

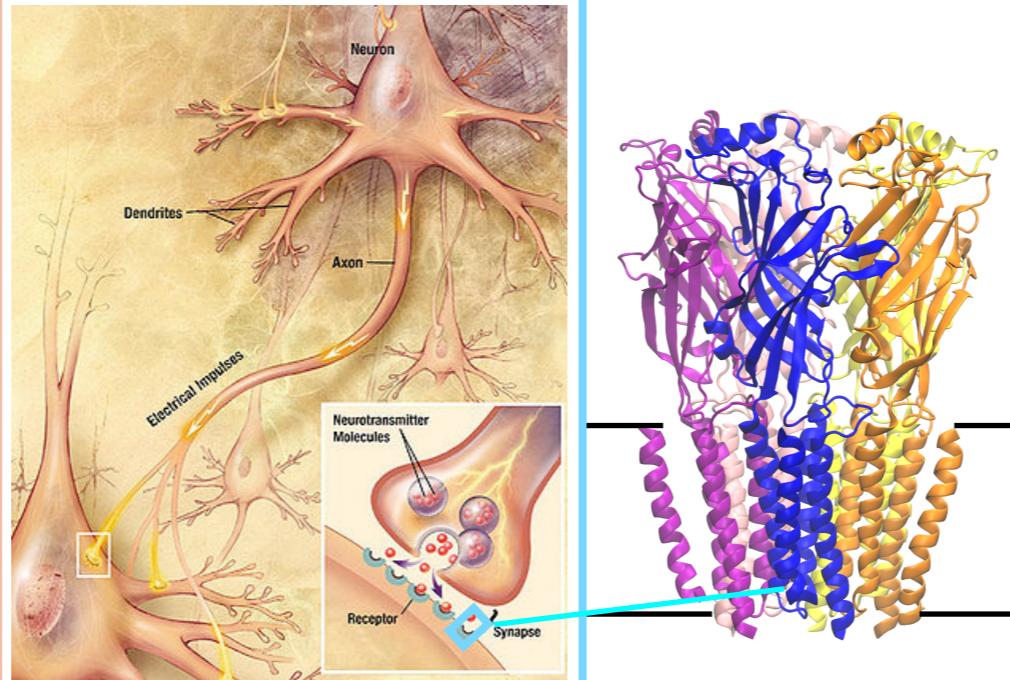
Ligand binding in membranes

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



motivation: pentameric gated ligand channels (pLGICs)

nicotinic acetylcholine receptor, GABAA receptor, glycine receptor, etc



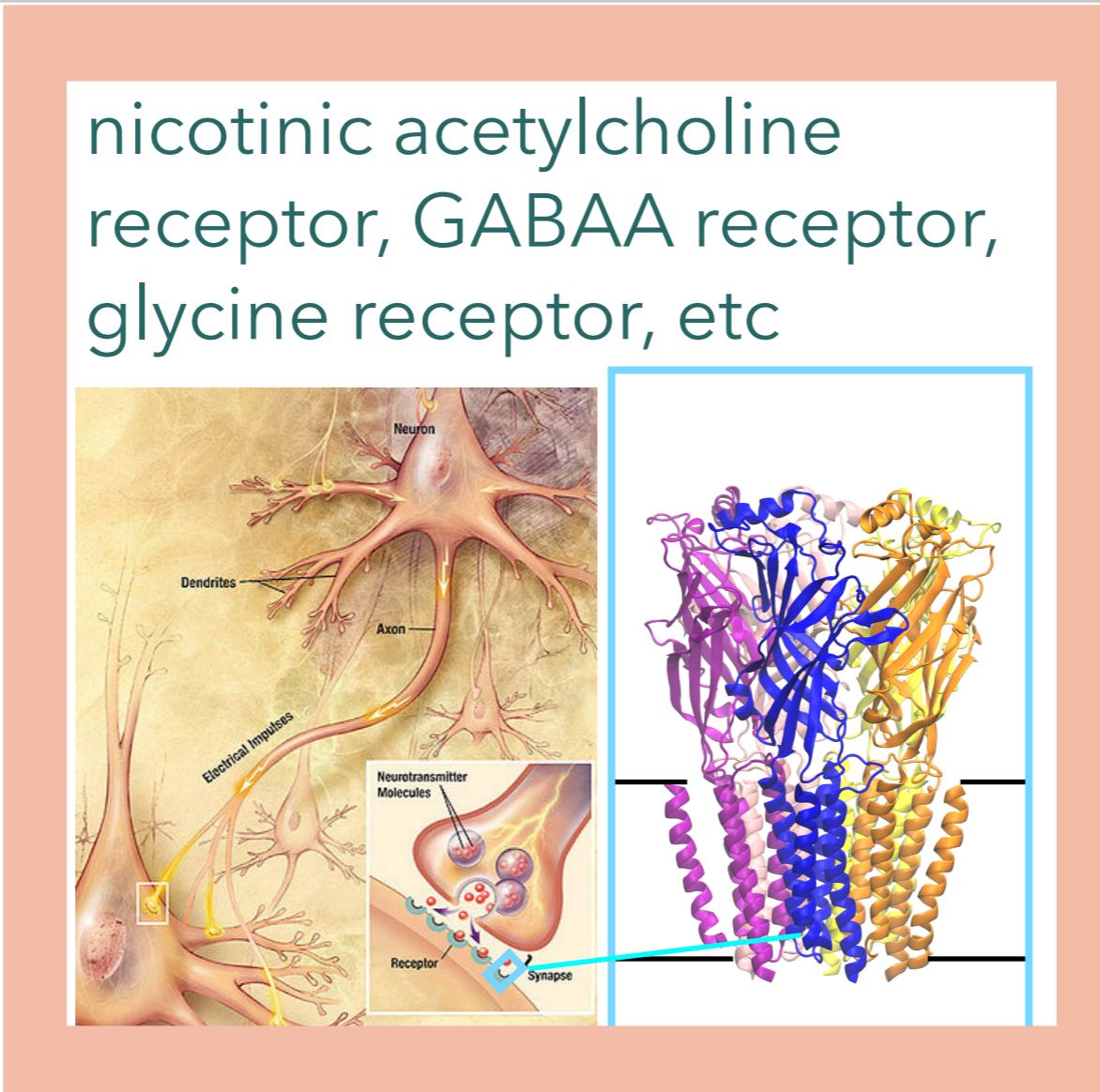
motivation: pentameric gated ligand channels (pLGICs)



recreation



nicotine



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The diagram illustrates a neuron with labels for "Neuron", "Dendrites", and "Axon". An inset shows "Electrical Impulses" traveling down the axon. Another inset shows "Neurotransmitter Molecules" being released from a vesicle at a "Synapse", interacting with a "Receptor". To the right, a 3D ribbon model of a pentameric gated ligand channel (pLGIC) is shown, composed of five subunits (one purple, four orange) forming a central pore.

general anesthetics



propofol



pentobarbital



chloroform

motivation: pentameric gated ligand channels (pLGICs)



recreation



nicotine

nicotinic acetylcholine receptor, GABA_A receptor, glycine receptor, etc

The diagram shows a neuron with Dendrites and an Axon. Electrical Impulses travel along the axon. At the synapse, Neurotransmitter Molecules are released from vesicles and bind to Receptors on the next neuron. A detailed inset shows the molecular structure of a pentameric GABA_A receptor channel embedded in a lipid bilayer membrane.

poisons



general anesthetics



propofol



pentobarbital



chloroform

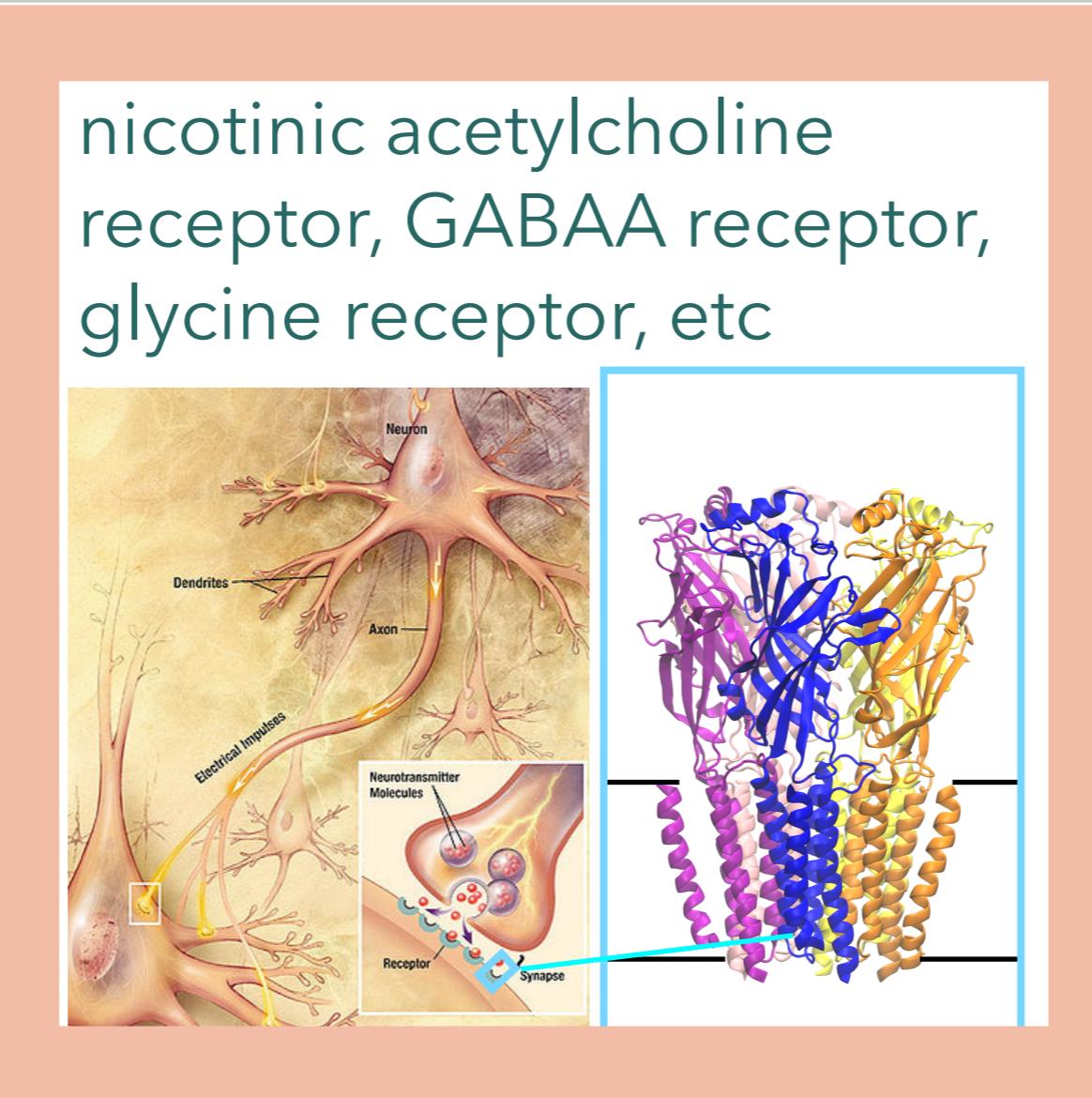
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sedatives



benzodiazepines



methaqualone

poisons



rat poison (TETS)



strychnine



curare



Fishberry (picrotoxin)

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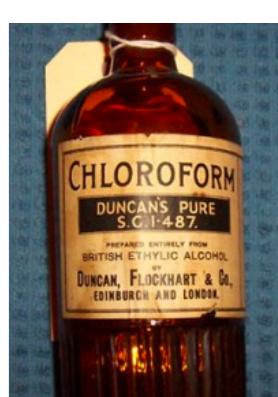
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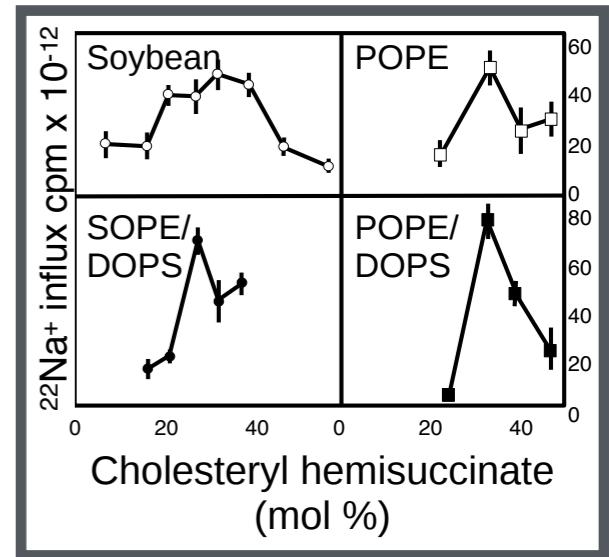


pentobarbital



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lipids



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

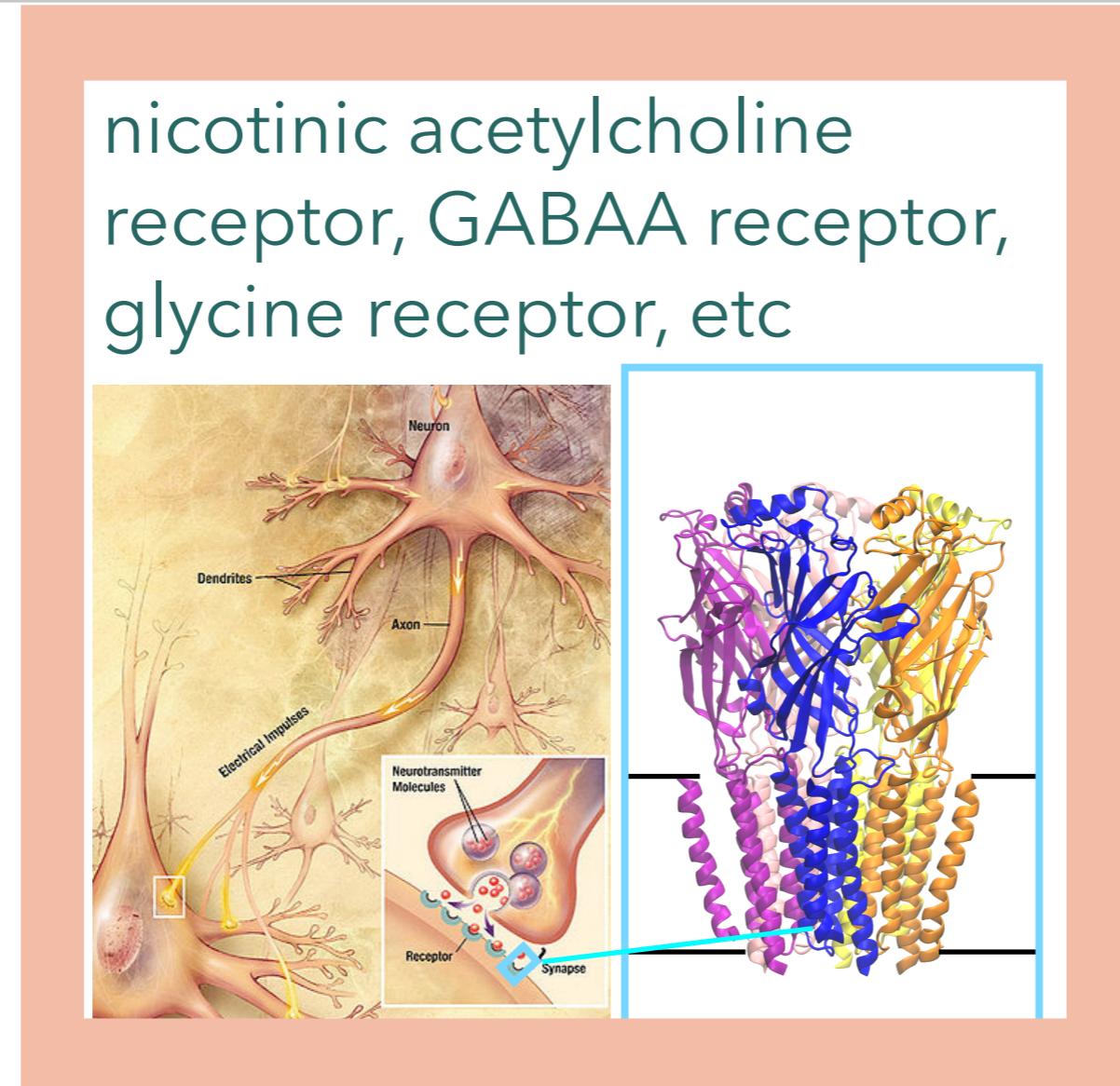


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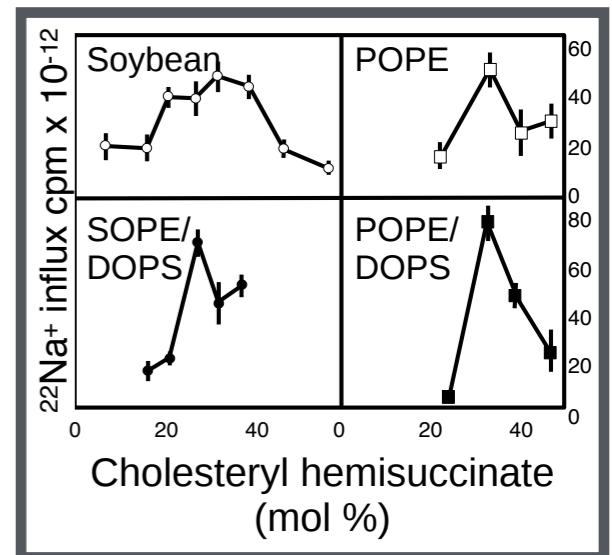


Fishberry (picrotoxin)

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lipids



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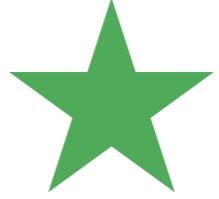
Detecting specific lipid binding

	Experiments	MD Simulation
Who	Lipid Species	Atomistic (AA)
Where	Binding Site	Coarse-grained (CG)
When	Affinity	

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Detecting specific lipid binding

Who

Lipid Species



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Experiments

Mass Spectrometry

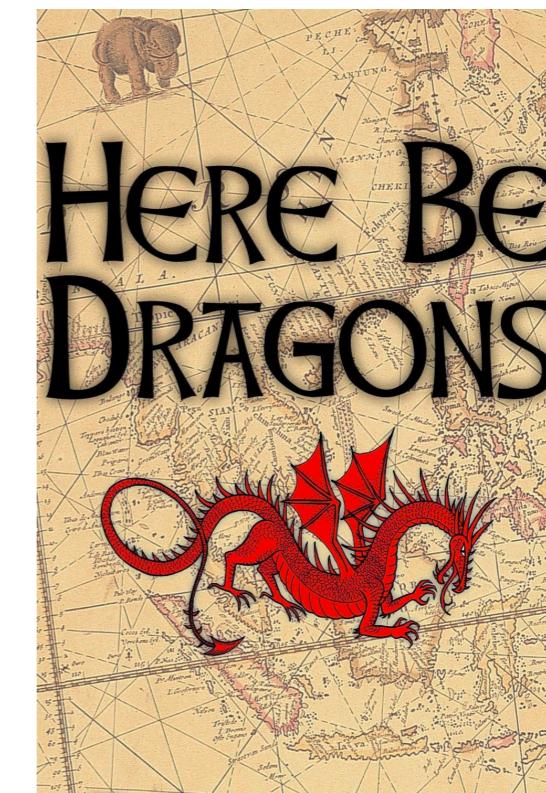
Soluble lipid-binding assay

Structural Biology

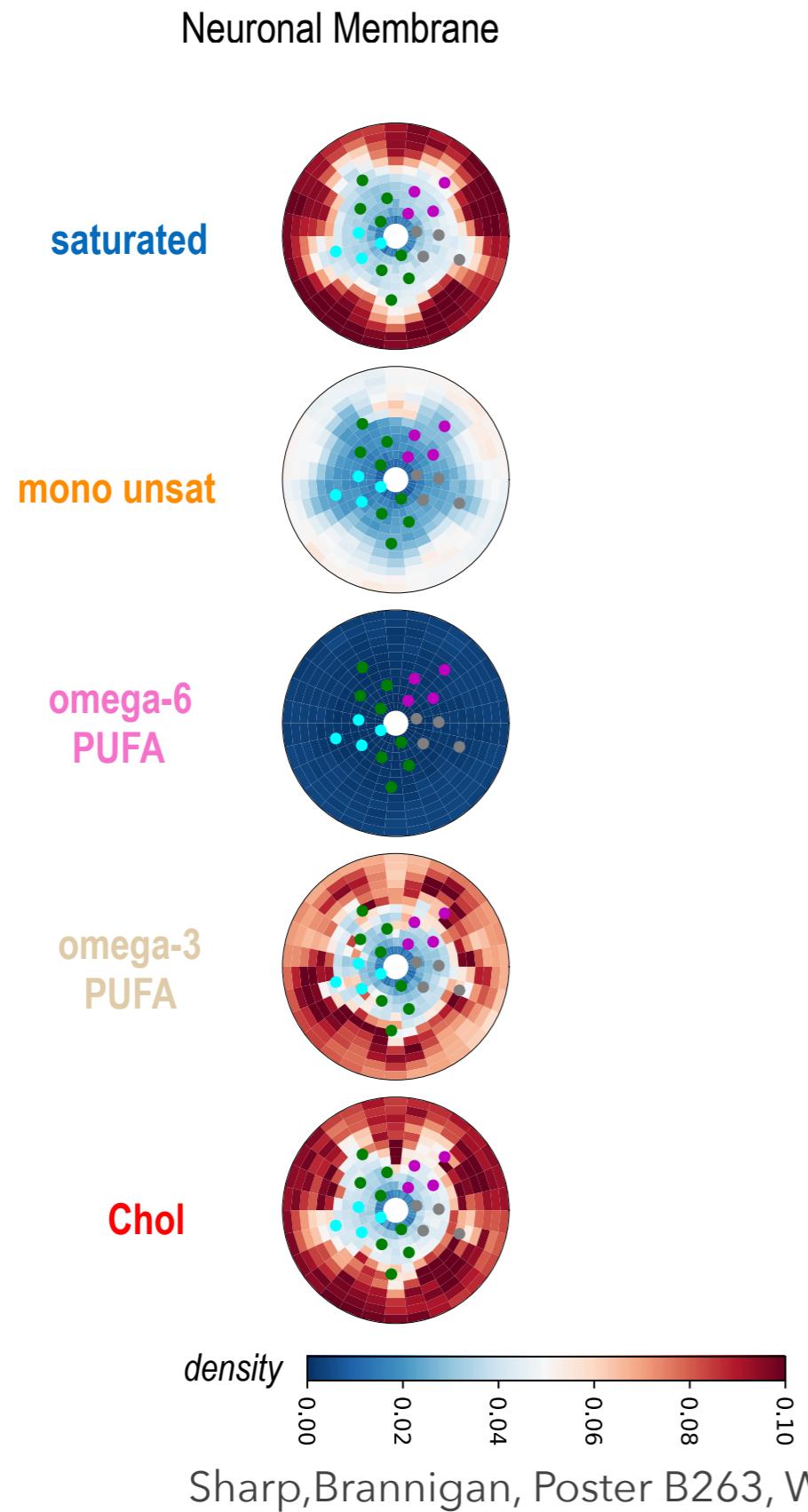
MD Simulation

Atomistic (AA)

