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Fluorescein applications as fluorescent probes for the detection of analytes

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ABSTRACT

Fluorescein derivatives are important fluorescent probes which can be used for detection and optical imaging. Fluorescein derivatives are usually constructed by introducing aldehyde groups or esterified onto fluorescein xanthene ring and benzene moiety. Typically, the research direction of connecting amino groups with fluorescein monoaldehyde is in hot. Because of their high activity, these derivatives can be complexed with the analytes to produce changes of colors and the increase or decrease of fluorescence intensity. This article reviewed fluorescein probes in the past two years according to classification of different analytes including metal ions, anions, small molecules and biological macromolecules. The synthesis methods, optical properties, possible mechanisms and applications of fluorescein probes are summarized. This article provides a reference for the screening of fluorescein probes with high sensitivity and biological detection and can propel their further applications in sensing and detection of analytes.

1. Introduction

Fluorescein is widely employed as a platform for various fluorescent probes and fluorescent labels because of their high-intensity emission peaks, high molar absorption coefficients and quantum yields in aqueous media [1]. Since Bayer first synthesized fluorescein in 1871, it has received much attention and has shown great promise in a variety of applications, especially in the field of smart sensors and bioimaging [2].

Over the last decade, amazing progress has been made in the use of fluorescent probes for the development of probes due to their unique photoluminescence properties [3]. Compared with traditional techniques including titrimetry, chromatography, electrochemistry, chemiluminescence and flow injection analysis, these probes open up a new avenue for fast-responding, highly sensitive, non-sample destructing and on-site analysis of specific targets [4]. A great variety of fluorophores including organic molecules, metal nanoclusters, semiconductor quantum dots (QDS), etc. with improved properties are increasingly available. A benefit of synthetic organic molecules is the ability to employ chemical approaches to control the properties and direct the position of the fluorophore. Applying organic synthesis enables efficient tailoring of the structure to obtain fluorescent probes for specific sensitive experiments. Accordingly, the development of preparative strategy toward functional fluorescein structure is highly desired.

Fluorescein has been frequently utilized as the fluorescent core due to its characteristic spirolactam structure, which can 'close–open' with an 'turn-on' fluorescence response in specific environments or as a result of specific events, and made it an excellent dye for the design of probes. Recently, functional fluorescence probes based on the fluorescein structure have attracted growing interest [5]. Functional fluorescence probes based on the fluorescein structure are available for modification by organic synthesis at two moieties: the xanthene ring and the benzoic acid moiety (hereafter called the benzene moiety). Its unique characteristic in every moiety is worth summarizing.

Herein, the past two-years' advances of fluorescein probes about the synthesis methods, optical properties, possible mechanisms and applications of fluorescein probes are summarized [6,7]. Common fluorescein probes mechanisms include the 'off–on' structure, fluorescence resonance energy transfer (FRET), photo induced electron transfer (PET) and chelation enhanced fluorescence (CHEF) [8,9]. This article reviewed fluorescein probes according to classifications of different analytes, including metal ions,

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anions, small molecules and biological macromolecules. It could facilitate the researchers to design and develop proper fluorescent probes in future work.

2. Fluorescein probes design mechanisms

Functional fluorescence probes based on the fluorescein structure are available for modification by organic synthesis at two parts: the xanthene ring and the benzene moiety. The two moieties are orthogonal to each other. Its unique characteristic in every moiety is worth summarizing. Rational design strategies based on the five positions of the two parts have allowed us to rapidly develop a wide range of fluorescein probes (Fig. 1).

The rational design of a chromogenic/ratiometric fluorescein probe is based on the abovementioned reaction with benzene moiety. In carboxyl of benzene moiety (1 site), fluorescein probes are synthesized through fluorescein hydrazine based on Schiff base reaction connecting with aldehyde containing compounds. Fluorescein moiety is used as the signaling unit and aldehyde containing compounds are used as the binding unit which is based on non-covalent supramolecular interaction. Due to the high activity of the Schiff base, fluorescein probes can provide more binding sites, which can improve the sensitivity for detection.

In the xanthene ring (2 site), fluorescein aldehyde was obtained through a Reimer–Tiemann reaction, which can be further used to prepare probes. The amino compounds can connect with fluorescein aldehyde based on Schiff base reaction. The probes as a result of the amino group contain a lone pair of electrons which can occur PET process within the molecule induces the quenching of fluorescence. The ions with an empty orbit can hinder the PET process, which leads to fluorescence recovery.

The above two species fluorescein probes have Schiff base structure, it can be divided into two types: bonding-signaling type probes and reactive fluorescein probes. Bonding-signaling type fluorescein probes produce an increase or decrease of fluorescence intensity due to electron transfer from amino group containing compounds to the xanthene ring. The ions with an empty orbit can hinder the electron transfer process, leading to fluorescence recovery. Due to the activity of the Schiff base, reactive fluorescein probes can facilitate a ring-opening of the fluorescein spirolactam and increase the rigidity of the molecular assembly. The purpose of detection can be achieved via the inhibition of C=N isomerization and the promotion of CHEF.

In hydroxyl of xanthene ring (3 site), fluorescein probes are synthesized through two phenolic hydroxyl groups of fluorescein based on esterification connecting with small organic molecules. The initial state of the third specie fluorescein probes is colorless and non-fluorescent due to the break of the π-conjugation. When a specific ion is added via an irreversible chemical reaction, ester bond breaking and small organic molecules are removed. The π-conjugation recovering leads to notable color and fluorescence changes. The transformation of the two states can achieve the goal of detection of different analytes.

In the benzene moiety (4 site), fluorescein probes are synthesized through fluorescein connecting with organic fluorophores or gold nanoparticles (AuNPs), there is an overlap between two fluorescence emissions. Fluorescein connecting with other fluorophores can be used as ratiometric fluorescein probes to detect analytes based on FRET process between fluorescein and other fluorophores. Ratiometric fluorescein probes allow a ratio of the fluorescence intensities at two wavelengths. After the recognition, the absorption in UV–vis spectra should be different, thus resulting in the color change. Fluorescence emission spectra are turned out to be an accurate and efficient method for detection of analytes. Fluorescein connecting with AuNPs based on FRET process induces the quenching of fluorescence. The fluorescence emission intensity of fluorescein is significantly quenched due to overlapping of its emission spectra and the absorption spectra of AuNPs at plasmon resonance wavelength. Some analytes can hinder the FRET process due to the reporter group's stronger binding capacity for the analyte than recognition group, which lead to fluorescence recovery.

In ortho position of hydroxyl group (5 site), fluorescein probes are synthesized through halogen substituted fluorescein based on substitution reaction connecting with small organic molecules. This can increase its complexation ability and selectivity to metal cations. Most of the probes constructed in this way are bonding-signaling type probes.

3. Detection application

3.1. Fluorescein probes detection of metal ions

Probes for metal ions based on the fluorescein structure are modified by the five positions. When the probes combined with metal ions, it can lead to the changes of colors and the increase or decrease of fluorescence intensity. Based on this, the purpose of detection can be achieved [10]. In aqueous solution and living cell, the fluorescein probes can be used to detect such as copper ions, zinc ions, mercury ions, gold ions, silver ions, palladium ions, iron ions, magnesium ions, cadmium ions and lead ions [11,12]. Detection of these ions has great significance for human health and environmental protection [13].

3.1.1. Fluorescein probes for copper ions

The safe concentration of Cu^{2+} in drinking water is limited to 31.48 μM [14], excessive Cu^{2+} have biologically toxic [15]. Therefore, there is a great demand for the development of fluorescein probes that can provide the selective analysis of Cu^{2+} with high sensitivity, low detection limit and quick response.

Imidazole is a π-electron-rich heteroaromatic molecule, its derivatives show excellent coordination properties toward metal ions. Helal et al. synthesized a bonding-signaling type fluorescein probe (F2) based on Schiff base reaction with fluorescein hydrazine and N-methylimidazole in 1 site. Owing to the electron donating nature of the methyl group on the imidazole ring, the electron-donating capability of imidazole increases, which makes F2 to produce a green fluorescence emission at 525 nm and specific selection for Cu^{2+}. Cu^{2+} being a paramagnetic cationic species, with open shell d-orbitals, quenches the fluorescence of F2 upon binding, due to the ET from imidazole to the Cu^{2+} that inevitably inhibits the ET

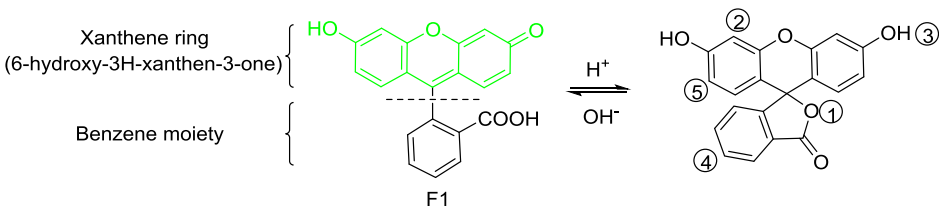

Fig. 1. Fluorescein fluorescence changes with pH and sites for modification.

between the fluorescein and imidazole [16] (Fig. 2). The limit of detection (LOD) for Cu^{2+} was 37 nM in H_2O/MeOH (4:1, v/v, 10 mM HEPES buffer, pH 7.4) solution. F2 could be applied to the determination of Cu^{2+} in living biological samples.

Based on the π-acceptor properties of the phenanthroline, which stabilize the Cu^{2+}, Mondal et al. synthesized a ratiometric fluorescein probe (F3) based on Schiff base reaction with fluorescein hydrazine and phenanthroline in 1 site. F3 can detect Cu^{2+} ions based on Cu^{2+}-induced FRET "off–on" mechanism (Fig. 3). FRET-based ratiometric fluorescent probes for Cu^{2+} have been developed, most of them are based on copper Cu^{2+} induced spiro-ring opening of rhodamine but there are a few fluorescein based FRET ratiometric fluorescent probes for Cu^{2+} [17]. The sensing of Cu^{2+} enhances the emission band of F3 at 506 nm based on FRET process between fluorescein as an acceptor and phenanthroline as a donor [17]. The enhancement of the fluorescence intensity is linear with Cu^{2+} concentration in the range from 0 to 520 μM with the LOD was 15.47 μM in CH_3CN/H_2O (8:2, v/v, 10 mM HEPES buffer, pH 7.4) solution. Furthermore, F3 is cell permeable and can be used to detect intracellular Cu^{2+} in Vero cells.

Fluorescein connecting small organic molecules in the ortho position of hydroxyl group (5 site) can increase its complexation ability and selectivity to metal cations. Fan et al. synthesized a fluorescein probe F4 which contains benzothiazole [18]. Fluorescein connects with benzothiazole in the ortho hydroxy point that can selectively detect Cu^{2+} and lead to the fluorescence quenching. Cu^{2+} can induce F4 to a spirolactone form, causing a colorimetric change and a dramatic decrease of the absorbance at 559 nm (Fig. 4). The decrease of the absorbance value was linear with Cu^{2+} concentration in the range from 0.03 to 2 μM. The LOD for Cu^{2+} is 7.48 nM in PBS buffer (pH 7.4). The binding ratio between F4 and Cu^{2+} is 2:1. The proposed method has an accuracy that can be applied in the determination of Cu^{2+} in tea and water samples.

Connecting the semi-rigid frame-work with fluorescein can provide specific binding sites to improve the detection sensitivity. Tachapermpon et al. synthesized a bonding-signaling type fluorescein probe F5 (a, b) based on Schiff base reaction and Borch reduction with fluorescein derivatives aldehyde (FL) and multidentate sulfide-containing ligands in 2 site. The fluorescence quenching at 516 nm could be attributed to the inherent quenching nature of Cu^{2+} [19]. F5 (b) was slightly inferior to F5 (a) in terms of sensitivity, due to the steric effect from two fluorescein moieties upon ions binding [19] (Fig. 5). The LOD for Cu^{2+} was 3.6 nM and 6.73 nM in acetonitrile/water solution (a 50:50 v/v, b v/v 40:60 v/v, 0.1 M PBS buffer, pH 7.2). Furthermore, F5 (a) can be used to detect intracellular Cu^{2+} in a HepG-2 cellular system.

Due to the high activity of the Schiff base, connecting the aldehyde containing compounds with fluorescein hydrazine can provide more binding sites, which can improve the sensitivity for detecting Cu^{2+}. Rathod et al. [20] synthesized a bonding-signaling type fluorescein probe F6 based on Schiff base reaction with fluorescein monoaldehyde and hydrazine hydrate in 2 site. The F6 complexation of Cu^{2+} ion resulted in the rapid change in color from colorless to deep yellow in acetonitrile, which could be attributed to the inherent quenching nature of Cu^{2+} (Fig. 6). The fluorescence intensity was distinctly quenched at 519 nm. The LOD for Cu^{2+} was 1.25 μM in Tris buffer (pH 7.4, with 1% dimethyl sulfoxide). F6 exhibits efficient binding for Cu^{2+} ion in water.

Connecting photochromic materials with fluorescein can effectively improve the sensitivity of probes and provides rapid response to metal ions [21]. Among the photochromic materials, diarylethene is widely used in fluorescein probes, because of its photoswitchable optoelectronic properties and overlapping of its emission spectra and the absorption spectra of fluorescein at plasmon resonance wavelength. Ma et al. synthesized a reactive fluorescein probe F7 via a triazole linkage diarylethene with fluorescein in 3 site. F7 offered a dual-controllable fluorescence switch triggered by both acid/base and light stimuli, and acted as a fluorescein probe for highly selective recognition of Cu^{2+} accompanied by the FRET from fluorescein to diarylethene [21]. When the F7 was treated with Cu^{2+} and $AsCH^{-}$, the fluorescence intensity at 541 nm was dramatically enhanced by 10-fold with remarkable fluorescence changed from dark to bright green due to Fenton reaction-assisted fluorescence signal amplification (Fig. 7). Significantly fluorescent quenching was observed in ring-closed isomer due to the FRET process between fluorescein moiety and the ring-closed diarylethene moiety. F7 provides an example for the design and construction of novel fluorescent probes based on photochromic diarylethene skeletons.

