

REVIEW

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The chronological evolution of small organic molecular fluorescent probes for thiols†

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Abnormal concentrations of biothiols such as cysteine, homocysteine and glutathione are associated with various major diseases. In biological systems, the structural similarity and functional distinction of these three small molecular thiols has not only required rigorous molecular design of the fluorescent probes used to detect each thiol specifically, but it has also inspired scientists to uncover the ambiguous biological relationships between these bio-thiols. In this minireview, we will discuss the evolution of small organic molecular fluorescent probes for the detection of thiols over the past 60 years, highlighting the potent methodologies used in the design of thiol probes and their particular applications in the semi-quantification of cellular thiols and real-time labelling. At the same time, the present challenges that limit their further application will be discussed. We hope that this minireview will promote future research to enable deeper insight into the crucial role of thiols in biological systems.

Received 8th September 2020
Accepted 13th November 2020

DOI: 10.1039/d0sc04960c

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Introduction

Detection of biological thiols has been going on for more than 100 years since the successive discoveries of cysteine (Cys), glutathione (GSH) and homocysteine (Hcy).^{1–3} During this time, it has been a real challenge, one that directly affects the accurate analysis of thiols, to make these optically inert molecules observable. Fortunately, thiols with nucleophilic reactivity are able to react efficiently with electron deficient systems such as benzoquinone and *N*-ethyl maleimide resulting in the formation of covalent bonds.^{3,4} Similarly, thiols can easily react with benzyl halide derivatives *via* nucleophilic substitution reactions.^{5,6} These specific covalent bonding processes enabled revolutionary technical support allowing the physiological and pathological roles of thiols to be seen.^{7–10} At the same time, the importance of thiols, especially in pathological processes, further increased the necessity to label and detect thiols quantitatively. An appealing use for the quantitative detection of thiols relates to homocystinuria, an inherited error in the metabolism of amino acids that results in an increased incidence of vascular injury and arterial thrombosis, discovered in 1962.^{11–14} Subsequently, a large number of studies reported that the increase in total blood Hcy content was related to the onset of cardiovascular and cerebrovascular diseases.¹⁵ Interestingly,

the concentration of total Cys in plasma was later found to be directly proportional to the concentration of total Hcy and, in the blood samples of patients with coronary heart disease, total Cys and total Hcy are significantly higher than normal levels.^{16,17} Today, the total concentration of Hcy in plasma is a clinical indicator of the risk of cardiovascular and cerebrovascular diseases. In another case, a higher GSH level was found to be associated with the resistance to Melphalan.¹⁸ Furthermore, Perry and co-workers reported the existence of higher levels of GSH in primary breast tumors compared with normal breast tissue and proposed the possibility that GSH levels act as a marker of breast cancer.¹⁹ Although the relation between these diseases and the concentrations of thiols were well studied, it is still unknown whether the observed thiol concentrations are the consequence or the cause of these diseases. Effective detection and labelling techniques are urgently needed to explore new areas of research.

Though the detection of thiols employing fluorescent probes emerged after 1960, it has encountered a renaissance in the past 20 years promoted not only by the significant improvement of laser scanning confocal microscopes, but also by the possibility that we can rationally design fluorescent probes to fulfil specific requirements. Recently, several excellent reviews have summarized the recent development of fluorescent probes for the detection of thiols.^{20–24} In this minireview, we will discuss the chronological evolution of application-oriented small molecular fluorescent probes for the detection of thiols over the last 60 years. We will also examine the present challenges and hope to inspire the design of future fluorescent probes to move from approaches that utilize trial and error to those that use design-based molecular engineering.

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† Electronic supplementary information (ESI) available: Reaction schemes for the fluorescent probes presented in this minireview and information on the reviews of the reported thiols. See DOI: 10.1039/d0sc04960c

Evolution of fluorescent probes for thiols

Studies of the chemical reactions of thiols are the overall basis for the design of fluorescent probes for thiols. The efficient capture of mercury ions by thiols and sulfur-containing proteins inspired the synthesis of the fluorescein-mercury compounds “di-acetoxymercurifluorescein and tetra-acetoxymercurifluorescein (1)” as fluorescent probes for the detection of thiols with a decreased fluorescent intensity initiated by the fluorescent thiol detection.^{25–27} This became the hallmark of fluorescent probes for thiols (Fig. 1).

The uses of fluorescent probes for the detection of thiols can be roughly divided into two types: I. quantitative analysis based on chromatographic separation in aqueous solution; II. *in situ* analysis of cellular thiols based on a specific reaction between the thiol and the probe.

For probes of the primary type, the distinct nucleophilic –SH moiety in biological thiols can readily react with electron-deficient centers and active halides *via* a nucleophilic addition (NAR) or a nucleophilic substitution reaction (NSR). For example, enabled by the specific reaction between a maleimide moiety and thiols in aqueous solution, a fluorescent probe based on a NAR mechanism was first reported by Kanaoka in 1964 (2).^{28–31} In this example, the thiols reacted quickly with the maleimide moiety *via* a NAR process which induced a turn-on fluorescent response. Specifically, for Cys, the amino moiety of the NAR product could further attack the amido bond to form a six-membered ring product *via* an intramolecular rearrangement.^{29,32} The corresponding fluorescence regulation mechanism was fully illustrated by the Wang group in 2016.³³ Over the past 40 years, other structures such as *N*-dansylaziridine (4),³⁴ Acrylodan (8)³⁵ and 4-oxobut-2-enoate derivatives (9),³⁶ and a variety of Michael acceptors (squaraine (12), 7-oxanorbornadiene, quinone, chromene and nitroolefin) were then reported to detect thiols *via* the one step NAR process.^{37–40}

