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# A highly effective “naked eye” colorimetric and fluorimetric curcumin-based fluorescent sensor for specific and sensitive detection of H<sub>2</sub>O<sub>2</sub> *in vivo* and *in vitro*†

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Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is involved in many important tasks in normal cell metabolism and signaling. However, abnormal levels of H<sub>2</sub>O<sub>2</sub> are associated with the occurrence of several diseases. Therefore, it is important to develop a new method for the detection of H<sub>2</sub>O<sub>2</sub> *in vivo* and *in vitro*. A turn-off sensor, 2,2-difluoro-4,6-bis(3-methoxy-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)styryl)-2H-1,3,2-dioxaborine (DFCB), based on curcumin was developed for the detection of H<sub>2</sub>O<sub>2</sub>. The DFCB, an orange-emitting sensor, was constructed by employing 2,2-difluoro-4,6-bis(4-hydroxy-3-methoxystyryl)-2H-1,3,2-dioxaborine (DFC) as the main carrier, and 2-(4-bromomethylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane as the recognition site. The recognition group on the DFCB sensor could be completely cleaved by H<sub>2</sub>O<sub>2</sub> to generate the intermediate DFC, which would lead to a colorimetric change from bright orange to light blue accompanying by a significantly quenched fluorescence, which could be seen by the naked eye. This sensor exhibited a highly specific fluorescence response to H<sub>2</sub>O<sub>2</sub>, in preference to other relevant species, with an excellent anti-interference performance. The sensor DFCB also possessed some advantages including a wide pH response range (6–11), a broad linear range (0–300 μM), and a low detection limit (1.31 μM). The sensing mechanism of the DFCB sensor for H<sub>2</sub>O<sub>2</sub> was verified by HRMS analysis, <sup>1</sup>H-NMR titration and DFT calculations. In addition, the use of the DFCB sensor was compatible with the fluorescence imaging of H<sub>2</sub>O<sub>2</sub> in living cells and zebrafish.

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## Introduction

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is one of the most important reactive oxygen species (ROS), and one of its key tasks is to activate immune cells and maintain the normal metabolism.<sup>1–4</sup> However, abnormal H<sub>2</sub>O<sub>2</sub> generation will cause irreversible damage to the organism, and may attack cellular biomolecules such as protein and DNA,<sup>5–10</sup> affect cell metabolism and proliferation, and cause cancer, abnormal amino acid modification, Alzheimer's disease and other diseases.<sup>11–13</sup> Due to the complex pathologies that arise in organisms, H<sub>2</sub>O<sub>2</sub> can cause oxidative stress and eventually lead to cell damage and

necrosis.<sup>14–16</sup> Therefore, to develop efficient methods for detecting H<sub>2</sub>O<sub>2</sub> is of great importance in disease prevention and medical diagnosis.

Existing quantitative detection methods, such as titrimetry, electrochemistry, spectrophotometry and so on, are relatively mature techniques.<sup>17–19</sup> Fluorescent sensor analysis has the advantages of fast response, strong selectivity, adaptability and microscopic properties, and it has been widely used for detecting some analytes in biological and environmental systems.<sup>20–23</sup> At present, there are many reported sensors for H<sub>2</sub>O<sub>2</sub> which use aryl borate or phenyl boric acid reactions to generate phenol, phenol oxidation to give quinone, oxonium or diketone to give acid, carbon–carbon double bond breaking, and so on.<sup>24–26</sup> In recent years, a limited number of fluorescent sensors have been widely used for monitoring H<sub>2</sub>O<sub>2</sub> in living organisms and cells.<sup>27–30</sup> However, some reported sensors for H<sub>2</sub>O<sub>2</sub> have suffered from some drawbacks, such as unsatisfactory sensitivity, narrow pH usage range, and so on. Therefore, it is necessary to design pH-compatible fluorescent sensors for detecting H<sub>2</sub>O<sub>2</sub> in biological systems.

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Curcumin, a natural product with a unique fluorescence nature, is found mainly in the rhizomes of the plant families Zingiberaceae and Araceae.<sup>31,32</sup> Curcumin has received much attention due to its wide range of health care functions and biological properties, such as antibacterial, anti-inflammatory, anti-infective, anti-cancer and liver protective activities. We propose that the curcumin-based fluorescent sensor may have low cytotoxicity.<sup>33–35</sup> Moreover, curcumin and its derivatives have a large conjugate structure, which could enhance the  $\pi$  electron delocalization. The curcumin and its derivatives are better to be emitted to generate outstanding long-wavelength fluorescence. The fluorescence sensor with a long-wavelength emission can effectively avoid interference with self-emission fluorescence, and is an ideal tool for *in vivo* imaging. In this case, some excellent curcumin-based fluorescent sensors have constructed.<sup>36–38</sup> Therefore, it is promising for the development of a new curcumin-based fluorescent sensor for  $\text{H}_2\text{O}_2$  detection.

It has been reported that  $\beta$ -dicarbonyl compounds which form a rigid structure with boron trifluoride ( $\text{BF}_3$ ), could enhance the fluorescence emission wavelength.<sup>39,40</sup> Based on this research,  $\text{BF}_3$  was purposefully added to curcumin to amplify its conjugation effect and sequentially modulate its fluorescence properties. In the research reported in this paper, a novel curcumin-based fluorescent sensor based on **DFCB** was developed. The quenching mechanism of **DFCB** on  $\text{H}_2\text{O}_2$  was confirmed by high resolution mass spectrometry (HRMS) analysis,  $^1\text{H}$ -NMR titration and DFT calculations. The bilateral ether bond of the **DFCB** sensor would be broken in the presence of  $\text{H}_2\text{O}_2$ , which leads to a significant change of the color which can be seen by the “naked eye”, and the spectral properties of the sensor. The **DFCB** sensor emitted orange fluorescence at 601 nm and exhibited a significantly quenched fluorescence response to  $\text{H}_2\text{O}_2$ . The **DFCB** sensor showed a high sensitivity and favorable selectivity for  $\text{H}_2\text{O}_2$  in PBS solution (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4). More importantly, the **DFCB** sensor could detect  $\text{H}_2\text{O}_2$  in HeLa cells as well as in living zebrafish, which confirmed that the **DFCB** sensor could be used to effectively monitor exogenous and endogenous  $\text{H}_2\text{O}_2$  in a biological system.

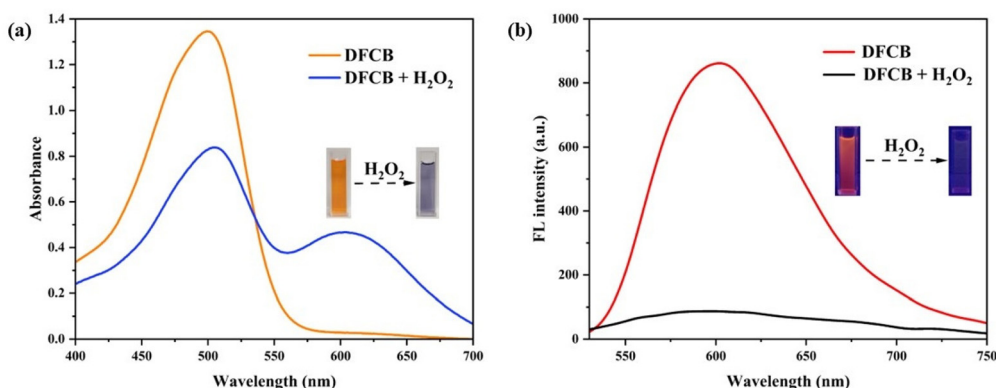
## Results and discussion

### Design and synthesis of the DFCB sensor

In this research, a new curcumin-based fluorescent sensor was constructed for  $\text{H}_2\text{O}_2$  detection. In this sensor, the curcumin unit is used logically as a highly effective fluorescent framework due to its lipophilic long-chain conjugate. After that, boron trifluoride diethyl etherate ( $\text{BF}_3 \cdot (\text{C}_2\text{H}_5)_2\text{O}$ ) was introduced into the curcumin framework to form the **DFC** compound, which can produce a strong electron push-pull effect. Next, the 2-(4-bromomethylphenyl)-4,4,5,5-tetramethyl-1,3,2-doxaborolane group was introduced into the molecule and used as a recognition group for  $\text{H}_2\text{O}_2$ . As expected, the sensor exhibited a significant fluorescence emission in the PBS solution (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4). After reaction with  $\text{H}_2\text{O}_2$ , the 2-(4-bromomethylphenyl)-4,4,5,5-tetramethyl-1,3,2-doxaborolane group of the **DFCB** sensor was selectively broken, allowing for the formation of the intermediate **DFC**, which would lead to a significantly quenched fluorescence emission. The chemical structure of the **DFCB** sensor was confirmed using  $^{13}\text{C}$ -NMR,  $^1\text{H}$ -NMR and HRMS analyses (see the ESI†).

