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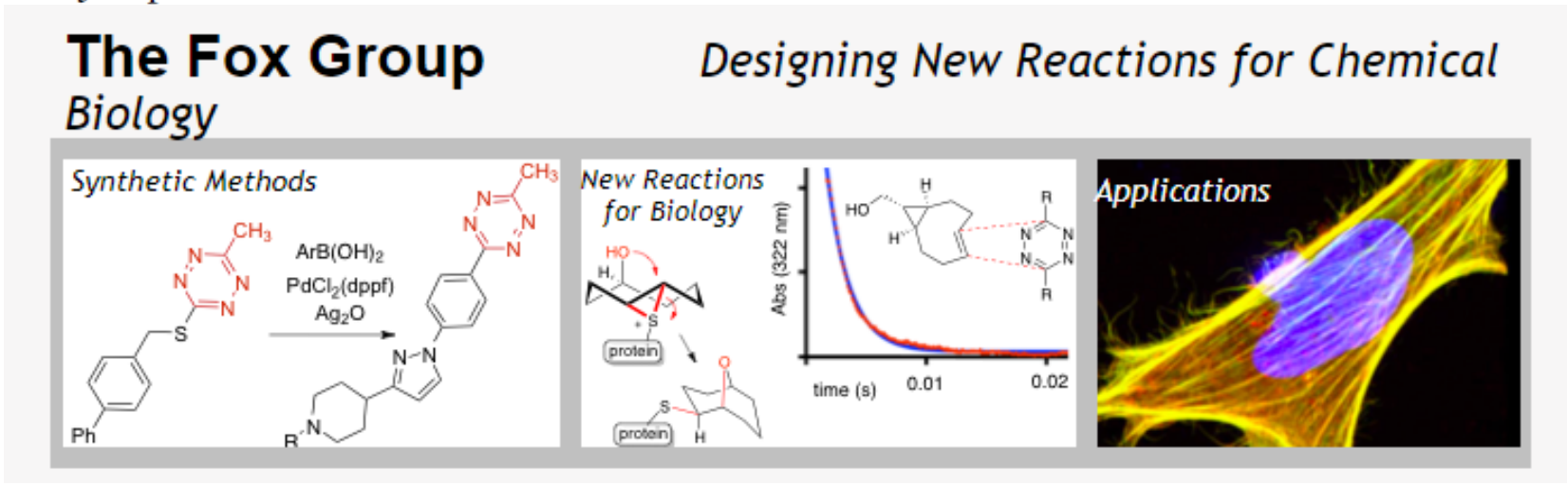
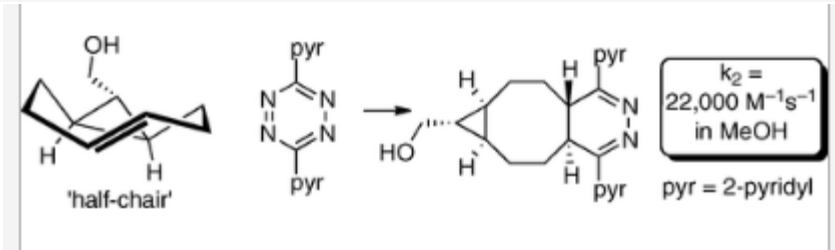
Article

Catalytic Activation of Bioorthogonal Chemistry with Light (CABL) Enables Rapid, Spatiotemporally Controlled Labeling and No-Wash, Subcellular 3D-Patterning in Live Cells Using Long Wavelength Light

Andrew Jemas, Yixin Xie, Jessica E. Pigga, Jeffrey L. Caplan, Christopher W. am Ende,* and Joseph M. Fox*





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University of Columbia, doctorate
MIT, postdoctor
2001- University of Delaware

J. Am. Chem. Soc. **2011**, 133, 9646

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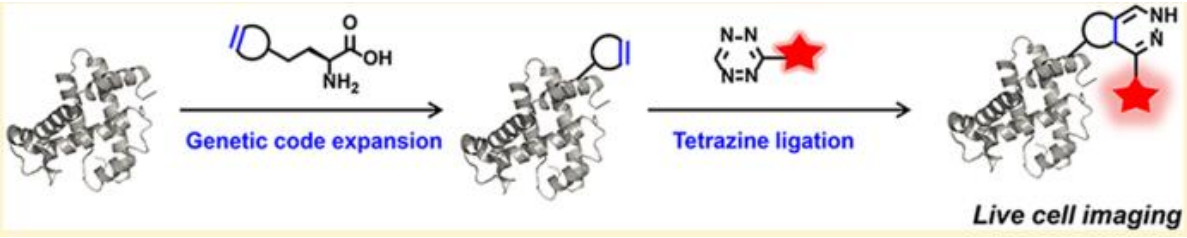
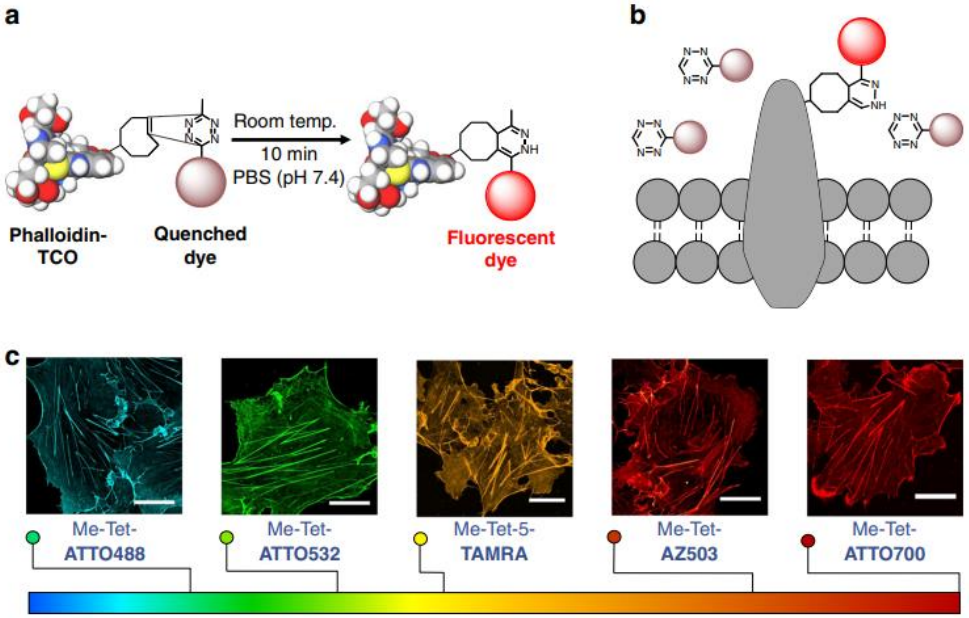
2022-05-05