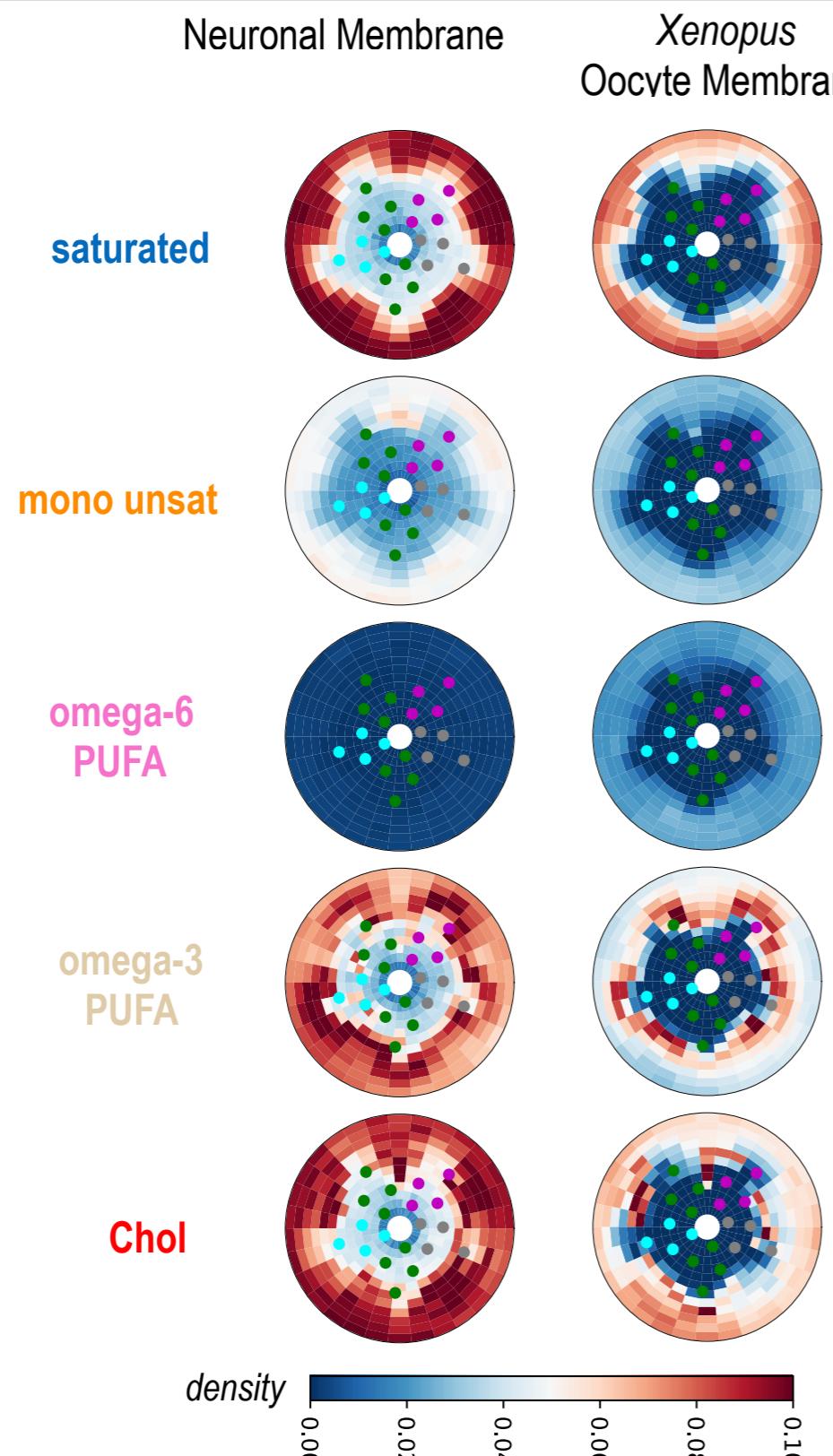
Coarse-grained (CG)



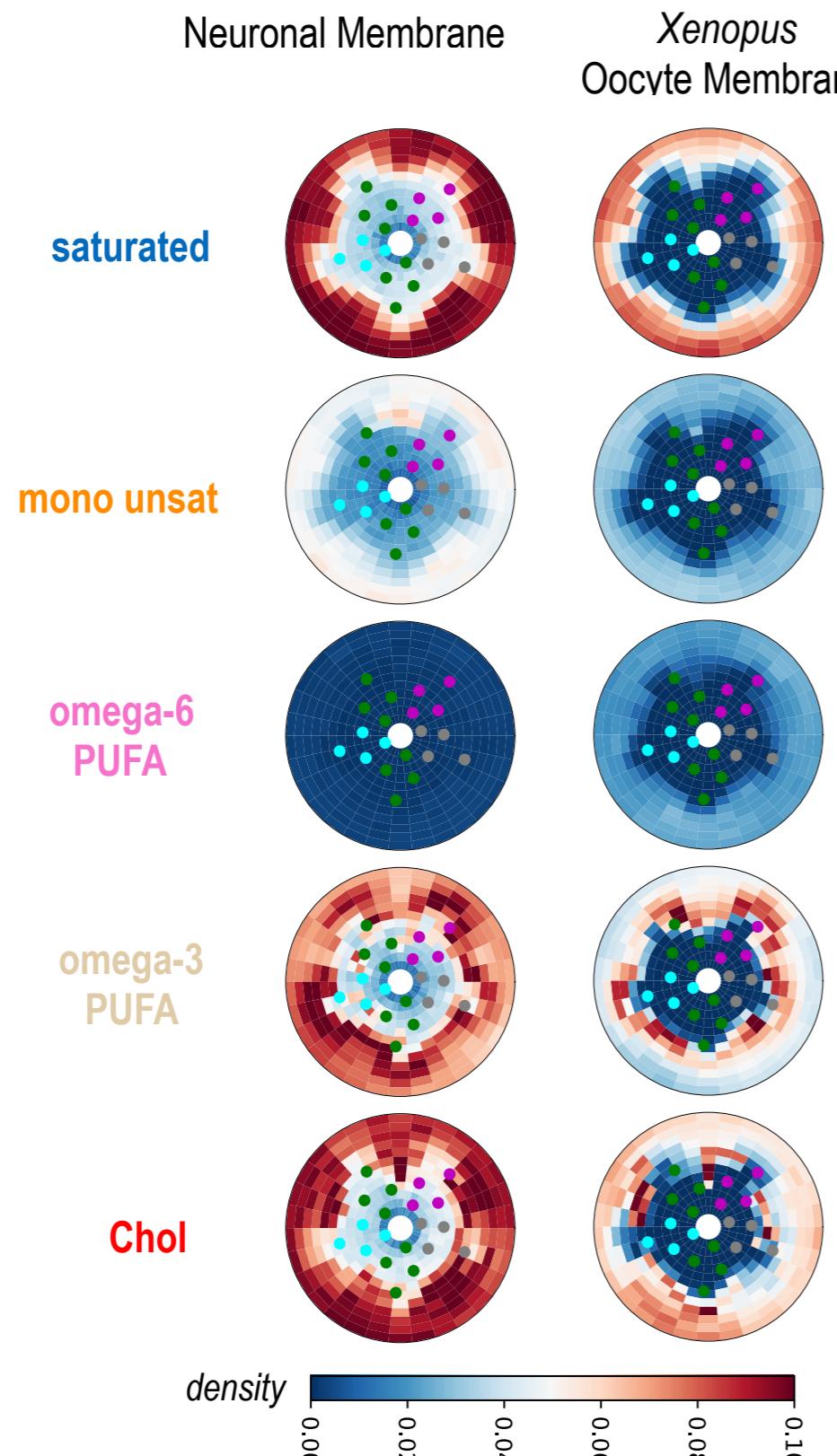
What's feasible in CG MD?



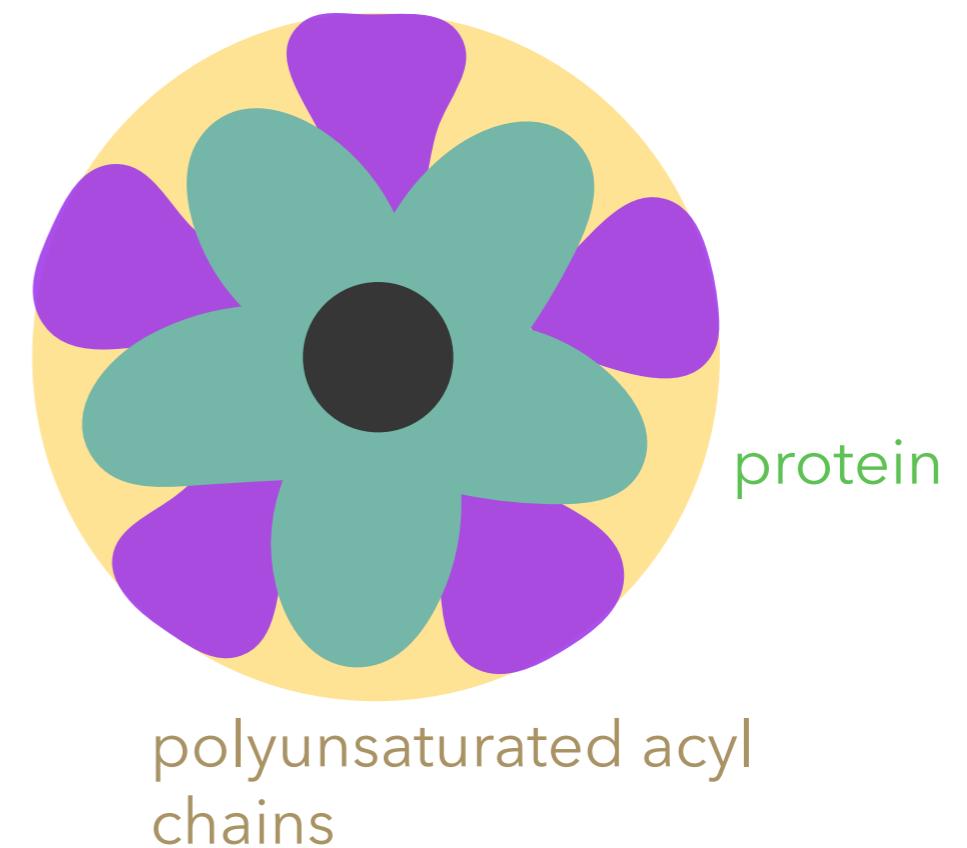
What's feasible in CG MD?



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cholesterol & saturated acyl chains



What's feasible in atomistic MD?

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Unfeasible

Demixing/domain formation

Lipid sorting

Lipid exchange

Effects of bulk membrane
composition

PMF of binding/unbinding

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Boundary lipid reorientation

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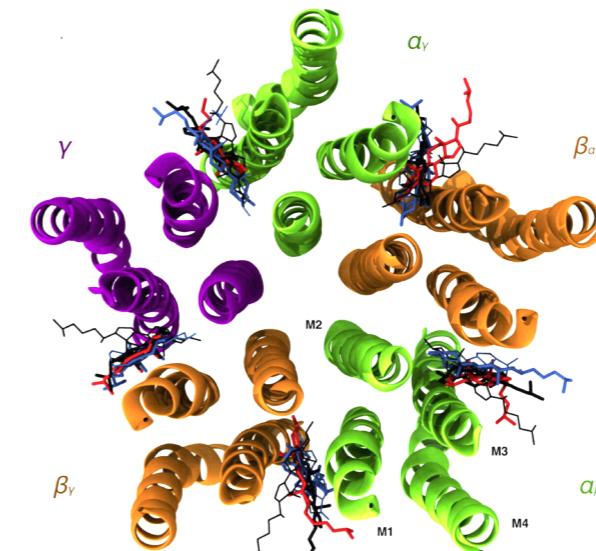
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Henin...Brannigan, 2014, Biophys J

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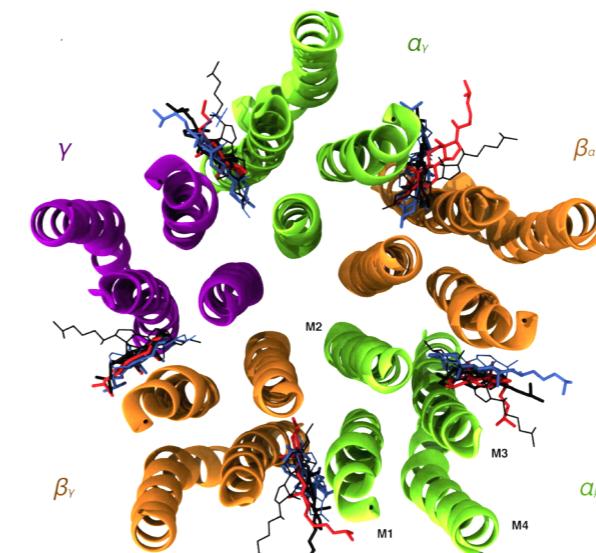
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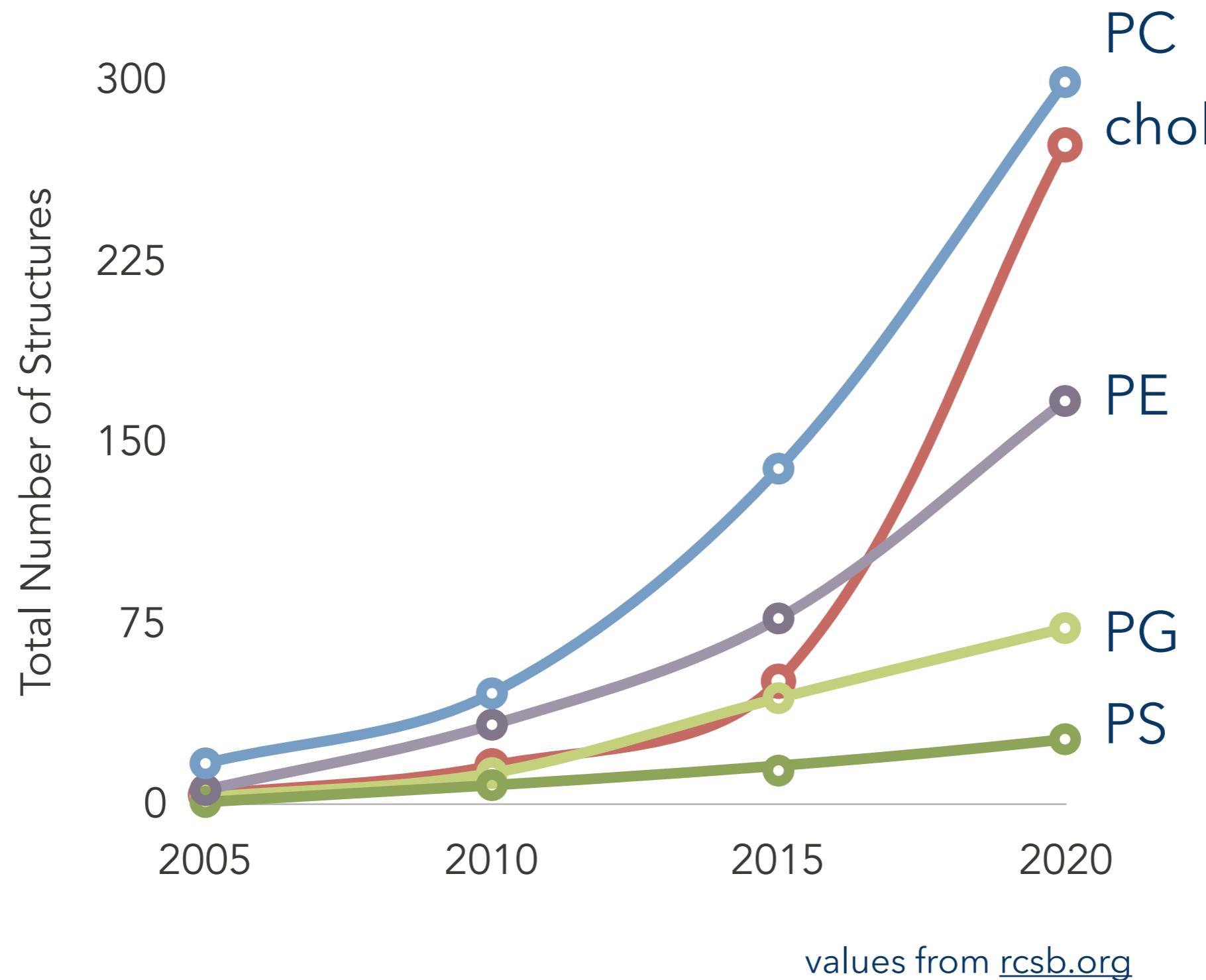
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But with a lipid-bound structure,
should be feasible to get an affinity
- and in a membrane environment!

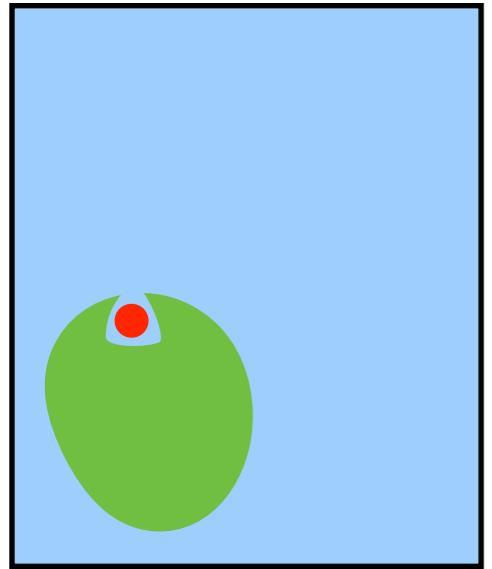
structures with resolved lipids



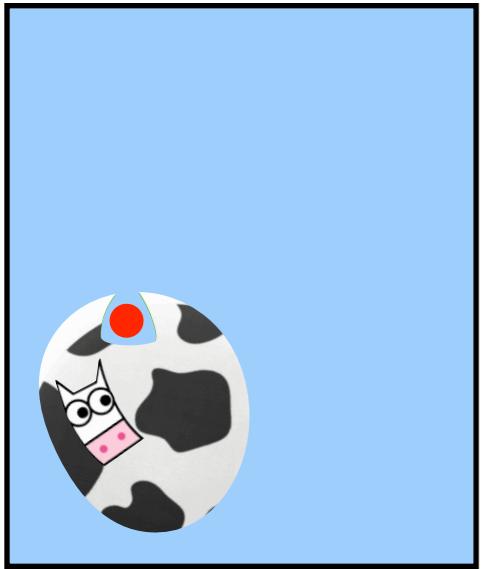
Two Big Questions

- 1) Is an affinity in the membrane environment even well-defined?
- 2) How do we do it?

