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FEATURE ARTICLE

Fluorescent molecular probes for the detection of chemical warfare agents and their mimics

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Owing to their direct toxic effects on human beings, animals, and plants, chemical warfare agents (CWAs) and their mimics have become widespread in chemical warfare and agriculture. The considerable concerns about their entry into biological systems and the residues in environment stimulate the development of rapid and sensitive methods for the detection and analysis of this family of compounds. In the progress of sensitive, selective, and fast responsive detection, fluorescent molecular probes have been widely used in the detection of CWAs in recent years. Here the recent reports on the design of fluorescent molecular probes and their advantages in the detection of CWAs were reviewed. Furthermore, the extensive interests accelerate the development of novel fluorescent molecular probes and detection techniques in this field.

Keywords chemical warfare agents, detection, fluorescent, molecular probes

1 Introduction

During the past decades, chemical warfare agents (CWAs) and organophosphates (OPs) pesticides have become widespread in chemical warfare and agriculture, owing to their high toxicity to the living organisms [1]. The severe toxicity of this family of compounds stems from their inhibition effect on the acetylcholinesterase, which induces the excessive accumulation of the acetylcholine and then causes fatal damage on the nervous system. Therefore, these compounds have become one of the most serious threats for lives and environment [2]. The considerable concerns about their entry into biological systems and the level of residues in environment stimulate the development of sensitive and selective detection and analysis of these chemical species. The molecular structures of several CWAs and their primary mimics are shown in Figure 1.

With the development of new techniques, the methods based on gas chromatography-mass spectrometry [3], high-performance liquid chromatography [4], electrochemistry [5,6], surface acoustic wave devices [7], enzymatic-based sensors [8–10], interferometry [11], and molecularly imprinted polymer (MIP) sensors [12] have been applied in the field of detection. However, the requirements of skilled training and large instrumentation in laboratory restrict their applications. On the other hand, by virtue of the rapid response, high sensitivity, low detection limit, and easy operation of the fluorescence property and characterization, the fluorescent method based on the design of fluorescent molecular probes has been extensively applied in the detection of highly toxic matters [13]. It has also been introduced into the detection of CWAs these years.

Based on the unique molecular structures of the CWAs, the electrophilic phosphorylation is usually utilized in the detection and analysis processes. Therefore, many fluorescent molecular probes applied in the detection of CWAs have been designed and synthesized. Generally, the phosphorus atom in the CWAs is electrophilic, and the P-X (X = F, Cl) bond can be cleaved by the nucleophilic reagents, hence the fluorescent molecular probes are usually composed of a nucleophilic receptor, a reporter, and sometimes a linker. The nucleophilic receptor (usually amino and hydroxyl groups) can selectively and sensitively recognize the target molecules. And the reporter usually is a fused-ring aromatic moiety (i.e. anthracene and pyrene) that provides the variety of fluorescent signals when the reaction between the fluorescent molecular

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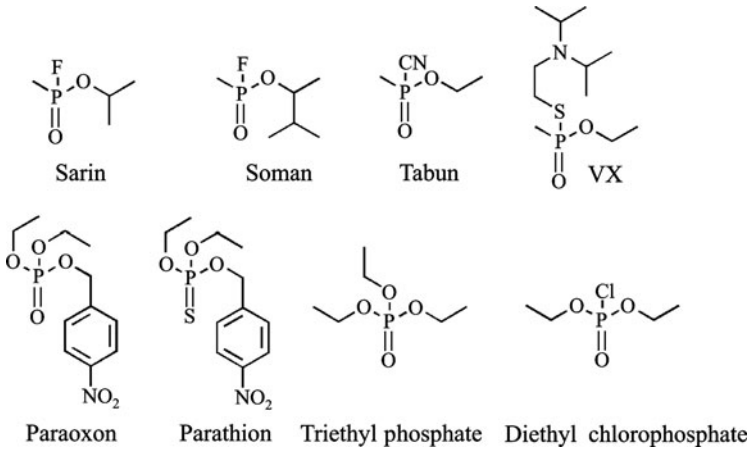
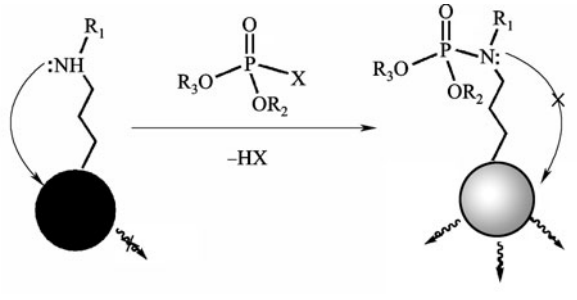


Figure 1 Molecular structures of several CWAs and their primary mimics.

probe and the target molecule takes place. And then the linker affords the association and the electronic communication between the receptor and the reporter. Following the reaction between the nucleophilic fluorescent molecular probes and the electrophilic phosphorous atom in the CWAs, the electronic transition between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of the reporter is modulated, and then the change of the emission wavelength and intensity results in the corresponding detection of the CWAs. Here, we overview the development of the fluorescent molecular probes for the detection of the CWAs and their mimics.

