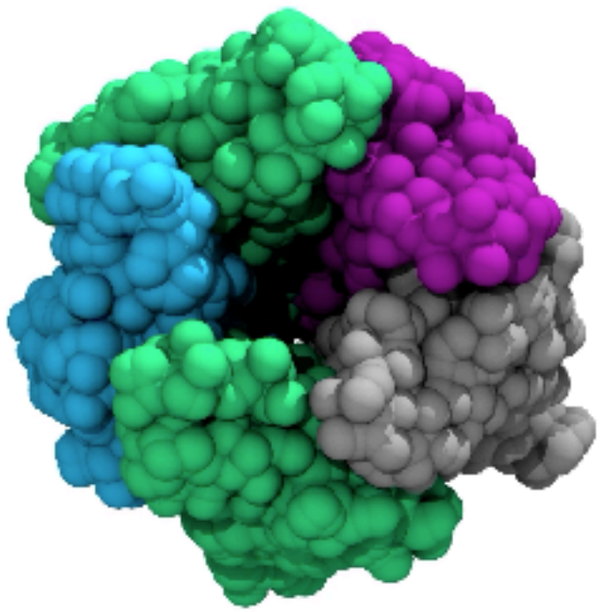
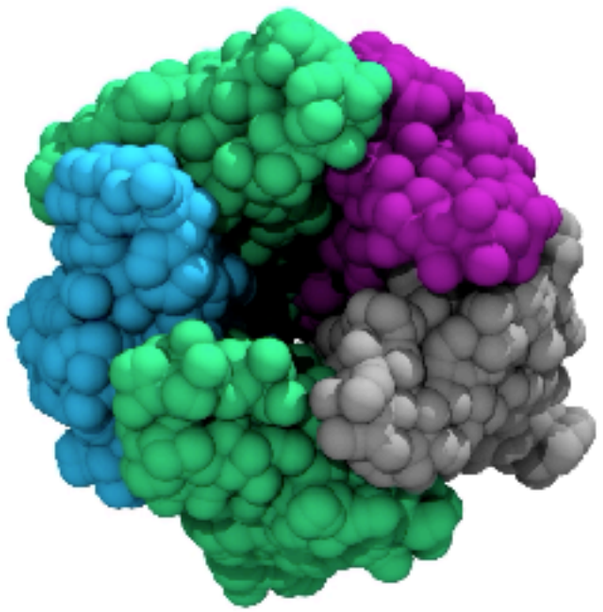


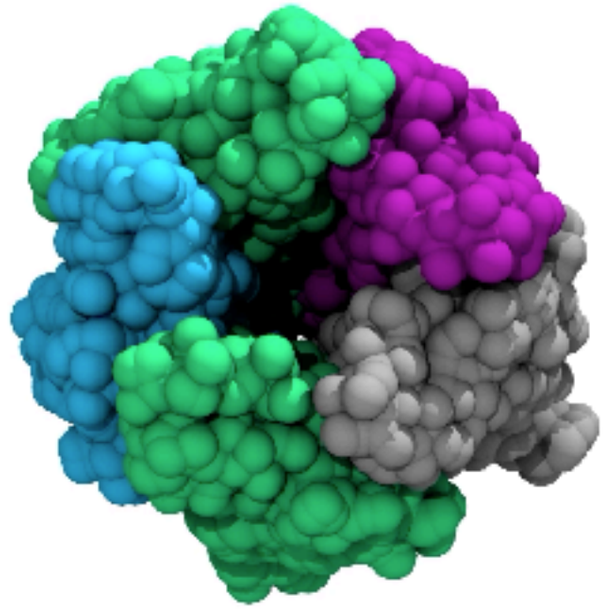
Committee Meeting 6/27/2019



Committee Meeting 6/27/2019

Nicotinic Acetylcholine Receptor

- Excitatory pentameric ligand gated ion channel gated by binding of acetylcholine and nicotine
    - Contributes to neuronal and muscular function by stimulating an action potential across post-synaptic membrane
- Found through out the central and peripheral nervous system
- Roll in neurological and physical disorders as well as addiction



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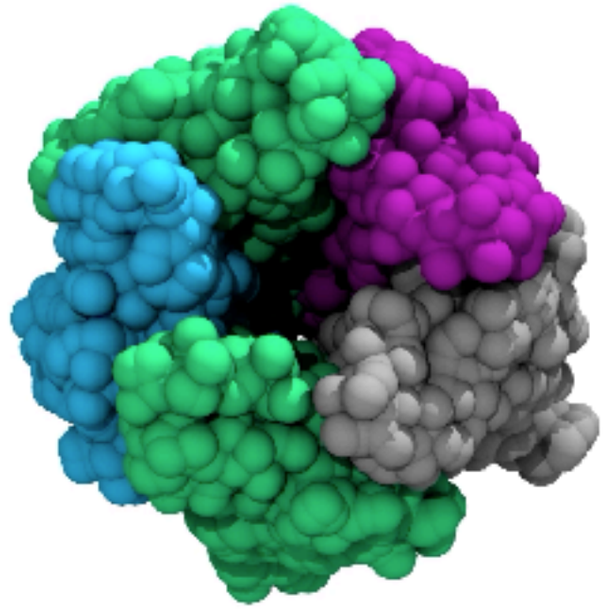


Background

- Little information on nicotinic acetylcholine receptor (nAChR)-lipid interactions in **post synaptic-like membranes**
- **Previous hypothesis**: nAChR is functional dependency on cholesterol suggesting nAChR partitions into cholesterol (CHOL) enriched domain
    - Marchand (2002), Zhu (2006), Campagna (2006) show nAChR clustering is dependent on lipid rafts

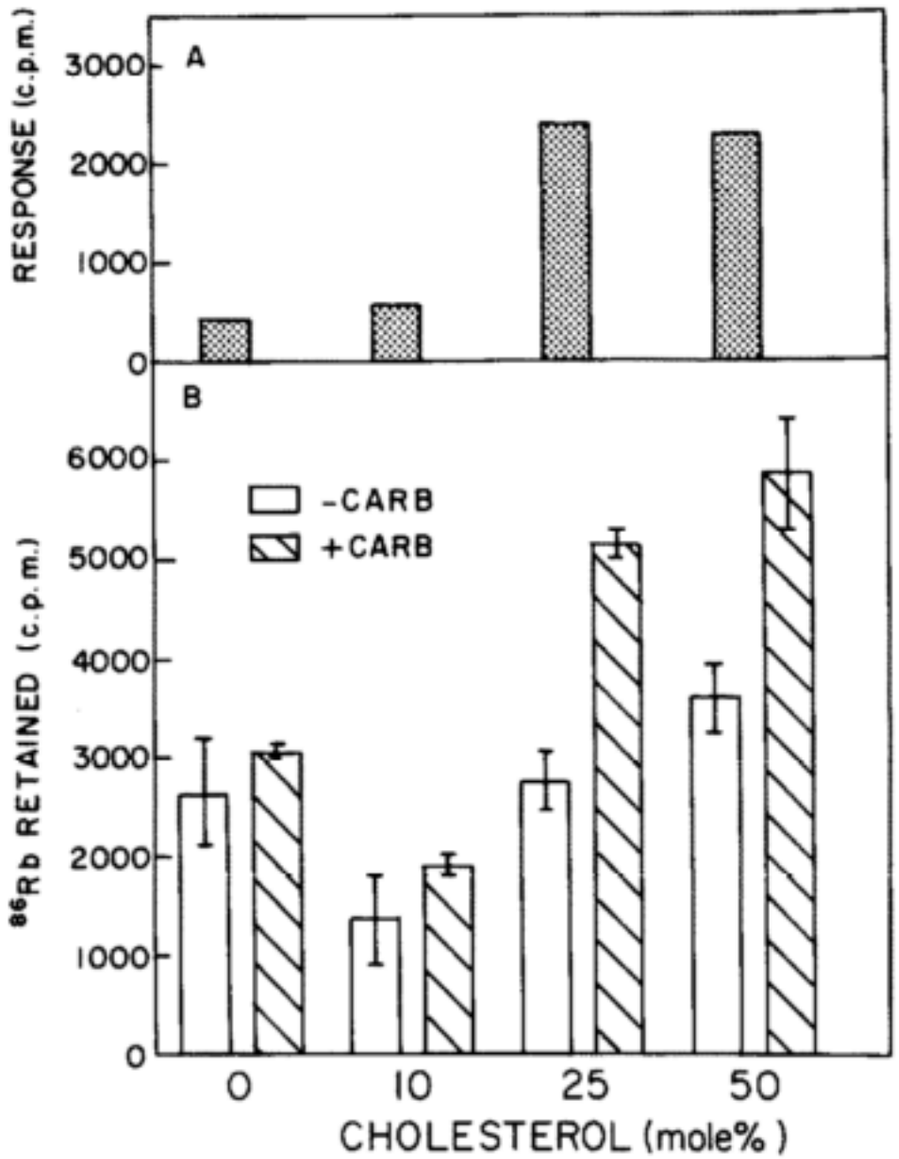


Fig.1. Effect of supplementing AChR-reconstituted vesicles with cholesterol on agonist-induced $^{86}Rb^{+}$ influx. (A) $^{86}Rb^{+}$ influx response to carb. (cpm(+ carb.)−cpm(− carb.)). (B) Total counts retained by Millipore filters. Counts obtained by filtering an equivalent amount of $^{86}Rb^{+}$ in the absence of membranes has been subtracted. The error bars indicate ± 1 SD (N = 4). The results are representative of 2 replicate reconstitution experiments.

nAChR in Experimental Membranes

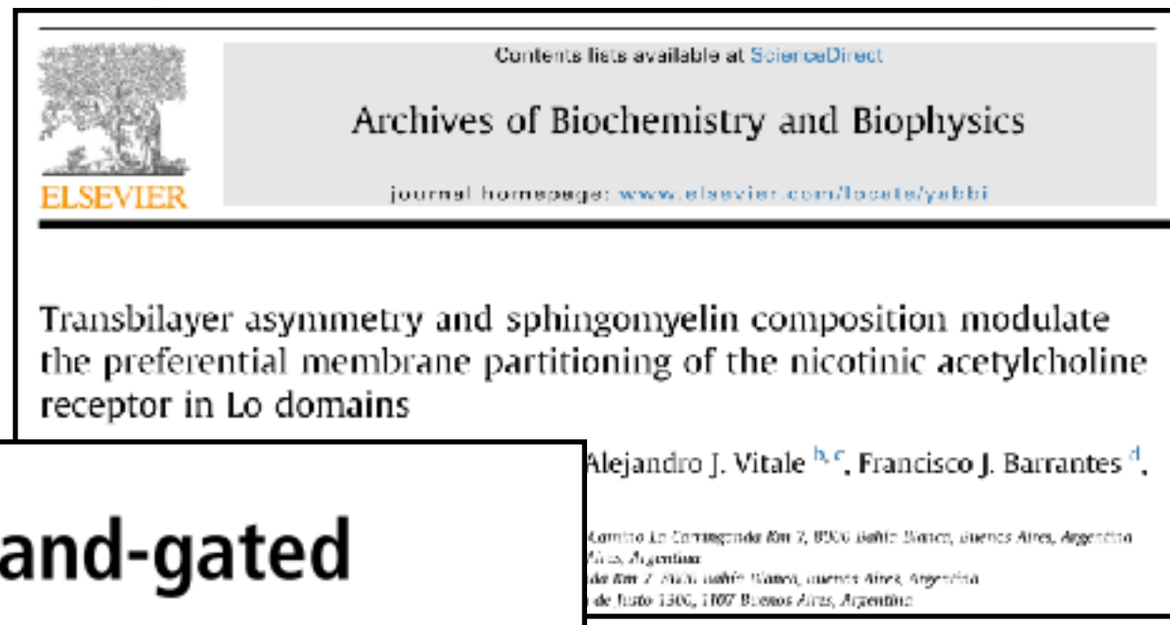
- nAChR tends to be placed in non-native membranes
  - Xenopus Oocyte
  - Experimental and computation membranes of DOPC/POPC
- Essentially: ***missing Polyunsaturated Fatty Acids (PUFAs)***

ASBMB

Partition profile of the nicotinic acetylcholine receptor in lipid domains upon reconstitution*

Vicente Bermúdez, Silvia S. Antollini,[1] Gaspar A. Fernández Nievas, Marta I. Aveldaño, and Francisco J. Barrantes

Instituto de Investigaciones Bioquímicas de Bahía Blanca, Consejo Nacional de Investigaciones Científicas y Técnicas, and UNESCO Chair of Biophysics and Molecular Neurobiology, Universidad Nacional del Sur, Bahía Blanca, Argentina



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Transbilayer asymmetry and sphingomyelin composition modulate the preferential membrane partitioning of the nicotinic acetylcholine receptor in Lo domains

Alejandro J. Vitale [b,c], Francisco J. Barrantes [d],

Camino La Carrindela Km 7, 8300 Bahía Blanca, Buenos Aires, Argentina
Aires, Argentina
de Km 7, 8300 Bahía Blanca, Buenos Aires, Argentina
de Julio 1305, 1107 Buenos Aires, Argentina

A gating mechanism of pentameric ligand-gated ion channels

Nicolas Calimet[a], Manuel Simoes[a], Jean-Pierre Changeux[b,c,1], Martin Karplus[a,d], Antoine Taly[e], and Marco Cecchini[a,1]

[a]Institut de Science et d'Ingénierie Supramoléculaires, Unité Mixte de Recherche 7006, Centre National de la Recherche Scientifique, Université de Strasbourg, F-67083 Strasbourg Cedex, France; [b]Centre National de la Recherche Scientifique, Unité de Recherche Associée 2182, F-75015 Paris, France; [c]Collège de France, F-75005 Paris, France; [d]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138; and [e]Institut de Biologie Physico-Chimique, Unité Propre de Recherche 9080, Centre National de la Recherche Scientifique, Université Paris 7-Diderot, 75005 Paris, France

Contributed by Jean-Pierre Changeux, July 29, 2013 (sent for review June 1,

Internal Dynamics of the Nicotinic Acetylcholine Receptor in Reconstituted Membranes†

John E. Baenziger,* Tim E. Darsaut, and Mary-Louise Morris

Department of Biochemistry, Microbiology, and Immunology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

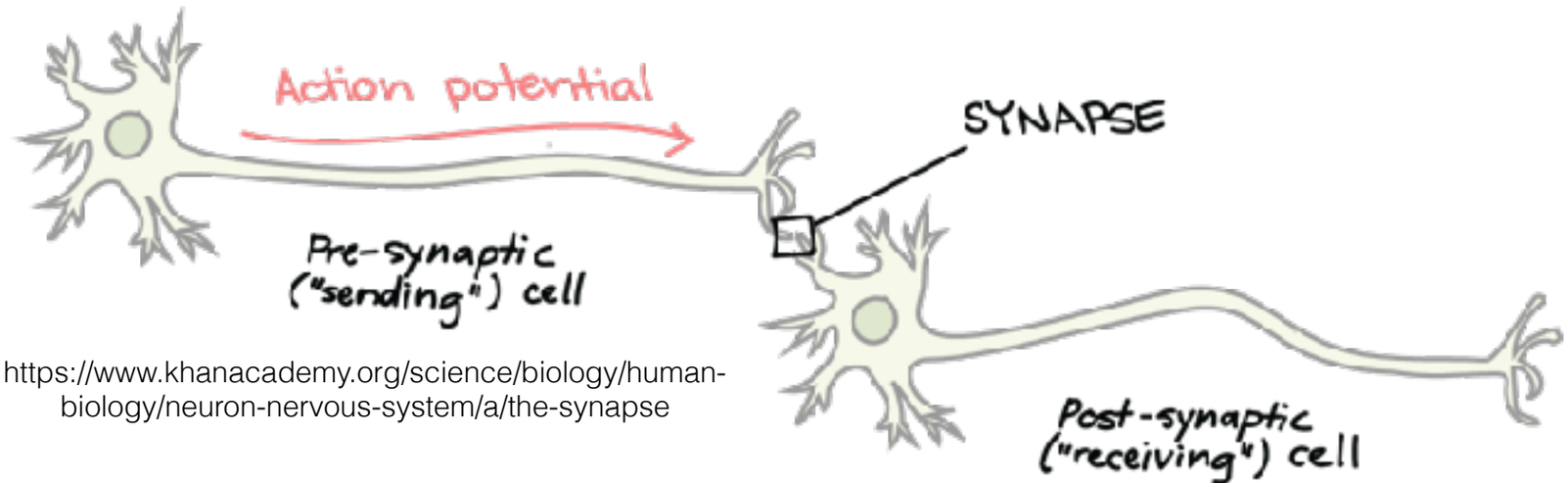
Received January 26, 1999; Revised Manuscript Received March 8, 1999

nAChR and Membrane Compositions

- Native nAChR membranes
  - *Torpedo* (similar to neuromuscular junction)

http://www.elasmodiver.com/MarbledTorpedoRay.htm

- Synaptic



https://www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/a/the-synapse

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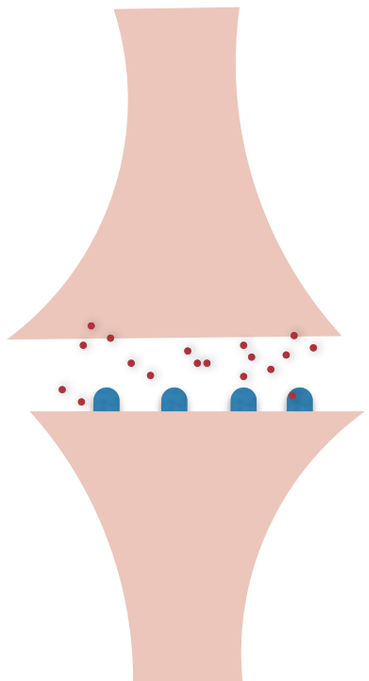

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JMN (Online)ISSN 1559-1166
DOI 10.1385/JMN/30:1-2:5

ORIGINAL ARTICLE

Nicotinic Acetylcholine Receptor Properties are Modulated by Surrounding Lipids

An In Vivo Study

Andrés Morales,*[*,1] Emilio de Juan,[1] Asia M. Fernández-Carvajal,[2] José Martinez-Pinna,[1] Juan Antonio Poveda,[2] José A. Encinar,[2] Isabel Ivorra,[1] and José Manuel González-Ros[2]

Addition of soybean lipids

Addition of synapse

Reference: *Biol. Bull.* **224:** 47–52. (February 2013)

Microtransplantation of Cellular Membranes From Squid Stellate Ganglion Reveals Ionotropic GABA Receptors

LUCA CONTI[1,2,*,†], AGENOR LIMON[1,3,*,†], ELEONORA PALMA[2,4], AND RICARDO MILEDI[1,3]

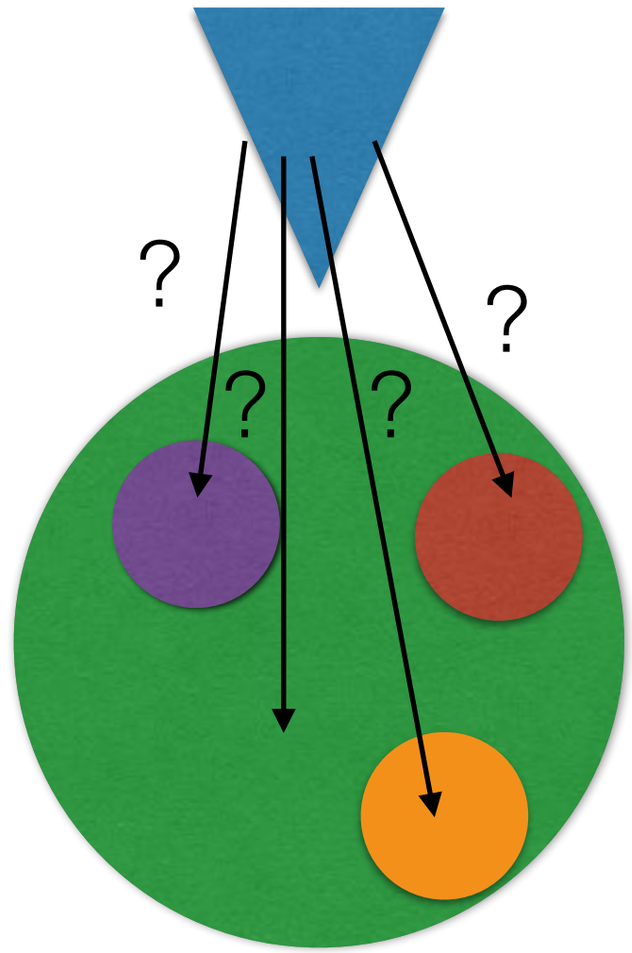
Previous Work

Main Question

- Where does nAChR partition within a native-like membrane with well-defined domains?

- Do nAChR "boundary lipids" just reflect the preferred domain composition, or are there additional preferences for specifically bound lipids?

- If so, what are the preferred boundary lipids, and can they bind so they are not even interacting with the surrounding domain?