At present, the fluorescein probes are more of connected fluorescein with nanomaterials. The reason is that nanomaterials can overcome the biological toxicity of organic probes. Gold nanoparticles (AuNPs) which can quench the fluorescence are widely used for FRET based probes. Hormozi-Nezhad and Taghipour synthesized a replacement fluorescein probe F8 with AuNPs and fluorescein isothiocyanate isomer I (FITC) in 4 site. AuNPs can effectively form Au–S covalent bonds through the binding of FITC to its thiol group. The probe detects Cu^{2+} based on FRET process between AuNPs as acceptor and FITC as donor [22]. The fluorescence of the FITC-AuNPs system was switched to 'turn-on' by adding D-Pencillamine (D-PC) to the solution (pH = 8.0) mixtures. D-PC can displace the FITC molecules on the NPs surface and make FITC molecules be released (Fig. 8). In this scenario, the chelating agent of the D-PC strongly interacts with Cu^{2+} [22]. Two linear calibration curves were obtained within the range of 1–9 nM and 10–40 nM. The LOD was 0.3 nM in Britton–Robinson buffer (0.2 M, pH 8). F8 was successfully used for the detection of copper ions in food and water samples.

Because of cysteine can induce increase of fluorescence intensity of FITC-AuNPs and can be catalytically oxidized by Cu^{2+}, Wang et al. synthesized a replacement fluorescein probe F9 with AuNPs and FITC in 4 site. Upon adding cysteine, FITC could be displaced from the surface of AuNPs leading to the recovery of fluorescence intensity. Cu^{2+} can catalyze O_2 oxidation of cysteine. The generated

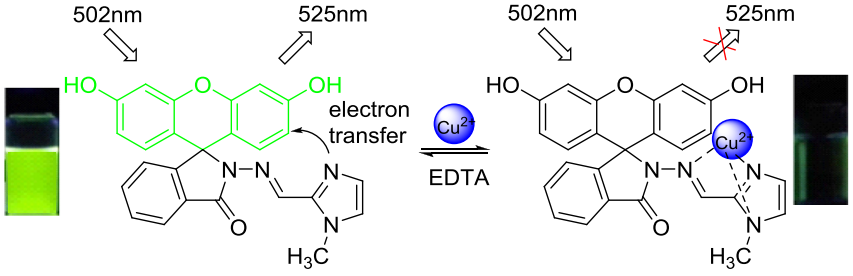

Fig. 2. Fluorescein probe F2 for Cu^{2+} detection.

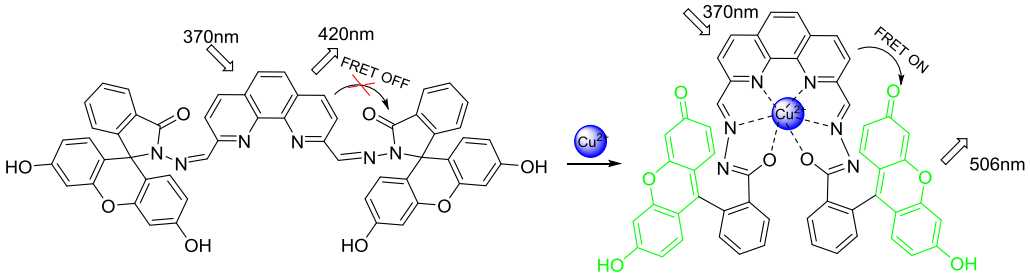

Fig. 3. Fluorescein probe F3 for Cu^{2+} detection.

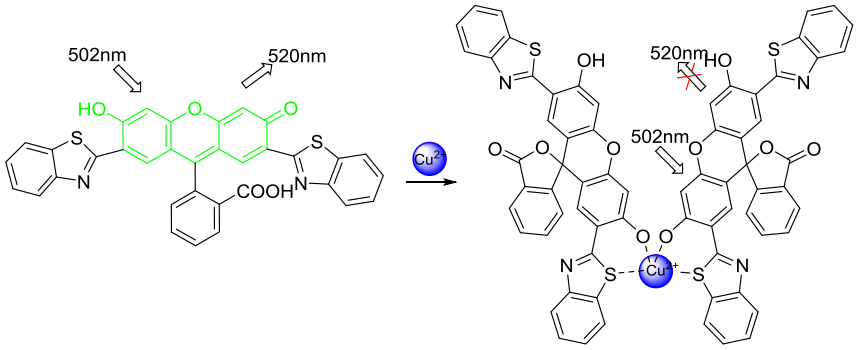

Fig. 4. Fluorescein probe F4 for Cu^{2+} detection.

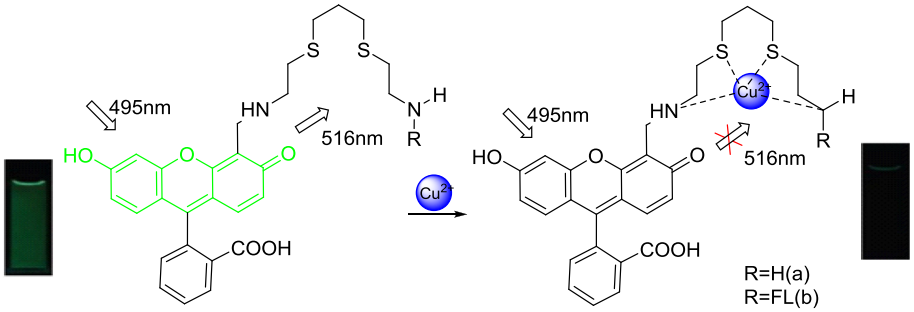

Fig. 5. Fluorescein probe F5 (a,b) for Cu^{2+} detection.

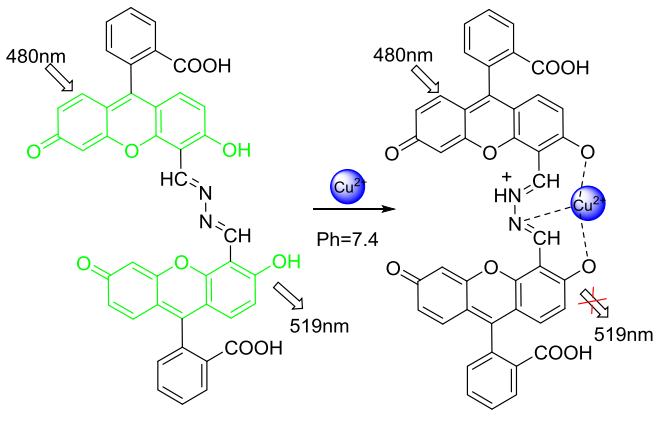

Fig. 6. Fluorescein probe F6 for Cu^{2+} detection.

disulfide cystine cannot remove FITC from AuNPs' surface (Fig. 9) [23]. Therefore, the recovery of fluorescence intensity was 95% weaker than that in the absence of Cu^{2+}. This method provided a linear relationship in the range of 1.0–17.0 nM. The LOD was 0.37 nM in Na_2HPO_4–NaH_2PO4 buffer (10 mM, pH 6.8). What's more, F9 provided a great potential application of the proposed method in real samples.

AuNPs and thiol on FITC are connected through Au–S covalent bonds. AuNPs provide more binding sites, which can improve the sensitivity for detecting Cu^{2+}. The probes which are composed by AuNPs have excellent characters such as low toxicity, sensitivity and specificity, which is better than traditional organic fluorescein probes.

3.1.2. *Fluorescein probes for zinc ions*

Zinc (Zn^{2+}) has excellent chemical and physical properties, including important roles in neuromodulation, DNA synthesis, gene expression. Bonding-signaling type probes are also used for the detection of Zn^{2+} [24,25]. Wang et al. synthesized a fluorescein probe F10 based on Schiff base reaction with fluorescein hydrazine and dihydroxybenzaldehyde in 1 site. The F10 binding with the Zn^{2+} can produce a CHEF effect with the increase of molecular rigidity (Fig. 10). It resulted in the enhancement of fluorescence and the change of color from colorless to yellow [26]. A near-linear correlation was obtained in the range of 0–15 μM. The LOD was

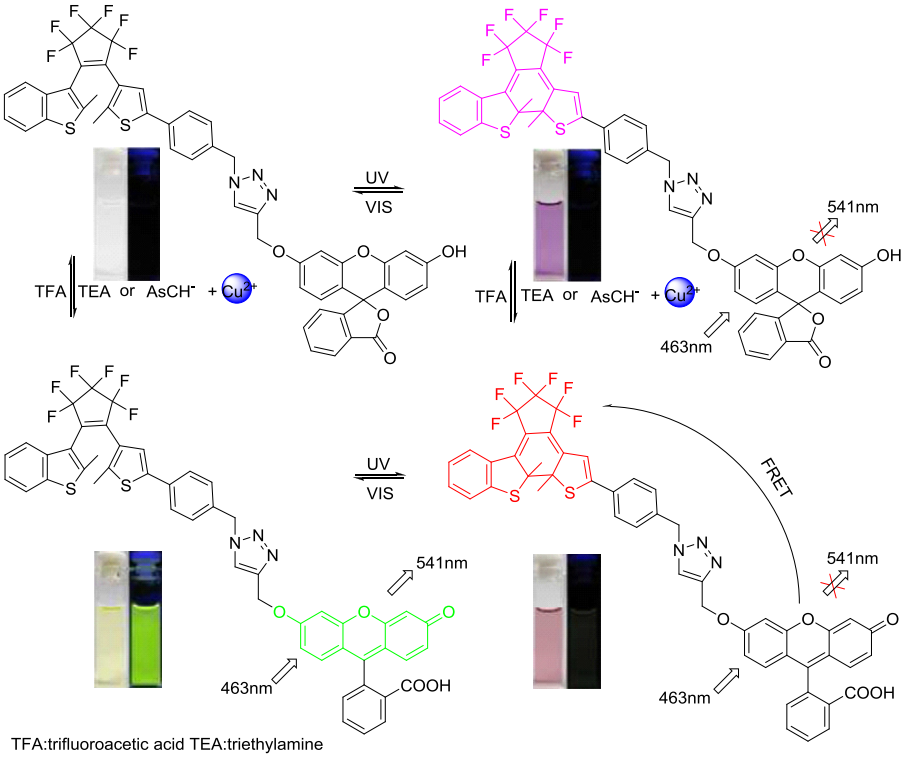

Fig. 7. Fluorescein probe F7 for Cu^{2+} detection.

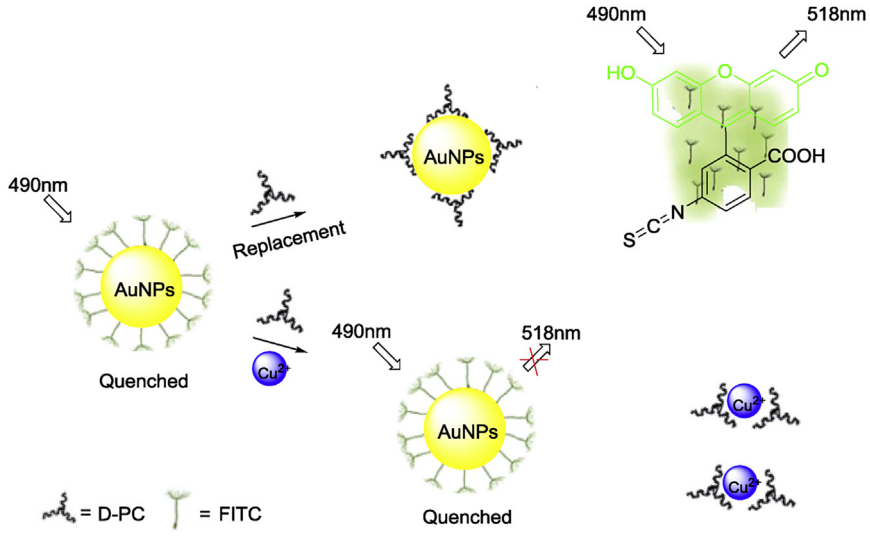

Fig. 8. Fluorescein probe F8 for Cu^{2+} detection.

10 μM in ethanol/HEPES buffer (1:1, v/v, pH 7.2). F10 detection of Zn^{2+} is reversible, EDTA can recover the fluorescence. Further the probe is applicable for imaging Zn^{2+} ions in live SMMC-7721 cells.

The spectral properties of probes (color) are changed because the detection process of reactive fluorescein probes generated optically active compounds with different new structures. Therefore, they are also called stoichiometry determination apparatus. Vidya et al. synthesized a reactive fluorescein probe F11 based on Schiff base reaction with fluorescein hydrazine and imidazole unit in 1 site [27]. After being treated with Zn^{2+}, the fluorescence of F11 at 530 nm was enhanced with a dramatic color change from colorless to yellowish green. This phenomenon is based on the fact that Zn^{2+} can induce F11 to be a ring-open structure (Fig. 11). The process of detecting Zn^{2+} with F11 is reversible, EDTA can recover the fluorescence. The LOD was 0.54 nM in HEPES buffer (10 mM, pH 7.4). Furthermore, the probe is applicable for imaging Zn^{2+} ions in HEK 293 cells.

