Is the lipid membrane druggable? Insights from MD simulations

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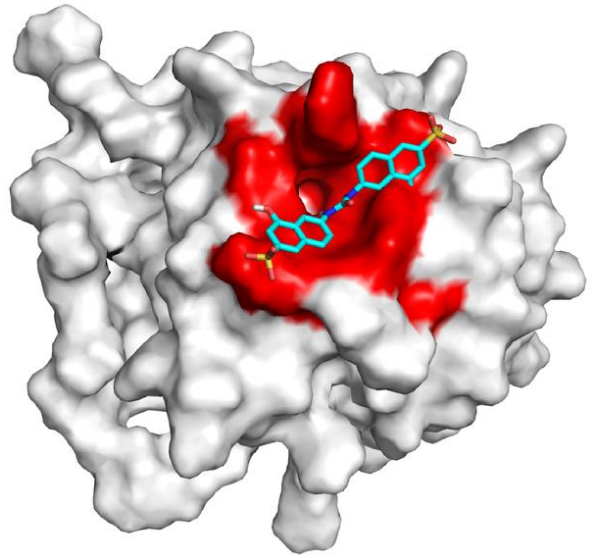
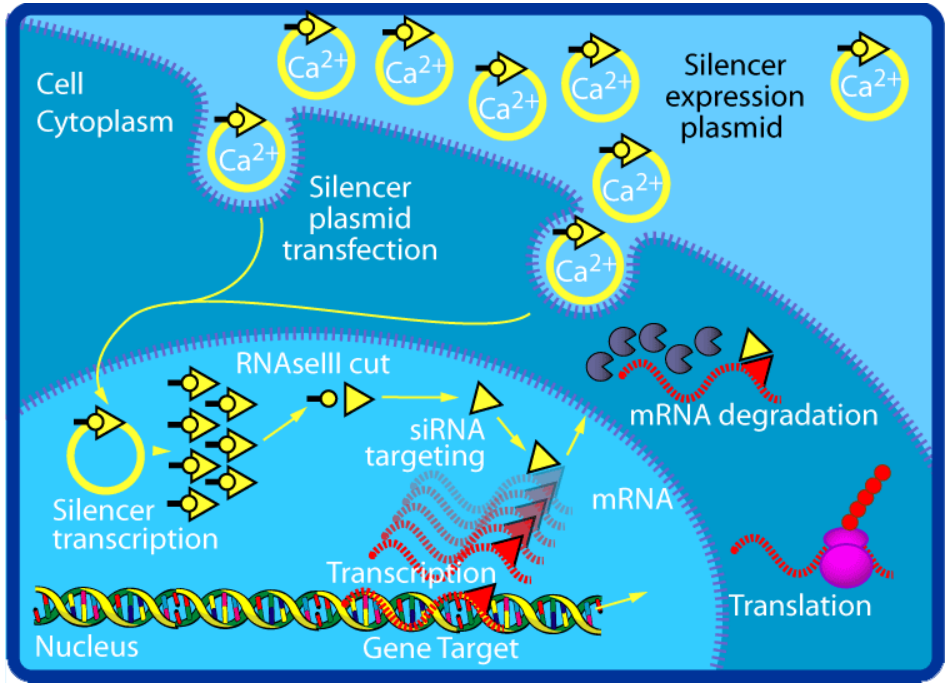
Common drug targets

- Most of drug targets are proteins
- DNA and RNA could also be targeted (siRNA, antisense RNA)

- Membranes are not considered as drug targets



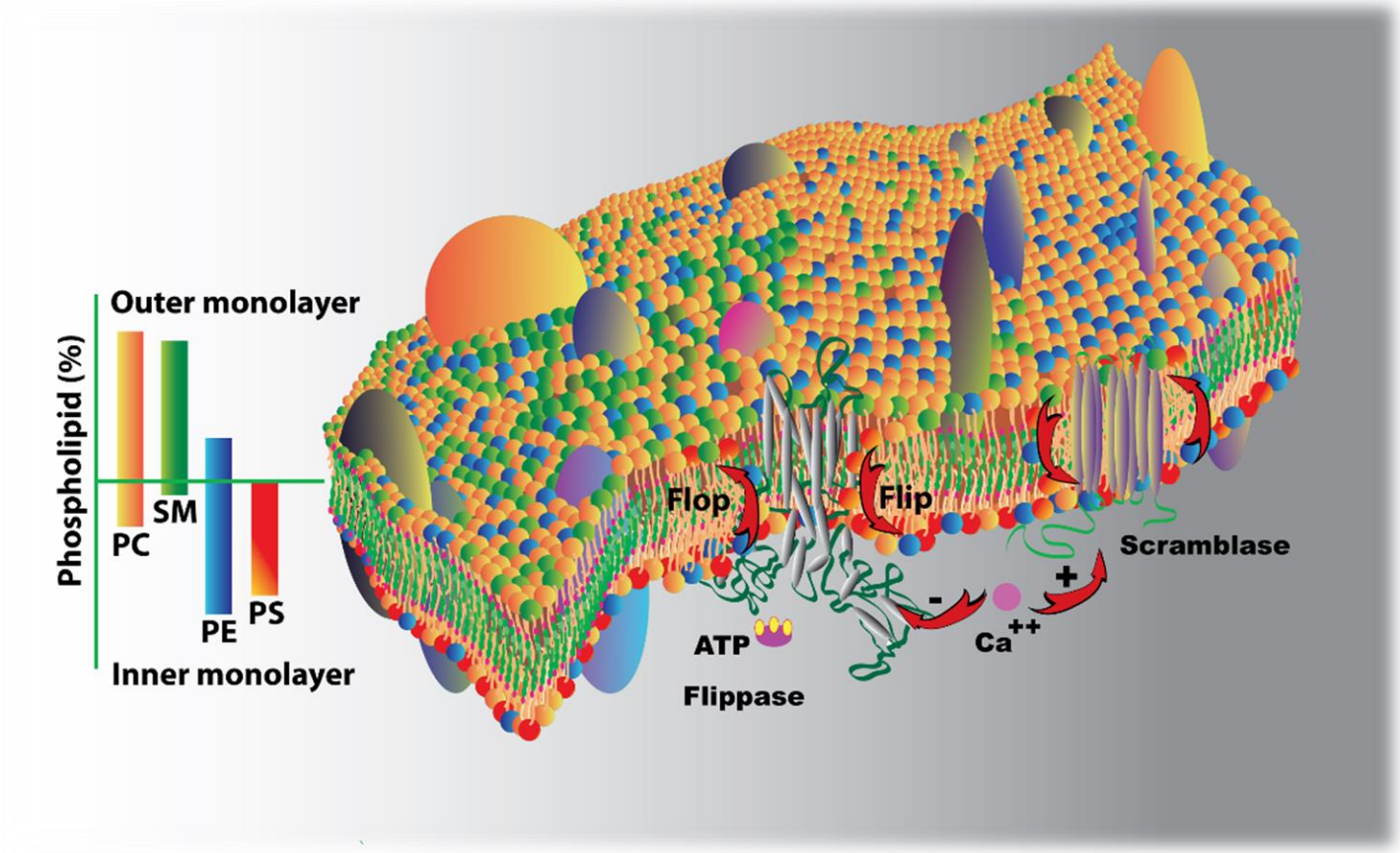


What is special about the membranes?

- No defined structure – the membranes are liquid crystals.
- Lipid composition is not pre-defined by genes but regulated dynamically.
- Composition may change in space and time.
- Tissue and cell type differences are relatively small.
- Different organelles have different compositions.

Crucial membrane properties

- Lipid composition
- Asymmetry of monolayers
- Curvature



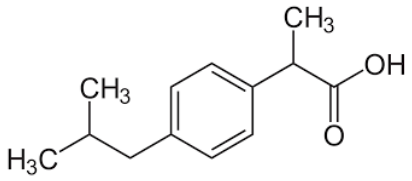
Membranotropic drugs

- Drug is membranotropic if it accumulates in the membrane.
- This is usually an accidental property, considered as a side effect.
- Membranes are considered as an unfortunate barrier for drug delivery. Membranotropism may help in this but has no value by itself.
- There are very few studies of membranotropic effects of the drugs.
- No structural similarity. The only common feature – amphiphilicity.

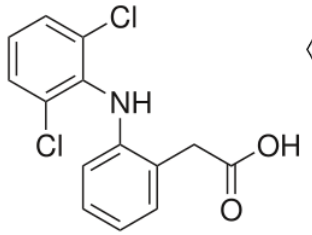
Known membranotropic drugs

Analgesic

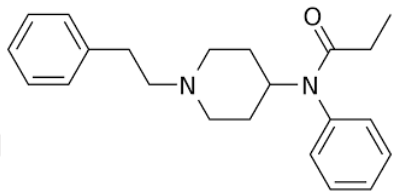
Ibuprofen



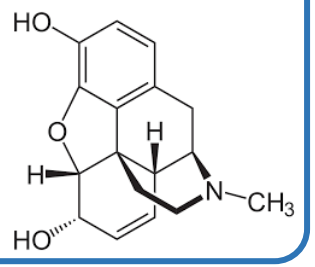
Diclofenac



Fentanyl

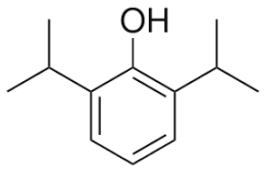


Morphine

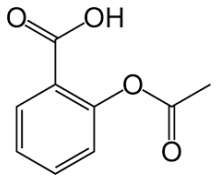


Anesthetic

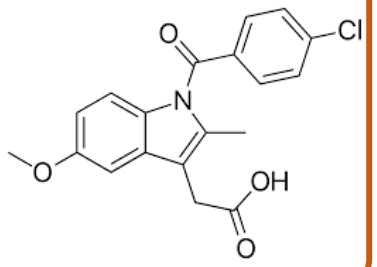
Propofol



Aspirin

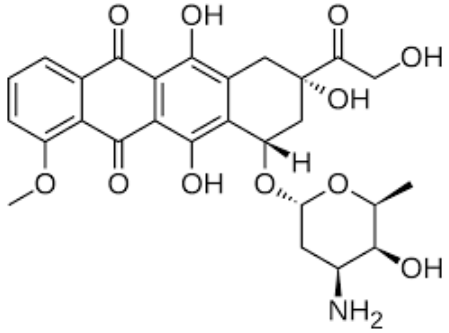


Indomethacin

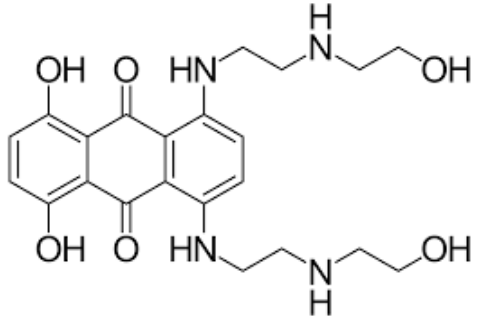


Antineoplastic

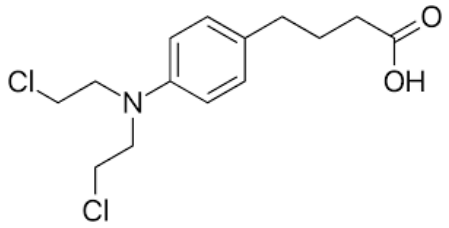
(whatever)rubicin



Mitoxanthrone

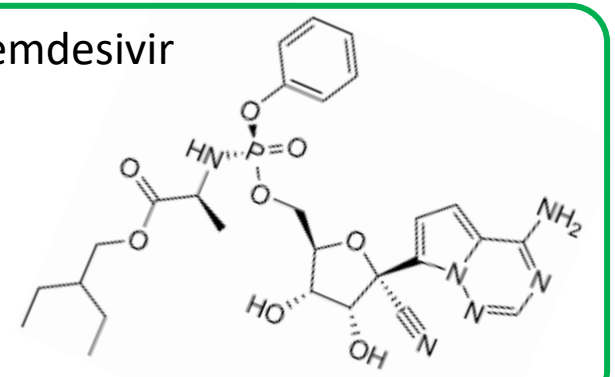


Chlorambucil



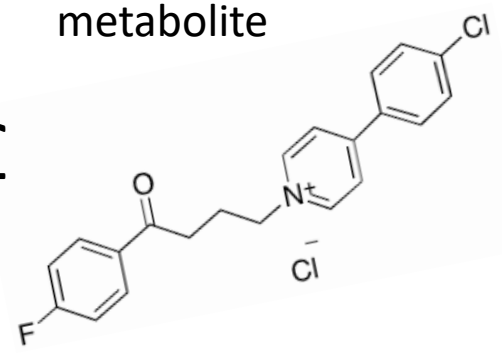
Antiviral

Remdesivir

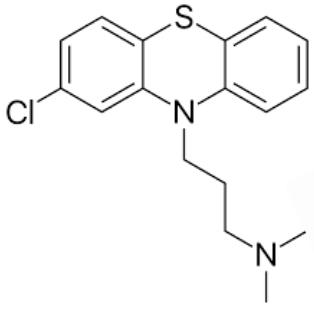


Antipsychotic

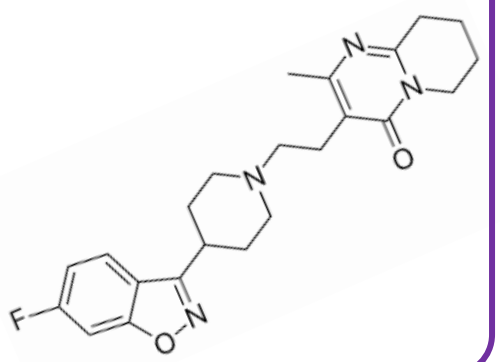
Haloperidol pyridium metabolite



Chlorpromazine

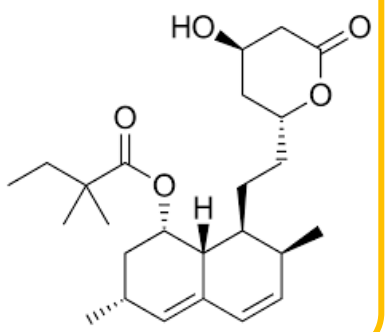


Risperidone



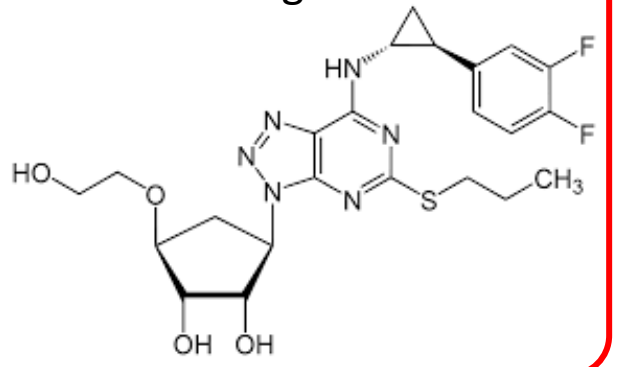
Lipid lowering

Simvastatin



Anti platelet

Ticagrelor

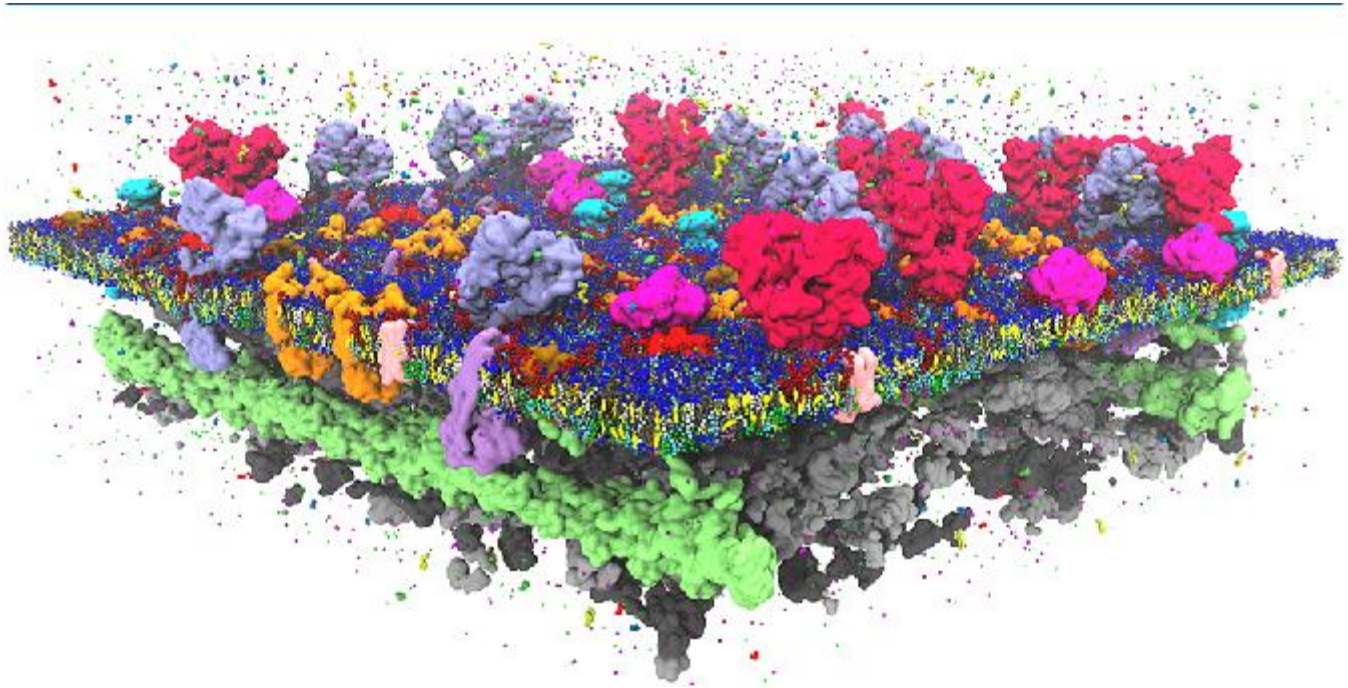


How membranotropic drags may act?

- Direct action on the plasma membrane
  - Change of physical properties (order, fluidity, phase separation).
  - Modulating passive diffusion of other drugs and metabolites.
- Indirect action on the membrane proteins
  - Changing the microenvironment for membrane proteins (raft-liking proteins).
  - Inhibiting multimerization of the membrane protein domains (receptors, ionic channels).
  - Interference with the membrane-exposed active sites (rotary ATPases).
  - Blocking the allosteric lipid binding sites.
- Dual action
  - The drug changes the microenvironment for its own target protein.

