

Organization and elasticity of membranes containing pentameric-ligand gated ion channels

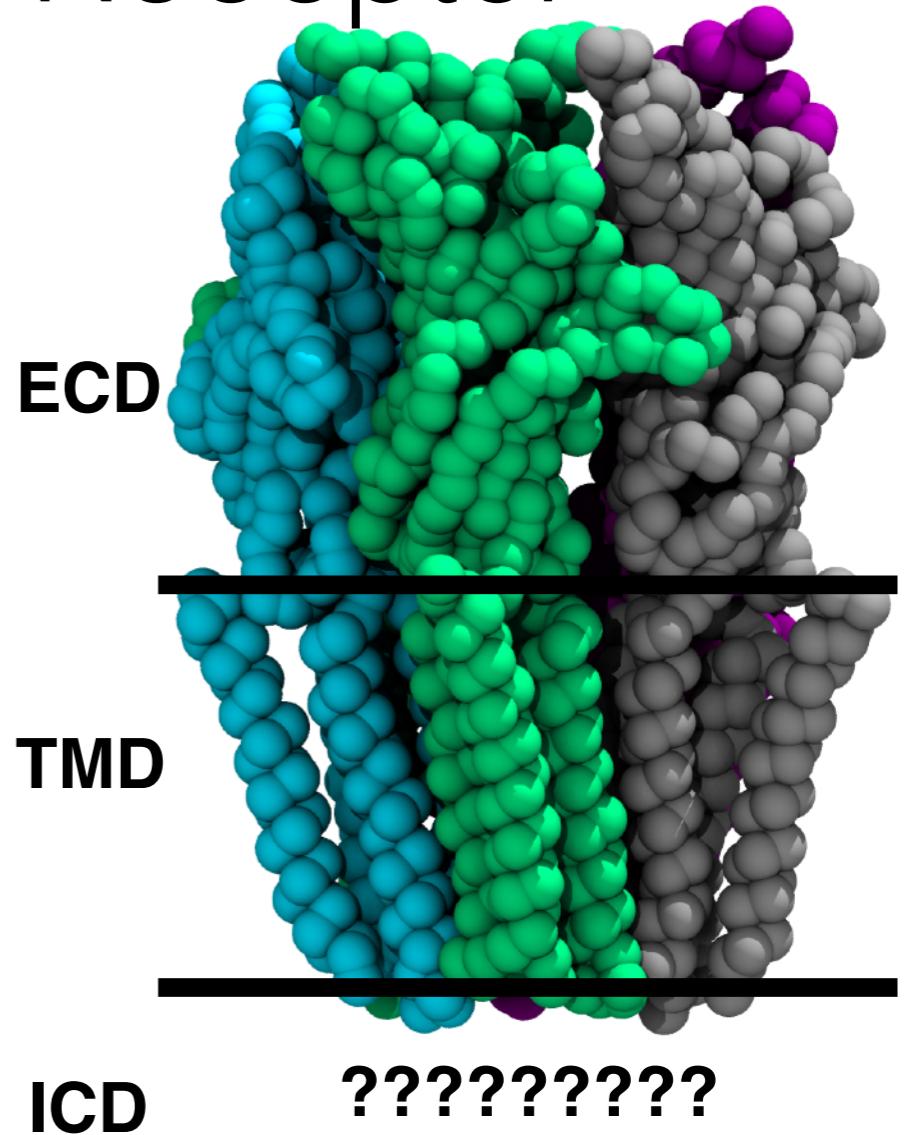
Liam Sharp

Outline of Talk

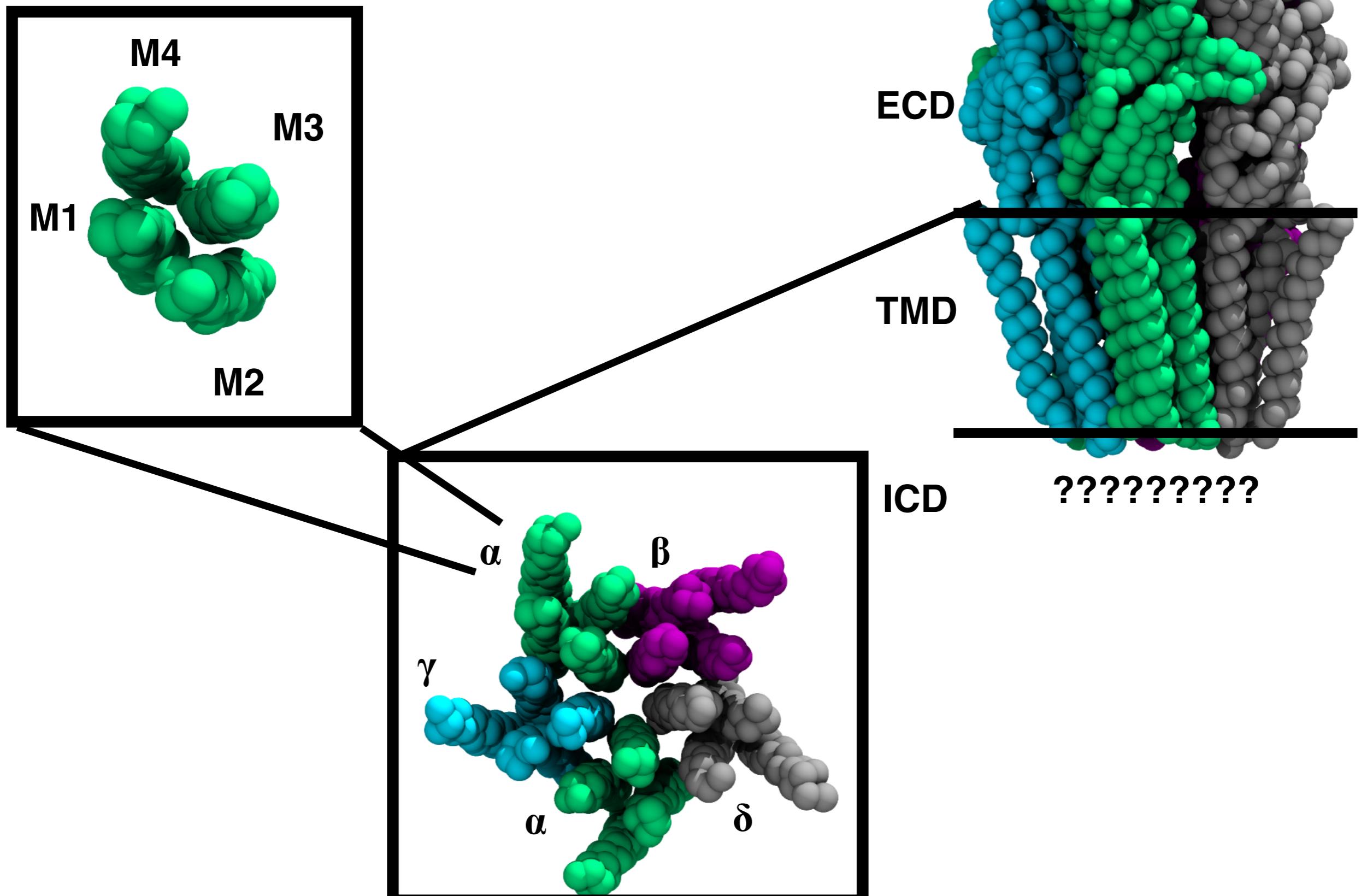
- Introduction
- Part 1: Preliminary Work
 - Methods
 - Results
 - Summary
- Part 2: Proposal
 - Aim 1
 - Aim 2
 - Aim 3

Nicotinic Acetylcholine Receptor (nAChR)

- 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

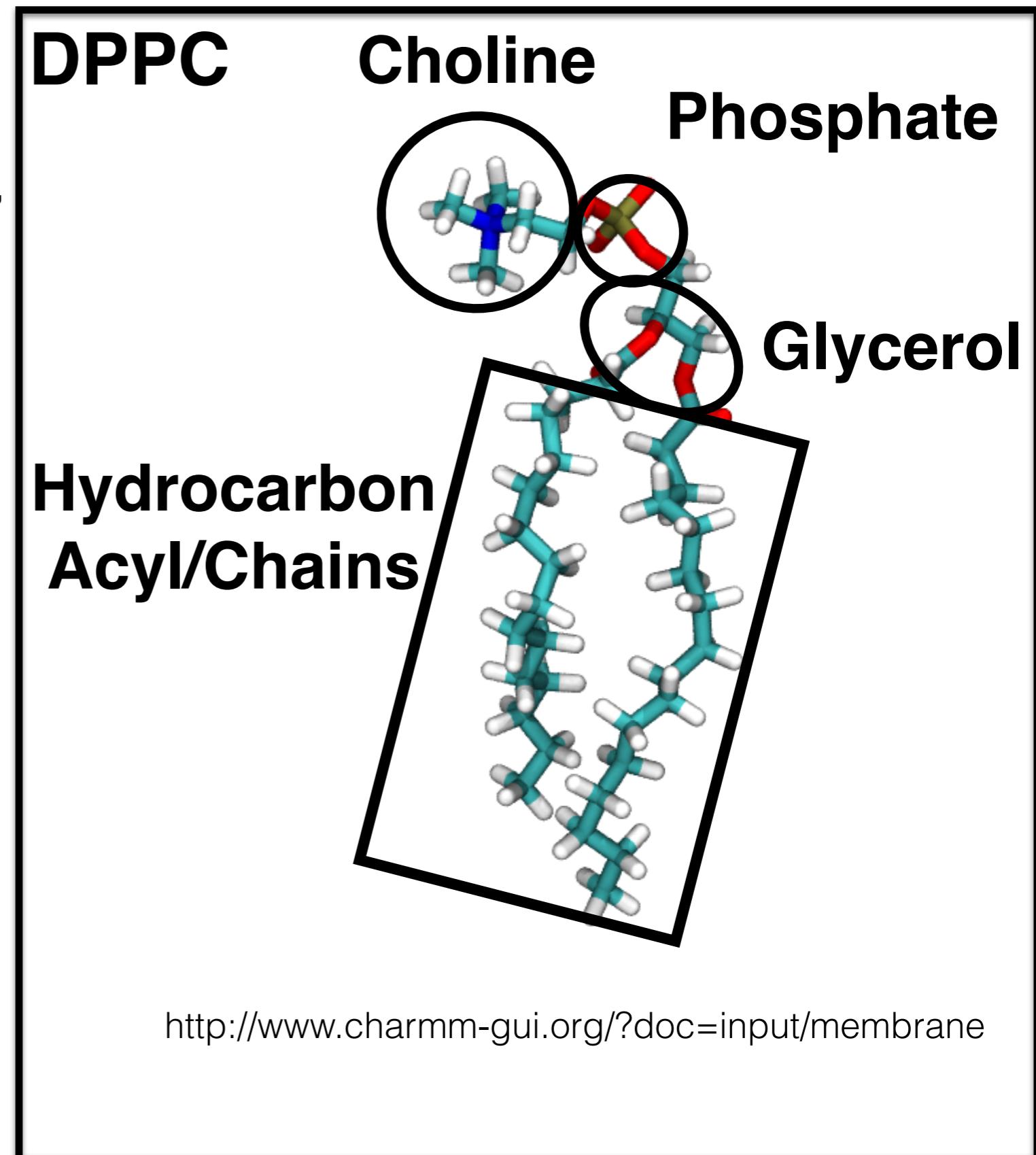


Nicotinic Acetylcholine Receptor (nAChR)



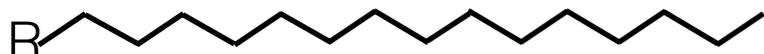
Phospholipids

- Hydrophilic Head
 - Choline (PC), ethanolamine (PE), serine (PS), phosphatidic acid (PA), inositol (PI)
 - Phosphate
- Glycerol backbone links the head group and acyl chains
- Hydrophobic Chains
 - Hydrocarbon chains of various lengths and hydrogen saturation
- Amphiphilic nature promotes spontaneous membrane formation



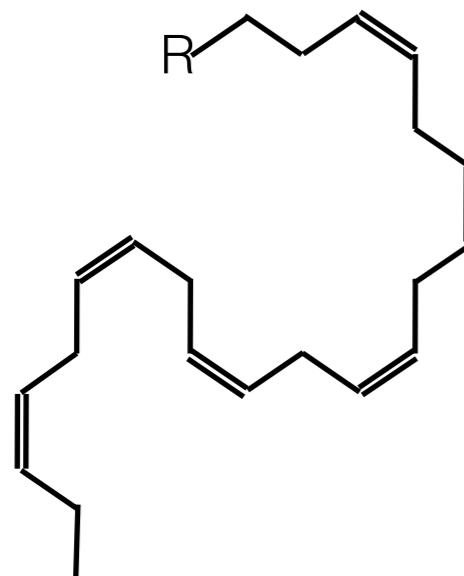
Phospholipids

Sat



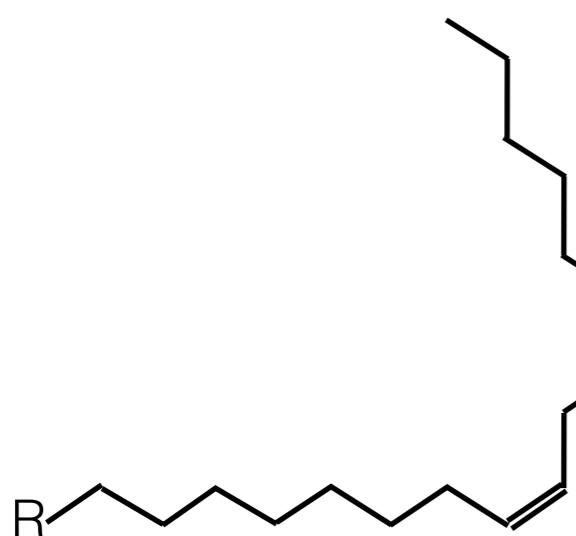
<https://pubchem.ncbi.nlm.nih.gov/compound/985#section=Top>

n-3



<https://pubchem.ncbi.nlm.nih.gov/compound/445580#section=Top>

n-6



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

- Focus on three acyl chains:

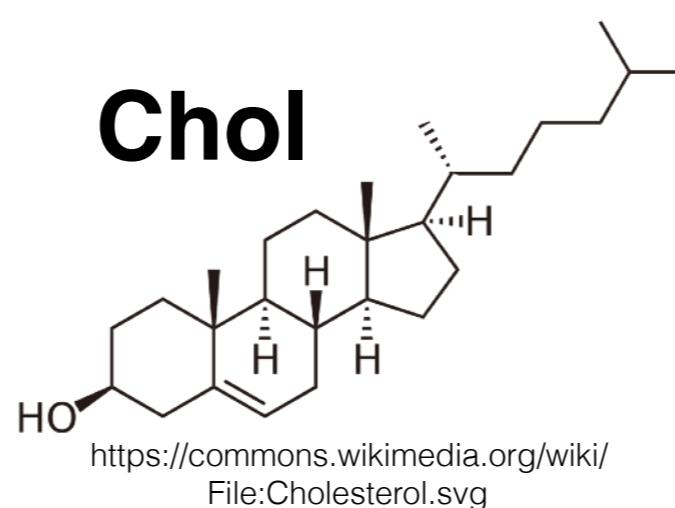
- Lipids with saturated fatty acid chains:
Palmitic acid 16:0 (PA)

- Lipids with poly-unsaturated fatty acid (PUFAs) chain:

- n-3
Docosahexaenoic acid 22:6 (DHA)

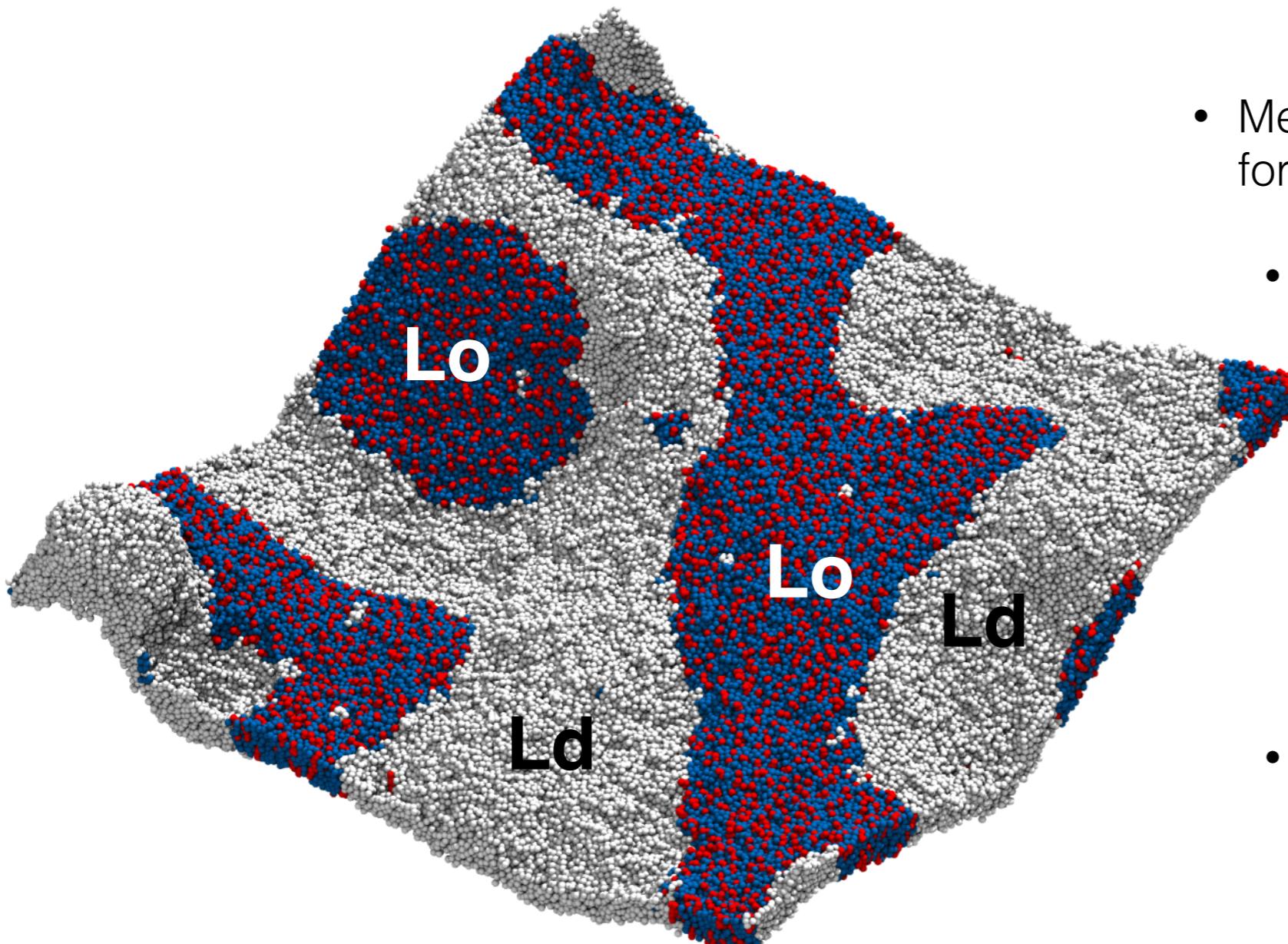
- n-6 Linoleic acid 18:2 (LA)

- Cholesterol



<https://commons.wikimedia.org/wiki/File:Cholesterol.svg>

Domain Formations



- Membranes with at least 3 lipids can form two distinct fluid phases
 - Liquid ordered (Lo):
 - Saturated Lipids and cholesterol
 - Stable, rigid, flat, low melting point
 - Historically called lipid rafts
 - Liquid disordered (Ld):
 - Unsaturated Lipids
 - Flexible, fluid, high melting point

Saturated Cholesterol Unsaturated

Domain Formations

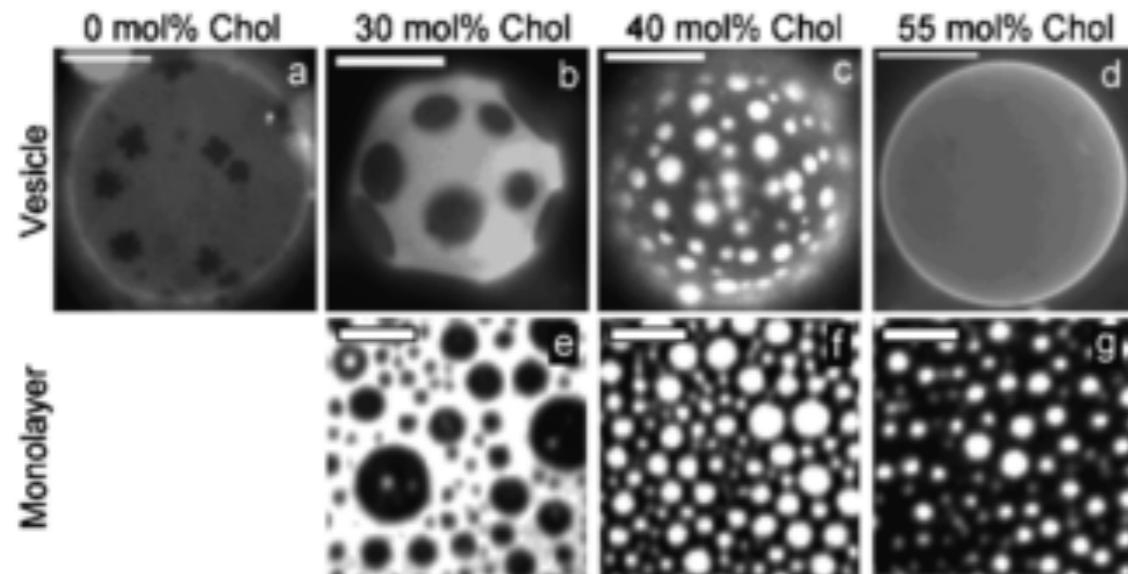


FIG. 1. Fluorescence micrographs of vesicles and monolayers of 1:1 di(18:1)PC/di(16:0)PC plus varying amounts of cholesterol. Scale bars are $20\text{ }\mu\text{m}$. Vesicles are $<5\text{ }^{\circ}\text{C}$ below their phase transition temperatures and exhibit either solid-liquid phase coexistence (a), liquid-liquid phase coexistence (b),(c), or no visible phase separation (d). Vesicle domains are not necessarily at equilibrium sizes and coalesce with time. Monolayers are at $(27 \pm 0.5)\text{ }^{\circ}\text{C}$ and below their phase transition surface pressures and exhibit liquid-liquid phase coexistence (e)–(g). Monolayer domain sizes do not significantly change on the time scale of experiments (minutes).

