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A highly selective fluorescence turn-on detection of hydrogen peroxide and D-glucose based on the aggregation/deaggregation of a modified tetraphenylethylene

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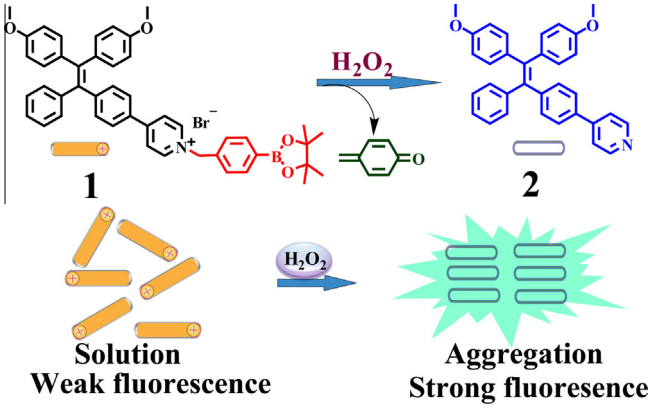
ABSTRACT

A new selective fluorescence turn-on detection of hydrogen peroxide was established by taking advantage of the aggregation induced-emission (AIE) behavior of tetraphenylethylene unit and the reaction of hydrogen peroxide toward the arylboronic ester group in compound **1**. Moreover, compound **1** was successfully utilized for the selective detection of D-glucose in aqueous solution.

Reactive oxygen species (ROS) as a class of radical or nonradical oxygen-containing species include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hydroxy radicals (·OH), superoxide anions (O_2^-), and nitric oxide.[1] In general, ROS are toxic to cells if the levels of ROS exceed the tolerable physiological range.[2] Among ROS species, hydrogen peroxide is the least reactive and mild oxidant. However, it can be transformed into strong oxidants. For example, H_2O_2 is readily converted to the highly reactive hydroxyl radical via the Fenton reaction.[3] Despite its hazardness to organisms, hydrogen peroxide is ubiquitous as it is a by-product of many metabolic reactions.[1d] Also, hydrogen peroxide is widely used for environmental and industrial applications such as drinking water purification.[4] Therefore, it is highly desirable to develop efficient and simple sensing systems to monitor H_2O_2 concentration level in not only living cells but also environment.

Up to now, a large number of sensors for H_2O_2 have been invented based on either electrochemical or optical methods.[5–7] Among them, fluorometry has caught more attention due to its higher sensitivity and can be applied in cells and tissue. For instance, pentafluorobenzenesulfonyl fluoresceins were utilized for sensing H_2O_2 based on the fact that the pentafluorobenzenesulfonyl group can be cleaved off by H_2O_2 via perhydrolysis and thus the fluorescence is enhanced.[6a] While the selectivity and sensitivity of these probes are excellent, most syntheses are usually time-consuming and laborious.

Herein we report a new fluorescence turn-on detection of H_2O_2 in aqueous solution with a new tetraphenylethylene (TPE) molecule **1** (Scheme 1) with *N*-4-(benzyl boronic pinacol ester) pyridinium bromide moiety. The sensing mechanism of **1** toward H_2O_2 in aqueous solution is illustrated in Scheme 1 and is explained as follows: (i) the pyridinium moiety may render **1** water-soluble. As a result it is anticipated that **1** is weakly emissive in aqueous



Scheme 1. The chemical structure of **1** and **2**, and the design rationale for the fluorescence turn-on detection of H_2O_2.

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solutions according to previous studies;[8–10] (ii) it is well known that H_2O_2 can stoichiometrically convert phenylboronic ester moiety into the phenol group.[5c,11] Thus, incubation of hydrogen peroxide with **1** should result in oxidation of the phenylboronic pinacolester, followed by hydrolysis and 1,6-elimination of *p*-quinone-methide to yield the *p*-pyridine substituted TPE (**2**, Scheme 1). Compound **2** is expected to show low solubility in aqueous solutions, thus aggregation will occur and turn on the fluorescence of TPE moiety based on the aggregation-induced emission (AIE) feature of TPE compounds.[8–10] In this way, compound **1** can be employed for the fluorescence turn-on detection of H_2O_2 in aqueous solutions. The results reveal that **1** exhibits high selectivity toward H_2O_2. Moreover, by combining the cascade enzymatic (oxidation of D-glucose with GOx to produce H_2O_2)[12] and chemical (oxidation of phenylboronic ester by H_2O_2) reactions, compound **1** is successfully utilized for the selective detection of D-glucose in aqueous solution.

