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Single near-infrared fluorescent probe with high- and low-sensitivity sites for sensing different concentration ranges of biological thiols with distinct modes of fluorescence signals†

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We describe a unique approach for the development of an interesting type of the fluorescent probes, which can show different modes of fluorescence signals to distinct concentration ranges of a target of interest. The key points for the design of the new type of the fluorescent probes include the judicious selection of the dye platforms and the corresponding high- and low-sensitivity sites. It is known that the normal concentrations of biological thiols have significant biological functions. However, up- or down-regulated concentrations of thiols may induce several diseases. Therefore, it is highly important to monitor the changes of thiol concentrations in living systems. Based on the proposed strategy, we engineer the novel NIR fluorescent probe, CHMC-thiol, which remarkably can display a turn-on signal to the low concentration range of thiols and a ratiometric response to the high concentration range of thiols for the first time. We anticipate that the intriguing strategy formulated herein will be widely useful for the development of concentration range-dependent fluorescent probes.

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Introduction

Small-molecular-weight biological thiols, including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), play a critical role in many biological processes. However, abnormal concentrations of Cys, Hcy and GSH are implicated in a variety of diseases, such as liver damage, skin lesions, slowed growth and edema.^{1–3} Therefore, it is highly important to monitor the changes of concentrations of thiols in living systems.

Although a large amount of fluorescent probes have been developed,^{4–7} among them only a few single fluorescent probes are capable of monitoring multiple targets in living systems with distinct fluorescence signals.⁸ A single fluorescent probe with the ability of sensing multiple targets in a consecutive manner imparts several advantages over the combination of several fluorescent probes, as it can avoid the problems such as cross-talk, different localizations, and different metabolisms induced by the combination use of several fluorescent probes. Nevertheless, to the best of our knowledge, there is no report yet describing a single fluorescent thiol probe for sensing the

different concentration ranges of thiols with distinct fluorescence signals. Thus, it is interesting to develop a probe exhibiting different modes of fluorescence signal for the different concentration ranges of thiols. Obviously, it is very challenging to design such intriguing concentration range-dependent fluorescent probes.

Aware of the great interest for the development of concentration range-dependent fluorescent probes, in particular with near-infrared (NIR) emission properties for potential applications in living animals, several factors should be considered in designing this type of probe. First, the NIR fluorescent dye should have two chemical handles, which can be readily modified to afford two interaction sites for the target of interest. Secondly, the two interaction sites should exhibit distinct sensitivity to the target of interest. This may provide the recognition basis (the interaction step in the overall sensing process). In other words, the probes should contain high and low sensitivity sites for the target of interest. Thirdly, mostly important and challenging, the high-sensitivity site of the probes should interact with the low concentration range of the target to provide one mode of fluorescence signal, while the low-sensitivity site of the probes should interact with the high concentration range of the target to give off the other mode of fluorescence signal. This involves the transduction of the recognition into the optical signal.

In 1992, Patonay *et al.* developed heptamethine cyanine dyes with a rigid chlorocyclohexenyl ring in the methine chain, which is favorable to increase the photostability and

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fluorescence quantum yields.⁹ The rigid structures also render the chloro group as a reactive site for chemical substitution at the central ring. Recently, our group has presented an approach for the regulation of the fluorescence of merocyanine dyes (**HD** NIR dyes).¹⁰ The dyes are advantageous over the traditional 7-hydroxycoumarin and fluorescein with maximal absorption and emission in the NIR region while retaining an optically tunable hydroxyl group. However, both the traditional chloro-substituted heptamethine cyanine dyes and the recently developed **HD** NIR dyes have only one chemical handle that can be transformed into a reactive site for a target of interest. To address this problem, we envisioned that by the hybridization of the chloro-substituted cyanine and **HD** NIR dye, we may afford an innovative type of NIR dye, **CHMC** (chloro-hydroxyl merocyanine), which, notably, contains a key chloro moiety and a critical hydroxyl group that can be readily transformed into two reactive sites for a target of interest (Fig. 1). We thus anticipated that the novel **CHMC** dye could be exploited as a powerful platform for the design of the concentration range-dependent NIR fluorescent probes for thiols.

