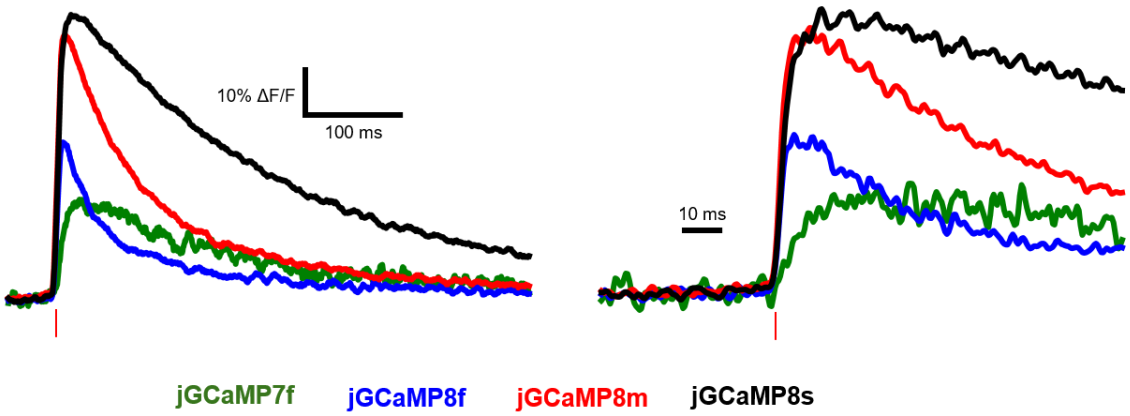
# jGCaMP8: Ultra-sensitive protein calcium sensors with fast kinetics

The Looger Lab and the GENIE Project Team at HHMI Janelia have developed a new suite of jGCaMP8 Calcium indicators, built upon the GCaMP scaffold. The jGCaMP8 sensors have fast kinetics without compromising sensitivity, setting a new standard for *in vivo* imaging. Sensors that have been extensively tested in mammalian neurons *in vivo* and *in vitro* are:

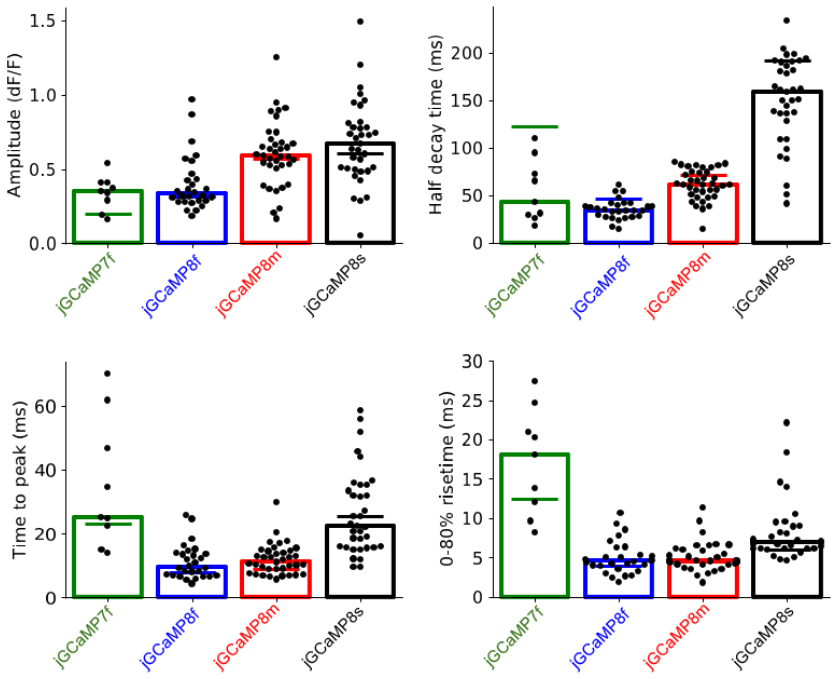
- **jGCaMP8f** (*fast*): 4x faster rise time, 2.5x faster decay time than jGCaMP7f
- **jGCaMP8m** (*medium*): almost 4x faster rise time and 3.5x more sensitive than jGCaMP7f
- **jGCaMP8s** (*sensitive*): 2x more sensitive than jGCaMP7s, >2x faster than jGCaMP7f (at 1 AP)

