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Highly efficient and selective red-emitting Ca^{2+} probe based on a BODIPY fluorophore

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ABSTRACT

A red-emitting Ca^{2+} probe based on difluoro-boron-dipyrromethene (BODIPY) fluorophore and 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,-*N*′,*N*′-tetra acetic acid (BAPTA) moiety was designed and synthesized. Four electron-donating 4-methoxyphenyl groups were introduced on BODIPY to make the emission of probe more bathochromic-shifted. Upon Ca^{2+} binding, the probe exhibits a significant increase of red fluorescence intensity (λ_{max} = 631 nm, Φ_F = 0.18), an excellent luminescence ON/OFF ratio (43-fold) and a detection limit of 39 μM. Furthermore, this probe shows desirable sensitivity and selectivity for Ca^{2+} over other metal ions, which could be potentially applied for Ca^{2+} detection.

1. Introduction

Calcium ions (Ca^{2+}) play an indispensable role in the physiological and biochemical functions of the organisms and cells.[1–3] It displays significant effect of skeletal growth, function of neurotransmitter release from neurons and contraction of all muscle cell types.[4,5] Therefore, as a general technique for measuring the Ca^{2+} signals and spatiotemporal fluctuations of free Ca^{2+} concentration in the living cells, fluorescent Ca^{2+} probe has attracted great interest in the past decade.[6–8]

The probes for Ca^{2+} detection are commonly composed of an ionophore moiety for chelating Ca^{2+} and a chromophore for determining their photophysical properties. For selective Ca^{2+} recognition, one of the most well-known Ca^{2+} chelating moiety, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,-*N*′,*N*′-tetraacetic acid (BAPTA), was employed in many previous works because of its high selectivity for Ca^{2+} over other metal ions.[9] Meanwhile, the fluorophores featured excellent photophysical properties, such as rhodamine, cyanine and difluoro-boron-dipyrromethene (BODIPY), were usually selected as chromophores in building fluorescent probes.[10] Among these, BODIPY fluorophores have attracted great interest in the past two decades due to their rich photophysical properties, such as strong ground-state absorption, intense fluorescent emission, high photoluminescence quantum yield, insensitivity to the pH and high chemical stability.[11] In addition, their photophysical properties can be readily tuned by structural modifications, which provide additional opportunities to meet the different requirements for diverse applications.[12,13]

In the past decade, great attention has been devoted into BODIPY fluorophores because they have been widely employed in the field of electronics and optoelectronics. However, highly sensitive Ca^{2+} probes based on BODIPY fluorophore as core structure are still limited. Johnsson group reported a BODIPY-based probe bearing BAPTA moiety (BOCA-1), which shows a 250-fold increase in green fluorescence intensity upon Ca^{2+} binding.[14] Suzuki et al. reported a near-infrared Ca^{2+} probe composed of a BODIPY-based KFL-fluorophore and a BAPTA binding moiety (KFCA), which shows a prominent ON/OFF ratio (120-fold) and intense NIR fluorescence emission (670 nm, Φ_F = 0.24).[15] In 2015, by introducing branched polyethylene glycol chains on BODIPY fluorophore, Gao group reported a class of Ca^{2+} probes based on PEG–BODIPY–BAPTA conjugates, which exhibit high sensitivity and selectivity for Ca^{2+}, and can monitor changes in the intracellular Ca^{2+} signal.[16] These previous works indicate that linking BAPTA on BODIPY fluorophore is expected to give rise to a photoinduced electron transfer (PET) from the electron-rich ion chelating moiety to the electron-withdrawing fluorophore moiety.[17] In addition, the fluorescence of BODIPY is likely quenched with

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absence of Ca^{2+} due to this PET process. Therefore, when the Ca^{2+} is chelating to BAPTA, the fluorescence intensity of the probe could be enhanced, because the PET process from the electron-donating ability of the BAPTA moiety to the fluorochrome is prohibited.[18]

In this work, we report the design and synthesis of a new BODIPY–BAPTA based Ca^{2+} probe (Scheme 1), which exhibits high efficiency and selectivity for Ca^{2+} detection. In order to make the emission more bathochromic-shifted, four electron-donating 4-methoxyphenyl groups were introduced on the BODIPY core of the probe. This probe exhibits a significant increase in red fluorescence intensity (631 nm) upon Ca^{2+} binding and a detection limit of 39 μM. This new BODIPY-based probe should become valuable tools for visualizing Ca^{2+} and rational design of optical chemosensors.

