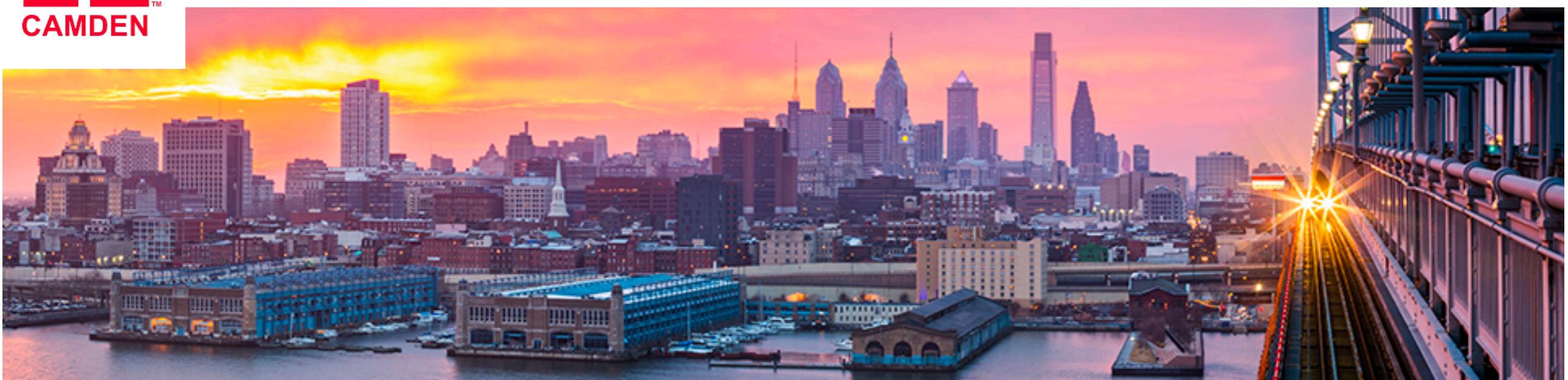


# Specifically-Bound Lipids: Prediction, Detection, and Effect of the Surrounding Membrane

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Center for Computational &  
Integrative Biology  
Rutgers University - Camden



# motivation: pentameric ligand-gated ion channels (pLGICs)



recreation



sedatives

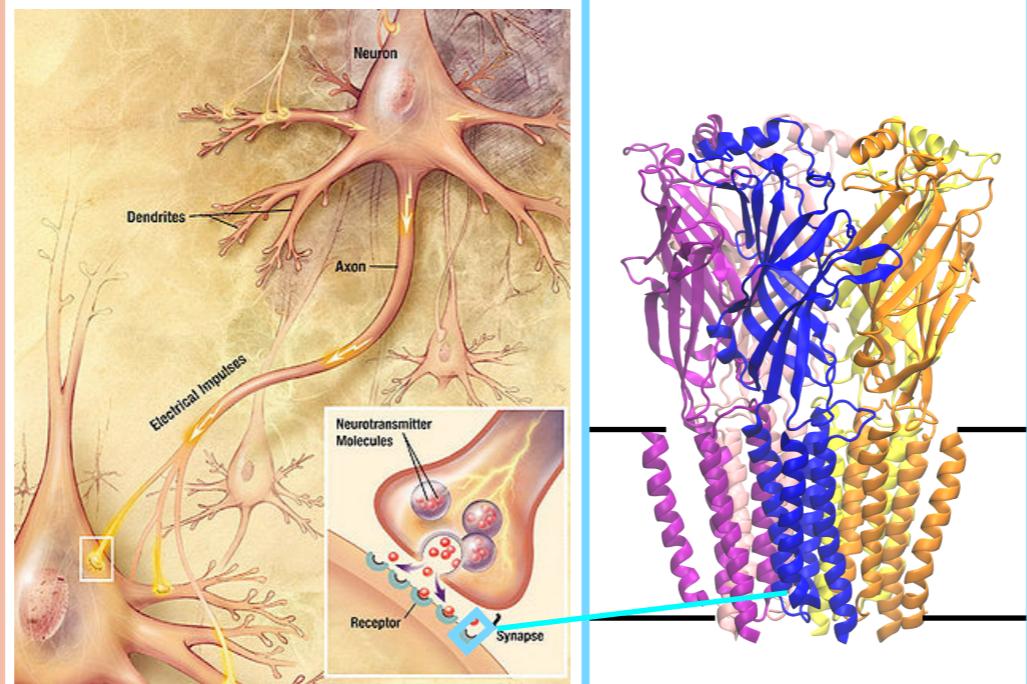


benzodiazapenes



methaqualone

nicotinic acetylcholine receptor, GABA<sub>A</sub> receptor, glycine receptor, etc



poisons



rat poison (TETS)



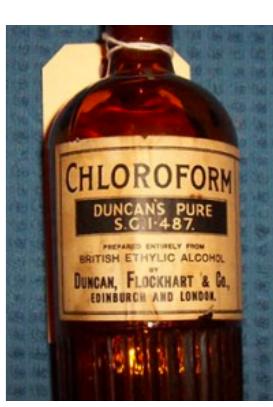
general anesthetics



propofol

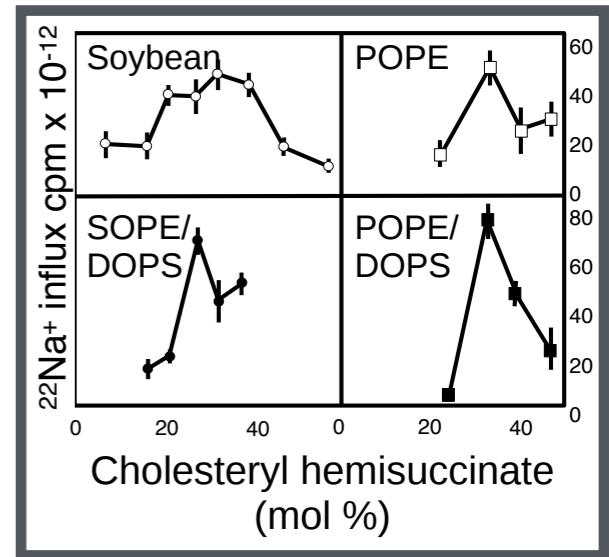


pentobarbital



chloroform

lipids



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



# pLGICs are very picky about lipids!

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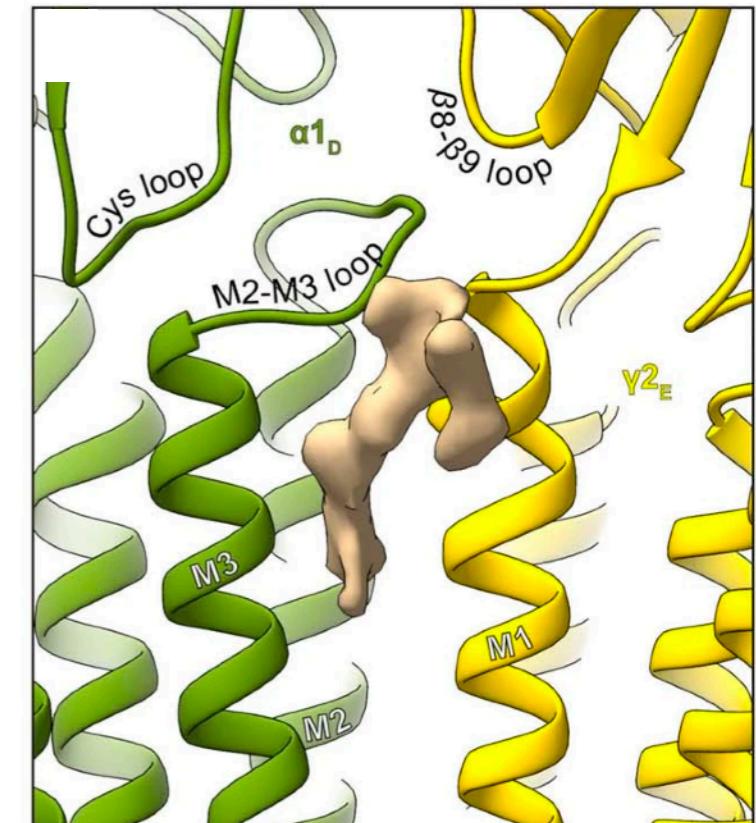
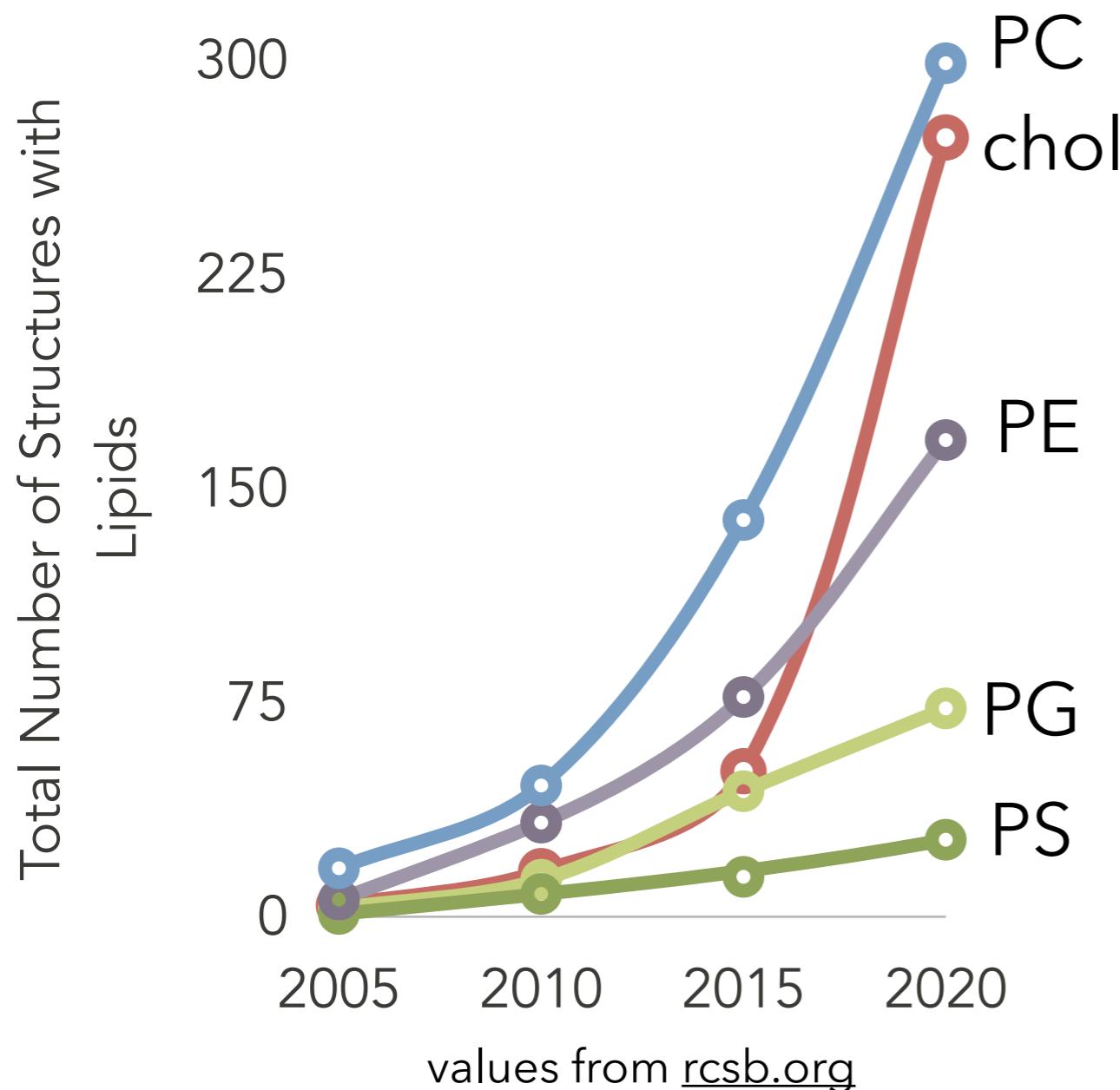


