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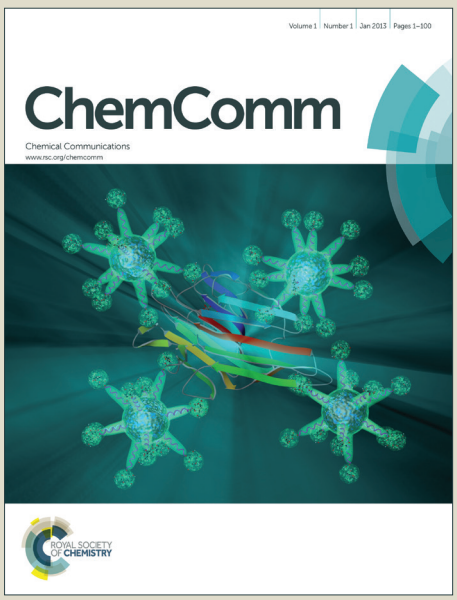

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ARTICLE TYPE

A FRET-ICT dual-quenching fluorescent probe with large *off-on* response for H_2S: synthesis, spectra and bioimaging

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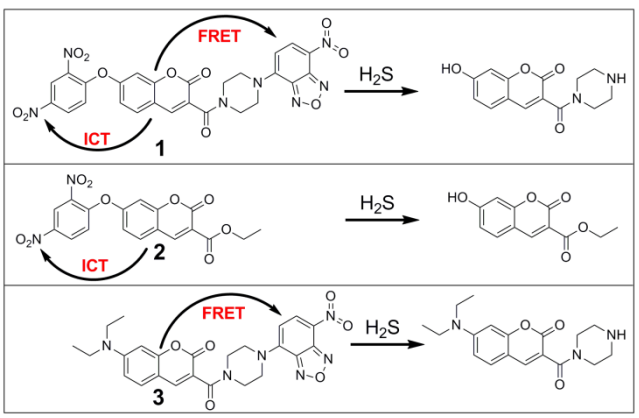
A FRET-ICT dual-quenching probe with large *off-on* fluorescent response upon H_2S treatment was reported. The probe can be used for bioimaging of endogenous H_2S in living cells.

Hydrogen sulfide (H_2S) is an important endogenous signalling molecule with multi-functions.[1-3] *In vivo*, H_2S can be enzymatic generated in many organs (heart, liver, kidney, brain, ileum, uterus, etc.) and tissues (connective tissues, adipose tissues, etc.).[2] Studies have shown that the endogenous concentration of H_2S is correlated with numerous diseases, including the symptoms of Alzheimer's disease, Down syndrome, diabetes and liver cirrhosis.[4] Despite H_2S has been recognized to be linked to numerous physiological and pathological processes, many of its underlying molecular events remain unknown. Therefore, it presents significant research value to develop efficient methods for detection of H_2S in living biological systems.

Traditionally, the major methods for H_2S detection are colorimetry, electrochemical assay, gas chromatography and sulfide precipitation.[5] However, these methods are destructive and require tedious preparation sequence. Recent work indicated that fluorescence-based methods are highly desirable and sensitive for *in-situ* and real-time visualization of H_2S in living biological systems.[6-11] These probes are majorly based on the specific H_2S-induced reactions, including reduction-based probes,[6-8] metal sulfide precipitation-based probes,[9] and nucleophilic-based probes.[10] Though the great success of these fluorescent H_2S probes,[6-11] we still need to develop advance probes with highly sensitivity, highly selectivity and fast-response properties for real-time detection of low concentration H_2S in biological samples. Recently, we reported a new fluorescent thiol probe with more than 400-fold turn-on response based a dual-quenching strategy.[12] In this work, we intend to develop highly sensitive fluorescent H_2S probes based on the dual-quenching strategy for the first time.