2 Fluorescent molecular probes for the detection of the CWAs in the solution, microbeads, and films

As the reaction of electrophilic substitution can easily take place when the nucleophilic reagents are present in the electrophilic system of CWAs, many fluorescent molecular probes based on the mechanism of electrophilic phosphorylation were designed and reported (Scheme 1). The alteration in the energy levels of HOMO and LUMO before and after electrophilic phosphorylation by CWAs generally induces the change in the intensity of the absorption and emission. Moreover, the fast response towards CWAs of these fluorescent molecular probes suggests the vital merit of this approach.



Scheme 1 The mechanism of the electrophilic phosphorylation between the CWAs and fluorescent molecular probes.

The first example that combines the superiority of this mechanism and the fluorescent molecular probes introduced into the detection of CWAs was developed by Meador's group (Figure 2) [14]. In N,N-bis(p-aminophenyl)-1,5-bis(p-(tetraethyleneglycoloxy)-phenyl)-anthracene-2,3,-6,7-tetracarboxyl bisimide **1**, the anthracene moiety acts as the reporter, and the amino group functions as the receptor. In DMF solution, the reaction between the amine and the acyl halides, such as acetyl chloride, thionyl chloride, and other mimics of CWAs, provides the dramatic enhancement of the fluorescence intensity at about 500 nm with excitation at 425 nm, which is ascribed to the suppression of the photo-induced electron transfer (PET) from the nitrogen atom to the anthracene. Additionally, as dispersed on a silica support, **1** shows the similar detection behavior towards gas-phase CWA mimics, which demonstrated its potential application as a solid-state sensor.

Another fluorescent molecular probe, fluoresceinamine **2** with the desired reactivity and fluorescence properties for the detection of CWAs, was reported by Walt's group (Figure 3) [15]. Upon reaction with the acyl and phosphoryl halides at pH 7.5 in the aqueous solution, the electron density of the nitrogen atom is decreased due to the electron withdrawing property of the phosphoryl group. Therefore, the original PET quenching effect on the fluorescein is inhibited, and the fluorescence is significantly enhanced. Specifically, the fluorescence enhancement was ascribed to the conversion of fluoresceinamine into phosphoramide when diethyl chlorophosphate (DCP) was introduced into the system. Furthermore, by adsorbing **2** onto the carboxylate-functionalized

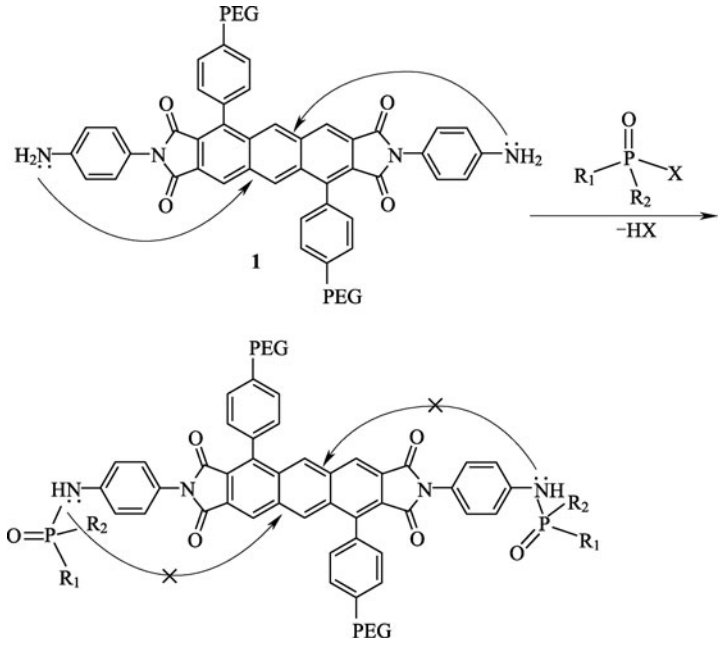


Figure 2 The molecular structure of probe **1** and the mechanism of detection.