typical membrane protein



pLGICs

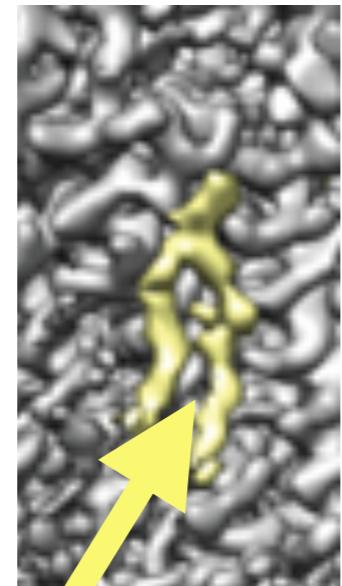
# Structures can tell us where lipids bind...



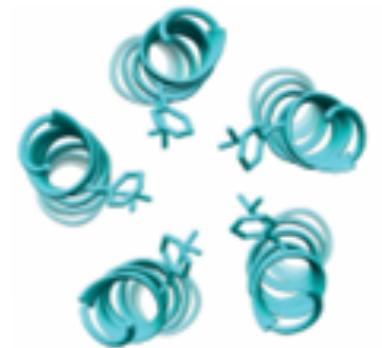
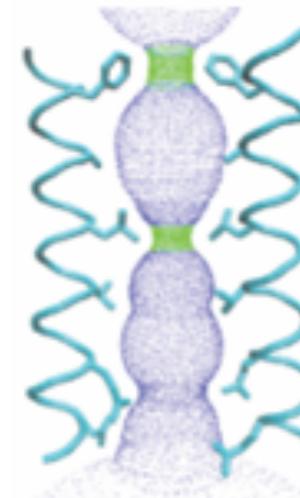
Kim...Hibbs, Nature, 2020

...but not (usually) who is binding.

# Case of the ELIC modulation site

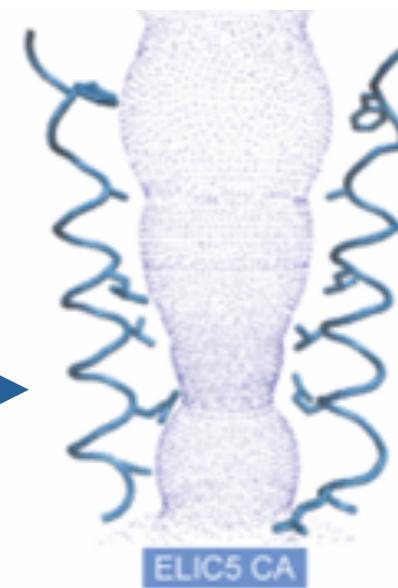


pre-active conformation



WT CA

wide-open conformation



ELIC5 CA

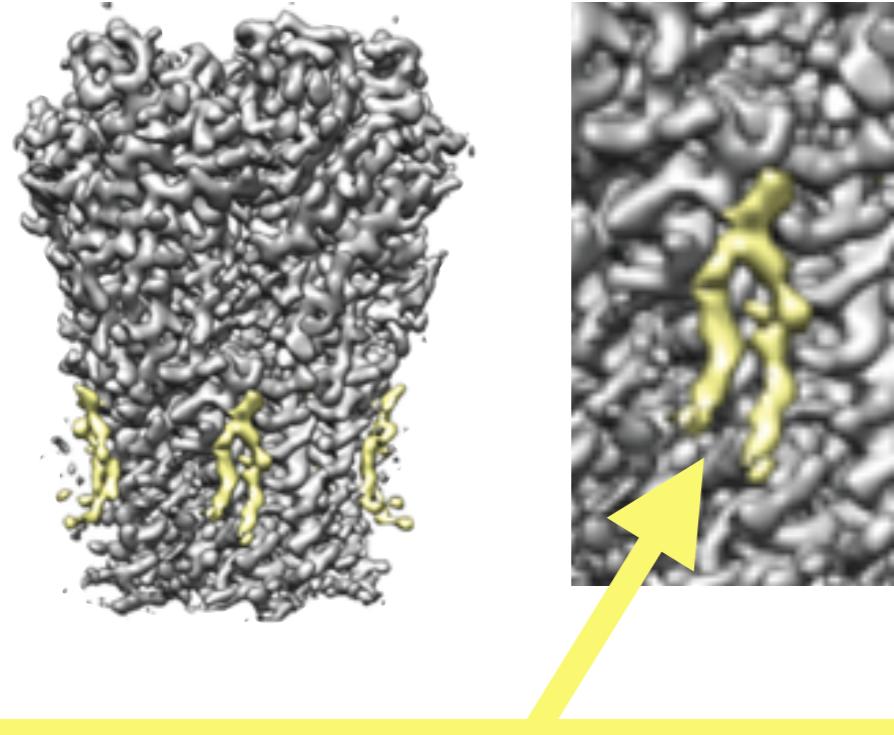
Question 2:  
Did they  
stabilize this  
conformation?



Wayland  
Cheng

John T. Petroff II, ... Ezry Santiago-McRae, ... Tom Joseph, Jérôme Hénin, Grace Brannigan & Wayland W. L. Cheng, Nature Communications 2022

