Lipid Modulation by Direct Binding of a Pentameric Ligand-Gated Cation Channel, ELIC

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Abstract

The Pentameric Ligand-Gated Ion Channel (pLGIC) protein family, which includes essential neurotransmitter receptors, is well-known to exhibit sensitivity to their local lipid environment. Erwinia Ligand-gated Ion Channel (ELIC) is a prokaryotic member of the pLGIC family. We recently demonstrated that ELIC selectively binds POPG over POPC or POPE, and that this affinity is state-dependent by computing relative free energies of binding. Furthermore, our SAFEP methodology was able to calculate absolute free energies of binding between membrane proteins and lipids. Here, we build upon those results to estimate the functional effects of lipid binding on ELIC by comparing the absolute binding free energies for both conducting (active) and non-conducting (inactive) conformations.

Pentameric Ligand-Gated Ion Channels (pLGICs)

- Ancient protein superfamily [1]
- High structural conservation [2]
- Chemoreceptive ion channels [2]
- Essential for bilaterian nervous systems[1]
- Known to be lipid-sensitive [3]
- Mechanism of lipid modulation is unknown[3]

Erwinia Ligand-gated Ion Channel (ELIC)

- Prokaryotic model pLGIC
- Homopentamer
- Shorter than Eukaryotic pLGICs
- Known POPG sensitivity [3]
- At least one binding site (Fig 1) [4]

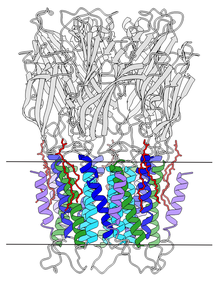


Fig 1: Erwinia Ligand-Gated Ion Channel (ELIC) side view. Cartoon representation of PDB ID 8D66. Membrane surfaces are indicated as black lines. The transmembrane helices (m1, m2, m3, and m4) are indicated in colors: dark blue, cyan, green, and purple, respectively.

ELIC Lipid Sensitivity

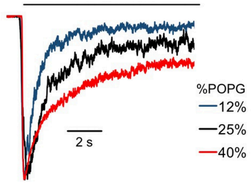


Fig 2: ELIC is sensitive to POPG concentration. Patch clamp recordings of ELIC in a POPC model membrane normalized to peak current. As POPG concentration is increased, desensitization is delayed. Peak currents also increase (Data not shown). Adapted from [5].

Free Energies of Binding from Molecular Dynamics Simulations using SAFEP

What is (SA)FEP?

FEP measures free energies from simulations:
- A thermodynamic cycle sums to 0 (fig 4)
- Alchemical transformation into a gas

Streamlined Alchemical FEP (SAFEP) improves convergence:
- The distance to bound configuration (DBC) (fig 3) quantifies the bound state
- DBC restraint prevents unbinding

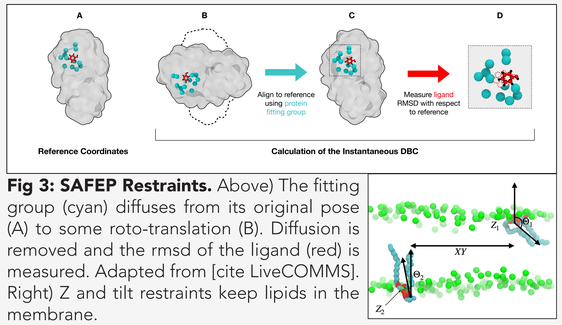


Fig 3: SAFEP Restraints. Above) The fitting group (cyan) diffuses from its original pose (A) to some roto-translation (B). Diffusion is removed and the rmsd of the ligand (red) is measured. Adapted from [cite LiveCOMMS]. Right) Z and tilt restraints keep lipids in the membrane.

Free Energies

The free energy of binding for each state is given by:

$$\Delta G_{\text{bind}} = \Delta G_{\text{bulk}} + \Delta G_{\text{restraints}} + \Delta G_{\text{DBC}} - \Delta G_{\text{site}}$$

Quantity	Free Energy of:	Method (Restraints)
ΔG_{BULK}	Decoupling POPG from the bulk	SAFEP (Tilt, Z, Distance)
ΔG_{SITE}	Decoupling POPG from the site	SAFEP (DBC)
ΔG_{REST}	Imposing the bulk restraints	-RT ln(V0/Vrest)
ΔG_{DBC}	Imposing the DBC restraint	Thermodynamic Integration

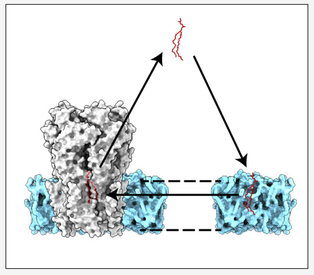


Fig 4: The thermodynamic cycle for moving a POPG molecule from the bulk to the site. POPG (red) is alchemically removed from each state: bound (left) and bulk (right). The free energy of each transformation is combined to estimate the free energy of transfer.