Classical Binding Assumptions



Classical Binding Assumptions

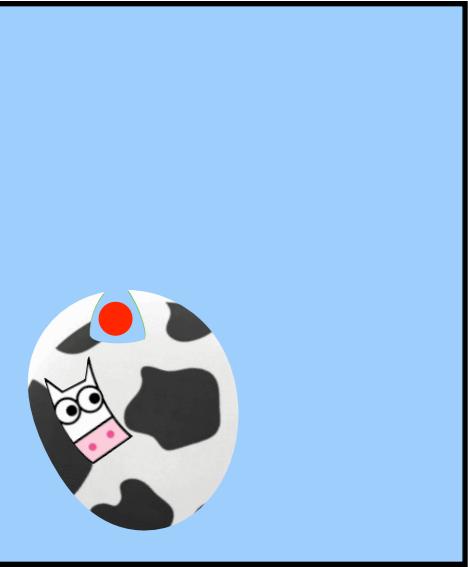


Classical Binding Assumptions

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



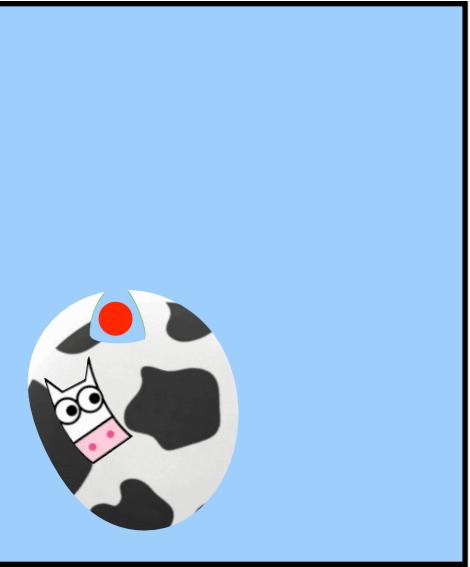
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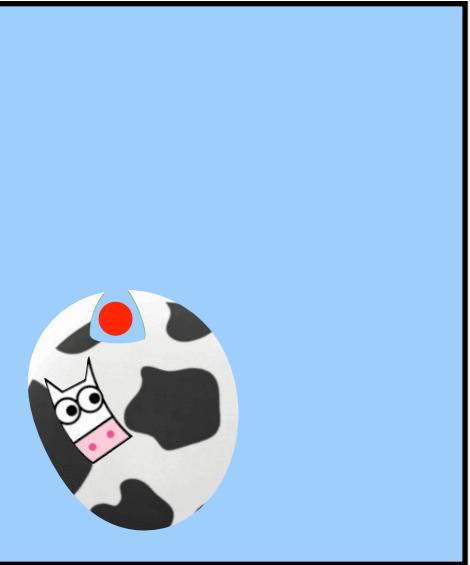
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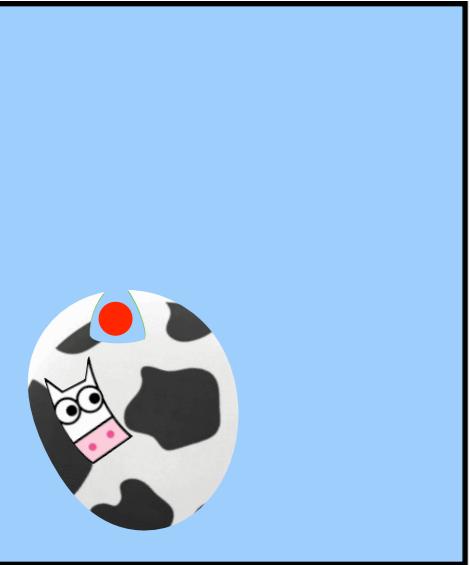


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homogenous bulk, **well-defined volume**
concentration

Classical Binding Assumptions

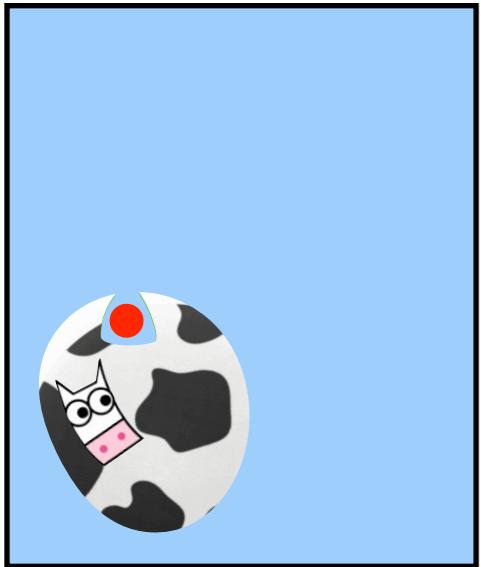


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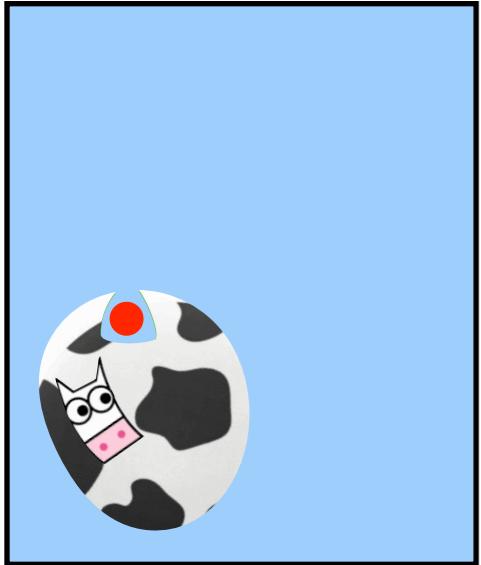
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ligand binding has no effect on **receptor-receptor
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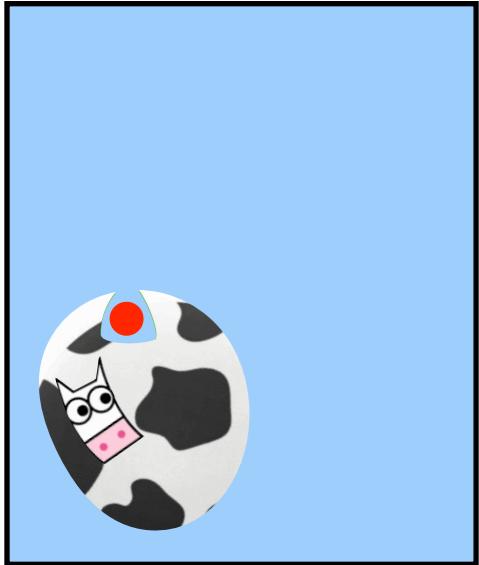


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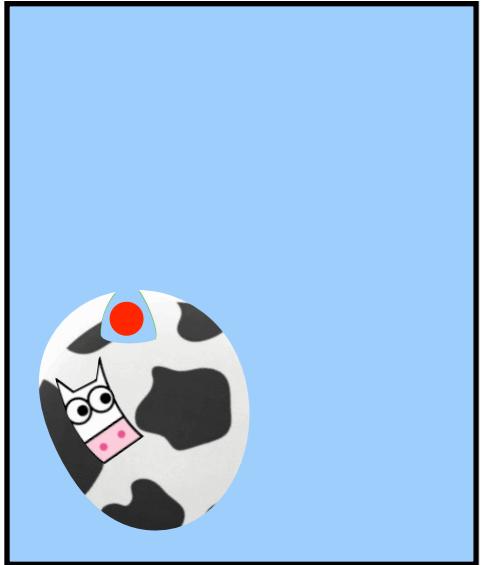
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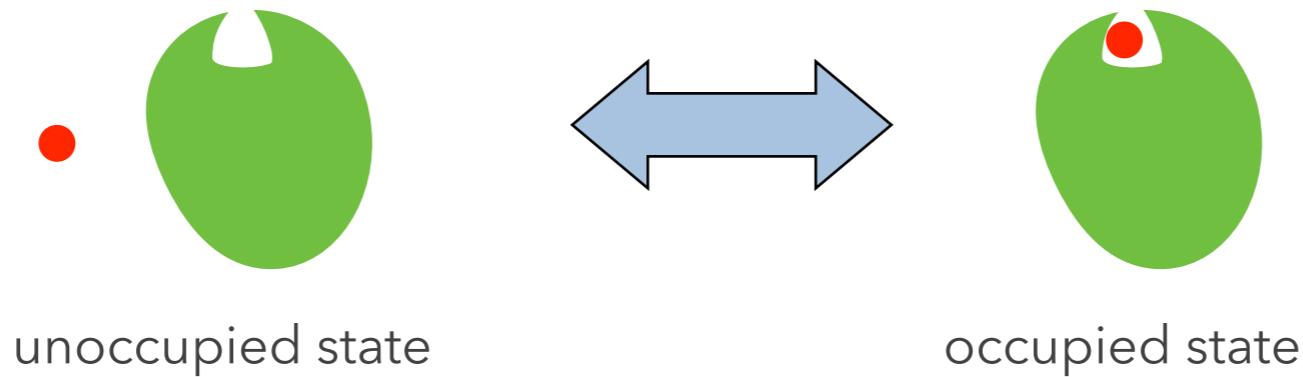
Equivalence between two state transition and reaction framework

Two-state
transition
framework

Reaction
framework

Equivalence between two state transition and reaction framework

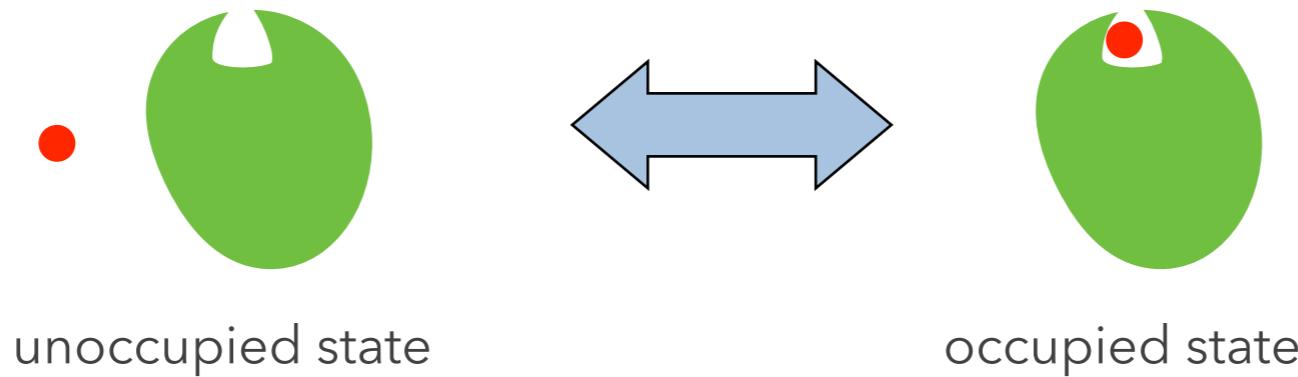
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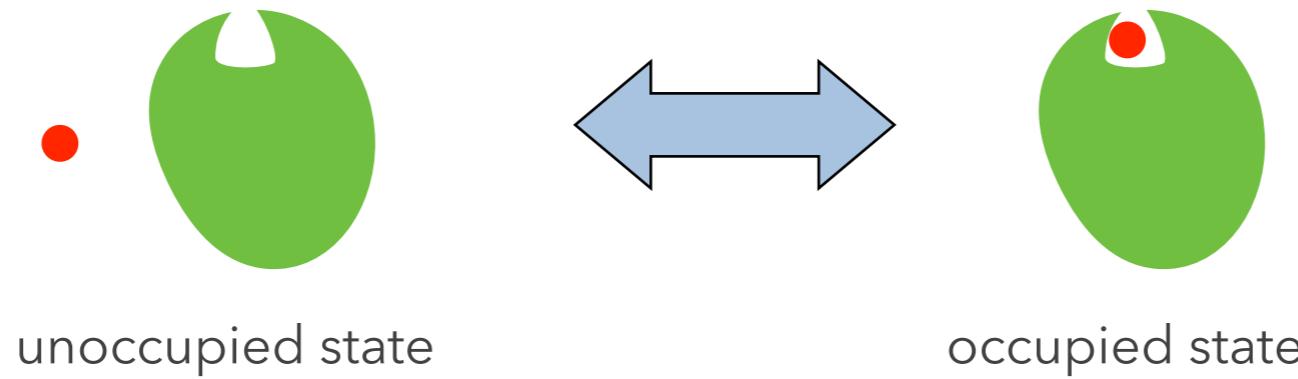
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Equivalence between two state transition and reaction framework

Two-state
transition
framework

unambiguous
in membrane



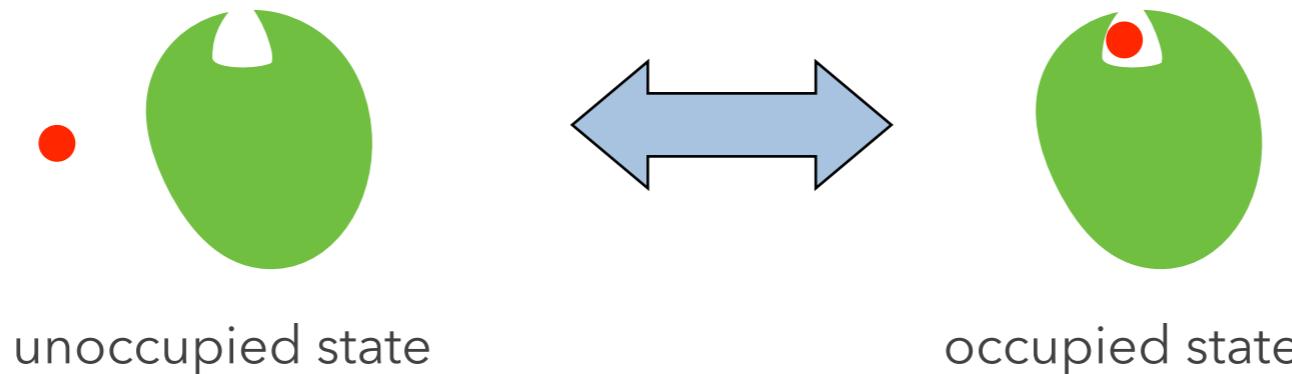
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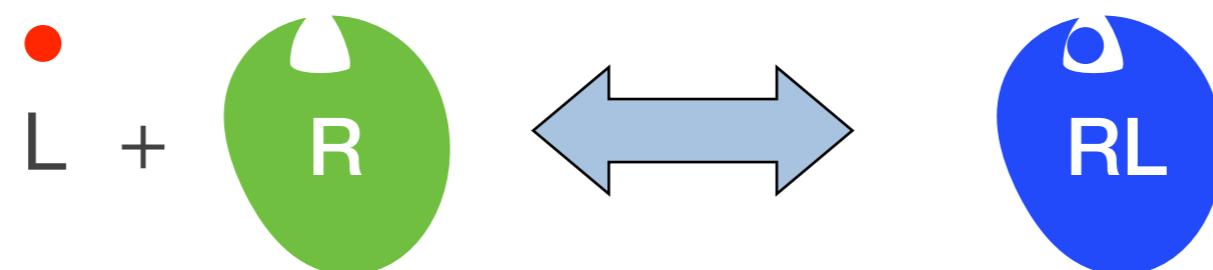
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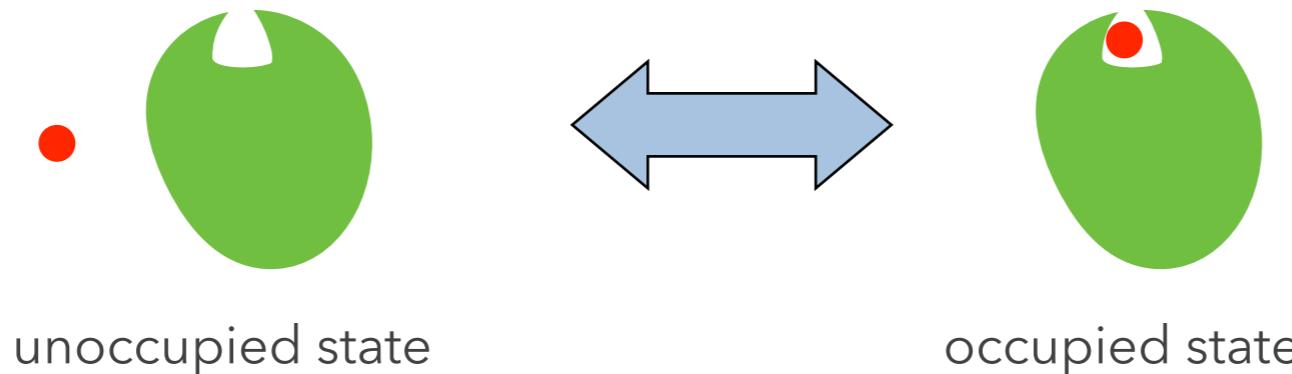


RL is a different molecular **species** than R or L

Equivalence between two state transition and reaction framework

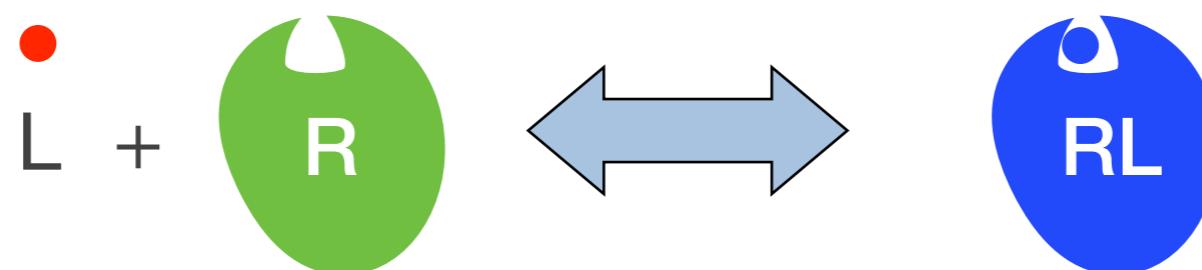
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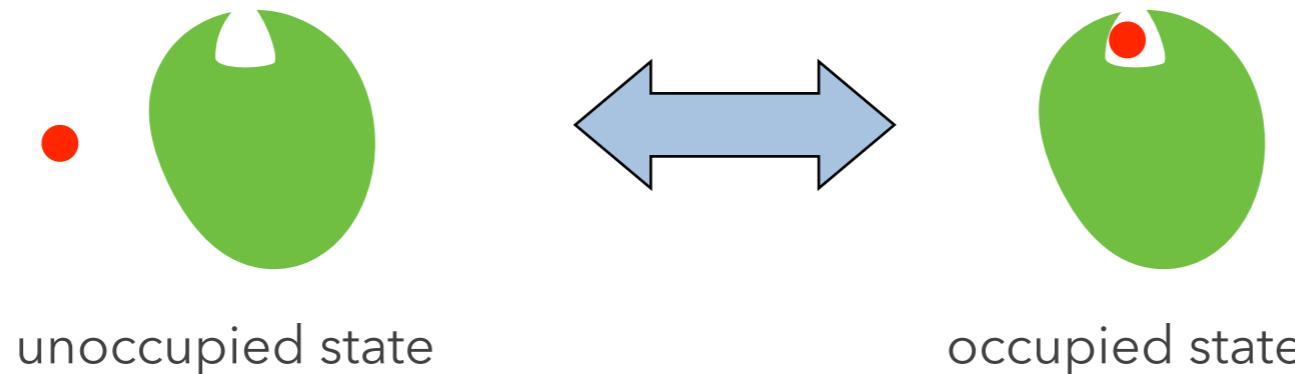
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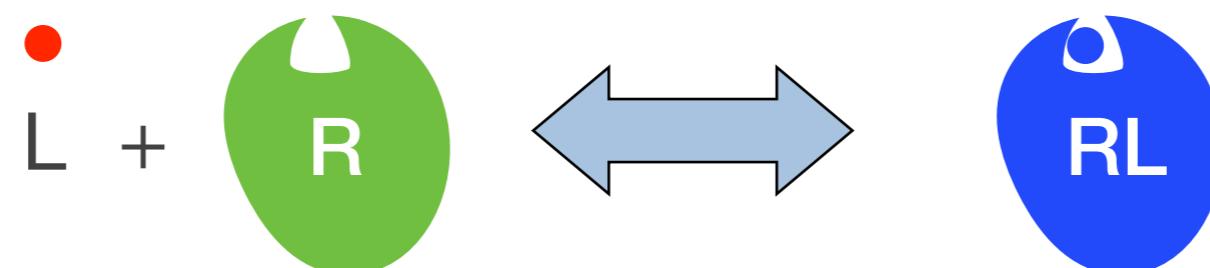
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Reaction
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multiple
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cancel, if careful



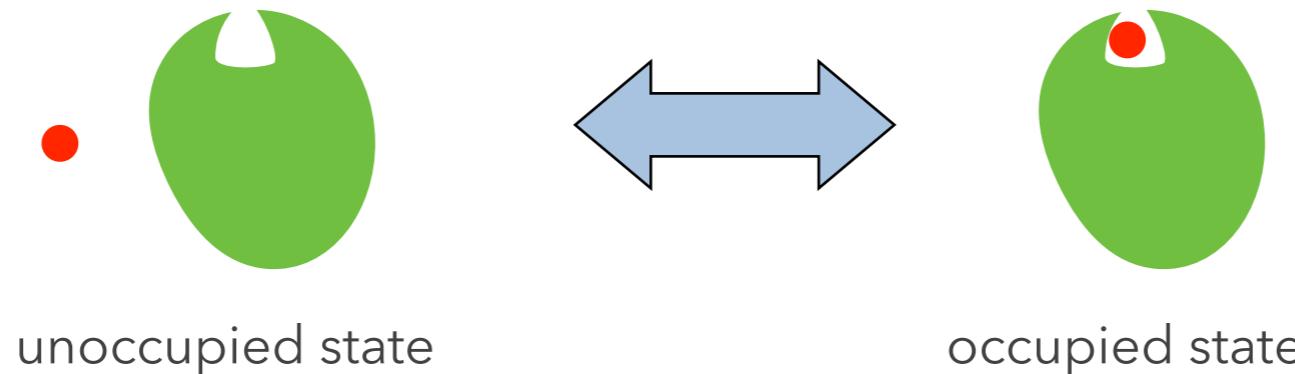
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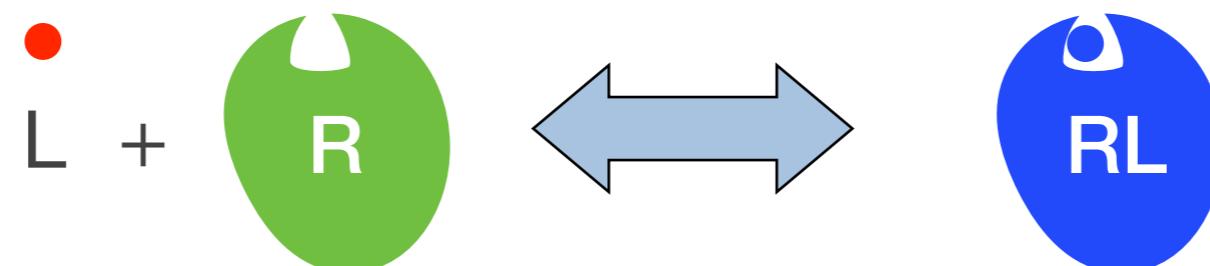
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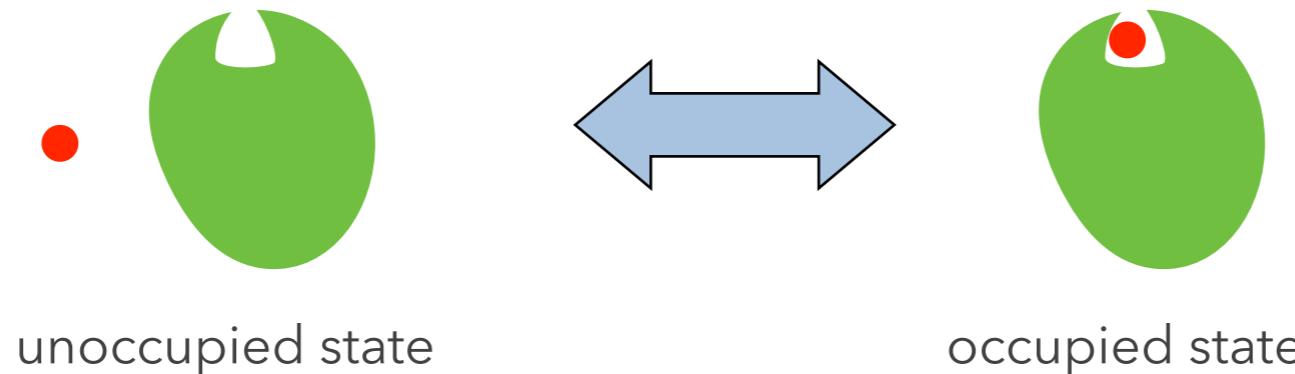
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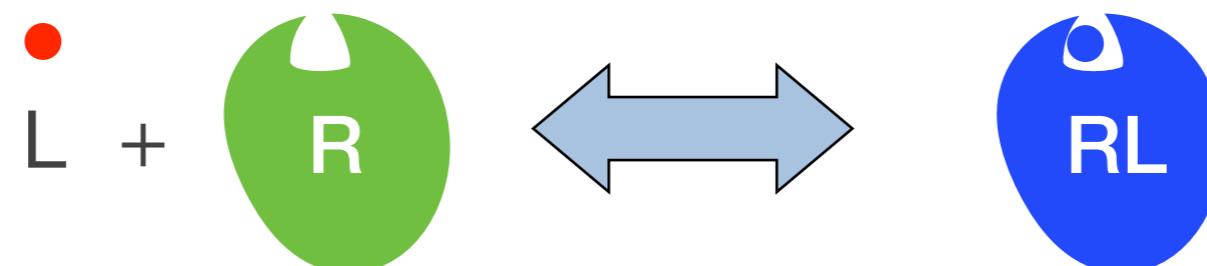
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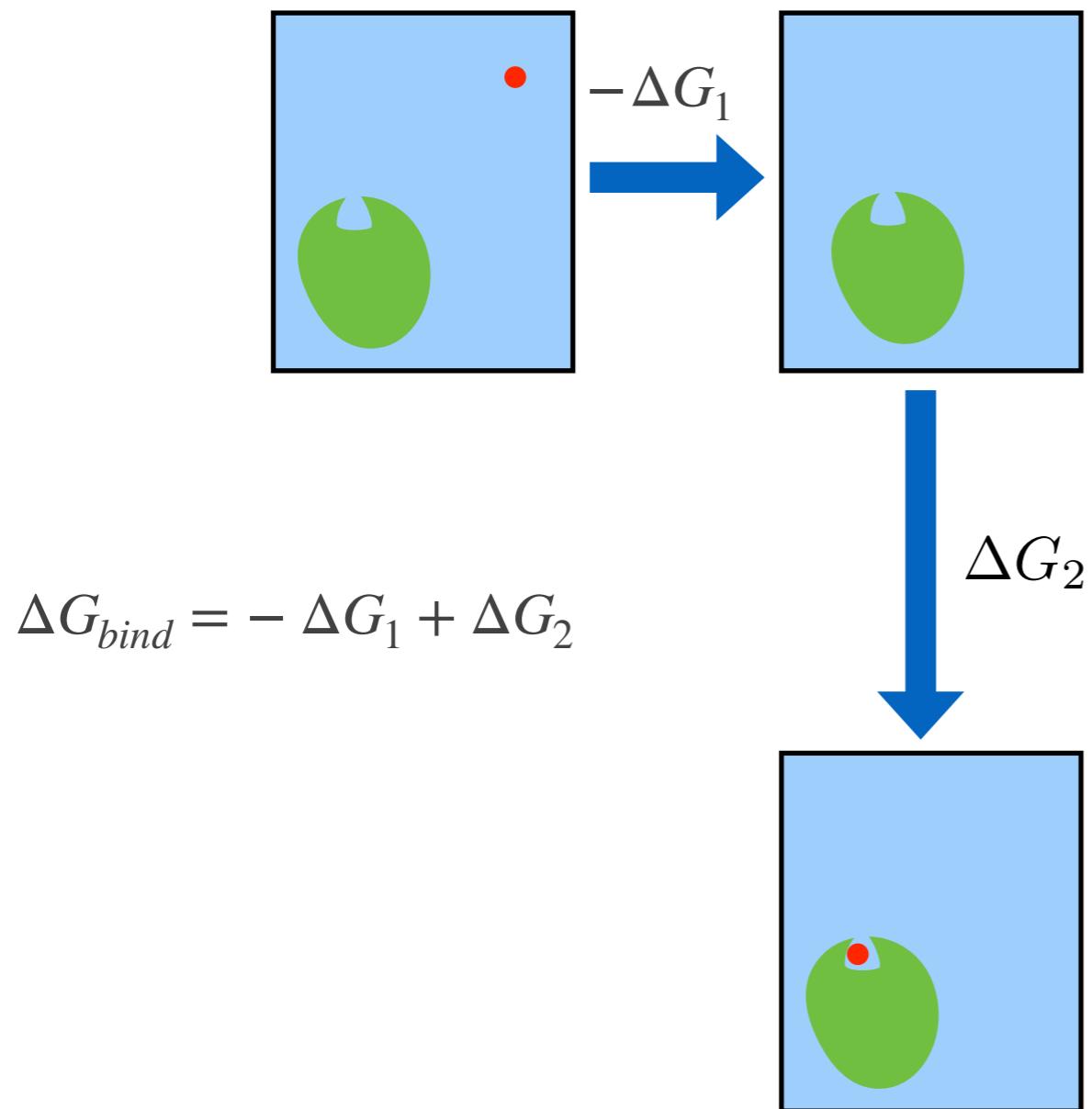
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Similar questions because

