ProteinMPNN-based Backbone Redesign and Conformational Biasing to Increase Membrane Targeting and Conductance of a Chimeric Ion Channel

Simon Pritchard[1]

1. *Alice Ting Lab, Department of Genetics, Stanford School of Medicine; skpritch@stanford.edu*

STATS326 - Generative Protein Modeling

Abstract

ProteinMPNN has revolutionized protein design in identifying evolutionarily favorable backbone mutations to create more stable proteins. Conformational Bias (CB) examines the structure-conditioned evolutionary scores of ProteinMPNN across conformational states to suggest point mutants capable of shifting a protein's allostery in favor of, or against, some state.

We begin by recapitulating the ProteinMPNN model. Retraining various hyper parameterized models on 27k protein structures from the Baker lab (replicating *Dauparas et al.*).

We then apply these models to Modular Antigen-Gated Ion Channels (MAGICs), an antigen-dependent ion channel system for greater context specificity in neuronal modulation. We perform ProteinMPNN-based backbone redesign of improve stability in the hopes of increasing membrane targeting, while also generating point mutants with CB to favor the open channel state and increase conductance.

Background and Motivation

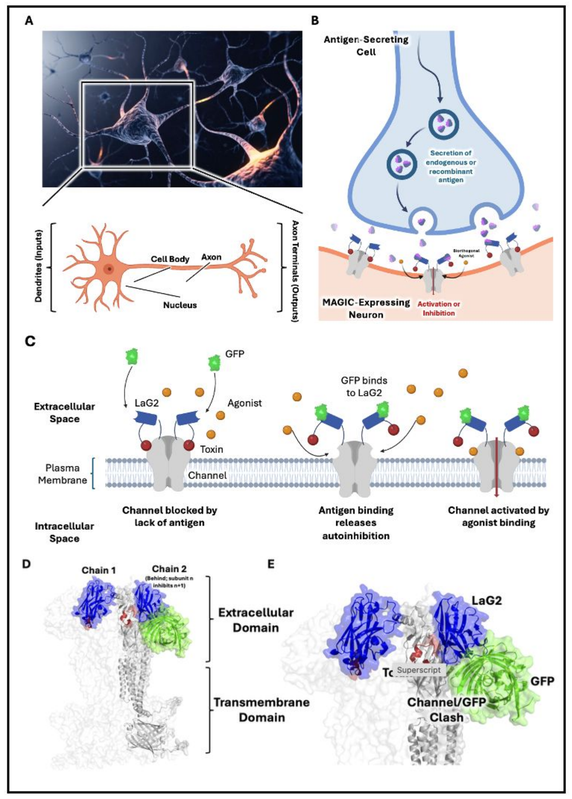

Figure 1. Modular Antigen-Gated Ion Channels (MAGICs) can be used to more precisely perturb neuronal systems. **a)** Neural systems are highly complex with many individual input and output nodes per neuron. **b)** A chimeric channel with activation conditioned on an antigen for activation would add context control for cell behavior modulation. **c)** The biorthogonal channel has an autoinhibitory domain (toxin-based) that prevents activation in the absence of some antigen (i.e GFP). Upon binding, the steric clash removes the autoinhibition and allows for activation by agonist. **d)** MAGICs are based on a biorthogonal homopentamer. **e)** The autoinhibitory domain is made up of an antigen-binding nanobody (i.e LaG2), and a toxic peptide that binds to the ligand-binding pocket. Upon antigen binding a steric clash occurs with the channel.

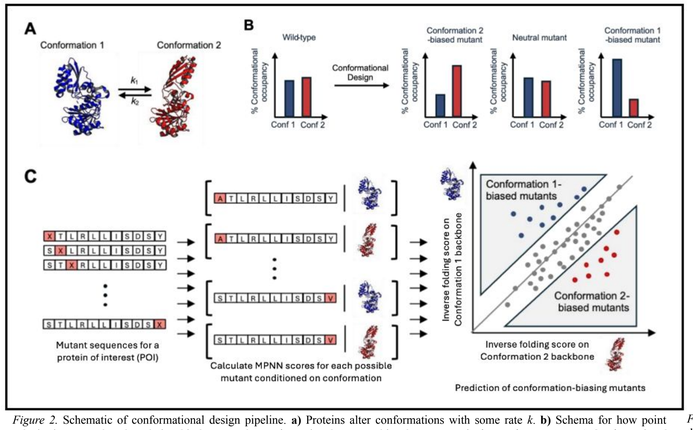

Figure 2. Schematic of conformational design pipeline. **a)** Proteins alter conformations with some rate *k*. **b)** Schema for how point substitutions can alter the relationship between the conformational states to bias the structure in favor of some state. **c)** Conformational design evaluates substitution mutations along the protein sequence and compares MPNN scores conditional on conformational state for biasing mutants.

ProteinMPNN Recapitulation

Table 1. Replication of Table 1 from *Dauparas et al.* Sequence recovery percentages and test perplexities for models with various modifications. Sequence recoveries and perplexities shown for both re-implementation and the original scores from Dauparas et al.

