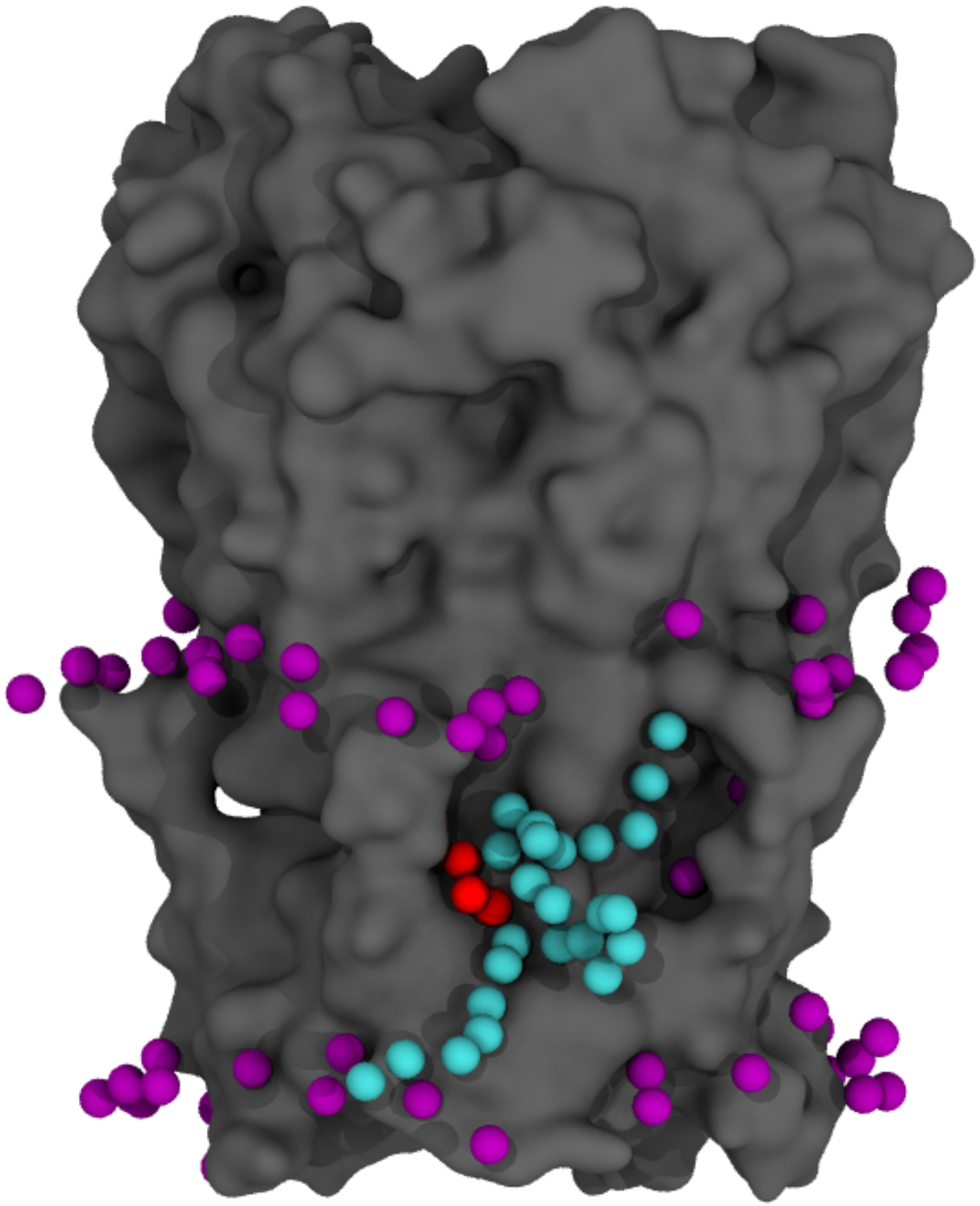
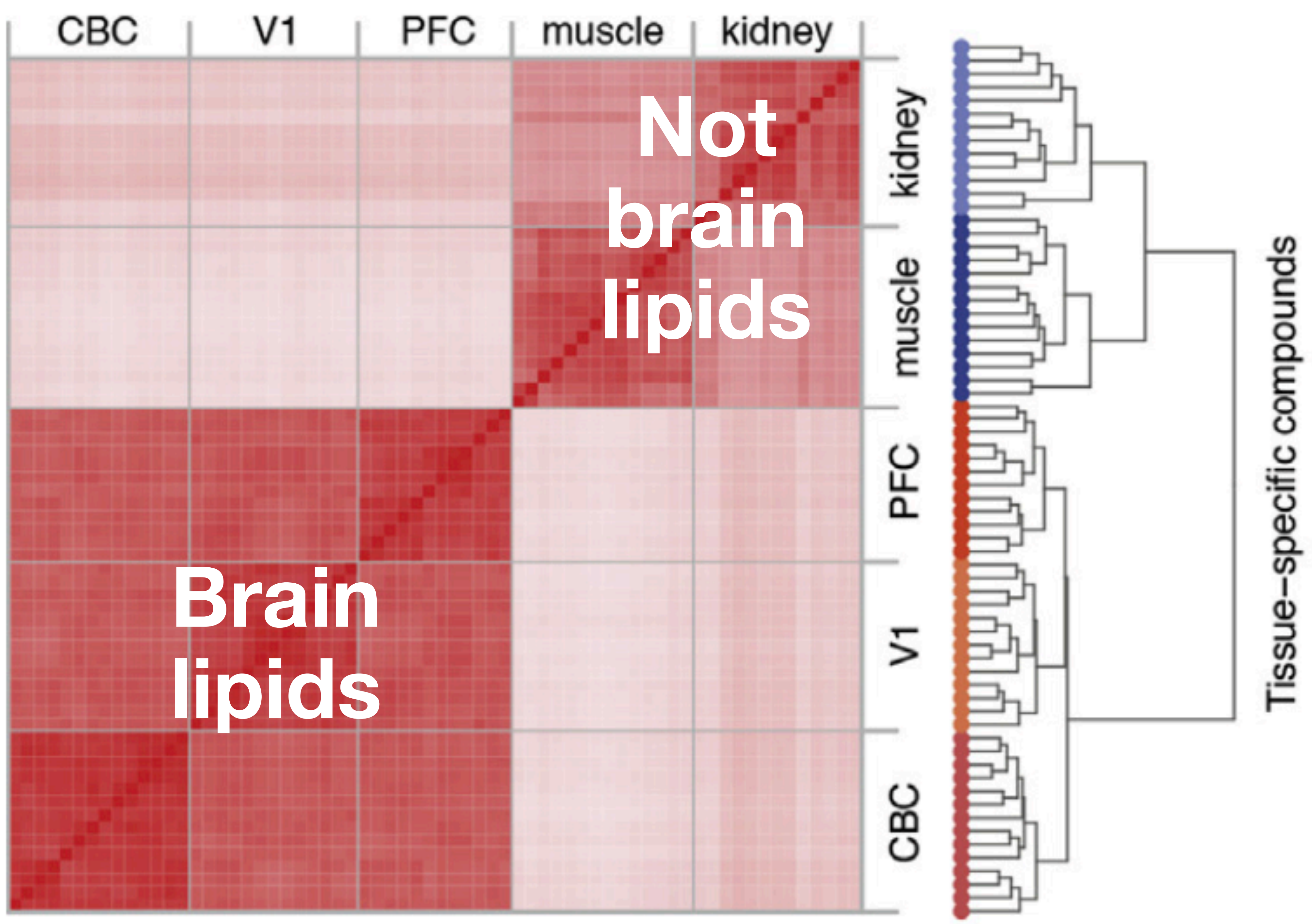
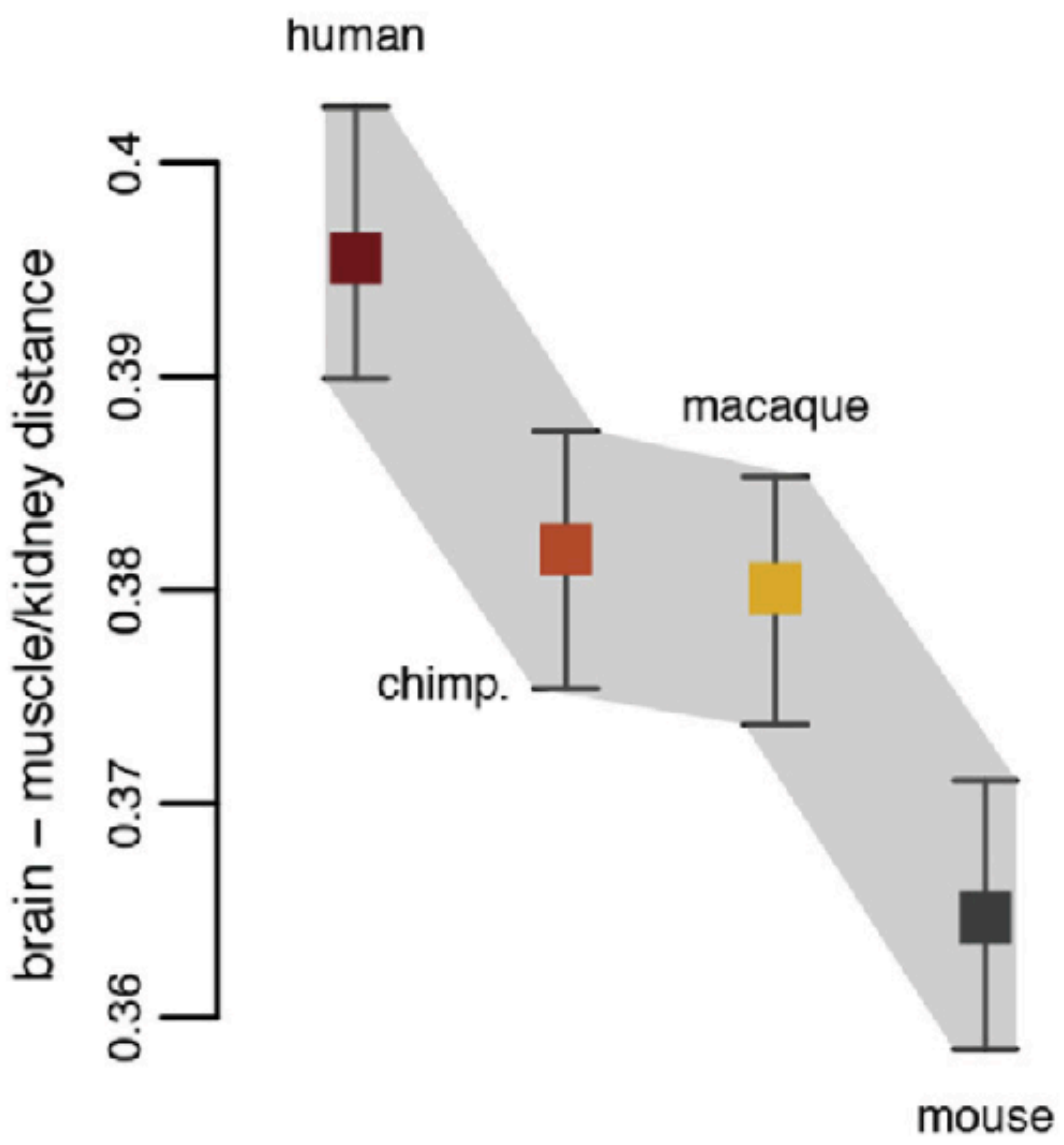
Cardiolipin stabilizes the resting conformation of ELIC5



Jesse Sandberg, Brandon Tan, Hanrui Xu, Wayland Cheng, & Grace Brannigan

Specific lipids allow our brains to function





Lipids are 1/2 brain's dry weight

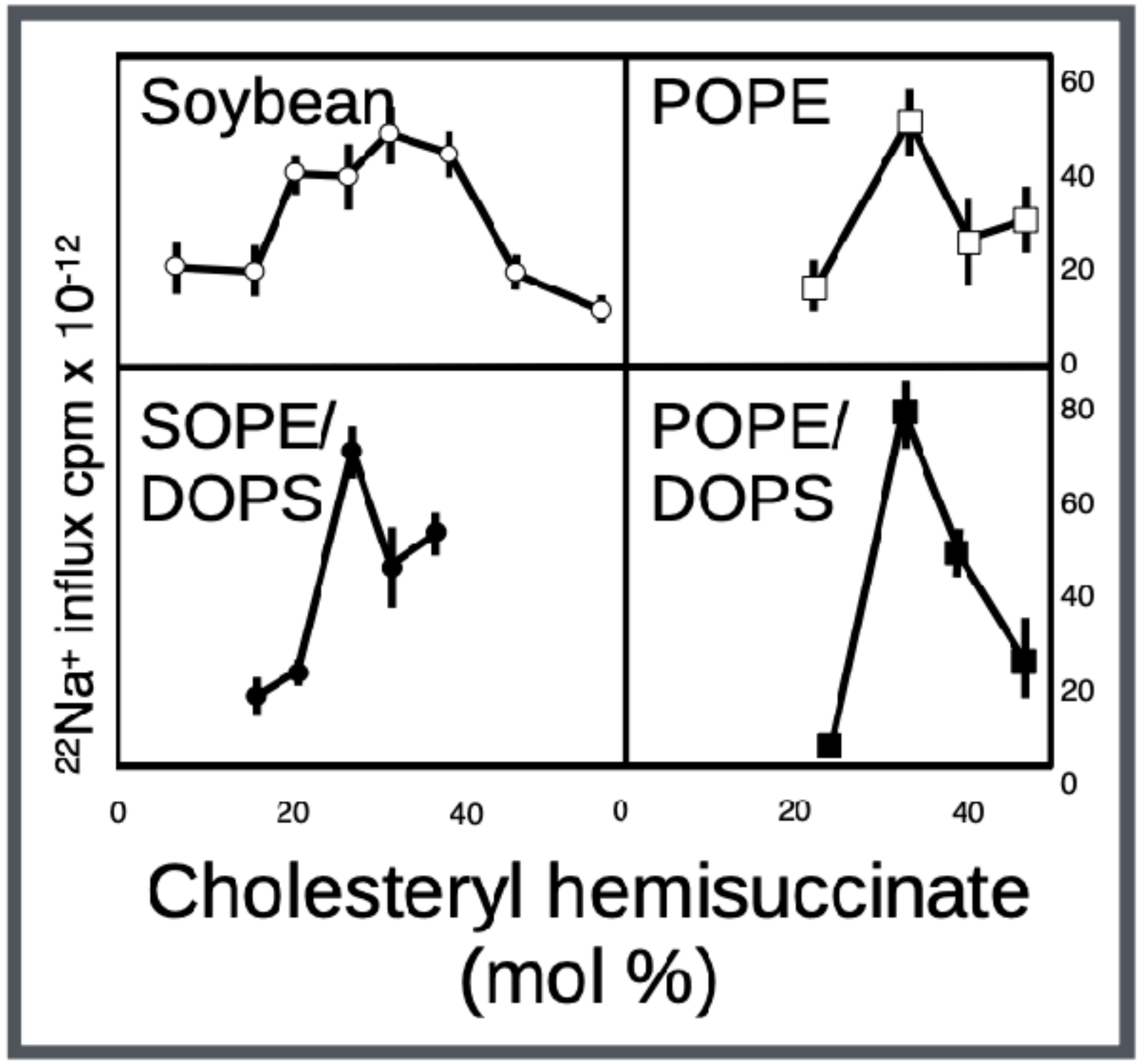
Brain requires different lipids than rest of body

