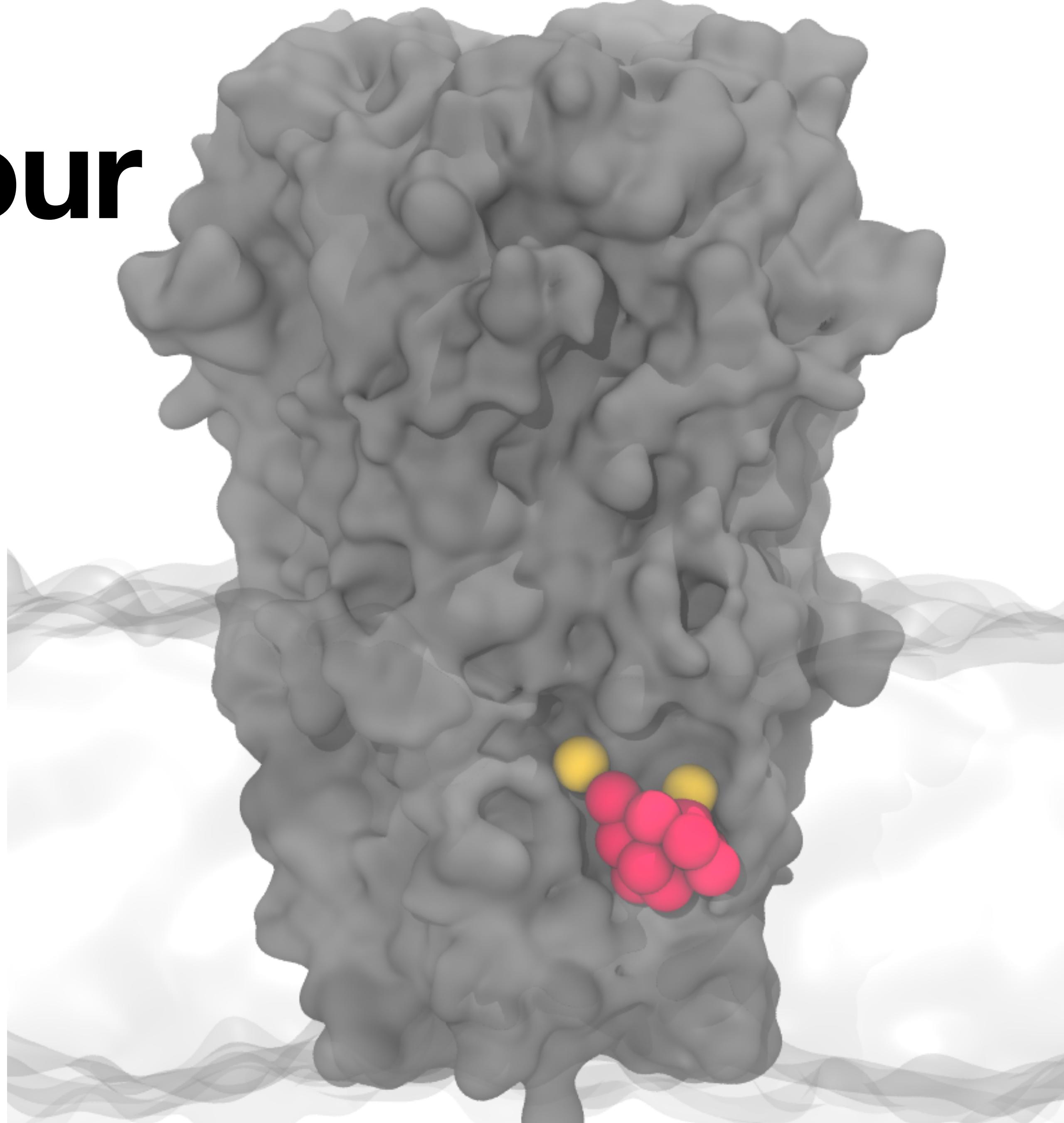


Have you had your fish oil today?

Measuring the binding affinity of DHA to the Nicotinic Acetylcholine Receptor with the Density-Threshold Affinity



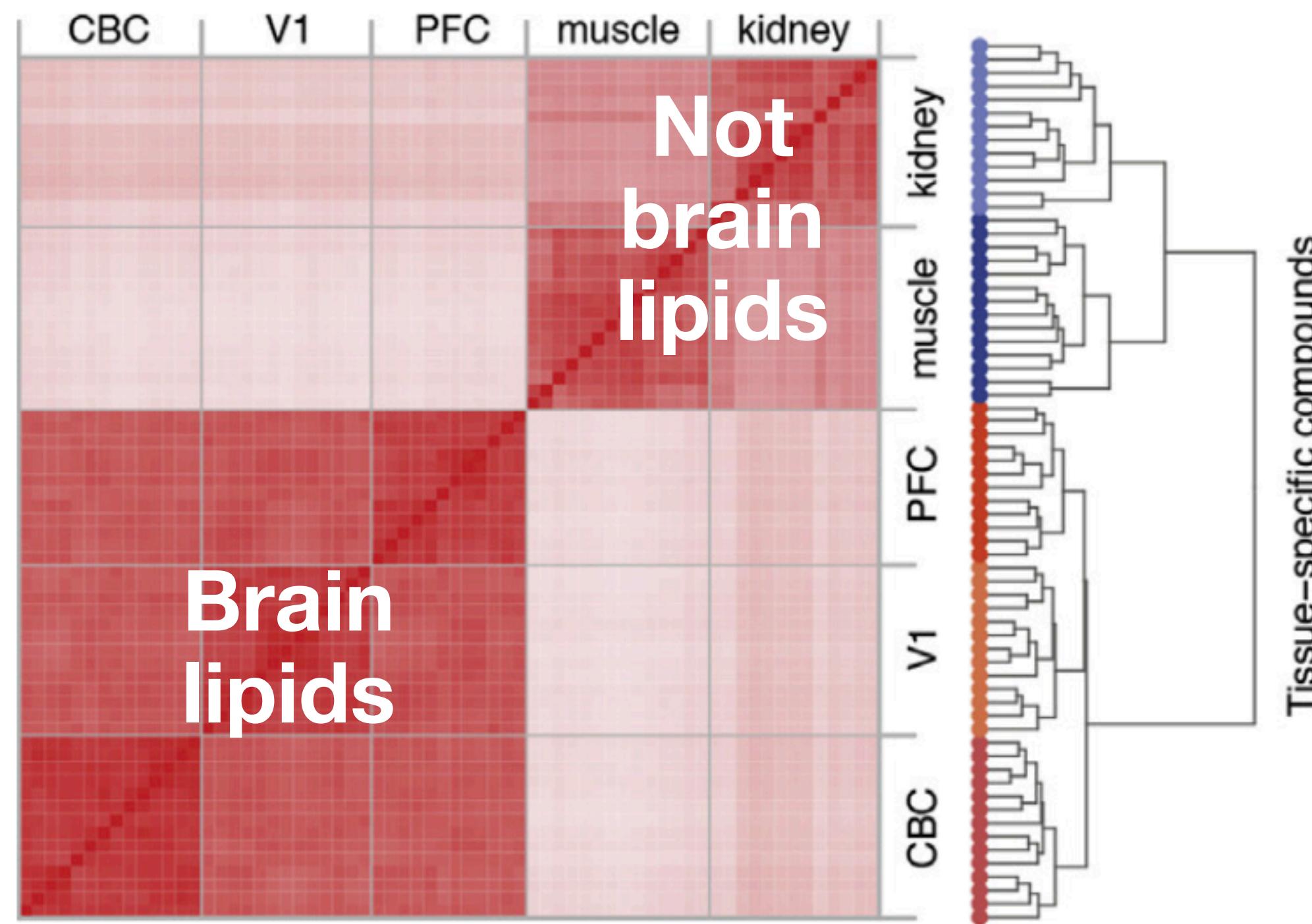
Jesse W Sandberg, Nour Awad,
Marie Prevost, Nathalie Barilone,
Pierre-Jean Corringer, Grace Brannigan



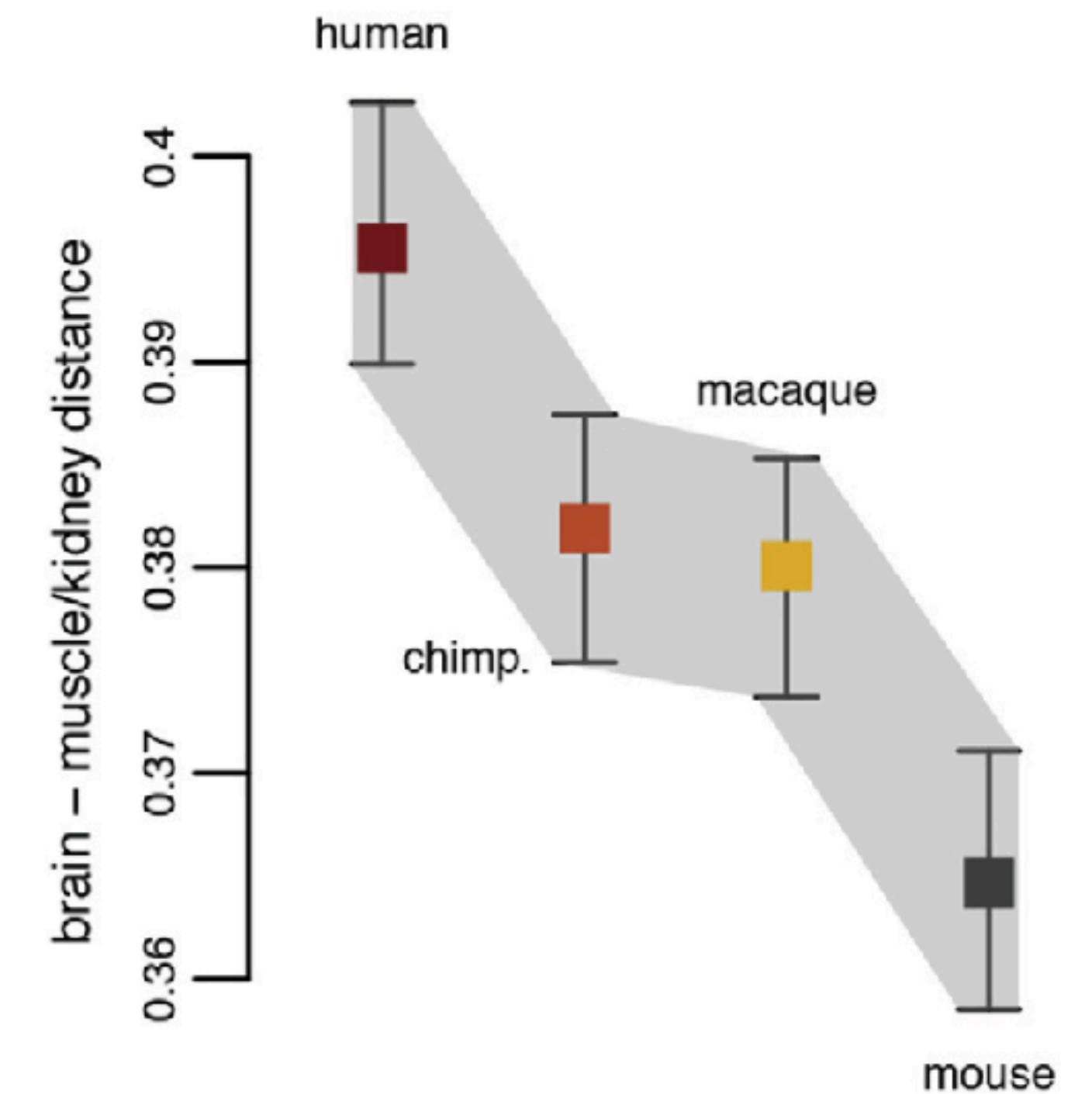
Specific lipids allow our brains to function



