SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ENVIRONMENTAL SCIENCES
BIOLOGICAL TECHNIQUES

Received
16 July 2013

Accepted
16 September 2013

Published
3 October 2013

Correspondence and requests for materials should be addressed to H.M.M. (mahm@iccas.ac.cn)

Sensitive detection of ozone by a practical resorufin-based spectroscopic probe with extremely low background signal

Yangyang Zhang, Wen Shi, Xiaohua Li & 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.

Ozone (O_3) has attracted much attention because of its key role in human health and disease, and its detection is of great importance for various biochemical studies as well as environmental evaluation. Here we develop a simple and practical spectroscopic off-on O_3 probe based on resorufin and the specific reaction of but-3-enyl with O_3. The probe shows an extremely low background spectroscopic signal, but reacts with O_3 producing a distinct color and fluorescence change. The detection limit of the probe for O_3 is 5.9 nM, which corresponds to an ozone concentration of 0.056 mg m^{-3} in air in this study and is lower than the international ambient air quality standard of 0.1 mg m^{-3}. More importantly, the proposed probe is worth popularizing, and its applicability has been successfully demonstrated on both the determination of O_3 in real ambient air samples and the imaging of O_3 in biological cells.

Ozone (O_3), one of reactive oxygen species (ROS), has attracted much attention because of its key role in human health and disease[1–5]. Recently, O_3 exposure has become a growing global health problem, especially in urban areas[6,7]. While O_3 in the stratosphere protects the earth from harmful ultraviolet light, tropospheric or ground-level O_3 is toxic and can damage the respiratory tract. Current evidence has demonstrated that ozone may be produced endogenously in inflammation and antibacterial responses of the immune system[8–10]. Moreover, cholesterol ozonolysis products are found to exist in clinical brain samples and likely trigger misfolding of protein in sporadic amyloid disease[11,12]. Thus, detection of O_3 would be helpful to various biochemical studies as well as environmental evaluation. Toward this end, several fluorescent probes for O_3 assay have been developed[5,13,14], among which indigo carmine is a classical one, but it can react with a number of ROS, suffering from poor selectivity[7]. Moreover, some of these probes employ cyanine skeletons that are known to have poor stability and high background fluorescence due to ready autoxidation and photooxidation[15]; the others have a relatively short analytical wavelength or require organic solvents as cosolvents, which may disturb the normal function of biomolecules and are unfavorable for biological studies[16]. On the other hand, chemiluminescent probes are also proposed to measure O_3[17–19]. However, false-positive ozone readings are often reported because of poor selectivity of chemiluminescence[18,19]. Therefore, spectroscopic probes with superior properties, such as high selectivity and sensitivity, and good stability and water solubility, are rather necessary for the detection of O_3 but are still rare to the best of our knowledge.

In this work, we have developed such a probe (**1**; Fig. 1) that has the above desired properties by incorporating the but-3-enyl group specific for O_3 into resorufin. We chose but-3-enyl as a recognition moiety because alkenes can be selectively cleaved by O_3 *via* ozonolysis[20–23], and resorufin as a fluorochrome due to its good stability and water-solubility as well as long analytical wavelength. Most notably, the fluorescence of resorufin is easily quenched *via* 7-hydroxy substitution[24–26]. Therefore, the designed **1**, in which its 7-hydroxy is substituted by but-3-enyl, is anticipated to show no or weak color and fluorescence; upon reaction with O_3, the resulting aldehyde (**2**) may undergo β-elimination[20–23] to yield the fluorescent resorufin (Fig. 1). As a result, the fluorescence of the reaction system could be turned on, which may provide the basis for the sensitive detection of O_3.

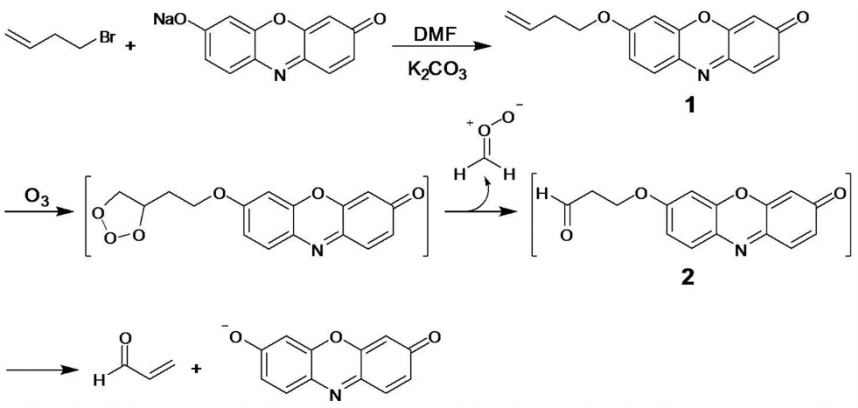

Figure 1 | Preparation of probe 1 and its reaction with O_3.

