Front. Chem. Sci. Eng. 2022, 16(10): 1425−1437
https://doi.org/10.1007/s11705-022-2163-1

REVIEW ARTICLE

Fluorescent probes and functional materials for biomedical applications

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Abstract Due to their simplicity in preparation, sensitivity and selectivity, fluorescent probes have become the analytical tool of choice in a wide range of research and industrial fields, facilitating the rapid detection of chemical substances of interest as well as the study of important physiological and pathological processes at the cellular level. In addition, many long-wavelength fluorescent probes developed have also proven applicable for *in vivo* biomedical applications including fluorescence-guided disease diagnosis and theranostics (e.g., fluorogenic prodrugs). Impressive progresses have been made in the development of sensing agents and materials for the detection of ions, organic small molecules, and biomacromolecules including enzymes, DNAs/RNAs, lipids, and carbohydrates that play crucial roles in biological and disease-relevant events. Here, we highlight examples of fluorescent probes and functional materials for biological applications selected from the special issues "Fluorescent Probes" and "Molecular Sensors and Logic Gates" recently published in this journal, offering insights into the future development of powerful fluorescence-based chemical tools for basic biological studies and clinical translation.

Keywords fluorescent probes, imaging, cell, biomedicine, biomolecules

Received December 15, 2021; accepted February 2, 2022

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1 Introduction

Fluorescence-based techniques have been extensively exploited in a number of research and industrial areas. The key agent to this technique is small-molecule fluorescent probes, which have proven advantageous in sensing and imaging applications due to their simplicity in design and synthesis, good sensitivity, selectivity, and minimal invasiveness when used for cell imaging [1,2]. Fluorescent probes have also been shown to be amenable to disease diagnosis and therapy; for example, the fluorescence-guided localization and stimulus-activated ablation of malignant tumors has been achieved *in vivo* [3–6]. With the rapid advancement of biophysical and analytical techniques, fluorescence-based agents have already become the most popular chemical tool for the detection, monitoring and tracking of biologically important species including ions [3], small molecules [7–9], reactive species and biomacromolecules including DNAs/RNAs, proteins, carbohydrates, and lipids, facilitating interdisciplinary studies at the interface of physics, chemistry, biology, and pharmaceutical and medical sciences [10]. The use of fluorescent probes in these basic research areas has led to the elaboration of a variety of previously unknown biological and diseases-relevant cellular processes at the molecular level [11,12].

Fluorescence is a photophysical phenomenon originating from the fast release of photonic energy of excited electrons as light, as they relax from the excited to ground state, and is typically observed for organic compounds with large conjugated structures [13]. Fluorescent

molecules, when modified with a receptor or reactive unit, can respond to a specific analyte of interest with a change in fluorescence intensity (fluorescence quenching or enhancement) or shift in fluorescence wavelength (ratiometric), thus achieving the fluorescence-based “sensing” of the analyte. In addition, by incorporating small-molecule fluorescent probes into functional material backbones, performance-enhanced (such as sensitivity, stability, and the ability to enter target cells more effectively) functional materials can be constructed to achieve the sensing as well as interruption of cellular processes with molecular precision, thereby aiding disease diagnosis and theranostics [14–16]. The following text will summarize recent progress in the development of fluorescent probes and functional materials for the detection and imaging of ions, reactive species, and biomacromolecules including enzymes DNAs/RNAs, lipids, and carbohydrates, and some other important small-molecular organic molecules at the cellular level and *in vivo* [17–19]. To date, several advanced methods for the analysis of binary images have been developed beyond the traditional image-processing and computer-vision approaches. Segmentation theory is widely exploited, and through clustering and probabilistic segmentation, convolutional networks for object recognition and semantic segmentation have been made possible. In addition, machine learning plays a vital role in image analysis, facilitating feature selection, classification, and the discovery of latent structures [20]. Examples are mainly selected from those published in recent special issues “Fluorescent Probes” (year 2020) and “Molecular Sensors and Logic Gates” (year 2021) published in this journal.

2 Fluorescence probes for metal ions

Fluorescent probes for metal ions including toxic metal ions and those that are essential to life have been extensively developed over the past 20 years. While real-time and accurate detection of toxic metal ions such as mercury and lead have proven useful for monitoring environmental pollution, the spatiotemporal tracking of the dynamic changes in the concentration and distribution of biologically important metal ions such as copper and zinc help advance our understanding of disease biology. Traditional methods for the analysis of ions in aqueous solution include liquid chromatography, atomic absorption, and mass spectrometry, which provide quantitative information of the analytes, and are costly and time-consuming. Fluorescent probes have been one of the most effective tools developed for the fast and sensitive detection of ions [21].

Detection of copper ions (mainly in the form of Cu^{2+}) is particularly important since unmonitored release can lead to environmental pollution and can be toxic to the human body. Significantly, the concentration of Cu^{2+} ions in drinking water must be below 2 $mg \cdot L^{-1}$ (30 $\mu mol \cdot L^{-1}$) as regulated by the World Health Organization. Although there are many reports on the selective detection of copper ions, most of them are based on coordination chemistry, which are susceptible to interference from competitive metal ions. To address this issue, Deng et al. [22] reported a “ligandless” *N*-cyclohexyl carbamate-based fluorene-*alt*-phenylene copolymer (PFPNCC) with good water solubility for the highly selective detection of Cu(II) (Fig. 1). The fluorescence of PFPNCC decreased gradually with increasing Cu(II) due to a photo-induced electron transfer process from the excited PFPNCC to Cu(II) in nucleophilic solvents such as *N*,*N*-dimethylformamide and dimethylsulfoxide. These findings provided a new strategy for developing polymer-based probes for various analytes.

Sasaki et al. [23] designed a focused fluorescent polymer sensor array based on two different carboxylic acid salts of polythiophenes, which was used to simultaneously detect 8 metal ions. Upon metal ion binding, the polythiophenes exhibited changes in fluorescence intensity or shifts in fluorescence emission wavelengths to different extents. The sensor array was also coated onto glass chips for the quantitative differentiation of metal ions in aqueous solution (Fig. 2). Similarly, a small library of C-bridged benzodiazoles

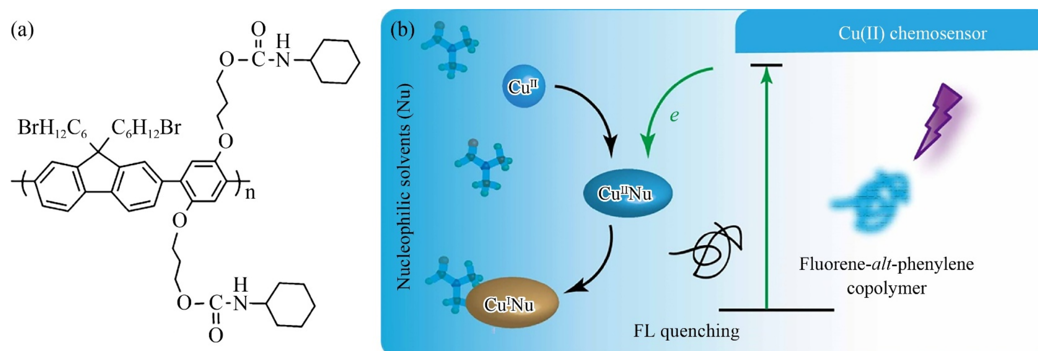

Fig. 1 (a) Structure of PFPNCC; (b) Plausible mechanism of the fluorescence quenching of PFPNCC in the presence of Cu^{2+} in nucleophilic solvents. Reprinted with permission from Ref. [22], copyright 2020, Springer Nature.

