



RUTGERS

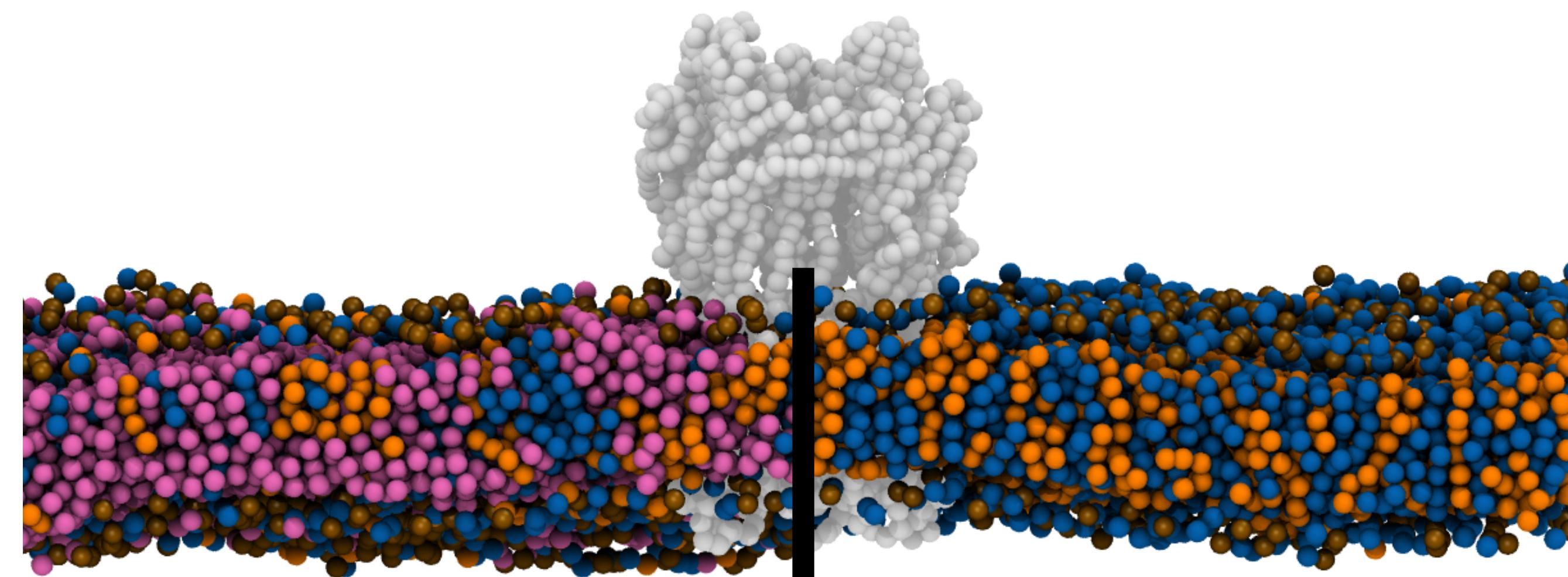
Camden College of
Arts and Sciences

RUTGERS

Center for Computational
and Integrative Biology

BOUNDARY LIPIDS OF PENTAMERIC LIGAND-GATED ION CHANNELS IN MODEL AND NATIVE MEMBRANES

Liam Sharp
Rutgers-Camden
Center for Computational and Integrative Biology
Mentored by Dr. Grace Brannigan



Outline

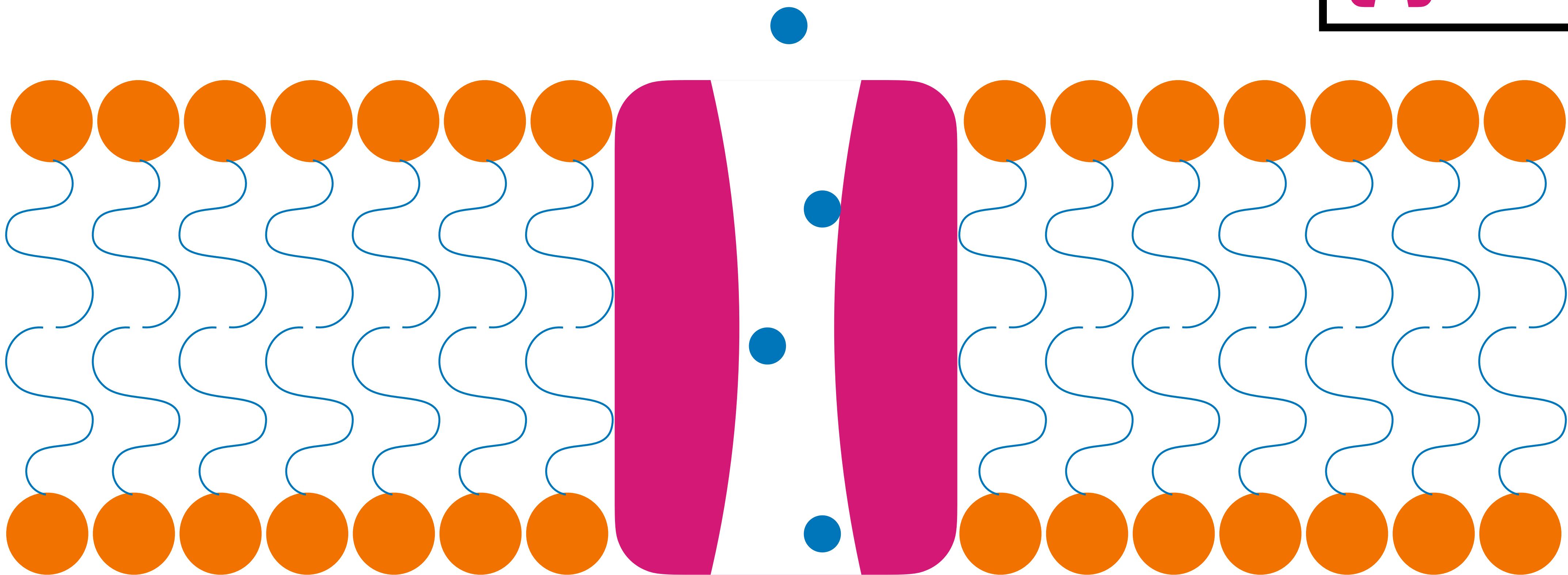
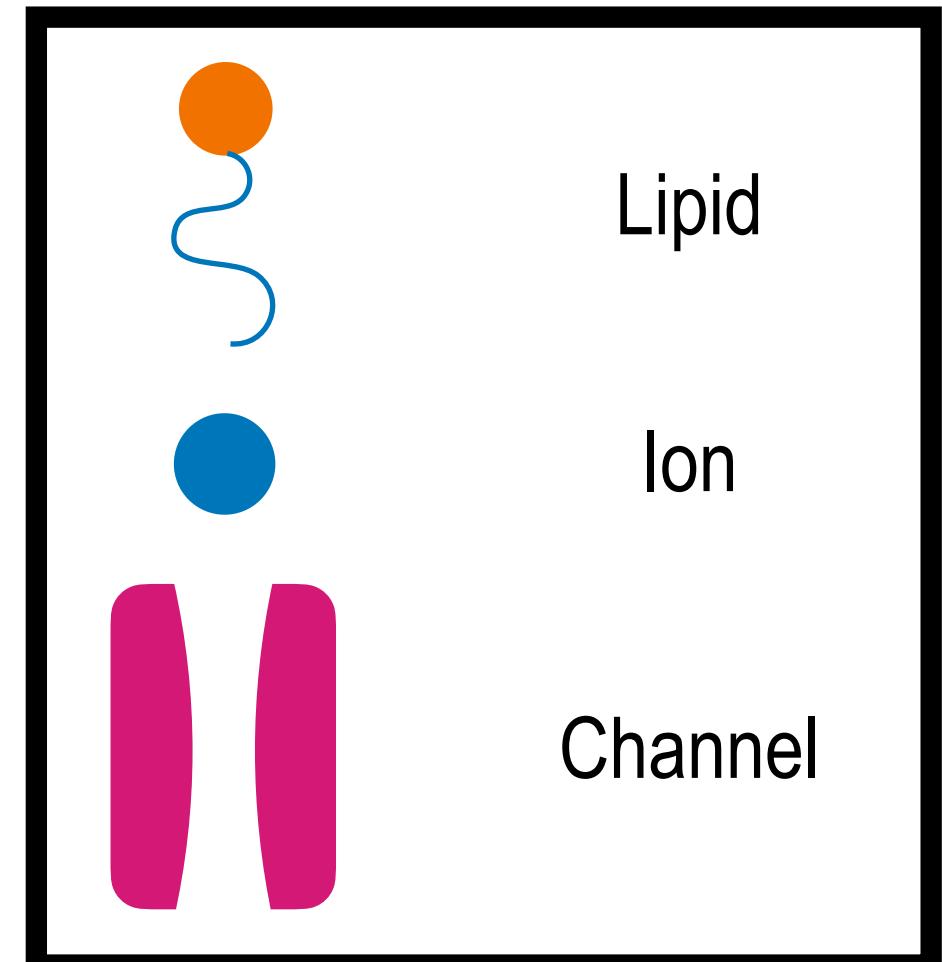
- **Introduction**
- Saturation, Sterols, and Domain-forming lipids: Identifying nAChR boundary lipids in PUFA-rich model membranes
- Lipid head-group charge: Boundary lipids for a bacterial sister channel in charged model membranes
- Putting it all together: Quantifying specific lipid-binding affinities in complex native-like membranes

Ion Channels

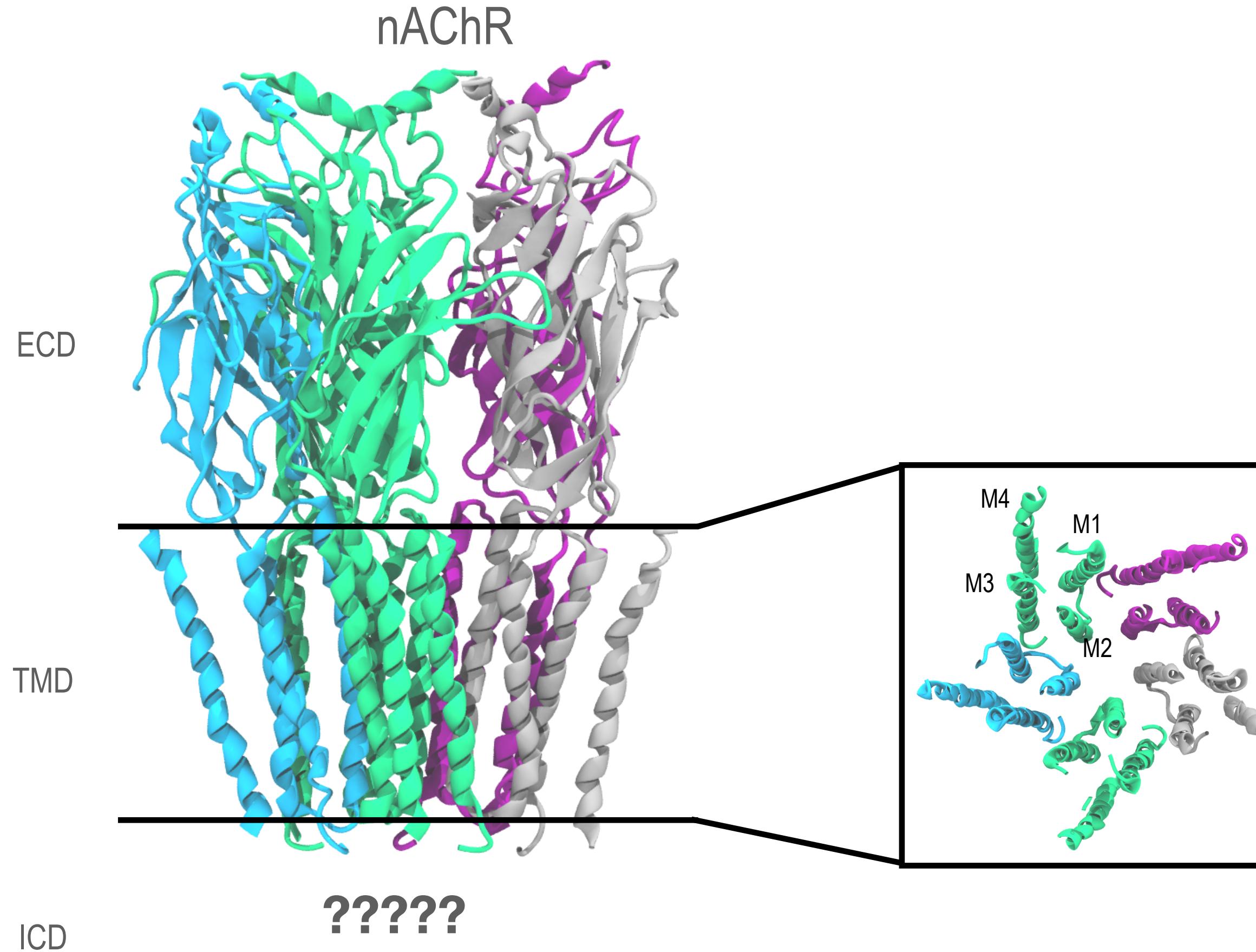
Transmembrane proteins

Passively conducts ions across membrane

Essential for various cellular functions



Pentameric Ligand Gated Ion Channels (pLGICs)

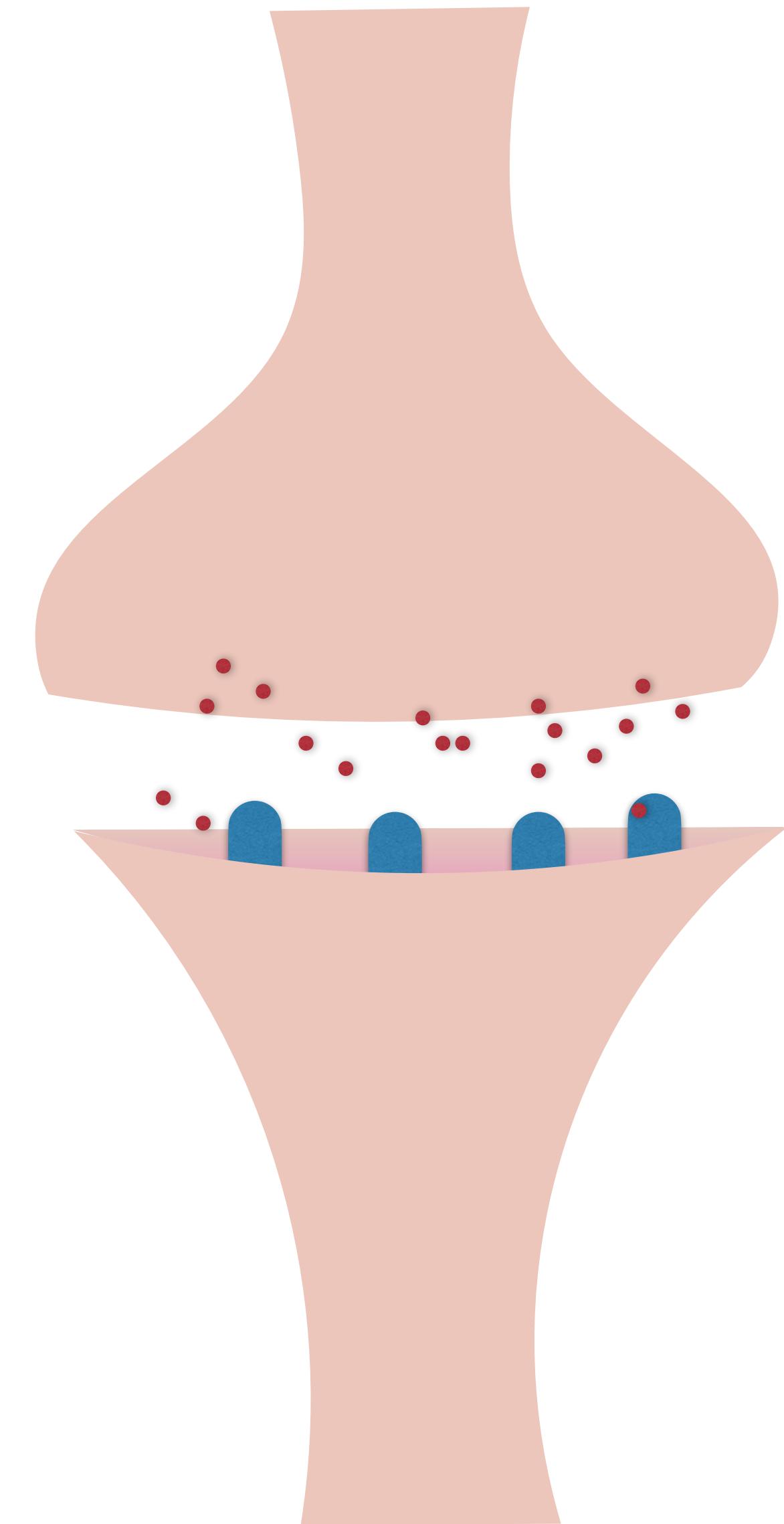


Pentameric ligand gated ion channels

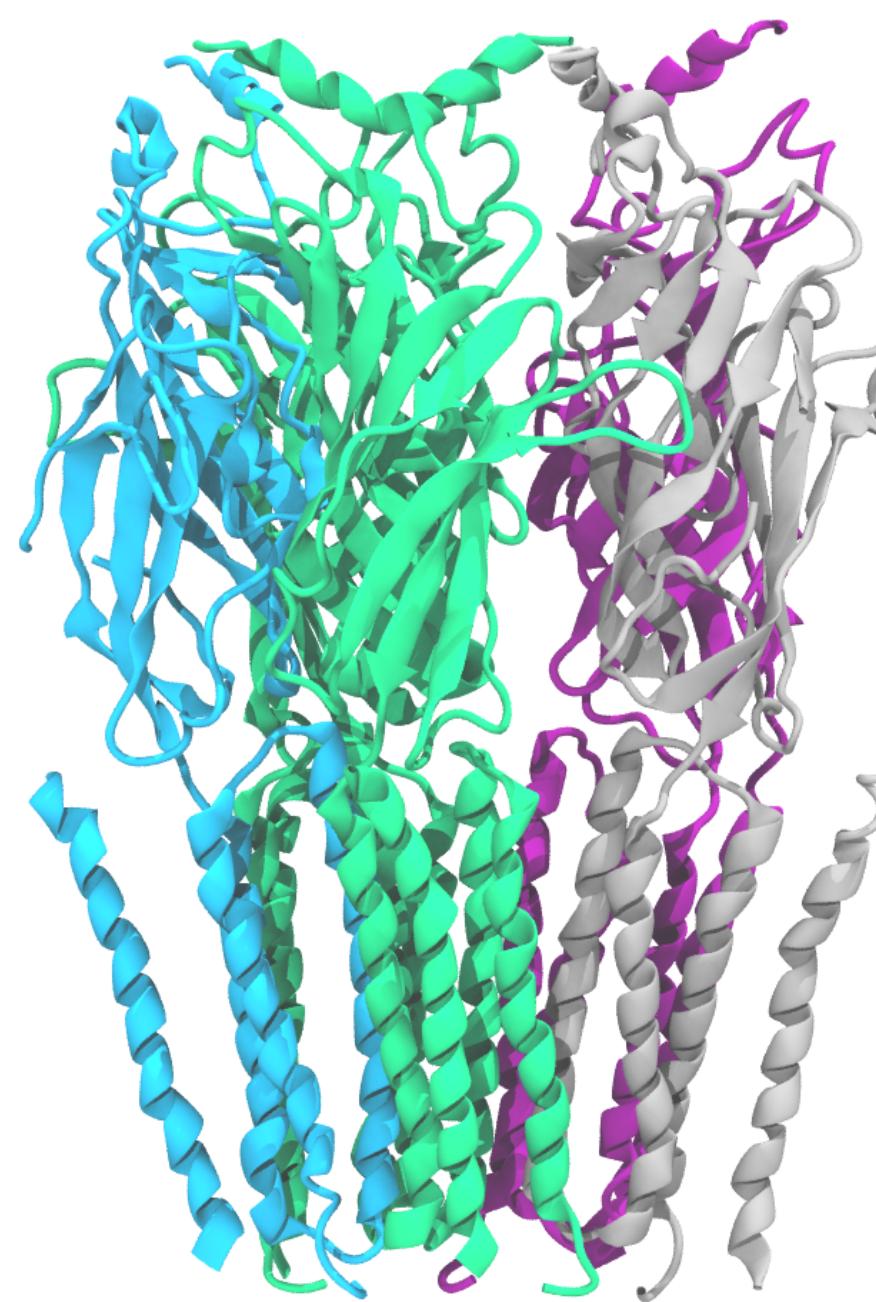
- Five subunits
- Three domains
 - Extra-Cellular (ECD)
 - Transmembrane (TMD)
 - Inter-Cellular (ICD)
- Open with the binding of specific small molecules
- pLGICs are structurally conserved

Where pLGICs are in Mammals

- pLGICs reside in the post synaptic membrane
- Responsible for stimulating and inhibiting action potentials



pLGICs role in physiology



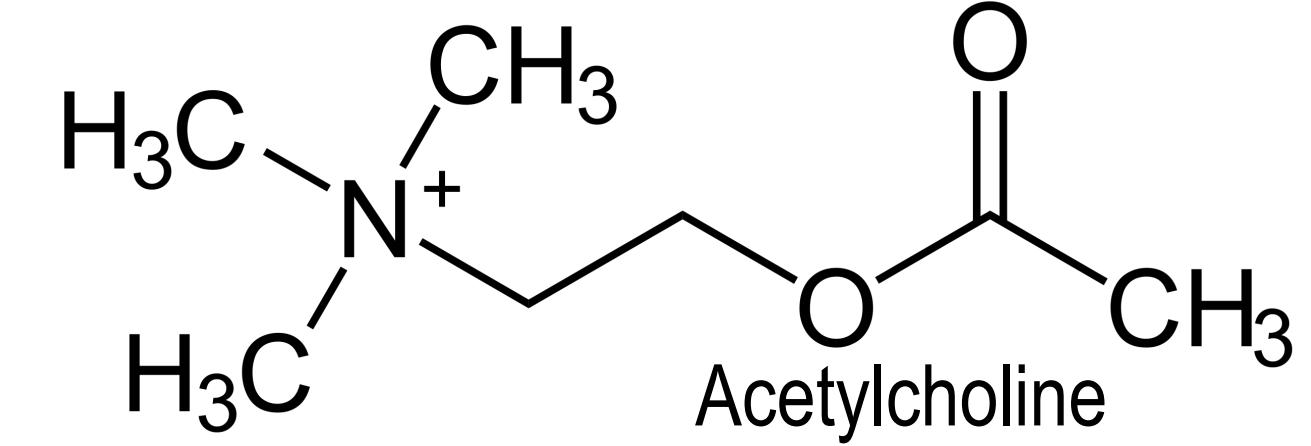
By stimulating or inhibiting action potentials along axons, pLGICs play a role in neurological function

- Cognition
- Learning
- Muscle Function

Improper function can play a role in neurological diseases and disorders:

- Bipolar Disorder
- Schizophrenia
- Depression
- Epilepsy
- Neuromuscular autoimmune diseases

Ligands and modulators

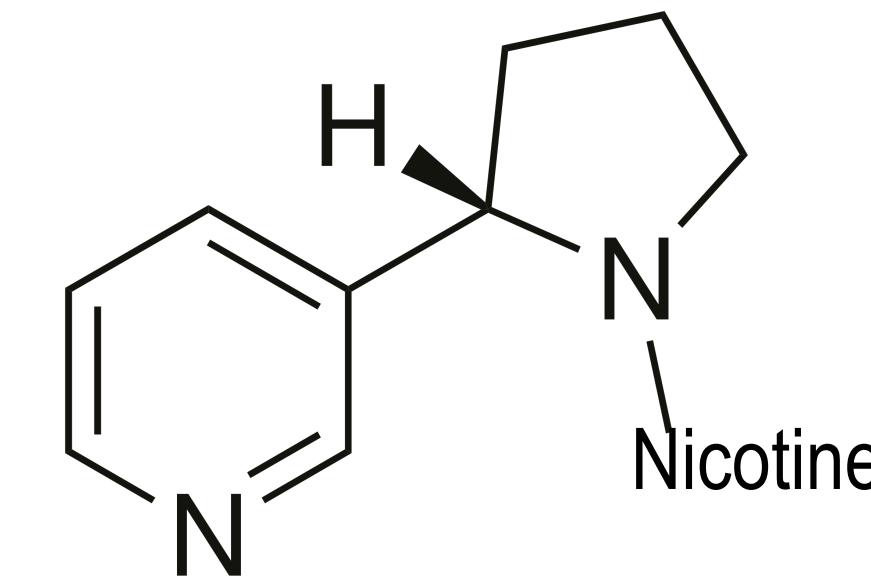




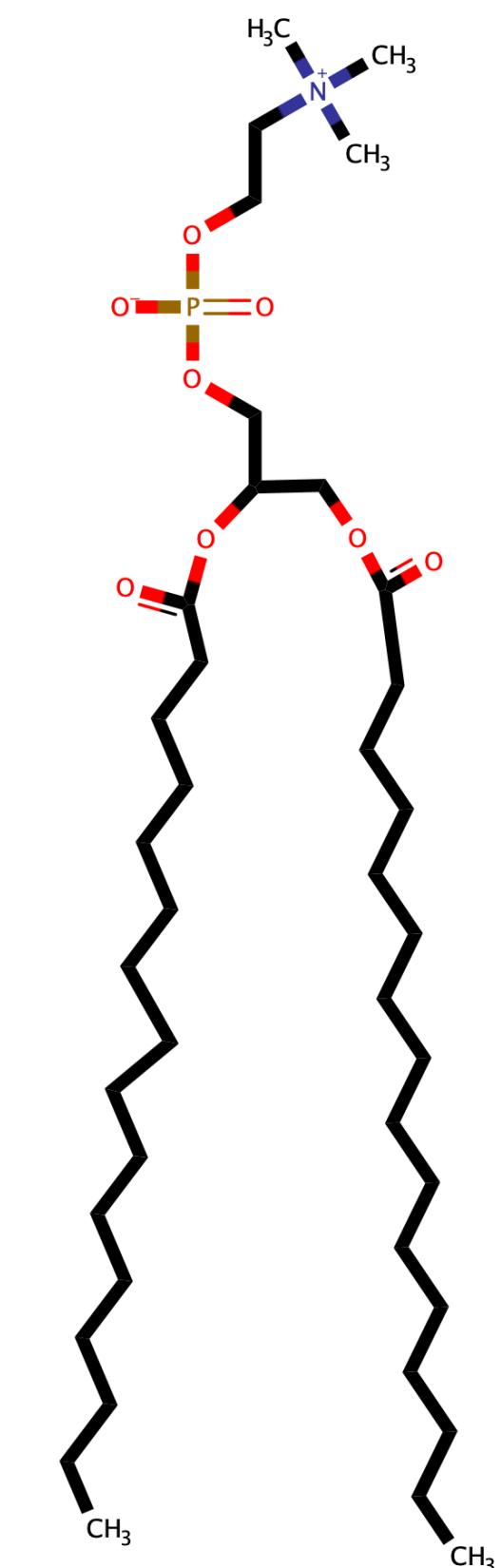
Neurotransmitters (Acetylcholine)

Drugs (Nicotine, alcohol, general anesthetics)

Lipids (Highly specific)



Dipalmitoylphosphatidylcholine (DPPC)



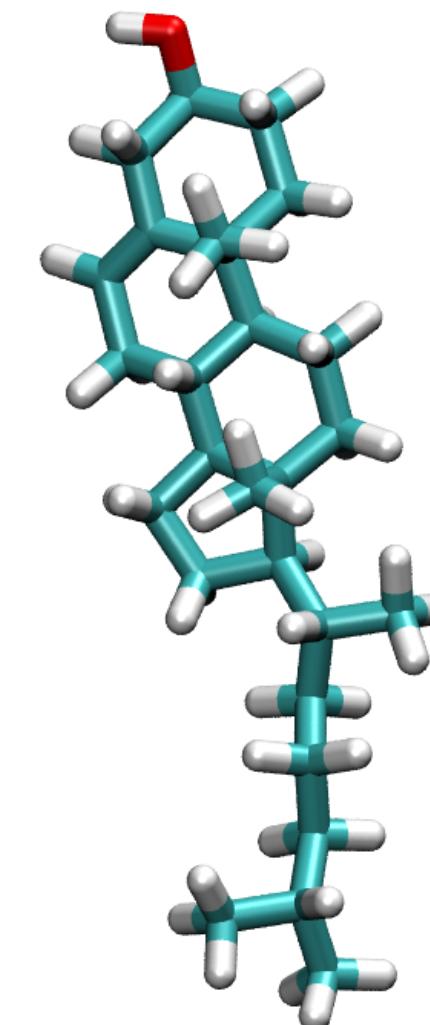
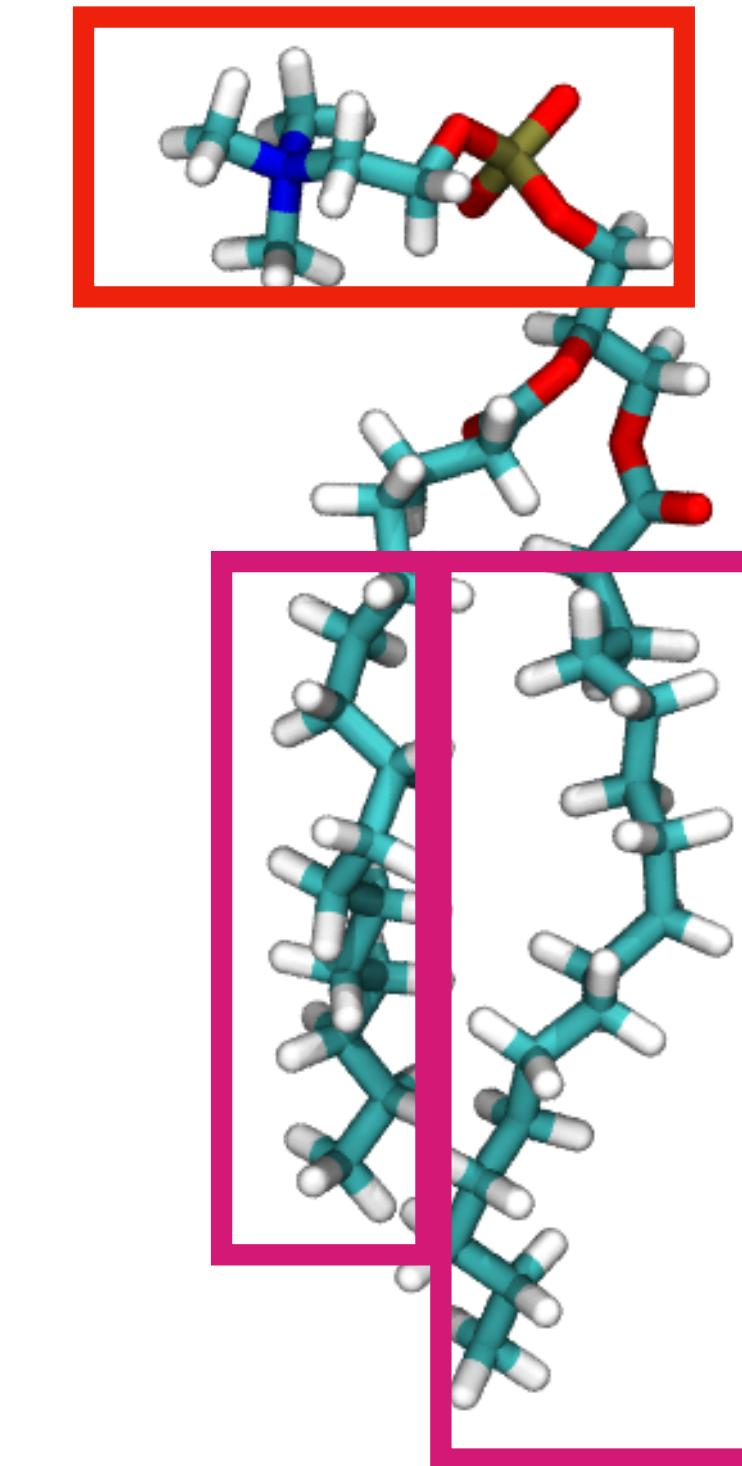
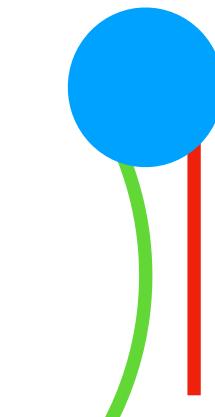
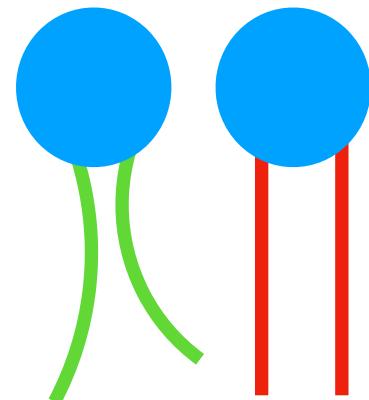
Lipids