Results

Spectroscopic response of 1 to O_3. The spectroscopic response of **1** to O_3 was studied. As shown in Fig. 2A, probe **1** displays a moderate absorption peak at 484 nm with a shoulder at around 400 nm, but nearly no absorption at 574 nm. However, reaction of **1** with O_3 gives a strong absorption band centered at 574 nm, concomitant with a distinct color change from nearly colorless to pink (see the inset of Fig. 2A), which may be useful for the simple detection of O_3 by the naked eye. Fig. 2B depicts the fluorescence change of **1**. As is seen, **1** itself shows a rather weak fluorescence around 585 nm, with a quantum yield of $\Phi \approx 0.003$ [27], which is ascribed to the alkylation of the 7-hydroxy group of resorufin. This extremely low background signal is desirable for sensitive detection. However, addition of O_3 leads to a 207-fold fluorescence enhancement, accompanied by a large fluorescence color change (see the inset of Fig. 2B). This fluorescence change is attributed to the release of the free resorufin (*vide infra*).

Optimization of experimental conditions. Reaction conditions (pH, buffer concentration and time) were examined in detail. The results showed that the most sensitive spectroscopic response of the probe to O_3 can be achieved in the ranges of pH 7–10, 30–200 mM of the phosphate buffer concentration, and 30–60 min of the reaction time (Supplementary Figs. S1–S3). As a result, a reaction medium of 50 mM phosphate buffer (pH 7.4) and a reaction time of 40 min at room temperature were chosen for the present system.

Analytical characteristics. Under the optimal conditions, **1** shows a gradual increase in the fluorescence intensity with the increase of O_3 concentration (Fig. 3), and a good linearity between the relative fluorescence intensity and the O_3 concentration was observed in the range of 1.0–30 μM O_3, with a linear equation of $\Delta I = 66.3 \times C\,(\mu M) + 22.6$ ($\gamma = 0.998$). The detection limit (3 S/m, in which S is the standard deviation of blank measurements, n = 11, and m is the slope of the linear equation) was determined to be 5.9 nM O_3, which corresponds to an ozone concentration of 0.056 mg m^{-3} in air in the present study (lower than the ambient air quality standard of 0.1 mg m^{-3} recommended by the World Health Organization). This detection limit is rather low, which is ascribed to the extremely low background signal of the probe.

Selectivity studies. To assess the specificity of the reaction, the fluorescence response of **1** to various commonly coexisting substances in both environmental and biological systems (in particular in air and cells) were examined in parallel under the same conditions. The examined substances included ROS, air pollutants emitted from motor vehicles (e.g., lead, palladium, platinum, nitric acid and sulfuric acid that are derived from nitrogen dioxide and sulfur dioxide)[28], and common cellular substances (e.g., $CaCl_2$, $MgCl_2$, glucose, vitamin C, amino acids, glutathione, and proteins)[29]. As shown in Fig. 4, only O_3 produces a large fluorescence enhancement, whereas the other substances do not show such a behavior. Moreover, the effects of these substances on the O_3 assay were also investigated (Supplementary Table S1), revealing that these substances at considerable concentrations hardly interfere with O_3 assay (error < 10%). These results indicate that **1** has a high selectivity for O_3. The possible reason for this may result from the fact that only O_3 can react with the terminal double bond of **1** to form a molozonide *via* a (3 + 2) cycloaddition[20–22], which undergoes retro (3 + 2) cycloaddition to yield aldehyde **2**, followed by β-elimination to release resorufin (Fig. 1).

