Electronic Supplementary Information for:

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

Wei Chen, Zhao Li, Wen Shi and Huimin Ma*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: mahm@iccas.ac.cn

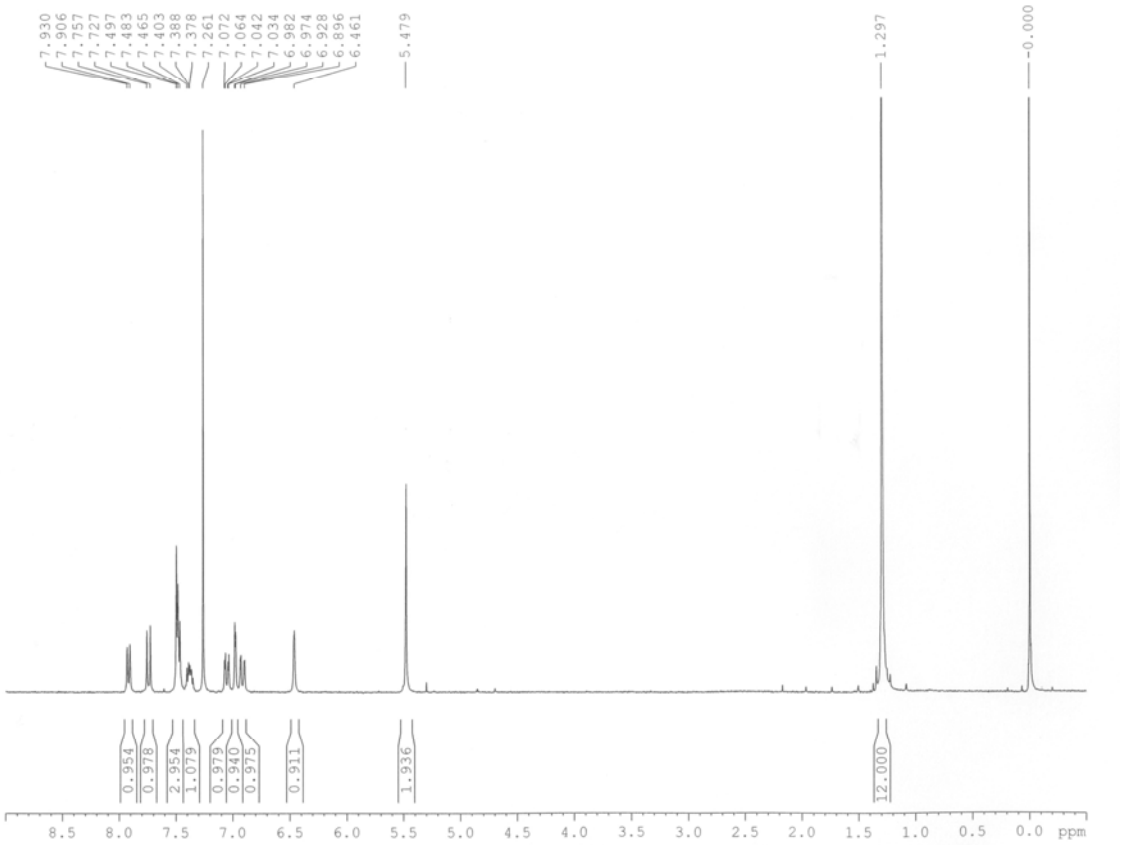
Reagents and apparatus

Resorufin sodium salt and benzoyl peroxide were obtained from Sigma-Aldrich, and Alfa Aesar, respectively. 2-Bromomethylphenylboronic acid pinacol ester was prepared following the reported procedure (Scrafton, et al., *J. Org. Chem.* **2008**, *73*, 2871–2874). Non-additive wheat flours and antimicrobial agent were obtained from local markets in China. Other chemicals used in this work were commercial products of analytical grade. Stock solution (1 mM) of **1** was prepared in acetonitrile. Stock solution (100 μg mL^{-1}) of BPO was prepared in ethanol, and stored in a refrigerator at 4 ^{o}C. Stock solutions of other substances were prepared in water. Deionized distilled water was used throughout.