Difference grows with evolutionary distance

Piomelli, et al., Nat. Rev. Neurosci., 2007

Bozek, et al., Neuron, 2015

Neurotransmitter receptors need specific lipids to function



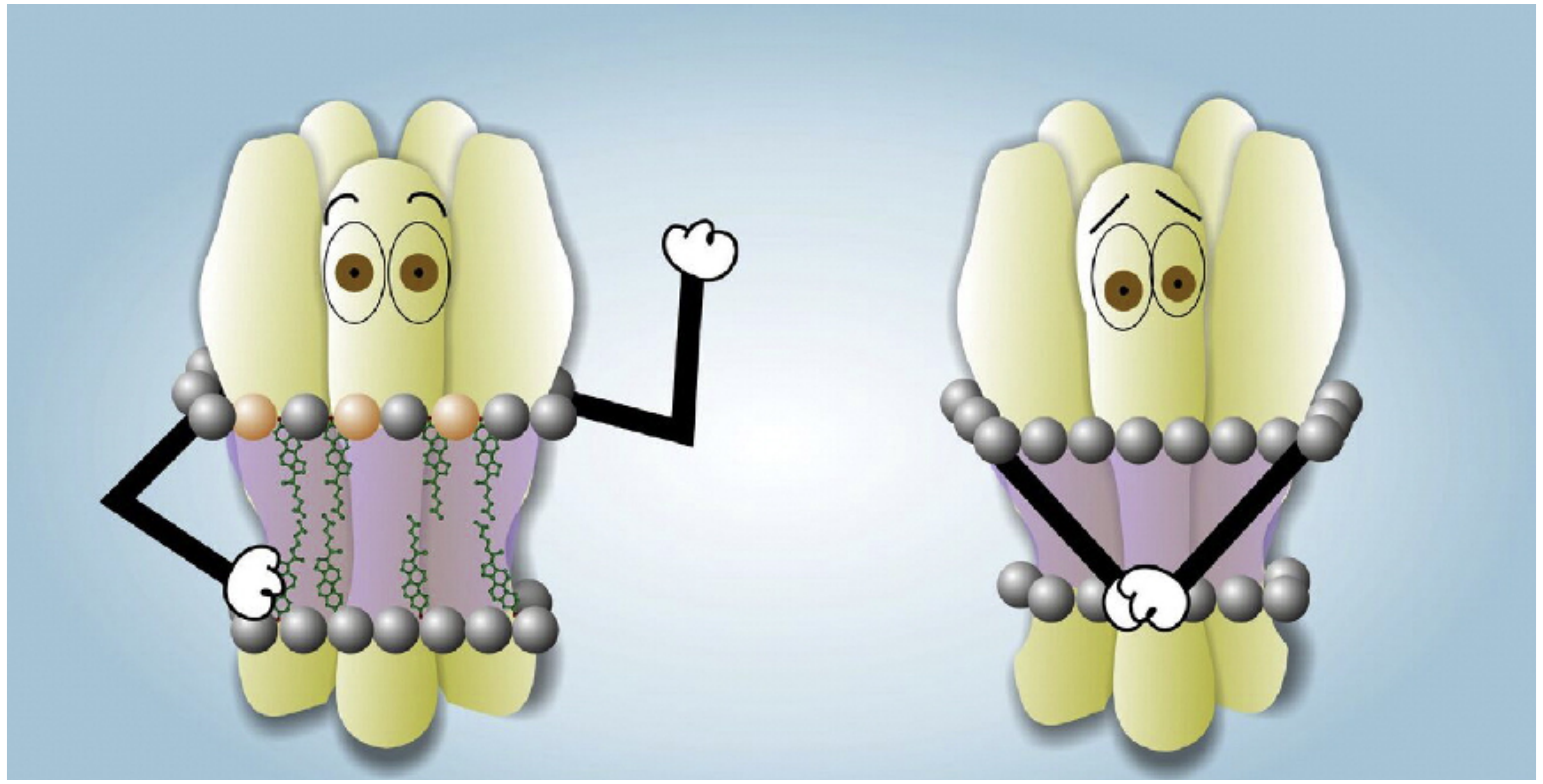
Ion channel has complex relationship between cholesterol, lipid composition

ELSEVIER
Biochimica et Biophysica Acta (BBA) - Biomembranes
Volume 1858, Issue 11, November 2016, Pages 2662-2670

Review

The lipid habitats of neurotransmitter receptors in brain

María Virginia Borroni [a], Ana Sofía Vallés [b], Francisco J. Barrantes [c]

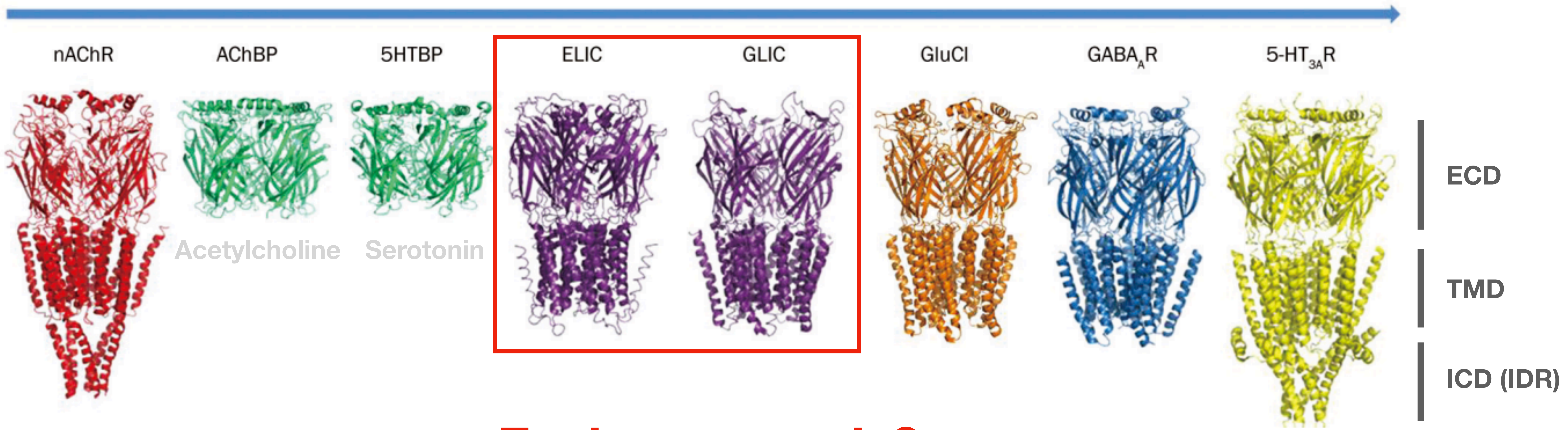


Criado, et al., J. Biol. Chem., 1984

Borroni, et al., BBA: Biomembranes, 2016

Many neurotransmitter receptors belong to pLGIC family

pLGIC entries to PDB
2005–2014

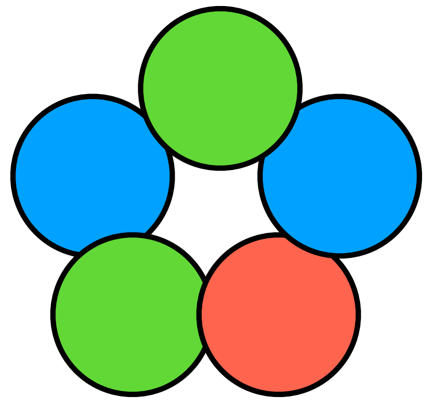


Acetylcholine

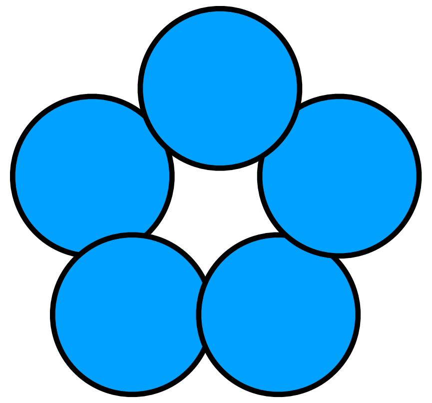
Easiest to study?