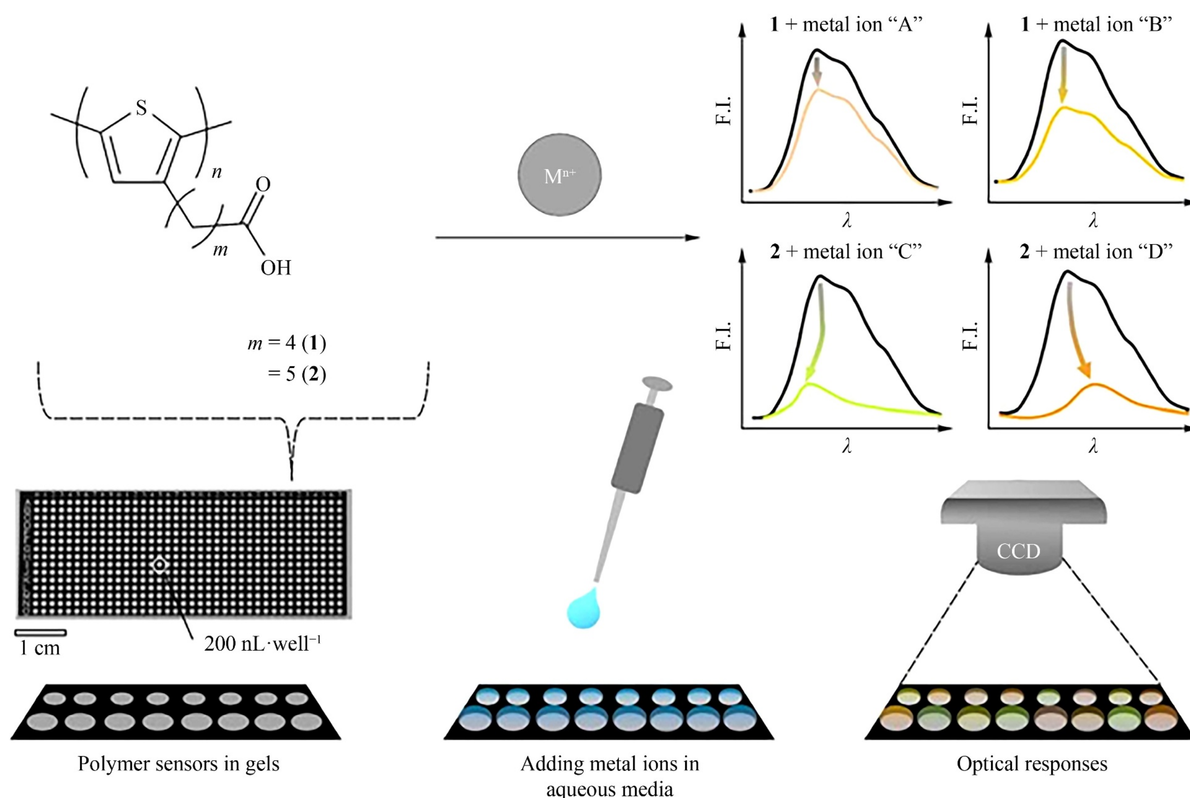

Fig. 2 Polythiophene-based minimized chemosensor array on glass chips designed for the simultaneous detection of different metal ions. Reprinted with permission from Ref. [23], copyright 2021, Springer Nature.

were synthesized by de Moliner et al. [24] as a new class of near-infrared (NIR) fluorescent dyes (Fig. 3), which were used in the construction of fluorescent probes able to discriminate Fe^{2+} and Fe^{3+} ions based on the differential aggregation profiles in aqueous solutions that contained Fe^{2+} or Fe^{3+} ions.

3 Fluorescence probes for reactive species and small molecules

Reactive oxygen species (ROS) are transient substances that are constantly being produced and consumed in all living creatures. In mammalian cells, ROS are a product of mitochondrial respiration and electron transfer, whose levels maintain the redox balance. However, abnormalities in ROS levels can cause cell dysfunction, leading to serious diseases including cancer, inflammation, diabetes, arthritis, and neurodegenerative diseases [25,26]. Superoxide anion ($O_2{\cdot}^-$) is a prototypical type of ROS with short half-life, high reactivity found at low concentrations in most cells and organs. Previously reported ROS probes are generally for a single analyte, and the development of multi-functional probes for the detection of more than one ROS that co-exist in the complicated cellular environment remains a challenging task. In addition, the ability to capture ROS selectively is demanding due to transient nature and short half-life and remains a significant challenge.

Chen et al [27] developed a method based on microchip electrophoresis laser-induced fluorescence (MCE-LIF) for the rapid and simultaneous measurement of $O_2{\cdot}^-$ and NO in the mitochondria of cells (Fig. 4). The presence of $O_2{\cdot}^-$ and NO converted non-fluorescent compounds 2-chloro-1,3-dibenzothiazolinecyclohexene (DBZTC) and 3-amino,4-aminomethyl-2',7'-difluorescein (DAF-FM) to the fluorescent products DBZTC oxide (DBO) and DAF-FM triazole (DAF-FMT), respectively. Based on the ratiometric changes of DBO and DAF-FMT in their respective electropherograms, the electropherographic peaks assigned to DBO and DAF-FMT were identified, which corresponded to the presence of $O_2{\cdot}^-$ and NO, respectively. In addition, the authors achieved the simultaneous tracking of the dynamic changes of $O_2{\cdot}^-$ and NO concentrations during resveratrol-induced cell apoptosis.

Hypochlorite (ClO^-), which is endogenously produced by the peroxidation of chloride ions in the presence of myeloperoxidase, plays a vital role in a variety of physiological and pathological events. Endogenous ClO^- in living cells, such as neutrophils, macrophages, mononuclear cells, may damage some invasive microorganisms when existing in excess, However, excessive ClO^- can also lead to a variety of human diseases such as cancer, cardiovascular disease, and neurodegeneration. Therefore, it's very important to monitoring intracellular levels of

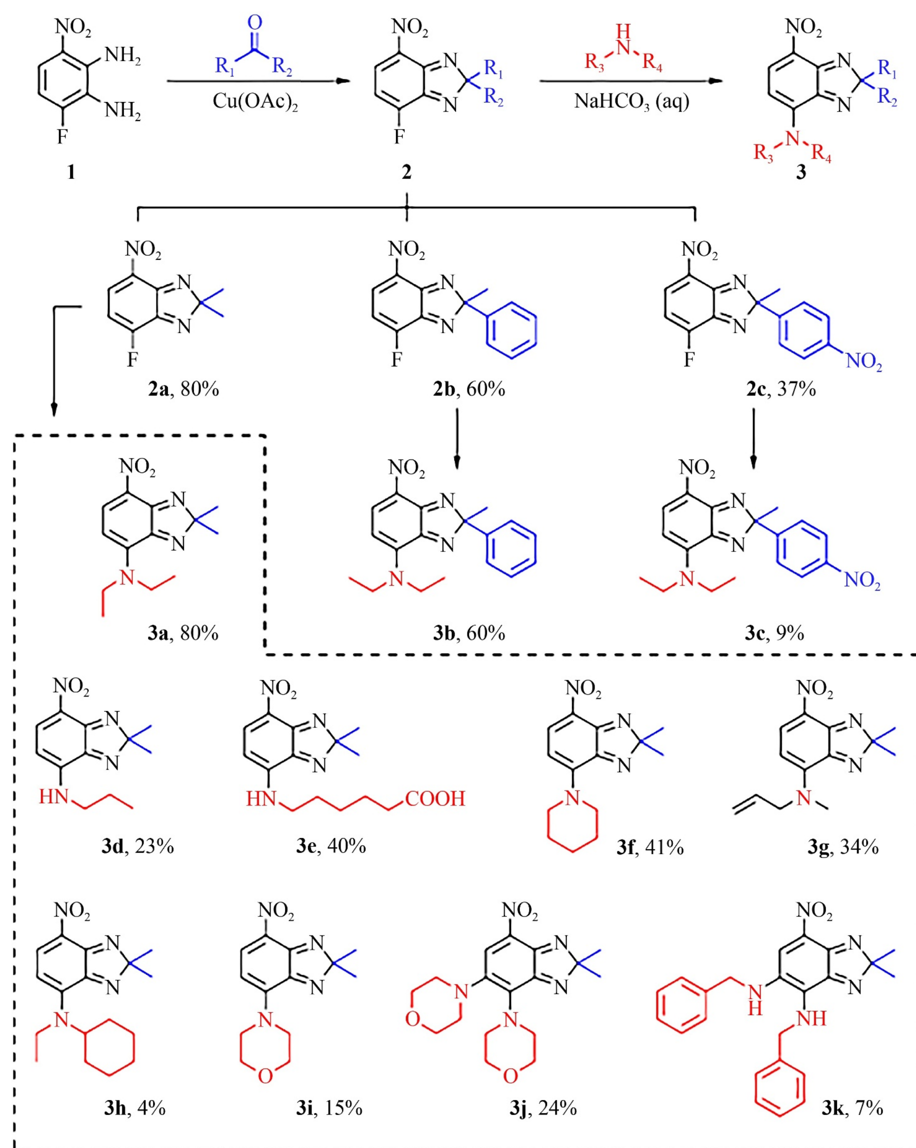

Fig. 3 Synthetic route to a focused library of C-bridged benzodiazoles. Reprinted with permission from Ref. [24], copyright 2021, Springer Nature.

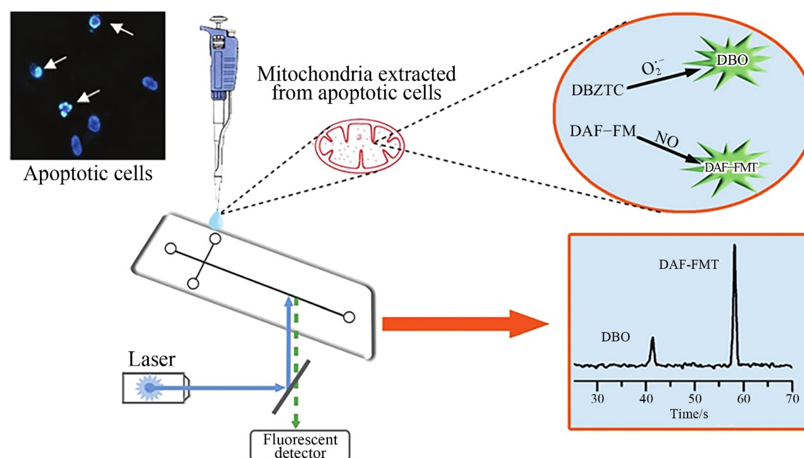

Fig. 4 Simultaneous determination of superoxide anion and NO in mitochondria of apoptotic cells based on MCE-LIF. Reprinted with permission from Ref. [27], copyright 2012, American Chemical Society.

