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Research Campus

Janelia Fluor® Family of Dyes

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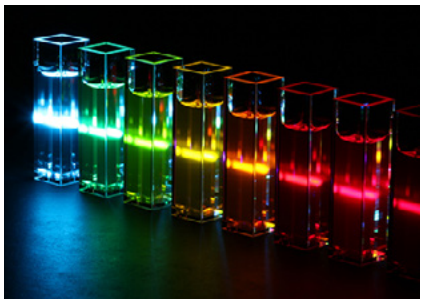
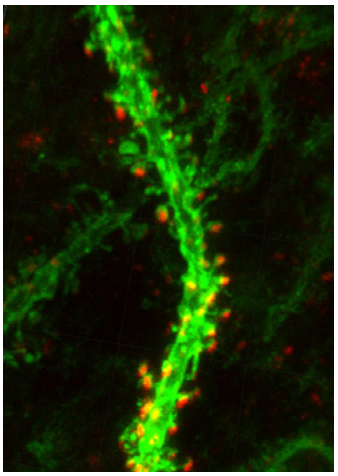
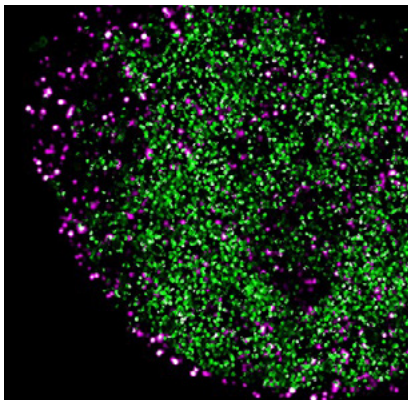






Janelia Fluor® Dyes Bright and Cell-Permeant Small-Molecule Fluorophores

Overview

Janelia Fluor® dyes revolutionize chemical imaging by combining **superior brightness, photostability, and cell permeability** into a versatile, easy-to-use fluorophore platform. Developed by simple azetidine substitution of classic dye structures, these labels are optimized for advanced microscopy techniques.

Key Advantages

- **High Quantum Yield and Brightness** Up to 2× brighter than traditional rhodamine dyes like TMR and Cy3.
- **Photostable for Extended Imaging** Longer fluorescence lifetimes enable more robust live-cell and single-molecule imaging.
- **Maintained Permeability and Size** Minimal structural changes retain excellent intracellular labeling properties.
- **Broad Wavelength Range** Covers UV to far-red; compatible with multiplexed experiments.

Spotlight Dyes

- **JF_{549}:** Bright red-orange dye for HaloTag/SNAP-tag labeling and super-resolution microscopy.
- **JF_{646}:** Far-red fluorophore ideal for deep imaging and minimal background fluorescence.

Applications

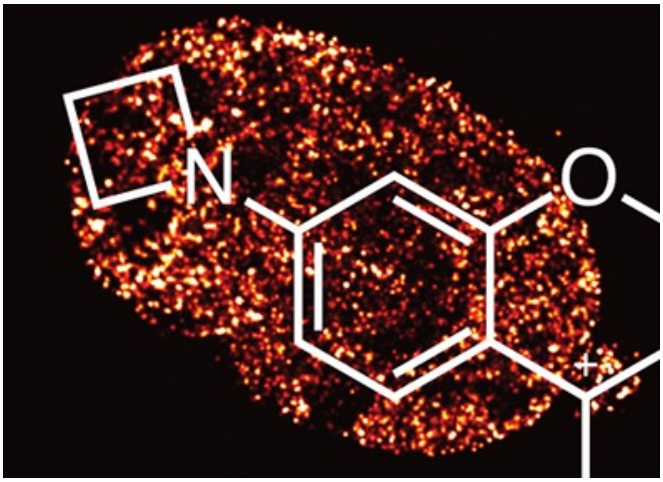
- Super-resolution imaging (*d*STORM, STORM)
- Single-molecule tracking in live cells
- Multiplexed imaging workflows
- Confocal, wide-field, and two-photon microscopy

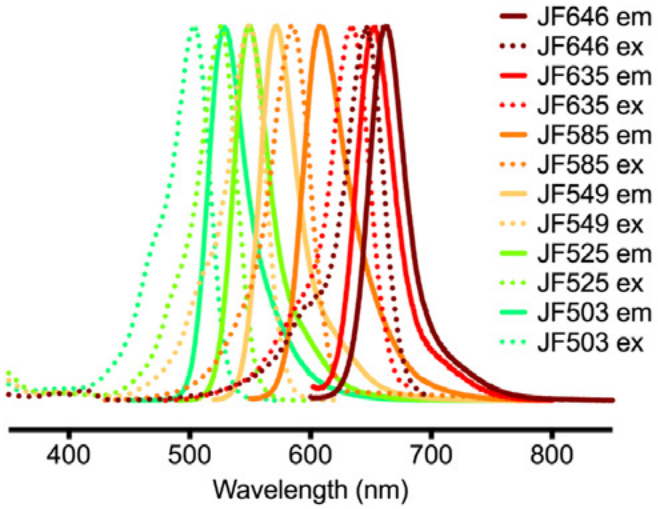
Reference: *Nat Meth.* (2015). https://doi.org/10.1038/nmeth.3256

IP Coverage: Certificate of Trademark Registration, Serial Number 86386733

U.S. Patents 9,933,417, 10,018,624, 10,161,932 and 10,495,632, JP6606096, CN106471067, AU2015240774, DE/FR/GB3126451, CA2944476

Janelia Tech ID: 2014-013





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JF_{479}

- $\lambda_{abs}/\lambda_{em}$: 479 nm/517 nm
- ε: 47,900 M^{-1} cm^{-1}
- Φ: 0.62
- *Structure:* NCH_3-containing rhodamine with 3,3-difluoroazetidine groups
- $K_{L\text{-}Z}$: 2.88

JF_{479} is a blue-green emitting fluorophore with spectral properties similar to GFP. It was derived from JF_{502} through 3,3-difluoroazetidine substitution, which decreased the $K_{L\text{-}Z}$ value from 4.33 to 2.88. The JF_{479}-HaloTag ligand has been synthesized and shows excellent labeling of nuclear and membrane proteins in cellular imaging experiments. Its short absorption wavelength allows for effective separation from green-excited dyes like JF_{525} in two-color imaging experiments, unlike the slightly longer wavelength JF_{503}. When bound to HaloTag, JF_{479} exhibits spectral properties nearly identical to enhanced GFP, making it an ideal small-molecule replacement for GFP in multicolor imaging.