GABA

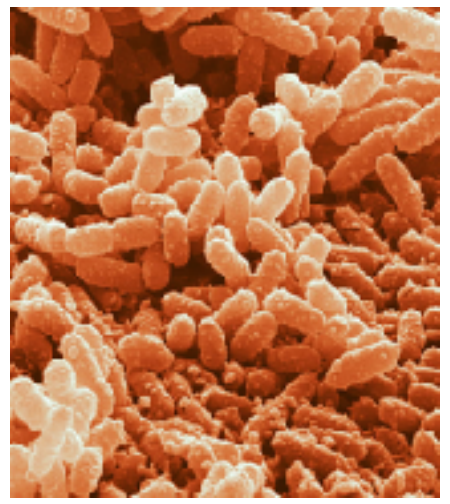
Serotonin



Heteromer



Homomer



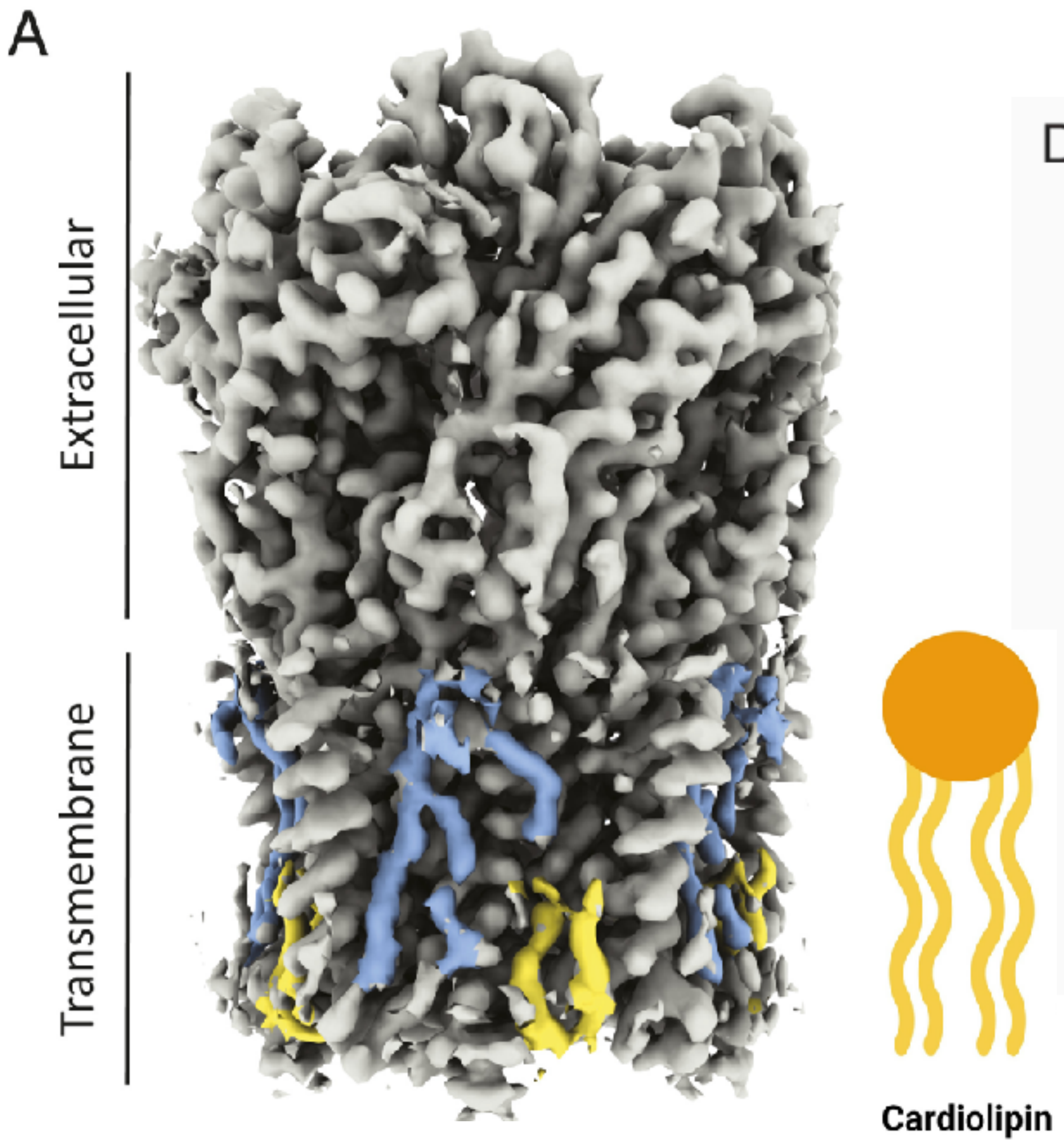
Prokaryotes

Eukaryotes

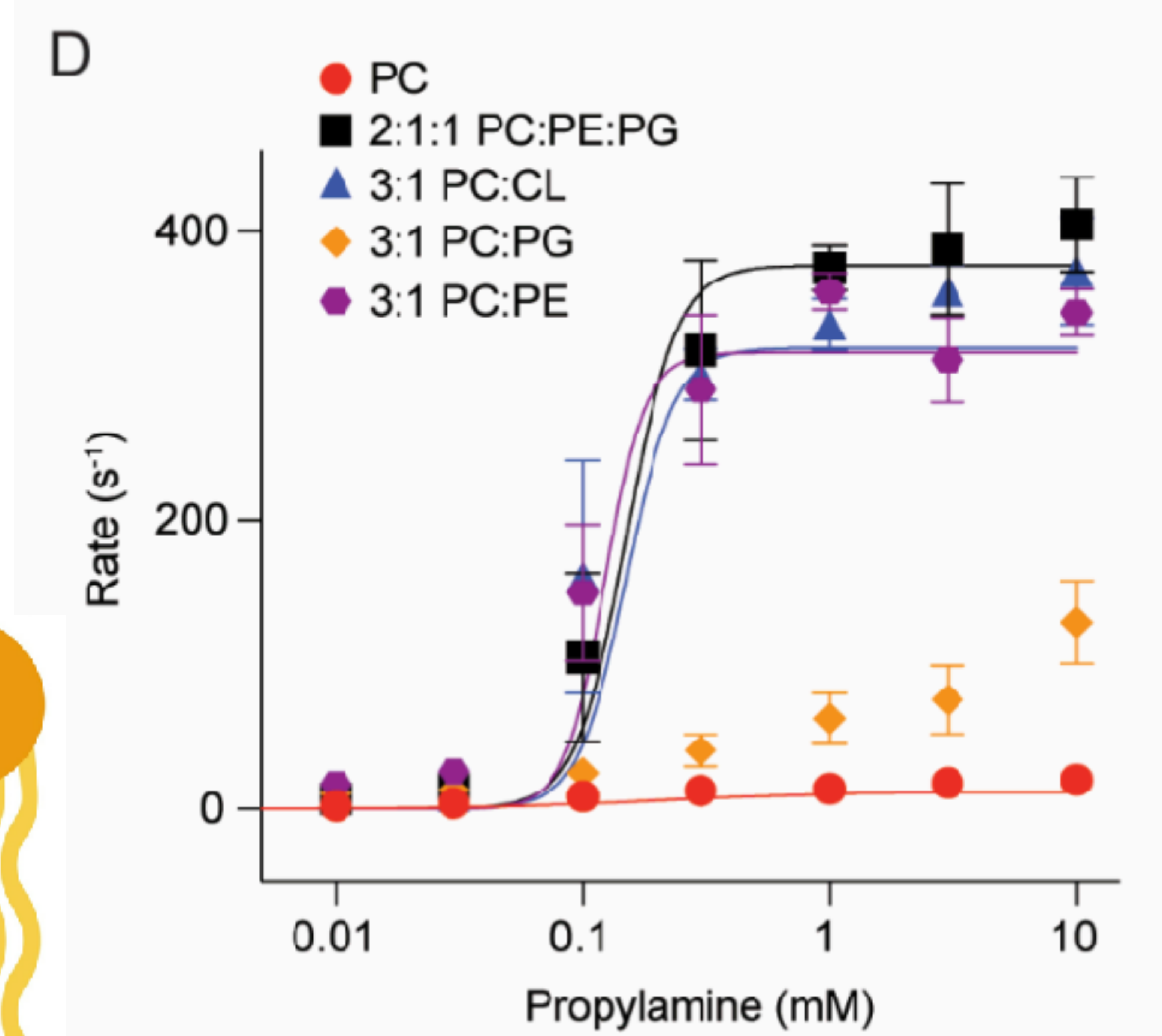
Wu, et al., Acta Pharm. Sin., 2015

ELIC is sensitive to lipids too

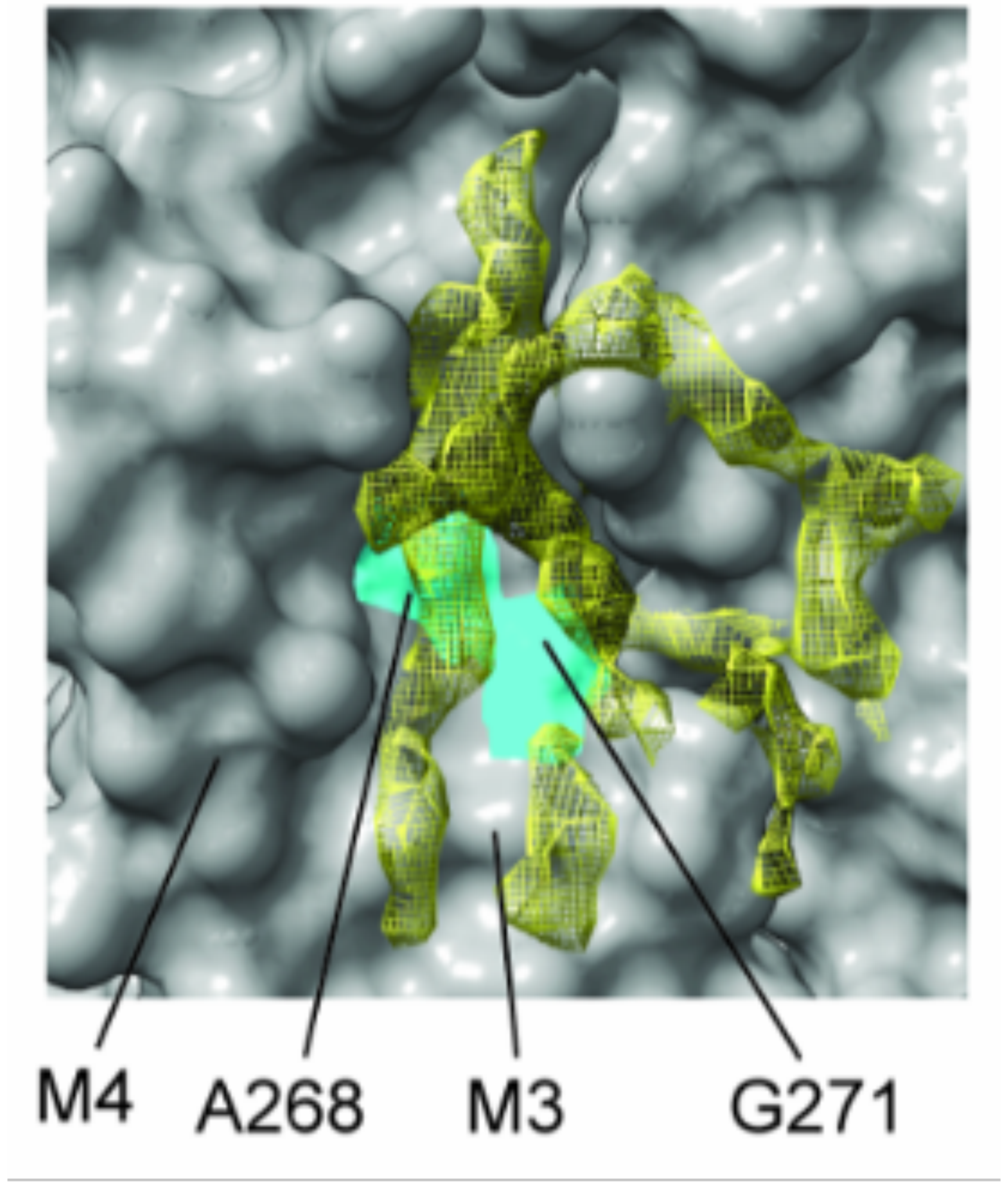
ELIC WT



ELIC5



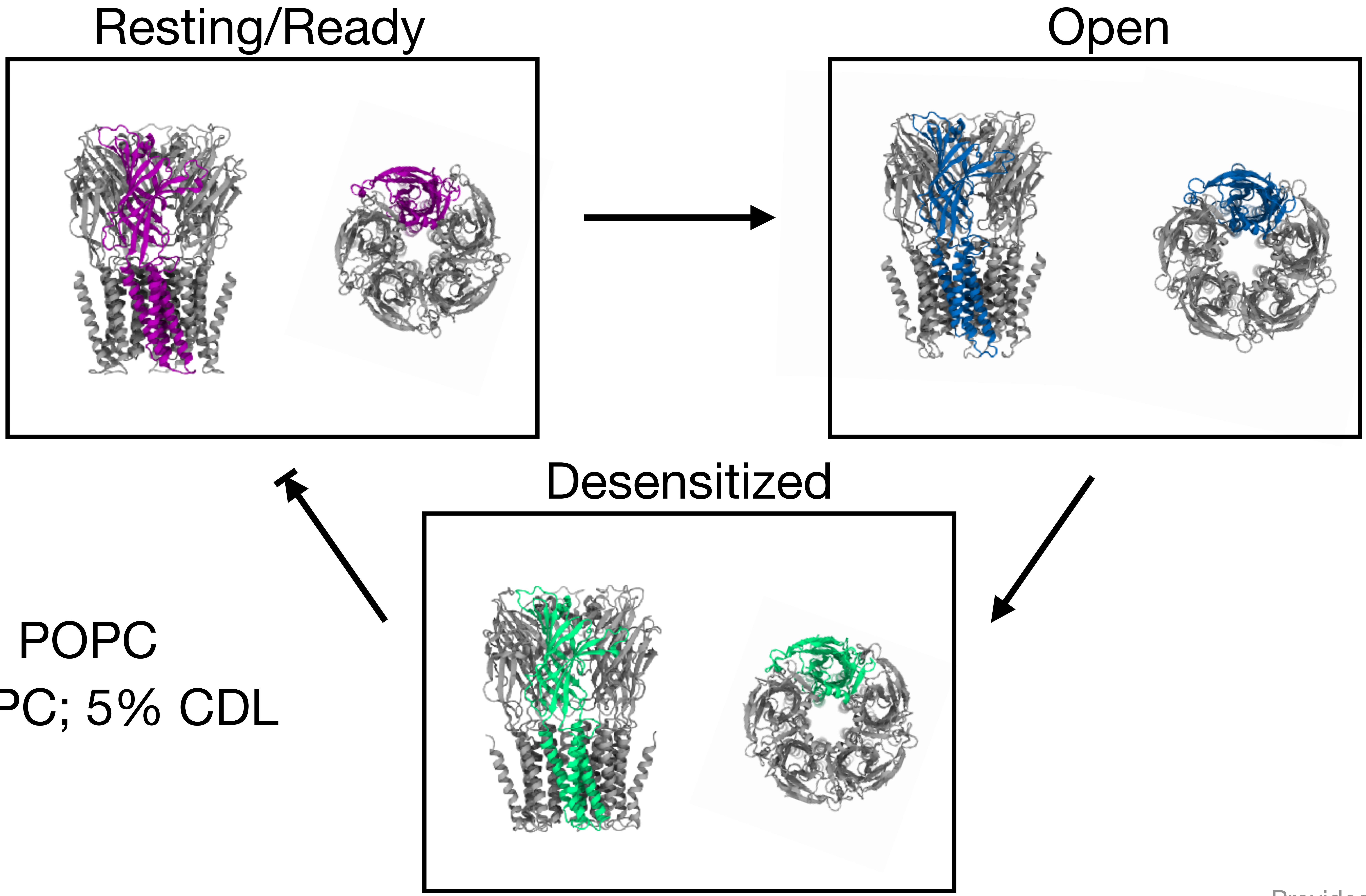
ELIC5



Kumar, Cymes, and Grosman, PNAS 2021

Provided by lab of Dr Wayland Cheng

Cardiolipin rescues function of ELIC5 in POPC



100% POPC
95% POPC; 5% CDL

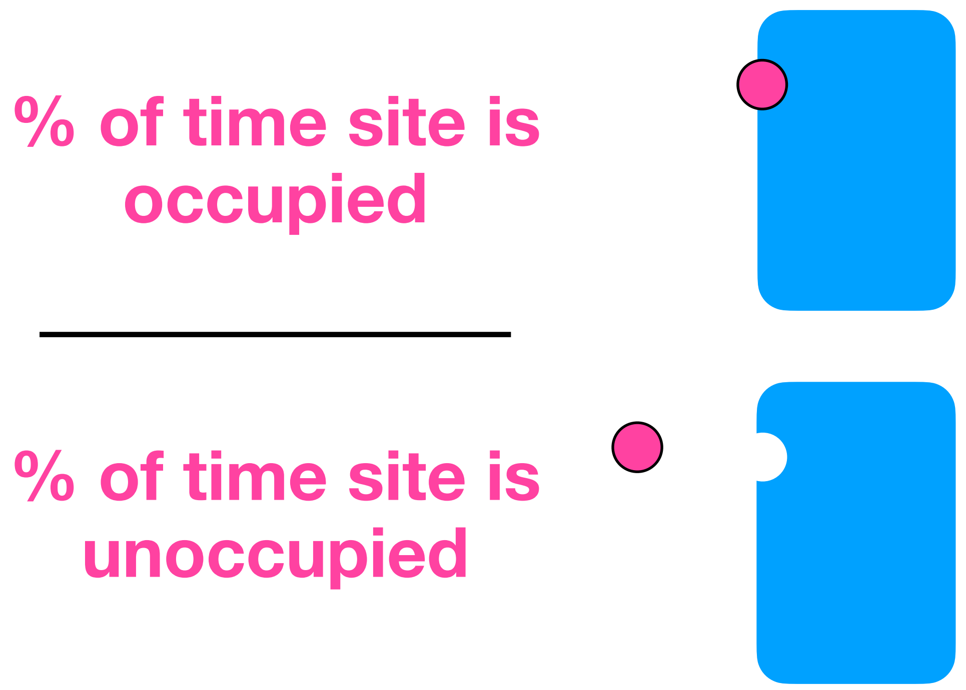
Provided by lab of Dr Wayland Cheng

Approach

- Simulate Resting and Desensitized ELIC5 structures in 95% POPC, 5% Cardiolipin (CL)
- Measure binding affinity of CL to M3/M4 outlet leaflet cleft in both simulations
- If CL binds preferentially, strong evidence of allosteric modulation

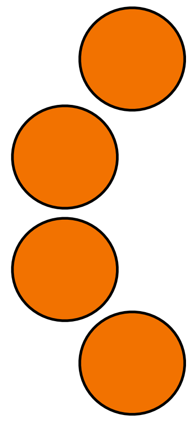
Conceptual hurdles to defining binding in hydrophobic contexts

$$\Delta G_{\text{bind}} = RT \ln K_d = -RT \ln \frac{P_{\text{occ}}}{P_{\text{unocc}}}$$

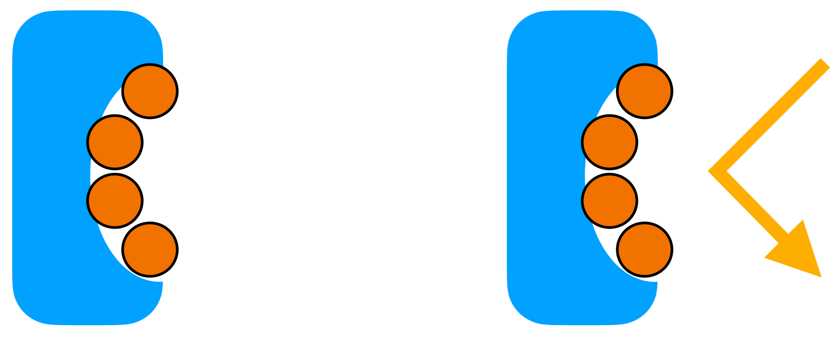


Superficial binding site

Flexible, chain-like ligand that is also the solvent



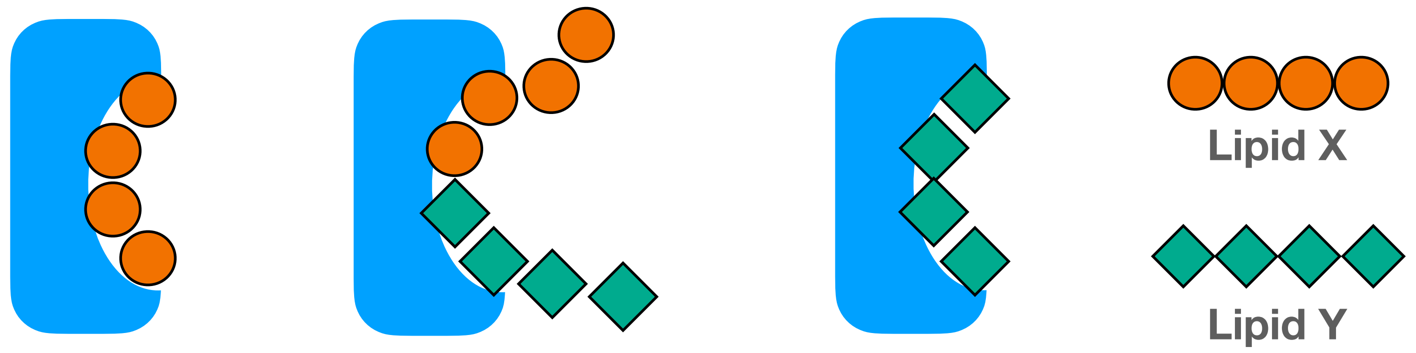
Problem 1: Ligand and/or Solvent?



