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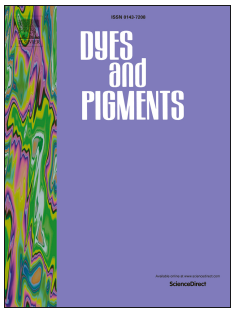

A near-infrared fluorescent probe for endogenous hydrogen peroxide real-time imaging in living cells and zebrafish

Xin Huang, Zhipeng Li, Zixin Liu, Chengchu Zeng, Liming Hu

PII: S0143-7208(18)32836-5

DOI: https://doi.org/10.1016/j.dyepig.2019.02.042

Reference: DYPI 7376

To appear in: *Dyes and Pigments*

Received Date: 24 December 2018

Revised Date: 24 February 2019

Accepted Date: 24 February 2019

Please cite this article as: Huang X, Li Z, Liu Z, Zeng C, Hu L, A near-infrared fluorescent probe for endogenous hydrogen peroxide real-time imaging in living cells and zebrafish, *Dyes and Pigments* (2019), doi: https://doi.org/10.1016/j.dyepig.2019.02.042.

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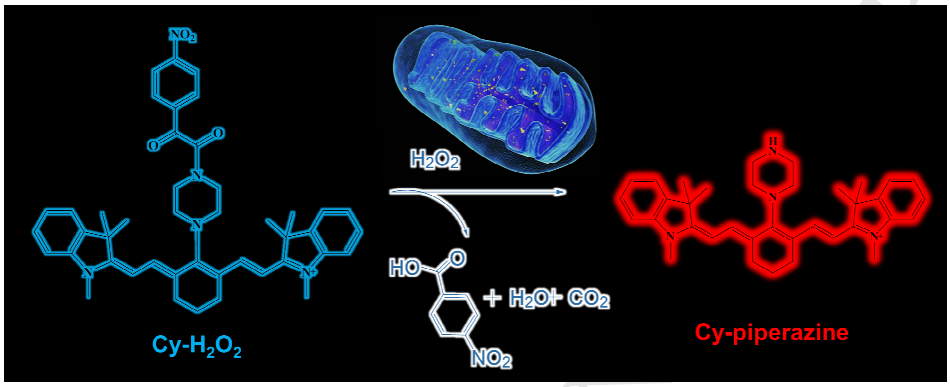
Xin Huang, Zhipeng Li, Zixin Liu, Chengchu Zeng and Liming Hu[*]



A near-infrared fluorescent probe for the detection of H_2O_2 in living cells and zebrafish rapidly.

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A near-infrared fluorescent probe for endogenous hydrogen peroxide real-time imaging in living cells and zebrafish

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Hydrogen peroxide
NIR
Fluorescence probe
Bioimaging

ABSTRACT

Hydrogen peroxide (H_2O_2) can be produced in mitochondria and plays a significant role in physiological metabolism. Overproduction of H_2O_2 is a hallmark of many diseases. Therefore, it is very important to develop a highly sensitive method for detecting H_2O_2 both *in vivo* and *in vitro*. Previously reported benzil-based fluorescence probes are superior to those based on boronate ester in terms of reaction selectivity. However, the near-infrared (NIR) probe with biocompatibility has been rarely reported for the detection of endogenous hydrogen peroxide and the real-time imaging in biological system. Hemicyanine skeleton has been proven to be effective scaffold for NIR fluorescent probes for non-invasive optical imaging *in vivo*. In this paper, a **Cy-H_2O_2** probe for real-time monitoring hydrogen peroxide in organisms was designed by modifying the NIR hemicyanine framework with benzil moiety. The results showed that **Cy-H_2O_2** exhibits high specificity and sensitivity, and has good water solubility and short response time (within 10 minutes) for detection of hydrogen peroxide *in vitro* and *in vivo*. The reaction mechanism was deduced by detecting product of the fluorescent probe reacting with hydrogen peroxide using HPLC. The probe shows a good linear relationship for the specific response to H_2O_2 within the concentration range of 0-7 μM and the detection limit is 65 nM. In addition, the probe **Cy-H_2O_2** has been successfully applied to H_2O_2 detection in living cells and zebrafish.

1. Introduction

Reactive oxygen species (ROS) are produced as a natural by-product of the normal metabolism of oxygen[1–6] and play a double effect in the organism. On one hand, the active oxygen is important in cell signalling, homeostasis, energy transformation and protection system.[3-7] On the other hand, excessive ROS concentrations may lead to damage of DNA or RNA, oxidations of polyunsaturated fatty acids in lipids or amino acids in proteins, oxidative deactivation of specific enzymes.[8]

Hydrogen peroxide, a major component of ROS, is an oxygen metabolite in living systems, which can cause cumulative oxidative damage by promoting oxidative stress.[9] It has been reported that H_2O_2 is used as a second messenger to regulate a wide range of serious diseases, including neurodegenerative disease and cancer.[10] Therefore, the monitoring of hydrogen peroxide is of great significance for the early diagnosis of related diseases.

Among the various analytical methods, real-time imaging based on fluorescence is considered to be the most sensitive tool owing to its sensitivity and simplicity.[11] Some fluorescent probes for detection of hydrogen peroxide have been reported, but there are some disadvantages, such as absorption and emission wavelengths in the ultraviolet-visible region[12] and long response time (>30 min)[13]. In addition, most of probes for detecting hydrogen peroxide are based on the unique reaction between H_2O_2 and boronate ester.[14] The recognition process of aromatic borate is susceptible to nitric oxide interference, which will affect the reliability of the detection.[15] Therefore, it is still necessary to develop NIR fluorescent probes with excellent selectivity for detecting hydrogen peroxide *in vivo* and *in vitro*.

