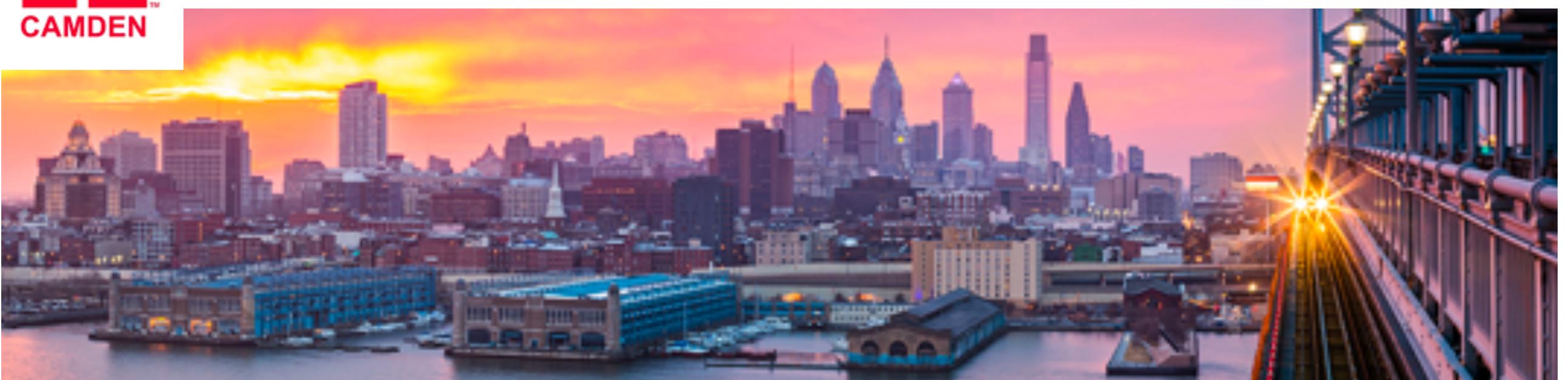


# Neuronal lipids: recent surprises and implications for mechanisms of anesthesia

Grace Brannigan  
Department of Physics  
Center for Computational and Integrative Biology  
Rutgers University-Camden



# Outline of Talk

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- I. Primer on MD
- II. Lipid accessibility and anesthetic selectivity
- III. Lipid specificity through spontaneous binding :  
model membranes and neuronal membranes
- IV. Lipid specificity through structural biology and free  
energy calculations

# Orientation

- pentameric ligand-gated ion channels (pLGICs, aka "Cys-Loop Receptors") critical for anesthesia.

- two long standing challenges:

- determining where GAs bind, determinants of affinity, ranking binding sites

- pLGICs are highly lipid sensitive

As of 2021:

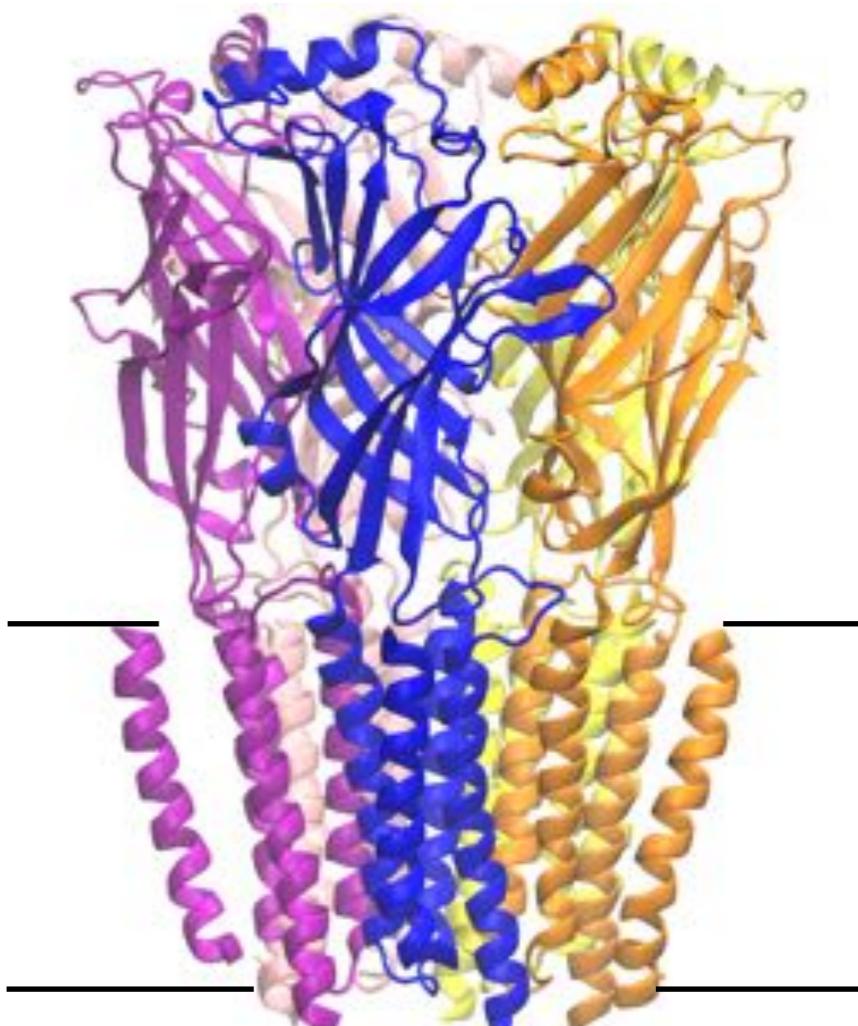
- many structures of pLGICs in complex with small molecules

- nearly all are in artificial environments

- in some cases lipids are also bound, but we don't know what the lipids are

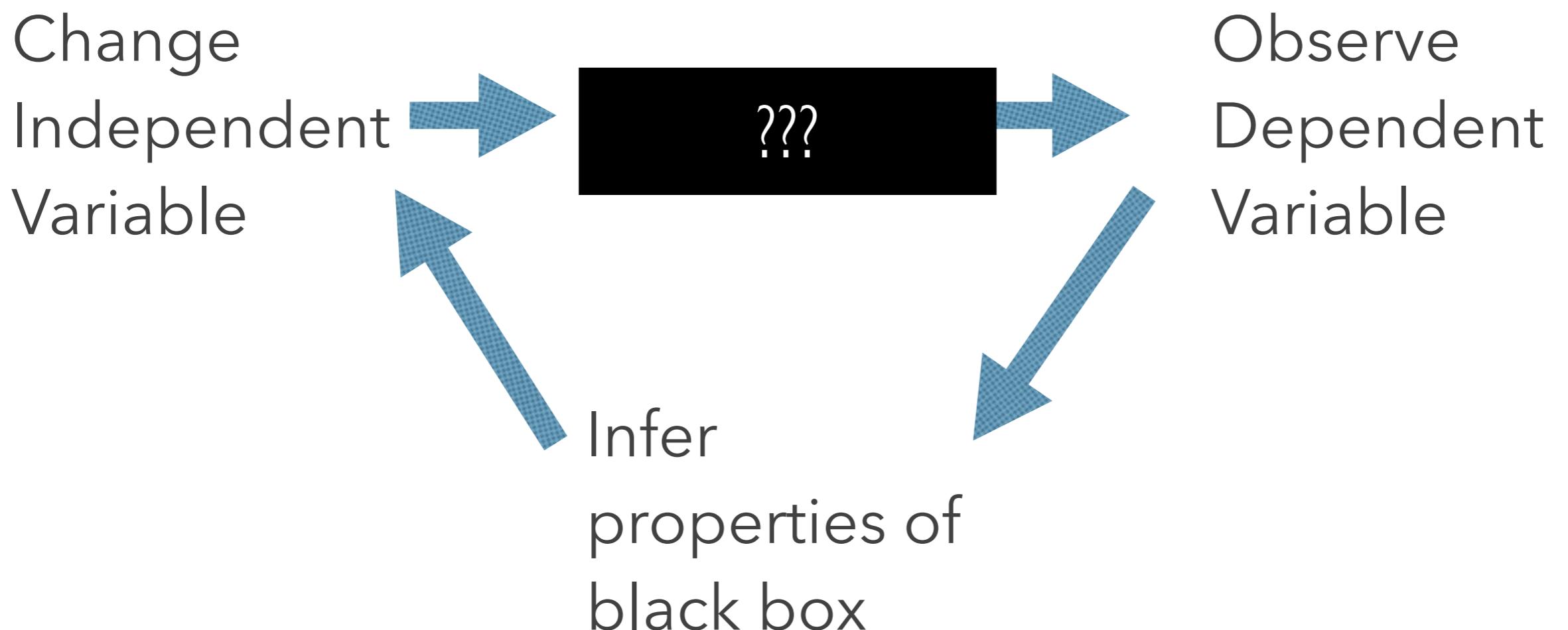
- Native MS:

- neuronal lipidome is not what we thought



# Classic Experimental Approach

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# Molecular Dynamics Study

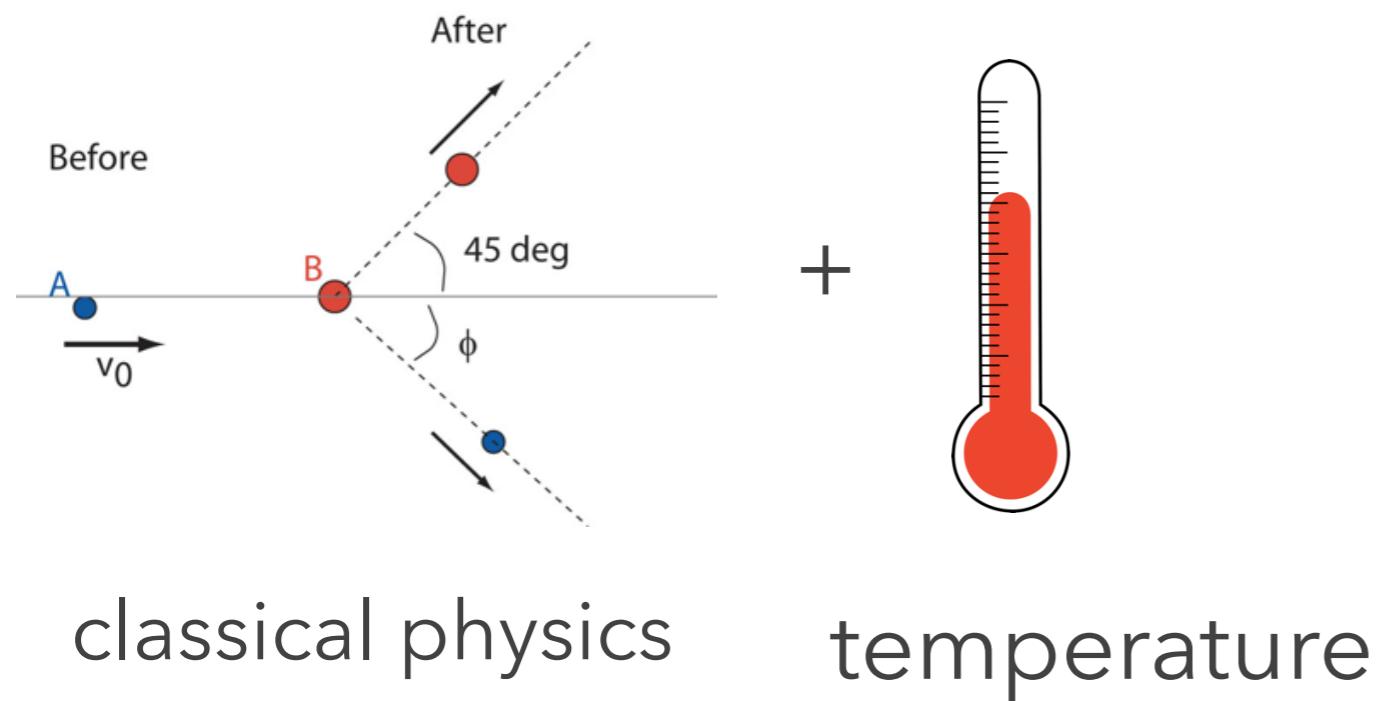


No black box!

**BUT** while energy of the system is easily accessible, entropy (and thus free energy) isn't.

Observe dependent variable (eventually)

# Molecular Dynamics Simulation



$$3k_B T = m \langle v^2 \rangle$$

↑      ↑  
temperature      average velocity squared

"Everything that living things do can be understood in terms of the jiggling and wiggling of atoms," - Richard Feynman, Lectures in Physics, 1963

# Computational Toolbox

- Fully-atomistic molecular dynamics simulation

more accurate

lipids cannot diffuse

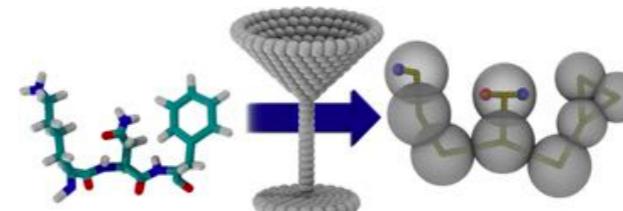
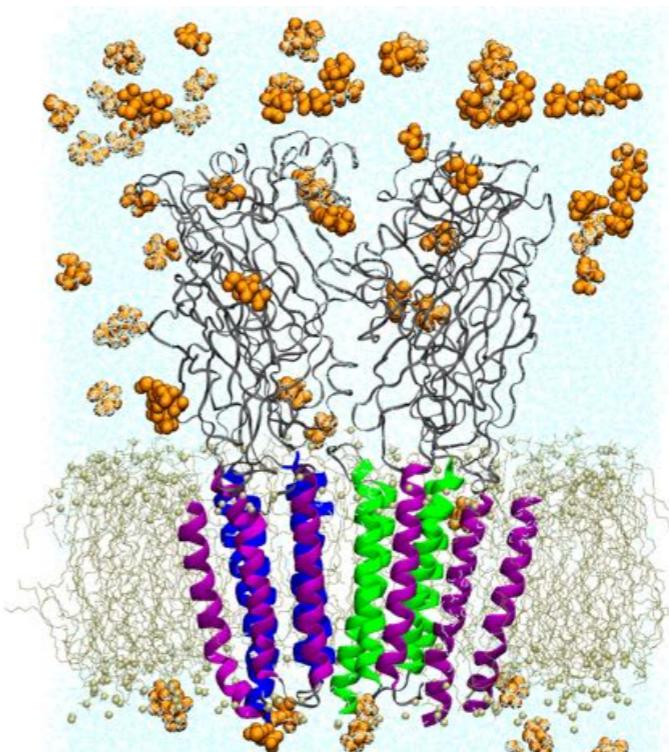
slow and expensive