Reaction mechanism. To prove the fluorescence response mechanism, the reaction products of **1** with O_3 were subjected to HPLC analysis. As shown in Supplementary Fig. S4, O_3, resorufin and the probe have a retention time of 2.59 min (curve B), 7.37 min

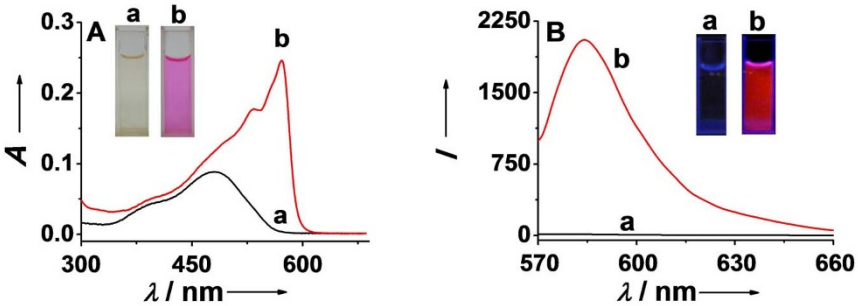

Figure 2 | Spectroscopic response of 1 to O_3. (A) Absorption spectra and (B) fluorescence emission spectra of 1 (10 μM) before (a) and after (b) reaction with O_3 (30 μM). The absorption spectra were measured with 50 mM phosphate buffer (pH 7.4) as a blank. The insets show the images of color and fluorescence changes of 1 before and after reaction with O_3.

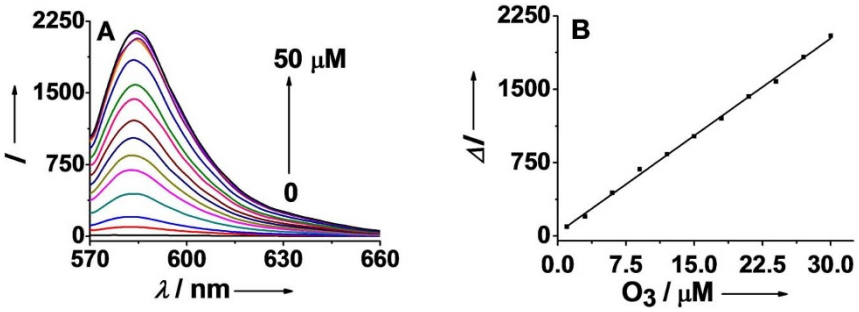

Figure 3 | Fluorescence response of 1 to O_3. (A) Fluorescence response of 1 (10 μM) to O_3 at varied concentrations (0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 40, 45 and 50 μM); $\lambda_{ex} = 550$ nm. (B) The plot of the relative fluorescence intensity (ΔI is the difference of fluorescence intensity of 1 in the presence and absence of O_3) against the O_3 concentration in the range of 1.0–30 μM. The measurements were performed in 50 mM phosphate buffer solution (pH 7.4) with $\lambda_{ex/em} = 550/585$ nm.

(curve C), and 17.07 min (curve D), respectively. Upon reaction with O_3 (curve E), the peak of the probe at 17.07 min decreases largely, concomitant with the appearance of a major peak at 7.37 min characteristic of resorufin. This clearly indicates that the reaction of **1** with O_3 generates free resorufin, thereby causing the generation of fluorescence (Fig. 1).

Detection of O_3 in ambient air samples. Our goals also included that probe **1** could be utilized to develop a simple method for convenient detection of O_3 in ambient air. To demonstrate this potential, we made the following experiments (Supplementary Fig. S5): the ambient air in a sealed box (40 cm × 30 cm × 30 cm) was irradiated by a UV-365 nm lamp for different periods of time (0–12 h), and then the air was sampled with a 50-mL syringe; after the air in the syringe was injected into a sealed test tube containing the phosphate buffer (pH 7.4) and probe **1**, followed by reaction for 40 min, the fluorescence of the reaction solution was measured (Fig. 5A). Note that the full absorption of O_3 from the O_3-containing air in the phosphate buffer of pH 7.4 requires 10 min at least (Supplementary Fig. S6); in our work 15 min was used to absorb O_3. For quantitative analysis of O_3 at lower levels, an additional calibration curve was constructed in the concentration range of 0.1–0.5 μM O_3 (Supplementary Fig. S7), and based on this curve the concentration of O_3 in the air irradiated under UV-365 nm lamp for 3–12 h (Fig. 5A) was determined to be 0.16–0.39 μM, corresponding to 1.5–3.7 mg m^{-3} (Table 1).

Detection of O_3 in ambient air from four different areas was further performed (see "Procedure for ozone assay in air" in Methods below). In brief, amber glass wide-mouth bottles containing water (typically 10 mL) were placed with the opening of the caps in different areas, naturally absorbing the ambient air for 8 h. The as-obtained samples were then subjected to fluorescence measurements according to the "General procedure for detection of O_3" in Methods below. The obtained results are shown in Fig. 5B. As is seen, the four places have different O_3 concentrations, among which the O_3 concentration in the air from the northwestern 4th ring road is the highest, which may arise from the heavy traffic in this area.

