

Accepted Manuscript

Title: BODIPY-Derived Piperazidine Fluorescent
Near-Neutral PH Indicator and Its Bioimaging

Author: Min Deng Chengduan Yang Deyan Gong Anam Iqbal
Xiaoliang Tang Weisheng Liu Wenwu Qin



PII: S0925-4005(16)30467-1
DOI: <http://dx.doi.org/doi:10.1016/j.snb.2016.04.003>
Reference: SNB 19977

To appear in: *Sensors and Actuators B*

Received date: 18-1-2016
Revised date: 31-3-2016
Accepted date: 1-4-2016

Please cite this article as: Min Deng, Chengduan Yang, Deyan Gong, Anam Iqbal, Xiaoliang Tang, Weisheng Liu, Wenwu Qin, BODIPY-Derived Piperazidine Fluorescent Near-Neutral PH Indicator and Its Bioimaging, *Sensors and Actuators B: Chemical* <http://dx.doi.org/10.1016/j.snb.2016.04.003>

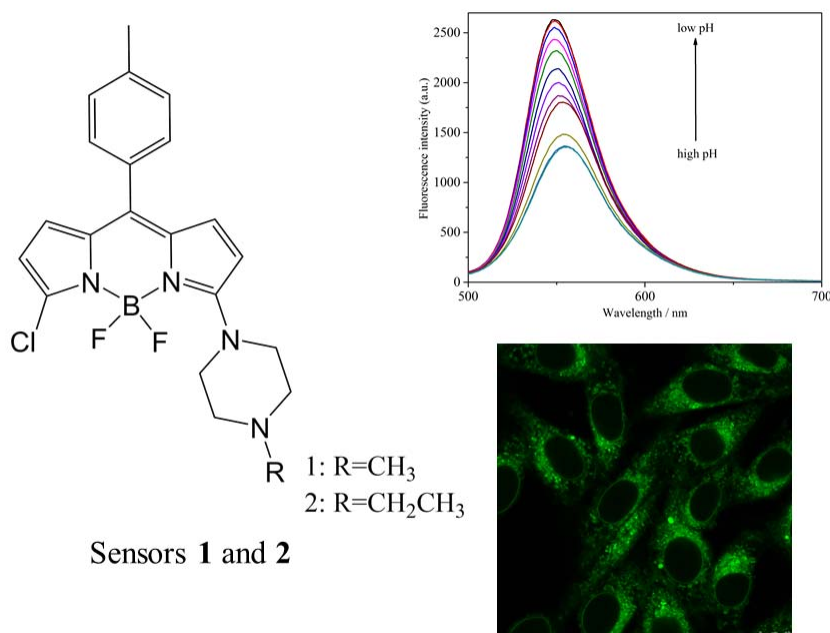
This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

BODIPY-Derived Piperazidine Fluorescent Near-Neutral PH Indicator and Its Bioimaging

Min Deng, Chengduan Yang, Deyan Gong, Anam Iqbal, Xiaoliang Tang, Weisheng Liu, and Wenwu Qin¹

Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province and State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China.

Graphical Abstract



¹ Corresponding author: Fax: +86-931-8912582 E-mail address: qinww@lzu.edu.cn

(W. Qin)

Highlights

- Two BODIPY derivative dyes **1** and **2** were synthesized and their crystal structures were obtained.
- Dyes **1** and **2** were with pK_a values near-neutral (around 6.5 and 6.8).
- Confocal microscopy experiments showed that **1** and **2** can be used in living cells.

Two new 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes (**1** and **2**) with piperazine subunits on position 3 or 5 were synthesized. Their absorption and steady-state fluorescence properties were investigated as a function of solvent. In water/MeCN (9:1, V/V) solution, the dyes show fluorescent enhancement upon increasing the acidity of the solution. They can be used in water/MeCN (9:1, V/V) solution as fluorescent pH probes excitable with visible light, with pK_a values near-neutral (around 6.5 and 6.8). Dyes **1** and **2** are readily taken up by biological cells in the cytosol where it has no obvious toxic effects.

Keywords: BODIPY; piperazine; near-neutral pH; biological cells;

1. Introduction

Fluorescent pH probes are obligato tools for measuring changes of intracellular proton concentrations[1]. Measurement of pH by fluorescence-based technique is well established for both imaging and sensing applications[1, 2]. Compared to most

other techniques, the fluorescence-based technique offers significant advantages owing to its generally nondestructive character, low background noise, high sensitivity and specificity, and the wide range of indicator dyes available[3-7]. To quantitatively determine pH, it is imperative to match the pK_a of the probe to the pH of the investigated system. Since the pH in the cytosol is close to neutral (generally between ~6.8 and 7.4), fluorescent probes with a pK_a around 7 are necessary for cytosolic pH determinations.

Due to their excellent characteristics, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene[8-10] (better known as difluoroboron *dipyrr*romethene or BODIPY) derivatives may be important beneficial fluorophores in the design of fluorescent sensors. The excellent qualities of BODIPY comprised relatively high fluorescence quantum yields and molar absorption coefficients, narrow emission bandwidths with high peak intensities, high elevated stability towards chemicals and light, and the extra feature of excitation / emission wavelengths in the visible region. Furthermore, the spectroscopic properties of BODIPY derivatives can be fine-tuned by synthetically introducing suitable substituents at the right positions of the BODIPY[8] core.

Difluoroboradiaza-*s*-indacenes with hydroxyaryl subunits have been reported as pH probes in aqueous-organic mixed media[11, 12]. These on / off pH probes showed a photo-induced electron transfer (PET) from the twisted and conjugatively uncoupled phenolate to the BODIPY subunit, causing fluorescence quenching at high pH. 8-(4'-Hydroxyphenyl) substituted BODIPY derivatives[11] sense the alkaline pH

range, while the tetrahydroxylated calix[4]arene derivative[12] is sensitive in the near-neutral pH range. In our previous work, we have synthesized and spectroscopically characterized two water-soluble, on/off pH indicators with pK_a around 7.5 based on *o*-chlorophenol linked to the *meso* (8-) position[13, 14] or via a vinyl bridge to the 3-position[15] of BODIPY. A series of pH indicators based on BODIPY with *meso*-anilino substituents have been described recently for imaging acidic endosomes in cancer cells.[16] These derivatives are almost nonfluorescent in basic media due to PET.

We chose N-methylpiperazine[17] and N-ethylpiperazine with the favorable pK_a around 9 to serve as pH-sensitive ligand for the near-neutral pH range. Substitution of piperazine will generally lead to altered the pK_a values. Due to the electron-withdrawing character of BODIPY, it is expected that conjugatively linking BODIPY fluorescent reporter subunit via an ethenyl bridge to piperazine will decrease the pK_a of piperazine in sensors **1** and **2** by approximately 1 unit compared to free piperazine[18].

2. Experimental section

2.1 Instrument and reagent

All chemical solvents and reagent were obtained from Bailingwei. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 and DRX-400/4 spectrometer with tetramethylsilane (0.00 ppm) as an internal standard and CDCl_3 as solvent. Chemical shift multiplicities are reported as s=singlet, d=doublet, t=triplet, q=quartet and m=multiplet. ^{13}C spectra were referenced to ppm with the CDCl_3 (77.67 ppm)

signal. Mass spectra were recorded in E.I. mode. Melting points were obtained with an X-4 precise micro melting point cryoscope (Beijing Fukai Instrument Co.) and are uncorrected. All measurements were performed at room temperature.

2.2 The synthesis of sensor 1

BODIPY derivatives **1** and **2** were prepared using the same procedure, starting from 3,5-dichloro-4,4-difluoro-8-(4-tolyl)-4-bora-3a,4a-diaza-*s*-indacene (**3**).

The synthetic route is depicted in Scheme 1. 150 mg (0.43 mmol) of compound **3** was dissolved in 25 ml acetonitrile and 0.5 ml of triethylamine was added to it. After 15 minutes, 48 μ l (0.43 mmol) of compound N-methylpiperazine was added to it. The reaction mixture was stirred at room temperature for 72 h. After solvent was removed under reduced pressure, the crude solid was purified by column chromatography on silica purified through column chromatography over silica (dichloromethane/methanol, 50/1, v/v) to obtain a reddish brown powder 130 mg of **1** with 75 % yield. Reddish solid, mp 127-128 °C; ^1H NMR (CDCl_3 , 400 MHz) δ 2.36 (s, 3H, H-a), 2.41 (s, 3H, H-b), 2.63 (t, 4H, $J = 4.8$ Hz, H-c), 3.99 (t, 4H, $J = 4.8$ Hz, H-d), 6.18 (d, 1H, $J = 4.0$ Hz, H-e), 6.246 (d, 1H, $J = 5.2$ Hz, H-g), 6.28 (d, 1H, $J = 4.0$ Hz, H-f), 6.848 (d, 1H, $J = 5.2$ Hz, H-h), 7.22 (d, 2H, $J = 8.0$ Hz, aromatic), 7.29 (d, 2H, $J = 8.0$ Hz, aromatic); ^{13}C NMR (CDCl_3 , 100 MHz): δ 21.45, 45.74, 50.29, 54.96, 113.03, 113.72, 119.02, 128.98, 129.50, 130.46, 131.35, 131.45, 131.55, 135.15, 135.64, 139.26, 162.01; MS (ESI) m/z calcd. For $\text{C}_{21}\text{H}_{22}\text{BClF}_2\text{N}_4$ 414.2; found 415.2 (M+1); 395.2 (M-F, 100%).

2.3 The synthesis of sensor 2

The crude solid was purified by column chromatography on silica purified through column chromatography over silica (dichloromethane/methanol, 50/1, v/v) to obtain a reddish brown powder 135 mg of **2** with 73 % yield. Reddish solid, mp 150-152 °C; ^1H NMR (CDCl_3 , 400 MHz) δ 1.12 (t, 3H, $J = 7.2$ Hz, H-a'), 2.41 (s, 3H, H-b), 2.49 (q, 2H, $J = 7.2$ Hz, H-a), 2.67 (t, 4H, $J = 4.8$ Hz, H-c), 4.00 (t, 4H, $J = 4.8$ Hz, H-d), 6.177 (d, 1H, $J = 4.0$ Hz, H-e), 6.25 (d, 1H, $J = 5.2$ Hz, H-g), 6.27 (d, 1H, $J = 4.0$ Hz, H-f), 6.844 (d, 1H, $J = 5.2$ Hz, H-h), 7.22 (d, 2H, $J = 8.0$ Hz, aromatic), 7.29 (d, 2H, $J = 8.0$ Hz, aromatic); ^{13}C NMR (CDCl_3 , 100 MHz): δ 11.87, 21.44, 50.41, 52.13, 52.80, 112.94, 113.78, 118.80, 128.96, 129.29, 130.46, 131.27, 131.34, 131.49, 135.20, 135.60, 139.20, 161.91; MS (ESI) m/z calcd. For $\text{C}_{22}\text{H}_{24}\text{BClF}_2\text{N}_4$ 428.2; found 429.2 (M+1, 100%).

< Scheme 1 >

2.4. Determination of K_a from Direct Fluorimetric Titration

The K_a values of **1** and **2** were determined in water/MeCN (9:1, V/V) solution at 25 °C by direct fluorometric titration as a function of pH using the fluorescence emission spectra. Nonlinear fitting of Eq.1[19, 20] to the steady-state fluorescence data F recorded as a function of $[\text{H}^+]$ yields values of K_a , the fluorescence signals $F_{\min}$ and $F_{\max}$ at minimal and maximal $[\text{H}^+]$, respectively, and n (the number of protons bound per fluorescent pH probe).

$$F = \frac{F_{\max} [\text{H}^+]^n + F_{\min} K_a}{K_a + [\text{H}^+]^n} \quad (1)$$

Because the fits of Eq. 1 to the fluorescence data F with n , K_a , $F_{\min}$, and $F_{\max}$ as

freely adjustable parameters always gave values of n close to 1, n was kept fixed at 1 in the final curve fittings, from which the estimated values of K_a are reported here. Equation 1 assumes (i) photo-excitation with light of constant incident spectral radiant power, (ii) that the absorbance A of the sample is small (≤ 0.1) and (iii) the rate of proton binding in the excited state can be neglected.

2.5. General method for measurements of photophysical properties

UV-vis absorption spectra were recorded on a Varian UV-Cary 5000 spectrophotometer and for the corrected steady-state emission spectra, a Hitachi F-7000 spectrofluorimeter was employed. Freshly prepared samples were used to perform all UV-vis absorption and emission measurements. For the determination of the fluorescence quantum yields ϕ_f of **1** and **2**, only dilute solutions with an absorbance below 0.1 at the excitation wavelength ($\lambda_{\text{ex}} = 480 \text{ nm}$) were used. Rhodamine 6G in water ($\phi_f = 0.76$) was used as fluorescence standard [21]. The ϕ_f values reported in this work are the averages of multiple (generally three), fully independent measurements. In all cases, correction for the solvent refractive index was applied.

All measurements were done at 25 °C with an air conditioner.

2.6. Crystal structure determination

Crystals of **1** were obtained by slow evaporation of the mixed solvent (dichloromethane/cyclohexane) (2:1, v/v) in air over one weeks, yielding clear dark orange block crystals with approximate dimensions of $0.18 \times 0.14 \times 0.11 \text{ mm}^3$ suitable for X-ray diffraction.

Crystals of **2** were obtained by slow evaporation of the ethyl acetate in air over two weeks, yielding clear dark violet block crystals with approximate dimensions of $0.34 \times 0.32 \times 0.31 \text{ mm}^3$ suitable for X-ray diffraction. The basic crystallographic data of **1** and **2** are compiled in Table S1.

The data for **1** and **2** was measured on a Bruker Smart APEX II CCD diffractometer at $155 \pm 10 \text{ K}$ and $297 \pm 10 \text{ K}$ respectively equipped with graphite-monochromatized Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$ and $\lambda = 1.54184 \text{ \AA}$). The structures were solved by direct methods. All non-hydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares methods on F^2 by using the program package SHELXS-97[22]. Hydrogen atoms were placed at calculated positions.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1443775 for **1** and CCDC 1443776 for **2**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/conts/retrieving.html).

2.7. Cell culture, fluorescence imaging and cytotoxicity assay

Cell Culture and fluorescence imaging. To determine the cell permeability of **1** or **2**, Baby Hamster Syrian Kindey (BHK) cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (fetal bovine serum). The cell lines were maintained in a humidified atmosphere containing 5% CO_2 at 37°C . After

removal of the culture medium, cells were incubated with **1** or **2** (10 μ M) in 1.0 mL of fresh culture medium for 2 h. Before imaging measurement, the cells were washed three times with PBS to remove the residual **1** or **2**. Cells were observed under an Olympus FV1000-IX81 laser confocal microscope.