- Coarse-grained MD simulation

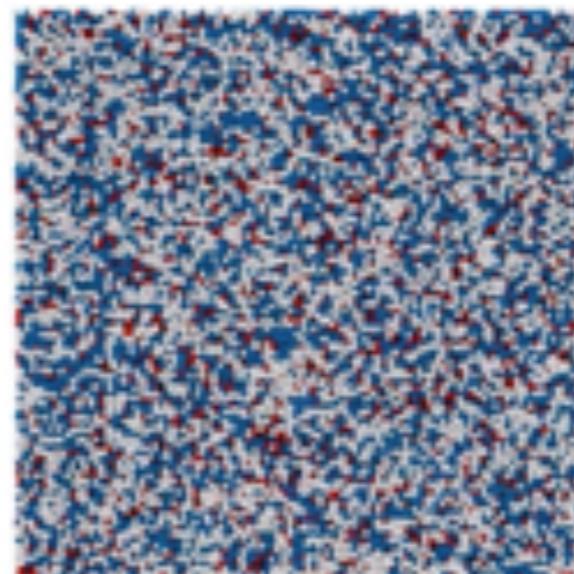
more efficient

lipids can easily diffuse over simulation times

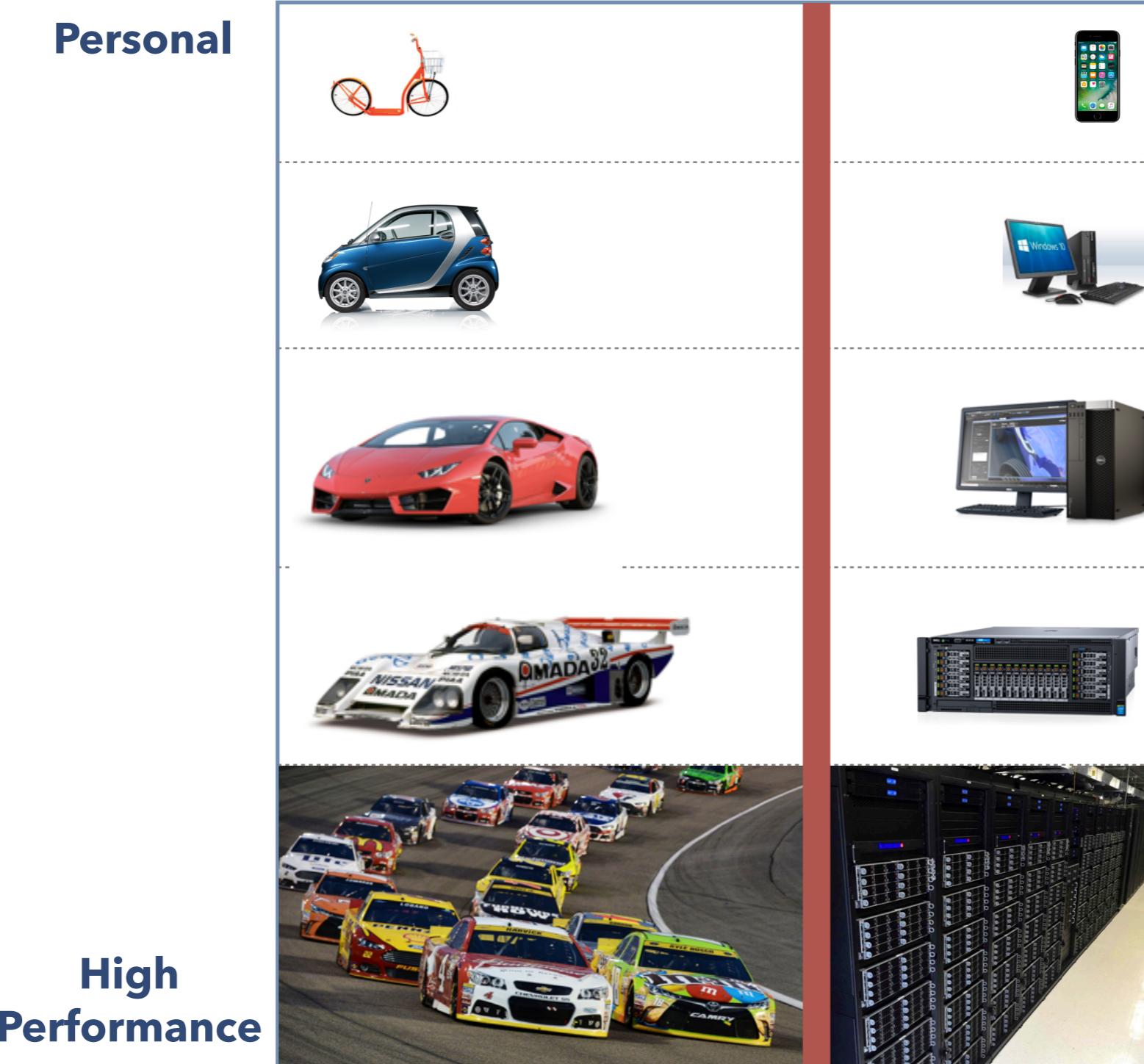
not appropriate for ligand binding or conformational change



de Jong...Marrink, JCTC, 2012

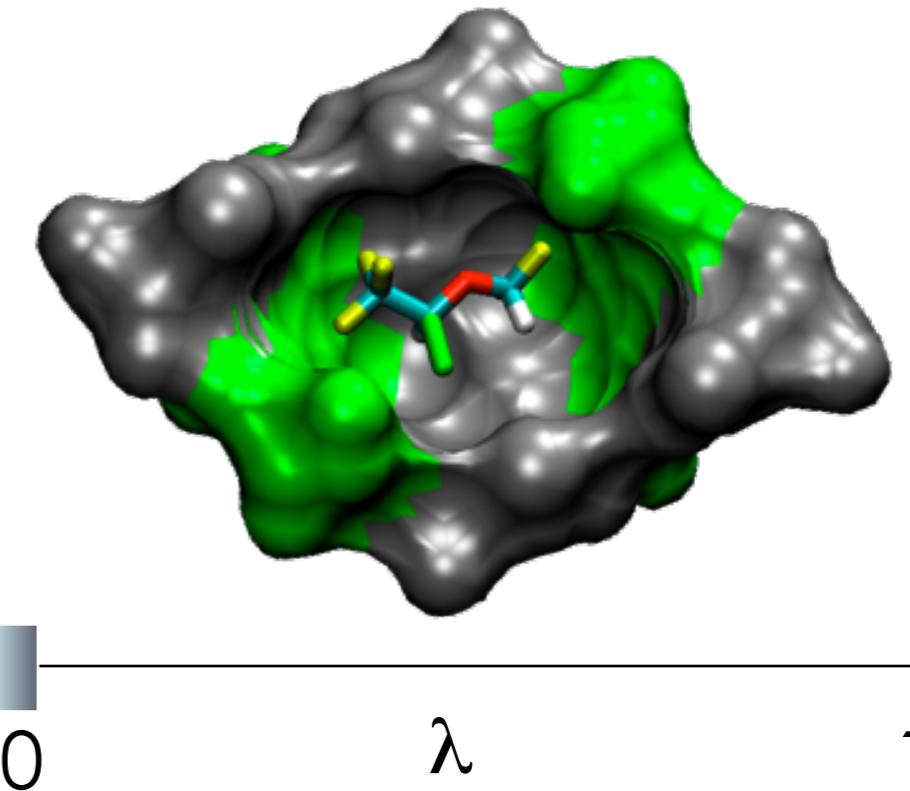


# Required Computing Power: PC vs HPC



# Binding Affinities: Free Energy Perturbation (FEP)

Intrinsically incorporates entropy, so we can calculate a **free** energy of binding



Make transformation gradual with coupling parameter  $\lambda$

$$H_\lambda = (1 - \lambda)H_X + \lambda H_Y$$

Use series of  $n$  windows with increasing  $\lambda_i$

$$\Delta G_{\lambda_i} = -k_B T \ln \langle e^{-(H_{\lambda_{i+1}} - H_{\lambda_i})/k_B T} \rangle_{\lambda_i}$$

Sum the windows:

$$\Delta G \sim \sum_i^n \Delta G_{\lambda_i}$$

# FEP works

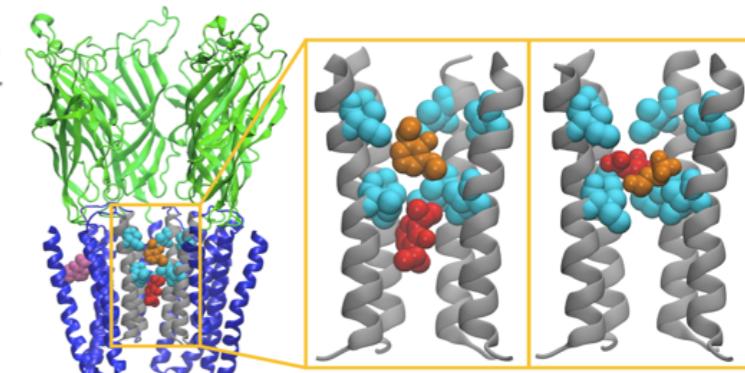
2012

OPEN  ACCESS Freely available online

PLOS COMPUTATIONAL BIOLOGY

## General Anesthetics Predicted to Block the GLIC Pore with Micromolar Affinity

David N. LeBard<sup>1,5</sup>, Jérôme Hénin<sup>2,5</sup>, Roderic G. Eckenhoff<sup>3</sup>, Michael L. Klein<sup>1</sup>, Grace Brannigan<sup>4\*</sup>



2015

## SCIENTIFIC REPORTS

OPEN

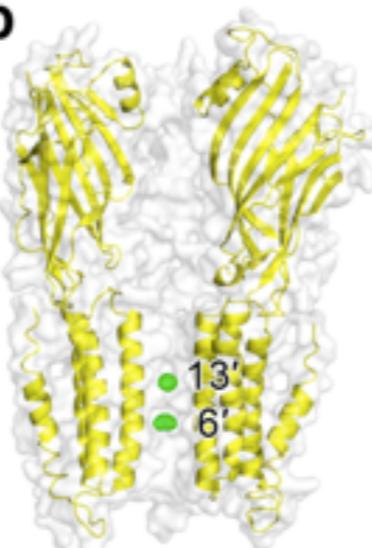
### Direct Pore Binding as a Mechanism for Isoflurane Inhibition of the Pentameric Ligand-gated Ion Channel ELIC

Received: 15 June 2015

Accepted: 10 August 2015

Published: 01 September 2015

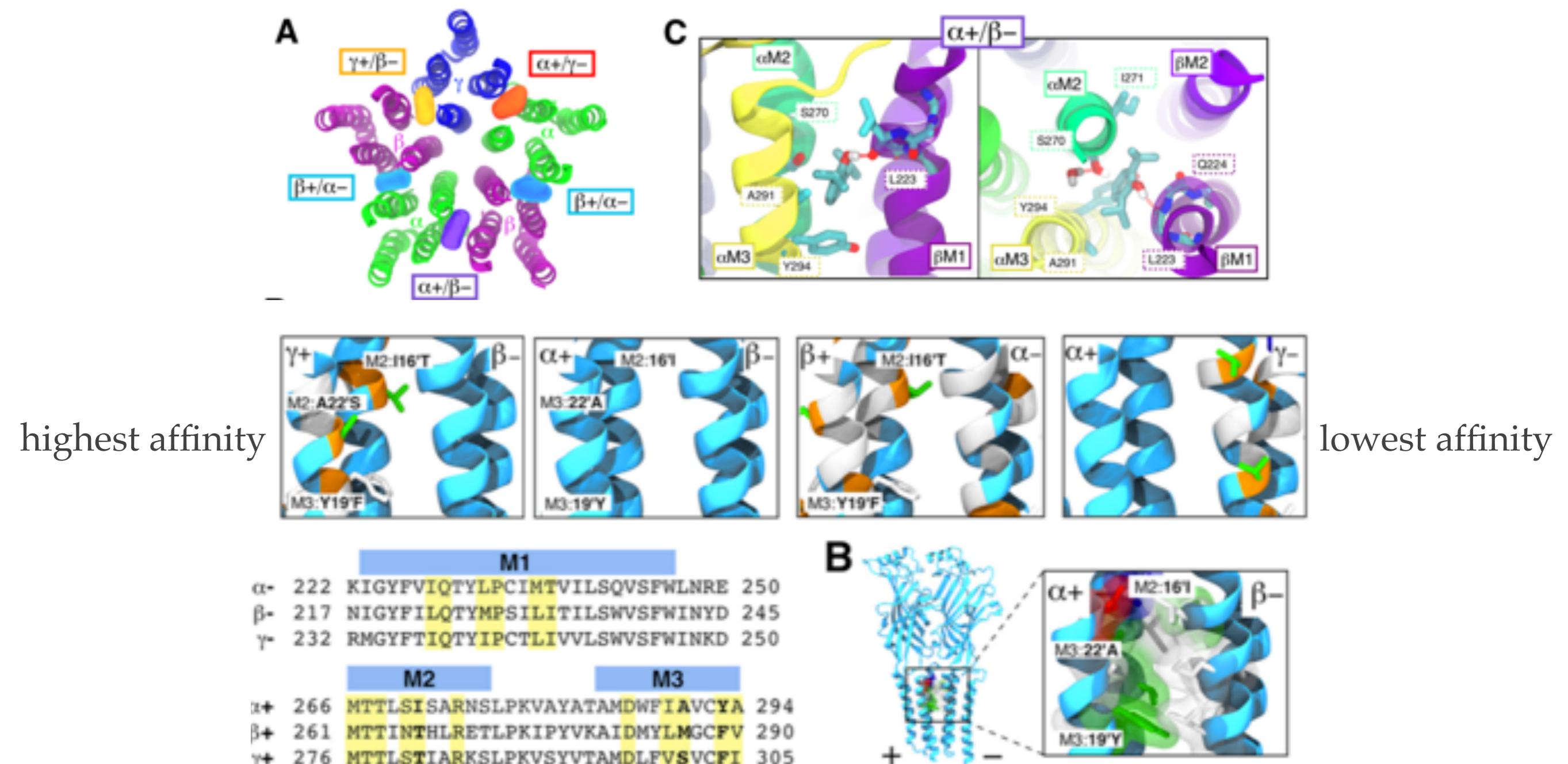
Qiang Chen<sup>1</sup>, Monica N. Kinde<sup>1</sup>, Palaniappa Arjunan<sup>1,2</sup>, Marta M. Wells<sup>1,4</sup>, Alina E. Cohen<sup>1</sup>, Yan Xu<sup>1,3,4</sup> & Pei Tang<sup>1,4\*</sup>



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## I. Effect of lipid specificity on anesthetic specificity

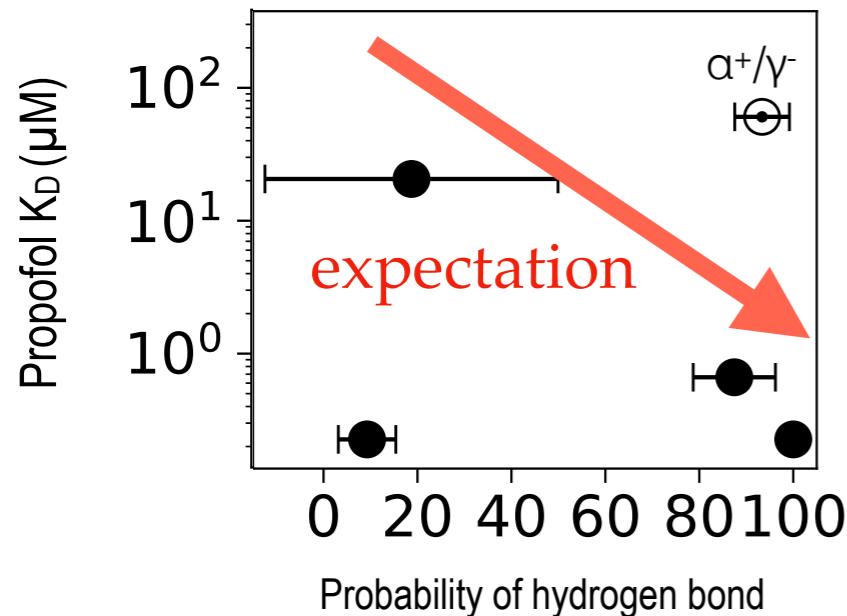
Preamble: propofol affinities seemed to be correlated to hydrogen bond propensity



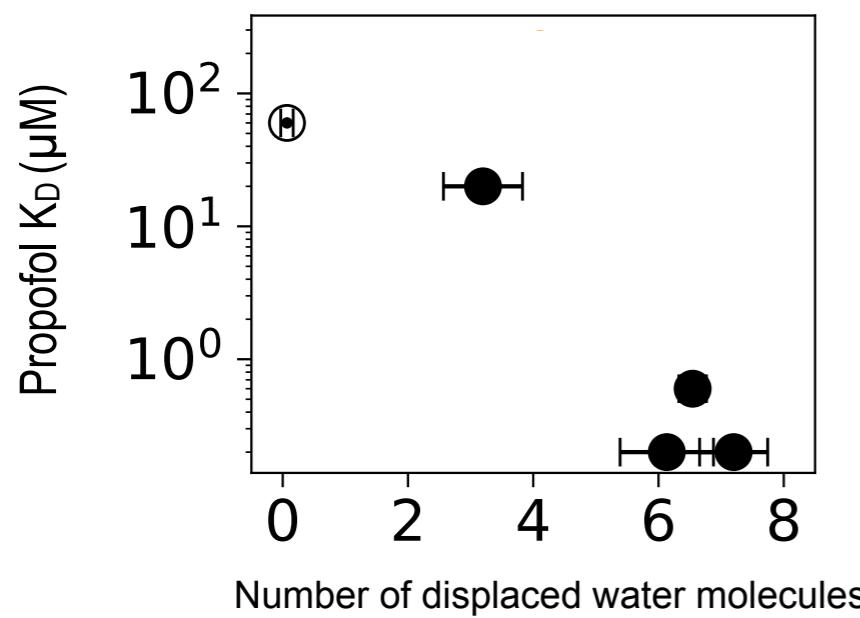
Woll, Murlidaran,...Brannigan, Garcia B., Eckenhoff, R. J. *Biol. Chem.* (2016).