ClO^- species [28,29]. The detection of ClO^- in living cells was achieved by Li et al. [30] using a redox-switchable UiO-type fluorescent sensor based on metal-organic frameworks (MOFs) synthesized by the direct ligand modification approach (Fig. 5). MOFs are porous materials formed by the self-assembly of inorganic metals with appropriate organic ligands. Due to their large specific surface area, high porosity, and adjustable pore structure, MOFs have been widely used for biosensing applications [31,32]. A *p*-methyloxyphenol-containing redox-switchable ligand termed H_2L-ol was mixed with $ZrCl_4$ and benzoic acids under solvothermal conditions to afford the MOF material UiO-68-ol. Upon the addition of ClO^-, the phenol group in UiO-68-ol was oxidized, leading to the transformation of H_2L-ol to H_2L-one resulting in fluorescence quenching. This MOF based material was used to detect intracellular ClO^- in RAW264.7 (Abelson leukemia expressed in mouse macrophage) cells.

Glucose is a diagnostic marker for diabetes, and the traditional approach to monitor glucose levels relies on glucose oxidase-based electrochemical sensors. However, this approach has some disadvantages in terms of the relatively low thermal stability of enzymes and stability issues when used under non-physiological conditions. Hashimoto et al. [33] developed a supramolecular system consisting of a phenylboronic acid-based fluorescent probe and γ-cyclodextrin (γ-CyD) for the recognition of glucose over other monosaccharides. The selective detection of glucose was achieved through the formation of pyrene- and anthanthrene-excimers in the hydrophobic cavity of γ-CyD upon binding with glucose in a stoichiometry of probe:glucose = 2:1 (Fig. 6). In a similar study, Xu et al. [34] developed a hydrogel system in which anthracene-modified boronic acid probes were embedded for the detection of monosaccharides. Based on the binding between boronic acid derivatives and carbohydrates, the boronic acid-containing hydrogel exhibited a ~16-fold fluorescence enhancement in the presence of fructose.

H_2S is an endogenous signaling molecule that modulates a myriad of physiological and pathological processes in the human body. A recent review by Zhou et al. [35] comprehensively summarized progress made in the construction of small-molecular fluorescent probes for the detection and imaging of H_2S in cells and *in vivo*. Shi et al. [36] synthesized a mitochondria-targeting ratiometric fluorescent probe. In the presence of H_2S, a Michael-type addition of HS^- to the vinyl bridge of the probe led to a ratiometric fluorescence change of the system (Fig. 7). The probe was used for imaging HeLa cells and nude

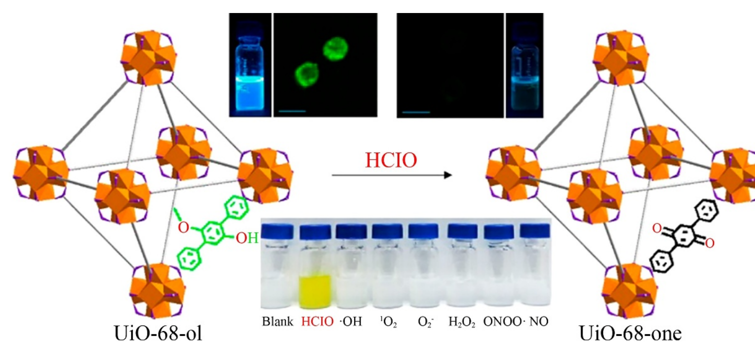

Fig. 5 Structure of a UiO-based MOFs probe for HClO detection and cellular imaging. Reprinted with permission from Ref. [30], copyright 2017, American Chemical Society.

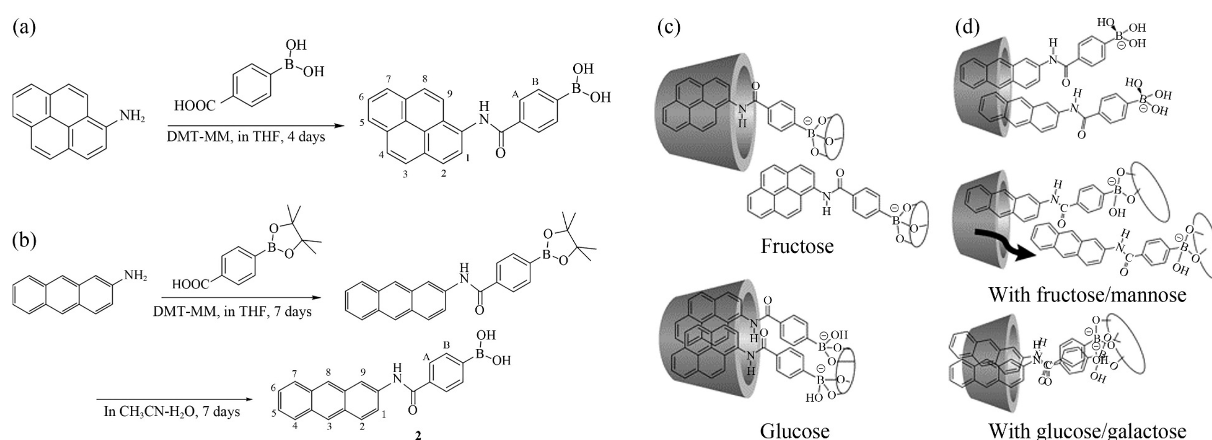

Fig. 6 (a, b) Synthesis of a pyrene-modified probe 1 and an anthracene-modified probe 2 for glucose detection. (c, d) Proposed binding modes of probes 1 and 2 with different saccharides in the presence of γ-CyD. Reprinted with permission from Ref. [33], copyright 2020, Springer Nature.

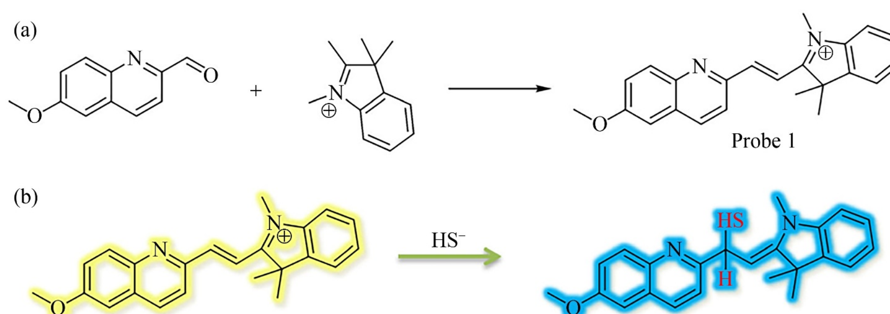

Fig. 7 (a) Synthesis of probe 1, and (b) its proposed sensing mechanism for HS^-. Reprinted with permission from Ref. [36], copyright 2021, Springer Nature.

mice on addition of exogenous H_2S a fast response time (within 30 s) was observed. Moreover, the probe was suitable for mitochondrial imaging in HeLa cells. Brito da Silva et al. [37] synthesized a fluorogenic H_2S probe based on azido-benzoxazole derivatives. When the azide group was reduced to an amine, the fluorescence of the probe was recovered due to the inhibition of the ESIPT (excited-state intramolecular proton transfer) process (Fig. 8). The probe was successfully used for the fluorescence imaging of T98G (human glioblastoma) cells.

A biothiol/thiophenol detection assay was developed by Xie et al. [38] using fluorescent probes based on resorufin and fluorescein modified with biothiol- and thiophenol-reactive groups, respectively (Fig. 9). The probes enabled the fluorescent imaging of both exogenously-added biothiols and thiophenol in HepG2 (human liver cancer) cells, and the imaging of endogenously generated biothiols in nude mice (Fig. 9). Similarly, a two-photon red-emitting fluorescent probe based on a naphthalene-BODIPY conjugate to which 2,4-dinitrobenzene-sulfonate was introduced as a thiophenol-reactive site was reported by Liu et al. [39]. The probe was suitable for the fluorescence imaging of thiophenol in live HeLa cells.