Application of Tetrazinetrans-Cyclooctene Ligation

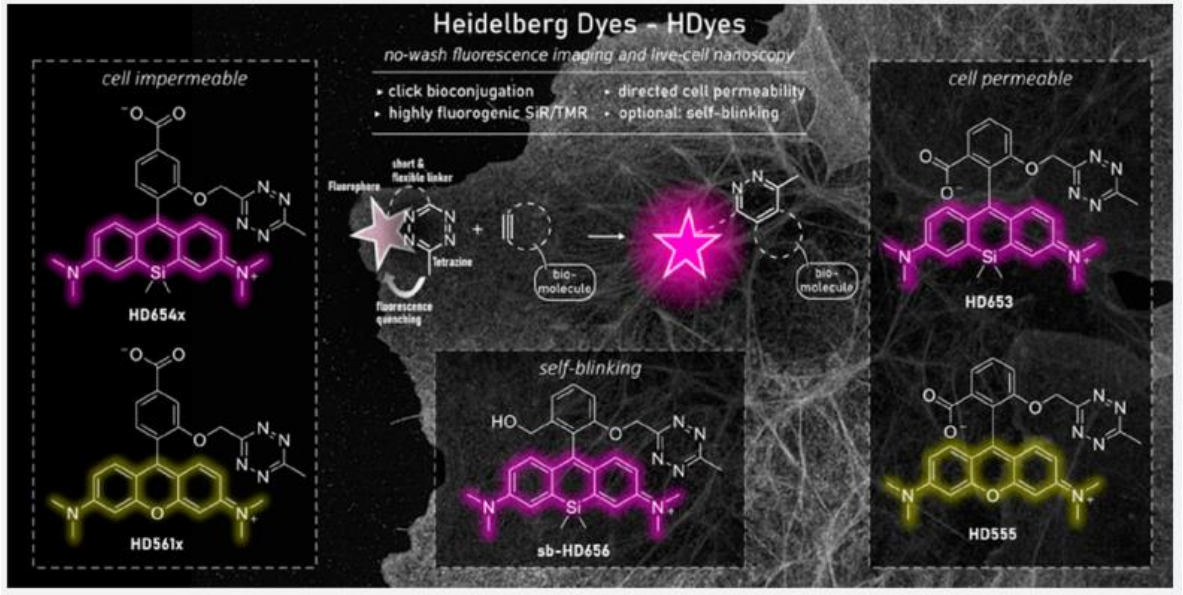
Site-Specific Bioorthogonal Labeling for Fluorescence Imaging of Intracellular Proteins in Living Cells



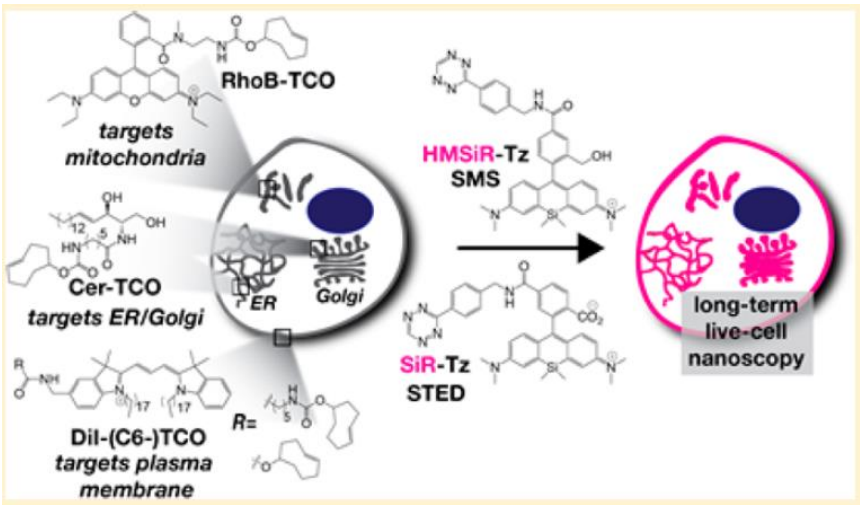
Bioorthogonal labeling with tetrazine-dyes for super-resolution microscopy



Bio-orthogonal Red and Far-Red Fluorogenic Probes for Wash-Free Live-Cell and Super-resolution Microscopy



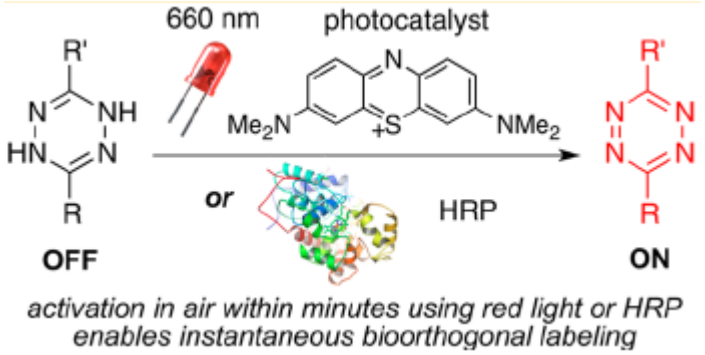
HIDE Probes: A New Toolkit for Visualizing Organelle Dynamics, Longer and at Super-Resolution



J. Am. Chem. Soc. **2015**, 137, 4602*; J. Am. Chem. Soc*. **2016**, 138, 14423; *Biochemistry* **2017**, 56, 5194; *Commun. Biol*. **2019**, 2, 261; *ACS Cent. Sci*. **2021**, 7, 1561

