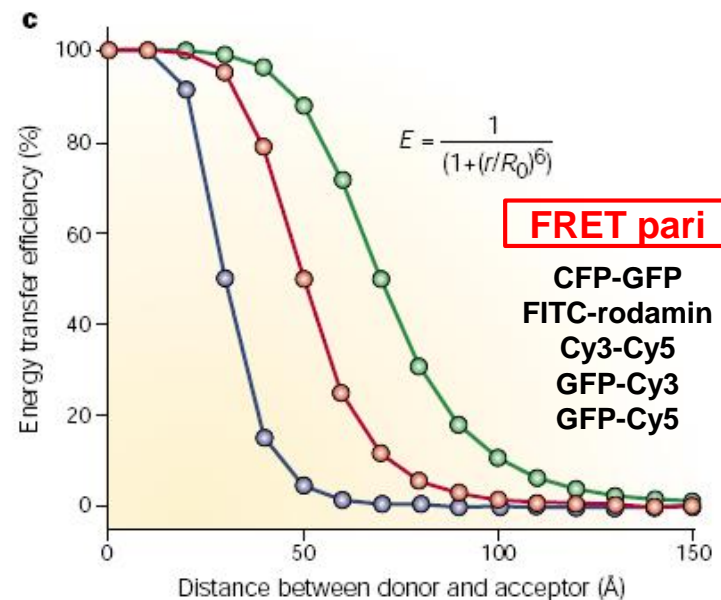
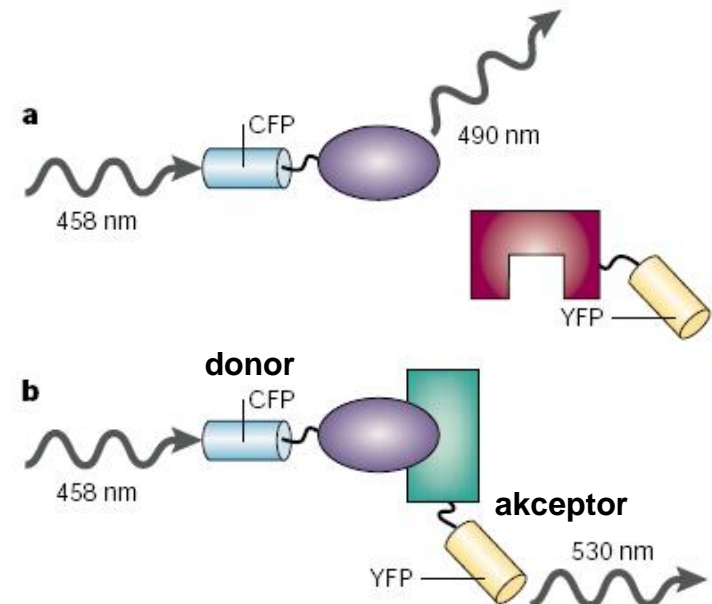
Conventional FRET
 Excitation Emission