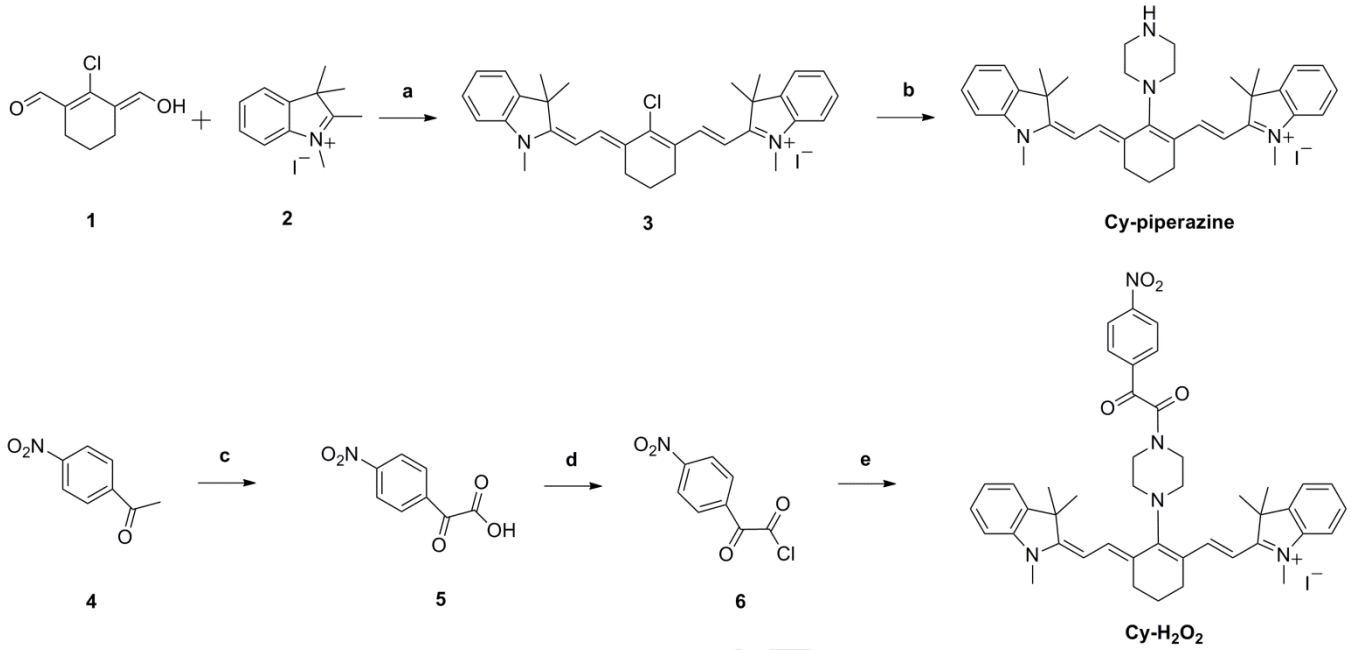
Herein, a benzil-based probe **Cy-H_2O_2** (**Scheme 1**) with enhanced fluorescence response to H_2O_2 was designed and synthesized. Piperazine-modified hemicyanine skeleton has NIR emission to avoid background interference.[16] Benzil (α-dibenzoyl) group, as a recognition moiety for H_2O_2 detection, is more specific and sensitive than aromatic borate.[17] The positive charge on the indole nitrogen atom makes the probe water-soluble.

Cyanine is a classical type of NIR fluorescent dye. We chose **Cy-piperazine** to design the probe, because such modified cyanine has recently become a very attractive fluorophore in this field. In particular, Sun et al. released a Chem Rev. detailing the application of substituted cyanines in the field of imaging.[18]

The probe **Cy-H_2O_2** itself shows a weak fluorescence. Under simulated physiological conditions, the reaction of **Cy-H_2O_2** with H_2O_2 leads to the rearrangement of benzil, followed by the decarboxylation, dehydration and thereby the release of dye **Cy-piperazine** (**Scheme 2**). Therefore, the probe has a "Turn-On" fluorescence response in monitoring H_2O_2 both *in*

