

Next-generation Janelia Fluor dyes do more than just shine

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Janelia Fluor dyes used to create multifunctional fluorophores that can be used for multiple purposes. Credit: Pratik Kumar

To neuroscientist Jason Vevea, Luke Lavis is not just a chemist. He's a modern-day magician.

For the past decade, Lavis, a senior group leader and head of Janelia's molecular tools and imaging research area, has been working with his lab to apply the magic of modern-day chemistry to old molecules, turning centuries-old rhodamine-based dyes into the Janelia Fluor dyes. The JF dyes, as they are known, are brighter and more stable than other dyes, and are available in a wide range of colors, making them indispensable to biologists worldwide for seeing molecules inside cells.

"Luke and his team are applying this new magic to these tried-and-true structures and creating a whole suite of spectrally tuned, best-in-class fluorophores that we can use," says Vevea, assistant member of the faculty at St. Jude Children's Research Hospital. "This makes Luke famous in the cell biology community as we rely heavily on microscopes. But then Luke starts going further."

After spending years perfecting the chemistry used to make the JF dyes and figuring out the rules behind how they get into living cells, Lavis and his team are now turning their attention to designing dyes for specific biological applications, including in [live animals](#).

Working with collaborating biologists like Vevea, Lavis and his team are creating dyes that can act as linkers for other ligands, using them to see brain-wide turnover of proteins in live animals and deploying the dyes as a time stamp to record the transcriptional history of cells. Janelia's Open Chemistry initiative, which provides many pre-commercial tools to

researchers around the world for free, helps drive these new innovations, Lavis says.

"We spent a long time developing the chemistry to make these dyes, and so now that we can make dyes really efficiently, we are using that chemistry to make useful derivatives," he says. "Since we have Open Chemistry, we are also able to get the dyes into researchers' hands, and just having these reagents around sparks new ideas."

Creating a multifunctional fluorophore

As a postdoc in HHMI Investigator Edwin Chapman's lab at the University of Wisconsin-Madison, Vevea dreamed of being able to isolate proteins in a cell at different time points to understand how cells and cellular components age. These insights could be important for understanding neurodegenerative diseases, like Alzheimer's and Parkinson's.

Vevea wanted to label and image proteins, and then extract these same proteins from the cell to characterize them. But with current methods and commercially available reagents, he couldn't do both actions at the same time.

Scientists use enzyme-based labeling tags, like the popular HaloTag, to study specific proteins in cells. These tags can be fused to a protein of interest and then act as a kind of Swiss Army knife to carry out different actions depending on what type of ligand is delivered to the tag.

Some ligands, like a JF dye, can be used to image the protein while other ligands, in principle, could be used to extract and purify the protein. But it wasn't possible to use two ligands at the same time, and the available commercial reagents that could be used to extract the protein of interest did not perform well in cells.

Vevea approached Lavis with an idea: Would it be possible to hang a ligand for purifying the protein onto the well-behaved JF dye attached to the HaloTag? This would allow Vevea to tag and image the protein, in addition to capturing and isolating it.

The result, described in a [preprint](#) on *bioRxiv*, is a multifunctional fluorophore that can be used for multiple purposes. Additionally, by attaching the affinity reagent to the JF dye, the research team improved the cellular compatibility of the affinity reagent above what it previously had alone.

This led to the hypothesis that, beyond using the system for imaging and purification, the JF dye could serve as a general linker for other types of ligands used for protein manipulation. This, in turn, could enable scientists to create new tools for biology using molecules previously considered difficult to work with.

"The dye is almost like a vehicle and all these small molecules are like passengers," says Pratik Kumar, a postdoc in the Lavis Lab and the first author of the new paper. "It is the perfect vehicle to take more passengers."

Seeing synapses across the brain

Down the hall from the Lavis Lab at Janelia, Boaz Mohar, a research scientist in the Spruston Lab, is using the JF dyes to track the turnover of proteins in the brains of live animals to zero in on which [brain regions](#) are involved in different activities.

Mohar and colleagues use a pulse-chase method to investigate the turnover of a synaptic protein. A HaloTag ligand containing a one-color JF dye (the pulse) is injected into a live mouse brain to label all the proteins of interest in a single color. After the mouse performs a task or

is manipulated in a way that is known to induce changes in the brain, it is again injected with a HaloTag ligand containing a different color JF dye (the chase).

Newly synthesized proteins are labeled with the chase color JF dye, allowing researchers to observe these new proteins. The relative amounts of pulse and chase proteins enable researchers to calculate the turnover rate of the protein. The team described the work in a [preprint](#) on *bioRxiv*.

Compared to existing techniques used to measure protein turnover, the group's method is better for detecting which brain regions are involved when an animal performs a task. This information helps researchers focus their attention on key regions of the brain in subsequent experiments involving individual neurons. Ultimately, the team hopes that enabling scientists to better identify brain regions involved in certain tasks or manipulations will one day lead to a better understanding of how to tailor disease treatments in humans.

Because there are so many different JF dyes, the team was able to find a dye that could cross the blood-brain barrier and work inside the brains of live animals, Mohar says.

"We are able, with these amazing JF dyes, to get to single input resolution, and now we will be able to answer questions that we weren't able to answer before about where things change on the single-cell level and on the single-region level," he says.

Generating a ticker tape

During the COVID-19 pandemic, Harvard University chemist Adam Cohen, a longtime collaborator with the Lavis Lab, and his postdoc, Dingchang Lin, set out on a similar mission to uncover new information

about brain-wide neural activity. Together, they wanted to see if they could use the JF dyes, attached to a HaloTag, to record a marker gene's activation history.

The researchers took advantage of an engineered protein fiber that could incorporate different fluorescent marks as it grew. To track the fiber's growth, the team added different color JF dyes, attached to HaloTags, at specific time points. As the fiber grew, the JF dyes formed colorful stripes that acted as a time-stamped ticker tape history of the fiber's growth. At the same time, a fluorescent [protein](#), also attached to the fiber, lit up when activated by a promoter of interest—for example, one associated with neuronal activity.

By correlating the JF dye time stamps with the fluorescence, researchers can obtain a recording of a gene activation in a cell. Like ecologists who use tree rings to understand a forest's climate history, biologists can use the ticker-tape record to understand a cell's gene expression history. The new method could be used to map the history of many different cellular processes at a single-cell level, including recording the activity in every neuron across the entire brain, says Lin, now an assistant professor at Johns Hopkins University and the first author of a *Nature Biotechnology* paper detailing the ticker-tape method.

The broad palette of bright, stable JF dyes, with their ability to cross the blood-brain barrier and get into cells, is central to the new method, Cohen says. The research also required a lot of pre-commercial dye, which Lavis and his team synthesized for Cohen's team as part of Janelia's commitment to collaboration and open science.

The collaboration between the two labs continues to grow, with Cohen's lab using Lavis's dyes in new and creative ways. Most recently the lab used the dyes in research examining dendritic biophysics and plasticity rules detailed in [several new preprints](#) posted to *bioRxiv*.

"Having access to these dyes was a game changer for us because it meant we could try stuff and think ambitiously," Cohen says. "That collaborative arrangement has made a huge difference for us. Luke has really been a model of how to disseminate these tools to the community, and that advances science overall."

More information: Dingchang Lin et al, Time-tagged ticker tapes for intracellular recordings, *Nature Biotechnology* (2023). [DOI: 10.1038/s41587-022-01524-7](https://doi.org/10.1038/s41587-022-01524-7)

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