Validation of the method. To evaluate the accuracy of the proposed method, a comparative study on the determination of O_3 in both standard solutions and sample solutions prepared from the air of the northwestern 4th ring road of Beijing was made with iodometric titration. As shown in Table 2, the analytical results of standard solutions from the two methods are in good agreement with each other using a Student's *t*-test[30], clearly indicating the validity of our probe. Furthermore, the content of O_3 in the sample solutions (see Methods below for details) is not detectable by the iodometric titration due to its low sensitivity, but can be determined to be 0.21 μM with the present sensitive fluorescence method.

The recovery of O_3 added into the sample solutions was examined. As shown in Supplementary Table S2, the recovery of O_3 determined by the present method ranges from 97 to 110%. The above results indicate that **1** can be used as a sensitive and selective probe for O_3 assay in real ambient air samples.

Application of the probe to cell imaging. First, the potential toxicity of **1** to cells was evaluated by using a standard MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay. The results (Supplementary Fig. S8) showed that the cell viability was not significantly changed upon treatment even with 10 μM **1** at 37°C for 24 h, indicating the low cytotoxicity and good biocompatibility of the probe. Then, the applicability of **1** for cell imaging was investigated. As shown in Fig. 6, the **1**-loaded HeLa cells

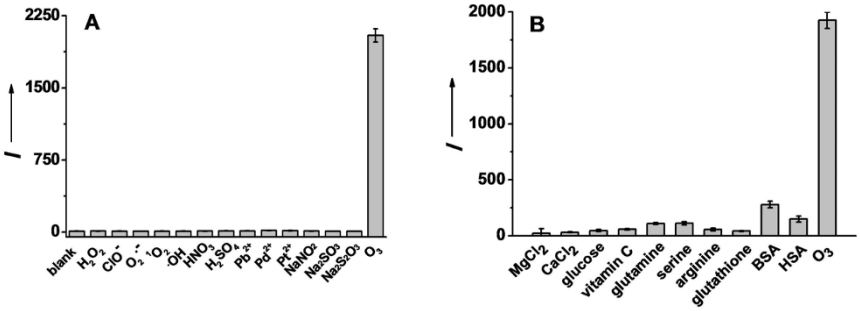

Figure 4 | Fluorescence responses of 1 (10 μM) to various substances. (A) ROS and air pollutants: blank (10 μM probe 1 only), H_2O_2 (100 μM), ClO^- (50 μM), $O_2\cdot^-$ (10 μM), $^1O_2$ (10 μM), ·OH (10 μM), HNO_3 (100 μM), H_2SO_4 (100 μM), Pb^{2+} (100 nM), Pd^{2+} (100 nM), Pt^{2+} (100 nM), $NaNO_2$ (250 μM), Na_2SO_3 (250 μM), $Na_2S_2O_3$ (250 μM) and O_3 (30 μM). (B) Common cellular substances: $MgCl_2$ (100 μM), $CaCl_2$ (100 μM), glucose (50 μM), vitamin C (10 μM), glutamine (50 μM), serine (50 μM), arginine (50 μM), glutathione (5 mM), bovine serum albumin (BSA, 100 μM), human serum albumin (HSA, 100 μM) and O_3 (30 μM). $\lambda_{ex/em} = 550/585$ nm. The results are the mean ± S.D. of three separate measurements.

