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A ratiometric fluorescent probe with excited-state intramolecular proton transfer for benzoyl peroxide†

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A novel ratiometric fluorescent probe A1, based on excited-state intramolecular proton transfer (ESIPT) mechanism, for the detection of benzoyl peroxide (BPO) was designed and synthesized. A1 was employed for determining BPO in food and pharmaceutical samples with good sensitivity and high selectivity.

Benzoyl peroxide (BPO), an important member of the reactive oxygen species (ROS) family, plays paradoxical roles in public health concern. On one hand, it has already been extensively explored for initiating polymerization, bleaching flour and treating acne.[1] On the other hand, exposure to excessive quantities of BPO is likely to pose risks to human health.[2] For example, BPO can act as a tumor promoter and is able to decompose into deleterious substances (*e.g.*, benzoic acid, biphenyl), inevitably leading to tissue damage and diseases. Besides, it is generally accepted that too high level of BPO in food would cause the degeneration of some essential nutrients. Thus, the maximum use level of BPO should be strictly regulated. Recently the European Union and China have prohibited BPO to be used in wheat flour due to the growing public concern on the abusive use of BPO.[3] Considering the potential risks of BPO and its easy access to human body by food intake or skin absorption, convenient and effective methods for BPO detection in real samples and biological settings are urgently desired.

Nowadays, small molecule-based fluorescent probes have received tremendous attention, due to their simple operation, high sensitivity and selectivity. Especially, some kinds of probes have been employed for the detection of ROS.[3] For example, Ma and co-workers developed a new resorufin-based probe which showed a fluorescent off-on response to BPO.[4] The probe was used for the detection of BPO in real samples with high sensitivity and selectivity. In addition, ratiometric small-molecule probes possessing inherent self-calibration achieved by measuring the intensity of the reacted and unreacted probes simultaneously, provide a more accurate and reliable way for fluorescent assay.[5] Herein, we are trying to design an excited-state intramolecular proton transfer (ESIPT)-based small-molecule probe for ratiometric measurement of BPO.

2-(Benzothiazol-2-yl)phenol and its derivatives can undergo ESIPT, convert to their corresponding keto tautomers and then produce exclusive keto emission accordingly.[6] On the contrary, the ESIPT process can be inhibited when the phenolic proton is replaced, giving intensively enol-like emission along with a distinct hypochromic shift. Hence, ratiometric fluorescent assay can be established *via* substitution of phenolic proton with a recognition unit which can specifically react with a species. Up to now, several ratiometric fluorescent probes based on 2-(benzothiazol-2-yl)phenol or its derivatives have been reported for determination of various substances, such as pyrophosphate, CN^-, H_2O_2 and *etc.*[7]

We synthesized a novel ratiometric probe for the detection of BPO by choosing 4-acetamino-2-(benzo[d]thiazol-2-yl)phenol **2** as an ESIPT fluorophore and *o*-substituted arylboronate **3** as a recognition unit. The hydroxyl group of **2** was protected by arylboronate in one step to obtain probe **A1** with a yield of 65% (Scheme 1). The structure of **A1** was confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (Fig. S1–3, ESI†).

The fluorescence response of **A1** to BPO is shown in Fig. 1(a). **A1** gives a single, blue emission centred at 423 nm. In addition, it shows a good stability within a wide pH range (see Fig. S4, ESI†). Upon addition of BPO (30 μM), a cyan emission band centred at 494 nm appears while the original emission band peaked at 423 nm almost fades out. The effects of the ethanol concentration and the reaction time on the determination of BPO were evaluated (see Fig. S5–6, ESI†) and the optimized fluorescent assay could be carried out in 20 mM PBS solution (pH 7.4) with 20% ethanol and 0.1% DMF at 37 °C for 60 min.

To evaluate the selectivity of **A1** towards BPO, we tested the fluorescence response of **A1** to various interferences, such as some common ions, amino acids, sugars and vitamins. As shown in Fig. 1(a), only negligible changes in the spectra were observed

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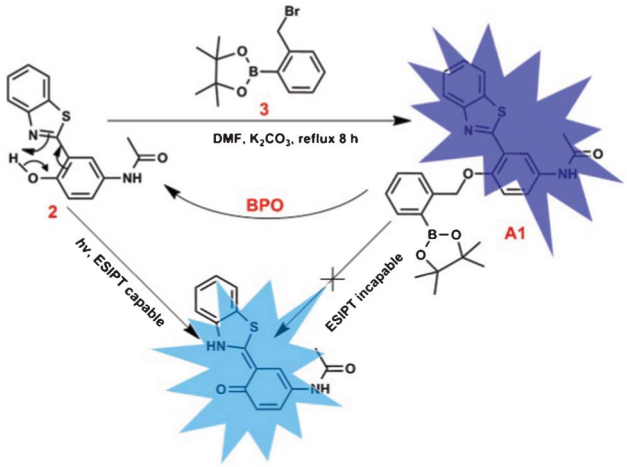

Scheme 1 Synthesis and the sensing mechanism of **A1**.