Cytotoxicity Test. BHK Cells were seeded at a density of 10^4 cells per well (100 μ L total volume/well) in 96-well assay plates for 24 h. Then, the as-prepared **1** or **2**, at the indicated concentrations (0, 5, 10, 15, 20, 25 μ g / ml), were added to the cell culture medium. Cells were incubated with **1** or **2** for 24 h. To determine toxicity, 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salts (MTS) was added to each well of the microtiter plate and the plate was incubated in the CO₂ incubator for additional 4 h. Absorbance values were determined with Bio-Rad model-680 microplate reader at 490 nm (corrected for background absorbance at 630 nm). The cell viability was estimated according to the following equation: cell viability (%) = mean of absorbance value of treatment group/mean absorbance value of control $\times$ 100%.

2.8. Theoretical calculations

The calculation was performed using the Gaussian 09 suite of programs[23]. The ground state structures of two compounds were based on the corresponding crystal structures and were not further optimized. The density functional theory (DFT) with Becke's three parameter hybrid functional with the Lee–Yang–Parr correlation functional (B3LYP) and 6–31+G(d) basis set were used to treat all atoms. The excited state related calculations were carried out with the time dependent density functional

theory (TD-DFT) with the ground state structure was obtained. There are no imaginary frequencies in frequency analysis, and the contours of the HOMOs and LUMOs were plotted to understand the nature of the excited state. The energy and oscillator strength for the twenty lowest singlet-singlet absorptions of each compound were obtained from the TD-DFT output files. The calculated absorption spectra were visualized directly with Gauss View 5.0.

3. Results and Discussion

3.1. Crystal structure of **1** and **2**.

As shown in Figure 1, the two planar pyrrole subunits and the boron atom constitute a plane for which the deviation of all non-hydrogen atoms is within the 0.005–0.070 Å range in the BODIPY ring system. The angle between the two pyrrole moieties is 10.43° (for **1**) and 7.45° (for **2**), which is near the average 5.79° (range 0°–18.1°) found in the Cambridge Structural Database (CSD, updated to October 2008). The two fluorine atoms are equidistant above and under the plane of the pyrrole moieties, and the F–B–F plane is 109.91° (for **1**) and 110.07° (for **2**) to the plane of the BODIPY core. The methyl phenyl ring at the meso-position makes an angle of 54.79° (for **1**) and 60.41° (for **2**) with the BODIPY plane, which is in the range of most BODIPY derivatives (40.3°–90°), but is smaller than the average value of 76.4° found in the CSD. The dihedral angles between piperzine ring and pyrroles is 41.15° (for **1**) and 41.80° (for **2**).

< Fig. 1 >

3.2. Spectroscopic properties **1** and **2**.

Sensors **1** and **2** were dissolved in several solvents respectively (cyclohexane, THF, MeCN) to investigate their solvatochromic properties. Figure 2 shows the normalized UV–vis absorption and fluorescence emission spectra of **1** dissolved in various solvents. The absorption spectrum is of comparable shape as those of the described BODIPY dyes[24-28], with an intense absorption band somewhat above 470 nm (is attributed to the 0–0 vibrational band of $S_0 \rightarrow S_1$ transition) in acetonitrile. Additionally, a weaker, broad band is found in the absorption spectra at around 340 nm. This broader and weaker absorption band is attributed to the 0–0 vibrational band of $S_0 \rightarrow S_2$ ($\pi - \pi^*$) transition. The main absorption band of **1** is at ~ 515 nm in cyclohexane, and a shoulder at the short wavelength side can be observed – centered at approximately 490 nm – which is assigned to the 0–1 vibrational band of the same transition. In more polar solvents no vibrational fine structure can be observed. The absorption spectra of **2** (Fig S1) is, in general, similar to those of **1** in the solvents used.

The probes **1** or **2** in various solvents shows an emission spectrum with a maximum at ~ 550 nm, shaped like the mirror image of the absorption spectrum, displaying the typical emission features of BODIPY[24-28]. The ϕ_f value for **1** and **2** are low in solvents (about 0.05) can be attributed to an efficient quenching via an excited-state ICT process from the nitrogen atom of piperazine to the strongly electron-deficient BODIPY acceptor. The absorption spectra are affected by solvent polarity; the maximum being blue-shifted (by ~ 44 nm) when the solvent is changed from cyclohexane (518 nm) to acetonitrile (474 nm). The maximum wavelength of the

fluorescence band is hardly affected by solvent polarity (Figure 2, Table 1): there is only a 7 nm difference between the wavelengths of the emission maxima $\lambda_{em(max)}$ in toluene (560 nm) and DMSO (553 nm). The blue shift of the absorption spectrum from cyclohexane to acetonitrile can hardly be attributed to a difference in solvent polarizability (Figure S2). If it is attributed to dipolar interactions, this blue shift suggests that the dipole moment of the ground state is larger than that of the excited state.[29]

< Fig. 2>

< Table 1>

3.3. Fluorimetric titrations of **1** and **2**.

Protonation of the piperazine function drastically alters the electron-donating properties of the tertiary amine group and consequently it is expected to switch off the ICT to the borondipyrromethene moiety. The changes of the absorption spectra of **1** as a function of pH are shown in Figure 3a. It is clear that changing the pH leads to several isosbestic points and is accompanied by a hypsochromic shift of the major absorption maximum of **1** (from 485 nm at pH 3.36 to 474 nm at pH 8.86) upon increasing the pH.

The fluorescence emission spectra of **1** in water/MeCN (9:1, V/V) solution are shown as a function of pH in Figures 3c. The fluorescence signals increase considerably at lower pH. The highest ϕ_f value (0.12, $\lambda_{ex} = 480$ nm) is found at low pH (pH 3.22). Binding of H^+ by the piperazine raises the energy of the intramolecular CT state and hence suppresses the formation of this state. Blocking this non-radiative

decay channel of the locally excited state leads to a large fluorescence enhancement.

Analogous absorption effects are found for **2** (Figure 3b), where the major absorption maximum shifts hypsochromically from 485 nm at pH 3.72 to 474 nm at pH 8.68. In analogy to **1**, the fluorescence emission maximum at ~550 nm of **2** remains almost unaffected by pH (Figure 3d). The fluorescence intensity increases significantly with decreasing pH. The highest ϕ_f value (0.13, $\lambda_{ex} = 480$ nm) is found at low pH (pH 3.82). Figure S3 and S4 displays the absorption and emission spectra of sensors **1** and **2** as a function of pH (7–12) in water/MeCN (9:1, V/V) solution. In alkaline conditions, the absorption and emission spectra hardly be influenced by pH in the range of 8–12.

< Fig. 3 >

The acidity constant K_a and the stoichiometry (n) of the binding of H^+ by the piperazine (tertiary amine) group of new fluorescent pH indicators in water/MeCN (9:1, V/V) solution were determined by fitting eq 1 to the fluorescence emission spectral data. The results of the direct fluorimetric titration using the emission data of Figure 3c ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 550$ nm) are displayed in Figure 4a and yield a pK_a value of 6.45 ± 0.05 and a well-defined 1:1 stoichiometry between **1** and H^+ , indicate the formation of indicative of ammonium ion. A similar titration using the emission spectra of Figure 3d ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 550$ nm) yield a pK_a value of 6.74 ± 0.11 and a well-defined 1:1 stoichiometry between **2** and H^+ (figure 4b). These results are in perfect agreement with the curve-fittings using different wavelength of sensor **1** (Fig S5, at 535 nm: $pK_a=6.43\pm0.06$, at 555 nm: $pK_a=6.52\pm0.06$) and sensor **2** (Fig S6

at 530nm: $pK_a=6.73\pm0.08$ at 560m: $pK_a=6.88\pm0.15$). The average pK_a obtained from Fig S7 are 6.50 ± 0.07 (**1**) and 6.80 ± 0.11 (**2**). These results show that the obtained pK_a values are independent of the selected wavelengths. All estimated n values are indicative of a 1 to 1 binding between dyes **1** or **2** and H^+ .

< Fig. 4>

3.4. Cell culture

For practical applications, it is necessary that the molecular probe described here is able to detect pH under biological conditions. To test whether **1** or **2** fulfills these additional requirements, measurements on live cells were performed. Based on the fluorescence intensity upon excitation at 488 nm of the BHK cells during dye loading (10 μ M), it can be concluded that **1** or **2** efficiently accumulates inside the cytosol, but is excluded from the cell nucleus (Fig. 5).

Moreover, cell cytotoxicity experiment of **1** or **2** was evaluated using BHK cell lines through the MTS assay. As shown in Fig S9, the **1** and **2** exhibited low cytotoxicity, which may be due to the low toxicity of BODIPY. Taken together, it is clearly demonstrated that the sensor is membrane-permeable, and the fluorescence change in the BHK cells was due to the synchronous presence of **1** and **2**. The result confirmed that the probe **1** or **2** can be used to image intracellular pH levels in living cells.

< Fig. 5>

4. Conclusion

We have synthesized two new 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene dyes

(1-2) with piperazine subunits. Their absorption and steady-state fluorescence properties were investigated as a function of solvent. In water/MeCN (9:1, V/V) solution, the dyes **1** and **2** undergo a reversible protonation-deprotonation reaction in the near-neutral pH range. They can be used in water/MeCN (9:1, V/V) solution as fluorescent pH probes excitable with visible light, with pK_a values were 6.5 ± 0.07 and 6.8 ± 0.11 . For cytosolic pH measurements, a better match between the pK_a of the indicator and pH (6.8–7.4) is desirable. Dyes **1** and **2** are readily taken up by biological cells in the cytosol where it is no obvious toxic effects.

Acknowledgments

The authors would like to thank the Natural Science Foundation of China (no. 21271094, 21371083). This work was supported by the National Science Foundation for Fostering Talents in Basic Research of the National Natural Science Foundation of China (Grant no. J1103307).

Supporting Information Available:

Compound characterization data, this material can be found in the Supporting Information.

References

- [1] R.P. Haugland, The handbook: a guide to fluorescent probes and labeling technologies: Molecular probes. 2005.
- [2] J. Han, K. Burgess, Fluorescent indicators for intracellular pH, Chem. Rev. 110(2009) 2709-2728.
- [3] J.-P. Desvergne, A.W. Czarnik, Chemosensors of ion and molecule recognition: Springer. Berlin. 1997.
- [4] X. Li, X. Gao, W. Shi, H. Ma, Design strategies for water-soluble small molecular chromogenic and fluorogenic probes, Chem. rev. 114(2013) 590-659.
- [5] G.K. Vegesna, J. Janjanam, J. Bi, F.-T. Luo, J. Zhang, C. Olds, et al., pH-activatable near-infrared fluorescent probes for detection of lysosomal pH inside living cells, J. Mater. Chem. B. 2(2014) 4500-4508.
- [6] Q. Wan, S. Chen, W. Shi, L. Li, H. Ma, Lysosomal pH Rise during Heat Shock Monitored by a Lysosome - Targeting Near - Infrared Ratiometric Fluorescent Probe, Angew. Chem. 126(2014) 11096-11100.
- [7] L. Fan, Q. Liu, D. Lu, H. Shi, Y. Yang, Y. Li, et al., A novel far-visible and near-infrared pH probe for monitoring near-neutral physiological pH changes: imaging in live cells, J. Mater. Chem. B. 1(2013) 4281-4288.
- [8] A. Treibs, F.H. Kreuzer, Difluoroboryl - Komplexe von Di - und Tripyrrylmethenen, Liebigs Ann. Chem. 718(1968) 208-223.
- [9] A. Loudet, K. Burgess, BODIPY dyes and their derivatives: syntheses and spectroscopic properties, Chem. Rev. 107(2007) 4891-4932.

- [10] G. Ulrich, R. Ziessel, A. Harriman, The chemistry of fluorescent bodipy dyes: versatility unsurpassed, *Angew. Chem., Int. Ed.* 47(2008) 1184-1201.
- [11] T. Gareis, C. Huber, O.S. Wolfbeis, J. Daub, Phenol/phenolate-dependent on/off switching of the luminescence of 4, 4-difluoro-4-bora-3a, 4a-diaza-s-indacenes, *Chem. Commun.* (1997) 1717-1718.
- [12] C.N. Baki, E.U. Akkaya, Boradiazaindacene-appended calix [4] arene: fluorescence sensing of pH near neutrality, *J. org. chem.* 66(2001) 1512-1513.
- [13] M. Baruah, W. Qin, N. Basaric, W.M. De Borggraeve, N. Boens, BODIPY-based hydroxyaryl derivatives as fluorescent pH probes, *J org chem*, 70(2005) 4152-4157.
- [14] W. Qin, M. Baruah, A. Stefan, M. Van der Auweraer, N. Boens, Photophysical Properties of BODIPY - Derived Hydroxyaryl Fluorescent pH Probes in Solution, *ChemPhysChem.* 6(2005) 2343-2351.
- [15] W. Qin, M. Baruah, W.M. De Borggraeve, N. Boens, Photophysical properties of an on/off fluorescent pH indicator excitable with visible light based on a borondipyrromethene-linked phenol, *J Photochem. Photobiol., A.* 183(2006) 190-197.
- [16] Y. Urano, D. Asanuma, Y. Hama, Y. Koyama, T. Barrett, M. Kamiya, et al., Selective molecular imaging of viable cancer cells with pH-activatable fluorescence probes, *Nat. med.* 15(2009) 104-109.
- [17] A. Castro, J.R. Leis, M.E. Peña, Decomposition of N-methyl-N-nitrosotoluene-p-sulphonamide in basic media: hydrolysis and transnitrosation reactions, *J. Chem. Soc., Perkin Trans. 2.* (1989) 1861-1866.
- [18] N. Boens, W. Qin, M. Baruah, W.M. De Borggraeve, A. Filarowski, N. Smisdom, et al., Rational Design, Synthesis, and Spectroscopic and Photophysical Properties of a Visible - Light - Excitable, Ratiometric, Fluorescent Near - Neutral pH Indicator Based on BODIPY, *Chem.—Eur. J.* 17(2011)

10924-10934.

[19] E. Cielen, A. Tahri, K. Ver Heyen, G.J. Hoornaert, F.C. De Schryver, N. Boens, Synthesis and spectroscopic characterisation of fluorescent indicators for Na⁺ and K⁺, J. Chem. Soc., Perkin Trans. 2. (1998) 1573-1580.

