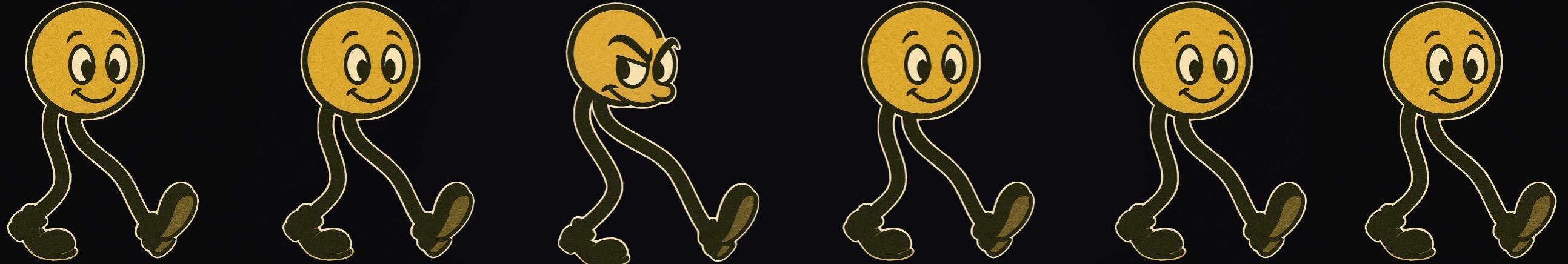




Statistical Mechanics of Protein Interactions

Grace Brannigan

Incoming Dispatch



Lipid noir

Forensic techniques for catching guilty lipids

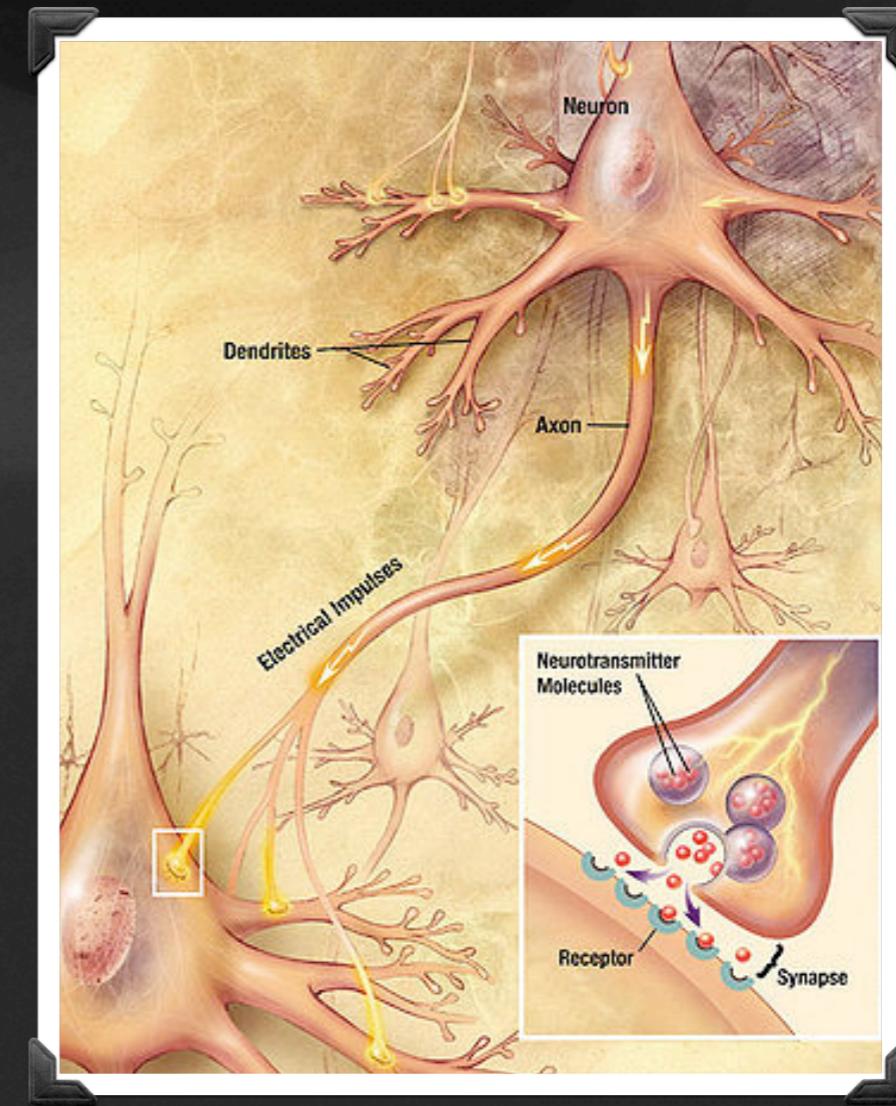
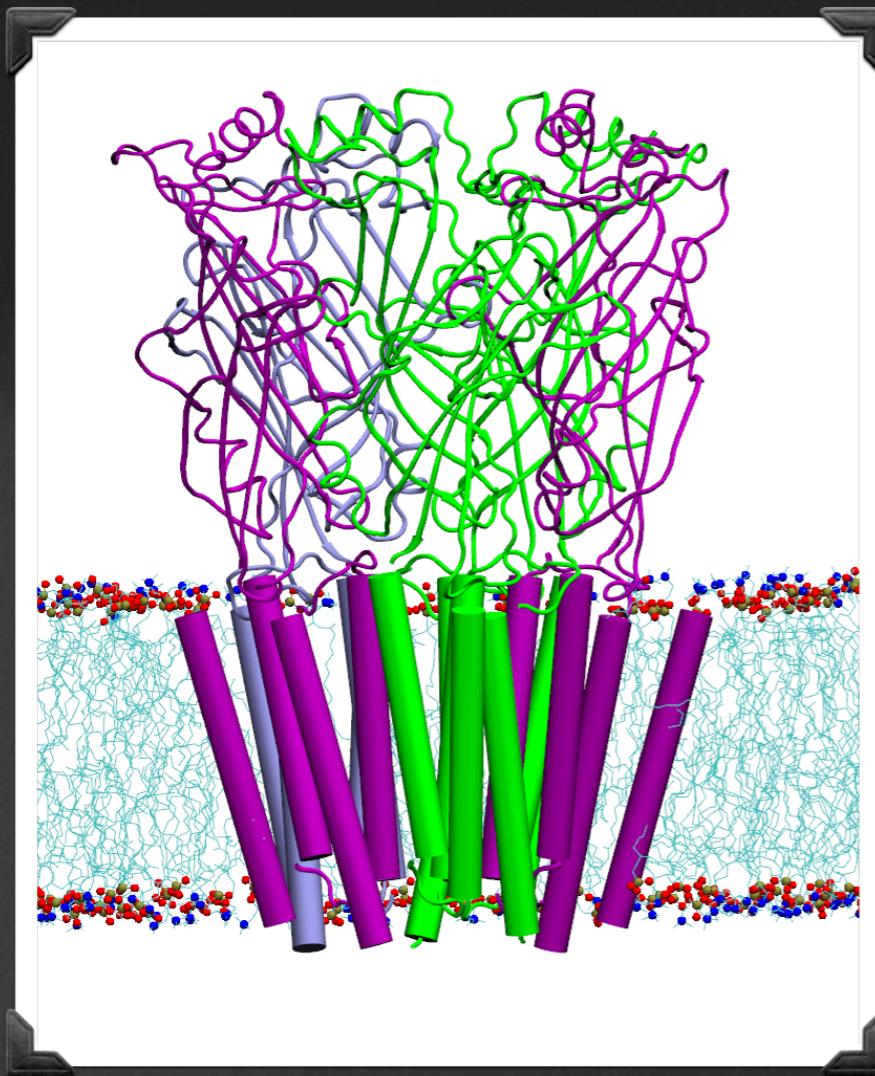
Grace Brannigan
Rutgers University - Camden

What do these scenes have in common?



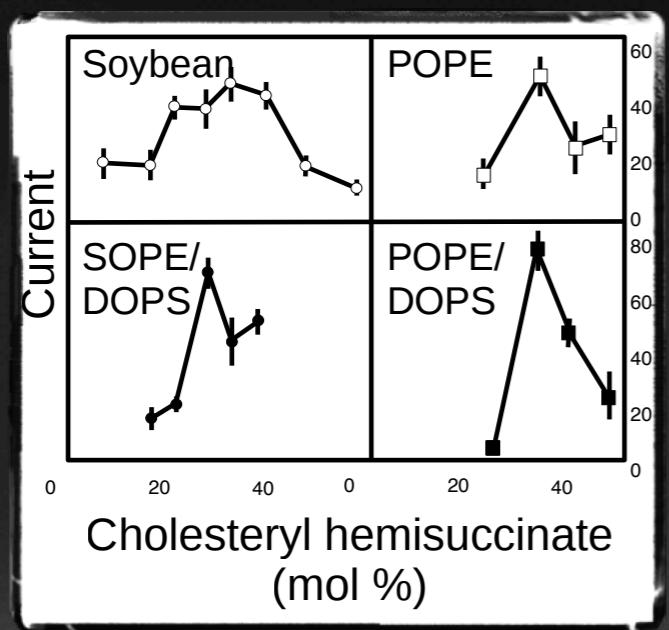
“pentameric ligand-gated ion channels” (pLGICs)

pLGICs: convert diffuse chemical signals into precise electric pulses



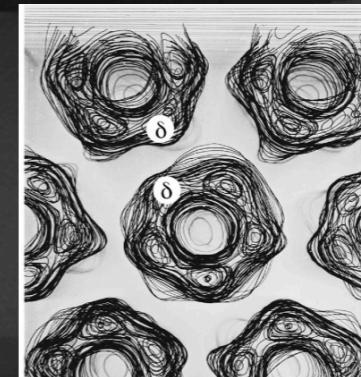
Cold Cases: Lipid modulation of pLGICs

1978-1985: first observations of modulation



Criado...Barrantes, 1984, J.Biol.Chem.

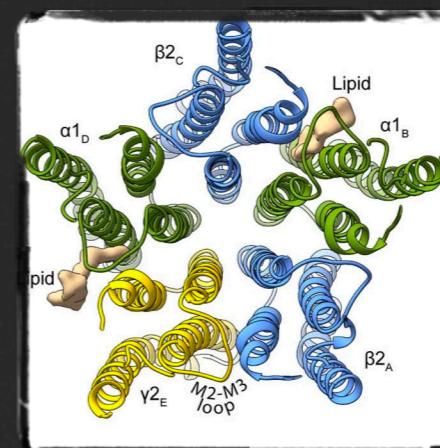
Structural biology lags
lipid-protein mechanisms



Brisson & Unwin, 1985

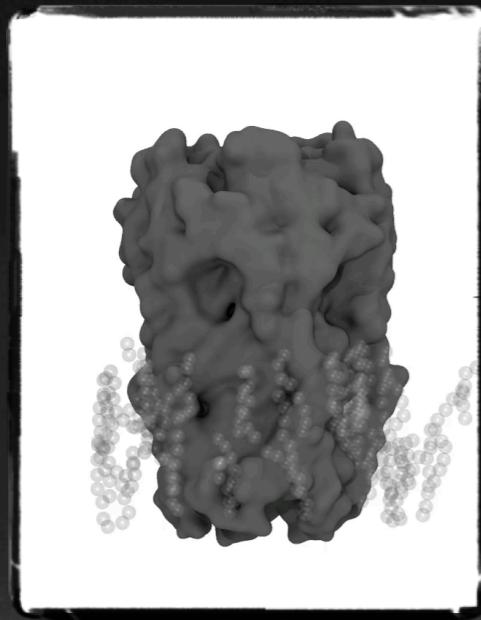
Weird modulation.

2020s: structural
signatures of the
guilty parties



Kim, et al, *Nature*, 2020, Extended Data Figure 5

What makes a guilty lipid in this court?



“Guilty”: specific and direct binding to proteins;
modulate them like ligands



“Not Guilty”: Boundary paralipidome
lipids paralipids(?) that diffuse through a
potential binding site



How do we quantify likelihood of guilt?

Kinetics: how long is an individual lipid bound?

Thermodynamics: how often is a member of that lipid species in the site?

Prosecuting in a pharmacology court

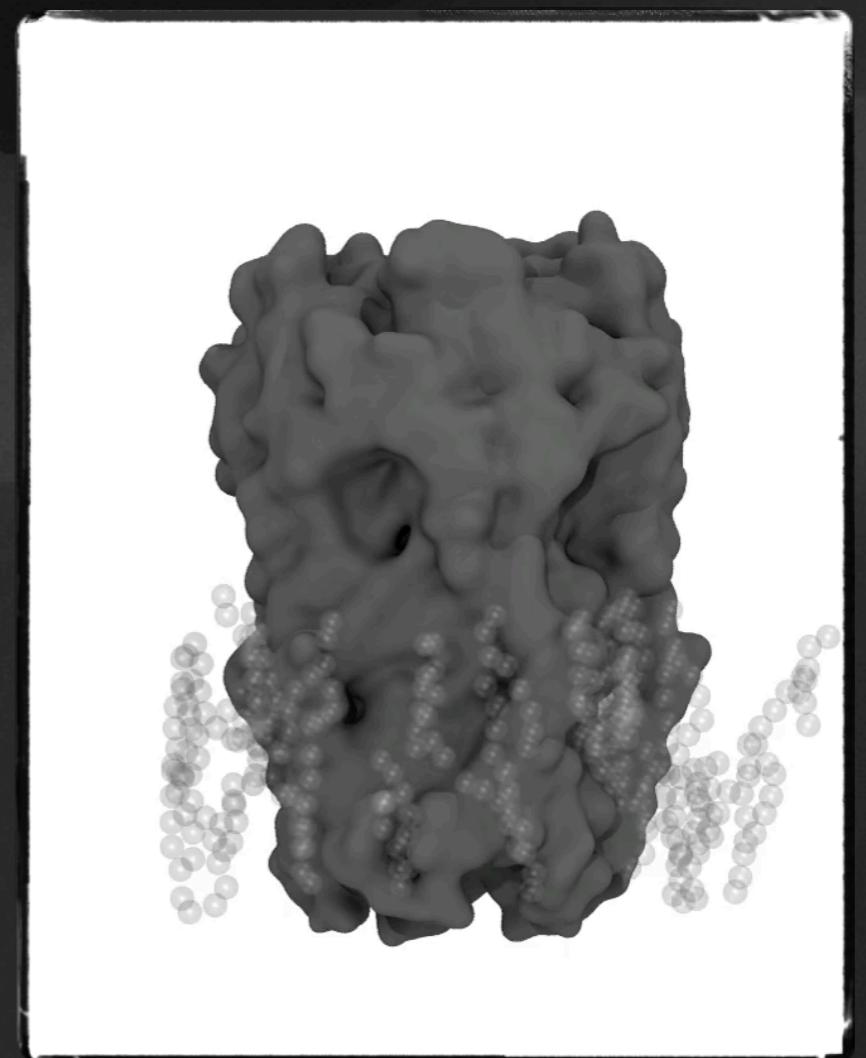
Need to build a **thermodynamic** case

Does the **binding pose** maintain interactions that will affect function?