$$\frac{[RL]}{[R]} = \frac{p_{occ}}{p_{unocc}}$$

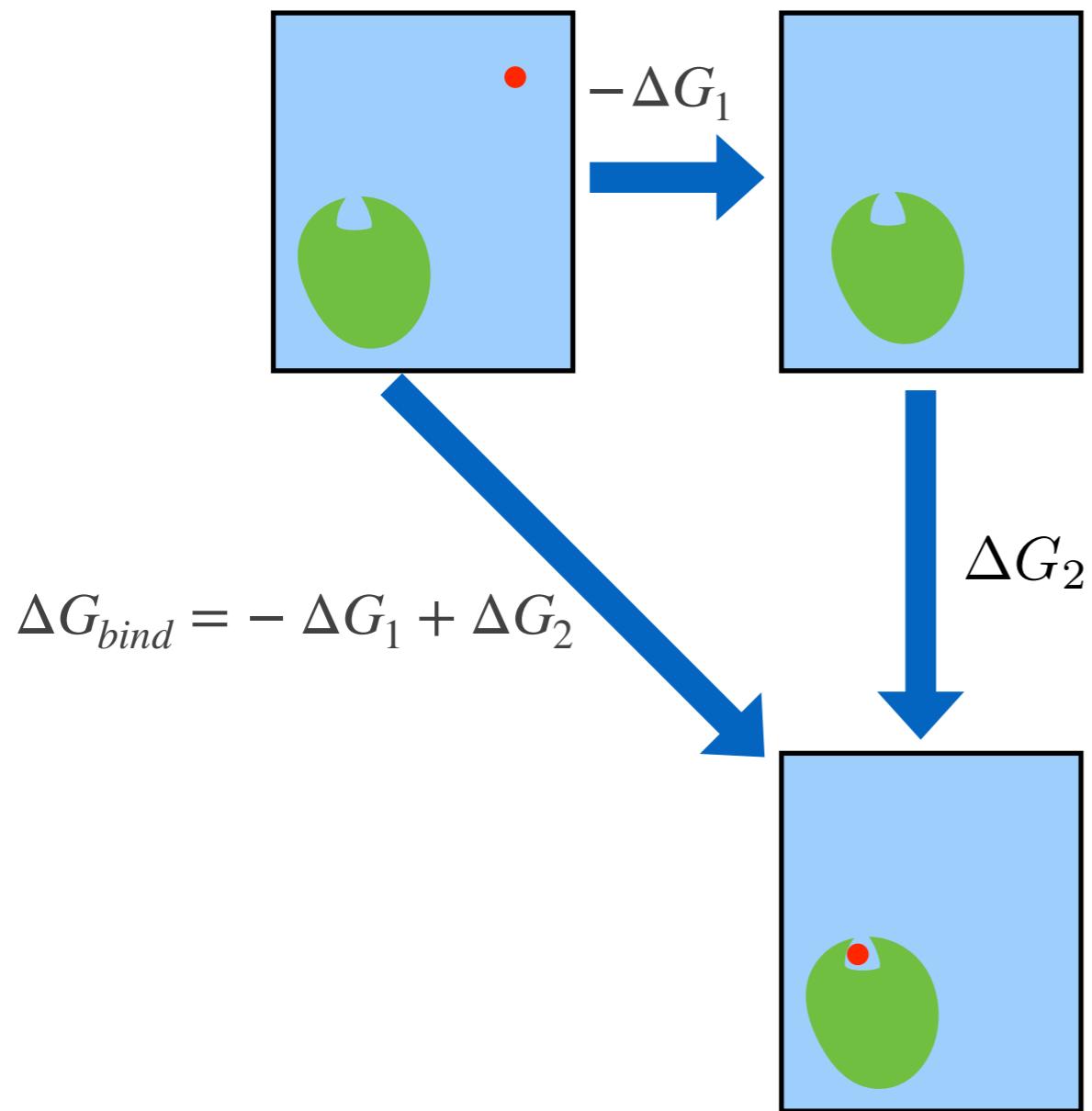
Ligand binding in AA-MD

Classic approach: Alchemical Free Energy Perturbation



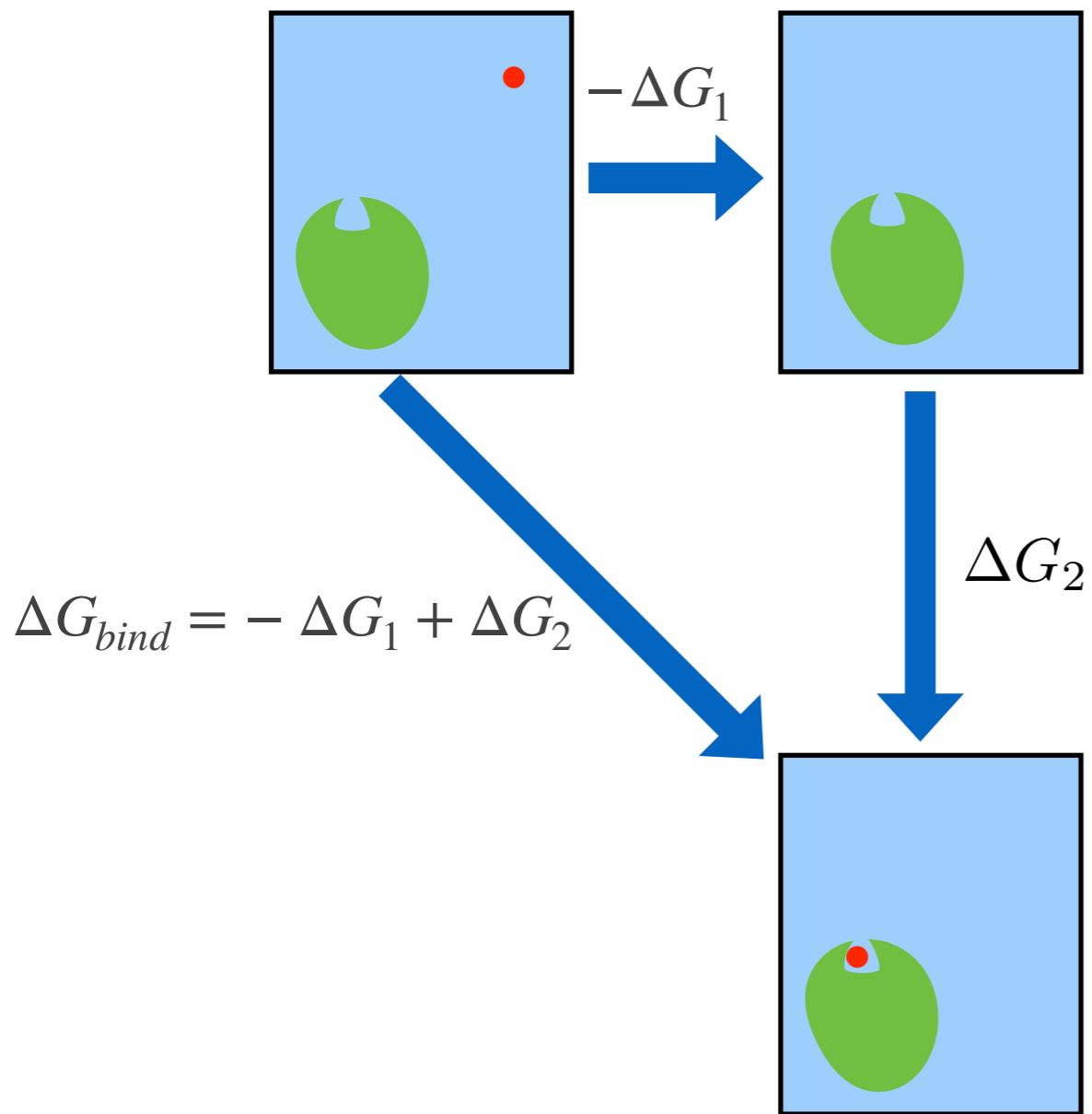
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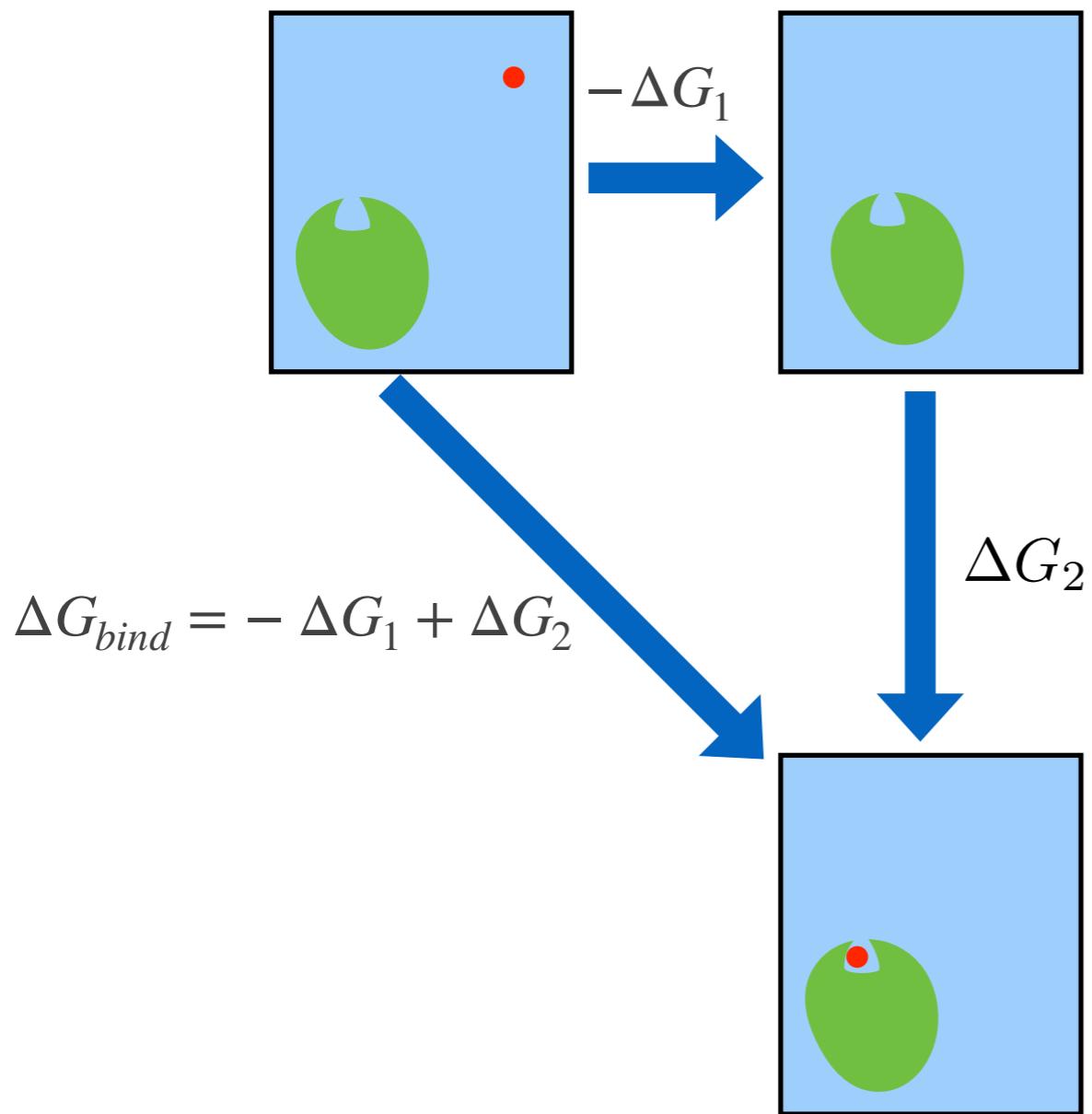
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Two-state transition:
No need to sample
unbinding path!

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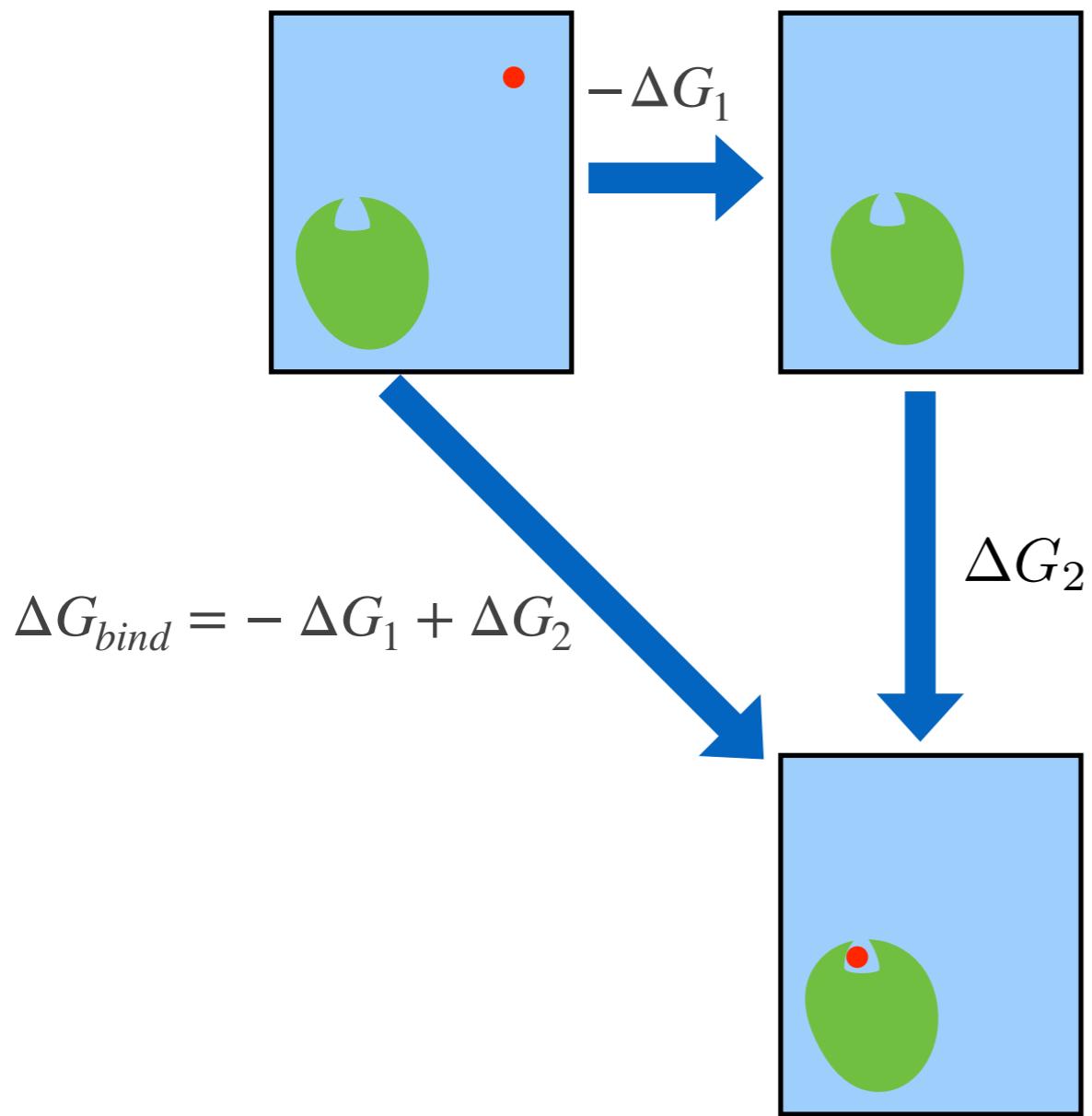


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Traditionally: run at
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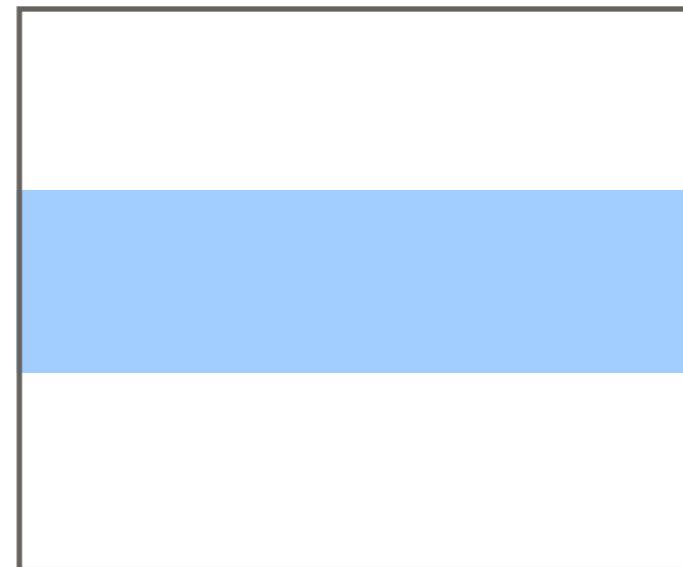
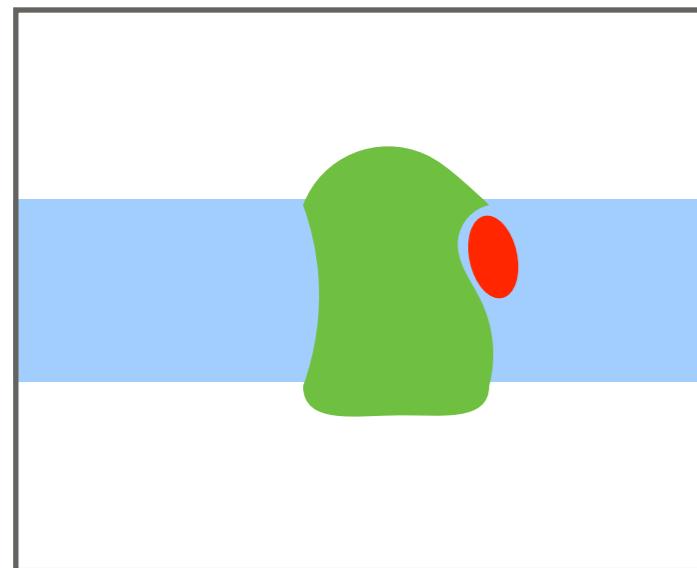


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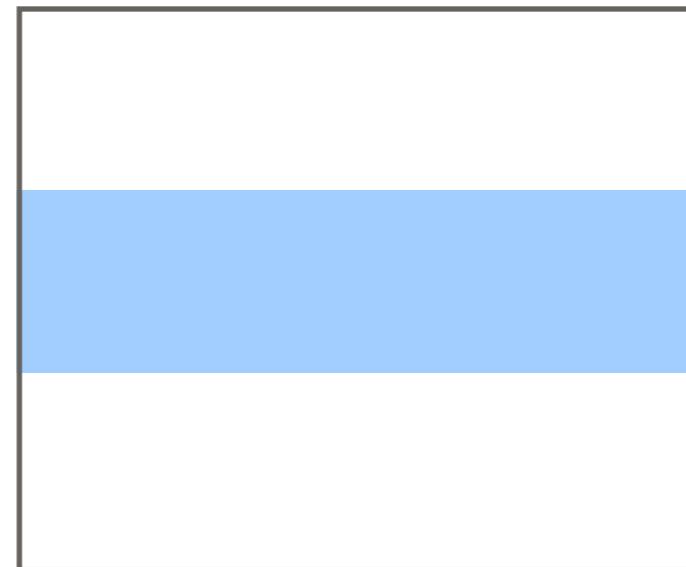
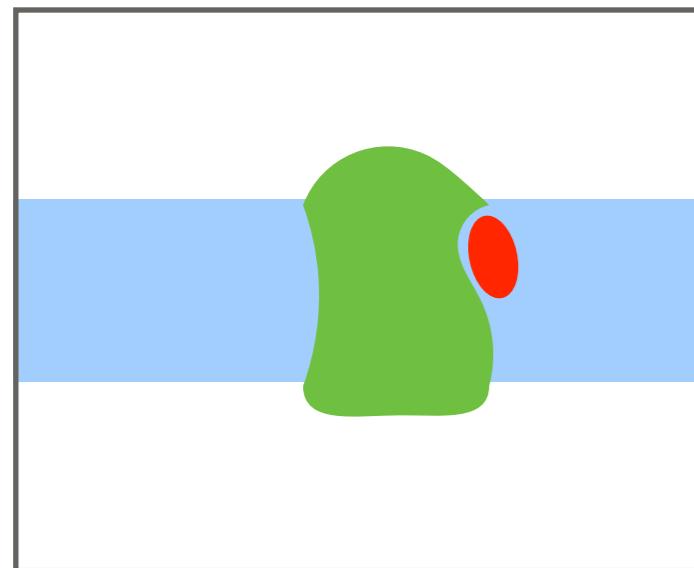
In a membrane, what would this look like?