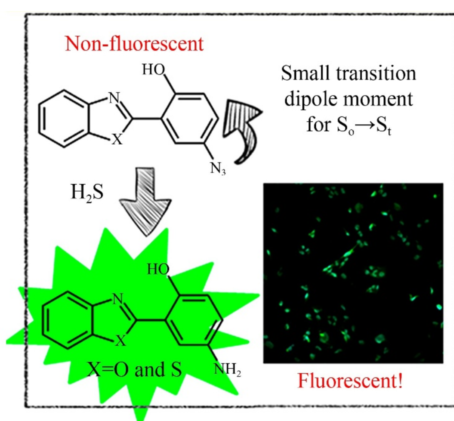

Fig. 8 Structure and biological application of azido-benzoxazole derivatives for the fluorogenic detection of H_2S based on ESIPT. Reprinted with permission from Ref. [37], copyright 2018, American Chemical Society.

4 Fluorescent probes for enzymes

Enzymes are essential catalytic species involved in the biochemical processes in all living organisms. Traditional analytical methods for enzymes are mainly immunosorbent and colorimetric assays, which are not suitable for sensing enzymes in live cells and *in vivo*. The exploitation of small-molecule fluorescent probes enables the effective tracking of the expression level as well as activity of enzymes involved in many biologically important processes. Therefore, the development of small-molecule based fluorescent probes for functionally diverse enzymes has become a topical research area [40]. For instance, Gurram et al. [41] used the commercially

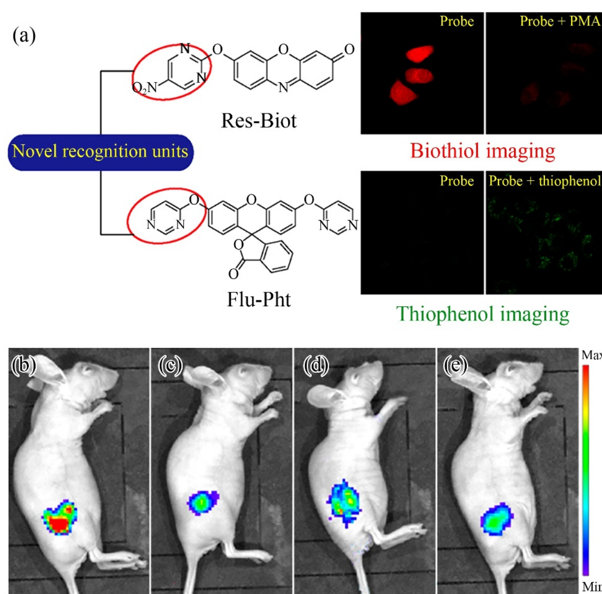

Fig. 9 (a) Structure and imaging application of Res-Biot and Flu-Pht for biothiols and thiophenol, respectively. (b–e) Fluorescence imaging of endogenous biothiols in nude mice. The mice were injected with (b) vehicle, (c) *N*-ethylmaleimide (1 mmol·L^{-1}, 200 μL), (d) lipopolysaccharide (1 mg·mL^{-1}, 200 μL), and (e) L-buthionine sulfoximine (BSO, 1 mmol·L^{-1}, 200 μL) followed by injection of Res-Biot (100 μmol·L^{-1}, 200 μL). Reprinted with permission from Ref. [38], copyright 2017, American Chemical Society.

available Nile Blue (NB) and Celecoxib (CCB) to produce a fluorescent probe, NB-C6-CCB, connected using an alkyl linkage. CCB is a selective inhibitor for cyclooxygenase-2 (COX-2). The probe was used for the selective detection of COX-2 in the Golgi apparatus of cancer cells. Based on their differences in expression level of COX-2, cancer and normal cells were effectively differentiated using the fluorescence from the probe localized in the Golgi apparatus (Fig. 10).

Odyniec et al. [42] designed a simple dual-responsive luciferase probe (PF3-GLC) for the simultaneous detection of β-glucoside enzyme (β-GLC) and H_2O_2 (Fig. 11). The "AND"-logic probe consisting of a fluorescein core as the fluorescence reporter and boronate ester and glucosyl group installed onto both sides of the fluorophore was used for the simultaneous sensing of β-GLC and H_2O_2 via a fluorescence "turn-on" signal. *In vitro* fluorescence dual-response of both CelTec2 (β-glucoside) and H_2O_2 was observed, indicating the potential of the probe for monitoring glucose metabolism.

Real-time imaging of UDP-glucuronosyltransferase 1A8 (UGT1A8, a UGT isoform involved in the biotransformation of environmental toxins and steroids) activity in living cells and tissues was achieved by Zhu et al. [43]. UGT1A8 adds a glucuronic acid to the fluorescent BDMP probe, based on the boron-dipyrromethene (BODIPY) scaffold, resulting in a strong fluorescence emission at 580 nm. The probe was used for the real-time imaging of endogenous UGT1A8 in HCT-15 (human colon cancer) cells and in LoVo (human colon cancer) tumor tissues (Fig. 12).

Xie et al. [44] developed a dual-function fluorescent "turn-on" probe termed KTLlip for the evaluation of lysine delipoylation. The probe acted as a substrate for recombinant NAD^+-dependent histone deacetylases Sirt1/Sirt2 to undergo a tandem delipoylation/intramolecular nucleophilic exchange reaction enhancing fluorescence (Fig. 13). KTLlip was able to measure the activity

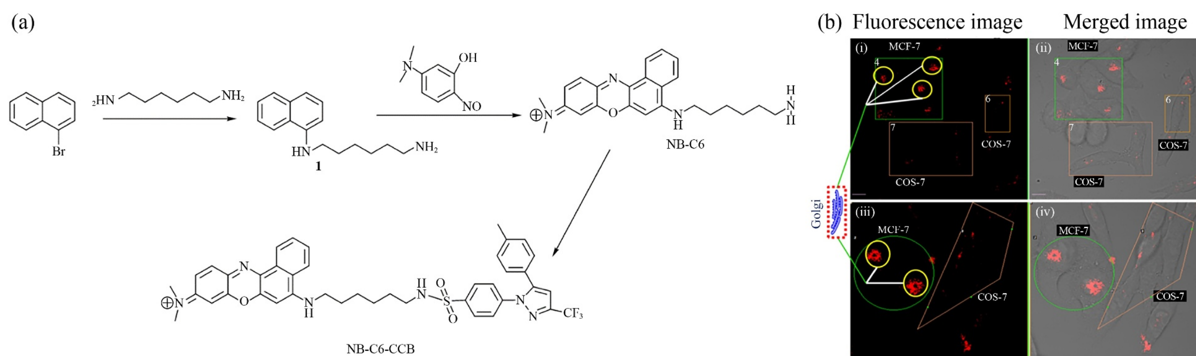

Fig. 10 (a) Synthesis of NB-C6-CCB. (b) Confocal microscopic images of (i) normal African green monkey kidney COS-7 cells and (iii) human breast cancer MCF-7 cells co-cultured following staining using NB-C6-CCB. Fluorescence images of (ii) co-cultured cancer (MCF-7) and (iv) normal cells (COS-7). Reprinted with permission from Ref. [41], copyright 2020, Springer Nature.

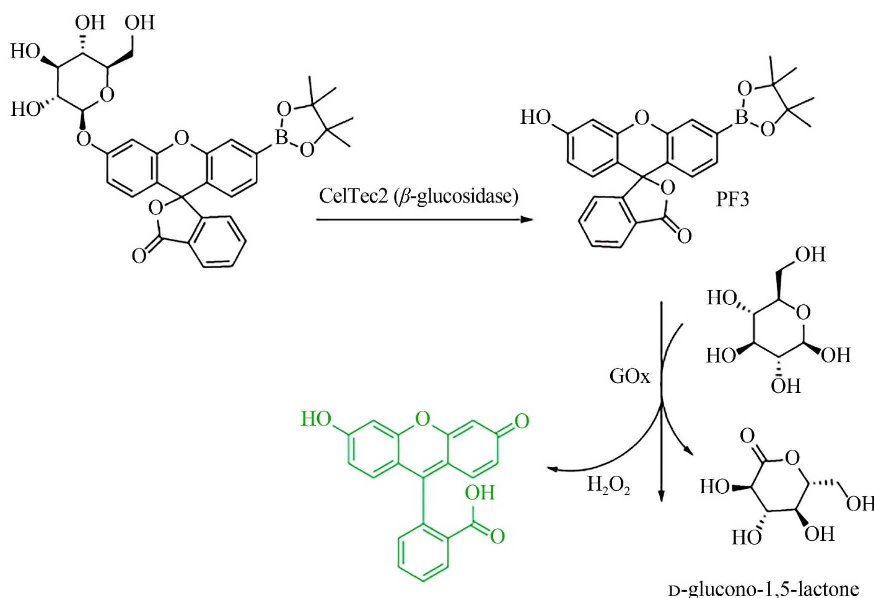

Fig. 11 Structure of a fluorescein-based dual-activated "AND"-logic probe and its sensing mechanism towards β-GLC and H_2O_2. Reprinted with permission from Ref. [42], copyright 2020, Springer Nature.