- Domain formation is observable experimentally

Organization in Lipid Membranes Containing Cholesterol

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(Received 13 June 2002; published 9 December 2002)

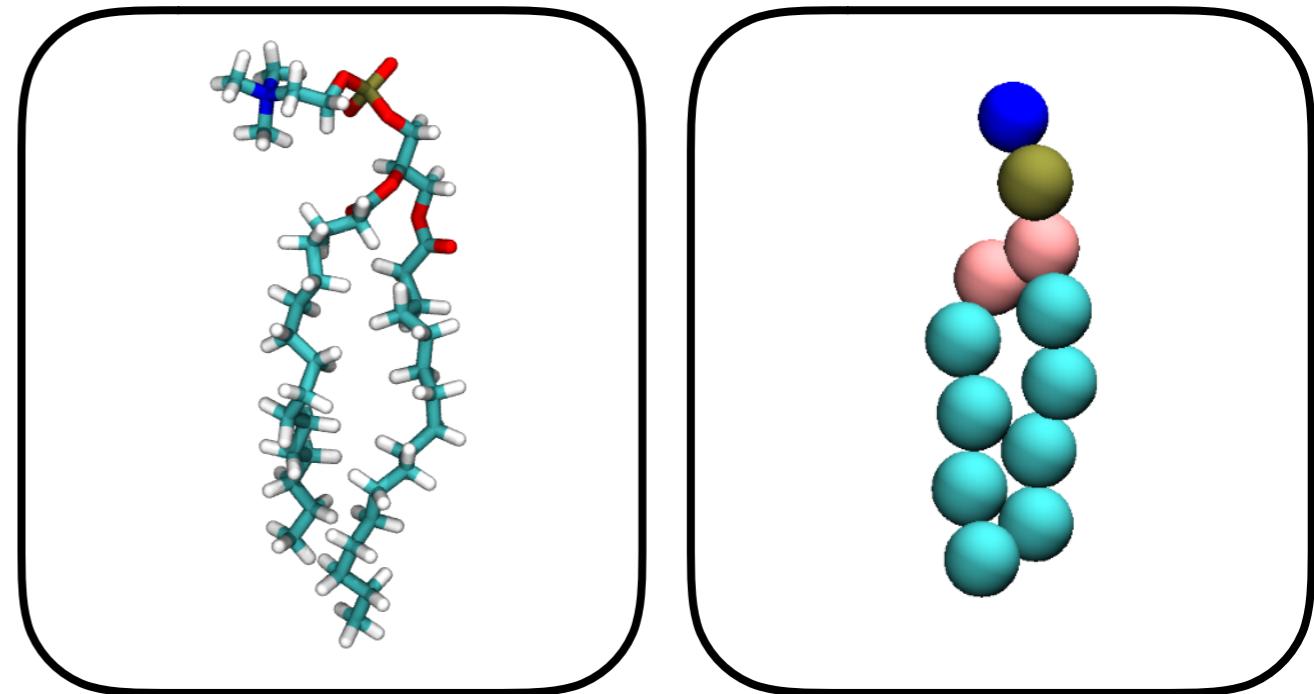
Part I

Preliminary Research:

- Method
- Can I confirm membrane domain formation?
- What are nicotinic acetylcholine receptors (nAChR) boundary lipids?
- If and where do lipids spontaneously 'embed' ?
- Is lipid binding subunit selective?

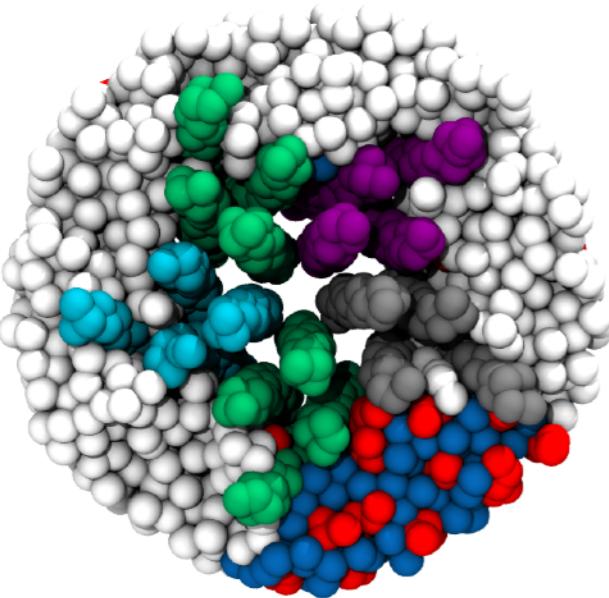
Methods

- Martini (ver 2.2) Coarse Grained Force Field
 - Fewer degrees of freedom
 - Decreased resolution
 - Longer time scales
 - Larger system
 - Domain Formation
- Martinize.py determine and converts secondary structure from atomistic to coarse grained
- Molecular Dynamics (Gromacs 5.0.6)
 - Gromacs maintains both secondary and tertiary structures
 - Harmonic bonds and elastic network



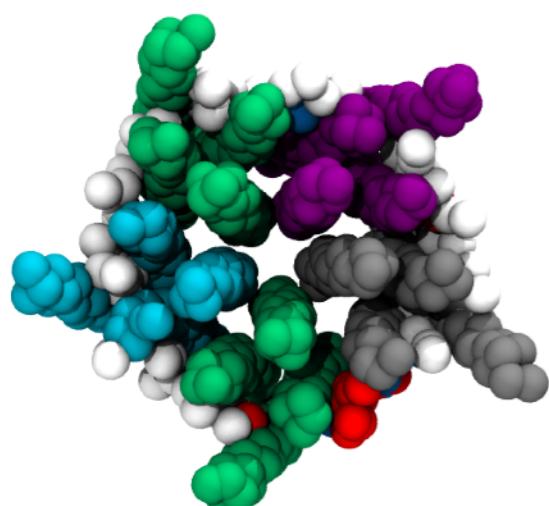
DPPC
Atomistic DPPC
 Coarse Grained

Methods: Why Coarse Grained?



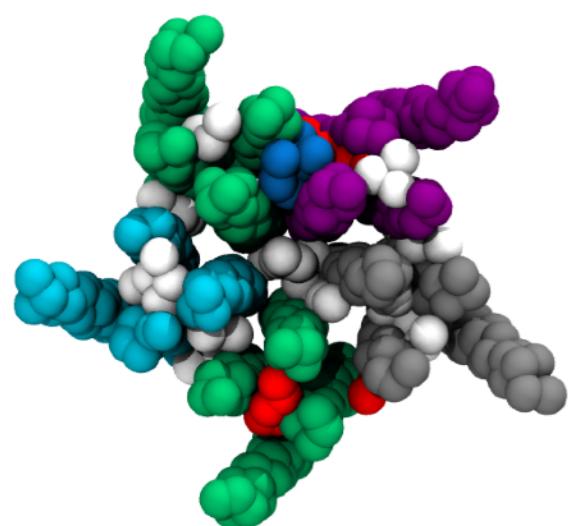
Boundary

- Coarse Grained molecular dynamic simulations let us see what optical microscopy cannot



Annular

- CG time scales allow for less expensive computation compared to atomistic



Embedded

- Easily observed domain formation and domain partitioning

This slide should show an example of functional dependence on cholesterol, like a graph from Criado et al or Dalziel et al. I think you have already done this in previous talks, haven't you?

Background

- Little information on nAChR-lipid interactions in **post synaptic-like membranes**
 - Confirm membrane domain formation
 - What are nicotinic acetylcholine receptors (nAChR) boundary lipids?
 - If and where do lipids spontaneously 'embed' ?
 - Is lipid binding subunit selective
- **Previous hypothesis:** nAChR is functional dependency on cholesterol suggesting nAChR partitions into cholesterol enriched domain
 - Marchand (2002), Zhu (2006), Campagna (2006) show nAChR clustering is dependent on lipid rafts

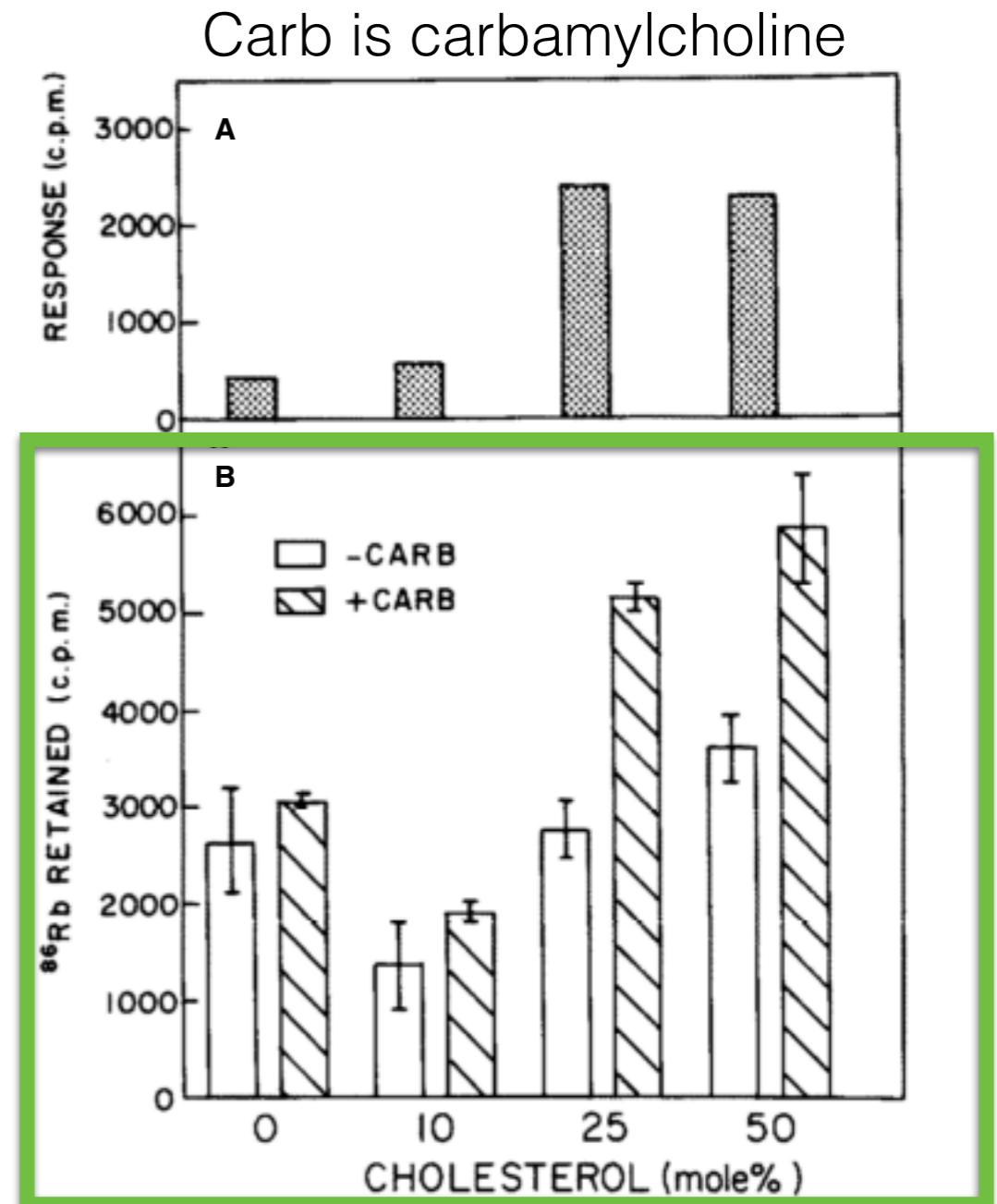


Fig.1. Effect of supplementing AChR-reconstituted vesicles with cholesterol on agonist-induced $^{86}\text{Rb}^+$ influx. (A) $^{86}\text{Rb}^+$ influx response to carb. ($\text{cpm}(+\text{ carb.}) - \text{cpm}(-\text{ carb.})$). (B) Total counts retained by Millipore filters. Counts obtained by filtering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

nAChR in Experimental Membranes

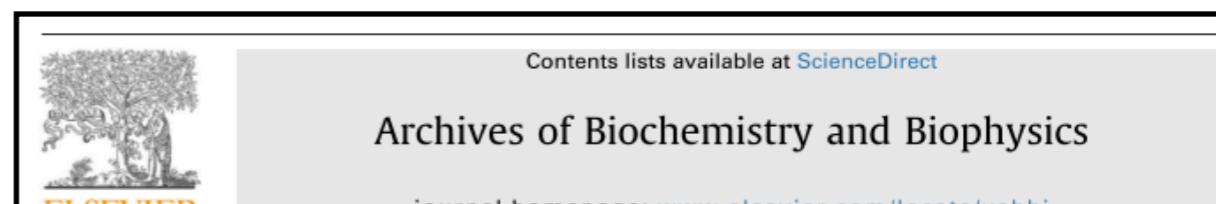
- nAChR tends to be placed in non-native membranes
 - Oocytes
 - Computation relies on DOPC/POPC
- Essentially: missing PUFAs



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

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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, Buenos Aires, Argentina



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

Vanesa L. Perillo ^{a,b,1}, Daniel A. Peñalva ^{a,b}, Alejandro J. Vitale ^{b,c}, Francisco J. Barrantes ^d, Silvia S. Antolini ^{a,b,*}

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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}

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^cCollège de France, F-75005 Paris, France; ^dDepartment of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA; ^eUnité de Biologie Physico-Chimique, Unité Propre de Recherche 9080, Centre National de la Recherche Scientifique, Paris, France

Internal Dynamics of the Nicotinic Acetylcholine Receptor in Reconstituted Membranes[†]

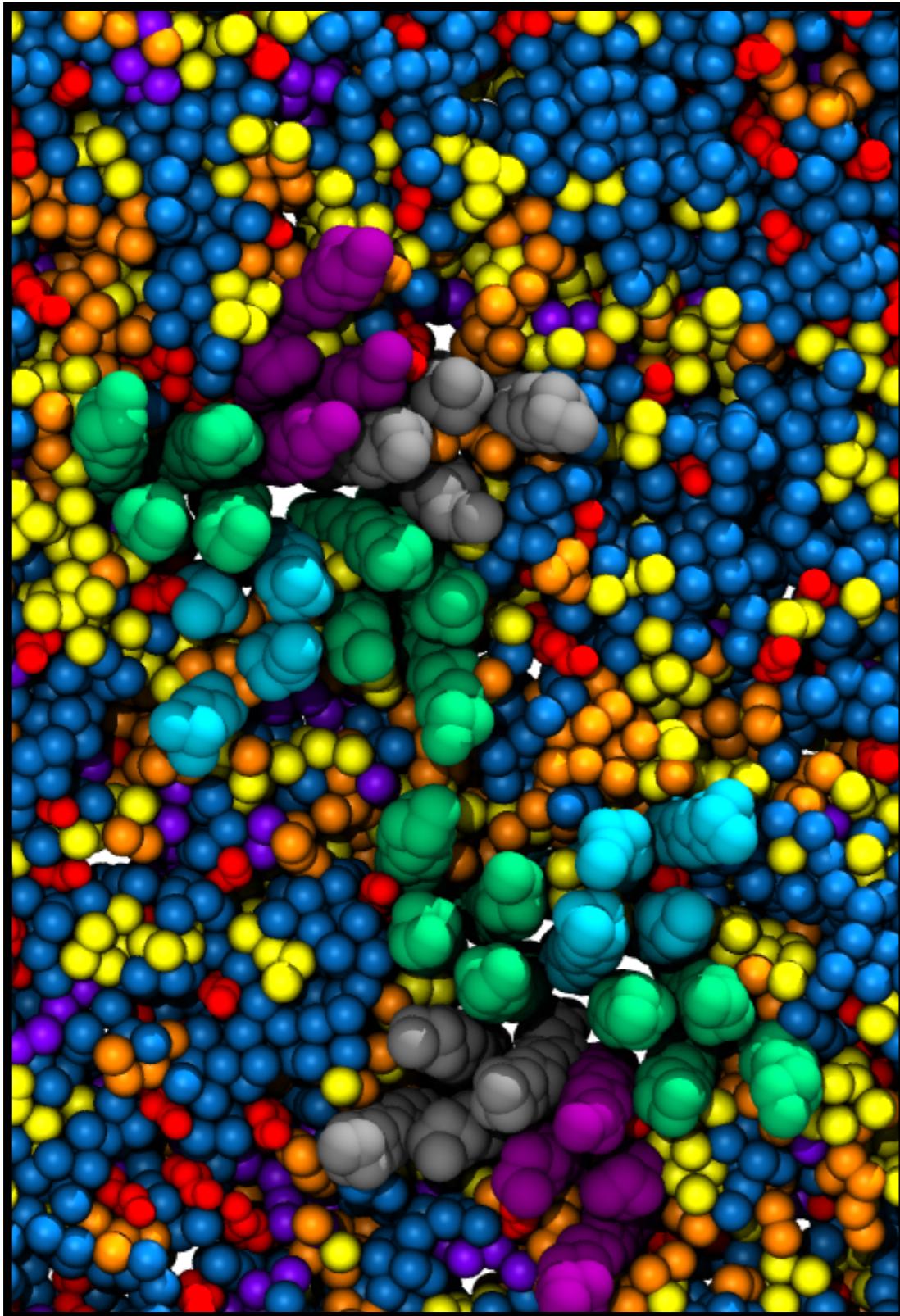
John E. Baenziger,* Tim E. Darsaut, and Mary-Louise Morris

ogy, and Immunology, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5
ry 26, 1999; Revised Manuscript Received March 8, 1999

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}

nAChR within Oocytes



- nAChR is frequently used in electrophysiology studies with *Xenopus* oocyte cell line
 - Rich in saturated- and sphingo-lipids
 - Poor in n-3 and cholesterol

nAChR and Membrane Compositions

- Native nAChR membranes

- Torpedo* (similar to neuromuscular junction)



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

| Saturation/Head Group | <i>Torpedo</i> | <i>Synapse</i> |
|-----------------------|----------------|----------------|
| n-0 | 59 | 52 |
| n-9 | 14 | 15 |
| n-7 | < 1 | < 1 |
| PUFA | 28 | 33 |
| — n-3 | — 19 | — 18 |
| — n-6 | — 9 | — 15 |
| PC | 43 | 43 |
| PE | 36 | 32 |
| PS | 13 | 13 |
| SM | 4 | 4 |
| PI | 3 | 8 |
| PA | 0 | 0 |
| Other | 1 | < 1 |
| Chol Mol Frac | 32 | 39 |

- Synaptic

- 1) W. Breckenridge, I. Morgan, J. Zanetta, G. Vincendon, Adult rat brain synaptic vesicles II. lipid composition, *Biochimica Et Biophysica Acta Bba - Gen Subj* 320(1973) 681–686.
- 2) C. Cotman, M. Blank, A. Moehl, F. Snyder, Lipid com- position of synaptic plasma membranes isolated from rat brain by zonal centrifugation, *Biochemistry-us* 8 (1969) 4606–4612.
- 3) F. Barrantes, The lipid environment of the nicotinic acetylcholine receptor in native and reconstituted mem brane, *Critical reviews in biochemistry and molecular...* (1989).
- 4) O. Quesada, F. Carol, M. Ferrer, S. J. O, F. Emily, J. Mercado, A. Dávila, R. Morales, L. J. A, Uncovering the lipidic basis for the preparation of functional nicotinic acetylcholine receptor detergent complexes for structural studies, *Sci Reports* 6 (2016) 32766.
- 5) W. G. Hill, N. M. Southern, M. Bryce, E. Potter, G. Apodaca, C. P. Smith, M. L. Zeidel, Isolation and characterization of the xenopus oocyte plasma mem- brane: a new method for studying activity of water and solute transporters., *Am. J. Physiol. Renal Physiol.* 289 (2005) F217–24
- 6) C. Regost, J. Arzel, M. Cardinal, G. Rosenlund, S. Kaushik, Total replacement of fish oil by soybean or linseed oil with a return to fish oil in Turbot (*Psetta maxima*), *Aquaculture*. 220 (2003) 737–747. doi:10.1016/s0044-8486(02)00655-5.
- 7) C.E. Whitman, R.L. Travis, Phospholipid Composition of a Plasma Membrane-Enriched Fraction from Developing Soybean Roots, *Plant Physiology*. 79 (1985) 494–498. doi:10.1104/pp.79.2.494.

nAChR and Membrane Compositions

| Lipid Comparison Table | | | | | |
|------------------------|---------------|---------------|------------|-----------------------|---------------|
| Saturation/Head Group | Torpedo (1,2) | Synapse (3,4) | Mammal (8) | Xenopus (5) Oocyte | Soybean (6,7) |
| n-0 | 59 | 52 | 53 | 46 | 19 |
| n-9 | 14 | 15 | 20 | 22 | 30 |
| n-7 | < 1 | < 1 | 13 | 14 | NA |
| PUFA | 28 | 33 | 14 | 17 | 51 |
| — n-3 | — 19 | — 18 | — 4 | — 6 | — 15 |
| — n-6 | — 9 | — 15 | — 10 | — 11 | — 36 |
| PC | 43 | 43 | 25 | 36 | 29 |
| PE | 36 | 32 | 37 | 22 | 27 |
| PS | 13 | 13 | 16 | 5 | 6 |
| SM | 4 | 4 | 14 | 26 | NA |
| PI | 3 | 8 | 7 | 7 | 6 |
| PA | 0 | 0 | 2 | 0 | 27 |
| Other | 1 | < 1 | 1 | 4 | 5 |
| Chol Mol Frac | 32 | 39 | 30 | 21 | 0 |

1)W. Breckenridge, I. Morgan, J. Zanetta, G. Vincendon, Adult rat brain synaptic vesicles II. lipid composition, *Biochimica Et Biophysica Acta Bba - Gen Subj* 320(1973) 681–686.