Unresolved mystery - why was the affinity for the  $\alpha^+/\gamma^-$  site so low? Still had hydrogen bonding partners!

# Why does propofol have such low affinity for the $\alpha/\gamma$ site?



more extensive sampling:  
correlation with hydrogen  
bonding is not consistent



better predictor: the  
number of displaced  
water molecules

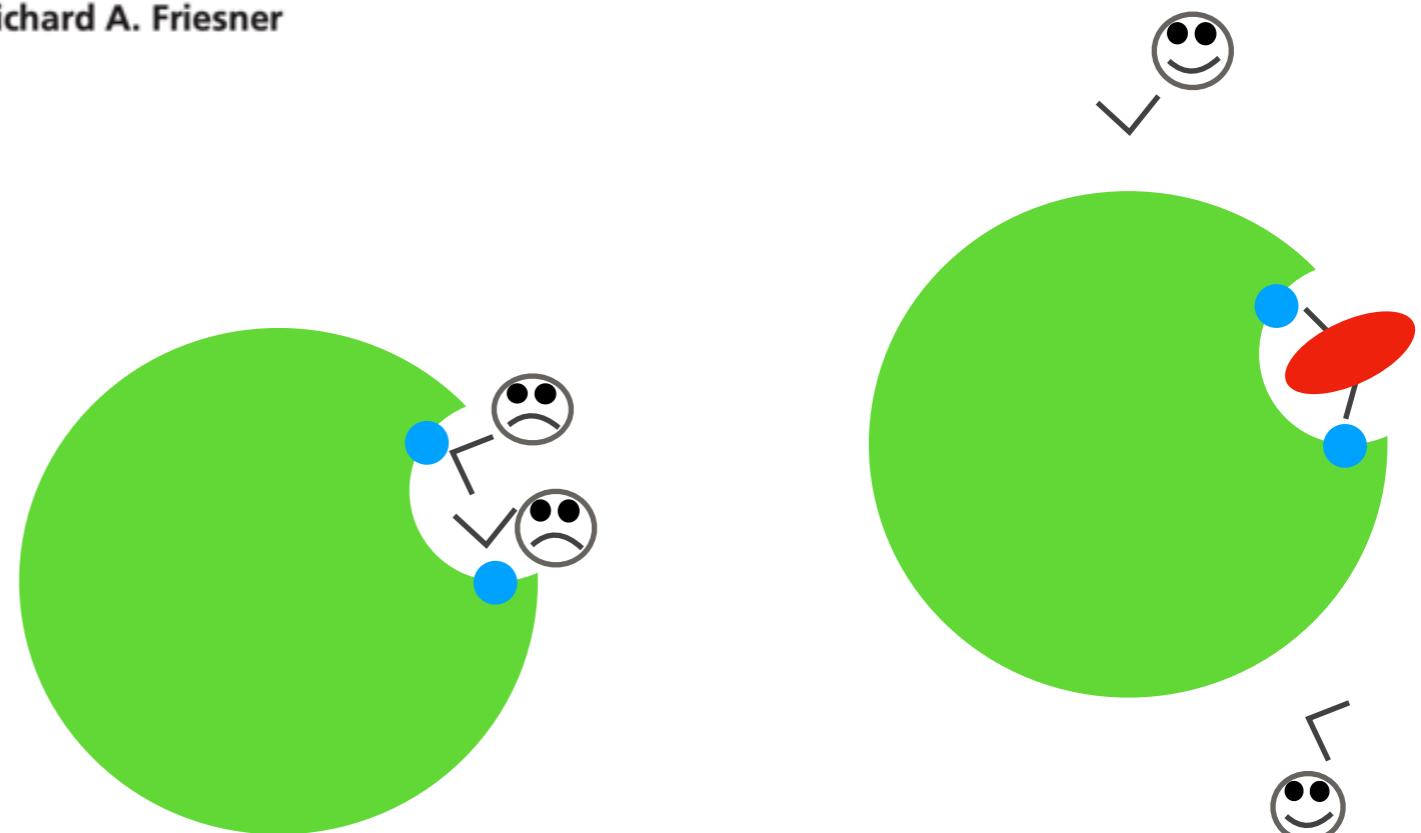
# Expected based on water entropy

## Motifs for molecular recognition exploiting hydrophobic enclosure in protein–ligand binding

Tom Young, Robert Abel, Byungchan Kim, Bruce J. Berne\*, and Richard A. Friesner

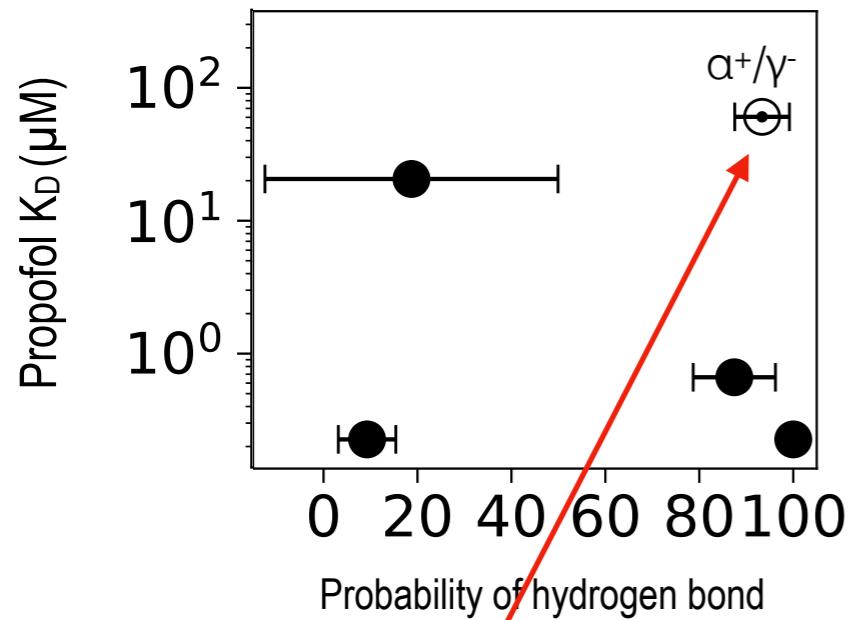
When a ligand binds to a solvated protein, water in the binding cavity is expelled into the bulk. Associated with this expulsion are enthalpic and entropic contributions to the free energy of binding that arise from the differences between the waters entropic and energetic properties in the bulk and its properties in the binding cavity. In many cases of protein–ligand binding, the energy of interaction between the water in the binding cavity and the protein is roughly comparable with the energy of interaction between the docked ligand and the protein. However, the entropy of structuring waters in the protein cavity has no equivalent mapping with the change in entropy from desolvating the ligand. This asymmetry suggests that the net contribution to the free energies of ligand binding when highly ordered waters are expelled from binding cavities is greater than when less-ordered waters are expelled. From a thermodynamic analysis of the principal hydration sites, we were able to characterize how the various molecular recognition motifs affect the excess chemical potentials of the solvating waters and verify the hypothesis of entropy-driven free energy liberation upon displacing the solvating waters structured by the molecular recognition motifs.

PNAS January 16, 2007 104 (3) 808-813

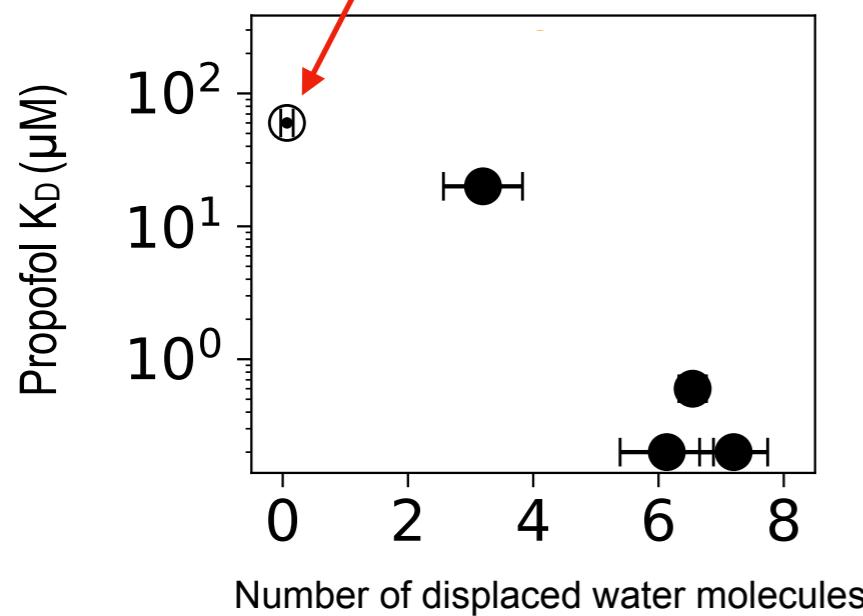


i.e. water in a  
**hydrophobic** or  
**amphipathic** cavity has  
only a few potential  
orientations.

# Are we splitting hairs?

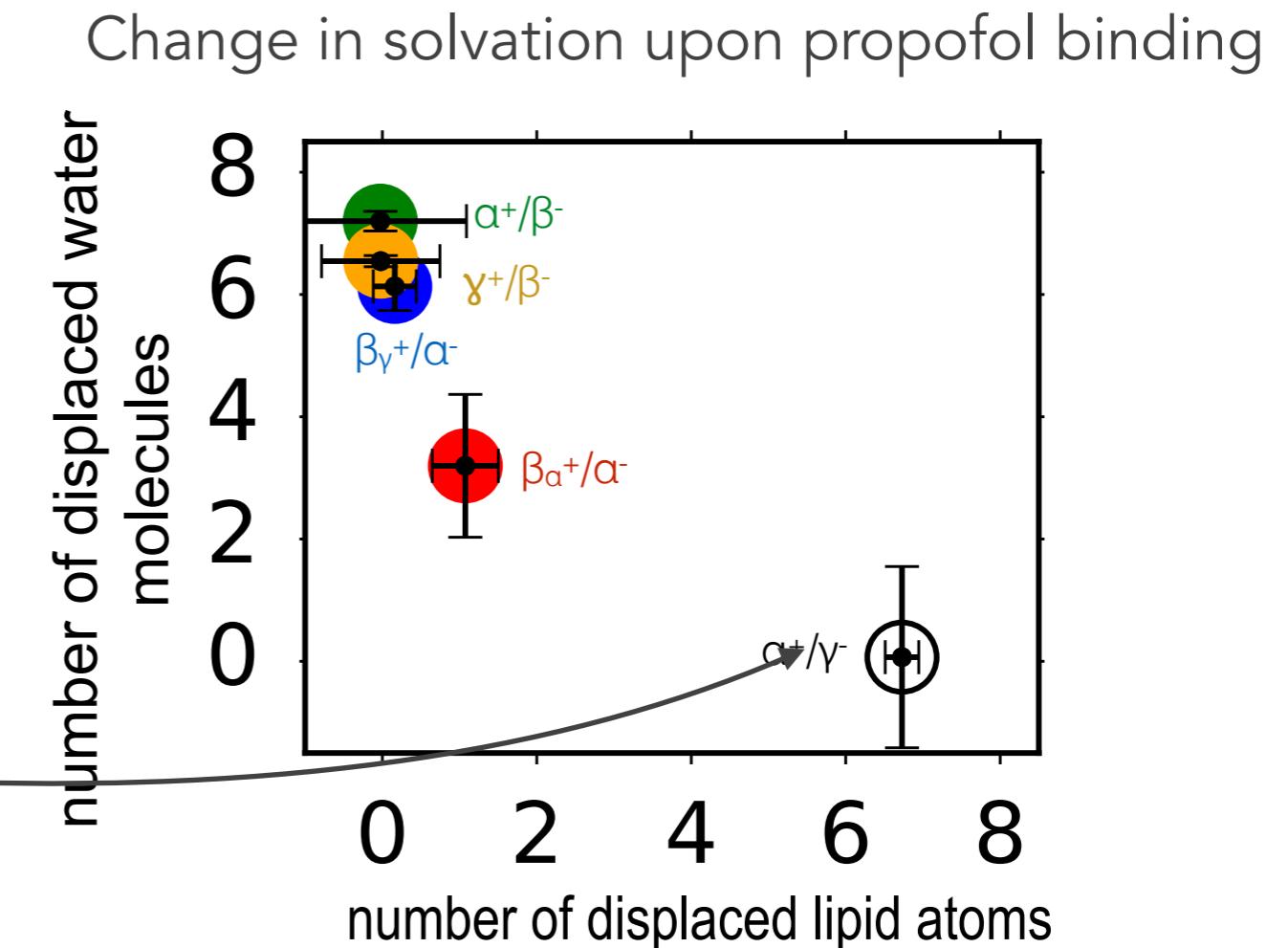
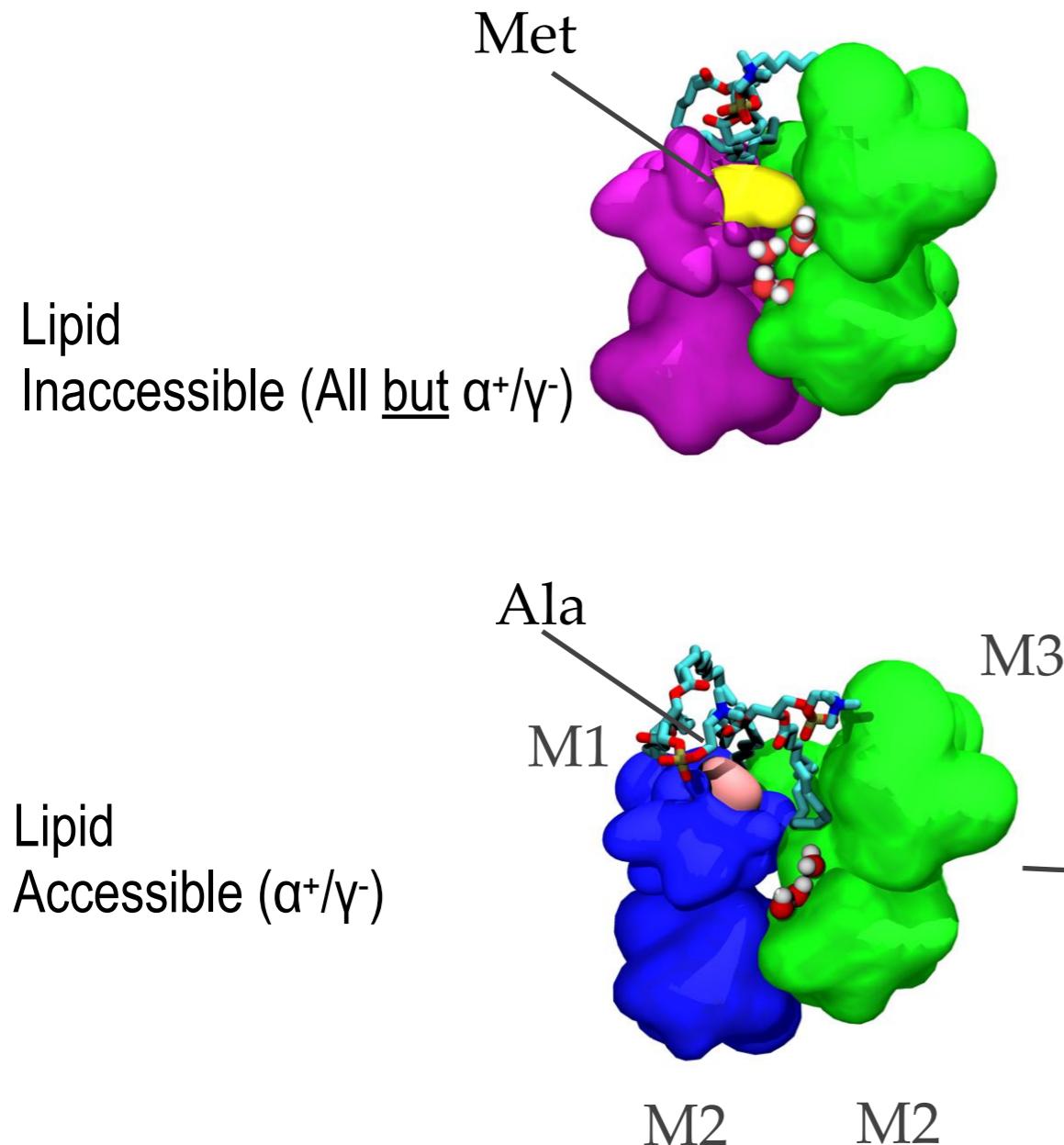


A site where the GA forms hydrogen bonds should be a water-filled apo site, right?



