# Forecasting Lipid Binding and Modulation of an Ion Channel from Simulation

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## Abstract

Membrane proteins are very sensitive (>5x gain of function) to their local lipid environment. The mechanisms that cause this sensitivity are not well understood. Our work involves computationally modeling a membrane protein to study how specific lipid binding may cause this sensitivity. Our system of interest is Erwinia ligand-gated ion channel (ELIC), a bacterial homolog of many neuronal proteins including n-acetylcholine receptor and GABAa receptor. We have extendend free energy perturbation (FEP) to phospholipid binding in a methodology we call SAFEP. From these simulations, we have been able to estimate the binding free energy of POPG to ELIC as well as make predictions of POPG modulation at a single-protein level. Our results are consistent with, and clarify, the experimental data available.

## Lipid Diversity Affects Membrane Function[1]

- Cell membranes have diverse compositions
- Membrane lipids differ widely

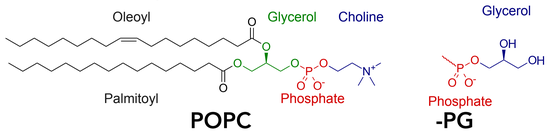


**Fig 1: Lipid Diversity.** Phospholipids can differ in their tail length and saturation (e.g. oleoyl, palmitoyl), or in their head group chemistry (e.g. choline, glycerol). POPC is a common mammalian lipid, while POPG is more common in bacterial membranes.

- Many membrane proteins have specific membrane requirements:
  - Cytochromes
  - ATPases
  - Ion Channels

## pLGICs are Essential Neuronal Proteins[2]

- Pentameric Ligand-gated Ion Channels
- Examples include[2]:
  - N-Acetylcholine receptor
  - $GABA_A$ receptor
  - $5\text{-}HT_3$ (serotonin) receptor
- Key role in neuron-neuron communication[2]:
  - Reside in post-synaptic membrane
  - Detect neurotransmitters
  - Initiate depolarization (action potential)
- ELIC is a bacterial model pLGIC [Fig 3][2,3]

## ELIC is Sensitive to POPG

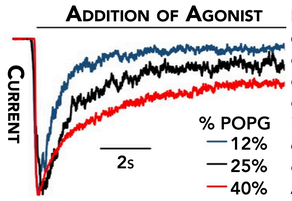


**Fig 2: ELIC is sensitive to POPG concentration.** Patch clamp recordings of ELIC in a POPC model membrane normalized to peak current. As POPG concentration is increased, desensitization is delayed. Peak currents also increase (Data not shown). Adapted from citation 4.