💡 Lipids are small amphiphilic molecules

💡 Two distinct classes are phospholipids and sterols

💡 Phospholipids are “modular”:

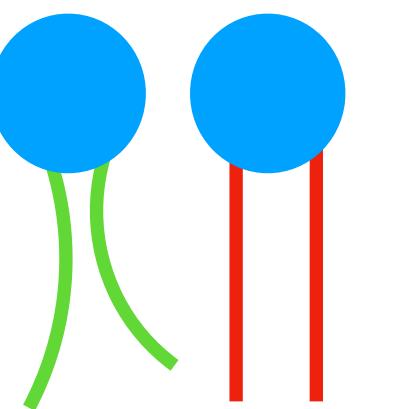
- Head group either neutral or anionic
- Acyl-Chains vary in length and saturation
 - Saturated lipids: straight and rigid
 - Unsaturated flexible with kinks
(See next slide!)
- Can be homo- or hetero-acidic



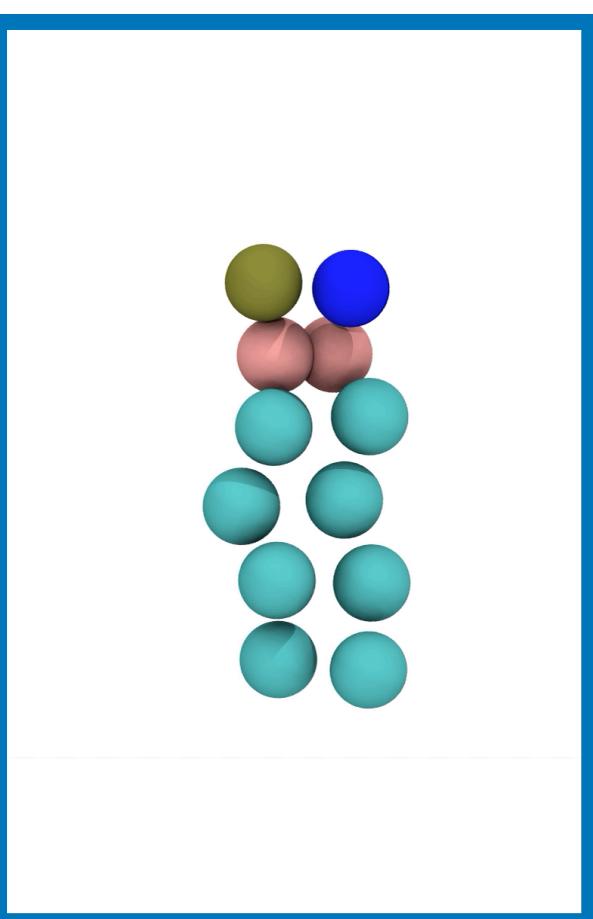
💡 Cholesterol

Lipids: Model Membranes

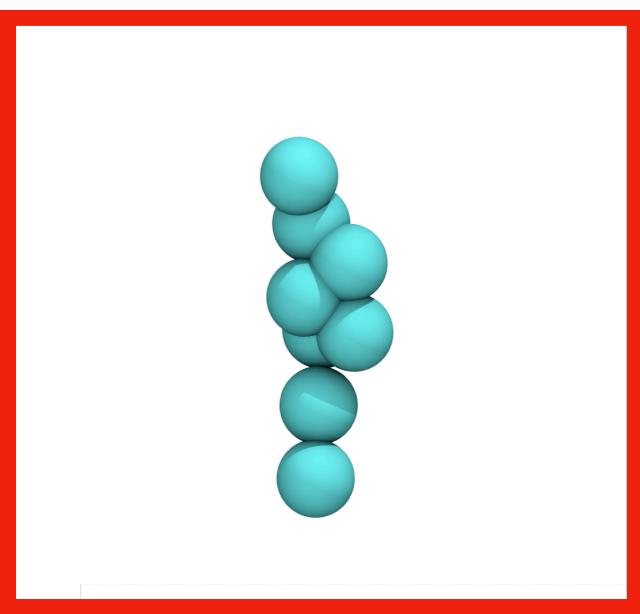
Homo-Acidic
Domain
Forming



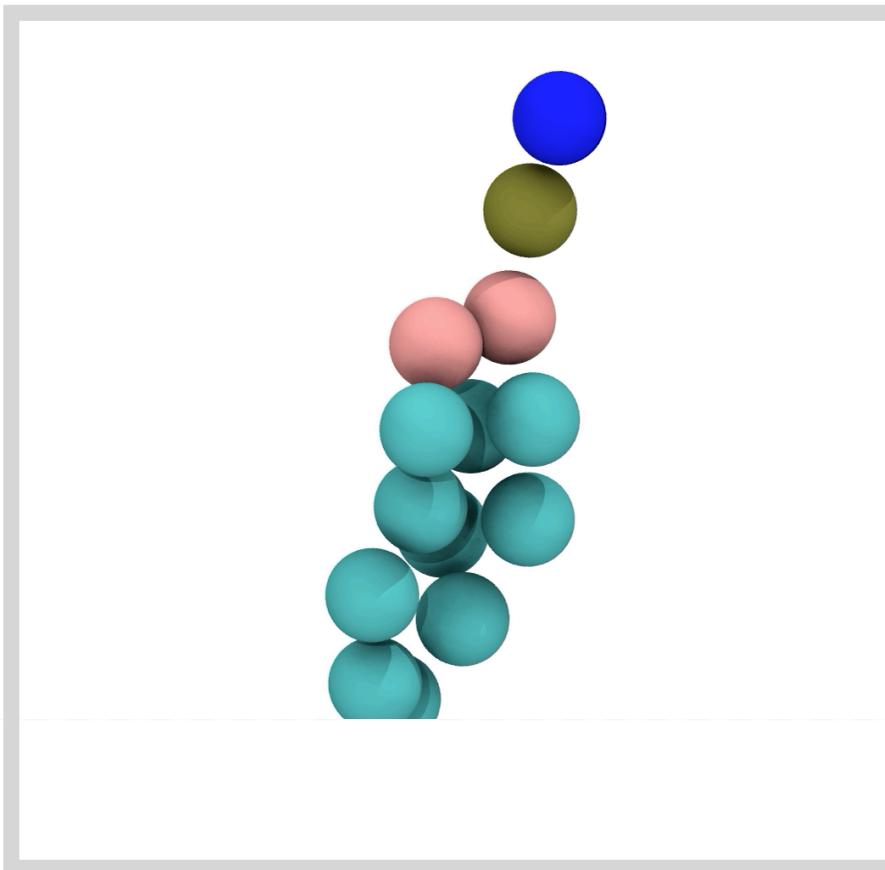
Saturated Chains



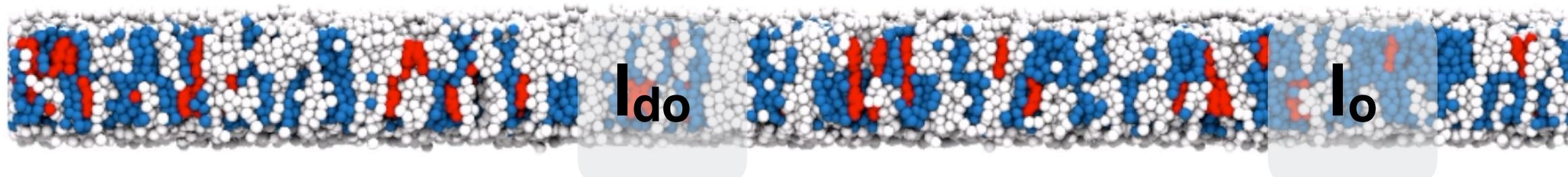
Cholesterol



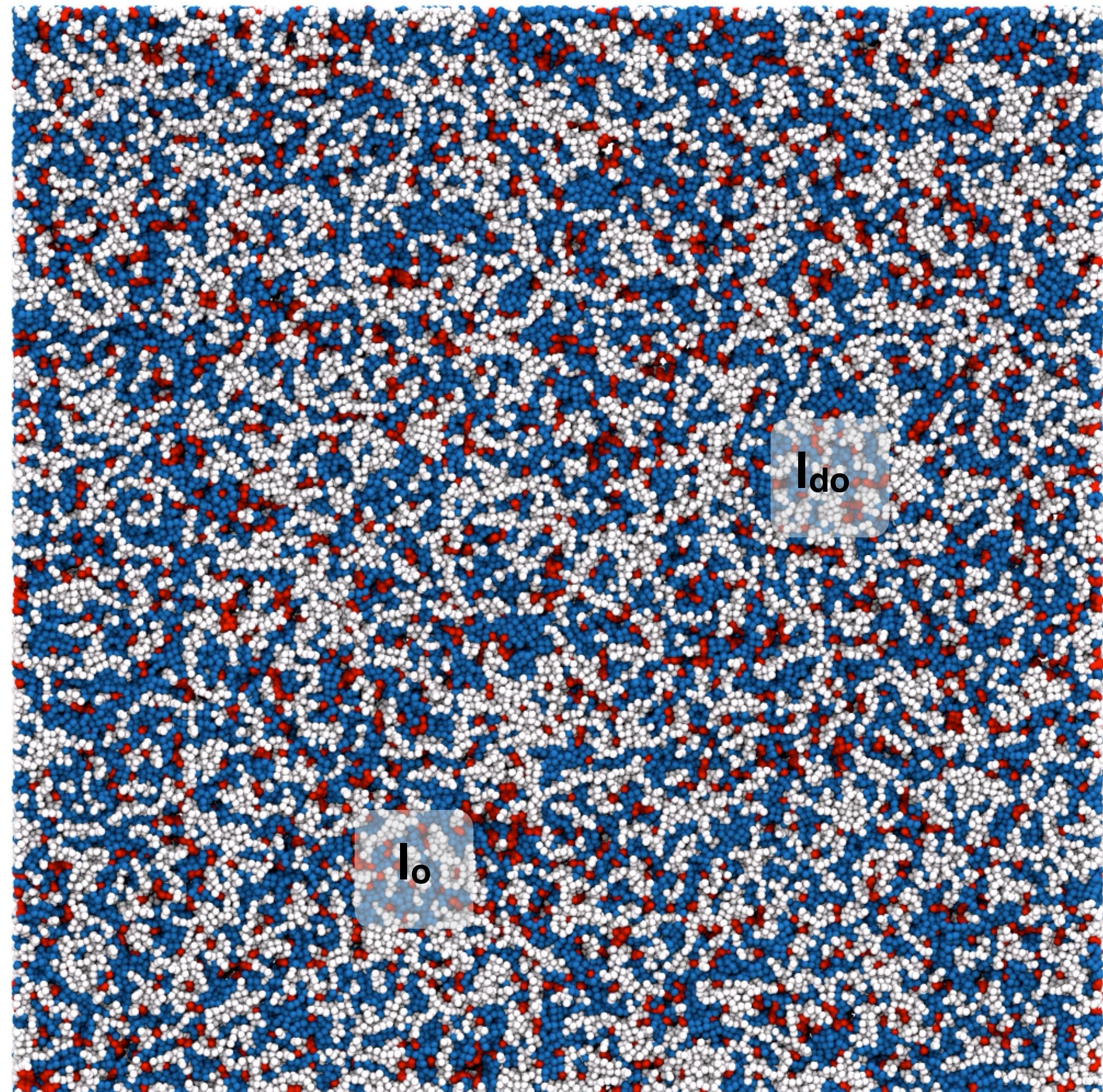
Polyunsaturated Chains



Model Membrane Side View



Model Membrane Extra-Cellular View

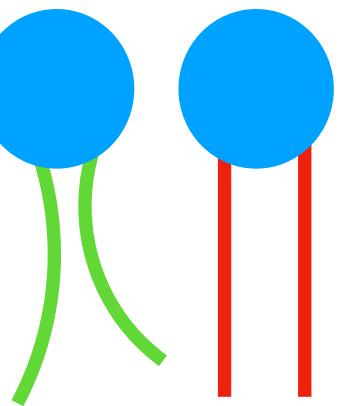


$\sim 2 \text{ us} \quad 75 \times 75 \text{ nm}^2$

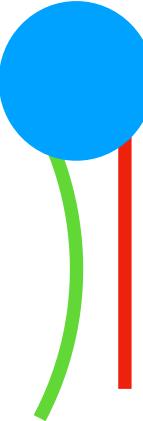
Lipids: Native Membranes

- Real membranes have tens to hundreds of lipids
- Most are hetero-acidic
- Leaflet asymmetry

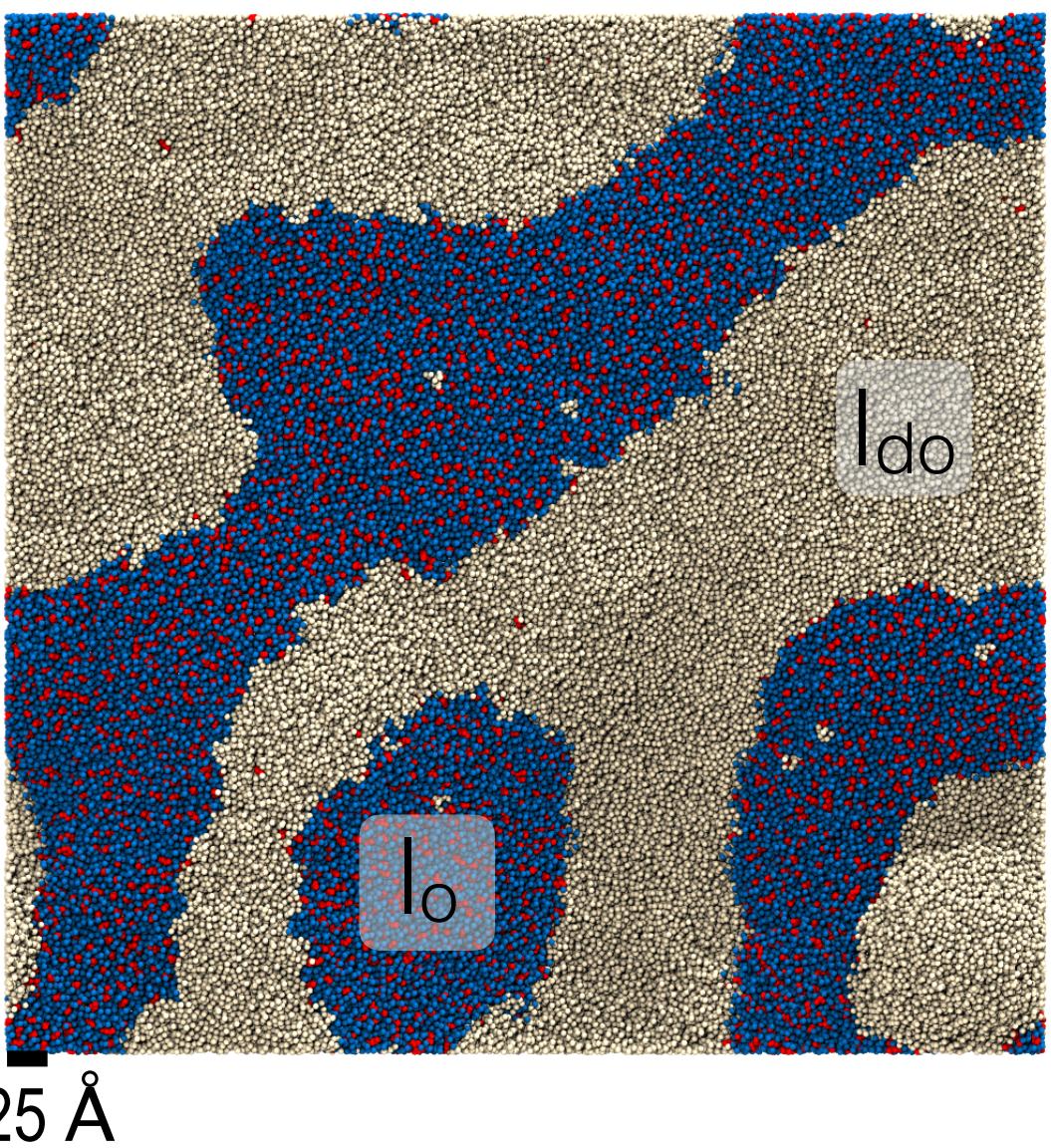
Homo-Acidic
Domain
Forming



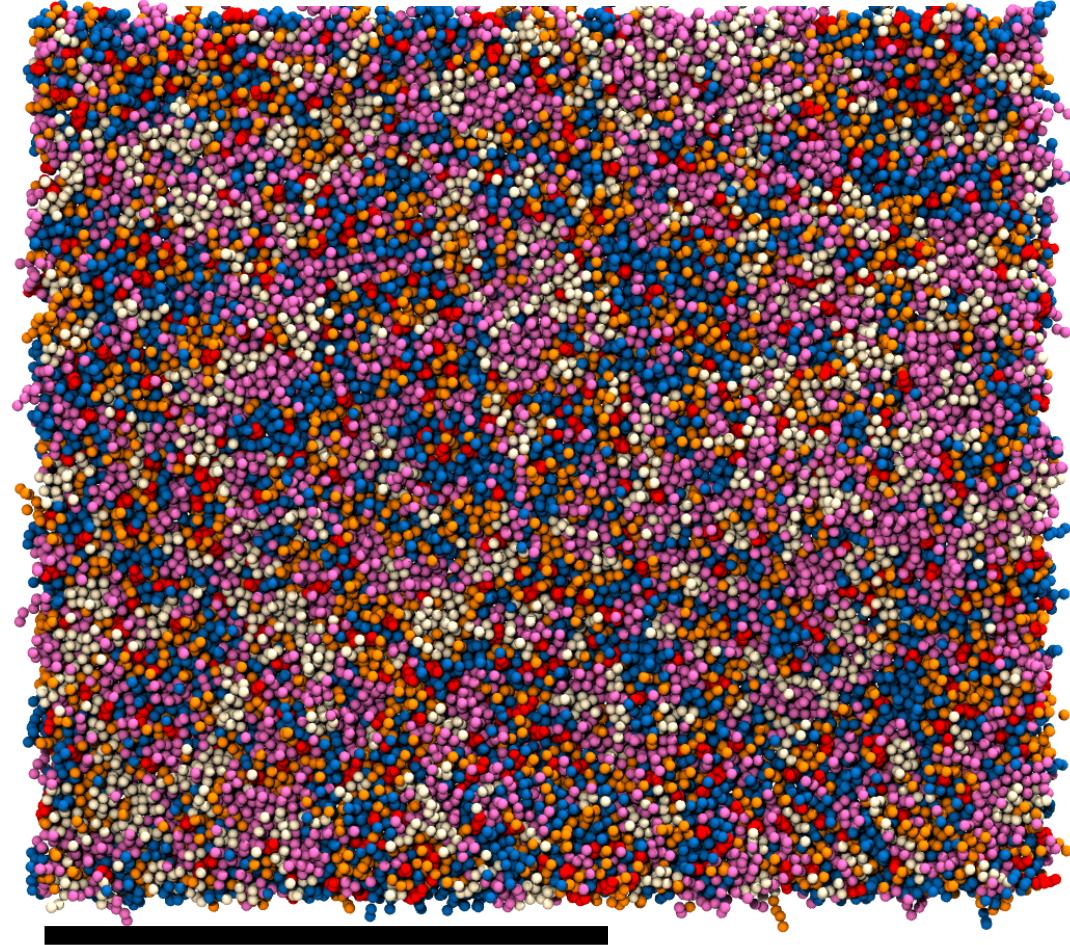
Hetero-Acidic
Non-Domain
Forming



Model Membrane



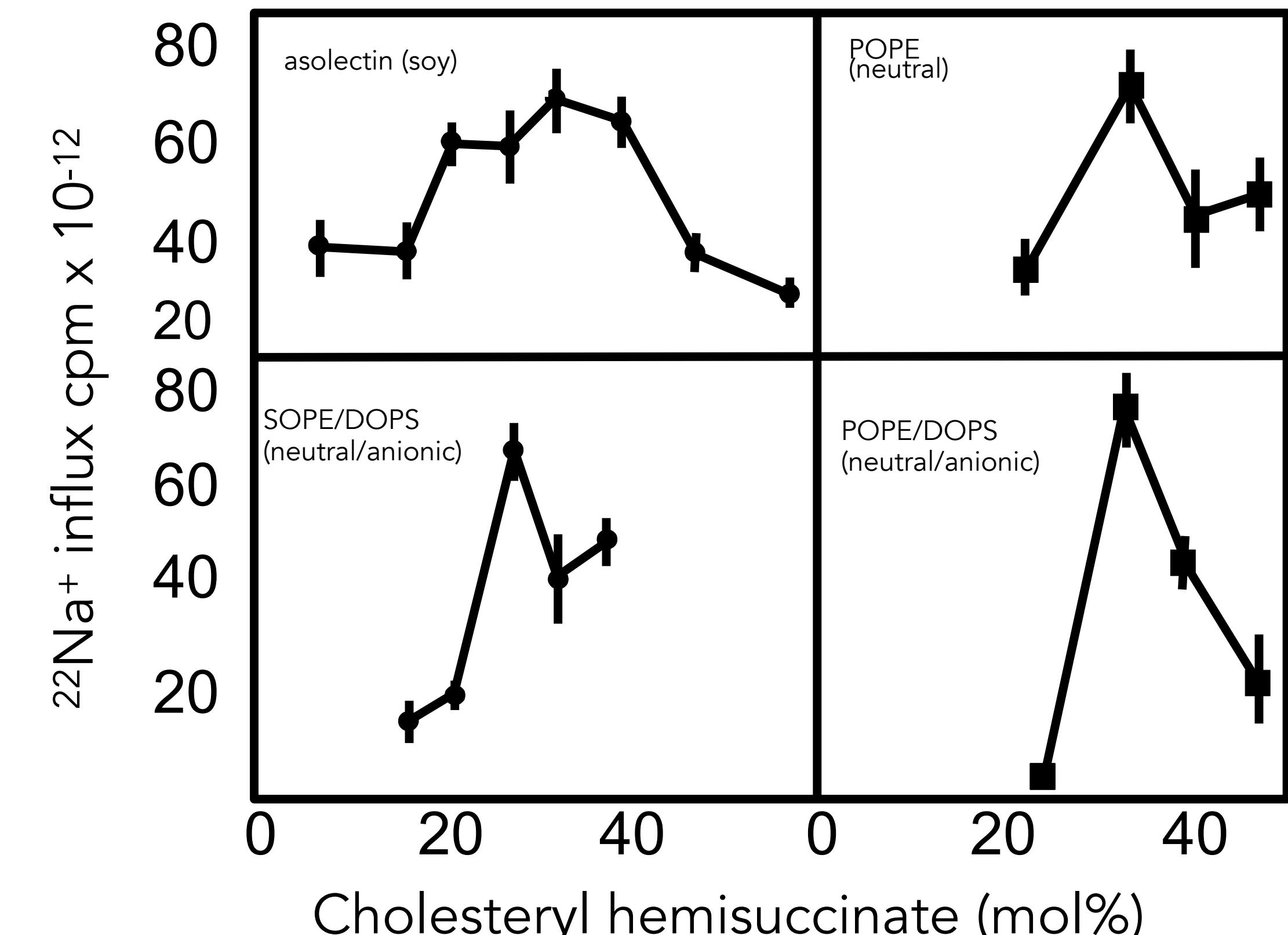
Native Membrane



25 Å ~15 species of lipid

Lipids modulate nAChR function

- Cholesterol is required for function (non-monotonic dependence)
- Anionic lipids are suggested for function

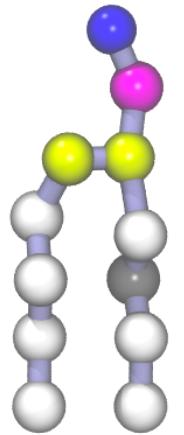


M. Criado, H. Eibl, and F. Barrantes, Biochemistry, 1982

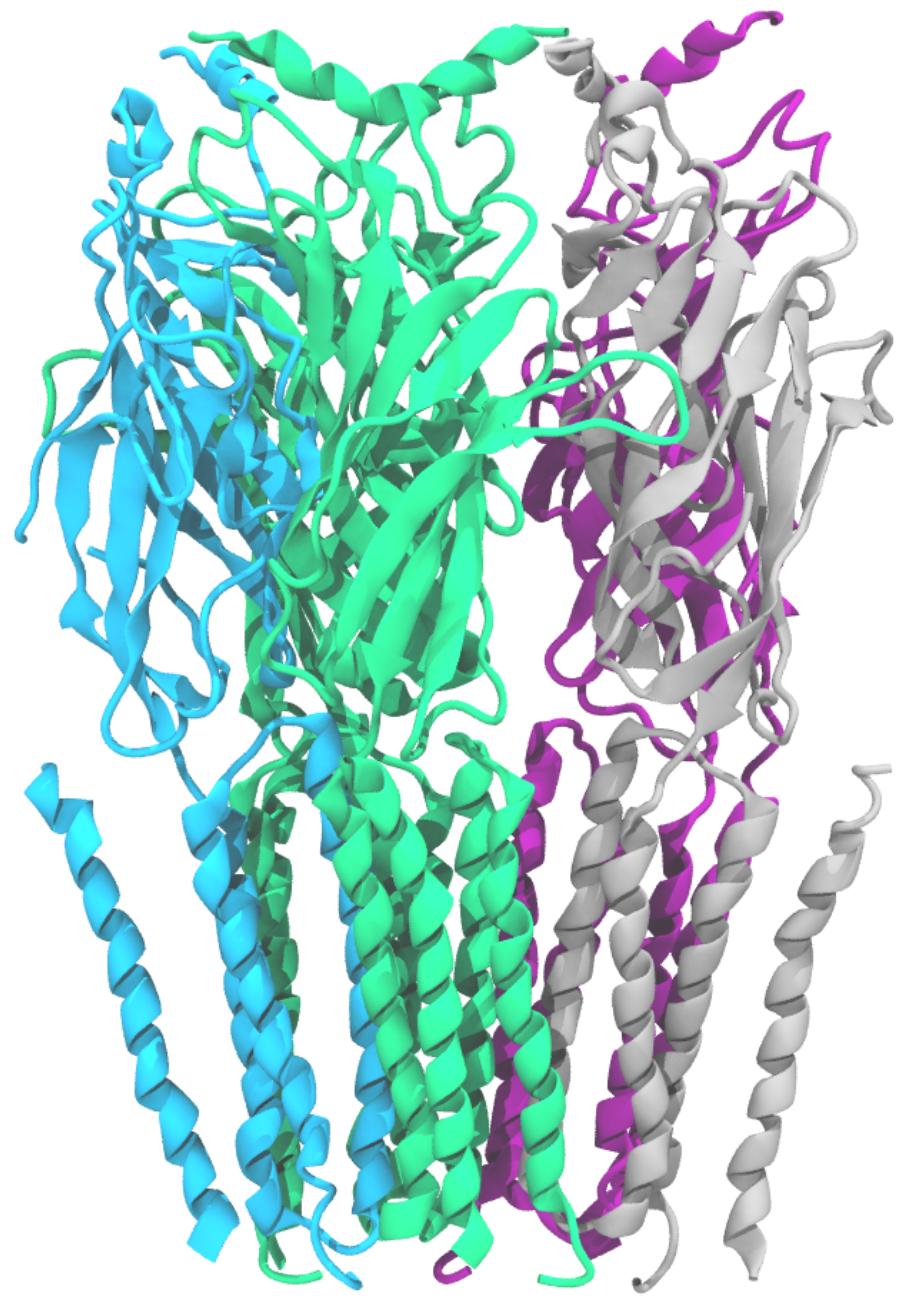
Lipid modulation mechanism

- Do modulating lipids indirectly or directly interact?
- If lipids interact directly where on the protein do they bind?
- How can we measure this lipid binding?

