Siyue Ma[a], Yaqi Wang[a], Mengyao She, Shen Wang, Zheng Yang, Ping Liu*, Shengyong Zhang and Jianli Li*

Design strategies and progress on xanthene-based fluorescent probe for metal ions

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Abstract: Metal ions play critical roles in numerous fundamental life processes. Hence, there is a great need to effectively monitor and image metal ions. Fluorescent probes are one of the most effective methods for measuring metal ions. In general, according to the different recognition mechanisms of fluorescent probes, they can be divided into two categories: reversible probes and irreversible probes. Among the various fluorophores, rhodamine and fluorescein, as the typical representatives of xanthene, have been paid much attention in biological imaging due to their high absorption coefficient, high fluorescence quantum yield, and water solubility. This review highlights the recent advances on chelation-based xanthene fluorescent probes that have been used for detecting metal ions. The focus has been on the design strategies to improve the selectivity and sensitivity of fluorescent probes by introducing different recognition moieties. Meanwhile, their recognition mechanism and applications are particularly highlighted.

Keywords: chelate; fluorescent probes; metal ions; xanthene.

[a]**Siyue Ma and Yaqi Wang:** These authors contributed equally to this work.

***Corresponding authors: Ping Liu and Jianli Li,** Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China, e-mail: liuping@nwu.edu.cn (Ping Liu); lijianli@nwu.edu.cn (J. Li)

Siyue Ma, Mengyao She and Shen Wang: Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China

Yaqi Wang: Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and Shaanxi Medical Devices Testing Centre, Xi'an, Shaanxi 710075, P.R. China

Zheng Yang: Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and School of Chemistry and Chemical Engineering, Xi'an University of Science and Technology, Xi'an, Shaanxi 710054, P.R. China

Shengyong Zhang: Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and School of Pharmacy, Fourth Military Medical University, Xi'an, Shaanxi 710032, P.R. China

Introduction

Metal ions play pivotal roles in numerous fundamental life processes (Carter et al. 2014, Properzi and Marcantoni 2014, Chen et al. 2015, Lee et al. 2015, Singha et al. 2015, Yin et al. 2015, Jung et al. 2016, Zhang et al. 2016b), including osmotic regulation, metabolism, biomineralization, and signaling. There are mainly two kinds of metal ions: (1) biologically essential metal ions, including Ca^{2+}, Fe^{3+}, Zn^{2+}, and Cu^{2+}, should be maintained within a suitable range to guarantee their normal biochemical functions and (2) biologically toxic metal ions, such as Hg^{2+} and Pb^{2+}, need to be detected with high sensitivity both *in vivo* and *in vitro* (Ye et al. 2013, Cui et al. 2015, Qian and Xu 2015). The change of these metal ion concentrations can affect the normal body and physiological functions directly (Figure 1). As a result, the detection and quantitative determination of these metal ions have emerged as the permanent research goal (Ding et al. 2015). Conventional methods, such as atomic absorption spectroscopy, high-performance liquid chromatography, and inductively coupled plasma-mass spectroscopy, have basic limitations in terms of cost, sample processing, and run times, which pose challenges or render them impractical for high-throughput clinical or research purpose. However, fluorescent sensing system has been recognized as one of the most efficient measures to monitor biological events *in vitro* and *in vivo* for its low-cost convenient pretreatment, rapid response, high sensitivity, and excellent selectivity (Zhou et al. 2011, Vendrell et al. 2012, Yang et al. 2013a, Li et al. 2014). In addition, when served as the reactant associated with the targeted analyte, it can provide a reliable fluorescent response, which can be detected using a fluorometer or even more conveniently under a portable UV lamp.

Until now, various fluorescent probes have been developed based on a diverse range of fluorophores, such

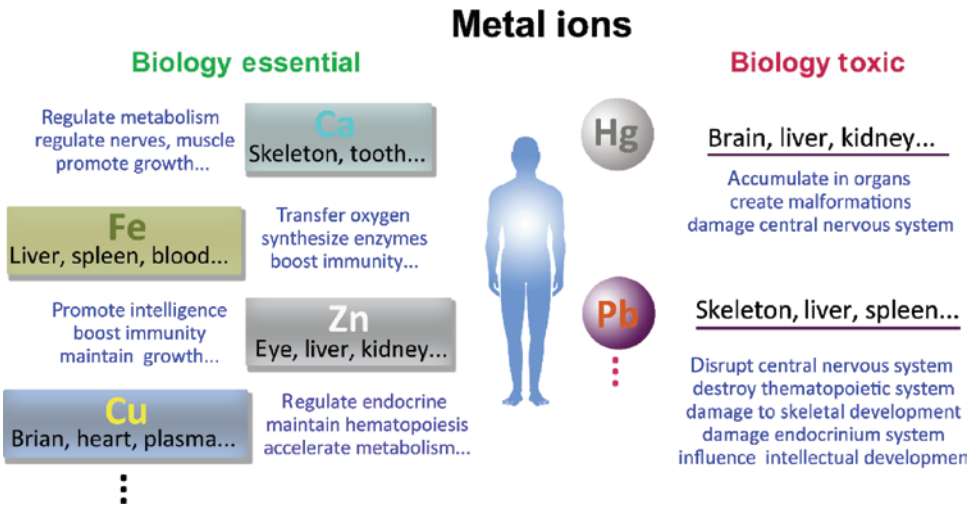


Figure 1: Effect of metal ions in the fundamental life processes.

as cyanine, BODIPY, xanthene, coumarin, pyrene, and 1,8-naphthalimide, with different excitation and emission wavelengths (Chen et al. 2012). In general, according to the different recognition mechanisms of these reported fluorescent probes, they can be divided into two categories: reversible and irreversible probes (Kartha et al. 2015, Moon et al. 2016). Reversible probes, usually called chelation-based probes, are typically based on the binding equilibrium between probe molecules and the target, and such processes of the coordination and release of the target result in the occurrence and disappearance of the reversible fluorescence signal. Irreversible probes are typically based on the extent of the chemical conversions between the probe molecules and the target, and such processes of the specific chemical reactions result in the fluorescence signal change, and the altered signals are maintained.

Rhodamine and fluorescein, as the typical representatives of xanthene, have been paid much attention in biological imaging due to their high absorption coefficient, high fluorescence quantum yield, and water solubility (Kim et al. 2008, Chen et al. 2010). Their carboxyl group can be easily converted to the spirolactam or spirolactone moiety, which could be used as a molecular scaffold to construct an excellent reversible fluorescent probe. Although such spirocyclic derivatives of rhodamine and fluorescein dyes are nonfluorescent, as shown in Figure 2, they will elicit the fluorescence emission via the ring-opening reaction of the corresponding spirocyclic (the spirocyclic C–X bond breaks, X = N, O, S) after the targeted metal ions are added (Shi and Ma 2008, Zhan et al. 2008, Huang et al. 2014b). If the metal ions are removed, the fluorescence signal will be recovered to its original state. Therefore, they have potential applications in detecting metal ions as reversible fluorescence probes. Especially, over the past decade, fluorescent probes based on xanthene have been focused on detecting those metal ions in living systems (Kikuchi 2010, Hayashi and Okamoto 2013, He et al. 2015). These efforts have uncovered many promising probes that have important applications (Li et al. 2015a, Lin et al. 2015, Tang et al. 2015, Fernandez and Vendrell 2016).

In addition, many researchers make an effort to the design strategies in developing a variety of highly selective and sensitive fluorescent probes that are of good applicability to biological systems. Generally speaking, for the probes derived from rhodamine B and 6G, the modification of molecules is most commonly concentrated on the N-terminus of spirolactam commonly, which could be linked to various receptors for metal ions. In contrast, the corresponding modification is most commonly concentrated on the hydroxyl group of the fluorescein derivate. Therefore, the aim of this review is to highlight the advances on xanthene-based fluorescent probes for metal ions, which cover mostly works published since 2011.

Xanthene fluorescent probes with the Schiff base structure

The most representative example of fluorescent probes based on xanthene for Cu^{2+} was reported by Czarnik's group in 1997, which attracts a great deal of attention (Dujols et al. 1997). Schiff base structural motif, a good

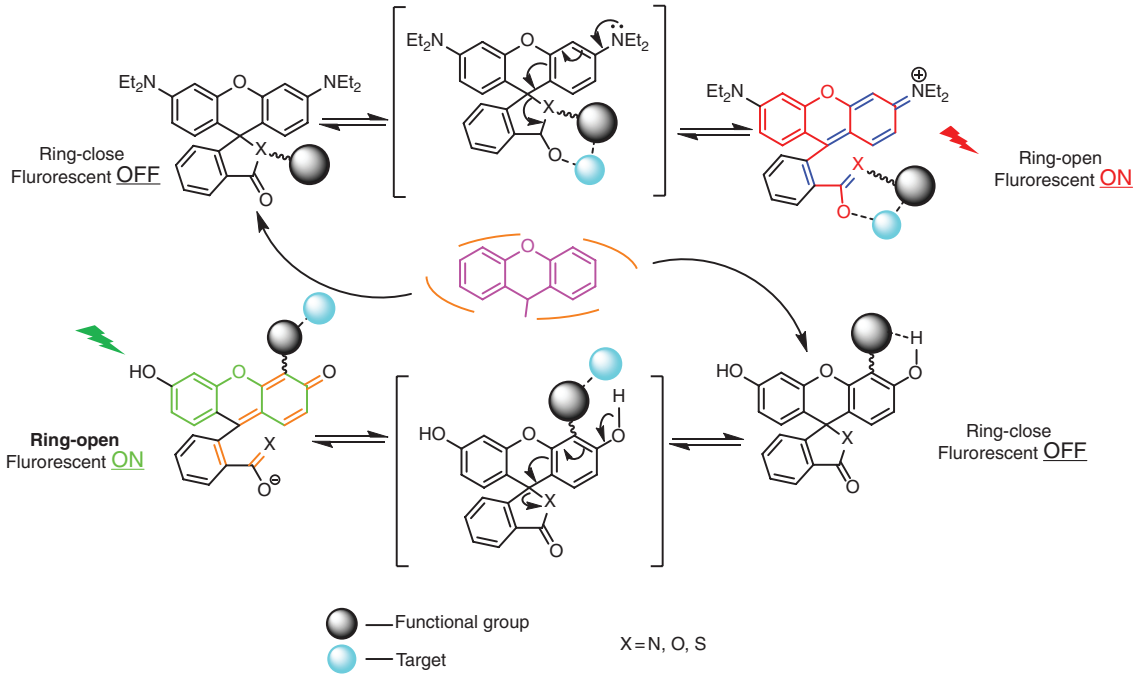


Figure 2: Recognition mechanism of fluorescent probes based on xanthene for detecting metal ions.

ligand, can be used for the identification of metal ions and quantitative analysis of the content of metal ions because of the electron-rich property. Hydrazone compounds, a kind of Schiff base compound obtained by modified hydrazide compound, have better biological activity and lower toxicity to organisms. As a result, based on previous work, lots of xanthene hydrazone probes are designed, improved, and synthesized by introducing different aldehydes or ketones.

Probes for Cu^{2+}

Most of the xanthene hydrazone probes could be applied in the identification of Cu^{2+} because of the good affinity of nitrogenous and oxygenous recognition moiety to Cu^{2+} according to the soft-hard acid-base theory. Copper ions, a kind of transition metal ions, play an essential role in a variety of fundamental physiological processes in organisms ranging from bacteria to mammals, and its distribution is strictly controlled *in vivo*. On the one hand, excess copper can cause a highly toxic state and lead to serious infant liver damage, such as Wilson's disease. On the other hand, the loss of copper homeostasis can result in Alzheimer's disease and Menkes disease. A comprehension of the physiological and pathological functions of Cu^{2+} in living cells is very significant (Liu et al. 2012b, Ge et al. 2013b, Goswami et al. 2014). Therefore, fluorescent probes for the detection of copper ion have been widely explored. Among them, the most interesting, selective, and sensitive probes are those that are based on xanthene hydrazone (Figure 3).

In 2011, Zhao et al. described the development of a rhodamine chromene-based fluorescence probe **1** to monitor the intracellular Cu^{2+} level in living HeLa cells, which exhibited a fluorescence response toward Cu^{2+} under physiological conditions with high sensitivity and selectivity (Liu et al. 2011). The fluorescence intensity was significantly increased by about 40-fold with 10 equivalents of added Cu^{2+}. The data from Job's method exhibited a maximum absorbance when the molecular fraction of **1** was close to 50%, which also suggests a 1:1 stoichiometry for the **1**-Cu^{2+} complex (Figure 4).