The synthesis of **1** started from monobromo-substituted tetraphenylethylene derivative (**3**) as shown in Scheme 2. Palladium-catalyzed Suzuki coupling reactions between compound **3** and 4-pyridinylboronic acid yielded compound **2**, which was allowed to react with 4-(bromomethyl) benzene boronic pinacol ester to afford **1** in 95.6% yield. The chemical structure of **1** was characterized with ^{1}H NMR, ^{13}C NMR, and mass spectra (detailed procedures are given in Supplementary data).[13]

As expected, compound **1** is soluble in water, and the HEPES buffer solution (10 mM, pH = 7.4) of **1** (5.0 μM) can be easily prepared. Figure 1 shows the fluorescence spectra of **1** before and after addition of different amounts of H_2O_2. As anticipated, the aqueous solution of **1** (5.0 μM) was almost non-emissive in the absence of H_2O_2 (see Fig. 1, curve a). However, after incubation with H_2O_2 at 37 °C the fluorescence intensity of **1** increased gradually concomitant with the emission peak progressively blue-shifting from 610 to 510 nm.[14] For instance, the fluorescence intensity at 510 nm (see Fig. 1, curve b) increased by more than 260 times after the solution of **1** was incubated with 150.0 μM of H_2O_2 for 30 min. In fact, the fluorescence quantum yield of the HEPES buffer solution (10 mM, pH = 7.4) of **1** (5.0 μM) increased from 0.006 to 0.121 (by reference to quinine hemisulfate monohydrate) after H_2O_2 (150.0 μM) was introduced. Such fluorescence enhancement can be distinguished with naked-eye as shown in the inset of Figure 1, where photos of solutions of **1** in the absence and presence of H_2O_2 under UV light (365 nm) illumination are displayed. Figure 2 depicts the plot of the relative fluorescence intensity ($I/I_0 - 1$) at 510 nm versus concentration of H_2O_2. Interestingly, the fluorescence intensity of **1** increases almost linearly with the concentration of H_2O_2 in the range of 10.0–110.0 μM as displayed in the inset of Figure 2. Accordingly, the detection limit of H_2O_2 was estimated to be 180.0 nM (n = 11 and S/N = 3).

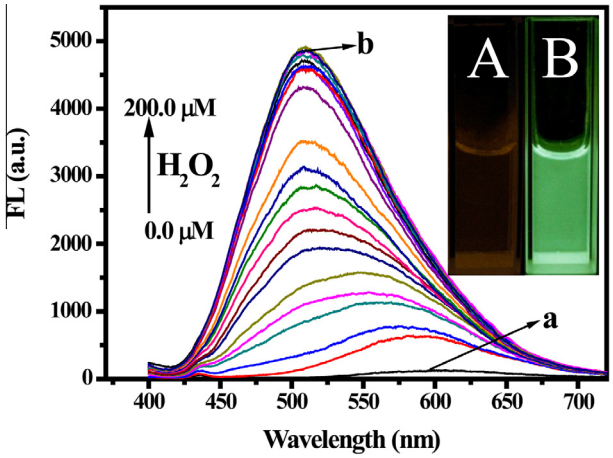


Figure 1. Fluorescence spectra of **1** (5.0 μM) after incubation with different amounts of H_2O_2; the reaction was performed at 37 °C for 60 min in 10 mM HEPES buffer at pH 7.4; the excitation wavelength was 390 nm; inset shows photos of the solution of 1 (5.0 μM) before (A) and after (B) incubation with 150.0 μM of H_2O_2 under UV light (365 nm).

