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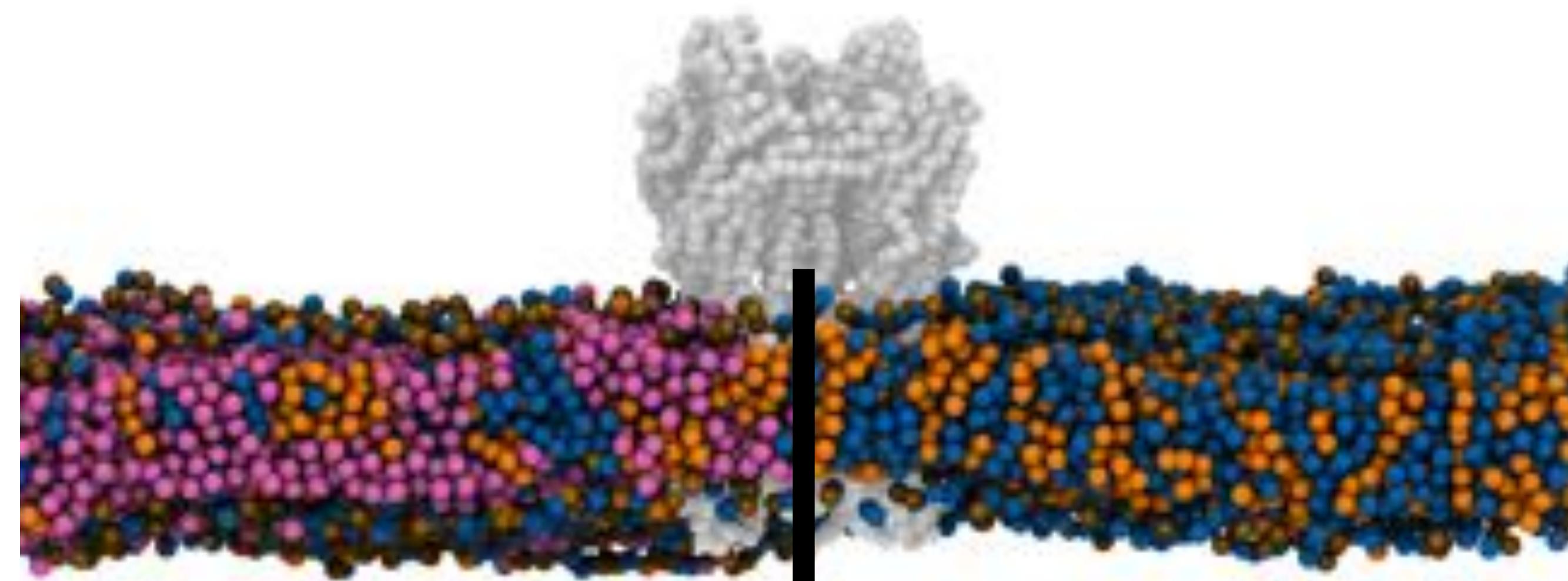
Camden College of  
Arts and Sciences

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Center for Computational  
and Integrative Biology

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

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Mentored by Dr. Grace Brannigan



# Outline

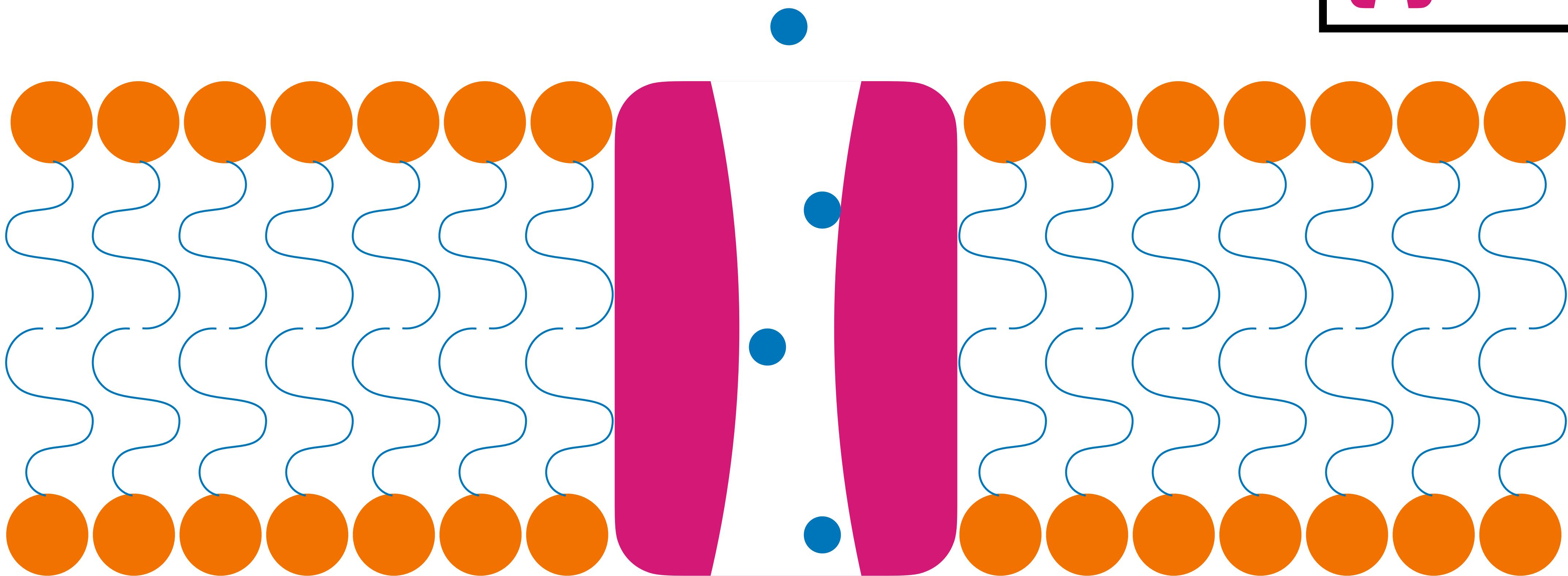
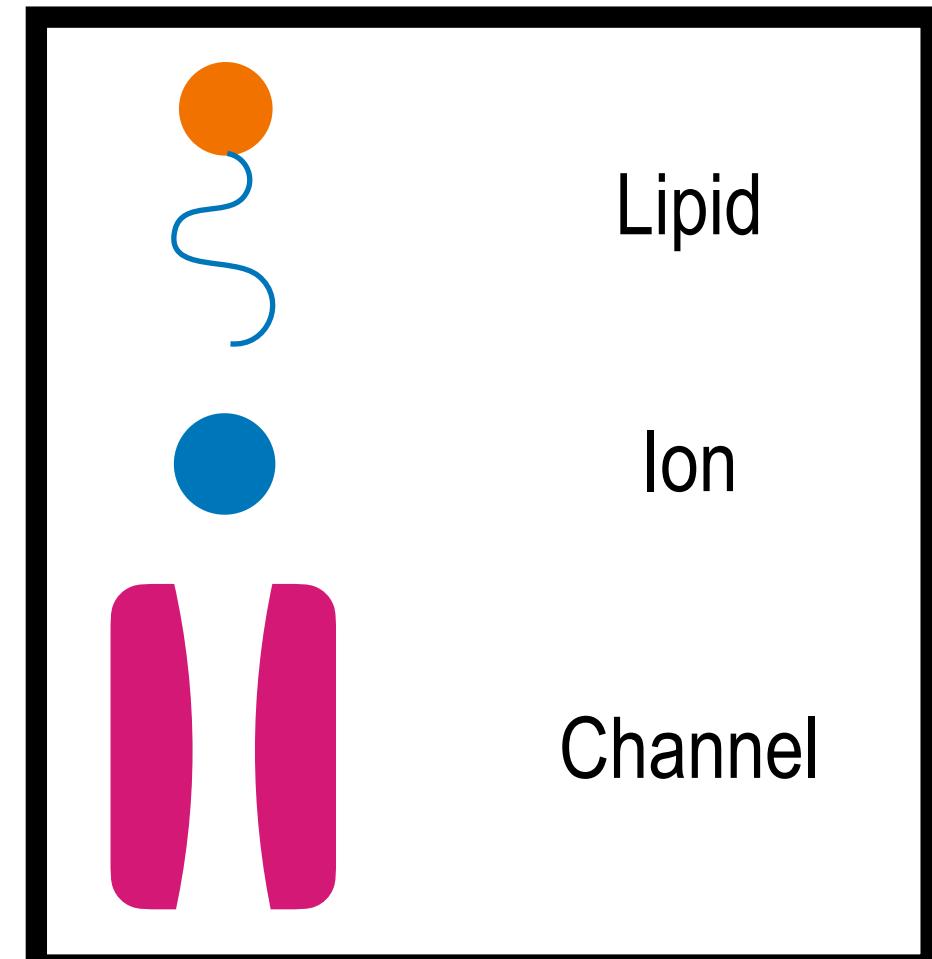
- **Introduction**
- Saturation, Sterols, and Domain-forming lipids: Identifying nAChR boundary lipids in PUFA-rich model membranes
- Lipid head-group charge: Boundary lipids for a bacterial sister channel in charged model membranes
- Putting it all together: Quantifying specific lipid-binding affinities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

# Ion Channels

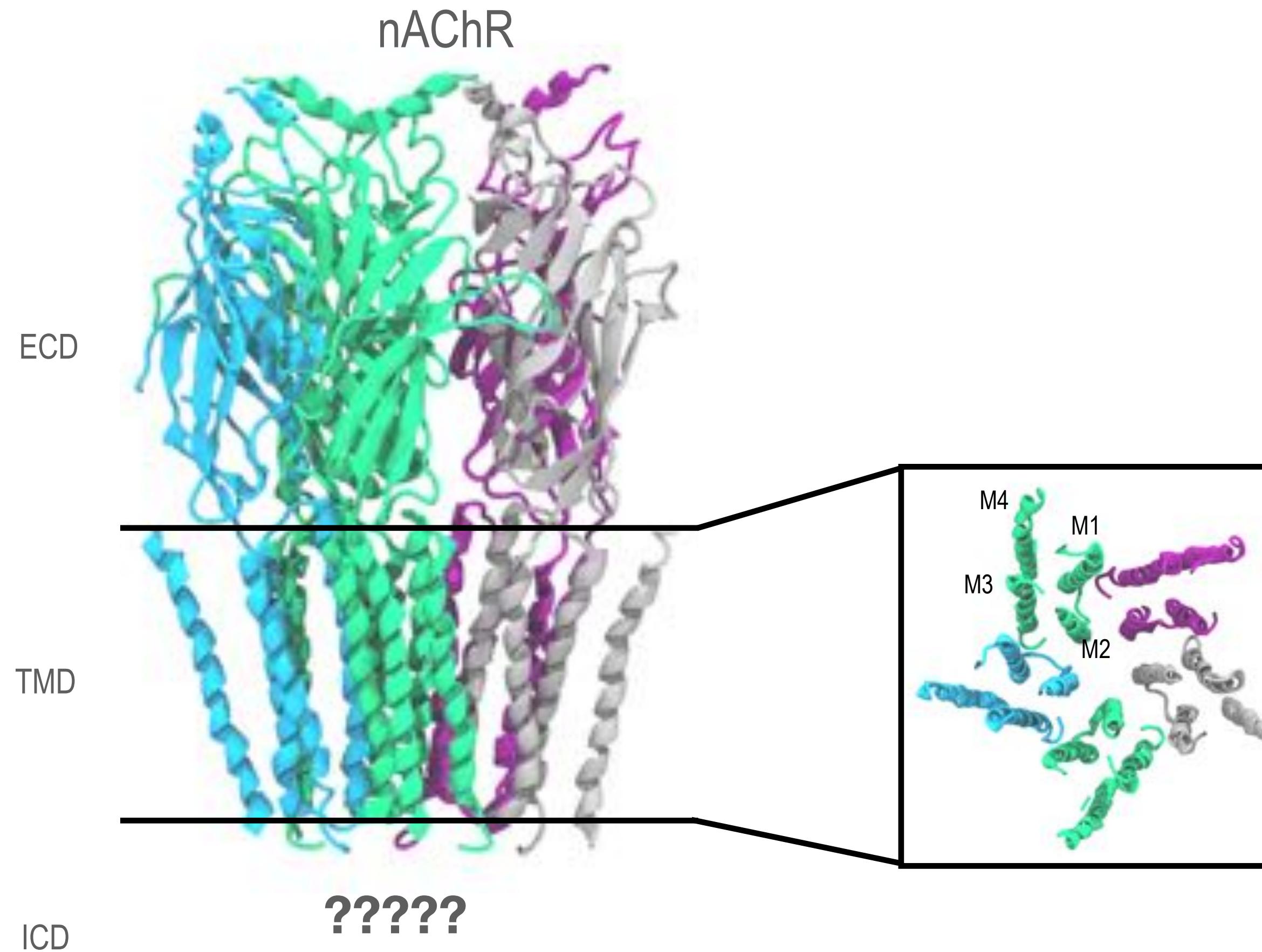
Transmembrane proteins

Passively conducts ions across membrane

Essential for various cellular functions



# Pentameric Ligand Gated Ion Channels (pLGICs)

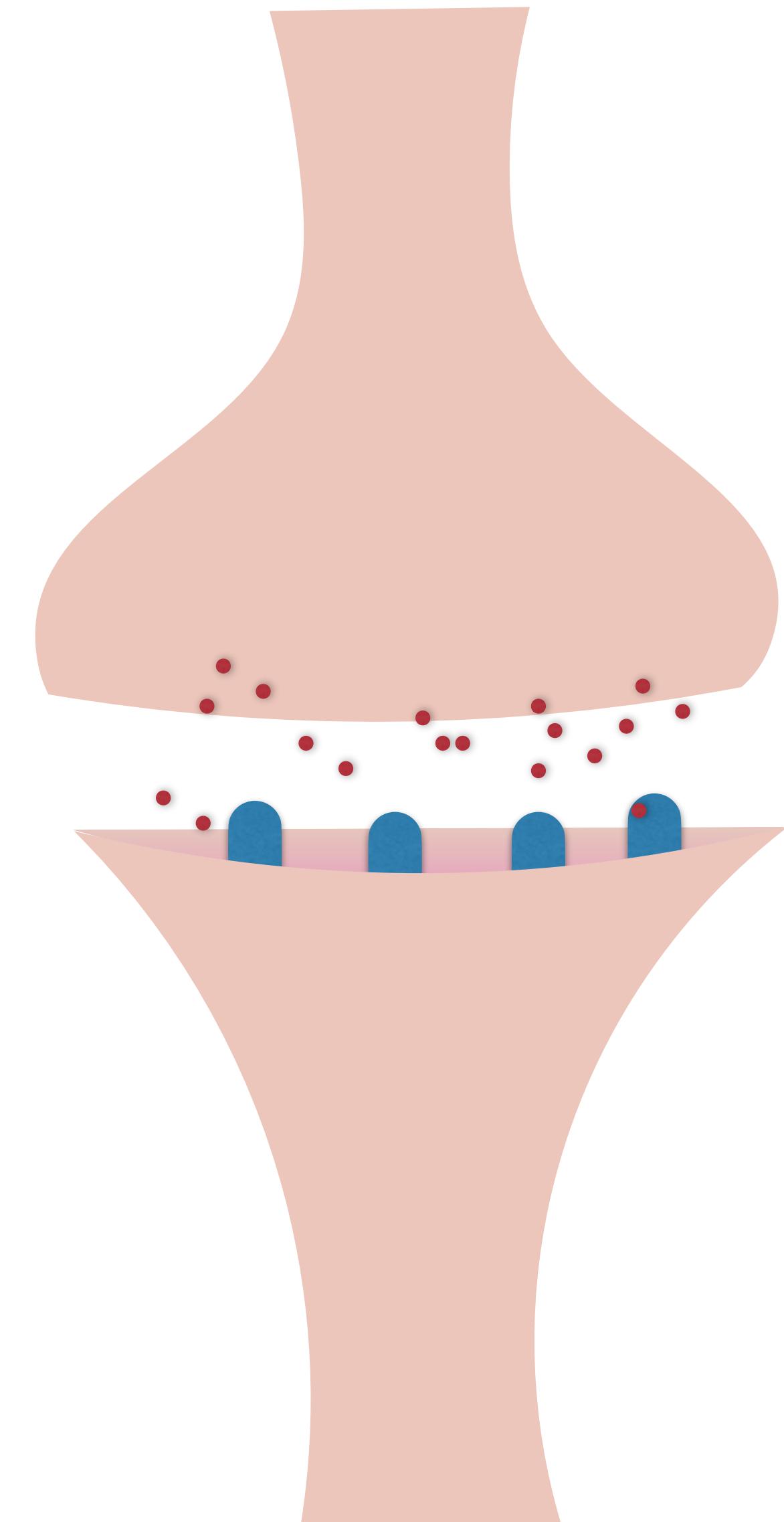


## Pentameric ligand gated ion channels

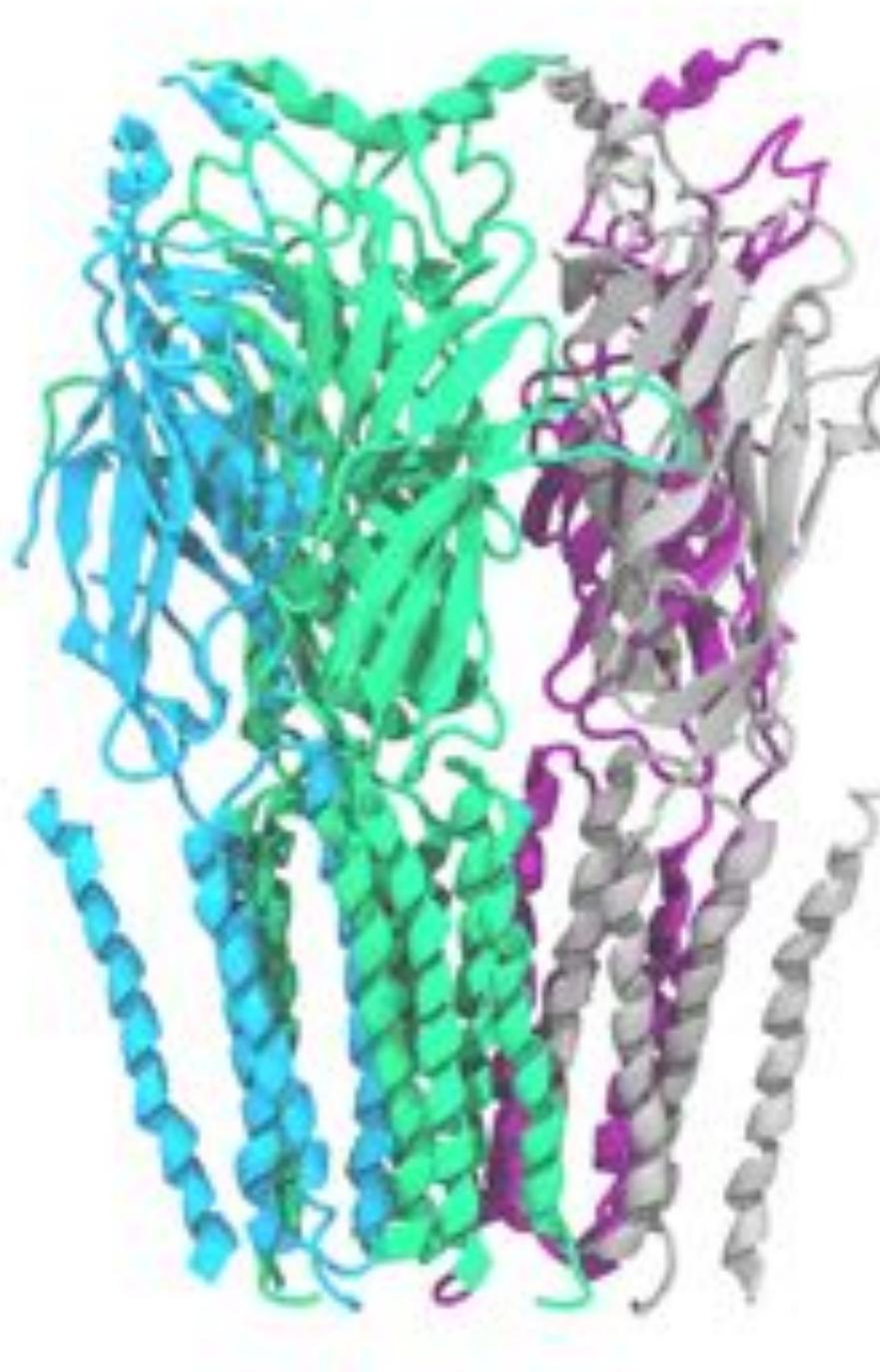
- Five subunits
- Three domains
  - Extra-Cellular (ECD)
  - Transmembrane (TMD)
  - Inter-Cellular (ICD)
- Open with the binding of specific small molecules
- pLGICs are structurally conserved

# 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:

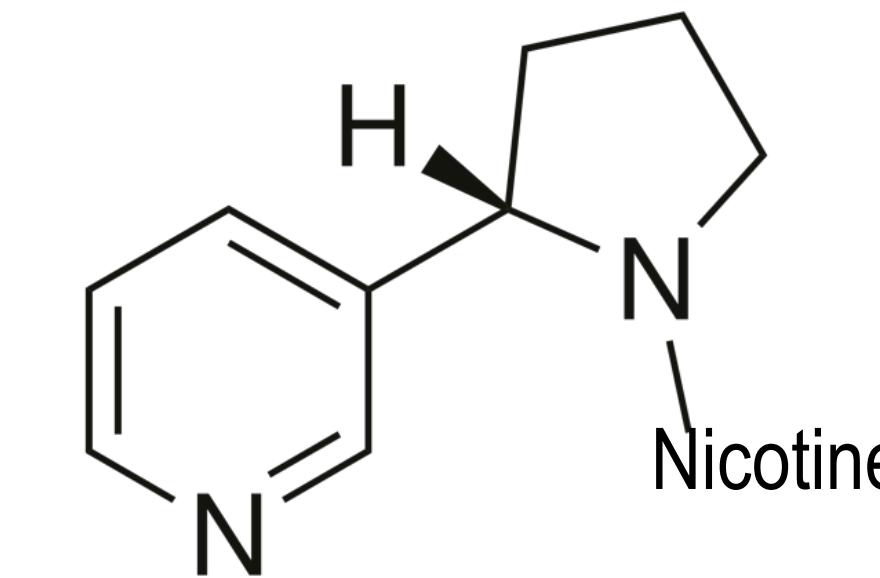
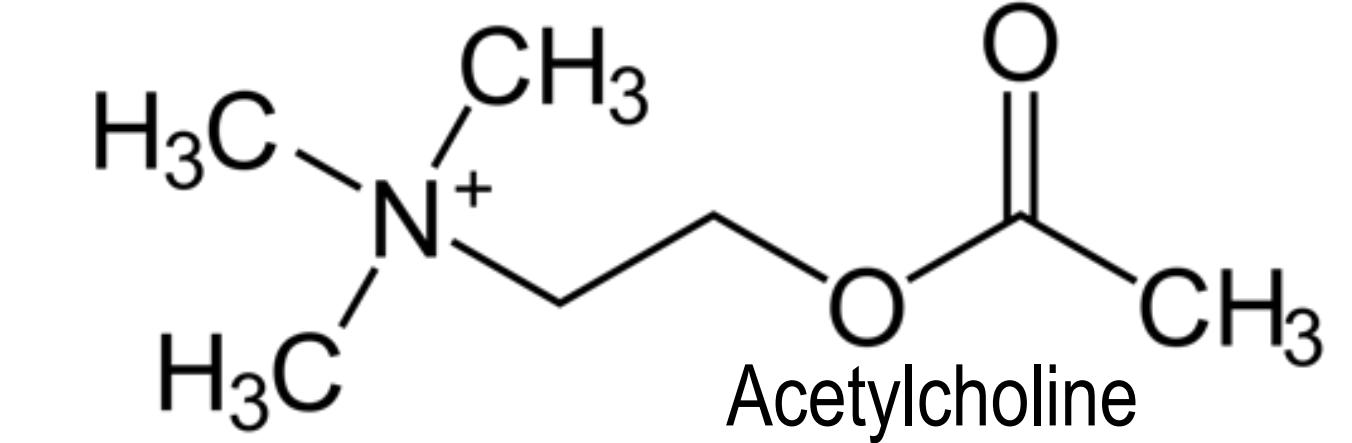
- Bipolar Disorder
- Schizophrenia
- Depression
- Epilepsy
- Neuromuscular autoimmune diseases

# Ligands and modulators

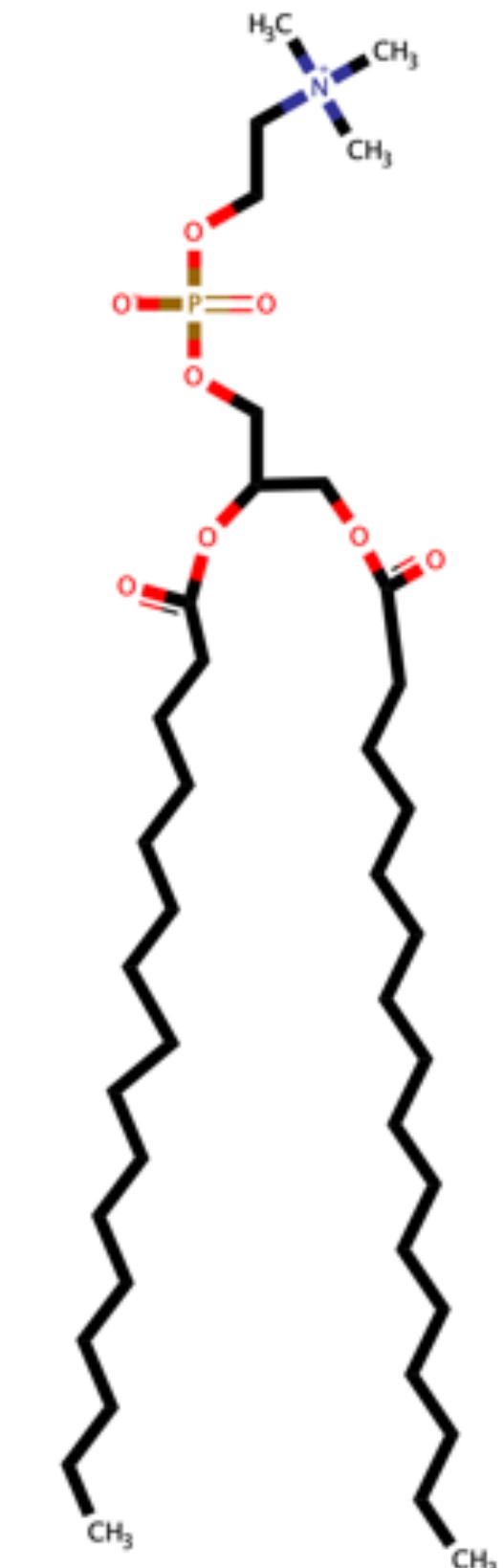
神经递质 (乙酰胆碱)

药物 (尼古丁, 酒精, 全身麻醉药)

脂质 (高度特异)



Dipalmitoylphosphatidylcholine (DPPC)



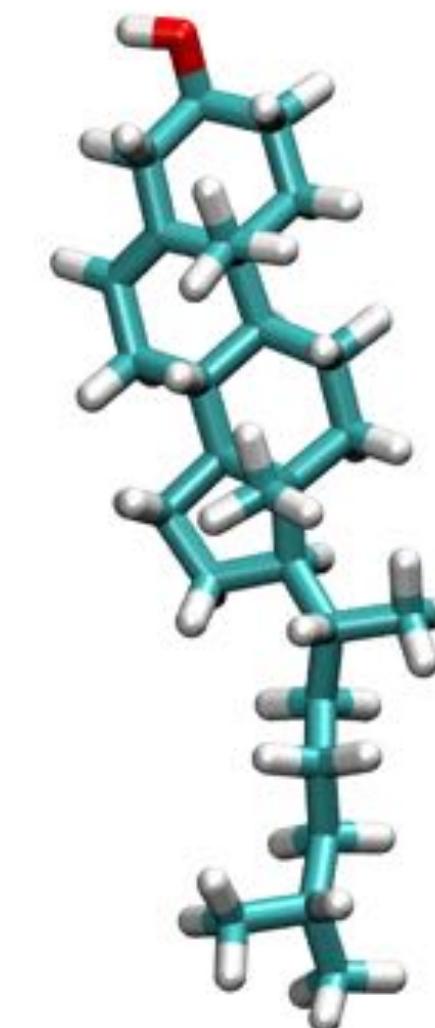
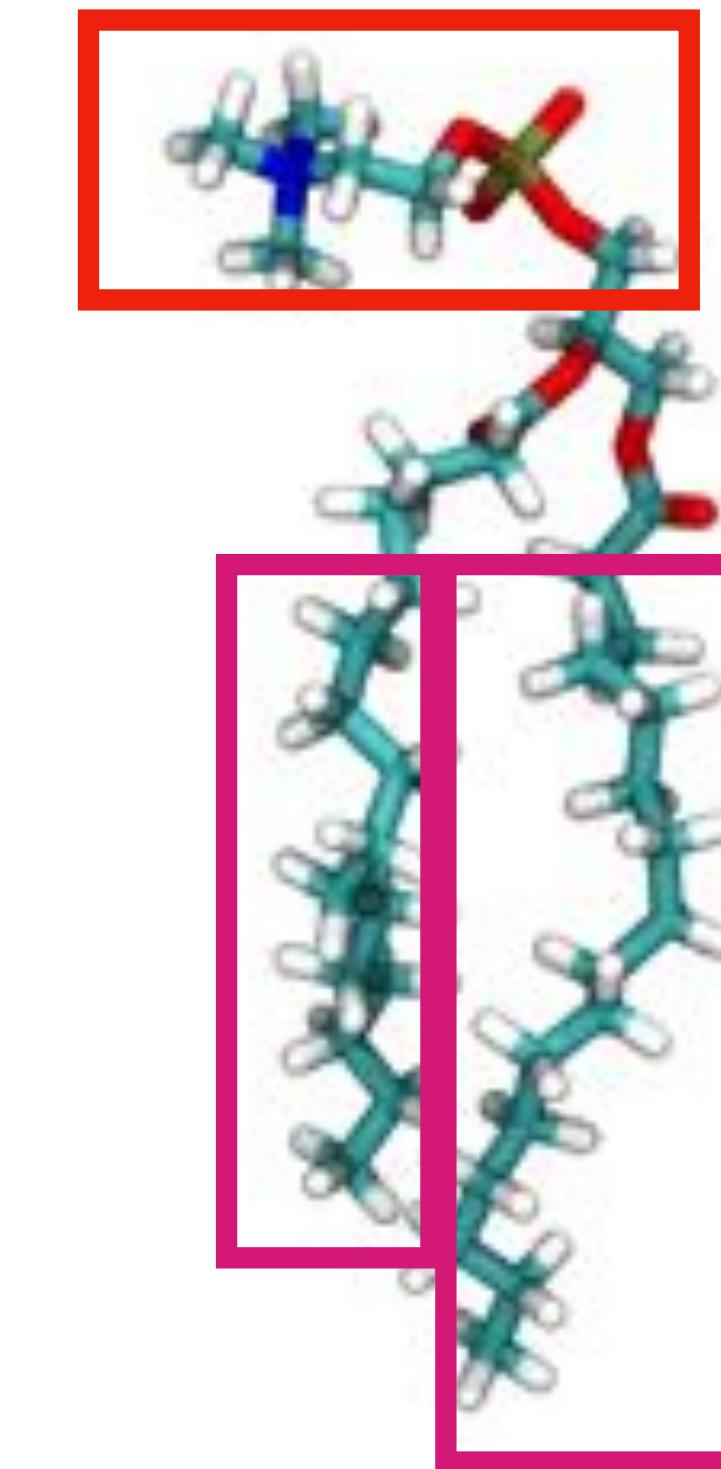
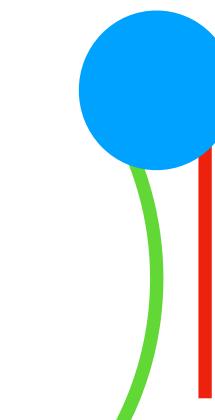
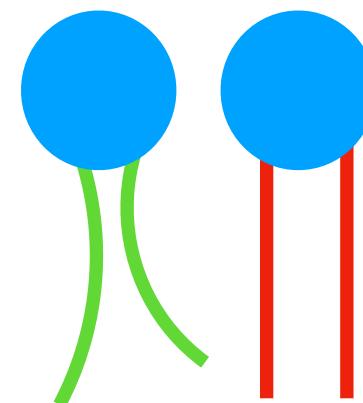
# Lipids

💡 Lipids are small amphiphilic molecules

💡 Two distinct classes are phospholipids and sterols

💡 Phospholipids are “modular”:

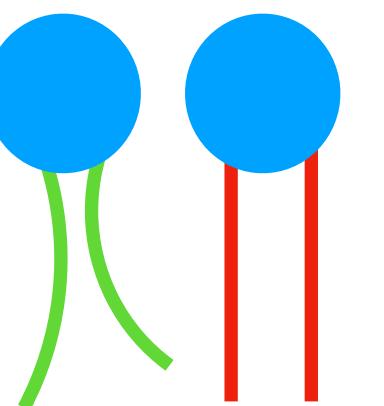
- Head group either neutral or anionic
- Acyl-Chains vary in length and saturation
  - Saturated lipids: straight and rigid
  - Unsaturated flexible with kinks  
(See next slide!)
- Can be homo- or hetero-acidic



