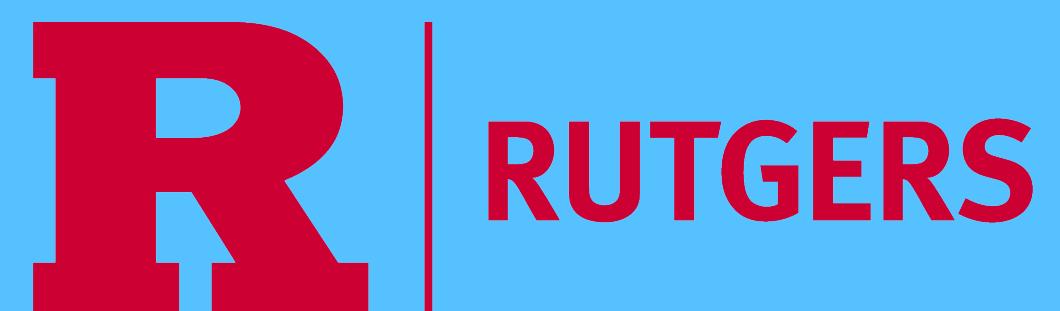


Measuring Lipid Binding Affinities in Unbiased CG-MD Using the Density-Threshold Affinity



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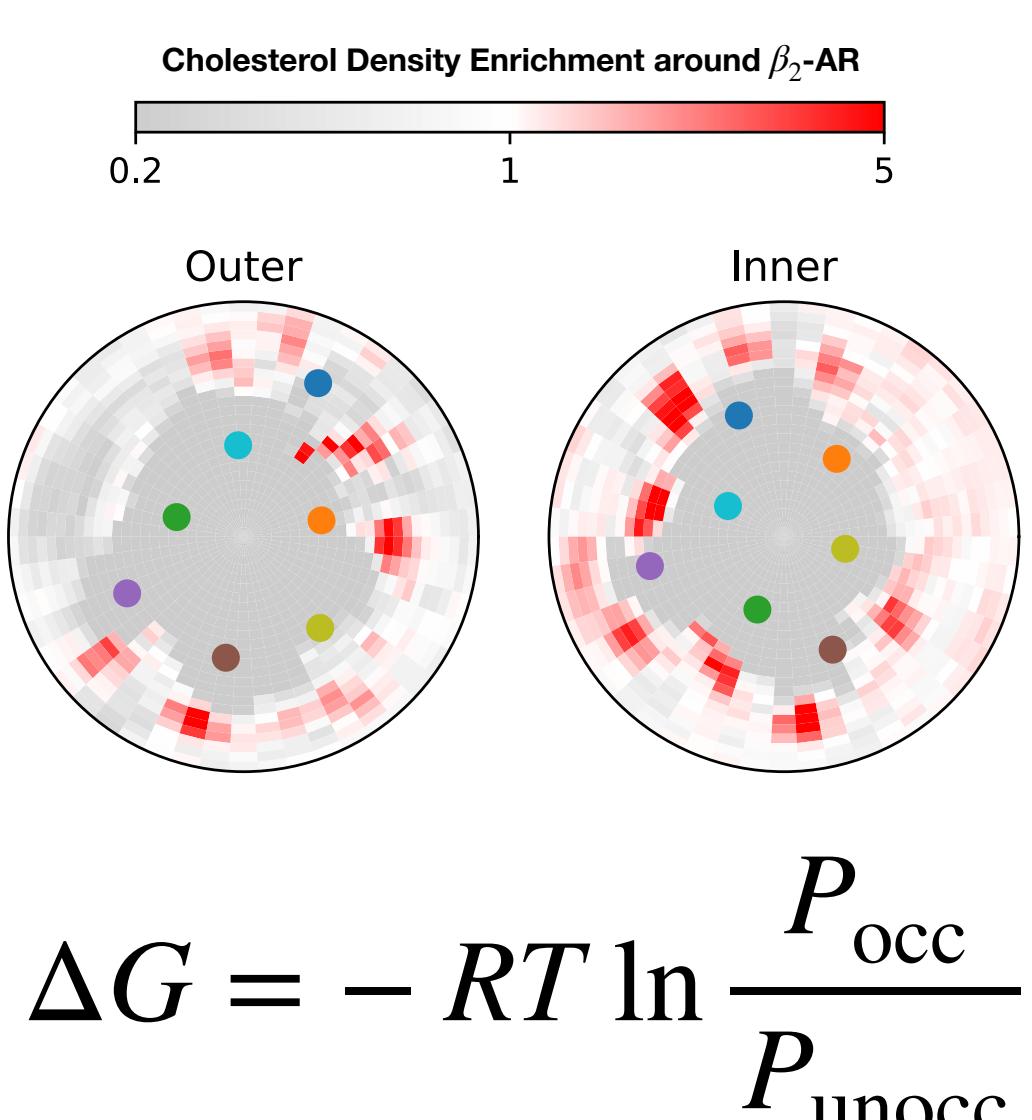
Read our protocol paper!¹

