

Computational prediction of specifically-bound lipids for pLGICs

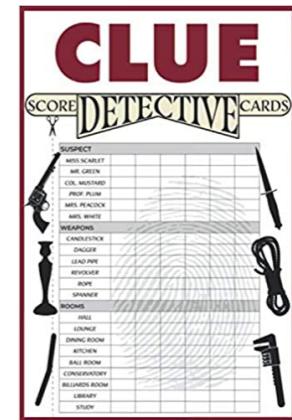
Grace Brannigan
Center for Computational &
Integrative Biology
Rutgers University - Camden



Two Stories

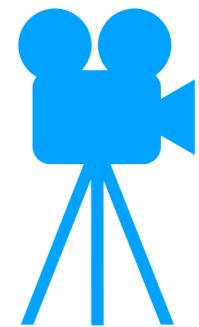
1. Identifying **lipid fragments** from structures

- atomistic resolution
- ELIC + model membranes
- new method: SAFEP
- bonus: state dependence!

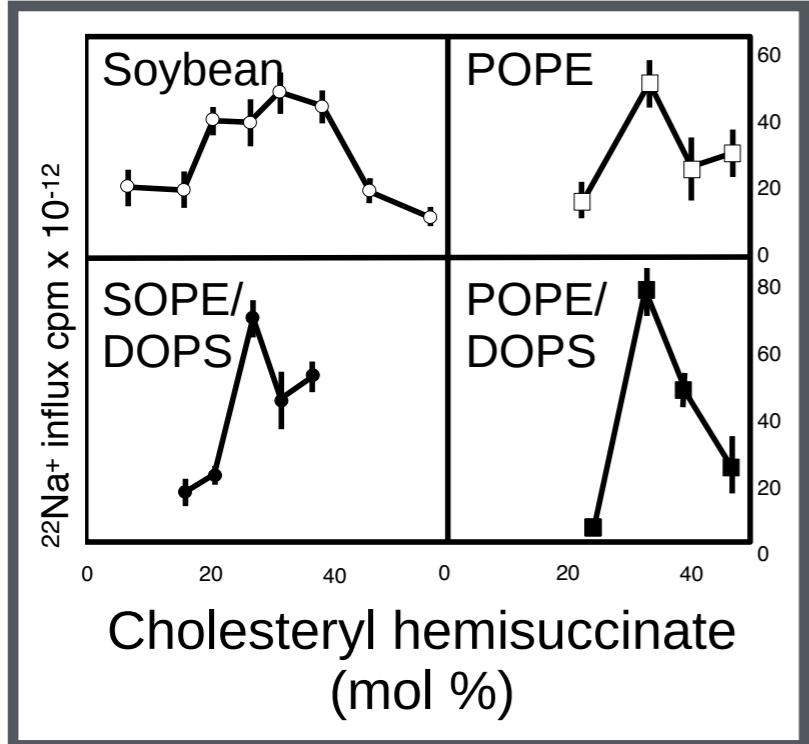


2. Quantifying lipid sorting in **complex quasi-native** membranes

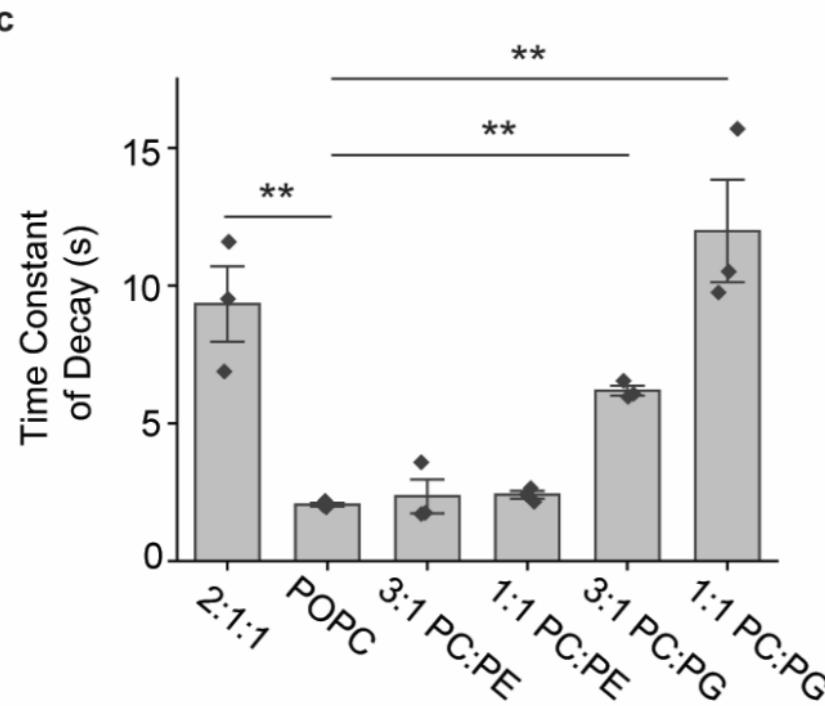
- coarse-grained resolution
- new method: density threshold affinity
- nAChR + neuronal membranes



motivation: pLGICs are very picky about lipids!



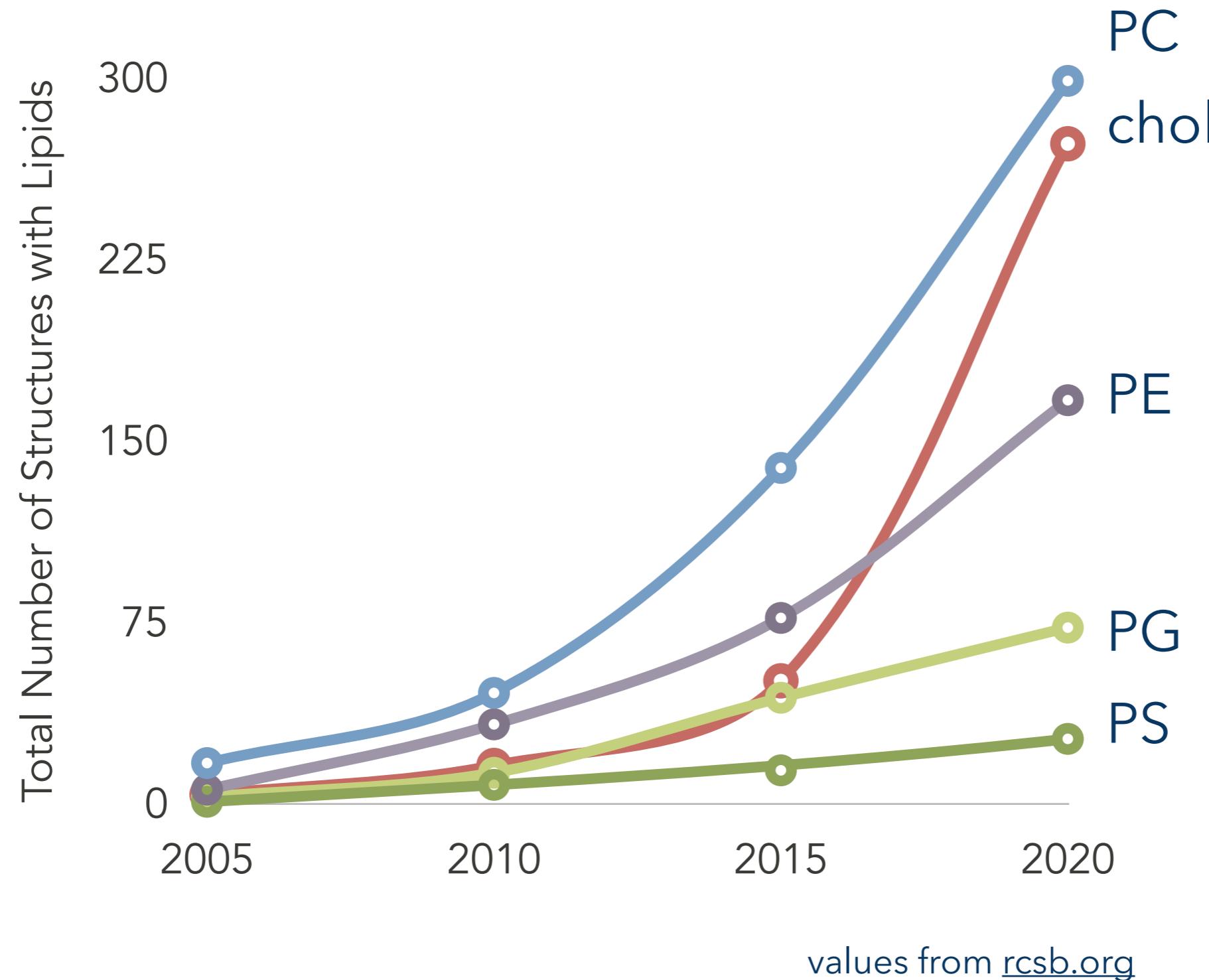
Criado...Barrantes, 1984, J.Biol.Chem.



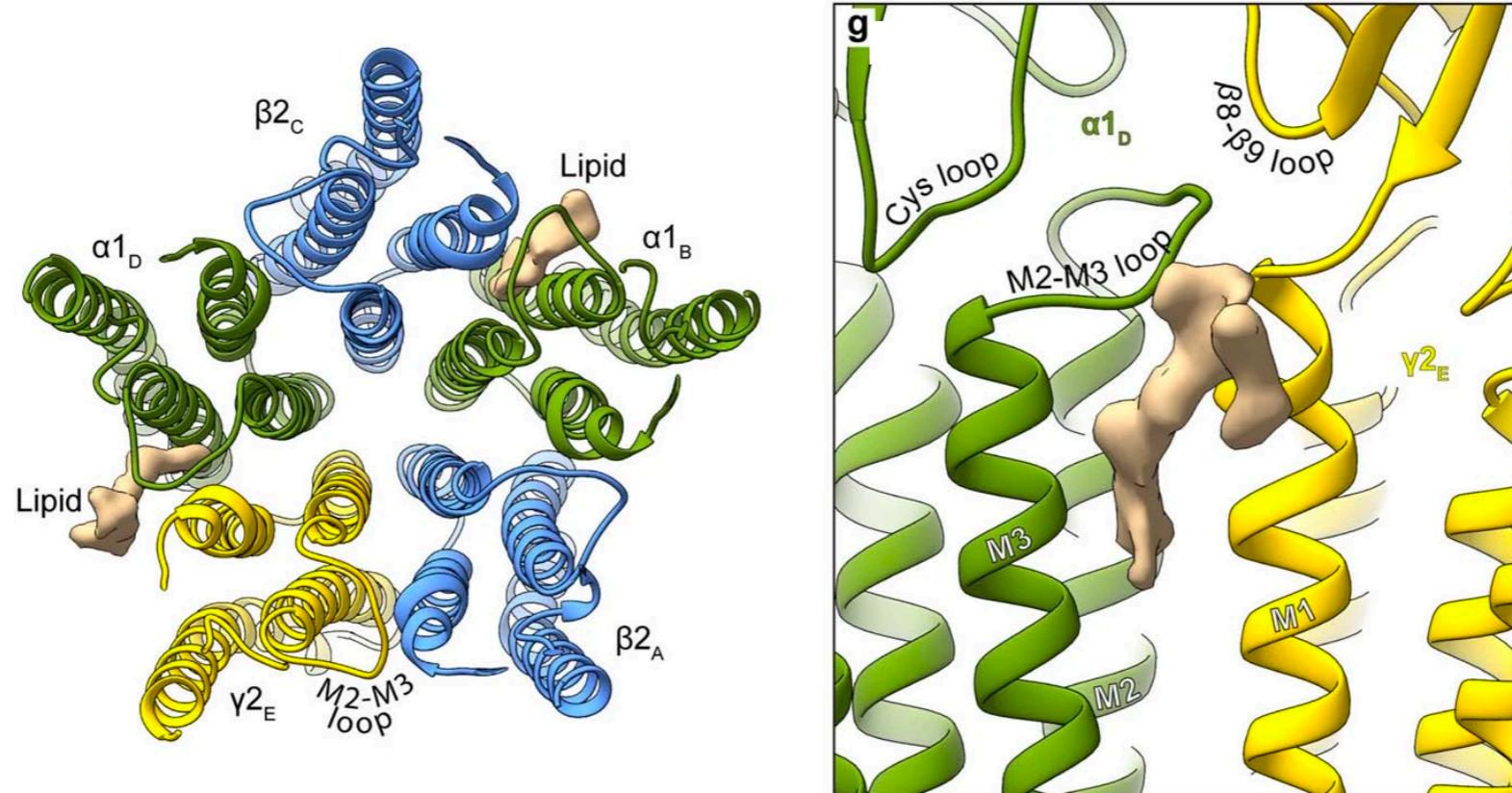
in preparation , collab with Wayland Cheng



Structures can tell us where lipids bind...