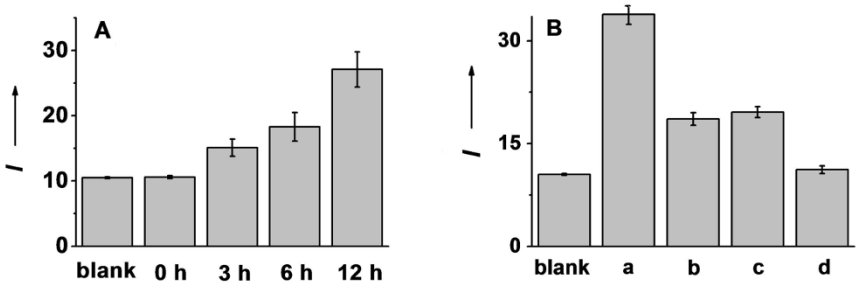

Figure 5 | Fluorescence intensity of 1 (10 μM) reacting with O_3 from air. (A) The air was irradiated by UV-365 nm lamp for different periods of time: 0 (control), 3, 6 and 12 h. (B) The air from four different places: (a) the northwestern 4th ring road of Beijing; (b) the garden of Institute of Chemistry, Chinese Academy of Sciences; (c) the outside of the laboratory window of Institute of Chemistry; (d) the inside of the laboratory window of Institute of Chemistry. Blank (another control) refers to the 50 mM phosphate buffer solution (pH 7.4) containing only probe 1. The measurements were made in 50 mM phosphate buffer solution of pH 7.4 with $\lambda_{ex/em}$ = 550/585 nm. The results are the mean ± S.D. of three separate measurements.

themselves exhibit neglectable intracellular background fluorescence (Fig. 6A), whereas the cells incubated with 5 μM O_3 for 30 min display a strong red fluorescence (Fig. 6B). On the other hand, HeLa cells treated with 0.05 mM of ethyl 4-vinylbenzoate (a specific scavenger of O_3)[8] produce a decreased fluorescence (Fig. 6C), and a higher concentration of ethyl 4-vinylbenzoate (0.1 mM) leads to a weaker fluorescence (Fig. 6D). This suggests that the fluorescence change in HeLa cells indeed arises from the cleavage reaction of **1** by O_3 releasing the free resorufin. Furthermore, to quantitatively compare the level of O_3 in the presence and absence of ethyl 4-vinylbenzoate, relative pixel intensity analysis was made by using ImageJ software (version 1.45 s, NIH), which revealed that the fluorescence intensity from the cells treated with 0.05 and 0.1 mM of ethyl 4-vinylbenzoate was decreased to 35% and 17%, respectively, compared with that (defined as 100%) in the absence of ethyl 4-vinylbenzoate (Supplementary Fig. S9). The above results indicate that the probe is cell membrane permeable, and can also be used to detect the change of O_3 in cells.

Discussion

As mentioned above, exposure to environmental O_3 is particularly a problem for those who suffer from respiratory ailments. These patients rely solely on community-based monitoring to adjust their indoor or outdoor activity so as to minimize their exposure. Because of this, development of a simple and sensitive method for O_3 assay is rather necessary for not only various biochemical studies but also air quality forecast.

By taking advantage of both the excellent fluorochrome of resorufin and the specific reaction of but-3-enyl with O_3, we have established such a simple and practical method with a detection limit of 0.056 mg m^{-3} in air (lower than the ambient air quality standard of 0.1 mg m^{-3} recommended by the World Health Organization) through designing a resorufin-based spectroscopic off-on probe. The probe shows an extremely low background spectroscopic signal, but reacts with O_3 producing a distinct color and fluorescence change, which makes the probe suitable for sensitive assay of O_3. This applicability has been successfully demonstrated on both the determination of O_3 in real ambient air samples and the imaging of O_3 in cells. We believe that the probe is worth popularizing, and may further find important analytical applications in practice.

Table 1 | The concentration of O_3 in the air irradiated by UV-365 nm lamp for different periods of time

Irradiation time (h)	0 (control)	3	6	12
O_3 (mg m^{-3})	<0.056 (detection limit)	1.5 ± 0.1[a]	2.1 ± 0.2[a]	3.7 ± 0.3[a]

[a]Mean of three determinations ± S.D.

Methods

Synthesis of 1. To a suspension of resorufin sodium salt (0.24 g, 1.0 mmol) in anhydrous DMF (5 mL), K_2CO_3 (0.21 g, 1.5 mmol) was added. After stirring for 30 min under Ar atmosphere, a solution of 4-bromo-1-butene (0.27 g, 2.0 mmol) in DMF (2 mL) was added dropwise. The resulting mixture was stirred at 40°C for 2 h, and then diluted with dichloromethane (50 mL). The reaction solution was washed three times with water (50 mL × 3) and brine (50 mL × 3) successively. The organic layer was separated and dried over $MgSO_4$. The solvent was removed by evaporation, and the residue was subjected to silica gel chromatography eluted with petroleum ether (b.p. 60–90°C)/ethyl acetate (v/v, 1 : 1), affording probe **1** as an orange solid (0.14 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$; Supplementary Fig. S10): δ 7.70 (d, J = 9.0 Hz, 1 H), 7.42 (d, J = 9.9 Hz, 1 H), 6.92–6.96 (m, 1 H), 6.81–6.86 (m, 2 H), 6.33 (d, J = 2.1 Hz, 1 H), 5.84–5.97 (m, 1 H), 5.14–5.24 (m, 2 H), 4.10–4.15 (m, 2 H), 2.57–2.64 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$; Supplementary Fig. S11): δ 186.3, 163.0, 149.8, 145.7, 145.5, 134.7, 134.2, 133.5, 131.6, 128.4, 117.7, 114.0, 106.7, 100.6, 68.2, 33.3. EI-MS (*m/z*): $[M]^+$ calcd. for $C_{16}H_{13}NO_3$, 267.09; found, 267 (Supplementary Fig. S12). Analysis (calcd., found for $C_{16}H_{13}NO_3$): C (71.90, 71.52), H (4.90, 5.08), N (5.24, 5.10).