[20] E. Cielen, A. Stobiecka, A. Tahri, G.J. Hoornaert, F.C. De Schryver, J. Gallay, et al., Synthesis and characterisation of Thio-H, a new excitation and emission ratioable fluorescent Ca²⁺/Mg²⁺ indicator with high brightness, J. Chem. Soc., Perkin Trans. 2. (2002) 1197-1206.

[21] J. Olmsted, Calorimetric determinations of absolute fluorescence quantum yields, J. Phys. Chem. 83(1979) 2581-2584.

[22] G. Sheldrick, SHELX-97, Program for crystal structure solution and refinement, University of Göttingen, Germany, (1997) 1456.

[23] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, et al., Gaussian 09, revision A. 1; Gaussian, Inc: Wallingford, CT. 2009.

[24] T.L. Arbeloa, F.L. Arbeloa, I.L. Arbeloa, I. Garcia-Moreno, A. Costela, R. Sastre, et al., Correlations between photophysics and lasing properties of dipyrromethene–BF₂ dyes in solution, Chem. Phys. Lett. 299(1999) 315-321.

[25] A. Costela, I. García-Moreno, C. Gomez, R. Sastre, F. Amat-Guerri, M. Liras, et al., Photophysical and lasing properties of new analogs of the boron-dipyrromethene laser dye PM567 in liquid solution, J. Phys. Chem. A. 106(2002) 7736-7742.

[26] V.M. Martinez, F.L. Arbeloa, J.B. Prieto, T.A. López, I.L. Arbeloa, Characterization of rhodamine 6G aggregates intercalated in solid thin films of laponite clay. 1. Absorption spectroscopy, J. Phys. Chem. B. 108(2004) 20030-20037.

- [27] V. Leen, V.Z. Gonzalvo, W.M. Deborggraeve, N. Boens, W. Dehaen, Direct functionalization of BODIPY dyes by oxidative nucleophilic hydrogen substitution at the 3- or 3,5-positions, *Chem. Commun.* 46(2010) 4908-4910.
- [28] A. Filarowski, M. Lopatkova, P. Lipkowski, M. Van der Auweraer, V. Leen, W. Dehaen, Solvatochromism of BODIPY-Schiff Dye, *J. Phys. Chem. B.* 119(2014) 2576-2584.
- [29] W. Qin, V. Leen, T. Rohand, W. Dehaen, P. Dedecker, M. Van der Auweraer, et al., Synthesis, spectroscopy, crystal structure, electrochemistry, and quantum chemical and molecular dynamics calculations of a 3-anilino difluoroboron dipyrromethene dye, *J. Phys. Chem. A.* 113(2008) 439-447.

Biographies

Min Deng received her B.S. degree from Lanzhou University, PR China, in 2014. She is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. Her research interests focus on developing fluorescent chemosensors.

Chengduan Yang received his B.S. degree from Weifang University, PR China, in 2013. He is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. Her research interests focus on developing fluorescent chemosensors.

Deyan Gong received his B.S. degree from Xi'an University of Technology, PR China

in 2014. He is currently a master candidate at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing fluorescent chemosensors.

Xiao-Liang Tang received his Ph.D. degree from Lanzhou University, PR China, in 2009. He is currently an associate professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing function materials and fluorescent chemosensors.

Wei-Sheng Liu received his Ph.D. degree from Lanzhou University, PR China, in 1996. He is currently a professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing function materials and fluorescent chemosensors.

Wen-Wu Qin received his Ph.D. degree from Lanzhou University, PR China, in 2003. He is currently a professor at College of Chemistry and Chemical Engineering, Lanzhou University, PR China. His research interests focus on developing fluorescent chemosensors

Figure captions

Fig. 1. ORTEP representation of compounds **1** (a) and **2** (b) with displacement ellipsoids at the 20% probability level.

Fig. 2: Normalized absorption (dash curves) and fluorescence spectra (solid curves) of sensor **1** in various solvents.

Fig. 3: Absorption spectra of sensors **1** (a) and **2** (b) in water/MeCN (9:1, V/V) solution as a function of pH. Corresponding fluorescence emission spectra c and d. ($\lambda_{\text{ex}} = 480 \text{ nm}$).

Fig. 4: The solid line represents the best fit of eq 1 with $n = 1$ to the fluorimetric titration data of sensors **1** (a) and **2** (b) obtained from the emission spectra of Figure 3c and 3d ($\lambda_{\text{ex}} = 480 \text{ nm}$).

Fig. 5: Confocal fluorescence images of BHK cells of the sensors **1** and **2**. The bright-field images of **1** (a) and **2** (c); the confocal fluorescence images of **1** (b) and **2** (d).

Table 1. Spectroscopic / Photophysical Data of sensors **1** and **2** in Different Solvents at 25 °C.

Scheme 1. Synthesis of sensors **1** and **2**.

Fig. 1

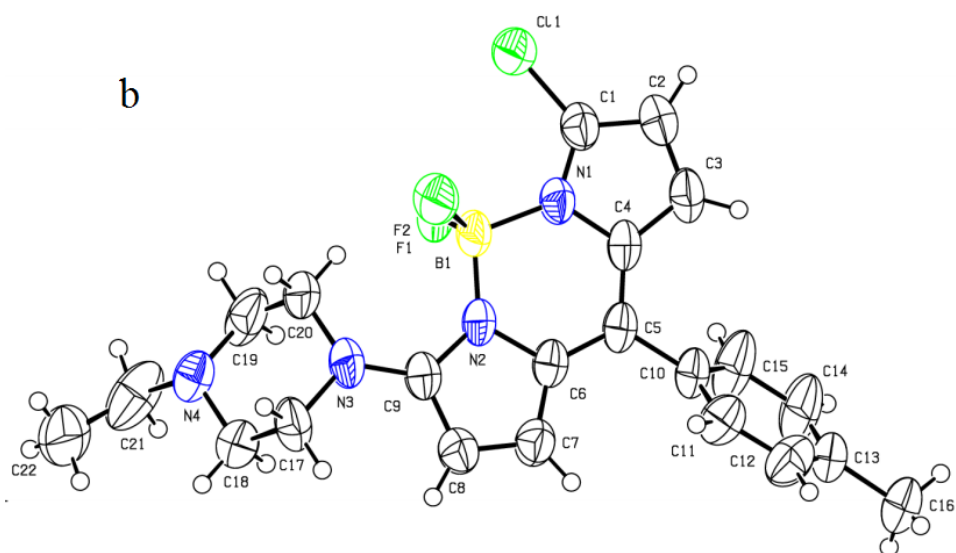
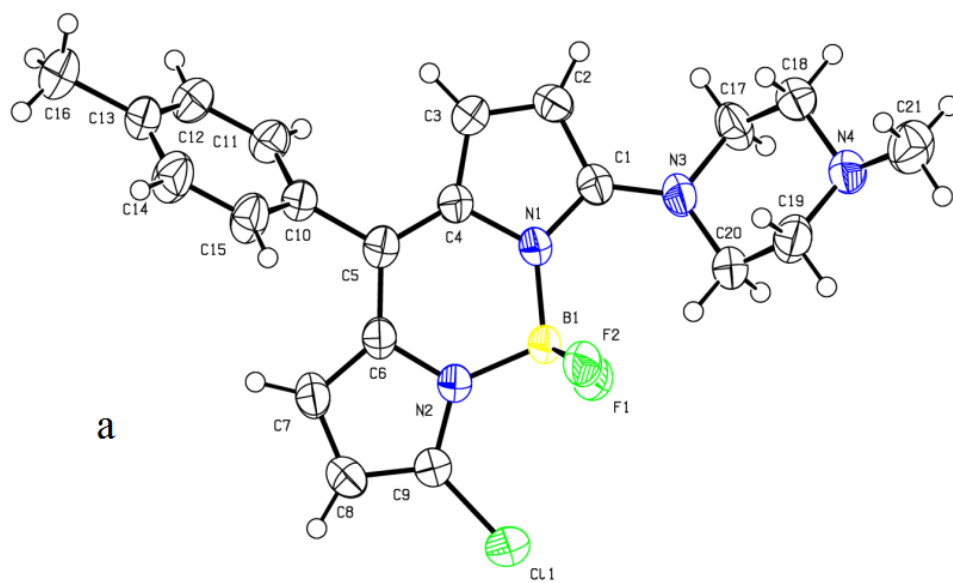
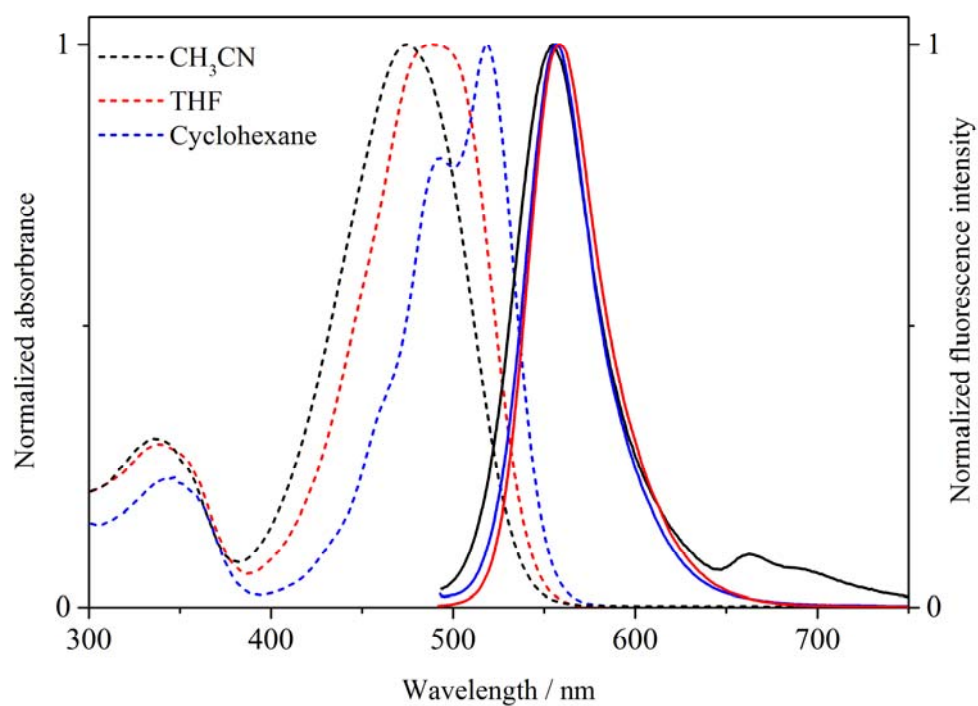
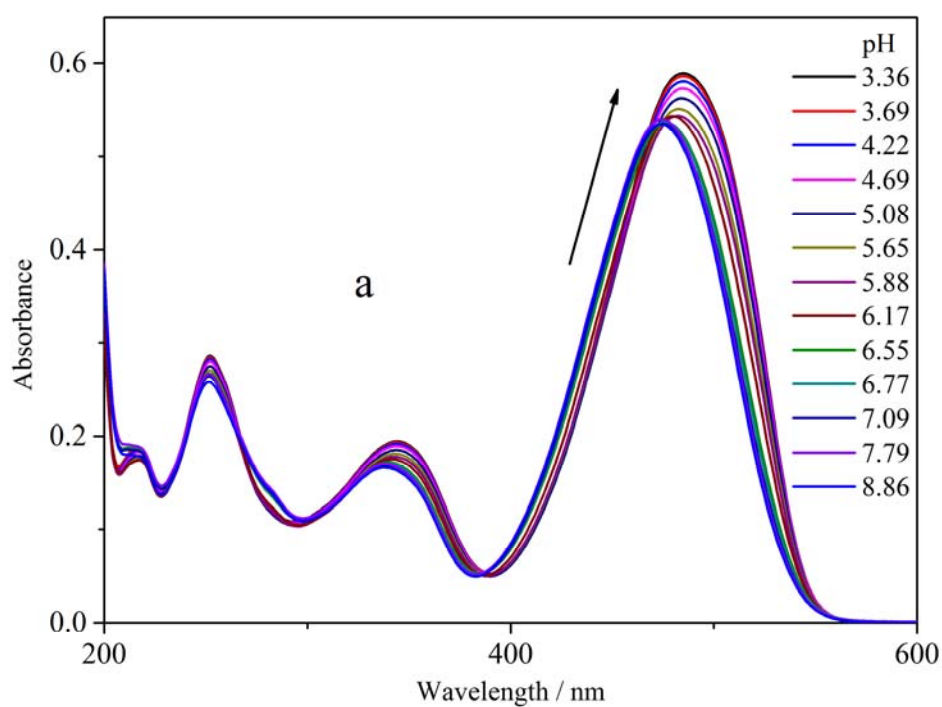
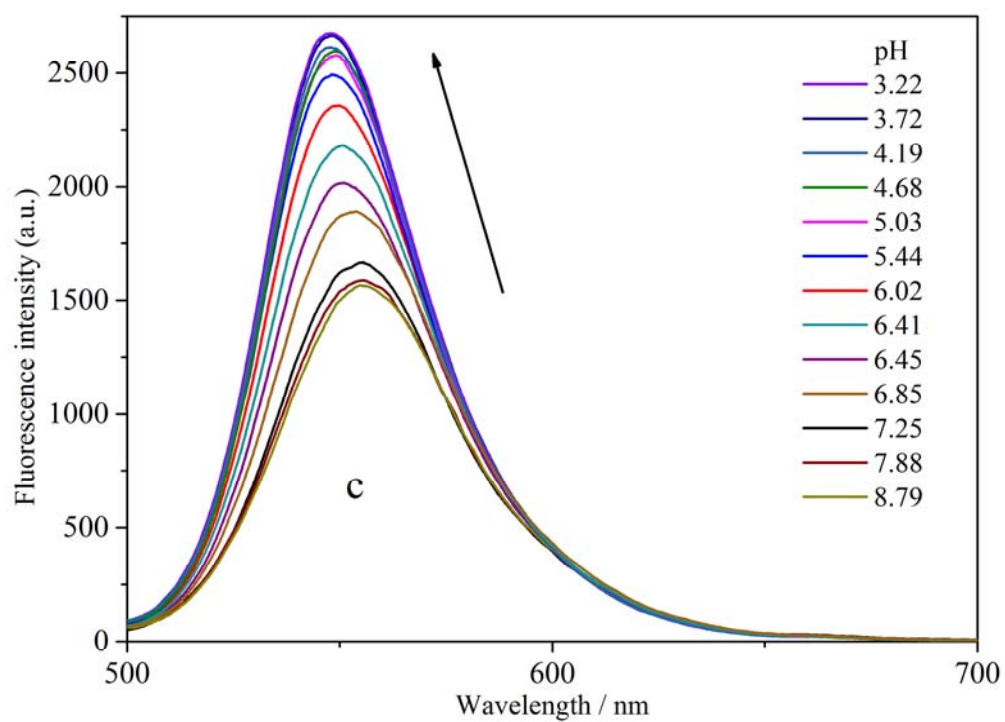
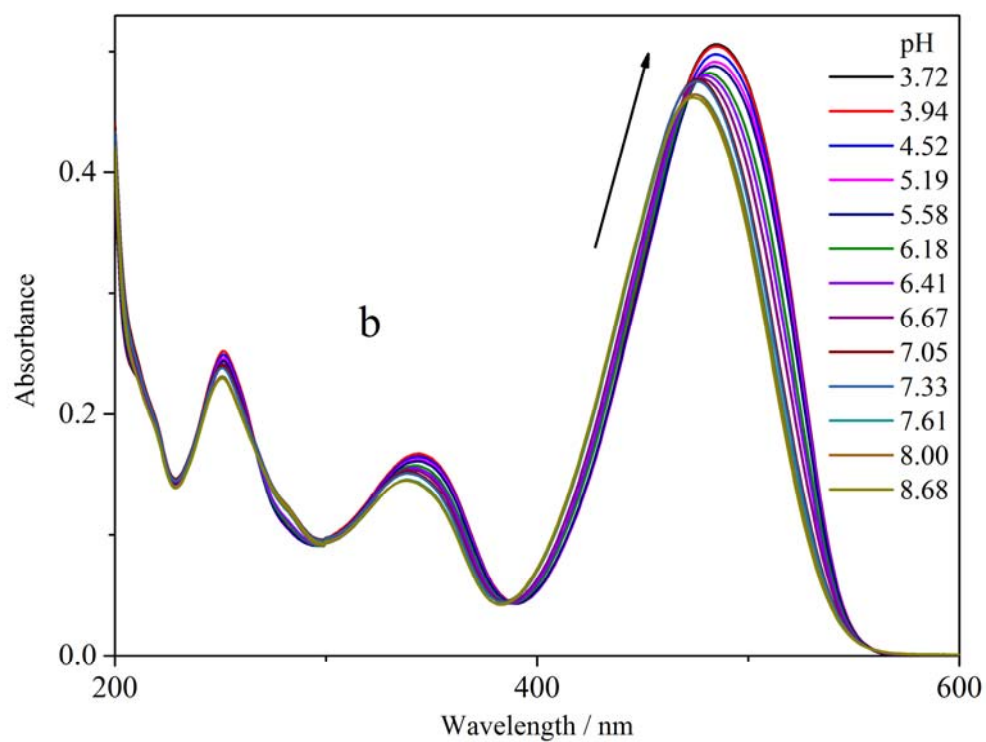
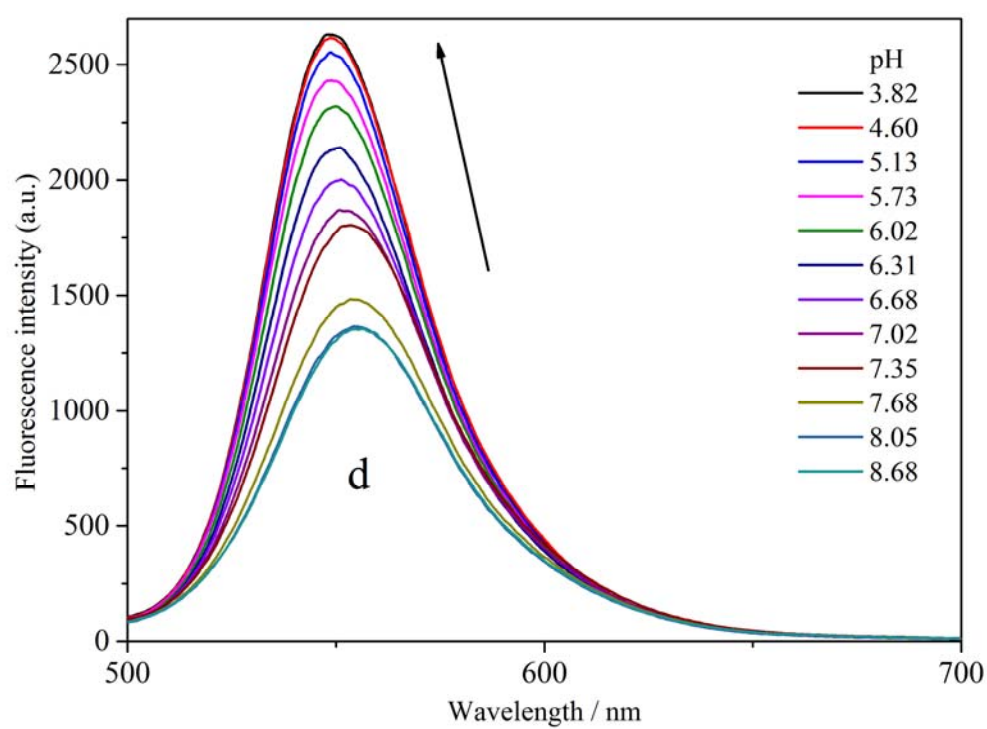
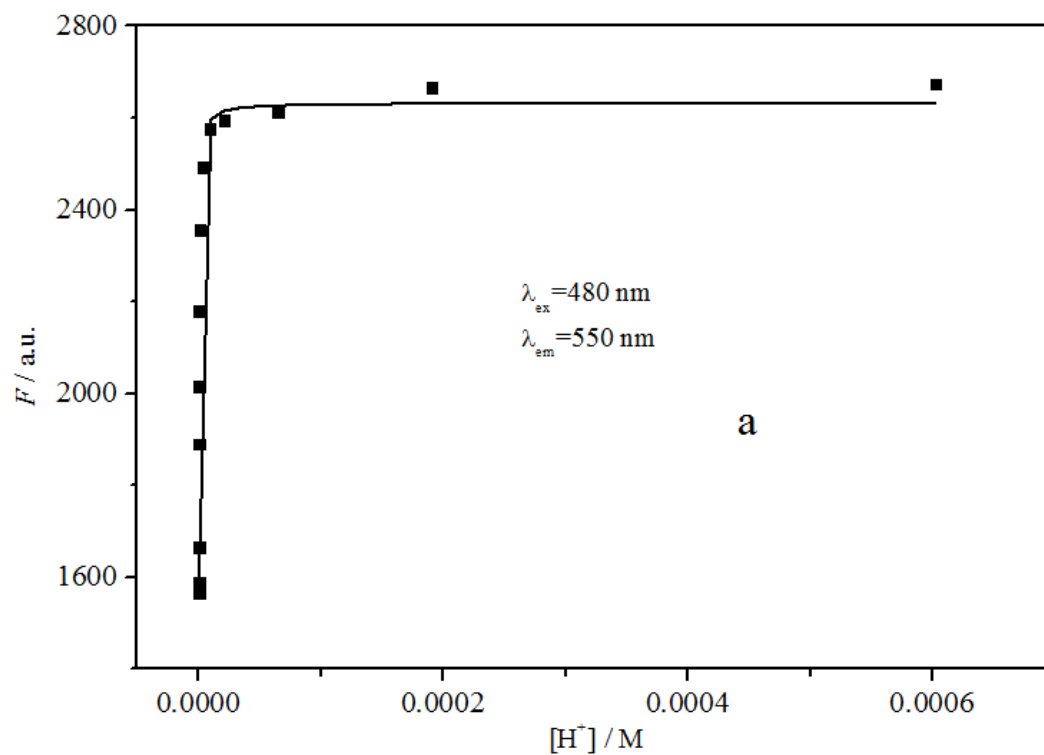


