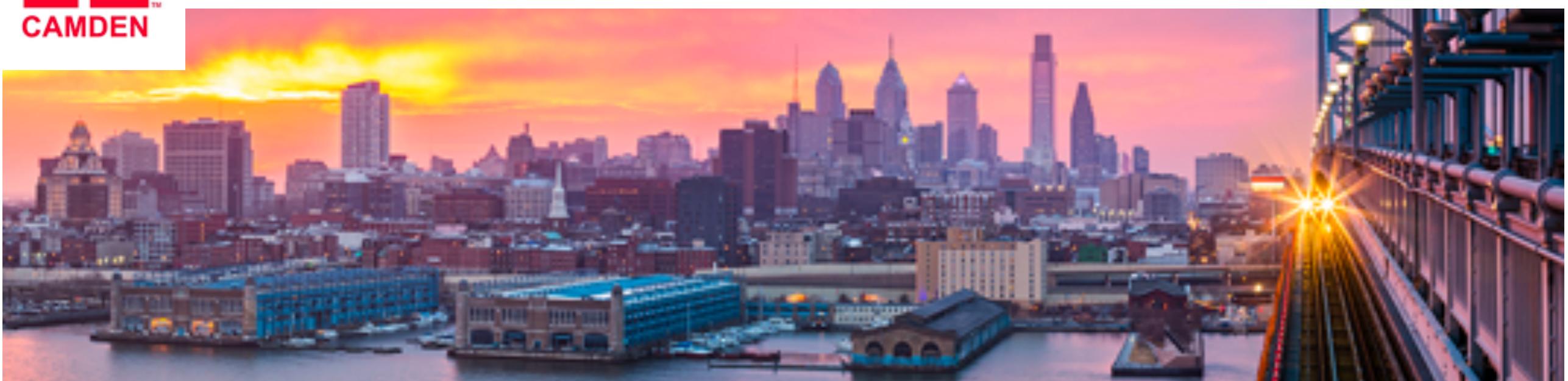


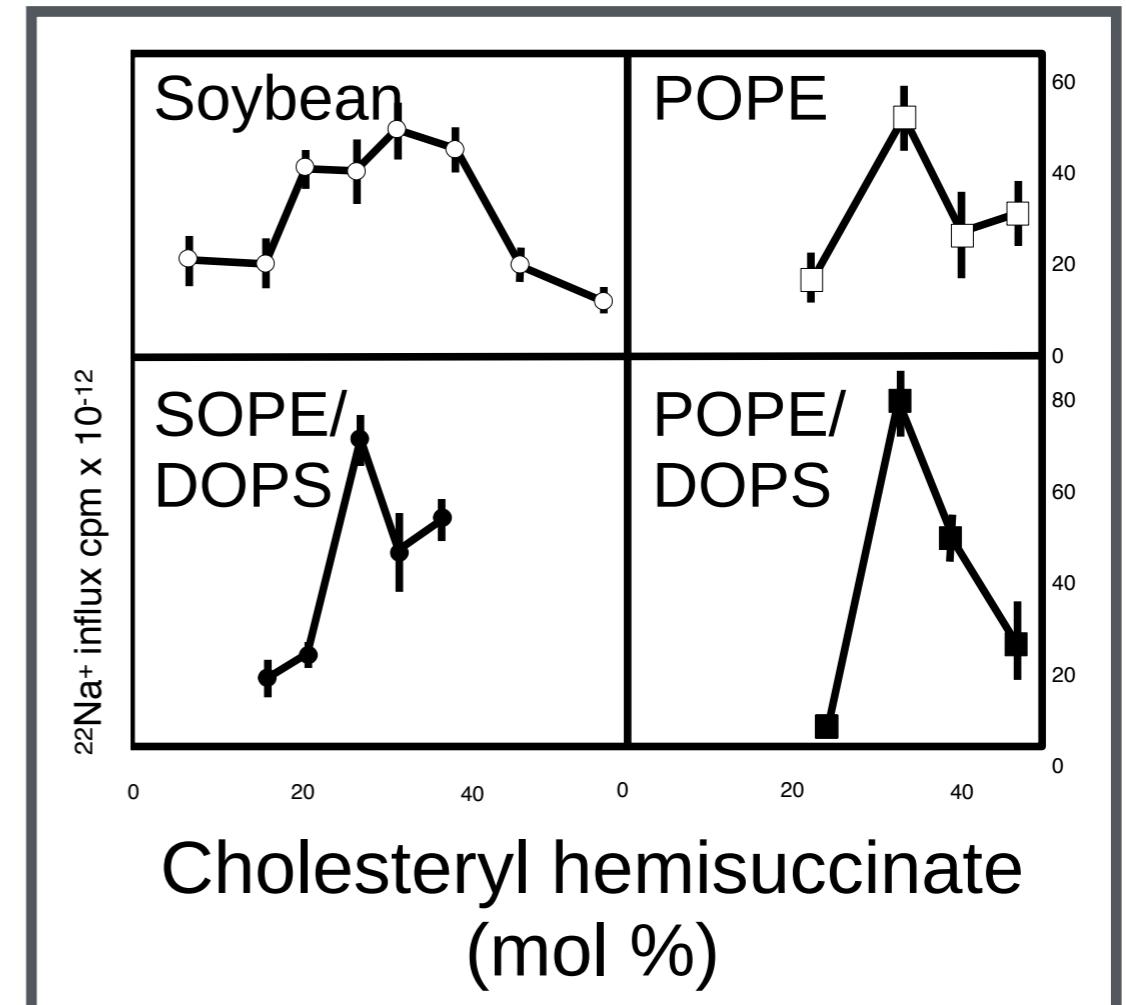
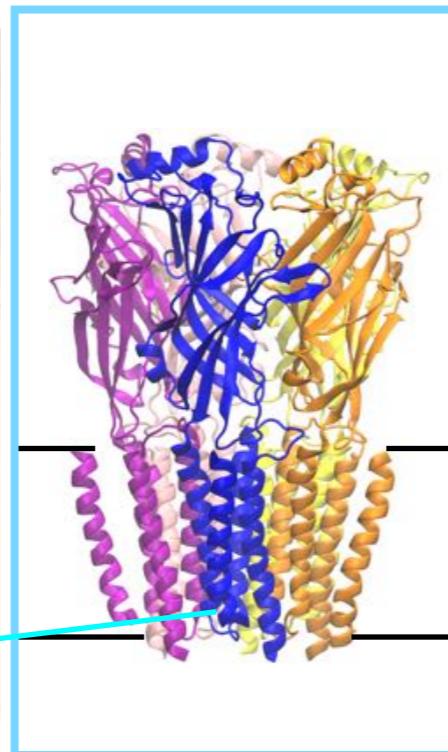
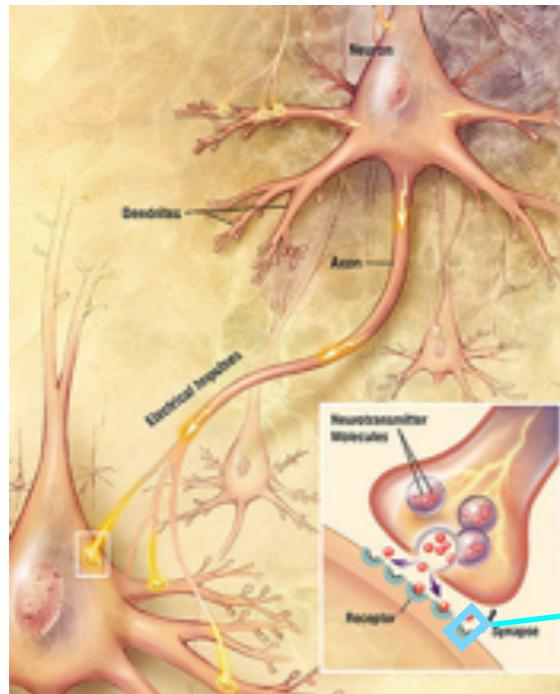
# Identifying structurally-resolved lipid fragments through molecular simulation

Grace Brannigan  
Center for Computational &  
Integrative Biology  
Rutgers University - Camden



