

Absolute binding affinities of phospholipids to ELIC by Streamlined Alchemical Free Energy Perturbation (SAFEP)



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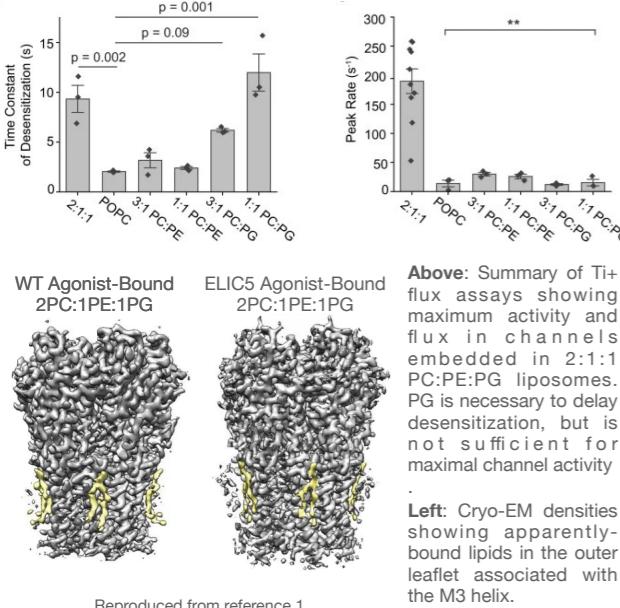


Abstract

An increasing number of cryo-EM structures of membrane proteins contain putative lipid binding sites with partially resolved lipid densities. In principle, these densities might be identified by computing the binding free energies of candidate ligands by alchemical free energy perturbation (FEP). In practice, however, these systems have several challenging features including lipid flexibility, system heterogeneity, and low binding affinity. Each of these confounds the distinction between the bound and unbound state: flexible molecules have large accessible volumes in conformation space; heterogeneous environments complicate the unbound state; and open, low-affinity sites mean that a "bound" ligand may still have considerably higher entropy than more in a more traditional, high-affinity binding pocket. Our SAFEP method seeks to address these problems by defining a single collective variable to define the bound state. This distance-from-bound configuration (DBC) metric reliably classifies ligand-protein conformations as "bound" or "unbound." SAFEP has been successfully applied to the computation of absolute binding affinities of cholesterol to three GPCRs and relative binding affinities of phospholipids to two mutants of ELIC based on structural data. We have extended SAFEP to the calculation of absolute binding affinities of phospholipids. This has required additional sampling of each state and refinement to the collective variables used, especially in the unbound state. While this is somewhat more computationally expensive than computing relative free energies, absolute affinities make functional questions more accessible and mitigate the error propagation inherent to relative binding affinities. It is our hope that these methods will aid in the interpretation of similar systems.

Introduction

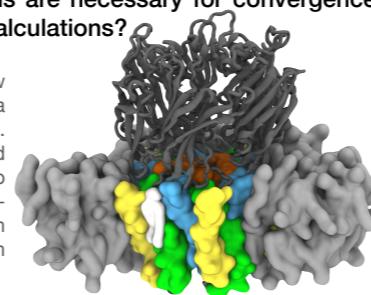
- pLGICs, pentameric ligand-gated ion channels, are found in bacteria, archaea, and metazoa
- pLGICs are neurotransmitter receptors in metazoa
- ELIC is a common prokaryotic model pLGIC
- Lipid sensitivity of pLGICs is well-documented
- Recent data suggest a dependence on both PG and PE even in the presence of agonist (1)
- Previous work:
 - relative FEP calculations in this system (1)
 - absolute FEP calculations with cholesterol bound to a GPCR (4)



Research Questions

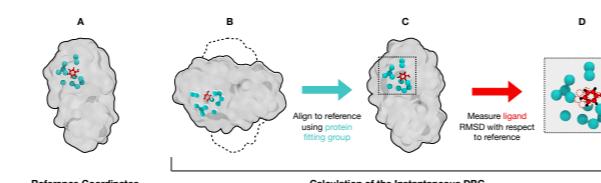
- What is the probability of POPG binding in a binary mixture with POPC?
- Does POPC behave as a solvent or a ligand in the M3 extracellular site?
- What modifications are necessary for convergence of absolute FEP calculations?

Right: A side-view rendering of ELIC WT in a POPC membrane. Membrane (gray and white) is cut away to show the transmembrane domain (colors). The ECD is in dark gray.