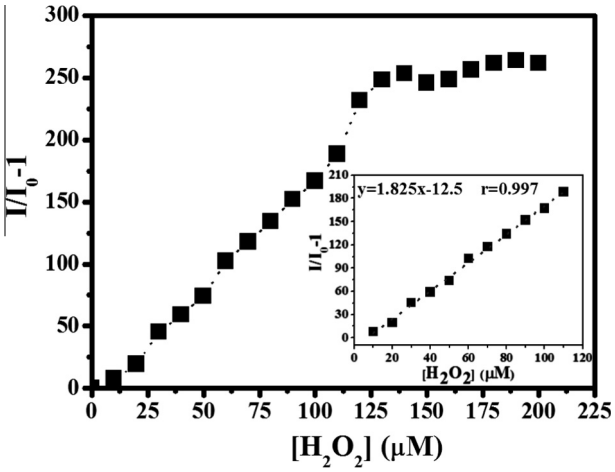
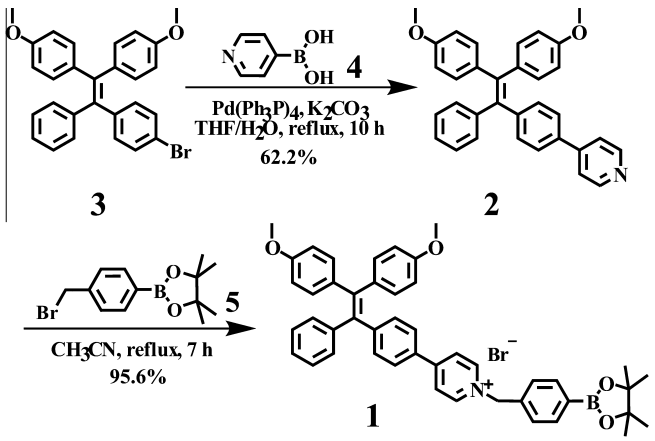


Figure 2. The plot of ($I/I_0 - 1$) at 510 nm versus the concentration of hydrogen peroxide; inset shows the linear relation for concentration of hydrogen peroxide in the range of 10.0–110.0 μM.

Moreover, fluorescence responses of **1** to other reactive oxygen species were examined. As depicted in Figure 3, significant fluorescence enhancement was observed only after incubation with H_2O_2. Other ROS, such as singlet oxygen, hydroxy radical, superoxide anion, and nitric oxide etc., induced only negligible fluorescence enhancement for **1** under the same conditions. Thus, compound **1** shows high selectivity toward H_2O_2.

As illustrated in Scheme 1, such fluorescence enhancement is attributed to the aggregation of compound **2** which is generated from the oxidation reaction of **1** with H_2O_2. The formation of fluorescent aggregates was confirmed by both confocal laser scanning microscopic (CLSM) and dynamic light scattering (DLS) studies. As displayed in Figure 4, there were almost no fluorescent aggregates for the solution of **1** (10.0 μM) before incubation with H_2O_2; however, fluorescent aggregates emerged after incubation with H_2O_2 (200.0 μM) based on the CLSM images. DLS data (see Fig. 5) also indicated the formation of aggregates of 200–600 nm for the solution of **1** (5.0 μM) after incubation with H_2O_2 (200.0 μM).



Scheme 2. Synthetic approach to compound **1**.

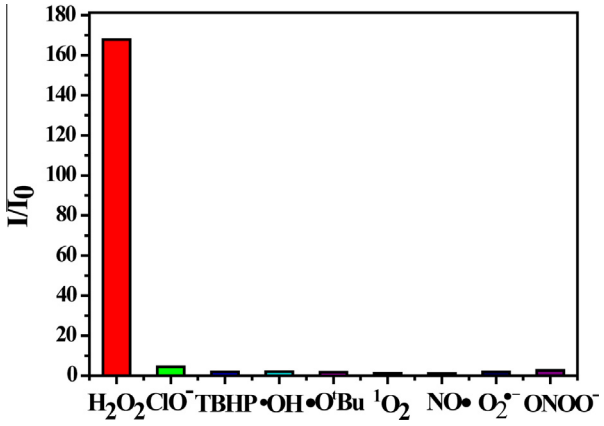


Figure 3. Variation of the relative fluorescence intensity at 510 nm of **1** (5.0 μM) after incubation with 100.0 μM of hydrogen peroxide and 200 μM of other reactive oxygen species; the reaction was performed at 37 °C for 60 min in HEPES buffer (10 mM, pH 7.4); the excitation wavelength was 390 nm.

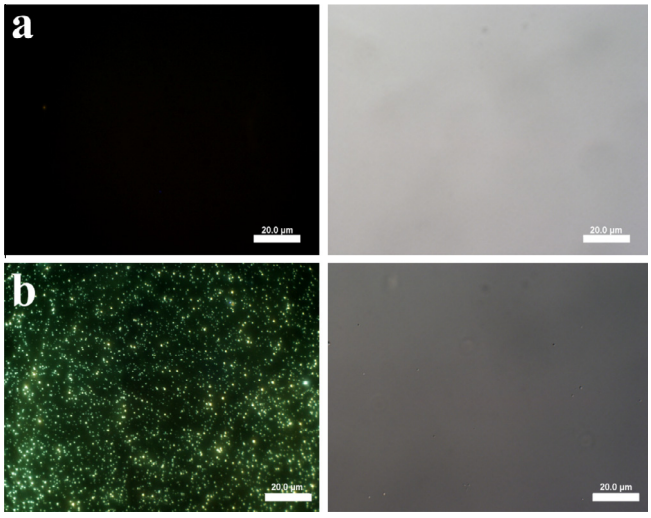


Figure 4. Fluorescence confocal laser scanning images of **1** (10.0 μM) before (a) and after (b) incubation with H_2O_2 (200.0 μM); the reaction was performed at 37 °C for 60 min in 10 mM HEPES buffer at pH 7.4. The scale bar represents 20 μm.

