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A new resorufin-based spectroscopic probe for simple and sensitive detection of benzoyl peroxide *via* deboronation†

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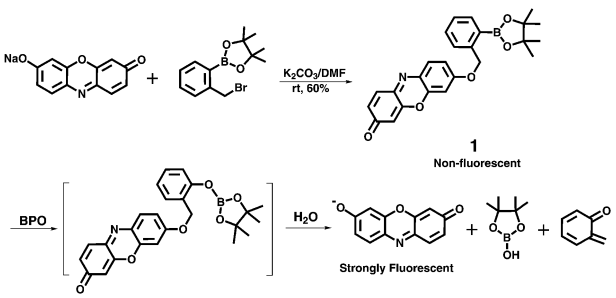
A new resorufin-based probe is developed, which exhibits a rapid and sensitive color and a fluorescence off–on response to benzoyl peroxide (BPO) in aqueous media containing 10% ethanol *via* deboronation. The probe has been applied to the simple detection of BPO in real samples such as wheat flour and antimicrobial agent.

Reactive oxygen species (ROS) have attracted much attention due to their key role in human health and disease.[1] Benzoyl peroxide (BPO), a strong oxidizer, is a ROS.[2] Unlike most of the other ROS, BPO has been extensively employed in our daily life, as flour bleaching agents,[3] antimicrobial agents to treat acne,[4] and as polymerization initiators in various industrial fields.[5] The wide use of BPO is receiving increasing concern because of its potential risks. According to the International Agency for Research on Cancer, BPO is not classified as a carcinogen, but the existing studies have showed that it may be a potential tumor promoter and can exert genotoxic effects on human peripheral lymphocytes *in vitro*.[6] Moreover, BPO can be decomposed into benzoic acid and other deleterious substances, like biphenyl and phenylbenzoate,[7] which may further evoke tissue damage and diseases. Consequently, the maximum usage level of BPO as either a flour additive or an antimicrobial agent is strictly controlled by safety regulations of the respective country. For instance, in 2009 Codex Alimentarius Commission set up a usage standard of BPO (<75 mg kg^{-1}) in wheat flour;[8] European Union and China even prohibited BPO to be used in wheat flour. Hence, the development of simple and convenient methods for the BPO assay is of great significance for the safety of food and pharmaceutical products.

Several methods have been proposed for detecting BPO, such as electrochemistry,[9] chemiluminescence,[10] UV-spectrophotometry,[11] HPLC,[12] and HPLC-MS.[7] However, most of these methods require a time-consuming sample pre-treatment and separation, and thus are inconvenient for the fast detection of BPO. To overcome this problem, a common but feasible strategy perhaps is to develop excellent spectroscopic probes and utilize their rapid and sensitive spectroscopic responses to BPO to accomplish the detection. Unfortunately, to the best of our knowledge, no fluorescent probes for BPO have been reported so far, with the exception of indirect fluorescence determination.[13] Herein we present the design, synthesis, and characterization of **1** (Scheme 1) as a new fluorescent probe for the BPO assay.



Scheme 1 Synthesis of **1** and its possible reaction with BPO.

In the construction of our probe, the key issue that we confront is the choice of an appropriate signaling unit and recognition unit so that a latent fluorescent probe with low fluorescence background could be prepared to afford high sensitivity.[14] Here we choose resorufin as a signaling unit because of its good water-solubility and easy fluorescence quenching *via* 7-hydroxy substitution.[15] On the other hand, Chang and co-workers have reported that H_2O_2 can efficiently and specifically trigger the deboronation of the probes bearing arylboronate.[16] We envision that BPO, having a similar property to H_2O_2, may also induce such a deboronation. Therefore, arylboronate is used as the recognition unit for BPO. As a result, the new probe **1** (Scheme 1) was synthesized in good yield (60%) by treating resorufin sodium salt with 2-bromomethylphenylboronic acid pinacol ester.