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JF_{503}

- $\lambda_{abs}/\lambda_{em}$: 503 nm/529 nm
- ε: 83,000 M^{-1} cm^{-1}
- Φ: 0.87
- *Structure:* Rhodol with 3,3-difluoroazetidine group
- $K_{L\text{-}Z}$: Not reported directly, but higher than JF_{479}

JF_{503} is a rhodol-based fluorophore that was created by replacing an azetidine substituent in JF_{519} with a 3,3-difluoroazetidine group, resulting in a ~15-nm blue-shift to better align with 488 nm excitation. The JF_{503}-HaloTag ligand has been synthesized and shows bright labeling of nuclear proteins in live cells. While it allows for efficient cellular labeling with loading kinetics similar to other 488-nm excited dyes, it has higher photostability compared to rhodamine 110 and trifluoroethylrhodamine derivatives. The JF_{503}-SNAP-tag ligand (20) has also been developed, enabling multicolor imaging applications. However, its longer absorption wavelength causes some spillover into the 532 nm excitation channel, making spectral separation challenging in certain multicolor applications compared to JF_{479}.

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JF_{525}

- $\lambda_{abs}/\lambda_{em}$: 525 nm/549 nm
- ε: 94,000 M^{-1} cm^{-1}
- Φ: 0.91
- *Structure:* Rhodamine with 3,3-difluoroazetidine groups
- $K_{L\text{-}Z}$: 0.068

JF_{525} was created by incorporating 3,3-difluoroazetidines into the JF_{549} scaffold, which decreased the $K_{L\text{-}Z}$ value from 3.5 to 0.068 and elicited a ~25 nm hypsochromic shift. This tuning strategy makes JF_{525} better suited for excitation with 514-532 nm light. The JF_{525}-HaloTag ligand shows improved cell permeability compared to JF_{549} derivatives, allowing faster intracellular labeling, and is blood-brain barrier permeant, making it useful for *in vivo* voltage imaging with Voltron. The JF_{525}-SNAP-tag ligand has also been developed for intracellular labeling, providing the first cell-permeable self-labeling tag ligand with excitation maximum near 532 nm. These properties make JF_{525} derivatives valuable for multicolor imaging applications where spectral separation is critical.

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JF_{526}

- $\lambda_{abs}/\lambda_{em}$: 526 nm/550 nm
- ε: 19,000 M^{-1} cm^{-1}
- Φ: 0.87
- *Structure:* Fluorinated rhodamine with 3,3-difluoroazetidine groups
- $K_{L\text{-}Z}$: 0.005

JF_{526} was created by combining two complementary strategies: direct fluorination of the xanthene system of JF_{552} and incorporation of 3,3-difluoroazetidines. This dual modification dramatically decreased the $K_{L\text{-}Z}$ value from 0.70 (JF_{552}) to 0.005, creating a highly fluorogenic dye. The low $K_{L\text{-}Z}$ value makes JF_{526} derivatives useful for no-wash cellular imaging applications where background fluorescence needs to be minimized. The JF_{526}-HaloTag ligand has been synthesized and can be used as a fluorogenic label. JF_{526} presents an example of how multiple tuning strategies can be combined to achieve specific photophysical properties for specialized imaging applications.

Janelia Fluor®/Blinking Janelia Fluor® 2014-013/2019-054

Continued

JF_{549}

- $\lambda_{abs}/\lambda_{em}$: 549 nm/571 nm
- ε: 101,000 M^{-1} cm^{-1}
- Φ: 0.88
- *Structure:* Rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 3.47

JF_{549} was the first member of the Janelia Fluor® dye series, created by incorporating four-membered azetidine rings into classic rhodamine structures. This modification substantially increased the quantum yield compared to tetramethylrhodamine (TMR). The JF_{549}-HaloTag ligand has become a standard for single-molecule imaging experiments due to its brightness and photostability. The JF_{549}-SNAP-tag ligand has also been synthesized, though it shows higher nonspecific staining compared to the fluorinated JF_{552}-SNAP-tag ligand. A photoactivatable version (PA-JF_{549}) has been developed for single-molecule localization microscopy and single-particle tracking applications, showing improved performance over fluorescent proteins like mEos3.2. JF_{549} serves as the foundation for many subsequent Janelia Fluor® dyes and continues to be widely used in cellular imaging applications.

Janelia Fluor® 2014-013

JF_{552}

- $\lambda_{abs}/\lambda_{em}$: 552 nm/575 nm
- ε: 95,000 M^{-1} cm^{-1}
- Φ: 0.83
- *Structure:* Fluorinated rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 0.70

JF_{552} was created through direct fluorination on the xanthene system of rhodamine dyes, which decreased the $K_{L\text{-}Z}$ value of JF_{549} from 3.5 to 0.70. This modification improves cell permeability compared to JF_{549} derivatives. The JF_{552}-HaloTag ligand shows better cell permeability than the JF_{549} analog, making it useful for live-cell imaging applications. The JF_{552}-SNAP-tag ligand exhibits lower nonspecific staining and faster live-cell labeling compared to the JF_{549}-SNAP-tag ligand, while maintaining comparable brightness and photostability in single-particle tracking experiments. These properties make JF_{552} derivatives particularly valuable for applications requiring high signal-to-background ratios in living cells.

Janelia Fluor® 2014-013

JF_{570}

- $\lambda_{abs}/\lambda_{em}$: 570 nm/593 nm
- ε: 83,600 M^{-1} cm^{-1}
- Φ: 0.63
- *Structure:* Sulfur-containing rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 2.24

JF_{570} is a sulfur-containing rhodamine derivative with azetidine groups, showing spectral properties red-shifted compared to JF_{549}. The JF_{570}-HaloTag ligand has been developed and shows less photostability compared to the fluorinated JF_{593}-HaloTag ligand in cellular imaging experiments. The sulfur bridge in the xanthene system causes a bathochromic shift in absorption and emission compared to the oxygen-containing JF_{549}.