In the same year, a new probe **2** based on the spirolactam form of rhodamine 101 hydrazone was reported by Xie's group, which exhibited a highly selective and sensitive response toward Cu^{2+} in aqueous solutions (Xie et al. 2011). The structural rigidity introduced by multiple n-propylene bridges of rhodamine 101 moieties can shift the absorption and emission maxima to longer wavelengths, with higher molar extinction coefficients and higher quantum yields compared to rhodamine B. The fluorescence of probe **2** can be detected in a low concentration (2×10^{-6} mol/l) of Cu^{2+}. The results revealed that probe **2** is a good colorimetric probe to Cu^{2+} in the red region (Figure 5).

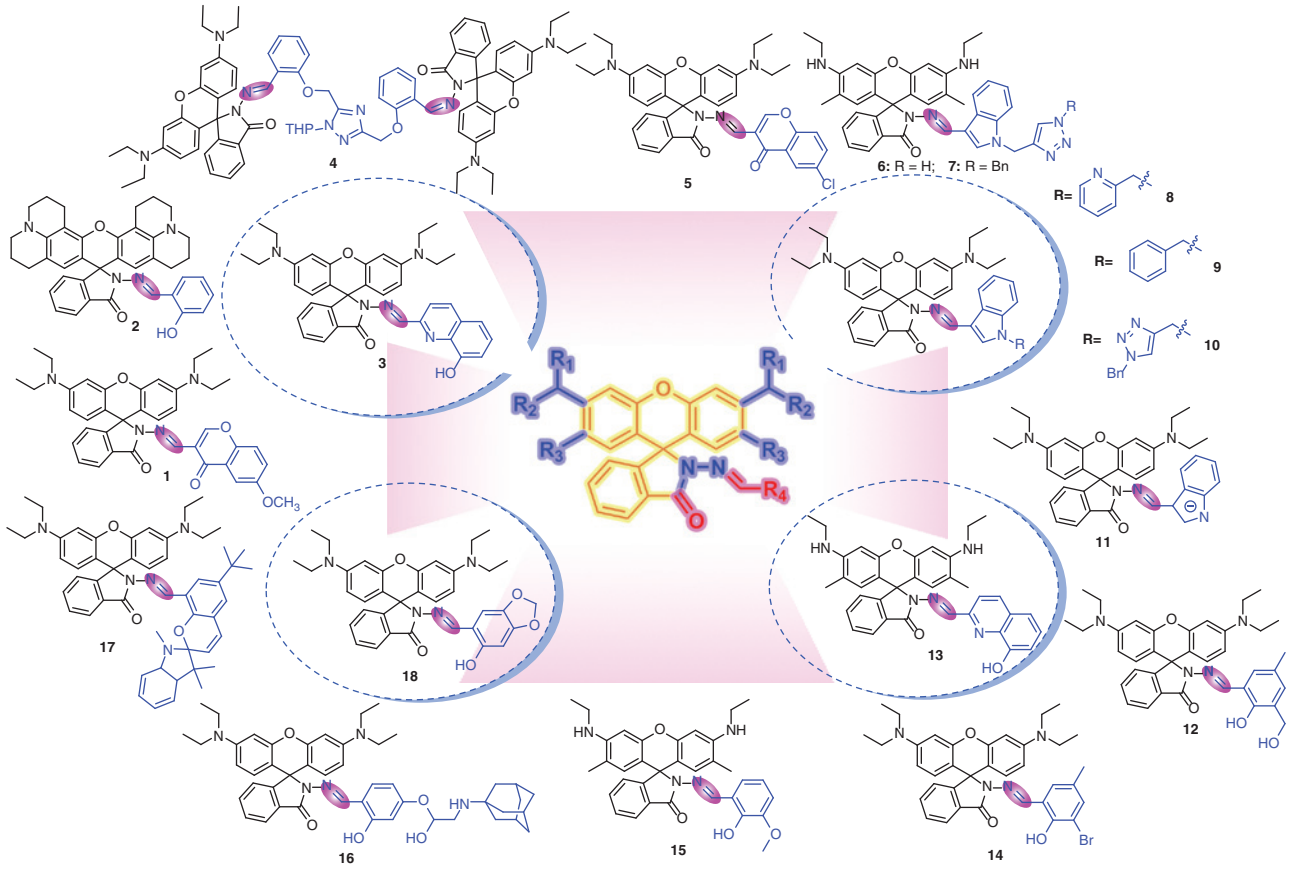


Figure 3: Probes **1–20** based on xanthene hydrazone for detecting Cu^{2+}.

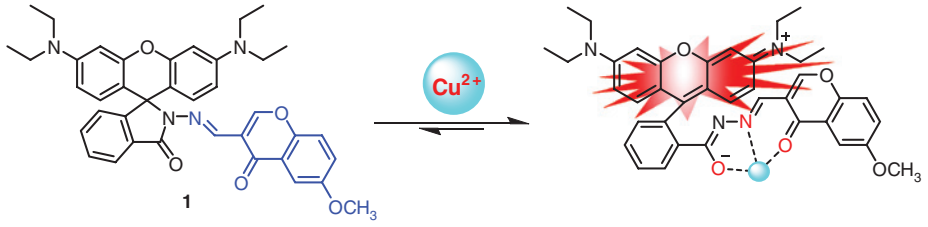


Figure 4: Recognition mechanism of probe **1** with Cu^{2+}.

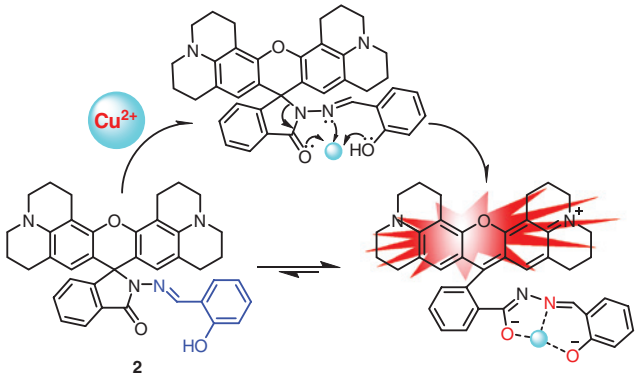


Figure 5: Recognition mechanism of probe **2** with Cu^{2+}.

8-Hydroxyquinoline-based ligands with extended conjugated fluorophores can form luminescent chelates with a number of metal ions. Therefore, a novel rhodamine-quinoline derivative-based probe **3** was designed and synthesized by Feng et al. which exhibited highly selective and sensitive colorimetric and "turn-on" fluorescent responses toward Cu^{2+} ions in aqueous solution (Feng et al. 2012). The good linear relationship was easily obtained from the fluorescence changes and the concentrations in the range of 20–120 mM. The fluorescence change was remarkable for the Cu^{2+} ion detection even in the presence of other metal ions, such as Ca^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, Ba^{2+}, Mg^{2+}, and Pb^{2+}.

In 2012, a new probe **4** based on rhodamine B with 1,2,4-triazole as subunit was synthesized and characterized by Zhang (Zhang et al. 2012). Because of the 1,2,4-triazole subunit containing lone electron pairs on N, the semirigid ligand could effectively chelate Cu^{2+} according to the ionic radius and limit the geometric structure of the complex. Probe **4** exhibited high selectivity and sensitivity for Cu^{2+} in ethanol/water (6:4, v/v) of pH 7.0 HEPES buffer solution and underwent ring opening mechanism, and a 2:1 metal-ligand complex was formed. Probe **4** displayed a linear response to Cu^{2+} in the range between 1.0×10^{-7} and 1.0×10^{-6} M with a detection limit of 4.5×10^{-8} M. Its capability of biological application was also evaluated and the results showed that probe **4** could be successfully employed as a Cu^{2+}-selective probe in the fluorescence imaging of living MCF-7 cells (Figure 6).

Zhao et al. described the development of a rhodamine chromene-based "turn-on" fluorescence probe **5** to monitor the intracellular Cu^{2+} level in living cells (Liu et al. 2012a). The new fluorescent probe with a chlorine group in chromene moiety exhibited good membrane-permeable property because the predicted lipophilicity of the present probe is strong, and a fluorescence response toward Cu^{2+} under physiological conditions with high sensitivity and selectivity facilitates the naked-eye detection of Cu^{2+} (Figure 7). The fluorescence quantum yield of **5** with 15 equivalents of Cu^{2+} was 0.26 at an excitation wavelength of 530 nm, which was higher than what was previously reported. The results showed a linear response range covering a concentration range of Cu^{2+} from 0.1×10^{-5} to 7.0×10^{-5} M and the correlation coefficient R is 0.9896. The detection limit was 2.3×10^{-7} M. The fluorescence intensity was remarkably increased upon the addition of Cu^{2+} within 1 or 2 min, whereas the other 16 metal ions (Na^{+}, Mg^{2+}, Al^{3+}, K^{+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Ba^{2+}, Pb^{2+}, Ag^{+}, and Cd^{2+}) caused no significant effect.

In 2013, Thennarasu et al. reported two new rhodamine-indole conjugates **6** and **7** (Chereddy et al. 2013b). They have provided evidence to show that both the sensitivity and the selectivity of probes **6** and **7** were tunable by choosing appropriate solvent systems. Compared to probe **6**, probe **7** had higher selectivity and sensitivity for Cu^{2+} and had excellent stability in physiological pH conditions. The limit of detection of Cu^{2+} (3×10^{-8} M) was lower than

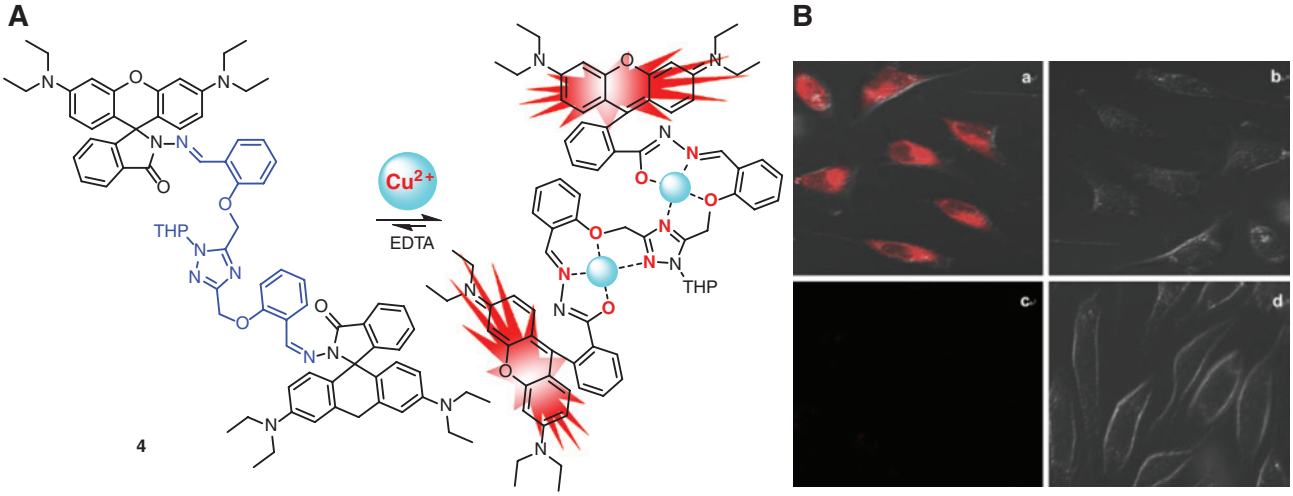


Figure 6: (A) Recognition mechanism of probe **4** with Cu^{2+} and (B) confocal fluorescence images in MCF-7 cells. Reprinted from Zhang et al. (2012).

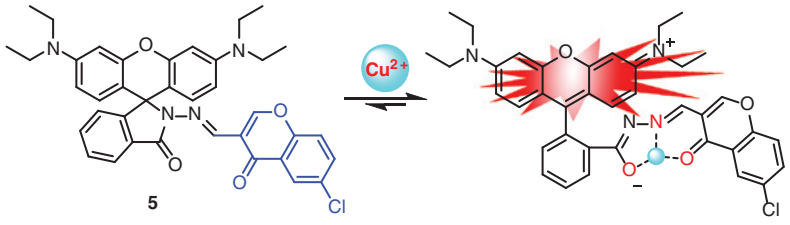


Figure 7: Recognition mechanism of probe **5** with Cu^{2+}.

the values reported for several other rhodamine-based Cu^{2+} probes. Moreover, probe **7** is not toxic up to ~10 mM and can be used for the detection of Cu^{2+} in live fibroblast cells. To explore the importance of the chelating ring size, nature, and orientation of coordinating atoms in the metal ion selective complex formation, they further synthesized three fluorescent probes **8–10** using a similar strategy (Chereddy et al. 2013a). The electrospray ionization-mass spectrometry (ESI-MS) data of the Cu^{2+} complexes of probes **8** and **9** suggested a 1:2 stoichiometry for both complexes. However, as the triazole ($pK_a = 0.3$) is slightly more basic than pyridine ($pK_a = 5.2$), probe **10** containing a triazole group instead of the pyridine group should favor the formation of the **10**-Cu^{2+} complex in a 1:1 stoichiometry. Meanwhile, the interference from Fe^{3+} ions observed in the cases of probes **8** and **9** was not observed in the case of probe **10**. Therefore, probe **10** is highly selective toward Cu^{2+}, forms a 1:1 Cu^{2+}-complex, and allows the spectrometric as well as naked-eye detection of Cu^{2+} in aqueous and biological samples. It is useful for the detection of intracellular Cu^{2+} levels in live keratinocyte cells because probe **10** is stable at physiological pH and is nontoxic to NIH 3T3 cells at low concentrations (Figure 8).