The fluorescein xanthene ring connected with benzoic acid in ortho hydroxy point (2 site) via an azo-bridge (−N═N−) introduced a benzoic acid and a fluorescein moiety to render a binding pocket [28]. Chantalakana et al. synthesized a bonding-signaling type fluorescein probe F12. This fluorescence quenching of the F12 is attributed to a PET process due to the presence of nitrogen lone pair electrons at the azo bridge (Fig. 12). The fluorescence

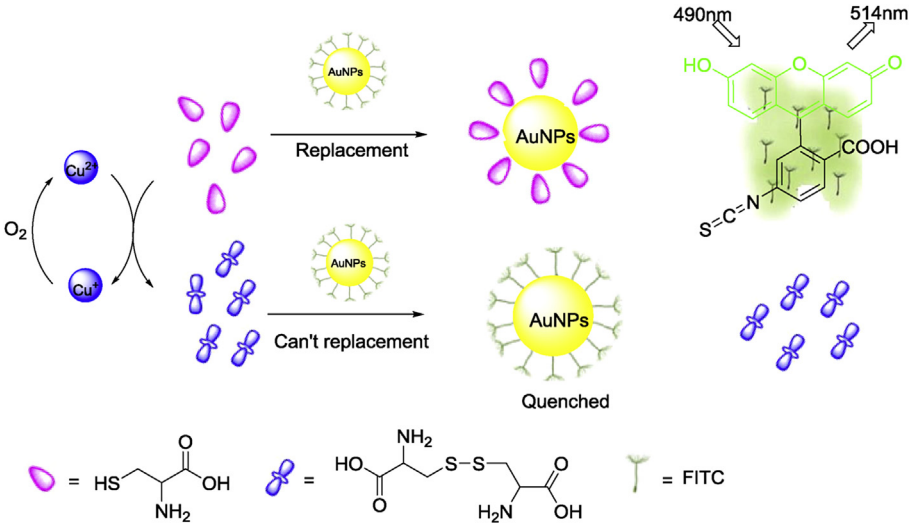

Fig. 9. Fluorescein probe F9 for Cu^{2+} detection.

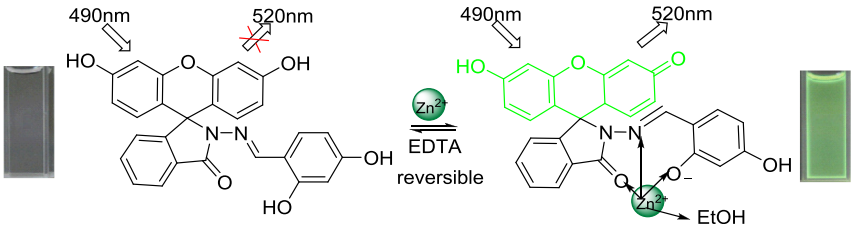

Fig. 10. Fluorescein probe F10 for Zn^{2+} detection.

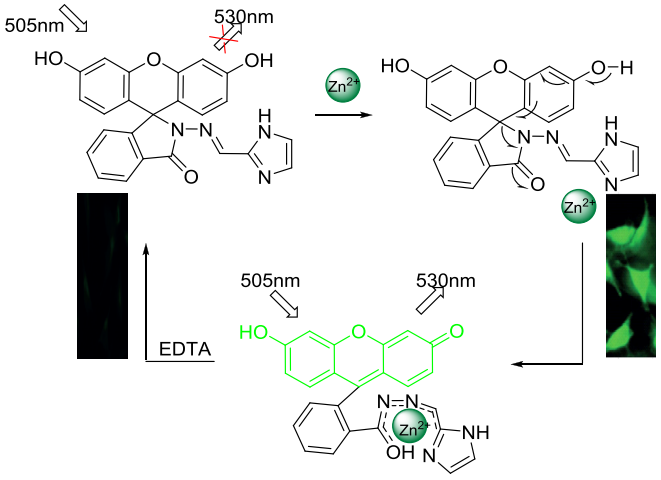

Fig. 11. Fluorescein probe F11 for Zn^{2+} detection.

intensity at 514 nm was restored upon addition of Zn^{2+}. The PET process was interrupted by the electron donating from nitrogen atoms to fluorescein upon Zn^{2+} coordination within the Zn^{2+} binding pocket. The LOD was 1.2 μM in CH_3CN–phosphate buffer (1:4, v/v, 10.0 mM, pH 7.4). F12 is also a promising platform for a variety of detection applications in environment and biological.

By comparison, it is found that the reactive probes can detect Zn^{2+} at nanomolar levels. Reactive probes are more accurate, so more of this type probes should be developed.

3.1.3. *Fluorescein probes for mercury ion*

Mercury ion (Hg^{2+}) can cause a set of severe lethal health effects, such as brain damage and other chronic disease [29]. Probes for the detection of Hg^{2+} are mainly focused on BODIPY, rhodamine and naphthalimide [30,31]. These probes are unsuitable for environmental and cellular applications due to poor water solubility. Water soluble fluorescein-based probes are synthesized to address these limitations. Fluorescein probes for the detection of Hg^{2+} are relatively few. Semi-rigid frame-work can be used for reorganizing binding sites of probes to make the size fit for specific analytes [32]. Using dithia-cyclic covalent modification of fluorescein could increase the complexation ability and selectivity to Hg^{2+}. Piyanuch et al. synthesized a bonding-signaling type fluorescein probe F13 based on an approach similar to host–guest supramolecular chemistry [32]. The fluorescein aldehyde and a dithia-cyclic were connected based on Schiff base reaction and Borch reduction in 2 site. The selective binding of F13 to Hg^{2+} provides the dual optical sensing through both fluorescence quenching at 515 nm and concurrent visual color changed from yellow to orange (Fig. 13). The LOD was 7.38 nM for Hg^{2+}, and provided a linear relationship in the range of 0–5 μM in Tris–HCl buffer–MeOH (95:5, v/v, pH 7.2) solution. Only a few fluorescent probes can be used to detect Hg^{2+} detection in the aqueous at nanomolar levels.

Bonding-signaling type probe can be used for the detection of Hg^{2+} at nanomolar levels. Wang et al. synthesized a bonding-signaling type fluorescein probe F14 by using thiol-DNA-functionalized AuNPs. In the presence of Hg^{2+}, F14 formed the hairpin structure of ssDNA which originates from thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination (Fig. 14). It resulted in notable fluorescence quenching due to the FRET process between the energy donor fluorescein and the energy acceptor AuNPs [33]. The LOD was 8 nM in Tris–CH_3COOH at (20 mM, pH 7.4). This probe was applied to monitor Hg^{2+} in tap water samples.

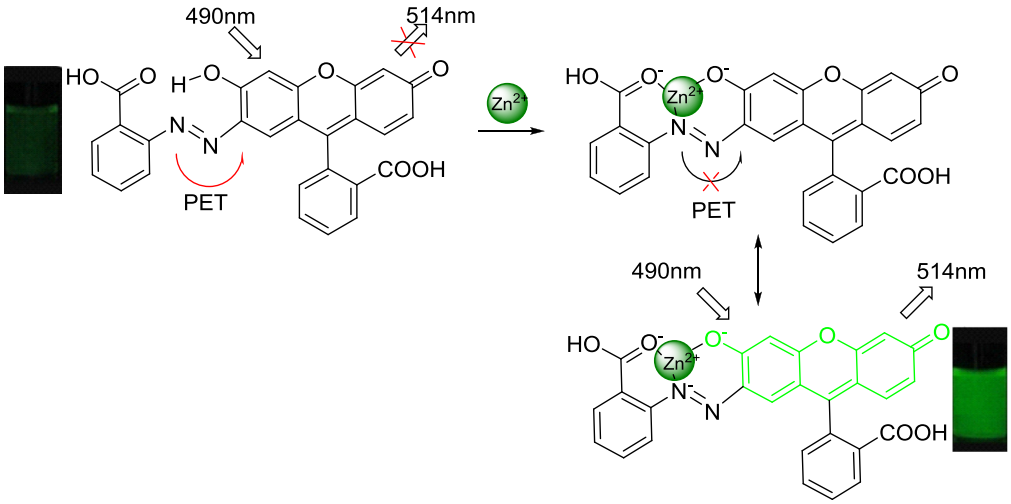

Fig. 12. Fluorescein probe F12 for Zn^{2+} detection.

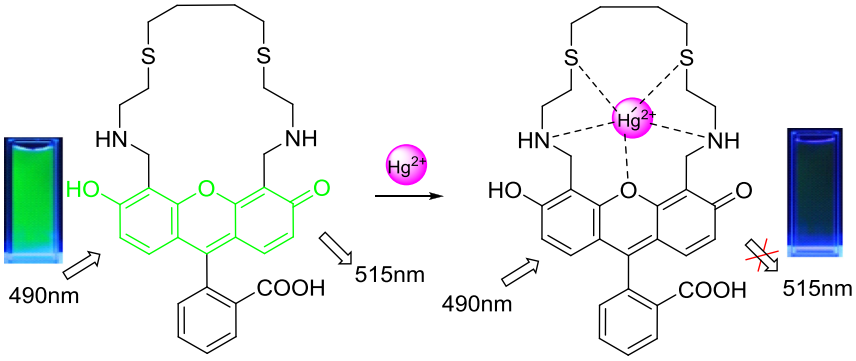

Fig. 13. Fluorescein probe F13 for Hg^{2+} detection.

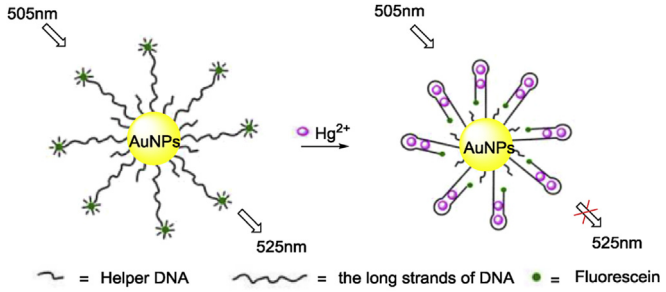

Fig. 14. Fluorescein probe F14 for Hg^{2+} detection.

3.1.4. *Fluorescein probes for gold ions*

Excessive gold ions (Au^{3+}) are highly toxic because it can bind to DNA strongly leading to their subsequent cleavage, and causing damage to the liver, kidneys, and the nervous system [34,35]. Using Au^{3+} promotes the hydrolysis and oxidability mechanism of C=N bond and can design reactive fluorescein probes. Kambam et al. synthesized a fluorescein probe F15 based on Schiff base reaction with fluorescein dicarboxaldehyde and 1-(pyridin-2-yl) hydrazine in 2 site. F15 could be induced to be a ring-open structure by Au^{3+}, causing a fluorescence enhancement at 523 nm. In the presence of Au^{3+}, F15 generated the 4′5′fluoresceindicarboxaldehyde which leads to the fluorescence enhancement [36] (Fig. 15). This is attributed to the irreversible C=N bond hydrolysis. A linear relationship between the fluorescence intensity and the concentrations of Au^{3+} is ranging from 0 to 60 μM. The LOD was 0.07 μM in HEPES buffer (10 mM, pH 7.4). Furthermore, the imaging experiments indicated that this probe is cell-permeable and can be used to detect Au^{3+} in MCF-7 cells.

Phosphine is a compound containing phosphorus. Although phosphine ligands are ubiquitous in transition metal chemistry, few reports of fluorescent phosphines exist that explore the effect of metal coordination on the photophysical properties of a phosphine-bound fluorescent group [37]. *Via* diphenylphosphino unit connected to fluorescein spirocyclic by an *o*-phenylene linker, Christianson and Gabbai synthesized a reactive fluorescein probe F16. Free F16 existences PET from the lone-pair-bearing phosphine cause the low emission. The oxidation of the phosphine deactivates the phosphorus-to-fluorophore PET leading to a resurrection of the fluorescent emission. This behavior toward Au^{3+} was the result of a combination of reduction of Au^{3+} and subsequent coordination to Au^{+} to form 1 equiv of phosphine oxide and 1 equiv of phosphine gold chloride complex phosphine fluorescein-Au per gold equivalent [37] (Fig. 16). A calibration curve for the addition of Au^{3+} to F16 in the sensing range shows a linear increase in fluorescence at 542 nm from 0 to 500 nM. The LOD was 76 nM in sodium phosphate buffer at (10 mM, pH 7.4, with 10 mM cetyltrimethylammonium chloride). The selectivity for Au^{3+} leads to a double 'turn-on' response that is based on both reaction and coordination, which is better than the other single response probes.

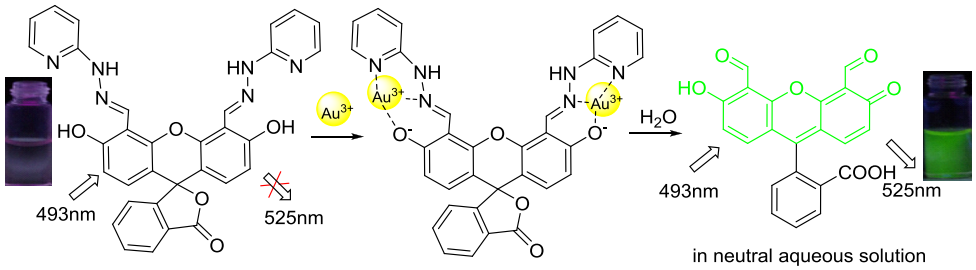

Fig. 15. Fluorescein probe F15 for Au^{3+} detection.

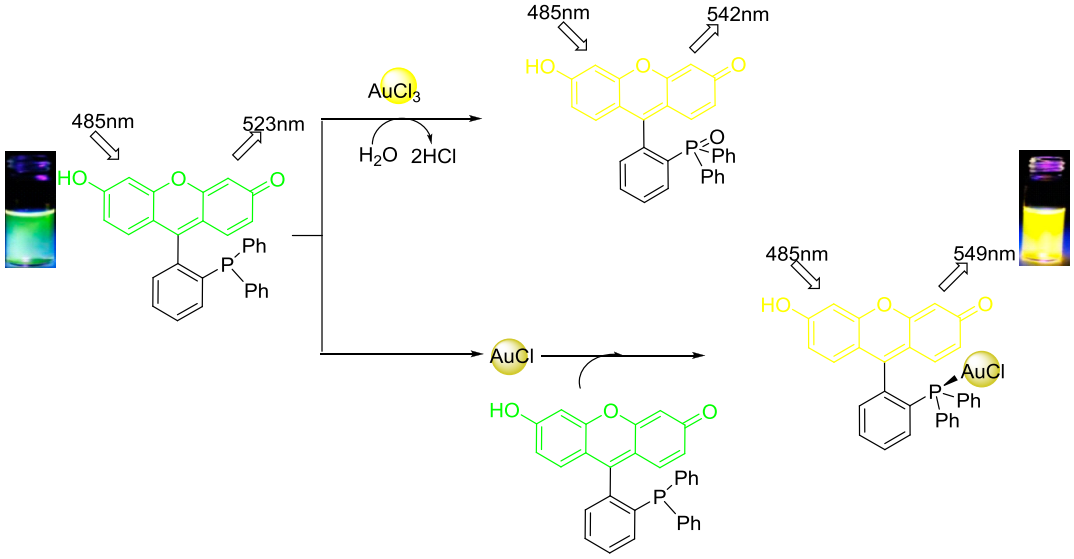

Fig. 16. Fluorescein probe F16 for Au^{3+} detection.

