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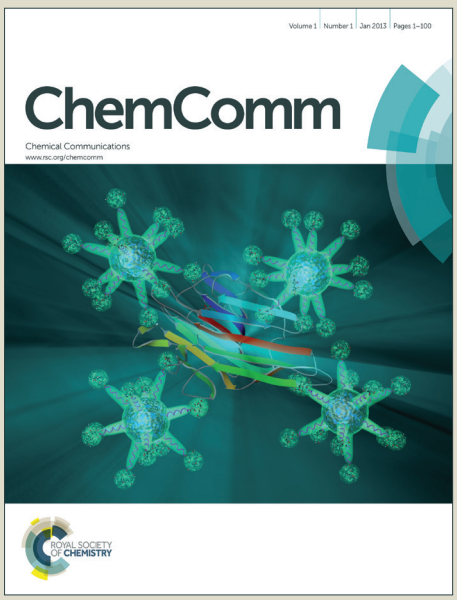

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A Fluorescent Probe for H_2S in vivo with Fast Response and High Sensitivity

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In this work, we design and synthesize a new near-infrared (NIR) ratiometric fluorescent probe FD-H_2S for the highly sensitivity (DL 68.2 nM) detection of H_2S with fast response (15 s), large emission shift (220 nm) and excellent enhancement (168-fold in ratiometric value). The probe could be applied to monitor and imaging exogenous or endogenous H_2S in live MCF-7 cells and in live mice with the fastest response.

Hydrogen Sulfide (H_2S), which is a foul-smelling gas and commonly perceived as a toxic industrial exhalation, has recently been found as an important endogenous gaseous signaling[1, 2] compound along with canbon monoxide[3] (CO) and nitric oxide[4] (NO). It can be produced from L-cysteine in reactions catalyzed by cystathionnine-β-synthase (CBS) or cystathionine-γ-lyase (CES)[2], and converted within different organs and tissues[5]. Whereas, most publications reported that H_2S concentration is in the range of 10-100 μM in blood[6], and lower endogenous H_2S concentrations in normal cells[7, 8]. As a signal molecule, H_2S regulates a broad array of physiological responses[7, 9-12] (vasodilation, cardioprotrction, inflammation, angiogenesis etc) to maintain cellular health. Furthermore, it acts as a scavenger or antioxidant of reactive oxygen species (ROS)[12]. However, studies had established that the abnormal level of H_2S in cells may cause many types of diseases including Down's syndrome, Alzheimer's disease, liver cirrhosis and diabetes[5, 13, 14]. Despite the many physiological effects of H_2S in cellular and whole animal studies, accurate molecular targets of H_2S are still being unraveled and remain an important goal. Therefore, monitoring cellular H_2S concentration is significant for biological research and clinical diagnoses[15]. So, it is of great vital to develop efficient methods for detecting H_2S in biosystem.

Many chemical methods have been developed to detect H_2S, such as colorimetric[16], gas chromatography[17], electrochemical analysis[18] and fluorescent probes[19-21]. Fluorescence imaging technology is regarded as a promising method for monitoring biological species in living cells owing to its high sensitivity, selectivity, in particular toward nondestructive characteristics[22-24]. In recent years, some fluorescent probes for H_2S have been reported. For example, Wang group used dansyl derivative **DNS-Az** as a chemodosimeter for H_2S rapid detection in blood based on the fluorescence intensity; Chang[25, 26] group synthesized a series of H_2S-responsive fluorescent probes based on azido-fluorescein or rhodamine, for investigations of H_2S in live cells; Lin[27, 28] group developed two cases of fluorescence probe for detecting H_2S with minutes level (<7min) response speed; very recently, Pluth[29] group reported firstly an azide-functionalized O-methylrhodol fluorophore **MeRho-Az** which exhibited 3D imaging with light sheet fluorescence microscopy (LSFM) to visualize H_2S in the intestinal tract of live transparent zebrafish. All these researches inspire us to develop a probe which could be used in real animal tissues with fast and sensitive fluorescence response.

