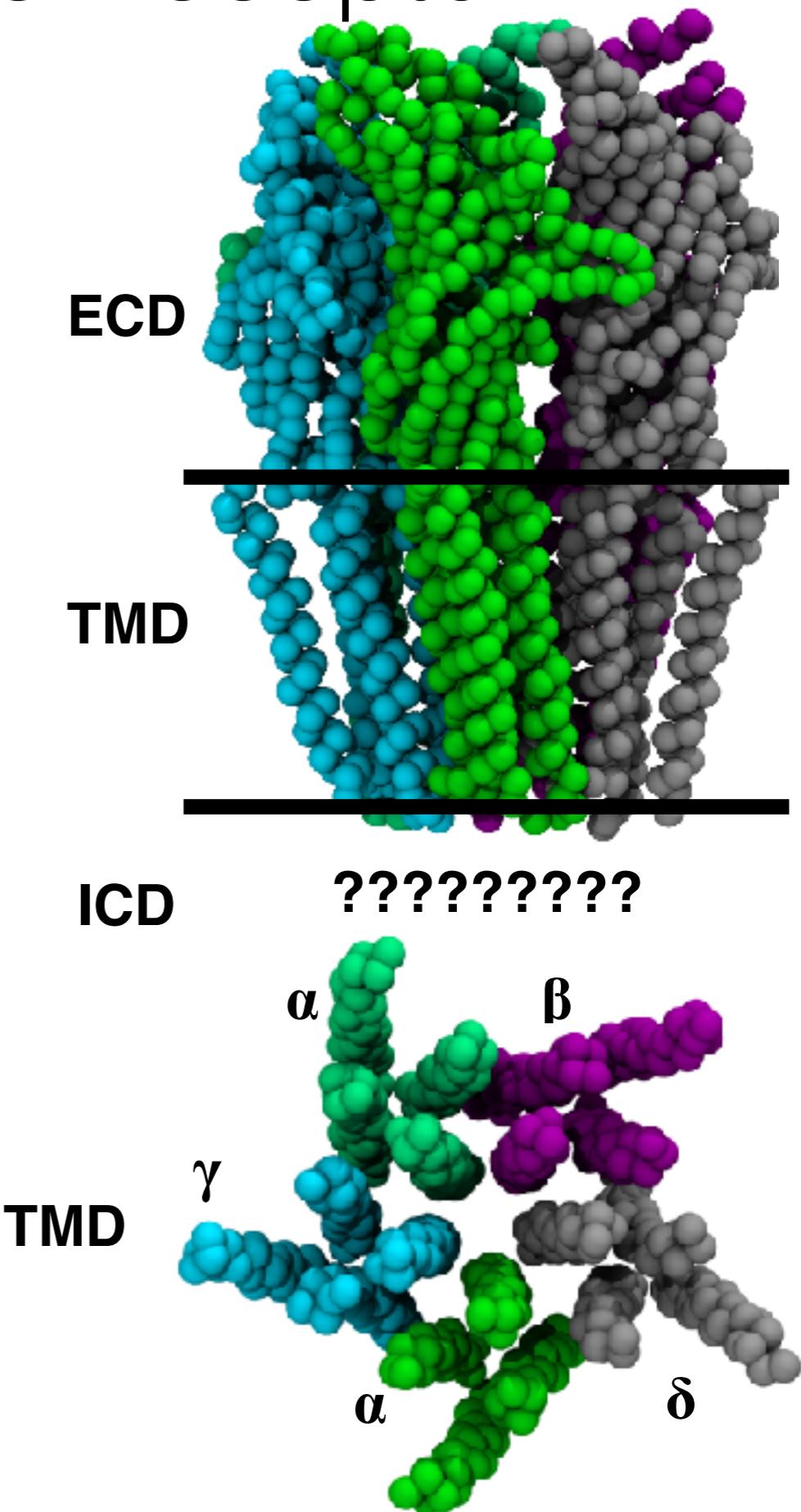


BOUNDARY LIPIDS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN QUASI-NATIVE MEMBRANES

Liam Sharp

Nicotinic Acetylcholine Receptor (nAChR)

- Pentameric ligand gated ion channel gated by binding of acetylcholine
- Found throughout the central and peripheral nervous system at the post synaptic terminal
- Role in neurological and physical disorders as well as addiction
- Contributes to neuronal and muscular function by stimulating an action potential after acetylcholine binds
- Extremely lipid sensitive



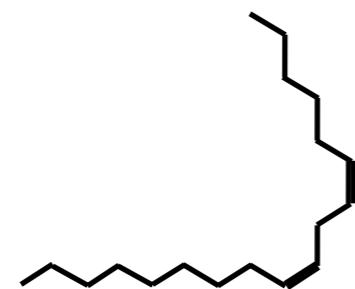
Phospholipids

- Hydrophilic Head
 - Choline, ethanolamine, serine, phosphatidic acid, inositol
 - Phosphate
 - Glycerol backbone
- Hydrophobic Chains
 - Hydrocarbon chains of various lengths and hydrogen saturation
- Amphiphilic nature promotes spontaneous membrane formation

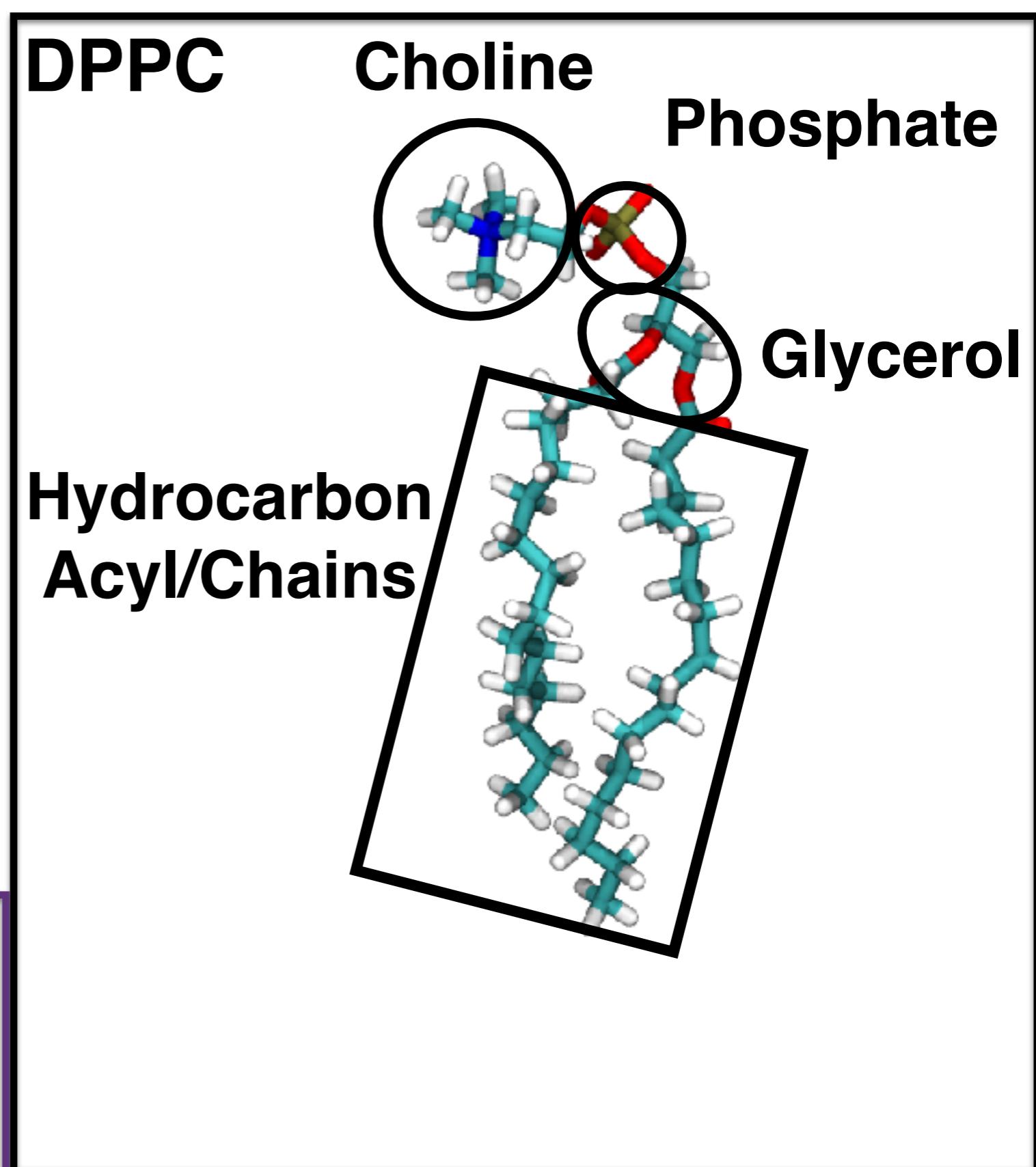
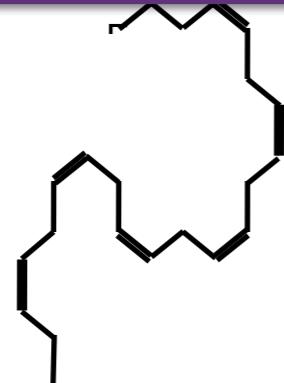
Sat



