

# Computational Prediction of Specifically Bound Lipids on Pentameric Ligand Gated Ion Channels



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## Abstract

Erwinia ligand gated ion channels (ELIC) are a common model system for the broader class of pentameric ligand gated ion channels (pLGICs) which play essential roles in cell signaling. Although pLGICs are activated by ligand binding, they are also modulated by specific interactions with bound lipids. Recent cryo-EM maps of ELIC in mixed model membranes containing POPC, POPG, and/or POPE reveal density corresponding to the glycerol backbone of bound lipids near the m3 and m4 helices. Due to lack of resolution of the lipid headgroup, however, it is not possible to identify which lipid species is bound from the cryo-EM data alone. Here we use all-atom molecular dynamics (MD) and streamlined alchemical free energy perturbations (SAFEP) to estimate the relative probability of each lipid species binding to a given protein conformation. We also describe methodological refinements that improve computational efficiency and convergence of free energy estimates.

## Summary

- The Erwinia ligand gated ion channel (ELIC) is a common model for pentameric ligand-gated ion channels (pLGICs)
- Ligand gating in ELIC may be modulated by specific interactions with bound lipids from the membrane<sup>1,2,3</sup>
- Functional and structural data show that a five-mutant (ELIC5) remains consistently open.
- ELIC5, therefore, is used as a proxy for the open state
- Cryo-EM maps show lipid density near the M3 and M4 helices of both ELIC5 and WT
- All-Atom Molecular Dynamics (MD) and Streamlined AFEP (SAFEP) were used to calculate the relative free energies of several states
- POPG was found to be the most likely lipid to bind to either state but favored the open state

## Primary Research Questions

- Which lipid is seen in the Cryo-EM maps?
- What is its relative affinity for the site?
- Do the bound lipids represent allosteric modulators of pore activity?
- If so, do they increase or decrease the likelihood of pore opening? By how much?

## Acknowledgments

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## Structure Determination

- ELIC5 was identified as a “wide-open” mutant - lacking the desensitization seen in wild type (WT) ELIC (unpublished data)
- Cryo-EM was used for structural comparisons and model creation (Fig 1-4)
- Lipid density can be seen in several of the resulting maps but head groups are not resolved