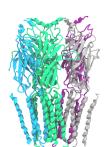
Indirect



Direct



Experimental methods to predict pLGIC-lipid interactions



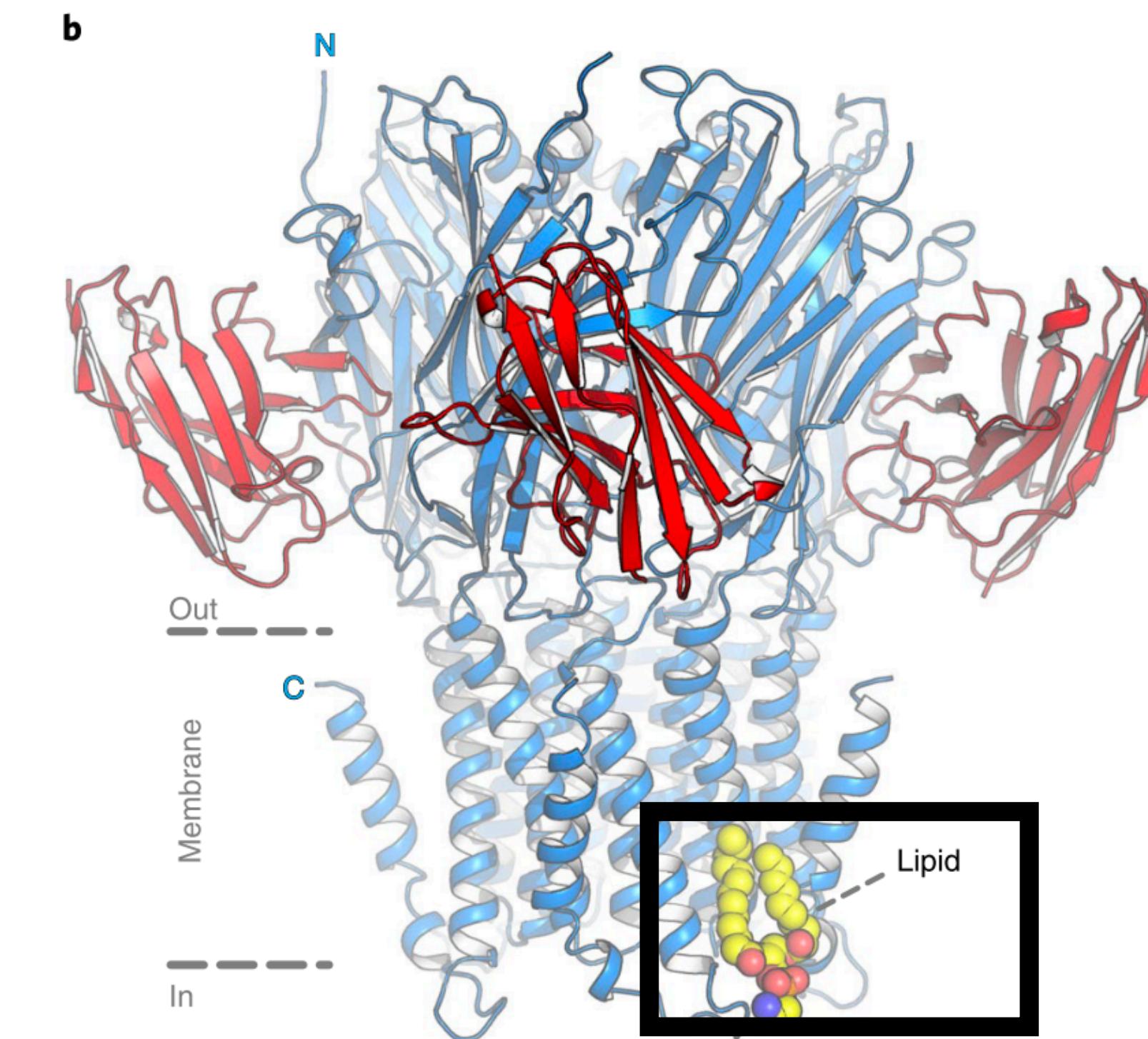
Structural Biology

- Cryo-EM, x-ray crystallography
- Where lipids interact



Interaction Based Methods

- Mass Spec, fluorescence quenching
- Estimate which lipids directly interacting



Functional Experiments

- Electrophysiology
- How lipids modulate a protein, which lipids modulate

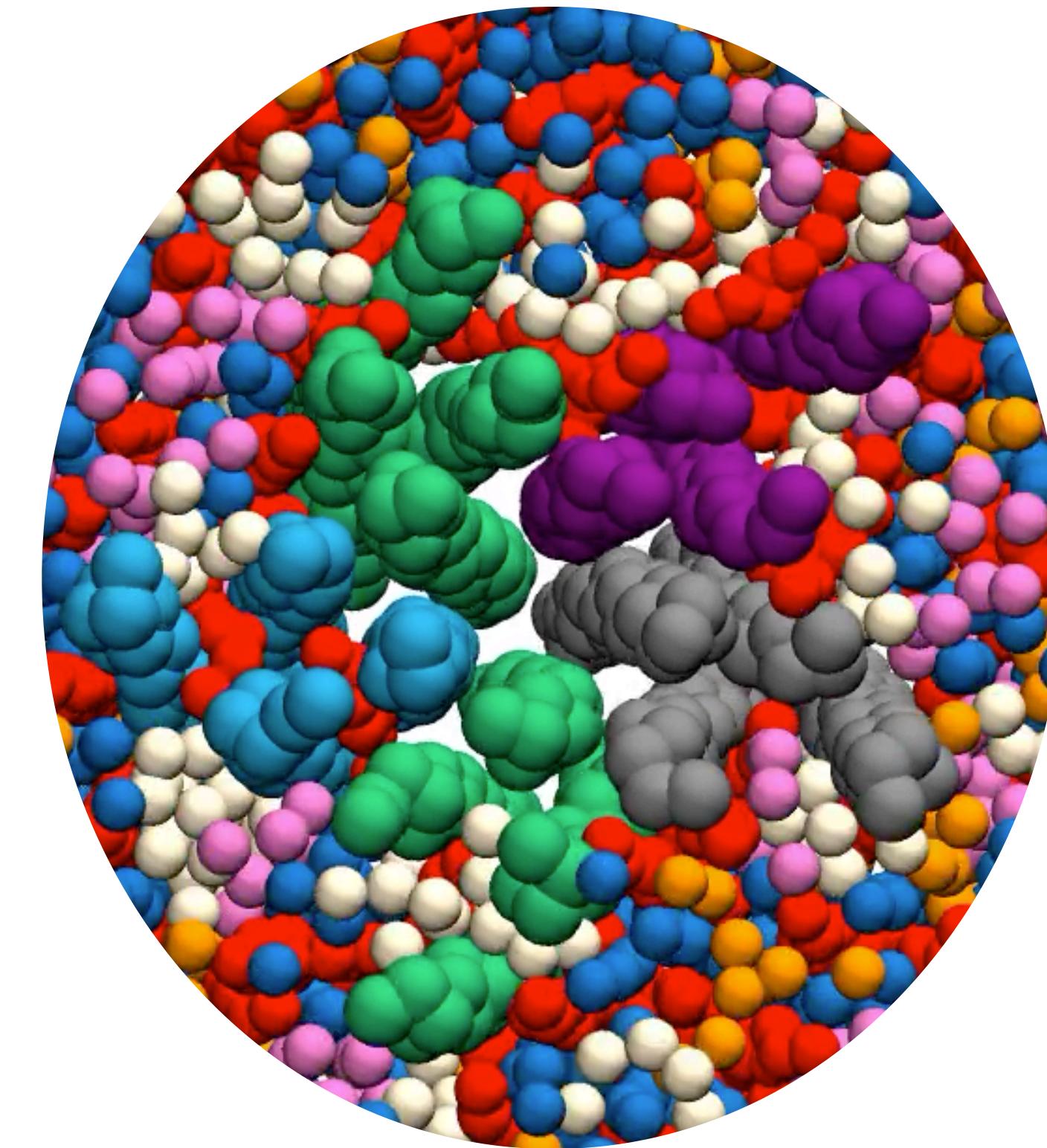
Camille M. Hénault...Woods...Brannigan... et al 2019 Nature Chemical Biology

A computational method to predict pLGIC-lipid interactions



Computational Biology: Molecular Dynamics (MD)

- Shows molecular interaction, where lipids bind, and how lipids bind
- Does not show function



What computational studies have been done to identify the boundary lipids?

Docking

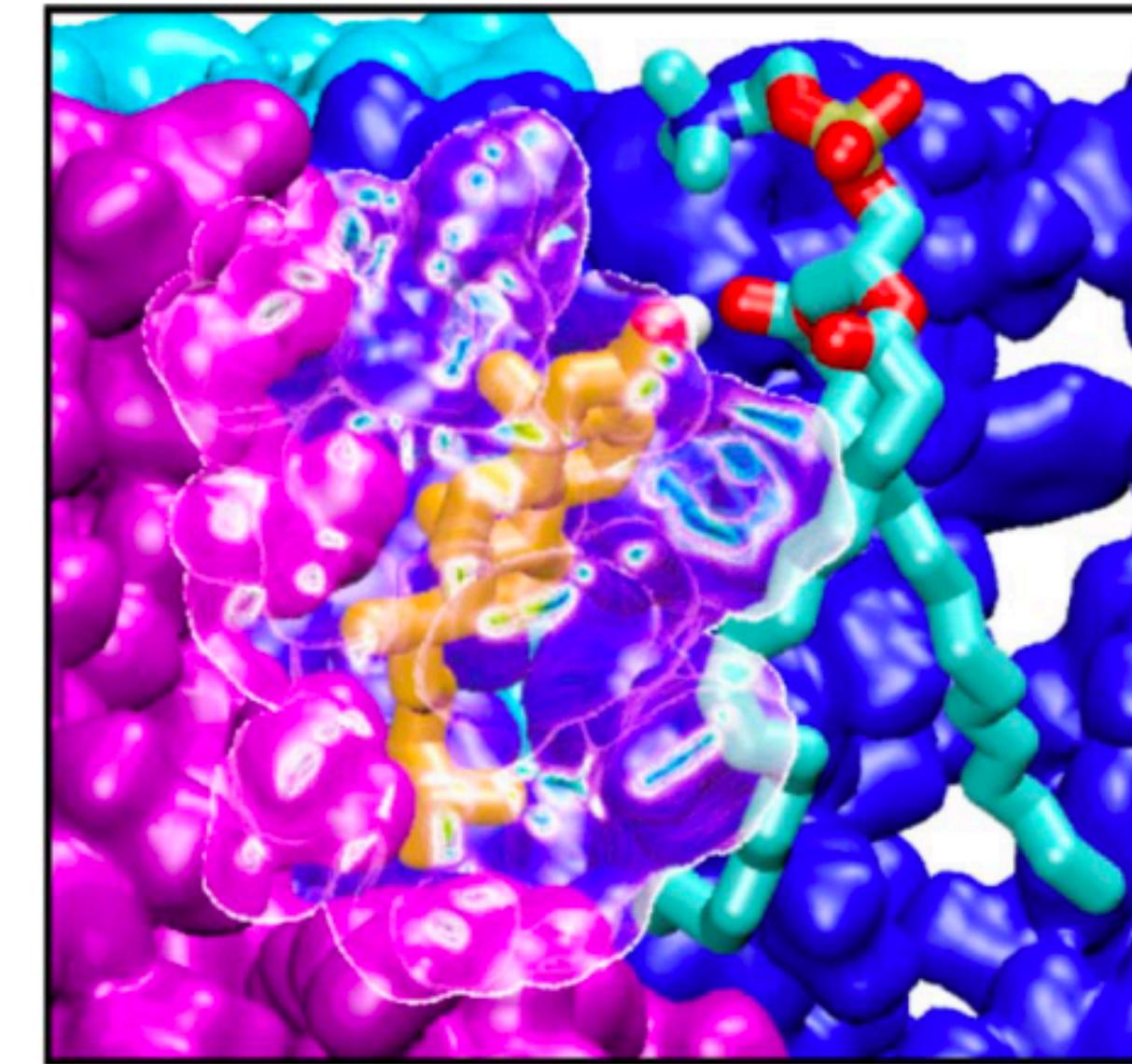
Atomistic MD simulations

- Frequently uses cholesterol or anionic lipids (i.e. missing realistic lipids)
- Computationally expensive for lipids to explore protein

No coarse-grained molecular dynamics simulations until 2019 (this is us!!)

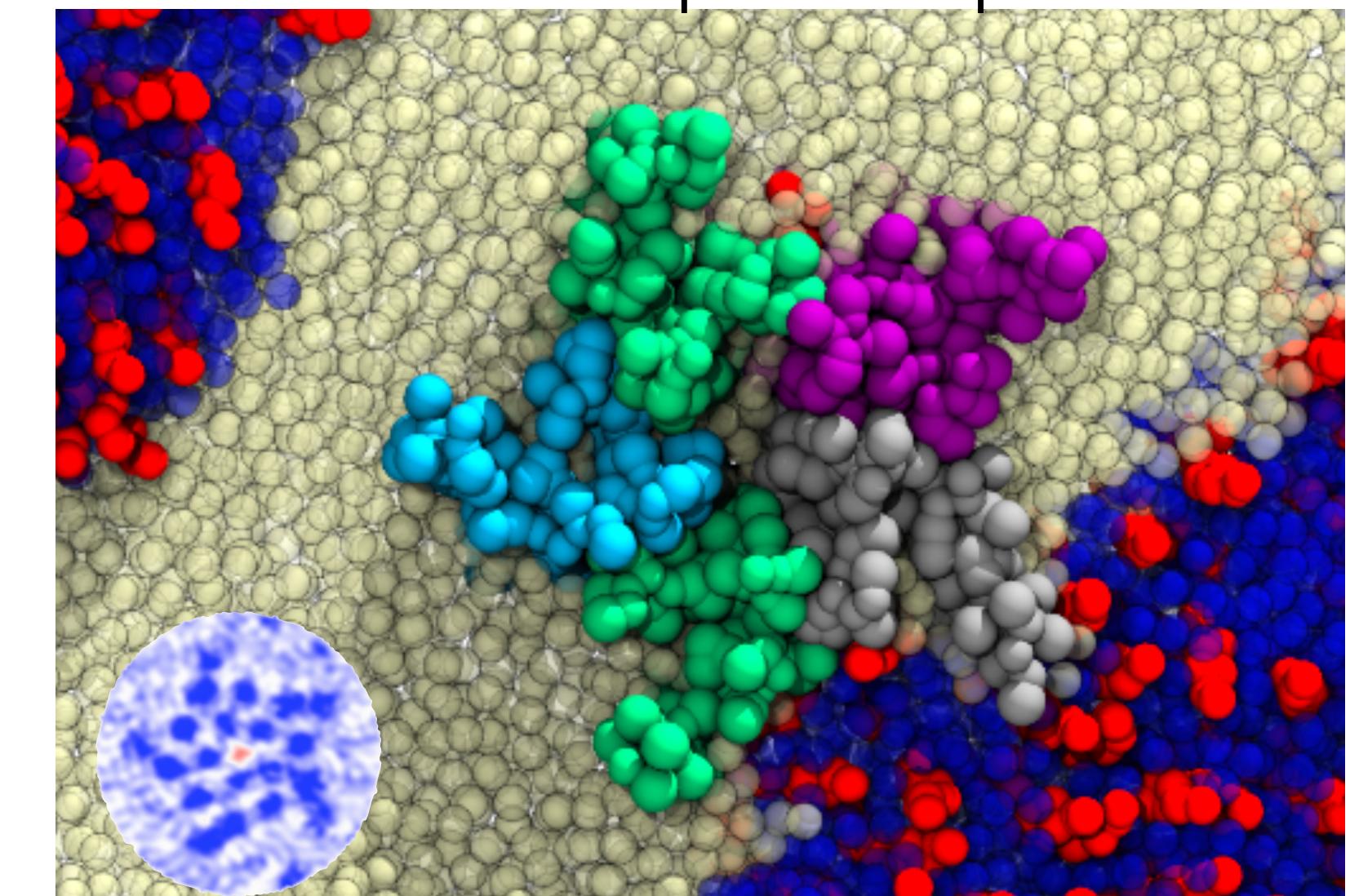
- Lower resolution but less expensive for lipid to explore the protein!

Atomistic: Shows every atom



Brannigan et al 2008, PNAS

Coarse-Grained: Multiple atoms per bead



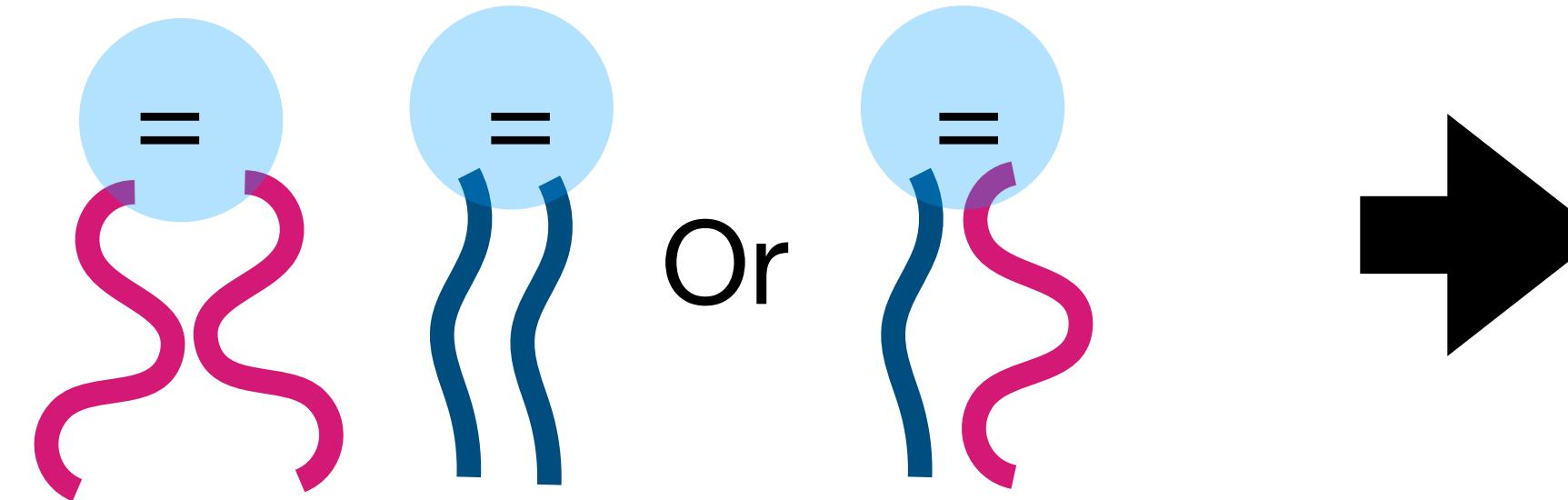
1 nm

Sharp et al 2019, BBA

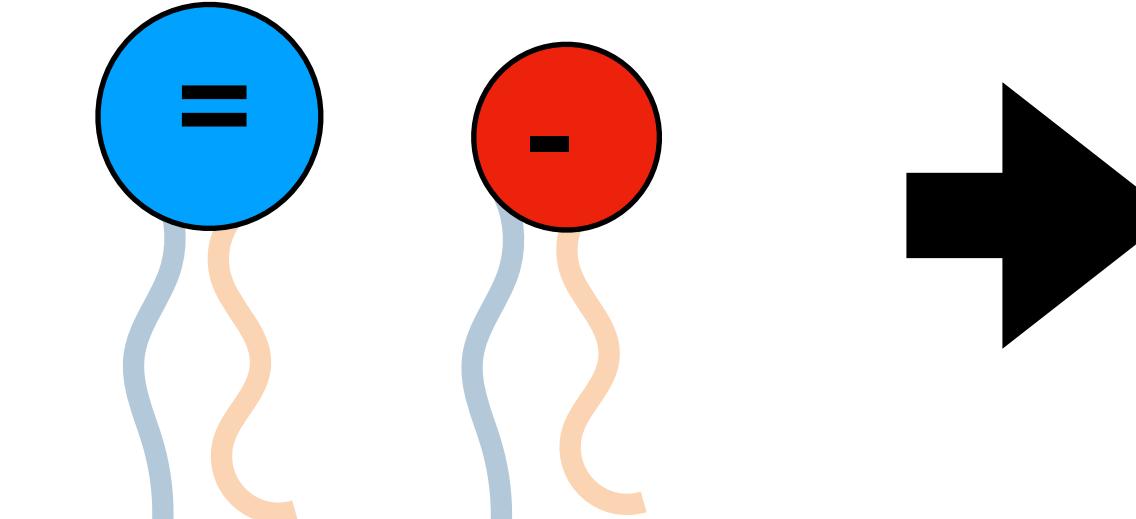
Goal of the thesis

- Identify specific lipid bind sites on pLGICs
- How do topological difference in lipids change lipid binding
- How does the bulk membrane composition change lipid binding

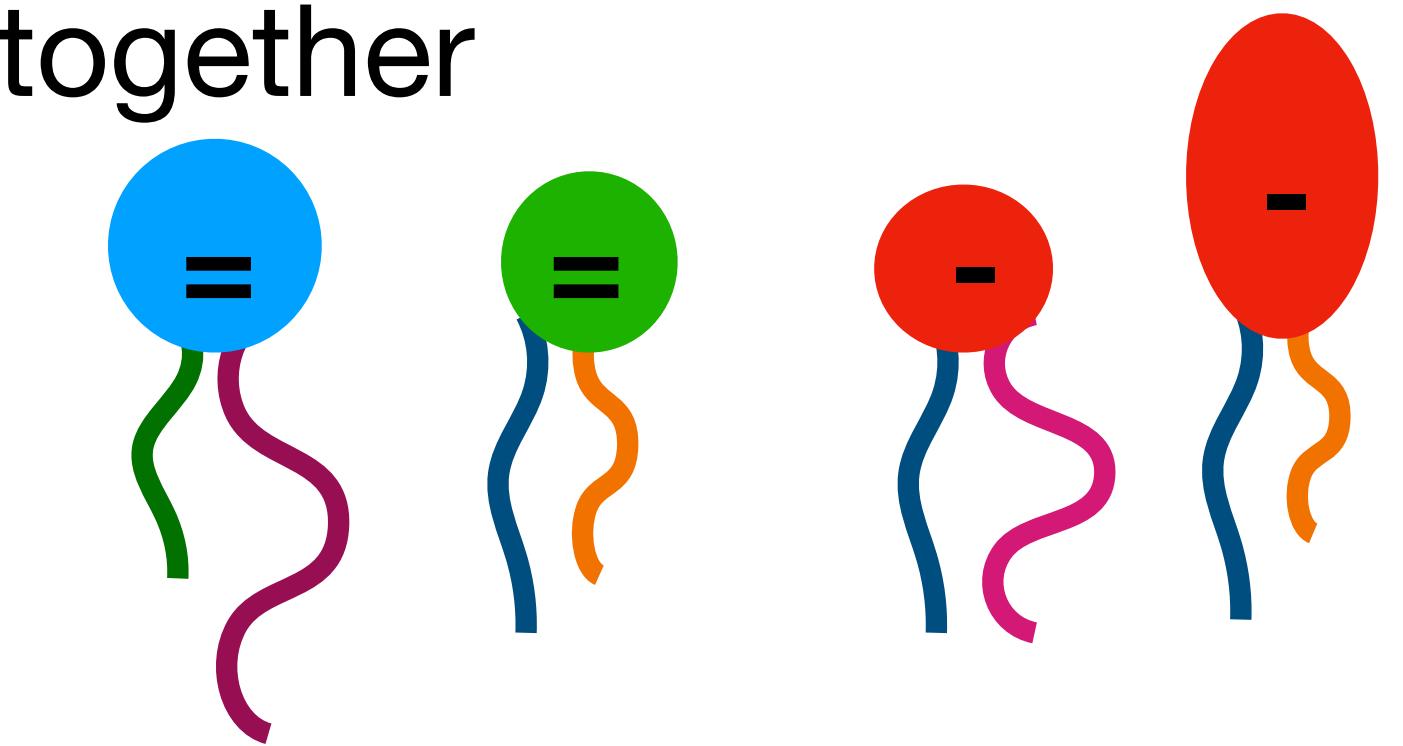
Section 1: Saturation, Sterols, and Domain-forming lipids



Section 2: Lipid head-group charge

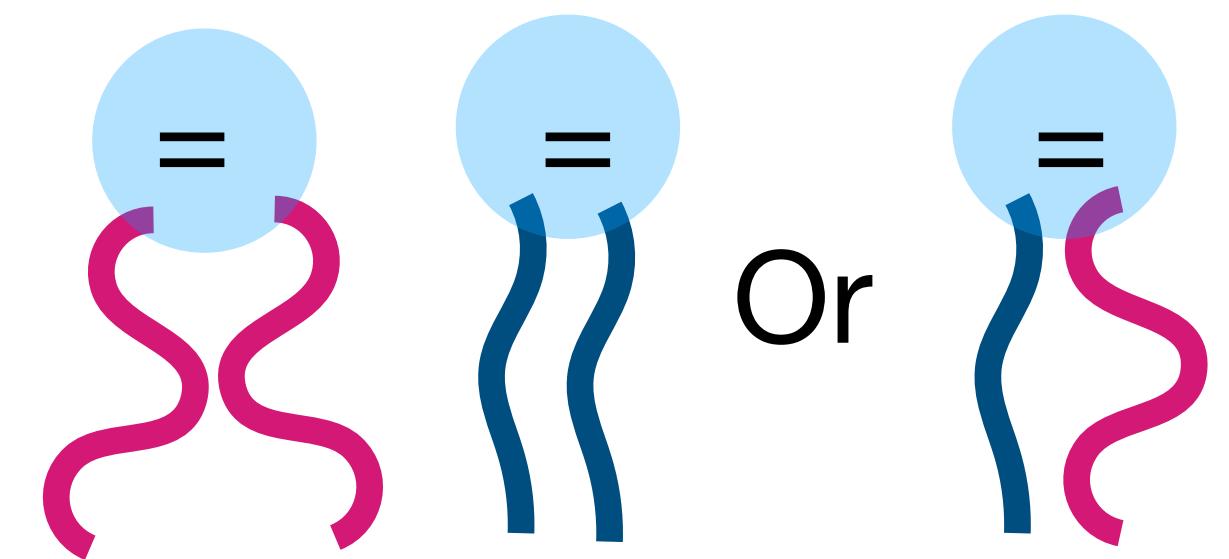


Section 3: Putting it all together



Outline

- Introduction
- **Saturation, Sterols, and Domain-forming lipids: Identifying nAChR boundary lipids in PUFA-rich model membranes**
- Lipid head-group charge: Boundary lipids for a bacterial sister channel in charged model membranes
- Putting it all together: Quantifying specific lipid-binding affinities in complex native-like membranes



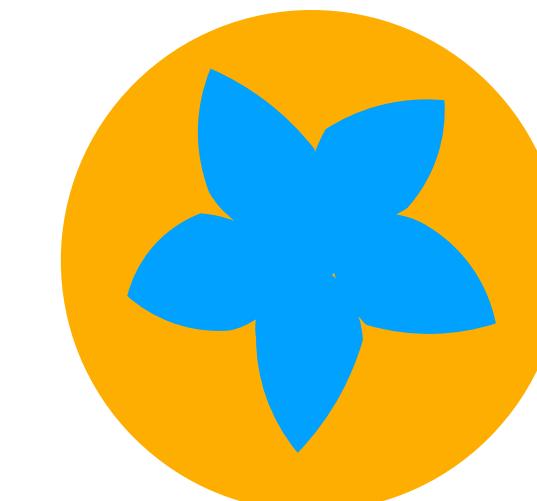
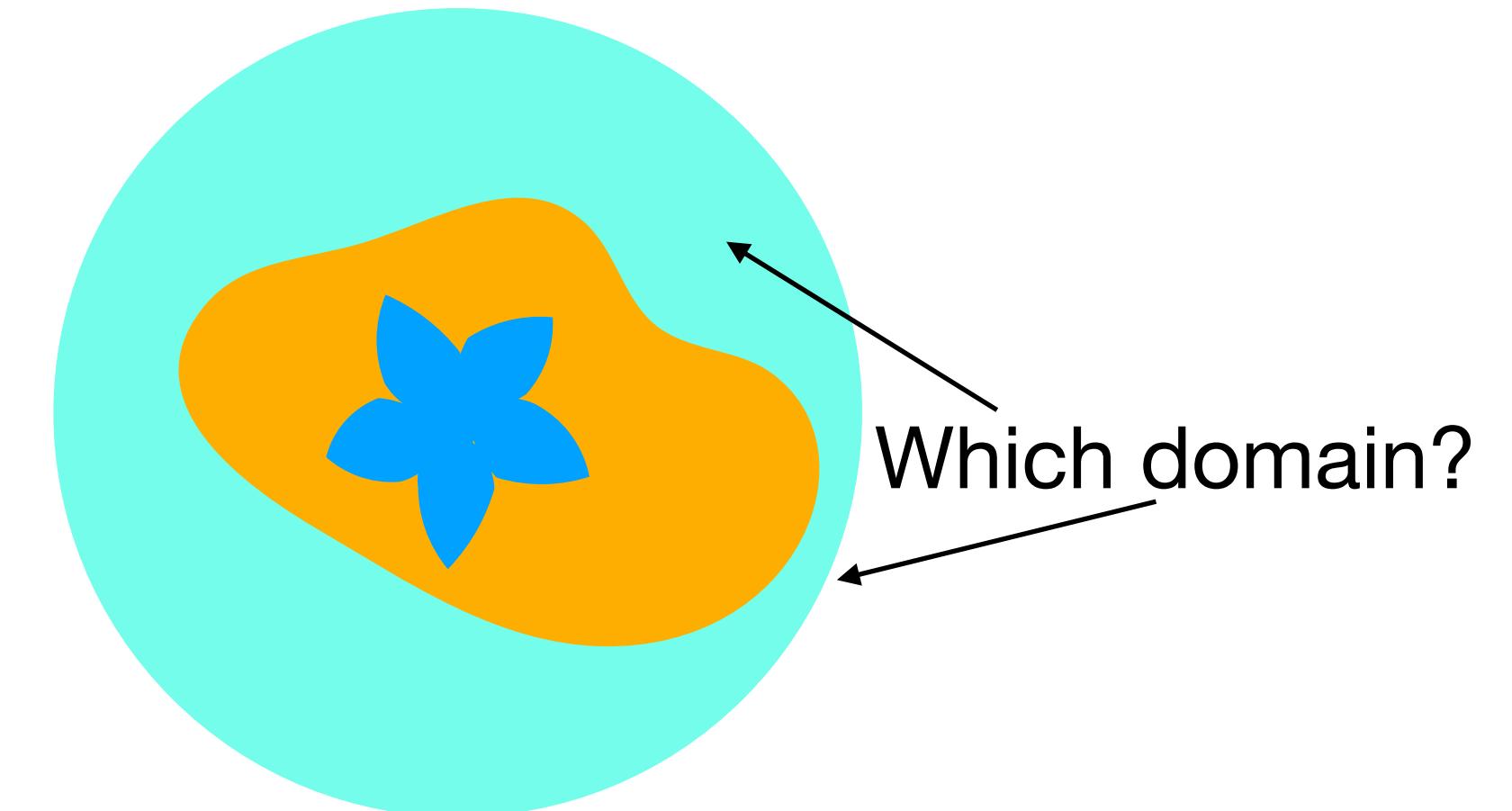
Or

nAChR in Ternary PUFA Rich Membranes

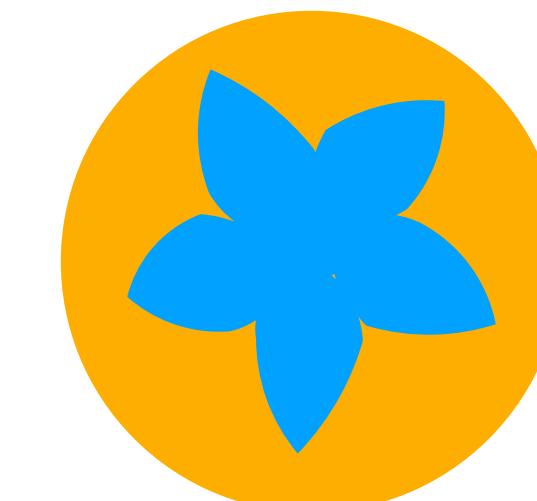
>If domains form, which domain does nAChR reside in?

What are the boundary lipids in PUFA rich membranes with homo- and hetero-acidic lipids?