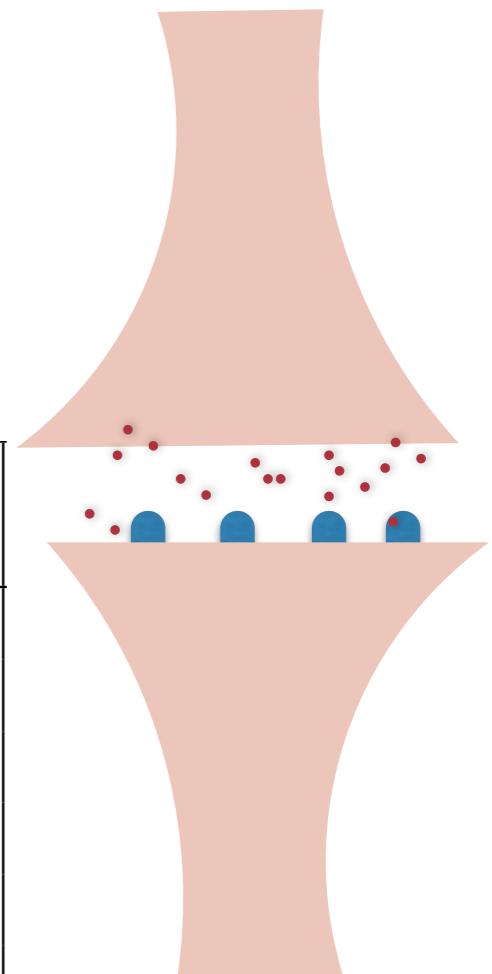
n-6



n-3



Nicotinic Acetylcholine Receptor (Membrane Comparison)



Saturation/Head Group	Torpedo (1,2)	Synapse (3,4)
n-0	59	52
n-9	14	15
n-7	< 1	< 1
PUFA	28	33
— n-3	— 19	— 18
— n-6	— 9	— 15

- 1) F. Barrantes, The lipid environment of the nicotinic acetylcholine receptor in native and reconstituted membrane, Critical reviews in biochemistry and molecular... (1989).
2) 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.
3) 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.
4) 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.
https://en.wikipedia.org/wiki/Electric_ray#/media/File:Torpedo_marmorata2.jpg

Nicotinic Acetylcholine Receptor (Membrane Comparison)

Saturation/Head Group	Torpedo (1,2)	Synapse (3,4)	Xenopus Oocyte (5)	Soybean (6,7)
n-0	59	52	46	19
n-9	14	15	22	30
n-7	< 1	< 1	14	NA
PUFA	28	33	17	51
— n-3	— 19	— 18	— 6	— 15
— n-6	— 9	— 15	— 11	— 36
PC	43	43	36	27
PE	32	36	22	25
PS	13	12	5	NA
SM	8	4	26	NA
PI	4	3	7	28
PA	< 1	0	0	20
Other	6	2	4	0
Chol Mol Frac	32	39	21	0

1)F. Barrantes, The lipid environment of the nicotinic acetylcholine receptor in native and reconstituted mem brane, Critical reviews in biochemistry and molecular... (1989).

2)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.

3)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.

4)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.

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.

Domain Formations

Review

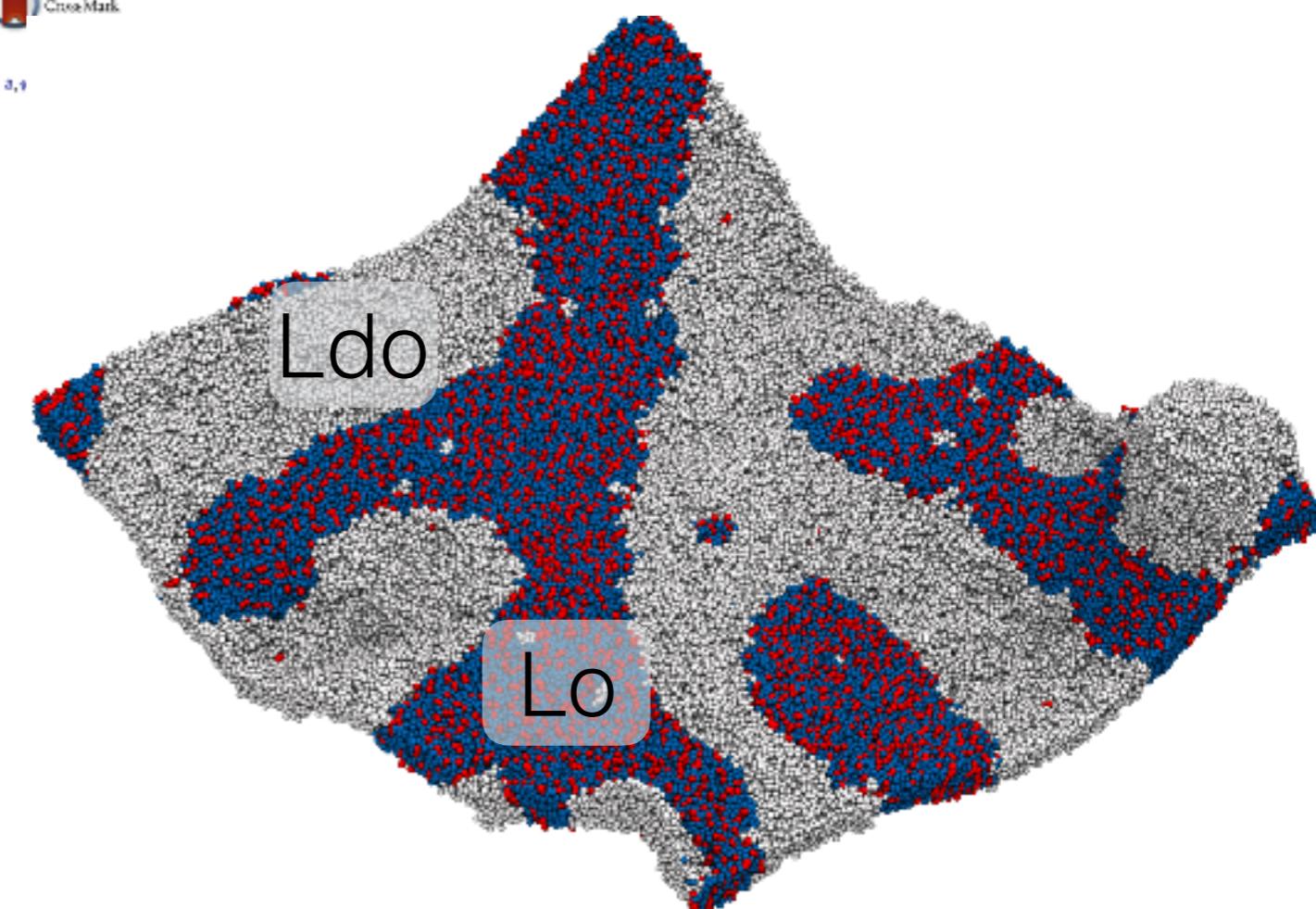
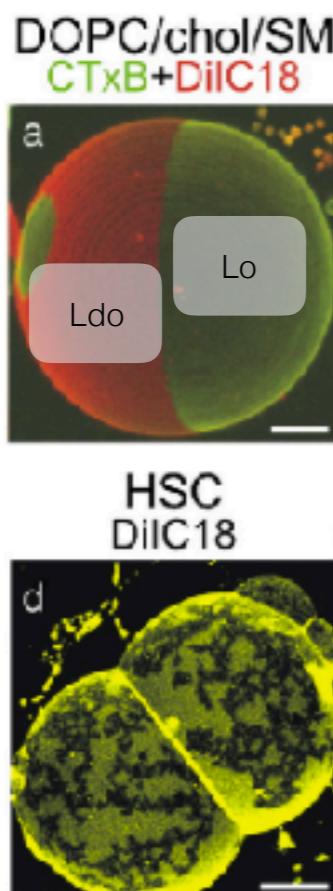
Recent progress on lipid lateral heterogeneity in plasma membranes:
From rafts to submicrometric domains



Mélanie Carquin ^{a,1}, Ludovic D'Auria ^{b,1}, Hélène Pollet ^a, Ernesto R. Bonagurzone ^b, Donationne Tyleca ^{a,*}

^a CEE Unit, de Duve Institute & Université Catholique de Louvain, UCL-BI, 75 05, Avenue Hippocrate, 75, B-1300 Brussels, Belgium

^b The Mueller Segregative Group at the Dept. Anatomy & Cell Biology, College of Medicine, University of Illinois, 928 S. Wood St., MC512, Chicago, IL 60612, USA