Specifically Bound?

Diffusing solvent?

Problem 2: Partial Occupancy

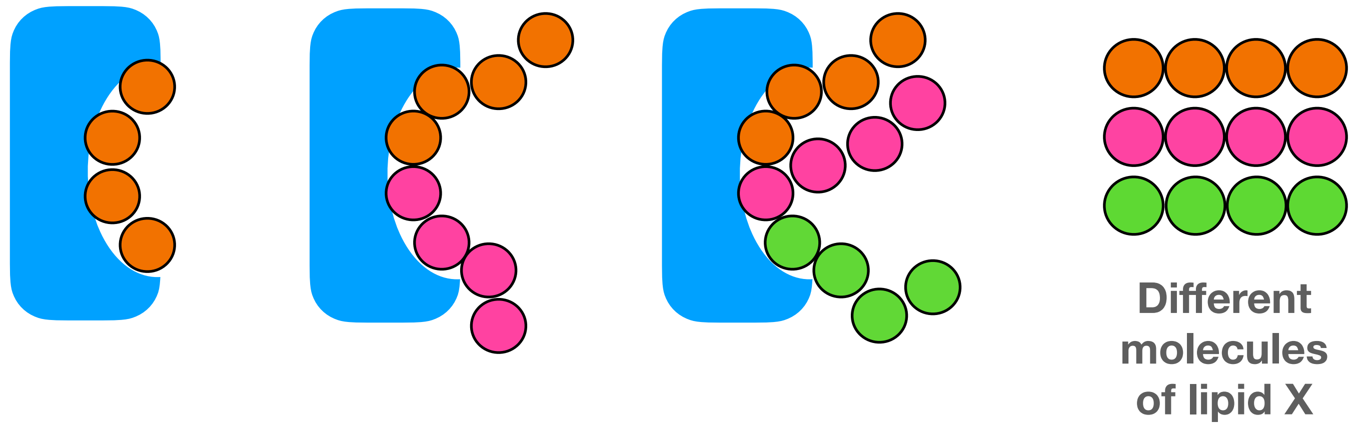


Occupied

Partially occupied?

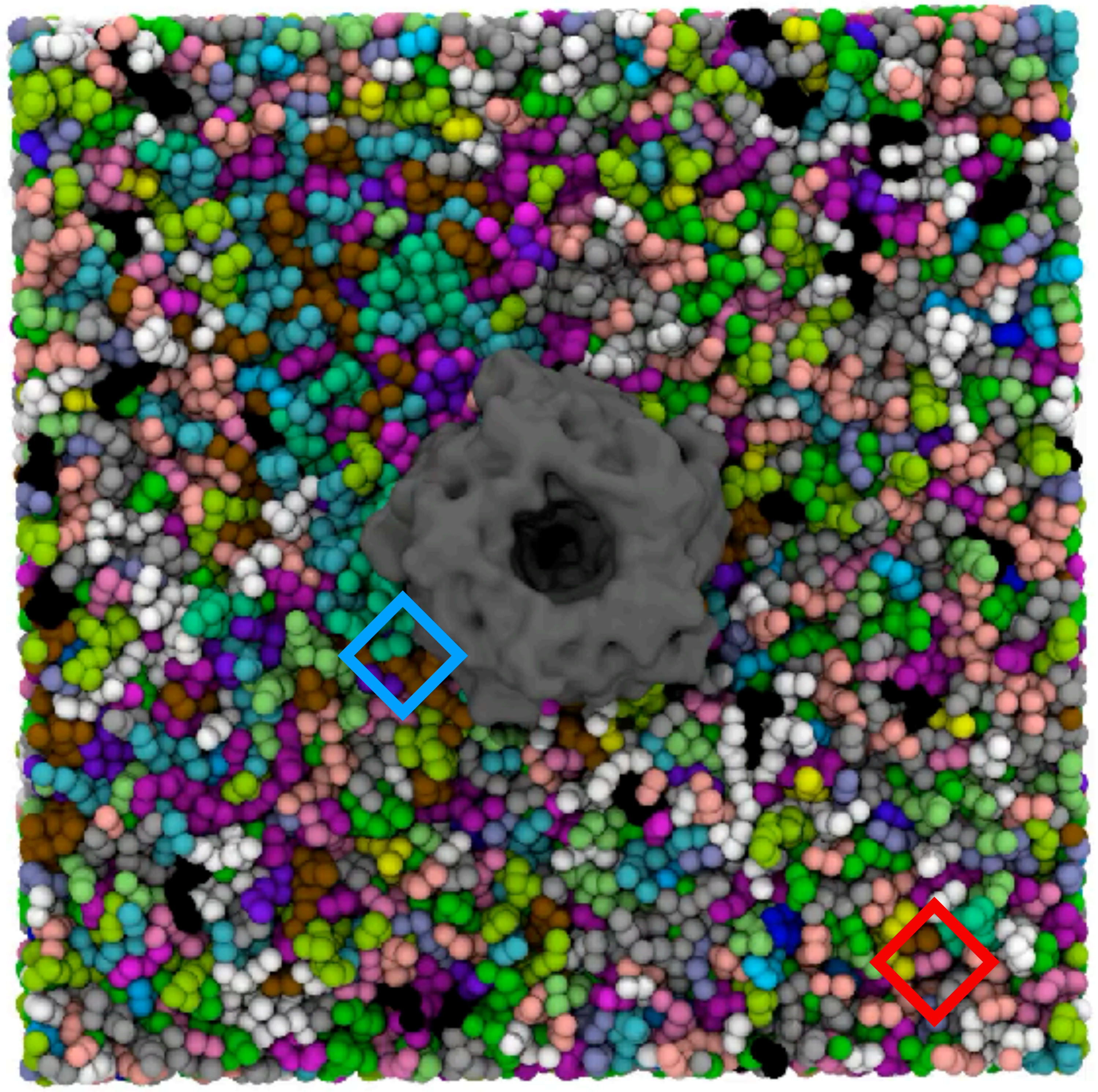
Unoccupied

Problem 3: Chemically Indistinct Ligands



Equally valid bound configurations

Density-Threshold Affinity circumvents all 3 problems



Count CL headgroup beads in designated site

Count CL headgroup beads in patch of “bulk” membrane

How much *more* likely am I to find
CL headgroup beads in site
rather than in bulk?

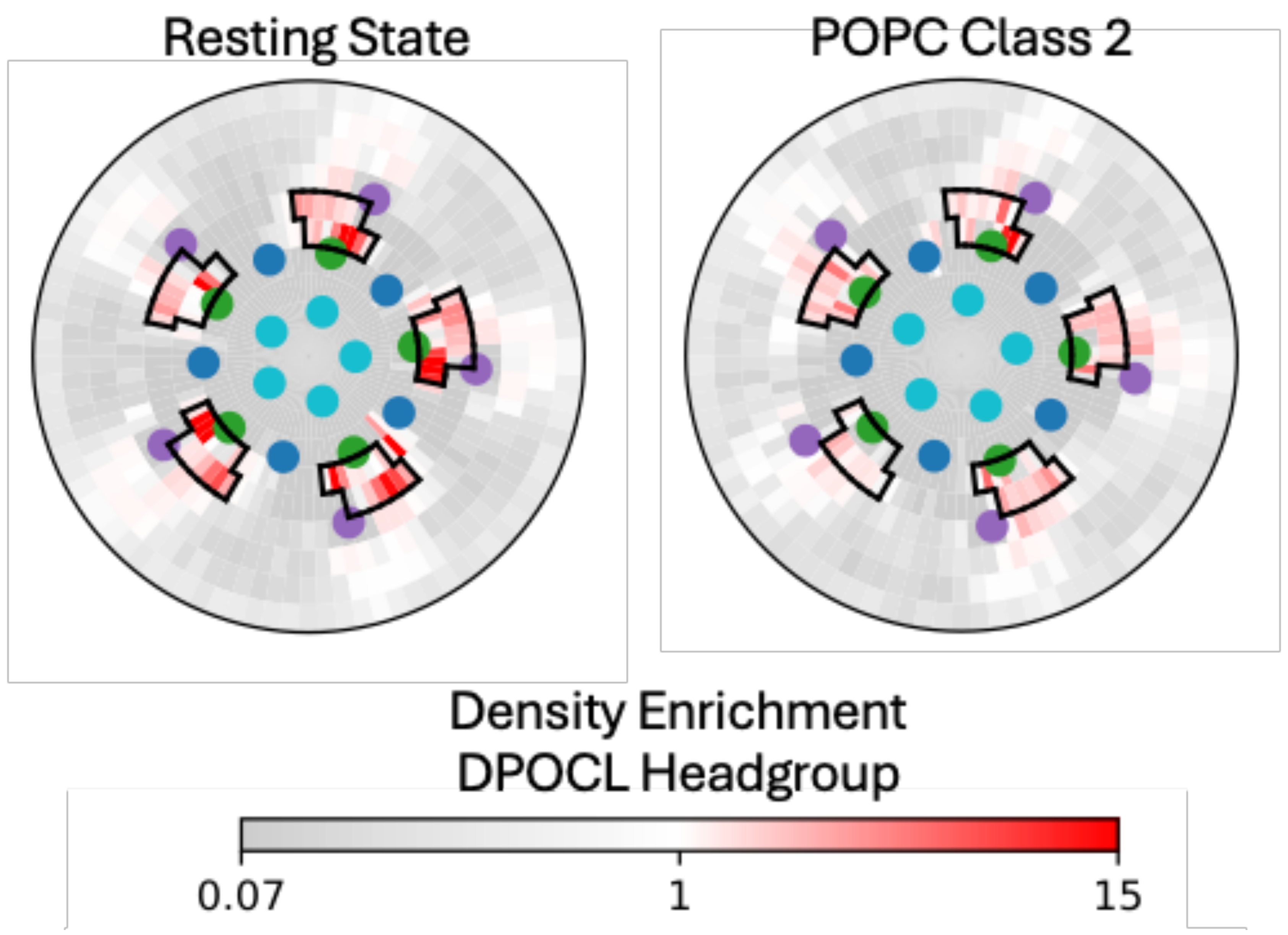
$$\Delta G_{\mathrm{bind}} = -RT \ln \frac{P_{\mathrm{occ}}}{(1 - P_{\mathrm{occ}})}$$

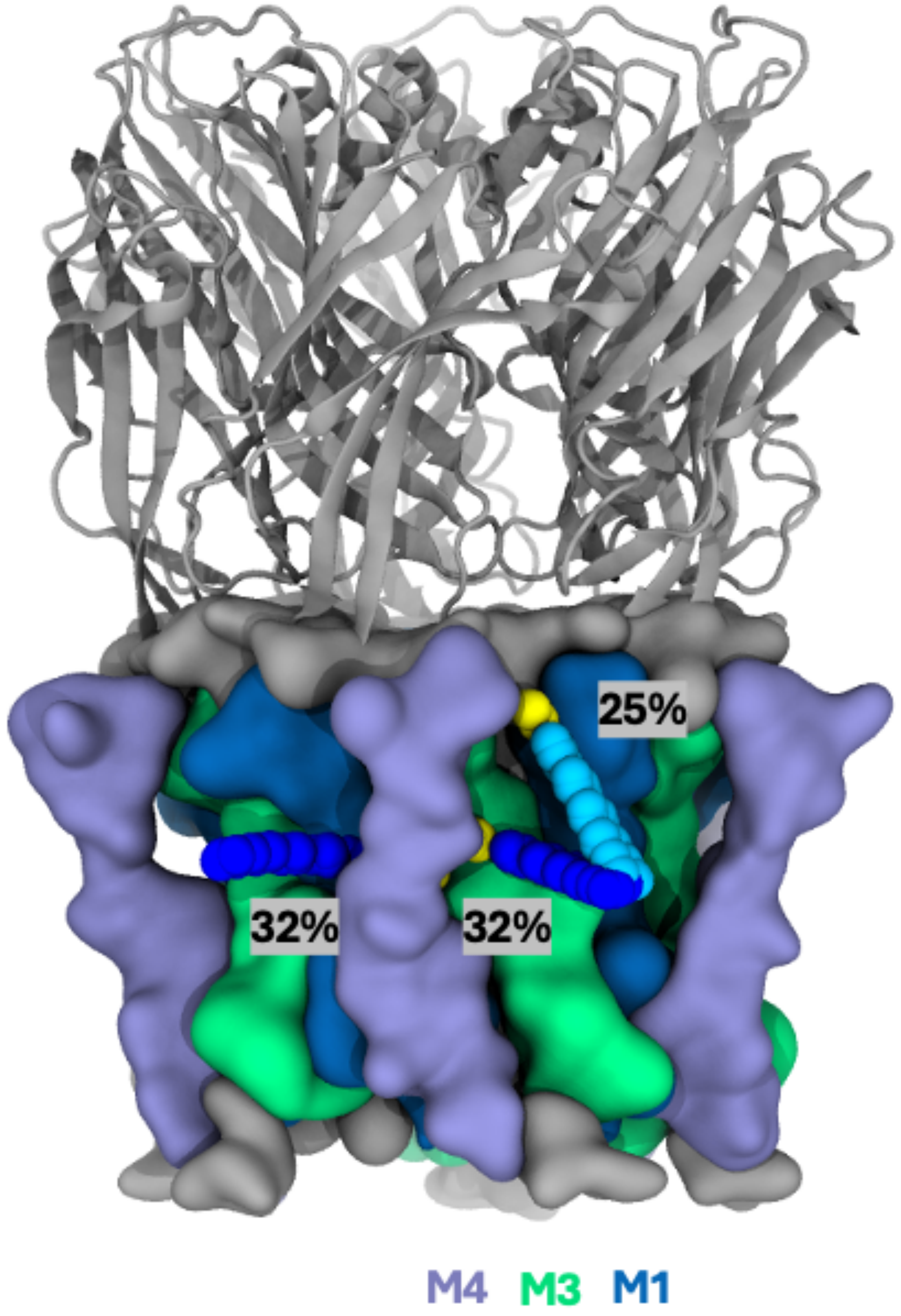
Problem 1:
Ligand and/or Solvent?