Selectivity on the level of lipids

- Cancer cells
  - have disrupted lipid asymmetry
    - Exposed PS on the outer leaflet.
    - Scrambled distribution of PE and PC (?)
  - Could be more or less curved than the benign membranes.
- Viral lipid envelopes
  - Usually originate from ER
    - Low SM, CR and Chol.
    - No asymmetry.
  - Uniform positive curvature.
- Bacterial membranes contain exotic lipids
  - Especially gram-positive ones.



Simulating realistic membranes

- Realistic composition
- Realistic asymmetry
- Realistic curvature

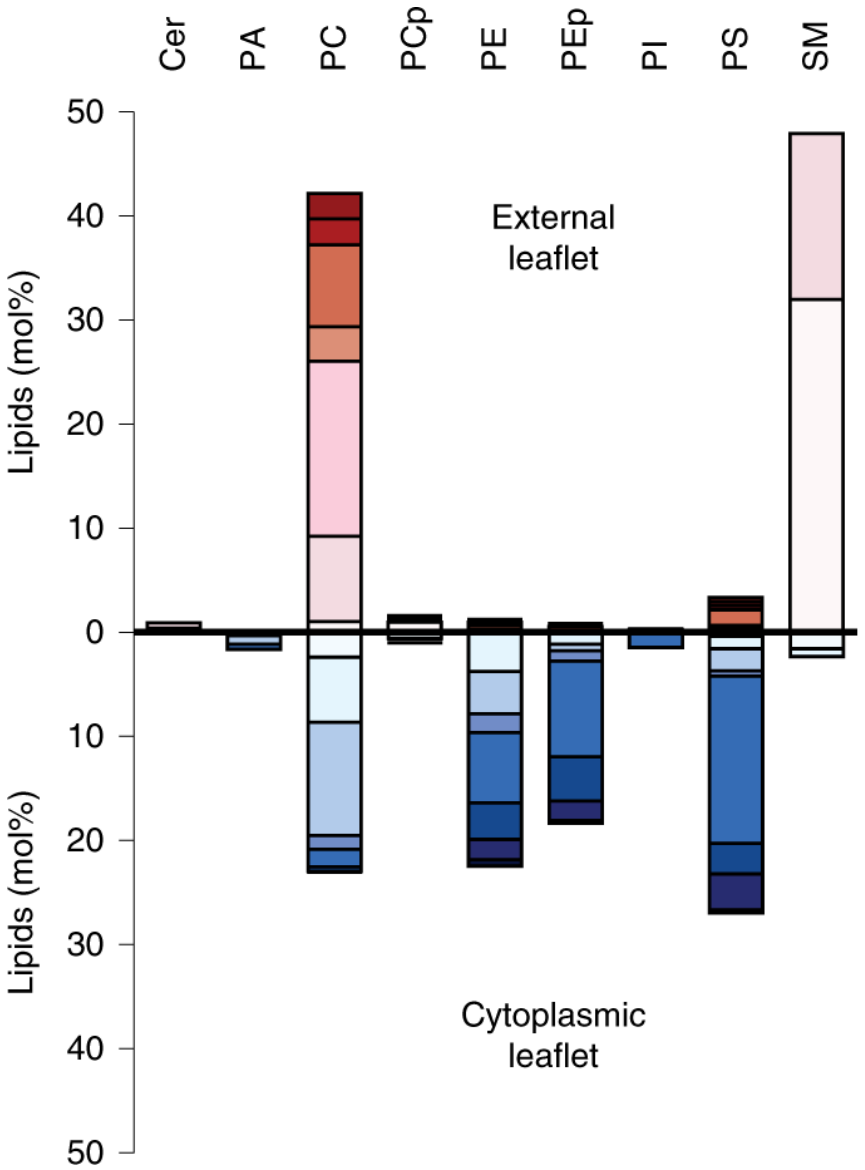
Realistic composition

- Classical MD force fields of the lipids is considered “done”.
- Most major lipids are ready.
- Many non-standard ones could be designed by combining building blocks.
- Tools like charmm-GUI allow fast creation of the membranes with almost any lipid composition (God bless the authors!).

Some remaining issues

- Bacterial lipids are often too crazy to be built from existing blocks.
- Heterogeneity of FA requires too large system size to have enough lipids of each kind.
- It is not clear how important is observed diversity of lipids for major membrane properties. Do you really need all 25 types of PC?

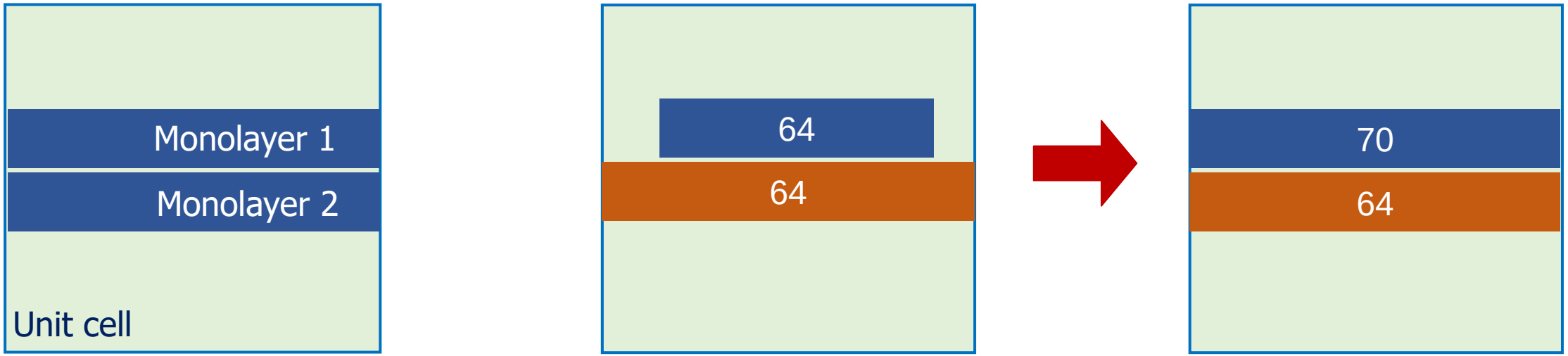
Realistic asymmetry



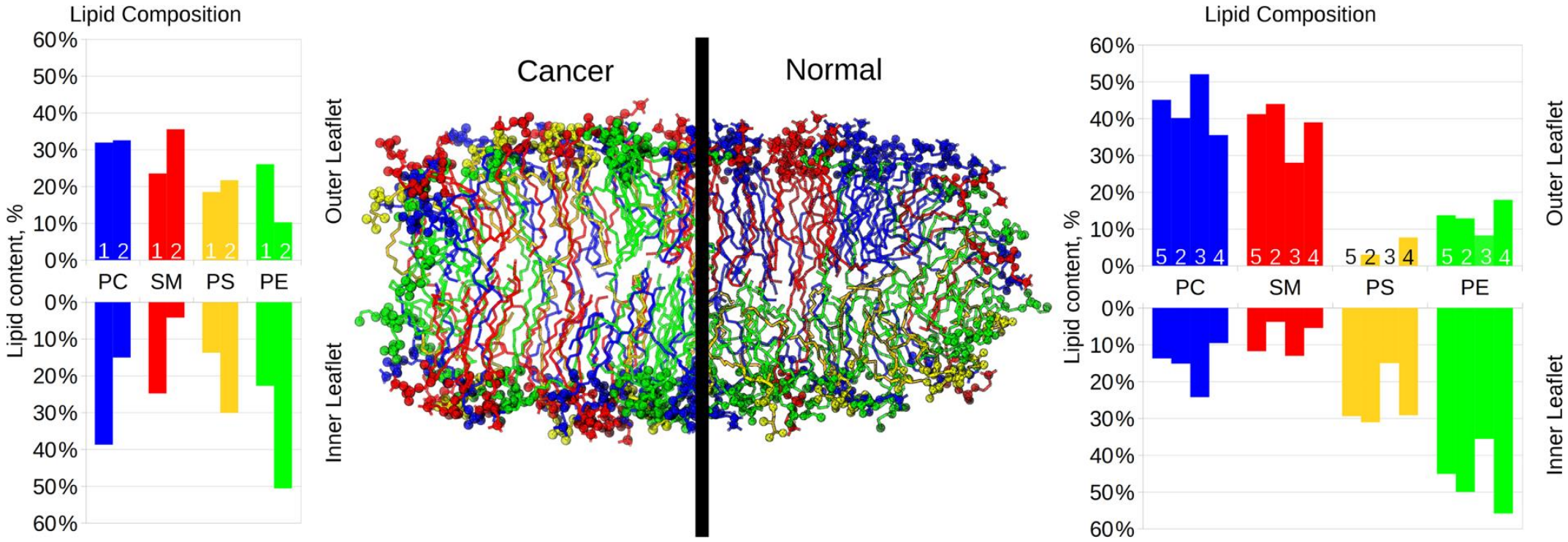
- Outer monolayer: neutral lipids and glycolipids, all SM and Cer.
- Inner monolayer: all anionic lipids, most of PE.
- Created by flippases and scramblases in energy consuming manner = very important for the cell.
- Experiments are complex.
- Distribution of cholesterol is extremely hard to measure.
- Very hard to measure dynamic aspects (lateral diffusion, flip-flop transitions, dynamic raft formation).
- Lack of resolution — no way to resolve atomic details in experiments.

Realistic asymmetry

- No problem to get almost any desired composition of monolayers.
- *Issue*: Monolayers have different areas per lipid but must be packed to the same unit cell.
- Partially resolved in tools like charmm-GUI by maintaining the library of pure bilayers with reference areas per lipid.
- Does not give exact equilibrium, fails in non-standard conditions…



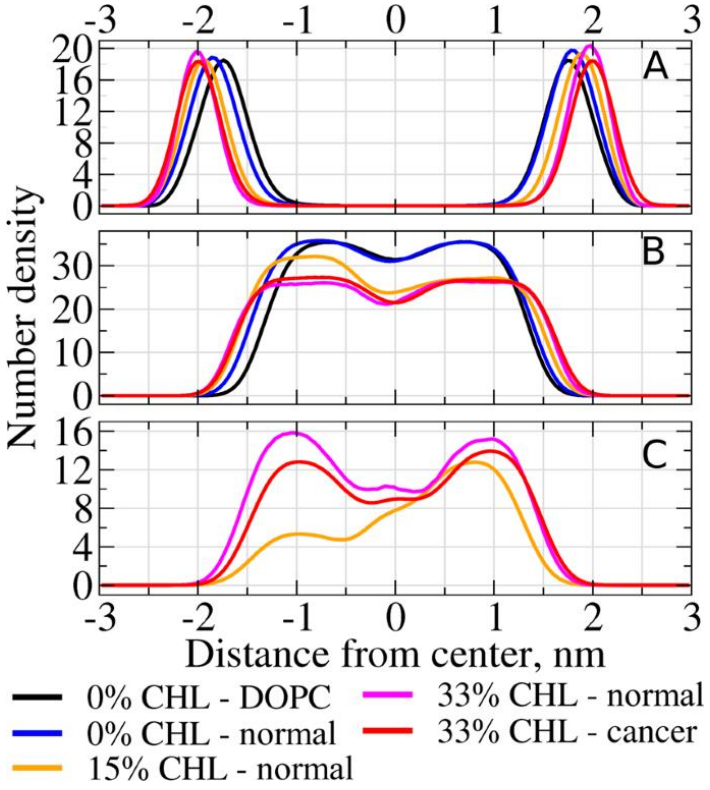
Asymmetry matters for drug delivery!

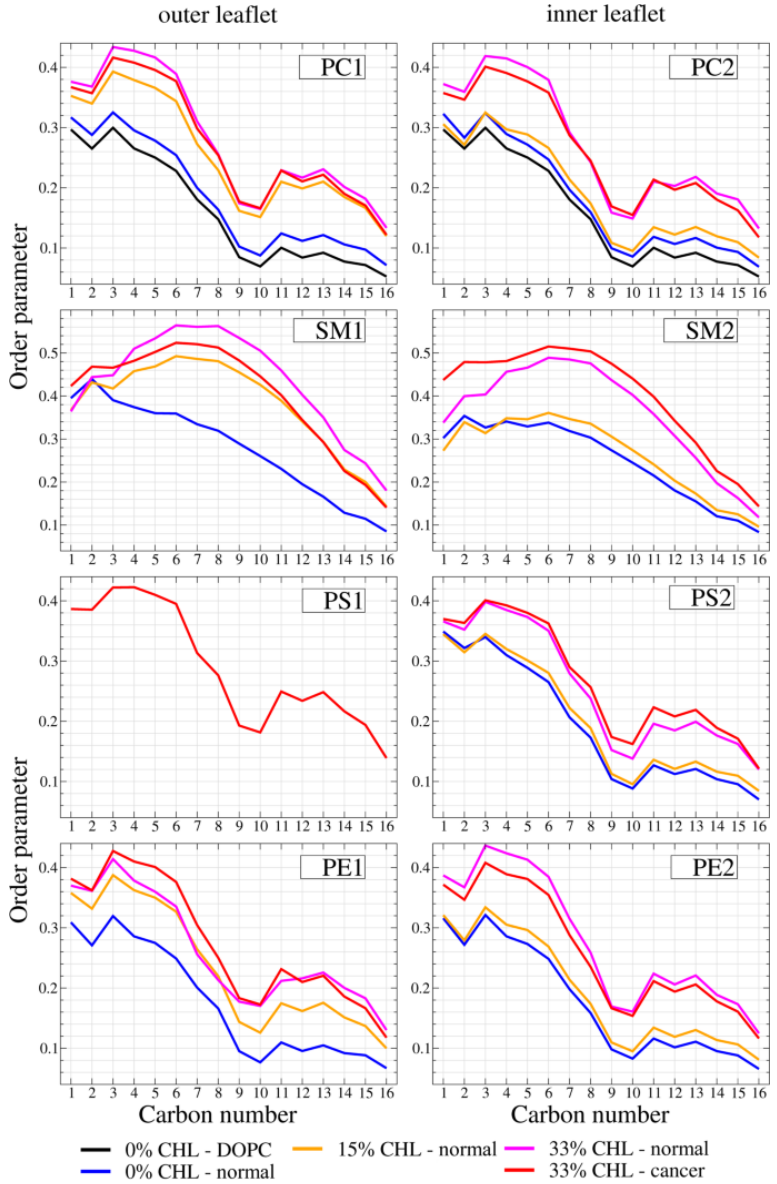


- Composition of cancer cell membranes is less asymmetric.
- PS is exposed on the outer leaflet.
- PC, PE and SM is scrambled partially.

T. Rivel, C. Ramseyer, S. Yesylevskyy, "The asymmetry of plasma membranes and their cholesterol content influence the uptake of cisplatin", 2019, Scientific reports 9 (1), 1-14

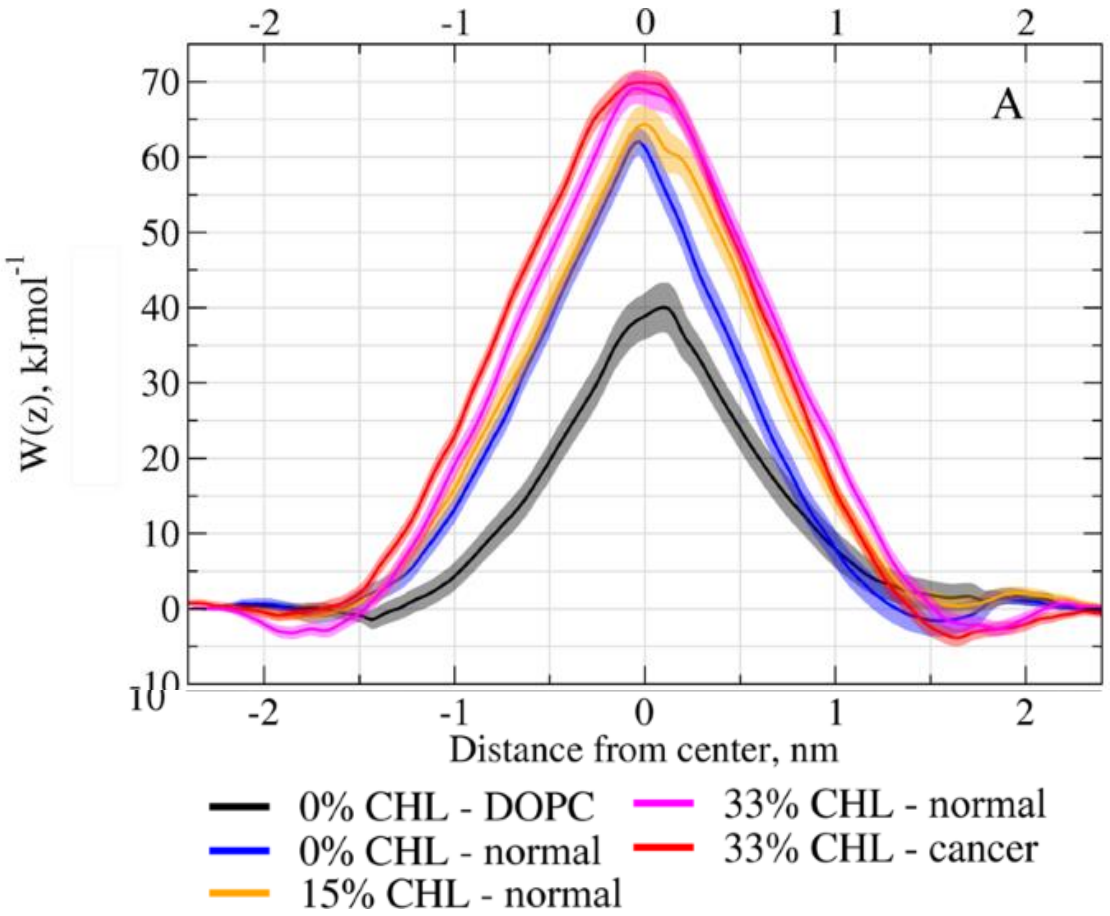
Asymmetry and the membrane properties





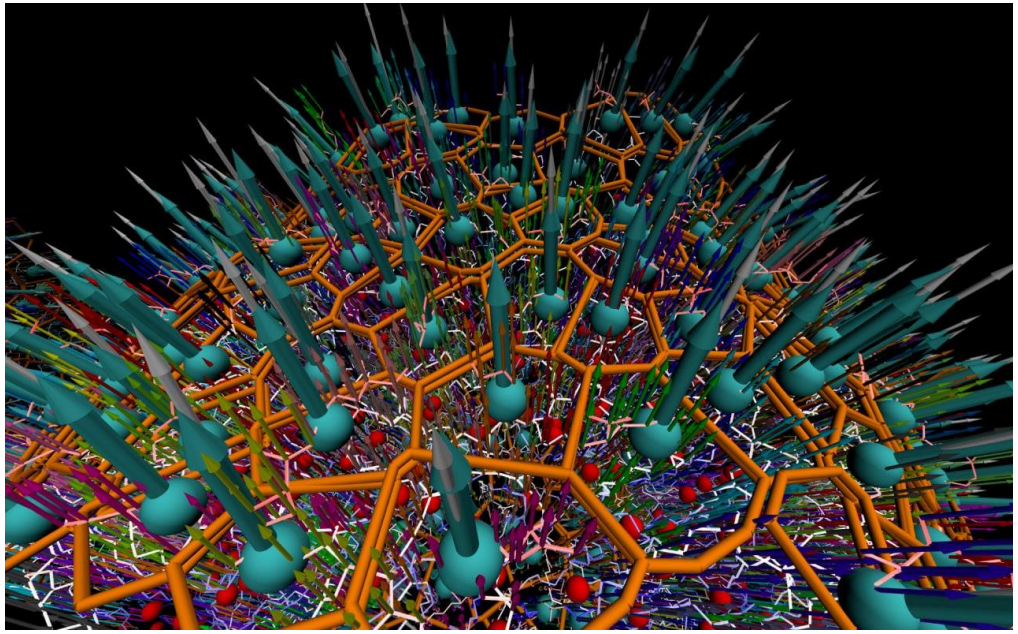
- The loss of asymmetry changes membrane properties, but not dramatically.
- Chol content matters much more.
- Is this enough to influence the membrane permeability?