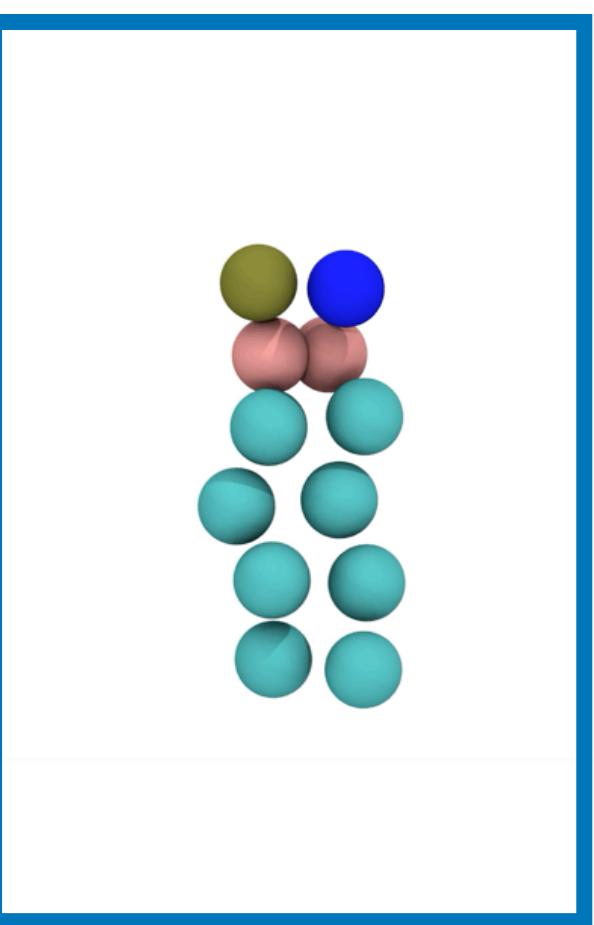
💡 Cholesterol

# Lipids: Model Membranes

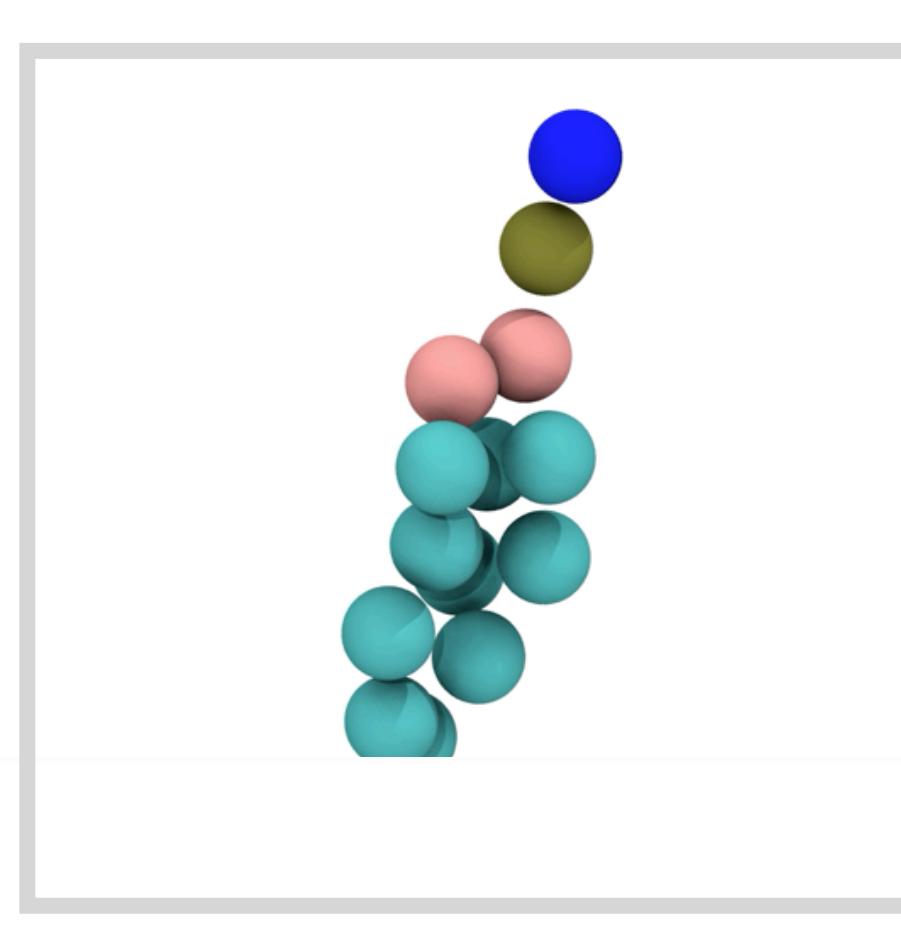
Homo-Acidic  
Domain  
Forming



Cholesterol

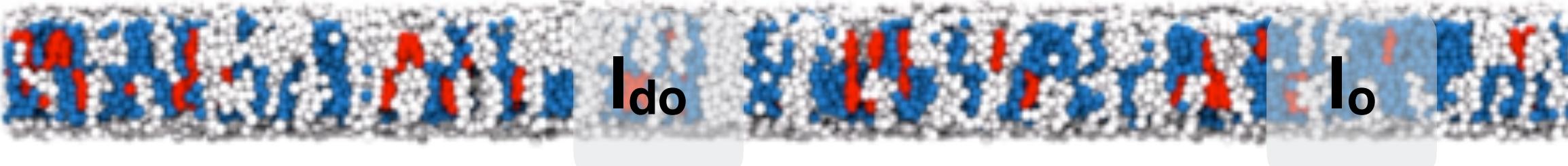


Saturated Chains

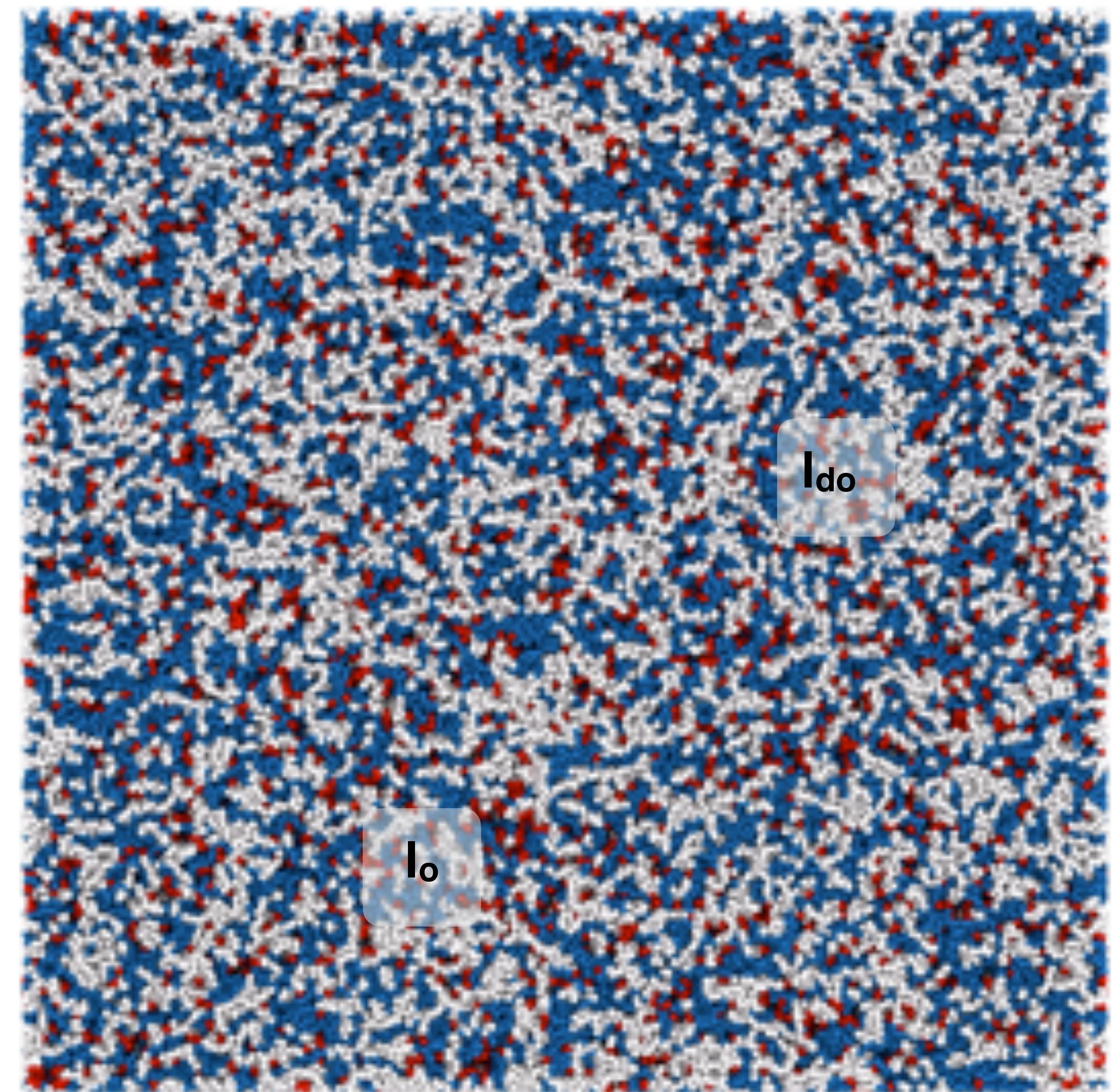


Polyunsaturated Chains

Model Membrane Side View



Model Membrane Extra-Cellular View

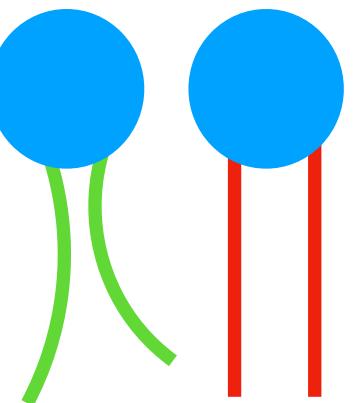


$\sim 2 \text{ us}$     $75 \times 75 \text{ nm}^2$

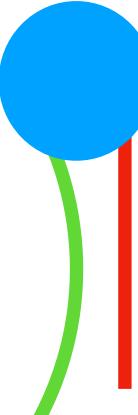
# Lipids: Native Membranes

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

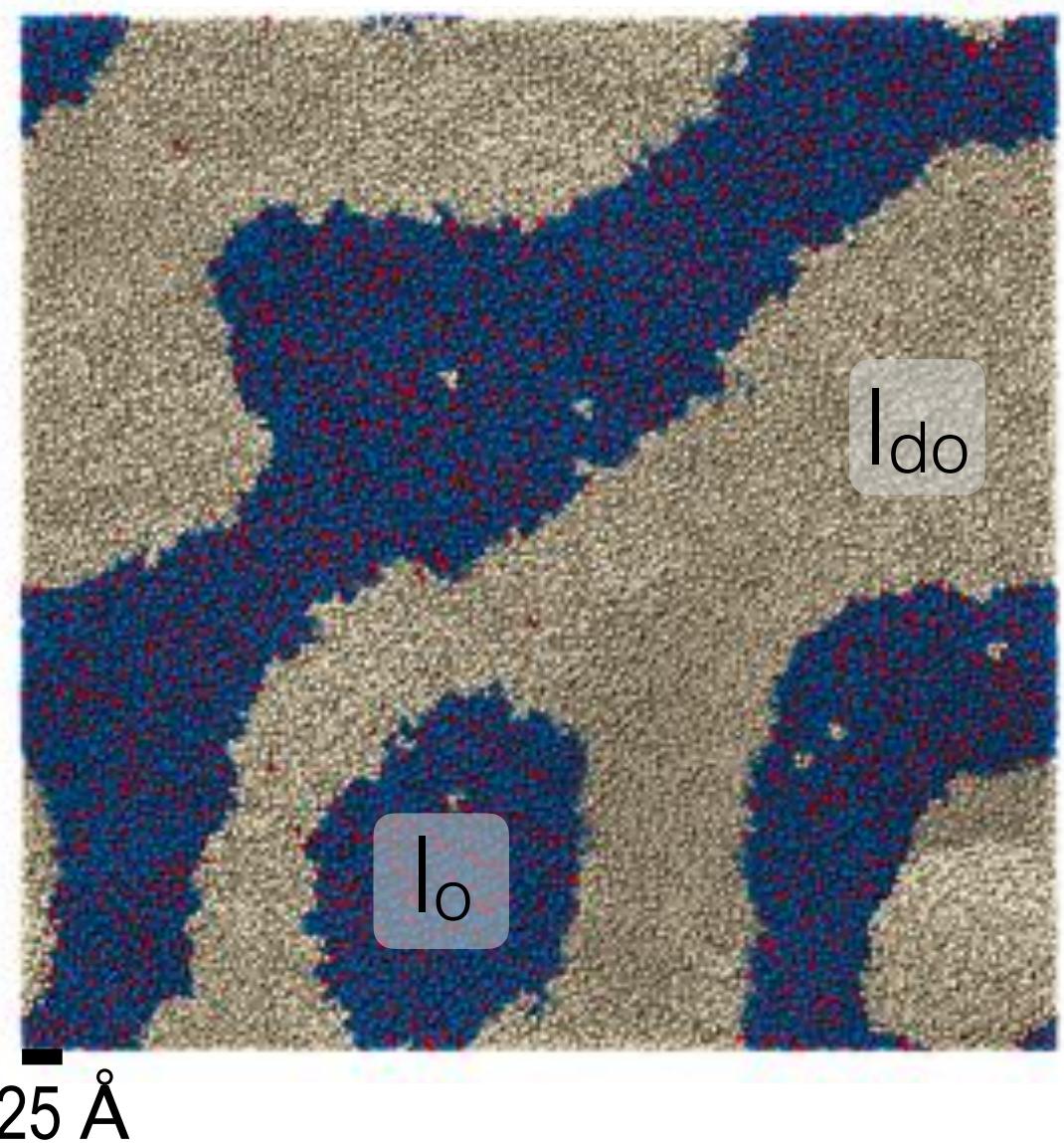
Homo-Acidic  
Domain  
Forming



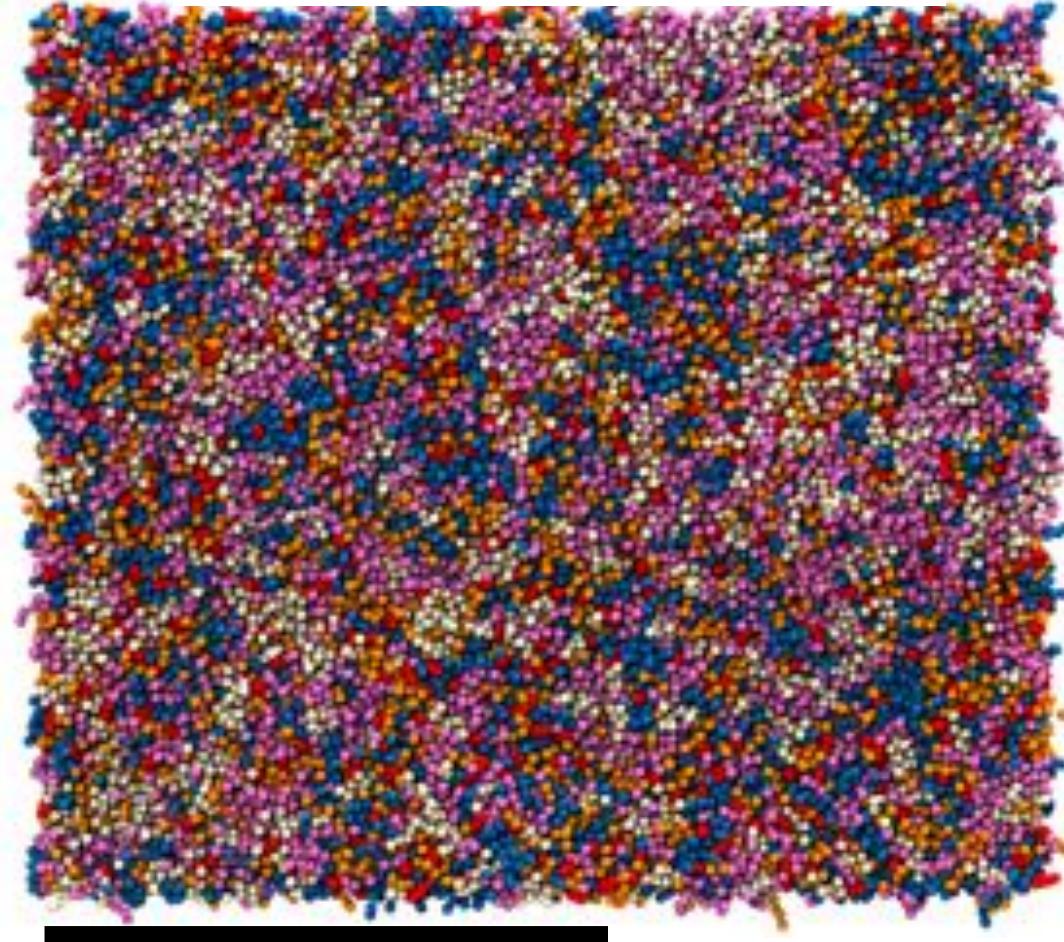
Hetero-Acidic  
Non-Domain  
Forming



Model Membrane

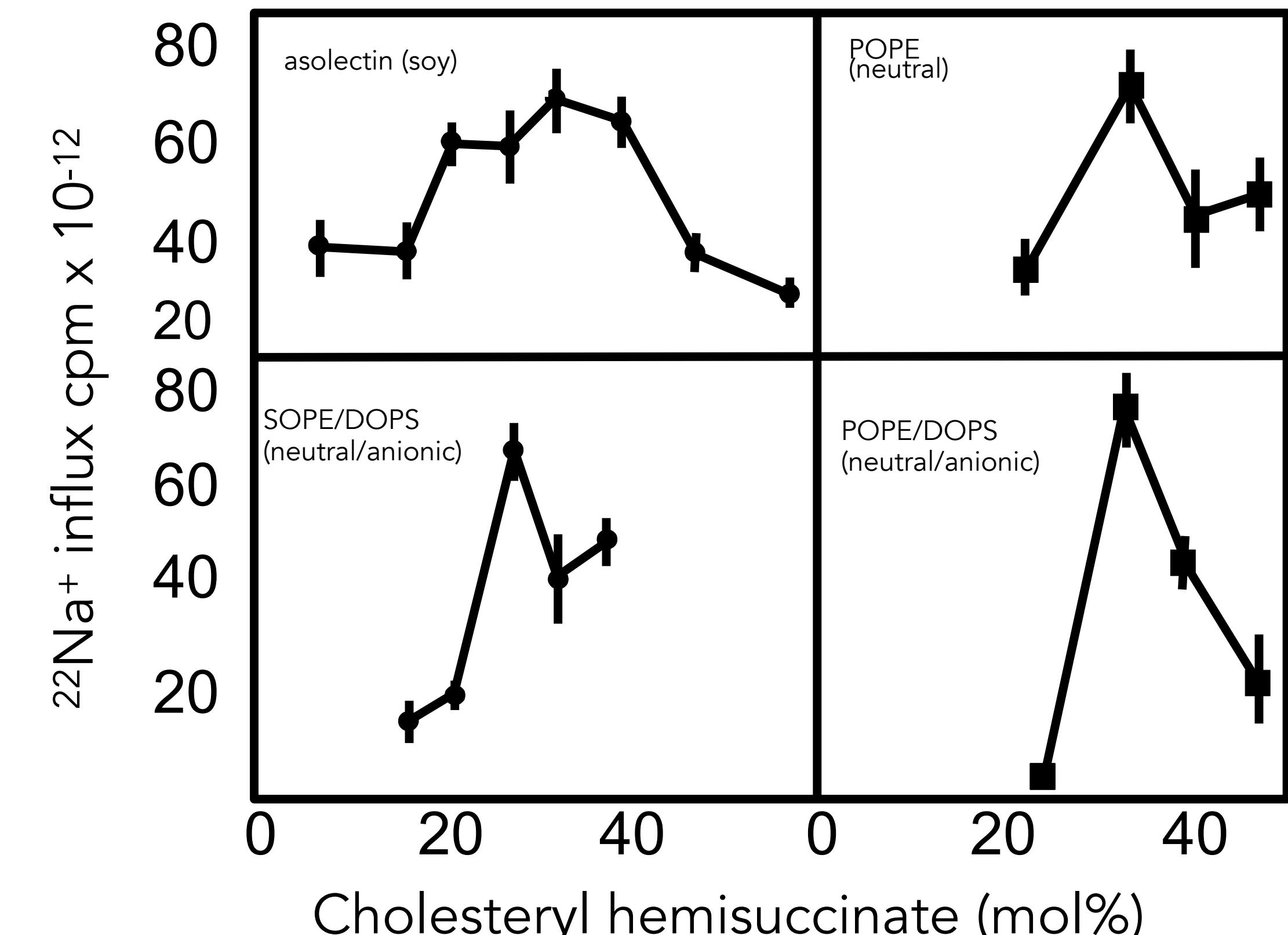


Native Membrane



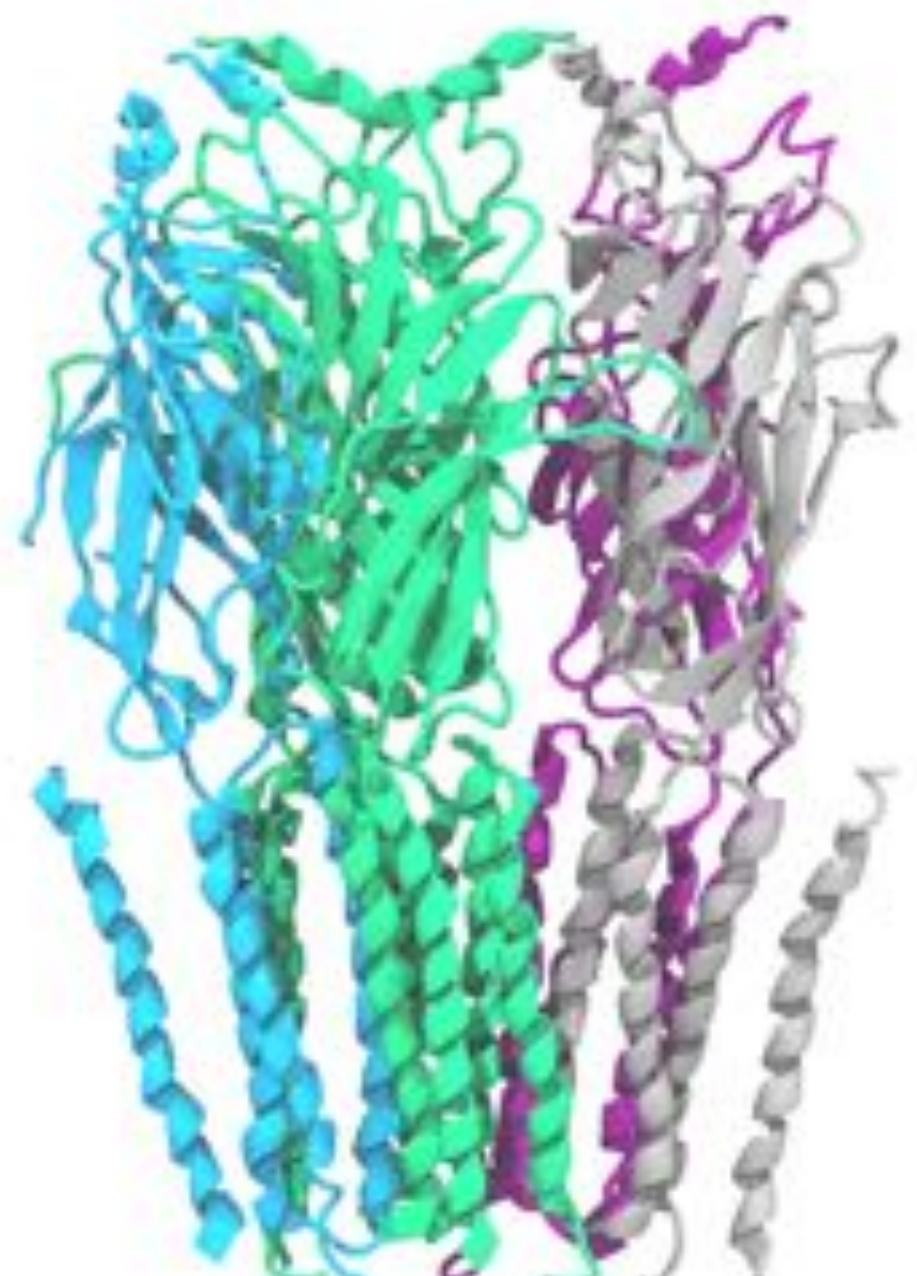
# Lipids modulate nAChR function

- 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

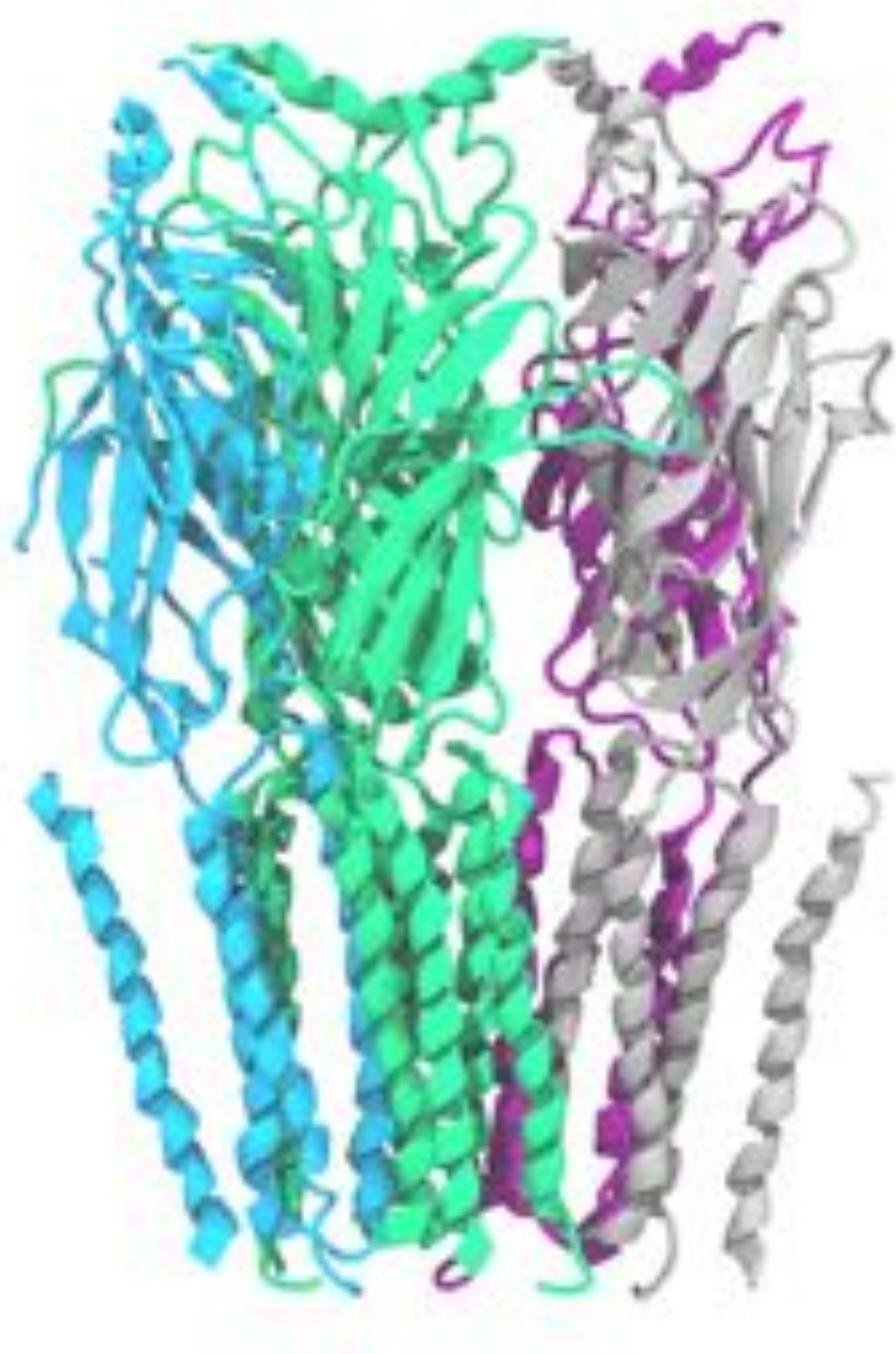
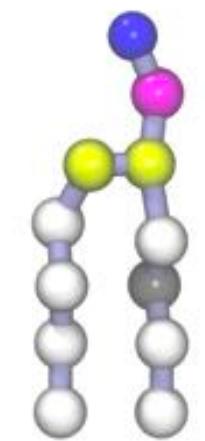


# Lipid modulation mechanism



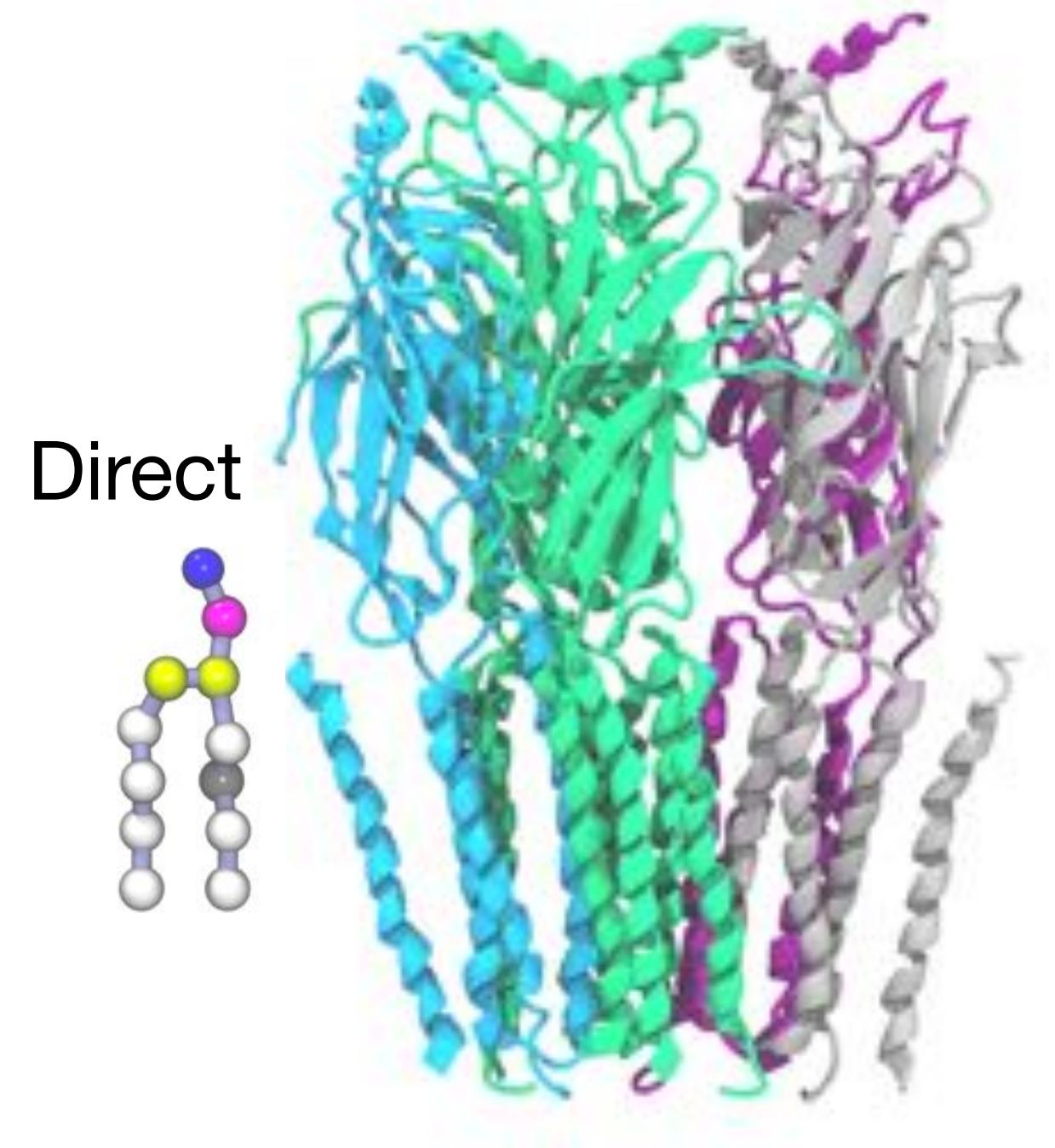
# Do modulating lipids indirectly or directly interact?

# Indirect



# 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?

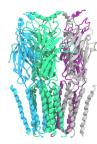


# Experimental methods to predict pLGIC-lipid interactions



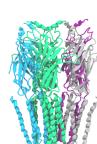
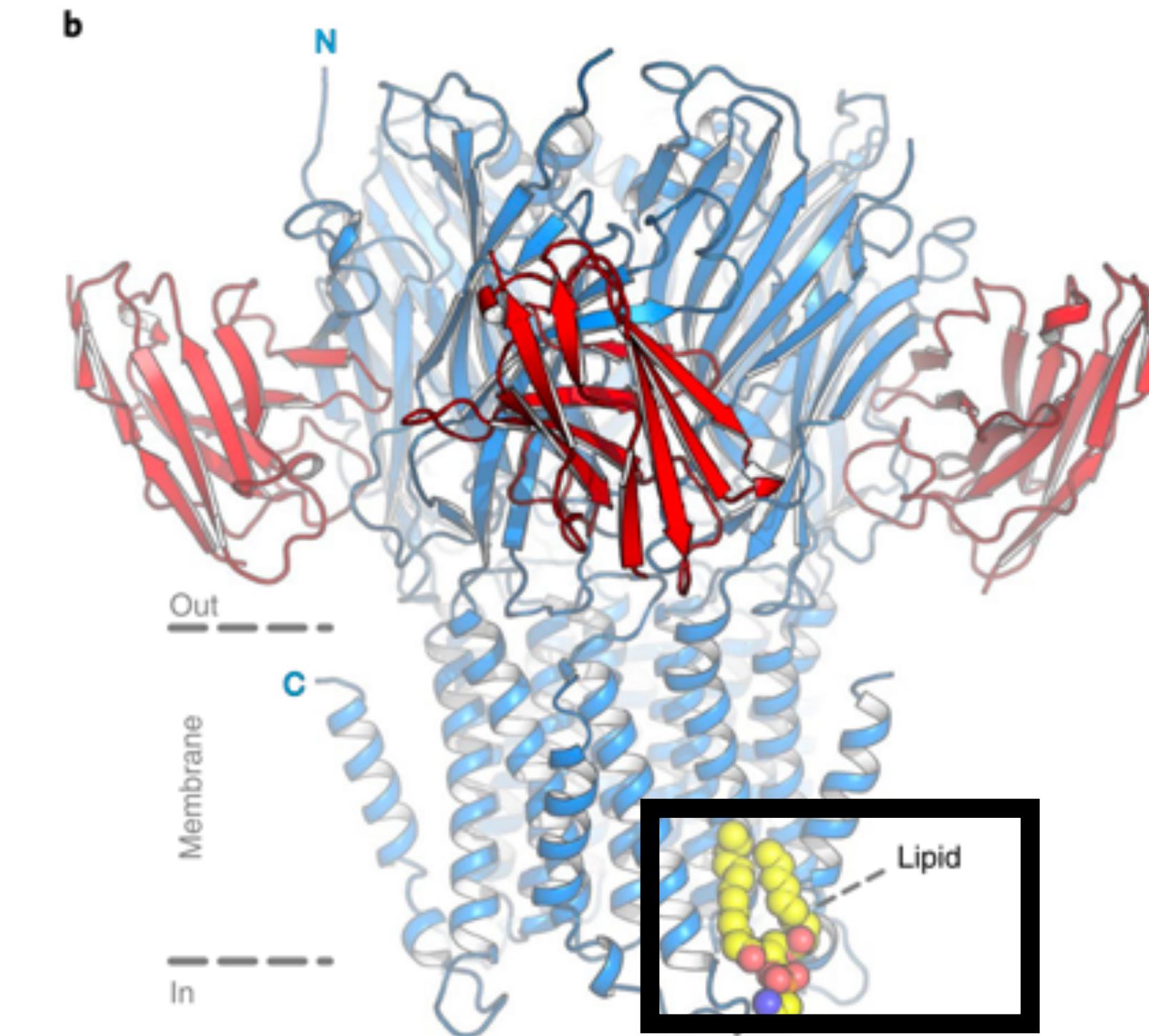
## Structural Biology

- 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**

# A computational method to predict pLGIC-lipid interactions

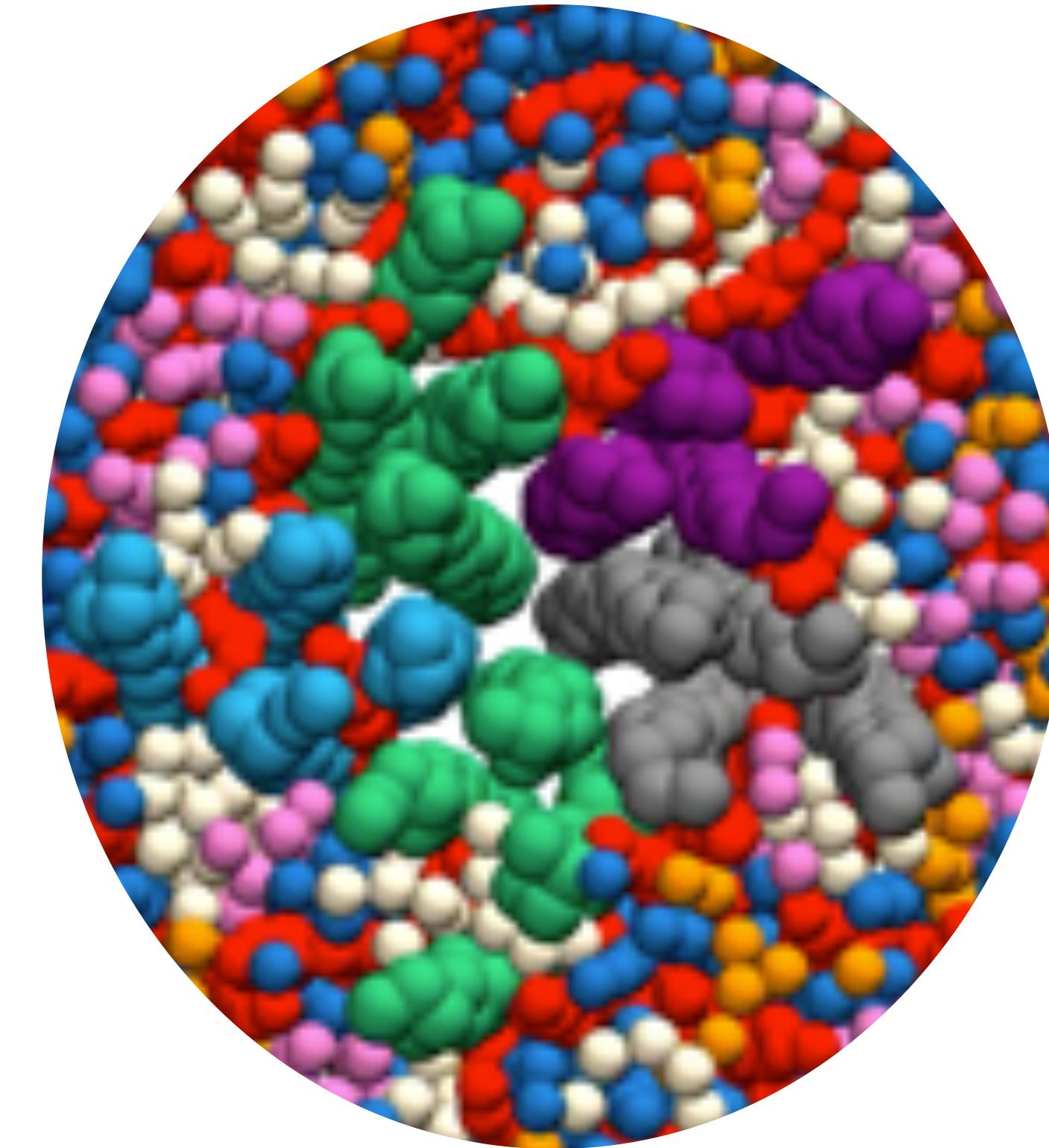


Computational Biology: Molecular  
Dynamics (MD)

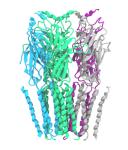
# A computational method to predict pLGIC-lipid interactions



Computational Biology: Molecular  
Dynamics (MD)

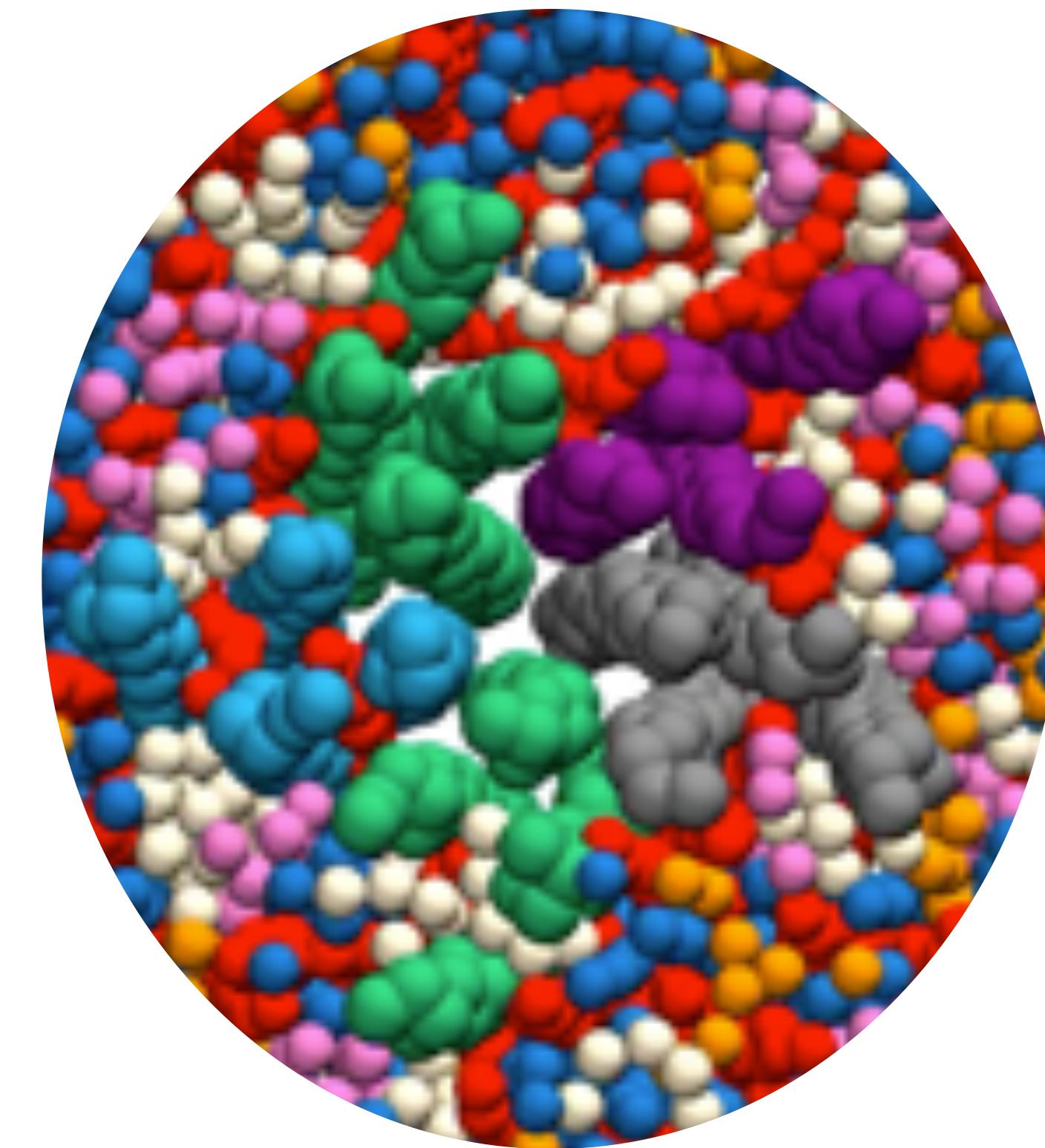


# A computational method to predict pLGIC-lipid interactions



## Computational Biology: Molecular Dynamics (MD)

- Shows molecular interaction, where lipids bind, and how lipids bind

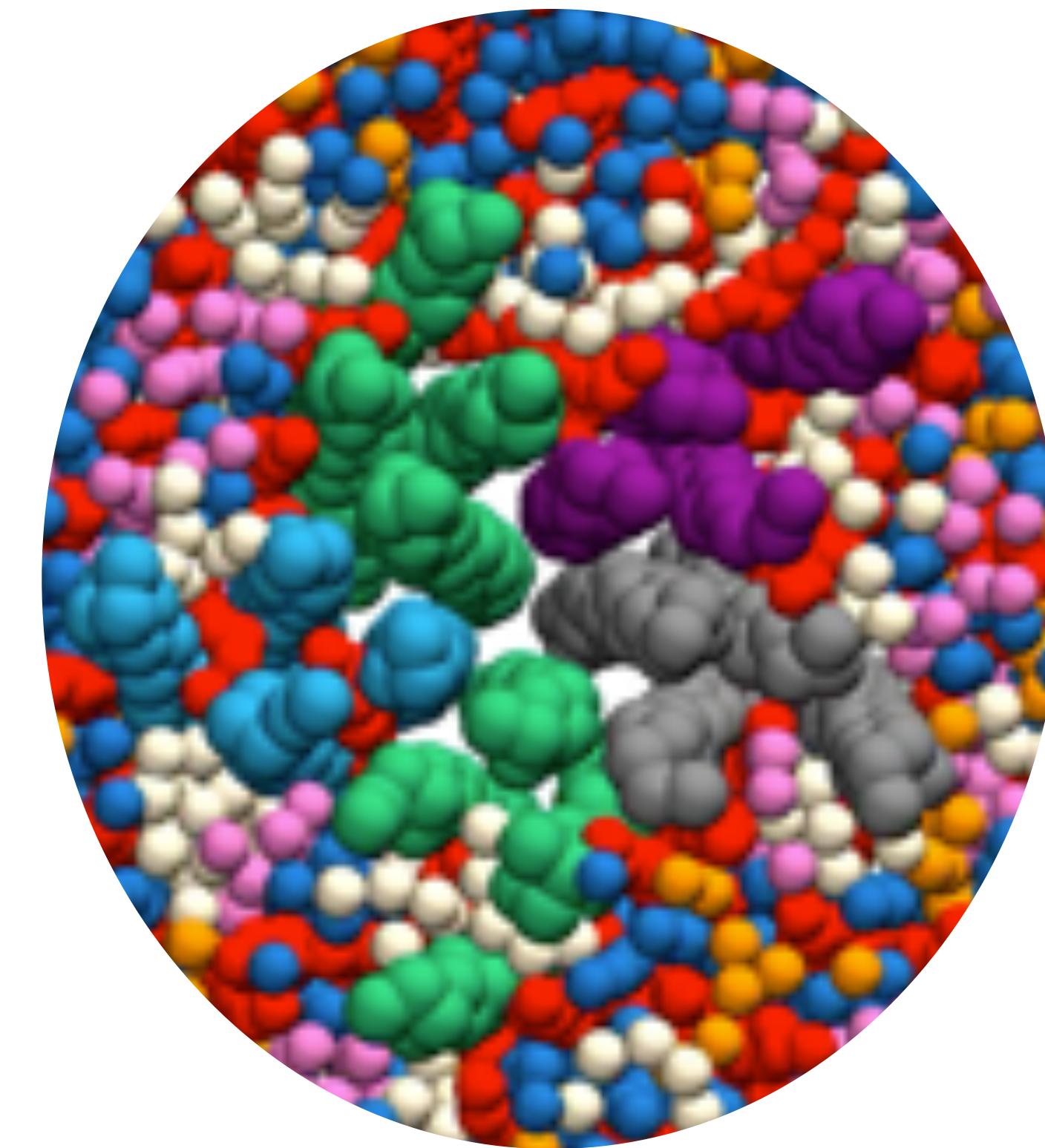


# A computational method to predict pLGIC-lipid interactions



## 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

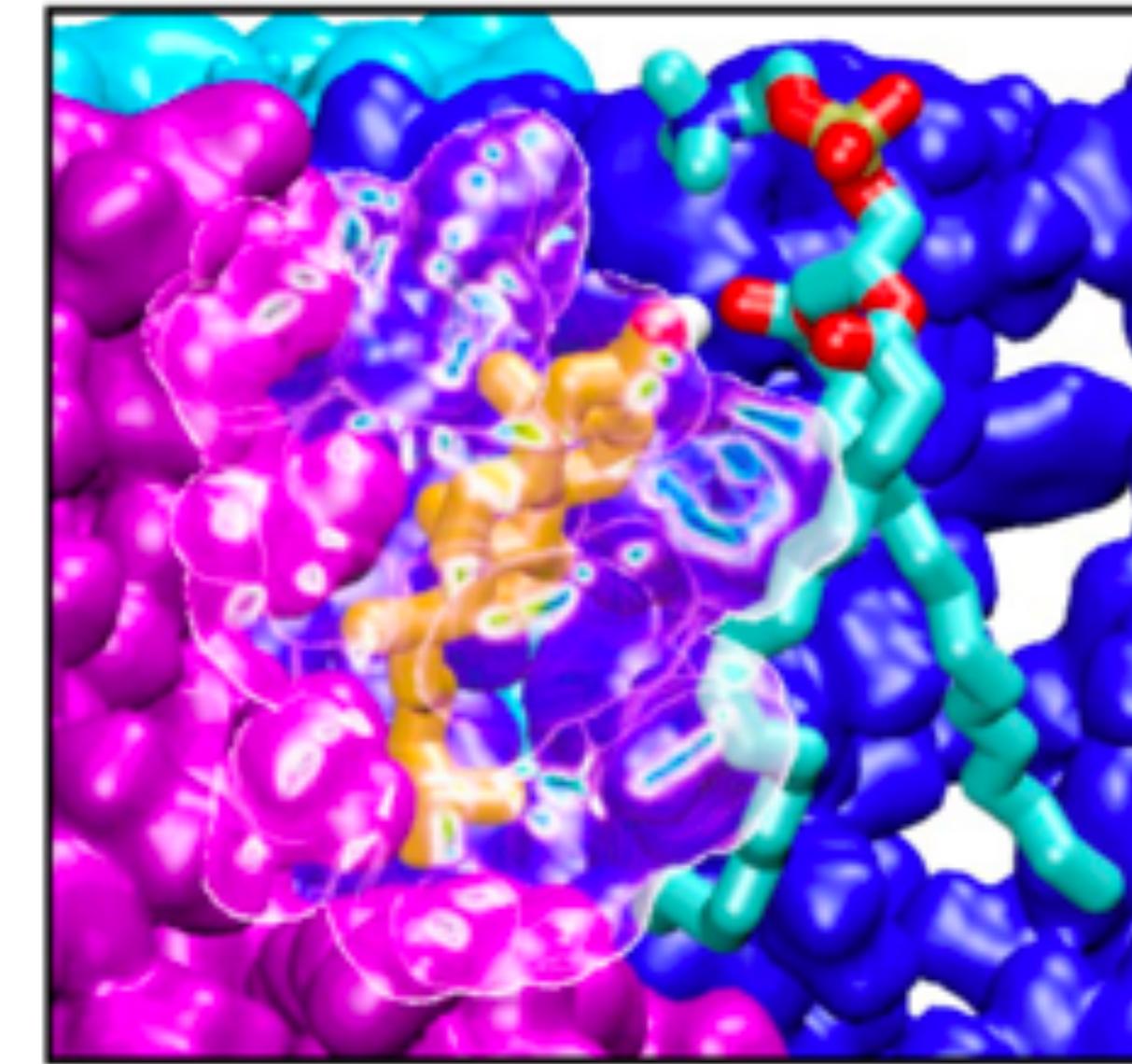
Atomistic MD simulations

- 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!!)