Based on the FRET mechanism, Das et al. have synthesized a new indole functionalized rhodamine probe **11**, which binds to Cu^{2+} with visually observable changes in their electronic and fluorescence spectral behavior (Kar et al. 2013). Probe **11** emitted only weak fluorescence at 490 nm when excited at 340 nm, whereas it resulted in a decrease in the fluorescence intensity at 490 nm and an increase in the fluorescence intensity at 587 nm upon the addition of Cu^{2+} to a solution containing **11** (Figure 9). These spectral changes are significant enough in the near-infrared (NIR) and visible regions of the spectrum and thus enable naked-eye detection. The spectral change is due to the formation of the **11**-Cu complex and the deprotonation of the indole unit occurs simultaneously with the addition of Cu^{2+}. The apparent binding constant for the formation of **11**-Cu complex was calculated by considering a 1:1 binding stoichiometry. Studies manifested that **11**-Cu complex is selective and fully reversible in the presence of sulfide anions. As determined by confocal fluorescence microscopy, probe **11** could also be used as an imaging probe for the detection of Cu^{2+} in HeLa cells.

In 2013, Long et al. developed a rhodamine-based fluorescent probe **12** for Cu^{2+} bearing the 8-hydroxyquinoline unit (Wang et al. 2013). Probe **12** formed a 1:1 complex with Cu^{2+} by cooperating the O and N of the 8-hydroxyquinoline unit and carbonyl O and N of rhodamine hydrazide unit. Therefore, it exhibited favorable features, including

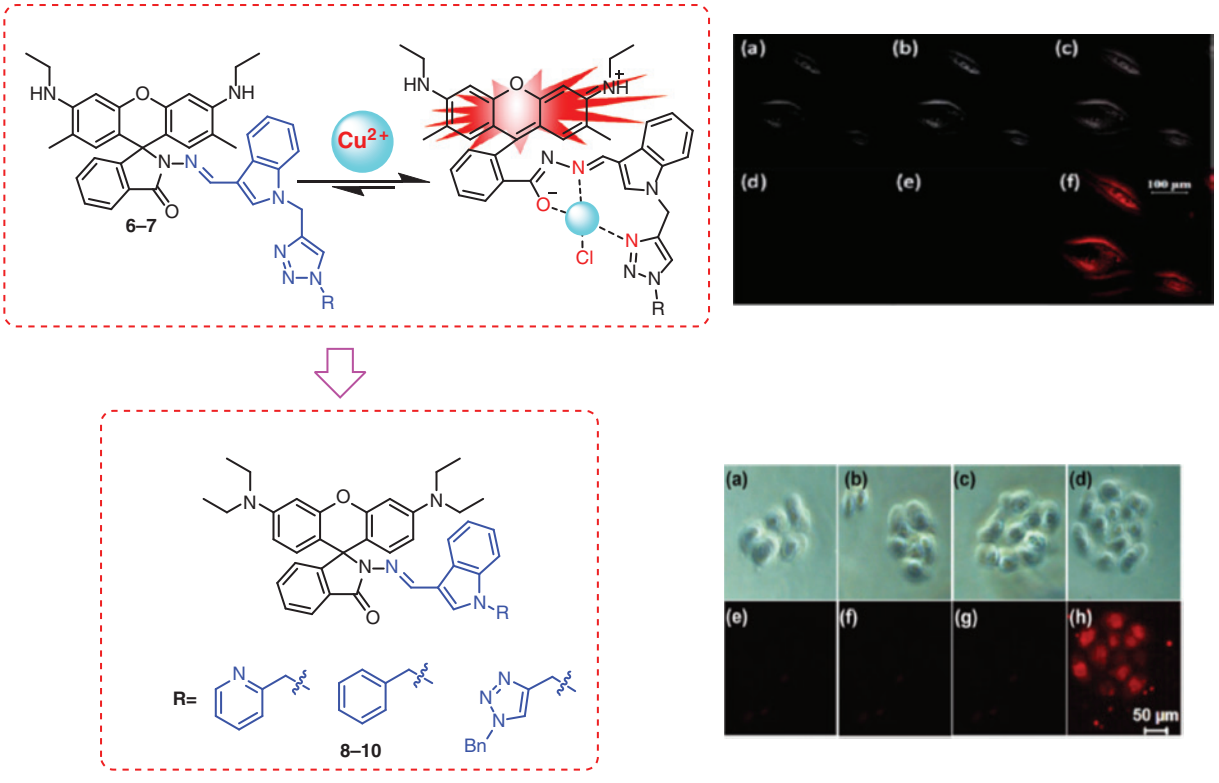


Figure 8: Fluorescent images of live keratinocyte cells incubated with probes **7** and **10**. Reprinted from Chereddy et al. (2013a,b).

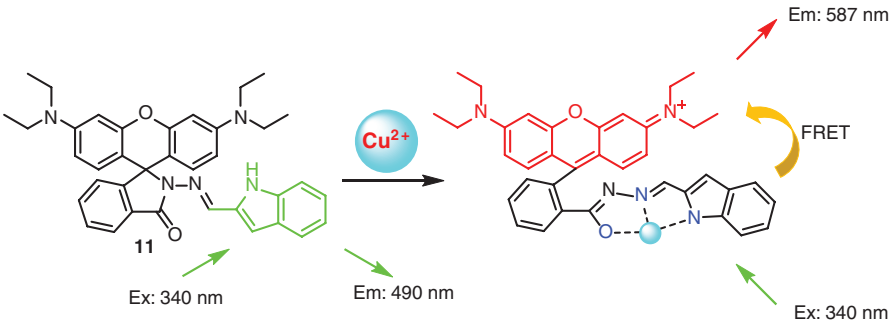


Figure 9: Cu^{2+}-induced FRET OFF→ON of probe **11**.

reversibility, high sensitivity with a large fluorescence enhancement (80-fold) and a low detection limit of 0.19 μM, high selectivity for Cu^{2+} over other heavy and transition metal (HTM) ions in Tris-HCl/ethanol (7:3, v/v, pH 7.4), working well at physiological pH, and good cell membrane permeability. Finally, probe **12** can be applied in the imaging of Cu^{2+} in living cells (Figure 10).

Tang et al. (2013) reported the synthesis and photophysical properties of a new rhodamine B-based probe **13**, which exhibited high selectivity, sensitivity, and rapid recognition behavior toward Cu^{2+} via colorimetric and fluorescent detection mode. The detection limit of probe **13** was evaluated to be 7.96×10^{-8} M. The Cu^{2+} recognition process is reversible and barely interfered by other coexisting metal ions, including Hg^{2+}, Ag^{+}, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Al^{3+}, Cr^{3+}, Mg^{2+}, K^{+}, and Na^{+}.

In 2014, Dong et al. synthesized a novel rhodamine fluorescent probe **14** by reacting rhodamine B hydrazide with 3-bromo-5-methylsalicylaldehyde, which has been developed as a new colorimetric probe for the selective and sensitive detection of Cu^{2+} (Zhang et al. 2014). Experimental results indicated that probe **14** can provide a rapid, selective, and sensitive response to Cu^{2+} with a linear dynamic range from 0.667 to 240 μmol/l. Common interferential ions did not show any interference on Cu^{2+} determination. It was anticipated that probe **14** can be a good candidate probe and has potential applications for Cu^{2+} determination. Besides, the proposed probe **14** exhibited the following advantages: a quick, simple, and facile synthesis (Figure 11).

In 2015, an "off-on" rhodamine-based fluorescent probe **15** for the selective detection of Cu^{2+} has been designed and synthesized by Mao et al. which showed a highly Cu^{2+}-selective fluorescence enhancement response in an aqueous pH 7 (Mao et al. 2015). Under optimum conditions, the increase of fluorescence intensity was linearly proportional to the concentration of Cu^{2+} over a wide range, and the limit of detection was 29 nM. The study indicated that a 1:1 stoichiometry complex was obtained by the copper ion chelated with vanillin-O, carbonyl-O, and rhodamine 6G hydrazide-N (Figure 12). Therefore, the present method could be applied for the analysis of Cu^{2+} in an aqueous system.

Yang et al. reported a damantane-modified salicylrhodamine B and β-cyclodextrin-modified Fe_3O_4@SiO_2 assembled by host-guest interactions, which induced novel inclusion complex magnetic nanoparticles (SFIC MNPs) colorimetric sensitive for Cu^{2+} (Zhang et al. 2015b). Probe **16** exhibited a clear color change from colorless to pink selectively and sensitively with the addition of Cu^{2+} in the experiments of UV-visible spectra, and the detection limit was measured up to 5.99×10^{-6} M in solutions of CH_3CN/H_2O (1:10). The SFIC MNPs were superparamagnetic

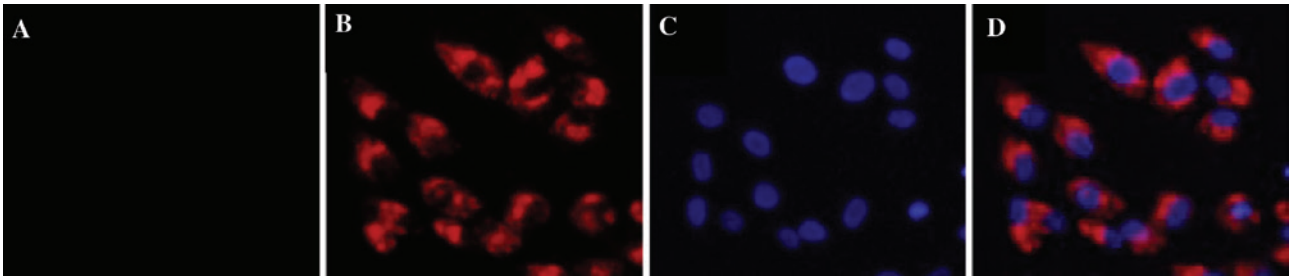


Figure 10: Fluorescence images of MG-63 cells. Reprinted from Wang et al. (2013).

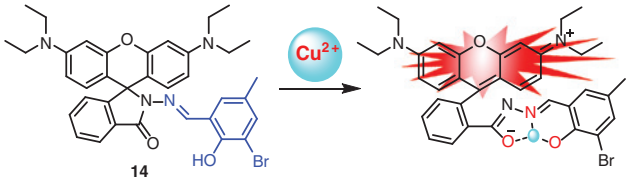


Figure 11: Recognition mechanism of probe **14** with Cu^{2+}.

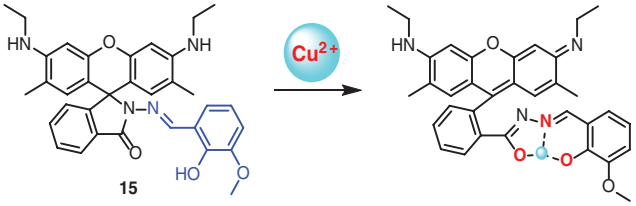


Figure 12: Recognition mechanism of probe **15** with Cu^{2+}.

according to magnetic measurements and could be separated and collected easily with a commercial magnet in 9 s. In addition, microspheres have also shown a good ability of separating for other ions from aqueous solutions due to a large number of hydroxyl groups on the surface (Figure 13).

Levels of lysosomal copper are closely related to the human body. However, there is one major limitation of detection methods for monitoring dynamic changes in copper pools that are unable to diagnostically assess the influence of copper accumulation on health status. Fortunately, Yang et al. reported a rhodamine fluorescent probe **17** activated by the presence of lysosomal Cu^{2+} in 2015 (Li et al. 2015b). Upon activation by lysosomal acidic pH, probe **17** bound with Cu^{2+} by spiropyran-based proton activation, promoting rhodamine ring-opening, which showed a "switched on" fluorescence signal (Figure 14). This strategy resolved some common challenges of chemical probes in biosensing, such as spatial resolution in cell imaging, solubility and stability in biological system, and interference from intracellular species. The new design, allowing one to track on a location-specific basis and visualizing lysosomal Cu^{2+}, could offer a potentially rich opportunity to examine copper physiology in both healthy and diseased states.

