Technology Offer

Photoactivable fluorescent dyes with hydrophilic caging groups and their use

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Abstract

This invention presents a series of photoactivatable fluorescent dyes characterized by their hydrophilic caging groups. These novel compounds are primarily designed for bioimaging applications, allow for controlled photoactivation at the desired wavelengths and exhibit enhanced solubility in aqueous environments. The technology facilitates high-precision imaging techniques, including super-resolution fluorescence microscopy and single-molecule tracking, by enabling precise spatial and temporal control over dye activation. Key to their utility, these photoactivatable labels possess enhanced biocompatibility and, in their activated form, are intact cell membrane-permeable and demonstrate fast binding kinetics to their intracellular targets, facilitating the detailed study of cellular processes and structures.

Advantages

- **Enhanced aqueous solubility:** The photoactivatable dyes are soluble in 100% water, eliminating the need for organic co-solvents and thus preserving the integrity of biological samples.
- **Controlled photoactivation and biocompatibility:** The compounds offer a precise control over the timing and location of dye activation with light. The photoactivation wavelength can be tuned by varying the substitution pattern of the protecting *ortho*-nitrobenzyl group. The fluorescent dyes released by photoactivation are intact cell-membrane permeant, stable in biological media, nontoxic and compatible with a variety of labeling strategies.
- **Photo-triggered cell-membrane permeability and fast labeling kinetics:** The photoactivatable dyes feature light-controlled cell-membrane permeability and labeling times on the order of seconds. This allows for spatially controlled, precise time stamping.
- **Excitation/emission wavelength tunability**: The technology offers the variety of excitation and emission wavelengths of the fluorophore to satisfy the equipment and imaging method constraints. This flexibility allows researchers to select the dyes according to their specific imaging requirements, including multi-color imaging experiments where signals from different fluorescent markers need to be distinctly resolved.
- **Superior imaging quality:** The hydrophilic caging groups and controlled activation reduce background noise, off-target binding and aggregation, significantly enhancing image clarity and detail.
- **Multiplexing capabilities**: The technology supports multiplexing with commercial fluorescent dyes and labels, enabling simultaneous observation of additional targets within the sample under investigation. This capability is crucial for studies requiring the visualization of interactions and dynamics between different biomolecules or cellular structures.
- **High photostability:** The photostability of both the caged and photoactivated labels prevents accidental uncaging and photobleaching under visible and infrared light. In STED imaging, resistance to two-photon activation by a high-power 775 nm laser ensures high contrast, improved resolution and enables longer imaging times.

Potential applications

- **Optical microscopy and bioimaging:** Versatile fluorescent labels and markers for FRET and BRET experiments with on-demand photoactivation, standard and advanced fluorescence microscopy and bioimaging studies.
- **Dynamic process monitoring:** Ideal for tracking dynamic biomolecular interactions and cellular processes in real time.
- **Nanoscale object localization:** Enables precise localization and study of nanoscale objects, down to single-molecule level.

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- **Fluorescent tagging and labeling:** Potential applications include protein, lipid and nucleic acid labeling and tracking, glycan analysis, flow cytometry, and as components in biosensors.
- **Receptor trafficking and cellular uptake studies:** Suitable for investigating receptor trafficking and cellular uptake mechanisms by live-cell fluorescence imaging.
- **Advanced super-resolution microscopy:** Compatible with STED, PALM, STORM, MINFLUX and MINSTED techniques for optical resolution below the diffraction limit.
- **Multiplex detection methods:** Photoactivation enables simultaneous detection of multiple targets per each region of interest within the sample, enhancing analytical capabilities.
- **Pulse labeling:** Temporal and spatially controlled cellular labeling for turnover, transport and time and space stamping studies.

Background

Previously reported and commonly used photoactivatable fluorescent dyes often suffer from low solubility in aqueous environments, poorly controlled activation and formation of non-fluorescent side products, leading to practical limitations of the labeling procedure and inferior imaging quality and resolution due to incomplete fluorescent labeling of the target structures. The hydrophilic caged fluorescent dyes of the present invention address all these challenges, providing a new improved tool for high-resolution imaging and biological research.