In general, it is very difficult to detect the concentration of H_2S instantaneously in vitro, as the response reaction always needs long time (such as 20 mins). It is important to explore fast and selective response reactions of fluorophore with H_2S, for probe-designing. In another aspect, few probes of live animal H_2S-imaging in biological studies exist due to the insurmountability challenges connected with sensibility and transitioning from cell culture to live animal tissues. In contrast, ratiometric NIR fluorescent probes endowing deep penetration and a self-calibration effect can greatly eliminate and interferences including micro-environment, probe concentration and instrument efficiency, are more favorable for H_2S monitoring and imaging in live animal tissues.

Keeping these considerations in mind, herein, we report a new near-infrared (NIR) ratiometric fluorescent chemosensor **FD-H_2S** for the highly selective and sensitivity detection of H_2S in vitro and in vivo. As we all know, flavylium derivative[30-33] is a commonly used fluorophore, and well-known for its NIR emission wavelength. Using the fluorophore and taking advantage of Michael addition of H_2S with accelerated rate, due to its strongly electron-withdrawing merit. We designed a sulfide-sensitive agent, **FD-H_2S**, in **Scheme S** (in ESI). We reasoned that the nucleophilic attack of HS^- (the main stable form of H_2S in the physiological condition) toward the

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benzopyrylium moiety of **FD-H_2S** will interrupt the π-conjugation, therefore leading to the decrease of the NIR emission. On the other hand, since **FD-H_2S** contains an intact 7-diethylaminecoumarin fluorophore, it can display coumarin emission peaks after adding H_2S. Therefore, two well-resolved emission peaks before and after the addition of H_2S could be obtained due to the distinct emission between **FD-H_2S** and the produced coumarin fluorophore, emabling the detection of H_2S with the efficient ratiometric sensing with highly selectivity over biothiols and other biologically related species, excellent sensitive (DL 68.2 nM), fast response (15 s) and large ratiometric fluorescent reponse (168-fold enhancement in ratiometric value with 220 nm emission shift). **FD-H_2S** was applied to monitor H_2S changes via fluorescence imaging in the presence of exogenous or endogenous H_2S in live MCF-7 cells, and firstly in mice with fast response.

With **FD-H_2S** probe in hand, initially, the spectroscopic properties (**Fig. S1**) of **FD-H_2S** (10 μM) were evaluated in 0.01 M PBS solution (acetonitrile /water = 3:7, pH 7.4). Free **FD-H_2S** has two intense absorption at 627 nm (ε=4.7×10^4 M^{-1} cm^{-1}) and 676 nm (ε=4.3×10^4 M^{-1} cm^{-1}) and a corresponding emission maximum at 707 nm with moderate fluorescent intensity as expected.

Upon the addition of NaHS (commonly exogenous donor of H_2S), the absorption peak at 627 nm and 676 nm decreased, while the peak at 425 nm increased accordingly (**Fig. S2**), which the colour of the solution turned from blue to slight yellow, enabling colorimetric detection of H_2S by the naked eyes. Furthermore, the absorbance ratio A_{425}/A_{625} and A_{425}/A_{676} have good linear correlation with NaHS concentration range from 0 to 260 μM (**Fig. S3**), and the detection limit (3σ/k) was calculated as low as 26.2 nM, 51.4 nM, respectively, much lower than previously reported probes. Thus, this colorimetric sensor can realize accurate quantitative detection for H_2S with low detection limit.

In the corresponding fluorescence spectra, the fluorescence emission intensity gradually increased at 487 nm (Φ_F = 0.005) and decreased at 707 nm (Φ_F = 0.024) (**Fig. 1a**) upon excitation at 430 nm. Such a large shift in emission (220 nm) make this probe favorable for dual emission ratiometric sensing with a drastic 168-fold enhancement in the emission intensity ratio (F_{487}/F_{707}) from 0.46 in the absence of NaHS to 77.34 in the presence of 122 equiv NaHS. In addition, the intensity ratios (F_{487}/F_{707}) were plotted as a function of NaHS concentration and a typical calibration graph was observed (**Fig. 1b**). Moreover, an excellent linearity was obtained between the fluorescence intensity of **FD-H_2S** and NaHS concentration in the range of 0-100 μM in the PBS buffer. The fluorescent detection limit (3σ/k) was 68.2 nM in PBS buffer, which also was much lower than most reported ratiometric and colorimetric H_2S probes.