Reimplementation / *Dauparas et al.*	Modification	Sequence Recovery (%)	Test Perplexity
Baseline	None	42.8/40.1	6.64/6.77
Experiment 1	Add N, Cα, C, Cβ, O distances	48.6/46.1	5.43/5.54
Experiment 2	Update encoder edges	44.1/42.0	6.28/6.37
Experiment 3	Combine 1 and 2	49.5/47.3	5.30/5.36
Experiment 4	Experiment 3 with agonistic decoding	50.2/47.9	5.21/5.25

Conformational Bias

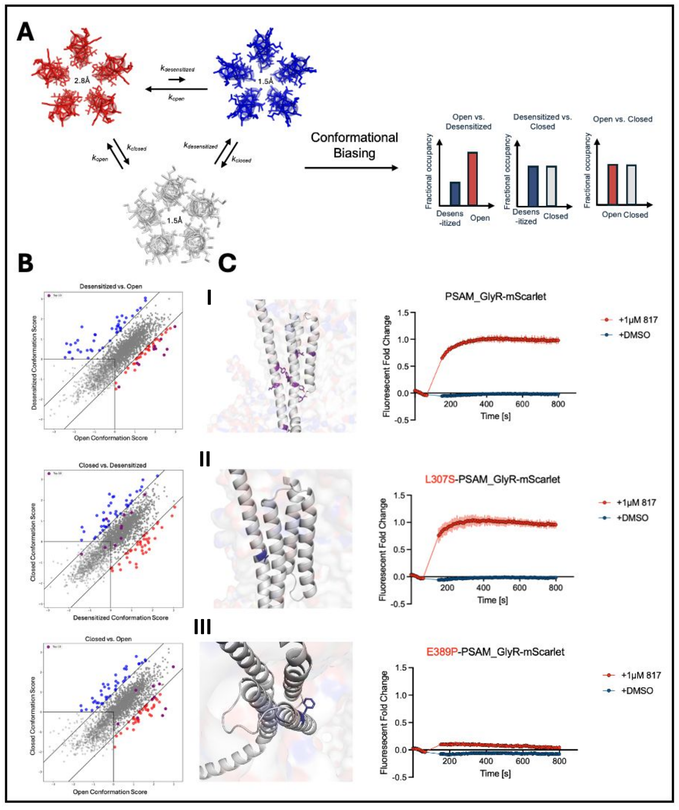

Figure 3. Conformational bias fails to generate channels with higher conductance; most point mutations result in complete LOF. **a)** Given open, desensitized, and closed channel states we seek to rebalance allostery in favor of open over desensitized, but not disrupting the equilibrium with closed. **c)** Scatterplots showing pairwise CB results, 10 selected mutations shown (purple). **c)** IPD with highlighted point mutant, membrane potential assay results for (I)WT chimeric channel, (II) L307S was the highest-functioning mutant, (III) E389P was characteristic of most point mutants selected.

Backbone Redesign

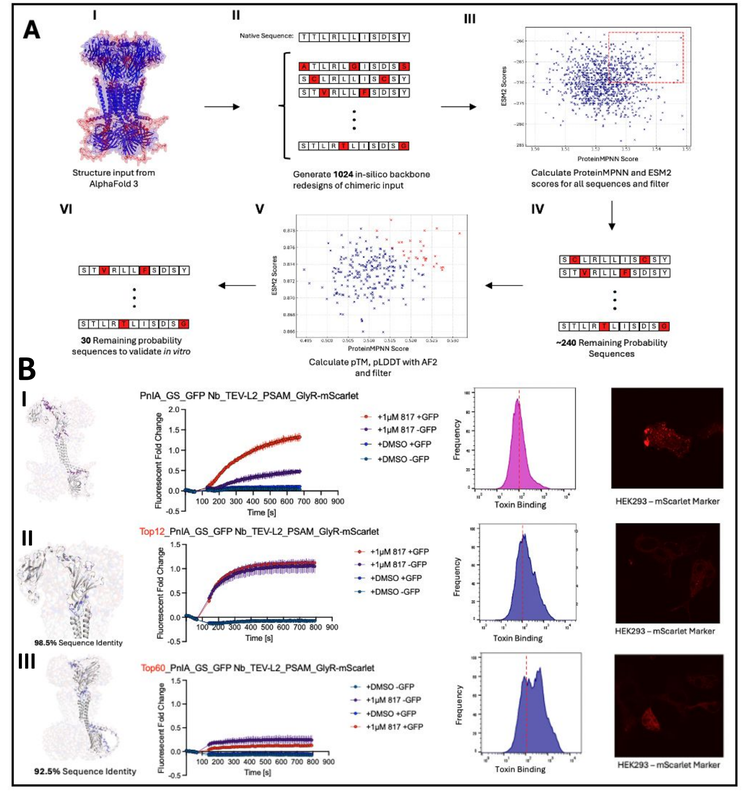

Figure 4. MPNN-based backbone redesign marginally improves membrane targeting at the cost of antigen-dependent conductance. **a)** Filtering design for MPNN-generated backbones. Top ~25% are selected based on MPNN and ESM2 scores, 30 scores are selected from the filtered set using AF2-generated pLDDT and pTM scores. **b)** MAGIC structure shown with potential or true mutation sites, MP functionality assay, FACS assay, and live-cell imaging for (I) WT inhibitory MAGIC channel, (II) backbone with the 12 highest point mutations, (III) backbone with the 60 highest point mutations. Top12 was the only redesigned channel that showed full conductance (but lost antigen-dependence). Higher levels of membrane-targeting are evidenced by the FACS results which use a membrane-impermeable toxin to measure surface presentation.

Conclusion

1. Evidently, and as suspected, we find the results from *Dauparas et al.*, to be highly reproducible. The increase in model performance is likely due to quality improvement in the Baker lab's dataset since publication.
2. We fail to see significant increases in conductance after applying the recommended point mutations. Worth noting is that the highest open state-biasing mutants were passed over due to their (theoretically) deleterious effects on the closed state.
3. We do observe marginal increases in the membrane targeting of backbone-redesigned MAGIC channels, but generally at the cost of functionality. Three more redesigns with ~86% sequence identity were tested and failed to activate upon agonist addition.
4. Inverse folding remains a worthwhile pathway for tool improvement but is by no means a magic wand. Pipeline optimization and larger mutant screens are necessary.