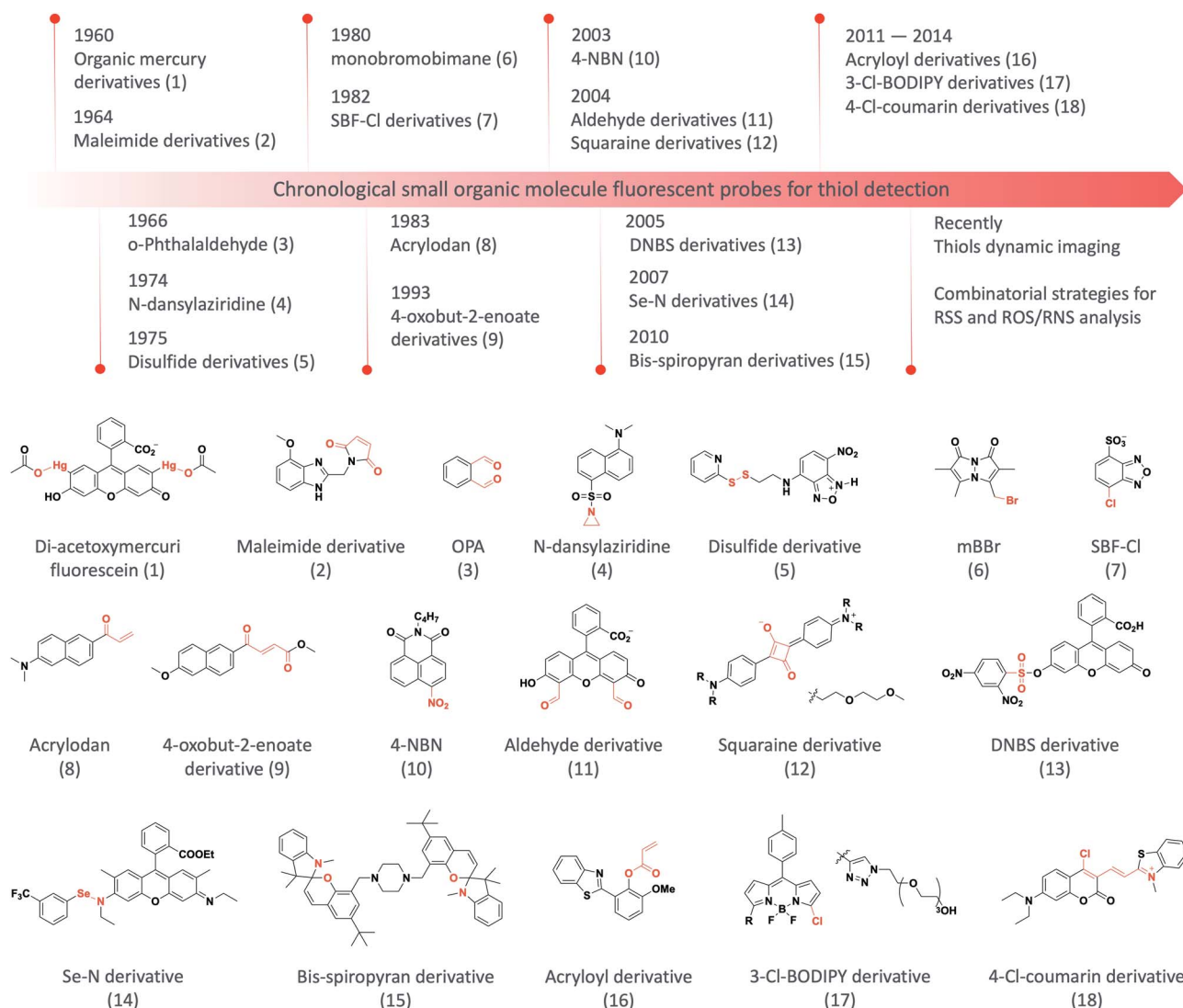


Fig. 1 Chronological evolution of small organic molecular fluorescent probes for the detection of thiols over the last 60 years and their representative molecular structures. The corresponding mechanisms for the reactions of these probes with thiols are presented in the ESI.†

O-phthalaldehyde (OPA, **3**) was found to react with both GSH and Hcy in aqueous solutions with appropriate pH conditions eliciting dual channel turn-on fluorescent responses and was first utilized for thiol detection by Cohn and coworkers in 1966.⁴¹ The formation of isoindole derivatives proceeded degradation of the reaction system.^{42,43} A fluorescent adduct of a naphthalene-based derivative and GSH allowed the quantification of GSH in serum to identify sepsis and further predicted mortality in patients with sepsis.⁴³ Furthermore, a similar reaction with OPA also proceeded with the co-existence of the thiols with other amino acids.⁴⁴