Problem 2:
Partial Occupancy

Problem 3:
Chemically Indistinct Ligands

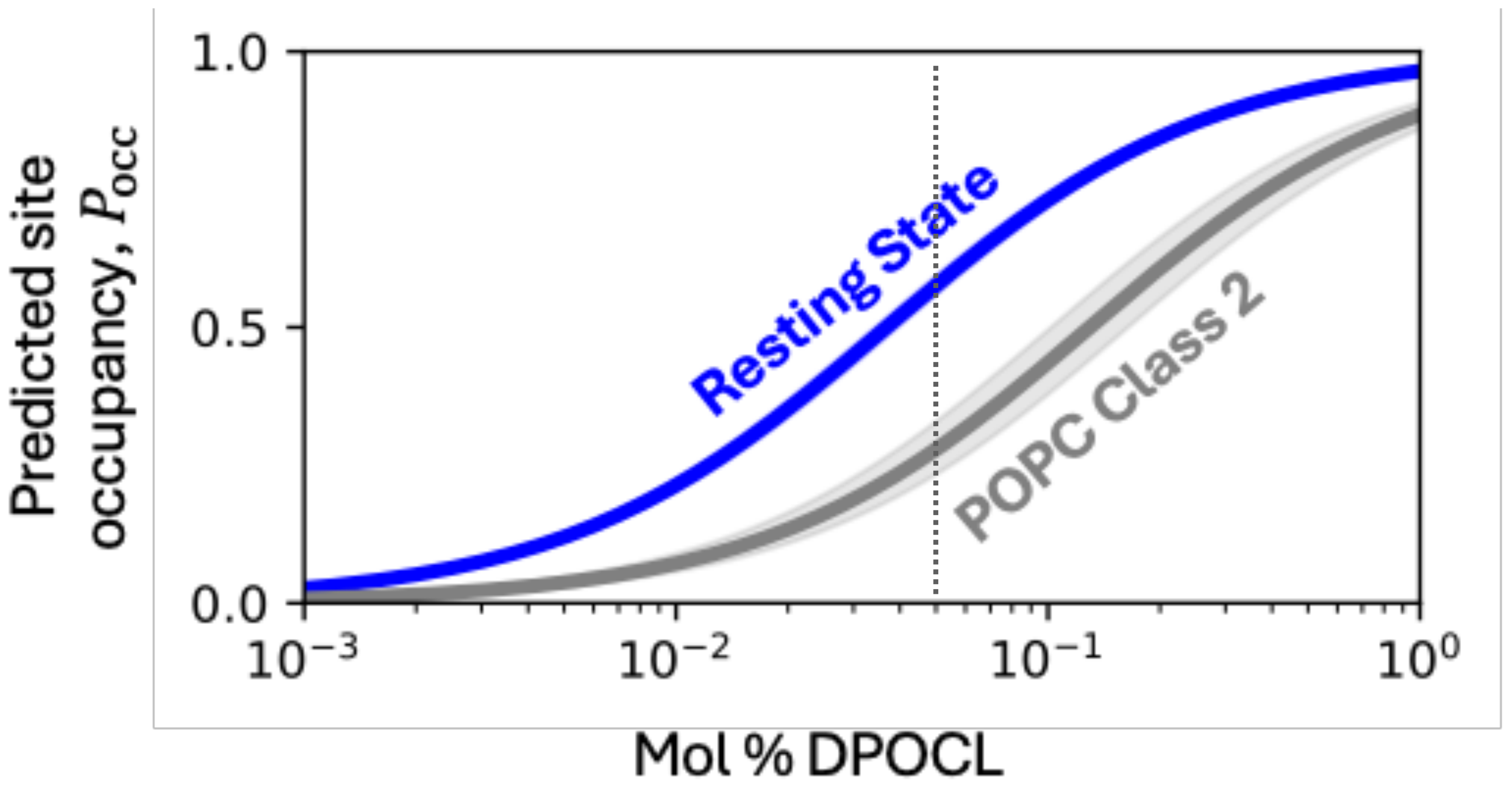
CL binds preferentially to Resting structure





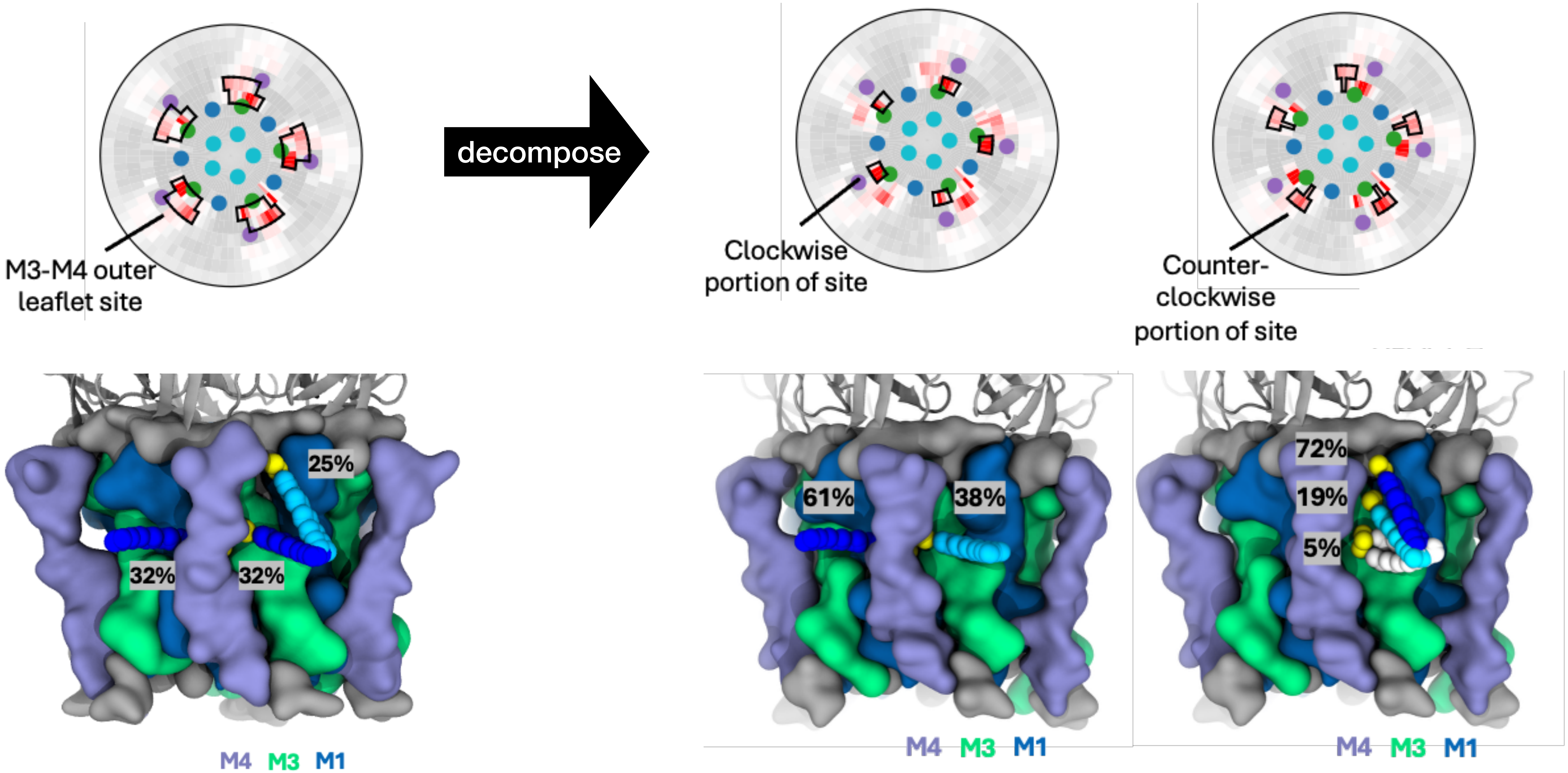
Binding Affinity
(kcal/mol)

Resting	Desensitized
-2.1. ± 0.1	-1.5. ± 0.3

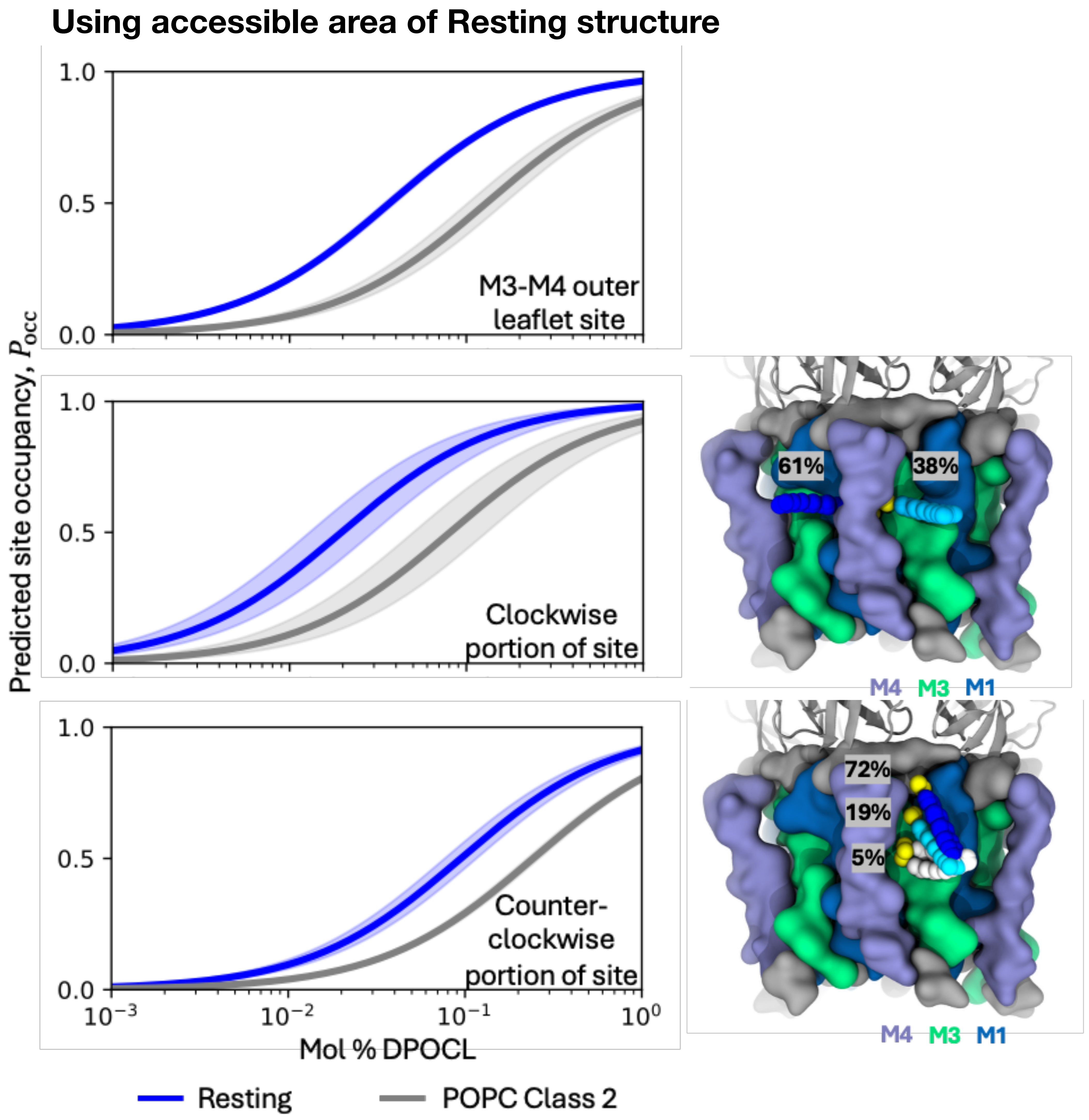


$$P_{occ} = \frac{x_B}{e^{\Delta G/RT} + x_B}$$

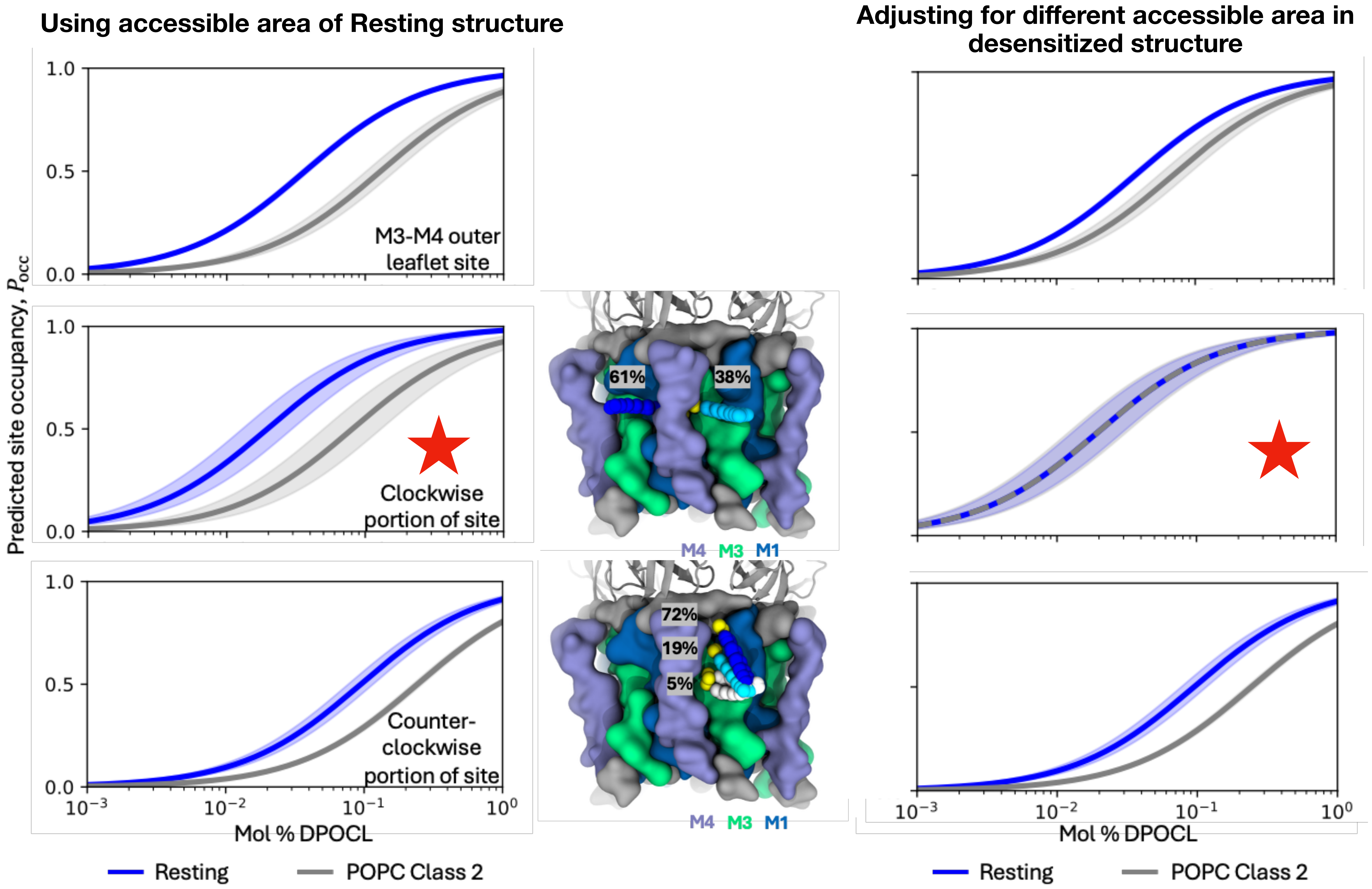
What if we made the site too big?