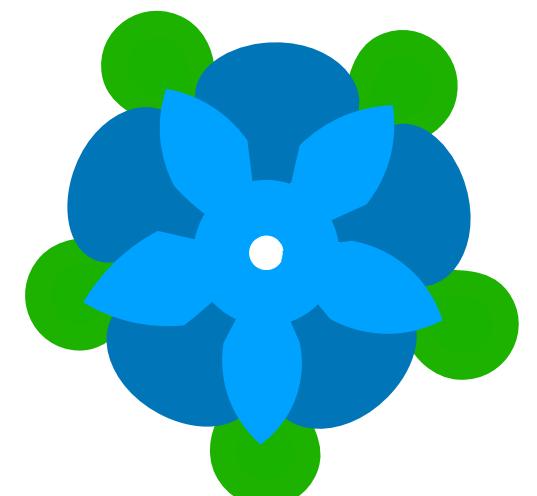
Are there preferential binding sites?



What are these lipids?



Or



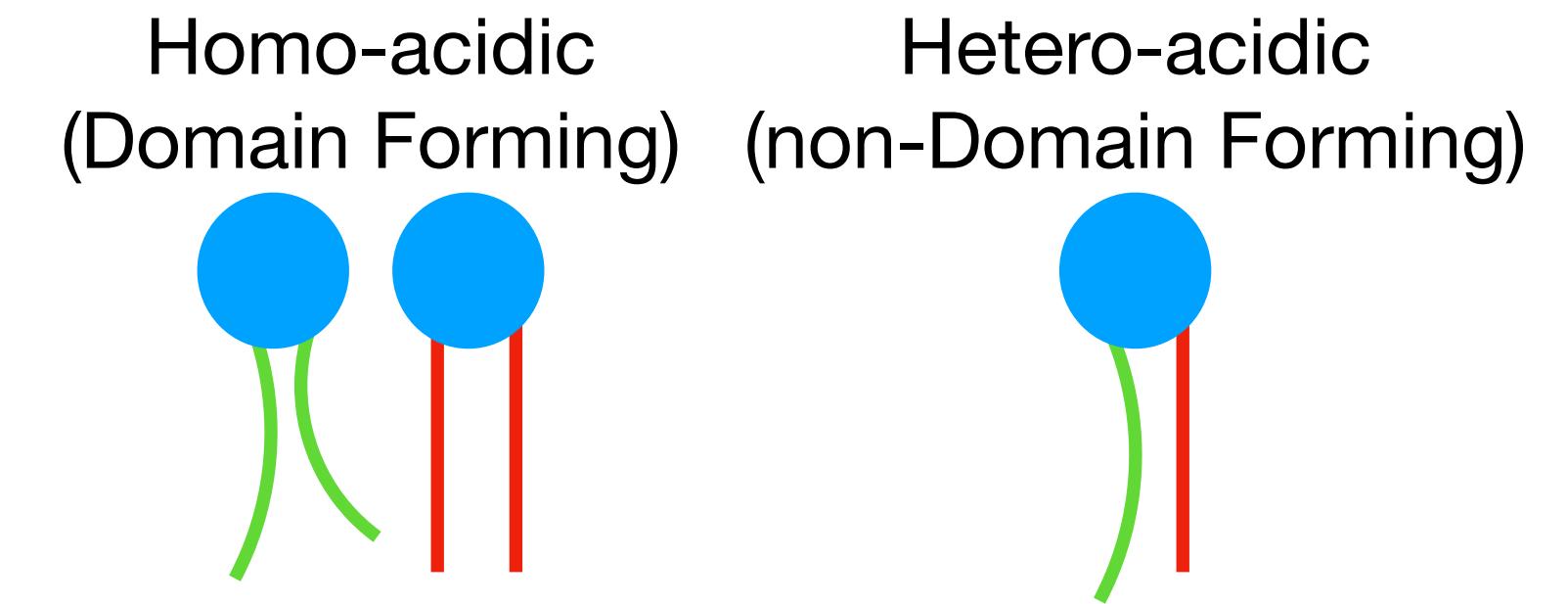
Approach

Simulate nAChR in various model domain-forming and non-domain forming mixtures

- Hetero-acidic lipids tend to be what you find in a living cell

Observe spontaneous domain partitioning

Quantify which lipids are most likely to occupy embedded and annular binding sites

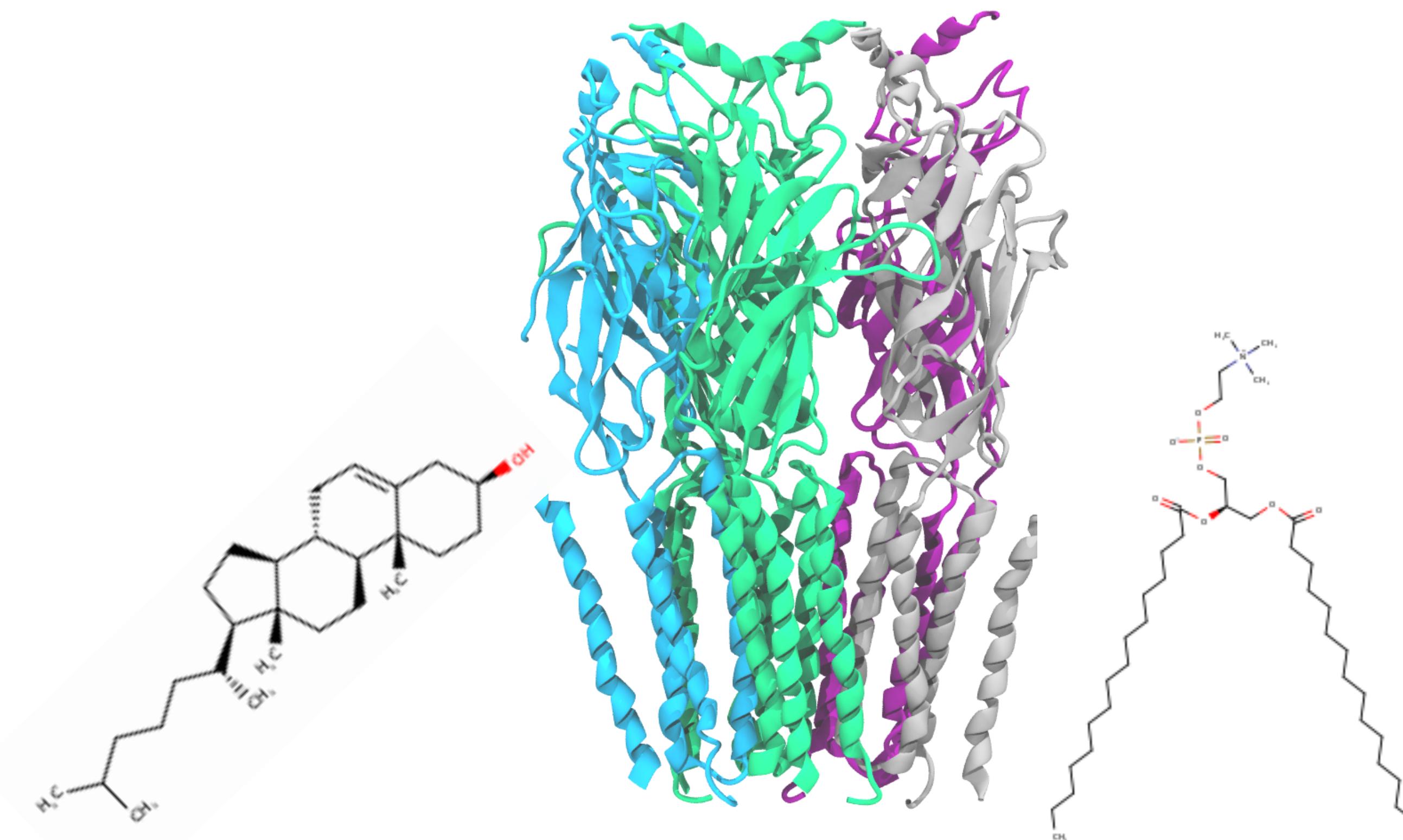


Methods

- Coarse grained molecular dynamics performed with Martini 2.2 and Gromacs 5.1.4
- Uses cryo-EM structure PDB 2BG9
- Membranes 25x25 nm² to 75x75 nm²

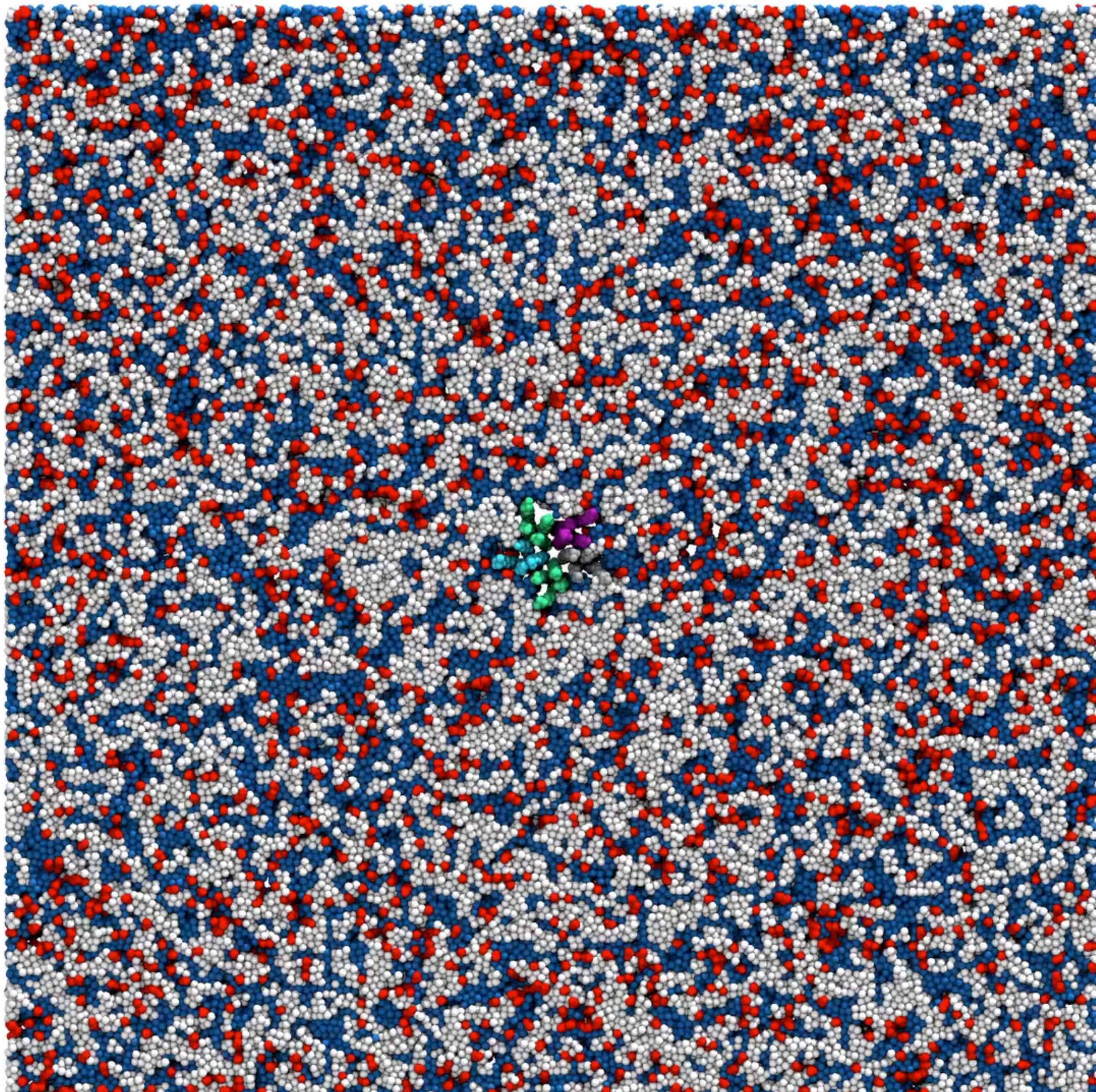
Hypothesis

Initial hypothesis: nAChR is functional dependency on cholesterol suggesting nAChR partitions into cholesterol enriched domain (Marchand et al 2002, Zhu et al 2006, Campagna 2006)



Homo-Acidic Model Membrane Visualization

- 💡 nAChR resides within unsaturated rich region (surprise!!)
- 💡 Hovers near raft forming domain

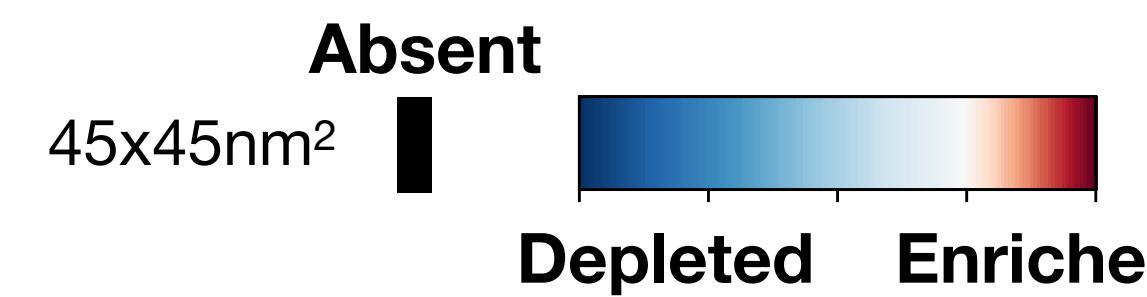
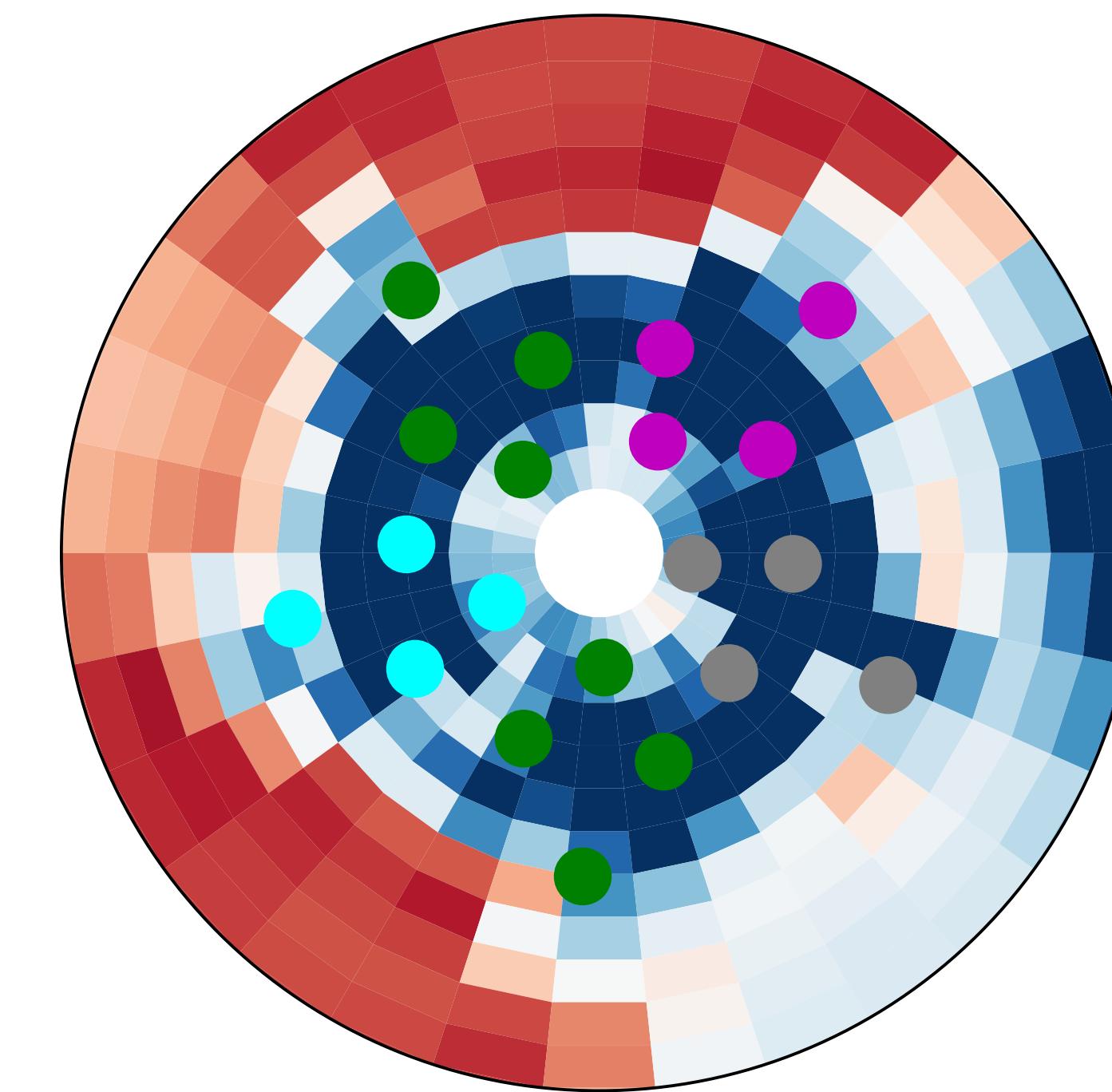


Saturated Cholesterol Unsaturated

~ 4 us

75x75nm²

Reading polar density plots

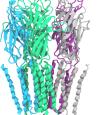


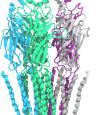
Woods, Sharp, Brannigan, 2019, Journal of Membrane Biology

Density enrichment in domain forming membranes

n-3 Ternary

Sat

 Strong enrichment of PUFAs in nAChR boundary

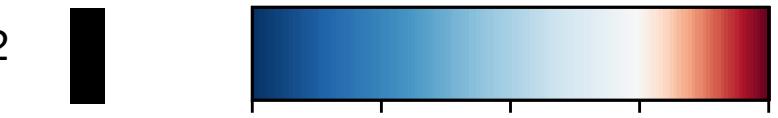
 PUFAs embed in protein

Chol

PUFA

Absent

45x45nm²



Depleted Enriched

Sharp et al, 2019, BBA

Density enrichment in domains vs non-domain forming membranes

Depleted

Enriched



25x25nm²

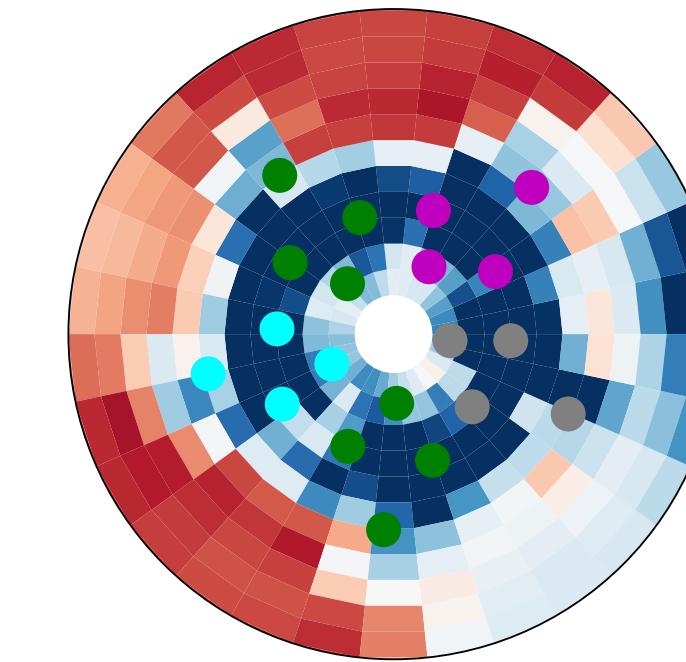
Hetero-Acidic

(non-Domain Forming)

Homo-acidic

(Domain Forming)

PUFA



Cholesterol

- When domains are removed:
 - PUFA enrichment decreases
 - Symmetric, highly-localized sites emerge
 - Most native lipids will be heteroacidic and restrict domain formation

Conclusion

• If domains form, which domain does nAChR reside in?

- PUFA rich domains

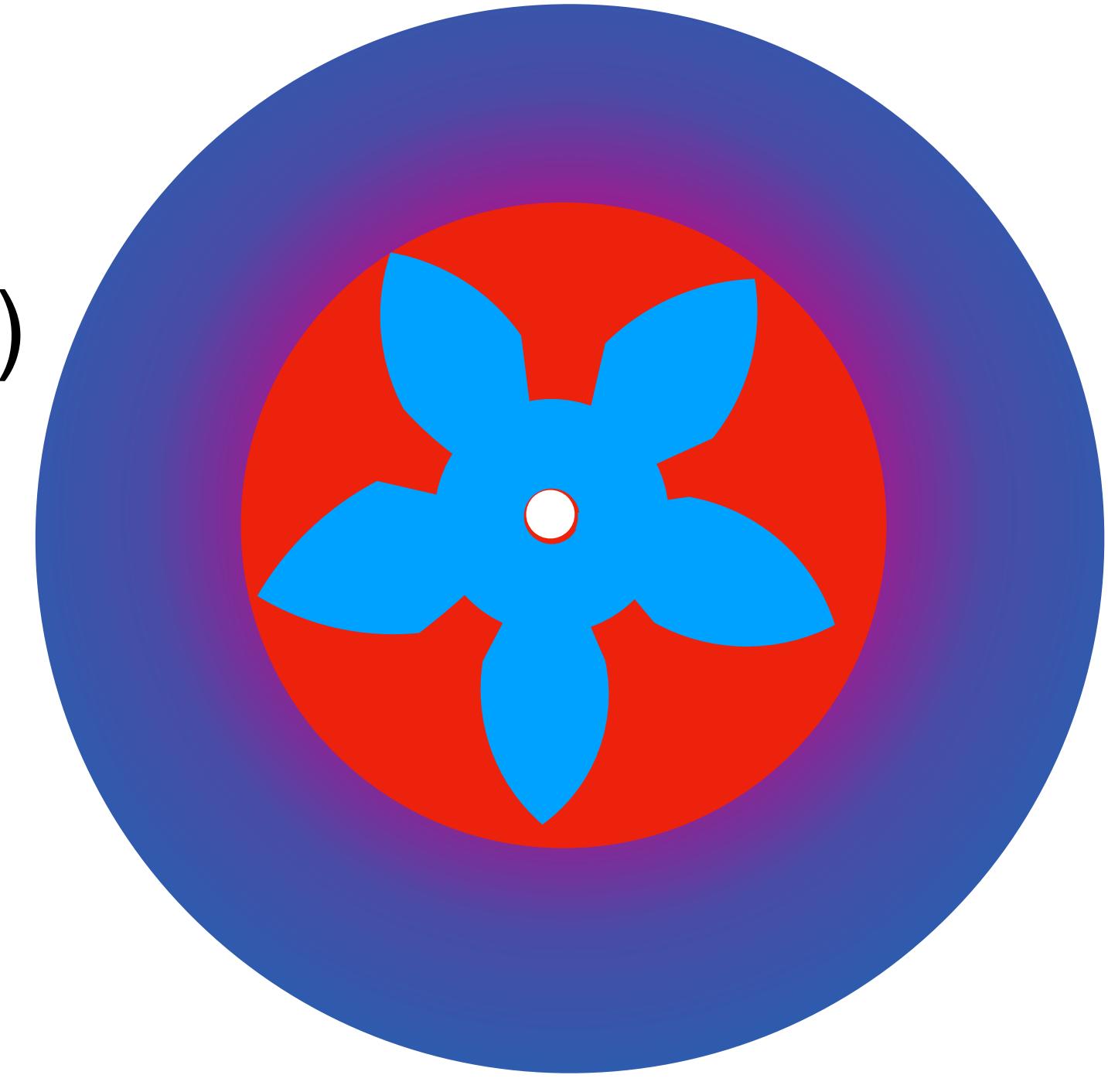
• What are the boundary lipids in PUFA rich membranes with homo- and hetero-acidic lipids?

- Homo-acidic: PUFA only
- Hetero-acidic: A mixture of everything, with localized sites

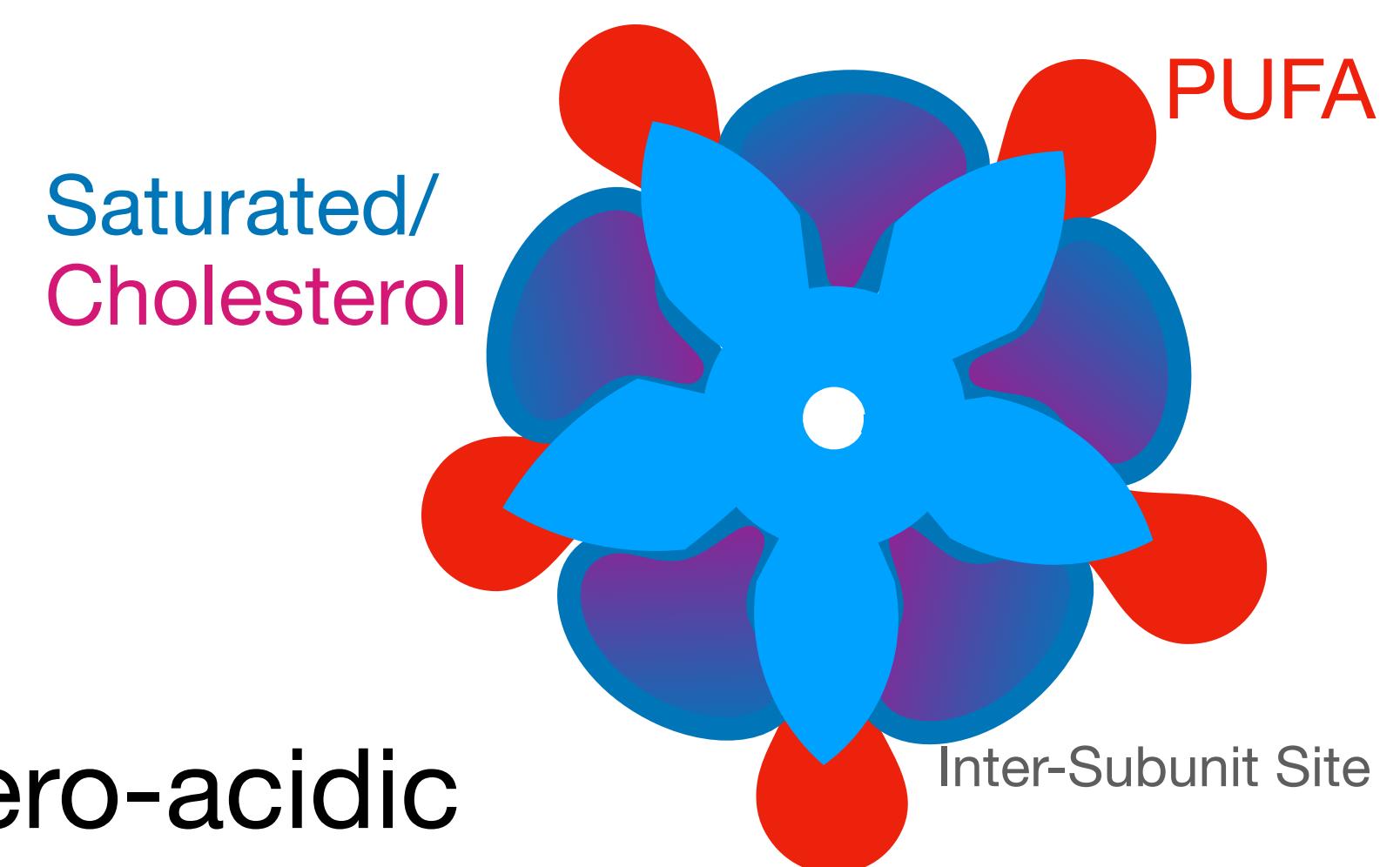
• Are there preferential binding sites?