General procedure for detection of O_3. Unless otherwise noted, all the measurements were made according to the following procedure. In a 10 mL test tube, 5 mL of 50 mM phosphate buffer (pH 7.4) and 100 μL of the stock solution of **1** were mixed, followed by addition of an appropriate volume of O_3 solution. The final volume was adjusted to 10 mL with the phosphate buffer, and the reaction solution was mixed well. After 40 min at room temperature, a 3-mL portion of the reaction solution was transferred to a quartz cell of 1 cm optical length to measure absorbance and fluorescence intensity/spectrum 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 O_3 was prepared and measured under the same conditions for comparison.

Procedure for ozone assay in air. Amber glass wide-mouth bottles containing water (typically 10 mL) were placed with the opening of the caps in four different areas [(a) the northwestern 4th ring road of Beijing; (b) the garden of Institute of Chemistry, Chinese Academy of Sciences; (c) the outside of the laboratory window of Institute of

Table 2 | Comparison of O_3 concentration determined by the proposed fluorescence method and iodometric titration

	Concentration of O_3 (μM)		
	standard solutions		sample solutions prepared from the air of the north-western 4th ring road of Beijing
	11.0	20.3	
The proposed fluorescence method	11.0 ± 0.3[a]	20.4 ± 0.1[a]	0.21 ± 0.03[a]
Iodometric titration	10.5 ± 0.2[a]	20.5 ± 0.3[a]	ND[b]

[a]Mean of three determinations ± S.D.
[b]Not detectable.

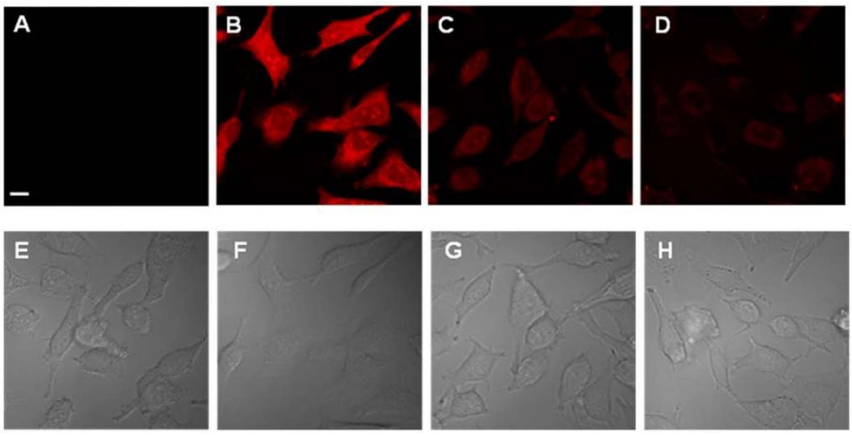

Figure 6 | Confocal fluorescence images of HeLa cells. (A) HeLa cells were incubated with 10 μM 1 for 30 min (control); (B) the 1-loaded HeLa cells were incubated with 5 μM O_3 for 30 min; (C) the 1-loaded HeLa cells were treated with 0.05 mM ethyl 4-vinylbenzoate for 30 min, and then incubated with 5 μM O_3 for 30 min; (D) the 1-loaded HeLa cells were treated with 0.1 mM ethyl 4-vinylbenzoate for 30 min, and then incubated with 5 μM O_3 for 30 min. The differential interference contrast (DIC) images of the corresponding samples are shown below (images E-H). Scale bar, 10 μm.