...but not who is binding.

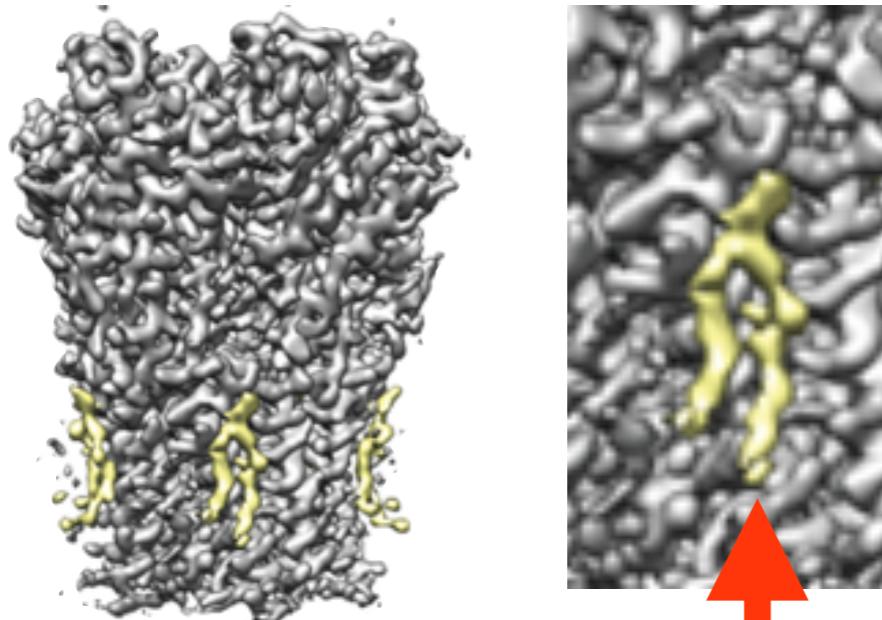
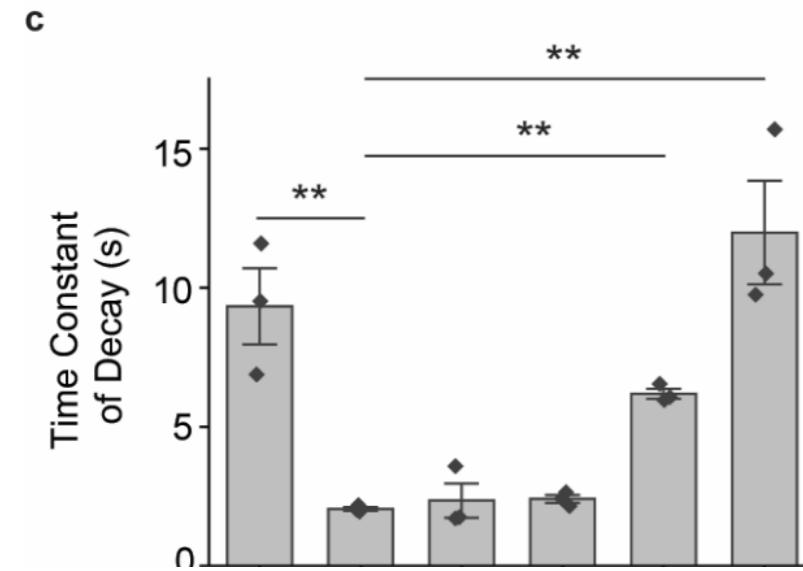


Kim...Hibbs, Nature, 2020

What about Mass Spec? well-- tells us **who** but not *where*.

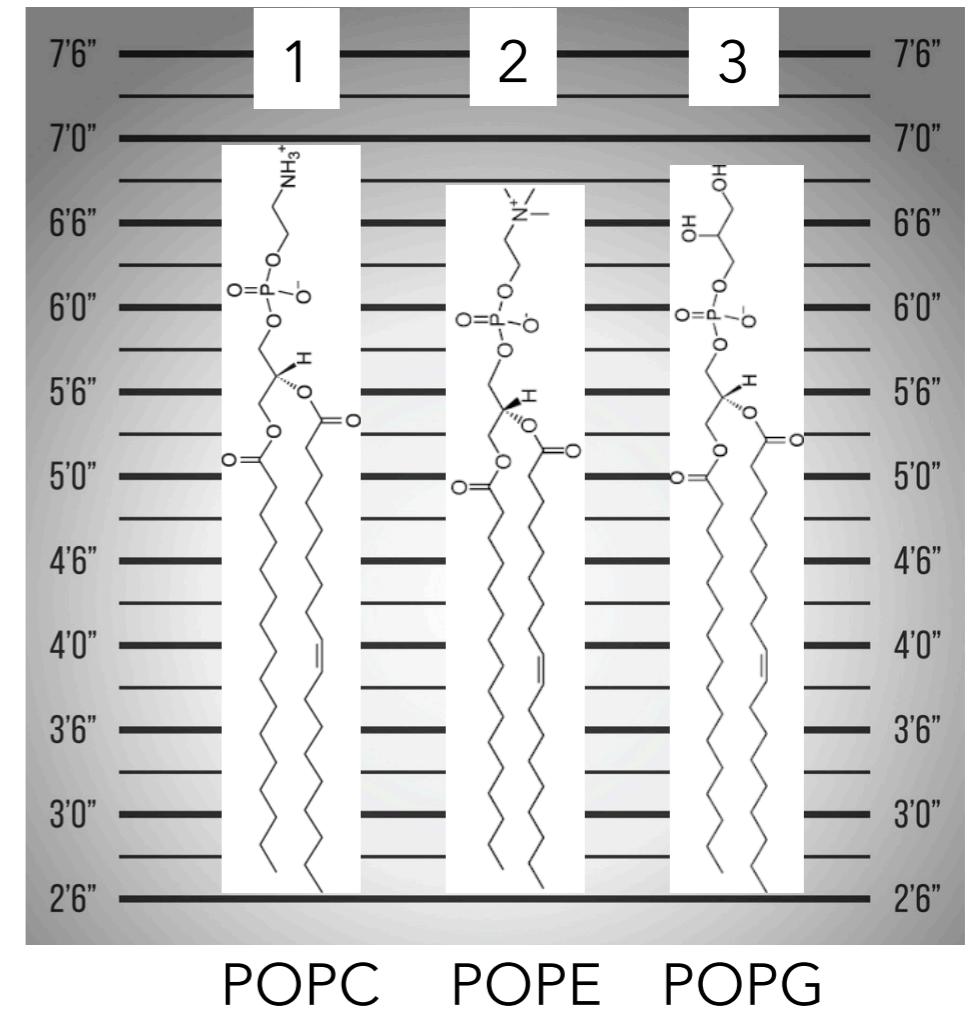
What about Coarse-grained MD? **who** and **where** but not *when*.

Case of the ELIC modulation site



in preparation , collab with Wayland Cheng

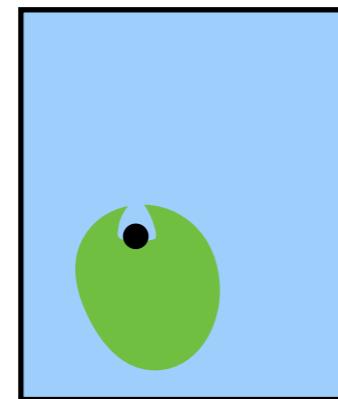
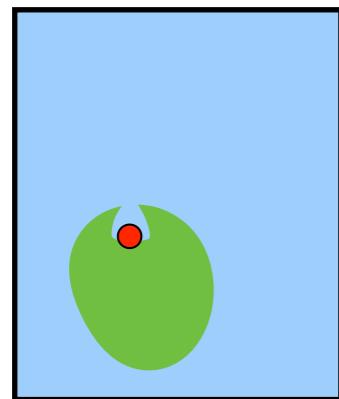
Suspects



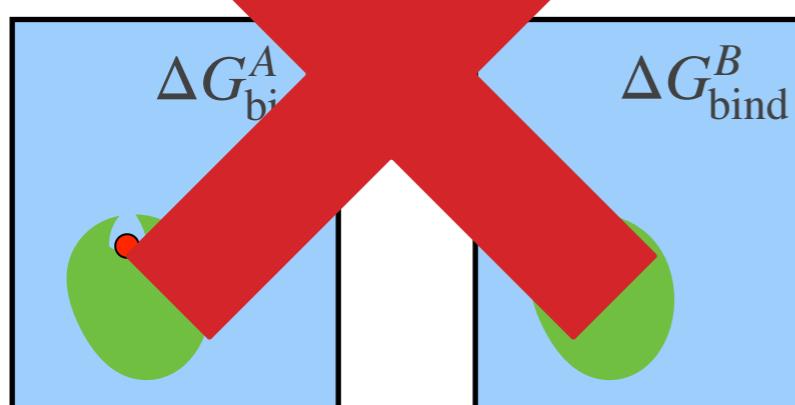
Plan: adapt a classic method for a new purpose

classic method: alchemical Free Energy Perturbation (FEP)

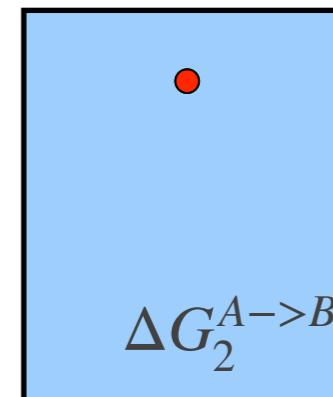
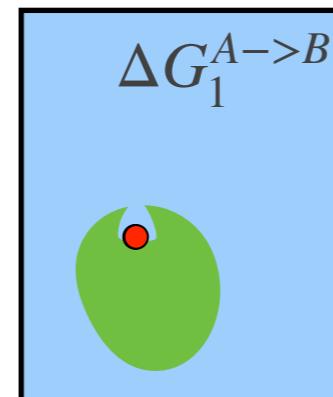
Drug-design question: what is the affinity difference between two ligands?



straightforward,
slow approach



alchemical FEP

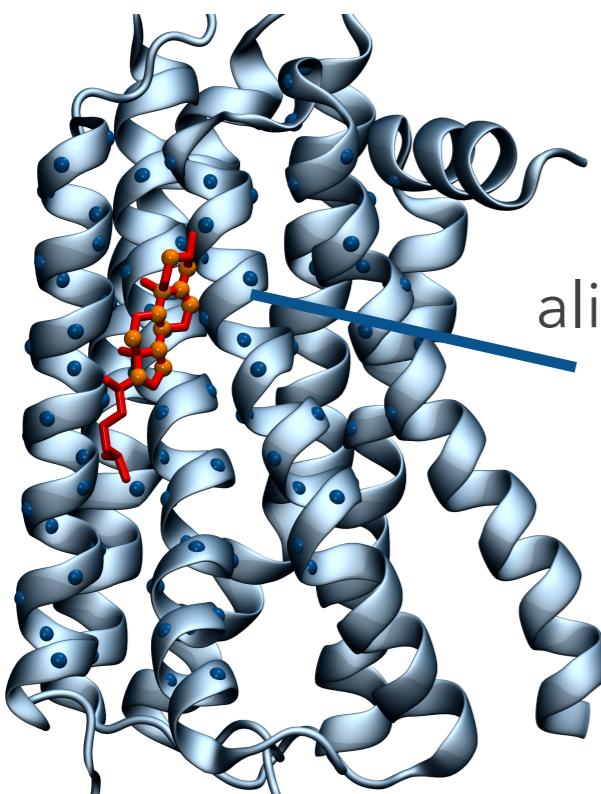


$$\Delta G_{\text{bind}}^A - \Delta G_{\text{bind}}^B = \Delta G_1^{A \rightarrow B} - \Delta G_2^{A \rightarrow B} < 0$$

Introducing: the DBC coordinate

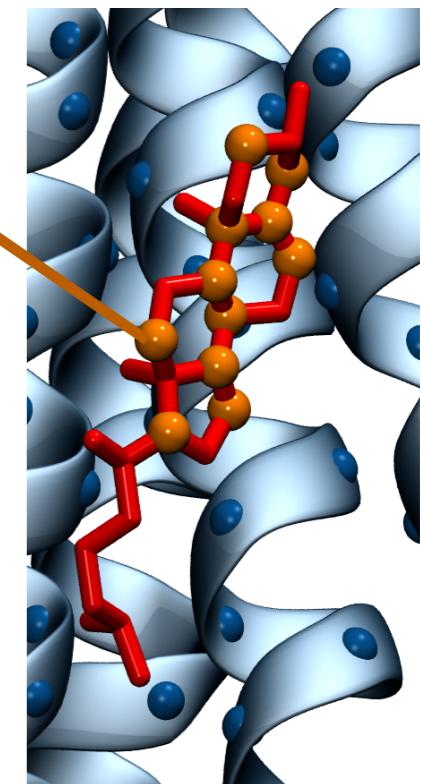
- distance-from-bound-configuration (DBC) coordinate: ligand RMSD in the site's frame of reference

- requires on-the-fly alignment in simulation software



...**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!