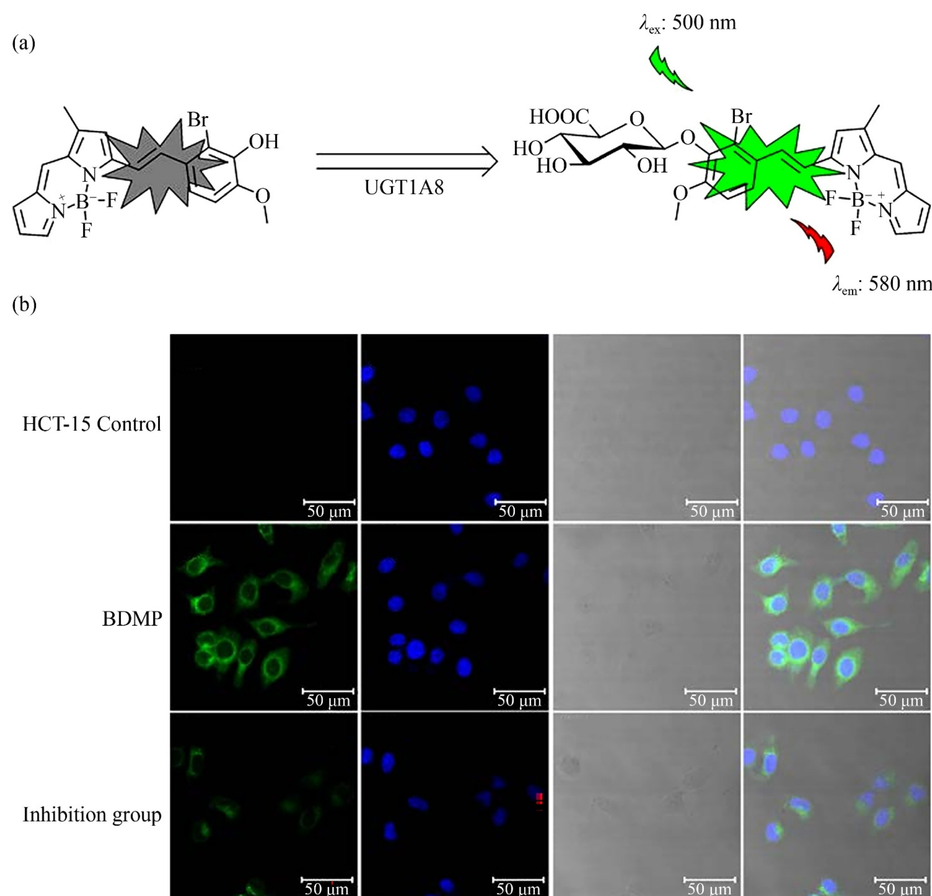

Fig. 12 (a) Schematic illustration of the addition of glucuronic acid to probe BDMP mediated by UGT1A8. (b) Confocal fluorescence images of HCT-15 cells with and without treatment with the BDMP probe and a UGT1A8 inhibitor. Reprinted with permission from Ref. [43], copyright 2021, Springer Nature.

of recombinant Sirt1/Sirt2 and could potentially be used for the screening of inhibitors of these enzymes, which may lead to the discovery of potential therapeutically active drugs. In addition, the probe was able to fluorescently label Sirt2 in a complex lysate environment.

5 Fluorescence probes for other biomolecules

The *in-situ* and real-time monitoring of mitochondrial DNA (mtDNA) damage was achieved by Feng et al. [45] (Fig. 14) using an environmentally sensitive fluorescent probe, MBI-CN consisting of 2-methyl-1*H*-benzo[*d*]imidazole and terephthalaldehyde. MBI was selected as the fluorophore because it can be easily implanted into the mtDNA double-stranded structure without causing DNA damage. However, when mtDNA is damaged, the malononitrile group in the MBI-CN could undergo degradation, and the resulting aldehyde-based product MBI-CHO with dual fluorescence emission exhibited a decreased fluorescence at both 437 and 553 nm.

A two-photon fluorescent probe that can be excited by light within the first biological optical window (650–950 nm) was developed by Kim et al. [46] (Fig. 15) based on the 6-acetyl-2-(dimethylamino) naphthalene skeleton for the imaging of amyloid-β (Aβ) plaques, a well-known biomarker for Alzheimer's disease (AD). The good tissue penetration depth of the probe facilitated the *in vivo* imaging of the Aβ plaques in an AD mouse model. Chen et al. [47] developed lysosome-targeting anticancer fluorescent agents for imaging zebra fish. Three probes based on an identical 4-(2-(bis(2-chloroethyl) amino) ethoxy) benzaldehyde skeleton were developed, and zebra fish embryos and larvae were used as models to validate the imaging capacity of the probes. In addition, these probes were shown to be selectively localized in the lysosomes of living cells and suitable for the *in vivo* tumor imaging of BxPC-3 (human pancreatic adenocarcinoma) tumor-bearing mice.

6 Conclusions

As effective chemical tools to selectively monitor biospecies associated physiological and pathological processes, fluorescent probes have been extensively developed facilitating the study of biochemistry, chemical

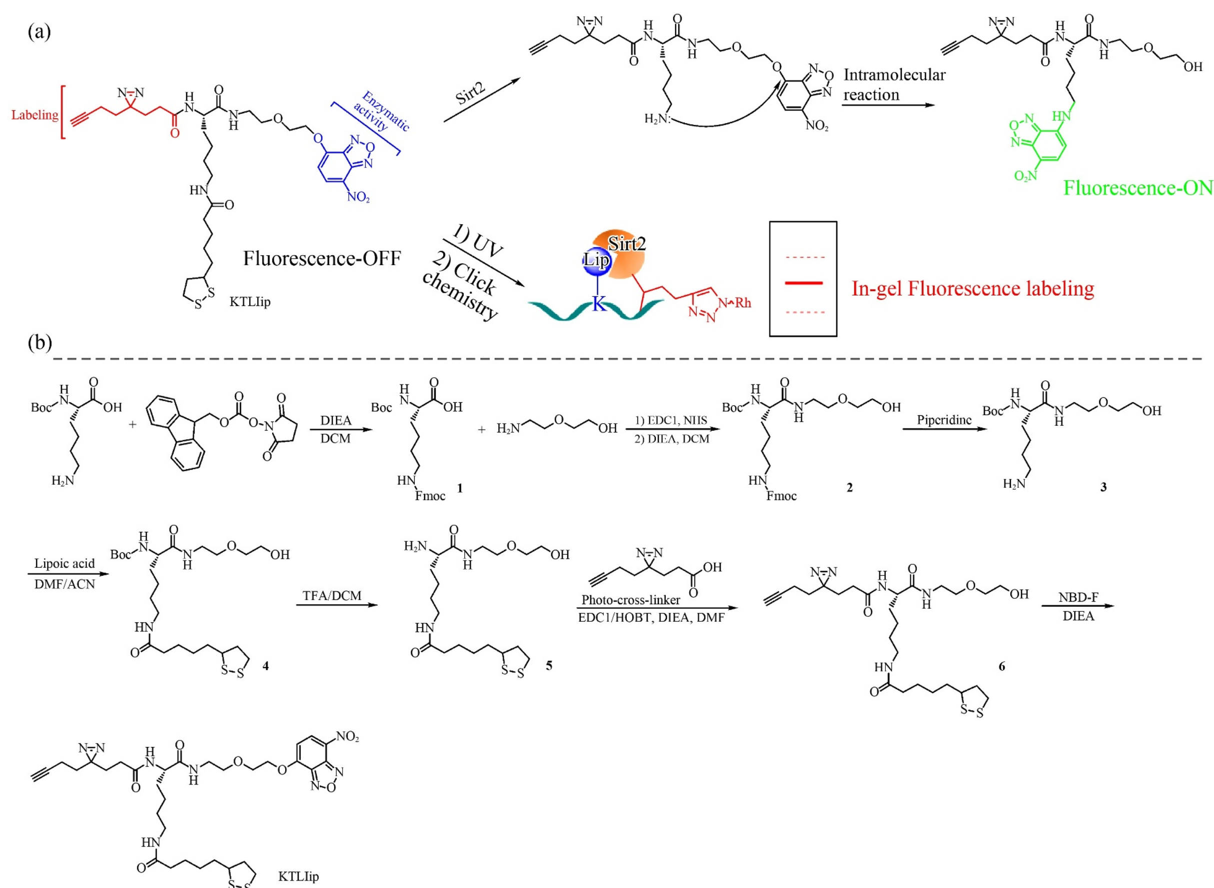

Fig. 13 (a) Schematic illustration of the mechanism of action of probe KTLlip for the detection and labeling of Sirt2. (b) Synthetic route for probe KTLlip. Reprinted with permission from Ref. [44], copyright 2021, Springer Nature.

biology, and biomedicine. Fluorescent probes with the ability to simultaneously react with two independent bio-species have been developed in recent years to overcome problems associated with single-response fluorescent probes [48–55]. Probes have also been developed that are able to monitor ions, reactive species, biomacromolecules, and small-molecule organic molecules which are important signaling molecules and are implicated in a range of physiological and pathological processes in living organisms [56–60]. In this short review, recent progresses towards the fluorescence-based sensing of several molecular targets have been discussed and summarized. We anticipate that this short report will encourage others to develop fluorescence probes and materials with improved characteristics suitable for monitoring biological systems.