Janelia Fluor® 2014-013

JF_{585}

- $\lambda_{abs}/\lambda_{em}$: 585 nm/609 nm
- ε: 1,500 M^{-1} cm^{-1} (free dye), 156,000 M^{-1} cm^{-1} (maximally absorbing form)
- Φ: 0.78
- *Structure:* Carborhodamine with 3,3-difluoroazetidine groups
- $K_{L\text{-}Z}$: <0.0001 (highly fluorogenic)

JF_{585} was developed by incorporating 3,3-difluoroazetidines into the JF_{608} scaffold, resulting in a blue-shift in spectra and a dramatic decrease in $K_{L\text{-}Z}$ to <0.0001, creating a highly fluorogenic dye. The JF_{585}-HaloTag ligand shows remarkable fluorogenicity with an 80-fold increase in absorption upon binding to HaloTag protein. This property makes it excellent for no-wash cellular imaging with high contrast.
The JF_{585}-SNAP-tag ligand has also been synthesized and functions effectively as a cellular label. In multicolor imaging applications, JF_{585} can be combined with green-excited dyes like JF_{503} and far-red dyes like JF_{646} for three-color experiments without washing steps. The orange emission of JF_{585} fills an important spectral window between green and far-red fluorophores.

Janelia Fluor® 2014-013

Continued

JF_{608}

- $\lambda_{abs}/\lambda_{em}$: 608 nm/631 nm
- ε: 99,000 M^{-1} cm^{-1}
- Φ: 0.67
- *Structure:* Carborhodamine with azetidine groups
- $K_{L\text{-}Z}$: 0.091

JF_{608} is a carborhodamine derivative where the xanthene oxygen in JF_{549} is replaced with a quaternary carbon, resulting in a ~60 nm bathochromic shift in spectral properties. The JF_{608}-HaloTag ligand has been developed but shows high background staining in no-wash cellular imaging experiments due to its intermediate $K_{L\text{-}Z}$ value. This limitation was addressed in the development of the more fluorogenic JF_{585}. The carborhodamine scaffold provides access to longer wavelength fluorophores while maintaining good brightness, though with a lower $K_{L\text{-}Z}$ value compared to the parent rhodamine dyes.

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JF_{635}

- $\lambda_{abs}/\lambda_{em}$: 635 nm/652 nm
- ε: ~400 M^{-1} cm^{-1} (free dye), 167,000 M^{-1} cm^{-1} (maximally absorbing form)
- Φ: 0.56
- *Structure:* Si-rhodamine with 3-fluoroazetidine groups
- $K_{L\text{-}Z}$: <0.0001 (highly fluorogenic)

JF_{635} was developed by incorporating 3-fluoroazetidine groups into a Si-rhodamine scaffold, creating a highly fluorogenic far-red dye. The JF_{635}-HaloTag ligand shows minimal absorption in aqueous solution but exhibits a >100-fold increase upon binding to HaloTag protein. This property makes it excellent for no-wash imaging with minimal background. The red-shifted spectral properties make JF_{635} useful for multicolor imaging applications and chemigenetic indicators. It serves as a building block for hybrid small molecule-protein sensors and voltage indicators like HASAP and HArclight. The JF_{635}-SNAP-tag ligand has also been developed, making it a versatile far-red label for various protein tagging systems.

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JF_{646}

- $\lambda_{abs}/\lambda_{em}$: 646 nm/664 nm
- ε: 5,600 M^{-1} cm^{-1} (free dye), 152,000 M^{-1} cm^{-1} (maximally absorbing form)
- Φ: 0.54
- *Structure:* Si-rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 0.0014

JF_{646} is a Si-rhodamine derivative where the xanthene oxygen in JF_{549} is replaced with a dimethylsilicon moiety, resulting in a ~100 nm bathochromic shift. The JF_{646}-HaloTag ligand shows a 21-fold increase in absorbance upon binding to HaloTag protein, making it moderately fluorogenic. A photoactivatable version (PA-JF_{646}) has been developed for super-resolution microscopy applications. The deuterated analog JFX_{646} shows improved properties with higher quantum yield when protein-bound and increased photostability. JF_{646} derivatives have been widely used for far-red cellular imaging applications where minimizing phototoxicity and increasing tissue penetration are important.

Janelia Fluor® 2014-013

JFX™ Dyes Brighter and More Photostable Deuterated Fluorophores

Overview

The JFX™ dye platform enhances the performance of Janelia Fluor® dyes by introducing **deuterium** at key auxochrome positions, boosting brightness and extending photostability without altering their spectral signatures or cell permeability.

Key Advantages

- **Higher Quantum Yield and Brightness** Deuterated dyes outperform their hydrogen-containing versions, delivering stronger fluorescence signals.
- **Superior Photostability** Slower photobleaching allows longer imaging sessions and better data quality.
- **Preserved Excitation/Emission Wavelengths** Retains 560 nm and 640 nm windows for easy integration into existing imaging setups.
- **Cell-Permeable and Versatile** Compatible with HaloTag®, SNAP-tag®, and traditional chemical conjugation strategies.

Spotlight Dyes

- **JFX_{549}:** Bright, deuterated orange-red dye for 560 nm excitation.
- **JFX_{554}:** Red-shifted variant offering even greater single-molecule tracking performance.
- **JFX_{646}:** Improved far-red analog for deep tissue and multiplexed imaging.
- **JFX_{650}:** Highest-performance far-red dye for demanding super-resolution and SPT experiments.