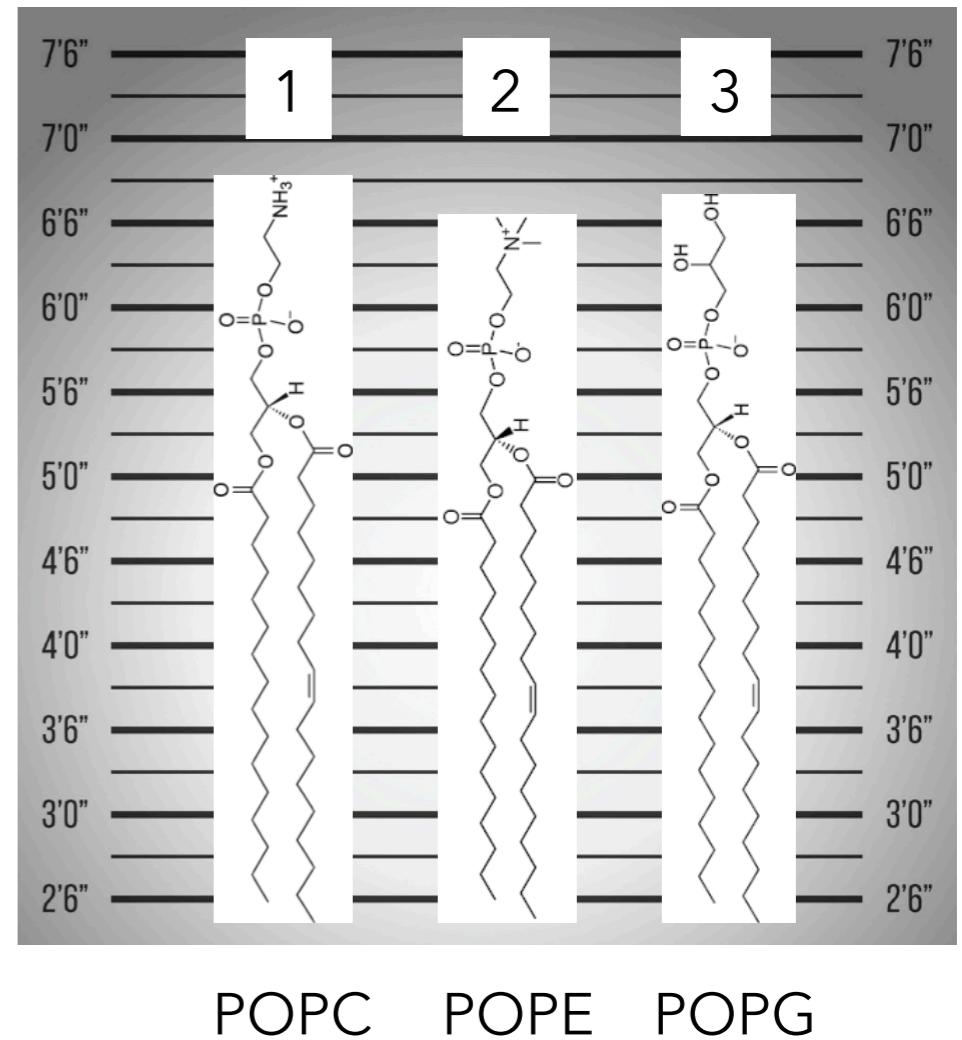
# Fragment identification



Question 1: Who is this?

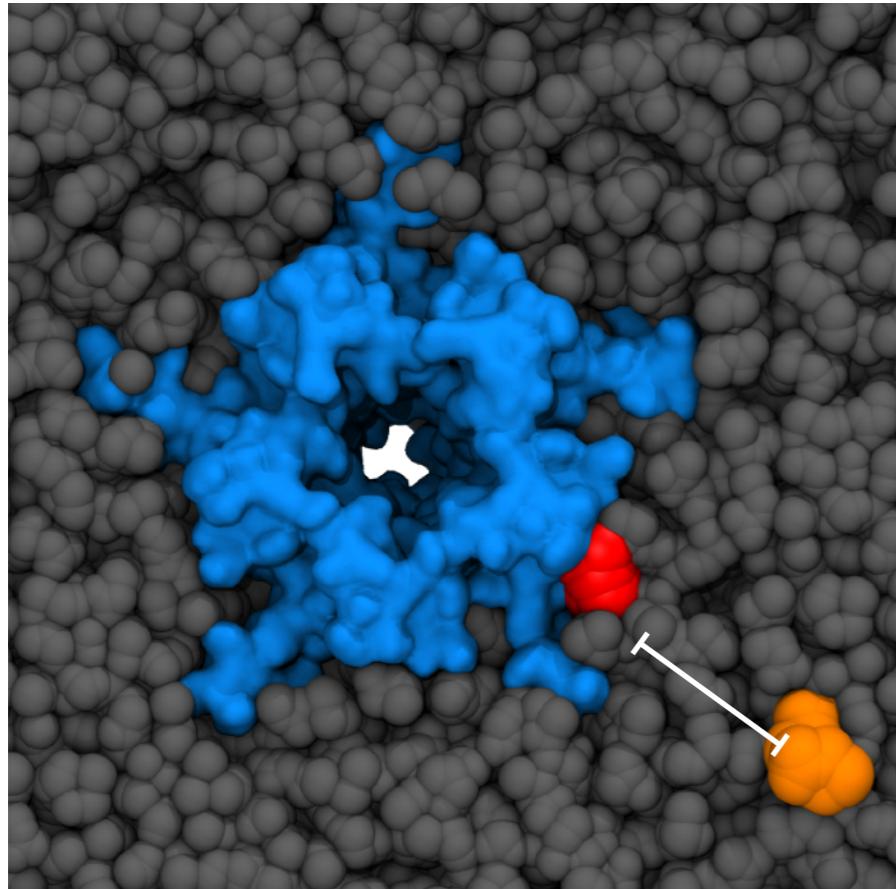
2:1:1 PC:PE:PG nanodisc

Suspects



we're a computational lab...so maybe we should use  
"computational microscopy"?

# Not so fast!!



in real time....how long would it take the **orange** lipid to exchange with the **red** lipid?

about 4 ms in lipid time or....

> 100 **years** of MD simulation time

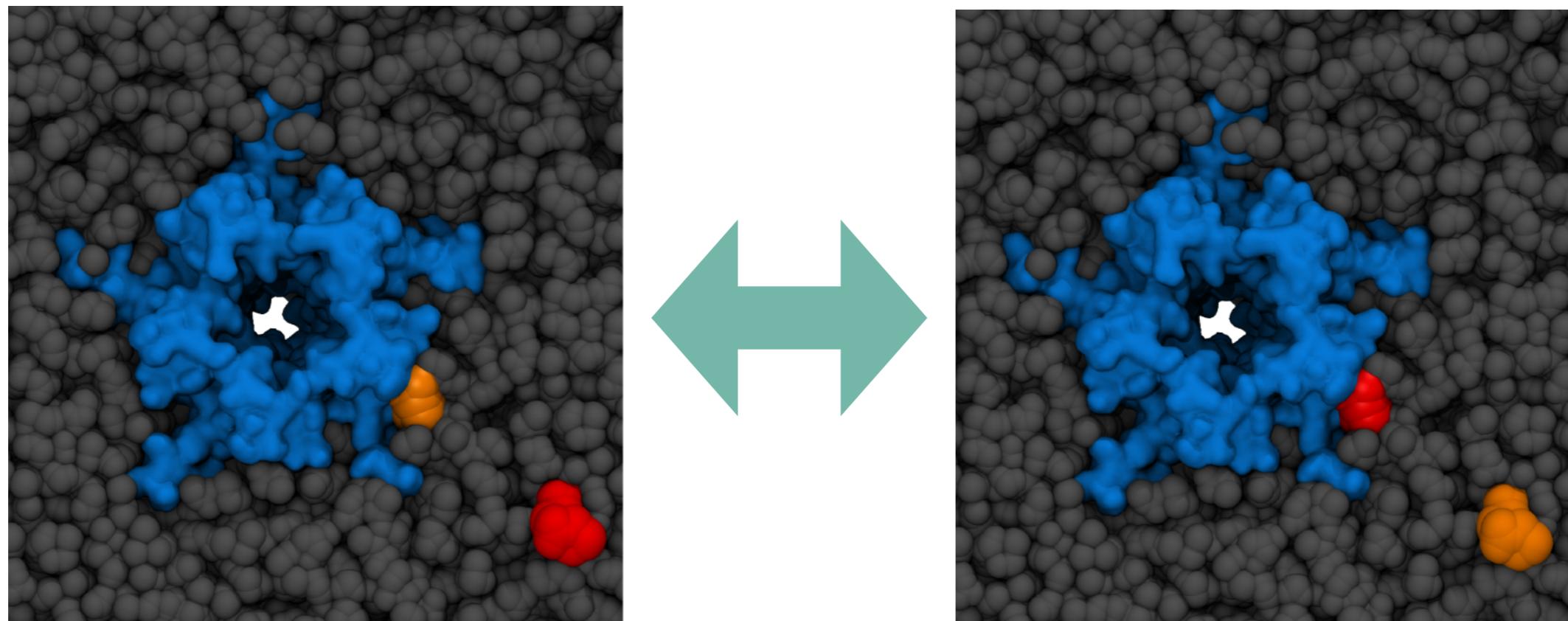
## options:

1. Enhanced spatial sampling?
2. Coarse-grained MD?
3. Avoid diffusion altogether?

# circumventing diffusion via FEP

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"all" we need to know: what is the free energy **difference** between these two states?



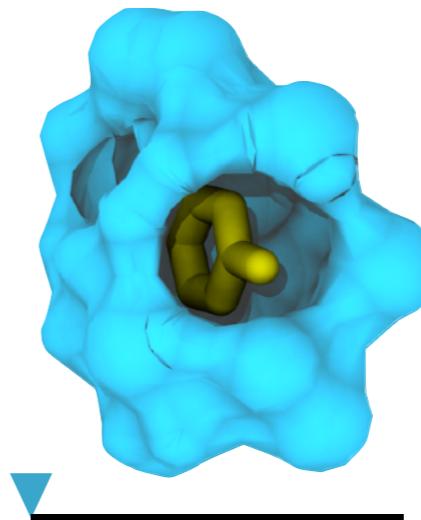
sounds like a job for....alchemical Free Energy Perturbation (FEP)!

