Picking a Molecular Lock
The Role of Lipids in Modulating a Bacterial Sensory Protein

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Intro - Ligand-gated Ion Channels

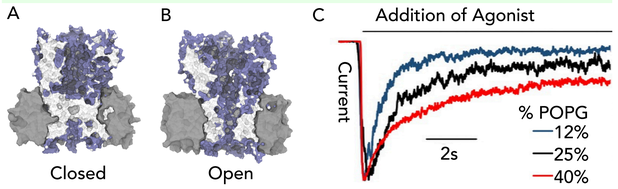

Fig 1: *Erwinia* Ligand-gated Ion Channel (ELIC). Cross sections of closed (A) and open (B) structures of ELIC (blue, solvent accessible, and white, interior) embeded in a POPC membrane (gray). Structures published in [2]. C) Patch clamp recordings of ELIC in a POPC:POPG model membrane. Peak currents are normalized. As POPG concentration is increased, desensitization is delayed. Peak currents also increase (Data not shown). Adapted from citation 4.

- Pentameric Ligand-gated Ion Channels (pLGICs) [3]:
  - Gated by small molecules
  - Many neurotransmitter receptors
  - Desensitize over time after initial opening
- *Erwinia* Ligand-gated Ion Channel (ELIC):
  - A bacterial model pLGIC
  - Function depends on POPG (Fig 2.C) and other lipids

Intro - Partially Resolved Lipids

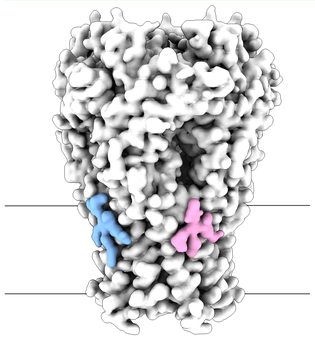

Fig 2: EM Density of ELIC. This density of Erwinia ligand gated ion channel (gray) is an example of a membrane protein with partially resolved lipid-like densities (blue and pink). Density obtained in PC:PG:PE 2:1:1 nanodiscs. The outer-most helix (M4) was not well-resolved and is hidden in this view. Density published in [2].

- The function of membrane proteins often depends on the local lipid environment. [1]
- Functionally relevant membranes may be untenable in nanodiscs or for cryo-EM generally
- EM densities of membrane proteins increasingly reveal bound lipid fragments of unknown identity. (e.g. [2])

Intro - Molecular Dynamics & FEP

- FEP: Free Energy Perturbation
  - Free energy method for physical simulations
  - Non-bonded interactions are weakened or strengthened in order to obtain the free energy difference
- Classical FEP is not well suited to superficial sites.

References

SAFEP Tutorial[6]

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3. Jaiteh, Taly, Hénin. PLOS ONE. 2014
4. Tong, [...], Brannigan, Cheng. eLife. 2019
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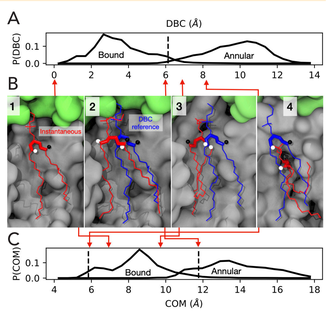
6. Santiago-McRae, Ebrahimi, Sandberg, Brannigan, Hénin. LiveCoMS. 2023

What Counts as a Bound Lipid in a Superficial Site? - SAFEP

- Binding to a superficial site introduces an ambiguity:
  - When is the lipid "bound" and when is it coincidentally in the site?
  - Or, equivalently, when is the site "occupied" and when is it simply filled with solvent?
- The Distance from Bound Configuration (DBC) (Fig 2):
  - RMSD of the most stably bound lipid atoms
  - In the protein frame of reference
  - Captures the fluctuations apparent in the cryo density
- Restraining the DBC:
  - Doesn't affect the bound ensembles
  - Can be corrected for in the gas phase

Fig 3: Comparison of collective variables for a bound lipid. Distributions and poses taken from a simulation of POPE bound to ELIC. A) the distribution of the DBC of POPE in both a bound and annular state. Red arrows indicate the location of each pose along the collective variable. B.1) The instantaneous lipid pose (red) with glycerol oxygens colored black and white to indicate orientation. B.2-4) The instantaneous lipid pose compared with the reference pose (blue). C) As in A, showing the distribution of the COM.



Why do we see Three Acyl Chains in the Cryo-EM? - Binding Modes

Fig 4: Side view of alternate binding modes. POPG occupies at least two binding modes: parallel (orange) and splayed (red). Comparison with experimental cryo-EM density (green). Binding modes were identified from one microsecond of unbiased MD simulation. POPG conformations were clustered by the quality threshold algorithm and the coordinates of each cluster were averaged to produce the reference coordinates (shown). Protein is shown in white.

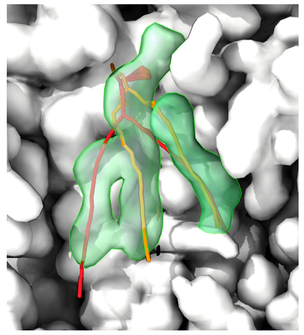
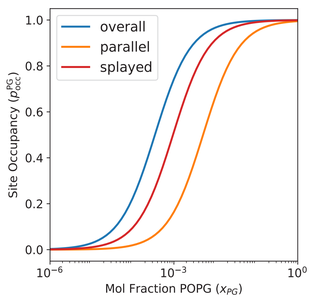


Fig 5: Site occupancy by POPG for each mode in the closed protein conformation. The parallel mode (orange) has the weakest affinity with an x50 of 10%. The splayed mode (red) has a higher affinity with an x50 of 0.5%. Together, these affinities approach the overall site (which includes both binding modes) affinity with an x50 of 0.01%.