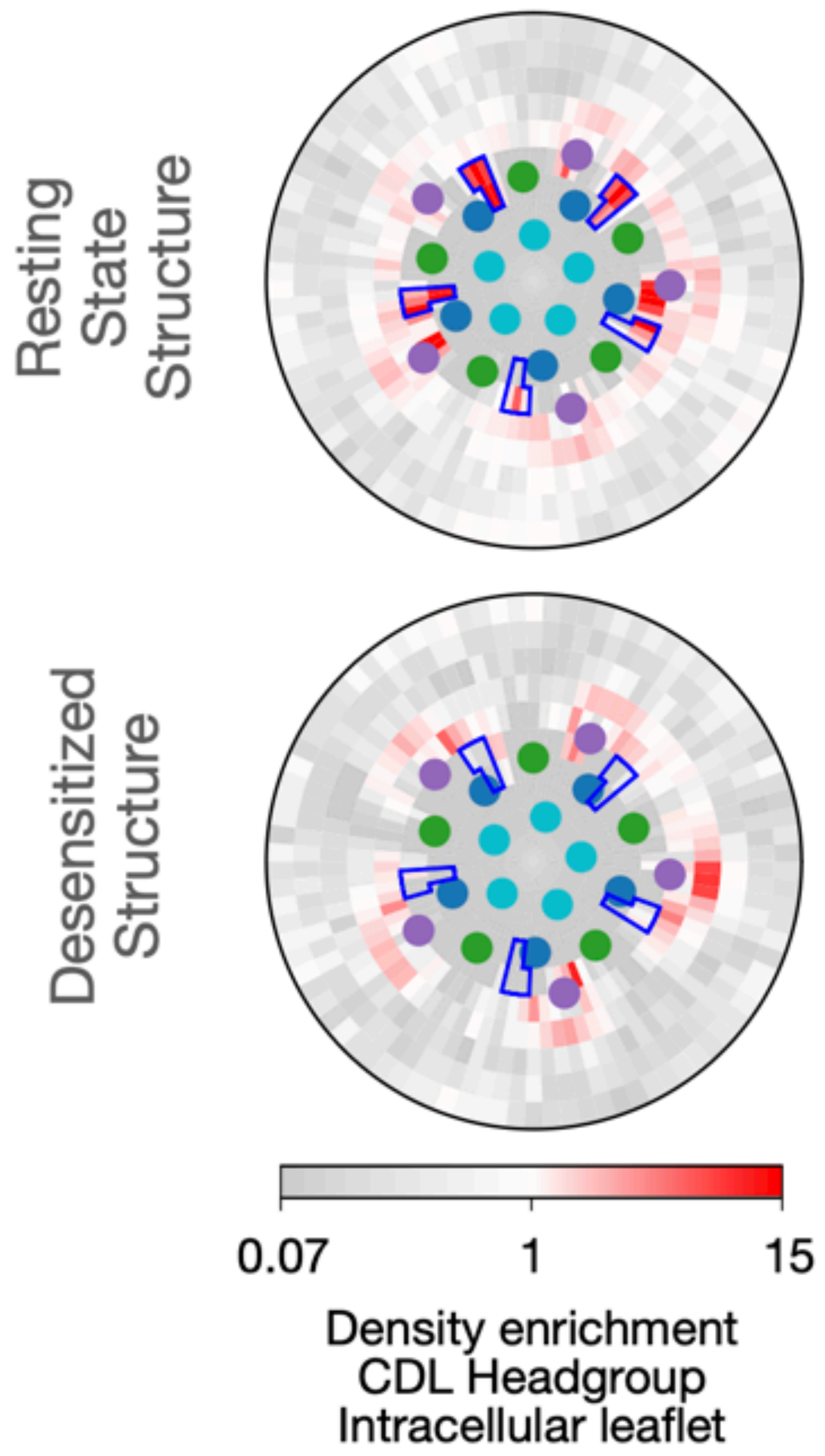
Results robust to site sub-selection

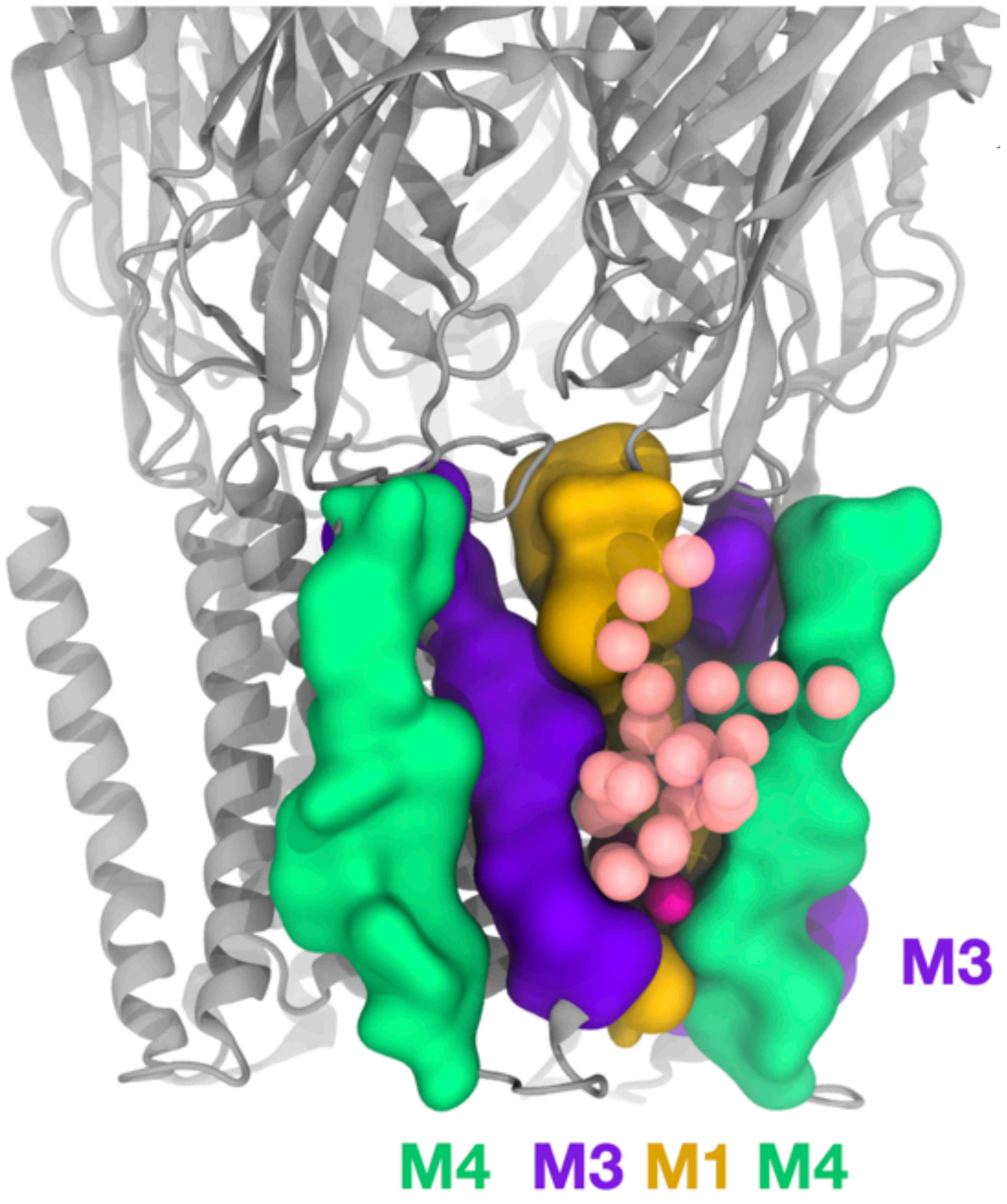


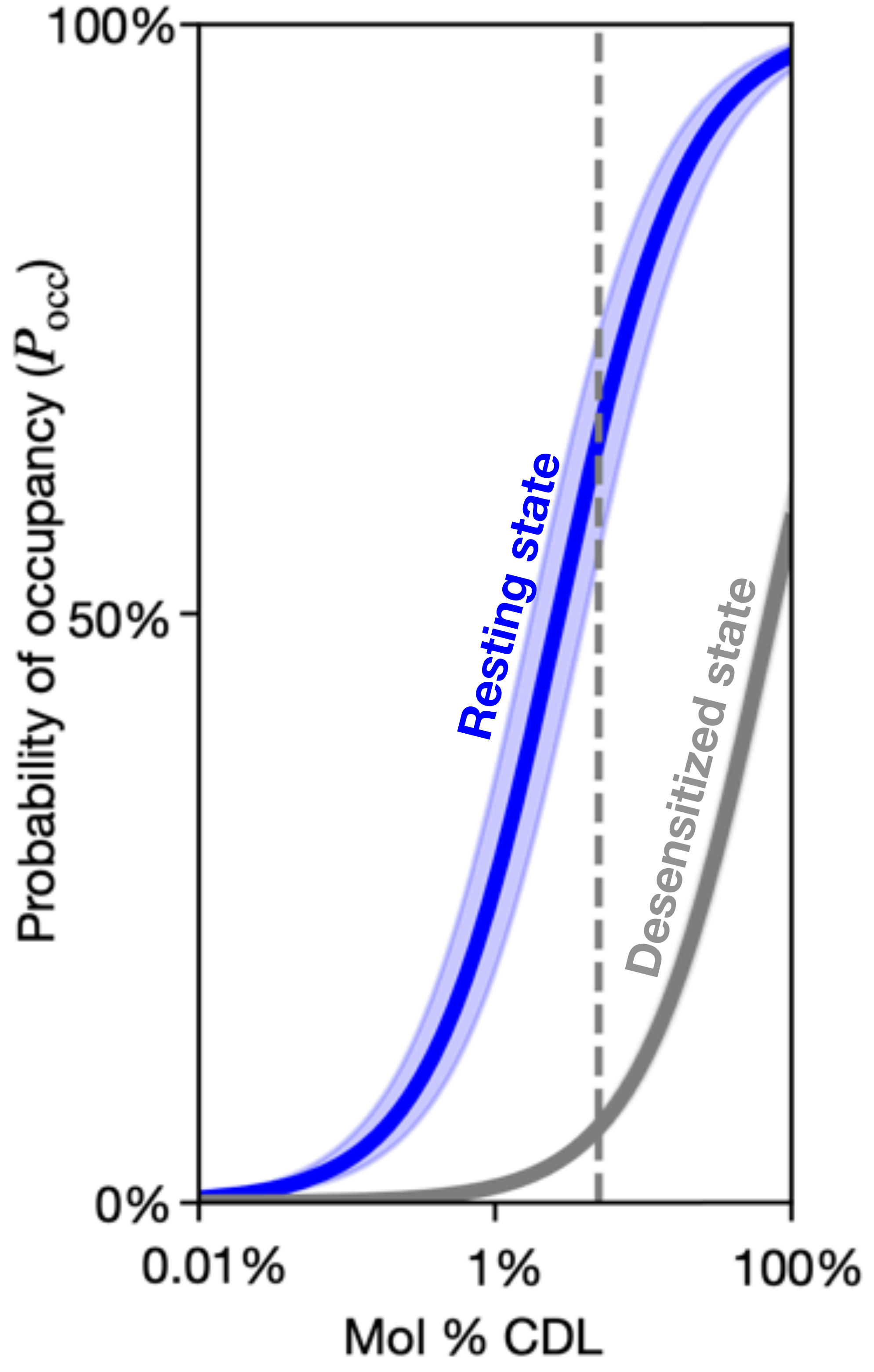
CW site difference entirely due to steric effects



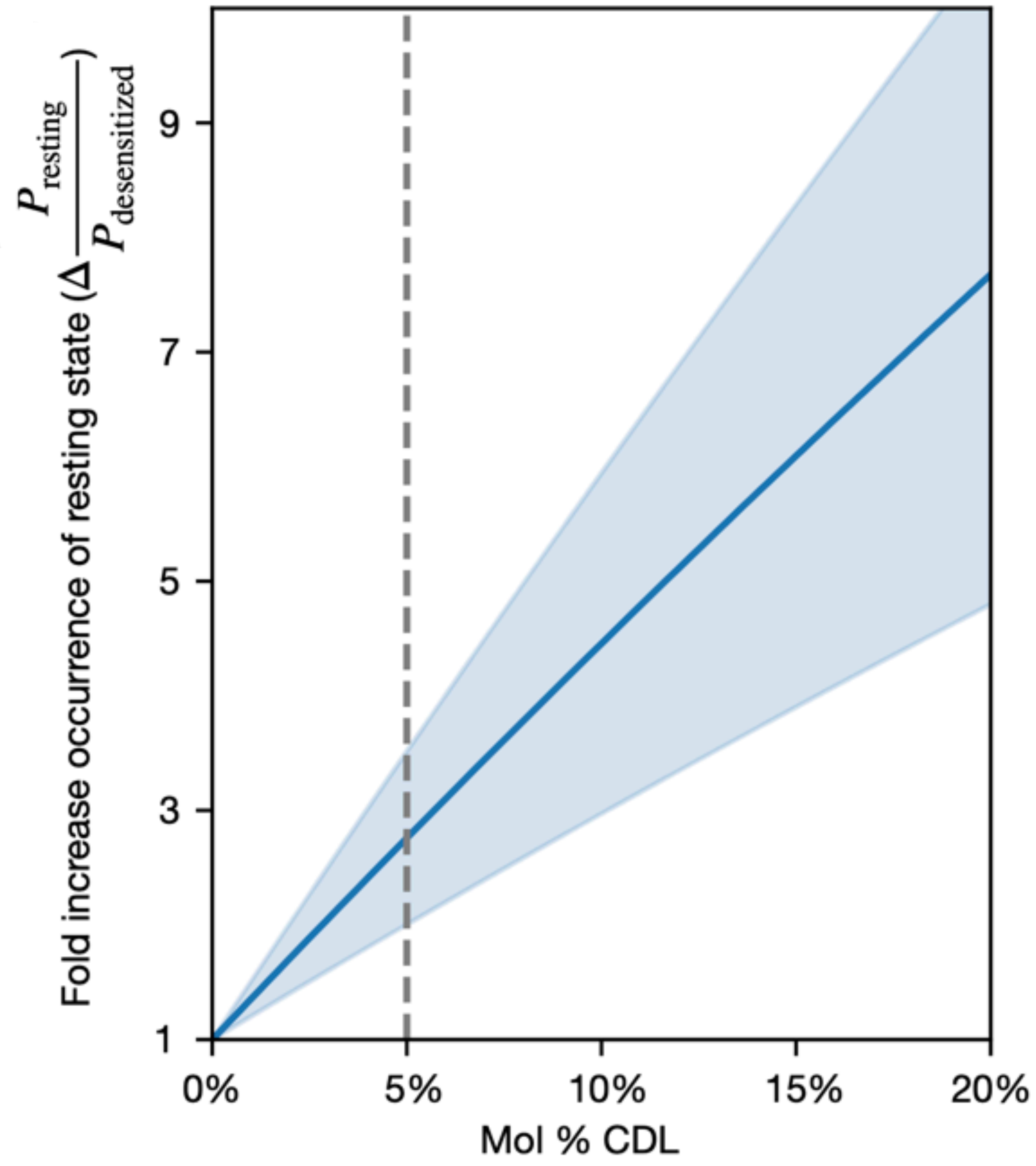
Inner leaflet binding site also identified