# motivation: mechanism of lipid modulation

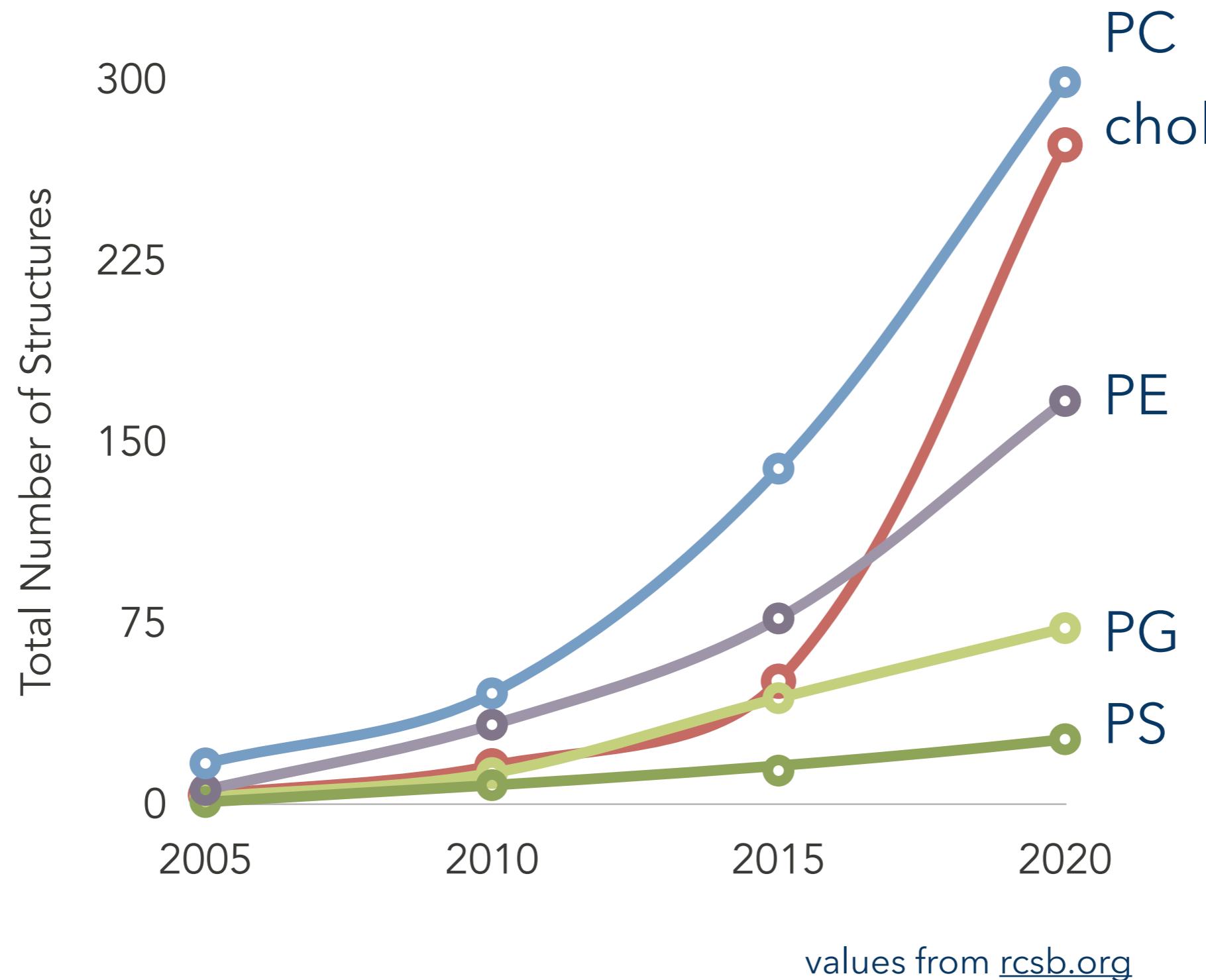
nicotinic acetylcholine receptor, GABA<sub>A</sub> receptor, glycine receptor, etc



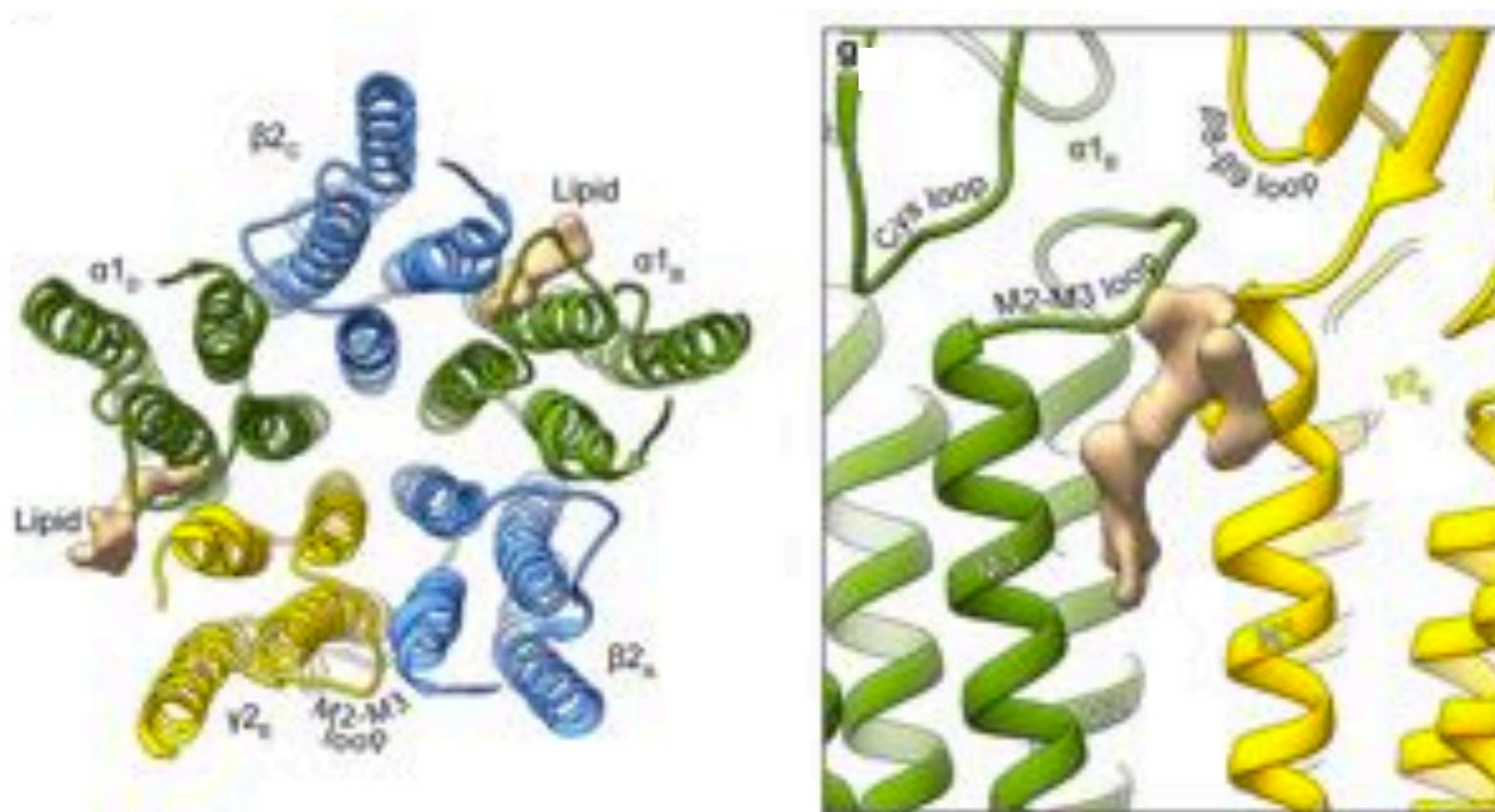
Criado...Barrantes, 1984, J.Biol.Chem.

Lipid sensitivity has been long established, but mechanism is still unknown

# structures with resolved lipids



# Problem: can't connect structure to function



Kim...Hibbs, *Nature*, 2020

Unless you can identify the bound lipid.

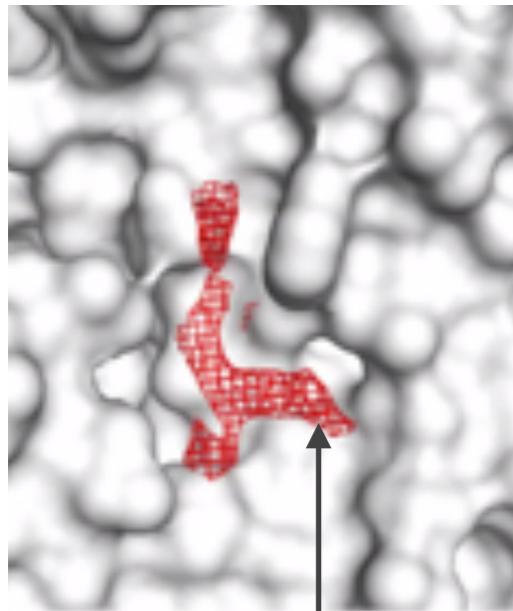
In a lipid mixture, usually you can't.