## In vivo performance

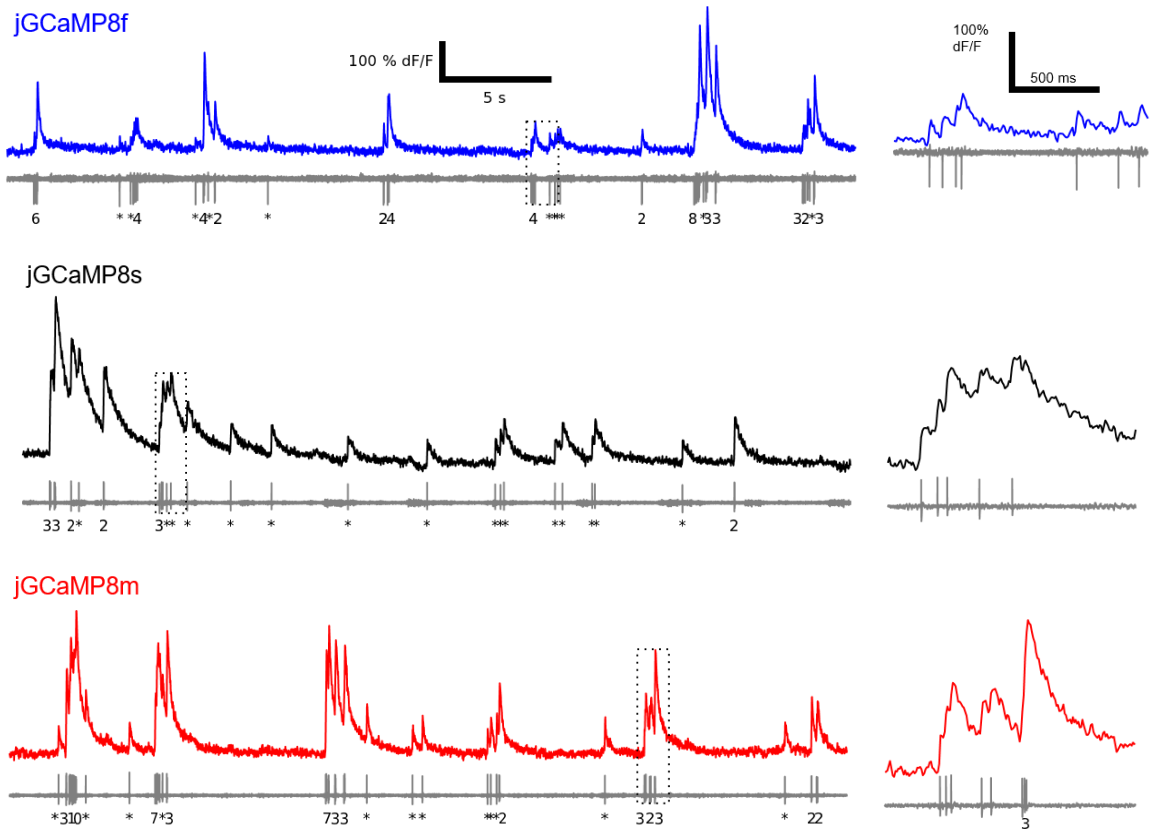
Below are representative traces based on simultaneous fluorescence and cell-attached electrophysiology in mouse visual cortex. Small fields of view were imaged in vivo at effective time resolutions of 2 ms




*Grand average of calcium transients elicited by single action potentials in vivo, aligned to action potential peak (red bar), resampled at 500 Hz. Right: zoom-in to rise kinetics*