What **lipid concentration** is required to get 50% occupancy of this site?

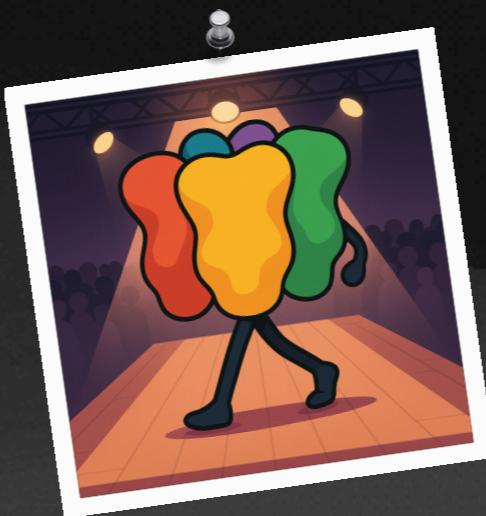
Do experiments **need** that **concentration** to see functional effects?

Is the lipid **enriched** in the site (more likely to be there than by diffusion alone)?



Two case studies

- ELIC POPG Modulation Site
 - Cryo-EM lipid fragment
 - Model Membrane
 - Fully atomistic



- nAChR boundary lipids paralipidome
 - Complex Membrane (neuronal membrane; xenopus oocyte)
 - Free energies from coarse-grained spontaneous binding



Case: modulation of ELIC by POPG



Client: Dr. Wayland
Cheng, Washington
University - St Louis



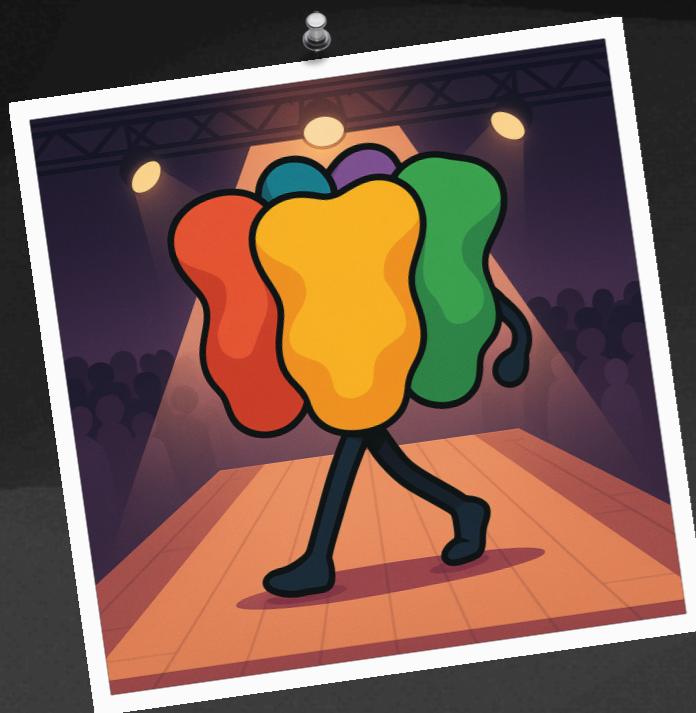
Lead detective:
Ezry Santiago-McRae



Consulting detective:
Dr. Jérôme Hénin, CNRS

Incident Report

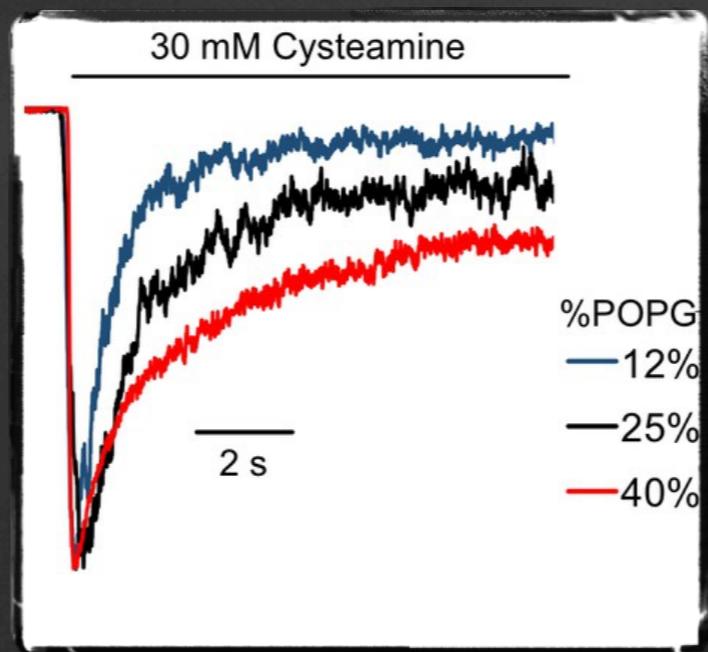
Protein



ELIC: a bacterial model member of the pLGIC family

Incident

Adding POPG lengthens the pulse duration, but not peak current



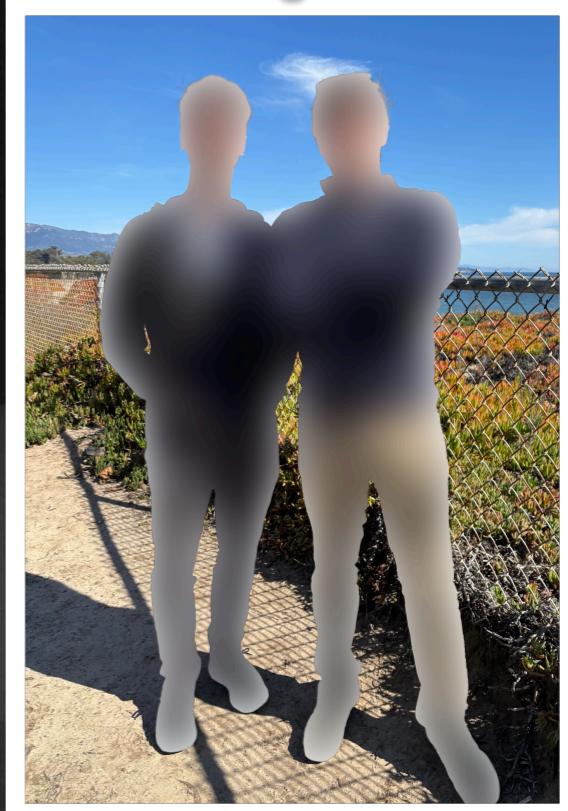
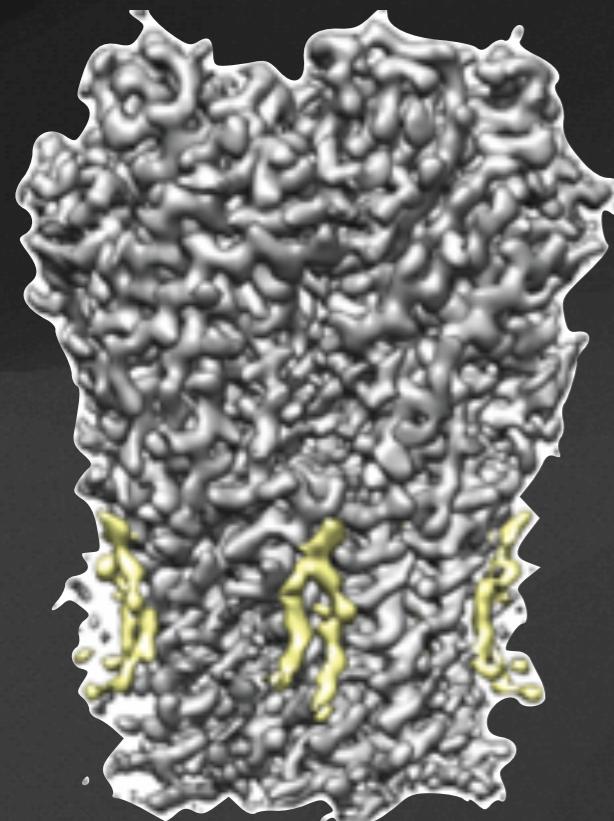
Tong, Petroff, Sharp, ... , GB, Cheng, eLife 2019

Is POPG binding directly to ELIC? Where?

Allosteric modulator? Does it stabilize the conducting state?

Grainy security footage provided by client

ELIC in a 2:1:1 PC:PG:PE nanodisc (cryo-EM)



Student 1

Student 2

Who is this?!?

What are they
doing there?!?

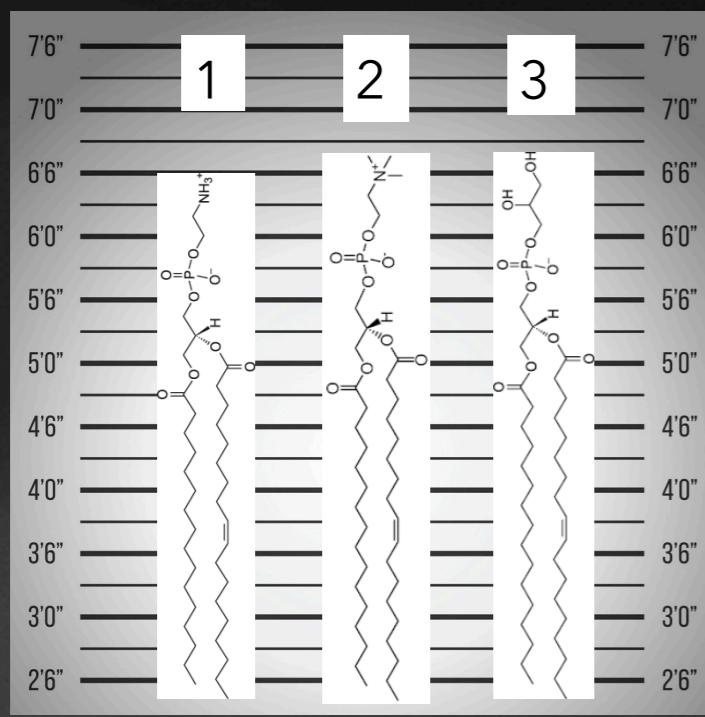
How strongly are
they binding?

Petroff, Santiago-McRae, ... Joseph, Hénin, GB, Cheng, Nat Comm 2022

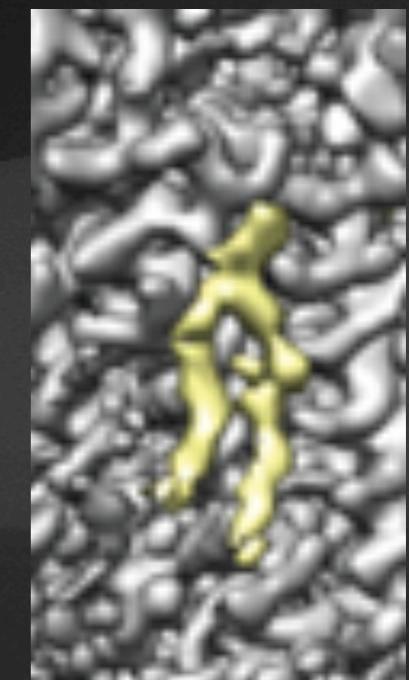
Suspect Identification

“Who is this?”

Lipids in the nanodisc



What is the relative probability that each of these lipids would occupy this specific binding pose?



Top suspect. Still, need to keep an open mind.

How a computational chemist would approach this problem

Preserve atomistic resolution

Forget about pathway

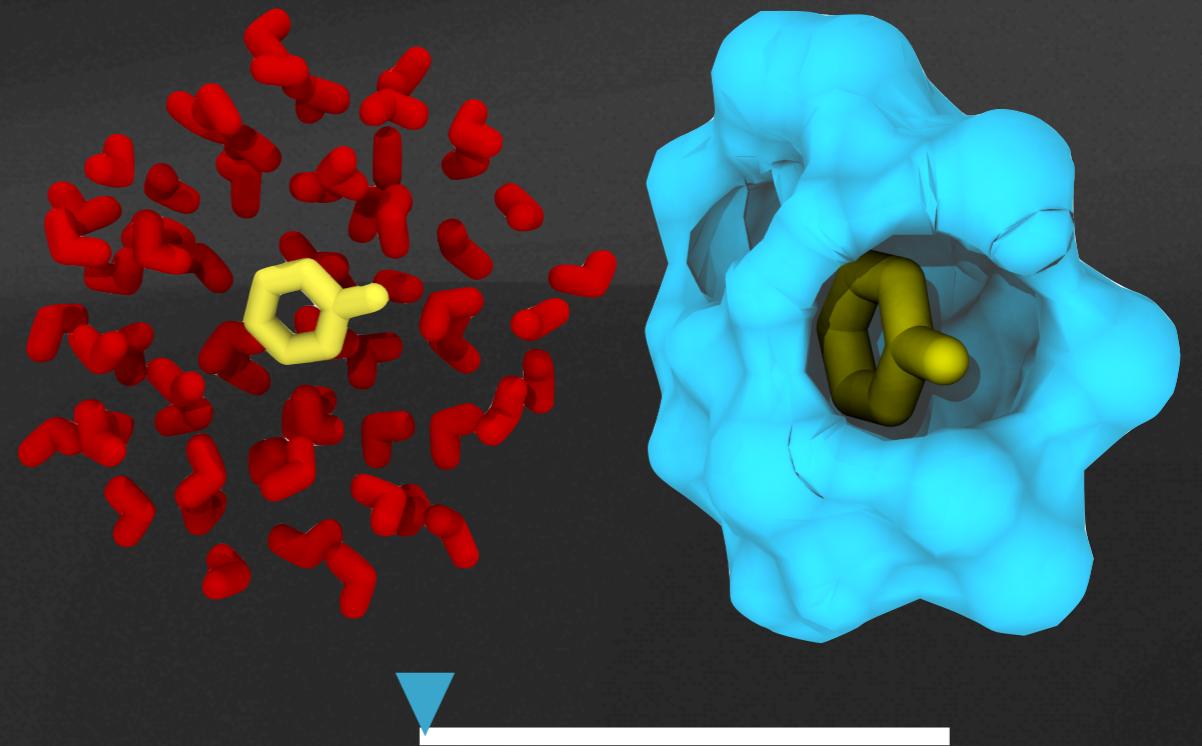
Relative Alchemical Free Energy Perturbation (FEP)