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7) C.E. Whitman, R.L. Travis, Phospholipid Composition of a Plasma Membrane-Enriched Fraction from Developing Soybean Roots, *Plant Physiology*. 79 (1985) 494–498. doi:10.1104/pp.79.2.494.

8) Helgi I. Ingólfsson, Manuel N. Melo, Floris J. van Eerden, Clément Arnarez, Cesar A. Lopez, Tsjerk A. Wassenaar, Xavier Periole, Alex H. de Vries, D. Peter Tielemans, Siewert J. Marrink,Lipid Organization of the Pla Membrane , 20014

ORIGINAL ARTICLE

Nicotinic Acetylcholine Receptor Properties are Modulated by Surrounding Lipids

An In Vivo Study

**Andrés Morales,^{*†} Emilio de Juan,¹ Asia M. Fernández-Carvajal,²
José Martínez-Pinna,¹ Juan Antonio Poveda,² José A. Encinar,²
Isabel Ivorra,¹ and José Manuel González-Ros²**

Addition of soybean
lipids

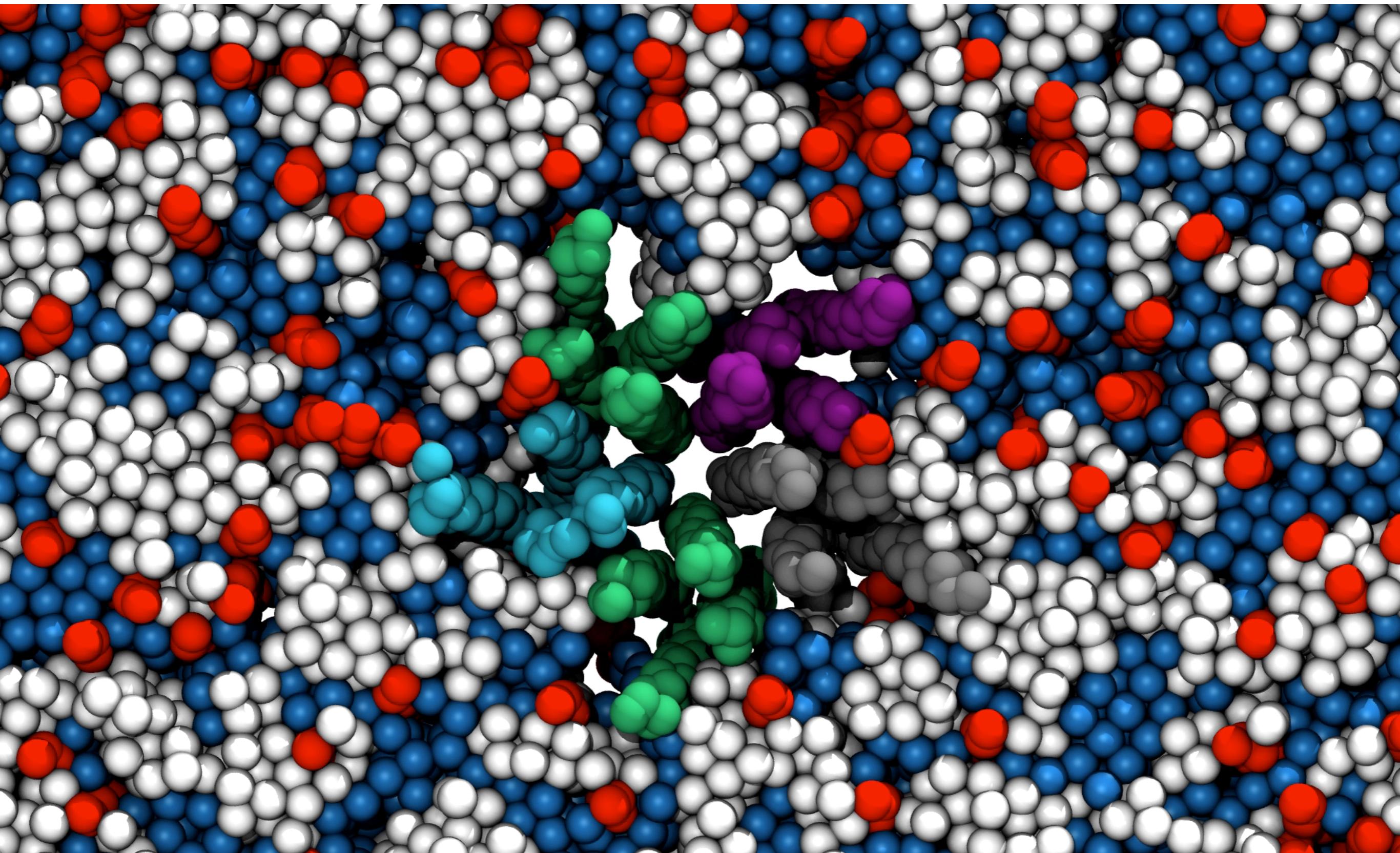
Reference: *Biol. Bull.* **224**: 47–52. (February 2013)
© 2013 Marine Biological Laboratory

Addition of synapse

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

LUCA CONTI^{1,2,*†}, AGENOR LIMÓN^{1,3,*†}, ELEONORA PALMA^{2,4}, AND RICARDO MILEDI^{1,3}

nAChR in quasi-Torpedo Membrane



Saturated Cholesterol Unsaturated

~ Initial 100 ns

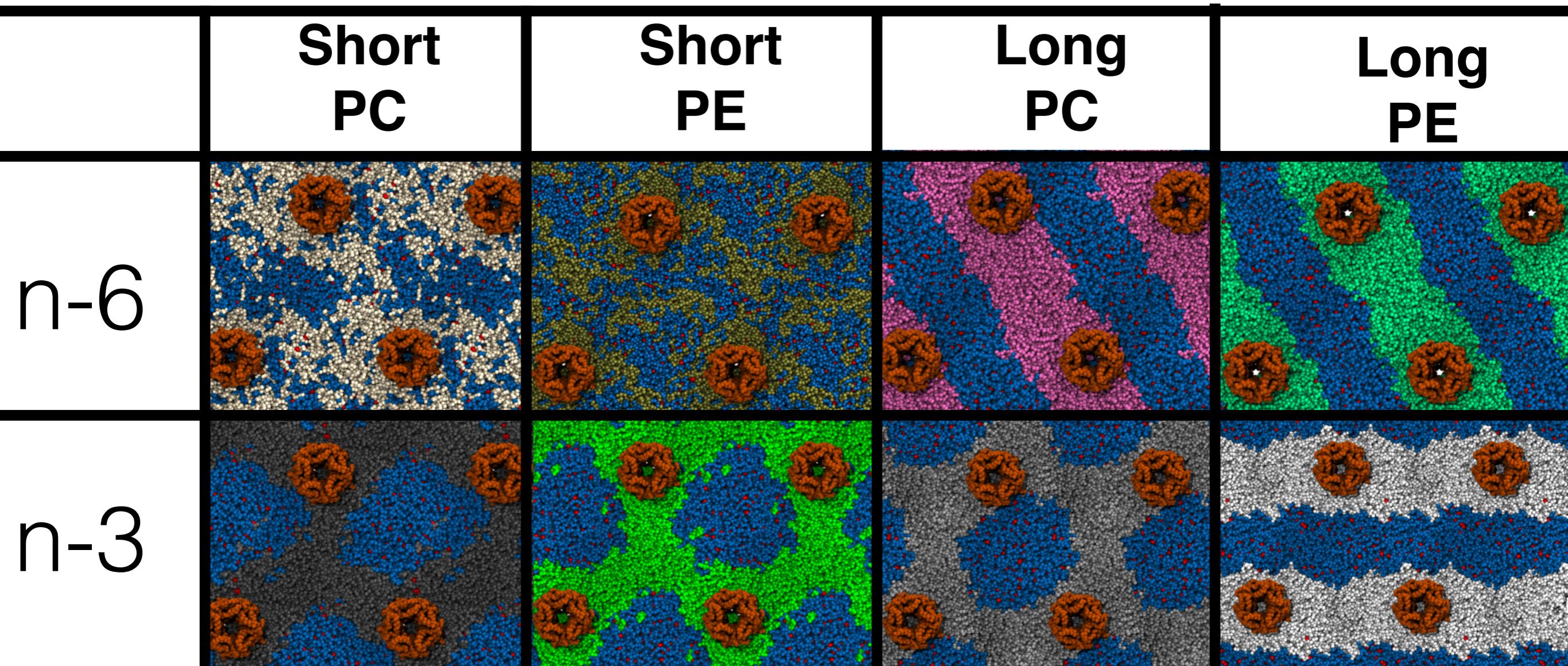
nAChR in quasi-*Torpedo* Membrane

Domain Formation and nAChR Partitioning

Final snapshot of simulations lasting 2 us

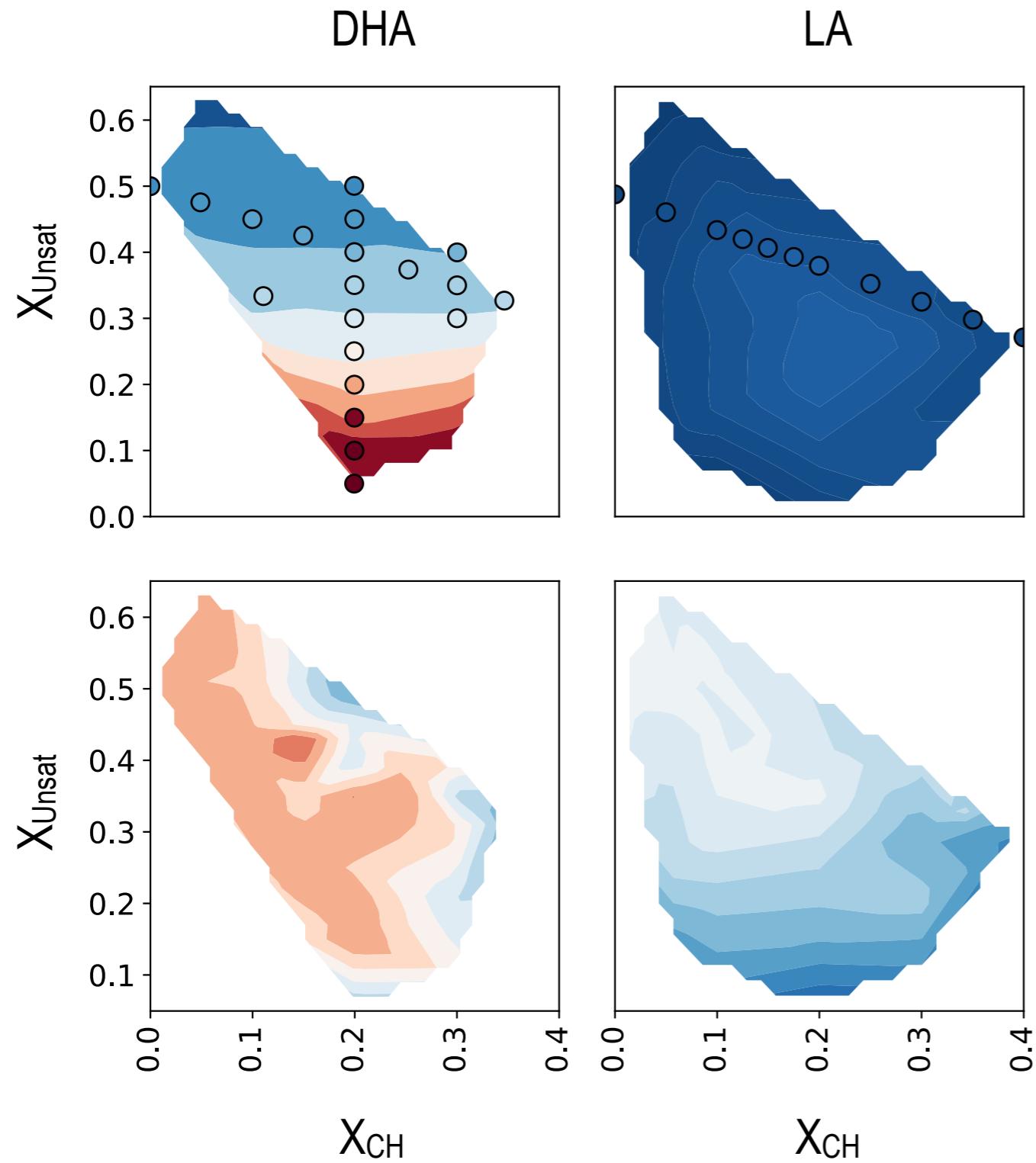
nAChR partitions into liquid-disordered domains

42.5% Saturated 15% Cholesterol 42.5%



nAChR in quasi-*Torpedo* Membrane

Domain Formation and nAChR Partitioning

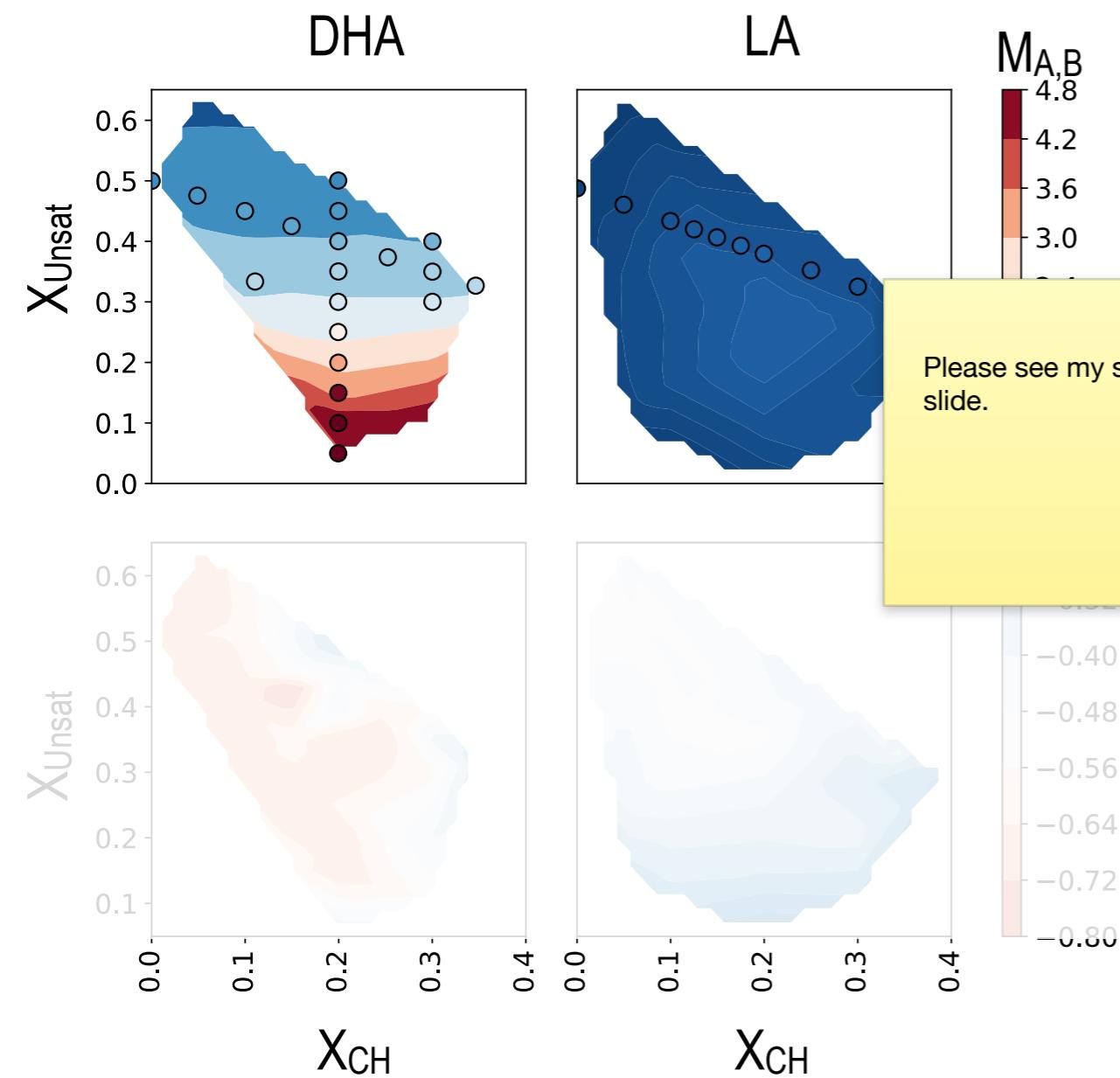


$$M_{A,B} \equiv \frac{1}{x_B} \left\langle \frac{nn_{A,B}}{nn_{tot}} \right\rangle - 1$$

$$Q_{sat} \equiv \frac{1}{x_{sat}} \left\langle \frac{b_{sat}}{b_{tot}} \right\rangle - 1$$

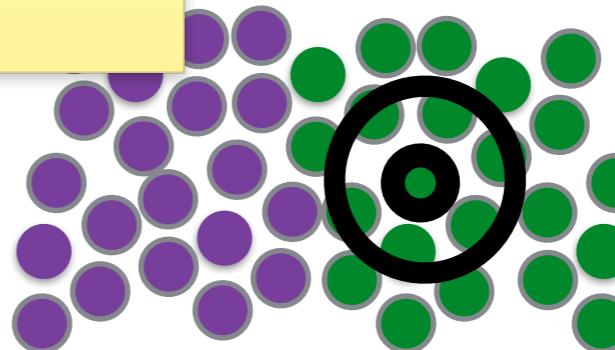
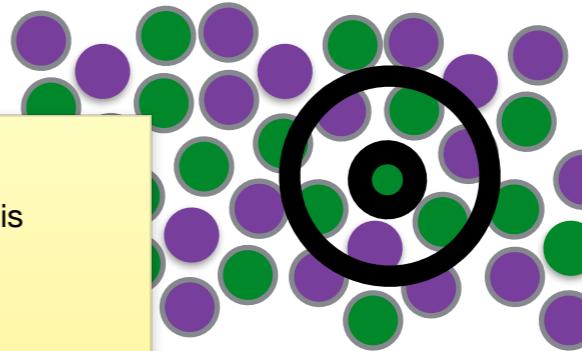
nAChR in quasi-*Torpedo* Membrane

Domain Formation and nAChR Partitioning



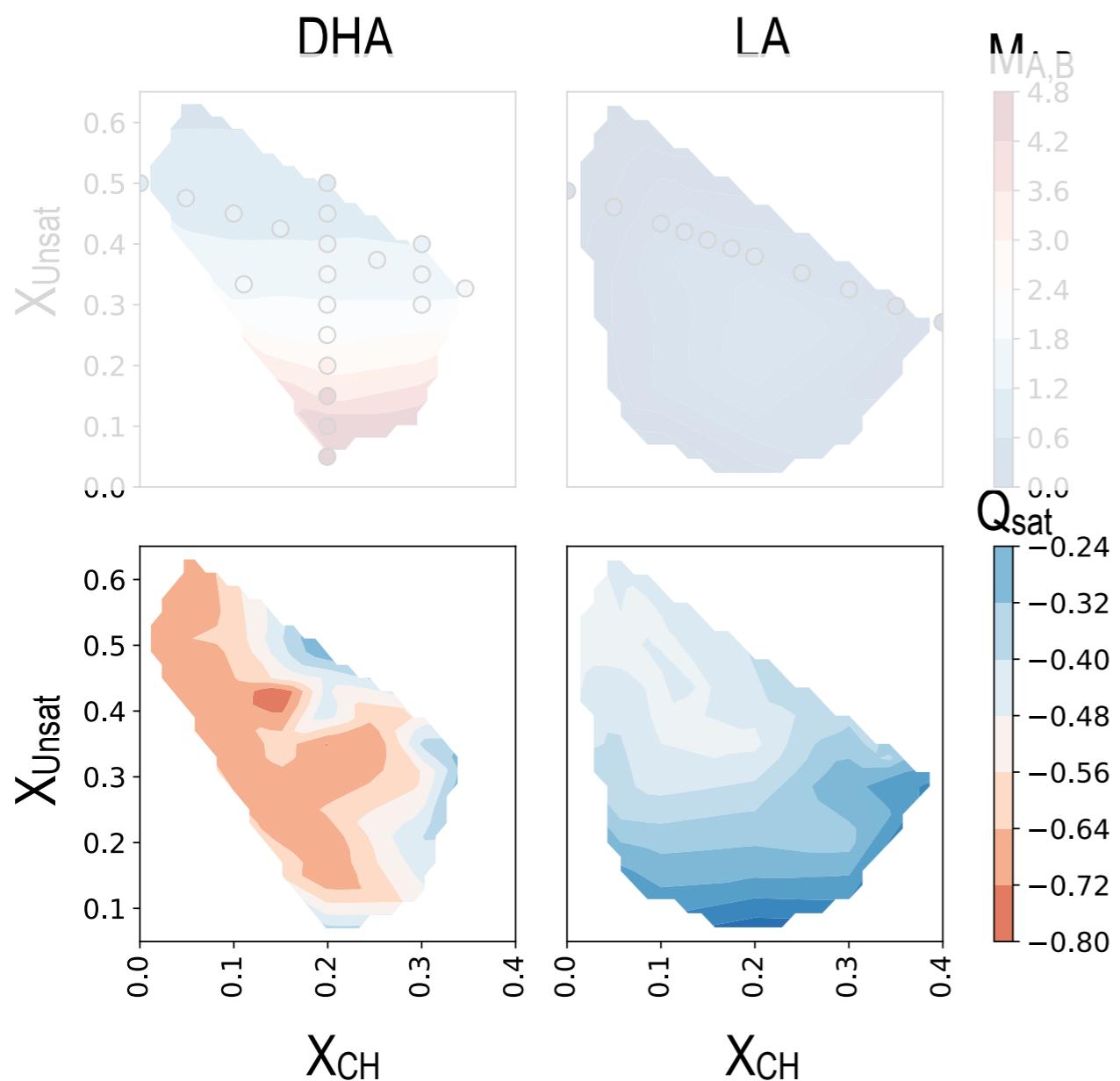
$$M_{A,B} \equiv \frac{1}{x_B} \left\langle \frac{nn_{A,B}}{nn_{tot}} \right\rangle - 1$$

Please see my slack note on this slide.



