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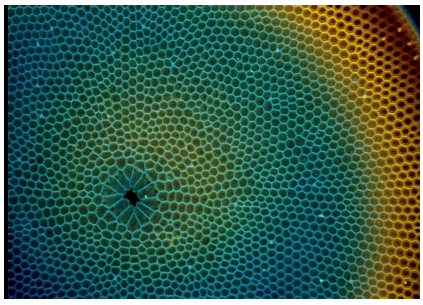
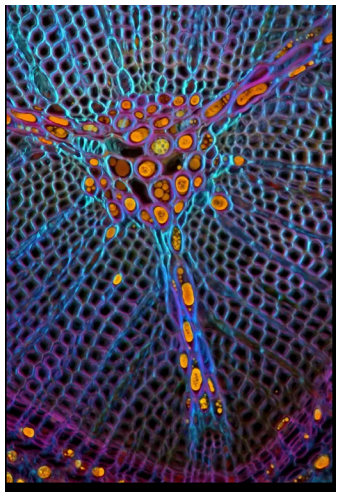
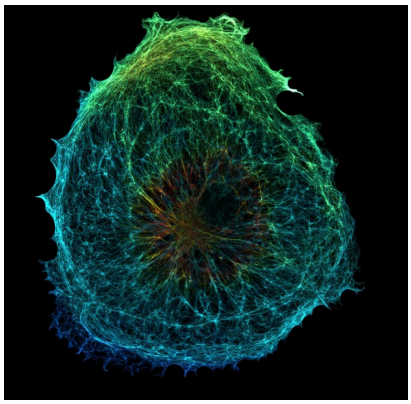
Research Campus

Janelia Genetically Encoded Reagents







GCaMP6 and jGCaMP7 Fluorescent Calcium Sensors

Immensely popular and proven technology in over 6,000 published studies:

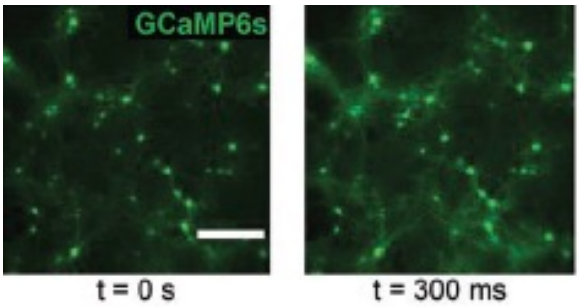
- Sensitivity, dynamic range, and kinetics that can exceed synthetic indicators and existing GECIs
- Several GCaMP6 and jGCaMP7 variants to select from, with overall brightness, rise/decay kinetics, and calcium affinity.

Advantages

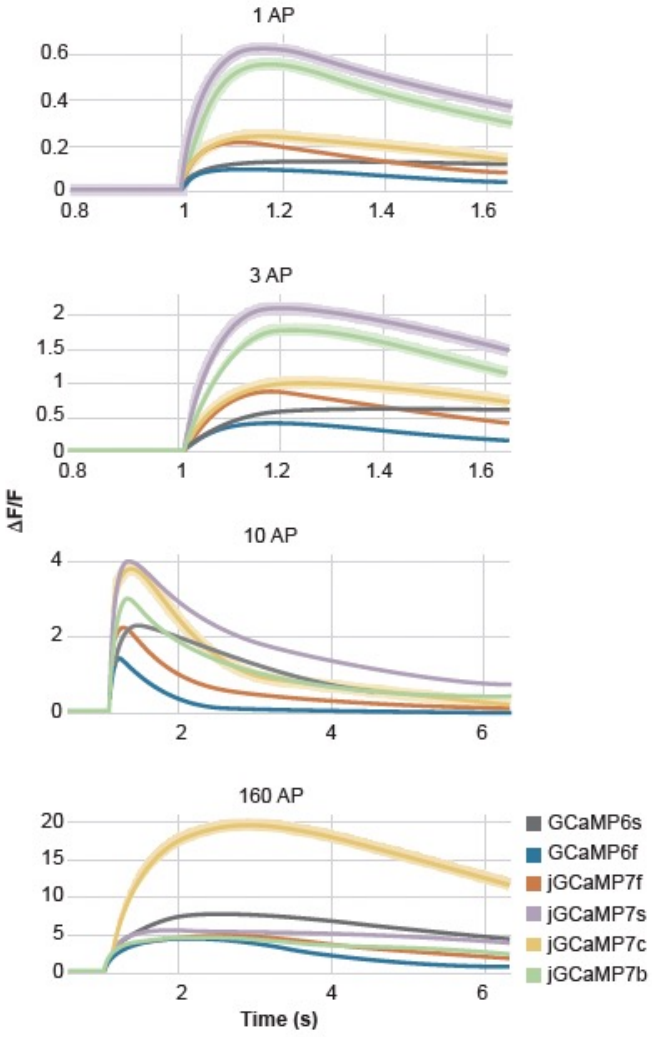
- Allow imaging cell activity long-term and *in vivo*, unlike calcium dyes, which are toxic to the cell
- GECIs can image neuronal populations for months
- Useful for genetically targeting expression.

Applications

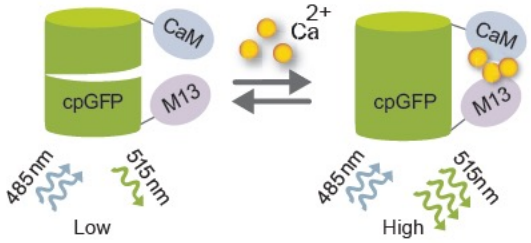
- Neuroscience research using *in vitro* or *in vivo* models
- Long-term functional imaging of neuronal activity and correlation with animal behavior
- Screening for G-protein coupled receptor (GPCR) or ion channel agonists and antagonists
- Cell, drosophila, zebrafish, mouse, and rat assays made possible through a multitude of variants and useful for imaging methods.



Signal changes for multiple GCaMP variants



Representative fluorescence dynamics of each variant.



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References

Chen, T.W., Wardill, T., Sun, Y., *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity (2013). *Nature*. doi.org/10.1038/nature12354

Dana, H., Chen, T.W., Hu, A., Shields, B.C., Guo, C., *et al. Thy1*-GCaMP6 Transgenic Mice for Neuronal Population Imaging *In Vivo* (2014). PLOS One. https://doi.org/10.1371/journal.pone.0108697

Grienberger, C., Konnerth, A., Imaging Calcium in Neurons (2012). *Neuron*. https://doi.org/10.1016/j.neuron.2012.02.011

Dana, H., Sun, Y., Mohar, B., *et al*. High-performance calcium sensors for imaging activity in neuronal populations and microcompartments (2019). *Nat Methods*. https://doi.org/10.1038/s41592-019-0435-6

Materials and US Patent nos. 9,488,642, 9,945,844, 9,518,980, 10,509,026, and EP 3540064, and EP 3713949 are available for license.

Tech ID: 2013-001

Available GCaMP6 Permutations (reference 1)

1. pAAV-CAG-GCaMP6s-WPRE-SV40
2. pAAV-CAG-GCaMP6m-WPRE-SV40
3. pAAV-CAG-GCaMP6f-WPRE-SV40
4. pAAV-Syn.GCaMP6s-WPRE-SV40
5. pAAV-Syn.GCaMP6m-WPRE-SV40
6. pAAV-Syn.GCaMP6f-WPRE-SV40
7. pGP-CMV-GCaMP6s
8. pGP-CMV-GCaMP6m
9. pGP-CMV-GCaMP6f
10. pAAV-CAG-Flex-GCaMP6s-WPRE-SV40
11. pAAV-CAG-Flex-GCaMP6m-WPRE-SV40
12. pAAV-CAG-Flex-GCaMP6f-WPRE-SV40
13. pAAV-Syn.Flex-GCaMP6s-WPRE-SV40
14. pAAV-Syn.Flex-GCaMP6m-WPRE-SV40
15. pAAV-Syn.Flex-GCaMP6f-WPRE-SV40

Available GCaMP7 Permutations (reference 4)

1. pGP-AAV-syn-jGCaMP7s-WPRE
2. pGP-AAV-syn-jGCaMP7b-WPRE
3. pGP-AAV-syn-jGCaMP7c-WPRE
4. pGP-AAV-syn-jGCaMP7f-WPRE
5. pGP-CMV-jGCaMP7s
6. pGP-CMV-jGCaMP7b
7. pGP-CMV-jGCaMP7c
8. pGP-CMV-jGCaMP7f
9. pGP-AAV-CAG-FLEX-jGCaMP7s-WPRE
10. pGP-AAV-CAG-FLEX-jGCaMP7b-WPRE
11. pGP-AAV-CAG-FLEX-jGCaMP7c-WPRE
12. pGP-AAV-CAG-FLEX-jGCaMP7f-WPRE
13. pGP-AAV-syn-FLEX-jGCaMP7s-WPRE
14. pGP-AAV-syn-FLEX-jGCaMP7b-WPRE
15. pGP-AAV-syn-FLEX-jGCaMP7c-WPRE
16. pGP-AAV-syn-FLEX-jGCaMP7f-WPRE

Available GCaMP Mouse Lines (provided by Jackson Labs)

1. C57BL/6J-Tg(Thy1-GCaMP6s)GP 4.3Dkim/J
2. C57BL/6J-Tg(Thy1-GCaMP6f)GP 5.17Dkim/J
3. C57BL/6J-Tg(Thy1-GCaMP6s)GP 4.12Dkim/J
4. C57BL/6J-Tg(Thy1-GCaMP6f)GP 5.11Dkim/J
5. C57BL/6J-Tg(Thy1-GCaMP6f)GP 5.5Dkim/J

Other variants may be available on request.

jGCaMP8 Fluorescent Calcium Sensors

The jGCaMP8 sensors have fast kinetics without compromising sensitivity, setting a new standard for *in vivo* imaging:

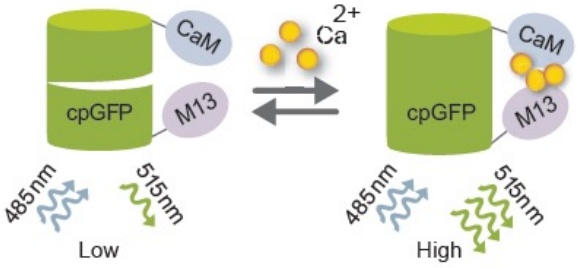
- jGCaMP8f (fast): 4x faster rise time, 2.5x faster decay time than jGCaMP7f
- jGCaMP8m (medium): almost 4x faster rise time and 3.5x more sensitive than jGCaMP7f
- jGCaMP8s (sensitive): 2x more sensitive than jGCaMP7s, >2x faster than jGCaMP7f (at 1 AP) see bit.ly/jgcamp8 for more information.