- One microsecond of unbiased simulation (Δ4C structure)
- POPG visited two distinct binding modes: splayed and parallel
- Together, these two binding modes correspond closely to the "three-legged" density observed the cryo-EM maps.

- Both binding modes had high affinity.
- Together, they contribute most of the affinity for the site which includes both binding modes and additional minor binding modes.

Are those Lipids Functionally Relevant? - Absolute Affinities

Fig 6: Schematic of Absolute Alchemical Binding Free Energy (ABFE) via SAFEP. The ligand (purple circle) starts the simulation either bound to the protein (A, green) or unbound (B) in the bulk (blue). To obtain the ΔG^0_{bind}, the free energy difference between the site-occupied (A) and unoccupied (B) states, a non-physical path is taken through the gas phase (C).

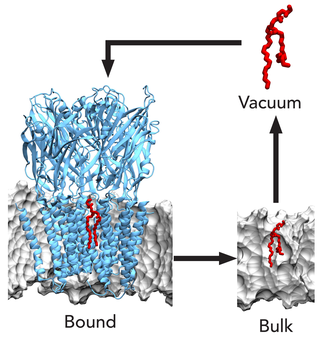
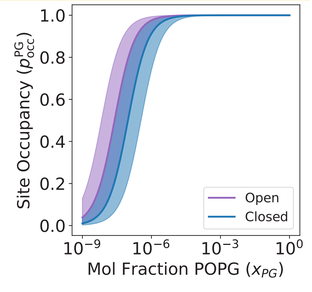


Fig 7: Site occupancy by POPG in a primarily POPC membrane. Occupancy probability of open (blue) and closed (orange). Shaded regions indicate ±1SEM. Occupancy probability can be expressed as a function of POPG mole fraction by

$$p_{\mathrm{occ}}(x_{\mathrm{PG}}) = \frac{x_{\mathrm{PG}}}{x_{\mathrm{PG}} + x_{50}}$$

where

$$x_{50} = e^{\beta \Delta G^{\circ}_{\mathrm{bind}}} \cdot x_{\mathrm{sim}}$$



- The whole lipid is decoupled to the gas phase
- This yields the absolute free energy difference of moving the lipid from the bulk to the site
- More expensive than RBFE, but more precise
- Applied to the highest affinity lipid, POPG (Fig 7)

- x_{50}: The mol fraction at which the site is 50% occupied
  - Open (Fig 1A): 10^{-5} % PG
  - Closed (Fig 1B): 10^{-3} % PG
- POPG occupies the site even at low mol fractions (Fig 7)
- Site occupancy is close to 100% under experimentally relevant conditions

Significance

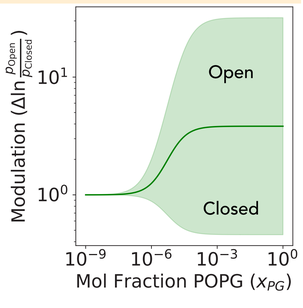
From bacteria to blue whales, all living things need to sense their environment. Whether that sensing is done by nerves or germs, it all comes down to molecular "noses" that recognize some external signal and convert it into an internal signal. In our nervous system, pLGICs (like the one pictured in blue) serve as those molecular noses to "smell" signals (neurotransmitters) sent between individual nerves. pLGICs are exquisitely sensitive to the molecules around them (like the lipid molecule shown in gold). Depending on those lipids, pLGICs become more or less sensitive to neurotransmitters, in turn making nerves more or less active. My research seeks to disentangle the mechanisms by which pLGICs and lipids interact to facilitate normal cellular function. By understanding these molecules, we can better understand how many organisms solve this problem of sensing and responding to their environment.

How Strong is the Modulation?

Fig 8: Log modulation of ELIC versus mole fraction of POPG Calculated by eqn 2. Greater values correspond to gain of function. Shaded region indicates ±1SEM. The relative open probability is a function of binding free energies

$$\Delta \ln \frac{p_{\mathrm{occ}}^{\mathrm{open}}}{p_{\mathrm{occ}}^{\mathrm{closed}}} = \frac{x_{\mathrm{PC}} + e^{-\beta \Delta G_{\mathrm{bind}}^{\mathrm{open}}} \cdot x_{\mathrm{PG}}}{x_{\mathrm{PC}} + e^{-\beta \Delta G_{\mathrm{bind}}^{\mathrm{closed}}} \cdot x_{\mathrm{PG}}}$$

Insets show the open (Fig 1B) and closed (Fig 1A) transmembrane domains.



- The differential affinity of POPG (Fig 7) for the open and closed states suggests allosteric modulation (Fig 8).
- Prediction: ELIC in 10% POPG will have approximately 15x higher open probability than in pure POPC

Conclusions

- Application:
  - A partially resolved lipid in two ELIC densities was identified as POPG (relative SAFEP).
  - POPG has affinity for at least two binding modes which combine to produce the experimental density.
  - POPG affinity is state dependent (absolute SAFEP) with an estimated 10 to 30 fold increase in open probability under experimentally relevant conditions.
- Methodology:
  - The DBC is an effective metric for quantifying the bound state of a lipid informed by the density.
  - Using SAFEP, we are now able to make quantitative predictions of phospholipid binding to superficial sites.

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