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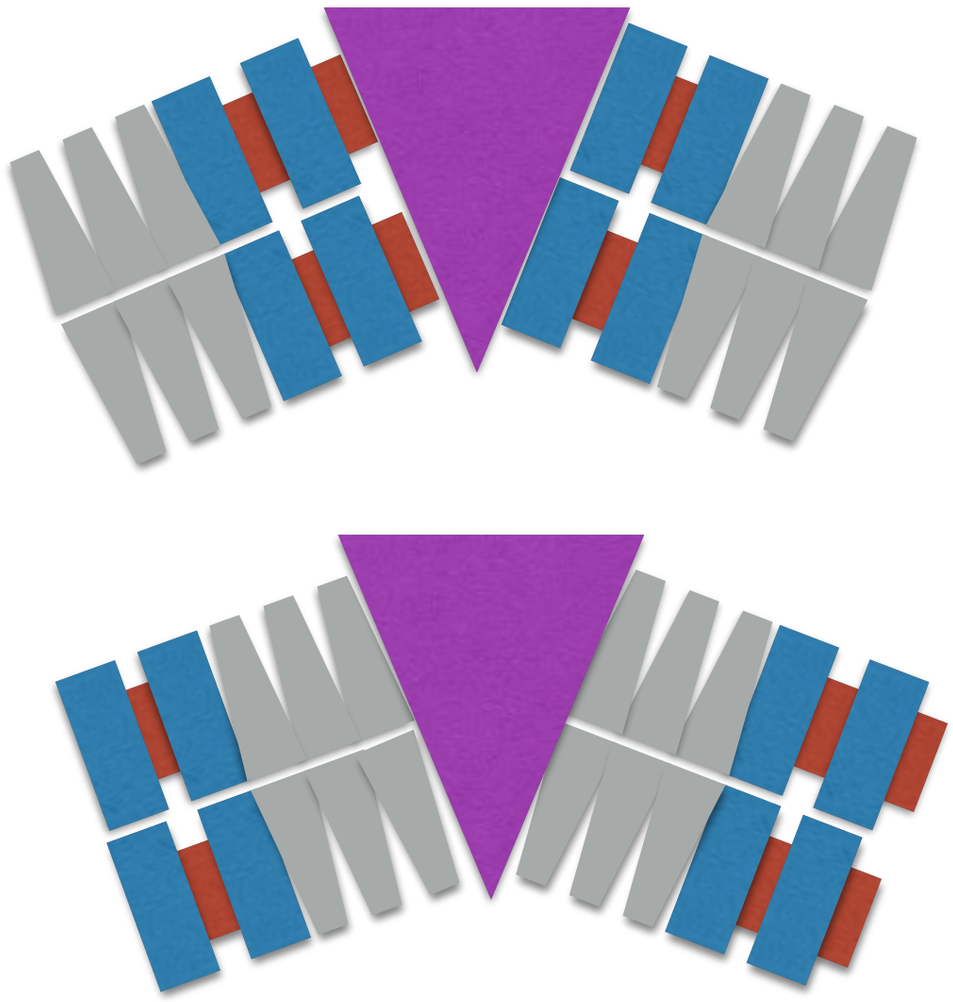

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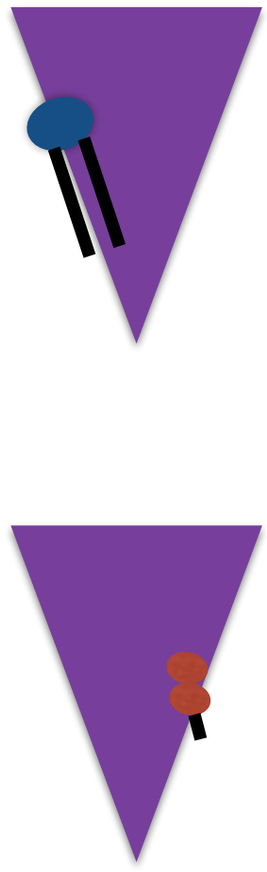
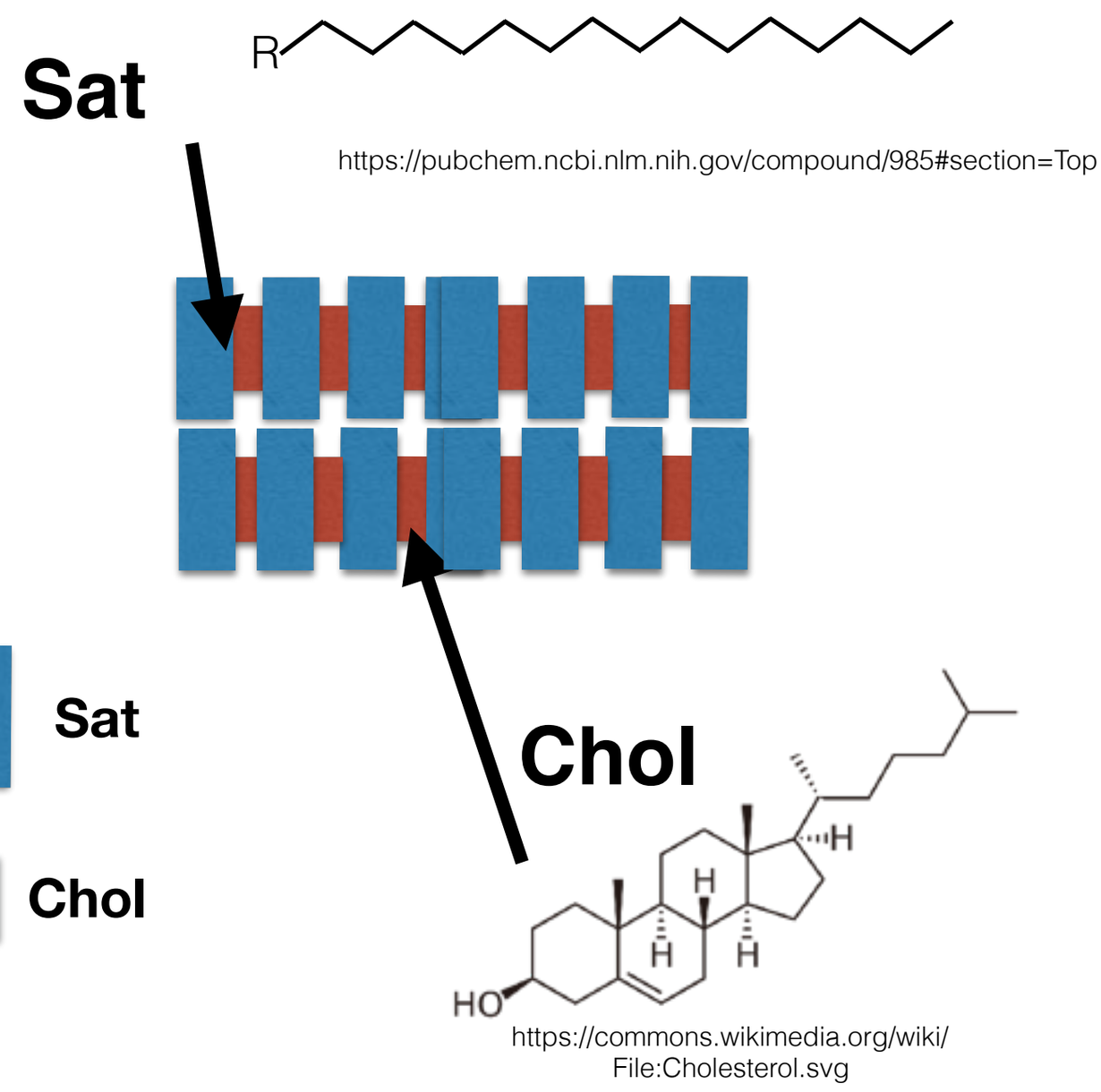


Recap of Preliminary Work: Simulations Series

- Simulating single nAChR in 3 membrane series with varying lipid ratios:
  - DPPC:CHOL



Recap of Preliminary Work: Simulations Series

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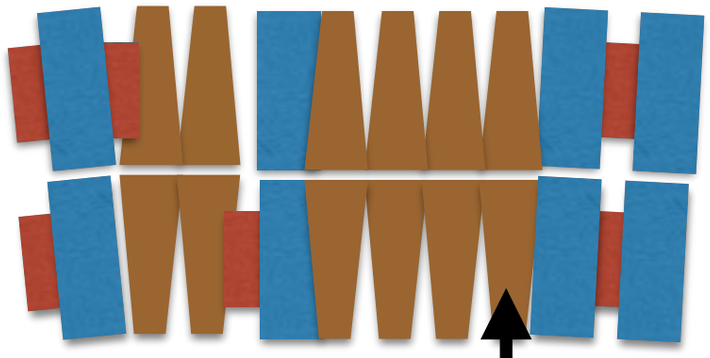

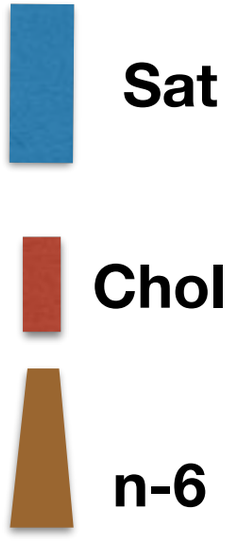

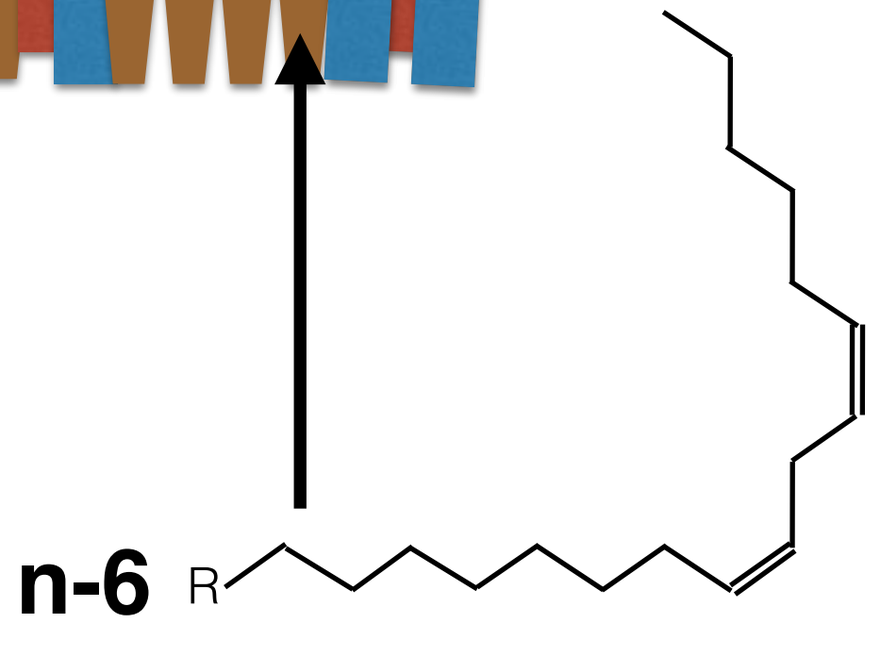

https://pubchem.ncbi.nlm.nih.gov/compound/5283446#section=Top

Recap of Preliminary Work: Simulations Series

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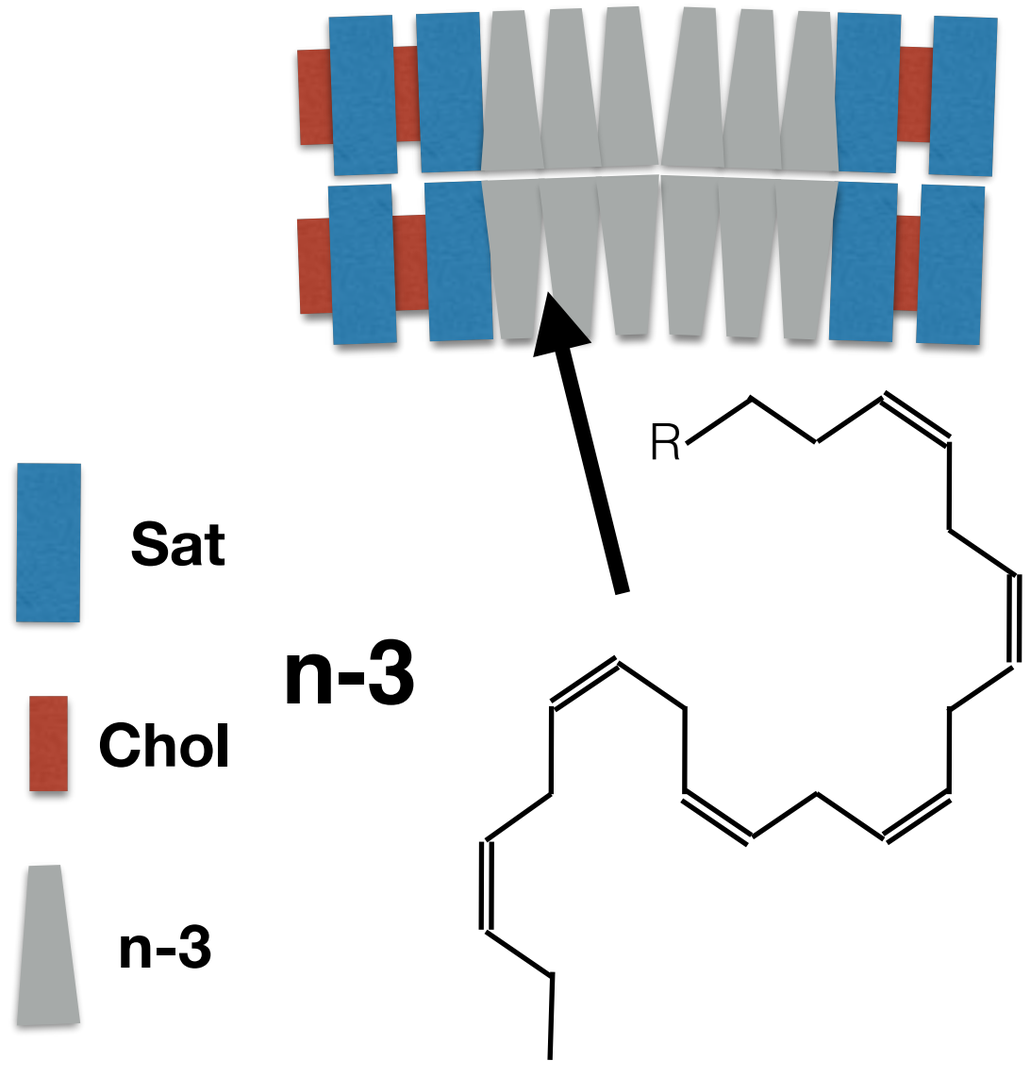

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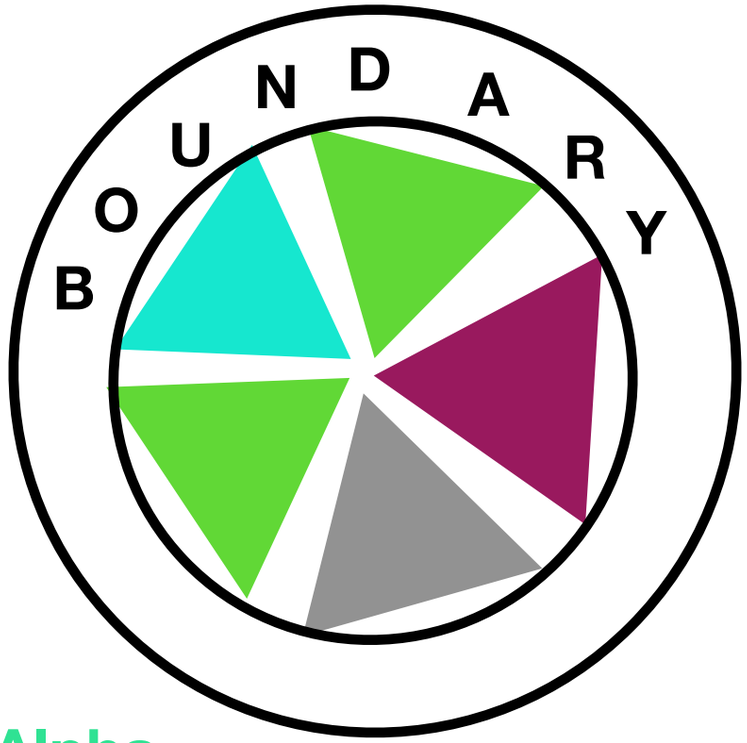
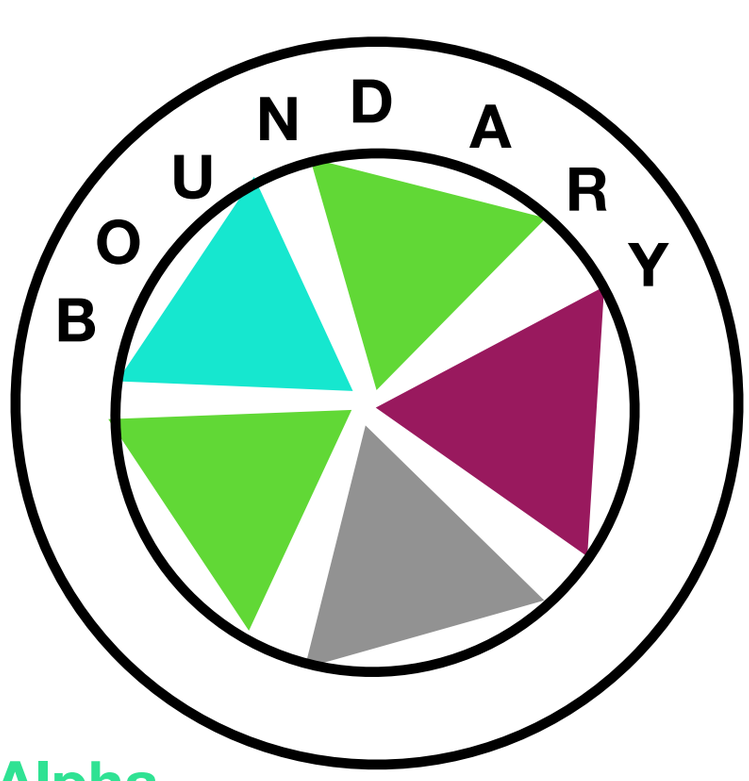
Recap of Preliminary Work: Binary Series Boundary Analysis



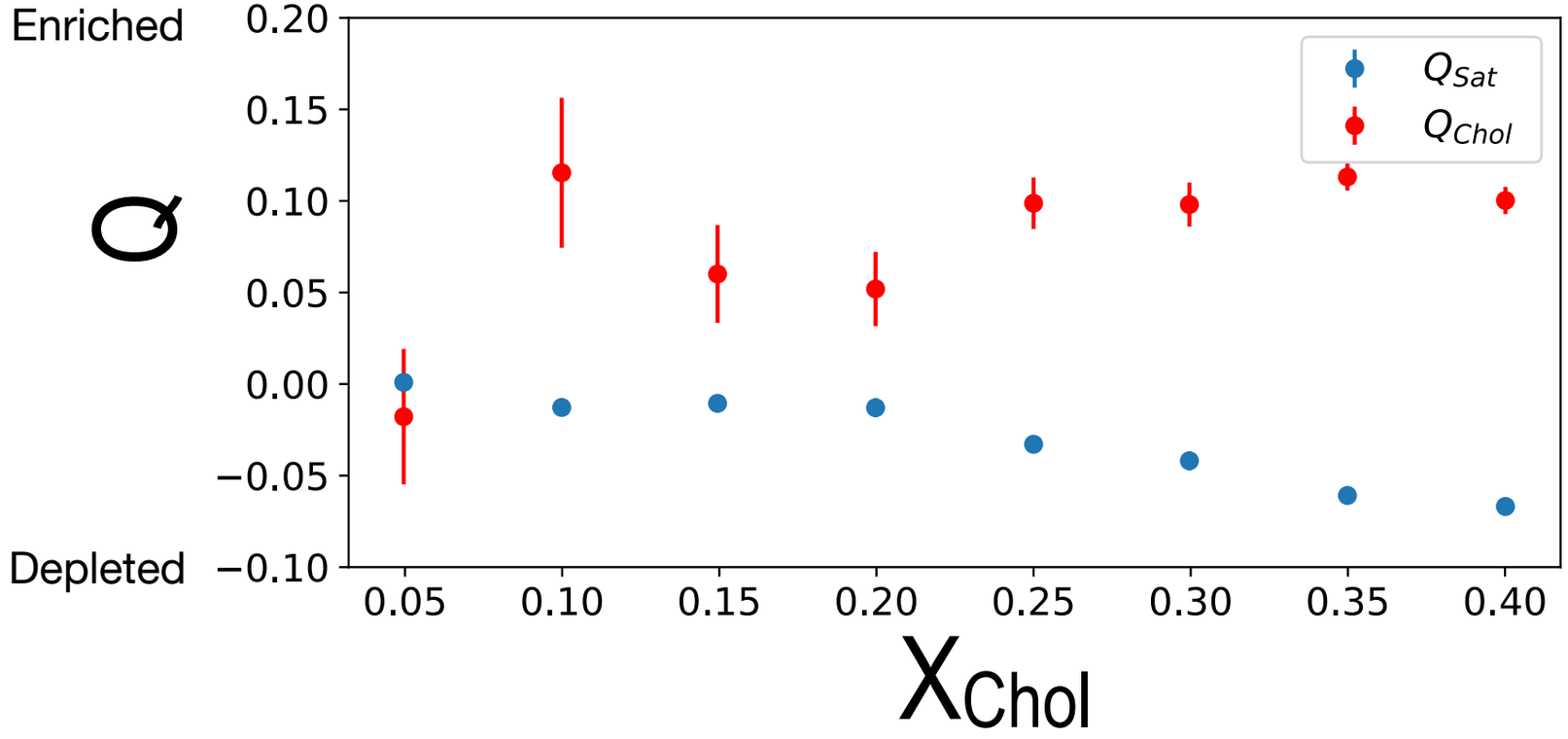
Alpha
Beta
Delta
Gamma

Recap of Preliminary Work: Binary Series Boundary Analysis

- Binary mixtures:
  Annulus enriched with cholesterol



Alpha
Beta
Delta
Gamma

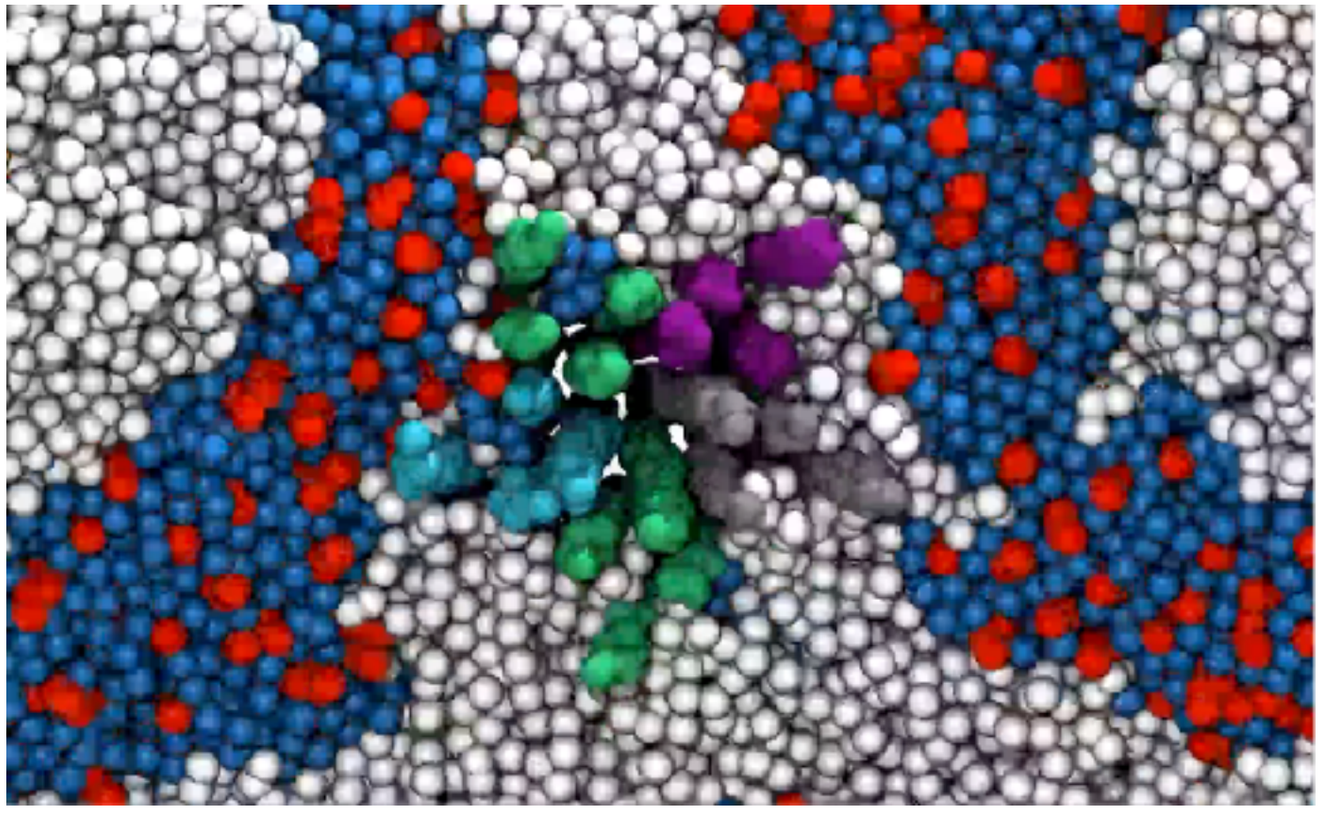


Recap of Preliminary Work: Ternary Simulation

- In ternary mixtures, expected nAChR to partition into CHOL enriched domain
    - Observed nAChR partition into CHOL depleted domain

PUFA Domain Formation

Alpha
Beta
Delta
Gamma



Saturated **Cholesterol** Unsaturated **~ Initial 100 ns**

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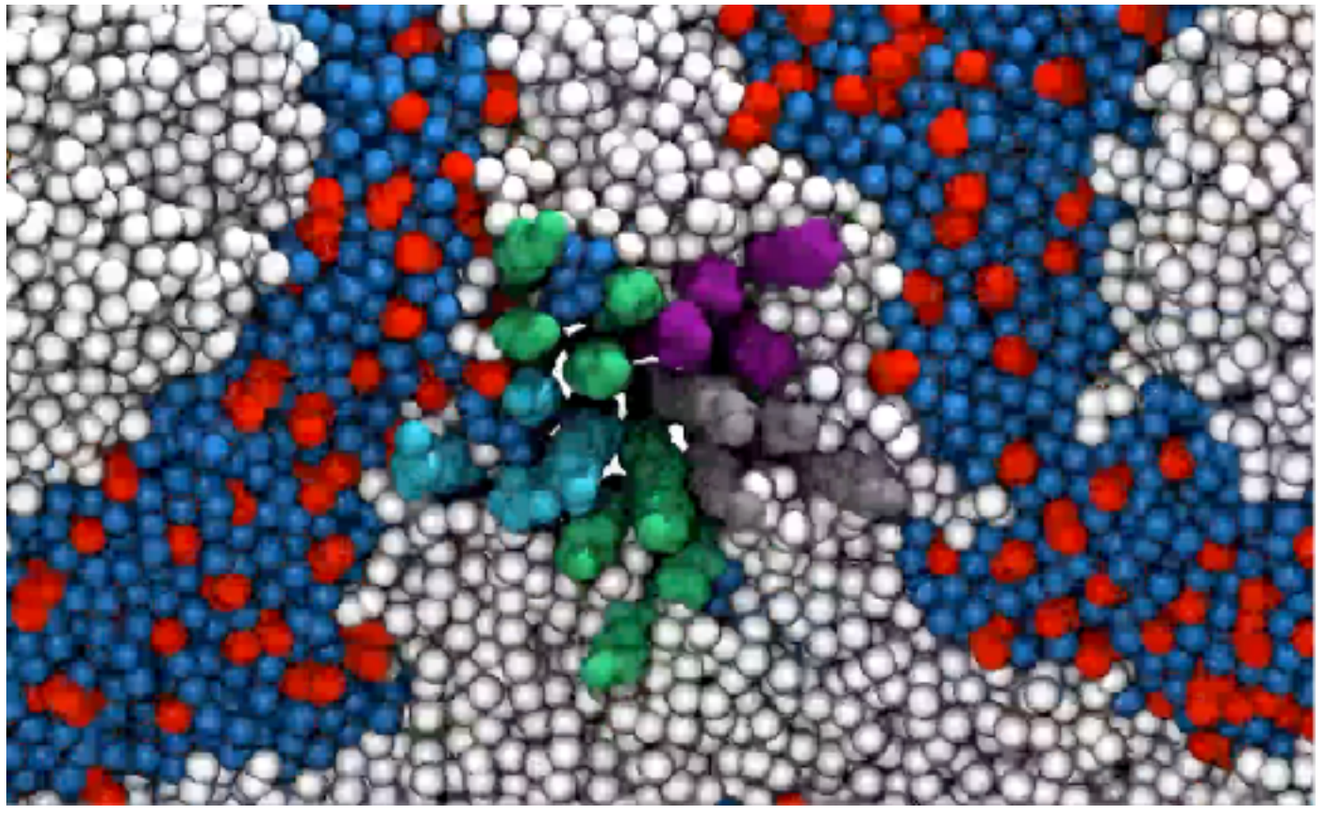