Advantages

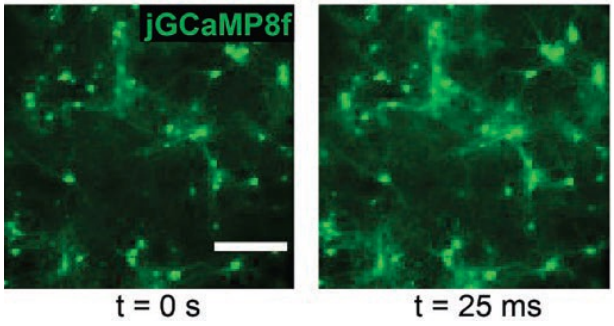
- Allow imaging cell activity long-term and *in vivo*, unlike calcium dyes that are toxic to the cell
- Useful for genetically targeting expression.

Applications

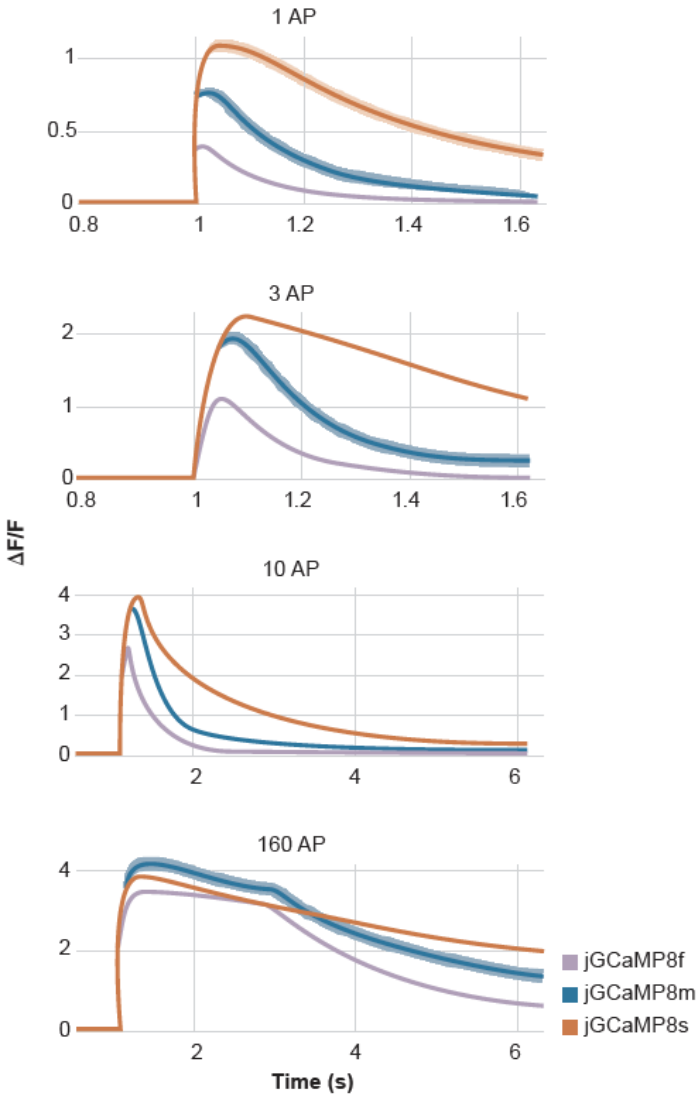
- Neuroscience research using *in vitro* or *in vivo* models
- Long-term functional imaging of neuronal activity and correlation with animal behavior
- Screening for G-protein coupled receptor (GPCR) or ion channel agonists and antagonists
- Cell, drosophila, zebrafish, mouse, and rat assays made possible through a multitude of variants and useful for imaging methods.



GCaMPs fluoresce on calcium binding



Signal changes for multiple jGCaMP8 variants



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References:

Zhang, Y., *et al*. jGCaMP8 Fast Genetically Encoded Calcium Indicators (2020). Janelia Research Campus. https://doi.org/10.25378/janelia.13148243.v4

Grienberger, C., *et al*. Imaging Calcium in Neurons (2012). *Neuron*. https://doi.org/10.1016/j.neuron.2012.02.011

Zhang, Y., *et al*. Fast and sensitive GCaM calcium indicators for imaging neural populations (2021). bioRxiv. https://doi.org/10.1101/2021.11.08.467793

Available jGCaMP8 Permutations (Reference 1):

1. pGP-CMV-jGCaMP8s
2. pGP-CMV-jGCaMP8m
3. pGP-CMV-jGCaMP8f
4. pGP-AAV-syn-jGCaMP8s-WPRE
5. pGP-AAV-syn-jGCaMP8m-WPRE
6. pGP-AAV-syn-jGCaMP8f-WPRE
7. pGP-AAV-syn-FLEX-jGCaMP8s-WPRE
8. pGP-AAV-syn-FLEX-jGCaMP8m-WPRE
9. pGP-AAV-syn-FLEX-jGCaMP8f-WPRE
10. pGP-AAV-CAG-FLEX-jGCaMP8s-WPRE
11. pGP-AAV-CAG-FLEX-jGCaMP8m-WPRE
12. pGP-AAV-CAG-FLEX-jGCaMP8f-WPRE
13. AAV-Syn-H2B-jGCaMP8s-WPRE
14. AAV-Syn-H2B-jGCaMP8m-WPRE
15. AAV-CamKIIa-jGCaMP8s-WPRE
16. AAV-CamKIIa-jGCaMP8m-WPRE
17. AAV-CamKIIa-jGCaMP8f-WPRE
18. AAV-mDlx-jGCaMP8s-WPRE
19. AAV-mDlx-jGCaMP8m-WPRE
20. AAV-mDlx-jGCaMP8f-WPRE
21. AAV-EF1a-jGCaMP8s-WPRE
22. AAV-EF1a-jGCaMP8m-WPRE
23. AAV-EF1a-jGCaMP8f-WPRE
24. pZac 2.1-GfaABC1D-lck-jGCaMP8s
25. pZac 2.1-GfaABC1D-lck-jGCaMP8m
26. pZac 2.1-GfaABC1D-lck-jGCaMP8f
27. AAV-syn-NES-jGCaMP8s-WPRE
28. AAV-syn-NES-jGCaMP8m-WPRE
29. AAV-syn-NES-jGCaMP8f-WPRE
30. AAV-syn-LifeAct-jGCaMP8s-WPRE
31. AAV-syn-LifeAct-jGCaMP8m-WPRE
32. AAV-syn-LifeAct-jGCaMP8f-WPRE
33. AAV-syn-axon-jGCaMP8s-WPRE
34. AAV-syn-axon-jGCaMP8m-WPRE
35. AAV-syn-axon-jGCaMP8f-WPRE
36. AAV-CAG-FLEX-NES-jGCaMP8s-WPRE
37. AAV-CAG-FLEX-NES-jGCaMP8m-WPRE
38. AAV-CAG-FLEX-NES-jGCaMP8f-WPRE
39. AAV-CAG-FLEX-LifeAct-jGCaMP8s-WPRE
40. AAV-CAG-FLEX-LifeAct-jGCaMP8m-WPRE
41. AAV-CAG-FLEX-LifeAct-jGCaMP8f-WPRE

jGCaMP8 Mouse lines:

1) Tetracycline-controlled transactivator protein (tTA)-dependent expression
   - JAX 037717 TetO-jGCaMP8s
2) Cre recombinase-dependent expression
   - JAX 037718 TIGRE2-jGCaMP8m-IRES-tTA2-WPRE
   - JAX 037719 TIGRE2-jGCaMP8s-IRES-tTA2
   - JAX 037952 TIGRE2-jGCaMP8s-IRES-tTA2-WPRE
   - JAX 039267 TIGRE2-RiboL1-jGCaMP8s-IRES-tTA2

Materials and patents, including US Patent 12,044,675, US patent application 18/744,923, EP4217496, CA 3,193,683, and AU 2021347266 are available for license.

Tech ID: 2020-014

jRCaMP1 and jRGECO1 Red Fluorescent Calcium Sensors

Calcium indicators are ideal for multicolor imaging and applications requiring greater depth in tissue than is possible with green variants. These Red GECIs enable dual-color imaging alongside green GECIs like GCaMP and are used with optogenetic effectors whose action cross-sections overlap with green GECIs.