In 2016, a new rhodamine probe **18** with a high selectivity for Cu^{2+} was synthesized by Wang et al. (Zhang et al. 2016a). The analytical results obtained by UV-vis and fluorescence spectrophotometry show that the linear range of probe **18** for Cu^{2+} is 0.50–20.00 and the limit of detection is 0.11 μM. The 1:1 stoichiometric structure between probe **18** and Cu^{2+} could be formed because the copper ions are cooperated with the O atom of introduced moiety, O atom of carbonyl, and N atom of rhodamine B hydrazide. Meanwhile, it can be easily seen by the naked eye that the pink color of a **18**-Cu^{2+} solution immediately disappeared after the addition of excess sodium EDTA, with the fluorescence intensity returning to the original state. Probe **18** can be employed as a reversible fluorescent probe for Cu^{2+} in drinking water and living HeLa cells.

In 2013, our group reported three new rhodamine Schiff base probes **19–21** (Yang et al. 2013b). The probes displayed remarkably Cu^{2+}-selective orange fluorescence

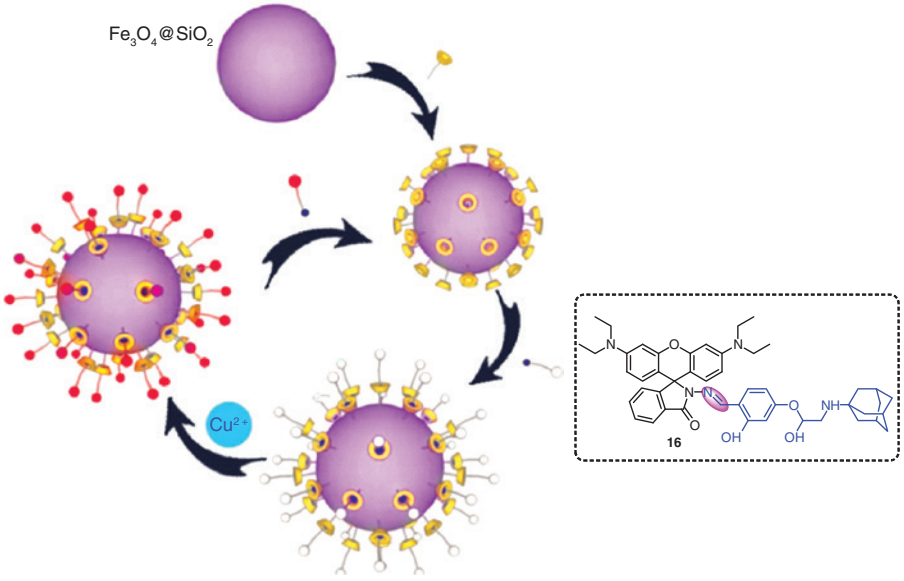


Figure 13: Chemical and schematic illustration of the preparation of SFIC MNPs probe **16** for Cu^{2+}.

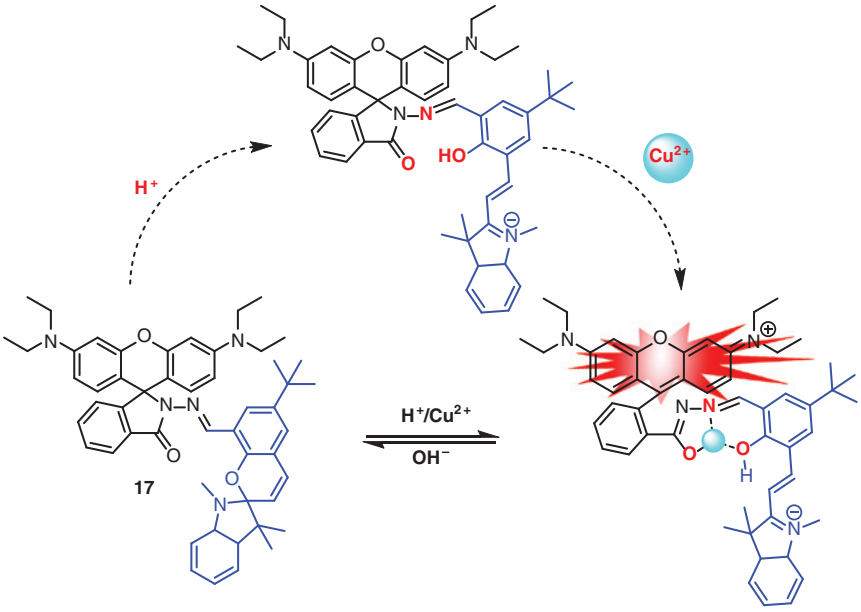


Figure 14: Recognition mechanism of probe **17** with Cu^{2+}.

and pink color switch over a wide range of tested metal ions in ethanol-PBS (5/5, v/v, pH 7.4) solution. Job's method was applied to study the binding stoichiometry of **19** and Cu^{2+}. The 2:1 stoichiometry is the binding mode of the probe and Cu^{2+} because the introduced cinnamyl aldehyde has no heteroatom. The coordination was also investigated to be a reversible process because the fluorescence disappeared when excess ethylenediamine was added to the colored solution of complex. Confocal laser scanning microscopy experiments revealed that the probes demonstrated the value for sensitive and selective detection of intracellular Cu^{2+} in living HeLa cells (Figure 15).

The above-mentioned strategy was subsequently extended that we have conducted comprehensive studies to explore the structure-property relationships of these probes **19–27**, which contain differently substituted cinnamyl aldehyde with a C=N Schiff base structural motif (She et al. 2016). Extensive work has been focused on the structure-property relationships of this probe model and has investigated the change of optical properties caused by different electronic effects and steric effect of the recognition group. More importantly, density functional theory (DFT) calculation simulates the ring-closed and ring-open forms of these modular Schiff base Cu^{2+} fluorescent probes. In addition, confocal laser scanning microscopy experiments suggested that all the probes would be a powerful tool for the sensitive and selective detection and mapping of Cu^{2+} adsorbed in environmental microbial systems. This approach provides a significant strategy for studying structure-property relationships and guiding the synthesis of probes with various optical properties (Figure 16).

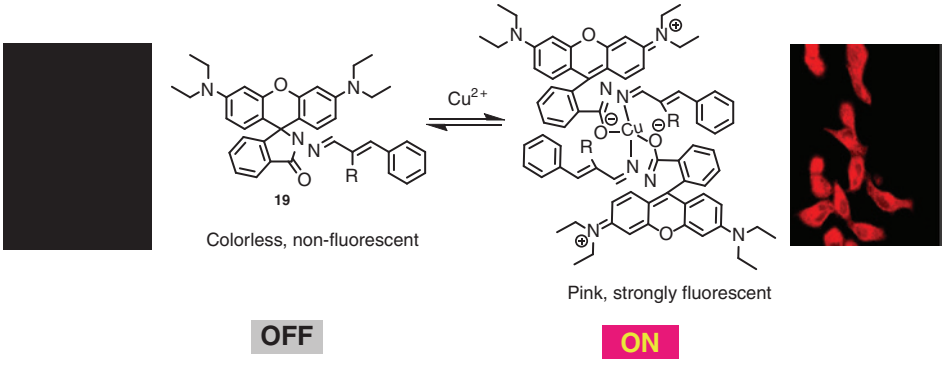


Figure 15: Proposed mechanism for the fluorescent changes upon the addition of Cu^{2+} and fluorescent images of HeLa cells incubated with probes. Reprinted from Yang et al. (2013b).

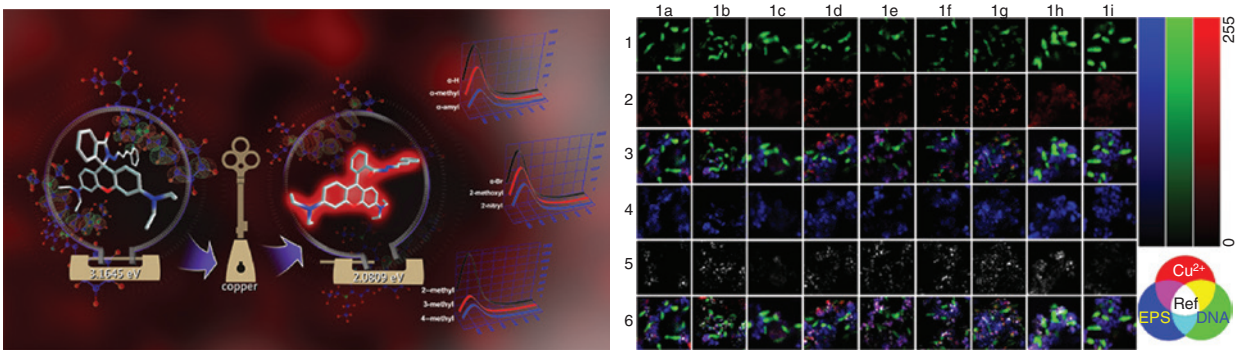


Figure 16: Schematic of the strategy for studying the structure-property relationships of nine modular Cu^{2+} fluorescent probes and single-cell scale maps showing the sorption of Cu^{2+} to cell-EPS-mineral aggregates. Reprinted from She et al. (2016).

Probes for detecting Hg^{2+}

To date, probes containing Schiff base structural motif have been developed successfully to detect Cu^{2+}. Therefore, there is a series of xanthene thiohydrazone probes that would be applied in detecting Hg^{2+} when the carbonyl oxygen atom is replaced by sulfur atom. Mercury ion is one of the most toxic metal ions. Mercury overload can cause serious damage to the brain, nervous system, endocrine system, and even the kidneys because of its toxic effects. Based on the soft-hard acid-base theory, Hg^{2+} is a representative example of soft acid and sulfur is a soft base. Therefore, a sulfur-based functional group must be a good candidate as the S is strong binding site for Hg^{2+}. Based on the above reasons, xanthene thiohydrazone probes, where the carbonyl oxygen atom is replaced by sulfur atom, were designed and synthesized (Figure 17).

In 2011, a novel fluorescent probe **28** has been reported by Jiang to detect Hg^{2+} in aqueous buffer solution, which demonstrated high selectivity for sensing Hg^{2+} with about 383-fold enhancement in fluorescence emission intensity and micromolar sensitivity ($K_d = 7.5 \times 10^{-6}$ mol/l; Wang et al. 2011a). In addition, in the presence of other metal ions, such as alkali and alkaline earth metal ions (K^+, Na^+, Mg^{2+}, and Ca^{2+}) and other transition metal ions (Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+}, Cr^{3+}, and Fe^{3+}), there was no evident fluorescence intensity enhancement because Hg^{2+} could be distinguished from other metal ions. Probe **28**

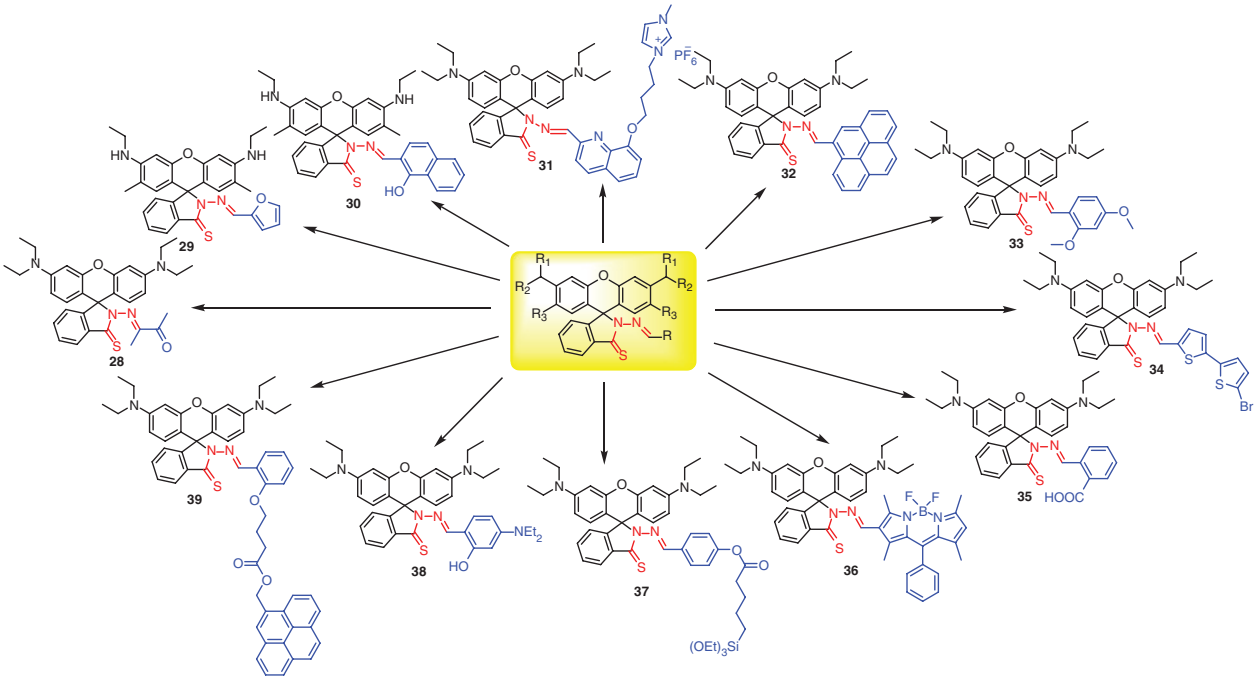
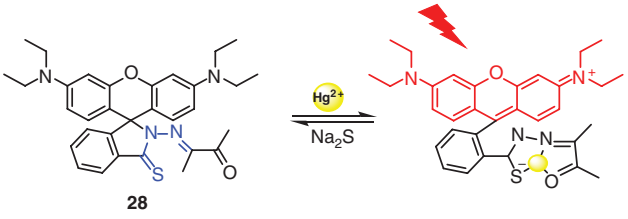


Figure 17: Probes based on rhodamine thiohydrazone for detecting Hg^{2+}.

is cell permeable and can visualize the changes of intracellular mercury ions in living cells using fluorescence microscopy (Figure 18).