How: Calculate free energy (Zwanzig equation) for a gradual chemical transformation in two different environments:

1. in solvent ($\Delta\Delta G_1$) and
2. in the binding cavity ($\Delta\Delta G_2$)

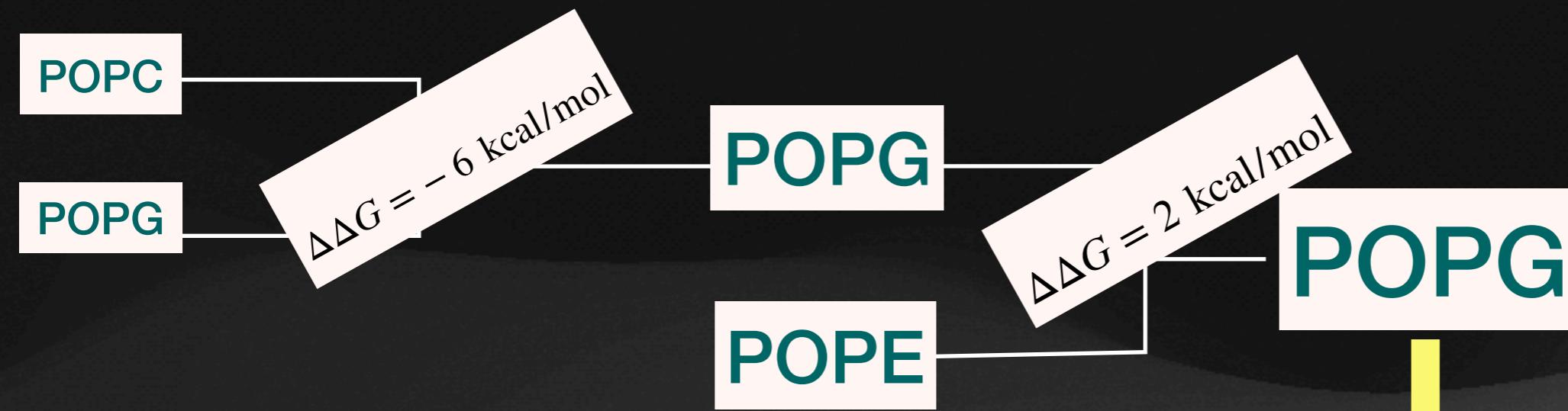
Output:

$$\Delta\Delta G_{bind} = \Delta\Delta G_2 - \Delta\Delta G_1$$



Transformation
Progression

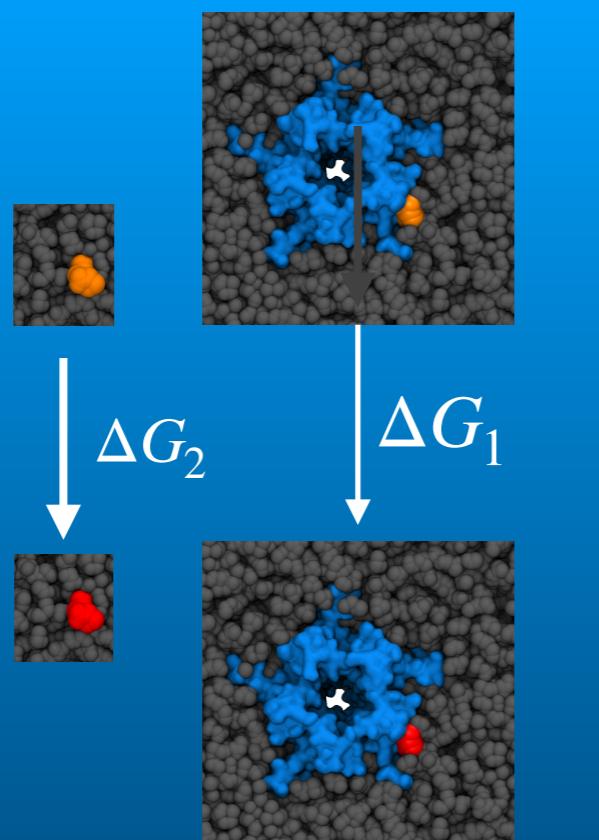
Lipid Single Elimination Tournament



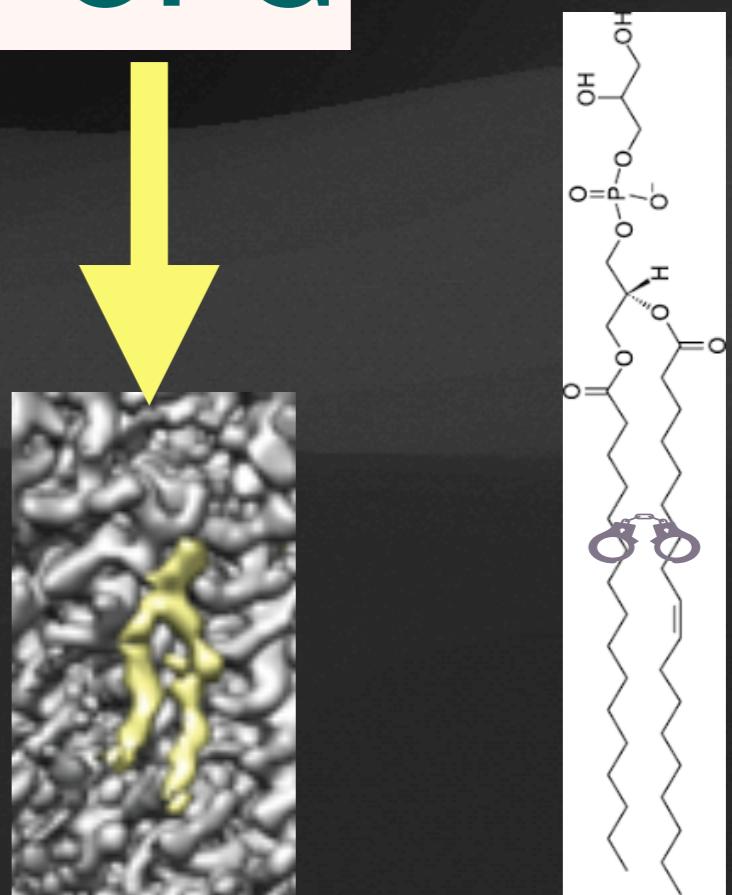
Head

To

Head

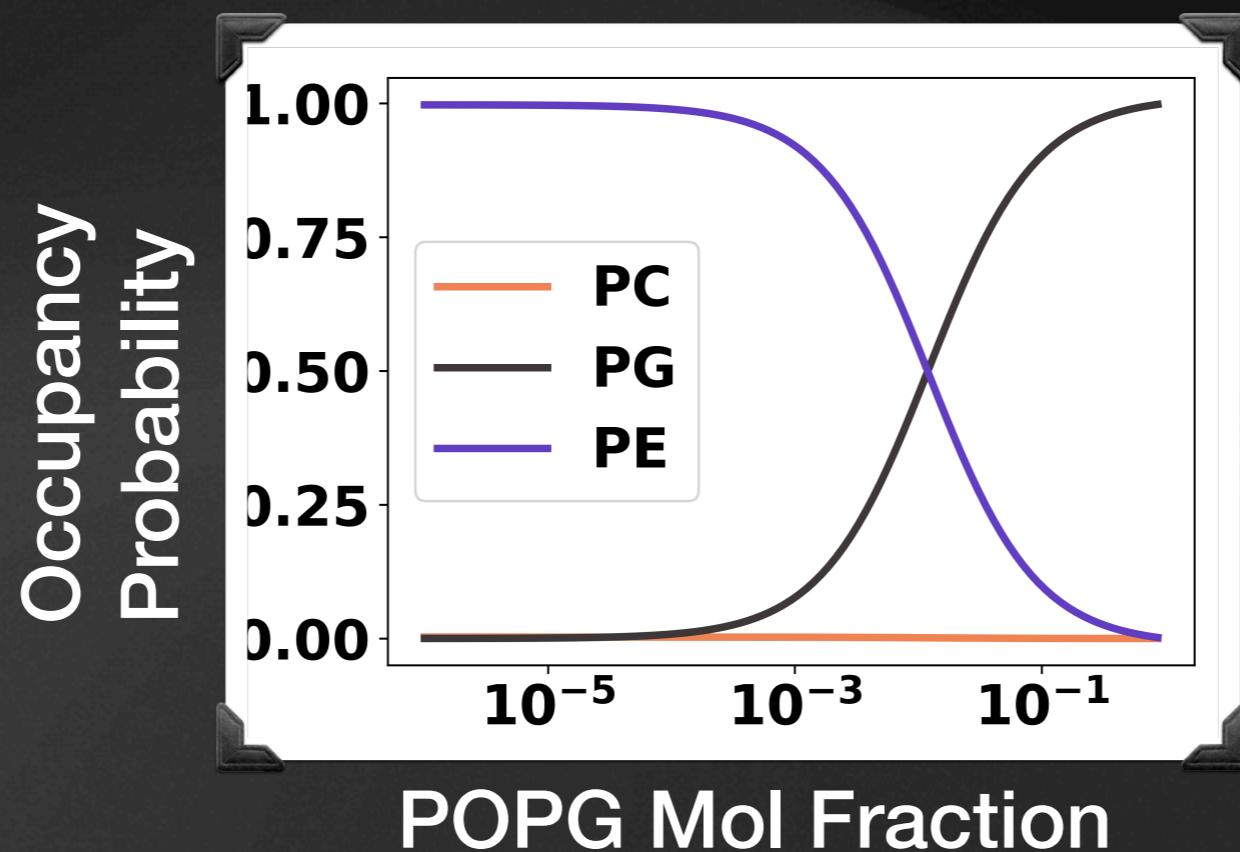


$$\Delta\Delta G_{bind} = \Delta\Delta G_2 - \Delta\Delta G_1 + \text{corrections}$$



How often will you find POPG in this site?

Can estimate occupancy titration if we assume that this specific binding mode is always occupied by POPC, POPG, and POPE



But we didn't like that assumption.

Absolute FEP: simple in theory

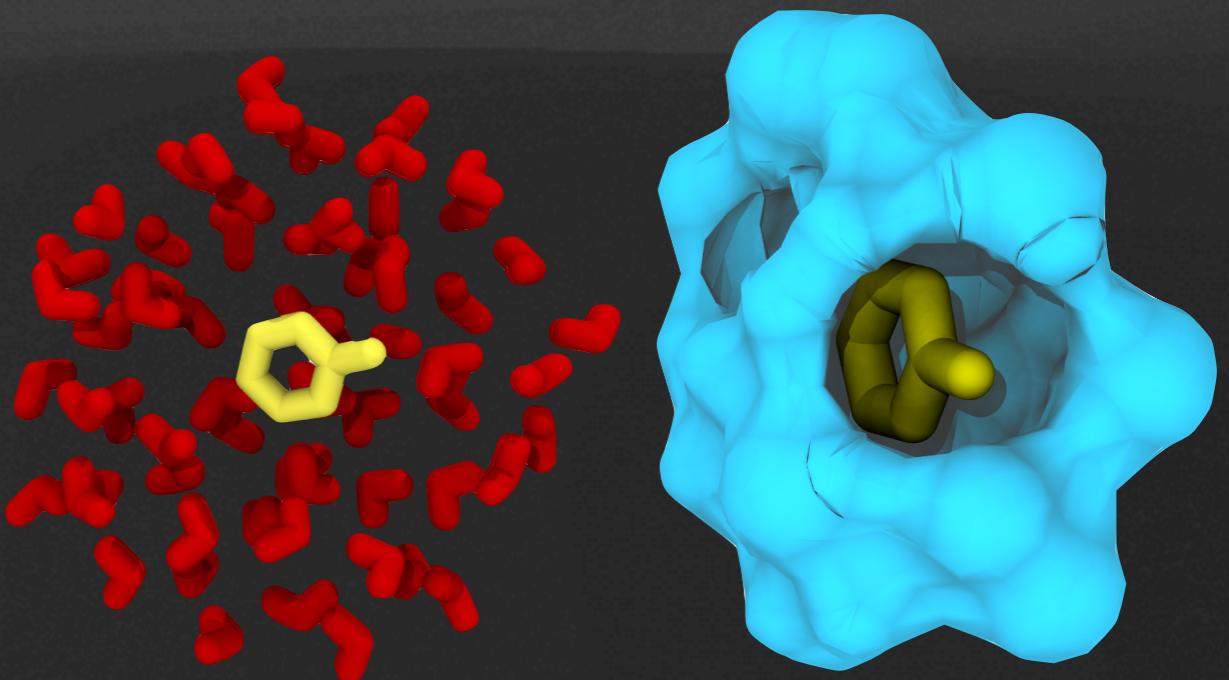
Absolute Alchemical Free Energy Perturbation (FEP)

How: Gradually decouple the ligand from the system

Use the Zwanzig equation to calculate the cost of decoupling:

1. in solvent (ΔG_1) and
2. in the binding cavity (ΔG_2)