Applications

- Single-particle tracking (SPT) and live-cell SMLM
- Intracellular imaging and super-resolution microscopy
- Long-duration confocal and two-photon fluorescence microscopy
- Analytical and diagnostic labeling technologies

Reference: *JACS Au.* (2021). https://doi.org/10.1021/jacsau.1c00006

IP Coverage: U.S. Patent Nos. 11,091,643, 11,787,946, and application 20240052169

Janelia Tech ID: 2017-043

JFX_{554}

- $\lambda_{abs}/\lambda_{em}$: 554 nm/576 nm
- ε: 114,000 M^{-1} cm^{-1}
- Φ: 0.80
- *Structure:* oxo-rhodamine with deuterated pyrrolidine groups
- $K_{L\text{-}Z}$: 4.96

JFX_{554} is a 560 nm-excited dye that contains a deuterated pyrrolidine in place of the azetidine typically found in Janelia Fluor® dyes. This structural motif further improves brightness and photostability while eliciting a slight red-shift. JFX_{554} is our brightest and most photostable dye with green-excitation in live-cell single-molecule imaging experiments.

Continued

JFX_{650}

- $\lambda_{abs}/\lambda_{em}$: 650 nm/667 nm
- ε: 17,600 M^{-1} cm^{-1}
- Φ: 0.53
- *Structure:* Silica-containing rhodamine with deuterated pyrrolidine groups
- $K_{L\text{-}Z}$: 0.0149

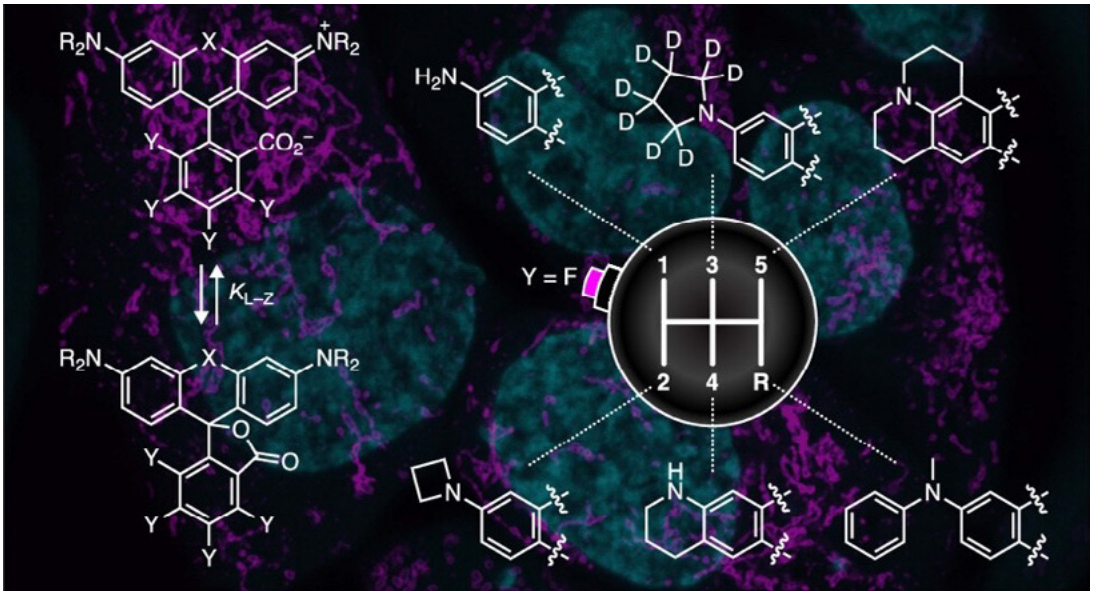
JFX_{650} is a 640 nm-excited dye that contains a deuterated pyrrolidine in place of the azetidine typically found in Janelia Fluor® dyes. This structural motif further improves brightness and photostability while eliciting a slight red-shift. JFX_{650} is our brightest and most photostable dye with far-red wavelength excitation in live-cell single-molecule imaging experiments.

JFX_{673}

- $\lambda_{abs}/\lambda_{em}$: 673 nm/690 nm
- ε: 156,000 M^{-1} cm^{-1}
- Φ: 0.42
- *Structure:* Silica-containing rhodamine with deuterated pyrrolidine and fluorine-substituted benzene groups
- $K_{L\text{-}Z}$: 1.56

JF_{673} is a fluorinated Si-rhodamine similar to JF_{669} but containing deuterated pyrrolidine auxochromes. The deuterium-containing substituents maintain brightness and bioavailability but increase photostability, compared to JF_{669}-based compounds. Like JF_{669}, JFX_{673} can be excited with far-red light (640-660 nm). This dye is useful for pulse-chase experiments *in vivo* and incorporation into chemigenetic sensors.

Red-Shifted and *Fluorinated* Janelia Fluor® Dyes
for Advanced Bioimaging



Overview

Fluorination and structural tuning of rhodamine scaffolds have yielded a powerful new class of **bright, red-shifted fluorescent dyes** for live-cell and super-resolution microscopy.

Key Advantages

- **Red/NIR Absorption and Emission** Fluorination shifts absorbance and emission by 15–25 nm, ideal for multi-plexed imaging and deep-tissue applications.
- **Enhanced Brightness and Stability** Improved extinction coefficients (ε) and stable fluorescence performance for single-particle tracking (SPT) and FLIM.
- **Improved Bioavailability** Tuning the lactone–zwitterion equilibrium (K_{L-Z}) ensures excellent membrane permeability and fluorogenic behavior.
- **Flexible Functionalization** New synthetic strategies (e.g., masked acyl cyanide chemistry) simplify attachment to tags like HaloTag or SNAP-tag.