# Detecting specific lipid binding

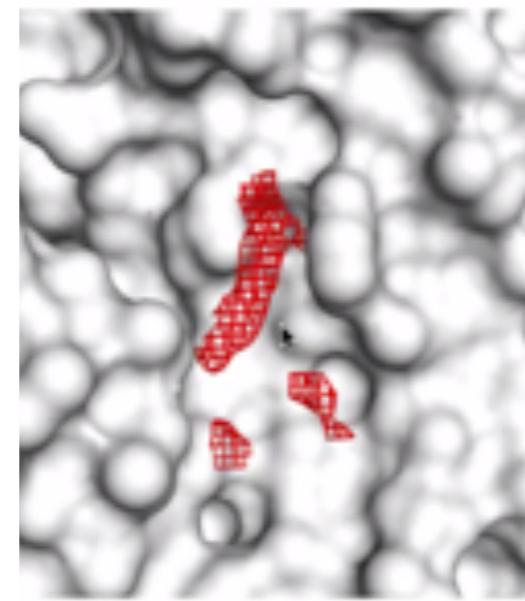
	Experiments	MD Simulation
Who	Lipid Species	
Where	Binding Site	
When	Affinity	
	Mass Spectrometry	
	Soluble lipid-binding assay	
	Structural Biology	
	Atomistic (AA)	
	Coarse-grained (CG)	

# Whodunit?

## Evidence



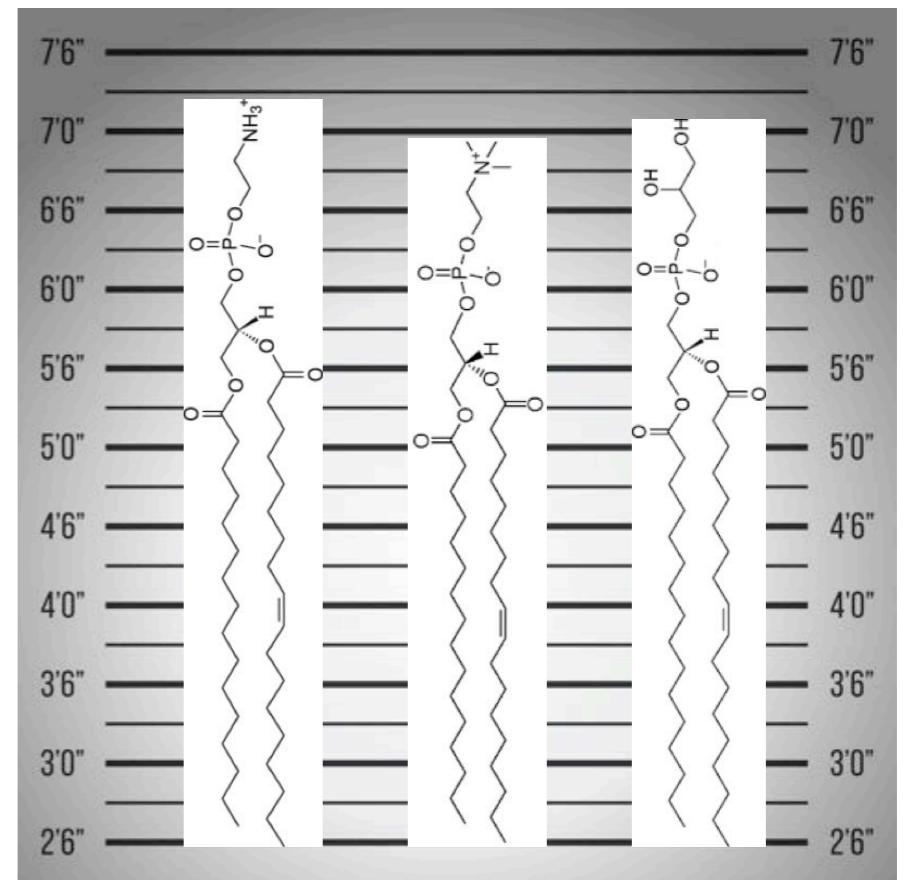
spotted in asolectin  
nanodiscs



and in POPC/POPE/  
POPG nanodiscs

Who is this?

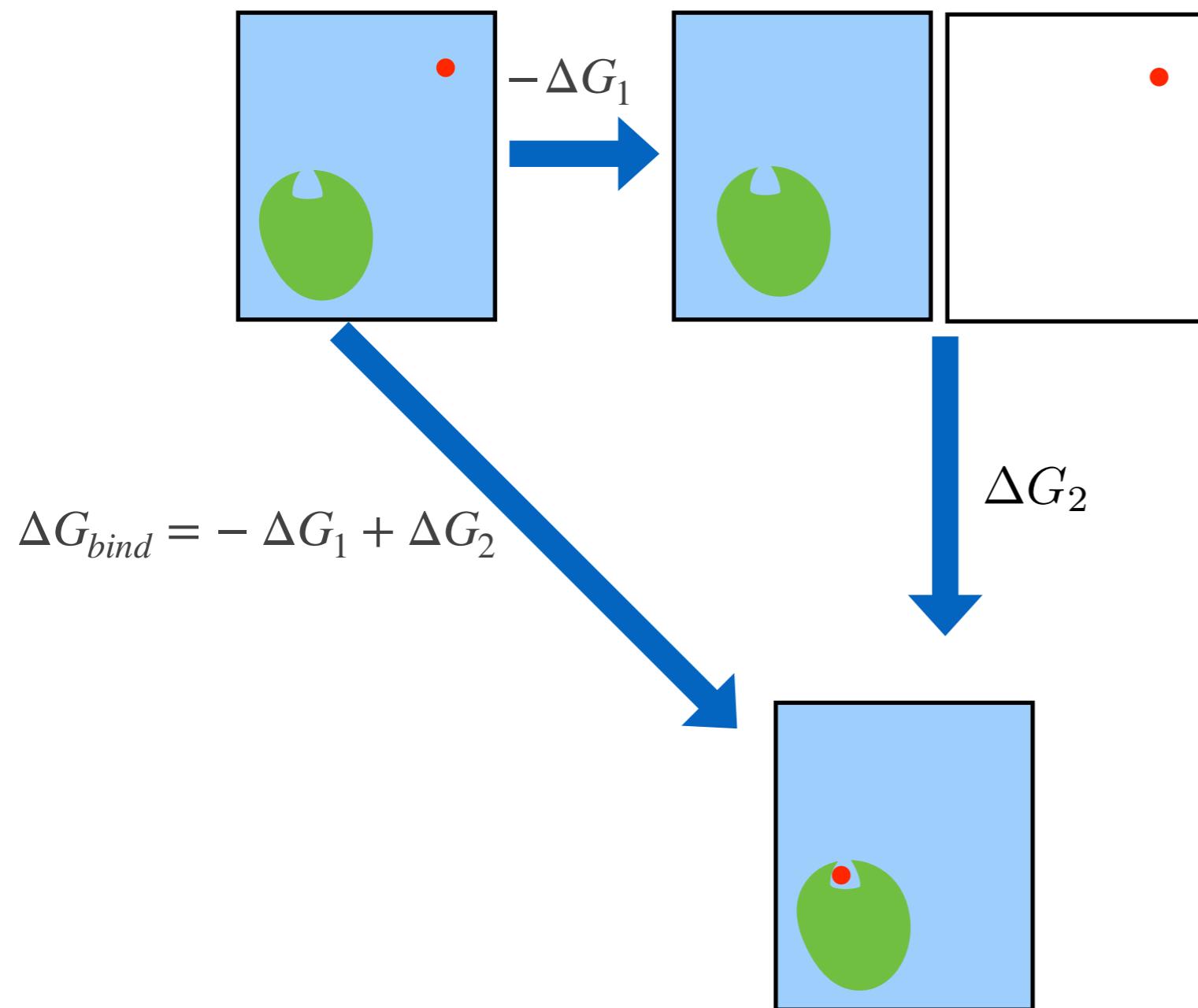
## Suspects



Goal: calculate relative affinities  
using atomistic simulation

# Ligand binding in AA-MD

Classic approach: Free Energy Perturbation (FEP)



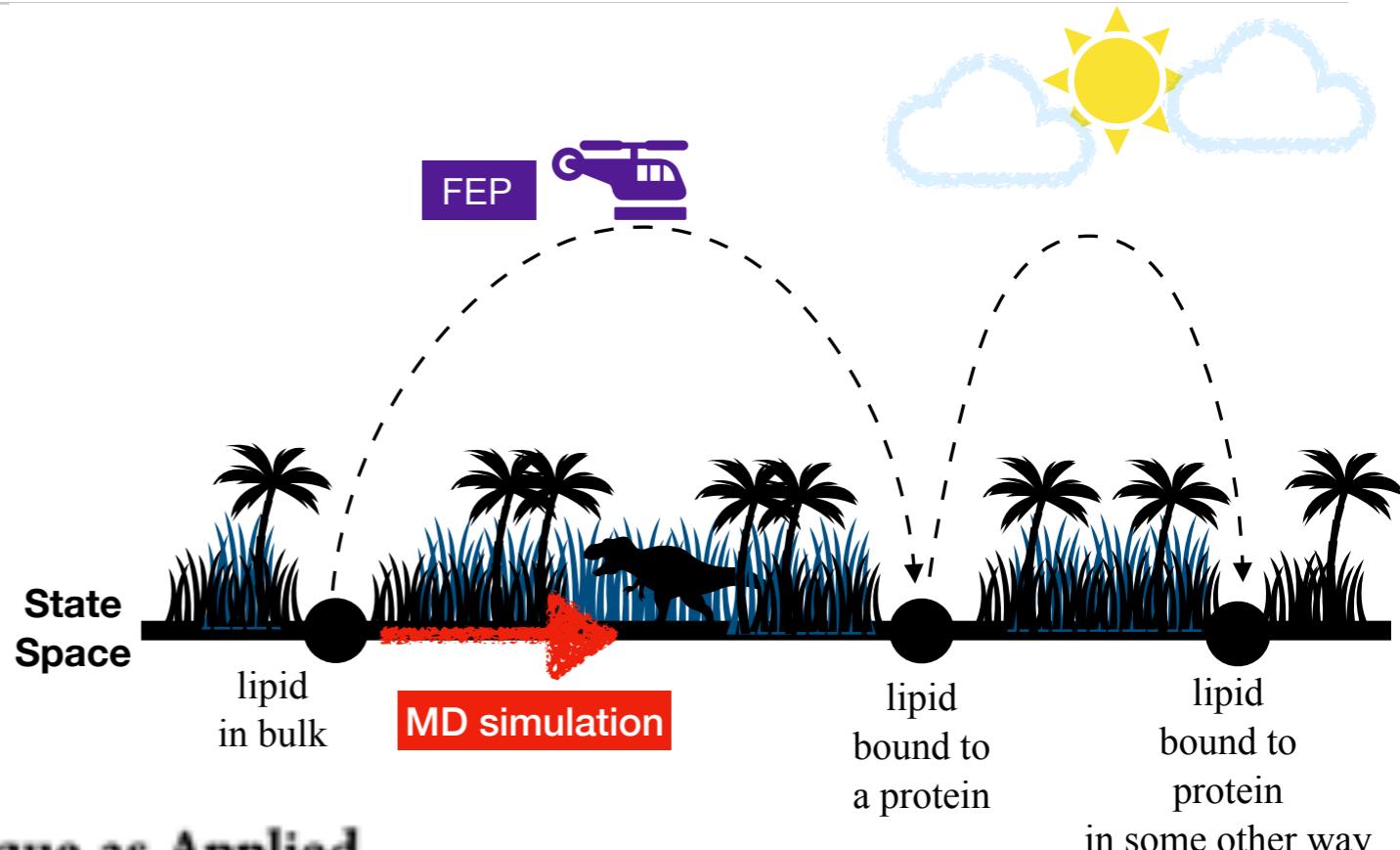
Two-state transition:  
**No need** to sample  
unbinding path!