Methodological Challenges

- FEP in membranes is notoriously challenging:
1. Membranes are highly heterogeneous
 2. Lipids have slow self-diffusion
 3. Binding sites are superficial
 4. Ambiguity between ligand and solvent molecules

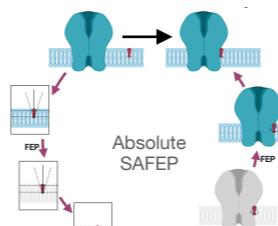


Above: the Distance from Bound Configuration (DBC) is defined as the internal RMSD of the ligand with respect to the binding pocket (the nearest N carbons to the bound ligand). Reproduced from reference 5

Absolute SAFEP

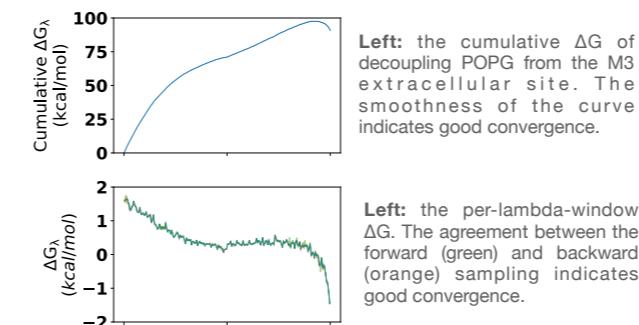
We define the occupied state as: a **given** lipid species has a **conformation** and **position consistent** with structural data.

- Bulk flat well restraints:
 - Tilt: the angle between the membrane normal and the lipid
 - Z-distance from other lipids in the same leaflet
- Site flat well restraint:
 - DBC (defined above)



$$\Delta G_r = RT \ln \left(\frac{1}{1 - \cos \Theta_r} \frac{V_{\text{target}}}{V_{\text{sim}}} \right)$$

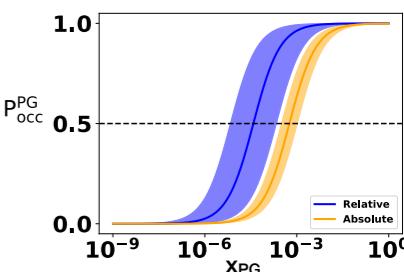
Results and Conclusions



Left: the cumulative ΔG of decoupling POPG from the M3 extracellular site. The smoothness of the curve indicates good convergence.
 Right: Probability of occupancy vs a titration of PG in a POPC membrane using relative SAFEP (blue) and absolute SAFEP (orange). Calculated as shown:

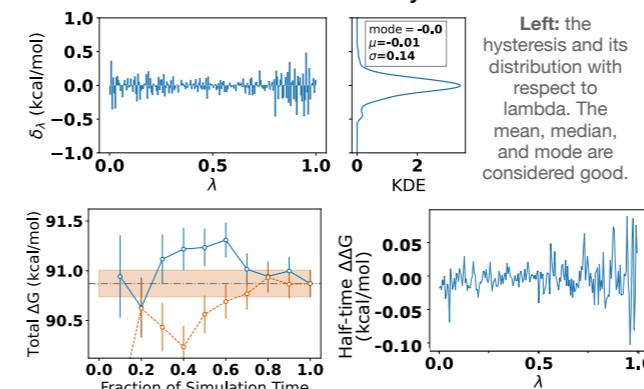
$$P_{\text{occ}} = \left(1 + e^{\frac{\Delta G}{RT}} \right)^{-1}$$

- Relative SAFEP (mutation) is inadequate for estimating the behavior of POPC mixtures
- Absolute SAFEP (decoupling), though more expensive, provides a much more precise estimate of POPG affinity for the M3 extracellular site



Convergence

- Error must be less than 1kcal/mol
- Forward-time and backward time samples should be within 1kcal/mol of each other by t=0.5



Above: the cumulative ΔG as a function of simulation time for both forward time (blue, solid) and backward time (orange, dashed). Above: the half-time $\Delta \Delta G$ vs window. The poorest convergence is seen in the final windows near $\lambda=1$ (fully decoupled)

Summary

- First successful ABFE calculation of a phospholipid
- WT agonist bound has PG x50 of 0.1% in a binary mixture with PC
- POPC in the M3 extracellular site is too disordered to contribute to the observed density
- POPC is consistent with a solvent in this case

← Open structure of ELIC Tutorial on bioRxiv →

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