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Fluorescent Imaging

A Dual-Response Fluorescent Probe Reveals the H_2O_2-Induced H_2S Biogenesis through a Cystathionine β-Synthase Pathway

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Abstract: The two signaling molecules H_2S and H_2O_2 play key roles in maintaining intracellular redox homeostasis. The biological relationship between H_2O_2 and H_2S remains largely unknown in redox biology. In this study, we rationally designed and synthesized single- and dual-response fluorescent probes for detecting both H_2O_2 and H_2S in living cells. The dual-response probe was shown to be capable of mono- and dual-detection of H_2O_2 and H_2S selectively and sensitively. Detailed bioimaging studies based on the probes revealed that both exogenous and endogenous H_2O_2 could induce H_2S biogenesis in living cells. By using gene-knockdown techniques with bioimaging, the H_2S biogenesis was found to be majorly cystathionine β-synthase (CBS)-dependent. Our finding shows the first direct evidence on the biological communication between H_2O_2 (ROS) and H_2S (RSS) in vivo.

Cellular redox balance plays an important role in various physiological processes.[1] Endogenous small molecules are involved in maintenance of intracellular redox homeostasis including the reactive oxygen species (ROS) and reactive sulfur species (RSS). Hydrogen peroxide (H_2O_2) is the best described signaling ROS molecule,[2] and its excessive production is implicated with various diseases including cancer, diabetes, cardiovascular, and neurodegenerative disorders.[3] Hydrogen sulfide (H_2S) as one of RSS and gasotransmitters has recently been demonstrated to exert protective effects including preservation of mitochondrial function, protection of neurons from oxidative stress, and inhibition of apoptosis.[4] H_2S is proposed to be produced from L-cysteine (L-Cys) by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MPST)/cysteine aminotransferase (CAT).[5] The endogenous H_2S level is correlated with numerous diseases, including the symptoms of Alzheimer's disease, Down syndrome, diabetes, and liver cirrhosis.[5]

It is intriguing that the redox atomosphere in the cell should be balanced to maintain the normal cell growth. The biobalance proposals include the possible mutual inducement on the production of ROS and RSS. Many attempts have been initiated to understand this redox biobalance in the molecular level.[6–8] For example, exogenous H_2S has been reported to exert an antioxidant effect against H_2O_2-induced oxidative stress and senescence in living cells.[6] CBS can be post-translationally activated in response to oxidative stress.[8] However, this biological regulation's pertinence to H_2S generation is not yet clear.[5a] Fluorescent probes should be excellent tools for real-time monitoring of these ROS and RSS in vivo.[9] To clarify the intricate biological relationship between H_2O_2 and H_2S, the development of a fluorescent probe that can selectively sense both the signaling molecules to give different fluorescent colors will be highly valuable. The dual-response probe can avoid inhomogenous intracellular distribution from two different probes and can be excitated by a single excitation for ratiometric imaging.

Chang et al. developed cell-trappable fluorescent probes for imaging endogenous H_2S production upon VEGF-stimulation, which was reported to be dependent on NADPH oxidase-derived H_2O_2 but not exogenous H_2O_2.[10] We have been interested in the biodetection of RSS for some time.[11] Our H_2S probes were developed for in situ visualizing cysteine-dependent H_2S biogenesis in living cells.[12] Herein, a series of single- and dual-response fluorescent probes were developed for live capture of H_2O_2 and/or H_2S selectively in vivo. Our bioimaging with RNA interference (RNAi) experiments revealed that both of exogenous and endogenous H_2O_2 molecules can induce CBS-dependent H_2S production in living cells, which provides the direct evidence on the crosstalk between H_2O_2 (ROS) and H_2S (RSS) in living human cells.

H_2O_2-mediated conversion of arylboronates to phenols developed by the Chang's group is a general reaction-based strategy for the design of H_2O_2 fluorescent probes;[13] and azido-based fluorescent probes have been developed to image H_2S in living cells.[10,11,14] To fluorescently sense both H_2S and H_2O_2, we synthesized a dual-response FRET (fluorescence resonance energy transfer) probe **1** that contained a pinacol boro-

