Russian Academy of Sciences

M.M. Shemyakin & Yu.A. Ovchinnikov Institute of Bioorganic Chemistry

COMPUTATIONAL DRUG DESIGN FOR MEMBRANE TARGETS: DIVING INTO COMPLEX DETAILS.

www.ibch.ru

Л.М.Б.С.

Roman EFREMOV

Laboratory of Biomolecular Modeling

efremov@nmr.ru
model.nmr.ru

The TEAM:

Л.М.Б.С.

Dmitry E. NOLDE	Ph.D.
Anton A. POLYANSKY	Ph.D.
Anton O. CHUGUNOV	Ph.D.
Pavel E. VOLYNSKY	Ph.D.
Andrey S. KUZNETSOV	Ph.D.
Yuri A. TROFIMOV	Ph.D.
Elena T. ALIPER	researcher
Nikolai A. KRYLOV	researcher

Computations: Supercomputer Center “Polytechnical”, St. Petersburg Polytechnic University.

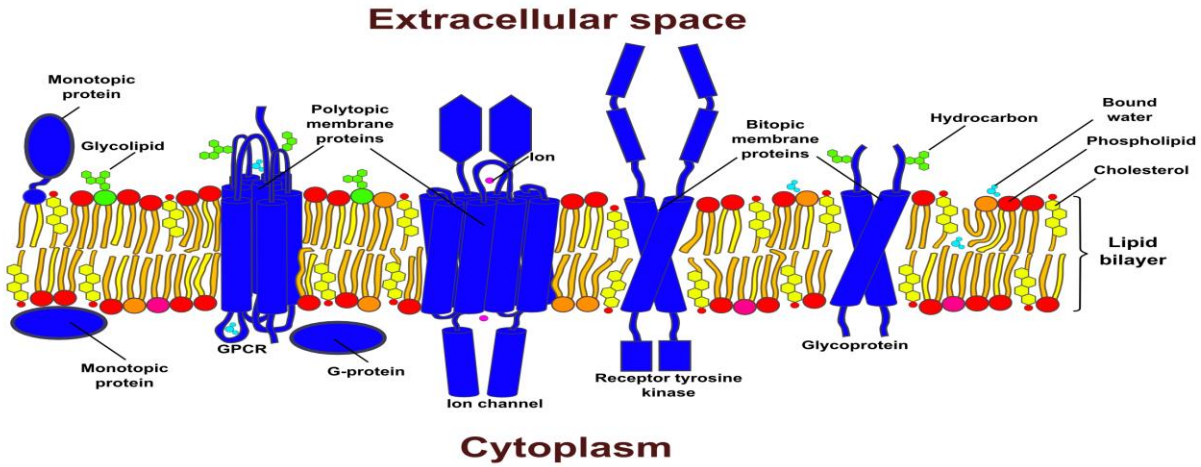
Grant sponsors: Russian Science Foundation;
Russian Foundation for Basic Research;
Ministry of Science and Higher Education.

Biomembranes as perspective pharmacological targets

Up to 70% of currently marketed drugs act either on membrane proteins or on membrane itself

Examples of potential targets:

- **G-protein coupled receptors (GPCRs);**
- **Transmembrane ion channels and transporters;**
- **Integral MPs involved in oligomerization upon their functioning (receptor tyrosine kinases, apoptotic proteins, *etc*.);**
- **Lipid bilayer of biomembranes (direct and indirect modifications of its properties can be vitally important for cell)**



OUTLINE:

1. Traditional flowchart of membrane proteins (MPs) modeling.

2. Why does the traditional approach require revision (diving into details)?

   2.1. Multistate organization of MPs;

   2.2. Oligomerization of MPs: signaling “orchestra” in action .

   2.2. Crucial role of membrane response: local / global effects, essential single lipids.

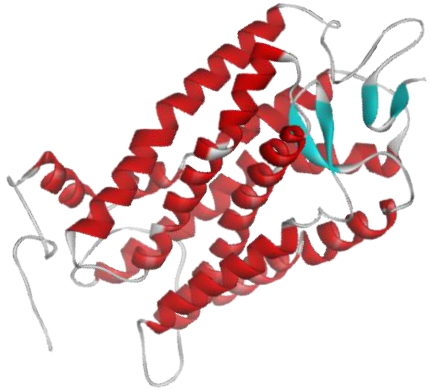
3. Examples:

   3.1. Receptor tyrosine kinases (RTKs);

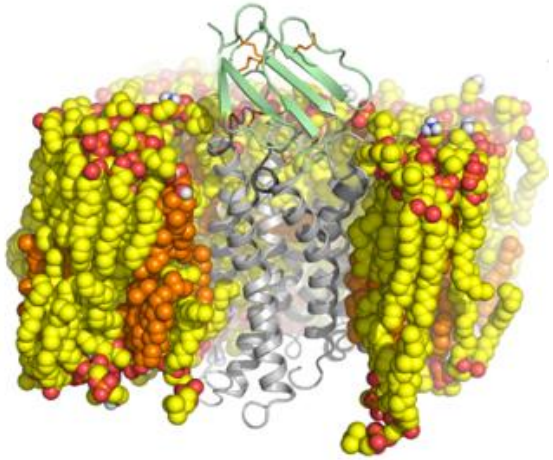
   3.2. Ion channels (TRPV family);

   3.3. SARS-Cov2 spike protein.

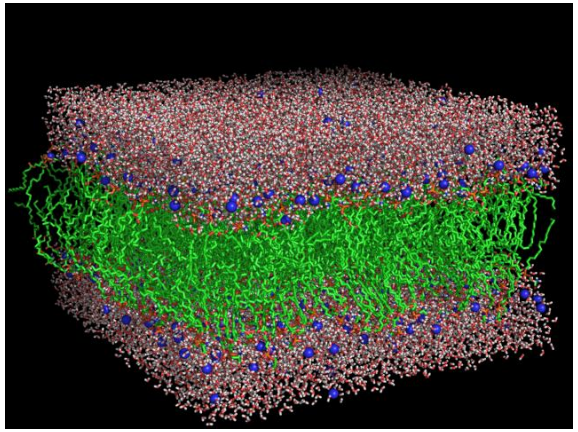
Point 1: Traditional flowchart of membrane proteins (MPs) modeling



Initial MP model:
Experiments (X-ray, cryo-EM, NMR);
Homology-built



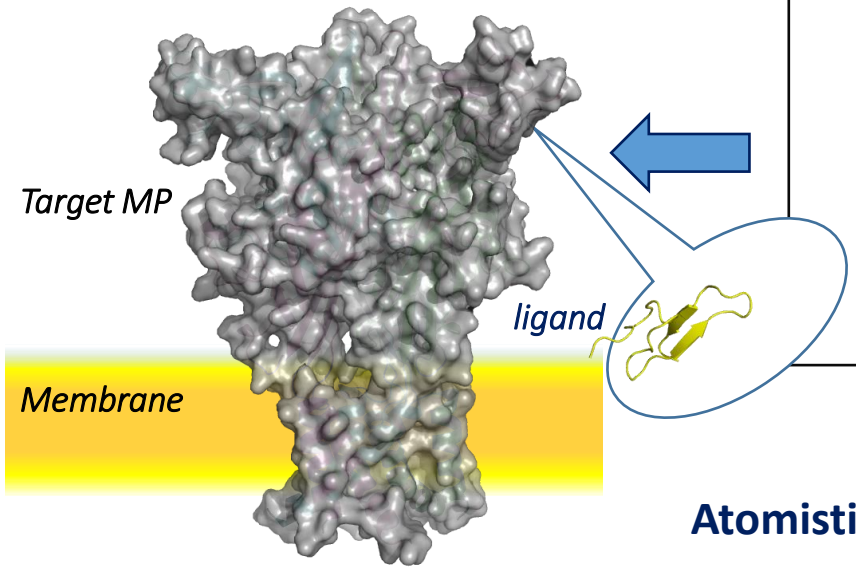
MP in a model membrane

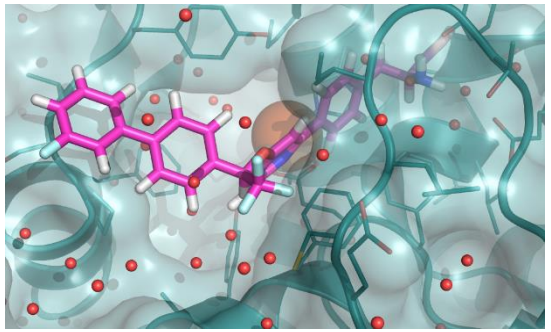


Membrane model:
Hydrated lipid bilayer

Conformational sampling
(MD, Monte Carlo (MC), etc.):

- **Clusters of MP's representative states;**
- **Overall stability of the system;**
- **"Membrane response" features,**
- **…**

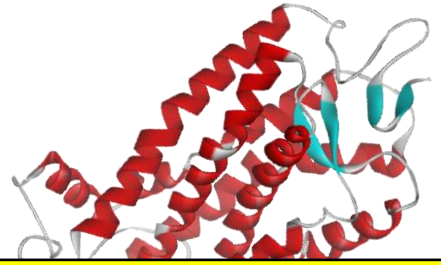


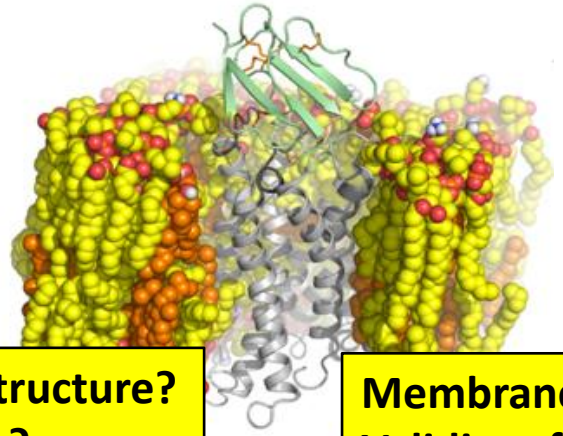


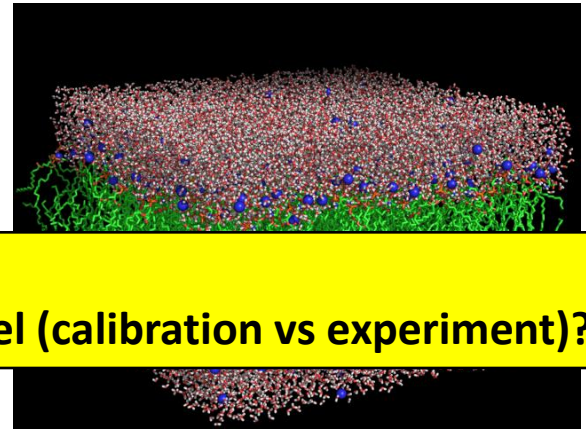
MP-ligand interactions:
MD/MC, docking, surface mapping, etc.