### Spectral properties of DFCB in response to $\text{H}_2\text{O}_2$

The optical properties of the **DFCB** sensor in the absence and presence of  $\text{H}_2\text{O}_2$  were determined using UV-vis absorption and fluorescence spectroscopy. As shown in Fig. 1a, the **DFCB** sensor had a sharp and strong absorption band at 503 nm. After adding 300  $\mu\text{M}$   $\text{H}_2\text{O}_2$  into the solution of the **DFCB** sensor, the absorbance at 503 nm decreased significantly, whereas a new absorption band appeared at 601 nm. At the same time, the color of the solution changed from bright orange to light blue. The previous data indicated that the **DFCB** sensor can serve as a colorimetric sensor for detecting  $\text{H}_2\text{O}_2$ . As shown in Fig. 1b, the fluorescence spectra of **DFCB** sensor had a strong fluorescence emission at 601 nm. However, after addition of 300  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , the fluorescence emission of the **DFCB** sensor decreased significantly, and the color of the fluorescence changed from bright orange to colorless. The fluorescence spectral results indicated that the **DFCB**



**Fig. 1** (a) The UV-vis absorption, and (b) the fluorescence spectra of the **DFCB** (100  $\mu\text{M}$ ) sensor before and after addition of  $\text{H}_2\text{O}_2$  (300  $\mu\text{M}$ ) in PBS solution (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4).  $\lambda_{\text{ex}}$  = 425 nm.

sensor could be used as a turn off fluorescence sensor for  $\text{H}_2\text{O}_2$  detection.

### Sensitivity of the DFCB sensor to $\text{H}_2\text{O}_2$

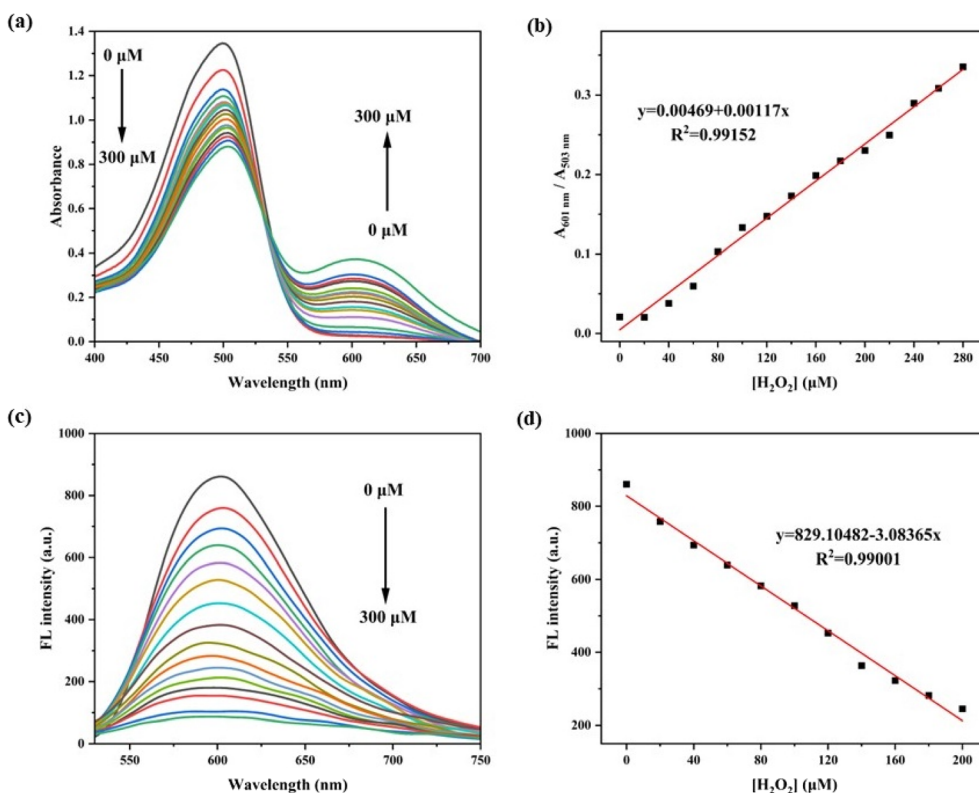
In order to explore the sensitivity of the DFCB sensor for detecting  $\text{H}_2\text{O}_2$ , the UV-vis absorption and fluorescence spectra of the DFCB sensor in response to different concentrations of  $\text{H}_2\text{O}_2$  were studied. As shown in Fig. 2a, with gradual addition of  $\text{H}_2\text{O}_2$  (0–300  $\mu\text{M}$ ), the absorption peak of sensor DFCB at 503 nm weakened gradually, whereas a new UV absorption peak appeared at 601 nm and was gradually enhanced. The absorption intensity ( $A_{601\text{ nm}}/A_{503\text{ nm}}$ ) of the DFCB sensor showed a good linear relationship with the  $\text{H}_2\text{O}_2$  concentration (0–300  $\mu\text{M}$ ), and the linear regression equation was  $y = 0.00469 + 0.00117x$  ( $R^2 = 0.99152$ ). These experimental data indicated that the DFCB sensor could be employed for the colorimetric quantitative detection of  $\text{H}_2\text{O}_2$ .

The fluorescence spectra of the DFCB sensor for detecting  $\text{H}_2\text{O}_2$  were measured. As shown in Fig. 2c, with the gradual addition of 0–300  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , the fluorescence emission peak of the DFCB sensor at 601 nm gradually weakened, and the fluorescence intensity of the DFCB sensor showed a linear relationship with the concentration of  $\text{H}_2\text{O}_2$  (0–300  $\mu\text{M}$ ). The linear regression equation was:  $y = 829.10482 - 3.08365x$  ( $R^2 = 0.99001$ ). According to the literature, the formula for the limit

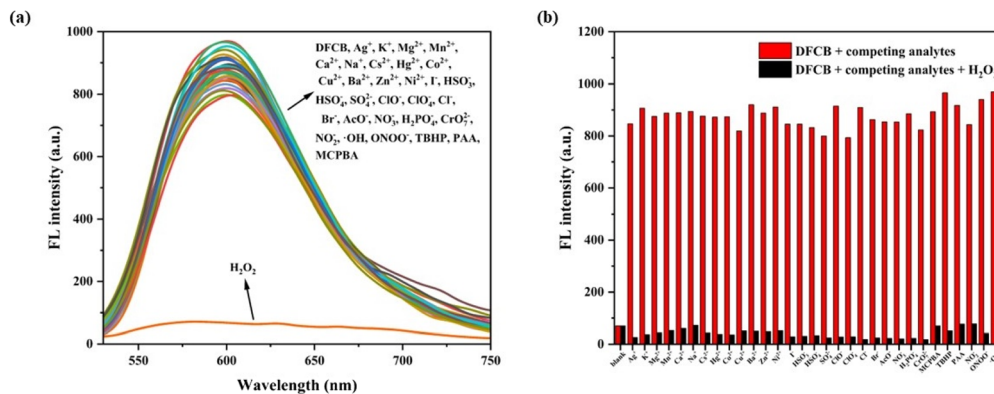
of detection was:  $\text{LOD} = 3\sigma/\kappa$ , and the detection limit was calculated to be 1.31  $\mu\text{M}$ ,<sup>11</sup> which was comparable to that of a previously reported sensor. Based on the UV-vis absorption and fluorescence spectroscopy, the DFCB sensor had high sensitivity and a low detection limit for  $\text{H}_2\text{O}_2$ .