- Only ascertainable in hetero-acidic: PUFAs occupy M4 and saturated and cholesterol occupy inter-subunits

Homo-acidic
(domain forming)

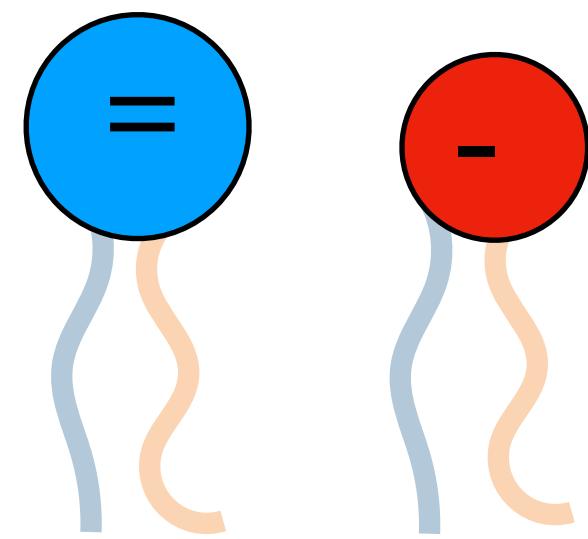


Hetero-acidic
(no domains)



Outline

- Introduction
- Saturation, Sterols, and Domain-forming lipids: Identifying nAChR boundary lipids in PUFA-rich model membranes
- **Lipid head-group charge: Boundary lipids for a bacterial sister channel in charged model membranes**
- Putting it all together: Quantifying specific lipid-binding affinities in complex native-like membranes



The effect of neutral and anionic lipids on ELIC in model membranes



Goal:

- Elucidate if charged phospholipids bind to ELIC
- Determine where they bind
- Determine which sites modulate function

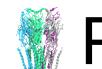
Approach

Experimental

 Giant Liposomes of POPC, POPE, and POPG at various compositions using:

- Native Mass Spectrometry to determine specific bound lipids and number of bound lipids
- Functional studies to test channel functionality in membrane compositions

Computational

-  Perform the role of computational microscopy to visualize lipid-ELIC binding
-  Simulate ELIC in model charged membranes
-  Predict lipid distributions around ELIC
-  2 Series of 15 coarse grained simulations of ELIC-lipid interaction run for 15 μ s each

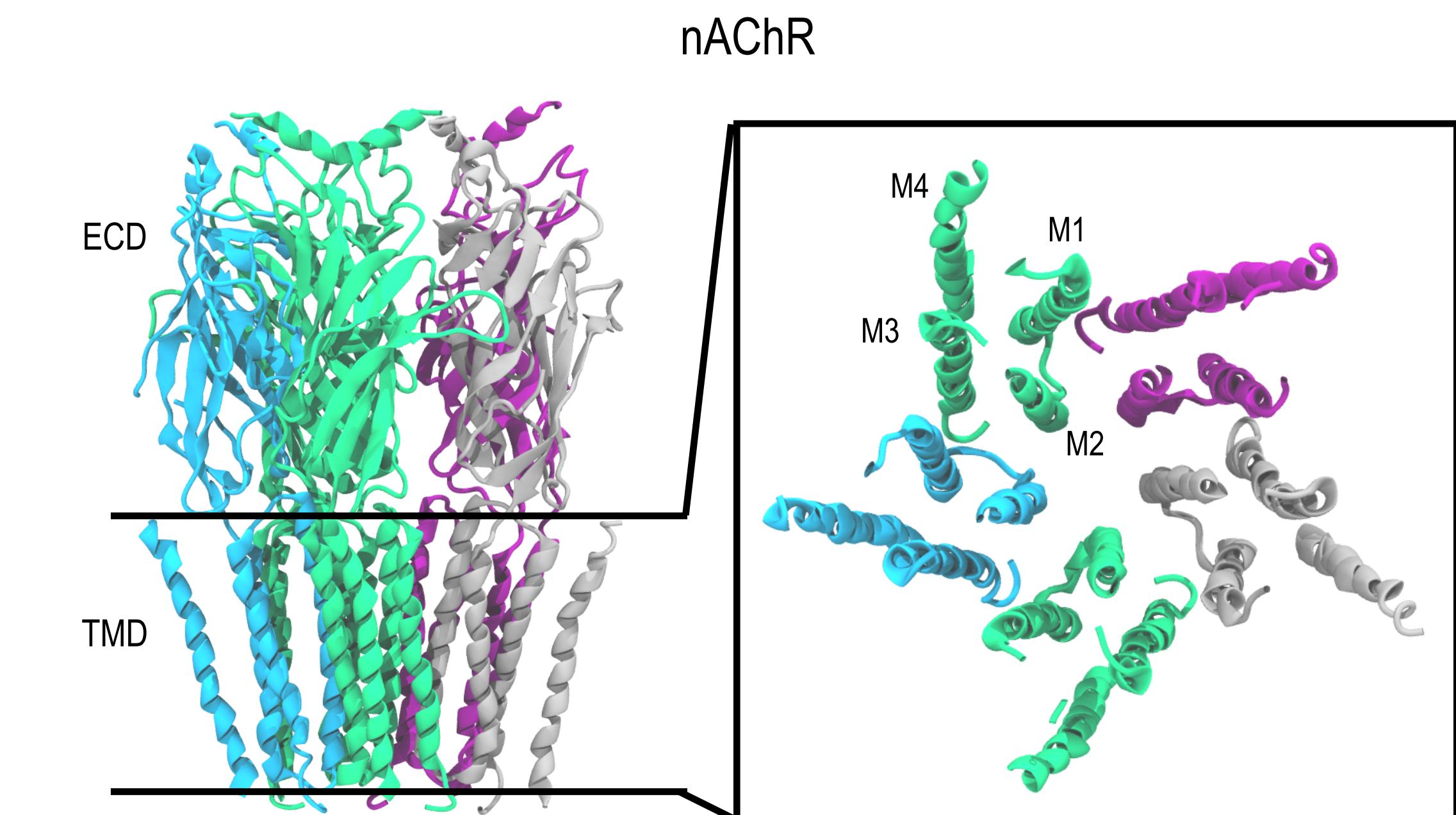
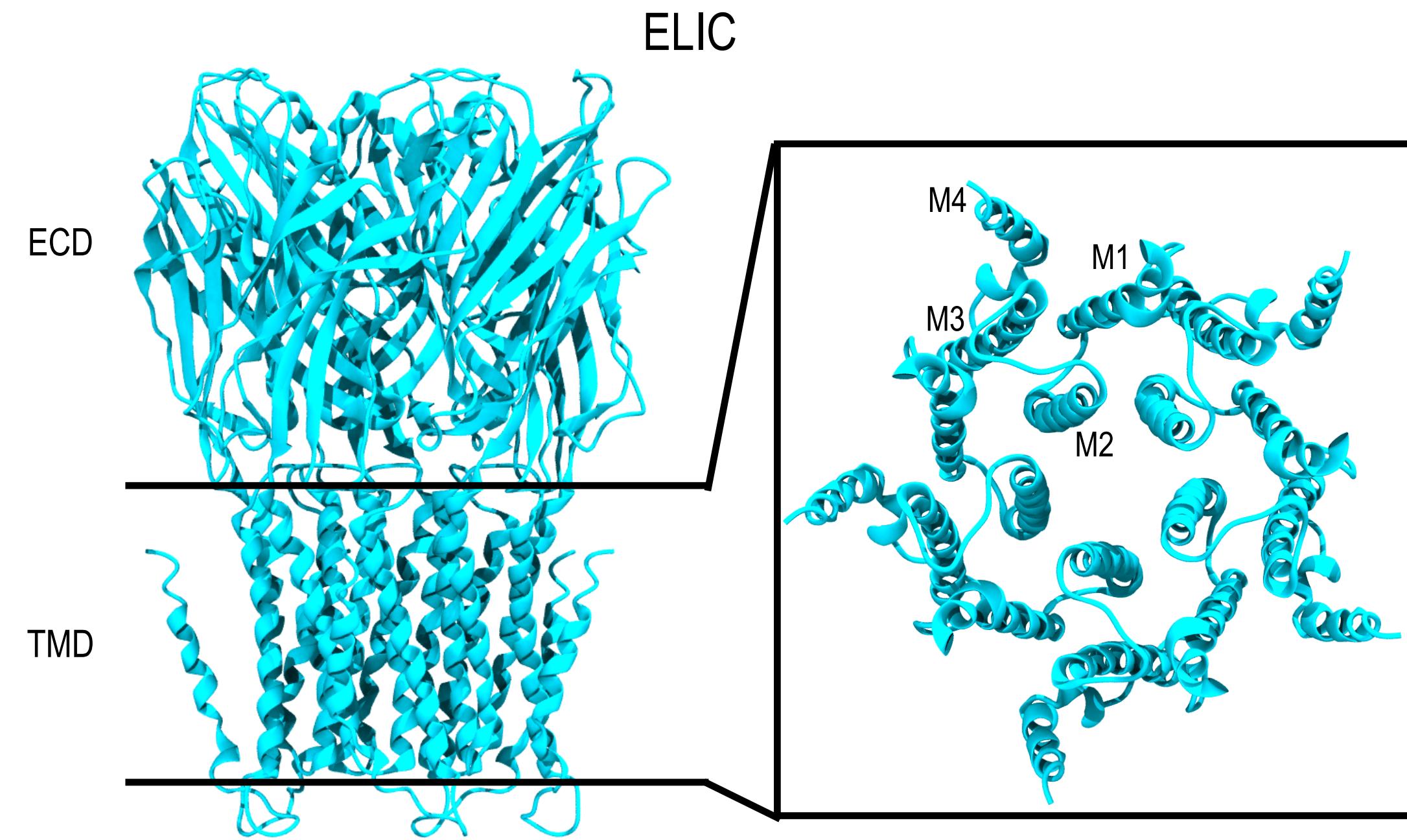
Methods

-  Coarse grained molecular dynamics performed with Martini 2.2 and Gromacs 2016.2
-  Membranes 25x25 nm²
-  Uses ELIC PDB 3RQW crystal structure

What do we expect?

💡 nAChR is known to be dependent on anionic lipids under some conditions

💡 ELIC is a bacterial channel in the same family as nAChR



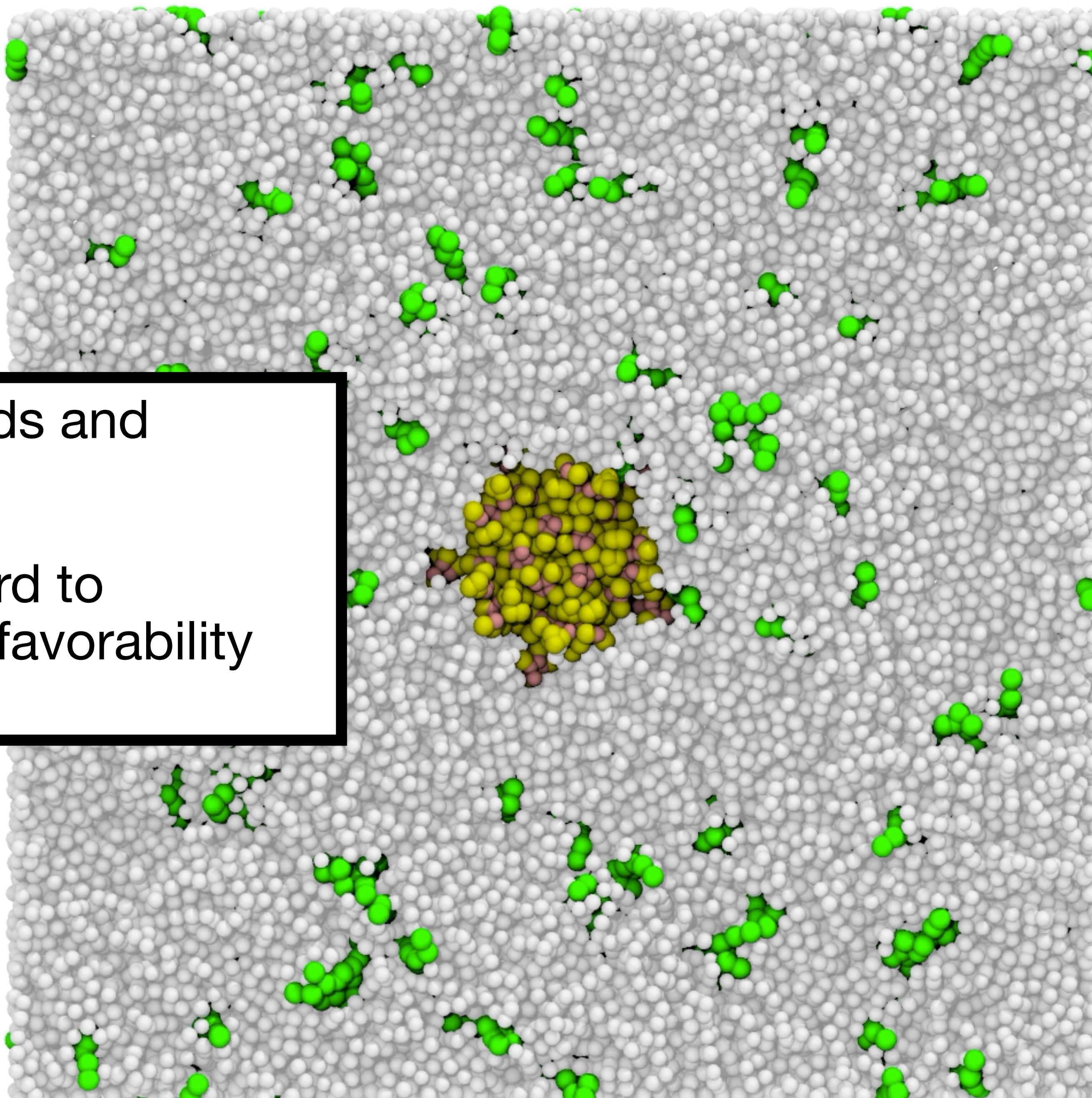
Visualization of results

85:15 POPE:POPG

Neutral

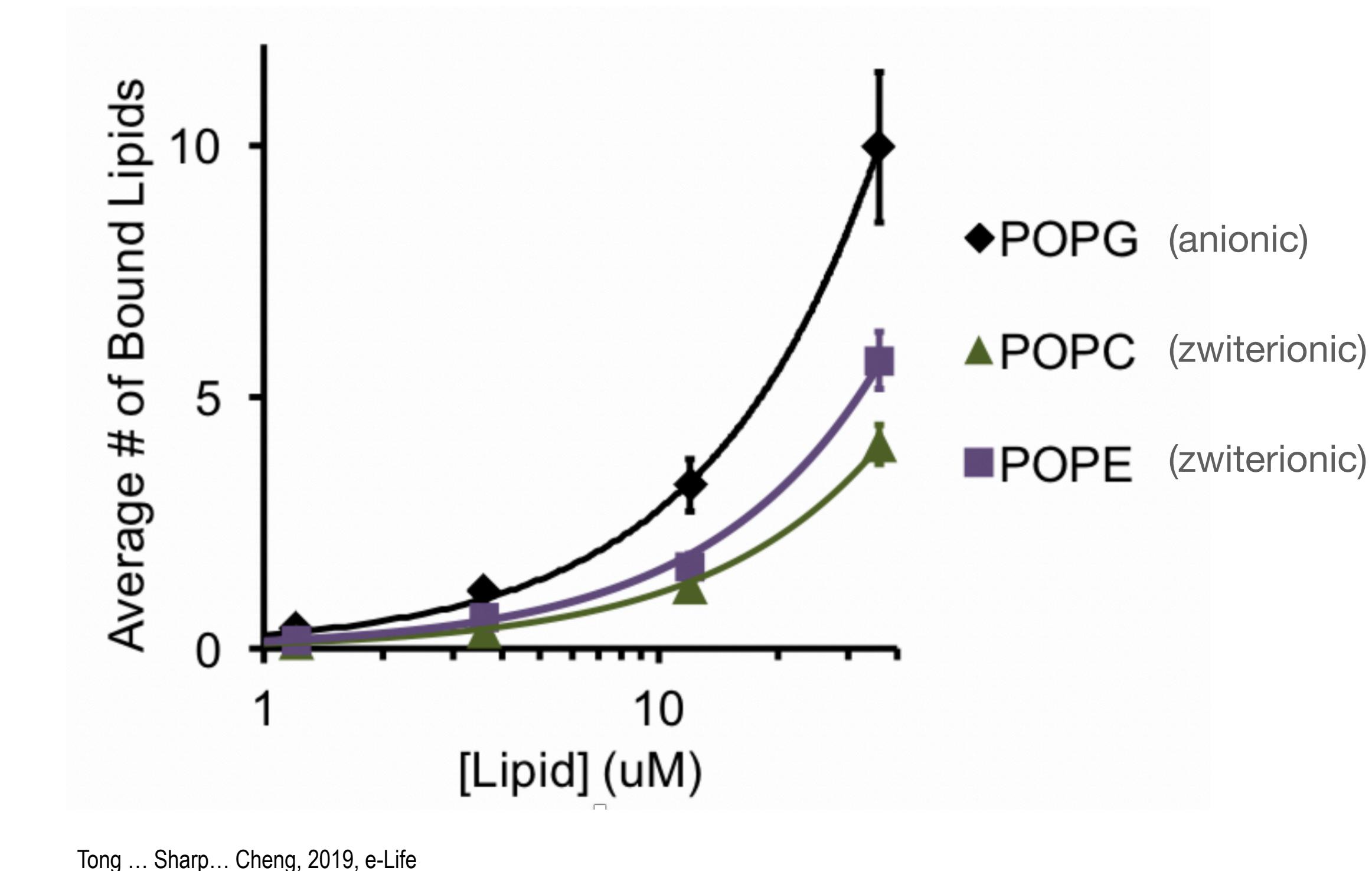
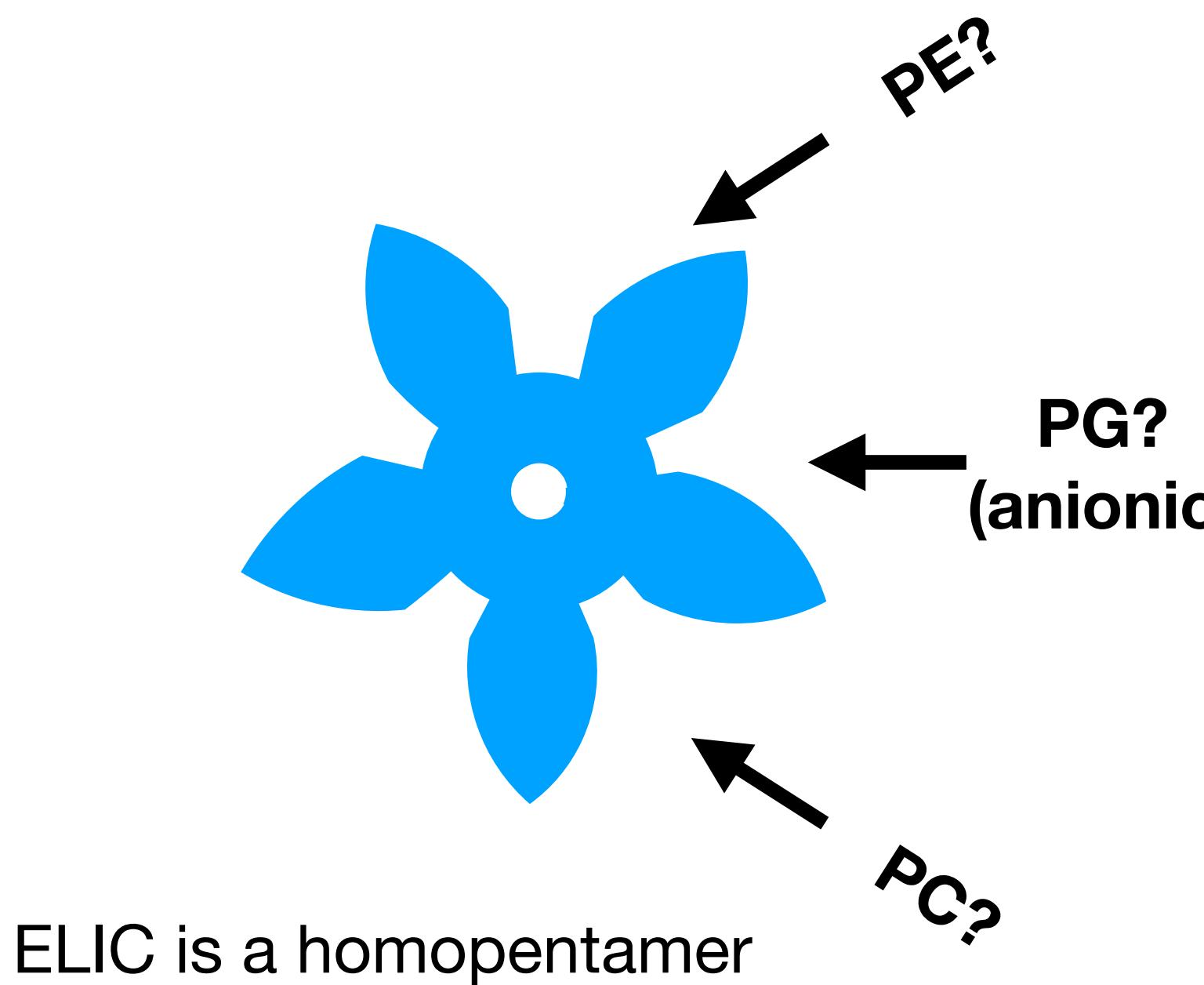
Anionic

- POP-G quickly binds and unbinds
- Not straight forward to determine PG/PE favorability visually



Mass Spec: No matter the concentration there is more PG

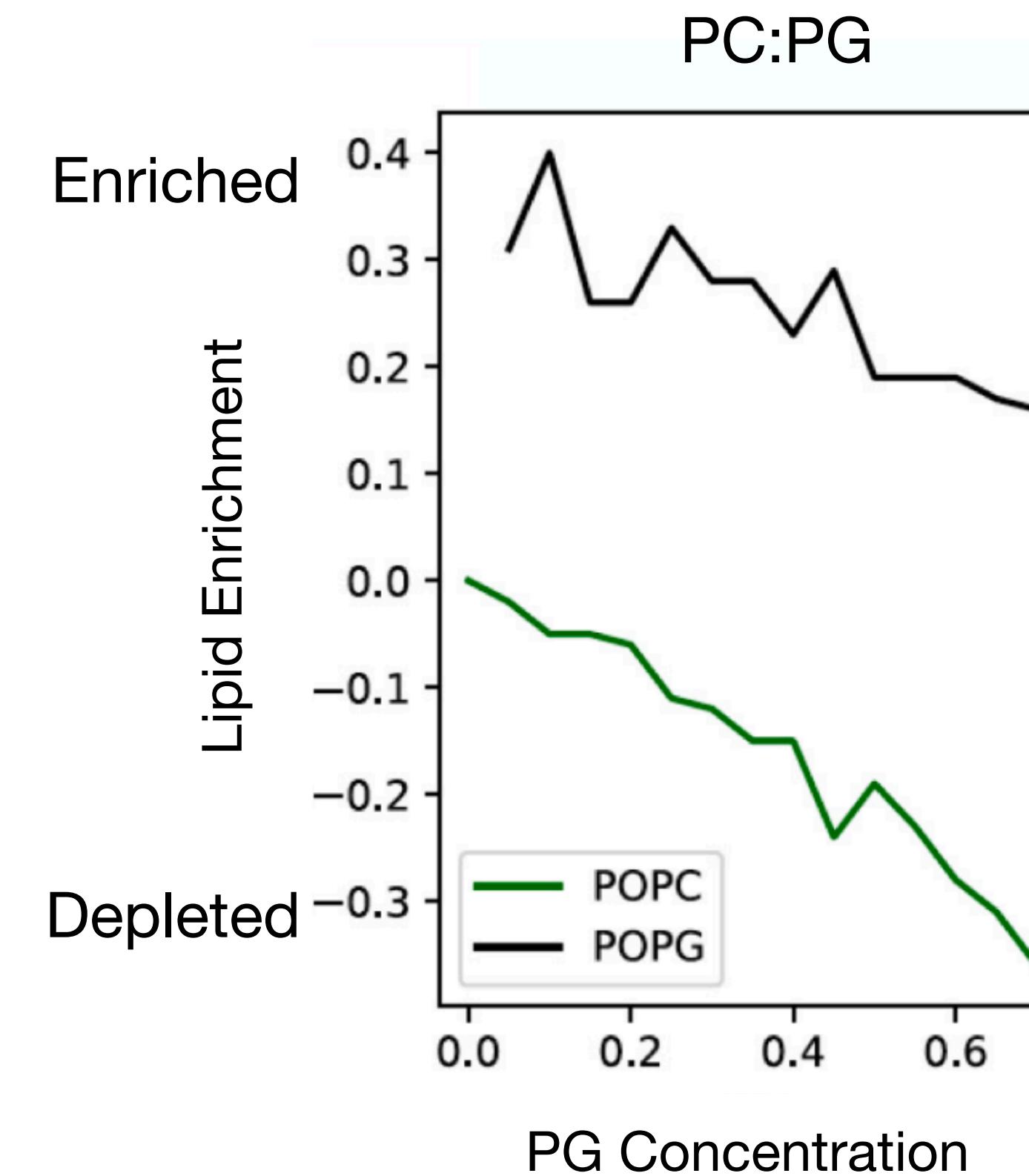
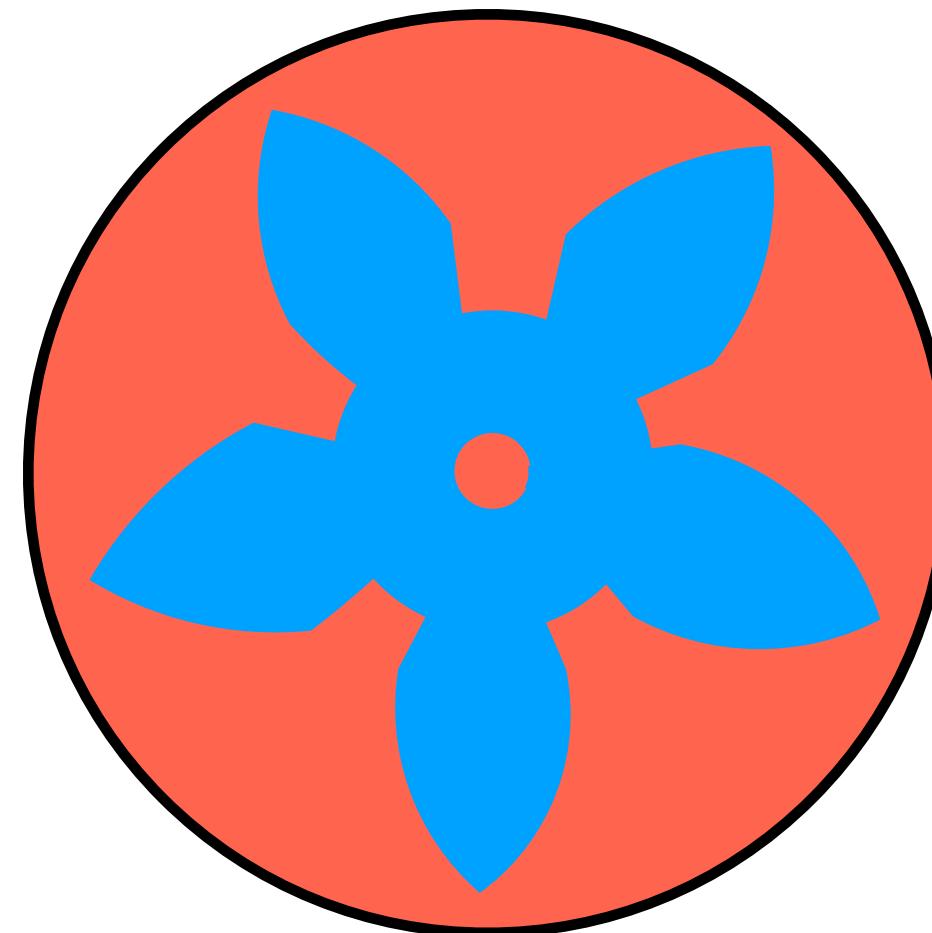
Bound PG > PE > PC
Cannot tell you where its bound



Tong ... Sharp... Cheng, 2019, e-Life

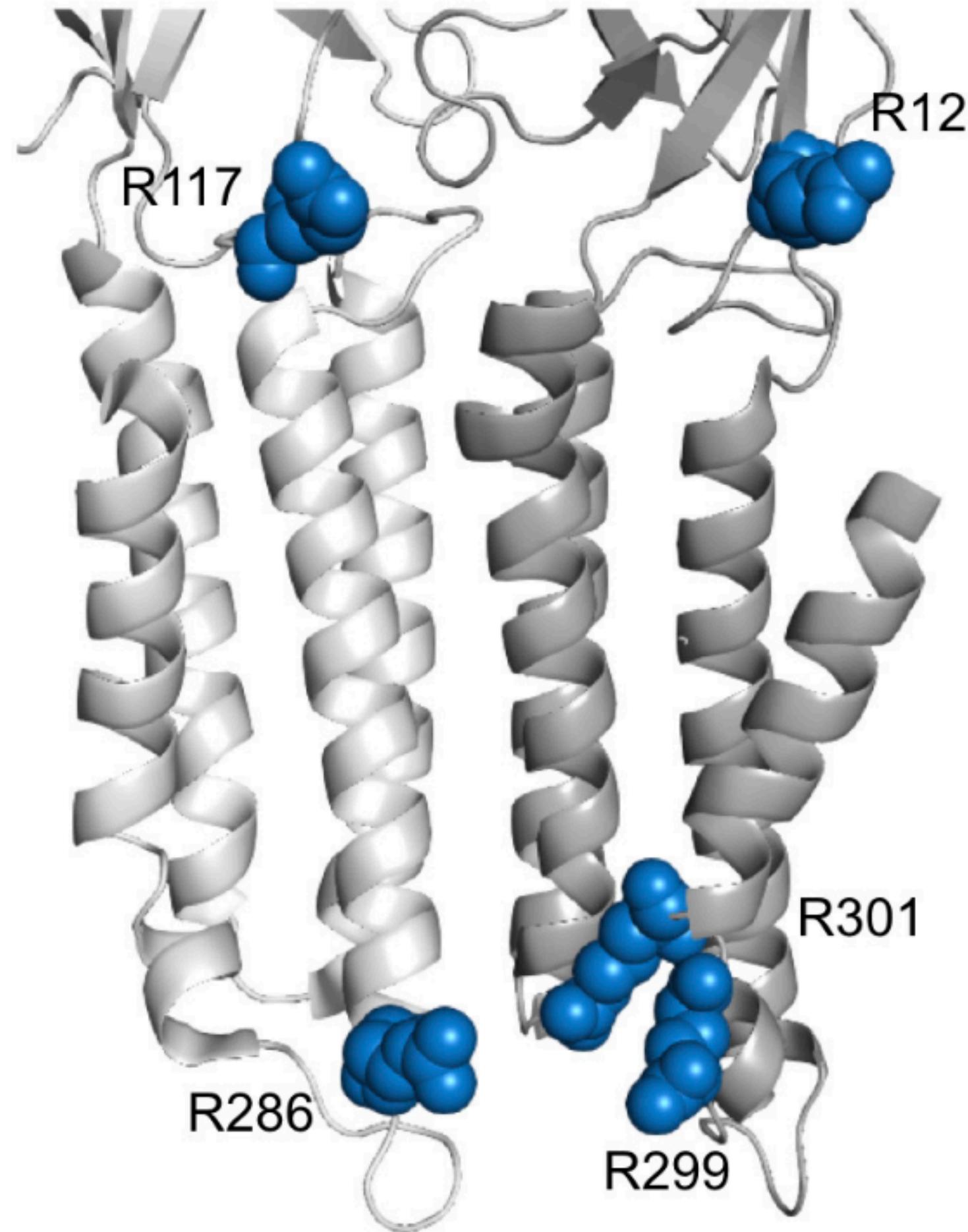
Computational: Simulations show PG enrichment in the boundary

- POPG shows greatest enrichment at low concentrations of itself
- Suggests specific site occupation
- Does not specify lipid binding sites



Tong ... Sharp... Cheng, 2019, e-Life

Experimental: PG binding is dependent on arginine residues

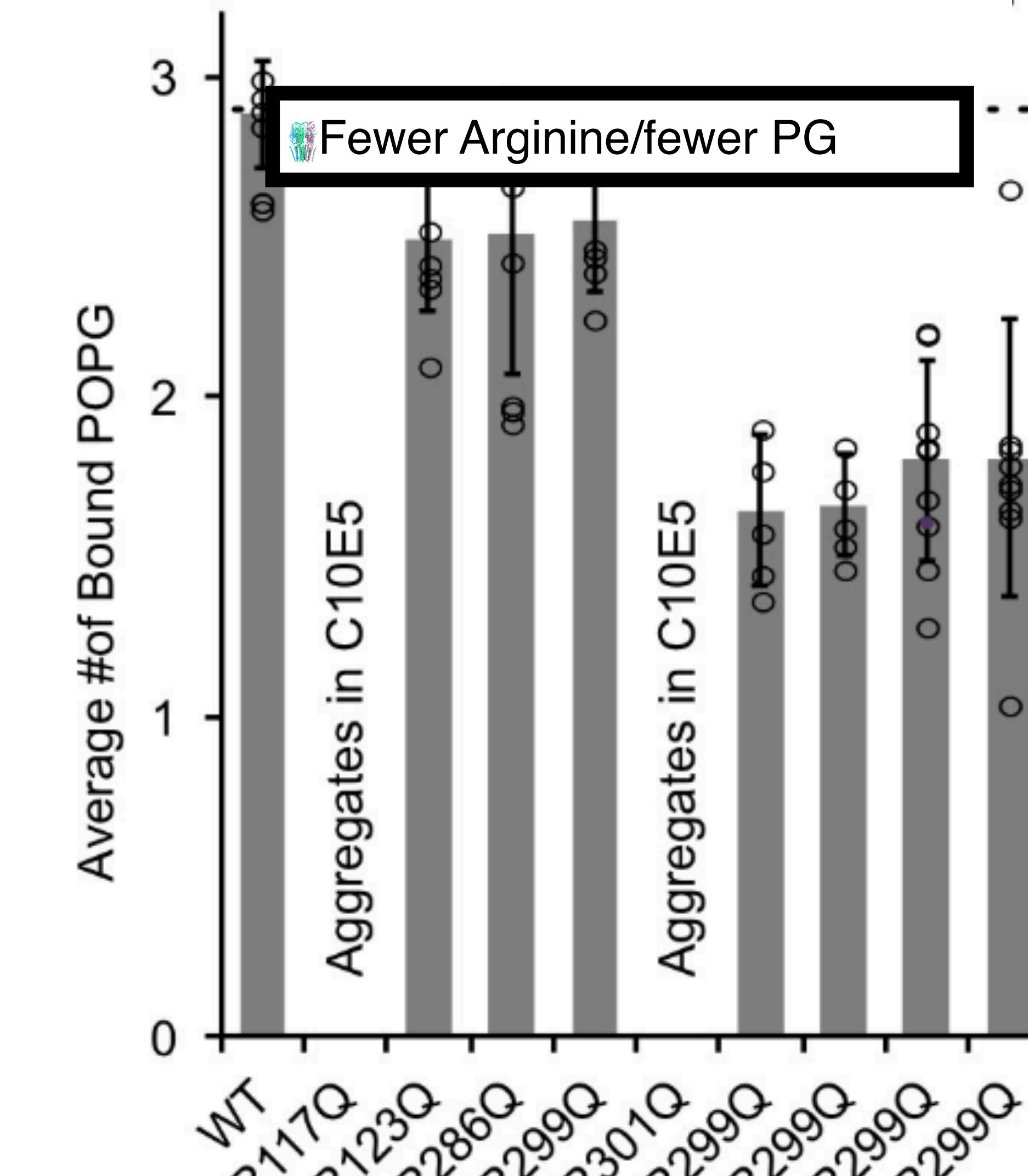


Arginine -> Glutamine

(+) -> (=)