Output: $\Delta G_{bind} = \Delta G_2 - \Delta G_1$



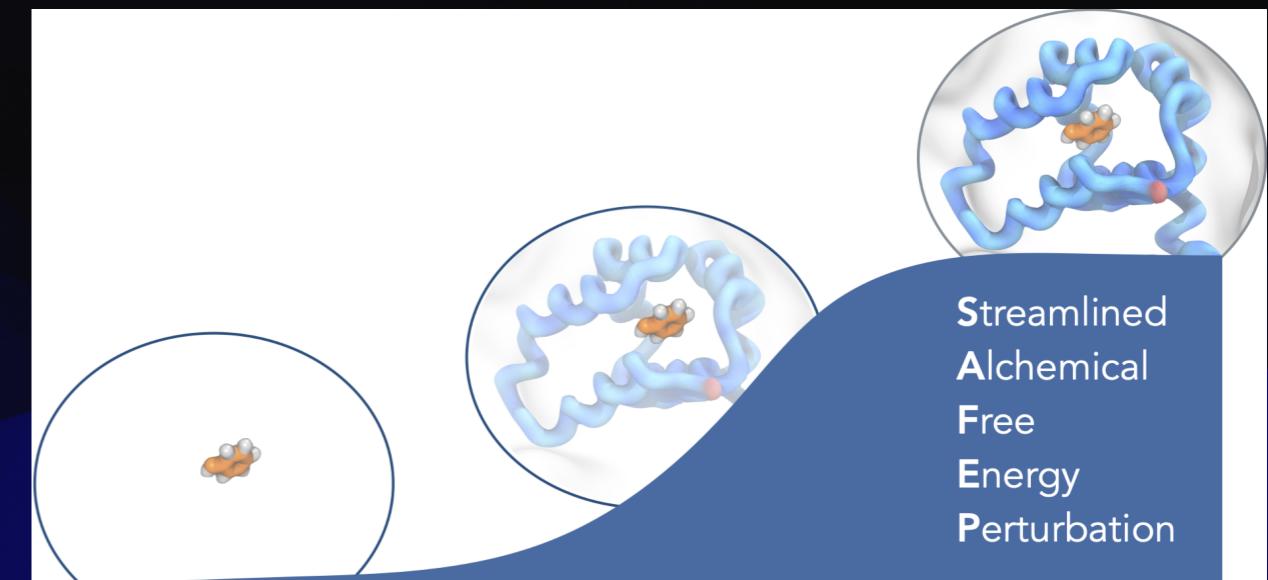
Decoupling Progression

Absolute FEP: painful in practice

- Computational chemists avoid absolute FEP if they can, and they have a lot of advantages over our situation
 - Enclosed binding cavity
 - Only a few possible ligand conformations
 - Solvent with much faster kinetics than the solute
- Insight: it's painful because it's usually carried out in an unnatural reference frame

Our solution: SAFEP

FEP but in a site-centered reference frame.



an absolute FEP implementation that works for lipids (and is simpler for a lot of aqueous proteins also)

A LiveCoMS Tutorial

**Computing Absolute Binding Affinities by Streamlined Alchemical Free Energy Perturbation (SAFEP)
[Article v1.0]**

Ezry Santiago-McRae^{1†}, Mina Ebrahimi^{2,3,4†}, Jesse W. Sandberg¹, Grace Brannigan^{1,5‡}, Jérôme Hénin^{3,4‡}

Testing SAFEP on something “simpler”

Cholesterol binding to GPCRs

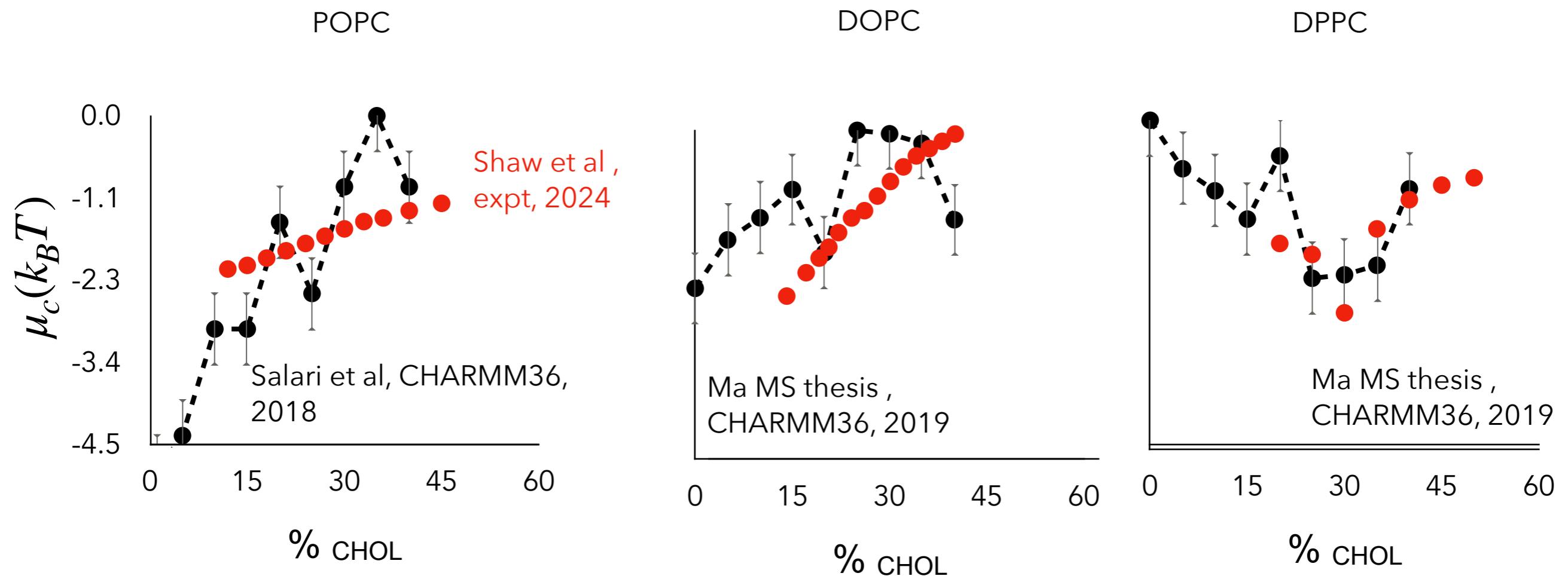
1. Decouple cholesterol from a binary cholesterol:POPC membrane at multiple concentrations
2. Decouple cholesterol from crystallographic sites on 3 different GPCRs
 - Beta2 adrenergic
 - 5HT-2B
 - Mu opioid
3. Combine to get virtual binding assay!

JCTC Journal of Chemical Theory and Computation Article
Cite This: *J. Chem. Theory Comput.* 2018, 14, 6560–6573 pubs.acs.org/JCTC

A Streamlined, General Approach for Computing Ligand Binding Free Energies and Its Application to GPCR-Bound Cholesterol

Reza Salari,^{†,‡} Thomas Joseph,^{†,§} Ruchi Lohia,[†] Jérôme Hénin,^{||,‡,¶} and Grace Brannigan^{*,†,‡,||,¶}

Step 1. Decouple cholesterol from membrane

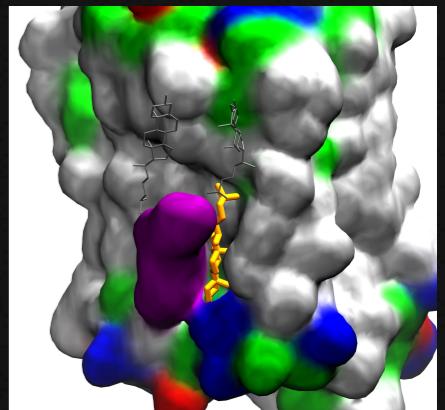
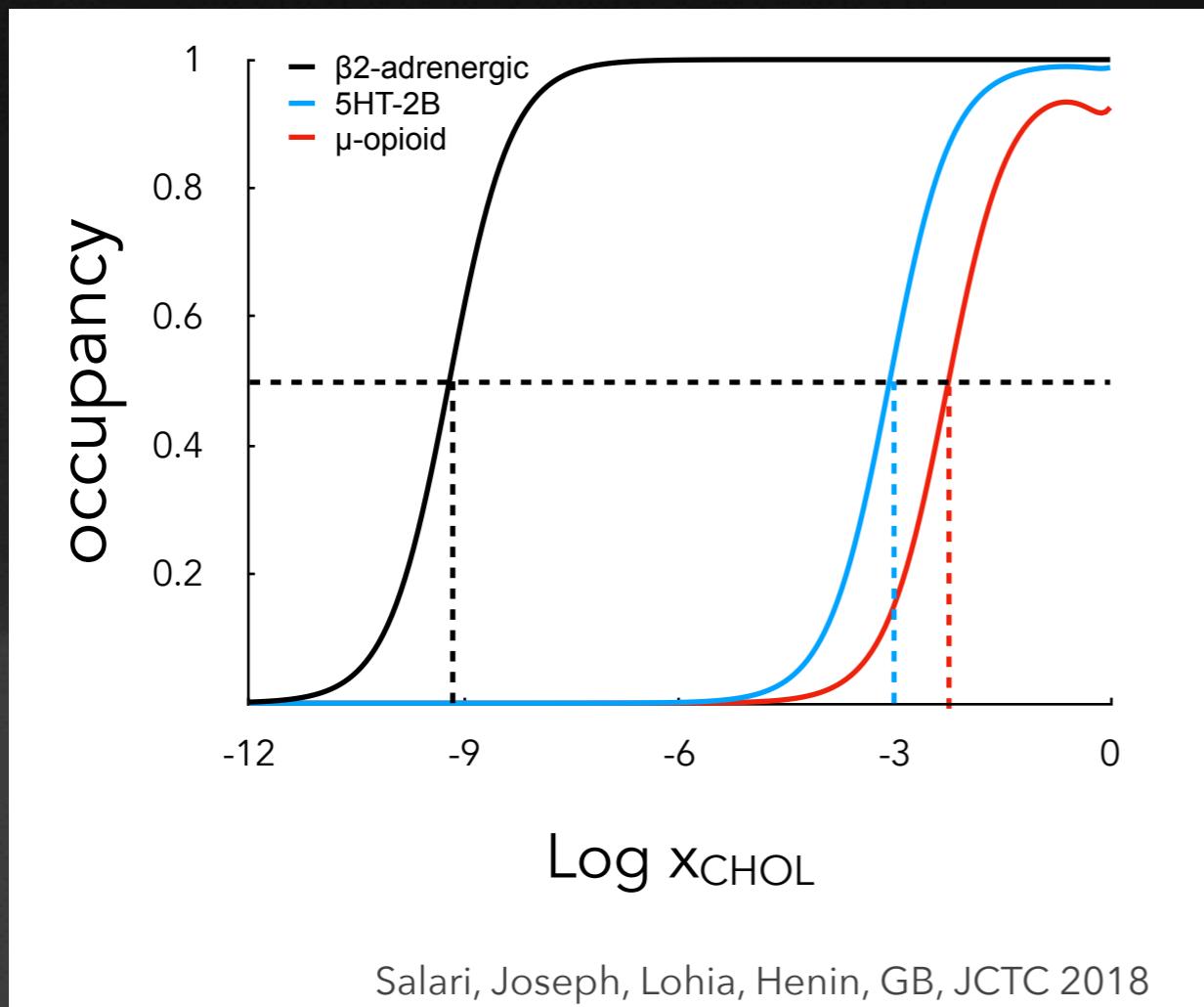


Wait a second...

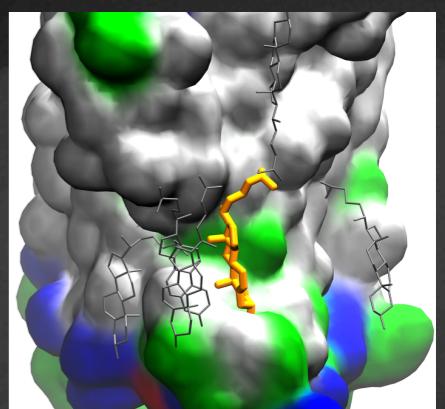
...that's the chemical potential of Charmm36
cholesterol in a membrane! Huh. Well, anyway...

Step 2. Decouple from site

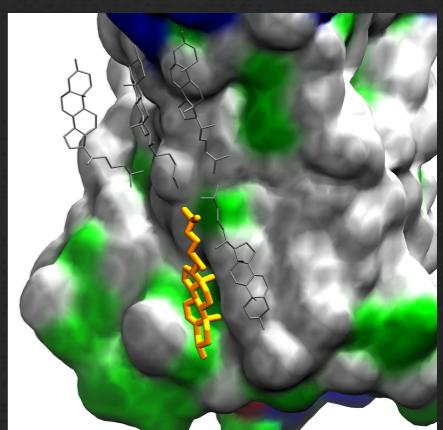
Combine to get binding curve



β_2 -Adrenergic
3D4S
 $x_{50} = 10^{-9}$



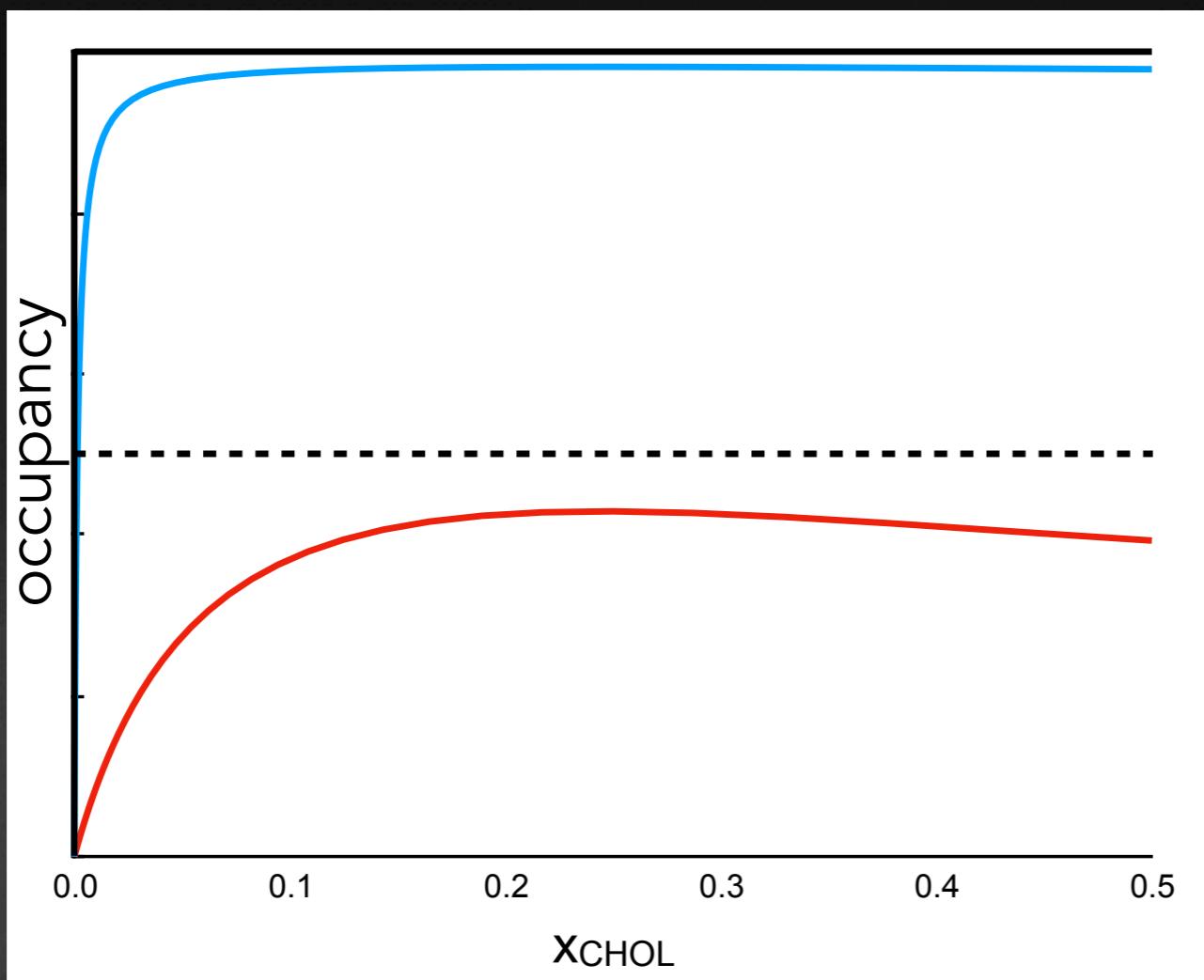
5-HT2B
4NC3
 $x_{50} = 10^{-3}$



μ -Opioid
5C1M
 $x_{50} = 10^{-2}$

...ok, that worked pretty well!