Adapting FEP to membranes: SAFEP

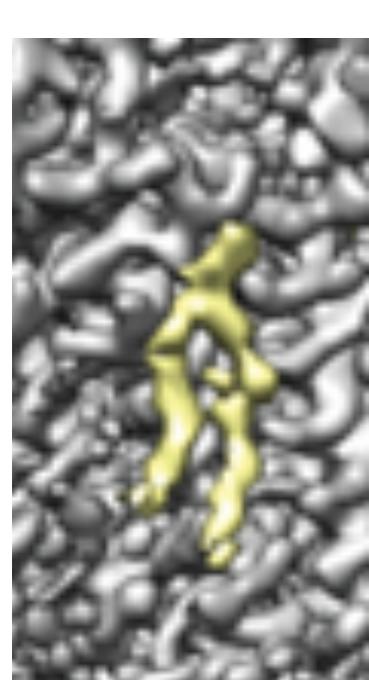
Streamlined **A**lchemical **F**ree **E**nergy **P**erturbation

FEP but in a site-centered reference frame.

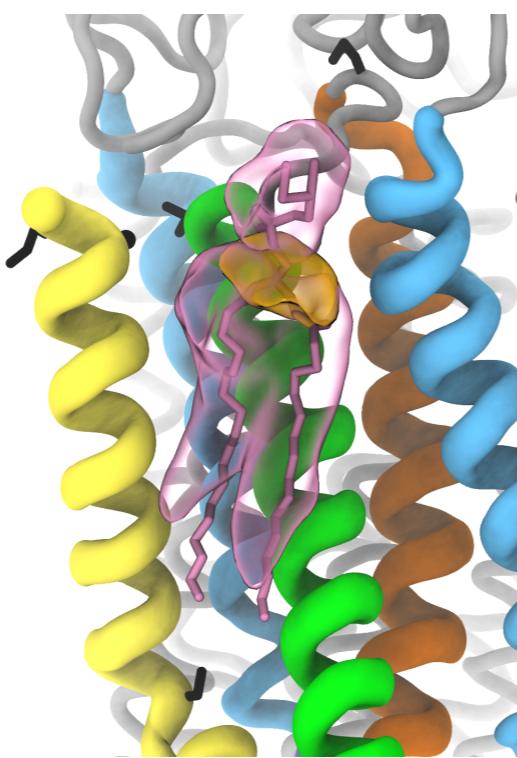
Switch to site-centered reference frame has to occur at every level, from implementation to interpretation - but it pays off.

- Implementation: new collective variable
- Interpretation: generalizeable theory

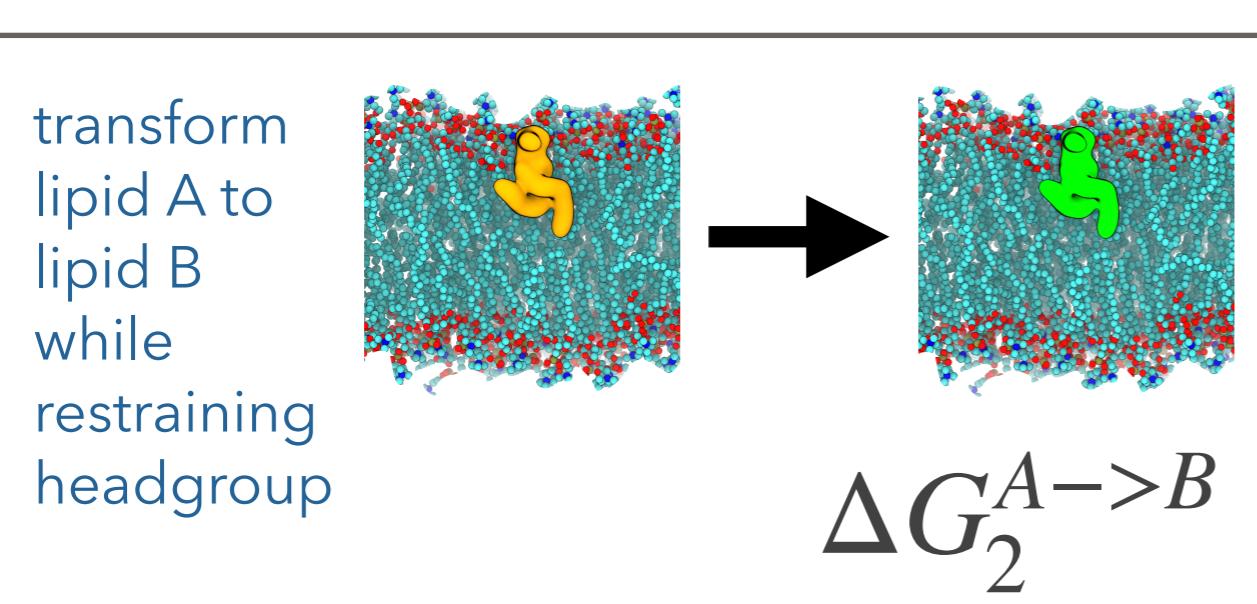
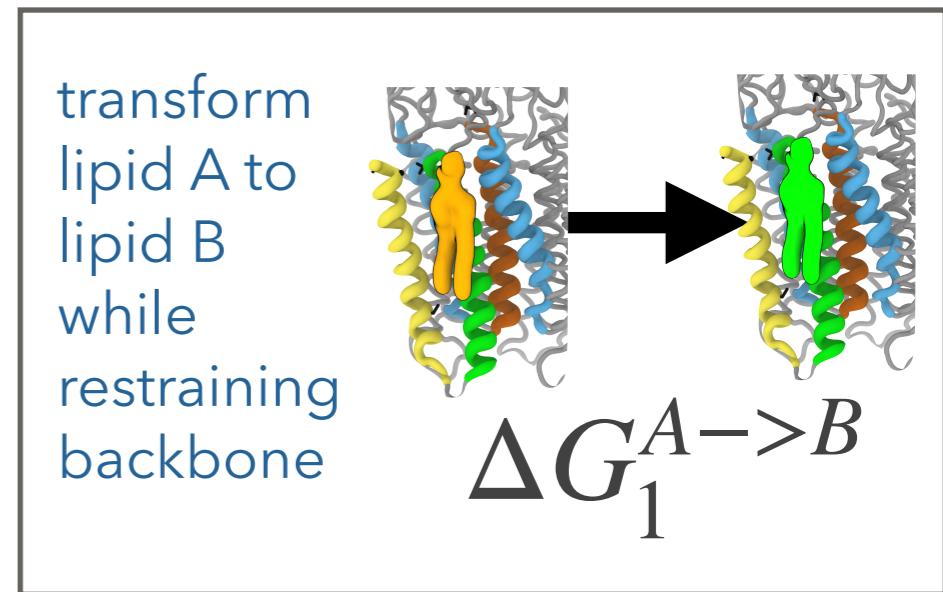
Using SAFEP to catch lipids



Model each lipid and then simulate with basic MD

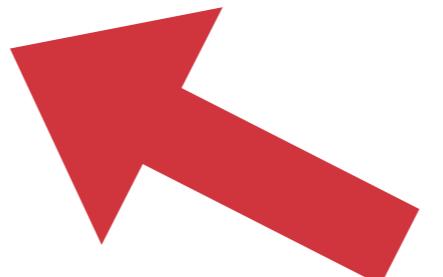
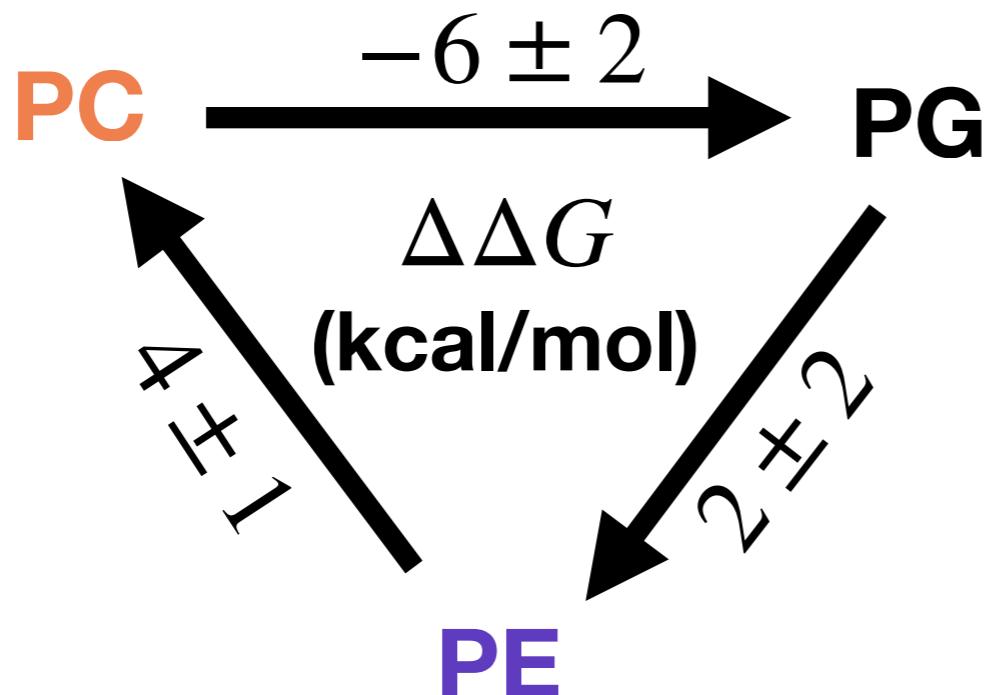


