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ORIGINAL ARTICLE

A BODIPY-Based Water-Soluble Fluorescent Probe for Naked Eye Detection of pH

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Abstract

In this study, a BODIPY-based water-soluble fluorescent chemosensor **BBP** has been synthesized using BODIPY as the fluorescence group and quinoline as the recognition group. **BBP** can be used for naked eye detection of pH in complete aqueous solution and it shows high specificity in a wide range of cations. The p*Ka* value is determined to be 2.94 and the fluorescence intensity is linearly related to pH in the range of 2.4–3.6.

Keywords BODIPY · Fluorescent Chemosensor · Naked eye · pH

Introduction

Changes in pH can interfere with cell growth, apoptosis and enzyme activity [1], resulting in the occurrence and development of a variety of diseases, such as cancers and Alzheimer's disease [2–5]. There are also a variety of methods for monitoring pH changes including fluorescent probes [6, 7], acid-base indicator titration [8], potentiometric titration [9] and so on. Fluorescent chemosensors used for detection of pH usually have low background noise and high selectivity and sensitivity [10–13]. However, it is noted that most sensors are tested under near neutral (pH 6.0–8.0) [14–16] or slightly acidic (pH 4.0–6.0) conditions [17–20], and there is a lack of sensors that are stable and applicable under extremely acidic conditions (below pH 4.0) [21, 22], especially sensors that are completely water-soluble.

BODIPY (4, 4-difluoro-4-bora-*3a*, *4a*-diaza-*s*-indacene, structural formula is shown in the following part of the dotted line in Scheme 1) has been revealed to be the most attractive as a fluorescent signaling unit in constructing fluorescent sensors toward heavy-metal ions, pH, solvent polarity, and small molecules because of its favorable characteristics such as high fluorescence quantum yield, high molar absorption coefficient, high fluorescence intensity, narrow emission band, and high stability in optical and chemical environment [23–32]. In recent years, BODIPY-based pH sensors have been reported [33–36] such as working in the region of near neutral pH 6.0–8.0 and detecting weak acidity within the pH range 4.0–6.0. However, theses BODIPY-based pH sensors still have some drawbacks such as poor naked eye recognition, poor water-soluble and stability. For these reasons, there is a practical need for the development of effective chemosensors for monitoring extremely acid levels under completely water-soluble.

In addition, some papers of scientists who study the relationship between structural modification and optical properties of BODIPY have been reported [37, 38]. Based on these researches, we synthesized a BODIPY-based fluorescent chemosensor **BBP**(N-(2-aminoethyl)-2-((5-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4 l4,5 l4-dipyrrolo[1,2-c:2′,1′-f][1,3,2]diazaborinin-10-yl)quinolin-8-yl)oxy)acetamide), which could sensitive detection pH due to the introduction of the quinoline. Sensor **BBP** used quinoline as a pH indicator in order to achieve naked eye detection of pH under extremely acidic conditions and completely water-soluble (See Scheme

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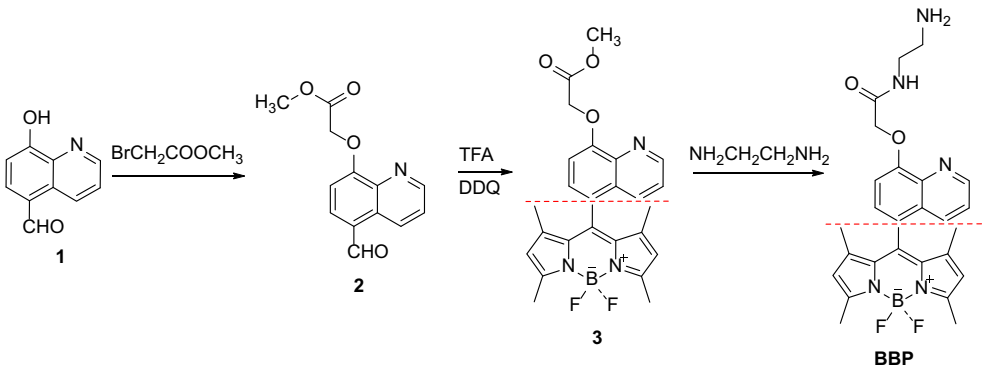
Scheme 1 Synthesis of sensor molecule **BBP**



1), which will be helpful for developing novel versatile fluorescent pH sensors with potential applications in chemical and biological fields.