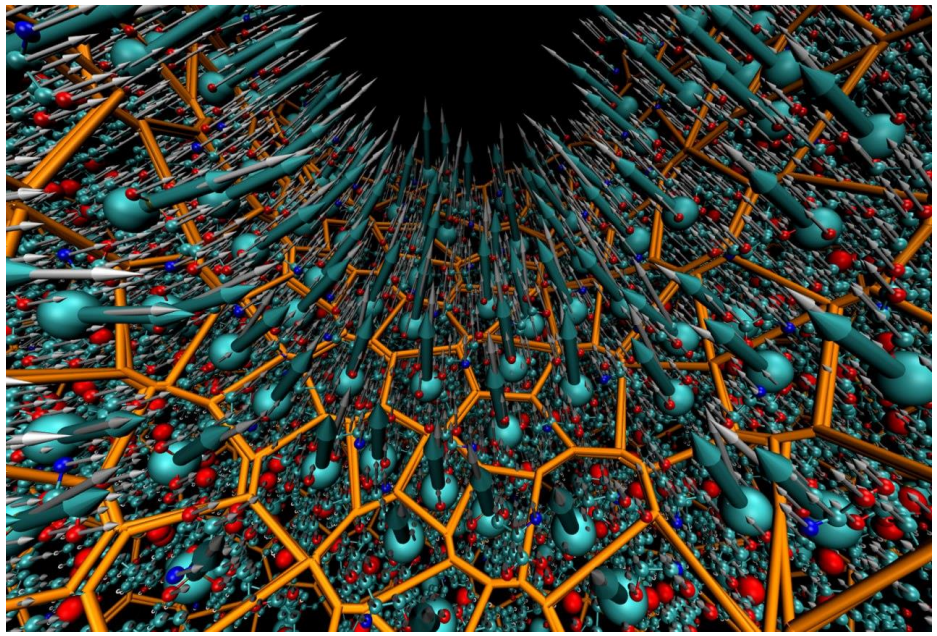
Asymmetry loss changes permeability!



System	Cholesterol content	Resistance, R, $s \cdot m^{-1}$	Permeability, P, $m \cdot s^{-1}$
DOPC	0%	$4.5 \pm 0.1 \cdot 10^{6}$	$2.2 \pm 0.05 \cdot 10^{-7}$
Normal	0%	$1.15 \pm 0.04 \cdot 10^{11}$	$8.7 \pm 0.3 \cdot 10^{-12}$
Normal	15%	$2.08 \pm 0.04 \cdot 10^{11}$	$4.8 \pm 0.1 \cdot 10^{-12}$
Normal	33%	$5.75 \pm 0.08 \cdot 10^{11}$	$1.74 \pm 0.03 \cdot 10^{-12}$
Cancer	33%	$6.3 \pm 0.2 \cdot 10^{12}$	$1.59 \pm 0.06 \cdot 10^{-13}$

- Despite minor apparent changes in PMFs and membrane properties the permeability for cisplatin changes by an order of magnitude in cancer cells due to the loss of asymmetry alone!



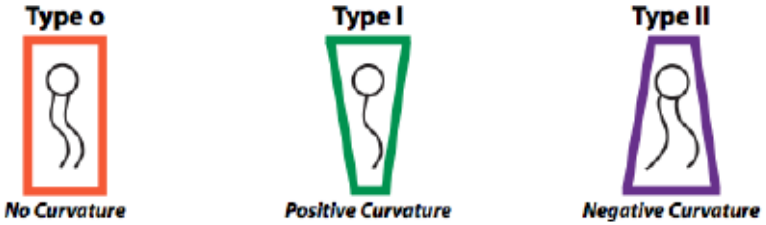


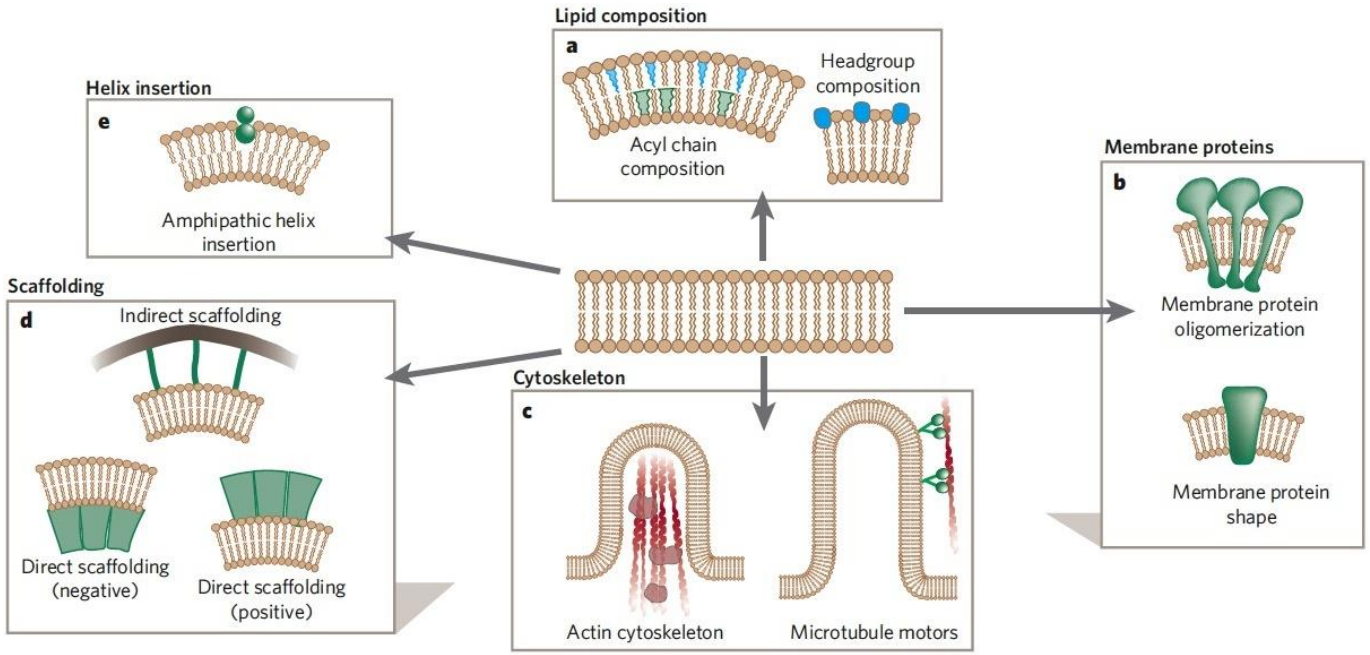
Simulating curved membranes

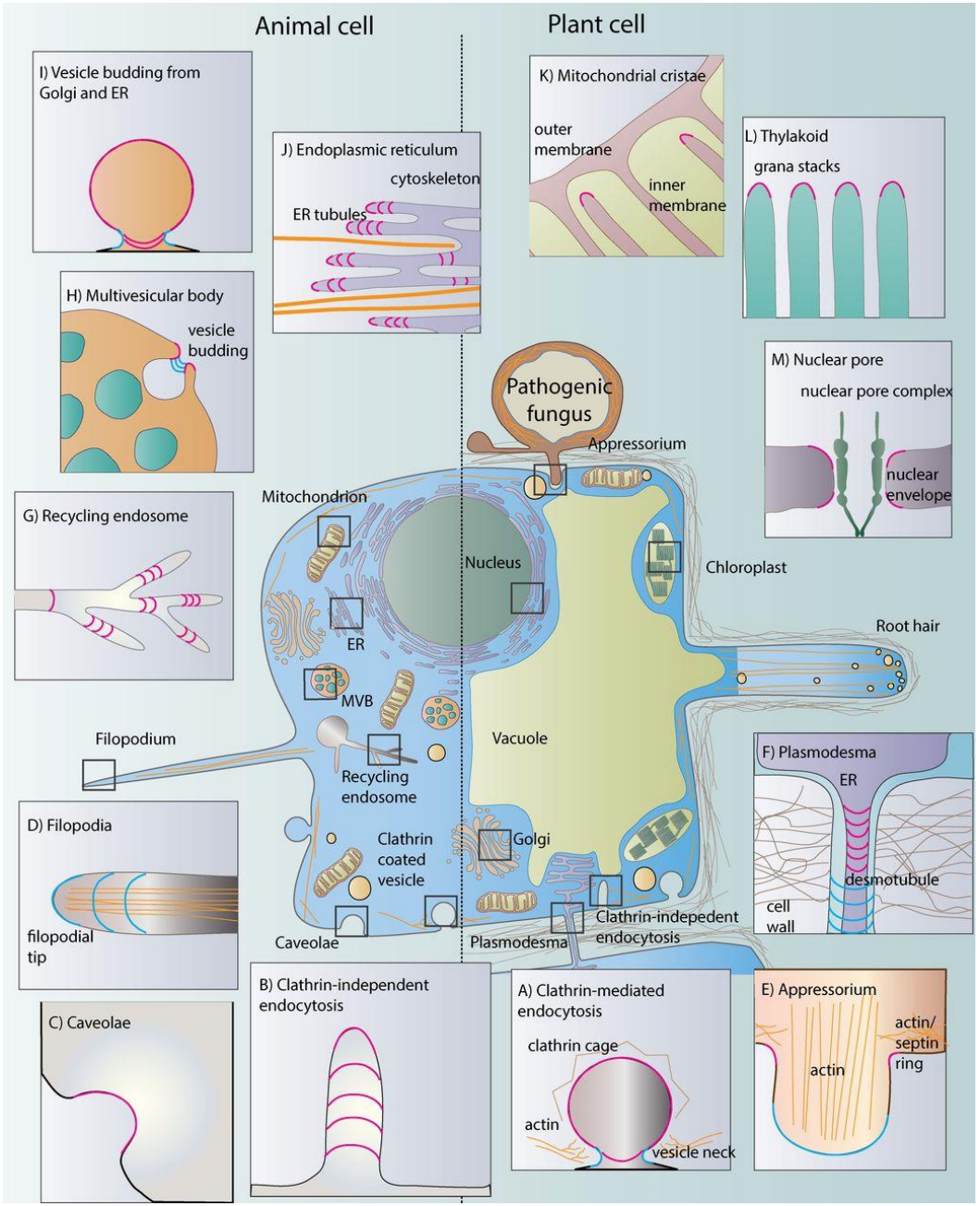
- Fighting the bending tension
- Maintaining the curvature
- Computing properties and permeability

Realistic curvature

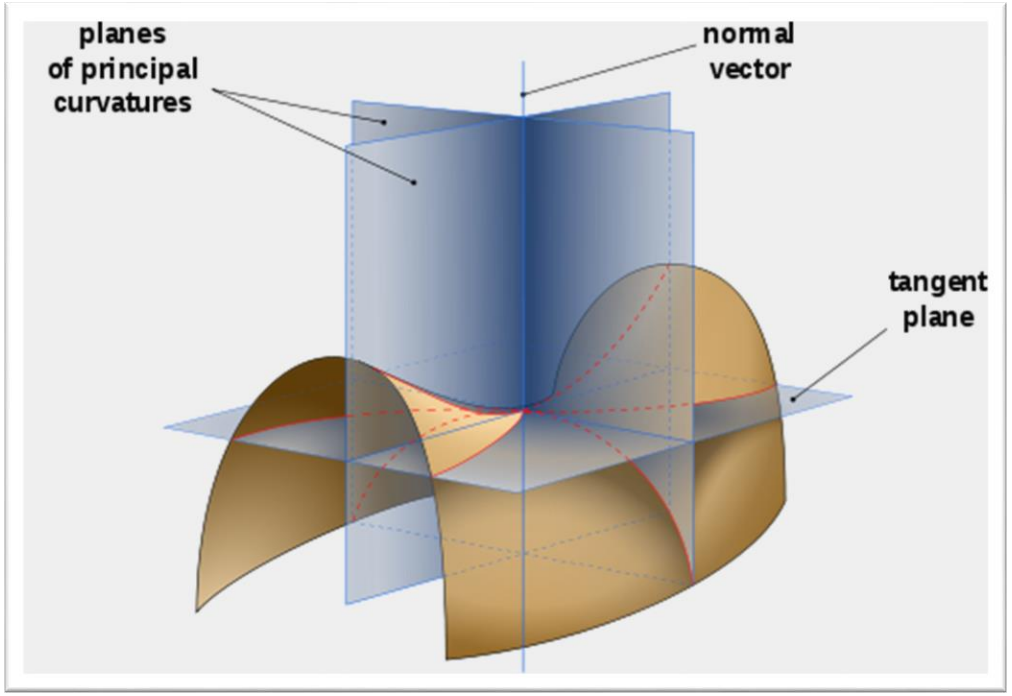
- Membrane bending is essential for cell functioning.
- Flat membranes are rare

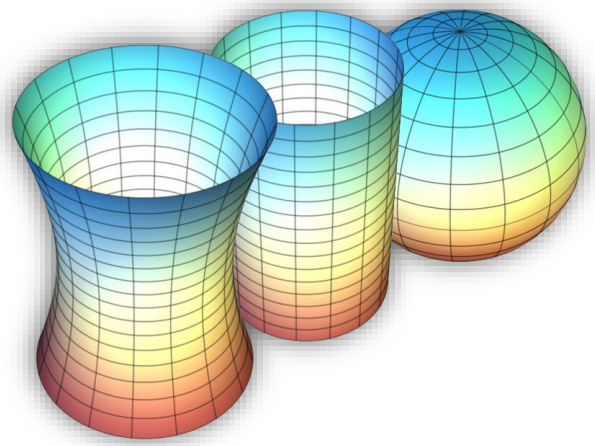


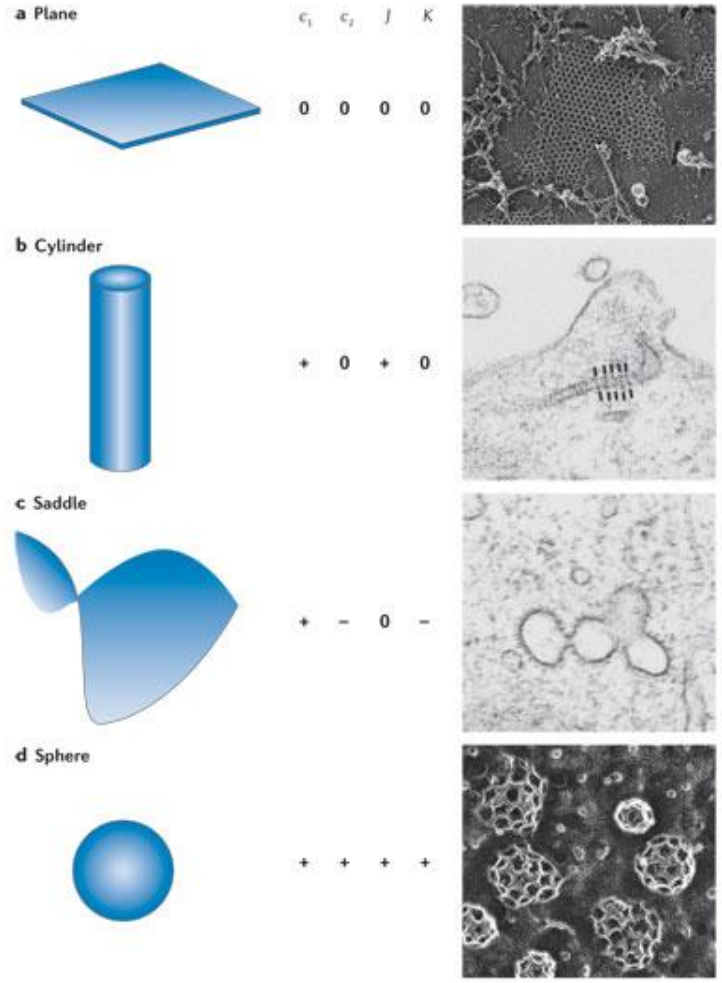




Math of the membrane curvature



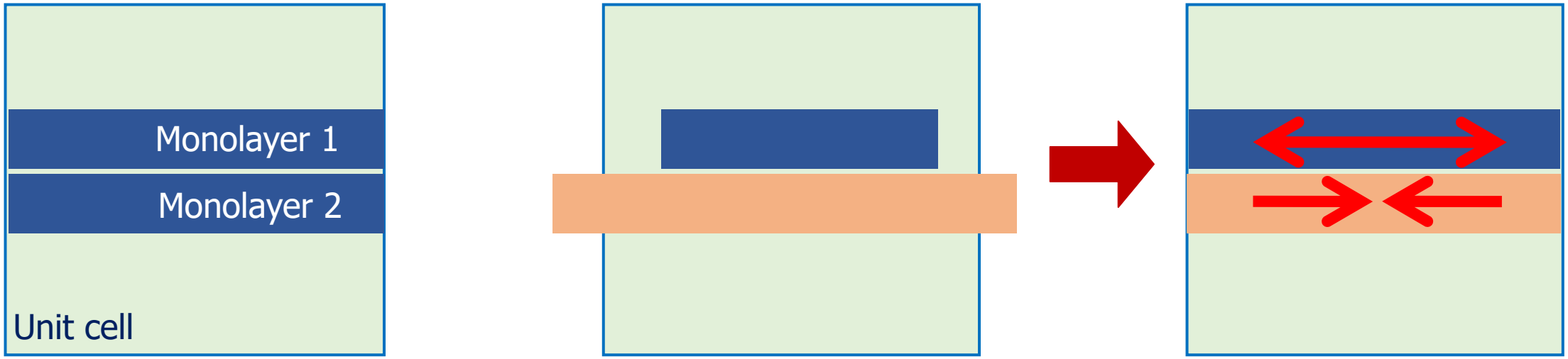




- k_1, k_2 – principal curvatures in perpendicular directions
- $K_M = (k_1 + k_2)/2$ - mean curvature
- $K_G = k_1 k_2$ - Gaussian curvature

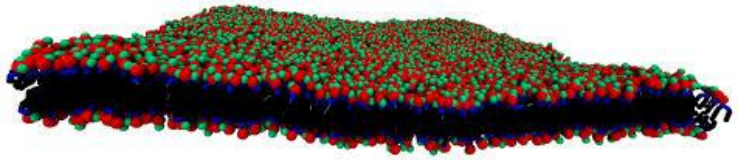
Issues with monolayer tension

- Monolayers with different areas must be packed to the same unit cell.
- This leads to mechanical stress for both monolayers — one contracts, another one expands.
- Unphysical condition, never observed in real membrane.