Disulfides (**5**),⁴⁵ monobromobimane (mBB, **6**) derivatives⁵ and 4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl) derivatives^{6,46,47} are a series of NSR-based fluorescent probes. 17 years after the first report of the "Ellman reagent", a fluorometric compound based on a disulfide bond was synthesized for the detection of thiols *via* interchange of the -S-S- and -SH groups.^{45,48,49} Today, disulfides species are one of the most important drug delivery systems that can be specifically activated at thiol-abundant sites. Moreover, combining a fluorophore and a drug with a disulfide bond allows the *in situ* visualization of drug release.^{50–52} Similar structures containing -Se-N- (**14**) or -Se-Se- moieties can also be used in the design of thiol probes.^{53–55} In contrast to the high specificity of disulfides for thiols, NBD-Cl was reported to react with both thiols (favorable) and non-thiol amino acids in weak alkaline solution which resulted in either non-fluorescent S-substituted products or highly fluorescent N-substituted products.^{6,56} The corresponding reaction mechanisms were confirmed in recent years to contain a successive aromatic nucleophilic substitution (S_NAr , one type of NSR process) and an intramolecular rearrangement.^{23,57} Sulfonic acid-substituted derivatives such as 4-chloro-7-sulfobenzo-furazan (SBF-Cl) (**7**)⁴⁷ and 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid (SBD-F)⁵⁸ displayed improved specificity toward thiols over other non-thiol amino acids in aqueous solution. The kinetics of the S_NAr process between one representative compound, 4-nitro-N-*n*-butyl-1,8-naphthalimide (4-NBN, **10**), and various thiols were studied by Triboni and co-workers. They indicated that the existence of micelles in aqueous solution such as hexadecyltrimethylammonium chloride (CTAC) caused an exponential acceleration of the reaction rate.⁵⁹ Similarly, 2,4-dinitrobenzenesulfonyl-modified phenol or aniline derivatives (DNBS, **13**) can react with thiols *via* an S_NAr process which generally induces the release of the corresponding phenol- or aniline-containing fluorophores and SO_2 (specifically, such molecules are used as SO_2 precursors to release SO_2 *via* the activation of cellular GSH).^{60,61} Sulfhydryl anions are the major nucleophilic constituents in these reactions. Thus, appropriate modification of these compounds can also furnish fluorescent probes for selenols or thiophenols.^{62–65} Furthermore, when mBB derivatives react with thiols they display turn-on fluorescent responses in aqueous solution which support the specific histological visualization of sulfhydryl proteins.^{66,67}

As discussed above, the fluorescent probes, at this stage, could generally react with all thiols and give similar fluorescent responses.^{53,60} The individual quantification of the three bio-

thiols requires further chromatographic separation. This fact makes it impossible to evaluate the different biological roles of cellular thiols *in situ* using the above probes. Further, due to the susceptibility to oxidation of biological thiols, cell lysis and further separation would magnify the test error. Fluorescent probes that can discriminate between the three bio-thiols might solve these limitations.

Fluorescent probes of the secondary type are those which could discriminate thiols with different fluorescent responses. This advance was realized utilizing insight into the differences between Cys, Hcy and GSH which mainly manifest in the following three aspects: I. they are small molecular thiols that have distinct cellular concentrations (c_{GSH} 1–10 mM, c_{Cys} 30–200 μ M, c_{Hcy} 9–13 μ M); II. the spacing between sulfhydryl and amino groups are different in each thiol; and III. the pK_a values of the sulfhydryl group induced distinct nucleophilic activities ($pK_a_{Cys} = 8.25$, $pK_a_{Hcy} = 8.87$ (10.0), $pK_a_{GSH} = 9.20$).^{68–70} Using these differences, we can obtain a signature for each thiol. For example, Cys displays the highest nucleophilic activity when compared with Hcy and GSH because of its prior deprotonation at physiological pH.^{38,49,71} Hcy favors oxidation by radicals with the formation of a stabilized α -amino carbon-centered radical *via* intramolecular hydrogen atom transfer.^{68,72,73} The tripeptide GSH is more easily influenced by steric hindrance^{74,75} and hydrogen bonds (**15**)^{76,77} during the reaction process.

Utilizing the difference in the spacings of the corresponding sulfhydryl and amino groups, fluorophore-conjugated aldehyde derivatives (**11**) were initially designed to detect Cys and Hcy over GSH.^{78–80} The reaction proceeded with the formation of a five-membered ring and six-membered ring for Cys and Hcy, respectively. Interestingly, α,β -unsaturated aldehydes can specifically detect Cys over Hcy and GSH.^{79,81} In 2011, the Strongin group reported the simultaneous detection of Cys and Hcy utilizing an acryloyl group (**16**) as the reaction site.⁸² Now, acryloyl modification has become a general molecular design for Cys-specific detection promoted by the more favorable formation of the seven-membered ring product (for the reaction with Hcy, the prospective product would be an eight-membered ring). For GSH specific detection, Yang and coworkers reported a series of chlorine-bearing BODIPY derivatives (**17**) in 2012.⁸³ A well-known reaction mechanism (S_NAr -rearrangement) inspired the successful development of active halogen derivatives able to discriminatively detect the three thiols.²³ In a recent study, a GSH S-substituted Si-rhodamine compound could be attracted by Cys to form a stable Cys N-substituted Si-rhodamine compound.⁸⁴ Another excellent probe design, reported by the Guo group in 2014, constructed three potential reaction sites in a single fluorescent probe (**18**) for the simultaneous discrimination of Cys and GSH *via* different emission channels.⁸⁵ A similar design strategy was later used for the simultaneous discrimination of Cys, Hcy and GSH using three emission channels.⁸⁶ These selective probes generally proceed *via* multi-stage reactions with thiols to produce different products with diacritical optical properties. Considering the natural concentration gradient of thiols in cells, the existence of a high concentration of GSH would consume an internalized probe



significantly and, thus, interfere with the semi-quantitative detection of Cys or Hcy in cells.^{84,87}