Experimental

Materials and Methods

Compounds **1** was synthesized as described in a previous study [39]. Other solvents and starting materials were purchased from Aladdin and Energy Chemical Reagents Ltd. Ultrapure water was used through all the tests. The melting points of intermediate and sensor **BBP** were measured on a WRS-C1 digital melting-point apparatus (Shanghai, China). The pH of all solutions was adjusted on a PHS-3C pH meter (Shanghai, China). The ^{1}H NMR (400 M) and ^{13}C NMR (100 M) spectra were recorded on a Bruker AVANCE III spectrometer (Switzerland) in $CDCl_3$ solution. The SEI-MS spectra of intermediate and sensor **BBP** were recorded on a Bruker Solarix XR Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometer. The UV-spectra of all samples were recorded on a UV-2602 spectrophotometer (Shanghai, China), and all fluorescence spectra were recorded on a HITACHIF-2500 spectrophotometer (Japan).

Synthesis

Preparation of Solid 2 173 mg of 1 mmol compound **1**, 229 mg of 1.5 mmol methyl bromoacetate and 500 mg of 3.6 mmol K_2CO_3 were dissolved in acetone (5 mL), and then the mixture was heated to reflux for 5 h. After that, the mixture was cooled to room temperature and filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (1% ~ 2% methanol in dichloromethane) to obtained solid **2.**

2: Yield 70%. Yellow white solid. Mp: 119.7 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) 3.84 (s, 3H), 5.08 (s, 2H), 7.04 (d, 1H, J = 8.0 Hz), 7.64 (dd, 1H, J_1 = 8.8 Hz, J_2 = 4.0 Hz), 7.97 (d, 1H, J = 8.0 Hz), 9.04 (dd, 1H, J_1 = 4.0 Hz, J_2 = 1.6 Hz), 9.67 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 10.18 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, TMS): δ (ppm) 53.6, 65.9, 107.5, 124.4, 125.5, 127.6, 133.8, 138.8, 139.8, 150.4, 158.5, 168.2, 191.8. HR-MS (ESI): m/z calcd for $C_{13}H_{12}NO_4$ [(M + H)$^+$]: 246.0761, found 246.0760 (Fig. S1-S3).

Preparation of Solid 3 Under argon, 0.49 g of 2 mmol solid 2, 0.38 g of 4 mmol 2, 4-dimethylpyrrole and 10 μL TFA were dissolved in 100 mL anhydrous CH_2Cl_2, and then the mixture was stirred at room temperature for overnight. A solution of DDQ (2,3-dichloro-5,6-dicyano-p-benzoquinone, 0.45 g, 2 mmol) in anhydrous CH_2Cl_2 was added, and the mixture was stirred for another 1 h. Then 3 mL triethylamine and 3 mL BF_3·OEt_2 were added and stirred for overnight. After that, the organic layer was washed with 100 mL of water, dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (20% acetone in hexane) to afford **3**.

3: Yield 32.8%. Red solid. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) 1.10 (s, 6H), 2.59 (s, 6H), 3.83 (s, 3H), 5.04 (s, 2H), 5.96 (s, 2H), 7.07 (d, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 8.0 Hz), 7.43 (dd, 1H, J_1 = 8.8 Hz, J_2 = 4.0 Hz), 8.11 (d, 1H, J = 8.8 Hz), 9.01 (d, 1H, J = 4.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, TMS): δ (ppm) 14.1, 14.7, 52.4, 66.2, 109.2, 121.4, 122.9, 125.5, 126.5, 128.2, 132.2, 133.4, 137.9, 140.1, 142.8, 150.4, 154.5, 156.1, 168.8. HR-MS (ESI): m/z calcd for $C_{25}H_{25}BF_2N_3O_3$ [(M + H)$^+$]: 464.1952, found 464.1953 (Fig. S4-S6).