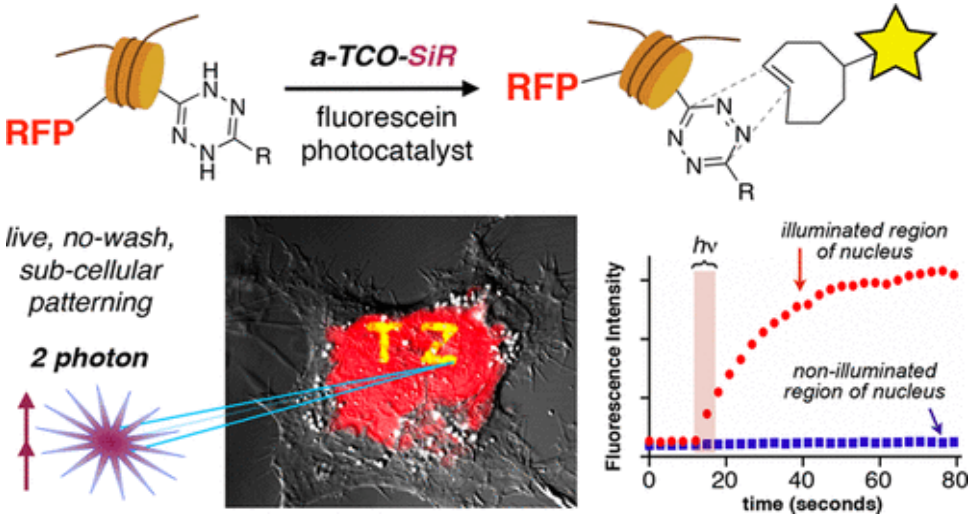
TOC & Research Progress

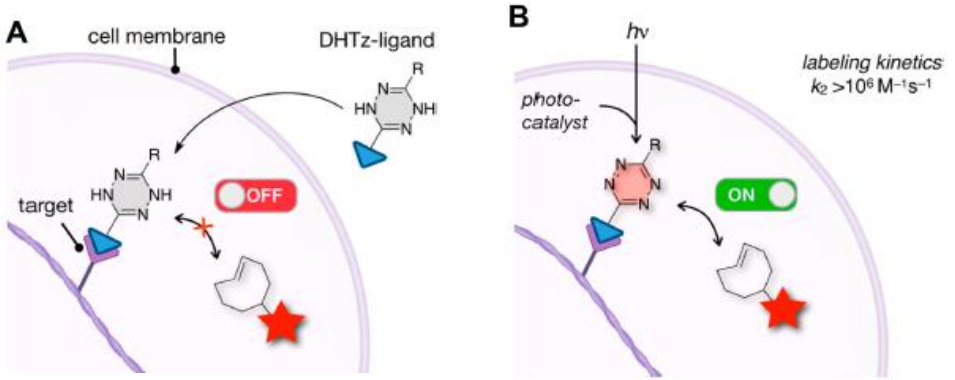
Rapid Bioorthogonal Chemistry Turn-on through Enzymatic or Long Wavelength Photocatalytic Activation of Tetrazine Ligation



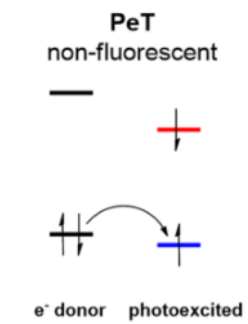
J. Am. Chem. Soc. **2016**, 138, 5978

Catalytic Activation of Bioorthogonal Chemistry with Light (CABL) Enables Rapid, Spatiotemporally Controlled Labeling and No-Wash, Subcellular 3D-Patterning in Live Cells Using Long Wavelength Light

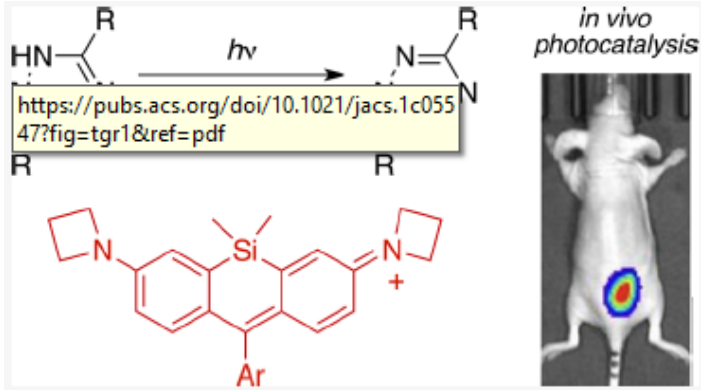




☐Synthesis and Evaluation of DHTz's with Improved Permeability, Stability, and Reactivity
☐Labeling Live Cells with CABL
☐CABL Improves the Efficiency of "Regular" Tetrazine Ligations



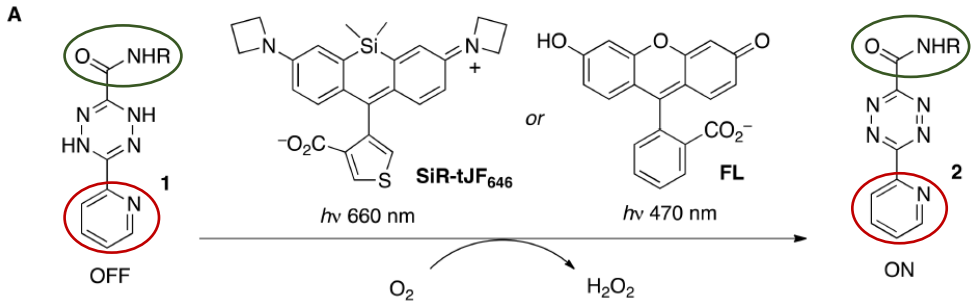
Enabling In Vivo Photocatalytic Activation of Rapid Bioorthogonal Chemistry by Repurposing Silicon Rhodamine Fluorophores as Cytocompatible Far-Red Photocatalysts