## Open or Closed? When and Why?

### ELIC's Pore can be Open or Closed to Ions

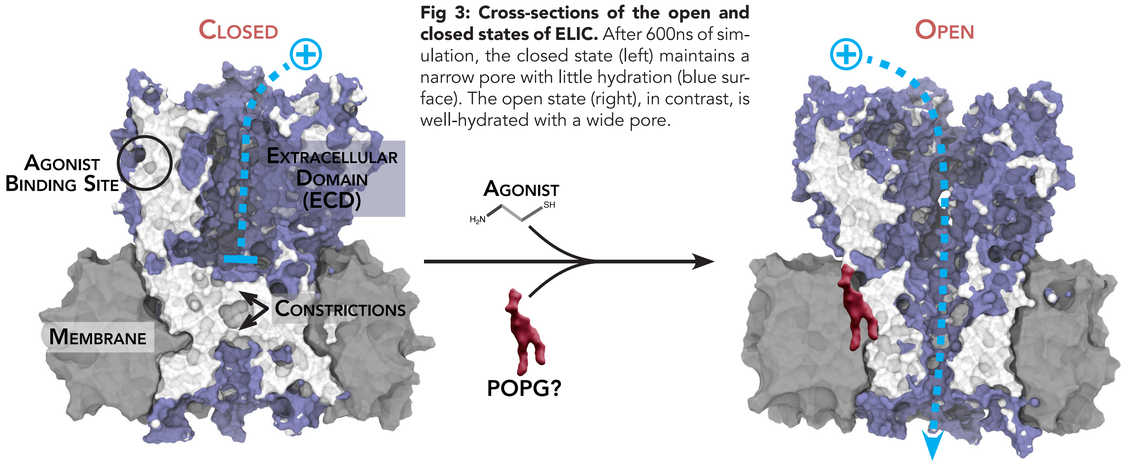


**Fig 3: Cross-sections of the open and closed states of ELIC.** After 600ns of simulation, the closed state (left) maintains a narrow pore with little hydration (blue surface). The open state (right), in contrast, is well-hydrated with a wide pore.

### The Open State has a Higher Affinity for POPG

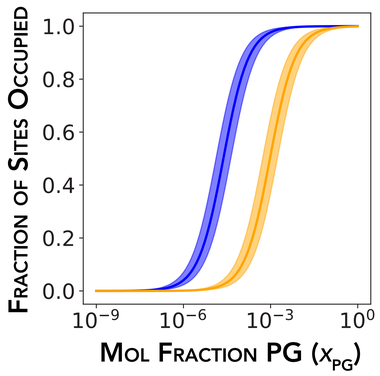


**Fig 4: Site occupancy by POPG in a primarily POPC membrane**. Occupancy probability of ELIC5/open (blue) and wild-type (orange). Shaded regions indicate 95% confidence intervals.

**Eqn 1:** Occupancy probability as a function of POPG mole fraction and binding affinity.

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

- Binding affinity for each state was computed by SAFEP simulations
- The open state has a much higher affinity for POPG than the closed state

### POPG Increases the Relative Open Probability by up to 30x

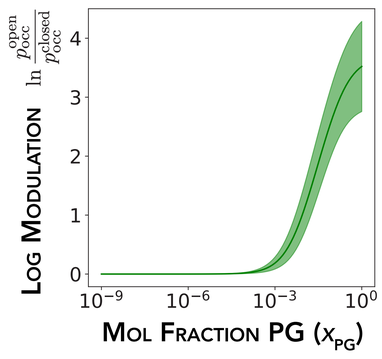


**Fig 5: Log modulation of ELIC versus mole fraction of POPG** Calculated by eqn 2. Greater values correspond to gain of function.Shaded region indicates 95% confidence interval.

**Eqn 2:** The relative open probability as a function of binding free energies.

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

- State-dependence suggests positive allosteric modulation
- Relative open probability is estimated by eqn 2
- Consistent with experiment:
  - Undetectable flux without POPG[4]
  - Compare with Fig 2

## Methods - SAFEP

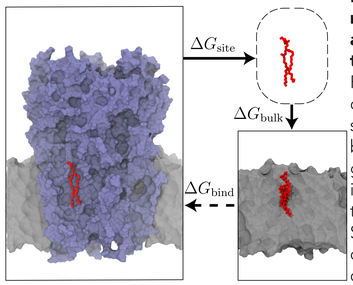


**Fig 6: The thermodynamic cycle for moving a POPG molecule from the bulk to the site**. POPG (red) is alchemically removed from each state: bound (left) and bulk (right). The free energy of each transformation is combined to estimate the free energy of transfer. Solid lines are computed directly. Dashed lines are derived.

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

- A molecular dynamics system is transformed into another over a simulation
- The free energy of transformation can be computed[5]
- SAFEP makes FEP in membranes possible:
  - Removes the need of a standard state
  - Introduces restraints that improve numerical convergence (not illustrated above)

## Summary

1. **Novel use of FEP on phospholipids via SAFEP**
2. **Quantitative Predictions:**
   - Under physiologically relevant conditions
   - Increasing POPG mol fraction
     → Increases site occupancy
   - Affinity is state-dependent
     → Increases open probability
   - Strong positive allostery
   - Consistent with experimental results

## References

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SAFEP Protocol[5]

## Acknowledgments

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