Lipids are 1/2
brain's dry weight

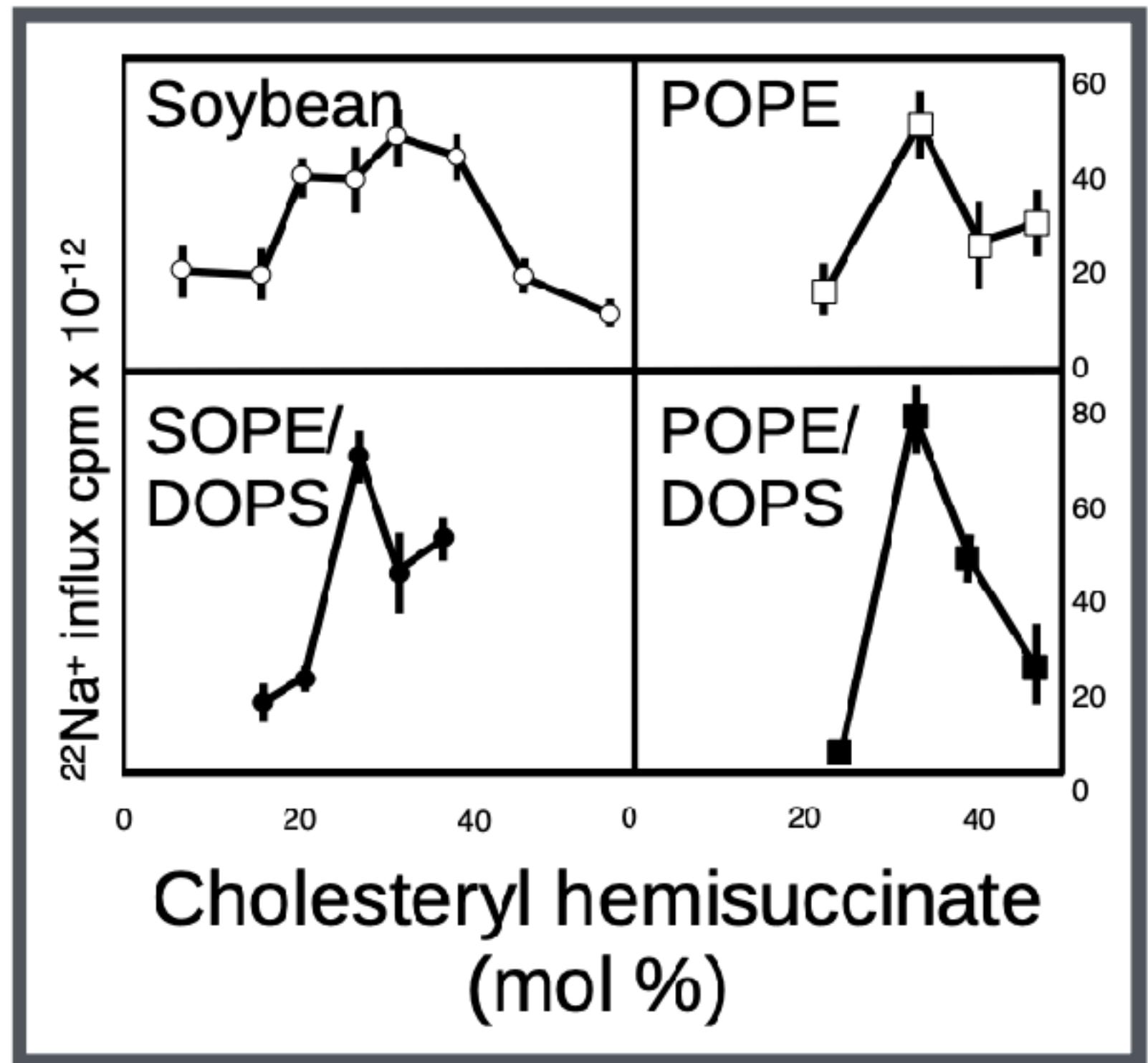


Brain requires different lipids than
rest of body



Difference grows
with evolutionary distance

Neurotransmitter receptors need specific lipids to function



Ion channel has complex relationship
between cholesterol, lipid composition



Biochimica et Biophysica Acta (BBA) -
Biomembranes

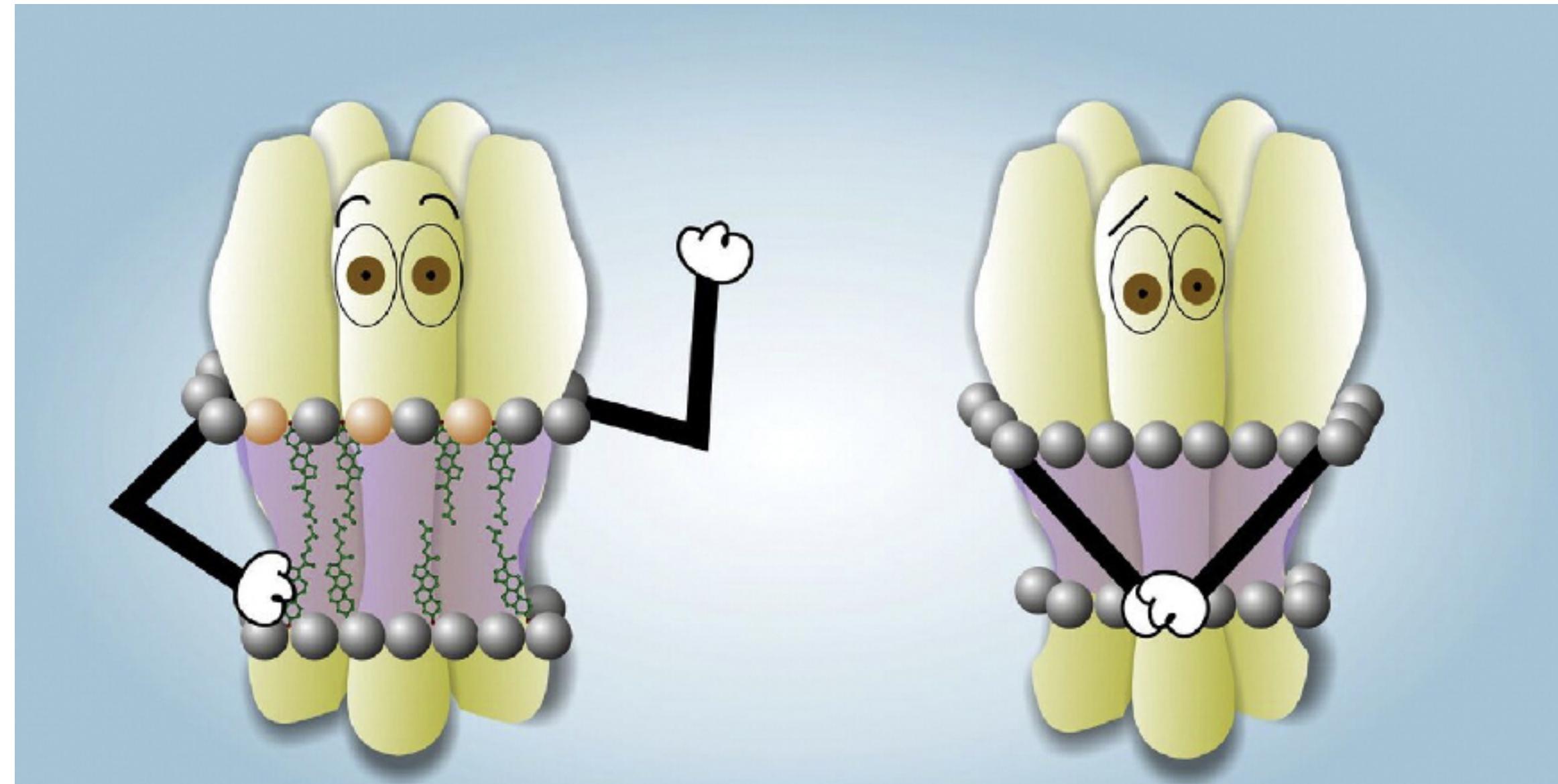
Volume 1858, Issue 11, November 2016, Pages 2662-2670