To conclude, we would like to provide some guidance regarding appropriate research areas where development of fluorescent probes with enhanced capabilities for biomedical applications is required. These improved probe systems should: (1) Be capable of imaging *in vivo* biomarkers and as such need to overcome the limited tissue penetration exhibited by visible fluorescence. One effective approach is to develop probes exhibiting long fluorescence emission wavelengths. Recent successful examples include the extension of emission wavelengths to the NIR-II region (1000–1700 nm), resulting in substantially enhanced imaging properties of organic dyes in terms of tissue penetration depth, spatial and temporal resolution, imaging contrast and biocompatibility [61]. Alternatively, multimodal imaging can be used to multiplex the tissue penetration advantages of imaging modalities such as computed tomography and magnetic resonance imaging, with the imaging precision of fluorescence [62]. (2) Be suitable for the quantitative analysis of trace amounts of endogenous metal ions, free radicals, anions, and enzymes in biological systems. As such, the judicious design of nanomaterial-based probes could be one solution to achieve this goal. For example, a dual-emission water-soluble g-C_3N_4@AuNCs-based nanomaterial probe was designed by Guo et al. [63] for the label-free, quantitative analysis of trace Fe^{2+} and Cu^{2+} in the presence of competing cations. (3) Be capable of multi-organelle-targeting and able to interrogate the spatial-temporal localization of biomarkers with sub-organelle precision [64]. We have reported a fluorescent glycoprobe-human serum albumin (HSA) hybrid system with reversibly tunable fluorescent properties as a super-resolution imaging agent for intracellular β-galactosidase (β-Gal) activity. Using this probe, we found that while β-

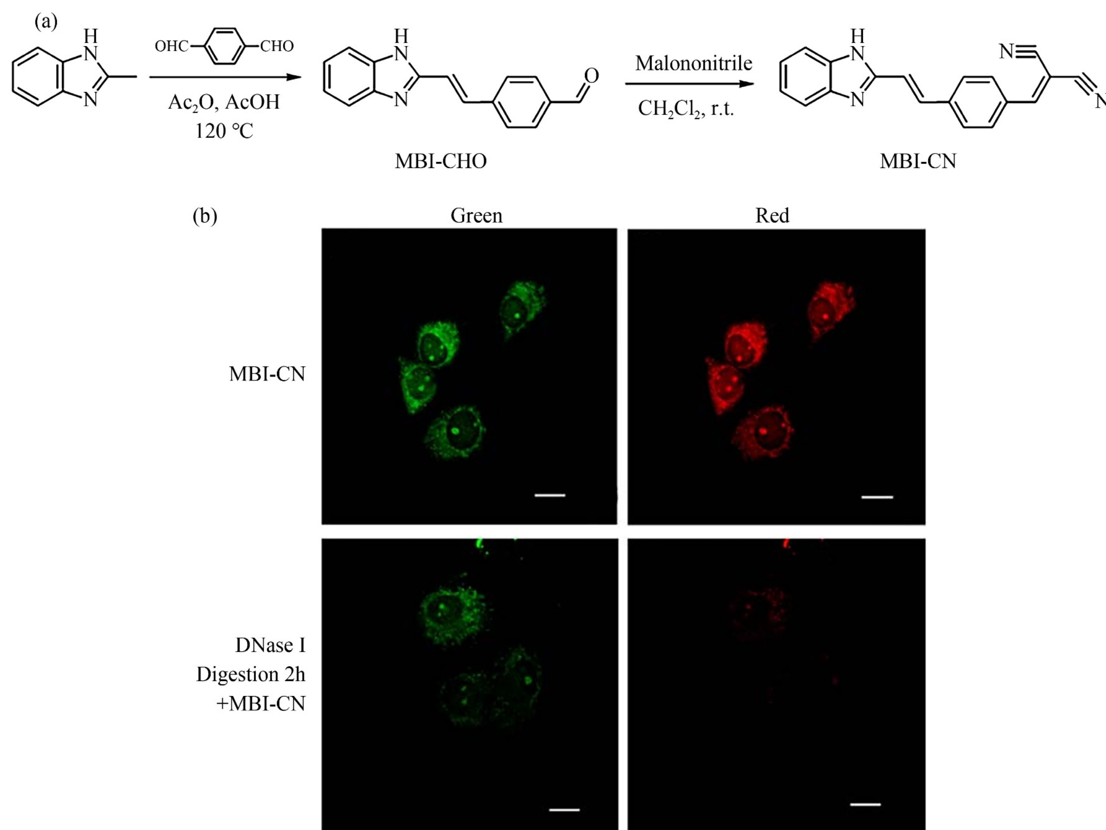

Fig. 14 Synthesis of MBI-CHO and MBI-CN, and the use of MBI-CN for imaging mtDNA degradation in cells. Reprinted with permission from Ref. [45], copyright 2021, Springer Nature.

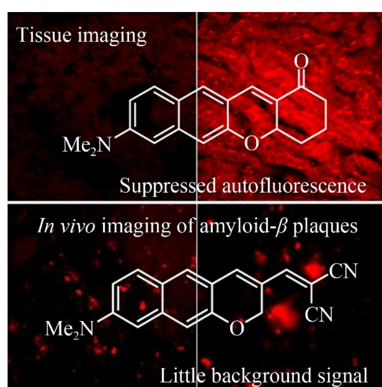

Fig. 15 Structures and biological imaging application of two-photon fluorescent dyes. Reprinted with permission from Ref. [46], copyright 2015, American Chemical Society.

Gal is evenly distributed in the cytoplasm of ovarian cancer cells, that the enzyme is specifically expressed in the lysosomes of senescent cells, thereby offering insight into the delineation of disease biology [65]. (4) Should have undergone extensive assessment of their biocompatibility to ensure non-toxicity and mildness towards the tissues undergoing clinical evaluation [66–68]. As such, the host−guest inclusion of probe molecules with HSA, an endogenous protein abundantly existing in the human blood, could represent a facile approach to minimize the toxicity of highly conjugated dyes [69].

Fluorescent probes with good water solubility are often required to enable high sensitivity, selectivity, and accurate imaging of endogenous biomarkers in living cells. The introduction of cationic units, anionic units or both, to organic molecules using simple chemical modifications is a particularly effective strategy that has been used to achieve this goal. For example, modification of quinoline-malononitrile using a sulfonate enabled Shao et al. [70] to substantial enhance the water solubility of their dye, thus improving the binding efficacy with Aβ aggregates. While, the addition of hydrophilic biomolecules such as carbohydrates to fluorescent dyes improves not only the water solubility, but in addition enhances the targeting towards specific cells with carbohydrate receptors [71−74].

Acknowledgements The authors thank the National Natural Science Foundation of China (Grant Nos. 21907030 and 22108077) and the International Cooperation Program of Shanghai Science and Technology Committee (Grant No. 19410712600). De-Tai Shi would like to thank the Natural Science Foundation of Jiangxi (Grant No. 20161BAB213067) and the Scientific Research Fund of Jiangxi Provincial Education Department (Grant No. GJJ170807). Tony D. James wishes to thank the Royal Society for a Wolfson Research Merit Award and the Open Research Fund of the School of Chemistry and Chemical Engineering, Henan Normal University for support (Grant No. 2020ZD01).