The spectroscopic response of **1** to BPO at varied concentrations was investigated in 20 mM pH 7.4 KH_2PO_4–Na_2HPO_4 buffer containing 10% (v/v) of ethanol (referred to the phosphate buffer). As shown in Fig. 1A, **1** displays a moderate absorption peak at 484 nm with a shoulder at around 400 nm, but nearly no absorption at 574 nm. Upon reaction with BPO, a strong absorption band centered at 574 nm is developed, and the absorbance at 574 nm is gradually increased with the increment of the BPO concentration (Fig. S1, ESI†), concomitant with a distinct color change from nearly colorless to pink (see the inset of Fig. 1A),

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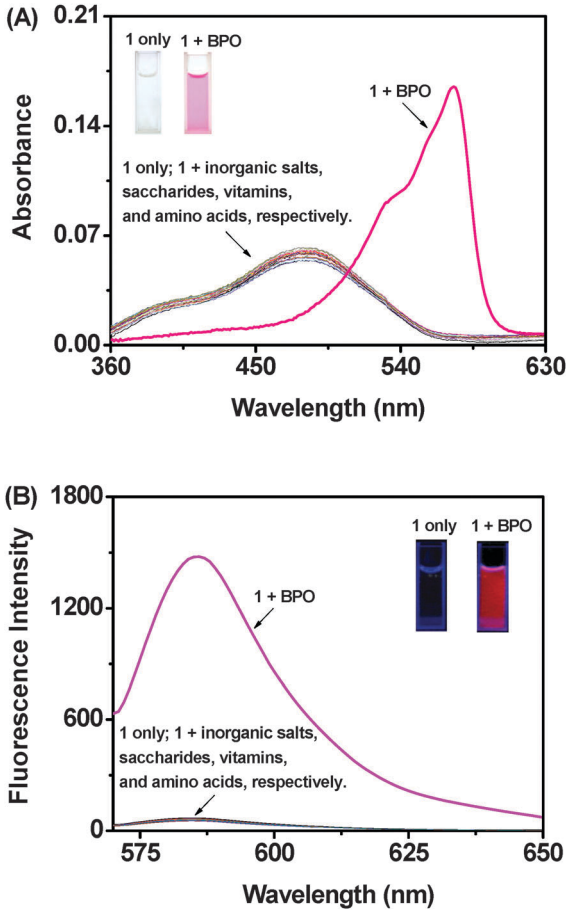

Fig. 1 (A) UV-vis and (B) fluorescence emission spectra (λ_{ex} = 550 nm) of **1** (3 μM) only and its reaction solutions with various species: 30 μM of BPO, $MgCl_2$, $CaCl_2$, $ZnSO_4$, $CuCl_2$, $Pb(AcO)_2$, $FeCl_2$, $FeCl_3$, $CrCl_3$, $CoCl_2$, $NiCl_2$, and $CdCl_2$; 1.5 mM of vitamin B_1, vitamin B_6, vitamin C, glucose, fructose, maltose, arginine, serine, glycine, NaF, NaBr, and NaI; 15 mM of NaCl and KNO_3. The insets in (A) and (B) depict the color and fluorescence change of the reaction systems in the absence and presence of BPO, respectively. The reactions were carried out for 15 min at room temperature in the phosphate buffer.

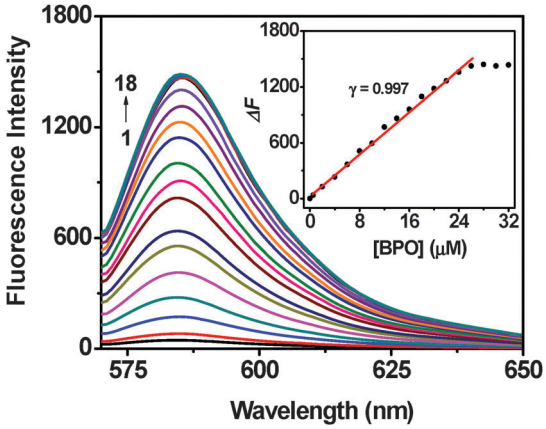

Fig. 2 Fluorescence emission spectra (λ_{ex} = 550 nm) of **1** (3 μM) with varied concentrations of BPO (0, 0.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32 μM BPO for curves 1–18, respectively). The inset depicts the plot of fluorescence increase value (ΔF) of the reaction system at $\lambda_{ex/em}$ = 550/585 nm against the corresponding reagent blank (without BPO). The reactions were carried out for 15 min at room temperature in the phosphate buffer.

which may be useful for the simple detection of BPO by the naked-eye.