### Selectivity of the DFCB sensor to $\text{H}_2\text{O}_2$

The selectivity and anti-interference properties of the DFCB sensor for the detection of  $\text{H}_2\text{O}_2$  were measured. As shown in Fig. 3a, the fluorescence spectra of the DFCB sensor after the addition of different analytes ( $\text{AcO}^-$ ,  $\text{Ag}^+$ ,  $\text{Ba}^{2+}$ ,  $\text{Br}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{ClO}^-$ ,  $\text{ClO}_4^-$ ,  $\text{Co}^{2+}$ ,  $\text{CrO}_7^{2-}$ ,  $\text{Cs}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{H}_2\text{O}_2$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{Hg}^{2+}$ ,  $\text{HSO}_3^-$ ,  $\text{HSO}_4^-$ ,  $\text{I}^-$ ,  $\text{K}^+$ , *meta*-chloroperoxybenzoic acid (MCPBA),  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\cdot\text{OH}$ ,  $\text{ONOO}^-$ , PAA,  $\text{SO}_4^{2-}$ , TBHP,  $\text{Zn}^{2+}$ ). After adding these analytes, the fluorescence intensity of the DFCB sensor was sharply quenched after the reaction with  $\text{H}_2\text{O}_2$ . In contrast, after the addition of the other analytes, the fluorescence intensity of the DFCB sensor exhibited no significant changes. These data demonstrated the superior selectivity of the DFCB sensor to  $\text{H}_2\text{O}_2$ . The anti-interference properties of the DFCB sensor for detecting  $\text{H}_2\text{O}_2$  were investigated. As shown in Fig. 3b, the fluorescence intensity of the DFCB sensor with  $\text{H}_2\text{O}_2$  was not affected by the presence of other analytes. Previous results had



**Fig. 2** (a) The UV-vis absorption spectra of the DFCB sensor (100  $\mu\text{M}$ ) with the addition of  $\text{H}_2\text{O}_2$  (0–300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ ,  $\text{pH} = 7.4$ ). (b) The linear fitting relationship between the  $\text{H}_2\text{O}_2$  concentration and the absorption intensity ratio ( $A_{601\text{ nm}}/A_{503\text{ nm}}$ ) of the DFCB sensor. (c) The fluorescence spectra of the DFCB sensor (100  $\mu\text{M}$ ) with the addition of  $\text{H}_2\text{O}_2$  (0–300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ ,  $\text{pH} = 7.4$ ). (d) The linear fitting relationship between  $\text{H}_2\text{O}_2$  concentration and the fluorescence intensity of the DFCB sensor at 601 nm.



**Fig. 3** (a) The fluorescence spectra of DFCB after the introduction of various analytes (300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4). (b) The fluorescence intensity of the DFCB sensor with  $\text{H}_2\text{O}_2$  at 601 nm in the presence of other analytes (300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4).  $\lambda_{\text{ex}}$  = 425 nm.

indicated that the DFCB sensor had excellent selectivity and anti-interference properties for  $\text{H}_2\text{O}_2$ .

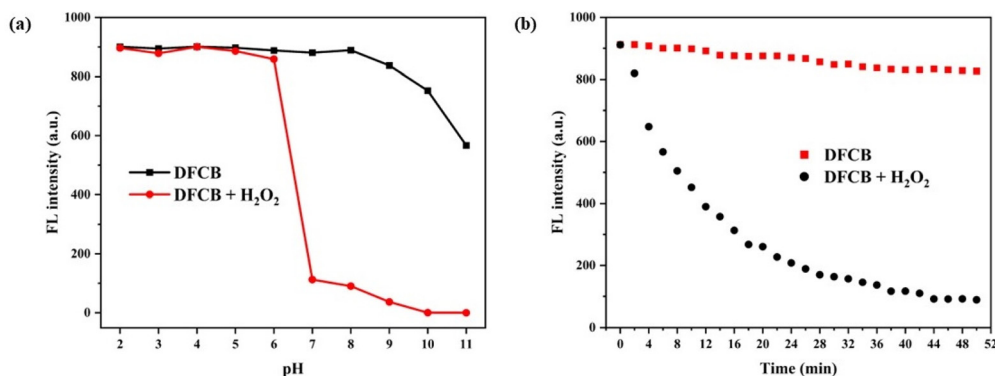
### Effects of pH and response time

The pH detection range determines whether the sensor can be applied in a biological environment. As shown in Fig. 4a, the DFCB sensor exhibited a high fluorescence intensity at 601 nm, which indicated its good stability. When the pH was located in the range of 2–6, the DFCB sensor showed no remarkable fluorescence change because the  $\text{H}_2\text{O}_2$  can react with acid to produce  $\text{H}_2\text{O}$  and  $\text{O}_2$  in an acidic environment. However, when the pH value increased to above 7, the fluorescence emission of the DFCB sensor was sharply quenched, which suggested that the DFCB sensor had an excellent detection ability over a wide pH range, and could be applied to living organisms. The response time of the DFCB sensor to  $\text{H}_2\text{O}_2$  was evaluated. As shown in Fig. 4b, after the addition of  $\text{H}_2\text{O}_2$ , the fluorescence intensity of the DFCB sensor dramatically decreased and reached saturation after 44 min, which suggested that the DFCB sensor can be employed for real-time monitoring of  $\text{H}_2\text{O}_2$  (Table 1).

### Detection mechanism

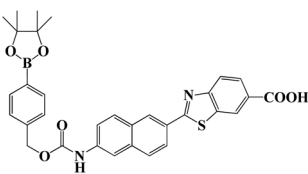
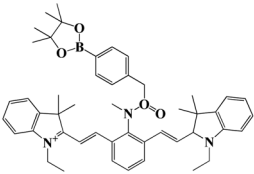
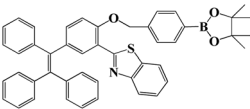
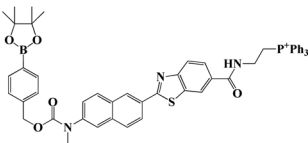
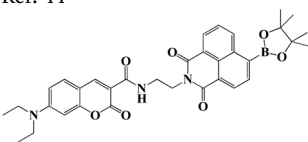
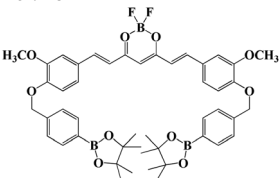
In order to demonstrate the reaction mechanism of DFCB to  $\text{H}_2\text{O}_2$ , HRMS analysis of the reaction product of the DFCB sensor with  $\text{H}_2\text{O}_2$  was performed. As shown in Fig. 5(a), it was observed that the main molecular ion peaks were recorded at an  $m/z$  value of 439.1144, which was consistent with the stable DFC ( $[\text{M} + \text{Na}]^+ = m/z$  439.1135). It was hypothesized that the boronate ester group of the DFCB sensor was oxidized and then dissociated fully to form the intermediate ( $[\text{M} + \text{K}]^+ = m/z$  665.1566) in the presence of  $\text{H}_2\text{O}_2$ , followed by quinoid dissociation to form stable DFC. In addition, the results of the HRMS analysis confirmed that the probable peak was recorded at an  $m/z$  value of 665.1585 when about 2 eq.  $\text{H}_2\text{O}_2$  was added. The HRMS analysis confirmed the logic of our preliminary analytical reaction mechanism.

To demonstrate the reaction mechanism between the DFCB sensor and  $\text{H}_2\text{O}_2$ , the  $^1\text{H}$ -NMR spectra of the DFCB sensor after reaction with different amounts of  $\text{H}_2\text{O}_2$  were recorded. As shown in Fig. 6, after adding  $\text{H}_2\text{O}_2$  into the  $\text{DMSO}-d_6$  solution of the DFCB sensor, the proton signal peaks ( $\text{H}_a$ ) of the phenylborate groups at 1.29 ppm disappeared with the



**Fig. 4** (a) Fluorescence intensity of the DFCB sensor (100  $\mu\text{M}$ ) at different pH values in the absence and presence of  $\text{H}_2\text{O}_2$  (300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4). (b) Response time of the DFCB sensor (100  $\mu\text{M}$ ) to  $\text{H}_2\text{O}_2$  (300  $\mu\text{M}$ ) in PBS buffer (containing 50%  $\text{CH}_3\text{CN}$ , pH = 7.4).