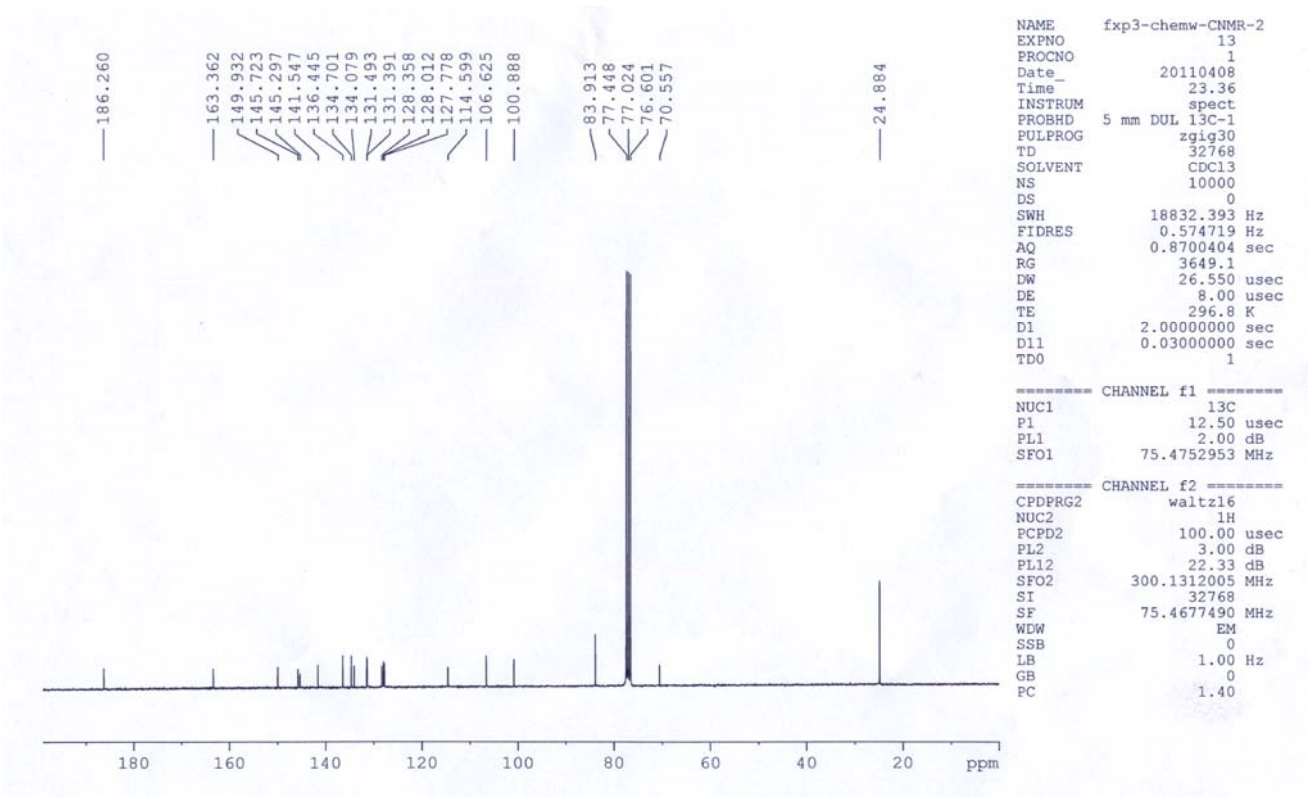
^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV-300 spectrometers with chemical shifts reported as ppm (in $CDCl_3$, TMS as internal standard). Electrospray ionization mass spectra (ESI-MS) were measured on an LC-MS 2010A instrument (Shimadzu, Kyoto, Japan). Fluorescence measurements were made on a Hitachi F-2500 spectrofluorimeter (Hitachi Ltd., Tokyo, Japan). Elemental analyses were carried out with a Flash EA 1112 instrument (Thermo Electron, Milan, Italy). High-performance liquid chromatography (HPLC) analyses were carried out with LC-20AT solvent delivery unit, SPD-20A UV-vis detector (Shimadzu, Japan) and Inertsil ODS-SP column (5 μm, 4.6 mm × 250 mm, GL Sciences Inc.).