Spotlight Dyes

- **JF_{657}:** Fluorinated analog of ATTO 647N; excels in live-cell imaging and SPT with superior brightness and localization stability.
- **JF_{698}:** Near-infrared absorbing dye designed for minimal background and high sensitivity imaging.
- **JF_{632} / JFX_{637}:** Fluorinated carborhodamines for advanced fluorescence microscopy and sensing.
- **Additional dyes: JF_{563}, JF_{610}, JF_{669}, JF_{593}**

Applications

- Super-resolution imaging (STED, SIM, SMLM)
- Single-particle tracking (SPT) in live cells
- Multicolor imaging panels
- FLIM-based biosensing of molecules like cAMP

Reference: *JACS.* (2023). https://doi.org/10.1021/jacs.3c05273

IP Coverage: US Patent Applications 17/116,987 and 18/893,747

Janelia Tech ID: 2020-006

Continued

JF_{559}

- $\lambda_{abs}/\lambda_{em}$: 559 nm/579 nm
- ε: 106,000 M^{-1} cm^{-1}
- Φ: 0.85
- *Structure:* Fluorinated rhodamine with 3-fluoroazetidine groups
- $K_{L\text{-}Z}$: 6.22

JF_{559} represents an intermediate tuning step, created by introducing a fluorine substituent on each azetidine ring of JF_{571}. This modification resulted in a $K_{L\text{-}Z}$ value (6.22) between those of JF_{549} (3.5) and JF_{571} (7.93), demonstrating the compatibility of different tuning strategies.
The JF_{559}-HaloTag ligand has been synthesized and shows effective labeling in live-cell applications. This dye exemplifies how the photophysical properties of rhodamines can be finely adjusted using complementary structural modifications, providing intermediate spectral and chemical properties for specific imaging needs.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

JF_{571}

- $\lambda_{abs}/\lambda_{em}$: 571 nm/590 nm
- ε: 101,000 M^{-1} cm^{-1}
- Φ: 0.78
- *Structure:* Fluorinated rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 7.93

JF_{571} is a fluorinated rhodamine derivative with azetidine groups. The fluorination of the pendant phenyl ring increased both the $K_{L\text{-}Z}$ value and λ_{abs} compared to JF_{549}. The JF_{571}-HaloTag ligand has been synthesized and shows improved photostability compared to the JF_{549}-HaloTag ligand in cellular imaging experiments. This dye demonstrates how fluorination of the pendant phenyl ring can be used to increase $K_{L\text{-}Z}$ values and red-shift absorption wavelengths while maintaining high quantum yields.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

JF_{593}

- $\lambda_{abs}/\lambda_{em}$: 593 nm/612 nm
- ε: 90,300 M^{-1} cm^{-1}
- Φ: 0.55
- *Structure:* Fluorinated sulfur-containing rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 6.06

JF_{593} is a fluorinated derivative of the sulfur-containing JF_{570}, created by incorporating fluorine atoms on the pendant phenyl ring. This modification increased both the $K_{L\text{-}Z}$ value (from 2.24 to 6.06) and the absorption wavelength. The JF_{593}-HaloTag ligand has been synthesized and shows improved photostability compared to the JF_{570}-HaloTag ligand in fixed cells. The combination of the sulfur bridge in the xanthene system and fluorination of the pendant phenyl ring creates a dye with orange-red excitation and emission properties.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

JF_{669}

- $\lambda_{abs}/\lambda_{em}$: 669 nm/682 nm
- ε: 112,000 M^{-1} cm^{-1}
- Φ: 0.37
- *Structure:* Fluorinated Si-rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 0.262

JF_{669} was developed by halogenating the pendant phenyl ring of JF_{646}, which substantially increased both the $K_{L\text{-}Z}$ value (from 0.0014 to 0.262) and the absorption wavelength. The JF_{669}-HaloTag ligand shows excellent cellular labeling properties with improved photostability compared to JF_{646}, and is blood-brain barrier permeant, enabling *in vivo* neuronal imaging after intravenous administration. This fluorophore demonstrates the versatility of the fluorinated phenyl ring as a synthetic handle for nucleophilic aromatic substitution reactions, enabling various derivatives including azides, nitriles, and amines. The JF_{669}-SNAP-tag ligand has also been developed, showing low nonspecific staining in live-cell applications.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

Continued

JF_{711}

- $\lambda_{abs}/\lambda_{em}$: 711 nm/732 nm
- ε: 12,400 M^{-1} cm^{-1}
- Φ: 0.17
- *Structure:* Phosphine oxide-containing rhodamine with 3-fluoroazetidine groups
- $K_{L\text{-}Z}$: ~0.001

JF_{711} combines a phosphine oxide-containing rhodamine scaffold with 3-fluoroazetidine groups to create a near-infrared fluorogenic dye. This combination strategy yields a fluorophore with a higher quantum yield (0.17) than JF_{722} (0.11) while maintaining a low $K_{L\text{-}Z}$ value of ~0.001, which is ideal for fluorogenic applications. The JF_{711}-HaloTag ligand shows a 5-fold increase in absorbance upon binding to HaloTag protein and exhibits higher brightness in fixed cells compared to JF_{722}-HaloTag ligand, making it useful for applications requiring high contrast and low background. The NIR excitation and emission properties make JF_{711} valuable for deep tissue imaging applications where minimizing autofluorescence and maximizing tissue penetration are important.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

JF_{722}

- $\lambda_{abs}/\lambda_{em}$: 722 nm/743 nm
- ε: 87,200 M^{-1} cm^{-1}
- Φ: 0.11
- *Structure:* Phosphine oxide-containing rhodamine with azetidine groups
- $K_{L\text{-}Z}$: 0.026

JF_{722} is a near-infrared dye created by incorporating a phosphine oxide moiety into the rhodamine scaffold with azetidine groups. This structural modification pushes the absorption and emission wavelengths well into the NIR region while maintaining reasonable brightness. The JF_{722}-HaloTag ligand does not show significant fluorogenicity due to its relatively high $K_{L\text{-}Z}$ value but demonstrates better loading kinetics in live cells compared to JF_{711}. This dye represents an important extension of the JF™ palette into the NIR region, where reduced phototoxicity and increased tissue penetration are advantageous for *in vivo* imaging. The phosphine oxide moiety provides an alternative strategy to silicon substitution for creating red-shifted rhodamine derivatives.

Trifluoro Janelia Fluor® JF3 dyes 2020-006

Bright, *Photoactivatable* Janelia Fluor® Dyes
for Super-Resolution Imaging

Overview

Photoactivatable versions of Janelia Fluor® dyes combine small-molecule fluorophores' strengths with the spatial and temporal control of photoactivation, ideal for advanced imaging workflows.