Fig. 2**Fig. 3**



**Fig. 4**

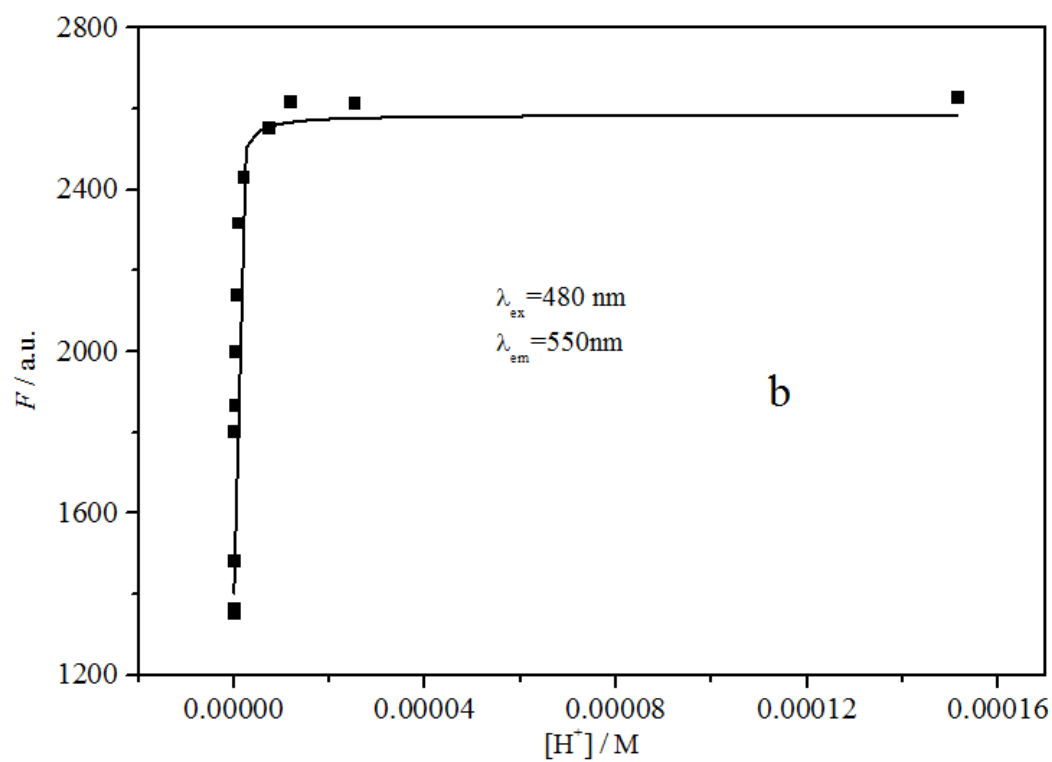
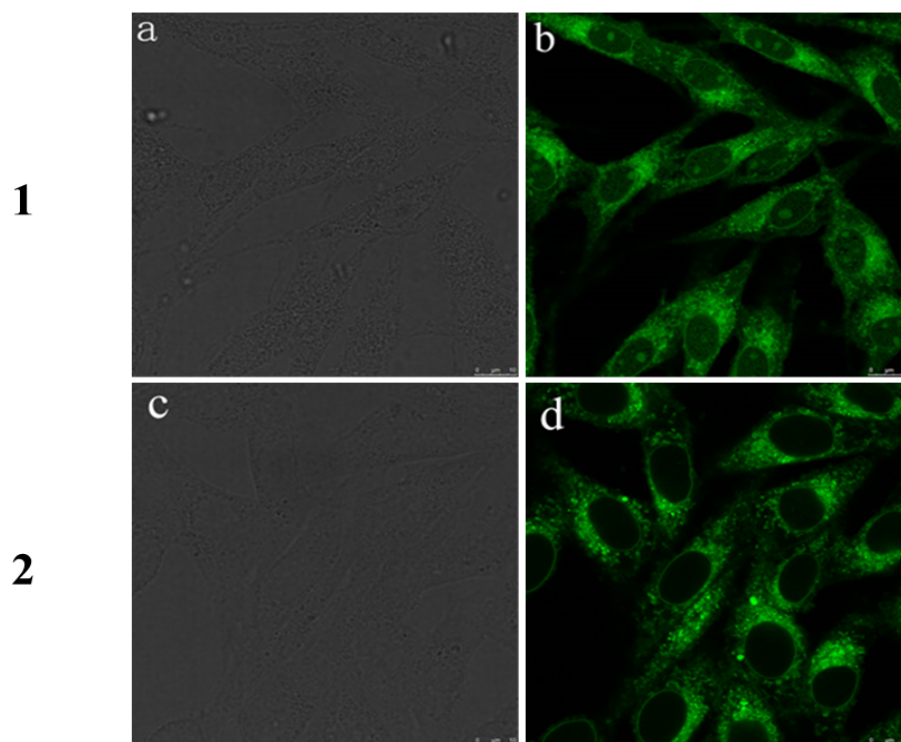


Fig. 5



Scheme 1

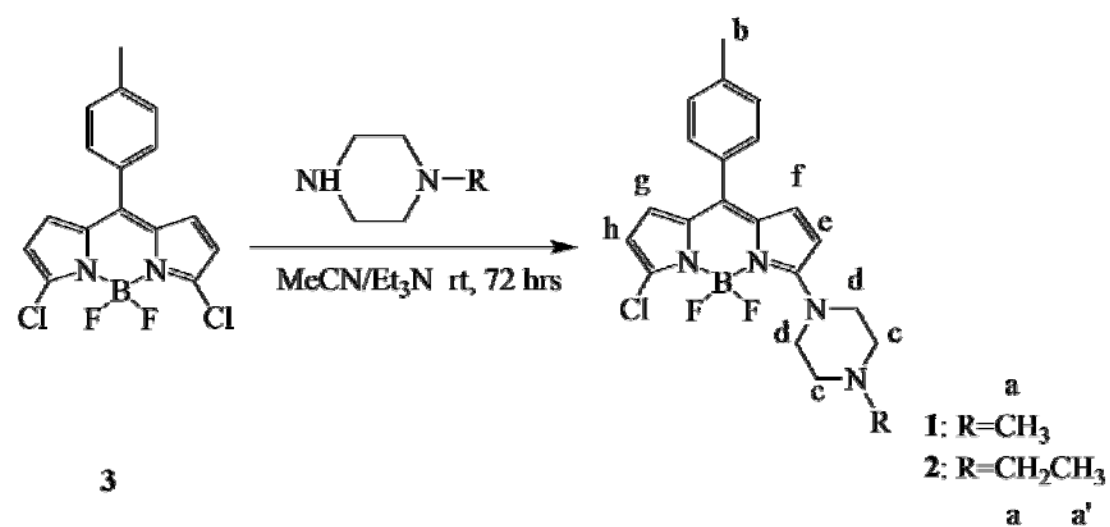


Table 1 Spectroscopic / Photophysical Data of sensors **1** and **2** in Different Solvents

at 25 °C

Solvent	$\lambda_{\text{abs}}(\text{max}) / \text{nm}$		$\lambda_{\text{em}}(\text{max}) / \text{nm}$		Φ_{f}	
	1	2	1	2	1	2
Cyclohexane	518	518	557	557	0.055±0.001	0.052±0.001
Toluene	511	511	560	560	0.053±0.002	0.057±0.002
Tetrahydrofuran	488	486	557	550	0.046±0.001	0.043±0.001
Dichloromethane	489	486	556	553	0.052±0.002	0.053±0.002
Dimethyl sulfoxide	484	485	553	554	0.052±0.001	0.049±0.002
Acetonitrile	474	474	556	556	0.047±0.003	0.055±0.001

Supporting Information

**BODIPY-Derived Piperazidine Fluorescent Near-Neutral pH
Indicator and Its Bioimaging**

Min Deng, Chenduan Yang, Deyan Gong, Anam Iqbal, Xiaoliang Tang, Weisheng Liu
and Wenwu Qin ²

*Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu
Province and State Key Laboratory of Applied Organic Chemistry, College of
Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R.
China.*

² Corresponding author: Fax: +86-931-8912582 E-mail address: qinww@lzu.edu.cn

(W. Qin)

Table S1. Basic Crystallographic Data for **1**–**2**. The data of **1** was measured with Mo $K\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$), while **2** was obtained with Cu $K\alpha$ radiation ($\lambda = 1.54178 \text{ \AA}$).