- **jRCaMP1a** Bright and photostable with no photoswitching, slow decay
- **jRCaMP1b** Bright and photostable, no photoswitching, less sensitive than jRCaMP1a
- **jRGECO1a** Most sensitive, fast kinetics, photoswitches under blue/green light, accumulates in endosomes
- **jRGECO1b** Sensitive, fast kinetics, less sensitive, less photoswitching than jRGECO1a

Advantages

- Best red GECIs available: superior signal, kinetics, and expression.
- Cheaper and allow longer cell imaging than small-molecule dyes.

Applications

- All uses for green indicator GCaMP, but at longer wavelengths: allowing deep imaging, imaging with less phototoxicity, and imaging in samples with high background fluorescence in the green channel.
- Dual-color imaging alongside blue, green, and yellow indicators or labels.
- Use alongside optogenetic effectors with minimal crosstalk between effector action spectra and GECI excitation spectra.
- Cell, brain slice, Drosophila, zebrafish, mouse, and rat assays enabled drug screening and basic science studies.

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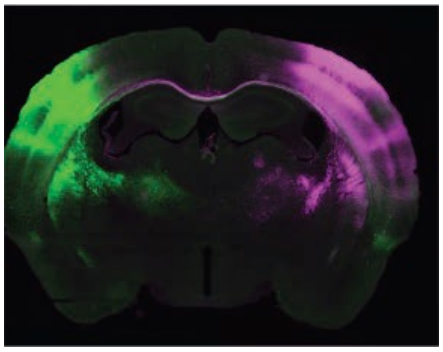
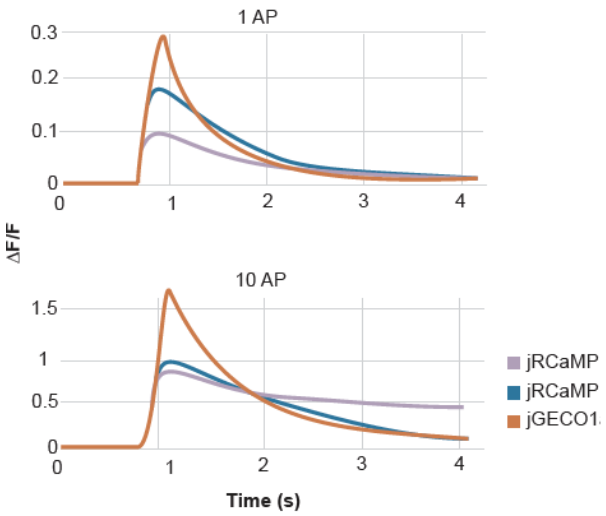
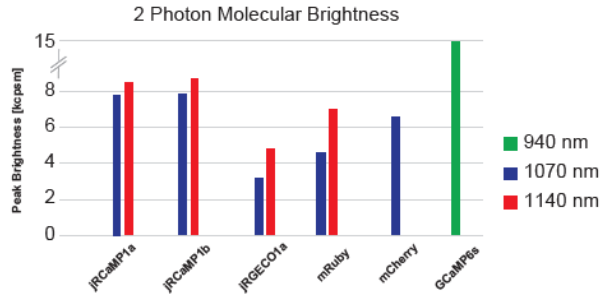
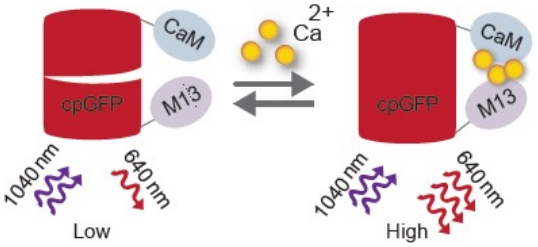


Two-color imaging in a mouse with jRGECO1a and GCaMP6







2-photon excitation of red GECIs with and without Ca2+

Available Permutations

1. pAAV-Syn-NES-jRGECO1a-WPRE-SV40
2. pAAV-Syn-Flex-NES-jRGECO1a-WPRE-SV40
3. pAAV-CAG-Flex-NES-jRGECO1a-WPRE-SV40
4. pCMV-NES-jRGECO1a
5. pRSET-NES-jRGECO1a
6. pSIV-syn-NES-jRGECO1a-IRES2-nls-GFP-WPRE
7. pAAV-Syn-NES-jRGECO1b-WPRE-SV40
8. pAAV-Syn-Flex-NES-jRGECO1b-WPRE-SV40
9. pAAV-CAG-Flex-NES-jRGECO1b-WPRE-SV40
10. pCMV-NES-jRGECO1b
11. pRSET-NES-jRGECO1b
12. pSIV-syn-NES-jRGECO1b-IRES2-nls-GFP-WPRE
13. pAAV-Syn-NES-jRCaMP1a-WPRE-SV40
14. pAAV-Syn-Flex-NES-jRCaMP1a-WPRE-SV40
15. pAAV-CAG-Flex-NES-jRCaMP1a-WPRE-SV40
16. pCMV-NES-jRCaMP1a
17. pRSET-NES-jRCaMP1a
18. pSIV-syn-NES-jRCaMP1a-IRES2-nls-GFP-WPRE
19. pAAV-Syn-NES-jRCaMP1b-WPRE-SV40
20. pAAV-Syn-Flex-NES-jRCaMP1b-WPRE-SV40
21. pAAV-CAG-Flex-NES-jRCaMP1b-WPRE-SV40
22. pCMV-NES-jRCaMP1b
23. pRSET-NES-jRCaMP1b
24. pSIV-syn-NES-jRCaMP1b-IRES2-nls-GFP-WPRE

Available Mouse Lines (provided by Jackson Labs)

1. Tg(Thy1-jRGECO1a)GP8.31Dkim/J (030526)
2. Tg(Thy1-jRGECO1a)GP8.20Dkim/J (030525)
3. C57BL/6J-Tg(Thy1-jRGECO1a)GP8.58Dkim/J (030527)
4. Tg(Thy1-jRGECO1a)GP8.62Dkim/J (030528)
5. B6;SJL-Tg(Thy1-jRGECO1a)GP8.5Kim/J (032010)

References

1. Dana, H., *et al*. Sensitive red protein calcium indicators for imaging neural activity (2016). *eLife*. https://doi.org/10.7554/eLife.12727

2. Dana, H., *et al*. Thy1 transgenic mice expressing the red fluorescent calcium indicator jRGECO1a for neuronal population imaging *in vivo* (2018). *PLoS One*. https://doi.org/10.1271/journal.pone.0205444

3. Turner-Evans, D., *et al*. Angular velocity integration in a fly heading circuit (2017). *eLIfe*. https://doi.org/10.7554/elife.23496

Material and US patents 9,644,007 and 10,053,492 are available for license.

Tech IDs: 2015-026 and 2015-052

jYCaMP Yellow Genetically Encoded Calcium Sensor

jYCaMP is a redshifted variant of jGCaMP7 capable of detecting single action potentials.

- Optimized for femtosecond lasers at fixed wavelengths above 1000 nm
- Ideal for two-photon microscopy
- Yellow variant of the high-performance calcium indicator jGCaMP7
- Outperforms its parent in mice and flies at excitation wavelengths above 1000 nm

Plasmids:

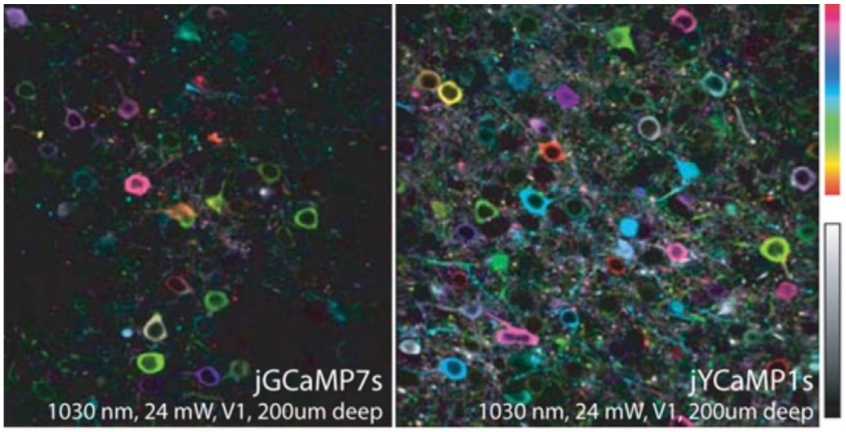
- pAAV-syn-FLEX-jYCaMP1s
- pAAV-syn-FLEX-axon-jYCaMP1s
- pAAV-syn-FLEX-jYCaMP1
- pAAV-syn-jYCaMP1
- pAAV-syn-jYCaMP1s

Reference:

Mohr, M.A., Bushey, D., Aggarwal, A., *et al*. jYCaMP: an optimized calcium indicator for two-photon imaging at fiber laser wavelengths (2020). *Nat Methods*. https://doi.org/10.1038/s41592-020-0835-7

jYCaMP materials and patents are available for license.

Tech ID: 2019-018



CaMPARI2 Photoconvertible Calcium Sensor

CaMPARI2 (Calcium Modulated Photoactivatable Ratiometric Integrator, 2nd generation) is a green-to-red photoconvertible protein construct that enables imaging of the integrated calcium activity of large populations of cells over defined time windows. Photoconversion depends on simultaneous light exposure and elevated calcium, marking active neuronal populations with single-cell and subsecond resolution.