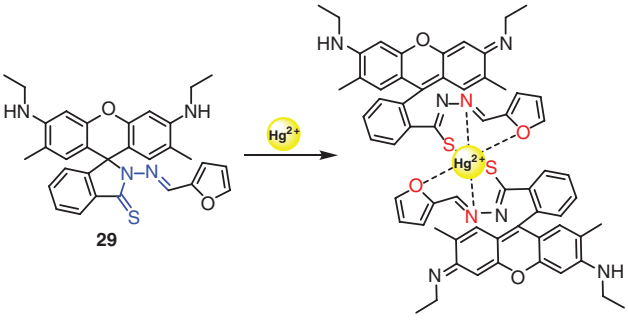
In the same year, Yang et al. reported a novel rhodamine-based highly sensitive and selective colorimetric "off-on" fluorescent probe **29** for Hg^{2+} ions designed and prepared using the well-known thiospirolactam rhodamine chromophore and furfural hydrazone as signal-reporting groups (Wang et al. 2011b). The photophysical characterization and Hg^{2+}-binding properties of probe **29** in neutral *N*,*N*-dimethylformamide (DMF) aqueous solution were also investigated. The signal change of probe **29** was based on a specific metal ion-induced reversible ring-opening mechanism of the rhodamine spirolactam. The response of probe **29** for Hg^{2+} ions was instantaneous and reversible. Moreover, this probe is applied for *in vivo* imaging in rat Schwann cells to confirm that it can be used as a fluorescent probe for monitoring Hg^{2+} in living cells with satisfying results, which further demonstrates its value of practical applications in environmental and biological systems (Figure 19).

Also in 2011, a novel fluorescent probe **30** based on rhodamine has been designed and synthesized by Li et al. for the detection of Hg^{2+} ions, which exhibited high sensitivity and selectivity over other metal ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, and Pb^{2+}) in aqueous solution and living cells (Wang et al. 2011c). Upon the addition of increasing concentrations of Hg^{2+}, a new emission band peaking at 555 nm appeared and developed, which could



28

Figure 18: Recognition mechanism of probe **28** with Hg^{2+}.



29

Figure 19: Recognition mechanism of probe **29** with Hg^{2+}.

be ascribed to the delocalized xanthene moiety of rhodamine group. The fluorescence response of probe **30** to other metal ions under the same condition was also investigated. There was no significant spectral change of probe **30** observed. It indicated that probe **30** could recognize Hg^{2+} from other metal ions even those that exist in high concentrations.

In 2012, Zhang et al. had a significant innovation in a new fluoroionphore-ionic liquid hybrid-based strategy to improve the performance of classic fluoroionphores via a synergistic extraction effect and realize simultaneous instrument-free detection and removal of heavy metal ions (HMIs; Jin et al. 2012). Hg^{2+} was chosen as a model HMI, and a rhodamine thiospirolactam was chosen as a model fluoroionphore to construct bifunctional fluoroionphore-ionic liquid hybrid probe **31**. The novel strategy provided a general platform for the highly sensitive detection and removal of various HMIs in aqueous samples and held promise for environmental and biomedical applications (Figure 20).

In 2013, Zhou et al. reported a novel rhodamine-pyrene-conjugated probe **32**, which exhibited lower detection limit, shorter response time, and advantageous reversibility (Chu et al. 2013). On the gradual addition of Hg^{2+} to a solution of probe **32**, a new fluorescence emission band centered at 594 nm appeared, which was due to the ring opening of the rhodamine thiospirolactam. Subsequently, the emission band changed with a blue shift in the maximum to 582 nm, which might be accounted for the intermolecular electron transfer between probe **32** and Hg^{2+}. Upon interaction with Hg^{2+}, the result showed a 1:1 stoichiometry for the Hg^{2+} complex accompanied with a weakened fluorescence resonance energy transfer (FRET) behavior. Moreover, probe **32** could be well applied in the water sample for the detection of Hg^{2+}.

A novel probe **33**, 3′,6′-bis(diethylamino)-2-((2,4-dimethoxybenzylidene)amino)spiro[isoindoline-1,9′-xanthene]-3-thione, was designed and synthesized in 2013 by Gao et al. (Liu et al. 2013). Probe **33** displayed highly selective and sensitive recognition of Hg^{2+}. Adding mercury ions into the aqueous solution, its fluorescence intensity was enhanced significantly, whereas its color was changed from colorless to pink. Therefore, a new fluorescence method of detection of Hg^{2+} was proposed. Satisfying results were obtained when the probe was applied in detecting Hg^{2+} in tap water, river water, and soil samples (Figure 21).

In 2014, Han et al. synthesized 2-carboxybenzaldehyde rhodamine B thiohydrazine, a fluorescent probe **34**, to recognize Hg^{2+} in DMF/H_2O (1:9, v/v) solution with high selectivity (Han et al. 2014). Most importantly, probe **34**

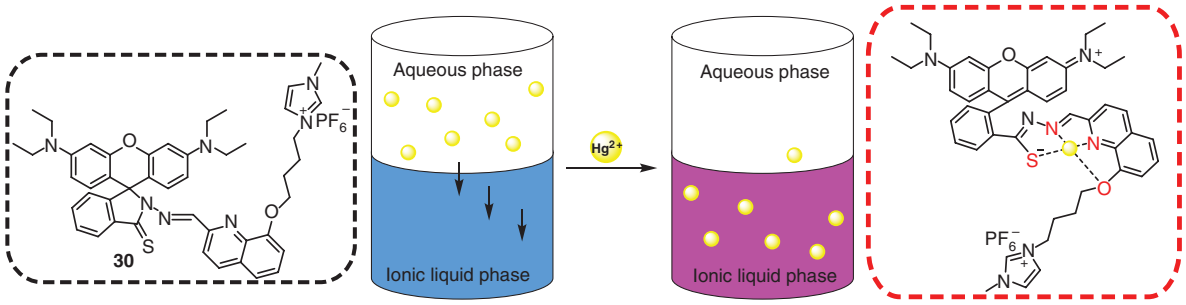


Figure 20: Structure, binding mechanism, and schematics of probe **31**-based bifunctional system for the removal and highly sensitive detection of HMIs via the synergistic extraction effect.

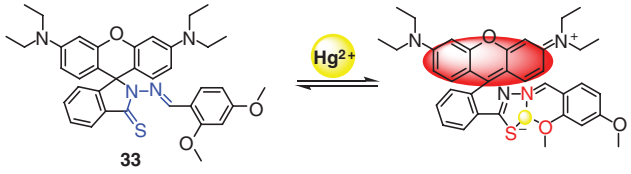


Figure 21: Recognition mechanism of probe **33** with Hg^{2+}.

could be employed to monitor Hg^{2+} in living cells using fluorescent imaging technique with satisfied results.

Emrullahoglu et al. described in 2014 the design and synthesis of a molecular probe **35** based on a rhodamine/BODIPY platform that displayed differential fluorescence responses toward Hg^{2+} and Au^{3+} and demonstrated its utility in intracellular ion imaging (Karakus et al. 2014). It exhibited a dual emission mode for the detection of Au^{3+} and a single emission mode for the detection of Hg^{2+} (Figure 22).

In the same year, a rhodamine-based optical probe **36** has been developed for the selective detection of Hg^{2+} in aqueous solution as well as in living cells by Guo et al. (Aydin et al. 2014). Live cell confocal imaging study demonstrates that probe **36** is also capable of imaging the presence of Hg^{2+} ions as well as its dynamic changes in live cells (Figure 23).

A core-shell structured inorganic-organic hybrid nanocomposite for Hg(II) sensing and removal was designed and fabricated in 2015 by Yang, where the core was composed of superparamagnetic Fe_3O_4 and the shell consisted of molecular silica sieve MCM-41 (Jiqu and Qixia 2015). A rhodamine-derived probe **37** was grafted onto the backbone of MCM-41 through a silane coupling reagent to control its loading content. This probe functionalized core-shell structure was confirmed and characterized by X-ray diffraction (XRD) analysis, electron microscopy images, IR spectra, thermogravimetry, and N_2 adsorption/desorption isotherms. It was found that the emission of

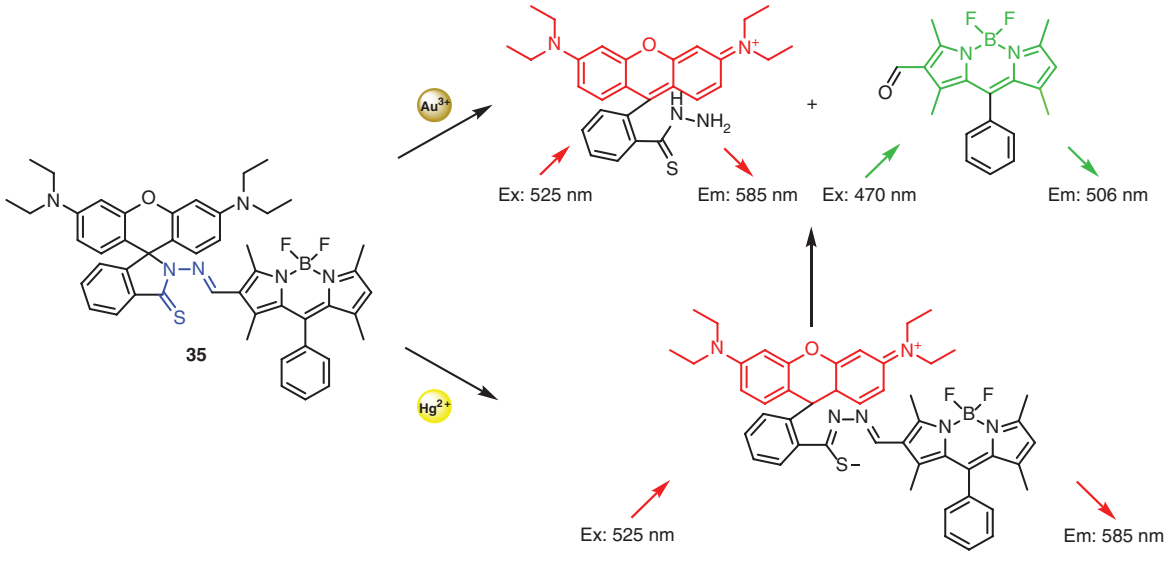


Figure 22: Recognition mechanism of probe **35** with Hg^{2+}.

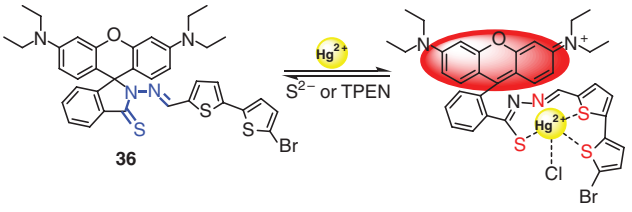


Figure 23: Recognition mechanism of probe **36** with Hg^{2+}.