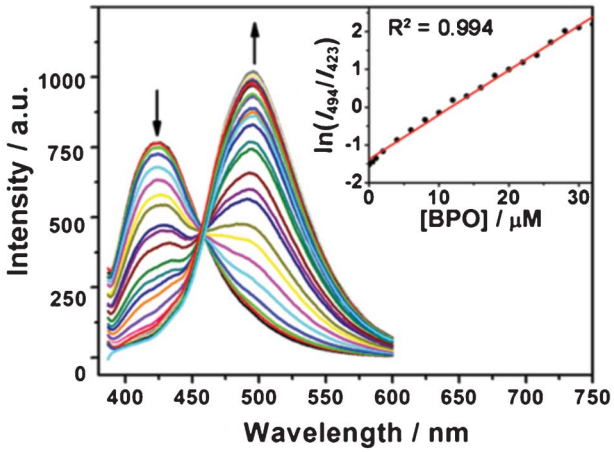

Fig. 2 Fluorescence spectra (λ_{ex} 377 nm) of **A1** (6 μM) towards various concentrations of BPO. Inset: The relationship between $\ln(I_{494}/I_{423})$ and the BPO concentration. The assay was carried out in 20 mM PBS solution (pH 7.4) with 20% ethanol and 0.1% DMF at 37 °C for 60 min.

upon the addition of various interferences. Also, **A1** exhibits high selectivity towards BPO over other ROS and some oxidizing agents (see Fig. 1(b) and (c)). Interestingly, BPO leads to a much more sensitive fluorescence change than that of H_2O_2 (see Fig. S7, ESI†), though H_2O_2 is very similar to BPO in many aspects. The finding is consistent with that of Ma's.[4] The significantly sensitive response of probe **A1** to BPO over H_2O_2 is probably attributed to (1) the effect of solvent, and (2) the structure of arylboronate (recognition unit). In this assay, ethanol not only acts as cosolvent for probe **A1** and BPO, but also promotes the reactivity of BPO through accelerating the decomposition of BPO into reactive intermediates.[8] On the other hand, the reactivity of H_2O_2 may be reduced by ethanol.[9] Additionally, the position of the substituted group at arylboronate also has an impact on sensing activity.[10] Still, the reason why the probe shows high selectivity for BPO over H_2O_2 remains unknown.

As depicted in Fig. 2, a decrease of the fluorescence intensity at 494 nm is concomitant with the increase at 423 nm upon addition of BPO. The fluorescence intensity ratio (I_{494}/I_{423}) increases with an increasing concentration of BPO. A linear relationship between $\ln(I_{494}/I_{423})$ and the concentration of BPO can be expressed by $\ln(I_{494}/I_{423}) = 0.1180 \times [\text{BPO}](\mu\text{M}) - 1.384$, within a concentration range of 0.5–30 μM ($R^2 = 0.994$). The detection limit was estimated ($3S/m$, $n = 11$) to be 80 nM.

To assess its practical application, **A1** was employed to quantify the content of BPO in wheat flour and antibacterial agent (Benzoyl Peroxide Gel, Laboratoires Galderma, France). For wheat flour samples, the recovery of BPO was within 95%–105% (listed in Table S1, ESI†). For antibacterial agent, the tested content of BPO is 4.6% while the indicative content is 5%. The results suggest that **A1** can be utilized for detection of BPO in real samples.

The possible mechanism of **A1** for selective response to BPO is illustrated in Scheme 1. Once the phenolic hydroxyl group is protected by arylboronate, **A1** is not able to undergo ESIPT and therefore only a blue enol-like emission at a shorter wavelength (423 nm) can be seen. On the contrary, after the arylboronate group is cleaved by BPO, the phenolic proton can be restored, leading to a cyan keto-emission at a relatively long wavelength (494 nm). To confirm the transformation of **A1** to **2**, the reaction was subjected to HPLC analysis. We found that a peak at 13.1 min