*Cell-wise analysis of calcium transients elicited by single action potentials in vivo. Black dots represent single cells, bars represent median of single cells, horizontal lines represent values calculated from the grand averages (calculated from previous figure).*



*(Previous page) Representative in vivo ground truth recordings of GCaMP8 sensors. Fluorescence traces were filtered with a Gaussian filter (sigma = 5 ms).*

## Cultured neuron screen performance

The following table summarizes the sensitivity and kinetics of the jGCaMP8 sensors in the GENIE field stimulation-based cultured neuron screen.

| | Max dF/F | Half-rise time (ms) | Time to peak (ms) | Half-decay time (ms) |
|---|---|---|---|---|
| jGCaMP7f (control) | 0.21±0.1 | 24.8±6.6 | 99.5±30.2 | 181.9±76.0 |
| **jGCaMP8f** | 0.41±0.12 | 7.1±0.74 | 24.8±6.1 | 67.4±11.2 |
| **jGCaMP8m** | 0.76±0.22 | 7.1±0.61 | 29.0±11.2 | 118.3±13.2 |
| **jGCaMP8s** | 1.11±0.22 | 10.1±0.86 | 57.0±12.9 | 306.7±32.2 |
| jGCaMP8.712* | 0.66±0.18 | 10.9±1.24 | 41.6±8.1 | 94.8±13.3 |

** jGCaMP8.712 is currently being tested and may be released at a later date*

## Characterization in purified protein

The jGCaMP8 indicators were purified for in vitro characterization of affinity, kinetics, and absorption.

| | Kd (nM) | Hill coeff. | Max dF/F | $k_{off}$ (fast) | $k_{off}$ (fast) % | $k_{off}$ (slow) | $k_{off}$ (slow) % |
|---|---|---|---|---|---|---|---|
| jGCaMP7f (control) | 150±2 | 3.10±0.16 | 31.0±1.1 | 7.34±0.12 | | | |
| **jGCaMP8f** | 334±18 | 2.08±0.22 | 78.8±9.7 | 37.03±0.75 | 91 | 1.37±3.49 | 9 |
| **jGCaMP8m** | 108±3 | 1.92±0.12 | 45.7±0.9 | 18.25±0.9 | | | |
| **jGCaMP8s** | 46±1 | 2.20±0.13 | 49.5±0.1 | 3.68±0.1 | | | |
| jGCaMP8.712* | 292±16 | 2.17±0.15 | 63.6±4.1 | 18.8±0.44 | | | |

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| | pKa, apo | pKa, sat | $EC_{sat}$($M^{-1}$ $cm^{-1}$) | $EC_{apo}$($M^{-1}$ $cm^{-1}$) |
|---|---|---|---|---|
| jGCaMP7f (control) | 8.68±0.13 | 6.71±0.06 | 52458 | 2783 |
| **jGCaMP8f** | 7.71±0.06 | 6.68±0.01 | 50759 | 1927 |
| **jGCaMP8m** | 7.40±0.02 | 6.68±0.07 | 49856 | 2249 |
| **jGCaMP8s** | 7.65±0.04 | 6.51±0.04 | 56960 | 2116 |
| jGCaMP8.712* | 9.01±0.13 | 6.83±0.06 | 41862 | 1358 |

** jGCaMP8.712 is currently being tested and may be released at a later date*

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**If you use these reagents in your work, please cite this document:**

Y. Zhang, M. Rózsa, D. Bushey, J. Zheng, D. Reep, G. J. Broussard, A. Tsang, G. Tsegaye, R. Patel, S. Narayan, J. X. Lim, R. Zhang, M. B. Ahrens, G. C. Turner, S. S.-H. Wang, K. Svoboda, W. Korff, E. R. Schreiter, J. P. Hasseman, I. Kolb, L. L Looger, *jGCaMP8 Fast Genetically Encoded Calcium Indicators* (2020). doi:10.25378/janelia.13148243.