Traditionally: run at  
one concentration,  
assume ideality

# Why get FEP to work in a membrane?

## Comparison in CG membranes

We then probed these sites using ABFE, using restraints and a thermodynamic cycle outlined in a recent study by Salari et al.<sup>46</sup> (see [Supporting Methods](#) and [Supporting Figure 3](#)). We obtained  $\Delta\Delta G_{\text{bind}}$  values of  $-5 \pm 2$ ,  $-8 \pm 1$ , and  $-2 \pm 1$  kJ mol<sup>-1</sup> for the three sites ([Figure 6C](#)), in reasonable agreement with the WTMetaD data. Importantly, we get an identical ranking of the sites between the techniques, with site 2 the highest energy and site 3 the lowest. Note that we were unable to probe these sites using PMF calculations, as the energies become swamped by background thermal fluctuations.



**Table 2. Computational Cost of Each Technique as Applied Here<sup>a</sup>**

Matthew Hansen, 2020

technique	system applied to here	required simulation time (μs)
PMF	Kir2.2, AAC, LeuT	50–75
FEP	Kir2.2, AAC, LeuT	25–40
ABFE	A <sub>2A</sub> R	35
WTMetaD	Kir2.2, AAC, LeuT, A <sub>2A</sub> R	120–190

<sup>a</sup>Reporting the simulation time used in each of the analysis measures here. Note that, as described in the Discussion, these values may overestimate the time required for convergence.

# Our Approach: SAFEP

---

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

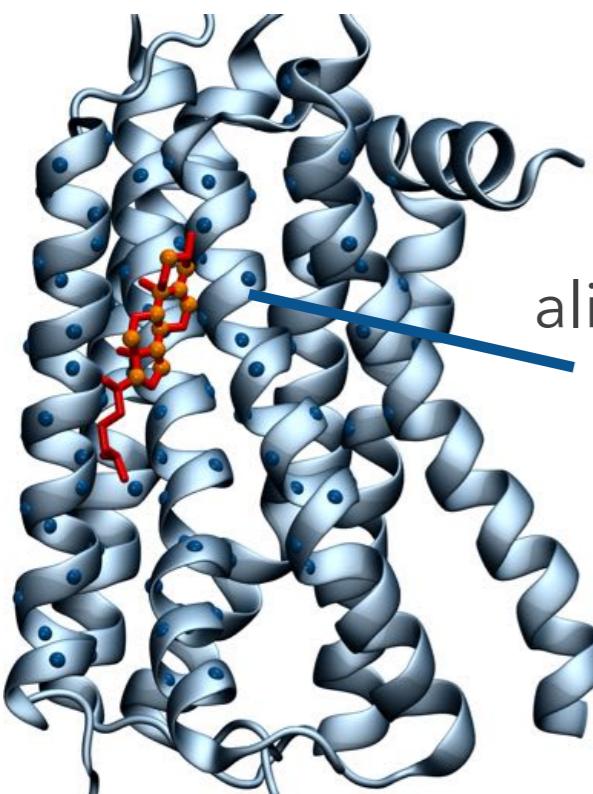
FEP but in a site-centered reference frame.

Switch to site-centered reference frame has to occur at every level, from implementation to interpretation - but it pays off.

- Implementation: new collective variable
- Interpretation: generalizeable theory

# Introducing: the DBC coordinate

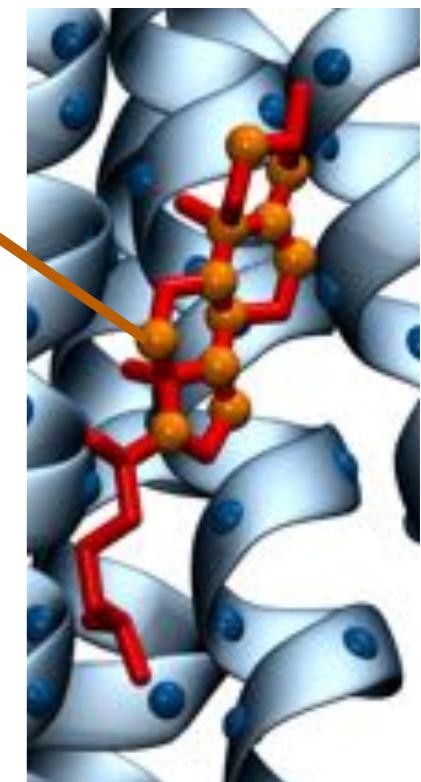
- distance-from-bound-configuration (DBC) coordinate: ligand RMSD in the site's frame of reference
- requires on-the-fly alignment in simulation software



aligns based on blue protein atoms...

...then calculates DBC using orange atoms

$$d = \left[ \sum_{l,\text{lig}} (\mathbf{x}'_l - \mathbf{x}^{\text{ref}}_l)^2 \right]^{\frac{1}{2}}$$



*Implemented in NAMD2.12  
and plugin for GROMACS*

User needs to choose protein atoms,  
ligand atoms, DBC tolerance. Simple!

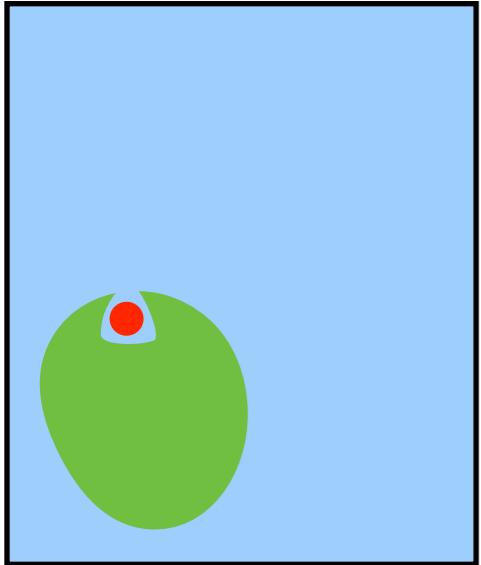
# Our Approach: SAFEP

---

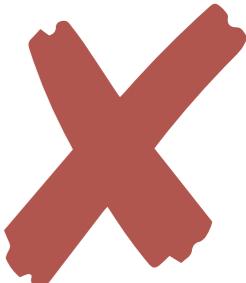
Main **interpretation** difference: anchored by occupancy probabilities, not standard binding affinities

- does require a general, more abstract, ligand-binding framework
- *bonus:* easy to incorporate **non-ideality!**

# Classical Binding Assumptions



ligand is **dilute** : no interactions between ligand



ligand is **abundant** : most ligand is free ligand



homogenous bulk, **well-defined volume**  
concentration



ligand binding has no effect on **receptor-receptor  
interactions**

# introducing: association coefficient $\kappa_A$

Sigmoidal binding assumes constant across concentrations

$$\frac{[RL]}{[R]} = K_A \gamma_L p_{\text{free}} [L]_{\text{tot}}$$

↓

constant by definition      constant for small  $[L]_{\text{tot}}$       only constant for large  $[L]_{\text{tot}}$

**problem:** Only true for intermediate concentrations. Rarely confirmed experimentally.  
 $K_A$  not useful outside infinitely dilute limit.