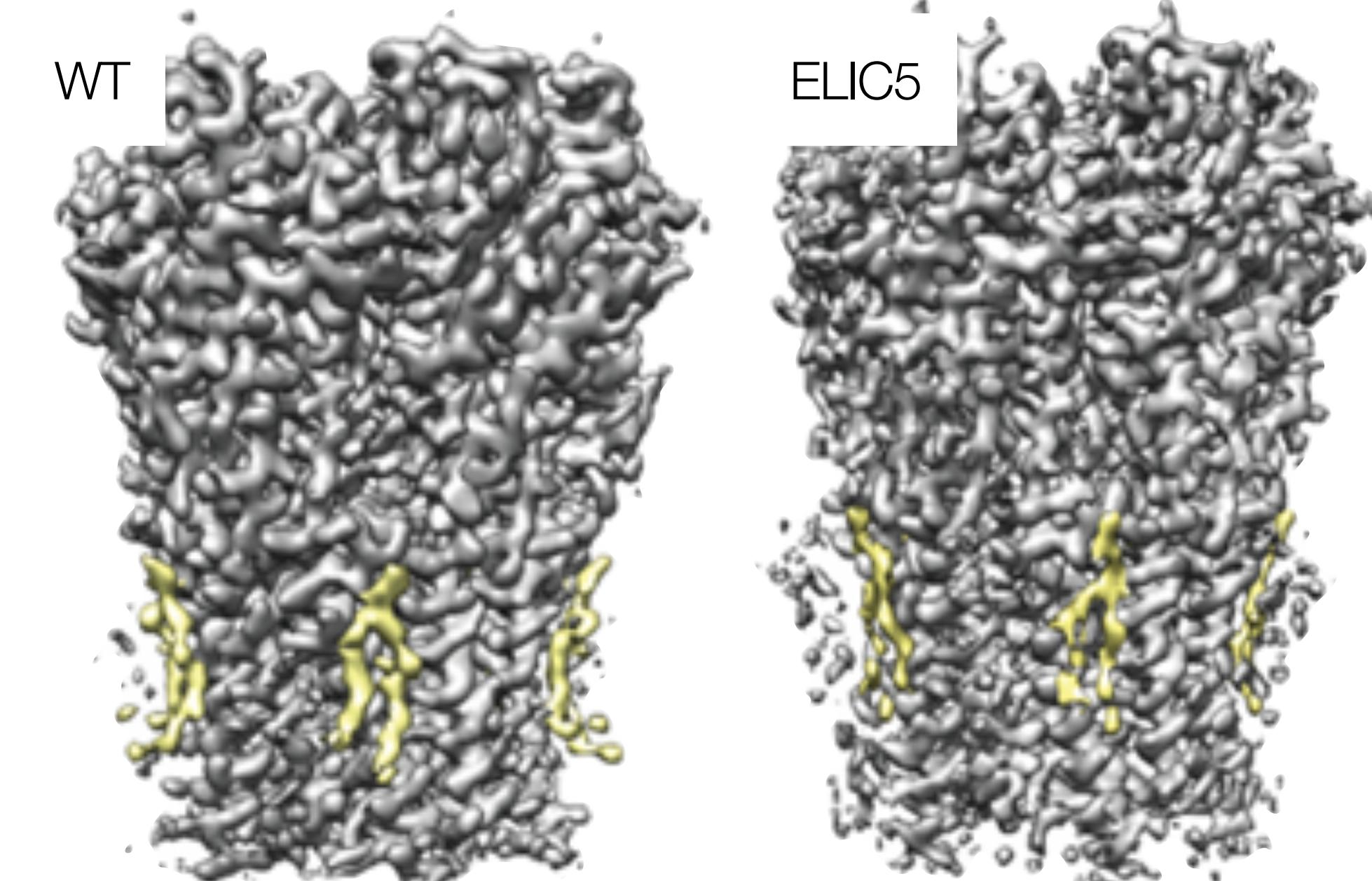


Figure 1: Cryo-EM maps of wild-type (WT, left) and a “wide-open” five-mutant (ELIC5, right), demonstrated lipid density in 2:1:1 PC:PG:PE model membrane nanodiscs (yellow).