Normal donor emission in dilute solution

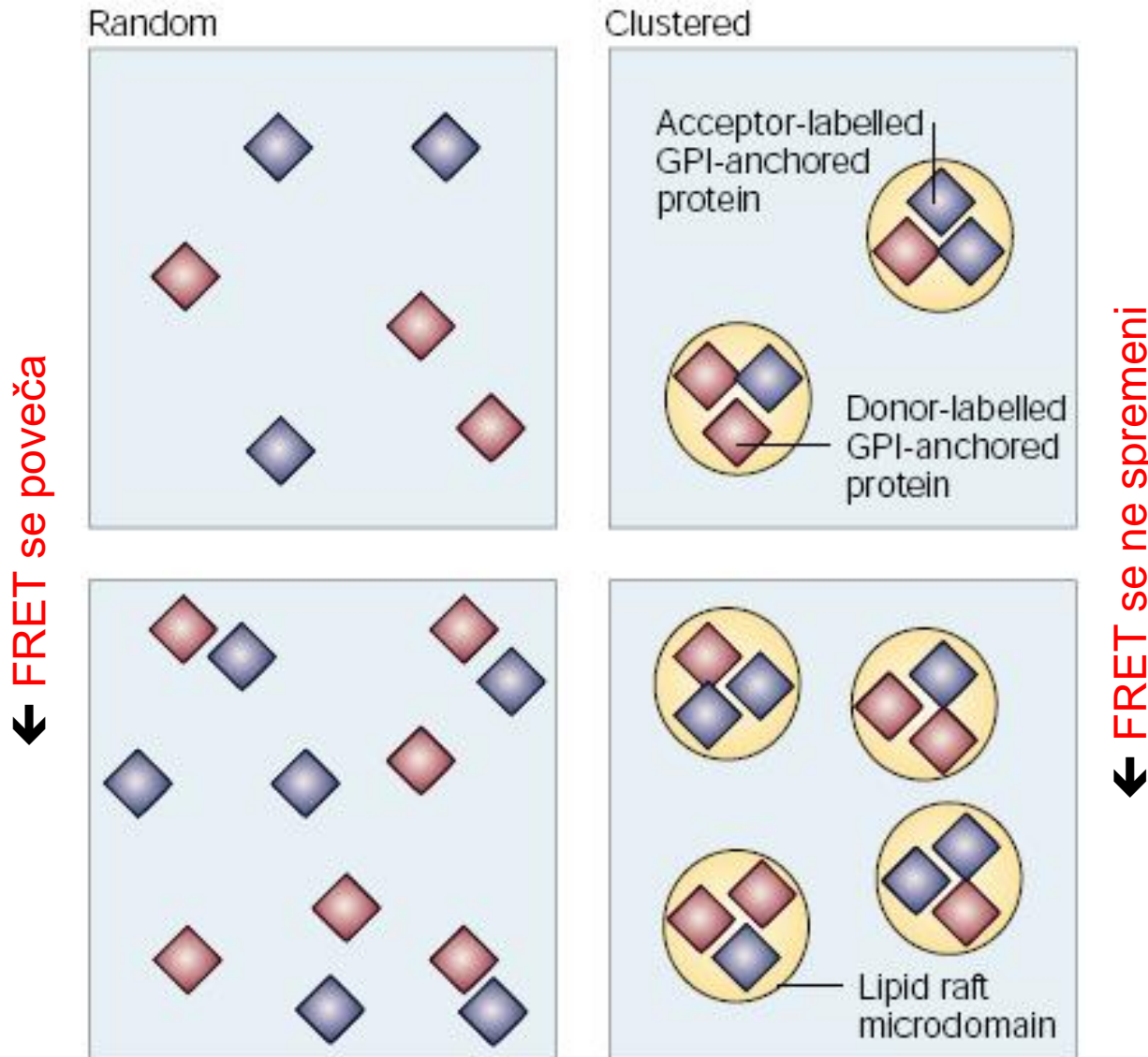


Red-shifted acceptor emission via donor-acceptor energy transfer in concentrated solution



FRET za detekcijo lipidnih raftov

FRET kot funkcija površinske gostote donorja in akceptorja



Lipidne in proteinske komponente raftov/kaveol

Lipids

Cholesterol

Sphingo-myelin

Glyco-sphingolipids (e.g., GM₁)

PIP₂

Proteins

Integral/Structural

Caveolins (Cav-1, -2, and -3)

Flotillins (FLO-1 and -2; aka, Reggies or Cavatellins)

LAT/PAG

MAL/BENE

Stomatins

VIP36

Acylated

Exoplasmic

GPI-linked proteins (e.g., Thy-1, alkaline phosphatase, folate receptor)

Cytoplasmic

Src-family tyrosine kinases (NRTKs)

G proteins

eNOS

H-Ras

Scavenger Receptors

CD 36

SRBI

RAGE

Other receptors

Receptors Tyrosine kinases (RTKs; e.g., EGF-R,

PDGF-R, Insulin-R)

Hepta-helical Receptors (e.g., Endothelin receptor)

Razporeditev in gibanje raftov sta odvisna od vrste celice

Polarizirana epitelijska celica (tesni stiki)

- akumulacija v apikalnem delu PM

Motorični nevron (citoskelet, zunajcelični matriks)

- akumulacija v aksonskem delu PM

Osteoklast (citoskelet, zunajcelični matriks)