The fluorescence change of **1** upon reacting with BPO is shown in Fig. 1B, which reveals that **1** itself has almost no emission at 585 nm due to the alkylation of the 7-hydroxy group of resorufin. This low background signal is extremely desirable for sensitive detection. Upon gradual introduction of BPO, an increase in fluorescence emission appears at 585 nm (Fig. 2), which resembles the fluorescence feature of resorufin, suggesting that the free fluorophore may be released. In addition, **1** is rather stable, because no considerable change in the fluorescence spectrum was observed after the probe's solution was stored at room temperature for two months.

The effect of pH of the reaction media varying from 4.8 to 8.3 was studied, revealing that pH 7.4 can be used for the present system (Fig. S2, ESI†). Because the water-insoluble BPO in different samples is usually extracted with an organic solvent such as ethanol,[10,11b,12c] the effect of ethanol unavoidably introduced into the detection system needs to be examined. The result showed that no noticeable fluorescence was produced when the ethanol volume fraction was too low (<0.2%), and 10% (v/v) of ethanol as a cosolvent may be used in this system (Fig. S3, ESI†). Time course studies indicated that the reaction of **1** with BPO at room temperature was fast, and the fluorescence increase could reach a plateau in 10 min (Fig. S4, ESI†). For the purpose of reproducibility, a reaction time of 15 min was employed in our experiments. Interestingly, under the same conditions H_2O_2 produces a slower and weaker fluorescence response than BPO (Fig. S4, ESI†), which may be ascribed to the presence of ethanol promoting the solubility and thereby the reactivity of BPO.

Under the conditions optimized above, the fluorescence increase value (ΔF) of **1** is directly proportional to the BPO concentration in the range of 0.5 to 26 μM (inset of Fig. 2), which gives a linear equation of $\Delta F = 39.1 + 56.2 \times$ [BPO] (μM) ($\gamma = 0.997$). The limit of detection[17] is 23 nM BPO, also showing a high sensitivity.

To assess the specificity of the reaction, the fluorescence response of **1** to various possible coexisting substances was examined in parallel under the same conditions. The tested substances included inorganic salts, vitamins, saccharides, amino acids and especially other important oxidants such as H_2O_2, $NaClO_4$, $NaClO_3$, NaClO, $NaNO_2$, $KBrO_3$, KIO_3, $KMnO_4$, $K_2Cr_2O_7$, KO_2, *tert*-butyl hydroperoxide, $FeCl_2$/H_2O_2, and $CuSO_4$/H_2O_2/ascorbic acid. As shown in Fig. 1, only BPO can produce a maximum absorption at 574 nm and a large fluorescence enhancement at 585 nm, whereas the other common non-oxidants do not show this behavior. More interestingly, compared to BPO, the other oxidants tested produce a much weaker fluorescence except H_2O_2 (Fig. S5, ESI†). Fortunately, BPO is often employed as a single additive. This implies that **1** possesses high selectivity for BPO over the other common substances. Besides, under the irradiation of a UV Hg lamp (360 nm), a distinct fluorescence color change of the reaction system in the absence and presence of BPO can also be produced (inset of Fig. 1B), which is convenient for the rapid detection of BPO.

To explore the spectroscopic response mechanism, the reaction products of **1** with BPO were subjected to ESI-MS and HPLC analyses. The ESI-MS spectrum of the reaction solution of **1** with BPO (Fig. S6, ESI†) shows a peak at m/z = 211.9 ($[M - Na]^-$), which is identical to that of resorufin. On the other hand, resorufin is further verified as a major final product by HPLC analysis. As shown in Fig. S7 (ESI†), the chromatographic peaks of resorufin, BPO, and **1** are located at 4.20 min (peak a in curve A), 16.51 min (peak b in curve B), and 19.60 min (peak c in curve C), respectively. After reaction with BPO, the chromatographic peak of **1** decreases markedly, and several new peaks appear (curve D), among which the major one at 4.20 min clearly indicates the generation of resorufin. Based on these data and the existing observation,[16] we propose that the reaction of **1** with BPO may proceed through the route depicted in Scheme 1: BPO oxidizes the moiety of phenylboronic acid pinacol ester in **1**, forming an unstable intermediate, which is followed by hydrolysis and 1,4-elimination of *o*-quinone-methide to release the resorufin.