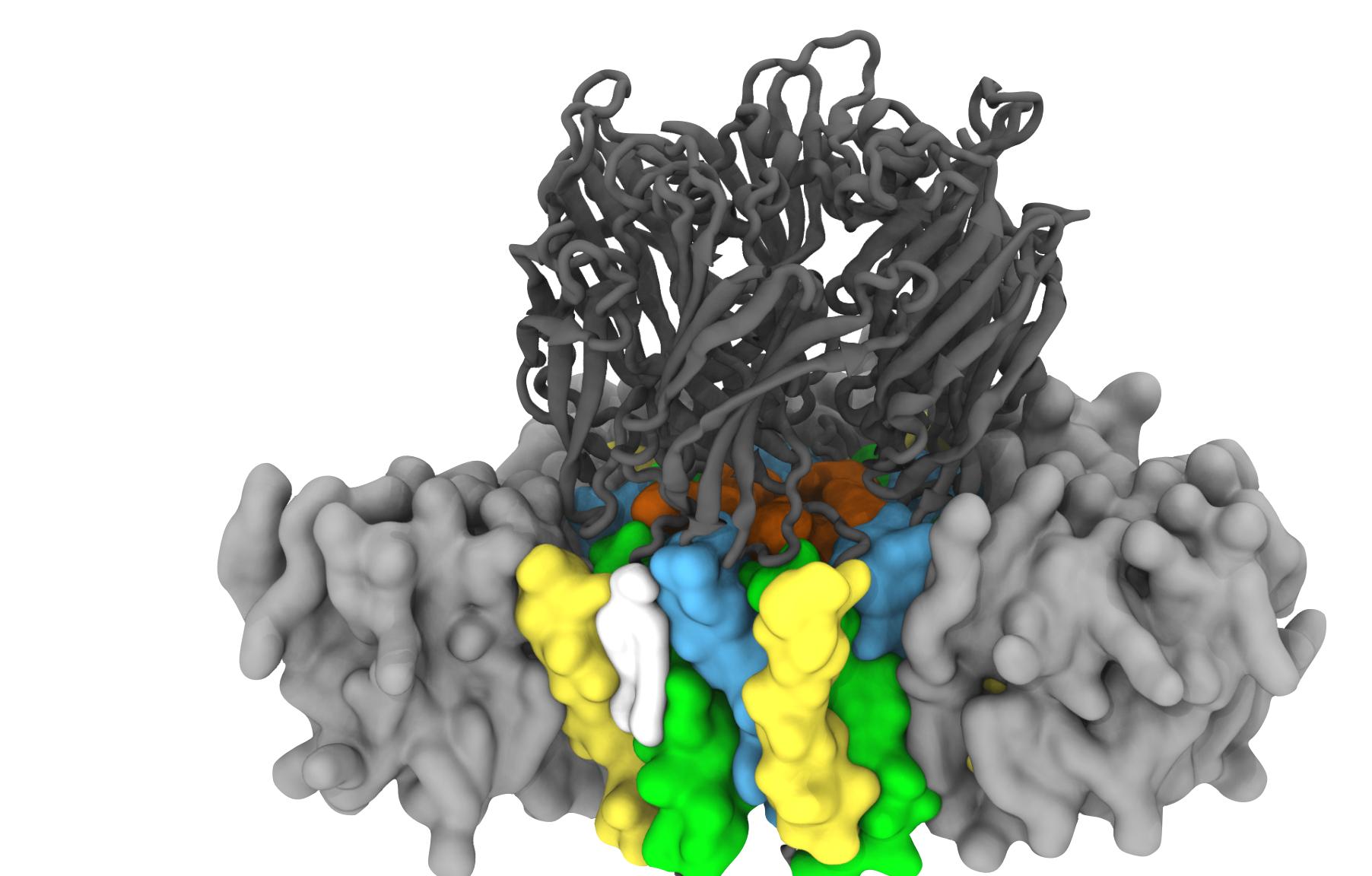


Figure 3: View of ELIC from within the membrane. Gray lipids are the POPC host membrane. White indicates a generic bound lipid.

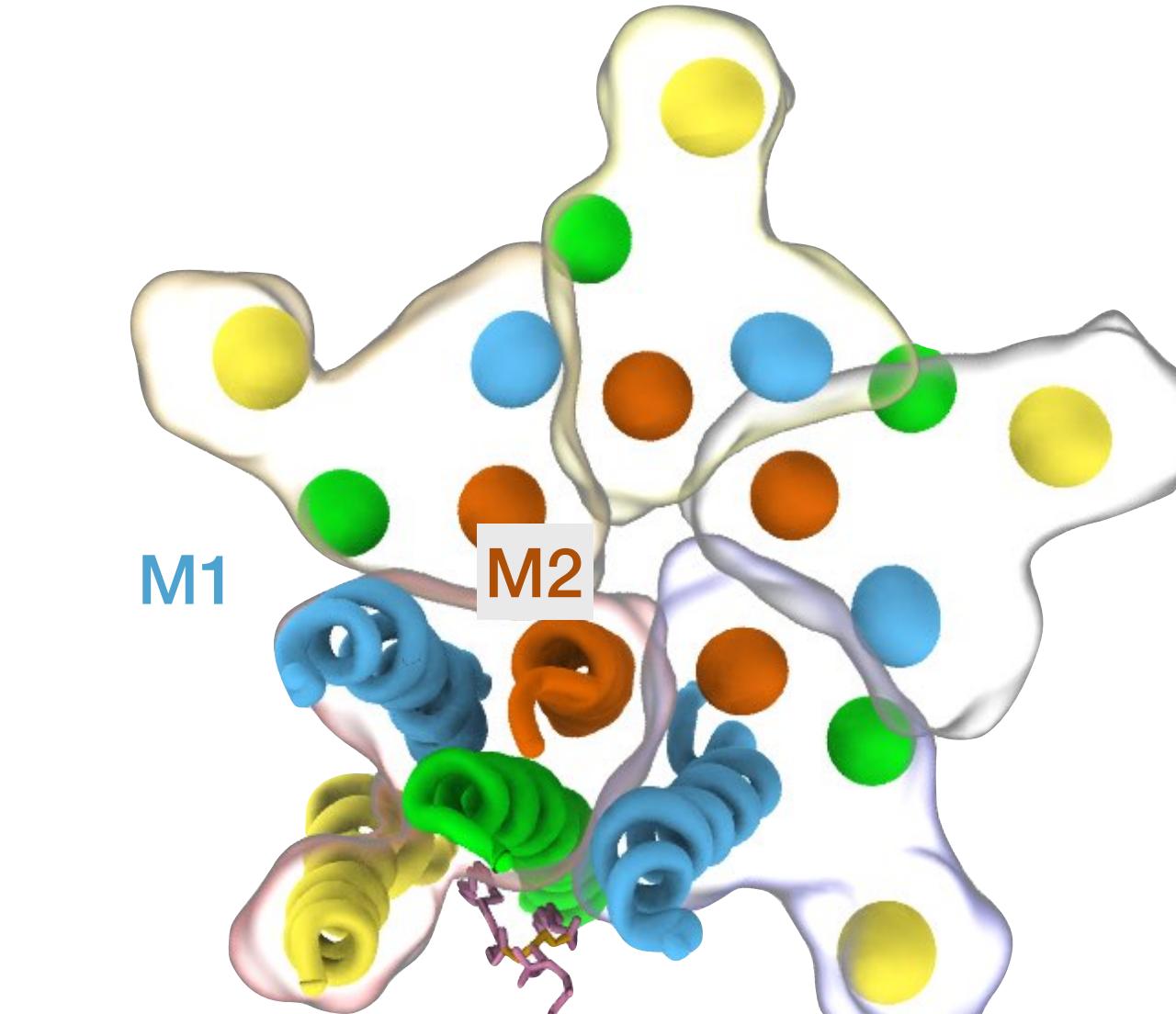


Figure 2: A top-down view of the ELIC WT transmembrane domain. Bound POPG is shown at the bottom.