- Fast kinetics
- Low photoconversion rate in low calcium conditions
- 100-fold difference in green-to-red switching in low- vs. high-calcium conditions

Plasmids:

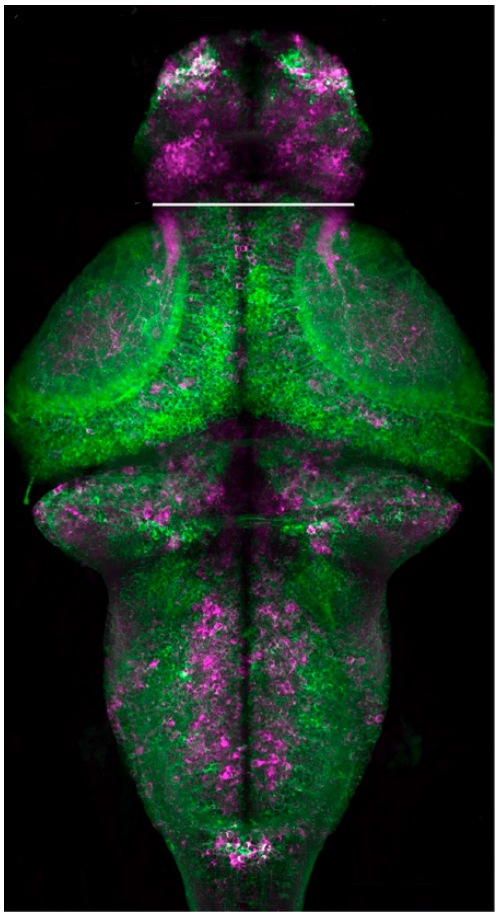
- pAAV_hsyn_NES-his-CaMPARI2-WPRE-SV40
- pAAV_hsyn_NES-his-CaMPARI2-F391W-WPRE-SV40
- pAAV_hsyn_NES-his-CaMPARI2-H396K-WPRE-SV40
- pAAV_hsyn_NES-his-CaMPARI2-F391W-G395D-WPRE-SV40
- pAAV_hsyn_NES-his-CaMPARI2-L398T-WPRE-SV40
- pTol2-HuC(elavl3)-CaMPARI2: pan-neuronal expression of CaMPARI2 in zebrafish, used to generate the CaMPARI2 zebrafish line at ZIRC (ZL13801)

Reference:

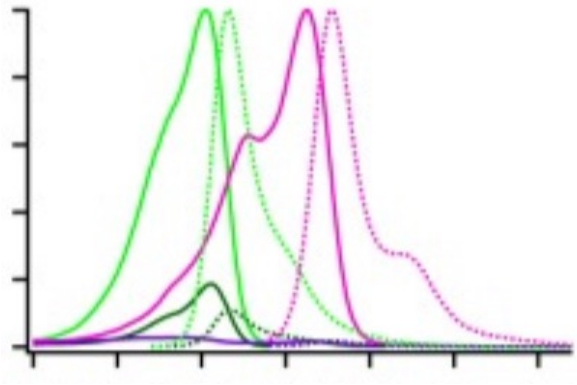
Moeyaert, B., *et al*. Improved methods for marking active neuron populations (2018). *Nat Commun*. http://doi.org/10.1038/*s41467-018-06935-2.*

Materials and US Patents 9,518,996 and 10,067,148 are available for license.

Tech IDs: 2013-016 and 2016-048



CaMPARI2 in a zebrafish



CaMPARI2 excitation (solid) and emission (dotted), before (green) and after (magenta) UV photoconversion.

rsCaMPARI Reversible Calcium Sensor

rsCaMPARI is a genetically encoded reversibly switchable fluorescent protein that:

- Enables spatiotemporally precise marking, erasing, and remarking of active neuron populations
- Works under brief, user-defined time windows of light exposure
- Kinetics are modulated by calcium concentration with blue light
- Reset the fluorescence with violet light
- Proof of action in freely swimming zebrafish

Reference:

Sha, F., Abdelfattah, A.S., Patel, R., Schreiter, E.R., Erasable labeling of neuronal activity using a reversible calcium marker (2020). *eLife*. https://doi.org/10.7554/eLife.57249

Materials and IP, including US Patent 12,072,340, are available for license.

Tech ID: 2018-008

Plasmids:

pRSET_ His-rsCaMPARI-mRuby3	Expresses rsCaMPARI in bacterial cells
pAAV-hsyn_ NES-His-rsCaMPARI-mRuby3	AAV plasmid for expressing rsCaMPARI in neurons, nucleus excluded
pAAV-hsyn_ NLS-His-rsCaMPARI-mRuby3	AAV plasmid for expressing rsCaMPARI in neurons, nucleus localized
pTol2-elavl3_ NES-rsCaMPARI-mRuby3	Pan-neuronal expression of rsCaMPARI in zebrafish, nucleus excluded

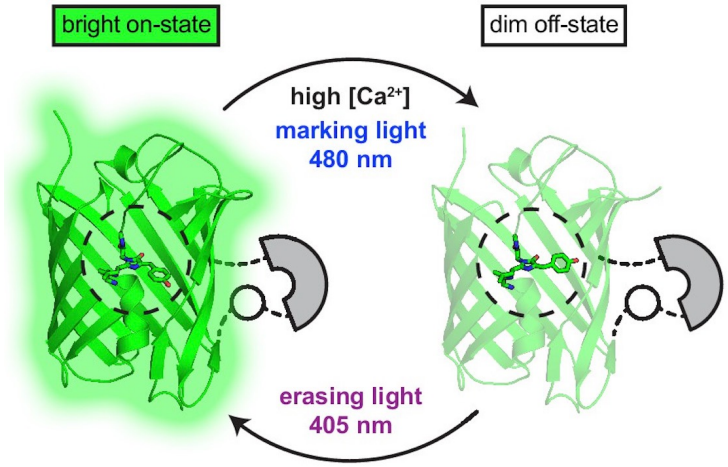


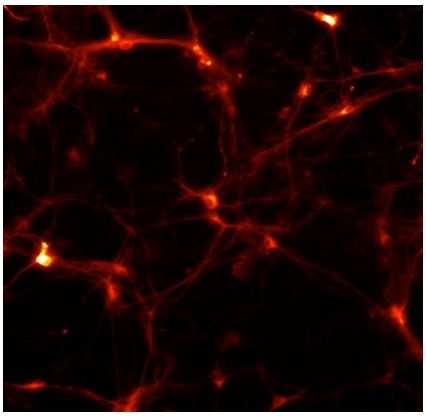
Illustration of the rsCaMPARI function.

HaloCaMP Chemigenetic Calcium Indicators Using HaloTag and Janelia Fluor® Dyes

HaloCaMP integrates circularly permuted HaloTag proteins with a range of Janelia Fluor® (JF™) dyes to form bright, modular calcium indicators for *in vitro* and in-cell imaging. This chemigenetic system offers far-red imaging, rapid labeling, and customizable neuroscience and cell biology readouts.

- *Red and Far-Red Imaging:* Enables imaging beyond GFP's spectral range, compatible with deep-tissue setups.
- *Multiple JF™ Dye Options*
  - **JF585** (orange): Highest $\Delta F/F_0$ (~23× *in vitro*)
  - **JF635, JF639** (far-red): Bright and neuron-compatible
  - **JF629**, **JF630:** Sensitive with low baseline for improved contrast
- *Neuron-Ready*

Expressed in hippocampal cultures and capable of reporting single action potentials.



Performance Highlights

Dye	Color	$\Delta F/F_0$	Kd (nM)	Notes
JF585	Orange	23.2	—	Highest ΔF/Fo *in vitro*
JF635	Far-Red	5–9	43–190	Strong neuronal performance
JF629	Far-Red	Moderate	—	Best with HaloCaMP1a
JF630	Far-Red	Moderate	—	Best with HaloCaMP1b

Applications

- Calcium imaging in primary neurons
- Customizable *in vitro* or *in vivo* indicators
- Voltage and other biosensing (HASAP, HArclight variants)
- Compatible with expanding dye palettes and multiplexed imaging

Plasmids:

- pCAG-HASAP1-ST: chemigenetic voltage indicator, soma targeted
- pAAV-synapsin-HaloCaMP1b-EGFP: chemigenetic calcium indicator
- pAAV-synapsin-HaloCaMP1a-EGFP: chemigenetic calcium indicator
- pCAG-HArcLight: chemigenetic voltage indicator
- pCAG-HASAP: chemigenetic voltage indicator

Reference:

Deo, C., *et al.* The HaloTag as a general scaffold for far-red tunable chemigenetic indicators (2021). *Nat Chem Biol*. http://doi.org/10.1038/s41589-021-00775-w

Materials and US Patents 11,708,397 and 12,275,767 are available for license.

Tech ID: 2018-016

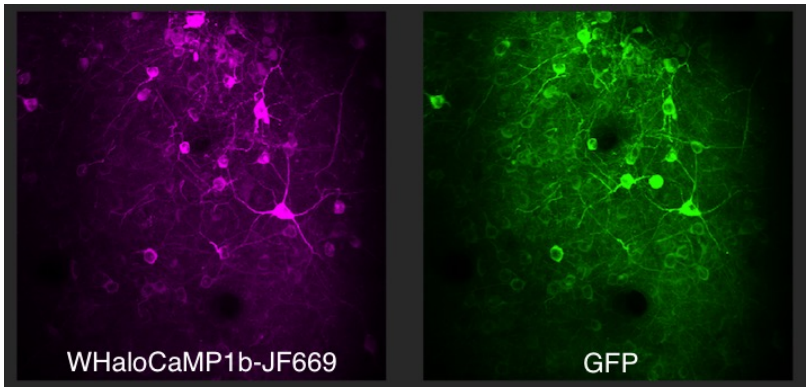
WHaloCaMP Modular, Bright, and Multiplexable Chemigenetic Calcium Indicators

WHaloCaMP sensors integrate the high-performance Janelia Fluor® dyes with genetically engineered HaloTag proteins for modular, tunable calcium imaging in living systems. This platform offers multiple dye options, works in live animals, and supports high-speed, multicolor, and fluorescence lifetime imaging. Fluorescence change in WHaloCaMP results from reversible quenching of the bound dye via a strategically placed tryptophan.