We discovered a FRET-based fluorescent H_2S probe based on selective thiolysis of NBD (7-nitro-1,2,3-benzoxadiazole) amine by H_2S to give a turn-on response.[13] Lin et al. reported a fluorescent probe for H_2S based on thiolysis of DNP (dinitrophenyl) ether.[10c] To obtain highly sensitive fluorescent probes for H_2S, we intend to install both NBD amine and DNP ether as quenching groups into one fluorophore. DNP and NBD can quench the fluorescence via intramolecular charge transfer (ICT)[10k] and fluorescence resonance energy transfer (FRET),[13] respectively. As a result, the fluorescence should be heavily quenched through FRET-ICT dual-quenching effects.[12] Consequently, the fluorescent turn-on fold of the probe after H_2S treatment can be significantly improved. To this end, we rational designed and synthesized a coumarin-based fluorescent H_2S probe **1** was with significant *off-on* response by using FRET-ICT dual-quenching effects (Scheme 1). The probe **1** is highly sensitive and selective towards H_2S in buffer, which can be used for bioimaging of endogenous H_2S in living cells.



Scheme 1. Chemical structures of fluorescent probes **1**-**3** and their reaction with H_2S.

1 and **2** were synthesized by conveniently coupling reactions and were well characterized by ^1H NMR, ^{13}C NMR and HR-MS (see ESI). As shown in Scheme 1, the coumarin fluorophore in **1** was dual-quenched by the FRET-ICT effects, while fluorophores in both **2** and **3** are quenched by single effect of ICT or FRET, respectively. We examined the emission spectra of probes **1**-**3** in PBS buffer (10 mM, pH 7.4). As shown in Fig. 1A, **1** showed significantly weak background fluorescence in PBS buffer, while **2** and **3** showed relative strong fluorescence under similar test condition. These results are consistent with our design that the coumarin fluorescence in **1** is heavily quenched through combined usage of FRET-ICT dual-quenching effects.

After reacting with 100 μM H_2S at room temperature overnight, a significant fluorescent *off-on* response was observed for **1** (Fig. 1B, Fig. S1), and the increase of maximal fluorescent intensity at

455 nm is more than 2000-fold for **1**. To our knowledge, this large *off-on* response is the biggest among H_2S probes in the literature.[6-11] The *off-on* response of **2** and **3** upon H_2S treatment (Fig. S2) was much smaller than that of **1** under similar condition. Therefore, we can draw the conclusion that the fluorescence turn-on fold upon reacting with H_2S for **1** is indeed greatly increased via dual-quenching effect, which agrees well with our initial hypothesis.

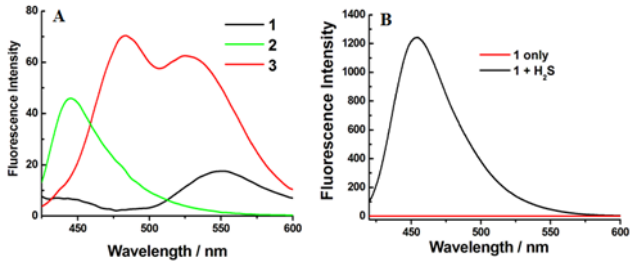


Fig. 1 (A) The fluorescence spectra of probes **1**-**3** (1 μM) upon excitation at 405 nm in PBS buffer. Slits: 10/5 nm. (B) Fluorescence response of **1** (1 μM) toward H_2S (100 μM) overnight. Slits: 2.5/5 nm.

We further examined the absorption spectra of probes in PBS buffer (Fig. S3). After reacting with H_2S, **1** and **2** displayed a decrease absorbance at 350 nm and an increase absorbance at 405 nm, respectively. H_2S-mediated cleavage of the electron-withdrawing DNP group releases oxygen donor at pH 7.4, increasing the push-pull character of the dye and resulting in large bathochromic shifts in the absorption and the ICT effect. The decrease absorbance at 510 nm (Fig. S3A) is due to the cleavage of NBD moiety from coumarin fluorophore.[13]

Encouraged by these preliminary results, we moved forward to study the reaction kinetics of the probes **1** and **2**. Both probes exhibited relative fast fluorescent increase upon H_2S treatment and showed comparable reaction rate (Fig. S4). The probe **3** showed faster reaction rate toward H_2S than that of **1** and **2**,[13] and the reaction kinetics of the dual-quenching probe **1** was mainly decided by the property of the slow reaction site DNP. A control experiment with only **1** or **2** was incubated in PBS buffer for the stability test. The results indicated that **2** was prone to slow hydrolysis at pH 7.4 (Fig. 2), while the fluorescence increase due to hydrolysis in **1** can be almost negligible, further indicating the advance of the dual-quenching probe **1**.