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Alchemical FEP

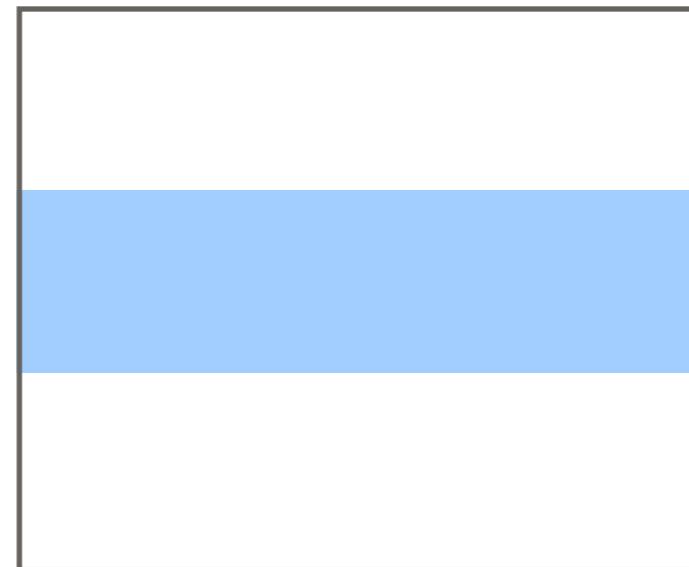
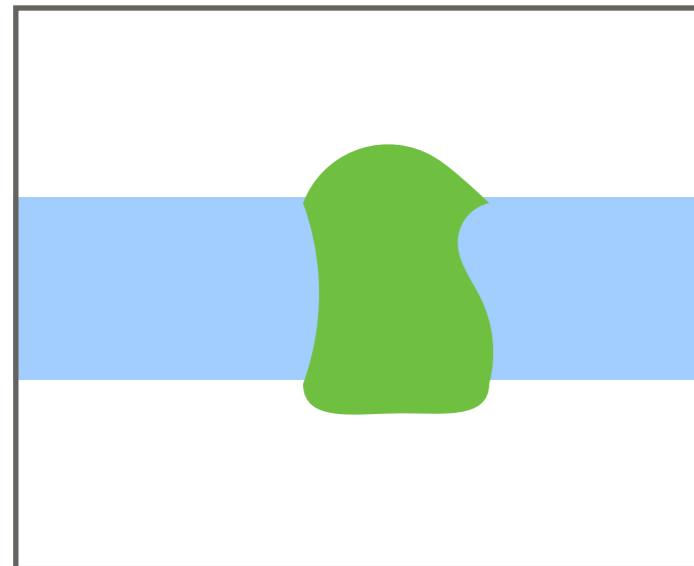


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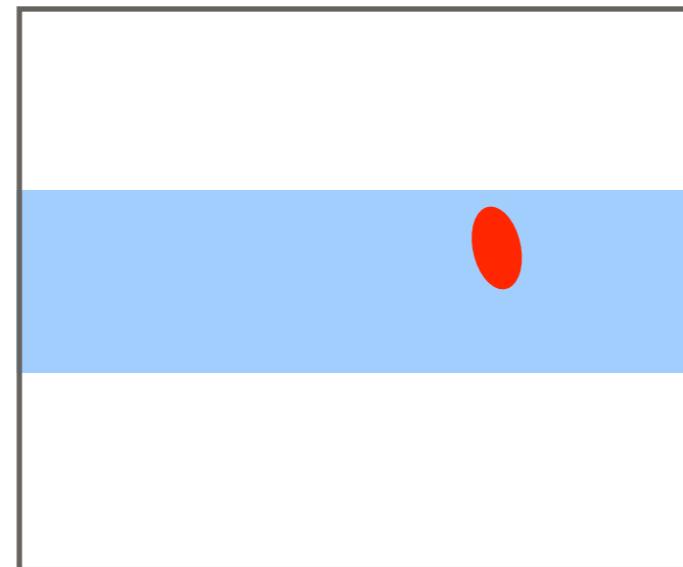
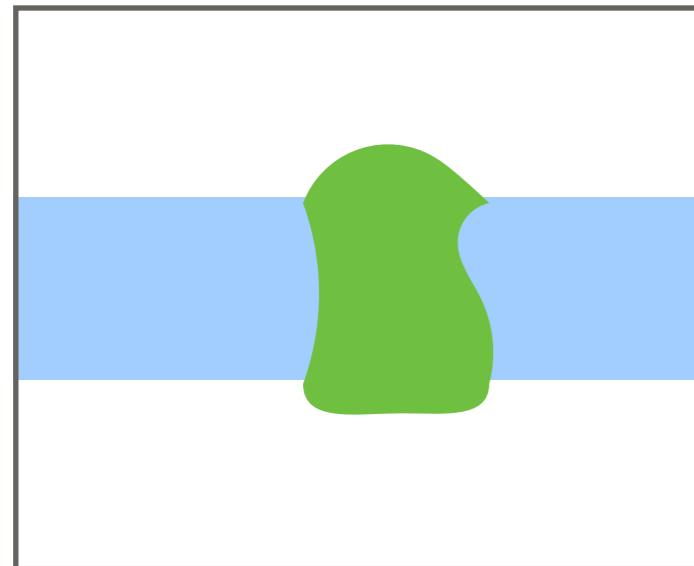


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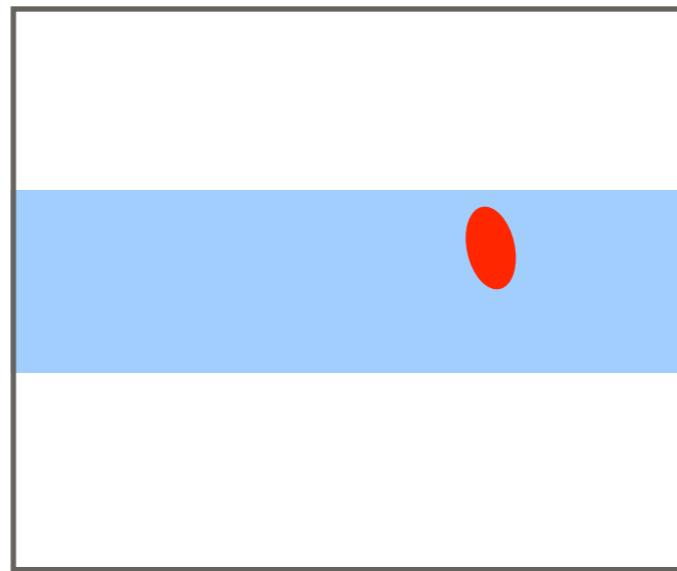
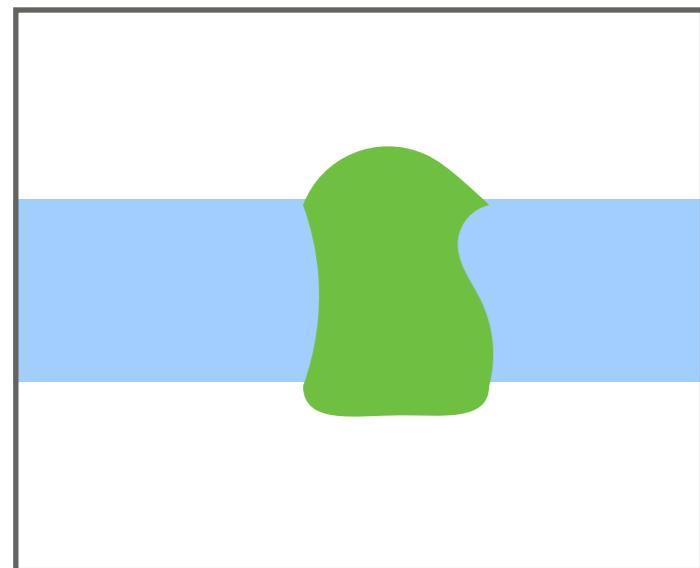


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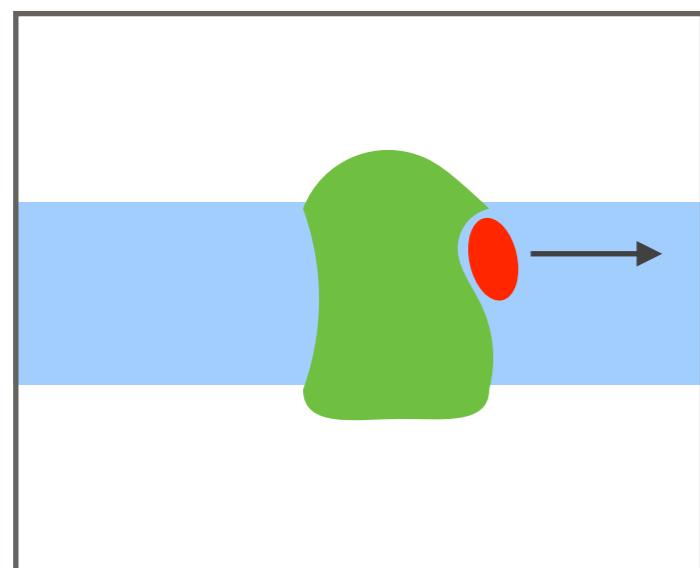
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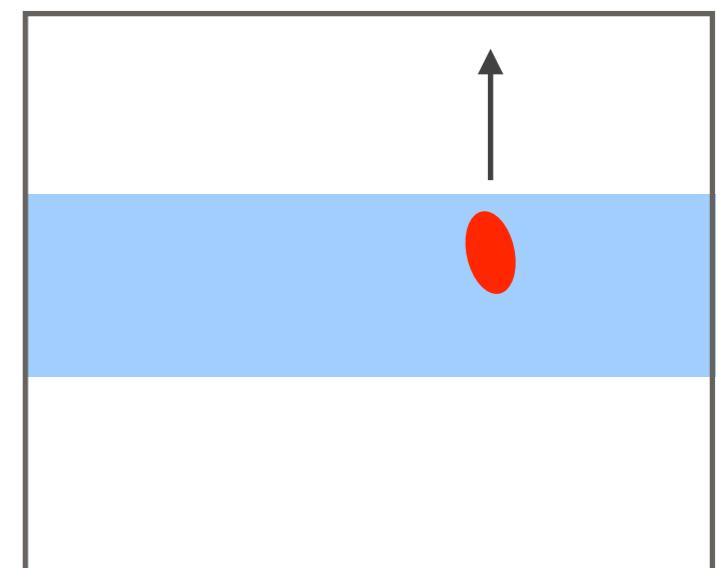
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In comparison...



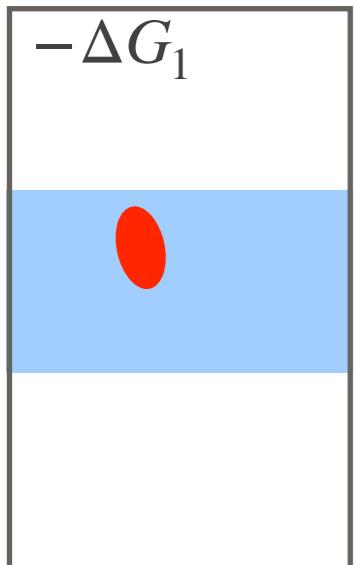
pathway dependent method
(ABF/Umbrella Sampling/Metadynamics/
Steered MD)

Works ok in coarse-grained simulations.
Unconvergeable in atomistic simulations.



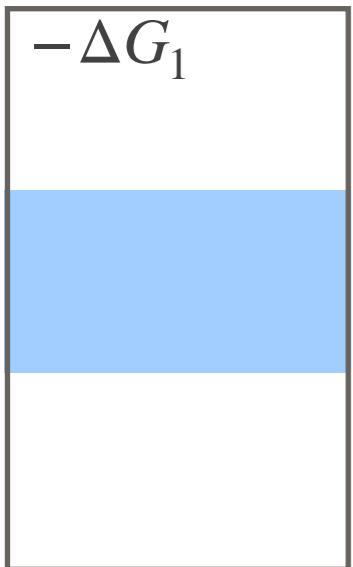
how to handle non-ideality

origin: ligand-ligand interactions in
bulk



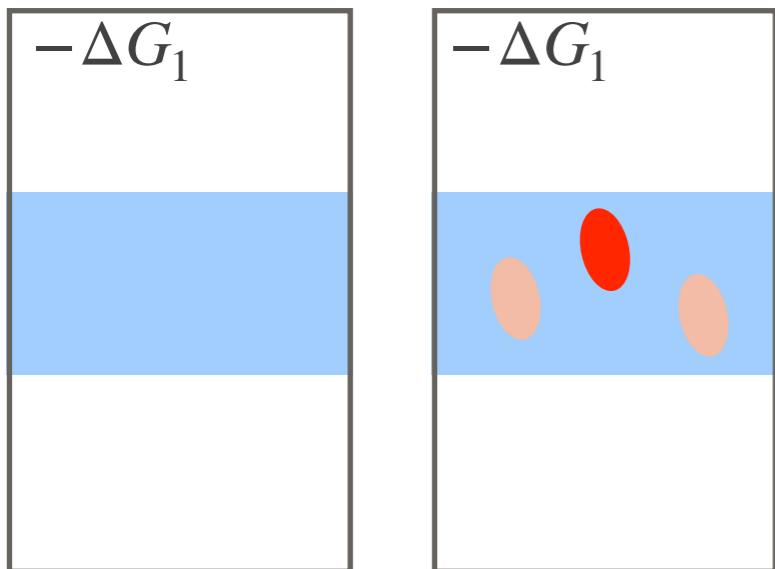
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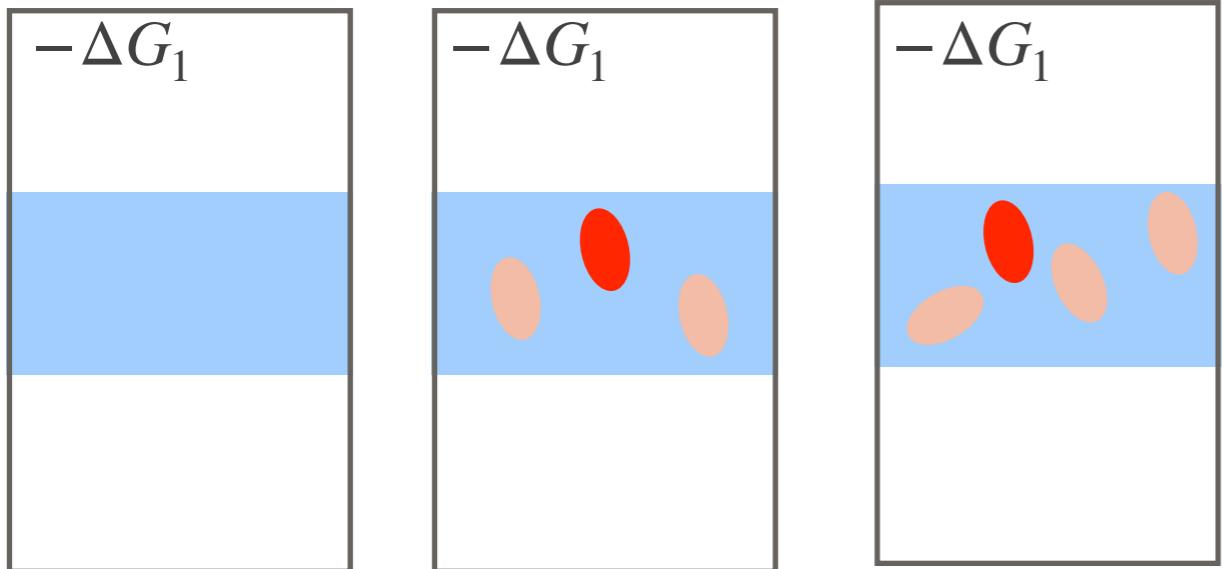
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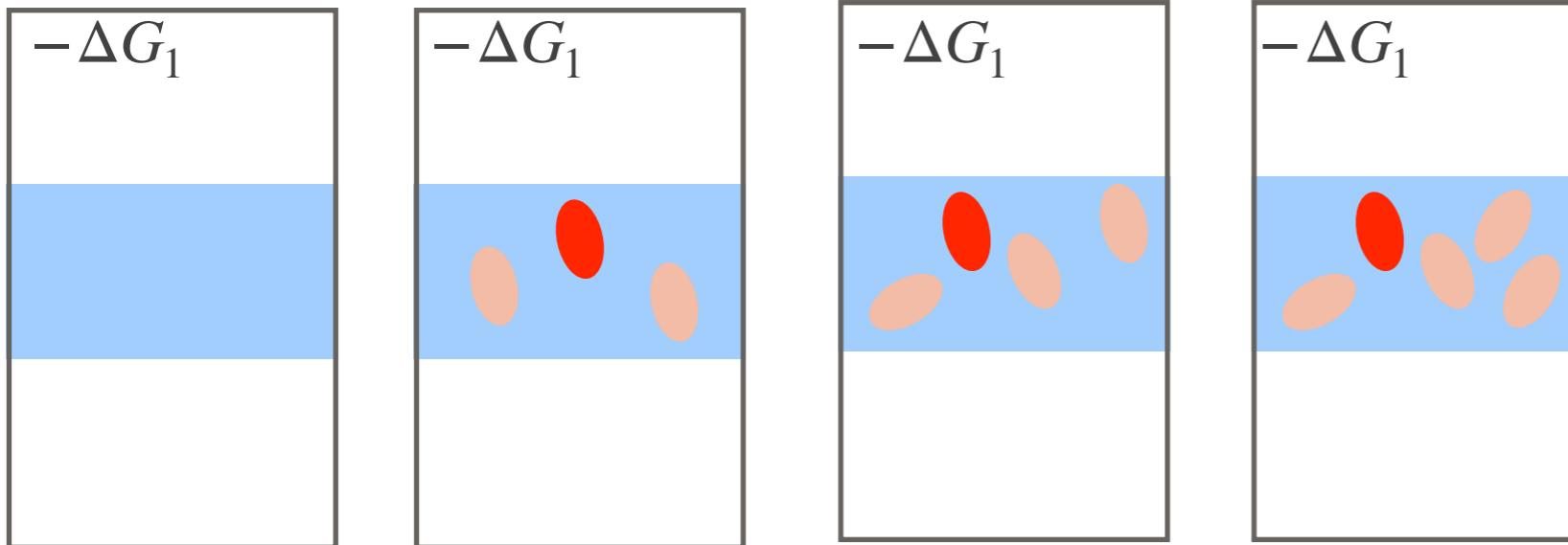
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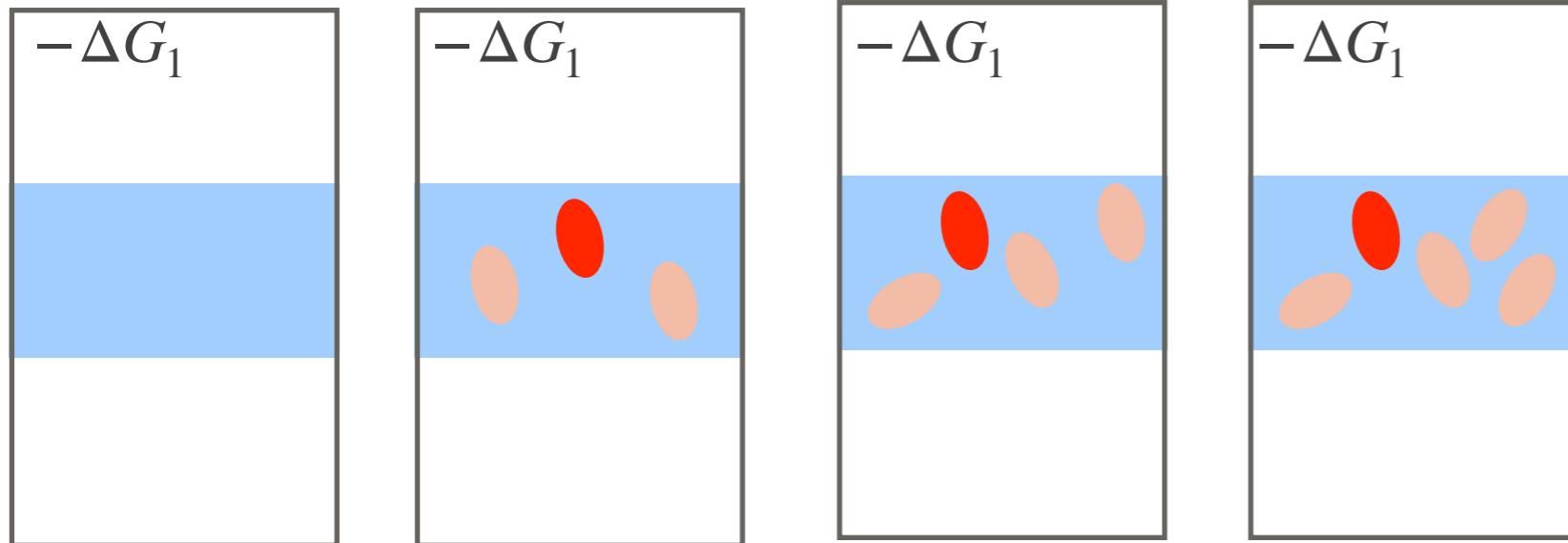
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origin: ligand-ligand interactions in
bulk