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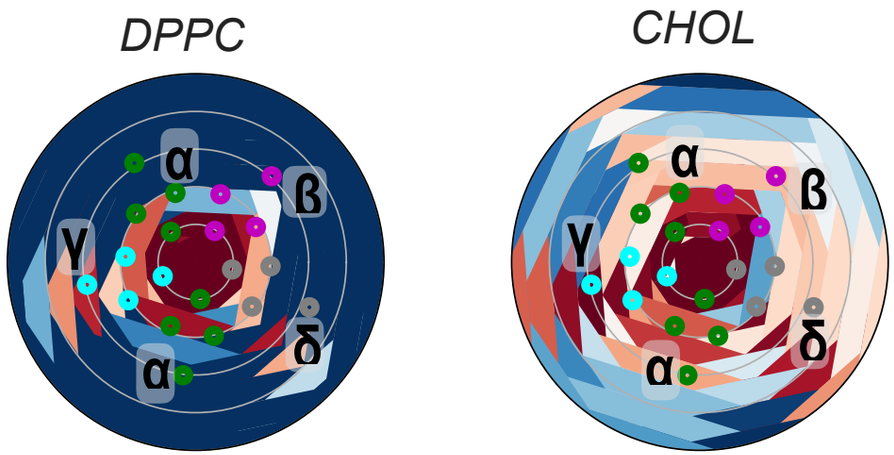
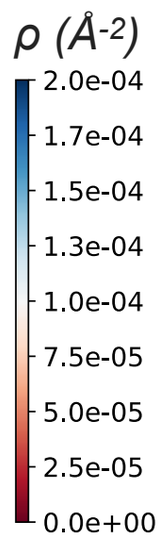
Recap of Preliminary Work: Density Plots Update

Last Year





Absolute Density

Alpha

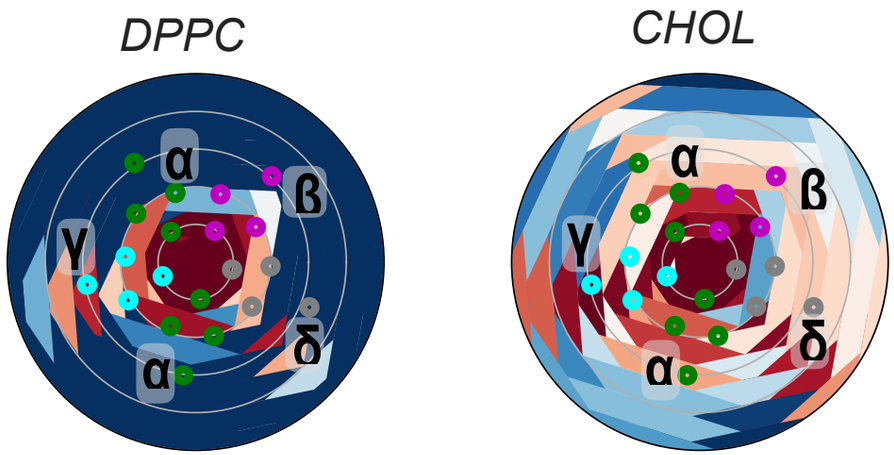
Beta

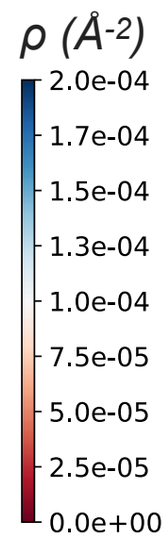
Delta

Gamma

Recap of Preliminary Work: Density Plots Update

Last Year





Absolute Density

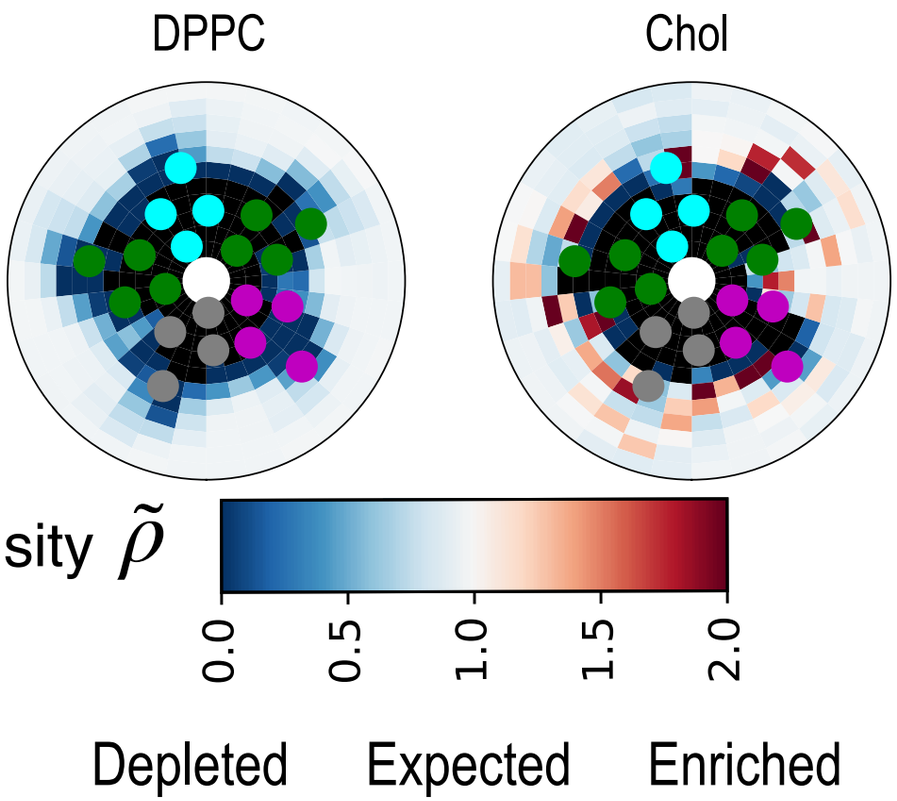
Alpha

Beta

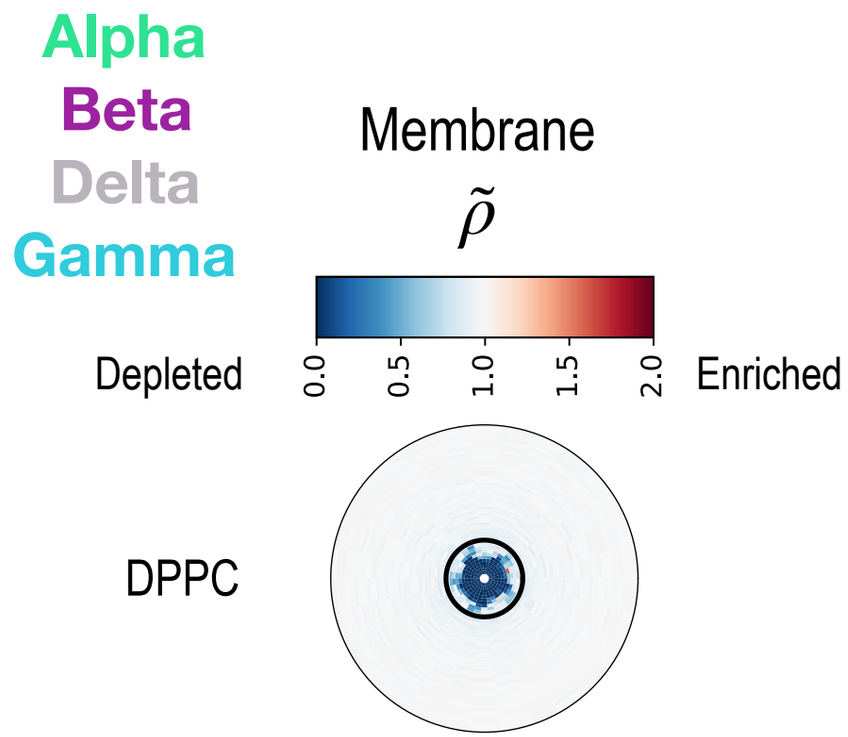
Delta

Gamma

This Year



Recap of Preliminary Work: Density Plots Binary Systems



Recap of Preliminary Work: Density Plots Binary Systems

Alpha
Beta
Delta
Gamma

Membrane

$\tilde{\rho}$

Depleted

0.0 0.5 1.0 1.5 2.0

Enriched

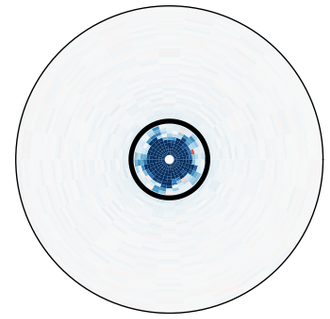
Annular region

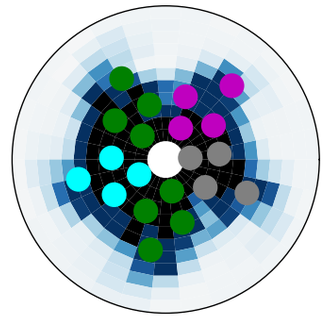
$\log \tilde{\rho}$

$\tilde{\rho} = 0$

-3.0 -2.0 -1.0 0.0 1.0

DPPC





Recap of Preliminary Work: Density Plots Binary Systems

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Beta
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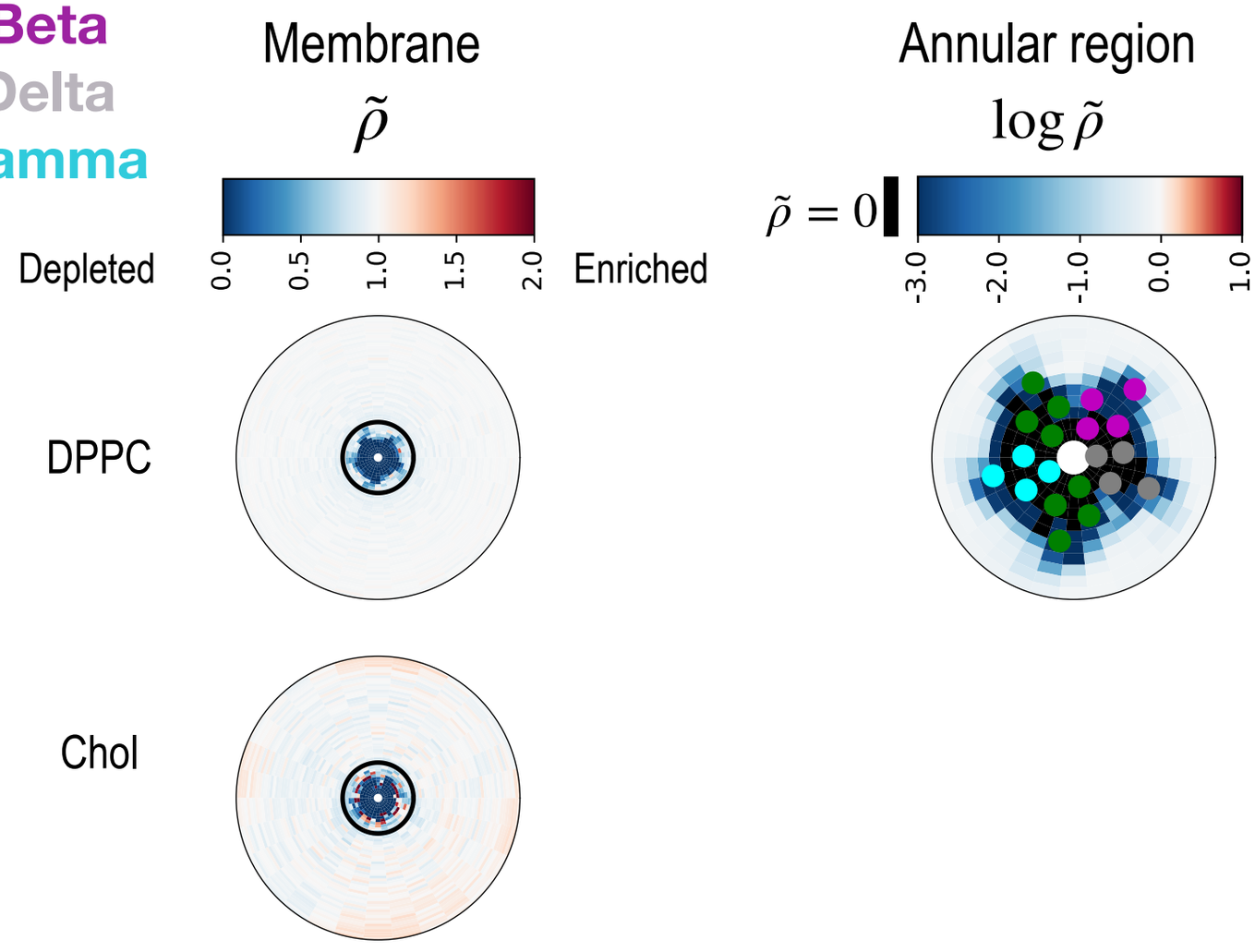
Gamma



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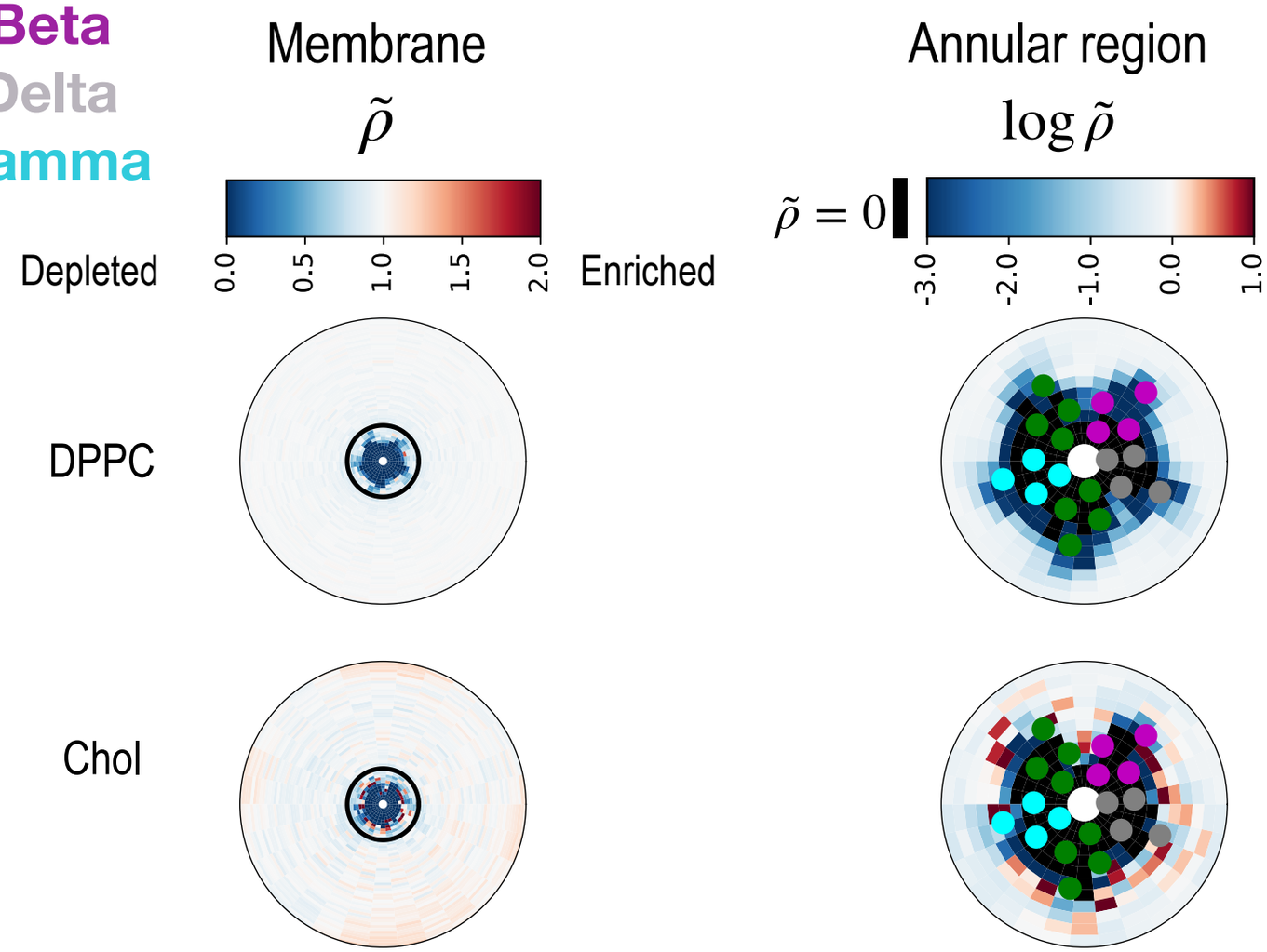
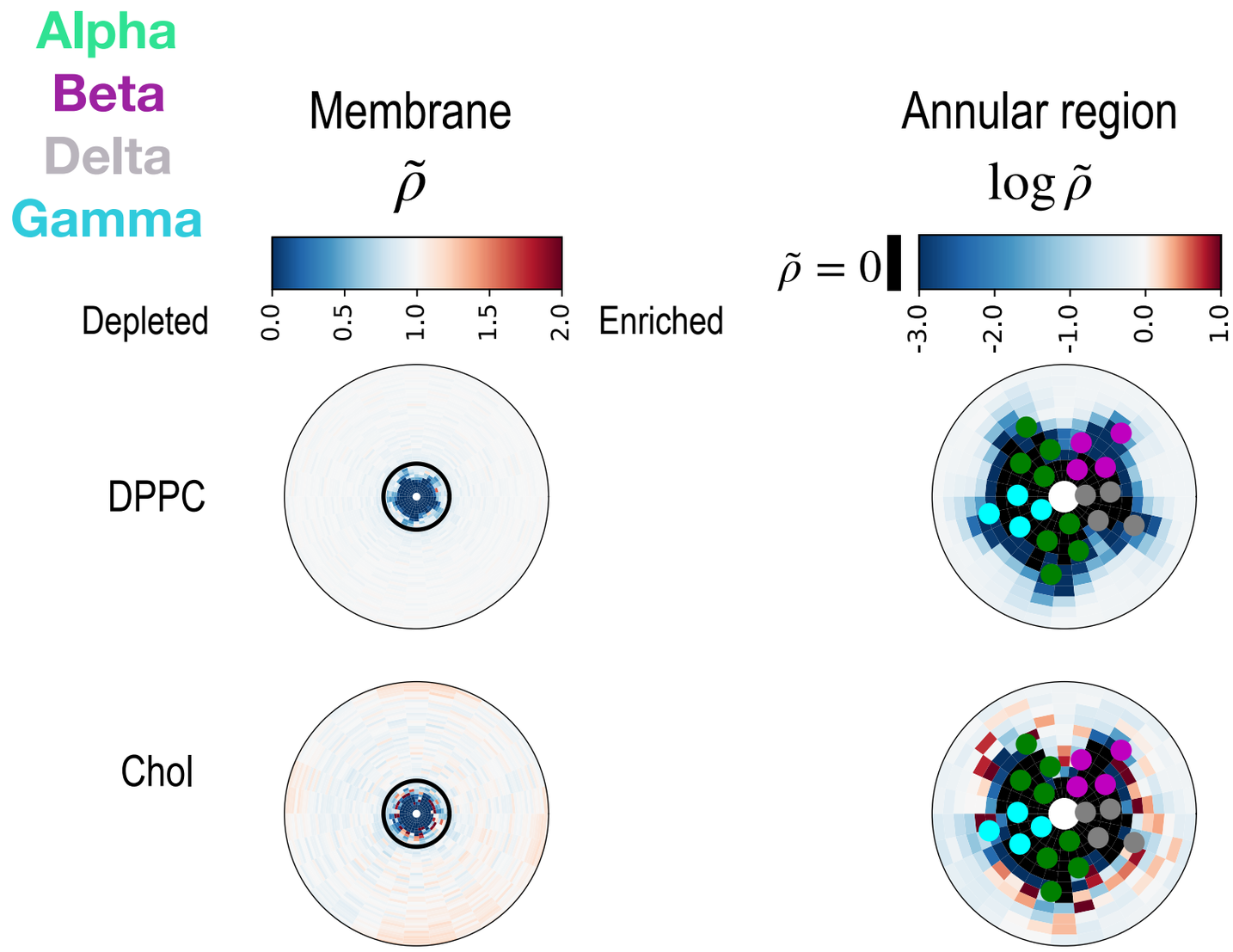
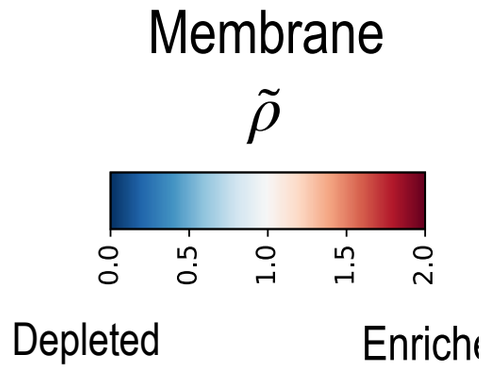


Recap of Preliminary Work: Density Plots Binary Systems

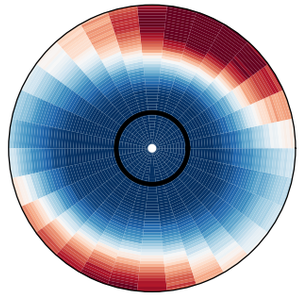
- Binary membrane:
  - Random mixing in the bulk membrane
  - CHOL enrichment around annulus and embedded



Recap of Preliminary Work: Density Plots Ternary Systems



DPPC



Ternary Mixture
with long n-3
PUFAs

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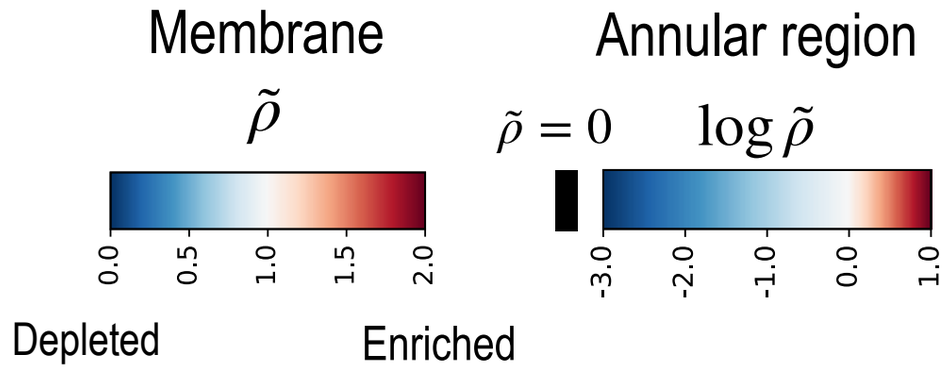