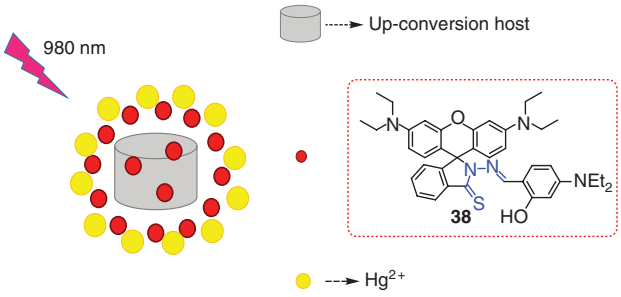


Figure 25: Recognition mechanism of probe **38** with Hg^{2+}.

this composite increased with increasing Hg^{2+} concentrations but was immune to other metal ions, showing good selectivity and high sensitivity toward Hg^{2+} ions. A linear Stern-Volmer curve was observed with short response time. In addition, this composite possessed good Hg^{2+} removing and recycling performance (Figure 24).

He et al. focused in 2015 on the Hg^{2+}-sensing behavior of rhodamine-derived probe **38** (Shen et al. 2015). The up-conversion NaYF4 nanocrystals were constructed and applied as the excitation host so that probe **38** could be lightened by the 980 nm excited up-conversion emission, aiming at better probe photostability. The efficient energy transfer between the up-conversion host and the probes was analyzed and confirmed by spectral analysis and emission decay lifetime comparison. It was found that the probe emission linearly increased with increasing Hg^{2+} where it was immune to other common metal ions, showing emission "turn-on" effect toward Hg^{2+} with good selectivity. The probe followed a simple complexation stoichiometry of 1:1 with Hg^{2+} ions (Figure 25).

A new rhodamine B-based fluorescent probe **39** containing pyrene moiety was designed and synthesized by Yao in 2016, which showed a colorimetric and fluorometric sensing ability for Hg^{2+} with high selectivity over other metal ions (Rui et al. 2016). The binding analysis using Job's plot suggested 1:1 stoichiometry for the complexes formed for Hg^{2+}. Probe **39** exhibited the linear fluorescence quenching to Hg^{2+} in the range of 0.3–4.8 μM (λ_{ex} = 365 nm) and 0.3–5.4 μM (λ_{ex} = 515 nm), and the detection limit was 0.72 μM. In addition, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay demonstrated that probe **39** had low cytotoxicity and was successfully used to monitor intracellular Hg^{2+} levels in living cells (Figure 26).

Xanthene fluorescent probes with amide structure

As the most abundant trace element, iron plays an obligatory role in many biochemical processes at the cellular level. The transfer, storage, and balance of Fe^{3+} are associated with living organisms. Either its excess or deficiency has a great influence on human and animal health. Iron at high level can lead to the formation of reactive oxygen species (ROS) by the Fenton reaction, which can impair lipids, nucleic acids, and proteins. Cellular toxicity caused by iron has been related to some serious diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. On the contrary, the scarcity of iron can cause anemia and breathing problems (Sahoo et al. 2012). Thus, many fluorescent probes for Fe^{3+} have been designed and synthesized in recent years (Figure 27).

In 2011, our group introduced benzothiazole moiety for the first time to synthesize a "turn-on" probe **40** based

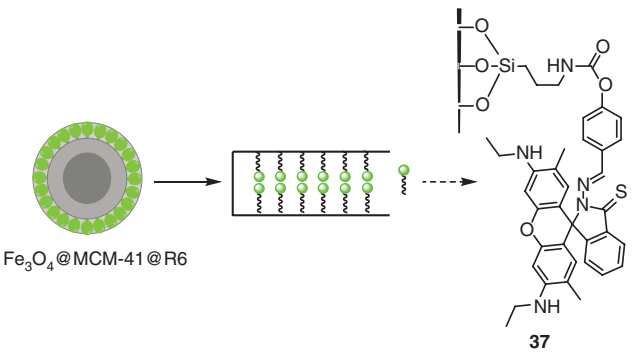


Figure 24: Design strategy for Fe_3O_4@MCM-41@R6.

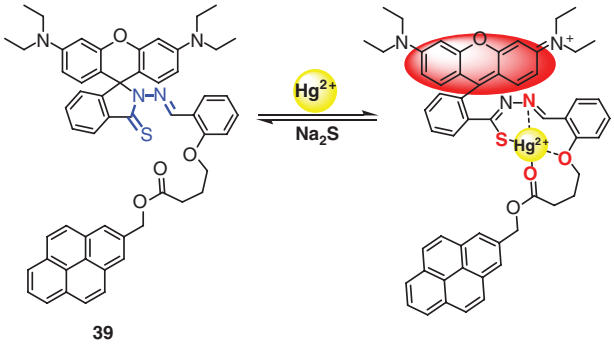


Figure 26: Recognition mechanism of probe **39** with Hg^{2+}.

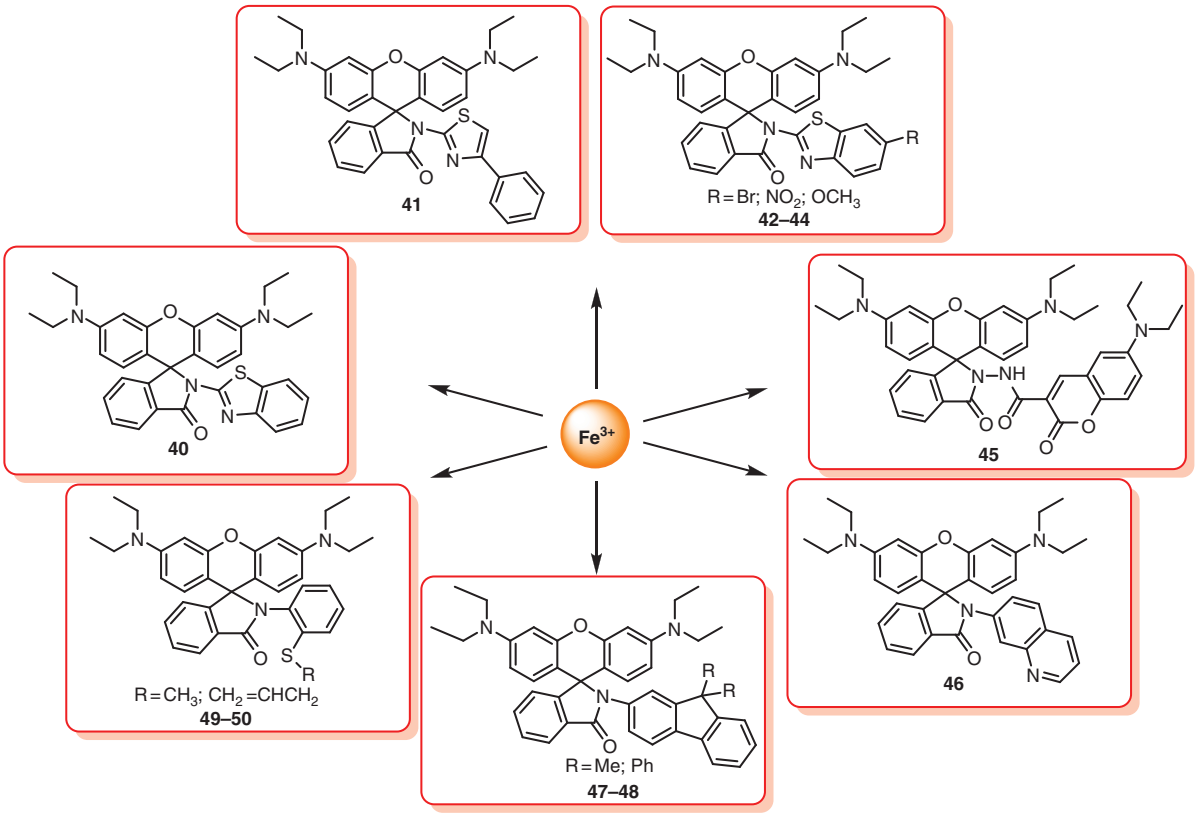


Figure 27: Probes **39–48** based on rhodamine with amine for detecting Fe^{3+}.

on rhodamine B exhibiting high sensitivity and selectivity toward Fe^{3+} in methanol solution (Yin et al. 2011). The addition of Fe^{3+} to the probe solution showed an approximately 193-fold enhancement in the fluorescence intensity at 580 nm when excited at 500 nm. The sensing mechanism was proposed to involve that the addition of Fe^{3+} induced the N atom of spirolactam to attack the C atom of carbonyl and the ring opening of the spirolactam of rhodamine because Fe^{3+} coordinates with N atom of benzothiazole of probe **40** by DFT calculations. Thereby, it is formed by the optimized geometry of the 1:1 1-Fe^{3+} complex. In an additional effort, in 2012, we respectively developed a probe **41** (She et al. 2012) and three probes **42–44** (Yang et al. 2012) based on rhodamine for the detection of Fe^{3+} and offered high selectivity and sensitivity toward Fe^{3+} over other metal ions such as Li^{+}, Na^{+}, K^{+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Hg^{2+}, Ag^{+}, Cu^{+}, Cu^{2+}, Fe^{2+}, and Cr^{3+}. These probes have a similar recognition mechanism toward Fe^{3+} that the coordination of Fe^{3+} is more inclined to occur at the N atom on the thiazole ring rather than at the O atom of the carbonyl moiety, which also can be supported by IR spectra that the amide carbonyl oxygen was actually not involved in the coordination. In addition, what deserves to be mentioned the most is that the single crystal structure of probe **44**-Fe^{3+} complex fully proved that recognition mechanism (Figure 28A). Upon coordination with Fe^{3+}, probes **41–44** displayed good brightness and fluorescent enhancement. A linear relationship was observed to exist between the relative fluorescence intensity of probes **41–44** and the concentration of Fe^{3+} in the range of 5–20 μM with a detection limit of 5 μM. Moreover, confocal laser scanning microscopy experiments have proven that probes **41–44** can be applied to monitor Fe^{3+} in living HeLa cells (Figure 28B).

In 2013, Zhao et al. have developed a new ratiometric fluorescence probe **45** based on rhodamine B and coumarin to monitor Fe^{3+} with high sensitivity and selectivity (Ge et al. 2013a). Upon the addition of Fe^{3+} to the aqueous solution of the probe, two fluorescence peaks at 580 and 460 nm were observed, which belong to rhodamine B and coumarin, respectively. This is a novelty design of the ratiometric probe of Fe^{3+} due to the CHEF process generated along with the PET process suppressed simultaneously. The fluorescence intensity at 580 nm was significantly increased by about 120-fold with 5 equivalents of Fe^{3+} added in aqueous solution (Figure 29).

In 2014, Qian et al. introduced rigid 8-aminoquinoline moiety and flexible 2-aminopyridine into rhodamine

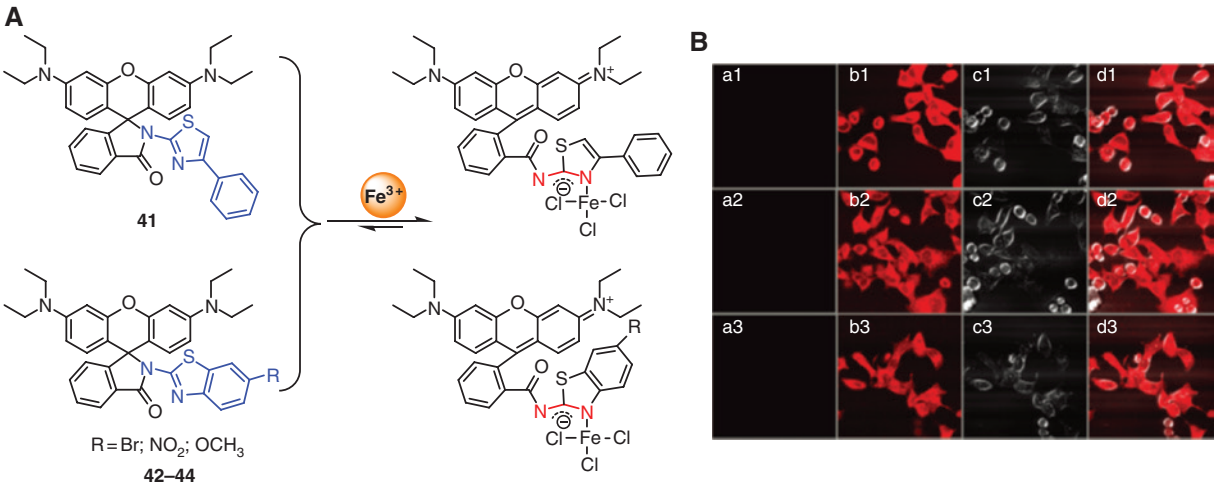


Figure 28: (A) Recognition mechanism of probes **41–44** with Fe^{3+} and (B) fluorescent images of HeLa cells incubated with 20 μM probes **42–44** for 30 min. Reprinted from Yang et al. (2012).