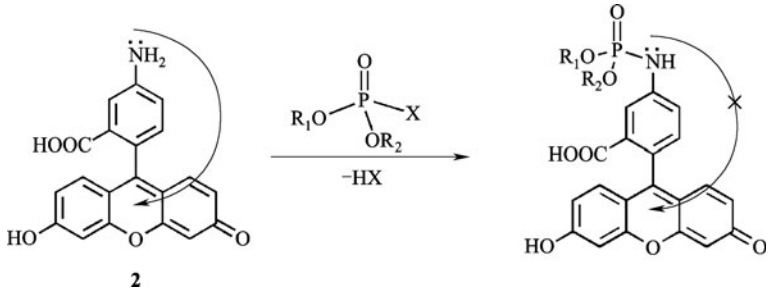
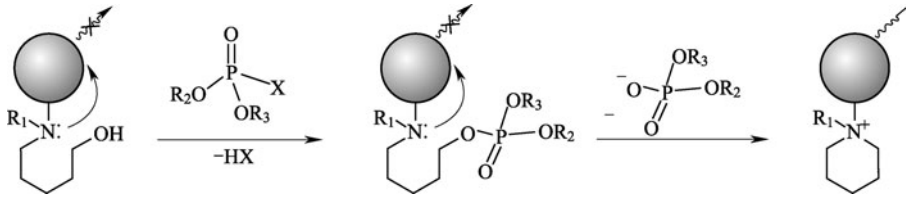


Figure 3 The molecular structure of probe **2** and the mechanism of detection.



Scheme 2 The mechanism of the electrophilic phosphorylation and elimination between the CWAs and fluorescent molecular probes.

polymer microbeads coated with poly(2-vinylpyridine), the fluorescent microbeads were obtained. Therefore, the fabrication of the probe **2** into microbeads can serve as an integrated microarray platform, which is a promising approach to develop a commercial sensor for in-situ detection.

The high reactivity of the phosphorylation ensures the sensitivity of this detection method. However, the detection is usually interfered by inorganic and organic acids since protons can also bind with the amino group. In order to rule out the influence of protons and thus to improve the selectivity, the reactions of electrophilic phosphorylation and then intramolecular cyclization are regularly utilized in the design of the fluorescent molecular probes for the detection of CWAs. Usually, there is a nucleophile group (hydroxyl and/or amine) as the receptor of the CWAs and a tertiary amine together with an appended fluorophore as the reporter in the fluorescent molecular probes (Scheme 2). When CWAs are introduced into the system, the hydroxyl group located near the tertiary amine is acylated, and then a rapid intramolecular N-alkylation takes place, which converts

the tertiary amine into a quaternary ammonium salt. If protons are introduced, they will attach on the tertiary amine and thus prohibit the cyclization. Due to the feasibility of the intramolecular cyclization after activation by the phosphorylation, the π-conjugation system of the fluorescent molecular probes will be extended, and the absorption and emission bands will be significantly bathochromically shifted. Furthermore, the improved rigidity of the fluorescent molecules will result in notable suppression of the non-radiative relaxation pathways, affording the improved sensitivity and selectivity. Many excellent results have been obtained based on this mechanism.

The first example exploiting this reaction into the detection of the CWAs was reported by the Pilato's group (Figure 4) [16]. The new probe **3**, a 1,2-enedithiolation platinum(II) complex with an appended alcohol, was designed and synthesized. Upon activated phosphates esters, **3** is converted to $[(dppe)Pt\{S_2C_2(CH_2CH_2\text{-}N\text{-}2\text{-}pyridinum)\}]^+$ (dppe = 1,2-bis(diphenylphosphino)ethane), producing the emission at 605 and 710 nm in the dichloromethane solution excited at 450 nm, which is assigned to the intraligand charge transfer from the thiolate to the heterocycle π^*. Furthermore, **3** was immobilized in the cellulose acetate/triethylcitrate films, and its response towards the phosphate esters was monitored. The optimal linear behavior between the intensity of the emission and the exposure time was obtained by modulating the concentration of the plasticizer. Therefore, the detection processes in the film afford improved feasibility for the in-situ detection of CWAs.