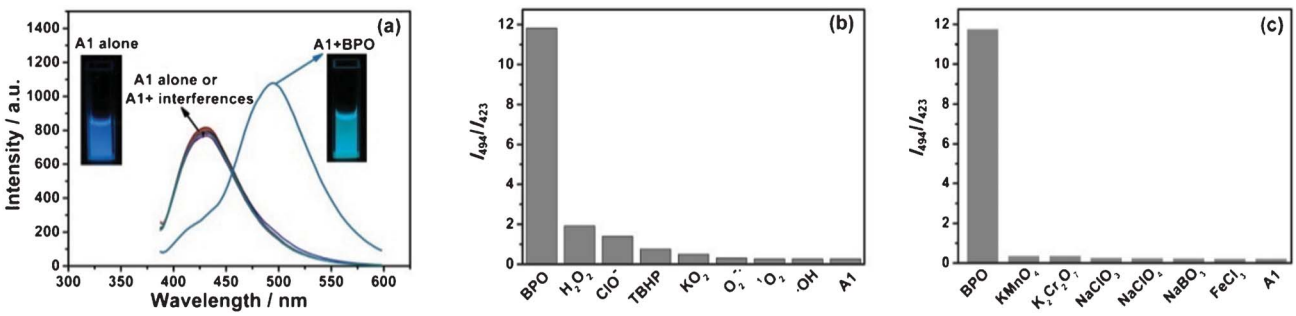

Fig. 1 (a) Fluorescence spectra of **A1** (6 μM) and upon addition of 30 μM BPO or various interferences including 30 μM of Zn^{2+}, Fe^{3+}, Fe^{2+} Co^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Cr^{3+}, Mn^{2+}; 1.5 mM of K^+, Na^+, NO_3^-, AcO^-, SO_4^{2-}, F^-, Cl^-, I^-, Br^-, glucose, fructose, saccharose, maltose, glycine, proline, glutamic acid, arginine, VB_1, VB_6, VB_{12}, V_C; (b) Emission ratio (I_{494}/I_{423}) of **A1** (6 μM) in the presence of 30 μM some ROS; and (c) oxidizing agents. The assay was carried out in 20 mM PBS solutions (pH 7.4) with 20% ethanol and 0.1% DMF at 37 °C for 60 min. (λ_{ex} 377 nm).

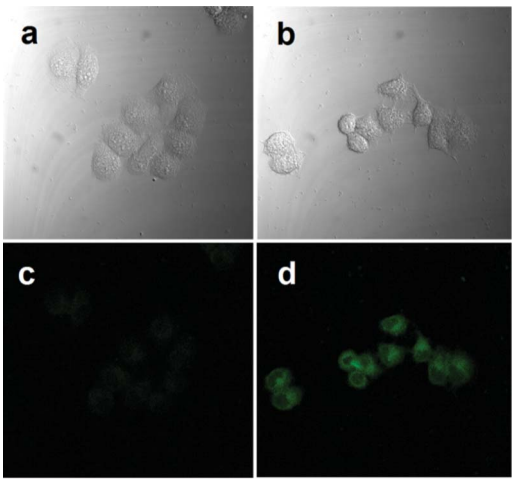

Fig. 3 Bright-field image (a) and the corresponding confocal fluorescence image (c) of HeLa cells after treated with **A1** (100 μM) at 37 °C for 60 min. Bright-field image (b) and the corresponding confocal fluorescence image (d) of HeLa cells pre-treated with 100 μM **A1** for 60 min and then incubated with BPO (100 μM) for 60 min (excited at 405 nm with emission at 505–530 nm).

which corresponds to **2** emerged after **A1** was treated with BPO (see Fig. S8, ESI†). To further verify the assumption, the eluates at 13.1 min were collected, and then analysed by HRMS. The molecular ion peak located at *m*/*z* 285.0691, is assigned to $C_{15}H_{13}N_2O_2S^+$, further confirming the formation of **2** (see Fig. S9, ESI†). Moreover, the optical changes (see Fig. S10, ESI†) together with reported literatures suggest that **A1** can transform to **2** after being treated with BPO, inducing a ratiometric spectral response towards BPO.

The imaging ability of **A1** for BPO in living cells was tested. As shown in Fig. 3, HeLa cells show extremely faint fluorescence after incubation with probe **A1** for 60 min. By contrast, in the presence of BPO, the fluorescence intensity is markedly increased. These results suggest that probe **A1** is cell membrane permeable and is able to react with BPO in living cells.

In summary, based on the ESIPT mechanism, we have developed a ratiometric fluorescent probe for BPO. The probe was employed for determination of BPO in real samples with high selectivity and good sensitivity. Moreover this new probe exhibits potential applications in fluorescent imaging of BPO in living cells.

Acknowledgements

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