Positive
Charge

Polar
Neutral

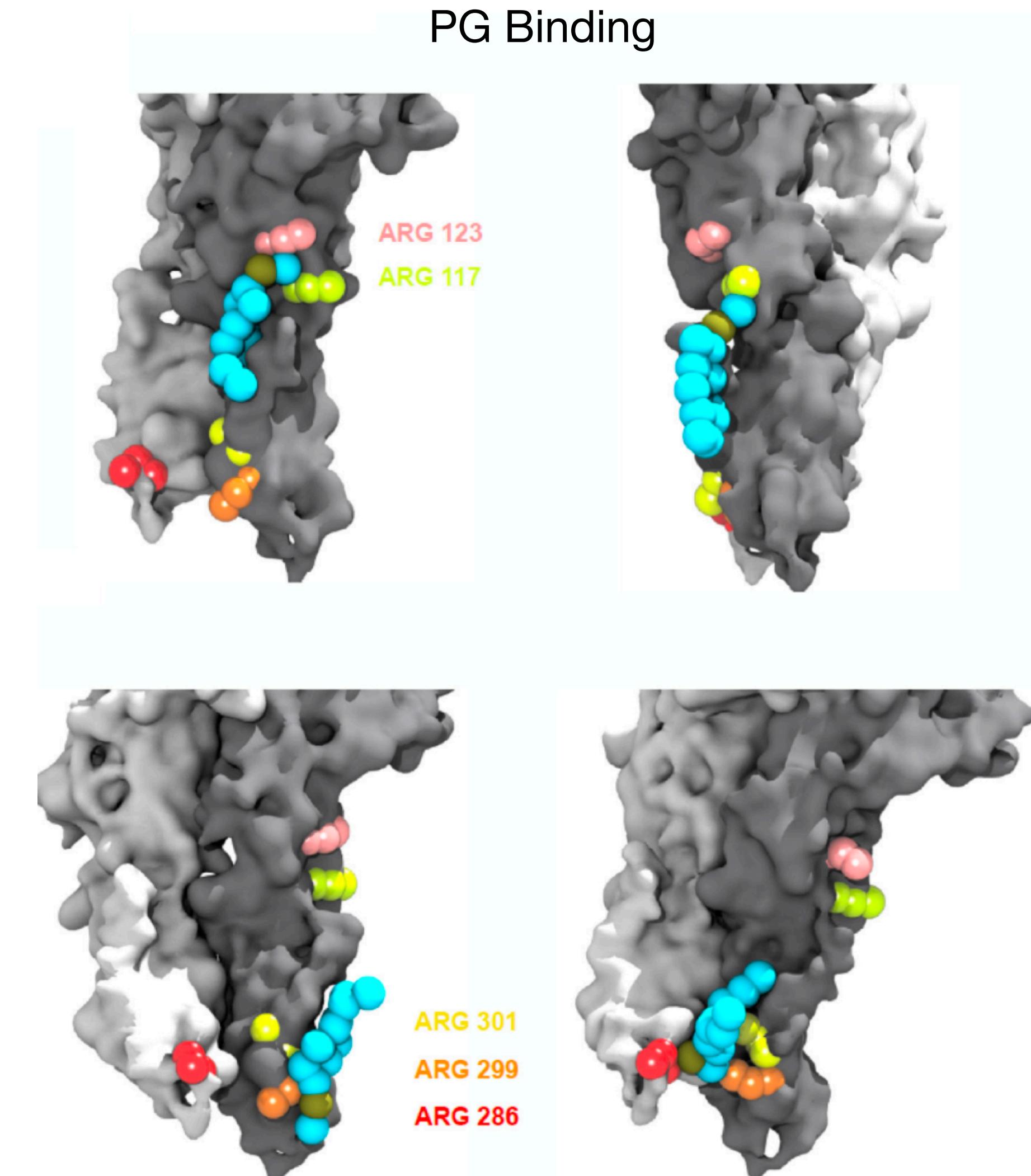
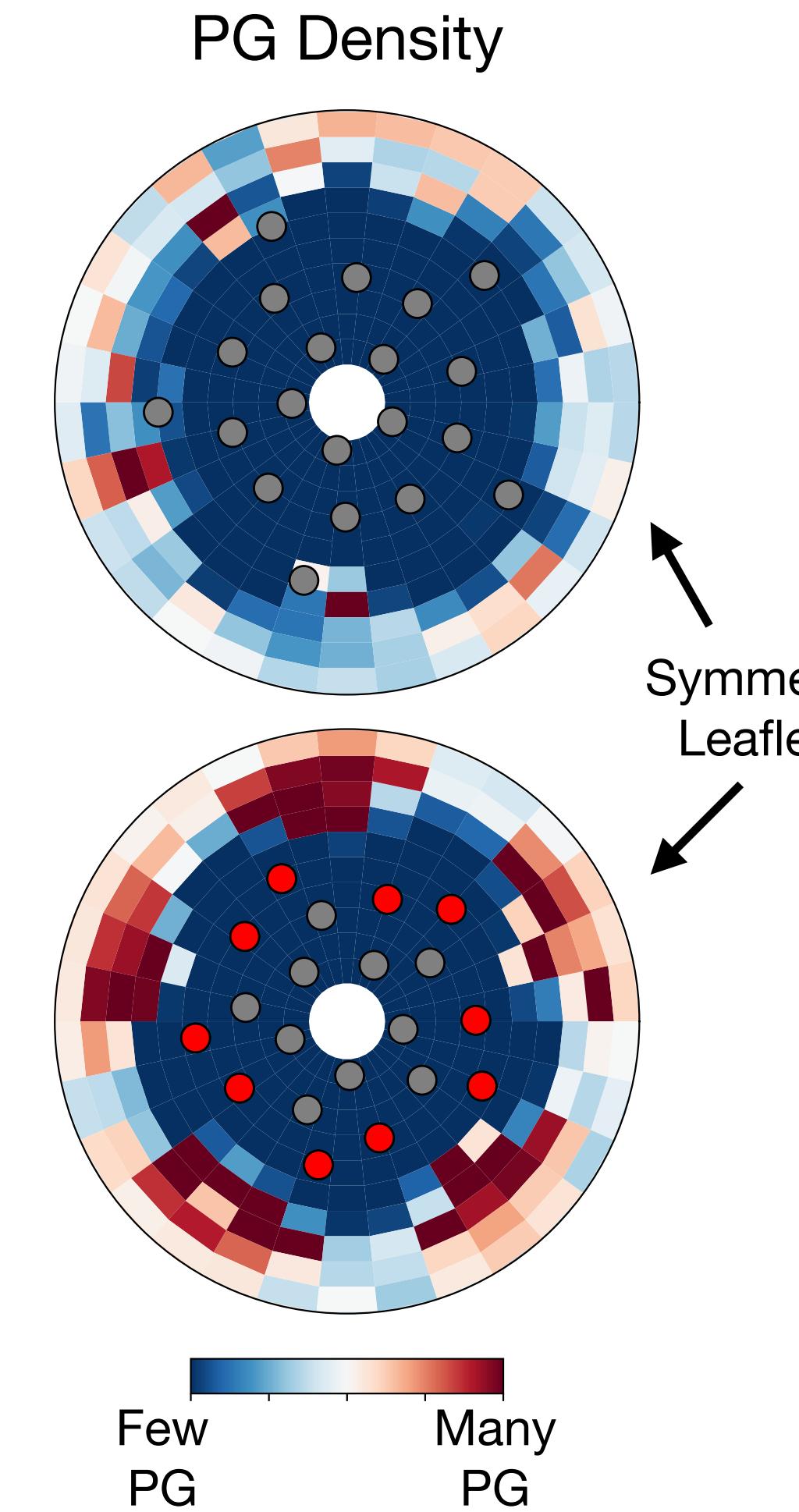
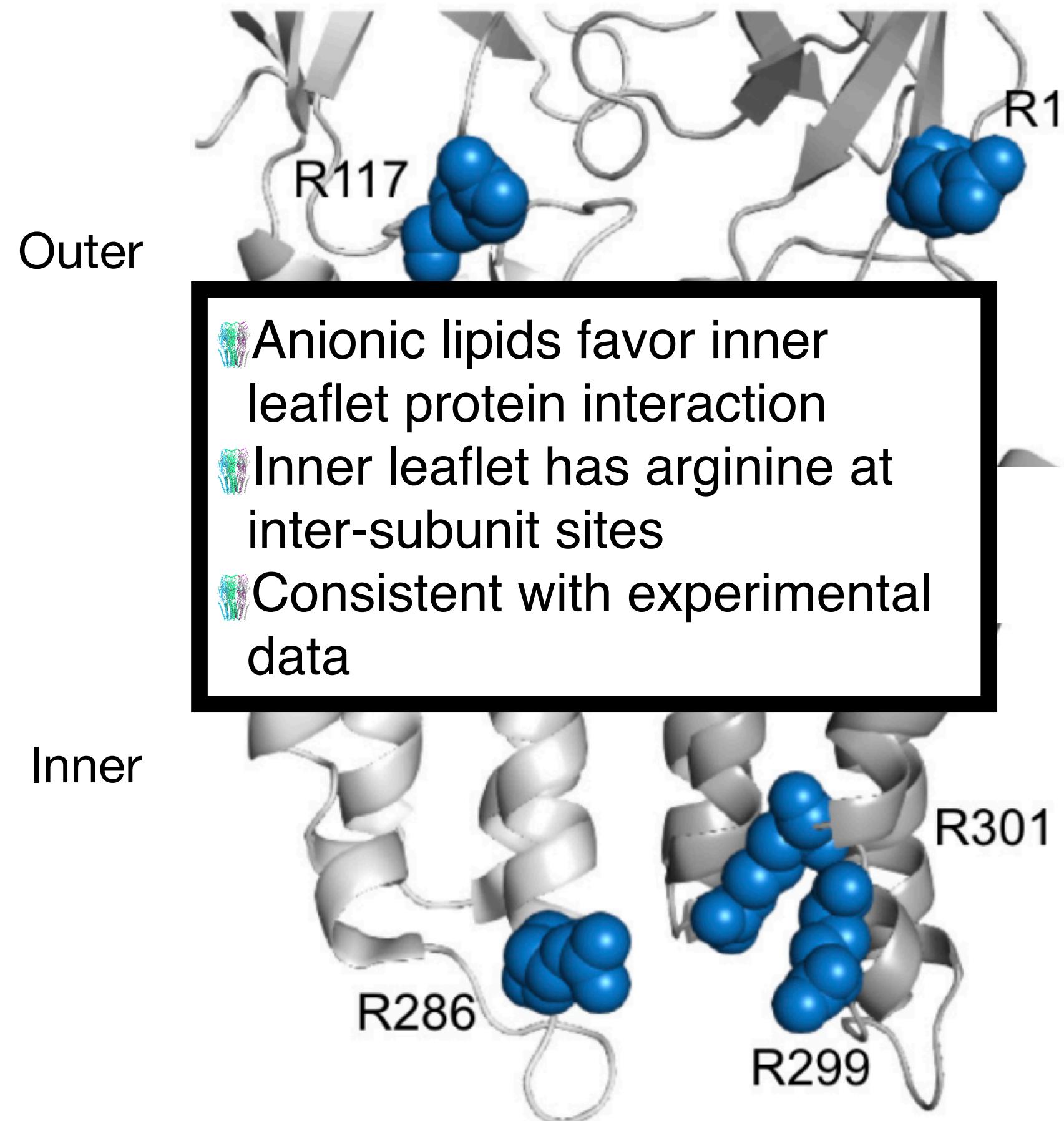


Single
Mutant

Double
Mutant

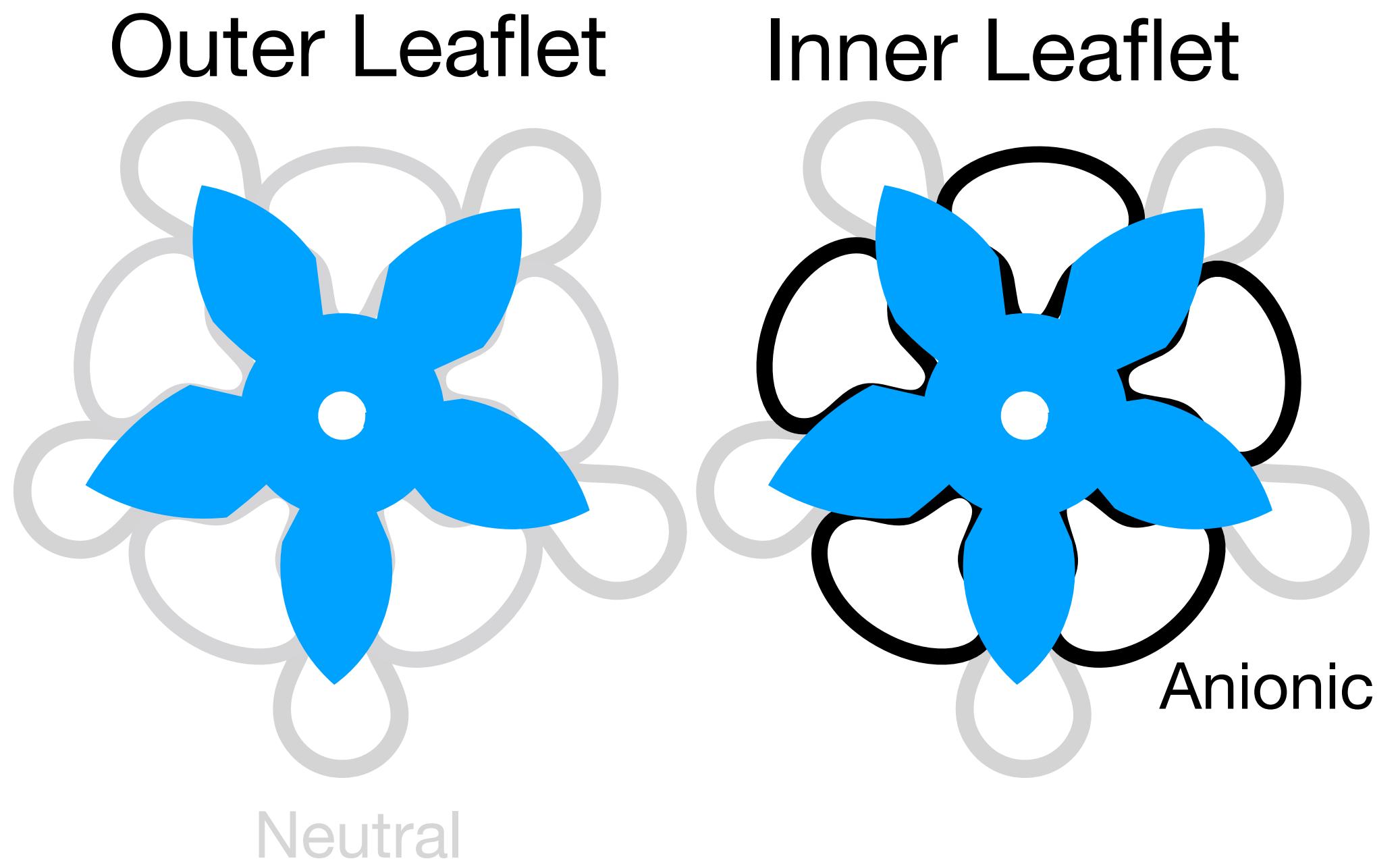
Tong ... Sharp... Cheng,
2019, e-Life

Computational: PG Density Related to Accessible Arginine



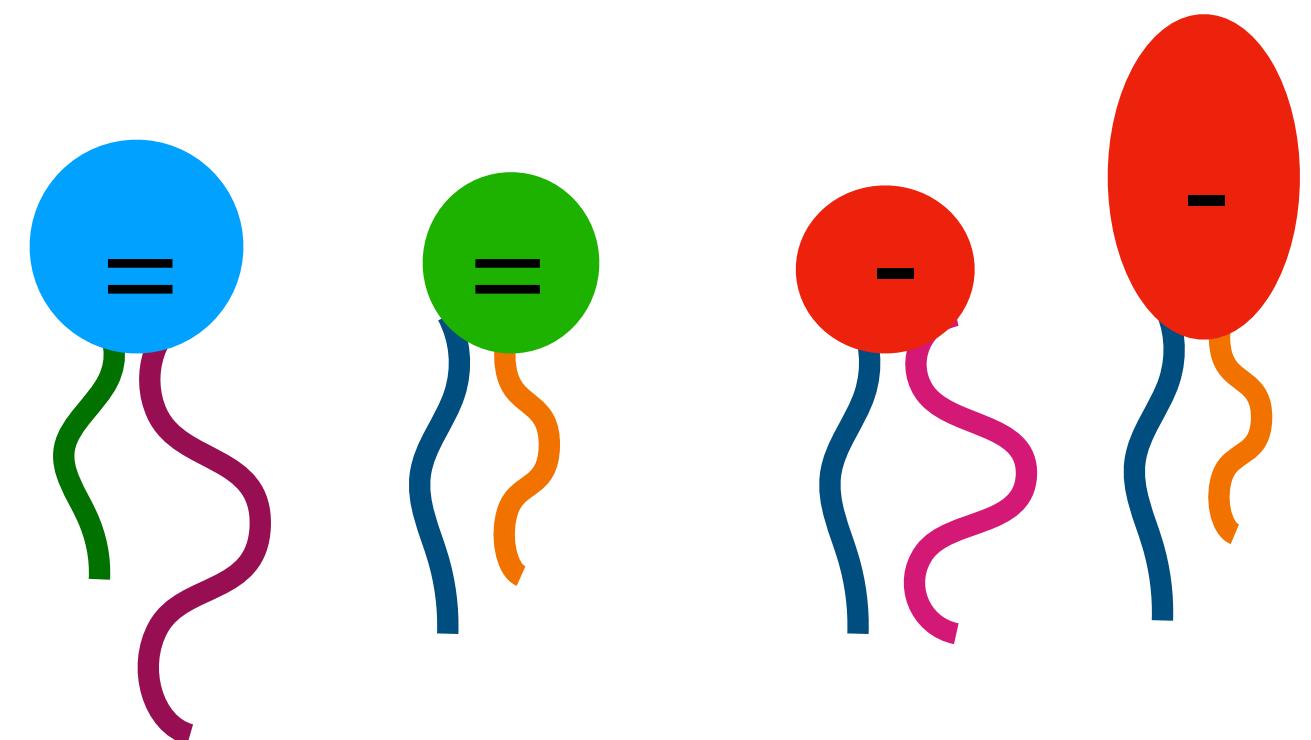
Conclusion

- Elucidate if charged phospholipids bind to ELIC
- ELIC boundary region is enriched in PG
- Determine where they bind
- PG tends to bind at inter-subunit sites in the inner leaflet to cationic amino acids
- Determine which sites modulate function
- Cationic amino acids play a role in both PG binding and ELIC function
- Simulations are consistent with experiments



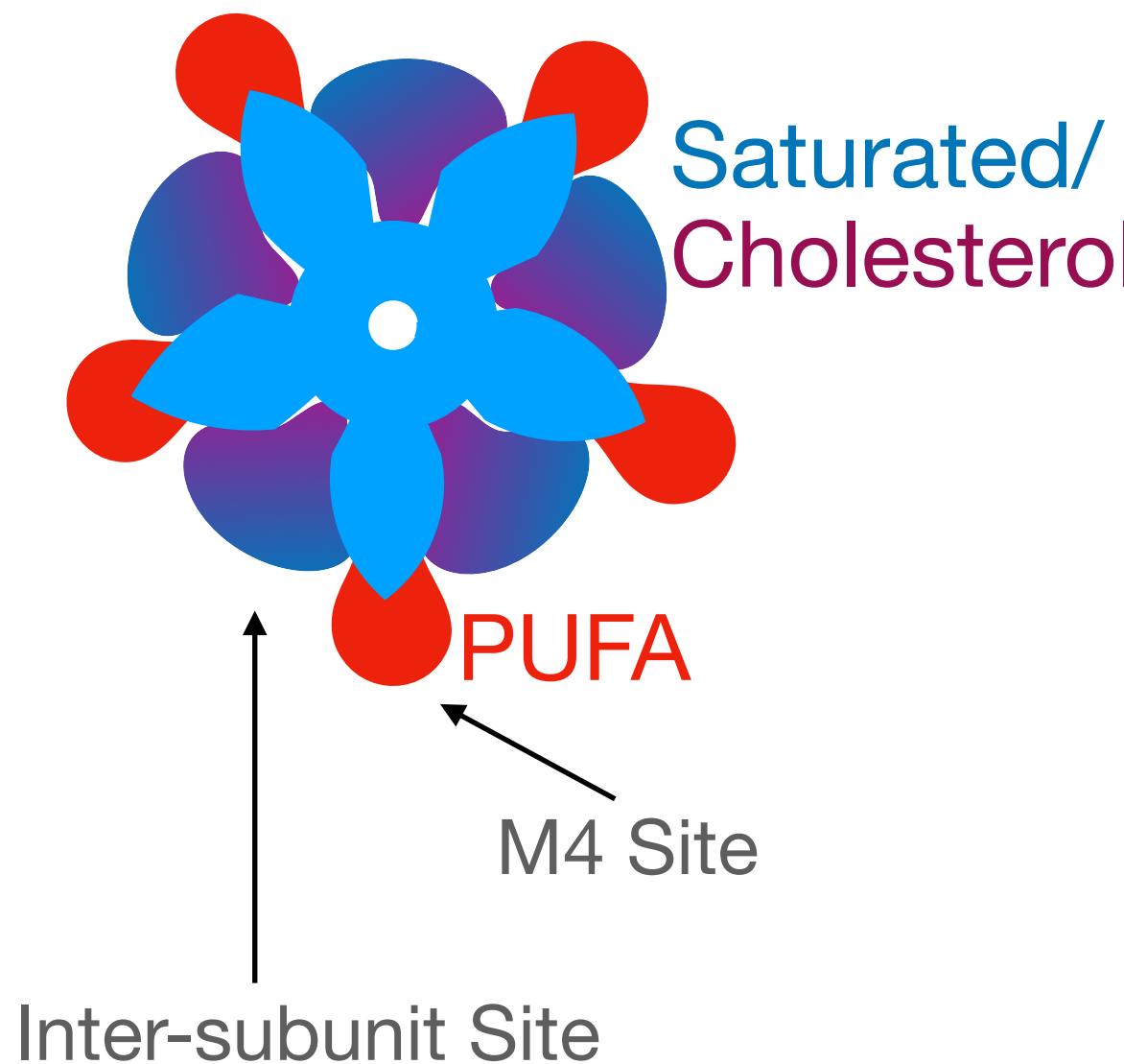
Outline

- Introduction
- Saturation, Sterols, and Domain-forming lipids: Identifying nAChR boundary lipids in PUFA-rich model membranes
- Lipid head-group charge: Boundary lipids for a bacterial sister channel in charged model membranes
- Putting it all together: Quantifying specific lipid-binding affinities in complex native-like membranes

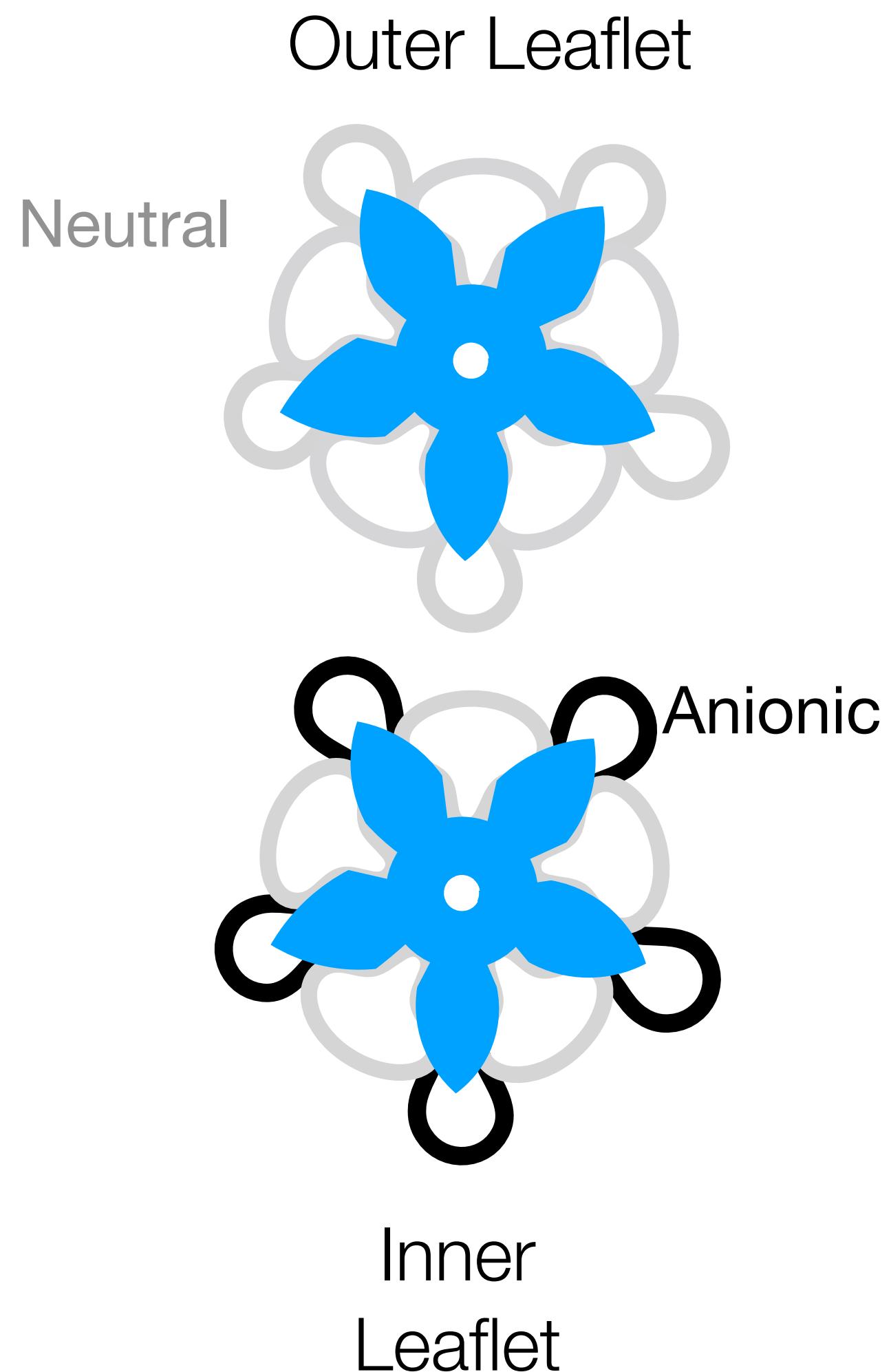


What have we learned so far?

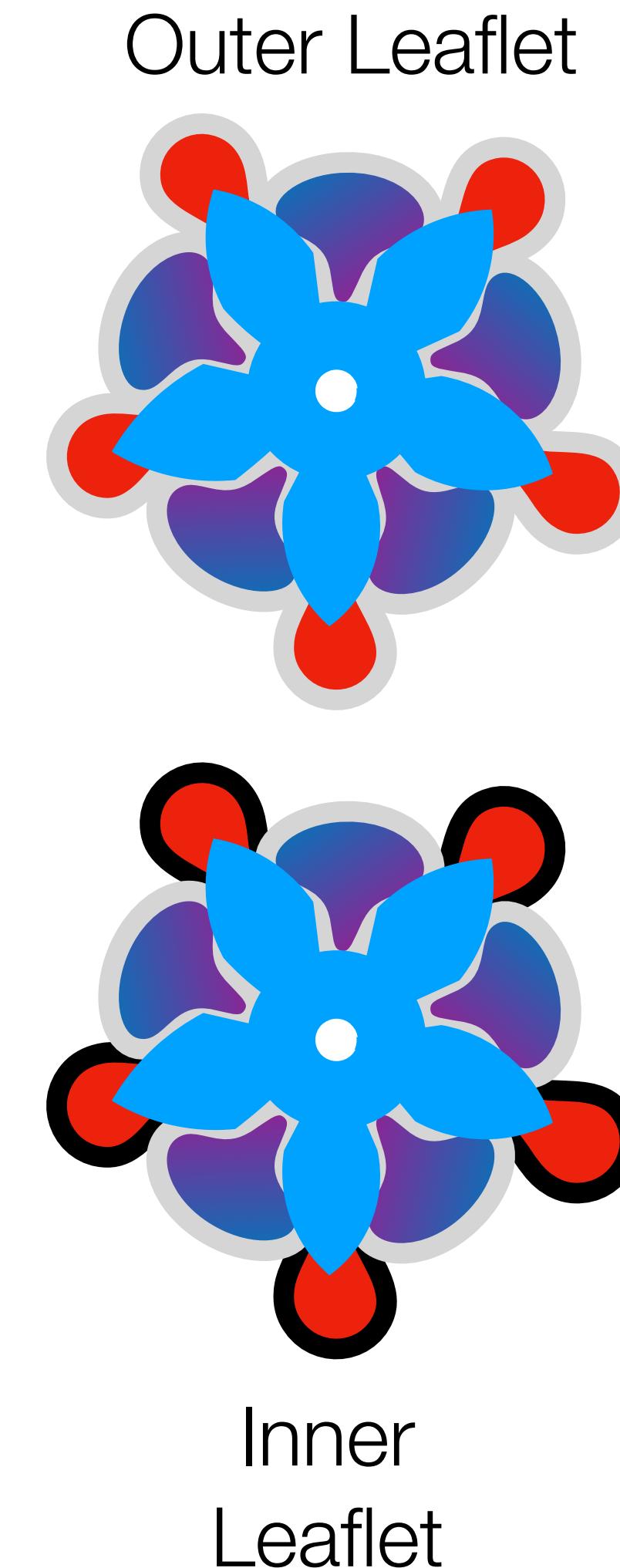
Section 1



Section 2



Combined ?