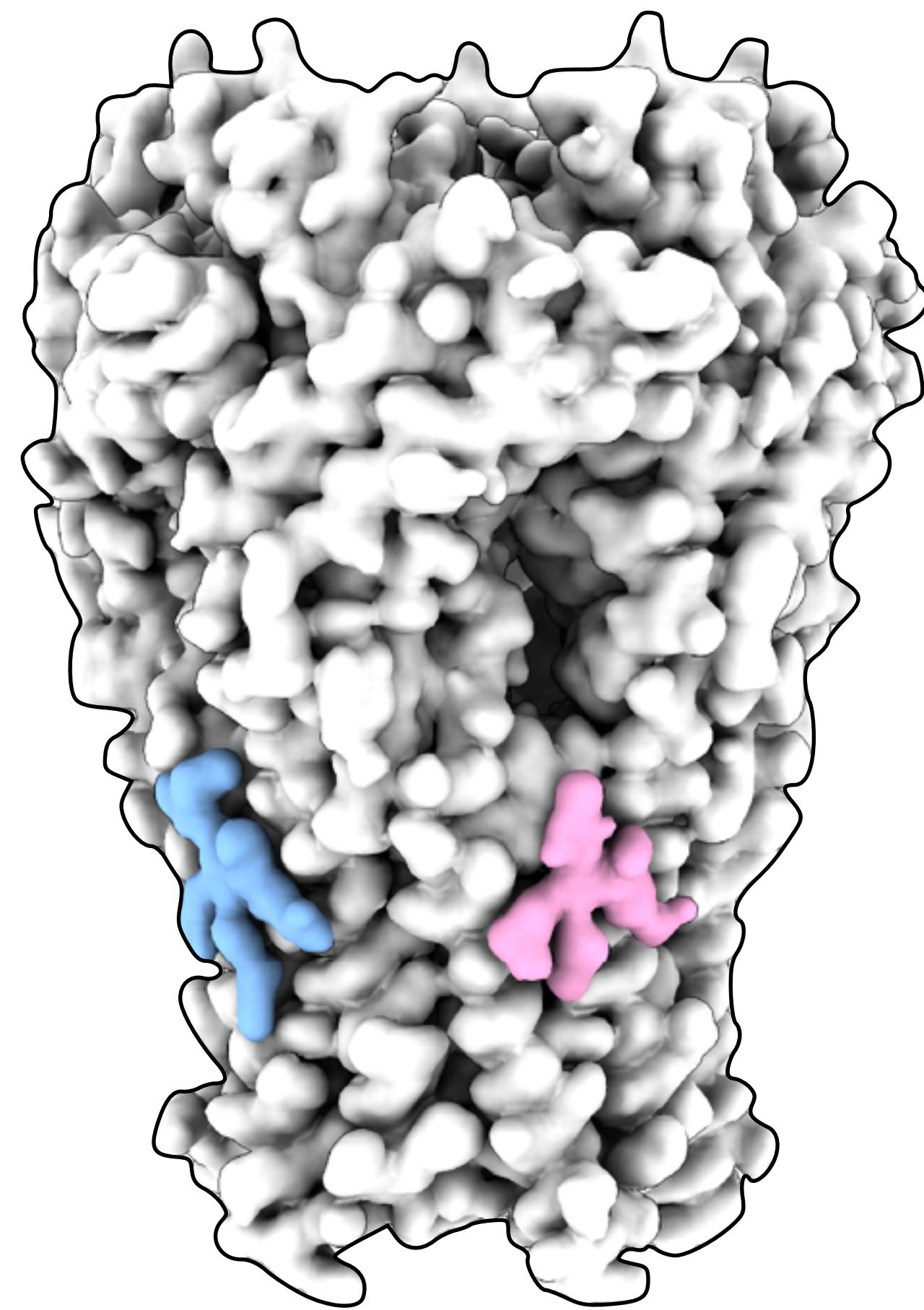
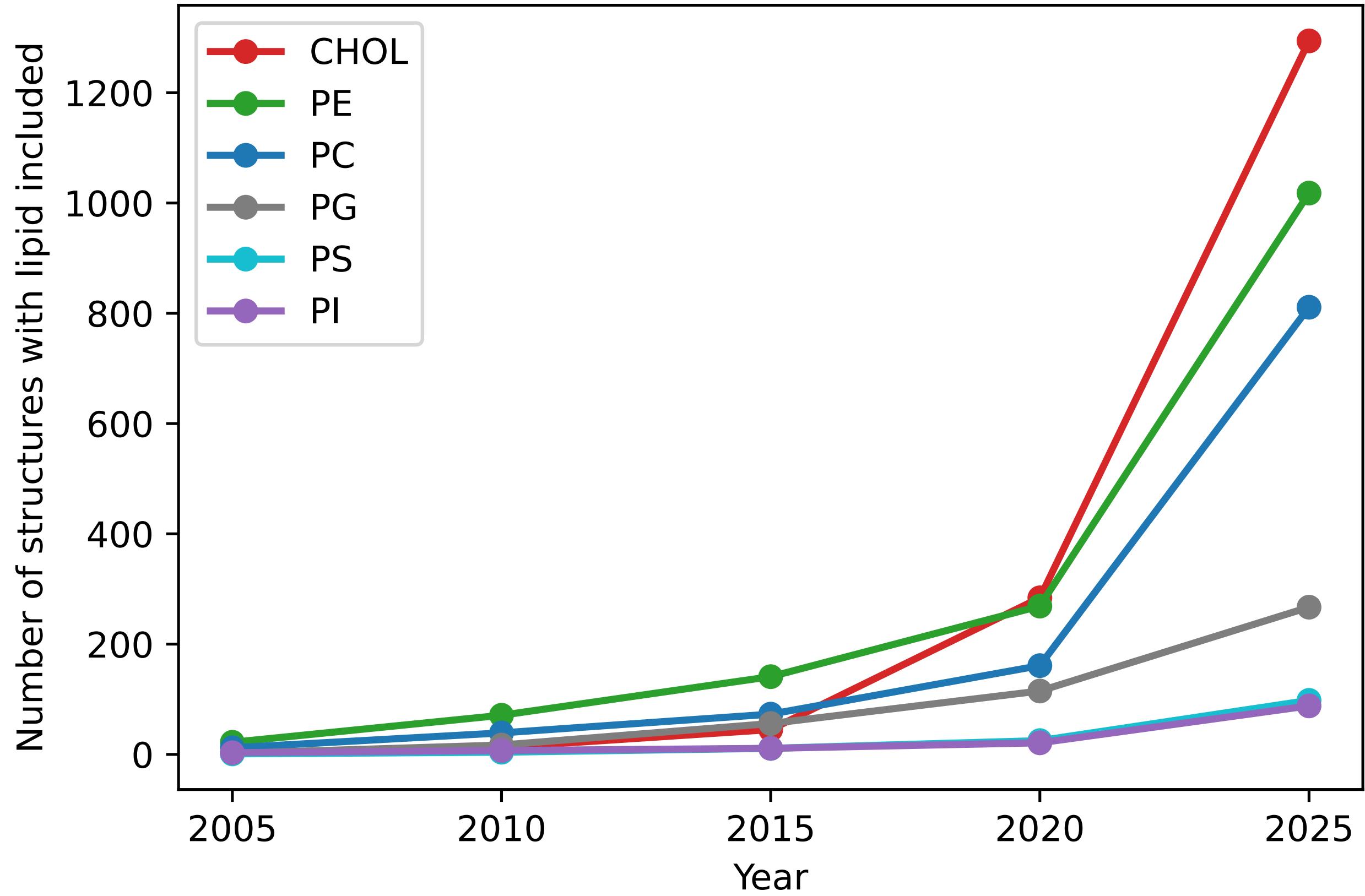
Review

The lipid habitats of neurotransmitter
receptors in brain

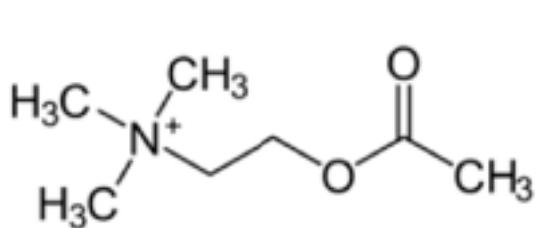
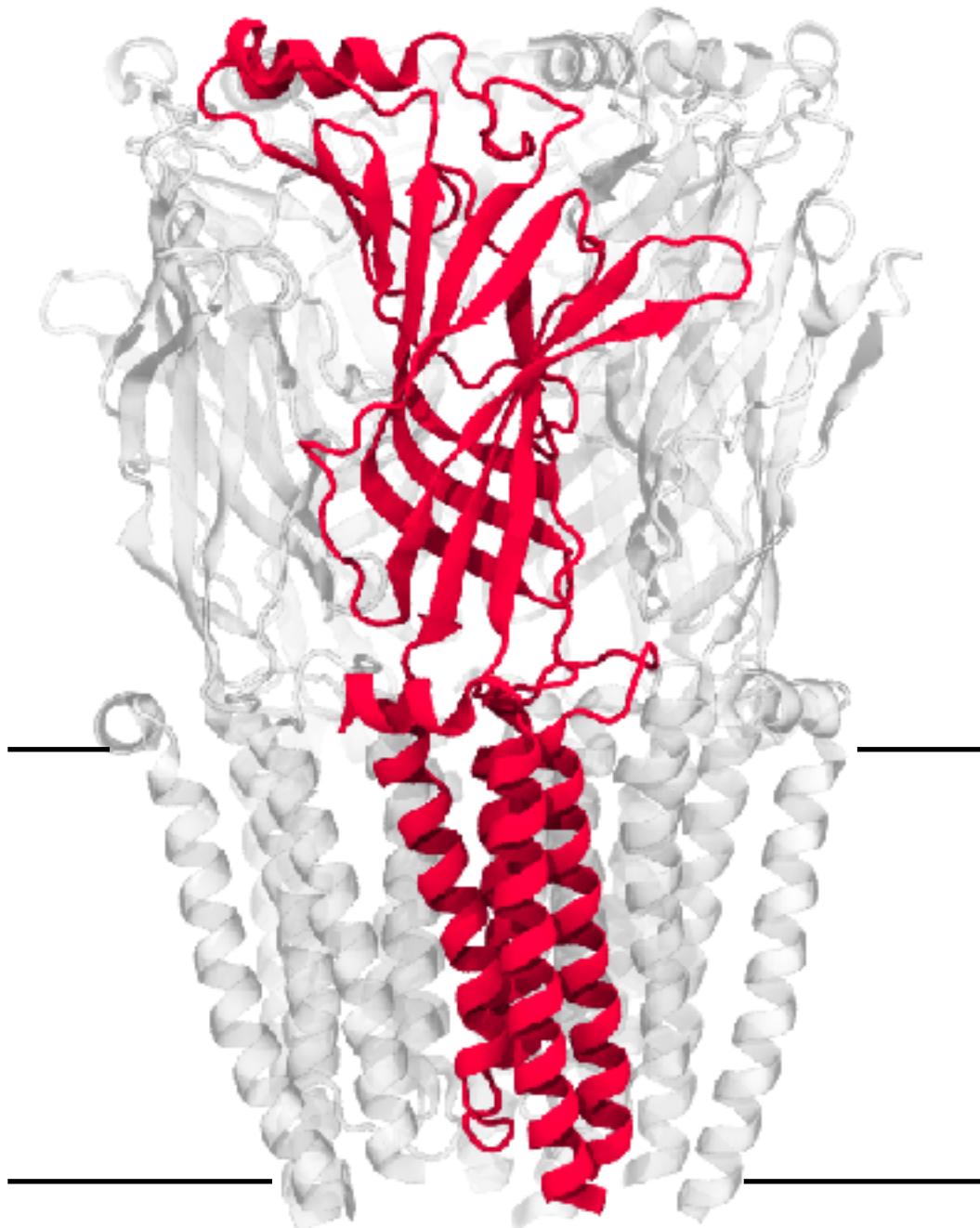
Maria Virginia Borroni ^a, Ana Sofia Vallés ^b, Francisco J. Barrantes ^c