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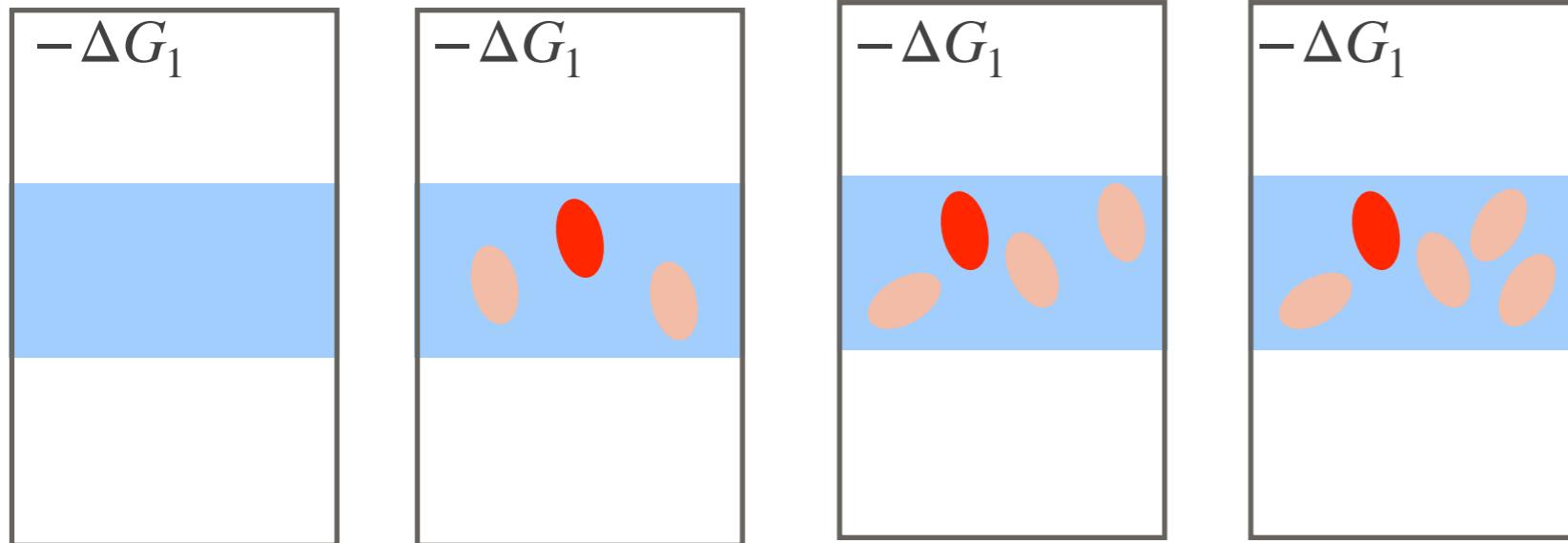
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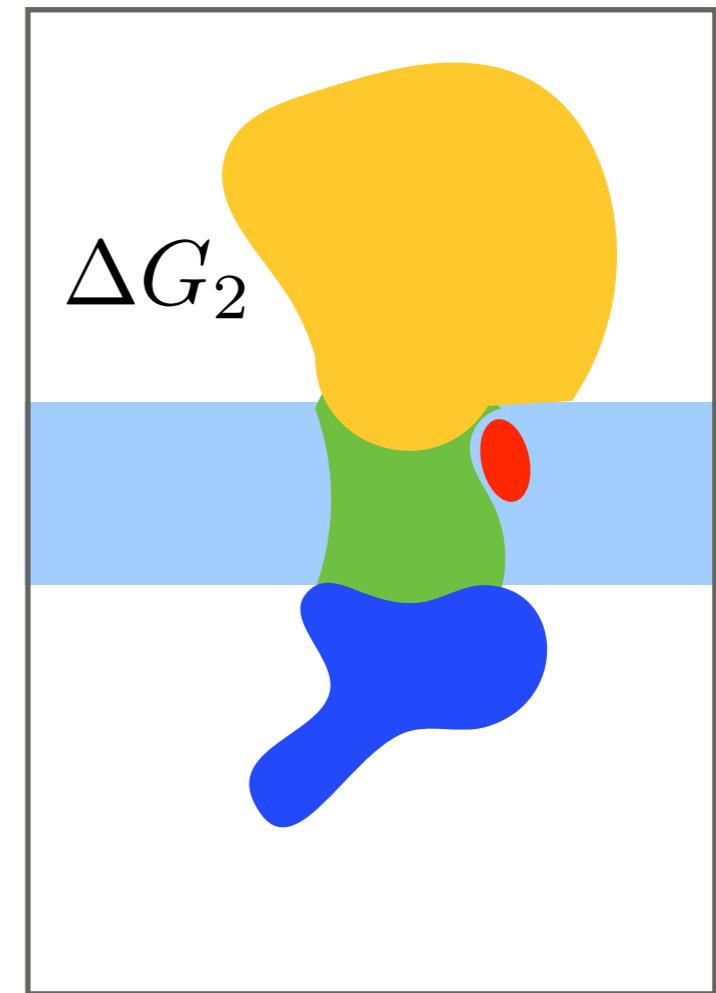
run small, fast simulations for
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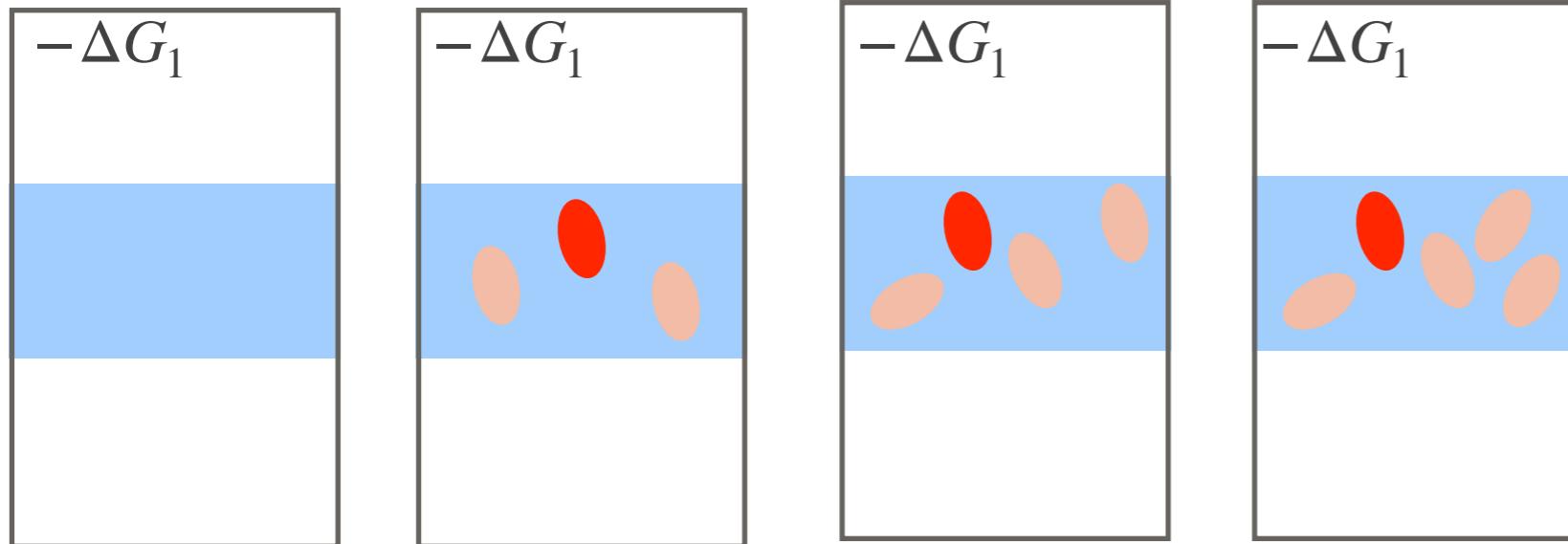
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run large, slow
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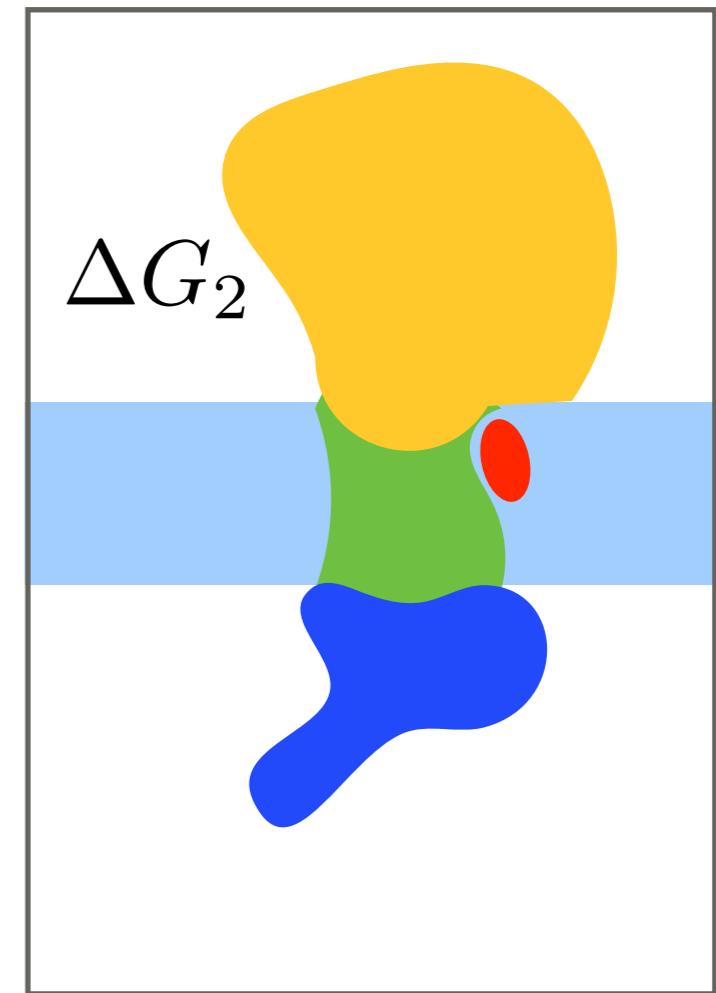
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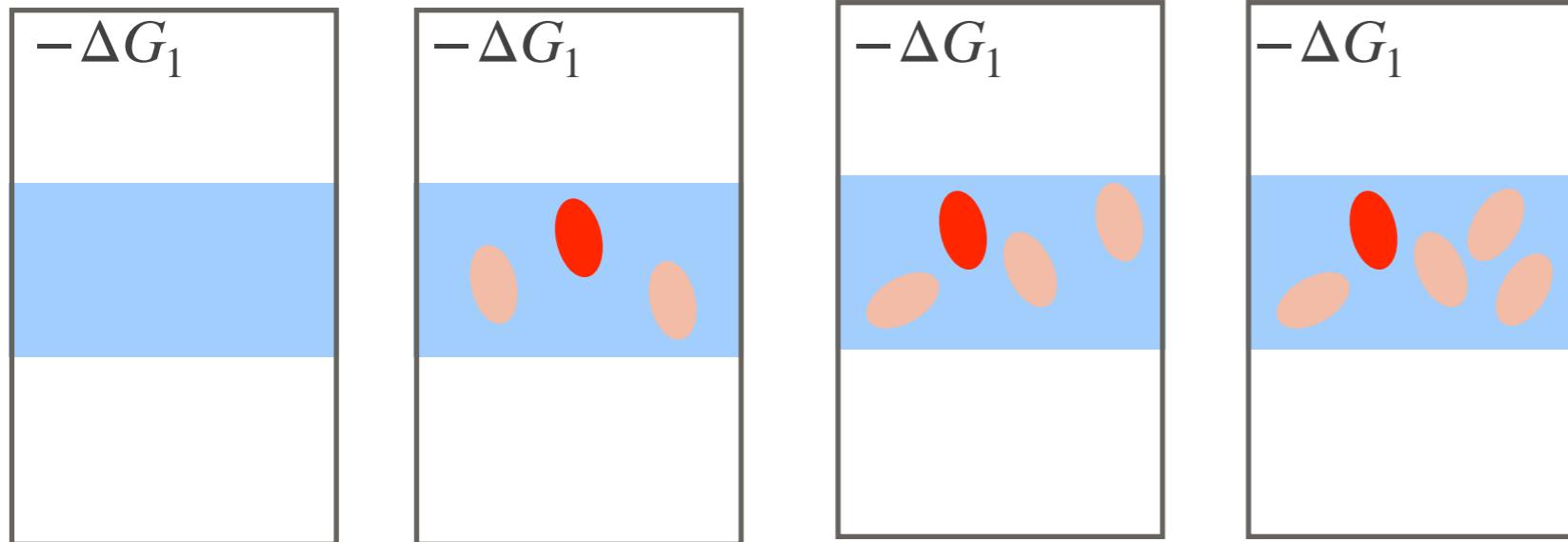
$$\kappa_A = \frac{e^{\Delta G_1}}{[L]_{tot}} e^{-\Delta G_2/RT}$$



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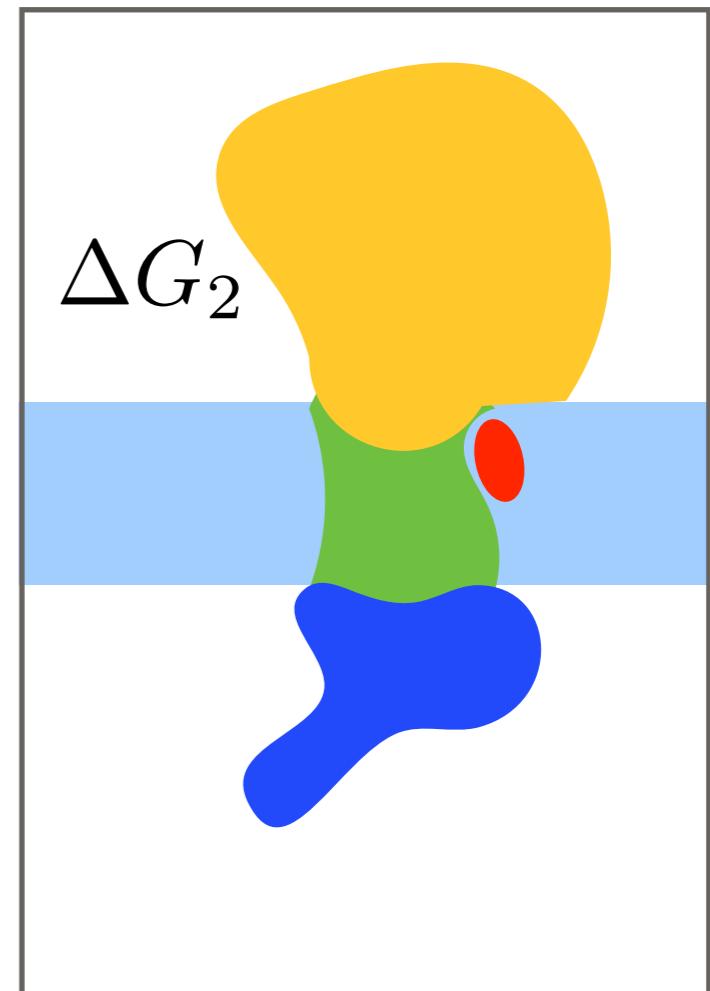
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run small, fast simulations for
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$$\kappa_A = \frac{e^{\Delta G_1}}{[L]_{tot}} e^{-\Delta G_2/RT}$$

note: also true for soluble systems but surprisingly unexplored



run large, slow
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introducing: association coefficient κ_A

Does the same job as K_A

$$\kappa_A = \frac{[RL]}{[R][L]_{tot}} \quad \Delta G_{bind} = -RT \ln \kappa_A [L]_{tot}$$

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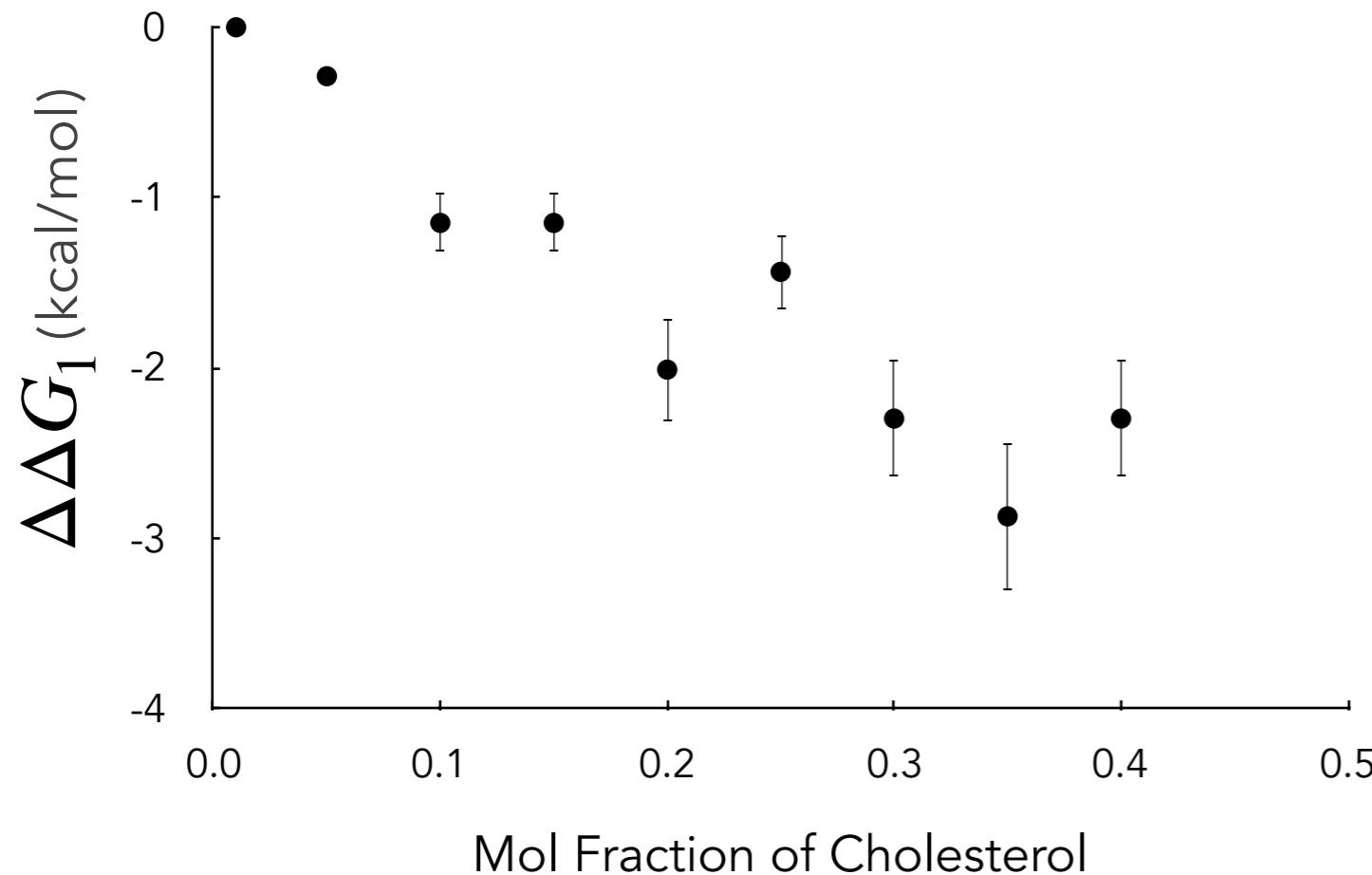
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- Reviewer 2 of Salari et al, JCTC 2018. Thank you!