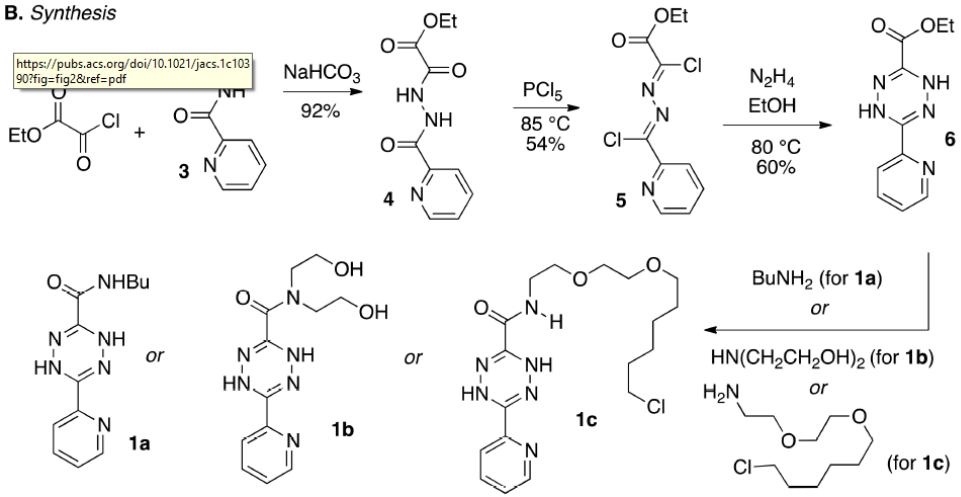


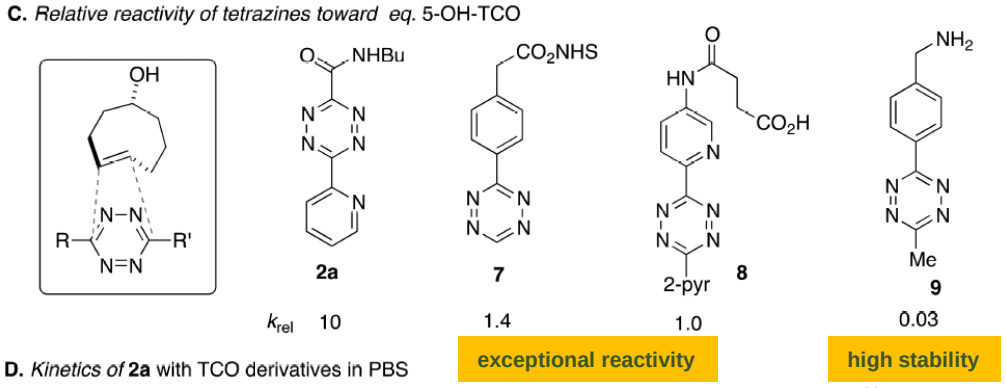
J. Phys. Chem. A **2014**, 118, 10622
J. Am. Chem. Soc. **2021**, 143, 10793

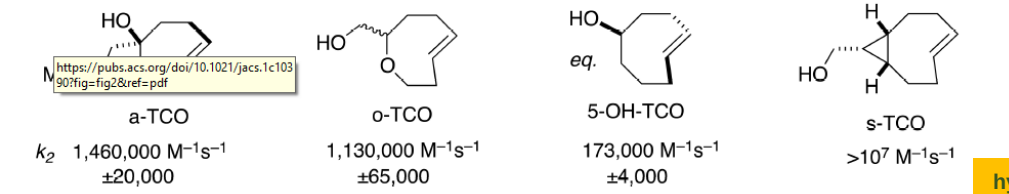
✓Fluorescence Imaging of Subcellular Targets in Live Cells Labeled by CABL
✓Live Cell, No-Wash Suborganelle Photopatterning
✓Spatiotemporally Resolved Labeling of Endogenous MAGL

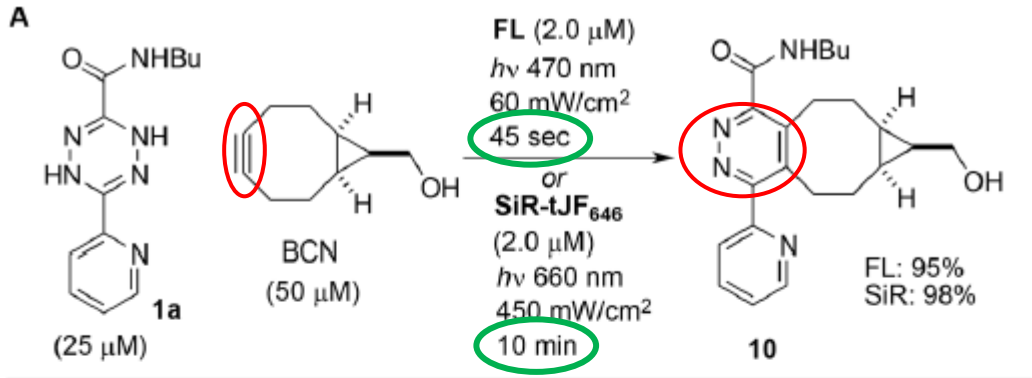
Synthesis and Evaluation of DHTz's with Improved Permeability, Stability, and Reactivity

