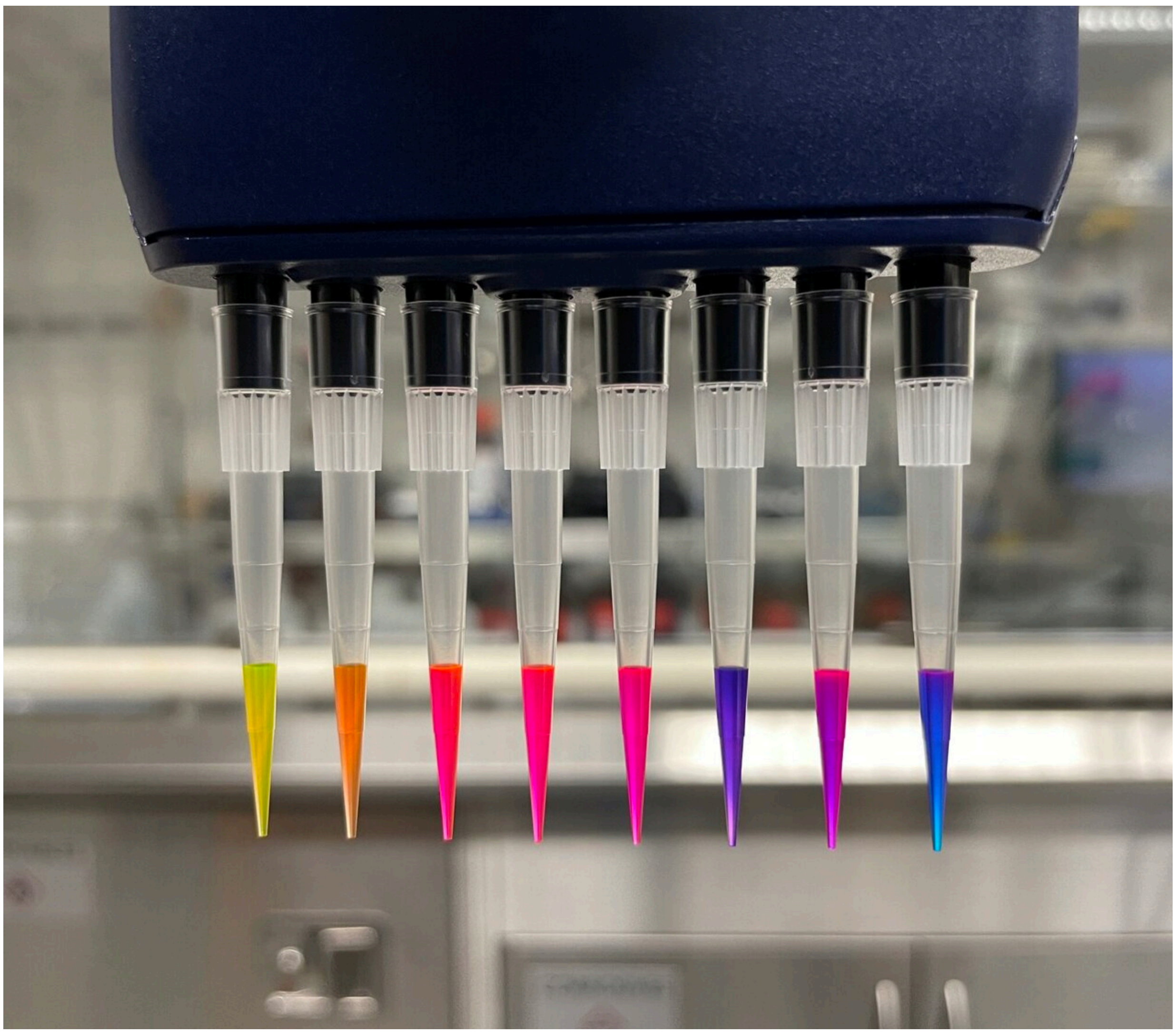
Researchers unveil comprehensive collection of rhodamine-based fluorescent dyes

December 8 2023



Rhodamine-based flourescent dyes developed at HHMI's Janelia Research Campus. Credit: Jonathan Grimm/HHMI Janelia Research Campus

When Senior Scientist Jonathan Grimm came to Janelia 13 years ago, he didn't know much about fluorescence or fluorescent dyes. But as an organic chemist who had been working in drug discovery at Merck, he certainly knew a thing or two about medicinal chemistry.

On a whim, Grimm and Janelia Senior Group Leader Luke Lavis decided to try using a mainstay medicinal chemistry reaction Grimm had picked up in the pharmaceutical industry to improve centuries-old dye chemistry. They thought this approach could allow access to completely new, previously inaccessible rhodamines—molecules Lavis had been working to make brighter and longer-lasting so they could be used to better image cells under powerful microscopes.