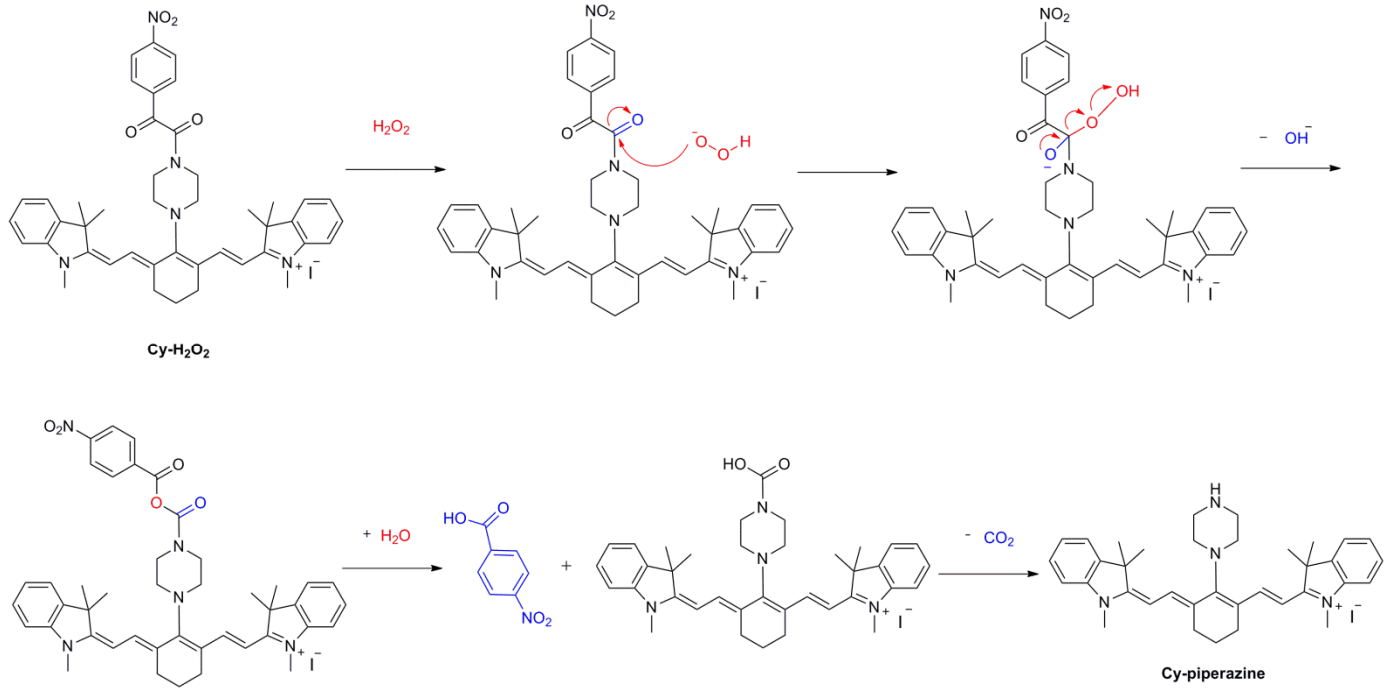
vitro and *in vivo*. The probe is not interfered by other ROS and biologically relevant species, shows high selectivity for hydrogen peroxide. In addition, the specific response of the probe to hydrogen peroxide exhibits a good linear relationship within the given concentration range and the detection limit is 65 nM. All of the above properties indicate that **Cy-H_2O_2** has latent capacity as a NIR fluorescence probe for high selectivity detection of hydrogen peroxide. Furthermore, **Cy-H_2O_2** can be applied to detect water samples in different concentrations of hydrogen peroxide. The fluorescent probe can also achieve real-time imaging in living cells and zebrafish.

Scheme 1. Synthesis of probe **Cy-H_2O_2**



Reagents and conditions: a. acetic anhydride, sodium acetate, y. 73%; potassium iodide aqueous solution, 70°C; b. piperazine, DMF, 90°C, y. 87%; c. SeO_2, pyridine, 110°C, y. 79%; d. dichloromethane, DMF, oxalyl chloride, reflux, y. 95%; e. Cy-piperazine, dichloromethane, triethylamine, rt, y. 74%.

Scheme 2. Proposed reaction mechanism



2. Results and discussion

2.1. Design and synthesis of the fluorescent probe ***Cy-H_2O_2***

Benzil group connected to fluorescent framework by a five-step reaction (**Scheme 1**). Firstly, there is almost unchange in the fluorescent properties of heptamethine cyanine (Cy7) after linking with piperazine. However, the steric hindrance of the

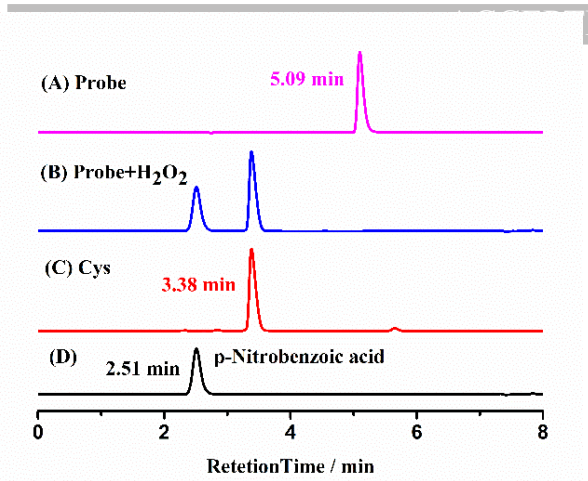


Figure 1. Several samples were measured by HPLC. (A) Probe; (B) Probe+H_2O_2; (C) Cy-piperazine.; (D) p-nitrobenzoic acid. (methanol/water (v/v=85/15) was used as the mobile phase.)

cyanine derivative is significantly lower than that of the Cy7, which makes the recognition group easy to connect. Secondly, the positive charge on the indole nitrogen atom improves hydrophilicity of the probe **Cy-H_2O_2**. Thirdly, the nitro group of the recognition moiety shuts down the doughty fluorescence of the conjugated system originating from the fluorophore. According to the above ideas, the fluorescence probe is designed and synthesized for detection of hydrogen peroxide with high selectivity and sensitivity.

*2.2. Reaction of **Cy-H_2O_2** with H_2O_2*

Piperazine derivative (**Cy-piperazine**), a new type of NIR fluorescent dye, has a maximum emission wavelength of 790 nm (λ_{ex}=730 nm).[19] However, the probe **Cy-H_2O_2** shows non-fluorescent due to Photoinduced electron transfer (PET) to the para nitro benzoyl moieties.[15,18] As explained in **Scheme 1**, the reaction between probe and H_2O_2 is the same as reported by Peng.[13] The nucleophilic attack of hydrogen peroxide destroys the amide bond of **Cy-H_2O_2** and then rearranges and releases the fluorescence substance **Cy-piperazine**, a Baeyer-Villiger type reaction.