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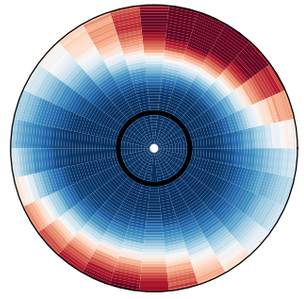
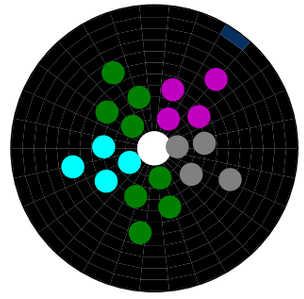
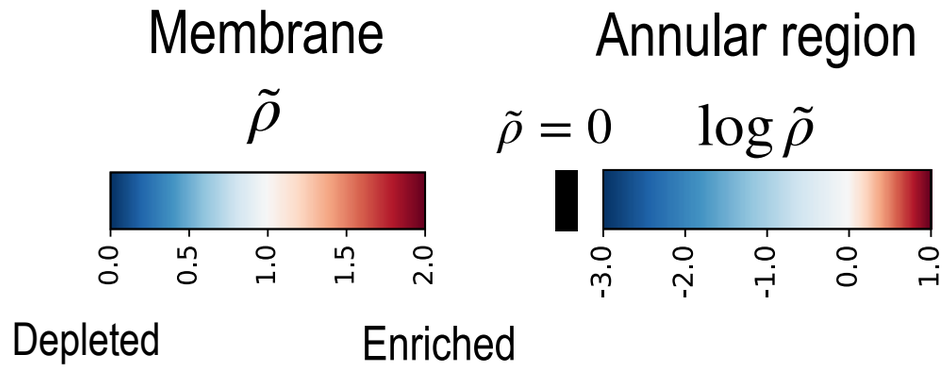




Ternary Mixture with long n-3 PUFAs

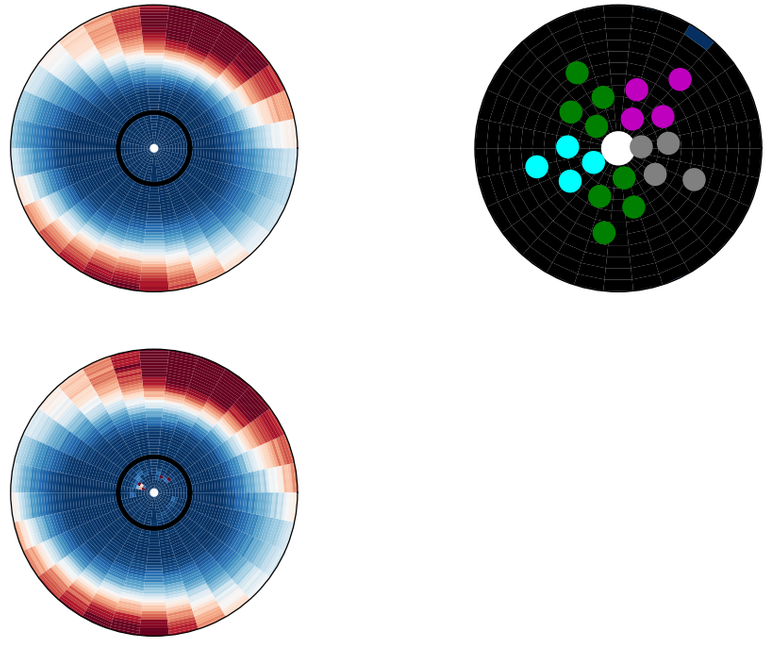
Recap of Preliminary Work: Density Plots Ternary Systems



Ternary Mixture with long n-3 PUFAs

DPPC

Chol



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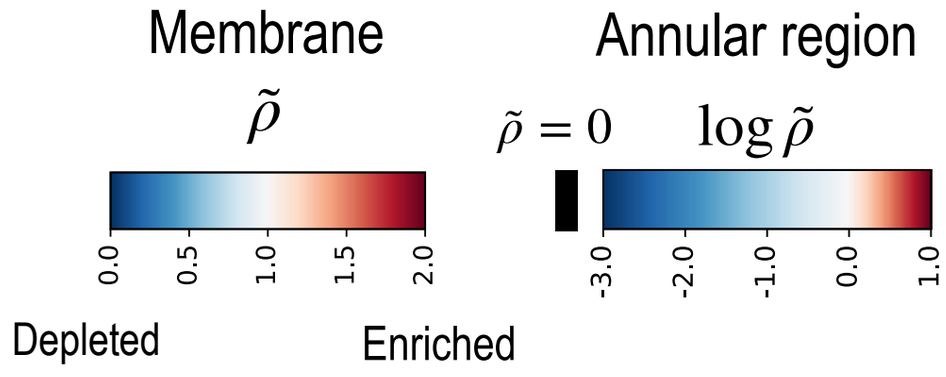
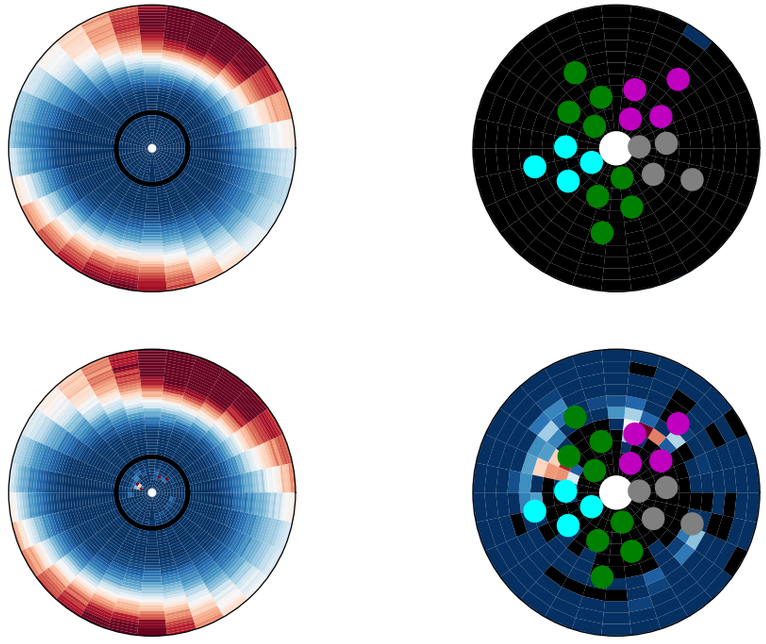


Ternary Mixture with long n-3 PUFAs

DPPC

Chol



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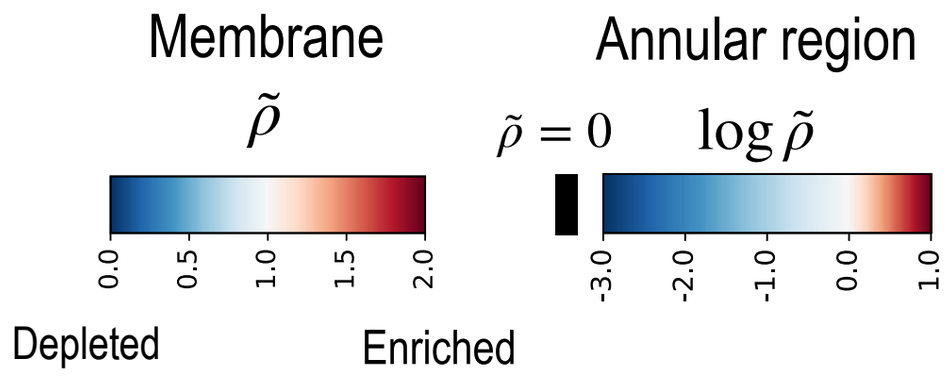
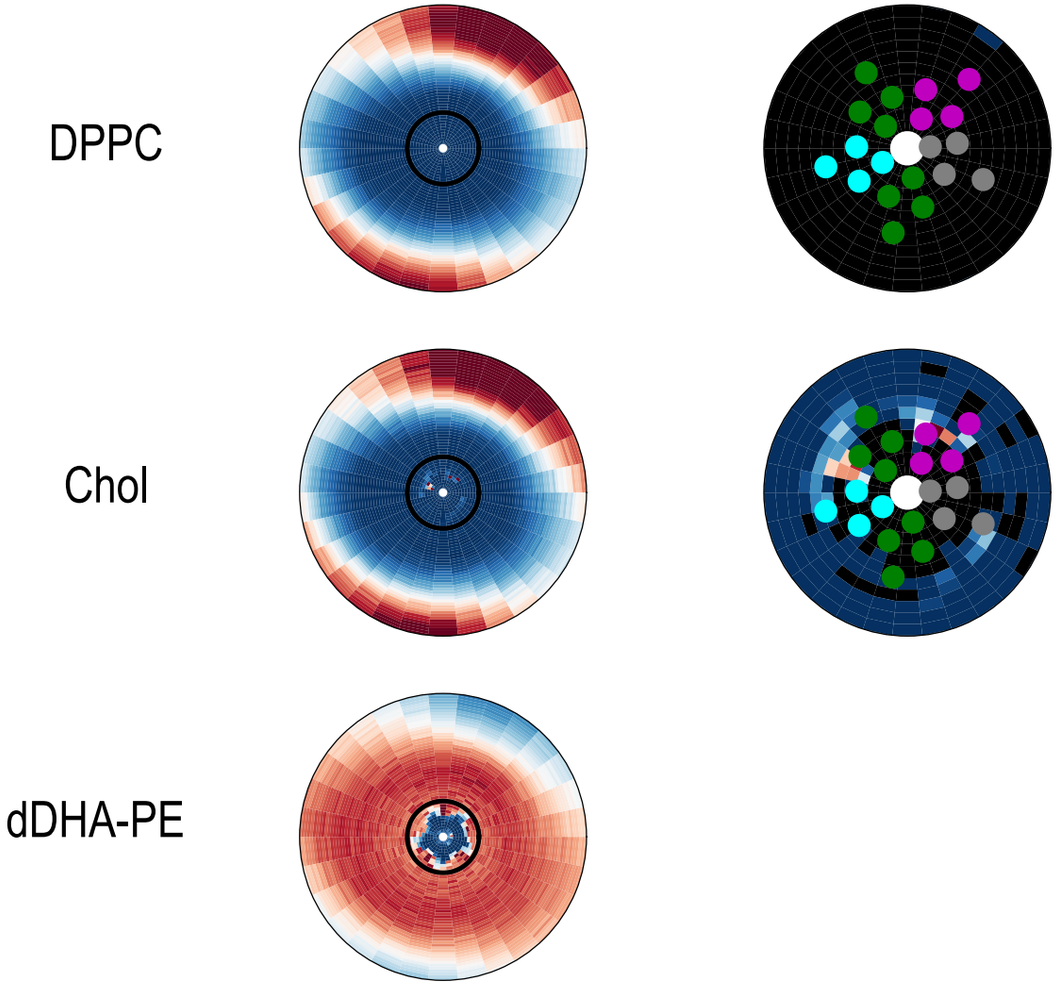
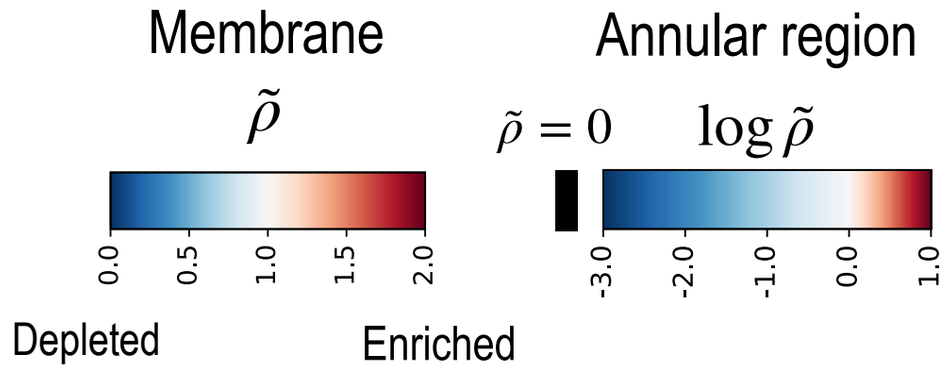


Ternary Mixture with long n-3 PUFAs



Recap of Preliminary Work: Density Plots Ternary Systems

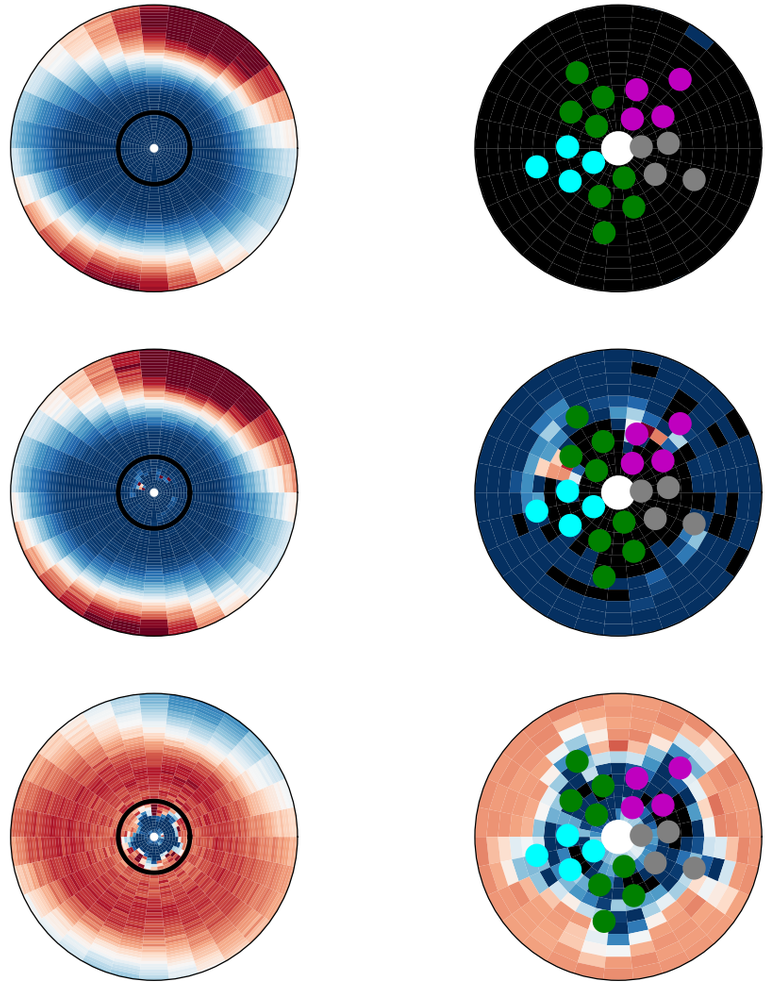


Ternary Mixture with long n-3 PUFAs

DPPC

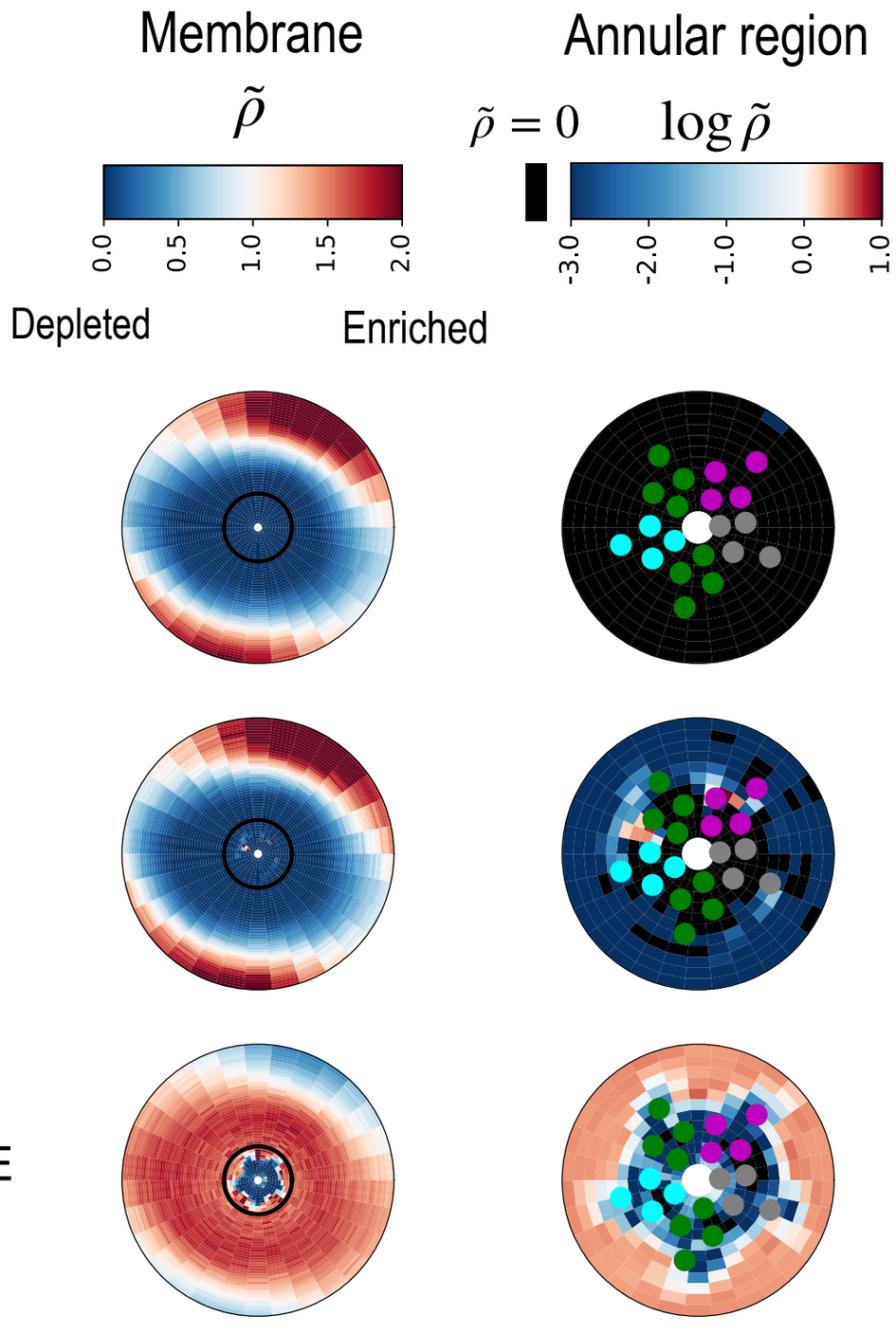
Chol

dDHA-PE



Recap of Preliminary Work: Density Plots Ternary Systems

- Ternary membrane:
  - Extensive domain formation
  - CHOL enriched domain forms bulk membrane
  - PUFA occupies annulus, and embedded region, some CHOL competition



Recap of Preliminary Work: Publication

- This work resulted in a publication

BBA · Biomembranes 1861 (2019) 887–896

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

Boundary lipids of the nicotinic acetylcholine receptor: Spontaneous partitioning via coarse-grained molecular dynamics simulation

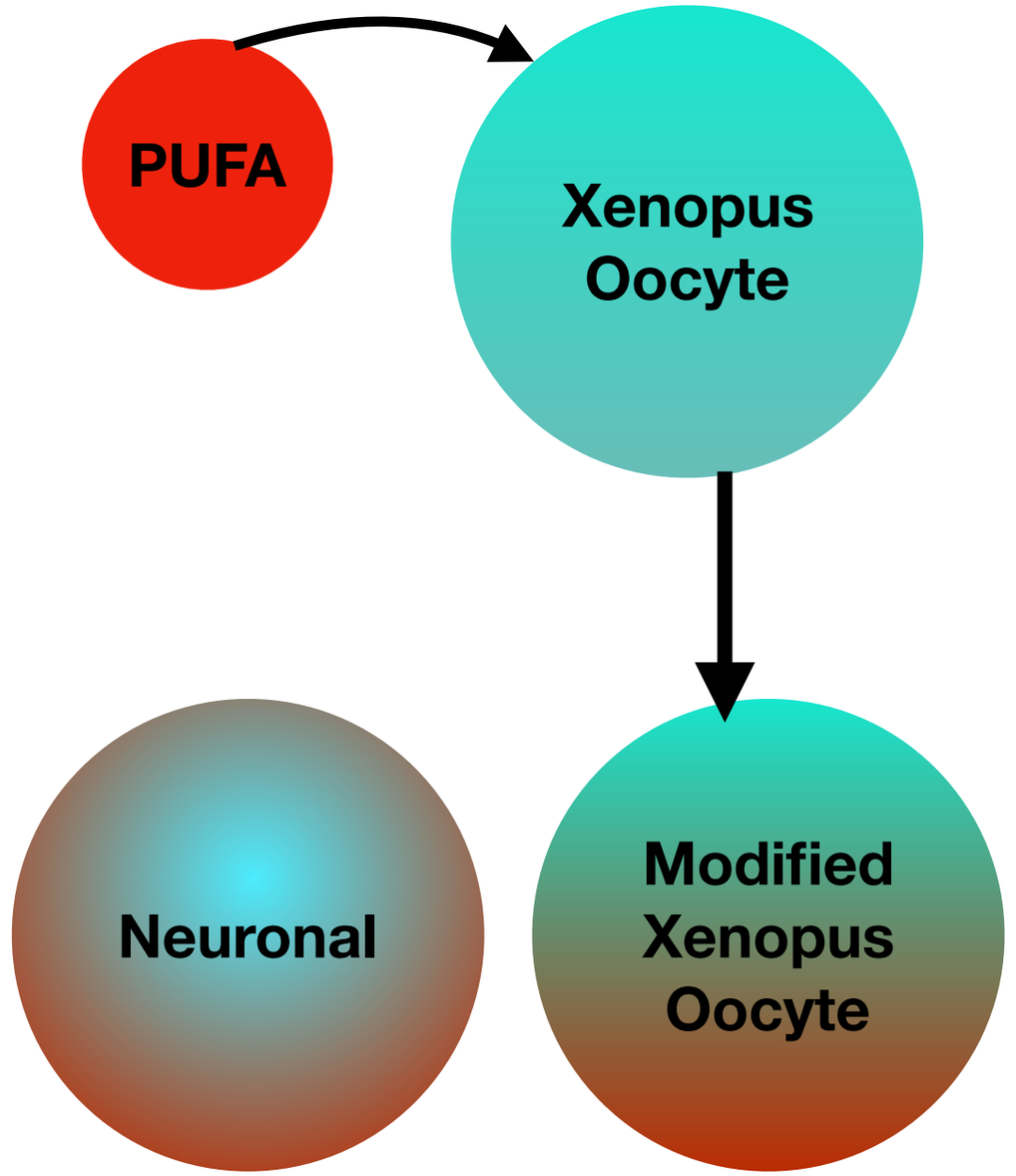
Liam Sharp[a], Reza Salari[a,1], Grace Brannigan[a,b,*]

[a] Center for Computational and Integrative Biology, Rutgers University-Camden, Camden, NJ, United States of America
[b] Department of Physics, Rutgers University-Camden, Camden, NJ, United States of America

In Progress

Aim 1

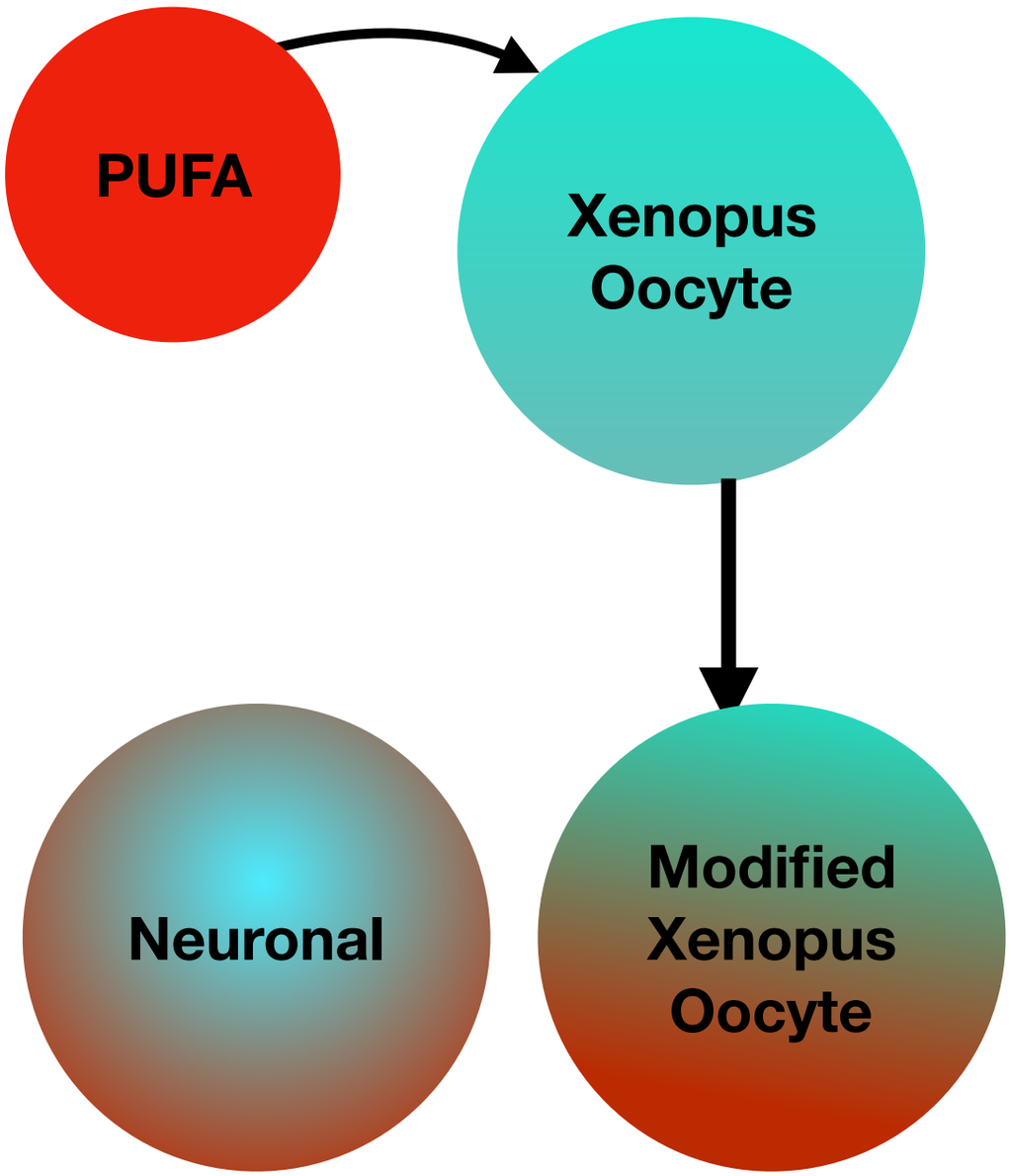
1. **Coarse-grained simulations of multiple subtypes of mammalian pLGICs in quasi-physiological membranes.** We observe nAChR partitioning into n-3 (DHA) rich domains with DHA-PE as nAChR's primary boundary lipid. *Xenopus* oocytes lipid composition are considerably different from neuronal membranes, but due to the observed affinity of DHA chains for nAChR , we believe adding small concentrations of n-3 is likely to restore the native boundary lipids. We will model various n-3 supplemented quasi-physiological membranes (such as oocytes) to predict those likely to provide a native local environment within an oocyte.