To evaluate the practical applicability of **1** for real samples such as wheat flour and antimicrobial agent, the possible interferences of main coexisting species were studied on the detection of BPO. The tolerable concentration was determined by the criterion at which a species gave a relative error of no more than 5% in recovery of 15 μM of BPO. The results (Table S1, ESI†) show that the coexisting species hardly interfere with the BPO assay. Then, real wheat flour and antimicrobial agent samples containing BPO were prepared (see ESI†), and the determination of BPO content in these samples was demonstrated. The results show that the determination can be completed in about 20 min due to not requiring a time-consuming separation, and satisfactory recovery of BPO even with a low content (50 mg kg^{-1}) can be obtained (Table S2, ESI†). This indicates that **1** is suitable for the rapid and sensitive analysis of BPO.

In conclusion, we have developed a new resorufin-based probe for BPO assay. The probe displays a rapid and sensitive color and a fluorescence off–on response to BPO. Moreover, the response of the probe is highly selective for BPO over other common substances, which makes it of great potential use in simple and quantitative detection of BPO in some real samples such as wheat flour and antimicrobial agent.

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Notes and references

1 (*a*) X. H. Li, G. X. Zhang, H. M. Ma, D. Q. Zhang, J. Li and D. B. Zhu, *J. Am. Chem. Soc.*, 2004, **126**, 11543–11548; (*b*) E. R. Stadtman, *Free Radical Res.*, 2006, **40**, 1250–1258; (*c*) E. W. Miller and C. J. Chang, *Curr. Opin. Chem. Biol.*, 2007, **11**, 620–625; (*d*) X. Q. Chen, X. Z. Tian, I. Shin and J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 4783–4804; (*e*) B. C. Dickinson and C. J. Chang, *Nat. Chem. Biol.*, 2011, **7**, 504–511; (*f*) X. Q. Chen, K. A. Lee, E. M. Ha, K. M. Lee, Y. Y. Seo, H. K. Choi, H. N. Kim, M. J. Kim, C. S. Cho, S. Y. Lee, W. J. Lee and J. Yoon, *Chem. Commun.*, 2011, **47**, 4373–4375.

2 (*a*) S. H. Ibbotson, M. N. Moran, J. F. Nash and I. E. Kochevar, *J. Invest. Dermatol.*, 1999, **112**, 933–938; (*b*) S. Fujisawa and Y. Kadoma, *Chemosphere*, 2006, **62**, 71–79.

3 B. P. Lamsal and J. M. Faubion, *LWT–Food Sci. Technol.*, 2009, **42**, 1461–1467.

4 S. R. Feldman, J. Tan, Y. Poulin, T. Dirschka, N. Kerrouche and V. Manna, *J. Am. Acad. Dermatol.*, 2011, **64**, 1085–1091.

5 K. Matyjaszewski and J. H. Xia, *Chem. Rev.*, 2001, **101**, 2921–2990.

6 (*a*) G. Bhasin, H. Kauser and M. Athar, *Arch. Toxicol.*, 2004, **78**, 139–146; (*b*) A. Yavuz, M. Topaktaş and E. S. İstifli, *Turk. J. Biol.*, 2010, **34**, 15–24.

7 Y. Abe-Onishi, C. Yomota, N. Sugimoto, H. Kubota and K. Tanamoto, *J. Chromatogr., A*, 2004, **1040**, 209–214.

8 X. J. Jia, Y. Wu and P. Liu, *Environ. Toxicol. Pharmacol.*, 2011, **31**, 479–484.

9 (*a*) C. Y. Wang and X. Y. Hu, *Anal. Lett.*, 2005, **38**, 2175–2187; (*b*) J. V. B. Kozan, R. P. Silva, S. H. P. Serrano, A. W. O. Lima and L. Angnes, *Biosens. Bioelectron.*, 2010, **25**, 1143–1148; (*c*) M. D. P. T. Sotomayor, I. L. T. Dias, G. O. Neto and L. T. Kubota, *Anal. Chim. Acta*, 2003, **494**, 199–205.