Preparation of Sensor BBP Under argon, 2.59 g of 10 mmol solid **3** and 0.6 g of 10 mmol fresh distilled ethylenediamine were dissolved in 15 mL anhydrous acetonitrile, and then the mixture was heated to reflux for 15 h. After that, the mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (20% methanol in dichloromethane) to obtained sensor **BBP.**

Sensor BBP Yield 51.5%. Red solid. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ (ppm) 1.09 (s, 6H), 1.26 (s, 2H), 2.59 (s,

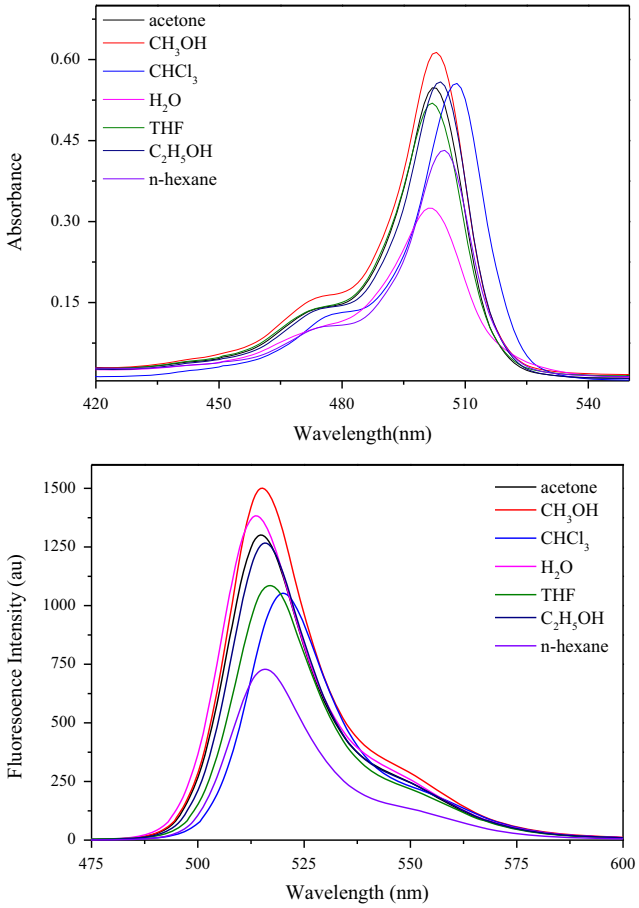


Fig. 1 UV and fluorescence spectras of sensor **BBP** in different solvents at a concentration of 5 μM, including acetone, methanol, chloroform, water, THF, ethanol and n-hexane

6H), 2.91 (t, 2H, $J = 6.0$ Hz), 3.48 (t, 2H, $J = 6.0$ Hz), 4.85 (s, 2H), 5.97 (s, 2H), 7.24 (d, 1H, $J = 8.0$ Hz), 7.44–7.49 (m, 2H), 8.16 (dd, 1H, $J_1 = 8.8$ Hz, $J_2 = 1.6$ Hz), 8.28 (s, 1H), 8.97 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 1.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, TMS): δ (ppm) 14.1, 14.7, 29.7, 41.5, 42.1, 69.4, 111.2, 121.6, 123.0, 126.2, 127.1, 128.4, 132.1, 133.9, 137.5, 140.1, 142.7, 150.3, 154.3, 156.3, 168.2. HR-MS (ESI): *m/z*

Table 1 Spectroscopic data of sensor **BBP** in various solvents at a concentration of 5 μM

Solvent	A	$\lambda_{abs\ max}$/nm	I	$\lambda_{em\ max}$/nm	Δν
methanol	0.61	502	1500	515	13
ethanol	0.56	504	1266	516	12
chloroform	0.55	508	1053	520	12
acetone	0.55	502	1301	515	13
THF	0.52	502	1085	517	15
n-hexane	0.43	505	728.1	516	11
water	0.33	502	1396	513	11