2. Results and discussion

2.1. Synthesis

The synthetic strategy of BODIPY–BAPTA conjugate probe (**CaRB**) was obtained according to Scheme 1. 2,4-Bis(4-methoxyphenyl)-1H-pyrrole (**1**) and 5-formyl-5′-methyl-BAPTA ethyl ester (**2**) were synthesized according to published procedures.[19,20] BAPTA aldehyde **2** was initially reacted with 2,4-Bis(4-methoxyphenyl)-1H-pyrrole **1** to afford a dipyrrole derivative intermediate that was oxidised with *p*-chloranil to a dipyrrole derivative intermediate, which was then reacted with $BF_3{\cdot}Et_2O$ to produce BODIPY–BAPTA ester **3**. Meanwhile, the synthesized ester **3** was transformed into the corresponding potassium salts *via* saponification with 10 equiv. of KOH under mild conditions in the mixture solvent of methanol and H_2O, and the mixture was stirred for 3 days. The subsequent neutralization with 1 M HCl aq. induced **CaRB.** The synthesized target compounds were characterized by ^1H NMR, ^{13}C NMR and MS.

2.2. Photophysical properties of probe

The probe **CaRB** was investigated toward the sensing abilities of Ca^{2+} in MeOH–MOPS buffer solution (1:1, v/v). As shown in Fig. 1, the initial solutions containing probes only exhibited very weak fluorescence ($\Phi_F = 0.009$). After 10 equiv of Ca^{2+} was added to the solutions containing probes (pH = 7.2, 5 μM), the fluorescent intensity increased sharply ($\Phi_F = 0.18$). The response are so rapid in that the fluorescence of probe with Ca^{2+} enhanced within 5 s.

As we can see in Fig. 2, the fluorescence intensity of **CaRB** without free Ca^{2+} solution was extremely neglectful to be distinguished from the baseline due to the PET process.[21,22] Conversely, when the **CaRB** probe was added in MeOH–MOPS buffer solution of Ca^{2+} (0–39 μM), it shows a gradually enhanced fluorescence. Especially, a 43-fold enhanced fluorescence which the maximum emission wavelength was located at 631 nm was observed when the probe solution was in presence of 39 μM Ca^{2+}. The lone pair of electrons with amino group of the BAPTA moiety is bound to Ca^{2+}. Therefore, Ca^{2+} coordination weakens the PET process and the fluorescent intensity of the probe is expected to increase. In addition, with the increasing of the concentration of Ca^{2+}, the fluorescence emitting wavelength showed a slight red shift (2 nm). This is attributed to the Ca^{2+} binding, which influenced the BAPTA amino nitrogen from donating electron density to the 8-position of BODIPY. Electron withdrawal or donation to the central carbon of BODIPY dyes are known to shift emission peaks to longer or shorter wavelengths.[9,23]

The Ca^{2+} probe was potentially interfered with the recognition of Ca^{2+} when it was exposed to ionic environments. The interference of other metal ions on calcium ions chelation should be explored. Fig. 3 displays the relatively selective and competitive measurements of **CaRB** to other metal ions. The results of the investigation indicate that Cu^{2+}, Fe^{2+}, Mg^{2+}, Ba^{2+}, Cr^{3+}, Al^{3+}, Fe^{3+}, K^+ and Na^+ slightly influenced the fluorescence intensity of **CaRB**. In contrast, the metal ions such as Co^{2+}, Pb^{2+}, Ni^{2+} and Cd^{2+} distinctly affected the fluorescent intensity, suggesting that Ca^{2+}