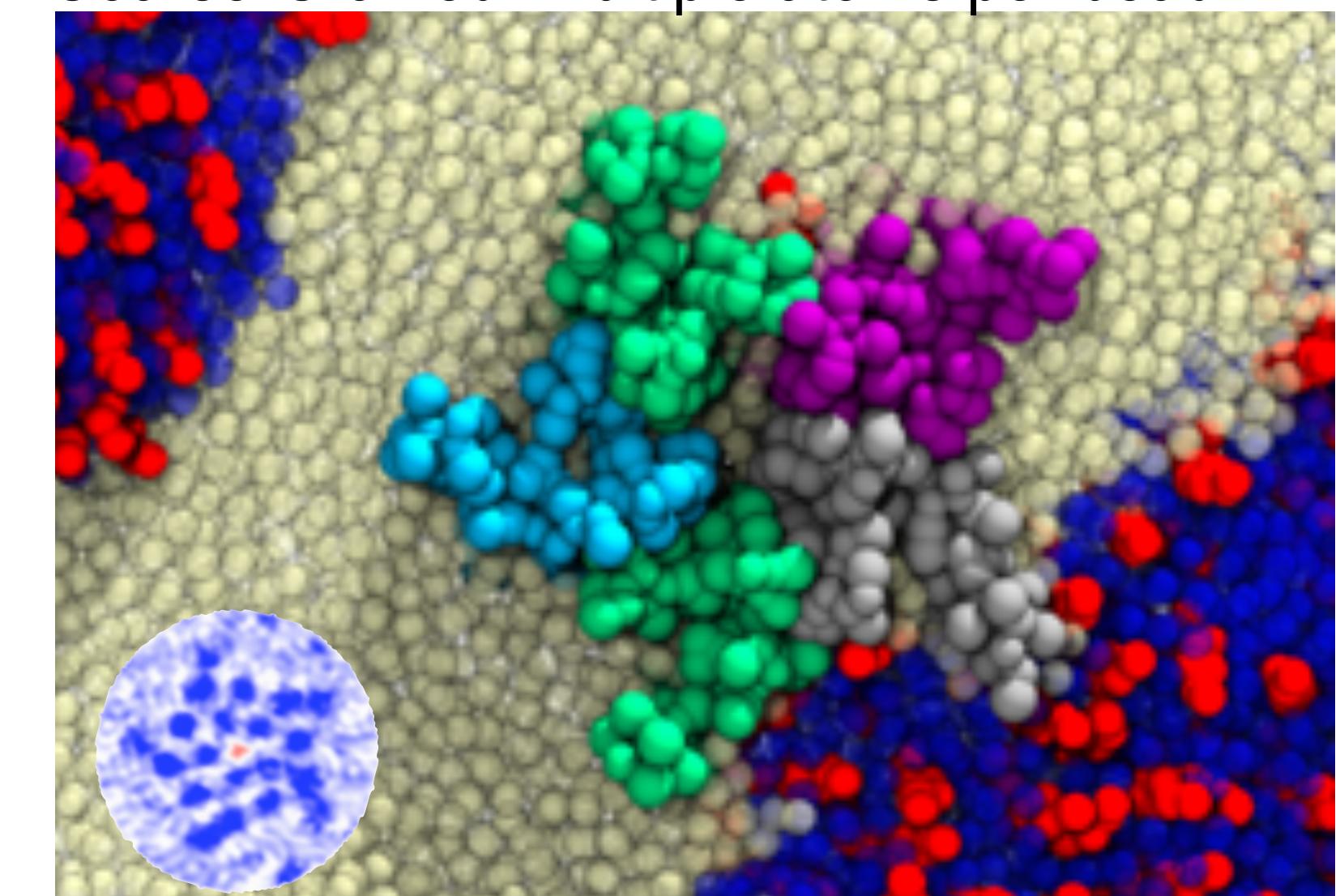
- Lower resolution but less expensive for lipid to explore the protein!

Atomistic: Shows every atom



Brannigan et al 2008, PNAS

Coarse-Grained: Multiple atoms per bead

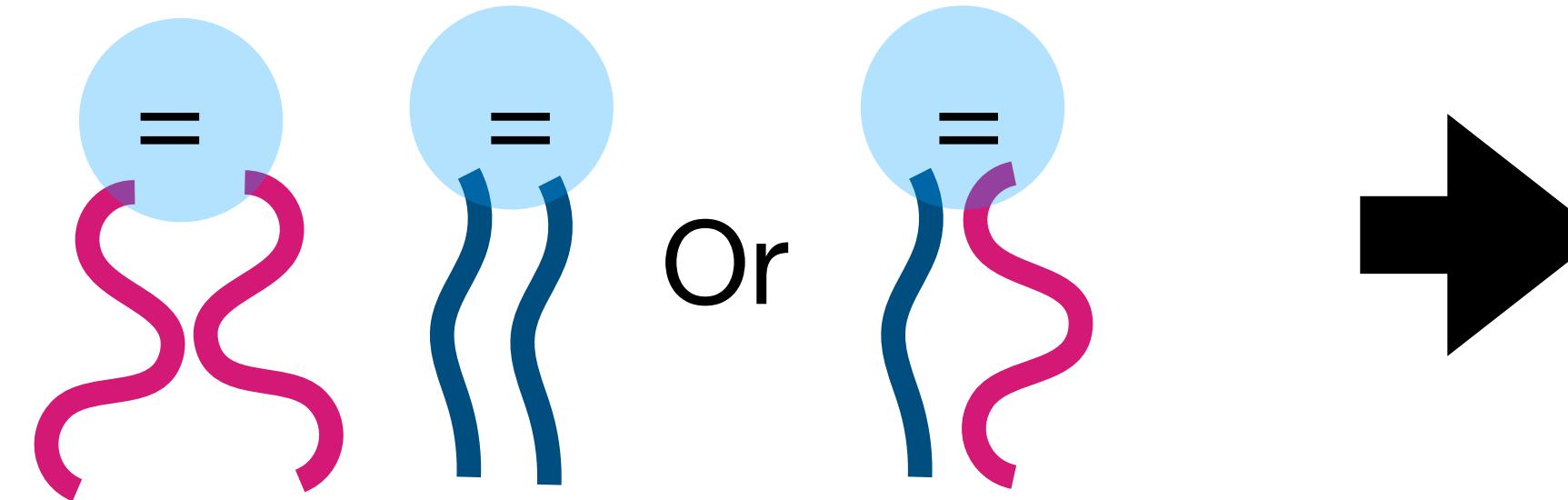


Sharp et al 2019, BBA

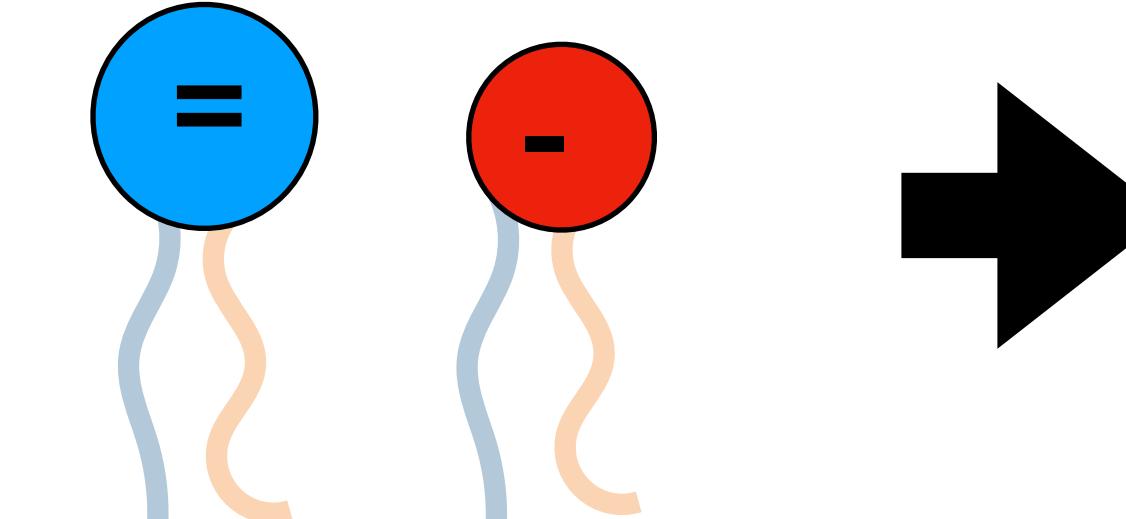
# Goal of the thesis

- 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

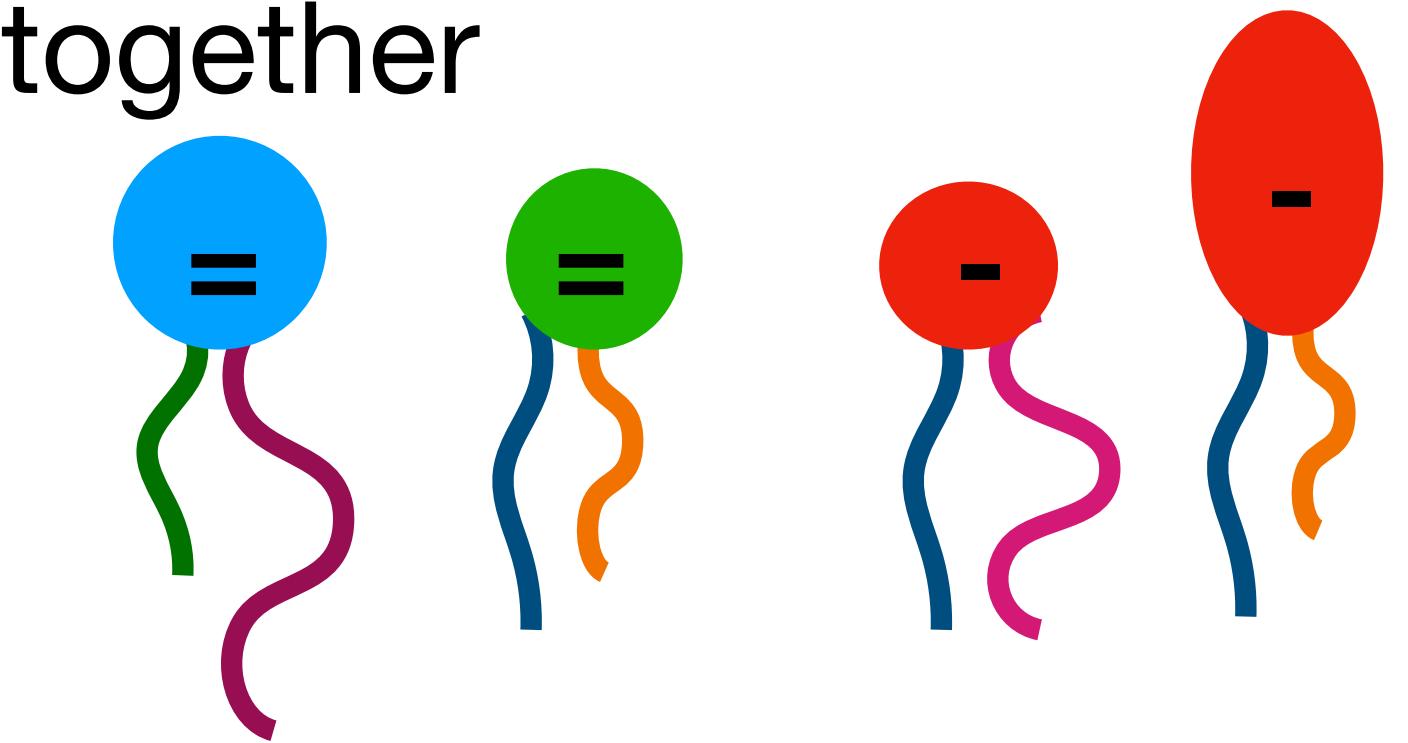
## Section 1: Saturation, Sterols, and Domain-forming lipids



## Section 2: Lipid head-group charge

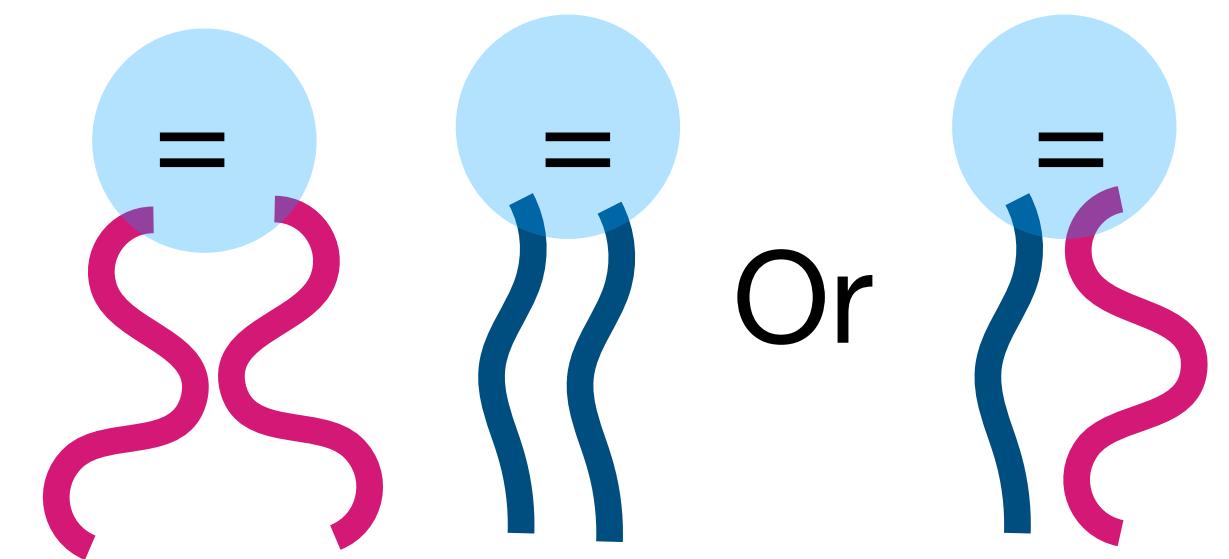


## Section 3: Putting it all together



# 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



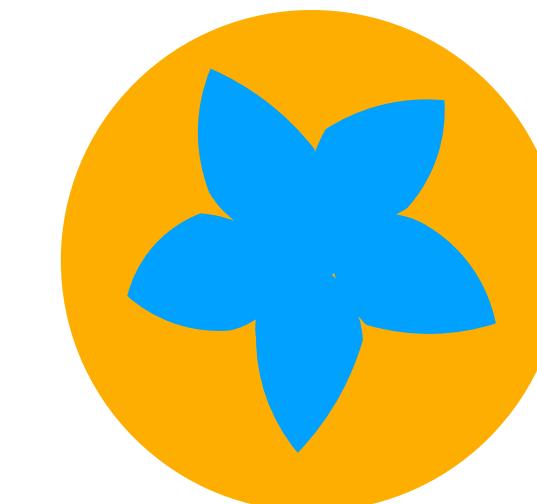
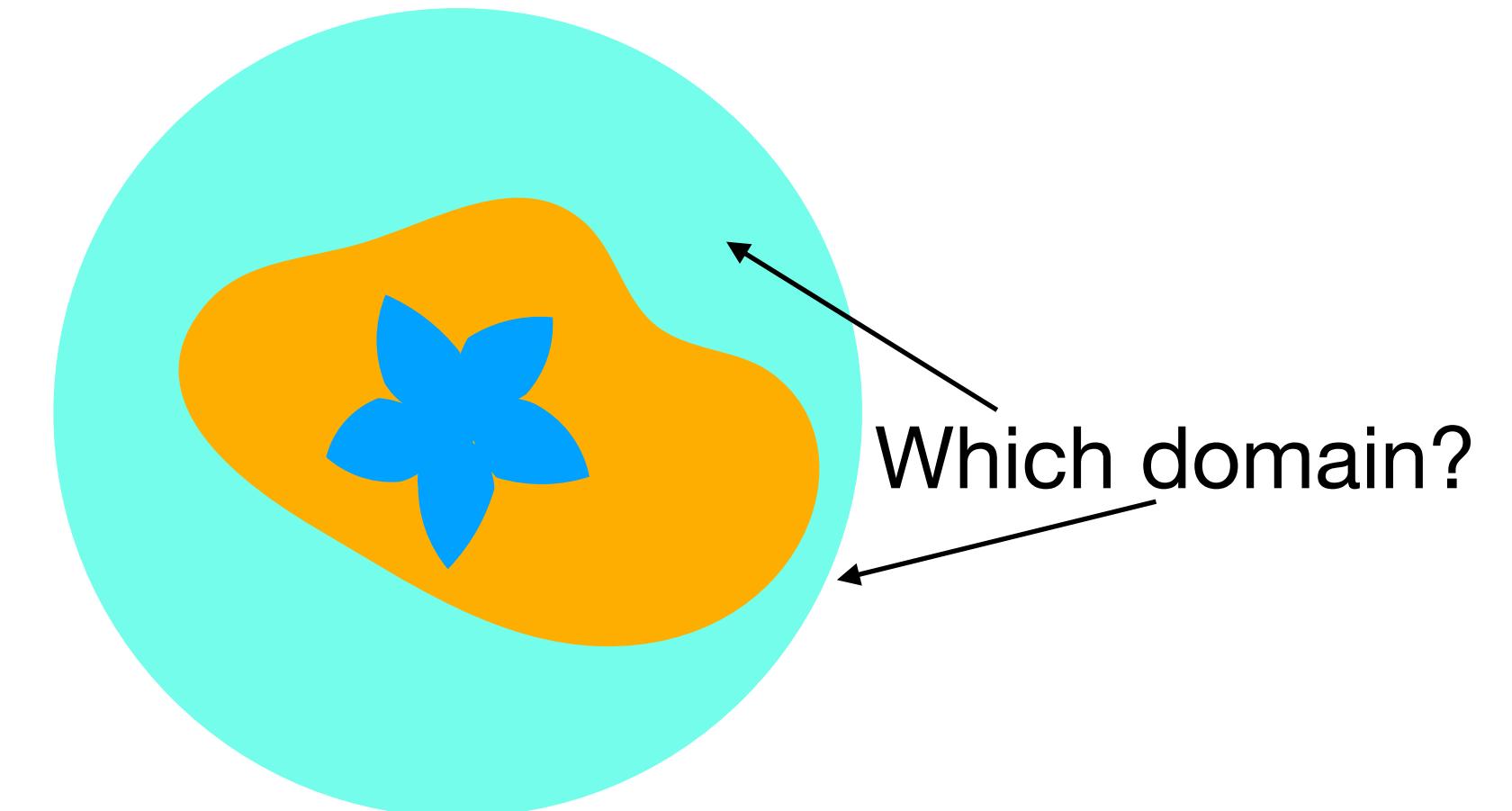
Or

# nAChR in Ternary PUFA Rich Membranes

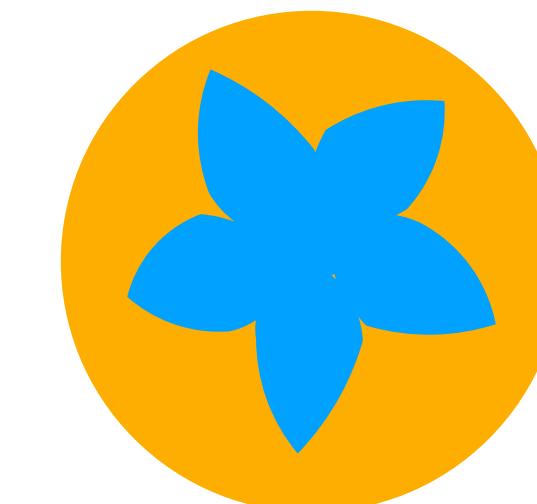
>If domains form, which domain does nAChR reside in?

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

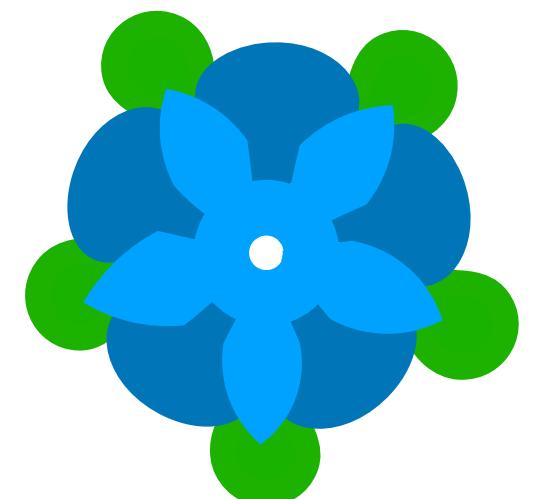
Are there preferential binding sites?



What are these lipids?



Or



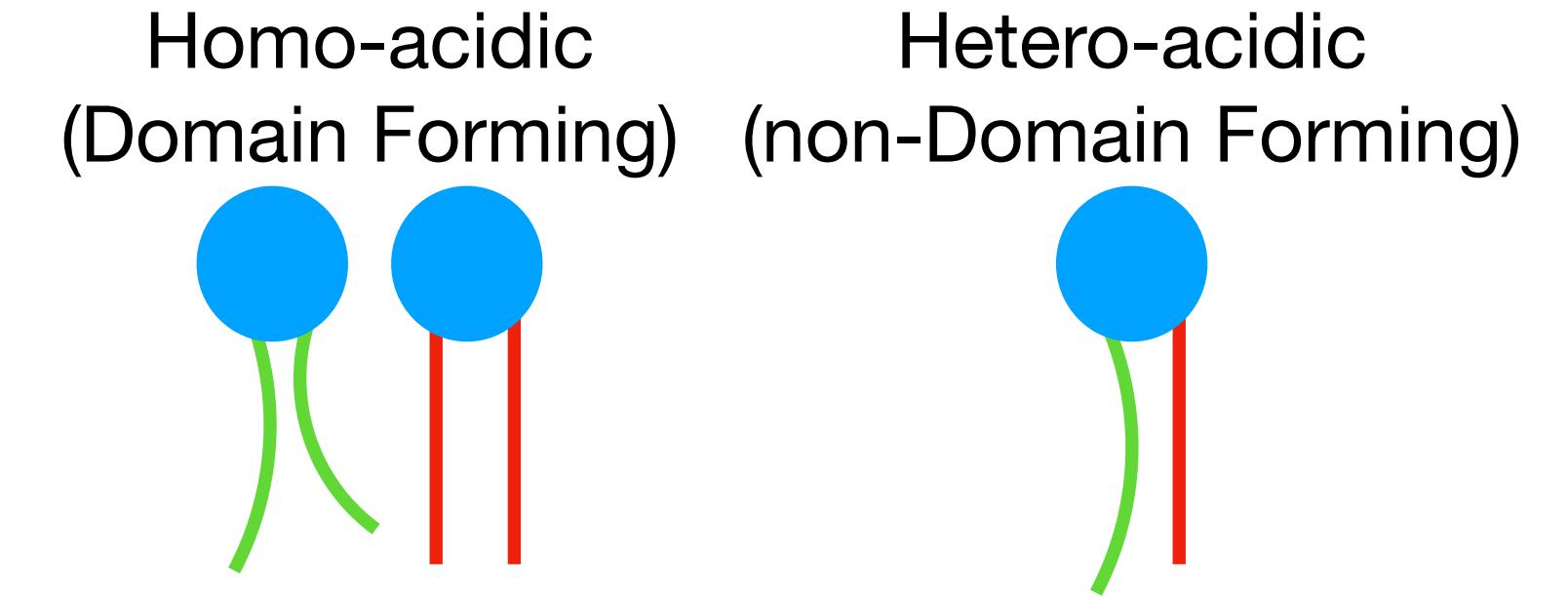
# Approach

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

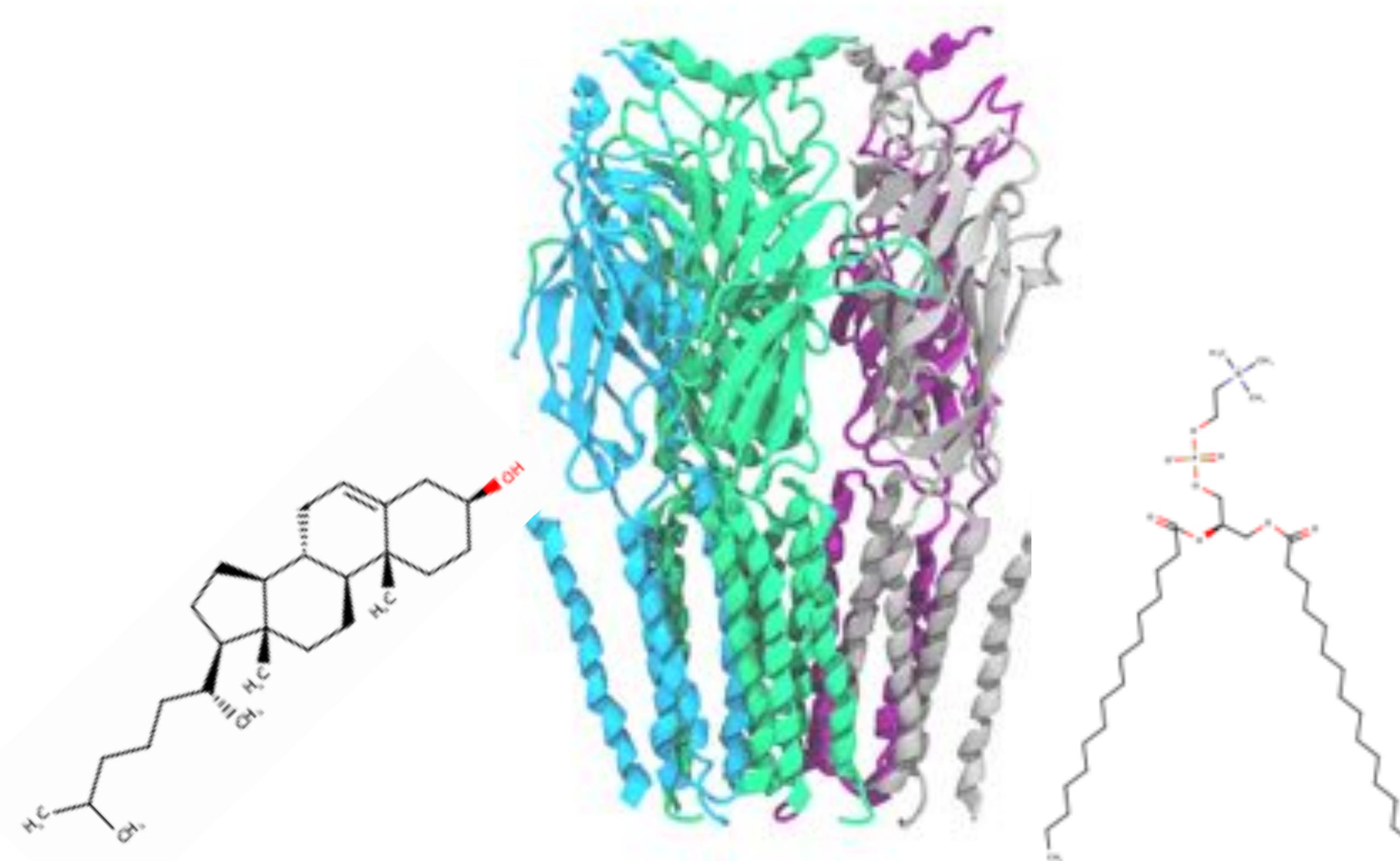


## Methods

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

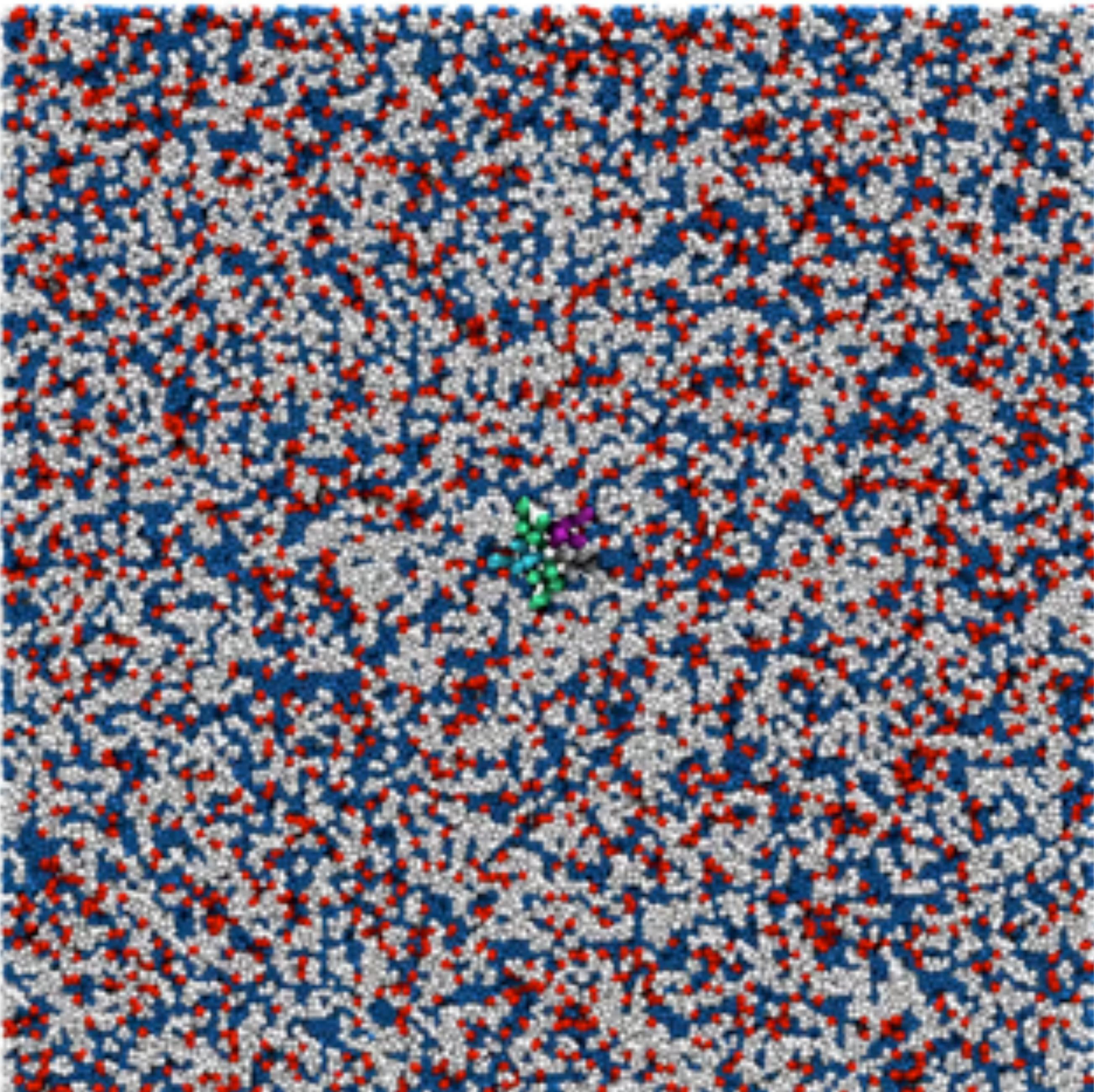
# Hypothesis

**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

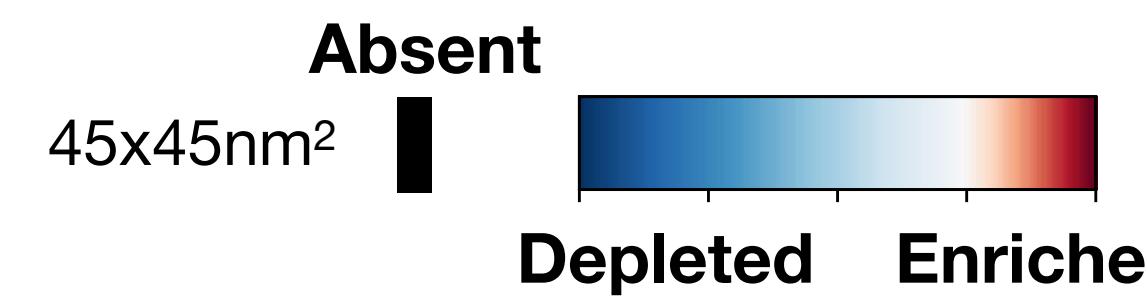
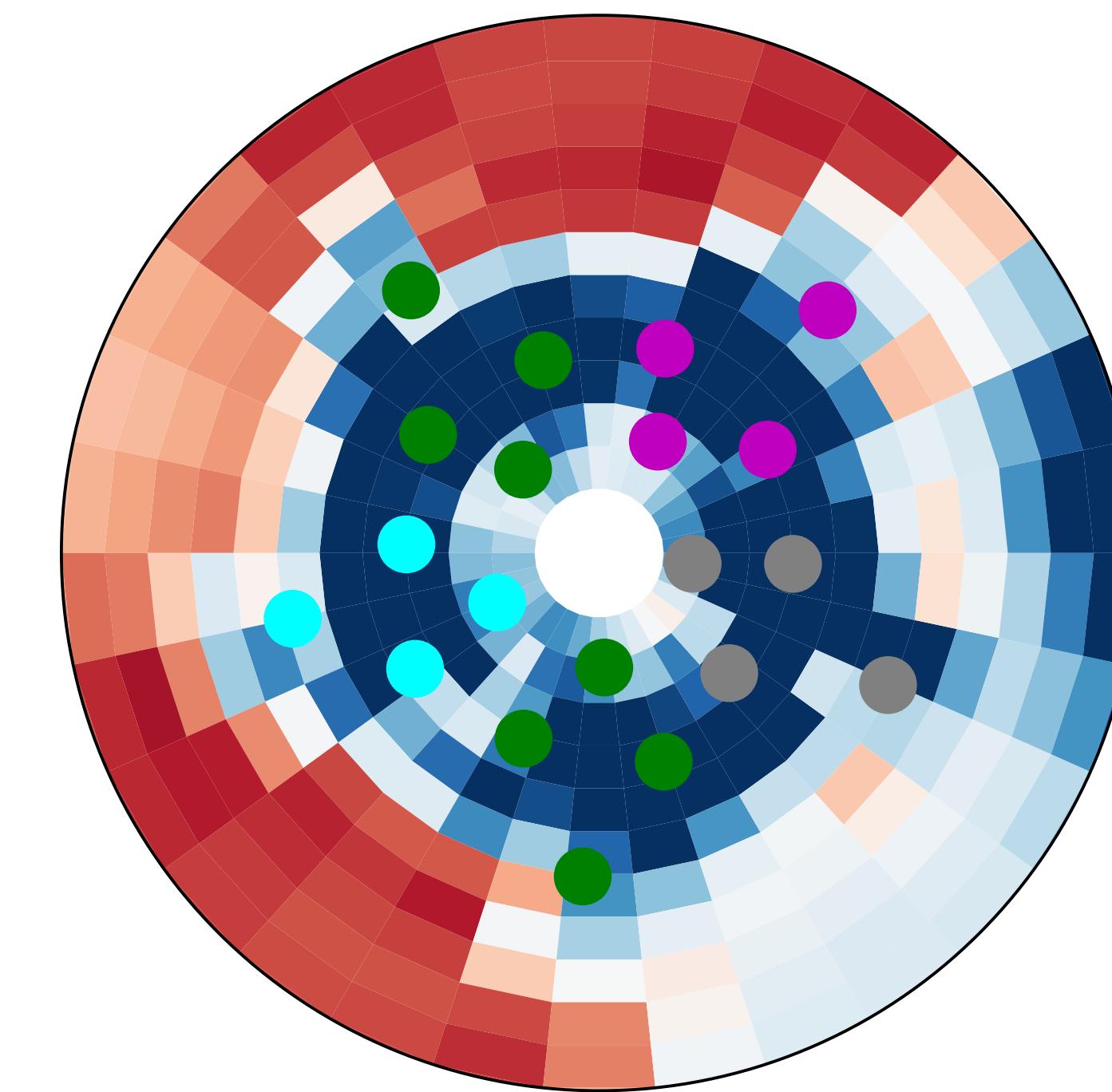


Saturated Cholesterol Unsaturated

~ 4 us

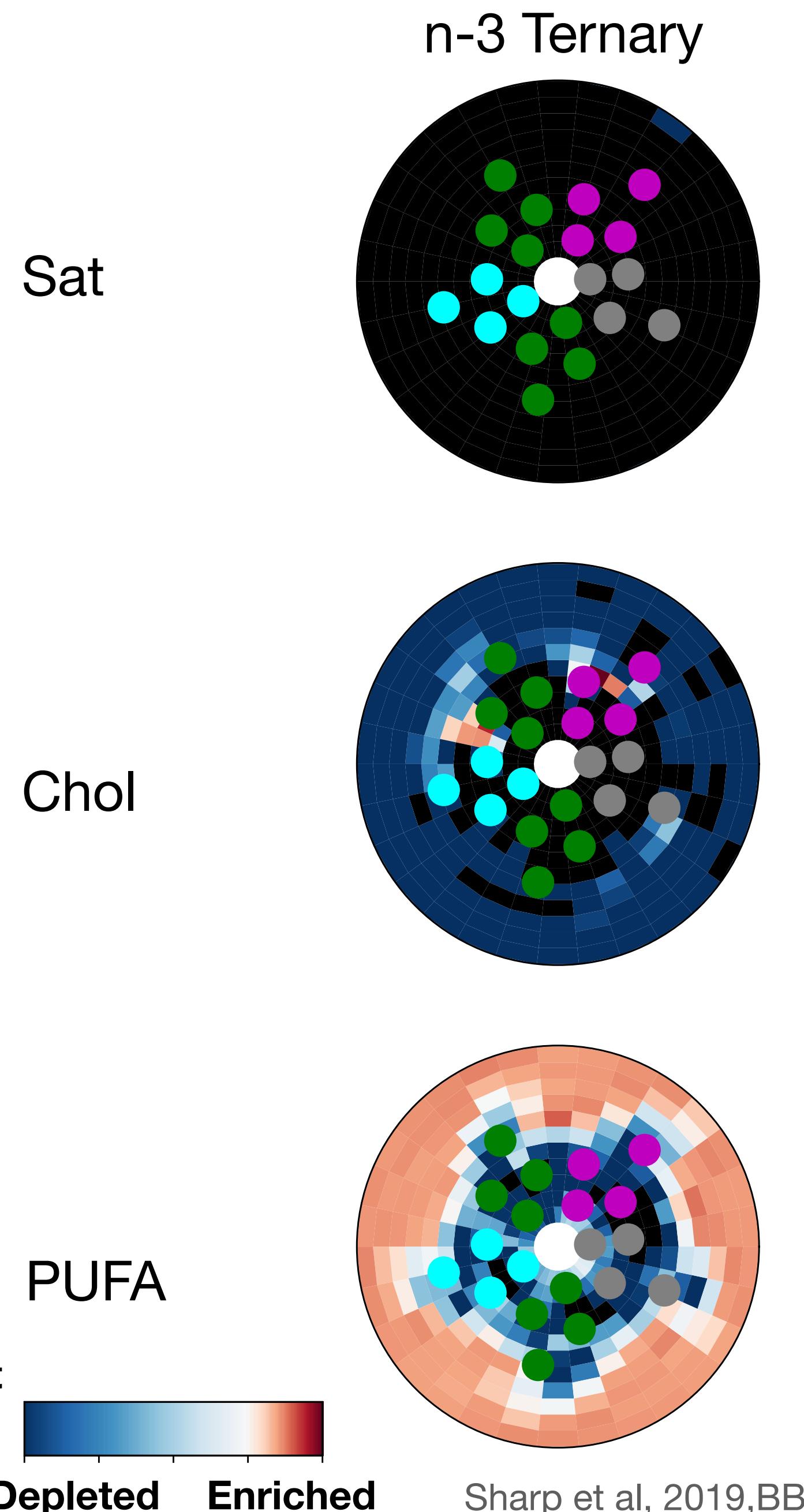
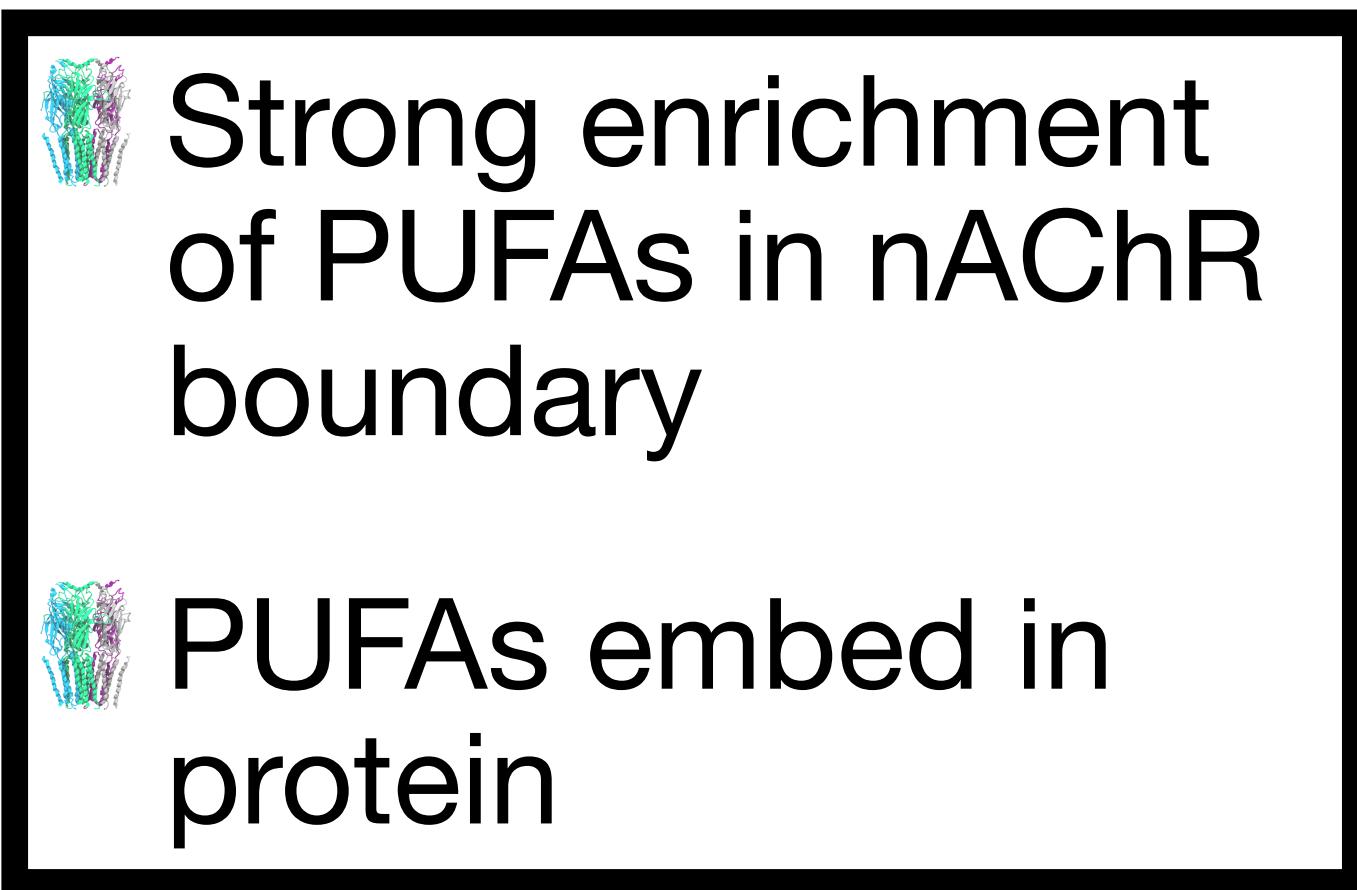
75x75nm<sup>2</sup>