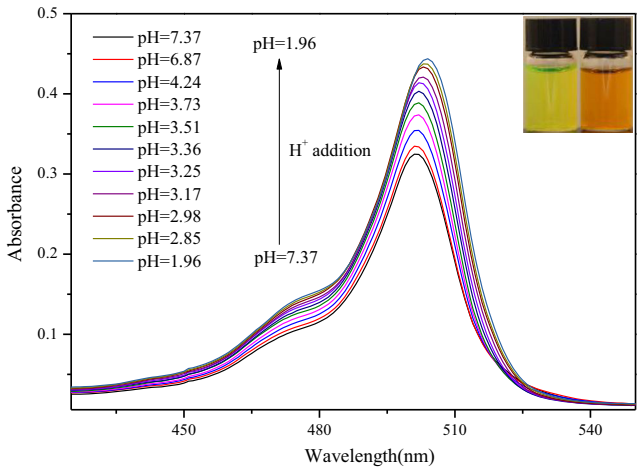


Fig. 2 The spectra changes of sensor **BBP** upon addition of H^+ ranging from 7.37 to 1.96 at room temperature. Inset: the color changed of sensor **BBP** (5 μM) in the absence and presence of H^+

calcd for $C_{26}H_{29}BF_2N_5O_2$ $[(M + H)^+]$: 492.2377, found 492.2373 (Fig. S7-S9).

General Procedure for Fluorescence Spectra Experiments

All tests were carried out in complete aqueous solution medium at room temperature. All fluorescence spectra were recorded at an excitation of 450 nm. The stock solutions of various metal ions (1×10^{-2} M) were prepared from chlorine salts in ultrapure water. The concentration of sensor **BBP** was 5 μM in all experiments. The slits of emission and excitation were 5 nm in all experiments.

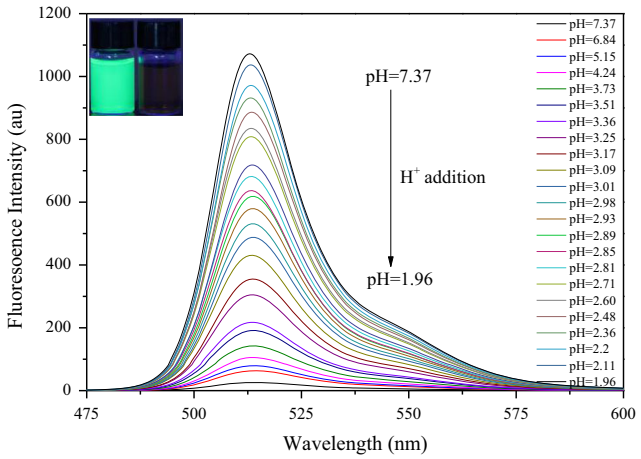


Fig. 3 Fluorescent intensity changes of sensor **BBP** upon addition of H^+ ranging from 7.37 to 1.96 at room temperature. Inset: the fluorescence changed of probe **BBP** (5 μM) in the absence and presence of H^+

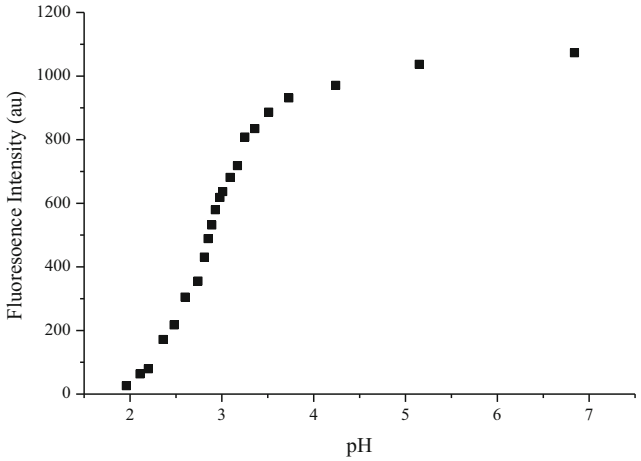


Fig. 4 A plot of fluorescence intensity of sensor **BBP** against pH from 7.37 to 1.96 in water solution. Excitation was at 450 nm and emission at 513 nm

Results and Discussion

Spectral Parameters of Sensor BBP in Different Solvents

The solvent effect of **BBP** was investigated at the same concentration, including acetone, methanol, chloroform, water, THF, ethanol and n-hexane. Figure 1 and Table 1 show that the maximum absorption wavelength is centered at 502–508 nm, which is mainly attributed to the S_0-S_1 transition of the BODIPY group; and the maximum emission wavelength is centered at 515–520 nm and shows a low Stokes shift (ca. 15 nm). Therefore, the **BBP** is less affected by the solvent polarity whether UV-vis absorption or fluorescence spectrum.