Synthesis of 1

To a solution of resorufin sodium salt (235 mg, 1 mmol) in DMF (10 mL) were added 2-Bromomethylphenylboronic acid pinacol ester (356.4 mg, 1.2 mmol) and K_2CO_3 (207.3 mg, 1.5 mmol). The reaction mixture was stirred at room temperature overnight, followed by evaporation under reduced pressure to give a violet-red residue. The residue was purified by silica-gel column chromatography with hexane/ethyl acetate (2:1, v/v) as the eluent, affording **1** as a saffron power (257.6 mg, yield 60%). The ^{1}H NMR and ^{13}C NMR spectra of **1** in $CDCl_3$ are given below. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.92 (d, 1H, J = 7.3 Hz), 7.74 (d, 1H, J = 8.9 Hz), 7.48 (m, 3H), 7.39 (m, 1H), 7.05 (m, 1H), 6.98 (d, 1H, J = 2.5 Hz), 6.91 (d, 1H, J = 9.8 Hz), 6.46 (s, 1H), 5.48 (s, 2H), 1.29 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 186.3, 163.4, 149.9, 145.7, 145.3, 141.5, 136.4, 134.7, 134.1, 131.5, 131.4, 128.4, 128.0, 127.8, 114.6, 106.6, 100.9, 83.9, 70.6, 24.9. ESI-MS: *m/z* 430.4 $[M+H]^+$, 452.3 $[M+Na]^+$. Elemental analysis, calcd. for $C_{25}H_{24}BNO_5$ (%): C 69.95, H 5.64, N 3.26; found: C 69.21, H 5.64, N 3.35.



^{1}H NMR spectrum of 1 in $CDCl_3$



^{13}C NMR spectrum of 1 in $CDCl_3$

General procedure for BPO detection

Unless otherwise noted, all the measurements were made in 20 mM pH 7.4 KH_2PO_4-Na_2HPO_4 buffer containing 10% (v/v) of ethanol (referred to the phosphate buffer) according to the following procedure. In a test tube, 2.5 mL of 24 mM pH 7.4 KH_2PO_4-Na_2HPO_4 buffer and 9 μL of the stock solution of **1** were mixed, followed by addition of a requisite volume of BPO sample solution. The final volume of the reaction solution was adjusted to 3 mL with an appropriate volume of ethanol or water, letting the concentration of ethanol to be 10% (v/v). After mixing and then standing for 15 min at room temperature, a 3-mL portion of the reaction solution was transferred into a 1-cm quartz cell to measure absorbance and fluorescence with $\lambda_{ex/em}$ = 550/585 nm and both excitation and emission slit widths of 10 nm. In the meantime, a blank solution containing no BPO was prepared and measured under the same conditions for comparison.

Sample preparation

The samples were prepared by mixing wheat flour (1 g) or gel-like antimicrobial agent (1 g)

with ethanol (5 mL) containing appropriate amounts of BPO in a tube. The sample was then sonicated for 2 min. After standing for 1 min, the sample was filtered through a disk filter (pore size: 0.45 μm), and 0.3 mL of the filtrate was subjected to the fluorescence analysis following the general procedure given above.

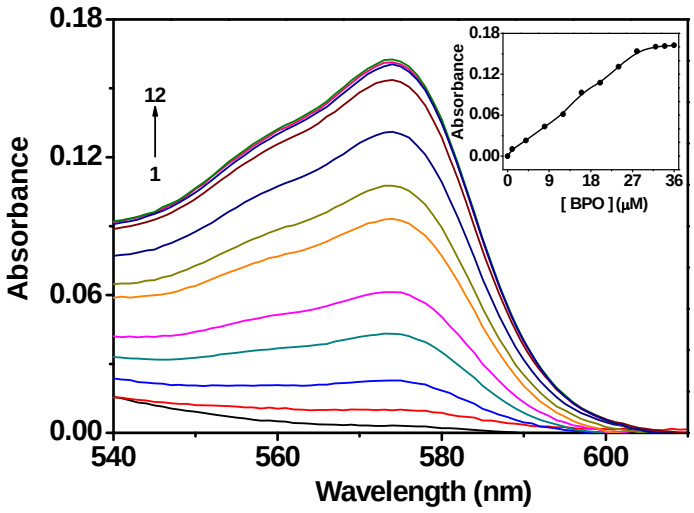


Fig. S1. UV–vis spectra of **1** (3 μM) reacting with varied concentrations of BPO (0, 1, 4, 8, 12, 16, 20, 24, 28, 32, 34, 36 μM of BPO for curves 1-12, respectively). The reactions were carried out for 15 min at room temperature in 20 mM pH 7.4 phosphate buffer.