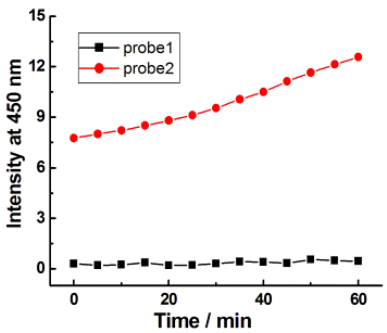


Fig. 2 Time-course experiments of 1 μM probes **1** and **2** in PBS buffer.

To gain detailed information about the sensitivity of **1**, the fluorescence intensity change was closely monitored by addition of various concentrations of H_2S into the probe (Fig. 3). The fluorescence intensity was linearly related to the concentration of H_2S from 0 to 100 μM (Fig. 3B). Specifically, the addition of even 2 μM H_2S into **1** could lead to more than 10-fold fluorescent increase. The detection limit was determined to be 0.28 μM (Fig. S5). These results clearly indicated that **1** has shown excellent sensitivity due to installation of the dual-quenching groups.

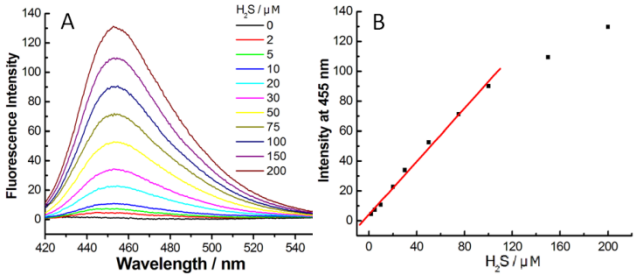


Fig. 3 Fluorescence response of **1** with different concentrations of H_2S (A, inset) and linear relationship (r = 0.9966) between fluorescence intensity and H_2S concentration (B).

In order to evaluate the specific nature of **1** for H_2S, it was incubated with various biological-related species in PBS buffer and the fluorescent increase at 455 nm was measured accordingly (Fig. 4). Among all the tested molecules, only biothiols showed noticeable fluorescence response. The fluorescence increase of biothiols (Cys and GSH), however, is limited and far below that of H_2S. As evidenced by these results, probe **1** is selective to H_2S for further biological applications. In addition, we also investigated the fluorescence response of probe **1** to H_2S under different pH values (Fig. S5). Results indicated that the probe can function over a wide range of pH from 6.0 to 8.5.

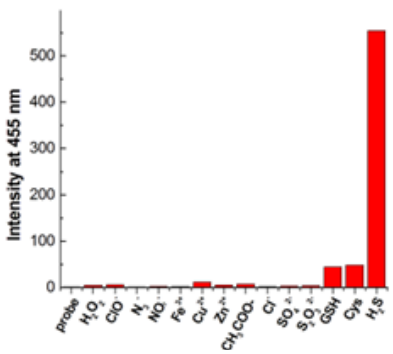


Fig. 4 Fluorescent intensity at 455 nm for **1** (1 μM) toward different species (1 mM) in PBS buffer. All reactions were performed in PBS (pH 7.4) at 37 ℃ for 30 min.