After selecting the platform, the next step is to choose the two interaction sites for thiols. With the above design criteria for the innovative fluorescent probes in mind, we proposed the new **CHMC-thiol** (Fig. 2) as a potential candidate probe that may show two modes of fluorescence signals towards low and high concentration ranges of thiols. It is known that the 2,4-dinitrobenzenesulfonate moiety is highly reactive towards thiols,^{3g,11} thus, it is reasonable to be selected as the high-sensitivity site for thiols. By contrast, the chloro group is less reactive to thiols, and it was chosen as the low-sensitivity site for thiols.^{2c,2f,12} From the optical point of view, the 2,4-dinitrobenzenesulfonate moiety is a notorious fluorescence quencher,^{3g,11} thus, we envisioned that interactions of the 2,4-dinitrobenzenesulfonate moiety of the probe **CHMC-thiol** with thiols may provide a turn-on mode in fluorescence. On the other hand, it is known that the nucleophilic substitution of the chloro moiety of chloro-substituted cyanine with heteroatoms (*i.e.* sulfur,¹² nitrogen^{9b}) may induce a ratiometric mode in fluorescence. We thus anticipated that, similarly, the nucleophilic substitution of the chloro moiety of the probe **CHMC-thiol** with thiols may induce a ratiometric mode in fluorescence. In this way, the unique



Fig. 1 The design of the novel **CHMC** dye as a representative platform for the concentration range-dependent NIR fluorescent probes by the hybridization of the chloro-substituted cyanine and **HD** NIR dye.

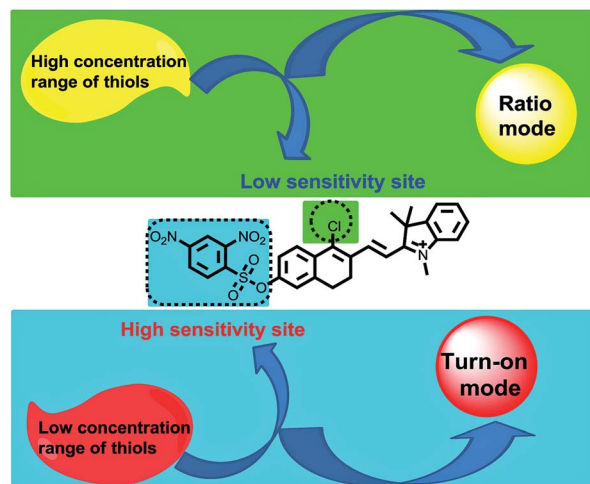


Fig. 2 Rational design of the smart fluorescent thiol probe, **CHMC-thiol** (**CHMC** as a platform for **CHMC-thiol**; the 2,4-dinitrobenzenesulfonate moiety is the high sensitivity site to provide a turn-on mode in fluorescence, and the chloro group is the low-sensitivity site to give off a ratiometric mode in fluorescence).

probe **CHMC-thiol** containing both the high-sensitivity site (the 2,4-dinitrobenzenesulfonate moiety) and the low-sensitivity site (the chloro moiety), may provide a turn-on mode and a ratiometric mode in fluorescence, respectively, upon interactions with thiols.

Results and discussion

Synthesis of the fluorescent probe **CHMC-thiol**

The synthesis of the smart probe **CHMC-thiol** was accomplished in four steps as shown in Scheme 1. Starting material **1** is commercially available. The intermediate aldehyde **2** was



Scheme 1 Synthesis of compounds **CHMC-thiol** and some controls **CHMC1,2**. Reagents and conditions: (a) DMF/ POCl_3 , 5 h; (b1) 1-butanol–benzene (7 : 3), indolium salt, 90 °C, 5 h; (b2) 1-butanol–benzene (7 : 3), benz[e]indolium salt, 90 °C, 5 h; (c) BBr_3 , dry CH_2Cl_2 , 0 °C, overnight. (d) Cs_2CO_3 , CH_2Cl_2 , 2,4-dinitrobenzenesulfonyl chloride, room temperature, 5 h.





Scheme 2 The proposed reaction mechanism of CHMC-thiol with biological thiols (represented by Cys) and the structures of the amino and sulfhydryl substituted controls 5 and 6.

normalized emission of the probe **CHMC-thiol** reaction with the low concentration range of Cys (Fig. S15a[†]) highly resembles that of **CHMC1** (Fig. S15b[†]). On the other hand, in the presence of a high concentration range of Cys, the chloro group in **CHMC-thiol** is initially replaced by the thiol group of Cys to produce the 4-sulfhydryl intermediate 3, and the subsequent intramolecular SNAr substitution of the sulfhydryl moiety by the amino group leads to the formation of the 4-amino product 4.^{2c,2f,12,15} This process is consistent with the results of mass spectrometry (Fig. S16[†]) and NMR analyses (Fig. S17[†]). To further confirm that the final product is the 4-amino product 4 instead of the 4-sulfhydryl intermediate 3, we further synthesized compounds 5 and 6 as the amino and sulfhydryl substituted controls, respectively (Scheme 2). The spectral studies indicate that the emission profile of the probe **CHMC-thiol** reaction with high concentration range of Cys highly resembles that of the control 5 instead of 6 (Fig. S18[†]). Thus, taken together, these data corroborate the sensing mechanism of **CHMC-thiol** in the presence of low and high concentration ranges of Cys as proposed in Scheme S2.[†]