$$P_{\mathrm{occ}} = \frac{x_B}{e^{\Delta G/RT} + x_B}$$



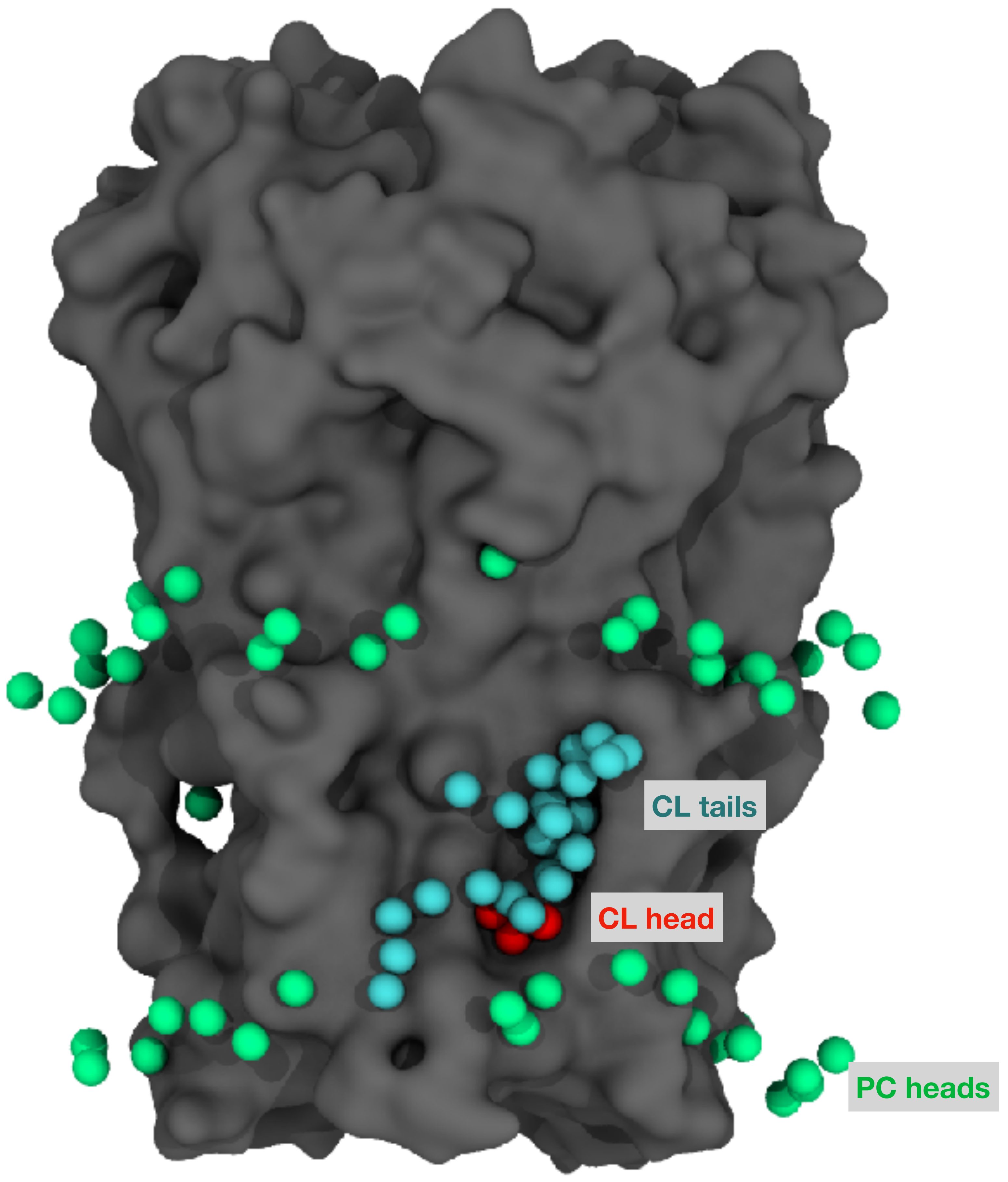
$$\Delta \frac{P_{\mathrm{A}}}{P_{\mathrm{B}}} = \frac{x_{\mathrm{PC}} + x_{\mathrm{CDL}} e^{-\Delta G_{\mathrm{A}}/RT}}{x_{\mathrm{PC}} + x_{\mathrm{CDL}} e^{-\Delta G_{\mathrm{B}}/RT}}$$

Summary:

- Cardiolipin binds preferentially to multiple sites on the Resting structure
- Cardiolipin stabilizes the Resting structure, potentially causing the rescue of function seen when CL is added to pure POPC membranes

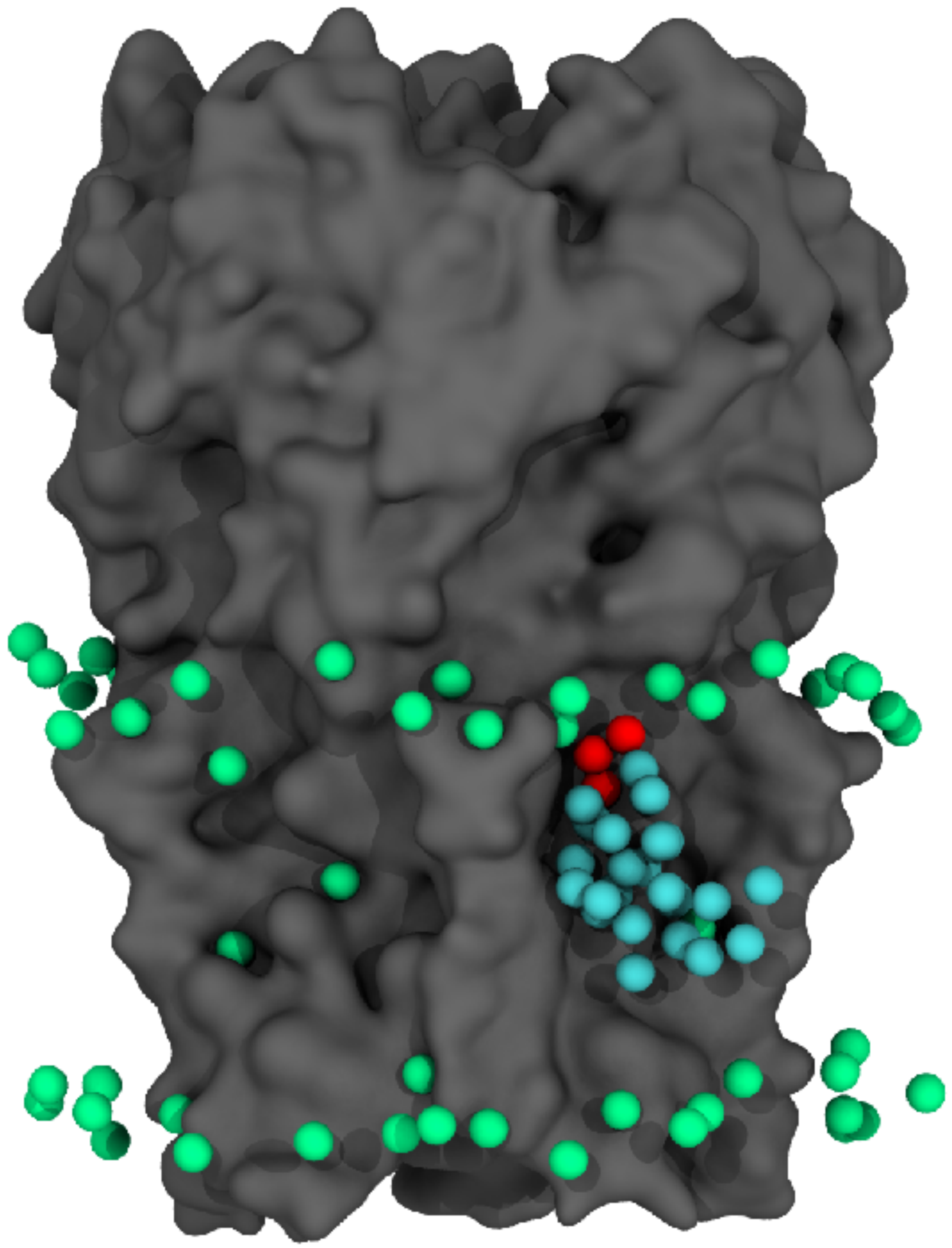
Subplot: trouble with leaflet sorting

We see some of this…



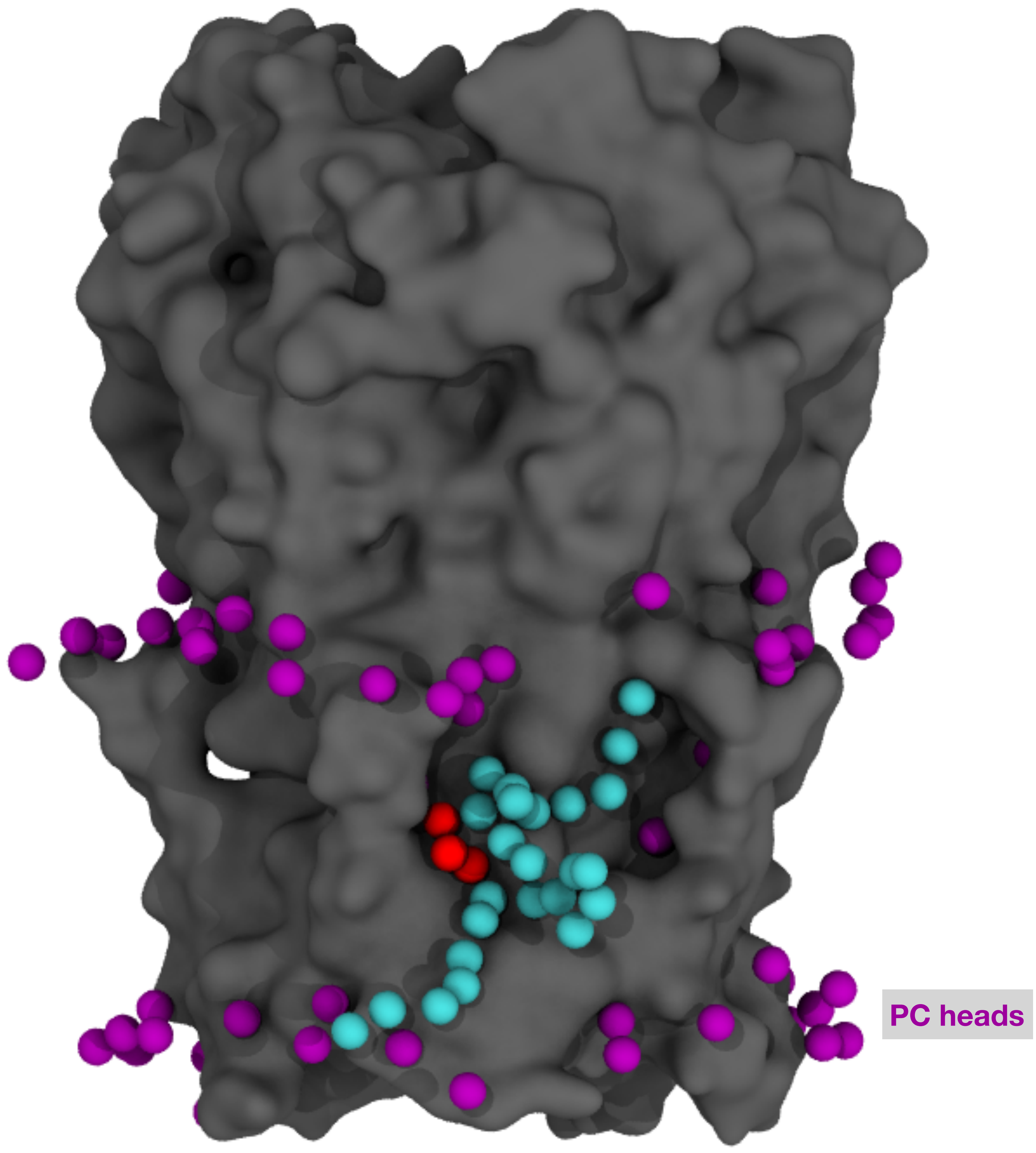
Inner leaflet M1/M4

…some of this…



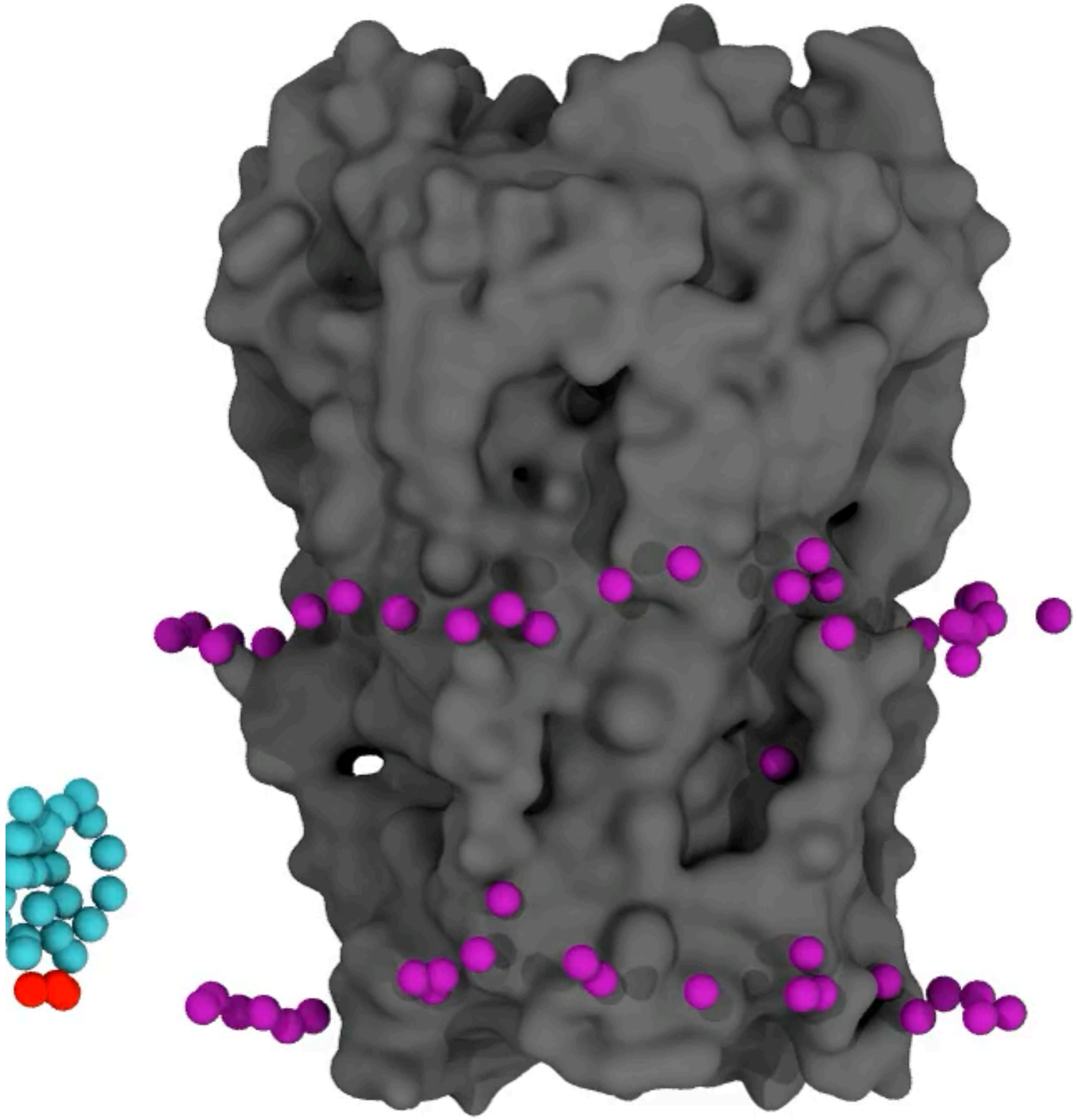
Outer leaflet M3/M4

…and a *lot* of this!