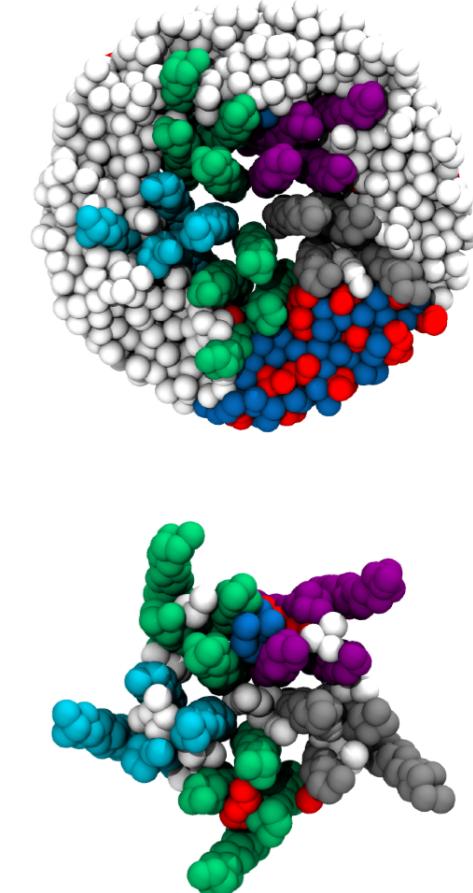
nAChR in quasi-*Torpedo* Membrane

Domain Formation and nAChR Partitioning



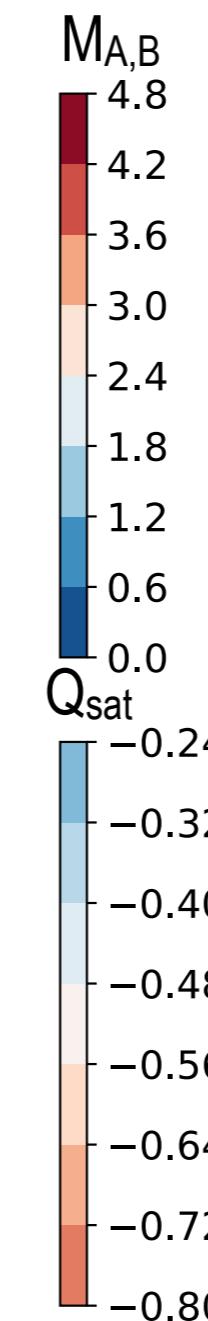
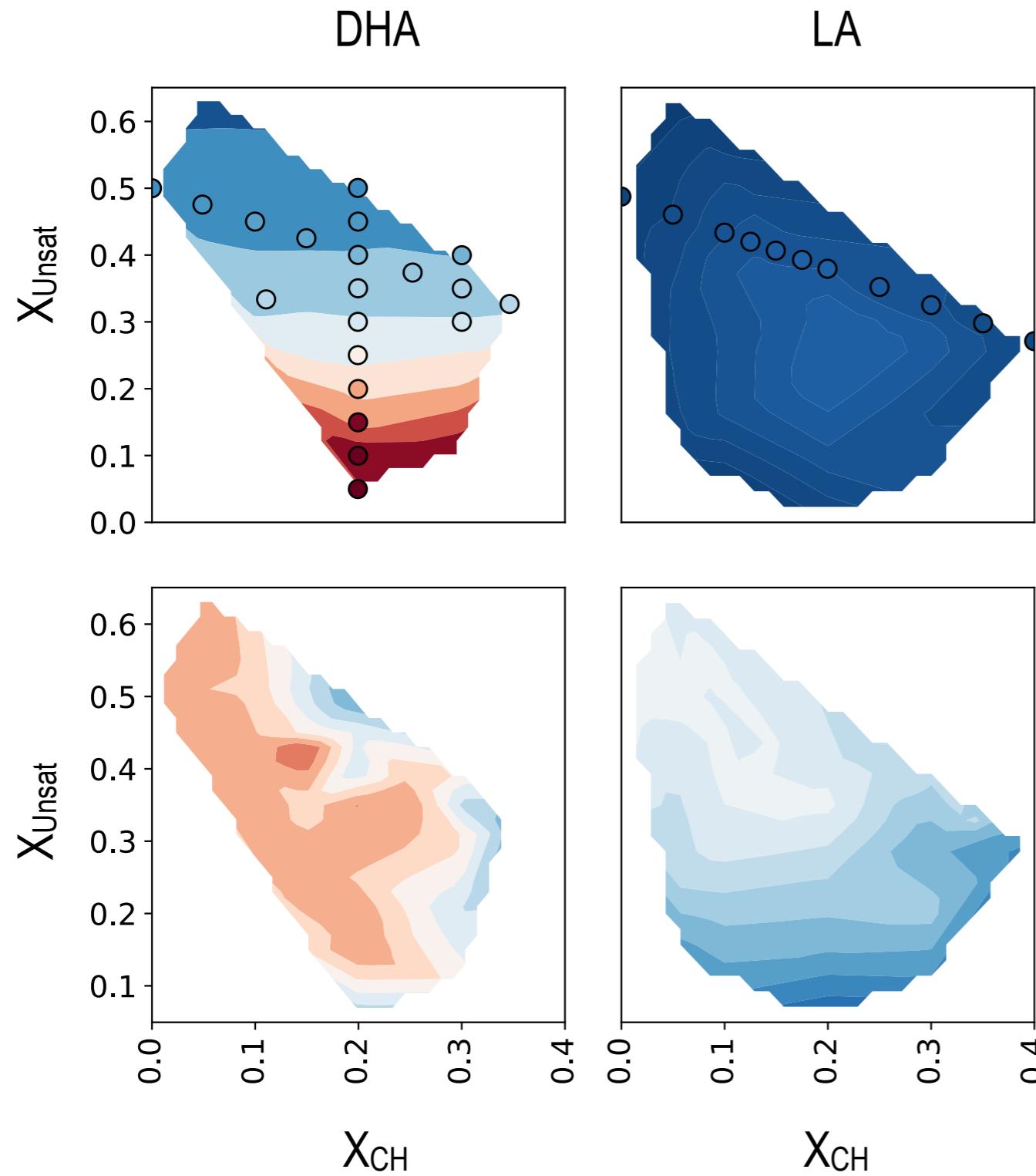
$$Q_{\text{sat}} \equiv \frac{1}{x_{\text{sat}}} \left\langle \frac{b_{\text{sat}}}{b_{\text{tot}}} \right\rangle - 1$$

- $Q > 0$ Boundary lipids enriched in DPPC
- $Q = 0$ Expected DPPC concentration in boundary lipids
- $Q < 0$ Boundary lipids depleted of DPPC



nAChR in quasi-*Torpedo* Membrane

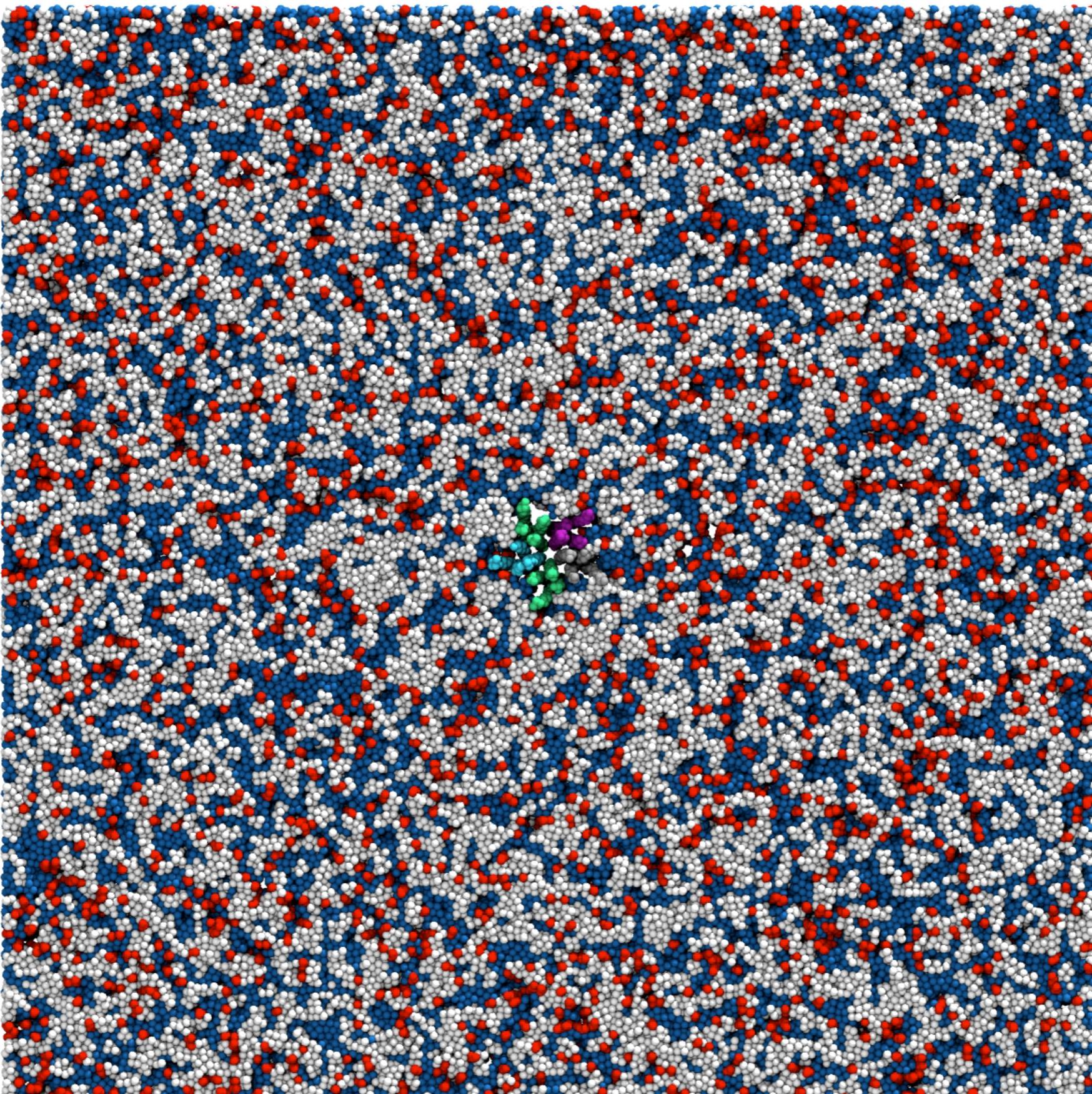
Domain Formation and nAChR Partitioning



$$M_{A,B} = \frac{\langle \eta_{A,B} \rangle}{x_B} - 1$$

$$Q_{sat} \equiv \frac{1}{x_{sat}} \left\langle \frac{b_{sat}}{b_{tot}} \right\rangle - 1$$

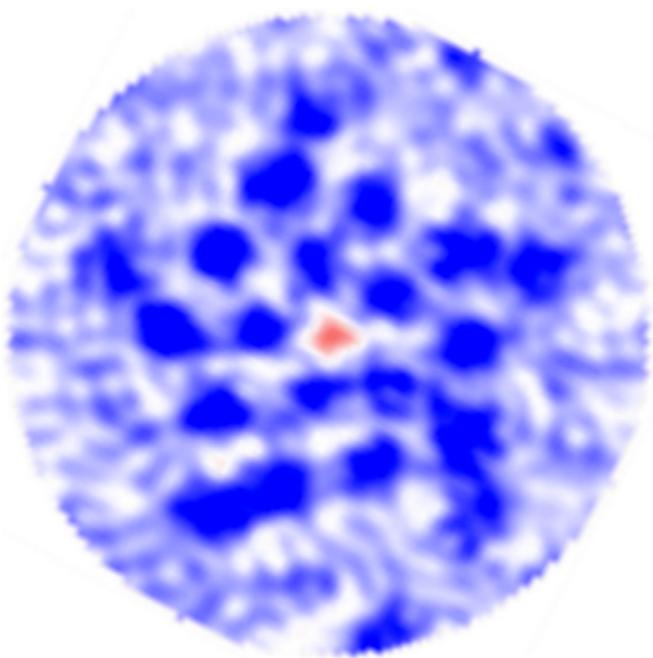
nAChR in quasi-*Torpedo* Membrane



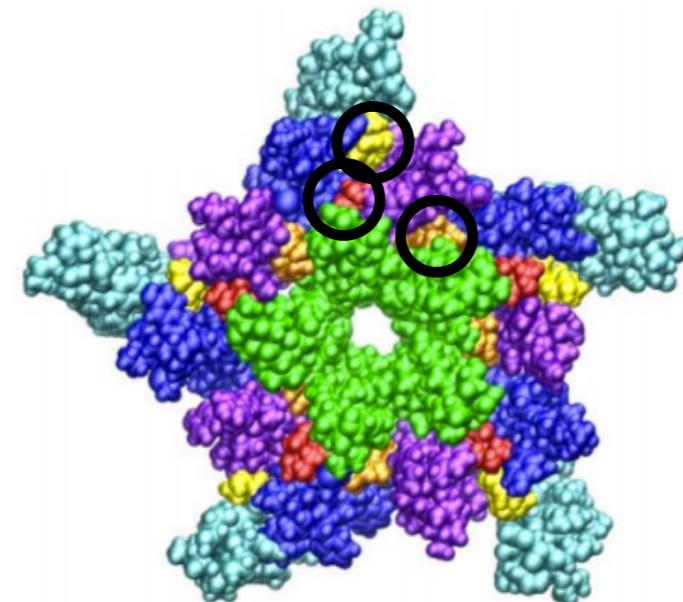
Saturated Cholesterol Unsaturated

$\sim 4 \text{ us}$

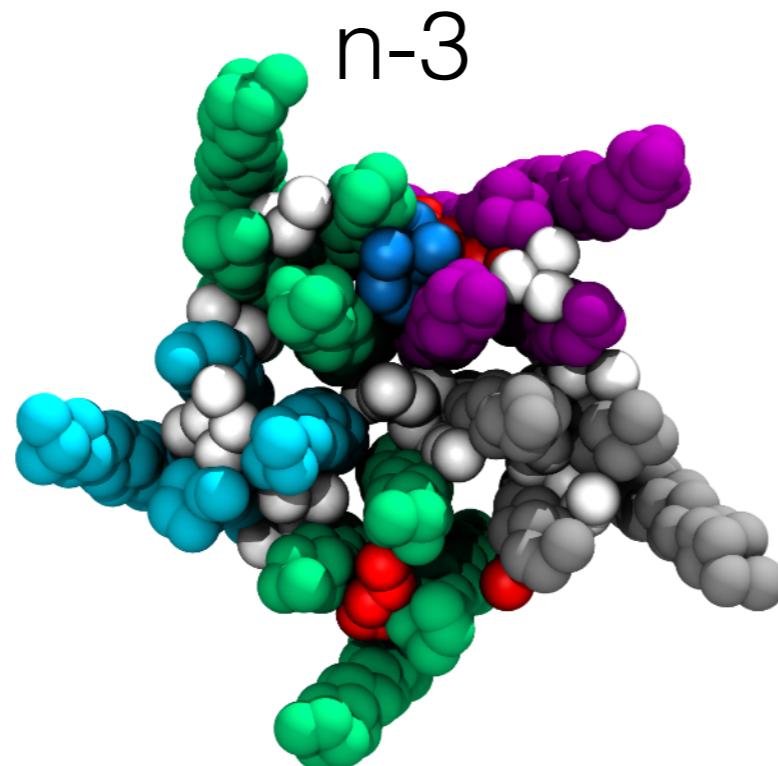
nAChR in quasi-*Torpedo* Membrane Lipid “Embedding” and Subunit Preference



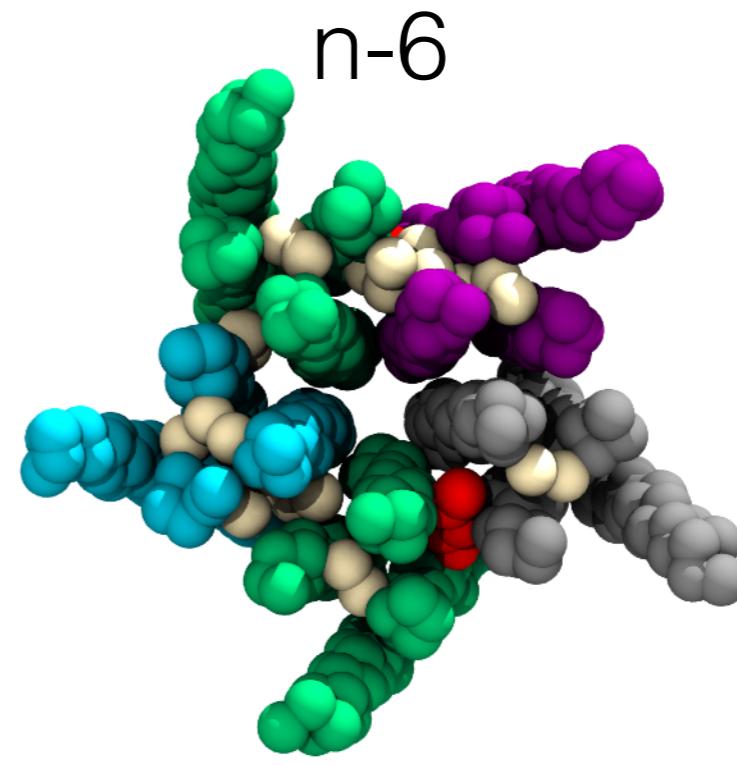
G. Brannigan, J. Hénin, R. Law, R. Eckeho , M. L. Klein,
Embedded cholesterol in the nicotinic acetylcholine receptor. 105 (2008) 14418–23.