Chemistry; (d) the inside of the laboratory window of Institute of Chemistry], naturally absorbing the air of the corresponding area for 8 h. Care was taken to ensure that the samples were not hit by direct sunlight. The as-obtained samples were then subjected to fluorescence measurements according to the above "General procedure for detection of O_3". For each place, experiments were done in triplicate.

Determination of ozone by iodometric titration. The determination of ozone by iodometric titration was carried out according to the known method[31]. Briefly, in a 100 mL flask, 20 mL of KI solution (100 μM) was mixed with 20 mL of the phosphate buffer (pH 6.0) containing O_3, followed by stirring for 30 min. Then, diluted H_2SO_4 was added dropwise to adjust the pH to 2.0. After 5 min, the solution was titrated with $Na_2S_2O_3$ solution (10 μM). When the color of the reaction solution became light yellow (near the end point), a few drops of starch solution were added, and the titration continued until the violet blue color disappeared due to the change of iodine to iodide.

Fluorescence imaging of ozone in HeLa cells. HeLa cells were grown on glass-bottom culture dishes (MatTek Co.) using Dulbecco's modified eagle media (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 μg mL^{-1} penicillin-streptomycin in a humidified 37°C, 5% CO_2 incubator. Before use, the adherent cells were washed three times with FBS-free DMEM. For O_3 imaging, the cells were incubated with 10 μM of **1** in DMEM at 37°C for 30 min, then with 5 μM of O_3 for 30 min, and finally washed three times with PBS (pH 7.4). Further researches were made by pretreatment of cells with ethyl 4-vinylbenzoate, a specific scavenger of O_3. Briefly, the cells were first incubated with 10 μM of **1** in DMEM at 37°C for 30 min, washed with PBS, and then incubated with ethyl 4-vinylbenzoate at different concentrations (0.05–0.1 mM) for 30 min. After that, the pretreated cells were incubated with 5 μM of O_3 for 30 min, and then washed three times with PBS (pH 7.4) for fluorescence imaging.