**Table 1** Comparison of other fluorescence sensors reported for specific detection of H<sub>2</sub>O<sub>2</sub>

| Sensors                                                                             | Response time | Detection limit (μM) | Linear range | pH range      | Application                       |
|-------------------------------------------------------------------------------------|---------------|----------------------|--------------|---------------|-----------------------------------|
|    | 120 min       | 4.0                  | 0–1 μM       | Not mentioned | Living mice                       |
| Ref. 41                                                                             |               |                      |              |               |                                   |
|    | 70 min        | 20                   | 0–10 μM      | 48            | Living mice                       |
| Ref. 42                                                                             |               |                      |              |               |                                   |
|    | 180 min       | 6.0                  | 0–100 μM     | 5–8           | Living cells                      |
| Ref. 43                                                                             |               |                      |              |               |                                   |
|    | 60 min        | 4.6                  | 0–1 mM       | 4–11          | Living cells                      |
| Ref. 44                                                                             |               |                      |              |               |                                   |
|   | 60 min        | 1.35                 | 0–200 μM     | 4–10          | Living cells and living zebrafish |
| Ref. 45                                                                             |               |                      |              |               |                                   |
|  | 44 min        | 1.31                 | 0–300 μM     | 6–11          | Living cells and living zebrafish |
| This work                                                                           |               |                      |              |               |                                   |

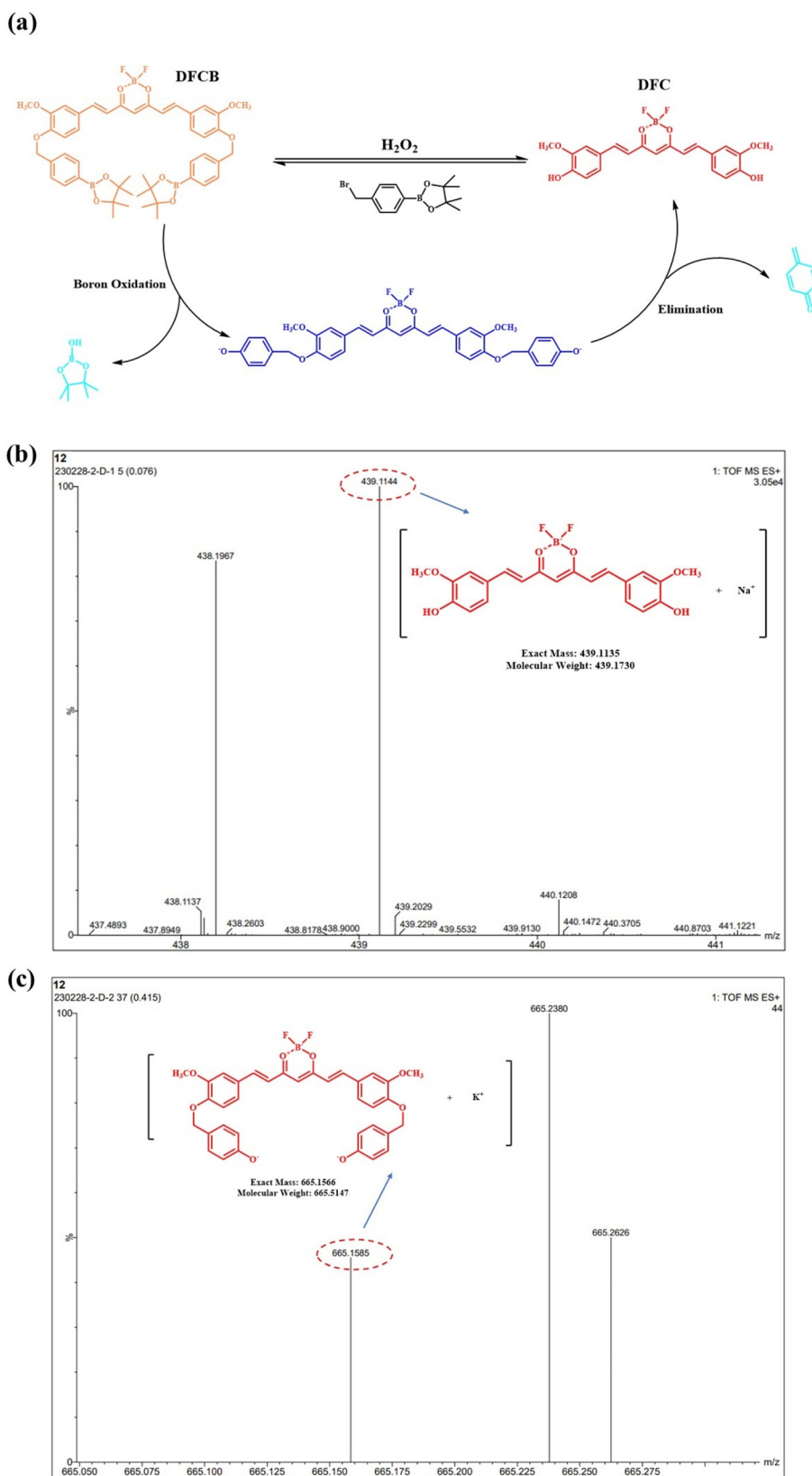
increase of the H<sub>2</sub>O<sub>2</sub> equivalent. At the same time, the proton signal peaks (H<sub>b</sub>) of the methylene units at 5.5 gradually disappeared, which indicated that the boronate ester group of the **DFCB** sensor can react with H<sub>2</sub>O<sub>2</sub> and was then removed. Based on HRMS analysis and <sup>1</sup>H-NMR titration, the sensing mechanism of **DFCB** on H<sub>2</sub>O<sub>2</sub> was selective boron oxidation which formed the intermediate, and the elimination reactions to release **DFC**, which might break the original structure of the sensor and would result in a significant weakening of the fluorescence emission at 601 nm.

The DFT calculation was carried out using the Gaussian 09 program to further reveal the optical changes of the **DFCB** sensor before and after the reaction with H<sub>2</sub>O<sub>2</sub>. Fig. 7 shows the spatial distribution and energy orbitals of the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) of the **DFCB** sensor and the **DFC** compound. The LUMO and HOMO of the **DFCB** sensor were

mainly distributed on the fluorine-boron core, the C=C bond, and the benzene ring, indicating the weak charge transfer effect of the **DFCB** sensor. However, the LUMO of the **DFCB** sensor was distributed on the fluorine-boron core and the C=C bond, whereas its HOMO was distributed on the phenol group, which would activate the charge transfer effect of the **DFCB** sensor after reaction with H<sub>2</sub>O<sub>2</sub>, and then it led to a red-shifted absorption spectra.

#### Imaging of **DFCB** in HeLa cells

The use of the **DFCB** sensor for imaging H<sub>2</sub>O<sub>2</sub> was investigated in living HeLa cells. Prior to the cell fluorescence imaging experiment, the cytotoxicity of the **DFCB** sensor was measured. As shown in Fig. S7 (ESI<sup>†</sup>), the **DFCB** sensor (0–4 μM) had no significant effect on the survival rate of the HeLa cells, and the cell survival rate was over 90%. This indicated that the **DFCB**



**Fig. 5** (a) The proposed sensing mechanism of DFCB with  $\text{H}_2\text{O}_2$ . (b) The HRMS spectra of the reaction of the DFCB sensor with 5 eq.  $\text{H}_2\text{O}_2$ . (c) The HRMS spectra of the reaction of the DFCB sensor with 2 eq.  $\text{H}_2\text{O}_2$ .