We also evaluated the capability of **CHMC-thiol** to detect other small-molecular-weight biological thiols (Hcy and GSH). The titrations of the novel probe **CHMC-thiol** (5 μM) with Hcy were conducted in PBS (pH 7.4, containing 10% EtOH as a cosolvent). The probe **CHMC-thiol** exhibits a similar behavior towards Hcy as found for Cys. Upon reaction with a low concentration range of Hcy, the fluorescence turn-on at 680 nm is due to the thiol-mediated removal of the 2,4-dinitrobenzenesulfonate moiety. However, on addition of a high concentration range of Hcy, a ratiometric response is observed owing to nucleophilic substitution followed by an intramolecular rearrangement (Fig. S19 and Scheme S1[†]). Hcy is a potential interferent at certain conditions for the detection of

Cys due to the structural and chemical similarity between them. In the presence of a low concentration range of GSH, the probe **CHMC-thiol** shows a similar trend to Cys and Hcy. However, the treatment of the probe with a high concentration range of GSH only produces the 4-sulfhydryl product due to the steric hindrance, which leads to a 10 nm redshift in the emission spectra (Fig. S20 and Scheme S2[†]). These results are in good agreement with those reported in the literature.^{2c,2f,12,15}

To examine the selectivity, the probe **CHMC-thiol** (5 μM) was treated with various biologically relevant species (e.g., representative anions, metal ions, reactive oxygen species, reactive nitrogen species, reducing agents) in aqueous buffer. As shown in Fig. 6, addition of representative anions (Cl^- , Br^- , I^- , AcO^- , N_3^- , CN^- , CO_3^{2-} , NO_2^-) at 1 mM, metal ions (K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+}) at 1 mM, reactive oxygen and nitrogen species (H_2O_2 , ClO^- , NO), and reducing agents (ascorbic acid, Na_2S) at biologically relevant concentrations induces no marked fluorescence enhancement at 680 nm. By contrast, upon treatment of thiols with the probe, a large fluorescence signal was observed at 680 nm (Fig. 6a). The fluorescence signals at 625 nm (Fig. 6b) are consistent with those at 680 nm (Fig. 6a). Thus, these data demonstrate that the probe **CHMC-thiol** has a high selectivity for thiols over other biological species tested at biologically relevant concentrations, suggesting that the probe **CHMC-thiol** is favorable for studies of biological thiols in biological systems.

Kinetic studies of the probe CHMC-thiol

The time course of the probe **CHMC-thiol** in the presence of thiols (represented by Cys) is displayed in Fig. 7. The response of the 2,4-dinitrobenzenesulfonate moiety (the high-sensitivity site)



Fig. 6 (a) Fluorescence responses of the probe **CHMC-thiol** (5 μM) to various biologically relevant species in aqueous buffer. Red bars represent the addition of an excess of the representative anions, metal ions, reactive oxygen species, reactive nitrogen species, reducing agents, small-molecule thiols. (Cl^- , Br^- , I^- , AcO^- , N_3^- , CN^- , CO_3^{2-} , NO_2^-) at 1 mM, metal ions (K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+}) at 1 mM, reactive oxygen and nitrogen species (H_2O_2 , OCl^- , NO , Na_2S) at 100 μM . Ascorbic acid, GSH, Hcy and Cys at 1 mM. (1) Cl^- , (2) Br^- , (3) I^- , (4) AcO^- , (5) N_3^- , (6) CN^- , (7) CO_3^{2-} , (8) NO_2^- , (9) Mg^{2+} , (10) K^+ , (11) Zn^{2+} , (12) Ca^{2+} , (13) H_2O_2 , (14) ClO^- , (15) NO , (16) ascorbic acid, (17) Na_2S , (18) GSH, (19) Hcy, (20) Cys. Excitation at 550 nm and emission at 680 nm; (b) fluorescence responses of the probe **CHMC-thiol** (5 μM) to various biologically relevant species in aqueous buffer. Cl^- , Br^- , I^- , AcO^- , N_3^- , CN^- , CO_3^{2-} , NO_2^-) at 1 mM, metal ions (K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+}) at 1 mM, reactive oxygen and nitrogen species (H_2O_2 , OCl^- , NO) at 100 μM . Ascorbic acid, GSH, Hcy and Cys at 1 mM. (1) Cl^- , (2) Br^- , (3) I^- , (4) AcO^- , (5) N_3^- , (6) CN^- , (7) CO_3^{2-} , (8) NO_2^- , (9) Mg^{2+} , (10) K^+ , (11) Zn^{2+} , (12) Ca^{2+} , (13) H_2O_2 , (14) ClO^- , (15) NO , (16) ascorbic acid, (17) Na_2S , (18) GSH, (19) Hcy, (20) Cys. Excitation at 550 nm and emission at 625 nm. Data were expressed as mean \pm SD of three experiments.