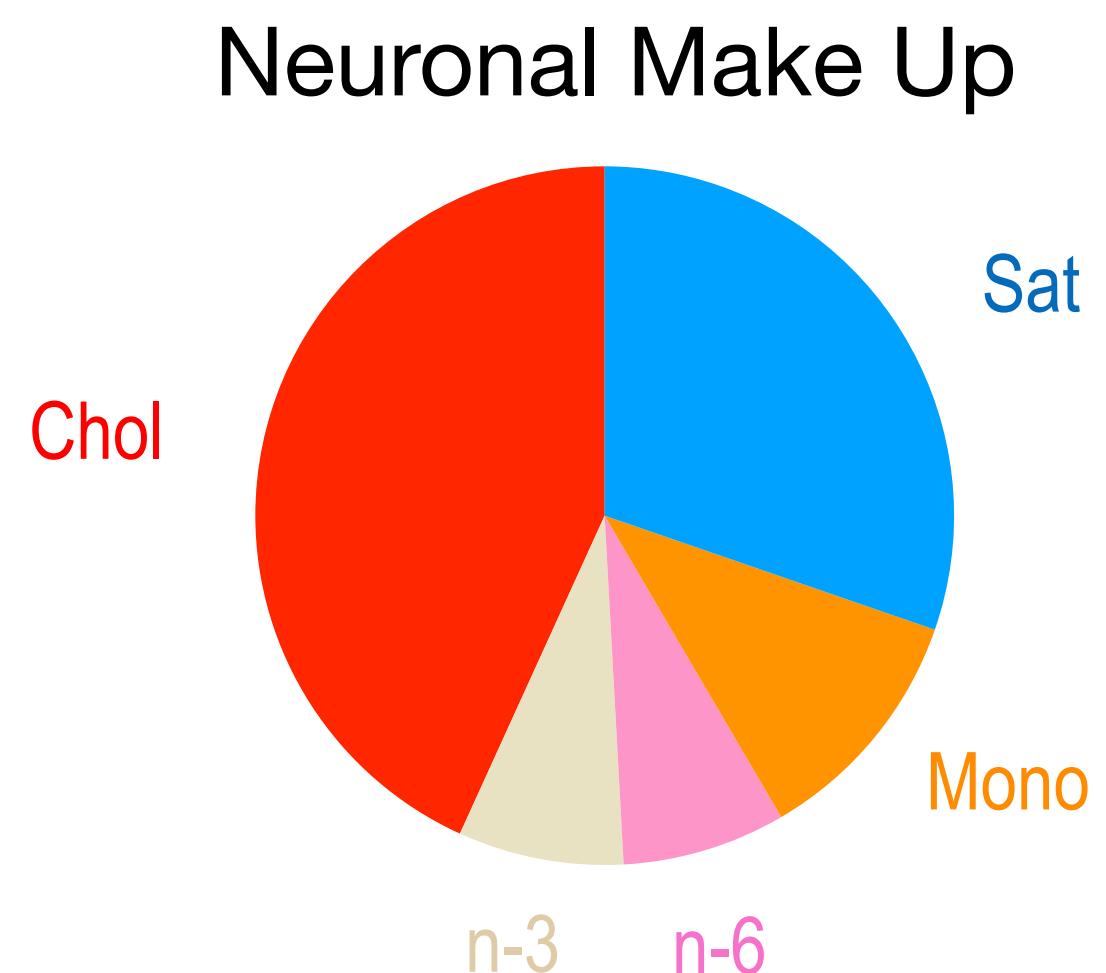
Goal

- What are the boundary lipids around nAChR in a native membrane?
- How are the lipids distributed?
- And can we calculate these lipid affinities?

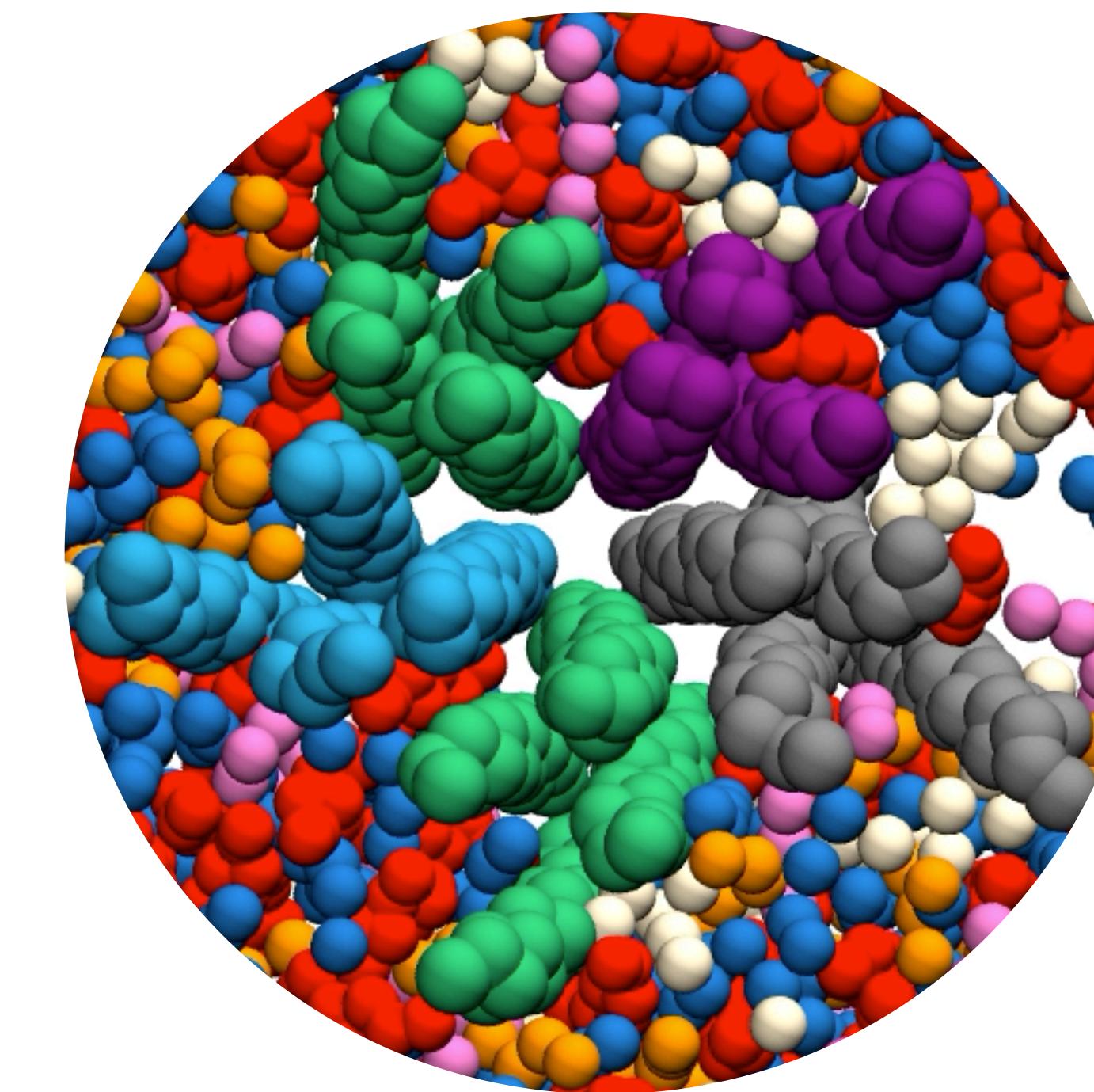
Approach

- Simulate nAChR in coarse grained neuronal membranes
(Ingólfsson 2017)
- Compare lipid distribution to previous analysis
- Determine lipids with the highest binding affinities for inter-subunit and M4 sites for both leaflets

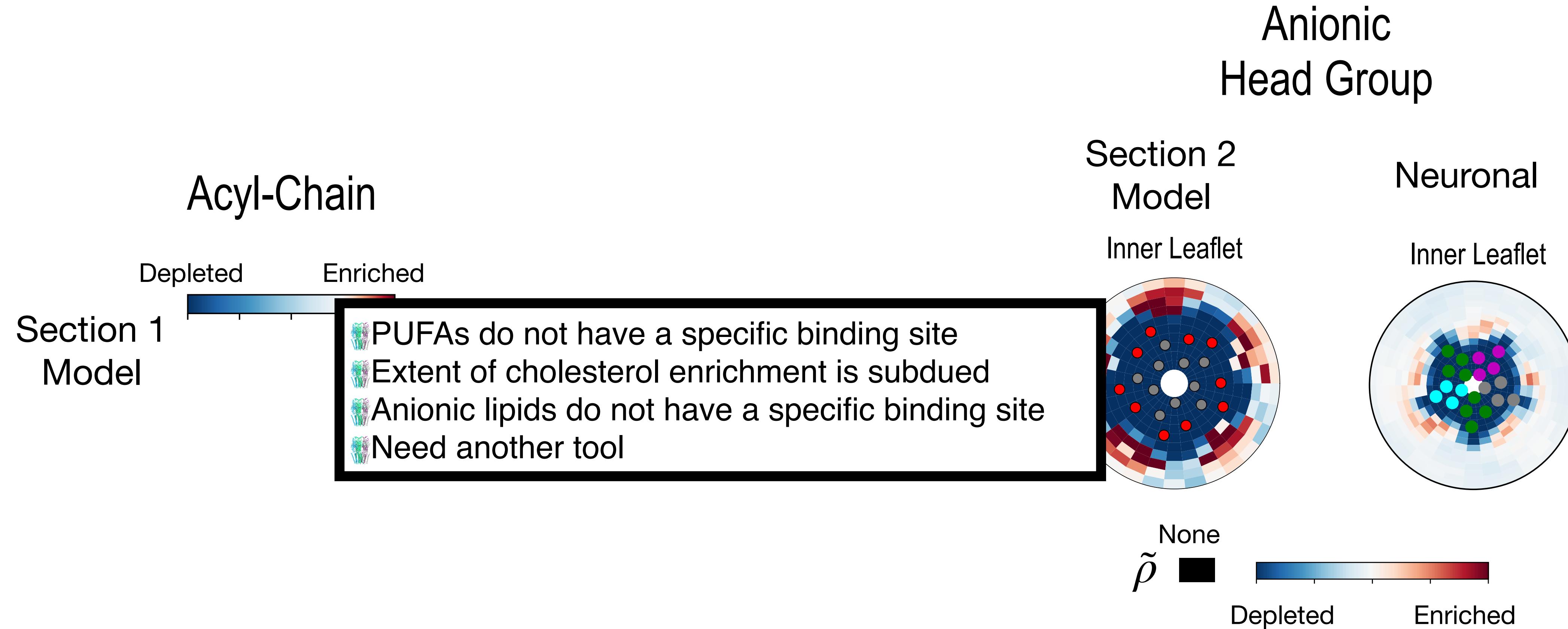
nAChR Synaptic Boundary Movie



Helgi I Ingólfsson et al, 2017

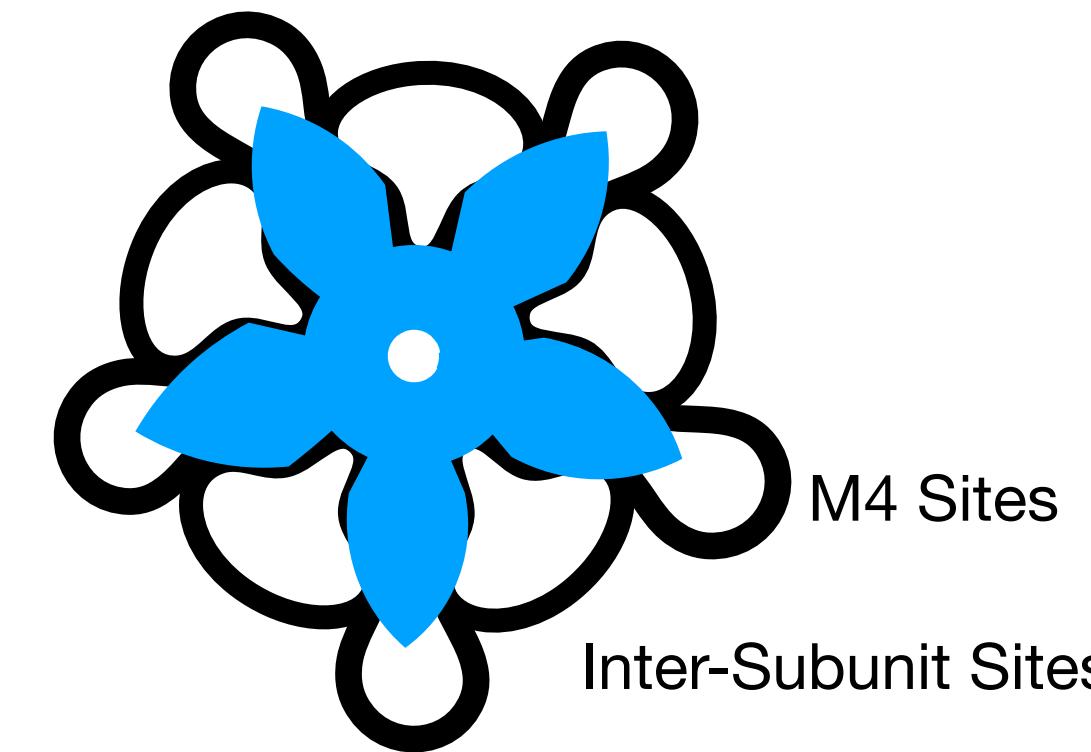


How do the density plots compare to previous results from model membrane



Lipid-nAChR Affinity, why do we want to determine this?

We have hypothesized these two regions of lipid occupation, the inter-subunit site and the M4 site

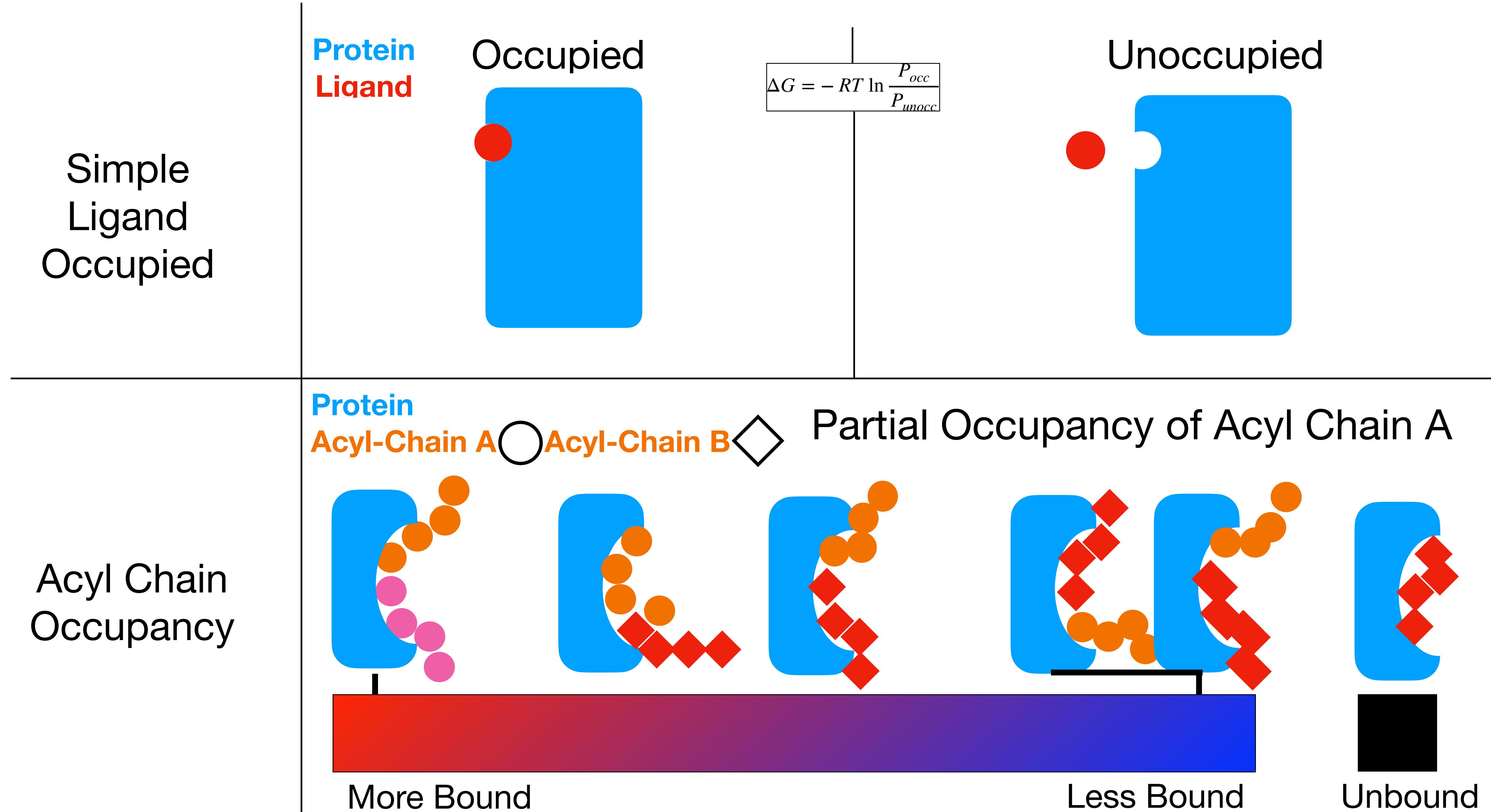


Dimensional reduction (2D distribution -> scalar), previously had used whole annulus and not individual sites

For individual sites, affinity makes more sense than enrichment

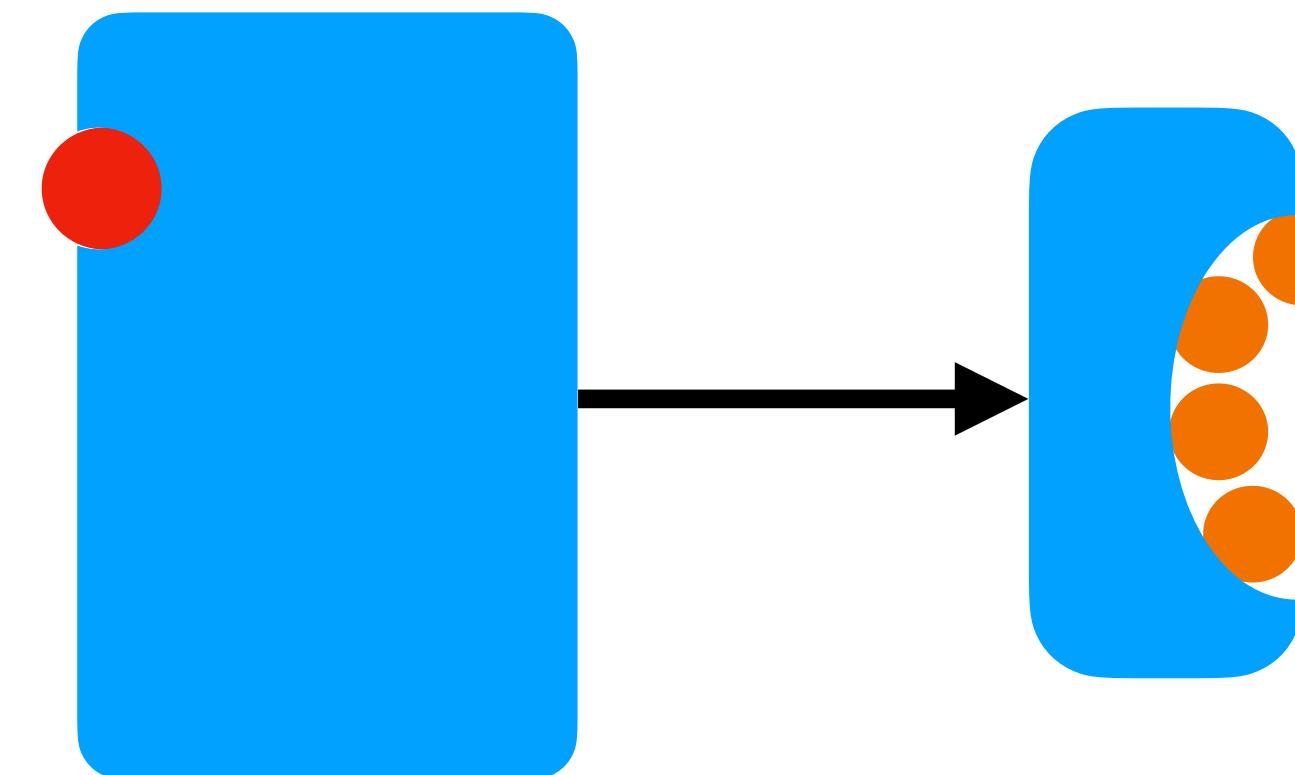
- Quantifies the free energy of binding a lipid (energetic information)
- Energetic information that can be used to predict densities in other membranes

Affinity calculations: Why is this a challenge for lipids?



What steps do we take to try and overcome the partial occupation issue?

What do we define as an affinity while using partial occupancy?



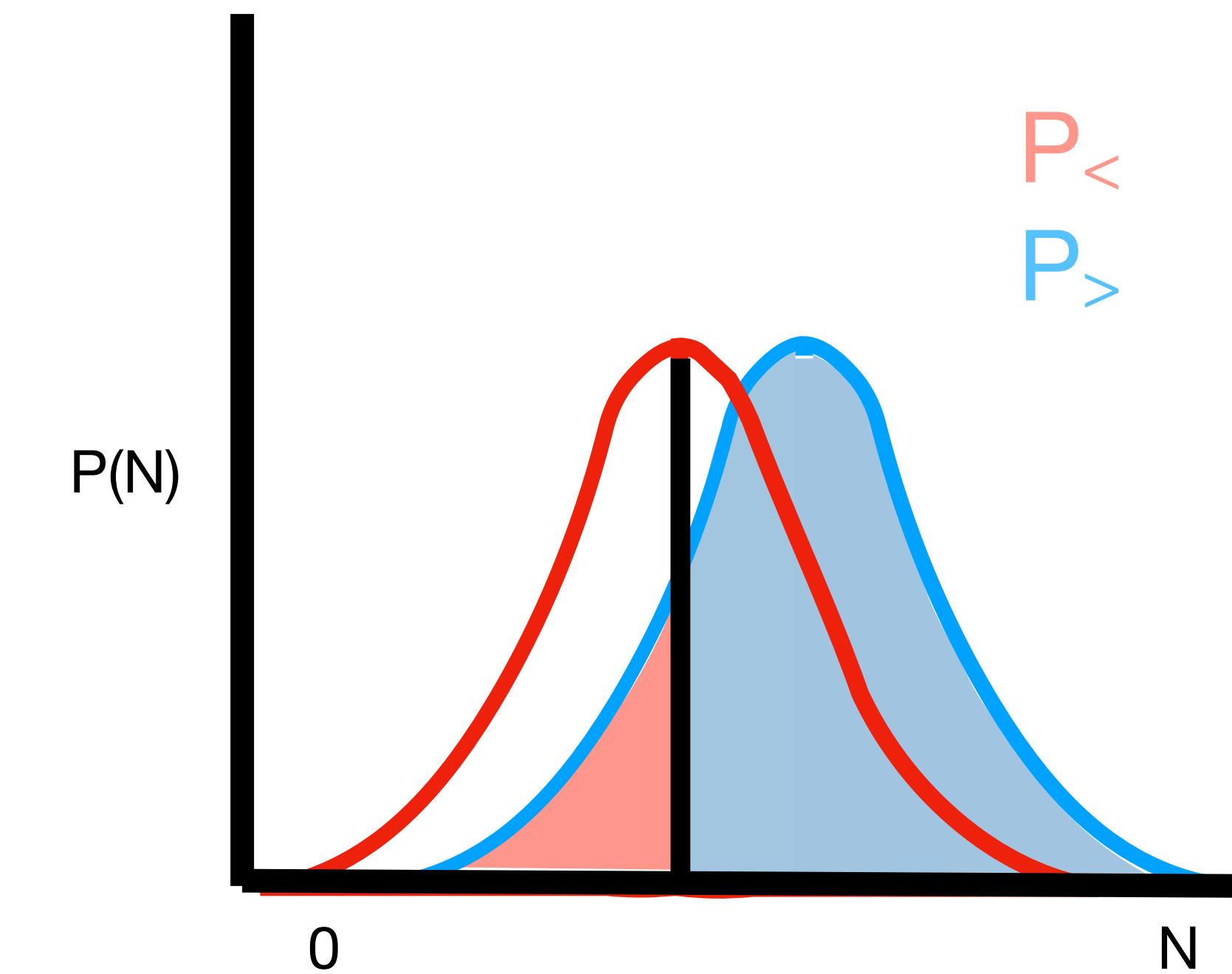
We derive the binding affinities by comparing two probability distributions

- Distribution of the number of lipid beads in a bulk area P_{bulk}
- Distribution of the number of lipid beads at an occupancy site P_{occ}

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

Affinity is derived from the overlap (or lack of overlap) of the two distributions

- Sum of P_{occ} less than and equal to the P_{bulk} peak is $P_<$
- Sum of P_{occ} greater than the P_{bulk} peak is $P_>$



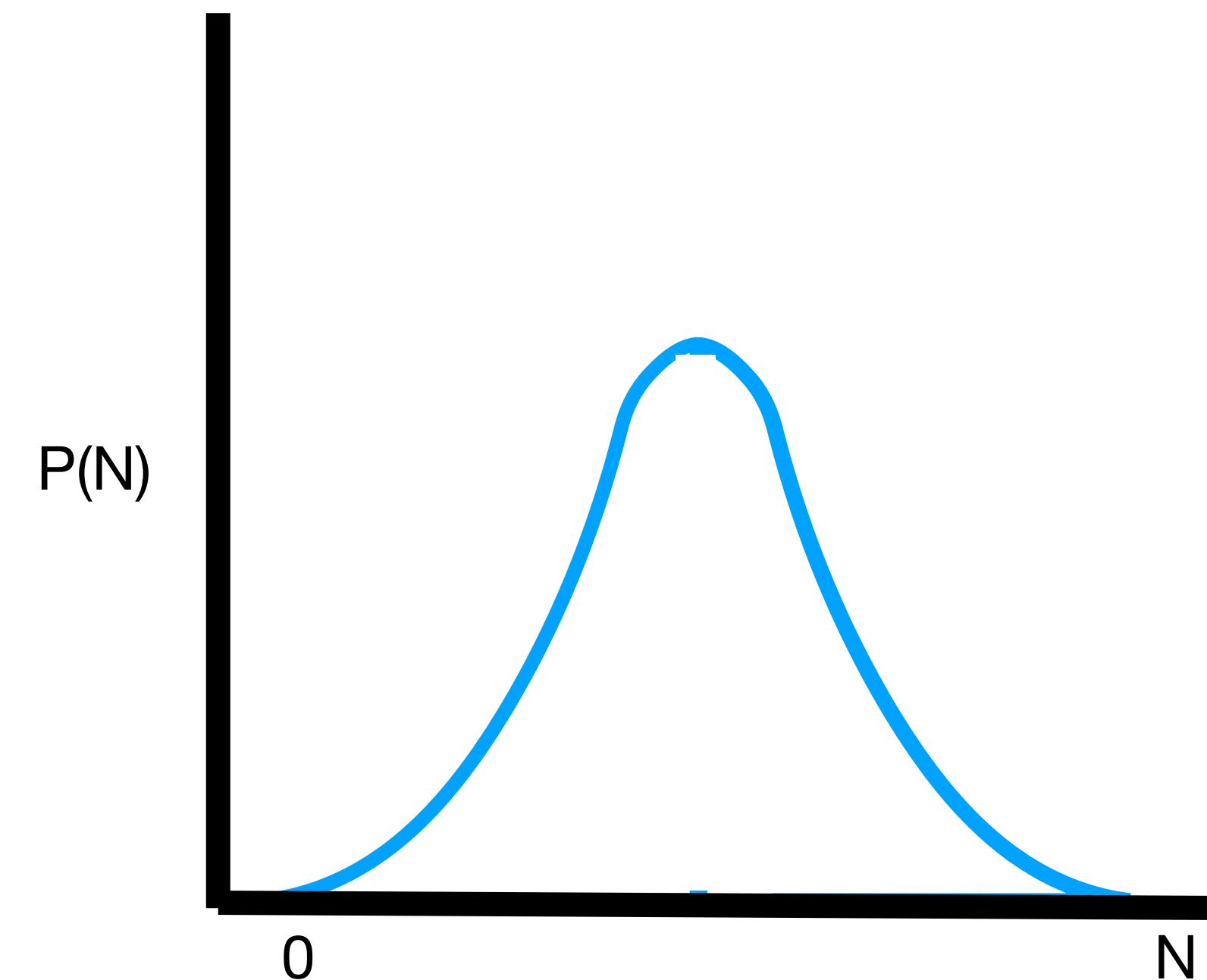
Partial Occupation and Affinity: How to calculate P_{occ}