3.1.5. *Fluorescein probes for silver ions*

Silver ions (Ag^{+}) are widely used in the electric industry, photographic and imaging industry leading to environmental pollution [38]. By modifying fluorescein hydrazine with 2-thiazolecarboxaldehyde and allyl bromide based on Schiff base reaction in 1 site and etherification reaction in 3 site, Sun et al. synthesized a reactive fluorescein probe (F17). By the polymerization of allyl ether, the probe can form the copolymer (Fig. 17) [38]. F17 could be induced to be a ring-open structure by Ag^{+}, causing the fluorescence enhancement at 505 nm. A near-linear correlation between fluorescence intensity and concentration of Ag^{+} is obtained over the range 10–60 μM. The LOD was 20 μM in water sample.

In order to simplify synthetic steps and improve the detection function, Shen et al. synthesized a reactive fluorescein probe (F18) based on Schiff base reaction with fluorescein hydrazine and 2-formylthiophene in 1 site. Hg^{2+} can induce F18 to form a ring-open structure, causing a colorimetric change and a fluorescence enhancement at 524 nm. The mechanism of sensing Ag^{+} is in agreement with the general knowledge of the unstable Schiff base, when Hg^{2+} and Ag^{+} are both present in a solution C=N tends to be broken (Fig. 18) [39]. Such that the spectrophotometric method can be utilized for the detection of Hg^{2+} in an ethanol–H_2O solution (3:2, v/v, HEPES buffer, 0.5 mM, pH 7.15) with a detection limit of 0.21 μM. After complexating with Hg^{2+}, F18 showed extremely high selectivity toward Ag^{+} with a LOD of 9 nM. F18 could be used as a fluorescein probe to detect Hg^{2+} and Ag^{+} in L-02 human liver cells. The probe–analyte complex formed by binding with analyte exhibits selectivity to another ion with high efficiency and lower detection limit. This type of probes provides a new idea for the future of fluorescein probes design.

3.1.6. *Fluorescein probes for other ions*

Fluorescein can be used to detect palladium ions though esterification and alkylation in 3 site. Kitley et al. synthesized a fluorescein probe (F19) based on etherification reaction with

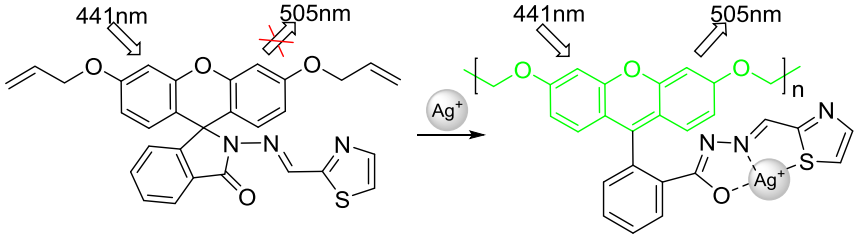

Fig. 17. Fluorescein probe F17 for Ag^{+} detection.

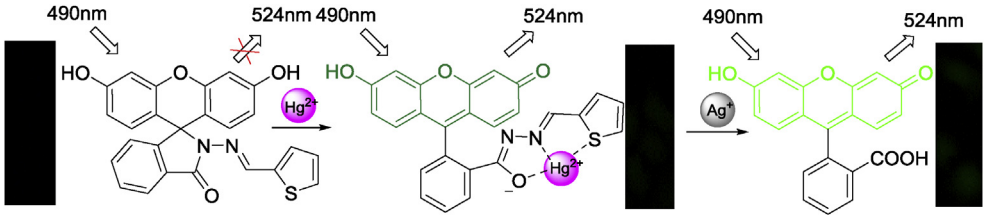

Fig. 18. Fluorescein probe F18 for Ag^{+} detection.

fluorescein and allyl bromide in 3 site [40] upon specific reaction with palladium and the resulting cleavage of the allyl ether, which can cause the fluorescence enhancement at 529 nm. The LOD was 4.6 nM with a linear range of 0–235 nM. Anchoring the probe to a solid support may be realized in the future, thereby providing more simple detection method in the practical application for detection of palladium ions (see Fig. 19).

According to the same mechanism with F19, Feng et al. synthesized a reactive fluorescein probe F20, Fig. 18, based on etherification reaction with fluorescein and allyl bromide in 3 site [41]. F20 based on the mechanism that CO can restore Pd^{2+} to Pd^{0}. The LOD was 29 nM and a linear range of 0–50 μM in 0.5% DMSO/PBS buffer (v/v, 10 mM, pH 7.4). The lipophilic property of fluorescein derivatives was greatly improved by esterification and alkylation. This kind of fluorescein probes have good cell membrane permeability, which can be applied to detect analytes in biological system (see Fig. 20).

The fluorescein probes for detecting Fe^{3+} have the advantages of convenient operation and high detection accuracy. Based on the similar structure with Ag^{+} detection, Gao et al. synthesized a reactive fluorescein probe (F21) based on Schiff base reaction with benzothiazole and fluorescein hydrazine in 1 site [42]. F21 could be induced to be a ring-open structure by Fe^{3+}, which can cause the fluorescence enhancement (Fig. 21). The LOD was 7.4 nM and a linear range of 0–10 μM in DMSO/H_2O (3:7, v/v, HEPES buffer, pH 7.2.) The probe detection sensitivity and detection limit of Fe^{3+} is higher than other types of probes [43].

Fluorescein probes with Schiff base structure in benzene moiety 1 site can also be used for the detection of Mg^{2+}. Li et al. synthesized a reactive fluorescein probe F22 for Mg^{2+} with fluorescein and 6-hydroxy-3-formylchromone [44]. Mg^{2+} can induce F22 to be a ring-open structure, which can cause the fluorescence enhancement at 504 nm (Fig. 22). The LOD was 0.2 nM and a linear range of 0.05–0.5 mM in ethanol solution. The binding ratio between the probe and Mg^{2+} is 2:1 which is different from the other fluorescein hydrazine type probes. Furthermore, F22 could be used for detecting Mg^{2+} in HeLa cells.

Fluorescein diarylethene derivative bridged by quinoline as a fluorescein probe to detect Cd^{2+} in aqueous solution with a dual channel mode. Piyanuch et al. synthesized a ratiometric fluorescein probe (F23) with fluorescein, diarylethene and quinolone in 1 site. F23 allows a ratio of the fluorescence intensities at two

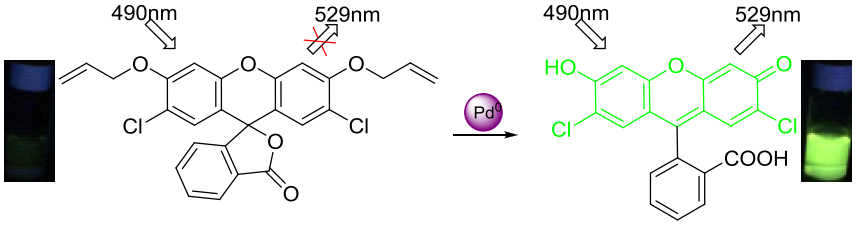

Fig. 19. Fluorescein probe F19 for Ag^{+} detection.

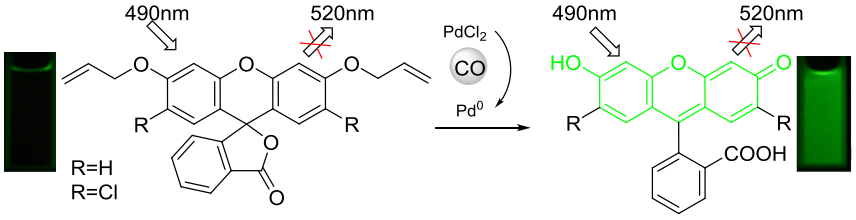

Fig. 20. Fluorescein probe F20 for CO detection.

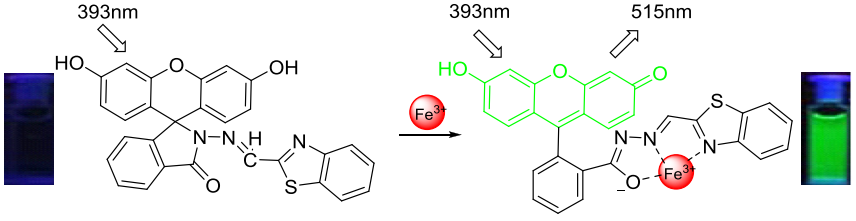

Fig. 21. Fluorescein probe F21 for Fe^{3+} detection.

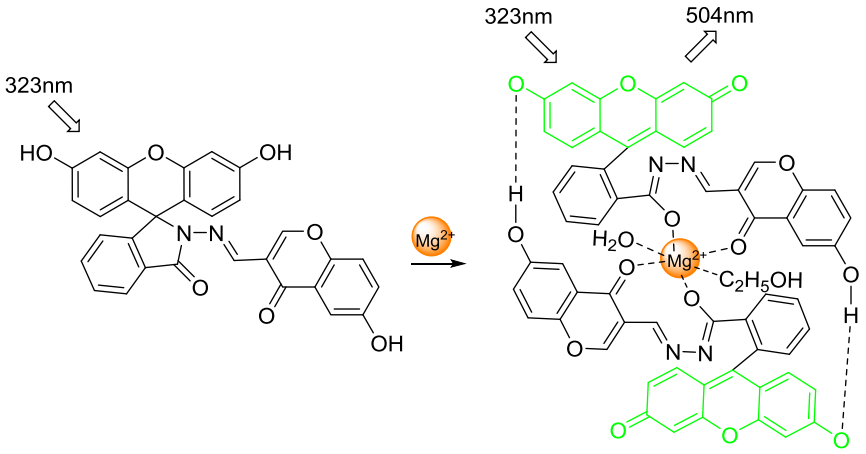

Fig. 22. Fluorescein probe F22 for Mg^{2+} detection.

wavelengths which is turned out to be an accurate and efficient method for detection of Cd^{2+}. Cd^{2+} can lead the F23 to form a spirolactam ring-open structure, an obvious red shift from 459 nm to 560 nm was observed. The emission intensity was enhanced by 8-fold with a concomitant fluorescence color change from dark to bright yellow (Fig. 23) [45]. Significantly fluorescent quenching was observed in ring-closed isomer due to the FRET processes between fluorescein moiety and the ring-closed diarylethene moiety. Finally, a molecularlogic circuit was constructed with both light and chemical stimuli as inputs and fluorescence intensity at 560 nm as output.

Fluorescein probes detection method can be used to achieve rapid detection of Pb^{2+}. Tang et al. synthesized a DNAzyme-based fluorescein probe (F24) with fluorescein, DNAzyme and Ethidium bromide (EB). When exciting fluorescein at 490 nM, then the FRET from donor fluorescein to acceptor EB occurs because of the desirable spectral overlap between fluorescein emission and EB absorption [46]. The FRET leads to the fluorescence of fluorescein decreasing greatly. In the presence of Pb^{2+}, the substrate is cleaved by DNAzyme, resulting in the FRET being broken (Fig. 24). The fluorescence of fluorescein increases dramatically at 520 nm. The LOD was 530 pM and a linear range of 0–100 nM in HEPES–Na buffer (0.1 M, pH 7.3). According to the Environmental Protection Agency (EPA), 0.07 μM of Pb^{2+} is the safety limit in drinking water [45]. This probe is sensitive and simple without any additional treatments, which provides a reference for other probes based on DNAzyme.

3.2. Fluorescein probes detection of anions

Anions play an important role in biological, chemical and environmental processes. Lack or excess can cause some problems in physiology or environment [47]. Fluorescein can modify through Schiff base reaction (1, 2 site) and esterification reaction (3 site). Fluorescein probes can complex or react with anions based on the unstable C=N bonds and ester bonds, which will result in

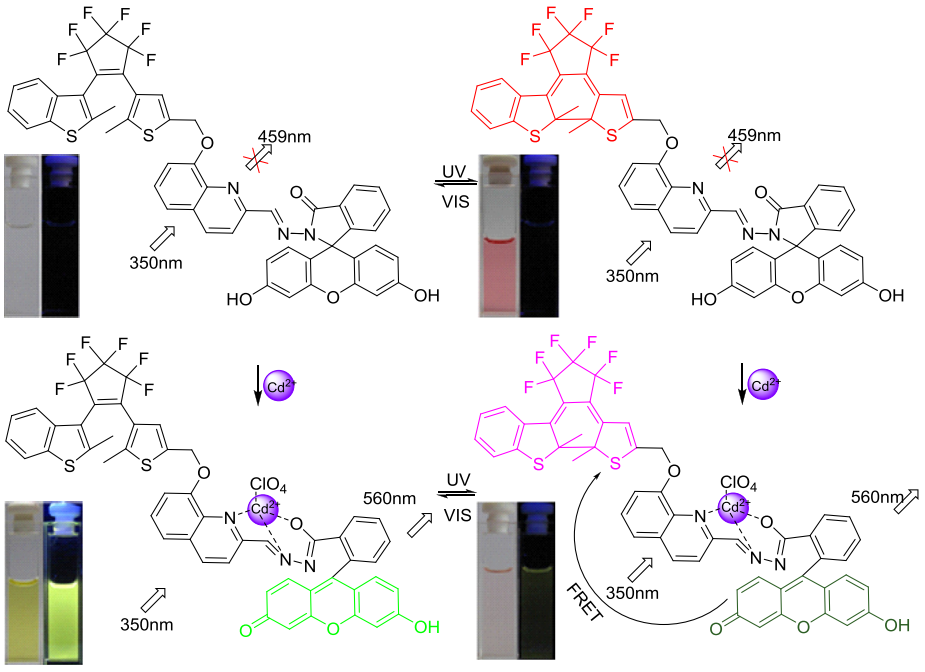

Fig. 23. Fluorescein probe F23 for Cd^{2+} detection.