10 (*a*) W. P. Yang, Z. J. Zhang and X. Hun, *Talanta*, 2004, **62**, 661–666; (*b*) W. Liu, Z. J. Zhang and L. Yang, *Food Chem.*, 2006, **95**, 693–698.

11 (*a*) I. Mori, H. Tominaga, Y. Fujita and T. Matsuo, *Anal. Lett.*, 1997, **30**, 2433–2439; (*b*) H. Xie, H. Y. Wang, L. Y. Ma and Y. Xiao, *Spectrochim. Acta, Part A*, 2005, **62**, 197–202; (*c*) A. Gupta, M. Gulati and N. K. Pandey, *Rasayan J. Chem.*, 2009, **2**, 649–654.

12 (*a*) A. I. Saiz, G. D. Manrique and R. Fritz, *J. Agric. Food Chem.*, 2001, **49**, 98–102; (*b*) P. Dehouck, E. V. Looy, E. Haghedooren, K. Deckers, Y. V. Heyden, E. Adams, E. Roets and J. Hoogmartens, *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.*, 2003, **794**, 293–302; (*c*) M. Wada, K. Inoue, A. Ihara, N. Kishikawa, K. Nakashima and N. Kuroda, *J. Chromatogr., A*, 2003, **987**, 189–195; (*d*) Q. H. Wang, W. Z. Shi and C. Y. Hou, *J. Food Process. Preserv.*, 2010, **34**, 414–424.

13 W. Chen, W. Shi, Z. Li, H. M. Ma, Y. Liu, J. H. Zhang and Q. J. Liu, *Anal. Chim. Acta*, 2011, **708**, 84–88.

14 (*a*) X. Q. Chen, M. Sun and H. M. Ma, *Curr. Org. Chem.*, 2006, **10**, 477–489; (*b*) J. X. Lu, C. D. Sun, W. Chen, H. M. Ma, W. Shi and X. H. Li, *Talanta*, 2011, **83**, 1050–1056; (*c*) A. Romieu, D. Tavernier-Lohr, S. Pellet-Rostaing, M. Lemaire and P.-Y. Renard, *Tetrahedron Lett.*, 2010, **51**, 3304–3308.

15 (*a*) M. Sun, D. H. Shangguan, H. M. Ma, L. H. Nie, X. H. Li, S. X. Xiong, G. Q. Liu and W. Thiemann, *Biopolymers*, 2003, **72**, 413–420; (*b*) W. Gao, B. Xing, R. Y. Tsien and J. Rao, *J. Am. Chem. Soc.*, 2003, **125**, 11146–11147; (*c*) S. Y. Kim and J. I. Hong, *Org. Lett.*, 2007, **9**, 3109–3112; (*d*) M. G. Choi, S. Cha, J. E. Park, H. Lee, H. L. Jeon and S. K. Chang, *Org. Lett.*, 2010, **12**, 1468–1471; (*e*) M. G. Choi, J. Hwang, S. Eor and S. K. Chang, *Org. Lett.*, 2010, **12**, 5624–5627.

16 (*a*) M. C. Y. Chang, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2004, **126**, 15392–15393; (*b*) E. W. Miller, A. E. Albers, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2005, **127**, 16652–16659; (*c*) E. W. Miller, O. Tulyathan, E. Y. Isacoff and C. J. Chang, *Nat. Chem. Biol.*, 2007, **3**, 263–267; (*d*) G. C. van de Bittner, E. A. Dubikovskaya, C. R. Bertozzi and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 21316–21321; (*e*) C. Chung, D. Srikun, C. S. Lim, C. J. Chang and B. R. Cho, *Chem. Commun.*, 2011, **47**, 9618–9620; (*f*) A. R. Lippert, G. C. Van de Bittner and C. J. Chang, *Acc. Chem. Res.*, 2011, **44**, 793–804.

17 (*a*) D. Feng, Y. Y. Zhang, T. T. Feng, W. Shi, X. H. Li and H. M. Ma, *Chem. Commun.*, 2011, **47**, 10680–10682; (*b*) C. D. Sun, W. Shi, Y. C. Song, W. Chen and H. M. Ma, *Chem. Commun.*, 2011, **47**, 8638–8640.