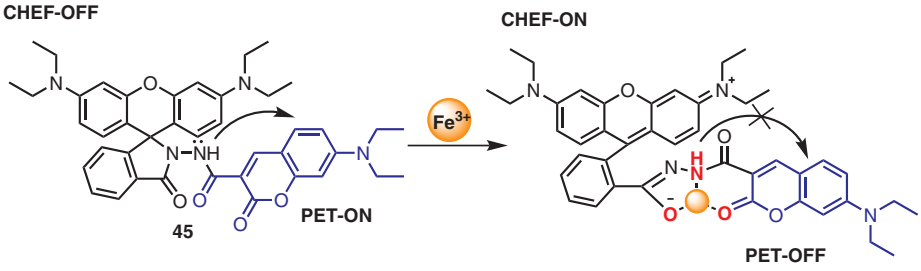


Figure 29: Proposed complex mechanism of **45**-Fe^{3+}.

chromophore to synthesize fluorescent probes to evaluate the effect of complexation with cations (Huang et al. 2014a). The experiments demonstrated that probe **46** with rigid 8-aminoquinoline moiety has higher selectivity and sensitivity for monitoring Fe^{3+} in aqueous solution. In the presence of Fe^{3+}, the O atom of carbanyl group is binding with Fe^{3+}, which promotes aggregation leading to a strong fluorescence at 590 nm. The recognition mode of probe **46** with Fe^{3+} was proven to be 2:1 on account that Fe^{3+} is bonded with quinolyl N and carbonyl O, which is concluded from Job's plot, 1D and 2D COSY H-H experiments (Figure 30). Finally, *in vivo* imaging demonstrated that probe **46** could be successfully applied as a bioimaging agent for monitoring Fe^{3+} in living cells.

Based on our previous work, in 2015, we presented two rhodamine-based probes **47** and **48** for the specific monitoring of Fe^{3+} in cellular systems with sufficient high selectivity and sensitivity (Ma et al. 2015). The fluorescence intensities were shown to be linearly related to the concentration of Fe^{3+} in the range of 0.9–20 μM for probe **47** and 5.0–20 μM for probe **48**. In addition, probe **47** was also found to be more sensitive with a detection limit of

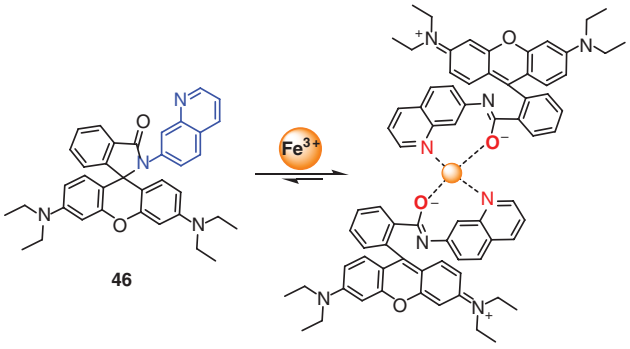


Figure 30: Recognition mechanism of probe **46** with Fe^{3+}.

0.9 μM than the 5.0 μM limitation of probe **48**, indicating the superior properties of probe **47**. A feasible mechanism was proposed that Fe^{3+} cooperated with the O atom of carbonyl and N atom of amide in the probe to form the 1:1 complexes. The titration of the complexes with ethylenediamine was also presented to determine the reversible nature of the binding process. This phenomenon likely resulted from the removal of Fe^{3+} from the binding complexes, resulting in the reconstitution of the spirolactam

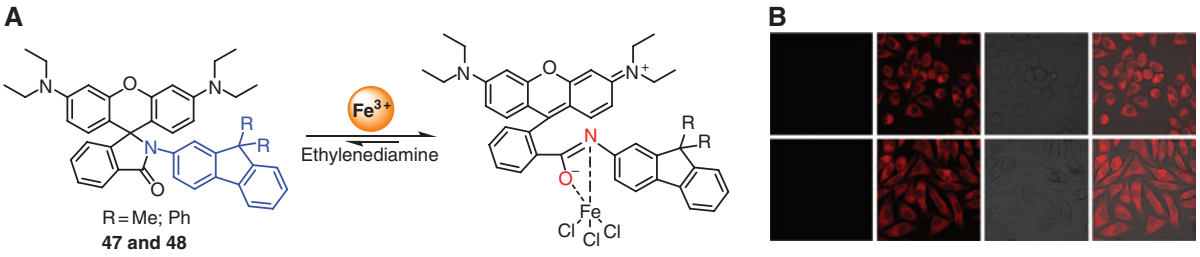


Figure 31: (A) Recognition mechanism of probes **47** and **48** with Fe^{3+} and (B) intracellular fluorescent imaging of probes **47** and **48** with Fe^{3+} in living L929 cells. Reprinted from Ma et al. (2015).

ring in the probes, thus indicating the reversible property of the probes. Finally, the obvious fluorescence image obtained from confocal laser scanning microscopy of the detection of Fe^{3+} in living L929 cells demonstrate that probes **47** and **48** could contribute to significant breakthroughs in understanding the critically important functions of Fe^{3+} in related cells and biological organs (Figure 31).

Yang et al. developed two probes **49** and **50** exhibiting prominent sensitive and selective response to Fe^{3+} more than other commonly coexistent metal ions in methanol/water (1:1, v/v) (Yang et al. 2015). The other metal ions include Na^{+}, K^{+}, Ca^{2+}, Cd^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, Hg^{2+}, and Cr^{3+}. An obvious fluorescent enhancement at about 580 nm was observed in the presence of Fe^{3+} and accompanied by significant color changes, which can be used for "naked-eye" detection. They studied the mechanism through IR and ^{1}H nuclear magnetic resonance (NMR) spectra, which revealed that the carbonyl oxygen was actually involved in coordination with Fe^{3+} and accompanied by the opening of the spirolactam ring. Moreover, confocal laser scanning microscopy experiments have proven that the probes were successfully used for fluorescence imaging in HepG2 cells (Figure 32).

Besides, there is a special structure that rhodamine binding with hydroxylamine forms hydroxamate, which has a strong chelation with Fe^{3+}. Fe^{3+} could remain in many proteins and enzymes because ferrichrome siderophores contain three hydroxamate units as binding sites.

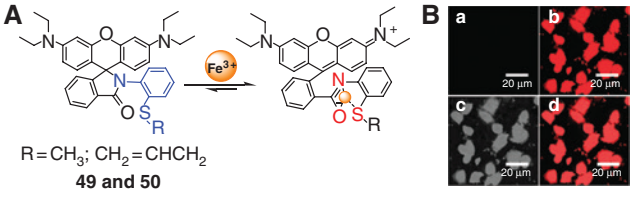


Figure 32: (A) Recognition mechanism of probes **49** and **50** with Fe^{3+} and (B) confocal fluorescence and bright-field images of HepG2 cells (scale bar, 20 mM). Reprinted from Yang et al. (2015).

The ferrichrome-Fe^{3+} complex is shown in Figure 33. We can easily find that Fe^{3+} could be cooperated with the O atoms of hydroxamate. Recently, some "turn-on" fluorescent probes have been developed based on the biomimetic hydroxamate binding unit coupled with rhodamine.

Hu et al. developed an acetyl rhodamine-hydroxamate fluorescent probe **51** to respond to Fe^{3+} ions (Hu et al. 2011). Upon mixing with Fe^{3+} in CH_3CN/H_2O (1:1, v/v), the spirolactam of **51** was opened, which resulted in the dramatic enhancement with 55 equivalents of added Fe^{3+}, both fluorescence and absorbance intensity as well as the color change of the solution. The detection limit of probe **51** for Fe^{3+} was estimated to be about 7.0×10^{-8} M ($S/N \geq 3$), which was sufficiently low for the detection of Fe^{3+} ions found in many chemical and biological systems. Confocal laser scanning microscopy experiments showed that probe **51** could be used to detect Fe^{3+} in living cells (Figure 34).

Xanthene fluorescent probes with 2,2-dipicolylamine (DPA) structure

Zn^{2+} is a biologically essential element that should be maintained within a suitable concentration range in living systems. The disorder of zinc metabolism has been

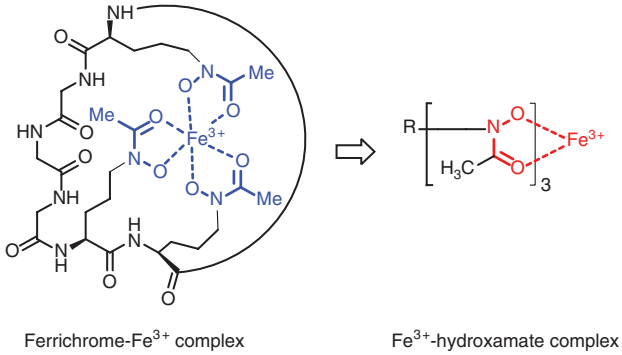


Figure 33: Ferrichrome-Fe^{3+} complex and Fe^{3+}-hyroxamate complex.

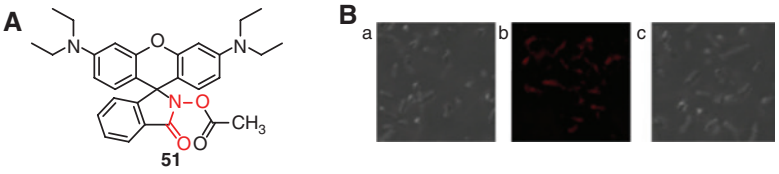


Figure 34: (A) Structure of probe **51** and (B) laser confocal scanning microscopy experiments. Reprinted from Hu et al. (2011).

correlated to epilepsy, diabetes, infantile diarrhea, and Alzheimer's disease because of its significance in many biological processes, for example, neural-signal transmissions and pathology, regulation of metalloenzymes, gene transcription, immune function, and mammalian reproduction. Therefore, there is an increasing demand for highly sensitive and selective analytical methods to detect Zn^{2+} ion.

Fortunately, a DPA unit can be developed as fluorescent probes for Zn^{2+} with advantages of tunable emission wavelengths, high selectivity, excellent sensitivity, and good cell permeability. A DPA unit is well known to be a classical receptor for zinc ions, which has been widely used for the design and development of various fluorescent probes for monitoring Zn^{2+} ion. Importantly, Lippard's group made a lot of effort in this filed (Chang et al. 2004, Nolan et al. 2004, Woodroofe et al. 2004, Goldsmith and Lippard 2006, Tomat et al. 2008). However, there is a major limit on the application of these small-molecule ratiometric probes in quantifying mobile zinc due to the complex syntheses, small dynamic ranges, and narrow gaps between the wavelengths. To overcome this challenge, subsequent studies on the design of fluorescent probes for zinc ions were performed by Lippard's group (Figure 35).

In 2014, Lippard et al. reported the synthesis and photophysical properties of probes **52** and **53**, which are the first ditopic resorufin-based probes for mobile zinc (Loas et al. 2014). Upon binding with Zn^{2+}, probes **52** and **53** exhibited 14- and 41-fold enhancements of their red fluorescence emission, respectively. The synthetic strategy employed in the design of the ditopic probes offers distinct advantages toward facile structural modifications of both the fluorophore and the Zn^{2+}-binding motifs. An envisioned homologous series will help improve the stability in solution, tune the photophysical and zinc-binding properties, and, of course, advance the understanding of the factors determining spontaneous localization in live cells (Figure 35).