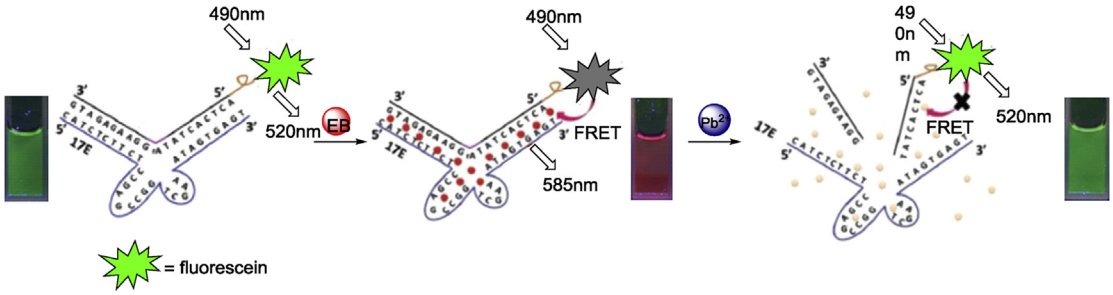

Fig. 24. Fluorescein probe F24 for Pb^{2+} detection.

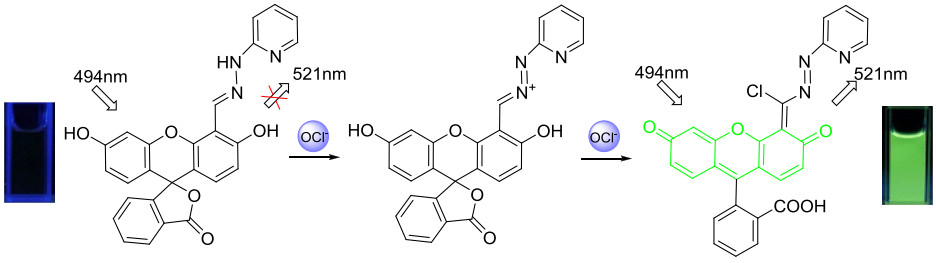

Fig. 25. Fluorescein probe F25 for OCl^- detection.

fluorescent enhancement or quenching. Fluorescein probes can be used to detect such as hypochlorite, sulfide, nitrate radical, fluoride ion and thiocyanate.

3.2.1. Fluorescein probes for hypochlorous acid

Hypochlorite anion (OCl^-) is an important reactive oxygen species (ROS) in living organisms. Many fluorescein probes for detecting hypochlorite have been developed based on the mechanism of the oxidization hydrolysis of hydrazide [47]. Wang et al. synthesized a fluorescein probe (F25) [48] based on Schiff base reaction with fluorescein monoaldehyde and 1-(pyridin-2-yl) hydrazine in 3 site. The hydrazine group of the probe first reacted with a molecule of OCl^- to cause protonation. Then, OCl^- continues to react with the imine structure of the probe, accompanied by deprotonation and chlorination processes (Fig. 25). This change extends the conjugation of F25, resulting in fluorescence enhancement at 521 nm. The LOD was 7.3 nM and a linear range of 0–30 μM in PBS buffer (10 mM, pH 7.4).

In order to reduce background fluorescence, Chen et al. synthesized a fluorescein probe (F26) based on condensation reaction with fluorescein bisaldehyde and 1, 8-diaminonaphthalene [49]. OCl^- will attack the C=N bond of F26 and cause the break of the C=N bond. The following nucleophilic attack by H_2O finally interrupted the PET mechanism by breaking donor 1, 8-diaminonaphthalene and acceptor fluorescein bisaldehyde linkage, leading to the enhancement of fluorescence (Fig. 26) [49]. Allows the LOD of 0.23 μM and a linear range of 0–10 μM in PBS solution (10 mM, pH 7.4, with 1% DMF). The probe is successfully utilized for hypochlorite in living macrophage cells upon stimulation.

Fluorescein hydrazine coalescent with aldehyde containing compounds in 1 site, which can be used for detection of OCl^-. Jin et al. synthesized a fluorescein probe (F27) based on Schiff base reaction with fluorescein hydrazide and 3, 4, 5-trihydroxybenzaldehyde. F27 could be induced to be a ring-open structure by OCl^- (Fig. 27) [50]. There was a linear range of 0.5–5 μM in methanol–PBS (5:5, v/v, pH 7.4) with LOD 0.5 μM. F27 successfully applied for quantitative detection of OCl^- in real life water samples, bovine plasma and *Rhodobacter ferrooxidans* sp. SW2 cells.

3.2.2. Fluorescein probes for other anions

Fluorescein has good hydrophilicity, poor affinity with membrane lipids because of two phenolic hydroxyl groups. Sulfur (SO_3^{2-}) play an important role in biological, industrial, and environmental processes [51]. The fluorescein probes can also be detected SO_3^{2-} based on the π-conjugation recovering which leads to notable color and fluorescence changes. Song et al. synthesized a reactive fluorescein probe (F28) based on dehydration condensation reaction with 2-chloro-5-nitro benzoic acid and fluorescein in 3 site [52]. F28 can be induced to be a ring-open structure by ester bond hydrolysis reaction via SO_3^{2-} and 2-chloro-5-nitro benzoic, causing the fluorescence enhancement at 520 nm (Fig. 28). The color of the

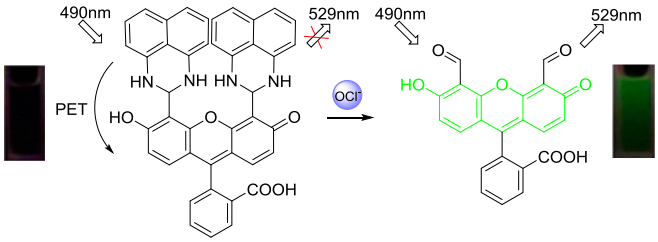

Fig. 26. Fluorescein probe F26 for OCl^- detection.

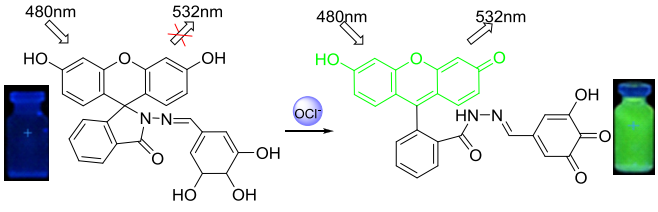

Fig. 27. Fluorescein probe F27 for OCl^- detection.

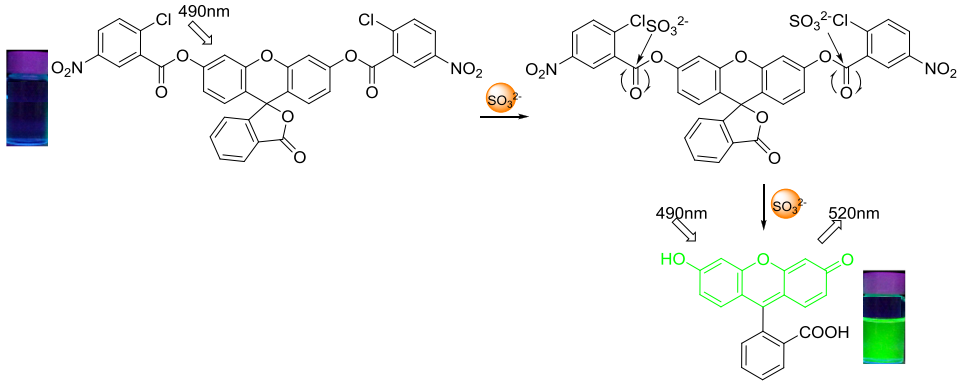

Fig. 28. Fluorescein probe F28 for SO_3^{2-} detection.

solution changed from colorless to yellow after the addition of SO_3^{2-}. There was a linear range of 0–100 μM in MeOH/Tris–HCl buffer (7:3, v/v, 1 mM, pH 7.0) with LOD 20 nM.

Introduction of active groups into the fluorescein structure in benzene moiety 4 site, the specific binding to the labeled molecules is improved. Fluorescein probes with active groups can be used to detect in organisms with high sensitivity and selectivity. FITC and small organic molecules are connected through amide reaction, which can also selectively detect ions with a lower detection limit. Fluoride ion (F^-) is of particular interest because of its broad use in preventing dental caries, and treatment of osteoporosis [53]. Zhang et al. synthesized a reactive fluorescein probe (F29) based on Amide reaction with 4-aminoantipyrine and FITC [53]. The F^- and AcO^- ions can be combined through oxygen (O) of fluorescein and sulfur, nitrogen site of 4-aminoantipyrine, and induced F29 formation a ring-open form causing a colorless-to-yellow color changes and a fluorescence enhancement at 538 nm and 542 nm, respectively (Fig. 29). There was a linear range of 0–250 μM in DMSO solution with LOD 0.63 nM. The proposed method offers good selectivity and accuracy that can be applied to the determination of F^- in water samples.

Thiocyanate (SCN^-) is a small anion by product of cyanide metabolism. Low levels of serum SCN^- may predispose them to inflammatory or inflammation-mediated diseases [54]. Song's group synthesized a simple and ultrasensitive replacement fluorescein probe (F30) of trace SCN^- based on the FRET between AuNPs and fluorescein [54]. AuNPs were explored as the efficient energy acceptors to substitute for fluorescein molecules acceptors and selected for establishing a FRET system as a fluorescence quencher. The fluorescence of fluorescein is significantly quenched when it is attached to the surface of AuNPs. Upon the addition of SCN^-, the fluorescence is turned on due to the competition action

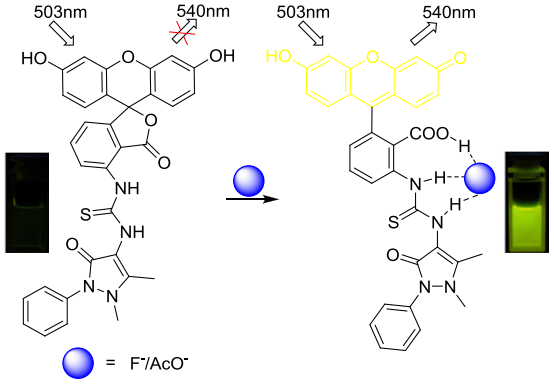

Fig. 29. Fluorescein probe F29 for F^- detection.

between SCN^- and fluorescein towards the surface of AuNPs, causing the fluorescence enhancement at 531 nm (Fig. 30). There was a linear range of 1–40 nM in PBS (10 mM, pH 7.0) with LOD 0.09 nM. F30 is successfully applied for the determination of SCN^- in milk products and saliva samples.

So far, the fluorescein probes for detecting anions are insufficient. Detection of anions also has same important significance as cations. Researchers can develop more fluorescein probe for anion in future.

3.2.3. *Fluorescein probes for both anions and cations*

Multifunctional probes are better because of it can detect both anions and cations. The main mechanism is that the combination of the fluorescein probes with one kind of ion leads to the quenching of fluorescence which can be recovered by another ion. The bifunctional fluorescein probes having biological and environmental relevance have been dramatic developed, owing to their low cost versatility and easy monitoring. It has already been reviewed that metal ions such as Al^{3+} quenched the fluorescence of fluorescein probes. Fluoride ions have high affinity towards the Al^{3+} and react with it to form a very stable $[AlFx]^{n-}$ species, which leads to the recovery of the fluorescence of fluorescein probes [55]. Diao et al. synthesized a reactive fluorescein probe (F31) based on Schiff base reaction with picolylamine and fluorescein monoaldehyde in 2 site. Due to the free picolylamine group did not have a lone pair of suitable energy for causing a sufficient PET process in the molecule, Free F31 has a strong fluorescence at 520 nm. Al^{3+} have an unfilled 3d shell and could strongly quench the emission of the fluorophore near it via electron transfer. In the presence of F^-, the fluorescence intensity and overall chemical structure of F31 were reconstructed since the abolishment of Al^{3+} (Fig. 31). F31 can fluorescent detection of Al^{3+} and F^- in concentration range of 0–10 μM and 0–30 μM, respectively. The LOD for Al^{3+} and F^- was 0.092 μM and 0.112 μM in Tris–HCl aqueous buffer (10 mM, pH = 7.0). Moreover, the "ON–OFF–ON" fluorescence changes upon the addition of Al^{3+} and F^- was applied in HeLa cell imaging.

Fluorescein probes suitable for simultaneous detection of multiple analytes are of particular importance for routine analysis in the field or contaminated site. FITC combining with other luminescent groups based on amide reaction can also ratiometrically detect both Hg^{2+} and F^-. Chereddy et al. synthesized a ratiometric fluorescein probe (F32) by rhodamine hydrazide and silyl ether modification FITC [9]. F32 selectively complexed with Hg^{2+} on the thiourea moiety which induced absorption and fluorescence properties of rhodamine hydrazide. F^- promoted the cleavage of silyl ether bond leading to the opening of fluorescein spirocyclic ring, and subsequently, the FRET from fluorescein moiety to rhodamine moiety (Fig. 32). The LOD was 51 nM and 5.4 nM,

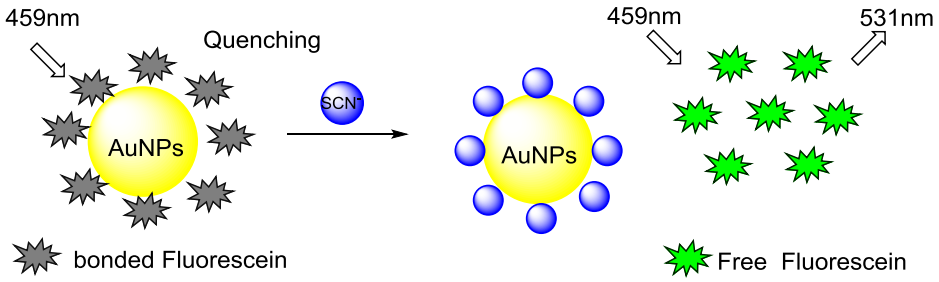

Fig. 30. Fluorescein probe F30 for SCN^- detection.