# Reading polar density plots

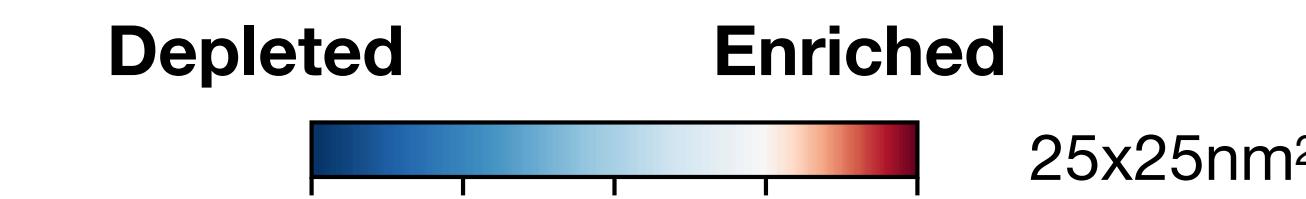


Woods, Sharp, Brannigan, 2019, Journal of Membrane Biology

# Density enrichment in domain forming membranes

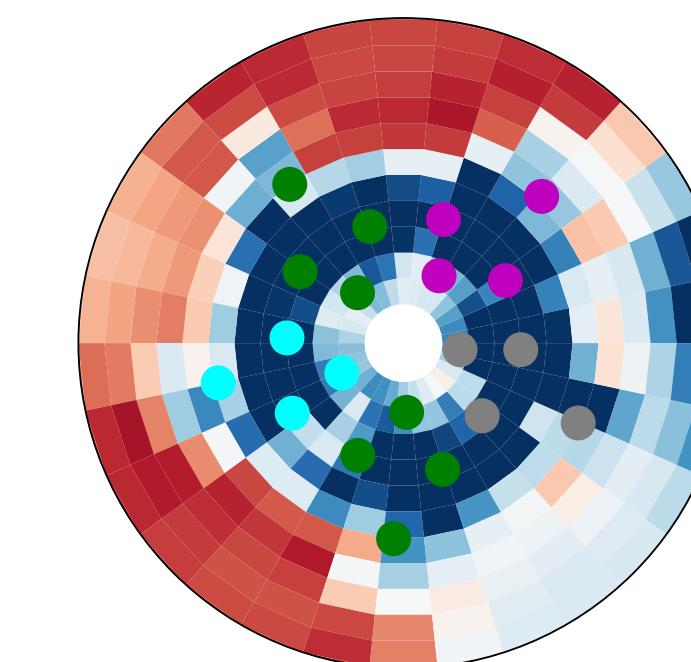
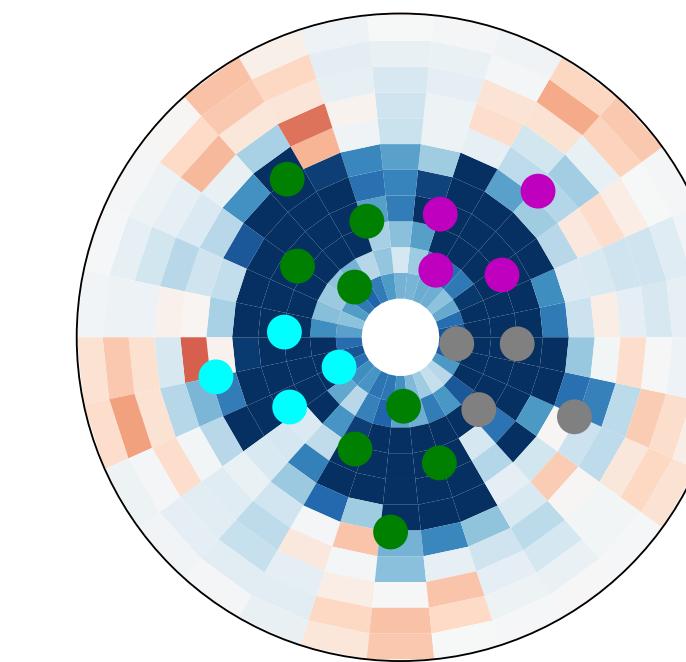


# Density enrichment in domains vs non-domain forming membranes

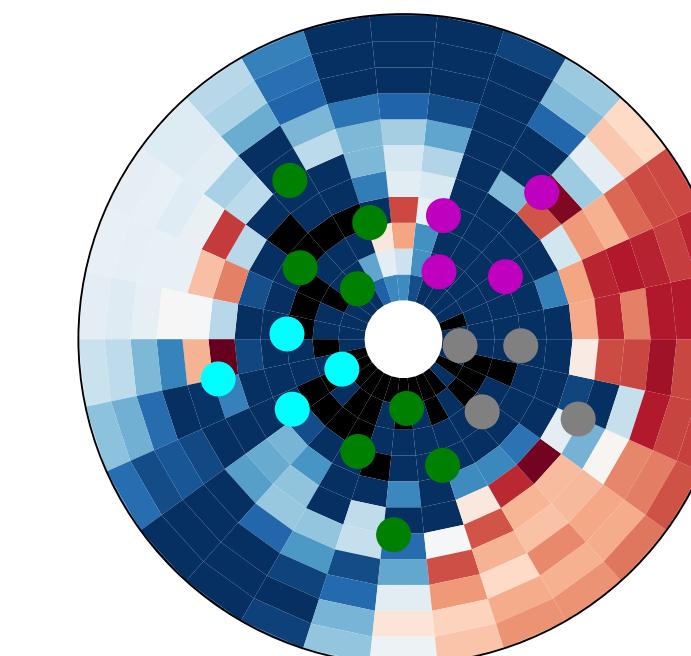
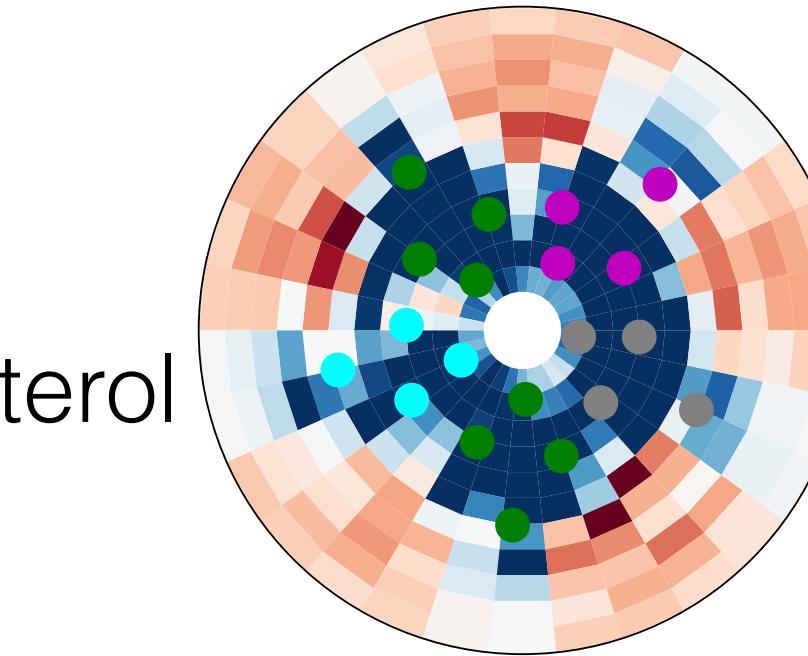


Hetero-Acidic  
(non-Domain Forming)

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

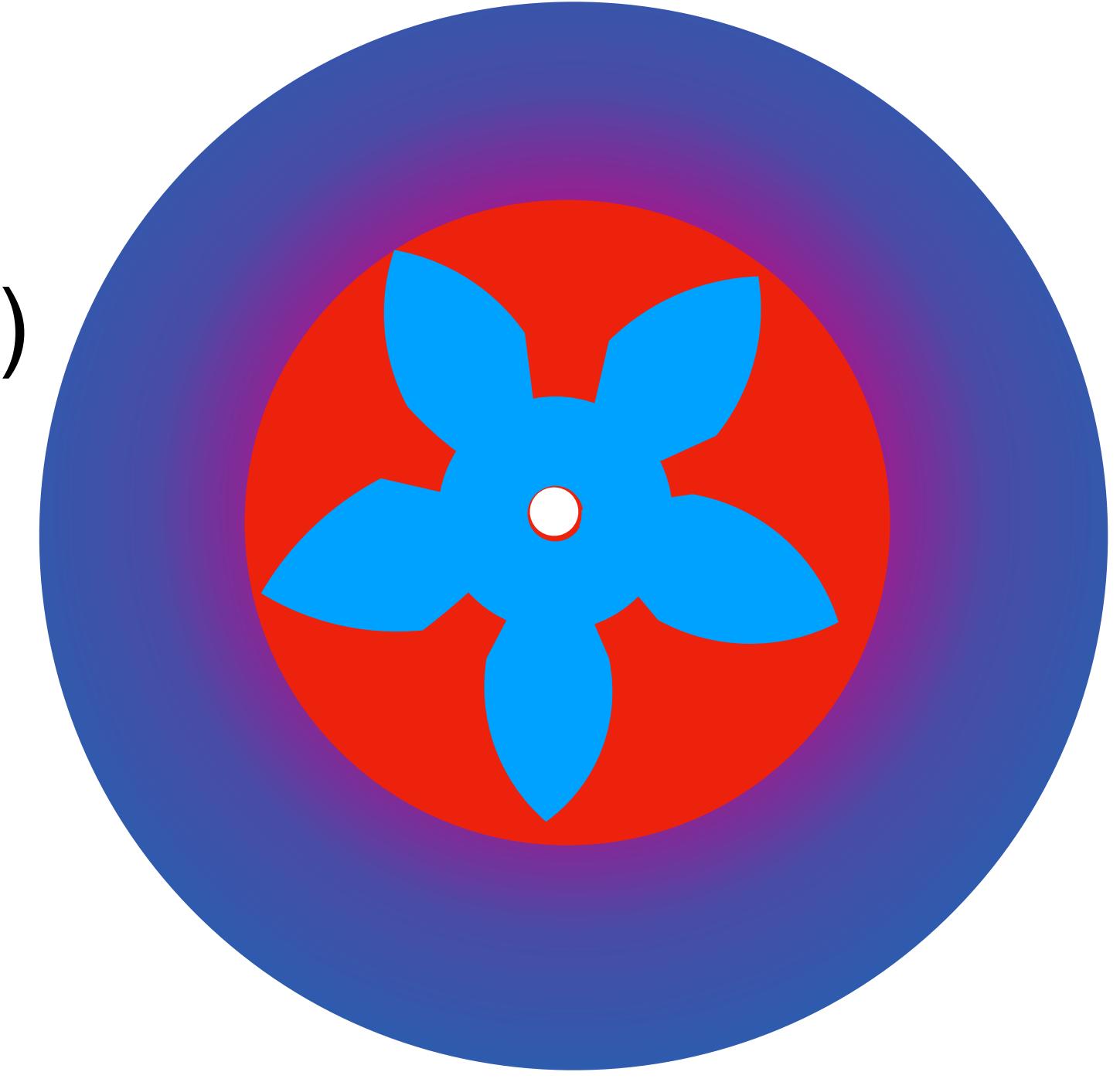
• What are the boundary lipids in PUFA rich membranes with homo- and hetero-acidic lipids?

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

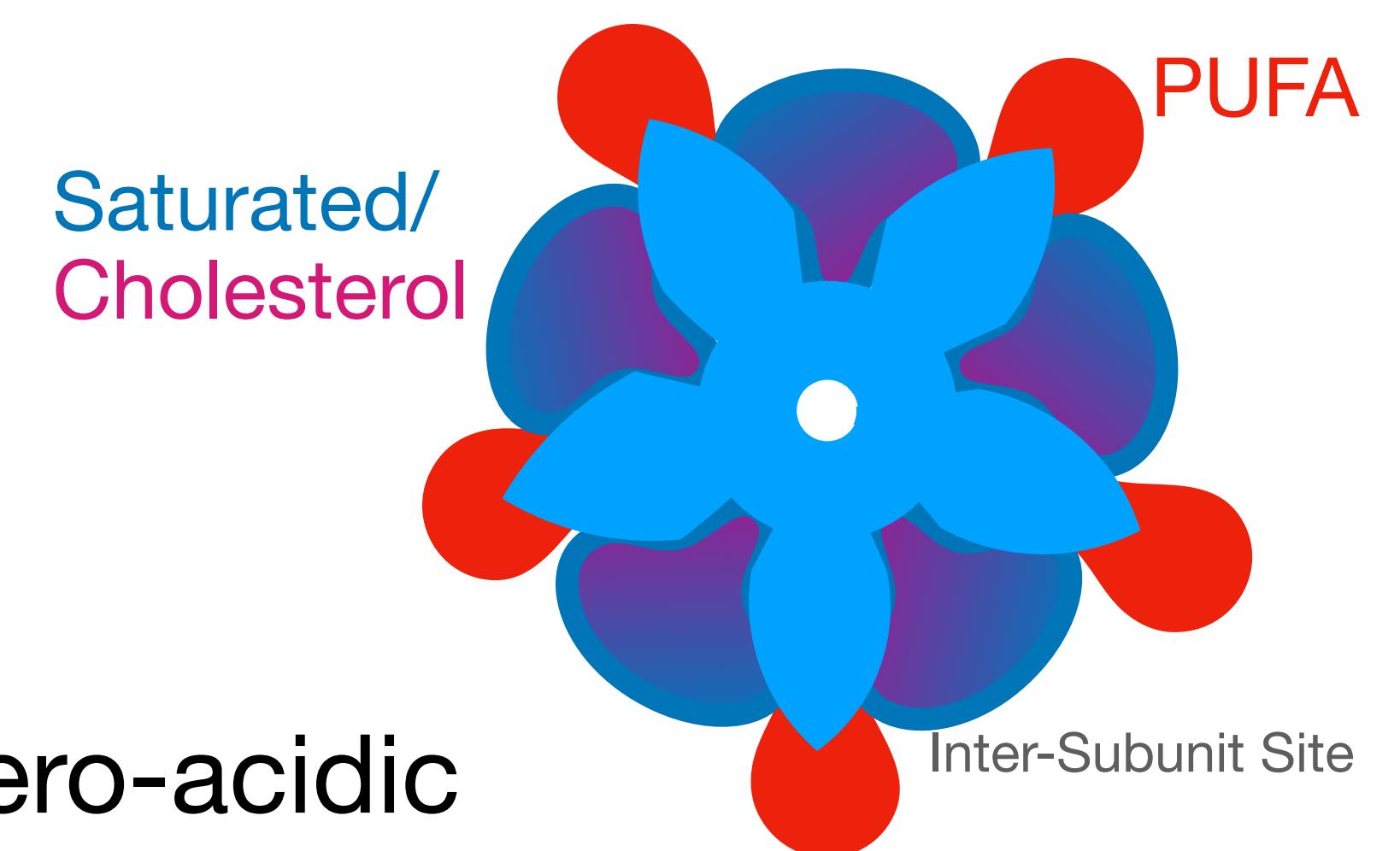
• Are there preferential binding sites?

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

Homo-acidic  
(domain forming)

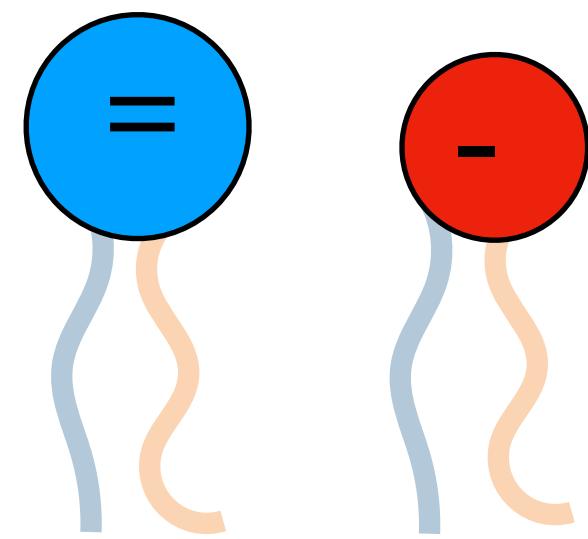


Hetero-acidic  
(no domains)



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# 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

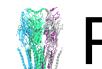
# Approach

## 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  $\mu$ s each

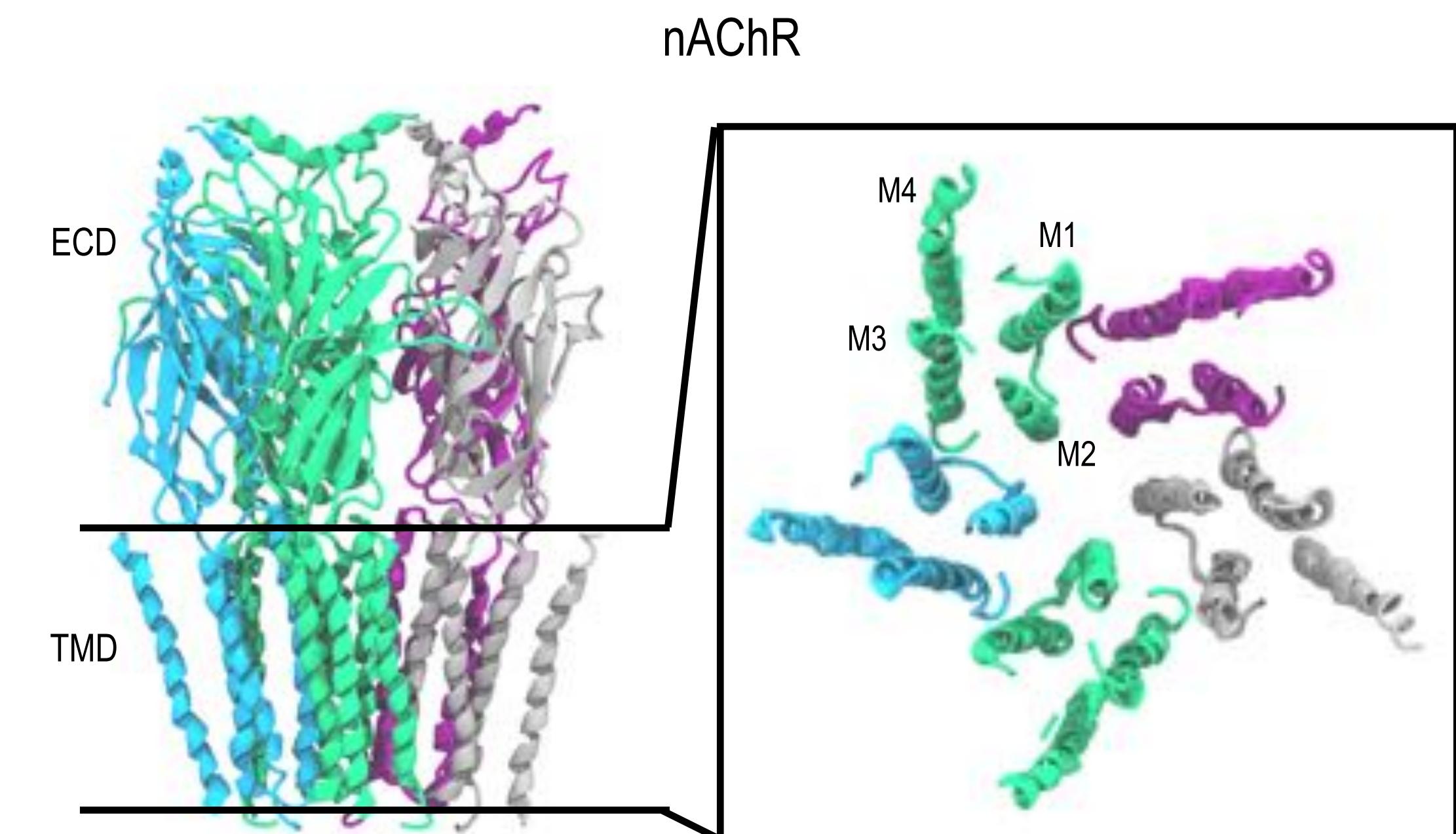
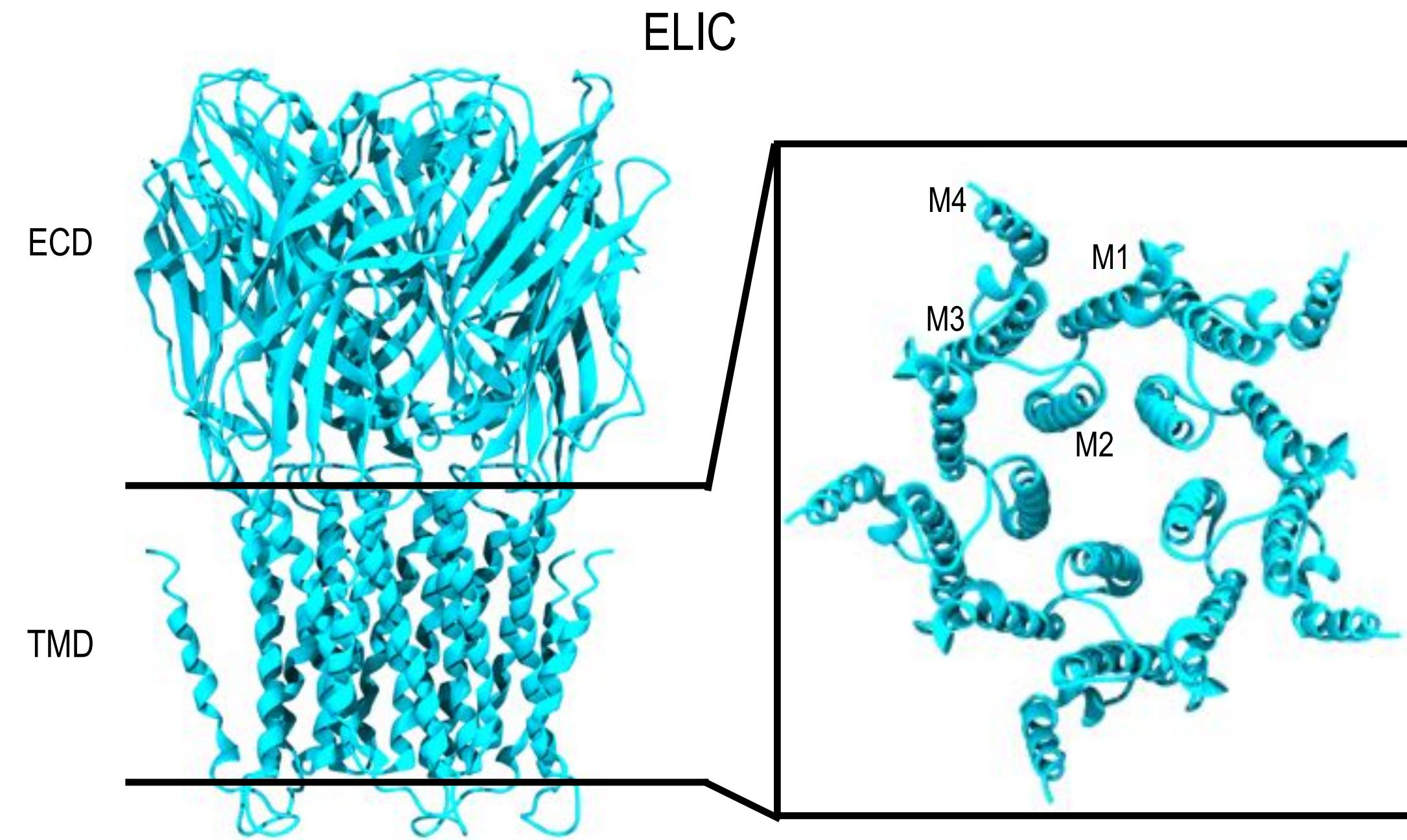
### Methods

-  Coarse grained molecular dynamics performed with Martini 2.2 and Gromacs 2016.2
-  Membranes 25x25 nm<sup>2</sup>
-  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



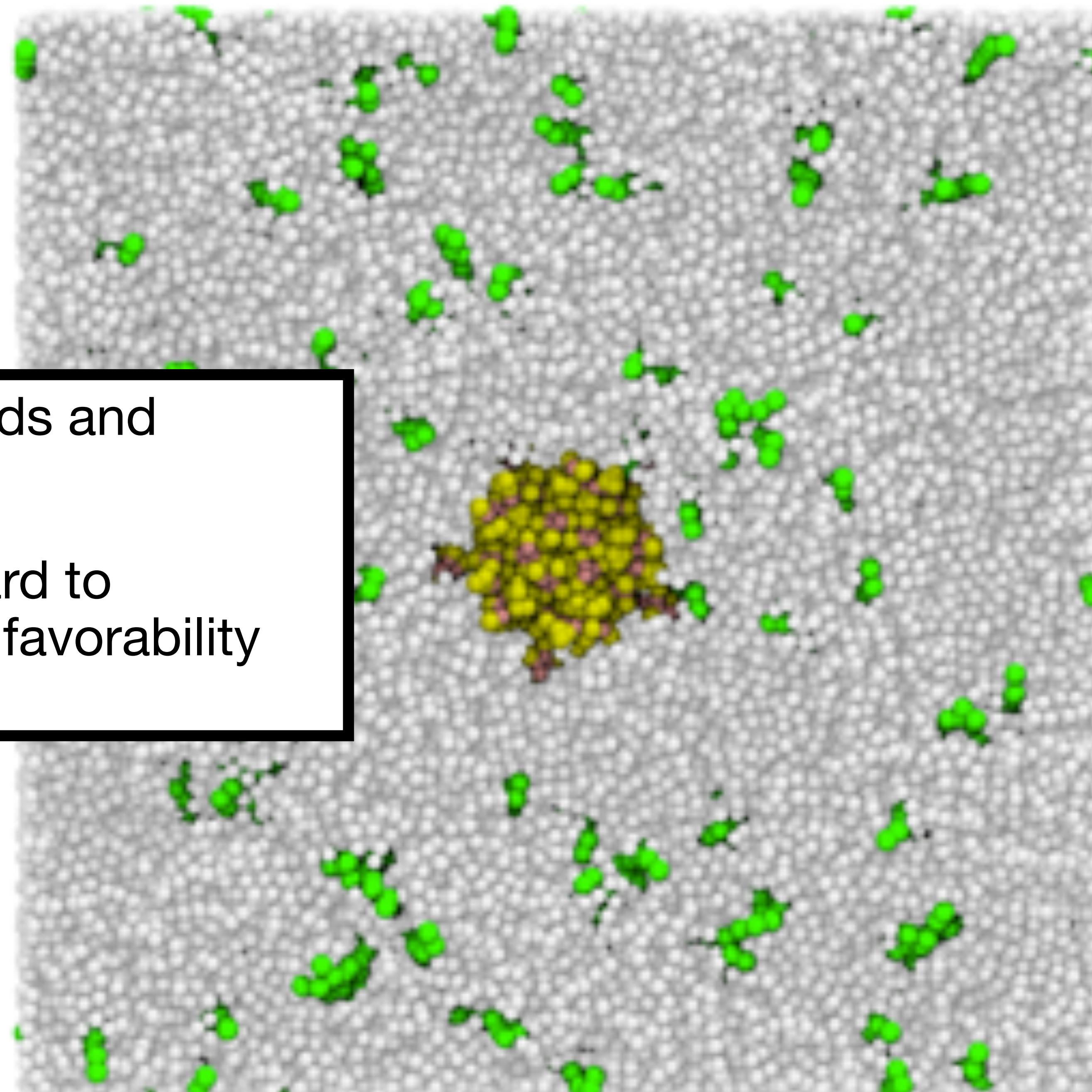
## Visualization of results

85:15 POPE:POPG

Neutral

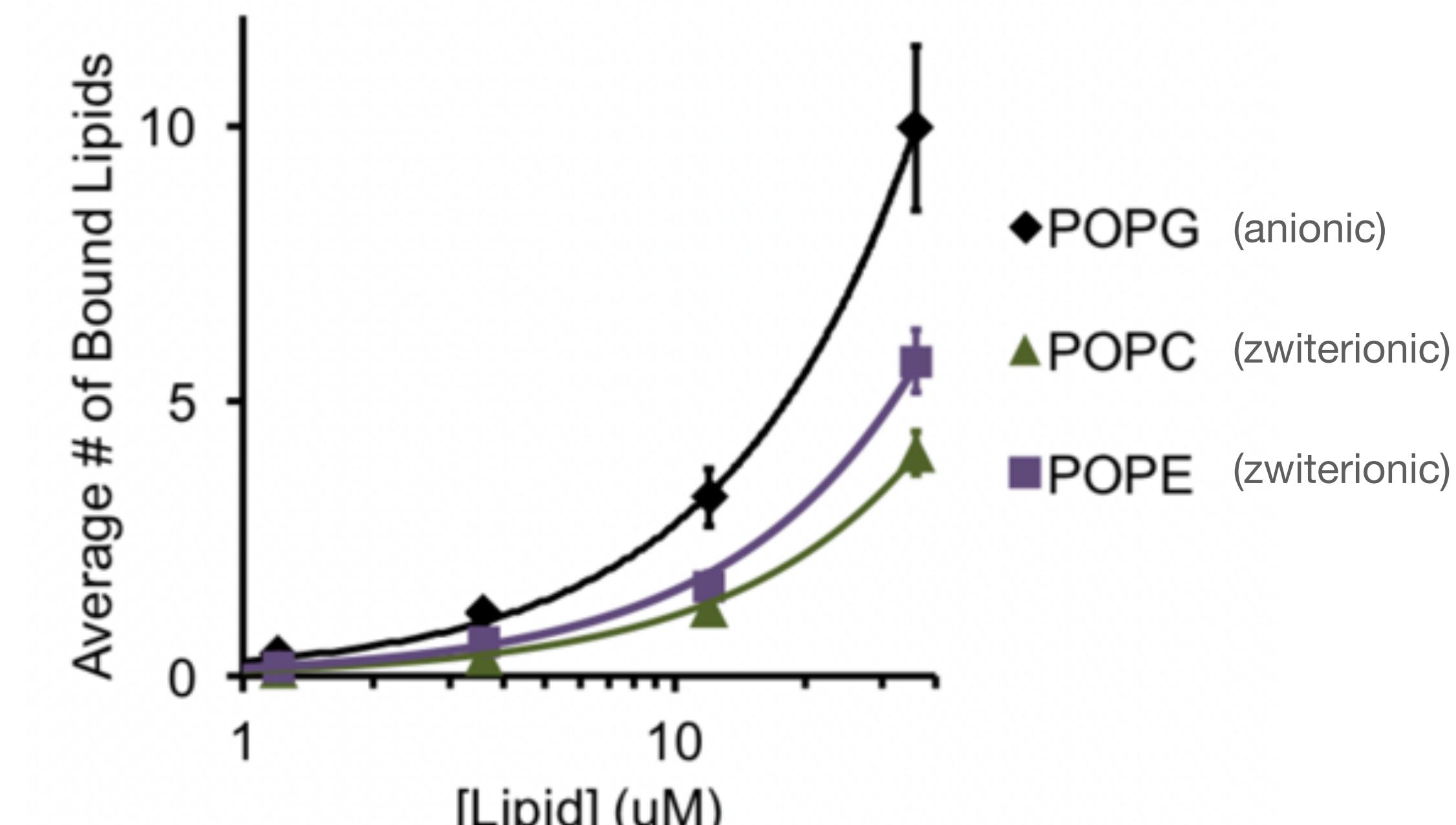
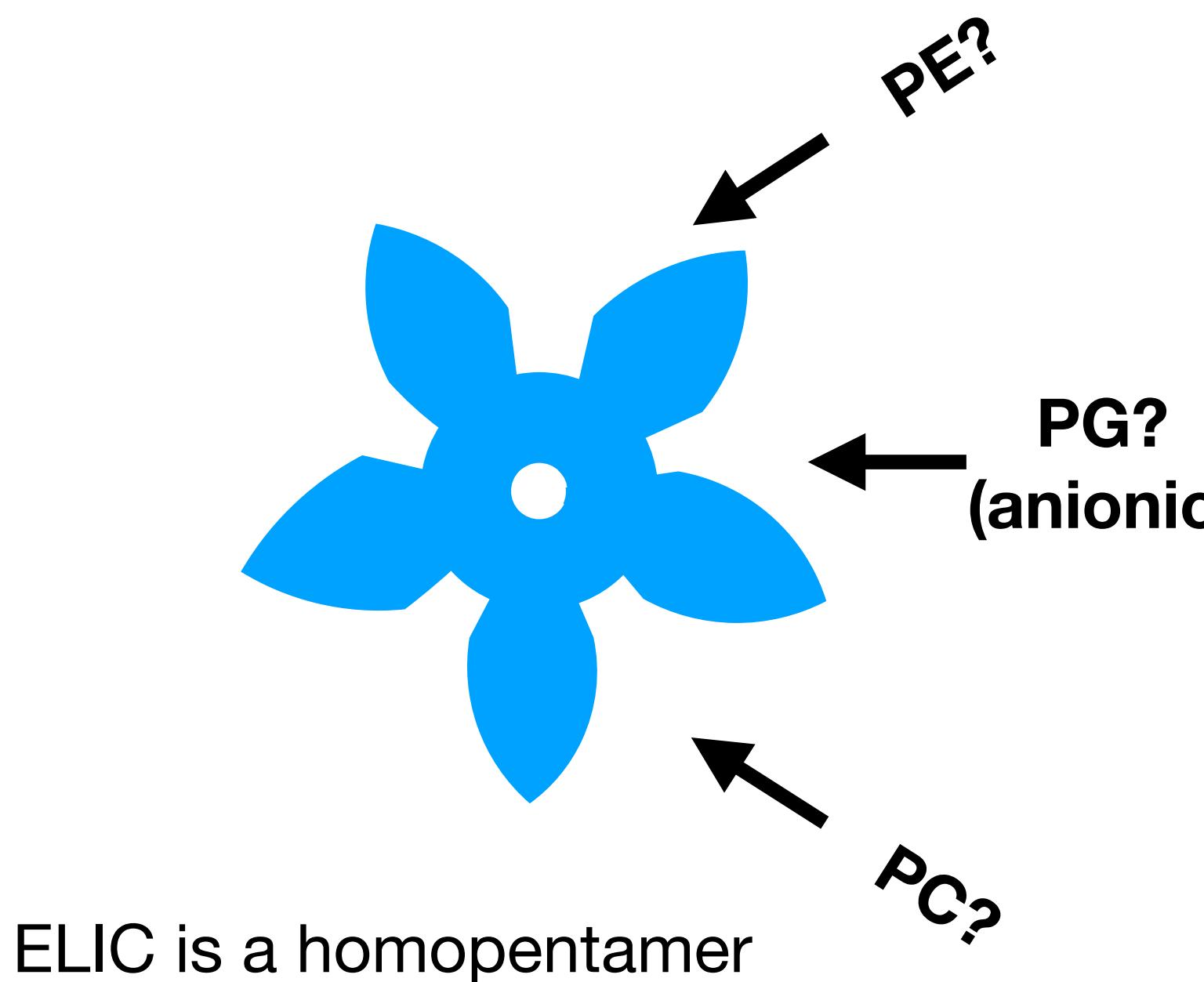
Anionic

- 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

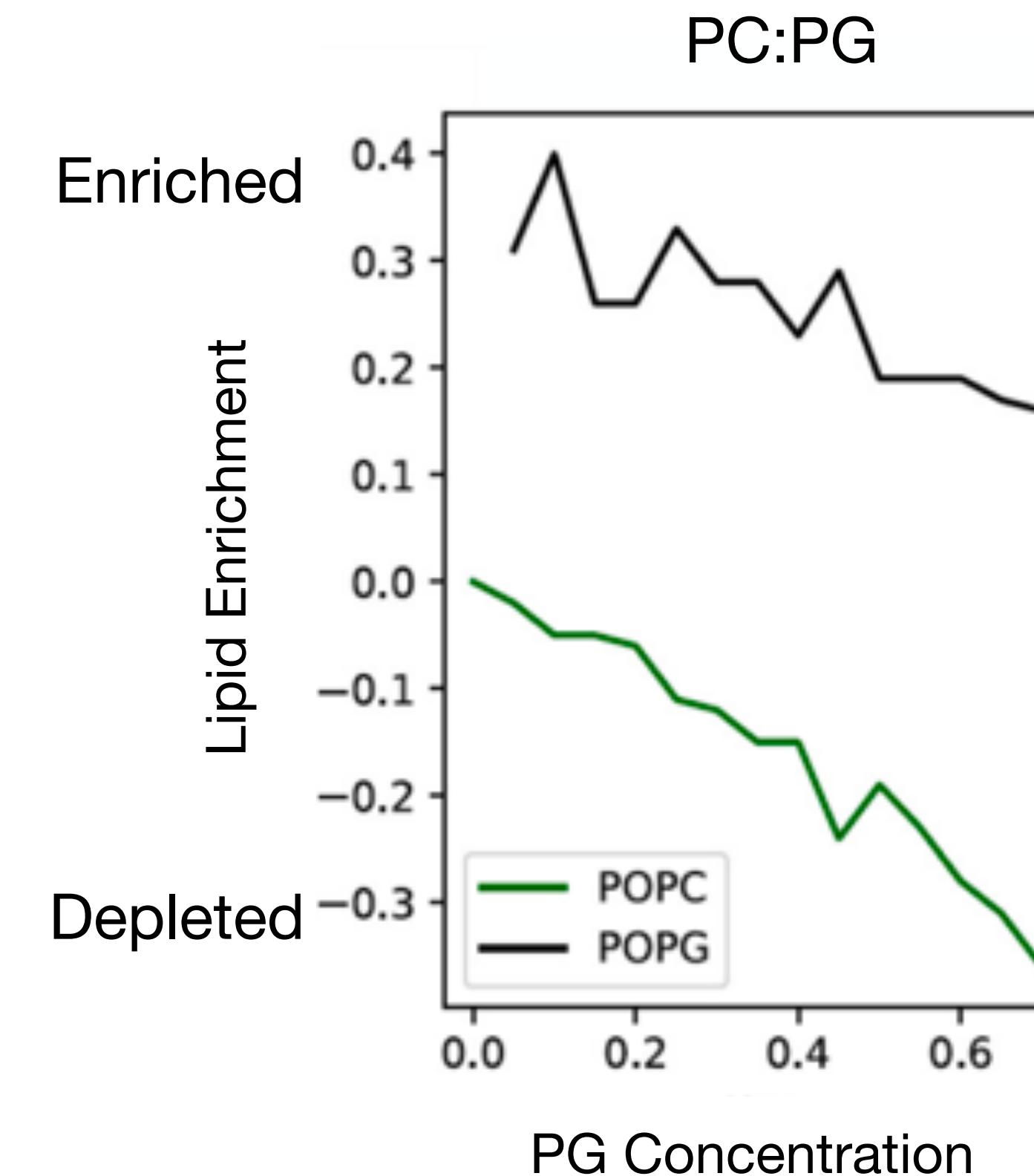
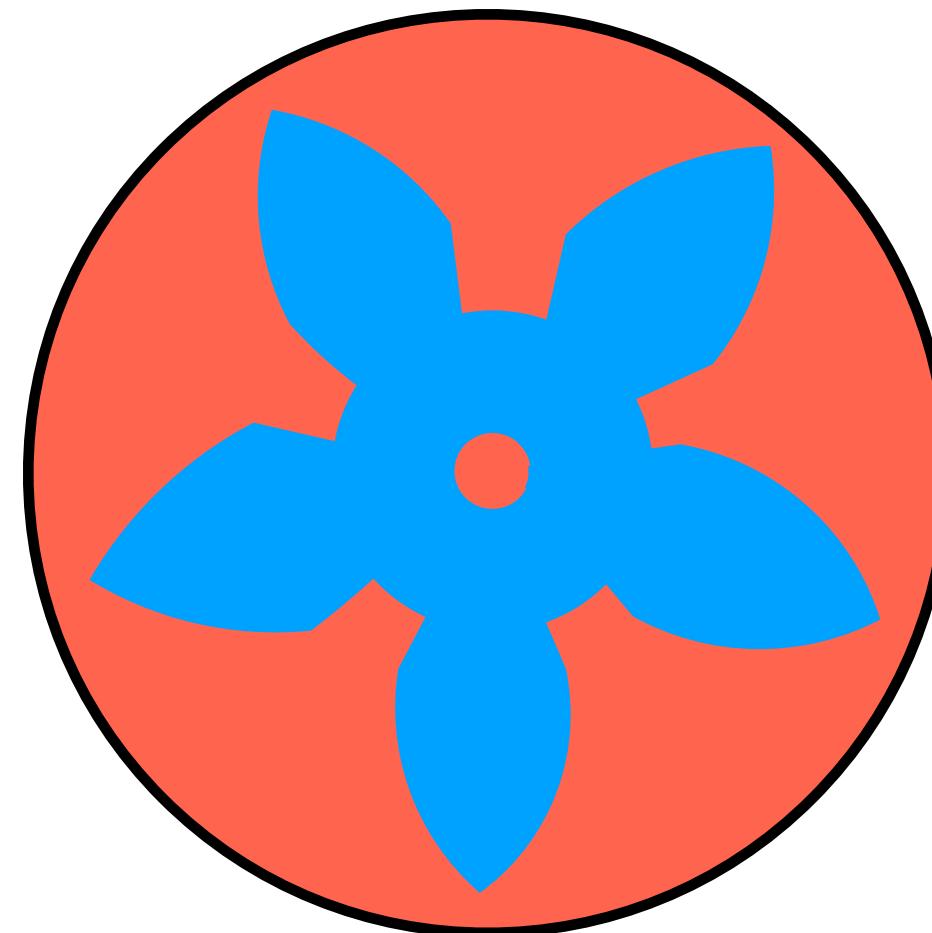
Bound PG > PE > PC  
Cannot tell you where its bound