As showed in **Fig. 1d**, the spectral response reaction could be completed within 15 s. The fast response implied that the probe would provide a rapid detection of H_2S.

According to H_2S (*pK*a 6.8) as a nucleophile, the spectroscopic properties of **FD-H_2S** changes after adding NaHS could be attributed to the formation of compound **FDB-H_2S**, which was verified by ^1H NMR titration experiment with different amounts of NaHS in DMSO-d_6/$CDCl_3$/CD_3OD (1/1/1, v/v/v) solution. In the presence of NaHS, the signal at 7.66 ppm (H_a) and 6.81 ppm (H_b), assigned to

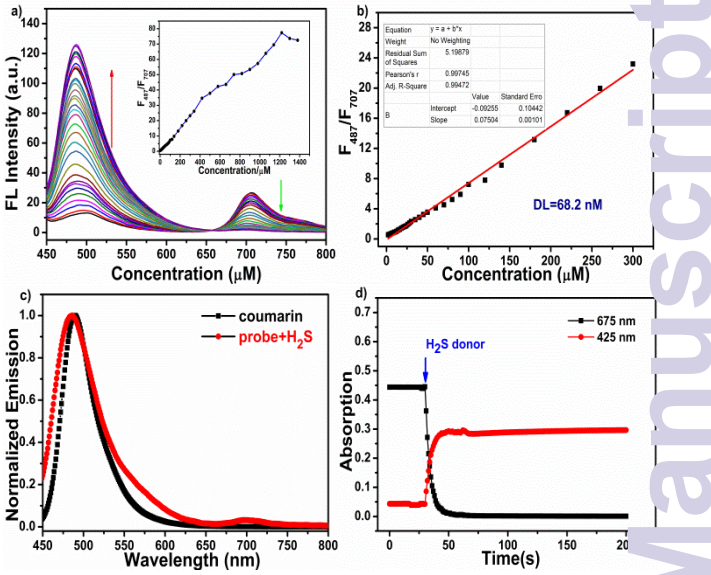

Fig. 1 The spectroscopic properties of **FD-H_2S** (10 μM) in PBS (0.01 M) solution (acetonitrile /water = 3:7, pH 7.4). (a) Fluorescence responses of **FD-H_2S** (10 μM) toward different concentrations of NaHS (titration concentration: 0-138 equiv of NaHS in aqueous solution), inset: the fluorescence intensity ratio (F_{487}/F_{707}) of **FD-H_2S** (10 μM) vs. increasing concentrations of NaHS; Each spectrum was obtained 2 min after NaHS addition. (b) Fluorescence intensity ratio (F_{487}/F_{707}) changes of **FD-H_2S** (10 μM) with 0-30 equiv of NaHS. (c) The normalized fluorescence emission of **FD-H_2S** (10 μM) with 100 equiv NaSH was compared with pure coumarin **3**. (d) Time dependence of absorption profiles of **FD-H_2S** (10 μM) with 100 equiv NaHS. λ_{ex} = 430 nm, slit: 5/10 nm.

the protons on the benzopyrylium moiety in **FD-H_2S**, were gradually decreased and finally disappeared while new peaks at 6.76 ppm and 3.83 ppm appeared and increased with increasing the addition amounts of NaHS (**Fig. S9**). This result clearly indicated the formation of **FDB-H_2S**. For a further confirmation, mass spectrometry analysis of the mixture of **FD-H_2S** and NaHS, and one apparent peak at m/z 475.68, corresponding to the hydrogenated product [**FDB-H_2S**+H]$^+$ (calcd 475.21 for $C_{28}H_{32}N_2O_3S^+$) was shown in MS data (**Fig. S10**). In addition, the normalization emission spectra of FDB-H_2S is highly similar to those of coumarin **3** (**Fig. 1c**), which further demonstrated the disruption of the π-electron conjugation in probe **FD-H_2S**. The results provide support for H_2S recognition mechanism depicted in **Scheme S1** (in ESI).