To demonstrate the biological applicability of probe **1**, we examined whether **1** can be used to detect intracellular H_2S in living cells. HEK293A and HeLa cells were treated with probe **1** (2 μM) for 30 min and then with different concentrations of Na_2S (50 and 200 μM) for another 30 min. Bright-field microscopy images show that the two cell types both retained good morphology after incubation with **1**. The fluorescent microscopy results (Fig. S6, S7) clearly indicated that probe **1** can be used for imaging exogenous H_2S in living cells in a concentration-dependent fashion. The cytotoxicity of the probe **1** was further evaluated using HEK293 by MTT assay (Fig. S9). The probe **1** did not show significant cytotoxicity at 0.5-2 μM range, implying that the probe is suitable for bioimaging of H_2S in living cells. To

test whether **1** could detect endogenous production of H_2S, cells were treated with Cys and then with **1**. As shown in Fig. 5, HEK293A cells with **1** displayed a fairly weak fluorescence under confocal fluorescence microscope (Fig. 5A). After using Cys to induce endogenous H_2S production,[10f] significantly fluorescent enhancement can be observed (Fig. 5B), implying that **1** can be used for bioimaging of endogenous H_2S in HEK293A cells. Above results suggest that **1** is cell-permeable and can react with intracellular H_2S efficiently.

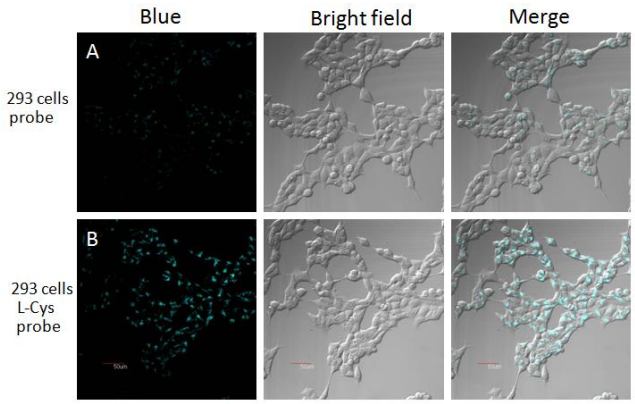


Fig. 5 Confocal microscopy images of intracellular H_2S detection in living HEK293A cells using **1**. HEK293 cells were incubated with (A) **1** (2 μM) for 30 min; (B) L-cysteine (200 uM) for 30 min and then **1** (2 μM) for 30 min. Emission was collected at blue channel (425-475 nm) with 405 nm excitation. Scale bar, 50 μm.

In summary, we report the first fluorescent H_2S probe based on FRET-ICT dual-quenching effects. Preliminary tests indicated that probe **1** has potential to image endogenous H_2S in living cells. The fluorescent enhancement of the probe upon H_2S treatment can reach more than 2000 folds, which is the biggest among probes in the literature. Compared with other DNP-based H_2S probes,[10] the probe **1** has large *off-on* response for H_2S and better stability toward hydrolysis. However, the FRET-based probes by Yuan[10n] and our group[11a] exhibited ratiometric behaviors for H_2S, which are more accurate in bioimaging than that of the turn-on probe. The development of fast-response and ratiometric H_2S probes based on the dual-quenching strategy is certainly deserved to perform. We believe that this design strategy could be employed as a general method for preparation of other highly sensitive and selective H_2S probes in future.