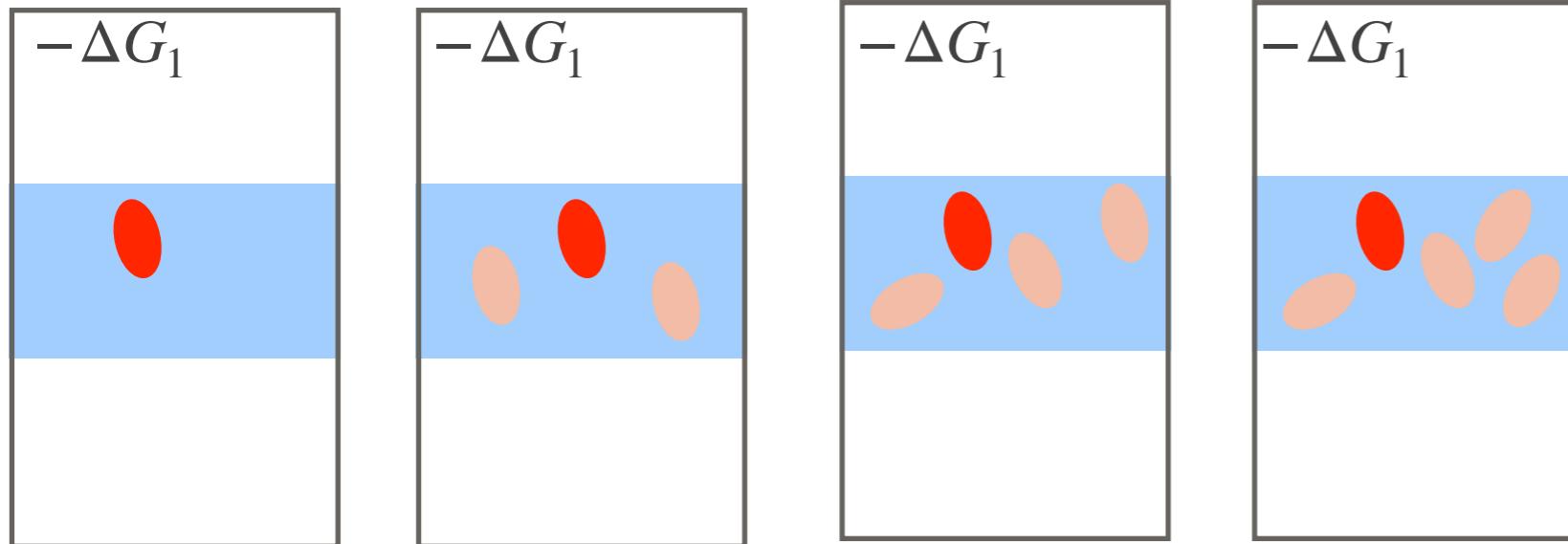
**solution:** Combine all this stuff together into one concentration dependent quantity ( $\kappa_A$ )  
drop assumptions, forget about infinitely dilute limit.

$$\Delta G_{\text{bind}} = -RT \ln \kappa_A [L]_{\text{tot}}$$

$$p_{\text{occ}} = \frac{\kappa_A [L]_{\text{tot}}}{1 + \kappa_A [L]_{\text{tot}}}$$

# how to handle non-ideality

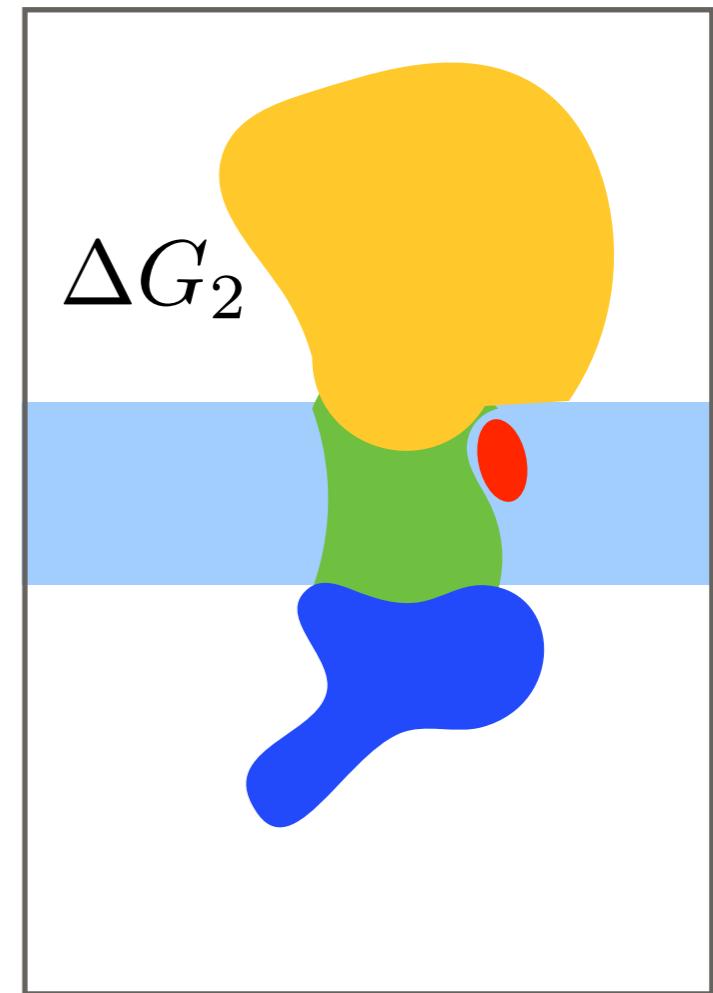
origin: ligand-ligand interactions in  
**bulk**



run small, fast simulations for  
each concentration

$$\kappa_A = \frac{e^{\Delta G_1}}{[L]_{tot}} e^{-\Delta G_2/RT}$$

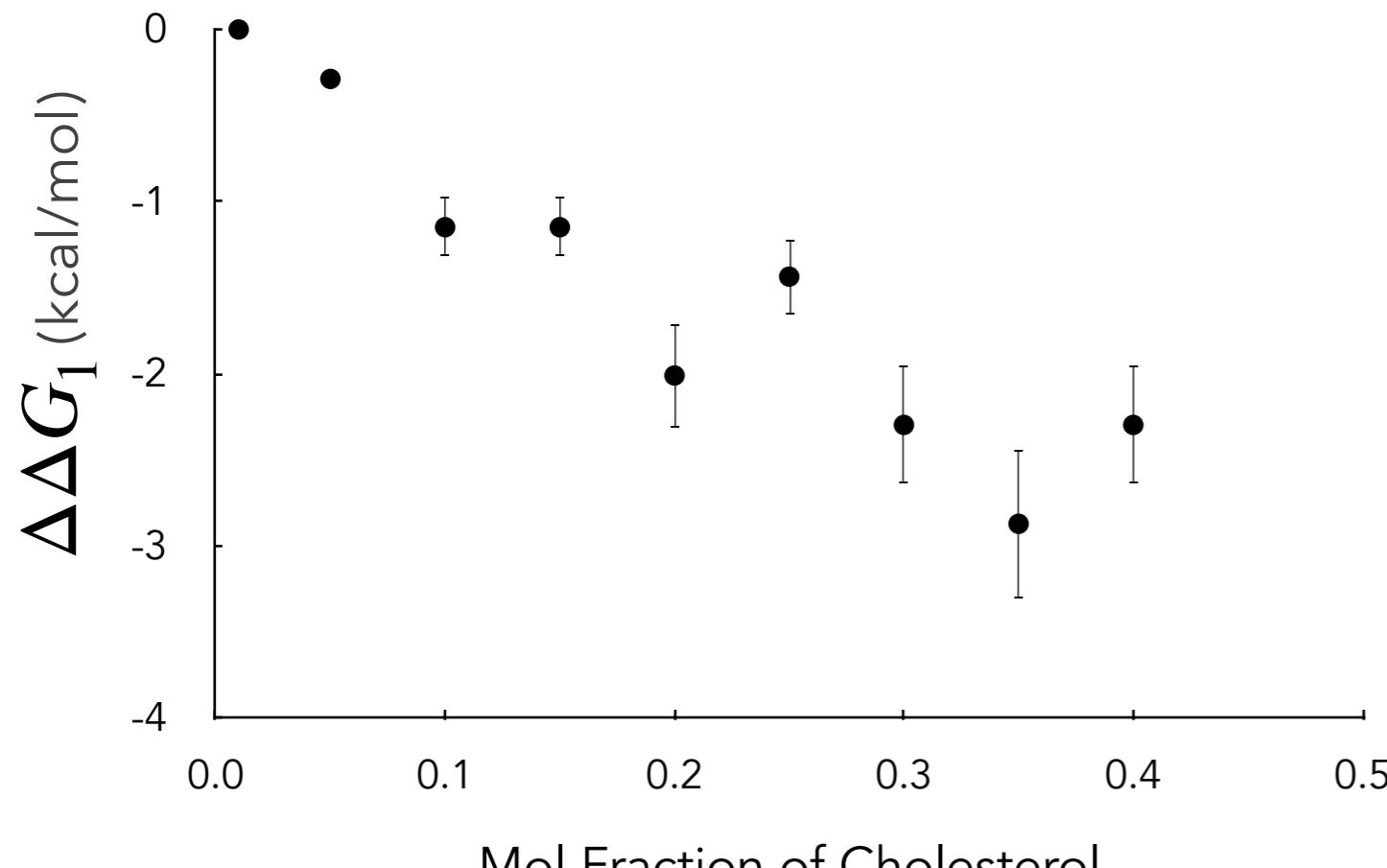
*note: also true for soluble systems but surprisingly unexplored*