G. Brannigan, J. Hénin, R. Law, R. Eckeho , M. L. Klein,
Embedded cholesterol in the nicotinic acetylcholine receptor. 105 (2008) 14418–23.



n-3



n-6

nAChR in quasi-*Torpedo* Membrane Lipid “Embedding” and Subunit Preference Experimental Comparison

IUCrJ

ISSN 2052-2525

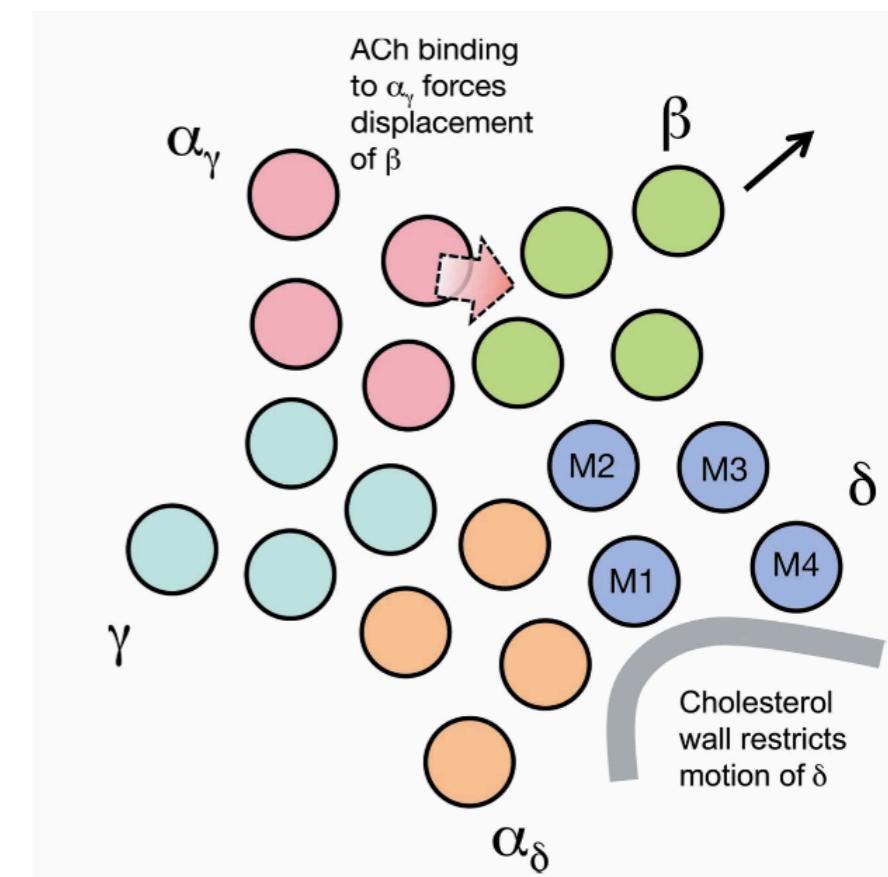
BIOLOGY | MEDICINE

Segregation of lipids near acetylcholine-receptor channels imaged by cryo-EM

Nigel Unwin*

MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, England. *Correspondence e-mail: unwin@mrc-lmb.cam.ac.uk

- Recent research suggests cholesterol rich domains near α_δ - δ subunit
- I commonly find cholesterol rich domains near α_δ and α_γ

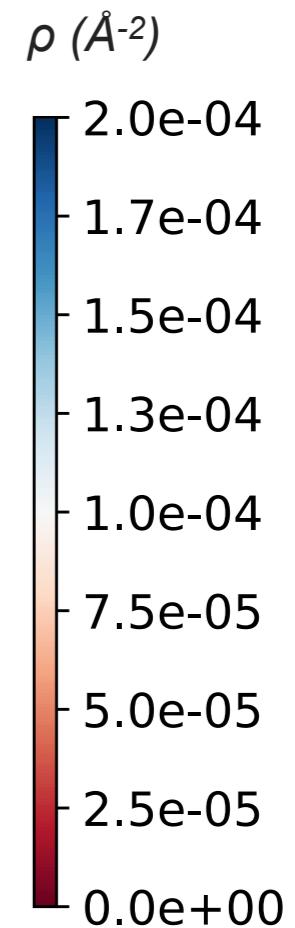
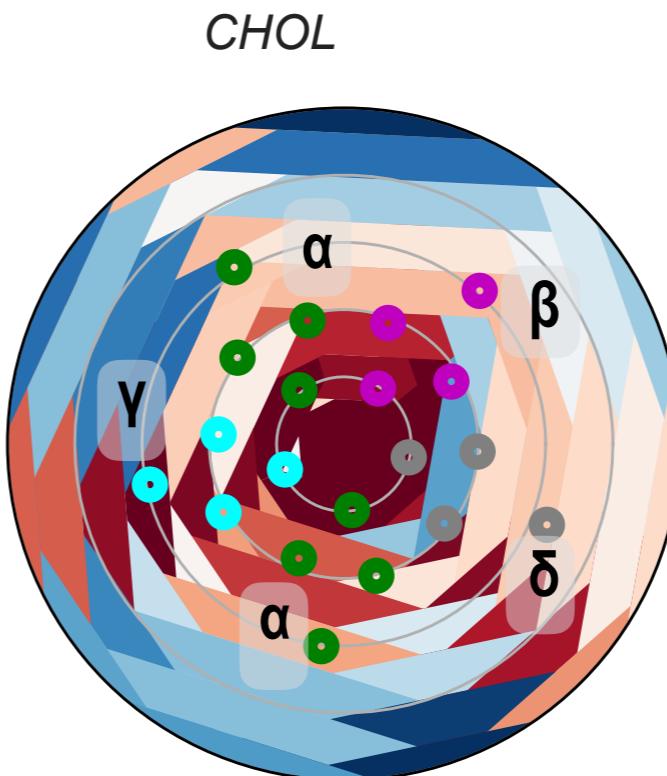
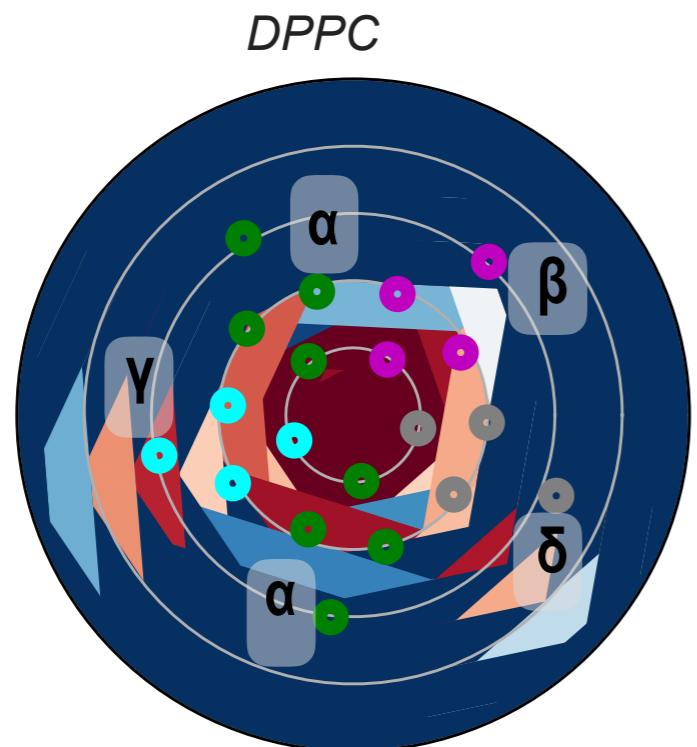


nAChR in quasi-*Torpedo* Membrane

Lipid “Embedding” and Subunit Preference

$$\rho_{B,i} = \left\langle \frac{n_{B,i}}{A_i} \right\rangle$$

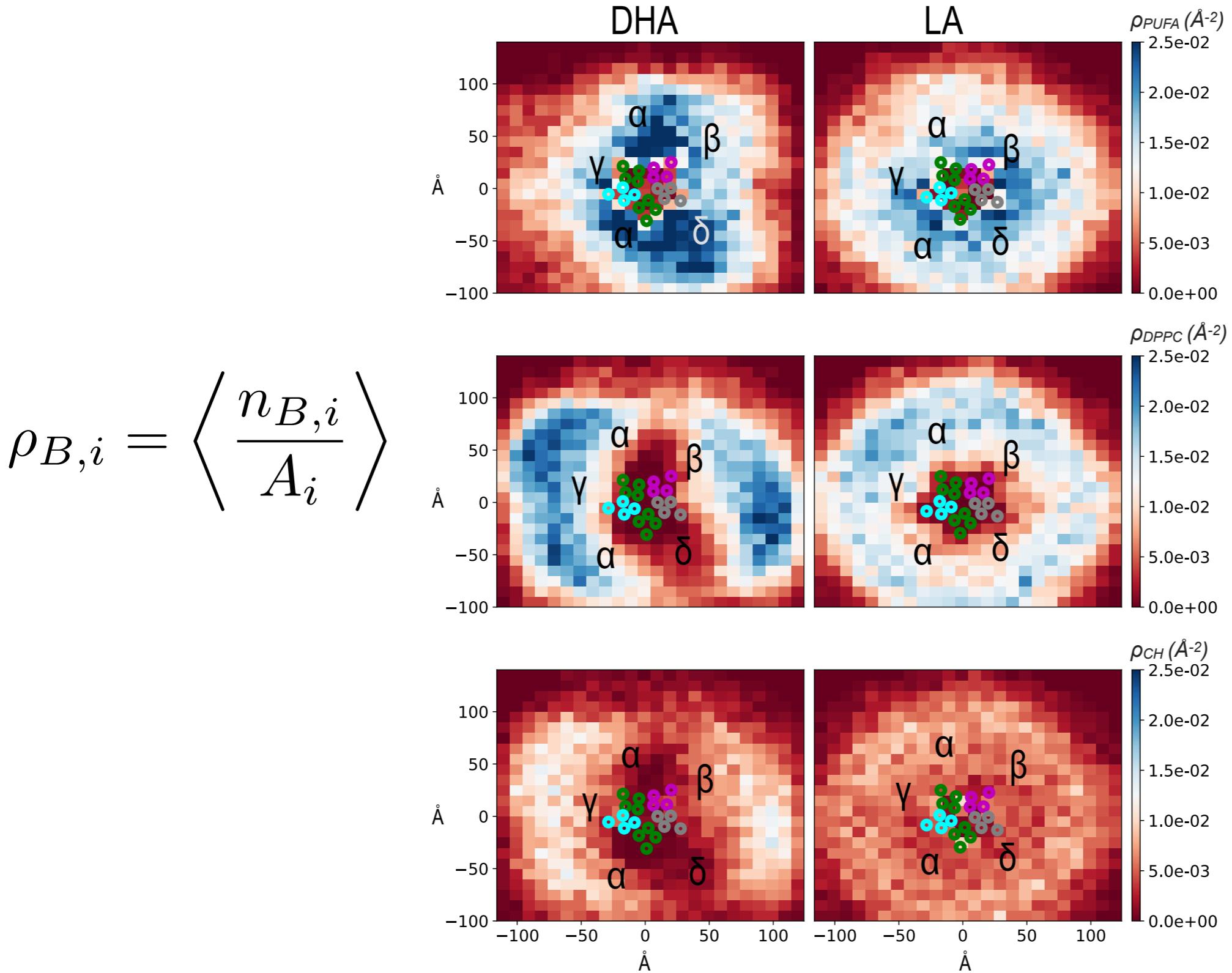
Binary



nAChR in quasi-*Torpedo* Membrane

Lipid “Embedding” and Subunit Preference

Ternary

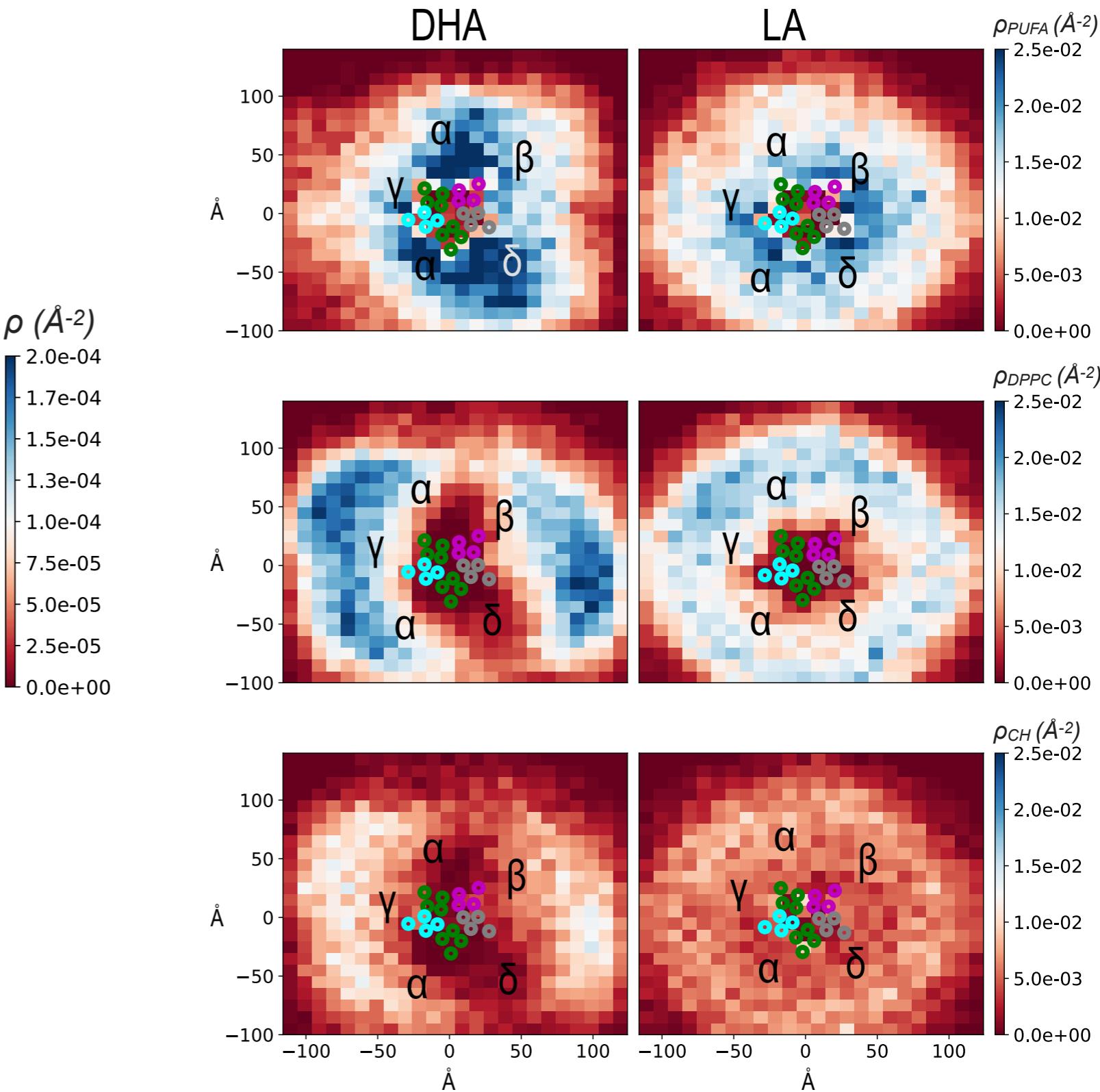
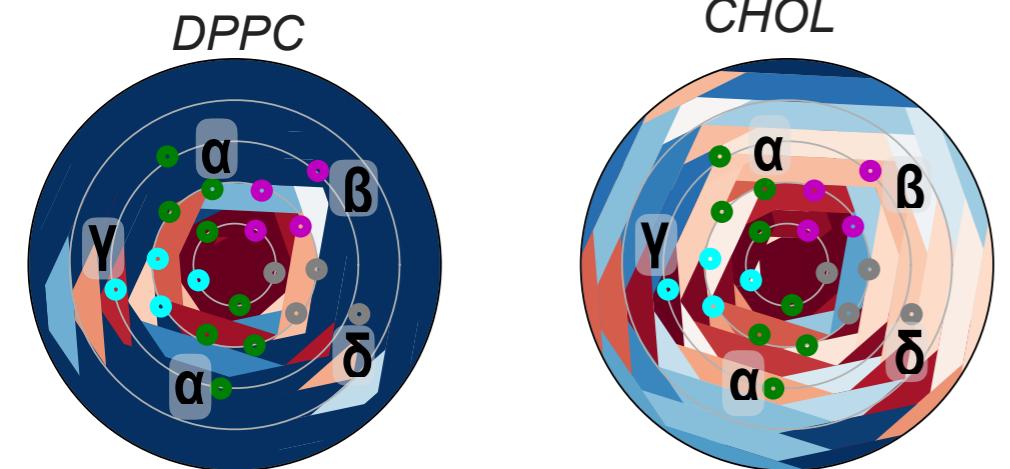


nAChR in quasi-*Torpedo* Membrane

Lipid “Embedding” and Subunit Preference

Ternary

Binary



Summary

- Model-native *Torpedo* membranes de-mix into liquid order and liquid disorder phases
- nAChR consistently partitions into cholesterol poor domains; which are abundant in long chained PUFAs suggesting an annular dependency for PUFAs
 - nAChR's orientation is similar when membrane size is increased
 - nAChR shows subunit-lipid preferences
 - Cholesterol, but to a greater extent DHA, embed throughout nAChR
- Cholesterol dependence may come from non-annular binding

Part II

Proposed Research for PhD Candidacy

- Aim 1: Coarse-grained simulations of multiple subtypes of mammalian pLGICs in quasi-physiological membranes
- Aim2: Investigation of the relative importance of pLGIC sequence versus shape in determining preferred lipid domain
- Aim3: Development and release of a user-friendly VMD plugin for measuring elastic parameters of heterogenous membranes

*Aim 1: Coarse-grained simulations
of multiple subtypes of mammalian
pLGICs in quasi-physiological
membranes*

Aim1

- *Xenopus* oocytes have significantly different membrane compositions compared to nAChR native membranes
- Supplementing oocytes with crude *Torpedo* membranes and/or soybean lipids have improved electro-physiologic testing

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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 Martínez-Pinna,¹ Juan Antonio Poveda,² José A. Encinar,²
Isabel Ivorra,¹ and José Manuel González-Ros²**

- Is there a specific lipid species and concentration that will allow nAChR local composition in oocytes to mimic that of native membranes?

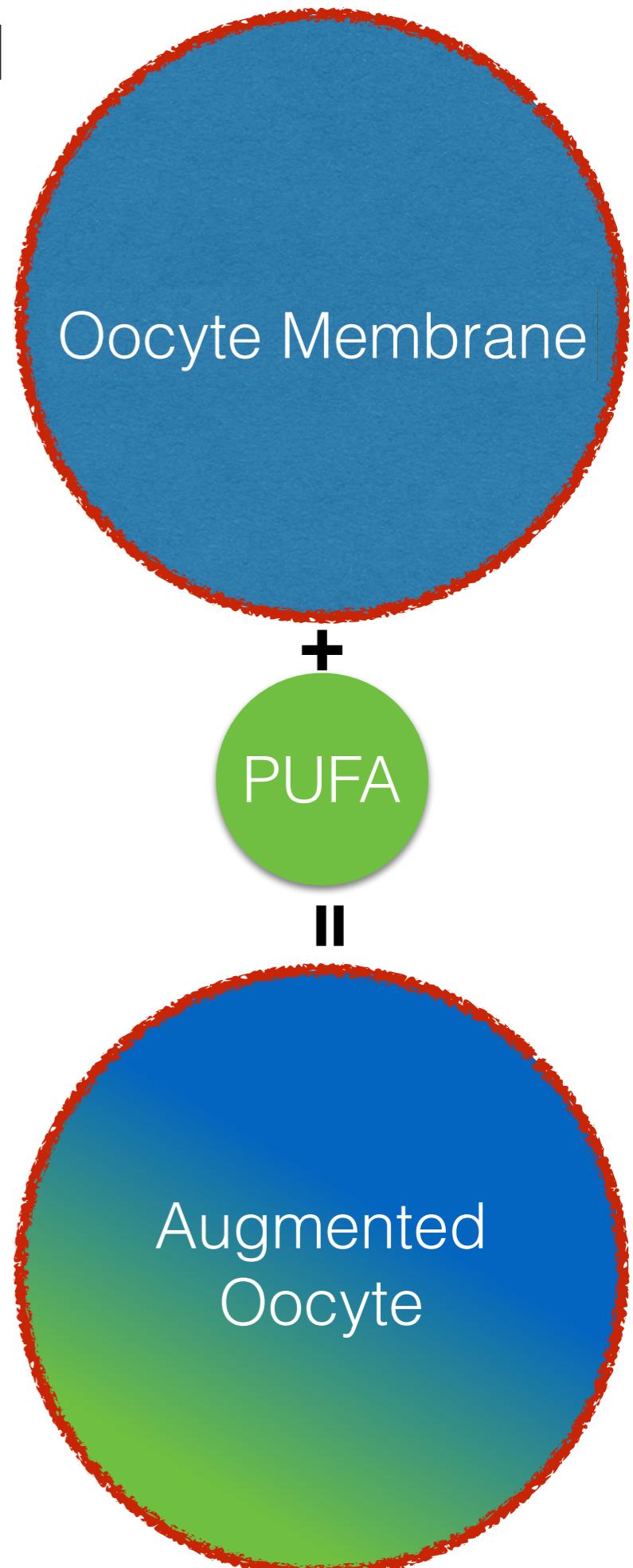
Aim1: Proposed Method

- **System Building:**

- Simulate quasi-oocyte membranes with ~ 10 proteins
- Supplement membrane PUFAs:
 - Potential target PUFAs:
 - ALA C18:3 (n-3)
 - EPE C20:5 (n-3)
 - DHA C22:6 (n-3)
 - C24:6 (n-3)