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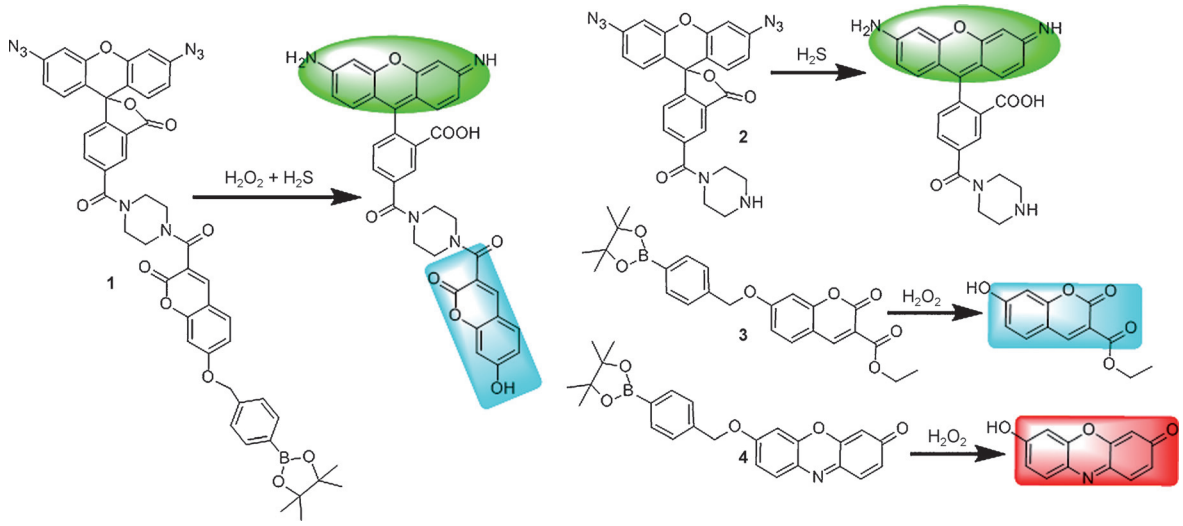

Figure 1. Structures of probes **1**–**4** and their reactions with H_2O_2 and/or H_2S.

nate moiety at the FRET donor (H_2O_2 trapper) and an azido moiety at the FRET acceptor (H_2S trapper) (Figure 1). It is noted that the H_2O_2 trapper should be at FRET donor, because we intend to observe the H_2O_2-induced H_2S production by FRET-based ratiometric imaging. Reaction of probe **1** with H_2O_2 or/and H_2S leads to a fluorescent turn-on response of FRET donor or/and acceptor, respectively. Compounds **2**–**4** were specific probes for H_2S or H_2O_2. Probe **1** could be conveniently prepared by successive coupling of azido-capped rhodamine **5**, piperazyl linker, and coumarin fluorophore **7** (Scheme S1, Supporting Information). Then the arylboronate was installed at the last step to **8** to produce two isomers, which could be purified by column chromatography. Probes **1**–**4** were synthesized and characterized by ^1H and ^{13}C NMR spectroscopy and HRMS (see the Supporting Information for detailed information).

Firstly, the UV/Vis absorption spectra were examined for probes **1**–**3** and their reactions with H_2O_2 and/or H_2S (Figure 2 and Figures S1 and S2, Supporting Information). After treatment with H_2O_2, the probe **3** exhibited a time-dependent increased absorption at 405 nm (Figure S1, Supporting Information); while no obvious absorption change was observed for that of H_2S (using Na_2S as an equivalent). We also observed the time-dependent absorption change at 500 nm for probe **2** through treatment with H_2S, but not with H_2O_2 (Figure S2, Supporting Information). These results indicated that the boronate-based and azide-based reaction sites have good selectivity toward H_2O_2 and H_2S, respectively. As expected, the probe **1** as a combination cassette of **2** and **3** displayed absorption changes at both 405 and 500 nm upon treatment with tandem H_2O_2 and H_2S (Figure 2A–C).

Probes **1**–**4** were further characterized by a series of fluorescent tests. Single-response probes **2**–**4** displayed a significant fluorescent turn-on response upon treatment with their specific activator (H_2O_2 or H_2S) in PBS buffer (Figures S3–S5, Supporting Information). For dual-response probe **1**, H_2O_2 elicited intense emission at 460 nm with excitation at 400 nm (Figure 2D), while nearly no emission at 525 nm was observed with excitation at 488 nm (Figure S6, Supporting Information). The treatment of **1** with H_2S led to turn-on fluorescence at 525 nm with excitation at 488 nm (Figure 2E), but no significant change with excitation at 400 nm (Figure S7, Supporting Information). The titrations of **1** with H_2O_2 or H_2S further supported the selectivity for both molecules (Figures S8 and S9, Supporting Information).