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

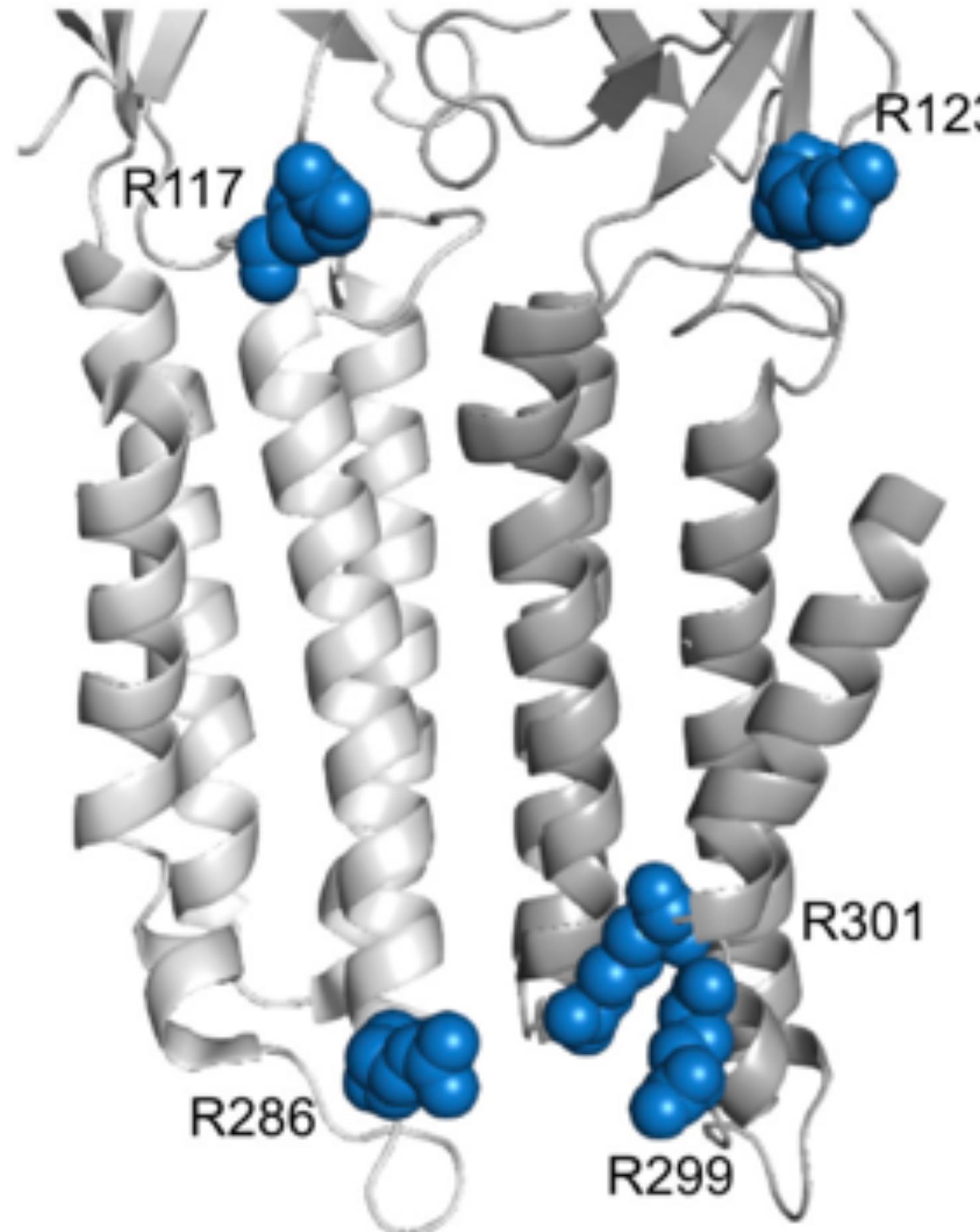
# Computational: Simulations show PG enrichment in the boundary

- 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

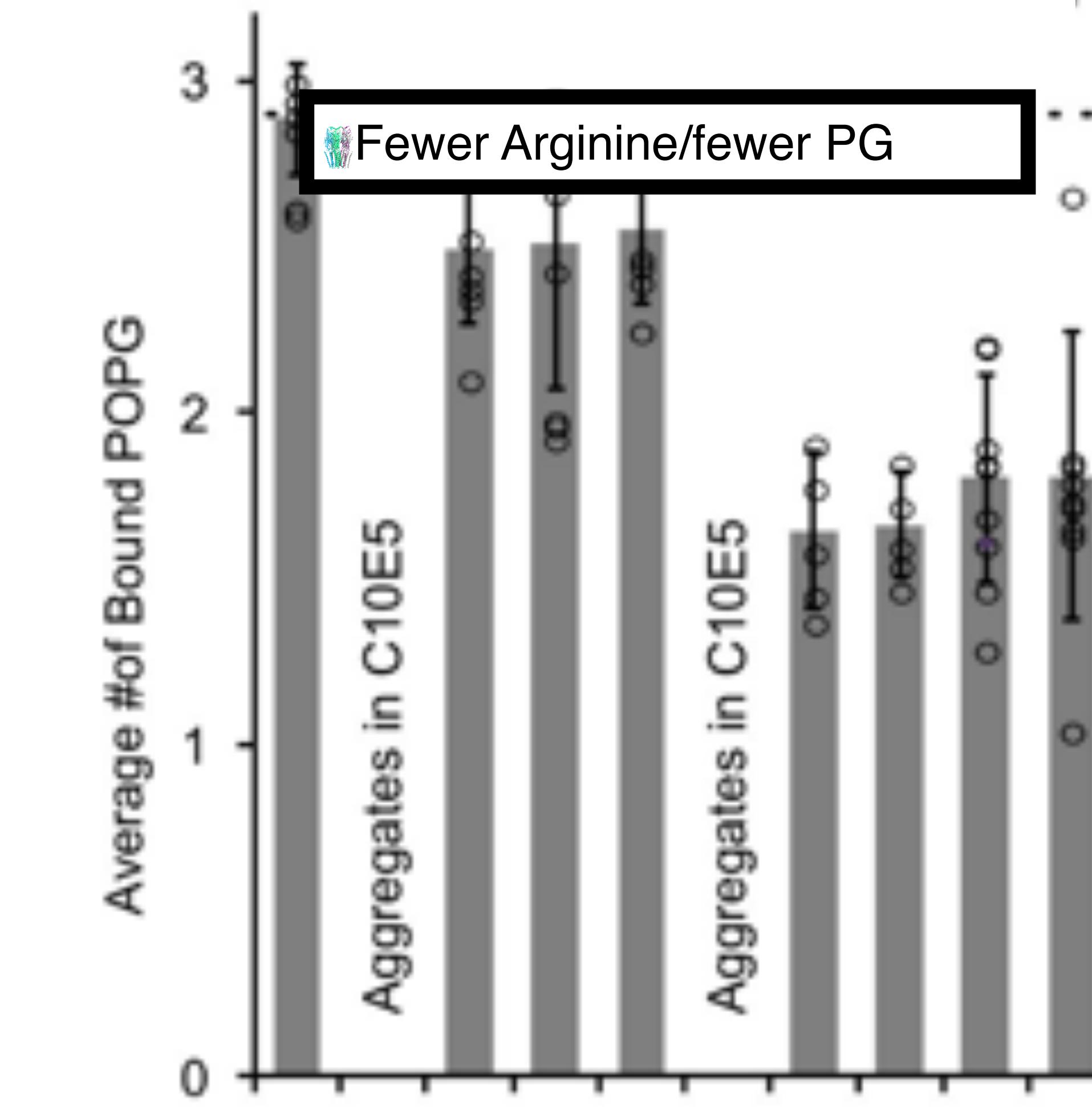


Arginine -> Glutamine

( + ) -> ( = )

Positive  
Charge

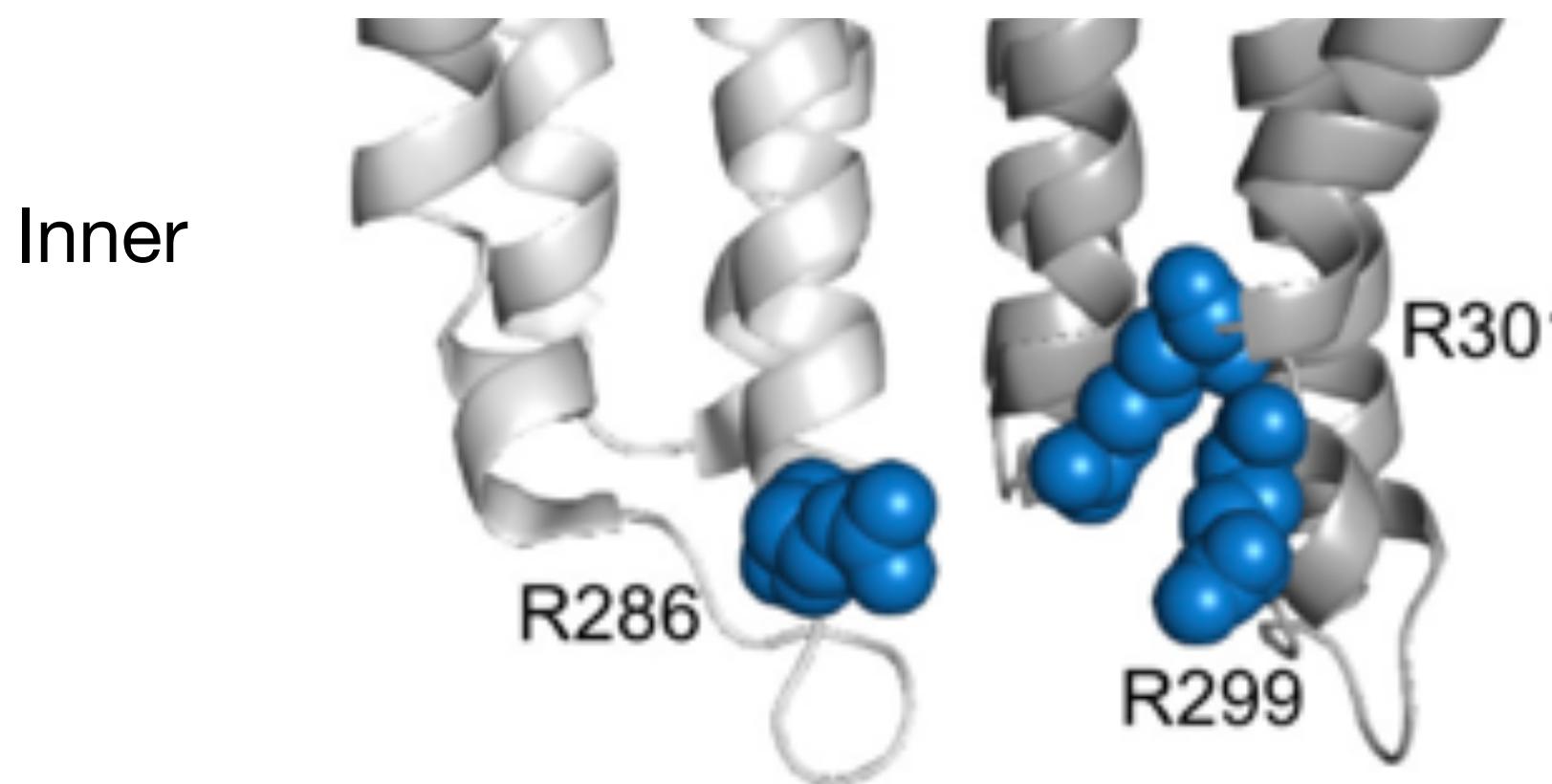
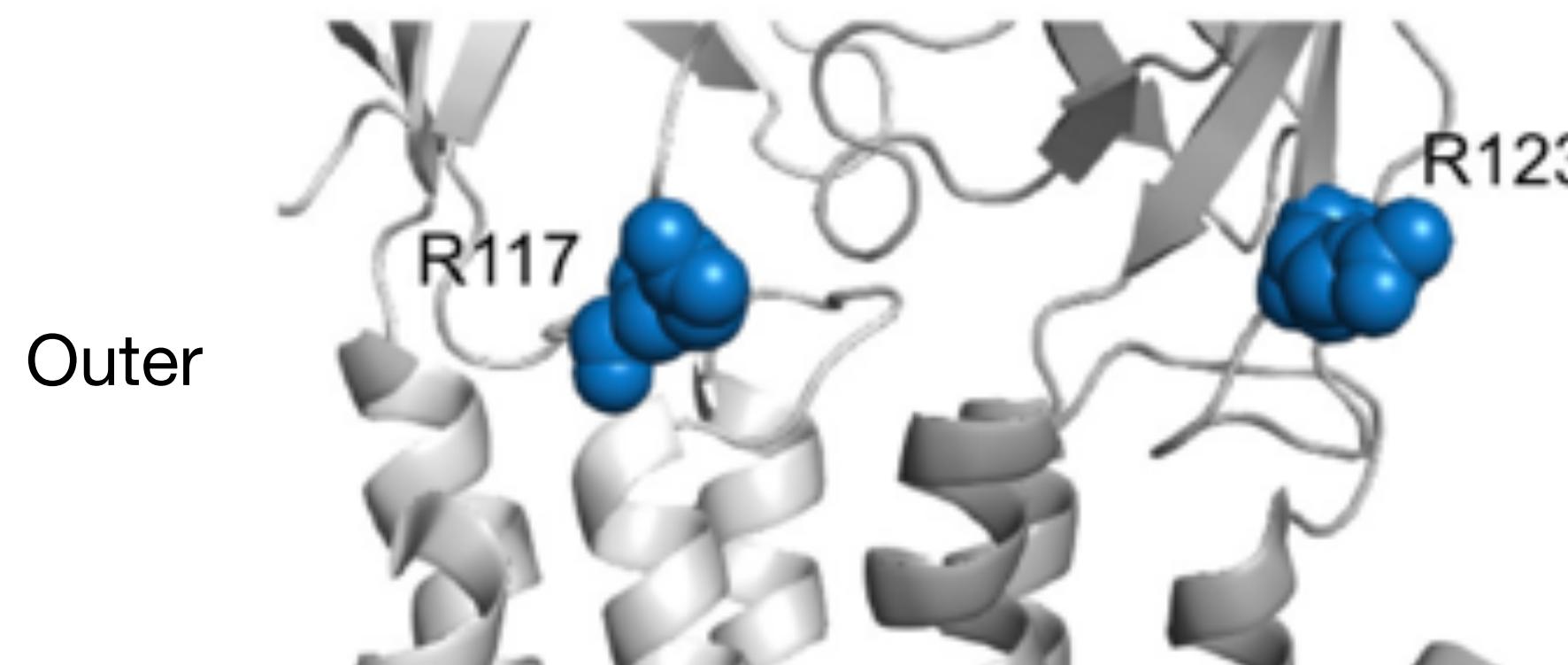
Polar  
Neutral



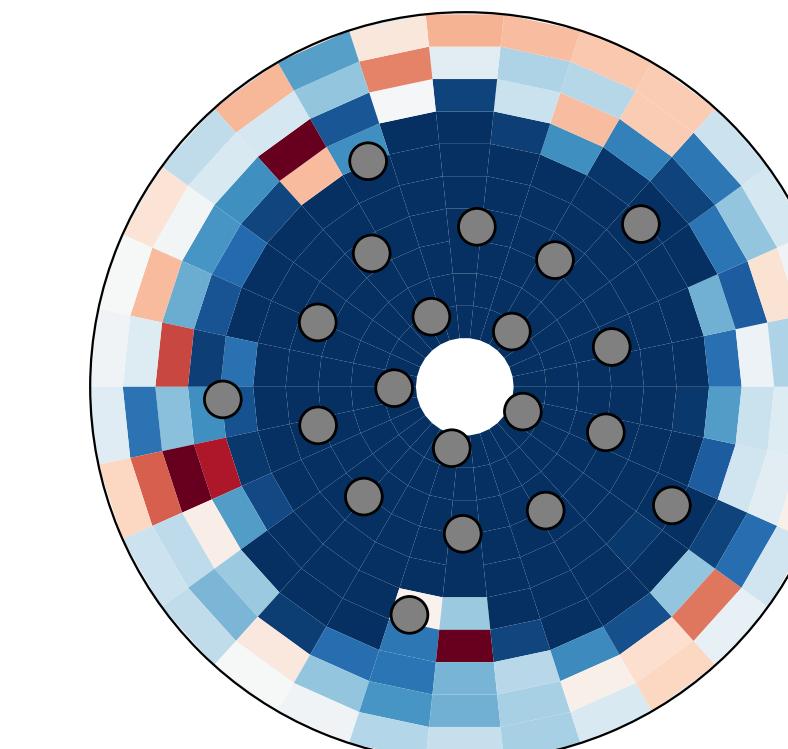
Single  
Mutant

Double  
Mutant

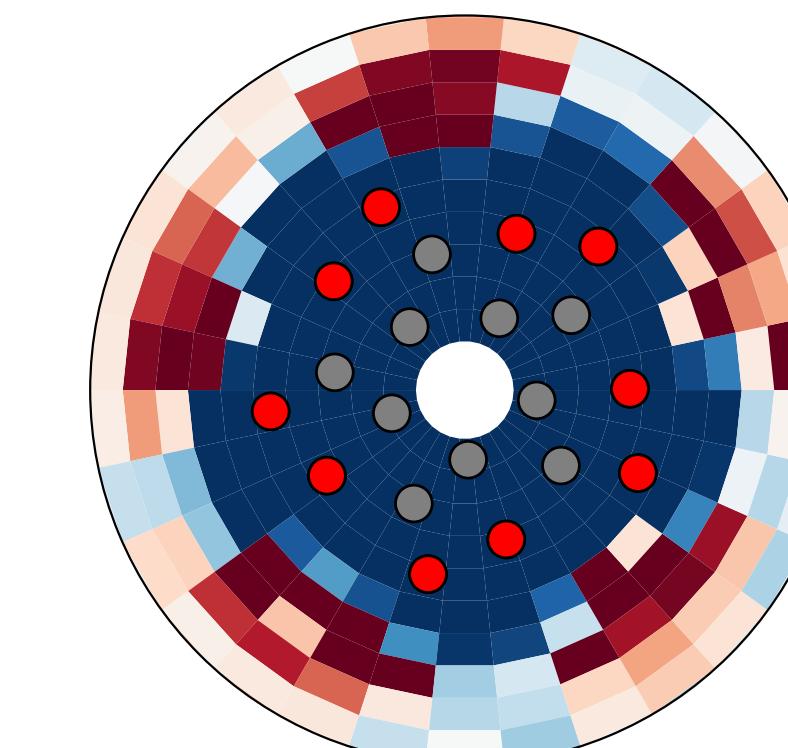
# Computational: PG Density Related to Accessible Arginine



PG Density

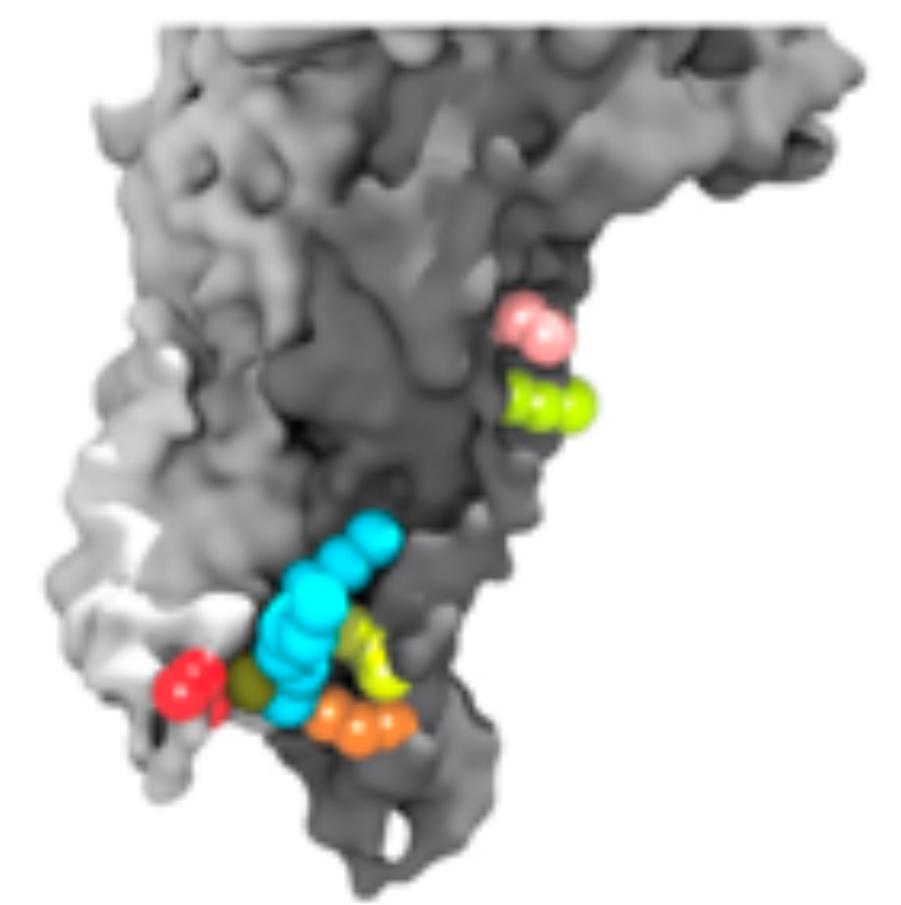
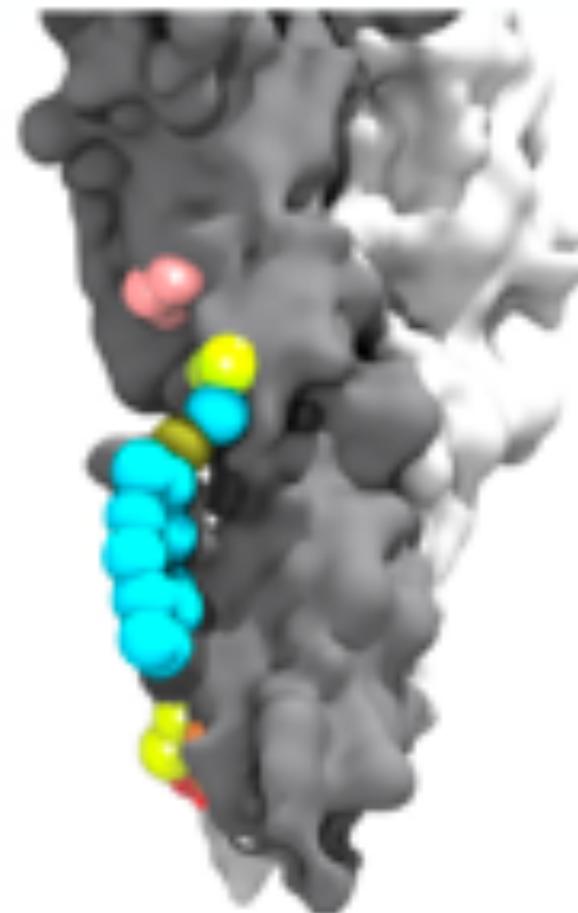
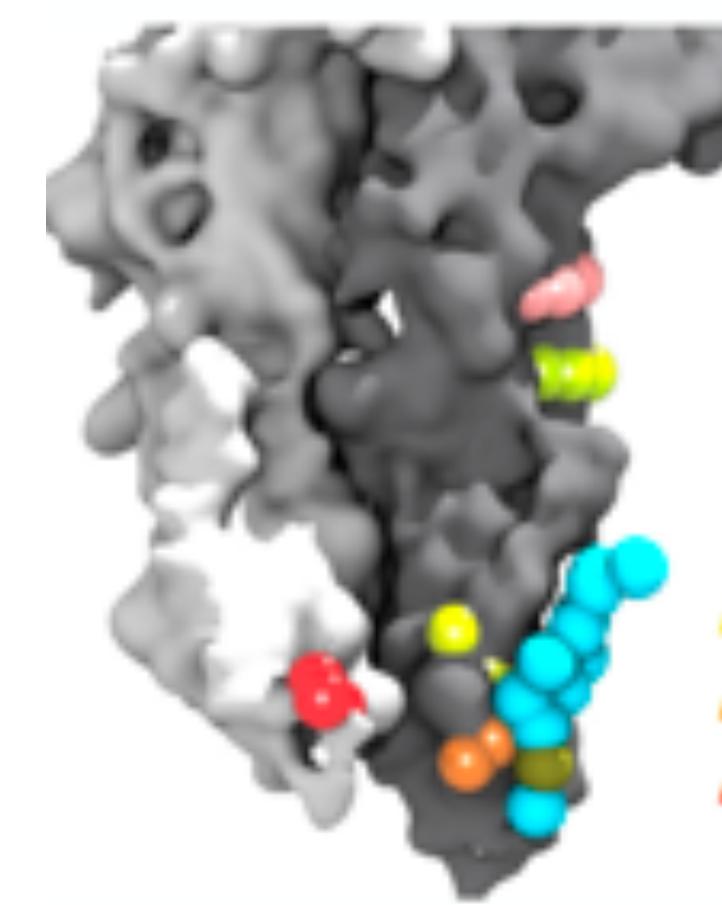


Symmetric Leaflets

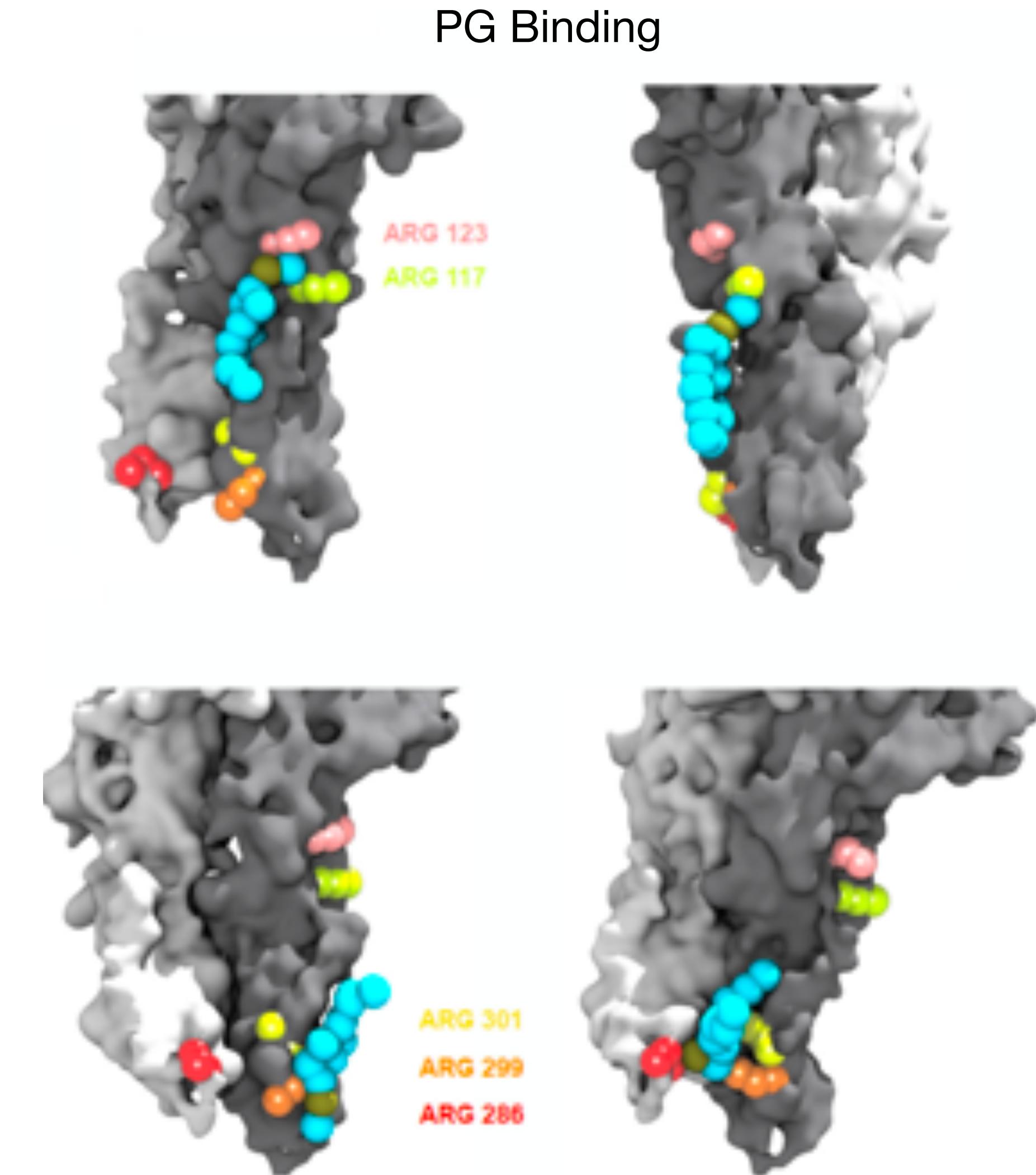
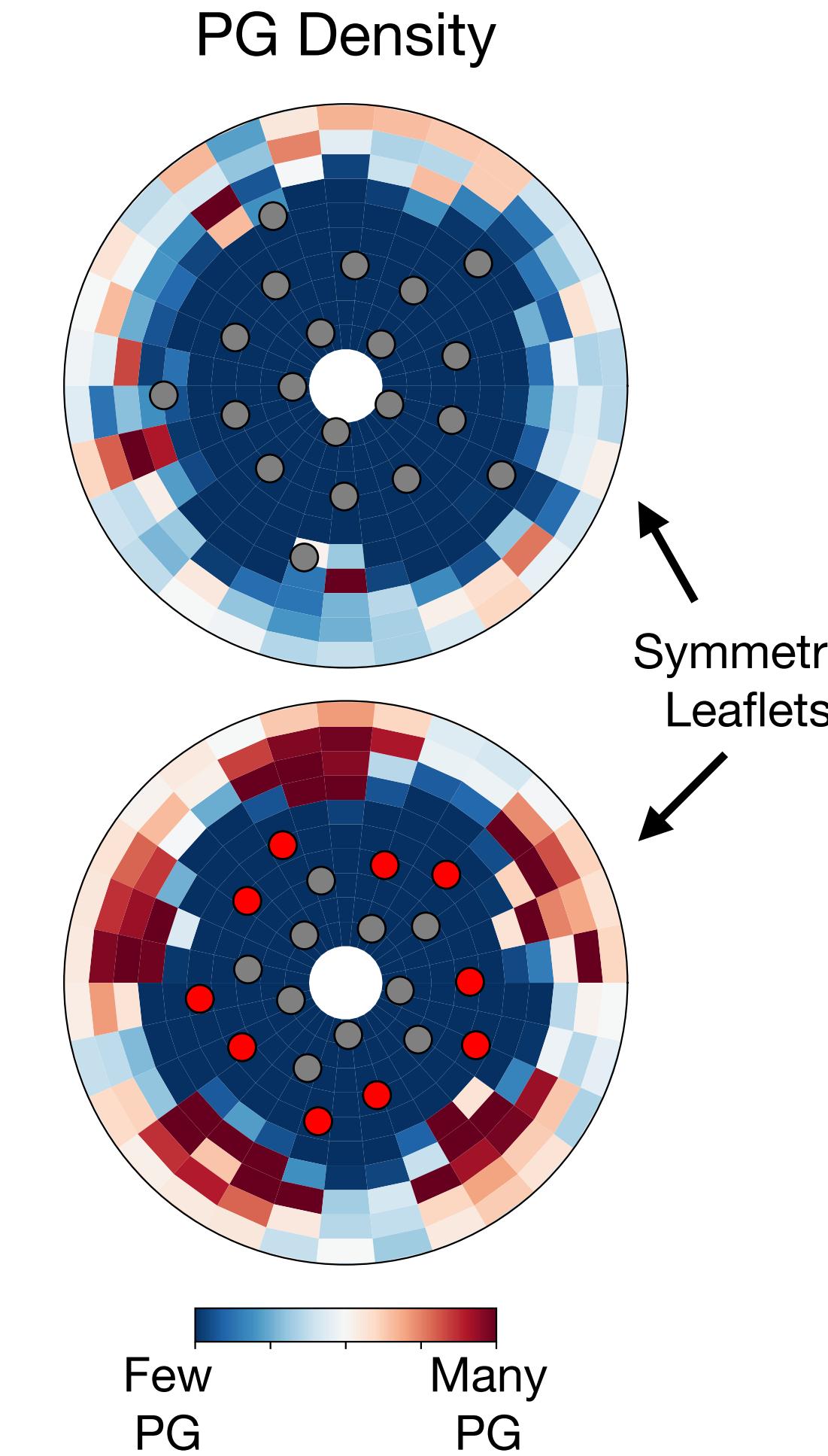
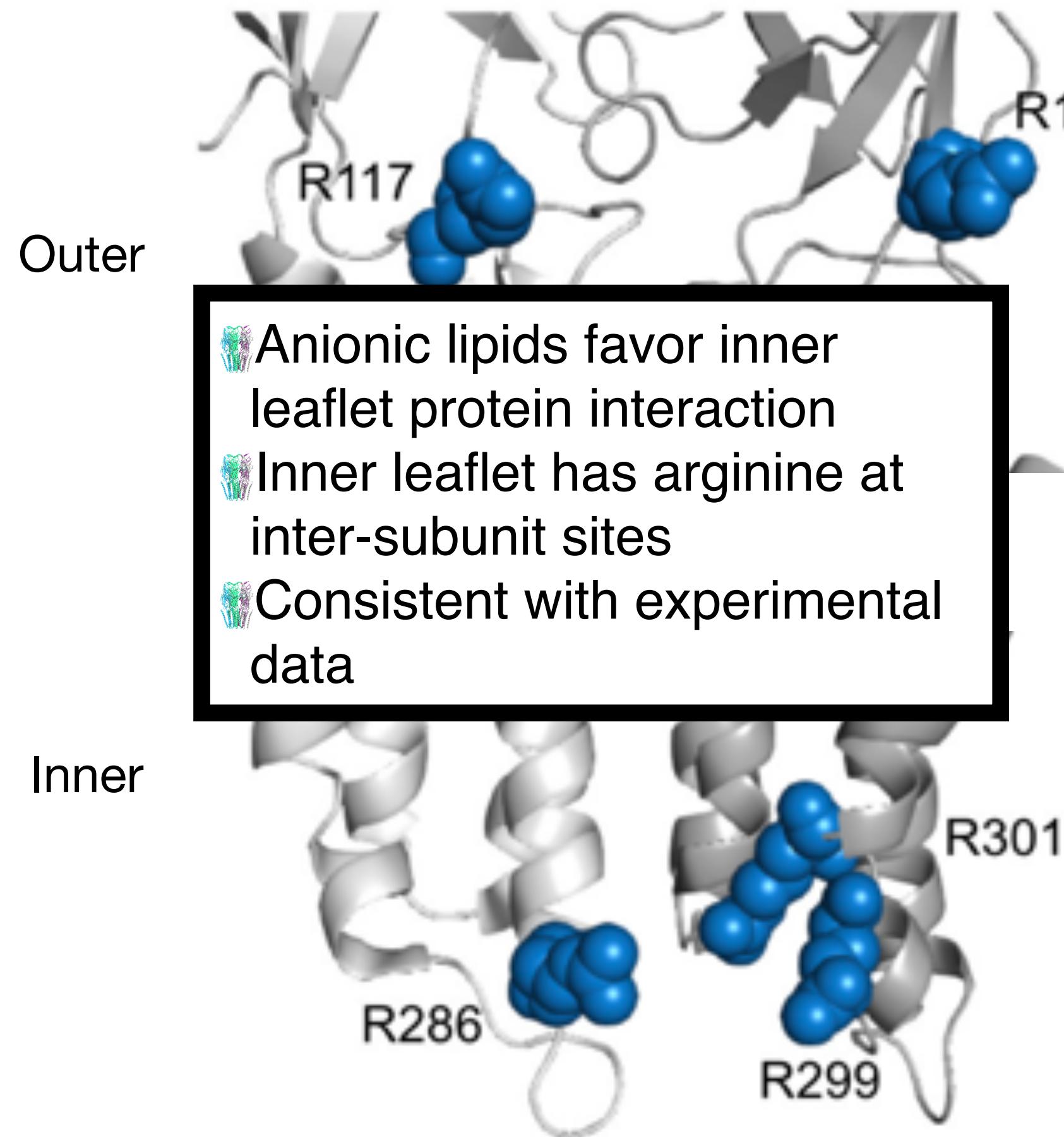