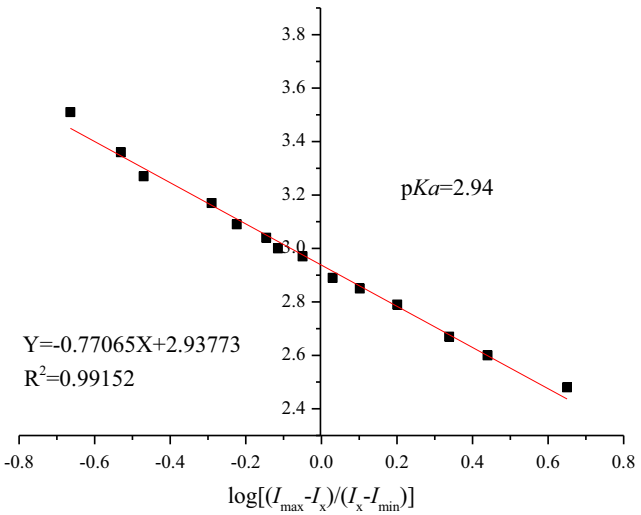


Fig. 5 Linear regression relationship between the pH value and log $[(I_{max}\text{-}I_x)/(I_x\text{-}I_{min})]$ in water solution. Excitation was at 450 nm and emission at 513 nm

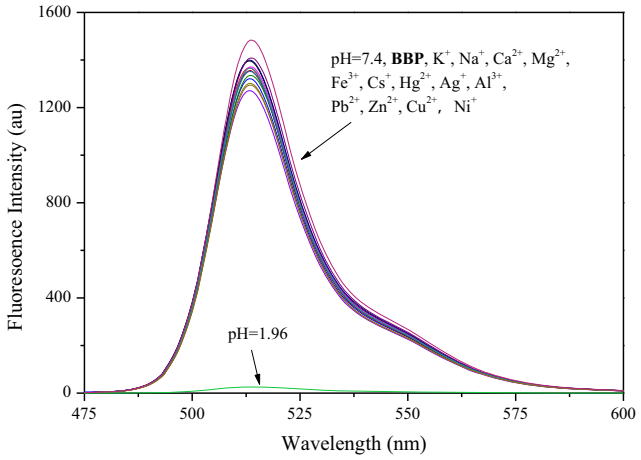


Fig. 6 Fluorescence responses of sensor **BBP** in the presence of different metal ions and H^+

The Absorbance Spectra Changes of Sensor BBP upon Addition of H^+

Figure 2 shows that the spectra of **BBP** change significantly with increasing H^+ concentration. The absorption spectra show a remarkable increase in absorbance and a slight red shift from 500 nm to 507 nm. The color of the **BBP** solution changes from yellow green to orange, indicating that **BBP** could be used for naked eye detection of H^+.

The Fluorescence Spectra Changes of Sensor BBP upon Addition of H^+

Figure 3 shows that **BBP** shows a marked fluorescence quenching response with the decrease in pH at 513 nm (λ_{ex} = 450 nm), and the quenching efficiency reaches a maximum of 98% $[(I_0\text{-}I)/I_0 \times 100\%]$ at pH = 1.96. Figure 4 shows that there is a significant linear relationship between fluorescence intensity and pH in the range of 2.48–3.51, indicating that **BBP** can be used to quantitatively detect H^+ within a certain pH range. According to the Henderson-Hasselbalch