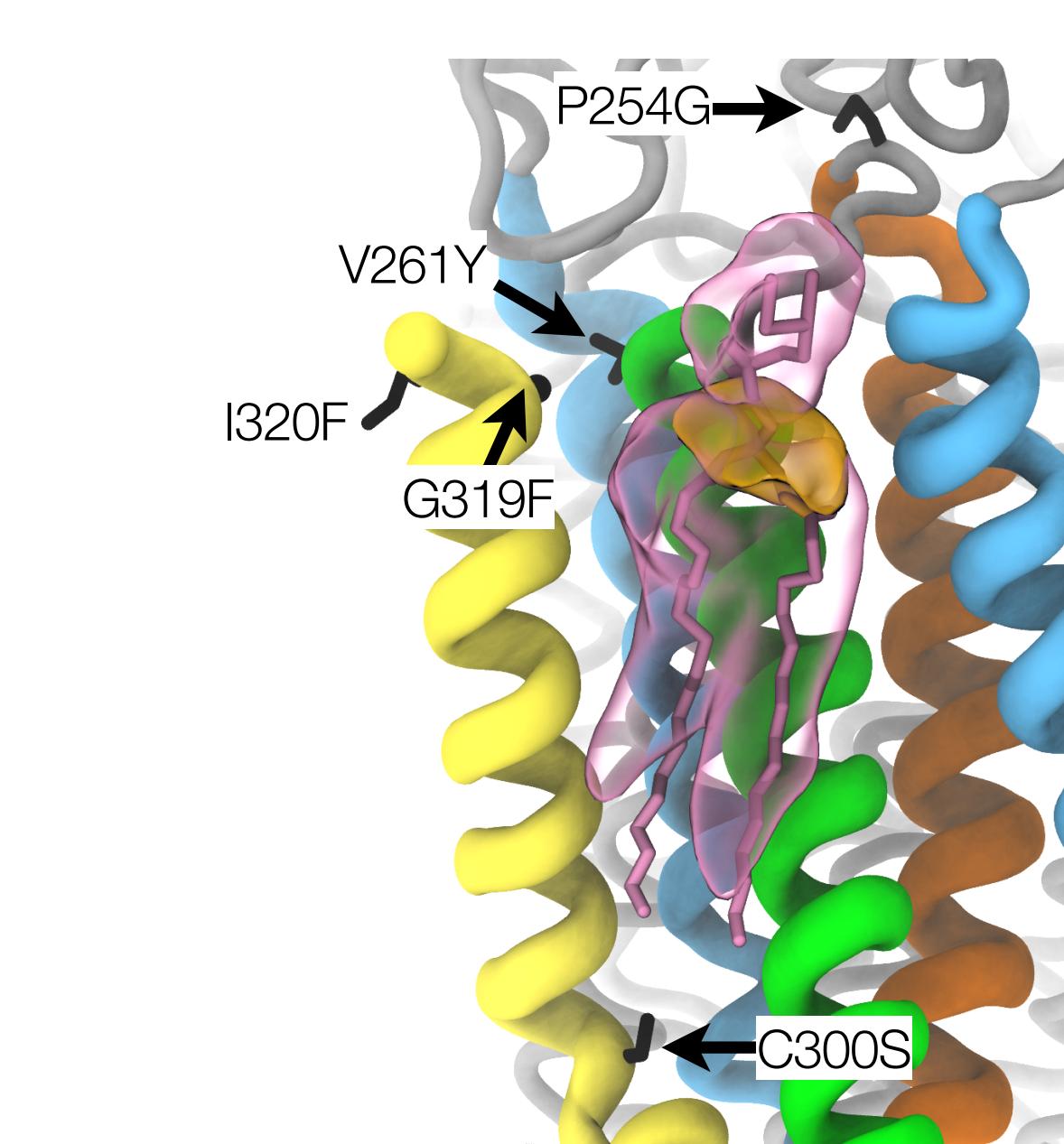


Figure 4: POPG in a bound configuration with WT ELIC. Mutated residues in ELIC5 are indicated in black. The unbiased simulated density of POPG is shown in transparent pink.

## Approach and Methods

- POPC, POPE, and POPG were compared using their free energies of mutation calculated by Streamlined Alchemical Free Energy Perturbations (SAFEP)<sup>4</sup> (Fig. 5) (CHARMM 36m, NAMD 2.14)
- Unbinding was prevented by a flat-bottom, RMSD restraint on the lipid's glycerol backbone
- These values were used to estimate relative free energy of binding, titration curves, and allosteric modulation.