Following the similar approach, the fluorescent molecular probes **4**, **5**, and **6** were designed by the Swager's group (Figure 5) [17]. The molecular design was based on the intramolecular cyclization reactions between the fluorescent molecular probes and CWAs to form the high delocalized systems. Due to the unfavorable cyclization of the thiophene and pyridinium in the probe **4**, probe **5** employed a phenyl ring instead of thiophene. The charge transfer between the phenyl group and the pyridinium and the favorable stereochemical orientation made the original design available in **5**. The emission intensity was remarkably enhanced upon the addition of DCP or diisopropylfluorophosphate (DFP) in the dichloromethane solution, which was ascribed to the extended delocalization system and the reduced degree of rotational freedom between the two rings. In order to improve the sensitivity of the detection, probe **6** was designed with a rigid naphthalene subunit instead of the phenyl group to promote

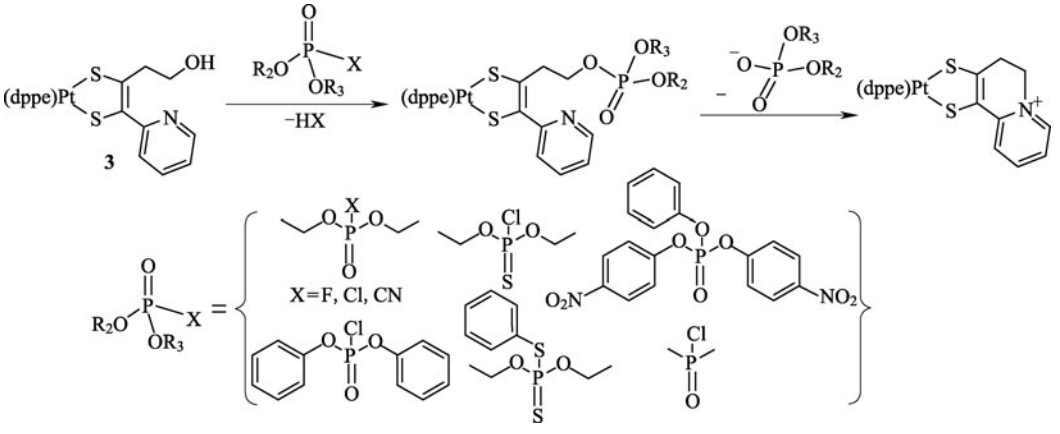


Figure 4 The molecular structure of probe **3** and the mechanism of detection.

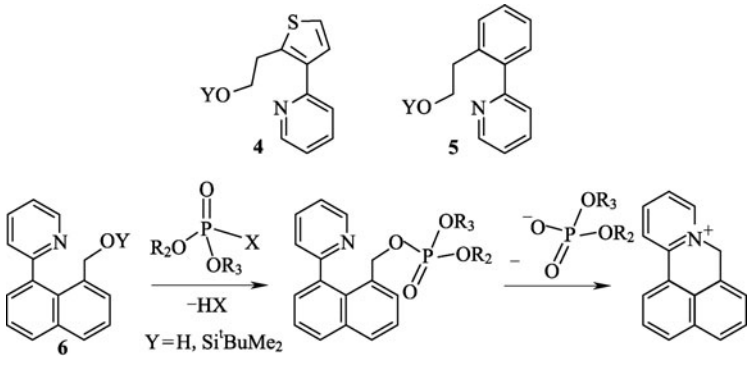


Figure 5 The molecular structure of probes **4**–**6** and the mechanism of detection.

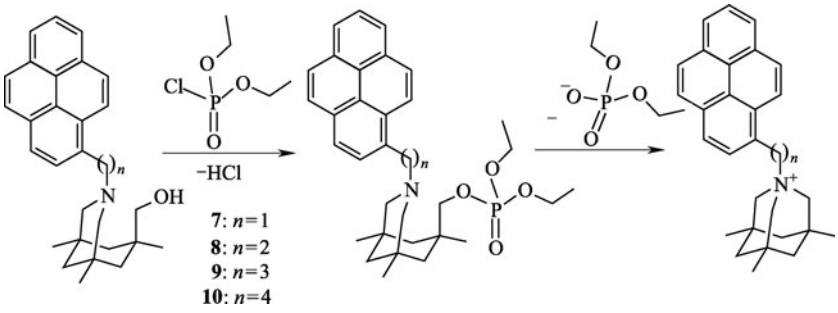


Figure 6 The molecular structure of probes **7**–**10** and the mechanism of detection.

the reaction rate to the CWAs. In addition, it was utilized to prepare a cellulose acetate film to detect DCP and DFP. The maximum emission wavelength shifts from 375 to 438 nm upon the cyclization within 5 minutes.

