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Technology Offer

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Cell-Penetrating Fluorescent Dyes with Secondary Alcohol Functionalities

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Background

Fluorescent dyes are widely used as indispensable markers in biology, optical microscopy, and analytical chemistry. The availability and the proper choice of the dye is a key factor to success of the entire labelling and imaging procedure. Due to superior brightness and photostability, synthetic dyes often represent an attractive alternative to fluorescent proteins.

Among the multitude of fluorophores reported so far, only rhodamines, carbopyronines, and silicon-rhodamines bearing a carboxyl in the *ortho*-position of the pendant aromatic ring provide specific vital labelling and perform well in super-resolution fluorescence microscopy. However, the spectral variety of photostable fluorescent dyes suitable for intracellular targeting and super-resolution imaging in living cells is quite limited, and only few of them are commercially available. Many “bright” dyes suitable for labelling of living cells are unpolar and only slightly soluble in aqueous media. In general, a “fatty” character of a dye is a drawback, because lipophilic dyes are “sticky” and often bind unspecifically with off-target structures. This leads to a fluorescent background which masks the target and reduces signal-to-noise ratio. As a remedy against “stickiness”, charged residues are often attached to dye cores. Additional ionic groups increase the polarity of fluorophores; thus, background emission is eliminated and the contrast of the image is improved. However, anionic dyes suffer from low or even negligible cell membrane permeation, while cationic dyes are often toxic or bind unspecifically. Furthermore, the presence of polar ionic groups restricts the synthetic flexibility, requires special protecting groups and poses severe limitations on the post-synthetic modifications of these dyes.

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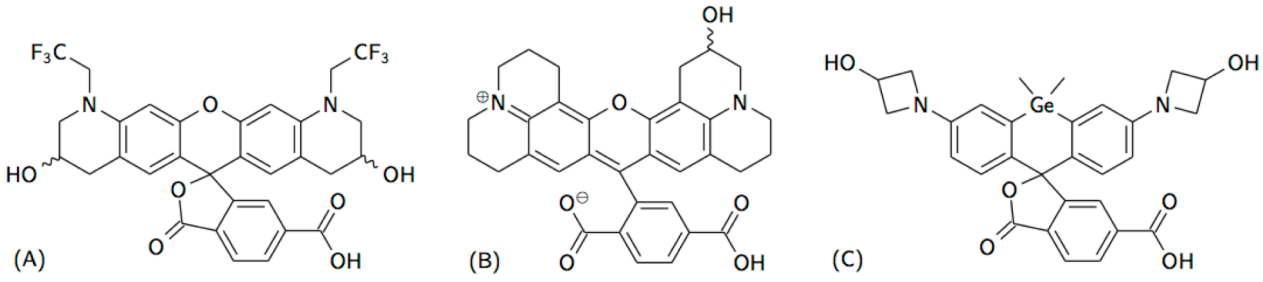
To address some of the above-stated limitations, hydroxyl groups are introduced into non-allylic and non-benzylic positions of the fluorescent dyes to increase polarity, improve solubility in water and prevent unspecific binding. The structures of some new dyes (emitting green and red light) are shown in scheme 1. The free carboxylic acid group in the dyes



Scheme 1: Examples of the hydroxylated cell-permeating fluorescent dyes with a reactive carboxylic acid group for coupling to site specific ligands. Absorption/emission maxima (aqueous PBS buffer) are 532/553 nm (A), 574/597 nm (B) and 631/651 nm (C), respectively. Extinction coefficients are in the range of 60 000–80 000 m^2/mol.

allows further modification, i. e. conjugation to small molecules (ligand, recognition unit) or to proteins. With these conjugates, not only cy£toskeleton proteins but also nuclear components have been stained specifically. In sub-micromolar concentrations – as recommended for imaging – the newly introduces dyes show no evidence of cytotoxicity.

The introduction of electron-withdrawing trifluoromethyl groups and/or hydroxyl groups (dye A in scheme 1) provokes only slight shifts in the absorption and emission spectra compared to the unfunctionalized fluorophores, whereas the equilibrium between the zwitterionic (open) and spirolactone (closed) forms shifts towards the latter, facilitating cell membrane penetration.

Apart from that, substitution of the oxygen atom in the xanthene backbone with a group 14 element atom (Si, Ge, Sn; dye C in scheme 1) leads to significant bathochromic shifts in the absorption and emission spectra. This enables the cross-talk-free two-color detection in living cells, including the STED super-resolution option. For instance, an optical resolution of about 60 nm was achieved by applying dye B and C from scheme 1 and using commercial 775 nm STED laser for the efficient “switching-off” of both dyes.

Advantages

- improved image quality due to reduced unspecific binding
- adjustable absorption and fluorescence spectra
- cell- and nucleus-permeant fluorescent dyes allow flexible single- and dual-color labelling in living cells (*in vitro* or *in vivo*)

Patent Information

EP patent application filed in September 2016.