run large, slow  
simulation for  
just one or two  
concentrations

# Quantifying non-ideality

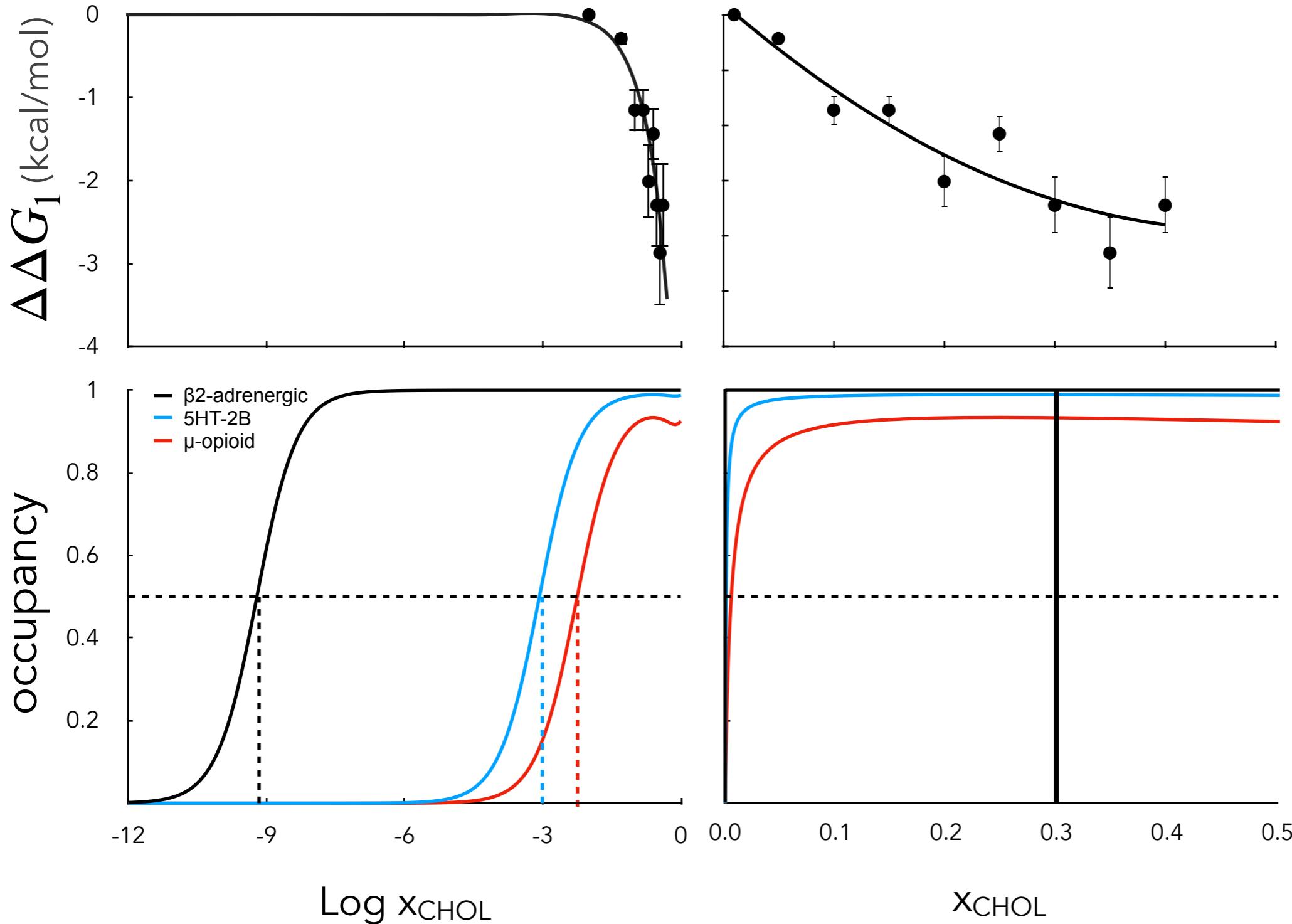
Free energy of cholesterol in POPC bilayer (relative to ideal-dilute)



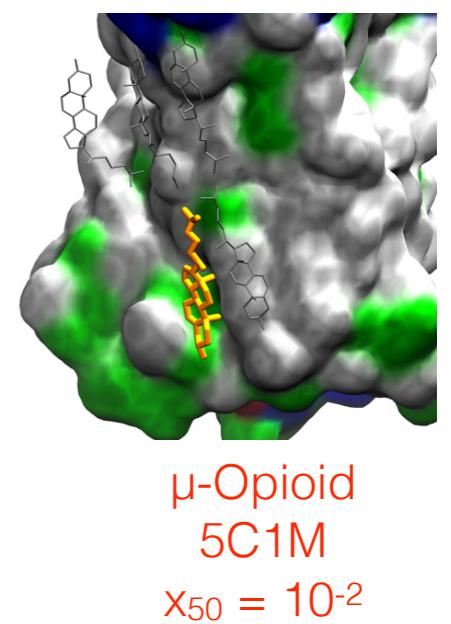
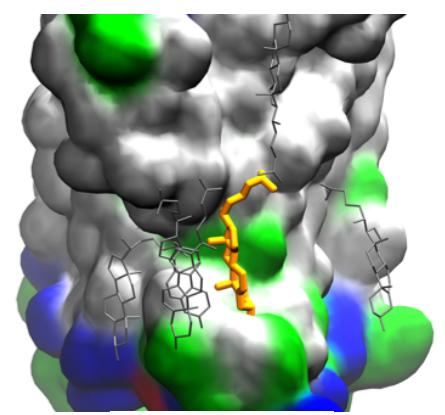
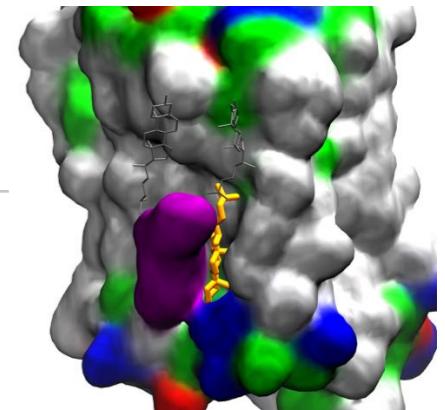
Salari, Joseph, Lohia, Henin, Brannigan, JCTC 2018

Surprising possible scenario : adding more cholesterol  
**reduces** cholesterol bound to membrane-protein

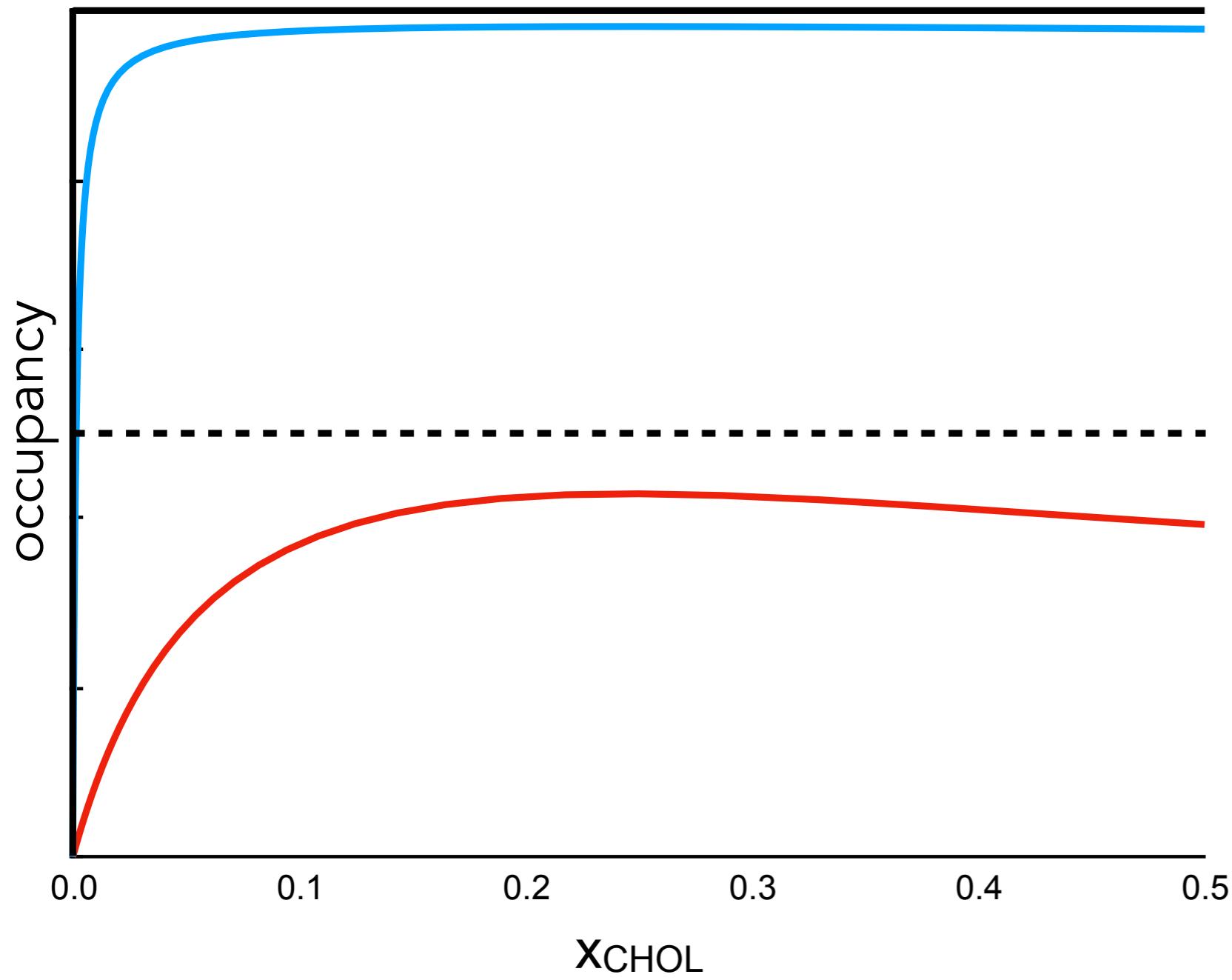
# proof of principle: virtual cholesterol binding assay



