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A highly selective ratiometric visual and red-emitting fluorescent dual-channel probe for imaging fluoride anions in living cells

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ABSTRACT

Recently, growing attention has been paid to the accurate determination of fluoride anion (F^-) in the environment and living systems for its toxicity and biological function investigation. In this paper, we developed a ratiometric visual and red-emitting fluorescent dual-channel probe (**1**) employed Si–O bond as a highly selective recognition receptor for imaging F^- in living cells. Probe **1** possesses a potential internal charge transfer (ICT) structure, and displays a large (158 nm) red-shifted absorption spectrum and the color changes from yellow to blue upon addition of F^- in the aqueous solution. In addition, probe **1** can be used to detect F^- quantitatively by the ratiometric absorption and turn-on fluorescence spectroscopy methods with excellent sensitivity. Finally, the results of its application to bioimaging of F^- in living cells show that probe **1** would be of great benefit to biomedical researchers for investigating the effects of fluoride in biological systems.

1. Introduction

Visual probes are widely developed because they have the capability to detect target analytes in the environment by the naked-eye, without the aid of any advanced instruments (Lin et al., 2010; Lan et al., 2012; Wang et al., 2013). Also, fluorescent probes are extensively exploited due to their high sensitivity, operational simplicity, and bioimaging analysis in living systems (Wu et al., 2011; Chen et al., 2012; Lee et al., 2013; Santos-Figueroa et al., 2013; Yang et al., 2013; Yuan et al., 2013b). Therefore, rational design of visual and fluorescent dual-channel probes for poisonous and biologically important species is of increasingly significant importance for their toxicity and biological function investigation in the environment and living systems.

Fluoride as a useful additive is commonly added to the toothpaste, pharmaceutical agents, and even drinking water for preventing dental caries and remedying osteoporosis (Horowitz, 2003; Cametti and Rissanen, 2009). However, excess intake of fluoride has been proven to result in fluorosis and urolithiasis (Arhima et al., 2004; Matsui et al., 2007). The U.S. Environmental Protection Agency (EPA) has set a maximum contaminant level (MCL) of 4 mg L^{-1} (4 ppm or 211 μM) and the Secondary Maximum Contaminant Level (SMCL) of 2 mg L^{-1} (2 ppm or 105 μM) in drinking water (Carton, 2006). Thus, the accurate determination of F^- is very important in the environment and living systems.

Despite advances in the development of visual or fluorescent probes for F^-, most of the reported examples still have some limitations in the determination and bioimaging (Hudnall et al., 2009; Kim et al., 2009; Hu et al., 2010; Bao et al., 2011; Cao et al., 2011; Lu et al., 2011; Zhang et al., 2011; Gai et al., 2012; Guo et al., 2012; Kim et al., 2012; Park et al., 2012; Du et al., 2013; Ke et al., 2013; Swamy et al., 2013; Xiong et al., 2013). First, some probes respond to F^- with the changes at the single wavelength intensity, which are prone to be disturbed by instrumental stability, sample environments and probe distribution. In contrast, ratiometric probes would eliminate most or all ambiguities by self-calibration of two wavelength intensities (Yang et al., 2009; Hu et al., 2010; Zhu et al., 2010; Yuan et al., 2011; Fan et al., 2013; Xiong et al., 2013). Additionally, the ideal ratiometric probe should display a large wavelength shift (> 80 nm) for practical use in the ratiometric determination (Lin et al., 2008; Chen et al., 2011; Xuan et al., 2012). Second, some probes based on hydrogen bonding or Lewis acid coordination have poor selectivity because they would be prone to be interfered from oxygen-containing basic anions, such as AcO^- and $H_2PO_4^-$ (Chen et al., 2008; Kumari et al., 2011). Third, most of the current probes could only be operated in organic solvents to detect tetrabutylammonium fluoride rather than inorganic fluoride salts (Kim and Hong, 2007; Bhosale et al., 2009; He and Yam, 2011; Lu et al., 2011; Cao et al., 2012; Im et al., 2013; Swamy et al., 2013). Lastly, most probes have short-wavelength excitation and emission spectra, which imposes difficulty on them in