Frames
Counts

23
10

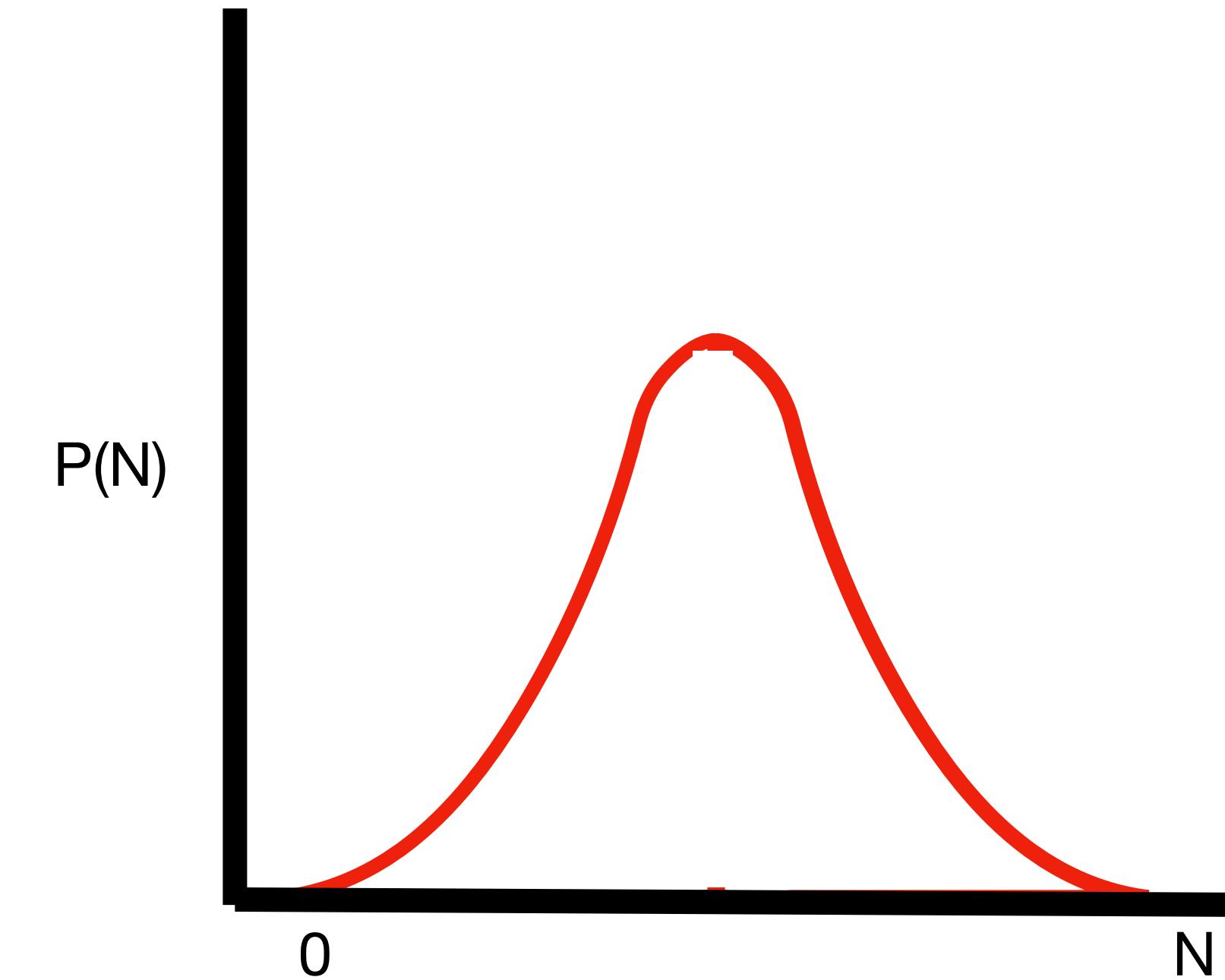
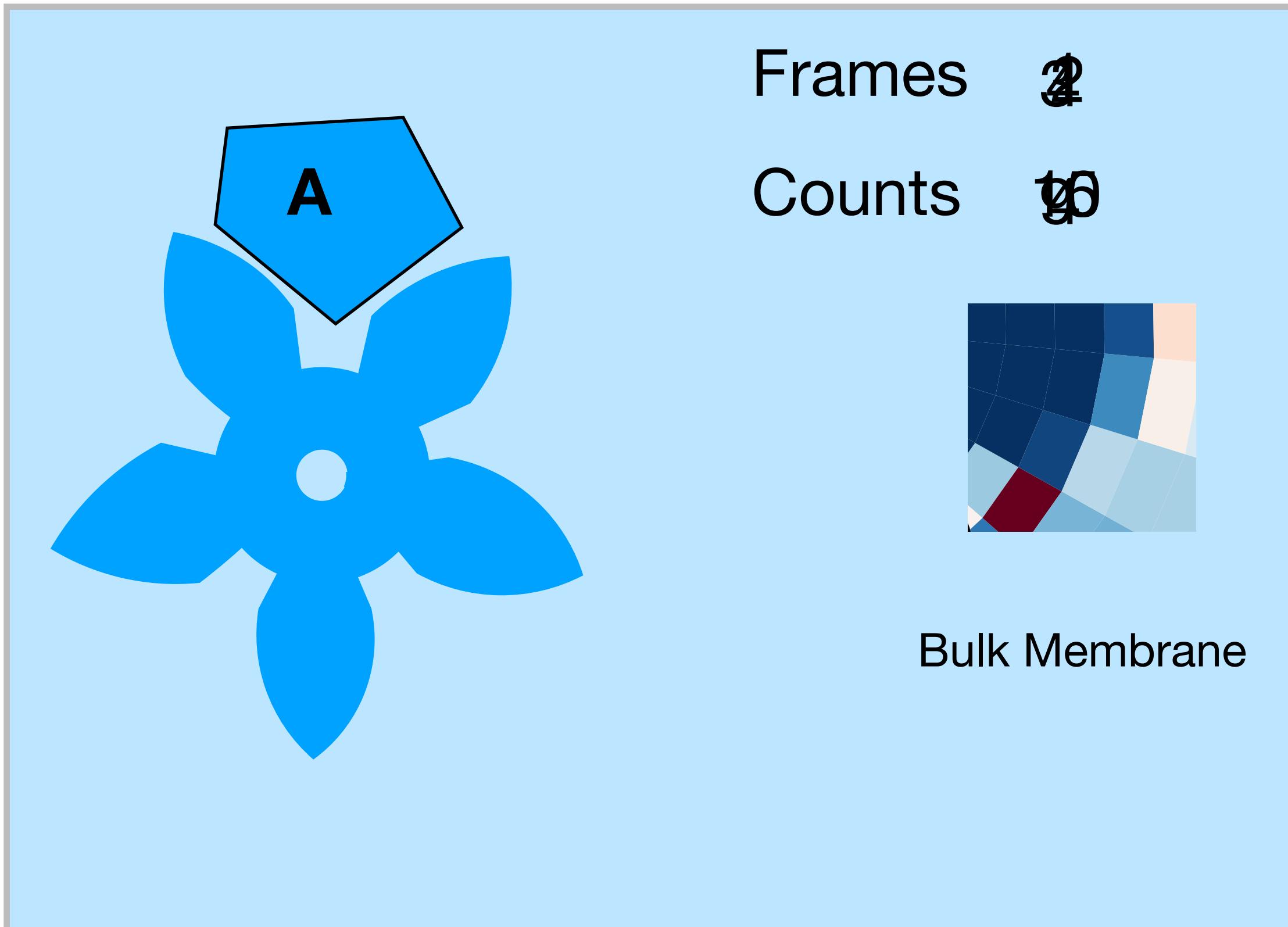


The occupancy site distribution P_{occ} is determined by counting the number of lipid beads within the area per frame



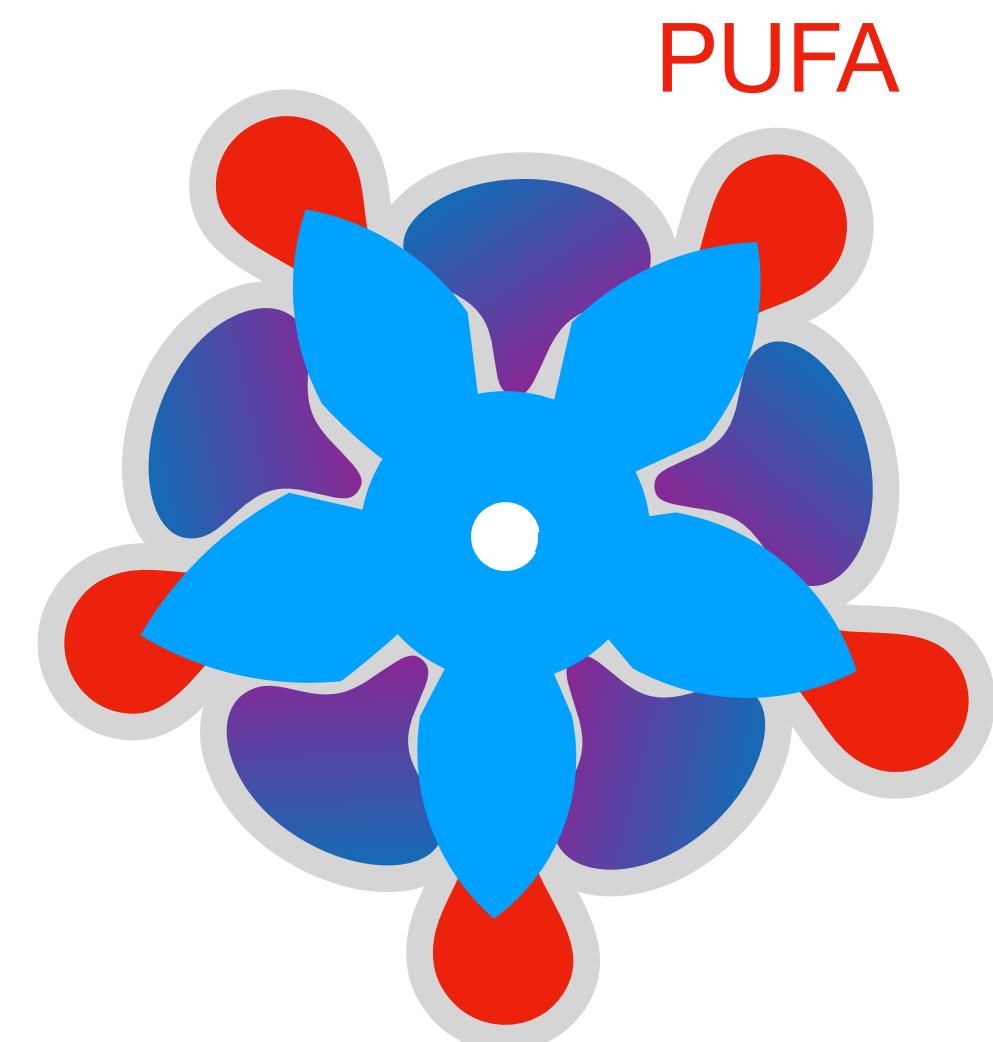
Partial Occupation and Affinity: How to calculate P_{bulk} ?

The bulk site distribution P_{bulk} is determined by counting the number of beads at a bulk area per frame

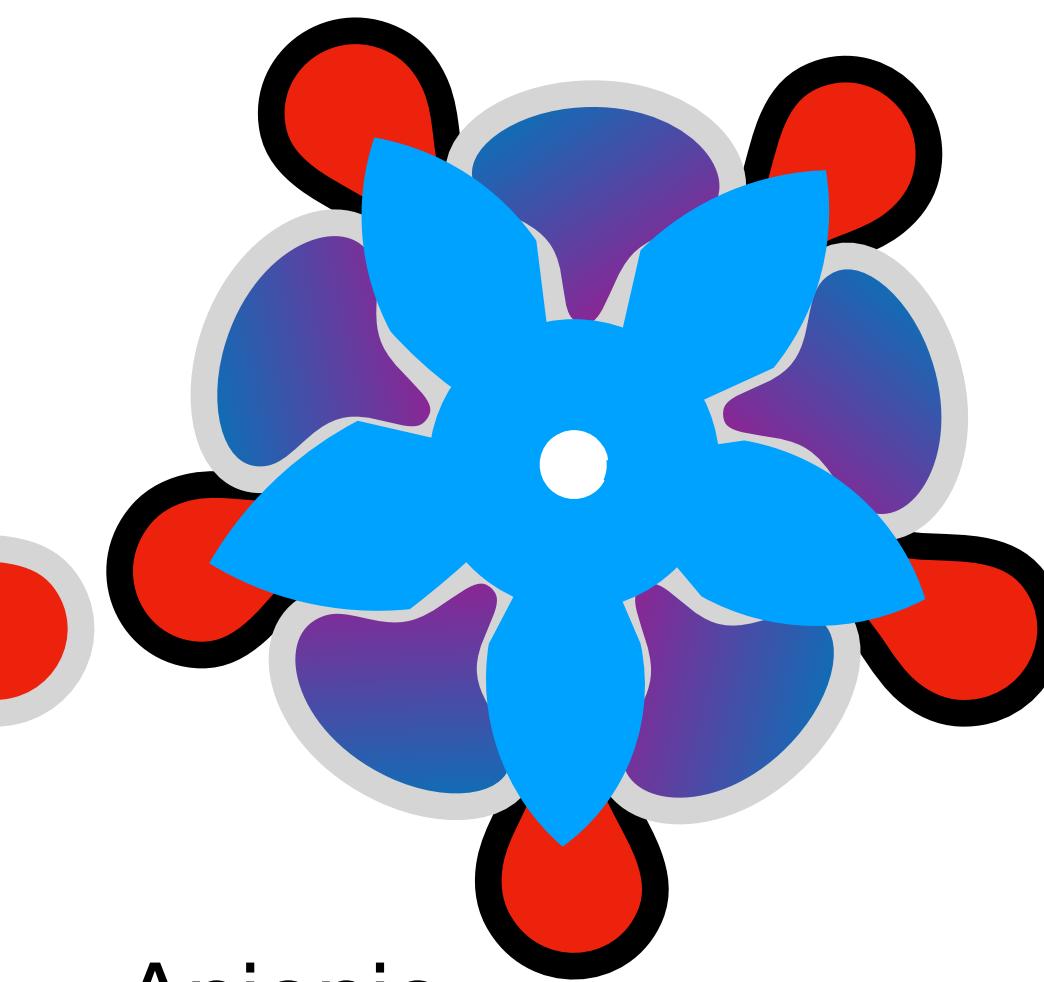


What do we expect

Outer Leaflet



Inner Leaflet



Neutral

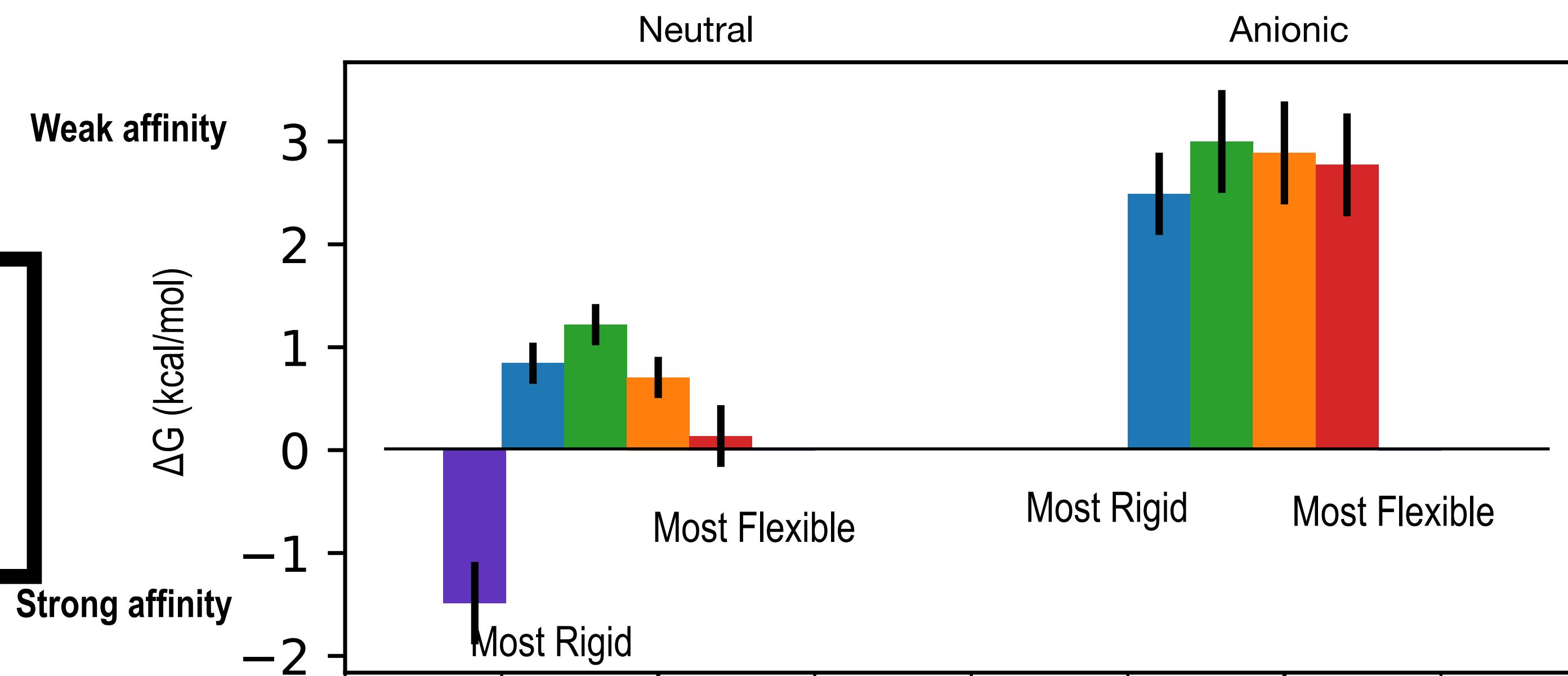
Anionic

Saturated/
Cholesterol

PUFA

Which lipids do inter-subunit sites in the outer leaflet prefer?

- Anionic lipids are unfavorable in the
- Phospholipids have a non-monotonic trend
 - n-3>n-6>saturated>monounsaturated
- Cholesterol has the strongest affinity

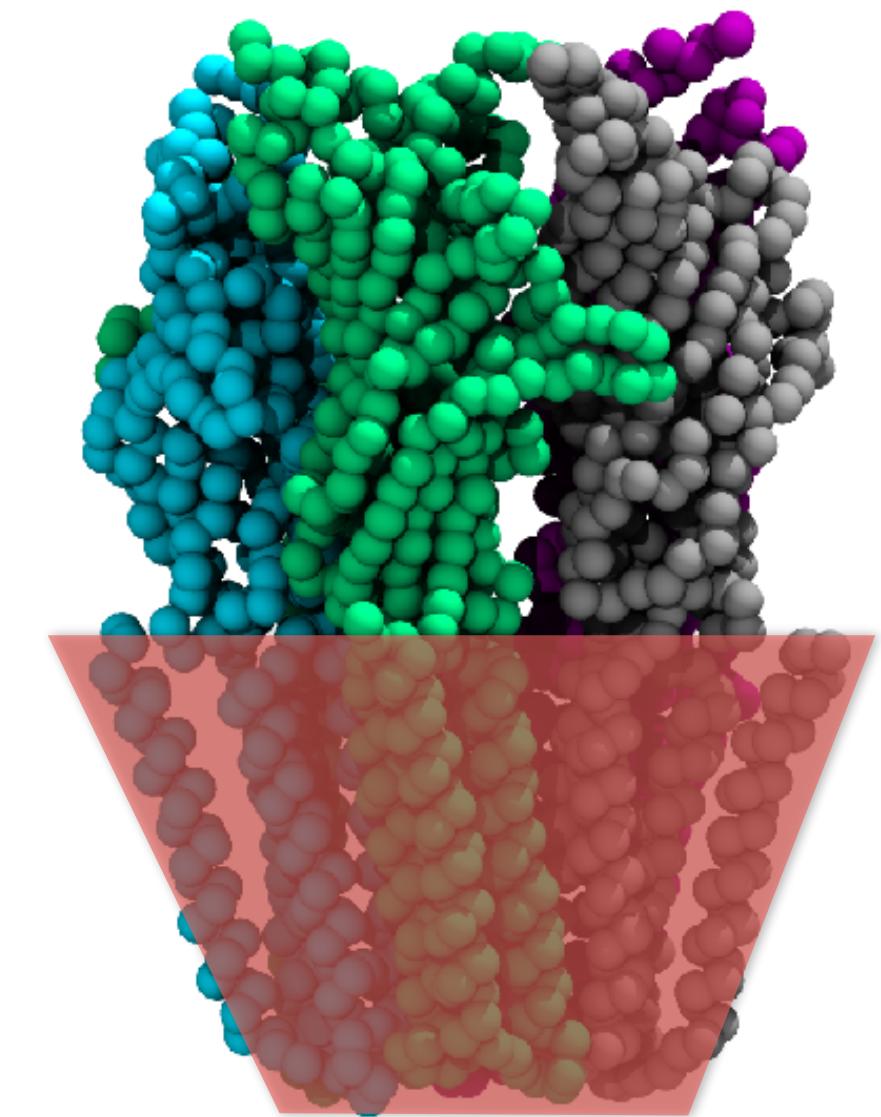
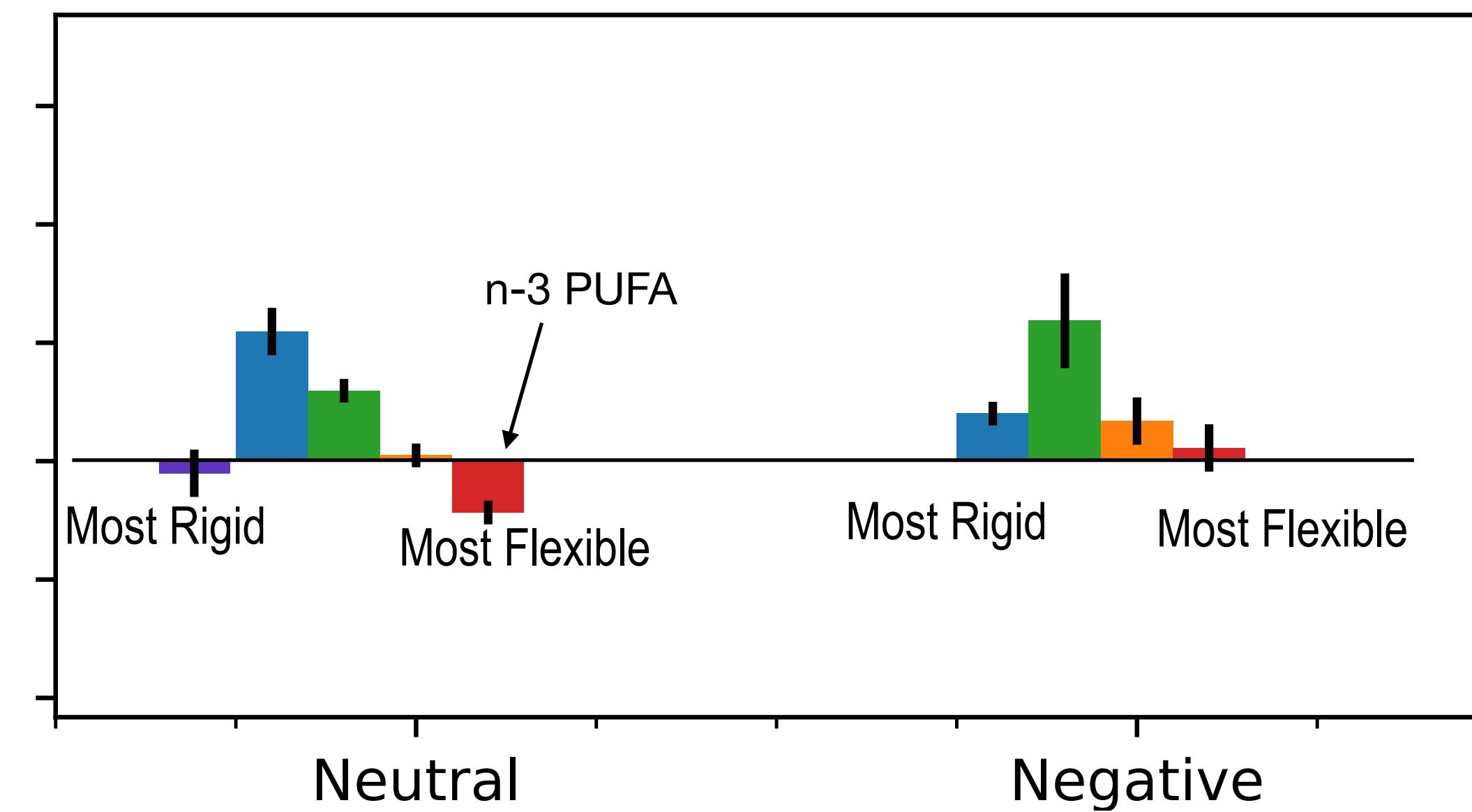


Which lipids do M4 sites in the outer leaflet prefer?

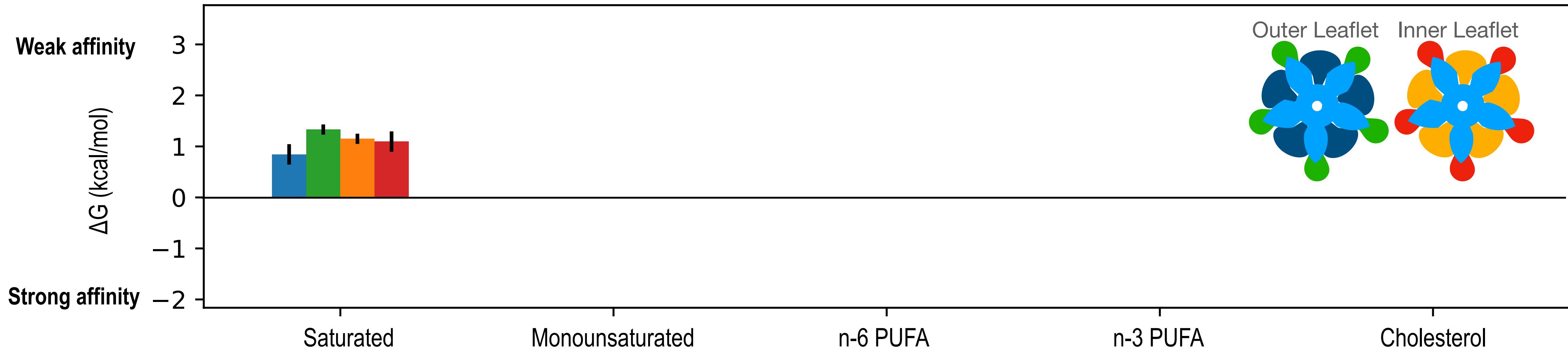
Weak affinity

Strong affinity

- Neutral phospholipid affinity strengths changes with chain flexibility
- Flexible anionic lipids have the strongest affinity

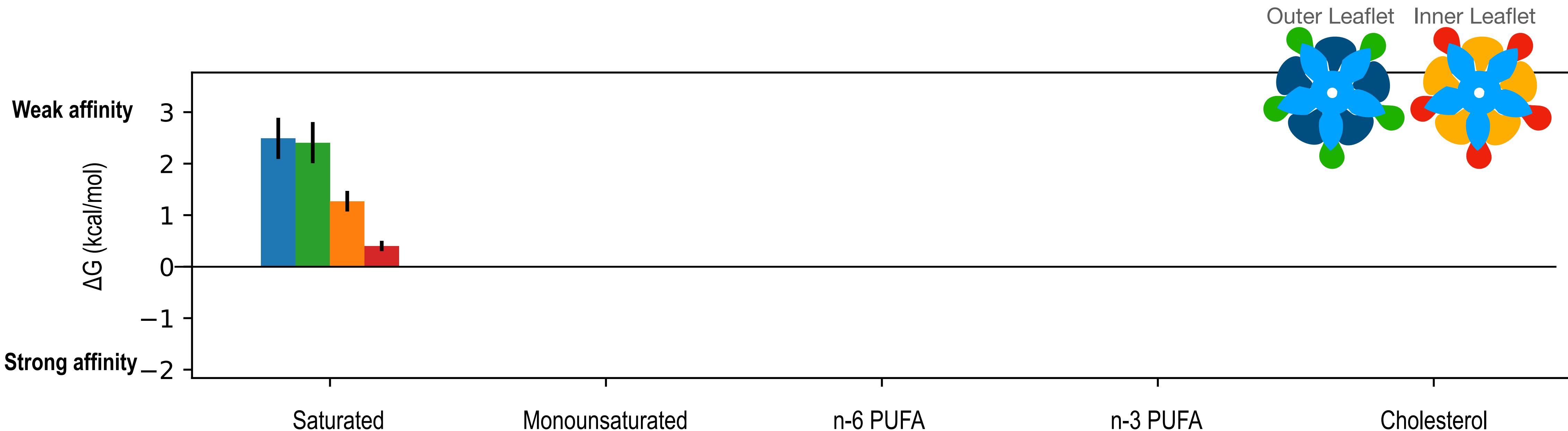


Which sites do neutral lipids prefer?



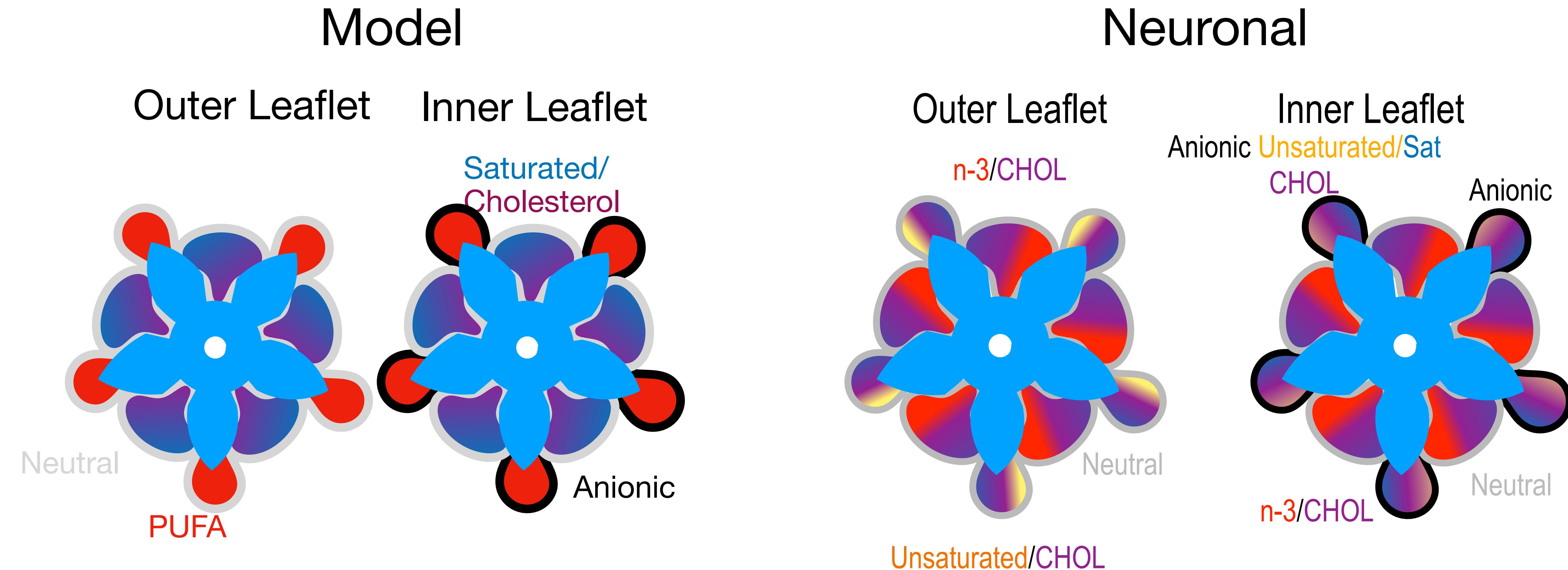
- Saturated lipids do not have a preferred site
- Monounsaturated lipids weakly prefer M4 sites in the inner leaflet
- Neutral PUFAs prefer M4 in the outer leaflet
- Cholesterol prefers inter-subunit sites in the outer leaflet

Which sites do anionic lipids prefer?



All anionic lipids prefer M4 sites in the inner leaflet

How do native membrane compare to model-membrane results



Conclusion

💡 What are the boundary lipids around nAChR in a native membrane?

- A little bit of everything
- Not limited to just one lipid

💡 How are the lipids distributed?

- PUFAs occupy M4 sites (stronger affinity in outer leaflet)
- Cholesterol occupies inter-subunit sites (stronger affinity in outer leaflet)
- All anionic lipids occupy M4 sites in the inner leaflet

Over all summary

- Model membranes serve a useful purpose as hypothesis building tools
- Signal in a model membrane can be exaggerated
- Coarse-grained simulations are a valuable complement to experimental techniques
- Our results could also aid interpretation of experiments in native membranes

Thank you for your time!

Dr. Grace Brannigan

Committee Members

Dr. Joseph Martin

Dr. Sean O'Malley

Dr. Jerome Hénin

Collaborator:

Dr. Wayland Cheng

Dr. John Petroff

Brannigan Lab:

Dr. Reza Salari

Shashank Sai

Dr. Sruthi Murlidaran

Dr. Ruchi Lohia

Rulong Ma

Kristen Woods

Jesse Sandberg

Anushriya Subedy*

Ezry St.Iago-McRae*

Connor Pitman

Jahmal Ennis

Dr. Tom Joseph*

The GMO Pugs (Success Circle 4)

Former Lab Members

*Honorary Lab Members



Office of Advanced Research
Computing



Computational resources: NSF
XSEDE Allocation NSF-MCB110149

Local cluster funded by NSF-
DBI1126052

Research Corporation, NIH
P01GM55876-14A1