	1	2
Cell system	Monoclinic	Monoclinic
Space group	$P2_1/n$ (number 14)	$P2_1/n$ (number 14)
a (Å)	10.7487(4)	11.2587(5)
b (Å)	10.0856(4)	10.2838(5)
c (Å)	19.0065(7)	18.6525(7)
α (°)	90	90
β (°)	102.121(4)	102.043(4)
γ (°)	90	90
V (Å ³)	2014.49(13)	2112.09(16)
Z	4	4
ρ_{calcd} (g/cm ³)	1.367	1.348
$2\theta_{\text{max}}$ (°)	52.64	139.38
μ (Mo $K\alpha$) (mm ⁻¹)	0.223	1.887
R [$F^2 > 2\sigma(F^2)$]	0.0594	0.0538
$wR(F^2)$	0.1589	0.1618
S	1.053	1.069
$\Delta\rho_{\text{max}}, \Delta\rho_{\text{min}}$	0.264, -0.391	0.377, -0.394

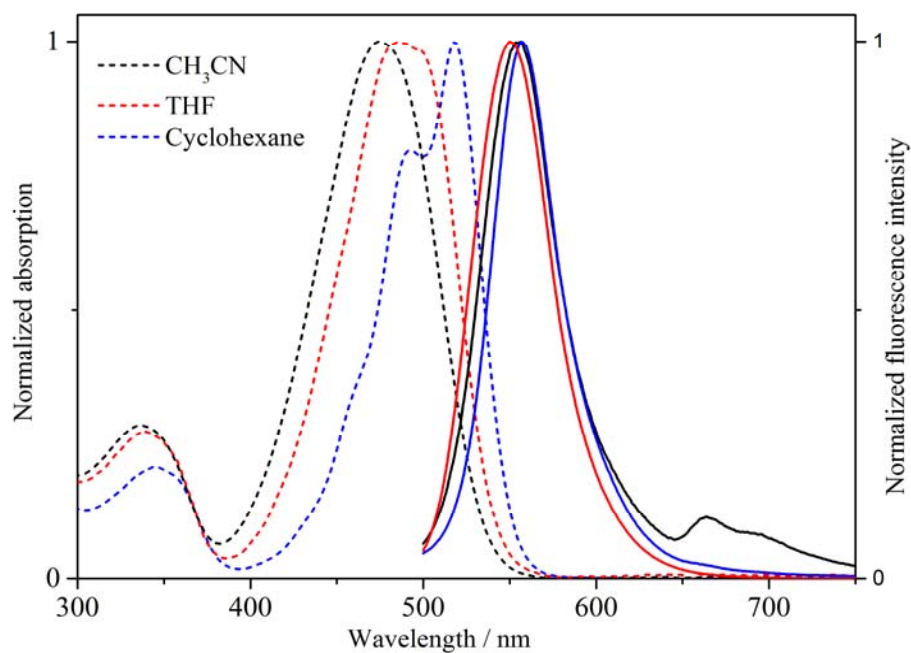


Fig S1: Normalized absorption (dash curves) and fluorescence spectra (solid curves) of sensor **2** in various solvents.

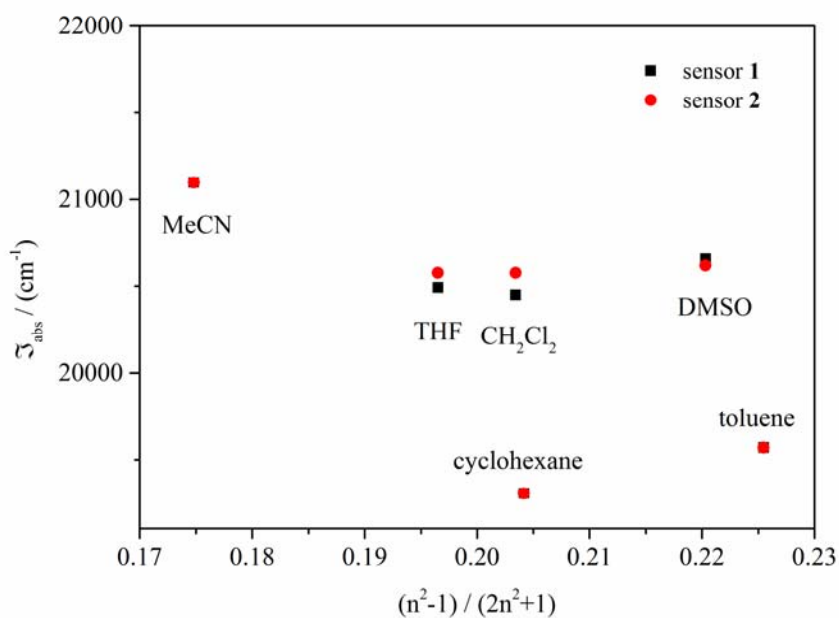


Figure S2: Plots of the absorption maxima for sensors **1-2** vs. $(n^2-1) / (2n^2+1)$ as a function of solvent. From left to right: MeCN, THF, CH_2Cl_2 , cyclohexane, DMSO, toluene.

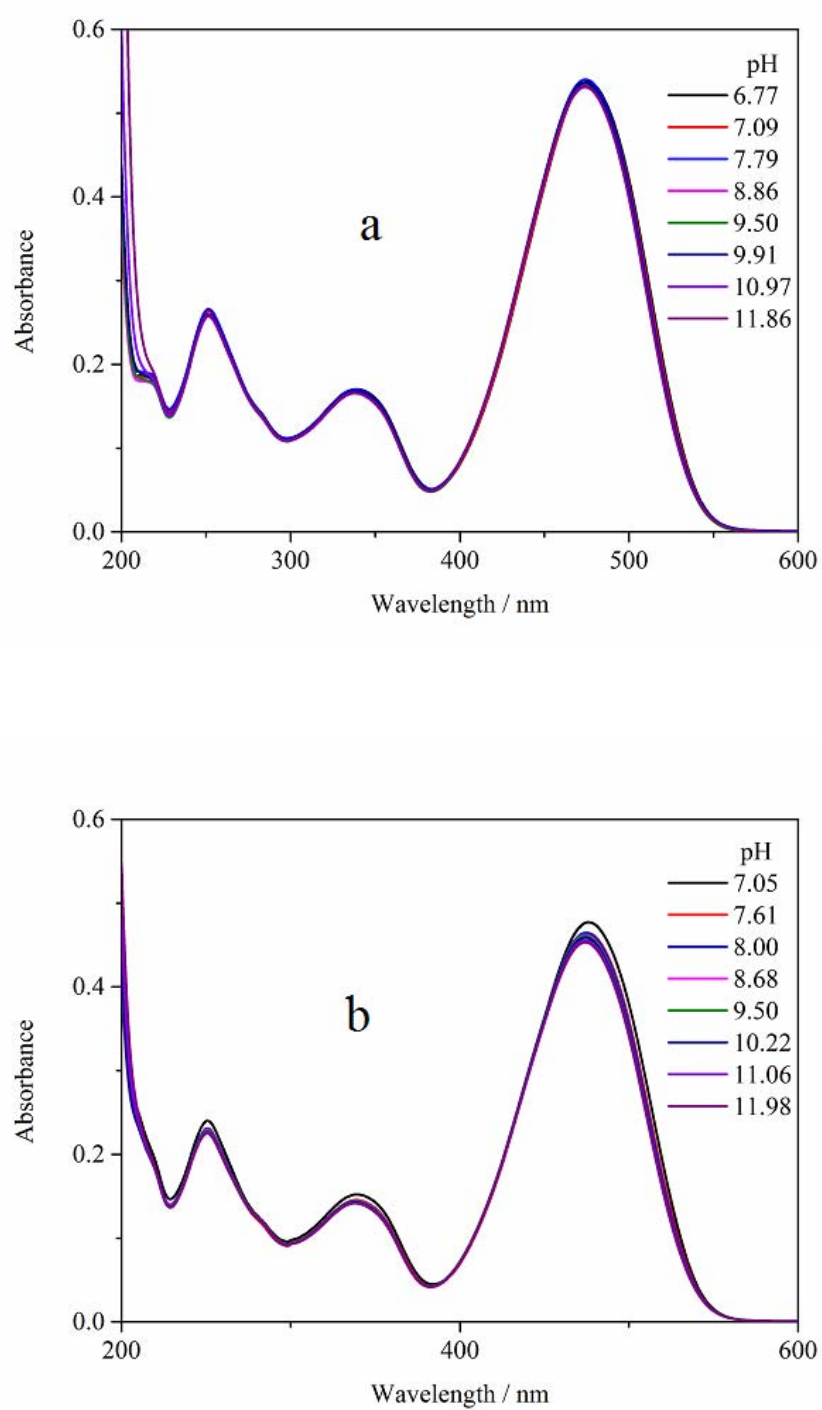


Fig S3: Absorption spectra of sensors **1** (a) and **2** (b) in water/MeCN (9:1, V/V) solution as a function of pH 7-12.

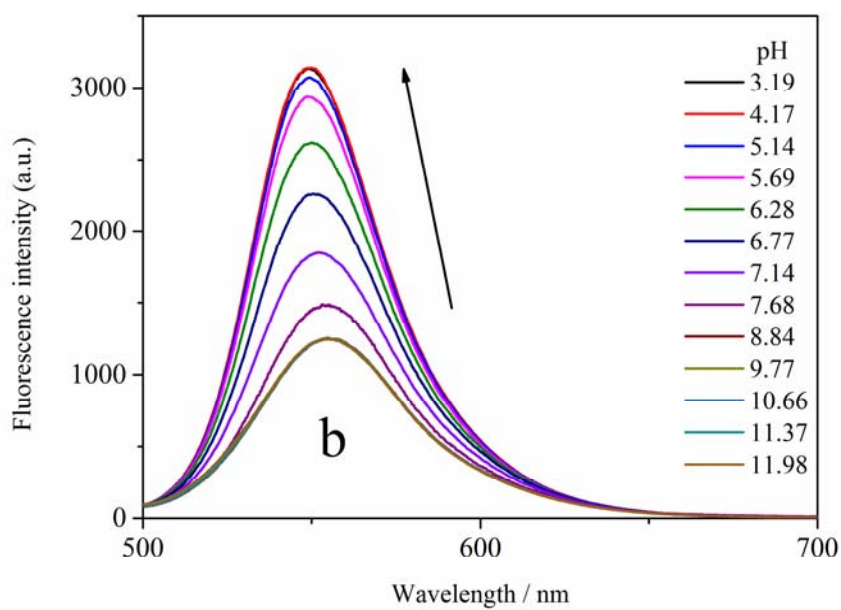
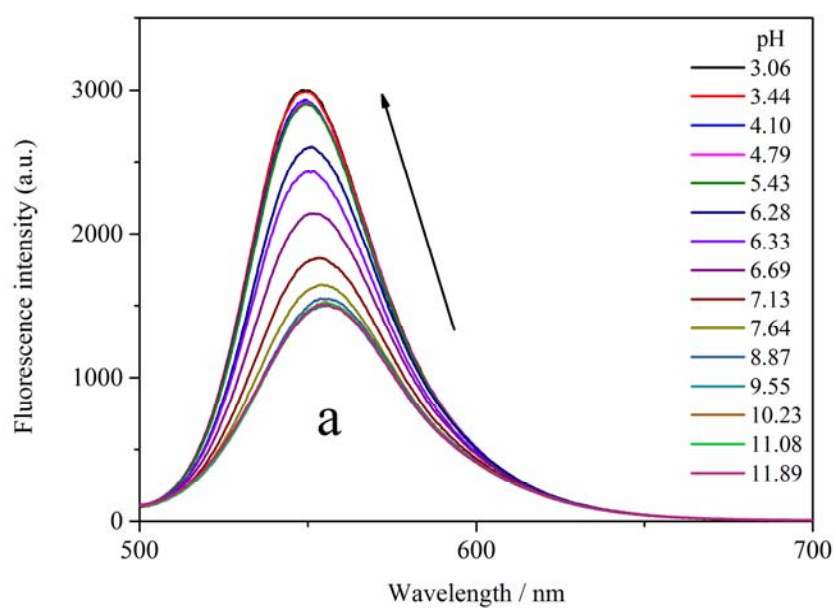


Fig S4: Fluorescence emission spectra of sensors **1** (a) and **2** (b) in water/MeCN (9:1, V/V) solution as a function of pH 3-12