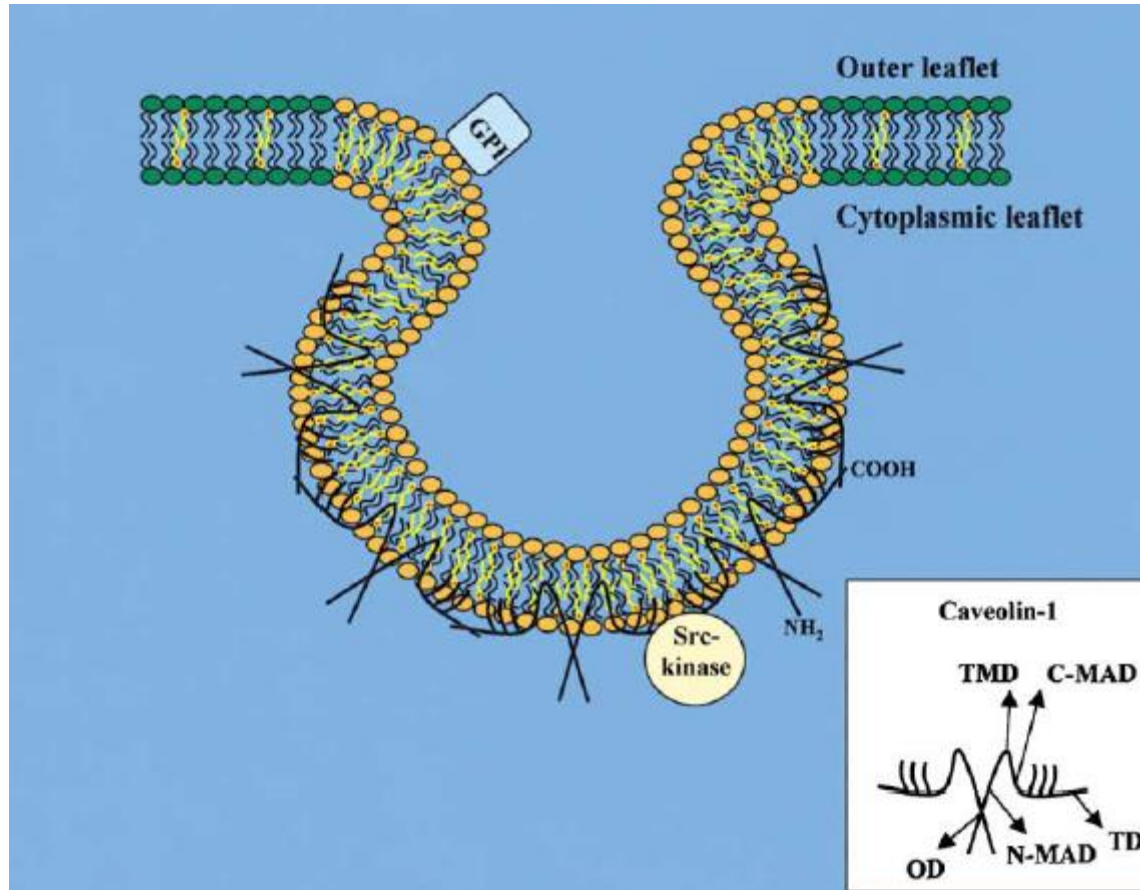
- asimetrična porazdelitev v PM

Limfocit in fibroblast

- enakomerna porazdelitev v PM

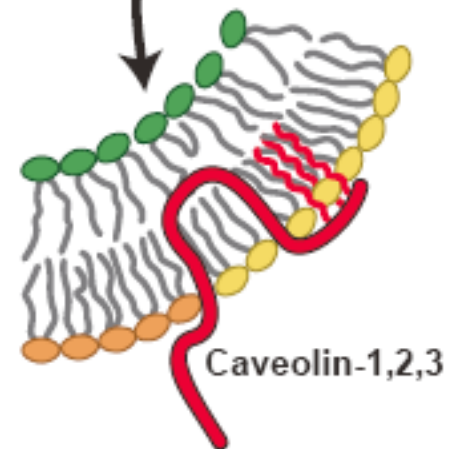
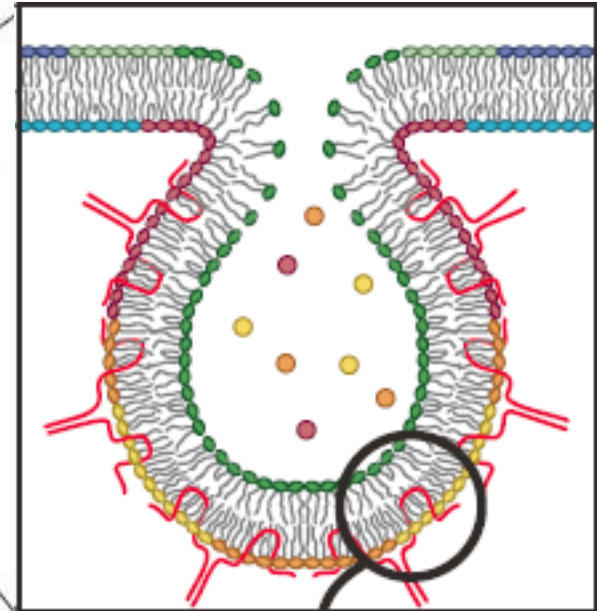
Kaveola (caveolae)

vrsta visoko-specializiranega lipidnega rafta



„Anatomija“ kaveole

Hu fibroblast



Kaveolini (Cav)

- Nujni za nastanek kaveole.
- Družina genov *Cav* je strukturno in funkcijsko ohranjena od črva (*C. elegans*) do človeka.
- Pri sesalcih: Cav-1 (α in β), Cav-2 in Cav-3 (21- bo 25-kDa).
- Integralni membranski proteini (tri-palmitoilirani).
- Cav-1 in Cav-2 se so-izražata, Cav-3 je mišično-specifičen.
- Polimerizirajo (14-16) in tako oblikujejo kaveolo.
- Vežejo holesterol, maščobne kisline in interagirajo s širokim naborom molekul, ki sodelujejo pri prenosu signalov (npr. RTyr kinaza, eNOS, G-proteini).
- Limfociti in nevroni jih ne vsebujejo.