Specific lipids bind to specific membrane proteins

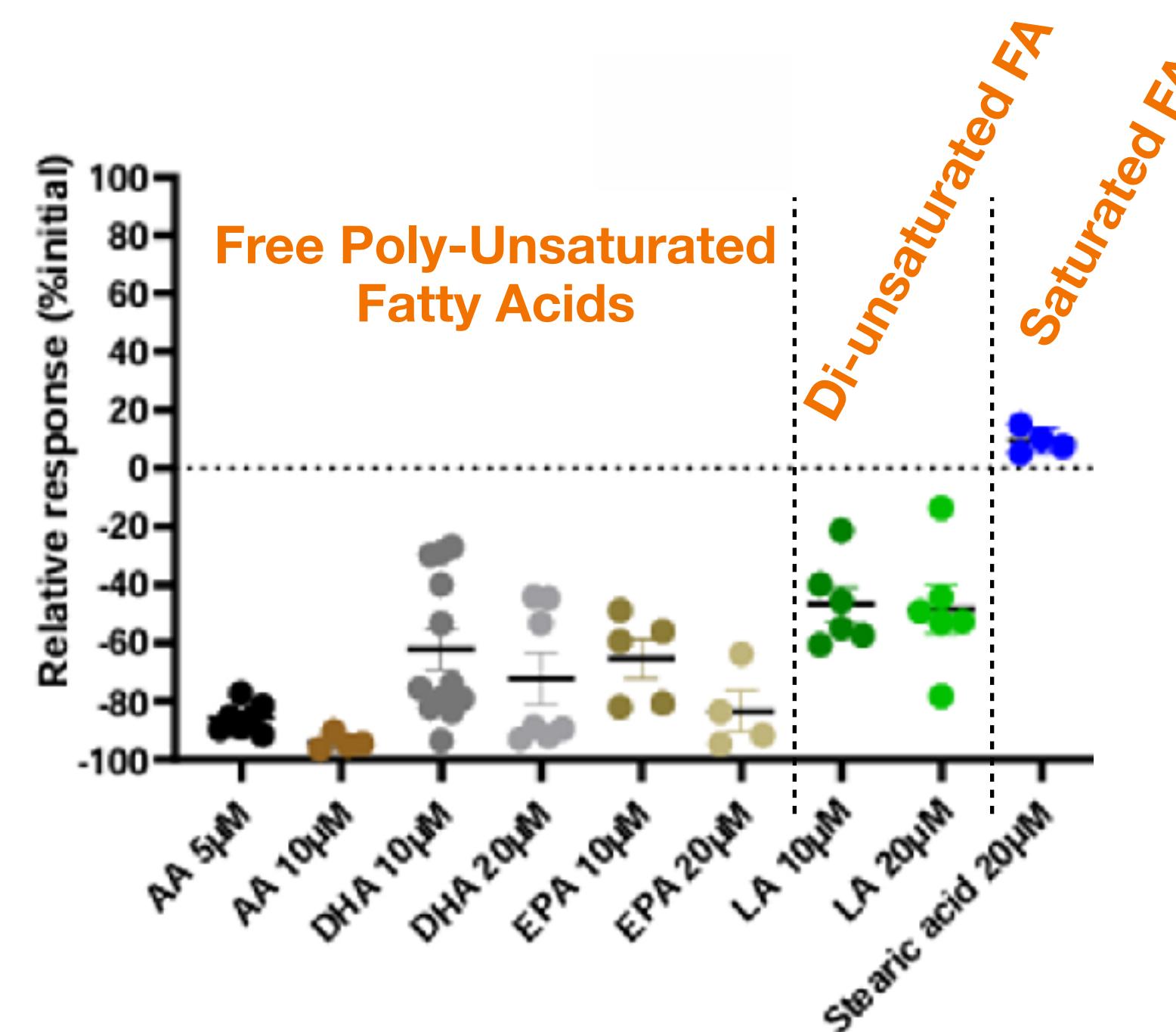


α -7 Nicotinic Acetylcholine Receptor is inhibited by fPUFAs



Nicotine

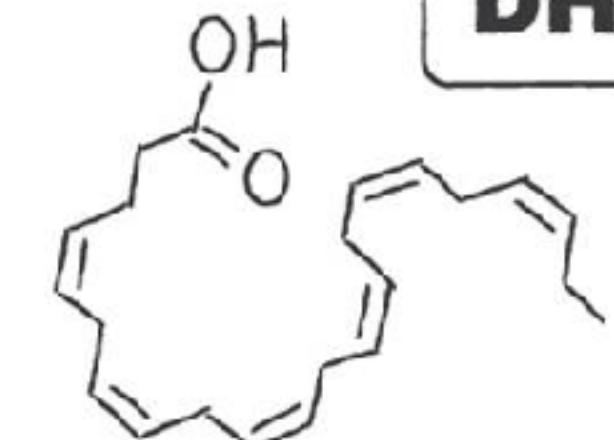
And many, many others...



Data courtesy of Corringer Lab



DHA



Docosahexaenoic Acid (DHA) (22:6, n-3)

Approach

1

**Simulate nAChR in membrane that mimics
Corringer lab experimental conditions
(*xenopus* oocyte)**

2

**Look for evidence of DHA binding to the
site they have identified from mutagenesis
and voltage clamp experiments**

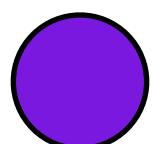
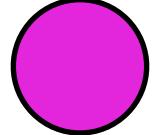
3

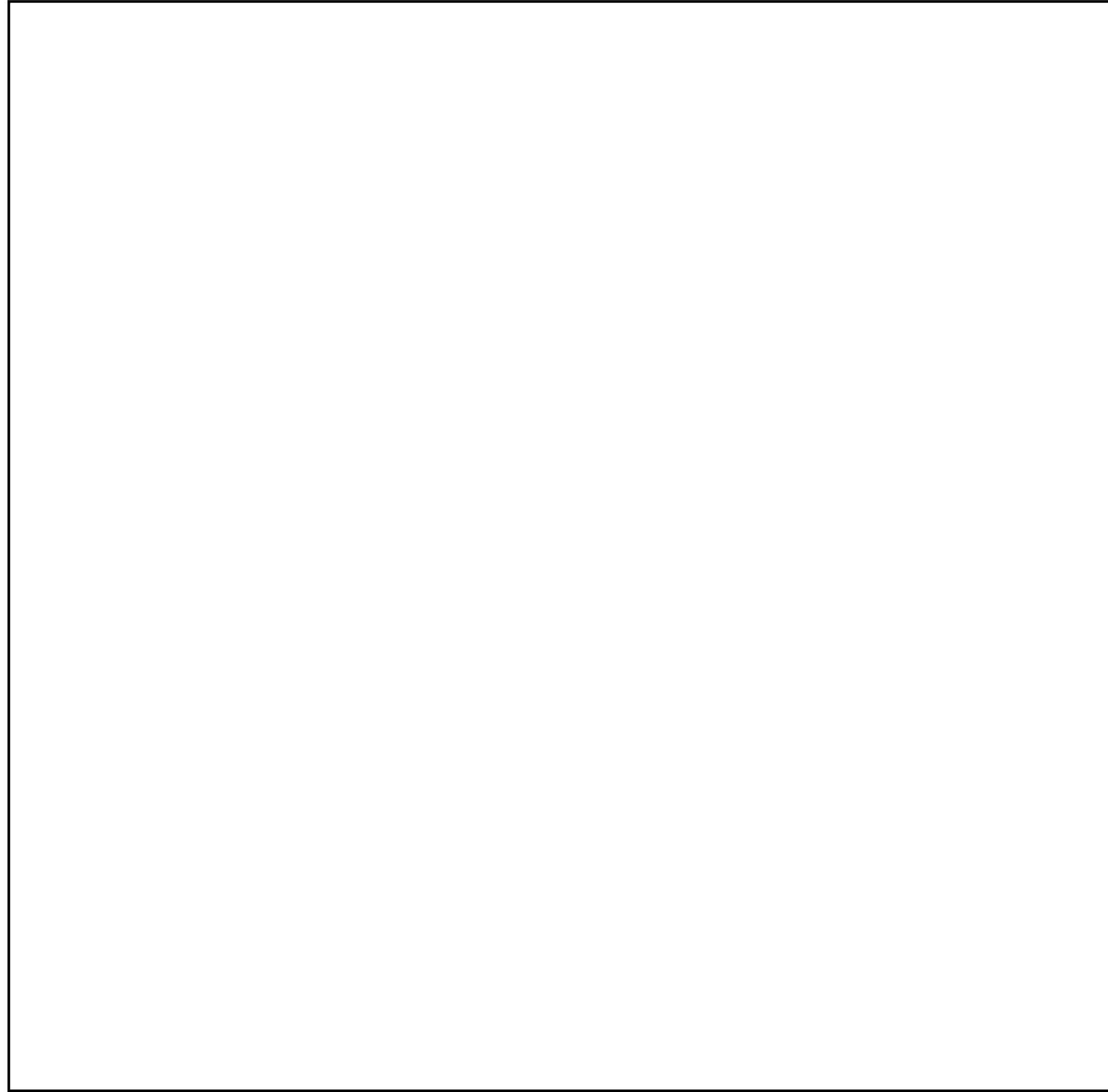
**Measure the binding affinity of
DHA to the nAChR**