Key Advantages

- **On-Demand Activation** Light-controlled activation (e.g., 405 nm) enables sparse labeling for PALM, sptPALM, and two-color experiments.
- **Exceptional Brightness** After activation, dyes retain the high quantum yields and photostability of standard JF™ dyes.
- **Live-Cell and Fixed-Cell Compatibility** Easily label intracellular targets via HaloTag and SNAP-tag systems.
- **Multicolor Capability** Distinct spectral windows (green–orange for PA-JF_{549}, far-red for PA-JF_{646}) enable simultaneous two-color imaging.

Spotlight Dyes

- **PA-JF_{549}:** Bright, fast-activating dye for single-molecule and high-precision localization microscopy.
- **PA-JF_{646}:** Red-shifted dye for deep-tissue and two-color super-resolution imaging alongside green/orange channels.

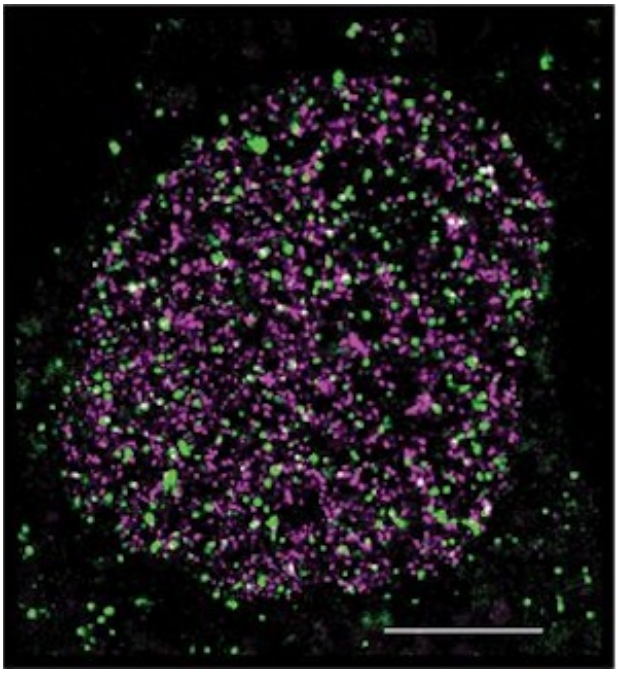
Applications

- Live-cell PALM and sptPALM
- Single-particle tracking of nuclear and cytoplasmic proteins
- Super-resolution imaging of mitochondria, chromatin, and cytoskeletal structures

Reference: *Nat Meth*. (2016). https://doi.org/10.1038/nmeth.4034

IP Coverage: US Patent 11,958,976, JP Application 2018-560894, EP Application EP17800333.1, CN 2017800283784

Janelia Tech ID: 2016-034



Bright and Targetable Red Ca^{2+} Indicators
with JF-BAPTA Technology

Overview

JF-BAPTA indicators merge the **fluorescence power of Janelia Fluor® dyes** with the Ca^{2+} sensitivity of BAPTA and the **genetic targeting** precision of HaloTag. This next-generation sensor platform delivers high-performance calcium imaging in both cytoplasmic and subcellular environments.

Key Features

- **High $\Delta F/F_0$ and Quantum Yield** Up to 15× signal change and quantum yields as high as 0.75.
- **HaloTag Targeting** Enables selective labeling and precise subcellular imaging.
- **Spectral Flexibility** JF_{549} and JF_{646} derivatives offer compatibility with GFP, optogenetics, and deep-tissue imaging setups.
- **Far-Red Imaging of the Primary Cilium** First small-molecule red calcium dye to successfully label and detect dynamic calcium fluxes in the primary cilium.

Spotlight Indicators

- **JF_{549}-BAPTA:** Bright red indicator for general Ca^{2+} imaging
- **HaloTag-JF_{549}-BAPTA:** Targetable red probe for high signal with subcellular control
- **HaloTag-JF_{646}-BAPTA:** Far-red, fluorogenic probe ideal for primary cilia and deep tissue imaging

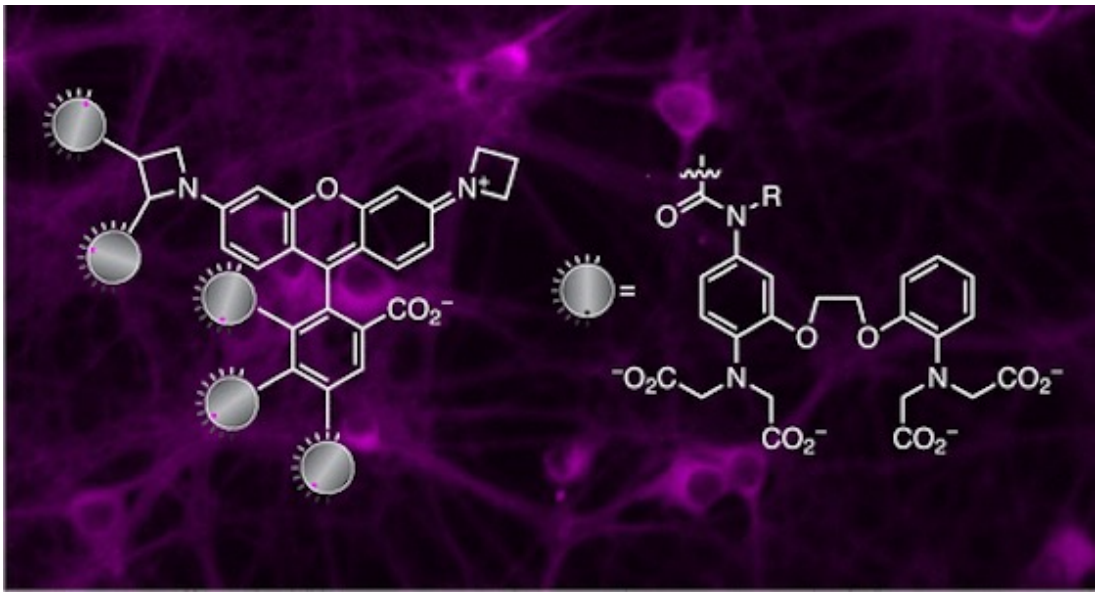
Applications

- Neural calcium imaging (single AP resolution)
- Primary cilium Ca^{2+} flux mapping
- Live-cell and multiplexed imaging
- Complementary with GFP and optogenetic tools