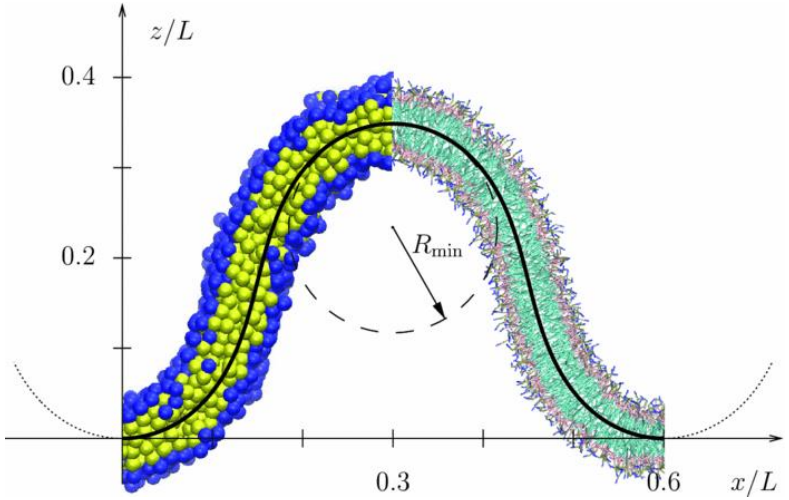


Existing approaches

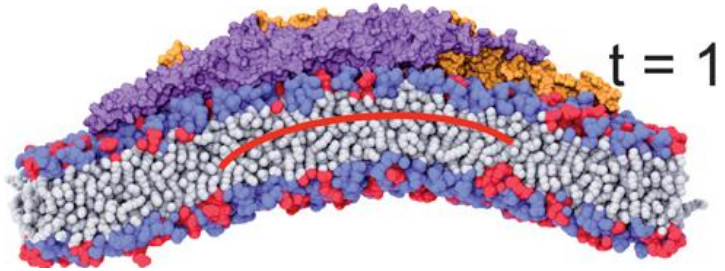
1. Spontaneous undulations.



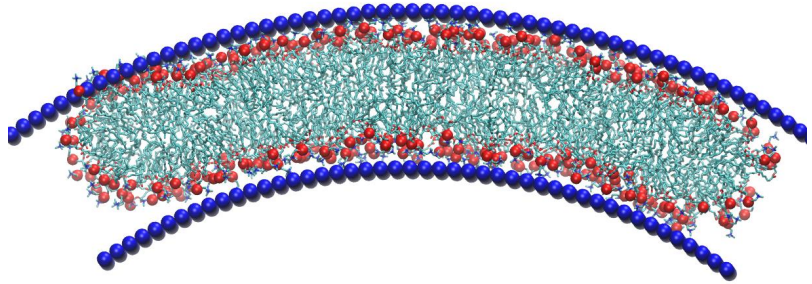
2. Membrane buckling.



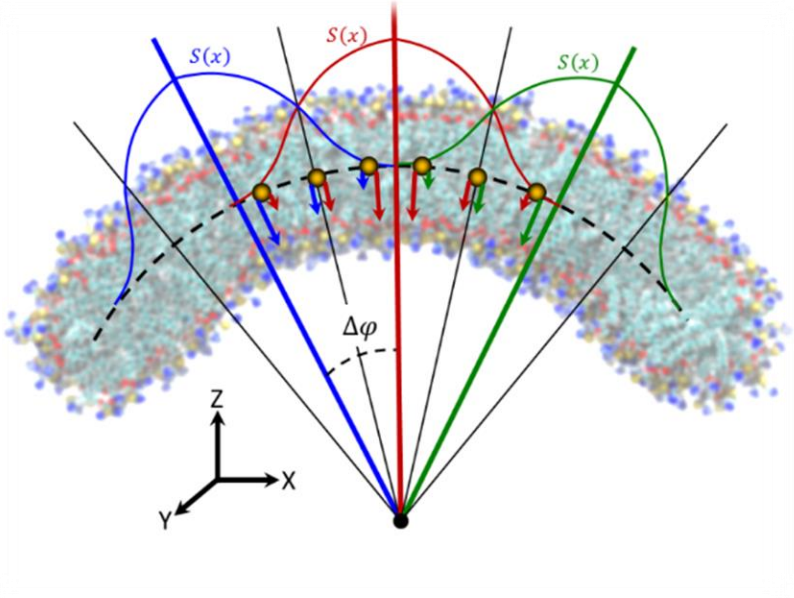
3. Protein-induces bending.



4. Scaffolding by dummy walls.



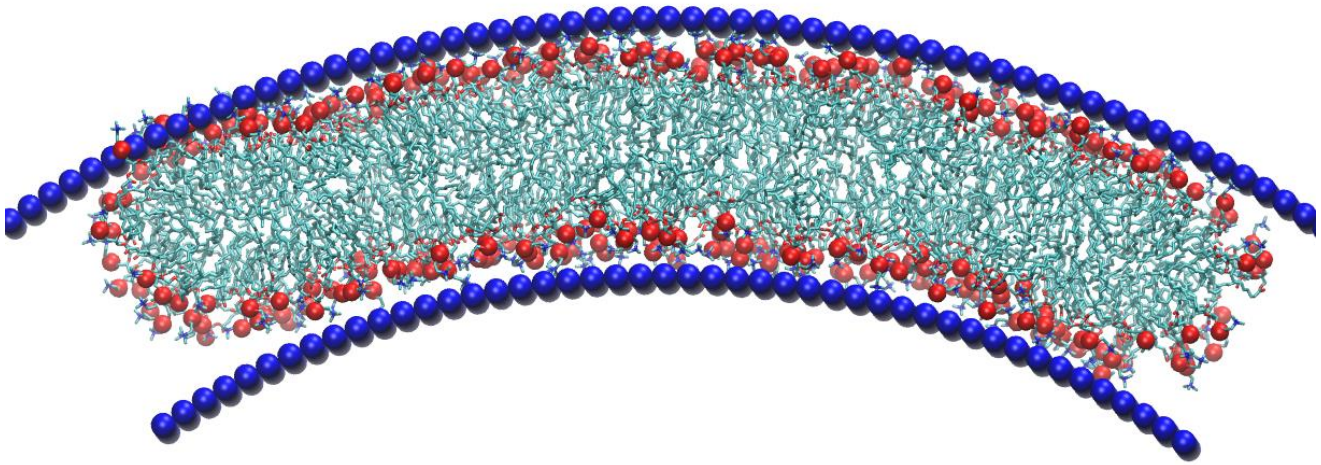
5. Enforcing curvature by external forces.



Existing approaches

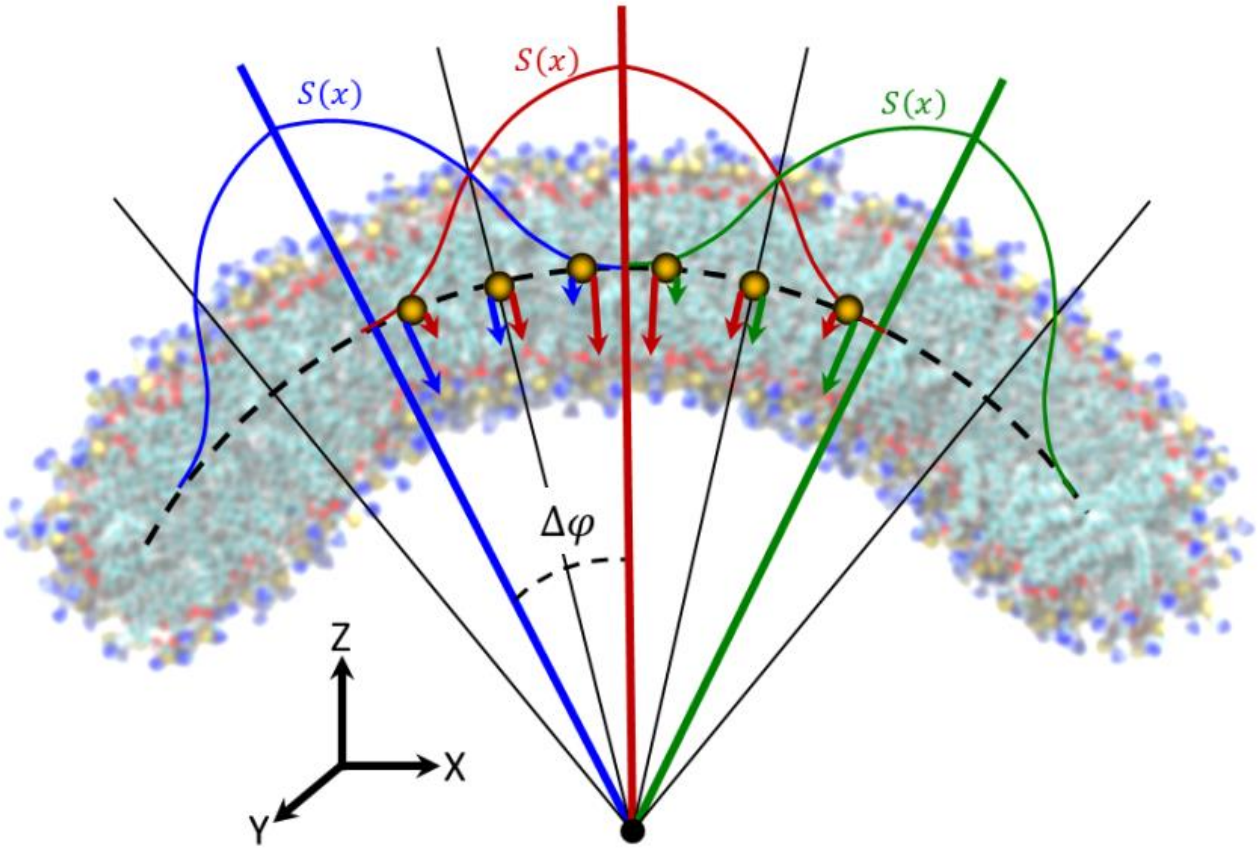
1. Spontaneous undulations.
   - No setup required. Require large systems. Long simulation times. Unpredictable. Large curvatures are very rare and transient.
2. Membrane buckling.
   - Easy to setup. Allows studying mechanical properties. Curvature induced by external compression. Strained elastic bending. Curvature is not uniform. Shape is complex (Euler elastica curve).
3. Protein-induces bending.
   - Mimics natural system. Only occurs under the protein. Non-uniform. Lipid diffusion and packing is affected.
4. Enforcing the curvature by external forces.
   - Curvature maintained without global strain or proteins. Uniform curvature. Methodologically complex. Requires either dummy particles of modification of the MD engine source code.

Solution #4: asymmetric bicelles with enforced curvature



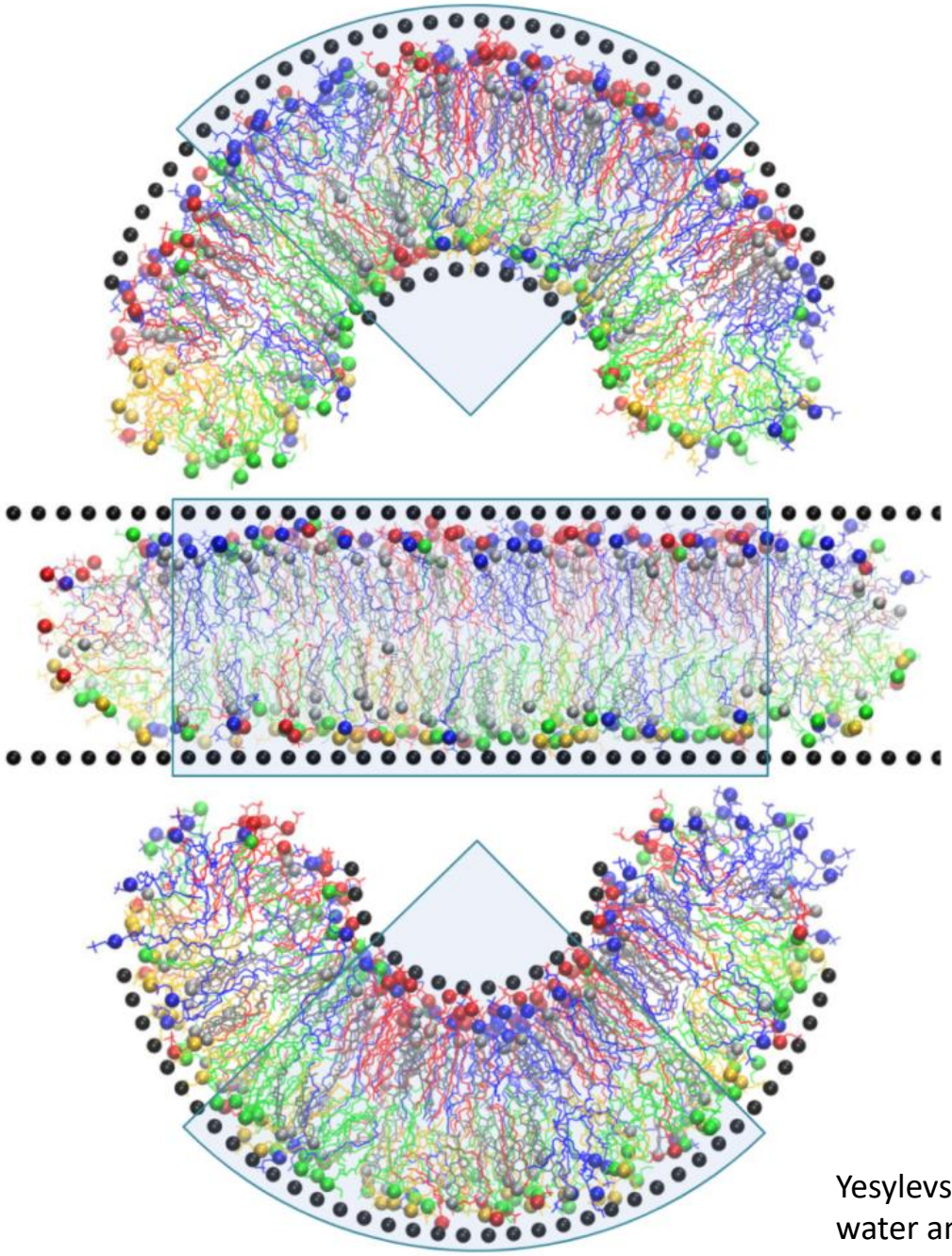
- Two shells of dummy particles interact only with the hydrophobic core of bilayer.
- Heads and water do not see dummy particles.
- Shape of the shells allow enforcing *any* given curvature to the whole bicelle.
- Fixed in space → easy to compute the PMFs

Solution #5: EnCurv: curvature enforced by radial pulling



- No dummy particles
- Generalization of COM pulling MD algorithm with more complex geometry
- System-agnostic and parameter-free (after initial tuning)
- Membrane is divided into overlapping sectors, which are pulled radially by external biasing force to minimize deviations from desired curvature
- Requires modification of MD engines (currently implemented in PLUMED)

S Yesylevskyy, H Khandelia, “EnCurv: Simple Technique of Maintaining Global Membrane Curvature in Molecular Dynamics Simulations”, 2021, Journal of Chemical Theory and Computation 17 (2), 1181-1193.