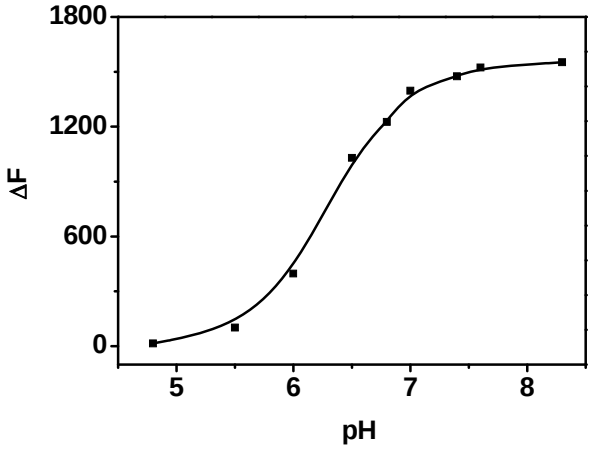


Fig. S2. Effect of pH on the fluorescence increase value (Δ*F*) of **1** (3 μM) with BPO (30 μM). The reactions were carried out for 15 min at room temperature in the phosphate buffer with different pH values. $\Delta F = F - F_0$, where F_0 and F are the fluorescence intensity before and after the addition of BPO, respectively.

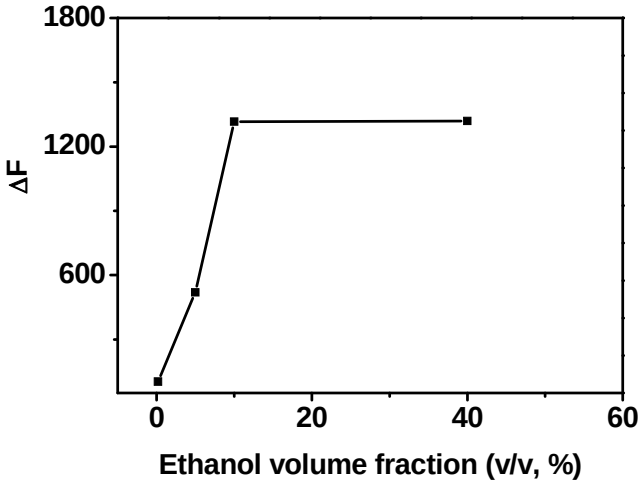


Fig. S3. Effect of ethanol volume fraction in pH 7.4 phosphate buffer on the fluorescence increase value (ΔF) of **1** (3 μM) with BPO (30 μM). The reactions were carried out for 15 min at room temperature in the phosphate buffer (a higher concentration of ethanol may lead to the precipitation of the phosphate buffer).

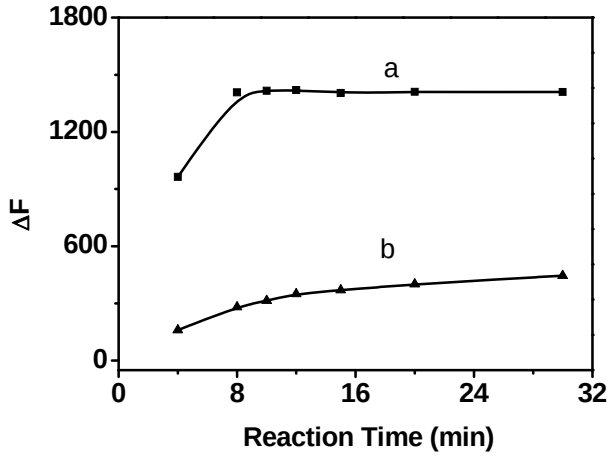


Fig. S4. Effect of reaction time on the fluorescence increase value (ΔF) of **1** (3 μM) reacting with 30 μM of BPO (curve a) and H_2O_2 (curve b). The reactions were carried out at room temperature in 20 mM pH 7.4 phosphate buffer.