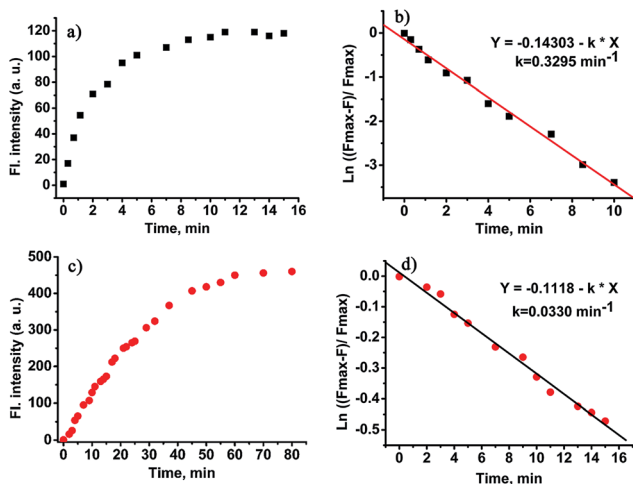


Fig. 7 (a) Reaction-time profiles of CHMC-thiol ($5.0 \mu\text{M}$) in the presence of $100 \mu\text{M}$ Cys (\blacksquare) at 680 nm ; (b) pseudo-first-order kinetic plot of the reaction of the probe CHMC-thiol ($5 \mu\text{M}$) with Cys ($100 \mu\text{M}$) at 680 nm in pH 7.4, 25 mM PBS. Slope = 0.3295 min^{-1} ; (c) reaction-time profiles of CHMC-thiol ($5.0 \mu\text{M}$) in the presence of $500 \mu\text{M}$ Cys (\blacksquare) at 625 nm ; (d) pseudo-first-order kinetic plot of the reaction of the probe CHMC-thiol ($5 \mu\text{M}$) with Cys ($500 \mu\text{M}$) at 625 nm in pH 7.4, 25 mM PBS. Slope = 0.0330 min^{-1} .

of CHMC-thiol to Cys is fast, and the reaction is complete within 10 min at 680 nm (Fig. 7a). The pseudo-first-order rate constant of the high-sensitivity site is calculated to be $k = 0.3295 \text{ min}^{-1}$ for Cys at 680 nm (Fig. 7b). By contrast, the response of the chloro group (the low sensitivity site) of CHMC-thiol to Cys is slower than the 2,4-dinitrobenzenesulfonate moiety, and the reaction is complete within 60 min at 625 nm (Fig. 7c). The pseudo-first-order rate constant of the second site is calculated to be $k = 0.033 \text{ min}^{-1}$ for Cys at 625 nm (Fig. 7d). The pseudo-first-order rate constant of the high sensitivity site is about 10 times that of the low sensitivity site. It is important to note that such a large difference in the pseudo-first-order rate constants of the two sensing sites is highly desirable for the development of concentration range-dependent fluorescent probes.

Imaging of the CHMC-thiol to different concentration ranges of thiols in live cells

Notably, the threshold of the low and high concentration ranges of the fluorescent probes CHMC-thiol is well within the physiological concentration range of Cys ($30\text{--}200 \mu\text{M}$).¹⁴ For preliminary fluorescence imaging applications, the probe CHMC-thiol was incubated with living HeLa cells pretreated with or without *N*-ethylmaleimide (as a thiol blocking agent). As shown in Fig. 8g, the cells treated only with the probe exhibit strong fluorescence in the red channel. However, when the cells are pretreated with *N*-ethylmaleimide, then incubated with CHMC-thiol, much weaker fluorescence in the red channel is observed (Fig. 8c). To further study the ability of the probe CHMC-thiol for imaging different concentration ranges of thiols in living cells, the cells were incubated with Cys ($100 \mu\text{M}$) for 30 min, and then treated with the probe CHMC-thiol ($5 \mu\text{M}$) for another 30 min. In such a case, the cells exhibit intense fluorescence in the orange