# two flavors of alchemical FEP

## Absolute

**How:** Gradually **turn off** the interactions between the ligand and everything else; calculate thermodynamic averages

**Output:**  $\Delta G$  of binding

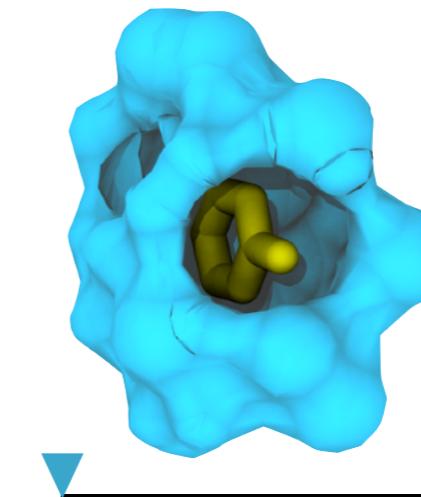


Decoupling Progression

## Relative

**How:** Gradually **transform** one chemical group into another; calculate thermodynamic averages

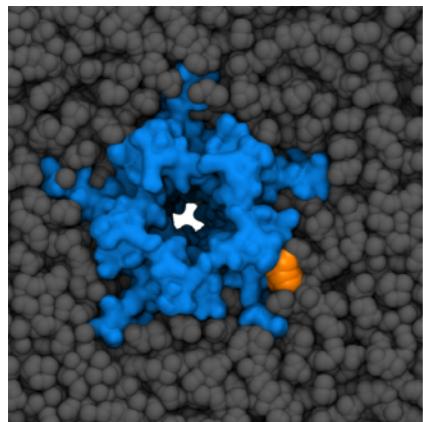
**Output:**  $\Delta\Delta G$  of binding (compare ligands)



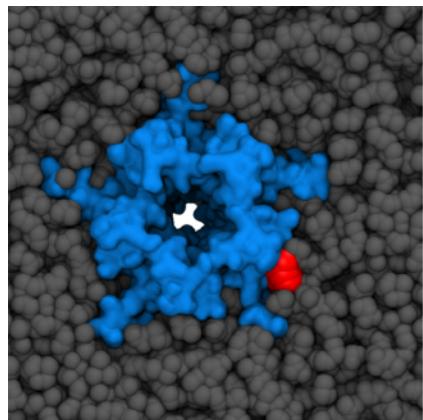
Transformation Progression

# initial plan: relative FEP

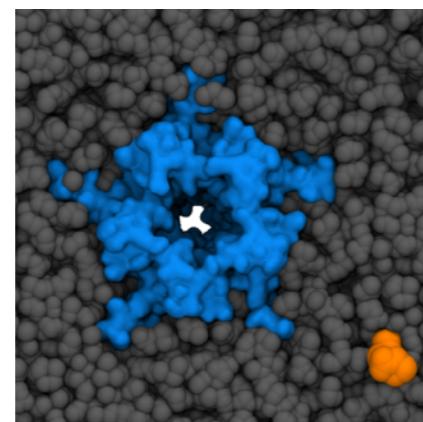
mutate  
in site



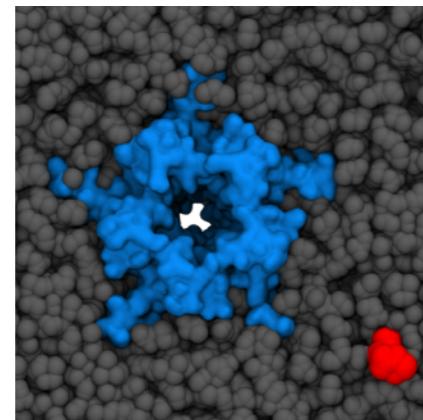
$$\downarrow \Delta G_1$$



mutate  
in bulk

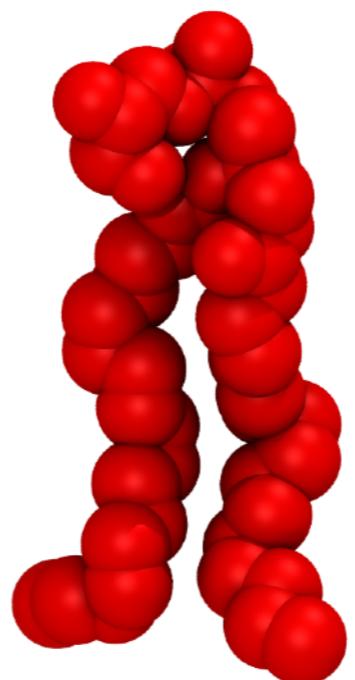


$$\downarrow \Delta G_2$$



If....

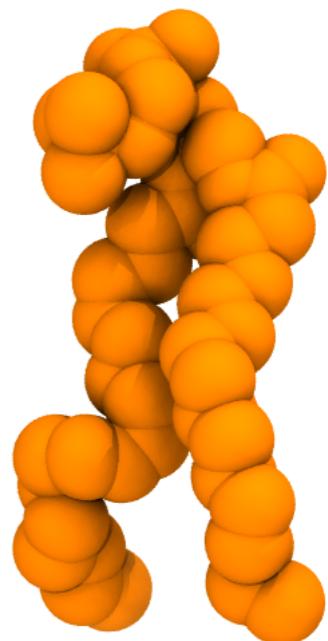
$$\Delta G_1 - \Delta G_2 > 0?$$



not guilty!

But if....

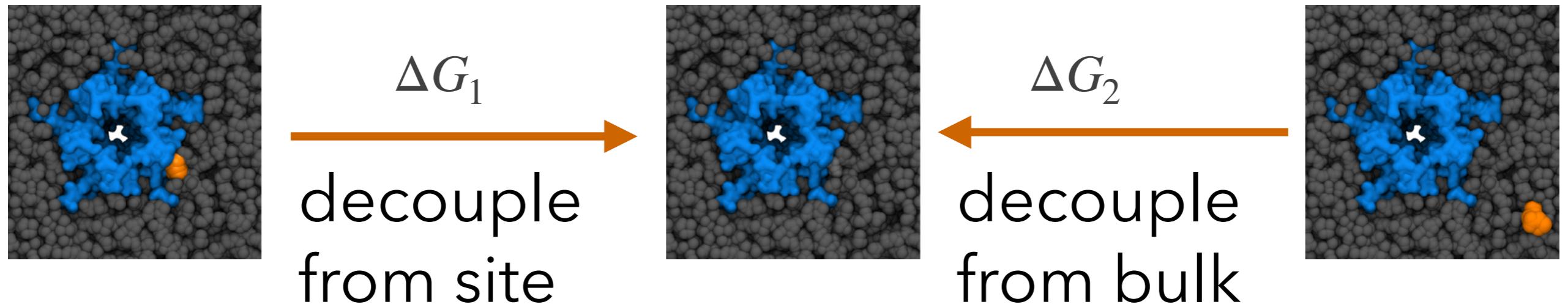
$$\Delta G_1 - \Delta G_2 < 0?$$



not guilty!

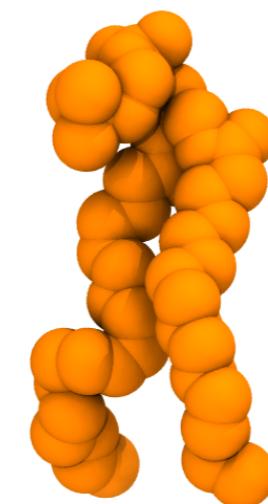
# alternate plan: absolute FEP

best option if one of our lipids is low affinity



If....

$$\Delta G_1 + \Delta G_2 \ll 0?$$



guilty if they  
have the  
opportunity!

# SAFEP: a FEP implementation that works in membranes

**S**treamlined **A**lchemical **F**ree **E**nergy **P**erturbation

FEP but in a site-centered reference frame.

**SAFEP** introduces the “distance-from-bound-configuration” (DBC): RMSD of the ligand in the site’s reference frame



## Tutorial

New Results

Follow this preprint

**Computing absolute binding affinities by Streamlined Alchemical Free Energy Perturbation**

Ezry Santiago-McRae, Mina Ebrahimi, Jesse W. Sandberg, Grace Brannigan, Jérôme Hénin

doi: <https://doi.org/10.1101/2022.12.09.519809>

This article is a preprint and has not been certified by peer review [what does this mean?].