Determine allowable deviation of the glycerol backbone

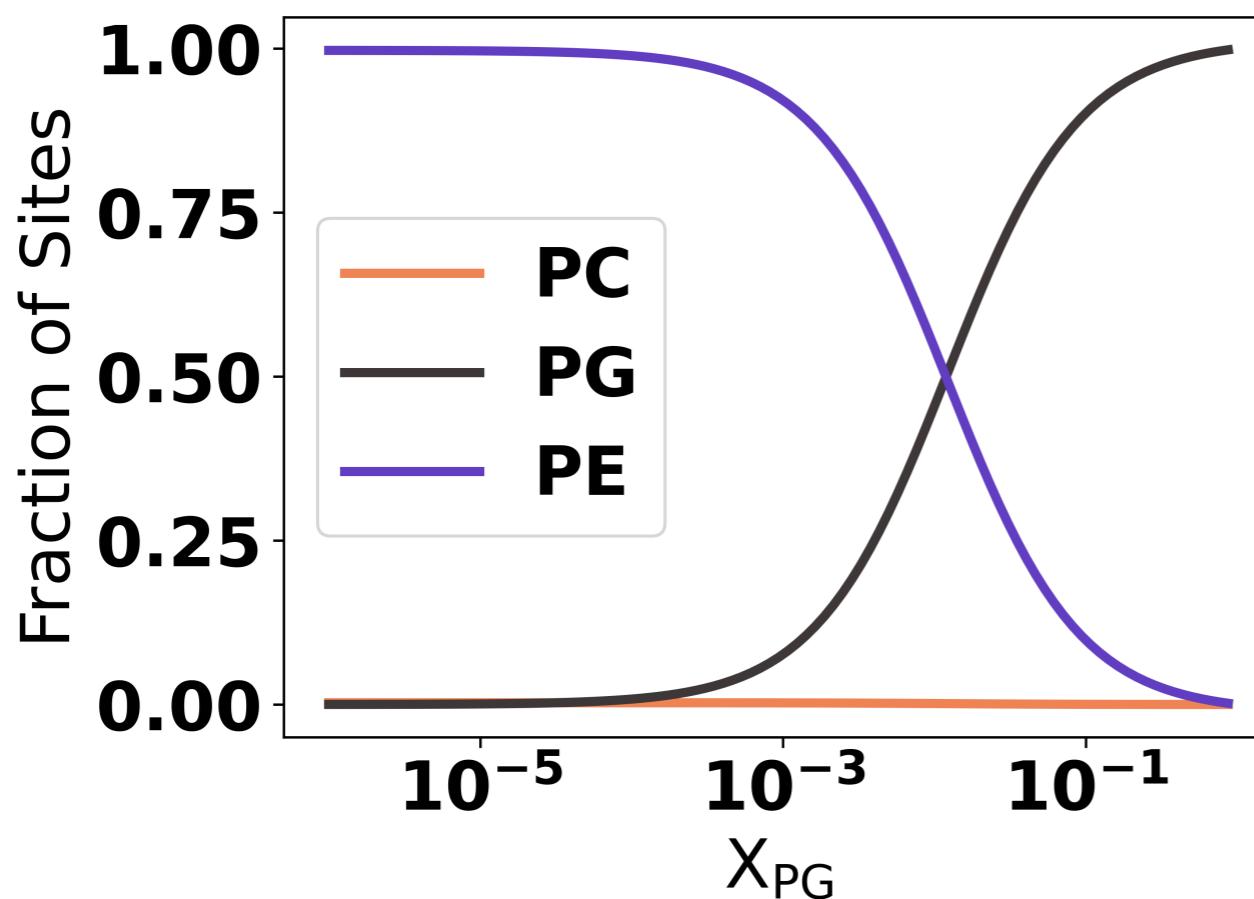


If $\Delta G_1^{A \rightarrow B} - \Delta G_2^{A \rightarrow B} < 0$,
then lipid B is more likely!

SAFEP applied to our ELIC suspects



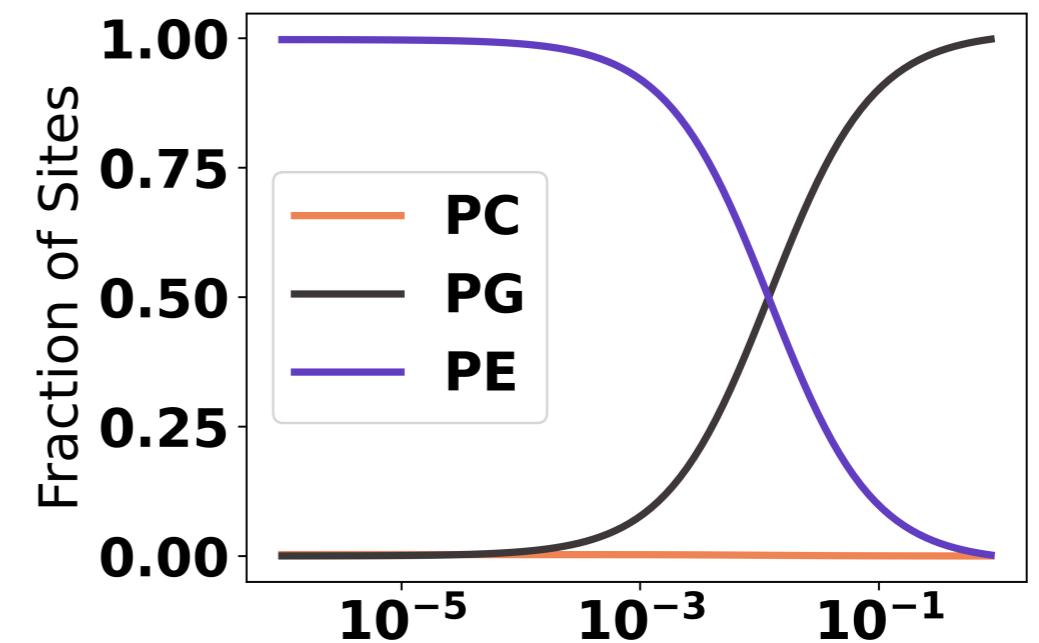
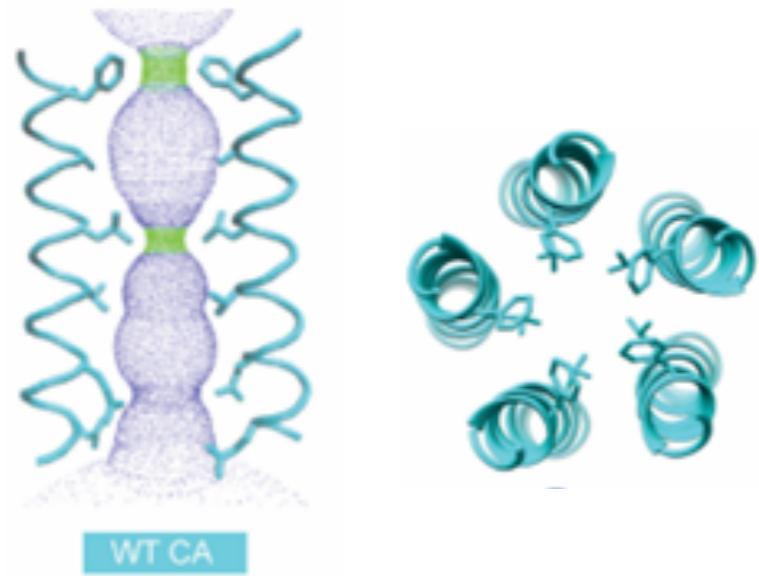
At the
crime
scene!



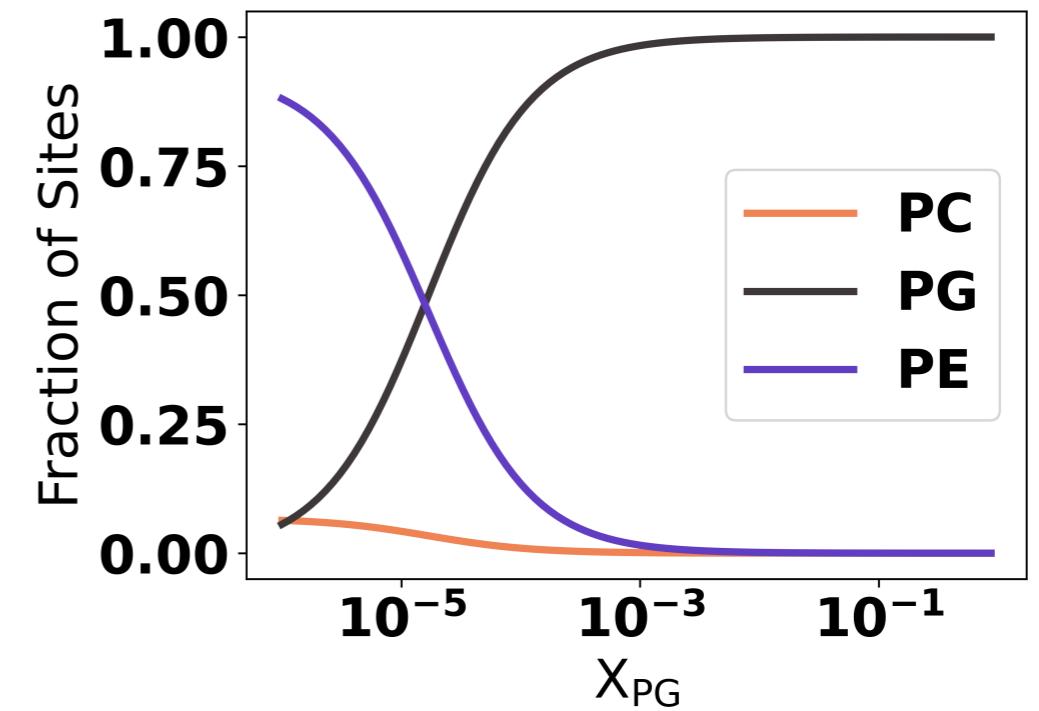
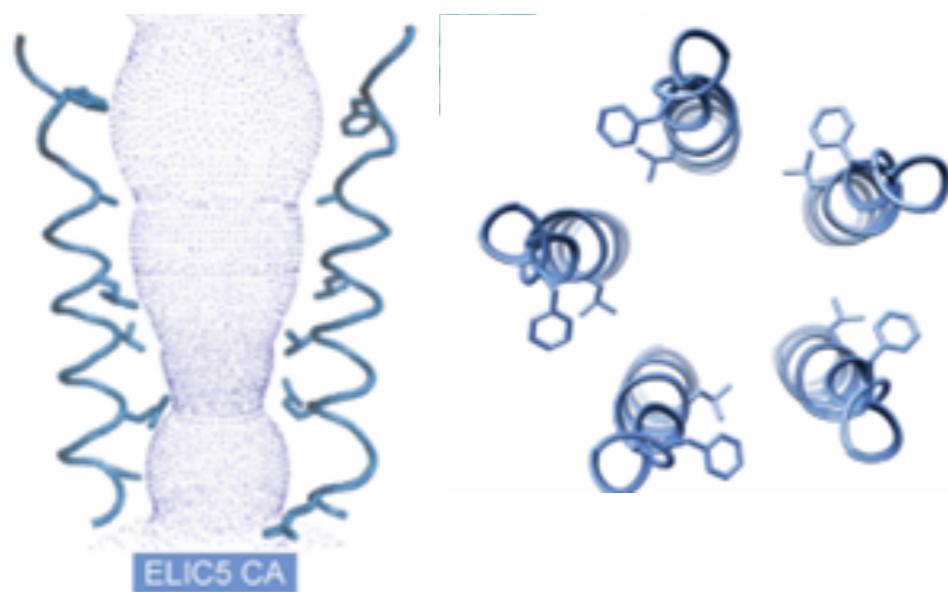
PG was in the outer
TMD binding
site....but did it do
anything?