The proposed mechanism was further validated by monitoring intermediate products by HPLC, **Figure 1**. Analysis of **Figure 1** shows that the retention time of products of the probe reacting with hydrogen peroxide was consistent with that of **Cy-piperazine** and p-nitrobenzoic acid respectively. In other words, fluorescence enhancement is caused by the probe **Cy-H_2O_2** reacting with hydrogen peroxide to release a strong fluorescent substance **Cy-piperazine**. Therefore, the probe has good selectivity and stability.

2.3. Fluorescent "Turn-On" responses to H_2O_2

The probe effect was tested in phosphate buffer solution (pH=7.4) and focused on the fluorescence turn-on reaction of **Cy-H_2O_2** to H_2O_2. Without the addition of H_2O_2, the solution of **Cy-H_2O_2** is colourless and hardly fluorescence. After adding H_2O_2 (10 μM), the reaction between H_2O_2 and the probe **Cy-H_2O_2** was almost complete in 7 minutes, and the fluorescent intensity increased more than 120-fold (**Figure 3A**). More importantly, the fluorescent intensity rose to a plateau value after 10 minutes (**Figure S5**), better than previously reported in the literature.[20] When the concentration of hydrogen peroxide is less than 7 μM, the fluorescent intensity of the probe has a good linear relationship with the concentration of H_2O_2 in optimal absorption wavelength (R^2 > 0.99, **Figure 2** and **Figure 3B**). The

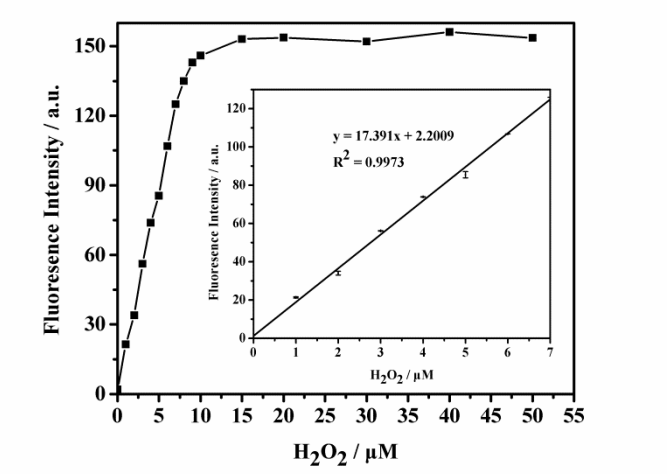


Figure 2. Calibration curve of fluorescence intensity (λ_{ex}=730 nm) in dependence of H_2O_2 concentrations. Data were acquired in PBS (pH=7.4). The equilibration time prior to luminescence measurement was 10 minutes. The data represents the average result of three independent experiments.

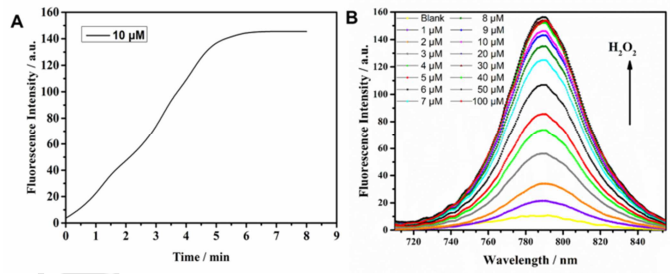


Figure 3. (A) The time-dependent fluorescence changes (λ_{ex}=730 nm), acquired from a mixture of probe **Cy-H_2O_2** (10 μM) and H_2O_2 (10 μM) at same volume in PBS (pH=7.4) solution at room temperature. With the increase of 790 nm intensity, the sample was collected with 1 acquisition every second. (B) Fluorescence intensity increase of probe **Cy-H_2O_2** (10 μM) upon the addition of H_2O_2 (0-100 μM) at same volume in PBS (pH=7.4) solution, λ_{ex}=730 nm, measured at 790 nm. The equilibration time prior to luminescence measurement was 10 minutes.

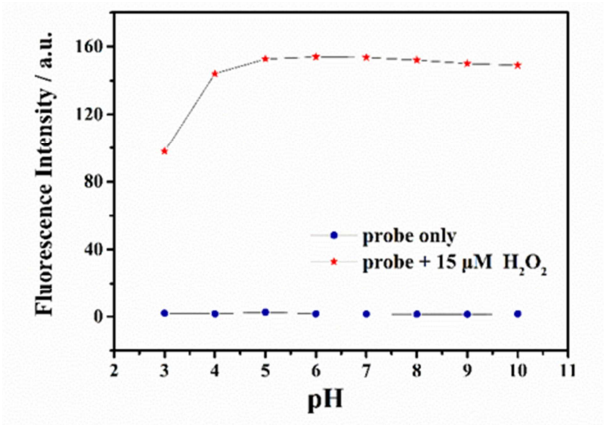


Figure 4. Fluorescence intensity of probe **Cy-H_2O_2** (10 μM) with and without the presence of H_2O_2 (15 μM) at same volume in water with different pH conditions (λ_{ex}=730 nm). The equilibration time prior to luminescence measurement was 10 minutes.

results indicate that **Cy-H_2O_2** can quantitatively identify the concentration of H_2O_2.

