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

Jesse W Sandberg[1] and Grace Brannigan[1,2]

[1]Center for Computational & Integrative Biology, *Rutgers University*, and [2]Dept. of Physics, *Rutgers University*

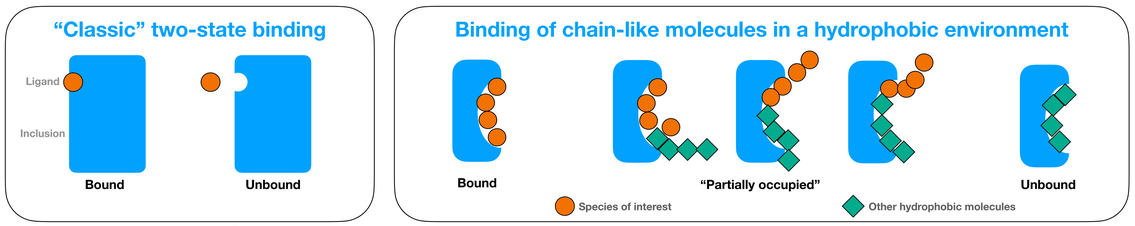
Read our protocol paper![2]

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.

Conceptual Problems When Defining Binding in Hydrophobic Contexts

Problem 1: Partial Occupancy

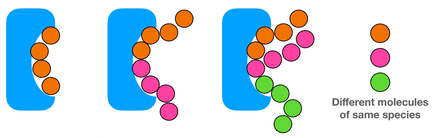


$$\Delta G = -RT \ln \frac{P_{\text{occ}}}{P_{\text{unocc}}} \qquad \Delta G = -RT \ln \frac{P_{\text{occ}}?}{P_{\text{unocc}}?}$$

Problem 2: Ligand and/or Solvent?

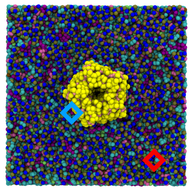
Is the molecule in the site because it is bound?
Or is it in the site because it is diffusing through?

Problem 3: Chemically Indistinct Ligands

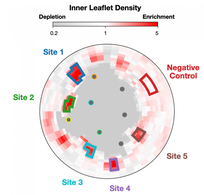


Equally valid bound configurations

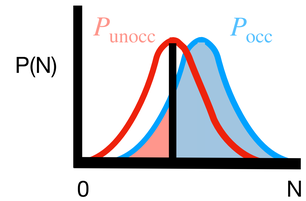
Solution: The Density-Threshold Affinity



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.

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

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

Cholesterol Affinity for GPCR: Binding Sites Identified & Ranked

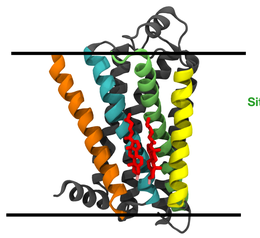


Fig 1A: The β_2 Adrenergic Receptor (β_2-AR, pdb id 3D4S)[3] with intracellular domain removed. Approximate membrane surface indicated by black lines. Cholesterol (red) are depicted in their crystallographic sites[3] as an illustration, but the structural cholesterol molecules were not coarse-grained alongside the protein.

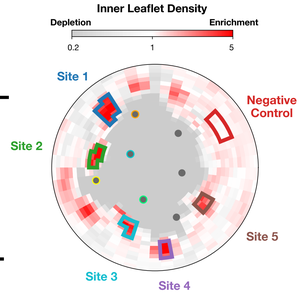


Fig 1B: Density enrichment of cholesterol in the inner leaflet within 2.5 nm of the protein center. Protein helices are indicated by grey circles, highlighted to match coloring in Fig 1A. Potential binding sites identified from this analysis are outlined in color and analyzed further on subsequent panels.

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

Fig 1C: Binding affinities (ΔG_{bind}) and standard error of the mean measured in each site. Standard error computed with 3 replicas. Analysis conducted over the second half of 10 μs simulations. Cells are colored to match site outlines in Fig 1B.