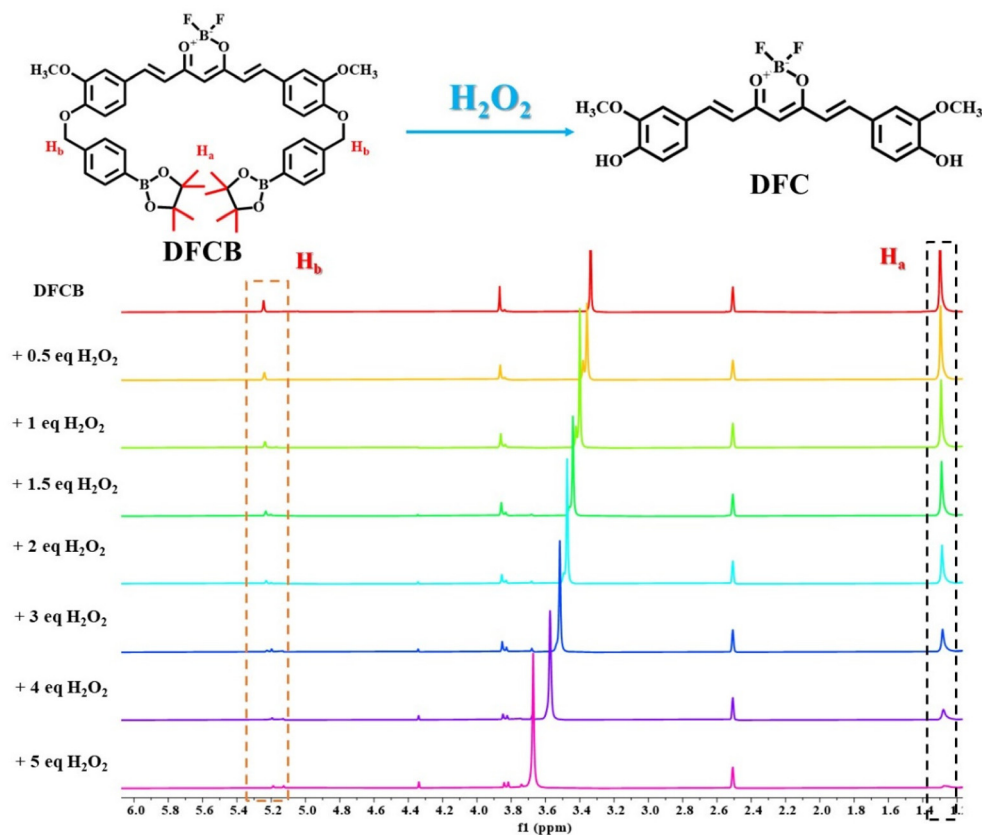


Fig. 6 The  $^1\text{H}$ -NMR spectra of the DFCB sensor before and after the reaction with different amounts of  $\text{H}_2\text{O}_2$ .

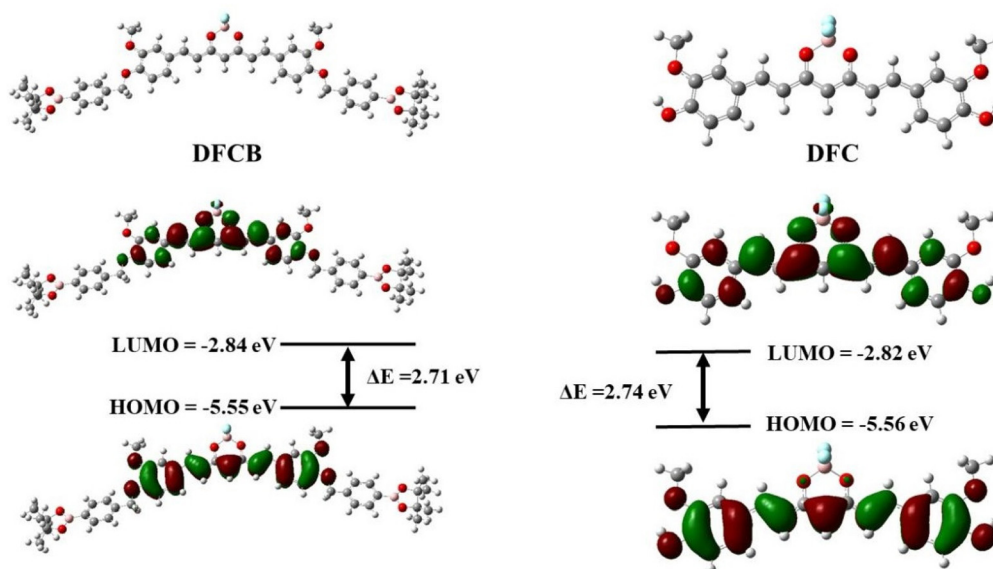
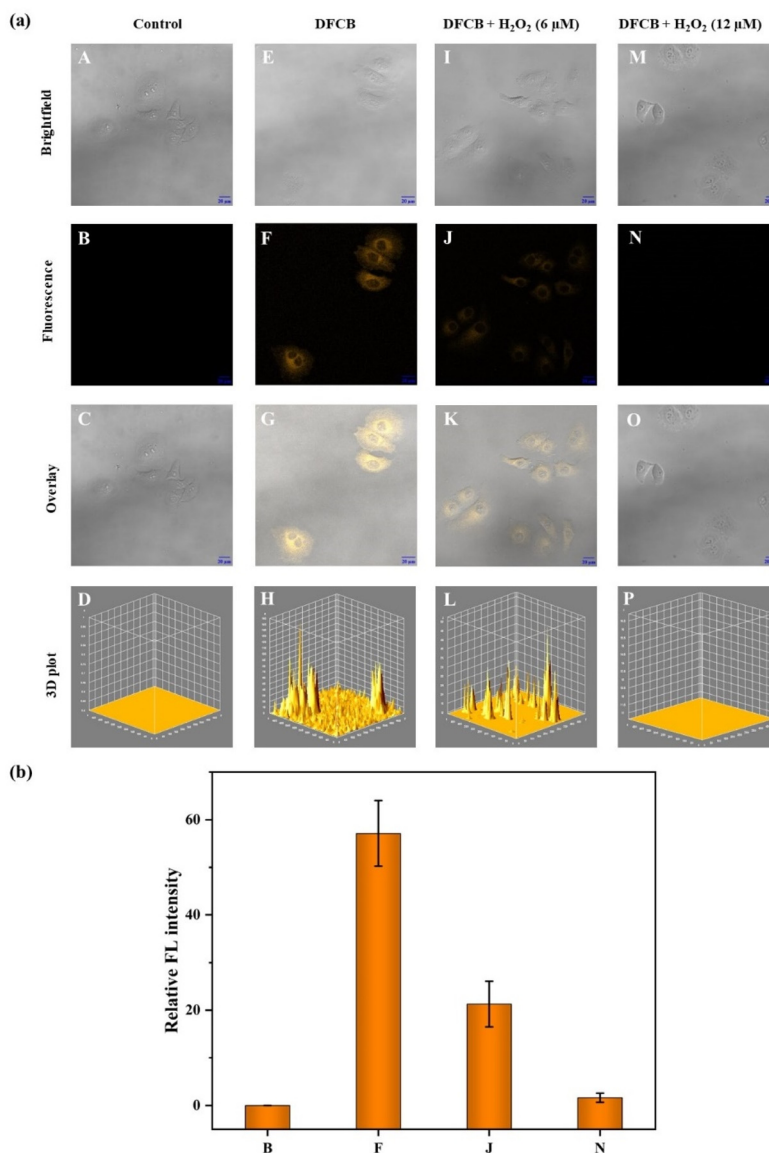


Fig. 7 Optimized geometric configurations and frontier molecular orbitals of the DFCB sensor and the DFC intermediate.

sensor was less cytotoxic to cells and that it could be applied in the cell imaging experiment.

Furthermore, the DFCB sensor was used for fluorescence imaging in living cells. As shown in Fig. 8, the control cells

showed no observable fluorescence emission. With the addition of the DFCB sensor, the HeLa cells emitted an orange fluorescence, which demonstrated that the sensor had good cell permeability and biocompatibility. After further incu-