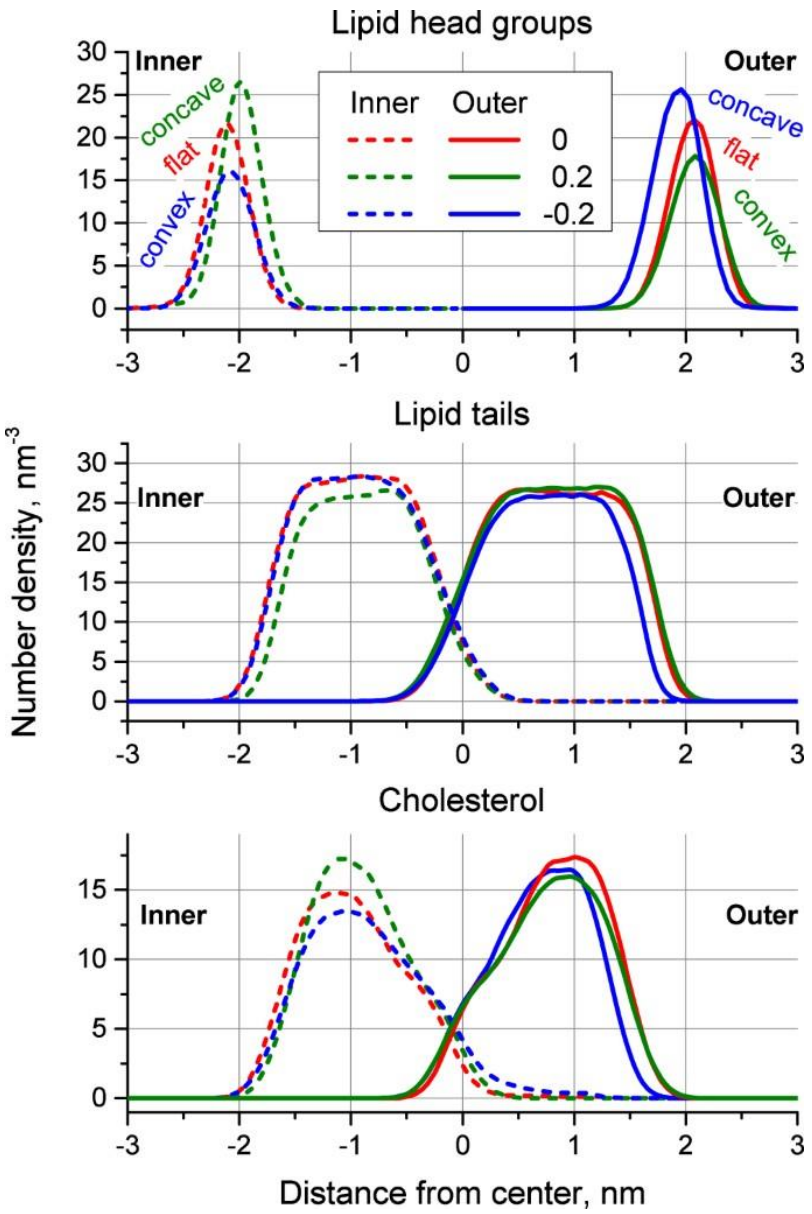
Curvature and drug permeability

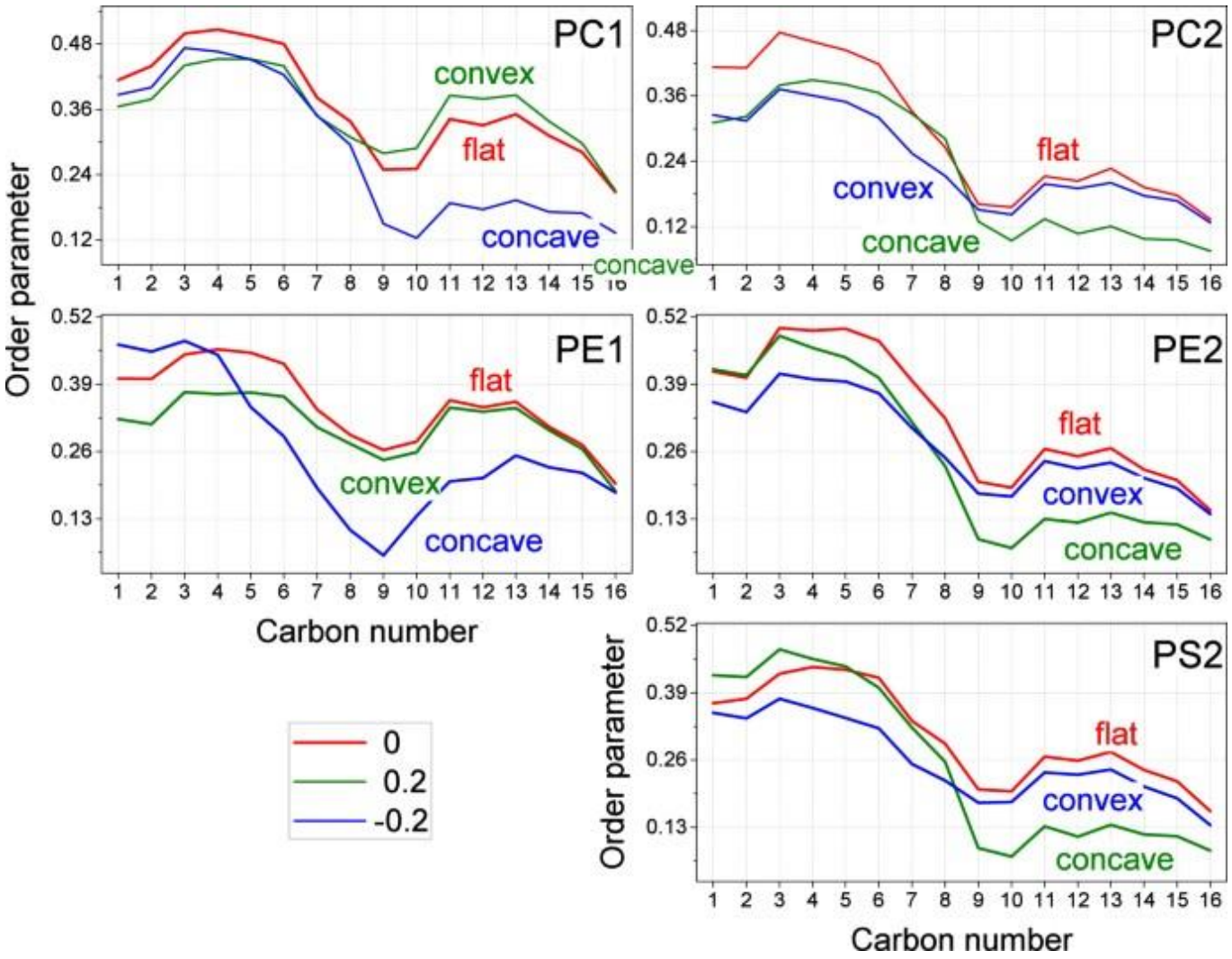
- Model of mammalian red blood cells plasma membrane.
- Permeability of water, ions, CPT and Gem studied.

Component	Outer monolayer	Inner monolayer
SM (sphingomyelin)	42	12
PC (1,2-dioleoyl-sn-glycero-3-phosphocholine)	46	14
PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine)	14	46
PS (1,2-dioleoyl-sn-glycero-3-phospho-L-serine)	0	30
Cholesterol	51	51

Yesylevskyy S, Rivel T, Ramseyer C. Curvature increases permeability of the plasma membrane for ions, water and the anti-cancer drugs cisplatin and gemcitabine. Scientific Reports. 2019;9(1):17214.

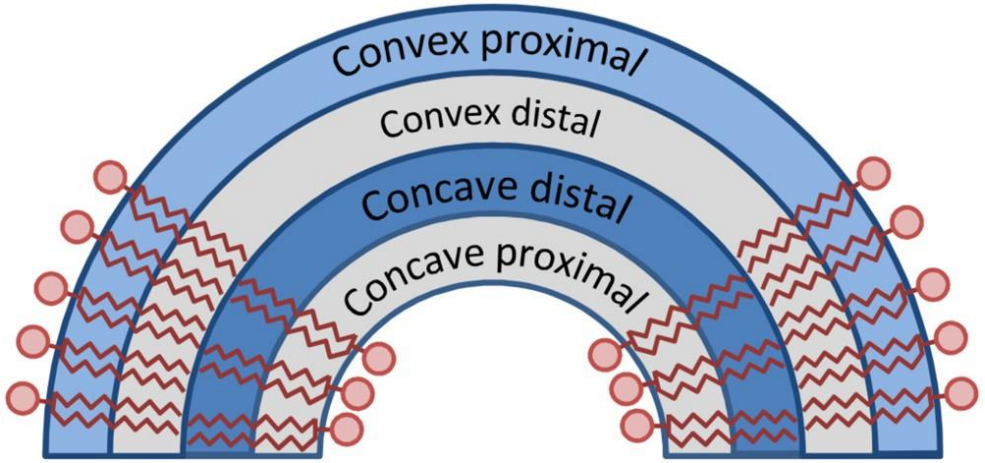
Curvature and the membrane properties

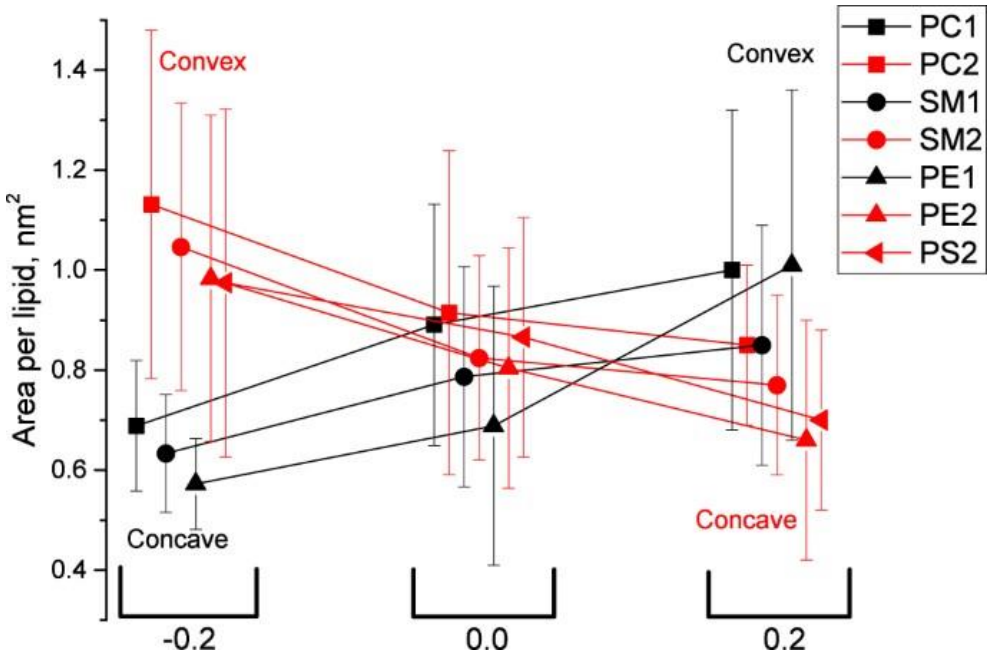




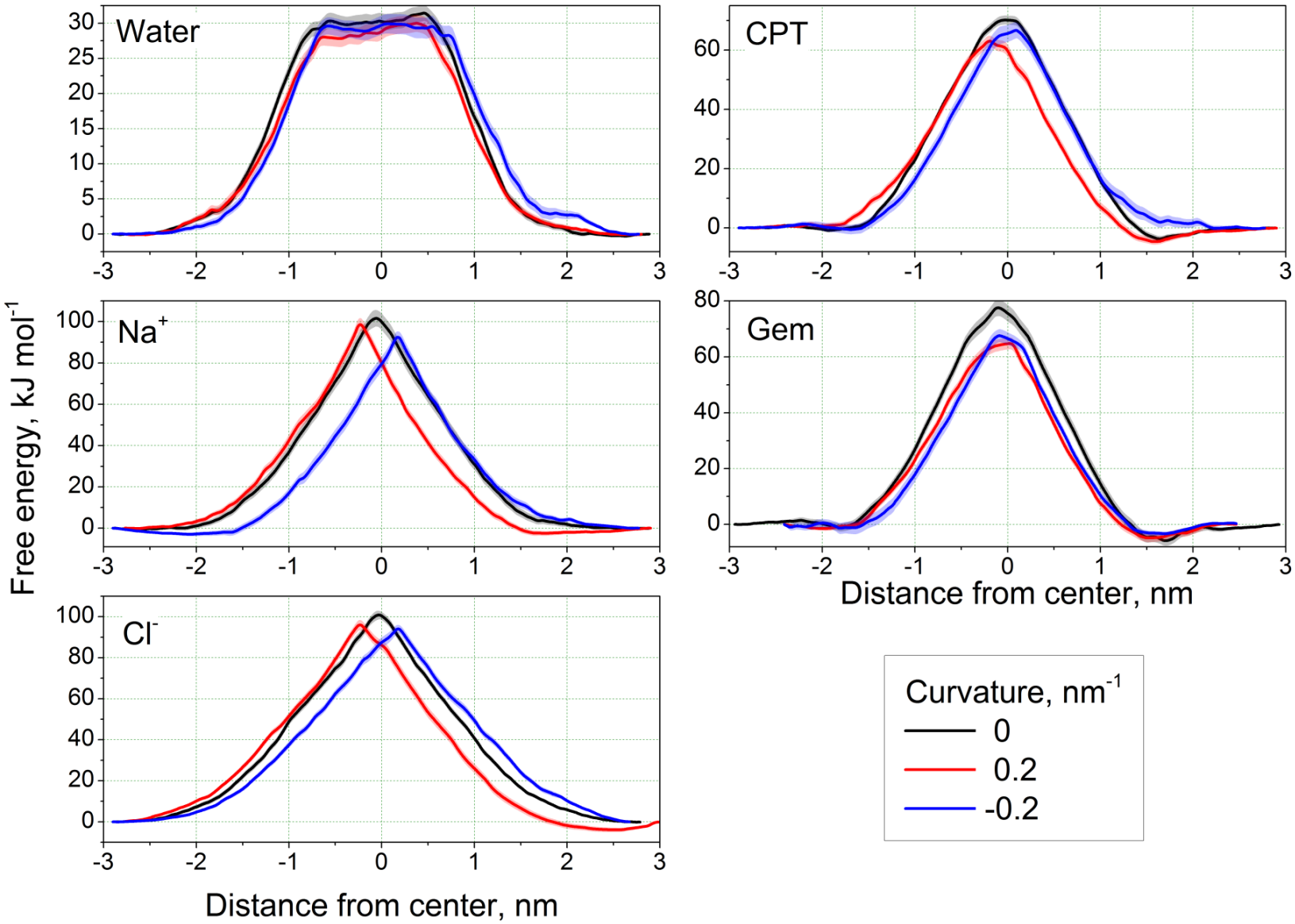
Curvature and the membrane properties

- The influence on membrane order is non-trivial and depends strongly on the monolayer and the location of carbon relative to the double bond.
- Shades of blue – amount of the negative change of membrane order.
- Area per lipid changes up to 30%.

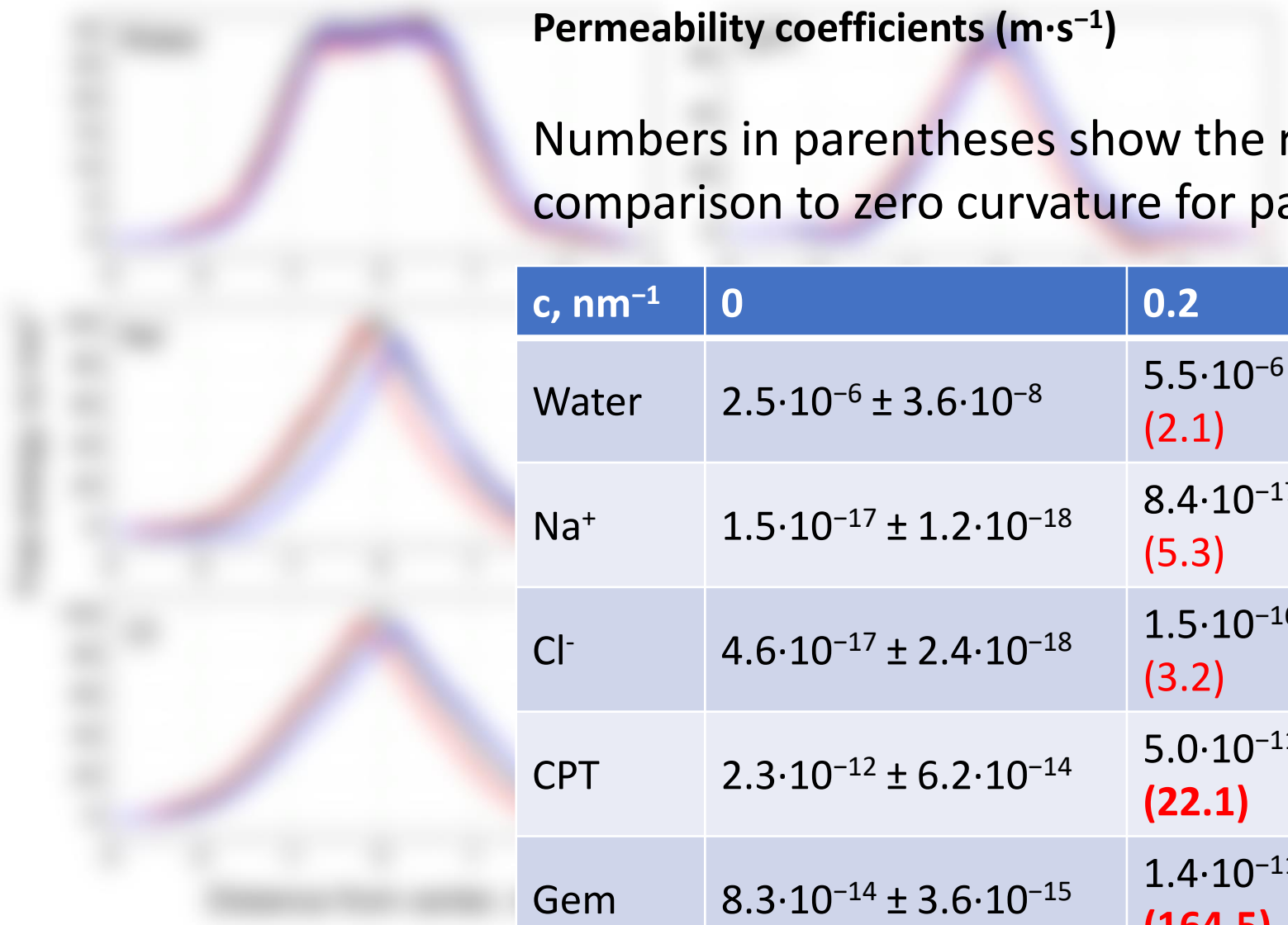




Curvature changes drug permeability!



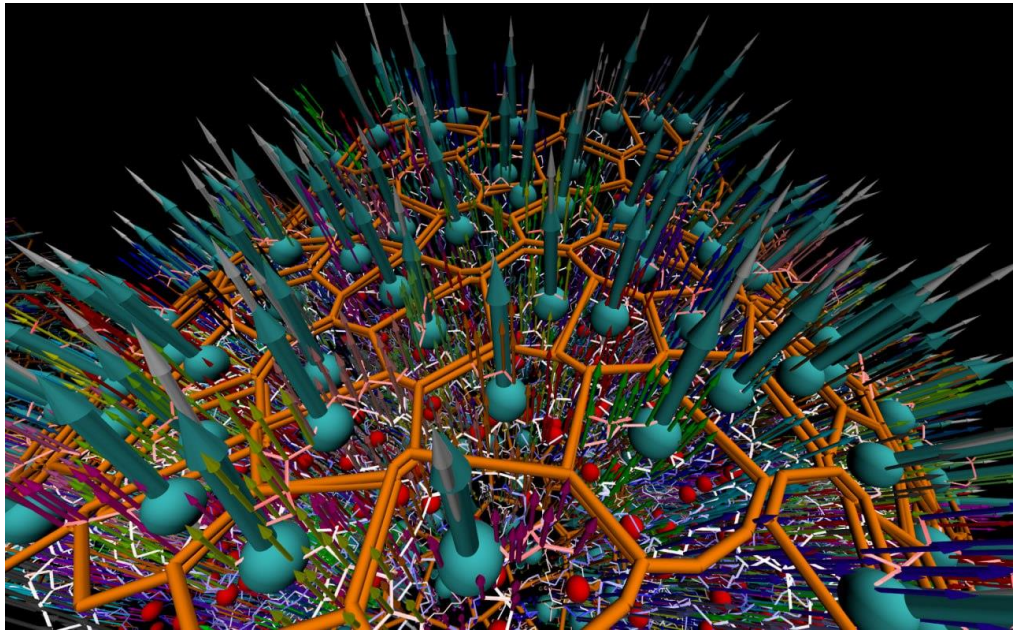
Curvature changes drug permeability!