Atomistic model of MP-ligand interaction as a basis for CADD

Point 1: Traditional flowchart of membrane proteins (MPs) modeling







Effect of experimental conditions on MP's structure?
How close is a model to the native structure?

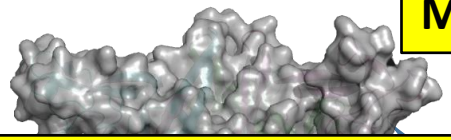
Membrane composition?
Validity of a membrane model (calibration vs experiment)?

MP in a model membrane

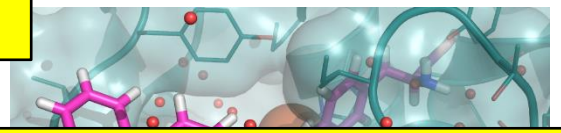
Initial MP model:
Experiments (X-ray, cryo-EM, NMR);
Homology-built

Membrane model:
Hydrated lipid bilayer

Well-known problems of limited sampling in atomistic simulations;
Missing key details in simple models (CG, GBSA, etc.)



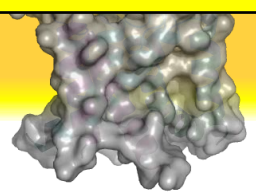
- Clusters of MP's representative states;
- Overall stability of the system;
- "Membrane response" features,
- …



Quality of ensemble docking:
Docking site?
Scoring function?
How to treat membrane in docking?

Quality of intermolecular interactions:
Charged states?
Effects of solvation?
Ions?

Membrane



MP-ligand interactions:
MD/MC, docking, surface mapping, etc.

How reliable is the model?

Atomisti… basis for CADD

Conclusion to this point:

The traditional approach to structure-based drug design for membrane protein targets is still an *extremely challenging procedure with no guaranteed success*!

The good news is that things are actually much worse!

Point 2:

Why does the traditional approach require revision (diving into details)?,

or:

Even simplest protein-membrane systems are not as “simple” as one can imagine…

Membrane proteins and oligomerization

Proteins that oligomerize

Protein kinase receptors

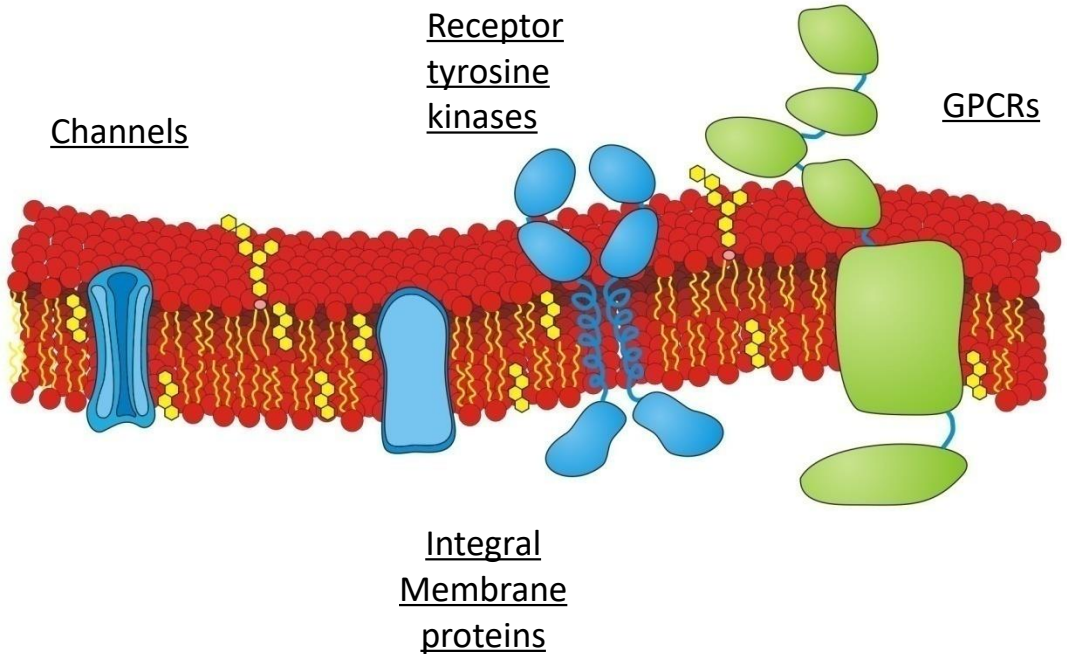
Immune membrane proteins

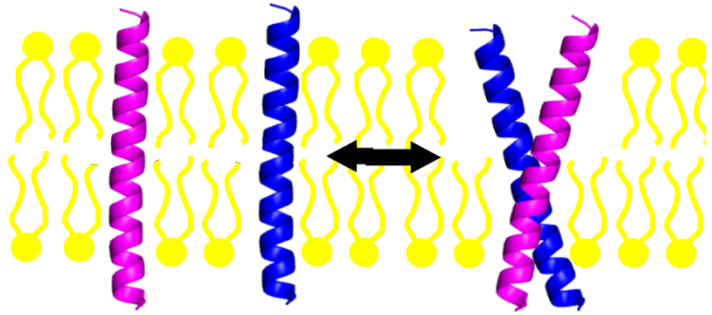
Integrins

Apoptotic proteins

Ligand- and voltage-gated channels

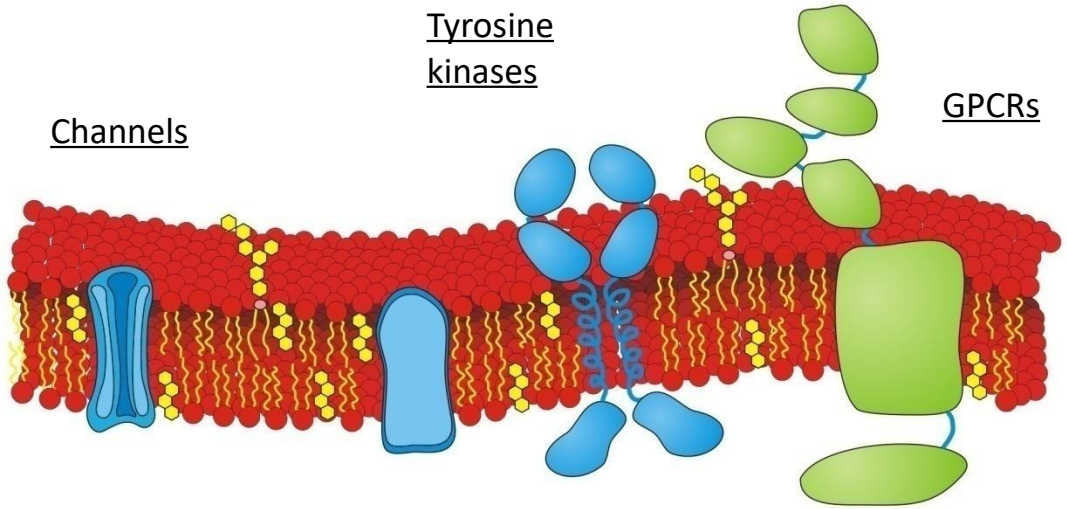
G-protein coupled receptors (GPCRs)

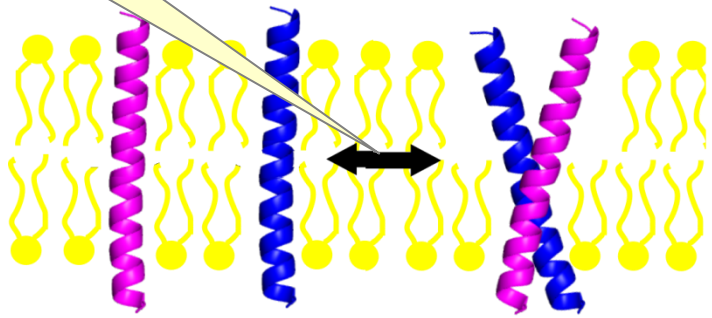




Membrane proteins and oligomerization

How do they dimerize?
(structure)