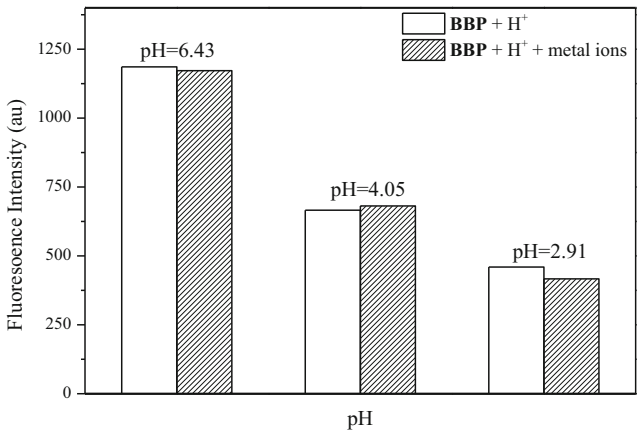


Fig. 7 Fluorescence maxima of sensor **BBP** at different pH

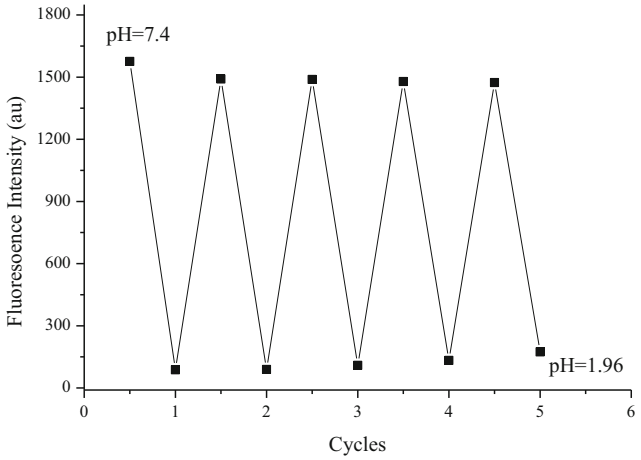


Fig. 8 Change in fluorescence intensity of **BBP** upon alternate addition of H^+ and OH^-

equation, $pH = pKa + \log [(I_{max}\text{-}I_x)/(I_x\text{-}I_{min})]$, where I_{max} is the maximum fluorescence intensity in a certain pH range, and I_x is the fluorescence intensity at a given pH, and I_{min} is the minimum fluorescence intensity in a certain pH range. Taking $\log[(I_{max}\text{-}I_x)/(I_x\text{-}I_{min})]$ as the abscissa and pH as the ordinate, the pKa value is obtained at pH = 2.94 from the intercept of trend line in Fig. 5. Thus, **BBP** can be used under extremely acidic conditions such as gastric acid, *Escherichia coli* and lysosomes.

The Fluorescence Response of Sensor BBP toward H^+

Quinoline-based derivatives have been widely used as fluorescent chemosensors due to their strong coordination ability with metal ions. In order to explore whether these metal ions can interfere with the detection of H^+, experiments were performed in a wide range of metal ions under neutral conditions (See Fig. 6). **BBP** shows no response to metal ions, indicating high specificity recognition of H^+ over a wide range of cations.

In order to investigate whether other metal ions interfere with the detection of H^+ under different pH conditions, experiments were performed at a pH of 6.43, 4.05 and 2.91 (See