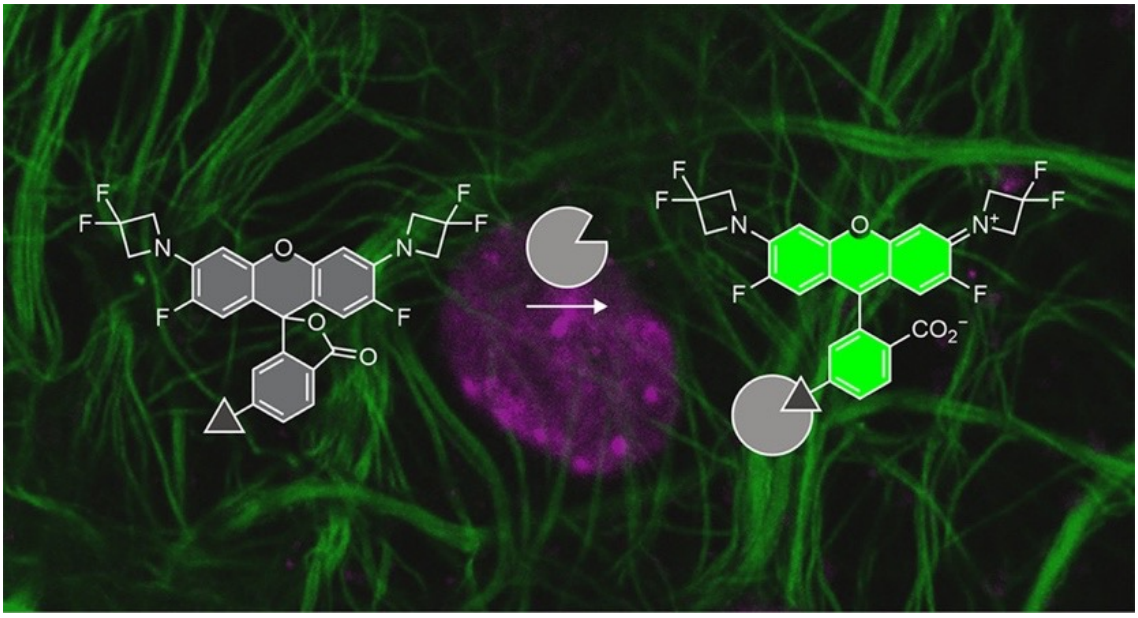
Reference: *JACS*. (2019). https://doi.org/10.1021/jacs.9b06092

Patent Coverage: US Patents 11,498,932 and 12,157,750

Janelia Tech ID: 2019-048



Spontaneously Blinking Labels
for Super-Resolution Imaging



Overview

This new generation of small-molecule fluorescent dyes, based on rational tuning of the lactone–zwitterion equilibrium (K_{L-Z}), delivers high-performance tools for biological imaging. Dyes engineered with K_{L-Z} values between 10^{-2} and 10^{-3} offer exceptional **cell permeability**, **fluorogenicity**, and compatibility with **super-resolution techniques**.

Key Benefits

- **No-Wash Fluorogenic Imaging** Dyes are non-fluorescent until bound to targets, reducing background and simplifying workflows.
- **Superior Cell Permeability** Lipophilic lactone states enhance membrane crossing, enabling live-cell intracellular labeling.
- **Exceptional Brightness and Stability** Structural optimizations (e.g., azetidine substitution) yield high quantum yields and robust photostability.
- **Advanced Microscopy Ready** Compatible with STED, SIM, lattice light-sheet, and SMLM imaging—including spontaneous blinking formats (e.g., HM-JF_{526}).

Highlighted Molecules

- **JF_{526}:** Bright green-emitting, highly fluorogenic dye for tagging proteins (HaloTag, SNAP-tag) and structures like microtubules, DNA, and lysosomes.
- **HM-JF_{526}:** Hydroxymethyl derivative enabling spontaneous blinking for high-precision localization microscopy in standard imaging buffers.
- **Other dye wavelengths are available:** JF_{646}b, JF_{639}b, JF_{639}b, JF_{630}b, JF_{629}b, JF_{626}b, and JF_{614}b.

Applications

- Live-cell microscopy
- No-wash multicolor imaging
- Single-molecule super-resolution (SMLM)
- Drug discovery and target engagement assays

Reference: *ACS Cent Sci.* (2019). https://doi.org/10.1021/acscentsci.9b00676

IP Coverage: US Patent Application 17/027,286

Janelia Tech ID: 2019-054

Photochromic and Spontaneously Blinking Dyes
Using Coumarin Switching

Overview

A versatile platform enabling the rational design of super-resolution imaging dyes by amide-coupling rhodamines to **coumarin** auxiliaries. Depending on the rhodamine's lactone–zwitterion equilibrium (K_{L-Z}), the dyes become either **photochromic** (405 nm-activatable) or **spontaneously blinking** at physiological conditions.

Key Advantages

- **Universal Switching Strategy** One chemical modification generates two classes of functional dyes for imaging.
- **Predictable Behavior** Dye blinking or photoactivation outcomes based on parent dye K_{L-Z} values.
- **Live-Cell and Fixed-Cell Compatibility** Works under normal biological conditions without specialized buffers.
- **Super-Resolution Ready** Optimized for PALM, SMLM, SOFI, and related single-molecule imaging modalities.

Spotlight Dyes

- **GB_{646}:** Si-rhodamine–coumarin dye activated by violet light (405 nm) for photochromic super-resolution imaging.
- **GB_{549}:** JF_{549}–coumarin dye that spontaneously blinks, eliminating the need for external switching buffers.