Abstract

It is now well established that lipids bind specifically to membrane protein transmembrane domains (TMDs) and may allosterically modulate protein function, but measuring a lipid binding affinity experimentally remains a challenge. Coarse-grained molecular dynamics (CG-MD) simulations have been used extensively to study lipid-protein interactions due to the enhanced lipid diffusion and the longer accessible time-scales afforded by a CG model. Nonetheless, a number of conceptual challenges arise when MD trajectories are analyzed, including how to define the ‘bound’ state, and how to differentiate between a bulk lipid versus a specifically bound lipid. Most solutions to these problems have relied on measuring residence times to calculate off-rates, but these quantities are difficult to compare across force fields as well as to experimental data. We previously introduced^{1,2} the Density-Threshold Affinity (DTA), a method for determining the binding affinity of a lipid for a defined binding site by measuring thermodynamic quantities in unbiased CG-MD. The DTA quantifies the excess density of a defined lipid species in a binding site, compared against a bulk membrane patch of equal area. In the present work, we show how the DTA can be used to quantitatively rank binding sites as well as to determine which lipid species will out-compete the other membrane components for a particular binding site.

Motivation

- Results from existing CG-MD techniques are difficult to quantitatively compare across lipids, sites, force fields, to experimental data, etc.
 - Density methods (see heat maps, right) are difficult to interpret, especially in complex membranes
 - Residence time methods are difficult to compare across forcefields due to differing kinetics
- Neither technique is comparable to experimental results, which report binding affinities (ΔG)
- FEP-based techniques are not straightforward to apply to branched, chain-like molecules

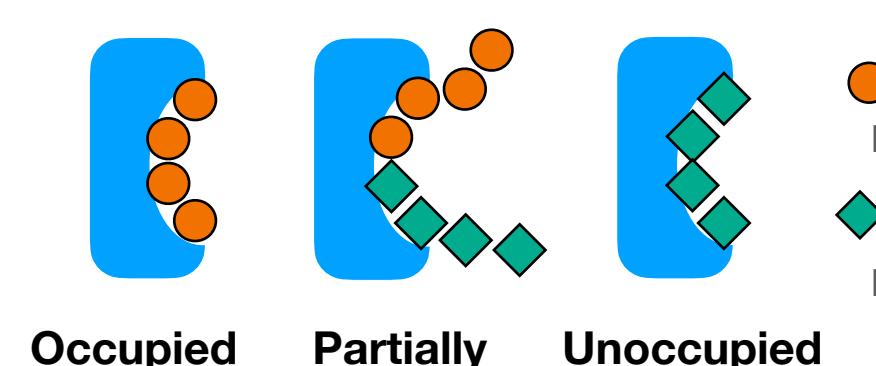


Some Problems When Defining Binding in Hydrophobic Contexts

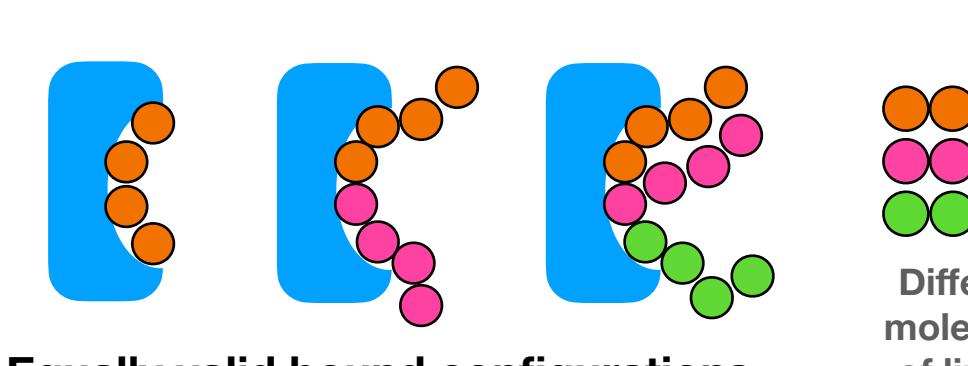
Problem 1: Ligand and/or Solvent?

Is the lipid in the site because it is bound?
Or because it is diffusing through?