Specific detection of Cys using its pK_a may surmount the consumption of the fluorescent probe by GSH observed in the aforementioned probe design. Through modulation of the electrophilic activity of the reaction site, Cys reacts with the fluorescent probe specifically as it has the highest nucleophilic activity of the three thiols. For instance, the reaction site of a coumarin conjugated α,β -unsaturated ketone could be readily modulated by the modification of electron donating groups. Thus the obtained fluorescent probes could detect Cys specifically *via* a one-step Michael addition reaction in the lysosomes⁸⁷ and mitochondria.⁸⁸ The superiority of these fluorescent probes was highlighted by the possibility to detect Cys reversibly which allowed the real time fluorescent imaging of cellular Cys dynamics.⁸⁹ Furthermore, the predictable reactivity gradient of the thiols was recently used for the simultaneous quantification of Cys and Hcy using a fluorescent probe which demonstrated the positive correlation of Cys and Hcy concentrations in human serum.⁹⁰

In the overall development process, improving the specificity of the fluorescent probes has always been the ambition to allow scientists to obtain more reliable and accurate results. The development of new reaction sites and new mechanisms for thiol fluorescent probes will constantly be a key research area in the following years.^{91,92}

Biological insight promoted by thiol probes

Utilizing these known thiol fluorescent probe design strategies, we can obtain the desired tools through rational molecular design. Fluorescent probes that have a specific response toward one of the biothiols allow us to evaluate concentration changes under different conditions without the potential interference caused by other species. For instance, Chen and coworkers utilized a diselenide-based GSH-specific probe to evaluate the GSH concentration changes in HepG2 and HL-7702 cells in response to hypothermia or hyperthermia. They demonstrated the higher temperature stress

resistance of HL-7702 cells (normal cells) over HepG2 cells (cancer cells).⁵⁵ Li and coworkers evaluated the intrinsic GSH levels in L02 and HepG2 cells and the changes seen following treatment with anti-cancer drugs. These changes suggested that GSH levels increase in normal cells to adapt to environmental stress.⁹³ Fluorescent probes that can react reversibly with thiols are a more favorable platform to visualize the analyte fluctuation in real-time and *in situ*. The presently reported reversible fluorescent probes were all based on Michael addition reaction processes. For these fluorescent probes, their photostability, cell compatibility and equilibrium constants with thiols are important parameters that affect their utility for long-term thiol analysis. Several excellent works have reported the real-time imaging of cellular GSH using reversible fluorescent probes and have illustrated unprecedented regulation of GSH dynamics under certain physiological conditions (19–21, Fig. 2).^{94–98}

The combination of multiple reaction sites for reactive sulfur species (thiols, SO_2 , H_2S , H_2S_2 , etc.) with successfully developed design strategies has provided fluorescent probes to detect different analytes simultaneously through different emission channels.^{38,99,100} For these probes, the most promising application was the *in situ* evaluation of correlations between two or more analytes and their cellular transformation due to spatio-temporal synchronization. In 2017, Yin and coworkers reported the visualization of cellular Cys metabolism to form SO_2 using fluorescent probe (22) based on two reaction sites.¹⁰¹ Subsequently, several examples have been reported to describe the dynamic thiol metabolism processes utilizing observable fluorescent methodology (23–26).^{102–106} Further, the simultaneous detection of thiols and reactive oxygen species or reactive nitrogen species have also been reported in recent years to evaluate the cellular crosstalk of these redox regulating species.³⁸

Among the currently reported thiol fluorescent probes, design strategies for the specific detection of Cys or GSH have been well studied. Although several fluorescent probes featuring specific responses toward Hcy have been reported,^{68,107–109} a general design strategy is still unestablished. The development of fluorescent probes for Hcy-specific

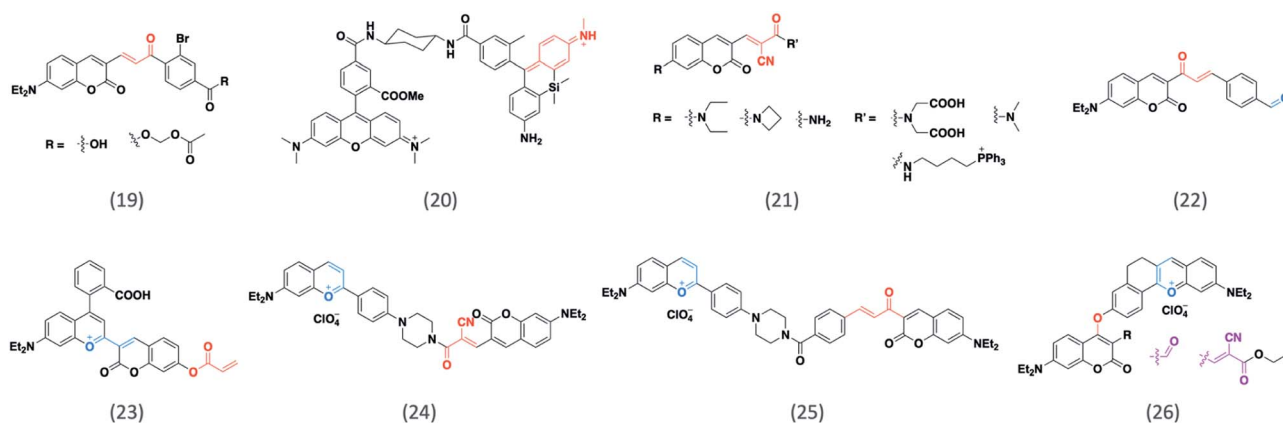


Fig. 2 19–21: Reversible fluorescent probes for dynamic GSH imaging; 22–26: Dual/multiple reaction site fluorescent probes for thiol metabolism imaging. The corresponding mechanisms for the reactions of these probes are presented in the ESI.†



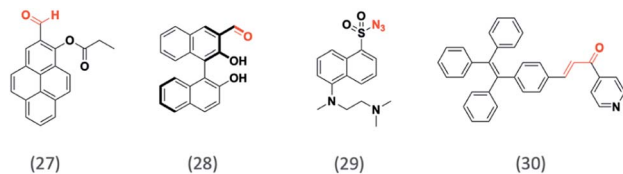


Fig. 3 Molecular structures of typical Hcy-specific fluorescent probes. The corresponding mechanisms for the reactions of these probes are presented in the ESI.†

detection is still urgently needed to help uncover the ambiguities of the pathological process of Hcy.