- **Spectral Flexibility** WHaloCaMP functions with JF™ dyes across the spectrum:
  - Green (JF494)
  - Orange (JF552)
  - Far-Red (JF669)
  - Near-IR (JF722)
- **Multiplexing Ready** Combine WHaloCaMP with GFP or red indicators (e.g., jRGECO1b, iGlucoSnFR) for 3-color imaging in zebrafish, flies, and mice.
- **Live-Animal Compatible** Effective *in vivo* labeling by retro-orbital injection of dye; functional in cortex, brainstem, retina, and more.
- **Quantitative Imaging** Enables FLIM (fluorescence lifetime imaging microscopy)-based calcium quantification via lifetime changes up to 2.1 ns.

Applications

- *In vivo* Ca^{2+} imaging in flies, zebrafish, and mice
- Two-photon and light-sheet imaging
- Astrocyte-neuron co-imaging
- Signal multiplexing with kinase and metabolite reporters



Performance Snapshot

Dye	Color	$\Delta F/F_0$	K_d (nM)	Bioavailable *In Vivo*?
JF494	Green	10×	71	Yes
JF552	Orange	4×	87	Yes
JF669	Far-red	7×	37	Yes
JF722	Near-IR	16× (*in vitro*)	26	No

Continued

WHaloCaMP plasmids	
p10xUAS-WHaloCaMP1a-EGFP	Drosophila expression with GAL4 driver
pCAG-WHaloCaMP1a-EGFP	Mammalian expression with CAG promoter
pTol2-elavl3-WHaloCaMP1a	zebrafish expression with elavl3 promoter (pan-neuronal expression)
pTol2-elavl3-WHaloCaMP1a-EGFP	zebrafish expression with elavl3 promoter (pan-neuronal expression)
pAAV-CaMKII-WHaloCaMP1a	AAV particles; CaMKII promoter
pAAV-CaMKII-WHaloCaMP1a-EGFP	AAV particles; CaMKII promoter
pAAV-synapsin-WHaloCaMP1a	AAV particles; synapsin promoter
pAAV-synapsin-WHaloCaMP1a-EGFP	AAV particles; synapsin promoter
pRSET-WHaloCaMP-eNOSpep-EGFP	E. coli expression
pRSET-WHaloCaMP1b-EGFP	E. coli expression
pTol2-elavl3-WHaloCaMP1a	E. coli expression
pTol2-elavl3-WHaloCaMP1a-EGFP	E. coli expression

Reference:

Farrants, H., *et al*. A modular chemigenetic calcium indicator for multiplexed *in vivo* functional imaging (2024). *Nat Methods*. https://doi.org/10.1038/s41592-024-02411-6

Materials and US Patent Application 18/318,997 are available for license.

Tech ID: 2022-012

iGlucoSnFR2 Genetically Encoded Glucose Sensor

iGlucoSnFR2 enables high-resolution glucose imaging using a circularly permuted GFP-based design optimized for cell biology and physiology research. It detects real-time glucose changes with high sensitivity and can be expressed in diverse model systems.

Key Benefits

- High Specificity: Strong selectivity for glucose over other sugars
- Large Fluorescence Changes: Detects dynamic shifts in glucose concentration (ΔF/F up to 2.3)
- Tunable Affinity: Sensors span from 1 µM to 10 mM K_d to match physiological ranges
- Versatile Formats: Available for cytosolic, surface, and secreted expression
- Ratiometric Capabilities: Co-expression with red fluorescent proteins allows motion correction

Applications

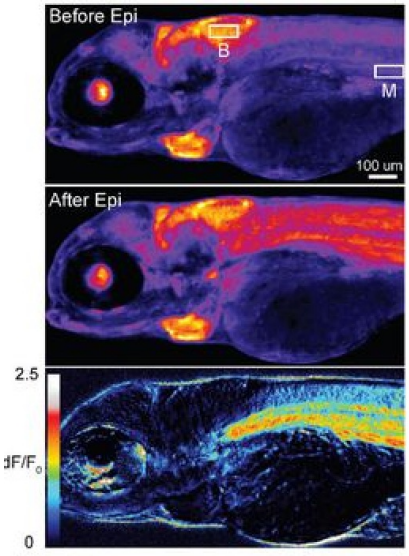
- Real-time metabolism imaging in neurons and astrocytes
- Hormonal response profiling (e.g., insulin, epinephrine)
- Transporter activity assays (e.g., Glut1 inhibition by cytochalasin B)
- Multiplexed studies with calcium or voltage indicators

Plasmids:

- pAAV.GFAP.(Cyto)iGlucoSnFR.mRuby2
  - Green glucose sensor with mRuby2 fusion, cytosolic
- pAAV.GFAP.(Cyto)iGlucoSnFR
  - Green glucose sensor, cytosolic
- pAAV.hSynap.(cyto)iGlucoSnFR-mRuby2
  - Green glucose sensor with mRuby2 fusion, cytosolic

Performance Snapshot

Variant	K_d (ATP)	ΔF/F	Applications
H66A/H348A	~2.3 mM	2.3×	Cytoplasmic tracking *in vitro*
L276V	~7.7 mM	~2.0×	Muscle and brain glucose
Additional variants	1 µM–10 mM	Tunable	Organelles, extracellular monitoring



Imaging organism-scale epinephrine responses. 5 dpf fish expressing both iGlucoSnFR (all cells, β-actin promoter) and jRGECO1a (muscle cells, α-actinin promoter). (Keller, J., et al.)

Reference:

Keller, J., *et al*. *In vivo* glucose imaging in multiple model organisms with an engineered single-wavelength sensor (2019). bioRxiv. https://doi.org/10.1101/571422

Materials and US Patents 11,162,942, 11,698,374, and 12,203,932 are available for license.

Tech IDs: 2021-027

iGluSnFR3 Genetically Encoded Glutamate Sensor

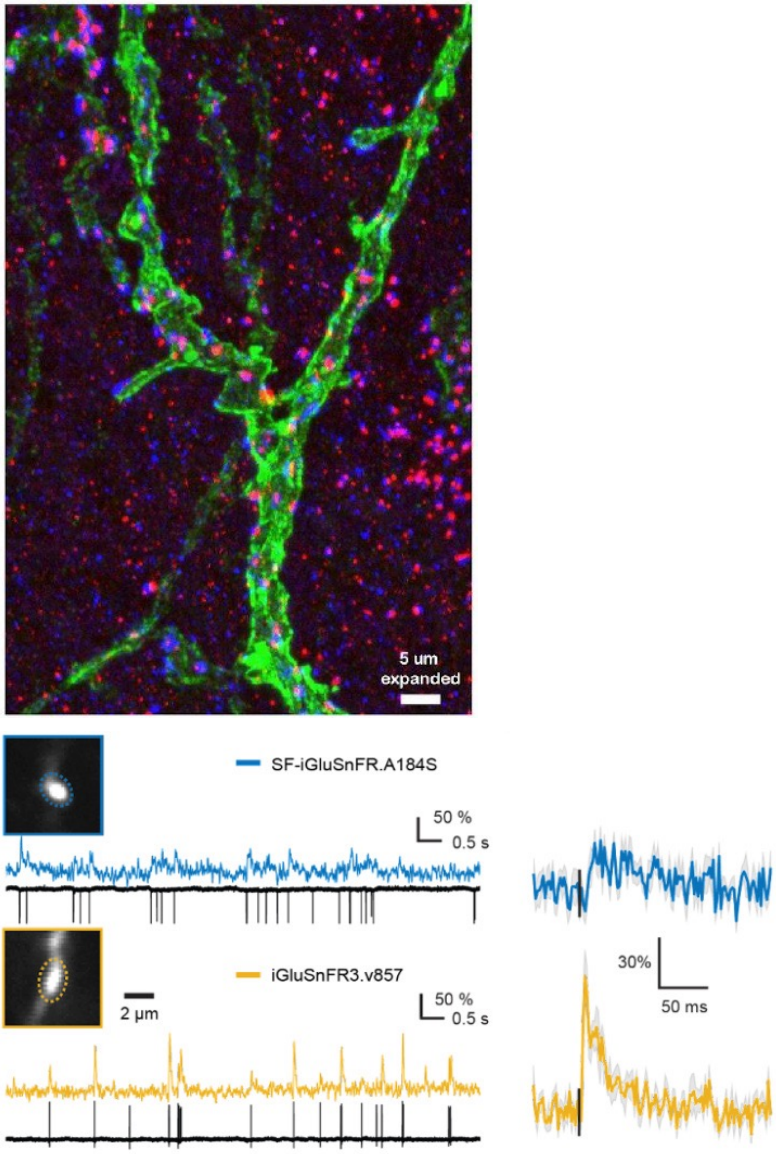
iGluSnFR3 is the highest-performing Glutamate-Sensing Fluorescent Reporter developed to image the most abundant neurotransmitter in the mammal brain with the greatest performance to date.