Fig. 8 Bright-field and fluorescence images of HeLa cells stained with the CHMC-thiol: (a–d) bright-field and fluorescence images of the cells incubated with *N*-ethylmaleimide, and then co-incubated with CHMC-thiol for 30 min; (e–h) bright-field and fluorescence images of cells incubated only with the probe for 30 min; (i–l) bright-field and fluorescence images of cells incubated with Cys for 30 min, and then treated with the probe for 30 min: the orange and red channels are corresponding to the emission windows of $580\text{--}640 \text{ nm}$, and $650\text{--}750 \text{ nm}$, respectively. Scale bar = $10 \mu\text{m}$.

channel (Fig. 8j). By contrast, the cells incubated with only the probe display almost no fluorescence in the orange channel (Fig. 8f). Thus, in the living cells, the probe shows a turn-on mode in the presence of the low concentration range of Cys (Fig. 8c and g) and a ratiometric mode in the presence of the high concentration range of Cys (Fig. 8f, g, j and k, and 9). Similar results are noted when the probe is treated with another type of cells, MCF-7 cells (Fig. S21[†]). These imaging data in the living cells are consistent with the results in solution (Fig. 5). Thus, as designed, the probe CHMC-thiol is cell membrane permeable and can report low and high concentration ranges of thiols with distinct modes of fluorescence signals in the living cells.

Imaging of CHMC-thiol to different concentration ranges of thiols in live animals

Based on the above studies, the prominent features of the probe CHMC-thiol include excellent NIR photophysical properties,

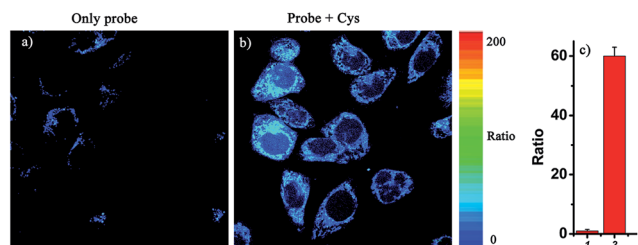


Fig. 9 Pseudo-colored ratiometric images ($F_{\text{orange}}/F_{\text{red}}$) of thiols in HeLa cells. (a) Ratio image ($F_{\text{orange}}/F_{\text{red}}$) of the cells incubated only with the probe CHMC-thiol ($5 \mu\text{M}$) for 30 min; (b) ratio image ($F_{\text{orange}}/F_{\text{red}}$) of the cells incubated with Cys ($100 \mu\text{M}$) for 30 min, and then treated with the probe ($5 \mu\text{M}$) for another 30 min; (c) average $F_{\text{orange}}/F_{\text{red}}$ intensity ratios in (a, b). The orange and red channels are corresponding to the emission windows of $580\text{--}640 \text{ nm}$, and $650\text{--}750 \text{ nm}$, respectively. Data are expressed as mean \pm SD of three experiments.



two reactive sites with different sensitivity and excellent selectivity. In particular, the threshold of the low and high concentration ranges of **CHMC-thiol** is well located in the physiological concentration range of Cys (30–200 μM).¹⁴ These favorable attributes encourage us to further investigate the suitability of the probe for detecting different concentration ranges of thiols with distinct modes of fluorescence signals in the context of living mice. The Kunming mice were divided into three groups (Fig. 10). One group was given saline (100 μL) in the peritoneal cavity, followed by intraperitoneal (i.p.) injection with **CHMC-thiol** (20 μM , in 20 μL DMSO). The second group was given an i.p. injection of *N*-ethylmaleimide (1 mM, in 100 μL saline), and followed by i.p. injection with **CHMC-thiol** (20 μM , in 20 μL DMSO) as the negative control experiment. The third group was given an i.p. injection of Cys (100 μM , in 100 μL saline), and followed by i.p. injection with **CHMC-thiol** (20 μM , in 20 μL DMSO). As shown in Fig. 10b, the mice treated with only the probe exhibit a much higher fluorescence readout (pseudocolor) than the mice treated with NEM (Fig. 10a) in the red channel. In addition, the mice treated with only the probe display a much higher fluorescence readout (pseudocolor) than the mice incubated with NEM and the probe (Fig. 10d and e) in the orange channel, but less than the mice incubated with Cys and the probe (Fig. 10f). Thus, the probe exhibits a turn-on mode to the low concentration range of Cys (Fig. 10a, b and g(1, 2)) and a ratiometric signal to the high concentration range of Cys (Fig. 10b, c, e, f and h). Notably, the imaging data in the living animals are consistent with both the results in the solution (Fig. 5) and the living cells (Fig. 8). Thereby, these results

demonstrate that the robust fluorescent probe **CHMC-thiol** can image the different concentration ranges of thiols with distinct modes of fluorescence signals in the living animals, for the first time.