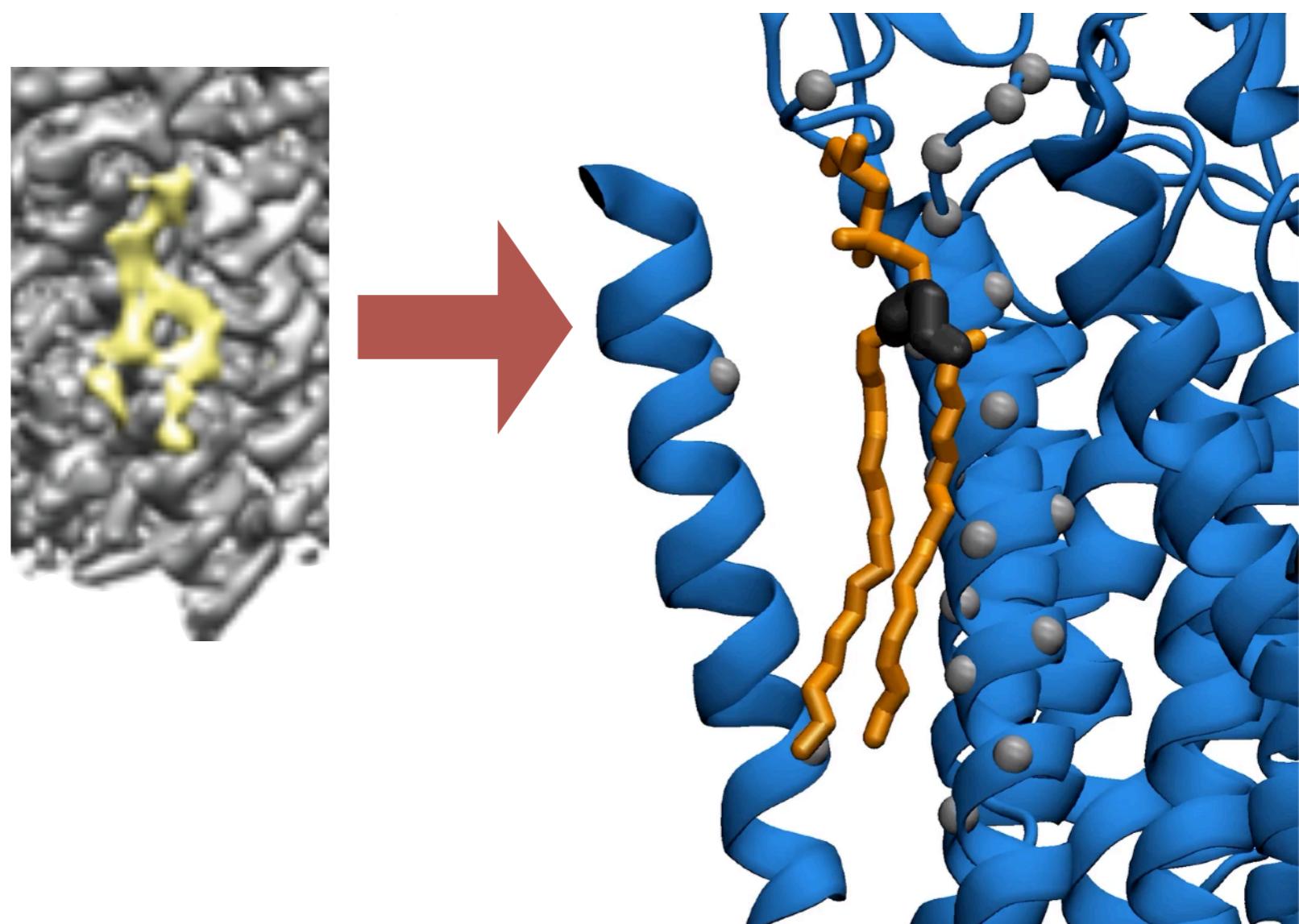
# Running FEP while maintaining DBC

---

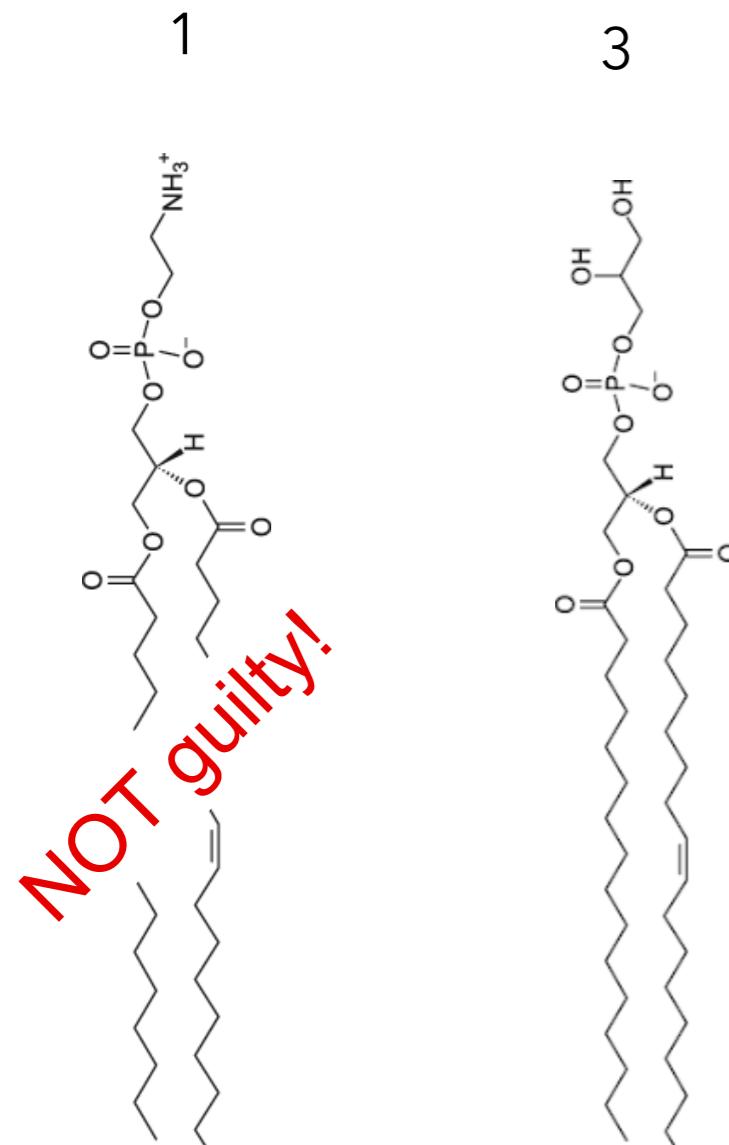
**bound configuration:** EM Density

**allowable distance-from-bound-configuration:** MD

**application of  
DBC restraint:**  
on-the-fly during  
FEP

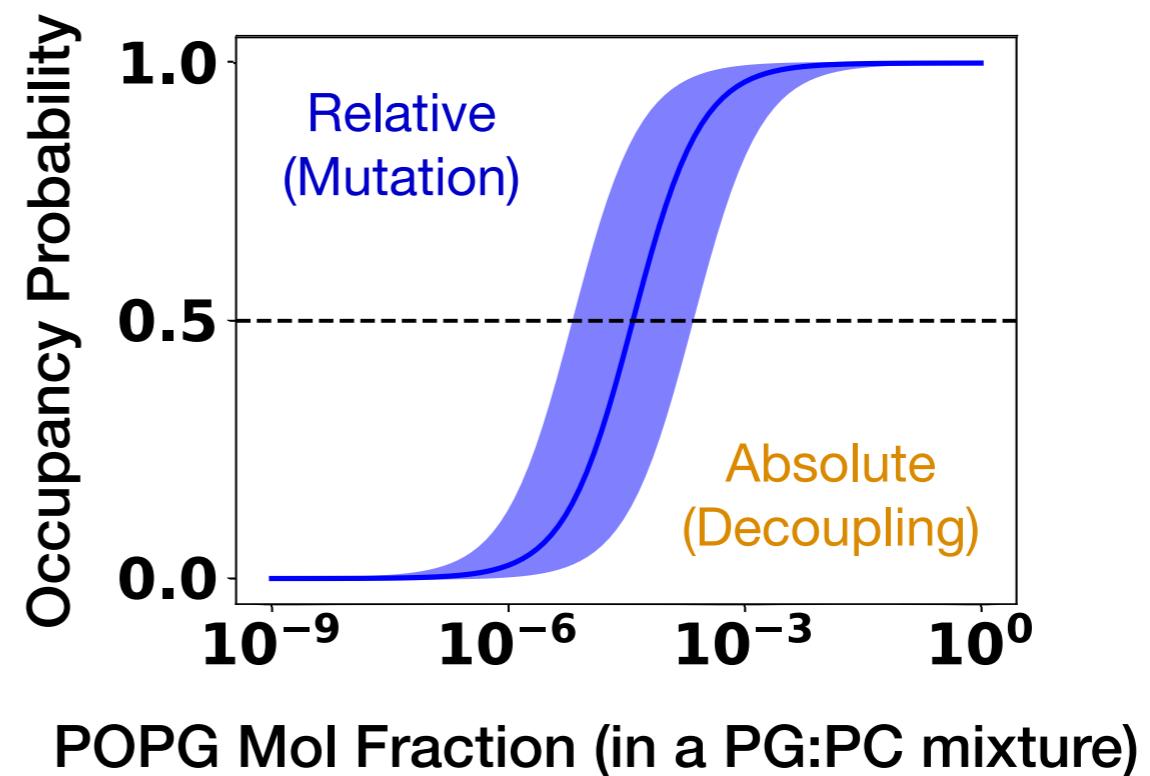