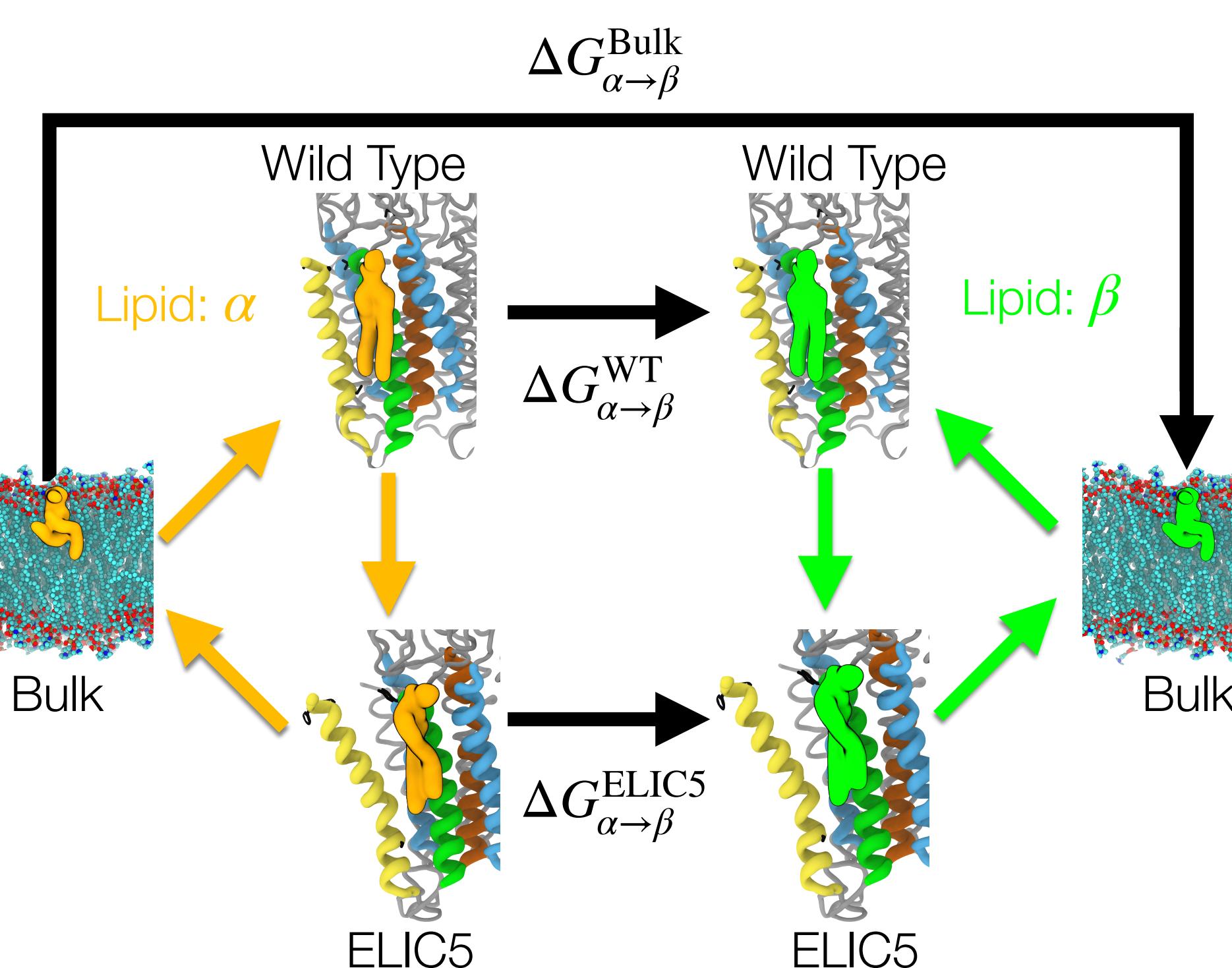


Figure 5: Map of thermodynamic cycles of interest. Free energies of replacement were calculated for each state (black arrows) (Bulk, WT, ELIC5). Lipid species are indicated by green or orange.

$$\Delta\Delta G_{\alpha \rightarrow \beta}^{\text{bulk} \rightarrow \text{WT}} = \Delta G_{\alpha \rightarrow \beta}^{\text{WT}} - \Delta G_{\alpha \rightarrow \beta}^{\text{bulk}}$$

Equation 1: The relative free energy of binding between any two lipids  $\alpha$  and  $\beta$  is the difference between the free energy to exchange the two lipids in the binding site vs in the bulk.