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References

1. Singha S, Yong W J, Sarkar S, Ahn K H. An endeavor in the reaction-based approach to fluorescent probes for biorelevant analytes: challenges and achievements. Accounts of Chemical Research, 2019, 52(9): 2571–2581
2. Erbas-Cakmak S, Kolemen S, Sedgwick A C, Gunnlaugsson T, James T D, Yoon J, Akkaya E U. Molecular logic gates: the past, present and future. Chemical Society Reviews, 2018, 47(7): 2228–2248
3. Sedgwick A C, Wu L, Han H-H, Bull S D, He X-P, James T D, Sessler J L, Tang B Z, Tian H, Yoon J. Excited-state intramolecular proton-transfer (ESIPT) based fluorescence sensors and imaging agents. Chemical Society Reviews, 2018, 47(23): 8842–8880
4. Wu D, Sedgwick A C, Gunnlaugsson T, Akkaya E U, Yoon J, James T D. Fluorescent chemosensors: the past, present and future. Chemical Society Reviews, 2017, 46(23): 7105–7123
5. Wu L, Sedgwick A C, Sun X, Bull S D, He X-P, James T D. Reaction-based fluorescent probes for the detection and imaging of reactive oxygen, nitrogen, and sulfur species. Accounts of Chemical Research, 2019, 52(9): 2582–2597
6. Kolanowski J L, Liu F, New E J. Fluorescent probes for the simultaneous detection of multiple analytes in biology. Chemical Society Reviews, 2018, 47(1): 195–208
7. Chan J, Dodani S C, Chang C J. Reaction-based small-molecule fluorescent probes for chemoselective bioimaging. Nature Chemistry, 2012, 4(12): 973–984
8. Wu L, Han H-H, Liu L, Gardiner J E, Sedgwick A C, Huang C, Bull S D, He X-P, James T D. ESIPT-based fluorescence probe for the rapid detection of peroxynitrite 'AND' biological thiols. Chemical Communications, 2018, 54(80): 11336–11339
9. Sedgwick A C, Gardiner J E, Kim G, Yevglevskis M, Lloyd M D, Jenkins A T A, Bull S D, Yoon J, James T D. Long-wavelength TCF-based fluorescence probes for the detection and intracellular imaging of biological thiols. Chemical Communications, 2018, 54(38): 4786–4789
10. Jiao X, Li Y, Niu J, Xie X, Wang X, Tang B. Small-molecule fluorescent probes for imaging and detection of reactive oxygen, nitrogen, and sulfur species in biological systems. Analytical Chemistry, 2017, 90: 533–555
11. Romieu A. "AND" luminescent "reactive" molecular logic gates: a gateway to multi-analyte bioimaging and biosensing. Organic & Biomolecular Chemistry, 2015, 13(5): 1294–1306
12. Wu D, Durán-Sampedro G, O'Shea D F. Synthesis and properties of water-soluble 1,9-dialkyl-substituted BF2 azadipyrromethene fluorophores. Frontiers of Chemical Science and Engineering, 2020, 14(1): 97–104
13. Zhang Y, Li S, Zhang H, Xu H. Design and application of receptor-targeted fluorescent probes based on small molecular fluorescent dyes. Bioconjugate Chemistry, 2021, 32(1): 4–24
14. Varandas P, Cobb A, Segundo M A, Silva E. Emergent glycerophospholipid fluorescent probes: synthesis and applications. Bioconjugate Chemistry, 2019, 31(3): 417–435
15. Singh H, Tiwari K, Tiwari R, Pramanik S K, Das A. Small molecule as fluorescent probes for monitoring intracellular enzymatic transformations. Chemical Reviews, 2019, 119: 11718–11760
16. Sedgwick A C, Han H-H, Gardiner J E, Bull S D, He X-P, James T D. The development of a novel AND logicbased fluorescence probe for the detection of peroxynitrite and GSH. Chemical Science, 2018, 9(15): 3672–3676
17. Jose D A, Sakla R, Sharma N, Gadiyaram S, Kaushik R, Ghosh A. Sensing and bioimaging of the gaseous signaling molecule hydrogen sulfide by near-infrared fluorescent probes. ACS Sensors, 2020, 5(11): 3365–3391
18. Tagit O, Hildebrandt N. Fluorescence sensing of circulating diagnostic biomarkers using molecular probes and nanoparticles. ACS Sensors, 2017, 2(1): 31–45
19. Sedgwick A C, Han H-H, Gardiner J E, Bull S D, He X-P, James T D. Long-wavelength fluorescent boronate probes for the detection and intracellular imaging of peroxynitrite. Chemical Communications, 2017, 53(95): 12822–12825
20. Nketia T A, Sailem H, Rohde G, Machiraju R, Rittscher J. Analysis of live cell images: methods, tools and opportunities. Methods, 2017, 115: 65–79
21. Lopez-Alled C M, Sanchez-Fernandez A, Edler K J, Sedgwick A C, Bull S D, McMullin C L, Kociok-Köhn G, James T D, Wenk J, Lewis S E. Azulene-boronate esters: colorimetric indicators for fluoride in drinking water. Chemical Communications, 2017, 53(93): 12580–12583
22. Deng W, Sun P, Fan Q, Zhang L, Minami T. Highly selective detection of copper(II) by a "ligand-free" conjugated copolymer in nucleophilic solvents. Frontiers of Chemical Science and Engineering, 2020, 14(1): 105–111
23. Sasaki Y, Lyu X, Zhang Z, Minami T. A minimized fluorescent chemosensor array utilizing carboxylate-attached polythiophenes on a chip for metal ions detection. Frontiers of Chemical Science and Engineering, 2022, 16(1): 72–80
24. de Moliner F, Biazruchka I, Konsewicz K, Benson S, Singh S, Lee J S, Vendrell M. Near-infrared benzodiazoles as small molecule environmentally-sensitive fluorophores. Frontiers of Chemical Science and Engineering, 2022, 16(1): 128–135
25. Odyniec M L, Sedgwick A C, Swan A H, Weber M, Tang T M S, Gardiner J E, Zhang M, Jiang Y B, Kociok-Kohn G, Elmes R B P, et al. 'AND'-based fluorescence scaffold for the detection of ROS/RNS and a second analyte. Chemical Communications, 2018, 54(61): 8466–8469
26. Maity S, Das S, Sadlowski C, Zhang J, Vegesnab G, Murthy N. Thiophene bridged hydrocyanine—a new fluorogenic ROS probe. Chemical Communications, 2017, 53: 10184–10187

27. Chen Z, Li Q, Sun Q, Chen H, Wang X, Li N, Yin M, Xie Y, Li H, Tang B. Simultaneous determination of reactive oxygen and nitrogen species in mitochondrial compartments of apoptotic HepG2 cells and PC12 cells based on microchip electrophoresis-laser-induced fluorescence. Analytical Chemistry, 2012, 84(11): 4687–4694
28. Winterbourn C C. Myeloperoxidase as an effective inhibitor of hydroxyl radical production implications for the oxidative reactions of neutrophils. Journal of Clinical Investigation, 1986, 78(2): 545–550
29. Sugiyama S, Okada Y, Sukhova G K, Virmani R, Heinecke J W, Libby P. Macrophage myeloperoxidase regulation by granulocyte macrophage colony-stimulating factor in human atherosclerosis and implications in acute coronary syndromes. American Journal of Pathology, 2001, 158(3): 879–891
30. Li Y, Yang S, Li Q Y, Ma J P, Zhang S, Dong Y. UiO-68-ol NMOF-based fluorescent sensor for selective detection of HClO and its application in bioimaging. Inorganic Chemistry, 2017, 56(21): 13241–13248
31. Tong P-H, Zhu L, Zang Y, Li J, He X-P, James T D. Metal-organic frameworks (MOFs) as host materials for the enhanced delivery of biomacromolecular therapeutics. Chemical Communications, 2021, 57: 12098–12110
32. Kumar P, Deep A, Kim K H. Metal organic frameworks for sensing applications. Trends in Analytical Chemistry, 2015, 73: 39–53
33. Hashimoto T, Kumai M, Maeda M, Miyoshi K, Tsuchido Y, Fujiwara S, Hayashita T. Structural effect of fluorophore on phenylboronic acid fluorophore/cyclodextrin complex for selective glucose recognition. Frontiers of Chemical Science and Engineering, 2020, 14(1): 53–60
34. Xu S, Sedgwick A C, Elfeky S A, Chen W, Jones A S, Williams G T, Jenkins T A, Bull S D, Fossey J S, James T D. A boronic acid-based fluorescent hydrogel for monosaccharide detection. Frontiers of Chemical Science and Engineering, 2019, 14(1): 112–116
35. Zhou L, Chen Y, Shao B, Cheng J, Li X. Recent advances of small-molecule fluorescent probes for detecting biological hydrogen sulfide. Frontiers of Chemical Science and Engineering, 2022, 16(1): 34–63
36. Shi Y, Huo F, Yue Y, Yin C. *N*-positive ion activated rapid addition and mitochondrial targeting ratiometric fluorescent probes for *in vivo* cell H_2S imaging. Frontiers of Chemical Science and Engineering, 2022, 16(1): 64–71
37. Brito da Silva C, Gil E, da Silveira Santos F, Morás A, Steffens L, Bruno Gonçalves P, Moura D, Lüdtke D, Rodembusch F. Proton-transfer-based azides with fluorescence off−on response for detection of hydrogen sulfide: an experimental, theoretical, and bioimaging study. Journal of Organic Chemistry, 2018, 83: 15210–15224
38. Xie X, Li M, Tang F, Li Y, Zhang L, Jiao X, Wang X, Tang B. Combinatorial strategy to identify fluorescent probes for biothiol and thiophenol based on diversified pyrimidine moieties and their biological applications. Analytical Chemistry, 2017, 89(5): 3015–3020
39. Liu H, Zhang X, Zhang J, Wang Q, Hu X, Wang P, Tan W. Efficient two-photon fluorescent probe with red emission for imaging of thiophenols in living cells and tissues. Analytical Chemistry, 2015, 87: 8896–8903
40. Zhang J, Chai X, He X-P, Kim H-J, Yoon J, Tian H. Fluorogenic probes for disease-relevant enzymes. Chemical Society Reviews, 2019, 48: 683–722
41. Gurram B, Li M, Fan J, Wang J, Peng X. Near-infrared fluorescent probe for fast track of cyclooxygenase-2 in Golgi apparatus in cancer cells. Frontiers of Chemical Science and Engineering, 2020, 14(1): 41–52
42. Odyniec M L, Gardiner J E, Sedgwick A C, He X-P, Bull S D, James T D. Dual enzyme activated fluorescein based fluorescent probe. Frontiers of Chemical Science and Engineering, 2020, 14(1): 117–121
43. Zhu M, Tian Z, Jin L, Huo X, Wang C, Cui J, Tian Y, Tian X, Feng L. A highly selective fluorescent probe for real-time imaging of UDP-glucuronosyltransferase 1A8 in living cells and tissues. Frontiers of Chemical Science and Engineering, 2022, 16(1): 103–111
44. Xie Y, Zhang J, Yang L, Chen Q, Hao Q, Zhang L, Sun H. A dual-function chemical probe for detecting erasers of lysine lipoylation. Frontiers of Chemical Science and Engineering, 2022, 16(1): 121–127
45. Feng B, Niu H, Zhai H, Shen C, Zhang H. *In-situ* hydrophobic environment triggering reactive fluorescence probe to real-time monitor mitochondrial DNA damage. Frontiers of Chemical Science and Engineering, 2022, 16(1): 92–102
46. Kim D, Moon H, Baik S, Singha S, Jun Y, Wang T, Kim K, Park B, Jung J, Mook-Jung I, Ahn K. Two-photon absorbing dyes with minimal autofluorescence in tissue imaging: application to *in vivo* imaging of amyloid-β plaques with a negligible background signal. Journal of the American Chemical Society, 2015, 137: 6781–6789
47. Chen X, Liu F, Chen B, Wu H, Li K, Xie Y, Kuang W, Li Z. Novel lysosome-targeted anticancer fluorescent agents used in zebrafish and nude mouse tumour imaging. Frontiers of Chemical Science and Engineering, 2022, 16(1): 112–120
48. Wu L, Huang J, Pu K Y, James T D. Dual-locked spectroscopic probes for sensing and therapy. Nature Reviews Chemistry, 2021, 5(6): 406–421
49. Wu L, Liu J, Li P, Tang B, James T D. Two-photon small-molecule fluorescence-based agents for sensing, imaging, and therapy within biological systems. Chemical Society Reviews, 2021, 50(2): 702–734
50. Yin J, Huang L, Wu L, Li J, James T D, Lin W. Small molecule based fluorescent chemosensors for imaging the microenvironment within specific cellular regions. Chemical Society Reviews, 2021, 50(21): 12098–12150
51. Wu L, Tian X, Groleau R R, Wang J, Han H-H, Reeksting S B, Sedgwick A C, He X-P, Bull S D, James T D. Coumarin-based fluorescent probe for the rapid detection of peroxynitrite 'AND' biological thiols. RSC Advances, 2020, 10(23): 13496–13499
52. Wang K, Feng B, Yang Y, Chen Y, Wang Y, Wang Y, Yang L, Jiang K, James T D, Zhang H. Dual-factor synergistically activated ESIPT-based probe: differential fluorescence signals to simultaneously detect α-naphthyl acetate and acid α-naphthyl