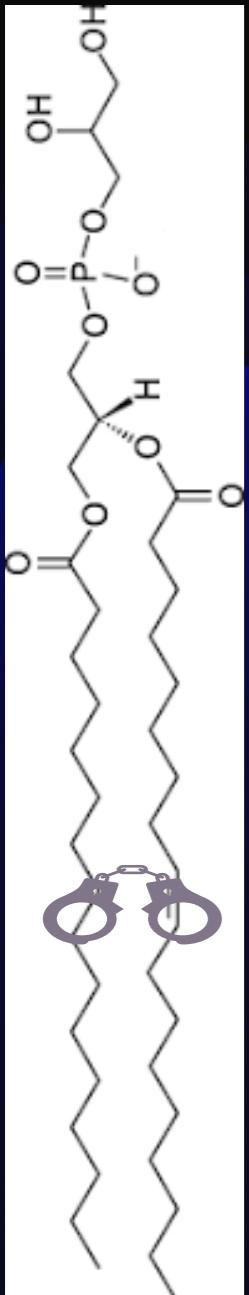
ASIDE: Suppose the affinity for the mu opioid site was just a little lower....



non-ideality of a
randomly
mixed bulk
membrane is
sufficient for
non-monotonic
binding +
functional
effects!!!!

Salari, Joseph, Lohia, Henin, GB, JCTC 2018

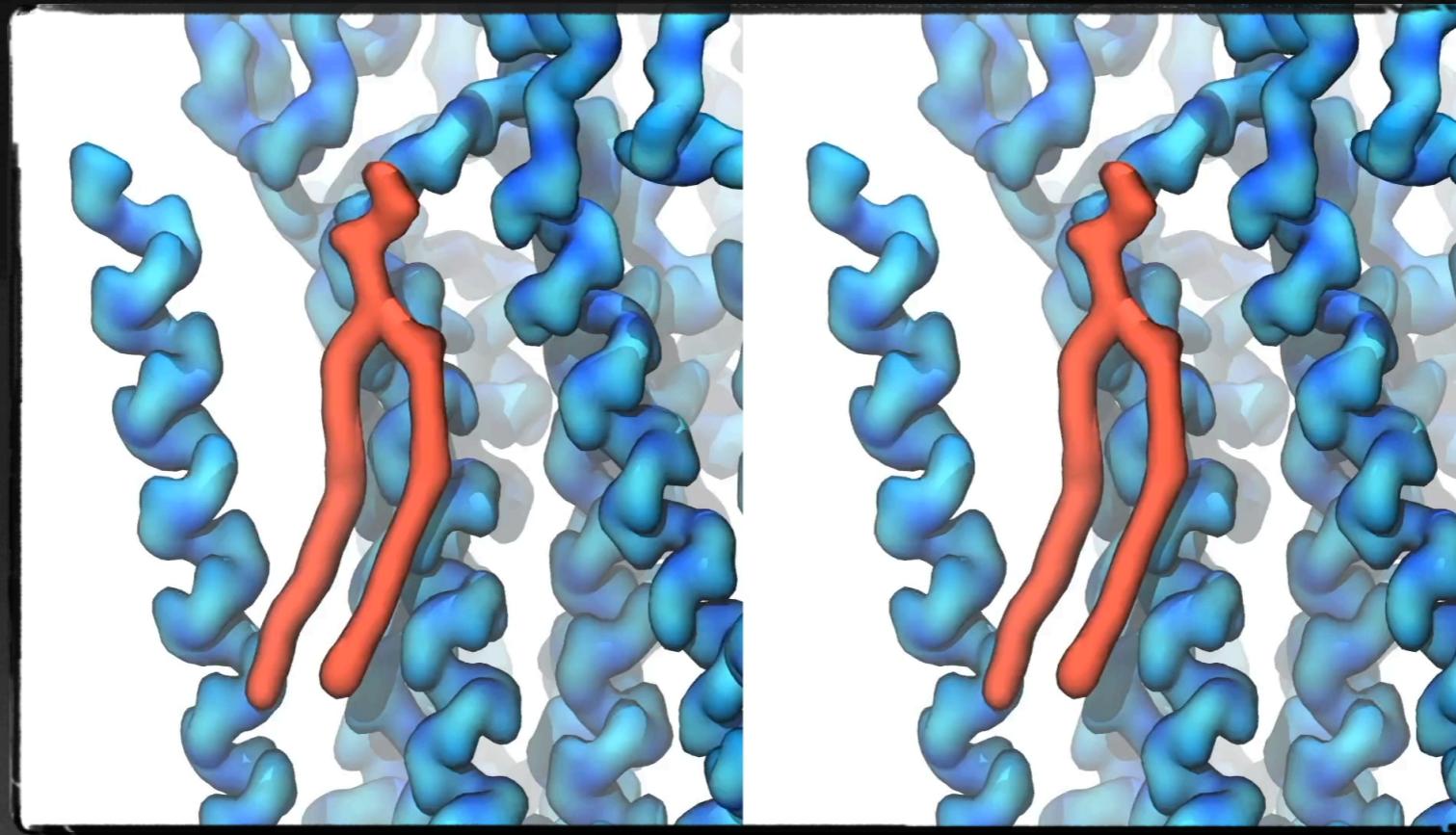
Back to the case!



Where we left off: POPG was found at a likely crime scene, but we didn't know how much POPG you would need OR if binding would do anything.

POPG was brought in for SAFEP questioning.

POPG under questioning



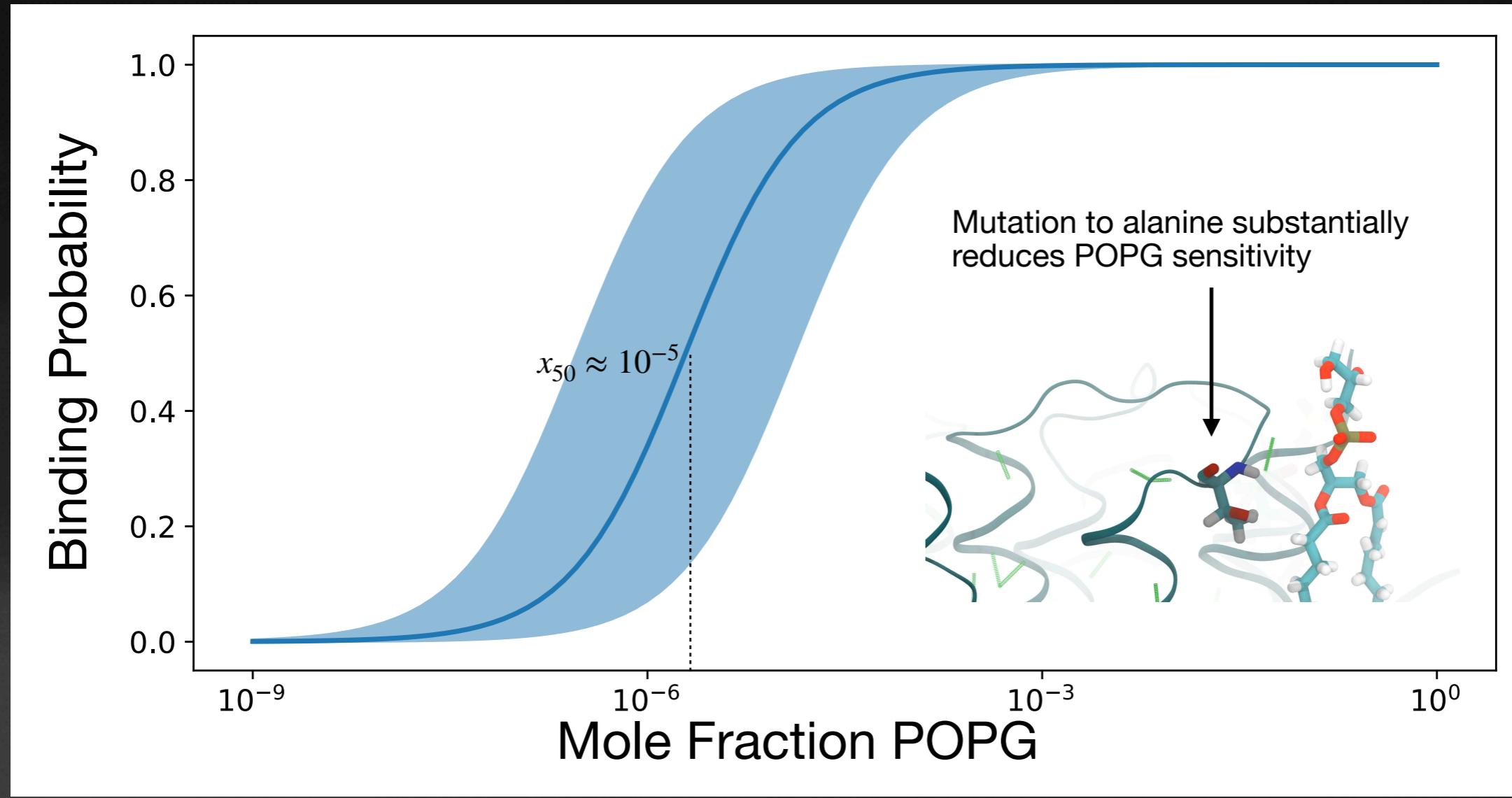
-Ezry St. Iago-McRae

Decoupling POPG from ELIC ($\Delta G = 92.3 \pm 0.6$ kcal/mol)

Audio represents size of free energy difference per window

Not shown: decoupling POPG from POPC ($\Delta G = 84.0 \pm 1.0$ kcal/mol)

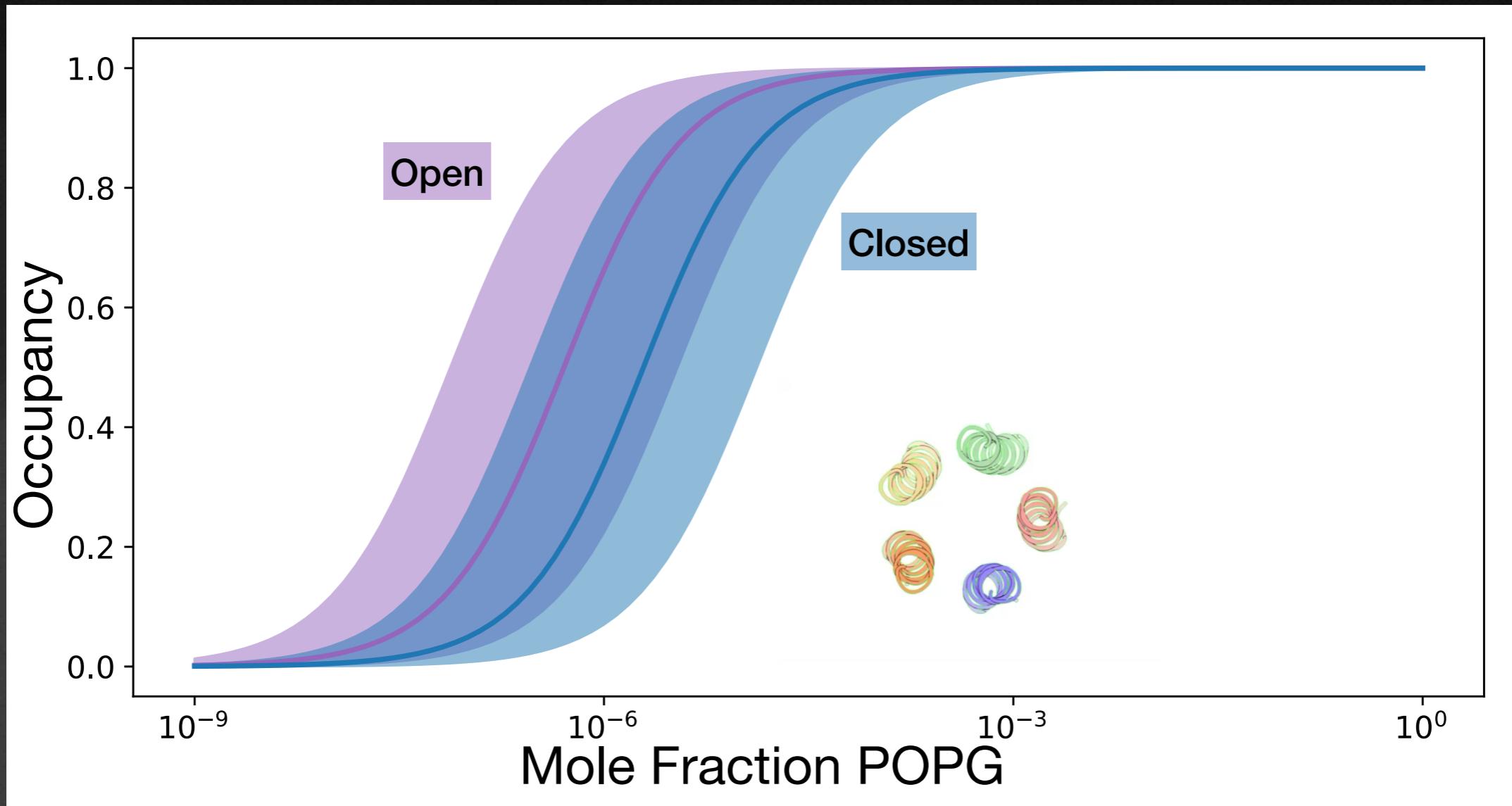
Would the site be occupied at experimental concentrations?



Prediction: only trace amounts of POPG are required for binding to 1 site (but 1 may not be sufficient for an effect)

Non-ideality: 20% POPG bulk is not significantly different
(but we are improving precision)

State dependent binding?