The fluorescent molecular probes **7**–**10** were designed and synthesized by Rebek's group (Figure 6) [18]. The receptor, i. e., hydroxyl group, was located in vicinity to the tertiary amine, which facilitated the intramolecular N-alkylation reaction. The fluorophore (pyrene) was linked to the nitrogen atom by the alkyl chains ranging from one (**7**) to four methylene units (**10**). The length of the alkyl chains brings significant influences in the detection of CWAs. With only one methylene linking the recognition unit and the reporter unit in probe **7**, the intermolecular substitution between the hydroxyl and DCP proceeds smoothly, and then the N-alkylization reaction causes the bonding of the lone-pair electrons in the amine, which brings a remarkable enhancement of the emission. With the increase of the distance between the nitrogen atom and the pyrene group, the emission of pyrene is less interfered by the lone-pair electrons on the nitrogen atom, and the detection performances of the probes **8**–**10** are not as prominent as that of **7**.

Using 2-(2-(dimethylamino)phenyl)ethanol as a building block, the molecular probe **11** (Figure 7), an azo dye, was designed and synthesized for the detection of CWAs [19]. As in the typical absorption spectra of azo dyes, **11** showed an absorption band at 410 nm which originated from the

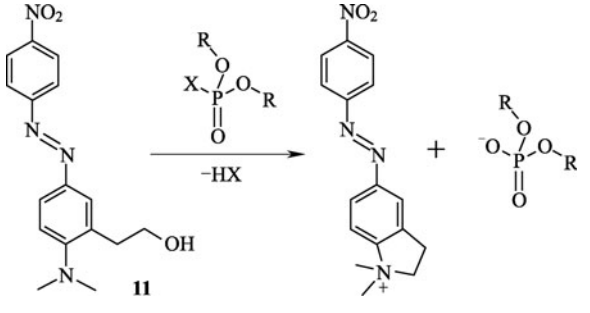


Figure 7 The molecular structure of probe **11** and the mechanism of detection.

charge-transfer from the aminophenyl to the nitrophenyl moiety. After the addition of DCP, the absorption band was hypsochromically shifted to 325 nm as a result of the anilinium group generated through the intramolecular cyclization process which was confirmed by NMR spectra, and exhibited the weakened electron-donating ability than that of the initial aminophenyl group.

The above results showed us the favorable selectivity for the detection of CWAs. However, much more effort should be devoted to the design and synthesis of the fluorescent molecular probes with higher detection efficiencies.

As the oxime moiety is a supernucleophile which can actively react with the electrophilic phosphor moieties of the CWAs [20], and coumarin is an excellent fluorescent reporter which can be excited in the visible region, the fluorescent supernucleophile probe **12** (Figure 8)**,** functionalized by oxime and coumarin, was designed and synthesized by Anslyn et al. [21]. In order to avoid the interference of proton, a nitrogen-phosphorus super-base, i.e., P_4-*t*-Bu base, was introduced into the initial system when the fluorescent behavior was studied. As the lone-pair electrons of the oximate anion quench the fluorescence of the coumarin through the PET process, the emission efficiency of the original DMSO solution of **12** with excess P_4-*t*-Bu base was low. The mechanism was confirmed by the control experiment of the analogues. When the mimic of CWA DFP was introduced into the system, upon the phosphorylation of oximate, the energy level of its orbital with lone-pair electrons was reduced which resulted in the inhibition of the PET process and the recovery of the emission. In order to study the kinetics of the reaction, the flow kinetic experiments were carried out. The rate constant k (slope) and the half-life were 1410 s^{-1} and 50 ms, respectively, which imply a quite rapid detection process. On the basis of the same approach, several analogous molecular probes were designed and synthesized.

From the above results, the significant results can be obtained in both solution and solid substrates. Due to the potential catastrophic effect of CWAs on the lives and environment, our group also focuses on the design and

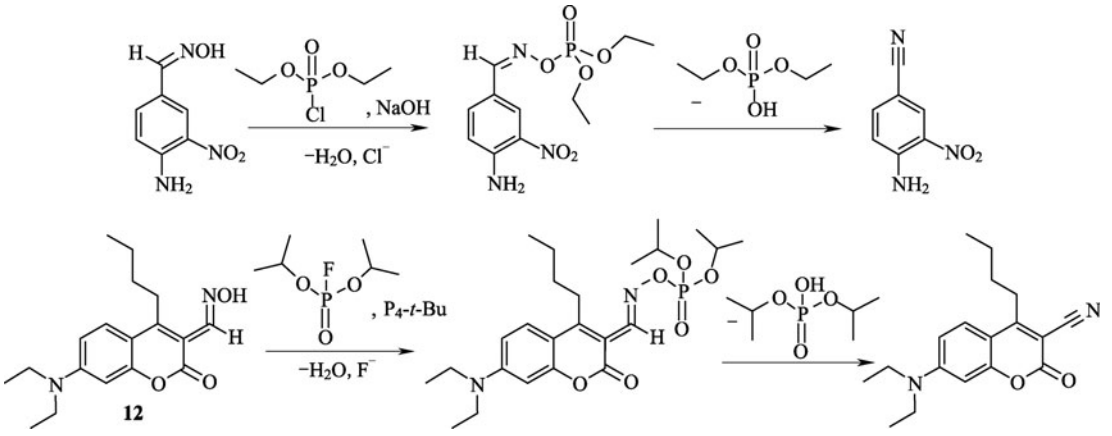


Figure 8 The molecular structure of probe **12** and the mechanism of detection.