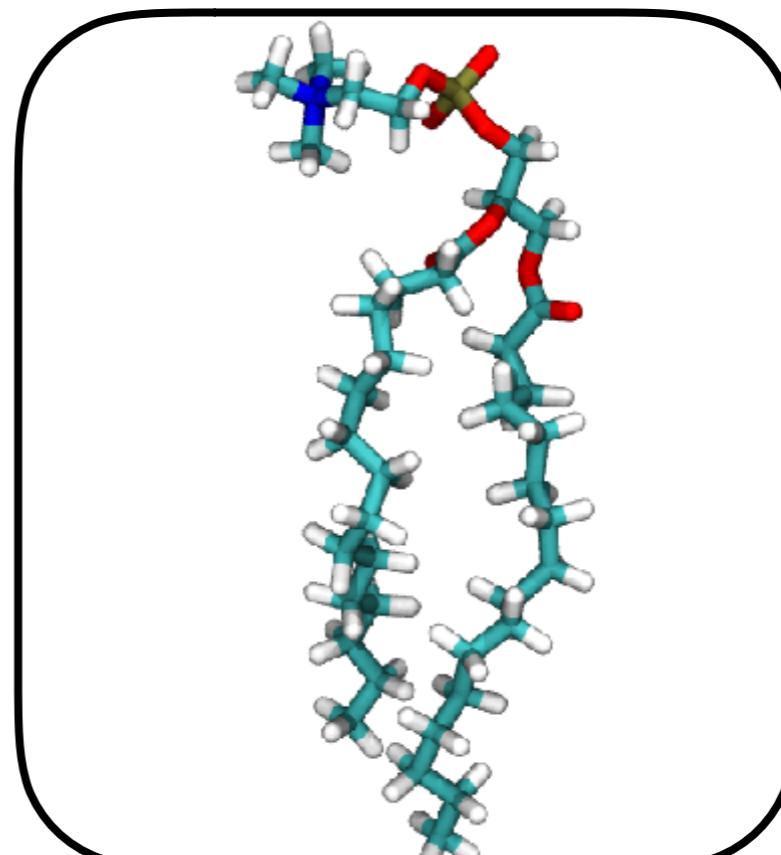


Saturated Cholesterol Unsaturated

Fig. 1. Visualization of phase coexistence in artificial and very specialized membranes. (a) Giant unilamellar vesicle (GUV) composed of DOPC:cholesterol:SM (1:1:1) and 0.1 mol% GM1. Labeling with Alexa 488-CTxB (GM1; green) and DilC18 (red) and examination at 23 °C. (b) GUV made of DOPC:cholesterol:SM (1:1:1). Labeling with Laurdan, fo, fluid-ordered; fd, fluid-disordered. (c) GUV made of POPC and 30% NCeramide (NCer) mixture. Labeling with Rhodamine DOPE and examination at 22 °C. (d) Human skin stratum corneum (HSC) lipid membrane. Labeling with DilC18 and examination at 25 °C. (e) Pig pulmonary surfactant membrane. Labeling with BODIPY-PC (green) and DilC18 (red) and examination at 36 °C. (f) PM sphere (PVS) derived from A431 cells. Labeling with Alexa 488-CTxB (GM1; green) and Alexa 566-transferrin (Tf; red) and examination at 37 °C. Notice Lo/Ld (a,b,c,f) vs solid-ordered (So)/Lc (c) or Sm/Sm (d) phase coexistence. DOPC, 1,2-dioleyl-sn-glycerol-3-phosphocholine; POPC, 1-palmitoyl-2-oleyl-sn-glycero-3-phosphoinositide; DOPE, 1,2-dioleyl-sn-glycero-3-phosphoethanolamine. All scale bars, 10 μm. Adapted with permission from: (a) [17]; (b) [43]; (c) [44]; (d) [18]; (e) [16]; (f) [47].

Methods

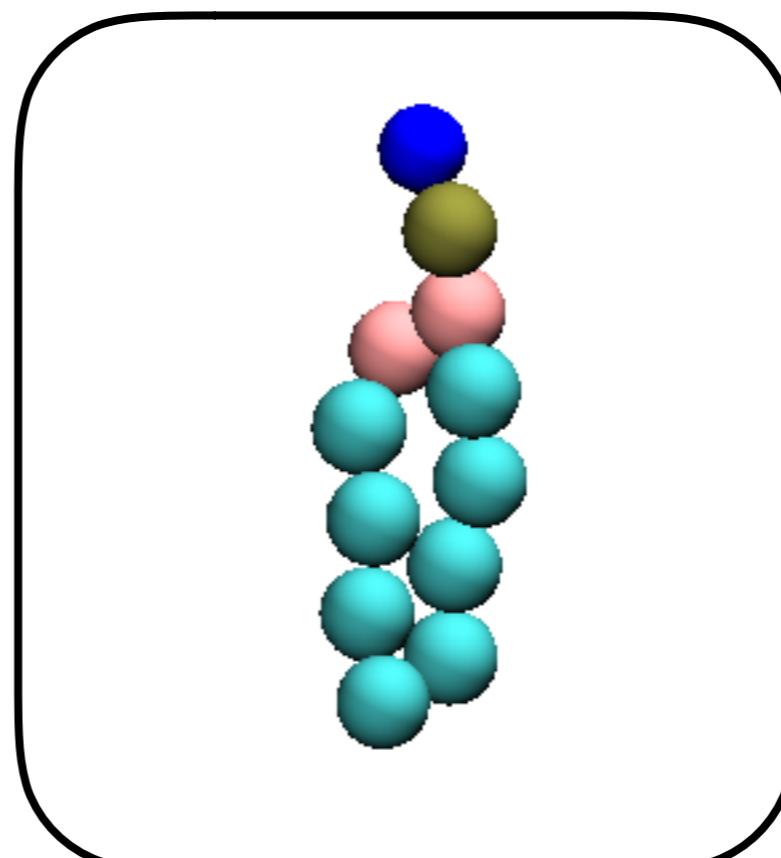
- Martini (ver 2.2) Coarse Grained Force Field
- `martinize.py` determine and converts secondary structure from atomistic to coarse grained
- `insane.py` places the protein in a randomized membrane
- Molecular Dynamics (GROMACS)
 - Harmonic restraints on secondary and tertiary structures



Head Group

**Glycerol
backbone**

Acyl chains



Head Group

**Glycerol
backbone**

Acyl chains

What We Know (Standing on the backs of Giants)

- nAChR-lipid interaction in a native membrane have not been well studied
- ~15% cholesterol must be present in model membranes to restore native function Fong and McNamee (1986)
- Experimental evidence in model membranes suggests the lo
- Asolectin (soy bean membrane) improves results Fong and McNamee (1985)
- Jones and McNamee (1987) showed cholesterol-nAChR direct interaction
- Brannigan et. al 2008 predicted cholesterol deep non-annular binding

Partition profile of the nicotinic acetylcholine receptor in lipid domains upon reconstitution^{1*}

Vicente Bermúdez, Silvia S. Antolini,¹ Gaspar A. Fernández Nieves, María I. Aveldano, and Francisco J. Barrantes

Instituto de Investigaciones Biogénicas de Bahía Blanca, Comisión Nacional de Investigaciones Científicas y Técnicas, and UNESCO Chair of Biofísics and Molecular Neurobiology, Universidad Nacional del Sur.



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,*}

^a Instituto de Investigaciones Biogénicas de Bahía Blanca (CONICET-UNSA), Camino La Carrangola Km 2, 8300 Bahía Blanca, Buenos Aires, Argentina

^b Universidad Nacional del Sur, Av. Alvaro 1253, 8000 Bahía Blanca, Buenos Aires, Argentina

^c Instituto Argentino de Tecnología (IATRA) IIA-ITGB, Lamina La Carrangola Km 2, 8300 Bahía Blanca, Buenos Aires, Argentina

^d Laboratory of Molecular Neurobiology, BIOMED UCA-CONECT, Av Moreau de Justo 1300, 1107 Buenos Aires, Argentina

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

Vol. 275, No. 2, Issue 2 January 14, pp. 777-785
Printed in U.S.A.

Effect of Membrane Lipid Composition on the Conformational Equilibria of the Nicotinic Acetylcholine Receptor^{*}

(Received for publication, July 20, 1999, and in revised form, November 3,

John E. Baenziger,[†] Mary-Louise Morris, Tim E. Darsaut, and Stephen E. Ryan

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

Annular and Nonannular Binding Sites for Cholesterol Associated with the Nicotinic Acetylcholine Receptor[†]

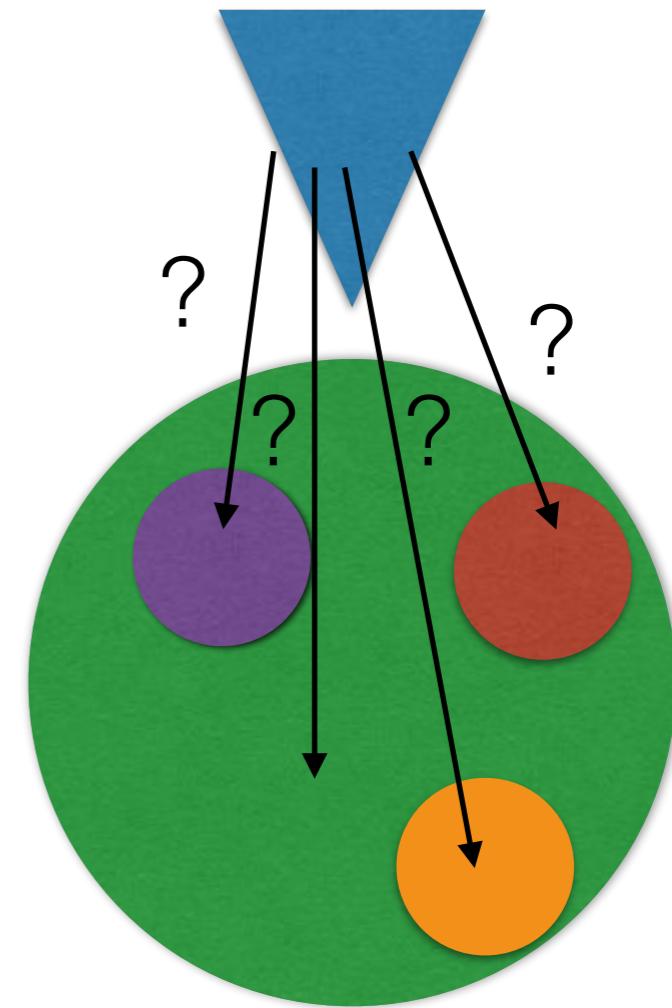
Owen T. Jones and Mark G. McNamee^{*}

Department of Biochemistry and Biophysics, University of California, Davis, Davis, California 95616