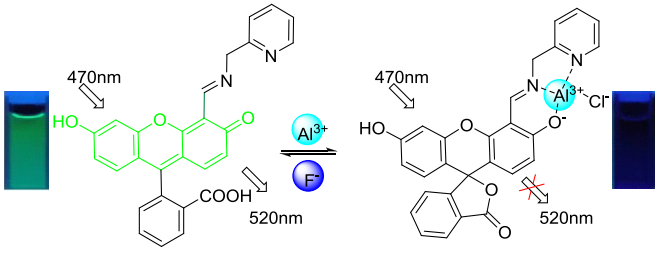

Fig. 31. Fluorescein probe F31 for F^- and Al^{3+} detection.

respectively for F^- and Hg^{2+} in Tris HCl–CH_3CN (1:1, v/v, 10 mM, pH 7.4). F32 can be used for the simultaneous and ratiometric detection of both Hg^{2+} and F^- ions in the both aqueous and W138 normal lung fibroblast cells.

3.3. Fluorescein probes detection of small molecule

Excess small molecules such as nitric oxide, hydrogen sulfide and reactive oxygen species can cause some diseases in physiology [56]. The lipophilic properties of fluorescein probes were improved greatly by esterification and alkylation in 3 site. Some small molecules such as amino acid, nitric oxide, hydrogen sulfide, reactive oxygen species and hydrazine can break the ester bond, which can make the fluorescein probes π-conjugation structure recovering and then leads to fluorescence changes. The fluorescein probes can be used to detect such as amino acid, nitric oxide, hydrogen sulfide, reactive oxygen species, hydrazine, phosphate and Adenosine triphosphate.

3.3.1. Fluorescein probes for amino acid

Thiol-containing molecules play essential roles in maintaining the appropriate redox status in physiological and pathological

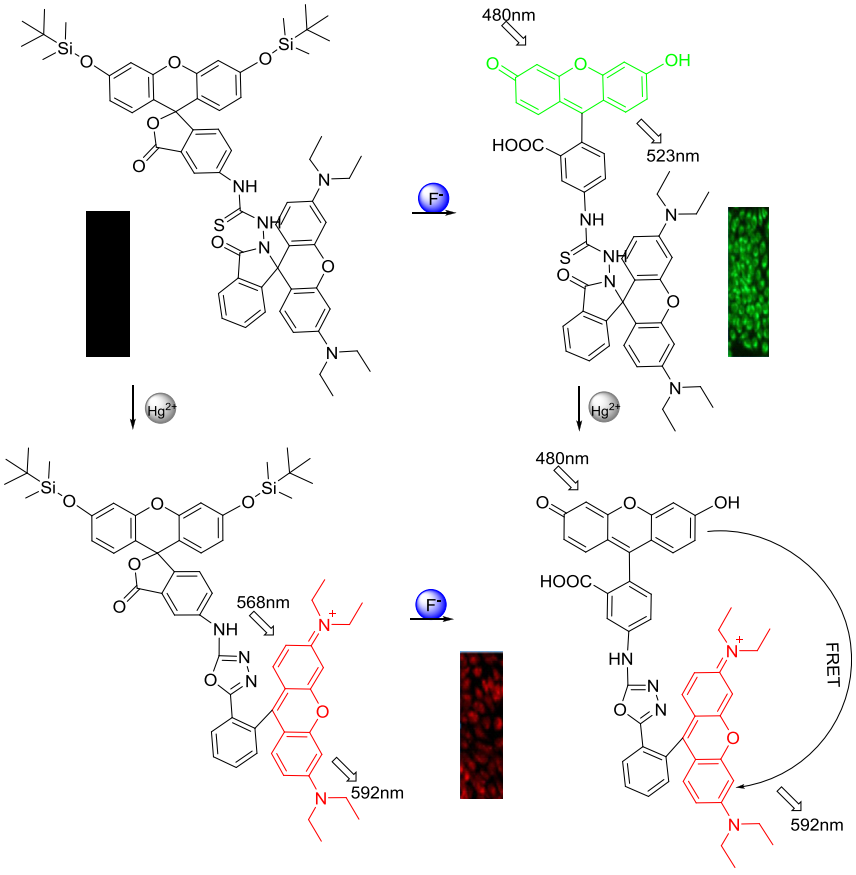

Fig. 32. Fluorescein probe F32 for Hg^{2+} and F^- detection.

processes. We will summarize and analyze various strategies for the design of fluorescein probes for rapid, selective and quantitative detection of thiols such as cysteine (cys), homocysteine (Hcy) and glutathione (GSH).

Fluorescein as donor or acceptor connecting with other fluorophores (rhodamine, BODIPY, coumarin) can be used to ratiometric fluorescent detect analytes based on FRET process between fluorescein and other fluorophores. FITC combining with amino-BODIPY dyes by amide reaction were used to ratiometric fluorescent detect Cys. Ma et al. synthesized a fluorescein ratiometric probe (F33) [57]. Acryloyl-FLTC as an acceptor and famino-BODIPY as a donor. The probe itself emits only through the BODIPY moiety. The Cys-promoted deacylation process produces the FRET, which emits green fluorescence from the fluorescein moiety. When the probe (10 mM) was treated with Cys (20 equiv), the BODIPY dye excited through the FRET (Fig. 33). A good linearity is observed between the ratio of peak heights at the two maximum wavelengths, I520 versus I452, and the concentration of Cys in the range of 10–600 mM. The LOD was 0.1 mM in CH_3CN–HEPES buffer (3:7, v/v, 10 mM, pH 7.4).

Based on the integration of fluorescein and coumarin, Peng et al. synthesized a chromogenic and ratiometric dual mode fluorescent probe (F34) [58]. All of fluorescence results indicate that F34 is highly selective for Cys over other amino acids, cations and anions. Among the amino acids including Hcy and GSH, this probe is highly selective for the detection of Cys. With the treatment of Cys, the turn-on fluorescence responses were observed at 540 nm by using two different excited wavelengths, especially accompanied by color changed from colorless to yellow (Fig. 34) The LOD was 0.084 μM in DMSO–PBS buffer (3:7, v/v, 10.0 mM, pH 7.4). The FRET probe was applied in bioimaging within HepG2 cells, and also applied to determine the level of cysteine in human blood plasma.

Different groups which are attached to the two phenolic hydroxyl groups of fluorescein in 3 site can construct asymmetric reactive fluorescein probes for RSH. Liu et al. synthesized a reactive fluorescein probe (F35) based on amide reaction between fluorescein methyl ester and 2, 4-dinitrobenzene sulfonyl (DNBS) [59]. DNBS can react with thiol and release the fluorescein which has strong fluorescence. F35 is in the state of open form which has a strong fluorescence when it reacts with thiol, causing the fluorescence enhancement at 521 nm (Fig. 35). The LOD 0.16 μM in DMSO/PBS solutions (3:7, v/v, 10 mM, pH = 7.4). All of properties prove F35 to be a good sensor for the selective detection of thiol. Moreover it shows potential application in bioimaging.

Similarly, modification of fluorescein with bromoacetyl ester and aldehyde can be used to detect Cys. Lee et al. designed a reactive fluorescein probe (F36) [60]. Initially nonfluorescent bromoacetyl fluorescein monoaldehyde was rapidly transformed into a strongly fluorescent molecule through the rapid ester bond cleavage reaction of F36 by the first Cys. The second Cys reaction with the aldehyde group formation a subsequent oxazolidine (Fig. 36). Upon addition of Cys, the enhancement at 528 nm of fluorescence intensity was linear. The LOD of Cys was determined to be 0.51 μM in DMSO/HEPES buffer (6:4, v/v, 0.1 M, pH 7.4). F36 was successfully applied for live cell imaging with tunicamycin, an inducer of cellular Cys.

F35, F36, F37 are asymmetric fluorescein probes with sensitivity and good membrane permeability, which can be used to detect intracellular small molecules. These new asymmetric structures study may open new doors to design probes for the small molecule detection.

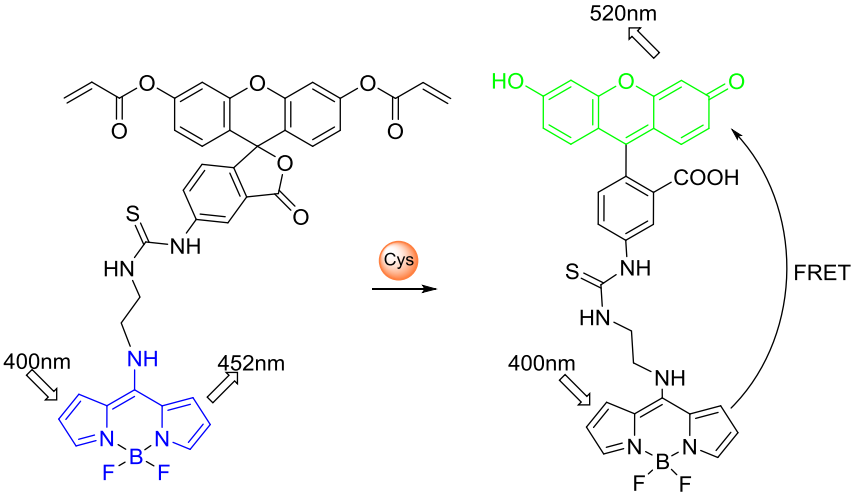

Fig. 33. Fluorescein probe F33 for Cys detection.

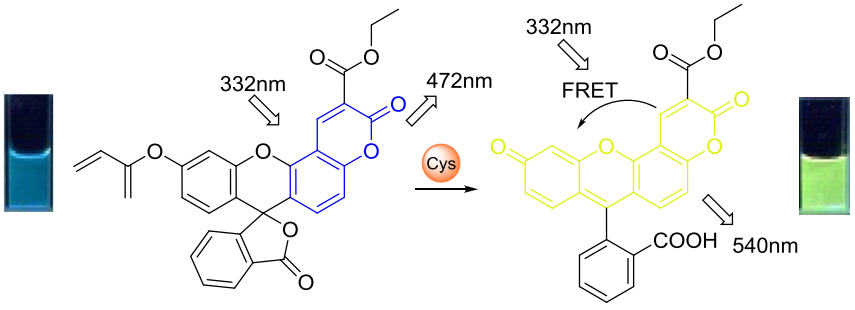

Fig. 34. Fluorescein probe F34 for Cys detection.

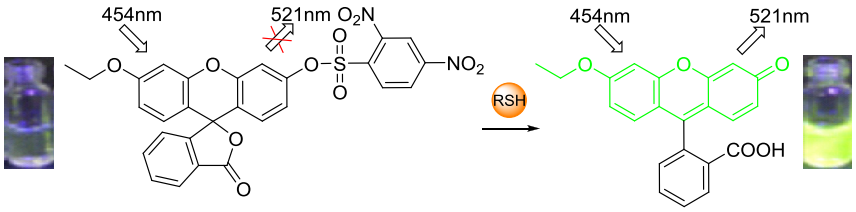

Fig. 35. Fluorescein probe F35 for RSH detection.

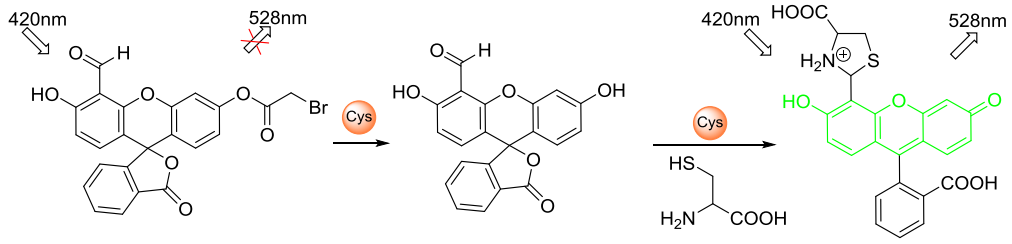

Fig. 36. Fluorescein probe F36 for Cys detection.

3.3.2. *Fluorescein probes for nitric oxide*

Nitric oxide (NO) is well-known to play an important role in regulating many physiological processes [61]. By combining with the nanoparticles to form a plurality of complexes, fluorescein derivatives (FL) can be used for the detection of NO. Jiang et al. synthesized replacement type probe (F37) via electrostatic interactions. Polyethylenimine-stabilized Fe_3O_4 magnetic nanoparticles were assembled with photosensitive nitric oxide (NO) donors, $Fe_4S_3(NO)_7^-$ (RBS) and CuFL complex consisting of fluorescein derivatives and Cu^{2+} to prepare Fe_3O_4–CuFL–RBS nanocomposites [61]. CuFL is responsible for fluorescence turn-off response and can be developed a probe for NO. CuFL reacts with NO and causing the remarkable fluorescence increase via nitrosation of secondary amine on ligands CuFL responds to NO selectively and yields N-nitrosated ligand (FL-NO). As prepared nanocomposites exhibited weak fluorescence and hardly released NO under light irradiation of 480 nm. The nanocomposites can release NO due to RBS photolysis under 365 nm light irradiation, while the released NO could conjugate with CuFL *in situ* to form FL-NO complex based on N-nitrosate reaction, which was verified by fluorescent increase of FL-NO (Fig. 37). The LOD was 6 nM and a linear range of 0.01–0.25 μM in PBS (10 mM, pH 7.0). Thus, Fe_3O_4–CuFL–RBS nanocomposites realized light-triggered NO release and turn-on fluorescence detection of NO *in situ*.