Conclusions

In summary, we have described a unique approach for the development of concentration range-dependent fluorescent probes. Based on the proposed strategy, we have synthesized the first concentration range-dependent NIR fluorescent probe, **CHMC-thiol**, which, interestingly, is capable of monitoring the low concentration range of thiols with a turn-on fluorescence signal and providing a ratiometric fluorescence response to the high concentration range of thiols. We have further demonstrated that the new probe can be employed to sense the changes in the thiol concentration ranges with distinct modes of fluorescence signals in both living cells and living animals. In addition, the innovative NIR dye, **CHMC**, is superior to the traditional chloro-substituted cyanine and the recently developed **HD** NIR dyes with retaining both the regulation sites of these two types of NIR dyes. We believe that **CHMC**, which intrinsically bears two chemical handles, may serve as a useful fluorescent platform for the design of the concentration range-dependent NIR fluorescent probes as powerful molecular tools for biological and clinical investigations in living systems.

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Notes and references

- (a) X. Chen, Y. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120; (b) C. Yin, F. Huo, J. Zhang, R. Martínez-Mañez, Y. Yang, H. Lv and S. Li, *Chem. Soc. Rev.*, 2013, **42**, 6032; (c) H. S. Jung, X. Chen, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2013, **42**, 6019; (d) L.-Y. Niu, Y.-Z. Chen, H.-R. Zheng, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *Chem. Soc. Rev.*, 2015, **44**, 6143.
- (a) O. Rusin, N. N. St Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian and R. M. Strongin, *J. Am. Chem. Soc.*, 2004, **126**, 438; (b) X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem., Int. Ed.*, 2011, **50**, 10690; (c) L. Y. Niu, Y. S. Guan, Y. Z. Chen, L. Z. Wu, C. H. Tung and Q. Z. Yang, *J. Am. Chem. Soc.*, 2012, **134**, 18928; (d) J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J.-H. Ryu and J. Yoon, *J. Am. Chem. Soc.*, 2014, **136**, 5351; (e) S. Y. Lim, K. H. Hong, D. I. Kim, H. Kwon and H. J. Kim, *J. Am. Chem. Soc.*, 2014, **136**, 7018; (f) J. Liu, Y. Q. Sun, Y. Huo, H. Zhang, L. Wang, P. Zhang and W. Guo, *J. Am. Chem. Soc.*, 2014, **136**, 574; (g) Z. Guo, S. W. Nam, S. Park and J. Yoon, *Chem. Sci.*, 2012, **3**, 2760.
- (a) X. Lou, Y. Hong, S. Chen, C. W. T. Leung, N. Zhao, B. Situ, J. W. Y. Lam and B. Z. Tang, *Sci. Rep.*, 2014, **4**, DOI: 10.1038/srep04272; (b) H. S. Hewage and E. V. Anslyn, *J. Am. Chem.*



Fig. 10 Representative fluorescent images (pseudo-color) of thiols (Cys) in the mice: (a) and (d) the mice were given an i.p. injection of *N*-ethylmaleimide and followed by injection with **CHMC-thiol**; (b) and (e) only the **CHMC-thiol** was injected in the peritoneal cavity of the mice; (c) and (f) the mice were given an i.p. injection of Cys and followed by an i.p. injection with **CHMC-thiol**. (g) Quantification of the fluorescence emission intensity from the abdominal area of the mice of groups a(1), b(2), c(3); d(4), e(5), f(6); (h) average $F_{\text{orange}}/F_{\text{red}}$ intensity ratios of the only probe group (1) and probe + Cys group (2). The mice were imaged with an excitation filter of 550 nm and the orange and red channels are corresponding to the emission windows of 580–640 nm, and 650–750 nm, respectively. Data were expressed as mean \pm SD of three experiments.