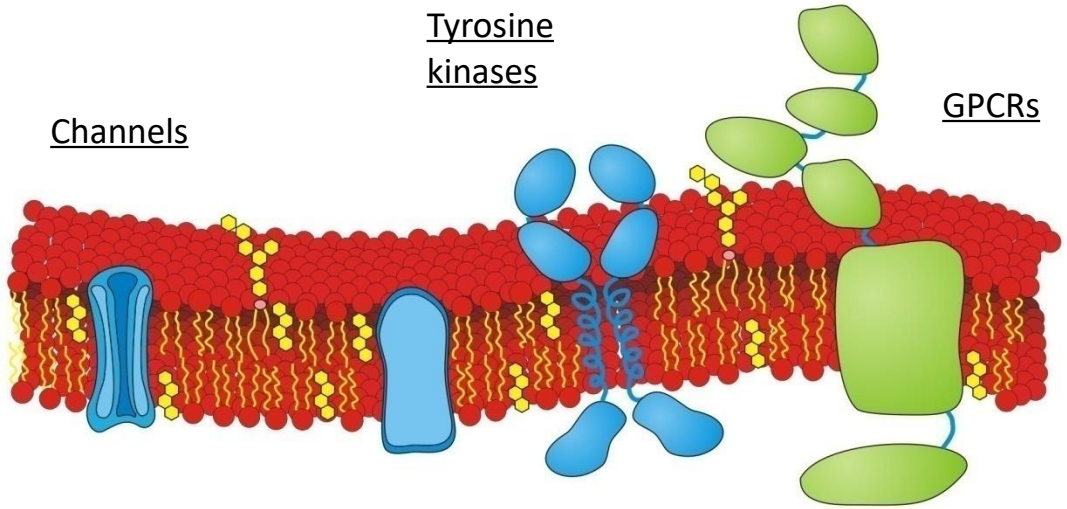


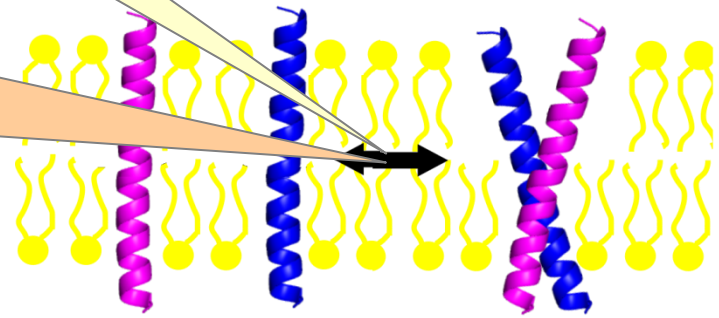


Membrane proteins and oligomerization

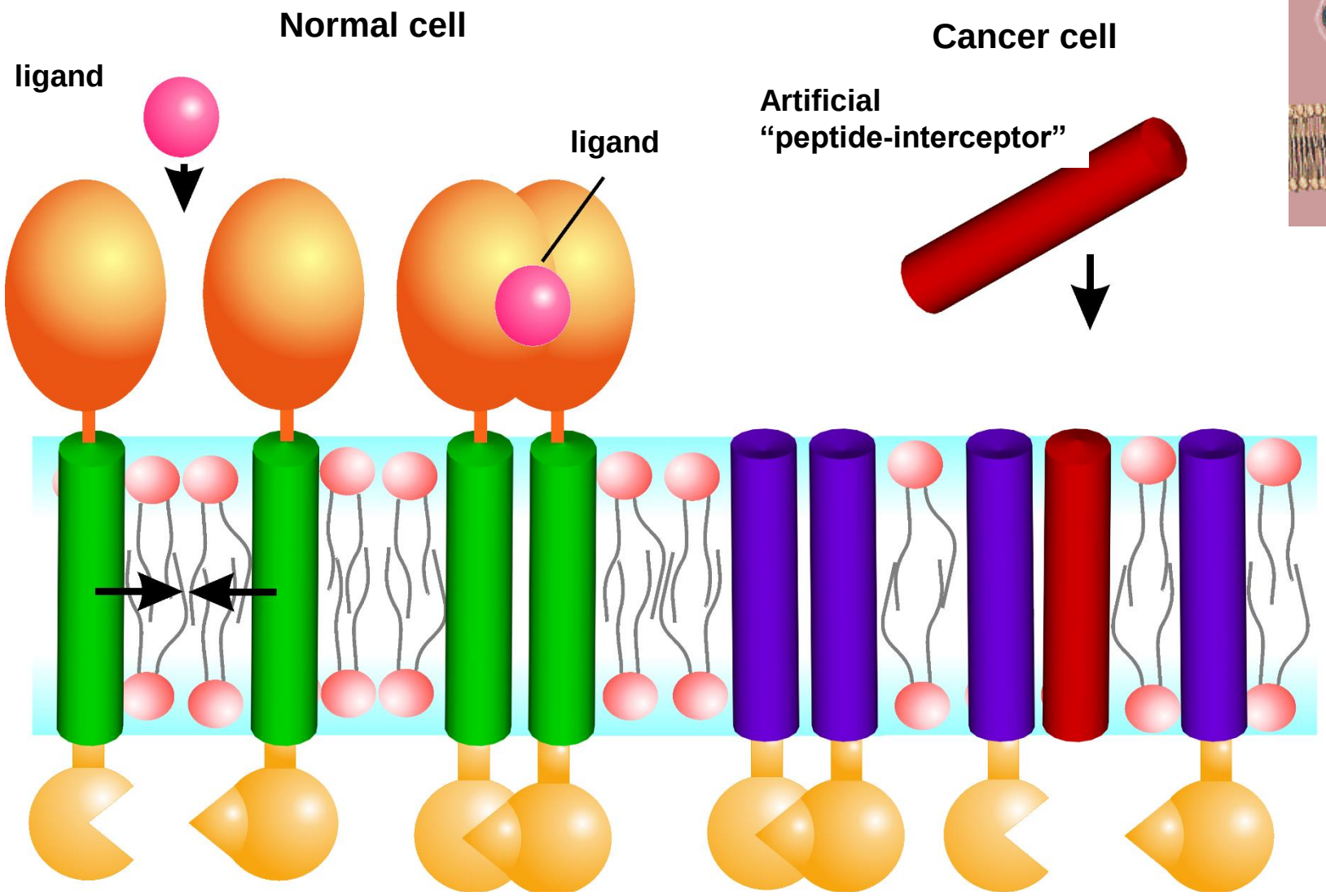
How do they dimerize?
(structure)

Why do they dimerize?
(free energy)

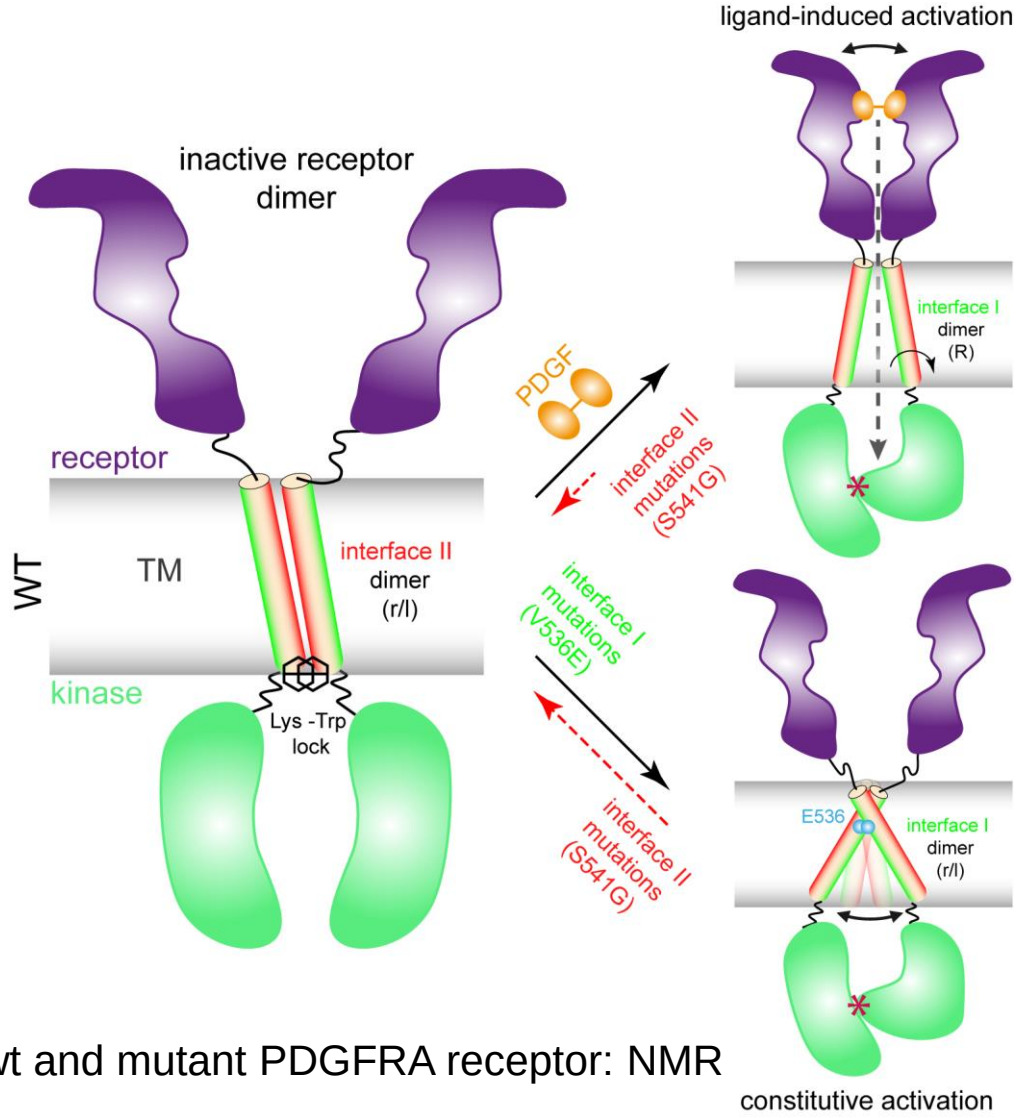




THE CONCEPT OF “PEPTIDES-INTERCEPTORS”