nAChR embedded in 22-component membrane

Extracellular view

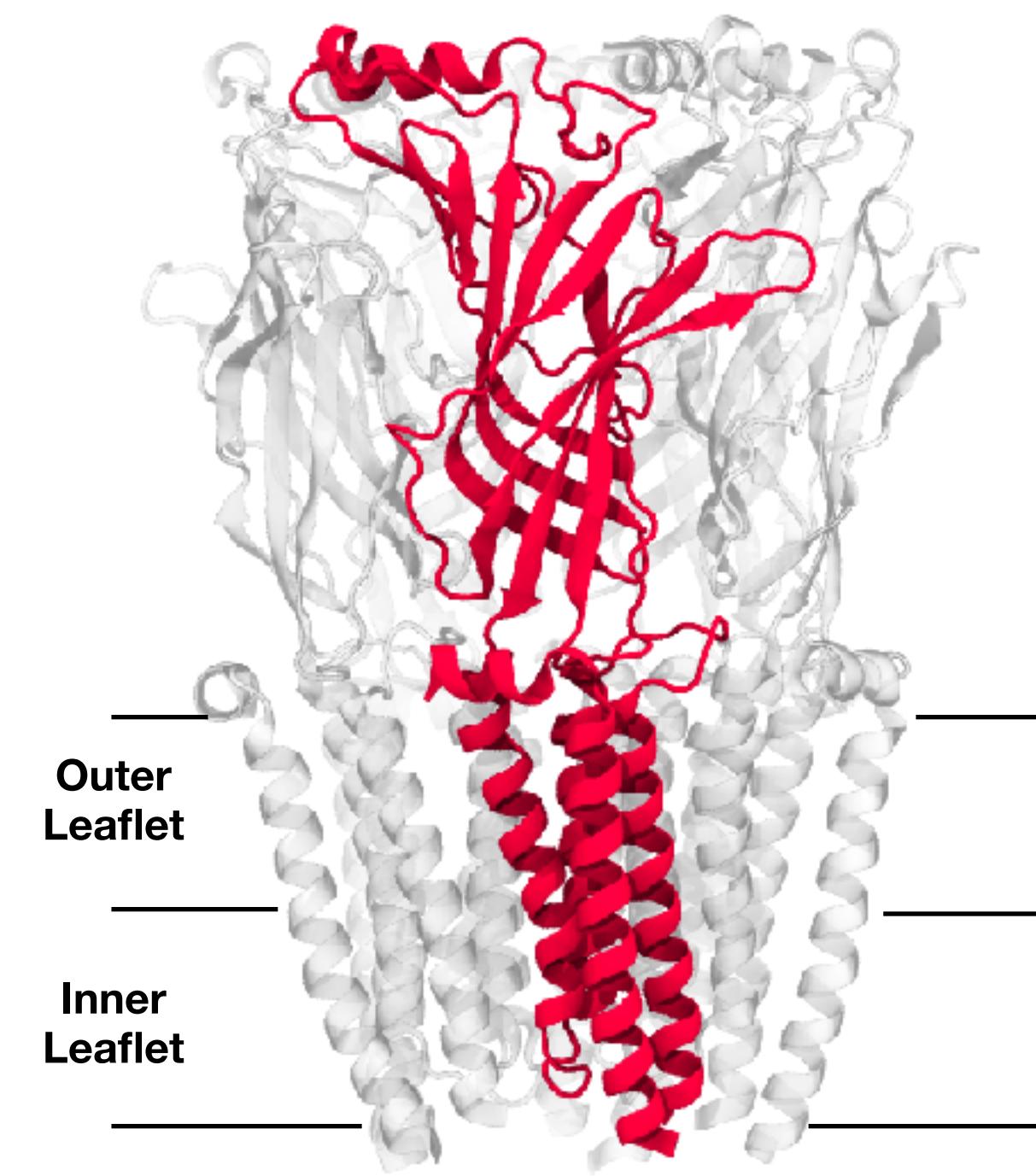
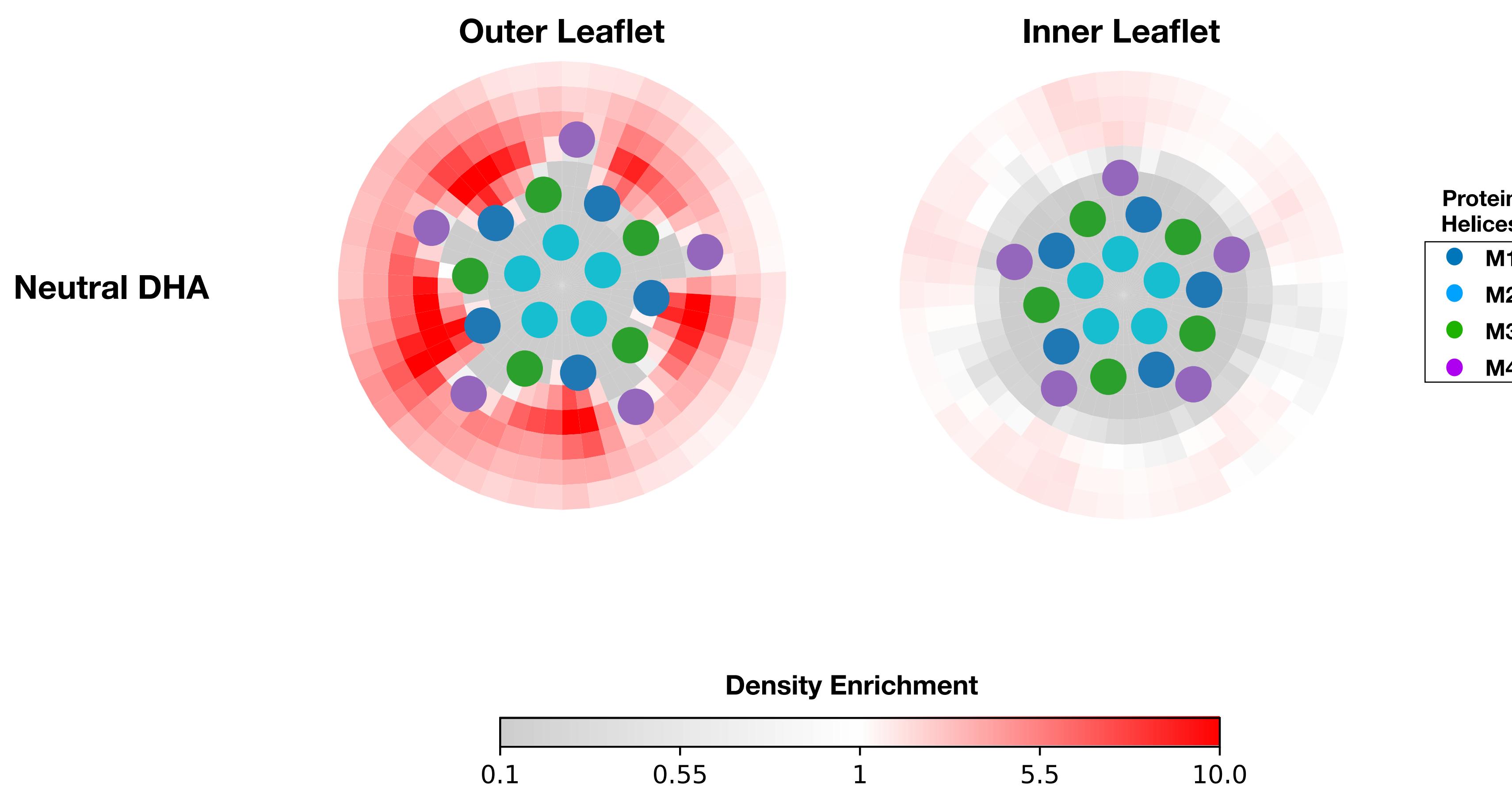
Same movie, showing DHA only

-  Neutral DHA
-  Charged DHA

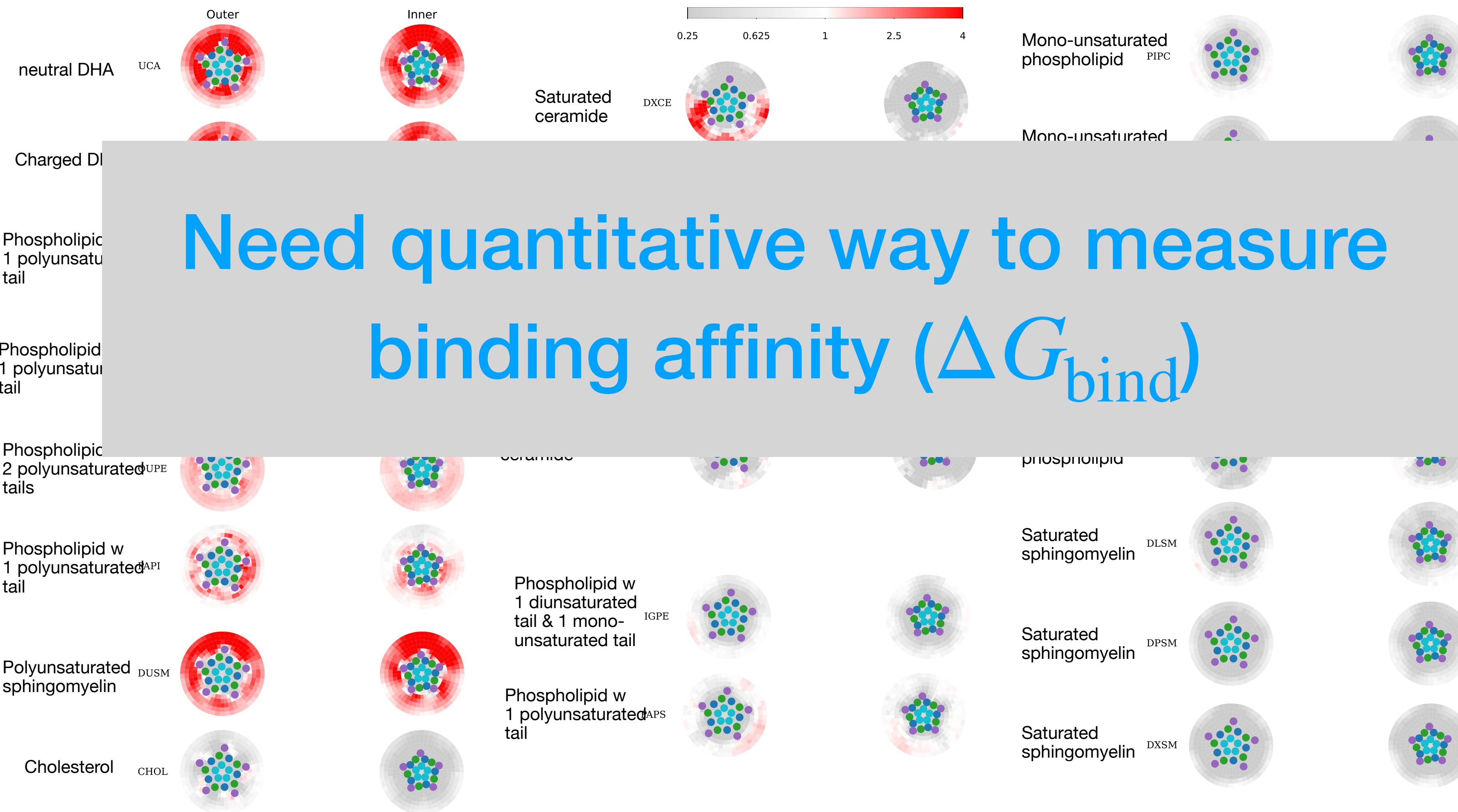


Intracellular view

DHA enriched in intersubunit site

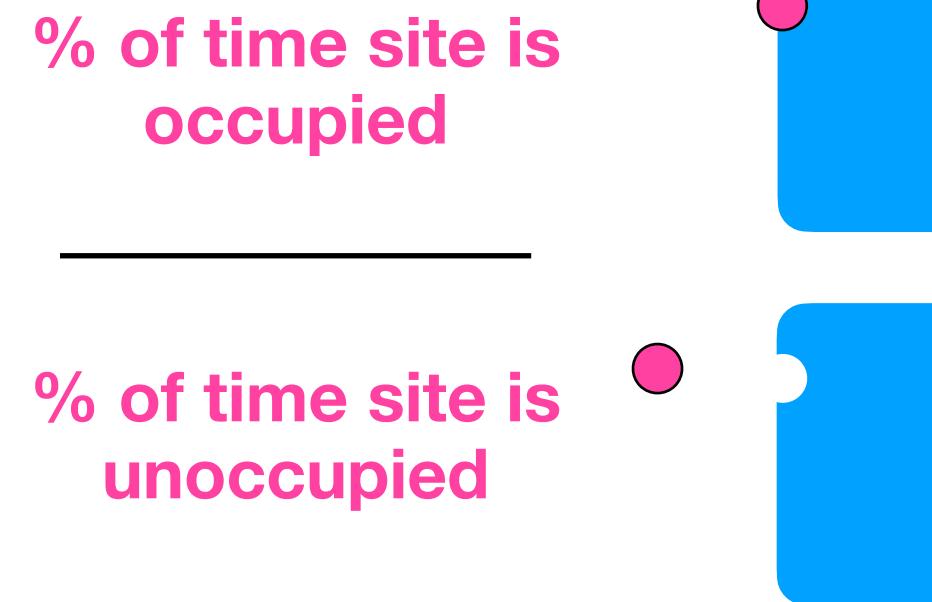


44 heat maps is too many heat maps



Conceptual hurdles to defining binding in hydrophobic contexts

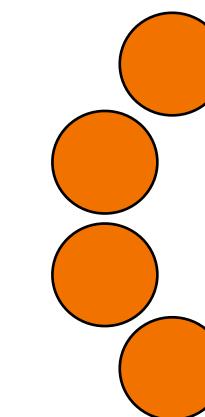
$$\Delta G_{\text{bind}} = RT \ln K_d = -RT \ln \frac{P_{\text{occ}}}{P_{\text{unocc}}}$$



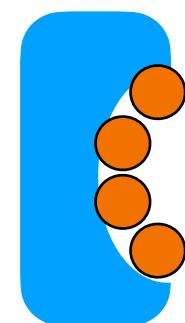
Superficial binding site



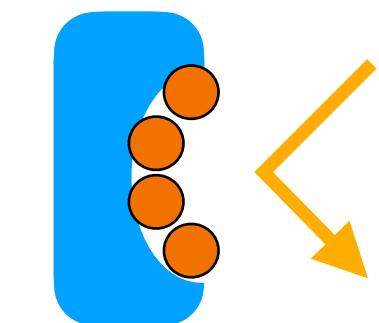
Flexible, chain-like ligand that is also the solvent



Problem 1: Ligand and/or Solvent?