2.4. Stability and Selectivity

In order to verify the stability of **Cy-H_2O_2** in various simulated biological fluids, the fluorescent intensity of **Cy-H_2O_2** in phosphate buffer at different pH was investigated in the presence or absence of the H_2O_2. As expected, the fluorescence

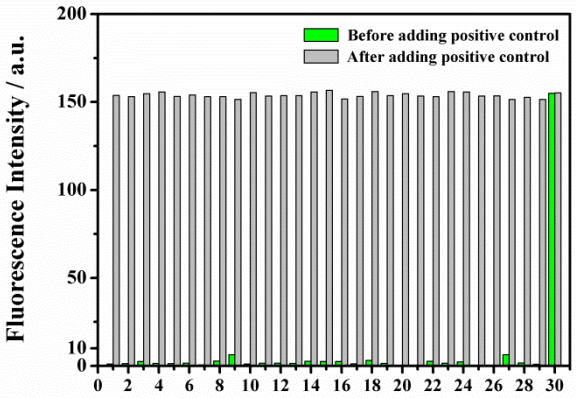


Figure 5. Fluorescence intensities of 10 μM **Cy-H_2O_2** (λex=730 nm) in the presence of various biological species: (1-30) Blank, Na^+, Mg^{2+}, NO_3^-, NH_4^+, Ca^{2+}, Fe^{2+}, $MnO_4^-(K^+)$, $Cr_2O_7^{2-}$, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, CH_3COO^-, I^-, $SO_4^{2-}(Cu^{2+})$, Proline, Alanine, N-acetylcysteine, Vitamin C, HgS, GSH , $S_2O_3^-$, SO_3^{2-}, ClO^-, ROO•, t-BuOOH, $ONOO^-$, •OH, NO• and H_2O_2. The equilibration time prior to luminescence measurement was 10 minutes. The green bars indicate the fluorescence response of the probe **Cy-H_2O_2** to the interfering substance (50 μM). Gray bars: then add 50 μM of H_2O_2.

signal was not enhanced without adding hydrogen peroxide in the detection system. Surprisingly, the fluorescence intensity of mixture which comprises same volume of 10 μM probe **Cy-H_2O_2** and 15 μM hydrogen peroxide was maintained at a high level in the pH range of 3-10(**Figure 4**). It is noteworthy that the probe **Cy-H_2O_2** has good permeability to the cell membrane, as the calculated log P of 3.47 obtained with the ALOGPS 2.1 programme.[21] According to the evaluation criteria, predicted value of log P is within the range of good transmembrane capability.[22]

The selectivity of the probe for H_2O_2 was subsequently investigated. A variety of interfering agents have been added to **Cy-H_2O_2** for anti-jamming performance testing, such as inorganic salts (Na^+, K^+, Mg^{2+}, NO_3^-, NH_4^+, Ca^{2+}, Fe^{2+}, MnO_4^-, $Cr_2O_7^{2-}$, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, CH_3COO^-, I^-, $SO_4^{2-}(Cu^{2+})$), amino acids (proline, alanine, N-acetylcysteine), reducing agents (vitamin C, HgS, GSH, $S_2O_3^{2-}$, SO_3^{2-}) and other ROS. As expected, the fluorescence of **Cy-H_2O_2** did not increase significantly after adding different interfering agents (**Figure 5**). The results indicated high selectivity of **Cy-H_2O_2** to H_2O_2.

2.5. Visualizations of H_2O_2 in living cells

The cytotoxicity of the probe **Cy-H_2O_2** was studied by using HeLa cells in the Methyl Thiazolyl Tetrazolium (MTT) assay.[23] The results were shown in **Figure S13**, the **Cy-H_2O_2** exhibited nearly noncytotoxicity.

Moreover, we tested the performance of **Cy-H_2O_2** *in vitro*. Cervical cancer cells HeLa showed faint fluorescence after 30 minutes of treatment with 10 μM **Cy-H_2O_2** at 37°C (**Figure 6A**). The cell image is the result of direct reaction of **Cy-H_2O_2** with H_2O_2 (10 μM), as shown in **Figure 6B.** Next, stimulation induces endogenous H_2O_2 production by Phobol 12-Myristate 13-Acetate (PMA) in living cells. PMA may trigger mitochondrial ROS production by activating PKC (protein kinase C).[24] As shown in **Figure 6C**, PMA stimulation resulted in a significant increase fluorescence intensity in cells. NAC (N-acetyl-L-cysteine) is an effective scavenger of ROS, which can scavenge H_2O_2 or inhibit its production in cells.[25] In order to confirm the change of intracellular H_2O_2, we pretreated cells with PMA for 30 minutes, and then incubated with NAC (1 mM) for 30 minutes (**Figure 6D**). The enhanced fluorescence intensity stimulated by PMA was due to the reaction of probes with H_2O_2 induced by oxidative stress. In addition, in the presence of NAC, the fluorescence signal was completely suppressed, verifying that the ability of NAC to resist ROS. Our results demonstrated that the probe **Cy-H_2O_2** is able to detect dynamic changes in endogenous H_2O_2 levels in living cells.