- We want to expand this aim to also include simulating realistic neuronal membranes with a diversity of proteins

Aim 1

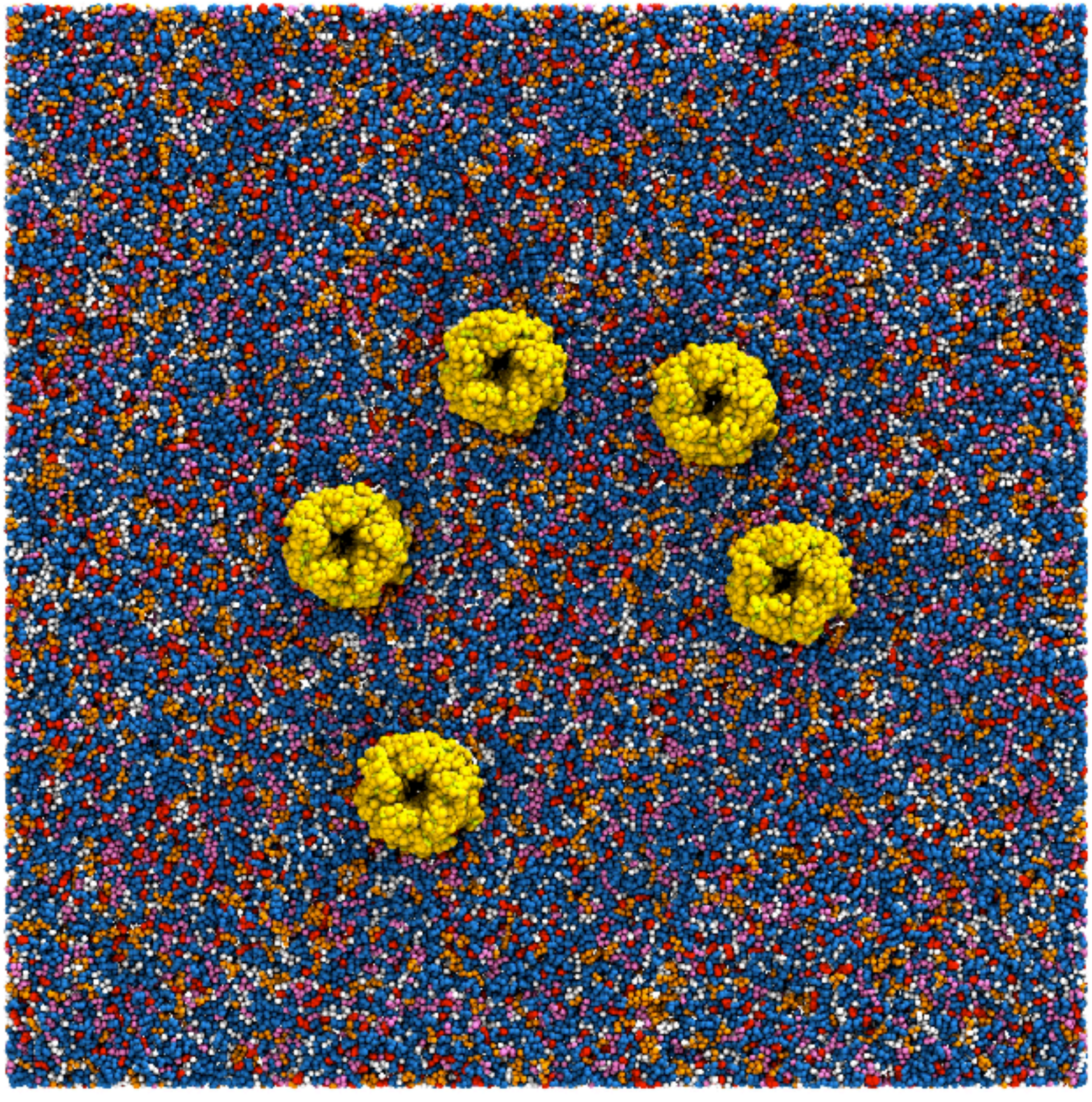
1. **Coarse-grained simulations of multiple subtypes of mammalian pLGICs in quasi-physiological membranes.** We observe nAChR partitioning into n-3 (DHA) rich domains with DHA-PE as nAChR's primary boundary lipid. *Xenopus* oocytes lipid composition are considerably different from neuronal membranes, but due to the observed affinity of DHA chains for nAChR , we believe adding small concentrations of n-3 is likely to restore the native boundary lipids. We will model various n-3 supplemented quasi-physiological membranes (such as oocytes) to predict those likely to provide a native local environment within an oocyte.



- We want to expand this aim to also include simulating realistic neuronal membranes with a diversity of proteins

Aim 1: Where things are at

Model
Xenopus
Oocyte



Sat

N

N7

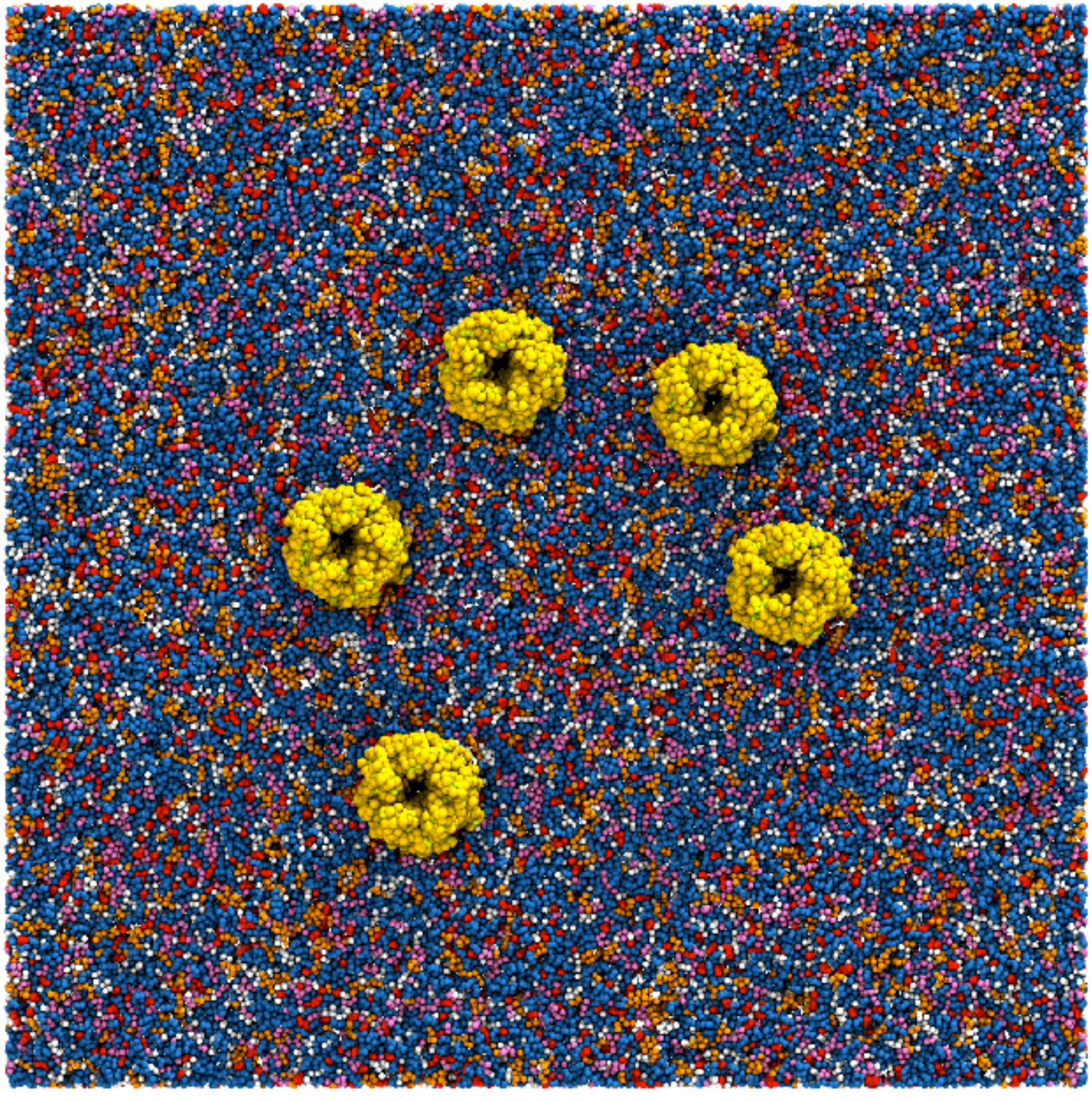
N6

N3

CHOL

Aim 1: Where things are at

Model
Xenopus
Oocyte



Sat

N

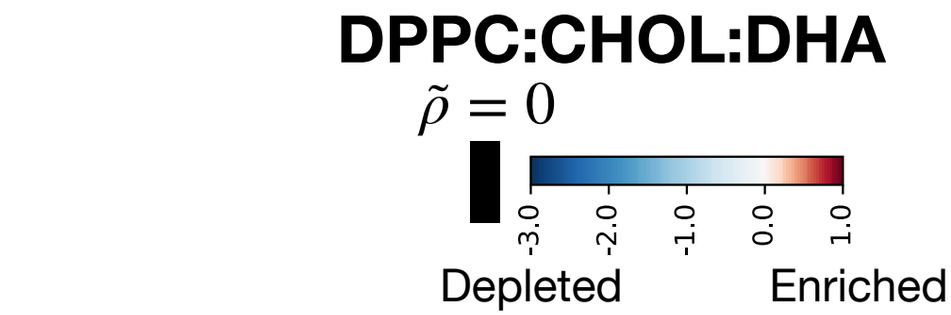
N7

N6

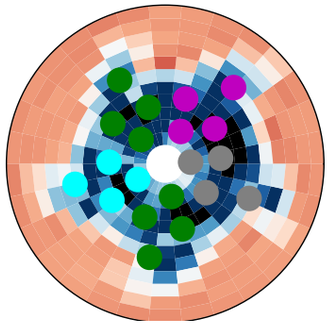
N3

CHOL

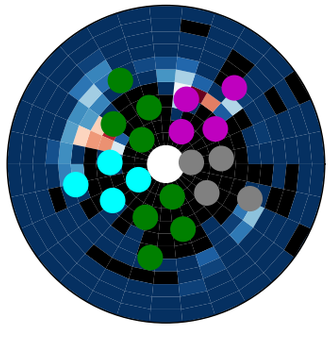
Aim 1: Single nAChR Xenopus Oocyte



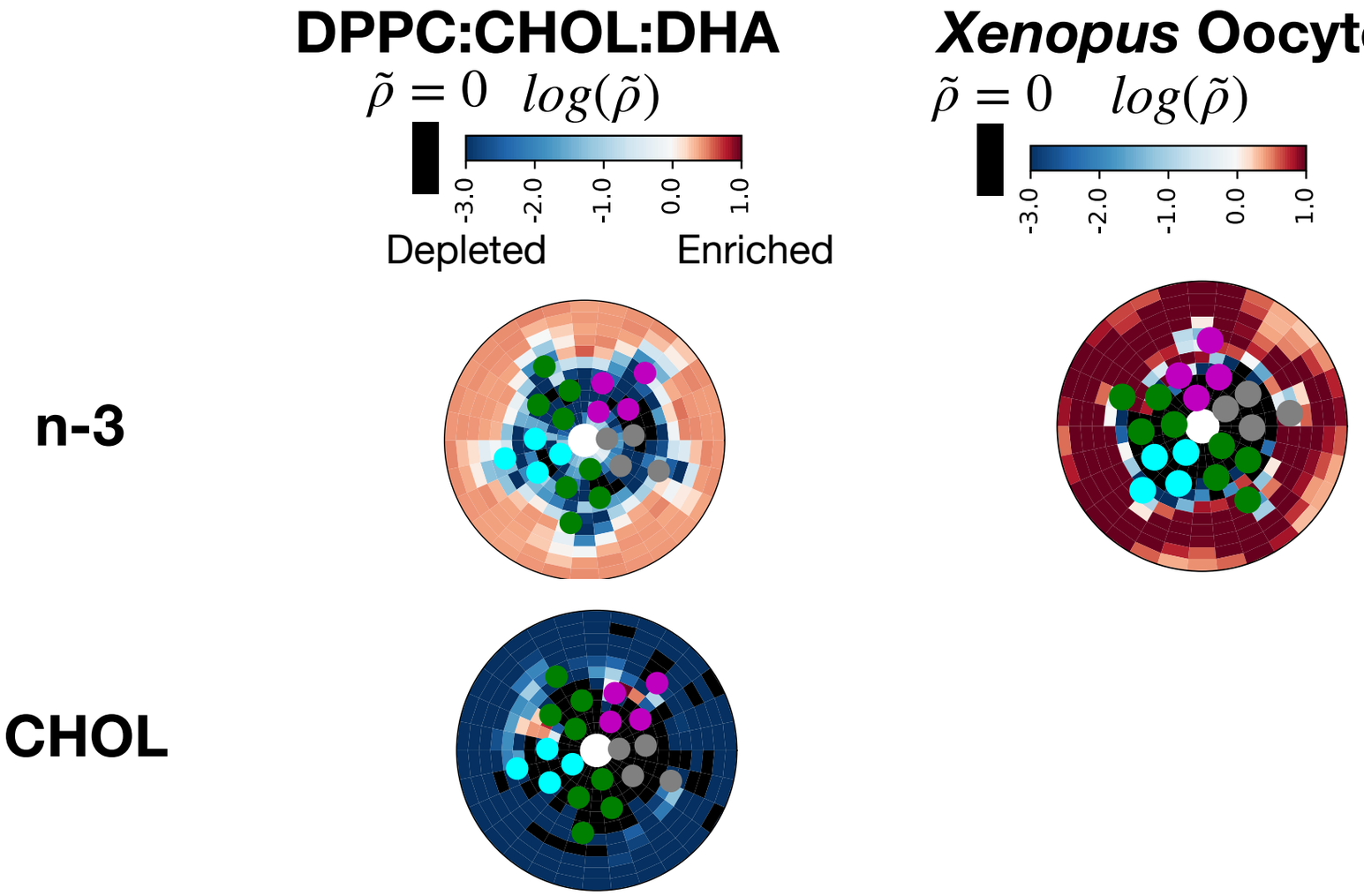
n-3



CHOL



Aim 1: Single nAChR Xenopus Oocyte



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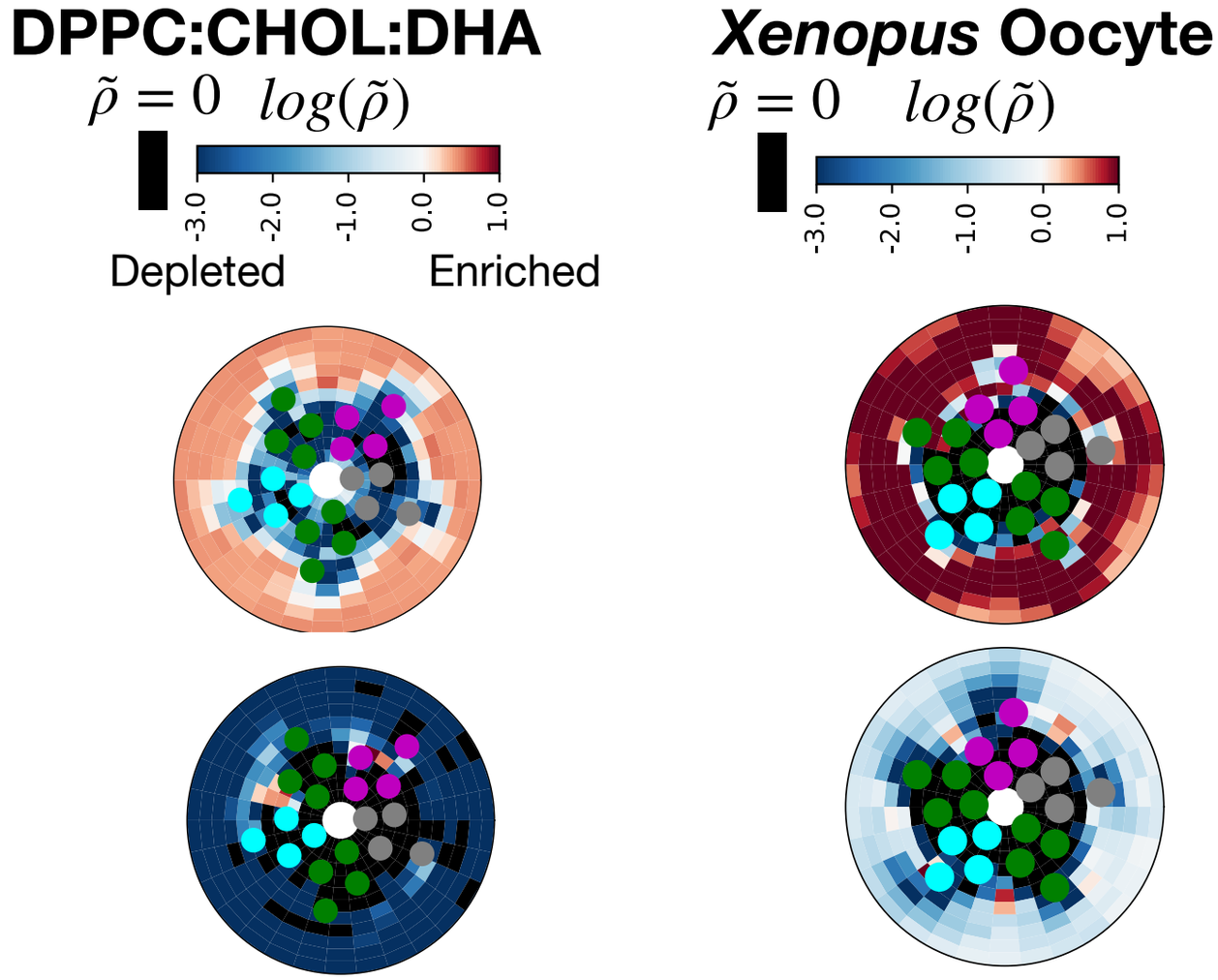
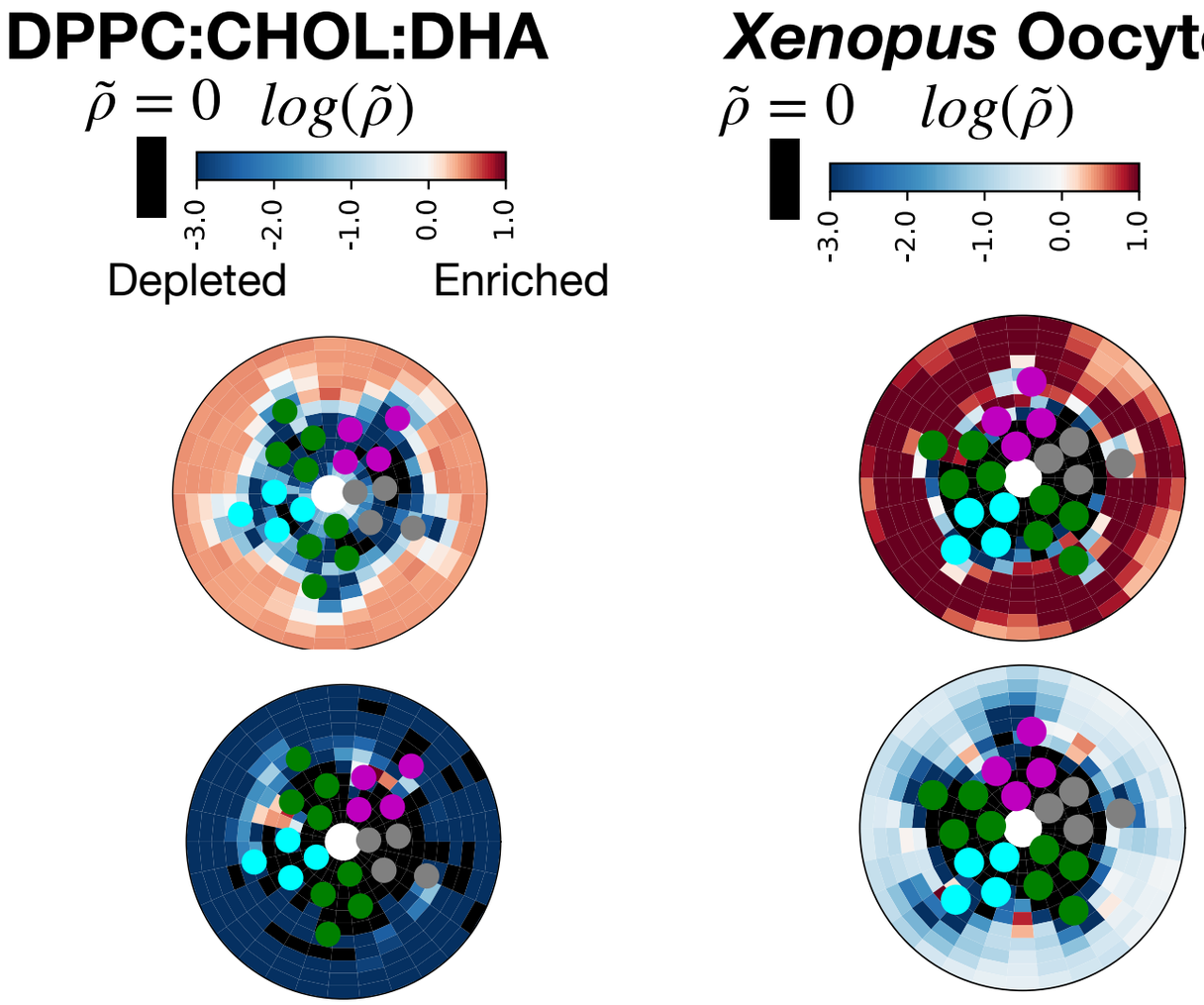


Aim 1: Single nAChR Xenopus Oocyte

- Boundary lipids follow similar trend to preliminary work
- Low n-3 PUFA and Chol concentration



Aim 1: What I'm still working on

- Choose synaptic proteins
    - Determine ~15 of the most common synaptic proteins with known structures
- Develop straight forward restraints for multiple species of protein (neuronal simulations)
- **Computer time (~ 168,000,000 hours of CPU time)**

Collaboration 1

Untangling direct and domain-mediated interactions between nicotinic acetylcholine receptors in DHA-rich membranes

Kristen Woods* · Liam Sharp* · Grace Brannigan†

Collaboration 1: Purpose of The research

- Goals: 1) How does membrane organization affect nAChR boundary lipids? 2) What role do domains play on protein clustering? 3) Are there subunit-subunit preferences?