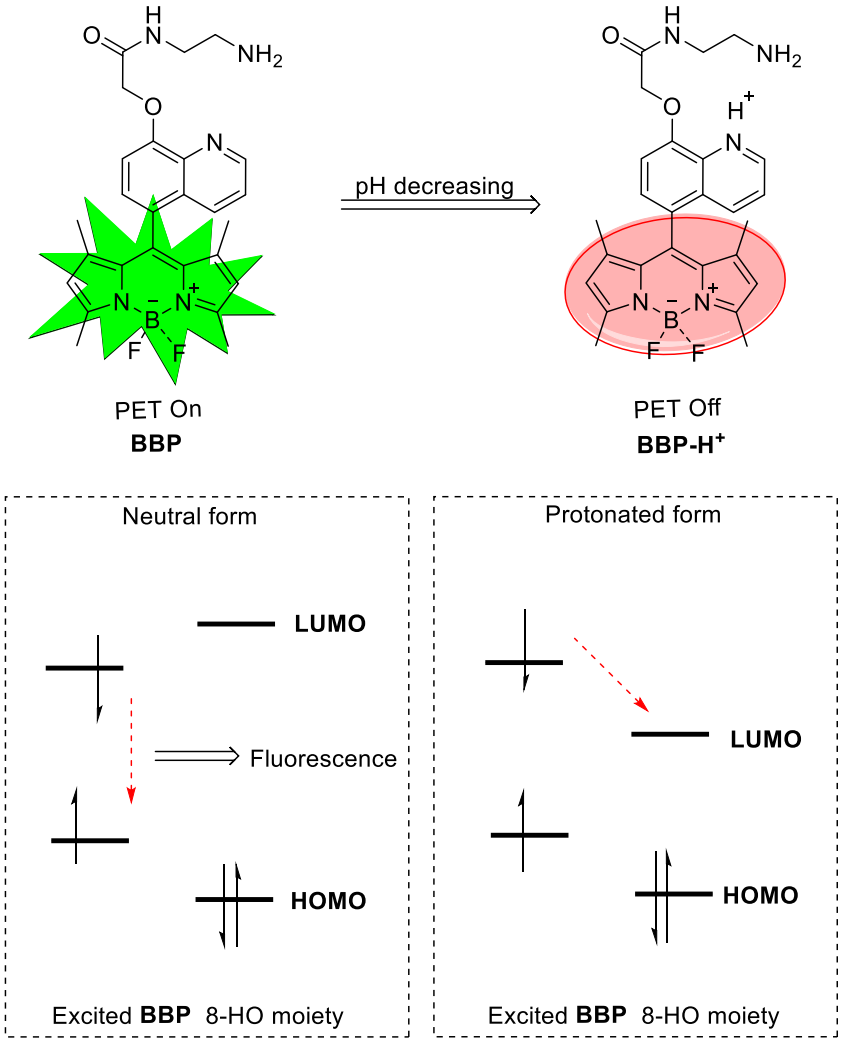


Fig. 9 The possible mechanism of **BBP** and H^+

Fig. 7). The results show that metal ions do not interfere with the recognition of H^+, indicating high selectivity of **BBP**.

Reversibility of Sensor BBP for H^+ and OH^-

The stability of **BBP** for the detection of H^+ was investigated using H^+ and OH^- as the input signal and fluorescence intensity as the output signal (See Fig. 8). After five cycles, the fluorescence response is still stable with the alternating addition of H^+ and OH^-, and the response time is less than 3 s. Thus, **BBP** has good stability in the detection of H^+.

The Possible Mechanism of Sensor BBP for H^+

Figure 9 shows that quinoline is an electron donor and BODIPY is an electron acceptor under neutral conditions, and the orbital energy of the quinoline group is higher than that of **BBP** in the excited state, resulting in fluorescence turn on. Under acidic conditions, the quinoline N atom is protonated in the form of NH^+, protonated quinoline acts as an electron acceptor and BODIPY acts as an electron donor, and the lowest unoccupied molecular orbital (LUMO) of the quinoline group is lower than that of **BBP** in the excited state**,** resulting in the transition of n to π^* is not allowed and fluorescence turn off. Therefore, **BBP** is a non-conjugated system by introducing ethylenediamine as a linking group, and its fluorescence spectra show obvious "on-off" fluorescence response and no shift of emission spectra with increasing H^+ concentration, and thus the PET (photoinduced electron transfer) mechanism is speculated.

Conclusions

In this study, we have successfully prepared a BODIPY-based sensor **BBP** using BODIPY as the fluorescence group and quinoline as the recognition group. **BBP** exhibits high specificity for H^+ over a wide range of cations under different pH conditions. With the alternate addition of H^+ and OH^- up to 5 times, the fluorescence intensity of **BBP** shows no significant attenuation and the color change only occurs within 3 s, indicating that **BBP** has good stability and high sensitivity for H^+ recognition. In the pH range of 2.48–3.51, the fluorescence intensity is linearly related to pH. The pKa value is obtained at 2.94. In conclusion, the sensor **BBP** can be used under extremely acidic conditions such as gastric acid, *Escherichia coli* and lysosomes.

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