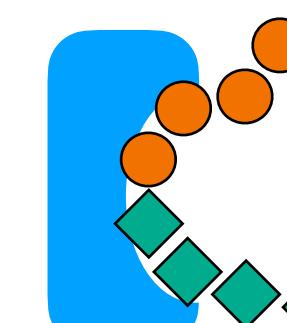
Specifically
Bound?



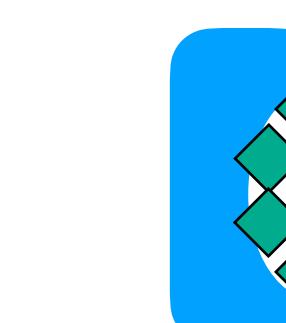
Diffusing
solvent?



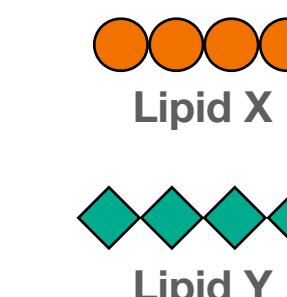
Occupied



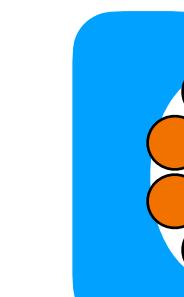
Partially
occupied?



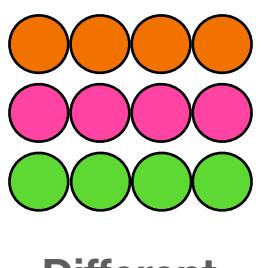
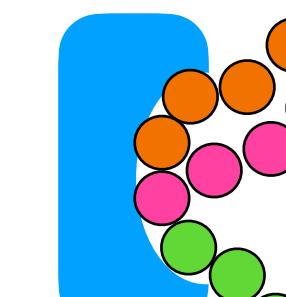
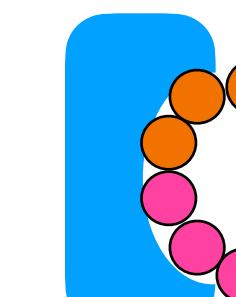
Unoccupied



Problem 3: Chemically Indistinct Ligands

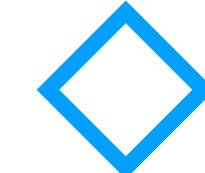


Equally valid bound configurations

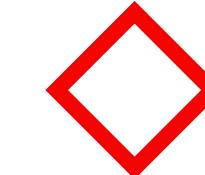


Different
molecules
of lipid X

Density-Threshold Affinity circumvents all 3 problems



Problem 1:
Ligand and/or Solvent?



Problem 2:
Partial Occupancy



Count DHA beads in designated site

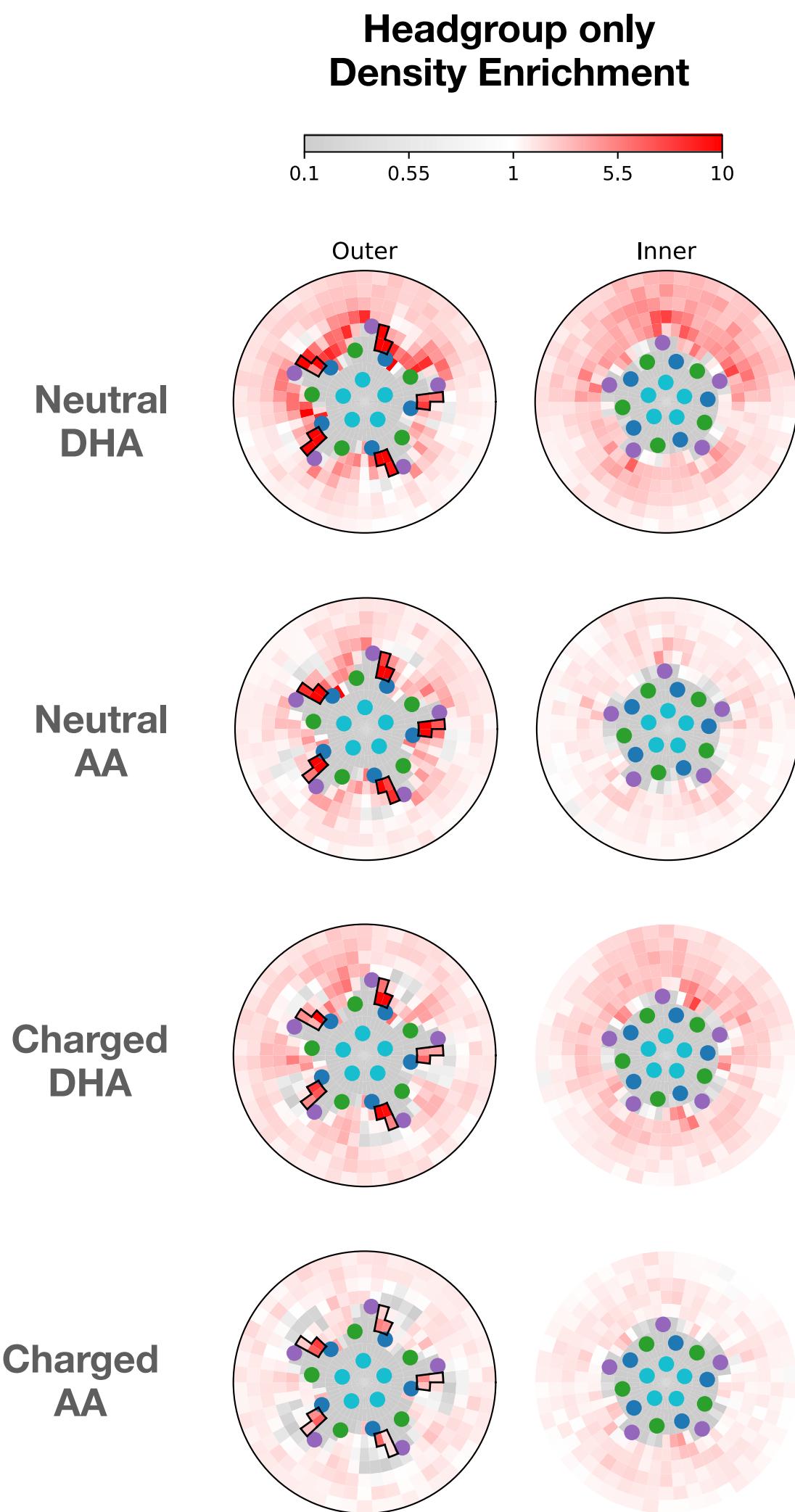
Count DHA beads in patch of “bulk” membrane

How much *more* likely am I to find DHA beads
in site rather than in bulk?

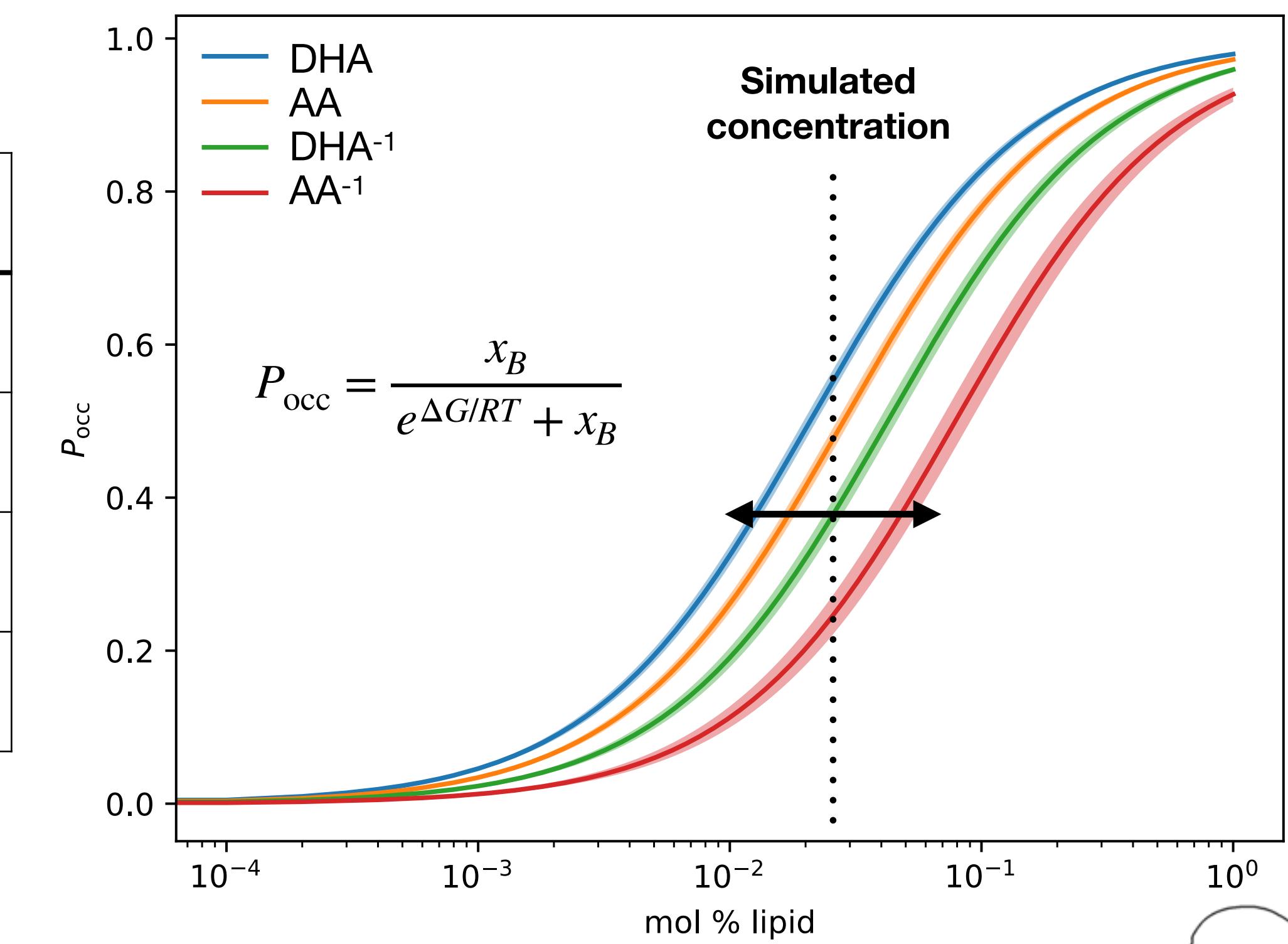
Problem 3:
Chemically Indistinct Ligands