next, check for state dependence

pre-active conformation

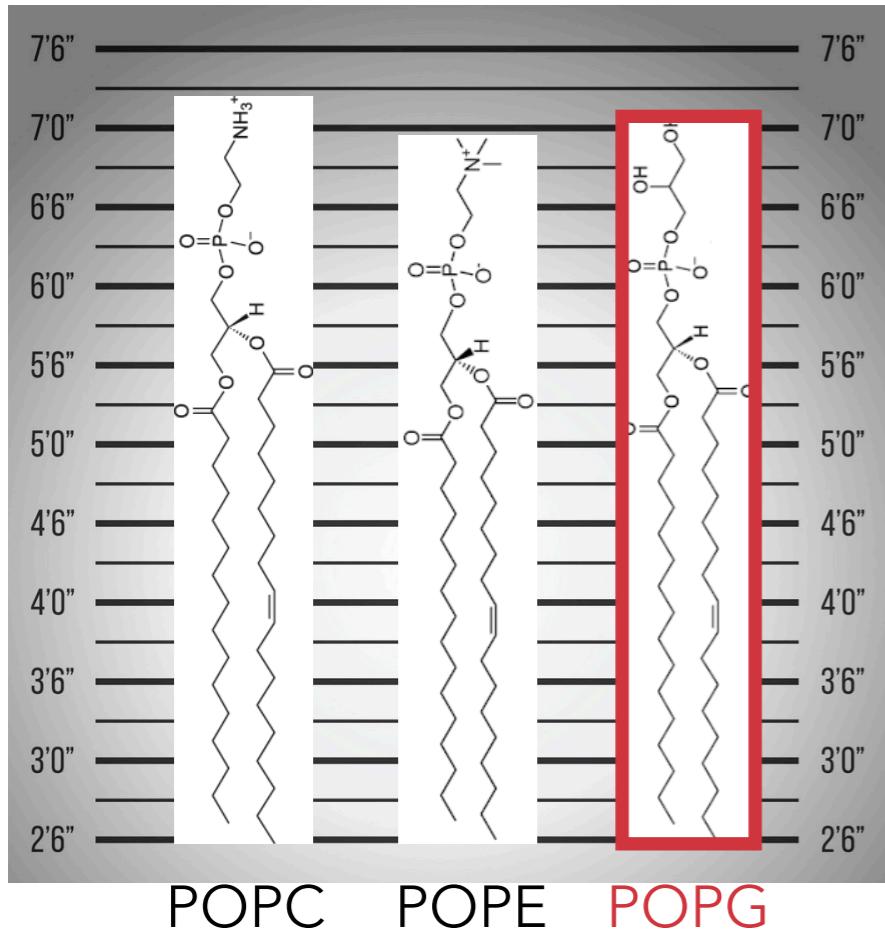


wide-open conformation

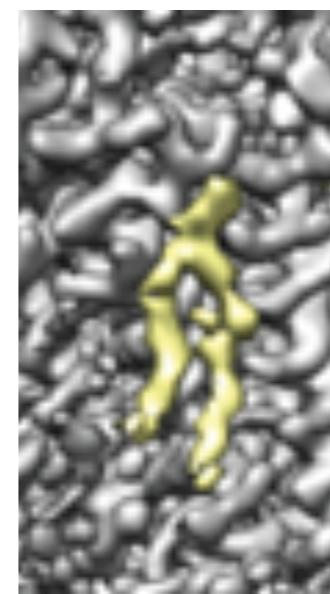


Summary

Who



Where



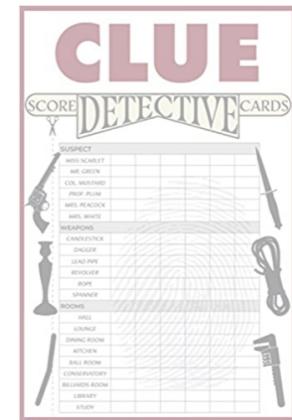
When

When you have
at least 1-5% PG

Two Stories

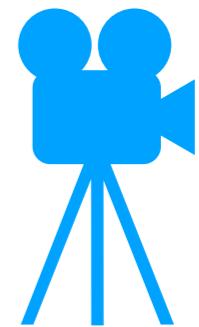
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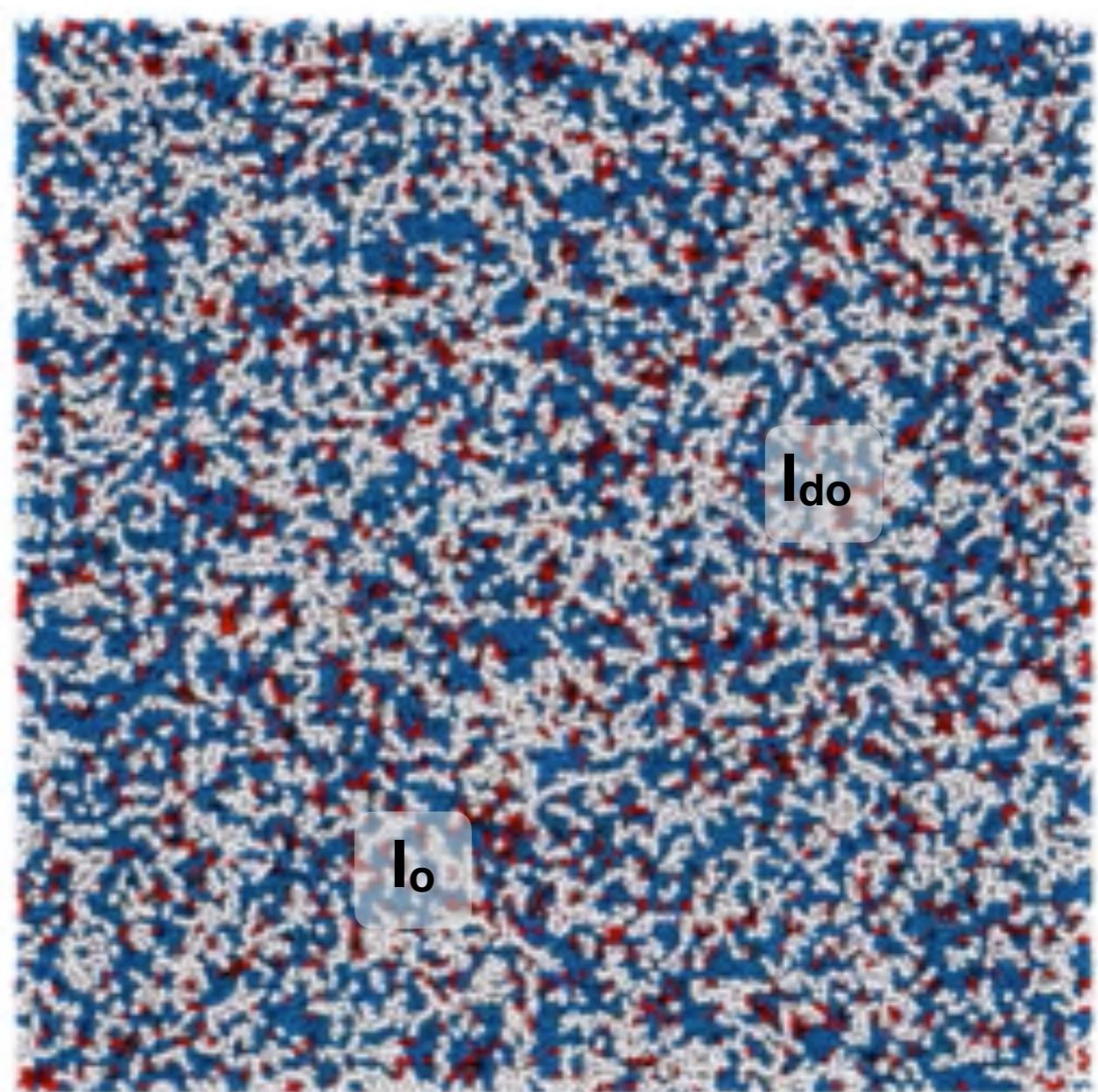
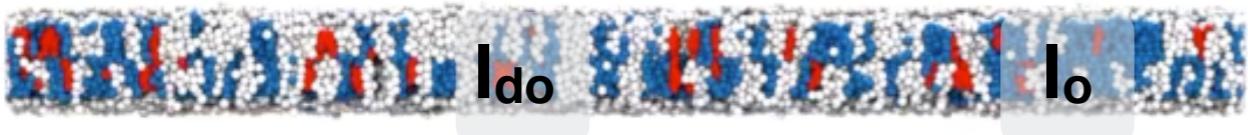
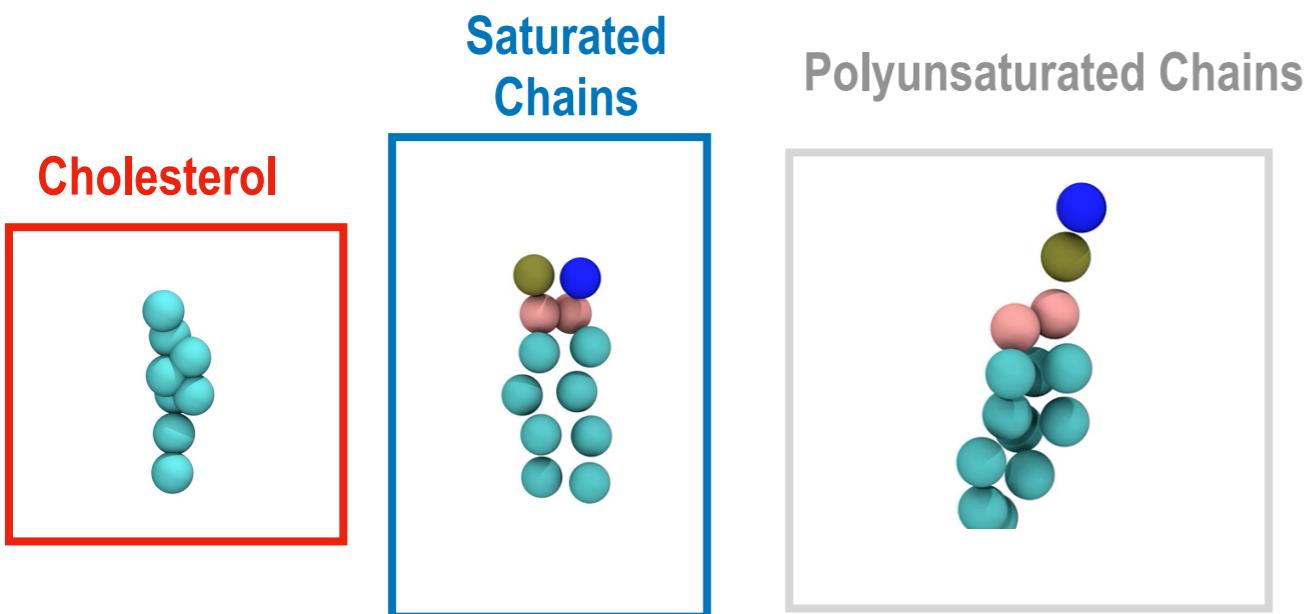
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- nAChR + neuronal membranes



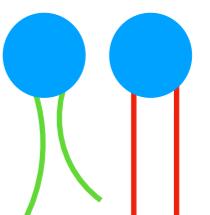
Computational Microscopy

via coarse-grained molecular dynamics simulation (MARTINI)