We then investigated the fluorescence response of **1** by addition of H_2O_2 followed by H_2S. As shown in Figure 2F, addition of H_2O_2 to **1** triggered a large fluorescence enhancement at 460 nm with excitation at 400 nm. After further addition of H_2S, a significant enhancement at 525 nm was observed. The decreased signal at 460 nm should be due to the FRET effect from donor to acceptor, and the FRET product has been identified by HRMS (see the Supporting Information). The fluorescent change (color) of **1** upon tandem treatment with H_2O_2 and H_2S can also be observed by the naked eye under a UV lamp (Figure 2G). Moreover, the time-dependent fluorescence response of **1** by addition of both H_2O_2 and H_2S was tested (Figure S10, Supporting Information). The results indicated that probe **1** can respond to the both signal molecules simultaneously to give different emission peaks. These results imply that **1** is a single-wavelength-exited dual-responsive probe, which can be used for detection of both H_2O_2 and H_2S simultaneously. The fluorescence response of **1** by addition of H_2S followed by H_2O_2 also gave dual-emission peaks (Figure S11, Supporting Information), though the treatment of **1** with H_2S could not lead to obvious turn-on fluorescence with excitation at 400 nm (Figure S7 and S11, Supporting Information). Therefore, the stimulant H_2O_2 should react with the FRET donor part of the dual-response probe for dual-emission detection.

To examine the selectivity, probe **1** was incubated with different ROS and RSS including NaOCl, *tert*-butyl hydroperoxide (TBHP), glutathione (GSH), homocysteine (Hcy), and Cys (Figure 2H,I). Subsequently the fluorescence signal was measured using different emission channels. The experimental results in-