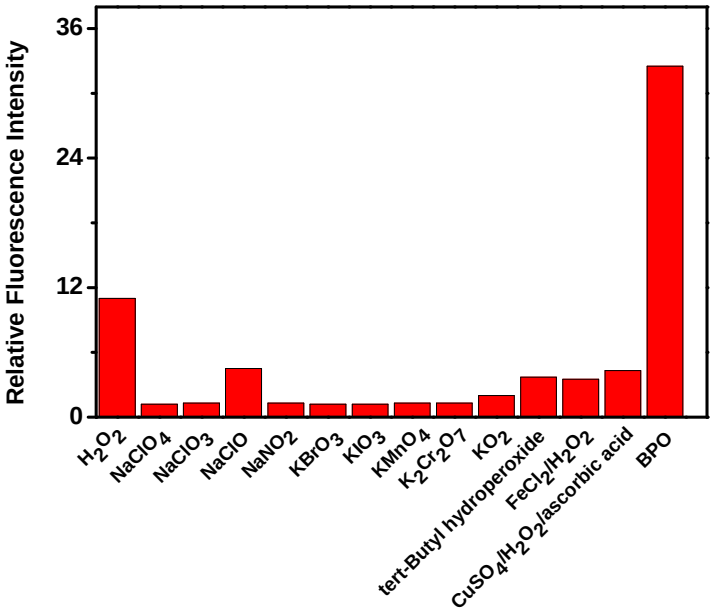


Fig. S5. The relative fluorescence intensity (F/F_0) of **1** (3 μM) reacting with different oxidants (30 μM). The reactions were carried out for 15 min at room temperature in 20 mM pH 7.4 phosphate buffer.

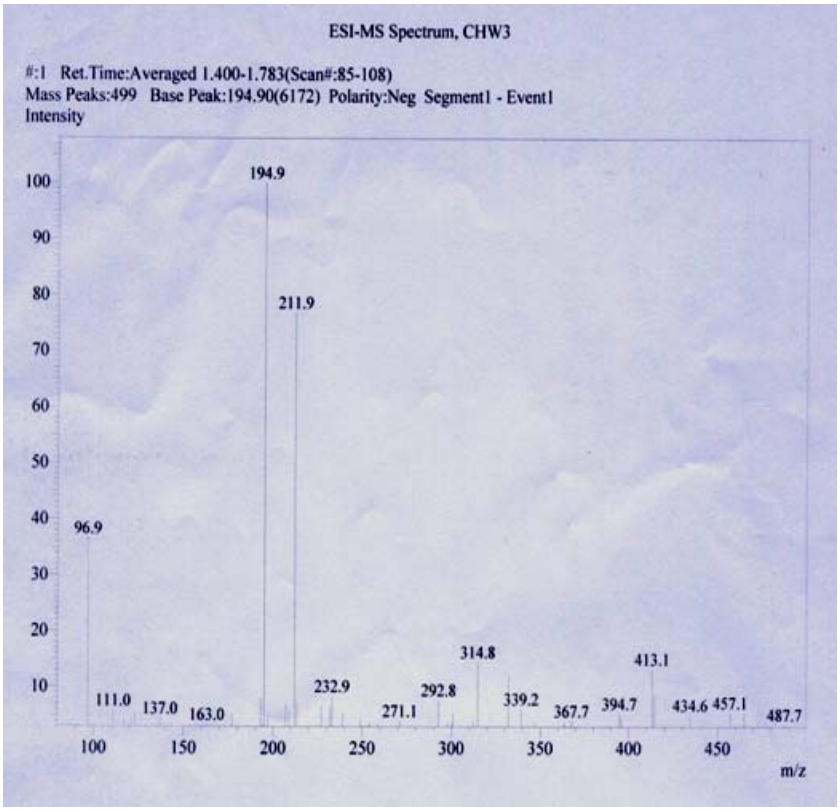


Fig. S6. ESI-MS spectrum of the reaction solution of **1** (100 μM) with BPO (200 μM).