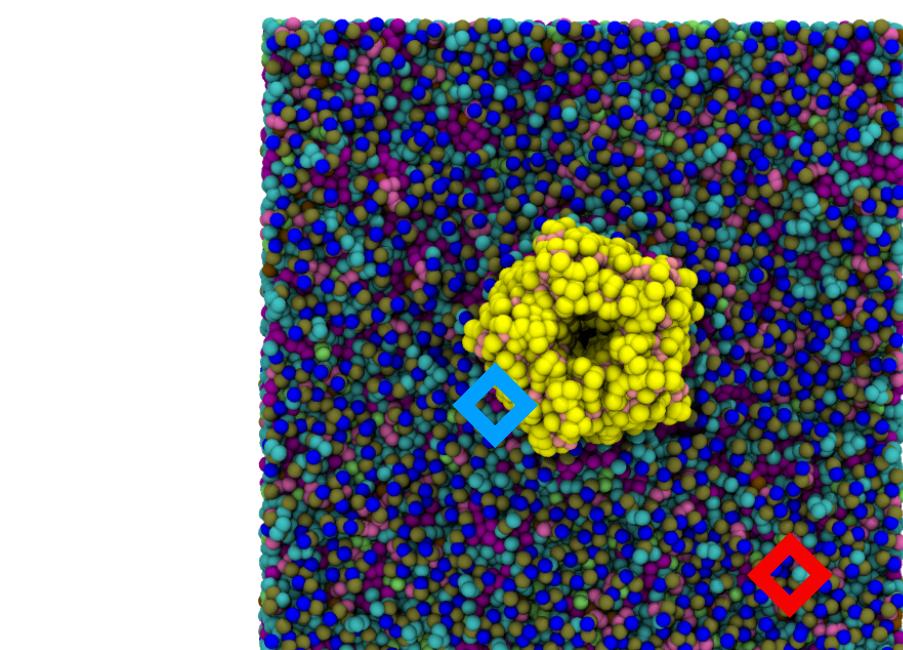
Problem 2: Partial Occupancy



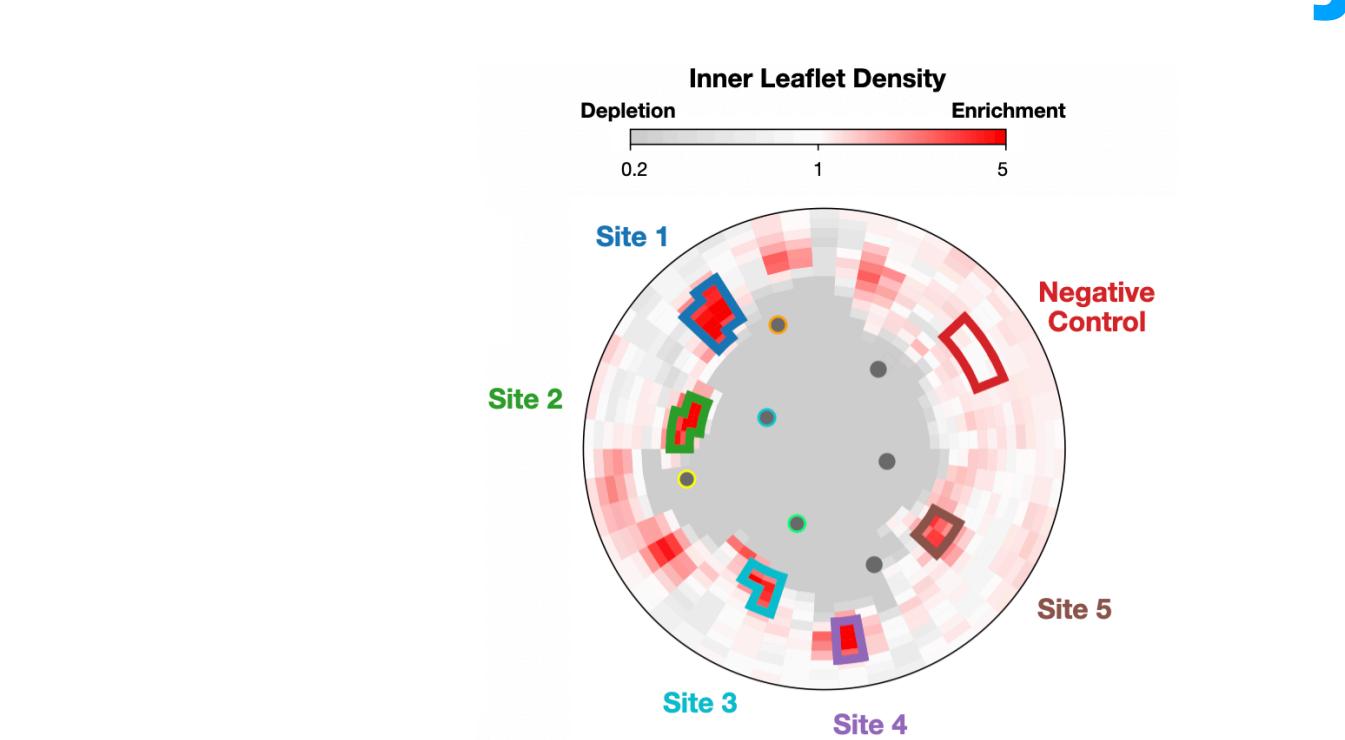
Problem 3: Chemically Indistinct Ligands