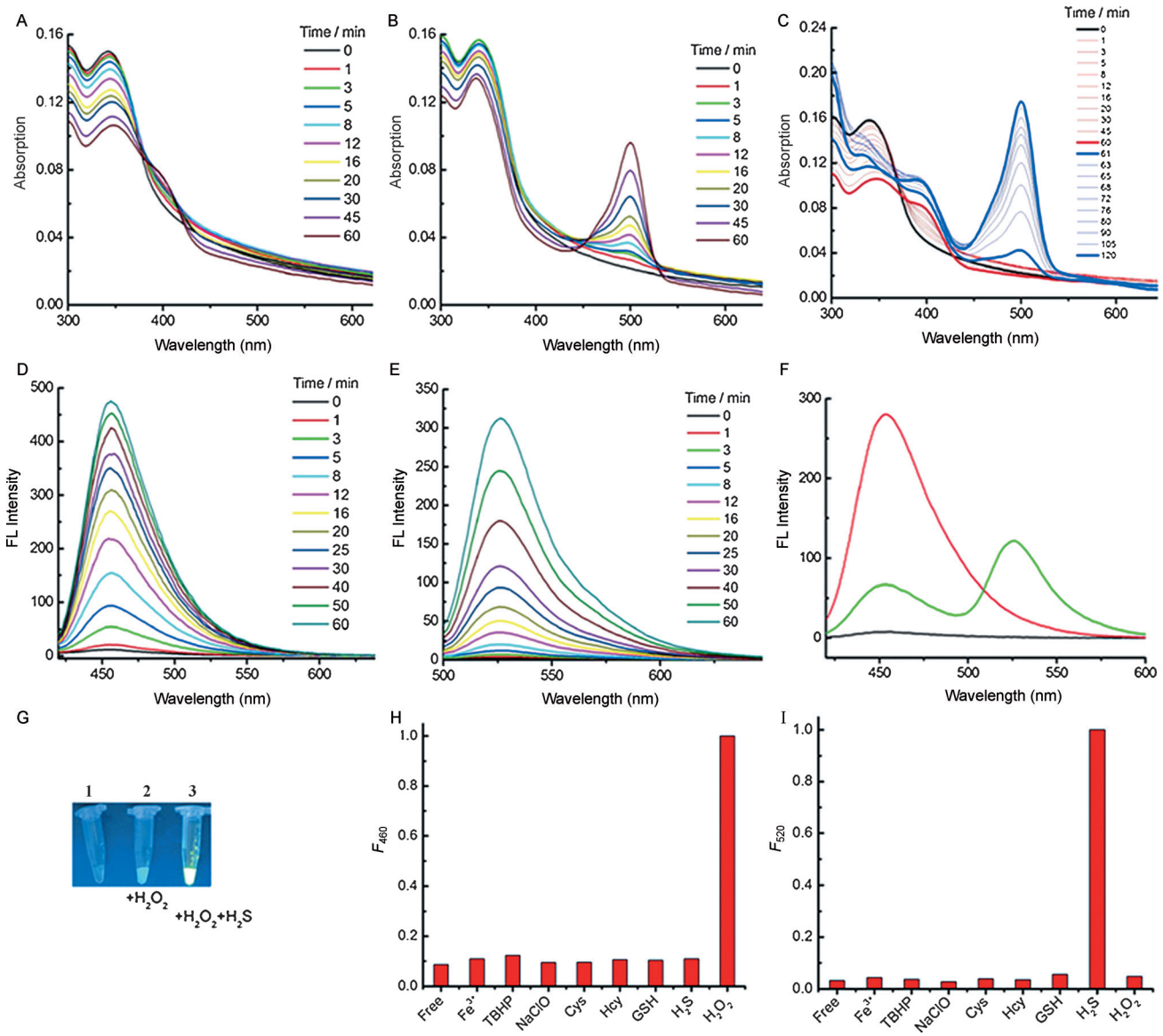

Figure 2. Fluorescence response of the probe **1** toward H_2O_2 and/or H_2S. Time-dependent absorption spectra of **1** (10 μM) in the presence of A) 1 mM H_2O_2 or B) 1 mM Na_2S or C) 1 mM H_2O_2 followed by 2 mM Na_2S. Time-dependent emission spectra of **1** (1 μM) upon reaction with D) 200 μM H_2O_2 or E) 200 μM H_2S or F) 100 μM H_2O_2 followed by 200 μM H_2S. The excitation wavelengths were 400, 488, 400 nm for (D–F), respectively. G) Photographs under UV lamp (365 nm) of **1** in the absence (lane 1) or presence of H_2O_2 (lane 2) or H_2O_2 followed by H_2S (lane 3). H,I) Relative fluorescence responses of **1** (1 μM) to various biologically relevant species (GSH, 5 mM; Cys, 1 mM; Hcy, 1 mM; Na_2S, 100 μM; $FeCl_3$, 100 μM; TBHP, 100 μM; NaClO, 100 μM; H_2O_2, 100 μM) in PBS (pH 7.4) with H) excitation at 400 nm and emission at 460 nm or I) excitation at 488 nm and emission at 520 nm.

dicated that **1** can be used to selectively detect H_2O_2 and/or H_2S at different emission wavelengths, making it a potential valuable tool for dual-color visualization of both H_2O_2 and H_2S.

To investigate the biological relationship between H_2O_2 and H_2S, human embryonic kidney 293 cells (HEK293) were selected as the model biological system, because endogenous H_2S could be produced from the cells.[12,15] Firstly, we tested the intracecullar fluorescence in the presence of probes and exogenous H_2S or H_2O_2. The addition of both **1** and H_2S resulted in a significant fluorescence increase at the green channel but not at the blue channel (Figure 3B), indicating that probe **1** could be used to image H_2S in living cells. When the living cells were treated with H_2O_2, fluorescent turn-on signals at both the blue and green channels were observed, implying that 1) H_2O_2 can enter into cells to trigger blue fluorescence of probe **1** and 2) H_2O_2 can directly induce endogenous production of H_2S from the living cells. The single-response probe **2** was further employed for quantification of the H_2O_2-induced H_2S production (Figure 3C and Figure S12, Supporting Information). The results indicated that higher concentration of exogenous H_2O_2 could trigger stronger green fluorescence inside the living cells, implying more endogenous H_2S molecules were produced from the living cells. It is well known that living organisms have evolved complicated molecular systems to combat oxidative pressure, including antioxidant metalloenzymes, an array of thiol-based redox couples and high concen-