Technology

The technology centers on photoactivatable triarylmethane fluorescent dyes (Figure 1), where the amino groups are protected by 2-nitrobenzyl carbamates. The additional substituents on the benzyl ring enable tuning to specific photoactivation/deprotection wavelengths while also enhance the hydrophilic properties of the dyes, and diverse substitution of the fluorophore core permits a selection of excitation and emission wavelengths for the fluorescent ligands released upon photoactivation. The label photoactivation triggers its cell-membrane permeability (Figure 2), which may be used for precise initiation of live-cell labeling with a ligand of choice (e.g., HaloTag). This strategic design allows for precise control over the activation of the dyes in time and space in aqueous sample environments, making them perfectly suited for high-resolution imaging applications and detailed biological studies.

Fig. 1

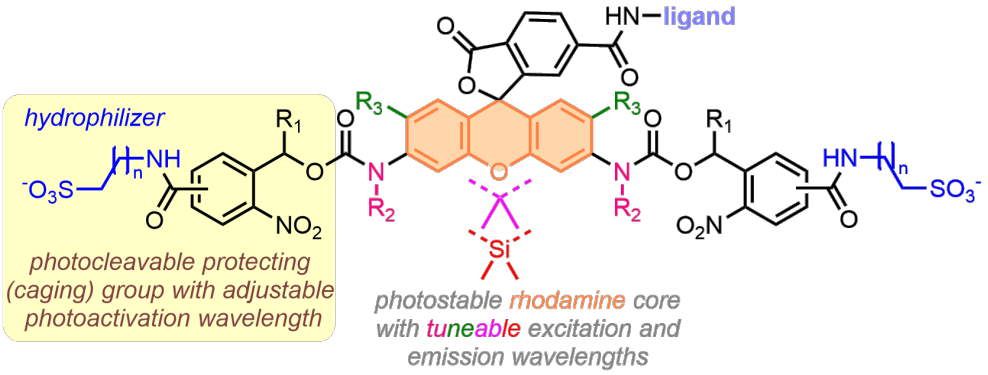

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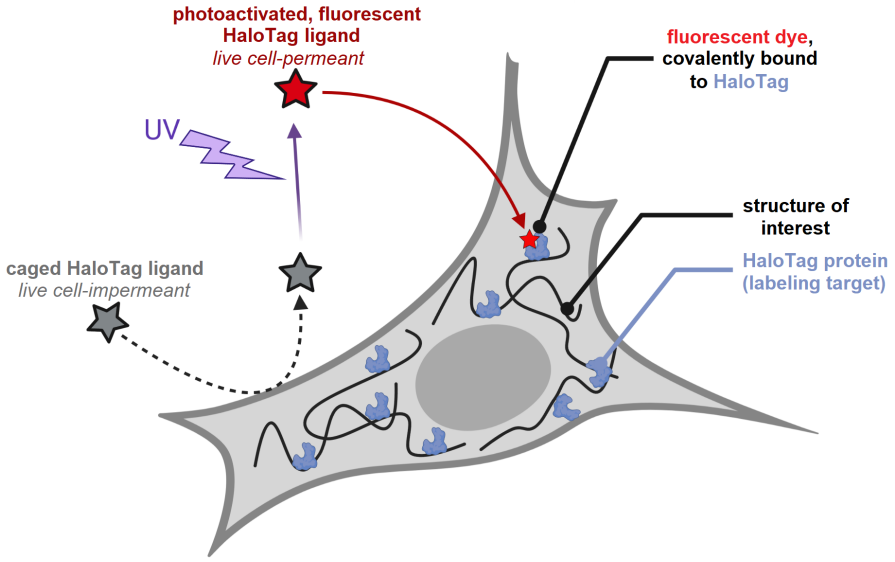
Fig. 2



Patent Information

EP application (EP3960817A1), US application (US20220064452A1)

Publications

A. N. Butkevich, M. Weber et al. Journal of the American Chemical Society 2021, 143, 18388-18393. >> https://doi.org/10.1021/jacs.1c09999

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