## Results and Discussion

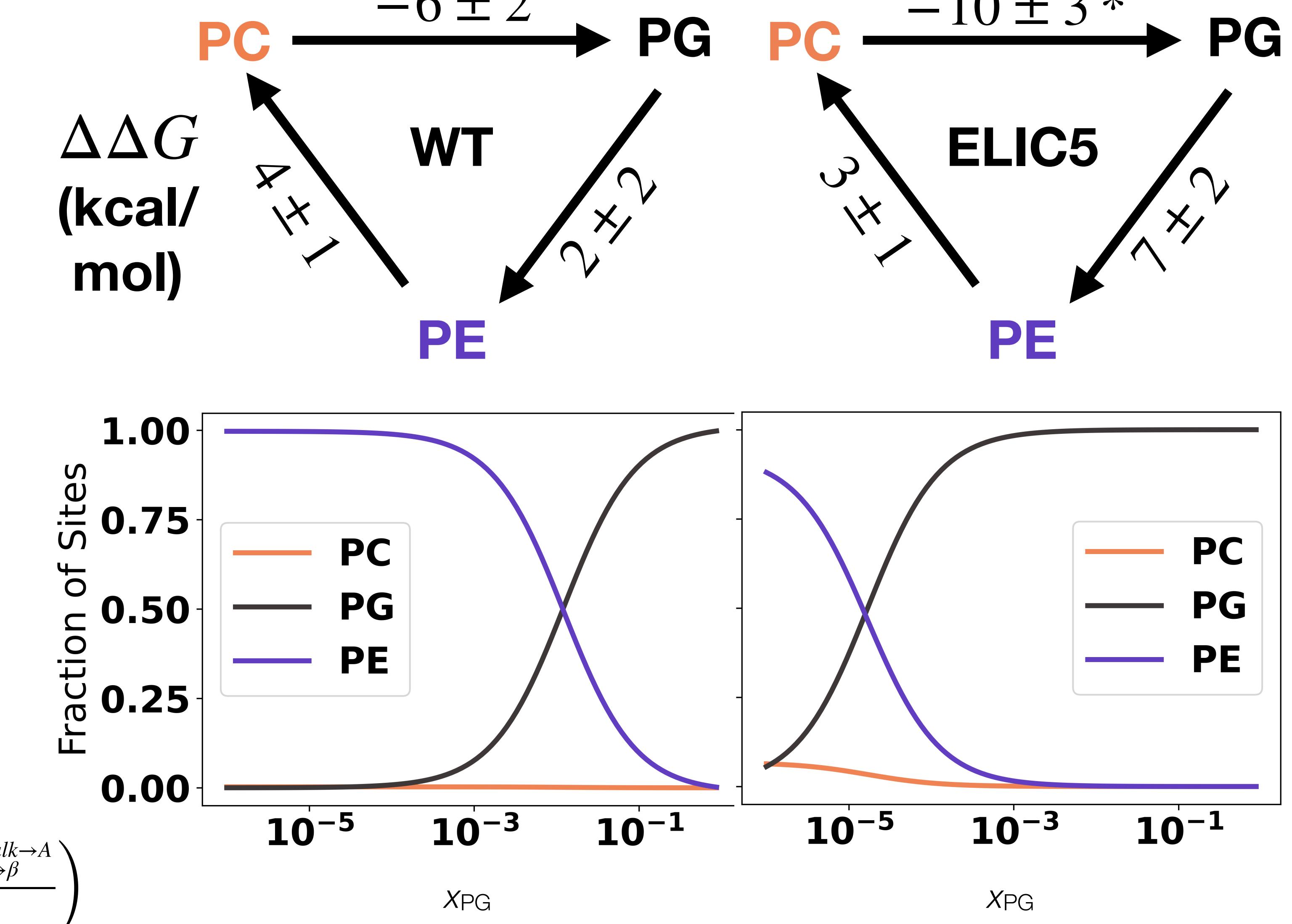


Figure 6: The change in free energy of replacing each lipid with another in the binding site of each ELIC form (Eq 1). Errors estimate standard error of the mean.  
\*Calculated from the sum of the other two legs

Figure 7: The fraction of site occupied by each lipid in a ternary mixture of 2:1:X PC:PE:PG as functions of the PG mol fraction (Equations 2 and 3). Each curve was calculated by ( $\alpha$ ) is WT or ELIC5:

$$P_{\alpha}^A = \left( 1 + \frac{P_{\beta}^A}{P_{\alpha}^A} + \frac{P_{\gamma}^A}{P_{\alpha}^A} \right)^{-1}$$

$$P_{\alpha \rightarrow \beta}^A = \frac{P_{\beta}^A}{P_{\alpha}^A} = \frac{x_{\beta}}{x_{\alpha}} \exp \left( -\frac{\Delta\Delta G_{\alpha \rightarrow \beta}^{\text{bulk} \rightarrow \text{A}}}{RT} \right)$$