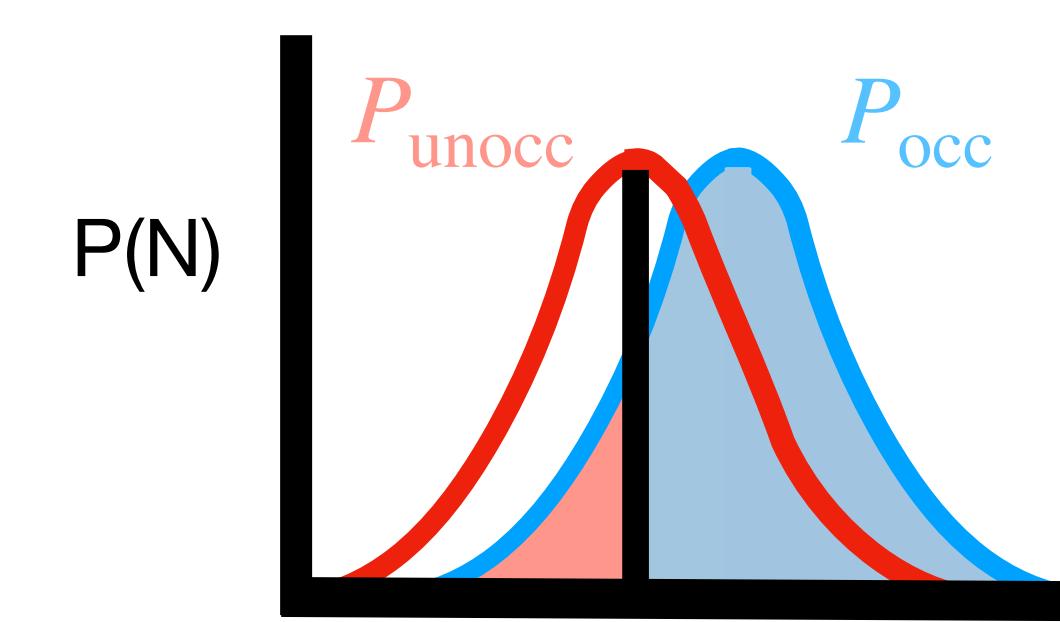
Solution: The Density-Threshold Affinity



What is the probability of finding an atom (or atoms) of interest in the selected binding site compared to finding it in a bulk patch of the same area?



Perform unbiased CG-MD simulation of system. Identify potential binding sites. One option is to measure lipid density enrichment and look for “hotspots.”