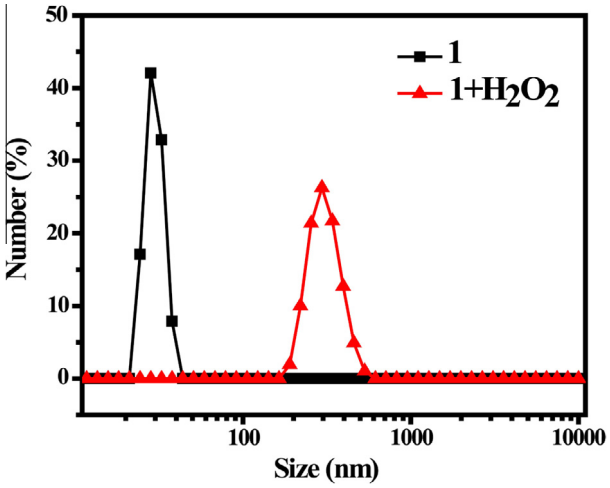


Figure 5. The DLS data of **1** (5.0 μM) before and after incubation with H_2O_2 (200.0 μM). The reaction between **1** and H_2O_2 was performed at 37 °C for 30 min in 10 mM HEPES buffer at pH 7.4 before the collection of DLS data.

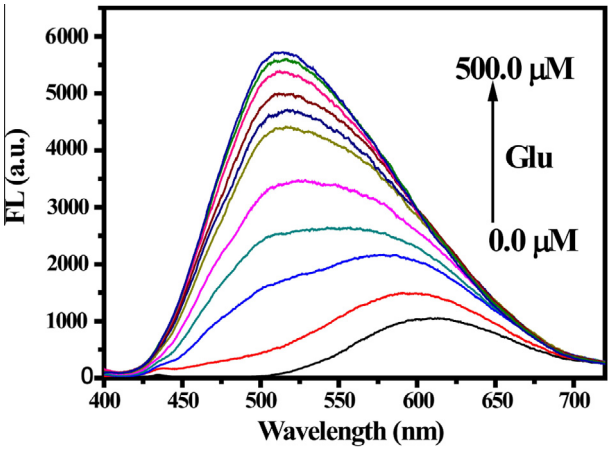


Figure 6. Fluorescence spectra of **1** (5.0 μM) after incubation with GOx (1.1 U/mL) and different concentrations of D-glucose; the reaction was performed at 37 °C for 90 min in 10 mM HEPES buffer at pH 7.4; the excitation wavelength was 390 nm.

The formation of **2** after incubation of **1** with H_2O_2 (see Scheme 1) is confirmed by the mass spectral (Fig. S2) and HPLC analysis (Fig. S3). The mass signal at $m/z = 470.3$, corresponding to the molecular weight of **2**+H^+, was detected after the solution of **1** was incubated with H_2O_2. Moreover, HPLC analysis also indicated the formation of **2** by comparing with the authentic sample of **2** (see Fig. S3).

In the following, we demonstrate the application of **1** for the selective detection of D-glucose. As reported previously,[12] D-glucose can be oxidized in the presence of GOx (glucose oxidase) to generate H_2O_2 which can further convert **1** into **2** (see Scheme 1). Therefore, it is anticipated the compound **1** can be utilized for the detection of D-glucose by taking advantage of the cascade enzymatic and chemical reactions. The ensemble HEPES buffer (10 mM, pH = 7.4) solutions containing **1** (5.0 μM), GOx (1.1 U/mL), and different amounts of D-glucose (0–0.5 mM) were incubated at 37 °C for 90 min. Then, the fluorescent spectrum of each solution was measured as shown in Figure 6. Obviously, the fluorescence intensity of the ensemble increased gradually after the addition of D-glucose; moreover, more fluorescence enhancement was observed by increasing the concentration of D-glucose in the ensemble solution. The fluorescence intensity ratio $(I/I_0 - 1)$ at 510 nm as the function of concentration of D-glucose is shown in Figure S6, where I and I_0 represent the fluorescence intensities of the ensemble solutions after and before incubation with D-glucose and GOx, respectively. In the concentration range of 0.05–0.25 mM, the fluorescence intensity ratio $(I/I_0 - 1)$ increases almost linearly with the concentration of D-glucose (see inset of Fig. S6). D-Glucose with concentration as low as 3.0 μM can be detected with this new fluorescent assay.