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Notes and references

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1 a) C. Szabó, *Nat. Rev. Drug Discovery*, **2007**, *6*, 917-935; b) L. Li, P. Rose, P. K. Moore, *Annu. Rev. Pharmacol. Toxicol.*, **2011**, *51*, 169-187.
2 a) B. Predmore, D. Lefer, G. Gojon, *Antioxid. Redox Signaling*, **2012**, *17*, 119-140; b) M. Whiteman, P. K. Moore, *J. Cell. Mol. Med*., **2009**, *13*, 488-507; c) H. Kimura, *Exp. Physiol.*, **2011**, *96*, 833-835.
3 a) G. D. Yang, L. Y. Wu, B. Jiang, W. Yang, J. S. Qi, K. Cao, Q. H. Meng, A. K. Mustafa, W. T. Mu, S. M. Zhang, S. H. Snyder, R. Wang, *Science*, **2008**, *322*, 587-590; b) L. F. Hu, M. Lu, Z. Y. Wu, P. T. Wong, J. S. Bian, *Mol. Pharmacol*., **2009**, *75*, 27-34; c) K. H. Kulkarni, E. M. Monjok, R. Zeyssig, G. Kouamou, O. N. Bongmba, C. A. Opere, Y. F. Njie, S. E. Ohia, *Neurochem. Res*., **2009**, *34*, 400-406; d) L. Li, M. Bhatia, P. K. Moore, *Curr. Opin. Pharmacol*., **2006**, *6*, 125-129; (e) H. Kimura, *Amino Acids*, **2011**, *41*, 113-121.
4 S. Fiorucci, E. Antonelli, A. Mencarelli, S. Orlandi, B. Renga, G. Rizzo, E. Distrutti, V. Shah, A. Morelli, *Hepatology*, **2005**, *42*, 539-548.
5 a) D. Jiménez, R. Martinez-Manez, F. Sancenon, J. V. Ros-Lis, A. Benito, J. Soto, *J. Am. Chem. Soc*., **2003**, *125*, 9000-9001; b) D. G. Searcy, M. A. Peterson, *Anal. Biochem.*, **2004**, *324*, 269-275; c) N. S. Lawrence, J. Davis, L. Jiang, T. G. J. Jones, S. N. Davies, R. G. Compton, *Electroanalysis*, **2000**, *18*, 1453-1460; d) J. Radford-Knoery, G. A. Cutter, *Anal. Chem.*, **1993**, *65*, 976-982.
6 a) A. R. Lippert, E. J. New, C. J. Chang, *J. Am. Chem. Soc.*, **2011**, *133*, 10078-10080; b) H. J. Peng, Y. F. Cheng, C. F. Dai, A. L. King, B. L. Predmore, D. J. Lefer, B. H. Wang, *Angew. Chem. Int. Ed.*, **2011**, *50*, 9672-9657; c) L. A. Montoya, M. D. Pluth, *Chem. Commun.*, **2012**, *48*, 4767-4769; d) S. Chen, Z. J. Chen, W. Ren, H. W. Ai, *J. Am. Chem. Soc.*, **2012**, *134*, 9589-9592; e) S. K. Das, C. S. Lim, S. Y. Yang, J. H. Han, B. R. Cho, *Chem. Commun.*, **2012**, *48*, 8395-8397; f) B. F. Chen, W. Li, C. Lv, M. M. Zhao, H. W. Jin, H. F. Jin, J. B. Du, L. R. Zhang, X. J. Tang, *Analyst*, **2013**, *138*, 946-951; g) W. Sun, J. L. Fan, C. Hu, J. F. Cao, H. Zhang, X. Q. Xiong, J. Y. Wang, S. Cui, S. G. Sun, X. J. Peng, *Chem. Commun.*, **2013**, *49*, 3890-3892; h) V. S. Lin, A. R. Lippert, C. J. Chang, *Proc. Natl. Acad. Sci. U.S.A.*, **2013**, *110*, 7131-7135; i) G. D. Zhou, H. L. Wang, H. L. Ma, X. Q. Chen, *Tetrahedron*, **2013**, *69*, 867-870.
7 For recent reviews: a) V. S. Lin, W. Chen, M. Xian, C. J. Chang, *Chem. Soc. Rev.*, **2015**, DOI: 10.1039/C4CS00298A; b) F. Yu, X. Han, L. Chen, *Chem. Commun.*, **2014**, *50*, 12234-12249.
8 a) F. B. Yu, P. Li, P. Song, B. S. Wang, J. Z. Zhao, K. L. Han, *Chem. Commun.*, **2012**, *48*, 2852-2854; b) Q. Q. Wan, Y. C. Song, Z. Li, X. H. Gao, H. M. Ma, *Chem. Commun.*, **2013**, *49*, 502-504; c) S. K. Bae, C. H. Heo, D. J. Choi, D. Sen, E. H. Joe, B. R. Cho, H. M. Kim, *J. Am. Chem. Soc.*, **2013**, *135*, 9915-9923; d) L. Zhang, S. Li, M. Hong, Y. Xu, S. Wang, Y. Liu, Y. Qian, J. Zhao, *Org. Biomol. Chem.*, **2014**, *12*, 5115-5125; e) B. Chen, P. Wang, Q. Jin, X. Tang, *Org. Biomol. Chem.*, **2014**, *12*, 5629-5633.
9 a) K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura, T. Nagano, *J. Am. Chem. Soc*., **2011**, *133*, 18003-18005; b) J. L. Long, L. P. Wang, D. Xie, Y. W. Zhan, *J. Luminescence*, **2013**, *139*, 40-46.
10 a) C. R. Liu, J. Pan, S. Li, Y. Zhao, L. Y. Wu, C. E. Berkman, A. R. Whorton, M. Xian, *Angew. Chem. Int. Ed.*, **2011**, *50*, 10327-10329; b) J. Liu, Y. Q. Sun, J. Y. Zhang, T. Yang, J. B. Cao, L. S. Zhang, W. Guo, *Chem. Eur. J.*, **2013**, *19*, 4717-4722; c) X. W. Cao, W. Y. Lin, K. B. Zheng, L. W. He, *Chem. Commun.*, **2012**, *48*, 10529-10531; d) Y. C. Chen, C. C. Zhu, Z. H. Yang, J. J. Chen, Y. F. He, Y. Jiao, W. J. He, L. Qiu, J. J. Cen, Z. J. Guo, *Angew. Chem. Int. Ed.*, **2013**, *52*, 1688-1691; e) Z. Xu, L. Xu, J. Zhou, Y. F. Xu, W. P. Zhu, X. H. Qian, *Chem. Commun.*, **2012**, *48*, 10871-10873; f) Y. Qian, J.