1. Dickinson, B. C. & Chang, C. J. Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat. Chem. Biol.* **7**, 504–511 (2011).
2. Feng, Q. *et al.* Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. *Free Radical Res.* **35**, 779–788 (2001).
3. Sytze van Dam, P. Oxidative stress and diabetic neuropathy: pathophysiological mechanisms and treatment perspectives. *Diabetes Metab Res. Rev.* **18**, 176–184 (2002).
4. Embola, C. W., Sohn, O. S., Fiala, E. S. & Weisburger, J. H. Induction of UDP-glucuronosyltransferase 1 (UDP-GT1) gene complex by green tea in male F344 rats. *Food Chem. Toxicol.* **40**, 841–844 (2002).
5. Garner, A. L. *et al.* Specific fluorogenic probes for ozone in biological and atmospheric samples. *Nat. Chem.* **1**, 316–321 (2009).
6. Schiermeier, Q. Atmospheric science: fixing the sky. *Nature* **460**, 792–795 (2009).
7. Dameris, M. Depletion of the ozone layer in the 21st century. *Angew. Chem. Int. Ed.* **49**, 489–491 (2010).
8. Wentworth, P. Jr. *et al.* Evidence for antibody-catalyzed ozone formation in bacterial killing and inflammation. *Science* **298**, 2195–2199 (2002).
9. Babior, B. M., Takeuchi, C., Ruedi, J., Gutierrez, A. & Wentworth Jr., P. Investigating antibody-catalyzed ozone generation by human neutrophils. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3031–3034 (2003).
10. Wentworth Jr., P. *et al.* Evidence for ozone formation in human atherosclerotic arteries. *Science* **302**, 1053–1056 (2003).
11. Salthammer, T. Formaldehyde in the ambient atmosphere: from an indoor pollutant to an outdoor pollutant? *Angew. Chem. Int. Ed.* **52**, 3320–3327 (2013).
12. Zhang, Q. H. *et al.* Metabolite-initiated protein misfolding may trigger Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4752–4757 (2004).
13. Takeuchi, K. & Ibusuki, T. Quantitative determination of aqueous-phase ozone by chemiluminescence using indigo-5,5-disulfonate. *Anal. Chem.* **61**, 619–623 (1989).
14. Xu, K. H. *et al.* A near-infrared fluorescent probe for monitoring ozone and imaging in living cells. *Chem. Commun.* **48**, 684–686 (2012).
15. Oushiki, D. *et al.* Development and application of a near-infrared fluorescence probe for oxidative stress based on differential reactivity of linked cyanine dyes. *J. Am. Chem. Soc.* **132**, 2795–2801 (2010).
16. Jia, J. & Ma, H. M. A water-soluble FRET probe for hypochlorous acid and its application to cell imaging. *Chinese Sci. Bull.* **56**, 3266–3272 (2011).
17. Jimenez, A. M., Navas, M. J. & Galan, G. Air analysis: determination of ozone by chemiluminescence. *Appl. Spectrosc. Rev.* **32**, 141–149 (1997).
18. Parrish, D. D. & Fehsenfeld, F. C. Methods for gas-phase measurements of ozone, ozone precursors and aerosol precursors. *Atmos. Environ.* **34**, 1921–1957 (2000).
19. Williams, E. J. *et al.* Comparison of ultraviolet absorbance, chemiluminescence, and DOAS instruments for ambient ozone monitoring. *Environ. Sci. Technol.* **40**, 5755–5762 (2006).
20. Criegee, R. Mechanism of ozonolysis. *Angew. Chem. Int. Ed.* **14**, 745–752 (1975).
21. Geletneky, C. & Berger, S. The mechanism of ozonolysis revisited by ^{17}O-NMR spectroscopy. *Eur. J. Org. Chem.* **8**, 1625–1627 (1998).
22. Schiaffo, C. E. & Dussault, P. H. Ozonolysis in solvent/water mixtures: direct conversion of alkenes to aldehydes and ketones. *J. Org. Chem.* **73**, 4688–4690 (2008).
23. Willand-Charnley, R., Fisher, T. J., Johnson, B. M. & Dussault, P. H. Pyridine is an organocatalyst for the reductive ozonolysis of alkenes. *Org. Lett.* **14**, 2242–2245 (2012).
24. Sun, M. *et al.* Simple Pb^{II} fluorescent probe based on Pb^{II}-catalyzed hydrolysis of phosphodiester. *Biopolymers* **72**, 413–420 (2003).
25. Chen, W., Li, Z., Shi, W. & Ma, H. M. A new resorufin-based spectroscopic probe for simple and sensitive detection of benzoyl peroxide *via* deboronation. *Chem. Commun.* **48**, 2809–2811 (2012).
26. Li, Z. *et al.* Nitroreductase detection and hypoxic tumor cell imaging by a designed sensitive and selective fluorescent probe, 7-[(5-nitrofuran-2-yl)-methoxy]-3*H*-phenoxazin-3-one. *Anal. Chem.* **85**, 3926–3932 (2013).
27. Chen, S. M., Chen, W., Shi, W. & Ma, H. M. Spectroscopic response of ferrocene derivatives bearing a BODIPY moiety to water: a new dissociation reaction. *Chem. Eur. J.* **18**, 925–930 (2012).
28. Ely, J. C. *et al.* Implications of platinum group element accumulation along U.S. roads from catalytic-converter attrition. *Environ. Sci. Technol.* **35**, 3816–3822 (2001).
29. Shi, W., Li, X. H. & Ma, H. M. A tunable ratiometric pH sensor based on carbon nanodots and its application to quantitatively measuring the intracellular pH of whole cells. *Angew. Chem. Int. Ed.* **51**, 6432–6435 (2012).
30. Jia, J. *et al.* Rhodamine B piperazinoacetohydrazine: a water-soluble spectroscopic reagent for pyruvic acid labeling. *Chem. Eur. J.* **16**, 6638–6643 (2010).
31. Rice, R. G. & Netzer, A. *Handbook of ozone technology and applications* (Vol. **2**), (Butterworth Publishers: Stoneham, MA, 1984, p.378).

Acknowledgements

This work is supported by grants from the NSF of China (Nos. 20935005, 21105104, 21275146, 21275147 and 21321003), 973 Program (Nos. 2011CB935800 and 2010CB933502), and the Chinese Academy of Sciences (KJCX2-EW-N06-01 and CMS-PY-201301).

Author contributions

Y.Y.Z. performed experiments. W.S. and X.H.L. assisted in data analysis. Y.Y.Z. and H.M.M. analysed data and wrote the paper. H.M.M. contributed to study design and overall supervision. All authors discussed the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Zhang, Y., Shi, W., Li, X. & Ma, H. Sensitive detection of ozone by a practical resorufin-based spectroscopic probe with extremely low background signal. *Sci. Rep.* **3**, 2830; DOI:10.1038/srep02830 (2013).