synthesis of fluorescent molecular probes towards the detection of the CWAs and their mimics. A derivative of the fluorophore 5-methoxy-2-(2-pyridyl)thiazole (MPT), the fluorescent molecular probe 2-(4-aminophenylethylyl)-5-methoxy-2-(2-pyridyl)thiazole 13 bearing a nucleophilic amino group was designed and synthesized (Figure 9) [22]. When acylated by propionyl chloride in acetonitrile, MPTEA exhibits a significant hypsochromic shift from 520 to 435 nm in the emission, which is ascribed to the intramolecular charge-transfer (ICT) processes from the amino group to the pyridine ring upon the acylation at the amino group [23]. Furthermore, the similar fluorescent behaviors are also observed in the addition of diethyl chlorophosphate. Further studies in the derivatives of MPT for the detection of CWAs and their mimics in the solution and microfluidic chips are under progress and will be published elsewhere.

3 Fluorescent molecular probes for the detection of the CWAs incorporated with the lanthanide complexes

Supramolecular systems are also involved in the detection of CWAs. A series of luminescent europium(III) complexes incorporated with the technique of MIP have exhibited fine selectivity for the detection of CWAs (Figure 10). The dithiobenzoate substituted tris(β-diketonate) europium(III) **14** was designed for the MIP [24], with the europium(III) compound acting as the polymerization substrate and the reactive site for pinacolyl methylphosphonate (PMP), the hydrolysis product of Soman. The luminescence titration of **14** with PMP or other analogues showed excellent results in sensitivity and selectivity for CWA detection, which proved the validity of the molecular design.

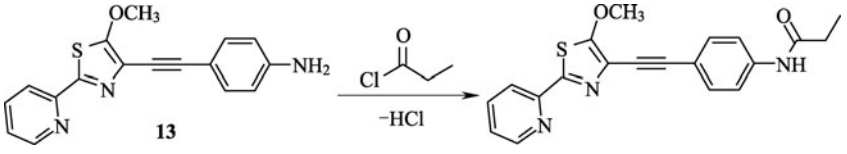


Figure 9 The molecular structure of probe **13** and the mechanism of detection.

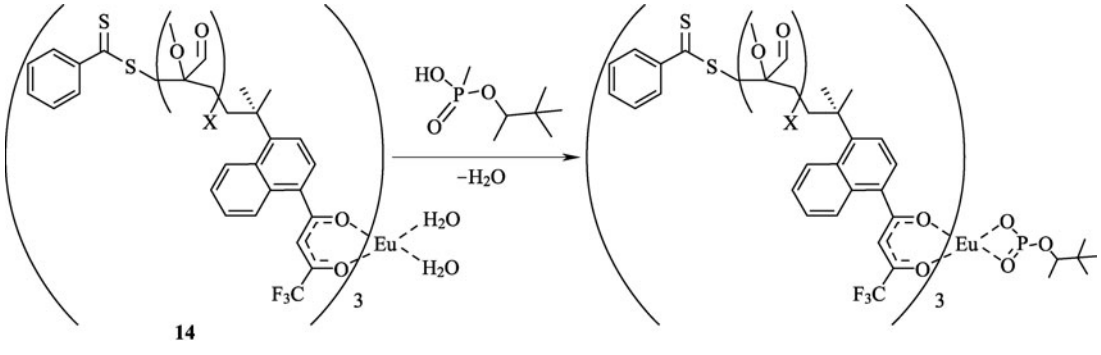


Figure 10 The molecular structure of probe **14** and the reaction mechanism of its detection for PMP.

Taking advantage of the binding affinity between the CWAs and the lanthanide ions, Rowan and Weder's group demonstrated a multifunctional sensor array for the metal cations and CWAs based on the fluorescent 2,6-bis(1'-methyl-benzimidazoly)pyridine ligands. The fluorescent molecular probes **15**–**18** are designed and synthesized to develop the metallo-supramolecular materials (Figure 11) [25]. Upon coordination with the La^{3+} ion, the fluorescence spectra of **15**–**18** showed a bathochromic shift from 380 to 420 nm and from 425 to 500 nm, respectively, together with a decreased emission intensity. However, when the aliphatic phosphate was introduced into the system, the original emission was recovered, affording the detection of CWAs. The sensor array based on the metal/ligand combination provided a simple and sensitive approach for the aliphatic organophosphates.