Karpus, O. Kabil, S. Y. Zhang, H. L. Zhu, R. Banerjee, J. Zhao, C. He, *Nat. Commun.*, **2011**, *2*, 495; g) W. Sun, J. L. Fan, C. Hu, J. F. Cao, S. G. Sun, F. L. Song, J. Y. Wang, X. Peng, J. *Org. Lett.*, **2013**, *15*, 4022-4025; h) X. Wang, J. Sun, W. Zhang, X. Ma, J. Lv, B. Tang, *Chem. Sci.*, **2013**, *4*, 2551-2556; i) Y. Qian, L. Zhang, S. T. Ding, X. Deng, C. He, X. E. Zheng, H. L. Zhu, J. Zhao, *Chem. Sci.*, **2012**, *3*, 2920-2923; j) L. Yuan, Q.-P. Zuo, *Sensor Actuat. B-Chem.*, **2014**, *196*, 151-155; k) T. Liu, Z. Xu, D. R. Spring, J. Cui, *Org. Lett.*, **2013**, *15*, 2310-2313; l) L. Yuan, Q.-P. Zuo, *Chem. Asian J.*, **2014**, *9*, 1544-1549; m) Z. Huang, S. Ding, D. Yu, F. Huang, G. Feng, *Chem. Commun.*, **2014**, *50*, 9185-9187; n) L. Yuan, F. Jin, Z. Zeng, C. Liu, S. Luo, J. Wu, *Chem. Sci.*, **2015**, DOI: 10.1039/C4SC03883E.

11 a) L. Wei, L. Yi, F. B. Song, C. Wei, B. F. Wang, Z. Xi, *Sci. Rep.*, **2014**, *4*, 4521; b) C. Wei, R. Wang, L. Wei, L. Cheng, Z. Li, Z. Xi and L. Yi, *Chem. Asian J.*, **2014**, *9*, 3586-3592.

12 H. Zhang, C. Zhang, R. Liu, L. Yi and H. Sun, *Chem. Commun.*, **2015**, *51*, 2029-2032.

13 C. Wei, L. Wei, Z. Xi, L. Yi, *Tetrahedron Lett.*, **2013**, *54*, 6937-6939.

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