# PG vs PC : who is more likely to bind?



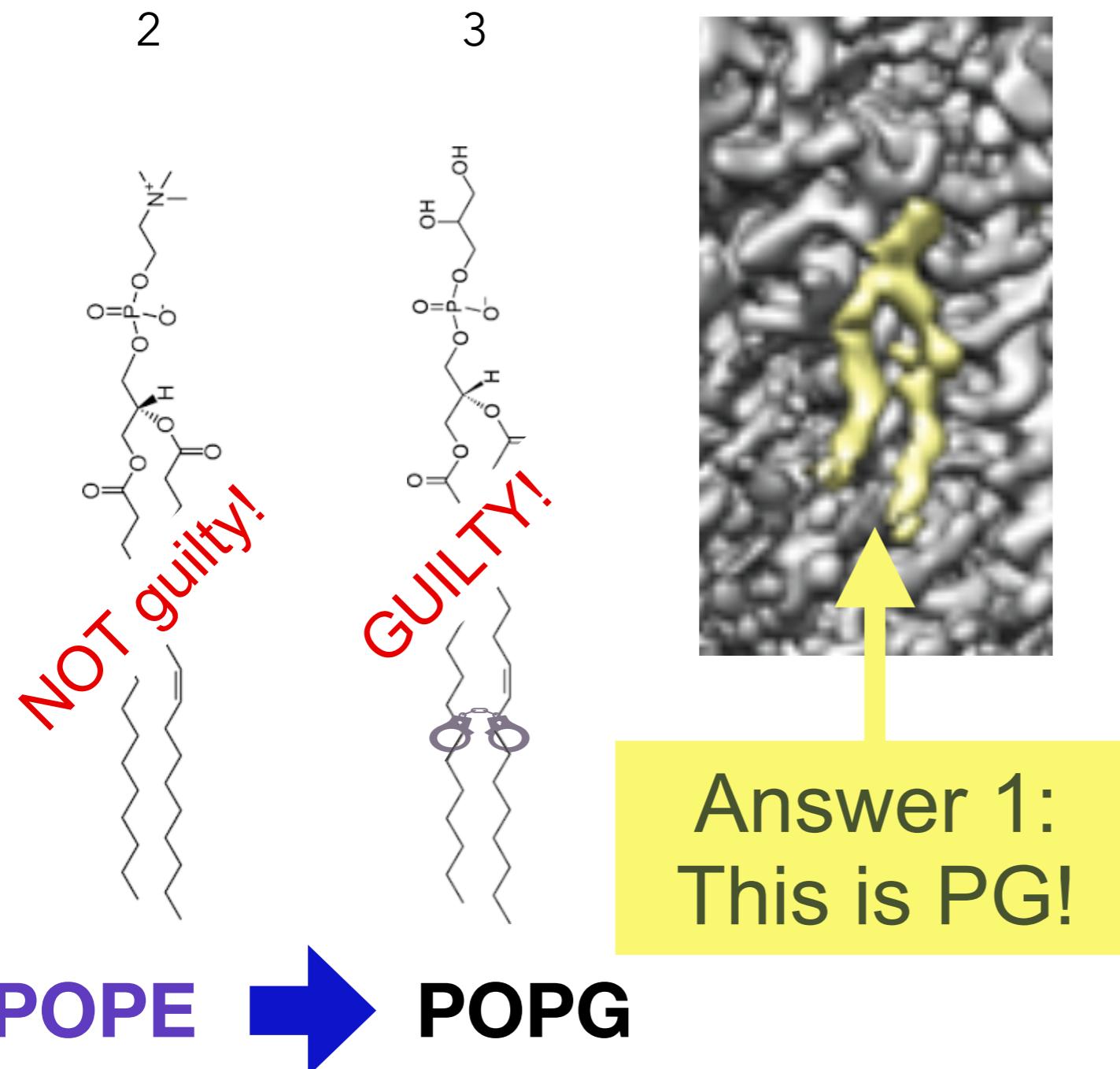
**POPC** → **POPG**

$$\Delta\Delta G = -6 \text{ kcal/mol}$$

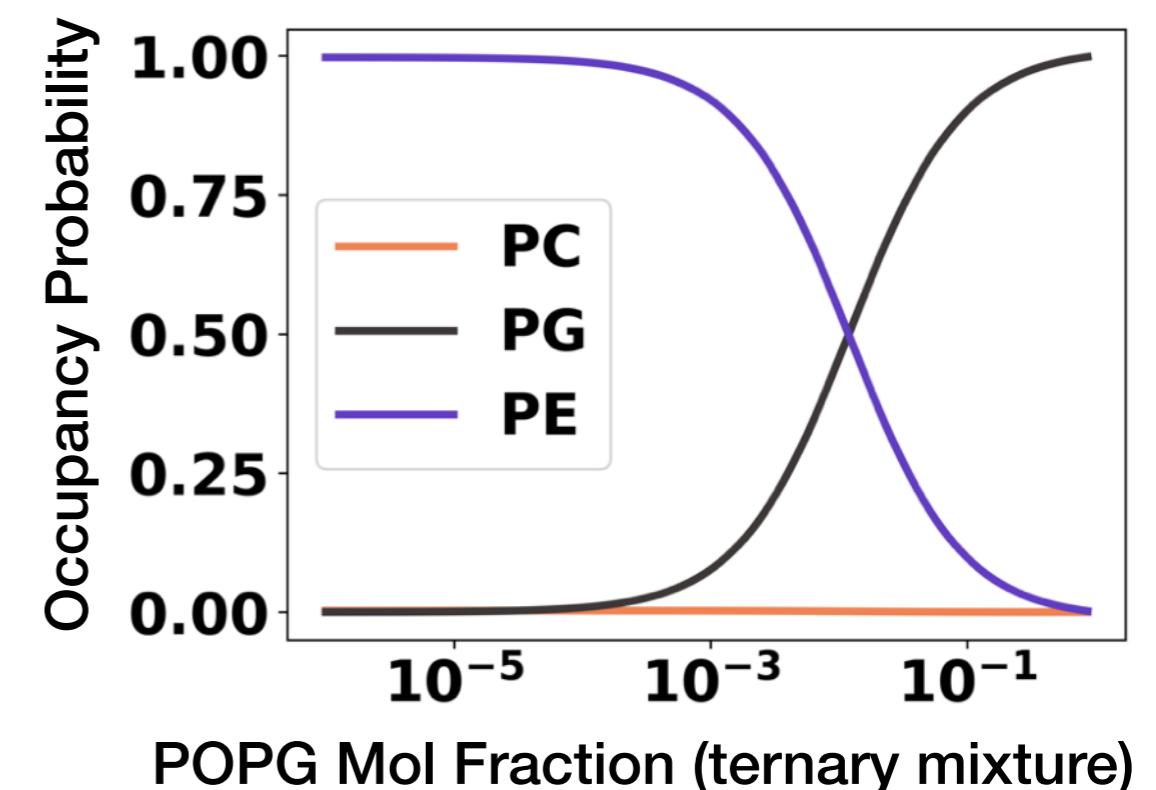


POPC is such a poor “ligand” that it can’t even be used as an endpoint in our relative affinity calculation