Quantifying non-ideality

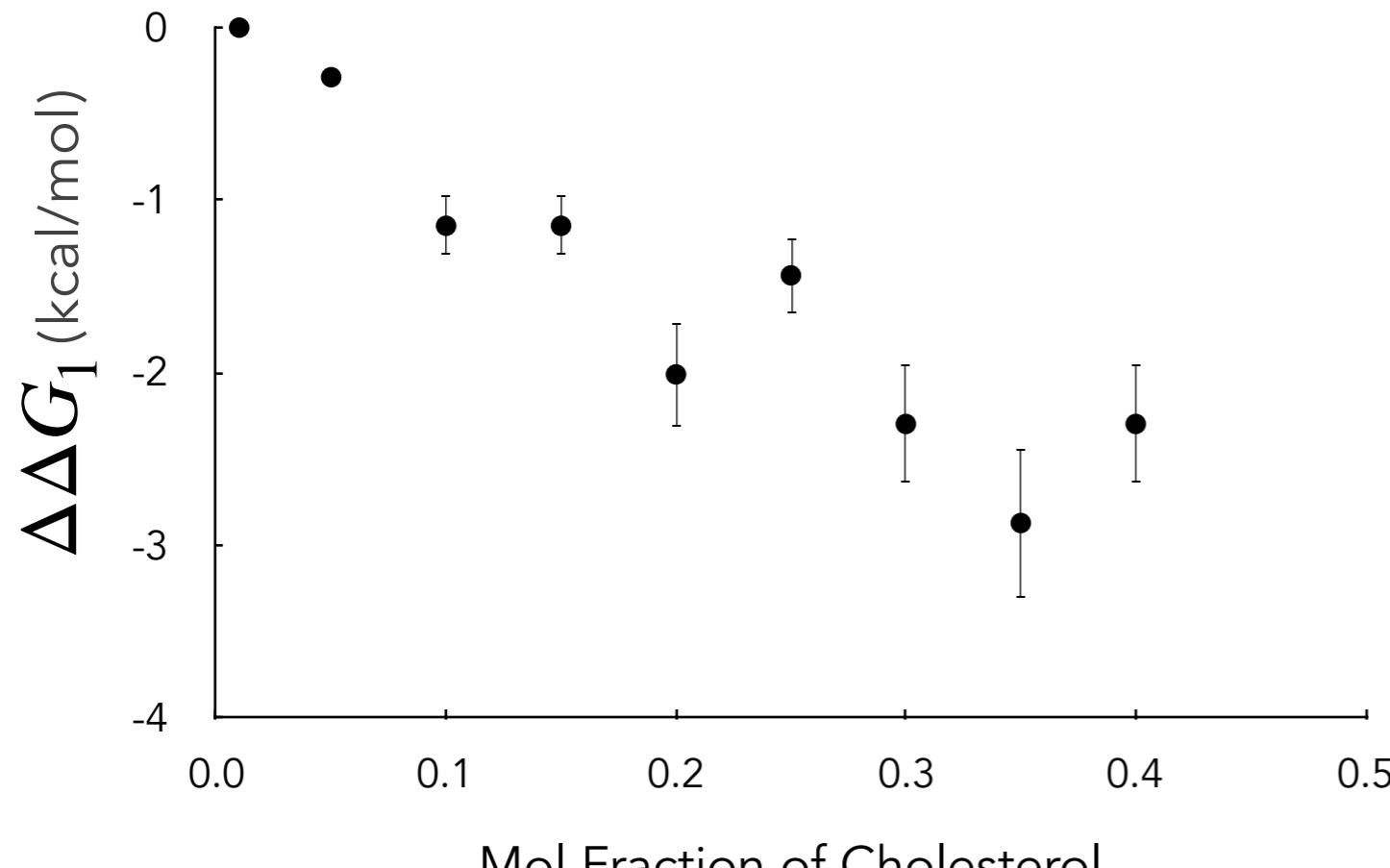
Free energy of cholesterol in POPC bilayer (relative to ideal-dilute)



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

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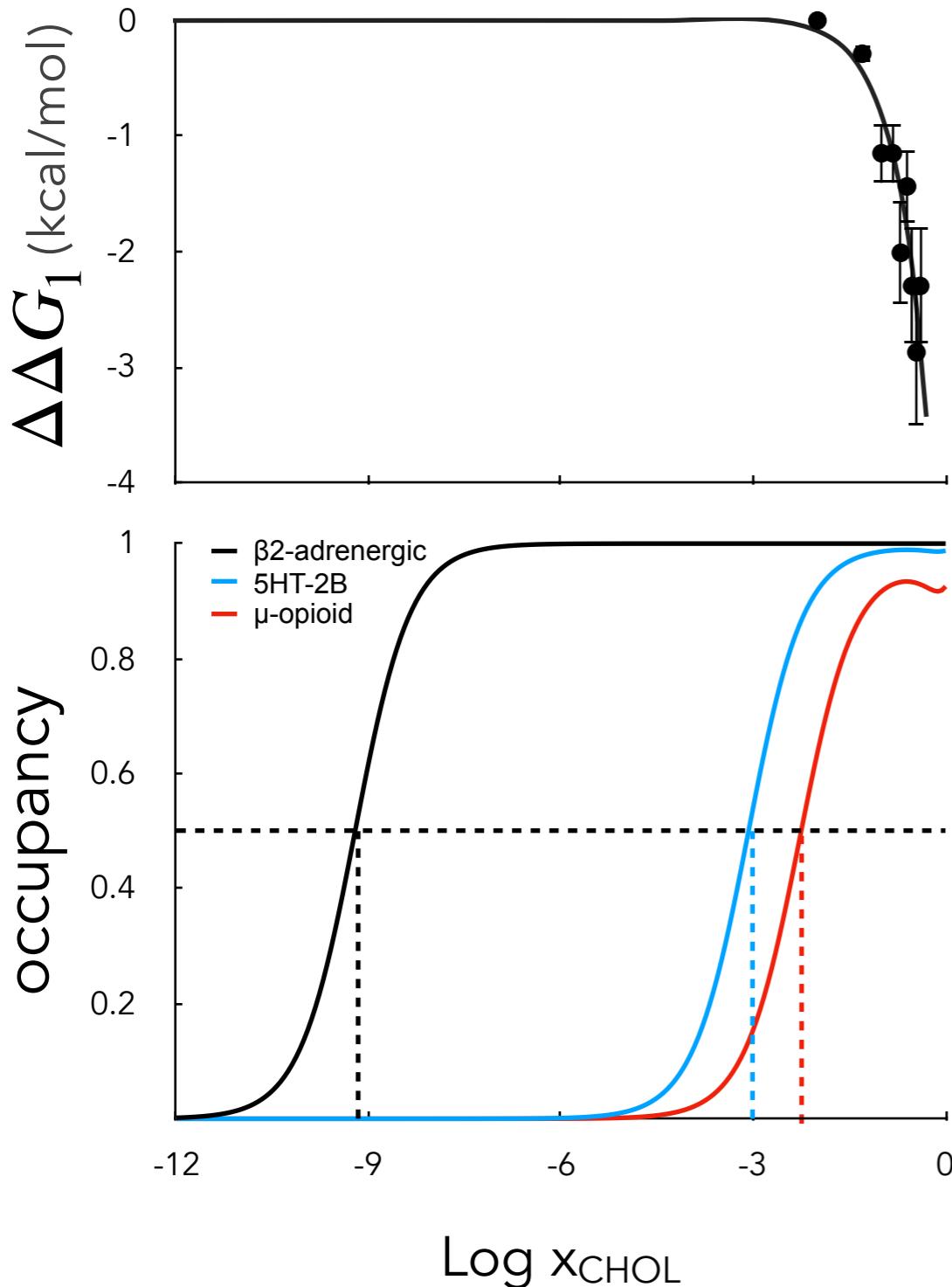
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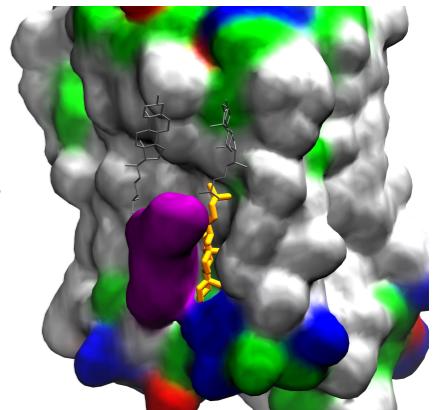
Salari, Joseph, Lohia, Henin, Brannigan, JCTC 2018

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

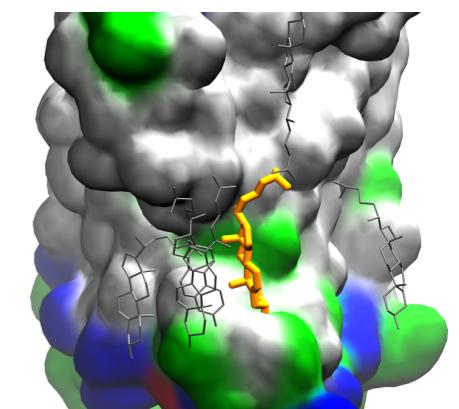
proof of principle: virtual cholesterol binding assay



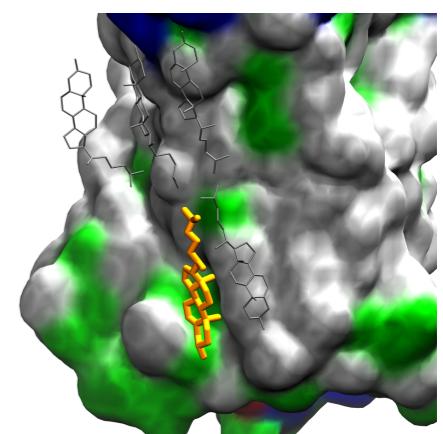
Salari, Joseph, Lohia, Henin, Brannigan, JCTC 2018



$\beta 2\text{-Adrenergic}$
3D4S
 $x_{50} = 10^{-9}$

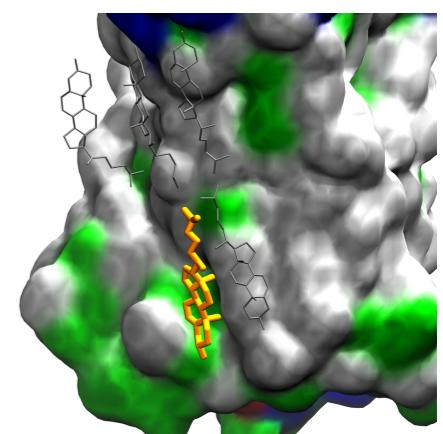
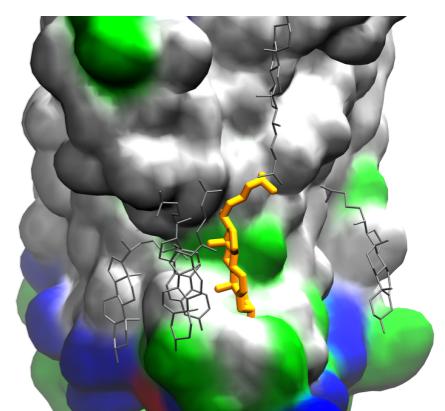
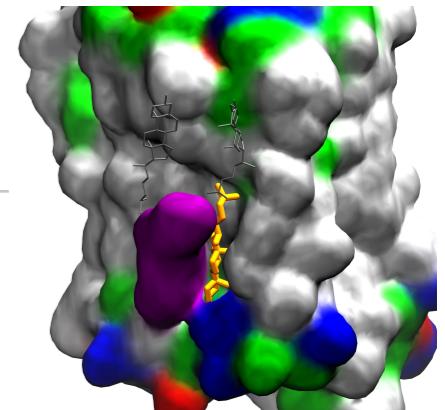
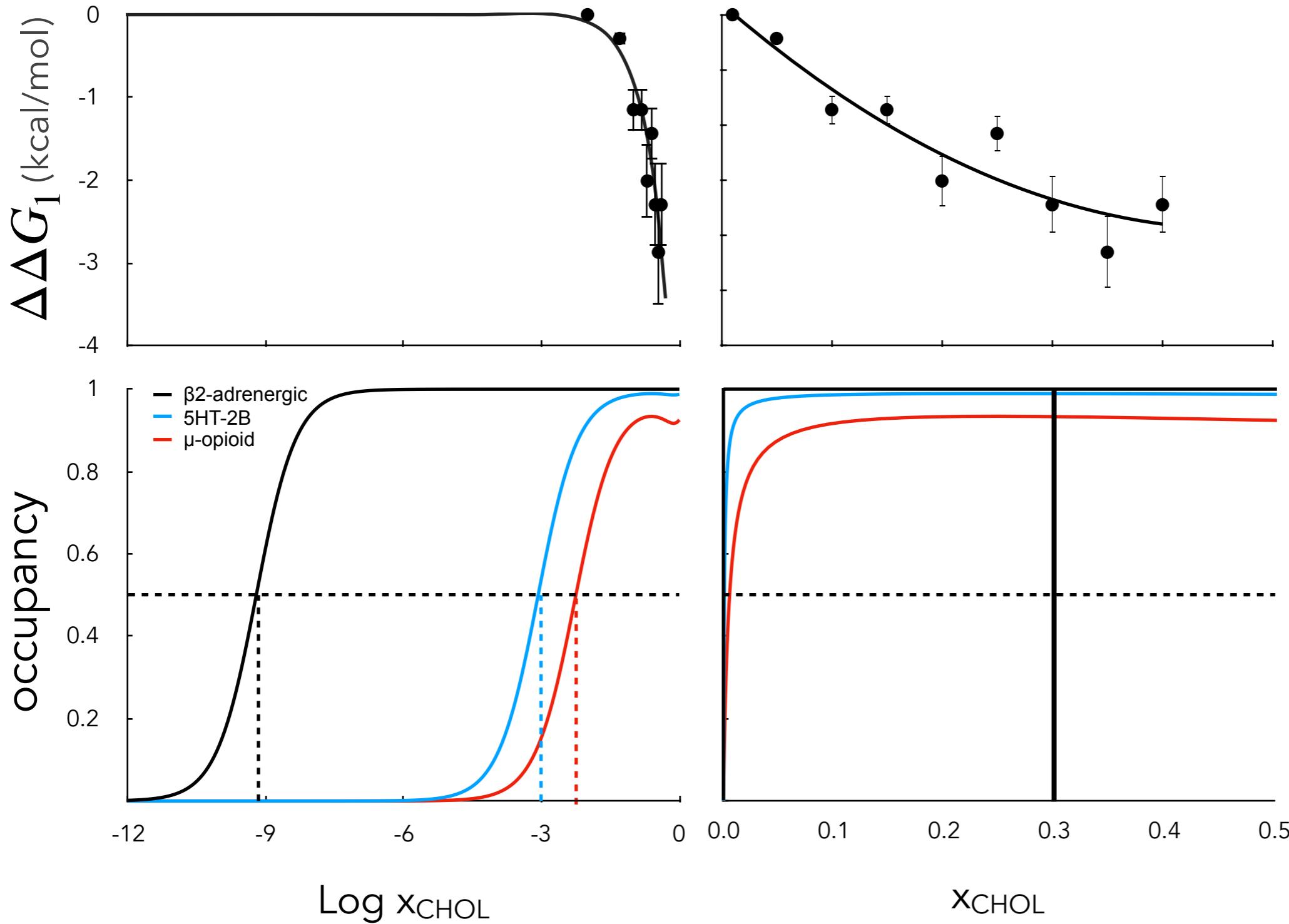