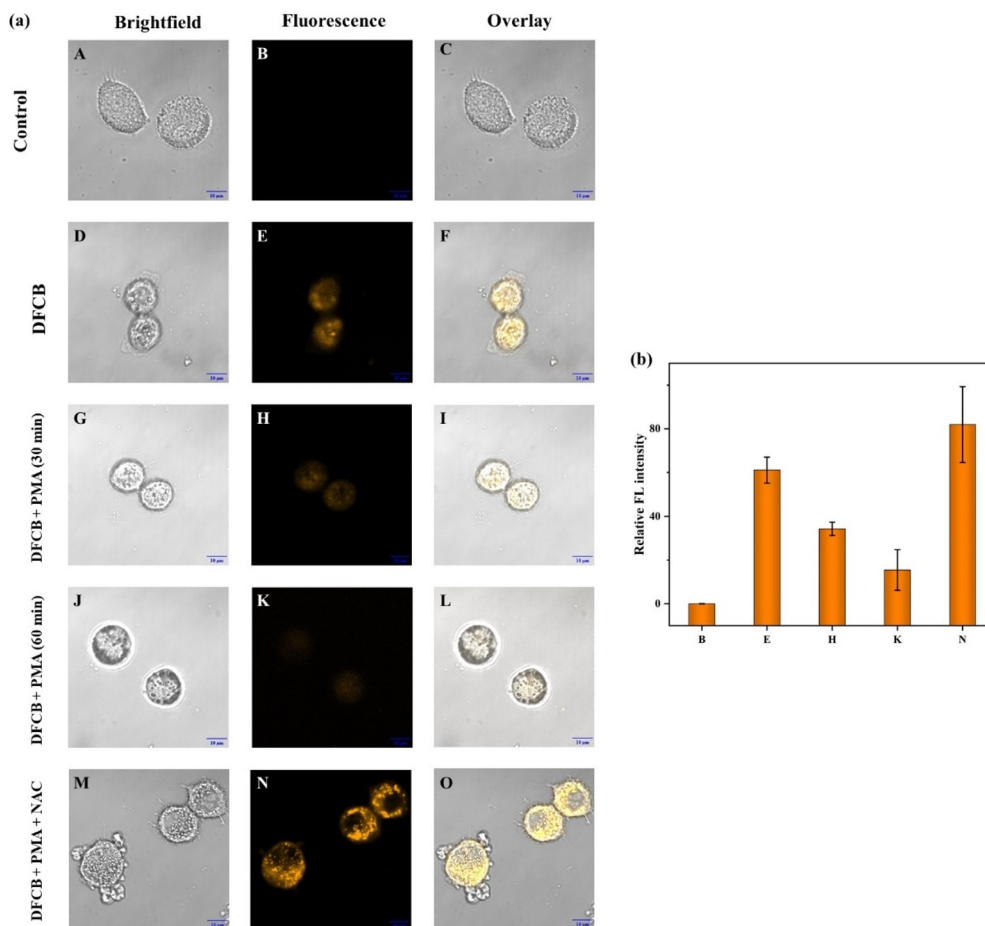


**Fig. 8** (a) Imaging of H<sub>2</sub>O<sub>2</sub> in HeLa cells: (A–C) only the HeLa cells were cultured for 1 h; (E–G) HeLa cells supplemented with the DFCB sensor (4 μM) were cultured for 1 h; (I–K) HeLa cells supplemented with the DFCB sensor (4 μM) and H<sub>2</sub>O<sub>2</sub> (6 μM) were cultured for 30 min; and (M–O) HeLa cells were cultured with the DFCB sensor (4 μM) and H<sub>2</sub>O<sub>2</sub> (12 μM) for 1 h. (D, H, L and P) are the corresponding 3D surface plots of the fluorescence images. (b) Quantitative intensity obtained from the fluorescent images of the HeLa cells.

bation with increasing concentrations of H<sub>2</sub>O<sub>2</sub> (6 and 12 μM), the fluorescence intensity of the cells gradually decreased and was completely quenched. The 3D surface plot and the relative fluorescence intensity were calculated to support the fluorescence quenching effect in living cells, which demonstrated that the DFCB sensor could be utilized for imaging exogenous H<sub>2</sub>O<sub>2</sub> *in vitro*.

The fluorescence labeling, with endogenous H<sub>2</sub>O<sub>2</sub>, was carried out in HeLa cells using the DFCB sensor. As shown in Fig. 9, in the presence of the free DFCB sensor (4 μM), there was significant fluorescence emission in the HeLa cells. Since phorbol myristate acetate (PMA, a ROS stimulant) could promote endogenous H<sub>2</sub>O<sub>2</sub> production, the HeLa cells dis-

played quenching which was supplemented with PMA (1 μg ml<sup>-1</sup>), and subsequently the cells were cultured for 30 min. If the cells were cultured with PMA for 60 min, the fluorescence intensity decreased further, which indicated that the DFCB sensor could detect endogenous H<sub>2</sub>O<sub>2</sub>. In addition, to verify that the endogenous H<sub>2</sub>O<sub>2</sub> production could cause the fluorescence quenching in the cells, the PMA-stimulated cells were further supplemented with *N*-acetyl-L-cysteine (NAC, a ROS scavenger, 1 mM) and then stained with the DFCB sensor. From Fig. 9(b) it was concluded that the average fluorescence intensity was enhanced after the removal of the endogenous H<sub>2</sub>O<sub>2</sub> from the HeLa cells. The previously described experimental imaging data confirmed the excellent imaging ability of the



**Fig. 9** (a) Imaging of  $\text{H}_2\text{O}_2$  in HeLa cells: (A–C) HeLa cells alone were cultured for 1 h and acted as the control; (D–F) HeLa cells supplemented with the DFCB sensor ( $4\ \mu\text{M}$ ) were cultured for 1 h; (G–I) the HeLa cells supplemented with PMA ( $1\ \mu\text{g}\ \text{ml}^{-1}$ ) were cultured for 30 min; (J–L) subsequently, the HeLa cells supplemented with PMA ( $1\ \mu\text{g}\ \text{ml}^{-1}$ ) were cultured for 60 min; and (M–O) the HeLa cells were cultured with PMA ( $1\ \mu\text{g}\ \text{ml}^{-1}$ ) and then incubated with NAC ( $1\ \text{mM}$ ), followed by staining with the DFCB sensor ( $4\ \mu\text{M}$ ) for 1 h. (b) The quantitative intensity was obtained from the fluorescent images of the HeLa cells.

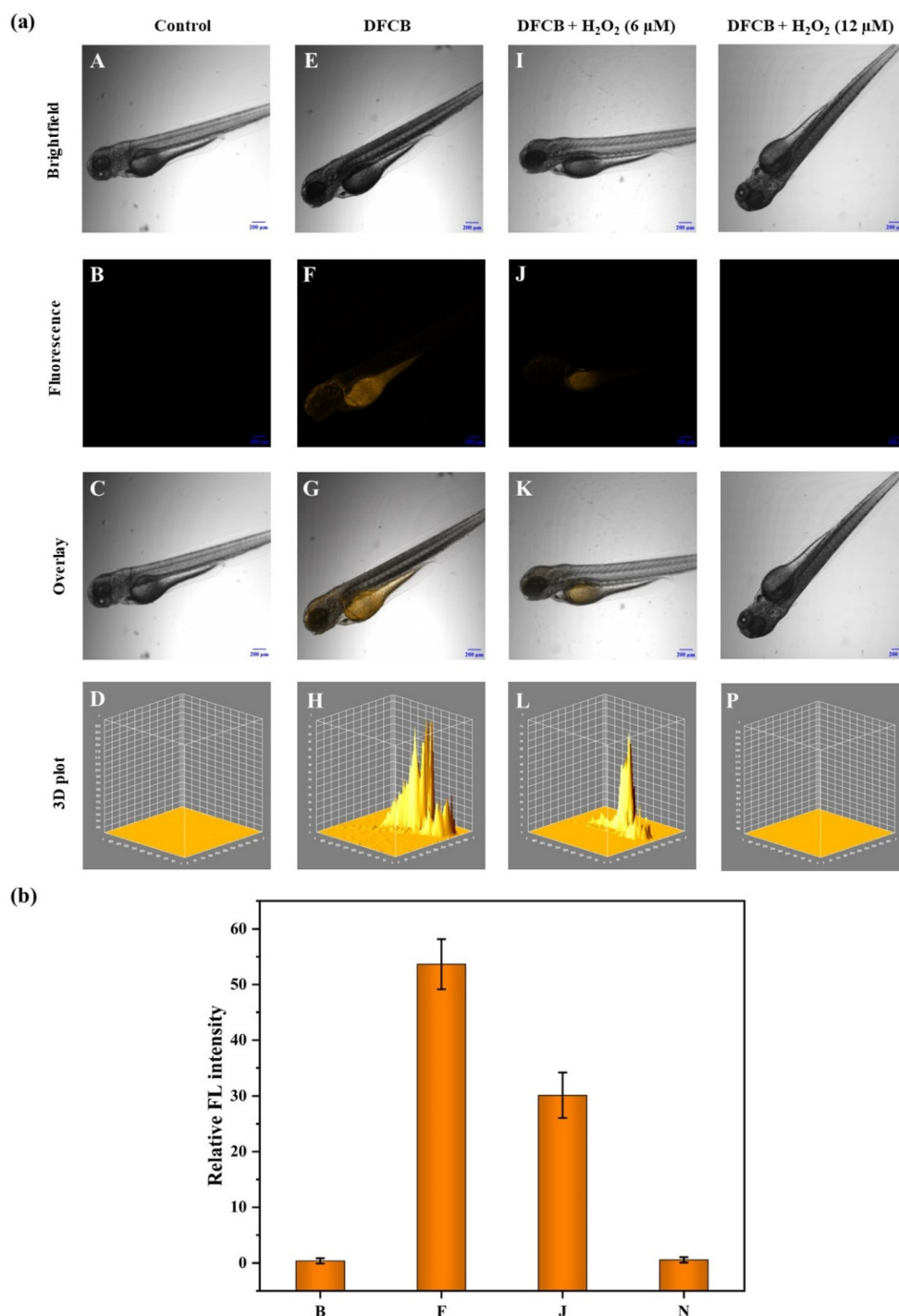
DFCB sensor for generating endogenous  $\text{H}_2\text{O}_2$ . In addition, the untreated HeLa cells were selected as the control group. Significant fluorescence emission could not be observed in the cells due to the absence of the DFCB sensor, and these experimental data confirmed that the DFCB sensor could be used to monitor endogenous  $\text{H}_2\text{O}_2$  production in living cells. All these cellular experiments confirmed that the DFCB sensor could image endogenous  $\text{H}_2\text{O}_2$  in living cells.

