

Grow the Glow: Design and Evolution of a Fluorescence-Based Genetically Encoded Biosensor for Glycine

Juliette Demers^{1,2}, Rochelin Dalangin², Marie-Eve Paquet^{2,3,4}

¹Department of Biochemistry, McGill University, Montreal, QC, Canada

²CERVO Brain Research Center, Université Laval, Quebec, QC, Canada

³Department of Anesthesiology and Intensive Care, Université Laval, Québec, QC, Canada

⁴Department of Microbiology, Biochemistry and Bioinformatics, Université Laval, Québec, QC, Canada

Keywords: Genetically Encoded Biosensor, Glycine, Neurotransmitter Dynamics, Protein Engineering, Structure-Guided Mutagenesis

Corresponding author email: juliette.demers@mail.mcgill.ca

Published: 03/25/2025

<https://doi.org/10.26443/msurj.v21i2.515>

Glycine is an amino acid that plays a crucial role in the development, functioning, and regulation of the central nervous system. It acts as the major inhibitory neurotransmitter in the brainstem, cerebellum, and spinal cord by modulating chloride ion levels in neurons. Additionally, glycine is a co-agonist required for the activation of excitatory NMDA receptors. Despite the importance of glycine in neurotransmission, much remains unknown about glycine dynamics *in vivo* due to the lack of appropriate monitoring tools. Fluorescence-based genetically encoded biosensors, composed of a fluorescent protein linked to a ligand-binding domain, provide the spatial (synaptic) and temporal (millisecond) resolution needed to monitor neurotransmitter dynamics. Although some existing biosensors can bind glycine, they suffer from low affinity and preferentially bind structurally similar molecules, such as L-proline and D-serine. Using these green fluorescent protein-based scaffolds, a glycine biosensor was designed by optimizing for physiological glycine affinity while minimizing binding of other amino acids. Combining structure-guided design and random mutagenesis, libraries of glycine biosensor variants were generated. Using a bacterial system, these libraries were screened for variants with increased glycine affinity, decreased affinity for other amino acids, and greater changes in fluorescence intensity upon ligand binding. Variants displaying desirable performance across these metrics were conserved for additional rounds of mutagenesis and screening. Preliminary screenings identified key mutations near the ligand-binding site and linker region that improve the biosensor's glycine affinity and change in fluorescence intensity. However, high-affinity off-target binding to amino acids structurally similar to glycine, such as L-alanine, remains a major challenge. To minimize challenges in off-target binding, future work will focus on transferring the screening process to a mammalian cell culture system, where optimization of membrane trafficking, folding, and functional performance in a physiologically relevant environment may be achieved.

© The Authors. This abstract is published under a CC-BY license:

<https://creativecommons.org/licenses/by/4.0/>