Received August 21, 1987; Revised Manuscript Received December 3, 1987

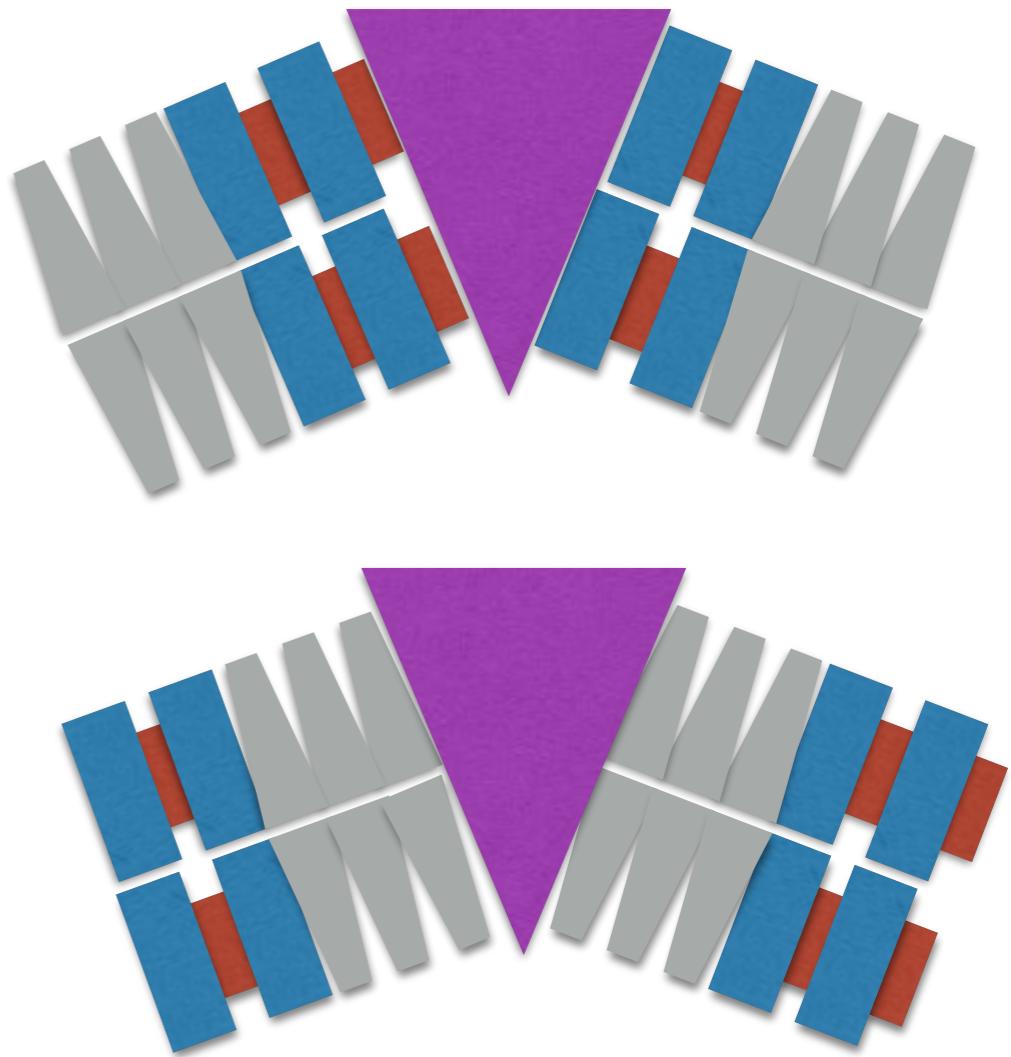
Main Question

- 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?



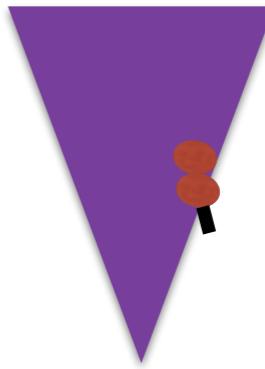
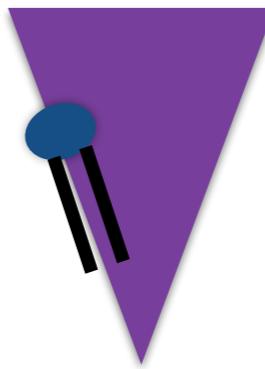
Main Question

- 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?

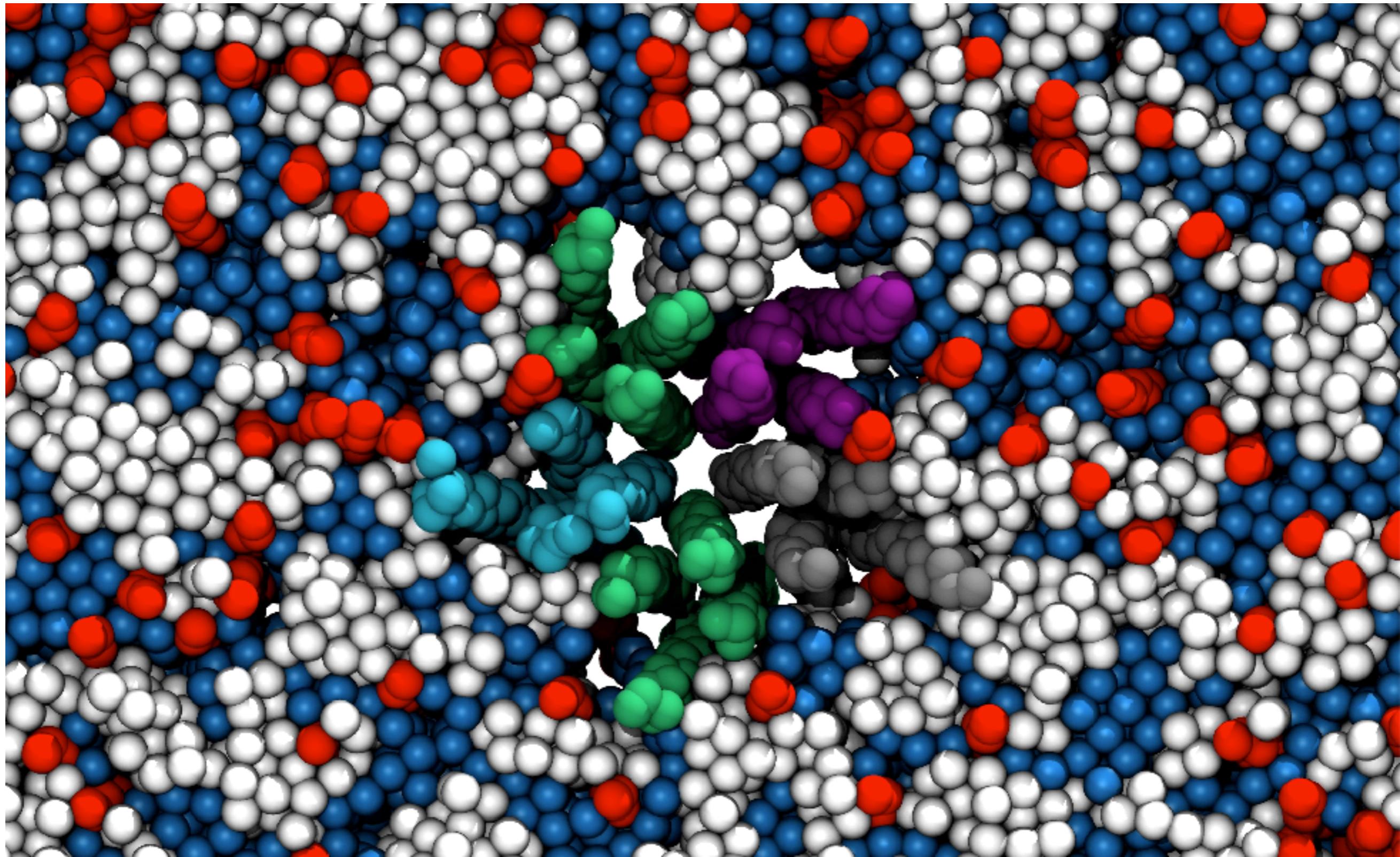


Main Question

- 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?



nAChR *Torpedo* Quasi-Native Membrane



Saturated Cholesterol Unsaturated

~ Initial 50 ns

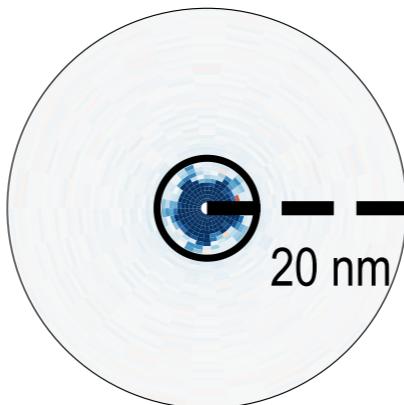
nAChR *Torpedo* Quasi-Native Membrane Domain Formation Across Three Compositions