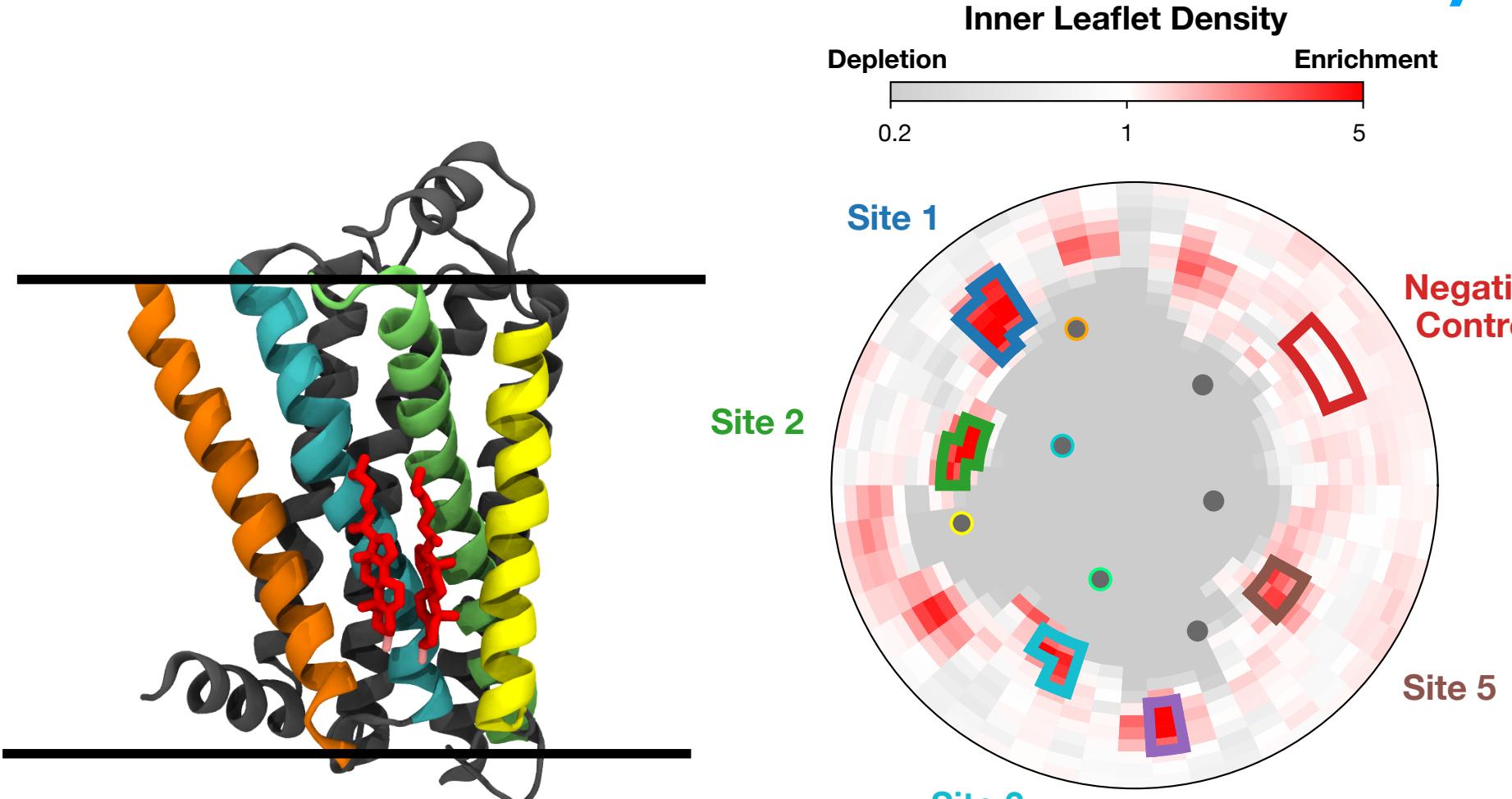


Construct probability distributions of finding N atoms in the site and in the bulk patch. Subdivide site distribution by peak of bulk distribution.

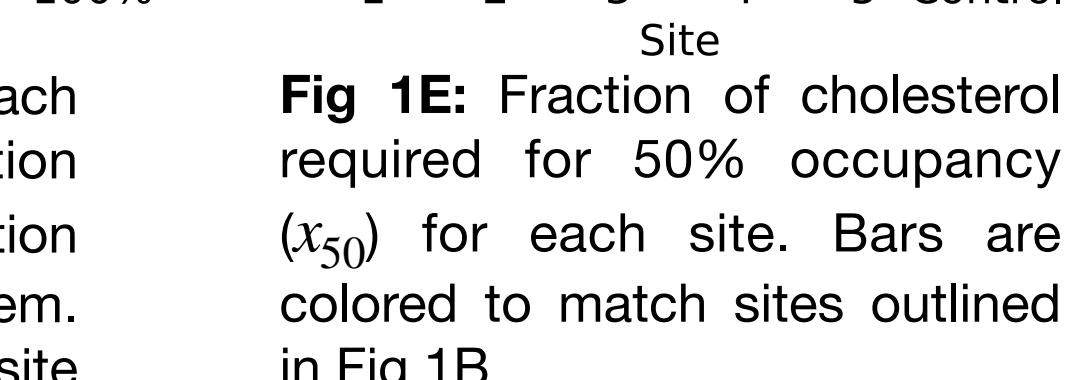
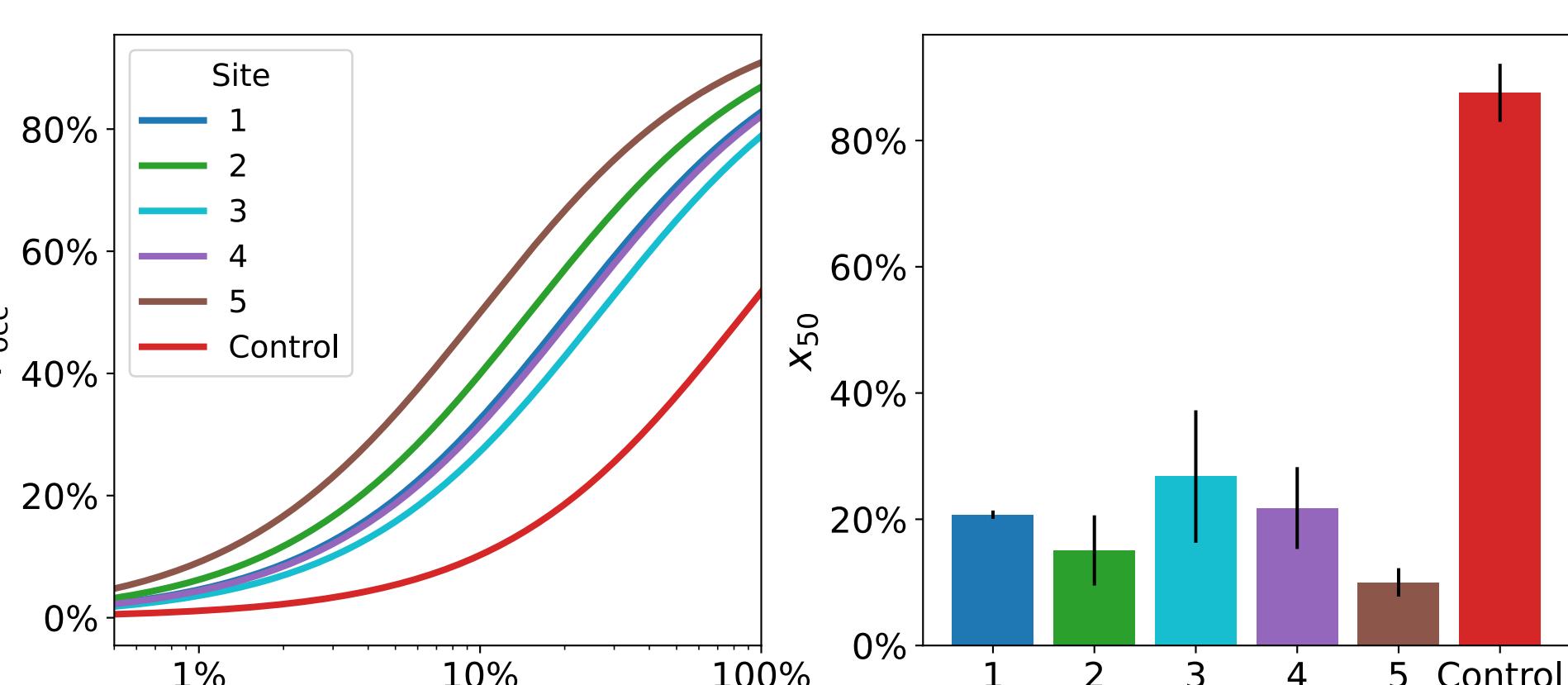
$$\Delta G = -RT \ln \frac{P_{occ}}{P_{unocc}}$$