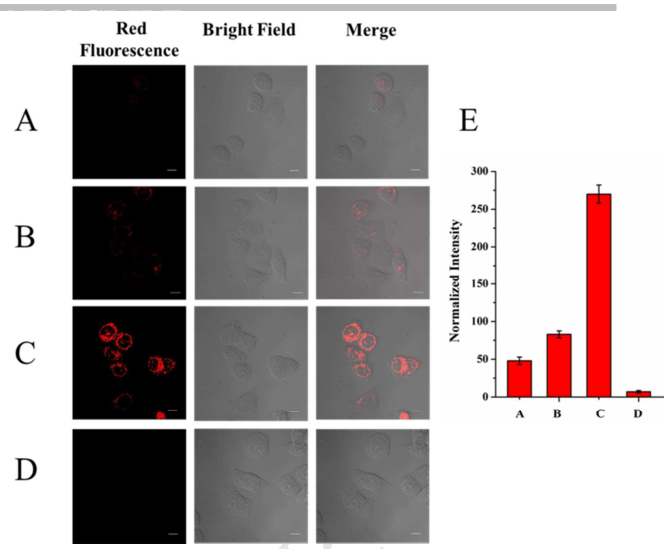


Figure 6. Confocal microscopic images and bright field images of HeLa cells exposed to PMA or NAC stimulation after incubation with the probe **Cy-H_2O_2** (10 μM) for 30 min. (A) HeLa cells were only loaded with **Cy-H_2O_2** (10 μM); (B) HeLa cells were loaded with **Cy-H_2O_2** (10 μM) and H_2O_2 (10 μM) at same volume; (C) The **Cy-H_2O_2**-preloaded cells were stimulated with PMA (1 mg/mL) for 30 min; (D) The **Cy-H_2O_2**-preloaded cells were treated with NAC (1 mM) after incubation with PMA (1 mg/mL); (E) Relative fluorescence intensities of (A)-(E). The images were collected from different emission channels (red channel: 750-800 nm) with the excitation wavelength at 730 nm. All the data express as mean ± SD of three experiments. Scale bar: 10 μm.

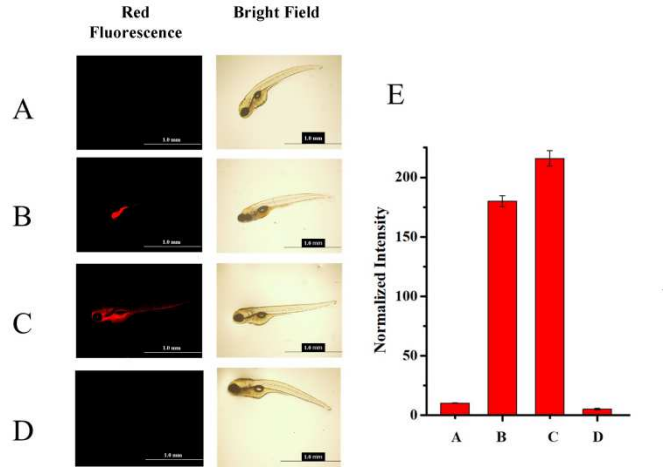


Figure 7. Microscopic images of zebrafish larvae. Fluorescent image of a zebrafish incubated with the probe (10 μg/mL) for 60 min. (**A**) Incubated for 1 hour; (**B**) Treated with APAP (3900 μM) for 24 h at 37°C for 1 h; (**C**) Incubated with 200 μM of H_2O_2 solution for 30 min. (**D**) Treated with NAC (1 mM) 30 min after (B); scale bar=1.0 mm.

2.6. Zebrafish imaging

The ability of the probe **Cy-H_2O_2** to monitor H_2O_2 *in vivo* was evaluated using a zebrafish as a carrier for the APAP (4-acetamido phenol) induced organ injury model.[13] Five-day-old larvae were cultured in the E3 medium contained different concentrations of APAP for 6 h, 12 h, 24 h, and 48 h,

respectively in 6-well microplate. Next, we washed the fishes three times with E3 medium to remove the remaining drugs. The fishes were then incubated in **Cy-H_2O_2** (10 μg/mL) E3 medium for 1 hour. Then, we washed the larvae three times with E3 medium and imaged under an OLYMPUS IX71 fluorescence microscope. The probe showed a response to endogenous hydrogen peroxide with emiting fluorescence, which mainly occurs in the abdomen of zebrafish (**Figures 7 and S15**). In addition, we also studied the imaging ability of the probe for exogenous H_2O_2 in zebrafish (**Figures 7 and S14**). Effect of the probe in zebrafish was consistent with living cells.

3. Experimental

3.1. Materials and instruments

All chemicals were purchased from J&K chemical reagent company without further purification except those with special instructions. Fluorescent spectra were recorded with a PerkinElmer LS55 fluorescence spectrophotometer with a 1 cm standard quartz cell. The fluorescence imaging of cells was performed with laser confocal fluorescence microscopy (Olympus IX 71, Japan). Mass data (ESI) was measured by quadrupole mass spectrometry. Samples for absorption and fluorescence measurements were contained in 1 cm × 1 cm quartz cuvettes.

3.2. Synthesis of ***Cy-H_2O_2***

Cy-piperazine.

Cy-piperazine was obtained as a deep-blue solid by the previously reported methods[26]. The total yield reached 64% through two-step synthesis process. (**Scheme 1**). **Cy-piperazine** was characterized by ^{1}H NMR, ^{13}C NMR and HR-MS (**Figures S7−S9**). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.61 (d, J = 13.0 Hz, 2H), 7.31 (t, J = 8.1 Hz, 4H), 7.11 (t, J = 7.4 Hz, 2H), 6.98 (d, J = 7.8 Hz, 2H), 5.70 (d, J = 13.0 Hz, 2H), 3.99 (s, 4H), 3.50 (s, 6H), 3.25 (s, 4H), 2.48 (t, J = 6.5 Hz, 4H), 1.89 – 1.80 (m, 2H), 1.66 (s, 12H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 21.73, 24.87, 29.03, 31.16, 36.51, 47.89, 56.68, 95.16, 108.90, 122.04, 123.30, 123.48, 128.38, 139.92, 140.64, 143.40, 168.94, 174.85. HRMS (ESI): *m/z* calcd. for $C_{36}H_{45}N_4^+$ $[M - I]^-$ 533.3639, found 533.3635.

2-(4-nitrophenyl)-2-oxoacetyl chloride (6).