PUFA species in mixture

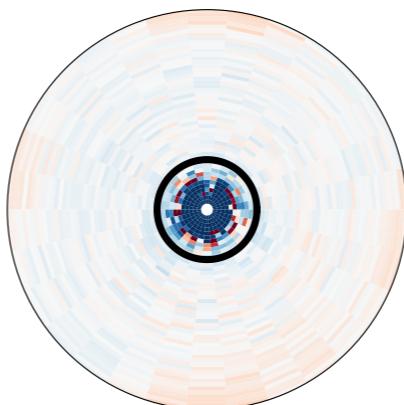
Lipid species calculated

Sat

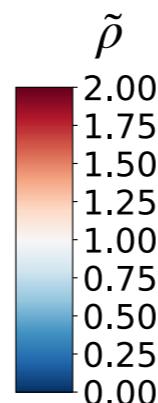
None



Chol



PUFA

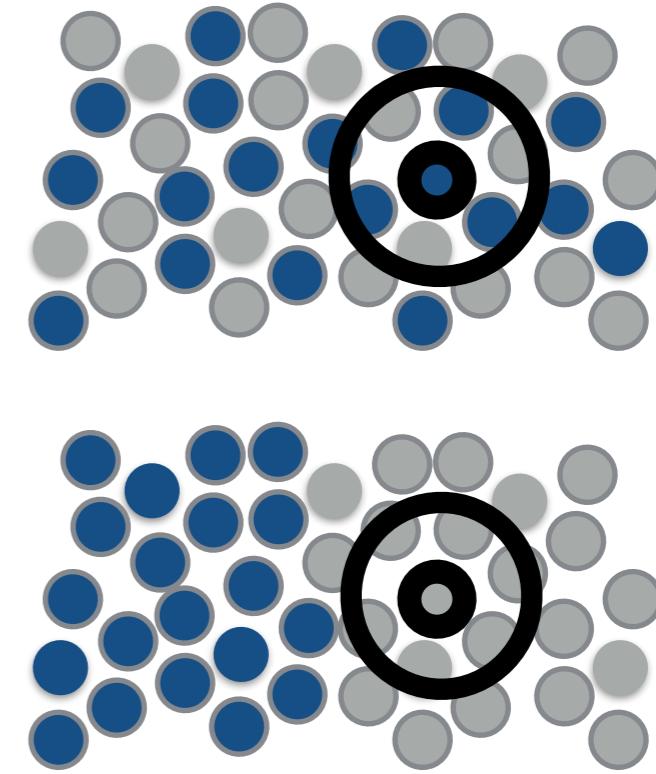
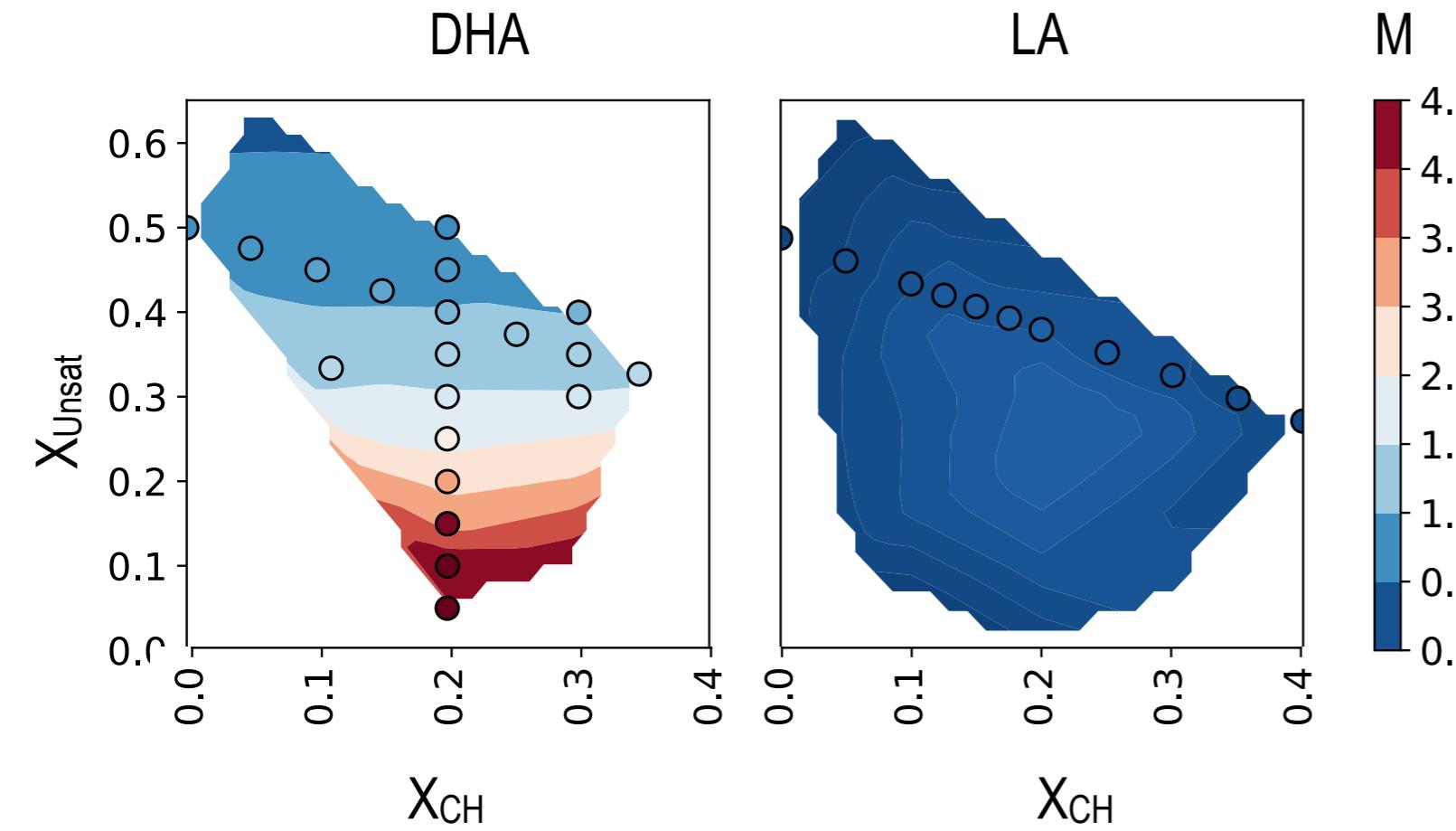


$$\rho_B(r_i, \theta_j) = \frac{\langle n_B(r_i, \theta_j) \rangle}{r_i \Delta r \Delta \theta}$$

$$\tilde{\rho}(r_i, \theta_j) = \frac{\rho_b(r_i, \theta_j)}{x_B s_b N_L / \langle L^2 \rangle}$$

nAChR *Torpedo* Quasi-Native Membrane PUFA Mixing

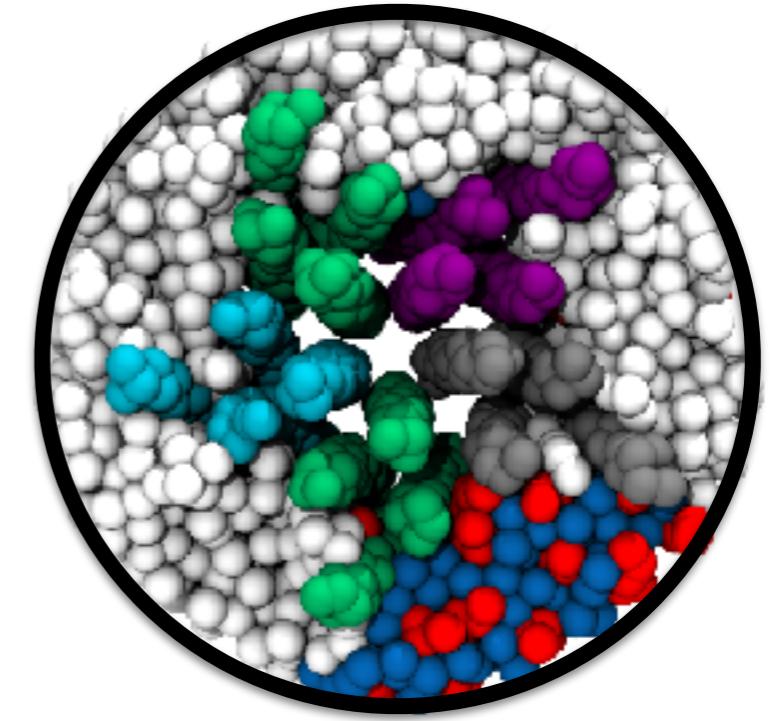
$$M_{A,B} = \frac{\langle n_{a,b} \rangle}{6x_b} - 1$$