In the 0.01 M PBS buffer (acetonitrile /water = 3:7, pH 7.4), the present probe was treated with a amount of ions (including K^+, Na^+, Mg^{2+}, Zn^{2+}, F^-, NO_2^-, NO_3^- and SCN^-), ROS (including NaClO and H_2O_2), reducing substances and biothiols (such as ascorbic acid, GSH and Cys) to examine its selectivity (**Fig. 2a**). Only NaHS could cause a dramatic increment of emission ratio F_{487}/F_{707} along with the clear change (**Fig. S5**), while others exhibited very minor changes in fluorescence behavior (**Fig. S6**). The coexistence of other analytes did not disturb with the reaction of NaHS with probe **FD-H_2S** (**Fig. S11**).

The influence of pH on fluorescent intensity ratio (F_{487}/F_{707}) was investigated by pH titration (**Fig. S7a**). **FD-H_2S** exhibited little changes in emission in the range of pH 4.5-9.5, which indicated that fluorescence imaging would not be disturbed by cellular pH micro environment. In physiological pH values (**Fig. S7b**), the probe could

keep stable for long time (over 60 mins), which is prerequisite for long term fluorescence imaging experiments.

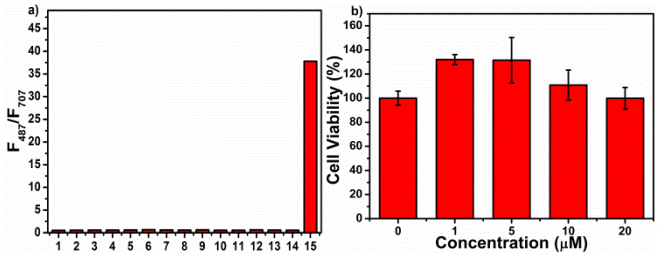

Fig. 2 (a) Fluorescence responses of **FD-H_2S** (10 μM) toward various biospecies (1 mM except for notation). 1, probe alone; 2, K^+; 3, Na^+; 4,Mg^{2+}; 5, Zn^{2+}, 6, F^-; 7,NO_2^-; 8, NO_3^-; 9, SCN^-; 10, ascorbic acid; 11,GSH; 12,Cys; 13, NaClO; 14, H_2O_2; 15, NaHS (0.40 mM). (b) Biological toxicity of **FD-H_2S** in living MCF-7 cells for 12 h. Cell viabilities were examined using Thermo Fisher Scientific. Error bar = RSD (n = 5).

In light of the above favorable spectroscopic properties of **FD-H_2S** for detecting H_2S, including high selectivity，high sensitivity and rapid response in physiological pH range, we studied its utility for monitoring H_2S in biological systems. Firstly, in order to assess the cytotoxicity of **FD-H_2S**, we performed 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assays in MCF-7 cells with 1, 5, 10, 20 μM probe **FD-H_2S** for 12 h, respectively. The obtained results clearly shown that probe **FD-H_2S** exhibited non-toxic to cultured cells under the experimental conditions at the concentration < 10 μM for 12 h (**Fig. 2b**).

Incubating MCF-7 cells with **FD-H_2S** (10 μM) for 30 min (**Fig. 3e-h**), weak fluorescence signals in green channel (460-510 nm) of confocal microscope FV1000 were collected, meanwhile, strongish fluorescence signals in red channel (655-755 nm) also were collected, which shown the remarkable membrane permeability of probe **FD-H_2S**. Then treated the cells with 200 μM NaHS at 37 °C for 20 min (**Fig. 3i-l**), the faint green fluorescence brightened (**Fig. 3i**) and the strongish red fluorescence diminished (**Fig. 3j**). The corresponding merged image of green channel and red channel were clearly distinguished, for the detection of exogenous H_2S in living MCF-7 cells.