4-nitrophenylglyoxylic acid (**5**) was obtained as a pale yellowish brown solid by the previously reported methods.[27] (**Scheme 1**) Then, 4-nitrophenylglyoxylic acid (39 mg, 0.2 mmol), oxaloyl chloride (53 μL, 0.6 mmol) and 3 mL dichloromethane were mixed and stirred for 2 minutes, and two drops of N, N-dimethylformamide (DMF) were added. Under the protection of nitrogen, the temperature rose to 45°C. After one hour, the mixture was cooled to room temperature. Then, the solvent and excess oxaloyl chloride were evaporated, affording the product (40.4 mg, 95%) as a light yellow transparent oil liquid.

Cy-H_2O_2.

Cy-piperazine (53 mg, 0.1 mmol), triethylamine (12 mL, 0.4 mmol) and 3 mL dichloromethane were mixed and stirred at 0°C. Then, **6** (40.4 mg, 0.19 mmol) was dissolved in 3 mL dichloromethane, and dripped slowly into above reaction solution. After stirred for 3 hours, the solvent was evaporated and dried under vacuum condition. Then, the product was purified by column chromatography over silica gel using dichloromethane/MeOH (V/V=20/1) as the eluent, affording the product (52.54 mg, 74%) as a pale blue solid. **Cy-H_2O_2** was characterized by ^{1}H NMR, ^{13}C NMR and HR-MS (**Figures S10−S12**). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 8.41 (d, J = 8.9 Hz, 1H), 8.42-8.23 (m, 3H), 7.84 (d, J = 13.7 Hz, 2H), 7.38 (s, 1H), 7.36 (s, 1H), 7.35 (s, 1H), 7.34 (d, J = 2.6 Hz, 1H), 7.19 (d, J = 7.4 Hz, 1H), 7.17 (s, 1H), 7.07 (s, 1H), 7.05 (s, 1H), 5.96 (s, 1H), 5.92 (s, 1H), 3.81-3.70 (m, 4H), 3.62 (s, 3H), 2.57 (t, J = 6.4 Hz, 4H), 1.93-1.82 (m, 4H), 1.75-1.66 (m, 7H), 1.25 (s, 6H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, ppm): δ 189.03, 170.59, 170.07, 164.44, 151.26, 143.11, 141.61, 140.24, 137.28, 131.43, 129.91, 128.64, 126.70, 124.26, 122.14, 109.81, 101.81, 98.38, 54.00, 53.53, 48.51, 31.99, 29.71, 28.10, 27.23, 25.57, 25.43. HRMS (ESI): *m/z* calcd. for $C_{44}H_{48}N_5O_4^+$ $[M]^+$ 710.3701, found 710.3708.

3.3. Cell cultures

The HeLa cells were provided by Chinese PLA General Hospital (Beijing, China). All biological samples were collected in accordance with the guidelines approved by the institutional review board of the Chinese PLA General Hospital. The HeLa cells were cultured on glass-bottom culture dishes (MatTek Co.) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) penicillin-streptomycin at 37°C in a humidified 5% CO_2 incubator. Before utilized, the adherent cells were washed three times with FBS free DMEM.

3.4. Zebrafish culture

Wild-type zebrafishes were purchased from Beijing University of Chinese Medicine. The juveniles were incubated in this laboratory. E3 culture medium contained 15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4 and 0.7 mM $NaHCO_3$ (pH=7.5). All fishes were incubated at 28°C in the experiment and all animal experiments were performed in full compliance with international ethics guidelines.

4. Conclusions

In summary, we have successfully constructed a fluorescent probe **Cy-H_2O_2**, which is selective and fast responding to hydrogen peroxide. The probe can not only detects water medium and living cells, but also detects endogenous and exogenous hydrogen peroxide in zebrafish with real-time operability and reliability. More importantly, **Cy-H_2O_2** displays NIR excitation and emission spectra, which is beneficial for fluorescence imaging *in vivo*. In addition, **Cy-H_2O_2** can be used to quantitatively detect and localize endogenous H_2O_2 production caused by drug oxidative damage which was mainly located at the abdomen in zebrafish. This provides an effective method for further monitoring of hydrogen peroxide in animals in real-time.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgments

The authors would like to acknowledge financial support from Beijing Key Laboratory of Environmental & Viral Oncology and Beijing Key Laboratory for Green Catalysis and Separation.

References and notes

1 H. J. Schiller, P. M. Reilly and G. B. Bulkley, *Crit. Care Med.*, 1993, **21**, S92; E. W. Childs, K. F. Udobi, J. G. Wood, F. A. Hunter, D. M. Smalley and L. Y. Cheung, *Shock*, 2002, **18**, 423.

2 A. R. Lippert, G. C. Van de Bittner and C. J. Chang, *Acc. Chem. Res.*, 2011, **44**, 793.

3 S. K. Yoo, T. W. Starnes, Q. Deng and A. Huttenlocher, *Nature* 2011, **408**, 109.

4 S. G. Rhee, *Science*, 2006, **312**, 1882; R. Zhang, J. Zhao, G. Han, Z. Liu, C. Liu, C. Zhang, B. Liu, C. Jiang, R. Liu, T. Zhao and M. Y. Han, *J. Am. Chem. Soc.*, **2016**, 138, 3769.

5 E. A. Veal, A. M. Day and B. A. Mol. Morgan, *Cell*, 2007, **26**, 1.

6 T. Finkel, M. Serrano and M. A. Blasco, *Nature*, 2007, **448**, 767.

7 N. Houstis, E. D. Rosen and E. S. Lander, *Nature*, 2006, **440**, 944.

8 M. T. Lin and M. F. Beal, *Nature*, 2006, **443**, 787; M. P. Mattson, *Nature*, 2004, **430**, 631.

9 B. C. Dickinson, C. Huynh and C. J. Chang, *J. Am. Chem. Soc.*, 2010, **132**, 5906; J. P. Fruehauf and F. L. Meyskens, *Clin. Cancer Res.*, 2007, **13**, 789.