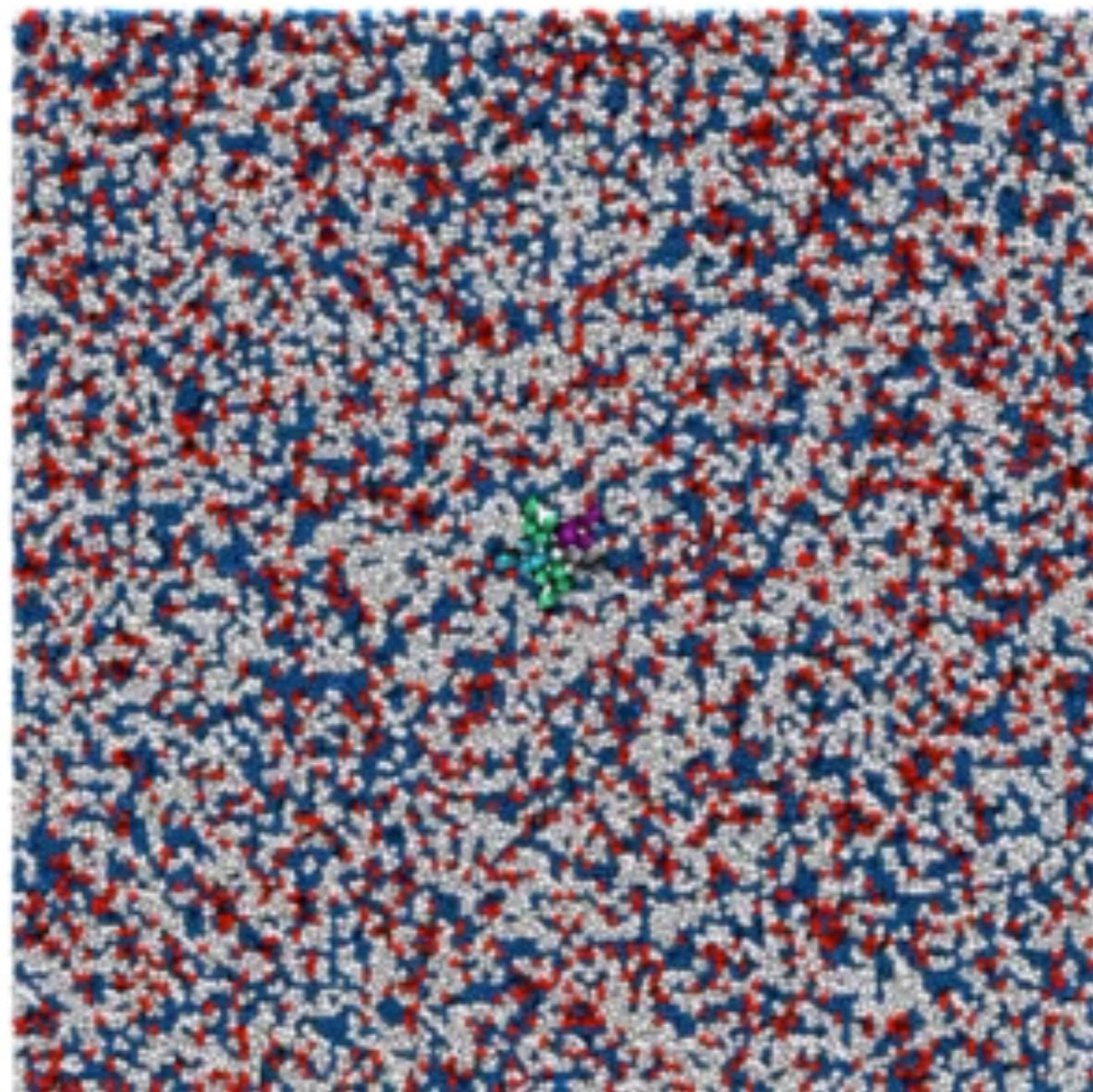


$\sim 2 \text{ us}$ $75 \times 75 \text{ nm}^2$

Homo-Acidic
Domain Forming

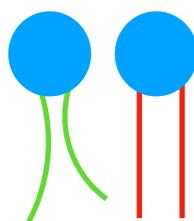


surprise: nAChR partitions to a cholesterol-poor domain

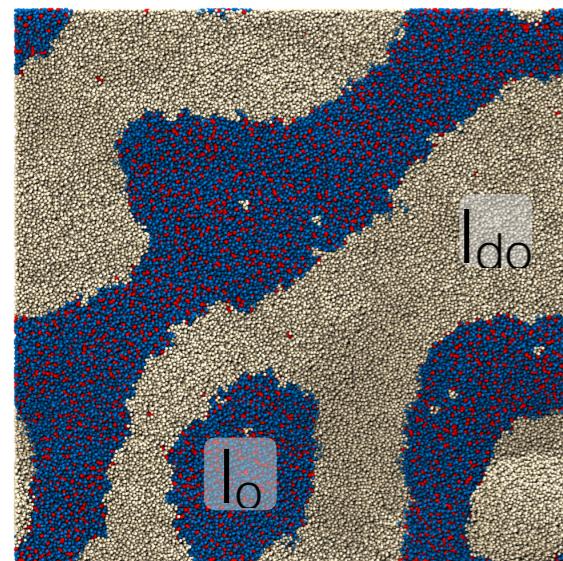


simulations in a quasi-native membrane

Homo-Acidic
Domain Forming



Model Membrane



25 Å

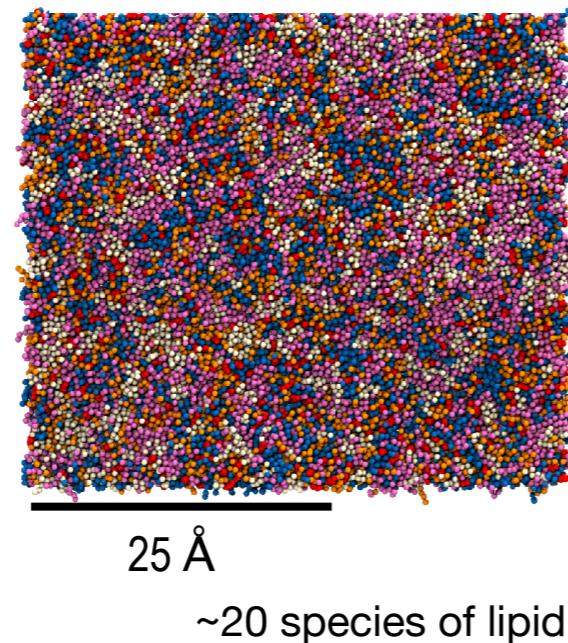
Sharp, Salari, Brannigan 2019

Woods, Sharp, Brannigan 2019

Hetero-Acidic
Non-Domain Forming



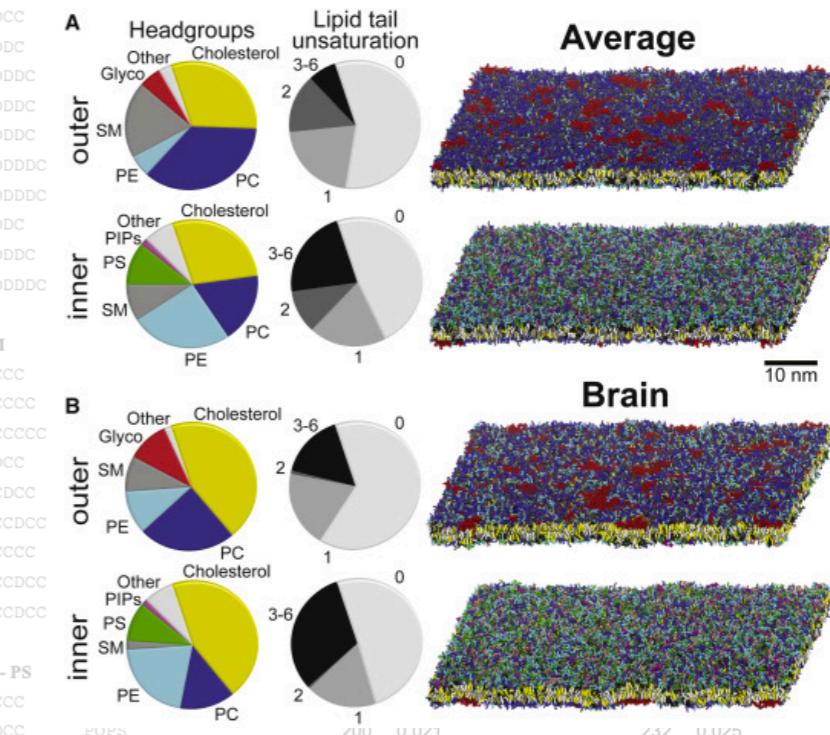
Native Membrane



Sharp, Brannigan 2021

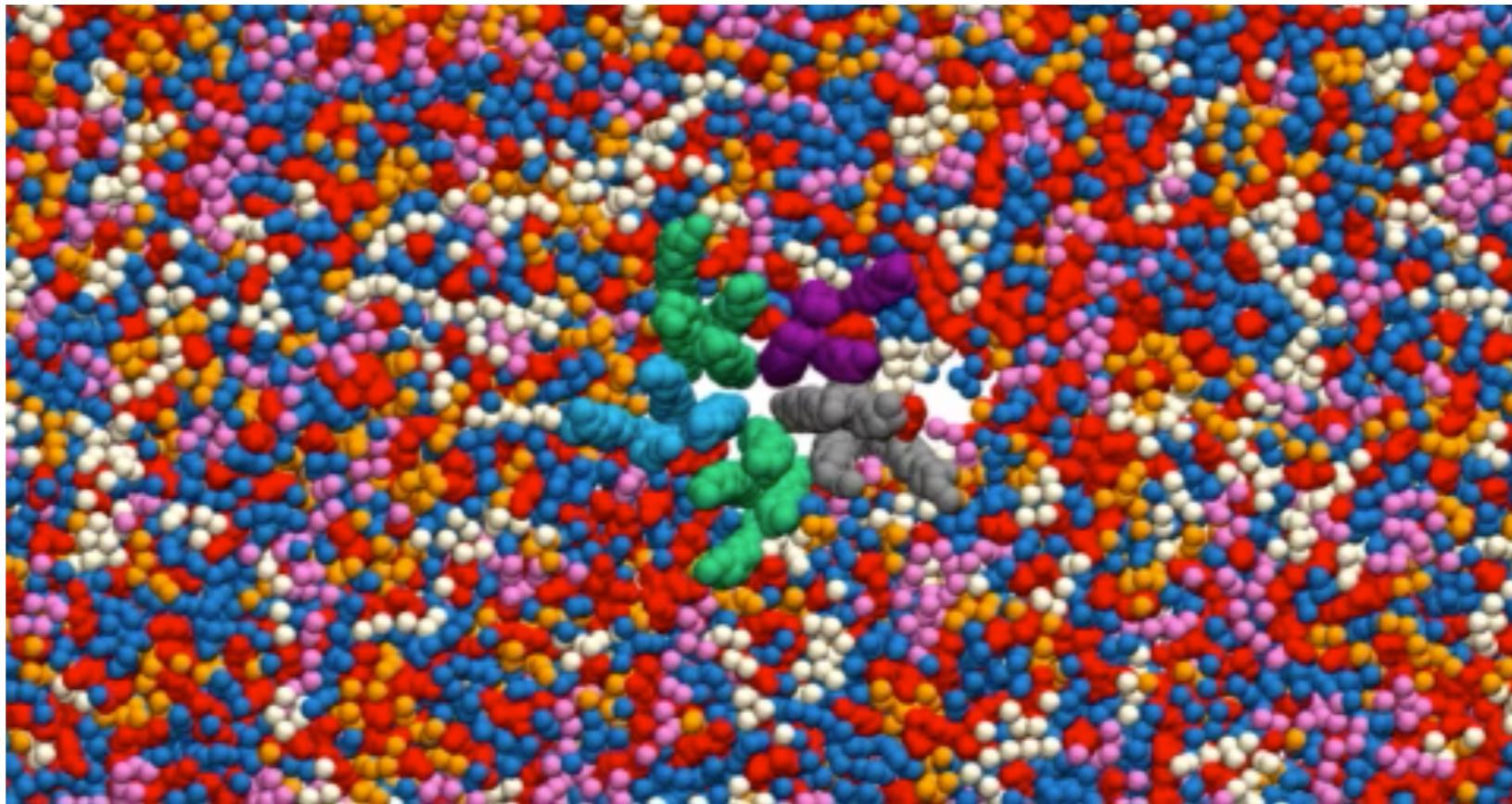
Table S1 Lipid composition^a

Lipid tail sn-1	Lipid tail sn-2	Acronym	Average	Brain						
			Outer leaflet count	Outer leaflet mole fraction	Inner leaflet count	Inner leaflet mole fraction	Outer leaflet count	Outer leaflet mole fraction	Inner leaflet count	Inner leaflet mole fraction
Phosphatidylcholine - PC			outer/inner ratio	0.69	0.31				0.65	0.35
CCCC	CCCC	DPPC								
CCCC	CDCC	POPC	1205	0.122	550	0.059	868	0.087	463	0.049
CDCC	CDCC	DOPC	106	0.011	49	0.005	221	0.022	118	0.012
CCCC	CDDC	PIP C	1772	0.179	810	0.087				
CCCC	CDDD	PFPC								
CCCC	CDDCC	PEPC	71	0.007	32	0.003				
CCCC	DDDDC	PAPC	283	0.029	129	0.014	463	0.046	247	0.026
DDDDC	DDDDC	DAPC	35	0.004	16	0.002				
CCCC	DDDDDC	PUPC	71	0.007	32	0.003	169	0.017	90	0.010
CDCC	CDDC	OIPC					59	0.006	32	0.003
CDCC	DDDDDC	OUPC					42	0.004	22	0.002
		Total:	3543	0.357	1618	0.173	2412	0.242	1288	0.136
Phosphatidylethanolamine - PE			outer/inner ratio	0.19	0.81				0.35	0.65
CCCC	CCDC	POPE	135	0.014	569	0.061	127	0.013	234	0.025
CDCC	CDCC									
CCCC	CDDC									
CCCC	CDDDC									
CCCC	DDDDC									
CCCC	DDDDDC									
CCCC	DDDDDC									
CDCC	CDDC									
CDCC	DDDDC									
CDCC	DDDDDC									
A	Headgroups	Cholesterol	Lipid tail unsaturation							
outer	Glyco		3-6	2	1	0				
outer	SM		2	1	0					
outer	PE		1	0						
inner	Other	Cholesterol	3-6	2	1	0				
inner	PIP s		2	1	0					
inner	PS		1	0						
inner	SM		0							
inner	PE		1	0						
B	Other	Cholesterol	Lipid tail unsaturation							
outer	Glyco		3-6	2	1	0				
outer	SM		2	1	0					
outer	PE		1	0						
inner	Other	Cholesterol	3-6	2	1	0				
inner	PIP s		2	1	0					
inner	PS		1	0						
inner	SM		0							
inner	PE		1	0						
Phosphatidylserine - PS										
CCCC	CCCC									
CCCC	CDCC									