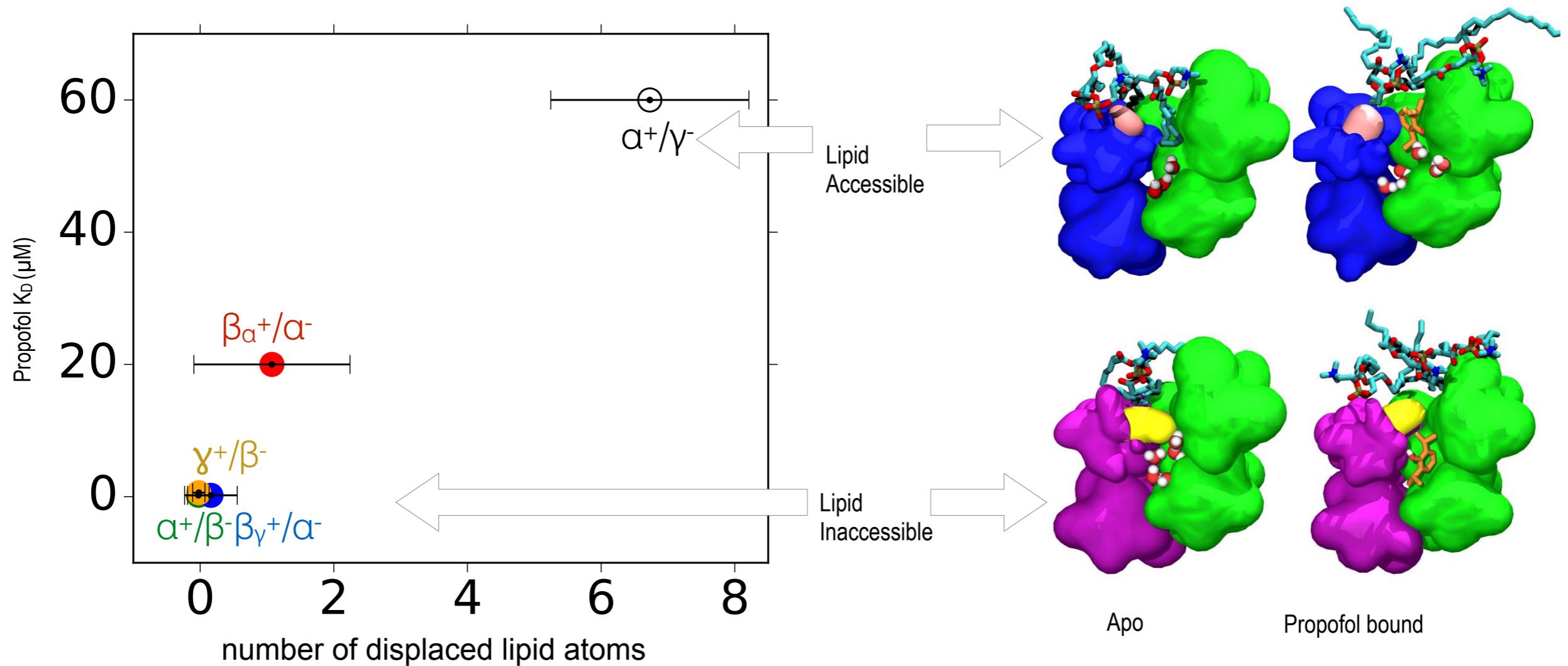
why?  
why aren't water molecules being displaced from this  $\alpha^+/\gamma^-$  site?

# GABA(A) receptors contain 1 lipid-accessible site

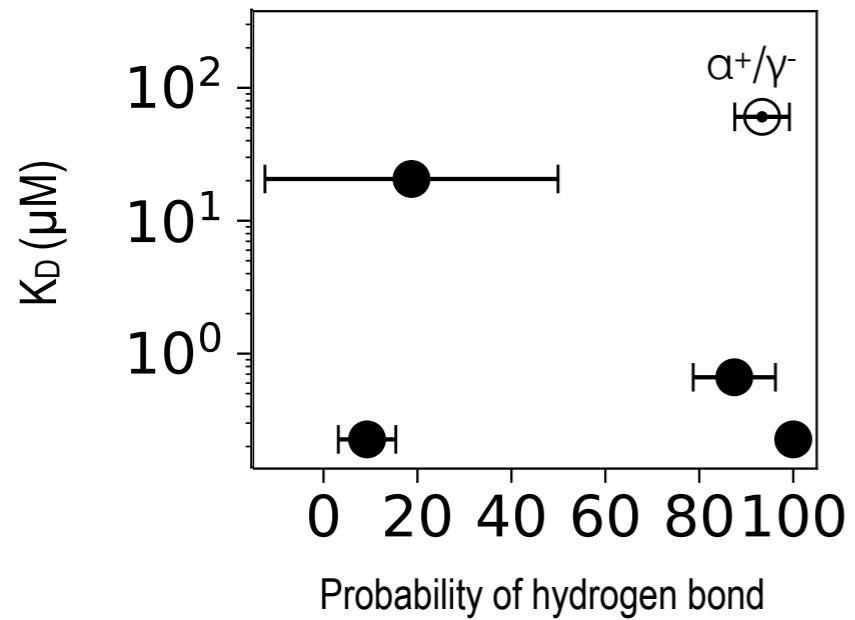


In the  $\alpha^+/\gamma^-$  site, lipids have already displaced the water molecules - anesthetic doesn't offer much benefit!

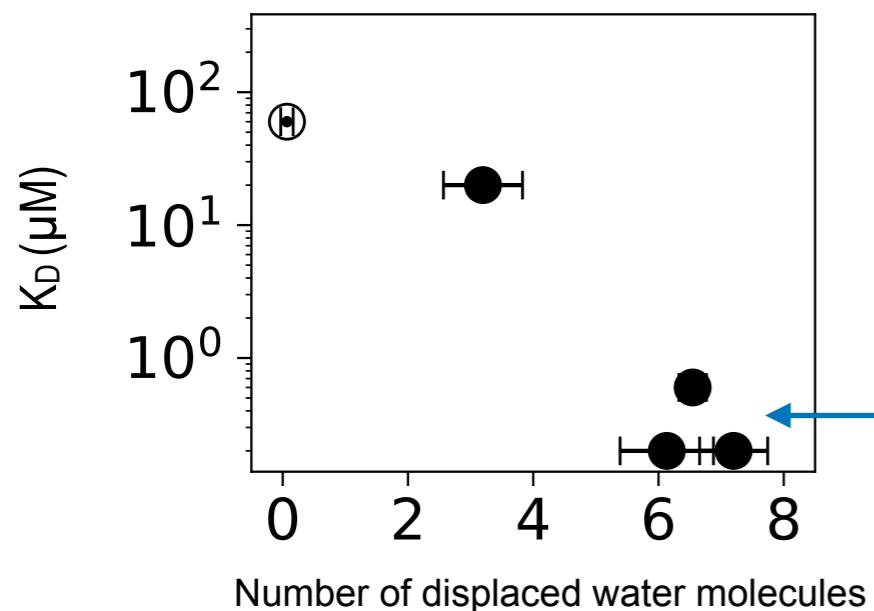
# Lipid-accessibility is an inverse predictor of affinity



# What about sevoflurane?



better correlation than propofol, but error is high on low affinity sites



sevoflurane is **too small** to reach this regime

# Summary

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- Water displacement works as a more general predictor than hydrogen bonding for propofol and sevoflurane/GABA(A)r intersubunit sites
- Lipid binding and accessibility predicts water displacement  
GAs aren't acting **vía** lipids - they are acting **like** lipids (by protecting the protein from water). If a lipid is already doing the job, the GA doesn't compete that well.

So, how could we start thinking about drugs that could compete with lipids?

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## II. Lipid specificity through spontaneous binding : model membranes and neuronal membranes

What are the determinants of lipid selectivity and affinity?

# Membrane Composition: Post-synaptic membranes are distinctive

	Torpedo <sup>a</sup>	Synapse <sup>b</sup>	Xenopus <sup>c</sup>	Mammalian <sup>d</sup>
PC	37	40	28	38
PE	44	35	17	22
PS + PI + PA	13	19	10	12
SM	3	5	20	20
Other PL	3	1	8	8
saturated	42	49	44	53
monounsaturated	26	15	39	20
polyunsaturated	32	35	17	27
Chol Mol Fract	35	30	21	29

Much more PE

Much less SM

Much more PUFA

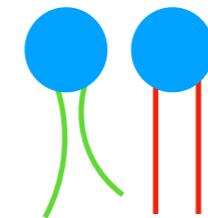
Some more CHOL

than most cultured cells

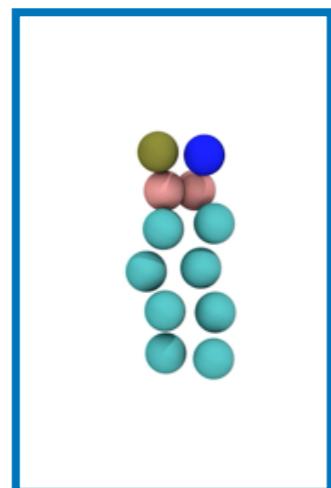
PUFA + CHOL + SAT .... should be great for domain formation!