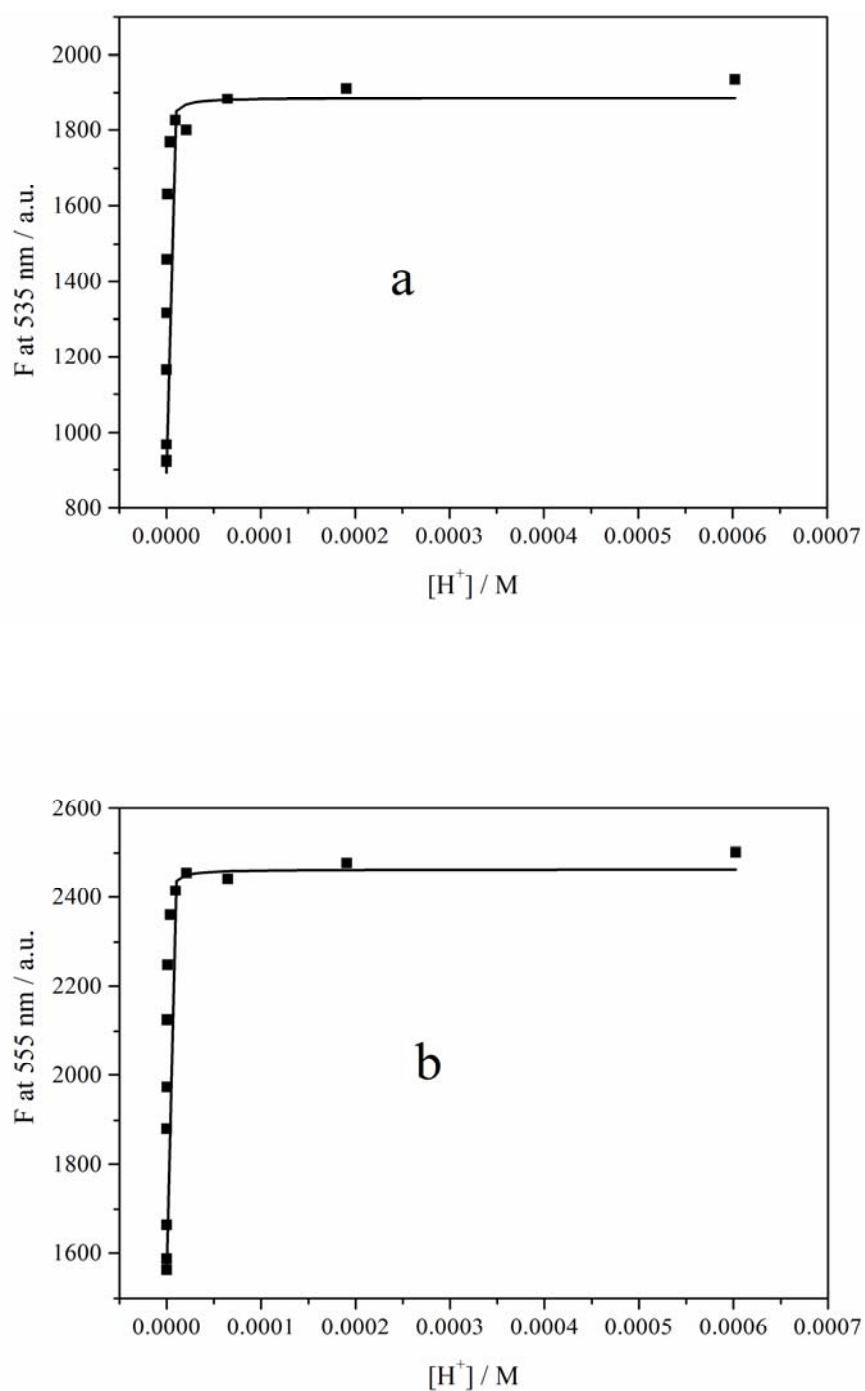


Fig S5: The solid line represents the best fit of eq 1 with $n = 1$ to the fluorimetric titration data of sensor **1** at 535 nm (a) and 555 nm (b) obtained from the emission spectra of Figure 3c ($\lambda_{\text{ex}} = 480$ nm).

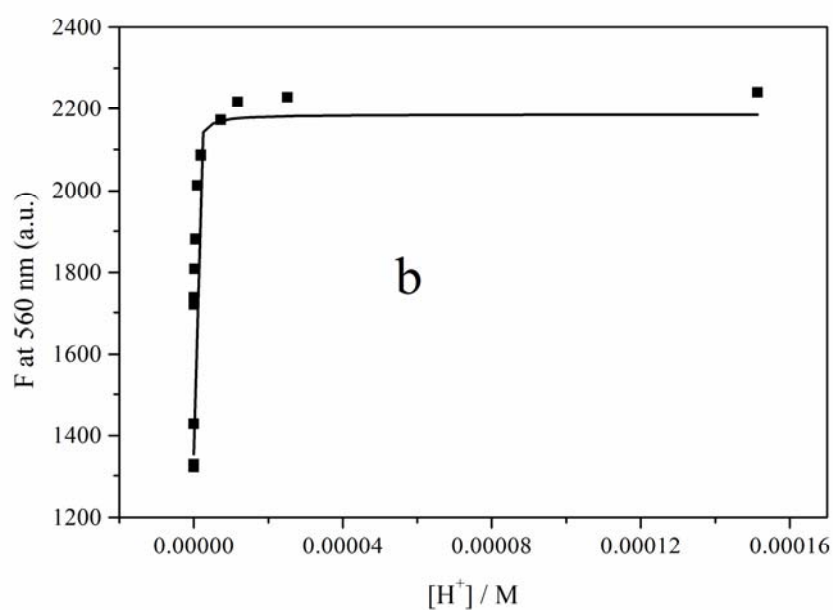
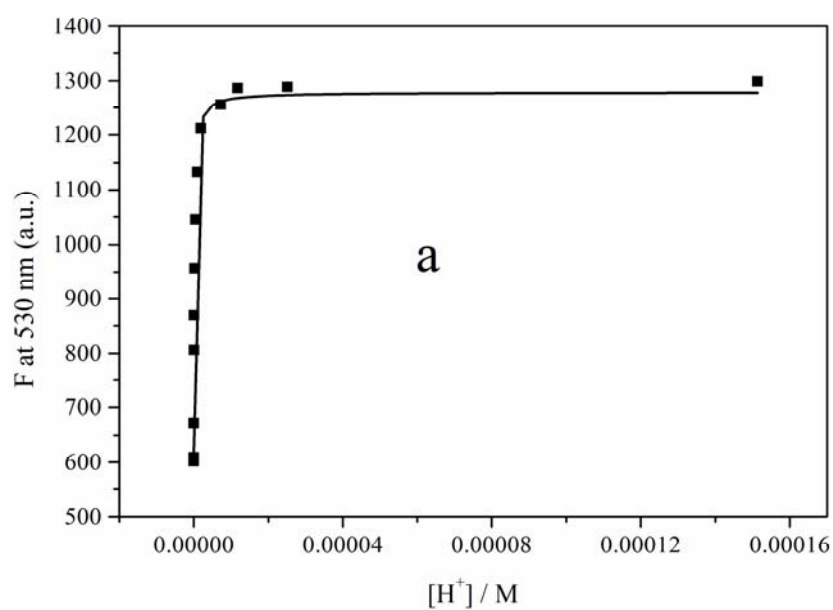


Fig S6: The solid line represents the best fit of eq 1 with $n = 1$ to the fluorimetric titration data of sensor **2** at 530 nm (a) and 560 nm (b) obtained from the emission spectra of Figure 3d ($\lambda_{\text{ex}} = 480$ nm).

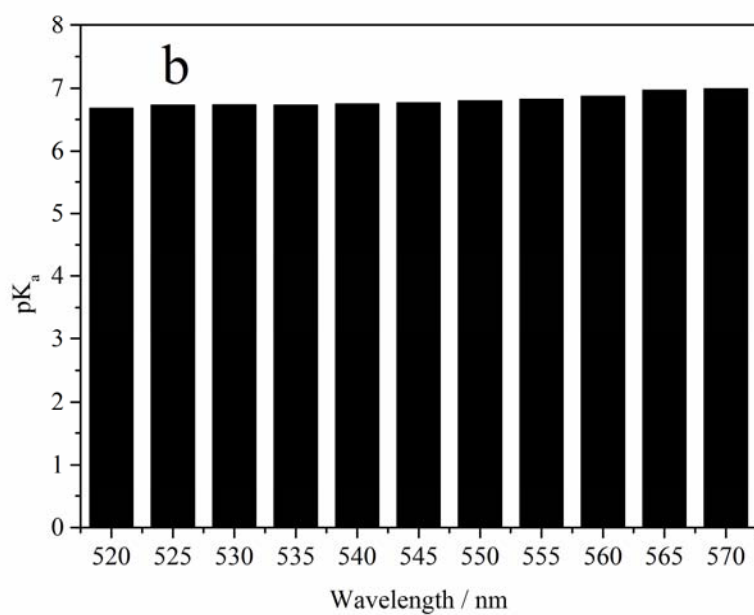
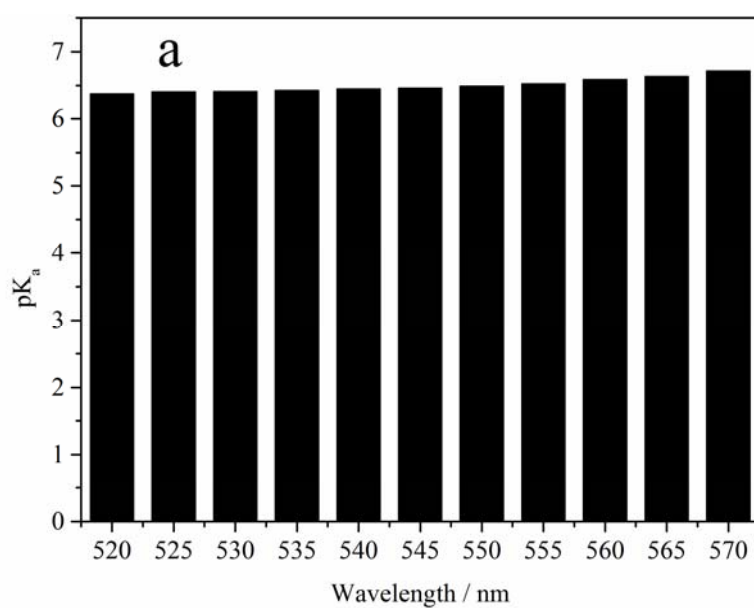


Fig S7: The pK_a of sensors 1 (a) and 2 (b) fitting at different wavelength.

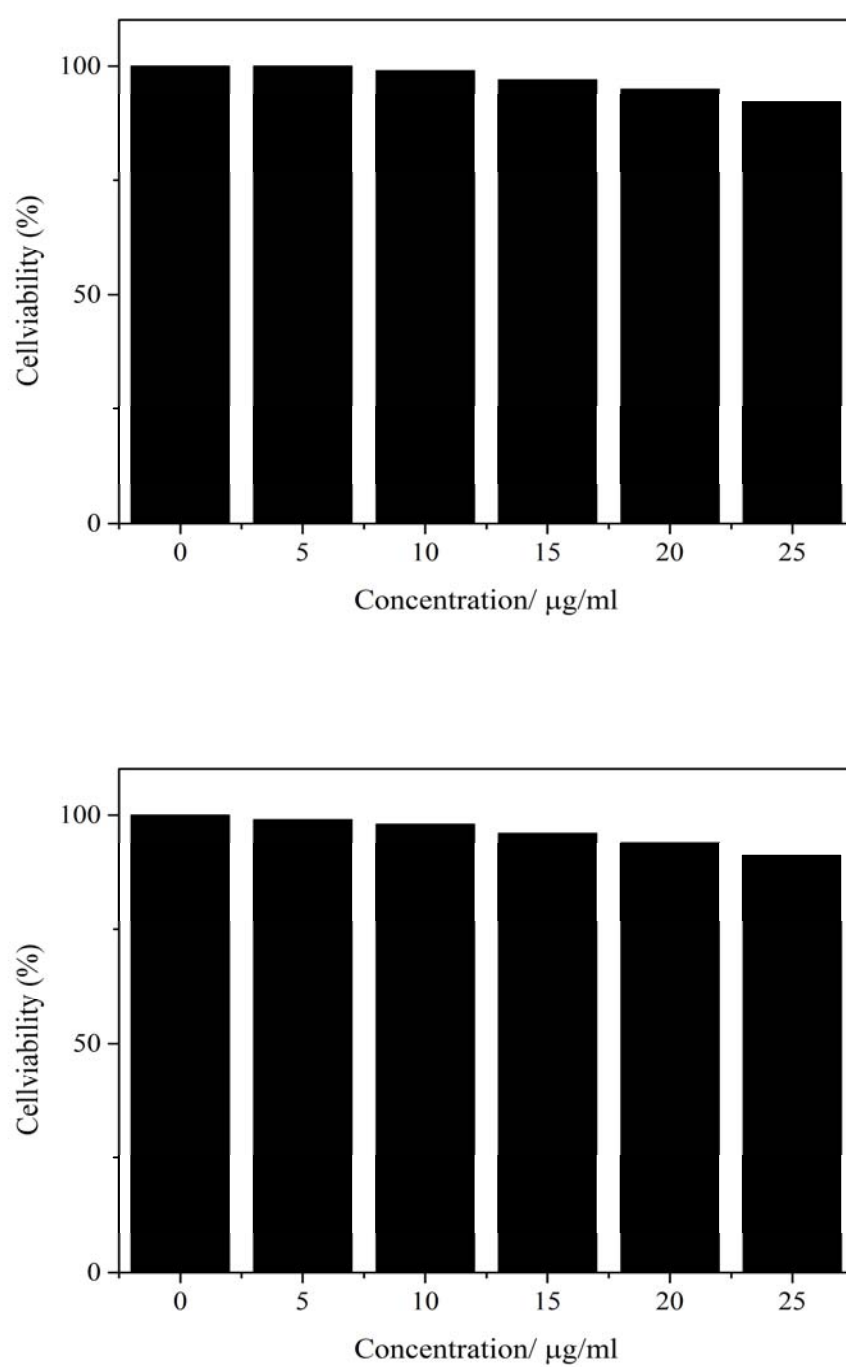


Fig S8: Effects of sensors 1 and 2 at varied concentrations on the viability of BHK cells.

Theoretical Calculations

For better understanding the photophysical property further, UV-vis absorption spectra corresponding transition energies of **1** and **2** were calculated and estimated with TD-DFT based on respective ground-state geometries. As shown in Figure 6a, calculated spectral curves of **1** and **2** look broadly similar in whole absorption wavelength range, and they are very consistent with the changes of UV-vis absorption spectra. The distributions of the molecular orbitals, HOMO and LUMO, are showed in Figure 6b, and they all are mainly localized on BODIPY core. The contribution to the lowest-lying singlet transitions (HOMO $\rightarrow$ LUMO) of both compounds respectively corresponding to their absorptions are mainly assigned to $\pi-\pi^*$ transitions, supported by UV-vis spectral profiles around 200–600 nm.