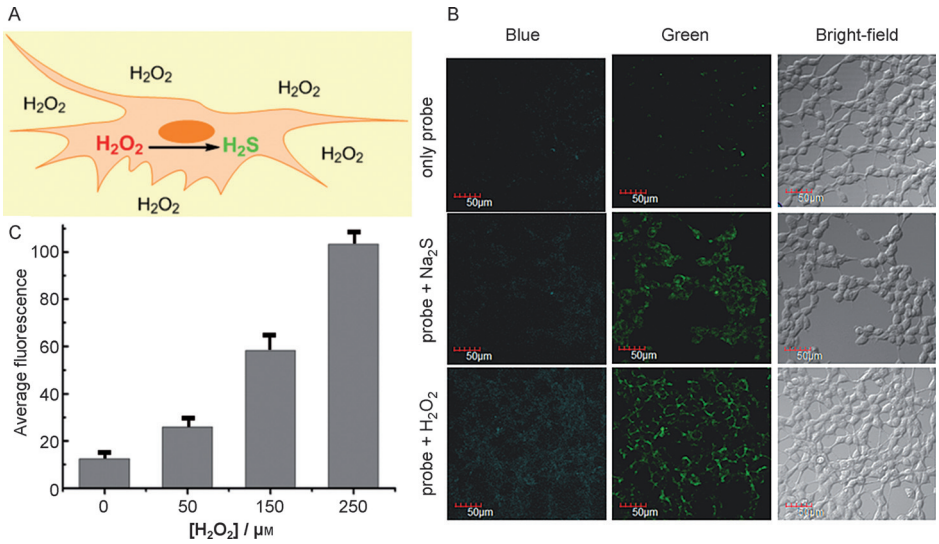

Figure 3. Exogenous H_2O_2 can induce endogenous H_2S production in living cells. A) Schematic drawing of exogenous H_2O_2 stimulation leads to H_2S production in cells. B) HEK293 cells were treated with **1**, **1** + Na_2S, and **1** + H_2O_2, respectively, and the imaging was taken immediately after media exchange with PBS buffer. Emission was collected at the blue (425–480 nm) and green channels (500–600 nm) with 405 and 488 nm excitation, respectively. C) HEK293 cells were preincubated with probe **2** for 30 min and then with H_2O_2 (0–250 μM) for 1 h. Data are expressed as the average fluorescence intensity of each image versus H_2O_2 concentration.

trations of GSH (1–10 mM) (RSS).[16] Herein, the observation of endogenous production of H_2S from living human cells by exogenous H_2O_2 stimulation should help to further understand the intracellular redox homeostasis.

Encouraged by the above results, we further tested whether endogenous H_2O_2 can induce H_2S biogenesis in living human cells. To demonstrate the usefulness of probe **1** for detection of endogenous H_2S, 200 μM GSH was added to the cells, which resulted in an obviously enhanced fluorescence at green channel (Figure 4B). The increased fluorescence signal could be attributed to endogenous generation of H_2S from GSH in living cells.[17] When cells pretreated by probe **4** were co-incubated with phorbol-12-myristate-13-acetate (PMA), an inducer for endogenous H_2O_2 production,[18] a marked red fluorescence increase was observed (Figure S13, Supporting Information), implying the PMA-induced endogenous H_2O_2 production. While cells were co-incubated with PMA and probe **1**, a fluorescence increase at both blue and green channels was observed (Figure 4B), just like the observation by addition of exogenous H_2O_2.

The turn-on response at the blue channel should be attributed to endogenous H_2O_2 production, while the turn-on signal at the green channel could be due to endogenous H_2S production. These results, for the first time, directly indicated that endogenous H_2O_2 can induce endogenous H_2S in living cells. To further verify this point, inhibition experiments were performed by using Propargylglycine (PPG), an analogue of Cys as a known inhibitor for CBS and CSE.[12] The PMA-induced green fluorescence was obviously decreased with addition of inhibitors, while the average fluorescence intensities at the blue channel are similar (Figure S14, Supporting Information). These results implied that PPG could inhibit PMA-induced H_2S biogenesis but not for H_2O_2 biogenesis.

In order to further analyze the PMA-induced endogenous H_2S production, ratiometric imaging of FRET-donor excitation-only was performed (Figure S15, Supporting Information). The results indicated that 1) endogenous H_2S could be produced through PMA stimulation; 2) such endogenous H_2S production could also be suppressed by enzyme inactivation using the