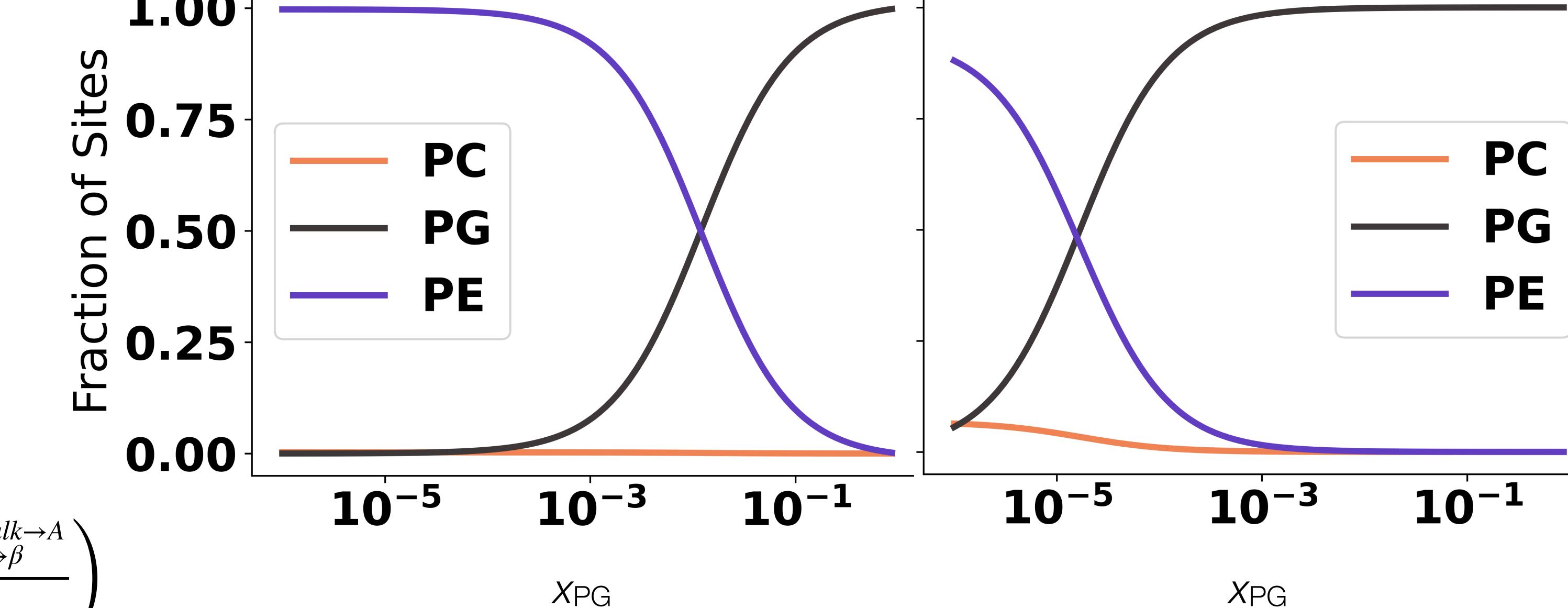


Figure 7: The fraction of site occupied by each lipid in a ternary mixture of 2:1:X PC:PE:PG as functions of the PG mol fraction (Equations 2 and 3). Each curve was calculated by ( $\alpha$ ) is WT or ELIC5:

$$\frac{P_{\text{ELIC5}}}{P_{\text{WT}}} = \frac{x_{\text{PC}} P_{\text{ELIC5}}^{\text{PC} \rightarrow \text{PG}} + x_{\text{PE}} P_{\text{ELIC5}}^{\text{PC} \rightarrow \text{PE}}}{x_{\text{PC}} P_{\text{WT}}^{\text{PC} \rightarrow \text{PG}} + x_{\text{PE}} P_{\text{WT}}^{\text{PC} \rightarrow \text{PE}}}$$

Where:  $P_{\text{WT}}$  is the probability of the pore adopting a non-conducting conformation,  $x_{\text{PC}}$  is the mole fraction of PC and  $P_{\alpha \rightarrow \beta}^A$  is the probability of  $\beta$  being bound relative to  $\alpha$ .

Blue indicates relative stabilization of the ELIC5 state. Arrows indicate values that were calculated explicitly. Other values were extrapolated.