When MCF-7 cells were stimulated with 300 μM L-cysteine (leading to an increased celluar H_2S concentration) at 37 °C for 30 min (**Fig. 3m-p**), and then treated with **FD-H_2S** (10 μM) for 30 min, eliciting bright fluorescence signal in the green channel (**Fig. 3m**) and weak fluorescence signal in the red channel (**Fig. 3n**) as expected, which implied that this probe also could monitor endogenesis H_2S in complex biological system. On the contrary, we also set a control experiment that MCF-7 cells were pre-incubated with 200 μM N-methylmaleimide (NMM) for 30 min to delete intracellular H_2S, and then treated with **FD-H_2S** (10 μM) at 37 °C for 30 min . As is shown in **Fig. 3a-d**, there were no noticeable fluorescence intensity in the green channel and obvious fluorescence intensity in the red channel. In addition, further to evaluate the practical value of our proposed probe **FD-H_2S**, the F_{Green}/F_{Red} could be signified the relative concentration of hydrogen sulfide. So, we used mathematical statistics methods to quantify different treatment of MCF-7 cells (**Fig. S20**). The results indicated: i) after inhibited by 200 μM NMM, the concentration of H_2S in the MCF-7 cells was decreased by 56%; ii) after cultured in exogenous 200 μM NaHS solution, the concentration of H_2S in the cells was increased by 85%; after incubated with L-cysteine, the concentration of H_2S was raised by 140% (**Fig. S20r**). Therefore, the mathematical statistics quantization may elucidate the relative concentration of basal exogenous and endogenesis H_2S in living MCF-7 cells.

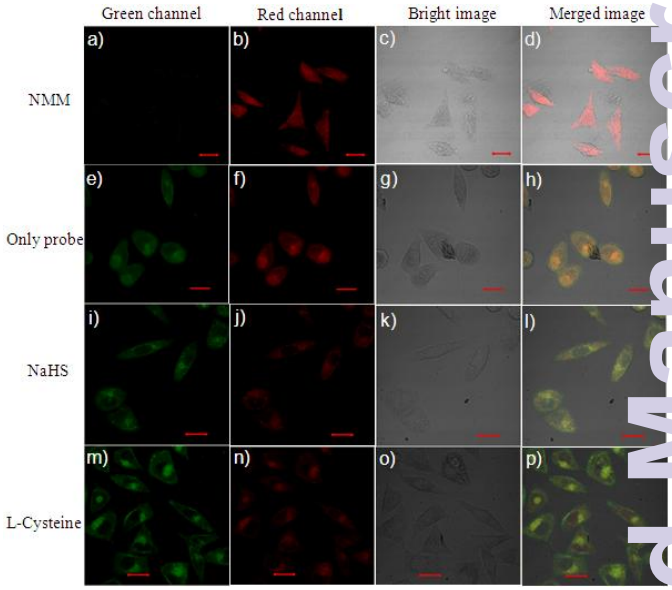

Fig. 3 Fluorescence image of H_2S in MCF-7 cells. the excitation wavelength was 405 nm and the emission was collected at 460-510 nm (green channel) and 655-755 nm (red channel), merged image generated from green to red channel: a-d) Cells were pre-incubated with 500 μM NMM at 37 °C for 60 min, and further incubated with **FD-H_2S** (10 μM) for 30 min. e-h) Cells were incubated with **FD-H_2S** (10 μM) at 37 °C for 30 min. i-l) Cells were pre-incubated with **FD-H_2S** (10 μM) for 30 min, and then treated with 200 μM NaHS at 37 °C for 20 min. m-p) Cells were pre-incubated with 300 μM L-Cysteine at 37 °C for 30 min, and then treated with **FD-H_2S** (10 μM) at 37 °C for 30 min. Scale bar = 20μm.

It is well known that many delocalized lipophilic cationic dyes possess an overall positive charge, which can be easily taken up and accumulate in the mitochondria of living cells. So, we sought to examine whether **FD-H_2S** can localize to the mitochondria. A commercially available, mitochondria-localizing dye (Mito Tracker Green FM) was employed for a co-localization study in MCF-7 cells (**Fig. S22a-b**). As shown in **Fig. S22** the fluorescence patterns of **FD-H_2S** and Mito Tracker Green FM signals overlapped perfectly (**Fig. S22d**) with a higher co-localization coefficient (Pearson's correlation) of 0.97 (**Fig. 22e**), and only minor the overlap of fluorescence pattern for lysosomes (**Fig. S21**). Hence, all results confirmed that **FD-H_2S** can specifically localize in mitochondria and be used to monitor H_2S in the mitochondria of living cells.