- Soc.*, 2009, **131**, 13099; (c) C. S. Lim, G. Masanta, H. J. Kim, J. H. Han, H. M. Kim and B. R. Cho, *J. Am. Chem. Soc.*, 2011, **133**, 11132; (d) M. Zhang, M. Yu, F. Li, M. Zhu, M. Li, Y. Gao, T. Yi and C. Huang, *J. Am. Chem. Soc.*, 2007, **129**, 10322; (e) B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666; (f) Y. H. Ahn, J. S. Lee and Y. T. Chang, *J. Am. Chem. Soc.*, 2007, **129**, 4510; (g) J. Shao, H. Sun, H. Guo, S. Ji, J. Zhao, W. Wu and T. D. James, *Chem. Sci.*, 2012, **3**, 1049; (h) R. Wang, L. Chen, P. Liu, Q. Zhang and Y. Wang, *Chem.–Eur. J.*, 2012, **18**, 11343; (i) F. Wang, Z. Guo, X. Li, X. Li and C. Zhao, *Chem.–Eur. J.*, 2014, **20**, 11471.
- 4 (a) L. D. Lavis and R. T. Raines, *ACS Chem. Biol.*, 2014, **9**, 855; (b) B. A. Smith, W. J. Akers, W. M. Leevy, A. J. Lampkins, S. Z. Xiao, W. Wolter, M. A. Suckow, S. Achilefu and B. D. Smith, *J. Am. Chem. Soc.*, 2010, **132**, 67; (c) K. Kundu, S. F. Knight, N. Willett, S. Lee, W. R. Taylor and N. Murthy, *Angew. Chem., Int. Ed.*, 2009, **48**, 299; (d) N. Karton-Lifshin, L. Albertazzi, M. Bendiko, P. S. Baran and D. Shabat, *J. Am. Chem. Soc.*, 2012, **134**, 20412; (e) W. Xuan, C. Sheng, Y. Cao, W. He and W. Wang, *Angew. Chem., Int. Ed.*, 2012, **51**, 2282; (f) X. Wu, M. Yu, B. Lin, H. Xing, J. Han and S. Han, *Chem. Sci.*, 2015, **6**, 798; (g) J. J. Hu, N.-K. Wong, S. Ye, X. Chen, M.-Y. Lu, A. Q. Zhao, Y. Guo, A. C.-H. Ma, A. Y.-H. Leung, J. Shen and D. Yang, *J. Am. Chem. Soc.*, 2015, **137**, 6837; (h) C. Huang, T. Jia, M. Tang, Q. Yin, W. Zhu, C. Zhang, Y. Yang, N. Jia, Y. Xu and X. Qian, *J. Am. Chem. Soc.*, 2014, **136**, 14237.
- 5 (a) K. Kiyose, H. Kojima and T. Nagano, *Chem.–Asian J.*, 2008, **3**, 506; (b) M. Lin, B. Fimmel, K. Radacki and F. Würthner, *Angew. Chem., Int. Ed.*, 2011, **50**, 10847; (c) G. M. Fischer, E. Daltrozzo and A. Zumbusch, *Angew. Chem., Int. Ed.*, 2011, **50**, 1406; (d) T. Hirayama, G. C. Van de Bittner, L. W. Grayb, S. Lutsenkob and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 2228; (e) M. Cui, M. Ono, H. Watanabe, H. Kimura, B. Liu and H. Saji, *J. Am. Chem. Soc.*, 2014, **136**, 3388; (f) A. T. Wrobel, T. C. Johnstone, A. Deliz-Liang, S. J. Lippard and P. Rivera-Fuentes, *J. Am. Chem. Soc.*, 2014, **136**, 4697.
- 6 (a) M. Schäerling, *Angew. Chem., Int. Ed.*, 2012, **51**, 3532; (b) Y. Yang, Q. Zhao, W. Feng and F. Li, *Chem. Rev.*, 2013, **113**, 192; (c) X. Li, X. Gao, W. Shi and H. Ma, *Chem. Rev.*, 2014, **114**, 590; (d) R. Guliyev, A. Coskun and E. U. Akkaya, *J. Am. Chem. Soc.*, 2009, **131**, 9007; (e) Y. Ueno, J. Jose, A. Loudet, C. Pérez-Bolívar, P. Anzenbacher Jr. and K. Burgess, *J. Am. Chem. Soc.*, 2011, **133**, 51; (f) E. Heyer, P. Retailleau and R. Ziessel, *Org. Lett.*, 2014, **16**, 2330; (g) F. Yu, P. Li, B. Wang and K. Han, *J. Am. Chem. Soc.*, 2013, **135**, 7674; (h) J. Ling, G. Naren, J. Kelly, T. S. Moody and A. P. de Silva, *J. Am. Chem. Soc.*, 2015, **137**, 3763.