??? leaflet M3/M4

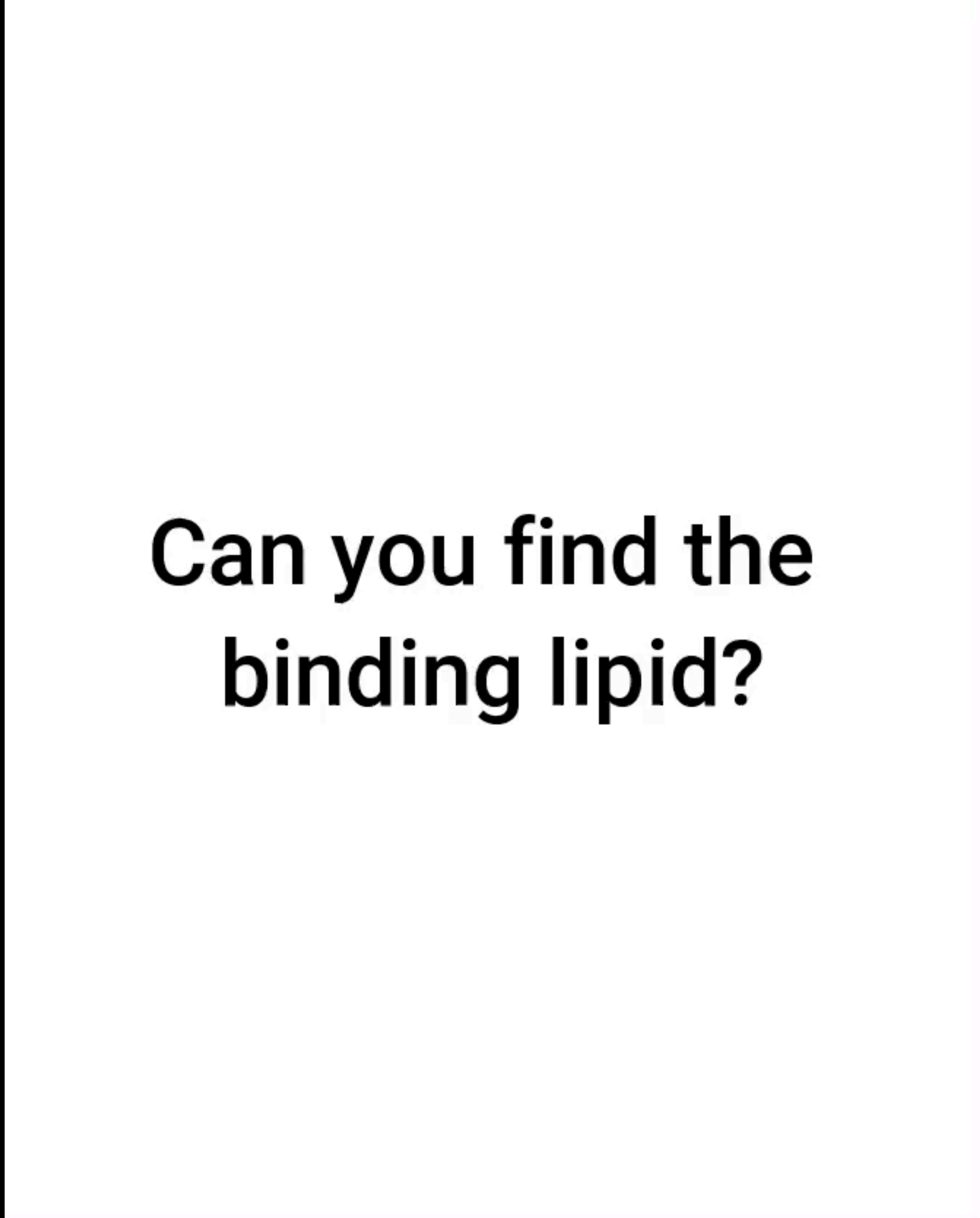
ELIC5 is a scramblase?



Can you find the binding lipid?

POPG molecules in a 2:1:1 POPC:POPE POPG membrane diffuse around and interact with ELIC5, an ‘open state` 5-mutant of the ELIC pentameric Ligand-Gated Ion Channel. Several binding events occur, but one stands out. Can you find the binding lipid?

This video shows the final 3 μs of a 10 μs trajectory.



Thank you!

Dr. Grace Brannigan
Dr. Liam Sharp
Ezry Santiago-McRae
Jahmal Ennis
Connor Pitman
Lindsey Riggs
Regina Salzer
Ryan Lamb
Asim Dave

Dr. Wayland Cheng
Dr. Brandon Tan
Dr. Hanrui Xu

ACCESS

OARC

Read our protocol paper!

DTA to date:

Initial publication
2021

Second usage
2022

Theory refined/
Protocol Published
2024

Experimental
collaborations
2024/2025

RESEARCH ARTICLE | MAY 13 2021

Spontaneous lipid binding to the nicotinic acetylcholine receptor in a native membrane

Special Collection: Special Collection in Honor of Women in Chemical Physics and Physical Chemistry

Liam Sharp; Grace Brannigan

Check for updates

+ Author & Article Information

J. Chem. Phys. 154, 185102 (2021)

https://doi.org/10.1063/5.0046333 Article history

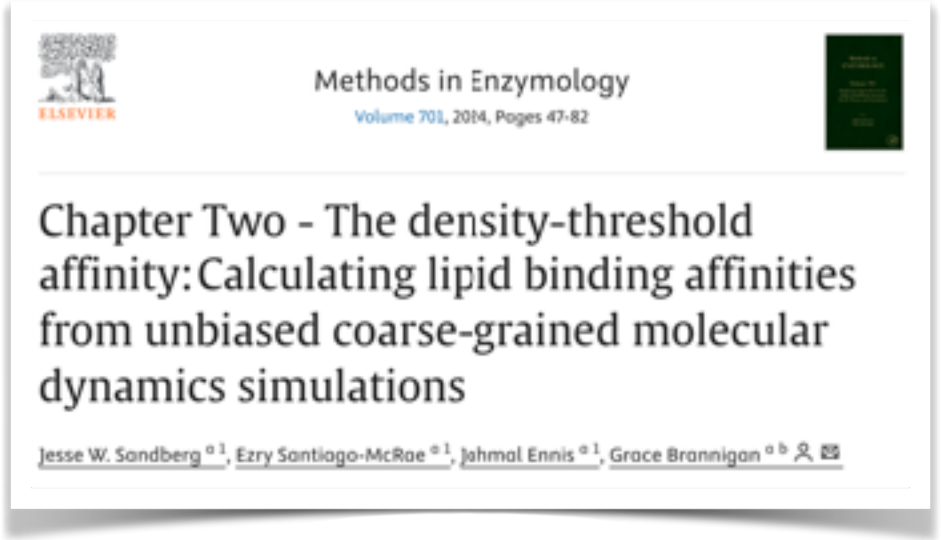
Research Article

Biochemistry and Chemical Biology, Structural Biology and Molecular Biophysics

Polyunsaturated fatty acids inhibit a pentameric ligand-gated ion channel through one of two binding sites

Noah M Dietzen, Mark J Arcario, Lawrence J Chen, John T Petroff II, K Trent Moreland, Kathiresan Krishnan, Grace Brannigan, Douglas F Covey, Wayland WL Cheng

Department of Anesthesiology, Washington University in St. Louis, United States; Department of Developmental Biology, Washington University in St. Louis, United States; Center for the Computational and Integrative Biology, Rutgers University, United States; Department of Physics, Rutgers University, United States; Department of Psychiatry, Washington University in St. Louis, United States; Taylor Institute for Innovative Psychiatric Research, Washington University in St. Louis, United States



ELSEVIER

Methods in Enzymology

Volume 701, 2024, Pages 47-82

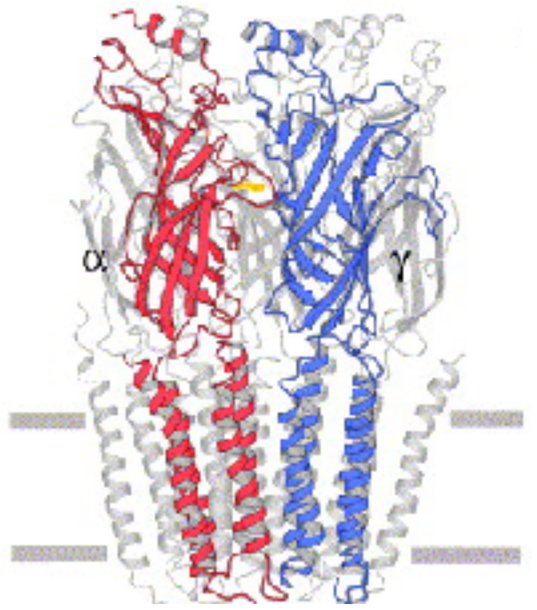
Chapter Two - The density-threshold affinity: Calculating lipid binding affinities from unbiased coarse-grained molecular dynamics simulations

Jesse W. Sandberg [a 1], Ezry Santiago-McRae [a 1], Jahmal Ennis [a 1], Grace Brannigan [a b]

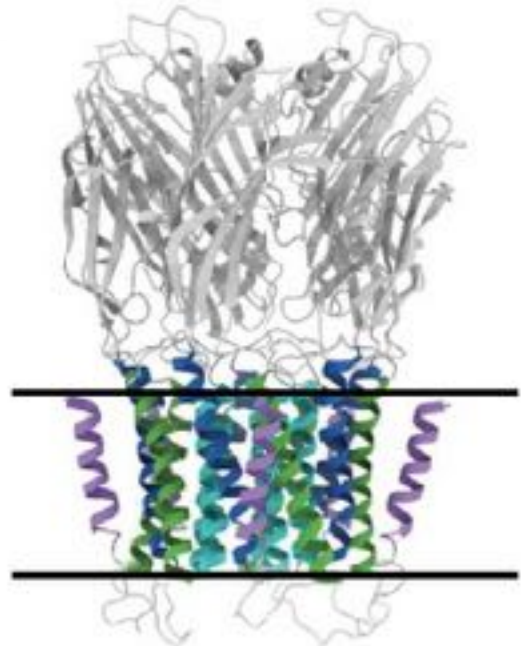
Pierre-Jean Corringer

Eric Klein

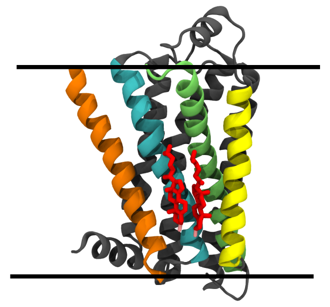
Wayland Cheng



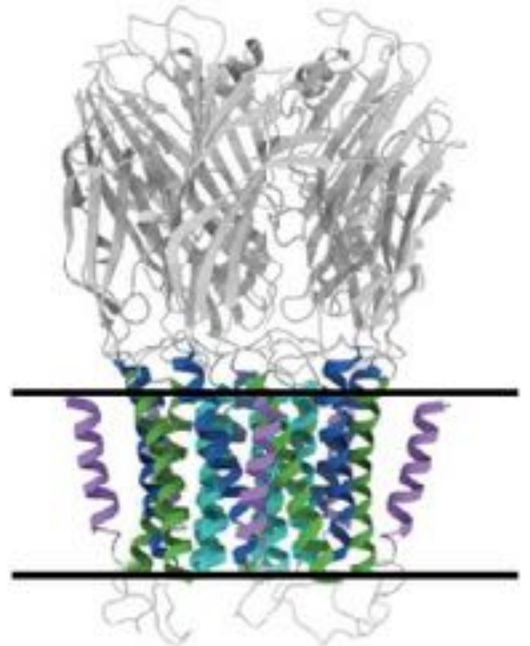
pLGIC:
Torpedo Nicotinic
Acetylcholine
Receptor



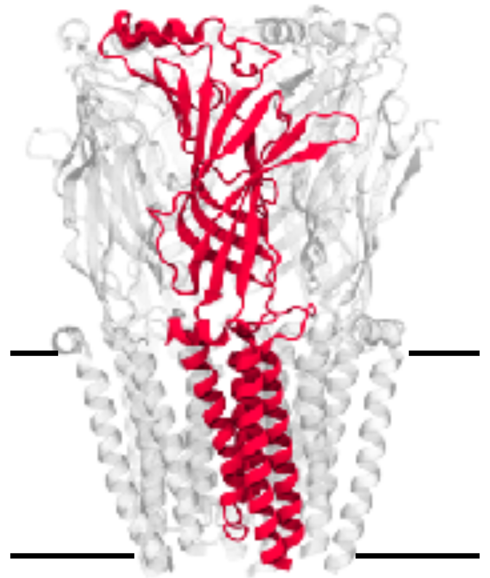
pLGIC:
Erwinia Ligand-gated
Ion Channel (ELIC)



GPCR:
β_2 Adrenergic
Receptor

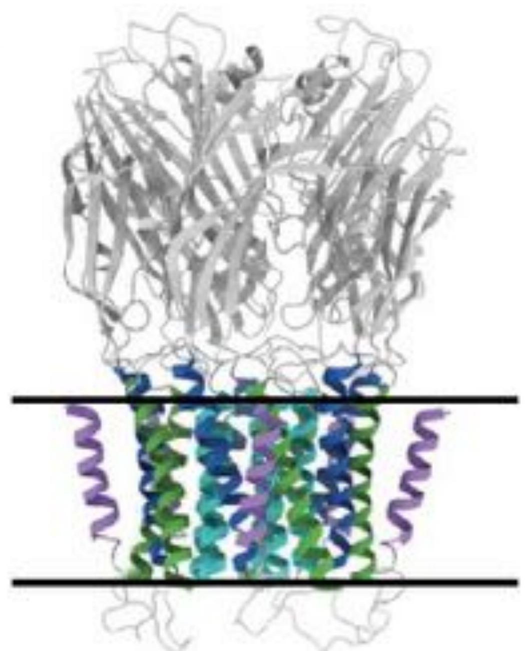


pLGIC:
ELIC



pLGIC:
α-7 Nicotinic
Acetylcholine
Receptor

OMP:
BAMa



pLGIC:
ELIC