Typically, as we mentioned above, the products of the reaction between deprotonated **11** with Cys or Hcy (five-membered ring compound for Cys, weak fluorescence; six-membered ring compound for Hcy, strong fluorescence) displayed distinct fluorescence properties at pH 6.0.¹⁰⁷ The appearance of photo-induced electron transfer in the Cys product, but not in the Hcy product caused these results and allowed Hcy-specific fluorescence detection. Similar fluorescent responses appeared in a tri-aldehyde fluorescein probe system.¹¹⁰ Density functional theory calculations of the electronic transitions in the probes facilitated the understanding of the differences observed in the fluorescence responses when utilizing the aldehyde derivatives **27** and **28** (Fig. 3).^{108,109} Another example is the preference of Hcy for reduction by dansyl azide analogue (**29**) when compared with the other thiols.⁶⁸ The formation of an amino substituted derivate causes a turn-on fluorescence response which realizes Hcy detection in serum. In a probe (**30**) detection system that utilizes aggregation-induced emission (AIE) the more hydrophobic Hcy NAR product displayed a unique aggregation-induced blue fluorescent emission.¹¹¹ This was despite all three bio-thiols being able to quench the fluorescent yellow emission in the detection solution. These examples might inspire potential progress in the future.

Conclusions

In this minireview, we have presented the evolution of small organic molecular fluorescent probes for the detection of thiols over the past 60 years. The differences between the three bio-thiols and the development of design strategies for different probes were discussed comprehensively. The present challenges that limit their further applications are discussed. We hope that this minireview will promote the future design of fluorescent probes to enable deeper insight into the crucial roles of thiols and that design strategies move from those based on trial and error to those that use design-based molecular engineering.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This study was performed in strict accordance with the Chinese guidelines for the care and use of laboratory animals and was

approved by the Institutional Animal Care and Use Committee of Scientific Research in Shanxi University (Taiyuan, China). We thank the National Natural Science Foundation of China (No. 21907062, 21775096), One hundred people plan of Shanxi Province, Shanxi Province "1331 project" key innovation team construction plan cultivation team (2018-CT-1), 2018 Xiangyuan County Solid Waste Comprehensive Utilization Science and Technology Project (2018XYSDJS-05), Shanxi Province Foundation for Returnees (2017-026), Shanxi Collaborative Innovation Center of High Value-added Utilization of Coal-related Wastes (2015-10-B3), the Shanxi Province Foundation for Selected (No. 2019), Innovative Talents of Higher Education Institutions of Shanxi, Scientific and Technological Innovation Programs of Higher Education Institutions in Shanxi (2019L0031), Key R&D Program of Shanxi Province (201903D421069), the Shanxi Province Science Foundation (No. 201901D111015), and Scientific Instrument Center of Shanxi University (201512).

References

- P. Klason and T. Carlson, *Ber. Dtsch. Chem. Ges.*, 1906, **39**, 738–742.
- C. C. Lucas and E. J. King, *Biochem. J.*, 1932, **26**, 2076–2089.
- B. E. S. Guzman, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1951, **11**, 201–266.
- E. Friedmann, D. H. Marrian and I. Simonreuss, *Br. J. Pharmacol. Chemother.*, 1949, **4**, 105–108.
- H. Gainer and N. S. Kosower, *Histochemistry*, 1980, **68**, 309–315.
- P. B. Ghosh and M. W. Whitehouse, *Biochem. J.*, 1968, **108**, 155–156.
- H. Stern, *Science*, 1956, **124**, 1292–1293.
- R. Benesch, R. E. Benesch, M. Gutcho and L. Laufer, *Science*, 1956, **123**, 981–982.
- C. E. Paulsen and K. S. Carroll, *Chem. Rev.*, 2013, **113**, 4633–4679.
- L. J. Alcock, M. V. Perkins and J. M. Chalker, *Chem. Soc. Rev.*, 2018, **47**, 231–268.
- N. A. J. Carson and D. W. Neill, *Arch. Dis. Child.*, 1962, **37**, 505–513.
- L. McDonald, C. Bray, C. Field, F. Love and B. Davies, *Lancet*, 1964, **283**, 745–746.
- T. Gerritsen and H. A. Waisman, *Science*, 1964, **145**, 588.
- L. A. Harker, S. J. Slichter, C. R. Scott and R. Ross, *N. Engl. J. Med.*, 1974, **291**, 537–543.
- J. L. Maddocks and J. MacLachlan, *Lancet*, 1991, **338**, 1043–1044.
- Y. Özkan, E. Özkan and B. Imşek, *Int. J. Cardiol.*, 2002, **82**, 269–277.
- Y. M. Go and D. P. Jones, *Free Radical Biol. Med.*, 2011, **50**, 495–509.
- S. C. Barranco, B. Weintraub, E. G. Beasley, K. K. MacLean, J. Shaeffer, N. H. Liu, K. Schellenberg and C. M. Townsend, *Cancer Res.*, 1990, **50**, 3614–3618.
- R. R. Perry, J. Mazetta, M. Levin and S. C. Barranco, *Cancer*, 1993, **72**, 783–787.