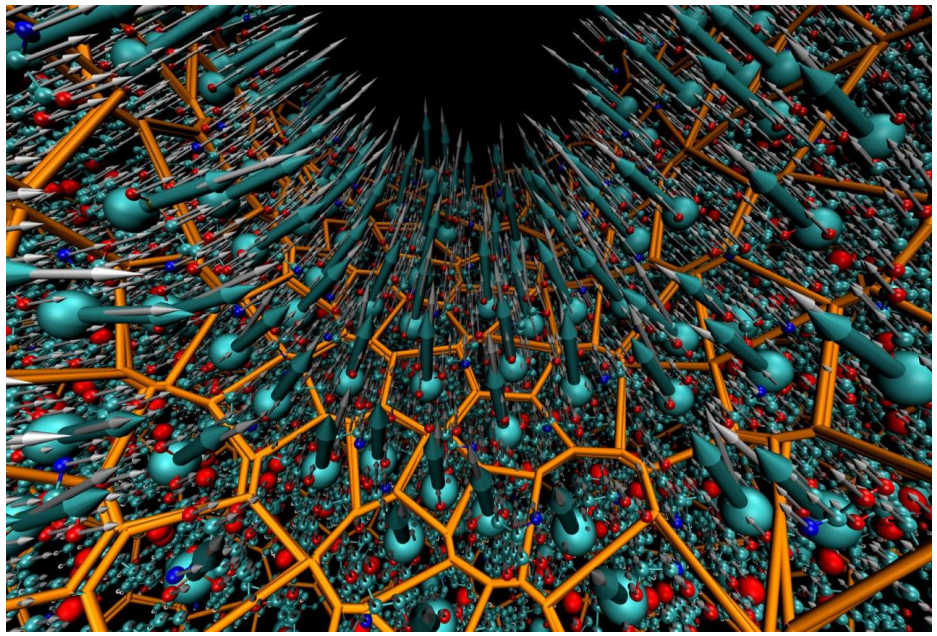


Permeability coefficients ($m \cdot s^{-1}$)

Numbers in parentheses show the ratio of permeability in comparison to zero curvature for particular ligand.

c, nm^{-1}	0	0.2	–0.2
Water	$2.5 \cdot 10^{-6} \pm 3.6 \cdot 10^{-8}$	$5.5 \cdot 10^{-6} \pm 7.2 \cdot 10^{-8}$ (2.1)	$3.7 \cdot 10^{-6} \pm 5.3 \cdot 10^{-8}$ (1.5)
Na^+	$1.5 \cdot 10^{-17} \pm 1.2 \cdot 10^{-18}$	$8.4 \cdot 10^{-17} \pm 6.9 \cdot 10^{-18}$ (5.3)	$1.7 \cdot 10^{-15} \pm 1.3 \cdot 10^{-16}$ (107.5)
Cl^-	$4.6 \cdot 10^{-17} \pm 2.4 \cdot 10^{-18}$	$1.5 \cdot 10^{-16} \pm 8.7 \cdot 10^{-18}$ (3.2)	$6.4 \cdot 10^{-16} \pm 3.3 \cdot 10^{-17}$ (13.8)
CPT	$2.3 \cdot 10^{-12} \pm 6.2 \cdot 10^{-14}$	$5.0 \cdot 10^{-11} \pm 1.6 \cdot 10^{-12}$ **(22.1)**	$7.0 \cdot 10^{-12} \pm 3.1 \cdot 10^{-13}$ (3.0)
Gem	$8.3 \cdot 10^{-14} \pm 3.6 \cdot 10^{-15}$	$1.4 \cdot 10^{-11} \pm 4.2 \cdot 10^{-13}$ **(164.5)**	$4.5 \cdot 10^{-12} \pm 1.5 \cdot 10^{-13}$ **(53.7)**



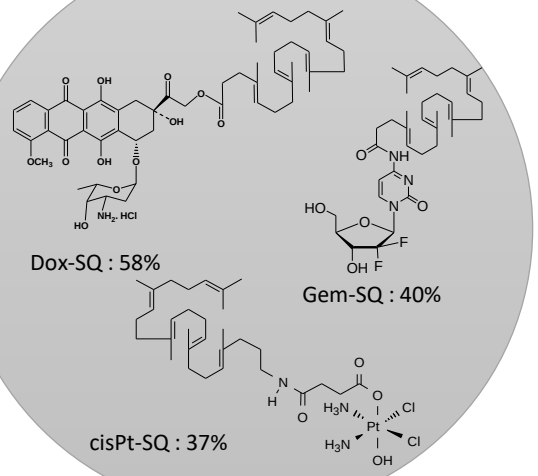


Making the membrane druggable

- Targeting cancer cell membranes by nanomedicines
- Computing drug-membrane interactions
- Asymmetry and curvature matters!

Membranotropic nanomedicines based on squalene

Anticancer drugs

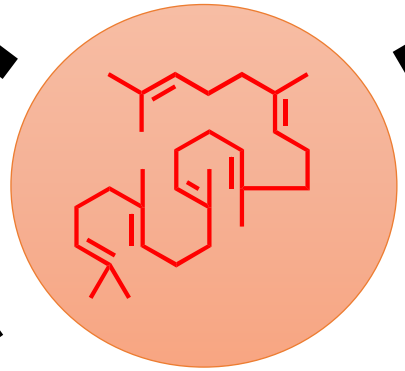


Dox-SQ : 58%

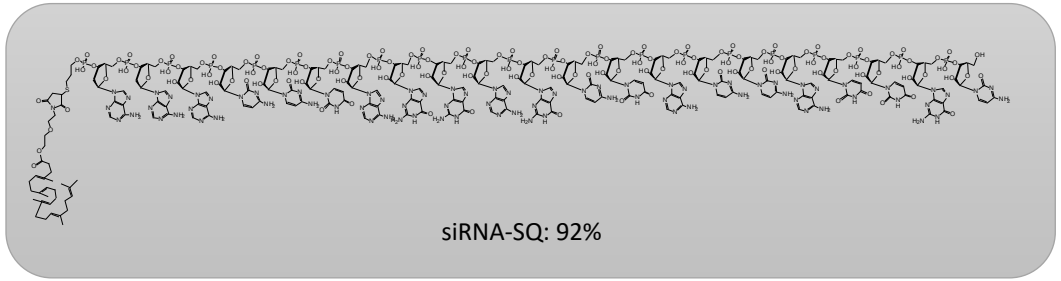
Gem-SQ : 40%

cisPt-SQ : 37%

SQUALENE

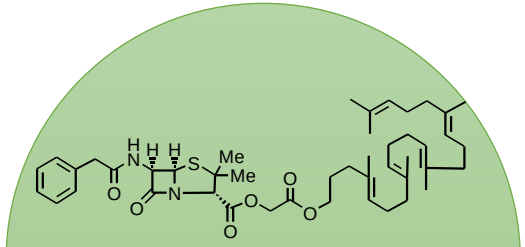


Anticancer siRNA

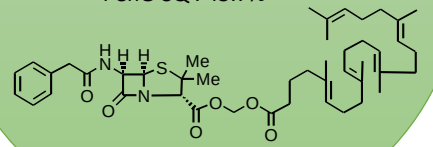


siRNA-SQ: 92%

Antibiotics

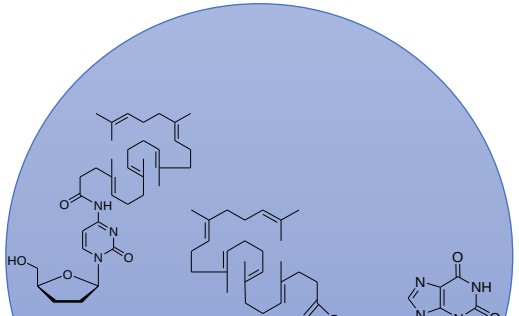


PenG-SQ : 43.7%



PenG-SQ pH : 43.8%

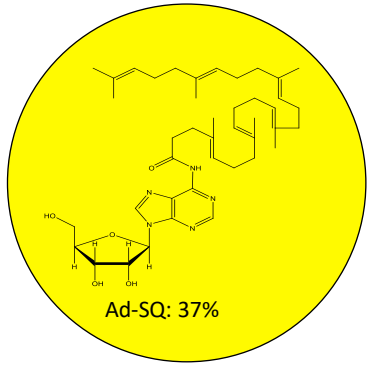
Antivirals



ddC-SQ : 35%

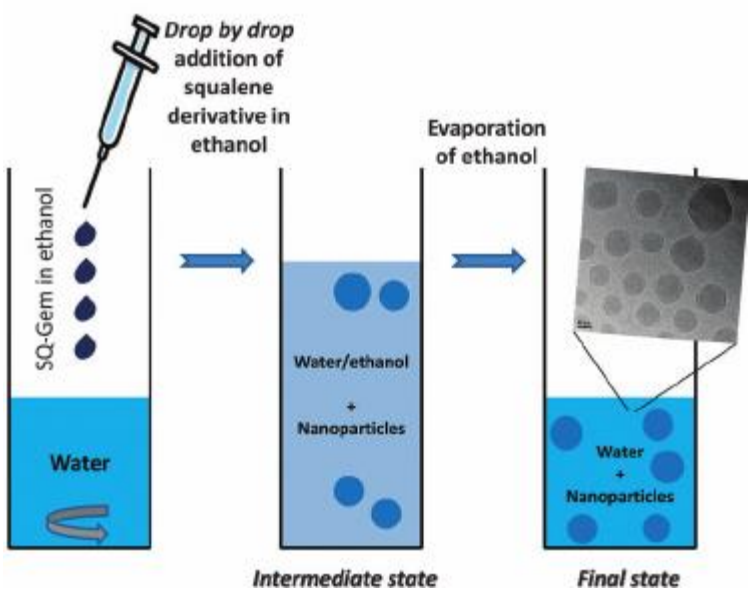
ddI-SQ : 39%

Neuroprotectors

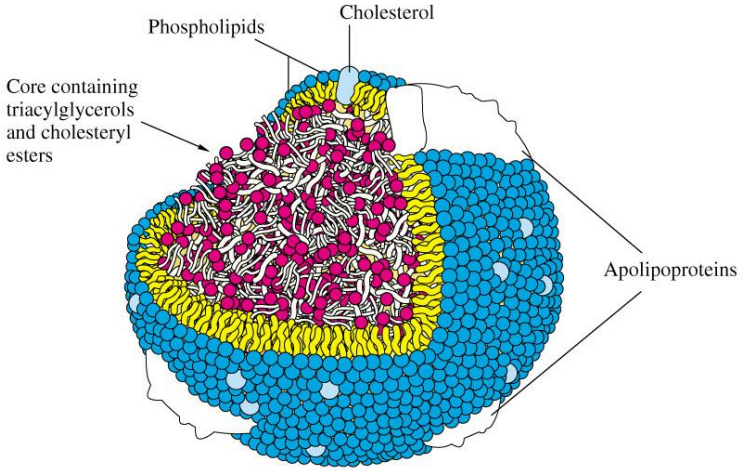


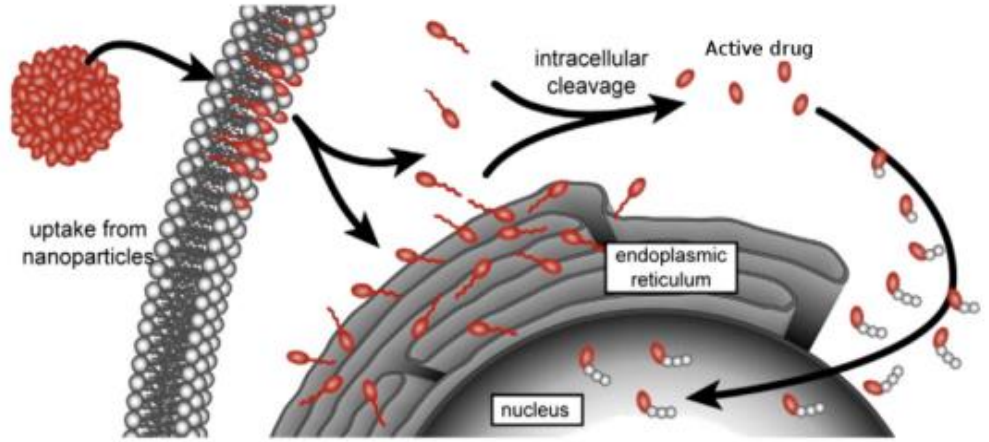
Ad-SQ: 37%

Membranotropic nanomedicines based on squalene



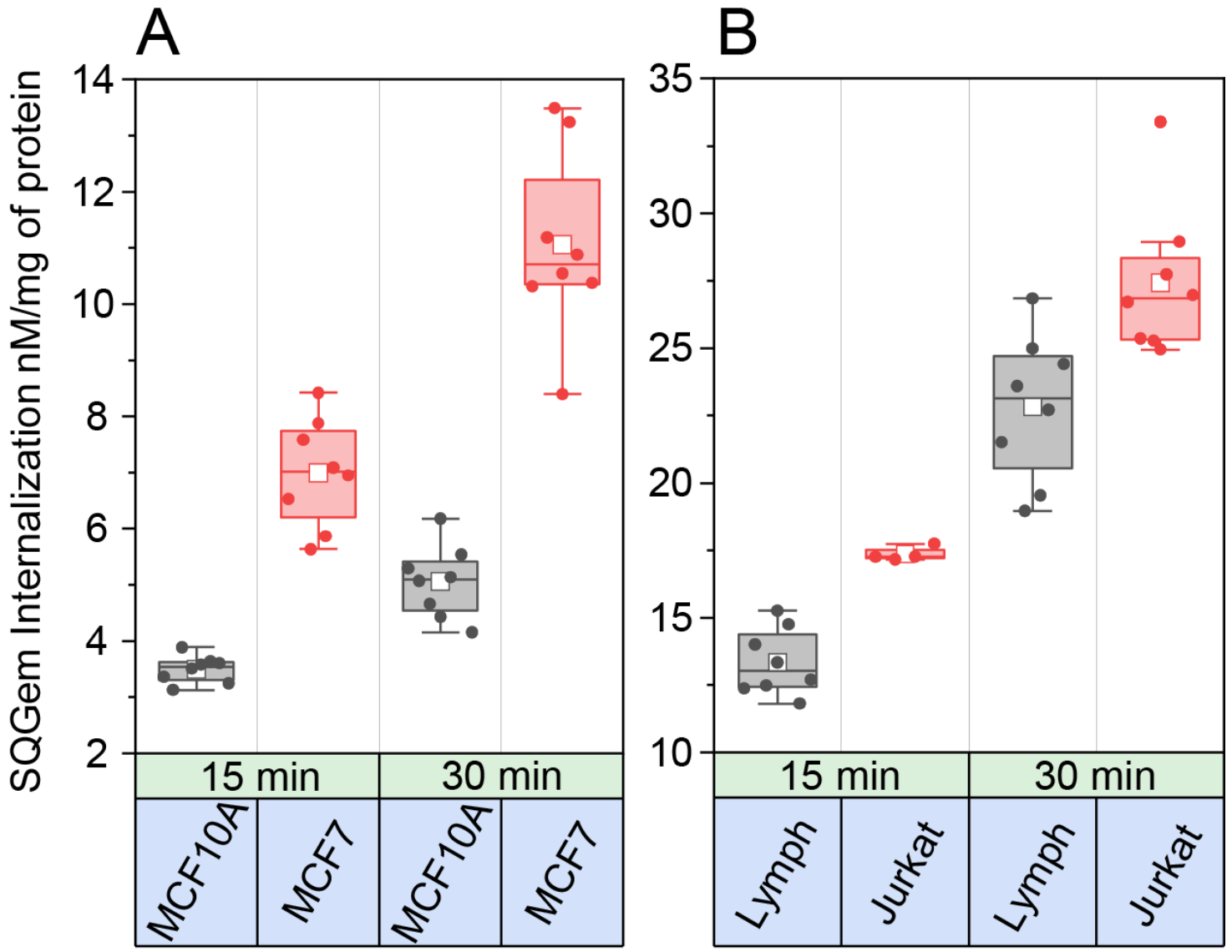
- Nanoparticles forms spontaneously in water.
- Transported by lipoproteins and albumin.
- Taken up by cells, incorporate into the plasma membranes.
- Act as a prodrug, drug is cleaved in the cell.





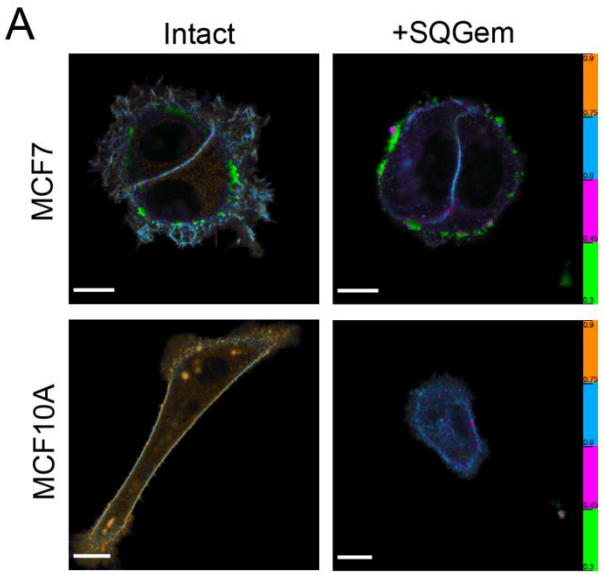
SQGem uptake by cancer and normal cells

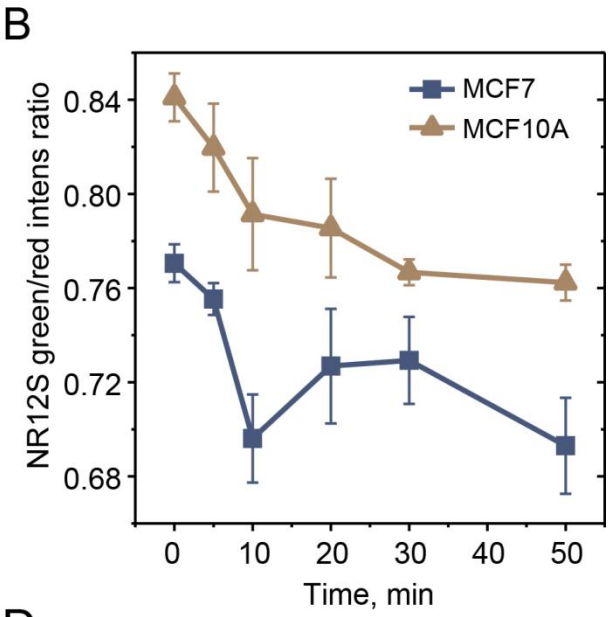
- Comparison of malignant and benign cell lines of the same origin (MFC7-MFC10, Jurkat-Lymphocites)
- Uptake of radioactive NPs measured.
- Cancer cells take up a lot more selectively!