PROBLEM-1: Dynamic oligomerization via multiple intermediate states

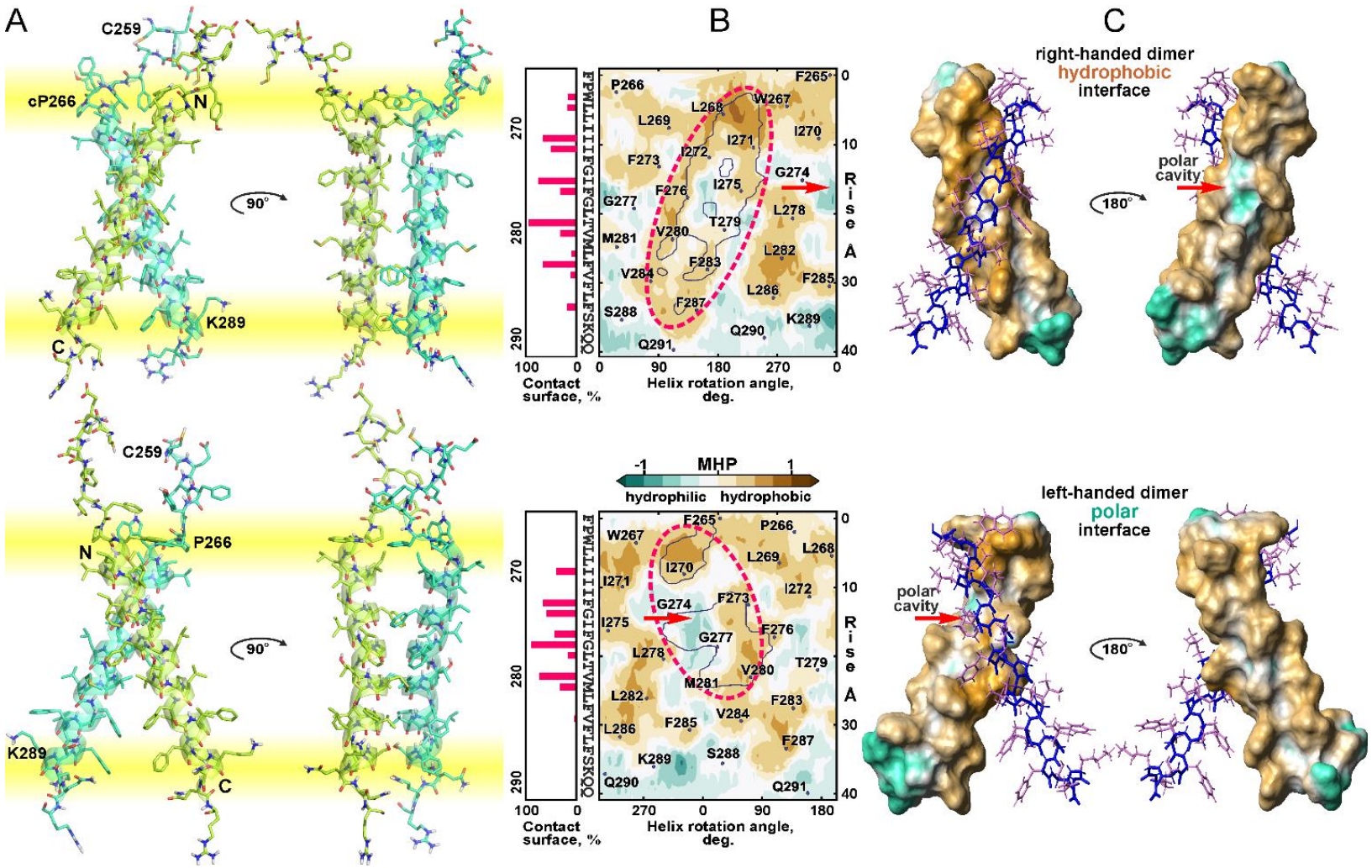


Polyansky A. et al. (2018) BBA – Gen. Subj.

Multiple states of TM domains in wt and mutant PDGFRA receptor: NMR and computational data.

CHALLENGE: One target – multiple conformations!

One target – multiple conformations



Alternative dimerization modes in TM domain of receptor hGHR: NMR and modeling results

hGHR - human growth hormone receptor

Bocharov E. et al. (2018) BBA – Gen Subj.

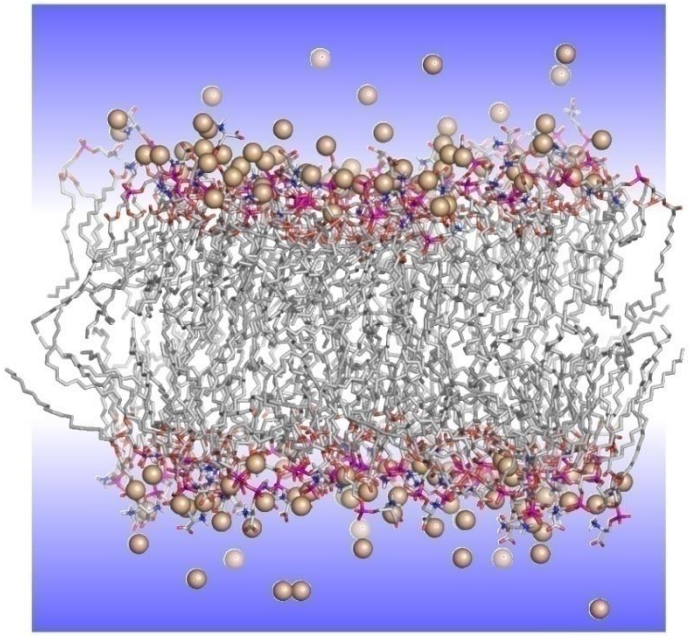
Point 3:

Crucial role of lipids in protein-membrane interactions:

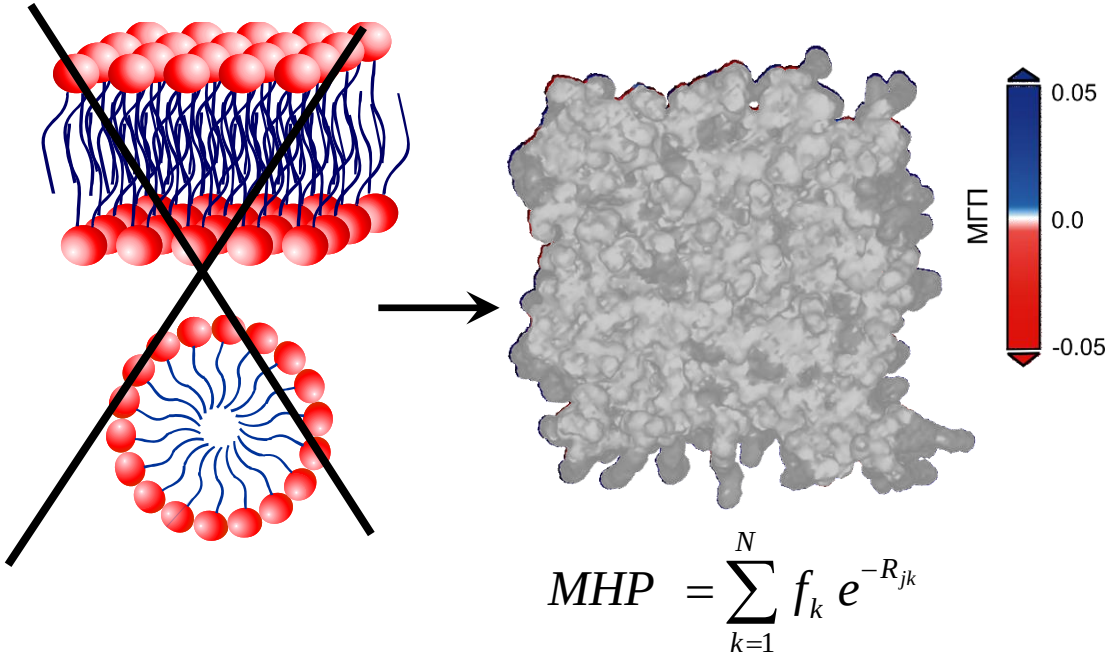
global / local effects + importance of single lipid molecules

PROBLEM-2:

Mosaic nature of the membranes



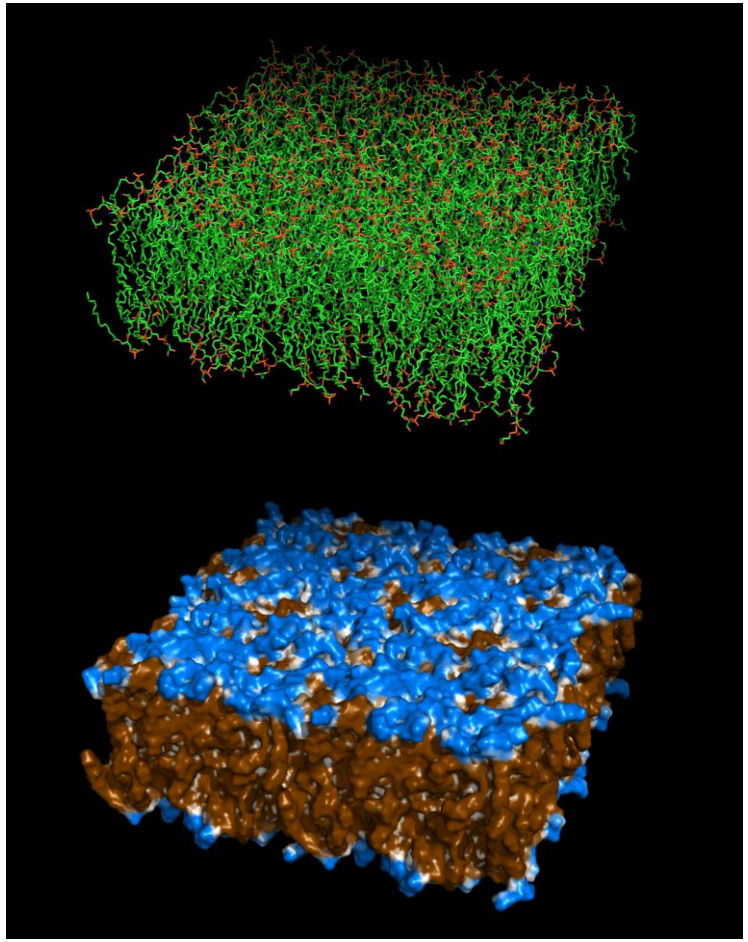
Hydrophobic organization of the surface

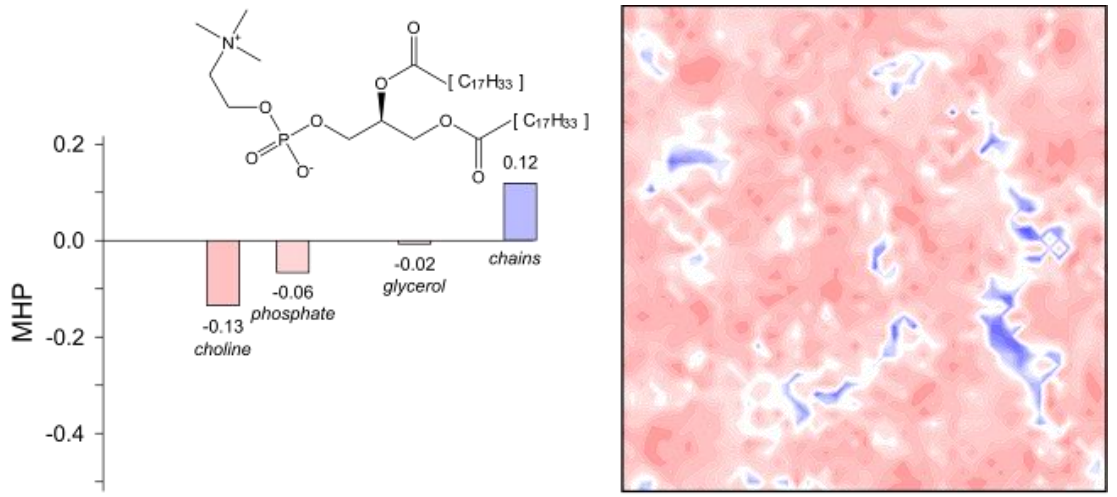


$$MHP = \sum_{k=1}^{N} f_k \, e^{-R_{jk}}$$

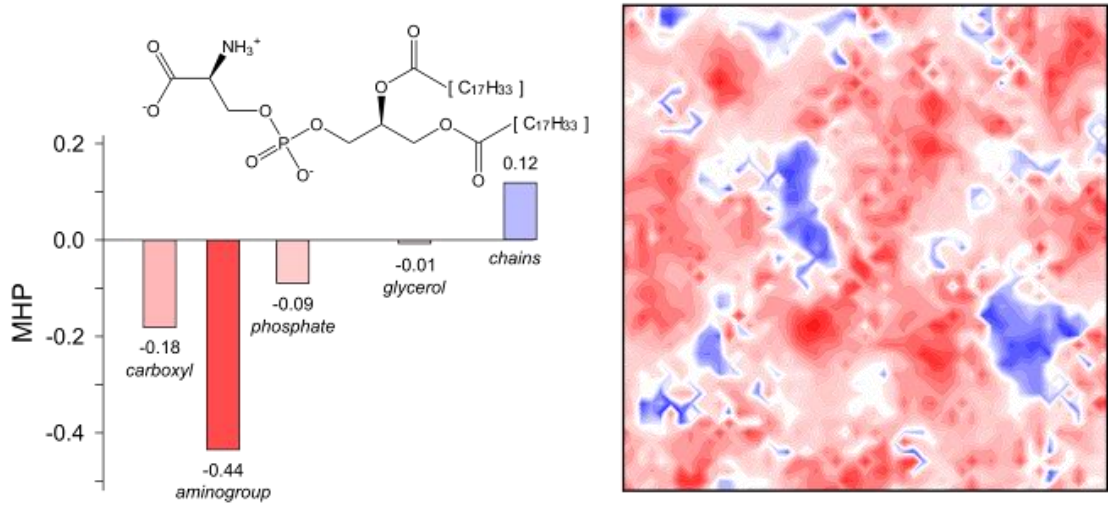
f_k- constant of atomic hydrophobicity, R_{jk}-distance between atom k and point j (Å), N – number of atoms

Heterogeneous hydrophobic / hydrophilic organization of surfaces in model lipid blayers





DOPC



DOPS

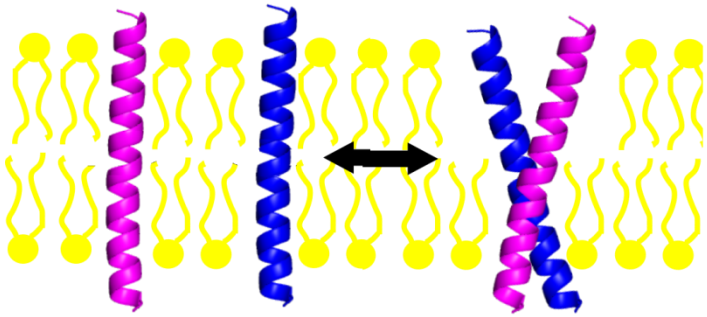
hydrophilic

hydrophobic

DOPC – dioleoyl-phosphatidylcholine (zwitterionic), DOPS – dioleoyl-phosphatidylserine (anionic).

Active role of membrane in oligomerization of MPs

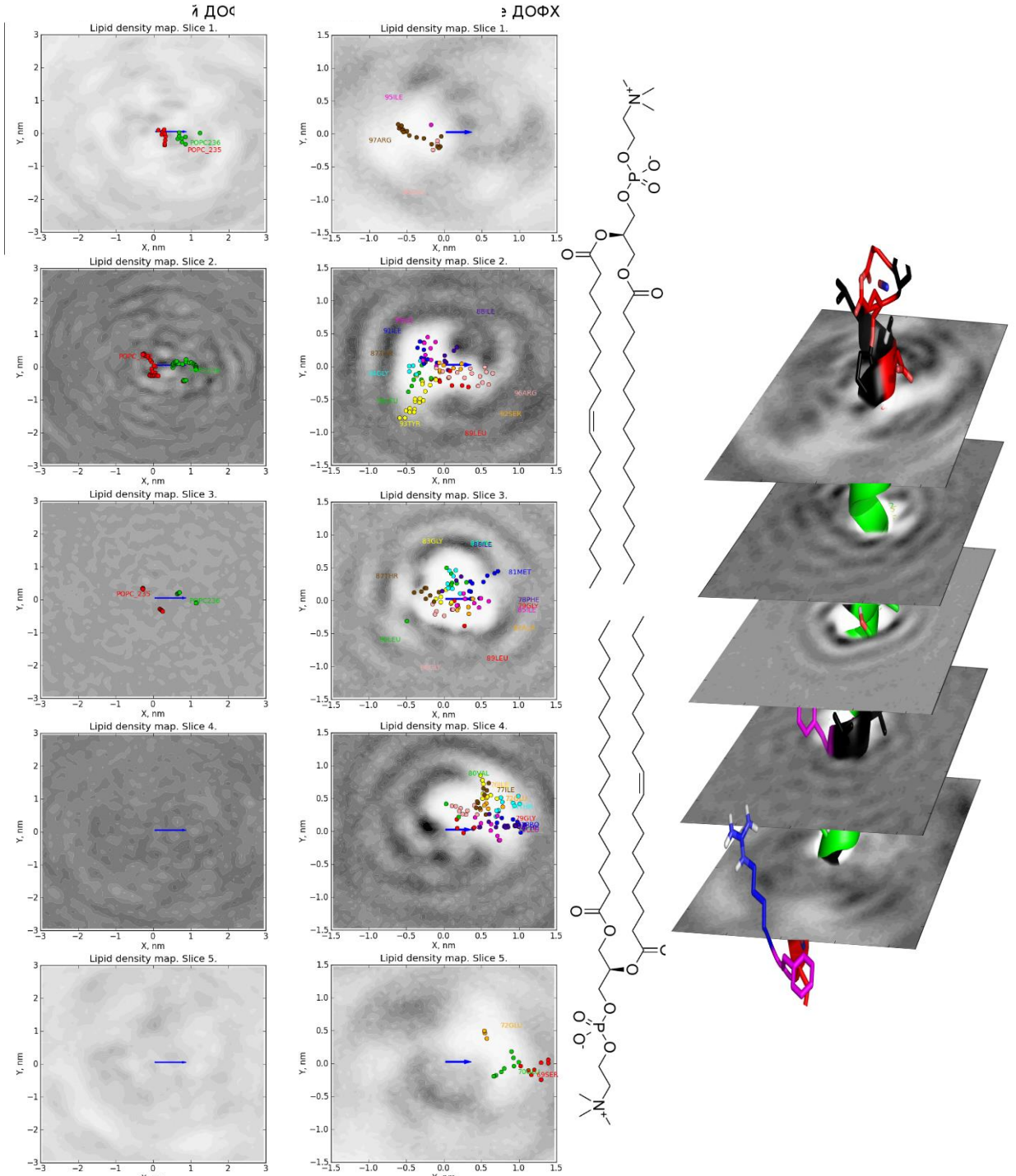
Membrane as a communicative medium (“Aether”), which promotes protein-protein interactions



What is a driving force of spontaneous helix-helix association?