- 20 R. Zhang, J. Yong, J. Yuan and Z. Ping Xu, *Coord. Chem. Rev.*, 2020, **408**, 213182.
- 21 J. Zhang, N. Wang, X. Ji, Y. Tao, J. Wang and W. Zhao, *Chem.-Eur. J.*, 2020, **26**, 4172–4192.
- 22 J. Dai, C. Ma, P. Zhang, Y. Fu and B. Shen, *Dyes Pigm.*, 2020, **177**, 108321.
- 23 Y. F. Kang, L. Y. Niu and Q. Z. Yang, *Chin. Chem. Lett.*, 2019, **30**, 1791–1798.
- 24 S. Lee, J. Li, X. Zhou, J. Yin and J. Yoon, *Coord. Chem. Rev.*, 2018, **366**, 29–68.
- 25 M. Wroński, *Anal. Bioanal. Chem.*, 1960, **175**, 432–436.
- 26 J. F. Lehman, L. P. Barrack and R. A. Lehman, *Science*, 1951, **113**, 410–412.
- 27 M. Wroński, *Anal. Bioanal. Chem.*, 1961, **180**, 185–188.
- 28 Y. Kanaoka, M. Machida, K. Tanizawa, Y. Ban, T. Sekine and Y. Soma, *Chem. Pharm. Bull.*, 1964, **12**, 127–134.
- 29 Y. Kanaoka, M. Machida and T. Sekine, *Biochim. Biophys. Acta*, 1973, **317**, 563–568.
- 30 T. Sekine, K. A. Kato, K. Takamori, M. Machida and Y. Kanaoka, *Biochim. Biophys. Acta, Gen. Subj.*, 1974, **354**, 139–147.
- 31 E. C. Klasen, *Anal. Biochem.*, 1982, **121**, 230–233.
- 32 Y. Kanaoka, M. Machida, Y. Ban and T. Sekine, *Chem. Pharm. Bull.*, 1967, **15**, 1738–1743.
- 33 X. Li, Y. Zheng, H. Tong, R. Qian, L. Zhou, G. Liu, Y. Tang, H. Li, K. Lou and W. Wang, *Chem.-Eur. J.*, 2016, **22**, 9247–9256.
- 34 W. H. Scouten, R. Lubcher and W. Baughman, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 1974, **336**, 421–426.
- 35 F. G. Prendergast, M. Meyer, G. L. Carlson, S. Iida and J. D. Potter, *J. Biol. Chem.*, 1983, **258**, 7541–7544.
- 36 P. Roveri, V. Cavrini and R. Gatti, *J. Liq. Chromatogr.*, 1993, **16**, 1859–1866.
- 37 J. V. Ros-Lis, B. García, D. Jiménez, R. Martínez-Mañez, F. Sancenón, J. Soto, F. Gonzalvo and M. C. Valdecabres, *J. Am. Chem. Soc.*, 2004, **126**, 4064–4065.
- 38 Y. Yue, F. Huo, F. Cheng, X. Zhu, T. Maffreyi, R. M. Strongin and C. Yin, *Chem. Soc. Rev.*, 2019, **48**, 4155–4177.
- 39 C. Yin, F. Huo, J. Zhang, R. Martínez-Mañez, Y. Yang, H. Lv and S. Li, *Chem. Soc. Rev.*, 2013, **42**, 6032–6059.
- 40 H. S. Jung, X. Chen, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2013, **42**, 6019–6031.
- 41 V. H. Cohn and J. Lyle, *Anal. Biochem.*, 1966, **14**, 434–440.
- 42 M. C. García Alvarez-Coque, M. J. Medina Hernández, R. M. Villanueva Camañas and C. Mongay Fernández, *Anal. Biochem.*, 1989, **178**, 1–7.
- 43 J. Li, Y. Kwon, K. S. Chung, C. S. Lim, D. Lee, Y. Yue, J. Yoon, G. Kim, S.-J. Nam, Y. W. Chung, H. M. Kim, C. Yin, J.-H. Ryu and J. Yoon, *Theranostics*, 2018, **8**, 1411–1420.
- 44 S. S. Simons and D. F. Johnson, *Anal. Biochem.*, 1977, **82**, 250–254.
- 45 T. Stuchbury, M. Shipton, R. Norris, J. P. Malthouse, K. Brocklehurst, J. A. Herbert and H. Suschitzky, *Biochem. J.*, 1975, **151**, 417–432.
- 46 M. W. Whitehouse and P. B. Ghosh, *Biochem. Pharmacol.*, 1968, **17**, 158–161.