10 L. Yuan, W. Lin, Y. Xie, B. Chen and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 1305.

11 Z. P. Li, Y. F. Wang, C. C. Zeng , L. M. Hu and X. J. Liang, *Anal. Chem*, 2018, **90**, 3666; Y. Sun, W. P. Cao, S. L. Li, S. B. Jin, K. L. Hu, L. M. Hu, Y. Y. Huang, X. Y. Gao, Y. Wu and X. J. Liang, *Sci. Rep.*, 2013, **3**, 3036; Z. P. Li, Y. F. Wang, Z. Xu, C. C. Zeng , L. M. Hu and X. J. Liang, *Sens Actuators B Chem.*, 2017, **242**, 189.

12 X. Xie, C. Yin, Y. Yue and F. Huo, Sens *Actuators B* Chem., 2018 , **267**, 76; P. Zhao, K. Wang, X. Zhu, Y. Zhou and J. Wu, *Dyes & Pigments*, 2018 , **155**, 143; Y. C. Jiang, D. Jin, Y. Li, X. L. Yan and L. G. Chen, *Res. Chem. Intermed.*, 2017, **43**, 2945.

13 J. Peng, X. Hou, F. Zeng and S. Wu, *Biosens. Bioelectron.*, 2017, **94**, 278; Wu G., Zeng F., Yu C., Wu S.and Li W., *J. Mater. Chem. B*, 2014, **2**, 8528; B. Dong, X. Song, X. Kong, C. Wang, Y. Tang, Y. Liu and W. Lin, *Adv. Mater.*, 2016, **28**, 8755.

14 E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652.

15 P. Wardman, *Free Radical Biol. Med., 2007, 43, 955;* M. Abo, Y. Urano, K Hanaoka., T. Terai, T. Komatsu and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629; J. Zielonka and B Kalyanaraman, *Free Radical Biol. Med.*, 2018, **128**, 3.

16 Y. Kun, Y. F. Biao and W. W. Zhang, *Biosens. Bioelectron.*,2015, **74**, 156; Y. Jiang, D. Jin, Y. Li, X. Yan and L. Chen, *Res. Chem. Intermed.*, 2017, **43**, 2945.

17 M. S. Purdey, H. J. Mclennan, M. L. Sutton-Mcdowall, D. W. Drumm, X. Zhang and P. K. Capon, *Sens Actuators B Chem.*, 2018, **262**, 750.

18 J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J.-H. Ryu and Y. Yoon. *J. Am. Chem. Soc.*, 2014, **136**, 5351; J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J.-H. Ryu and Y. Yoon. *Nature Protocols*, 2015, **10**, 1742; W. Sun, S. Guo, C. Hu, J. Fan and X. Peng. *Chem Rev.*, 2016, **116**, 7768.

19 J. C. Xiong, L. L. Xia, Q. L. Huang, J. X. Huang, Y. Q. Gu and P. Wang, *Talanta*, 2018, **184**, 109.

20 F. Rezende, R. P. Brandes and K. Schröder, *Antioxid Redox Sign*, 2018, **29**, 585.

21 I. V. Tetko, and V. Y. Tanchuk, *J. Chem. Inf. Comput. Sci.*, 2002, **42**, 1136.

22 M. Kamiya, D. Asanuma, E. Kuranaga, A. Takeishi, M. Sakabe, M. Miura, T. Nagano and Y. Urano, *J. Am. Chem. Soc.*, 2011, **133**, 12960.

23 X. Huang, Z. P. Li, T. T. Cao, Q. Cai, C. C. Zeng, H. Fu and L. M. Hu, *Rsc Adv.*, 2018, **8**, 14603.

24 M. Alasubramanyam, i A. Koteswar, R. Kumar, S. Monickaraj, J. Maheswari and V. Ohan, *J. Biosci.*, 2003, **28**, 715.

25 B. C. Dickinson, J. Peltier, D. Stone, D. V. Schaffer and C. J. Chang, *Nat. Chem. Biol.*, 2011, **7**, 106; V. S. Lin, B. C. Dickinson and C. J. Chang, *Methods Enzymol.*, 2013, **526**, 19.

26 T. Myochin, K. Kiyose, K. Hanaoka, H. Kojima, T. Terai and T. Nagano, *J. Am. Chem.* Soc., 2011, **133**, 3401; J. J. Zhang, B. F. Yu, L. L. Ning, X. Y. Zhu, J. X. Wang, Z. J. Chen, X. Y. Liu, X. J. Yao, X. Y. Zhang and H. X. Zhang, *Eur. J. Org. Chem.*, 2015, **2015**, 1711; L. Yu, T. T. Li, Q. L. Wang, L. Li and L. G. Chen, *Res Chem Intermed*, 2014, **40**, 1469.

27 C. Wang, J. Qiao, X. Liu, H. Song, Z. Sun and W. Chu, *J. Org. Chem.*, 2018, **83**, 1422.

Supplementary Material

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Highlights

- A near-infrared probe **Cy-H_2O_2** has been developed for the detection of **H_2O_2**.
- The sensor has an instantaneous response to **H_2O_2**, which respond within 10 min.
- The probe presents a good linear relationship and the detection limit is 65 nM.
- We successfully applied the probe to detect H_2O_2 in living cells and zebrafish.
- The probe provides an adjunct to the study of ROS-related diseases.