# Domain Formation in Model Membranes

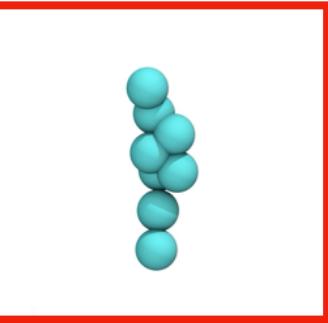
Homo-Acidic  
Domain Forming



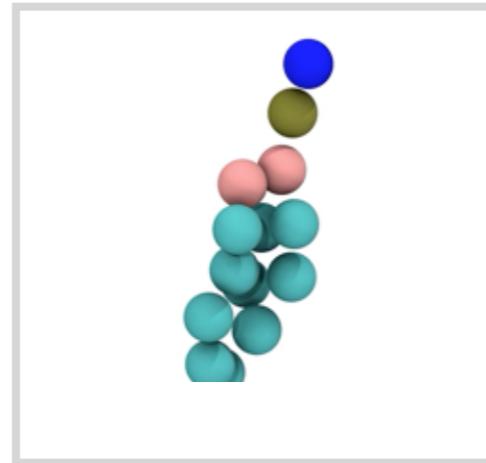
Saturated  
Chains



Cholesterol



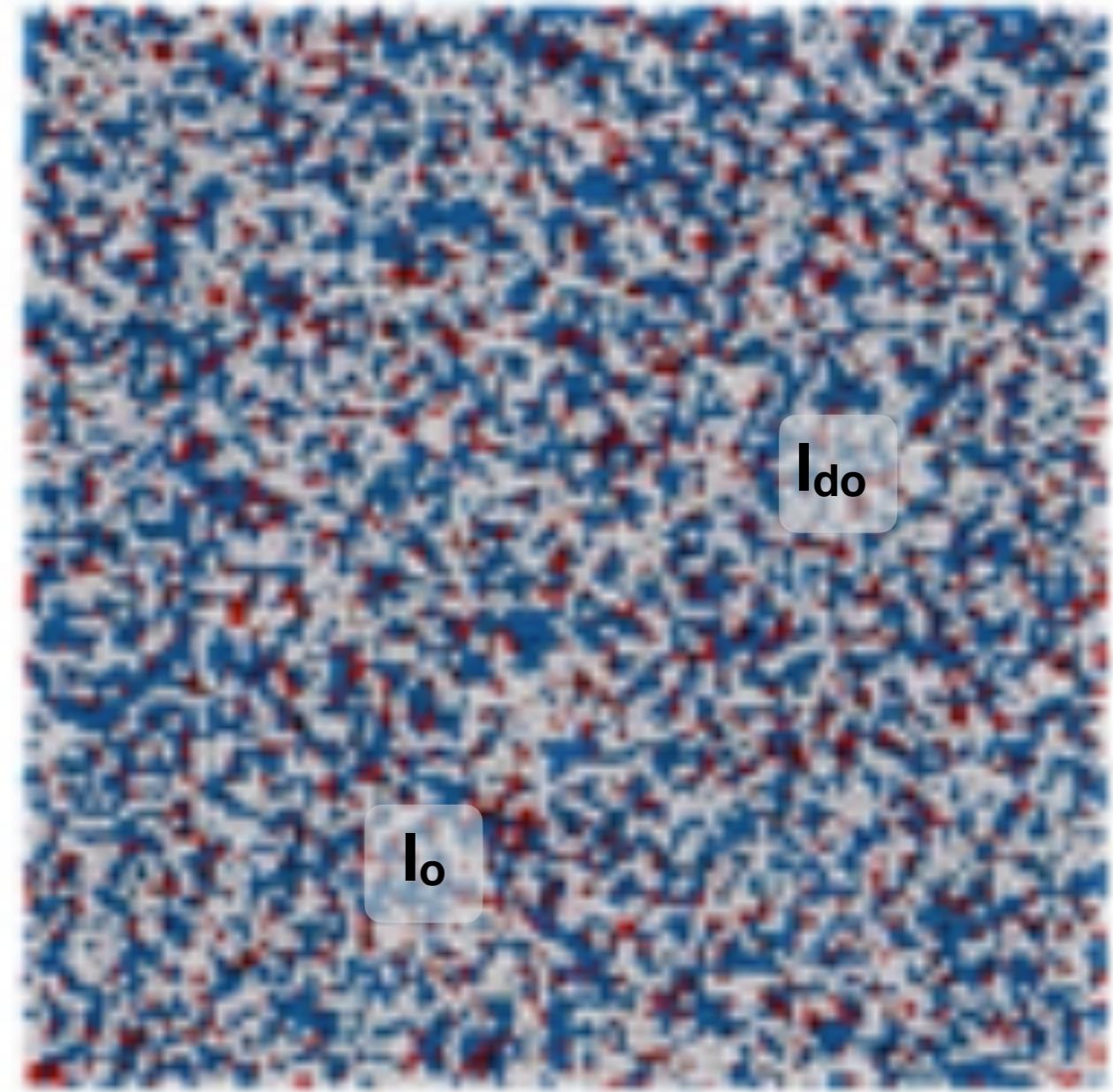
Polyunsaturated  
Chains



Model Membrane Side View



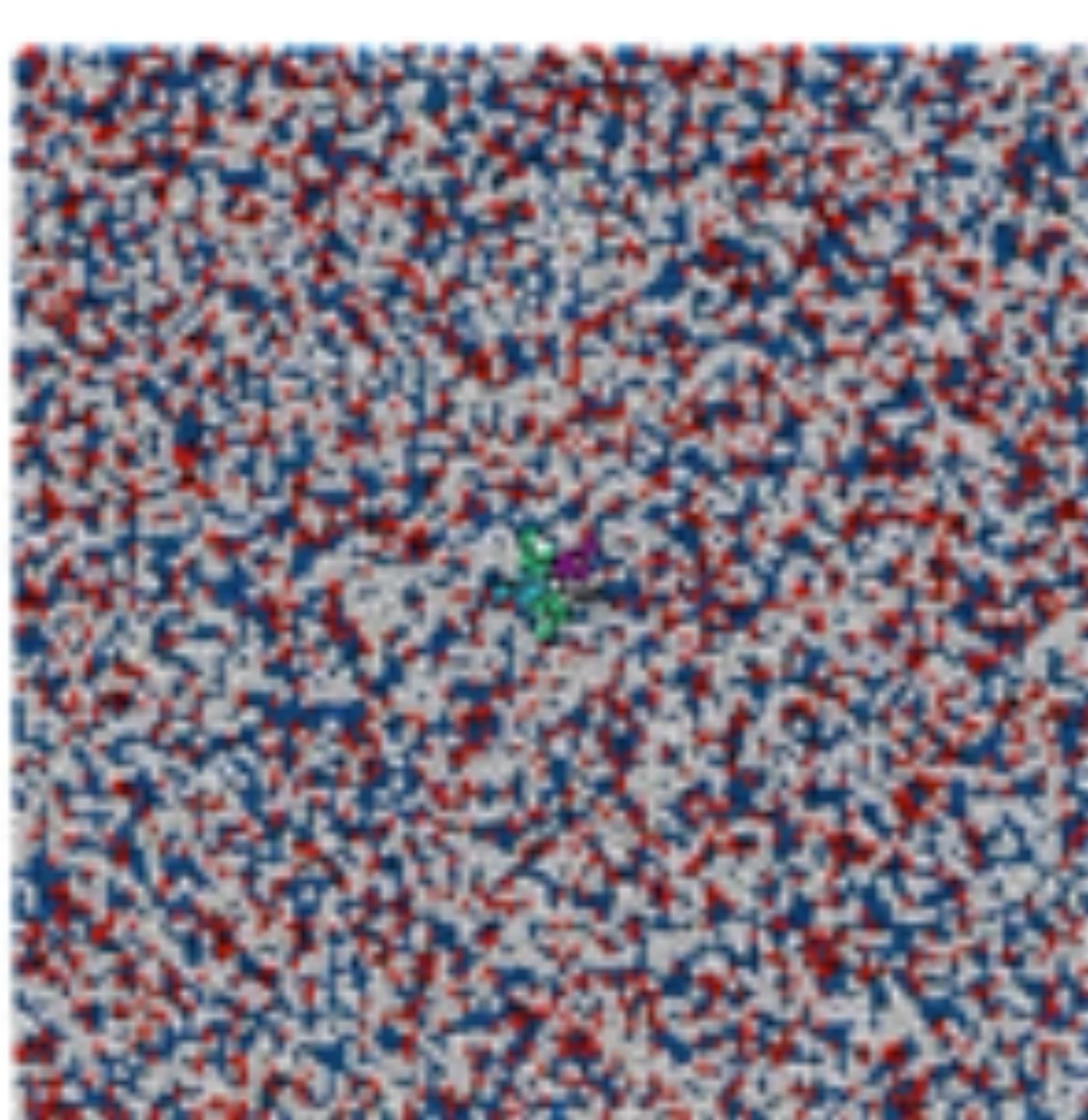
Model Membrane Extra-Cellular View



~ 2 us    75x75nm<sup>2</sup>

# And the pLGIC partitions into the....

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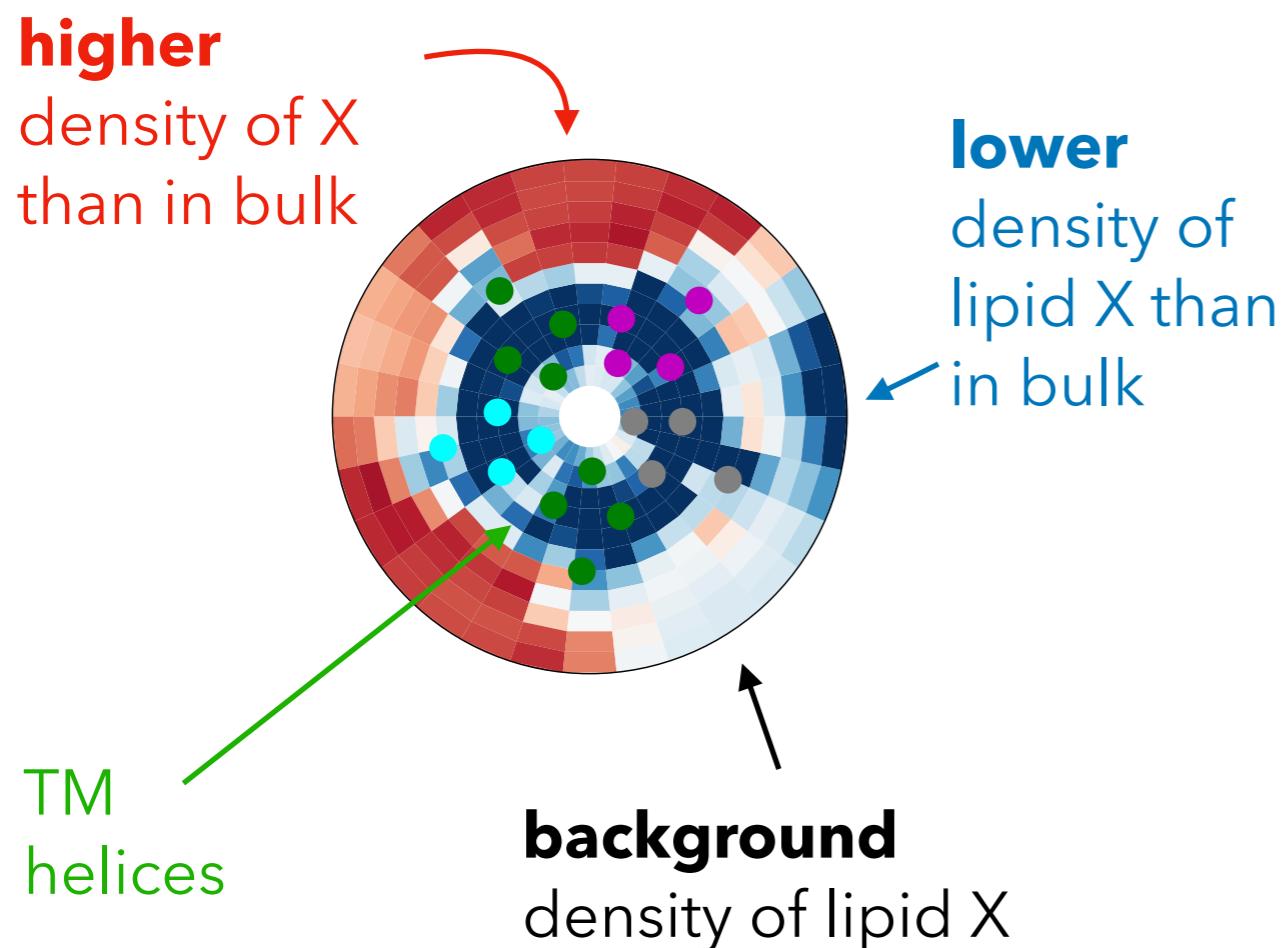


...liquid  
**disordered**  
phase?!?

In retrospect,  
makes sense:  
membrane  
deformations by  
cone-shaped  
protein are less  
costly in more  
flexible domain

# Quantifying lipid densities

For a given lipid species X



Sat

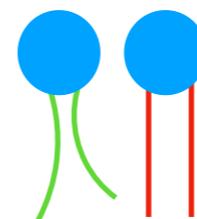
Chol

PUFA

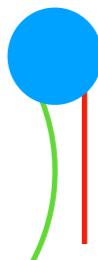
# Are domains required for specific binding?

- Recent MS results: most plasma membrane lipids are heteroacidic: saturated and unsaturated chains in the same lipid.
- Can't separate into domains!

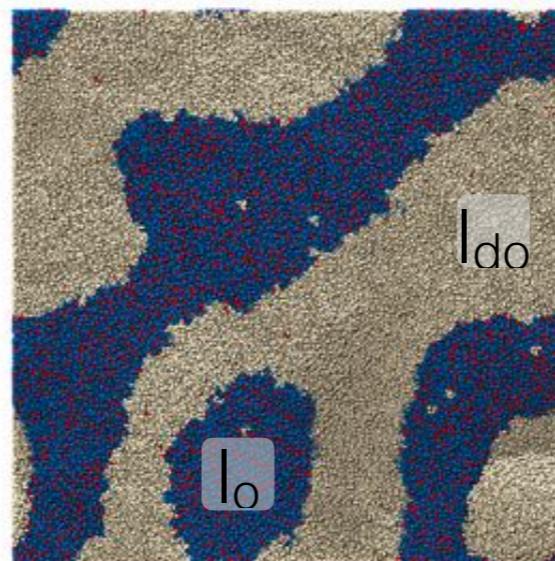
Homo-Acidic  
Domain Forming



Hetero-Acidic  
Non-Domain Forming



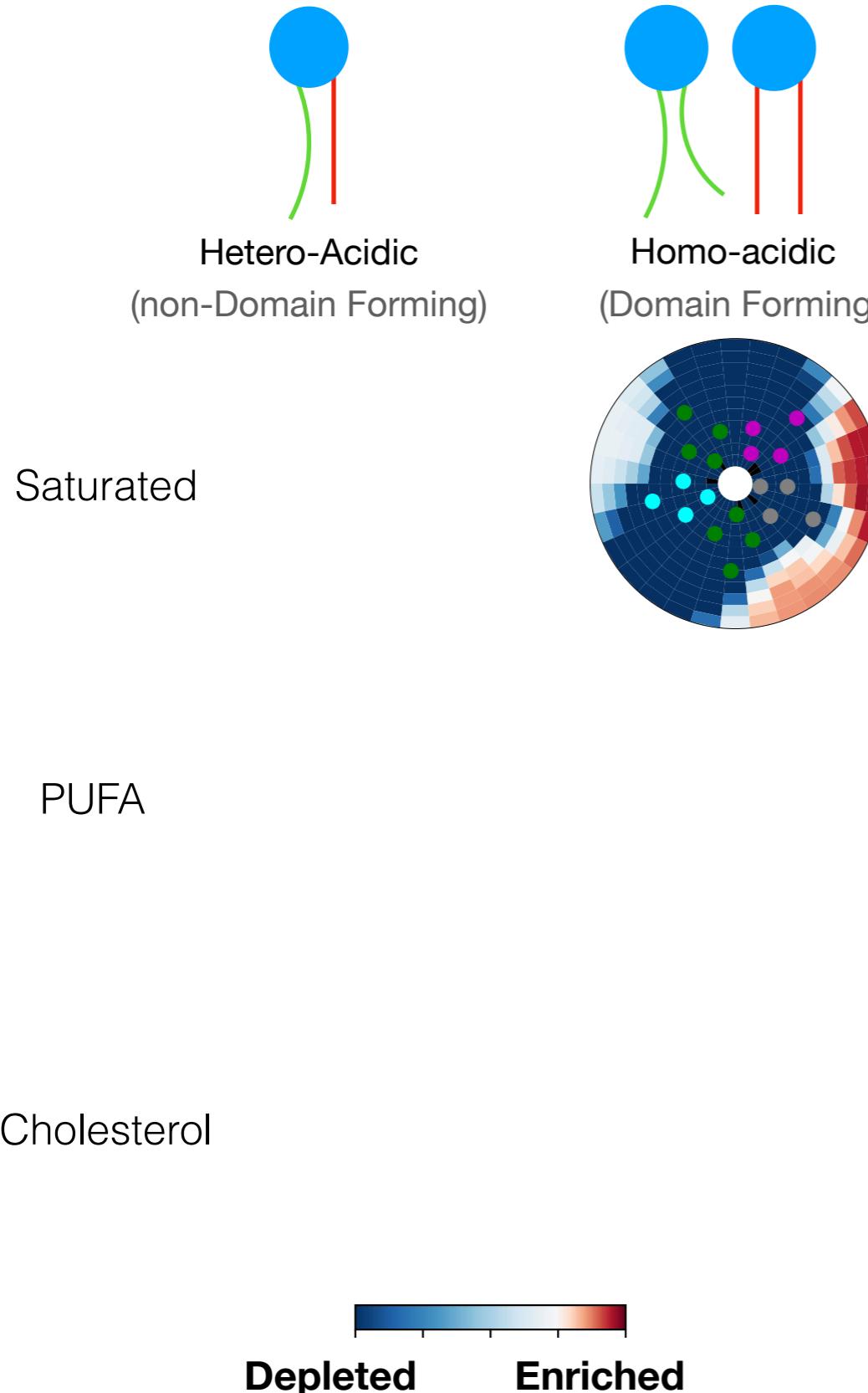
Model Membrane



Native Membrane



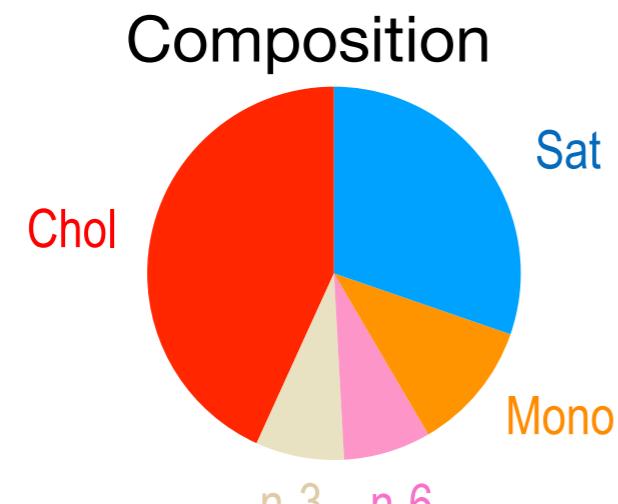
# Sorting Hetero vs. Homoacidic lipids



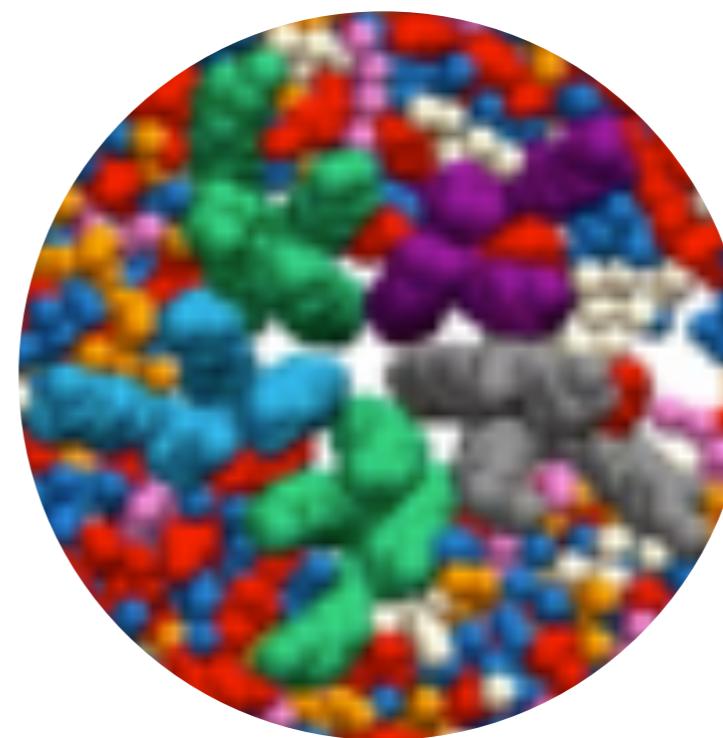
When domains are removed:  
PUFA enrichment decreases  
Symmetric, highly-localized  
sites emerge