NO one-electron reduced and protonated product, nitroxyl (HNO) has recently been found to exhibit distinct chemical properties and biological functions [62]. Based on amide reaction of coumarin piperazine, 2-(diphenylphosphanyl) benzoic acid and 5(6)-carboxyfluorescein, fluorescein probe can be used for the detection of HNO. Zhang et al. synthesized a ratiometric fluorescein probe (F38) [62]. In the FRET system fluorescein is an acceptor and coumarin is a donor. The reaction between phosphine and HNO generated aza-ylide intermediate. Aza-ylide will attack the adjacent electrophilic ester in an intramolecular manner, leading to the release of alcohol and "turn-on" fluorescence at 517 nm (Fig. 38) [62]. The color of the solution changed from blue to green after the addition of HNO. There was a linear range of 0–100 μM in PBS buffer (10 mM, pH 7.4) with LOD 1.4 μM. Bioimaging studies also demonstrated that F38 can be used to detect HNO in HeLa cells.

Boronate fluorescein probes have emerged recently as a versatile tool for the detection of reactive oxygen and nitrogen species. Sikora et al. synthesized a reactive fluorescein mono-boronate

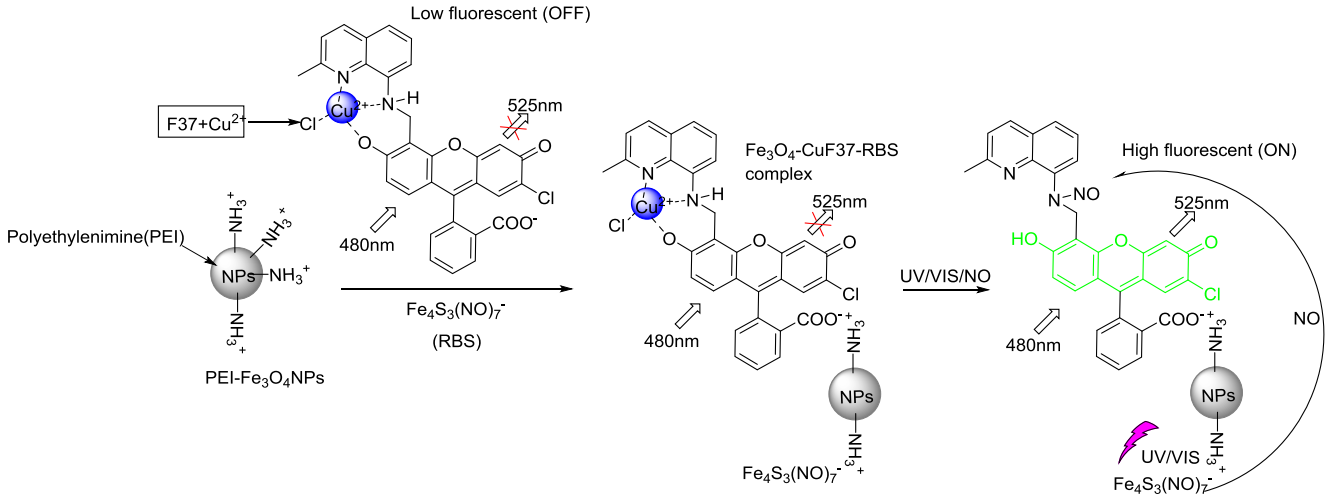

Fig. 37. Fluorescein probe F37 for NO detection.

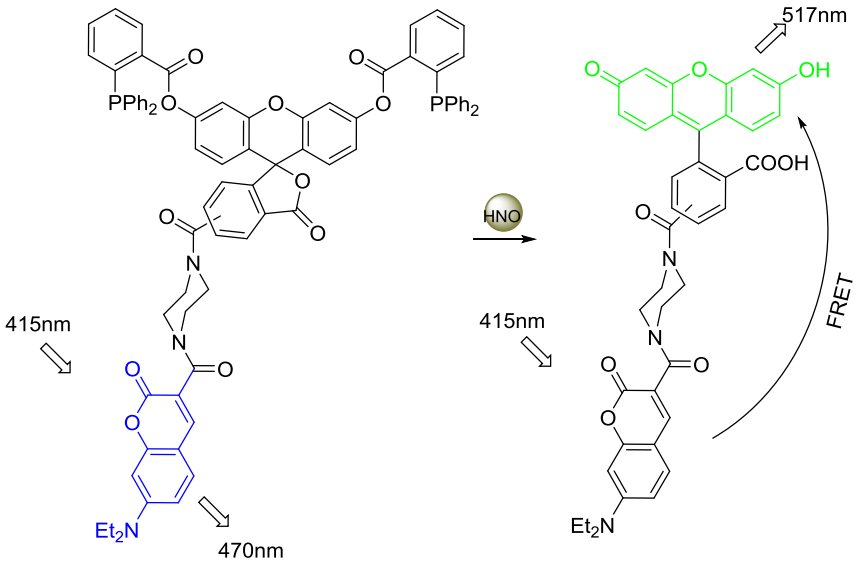

Fig. 38. Fluorescein probe F38 for HNO detection.

probe (F39) [63], a 4-(pinacol boronate) benzyl derivative of fluorescein methyl ester. Kinetics analysis of the oxidative conversion of F39 has shown that F39 oxidation is a two-step reaction. The first step is reaction of oxidant with boronate group leading to the corresponding phenol (Fig. 39). The second step is quinone methide elimination leading to fluorescence intensity enhancement at 518 nm. F39 can be used to detect $OONO^-$ in EAhy.926 cells culture experiments.

3.3.3. *Fluorescein probes for reactive oxygen species*

Reactive oxygen species (ROS) including H_2O_2, $O_2^{\cdot-}$, and OH•, which are important signal transduction molecules can be used in a wide range of physiological functions to regulate but excessive will lead to oxidative stress [64]. Fluorescein modified by organic synthesis at 3, 4 site can be used for detection of ROS.

It is difficult to detect H_2O_2 with conventional probes due to the low concentration and short lifetime of H_2O_2. Ratiometric fluorescein probes allow a ratio of the fluorescence intensities at two wavelengths, which provides an accurate and efficient method for the intracellular detection of H_2O_2. Qiao et al. synthesized a ratiometric probe (F40) via self-assembled polymeric nanoprobes for FRET-based ratiometric detection of mitochondrial H_2O_2 in living cells [64]. F40 was consisting of a self-assembled polymeric micelle as the carrier, tetraphenylethene (TPE) as the donor, fluorescent boronate as the H_2O_2-responsive acceptor and triphenylphosphonium as a mitochondria-targeted moiety. In the presence of H_2O_2, the reaction of F40 with H_2O_2 transformed the boronates into phenols with concomitant lactone opening, resulting in a green fluorescent fluorescein product with an absorption band of 400–525 nm (Fig. 40). The LOD was 0.95 μM in HEPES buffer (20 mM, pH 7.0). The F40 can detect both exogenous and endogenous mitochondrial H_2O_2 changes in living cells.

Based on dehydration condensation reaction, aryl-triflate was connected with fluorous substituted in 3 site, which can be used to detect $O_2^{\cdot-}$. Hu et al. synthesized a reactive fluorescein probe (F41) [65] which could be induced to be a ring-open structure by ester bond hydrolysis reaction via $O_2^{\cdot-}$ and aryl-triflate, causing the fluorescence enhancement at 534 nm (Fig. 41). The LOD was 23 nM with linear range of 0–12 μM in potassium phosphate buffer (0.1 M, pH 7.4, with 0.1% DMF). Additionally, F41 can be efficiently applied to visualize $O_2^{\cdot-}$ in the intact live zebrafish embryos.

3.3.4. *Fluorescein probes for hydrogensulfide*

Hydrogensulfide (H_2S), a kind of toxic gas, is endogenous gas transmitter [66]. Fluorescein probes can be designed through the mechanism that H_2S promotes the hydrolysis of ester bond, which leads to the π-conjugation recovering and change of fluorescence (Fig. 42). Due to high membrane permeability of the esterified probes in 3 site, it is a significant breakthrough for visualizing endogenous H_2S within living biological samples and opens up further opportunities to study its cellular biochemistry. Wu et al. synthesized a reactive fluorescein probe (F42) based on substitution reaction between hydroxyl groups of fluorescein and 2-thiophenecarbonyl chloride [67]. F42 demonstrates high selectivity and sensitivity with a linear detection range from 20 to 100 μM of H_2S in PBS buffer (20 mM, pH 7.4, with 1% CH_3COCH_3) and biological bovine plasma systems.

The introduction of the ortho aldehyde group shifted the traditional intermolecular thiolysis reaction to an intramolecular reaction, which accelerating the nucleophilic reaction. Zhang

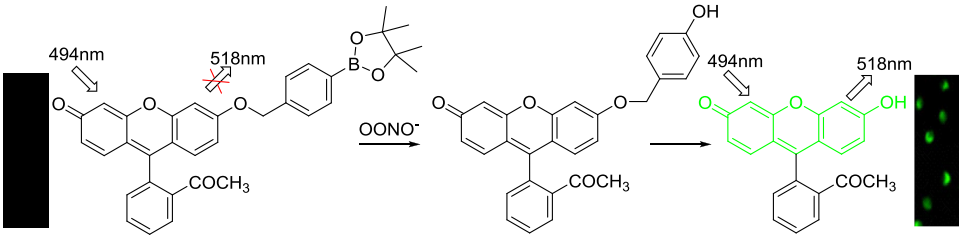

Fig. 39. Fluorescein probe F39 for $OONO^-$ detection.

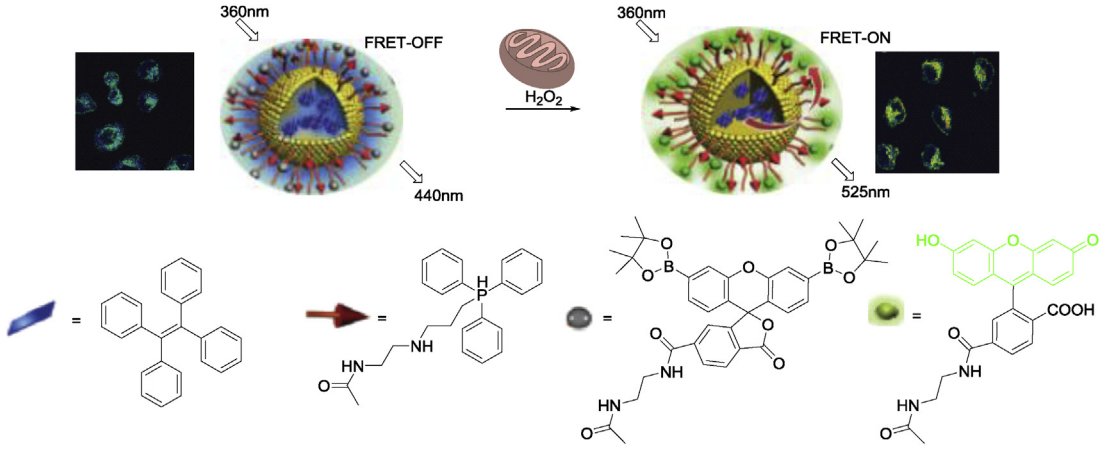

Fig. 40. Fluorescein probe F40 for H_2O_2 detection.

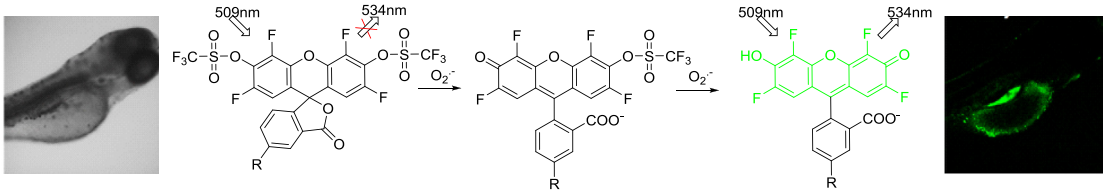

Fig. 41. Fluorescein probe F41 for $O_2^{\cdot-}$ detection.

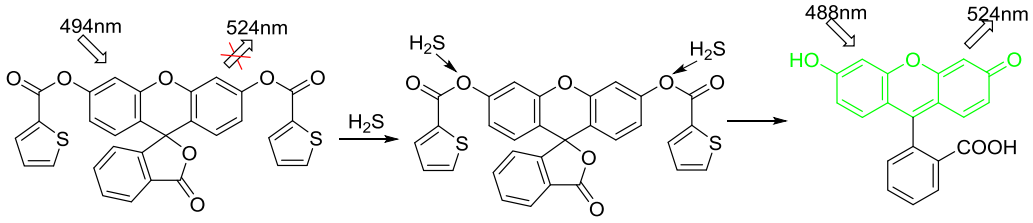

Fig. 42. Fluorescein probe F42 for H_2S detection.

et al. synthesized a reactive fluorescein probe (F43) based on substitution reaction between hydroxyl groups of fluorescein dicarboxaldehyde and 2, 4-dinitrobenzene sulfonyl chloride in 3 site [68]. F43 could be induced to be a ring-open structure by nucleophilic reaction between benzenesulfonate and H_2S which caused the fluorescence enhancement at 510 nm and the color of the probe solution turned to yellow (Fig. 43). The LOD was 0.024 μM with a near-linear on the range of 0–10 μM in HEPES buffer (50 mM, pH 7.4). Because of the presence of ester bond, the probe has good membrane permeability. Confocal laser scanning micrographs of HeLa cells incubated with F43 confirmed that F43 is the cell permeable and can successfully detect H_2S in living cells.