Finally, the above desirable results encouraged us the suitability of the probe for monitoring H_2S in the context of living animals. As the autofluorescence produced in short wavelengths in vivo, the green channel of the probe might be interfered. Therefore, we just tested the images in red channel. First, a PBS/DMSO/CH_3CN (28:12:1, v/v/v) solution (100 μL) containing **FD-H_2S** (50 μM) was introduced by peritoneal cavity injection into shaved living mice; a bright fluorescent signal was collected immediately (**Fig. 4b**). Then a 100 μL PBS solution containing NaHS (150 μM) was peritoneal cavity injected, and it was clear that the fluorescence signal was weaker with 1 min incubation (**Fig. 4c**). Moreover, **Fig. 4d** shows that there was almost no fluorescence signal with only 6 min corresponding with in vitro (**Fig. S4**), and the fluorescence intensity keep

unchanged as the time went on (**Fig. 4f-h**). In contrast, when shaved living mice were injected into peritoneal cavity with a PBS/DMSO/CH_3CN (28:12:1, v/v/v) solution (150 μL) containing **FD-H_2S** (80 μM), however, no obvious change in red channels was observed up to nearly 1 hour (**Fig. S23a-l**), implying that the weaken red channel was not caused by organism circulation or photobleaching. To the best of our knowledge, these results demonstrate that the new NIR probe **FD-H_2S** is the fastest response towards H_2S in the living animals.

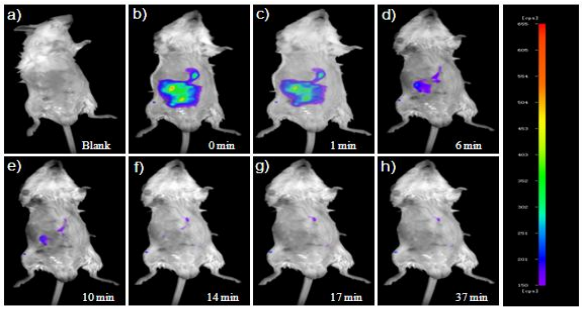

Fig. 4 Representative fluorescent images of H_2S in mice. (a) Without any treatment of mouse was as control; (b) only the probe **FD-H_2S** was injected in the peritoneal cavity of the mouse; (c) The probe was injected in the peritoneal cavity of the mouse and injected with NaHS. Images were taken after incubation of different times (1 min (c), 6 min (d), 10 min (e), 14 min (f), 17 min (g), 37 min (h)) with the excitation filter at 630 nm and the emission filter set at 700 ± 20 nm.

Conclusions

In summary, we have developed the probe **FD-H_2S**, which has an excellent linear ratiometric fluorescence rapid response to H_2S with ultra-low detection limit. The ratiometric probe displays 220 nm blue shift in emission upon reaction with H_2S, which contributes to the observation of dual-channel fluorescence signal changes with high resolution. Furthermore, **FD-H_2S** localizes in mitochondria via the co-localization experiment. We also use the mathematical statistics of quantization for elucidating the relative concentration of basal, exogenous and endogenesis H_2S in biological systems. In red channel, the probe is the fastest response towards H_2S in living mice. We expect that this probe to be useful in chemical and biological applications.