- All simulations were run with 2:2:1 PUFA:Sat:Chol

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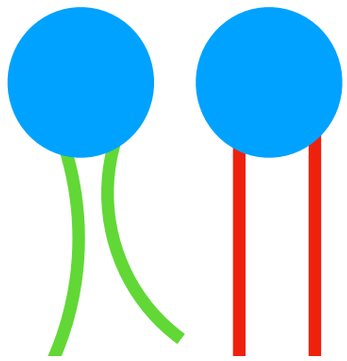
- Four series using homoacids (DPPC and DHA-pC) and Chol, with 1 to 4 proteins



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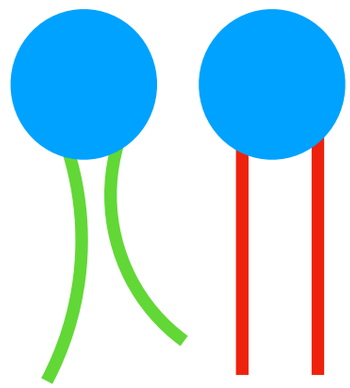
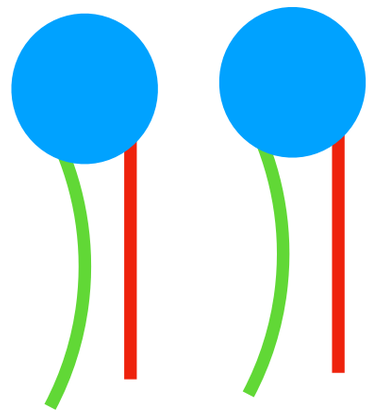
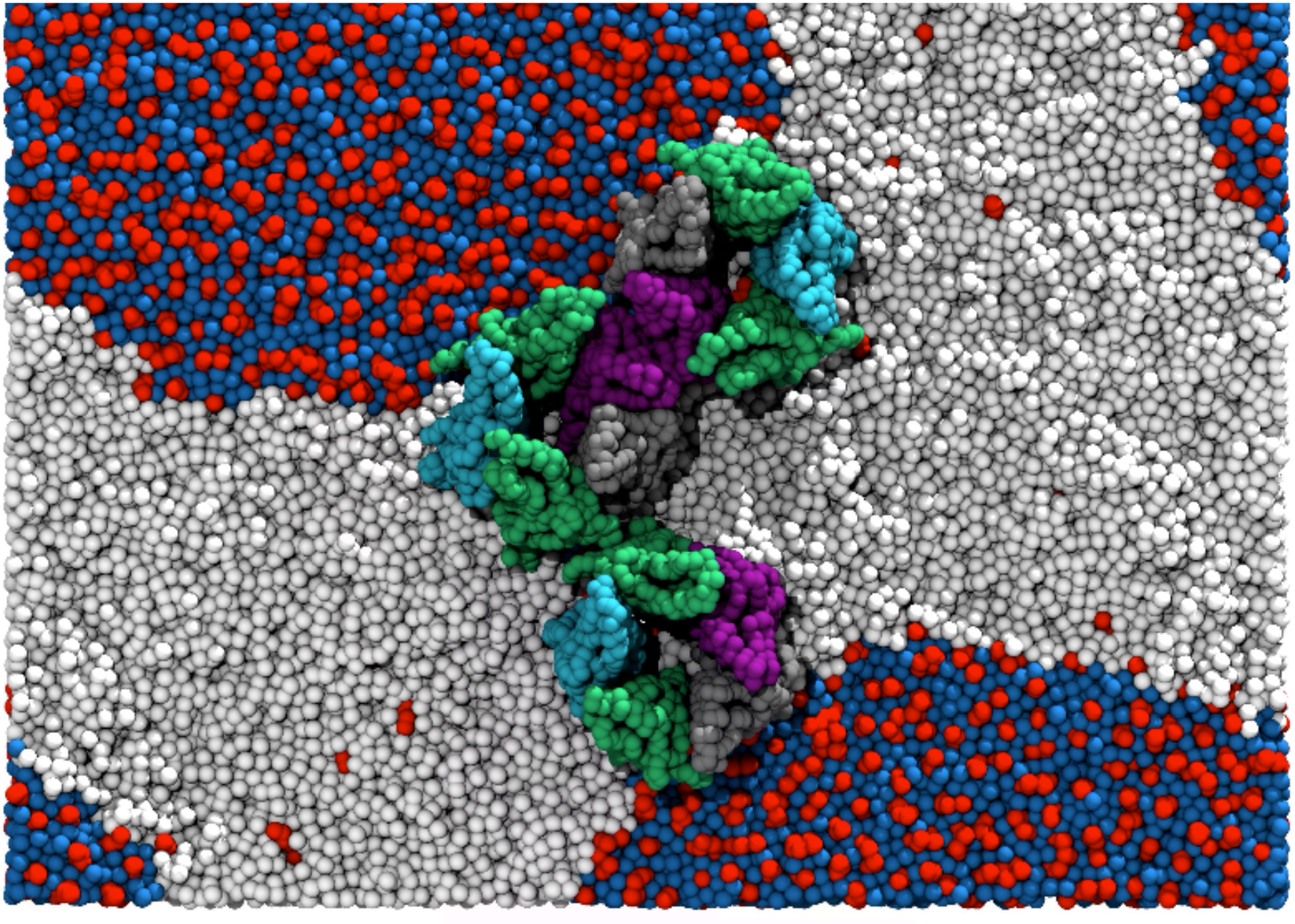


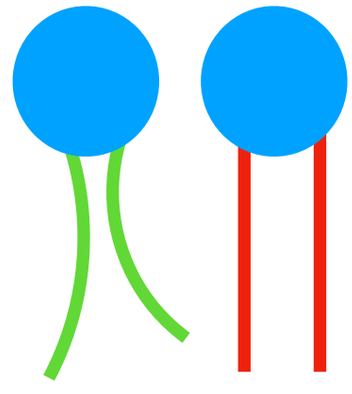
- Four series using heteroacids (PDPC) and Chol, with 1 to 4 proteins



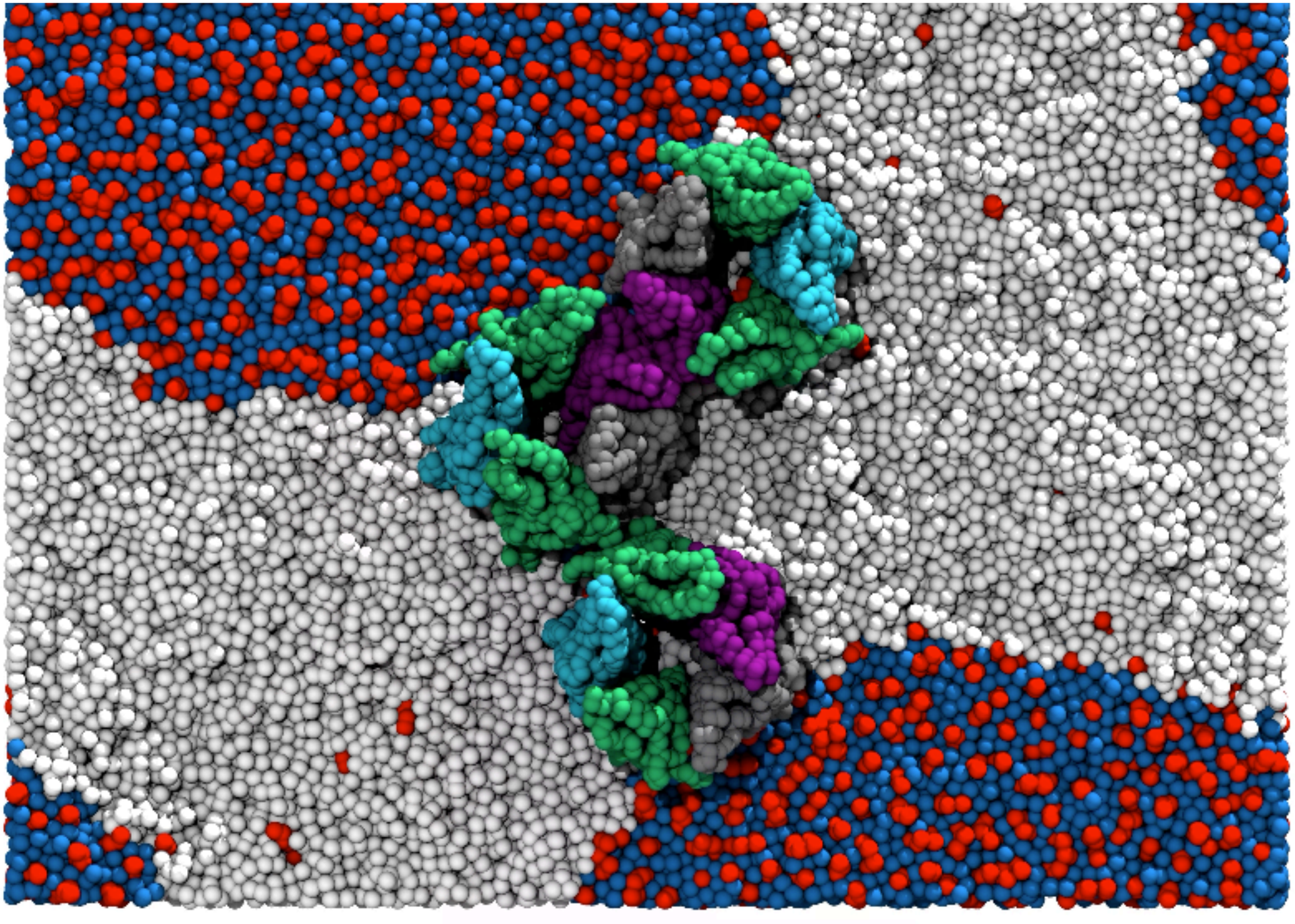
Collaboration 1: Purpose of The research



Homoacids Membrane



Collaboration 1: Purpose of The research



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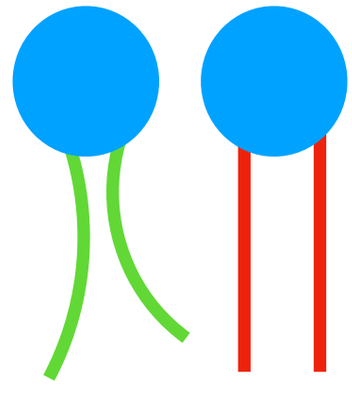

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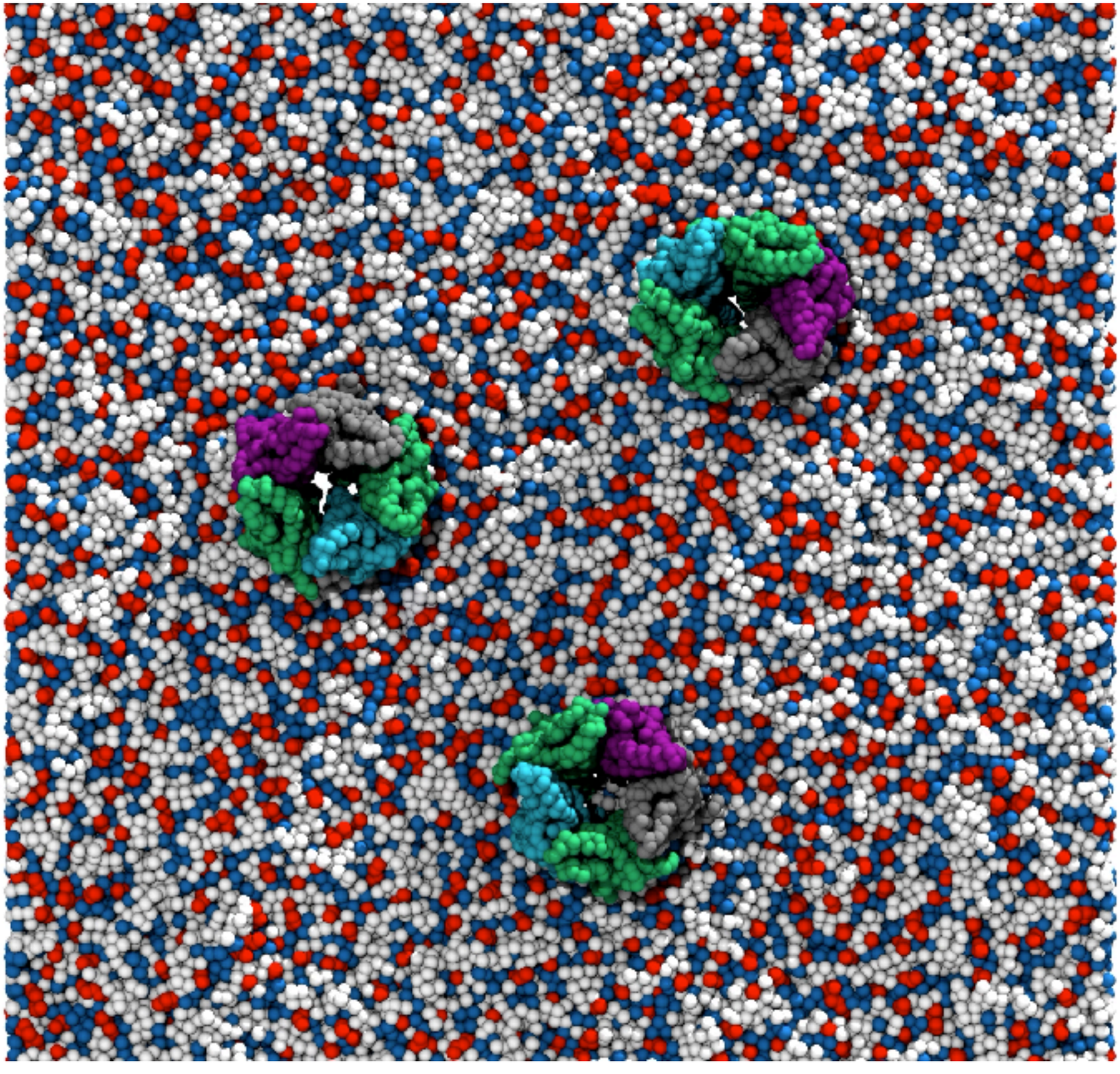
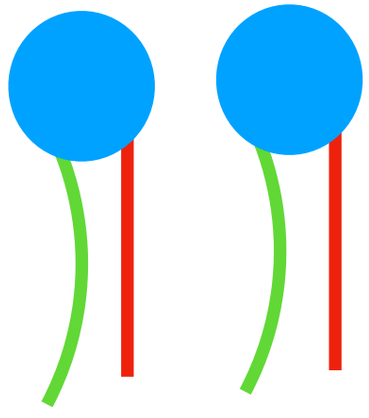
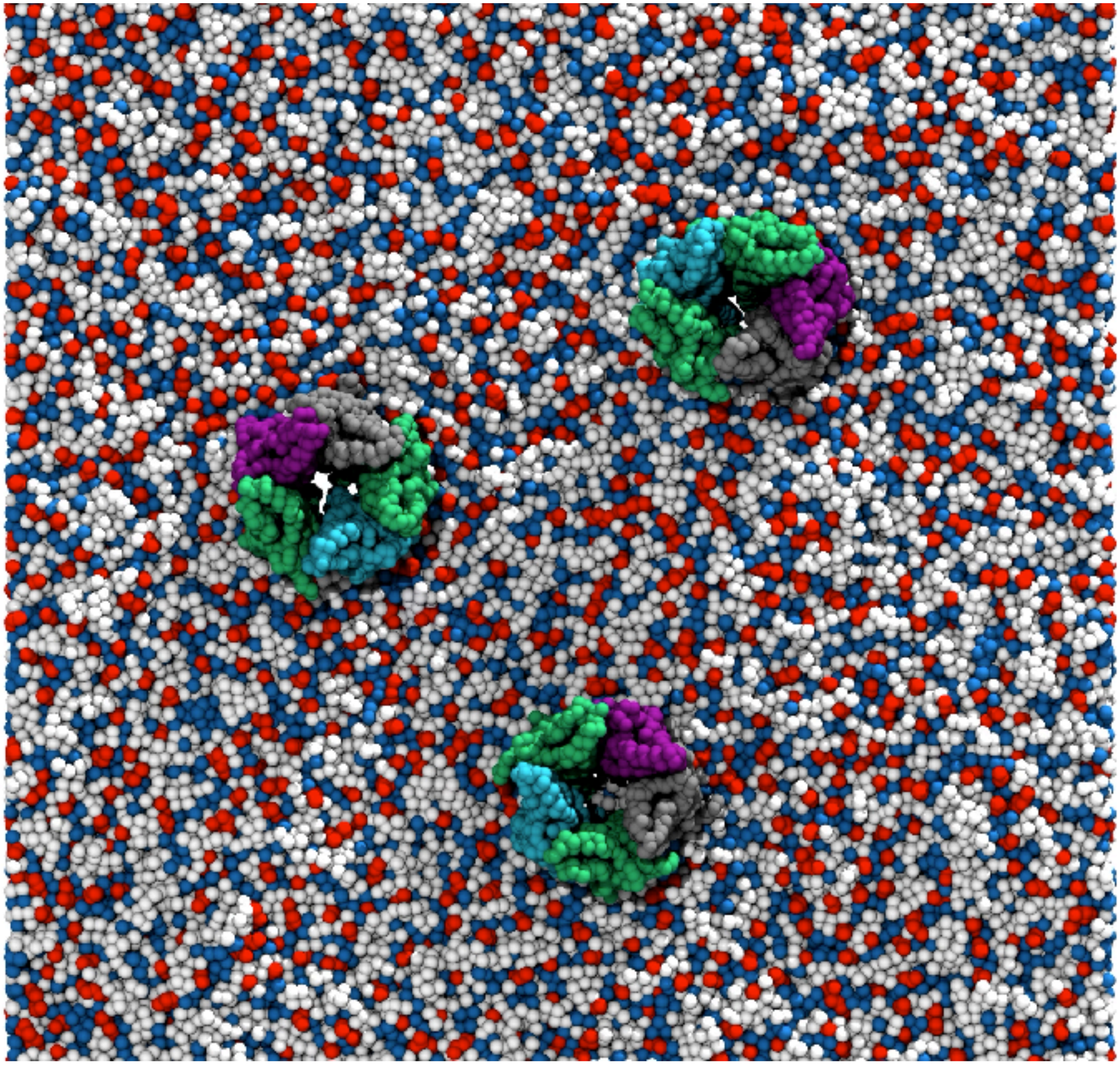


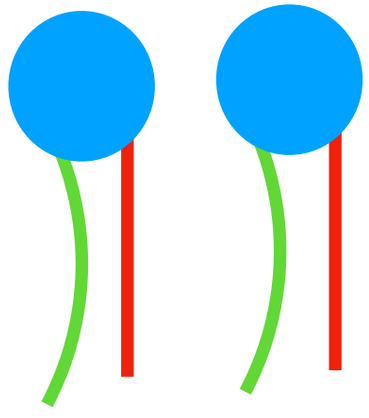
Hetero Membrane



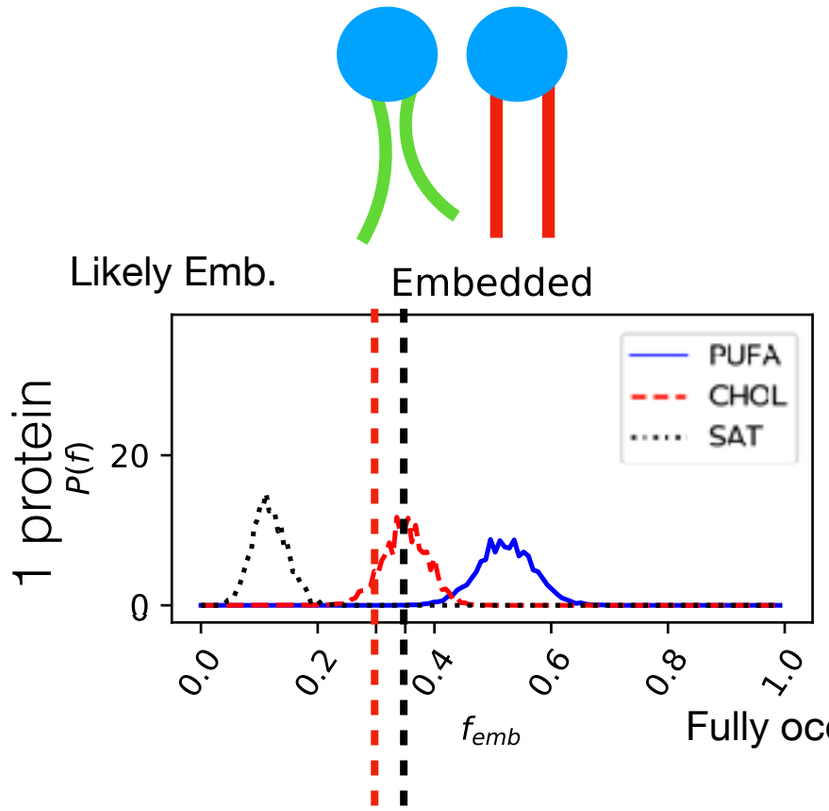
Collaboration 1: Purpose of The research



Hetero Membrane



Collaboration 1: Probability of Lipid Species Fraction Embedded and Annular Lipid Species (Homoacid)



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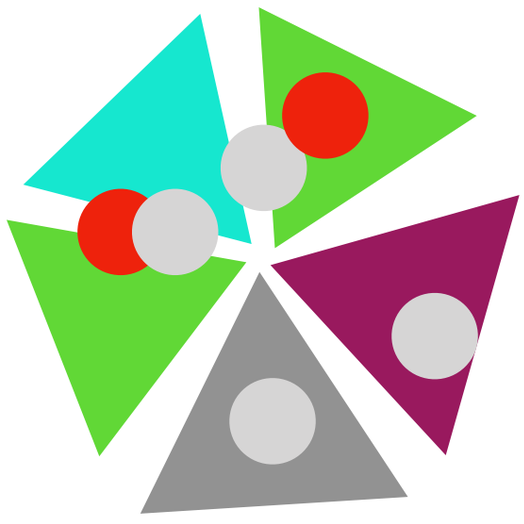
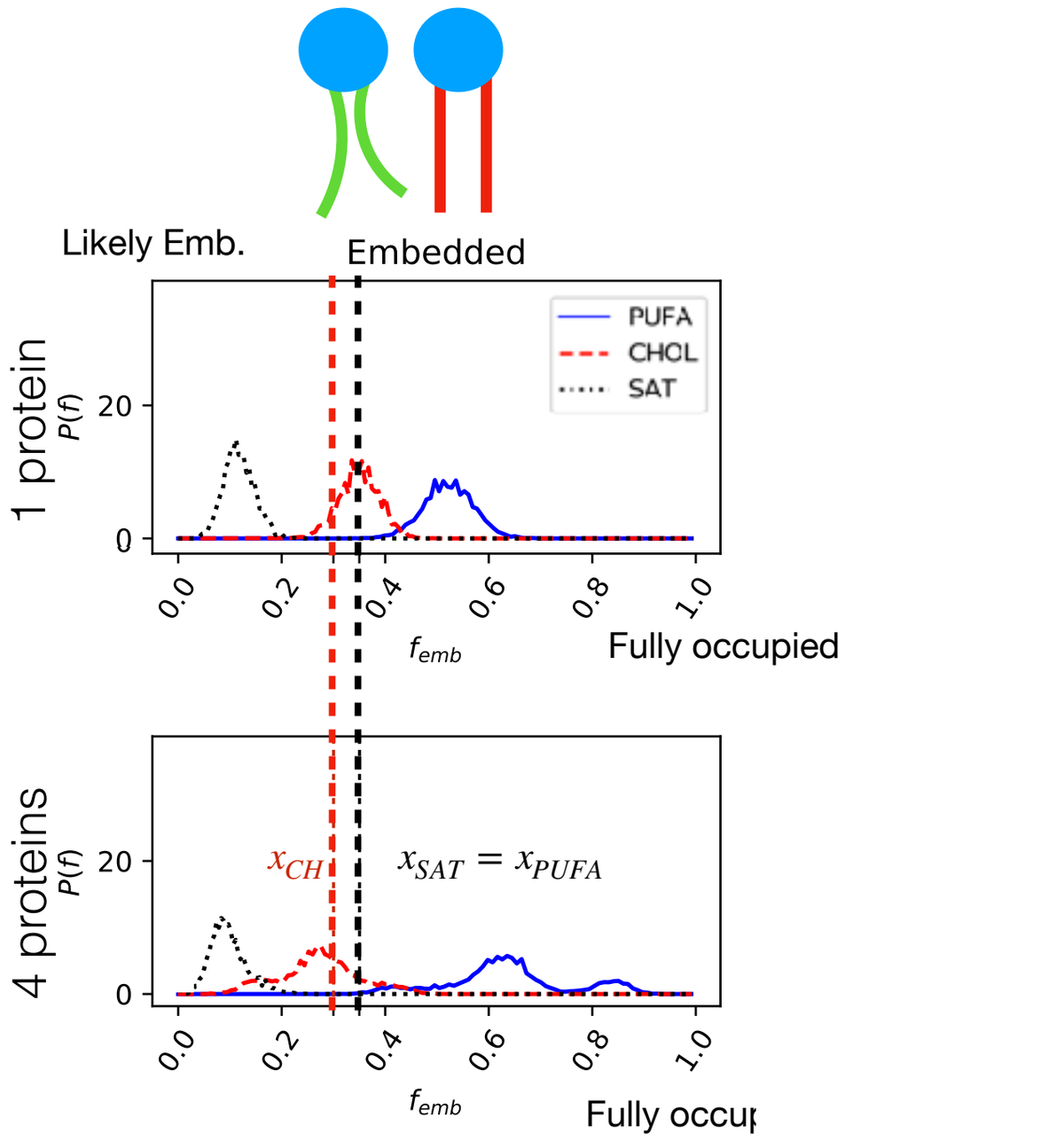