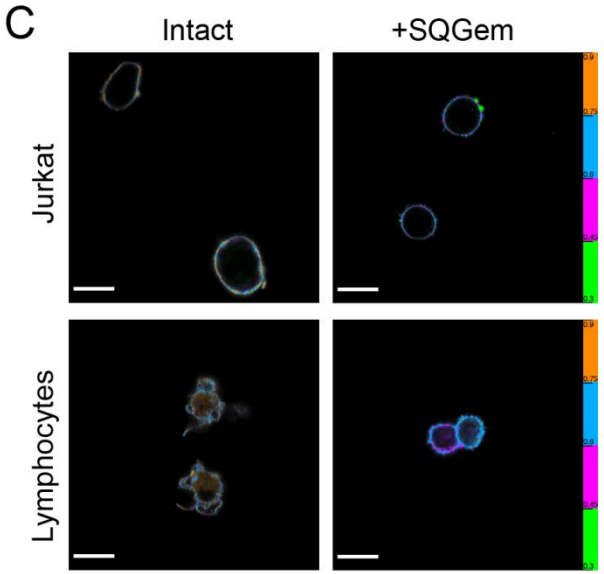


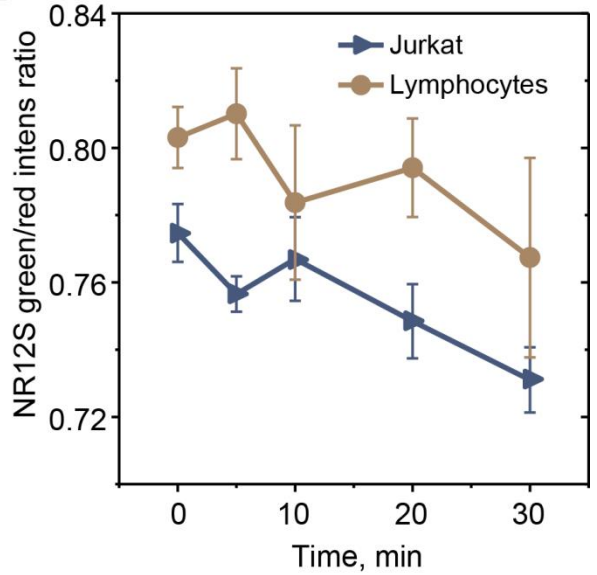
SQGem uptake by cancer and normal cells

- SQGem incorporates directly into the plasma membrane.
- Accumulates more in cancer cell membranes.
- The membrane order decreases upon incorporation, which is detected by the fluorescent order-sensitive probes.

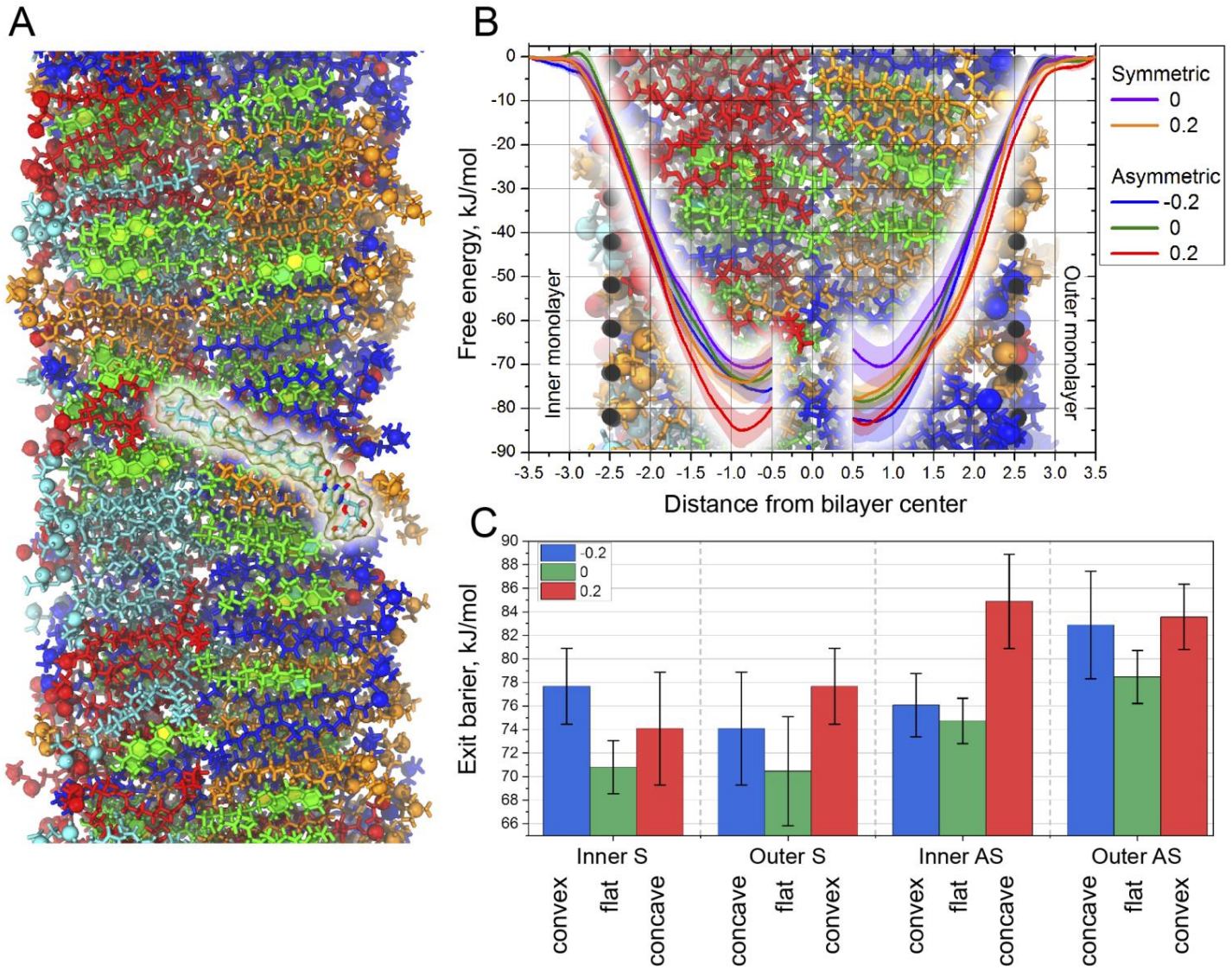






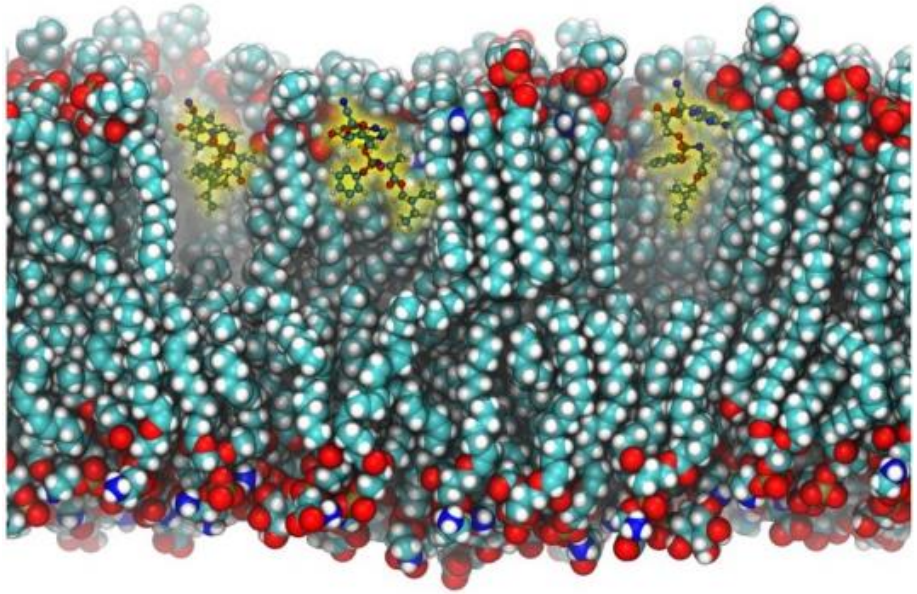


SQGem membrane insertion is sensitive to both asymmetry and curvature



- PMFs of extracting SQGem from each monolayer.
- Asymmetric (benign) vs. symmetric (cancer) membrane.
- Flat vs. curved (concave vs. flat vs. convex monolayers).
- Diffusion to cytoplasm is faster in symmetric membrane (regions of negative curvature would contribute equally, the regions of negative and curvature would favor symmetric membrane)

→ better uptake in cancer cells.

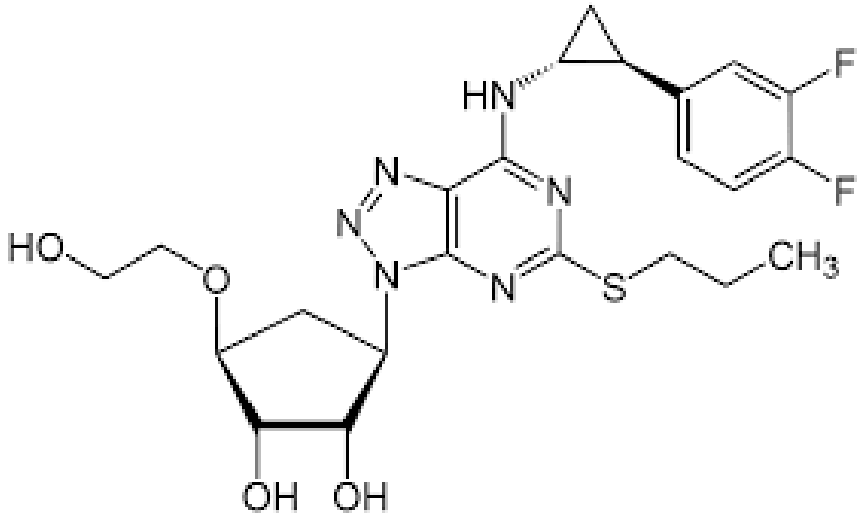


Unusual membrane effects of existing drugs

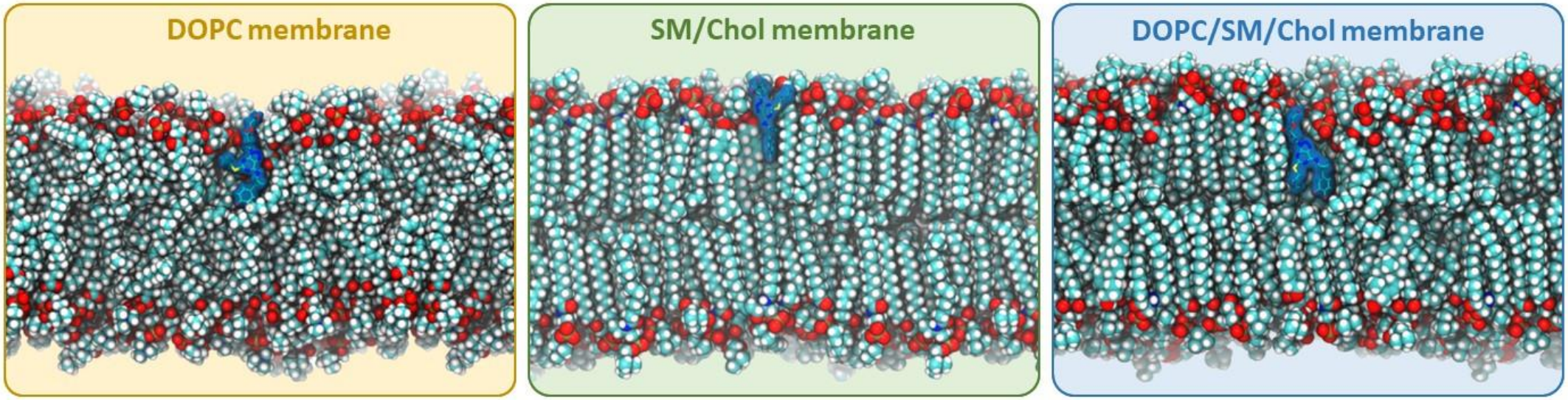
- Approved drugs could be membranotropic (surprise!)
- These effects may lead to positive and negative side effects
- Not studied at all…

Case #1: Ticagrelor

- Very widespread anti-platelet drug.
- Non-covalent inhibitor of P2Y12 platelet receptor of ADP. Prevents platelet activation.
- Never considered as membranotropic (despite being obviously amphiphilic).

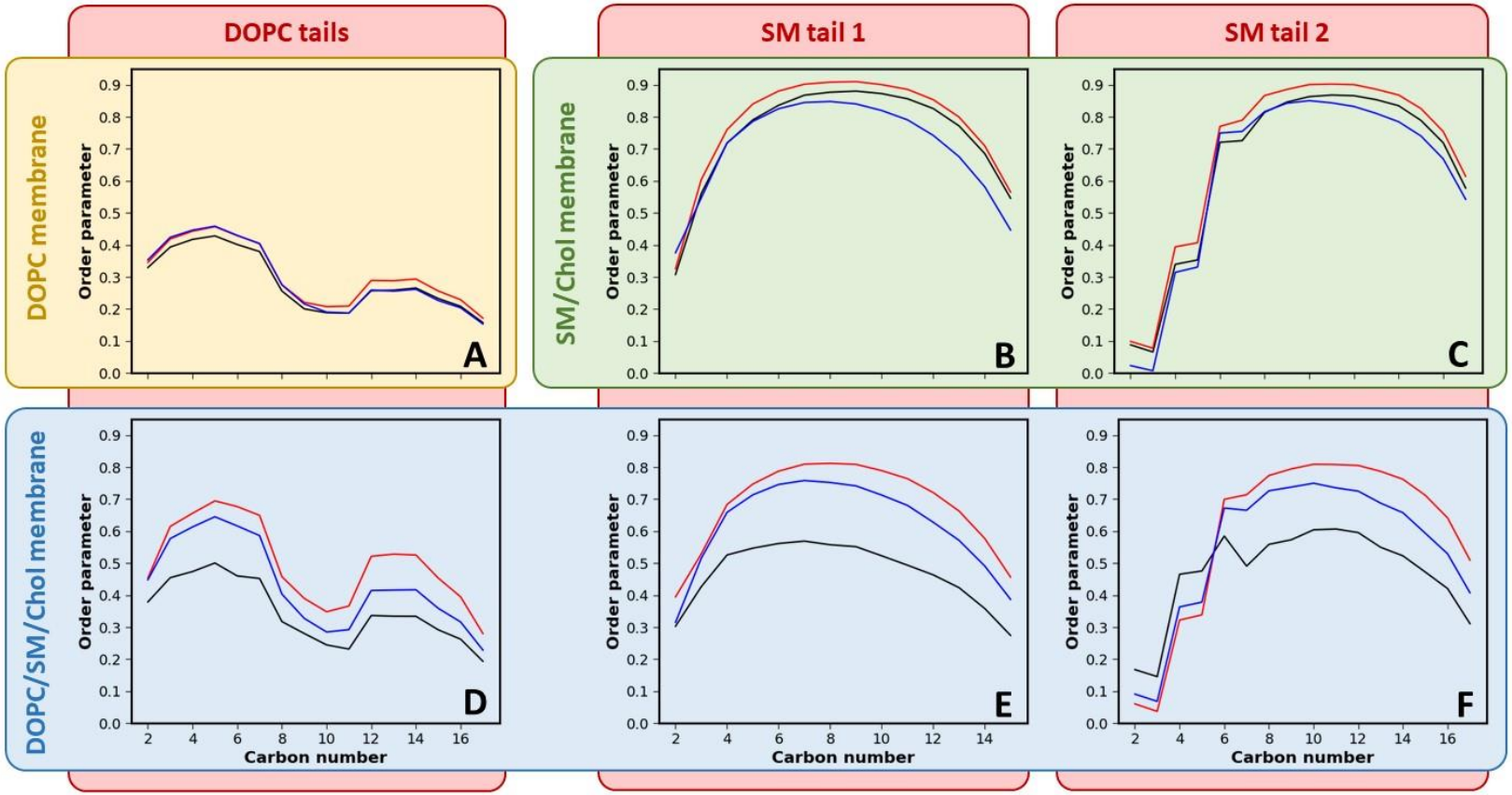


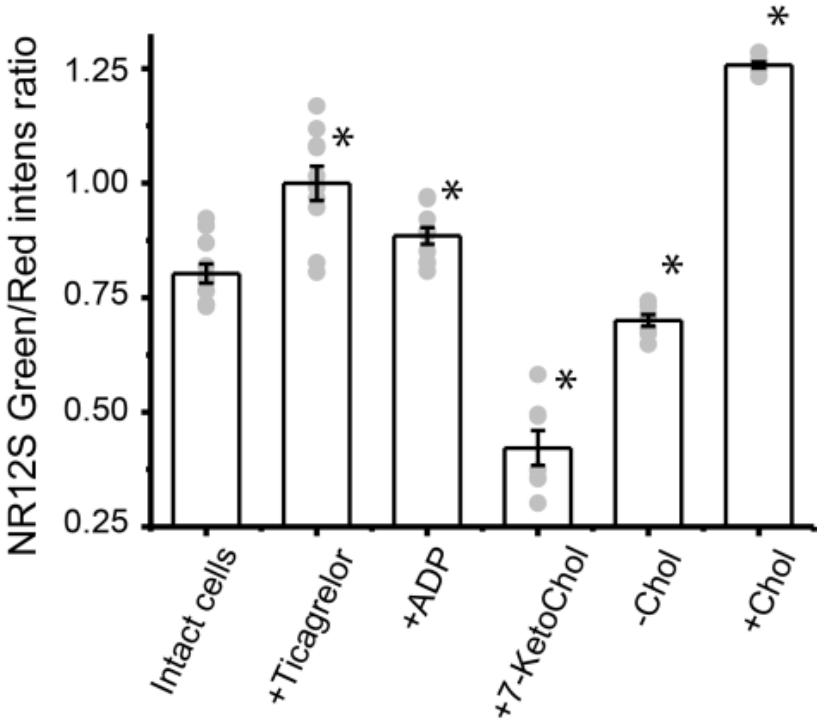
MD simulation of ticagrelor in membranes



- Model membranes to mimic ordered, disordered and mixed phases.
- Incorporation of TIC changes the lipid ordering → comparable with experiments with the fluorescent probes on model vesicles.
- Also compared with experiments on the whole platelets.

