NU Tech
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(B-30)

Photoresistant Fluorescent Dyes for Bioimaging

Background:

Degradation of fluorescent dyes when exposed to light, or photofading, often poses a problem in bioimaging by fluorescence microscopy. In particular, when applying super-resolution techniques, such as simulated emission depletion (STED) microscopy, the intense laser beams required for STED provoke rapid photofading of fluorescent probes. Photobleaching significantly limits the performance and practical utility. Even the most advanced photostable fluorophores currently available suffer from this limitation.

Technology Overview:

Researchers at Nagoya University have developed novel fluorescent dyes, based on a benzophosphole scaffold. C-Naphox and its analogues, along with NHS or maleimide derivatives for conjugation, constitute a series of photoresistant fluorescent dyes, Phox BrightTM, with a wide range of emission wavelength (ca. 440 - 610 nm), which allow for multi-colour imaging.

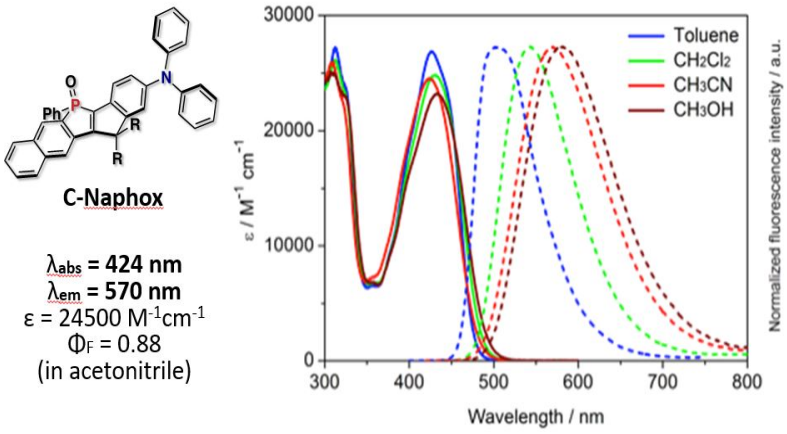

Figure 1: Molecular structure and photophysical data of a representative Phox BrightTM dye, *C-Naphox*

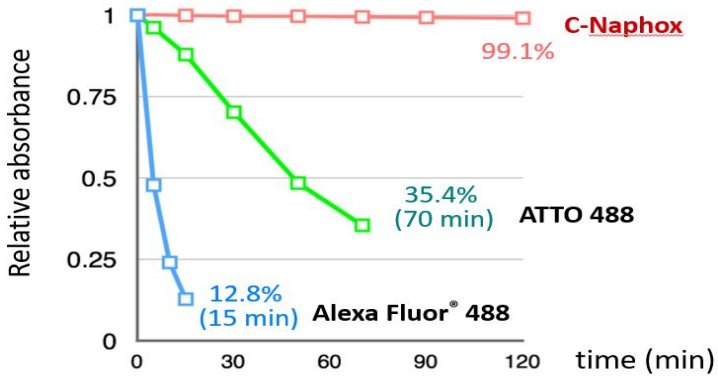

Figure 2: Photostability of C-Naphox, Alexa Fluor 488 and Atto 488 in *DMSO/HEPES buffer solutions (300 W Xe lamp (325 nm))* Xe lamp (325mm)

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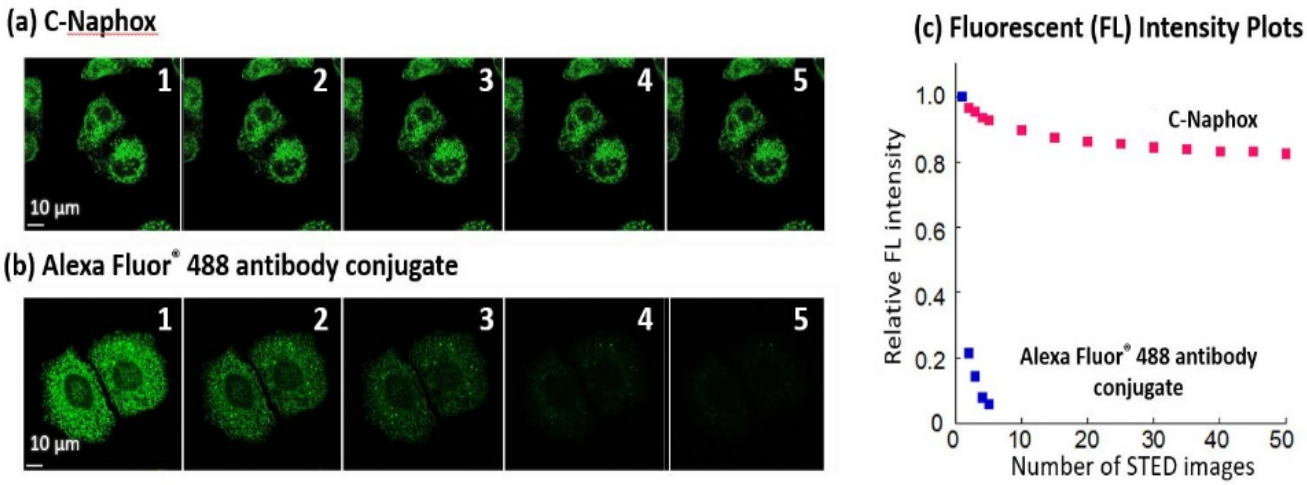

Repeated five STED images of cells stained with (a) C-Naphox and (b) Alexa Fluor® 488-conjugated anti-KDEL antibody. (c) Plots of normalized intracellular fluorescence intensity plots as a function of the number of recorded STED images. Normalization against the initial fluorescence intensity.
Irradiation sources: a tunable white-light excitation laser (488 nm, 80 MHz, output power 70%, AOTF 80%) and a CW-STED laser (592 nm CW laser, output power 95%, AOTF 100%) were used.

Figure 3: Photostability of C-Naphox and Alexa Fluor 488 under repeated STED imaging

Benefits:

- Exceptionally high resistance to photobleaching (Figure 1)
- Intense fluorescence (Figure 2)
- Large Stokes shift (Figure 3)

Further Details:

Wang et al., Angew. Chem. Int. Ed. 2015, 54, 15213–15217
Figure 3: Photostability of C-Naphox and Alexa Fluor 488 under repeated STED imaging

Potential Applications:

Super-resolution bioimaging
In-depth z-axis 3D imaging
Other uses of fluorescent dyes, where photobleaching causes a problem

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