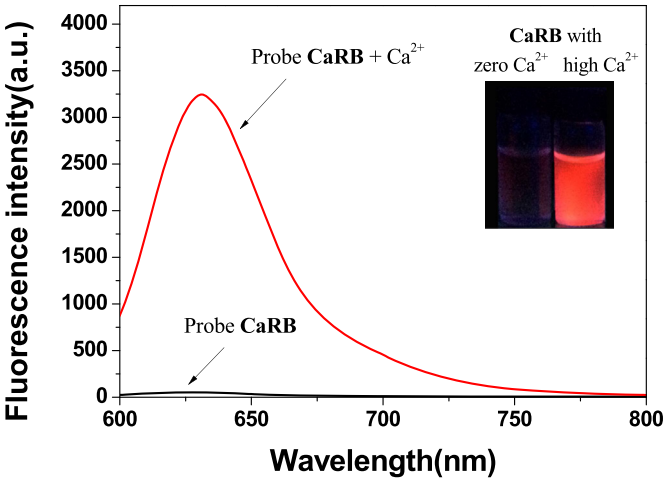


Fig. 1. Fluorescent spectral change of 5.0 μM probe **CaRB** in presence of 50.0 μM Ca^{2+}(MeOH–MOPS buffer solution, 1:1, v/v). The inset shows the photographs of **CaRB** in the absence of Ca^{2+} and in the presence of free Ca^{2+} under UV irradiation (365 nm).

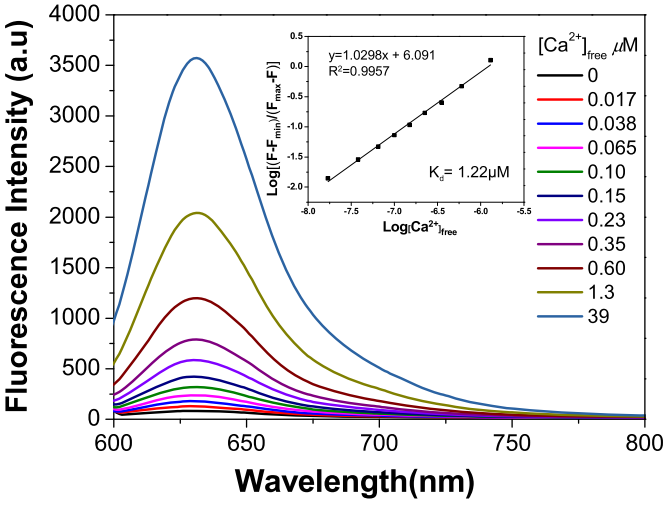
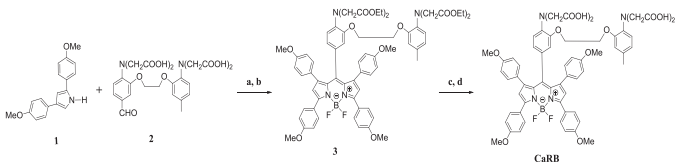


Fig. 2. Emission spectra of **CaRB** (1 μM) at varying Ca^{2+} concentrations (0–39 μM) in MeOH–MOPS buffer solution (1:1, v/v) containing 3-(*N*-morpholino) propanesulfonic acid (30 mM), KCl (100 mM) and ethyleneglycol tetraacetic acid (EGTA; 10 mM) at pH 7.2 and 20 °C. Excitation wavelength was 584 nm. Inset: Determination of the K_d value of **CaRB** using a plot of log $[(F-F_{min})/(F_{max}-F)]$ against $\log[Ca^{2+}]_{free}$ (M).



Scheme 1. Synthesis of **CaRB.** (a) DDQ, TFA, CH_2Cl_2, r.t.; (b) DIETA, $BF_3{\cdot}OEt_2$, CH_2Cl_2, r.t.; (c) KOH, MeOH, H_2O, r.t.; (d) HCl, H_2O, r.t..