- 7 (a) M. H. Lee, B. Yoon, J. S. Kim and J. L. Sessler, *Chem. Sci.*, 2013, **4**, 4121; (b) X. Wang, L. Cui, N. Zhou, W. Zhu, R. Wang, X. Qian and Y. Xu, *Chem. Sci.*, 2013, **4**, 2936; (c) C. Liu, W. Chen, W. Shi, B. Peng, Y. Zhao, H. Ma and M. Xian, *J. Am. Chem. Soc.*, 2014, **136**, 7257; (d) Z. Huang, S. Yu, K. Wen, X. Yu and L. Pu, *Chem. Sci.*, 2014, **5**, 3457; (e) D. R. Rooker and D. Buccella, *Chem. Sci.*, 2015, **6**, 6456; (f) Y. You and W. Nam, *Chem. Sci.*, 2014, **5**, 4123; (g) Y.-J. Huang, W.-J. Ouyang, X. Wu, Z. Li, J. S. Fossey, T. D. James and Y.-B. Jiang, *J. Am. Chem. Soc.*, 2013, **135**, 1700; (h) J. Zhou, L. Li, W. Shi, X. Gao, X. Li and H. Ma, *Chem. Sci.*, 2015, **6**, 4884; (i) Q. Zhao, X. Zhou, T. Cao, K. Y. Zhang, L. Yang, S. Liu, H. Liang, H. Yang, F. Li and W. Huang, *Chem. Sci.*, 2015, **6**, 1825.
- 8 (a) H. Komatsu, T. Miki, D. Citterio, T. Kubota, Y. Shindo, Y. Kitamura, K. Oka and K. Suzuki, *J. Am. Chem. Soc.*, 2005, **127**, 10798; (b) L. Yuan, W. Lin, Y. Xie, B. Chen and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 1305; (c) X. Gao, X. Li, L. Li, J. Zhou and H. Ma, *Chem. Commun.*, 2015, **51**, 9388.
- 9 (a) L. Strekowski, M. Lipowska and G. Patonay, *J. Org. Chem.*, 1992, **57**, 4578; (b) X. Peng, F. Song, E. Lu, Y. Wang, W. Zhou, J. Fan and Y. Gao, *J. Am. Chem. Soc.*, 2005, **127**, 4170.
- 10 L. Yuan, W. Lin, S. Zhao, W. Gao, B. Chen, L. He and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 13510.
- 11 (a) H. Maeda, H. Matsuno, M. Ushida, K. Katayama and K. Saeki, *Angew. Chem., Int. Ed.*, 2005, **44**, 2922; (b) J. Zhang, A. Shibata, M. Ito, S. Shuto, Y. Ito, B. Mannervik, H. Abe and R. Morgenstern, *J. Am. Chem. Soc.*, 2011, **133**, 14109; (c) W. Jiang, Q. Fu, H. Fan, J. Ho and W. Wang, *Angew. Chem., Int. Ed.*, 2007, **46**, 8445; (d) X. Liu, W. Zhang, C. Li, W. Zhou, Z. Li, M. Yu and L. Weia, *RSC Adv.*, 2015, **5**, 4941.
- 12 (a) L.-Y. Niu, H.-R. Zheng, Y.-Z. Chen, L.-Z. Wu, C.-H. Tung and Q.-Z. Yang, *Analyst*, 2014, **139**, 1389; (b) X. Wang, J. Lv, X. Yao, Y. Li, F. Huang, M. Li and B. Tang, *Chem. Commun.*, 2014, **50**, 15439.
- 13 The pK_a was calculated according to the Henderson–Hasselbalch-type mass action equation ($\log[(F_{\max} - F)/(F - F_{\min})] = pK_a - pH$).
- 14 (a) T. K. Chung, M. A. Funk and D. H. Baker, *J. Nutr.*, 1990, **120**, 158; (b) S. Park and J. A. Imlay, *J. Bacteriol.*, 2003, **185**, 1942.
- 15 (a) J. Liu, Y.-Q. Sun, H. Zhang, Y. Huo, Y. Shi and W. Guo, *Chem. Sci.*, 2014, **5**, 3183; (b) Y. Kim, S. V. Mulay, Mi. Choi, S. B. Yu, S. Jon and D. G. Churchill, *Chem. Sci.*, 2015, **6**, 5435; (c) F. Wang, L. Zhou, C. Zhao, R. Wang, Q. Fei, S. Luo, Z. Guo, H. Tian and W.-H. Zhu, *Chem. Sci.*, 2015, **6**, 2584.