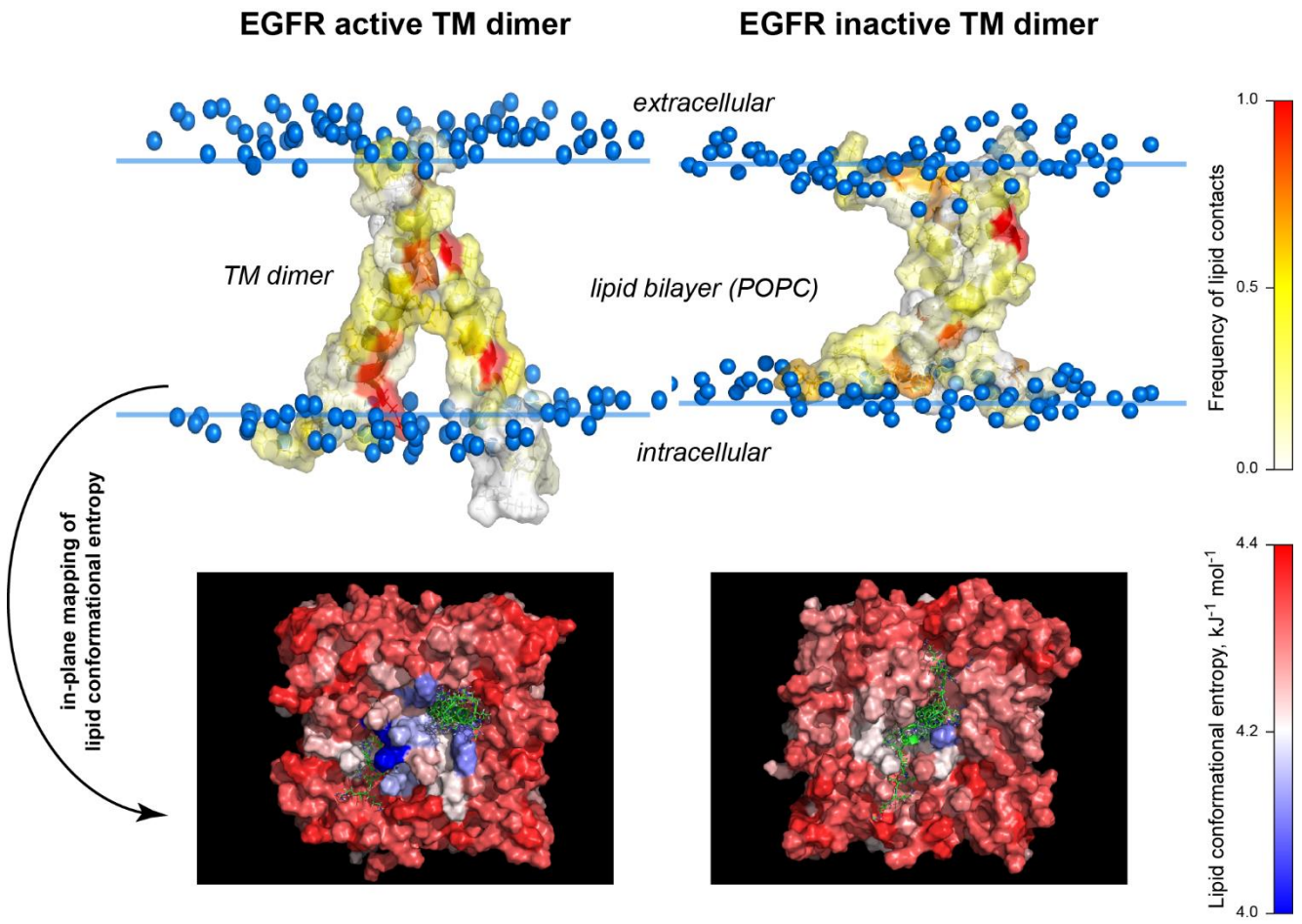
Possible answers:

- **Protein sequence;** +
- **Membrane;** +
- **Water;** +



Kuznetsov A.S. *et al.* (2015) ***J. Chem. Theory & Comput.*** 11: 4415

TM domains of some receptors in active and ground state interact with surrounding lipids in a different way



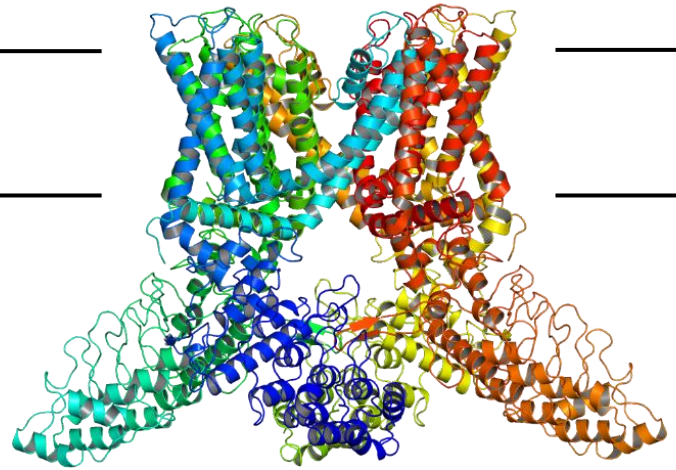
Future work: role of lipids in receptor activation/inactivation…

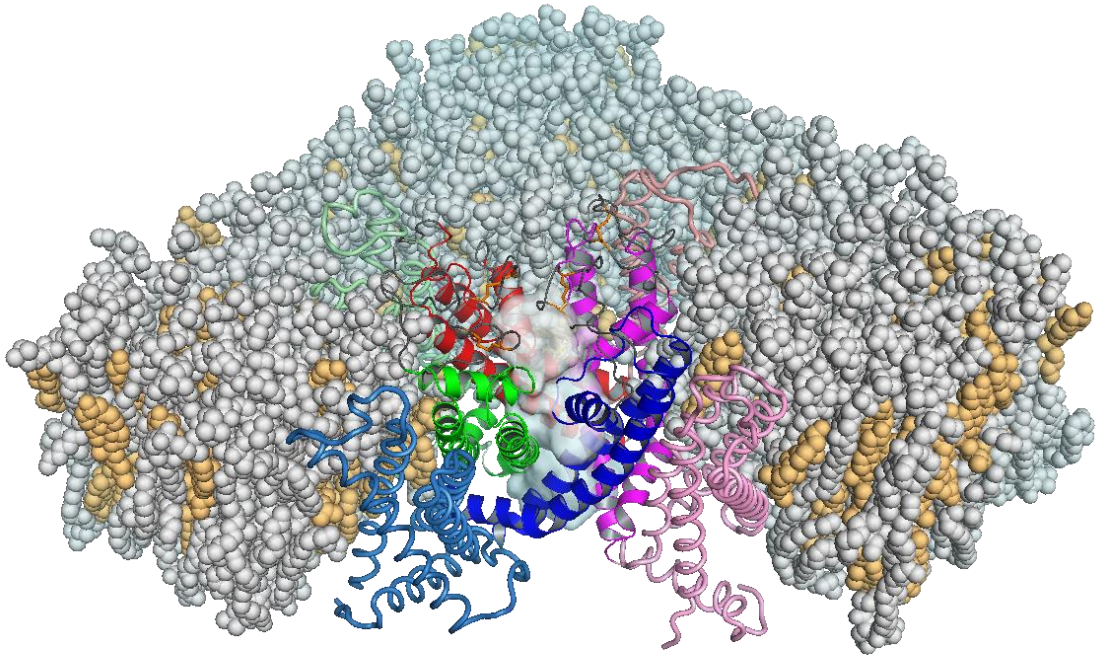
Bocharov E. *et al.* (2017) ***Biochemistry*** 56:1697

Emerging role of single lipids in protein structure and functioning:

a case of TRPV ion channels

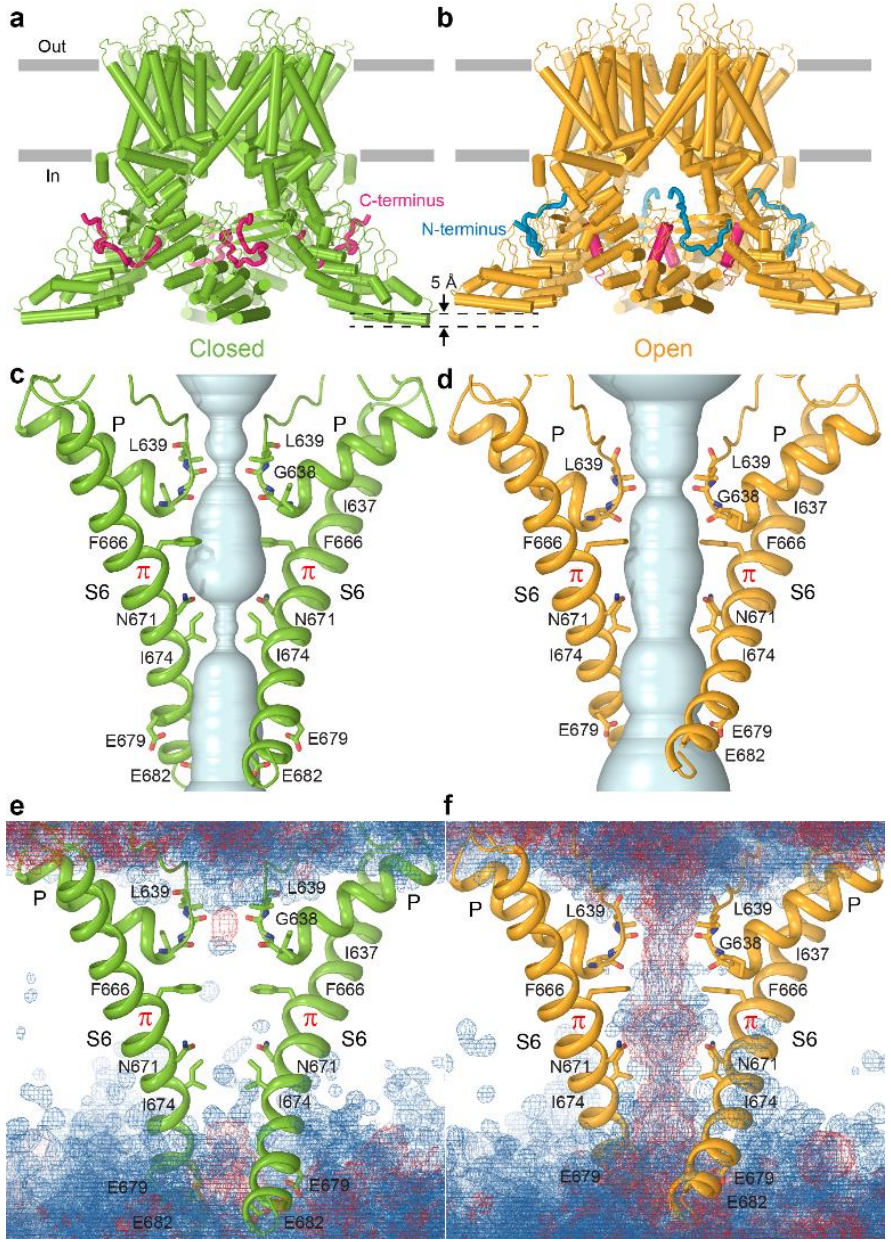
membrane





TRPV3 - temperature-sensitive TRP channel: opening in response to heat

Closed state



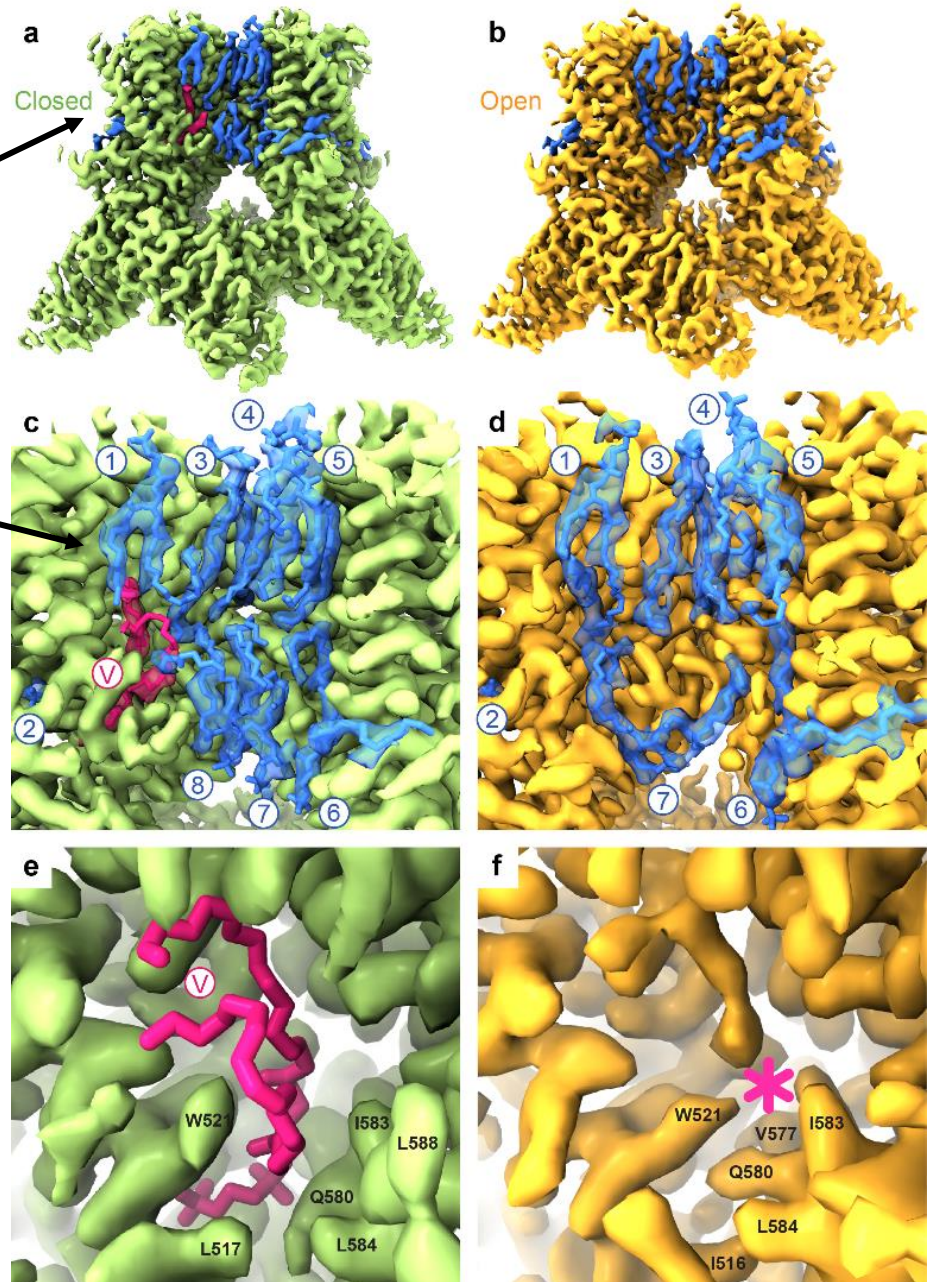
Heat-open state

Nadezhdin K. et al. (2021)
Nat. Struct. Mol. Biol. 28:564.

Bound lipids in Cryo-EM structures of TRPV3: what's about their role?

Closed and heat-open TRPV3 channel in a nanodisk

Tightly bound lipids in the specific sites on the protein surface

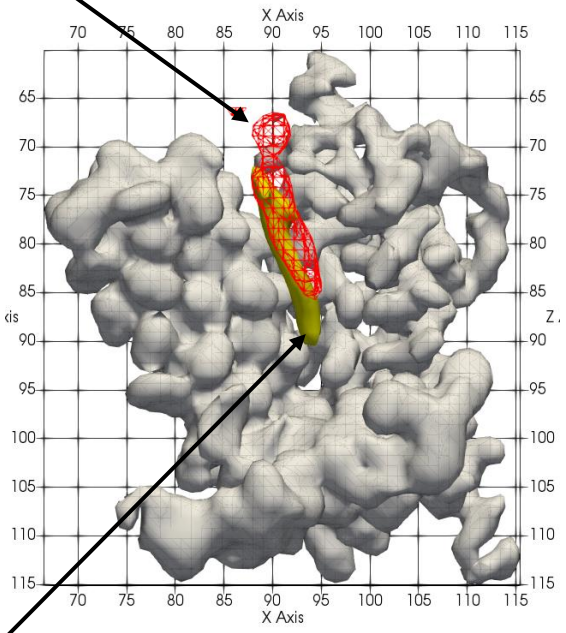


Nadezhdin K. et al. (2021) ***Nat. Struct. Mol. Biol.*** 28:564.

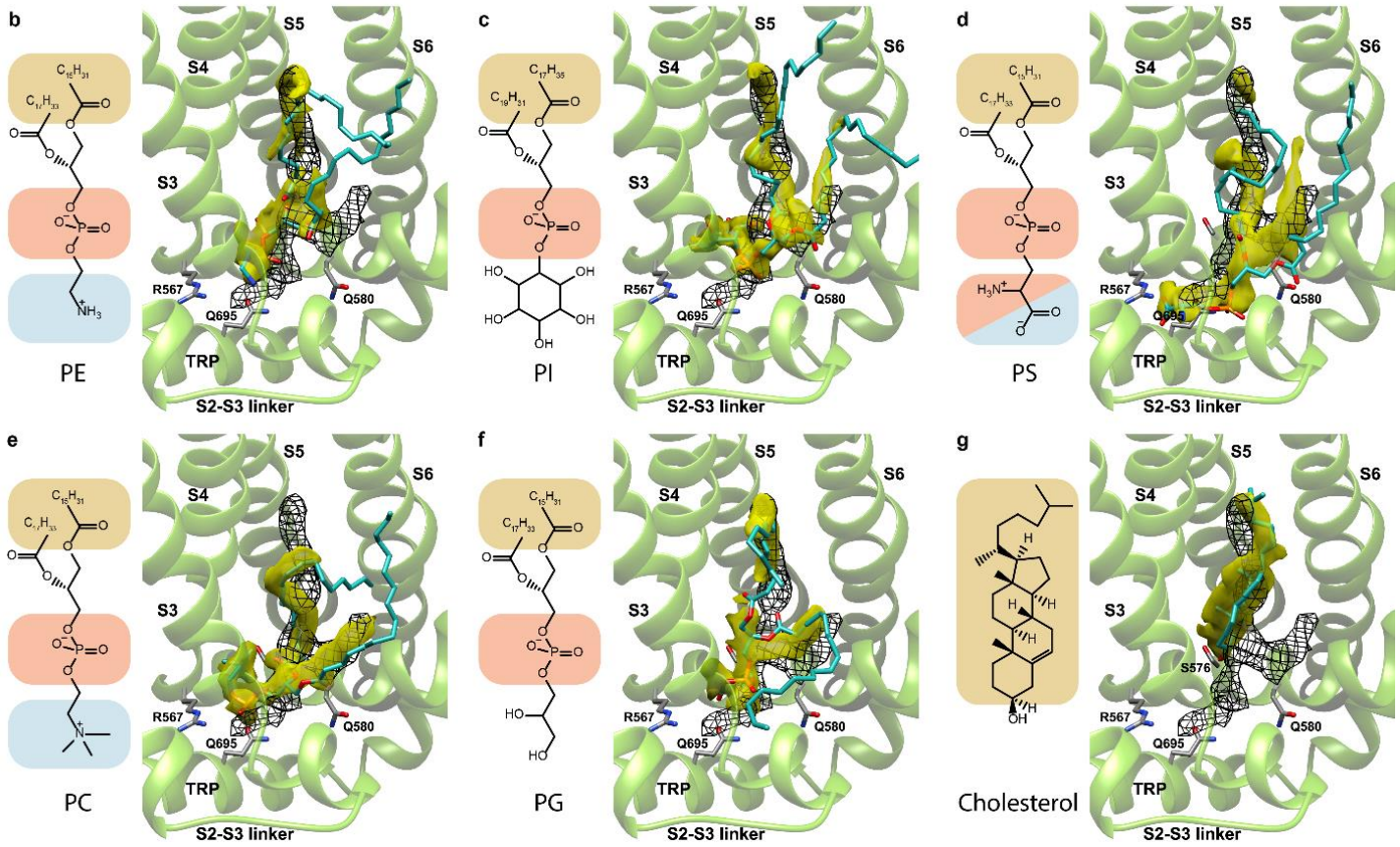
Molecular modeling permits correct assignment of lipid densities observed by Cryo-EM

MD-averaged density distributions of different types of bound lipids in TRPV3

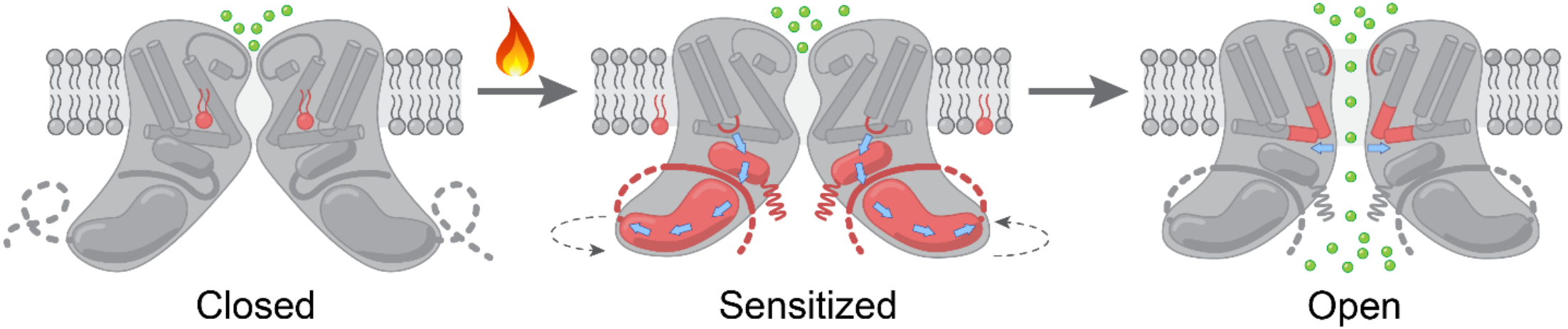
Cryo-EM



MD



Mechanism of TRPV3 temperature activation:

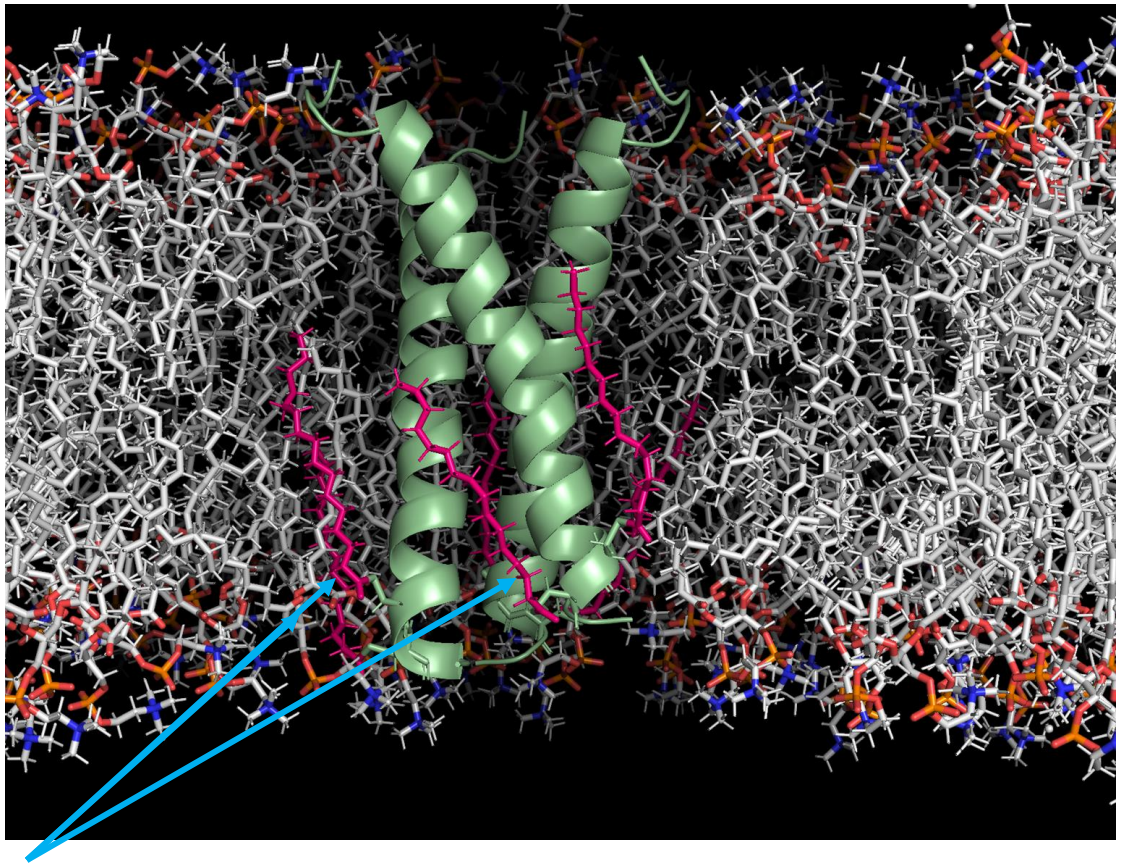


Nadezhdin K. et al. (2021) ***Nat. Struct. Mol. Biol.*** 28:564.

SARS-CoV-2 SPIKE TRANSMEMBRANE DOMAIN (TMD): *In silico* MODEL OF A HOMO-TRIMER

Important role of palmitoylated Cys residues in structural/dynamic behavior of TMD

POPC bilayer



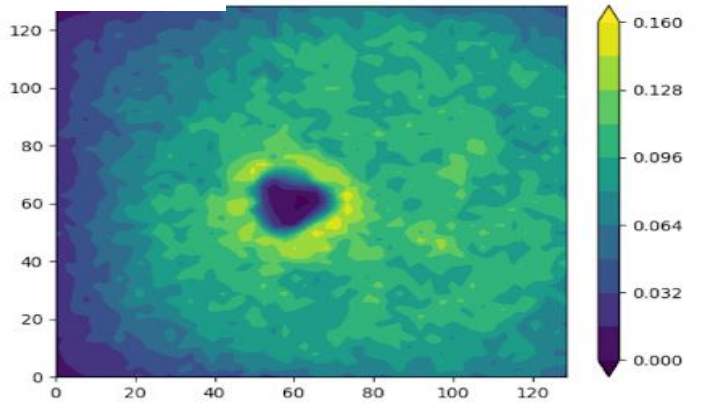
palmitoylated Cys residues

INITIAL GUESS:

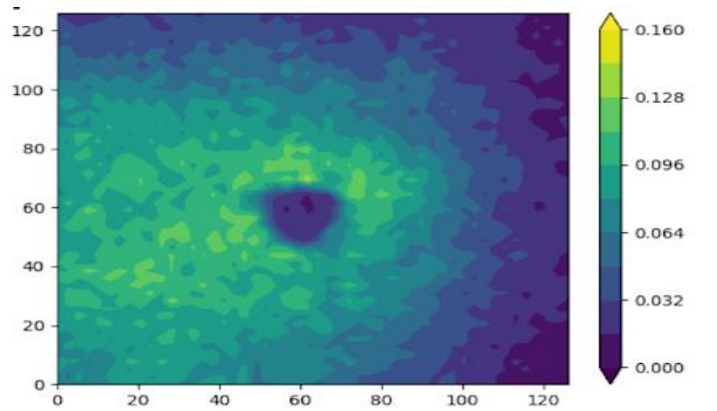
Palmitoylation promotes stability of TMD in the spike protein

Order parameters of acyl chains of lipids

No palmitoylation

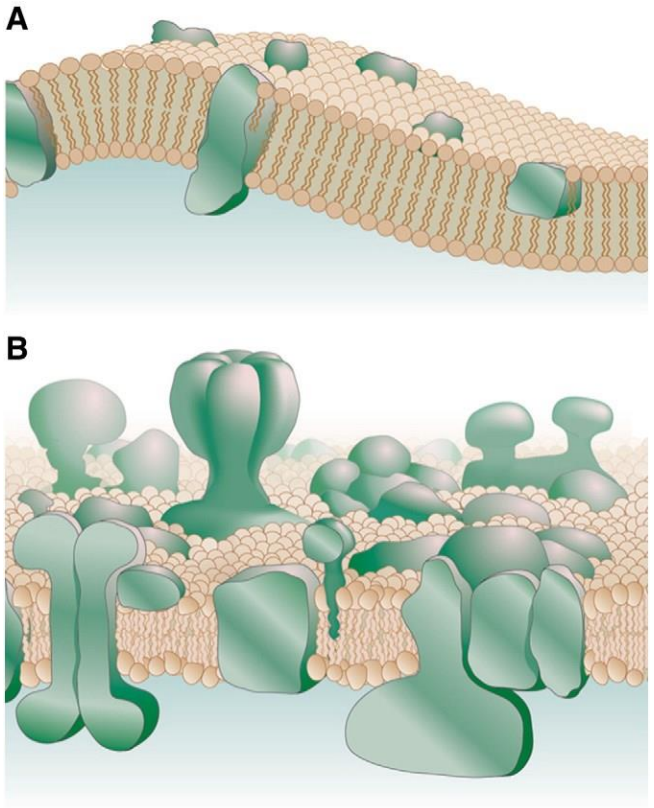


Palmitoylation of 2 Cys



Specific features of membrane proteins as pharmacological targets:

S.J. Singer & G.L. Nicolson, 1972



D.M. Engelman, 2005

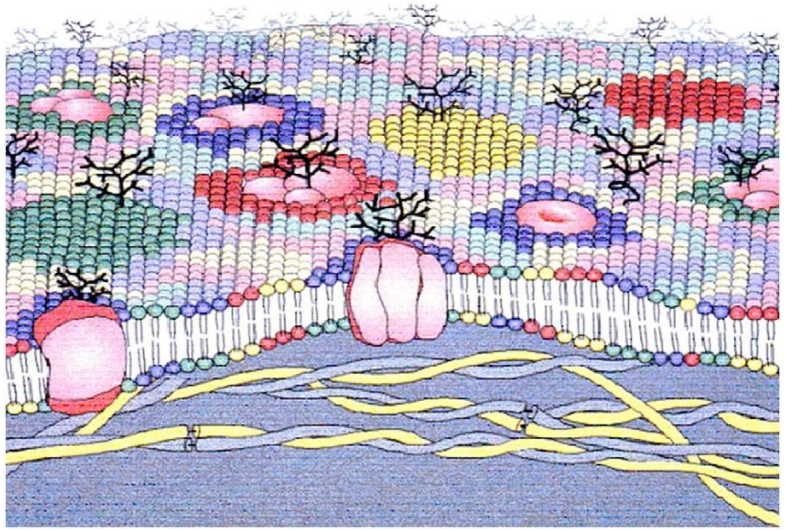
Current challenges in CADD aplications:

Dynamic oligomerization;

Functioning in “orchestra” (especially for signaling receptors);

Specific edging with lipids/water/ions;

Crowding effects.



P.V. Escribá et al., 2008

CONCLUSIONS:

Membrane proteins and surrounding lipids are “born to be together”:

- Membrane lipids regulate the behavior of membrane proteins in a wide range;
- Effects of lipids on the structure/functioning of membrane proteins have both, global and local character;
- Especially strong effects are often caused by annular lipids;
- Even single lipids can induce global rearrangements in membrane proteins

  (example: TRPV channels)
- Atomistic modeling perfectly complements the most powerful experimental techniques;
- Rational design of lipid-modulators opens new perspectives in control of cell functioning