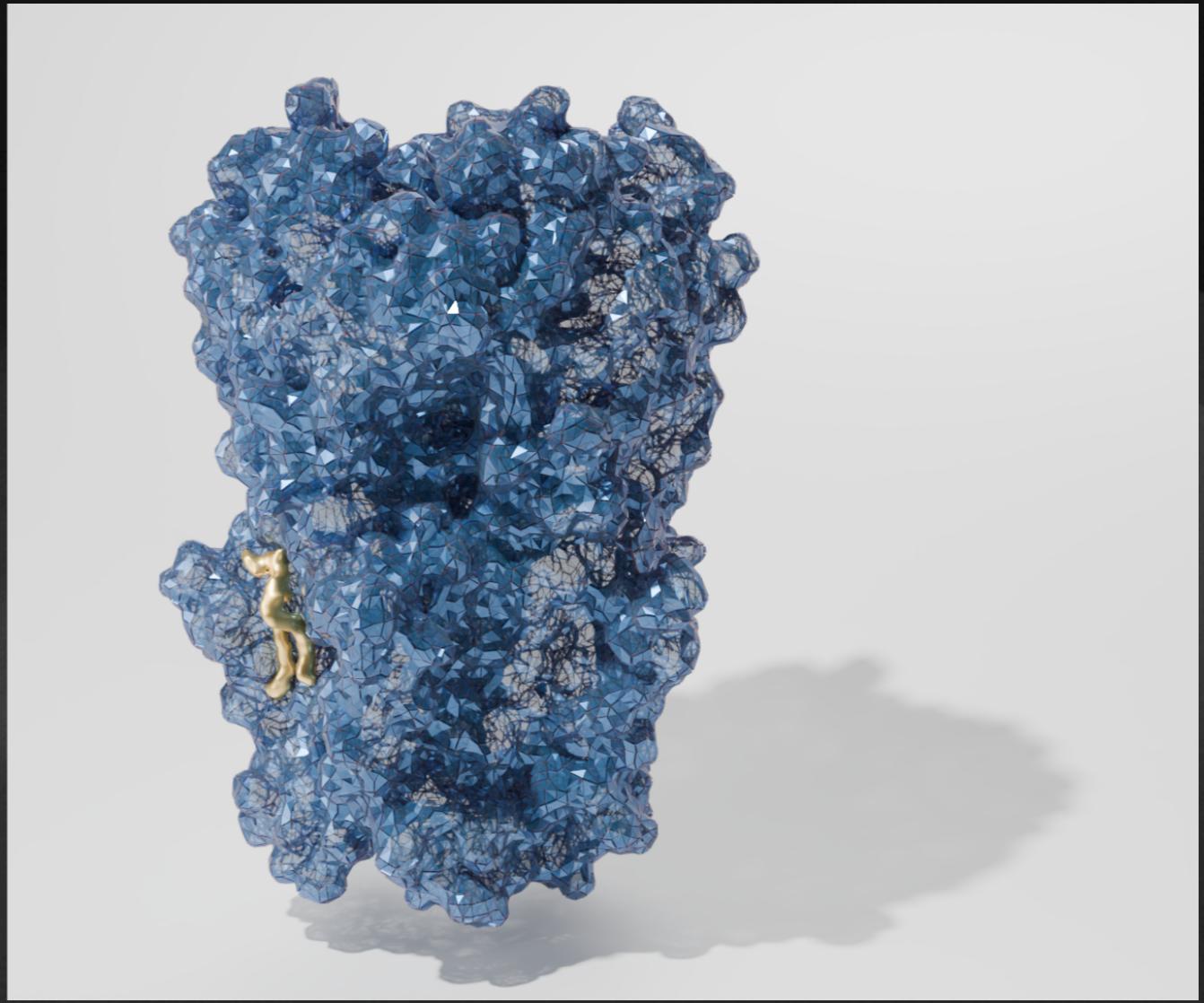


Yes! POPG seems to stabilize the open state.

Closing Statement

Used SAFEP to show that....

- POPG binds with high affinity to this modulatory site : it was at the scene!
- POPG outcompetes POPE, while POPC is an innocent bystander
- POPG binds with higher affinity to the open state than the closed state: not a victimless crime!
- Consistent with cryo-EM and functional data



Humans of the jury, I urge you to find POPG guilty of modulation by direct binding!

Case: finding boundary lipids of the nicotinic acetylcholine receptor in native membranes



Lead detectives: **Dr. Liam Sharp**

**Jesse
Sandberg**

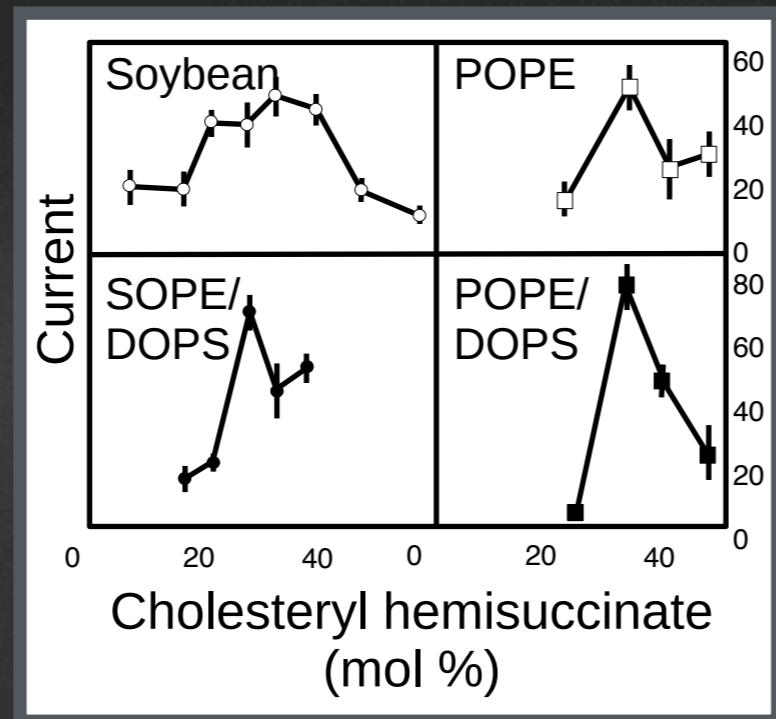
Case Report

Subject



nAChR: the
nicotinic
acetylcholine
receptor

Non-monotonic cholesterol sensitivity

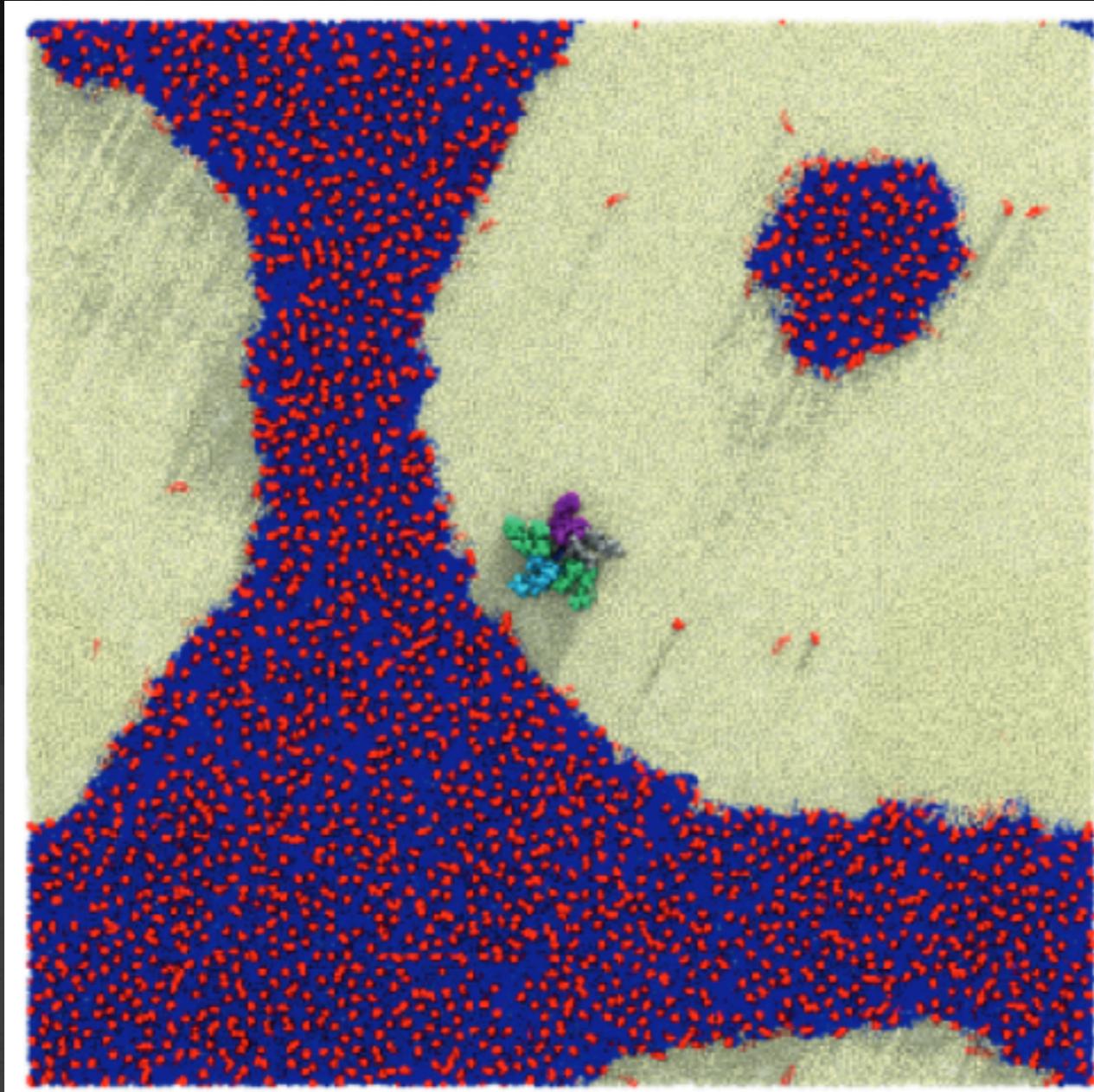


Criado...Barrantes, 1984,
J.Biol.Chem.
+ ~100 other papers

Is nAChR
cholesterol
sensitivity tied to
Liquid-ordered
domain
partitioning?

Are there
specific sites for
phospholipids?

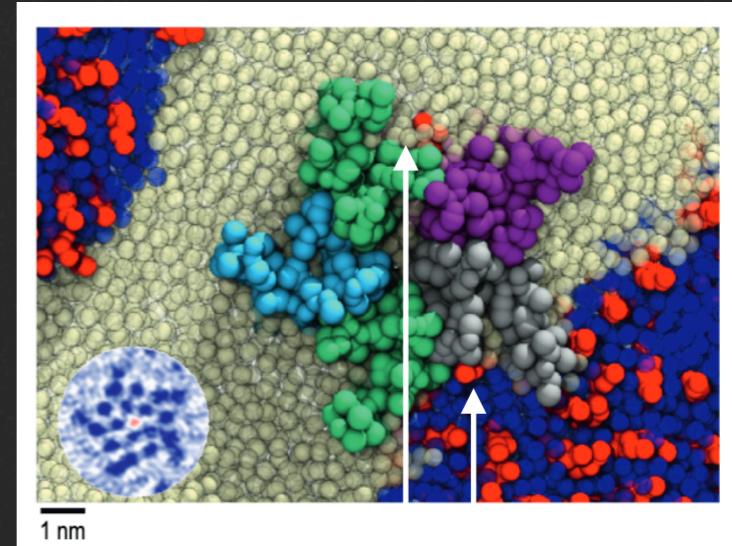
Surprise: Martini nAchr partitions to a cholesterol-poor domain