Few PG      Many PG

PG Binding



# 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

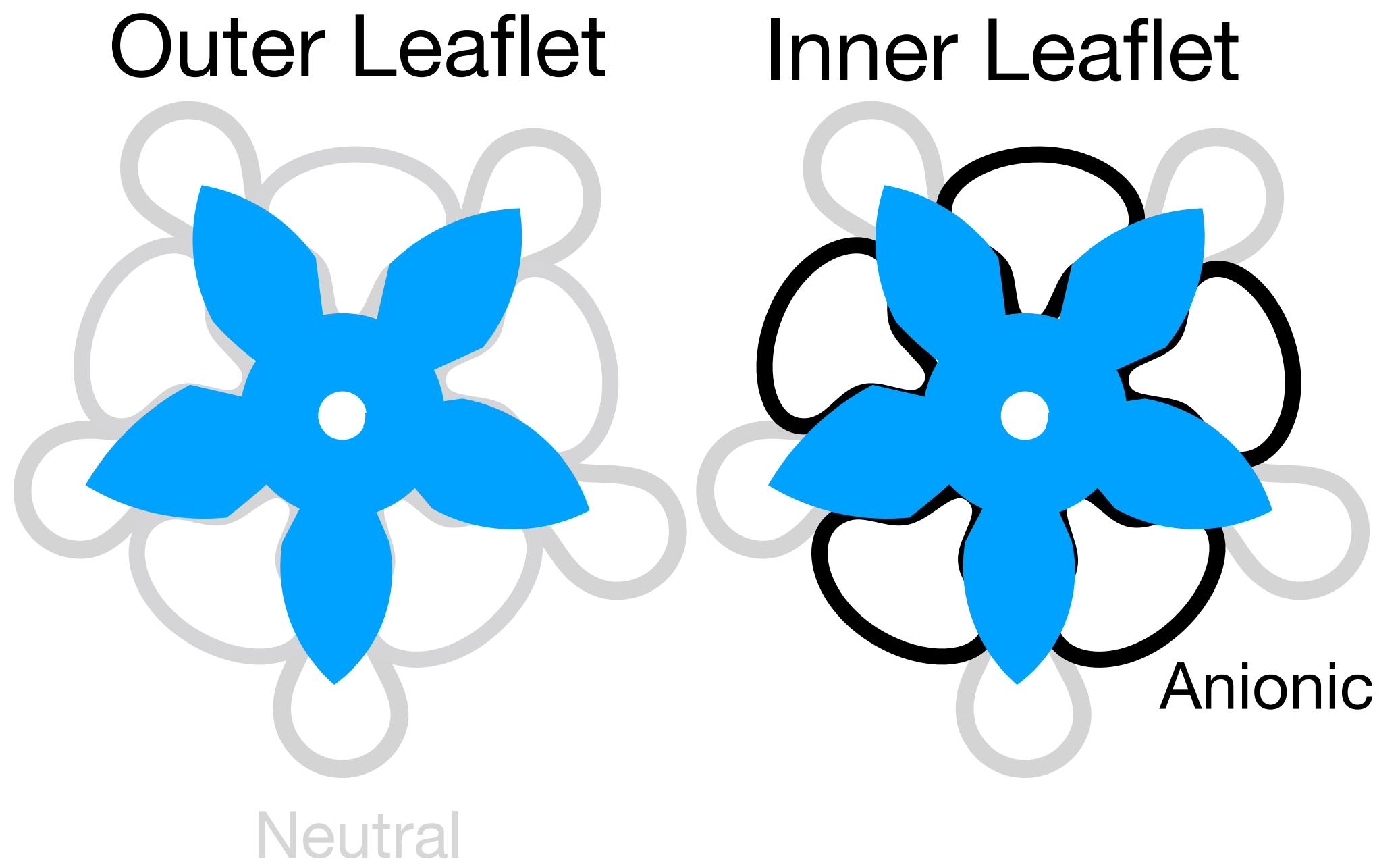
Determine which sites modulate function

Cationic amino acids play a role in both PG binding and ELIC function

Simulations are consistent with experiments

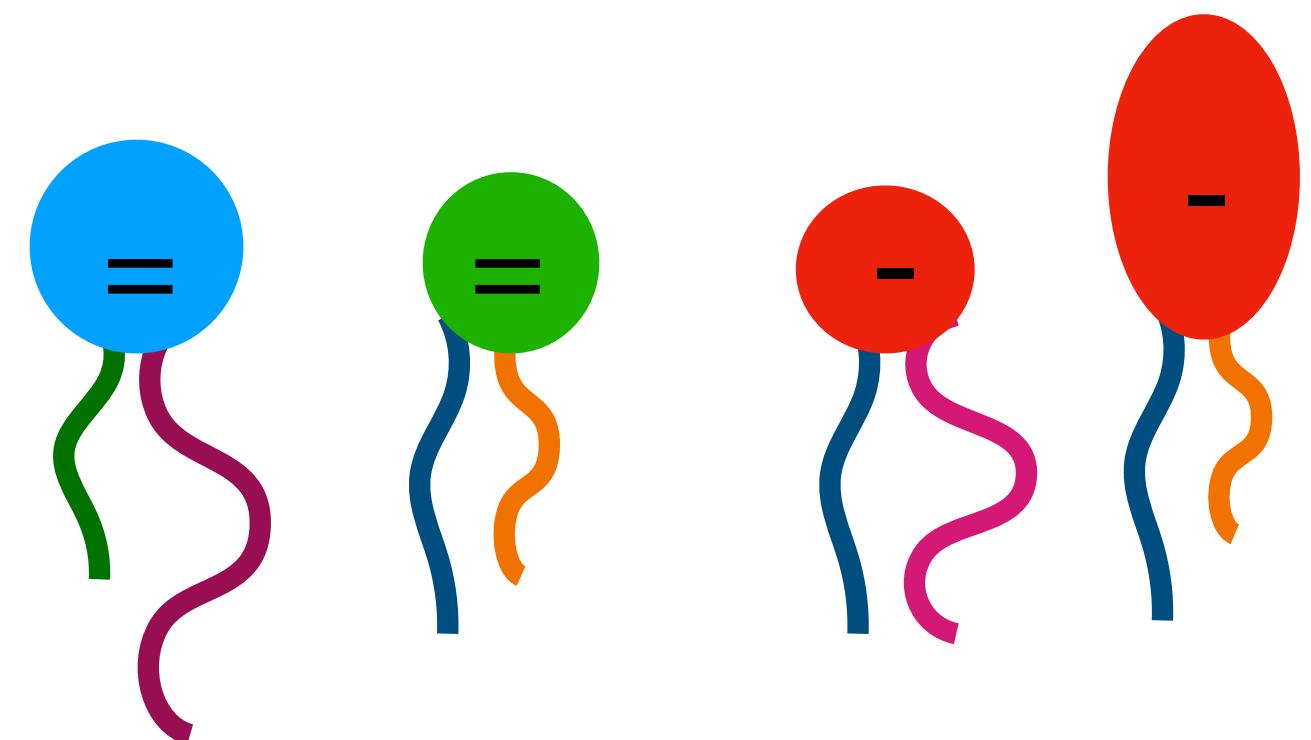
# Conclusion

- 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



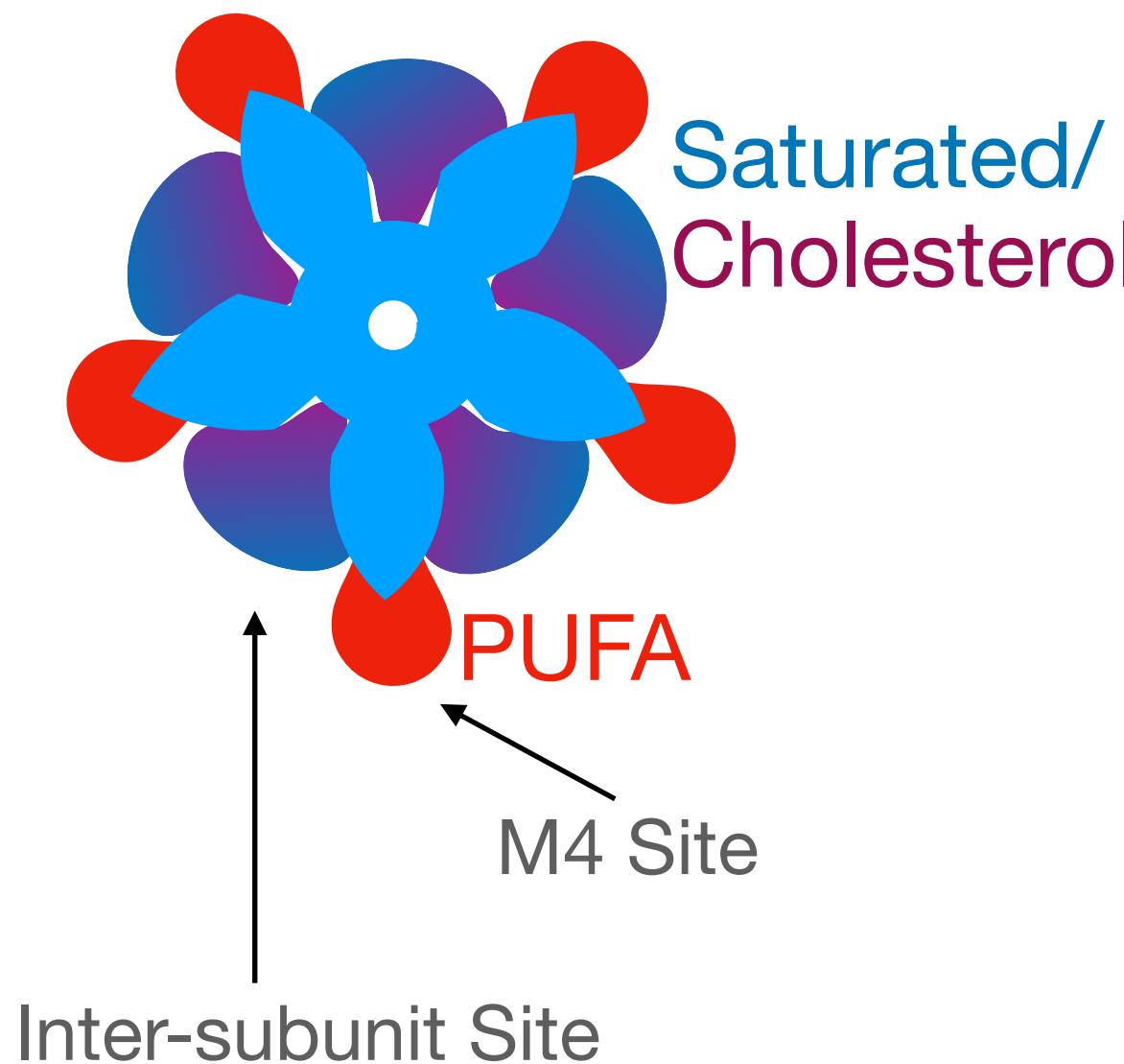
# Outline

- 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

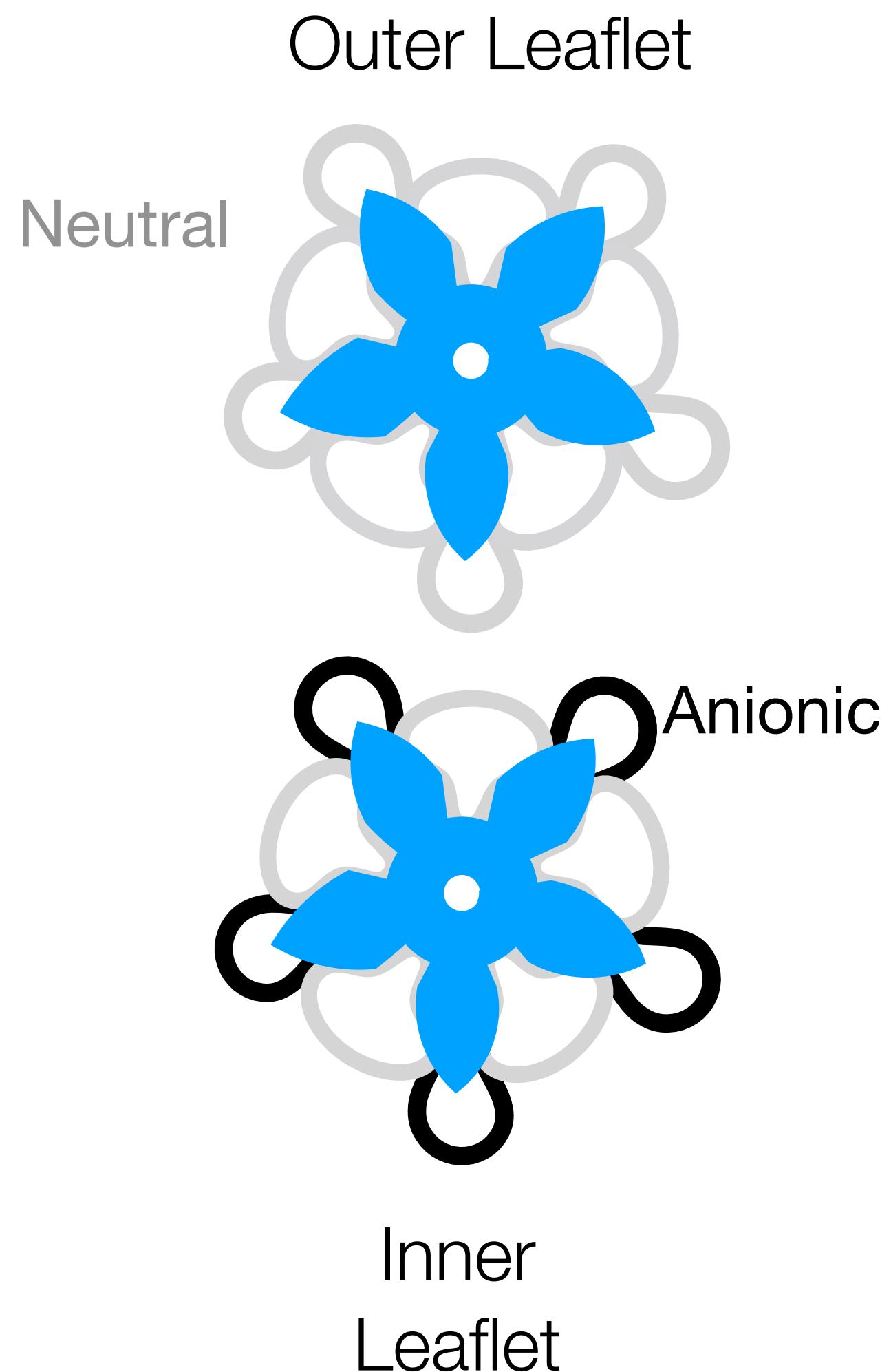


# What have we learned so far?

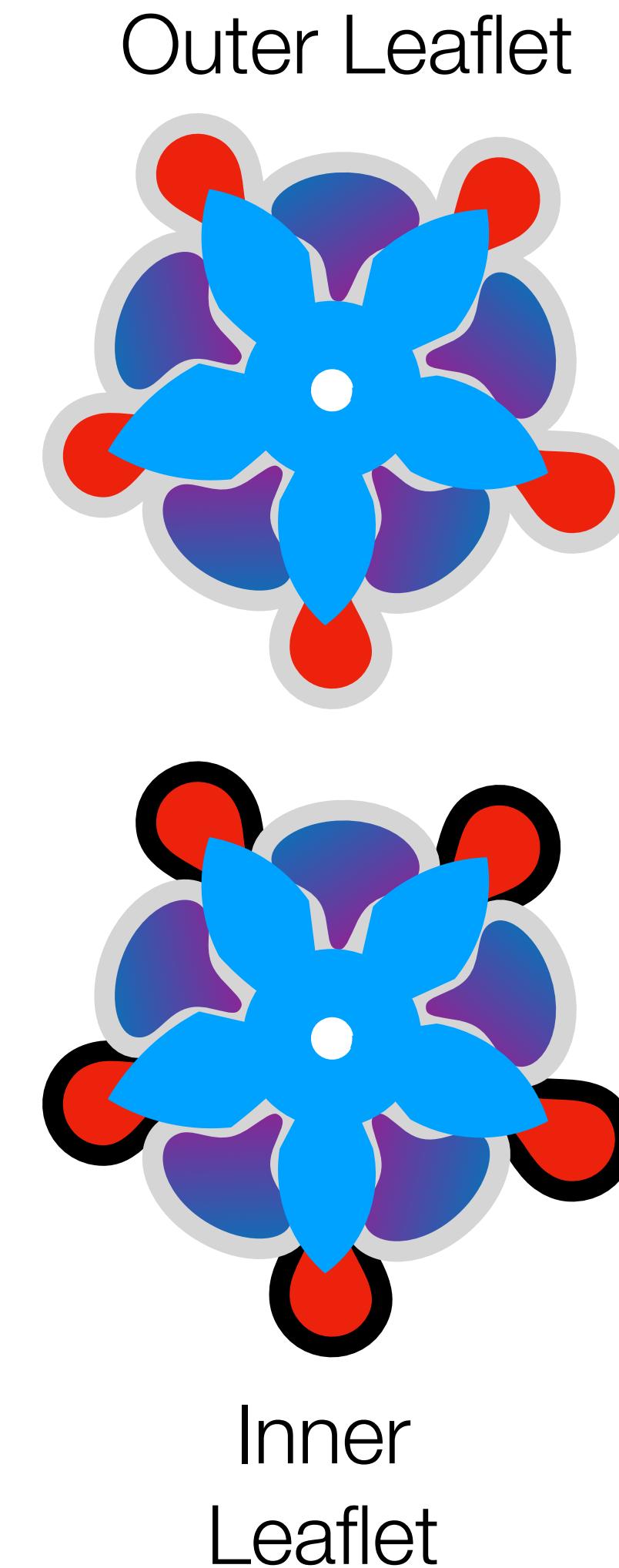
## Section 1



## Section 2



## Combined ?



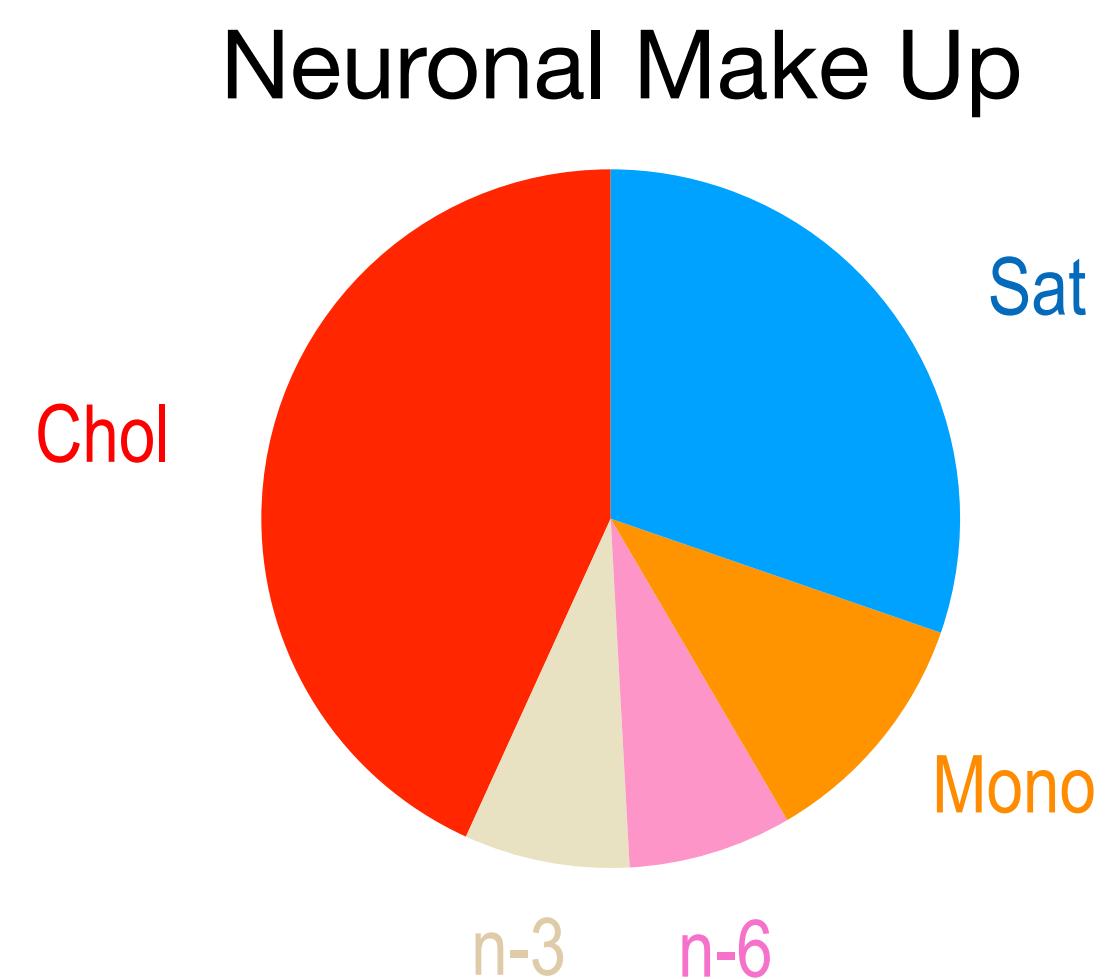
# Goal

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

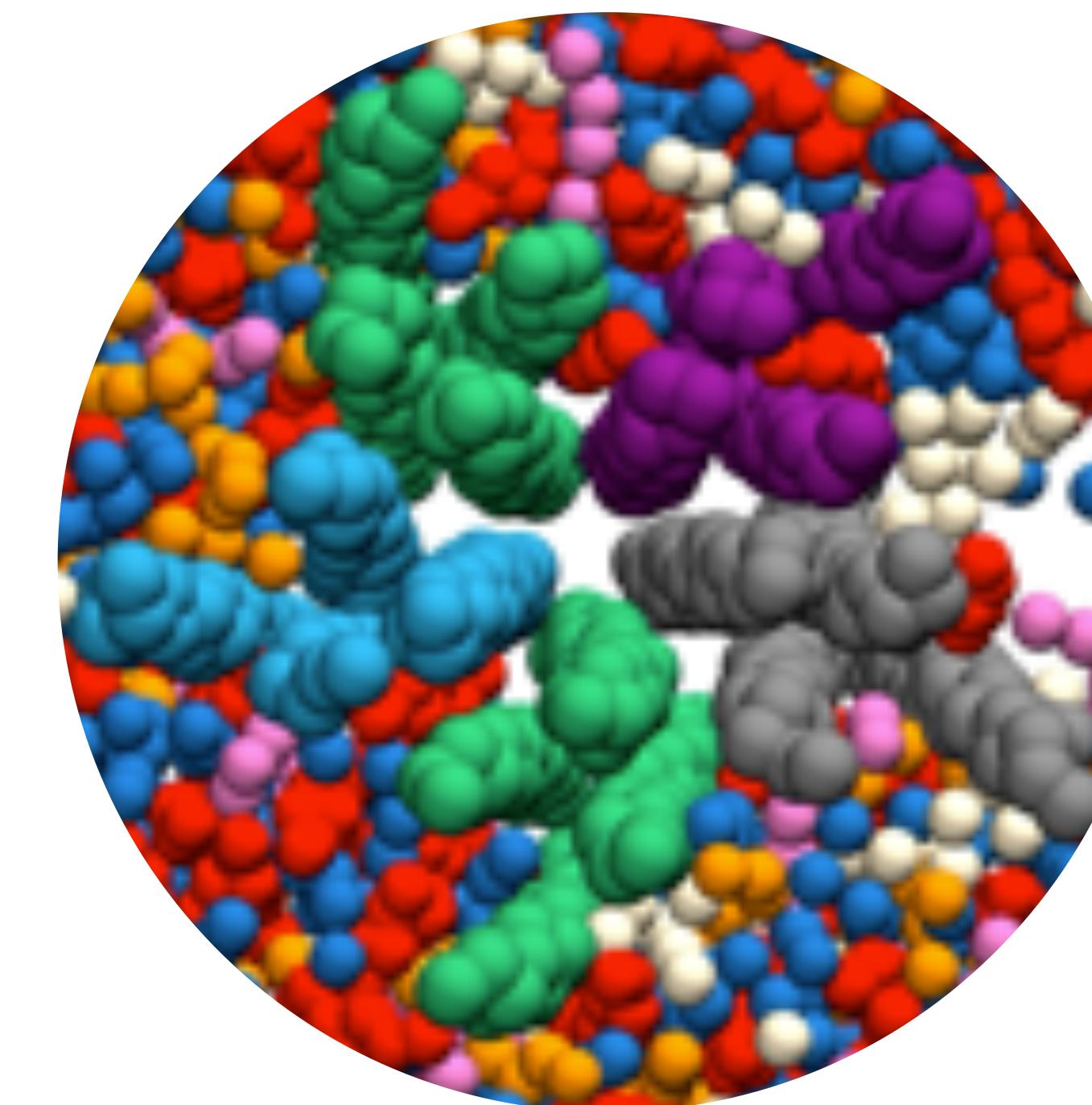
# Approach

- 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

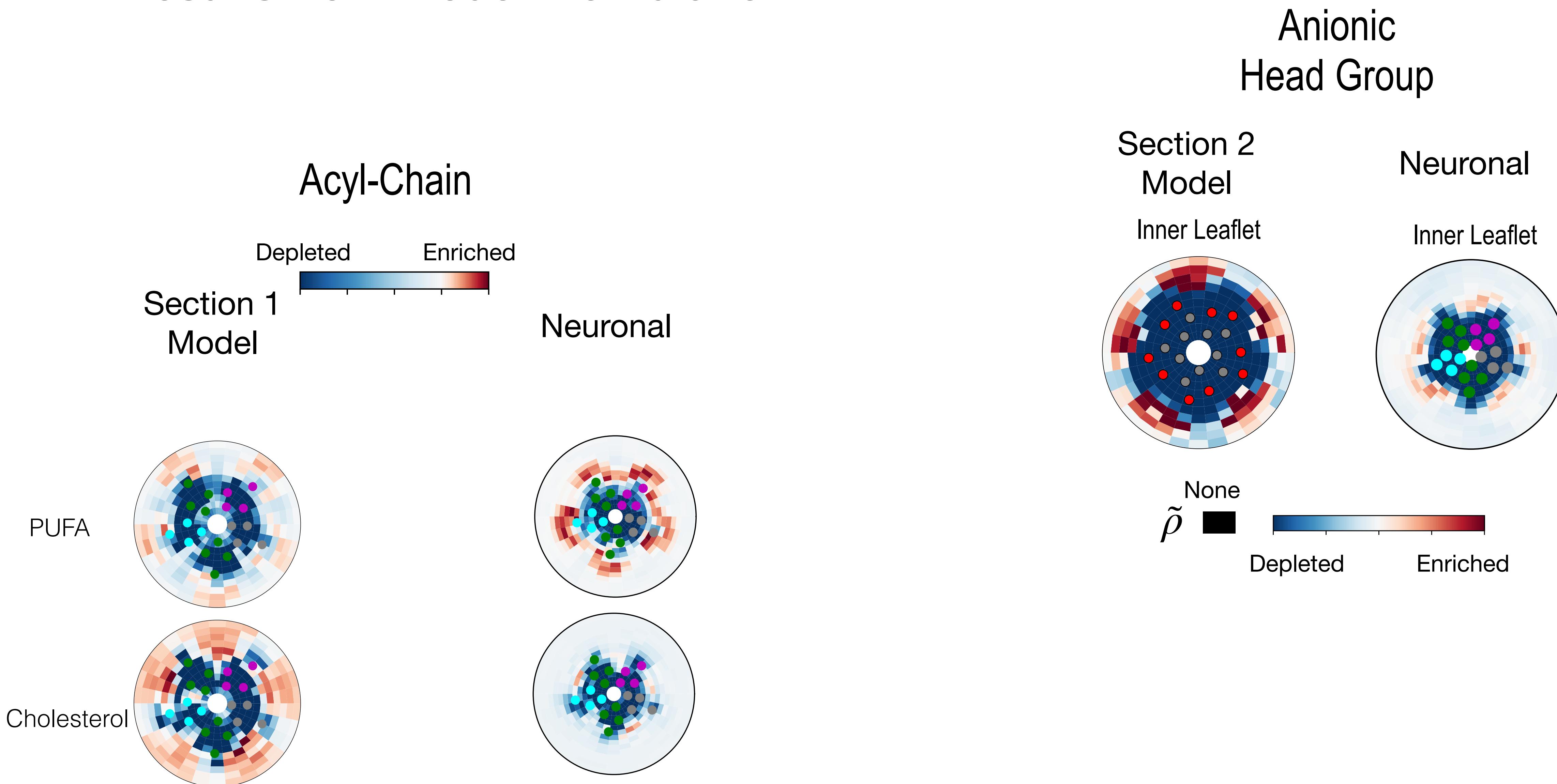
# nAChR Synaptic Boundary Movie



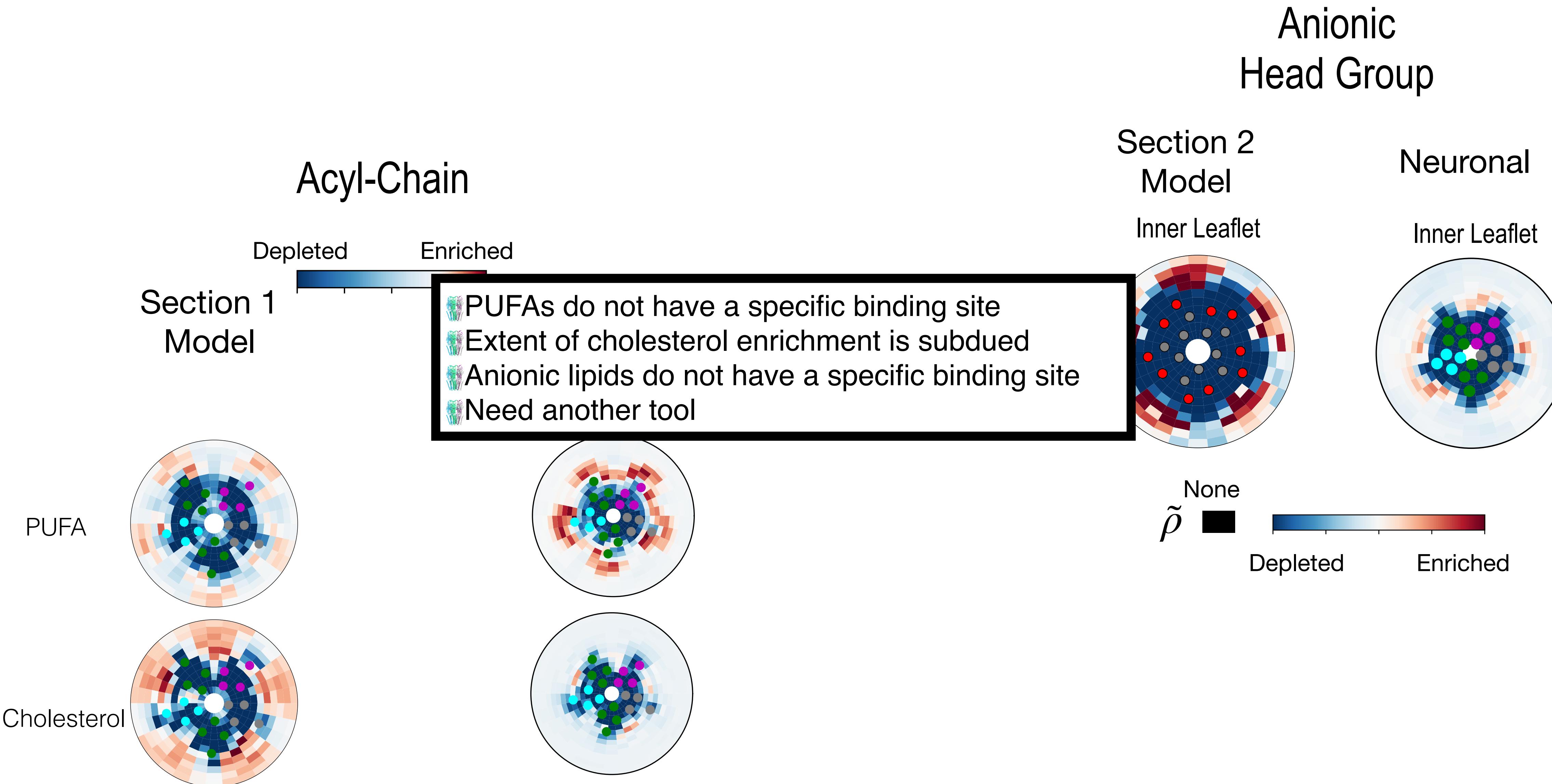
Helgi I Ingólfsson et al, 2017



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

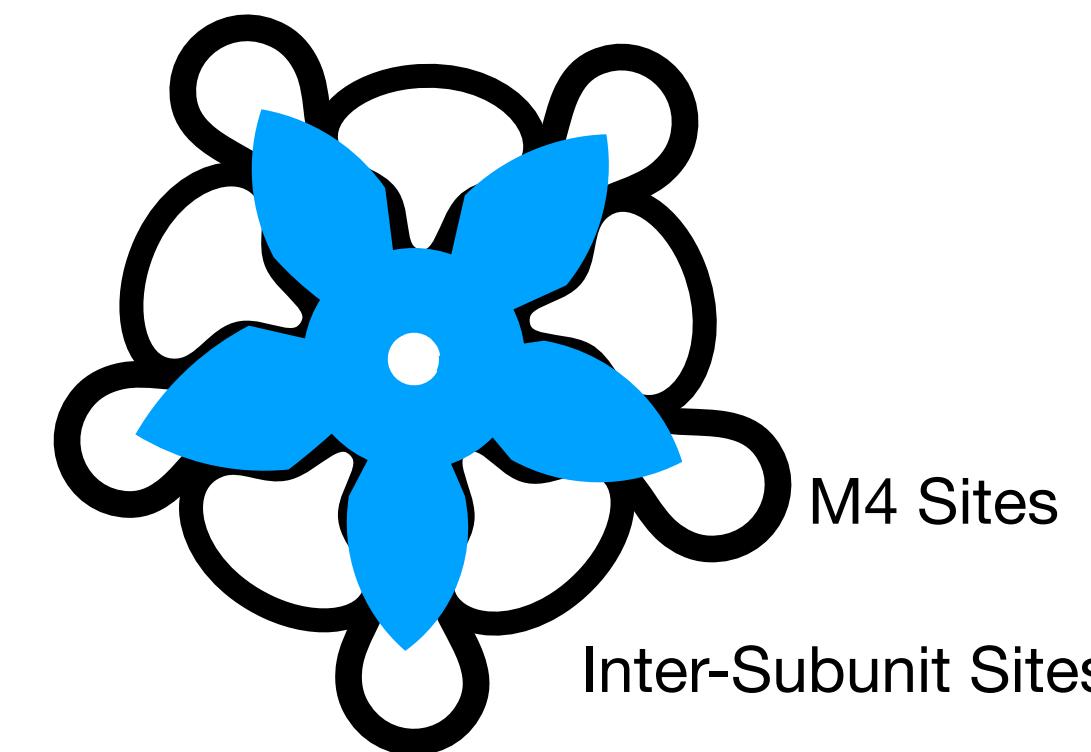


# How do the density plots compare to previous results from model membrane



# 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

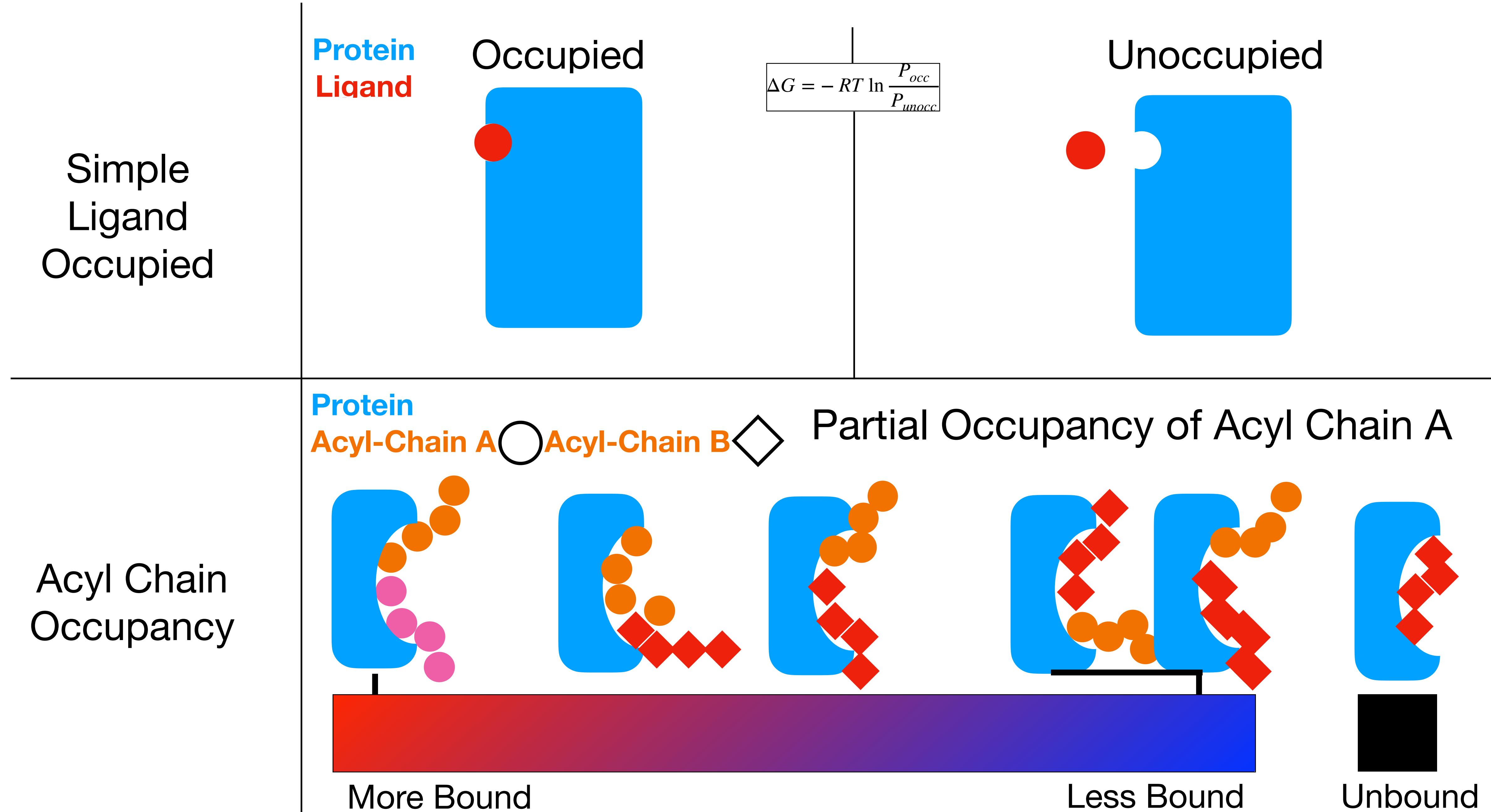


Dimensional reduction (2D distribution -> scalar), previously had used whole annulus and not individual sites

For individual sites, affinity makes more sense than enrichment

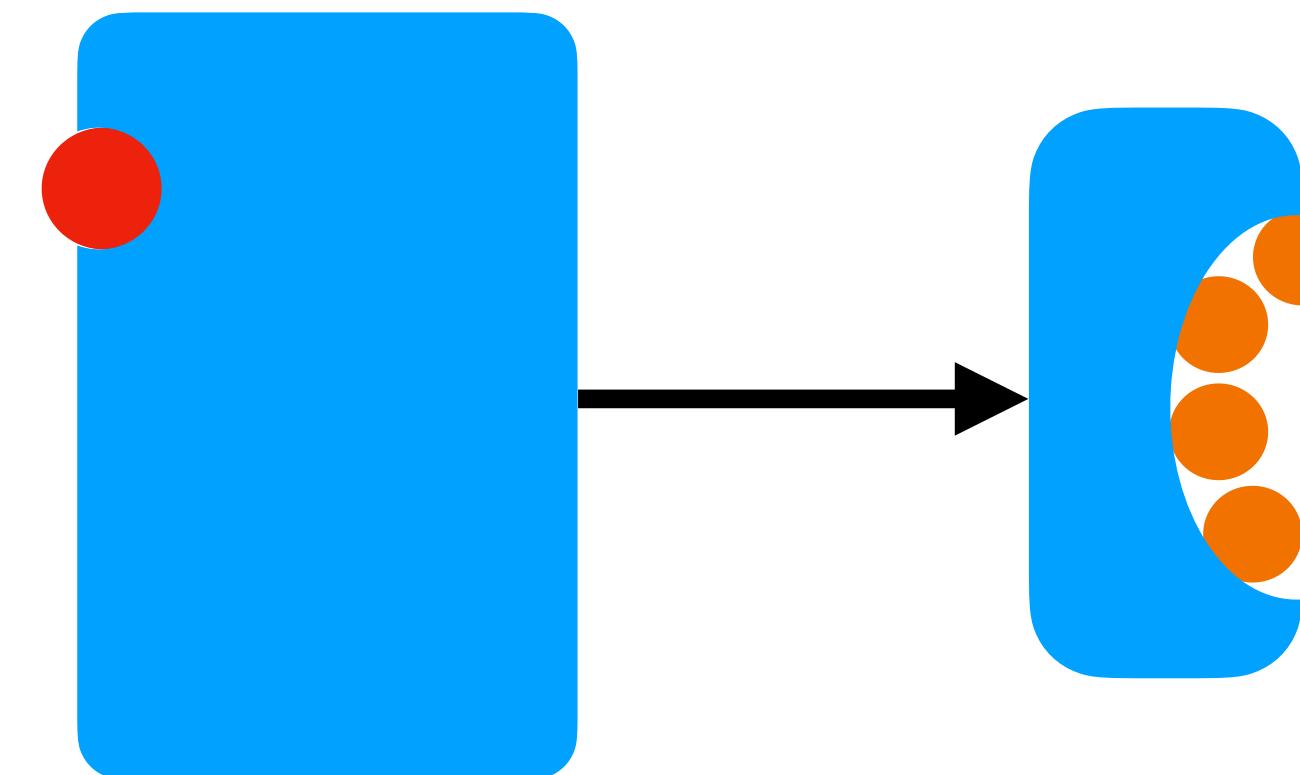
- Quantifies the free energy of binding a lipid (energetic information)
- Energetic information that can be used to predict densities in other membranes

# 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?



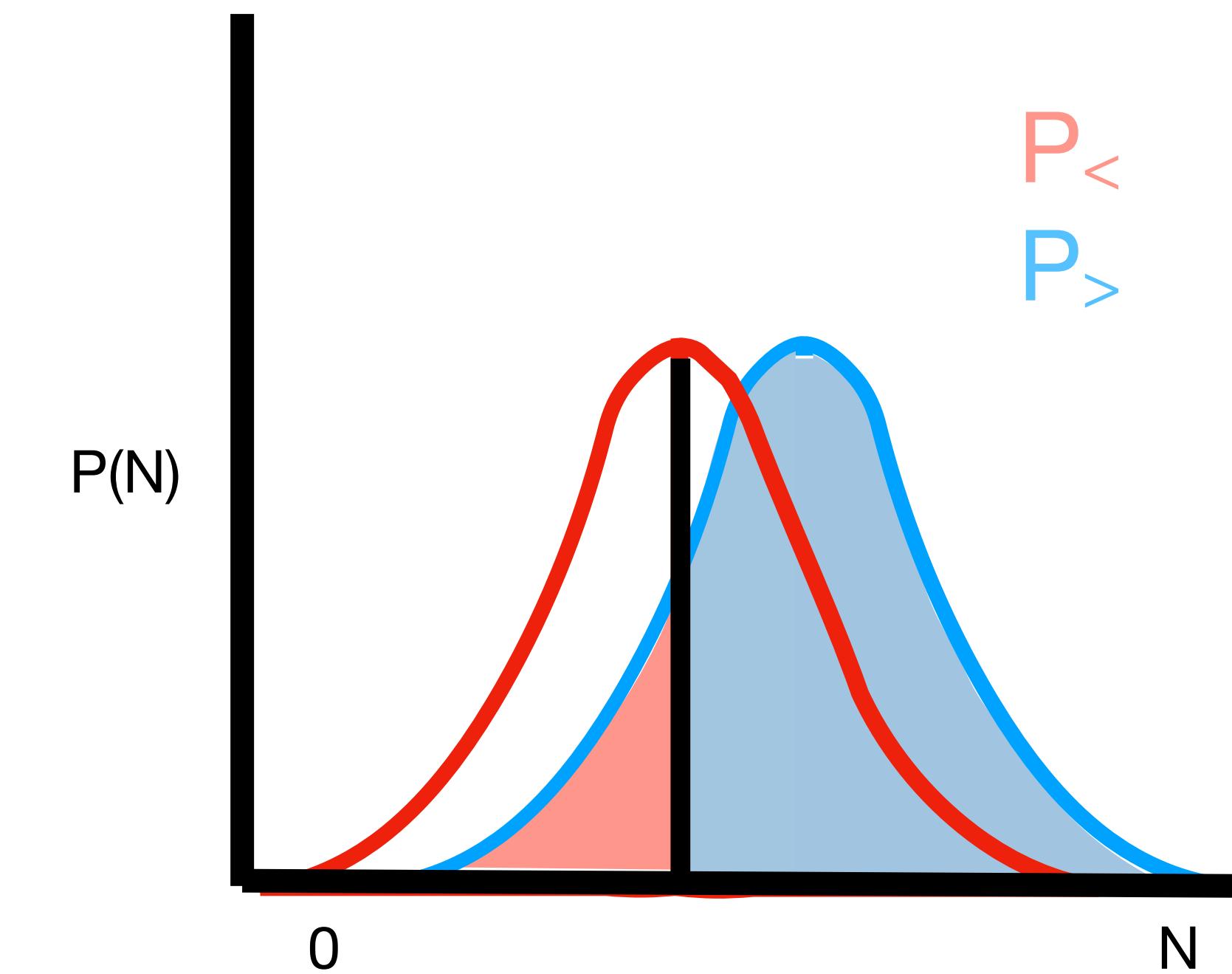
We derive the binding affinities by comparing two probability distributions