Measure ΔG of ligand for site. Compare multiple sites or compare multiple lipids within same site.
(See applications below)

Cholesterol Affinity for β_2 -AR: Binding Sites Identified & Ranked



Site	ΔG_{bind} (kcal/mol)
1	-1.0 ± 0.1
2	-1.2 ± 0.3
3	-0.8 ± 0.3
4	-1.0 ± 0.2
5	-1.5 ± 0.2
Control	-0.1 ± 0.1



Force Field: Martini 2.2
Simulation Software: GROMACS 2018
Membrane composition: 70% POPC, 30% Cholesterol

Differential Binding of Lipid Tails to nAChR Intersubunit Site in Oocyte-Mimetic Membrane

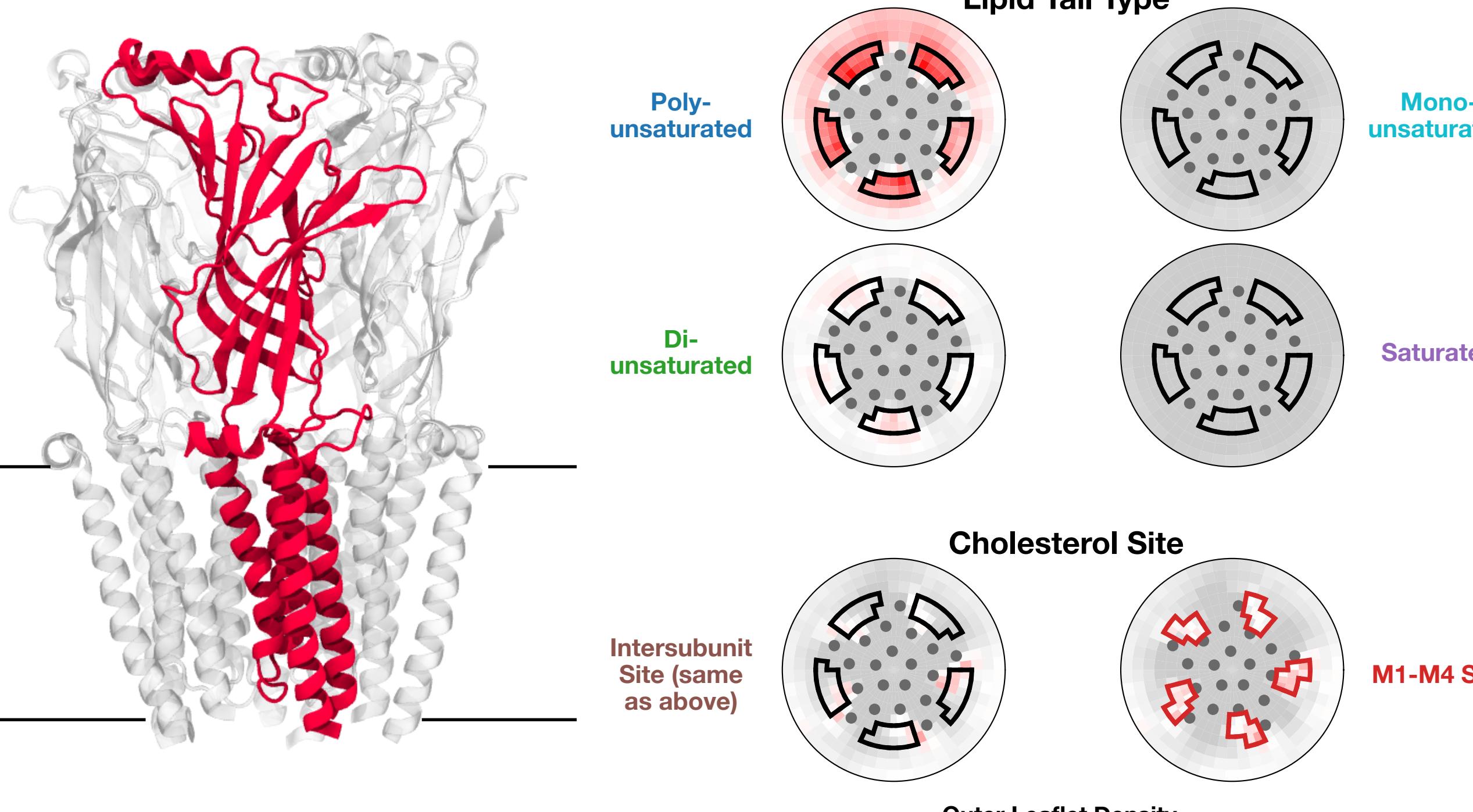


Fig 2A: The α -7 nicotinic acetylcholine receptor (nAChR) (pdb id 8v89)⁴ with intracellular domain removed, shown in membrane/lateral view. Black bars indicate approx. membrane position.