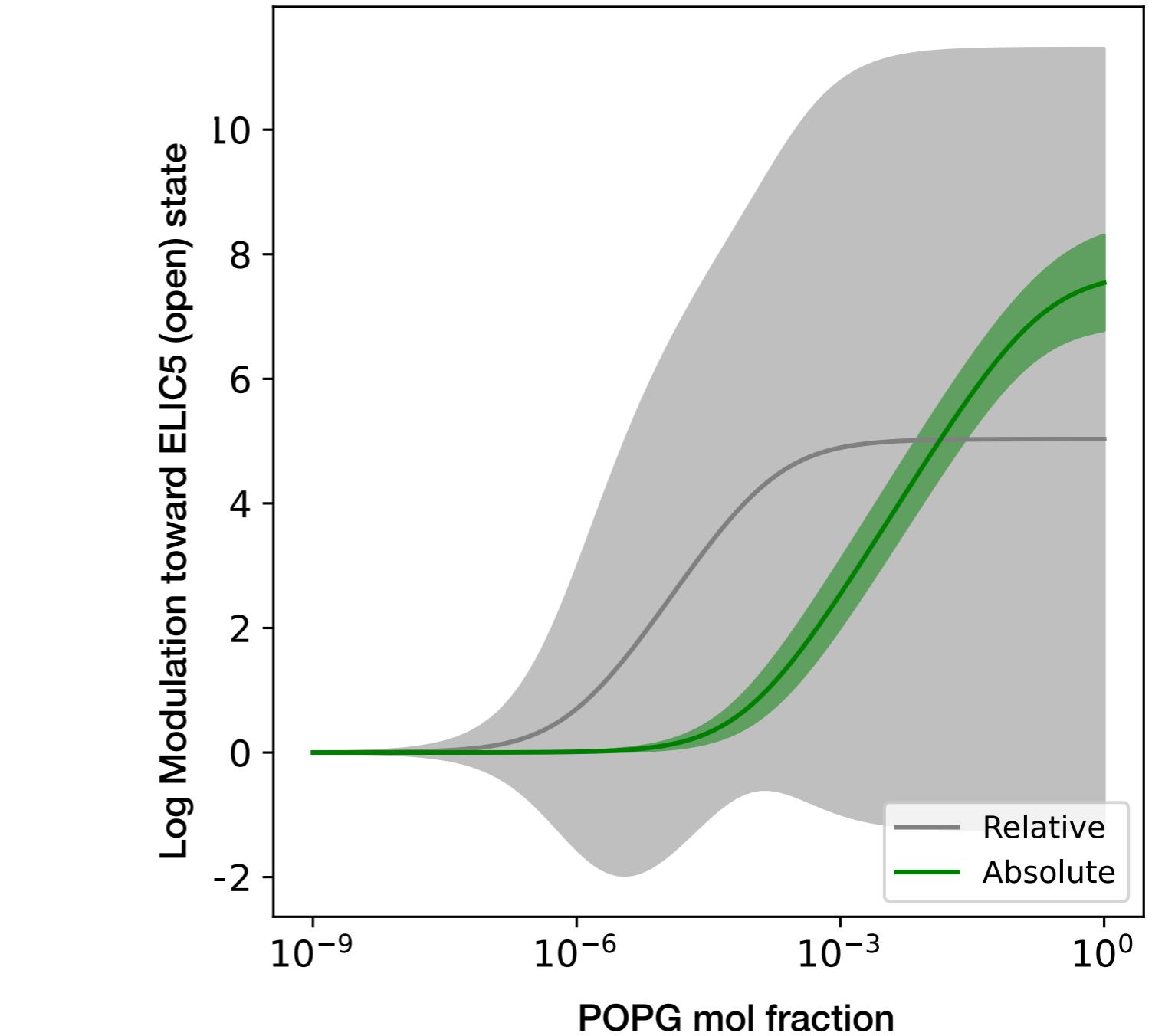
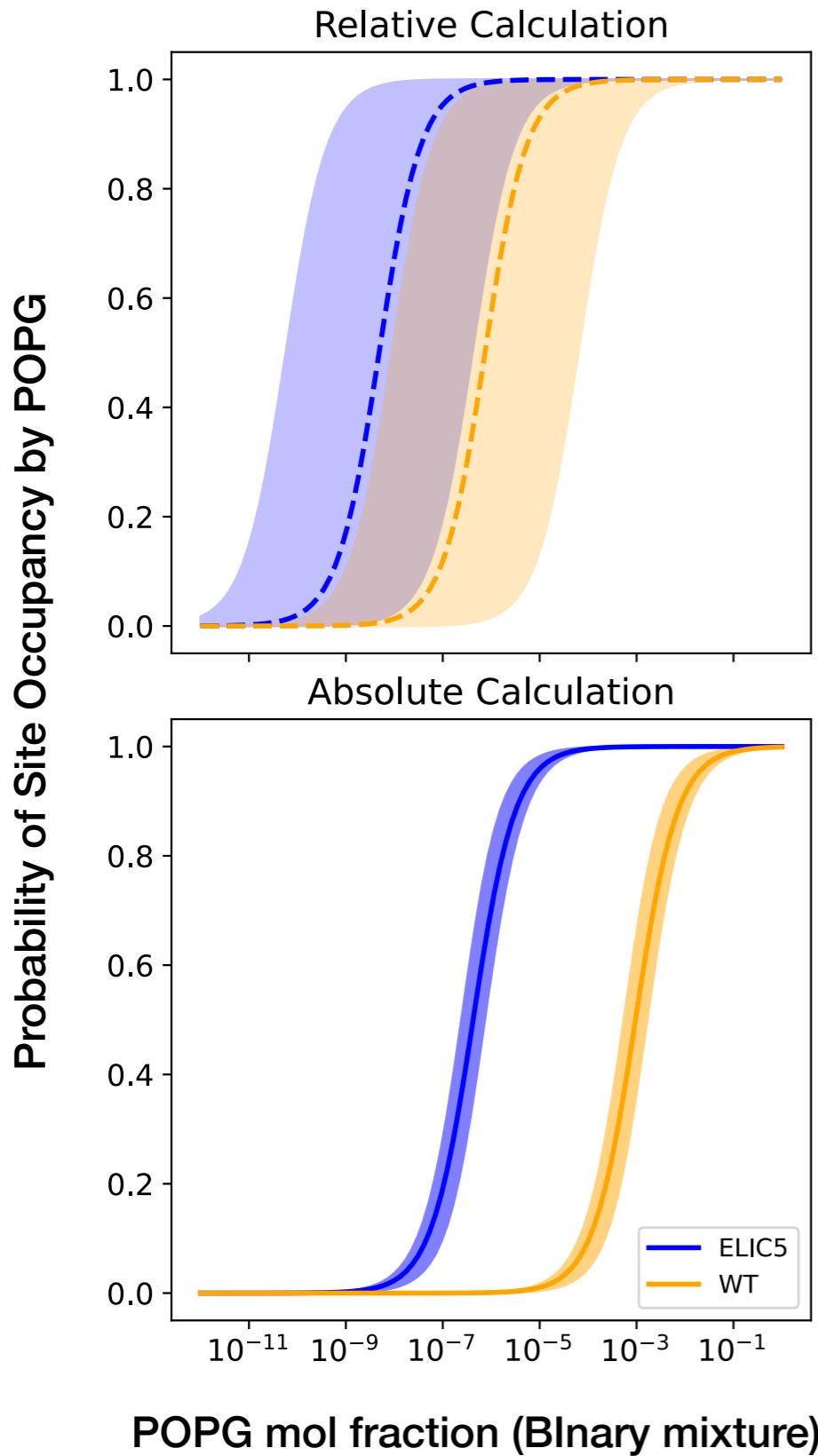
# PE vs PG : who is more likely to bind?



$$\Delta\Delta G = -2 \text{ kcal/mol}$$

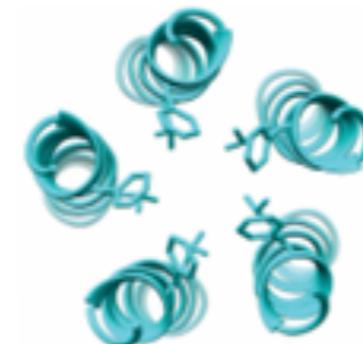
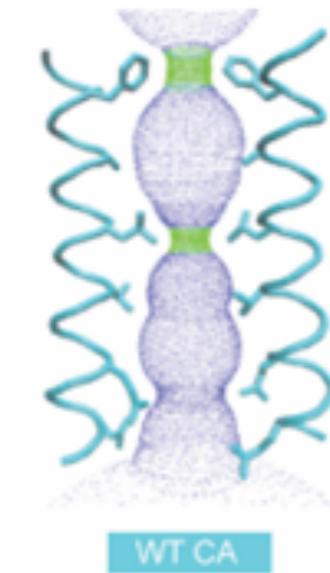


# state dependence (binary mixture)

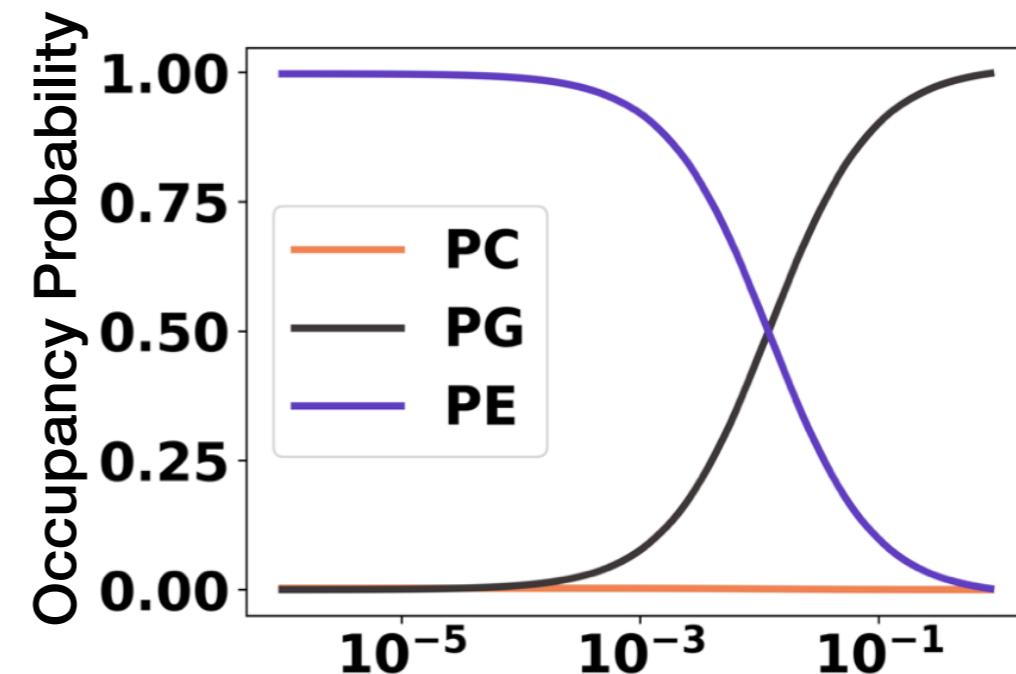


absolute FEP: POPG causes gain of function  
relative FEP: ???