Plasmids:

- pAAV.hSyn.iGluSnFR3.v857.IgK-NGR
- pAAV.hSyn.FLEX.iGluSnFR3.v857.IgK-NGR
- pAAV.hSyn.FLP-FRT.iGluSnFR3.v857.SGZ
- pAAV.hSyn.FLP-FRT.iGluSnFR3.v857.GPI
- pAAV.CAG.FLEX.iGluSnFR3.v857.PDGFR
- pAAV.CAG.FLEX.iGluSnFR3.v857.GPI
- pAAV.GFAP.iGluSnFR3.v857.GPI
- pAAV.GFAP.iGluSnFR3.v857.SGZ
- pAAV.GFAP.iGluSnFR3.v857.PDGFR
- pAAV.CAG.iGluSnFR3.v857.GPI
- pAAV.CAG.iGluSnFR3.v857.SGZ
- pAAV.CAG.iGluSnFR3.v857.PDGFR
- pAAV.hSyn.iGluSnFR3.v857.GPI
- pAAV.hSyn.iGluSnFR3.v857.SGZ
- pAAV.hSyn.iGluSnFR3.v857.PDGFR
- pRSET iGluSnFR3 v82
- pRSET iGluSnFR3 v857
- pAAV.hSyn.FLEX.iGluSnFR3.v82.GPI.codonopt
- pAAV.hSyn.FLEX.iGluSnFR3.v857.SGZ.codonopt
- pAAV.hSyn.FLEX.iGluSnFR3.v857.GPI.codonopt
- pAAV.hSyn.FLEX.iGluSnFR3.v857.PDGFR.codonopt

Reference:

Aggarwal, A., Liu, R., Chen, Y., *et al*. Glutamate indicators with improved activation kinetics and localization for imaging synaptic transmission (2023). *Nat Methods*. https://doi.org/10.1038/s41592-023-01863-6



2P imaging of individual boutons and simultaneous cell-attached recordings from representative neurons labeled with SF-iGluSnFR.A184S (top, 'A184S') and v857 (bottom). And spike-triggered averages for isolated APs measured with v857 (bottom) and A184S (top). (Aggarwal et al.*)*

Materials and US Patent 10,060,920 are available for license.

Tech ID: 2021-017

iGluSnFR4 Genetically Encoded Glutamate Sensors

Ultra-sensitive, Synapse-resolved Glutamate Indicators with Tunable Kinetics

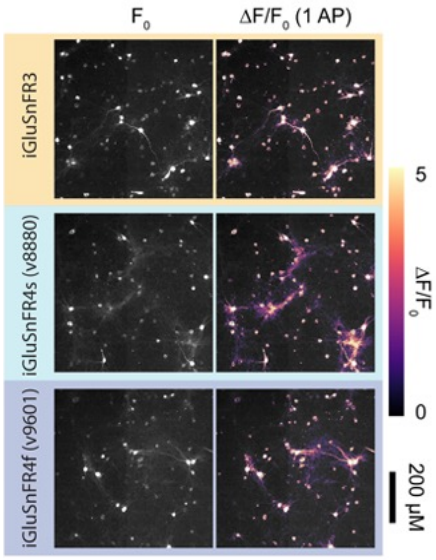
iGluSnFR4 is a genetically encoded glutamate sensor developed through high-throughput, structure-guided mutagenesis of the iGluSnFR3 scaffold. Two new variants—iGluSnFR4f (fast decay) and iGluSnFR4s (slow decay)—deliver significantly improved sensitivity, brightness, and temporal control for detecting glutamate release at single-synapse resolution *in vivo*. These tools address key limitations in tracking synaptic input dynamics, enabling simultaneous imaging of large synapse populations and rapid neurotransmitter release.

What's New in iGluSnFR4 vs iGluSnFR3?

- **Higher sensitivity:** Up to 4.7× higher signal-to-noise (SNR) for single vesicle events
- **Faster activation:** Both 4f and 4s respond in <2 ms
- **Tunable decay:**
  - iGluSnFR4f: 26 ms decay for rapid synaptic dynamics
  - iGluSnFR4s: 153 ms decay for integration and fiber photometry
- **Improved photostability and detectability**
- **Greater spatial resolution:** Reduced crosstalk and sharper dendritic spine localization
- **Better performance under standard (30 Hz) video-rate two-photon imaging**

Applications

- **Synapse-specific recording** in cortex, hippocampus, and thalamus
- **Fast glutamate release detection** during whisking and visual processing
- **Fiber photometry** in deep brain regions (e.g., VTA)
- **Functional connectomics** and mapping synaptic input diversity



Images of cultures expressing iGluSnFR3, iGluSnFR4s, and iGluSnFR4f at baseline (F_0, left), and peak fluorescence change ($\Delta F/F_0$, right) following a single field stimulus. (Aggarwal, A., et al.*)*

Performance Highlights

Variant	$\Delta F/F_0$	Decay (ms)	Best For
iGluSnFR4f	↑↑	26	Fast synaptic dynamics
iGluSnFR4s	↑↑	153	Population activity, photometry
iGluSnFR3	Base	29	Previous gold standard

Continued

Plasmids

- pAAV-syn-FLP-FRT-iGluSnFR4f-NGR-WPRE
- pAAV-syn-FLP-FRT-iGluSnFR4s-NGR-WPRE
- pAAV-CAG-iGluSnFR4f-NGR-WPRE
- pAAV-CAG-iGluSnFR4s-NGR-WPRE
- pAAV-CAG-iGluSnFR4f-PDGFR-WPRE
- pAAV-CAG-iGluSnFR4s-PDGFR-WPRE
- pAAV-CAG-flex-iGluSnFR4f-PDGFR-WPRE
- pAAV-CAG-flex-iGluSnFR4s-PDGFR-WPRE
- pAAV-GFAP-iGluSnFR4f-PDGFR-WPRE
- pAAV-GFAP-iGluSnFR4s-PDGFR-WPRE
- pRSET-iGluSnFR4f
- pRSET-iGluSnFR4s
- pAAV-syn-flex-iGluSnFR4f-NGR-WPRE
- pAAV-syn-flex-iGluSnFR4s-NGR-WPRE
- pAAV-syn-flex-iGluSnFR4f-PDGFR-WPRE
- pAAV-syn-flex-iGluSnFR4s-PDGFR-WPRE
- pAAV-syn-iGluSnFR4f-NGR-WPRE
- pAAV-syn-iGluSnFR4s-NGR-WPRE
- pAAV-syn-iGluSnFR4f-PDGFR-WPRE
- pAAV-syn-iGluSnFR4s-PDGFR-WPRE

Reference:

Aggarwal, A., *et al*., Glutamate indicators with increased sensitivity and tailored deactivation rates. (2025) bioRxiv. http://doi.org/10.1101/2025.03.20.643984

Materials and US Patent 10,060,920 are available for license.

Tech ID: 2021-017

iGABASnFR2 Genetically Encoded GABA Sensor

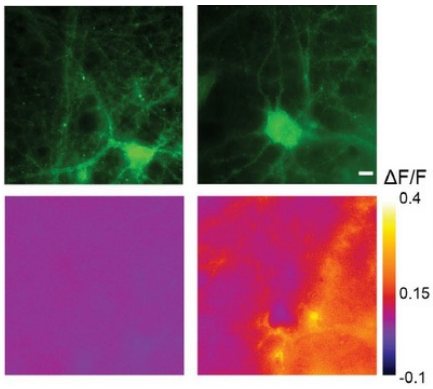
iGABASnFR2 is the current generation of GABA-Sensing Fluorescent Reporters. Other sensor technologies, such as iGluSnFR3, jGCaMP8, and jRGECO, are optimized for excitatory synaptic transmission and action potentials. iGABASnFr2 complements these technologies with the ability to detect inhibitory synaptic transmission and inhibitory post-synaptic currents.

- Positive-going version (iGABASnFR2) and negative-going version (iGABASnFR2n)
- iGABASnFR2: 4x higher sensitivity to APs than iGABASnFR, iGABASnFR2n: 2x higher sensitivity to APs than iGABASnFR.
- Both enable single-AP high-speed imaging
- Enables detection of signal in individual dendrites and synaptic boutons
- Higher affinity for GABA (iGABASnFR2: EC50: 6.4±0.2 µM, ~7x tighter than iGABASnFR, ΔF/ Fmax=0.5±0.05, ~2x higher than iGABASnFR)

References:

Kolb, I., *et al*. Optimization of genetically encoded GABA indicator (2022). Janelia Research Campus. https://doi.org/10.25378/ janelia.19709311.v3

Kolb, I., *et al*. iGABASnFR2: Improved genetically encoded protein sensors of GABA (2025). BioRxiv. https://doi.org/10.1101/2025.03.25.644953



High magnification imaging of iGABASnFR dynamics in response to 5 AP stimulation.

Plasmids:

Name	Expression	Change
pGP-RSET-iGABASnFR2(no bind)-6xHis	Bacterial	None
pGP-RSET-iGABASnFR2n-6xHis	Bacterial	Negative
pGP-RSET-iGABASnFR2-6xHis	Bacterial	Positive
pGP-AAV-CAG-iGABASnFR2n-WPRE	AAV-mediated	Negative
pGP-AAV-CAG-iGABASnFR2-WPRE	AAV-mediated	Positive
pGP-AAV-CAG-flex-iGABASnFR2(no bind)-WPRE	AAV-mediated	None
pGP-AAV-CAG-flex-iGABASnFR2n-WPRE	AAV-mediated	Negative
pGP-AAV-CAG-flex-iGABASnFR2-WPRE	AAV-mediated	Positive
pGP-AAV-syn-flex-iGABASnFR2(no bind)-WPRE	AAV-mediated	None
pGP-AAV-syn-flex-iGABASnFR2n-WPRE	AAV-mediated	Negative
pGP-AAV-syn-flex-iGABASnFR2-WPRE	AAV-mediated	Positive
pGP-AAV-syn-iGABASnFR2(no bind)-WPRE	AAV-mediated	None
pGP-AAV-syn-iGABASnFR2n-WPRE	AAV-mediated	Negative
pGP-AAV-syn-iGABASnFR2-WPRE	AAV-mediated	Positive
pGP-AAV-GFAP-iGABASnFR2(no bind)-WPRE	AAV-mediated	None
pGP-AAV-GFAP-iGABASnFR2n-WPRE	AAV-mediated	Negative
pGP-AAV-GFAP-iGABASnFR2-WPRE	AAV-mediated	Positive
pGP-CAG-iGABASnFR2(no bind)-WPRE-bGH-polyA	Mammalian	None
pGP-CAG-iGABASnFR2n-WPRE-bGH-polyA	Mammalian	Negative
pGP-CAG-iGABASnFR2-WPRE-bGH-polyA	Mammalian	Positive

Materials and US Patent 11,698,374 are available for license.