# simulations in a quasi-neuronal membrane

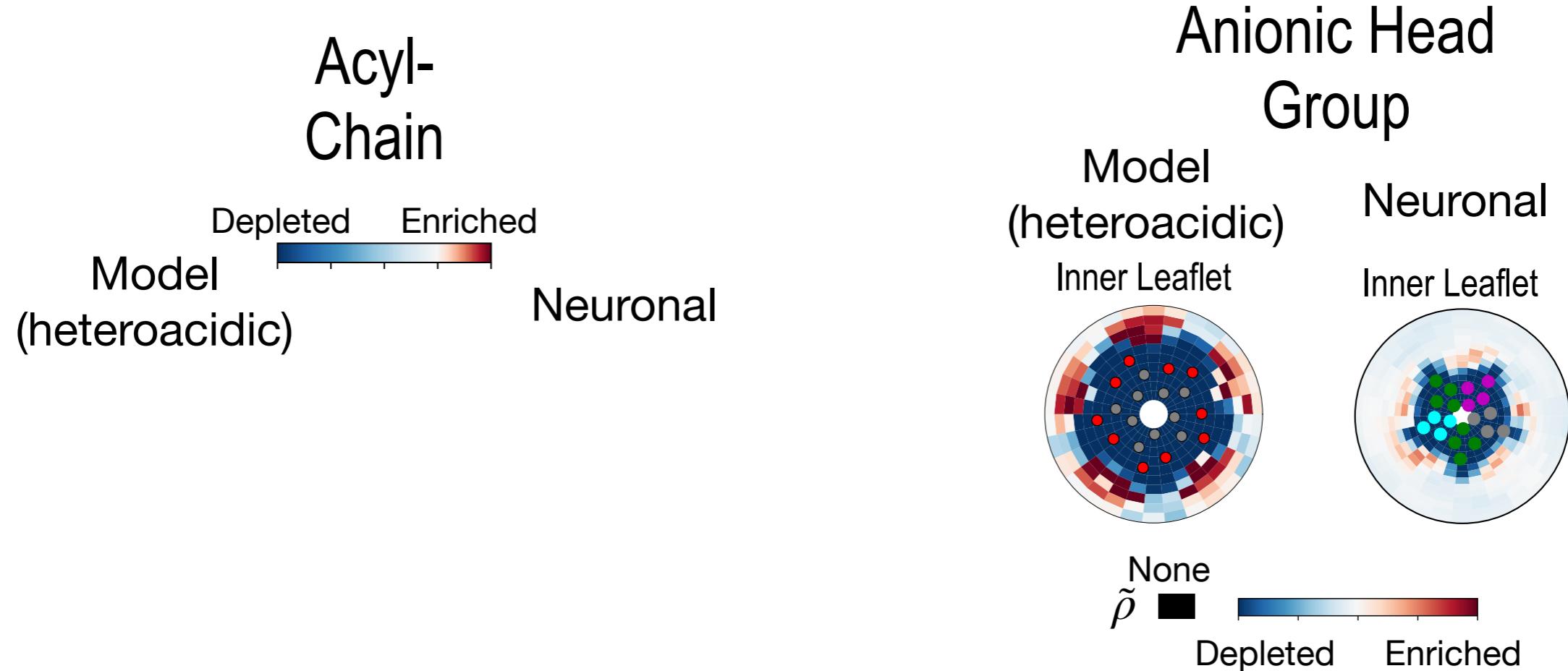
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Helgi I Ingólfsson et al, 2017



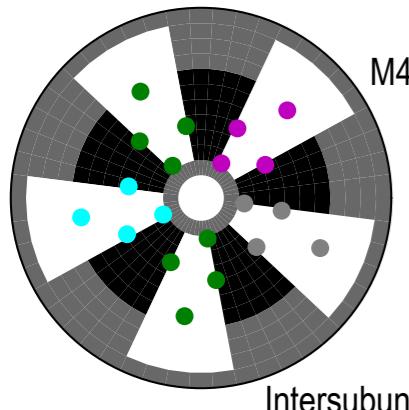
# Neuronal vs. Heteroacidic Model



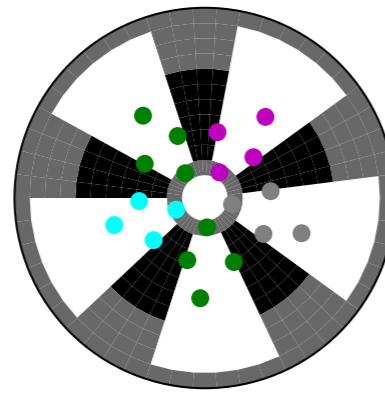
Specific sites are more localized in neuronal membranes, but distribution patterns are consistent

# Quantifying binding free energies from densities

Outer Leaflet



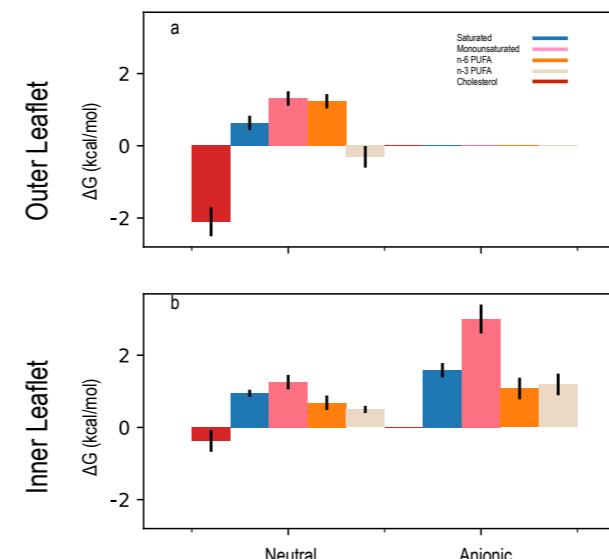
Inner Leaflet



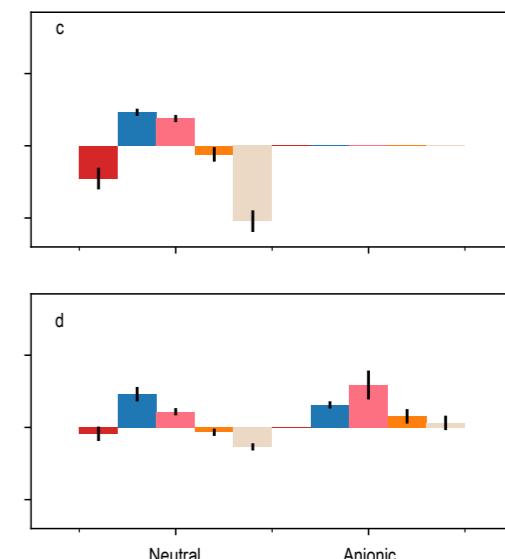
Intersubunit

## Site-centric

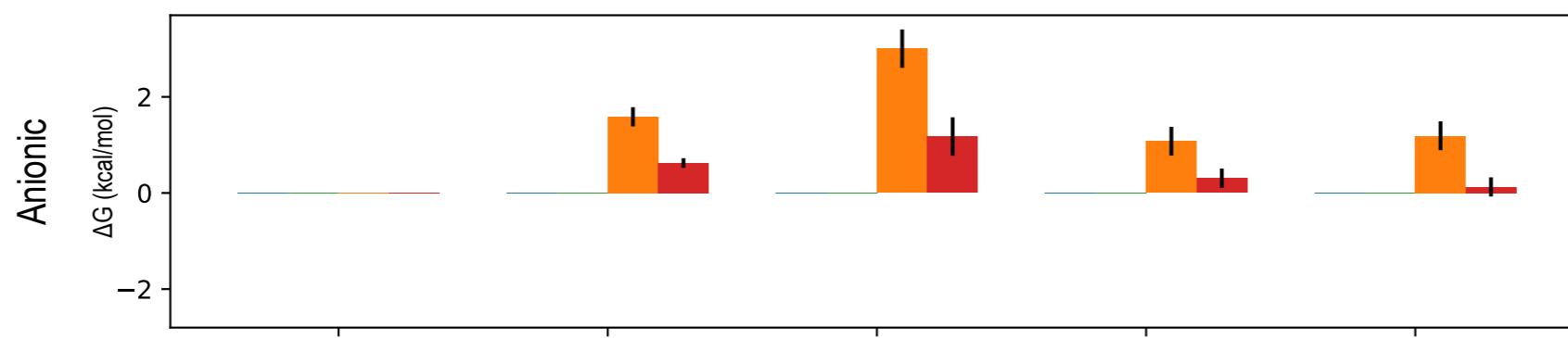
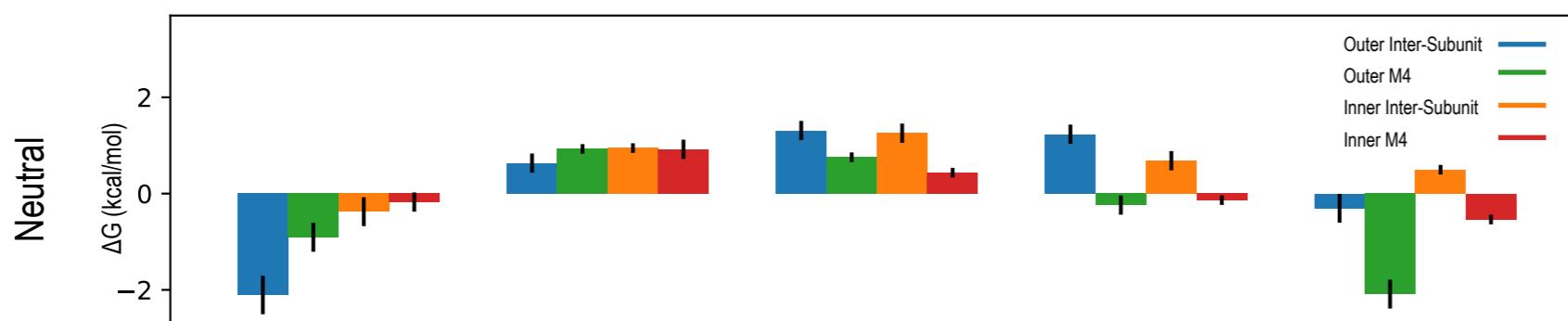
Inter-Subunit Site



M4 Site

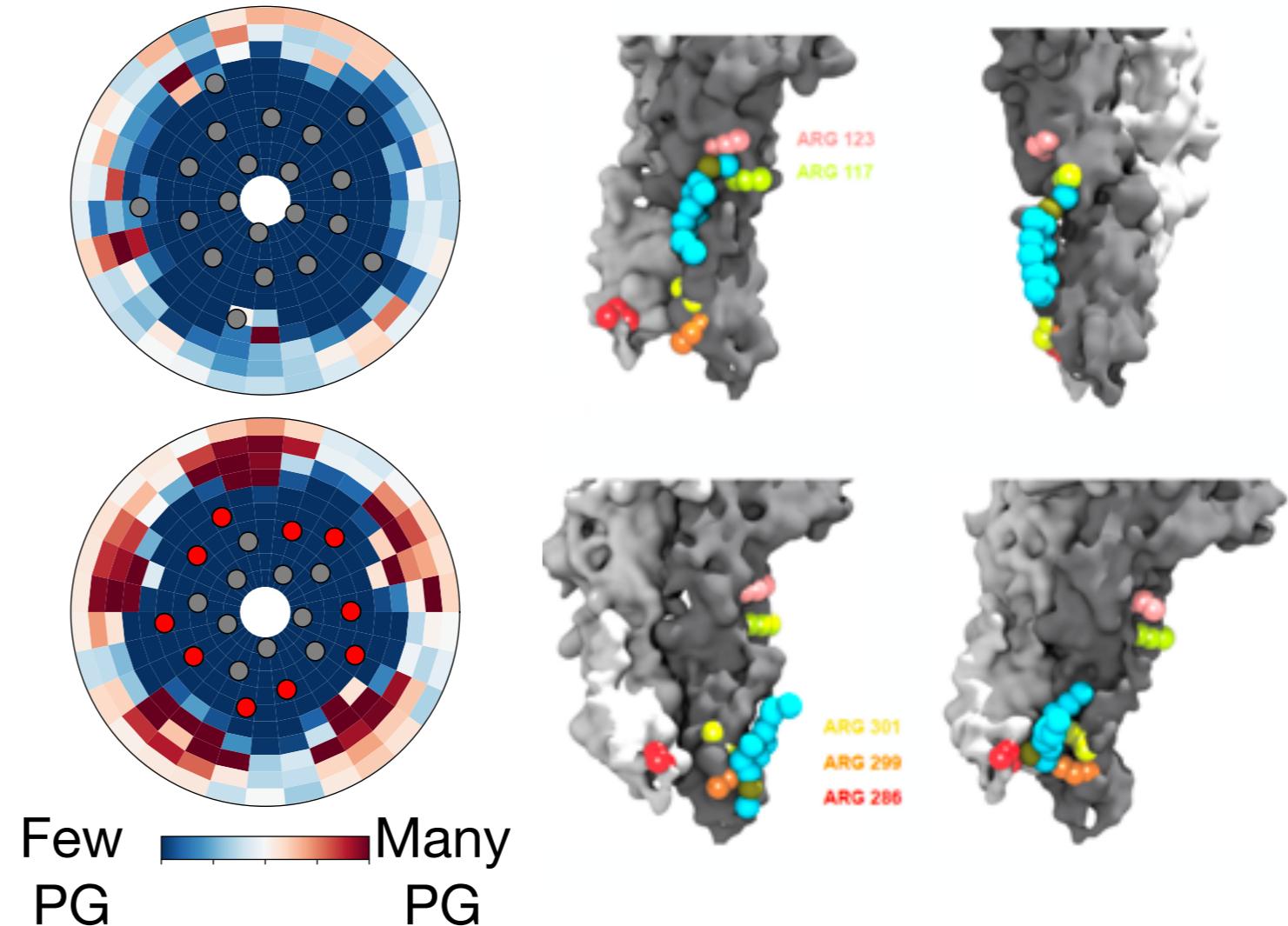
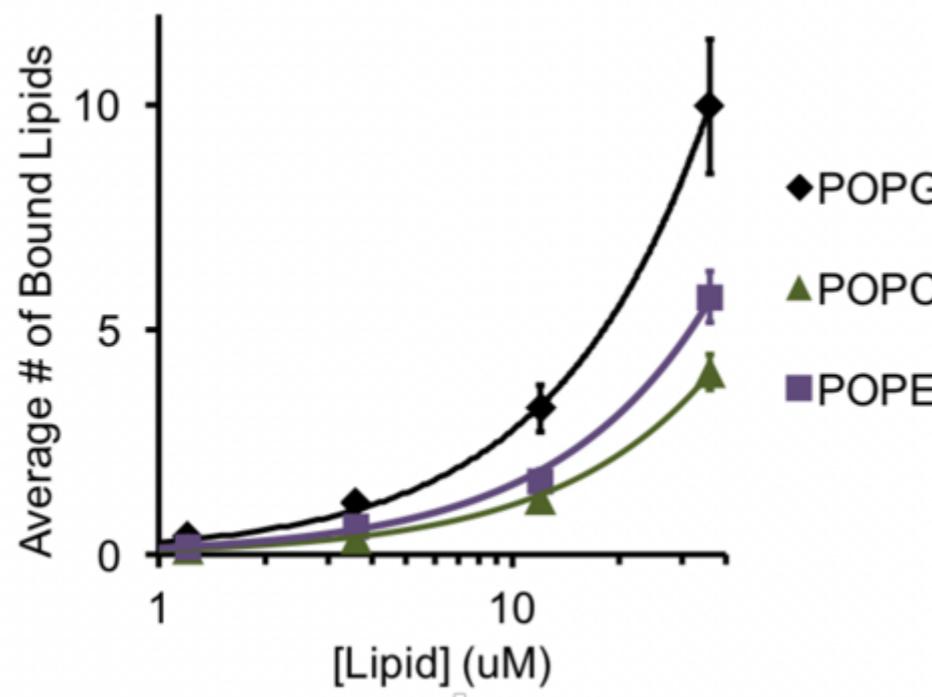