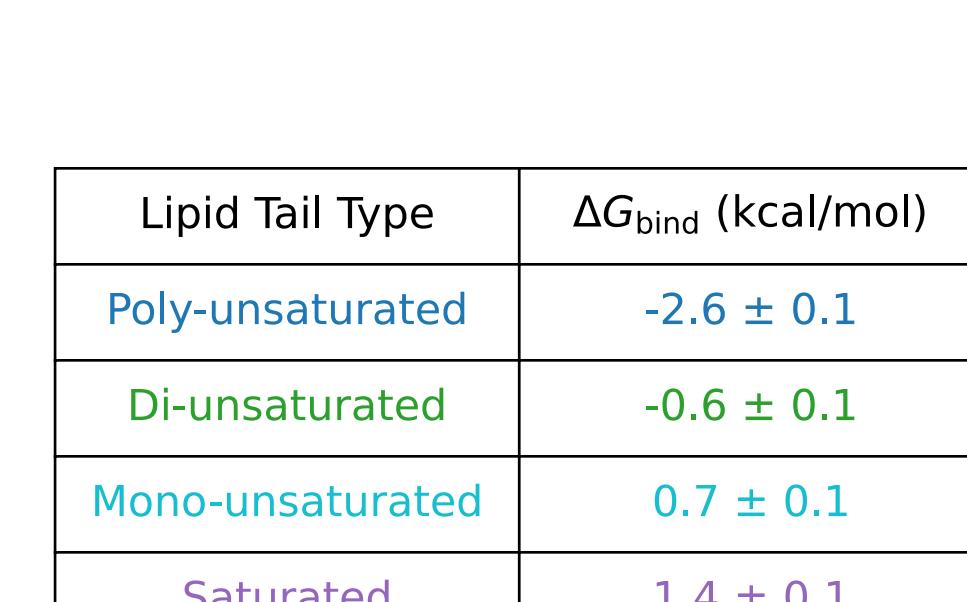


Fig 2B: Density enrichment for different lipid tail types (top) and of cholesterol (bottom). Approximate helix positions indicated by grey dots. The intersubunit site (black) is analyzed for all lipid types present in the system. An M1-M4 helix site for cholesterol is also analyzed.

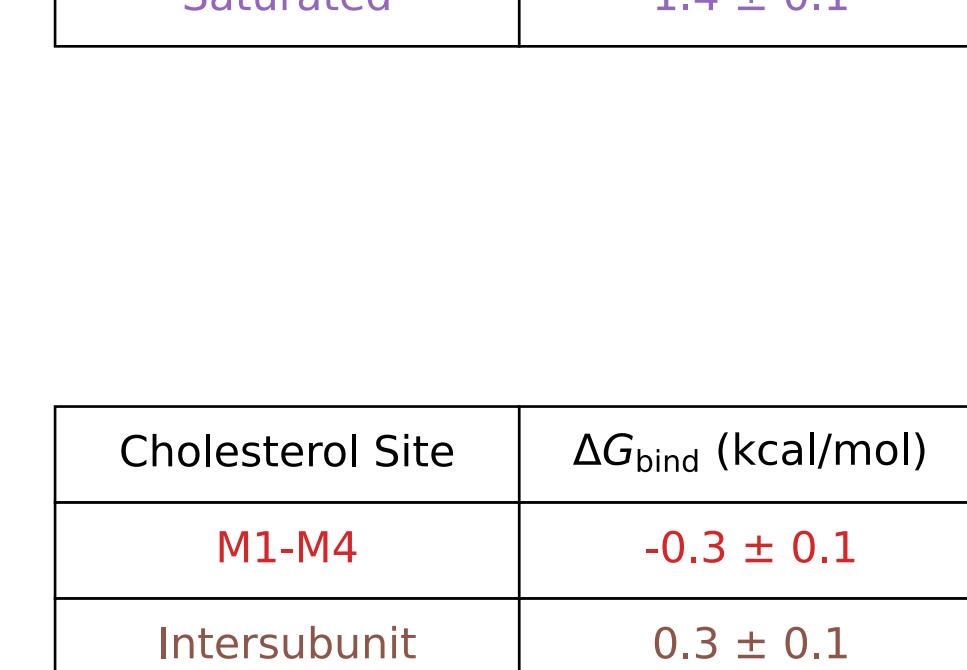


Fig 2C: Binding affinities (ΔG_{bind}) and standard error of the mean measured in each site. The five symmetric sites are treated as independent replicas and standard error is computed with N=20 (5 subunits × 4 replicas). Analysis conducted over the second half of 10 μ s simulations.