Synaptic/Torpedo



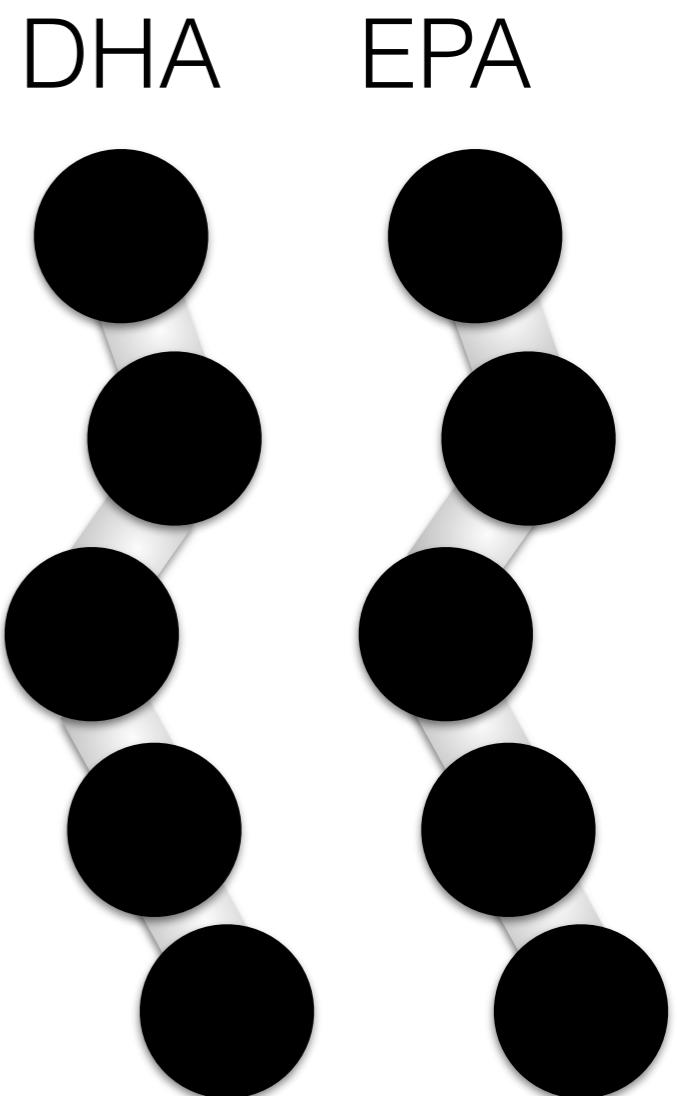
Aim1: Proposed Method

- **Potential Issue:**

- Martini maps 4 heavy atoms to a single bead
- Martini lipid parameters have limited n-3 due to this

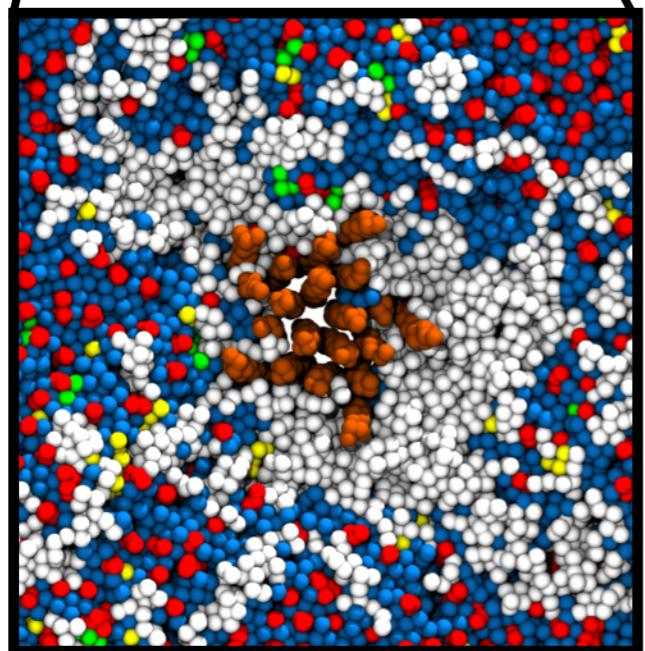
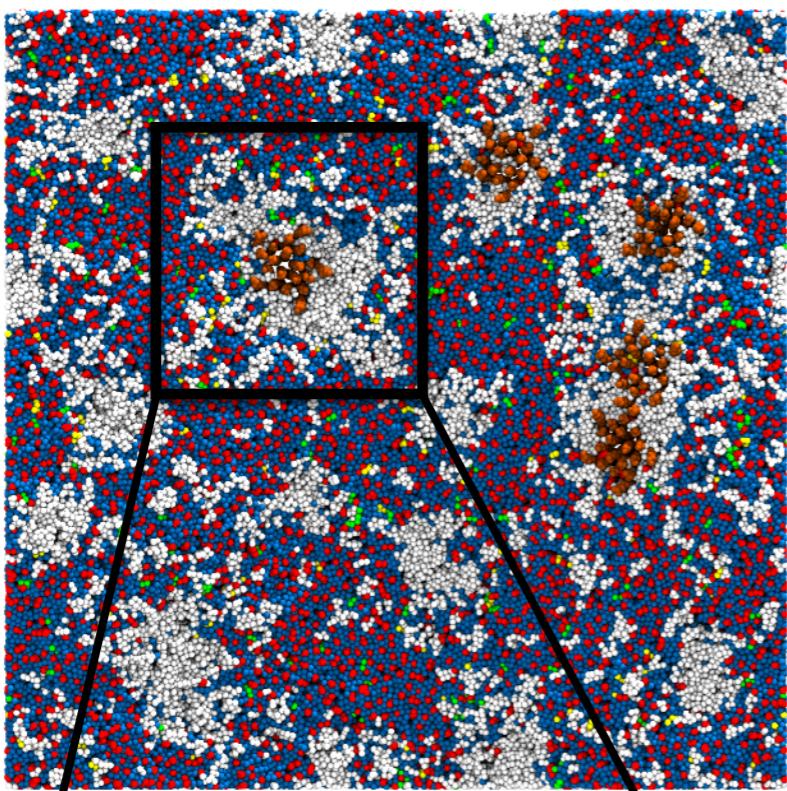
- **Potential Solution:**

- Martini allows to build custom lipid parameters: `lipid-martini-itp-v05.py`
- **Example** DHA and EPA share identical Martini structure, but have different properties for biological function
- Should be able to construct mimic of EPA using Martini

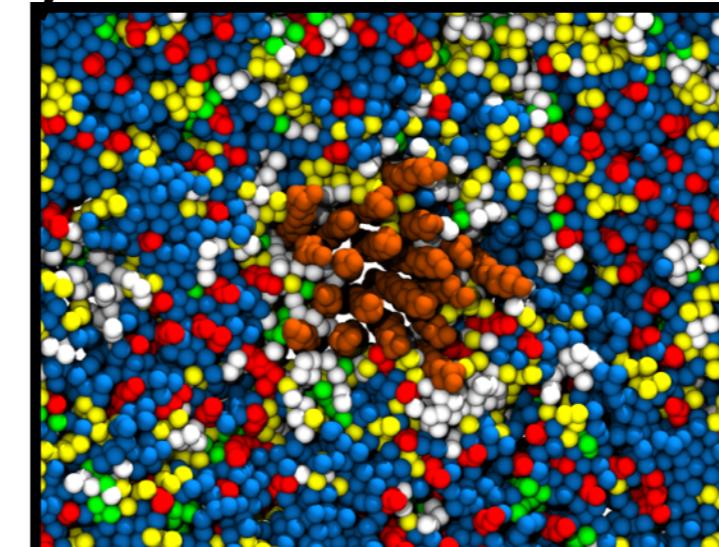
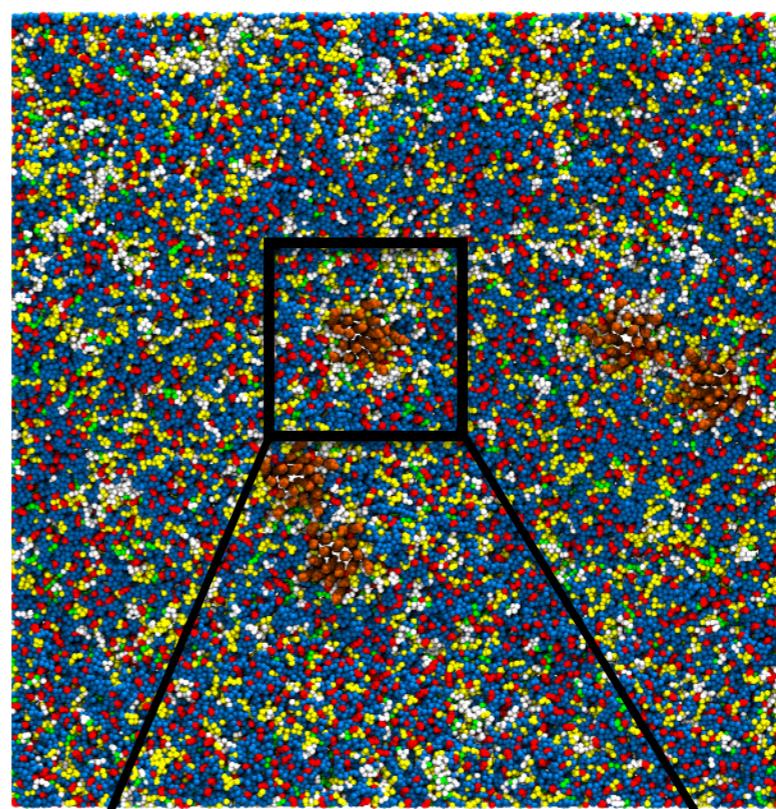


Aim1: quasi-Synaptic and quasi-Oocyte Controls

Synaptic

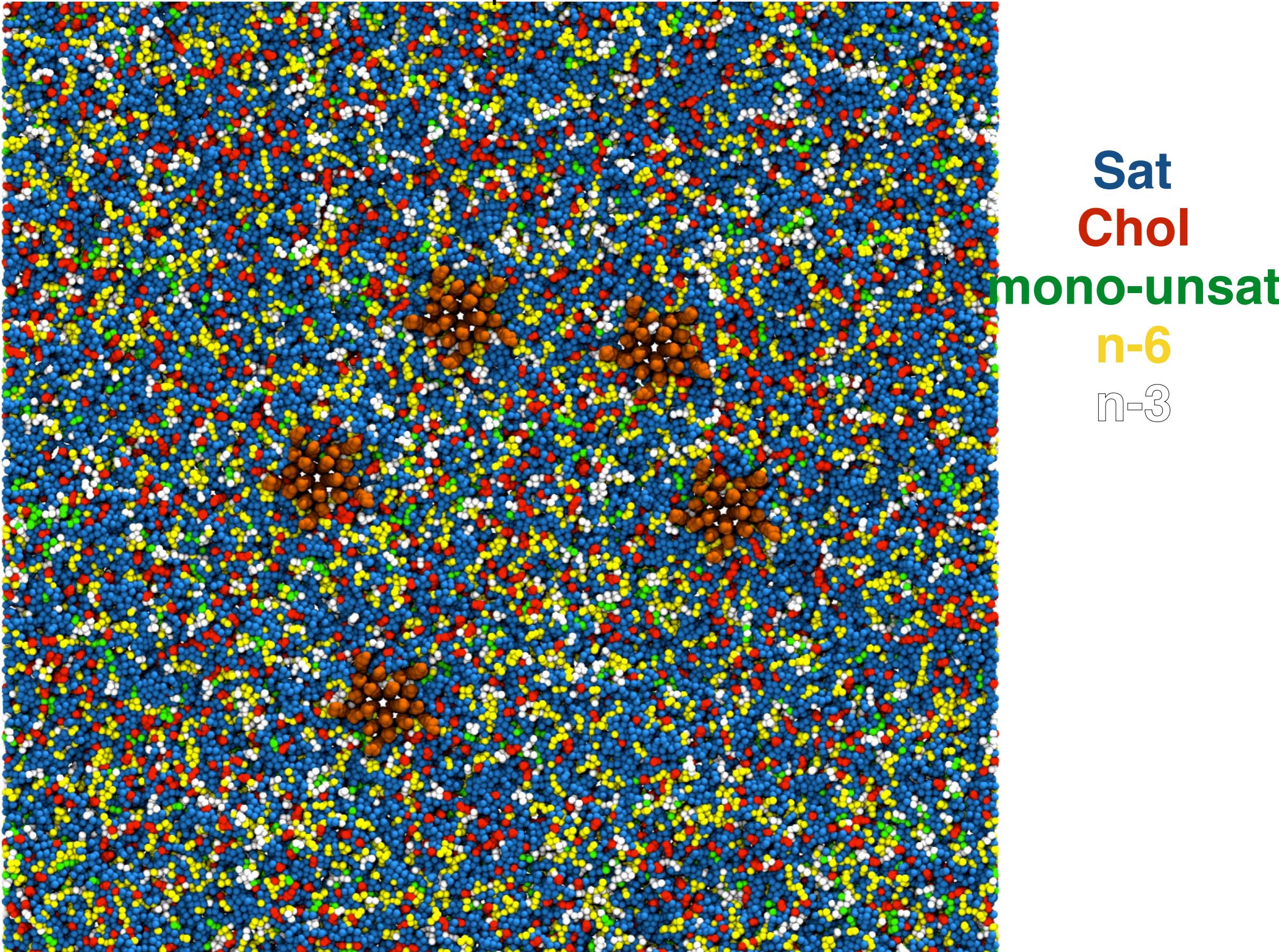


Oocyte

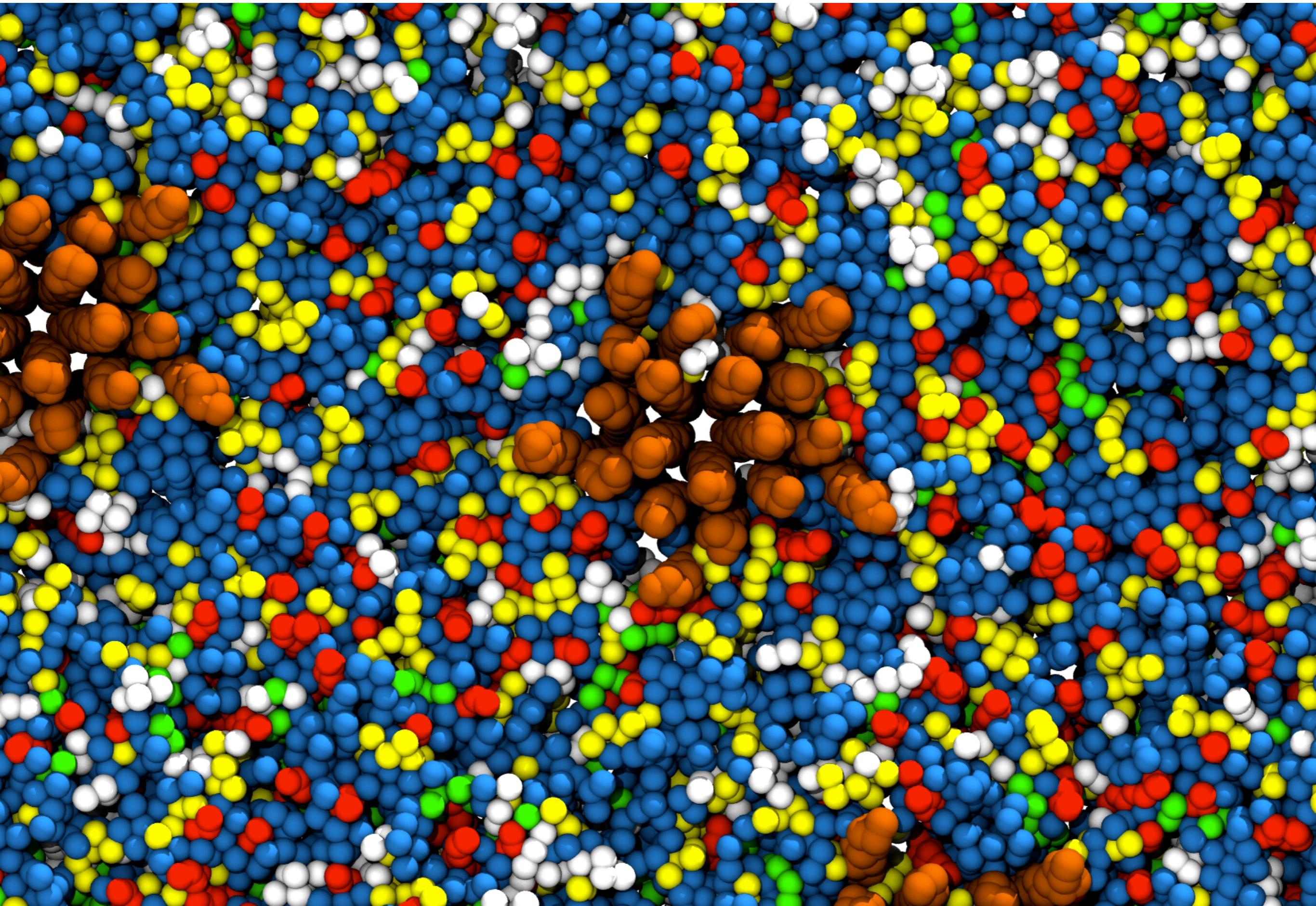


Sat
Chol
mono-unsat
n-6
n-3

Aim1: quasi-Oocyte



Aim1: quasi-Oocyte



Aim2: Investigation of the relative importance of pLGlC sequence versus shape in determining preferred lipid domain

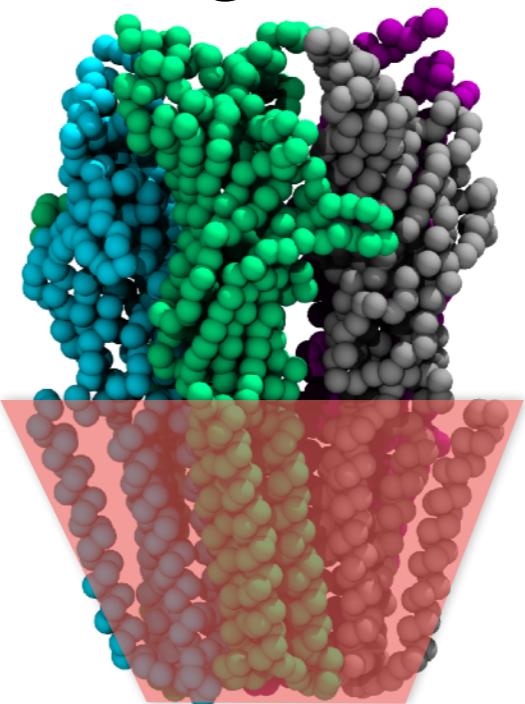
Aim2

- Why does nAChR partition into the Ld phase?
 - Based on preliminary work nAChR shows subunit-lipid preference
 - pLGIC have an approximate “cone” shape, theoretically, not optimal for Lo phases
- Can we determine which may play a greater role?
- What is the effect on membrane organization?

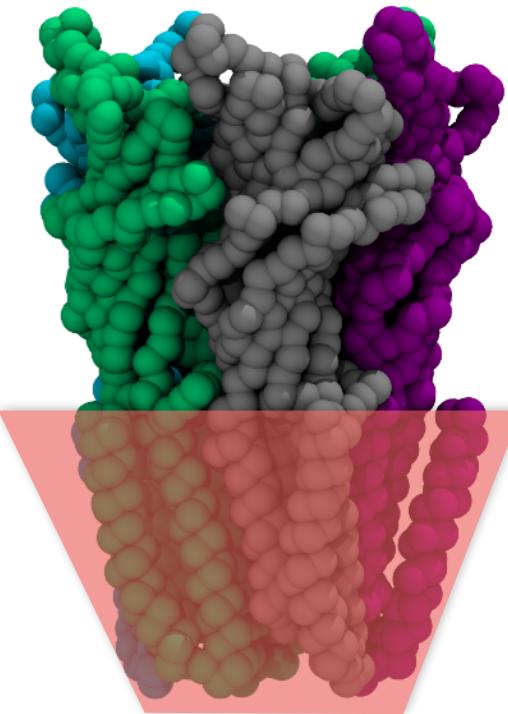
Aim 2

- pLGIC have high sequence diversity but conserved structures
 - I hypothesize partitioning to be dependent on sequence and structure
 - Which provides a greater role?