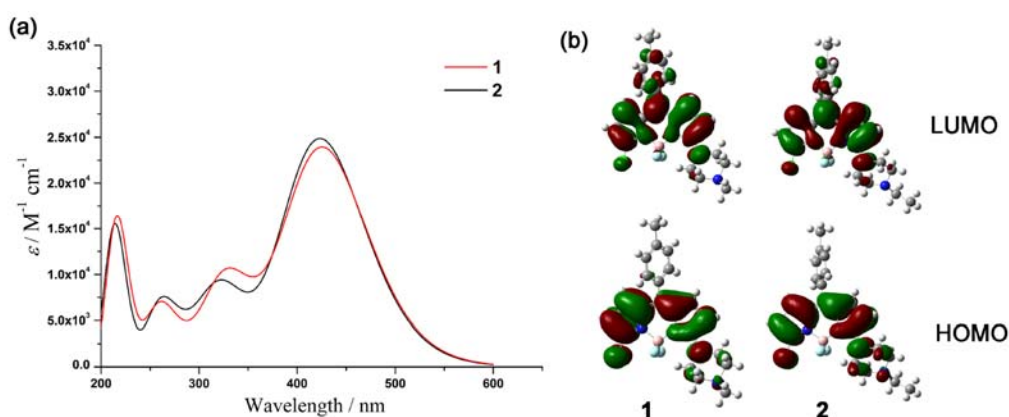


Fig S9: (a) Theoretical UV-vis absorption spectra of **1** and **2**. (b) HOMO and LUMO distributions of **1** and **2**.

Table S2. Spectroscopic Properties Estimated on the Basis of DFT/B3LYP/6-31g* Calculations.

molecule	E_{abs} theory (eV)	E_{abs} exp (eV)
1	2.90	2.62
2	2.92	2.62

Table S3. DFT optimized coordinates for sensor **1** in the ground state.

Number	Atom	Coordinates		
		X	Y	Z
1	Cl	-0.2940000	4.5720000	-0.0820000
2	F	-1.5980000	1.9310000	-1.1250000
3	F	-1.4120000	1.6530000	1.1270000
4	N	-0.6570000	-0.1340000	-0.3260000
5	N	0.5830000	2.0270000	-0.1160000
6	N	-2.9180000	-0.9700000	-0.6150000
7	N	-5.5540000	-1.4050000	0.4090000
8	C	-1.5860000	-1.1120000	-0.5750000
9	C	-0.9060000	-2.3700000	-0.7380000
10	H	-1.3050000	-3.1840000	-0.9440000
11	C	0.3970000	-2.1640000	-0.5400000
12	H	1.0560000	-2.8210000	-0.5570000
13	C	0.6170000	-0.7860000	-0.3000000
14	C	1.7780000	-0.0940000	-0.1160000
15	C	1.7840000	1.3350000	-0.0470000
16	C	2.8210000	2.2550000	-0.0140000
17	H	3.7300000	2.0550000	0.0240000
18	C	2.2640000	3.5270000	-0.0460000
19	H	2.7220000	4.3370000	-0.0350000
20	C	0.9050000	3.3520000	-0.0970000
21	C	3.0820000	-0.8050000	0.0170000
22	C	3.5560000	-1.6940000	-0.9200000
23	H	3.0500000	-1.8770000	-1.6790000
24	C	4.7820000	-2.3230000	-0.7440000
25	H	5.0740000	-2.9330000	-1.3830000
26	C	5.5770000	-2.0680000	0.3500000
27	C	5.1030000	-1.1740000	1.2760000
28	H	5.6220000	-0.9840000	2.0250000
29	C	3.8800000	-0.5470000	1.1340000
30	H	3.5860000	0.0500000	1.7840000
31	C	6.9150000	-2.7420000	0.5460000
32	H	7.5050000	-2.1520000	1.0200000
33	H	7.2950000	-2.9560000	-0.3100000
34	H	6.7950000	-3.5490000	1.0520000
35	C	-3.7760000	-2.0460000	-1.1230000
36	H	-3.2260000	-2.7890000	-1.4160000
37	H	-4.2830000	-1.7260000	-1.8860000
38	C	-4.7290000	-2.5190000	-0.0480000
39	H	-5.2960000	-3.2230000	-0.4000000
40	H	-4.2250000	-2.8810000	0.6990000
41	C	-4.6970000	-0.3580000	0.9240000

42	H	-4.1950000	-0.6990000	1.6820000
43	H	-5.2440000	0.3790000	1.2360000
44	C	-3.7340000	0.1470000	-0.1230000
45	H	-4.2260000	0.5430000	-0.8580000
46	H	-3.1600000	0.8290000	0.2600000
47	C	-6.5340000	-1.8430000	1.3970000
48	H	-7.1240000	-2.4870000	0.9990000
49	H	-7.0430000	-1.0870000	1.7000000
50	H	-6.0810000	-2.2420000	2.1420000
51	B	-0.8220000	1.3930000	-0.1070000

Table S4. DFT optimized coordinates for sensor **2** in the ground state.

Number	Atom	Coordinates		
		X	Y	Z
1	Cl	-0.2675360	4.6528090	0.0354490
2	F	1.1027810	1.7889020	1.0059200
3	F	1.1092380	2.1331930	-1.2409390
4	N	-0.9934340	2.0626320	-0.0929280
5	N	0.3729860	-0.0180990	-0.4287090
6	N	2.6630230	-0.7009010	-0.7915660
7	N	5.2973310	-0.9289690	0.3141480
8	C	-1.3896370	3.3481920	0.0013980
9	C	-2.7511730	3.4439860	0.1152130
10	H	-3.2534030	4.2237310	0.1852480
11	C	-3.2300820	2.1429700	0.1031070
12	H	-4.1221930	1.8893450	0.1643370
13	C	-2.1456570	1.2870190	-0.0141300
14	C	-2.0600750	-0.1268060	-0.1343460
15	C	-0.8535560	-0.7366680	-0.3709970
16	C	-0.5743880	-2.0970120	-0.6213310
17	H	-1.1988680	-2.7860070	-0.6320160
18	C	0.7472150	-2.2299530	-0.8399850
19	H	1.1918520	-3.0198250	-1.0463180
20	C	1.3483470	-0.9325460	-0.6981460
21	C	-3.3053880	-0.9255200	0.0191330
22	C	-3.8037450	-1.7215140	-0.9701120
23	H	-3.3554700	-1.7807350	-1.7831190
24	C	-4.9678790	-2.4414900	-0.7757180
25	H	-5.2921050	-2.9676260	-1.4709130
26	C	-5.6640840	-2.4044250	0.4147880
27	C	-5.1821590	-1.5742880	1.3744830
28	H	-5.6394960	-1.5051140	2.1810050
29	C	-4.0306670	-0.8282340	1.1902320
30	H	-3.7425130	-0.2547240	1.8630110
31	C	-6.9038750	-3.2453420	0.6487420
32	H	-7.5848930	-2.7045520	1.0536130
33	H	-6.6882290	-3.9778170	1.2312490
34	H	-7.2209990	-3.5855590	-0.1901650
35	C	3.5987990	-1.7420330	-1.2254780
36	H	4.1019980	-1.4270360	-1.9932080
37	H	3.1041590	-2.5318070	-1.4929530
38	C	4.5512590	-2.0970100	-0.1077560
39	H	4.0515290	-2.4516240	0.6439060
40	H	5.1671560	-2.7828160	-0.4113720
41	C	4.3746010	0.0837810	0.7500690

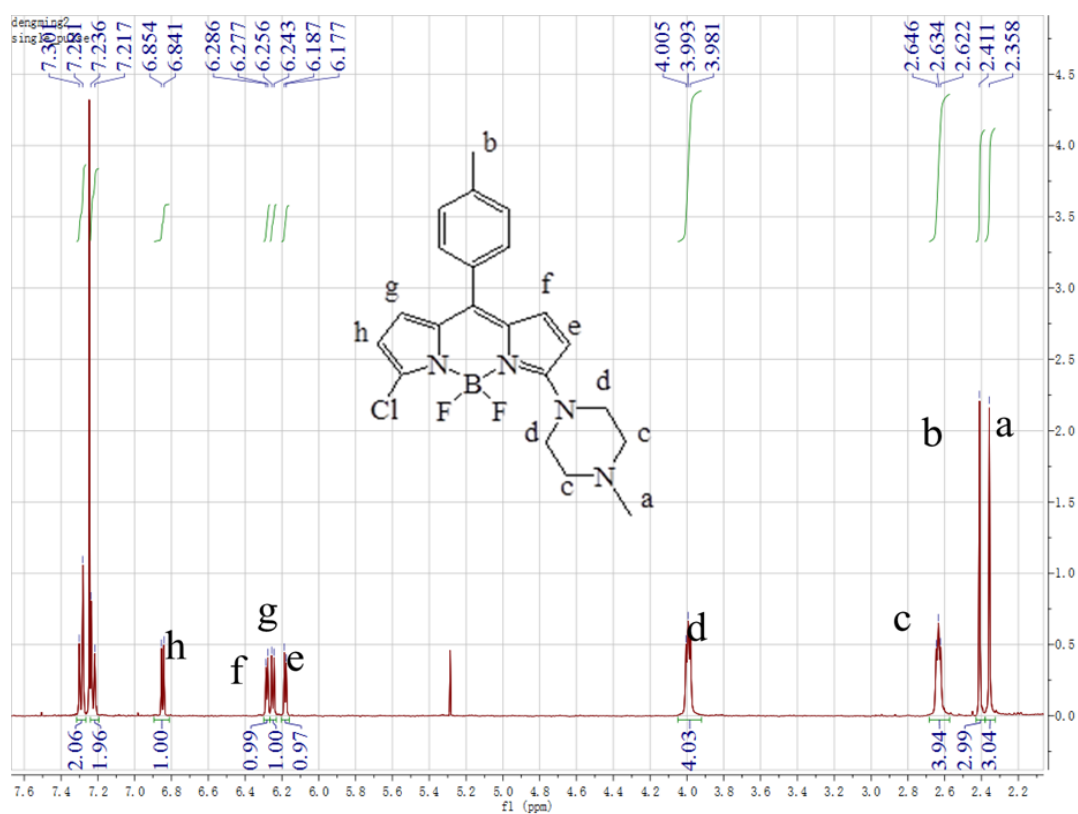
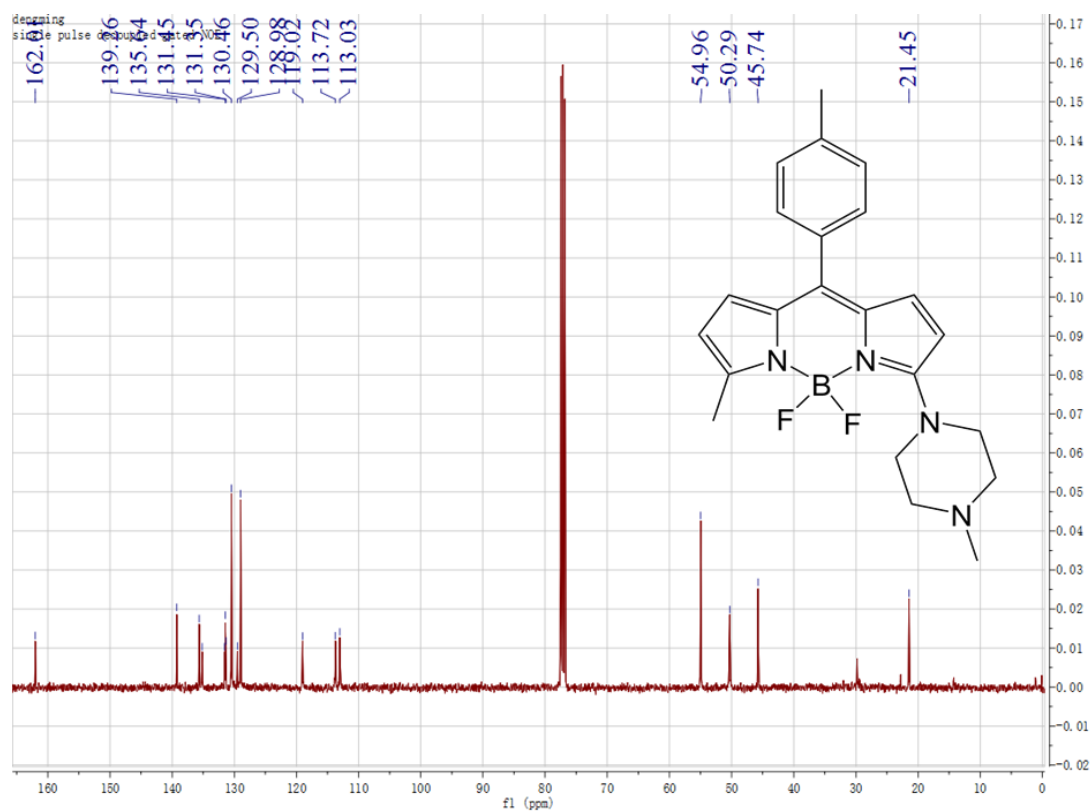
42	H	4.8688240	0.8663800	1.0396280
43	H	3.8675550	-0.2479130	1.5078620
44	C	3.4299440	0.4722500	-0.3467590
45	H	2.8220050	1.1570680	-0.0270010
46	H	3.9286380	0.8375880	-1.0938770
47	C	6.3101100	-1.2737980	1.3488590
48	H	5.9017930	-1.8608240	2.0039100
49	H	6.5796930	-0.4630240	1.8077440
50	C	7.5277130	-1.9426270	0.7886200
51	H	8.1160090	-2.1898350	1.5057210
52	H	7.9798120	-1.3382450	0.1967540
53	H	7.2657080	-2.7295170	0.3039390
54	B	0.4516620	1.5155310	-0.1880720