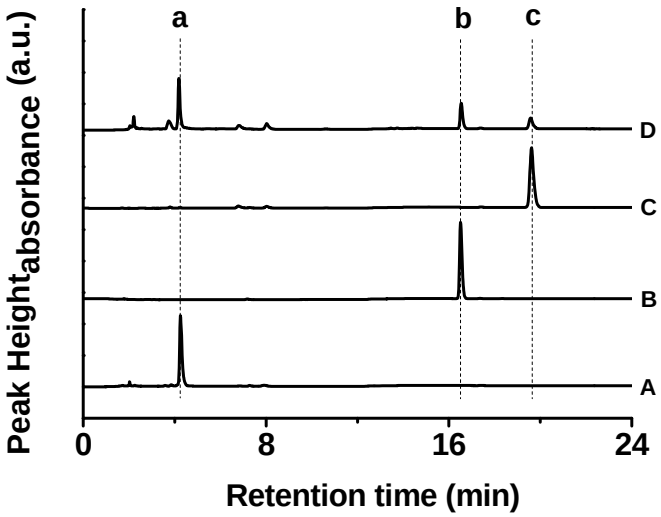


Fig. S7. HPLC chromatograms of different reaction systems in the phosphate buffer. (A) 100 μM resorufin; (B) 200 μM BPO; (C) 100 μM **1**; (D) 100 μM **1** + 200 μM BPO. The assignment of the peaks: (a) 4.20 min, resorufin; (b) 16.51 min, BPO; (c) 19.60 min, probe **1**.

Table S1. Recovery of BPO (15 μM) in the presence of various coexisting species

Species	Concentration (μM)	Molar ratio of the added species to BPO	Recovery (%)
NaCl	15000	1000	97.6
KNO_3	15000	1000	98.3
$CaCl_2$ [a]	30	2	96.5
$MgCl_2$ [a]	30	2	97.5
$NaClO_3$	1500	100	102.8
$KBrO_3$	1500	100	96.6
KIO_3	1500	100	95.0
$NaClO_4$	1500	100	99.8
$NaNO_2$	1500	100	95.7
NaF	1500	100	96.8
NaBr	1500	100	101
NaI	1500	100	100.7
$ZnSO_4$ [a]	30	2	96.4
$CuCl_2$ [a]	30	2	99.9
$Pb(AcO)_2$ [a]	30	2	97.2
$FeCl_2$ [a]	30	2	101.1
$FeCl_3$ [a]	30	2	98.9
$CrCl_3$ [a]	30	2	100.9
$CoCl_2$ [a]	30	2	101
$NiCl_2$ [a]	30	2	97.3
$CdCl_2$ [a]	30	2	97
Glucose	1500	100	99.6
Fructose	1500	100	97.6
Maltose	1500	100	103.6
Arginine	1500	100	97.5
Serine	1500	100	102.2
Glycine	1500	100	103.4
Vitamin B_1	1500	100	96.2
Vitamin B_6	1500	100	99.9
Vitamin C	1500	100	101.7
Carbopol	7500	500	97.4

[a] Higher concentrations of these species led to the precipitation of metal salts.

Table S2. Determination of BPO content in wheat flour and antimicrobial agent

Sample	BPO added ($mg\ kg^{-1}$)	BPO found by proposed method[a] ($mg\ kg^{-1}$)	Recovery (%)
Wheat flour A	0	< 0.28 (detection limit) [b]	–
Wheat flour B	50	51.4 ± 1.0	102.8 ± 1.9
Wheat flour C	100	103.0 ± 1.7	103.0 ± 1.7
Wheat flour D	200	197.1 ± 7.6	98.7 ± 3.7
Antimicrobial agent A	0	< 0.28 (detection limit) [b]	–
Antimicrobial agent B	50	49.8 ± 2.2	99.4 ± 4.3
Antimicrobial agent C	100	98.4 ± 2.3	98.4 ± 2.3
Antimicrobial agent D	200	200.4 ± 3.1	100.2 ± 1.5

[a] Mean of three determinations ± standard deviation.

[b] In this method the detection limit of 23 nM BPO corresponds to 0.28 $mg\ kg^{-1}$.