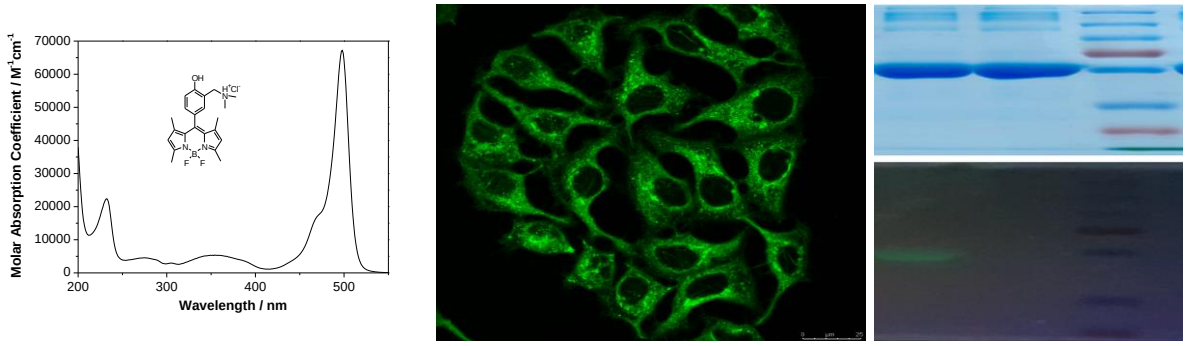
NEW PHOTOACTIVABLE BODIPY FLUORESCENT LABEL FOR PROTEINS

INVENTION

Researchers from the Ruđer Bošković Institute (Croatia) have developed new fluorescent BODIPY dyes that are substituted with photochemically reactive substituents, allowing for the photochemical staining of proteins and the use in fluorescent microscopy. The photochemical activation is based on the photodeamination reaction whereupon reactive intermeidates - quinone methides are formed.



Left figure shows absorption spectrum of BODIPY dye N2 in CH_3CN; Middle figure : confocal image of live MCF-7 cells stained with N2 (c = 10^{-6} M, λ_{exc} = 488 nm, λ_{em} = 500-550 nm; Right image: gel after deanturing electrophoresis; left line bovine serum albumin treated with N2 and irradiated at 350 nm; middle line, not irradiated, right line mark; upper gel stained with Comassie blue).

APPLICATION

Fluorescent photoactivable dyes can be used in biological experiments for visualization of cells and cell organelles in fluorescence microscopy, as well as in photoactivable staining of proteins prior to denaturing gel electrophoresis.

For example, BODIPY dye (N2) has a maximum of absorption at 500 nm (ε≈65000 $M^{-1}cm^{-1}$), and can be easily excited bay a laser at 488 nm. Fluorescence maximum is at 510 nm, and fluorescence quantum yield in aqueous solution Φ_f =0.25-0.30. The molecule is photochemically stable upon excitation with visible light, but upon excitation with UV light (350 nm), photodeamination to quinone methide takes place with efficiency of Φ_R = 0.19±0.03. Thus, irradiation of proteins in the presence of N2 with 350 nm light leads to photoattachment of the dye, which can be visualized by its fluorescence after gel denaturing elelctrophoresis.

ADVANTAGES OF PHOTOACTIVABLE BODIPY DYES

1) Bright fluorescent BODIPY dye which can be photoactivated upon excitation with UV-light, allowing spatial and temporal control of the activation;

2) Photostability of the dye upon excitation with visible light, compatible with high power lasers, enabling fluorescence readout without the dye bleaching or detachment from protein;

3) Non cytotoxic in the concentration used for labeling and microscopy.

STAGE OF DEVELOPMENT

The method is a proof-of-concept validated for several BODIPY dyes.

OPPORTUNITY

The BODIPY dyes are available for licensing. The RBI is seeking companies ready to start marketing these BODIPY dyes. The RBI research group is willing to support activities needed to produce the final products and to help shorten the time to market.

IP STATUS - granted patent in US, UK, Germany and France.

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