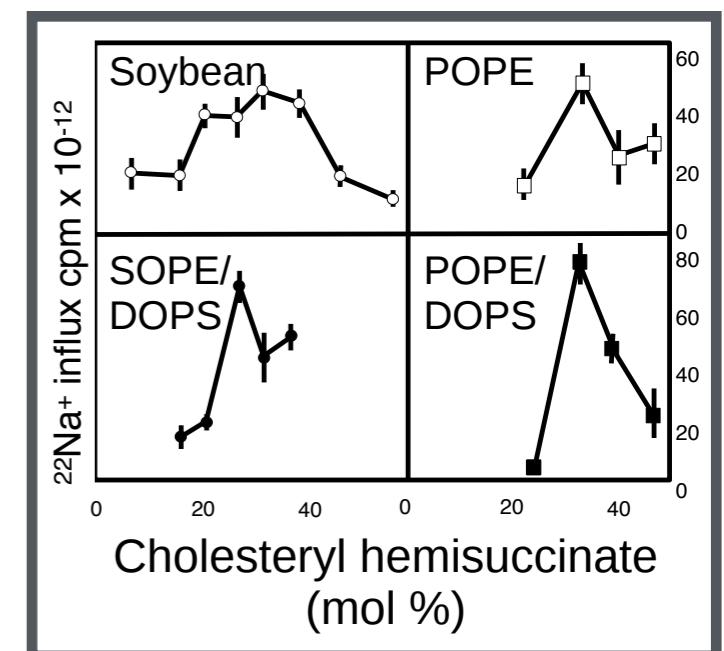
Salari, Joseph, Lohia, Henin, Brannigan, JCTC 2018



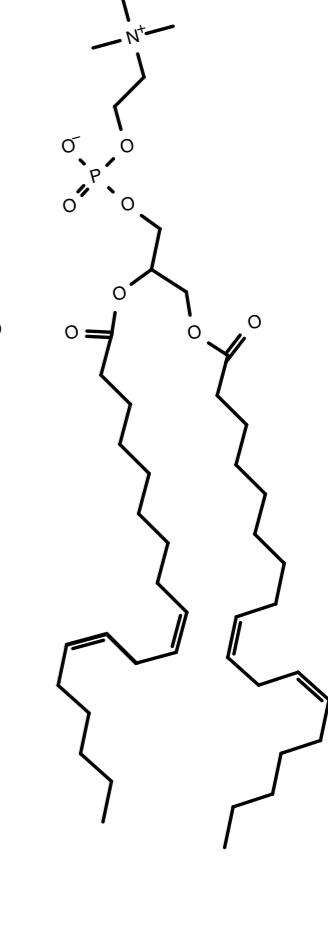
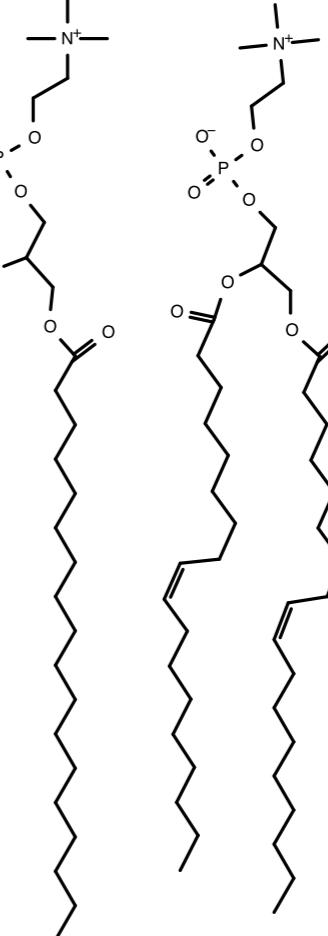
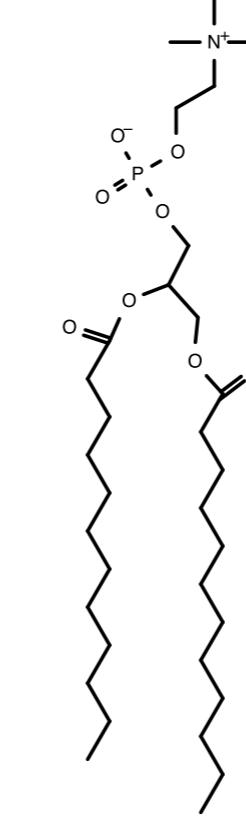
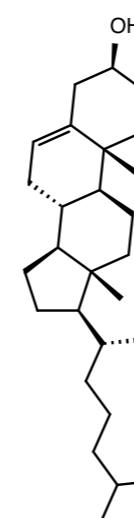
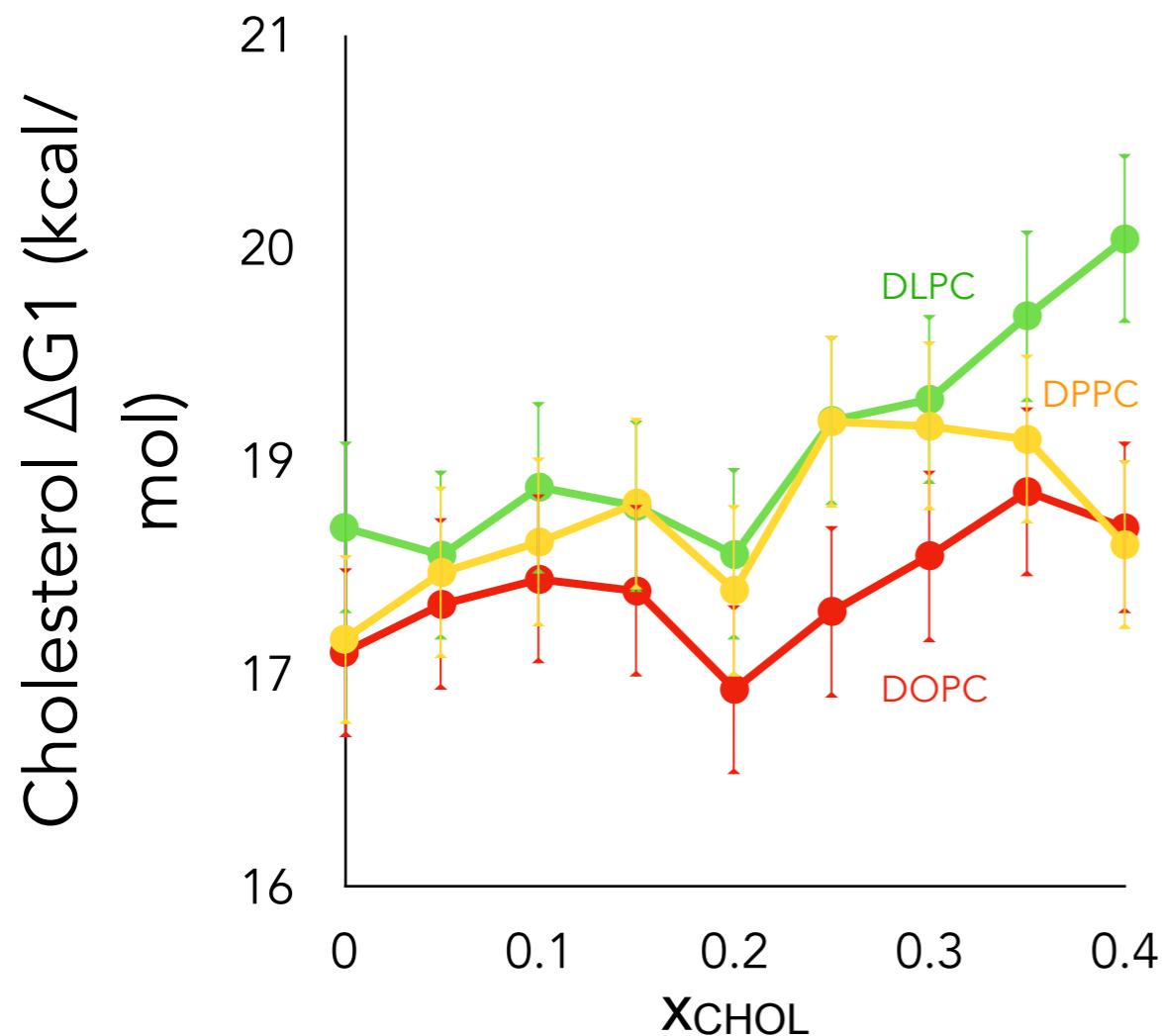
# surprising sensitivity