Tech ID: 2021-020

iAchSnFR Genetically Encoded Acetylcholine Sensor

A fast genetically encoded fluorescent sensor for faithful *in vivo* acetylcholine detection in mice, fish, worms, and flies

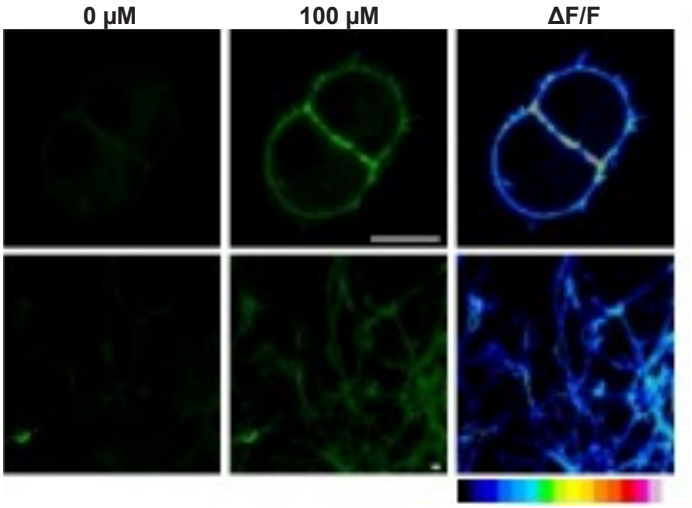
- Acetylcholine receptors are among the most diverse neurotransmitter receptor families
- Large fluorescence changes (up to 12-fold)
- Millisecond-level rapid rise and decay kinetics
- Insensitivity to most cholinergic drugs
- Available in green (default, GFP-based) and yellow (Venus) versions
- Optional red fluorescent protein tag (mRuby2) for normalization

Plasmids:

- pAAV-hSynap-iAChSnFR
- pAAV-hSynap-iAChSnFR-NULL
- pAAV-hSynap-iAChSnFR-Venus
- pAAV-hSynap-iAChSnFR-Venus-NULL
- pAAV-hSynap-iAChSnFR-mRuby2
- pAAV-CAG-iAChSnFR
- pAAV-CAG-iAChSnFR-NULL
- pAAV-CAG-FLEX-iAChSnFR
- pAAV-CAG-FLEX-iAChSnFR-NULL
- pAAV-CAG-FLEX-iAChSnFR-Venus
- pAAV-CAG-FLEX-iAChSnFR-Venus-NULL
- pAAV-CAG-FLEX-iAChSnFR-mRuby2
- pAAV-ZAC-GFAP-iAChSnFR
- pAAV-ZAC-GFAP-iAChSnFR-NULL
- pAAV-ZAC-GFAP-iAChSnFR-mRuby2

Reference:

Borden, P.M., *et al*. A fast genetically encoded fluorescent sensor for faithful in vivo acetylcholine detection in mice, fish, worms, and flies (2020). bioRxiv. https://doi.org/10.1101/2020.02.07.939504



Representative images of HEK293 cells and hippocampal neuron cultures in buffer alone, with 100 µM acetylcholine, and transformation of ΔF/F as a heat map. (Borden et al.*)*

Materials and IP including US Patent 11,698,374 are available for license.

Tech ID: 2021-024

iATPSnFR2 High-Performance Genetically Encoded Sensor for Intracellular ATP

iATPSnFR2 is a circularly permuted GFP-based ATP sensor engineered for rapid, high-sensitivity detection of ATP levels in living cells. With ratiometric readout options and subcellular targeting flexibility, it's ideal for neuroscience, cell biology, and metabolism research, and drug screening.

- High Signal: ΔF/F ~12, optimized for live-cell imaging
- Tunable Affinity: Three variants have ATP K_d values of 4 μM, 16 μM, and 500 μM
- Optional Ratiometric Output: HaloTag, mIRFP, and mCherry fusion options for normalization
- Fast Response: Fluorescence changes complete in <2 sec
- Subcellular Precision: Reveals ATP consumption in cytosol, mitochondria, or single synaptic boutons
- Compatible with JFX650 and other HaloTag ligands

Applications

- ATP tracking during neuronal firing
- Mitochondrial metabolism imaging
- Synaptic energy profiling
- Metabolic stress assays (e.g., 2dOG, oligomycin)

Plasmids:

- Bacterial expression / *in vitro* purification
  - pNoRBP.iATPSnFR2
  - pNoRBP.iATPSnFR2.HaloTag
  - pNoRBP.iATPSnFR2.mIRFP
- Cytosolic expression in neurons
  - pAAV.hSynapsin.(cyto).iATPSnFR2.HaloTag
  - pAAV.hSynapsin.(cyto).iATPSnFR2.mIRFP-670nano3
  - pAAV.hSynapsin.(cyto).iATPSnFR2.mCherry

Continued

Performance Snapshot

Variant	K_d (ATP)	ΔF/F	Application Target
A95A.A119L	530 μM	~12	General cellular ATP observation
A95K	16 μM	~11	Low [ATP] systems
S29W.A95K	4 μM	~12	Low [ATP] compartments, *in vitro* studies

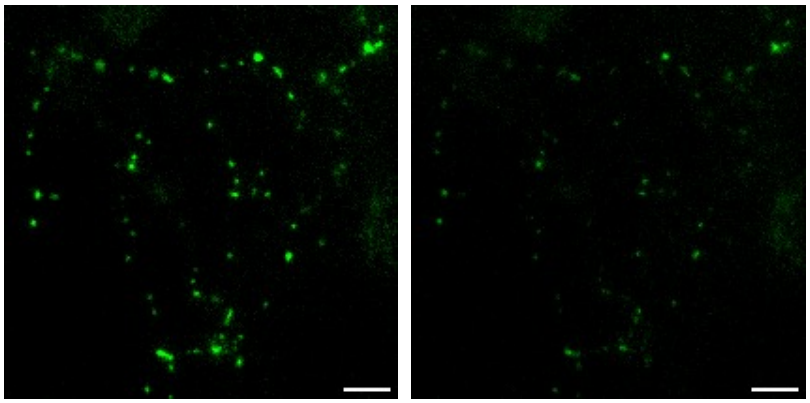


Figure 1. iATPSnFR2.HaloTag-JFX650 before and after inhibition of glycolysis in cultured neurons.

- Cytosolic expression in glial cells
  - pAAV.GfaABC1D.(cyto).iATPSnFR2.HaloTag
  - pAAV. GfaABC1D.(cyto).iATPSnFR2.mIRFP-670nano3
  - pAAV. GfaABC1D.(cyto).iATPSnFR2.mCherry
- Cytosolic expression in most mammalian cells
  - pAAV.CAG.(cyto).iATPSnFR2.HaloTag
  - pAAV.CAG.(cyto).iATPSnFR2.mIRFP670nano3
  - pAAV.CAG.(cyto).iATPSnFR2.mCherry
- Cytosolic expression in mammalian cells with Cre recombinase dependence
  - pAAV.CAGFLEX.(cyto).iATPSnFR2.HaloTag
  - pAAV.CAGFLEX.(cyto).iATPSnFR2.mIRFP-670nano3
  - pAAV.CAGFLEX.(cyto).iATPSnFR2.mCherry
- Mitochondrial expression in neurons
  - pAAV.hSynapsin.(mito).iATPSnFR2.HaloTag
  - pAAV.hSynapsin.(mito).iATPSnFR2.mIRFP-670nano3
  - pAAV.hSynapsin.(mito).iATPSnFR2.mCherry
- Mitochondrial expression in glial cells
  - pAAV.GfaABC1D.(mito).iATPSnFR2.HaloTag
  - pAAV. GfaABC1D.(mito).iATPSnFR2.mIRFP-670nano3
  - pAAV. GfaABC1D.(mito).iATPSnFR2.mCherry
- Mitochondrial expression in most mammalian cells
  - pAAV.CAG.(mito).iATPSnFR2.HaloTag
  - pAAV.CAG.(mito).iATPSnFR2.mIRFP670nano3
  - pAAV.CAG.(mito).iATPSnFR2.mCherry
- Mitochondrial expression in mammalian cells with Cre recombinase dependence
  - pAAV.CAGFLEX.(mito).iATPSnFR2.HaloTag
  - pAAV.CAGFLEX.(mito).iATPSnFR2.mIRFP670nano3
  - pAAV.CAGFLEX.(mito).iATPSnFR2.mCherry
- Synaptic expression in neurons [on synaptosomes, facing cytosol]
  - pAAV.hSynapsin.(synapto).iATPSnFR2.HaloTag
  - pAAV.hSynapsin.(synapto).iATPSnFR2.mIRFP670nano3
  - pAAV.hSynapsin.(synapto).iATPSnFR2.mCherry

Reference:

Marvin, J., *et al*. iATPSnFR2: A high-dynamic-range fluorescent sensor for monitoring intracellular ATP (2024). PNAS. https://doi.org/10.1073/pnas.2314604121

Materials and US Patent Application 18/647,657 are available for license.