Similarly, another series of supramolecular fluorescent probes **19**–**27**, based on the affinity between terpyridine and lanthanide, was explored by Tew et al. (Figure 12) [26]. Because of the smaller binding constant between terpyridine and lanthanide, and the inherent binding between lanthanide and CWAs, the emissions of the terpyridine-lanthanide complexes at 620 nm were quenched by the SOS (Soman surrogate) or SAS-Cl (Sarin surrogate), but many other organophosphates have nearly no effect, indicating the notable selectivity of the current probes. Besides, the detection limit of the SAS-Cl is about 6 ppb, which is much lower than the immediately dangerous-to-life level of Sarin. Recently, by incorporating a derivative of pyrene imine into polystyrene films [27], the same group developed the optical films and fibers for the detection of CWAs.

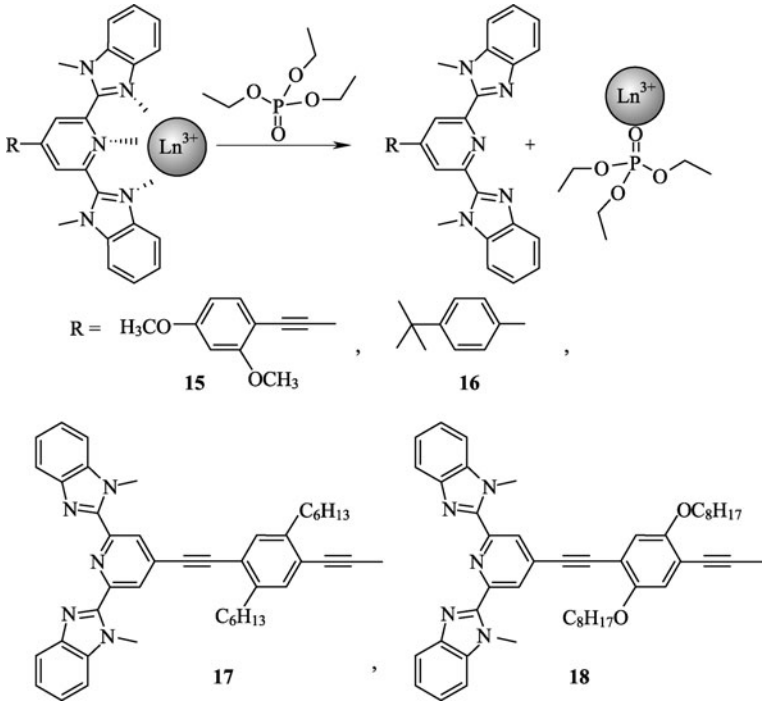
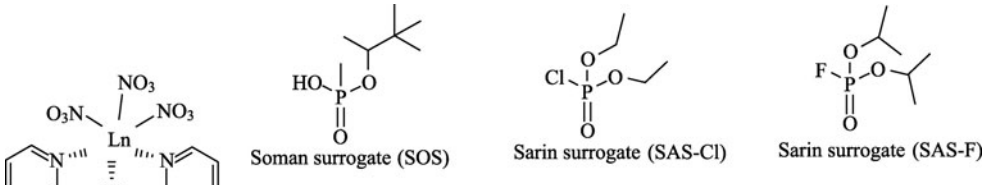


Figure 11 The molecular structures of probes **15**–**18** and the mechanism of detection.



19: R=H, Ln=Eu^{III}; **20**: R=H, Ln=Tb^{III}; **21**: R=H, Ln=Dy^{III};
22: R=$O(CH_2)_3CH_3$, Ln=Eu^{III}; **23**: R=$O(CH_2)_3CH_3$, Ln=Tb^{III};
24: R=$O(CH_2)_3CH_3$, Ln=Dy^{III}; **25**: R=MMA based polymer, Ln=Eu^{III},
26: R=MMA based polymer, Ln=Tb^{III}; **27**: R =MMA based polymer, Ln=Dy^{III}.

Figure 12 The molecular structures of probes **19**–**27** and the CWA mimics in the detection.

