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A single-wavelength genetically encoded glutamate sensor for in vivo neurotransmitter observation.

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We have developed a number of high signal-to-noise single-wavelength genetically encoded biosensors for key analytes. Each sensor in made from the insertion of a circularly permuted fluorescent protein (cpFP) into a bacterial periplasmic binding proteins. The periplasmic binding protein superfamily includes scaffolds specific for a number of analytes whose visualization would be critical to the reverse engineering of complex systems such as neural networks, biosynthetic pathways, and signal transduction cascades. The best characterized sensor that we have [so far] is for glutmate. Glutamate is the most abundant excitatory neurotransmitter in vertebrates and the primary signaling molecule at the neuromuscular junction in invertebrates. Commonly used methods for studying glutamate signaling in living cells and organisms are invasive and suffer from poor spatial and temporal resolution. Our intensity-based glutamate-sensing fluorescent reporter ("IntenseGluSnFR"), constructed from a bacterial glutamate-binding protein and circularly permuted GFP, has signal-to-noise ratio and kinetics adequate for in vivo imaging. We systematically engineered IntenseGluSnFR in vitro to maximize its fluorescence change, and subsequently validated its utility for visualizing glutamate release by neurons and astrocytes in increasingly intact neurological systems. Hippocampal culture showed that IntenseGluSnFR can detect single field stimulus-evoked glutamate release. In mouse retina in vitro, two-photon fluorescence imaging showed neuronal responses to light stimuli consistent with tonic glutamate signaling. In mouse visual cortex in vivo, spontaneous activity produced phasic glutamate signals consistent with single release events. And in C. elegans, glutamate signals preceded and predicted post-synaptic calcium transients. IntenseGluSnFR enables a new generation of functional imaging experiments by making observable the precise spatio-temporal localization of neurotransmitters in vivo.

Biography

Jonathan Marvin used computational protein engineering to develop biosensors during his PhD at Duke University. As a postdoc and senior scientist at Genentech and ImClone Systems, he used phage display to engineer antibodies. At Janelia Farm, he uses both computational and library-based protein engineering to develop new biosensors.

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