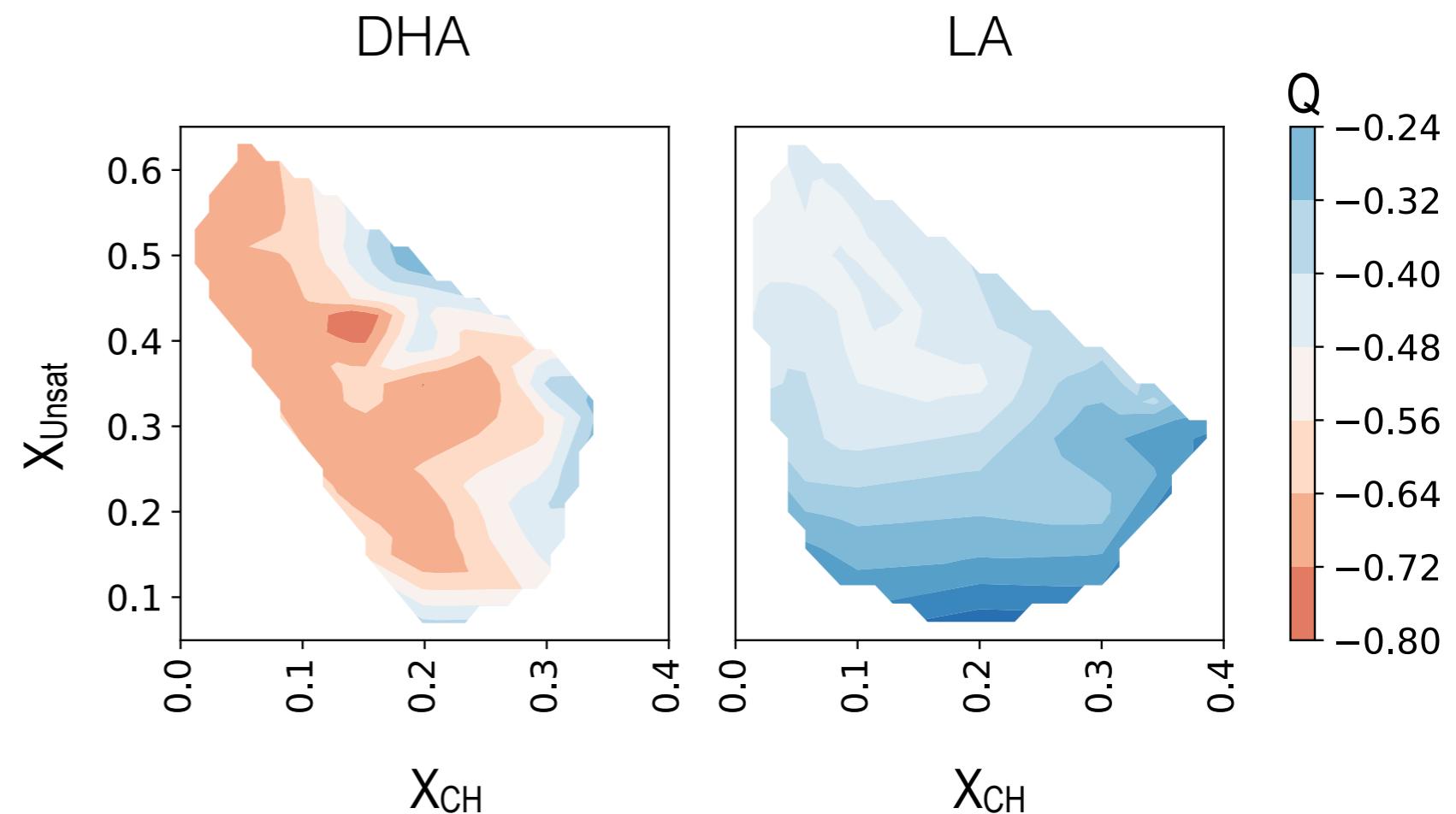
- Domain formation is independent of a protein
- nAChR may affect how well defined a domain is

nAChR *Torpedo* Quasi-Native Membrane Boundary Lipids

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



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



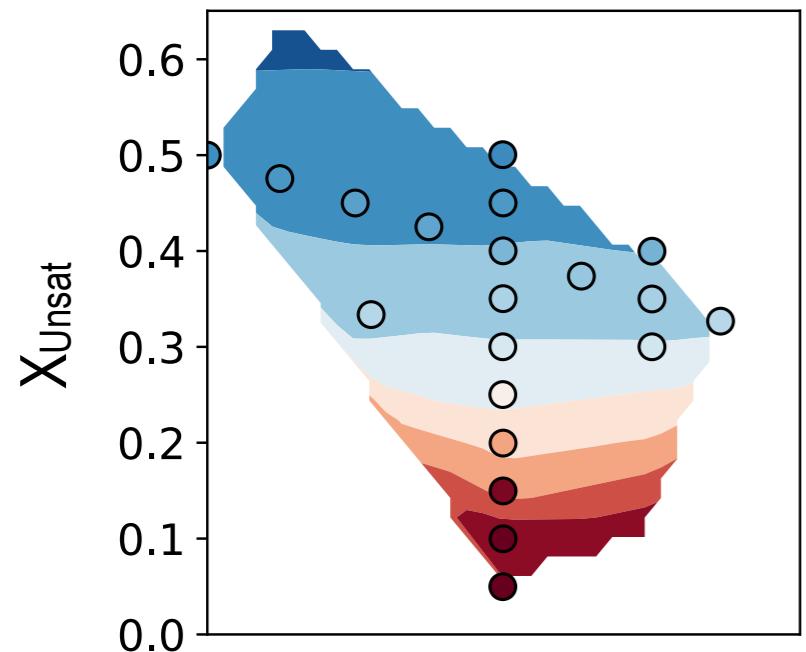
nAChR *Torpedo* Quasi-Native Membrane Mixing-Boundary Environment Relationship

- What effect does domain formation have on boundary lipid enrichment/depletion?
 - Lipid-lipid interaction forms domains and the protein picks a domain
 - Should lead to very similar M vs Q trends
 - Protein-lipid interaction where the protein surrounds itself with lipids it wants regardless of their placement
 - Should lead to more independent M vs Q trends

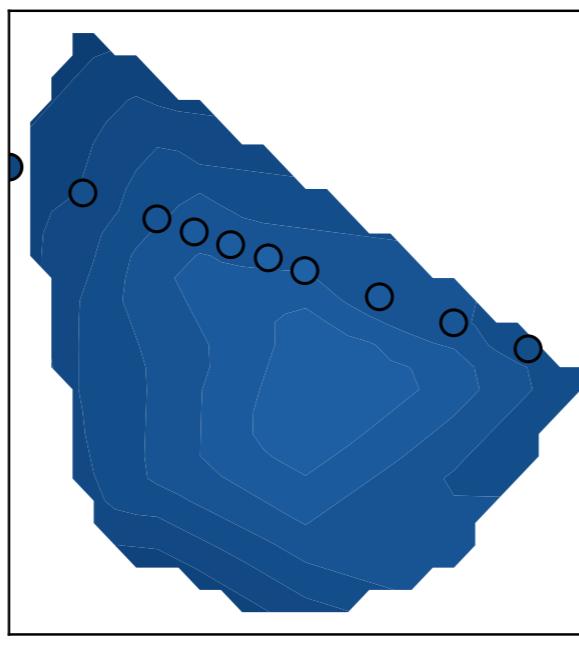
nAChR *Torpedo* Quasi-Native Membrane Mixing-Boundary Environment