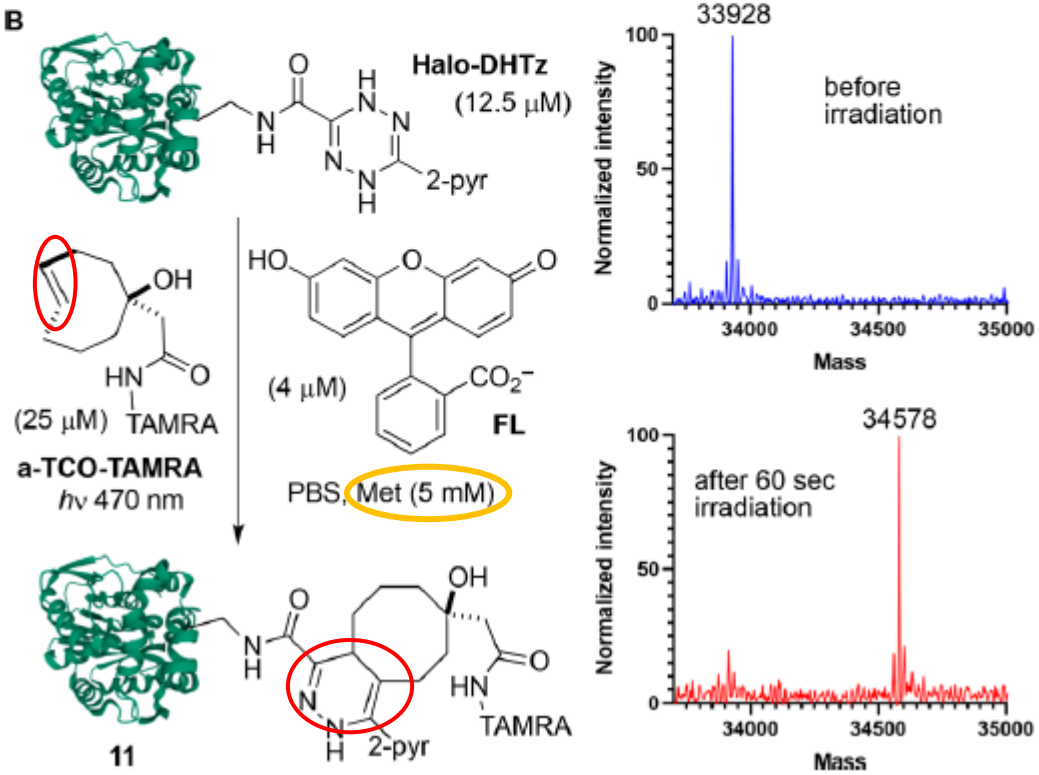










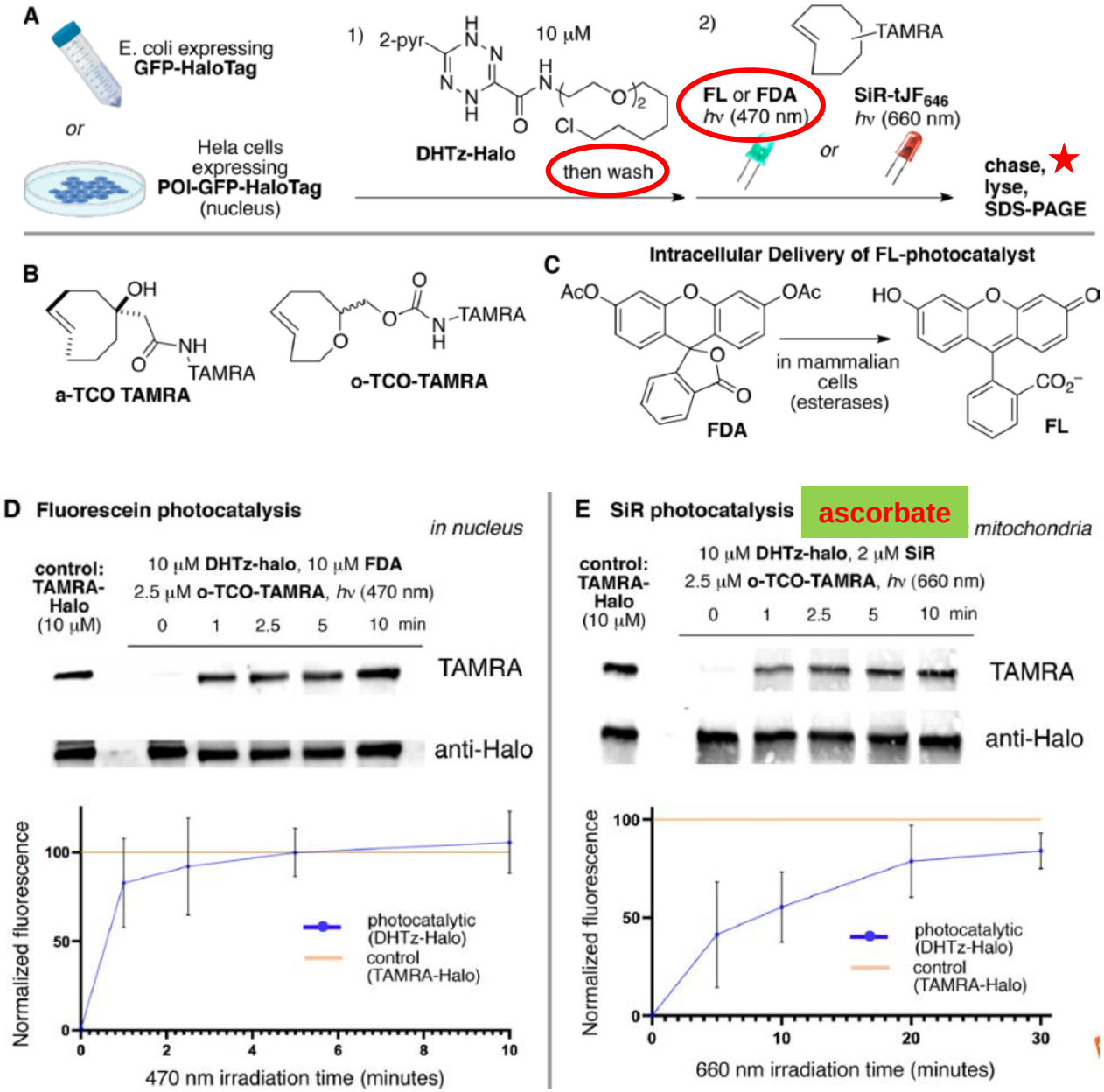


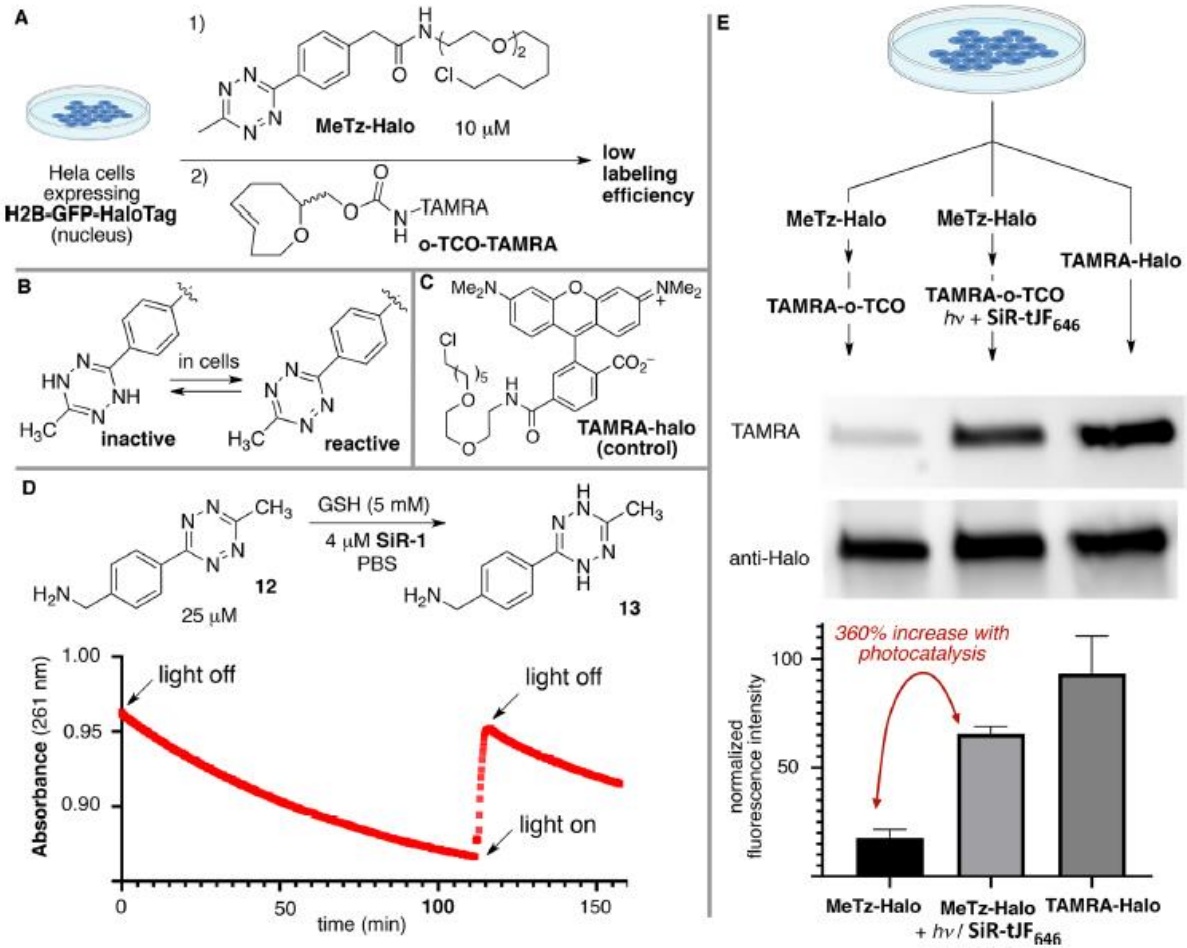
hydrophilicity and improved permeability