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

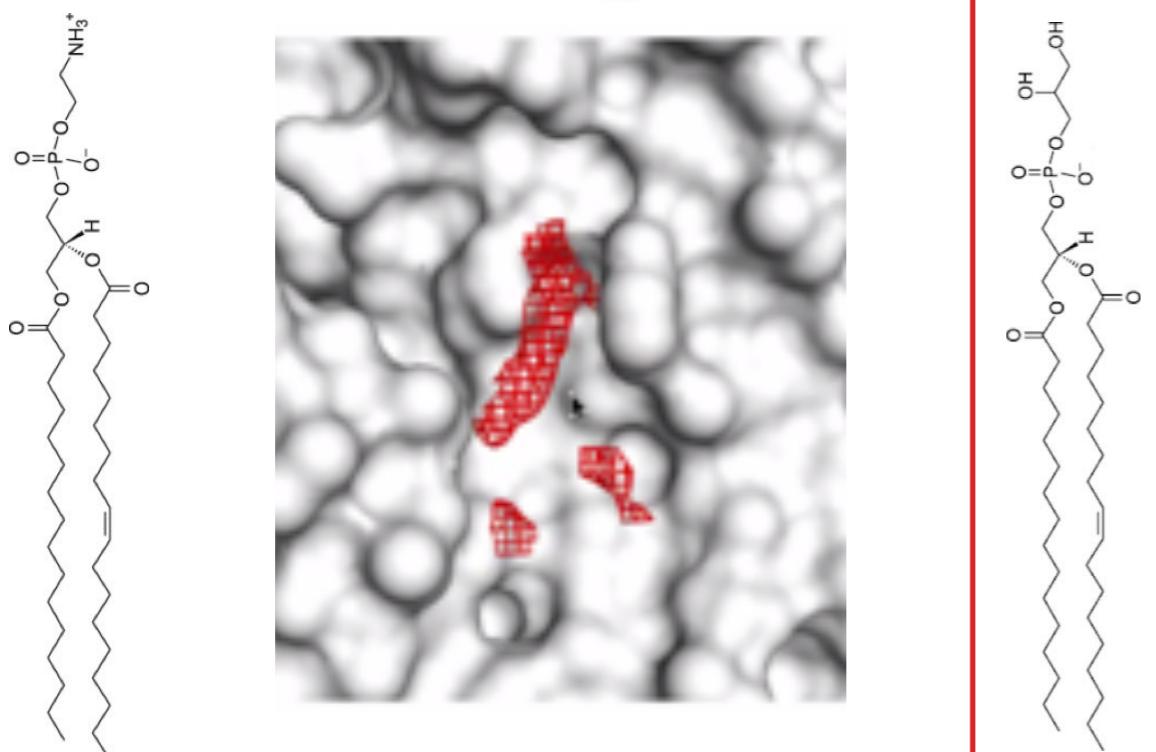
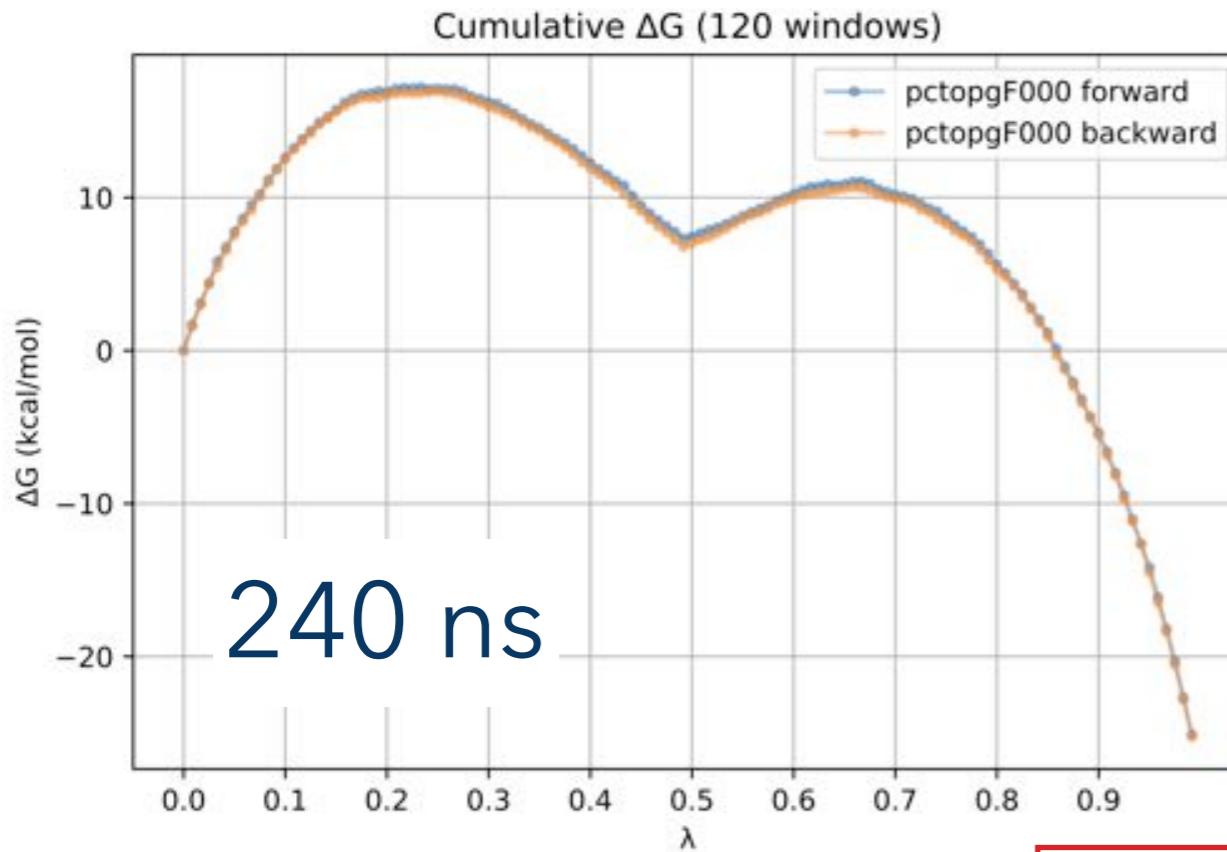


Aside: FEP is great for partitioning calculations



phospholipid

# 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.

# Summary

- SAFEP can be used to determine binding free energies for both rigid and flexible lipids with atomistic resolution
- SAFEP can distinguish between lipid headgroups in bound structures (next step: acyl chains)
- Non-ideality is sufficient to explain non-monotonic assay results

JCTC  
Journal of Chemical Theory and Computation

Article

Cite This: *J. Chem. Theory Comput.* 2018, 14, 6560–6573

[pubs.acs.org/JCTC](https://pubs.acs.org/JCTC)

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

Reza Salari,<sup>†,‡</sup> Thomas Joseph,<sup>†,§</sup> Ruchi Lohia,<sup>†</sup> Jérôme Hénin,<sup>||,‡,¶</sup> and Grace Brannigan\*,<sup>†,‡,||,¶</sup>

Original method -  
NAMD inputs are in SI

JCTC  
Journal of Chemical Theory and Computation

Article

Cite This: *J. Chem. Theory Comput.* 2019, 15, 5727–5736

[pubs.acs.org/JCTC](https://pubs.acs.org/JCTC)

## Insights into Membrane Protein–Lipid Interactions from Free Energy Calculations

Robin A. Corey,<sup>†</sup> Owen N. Vickery,<sup>†</sup> Mark S. P. Sansom,<sup>¶</sup> and Phillip J. Stansfeld\*,<sup>¶</sup>

GROMACS  
implementation in SI

# Detecting specific lipid binding

	Experiments	MD Simulation
Who	Lipid Species	
Where	Binding Site	
When	Affinity	
	Mass Spectrometry	
	Soluble lipid-binding assay	
	Structural Biology	
	Atomistic (AA)	
	Coarse-grained (CG)	

# Acknowledgments

## Contributing Group Members

*Dr. Reza Salari*

*Dr. Thomas Joseph*

*Dr. Mark Arcario*

*Dr. Ruchi Lohia*

*Dr. Liam Sharp*

*Kristen Woods*

*Rulong Ma*

Reviewers of Salari et al, JCTC 2018

## 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 Ullens (KU Leuven)



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