MD simulation of ticagrelor in membranes

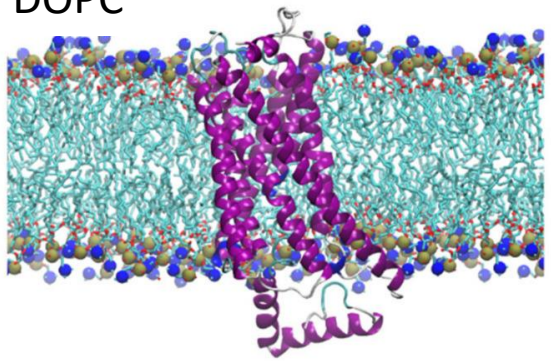




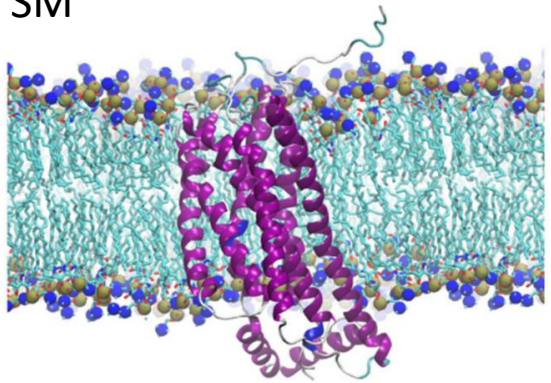
- Ticagrelor accumulates in the membranes.
- Ticagrelor changes the lipid order in non-trivial composition-dependent and concentration-dependent manner.

Ticagrelor receptor is sensitive to lipid order

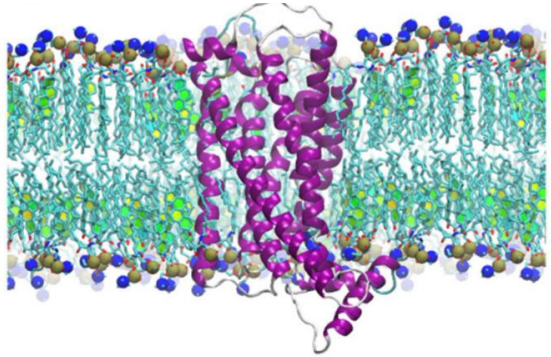
DOPC

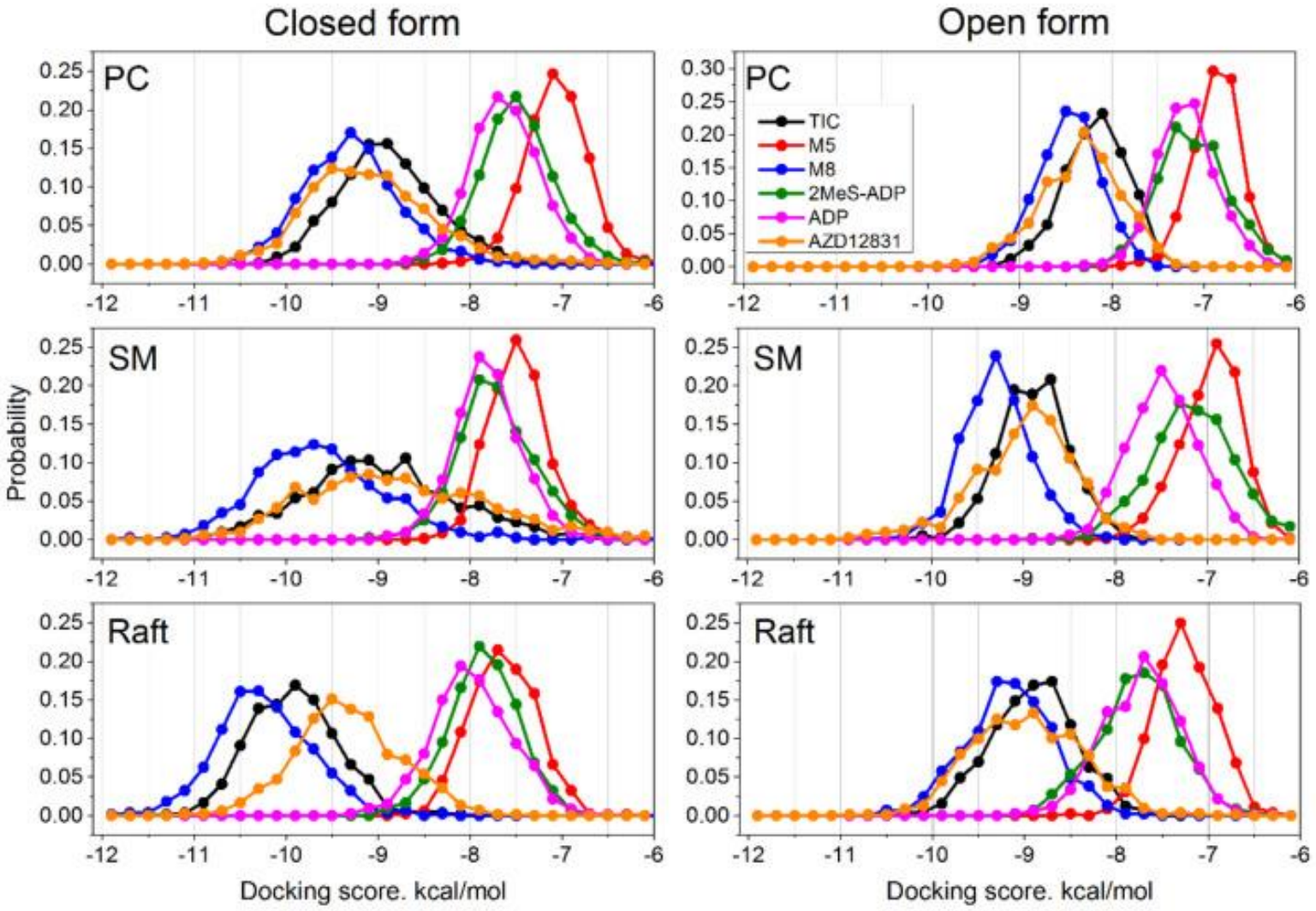


SM



SM/CHOL (“raft”)





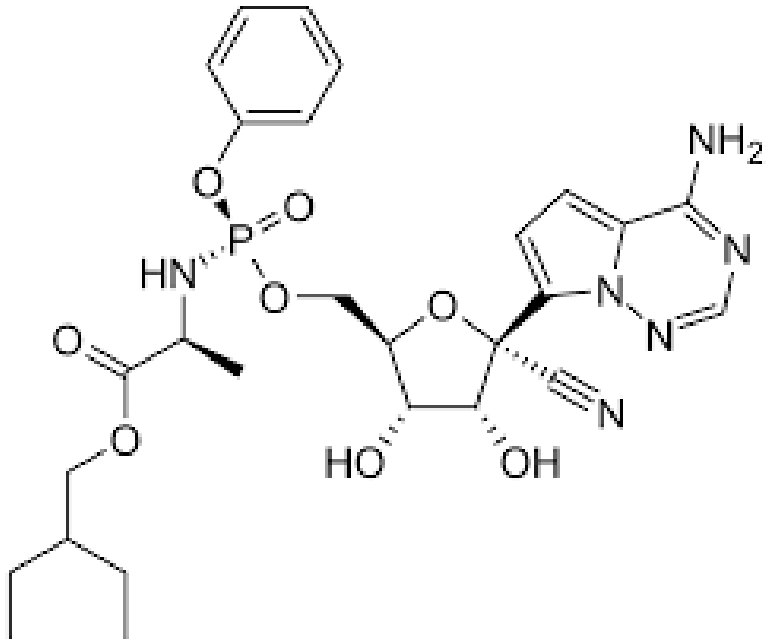
Haghighi F, Yesylevskyy S, Davani S, Ramseyer C. Membrane Environment Modulates Ligand-Binding Propensity of P2Y12 Receptor. Pharmaceutics. 2021 Apr 9;13(4):524.

Is ticagrelor a dual-action drug?

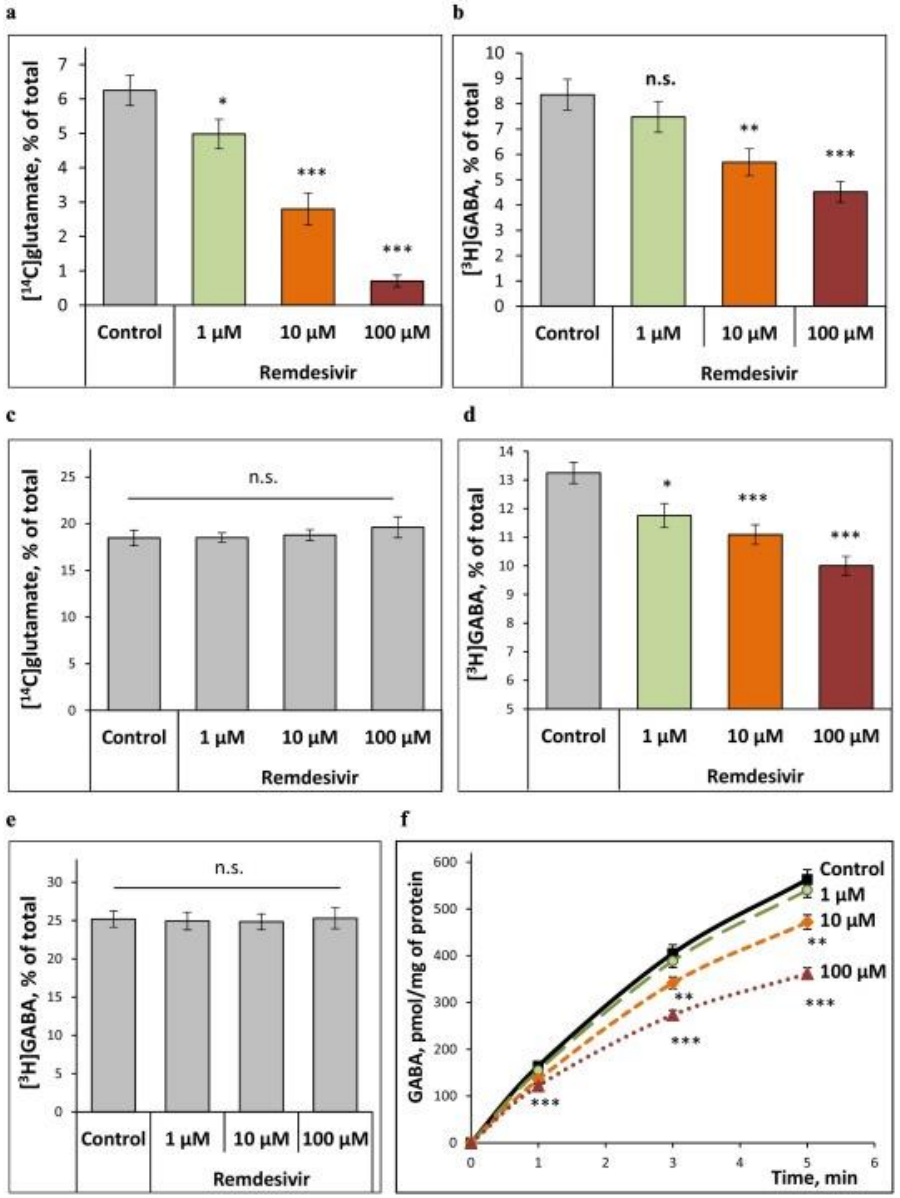
- Ticagrelor changes the membrane ordering significantly.
- It’s own receptor P2Y12 is sensitive to the changes of order.
- Can ticagrelor modulate affinity of *it’s own target protein* by acting on the *lipid bilayer*?
  - Dual action of the drug?
  - Never described before.
  - Could have practical implications for drug design.

Case #2: Remdesivir

- Antiviral drug, which become famous during COVID pandemic.
- Widely studied as possible COVID cure (kind of works, but ineffective in clinics, currently not used).
- Never considered as membranotropic (despite being obviously amphiphilic).



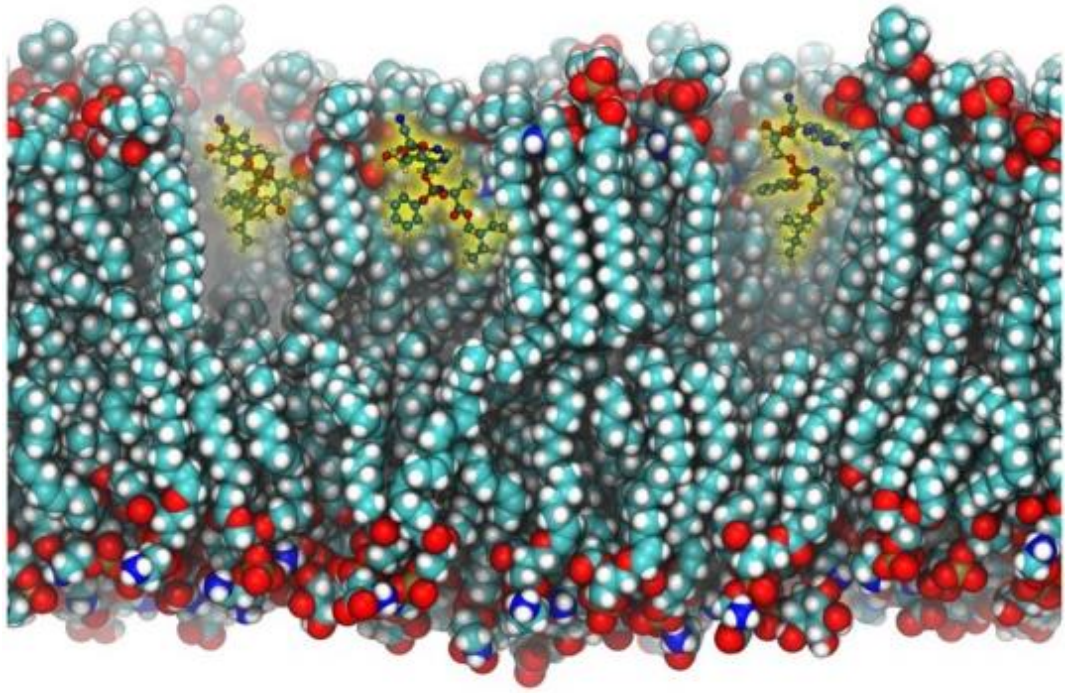
Remdesivir alters release of neurotransmitters

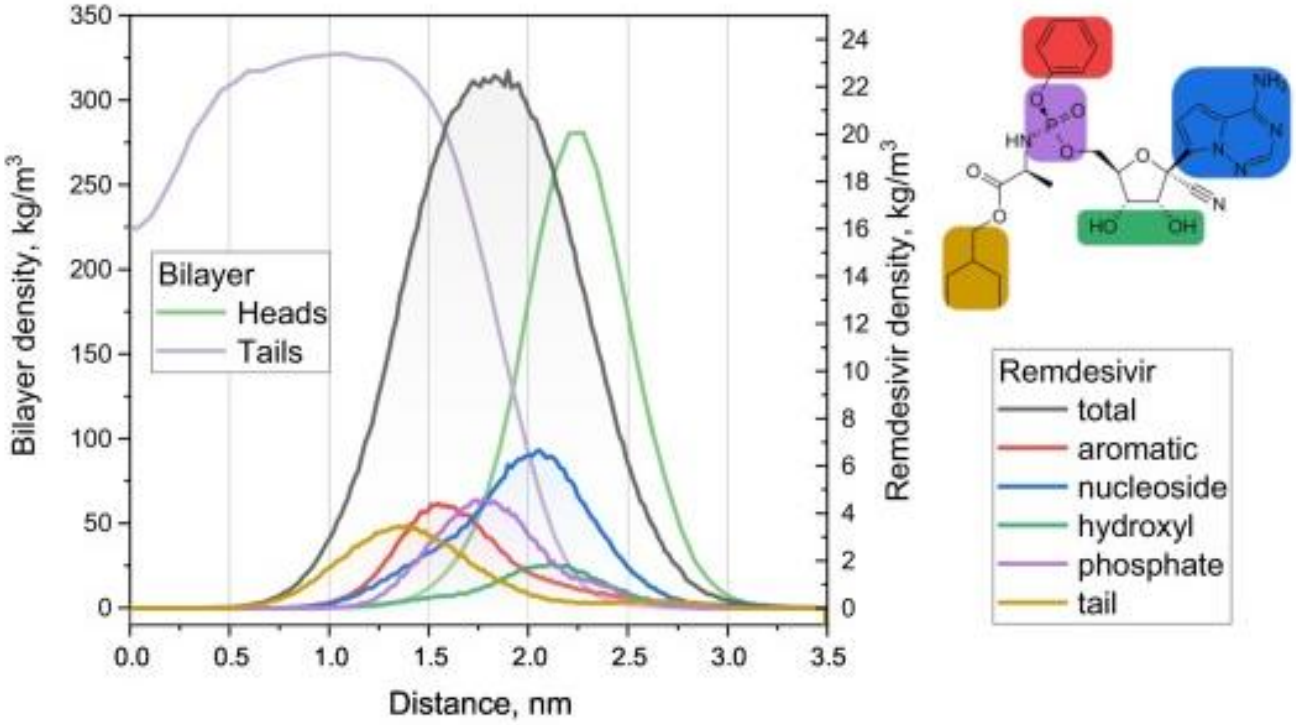


- Decreases exocytotic release of L-glutamate and GABA in nerve terminals.
- Decreases synaptic vesicle acidification in nerve terminals.
- Dosage should be controlled to prevent possible adverse neuromodulatory action!
- The effect is shown to be related to direct membrane accumulation → another unexpected membranotropic drug.

Biochimica et Biophysica Acta (BBA) - Biomembranes. 2022 Aug 1;1864(8):183945.

MD simulations confirm membrane accumulation





- Remdesivir behaves as a typical membranotropic compound.
- Changes membrane ordering and local propertied.
- Possible direct effects on viral membrane envelopes?

Conclusions

- Membranes should be considered as the first-class entities in drug design.
  - It is not possible to treat them only as an unfortunate permeation barrier.
- Lipid membranes are druggable (without involving proteins).
  - New treatment modalities for cancer and precision medicine.
- Existing drugs may have unexpected membranotropic effects.
  - Drug repurposing and off-target usage.
  - Unknown adverse effects.
  - Possible dual action on the membrane and protein receptors.
- Membrane asymmetry and curvature matters
  - Drug permeability is sensitive to asymmetry and curvature.
  - MD simulations of the flat membranes are not enough! (another layer of complexity)


Wake
UP
AND
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your attention!
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