Sharp, Salari, GB, BBA-Biomembranes 2019

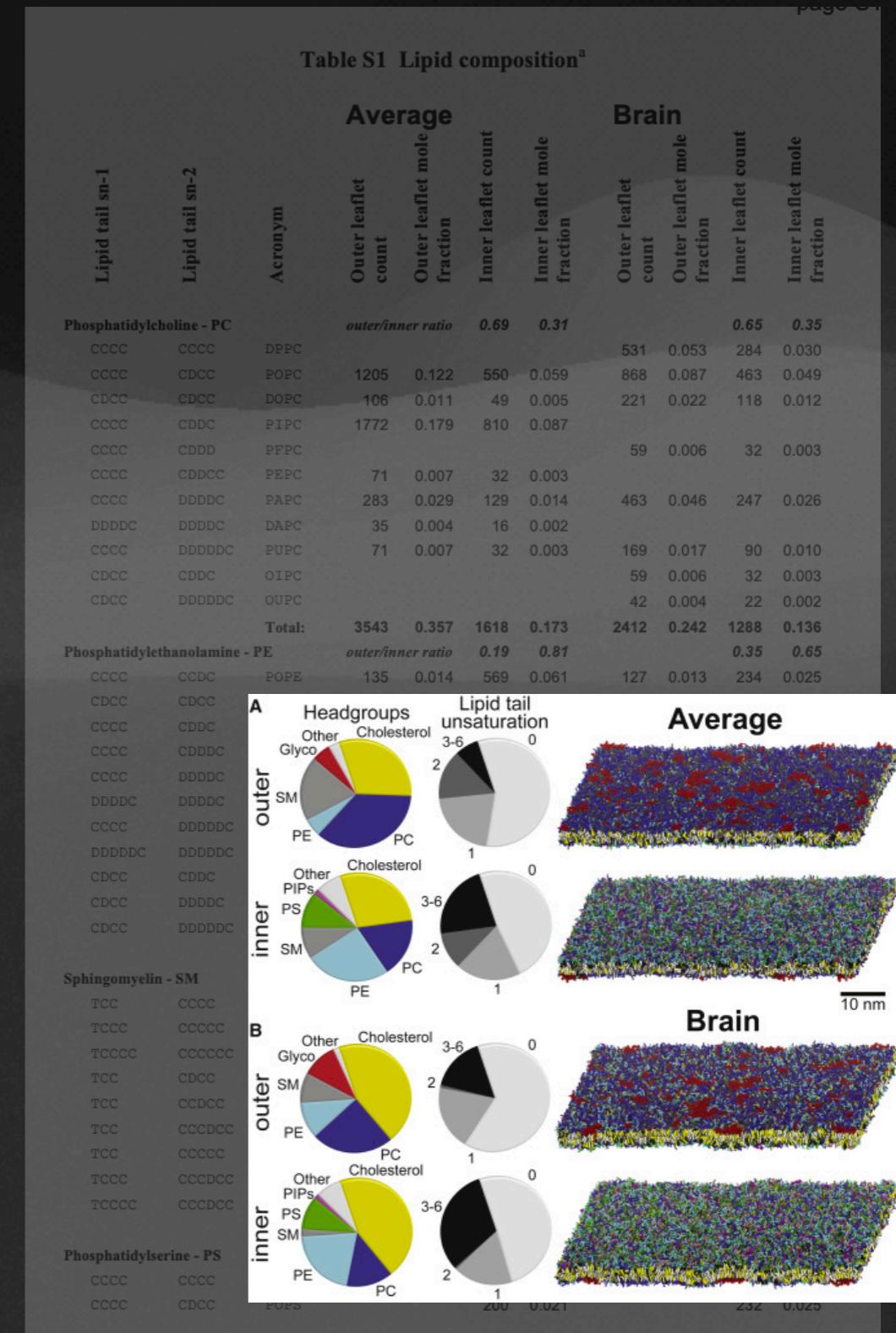
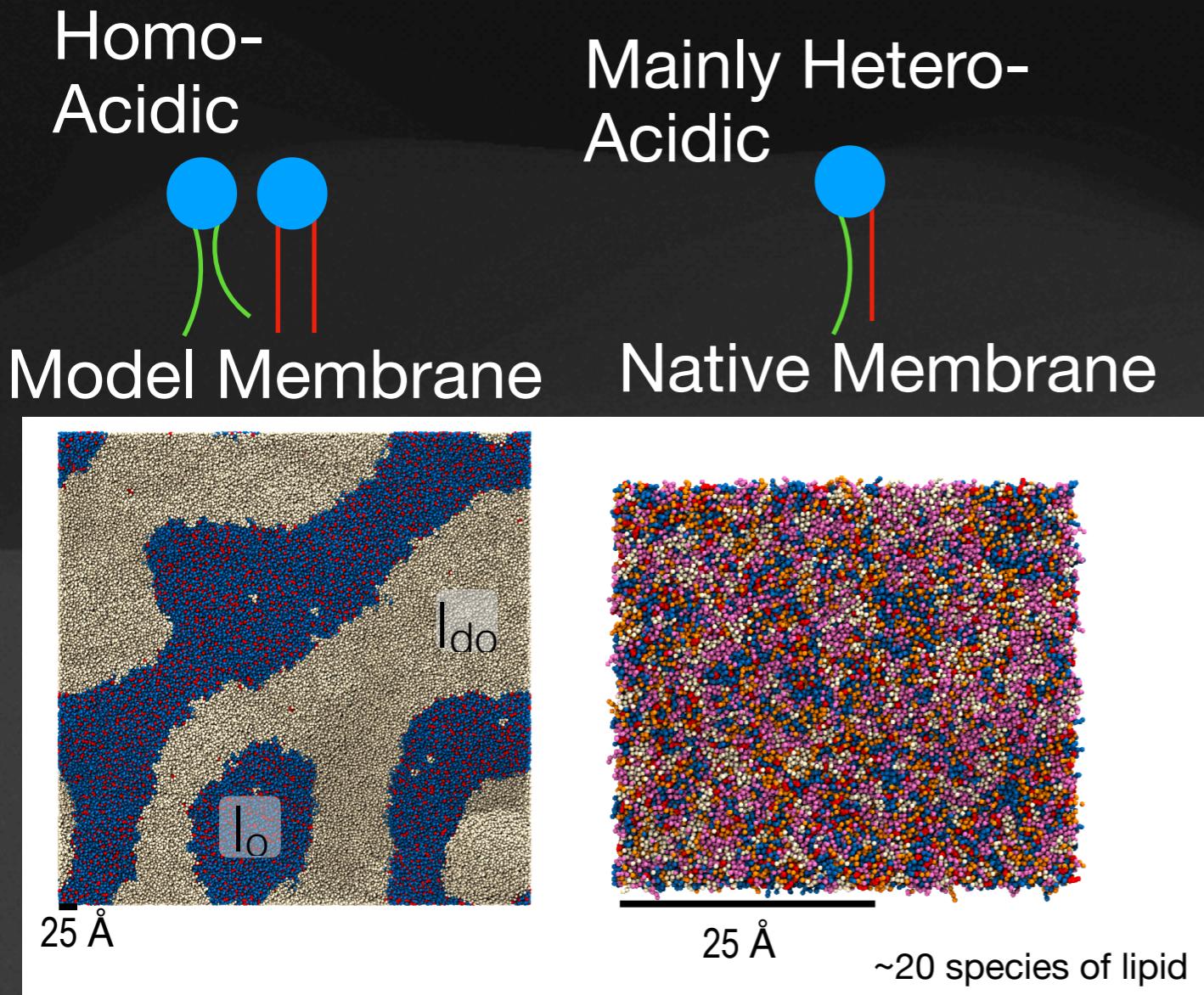
Paraphrasing Sarah Keller

Of course it does!
It's cone-shaped and
pointy. No liquid ordered
domain is going to
accept that.

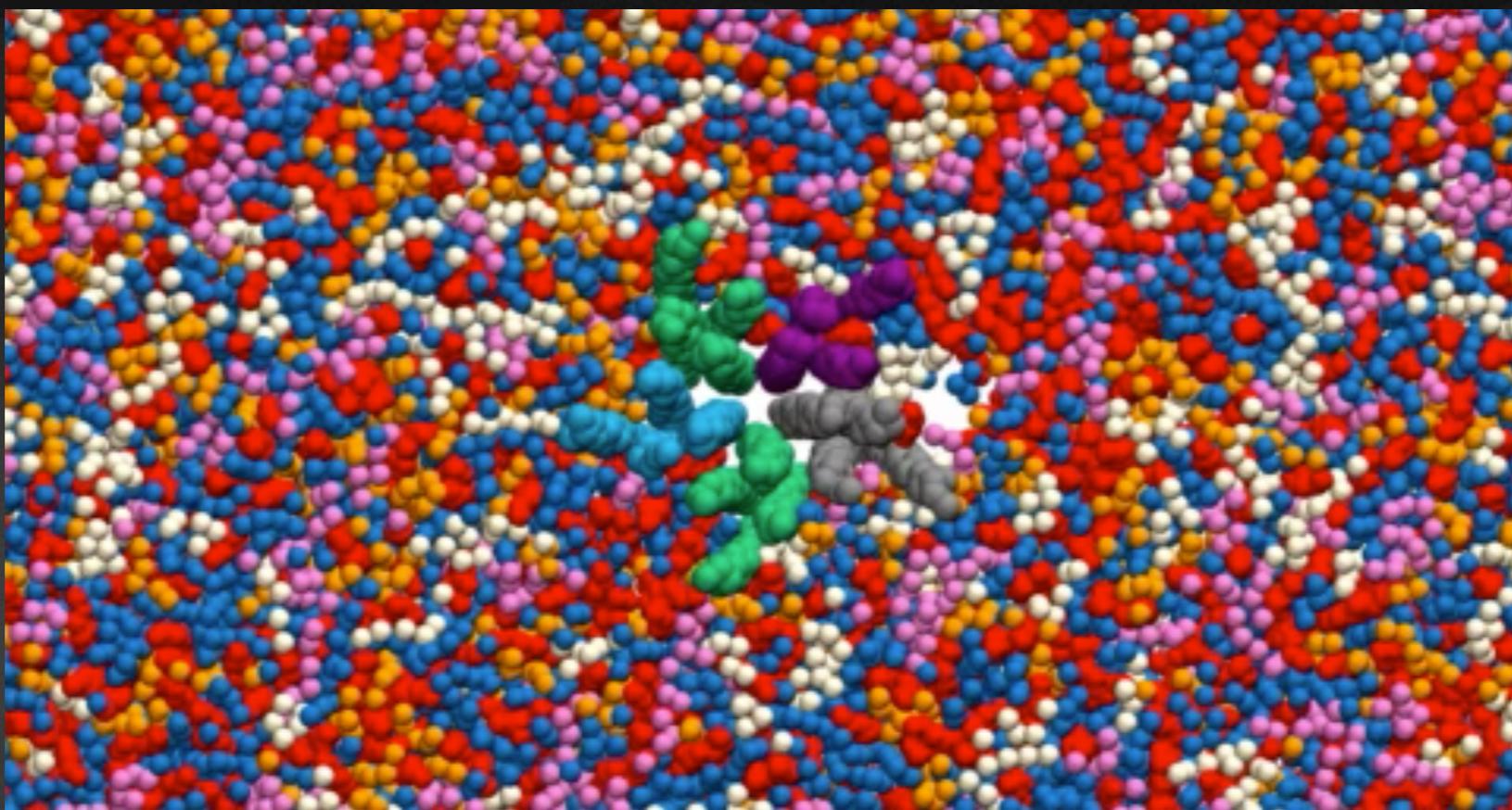


Still maintains interactions with cholesterol!