3.3.5. *Fluorescein probes for hydrazine*

Hydrazine (NH_2NH_2) is extremely toxic and can be easily absorbed by oral, dermal and inhalation exposure routes [69]. The fluorescein probe for detection of NH_2NH_2 is very rare. The fluorescein probe detects NH_2NH_2 because it can promote the hydrolysis of ester bond, which leads to fluorescence changes. Li et al. synthesized a reactive fluorescein probe (F44) [69] based on condensation reaction with fluorescein and *p*-nitrobenzoic acid in 3 site. F44 exhibited high selectivity and excellent sensitivity towards hydrazine with a detection limit as low as 46 nM in PBS–DMSO (1:4, v/v, 10 mM, pH = 7.4) solution. F44 selectively reacts with NH_2NH_2 in a physiological environment, leading to a fluorescence enhancement response along with the color changed from colorless

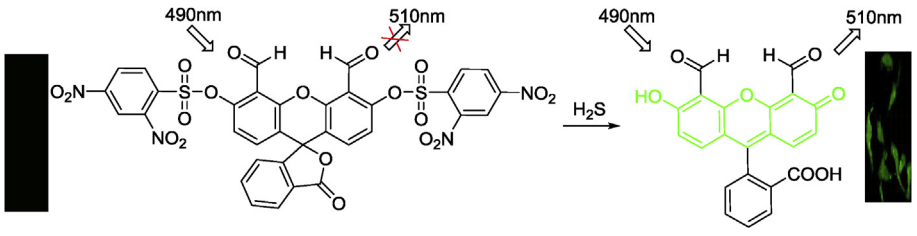

Fig. 43. Fluorescein probe F43 for H_2S detection.

to yellow, allowing colorimetric detection of hydrazine (Fig. 44). Furthermore, F44 was successfully applied for visualizing hydrazine in living cells.

3.3.6. Fluorescein probes for inorganic phosphate

(Inorganic phosphate) Pi is ubiquitous in biological systems. The increasing of the level of Pi has been implicated with abnormal renal function. Meng et al. synthesized a bonding-signaling type fluorescein probe (F45) [70] based on Schiff base reaction between fluorescein monoaldehyde and aminopyridine in 2 site. Then the compound can combine Fe^{3+} ions through the oxygen site of fluorescein and nitrogen site of aminopyridine constitute F45–Fe^{3+}. Fluorescence of F45 was effectively quenched due to the paramagnetic nature of Fe^{3+} ions, and the decomplexing of Fe^{3+} in the presence of Pi could lead to a fluorescence restoration (Fig. 45). Thus, the non-fluorescence F45–Fe^{3+} could be employed as the platform for the detection of Pi. The detection limit of 300 nM with near-linear in the range of 0–2.5 μM for Pi in HEPES buffer (THF:H_2O = 3:7, v/v, 20 mM, pH = 7.4). This probe provided a new method for the Pi detection in aqueous solution and living system.

3.3.7. Fluorescein probes for adenosine triphosphate

Adenosine triphosphate (ATP), as a major carrier of chemical energy in living species, is a mediator of chemosensory transduction in the central nervous system [71]. Aptamers are short, single-stranded artificial DNA that exhibit high affinity specifically to target molecules, which was considered to be effective detection and diagnostic tools [71]. Wen et al. synthesized a fluorescein probe (F46) [71] via exonuclease III (Exo III)-aided amplification assay based on a graphene platform for sensitive detection of adenosine triphosphate (ATP) [71]. This system consists of FITC-labeled ATP aptamers, graphene and Exo III. In the presence of target ATP, the target molecule binding the aptamer leads to the formation of aptamer target complexes. Subsequently, Exo III selectively catalyzes the stepwise removal of mononucleotides from aptamer target complexes, resulting in the removal of FITC and the release of target ATP. The released target ATP then binds with another FITC-labeled ATP aptamer probe, and the cycle starts a new, resulting in the continuous cleavage of aptamer target complexes. Finally, graphene is added to quench the fluorescence of rudimental FITC-labeled aptamer probes, and the fluorescence from the free FITC is detected (Fig. 46). The LOD is 5 nM with a linear range of 10–1000 nM. The present method was successfully applied for the detection of ATP in human serum.

3.4. Fluorescein probes detection of biological macromolecules

Biological macromolecules play an important role in biological processes, lack or excess will cause some problems in physiology [72]. Due to fluorescein phenolic hydroxyl groups connected

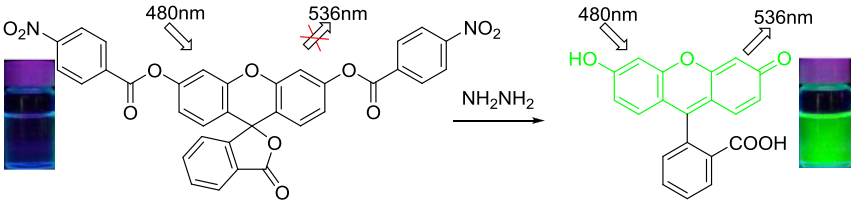

Fig. 44. Fluorescein probe F44 for NH_2NH_2 detection.

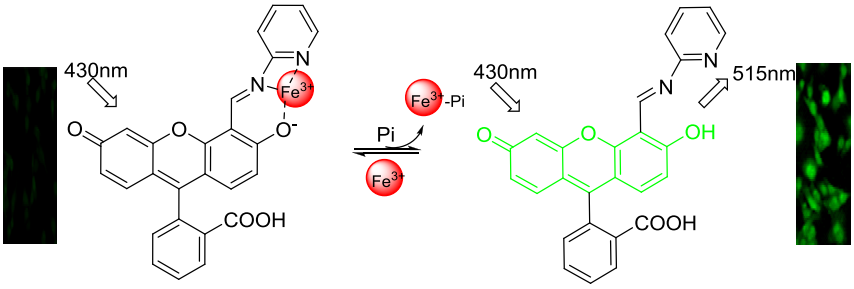

Fig. 45. Fluorescein probe F45 for Pi detection.

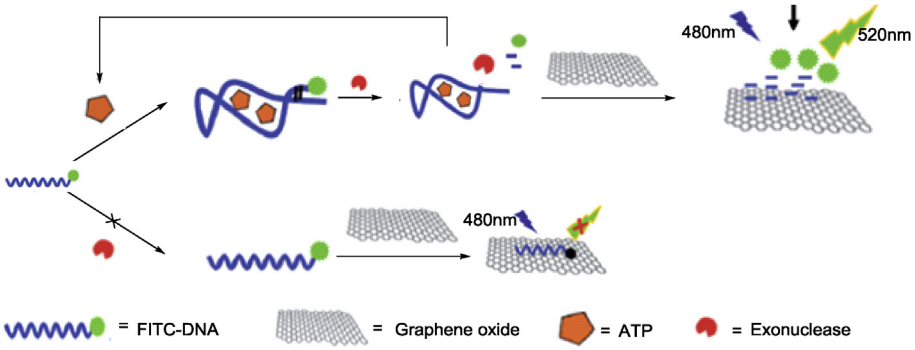

Fig. 46. Fluorescein probe F46 for ATP detection.

organism that can be hydrolyzed by enzymes, causing a fluorescence recovery. This mechanism can be used to detect enzymes. Chen et al. synthesized a kind of reactive fluorescein probe (F47) [73] based on the connection between cephalosporin and Tokyo Green fluorescein via benzyl ether linker in 3 site. The cephalosporin structure in F45 could be recognized and hydrolyzed by Bla (β-lactamase). The opening of the β-lactam ring would trigger spontaneous fragmentation, which leads to the cleavage of the ether bond at the 3′-position and subsequent release of free Tokyo Green, with a fluorescence emission at 525 nm (Fig. 47). The recovery of the fluorescence of Tokyo Green indicates the existence of the Bla. This approach allows rapid screening of a broad spectrum of Blas in real milk samples within 15 min without any pretreatment. Combined with the immuno-magnetic separation, it achieved sensitive and quantitative detection of Bla (10–5 U/mL), which provides a universal platform for screening and determining Blas in complex samples with high efficiency and accuracy.

Aggregation-induced emission (AIE) dots as the FRET donors can avoid the limitations of the donors consisting of aggregation-caused quenching fluorophores in nanoparticle based FRET systems. Wu's group discovered a ratiometric fluorescein probe (F48) [74] for carboxylesterase (CaE) detection. Upon enzymatic reaction, electrostatic interaction between the cationic TPE-N+ dots and the enzymatic reaction product the negatively charged fluorescein molecules allows the FRET process to proceed, thus affording the ratiometric fluorescence CaE assay, causing the fluorescence enhancement at 520 nm (Fig. 48). The detection limit was 0.26 U/L with a linear range of 0–50 U/L in PBS (1 mM, pH 7.4). This strategy may provide a new and effective approach for establishing new FRET systems and developing other enzyme assays.

4. Conclusion

This article reviewed fluorescein probes in the past two years according to classification of different analytes including metal ions, anions, small molecules and biological macromolecules. Functional fluorescence probes based on the fluorescein structure are available for modification by organic synthesis at five positions: the xanthene ring 2, 3, 5 sites and the benzene moiety 1, 4 sites. Schiff base fluorescein probes are achieved through fluorescein derivatives based on Schiff base reaction in benzene moiety (1 site) and xanthene ring (2 site). Due to the high activity of the Schiff base, fluorescein probes can provide more binding sites, which can improve the sensitivity for detection of ions. The probes which are

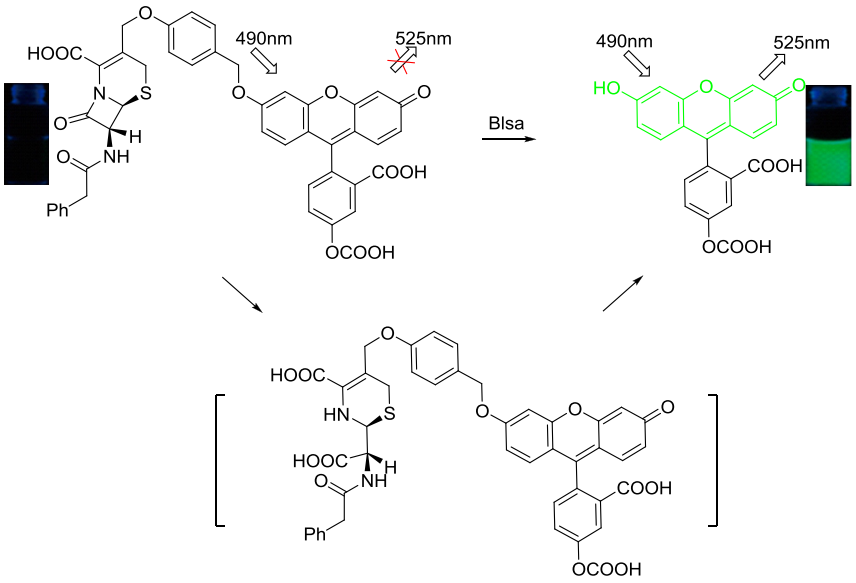

Fig. 47. Fluorescein probe F47 for BLa detection.

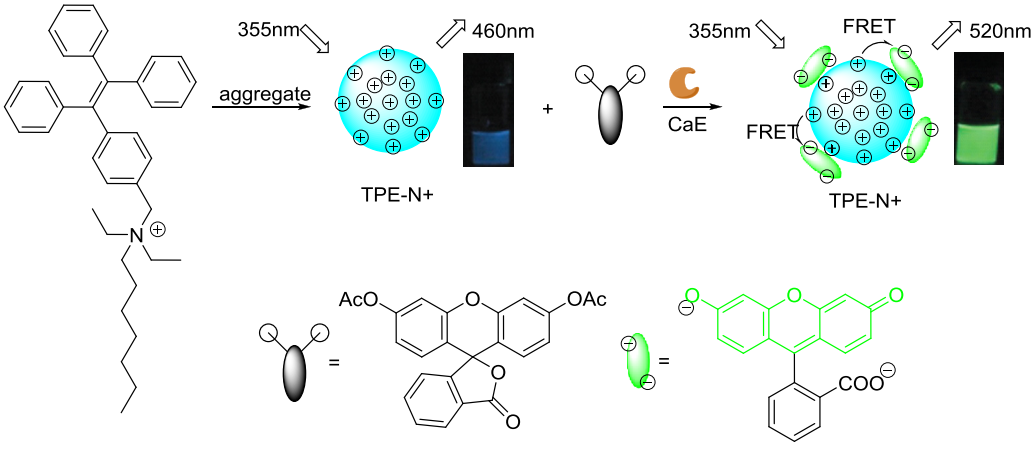

Fig. 48. Fluorescein probe F48 for CaE detection.

used for the detection of ions have the advantages of simplicity, high efficiency and excellent selectivity. Lipophilic fluorescein probes are synthesized through two phenolic hydroxyl groups of fluorescein based on esterification or alkylation connecting with small organic molecules in xanthene ring 3 site, which can be used for the detection of small molecules and biological macromolecules. The probes have the advantages of wide range of detecting substances and good permeability of the cell membrane, and they have a practical value in quantitative detection of toxic substances in biological systems and imaging. Fluorescein connected with other fluorophores or AuNPs in benzene moiety 4 site, which can build ratiometric fluorescein probes or AuNPs fluorescein probes separately. Ratiometric fluorescein probes allow a ratio of the fluorescence intensities at two wavelengths, and are turned out to be an accurate and efficient method for detection of analytes. AuNPs fluorescein probes are better than the traditional organic fluorescein probe and has low toxicity.

At the same time, fluorescein probes still have some problems that limit their further applications, such as the recognition process needs to be done in the solution with high organic solvent content. The toxicity of the organic solvent leads to the destruction of the biological system, and the detection accuracy of the bonding-signaling type probes is not as good as that of the reactive probes. Most of the detected analytes focus on the ions and the detection of organic small molecules, biological macromolecules are relatively few. Therefore, the direction of future research is to develop practical reactive probes which can be used for detection of small molecules and biological macromolecules in water. It has important scientific significance and application prospect in the field of industrial production of disease diagnosis and treatment.

Acknowledgments

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