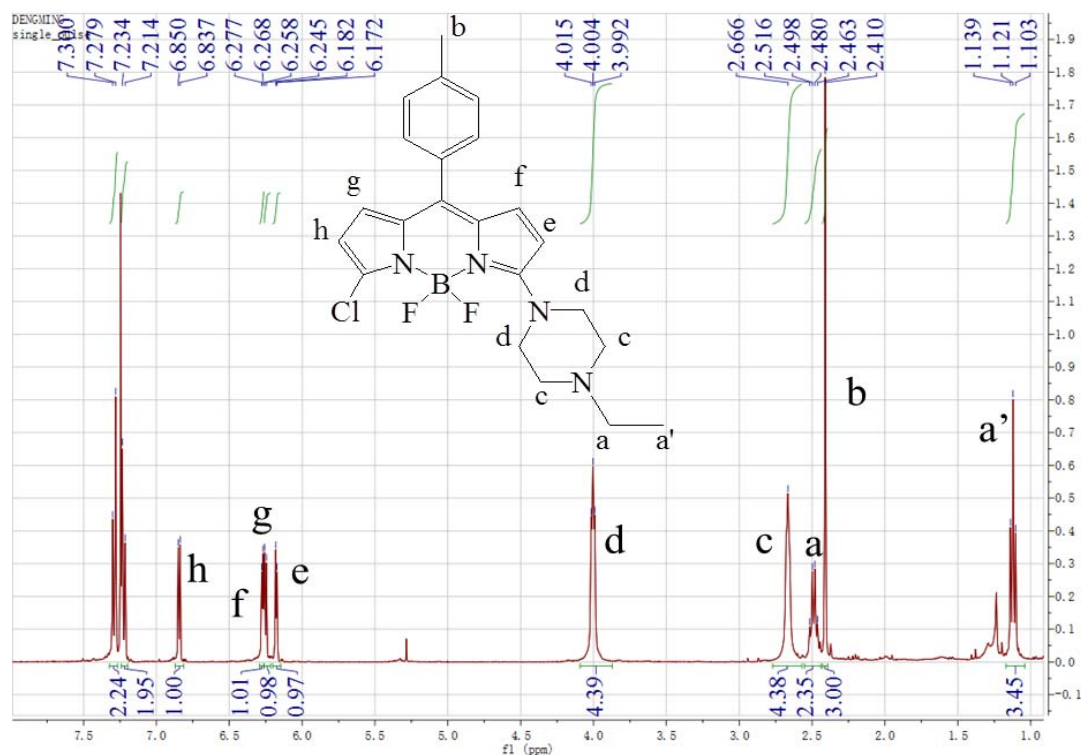
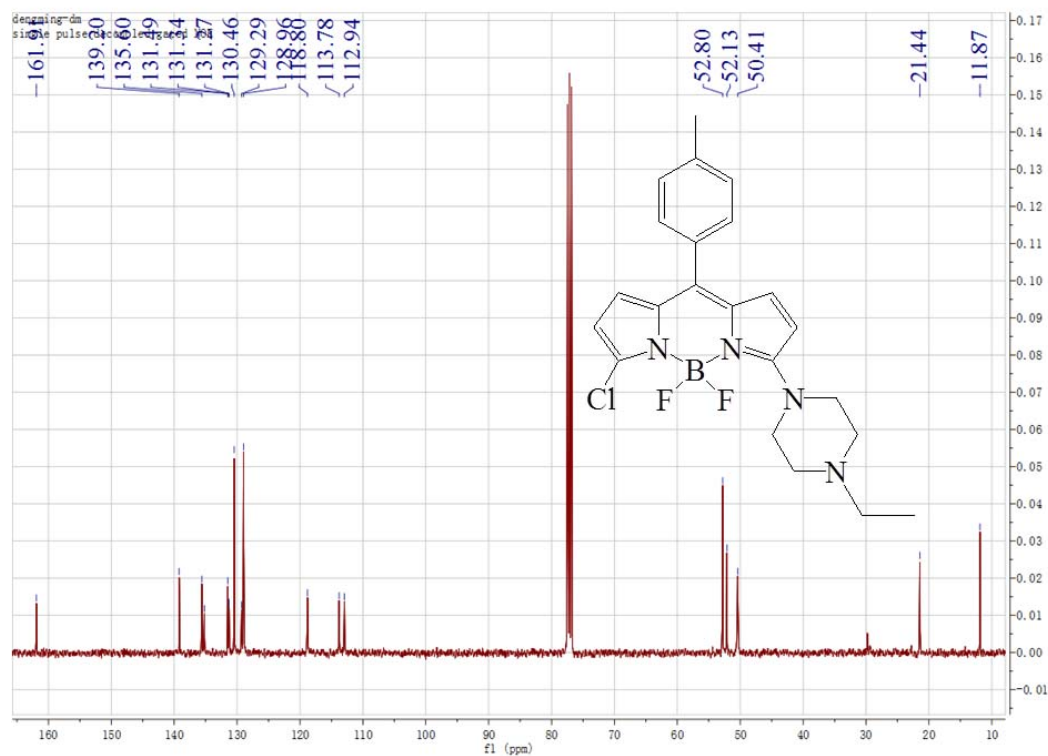
Table S5. TD-DFT calculated singlet absorption data for sensor **1** (Selected absorption wavelength > 200 nm).

State (<i>E</i> /nm, o.s.)	transition	coefficient	state (<i>E</i> /nm, o.s.)	transition	coefficient
S1 (426.64, 0.5820)	108 ->109	0.70659	S13 (219.91, 0.0745)	100 ->109	0.12831
	108 ->109	-0.11991		106 ->111	-0.17846
S2 (371.52, 0.0218)	107 ->109	0.70178		107 ->111	0.62871
				108 ->112	0.21036
S3 (329.36, 0.2222)	102 ->109	0.14213	S14 (218.26, 0.0675)	100 ->109	0.51344
	105 ->109	0.29168		106 ->111	-0.10781
	106 ->109	0.62075		107 ->111	-0.26130
S4 (328.44, 0.0216)	105 ->109	0.63597		108 ->112	0.28075
	106 ->109	-0.29299		108 ->113	-0.23551
S5 (296.96, 0.0019)	104 ->109	0.62972	S15 (216.02, 0.1653)	100 ->109	-0.32383
	108 ->110	-0.30503		105 ->110	0.26021
S6 (294.47, 0.0032)	103 ->109	0.21159		106 ->111	-0.19789
	104 ->109	0.27894		107 ->111	-0.15331
	108 ->110	0.60332		108 ->112	0.45173
S7 (291.55, 0.0125)	102 ->109	-0.13133		108 ->113	0.20055
	103 ->109	0.63672	S16 (214.51, 0.0845)	105 ->110	0.64869
	104 ->109	-0.11501		108 ->112	-0.22824
	108 ->110	-0.19752	S17 (210.40, 0.0012)	99 ->109	0.68198
	108 ->111	0.12600		108 ->114	-0.11965
S8 (281.36, 0.0305)	103 ->109	-0.11603	S18 (209.81, 0.0060)	99 ->109	0.13832
	108 ->111	0.69159		104 ->110	-0.11099
S9 (259.43, 0.1547)	102 ->109	0.64948		105 ->111	-0.14705
	103 ->109	0.14240		106 ->111	0.39478
	106 ->109	-0.12772		108 ->112	0.13839
	108 ->112	0.15564		108 ->113	0.16078
S10 (238.90, 0.0003)	101 ->109	0.70009		108 ->114	0.44682
			S19 (208.47, 0.0022)	105 ->111	0.34771
S11 (230.09, 0.0044)	104 ->111	-0.25993		106 ->111	-0.30866
	106 ->110	-0.44027		108 ->112	-0.15962
	107 ->110	0.46870		108 ->114	0.46017
S12 (225.62, 0.0014)	103 ->110	0.10805	S20 (208.01, 0.0126)	103 ->110	0.12493
	104 ->111	0.22167		105 ->111	0.54324
	106 ->110	0.38730		106 ->110	-0.10493
	107 ->110	0.52804		106 ->111	0.27987
				108 ->112	0.16282
				108 ->113	-0.19489

Table S6. TD-DFT calculated singlet absorption data for sensor 2 (Selected absorption wavelength > 200 nm).

State (<i>E</i> /nm, o.s.)	transition	coefficient	state (<i>E</i> /nm, o.s.)	transition	coefficient
S1 (424.57, 0.6025)	112 ->113	0.70387	S13 (219.91, 0.0141)	111 ->115	0.69323
	112 ->113	-0.12119			
S2 (374.27, 0.0272)	111 ->113	0.69909	S14 (217.49, 0.0269)	104 ->113	0.62272
				112 ->116	0.12535
S3 (325.46, 0.1172)	106 ->113	0.12862		112 ->117	0.26638
	109 ->113	0.49869	S15 (215.33, 0.2202)	109 ->114	0.12758
	110 ->113	-0.47475		110 ->115	0.16207
S4 (321.23, 0.0930)	106 ->113	-0.10015		112 ->116	0.56605
	109 ->113	0.49029		112 ->117	-0.20127
	110 ->113	0.48520		112 ->118	0.22973
S5 (297.31, 0.0031)	106 ->113	0.10090	S16 (212.88, 0.0291)	104 ->113	0.12917
	108 ->113	0.67835		109 ->114	-0.28943
	112 ->114	0.14294		110 ->115	-0.14899
S6 (293.19, 0.0041)	107 ->113	0.26925		112 ->116	-0.14982
	112 ->114	0.63684		112 ->117	-0.19551
S7 (290.60, 0.0241)	107 ->113	0.62477		112 ->118	0.53908
	108 ->113	0.11784	S17 (212.00, 0.0770)	109 ->114	0.61471
	112 ->114	-0.26073		112 ->116	-0.20736
	112 ->115	0.10478		112 ->118	0.21374
S8 (280.32, 0.0280)	112 ->115	0.69563	S18 (210.45, 0.0004)	103 ->113	0.68933
S9 (260.60, 0.1619)	106 ->113	0.65002	S19 (206.64, 0.0091)	102 ->113	-0.13290
	107 ->113	0.11421		107 ->114	-0.14481
	110 ->113	0.15403		107 ->115	0.10066
	112 ->116	0.15236		108 ->114	-0.13301
S10 (237.67, 0.0001)	105 ->113	0.70020		110 ->114	-0.10866
				110 ->115	0.58320
S11 (228.79, 0.0010)	108 ->115	0.14143		112 ->116	-0.19553
	110 ->114	0.22821		112 ->118	0.11912
	111 ->114	0.64889	S20 (205.87, 0.0195)	109 ->115	0.64585
S12 (224.15, 0.0022)	107 ->114	-0.15741		112 ->117	0.18442
	108 ->115	0.32983			
	110 ->114	0.50917			
	111 ->114	-0.27841			

^1H NMR (CDCl_3 , 400 MHz) spectrum of sensor **1** ^{13}C NMR (CDCl_3 , 400/4 MHz) spectrum of sensor **1**

^1H NMR (CDCl_3 , 400 MHz) spectrum of sensor 2 ^{13}C NMR (CDCl_3 , 400/4 MHz) spectrum of sensor 2

MS Spectrum of **1** (M+1) (the molecular weight of **1** is 414.2).

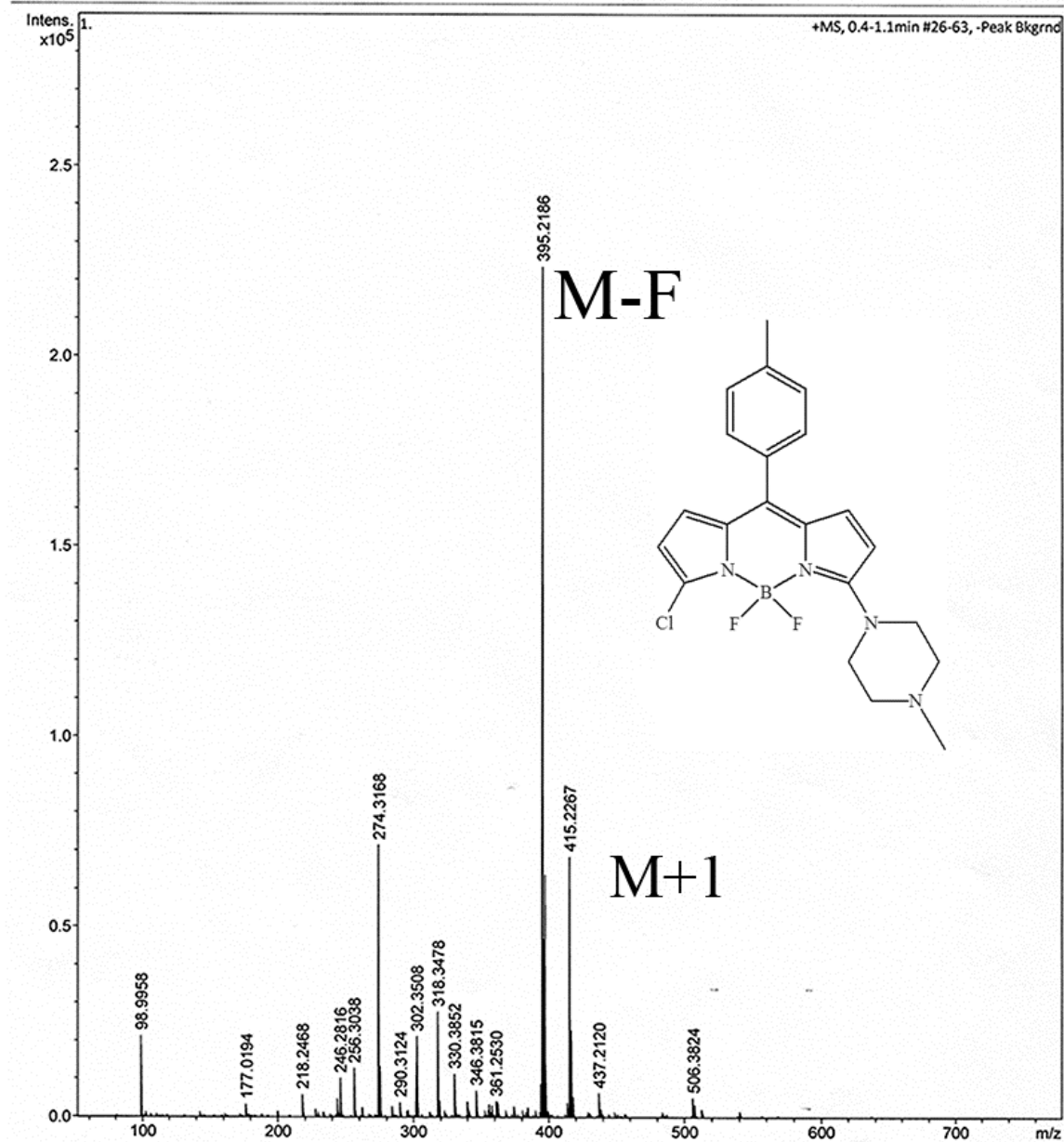
Generic Display Report

Analysis Info

Analysis Name D:\Data\yangy\new\GUOYALI150508_20_01_2317.d
Method POS_100-1200_For LC.m
Sample Name GUOYALI150508
Comment

Acquisition Date 5/8/2015 12:04:34 PM

Operator LZU
Instrument micrOTOF



MS Spectrum of **2** (M+1) (the molecular weight of **2** is 428.2).