#### Bioimaging in zebrafish

The DFCB sensor was further used to detect  $\text{H}_2\text{O}_2$  *in vivo*. As shown in Fig. 10, zebrafish treated with the DFCB sensor ( $4\ \mu\text{M}$ ) exhibited an intense orange fluorescence, suggesting that the DFCB sensor possessed excellent cell permeability. However, the fluorescence intensity of the DFCB sensor gradually decreased until it was completely quenched by the increase of  $\text{H}_2\text{O}_2$  concentration (6 and  $12\ \mu\text{M}$ ). Furthermore, the fluorescence imaging phenomenon in living zebrafish was supported by the 3D surface and the relative fluorescence intensity plots. The previous experimental results

showed that the DFCB sensor can detect exogenous  $\text{H}_2\text{O}_2$  in organisms.

As shown in Fig. 11, the zebrafish incubated with the DFCB sensor ( $4\ \mu\text{M}$ ) emitted an orange fluorescence unlike the control group, which confirmed that zebrafish cannot emit fluorescence by themselves. With the addition of PMA ( $1\ \mu\text{g}\ \text{ml}^{-1}$ ) for different times, the emission fluorescence of the DFCB sensor in zebrafish was gradually quenched, which indicated that the PMA supplement could stimulate the production of endogenous  $\text{H}_2\text{O}_2$  in zebrafish. Compared with the zebrafish with the DFCB sensor, the fluorescence intensity was stronger in the zebrafish which was treated with NAC ( $1\ \text{mM}$ ) and then cultured with the DFCB sensor. These experimental results confirmed that the DFCB sensor had good potential for use in biological imaging and in the detection of endogenous  $\text{H}_2\text{O}_2$ . In addition, the relative fluorescence intensity chart was used to support the biological imaging experiments. These experimental data indicated that the DFCB sensor can detect exogenous and endogenous levels of  $\text{H}_2\text{O}_2$  in biological systems.



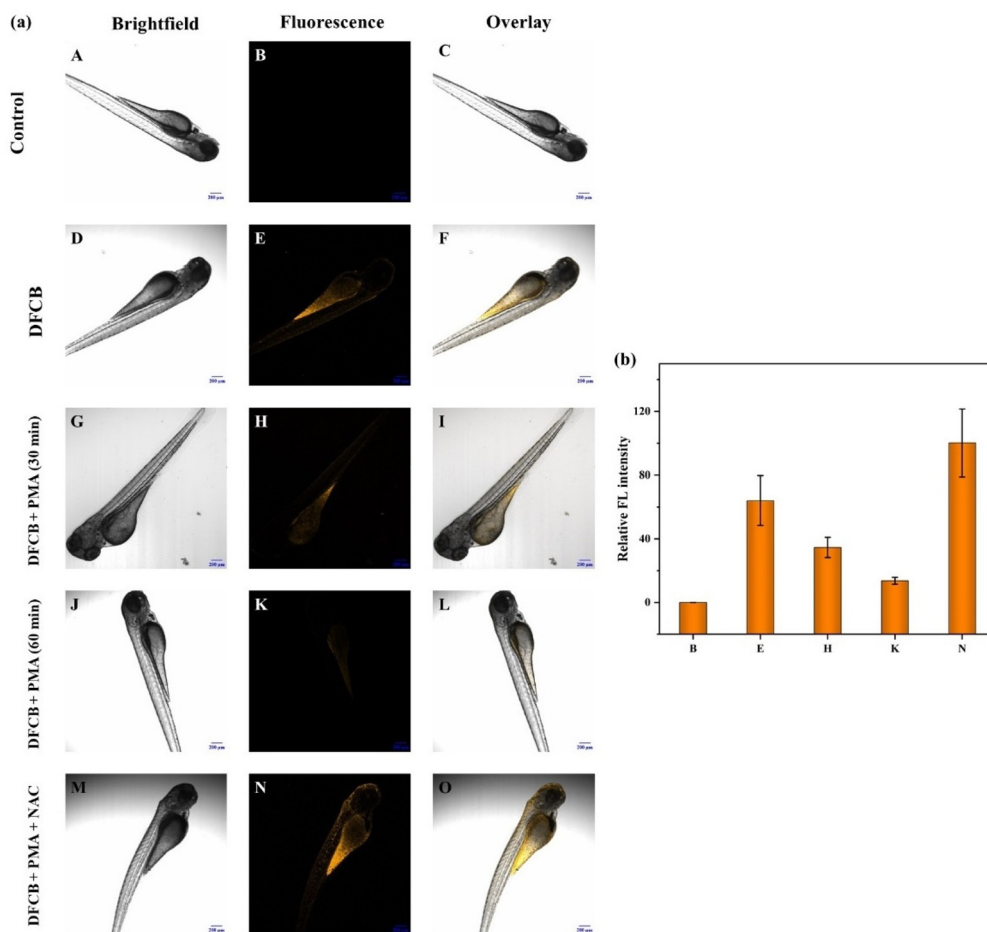
**Fig. 10** (a) Imaging of H<sub>2</sub>O<sub>2</sub> in zebrafish: (A–C) zebrafish alone were cultured for 1 h and acted as the control; (E–G) zebrafish supplemented with the DFCB sensor (4 μM) were cultured for 1 h; (I–K) zebrafish supplemented with the DFCB sensor (4 μM) and H<sub>2</sub>O<sub>2</sub> (6 μM) were cultured for 30 min; and (M–O) zebrafish were cultured with the DFCB sensor (4 μM) and H<sub>2</sub>O<sub>2</sub> (12 μM) for 1 h. (D, H, L and P) are the corresponding 3D surface plots of the fluorescence images. (b) Quantitative intensity was obtained from fluorescent images of the zebrafish.

## Experiments

### Instruments and materials

The chemical reagents used in the experiments are normally available commercially and were used directly. Curcumin was

purchased from the Shanghai Titan Technology Company. The BF<sub>3</sub>·(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>O was obtained from the Shanghai Lingfeng Chemical Reagent Company. The 2-(4-bromomethylphenyl)-4,4,5,5-tetramethyl-1,3,2-doxaborolane was obtained from the Shanghai Haohong Biomedical Technology Company. The



**Fig. 11** (a) The imaging of H<sub>2</sub>O<sub>2</sub> in zebrafish: (A–C) zebrafish alone were cultured for 1 h; (D–F) zebrafish supplemented with the DFCB sensor (4  $\mu$ M) were cultured for 1 h; (G–I) zebrafish supplemented with PMA (1  $\mu$ g ml<sup>-1</sup>) were cultured for 30 min; (J–L) subsequently, the zebrafish supplemented with PMA (1  $\mu$ g ml<sup>-1</sup>) were cultured for 60 min; and (M–O) the zebrafish were cultured with PMA (1  $\mu$ g ml<sup>-1</sup>) and then incubated with NAC (1 mM), followed by staining with the DFCB sensor (4  $\mu$ M) for 1 h. (b) Quantitative intensity obtained from fluorescent images of zebrafish.

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on a AV-600 NMR spectrometer (Bruker). The HRMS was carried out on a JMS-800D mass spectrometer (Jeol). The UV-vis absorption spectra were recorded on a UV-2450 spectrometer (Shimadzu). The fluorescence emission spectra were recorded using a LS55 fluorescence spectrometer (PerkinElmer).