Site Occupancy

Probabilities of site occupation are given by:

$$p_{\text{occ}} = \frac{x_{\text{PG}}}{e^{\frac{-\Delta G_{\text{bind}}}{RT}} + x_{\text{PG}}}$$

Where x_{PG} is the mole fraction of PG in a POPC membrane

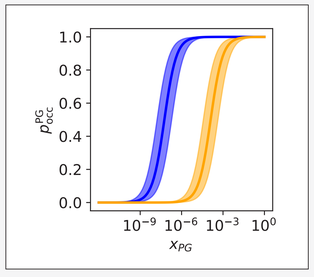


Fig 5: Site occupancy by POPG in a primarily POPC membrane. Occupancy probability of ELIC5/open (blue) and wild-type (orange). Shaded regions indicate 95% confidence intervals.

Functional Modulation

Because affinity is state-dependent, modulation can be obtained by:

$$\frac{p_{\text{occ}}^{\text{open}}}{p_{\text{occ}}^{\text{closed}}} = \frac{\Delta G_{\text{bind}}^{\text{open}} \cdot x_{\text{PG}} + 1}{\Delta G_{\text{bind}}^{\text{closed}} \cdot x_{\text{PG}} + 1}$$

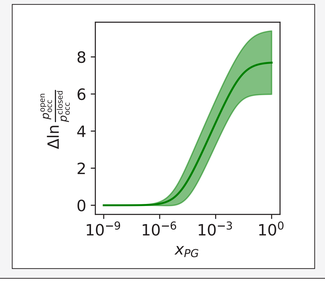


Fig 6: Log modulation of ELIC versus mole fraction of POPG Shaded region indicated 95% confidence interval.

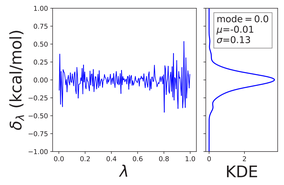
Implementation and Convergence

- All simulations were run in NAMD2.14 using CHARMM36m with WYF corrections
- In the bulk, two lipids were decoupled to preserve leaflet number symmetry

Site Hysteresis

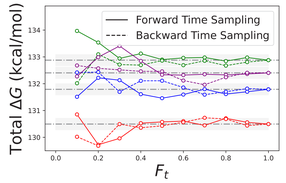
$$\delta_{\lambda,i} \equiv \Delta G_{i+1,i} - \Delta G_{i,i+1}$$

Fig 6: Hysteresis. Forward-backward sampling difference ($\delta_{\lambda i}$) for POPG decoupled from the site. ΔG calculated by exponential averaging.



Bulk Convergence

Fig 7: Convergence of ΔG. Comparison of simulation time subsampling ΔG_{bulk}. The F_t is the fraction of the simulation used to calculate ΔG taken from the start of the simulation (Forward) or from the end of the simulation (Backward).



Summary

Membrane proteins may be generally modulated by direct binding of lipids
- Demonstrated in ELIC with POPG

Absolute SAFEP was successfully applied to a phospholipid (at atomistic resolution)
- In both a bound and bulk conformation
- In both an open (active) and closed (inactive) protein conformation

POPG has strong affinity for the site
- Affinity is state-dependent
- Strong positive allostery under experimental conditions

50% occupancy in a POPC membrane:
- 10^{-4}% POPG (closed)
- 10^{-6}% POPG (open)

References

1. Jaiteh, Taly, Henin. PLOS ONE. 2016
2. Howard. JMB. 2021
3. Thompson, Baenziger. Biochimica et Biophysica Acta-Biomembranes. 2020
4. Petroff, [...], Hénin, Brannigan, Cheng. Nat Comms. 2022.
5. Tong, [...], Brannigan, Cheng. eLife. 2019
6. Santiago, Ebrahimi, Sandberg, Brannigan, Hénin. LiveCoMS. 2023

SAFEP Protocol[6]

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