Identifying Phospholipids in Structures: POPG is an Allosteric Modulator of a pLGIC

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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 ystems 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.

Research Questions

- What is the probability of POPG binding to the M3 site?
- Can we predict that probability computationally?
- Does POPC bind specifically to the M3 site?
- How strong is POPG modulation in the site?

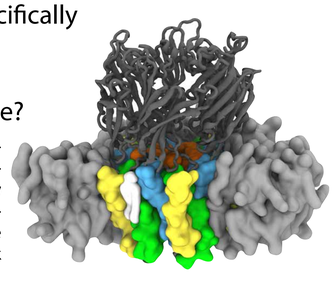


Figure 3: Three-quarter view rendering of ELIC WT. POPC membrane (gray) is cut away to show the transmembrane domain (colors) and bound lipid (white). The extracellular domain is in dark gray.

POPG Binds to ELIC in a POPC:POPG Mixture

- Free energies of binding estimated using SAFEP
- Estimated equilibrium properties:

Relative SAFEP estimates:
- ELIC5 PG $\log(x_{50})$ = -8 ± 2 mol%
- WT PG $\log(x_{50})$ = -6 ± 2 mol%

Absolute SAFEP estimates:
- ELIC5 PG $\log(x_{50})$ = -6.4 ± 0.5 mol%
- WT PG $\log(x_{50})$ = -3.0 ± 0.3 mol%

- Binding is state-dependent
- Relative FEP assumes POPC binds BUT...
- POPC's low affinity yields poor convergence

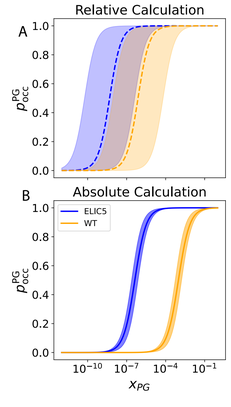


Figure 6: The probability of site occupancy by POPG as a function of POPG mol fraction calculated by:

$$\Delta G_{bind}^{PG}(x_{PG}) = \Delta G_{bind}^{PG} - RT \ln \frac{x_{PG}^{sim}}{x_{PG}}$$

$$p_{occ}^{PG}(x_{PG}) = \left(e^{\left(\Delta G_{bind}^{PG}(x_{PG})\right)} + 1\right)^{-1}$$

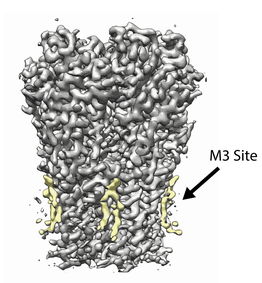
A) Results of the relative calculations with ELIC5 (blue) and WT (orange). B) Results of the absolute calculations colored as in A. Filled regions indicate ± 1 SEM.

Background

Pentameric Ligand-Gated Ion Channels, pLGICs:
- Found in bacteria, archaea, and metazoa (1)
- Neurotransmitter receptors in metazoa (1-5)
- Sensitive to the lipid environment (2-5)

ELIC, a common model pLGIC:
- Opened by GABA and GABA-analogues (2,3)
- Native agonist is not known (2,3)

Previously we determined:
- The identities of lipid fragments using SAFEP (2)
- POPG modulates activity via an extracellular M3 site in model membranes (2)



WT Agonist-Bound
2PC:1PE:1PG

Figure 1: Cryo-EM density showing apparently-bound lipids (yellow) in the outer leaflet associated with the M3 helix. Reproduced from reference 2.

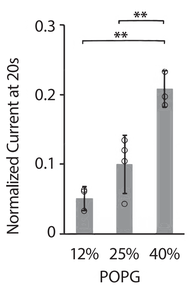


Figure 2: Electrophysiology statistics demonstrating POPG dependence of ELIC in GUVs. Reproduced from reference 3.

Methodology

Alchemical Free Energy Perturbation (FEP)

1. Bypasses (un)binding
2. Non-physical (alchemical) intermediate state
3. Two common paths (fig. 4)

Challenges for FEP in lipid membranes:

1. Highly heterogeneous
2. Slow self-diffusion
3. Superficial binding sites
4. Ambiguity between ligands and solvents

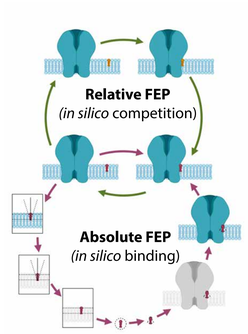
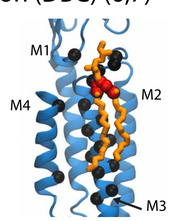


Figure 4: A summary of the thermodynamic cycles of both relative FEP (green, upper arrows) and absolute FEP (red, lower arrows). Bulk lipids are light blue, test lipids are orange and red, non-interacting molecules are gray.

Streamlined Alchemical Free Energy Perturbation (SAFEP) (6,7)
- Occupied state defined as: a given lipid species with a conformation and postion consistent with the structural data.
- Distance-to-Bound-Configuration (DBC) (6,7)

Figure 5: Distance-to-Bound-Configuration (DBC) is defined as the internal RMSD of a subset (red) of the ligand atoms (orange) with respect to a subset of protein atoms (black beads). Only one subunit of ELIC WT is shown for clarity (blue cartoon).



POPG is a Positive Allosteric Modulator

- Activity modulation was estimated from the state-dependent binding.

Relative SAFEP estimates:
- EC_{50} between $x_{PG}=10^{-6}$ and 1
- Maximum enhancement between 10^{-2} and 10^{11}

Absolute SAFEP estimates:
- EC_{50} between $x_{PG}=10^{-3}$ and 10^{-2}
- Maximum enhancement between 10^{6} and 10^{8}

- POPG is a strong positive modulator in the M3 site
- Relative FEP is too imprecise to be useful here

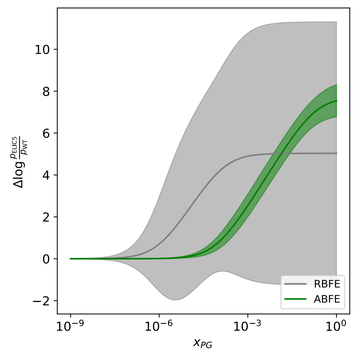


Figure 7: Modulation of ELIC by POPG relative to a pure POPC membrane computed by:

$$K_M = e^{\left(-\beta \Delta G_{bind}^{M}\right)}$$

$$\Delta \log \frac{p_{ELIC5}}{p_{WT}}(x_{PG}) = \frac{K_{ELIC5}\, x_{PG} + 1}{K_{WT}\, x_{PG} + 1}$$

Results of the relative calculation are in gray. The absolute results are in green. Filled regions indicate ± 1 SEM.

Summary

- First successful computational prediction of phospholipid binding affinities at atomistic resolution
- Both WT and ELIC5 have strong affinity for POPG in a POPC:POPG membrane
- POPC in the M3 site is too disordered to contribute to the observed density - i.e. a solvent
- POPG is a strong positive modulator in the M3 site consistent with experiments

← Open Structure of ELIC (2)
SAFEP Tutorial (7) →

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