- 47 J. L. Andrews, P. Ghosh, B. Ternai and M. W. Whitehouse, *Arch. Biochem. Biophys.*, 1982, **214**, 386–396.
- 48 G. L. Ellman, *Arch. Biochem. Biophys.*, 1958, **74**, 443–450.
- 49 K. Brocklehurst, *Int. J. Biochem.*, 1979, **10**, 259–274.
- 50 X. Wu, X. Sun, Z. Guo, J. Tang, Y. Shen, T. D. James, H. Tian and W. Zhu, *J. Am. Chem. Soc.*, 2014, **136**, 3579–3588.
- 51 M. Ye, X. Wang, J. Tang, Z. Guo, Y. Shen, H. Tian and W. H. Zhu, *Chem. Sci.*, 2016, **7**, 4958–4965.
- 52 R. Hong, G. Han, J. M. Fernández, B. J. Kim, N. S. Forbes and V. M. Rotello, *J. Am. Chem. Soc.*, 2006, **128**, 1078–1079.
- 53 B. Tang, Y. Xing, P. Li, N. Zhang, F. Yu and G. Yang, *J. Am. Chem. Soc.*, 2007, **129**, 11666–11667.
- 54 X. Han, F. Yu, X. Song and L. Chen, *Chem. Sci.*, 2016, **7**, 5098–5107.
- 55 X. Han, X. Song, F. Yu and L. Chen, *Chem. Sci.*, 2017, **8**, 6991–7002.
- 56 K. Imai and Y. Watanabe, *Anal. Chim. Acta*, 1981, **130**, 377–383.
- 57 L. Y. Niu, H. R. Zheng, Y. Z. Chen, L. Z. Wu, C. H. Tung and Q. Z. Yang, *Analyst*, 2014, **139**, 1389–1395.
- 58 K. Imai, T. Toyo'oka and Y. Watanabe, *Anal. Biochem.*, 1983, **128**, 471–473.
- 59 E. R. Triboni, M. J. Politi, I. M. Cuccovia, H. Chaimovich and P. Berci Filho, *J. Phys. Org. Chem.*, 2003, **16**, 311–317.
- 60 H. Maeda, H. Matsuno, M. Ushida, K. Katayama, K. Saeki and N. Itoh, *Angew. Chem., Int. Ed.*, 2005, **44**, 2922–2925.
- 61 Y. Liu, K. Li, K. X. Xie, L. L. Li, K. K. Yu, X. Wang and X. Q. Yu, *Chem. Commun.*, 2016, **52**, 3430–3433.
- 62 H. Maeda, K. Katayama, H. Matsuno and T. Uno, *Angew. Chem., Int. Ed.*, 2006, **45**, 1810–1813.
- 63 B. Zhang, C. Ge, J. Yao, Y. Liu, H. Xie and J. Fang, *J. Am. Chem. Soc.*, 2015, **137**, 757–769.
- 64 W. Jiang, Q. Fu, H. Fan, J. Ho and W. Wang, *Angew. Chem., Int. Ed.*, 2007, **46**, 8445–8448.
- 65 Y. Yue, F. Huo, Y. Zhang, J. Chao, R. Martínez-Mañez and C. Yin, *Anal. Chem.*, 2016, **88**, 10499–10503.
- 66 R. C. Fahey, G. L. Newton, R. Dorian and E. M. Kosower, *Anal. Biochem.*, 1981, **111**, 357–365.
- 67 K. Wang, H. Peng and B. Wang, *J. Cell. Biochem.*, 2014, **115**, 1007–1022.
- 68 H. Peng, K. Wang, C. Dai, S. Williamson and B. Wang, *Chem. Commun.*, 2014, **50**, 13668–13671.
- 69 A. V. Glushchenko and D. W. Jacobsen, *Antioxid. Redox Signaling*, 2007, **9**, 1883–1898.
- 70 U. Reddy, H. Agarwalla, N. Taye, S. Ghorai, S. Chattopadhyay and A. Das, *Chem. Commun.*, 2014, **50**, 9899–9902.
- 71 Y. Yue, F. Huo, X. Li, Y. Wen, T. Yi, J. Salamanca, J. O. Escobedo, R. M. Strongin and C. Yin, *Org. Lett.*, 2017, **19**, 82–85.
- 72 W. Wang, J. O. Escobedo, C. M. Lawrence and R. M. Strongin, *J. Am. Chem. Soc.*, 2004, **126**, 3400–3401.
- 73 L. Hakuna, J. O. Escobedo, M. Lowry, A. Barve, N. McCallum and R. M. Strongin, *Chem. Commun.*, 2014, **50**, 3071–3073.
- 74 K. Purkait, S. Chatterjee, S. Karmakar and A. Mukherjee, *Dalton Trans.*, 2016, **45**, 8541–8555.



