Listing of Methods

Hires SA, Zhu Y, Tsien R (2008). Optical measurement of synaptic glutamate spillover and reuptake by linker optimized glutamate-sensitive fluorescent reporters
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Introduction to article

The classical junction between one nerve and another or one nerve and a muscle is the synapse. Neurotransmitter is released from the cell on one side of the synapse and detected by a receptor on the membrane on the opposite side. The neurotransmitter glutamate also excites other nearby cells, by spillover of glutamate from the synapse. To understand how glutamate spillover works, it would be helpful to be know how much glutamate is present and at what times during and after nervous stimulation. Hires et al devised a means to measure glutamate in real time.

Experiments and methods

- **Fig. 1a:** Genetic map of glutamate sensors. Nature of CFP, GltI, and Citrine. Nature of His6 tag. Nature of Ig signal peptide. Nature of PDGFR.
- **Fig. 1b**: Overall working of glutamate sensors
- **Fig**. **1c-e:** Measurement of glutamate by fluorescence spectroscopy.
- **Fig**. **2a,b:** Expression of glutamate sensor in HEK293 cells, cloned into AKAR2-pRSET$_B$ PKA reporter for one sensor and pDisplay for the other. Introduced into the cells by transfection. Expression detected by fluorescence microscopy using FRET channel.
- **Fig. 2a,c,d,e:** Optimization of glutamate sensor by in vitro mutagenesis and screening of a linker truncation library, measured by fluorescence microscopy.
- **Fig. 2f,g:** In vitro measurement of glutamate with optimized sensor, fluorescence spectroscopy.
- **Fig. 3:** In vivo measurement of glutamate in dendrites after synaptic release by fluorescence microscopy
- **Fig. 4:** Calibration of glutamate measurements by averaging and normalization.
- **Fig. 5:** Time course of glutamate presence by quantitation of fluorescence microscopy