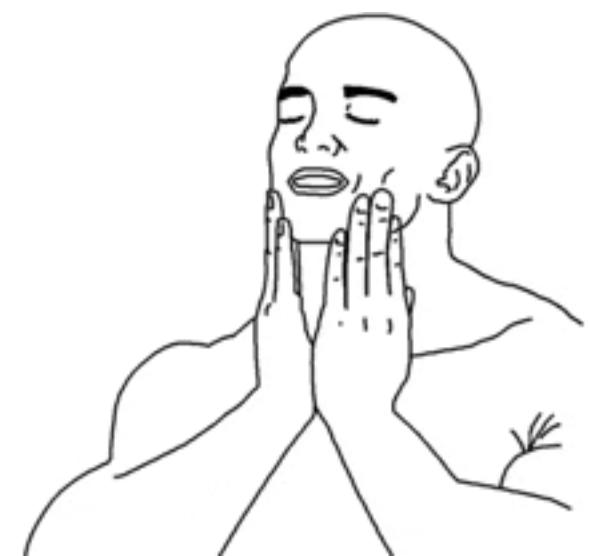
DTA allows for easy comparisons



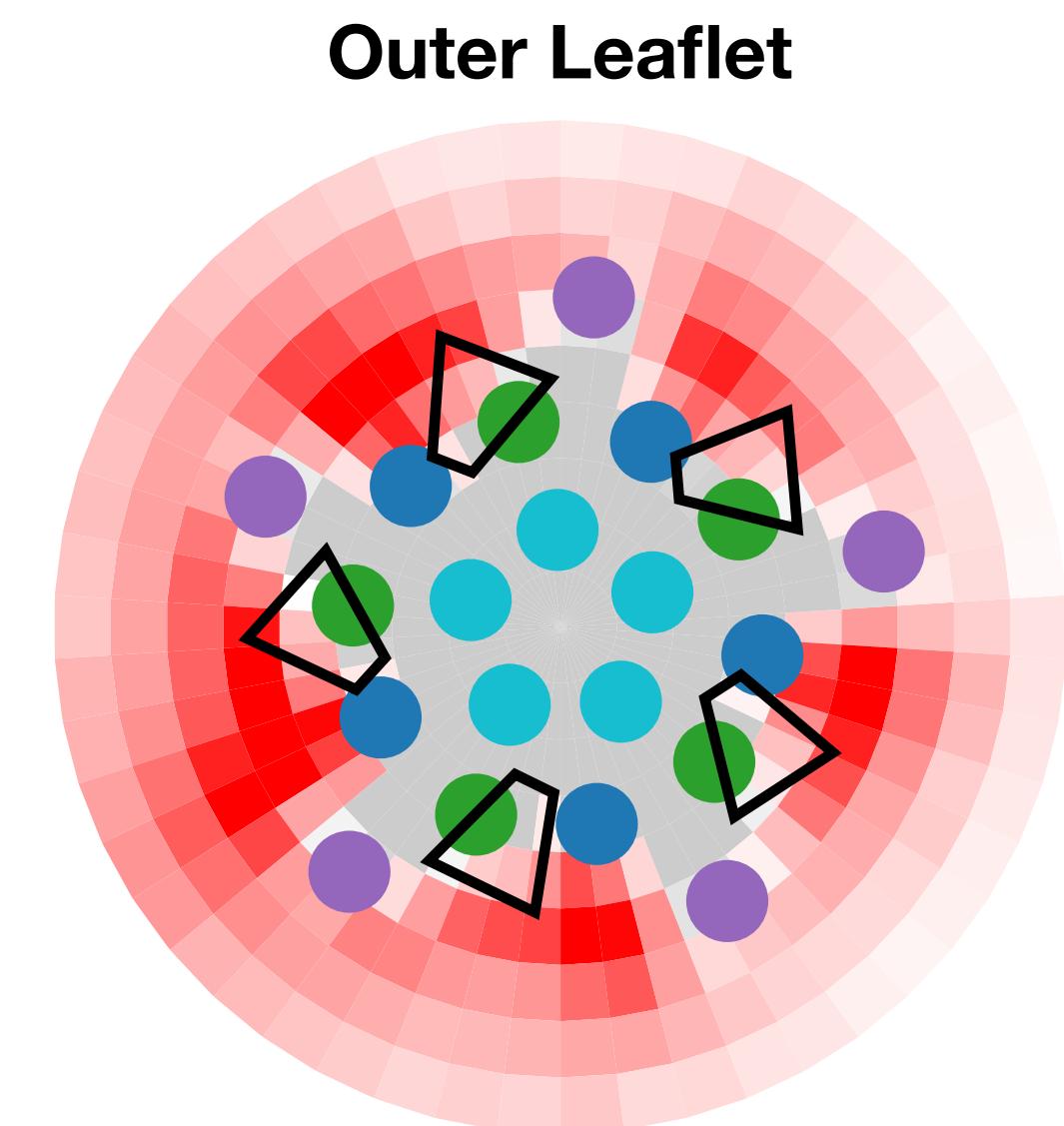
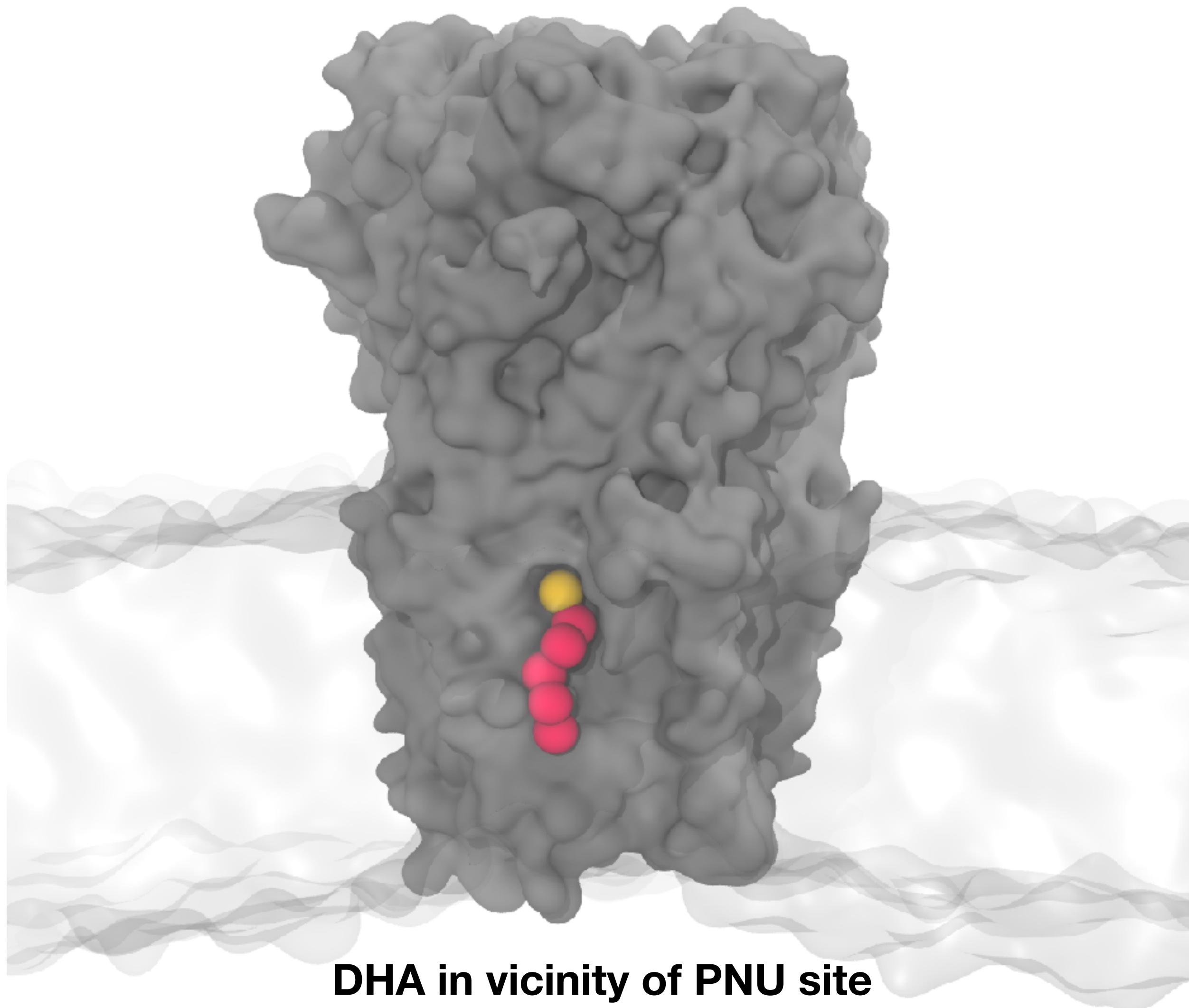
	ΔG_{bind} (kcal/mol)
Neutral DHA	-2.5 +/- 0.1
Neutral AA	-2.3 +/- 0.1
Charged DHA	-2.0 +/- 0.1
Charged AA	-1.6 +/- 0.2