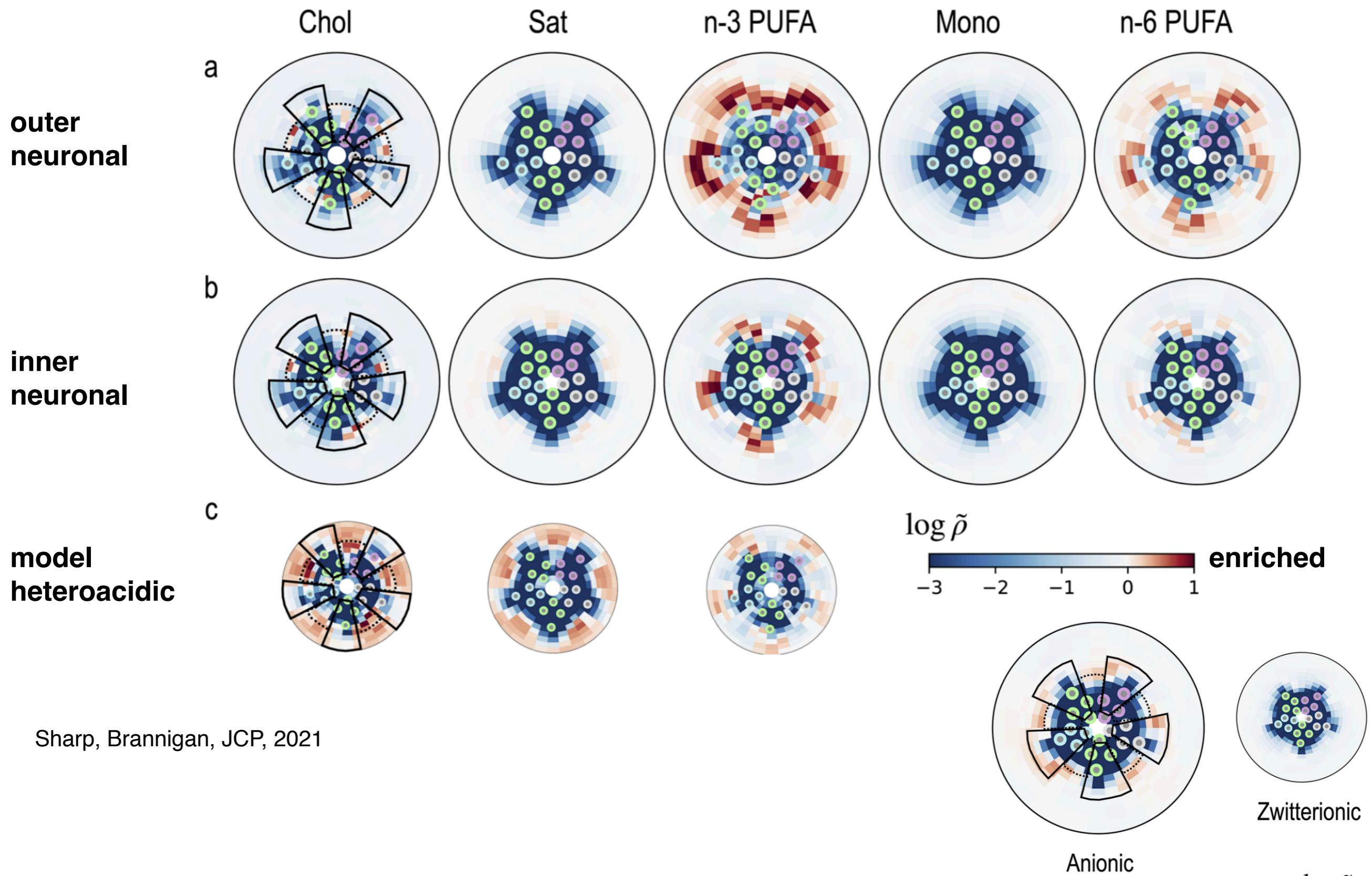


Helgi I Ingólfsson et al, 2017

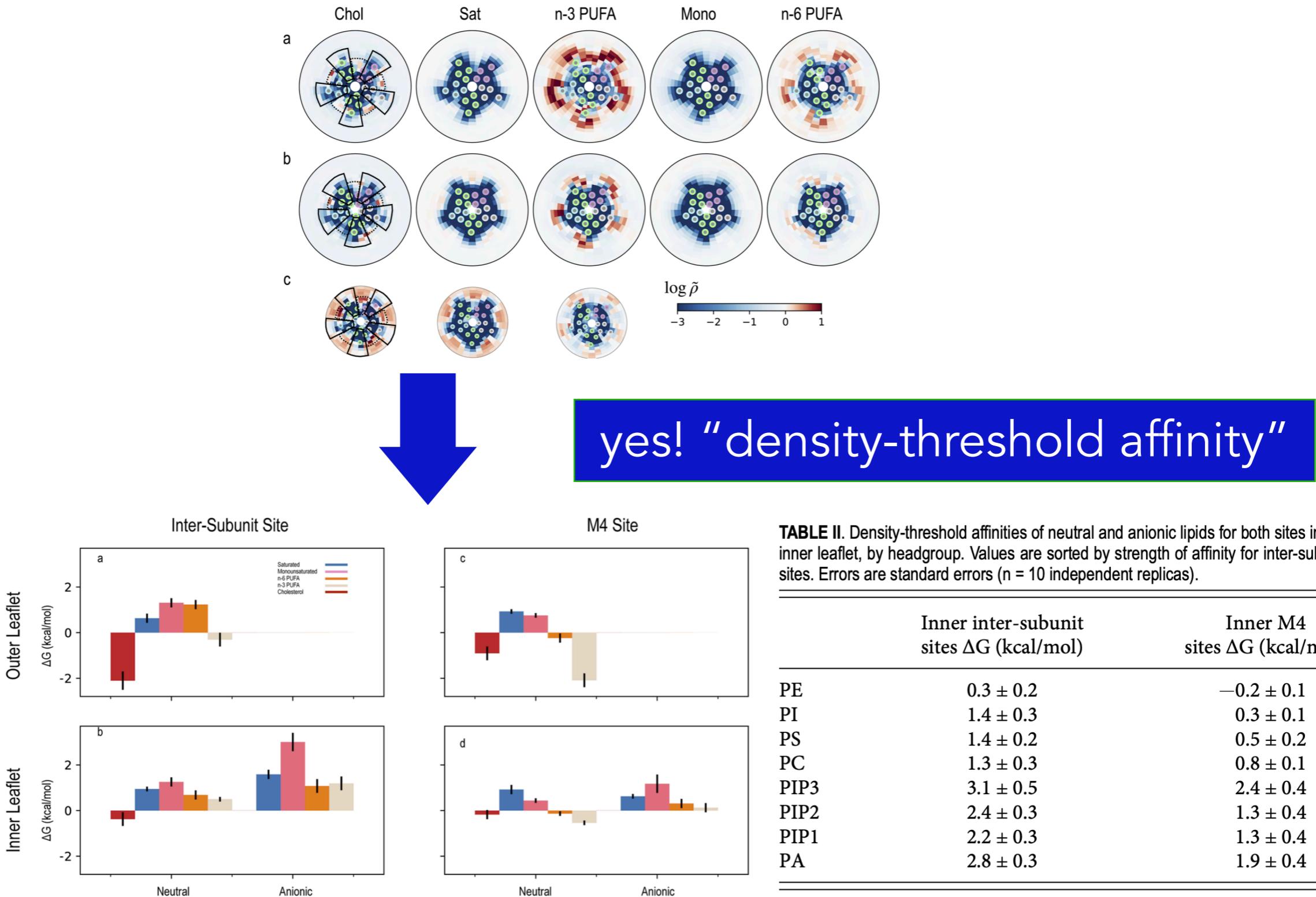
nAChR in a post-synaptic membrane



boundary lipids: post-synaptic membrane

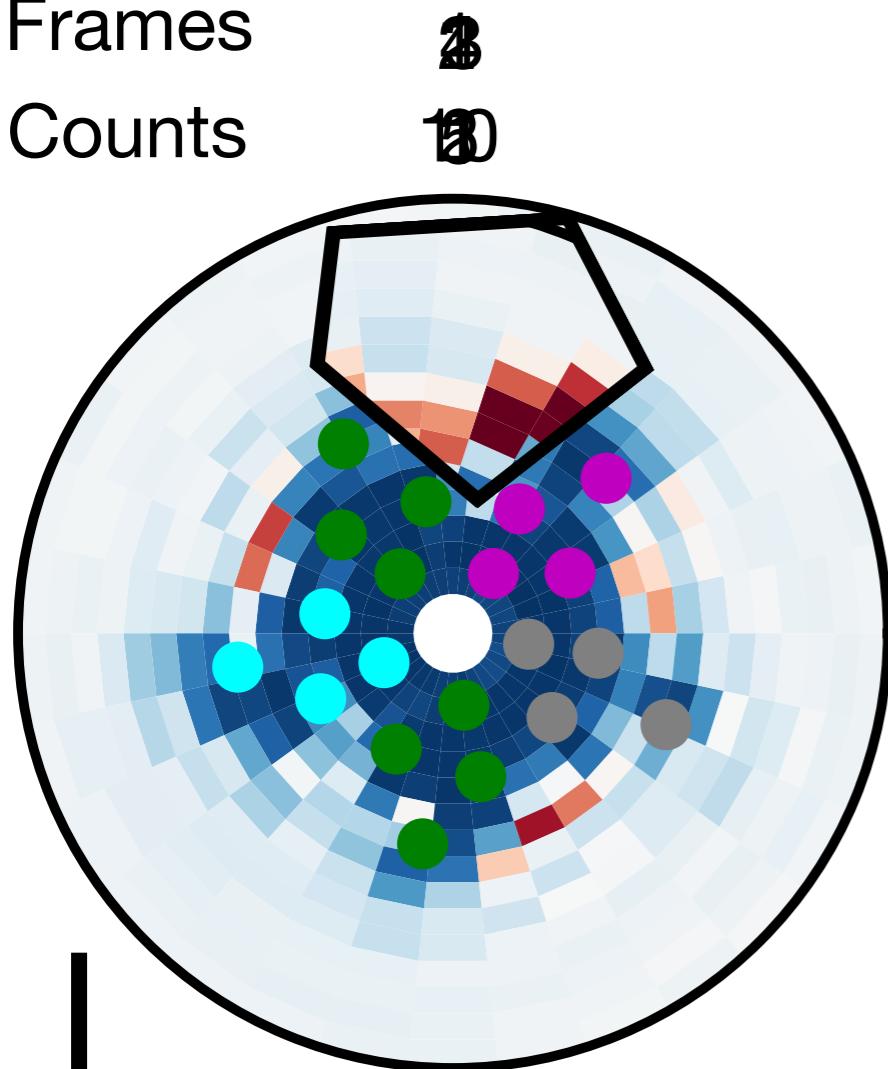


can we convert all these maps to numbers?