- 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

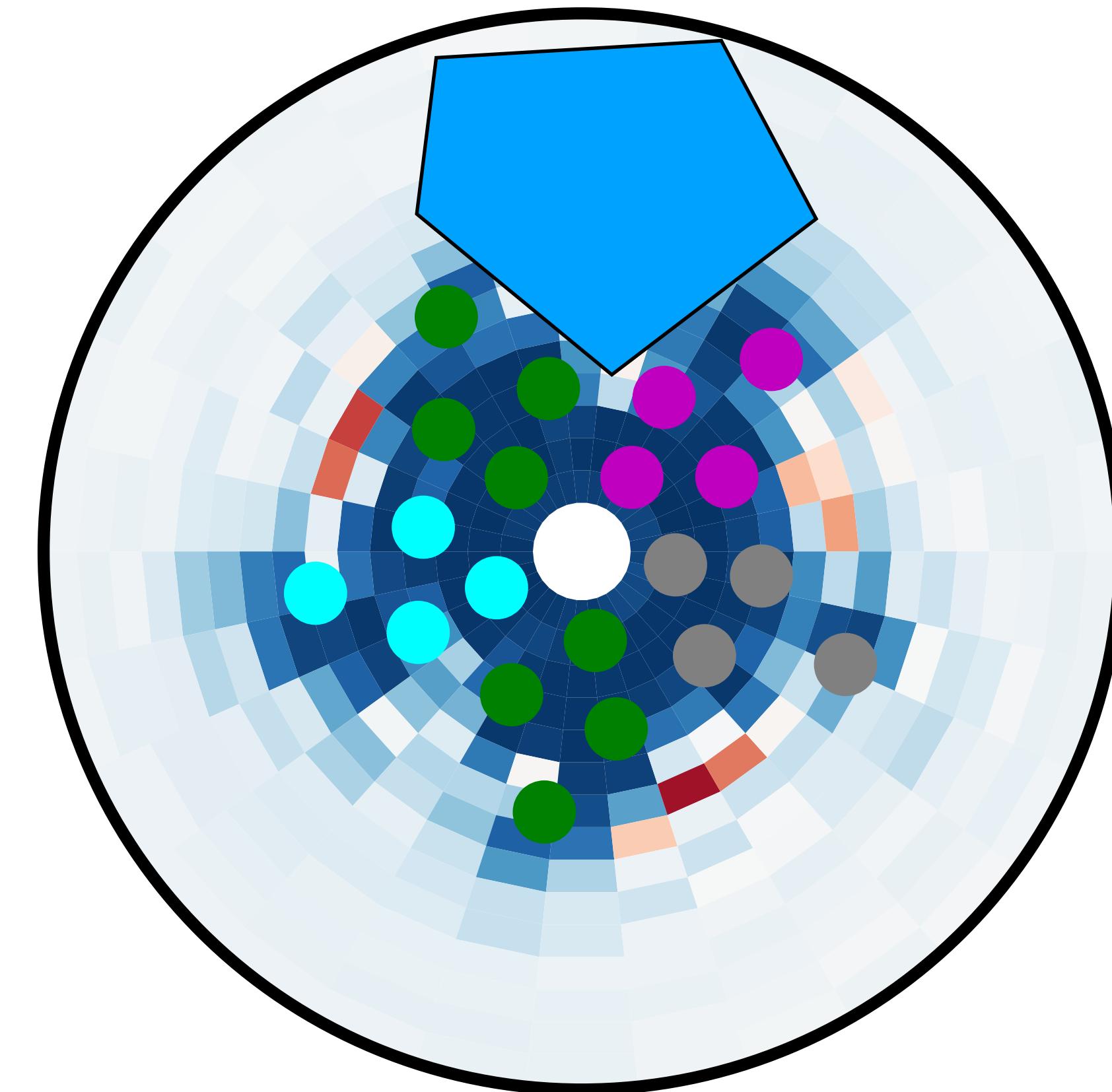
- Sum of  $P_{occ}$  less than and equal to the  $P_{bulk}$  peak is  $P_<$
- Sum of  $P_{occ}$  greater than the  $P_{bulk}$  peak is  $P_>$



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

Frames

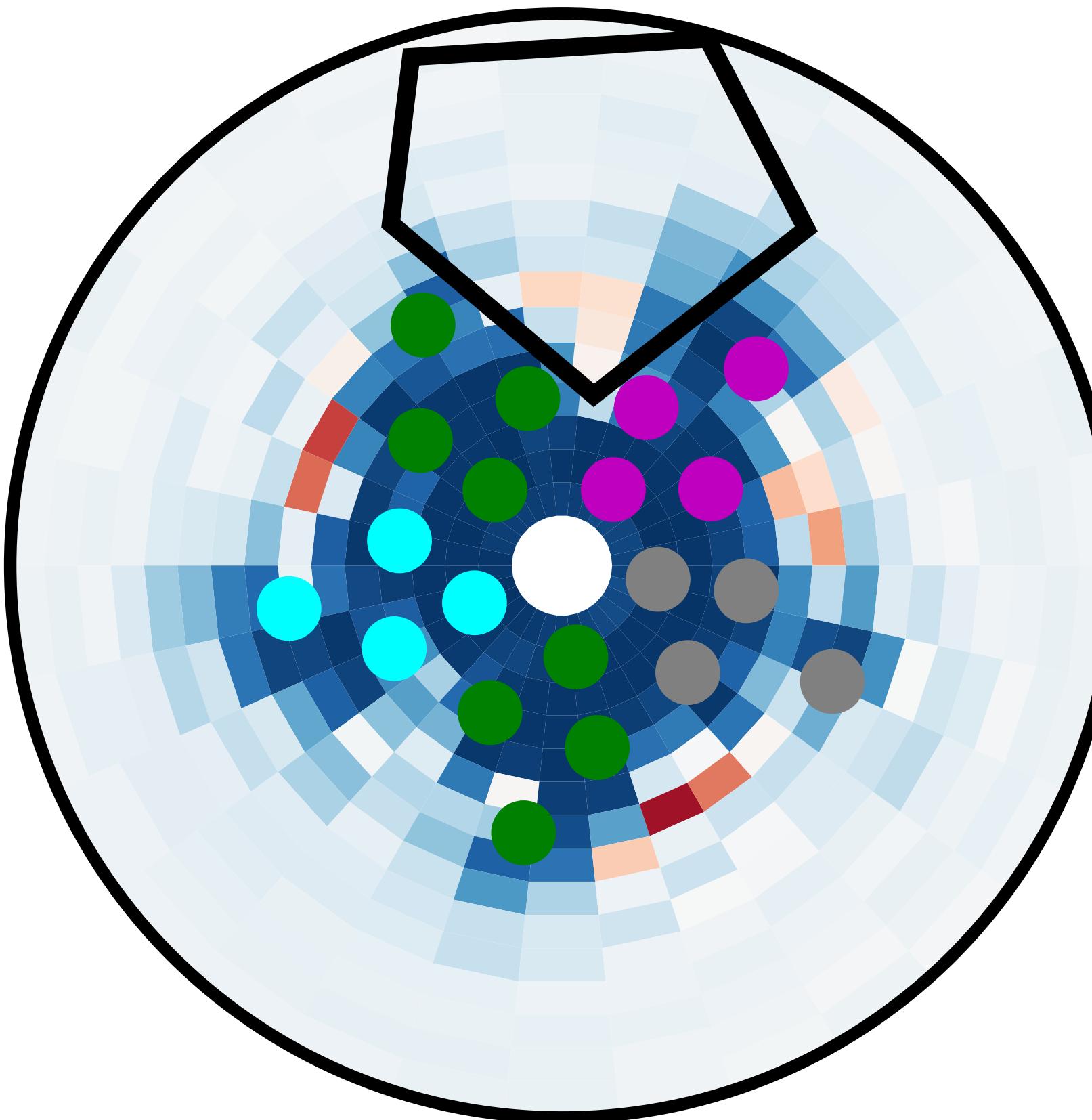
Counts



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

Frames  
Counts

1  
2



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 $P_{occ}$

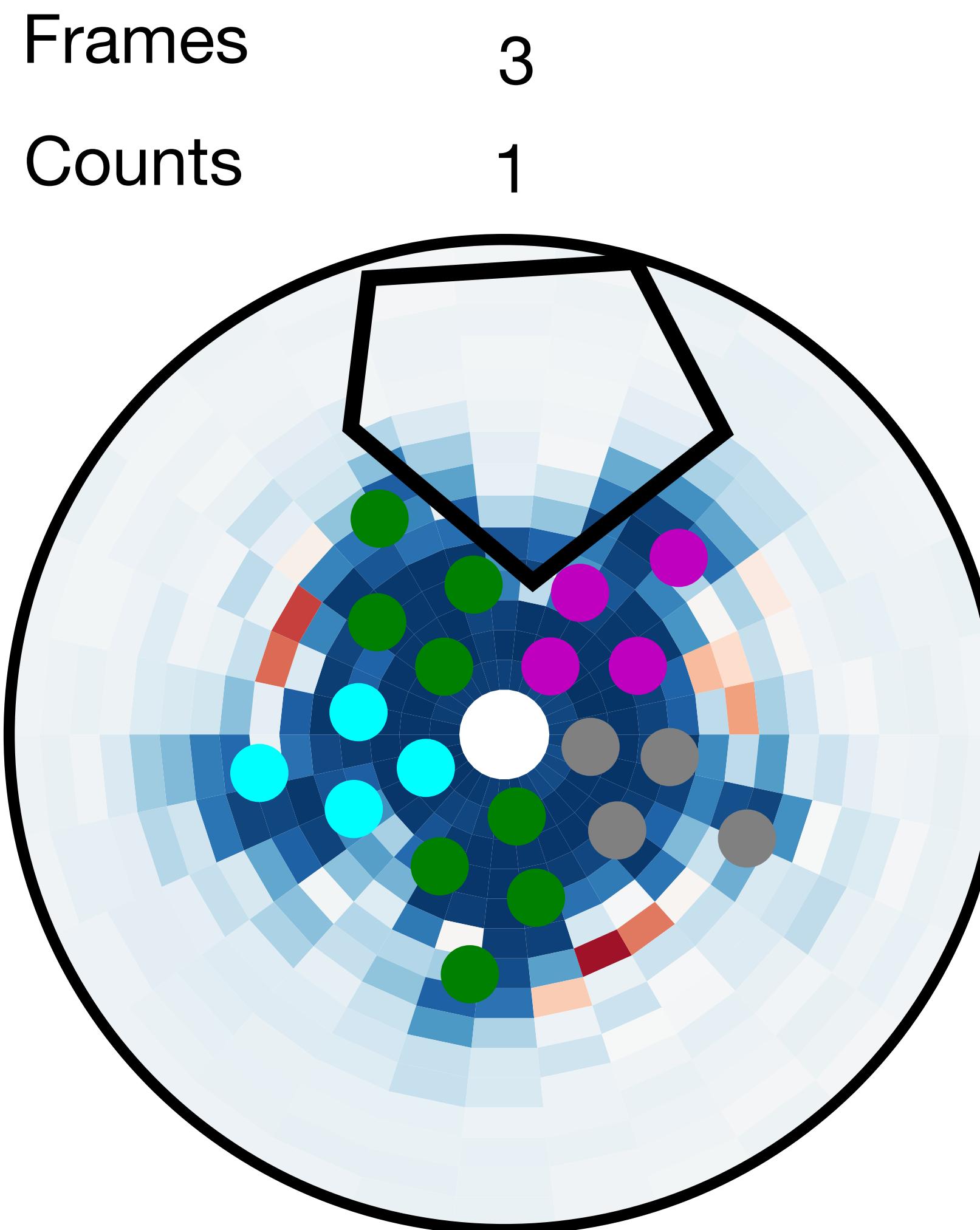
Frames  
Counts

2  
10



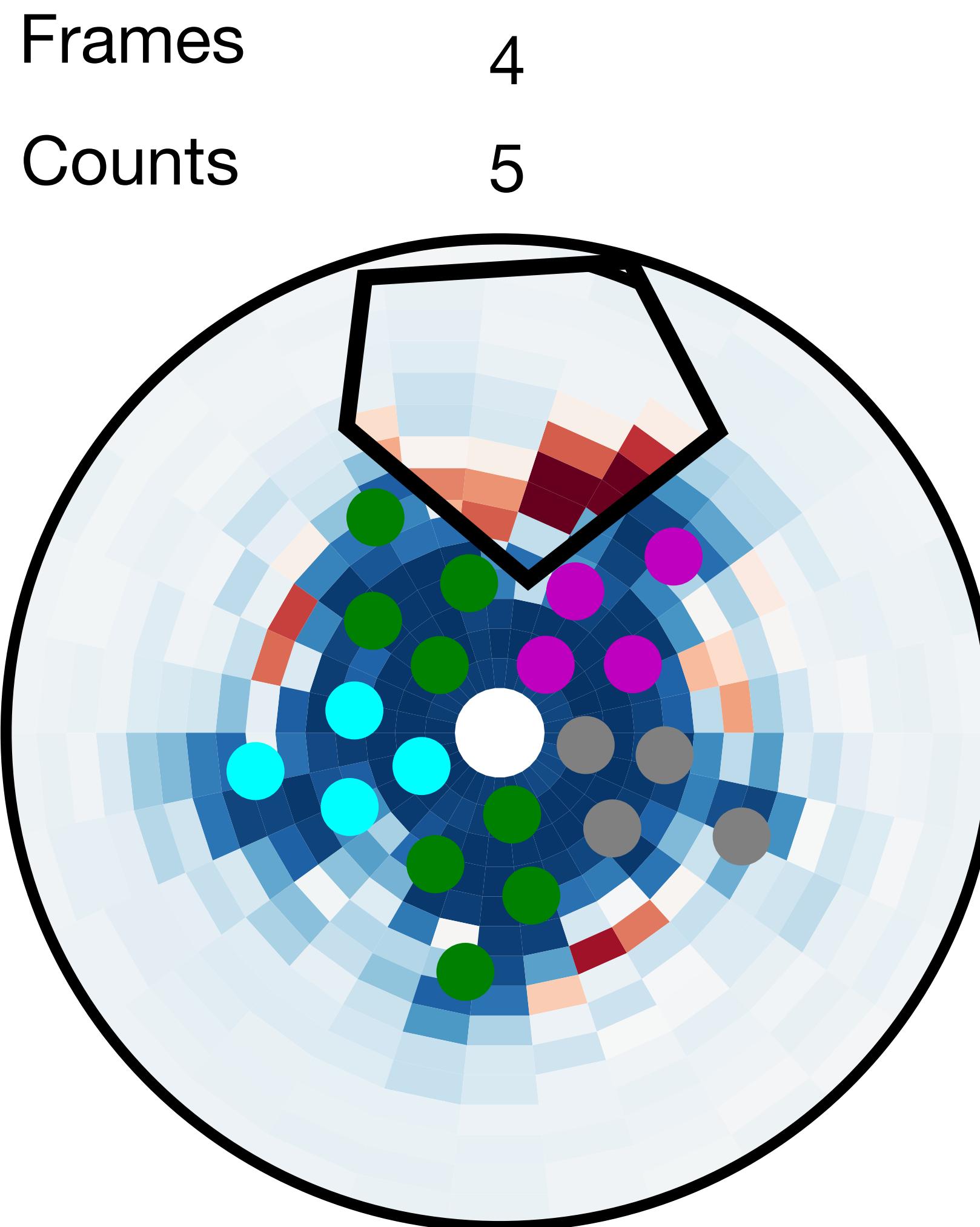
The occupancy site distribution  $P_{occ}$  is determined by counting the number of lipid beads within the area per frame

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



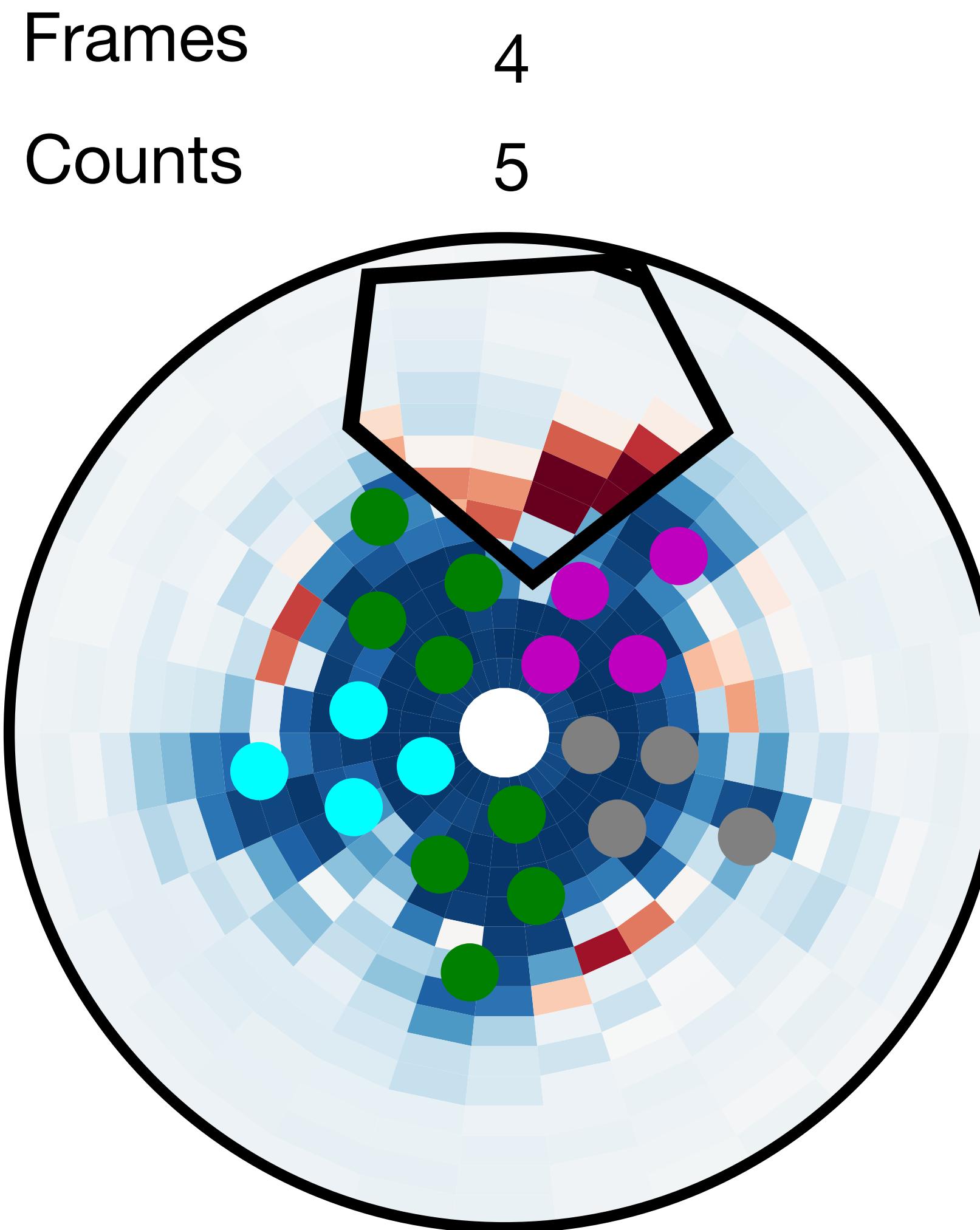
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# Partial Occupation and Affinity: How to calculate $P_{occ}$

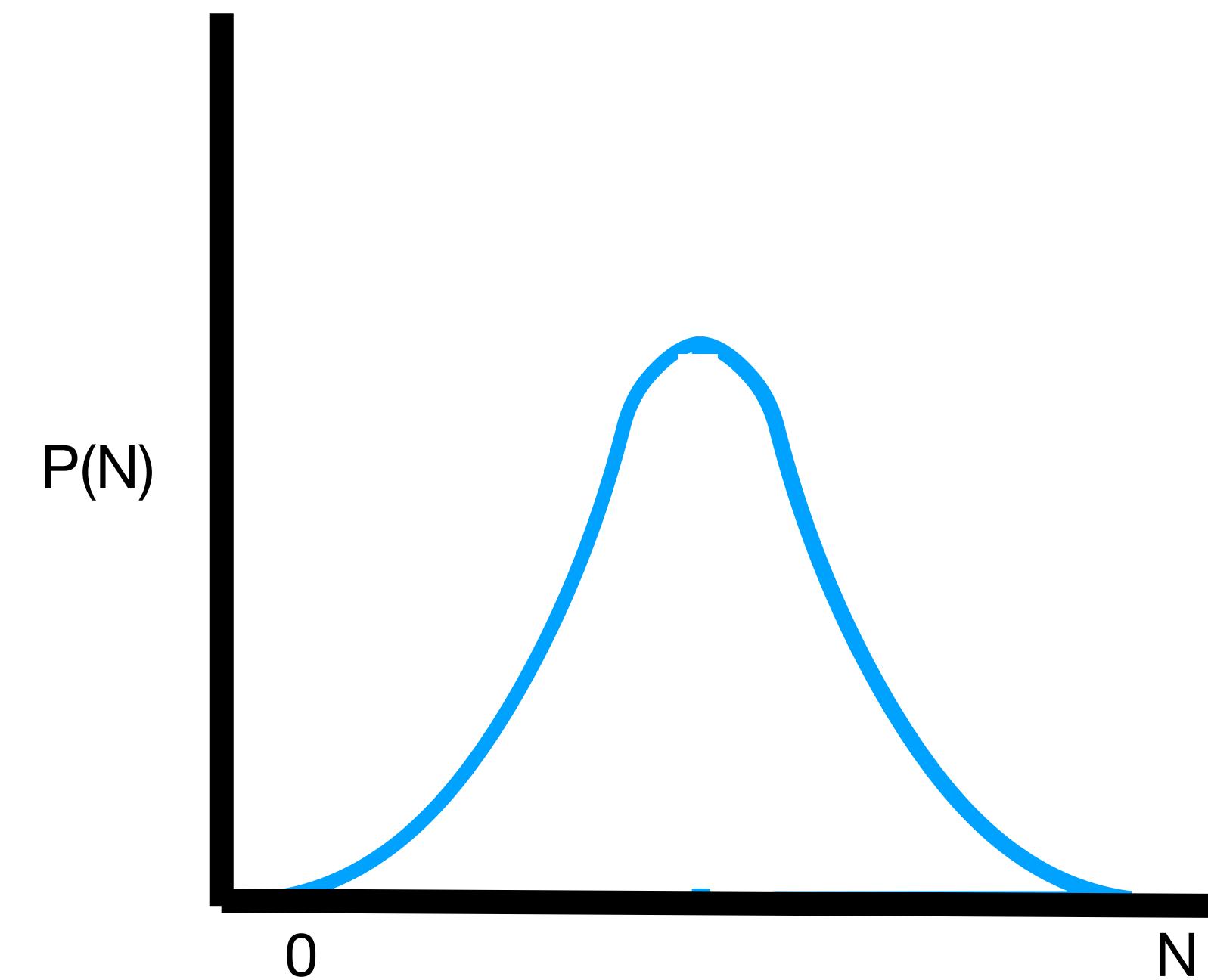


The occupancy site distribution  $P_{occ}$  is determined by counting the number of lipid beads within the area per frame

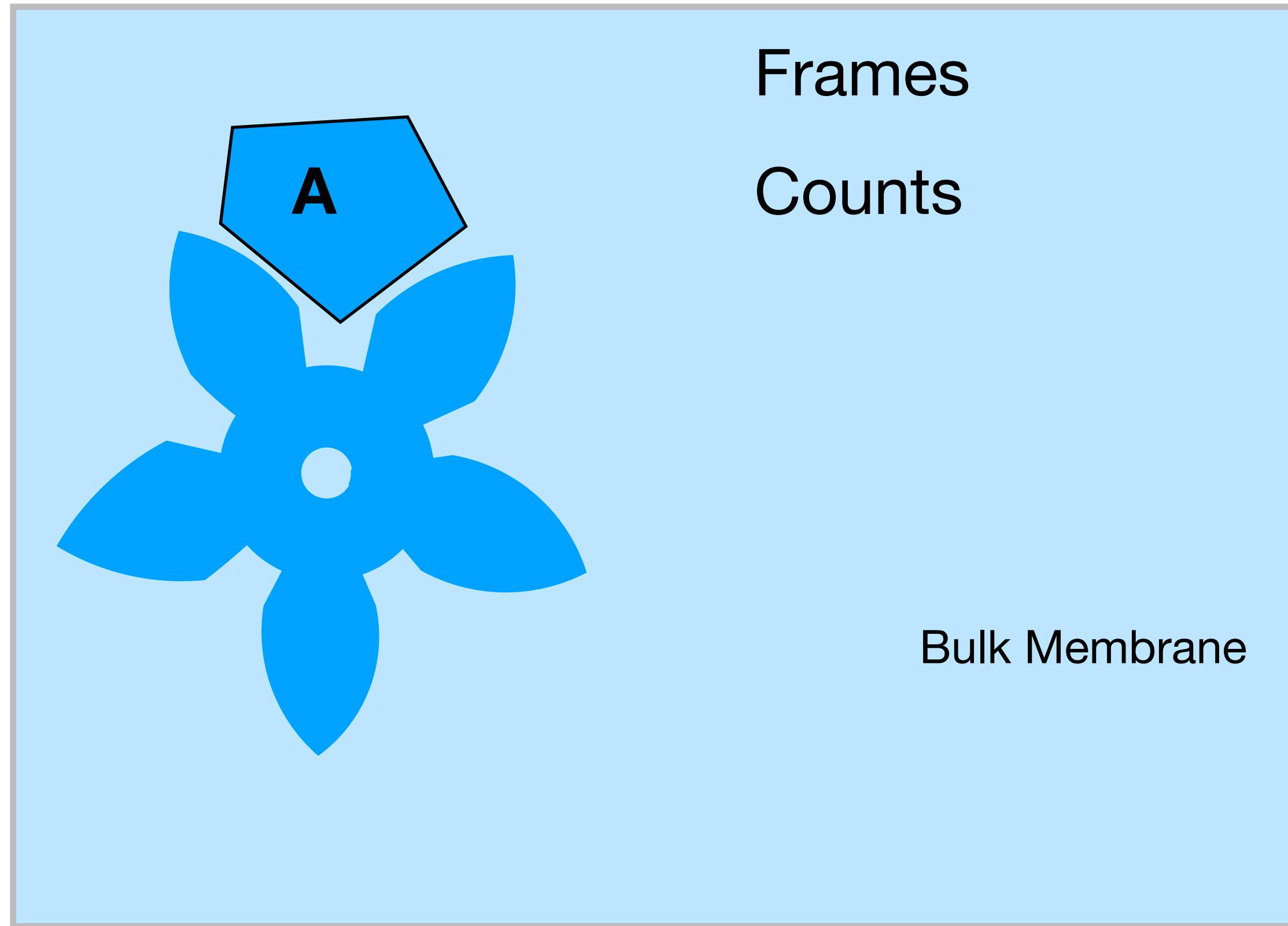
# Partial Occupation and Affinity: How to calculate $P_{occ}$



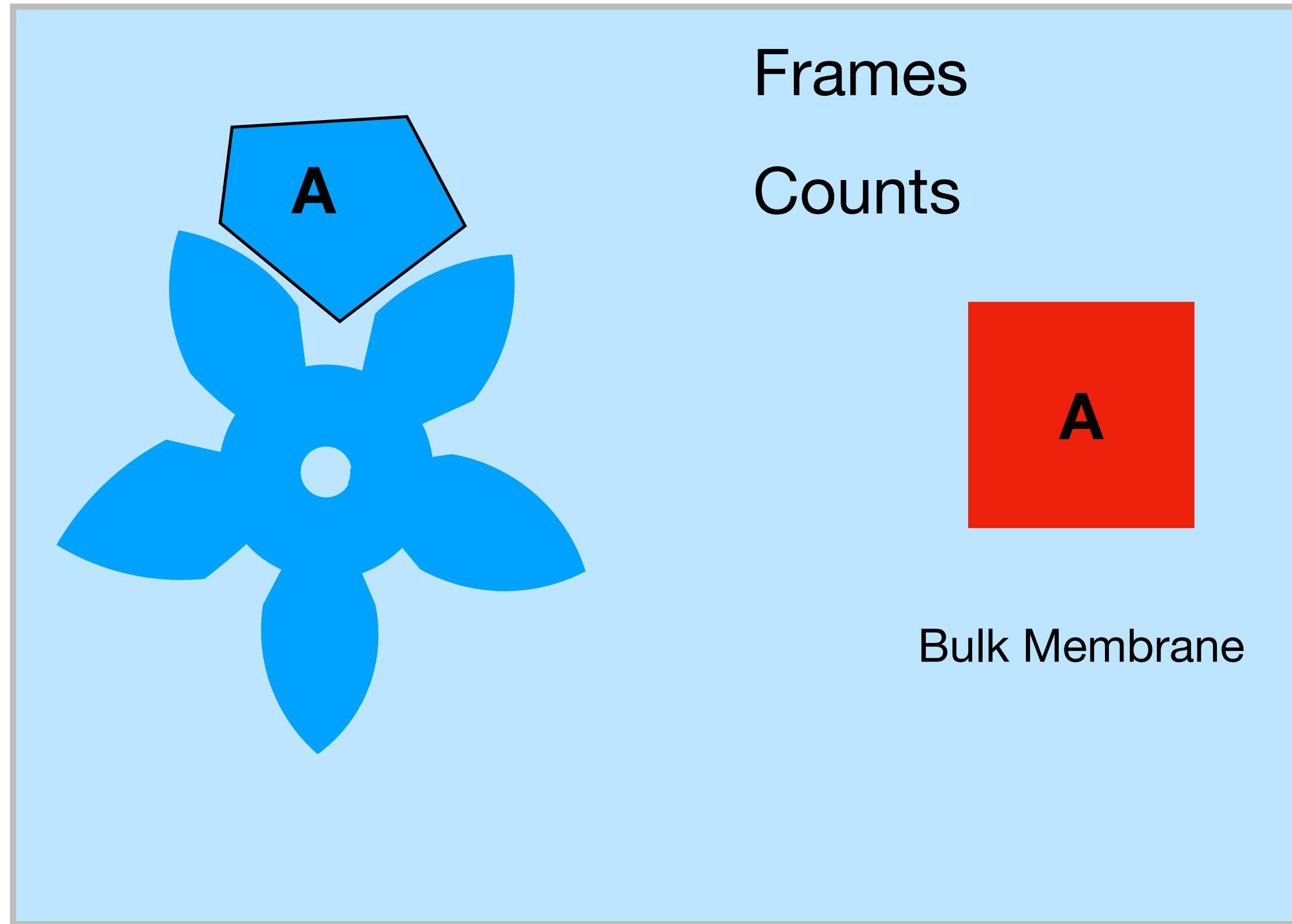
The occupancy site distribution  $P_{occ}$  is determined by counting the number of lipid beads within the area per frame



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

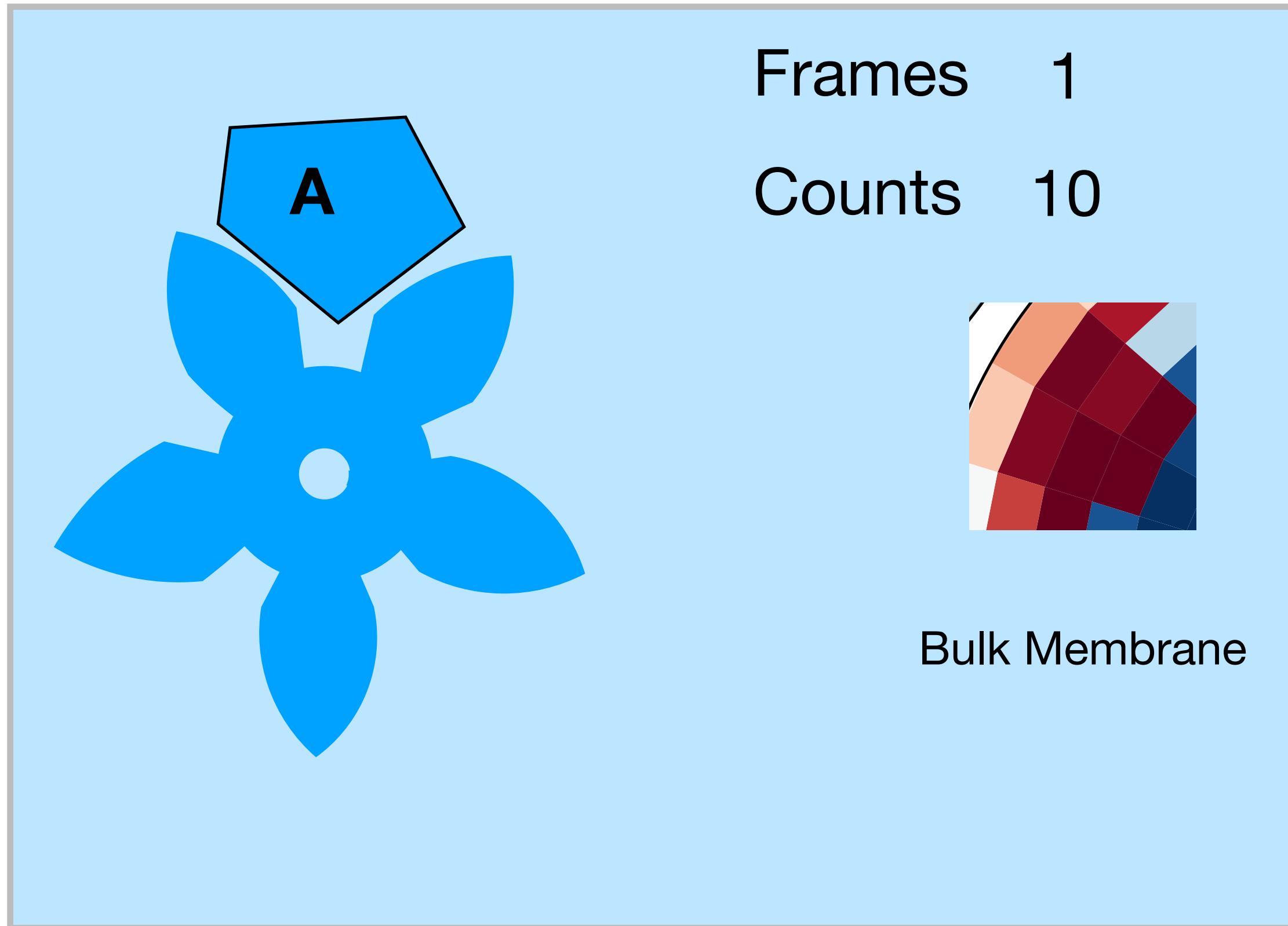


# Partial Occupation and Affinity: How to calculate $P_{bulk}$ ?



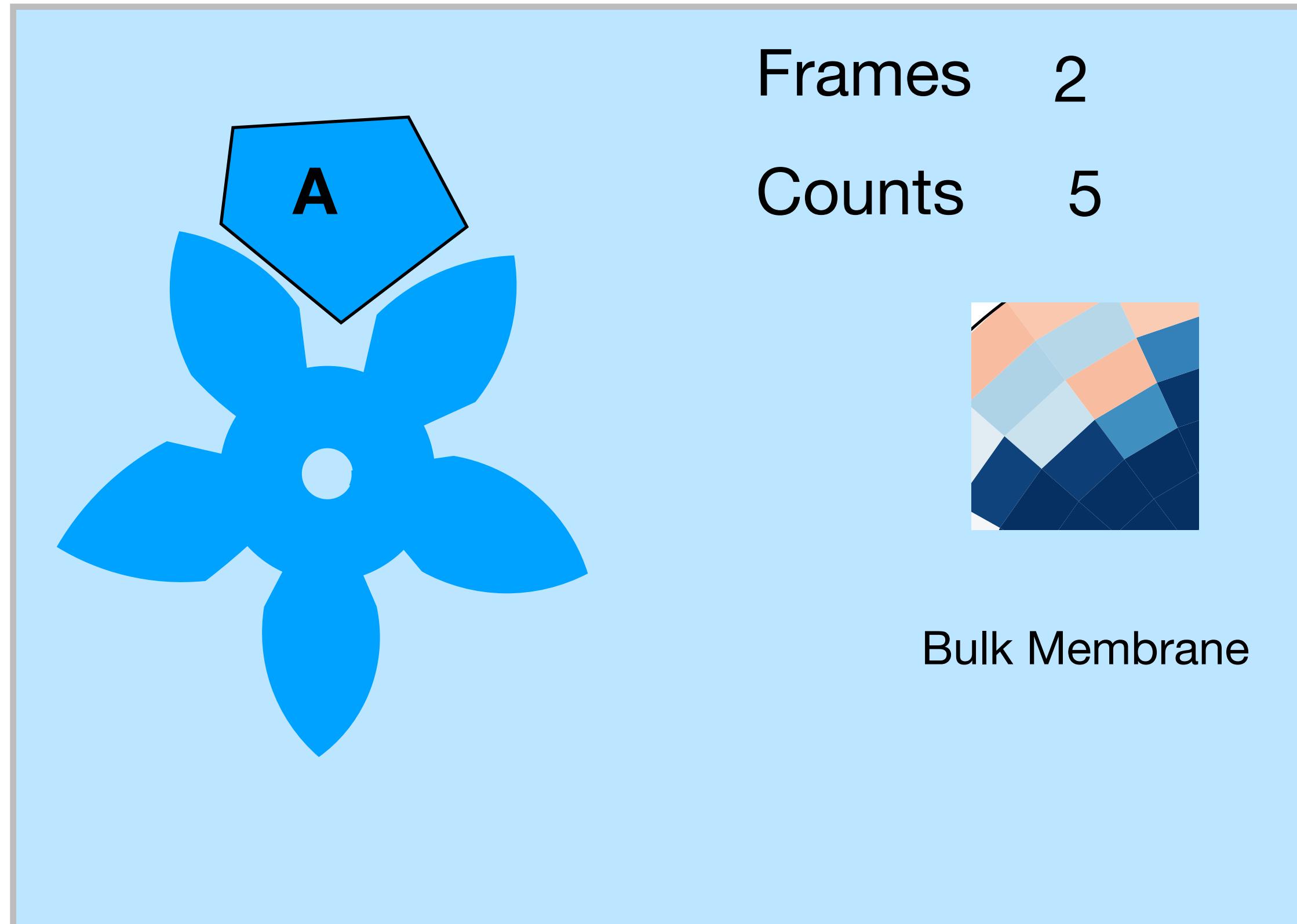
# Partial Occupation and Affinity: How to calculate $P_{bulk}$ ?