Relationship

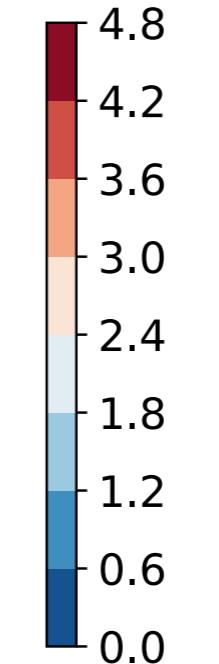
DHA



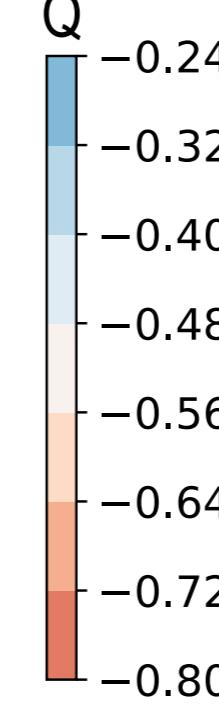
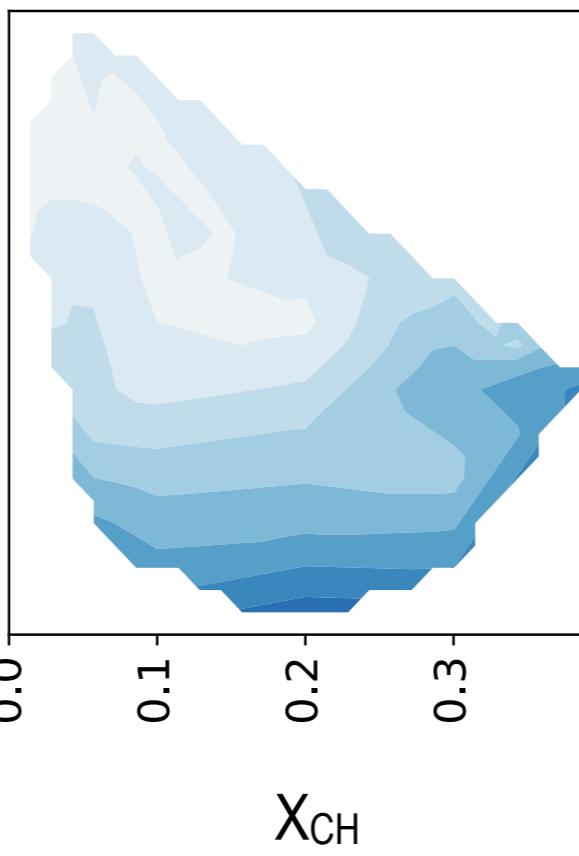
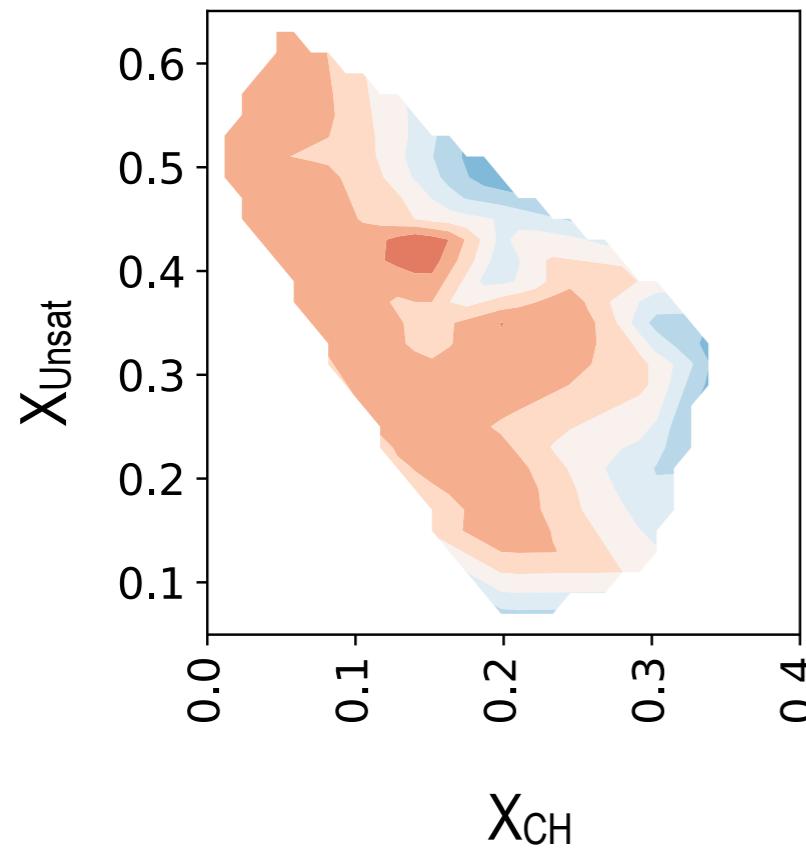
LA



M



Looks most
similar to option 2



nAChR *Torpedo* Quasi-Native Membrane Embedded Lipids

PUFA species in mixture

Lipid species calculated

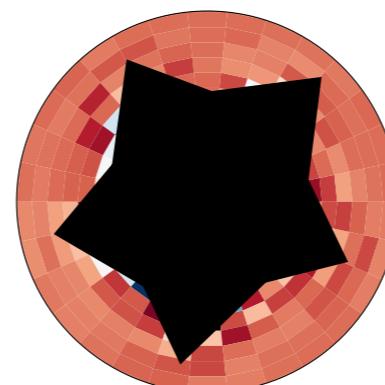
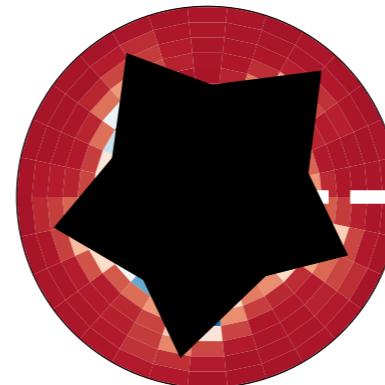
Sat

Chol

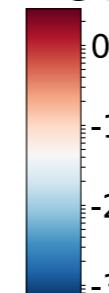
PUFA

None

5 nm



$\log \tilde{\rho}$



NaN
value

$$\rho_B(r_i, \theta_j) = \frac{\langle n_B(r_i, \theta_j) \rangle}{r_i \Delta r \Delta \theta}$$

$$\tilde{\rho}(r_i, \theta_j) = \frac{\rho_b(r_i, \theta_j)}{x_B s_b N_L / \langle L^2 \rangle}$$

Lipid Embedded pLGICs

Crystal structure and dynamics of a lipid-induced potential desensitized-state of a pentameric ligand-gated channel

Sandip Basak^{1†}, Nicolaus Schmandt^{2†‡}, Yvonne Gicheru^{1†}, Sudha Chakrapani^{1*}

¹Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA
²Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio, USA

LETTER

doi:10.1038/nature13669

nature
structural &
molecular biology

X-ray structures of GluCl in *apo* states reveal a gating mechanism of Cys-loop receptors

Thorsten Althoff^{1†*}, Ryan E. Hibbs^{1†*}, Surajit Banerjee² & Eric Gouaux^{1,3}

Crystal structures of a GABA_A-receptor chimera reveal new endogenous neurosteroid-binding sites

Duncan Laverty¹, Philip Thomas¹, Martin Field¹, Ole J Andersen², Matthew G Gold¹, Philip C Biggin², Marc Gielen^{1,3} & Trevor G Smart¹

Plug

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

L. Sharp^a, R. Salari^{a,b}, G. Brannigan^{a,c}

^a*Center for Computational and Integrative Biology, Rutgers University-Camden, Camden, NJ*