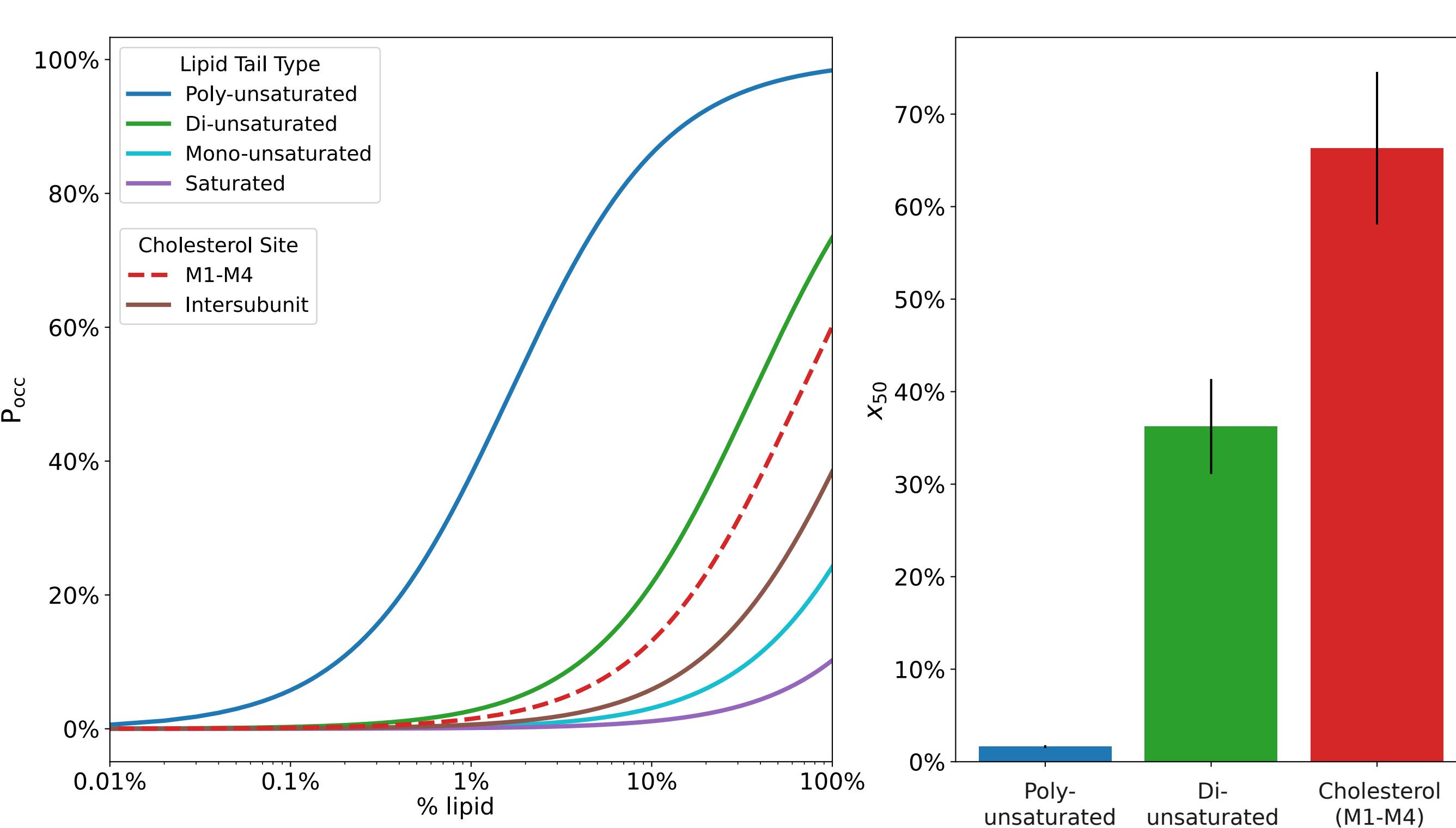


Fig 2D: Titration curve for all lipids in the intersubunit site (solid lines) and for cholesterol in the M1-M4 site (dashed line).

Force Field: Martini 2.2
Simulation Software: GROMACS 2024
Membrane composition: 22-species oocyte-mimetic derived from Hill, et al.⁵

Specific Findings

- Cholesterol binds favorably to all five sites identified in the inner leaflet of the β_2 -AR. Sites 1 and 2 correspond to the sites identified in Hanson, et al.³ Site 5 corresponds to a density previously observed in Cang, et al.⁶ and Manna, et al.⁷
- Polyunsaturated tails are expected to outcompete all other tail types in the outer leaflet intersubunit region of the nAChR when in a *xenopus* oocyte. Cholesterol is expected to occupy a different site (M1-M4) more than 40% of the time in this membrane composition.

Accessible Research Questions

- Which site does lipid X prefer? Does lipid X outcompete lipid Y?
What concentration of lipid X is needed in order to bind?
Is lipid X an allosteric modulator?

References

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