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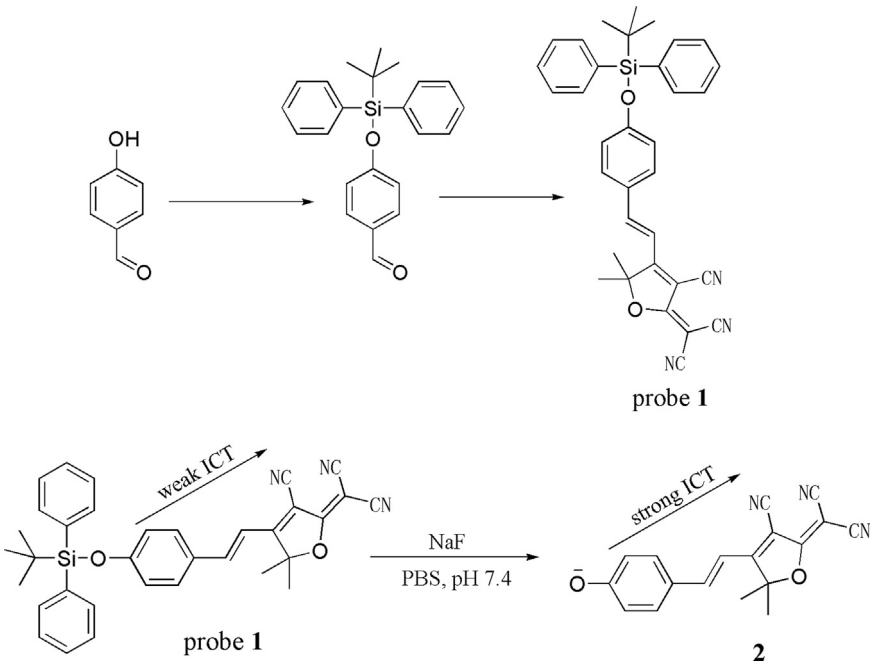

Scheme 1. The synthesis of probe **1** and the reaction mechanism of probe **1** with F^-.

the bioimaging of F^- in the living systems because strong absorption and autofluorescence of biomolecules in the short-wavelength region lead to low signal-to-noise ratios (Yuan et al., 2012a; Yuan et al., 2012b). Moreover, light in the short-wavelength region has limited tissue penetration for it is easily scattered and well-absorbed by biomolecules (Yuan et al., 2013a). A possible solution to the above-mentioned problems is to develop fluorescent probes with emission wavelengths in the far-red to near-infrared (NIR) (>600 nm) region (Sun et al., 2012; Yuan et al., 2013a).

Based on above considerations, we herein developed a highly selective ratiometric visual and red-emitting fluorescent dual-channel probe **1** (Scheme 1, **1**) with 158 nm absorption shift for detecting fluoride in aqueous solution and living systems. Probe **1** was constructed using a strong electron-withdrawing group, 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF), and a potential strong electron-donating group phenol derivative. Obviously, probe **1** possesses a latent internal charge transfer (ICT) structure. Additionally, to enhance the selectivity of probe, we chose a tert-butyldiphenylsilyl (TBDPS) moiety as the specific reaction site for F^- (Cametti and Rissanen, 2009; Zhu et al., 2011). The amphipathic structure of probe **1** containing the relatively hydrophilic TCF and lipophilic TBDPS would be favorable for both cell permeability and intracellular fluorescence imaging (Zhu et al., 2011). Fluoride-mediated cleavage of the Si–O bond releases a stronger phenolate donor, increasing the push–pull character of the probe and resulting in a higher quantum yield and a large bathochromic shift in the emission and absorption spectra. The reaction mechanism of probe **1** with fluoride is shown in Scheme 1.

2. Experimental

2.1. Materials and instrumentations

All chemicals used in this paper were obtained from commercial suppliers and used without further purification. 4-(tert-butyldiphenylsilyloxy)benzaldehyde and 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) were synthesized according to known procedures (Zhu et al., 2011; Moerner et al., 2005). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography.

^{1}H NMR and ^{13}C NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in $CDCl_3$, TMS as internal standard). Electrospray ionization (ESI) mass spectra were measured with an LC-MS 2010A (Shimadzu) instrument. Absorption spectra were recorded on a UV-3101PC spectrophotometer. Fluorescence emission spectra were measured on Perkin-Elmer Model LS-55. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2.2. General procedure for analysis

Parent stock solutions (100 mM) of F^- and other anions were prepared in ultra-pure water. Parent stock solutions of probe **1** (1 mM) were prepared in dimethyl sulphoxide (DMSO). Test solutions were prepared by placing 25 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each stock solution, and then diluting the solution to 10 mL with the mixture of ethanol and ultrapure water (7:3, v/v) containing phosphate buffered saline (PBS, 5 mM, pH 7.4). All measurements were made at room temperature (25 °C). All spectra were obtained in a quartz cuvette (path length = 1 cm).

2.3. Cell culture and fluorescence imaging

HeLa cells (gifted from the center of cells, Peking Union Medical College) were cultured in culture media (DMEM/F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 mg mL^{-1} of streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2. HeLa cells were seeded in a 96-well plate at a density of 10^4 cells per well in culture media. After 24 h, the cells were incubated with 10 μM probe **1** in culture media for 30 min at 37 °C, and then they were incubated with NaF (10 mM) for another 30 min.

Fluorescence imaging of living HeLa cells was observed under a confocal fluorescence microscope (excitation light source: 543 nm; Nikon C1-Si).