Labeling Live Cells with CABL

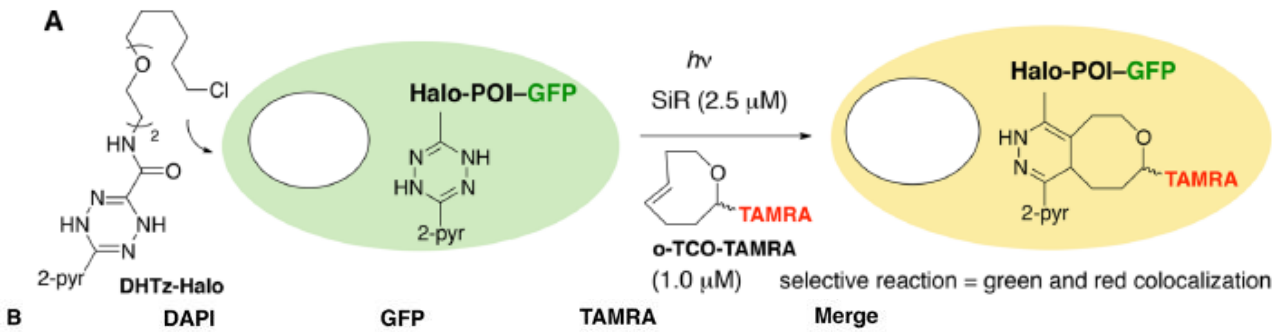
Improves the Efficiency of "Regular" Tetrazine Ligations

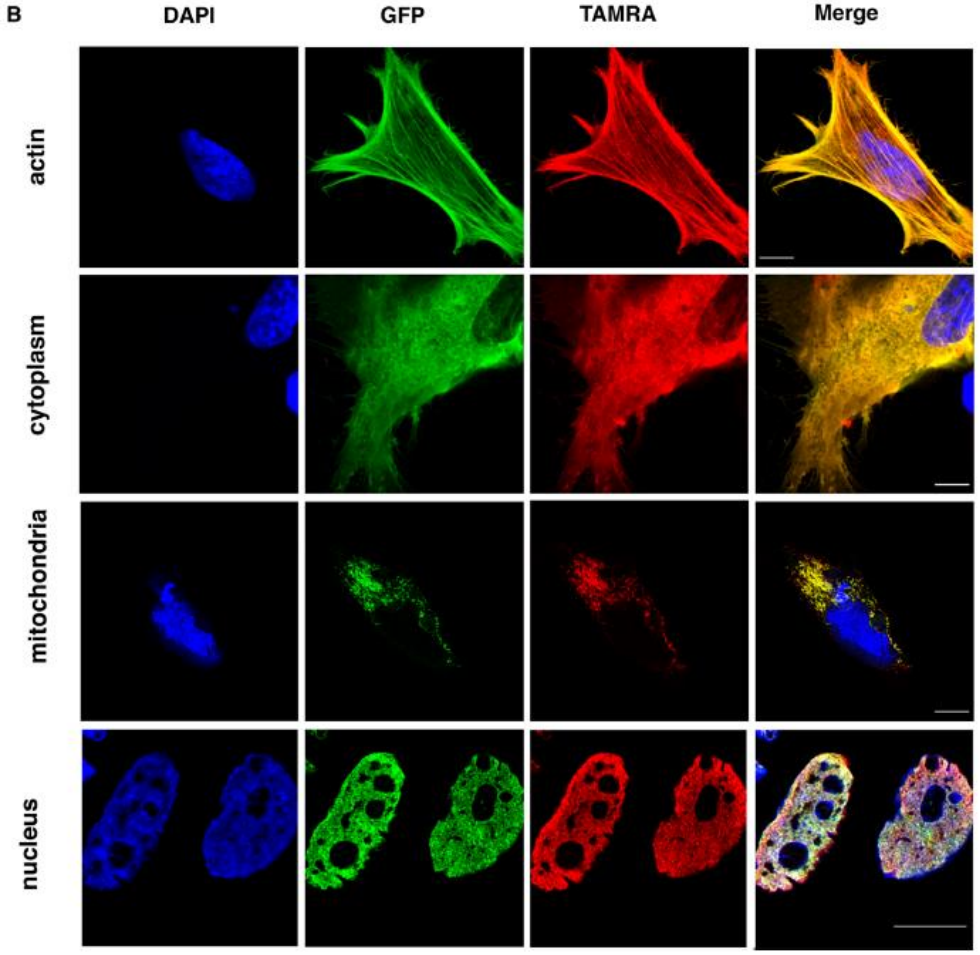
Other successful sensitizers (irradiation wavelength): acridine orange (528 nm), coomassie brilliant blue (528 nm), rhodamine B (590 nm), BODIPY (475 nm), safranin (528 nm), phenol red (528 nm), and carboxylfluorescein (528 nm).

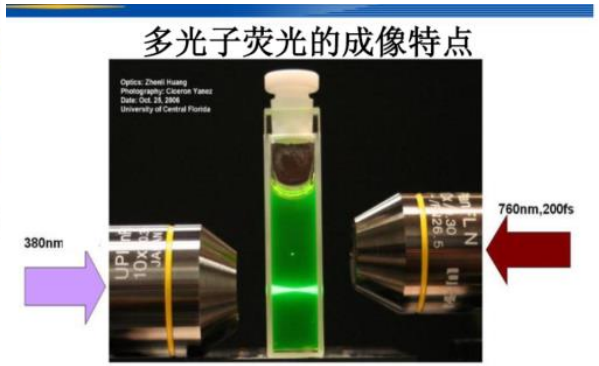




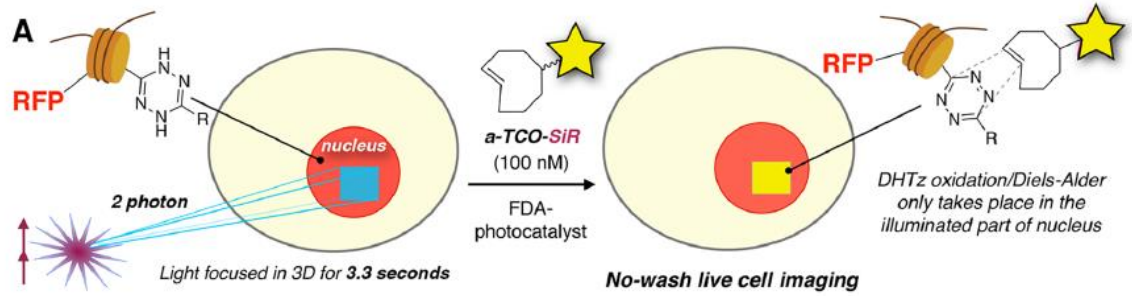
Fluorescence Imaging of Subcellular Targets in Fixed Cells