Zhang et al. devised a strategy for repurposing existing intensity-based probes for quantitative applications (Zhang et al. 2015a). Using solid-phase peptide synthesis, they conjugated a zinc-sensitive derivative and a zinc-insensitive 7-hydroxycoumarin derivative onto opposite

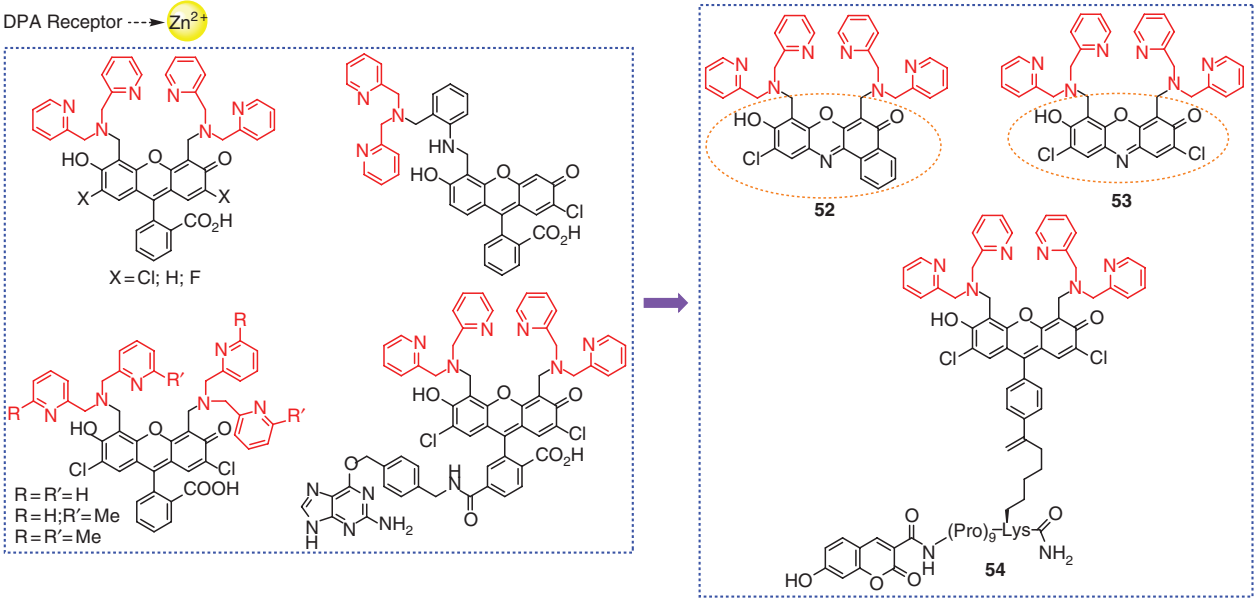


Figure 35: Xanthene fluorescent probes with DPA structure for detecting Zn^{2+}.

ends of a rigid P9K peptide scaffold to design a ratiometric fluorescent probe **54** for mobile zinc (Figure 35). Probe **54** could be applied to quantify the mobile zinc in epithelial prostatic cells and showed that both normal and tumorigenic cells maintain buffered mobile zinc levels even when challenged with exogenous zinc. Besides, it was used to measure mobile zinc levels in human seminal plasma and reveal a positive correlation between the total and mobile zinc levels.

Xanthene fluorescent probes with 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid moiety (BAPTA) structure

Since Tsien reported the first intracellular calcium sensor (Tsien 1980), BAPTA binding unit has been employed along with a series of fluorophores to construct fluorescent calcium probes. Based on the high selectivity of the BAPTA ligand, an optically controlled Ca^{2+} probe was developed to mimic natural calcium oscillations (Wu et al. 2011). Probe **55**, a spiroamido-rhodamine derivative of BAPTA, underwent cycles of reversible transitions between a colorless closed state and a fluorescent open ring (Figure 36). The closed-state exhibited a high affinity for Ca^{2+} (K_d = 509 nM) with excellent selectivity over Mg^{2+} (K_d = 19 mM). The open isomer had a 350-fold lower Ca^{2+} affinity (K_d = 181 μM), whereas the Mg^{2+} affinity was not significantly affected (K_d = 14 mM).

Although BAPTA is a highly selective ligand for calcium, the sensitivity of probes possessing the moiety still needs to be improved. By considering the NIR response of Si-rhodamine, it was used by Nagano et al. to construct a BAPTA-based Si-rhodamine calcium probe **56** and its cell-permeable derivative **57** (Figure 37; Egawa et al. 2011). Probe **56** displayed red fluorescence at 660–670 nm when it was complexed with calcium ions in MOPS buffer solution containing KCl and ethylene glycol tetracetic acid (pH 7.2). In addition, probe **56** exhibited high selectivity to calcium without interference from other metal ions. Furthermore, its high tissue penetration ability, low background autofluorescence, and phototoxicity suggested that probe **56** is applicable in the biological systems and demonstrated that it could be used in monitoring the action potentials of calcium increase in neuronal cell body. Moreover, probe **57** has high cell membrane permeability to make it useful for multicolor fluorescence imaging of action potentials in brain slices loaded with sulforhodamine 101 and potentially applicable to neuroscience studies.

The concentration change of calcium that is closely correlated to many physiological phenomena and the ability of simultaneously monitoring cytoplasmic calcium and other metal ions or proteins are of importance in studies of biological signaling pathways. Hanaoka et al. designed a series of Si-rhodamine-based calcium probes **58–60** based on a PET mechanism (Figure 37; Egawa et al. 2013). These probes, in which a benzo-amide unit is linked

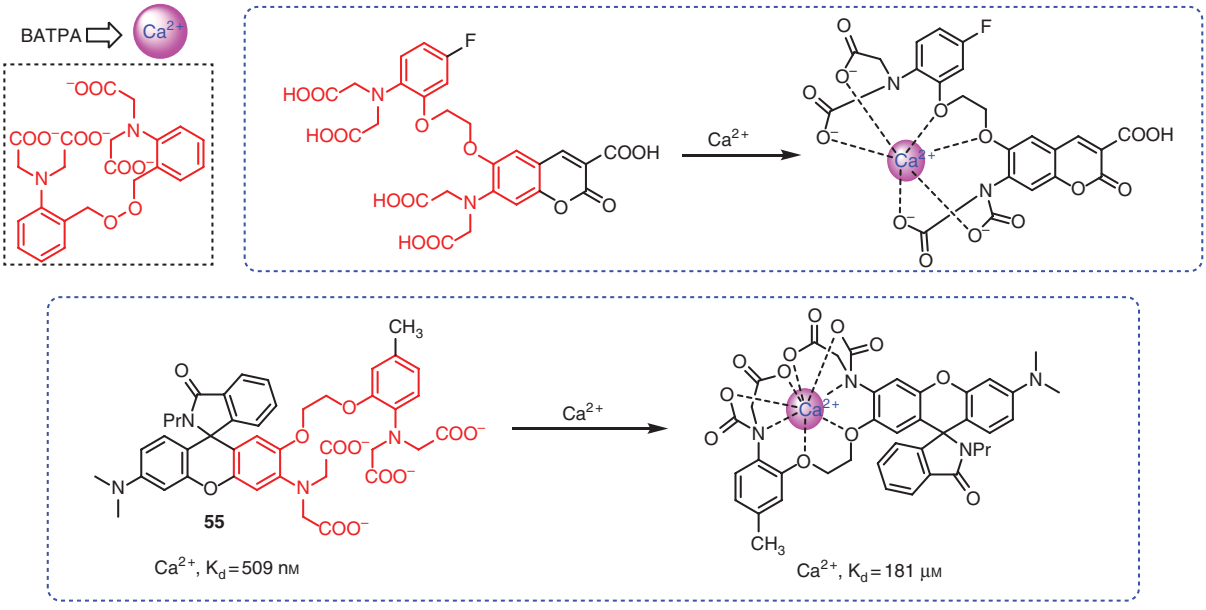
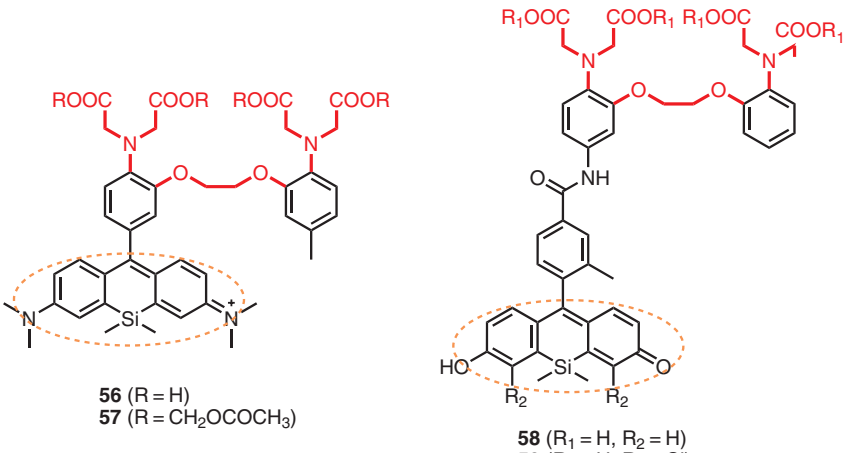


Figure 36: Development of BAPTA structure for Ca^{2+}.



56 (R = H)
57 (R = CH_2OCOCH_3)

58 (R_1 = H, R_2 = H)
59 (R_1 = H, R_2 = Cl)
60 (R_1 = CH_2OCOCH_3, R_2 = Cl)

Figure 37: The structure of probe **56–60**.

to BAPTA, were employed to study protein-expressing animals and cultured cells. Moreover, fluorescence images of histamine-induced calcium oscillations in HeLa cells using these probes were employed to visualize changes in cytoplasmic calcium concentration.

Conclusion and perspectives

In this review, representative examples of metal ion fluorescent probes based on xanthene reported from 2011 to 2016 were discussed. In particular, the design strategies, recognition mechanism, and application of such fluorescent probes were illustrated. Xanthene, as a molecular scaffold that was employed to design novel probes for the selective recognition of metal ions, have become a very attractive sensing system for intracellular detection. Current efforts on xanthene fluorescent probes have been focused on improving the sensitivity and selectivity, expanding the family of detectable metal ions, developing new sensing mechanisms, and further developing in biochemical and biomedical sciences, clinical medicine, and so on.

Although a large variety of fluorescent probes that have high sensitivity and specificity for the detection of metal ions have been developed, what remains lacking is the practical application of the probes. Because metal ions are very important in living organisms, new probes that are compatible with *in vivo* imaging are urgently required. Therefore, future efforts in this area need to be addressed in the design of such type of probes that can be successfully employed in detecting and monitoring targeted metal ions in *in vivo* imaging and in the environment. In addition, more efforts should be directed to the areas of calculation. It is expected that the future development of metal ion probes will become more theory application driven. A rapid development in the research of fluorescent metal ion probes is thus anticipated in the coming years. We hope that this review entices the scientific community into designing ever more highly sensitive and selective fluorescent probes for metal ions and might educate students new to the field as well as provide guidance in the selection of appropriate molecular modes for future applications in the environment and bioimaging.

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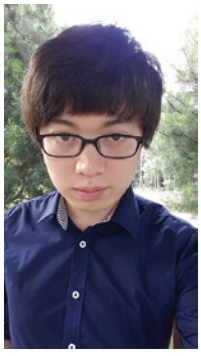
Bionotes

Siyue Ma
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China

Siyue Ma received her BS from the School of Chemistry, Chemical Engineering and Materials, Heilongjiang University, P.R. China, in 2014. She is currently a PhD student at the College of Chemistry and Materials Science, Northwest University, P.R. China. Her research interests focus on developing fluorescent chemosensors.

Yaqi Wang
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and Shaanxi Medical Devices Testing Centre, Xi'an, Shaanxi 710075, P.R. China

Yaqi Wang is a postdoctoral student at Northwest University as well as a section chief at Shaanxi Medical Devices Testing Centre, P.R. China. He received his PhD in biochemistry and molecular biology from Fudan University in 2012. His current research interests include synthesis and research methods for organic substances, fluorescent molecular devices, activity-based probes, and detection of techniques for medical devices.



Mengyao She
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China

Mengyao She received his BS from the College of Chemistry and Materials Science, Northwest University in 2012. He is currently a PhD student at the same college. His research interests focus on developing fluorescent chemosensors.

Shen Wang
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China

Shen Wang received his BS from the College of Chemistry and Chemical Engineering, Hubei University, P.R. China, in 2015. He is currently a master's candidate at the College of Chemistry and Materials Science, Northwest University. His research interests focus on developing fluorescent chemosensors.

Zheng Yang
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and School of Chemistry and Chemical Engineering, Xi'an University of Science and Technology, Xi'an, Shaanxi 710054, P.R. China

Zheng Yang is now working in Xi'an University of Science and Technology, P.R. China. He received his PhD in organic chemistry from Northwest University in 2015. His research interests focus on the synthesis and research methods for organic substance, functional biological fluorescent sensors, and organic chemical energy.

Ping Liu
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China, **liuping@nwu.edu.cn**

Ping Liu is a professor at Northwest University. She received her PhD in inorganic chemistry from Northwest University in 2005. Her current research interests include the synthesis and study of new ligands and their functional complexes.

Shengyong Zhang
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China; and School of Pharmacy, Fourth Military Medical University, Xi'an, Shaanxi 710032, P.R. China

Shengyong Zhang is an academician of the National Academy of Engineering. He received his PhD in chemistry from Universite Paris-Sud in 1982. He is now a professor at both Fourth Military Medical University and Northwest University. His research interests include asymmetric catalysis, synthesis of chiral drugs, resolution of racemic compounds, analysis, and separation and determination of optical purity of chiral compounds.

Jianli Li
Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of the Ministry of Education and College of Chemistry and Materials Science, Northwest University, Xi'an, Shaanxi 710127, P.R. China, **lijianli@nwu.edu.cn**

Jianli Li is a professor at Northwest University. He received his PhD in organic chemistry from Northwest University in 2007. His current research interests include synthesis and research methods for organic substance, fluorescent molecular devices, chemical and biological sensors, and molecular recognition.