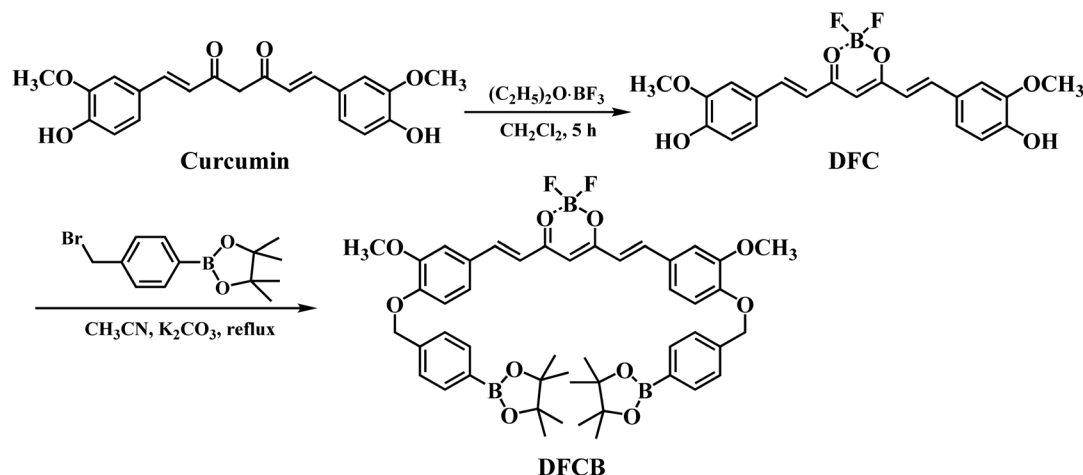
#### Synthesis of compound DFC

Curcumin (1 mmol) was dissolved in 30 mL of CH<sub>2</sub>Cl<sub>2</sub> at ambient temperature, and BF<sub>3</sub>·(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>O (2.4 mmol) was added dropwise. The reaction was continued for 5 h, and monitored with thin layer chromatography. After concentrating and removing half of the solvent, the reaction mixture was placed in a refrigerator for crystallization to take place. The red solid product DFC was obtained by filtering, washing with CH<sub>2</sub>Cl<sub>2</sub>, and drying, with a yield of 98%. <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 10.09 (s, 2H), 7.92 (d, *J* = 15.5 Hz, 2H), 7.47 (s, 2H), 7.34 (d, *J* = 10.3 Hz, 2H), 7.01 (d, *J* = 15.6 Hz, 2H), 6.87 (s, 2H), 6.45 (s, 1H), 3.85 (s, 6H). <sup>13</sup>C-NMR (151 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 178.89, 151.52, 148.35, 147.14, 126.16, 125.44, 118.03, 116.13, 112.56,

101.29, 55.94. HRMS (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>21</sub>H<sub>19</sub>BO<sub>6</sub>F<sub>2</sub>Na + H<sup>+</sup>, 439.1140; found, 439.1141.

#### Synthesis of the DFCB sensor

The DFC (1 mmol) and 2-(4-bromomethylphenyl)-4,4,5,5-tetramethyl-1,3,2-doxaborolane (2.4 mmol), and K<sub>2</sub>CO<sub>3</sub> (2.4 mmol) were dissolved in 8 mL of CH<sub>3</sub>CN, and the reaction mixture was then refluxed for 6 h. The reaction mixture was kept in cryogenic refrigerator overnight, and the brown precipitate was filtered to obtain the crude DFCB. The crude DFCB was dissolved in ethyl acetate, and washed with deionized water until the pH became neutral. After drying with MgSO<sub>4</sub>, followed by filtration, and distillation of the solvent, the DFCB was finally obtained, with a yield of 53% (Scheme 1). <sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 7.96 (d, *J* = 15.6 Hz, 2H), 7.70 (d, *J* = 7.6 Hz, 4H), 7.53 (s, 2H), 7.44 (dd, *J* = 17.0, 8.1 Hz, 6H), 7.17–7.07 (m, 4H), 6.52 (s, 1H), 5.23 (s, 4H), 3.86 (s, 6H), 1.29 (s, 24H). <sup>13</sup>C-NMR (151 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 179.43, 158.87, 158.65, 158.42, 158.20, 151.59, 149.70, 147.12, 144.36, 140.22, 134.91, 127.77, 127.32, 125.34, 124.51, 119.42, 117.70, 115.75, 113.67, 111.96, 84.00,



**Scheme 1** The synthesis route for the DFCB sensor.

70.03, 56.12, 24.95. HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{47}H_{53}B_3O_{10}F_2Na + H^+$ , 871.3784; found, 871.3794.

### Spectral measurements

The stock solution of DFCB (1 mM) was prepared in  $CH_3CN$ . The stock solutions of different metal ions (10 mM) ( $Ag^+$ ,  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Co^{2+}$ ,  $Cs^+$ ,  $Cu^{2+}$ ,  $Hg^{2+}$ ,  $K^+$ ,  $Mg^{2+}$ ,  $Mn^{2+}$ ,  $Na^+$ ,  $Ni^{2+}$ ,  $Zn^{2+}$ ), anions ( $AcO^-$ ,  $Br^-$ ,  $Cl^-$ ,  $ClO^-$ ,  $ClO_4^-$ ,  $CrO_7^{2-}$ ,  $H_2PO_4^-$ ,  $HSO_3^-$ ,  $HSO_4^-$ ,  $I^-$ ,  $NO_3^-$ ,  $NO_2^-$ ,  $SO_4^{2-}$ ) and ROS (MCPBA,  $\cdot OH$ ,  $ONOO^-$ , PAA, TBHP) were prepared in deionized water. The UV-vis absorption and fluorescence spectra of the samples were recorded in PBS solution (50%  $CH_3CN$ , pH = 7.4) at room temperature. For the fluorescence spectroscopy measurements, the excitation wavelength was  $\lambda_{ex} = 425$  nm, and the slit width was  $\lambda_{ex}/\lambda_{em} = 5$  nm/5.5 nm.

### HeLa cell and zebrafish imaging

The HeLa cells were cultured in DMEM containing 10% fetal bovine serum (FBS) with 5%  $CO_2$  at 37 °C. Next, the cells were divided into four groups. Except for group 1, the other cells from groups 2 to 4 were incubated in 4  $\mu M$  DFCB solution for 1 h at 37 °C, and then incubated with different concentrations of  $H_2O_2$  (0, 6, 12  $\mu M$ ) for another 30 min. Fluorescence images of the HeLa cells were recorded using a laser confocal fluorescence microscope in the orange channel ( $\lambda_{ex} = 405$  nm,  $\lambda_{em} = 450$ –650 nm).

Four-day-old zebrafish were randomly divided into three groups. Each group was incubated with 4  $\mu M$  of the DFCB sensor for 30 min, and then incubated with different concentrations of  $H_2O_2$  (0  $\mu M$ , 6  $\mu M$ , and 12  $\mu M$ ) for 44 min. After washing the zebrafish three times with the PBS solution, the fluorescence images of the zebrafish were obtained using a laser confocal fluorescence microscope in the orange channel ( $\lambda_{ex} = 405$  nm,  $\lambda_{em} = 450$ –650 nm).

## Conclusions

In summary, a new curcumin-based fluorescent DFCB sensor was designed and synthesized for use in  $H_2O_2$  detection. The DFCB sensor emitted an intense orange fluorescence and exhibited a significant quenched fluorescence response to  $H_2O_2$ . The color of the DFCB sensor solution changed from orange to blue in the presence of  $H_2O_2$ , when the solution was viewed with the naked eye. The DFCB sensor could selectively detect  $H_2O_2$  and showed a good anti-interference performance. Moreover, the DFCB sensor exhibited the advantages of a low detection limit (1.31  $\mu M$ ), wide linear range (0–300  $\mu M$ ), and broad pH detection range (6–11). Furthermore, the DFCB sensor with low cytotoxicity and good biocompatibility was successfully used for imaging endogenous and exogenous  $H_2O_2$  in living HeLa cells, as well as in living zebrafish. We predict that the results of this research will have the potential to be used in future for monitoring the physiological and pathological effects of  $H_2O_2$  *in vivo*.

## Author contributions

Wenhao Du: investigation, methodology and writing – original draft. Zheyu Shen: formal analysis. Yueying Liang: software. Shuai Gong: investigation. Zhiyuan Meng: software. Mingxing Li: data curation. Zhonglong Wang: writing – review and editing. Shifa Wang: project administration.

## Ethics declarations

### Ethics approval

All the animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Nanjing University and approved by the Animal Ethics Committee of China.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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