$5\text{-HT}2\text{B}$
4NC3
 $x_{50} = 10^{-3}$

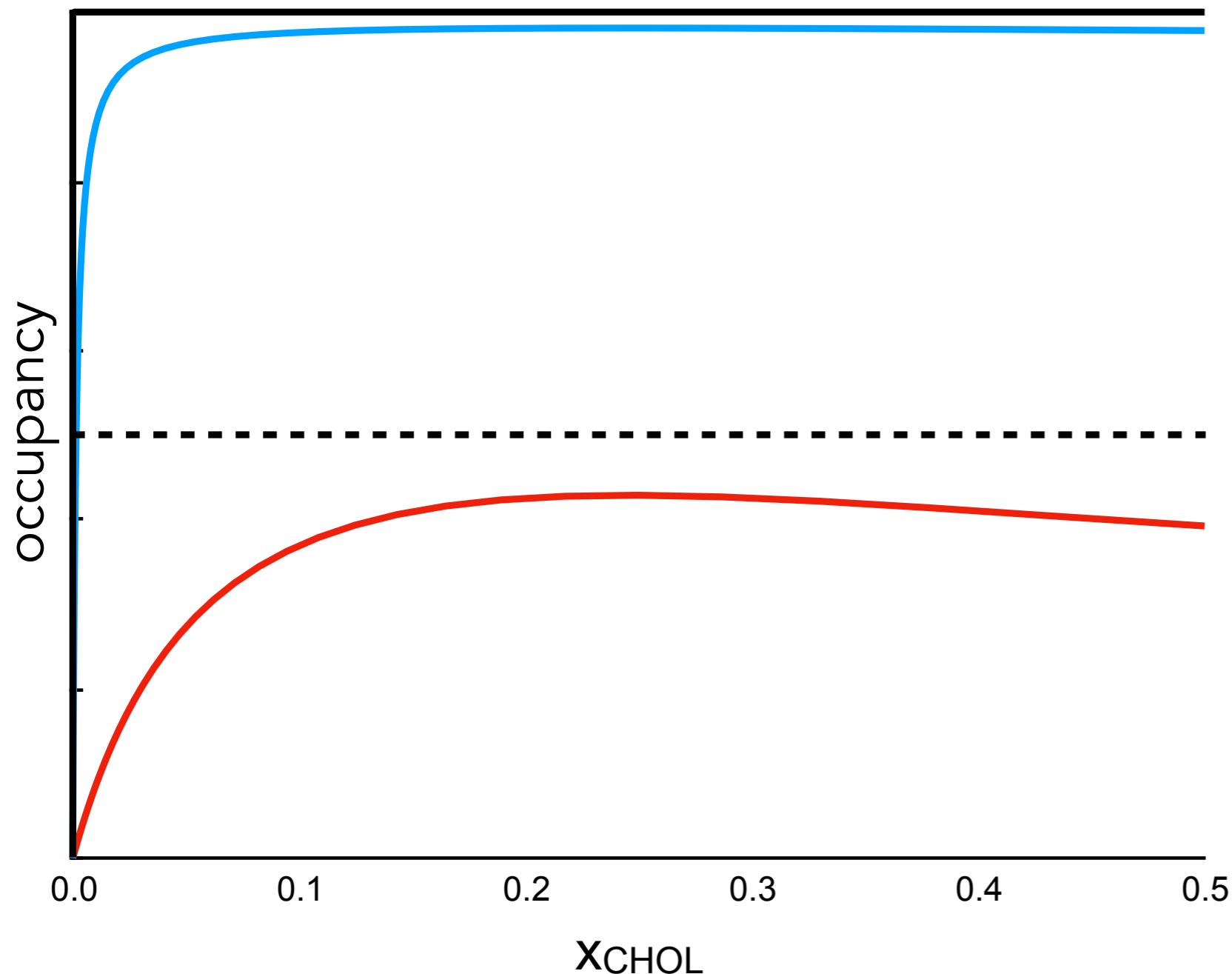


$\mu\text{-Opioid}$
5C1M
 $x_{50} = 10^{-2}$

proof of principle: virtual cholesterol binding assay

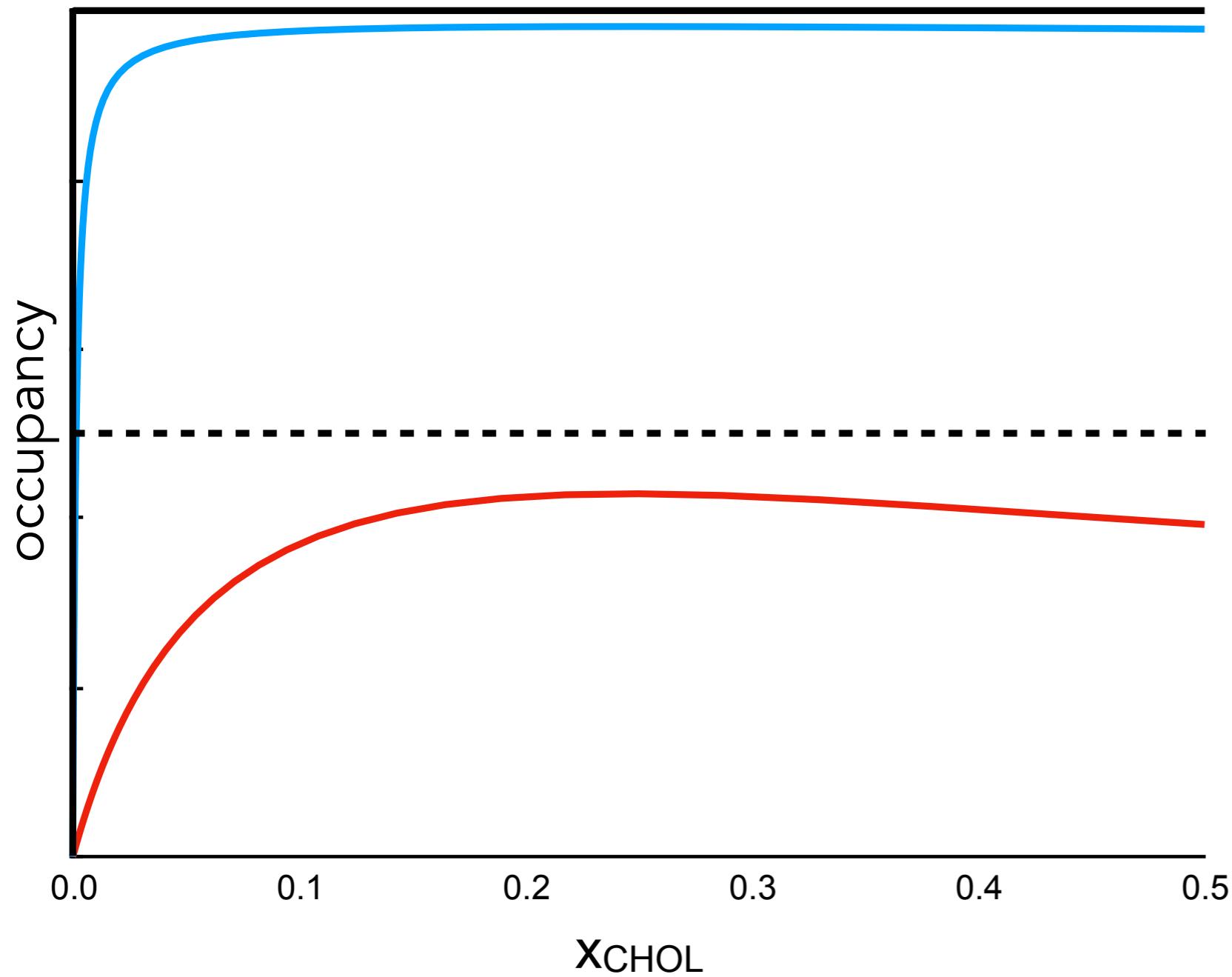


surprising sensitivity

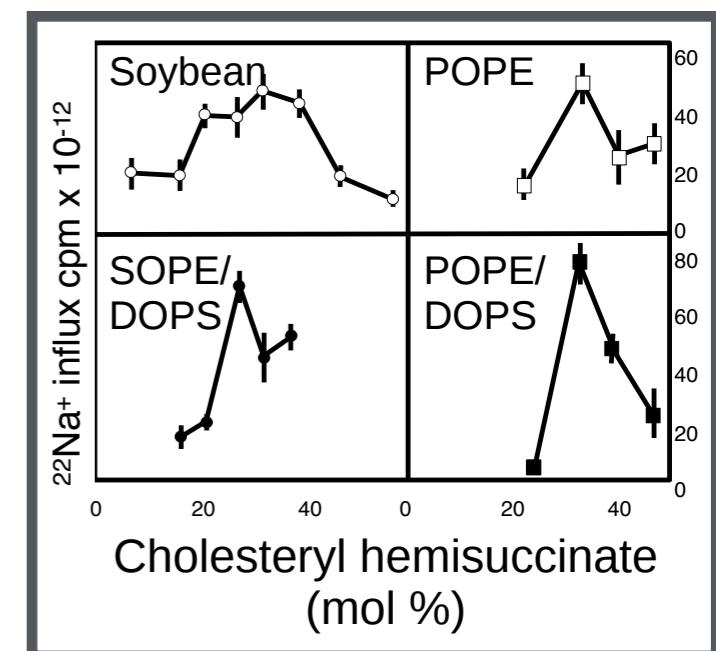


non-ideality of a
randomly mixed
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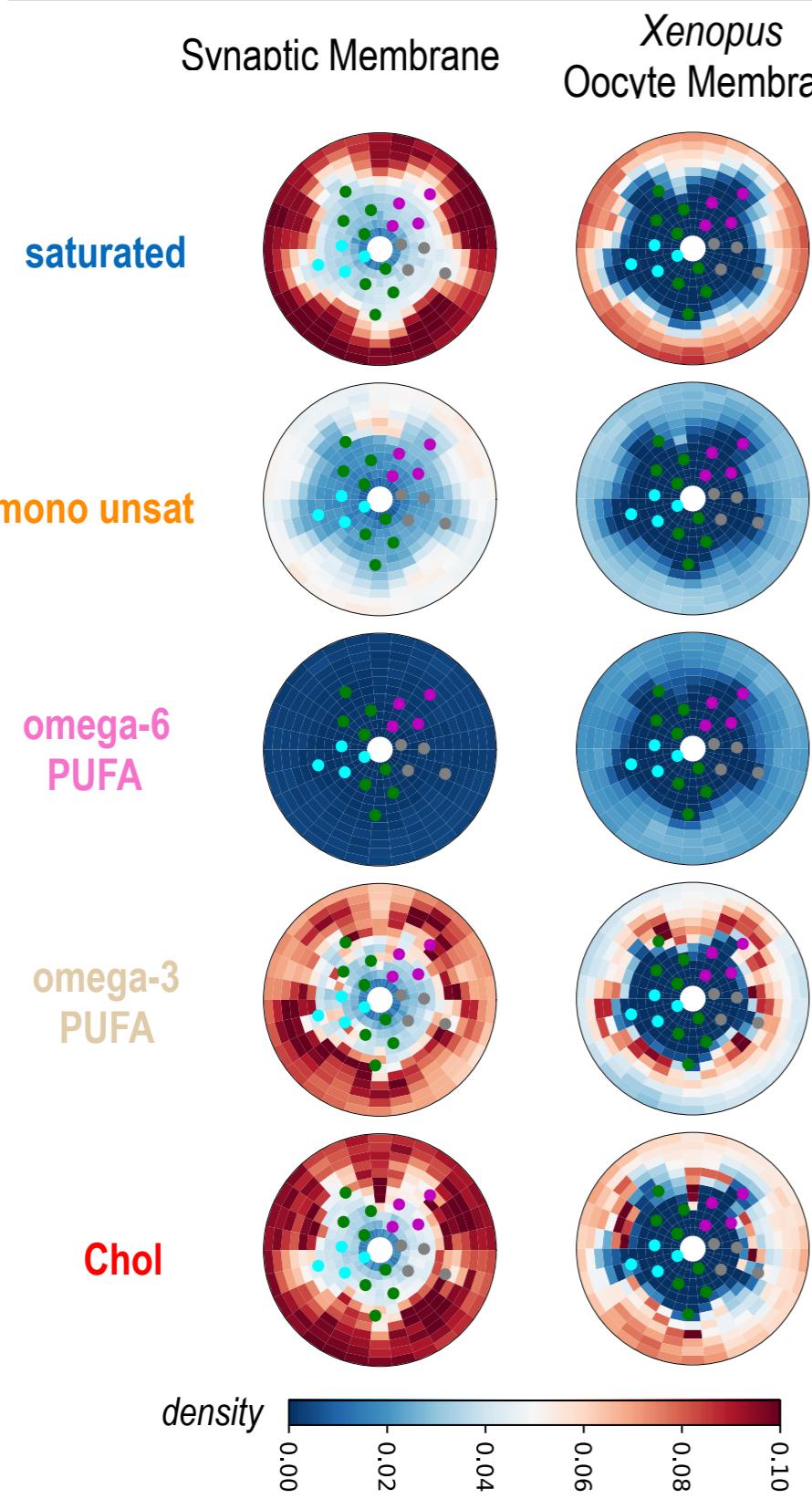
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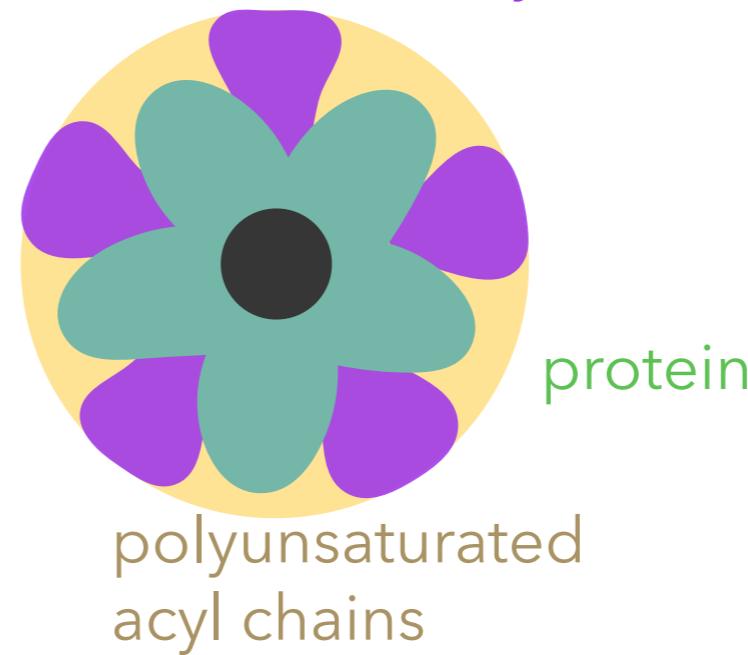
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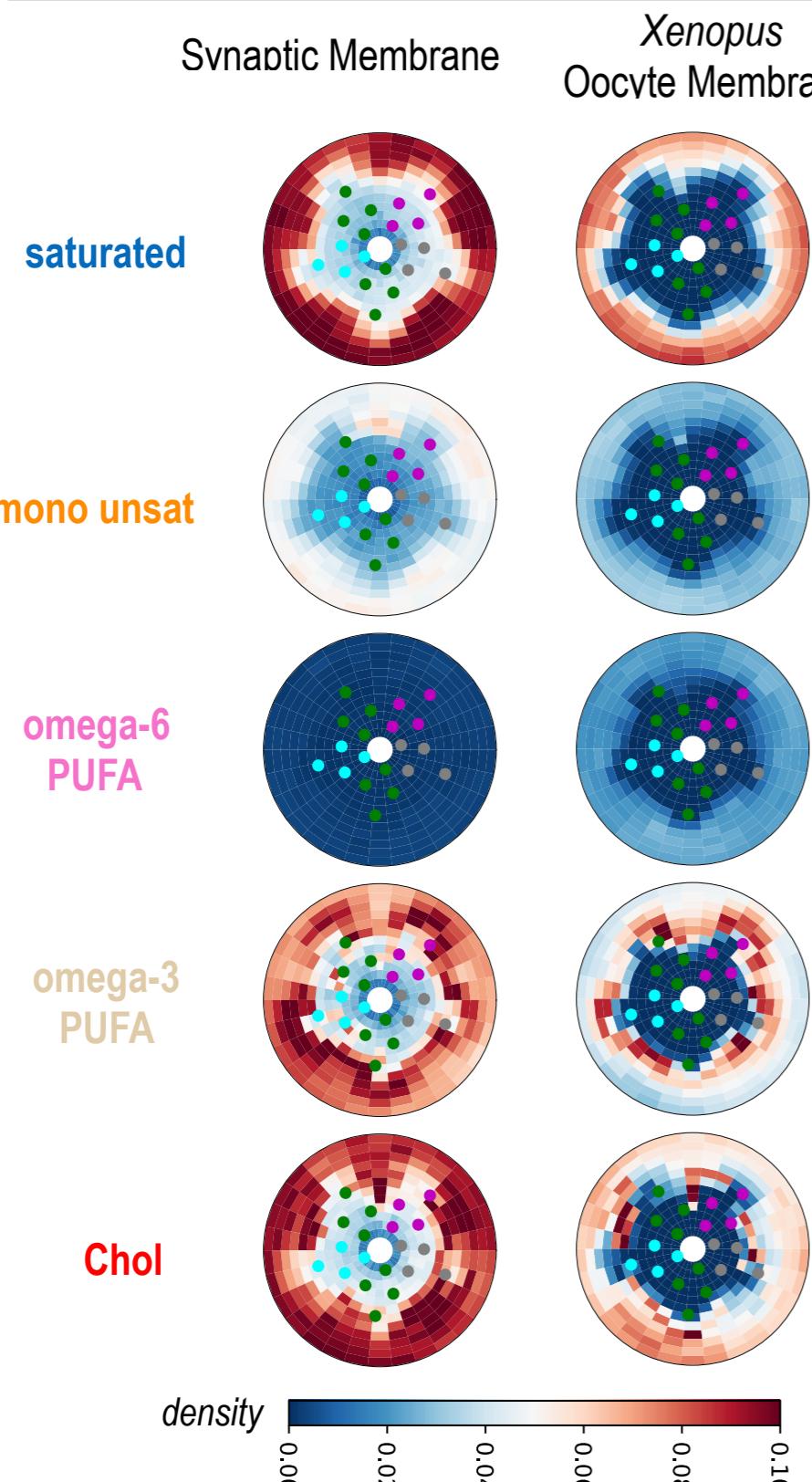
back to pLGICs



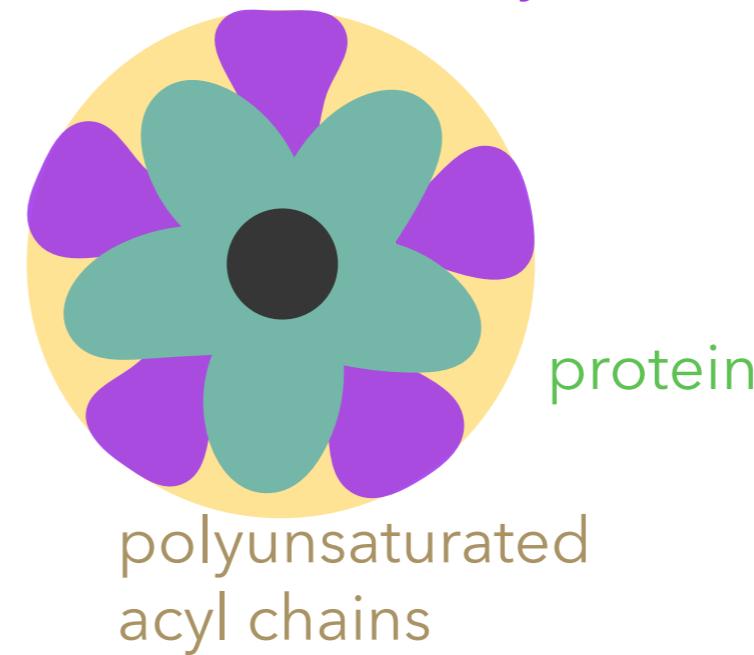
cholesterol &
saturated acyl chains



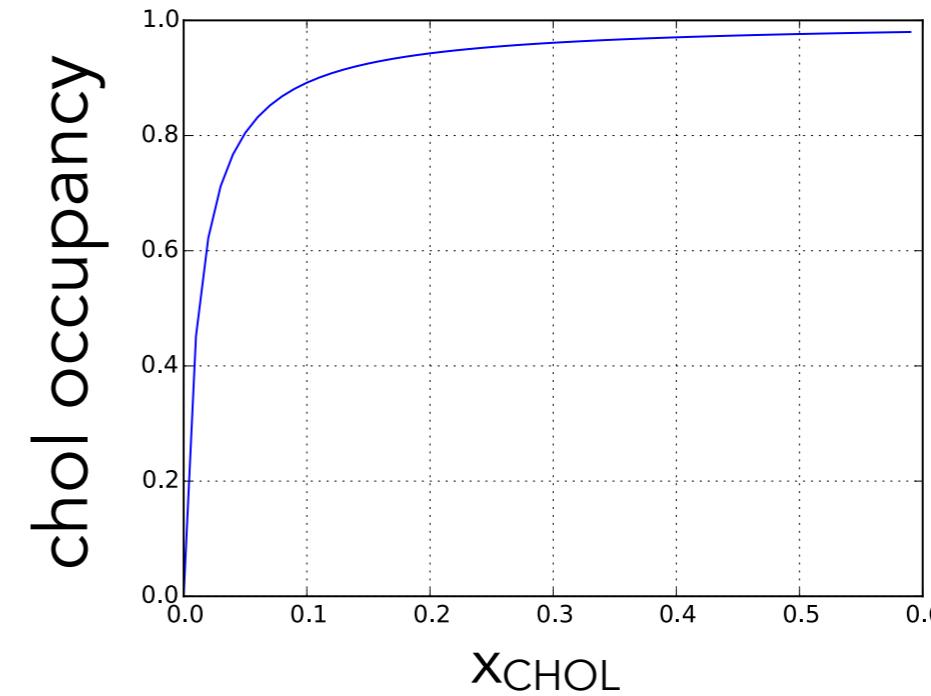
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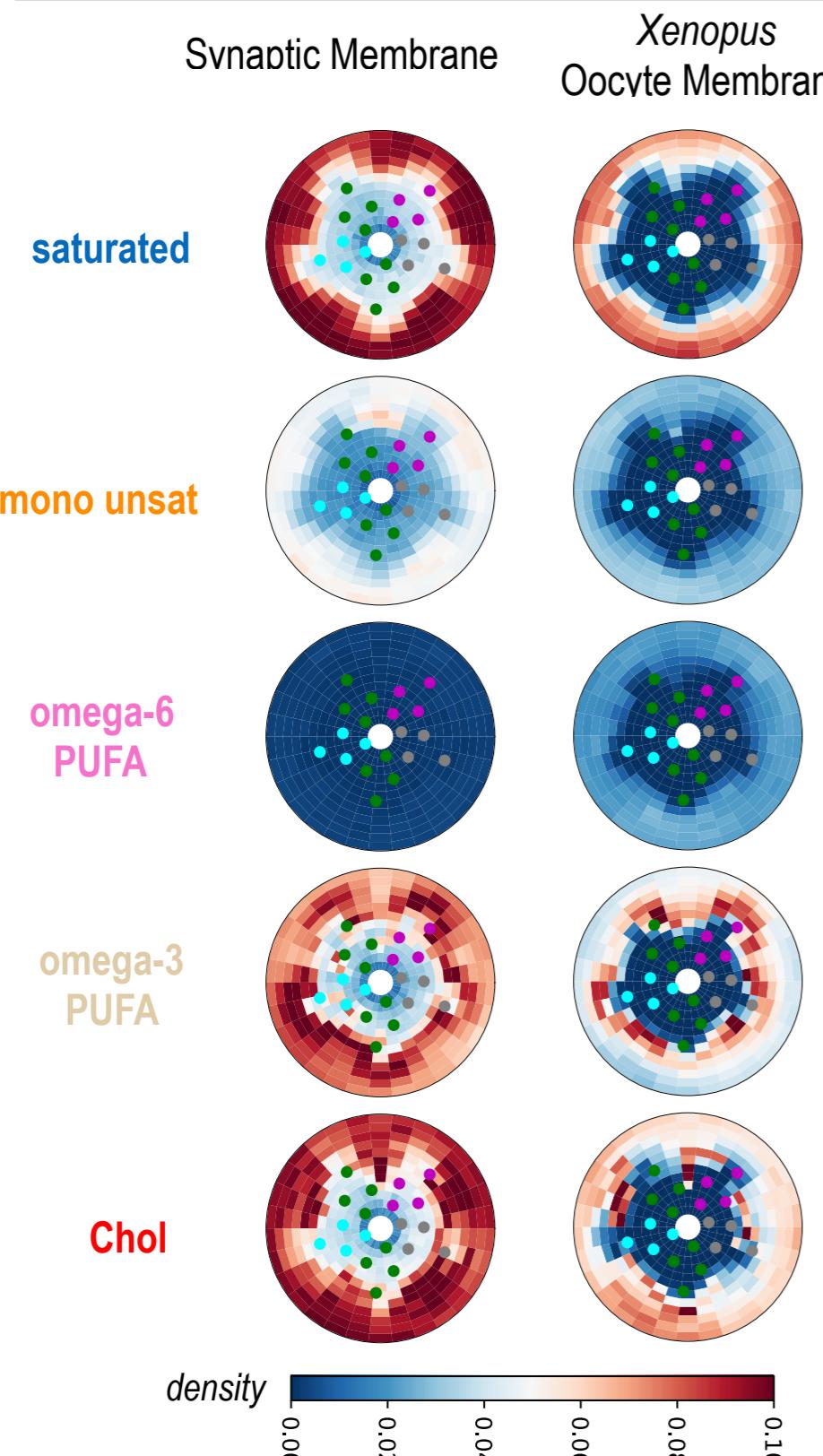
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predicted intersubunit binding in POPC



back to pLGICs

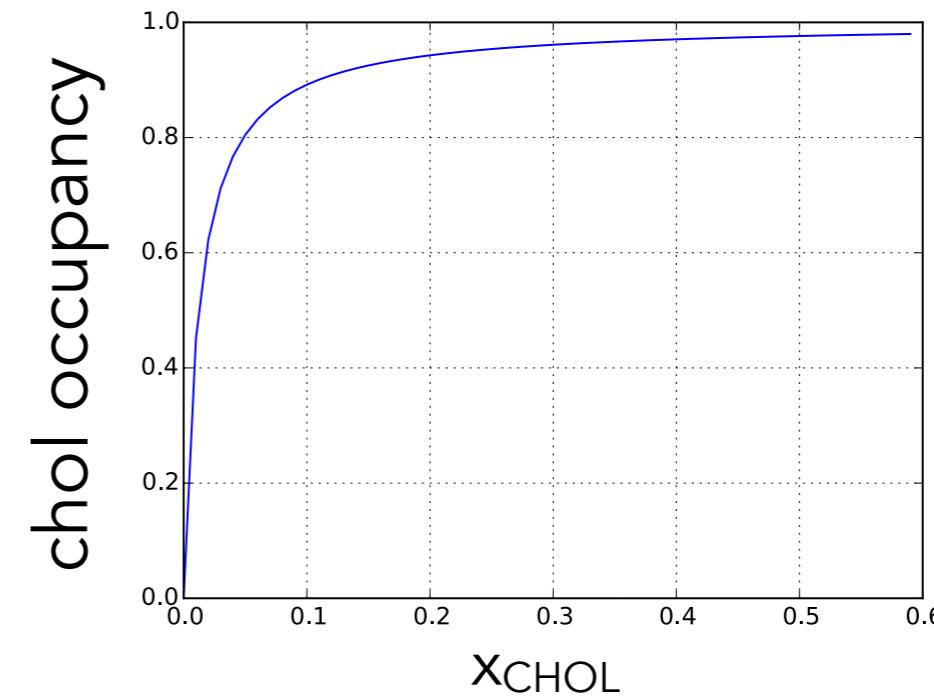


cholesterol &
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Supported,
so far!

predicted intersubunit binding in POPC



Detecting specific lipid binding

Who

Lipid Species



Where

Binding Site



When

Affinity



Experiments

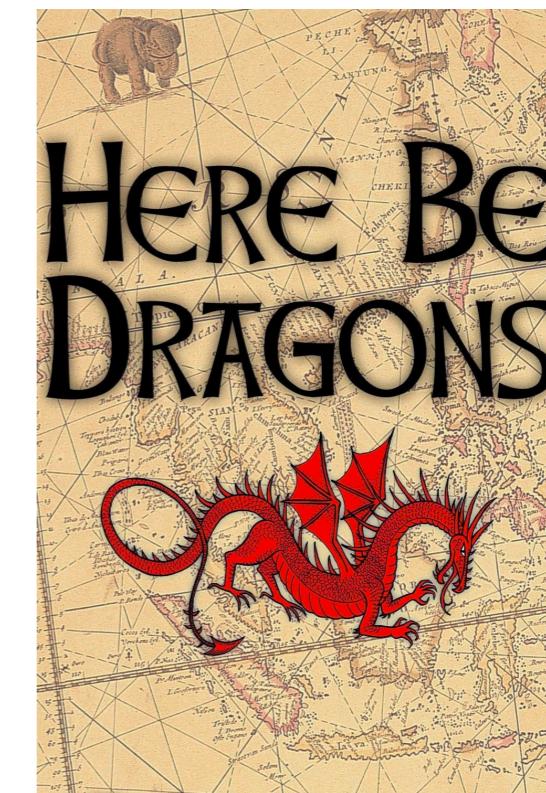
Mass Spectrometry

Soluble lipid-binding assay

Structural Biology

MD Simulation

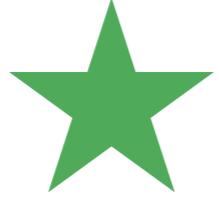
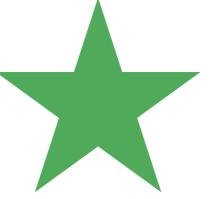
Atomistic (AA)



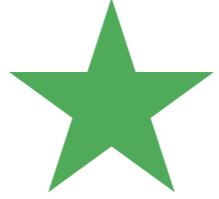
Coarse-grained (CG)



Detecting specific lipid binding

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

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Detecting specific lipid binding

	Experiments	MD Simulation
Who	Lipid Species	Atomistic (AA)
Where	Binding Site	Coarse-grained (CG)
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The table compares experimental methods and MD simulation across three categories: Who, Where, and When. The methods listed are Mass Spectrometry, Soluble lipid-binding assay, Structural Biology, Atomistic (AA), and Coarse-grained (CG). Green stars indicate the method is applicable, while a checkmark indicates it is not.

- Who:** Mass Spectrometry (star), Soluble lipid-binding assay (checkmark), Structural Biology (checkmark), Atomistic (AA) (checkmark), Coarse-grained (CG) (star).
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Detecting specific lipid binding

	Experiments			MD Simulation	
	Mass Spectrometry	Soluble lipid-binding assay	Structural Biology	Atomistic (AA)	Coarse-grained (CG)
Who	Lipid Species				
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- Next up : polyunsaturated fatty acids!

Acknowledgments

Group Members

coarse-grained
results -

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Kristen Woods

AFEP Methods

Dr. Reza Salari

**Dr. Thomas
Joseph**

Dr. Ruchi Lohia

Reviewers of Salari et al, JCTC 2018,

Sharp et al, BBA-Biomembranes 2019,

Woods et al, JMB 2019

AFEP in pLGICs

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Dr. Sruthi
Murlidaran

non-ideality in
membranes

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Liam Sharp

Rulong Ma

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