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



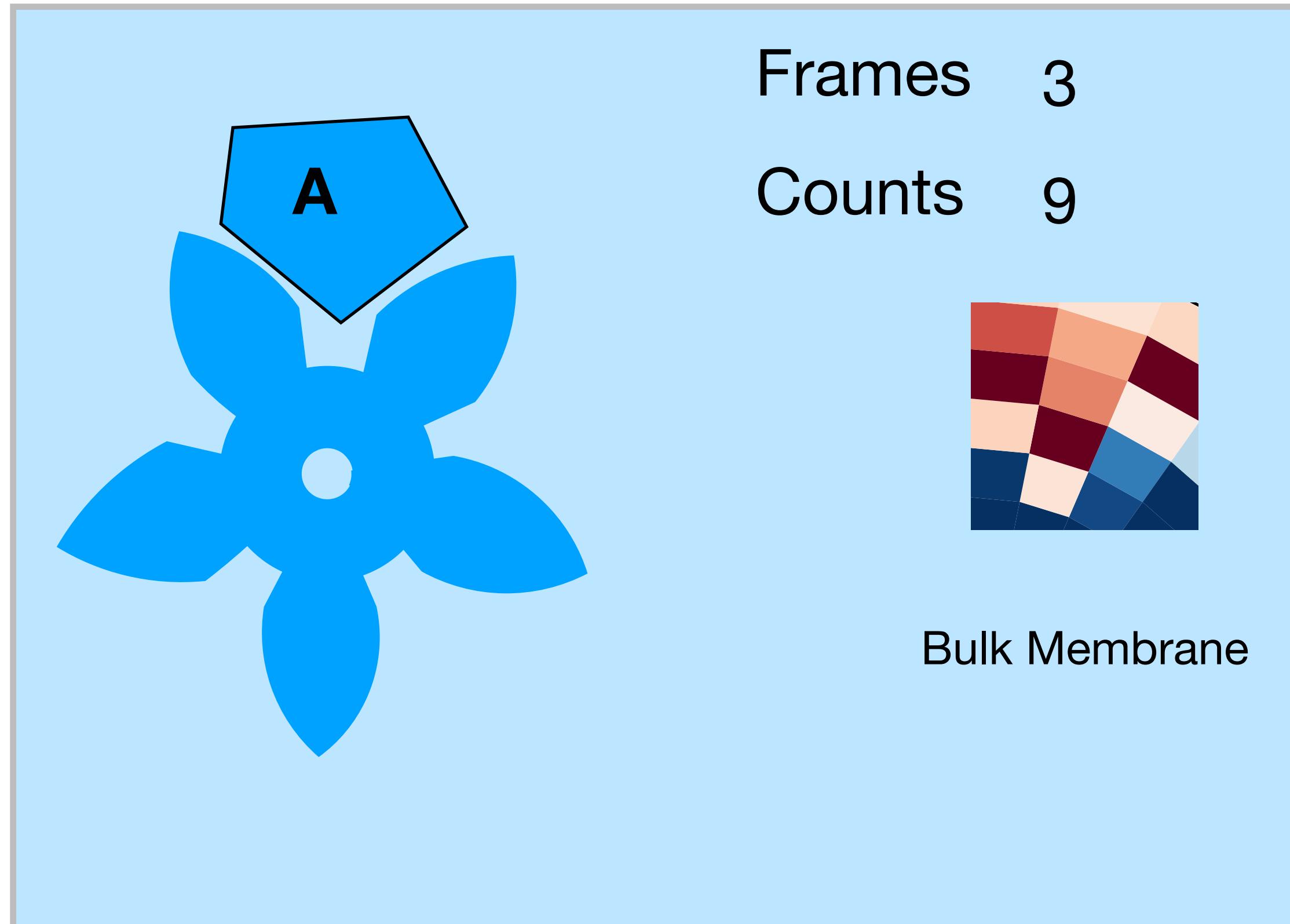
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The bulk site distribution  $P_{bulk}$  is determined by counting the number of beads at a bulk area per frame



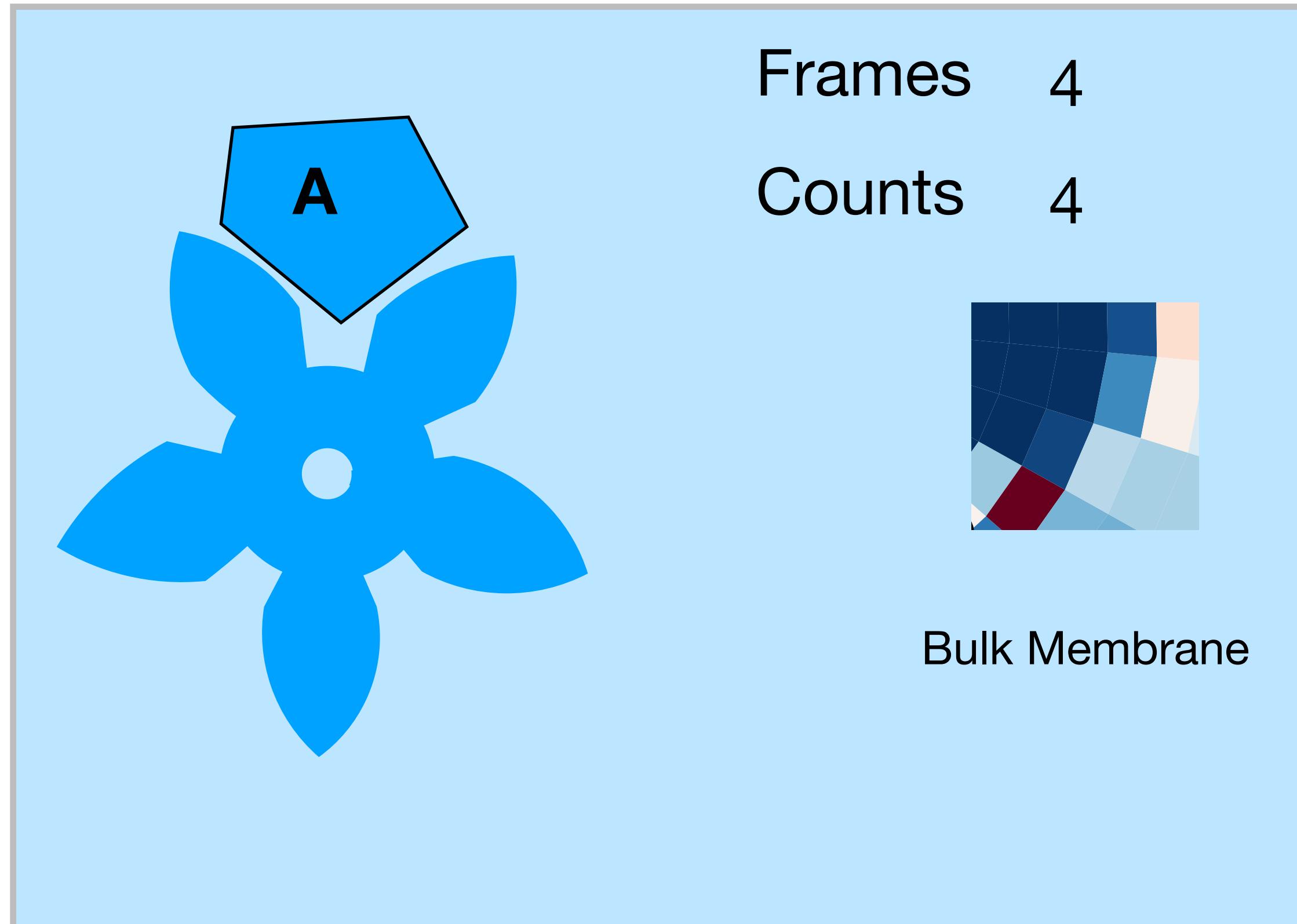
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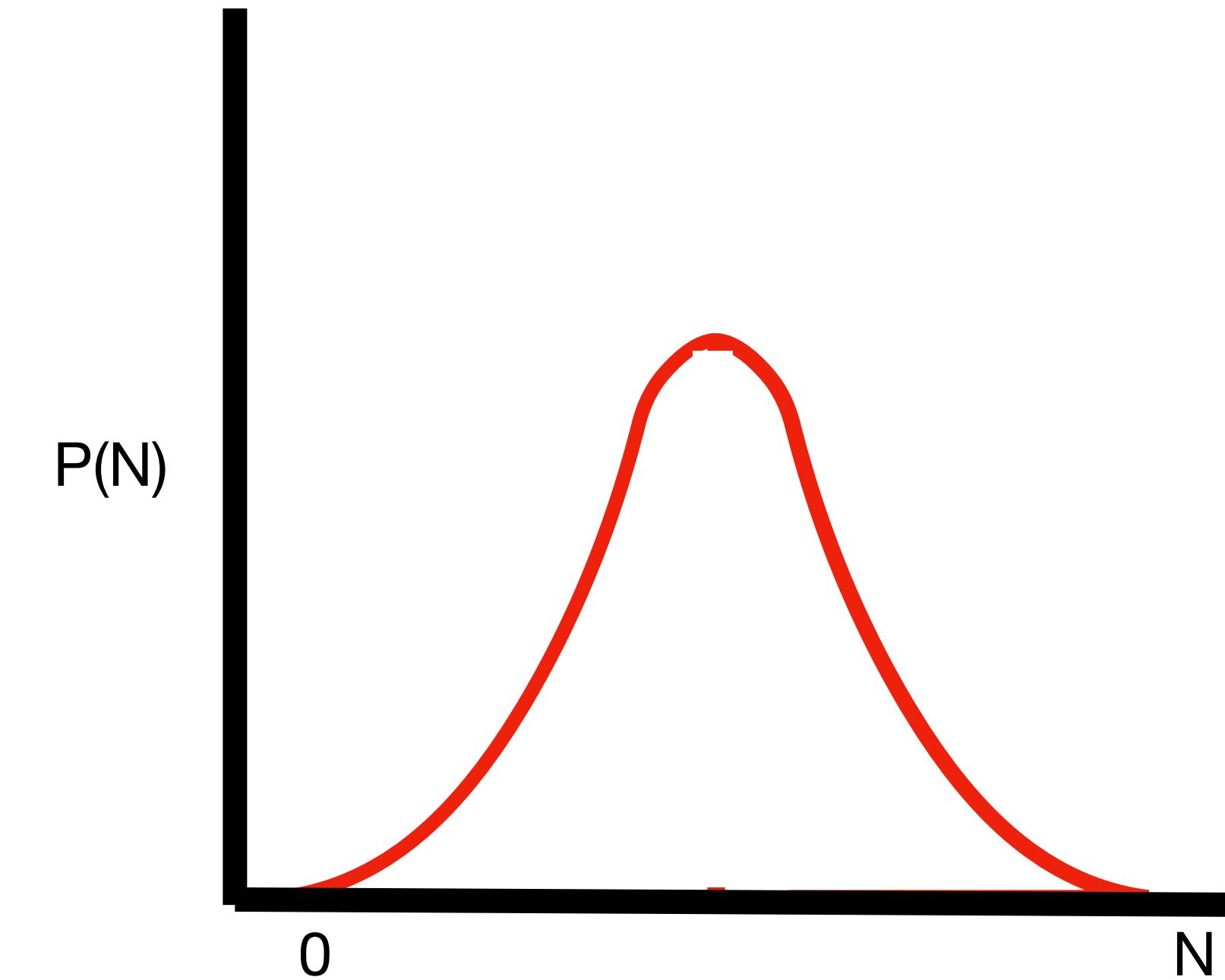
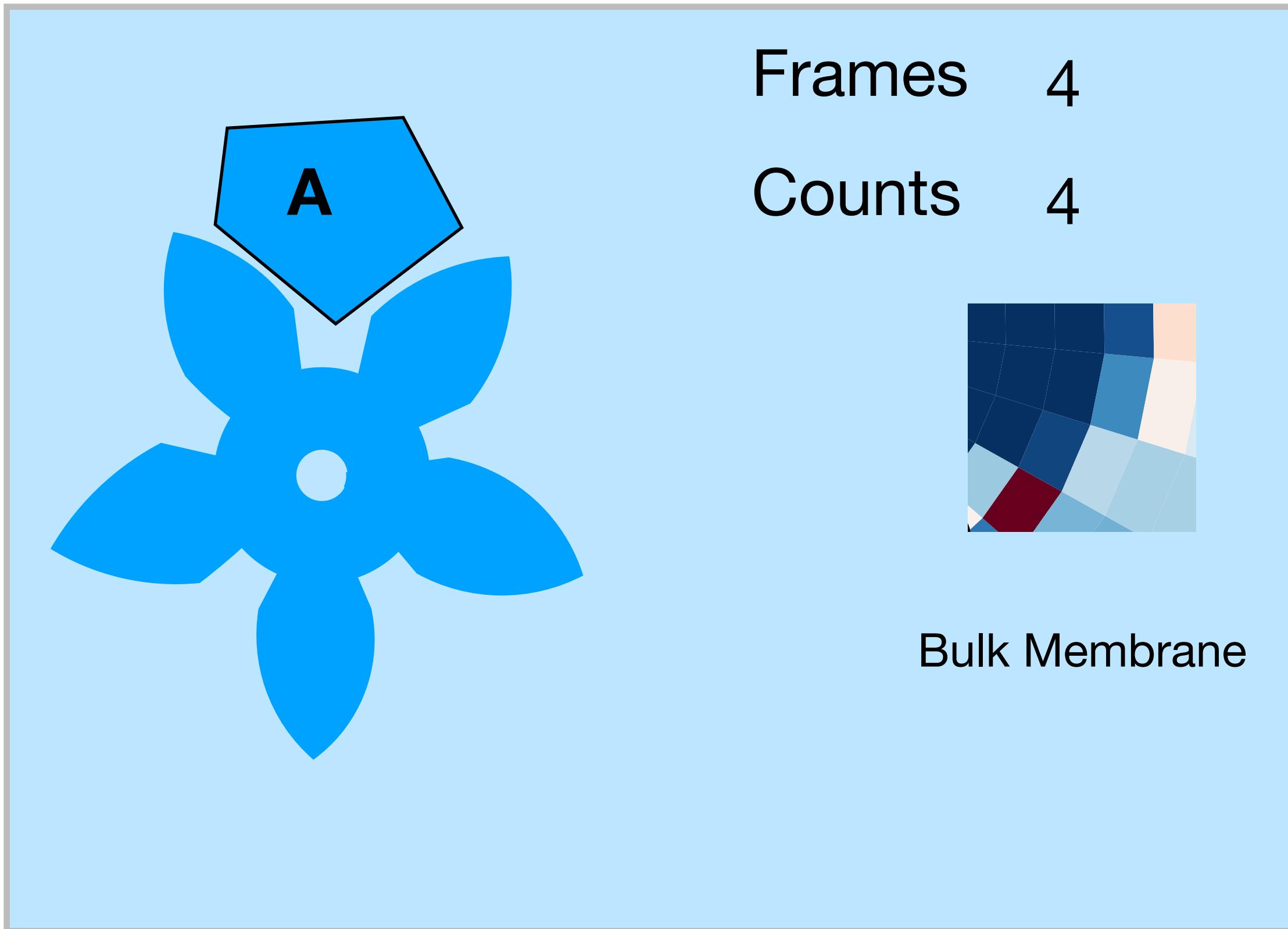
# Partial Occupation and Affinity: How to calculate $P_{bulk}$ ?

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



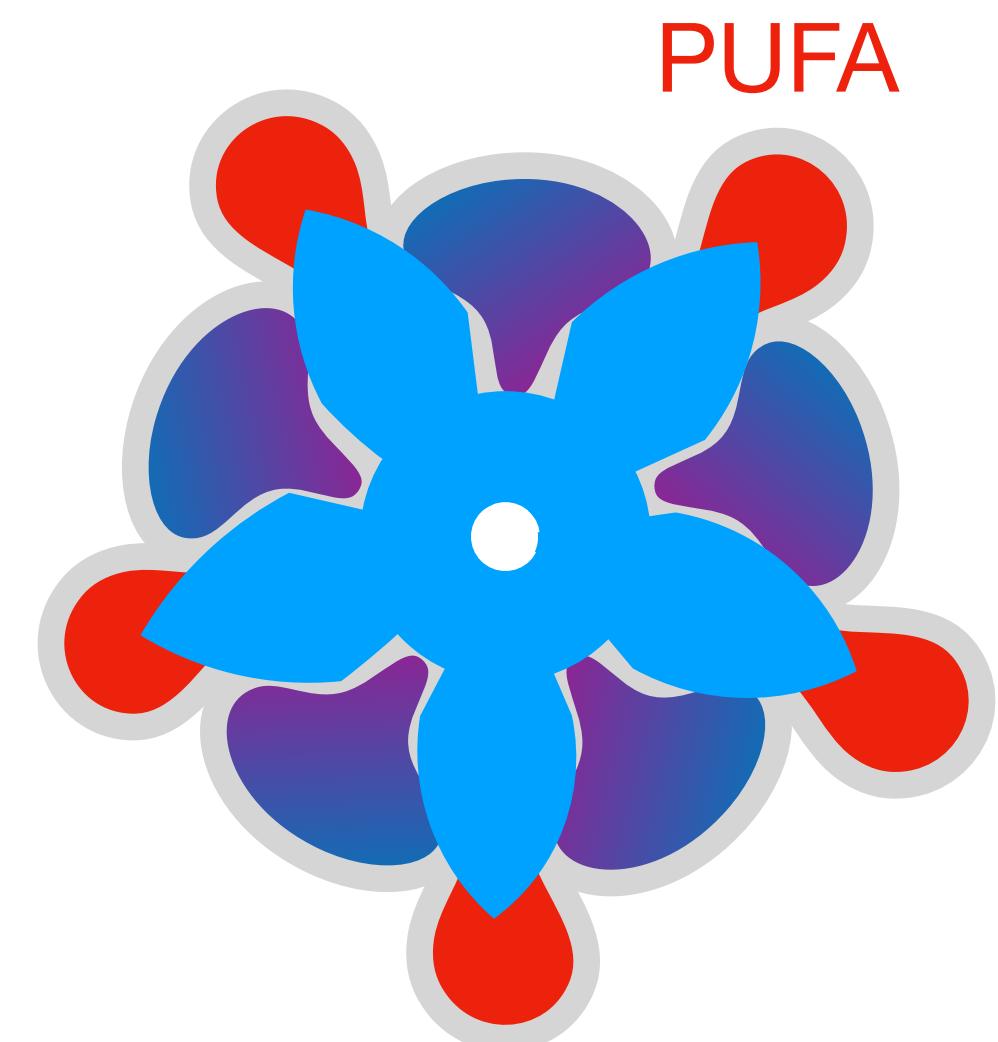
# Partial Occupation and Affinity: How to calculate $P_{bulk}$ ?

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



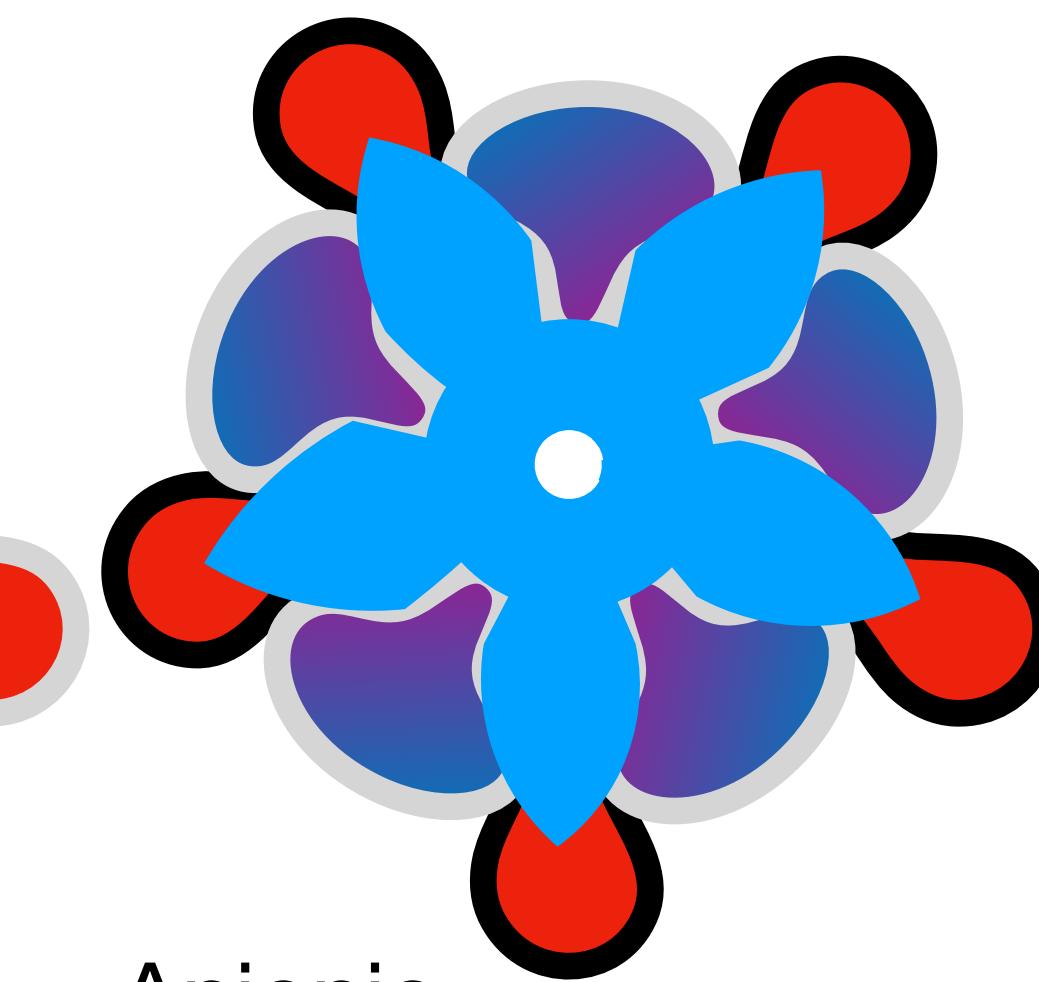
# What do we expect

Outer Leaflet



Neutral

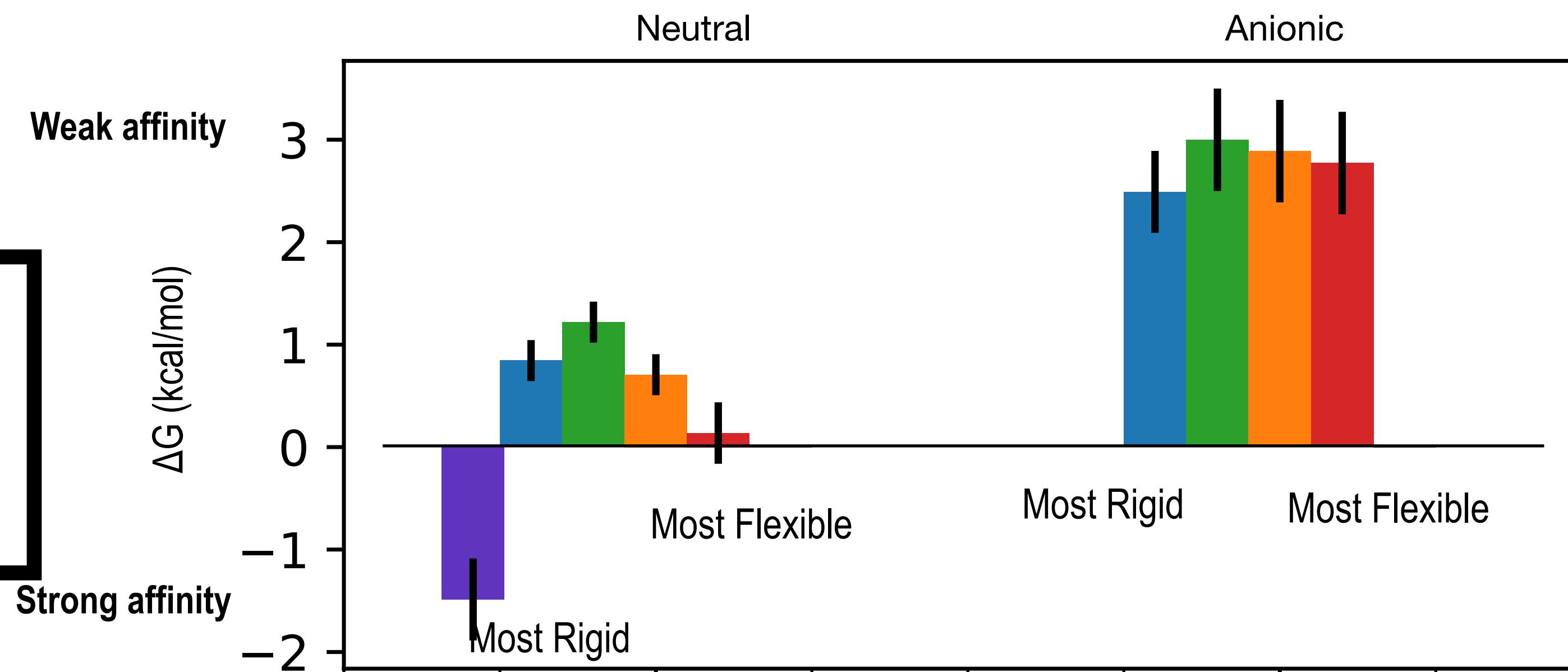
Inner Leaflet



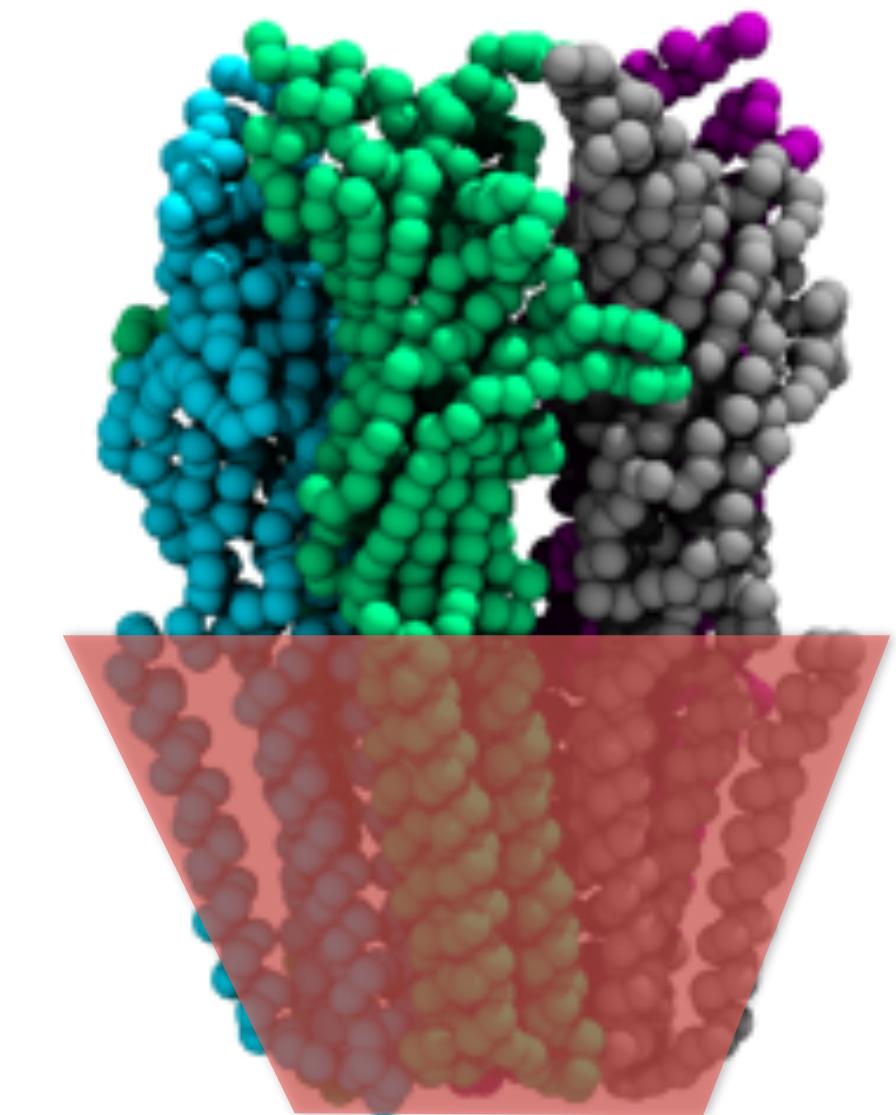
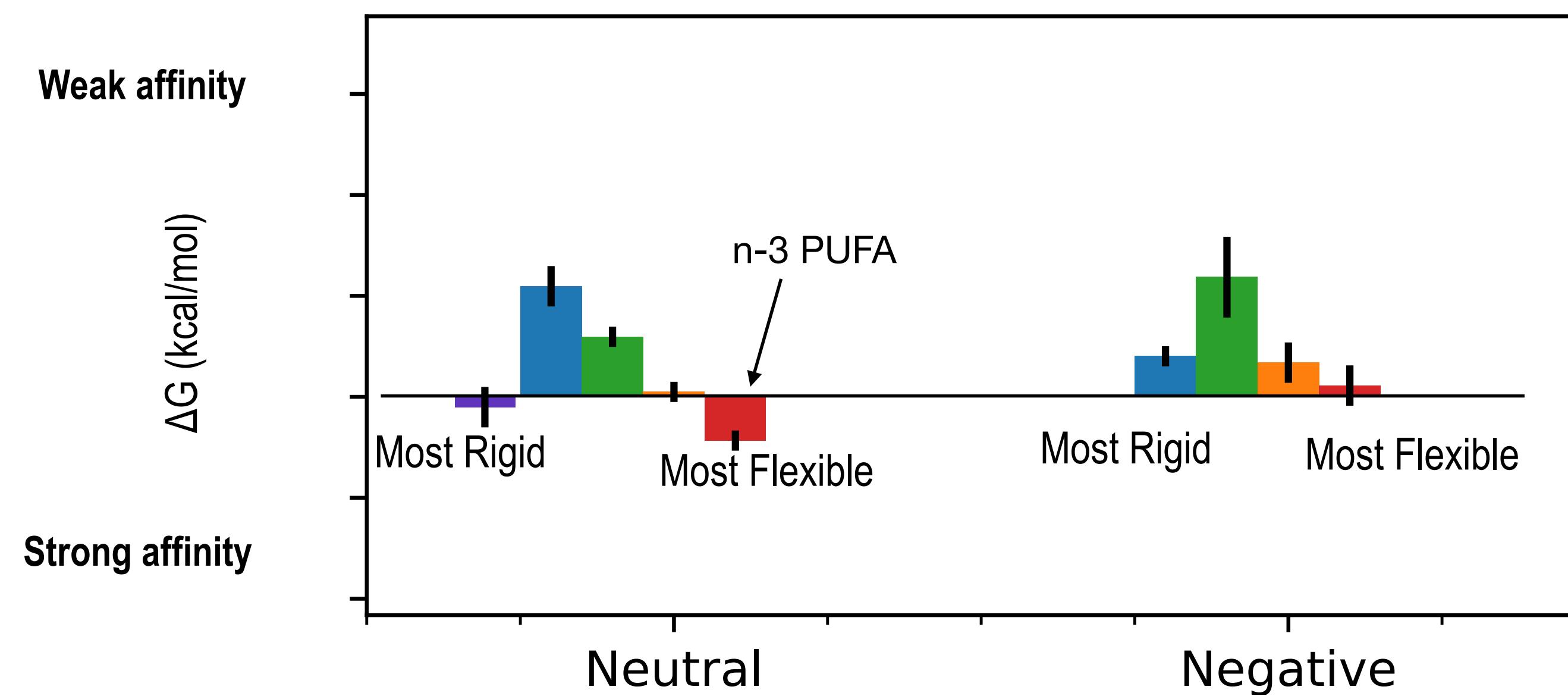
Saturated/  
Cholesterol

# 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?

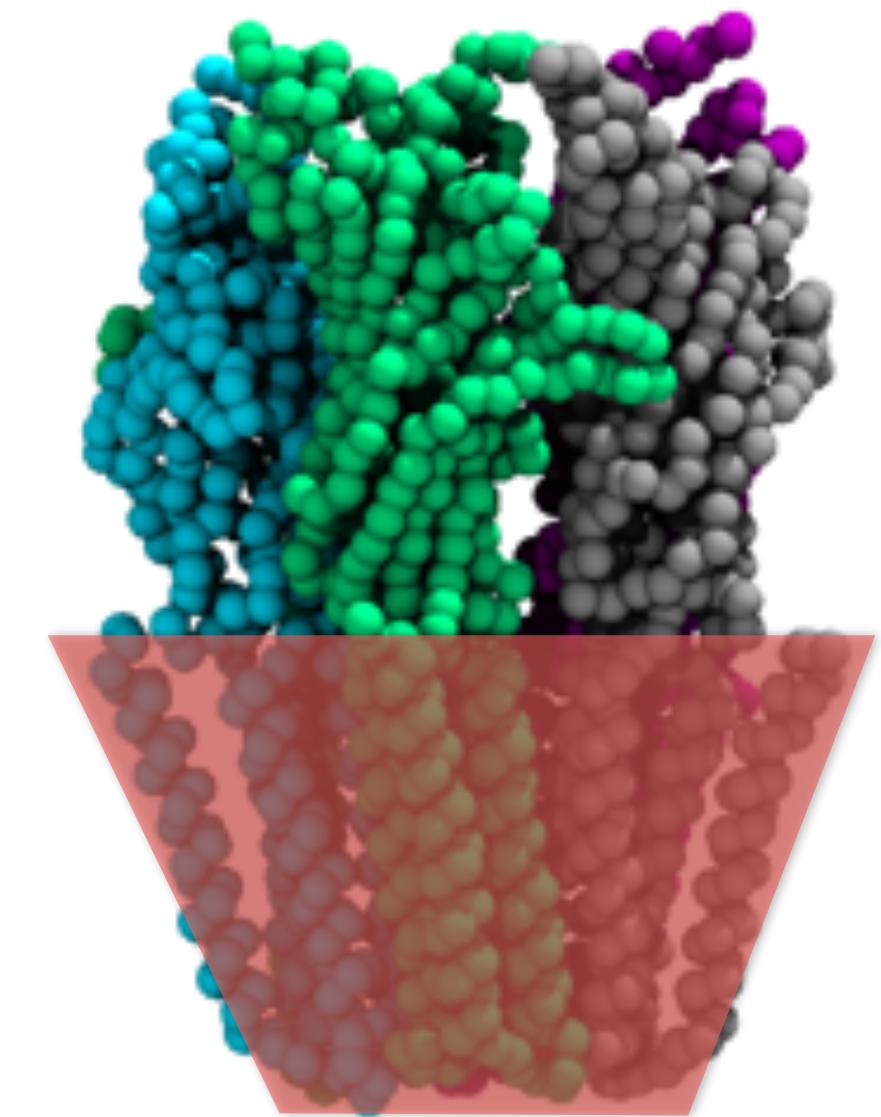
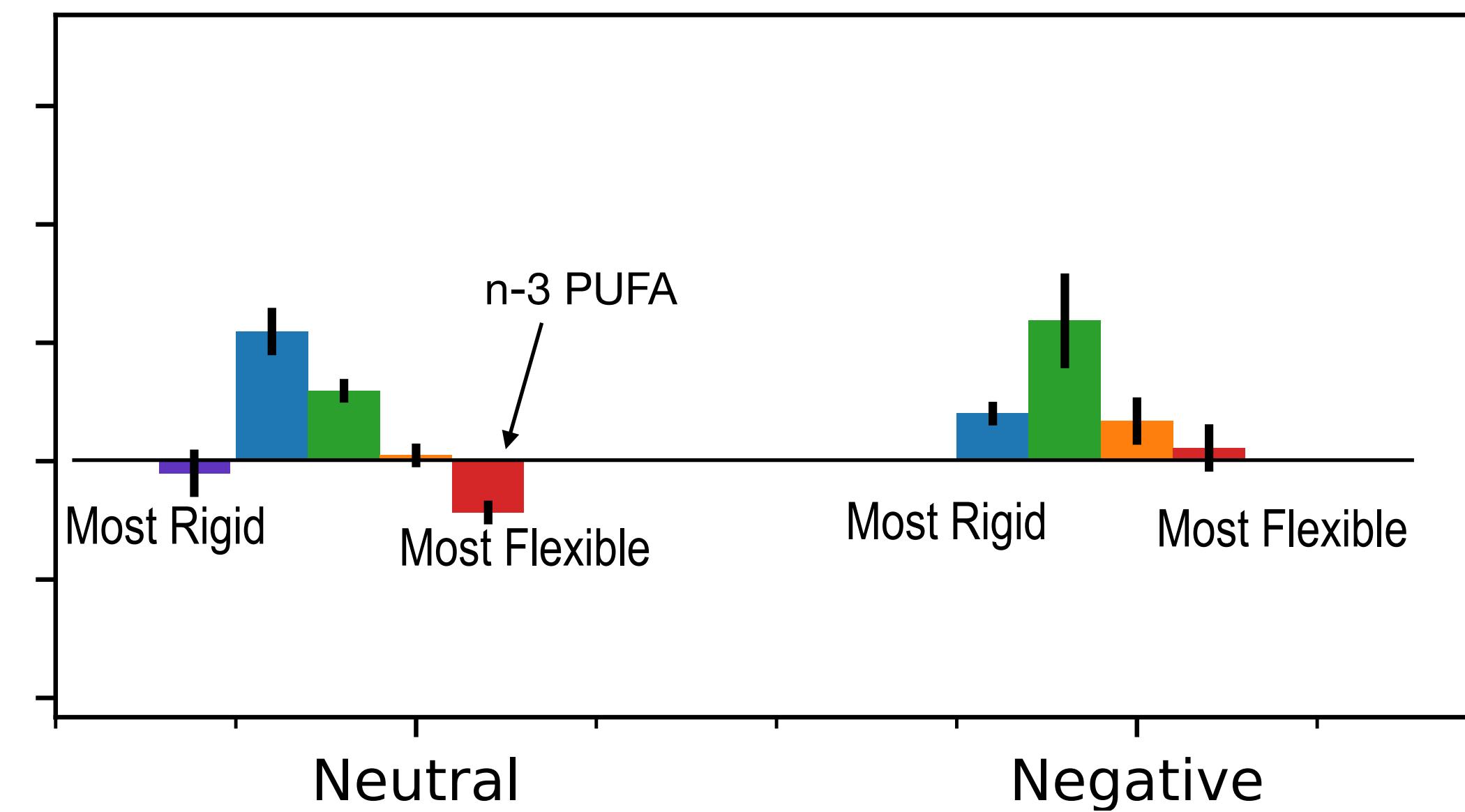


# Which lipids do M4 sites in the outer leaflet prefer?

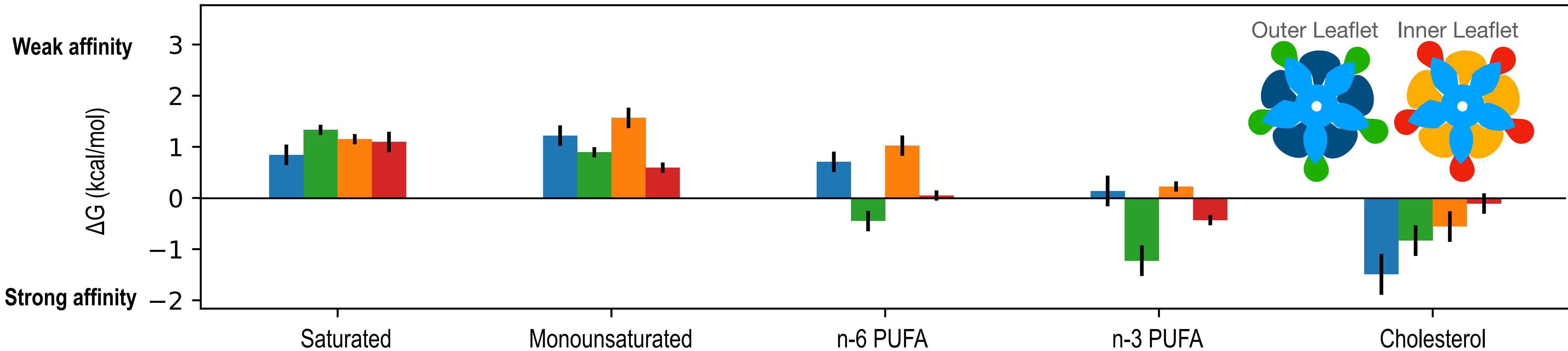
Weak affinity

Strong affinity

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

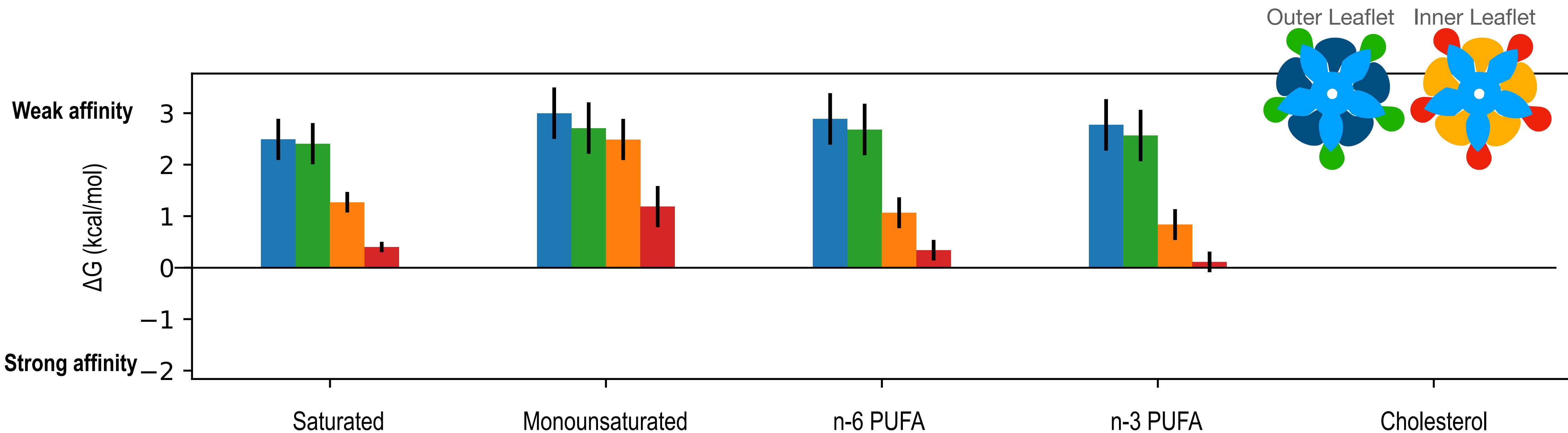


# Which sites do neutral lipids prefer?



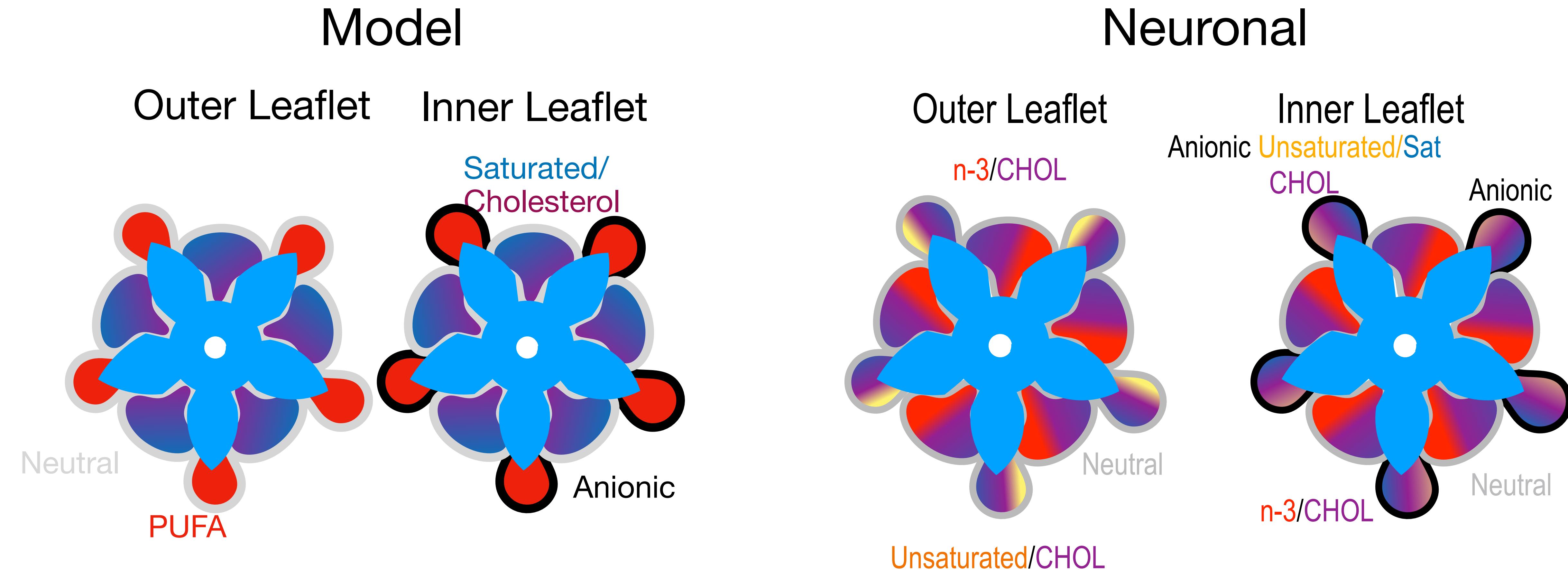
- Saturated lipids do not have a preferred site
- Monounsaturated lipids weakly prefer M4 sites in the inner leaflet
- Neutral PUFAs prefer M4 in the outer leaflet
- Cholesterol prefers inter-subunit sites in the outer leaflet

# 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

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Dr. Jerome Hénin

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Dr. Tom Joseph\*

The GMO Pugs (Success Circle 4)

## Former Lab Members

\*Honorary Lab Members



Office of Advanced Research  
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Computational resources: NSF  
XSEDE Allocation NSF-MCB110149

Local cluster funded by NSF-  
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Research Corporation, NIH  
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