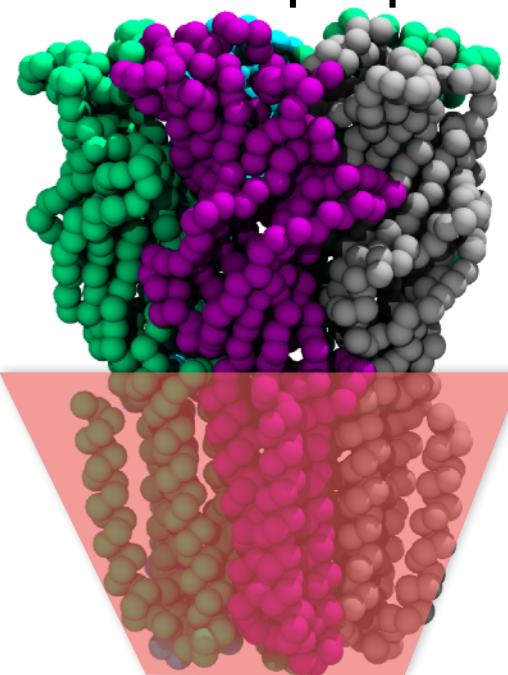
nAChR
2bg9



GLIC
4ilb



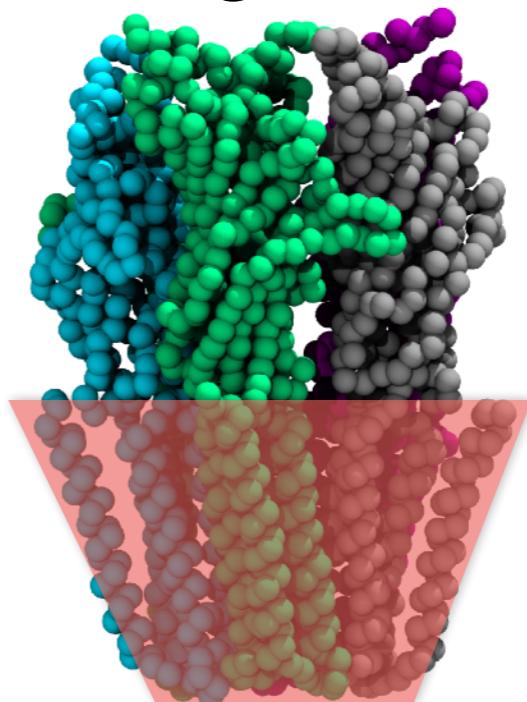
GABA(A)
2α₁2β₃γ₂



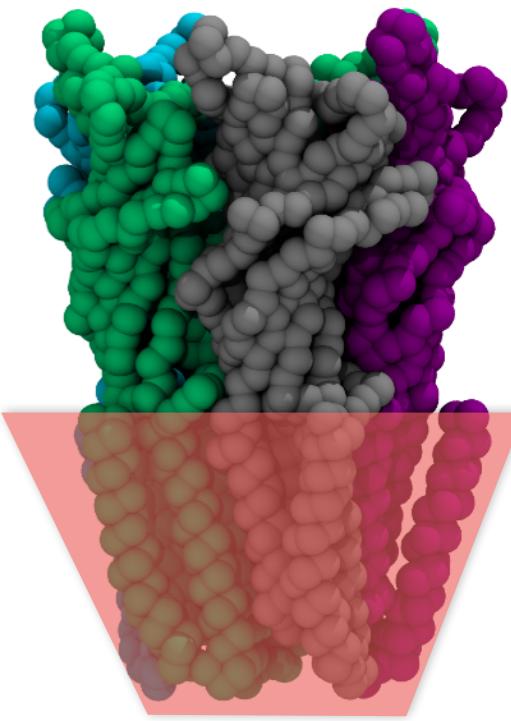
Aim 2

- nAChR:GLIC- ~19% Identity
- GABA(A):GLIC- ~21% Identity
- nAChR:GABA(A)- ~20% Identity
 - Alignment using Jalview, Muscle algorithm

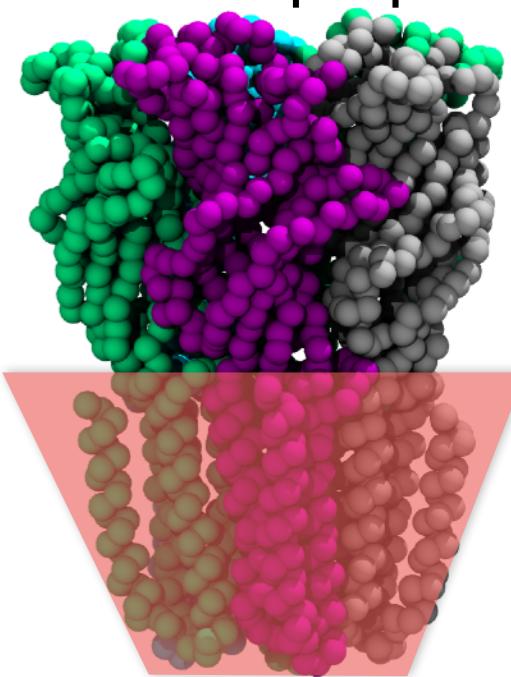
nAChR
2bg9



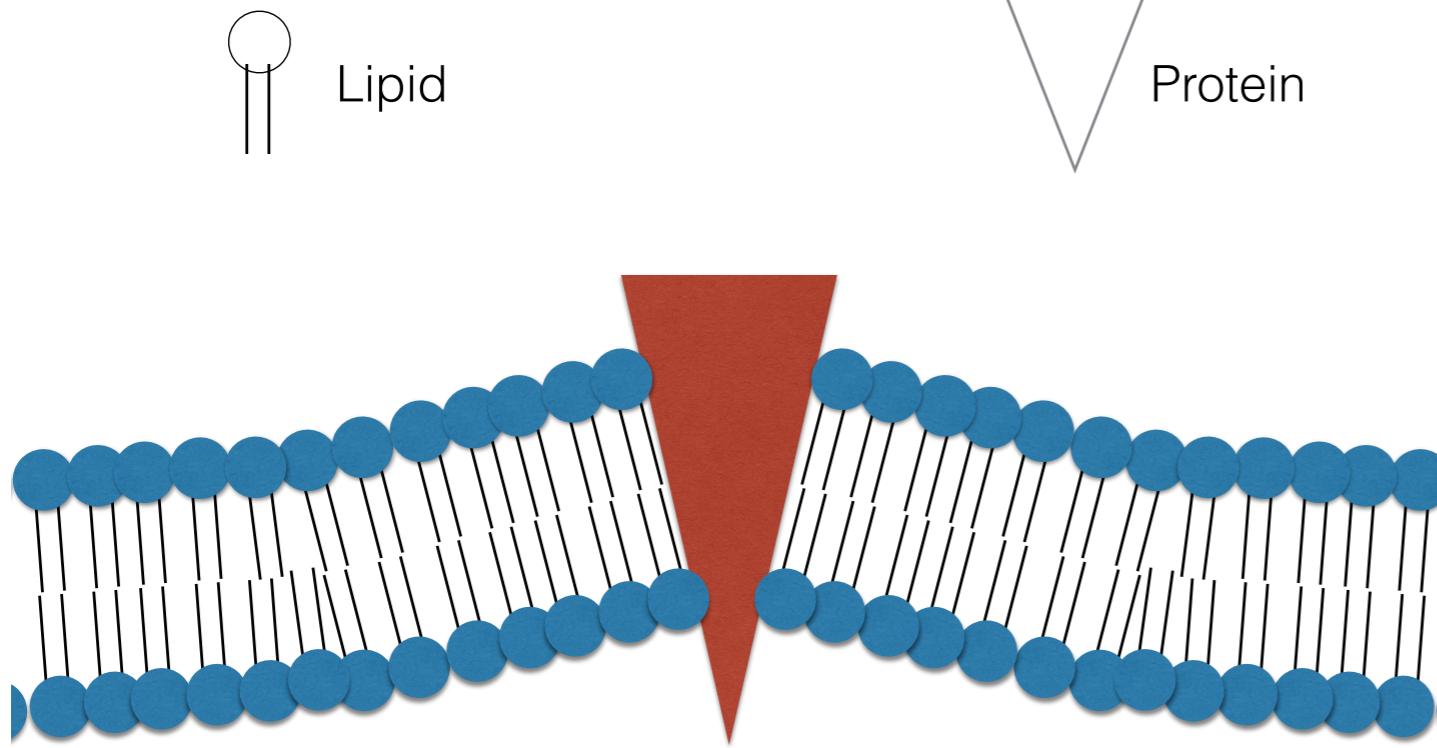
GLIC
4ilb



GABA(A)
2a₁2β₃γ₂



Aim 2 Role for Elasticity : Stiff membranes resist deformation



Inclusions in membranes

Mark Goulian

Recent work on membrane-inclusion and inclusion-inclusion interactions has focused on models of the membrane based on curvature elasticity. The resulting deformation profiles are non-monotonic and lead to either attractive or repulsive interactions. In addition, considerations of thermal membrane fluctuations and the membrane-inclusion contact angle have led to predictions of new long-range interactions.

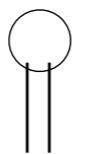
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Current Opinion in Colloid & Interface Science 1996, 1:358-361

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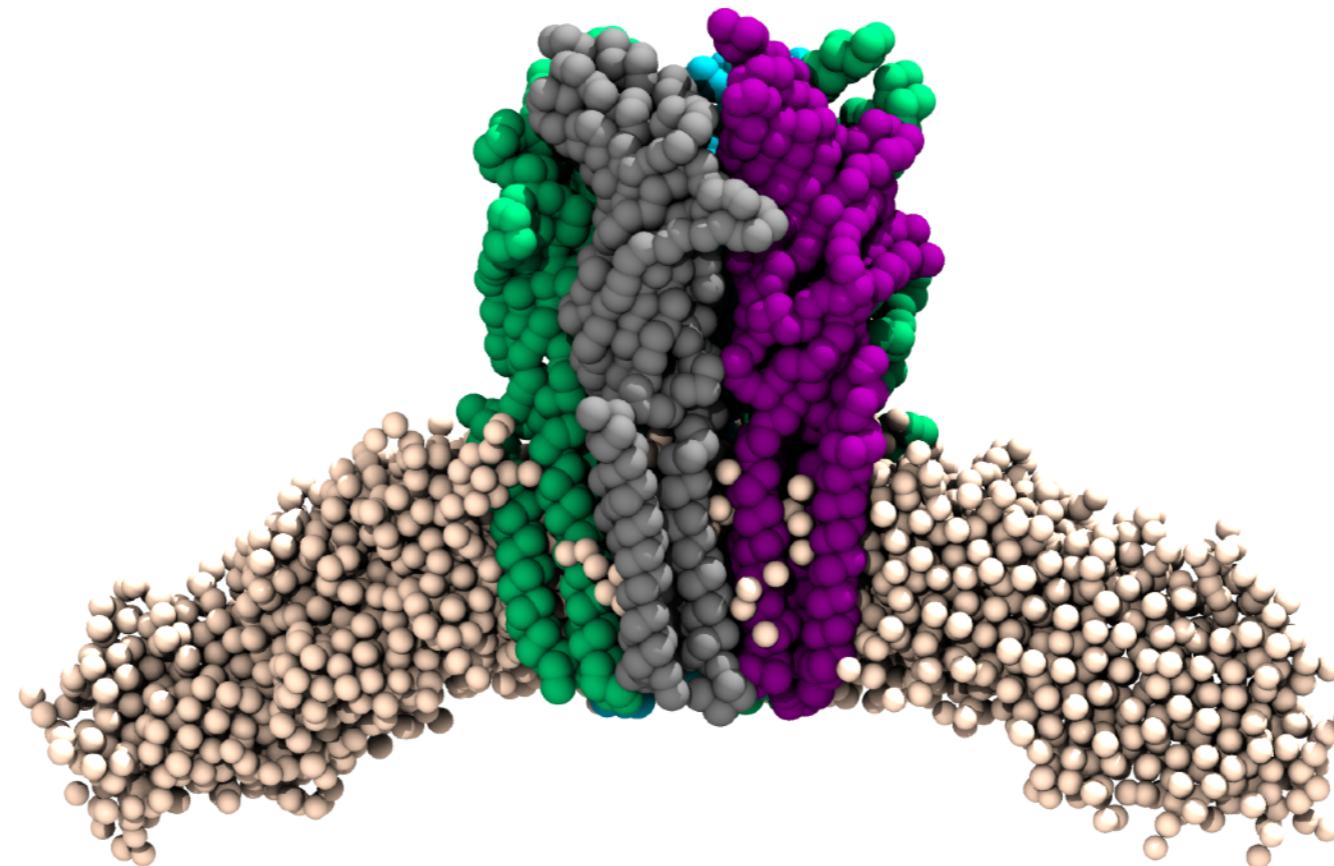
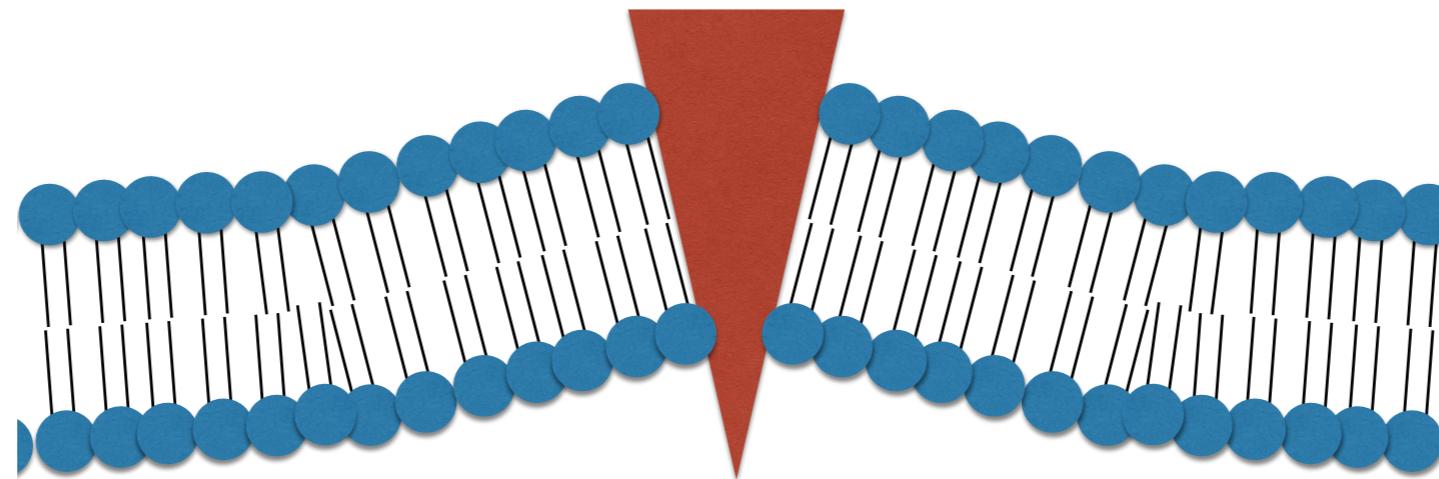
Aim 2 Role for Elasticity : Stiff membranes resist deformation



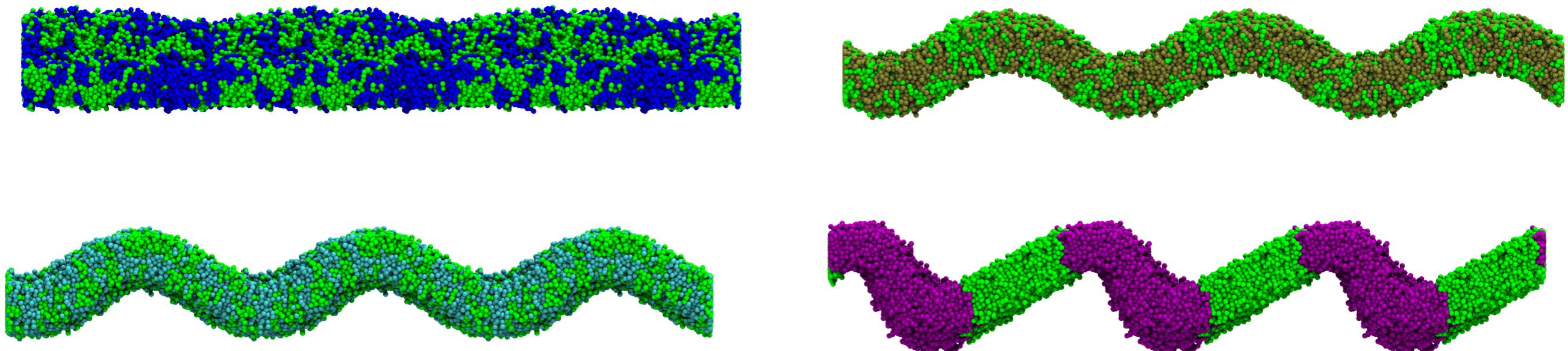
Lipid



Protein



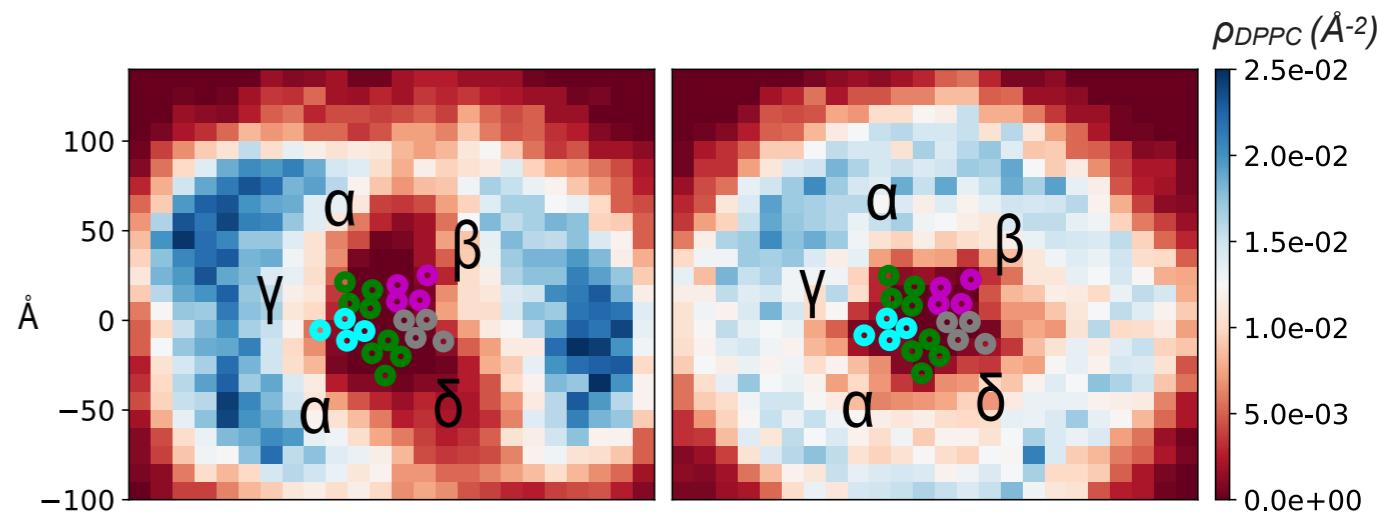
Aim 2 Role for Elasticity : Stiff membranes resist deformation



- Above: binary mixtures of saturated lipids (green) and PUFAs (other color)
- As PUFAs get longer or more unsaturated, undulations become greater
 - Final image shows flat Lo and curved Ld phases

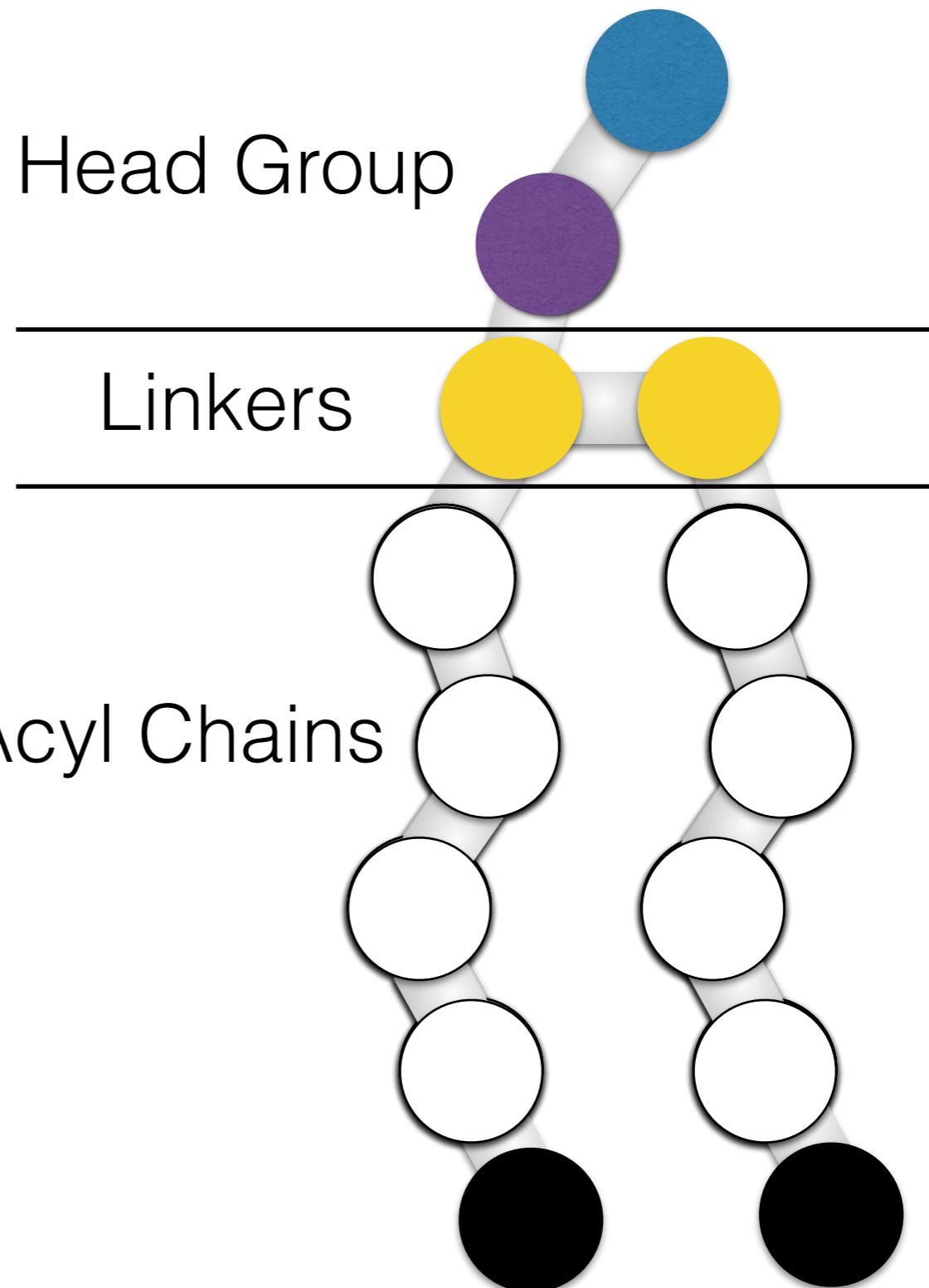
Aim 2: Proposed Method

- Approach to test effect of sequence:
 - Measure Q_{sat} for other pLGICs (GABA(A)r homopentamer, a4b2 nAChR, GLIC)
 - Measure Q_{sat} for nAChR containing only α and/or γ subunits, which preferred to face liquid ordered domain
- Calculate average distance from domain boundary for homopentamers vs heteropentamers
- If non-PUFAs dominate boundary shell with other pLGIC, partitioning shows strong dependency on sequence