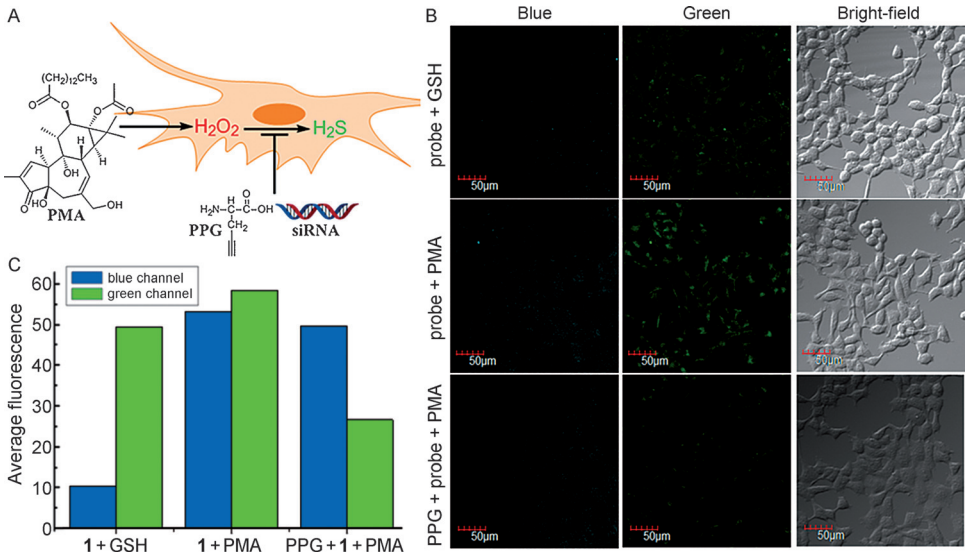

Figure 4. Images of PMA-induced endogenous H_2O_2 and H_2S in living cells. A) Schematic drawing indicates that PMA can induce biogenesis of both H_2O_2 and H_2S inside cells, and the endogenous production of H_2S can be inhibited by PPG. B) Dual-channel imaging of cells in the presence of probe **1** and stimulants as indicated on the left side. Cells were preincubated with DL-PPG (50 μg mL^{-1}) for 20 min or not, and then incubated with PMA (1 μg mL^{-1}) and probe **1** (1 μM) for 60 min without media exchange. After being washed with PBS, cells were used for imaging immediately. Emission was collected at the blue (425–480 nm) and green channels (500–600 nm) with 405 and 488 nm excitation, respectively. C) The average fluorescence intensity from the blue and green channels of probe **1** in the absence or presence of stimulants.

PPG inhibitor; and 3) endogenous H_2O_2 can induce enzymatic H_2S production in living cells. Furthermore, because PMA can trigger endogenous H_2O_2 production during cell signaling to result in an inflammatory response,[18] the PMA-induced H_2S production could help in the understanding of the anti-inflammatory role of H_2S-releasing drugs at a molecular level. H_2S-releasing drugs may serve as a treatment for PMA- and oxidative-induced inflammation.

The H_2S biogenesis pathways include CBS, CSE, and 3MPST/CAT.[5] By combining the sequence-specific gene-knockdown technology (RNAi) with bioimaging, we seek to gain a better understanding about the pathway for the H_2O_2-induced H_2S biogenesis. CBS, CSE, and 3MPST genes were specifically silenced by using three designed siRNA sequences with the efficiency of above 80% (Figure S16, Supporting Information).[19] Subsequently, these gene-silencing cells were used for bioimaging studies with probe **1**. No significant increase of green fluorescence was observed in the PMA treatment series in CBS-knockdown cells, implying no endogenous H_2S production during PMA stimulation. But for CSE- or 3-MPST-knockdown cells, still significant green fluorescence signals were observed (Figure 5A and Figure S17, Supporting Information). These results implied that H_2O_2-induced H_2S biogenesis may largely depend on the CBS pathway. Consideration of the substrate of CBS in vivo, L-Cys should be involved in the H_2O_2-induced H_2S production under oxidative stress (Figure 5B). Apart from that, we also treated living cells with a high concentration Na_2S or Cys and found no obvious H_2O_2-triggering fluorescence based on probe **4** (Figure S18, Supporting Information).

In summary, based on two selective chemical reactions, a series of single- and dual-response fluorescent probes were rationally designed and synthesized to investigate the biological relationship between H_2O_2 and H_2S. The endogenous production of H_2O_2 and H_2S can be imaged simultaneously with a dual-response fluorescent probe. Our finding shows the first direct evidence on the crosstalk between H_2O_2 (ROS) and H_2S (RSS) in vivo and the H_2O_2-induced H_2S production was found to be CBS-dependent. Consideration of the irreversible reactivity of the dual-response probe **1**, the development of dual-detection probes based on reversible reactions is still needed. It is well known that H_2S could exert its biological action through modulation of $[Ca^{2+}]$ and intracellular pH as well as interacting with other signaling molecules.[20] The development of dual- and multiresponse fluorescent probes should serve as invaluable tools to investigate the possible link between H_2S and other biological molecules in vivo at the molecular level.

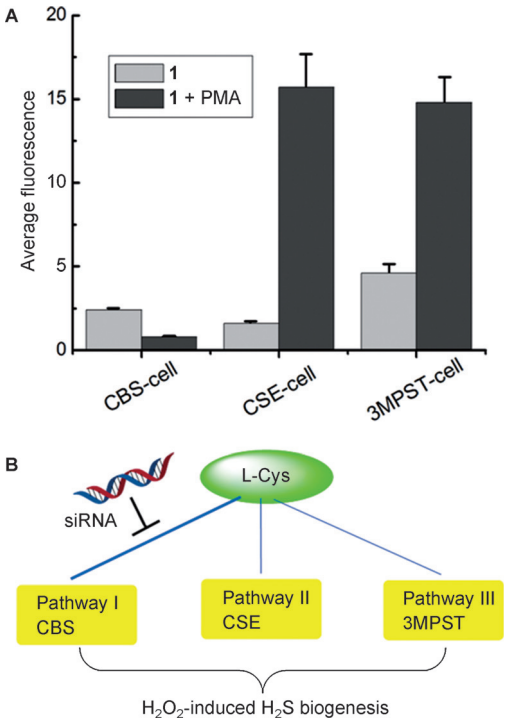

Figure 5. Regulation of the H_2O_2-induced H_2S biogenesis by RNAi based on fluorescent imaging analysis. A) The gene-knockdown HEK293 cells were named as CBS-, CSE-, and 3MPST-cell, respectively. Cells were treated with **1** or **1** + PMA for 1 h, respectively, and imaged immediately after media exchange with PBS buffer. Data are expressed as the mean fluorescence intensity at green channel (500–600 nm). B) Schematic representation indicates that the H_2O_2-induced H_2S biogenesis in living cells is majorly CBS-dependent with L-Cys as the sources.