## Lipid-centric



# Expt Validation in ELIC

## Native Mass Spec (ELIC)



# Second Summary

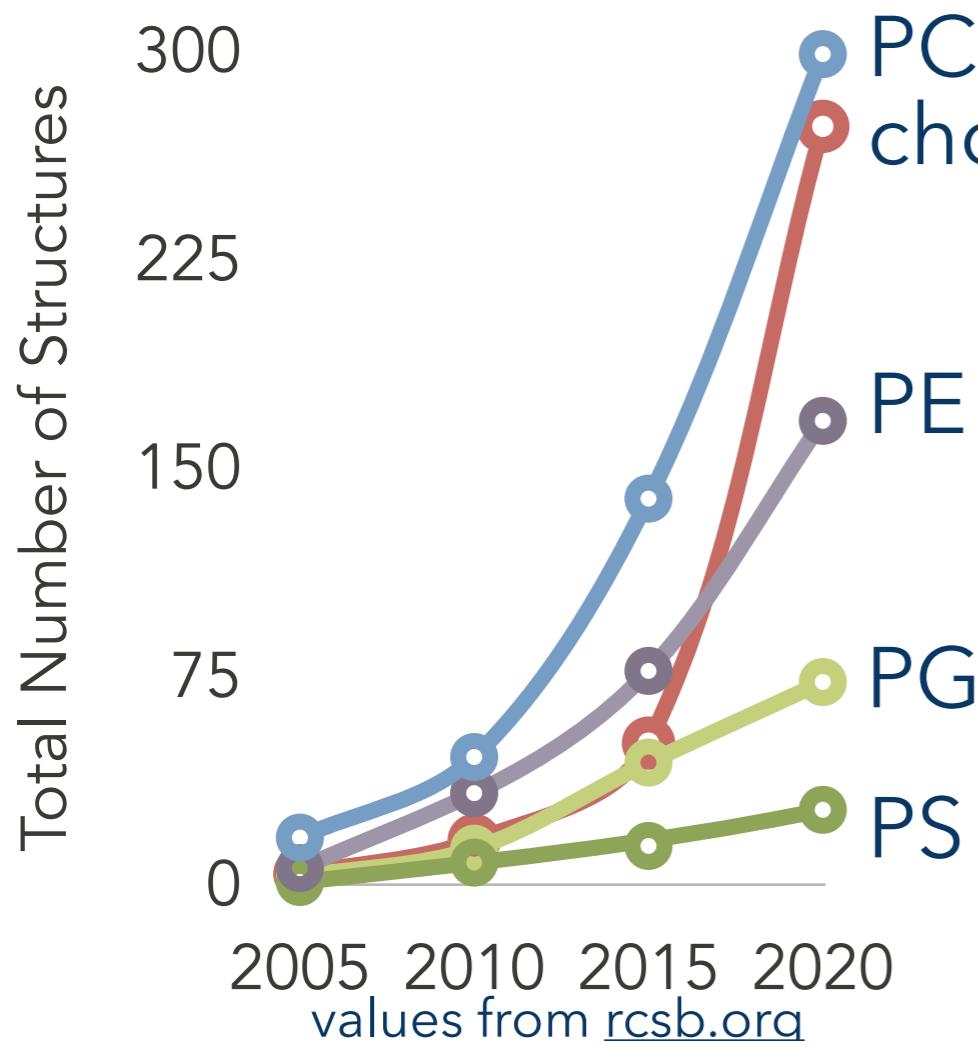
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- Heteroacidic lipid mixtures (particularly SDPC/cholesterol) reproduce the distribution of acyl chains in neuronal membranes
- Distribution of anionic headgroups is dependent upon sequence:  
nAChR anionic headgroups only bind to the inner leaflet TMD, while ELIC has occupancy in both leaflets
- Cholesterol prefers the intersubunit site in the outer leaflet; PUFAs prefer to pack around the M4 helix

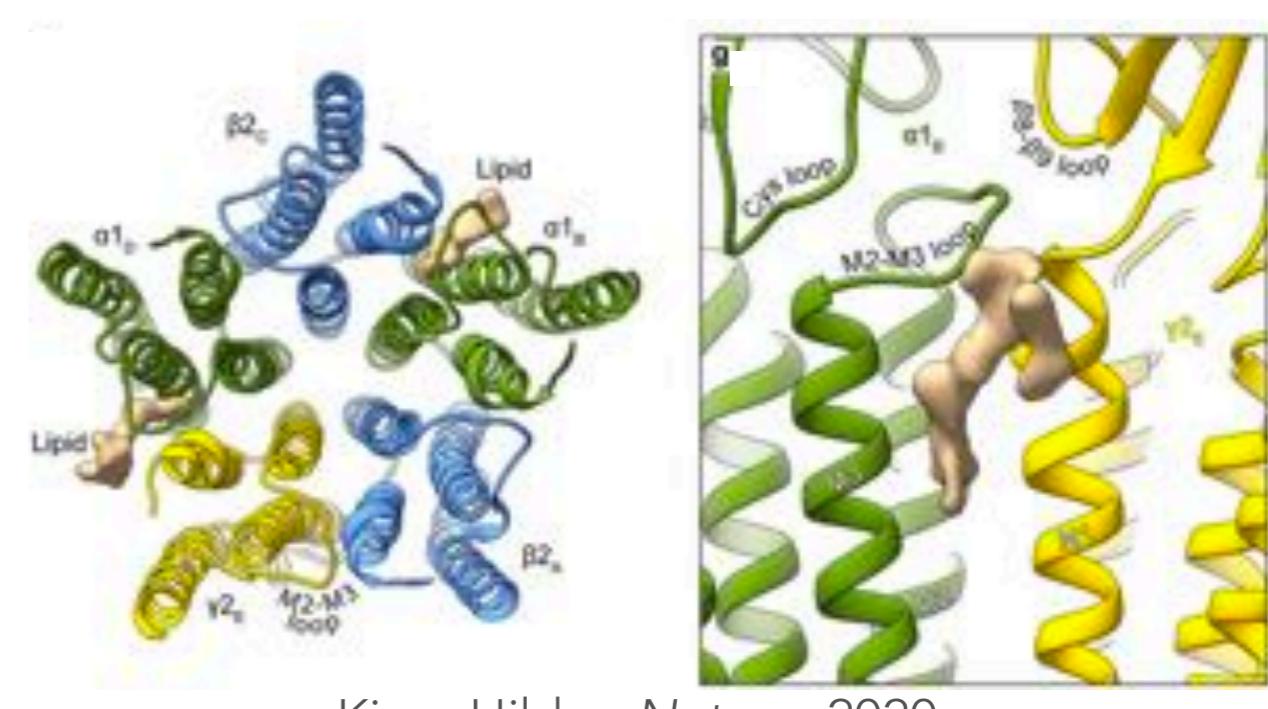
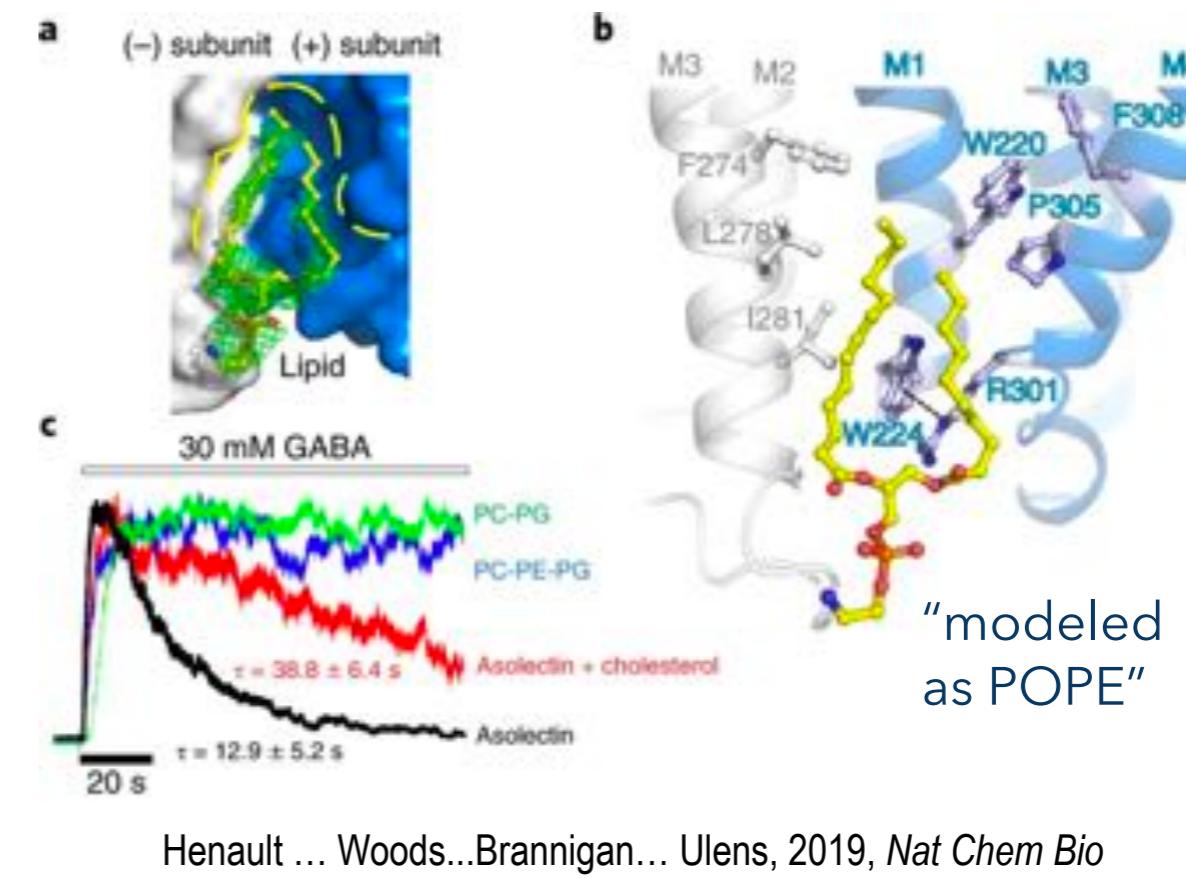
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### III. Lipid specificity through structural biology and free energy calculations (higher-resolution)

# structures with resolved lipids



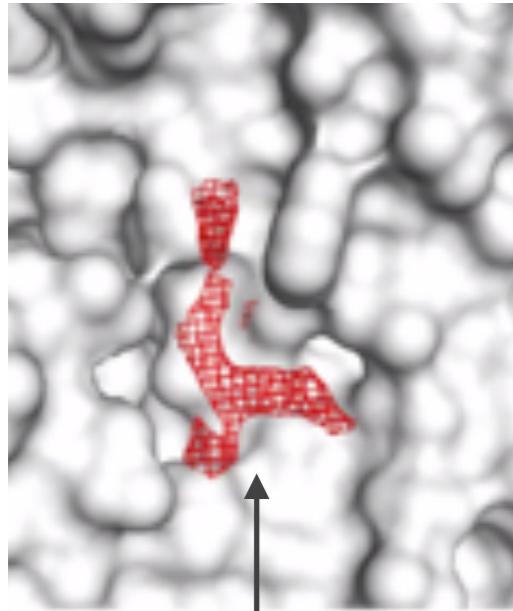
Problem: you can't usually identify the bound lipid.



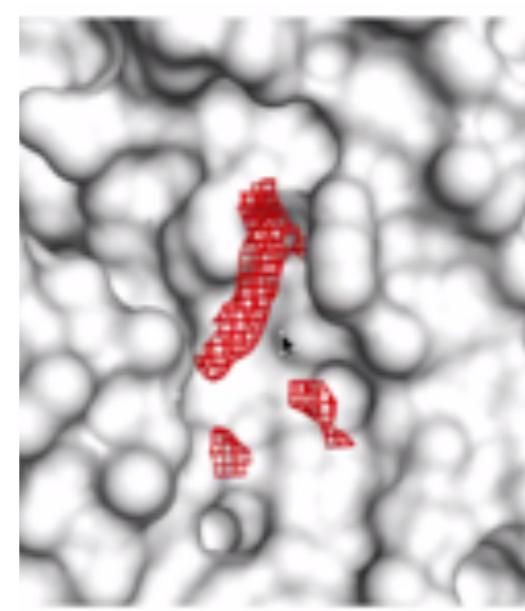
# Whodunit?

## Evidence

Courtesy of Dr. Wayland Cheng



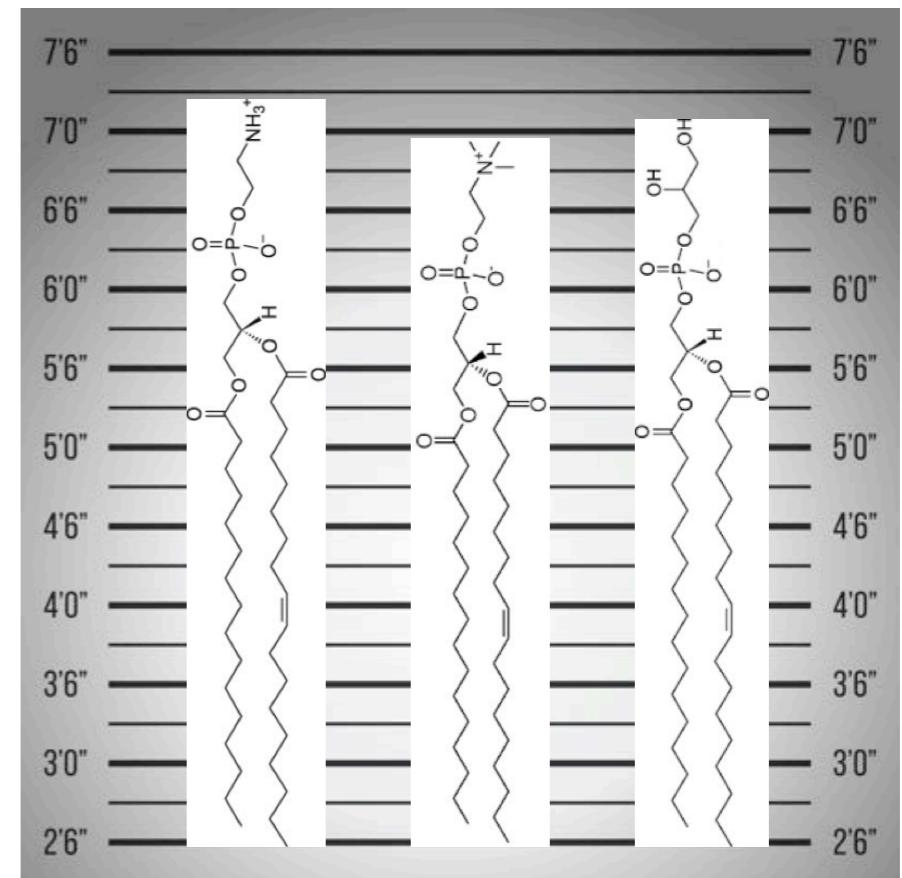
spotted in asolectin  
nanodiscs



and in POPC/POPE/  
POPG nanodiscs

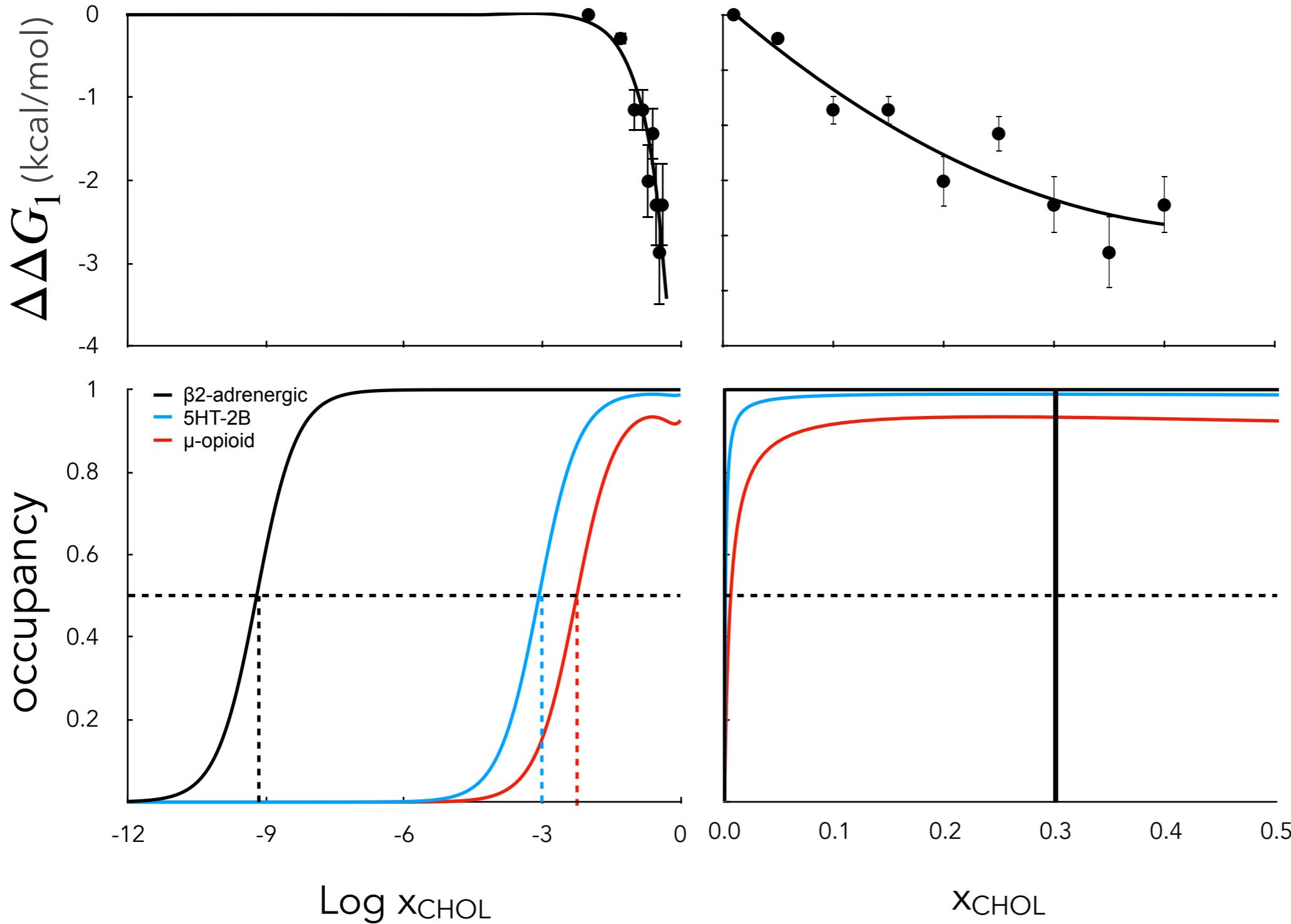
Who is this?