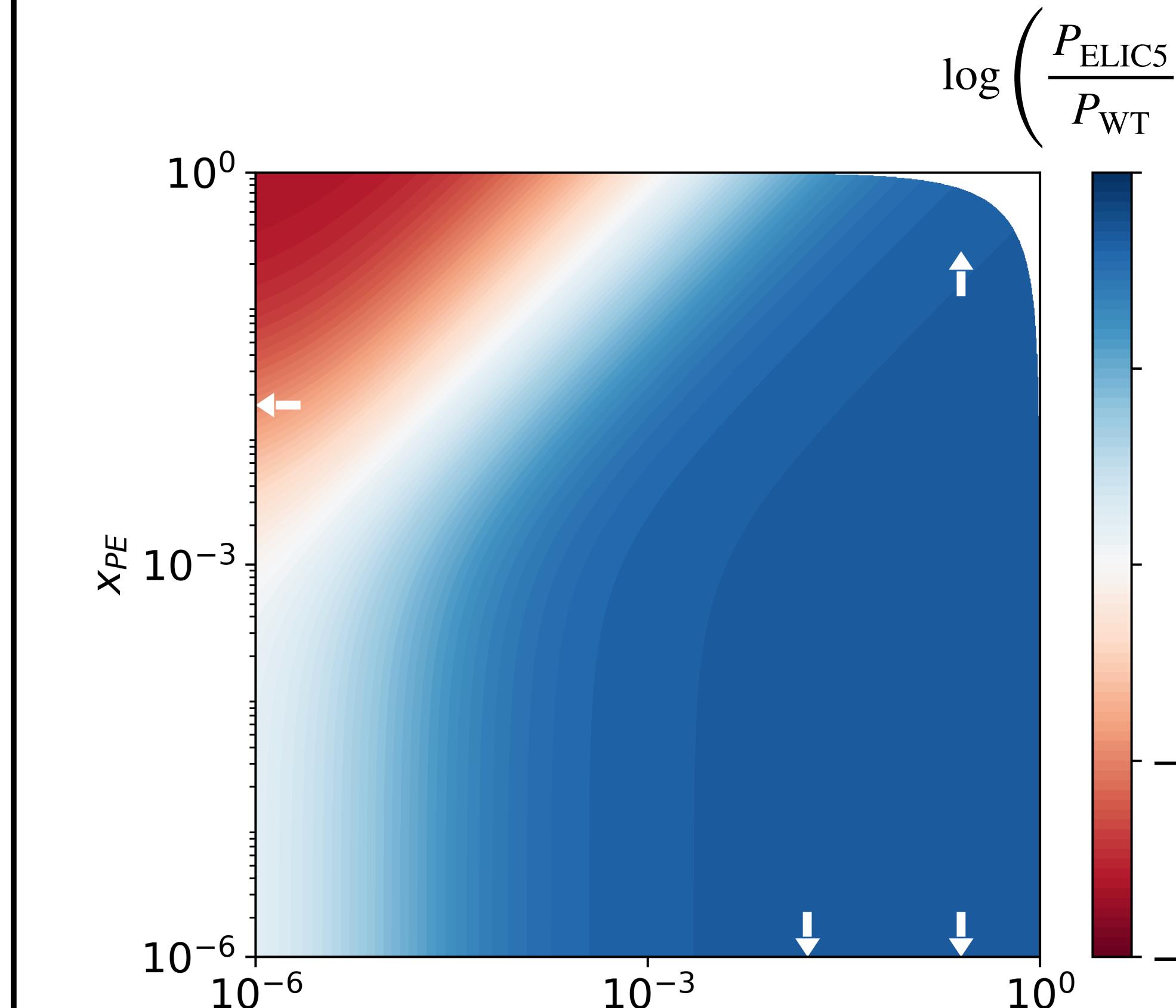


Figure 8: Relative stability of the ELIC5 conformation vs the WT conformation as a function of PE and PG mole fractions.

- In the 2:1:1 membrane, the occupancy rank order is: PG > > PE > PC (Fig 6)
- In a 2:1:X membrane, the site will be occupied by PE more than 95% of the time (Fig 7) even at low concentrations of PG ( $x_{\text{PG}} < 10^{-3}$  for WT,  $x_{\text{PG}} < 10^{-6}$  for ELIC5).
- Bound POPG stabilizes the open ELIC5 conformation (Eq. 2, Fig 8)

## Conclusions

- POPG has a high affinity for this binding site regardless of conformation, and occupies 50% of the binding sites at a mol fraction of  $x_{50} = 10^{-3}$  for the non-conducting WT conformation in a ternary mixture of POPC, POPE, and POPG.
- The POPG binding curve shifts significantly left for the open ELIC5 conformation, suggesting that POPG stabilizes the open state.
- Together, these data suggest that POPG binds as an allosteric modulator in the site identified by the Cryo-EM maps
- Bound POPG increases the likelihood of pore opening compared to PE or PC

## References

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