Acknowledgements

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Keywords: fluorescent imaging · fluorescent probes · hydrogen peroxide · hydrogen sulfide · redox homeostasis

[1] a) A. Banjac, T. Perisic, H. Sato, A. Seiler, S. Bannai, N. Weiss, P. Kölle, K. Tschoep, R.-D. Issels, P.-T. Daniel, M. Conrad, G.-W. Bornkamm, *Oncogene* **2008**, *27*, 1618–1628; b) L. Sullivan, N. Chandel, *Cancer Metab.* **2014**, *2*, 17; c) K.-M. Holmström, T. Finkel, *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 411–421; d) C. E. Paulsen, K. S. Carrol, *Chem. Rev.* **2013**, *113*, 4633–4679.

[2] M. Reth, *Nat. Immun.* **2002**, *3*, 1129–1134.

[3] a) K. Krohn, J. Maier, R. Paschke, *Nat. Clin. Pract. Endocrinol. Metab.* **2007**, *3*, 713–720; b) M. Lin, M, Beal, *Nature* **2006**, *443*, 787–795; c) G. F. Z. da Silva, L.-J. Ming, *Angew. Chem. Int. Ed.* **2007**, *46*, 3337–3341; *Angew. Chem.* **2007**, *119*, 3401–3405.

[4] a) G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A. Mustafa, W. Mu, S. Zhang, S. Snyder, R. Wang, *Science* **2008**, *322*, 587–590; b) J. Elrod, J. Calvert, J. Morrison, J. Doeller, D. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabo, H, Kimura, C.-W. Chow, D. Lefer, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 15560–15565; c) G. Kolluru, X. Shen, S. Bir, C. Kevil, *Nitric Oxide* **2013**, *35*, 5–20.

[5] a) O. Kabil, R. Banerjee, *Antioxid. Redox Signaling* **2014**, *20*, 770–782; b) S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah, A. Morelli, *Hepatology* **2005**, *42*, 539–548.

[6] a) S. Taniguchi, L. Kang, T. Kimura, I. Niki, *Br. J. Pharmacol.* **2011**, *162*, 1171–1178; b) Y. Wen, H. Wang, S. Kho, S. Rinkiko, S. Sheng, H. Shen, *PLOS one* **2013**, *8*, e53147; c) Y. Luo, X. Liu, Q. Zheng, X. Wan, S. Ouyang, Y. Yin, X. Sui, J. Liu, X. Yang, *Biochem. Biophys. Res. Commun.* **2012**, *425*, 473–477.

[7] a) R. Suo, Z. Zhao, Z. Tang, Z. Ren, X. Liu, L. Liu, Z. Wang, C. Tang, D. Wei, Z. Jiang, *Mol. Med. Rep.* **2013**, *7*, 1865–1870; b) Z. Xu, X. Wang, D. Xiao, L. Hu, M. Lu, Z. Wu, J. Bian, *Free Radical Biol. Med.* **2011**, *50*, 1314–1323.

[8] E. Mosharov, M. Cranford, R. Banerjee, *Biochemistry* **2000**, *39*, 13005–13011.

[9] For recent reviews: a) J. Chan, S. Dodani, C. Chang, *Nat. Chem.* **2012**, *4*, 973 – 984; b) X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 2120 – 2135; c) M. Schäferling, *Angew. Chem. Int. Ed.* **2012**, *51*, 3532 – 3554; *Angew. Chem.* **2012**, *124*, 3590 – 3614; d) S. I. Dikalov, D. G. Harrison, *Antioxid. Redox Signaling* **2014**, *20*, 372 – 382; e) B. C. Dickinson, D. Srikun, C. J. Chang, *Curr. Opin. Chem. Biol.* **2010**, *14*, 50 – 56; f) V. Lin, W. Chen, M. Xian, C. Chang, *Chem. Soc. Rev.* **2015**, *44*, 4596 – 4618; g) L. Yuan, W. Lin, Y. Xie, B. Chen, S. Zhu, *J. Am. Chem. Soc.* **2012**, *134*, 1305 – 1315; h) F. Yu, X. Han, L. Chen, *Chem. Commun.* **2014**, *50*, 12234 – 12249.