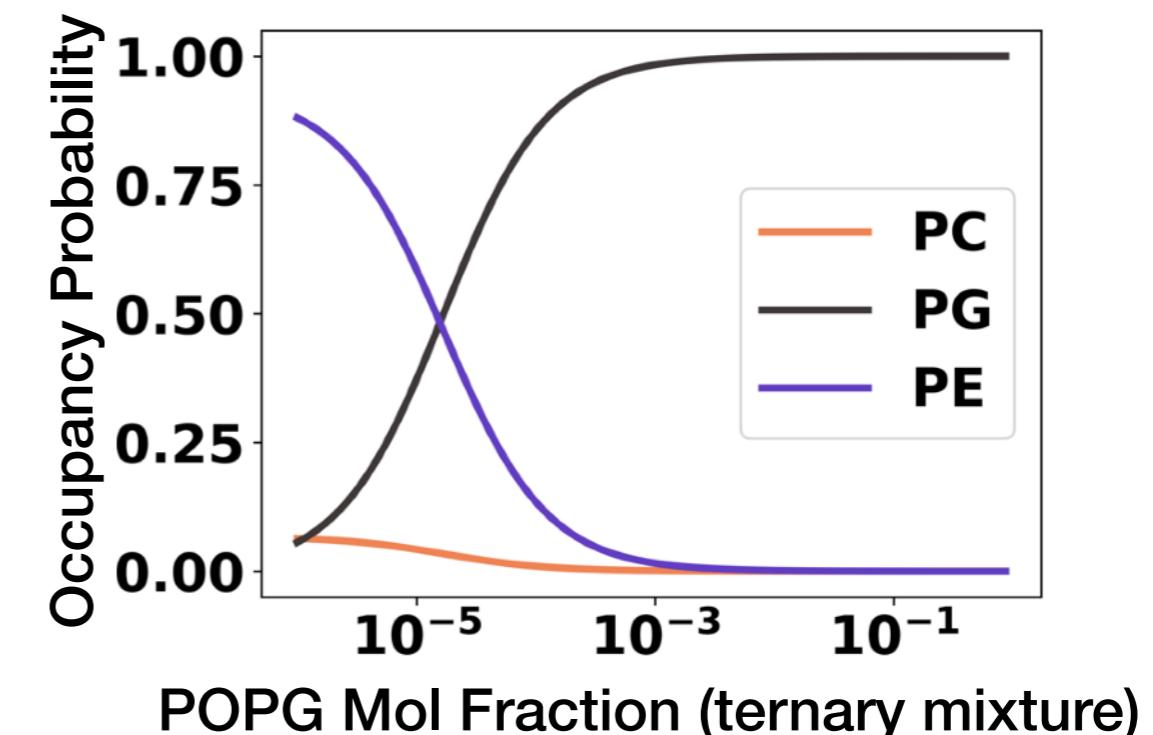
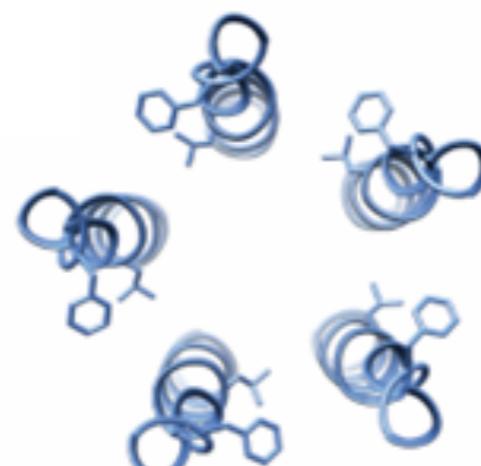
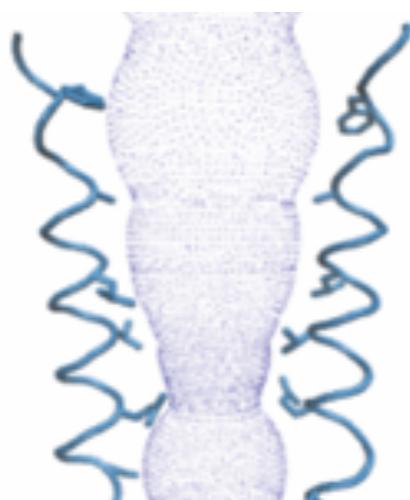
# state dependence (ternary mixture)



pre-active conformation



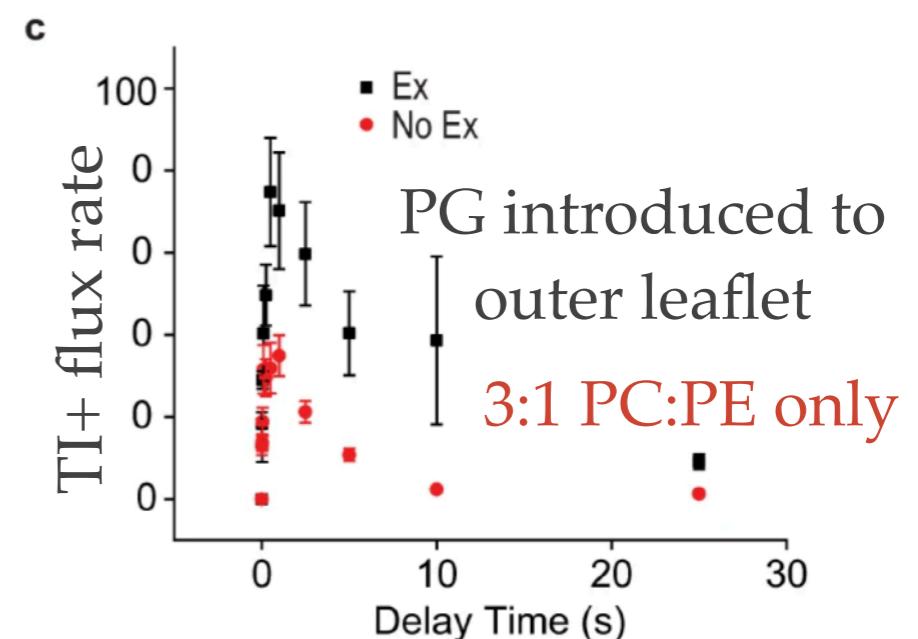
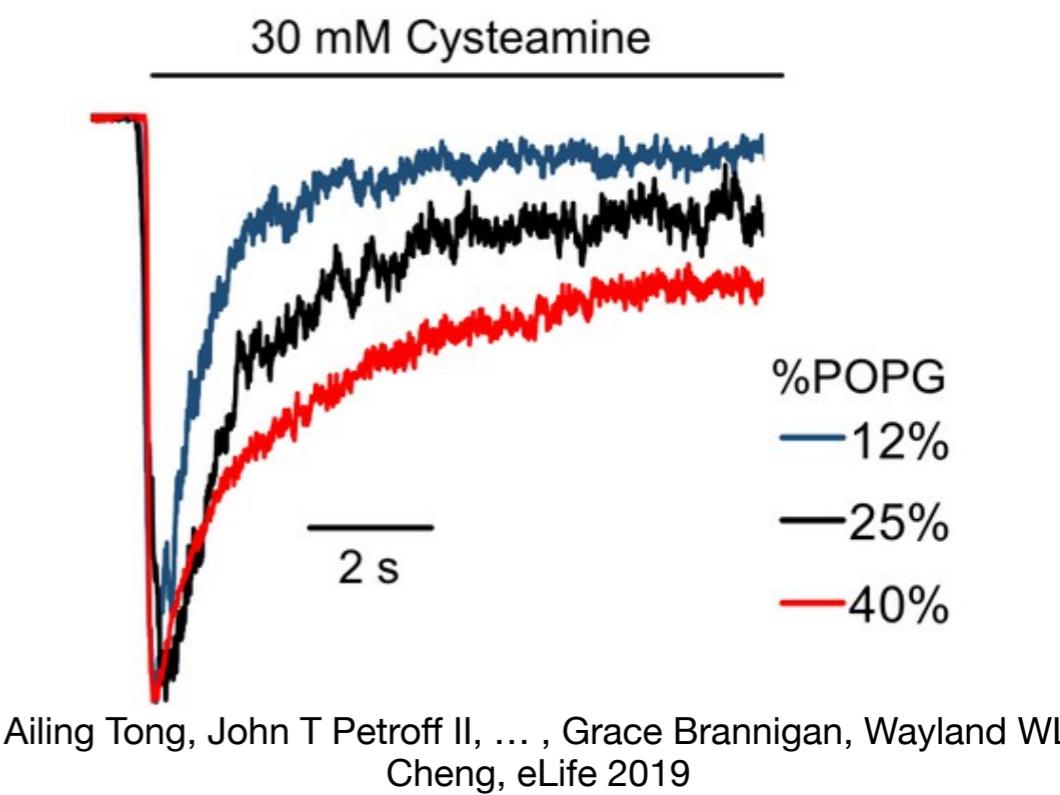
wide-open conformation



Answer 2: Yes, PG stabilizes the wide-open conformation!

# Summary

- Used SAFEP to identify lipid fragments in structures of ELIC
  - PE and PG can both occupy the observed binding mode; PG starts out-competing PE at less than 1:10 PG:PE
  - Absolute affinity calculations were as straightforward as relative calculations, and less risky
- Also used SAFEP to calculate state dependence of lipid binding
  - yes, PG has a higher affinity for the wide open ELIC5 conformation!
  - Consistent with functional data



# Acknowledgments

**Ezry St. Iago-McRae (Rutgers) - Poster Thursday!!**



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**Dr. Mark Arcario (Washington University - St Louis)**

**Jesse Sandberg (Rutgers)**



Brannigan Lab