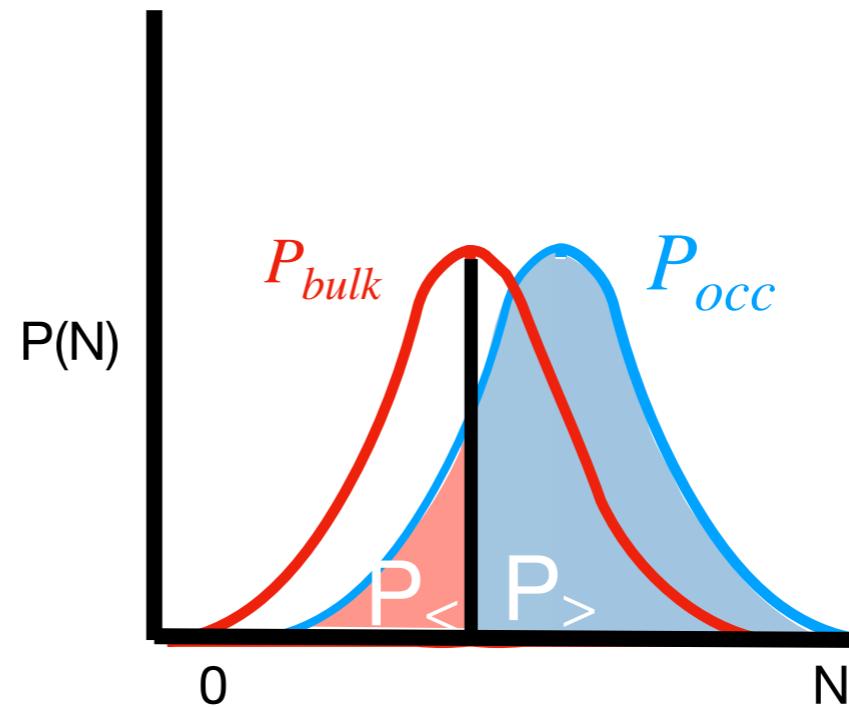


density threshold affinity

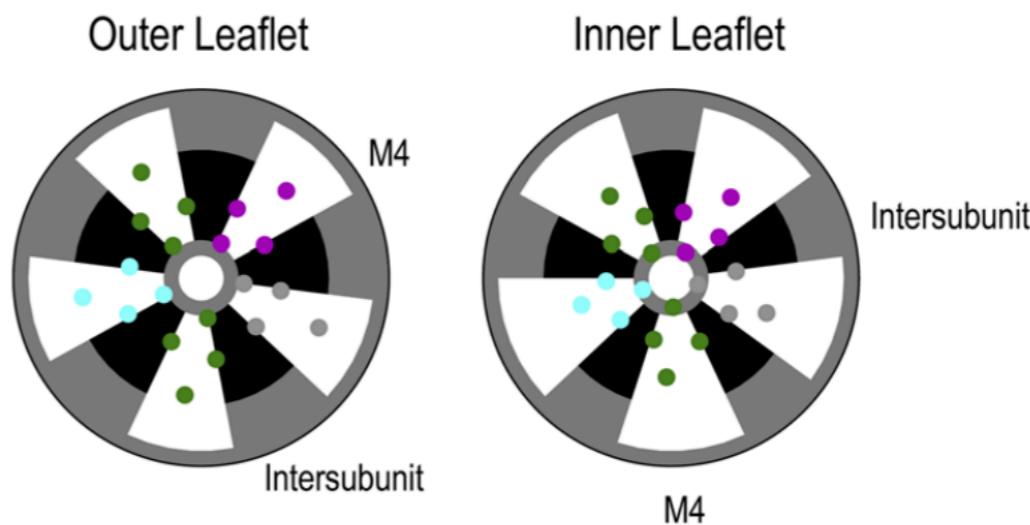
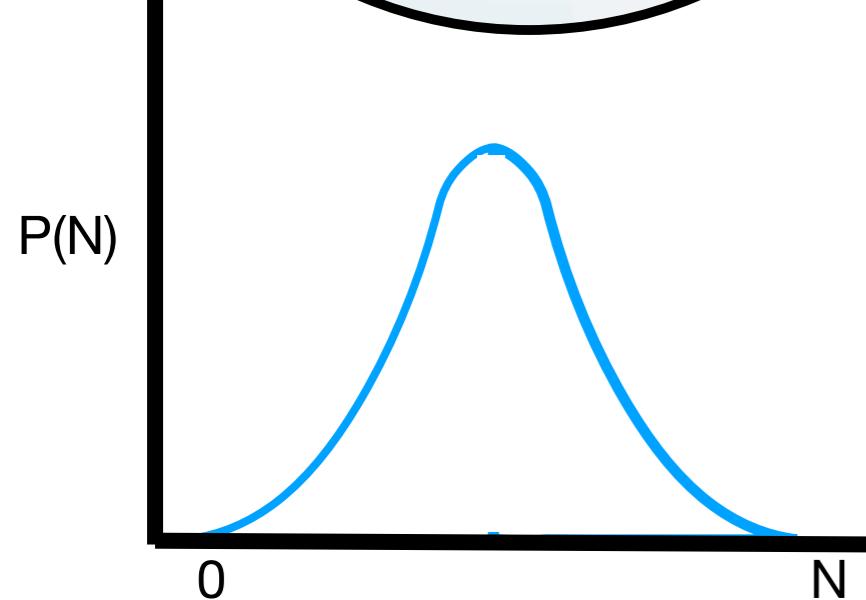
Frames
Counts



2
10

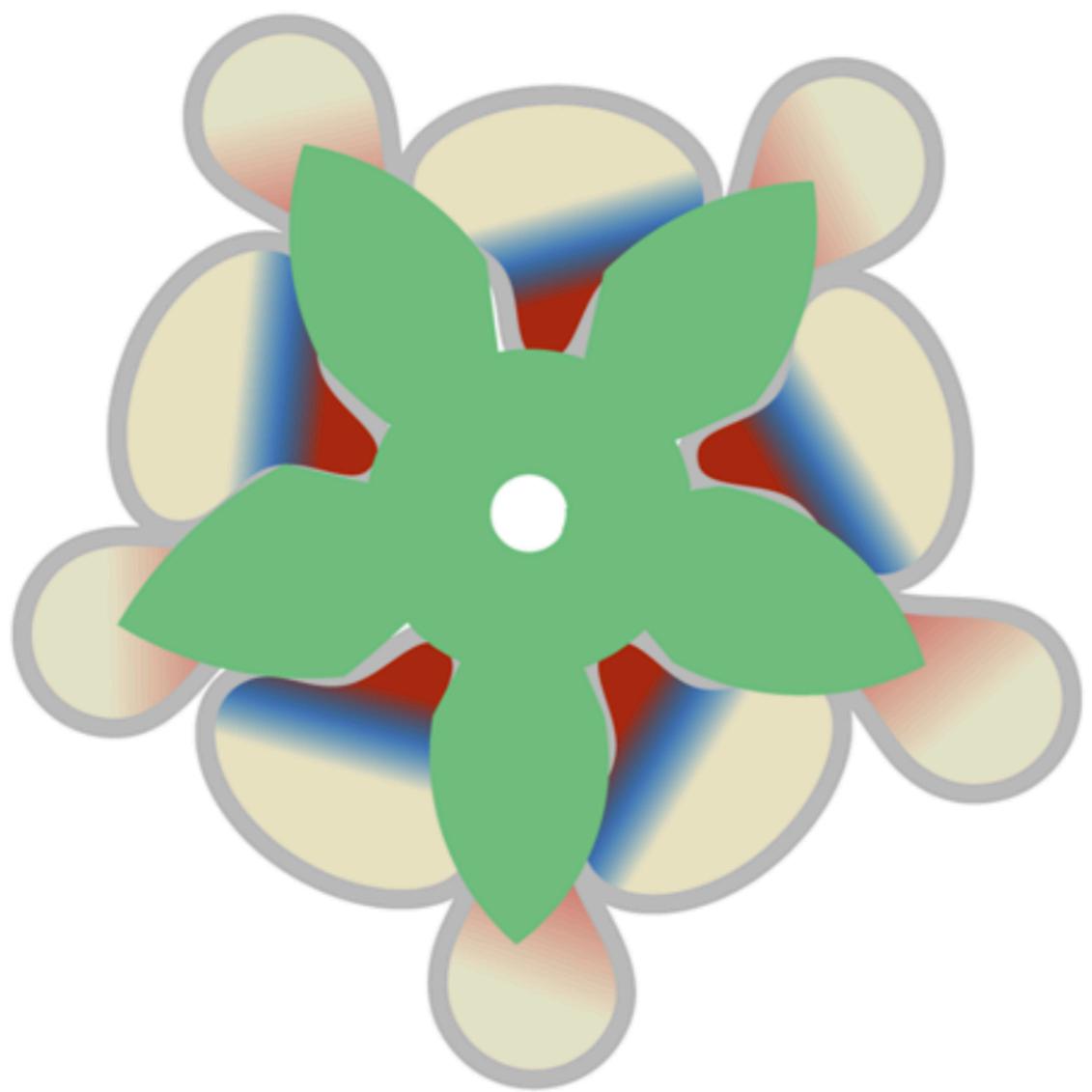


$$\Delta G = -RT \ln \frac{P_>}{P_<}$$

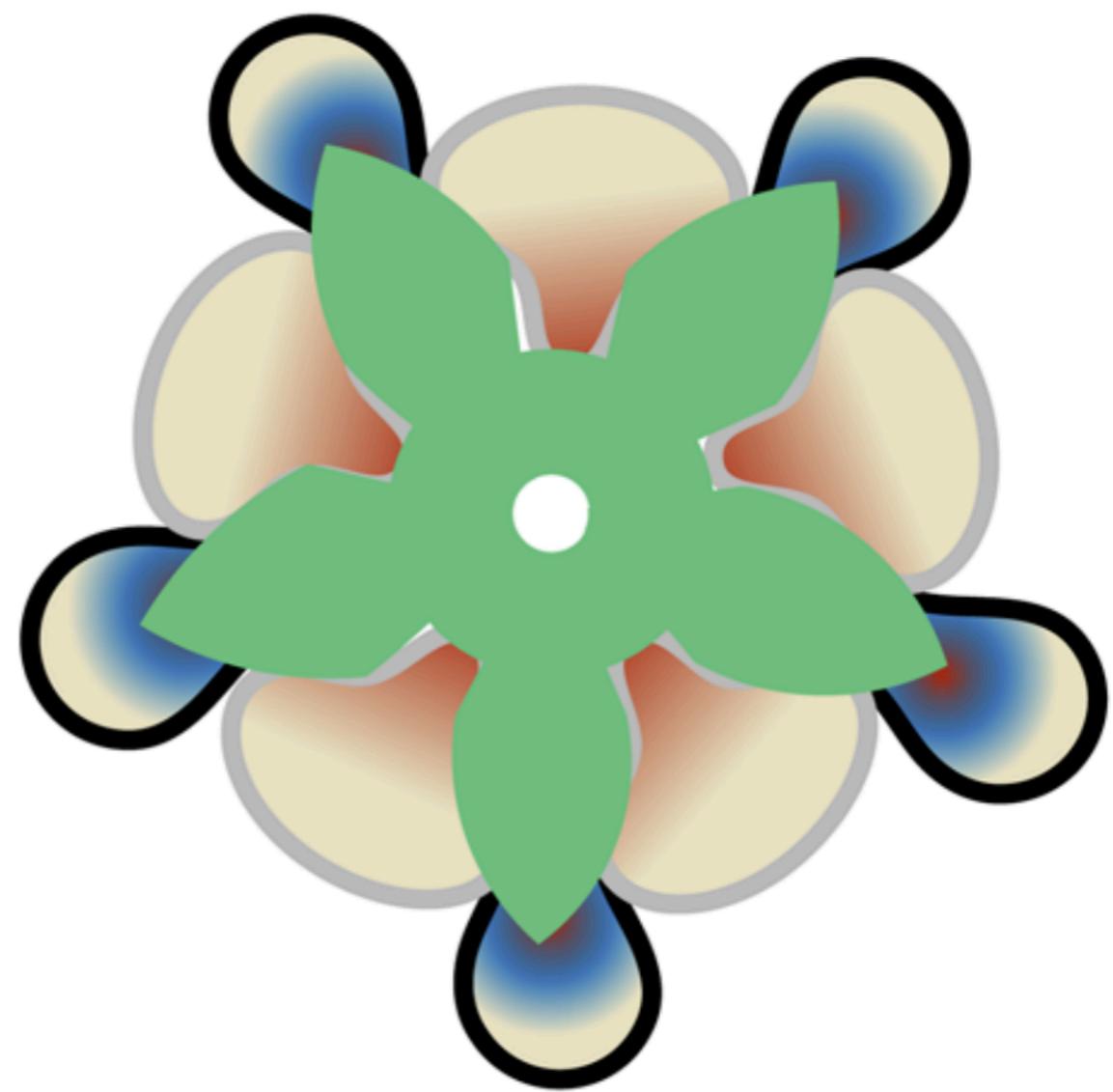


outcome: nAChR boundary lipids in a quasi-neuronal membrane

Outer Leaflet



Inner Leaflet



PUFA

Saturated

Cholesterol

Neutral

Anionic

Part II: Summary

- nAChRs bind cholesterol **but avoid** cholesterol-rich domains
 - We now know that domain formation in post-synaptic membrane is unlikely anyway
- Densities can yield affinities!
- Lipid selectivity in a quasi-native neuronal membrane:
 - Outer leaflet:
 - rigid groups (chol and saturated chains) occupy the concave regions
 - flexible chains (PUFAs) pack the convex regions
 - Inner leaflet:
 - saturated acyl chains shift from concave regions to convex regions (pack around M4)
 - Anionic lipids occupy M4 sites in the inner leaflet

Acknowledgments

SAFEP Team

Ezry St. Iago-McRae

Dr. Jérôme Hénin, (CNRS-IPBC France)

Dr. Thomas Joseph
(University of Pennsylvania)

Dr. Mark Arcario
(Washington University - St Louis)

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XSEDE

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

Dr. Jérôme Hénin, (CNRS-IPBC France)

Dr. Thomas Joseph (University of Pennsylvania)



nAChR lipid sorting

Dr. Liam Sharp (University of Delaware)

Tools now maintained and developed by **Jesse Sandberg**