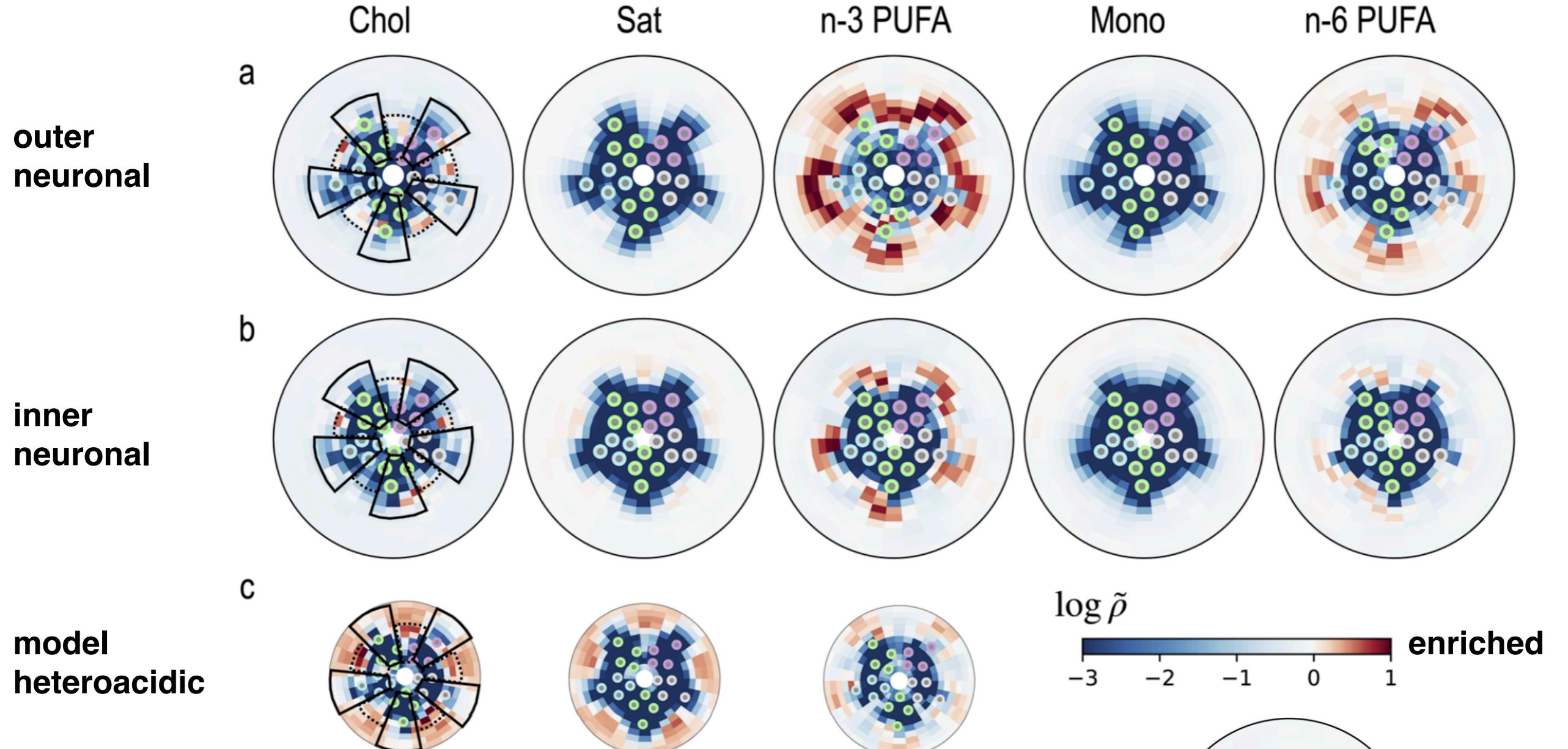
Moving to a quasi-native membrane



Well this is messy

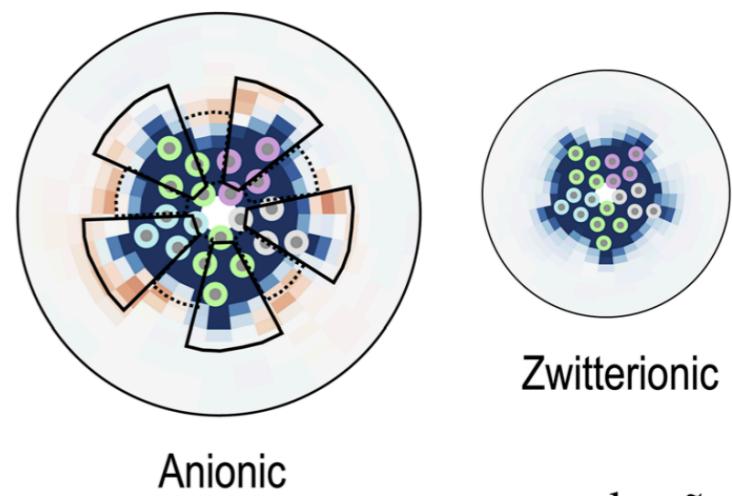


Quantifying density enrichment



Sharp, GB, J. Chem Phys., 2021

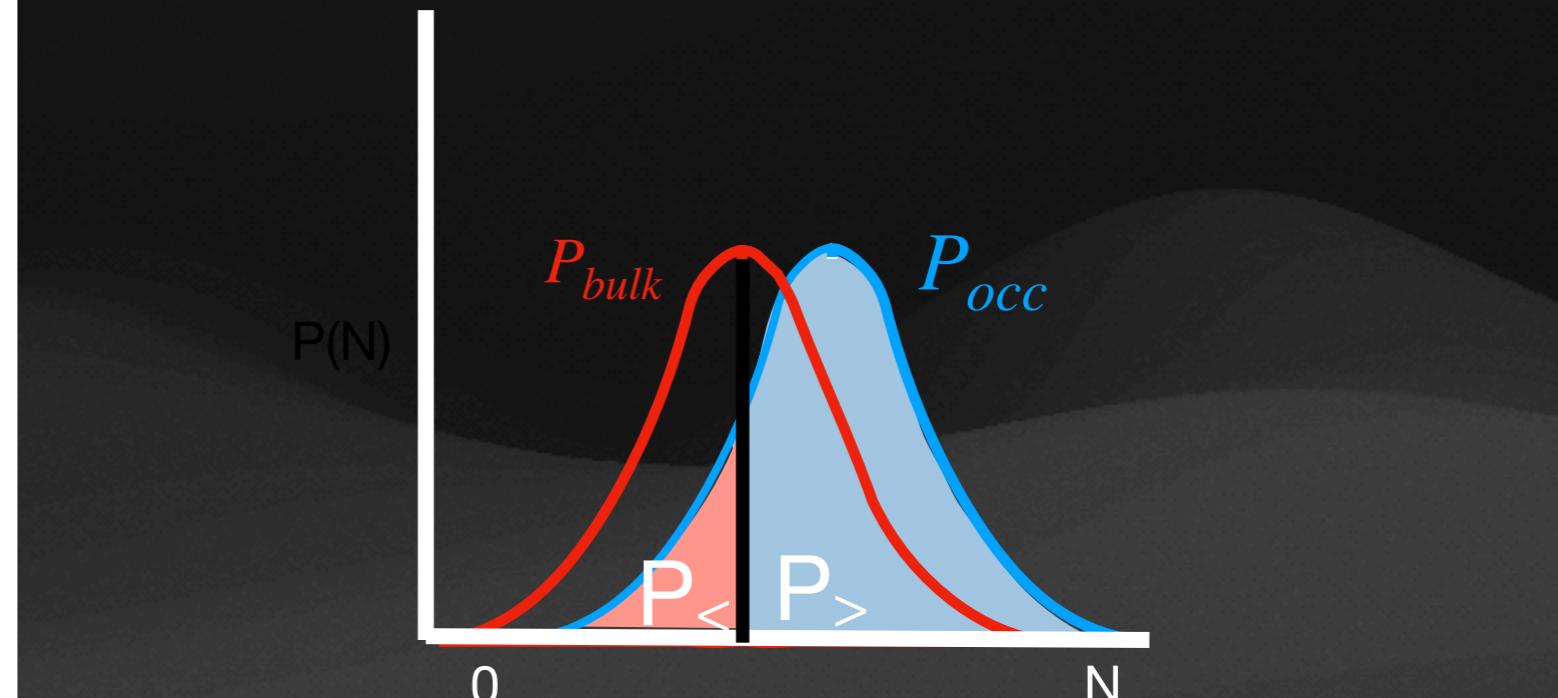
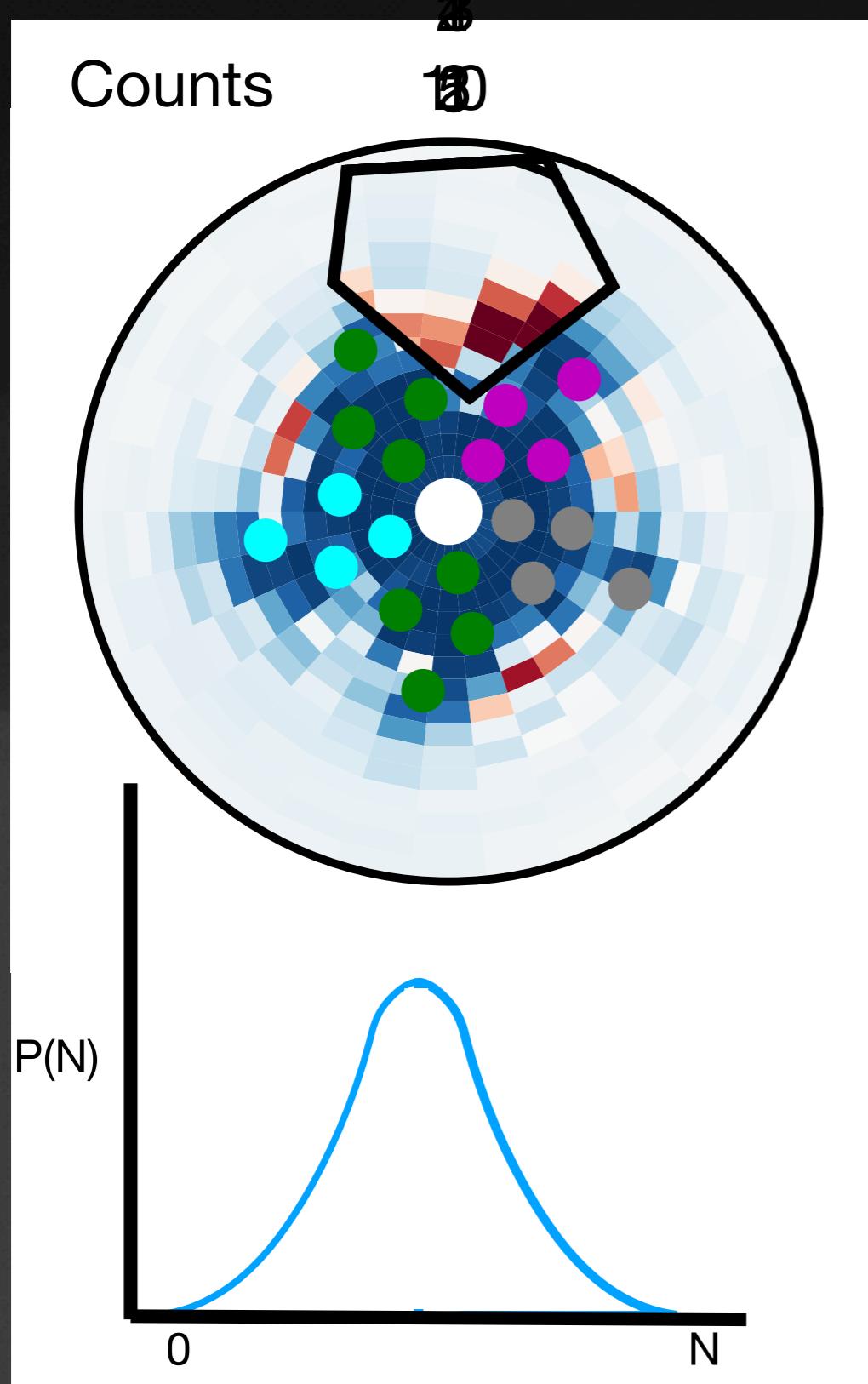
Can we convert these to scalars?



Anionic

Zwitterionic

“Density-threshold affinity”



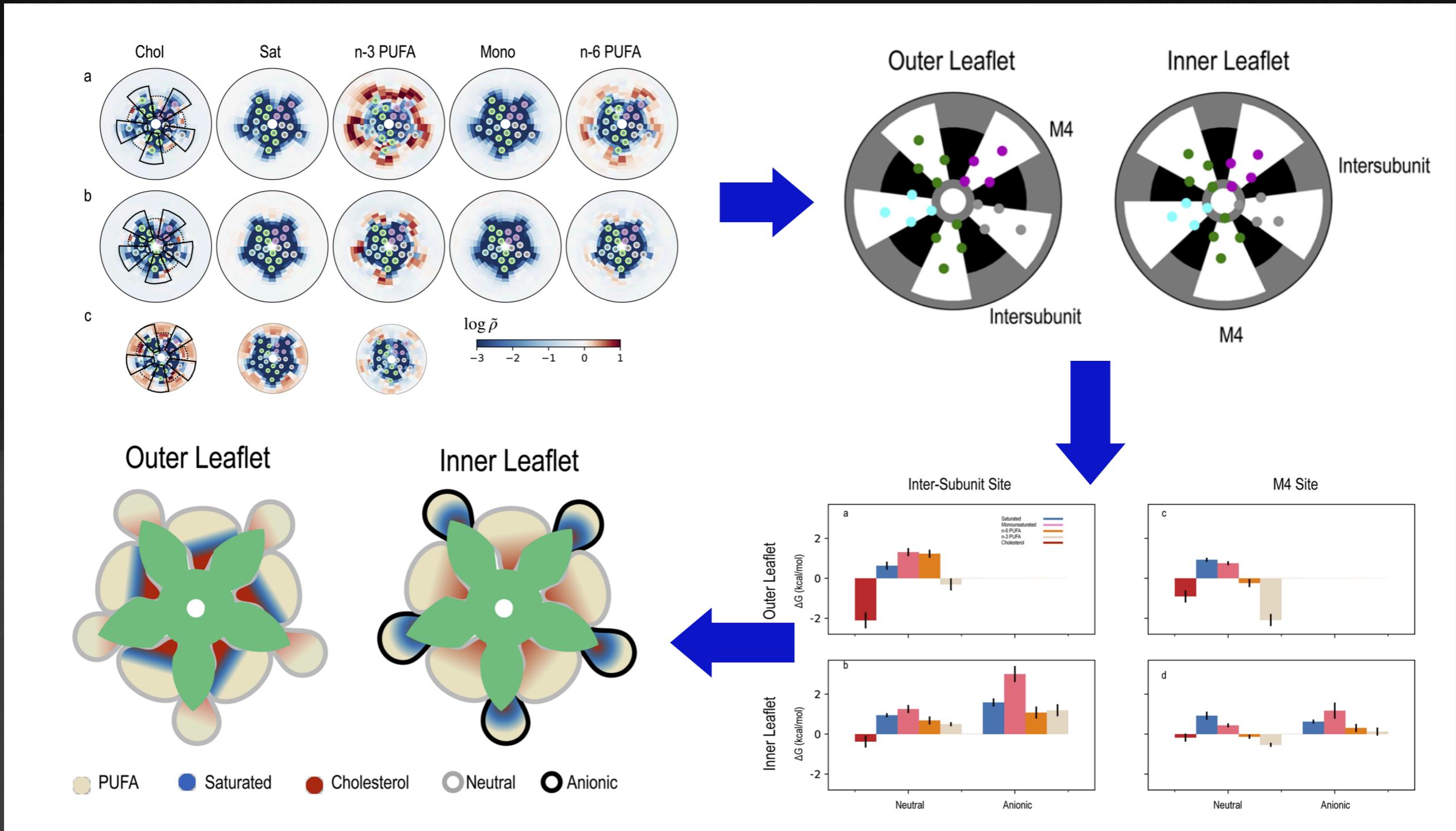
$$\Delta G = -RT \ln \frac{P_>}{P_<}$$

First Application: Sharp, Brannigan, JCP, 2021

General Methods Paper: Sandberg,
Santiago-McRae, Ennis, GB, MIE, 2024

https://github.com/BranniganLab/density_threshold_affinity

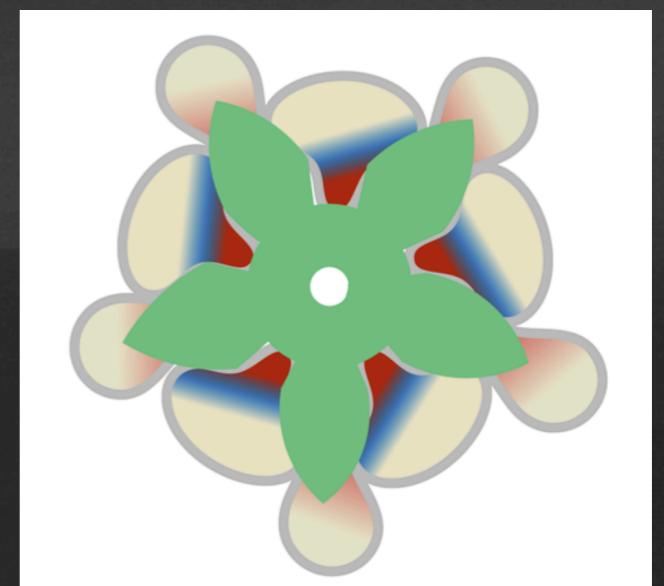
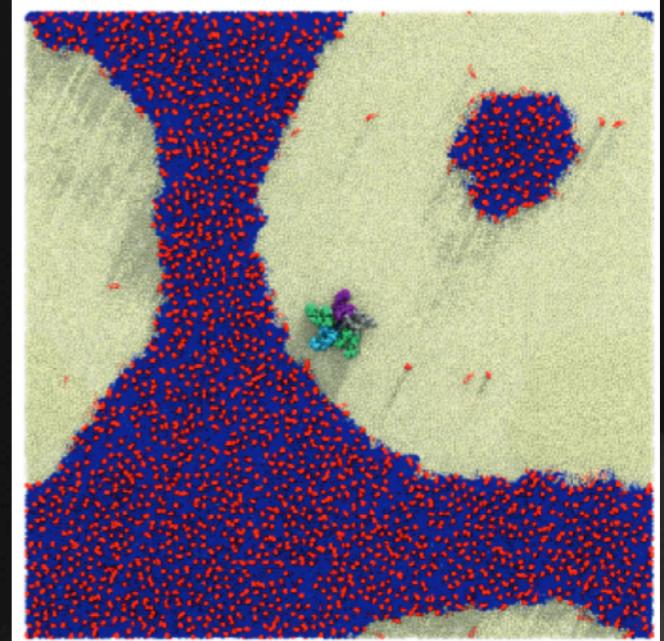
Using DTA to construct the mean-field paralipidome



Closing Statement

Martini 2 + DTA suggests that:

- Liquid ordered domains do not welcome pLGICs!
- Concave, intersubunit sites are a haven for cholesterol escapees.
- Bound cholesterol is a bad influence — draws in saturated chains for the next shell!
- Saturated chains drag along their PUFA partners, who help insulate the proper membrane from the packing and curvature defects caused by the nAChR!
- pLGICs are troublemakers!!!



Now using DTA for...

Supporting evidence for ELIC cases (w Wayland Cheng, Wash U-St Louis)

FFA binding to nAChR in oocyte membranes (w/ Pierre-Jean Corringer, Pasteur Institute)

Ceramides binding to BAM A (Jahmal Ennis w/ Eric Klein, RU-C)



Methods in Enzymology
Available online 4 April 2024
In Press, Corrected Proof  [What's this?](#)

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}  

<https://authors.elsevier.com/a/1is%7EXHRzCT-YQ>



Acknowledgments



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Wayland Cheng and Lab
(Washington University - St. Louis)