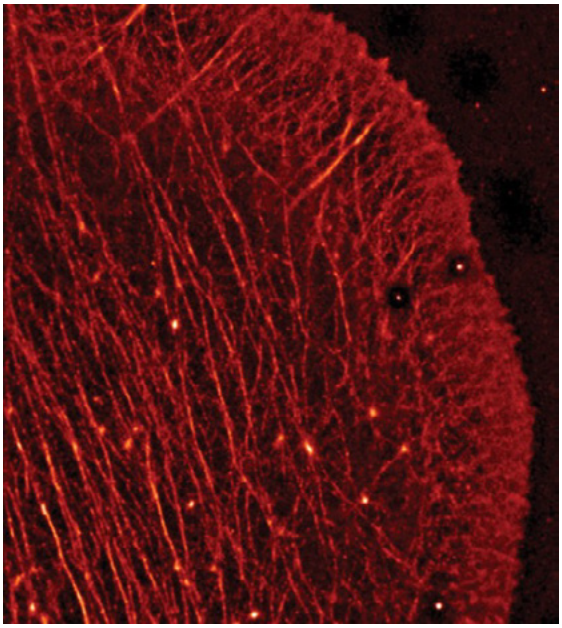
Applications

- Single-molecule localization microscopy (SMLM)
- Super-resolution imaging in live and fixed cells
- High-density molecular mapping
- Multicolor blinking microscopy

Reference: *bioRxiv.* (2024). https://doi.org/10.1101/2024.05.12.593749

IP Coverage: US Patent 11,067,566

Janelia Tech ID: 2017-026



Biotin-Janelia Fluor® Conjugates

Multifunctional Probes for Imaging and Affinity Capture

Overview

Biotin-JF™ conjugates are cell-permeable, fluorescent ligands designed for **dual-purpose applications**: live-cell imaging and biochemical purification using the HaloTag platform.

Key Benefits

- **Live-Cell Compatible** Rhodamine-derived scaffolds ensure efficient intracellular labeling and visualization.
- **Bright and Fluorogenic** Enhanced brightness upon HaloTag binding enables high-contrast imaging.
- **Affinity Tag Built-In** treptavidin-compatible biotin moiety allows pulldown of HaloTag-tagged compartments.
- **Outperforms Commercial Ligands** Demonstrated superior capture and permeability versus existing biotin-HaloTag compounds.

Spotlight Ligands

- **JF_{646}-biotin:** Bright far-red fluorogenic probe; excellent for mitochondrial capture
- **JF_{635}-biotin:** Red-shifted probe with high labeling efficiency
- **JF_{608}-biotin:** Balanced option for multi-organelle imaging
- **JF_{549}-biotin:** Selective labeling of cell-surface due to impermeability

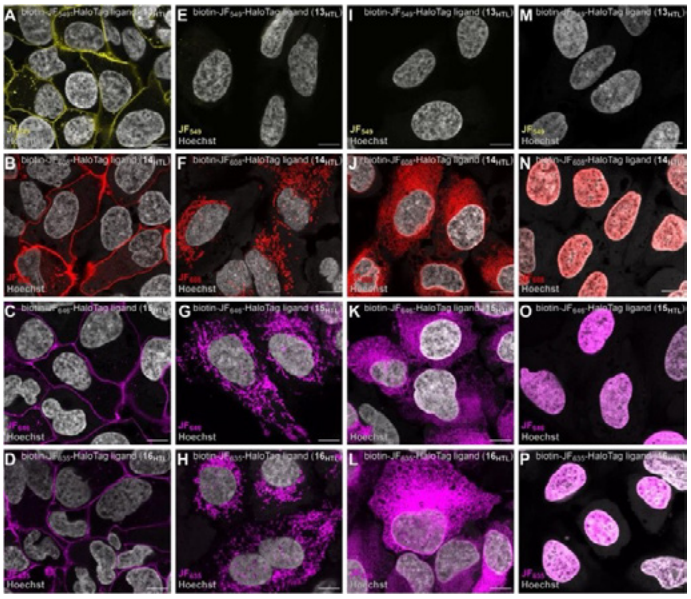
Applications

- Fluorescent labeling of intracellular HaloTag proteins
- Streptavidin-mediated pulldown of labeled organelles (e.g., mitochondria)
- Proteomic profiling, cell sorting, or target validation
- High-content imaging followed by molecular capture

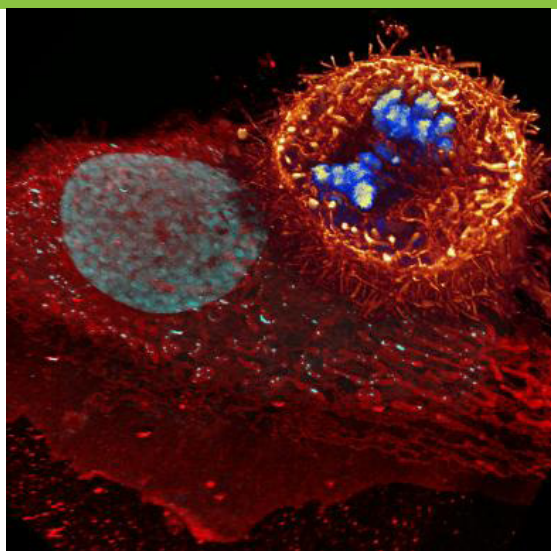
Reference: *bioRxiv.* (2022). https://doi.org/10.1101/2022.07.02.498544

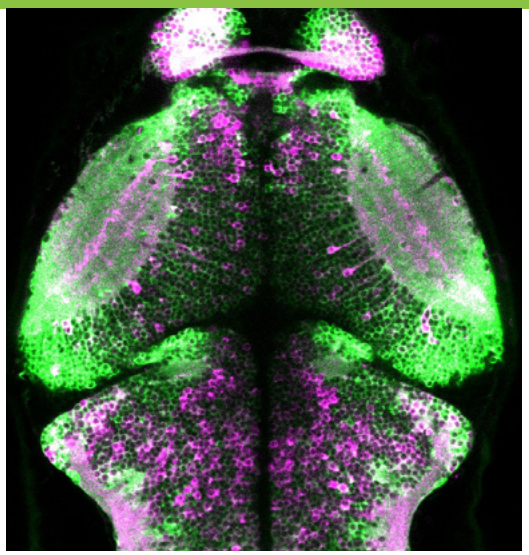
IP Coverage: US Patent Application 18/390,289

Janelia Tech ID: 2023-001



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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