Alpha
Beta
Delta
Gamma

Expected Chol Fraction

Expected Phospholipid Fraction

Collaboration 1: Probability of Lipid Species Fraction Embedded and Annular Lipid Species (Homoacid)



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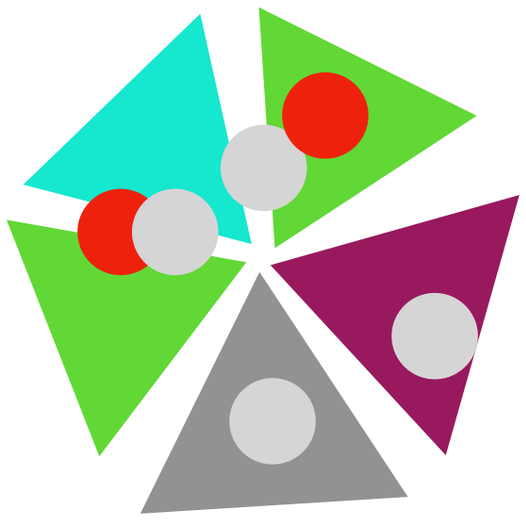

Alpha
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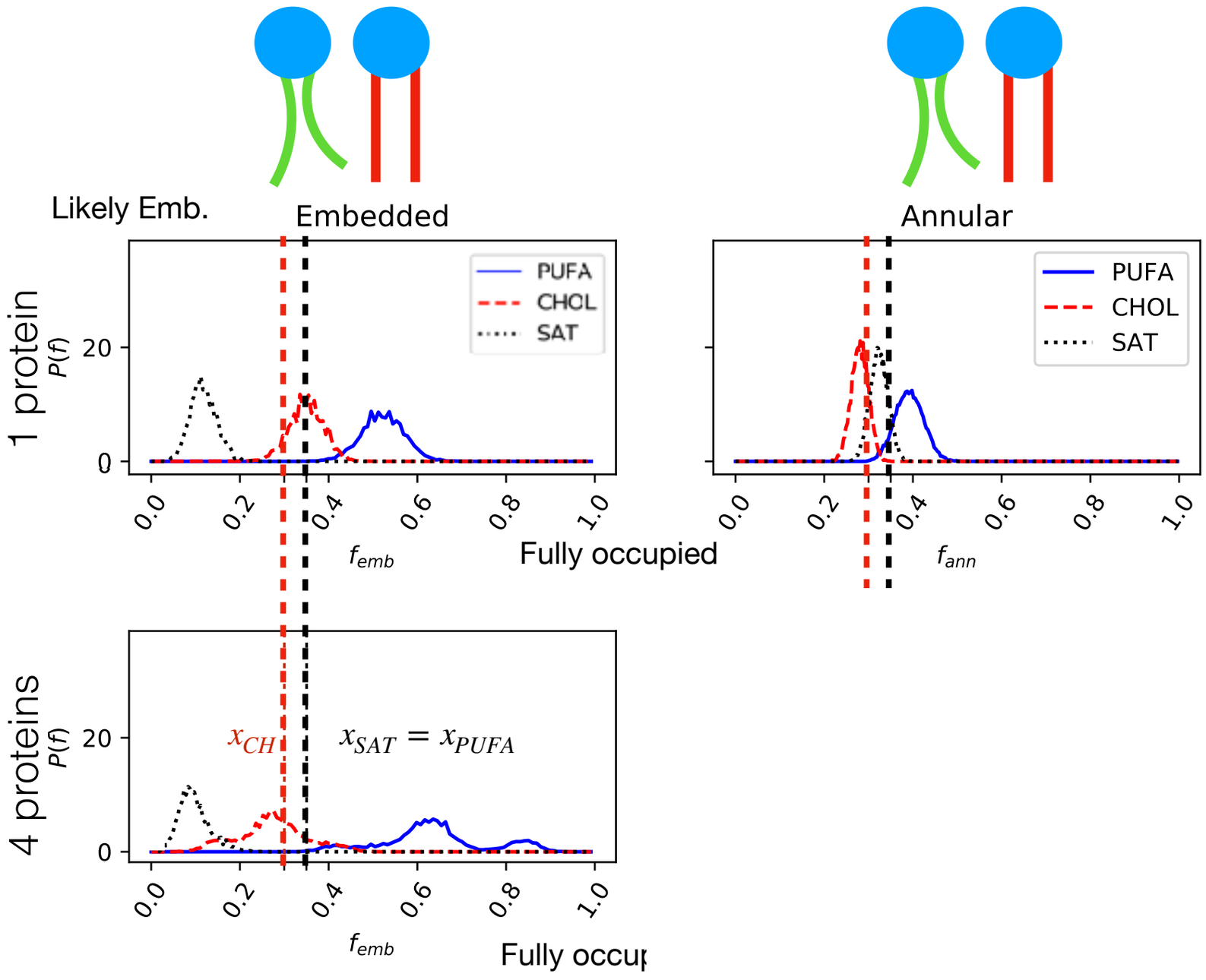


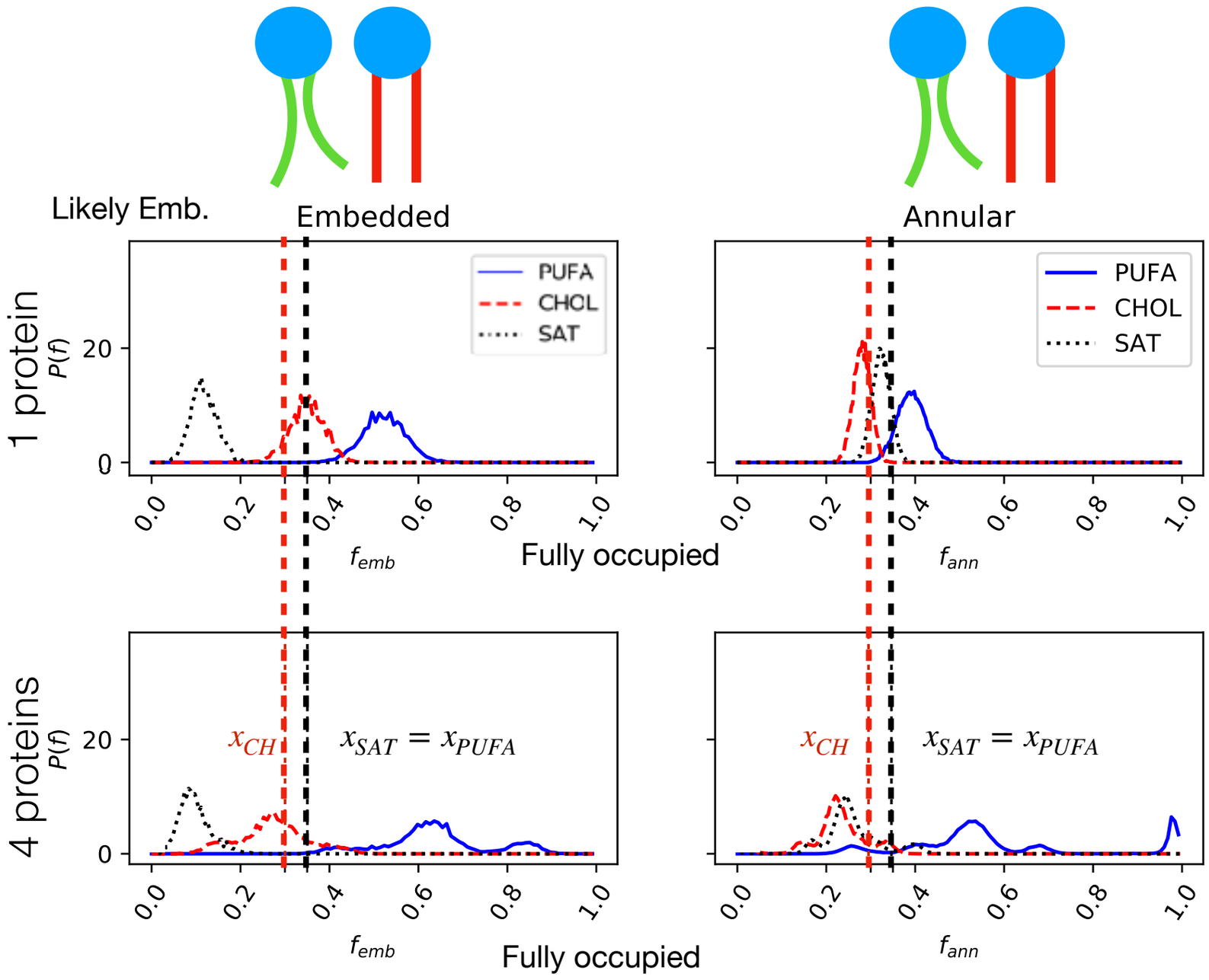
Expected Chol Fraction

Expected Phospholipid Fraction

Collaboration 1: Probability of Lipid Species Fraction Embedded and Annular Lipid Species (Homoacid)

Annular

Alpha
Beta
Delta
Gamma

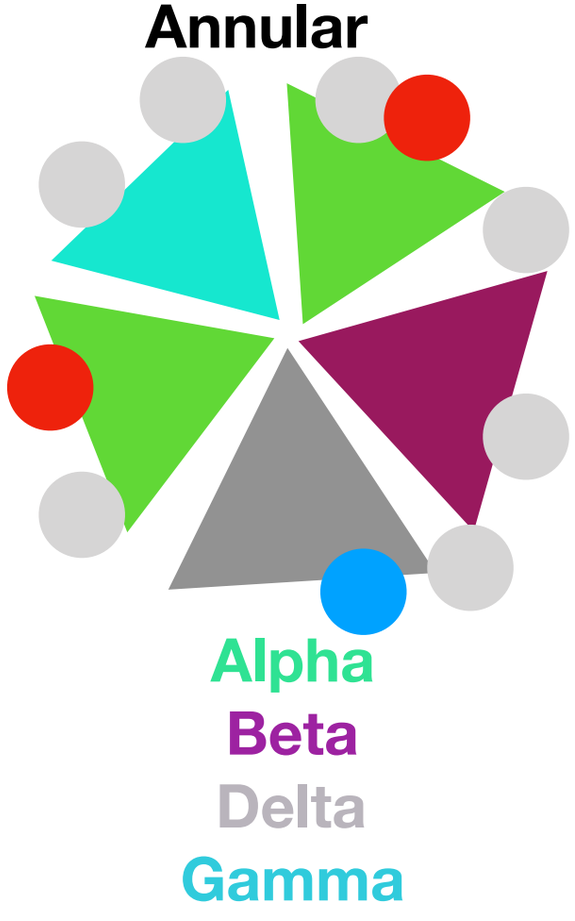


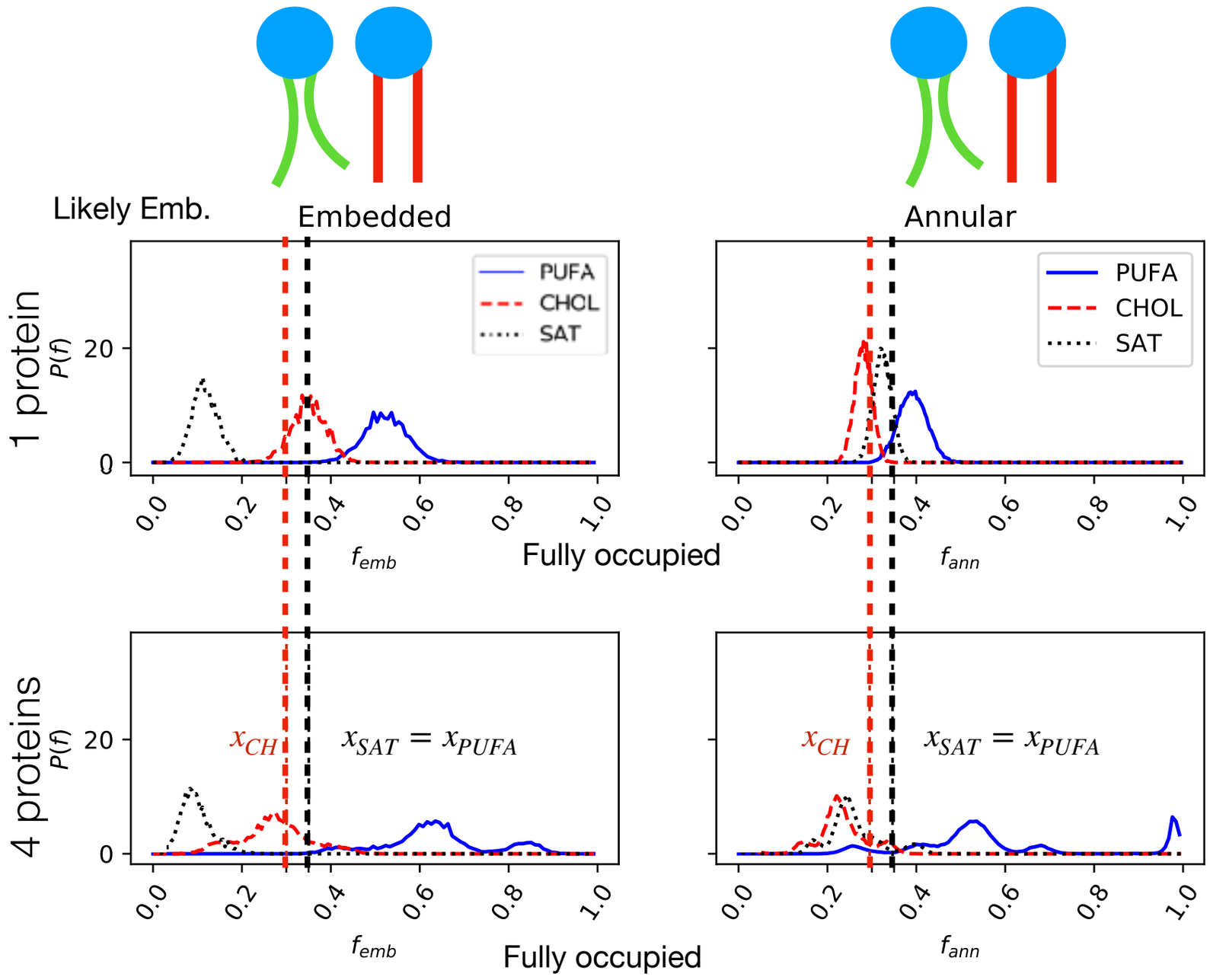
Expected Chol Fraction

Expected Phospholipid Fraction

Collaboration 1: Probability of Lipid Species Fraction Embedded and Annular Lipid Species (Homoacid)

