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Invited paper

# A new pyrene-based fluorescent probe with large Stokes shift for detecting hydrogen peroxide in aqueous solution and living cells

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ABSTRACT

Hydrogen peroxide ($H_2O_2$) plays an important role in signal transduction and therapy of serious diseases. In this work, we have developed a new pyrene-based fluorescent probe **Py-Boe**, which possesses large Stokes shift and achieves detection of $H_2O_2$ in aqueous solution. The probe **Py-Boe** exhibits excellent photostability, high sensitive and selectivity. Taking advantage of these properties, the probe **Py-Boe** achieves successfully imaging of exogenous and endogenous $H_2O_2$ in living RAW 264.7 cells. We expect that this dye with large Stokes shift may open an avenue to construct novel fluorescent probe with large Stokes shift for biological application.



## 1. Introduction

Reactive oxygen species (ROS, e.g. $H_2O_2$, $ClO^-$, $^1O_2$ and $O^{2-}$) are formed as a natural byproduct of the normal metabolism of oxygen in living organisms [1,2]. The overproduction of ROS is involved in the general phenomenon of oxidative stress that is implicated in aging and death [3–6]. Among them, hydrogen peroxide ($H_2O_2$), a major ROS, plays an important role in cell growth, proliferation, host defense, and signaling pathways under physiological conditions [7–12]. But excessive $H_2O_2$ generation leads to the pathogenesis of many disorders, such as inflammatory disease, cardiovascular disease, Alzheimer's disease, and cancer [13–17]. Consequently, it is pretty important to monitor $H_2O_2$ in living organisms.

Compared to other techniques, fluorescence probes have become more powerful tool to detect targets in biological imaging field [18–24]. Because the fluorescent probe needs high sensitivity, high selectivity, and useful applications in the biology, chemistry and medicine. Thus, development of $H_2O_2$ fluorescent probe have been arousing people's interest. Up to present, there are various fluorescent $H_2O_2$ probes based on rhodamine, naphthalimide, bodipy and so on have been reported [25]. However, little attention had been paid on pyrene as fluorescent probe with large Stokes shift for detecting $H_2O_2$ in aqueous solution and living cells. Thus, the aim of our work is to design a fluorescent probe based on pyrene for high sensitive and selective detecting $H_2O_2$ in aqueous solution and living cells.

Herein, we introduce pyrene-based fluorophore as a novel fluorescent dye for developing a fluorescent probe 4,4,5,5-tetramethyl-2-(4-((pyren-1-yloxy)methyl)phenyl)-1,3,2-dioxaborolane (**Py-Boe**) with large Stokes shift and achieve high sensitive and selective detection of $H_2O_2$ in aqueous solution and living cells. We select pyrene-based derivative as fluorescent platform by careful structural modifications and the phenyl boronic ester serves as the recognition site of $H_2O_2$, respectively. Furthermore, **Py-Boe** exhibits ideal properties and achieved detection of $H_2O_2$ in aqueous solution and living cells (Scheme 1).

## 2. Experimental

### 2.1. Material and instrumentation

All reagents and chemicals were purchased from commercial suppliers and used without further purification. The solvents used in the spectral measurement are chromatographic grade. Spectroscopic measurements were performed in pH 7.4 PBS buffer solution. Thin layer chromatography (TLC) analyses were obtained on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from Qingdao Ocean Chemicals.

Nuclear magnetic resonance spectra ($^1H$ and $^{13}C$) were obtained on an AVANCE III 400 MHz Digital NMR Spectrometer. The high

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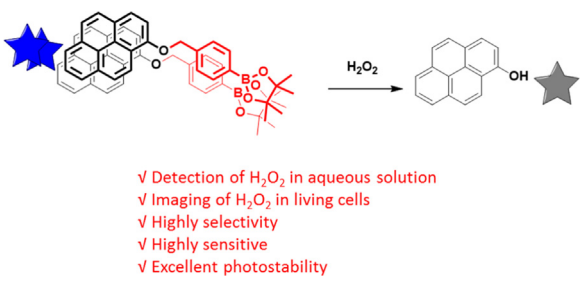



**Scheme 1.** Sensing process of probe **Py-Boe** to H2O2 and ideal properties.

resolution mass spectrometry (HRMS) spectra were recorded on Agilent Technologies 6510 Q-TOF LC/MS or ThermoFisher LCQ Fleet. Milli-Q water (18.2 MU cm) and spectroscopic pure solvents were used in spectral experiments. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer. Cell imaging experiment of **Py-Boe** was excited and collected through Nikon A1MP confocal fluorescent microscope. Transmission electron microscopic (TEM) images were recorded using a FEI Tecnai G2 F20 high-resolution transmission electron microscope operating at 200 kV. The particle size distribution measurements were conducted on a Microtrac S3500 analyzer.