The result was the start of what would become the now ubiquitous and indispensable Janelia Fluor dyes, bright, photostable, cell-permeable fluorescent probes that allow biologists to see the molecules inside cells. More than a decade after they were first unveiled, these fluorescent dyes that span the color spectrum have become a staple of biology labs worldwide.

Using a similar approach, Grimm, Lavis, and their collaborators have now released the culmination of their years of work in the *Journal of the American Chemical Society*: a comprehensive collection of additional rhodamine-based fluorescent dyes—a whole new set of far-red shifted dyes that can penetrate deeper into tissue and are good for in vivo imaging, making them vitally important for biologists.

The team also shared their approach—the novel chemistry they developed to synthesize the dyes and insights that provide a roadmap for designing future probes.

"Along the way, we applied or modified or came up with totally new ways to make rhodamines that have a pretty broad scope, and that enabled us to make so many dyes relatively quickly," Grimm says. "This is probably the most comprehensive work we've done with rhodamines so far."

Creating a comprehensive collection

The latest project started at the onset of the COVID-19 pandemic in early 2020. The team had just released research detailing the novel chemistry they used to expand the Janelia Fluor dye palette.

Next, they wanted to see if they could apply what they learned about optimizing the Janelia Fluor dyes to other types of rhodamine-based dyes while also further improving the chemistry used to synthesize them.

As the world shut down, Grimm and Lavis planned new chemistry—including completely novel chemical reactions—that sought to rationally incorporate the lessons learned from the Janelia Fluor dyes into other classic but suboptimal rhodamines.

A few months later, Grimm got back into the lab and began seeing if their work on paper could translate to the real—and sometimes unpredictable—world of organic chemistry. With COVID precautions in place, Grimm worked alone in the lab, optimizing the chemistry and creating the first new dyes.

"It probably would have happened anyway, but for better or for worse, when there is nothing else to focus on, or the things to focus on were bad—as 2020 was for everybody—chemistry was a nice distraction," Grimm says.

The new research lays out the culmination of the team's work over the past three-plus years. Unlike the traditional Janelia Fluor dyes, which are characterized by an appendage called an azetidine ring, the other rhodamine-based dyes have different substituents protruding from other parts of their molecular structures.

Armed with knowledge from optimizing the JF dyes, the team modified these other areas on the older rhodamine dyes to alter their color, brightness, photostability, cell permeability, and other characteristics.

The result is a whole new set of rhodamine-based dyes for imaging. The team was also able to devise several new ways to make classic rhodamine dyes, enabling them to create dozens of functional versions relatively quickly.

"We had known for a long time how changing the functionality on the 'top' of the molecule affects the colors of the fluorophores, but we also figured out that this strongly affects the chemical properties of the dye," Lavis says. "We exploited that in different ways to make bright, red-shifted imaging agents."

The final chapter

While this isn't the end of the story for rhodamine dyes, the work is likely moving in a different direction. Now the team is focused on designing reagents that are specifically tailored for use by their biologist collaborators, working to build the very best tools they can with the knowledge they've gained.

"We can make any rhodamine dye we would ever want with this chemistry, and so the big question is what do we make next," Lavis says. "It's not what can we make but what should we make."

Grimm says developing this expansive set of rhodamines, which took over a decade, is a testament to HHMI Janelia's support of long-term efforts that are beneficial to the wider scientific community. Having permanent staff scientist positions at Janelia also enables Grimm and other senior scientists to provide continuity to a large project like the Janelia Fluor dyes. Four of the researchers on the most current publication were also on the very first rhodamine dye paper the lab published in 2011.

For Grimm, it also means he gets to do what he loves—be in the lab, do chemistry, and create tools that are useful to biologists. And, more than 13 years later, he's also learned a thing or two about fluorescent dyes.

"It is very satisfying to have this timeline of papers that show all that we've done over the years, and it all started with just one random reaction based on a little calculation that Luke did, which itself was enabled by a synthetic method that we just happened to pursue, on a whim, simply to make dye synthesis a little easier," Grimm says. "Even if a calculation looks great, it doesn't always pan out that way. In this case, it was dead on, and it certainly paid off."

More information: Jonathan B. Grimm et al, Optimized Red-Absorbing Dyes for Imaging and Sensing, *Journal of the American Chemical Society* (2023). DOI: 10.1021/jacs.3c05273

Provided by Howard Hughes Medical Institute

Citation: Researchers unveil comprehensive collection of rhodamine-based fluorescent dyes (2023, December 8) retrieved 27 April 2024 from https://phys.org/news/2023-12-unveil-comprehensive-rhodamine-based-fluorescent-dyes.html