In order to demonstrate the selectivity of this fluorescence detection of D-glucose, the fluorescence spectra of compound **1** were also recorded after separate incubation with D-mannose, D-fructose, and D-galactose in the same way as for D-glucose. As depicted in Figure 7, a remarkable fluorescence enhancement was only observed for **1** after incubation with GOx and D-glucose. This is understandable by considering the fact that GOx can selectively oxidize D-glucose to generate H_2O_2.

In summary, we have successfully established a selective fluorescence turn-on detection of H_2O_2 by making use of the abnormal fluorescent behavior of TPE compounds and the oxidation of the phenylboronic pinacol ester group by H_2O_2. This fluorometric turn-on detection of H_2O_2 possesses the following features: (1) **1**

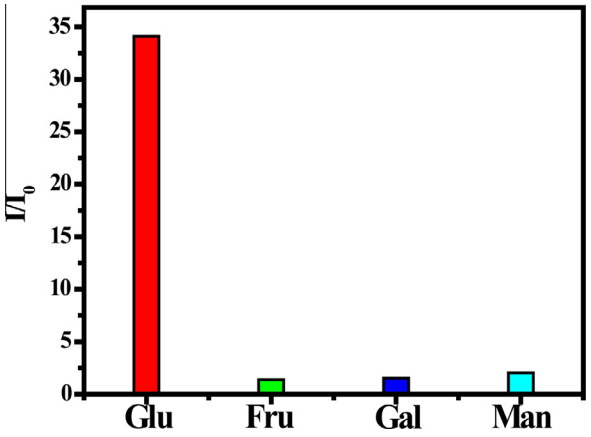


Figure 7. Variation of the relative fluorescence intensity at 510 nm of **1** (5.0 μM) after incubation with GOx (1.1 U/mL) and 250.0 μM of D-glucose or 250.0 μM of D-fructose/galactose/mannose; the reaction was performed at 37 °C for 90 min in 10 mM HEPES buffer at pH 7.4; the excitation wavelength was 390 nm.

is easily synthesized; (2) the determination procedure can be carried out in aqueous solutions; (3) H_2O_2 with concentration as low as 180 nM can be detected and interferences from other ROS are negligible. Moreover, the ensemble of compound **1** and GOx was successfully employed for the detection of D-glucose with good sensitivity and selectivity by utilizing the cascade enzymatic and chemical reaction.

Acknowledgments

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Supplementary data

Supplementary data (synthesis and characterization; UV spectra, DLS data and relevant data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.01.056.

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13. *Characterization data for compound* ***1****:* ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.15 (d, J = 4.0 Hz, 2H), 8.48 (d, J = 4.0 Hz, 2H), 7.91 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.26–7.11 (m, 5H), 7.00 (d, J = 4.0 Hz, 2H), 6.93 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.0 Hz, 2H), 6.74–6.69 (t, J = 8.0 Hz, 4H), 5.85 (s, 2H), 3.68 (s, 6H), 1.28 (s, 12H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.1, 158.0, 154.3, 148.4, 144.7, 143.2, 141.7, 137.6, 137.5, 135.2, 135.1, 132.2, 132.1, 132.0, 130.8, 128.1, 128.0, 127.7, 126.6, 124.3, 113.4, 113.2, 83.9, 62.1, 24.6; HR-MS (ESI, positive): calcd for $C_{46}H_{45}BNO_4$ $[M-Br^-]^+$: 686.3454; found: 686.3441.
14. Our previous studies indicated that the absorption and fluorescence of tetraphenylethylene derivatives could be tuned by intramolecular electron-donor (D) and acceptor (A) interactions; strong intramolecular D–A interaction could yield red-shifts for both absorption and fluorescence spectra (see, Gu, X.; Yao, J.; Zhang, G.; Zhang, C.; Yan, Y.; Zhao, Y.; Zhang, D. *Chem. Asian J.* **2013**, *8*, 2362). Compared with pyridium unit, pyridine unit possesses weaker electron-accepting ability, leading to weak intramolecular D–A interaction within **2** and the fluorescence blue-shift after pyridium moiety in **1** is transformed into pyridine in **2**.