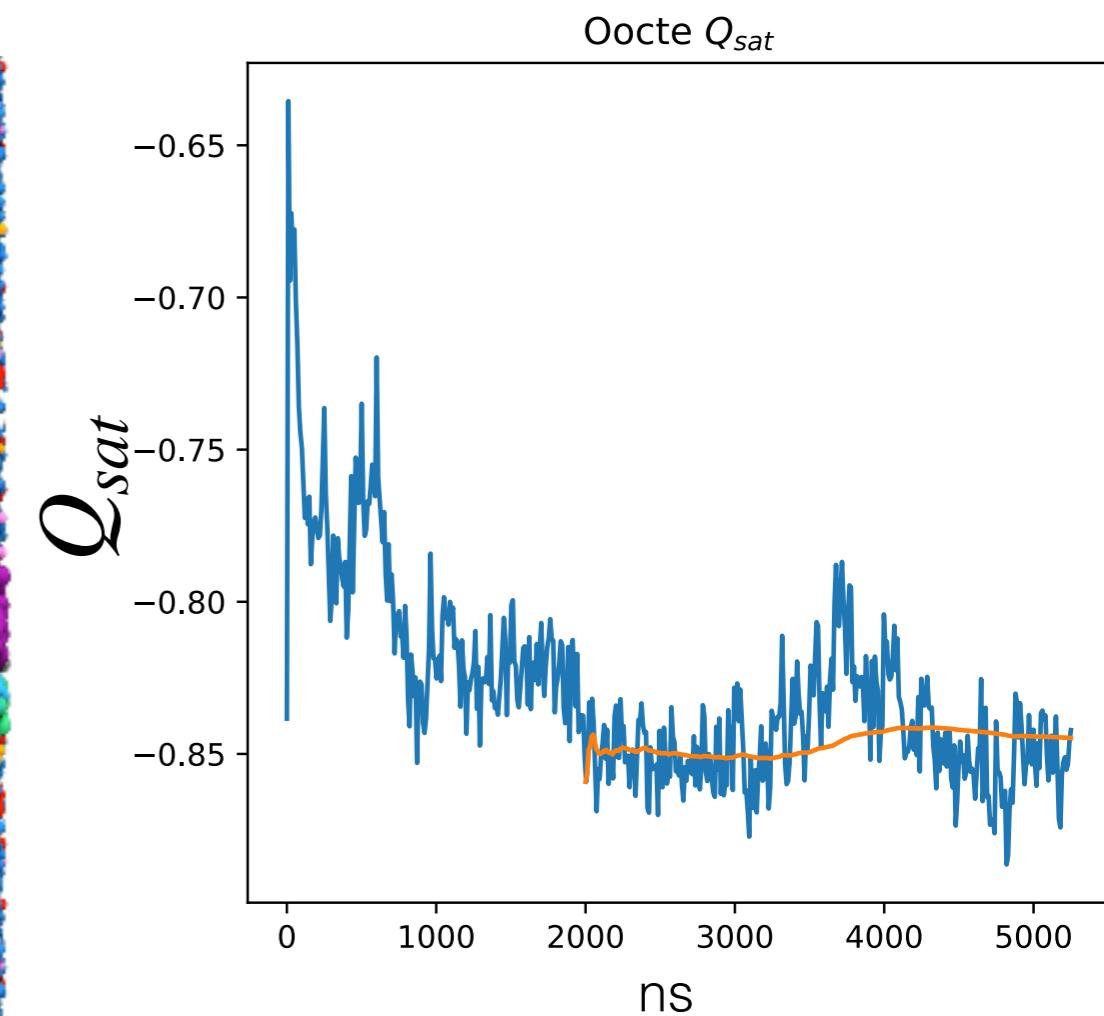
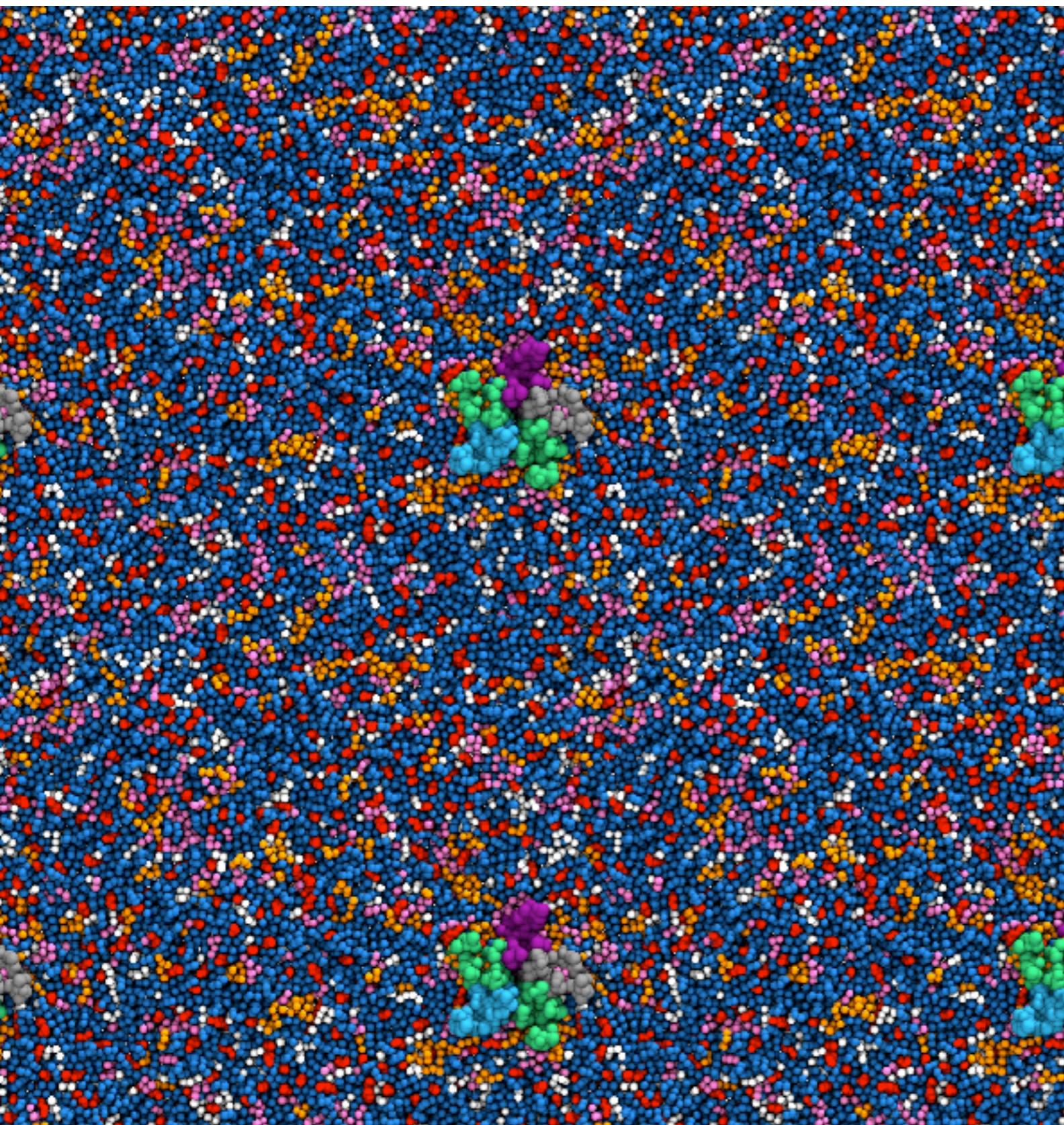
^b*Now at Washington University School of Medicine in St Louis*

^c*Department of Physics, Rutgers University-Camden, Camden, NJ*

Submitted to BBA (Currently working on revisions)

Can be found: <https://arxiv.org/abs/1807.11090>

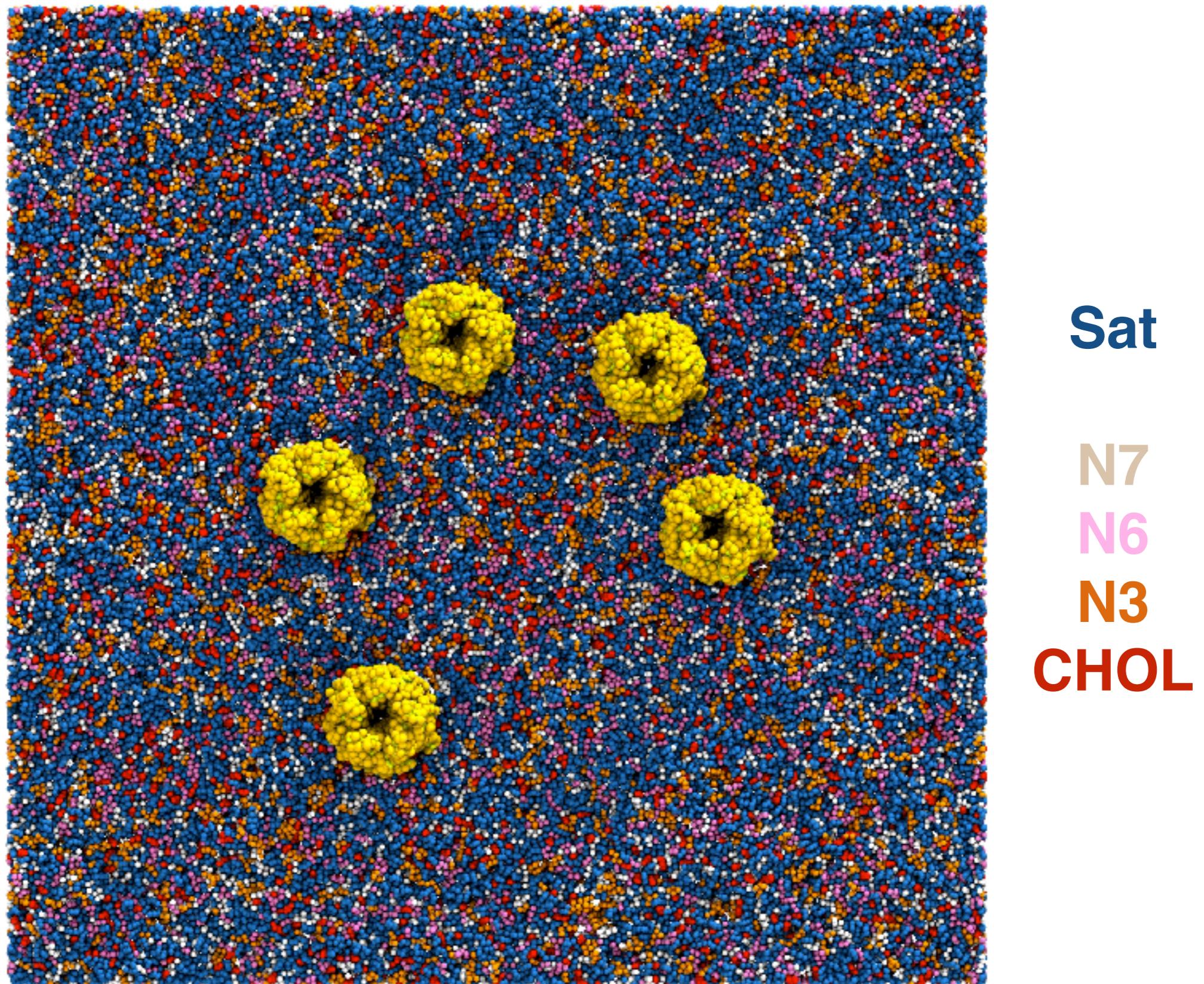
nAChR within Quasi-Xenopus Oocyte Bilayer



Sat $\langle Q_{sat} \rangle = -.841$

N7
N6
N3
CHOL

Multiple nAChR within Quasi-Xenopus Oocyte Bilayer



Summary

- Where does nAChR partition within a native-like membrane with well-defined domains?
 - Liquid disorder phase
- Do nAChR "boundary lipids" just reflect the preferred domain composition, or are there additional preferences for specifically bound lipids?
 - nAChR “boundary lipids” mostly reflect its domain composition, however cholesterol still plays a factor
- If so, what are the preferred boundary lipids, and can they bind so they are not even interacting with the surrounding domain?
 - PUFAs and cholesterol both interact directly with nAChR, with a affinity for PUFA non-annular binding
 - Within *Xenopus* oocytes nAChR favors PUFAs and cholesterol over other lipids (trend continues for neuronal and torpedo membranes)
- Read our paper!

Acknowledgment

- Dr. Brannigan
- **Committee Members**
 - Dr. Martin
 - Dr. O'Malley
 - Dr. Hénin
- **Brannigan Lab:**
 - **Sruthi**
 - Ruchi
 - Kristen
 - **Shashank**
 - **Dr. Salari**
 - Rulong



- Computational resources: NSF XSEDE Allocation NSF-MCB110149
- Local cluster funded by NSF-DBI1126052
- Research Corporation, NIH P01GM55876-14A1