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FIRST REACTIONS

Bright Dyes Bring Biology into Focus

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New synthetic approach broadens accessibility to dyes for advanced molecular imaging.

Major advances in microscopy have revolutionized our view of the cell, allowing us to see what was previously not visible, now with unprecedented resolution. This would not be possible without an impressive portfolio of bright, modular, and photostable fluorescent dyes. Among these are xanthene-based dyes which includes fluorescein, a versatile FDA-approved reagent first synthesized more than a century ago. Since then, coarse-tuning by substitution of the endocyclic xanthene oxygen with carbon, silicon, and phosphorus has expanded this family to include members with emission wavelengths spanning the entire visible spectrum. However, fine-tuning of specific photophysical properties remains a profound challenge owing to lengthy, nonmodular, and low-yielding synthetic approaches. In this issue, a group led by Lavis, from Janelia Research Campus, has established a powerful divergent strategy to enable the assembly of an assortment of xanthene dyes with unique properties primed to further push the boundaries of molecular imaging (Figure 1, Figure 2).[1]

Previously, Lavis reported the synthesis of Janelia Fluor 646 (JF_{646}),[2] a bright silicon-containing xanthene dye that was immediately adopted by the field for super-resolution microscopy. However, its synthesis was low-yielding (4.6% over 11 steps) which hampered further development. The major bottleneck was indeed the preparation of a key silicon–xanthone building block since it accounts for a majority of the synthesis and involves chemistry that is typically difficult to reproduce (at least in our hands). This was followed by metal–halogen exchange chemistry using an appropriate aryl halide precursor to assemble the final dye scaffold. However, the electrophile and nucleophile are often electronically mismatched resulting in another low-yielding step (Figure 2). Lavis's innovative solution drew inspiration from the Friedel–Crafts reaction, which was originally used to synthesize fluorescein.[3] Starting from bis(bromoaryl)-silane, a sequence involving lithium–bromide exchange, magnesium transmetalation, and electrophilic addition onto anhydrides or esters directly yields the Si-xanthene scaffold with high overall conversions (30–60% yield).[1]

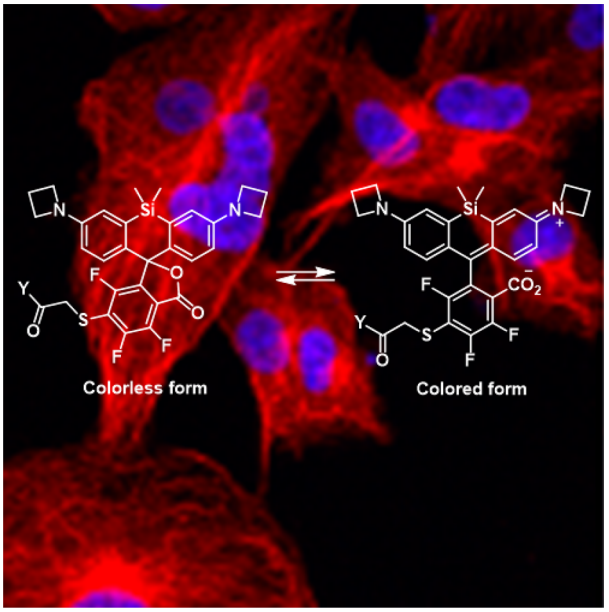

Figure 1. Cellular imaging with JF_{669} analog prepared using the new synthetic approach. Y = tubulin antibody.

> The new fluorinated series exhibited pK_a values as low as 6.08, meaning that they will be highly fluorescent at pH 7.4.

With this new approach in hand, Lavis and co-workers assembled a remarkable panel of dyes to improve their general utility for various biological applications. In one example, they developed fluorinated congeners of silicon-fluorescein[2] (pK_a = 8.27) since at physiological pH, a large proportion of this dye exists in a protonated and colorless form. The new fluorinated series exhibited pK_a values as low as 6.08, meaning that they will be highly fluorescent at pH 7.4. Furthermore, Lavis demonstrated the generality of his approach by preparing an extensive series of

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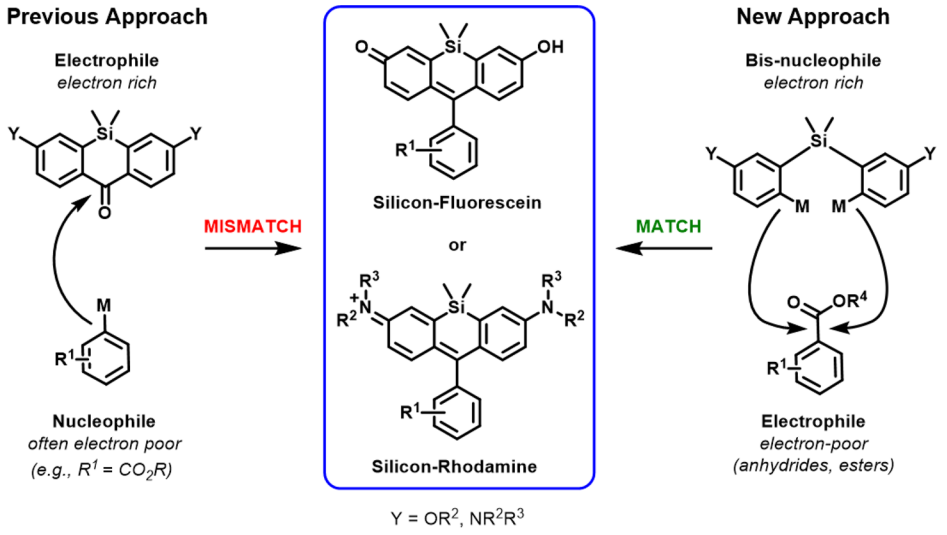

Figure 2. Synthetic approaches for Si-fluoresceins and Si-rhodamines.

Although xanthenes are age-old dyes, their evolution has been hindered by synthetic limitations. The work by Lavis and co-workers has laid the groundwork to overcome this challenge.

silicon-rhodamines with various amino substituents, including analogues of JF_{646} mentioned above. Interestingly, the diversity in substitutions made possible by this approach allows for considerable flexibility in fine-tuning key optical properties of the dye (e.g., excitation maxima). Additionally, the versatility of this approach made it possible to easily access JF_{669}-thio-HaloTag ligand, a cell permeable nuclear stain for super-resolution microscopy. Finally, a new lipid probe for multicolor and deep tissue imaging was successfully developed for visualizing internal membrane structures.[4]

As new microscopy techniques take center stage in biological discovery, it will be crucial that the development of new fluorophores keep pace. Although xanthenes are age-old dyes, their evolution has been hindered by synthetic limitations. The work by Lavis and co-workers has laid the groundwork to overcome this challenge. In addition to the spectacular collection of dyes highlighted in this article, we anticipate this methodology can be employed to also develop asymmetric silicon-rhodamines (different amino substituents), silicon-rhodols, and silicon-naphthofluorescein (dyes with extended conjugation). Moreover, we envision that this new approach will be widely used by those in the field interested in developing organelle-specific stains, reagents for super-resolution microscopy, or analyte responsive probes.

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