### 2.2. Preparation of samples and test solution

The stock solution of the probe **Py-Boe** was prepared at 1 mM in DMF. The different pH (4.0–9.0) PBS solutions were prepared. The solutions of various testing species such as NaCl, KCl, $CuCl_2$, $FeCl_3$, glutathione (GSH), $H_2O_2$, homocysteine (Hcy), cysteine (Cys), $NaNO_2$, $NaNO_3$, $Na_2SO_3$, t-butylhydroperoxide, peroxide *tert*-butyl ether were prepared in 50 mM from in Milli-Q water solution.

### 2.3. Cell culture and image experiment

RAW 264.7 cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% $CO_2$ incubator at 37 °C. Before the imaging experiments, RAW 264.7 cells were subcultured and seeded in the glass bottom culture dishes (Nest) at a density of $1 \times 10^5$/mL. The cells were placed on glass coverslips and allowed to adhere for 24 h. When the cells reached about 70% confluence, they were then used to the imaging experiments.

The PMA control experiment: Firstly, for the experimental group, the culture medium of the cells was changed to a fresh medium containing 8 μM **Py-Boe**, and then incubated for 30 min. Subsequently, the medium was removed and washed three times with PBS to remove the excess probe. Secondly, we carried out the control experiment, in which the culture medium of the cells was changed to a fresh medium containing PMA (3 μg/mL) and **Py-Boe** (8 μM) incubated for 3 h. Then, the medium was removed and washed three times with PBS to remove the excess PMA and **Py-Boe**. Finally, the confocal imaging was carried out ($\lambda_{ex}$ = 404 nm, $\lambda_{em}$ = 425–475 nm)

### 2.4. Cell viability evaluated by MTT assay

Viability of the cells was assayed using cell proliferation Kit I with the absorbance of 492 nm being detected using a Perkin-Elmer Victor plate reader. Eight thousand cells were seeded per well in a 96-well plate. After overnight culture, various concentrations of **Py-Boe** were added into the 96-well plate. After 3 h treatment, 20 μL of MTT solution (5 mg/mL in phosphate buffer solution) was added into the each well. After 8 h incubation at 37 °C, 200 μL DMSO was added to dissolve the purple crystals. After 20 min incubation, the optical density readings at 492 nm were taken using a plate reader.

### 2.5. Synthesis of the probe Py-Boe

1-Hydroxypyrene (0.11 g, 0.5 mmol) was added into a flask containing a mixture of 4-bromomethylphenyl) boronic acid (0.15 g, 0.5 mmol), $K_2CO_3$ (0.07 g, 0.5 mmol), and 10 mL of DMF with nitrogen at room temperature for 6 h, then poured into $H_2O$ (500 mL) and extracted with EtOAc. The organic phase was separated, dried with $MgSO_4$, and removed by vacuum distillation. The product **Py-Boe** was obtained as a yellow solid with a yield of 60% after purified by column chromatography with ethyl acetate/petroleum ether (3:1, v/v) as eluent. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.44 (d, $J$ = 9.2 Hz, 1H), 8.27–8.19 (m, 3H), 8.16 (d, $J$ = 9.2 Hz, 1H), 8.10–7.97 (m, 3H), 7.84 (d, $J$ = 8.5 Hz, 1H), 7.76 (d, $J$ = 8.0 Hz, 2H), 7.65 (d, $J$ = 8.0 Hz, 2H), 5.57 (s, 2H), 1.31 (s, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 152.71, 140.32, 135.17, 131.73, 131.70, 127.26, 126.64, 126.53, 126.16, 125.89, 125.51, 125.44, 125.17, 124.94, 124.36, 124.28, 121.35, 120.63, 109.64, 83.90, 77.40, 77.08, 76.76, 70.83, 24.93. HRMS ($m/z$): [M+H] calcd for $C_{29}H_{27}BO_3$: 434.2053; found: 435.2211.

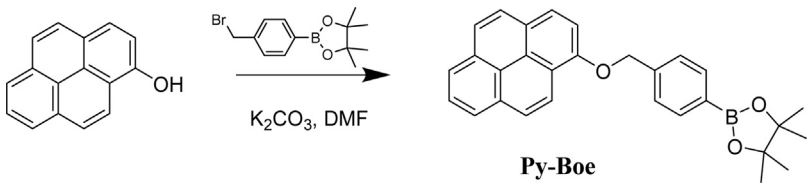
## 3. Results and discussion

### 3.1. Synthesis

The synthesis process of **Py-Boe** was provided in Scheme 2. The products were characterized by $^1$H NMR, $^{13}$C NMR and HRMS in Supporting information. Pyrene was used as the fluorescent platform and the phenyl boronic ester served as the recognition site of $H_2O_2$.

### 3.2. Optical physical properties of *Py-Boe*

With the probe **Py-Boe** in hand, we set out to investigate the spectral changes of the probe **Py-Boe** in different solutions. The




**Scheme 2.** Synthesis of the probe **Py-Boe.**