

Structure Guided Genetically Encoded Voltage Indicator Engineering

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Short Abstract — Genetically encoded voltage indicators (GEVIs) are protein sensors that transform cell membrane voltage signals into fluorescence changes. They are promising tools for simultaneously recording from large populations of neurons with cell type specificity. However, currently- available GEVIs have insufficient brightness, photostability, response amplitude and kinetics. To overcome these limitations, we used structural approaches to identify the important residues for voltages sensing, mutated these positions, and screened for variants with improved properties. We also used these screening results in a machine learning approach to refine our strategy for further improvements.

Keywords — Structure guided screening, machine learning, Genetically encoded voltage indicators

I. BACKGROUND

MONITORING neuronal collaborations in circuits in vivo is a central goal in neuroscience but remains challenging. A critical technology gap is the lack of tools that can quantitatively monitor neuronal electrical (voltage) dynamics with single-cell or even subcellular resolution from a large and genetically-defined populations of neurons. Genetically encoded voltage indicators (GEVIs) are a promising solution to achieving this goal, yet current versions exhibit insufficient in kinetics, response amplitude, brightness and photostability for detecting fast voltage transients in vivo [1].

Accelerated Sensor of Action Potentials 1 (ASAP1) [2] is a GFP-based GEVIs with a circularly permuted GFP (cpGFP) inserted in the voltage sensitive domain. ASAP1 is a suitable starting templates for further GEVI improvements because they have fast (millisecond-timescale) kinetics and compatibility with two photon imaging methods (ASAP2s) [3]. However, its sensitivity (response amplitude) to voltage transients remains small, motivating further optimization.

Since it is impossible to screen the entire sequence space, we turned to semi-rational screening methods, supported by the accumulation of sequence and structural data, and improvements in machine learning algorithms. For example, 3D structural prediction using Rosetta [4] and existing physical model on voltage-sensing domain (VSD) movements

during membrane depolarization [5] suggest promising mutation sites. By targeting specific residues, we can focus our screening efforts on smaller and more productive screening libraries. Performing machine learning on screening results will reveal the relative importance of each position in ASAP and guided the rational design of new variants [6].

II. RESULTS

A. Structure-guided GEVI screening at single positions

We used 3D structure alignment to locate amino acids predicted to be important in physical models of orthologous voltage-sensing domain. Mutating these residues in voltage indicator ASAP1 led to mutants with larger sensitivity.

B. Structure-guided optimization of interacting residues

Using data from single-position screening, we figured out the positive and negative mutations at each important site. We did multi-position screening to combine the most promising variants and determined whether mutations produced additive, subtractive, or synergistic effects on indicator performance. We applied machine learning algorithms to quantify the relative importance of each variant on individual performance metrics: kinetics, response amplitude, and brightness.

III. CONCLUSION

Using semi-rational protein engineering and high-throughput screening pipeline, we developed genetically encoded voltage indicators with improved properties, which better meet the need for large-population voltage dynamics quantification in neuroscience.

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