- 75 Y. Qing, M. D. Liu, D. Hartmann, L. Zhou, W. J. Ramsay and H. Bayley, *Angew. Chem., Int. Ed.*, 2020, 15711–15716.
- 76 J. Yin, Y. Kwon, D. Kim, D. Lee, G. Kim, Y. Hu, J. H. Ryu and J. Yoon, *J. Am. Chem. Soc.*, 2014, **136**, 5351–5358.
- 77 N. Shao, J. Jin, H. Wang, J. Zheng, R. Yang, W. Chan and Z. Abliz, *J. Am. Chem. Soc.*, 2010, **132**, 725–736.
- 78 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**, 438–439.
- 79 W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949–15958.
- 80 F. Tanaka, N. Mase, C. F. Barbas and C. F. B. Iii, *Chem. Commun.*, 2004, **4**, 1762–1763.
- 81 H. Li, J. Fan, J. Wang, M. Tian, J. Du, S. Sun, P. Sun and X. Peng, *Chem. Commun.*, 2009, 5904–5906.
- 82 X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem., Int. Ed.*, 2011, **50**, 10690–10693.
- 83 L. Niu, Y. Guan, Y. Chen, L. Wu, C. Tung and Q. Yang, *J. Am. Chem. Soc.*, 2012, **134**, 1–24.
- 84 J. Liu, M. Liu, H. Zhang, X. Wei, J. Wang, M. Xian and W. Guo, *Chem. Sci.*, 2019, **10**, 10065–10071.
- 85 J. Liu, Y. Q. Sun, Y. Huo, H. Zhang, L. Wang, P. Zhang, D. Song, Y. Shi and W. Guo, *J. Am. Chem. Soc.*, 2014, **136**, 574–577.
- 86 G. Yin, T. Niu, T. Yu, Y. Gan, X. Sun, P. Yin, H. Chen, Y. Zhang, H. Li and S. Yao, *Angew. Chem., Int. Ed.*, 2019, **58**, 4557–4561.
- 87 Y. Yue, F. Huo, P. Yue, X. Meng, J. C. Salamanca, J. O. Escobedo, R. M. Strongin and C. Yin, *Anal. Chem.*, 2018, **90**, 7018–7024.
- 88 Y. Yue, F. Huo, X. Pei, Y. Wang and C. Yin, *Anal. Chem.*, 2020, **92**, 6598–6603.
- 89 E. H. Krenske, R. C. Petter and K. N. Houk, *J. Org. Chem.*, 2016, **81**, 11726–11733.
- 90 Y. Yue, F. Huo, Y. Wang, K. Ma, X. Li and C. Yin, *Chem. Commun.*, 2020, **2**, 9146–9149.
- 91 Y. Xu, L. Zhang, Y. Liu, Z. Jin, Q. Zhao, F. Yang and D. Xiao, *Biosens. Bioelectron.*, 2016, **77**, 182–187.
- 92 Z. Mao, J. Liu, T. S. Kang, W. Wang, Q. Bin Han, C. M. Wang, C. H. Leung and D. L. Ma, *Sci. Technol. Adv. Mater.*, 2016, **17**, 109–114.
- 93 Y. Jiang, J. Cheng, C. Yang, Y. Hu, J. Li, Y. Han, Y. Zang and X. Li, *Chem. Sci.*, 2017, **8**, 8012–8018.
- 94 X. Jiang, Y. Yu, J. Chen, M. Zhao, H. Chen, X. Song, A. J. Matzuk, S. L. Carroll, X. Tan, A. Sizovs, N. Cheng, M. C. Wang and J. Wang, *ACS Chem. Biol.*, 2015, **10**, 864–874.
- 95 X. Jiang, J. Chen, A. Bajić, C. Zhang, X. Song, S. L. Carroll, Z. L. Cai, M. Tang, M. Xue, N. Cheng, C. P. Schaaf, F. Li, K. R. MacKenzie, A. C. M. Ferreón, F. Xia, M. C. Wang, M. Maletić-Savatić and J. Wang, *Nat. Commun.*, 2017, **8**, 16087.
- 96 K. Umezawa, M. Yoshida, M. Kamiya, T. Yamasoba and Y. Urano, *Nat. Chem.*, 2017, **9**, 279–286.
- 97 Z. Liu, X. Zhou, Y. Miao, Y. Hu, N. Kwon, X. Wu and J. Yoon, *Angew. Chem., Int. Ed.*, 2017, **56**, 5812–5816.
- 98 J. Chen, X. Jiang, C. Zhang, K. R. Mackenzie, F. Stossi, T. Palzkill, M. C. Wang and J. Wang, *ACS Sens.*, 2017, **2**, 1257–1261.
- 99 L. He, X. Yang, K. Xu, X. Kong and W. Lin, *Chem. Sci.*, 2017, **8**, 6257–6265.
- 100 L. Jia, L.-Y. Niu and Q.-Z. Yang, *Anal. Chem.*, 2020, 10800–10806.
- 101 Y. Yue, F. Huo, P. Ning, Y. Zhang, J. Chao, X. Meng and C. Yin, *J. Am. Chem. Soc.*, 2017, **139**, 3181–3185.
- 102 B. Xu, H. Zhou, Q. Mei, W. Tang, Y. Sun, M. Gao, C. Zhang, S. Deng and Y. Zhang, *Anal. Chem.*, 2018, **90**, 2686–2691.
- 103 X. Yang, W. Liu, J. Tang, P. Li, H. Weng, Y. Ye, M. Xian, B. Tang and Y. Zhao, *Chem. Commun.*, 2018, **54**, 11387–11390.
- 104 W. Zhang, F. Huo, F. Cheng and C. Yin, *J. Am. Chem. Soc.*, 2020, **142**, 6324–6331.
- 105 T. Li, F. Huo, J. Chao and C. Yin, *Chem. Commun.*, 2020, **56**, 11453–11456.
- 106 Y. Huang, Y. Zhang, F. Huo, J. Chao, F. Cheng and C. Yin, *J. Am. Chem. Soc.*, 2020, **142**, 18706–18714.
- 107 A. Barve, M. Lowry, J. O. Escobedo, K. T. Huynh, L. Hakuna and R. M. Strongin, *Chem. Commun.*, 2014, **50**, 8219–8222.
- 108 Y. W. Wang, S. B. Liu, W. J. Ling and Y. Peng, *Chem. Commun.*, 2016, **52**, 827–830.
- 109 H. Y. Lee, Y. P. Choi, S. Kim, T. Yoon, Z. Guo, S. Lee, K. M. K. Swamy, G. Kim, J. Y. Lee, I. Shin and J. Yoon, *Chem. Commun.*, 2014, **50**, 6967–6969.
- 110 A. Barve, M. Lowry, J. O. Escobedo, J. Thainashmuthu and R. M. Strongin, *J. Fluoresc.*, 2016, **26**, 731–737.
- 111 N. Zhao, Q. Gong, R. X. Zhang, J. Yang, Z. Y. Huang, N. Li and B. Z. Tang, *J. Mater. Chem. C*, 2015, **3**, 8397–8402.