acetate esterase. Analytical Chemistry, 2021, 93(43): 14471–14480

53. He X-P, Hu X-L, James T D, Yoon J, Tian H. Multiplexed photoluminescent sensors: towards improved disease diagnostics. Chemical Society Reviews, 2017, 46: 6687–6696
54. Rai A, Singh A K, Tripathi K, Sonkar A K, Chauhan B S, Srikrishna S, James T D, Mishra L. A quick and selective rhodamine based “smart probe” for “signal-on” optical detection of Cu^{2+} and Al^{3+} in water, cell imaging, computational studies and solid state analysis. Sensors and Actuators B: Chemical, 2018, 266: 95–105
55. Murfin L C, Weber M, Park S J, Kim W T, Lopez-Alled C M, McMullin C L, Pradaux-Caggiano F, Lyall C L, Kociok-Köhn G, Wenk J, Bull S D, et al. Azulene-derived fluorescent probe for bioimaging: detection of reactive oxygen and nitrogen species by two-photon microscopy. Journal of the American Chemical Society, 2019, 141(49): 19389–19396
56. Yang M, Fan J, Du J, Peng X. Small-molecule fluorescent probes for imaging gaseous signaling molecules: current progress and future implications. Chemical Science, 2020, 11: 5127–5141
57. Tran F H, Zheng J J. Modulating the wnt signaling pathway with small molecules. Protein Science, 2017, 26: 650–661
58. Dong B L, Kong X Q, Lin W Y. Reaction-based fluorescent probes for the imaging of nitroxyl (HNO) in biological systems. ACS Chemical Biology, 2018, 13: 1714–1720
59. Zhou Y Q, Wang X, Zhang W, Tang B, Li P. Recent advances in small molecule fluorescent probes for simultaneous imaging of two bioactive molecules in live cells and *in vivo*. Frontiers of Chemical Science and Engineering, 2022, 16(1): 4–33
60. Su Y B, Yu B, Wang S, Cong H, Shen Y. NIR-II bioimaging of small organic molecule. Biomaterials, 2021, 271: 120717–120739
61. Yang Q, Ma H, Liang Y, Dai H. Rational design of high brightness NIR-II organic dyes with S-D-A-D-S structure. Accounts of Materials Research, 2021, 2: 170–183
62. Zhao J, Chen J, Ma S, Liu Q, Huang L, Chen X, Lou K, Wang W. Recent developments in multimodality fluorescence imaging probes. Acta Pharmaceutica Sinica B, 2018, 8: 320–338
63. Guo X, Huang J, Wang M, Wang L. A dual-emission water-soluble g-C_3N_4@AuNCs-based fluorescent probe for label-free and sensitive analysis of trace amounts of ferrous(II) and copper(II) ions. Sensors and Actuators B: Chemical, 2020, 309: 127766–127776
64. Han H-H, Tian H Jr. , Zang Y, Sedgwick A C, Li J, Sessler J L, He X-P, James T D. Small-molecule fluorescence-based probes for interrogating major organ diseases. Chemical Society Reviews, 2021, 50: 9391–9429
65. Chai X, Han H-H, Sedgwick A C, Li N, Zang Y, James T D, Zhang J, Hu X-L, Yu Y, Li Y, et al. Photochromic fluorescent probe strategy for the super-resolution imaging of biologically important biomarkers. Journal of the American Chemical Society, 2020, 142: 18005–18013
66. Yang F, Zhang Q, Huang S, Ma D. Recent advances of near infrared inorganic fluorescent probes for biomedical applications. Journal of Materials Chemistry B: Materials for Biology and Medicine, 2020, 8: 7856–7879
67. Miao Q, Yeo D C, Wiraja C, Zhang J, Ning X, Xu C, Pu K. Near-infrared fluorescent molecular probe for sensitive imaging of keloid. Angewandte Chemie International Edition, 2018, 57: 1256–1260
68. Chen W, Xu S, Day J J, Wang D, Xian M. A general strategy for development of near-infrared fluorescent probes for bioimaging. Angewandte Chemie International Edition, 2017, 56: 16611–16615
69. Fu Y, Han H-H, Zhang J, He X-P, Feringa B L, Tian H. Photocontrolled fluorescence “Double-Check” bioimaging enabled by a glycoprobe—protein hybrid. Journal of the American Chemical Society, 2018, 140: 8671–8674
70. Shao A, Guo Z, Zhu S J, Zhu S, Shi P, Tian H, Zhu W. Insight into aggregation-induced emission characteristics of red-emissive quinoline-malononitrile by cell tracking and real-time trypsin detection. Chemical Science, 2014, 5: 1383–1389
71. Thomas B, Yan K-C, Hu X-L, Donnier-Maréchal M, Chen G-R, He X-P, Vidal S. Fluorescent glycoconjugates and their applications. Chemical Society Reviews, 2020, 49: 593–641
72. He X-P, Zang Y, James T D, Li J, Chen G-R, Xie J. Fluorescent glycoprobes: a sweet addition for improved sensing. Chemical Communications, 2017, 53: 82–90
73. Dou W-T, Wang X, Liu T T, Zhao S W, Liu J-J, Yan Y, Li J, Zhang C-Y, Sedgwick A C, Tian H, Sessler J L, Zhou D-M, He X-P. A homogeneous high-throughput array for the detection and discrimination of influenza A viruses. Chem, 2022, doi: org/10.1016/j.chempr.2022.03.012 (in press)
74. Dou W-T, Han H-H, Sedgwick A C, Zhu G-B, Zang Y, Yang X-R, Yoon J Y, James T D, Li J, He X-P. Fluorescent probes for the detection of disease-associated biomarkers. Science Bulletin, 2022, 67: 853–878