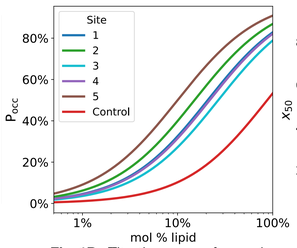


Fig 1D: Titration curve for each site calculated from equation below, where x_B is the mol % of lipid. Lines are colored to match site outlines in Fig 1B.

$$P_{\text{occ}} = \frac{x_B}{e^{\Delta G/RT} + x_B}$$

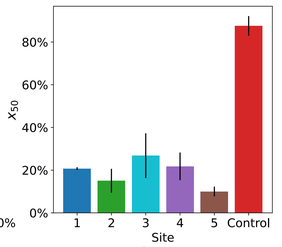


Fig 1E: Mole percentage at which the site is expected to be occupied 50% of the time (x_{50}). Bars are colored to match sites outlined in Fig 1B.

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

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

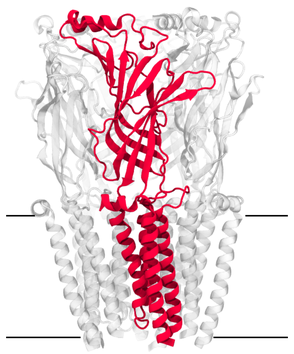


Fig 2A: The α-7 nicotinic acetylcholine receptor (nAChR) (pdb id 8v89)[4] with intracellular domain removed, shown in membrane/lateral view. Black bars indicate approx. membrane position.

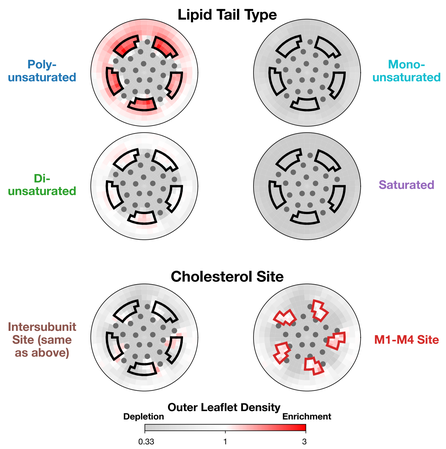


Fig 2B: Density enrichment for different lipid tail types (top) and of cholesterol (bottom). Approximate helix positions indicated with grey dots. The intersubunit site (black) is analyzed for all lipid types present in the system. An M1-M4 helix site for cholesterol is also analyzed.

Lipid Tail Type	ΔG_{bind} (kcal/mol)
Poly-unsaturated	-2.6 ± 0.1
Di-unsaturated	-0.6 ± 0.1
Mono-unsaturated	0.7 ± 0.1
Saturated	1.4 ± 0.1

Cholesterol Site	ΔG_{bind} (kcal/mol)
M1-M4	-0.3 ± 0.1
Intersubunit	0.3 ± 0.1

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 μs simulations.

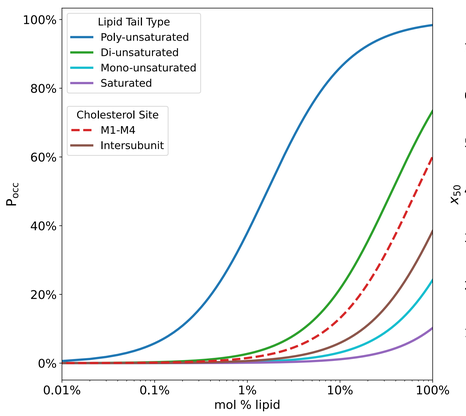


Fig 2D: Titration curve for all lipids in the intersubunit site (solid lines) and for cholesterol in the M1-M4 site (dashed line). Equation provided below Fig 1D.

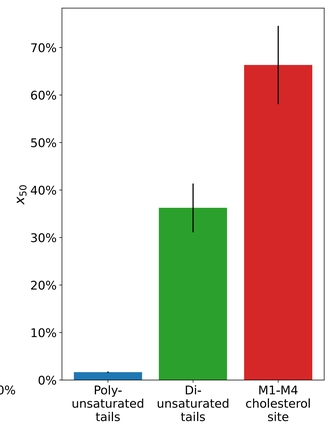


Fig 2E: Mole percentage at which the site is expected to be occupied 50% of the time (x_{50}).

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 ligand prefer? **Does ligand A outcompete ligand B?**

What concentration do I need in order to bind?

Is ligand an allosteric modulator?

References

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2. Sandberg, et al., Meth. In Enz., 2024
3. Hanson, et al., Structure, 2008
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5. Hill, et al., Am. J. Physiol., 2005
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