PUFA occupies majority of protein and annulus regardless of protein number

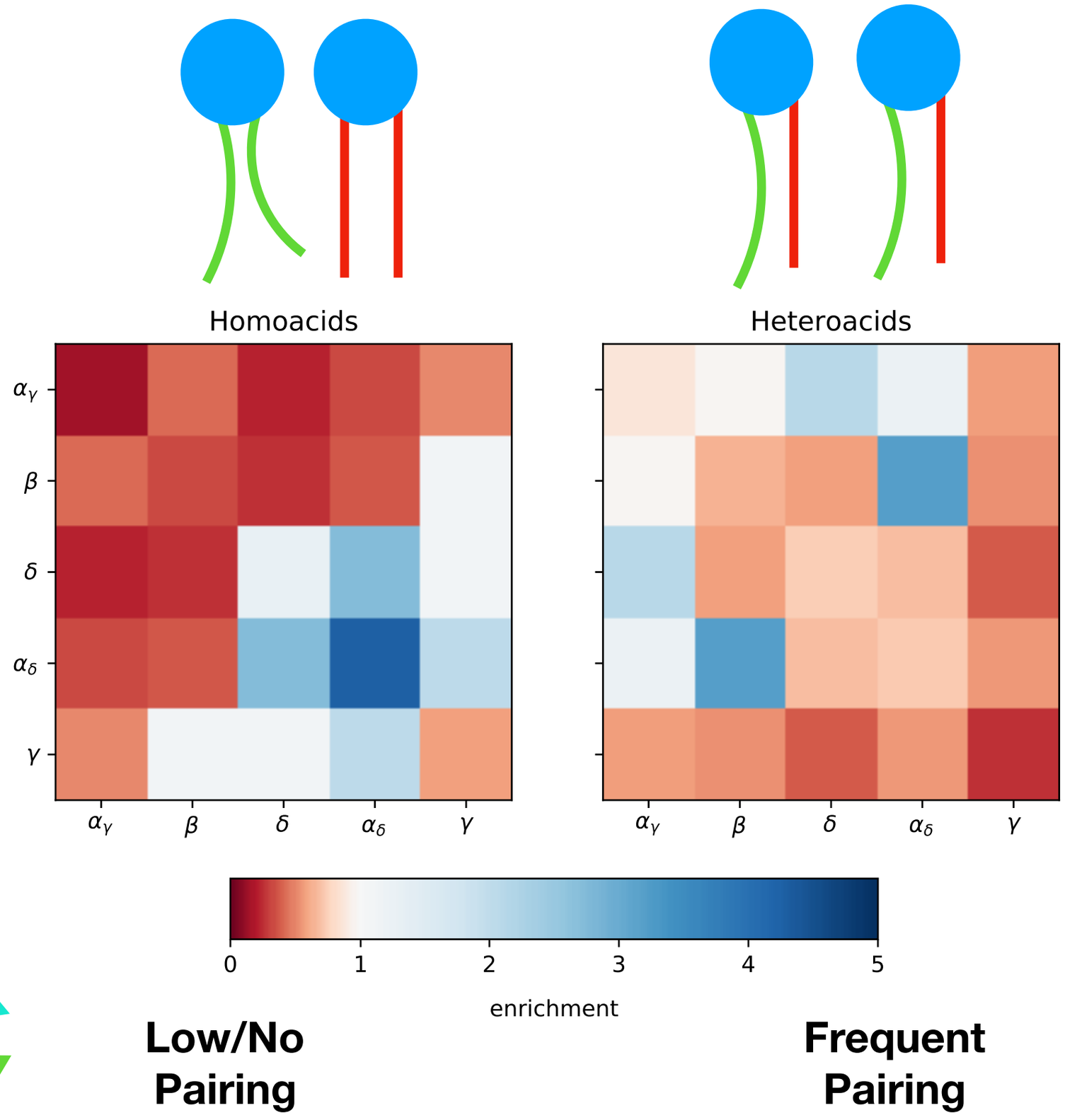


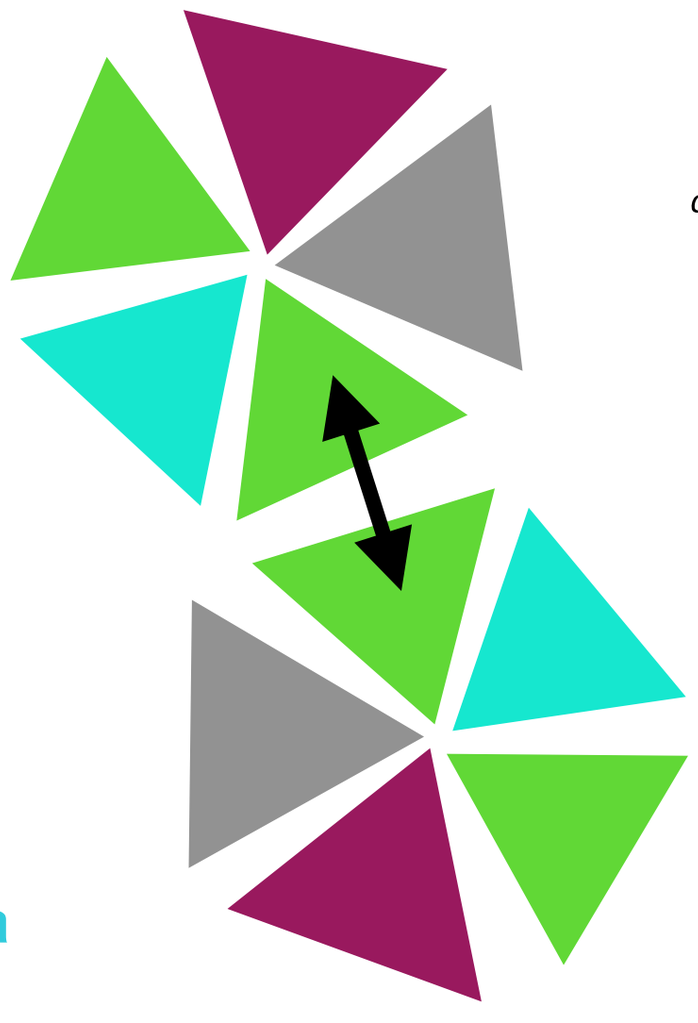


Expected Chol Fraction

Expected Phospholipid Fraction

Collaboration 1: Effect of Dimerization and Domain formation





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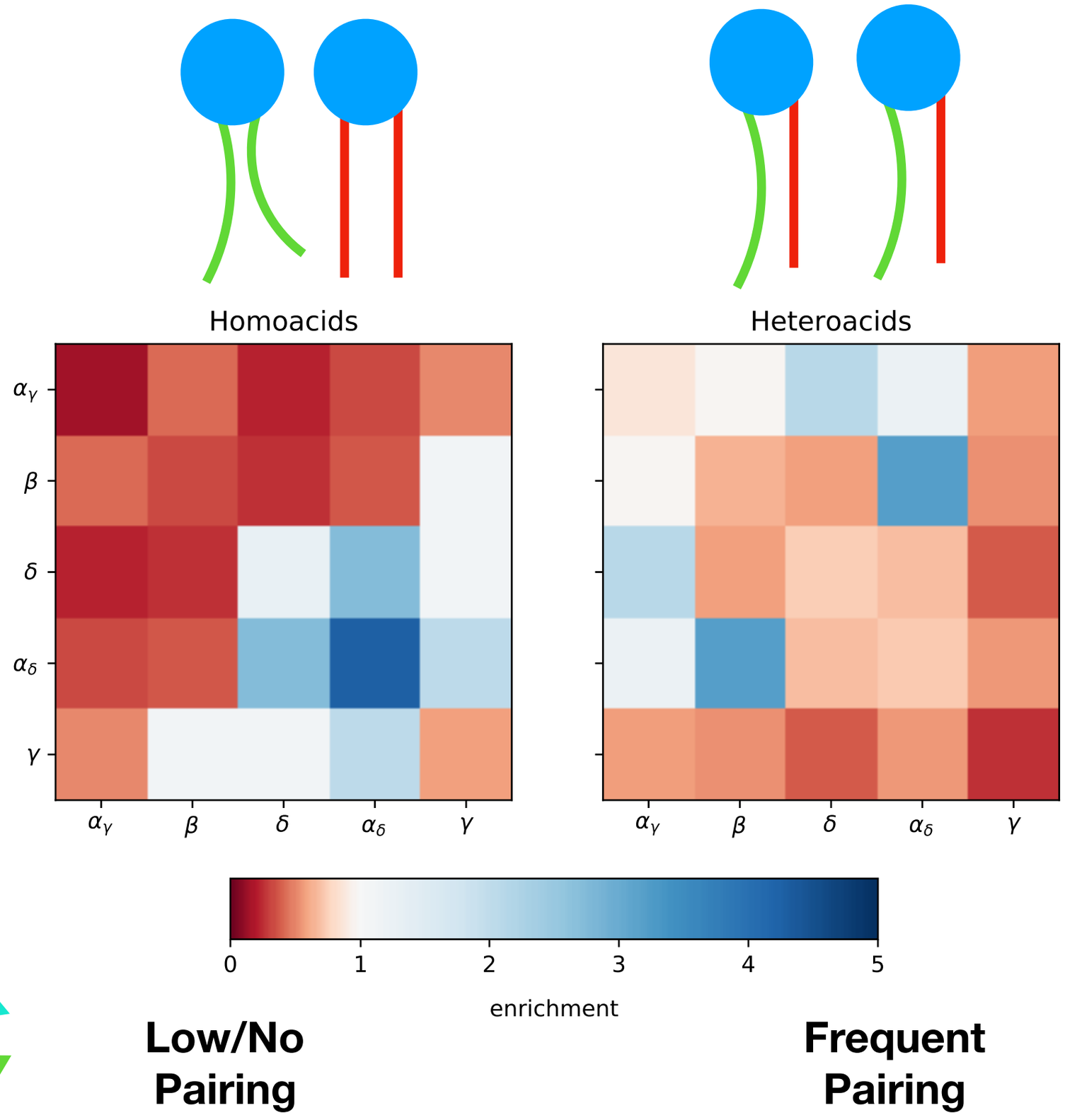
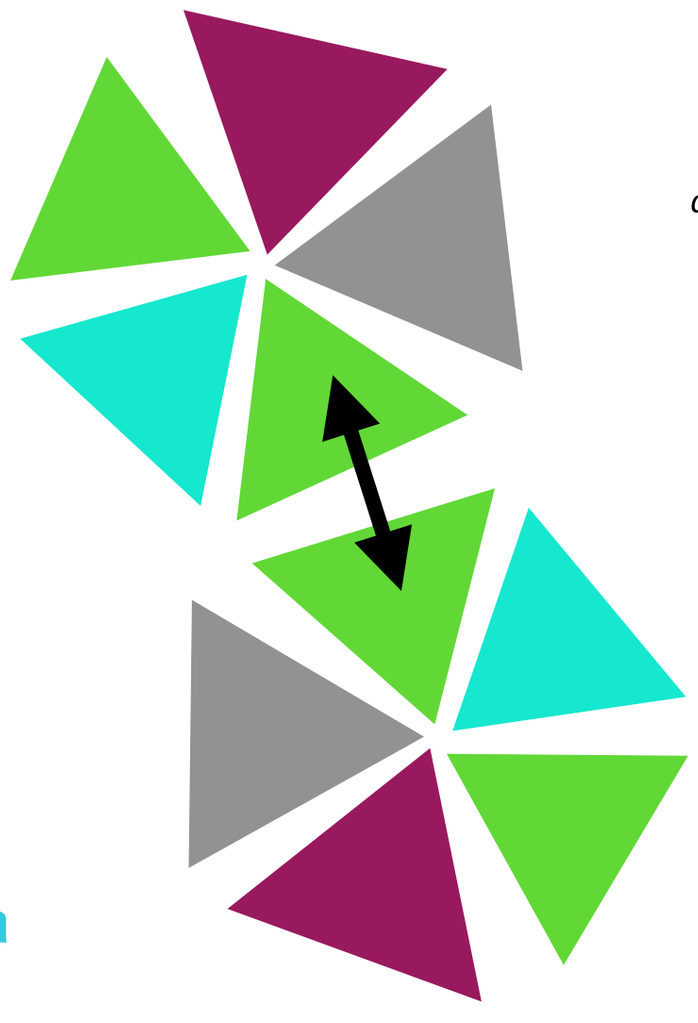




Collaboration 1

Untangling direct and domain-mediated interactions between nicotinic acetylcholine receptors in DHA-rich membranes

Kristen Woods* · **Liam Sharp*** · **Grace Brannigan†**

In preparation for resubmission

Collaborative with Dr. Cheng (Washington State at St. Louis):

Direct Binding of Phosphatidylglycerol at Specific Sites Modulates Desensitization of a Pentameric Ligand-Gated Ion Channel

[1]Ailing Tong, [1]John T. Petroff II, [2]Fong F. Hsu, [4]Liam Sharp, [3,4]Grace Brannigan, [1,*]Wayland W. L. Cheng

From the Departments of [1]Anesthesiology, and [2]Internal Medicine, Mass Spectrometry Resource, Division of Endocrinology, Diabetes, Metabolism, and Lipid Research, Washington University in St. Louis, MO, USA, and the [3]Department of Physics and [4]Center for Computational and Integrative Biology, Rutgers University, Camden, NJ, USA.

Collaboration 2: Purpose

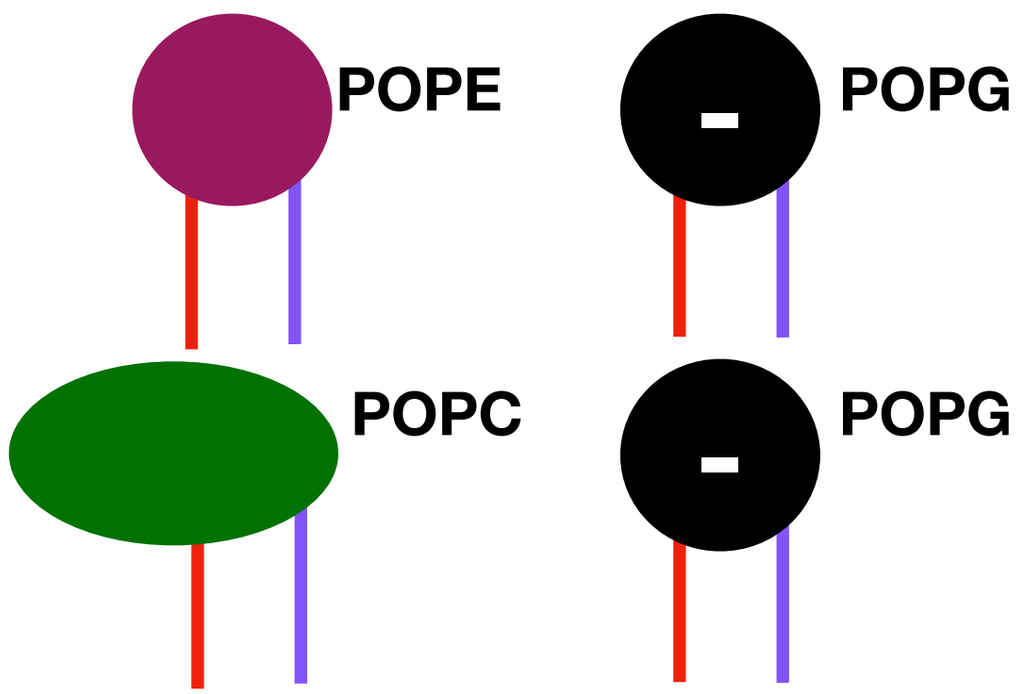
- Purpose: 1) Elucidate if phospholipids bind to ELIC 2) If phospholipids bind, do they modulate ELIC functionality

- Coarse Grained MD is used to better model lipid-ELIC interactions

- 2 Series of 15 simulations run for 15 μs each

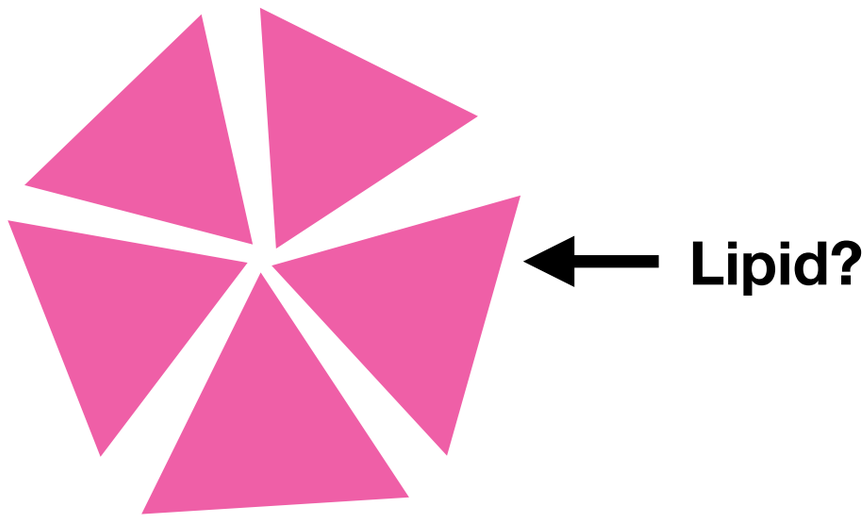
  - Series 1: POPE and POPG

  - Series 2: POPC and POPG



Collaboration 2: Purpose

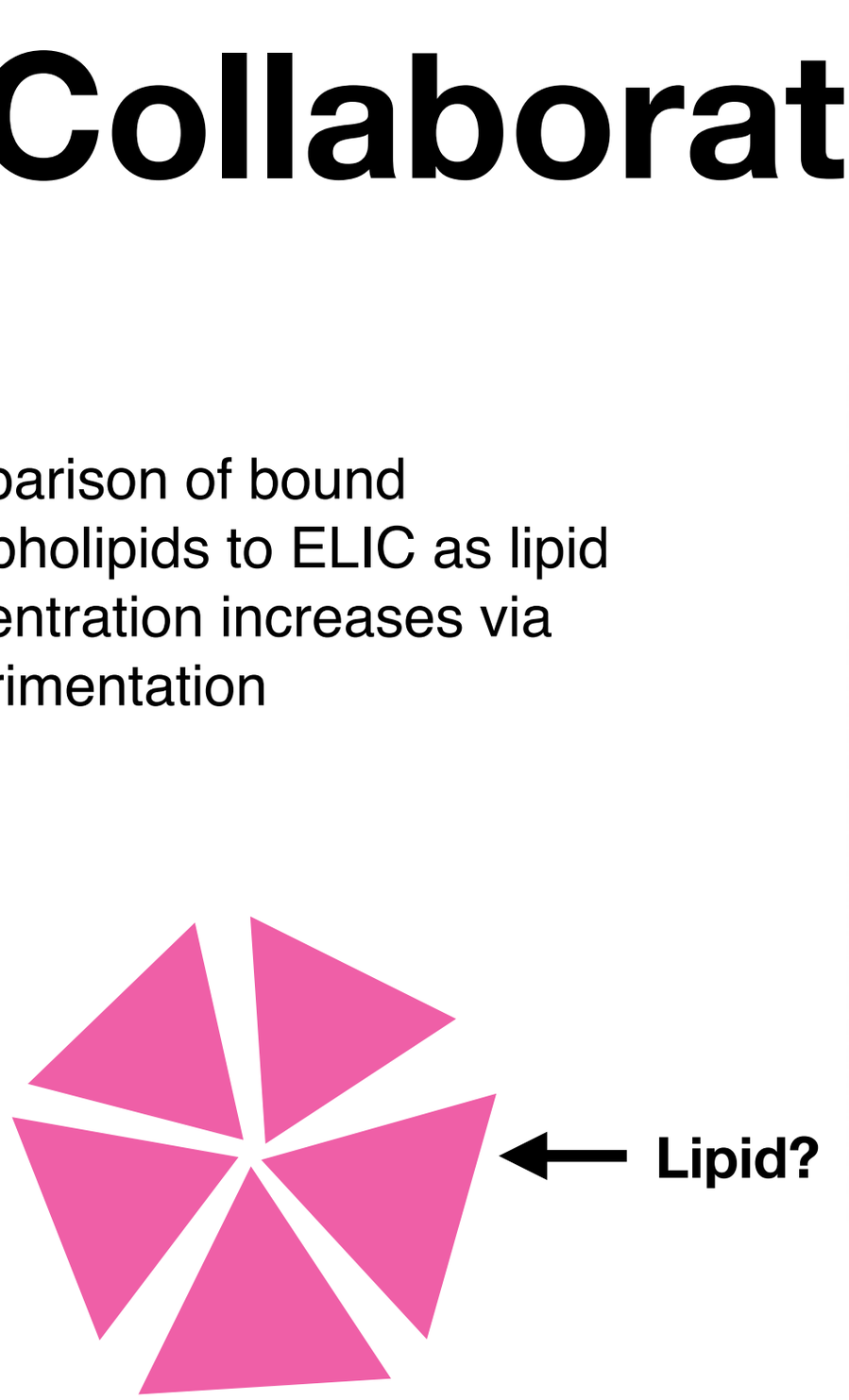
Comparison of bound phospholipids to ELIC as lipid concentration increases via experimentation

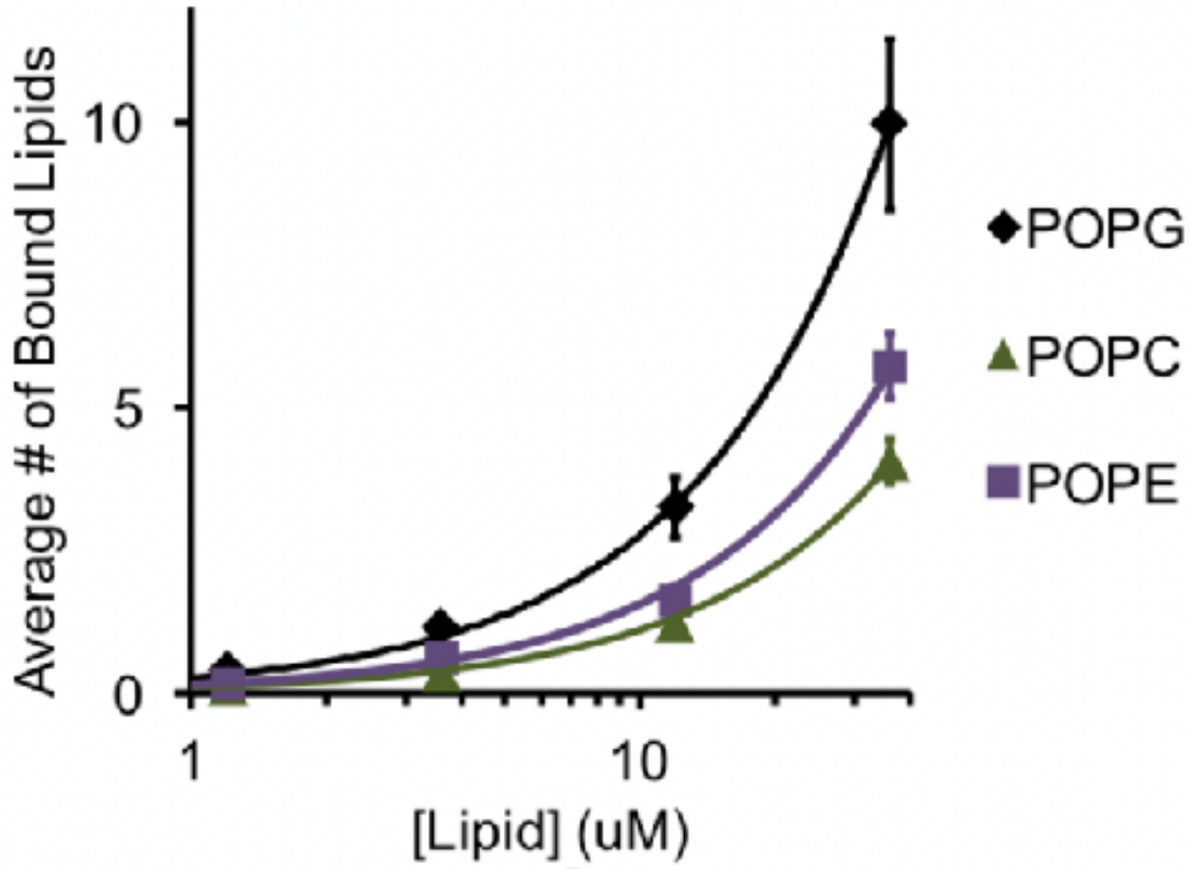


ELIC has a monopentamer

Collaboration 2: Purpose

Comparison of bound phospholipids to ELIC as lipid concentration increases via experimentation



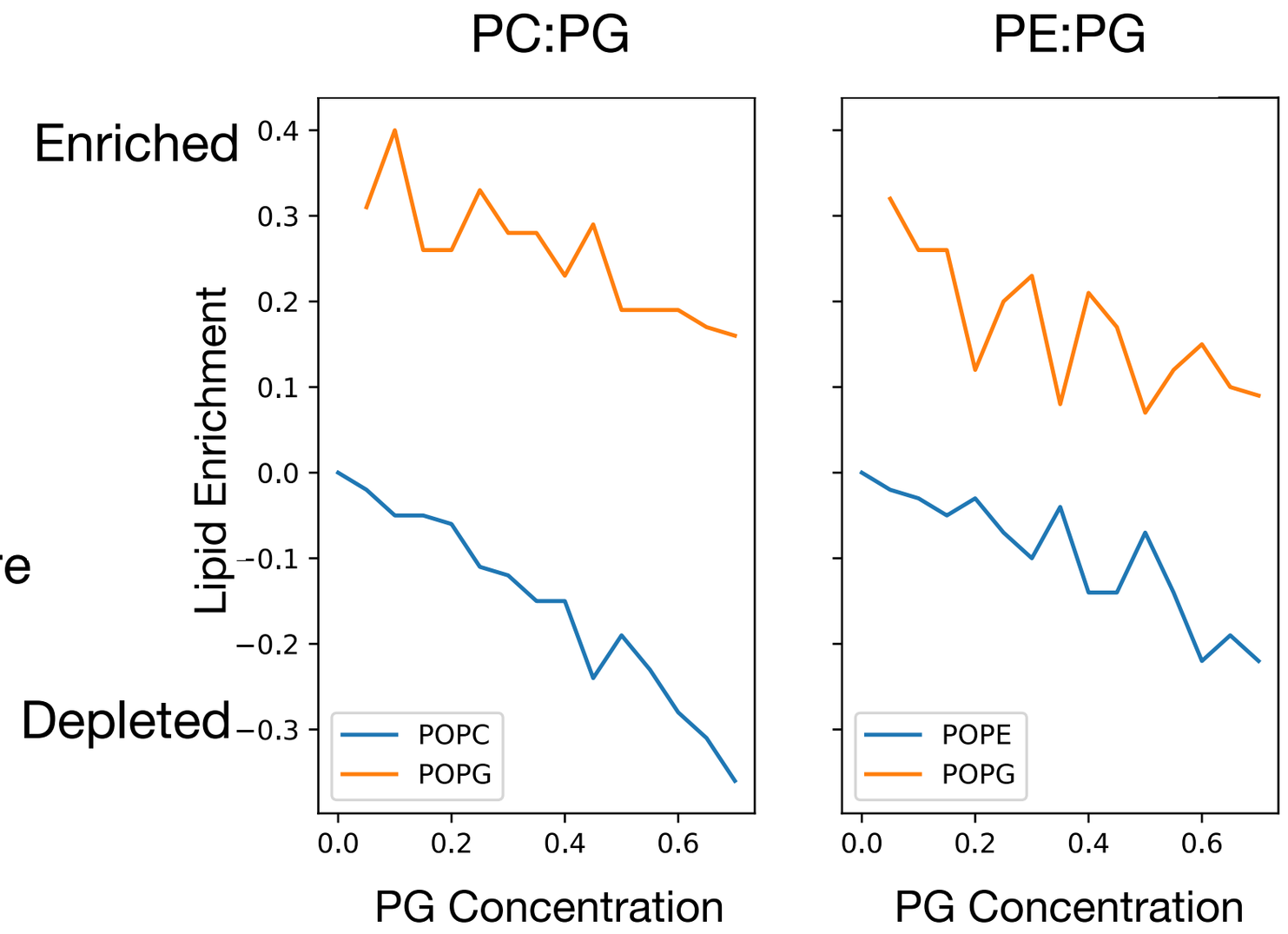


Direct Binding of Phosphatidyglycerol at Specific Sites Modulates Desensitization of Pentameric Ligan-Gated Ion Channels

ELIC has a monopentamer

Collaborative with Dr. Cheng (Washington State, Lt. Luis): *Direct Binding of Phosphatidyglycerol at Specific Sites Modulates Desensitization of Pentameric Ligan-Gated Ion Channels*

- POPG shows greatest enrichment at low concentrations of itself
  - Does not mean there are less lipids
  - More PC or PE

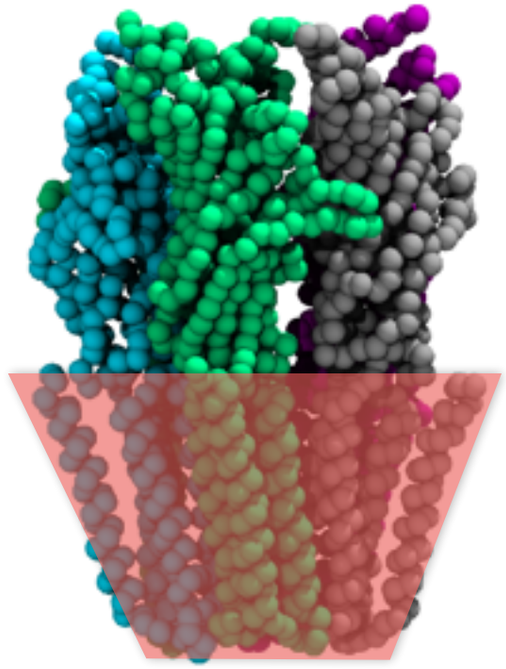


Future Work

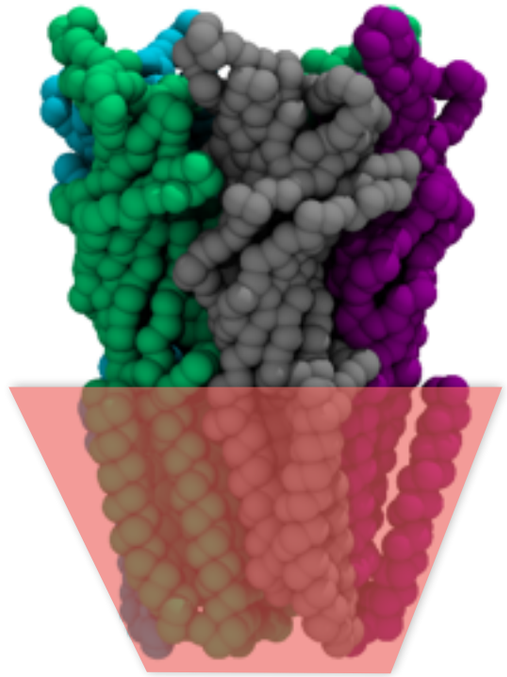
Aim 2

- **Investigation of the relative importance of pLGIC sequence versus shape in determining preferred lipid domain** This can be tested comparing effects on partitioning profiles upon mutation of lipid facing residues versus adjustments in membrane lipid composition. If the effect of the protein's sequence is measured to be greater than its shape, it is likely that pLGICs will display significant variation in partitioning behavior and annular lipid preferences. If the reverse is observed, it is likely that overall pLGIC shape and relative flexibility of domains drives partitioning; which may be shared across all pLGIC.

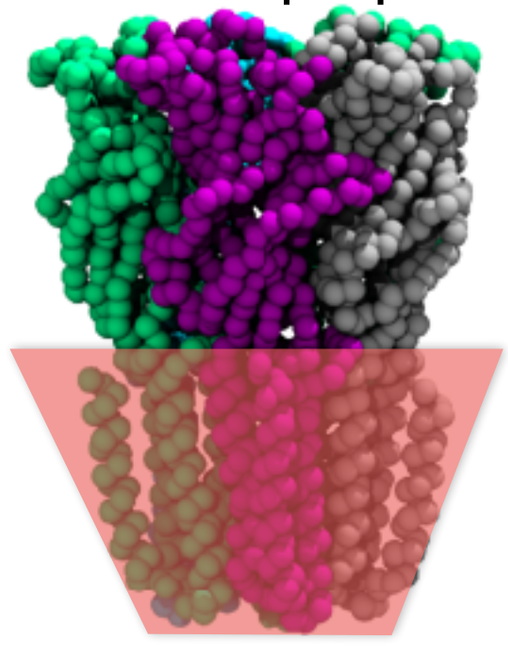
nAChR
2bg9



GLIC
4ilb



GABA(A)
$2\alpha_1 2\beta_3\gamma_2$



Thank you for your time