[10] V. Lin, A. Lippert, C. Chang, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 7131 – 7135.

[11] a) L. Yi, H. Li, L. Sun, L. Liu, C. Zhang, Z. Xi, *Angew. Chem. Int. Ed.* **2009**, *48*, 4034 – 4037; *Angew. Chem.* **2009**, *121*, 4094 – 4097; b) C. Wei, L. Wei, Z. Xi, L. Yi, *Tetrahedron Lett.* **2013**, *54*, 6937 – 6939.

[12] a) L. Wei, L. Yi, F. Song, C. Wei, B. Wang, Z. Xi, *Sci. Rep.* **2014**, *4*, 4521; b) C. Wei, R. Wang, L. Wei, L. Cheng, Z. Li, Z. Xi, L. Yi, *Chem. Asian J.* **2014**, *9*, 3586 – 3592; c) C. Wei, Q. Zhu, W. W. Liu, W. B. Chen, Z. Xi, L. Yi, *Org. Biomol. Chem.* **2014**, *12*, 479 – 485; d) C. Zhang, L. Wei, C. Chao, J. Zhang, R. Wang, Z. Xi, L. Yi, *Chem. Commun.* **2015**, *51*, 7505 – 7508.

[13] A. Lippert, G. Van de Bittner, C. Chang, *Acc. Chem. Res.* **2011**, *44*, 793 – 804.

[14] a) H. Zhang, P. Wang, G. Chen, H. Cheung, H. Sun, *Tetrahedron Lett.* **2013**, *54*, 4826 – 4829; b) A. R. Lippert, E. J. New, C. J. Chang, *J. Am. Chem. Soc.* **2011**, *133*, 10078 – 10080; c) J. Cao, R. Lopez, J. M. Thacker, J. Y. Moon, C. Jiang, S. N. S. Morris, J. H. Bauer, P. Tao, R. P. Mason, A. R. Lippert, *Chem. Sci.* **2015**, *6*, 1979 – 1985; d) M. D. Hammers, M. J. Taormina, M. M. Cerda, L. A. Montoya, D. T. Seidenkranz, R. Parthasarathy, M. D. Pluth, *J. Am. Chem. Soc.* **2015**, DOI: 10.1021/jacs.5b04196.

[15] a) V. Telezhkin, S. Brazier, S. Cayzac, C. Müller, D. Riccardi, P. Kemp, *Adv. Exp. Med. Biol.* **2009**, *648*, 65 – 72; b) F. Sekiguchia, Y. Miyamoto, D. Kanaoka, H. Ide, S. Yoshida, T. Ohkubo, A. Kawabata, *Biochem. Biophys. Res. Comm.* **2014**, *445*, 225 – 229.

[16] a) I. Zelko, T. Mariani, R. Folz, *Free Radical Biol. Med.* **2002**, *33*, 337 – 349; b) M. Alfonso-Prieto, X. Biarnés, P. Vidossich, C. Rovira, *J. Am. Chem. Soc.* **2009**, *131*, 11751 – 11761; c) H. Sies, *Free Radical Biol. Med.* **1999**, *27*, 916 – 921; d) R. Banerjee, *J. Biol. Chem.* **2012**, *287*, 4397 – 4402.

[17] Y. Qian, J. Karpus, O. Kabil, S. Zhang, H. Zhu, R. Banerjee, J. Zhao, C. He, *Nat. Commun.* **2011**, *2*, 495.

[18] S. Rhee, *Science* **2006**, *312*, 1882 – 1883.

[19] a) L. Wei, Z. Zhu, Y. Li, L. Yi, Z. Xi, *Chem. Commun.* **2015**, *51*, 10463; b) S. Bhattacharyya, S. Saha, K. Giri, I. Lanza, K. Nair, N. Jennings, C. Rodriguez, G. Lopez-Berestein, E. Basal, A. Weaver, D. Visscher, W. Cliby, A. Sood, R. Bhattacharya, P. Mukherjee, *PLOS One* **2013**, *8*, e79167; c) P. Manna, S. Jain, *J. Cell. Biochem.* **2013**, *114*, 2334 – 2345.

[20] a) S. Lee, Y. Cheng, P. Moore, J. Bian, *Biochem. Biophys. Res. Commun.* **2007**, *358*, 1142 – 1147; b) S. Lee, Y. Hu, L. Hu, Q. Lu, G. Dawe, P. Moore, P. Wong, J. Bian, *Glia* **2006**, *54*, 116 – 124; c) D. Scuffi, C. Álvarez, N. Laspina, C. Gotor, L. Lamattina, C. García-Mata, *Plant Physiol.* **2014**, *166*, 2065 – 2076.

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