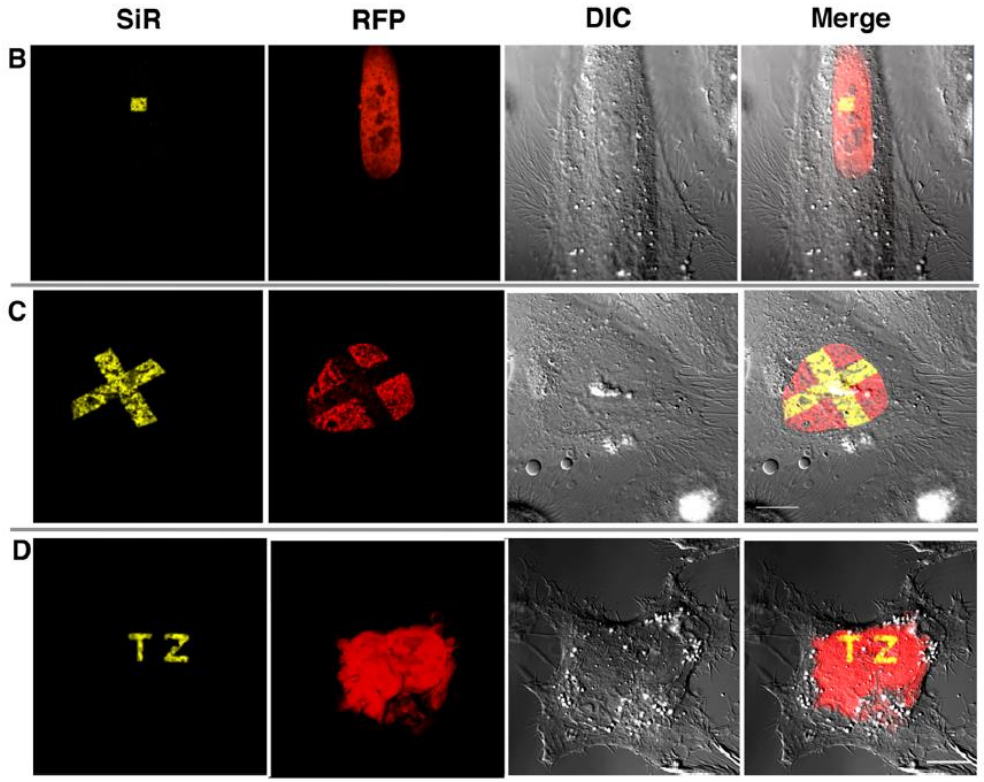




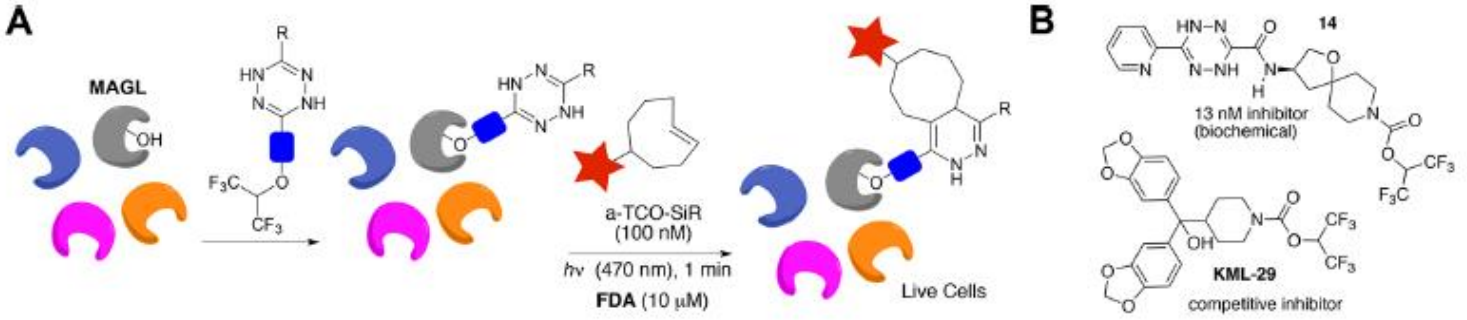


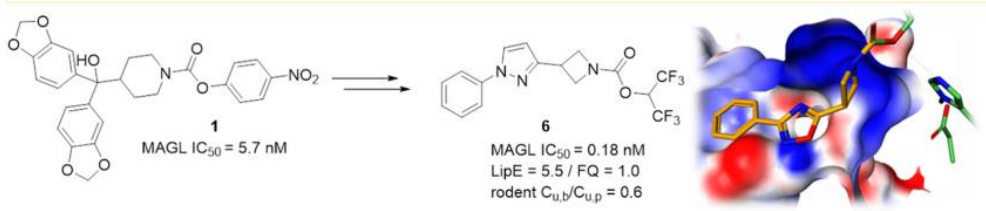
Live Cell, No-Wash Suborganelle Photopatterning





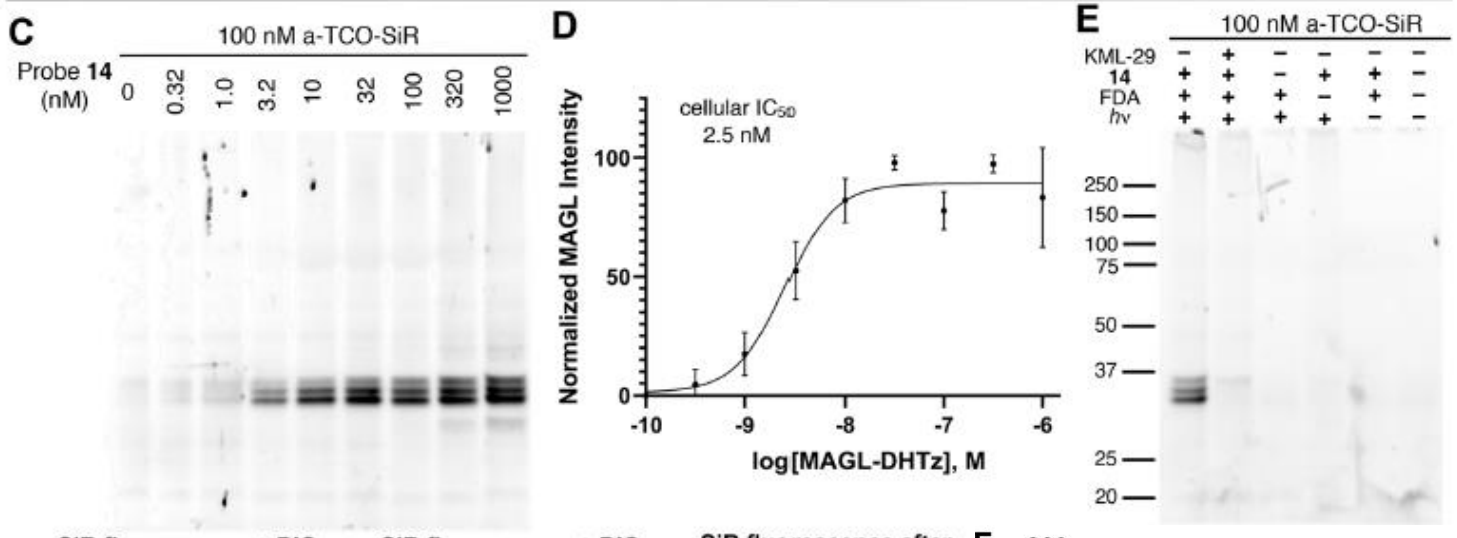
Spatiotemporally Resolved Labeling of Endogenous MAGL.