Aim 2: Proposed Method

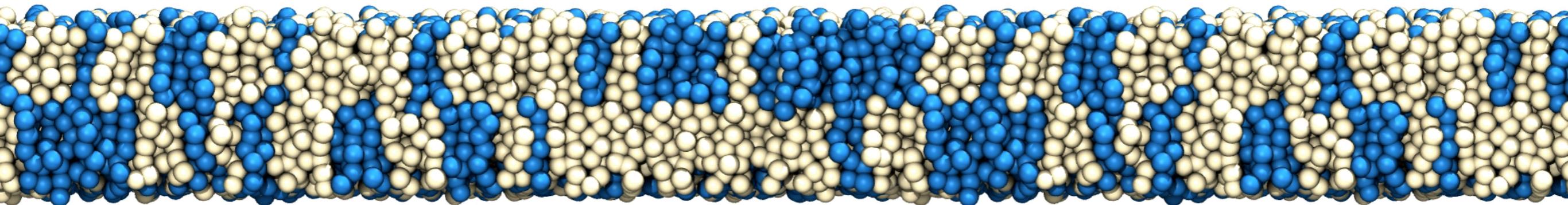
- Approach to test role of domain flexibility:
 - Double bonds add flexibility to acyl chains
 - Varying saturation and chain length will alter membrane elasticity
 - Using lipid-martini-itp-v05.py we can make custom lipids
- If (for example) Ld phase is made more flexible and a nAChR partitions into Lo
 - pLGIC shows strong dependency on membrane elasticity



Aim3: Development and release of a user-friendly VMD plugin for measuring elastic parameters of heterogenous membranes

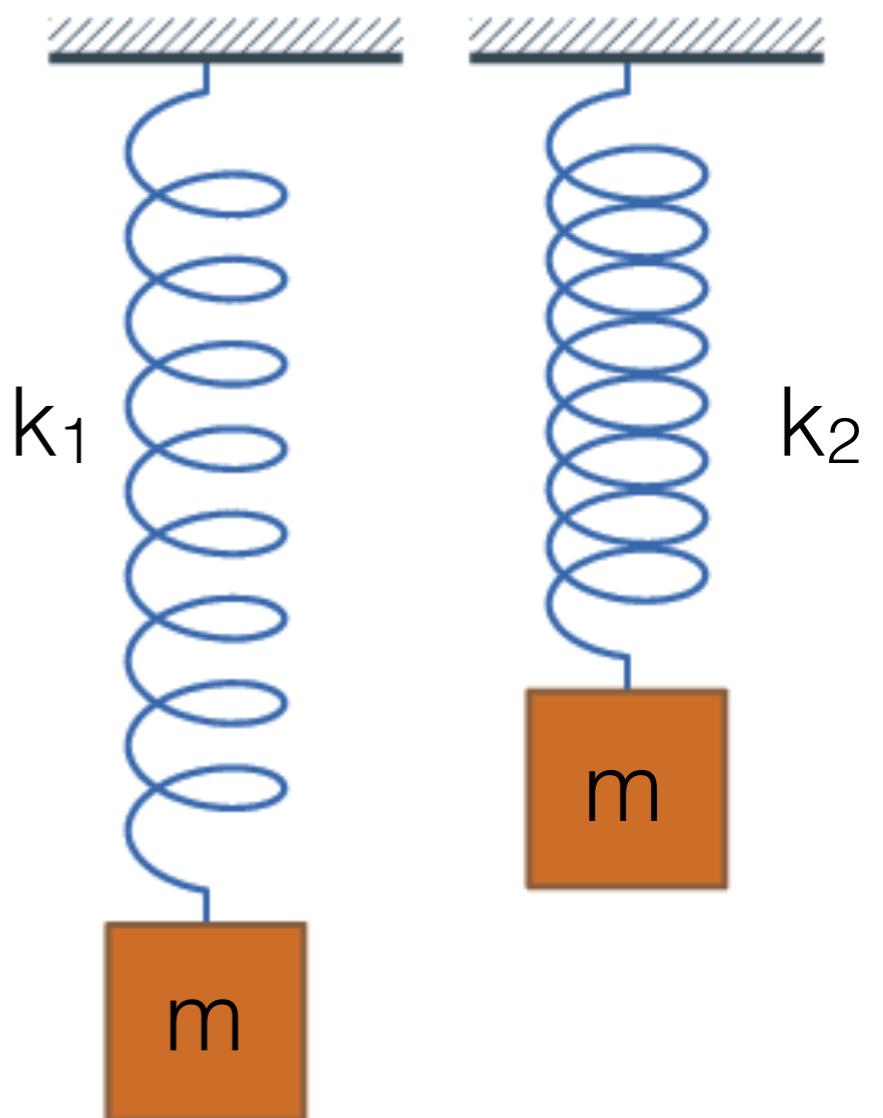
Aim 3: Tool development for quantifying membrane elasticity

- Aim: Measure the bending modulus (k_c) of a membrane
 - Composition independent
 - Measure by thermal fluctuations (example below)



Aim 3: Tool development for quantifying membrane elasticity

- The bending modulus (k_c) is analogous to the spring constant k , but for membrane curvature rather than spring length



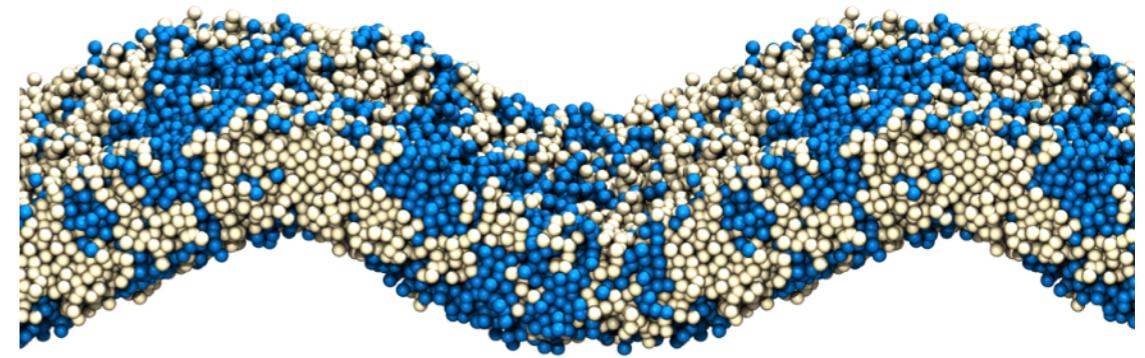
https://www.sciencebuddies.org/science-fair-projects/project-ideas/Phys_p064/physics/simple-harmonic-motion-springs

Aim 3: Tool development for quantifying membrane elasticity

- Significant work has already been put into the physics and math
- VMD provides a versatile set of tools and selection commands
- Open the door to elasticity quantitative analysis to those not versed in Fourier Spectrum analysis and lower level programming languages

Aim3: Proposed Method

- Measure k_c from thermal undulations, given “Helfrich Hamiltonian” for membrane free energy.



Please consider Problem 7 from the homework again, and read over it carefully, including my “clarifications”.

The expression you had in lines 1,2,4 is really the free energy *per unit area*. Let's call it lower case f here.

(The curvature in line 3 was really the local curvature, so left hand side of your line 3 should -technically- be $H(r)$. We just don't normally go so far as to write that way.)

For the total free energy of the membrane you need to *integrate* over the whole area to get the total free energy.

That integration is the only reason you can put in terms of Fourier series. Also, it's why K drops out.

So the equations I think you should have for this slide are shown on the right, please let me know if you don't understand them.

$$f = \frac{k_c}{2} (H - c_0)^2 + k_G K$$

$$F = \int_A dA f = \int_A dA \frac{k_c (H - c_0)^2}{2} + k_G K$$
$$c_0 = 0$$

$$\int_A dA K = 0$$

$$F = \frac{k_c}{2} \int_A dA H^2$$

H- mean curvature

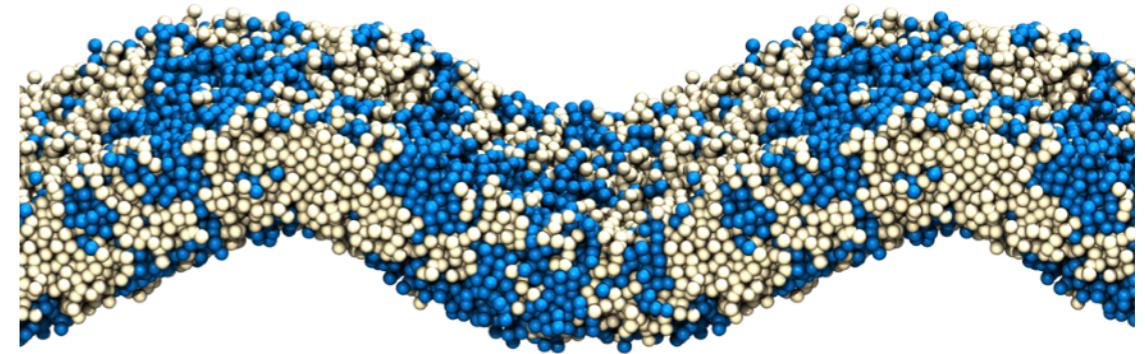
K- Gaussian curvature

c_0 - spontaneous curvature

k_c - bending modulus

k_G - Gaussian modulus

Aim3: Proposed Method



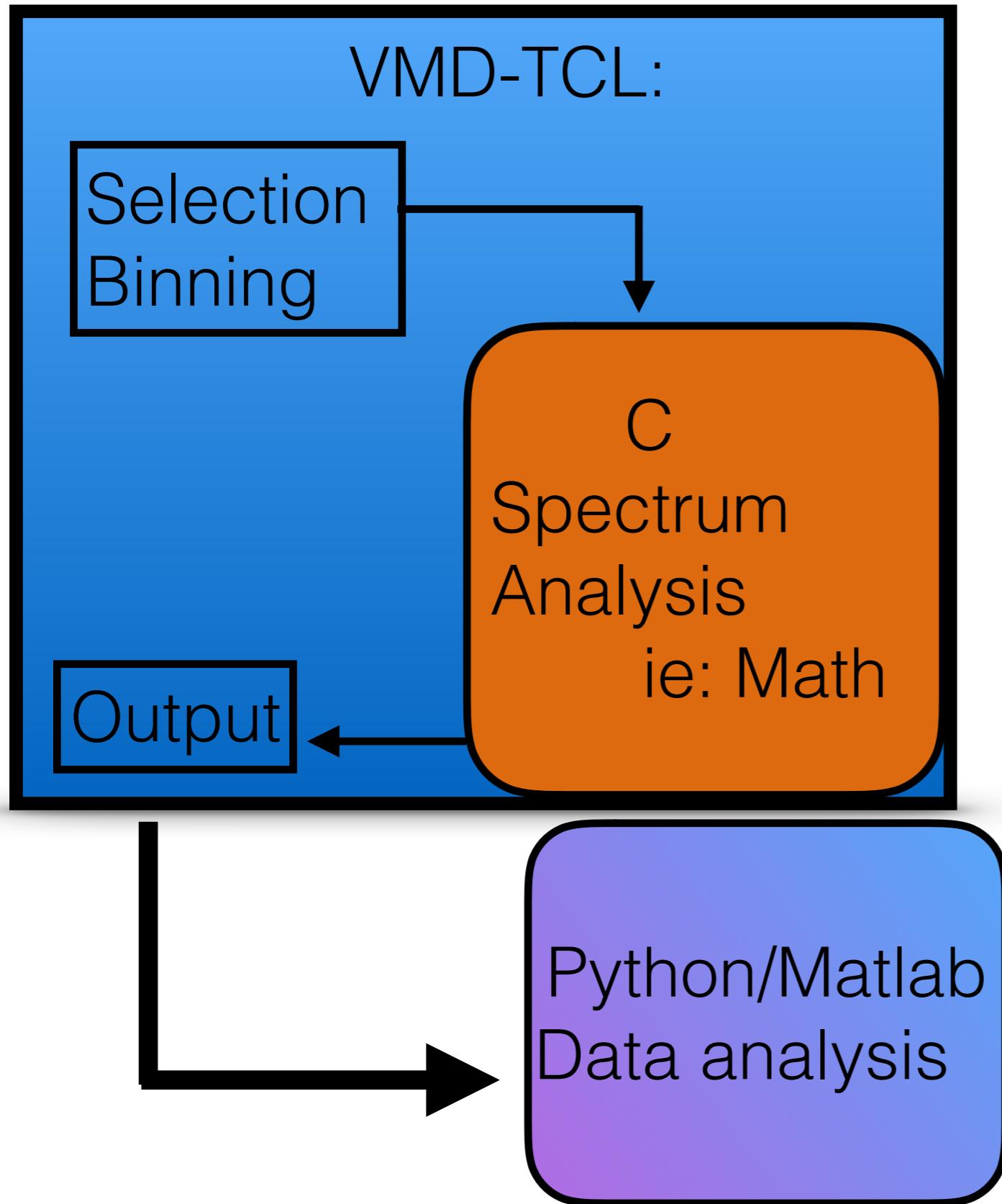
- Approximate the mean curvature using the Monge gauge :

$$H = \nabla^2 h(x, y)$$

- Calculate fluctuation spectrum using Fourier transform using q (the angular frequency)

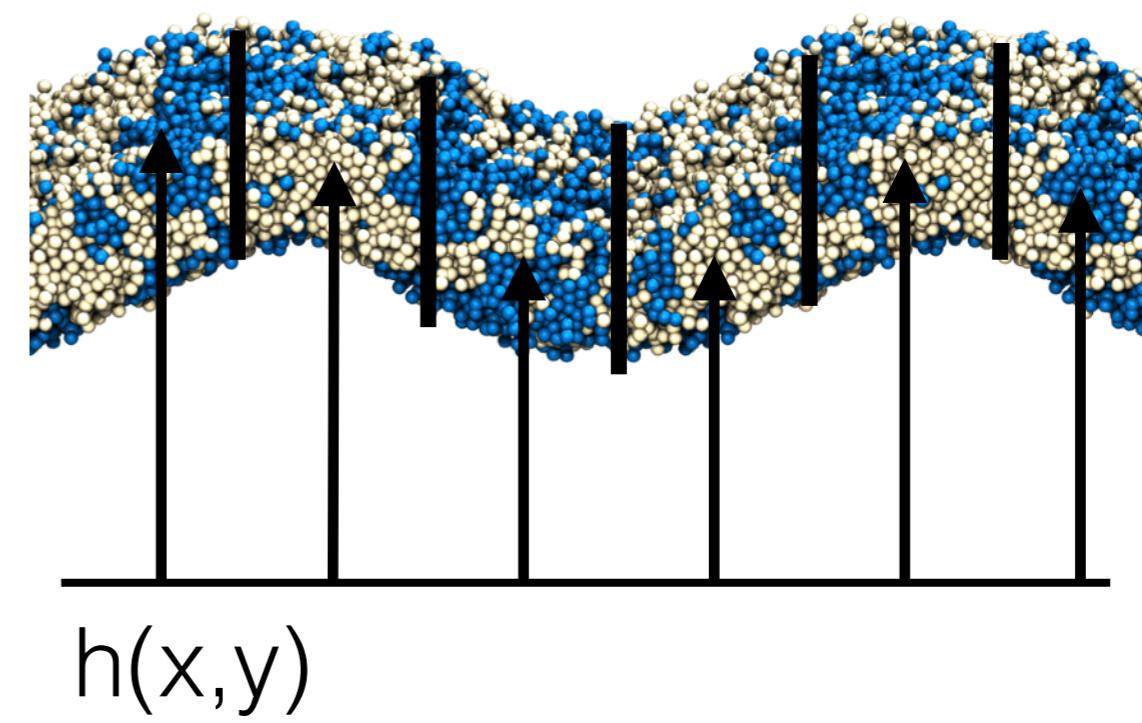
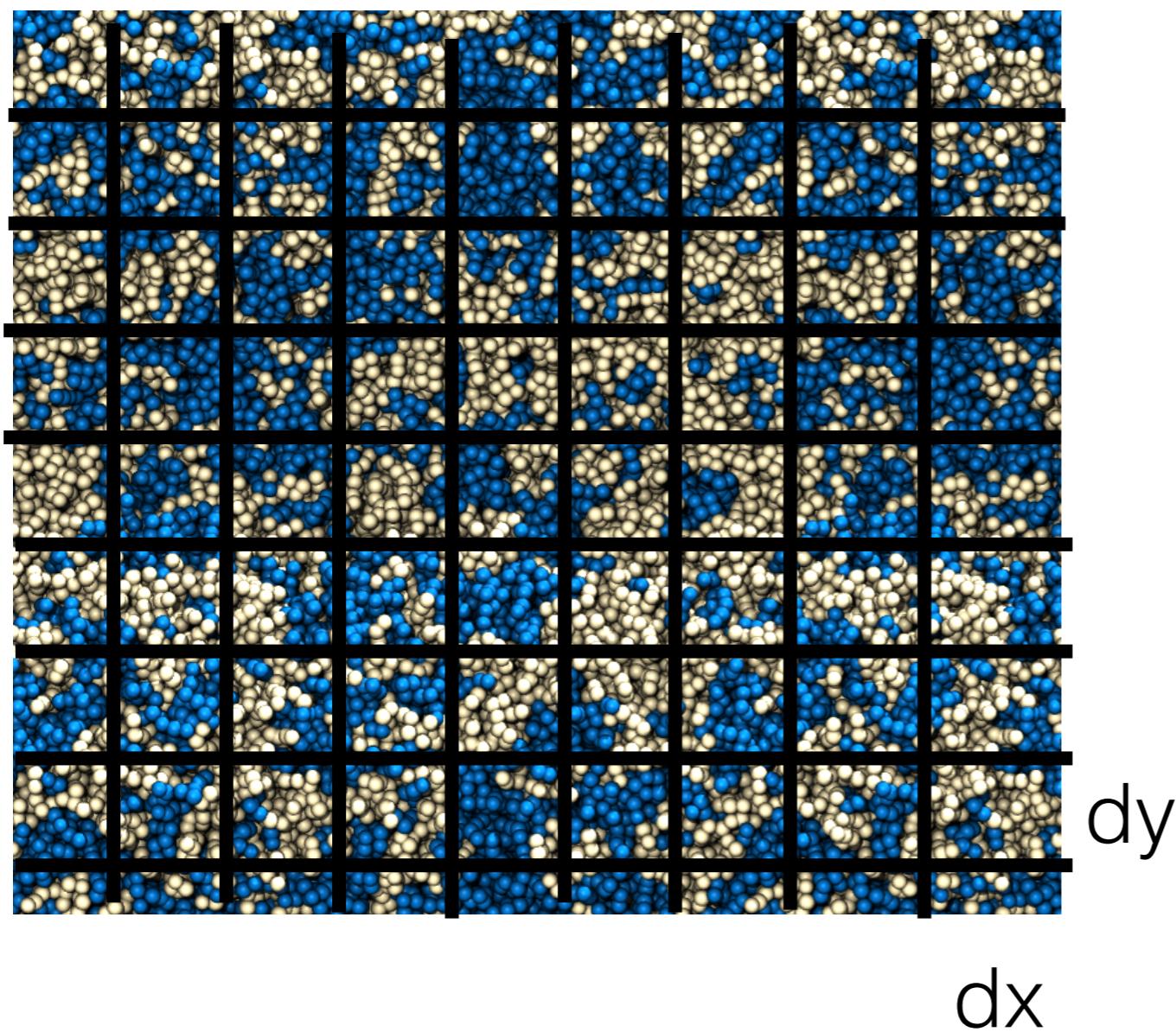
$$\langle |h_q|^2 \rangle \sim \frac{k_B T}{k_c |q^4|}$$

Aim3: Proposed Method



- Package will be:
 - tk terminal usable (VMD's terminal)
 - GUI support
 - Allow for:
 - custom bin area
 - adjustable frame inclusion
 - region of inclusion

Aim3: Proposed Method



Relationship between Aim 2 and Aim 3

- In Aim 2 I want to determine to what extent, if any, the elasticity of the membrane effects protein partitioning
- The tools to be built in Aim 3, will allow me to quantify the elastic nature of various membranes

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 - Dr. Salari
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 - Local cluster funded by NSF-DBI1126052
 - Research Corporation, NIH P01GM55876-14A1

Thank you for your time!