Tech ID: 2023-007

CASFISH DNA labeling with CRISPR/Cas9

CASFISH is a novel method to rapidly detect DNA sequences more sensitively than standard FISH and without denaturation, and the optimized plasmid to implement the method easily. CRISPR with inactivated Cas9 protein detects endogenous DNA sequences in fixed and unfixed material. Researchers can use the CASFISH method to detect short DNA sequences in cells *in situ* through a designed complex of Cas9 protein modified to only bind DNA with single-guide RNA. It can be labeled with any means: fluorescent proteins, dyes, quantum dots, gold particles, or other analytes consistent with microscopy or other appropriate detection methods. CASFISH provides researchers with simpler, more efficient, and robust *in situ* imaging of cellular DNA.

- Provides detection and visualization of cellular DNA for gene expression research
- Can be used directly on tissues for the rapid diagnosis of disease
- Rapidly detects DNA sequences more sensitively and without denaturation
- Reduces both the deleterious effects and inconvenience of sample preparation
- Does not require the use of chemicals, heat, or fixation

Plasmids:

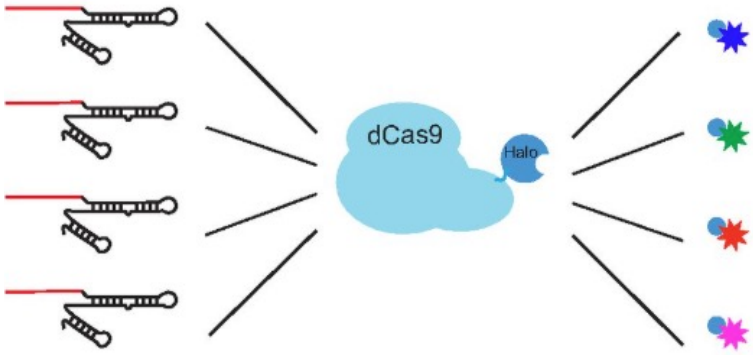
- pET302-6His-dCas9-Halo

Reference:

Deng, W., *et al.* CASFISH: CRISPR/Cas9-mediated *in situ* labeling of genomic loci in fixed cells (2015). PNAS. http://doi.org/10.1073/pnas.1515692112

Materials and IP including US 11,174,506 and US 11,174,507, EP3207131, CN107208086 are available for license.

Tech ID: 2014-041



CASFISH Imaging Strategy

FLInChR Imaging Strategy

Optical inhibitor of neuronal activity in Drosophila

FLInChR is an engineered variant of a light-gated opsin, channelrhodopsin, that is a potent optical inhibitor of neuronal activity. It is useful for optogenetic inactivation of neurons, or any mammalian cell, expressing the channels both *in vitro* and *in vivo*.

Lasers achieve photo stimulation of FLInChR *in vitro* and *in vivo*, as described in Brown *et al.*

Plasmids:

FLInChR plasmid has the fusion signal sequence and transmembrane domain of Neurexin 1B-delta to ChR2E123T/T159C for inversion ChR2 to an optogenetic inhibitor.

• AAV-CAG-FLInChR-mVenus

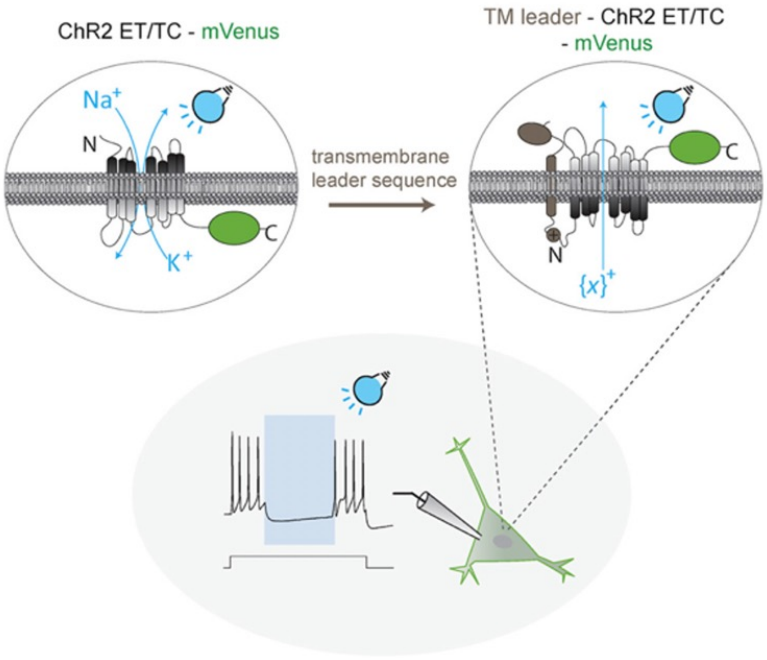
Reference:

Brown, J., *et al.* Expanding the Optogenetics Toolkit by Topological Inversion of Rhodopsins (2018). *Cell*. http://doi.org/10.1016/j.cell.2018.09.026

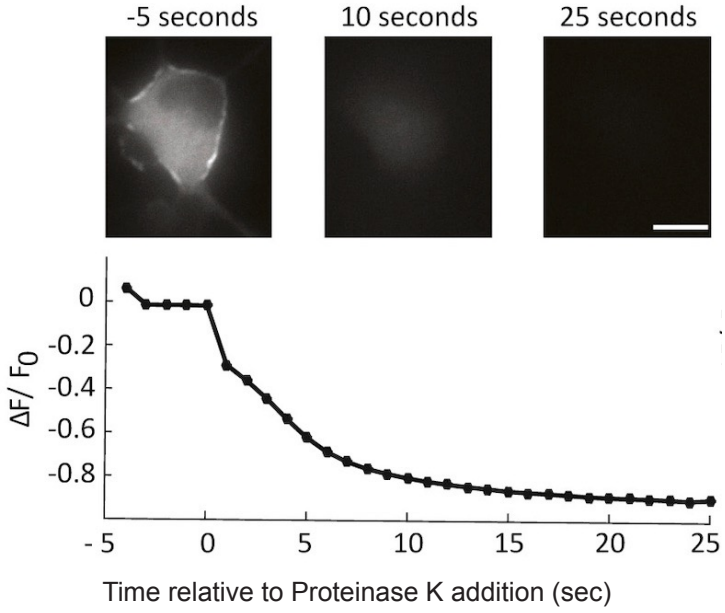
Material and US Patent 12,162,921 are available for license.

Tech ID: 2018-022

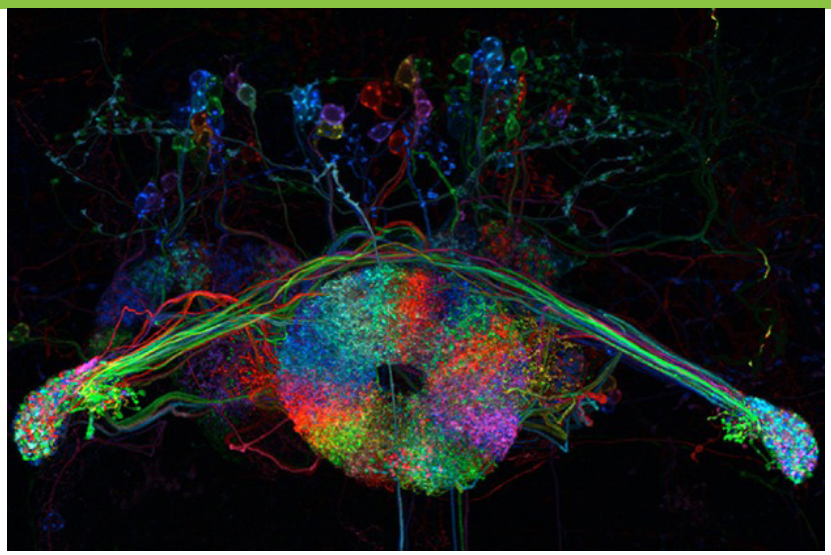
Novel opsin engineering through topological inversion

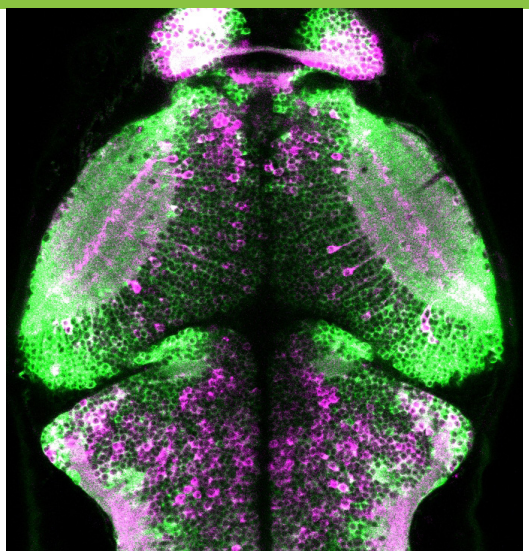


FLInChR response



About Janelia Research Campus





The Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia, is an innovative research center that advances scientific fields by breaking through technical and intellectual barriers.

Janelia's integrated teams of lab scientists and tool-builders pursue a focused set of scientific questions with potential for transformative impact. The campus operates in broad research areas that incorporate diverse expertise across science and engineering, including Mechanistic Cognitive Neuroscience, 4D Cellular Physiology, and the AI@HHMI initiative.

Since opening in 2006, Janelia scientists have made numerous biological advances, including foundational analysis of the neural connections and computations underlying behavior. The campus has distributed thousands of research tools to laboratories worldwide, including Janelia Fluor® Dyes, GCaMP calcium sensors, and GAL4 driver lines. This widespread tool sharing demonstrates Janelia's commitment to open science and collaborative research.

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