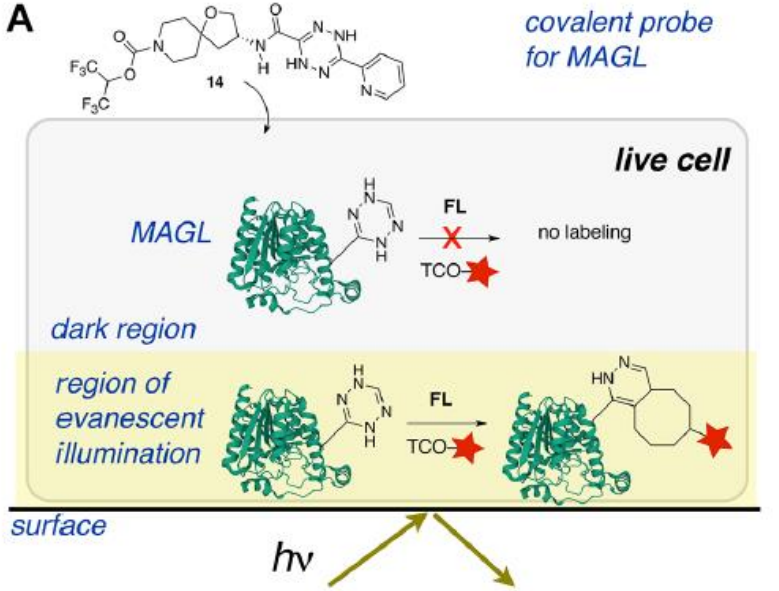


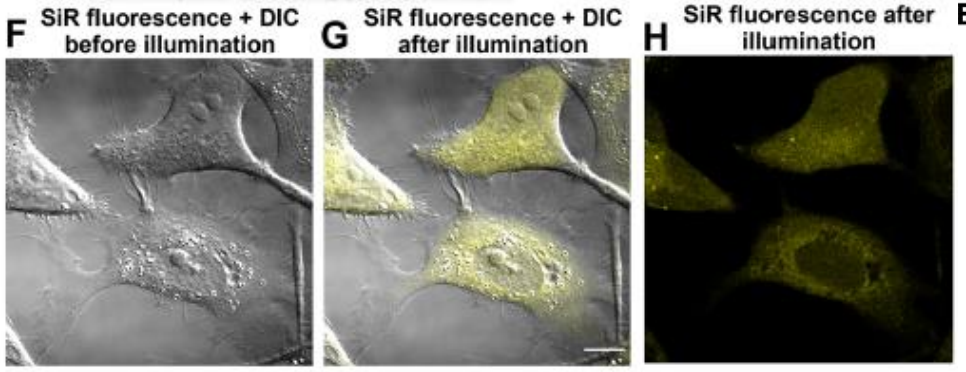


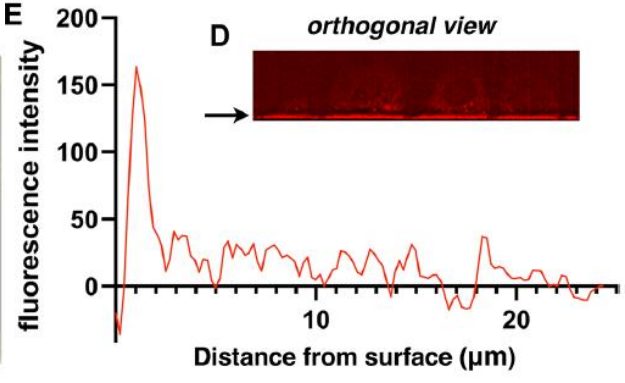
J. Med. Chem. **2017**, 60, 9860

9 (125 μM) was then added to quench any unreacted a-TCO-SiR









HeLa cells expressing HaloTag-H2B-mCherry and labelled with **DHTz-Halo**. Cells were treated with **a-TCO-SiR** (100 nM) and **FDA** (10 μM). The area circled in yellow (lower left) was irradiated with 3.3 second pulses of 880 nm light at 10% laser power, and the resulting increase in fluorescence as **a-TCO-SiR** is recruited to the nascent Tz is clearly visible. (Scale bar=10 μm)

HeLa cells expressing HaloTag-H2B-mCherry and labelled with **DHTz-Halo**. Cells were treated with **a-TCO-SiR** (100 nM) and **FDA** (10 μM). A square patch of a single nucleus is irradiated with a single pulse of 880 nm light at 25% laser power, leading to a rapid recruitment of **a-TCO-SiR** (Scale bar=10 μm)

HeLa cells expressing HaloTag-H2B-mCherry and labelled with **DHTz-Halo**. Cells were treated with **a-TCO-SiR** (100 nM) and **FDA** (10 μM). An “X” shape irradiated across the entirety of a single cell with a single pulse of 880 nm light at 25% laser power, leading to a rapid recruitment of **a-TCO-SiR** in the nucleus alone. This demonstrates that no off-target labeling was observed from non-specific binding to proteins not labeled with **DHTz-Halo**. (Scale bar=10 μm)

A z-stack was generated from the cell shown in **Fig. 7D**, demonstrating that CABL possesses a high degree of both axial and lateral resolution.

PC3 cells were labeled with probe **14** for one hour, then treated with **a-TCO-SiR** (100 nM) and **FDA** (10 μM). The cells were then exposed to pulses of 480 nm light at 2% laser power at regular intervals, demonstrating recruitment of **a-TCO-SiR** to endogenous MAGL. (Scale bar=10 μm)

Light-activated tetrazines enable live-cell spatiotemporal control of bioorthogonal reactions