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Notes and references

1. M. M. Gadalla and S. H. Snyder, *J. Neurochem.*, 2010, **113**, 14-26.
2. N. Sen, B. D. Paul, M. M. Gadalla, A. K. Mustafa, T. Sen, R. Xu, S. Kim and S. H. Snyder, *Mol. Cell*, 2012, **45**, 13-24.
3. H. P. Kim, S. W. Ryter and A. M. Choi, *Annu. Rev. Pharmacol. Toxicol.*, 2006, **46**, 411-449.
4. H. Yu, Y. Xiao and L. Jin, *J. Am. Chem. Soc.*, 2012, **134**, 17486-17489.
5. W. Yang, G. Yang, X. Jia, L. Wu and R. Wang, *J. Physiol.*, 2005, **569**, 519-531.
6. H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. Wang, *Angew. Chem., Int. Ed.*, 2011, **50**, 9672-9675.
7. N. L. Whitfield, E. L. Kreimier, F. C. Verdial, N. Skovgaard and K. R. Olson, *Am. J. Pathol.*, 2008, **294**, R1930-R1937.
8. J. Furne, A. Saeed and M. D. Levitt, *Am. J. Pathol.*, 2008, **295**, R1479-R1485.
9. J. W. Calvert, S. Jha, S. Gundewar, J. W. Elrod, A. Ramachandran, C. B. Pattillo, C. G. Kevil and D. J. Lefer, *Circ. Res.*, 2009, **105**, 365-374.
10. C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Módis, P. Panopoulos, A. Asimakopoulou, D. Gerö, I. Sharina and E. Martin, *Proc. Natl. Acad. Sci.*, 2012, **109**, 9161-9166.
11. R. C. Zanardo, V. Brancaleone, E. Distrutti, S. Fiorucci, G. Cirino and J. L. Wallace, *FASEB J.*, 2006, **20**, 2118-2120.
12. V. S. Lin, W. Chen, M. Xian and C. J. Chang, *Chem. Soc. Rev.*, 2015, **44**, 4596-4618.
13. K. Eto, T. Asada, K. Arima, T. Makifuchi and H. Kimura, *Biochem. Biophys. Res. Commun.*, 2002, **293**, 1485-1488
14. D. Jiménez, R. Martínez-Máñez, F. Sancenón, J. V. Ros, A. Benito and J. Soto, *J. Am. Chem. Soc.*, 2003, **125**, 9000-9001.
15. X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120-2135.
16. M. N. Hughes, M. N. Centelles and K. P. Moore, *Free Radical Biol. Med.*, 2009, **47**, 1346-1353.
17. J. Radford-Knoery and G. A. Cutter, *Anal. Chem.*, 1993, **976-982**.
18. D. G. Searcy and M. A. Peterson, *Anal. Biochem.*, 2004, **324**, 269-275.
19. F. Yu, P. Li, P. Song, B. Wang, J. Zhao and K. Han, *Chem. Commun.*, 2012, **48**, 2852-2854.
20. W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, S. Cui, S. Sun and X. Peng, *Chem. Commun.*, 2013, **49**, 3890-3892.
21. D. Zhang, W. Chen, J. Kang, Y. Ye, Y. Zhao and M. Xian, *Org. Biomol. Chem.*, 2014, **12**, 6837-6841.
22. J. Fan, H. Mu, H. Zhu, J. Wang and X. Peng, *Analyst*, 2015, **140**, 4594-4598.
23. X. Wang, Z. Guo, S. Zhu, H. Tian and W. Zhu, *Chem. Commun.*, 2014, **50**, 13525-13528.
24. H. Zhu, J. Fan, J. Wang, H. Mu and X. Peng, *J. Am. Chem. Soc.*, 2014, **136**, 12820-12823.
25. A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078-10080.
26. V. S. Lin, A. R. Lippert and C. J. Chang, *Proc. Natl. Acad. Sci.*, 2013, **110**, 7131-7135.
27. K. Zheng, W. Lin and L. Tan, *Org. Biomol. Chem.*, 2012, **10**, 9683.
28. X. Cao, W. Lin, K. Zheng and L. He, *Chem. Commun.*, 2012, **48**, 10529.
29. M. D. Hammers, M. J. Taormina, M. M. Cerda, L. A. Montoya, D. T. Seidenkranz, R. Parthasarathy and M. D. Pluth, *J. Am. Chem. Soc.*, 2015.**137**, 10216-10223.
30. B. Eberhard, *Anal. Chem.*, 1986, **58**, 384-387.
31. H. Chen, W. Lin, W. Jiang, B. Dong, H. Cui and Y. Tang, *Chem Commun.*, 2015, **51**, 6968-6971.
32. J. Liu, Y. Sun, J. Zhang, T. Yang, J. Cao, L. Zhang and W. Guo, *Chemistry - A European Journal*, 2013, **19**, 4717-4722.
33. H. Lv, X.-F. Yang, Y. Zhong, Y. Guo, Z. Li and H. Li, *Anal. Chem.*, 2014, **86**, 1800-1807.