## Suspects

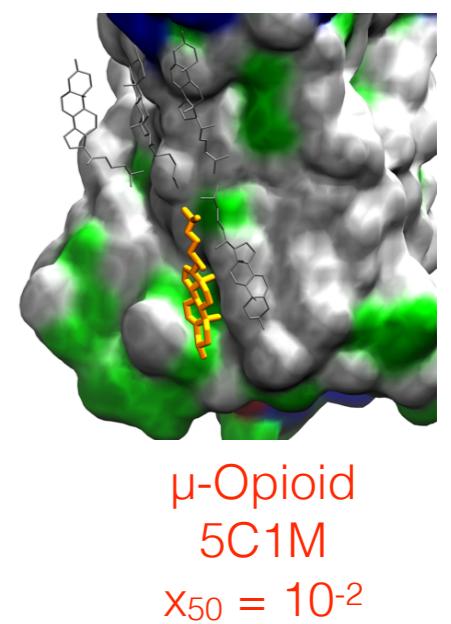
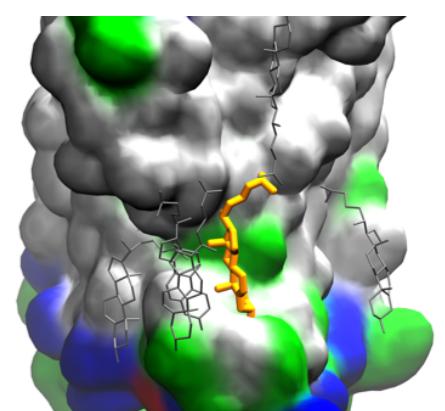
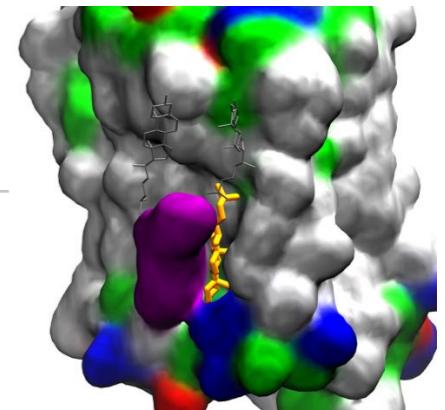


Goal: identify the lipid using SAFEP (the same approach we used to calculate GA binding affinities)

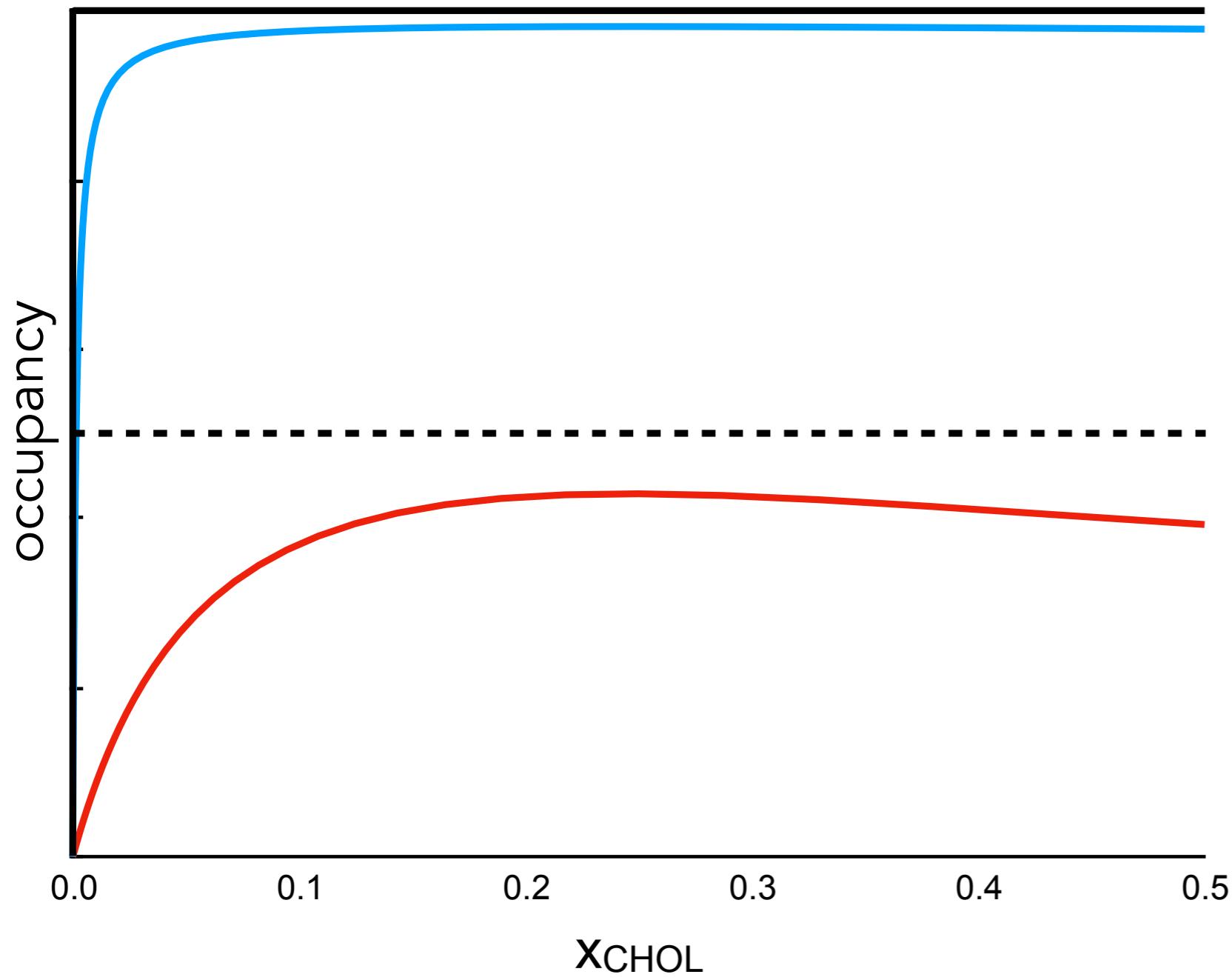
# proof of principle: virtual cholesterol binding assay



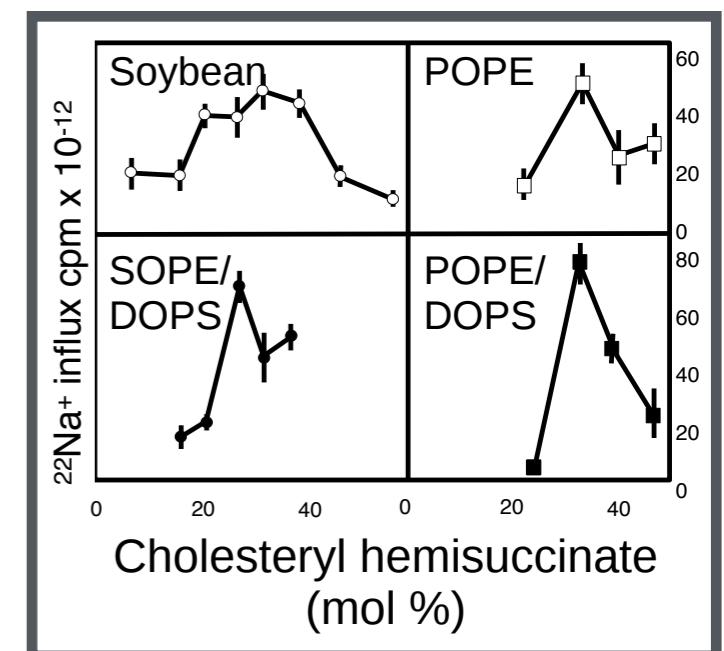
Salari, Joseph, Lohia, Henin, Brannigan, JCTC 2018



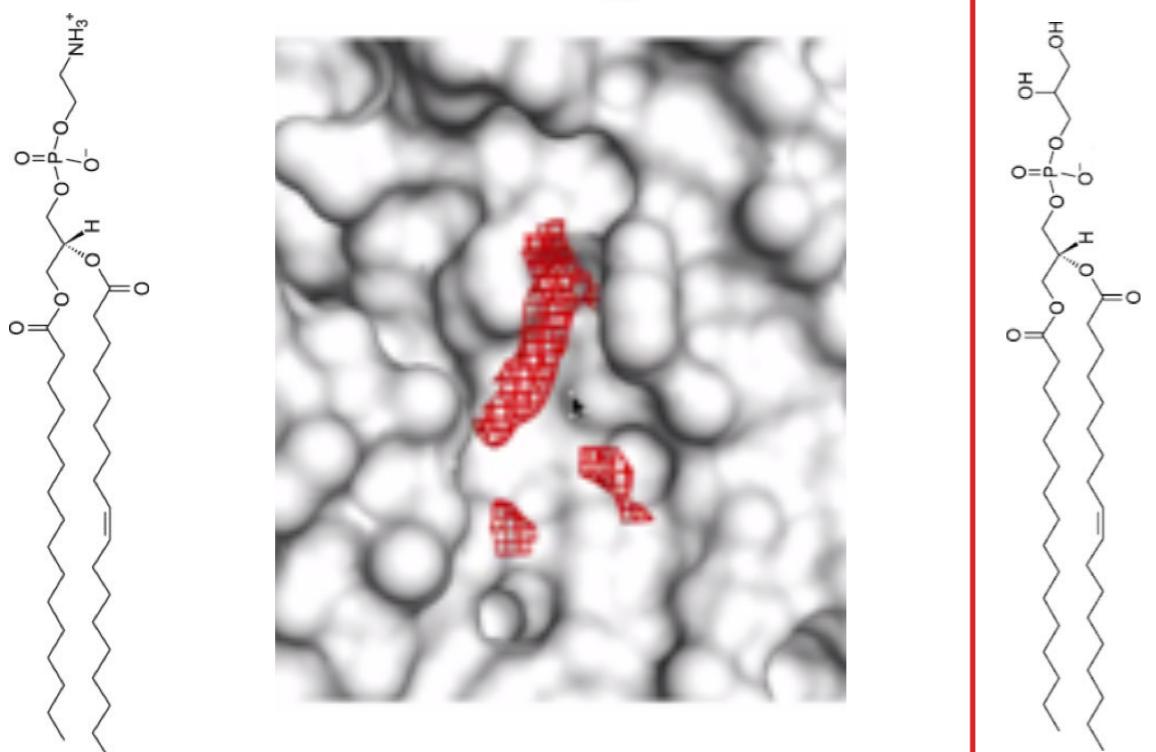
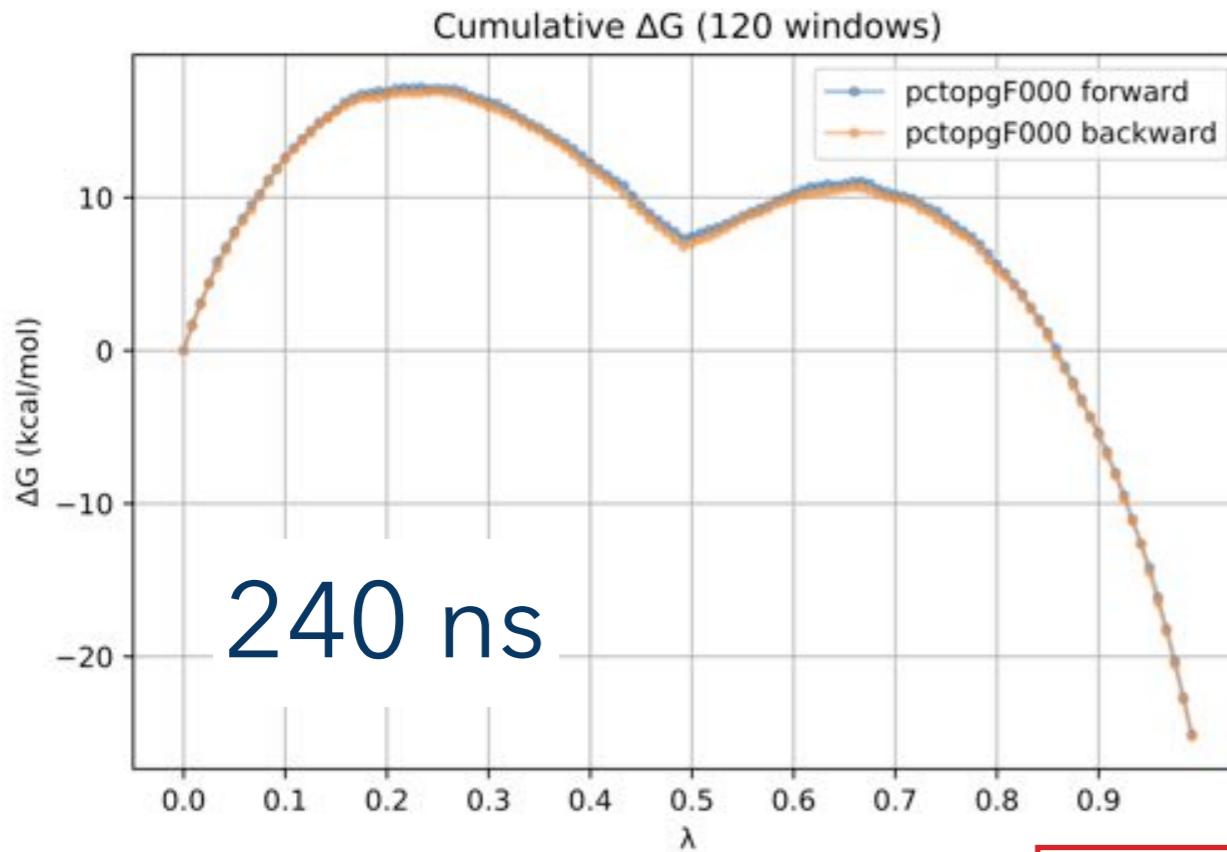
# surprising sensitivity



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



# But can SAFEP converge for a flexible lipid?



Yes!

$$\Delta\Delta G_{protein} = -23.6 \text{ kcal/mol}$$

$$\Delta\Delta G_{membrane} = -16.6 \text{ kcal/mol}$$

$$\Delta\Delta G = -7.0 \text{ kcal/mol}$$

$$\frac{P_{PG}}{P_{PC}} = e^{-\Delta\Delta G/k_B T} = 10^5$$

Conclusion: For equal amounts of POPC and POPG, you are  $10^5$  times more likely to find POPG in this site than POPC.

# Final Summary

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- Here, the most general predictor of intersubunit site selectivity is water displacement
  - drugs that bind to lipid-facing sites need to successfully compete with lipids
  - what those lipids are will vary with experimental approach, cell type, individual
  - in real cells, proteins will likely have access to full range of lipids (less segregation into rafts than we expected)
- Two approaches for quantifying the likelihood of lipids binding to individual sites under certain conditions
  - affinities from spontaneous binding: rigid lipids bind to concave regions, flexible lipids to convex regions
  - SAFEP allows us to increase resolution: identify lipids in structures

# Acknowledgments

## Contributing Group Members

*Dr. Reza Salari*

*Dr. Thomas Joseph*

*Dr. Mark Arcario*

*Dr. Sruthi Murlidaran*

*Dr. Ruchi Lohia*

*Dr. Liam Sharp*

*Kristen Woods*

*Rulong Ma*

## Collaborators

*Dr. Jérôme Hénin, (CNRS-IPBC France)*

*Dr. Wayland Cheng (Washington University - St. Louis)*

*Dr. Thomas Joseph (University of Pennsylvania)*

*Dr. Roderic Eckenhoff (University of Pennsylvania)*

*Dr. John Baenziger (University of Ottawa)*

*Dr. Chris Ulens (KU Leuven)*



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