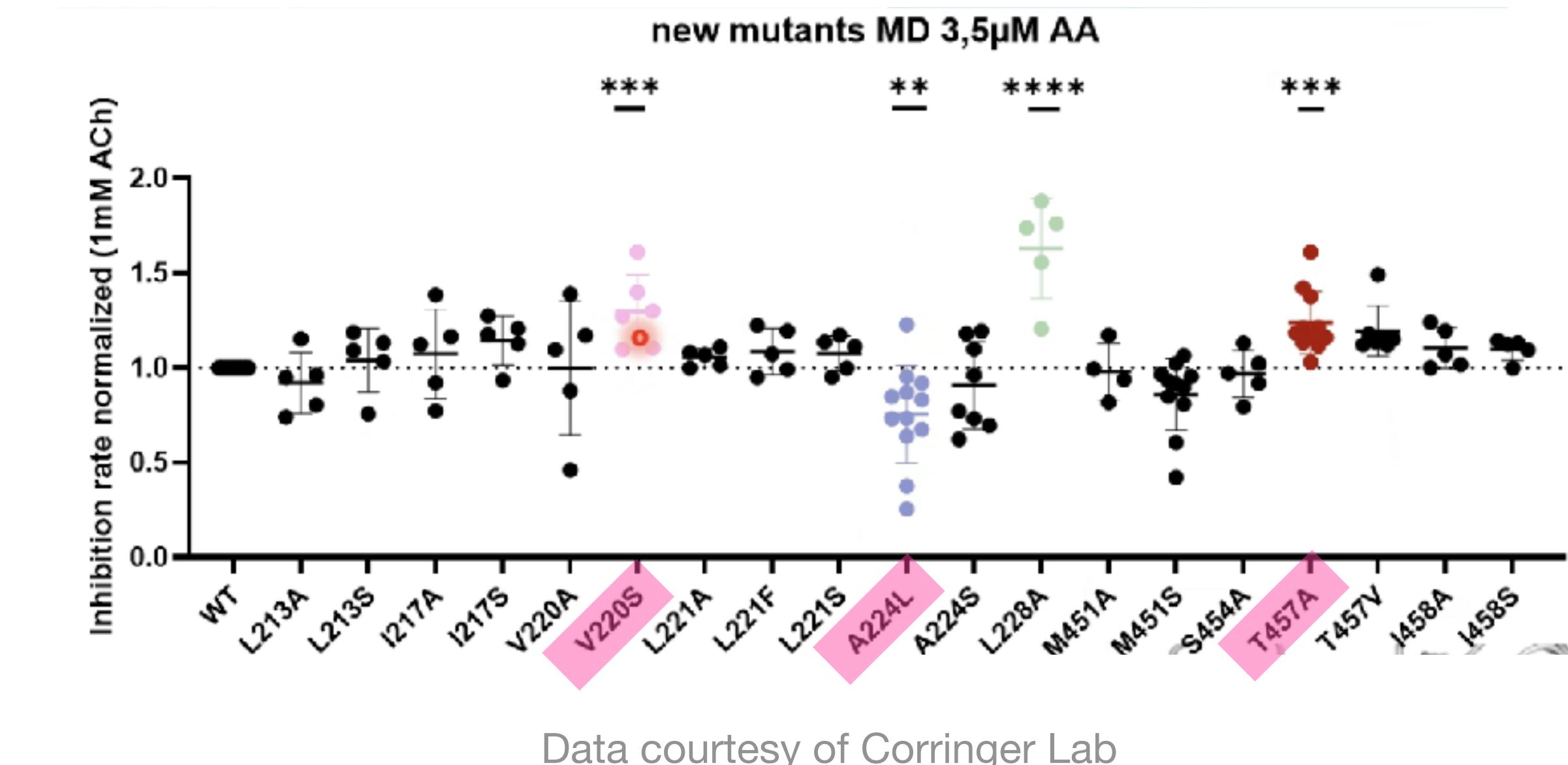
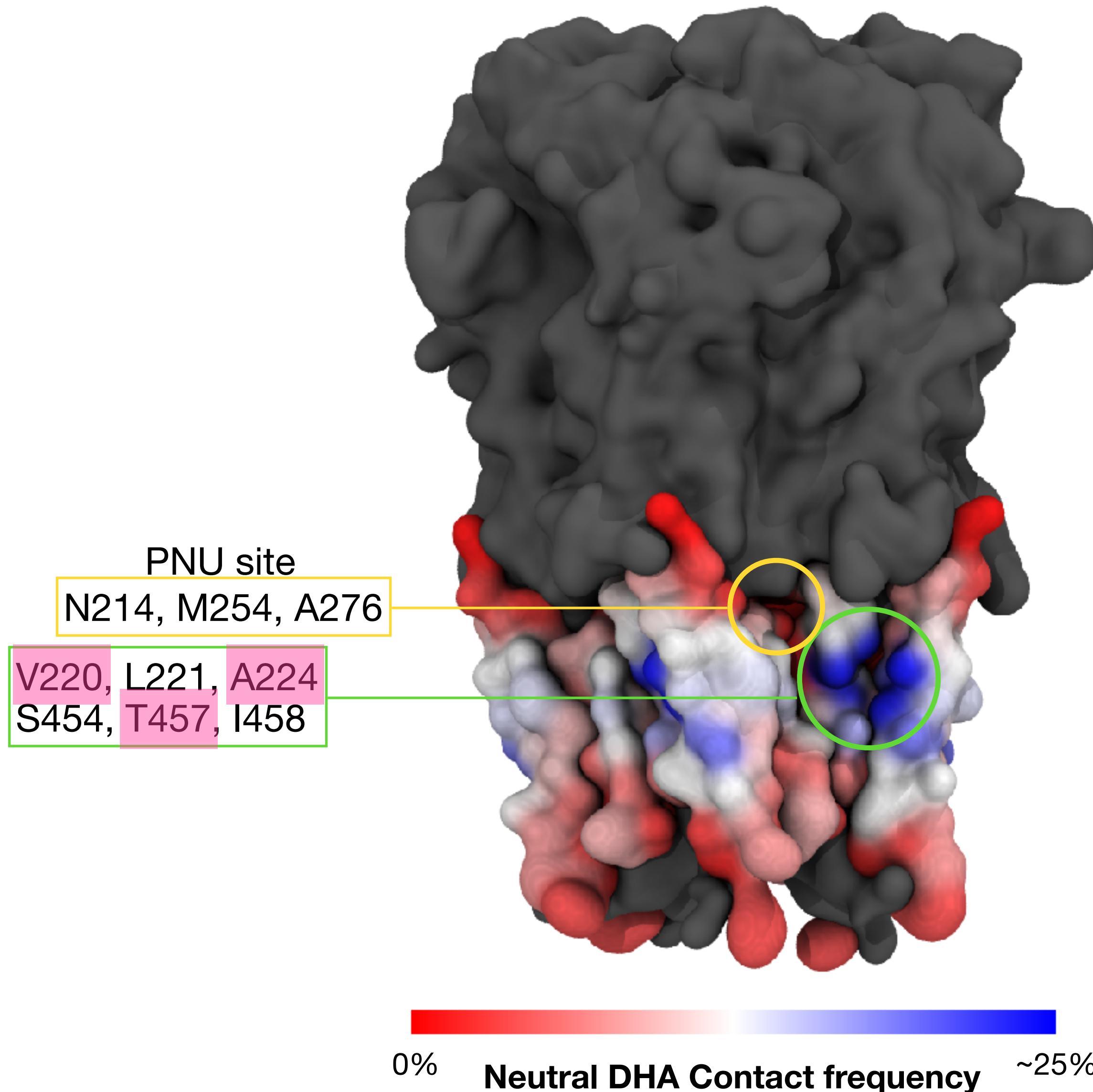
One figure instead of 8 heat maps!

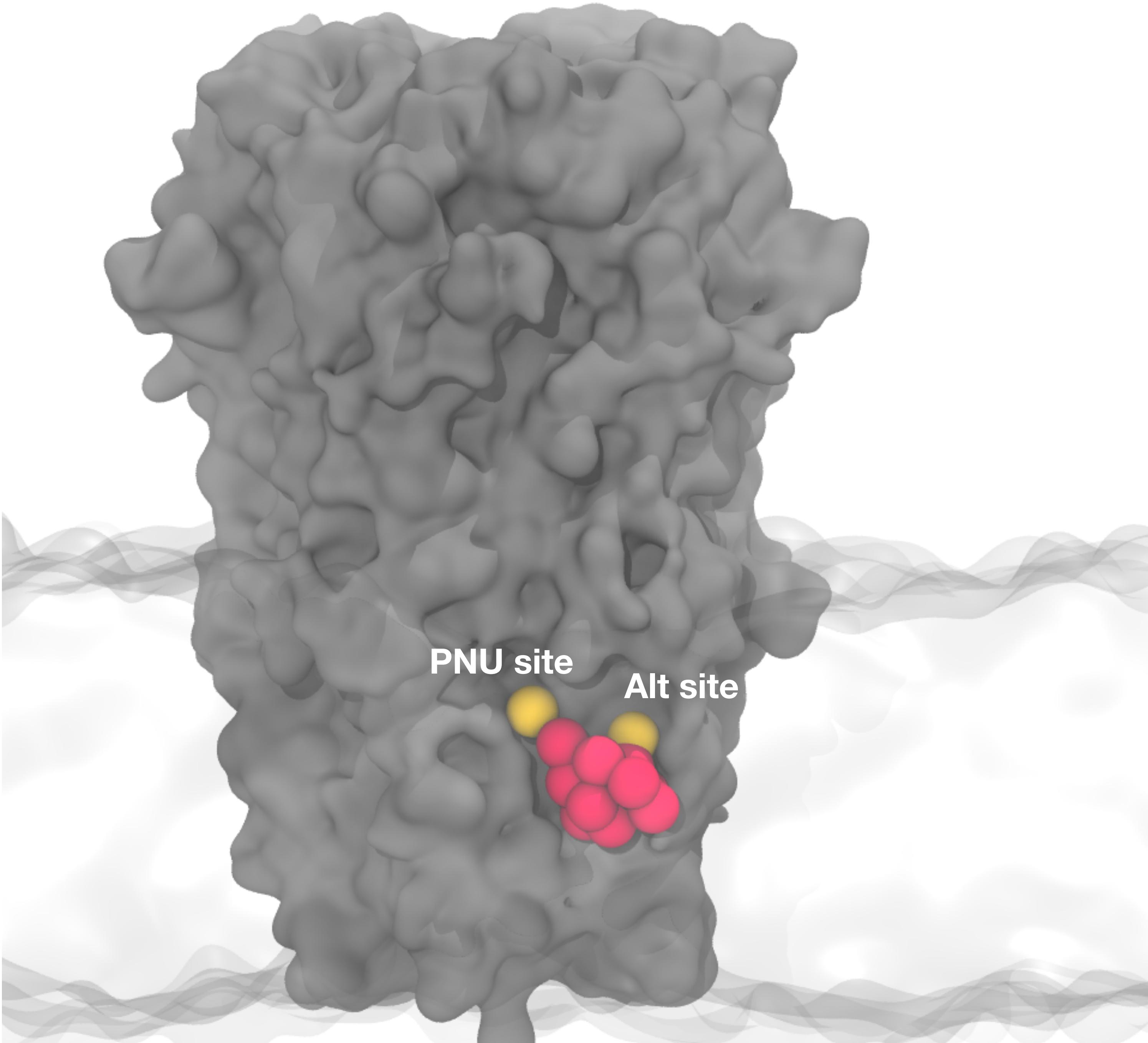


“Can you measure the PNU site?”



Contact frequency indicates potential alternate site





Conclusion

- nAChR binds fPUFAs in two distinct intersubunit sites
- We computationally predicted a functionally significant additional binding site
- We are currently measuring the ΔG_{bind} of DHA to both the PNU site as well as the additional site we identified
- Density-Threshold Affinity is an experimentally-validated method for calculating binding affinities from unbiased CG-MD membrane simulations

Problem 1:
Ligand and/or Solvent?



Problem 2:
Partial Occupancy



Problem 3:
Chemically Indistinct Ligands



Thank you!

Dr. Grace Brannigan

Dr. Liam Sharp

Ezry Santiago-McRae

Jahmal Ennis

Connor Pitman

Lindsey Riggs

Regina Salzer

Ryan Lamb

Asim Dave



Dr. Pierre-Jean Corringer

Dr. Nour Awad

Dr. Marie Prevost

Nathalie Barilone



Read our protocol paper!

