An Integrative Computational Approach for Engineering Genetically Encoded Voltage Indicators

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***Short Abstract* — Neurons compute by regulating the electrical potential (voltage) across their plasma membrane. Promising tools to quantitatively monitor voltage dynamics are fluorescent biosensors called Genetically Encoded Voltage Indicators (GEVI). However, currently available GEVIs are limited by combinations of factors including sensitivity, kinetics, brightness, photostability and compatibility with two-photon microscopy. The size of the sequence space precludes saturation mutagenesis across all GEVI residues, motivating the development of quantitative approaches to guide mutagenesis to those residues most likely to tune GEVI performance. Here we report a novel computational method to identify critical biosensor residues and demonstrate its application for improving voltage indicators. We anticipate that this methodology will be of broad utility for biosensor engineering.**

***Keywords* — Genetically encoded voltage indicator, ASAP, Hidden Markov Model, evolutionary trace, electrostatic interaction, covariance**

I. Background

MONITERING neural electrical activity *in vivo* with single neuron resolution is a longstanding goal in neuroscience, but with the fast development of Genetically Encoded Voltage Indicators (GEVIs), we are getting ever closer to achieving that goal. However, aside from deficits in sensitivities and kinetics, many existing GEVIs have poor performance under two-photon microscopy, which limits their application for deep tissue imaging. Indicators of the Accelerated Sensor of Action Potentials (ASAPs) family, on the other hand, shows moderate sensitivity and millisecond-timescale kinetics under both one-photon and two-photon microscopy. The most recent variant of ASAP family-ASAP2s demonstrates higher signal-to-noise ratio than ASAP1 at all frequencies but also shows slower kinetics than ASAP1 and thus has room for further improvement [1].

Here we report a computational approach to guide mutagenesis of the voltage sensing domain (VSD) of ASAPs and thus improve their sensitivity and kinetics systematically, enabling their application in mammalian models *in vivo*.

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II. Results

While the crystal structures of ASAP and its underlying VSD are not currently available, the structure of a homologous domain from the sea squirt *Ciona intestinalis* has recently been solved. However, homology modeling the ASAP VSD is complicated by repeated patterns of positively-charged arginines in the fourth transmembrane helix, which makes it challenging to determine the correct alignment between the sea squirt and the ASAP VSD. Here we choose Hidden Markov Model, a statistical model that shows promising VSD alignment results previously [2].

Second, although crystallized structure for Ciona VSD is solved, it remains a challenge to localize functionally important sites [3]. Here we use evolutionary trace (ET), a method capable of distinguishing functionally essential residues from residues that regulate specific functional features [3], to locate candidate residues for mutation.

Third, VSD is distinct from other domain in that electrostatic interaction dominates preferable resting and depolarized conformation. Therefore, an adequate model of electrostatic interaction is required to predict the direction and magnitude of point mutation. Here we propose a model that considers VSD charged residues in the surrounding electric field.

Fourth, single-residue mutation may disrupt the intramolecular interaction and thus affect protein folding. Here we include covariance analysis to retain proper folding.

With this integrative computational approach, we successfully identified residues that tune ASAP sensitivities and kinetics.

III. Conclusion

This integrative computational approach proves to be an effective method to decrease variant library size and to identify variants with desired features. We can generalize this approach to the engineering of other GEVIs to obtain improved variants.

References

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