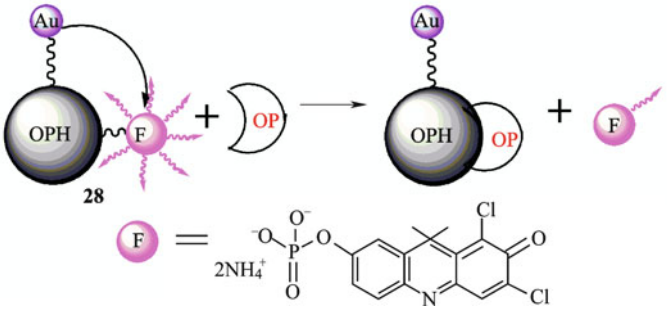
The series of surpramolecular probes based on the lanthanide ions provided us the material for the sensitive and selective detection of CWAs and their mimics. But the problems of incorporating proper organic moieties, simple synthesis processes, and easy operation should still be taken into our consideration.

4 Fluorescent molecular probes for the detection of the CWAs incorporated with gold nanoparticles

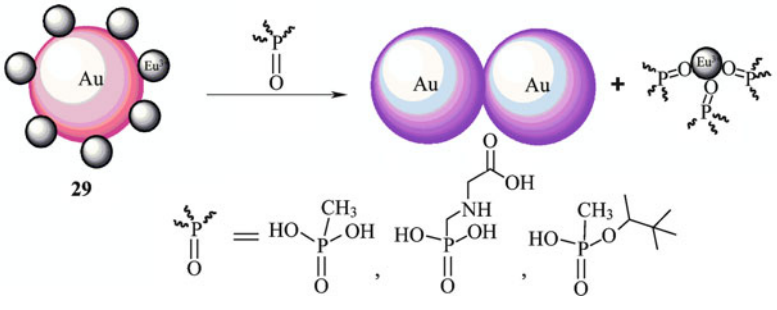
Recently, the research on gold nanoparticles has become one of the promising fields in the interdisciplinary study. Depending on the unique property of the surface enhancement of fluorescence, gold nanoparticles, as a part of supramolecular probe, have also been introduced into the fluorescent detection of CWAs (Scheme 3) [28]. Simonian et al. reported that, in the gold nanoparticle-attached supramolecular system **28**, there was a phosphate derivative of a fluorophore which was attached to the receptor of the CWAs (organophosphorous hydrolase, OPH). After the addition of the CWA mimics (paraoxon) into the system, the binding of the paraoxon with the OPH released the fluorophore from OPH, which results in the "turn-off" of the emission enhanced by gold nanoparticles in the initial state.

The molecular fluorescent probe **29** based on the surface enhancement of fluorescence of gold nanoparticle was presented by Ray's group (Scheme 4) [29]. The solution of Eu(III) ions exhibited five emission bands respectively at about 458, 535, 555, 590, and 615 nm upon the excitation at 395 nm. When the gold nanoparticles were introduced, the emissions at 535, 555, and 590 nm were quenched, but the emission intensity at 615 nm was increased. The emission of the fluorescent Eu(III) ions bound to the gold nanoparticles through the electromagnetic interaction exhibits a strong enhancement. While upon the addition of the CWAs mimics, the emission underwent a "turn-off" process which came from the release of Eu(III) ions from the gold nanoparticles and the inhibition of the surface enhanced fluorescence.

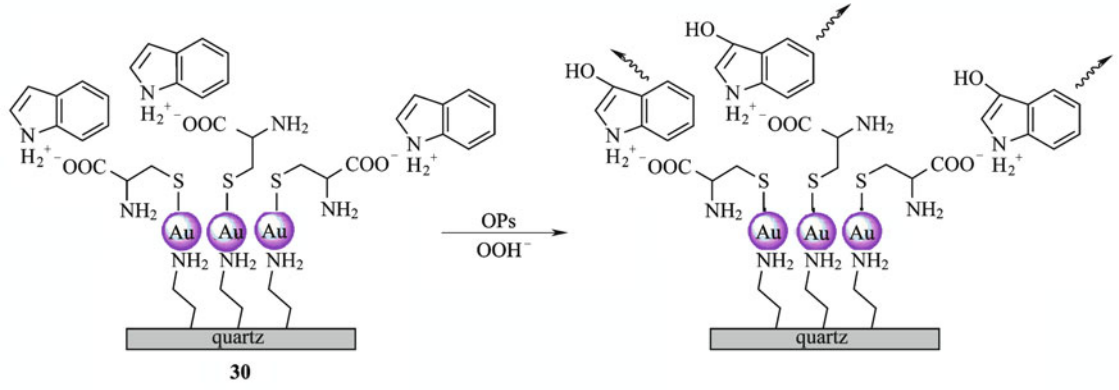
Sun et al. prepared a multilayer and interfacial sensing system for the detection of CWAs (Scheme 5) [30]. The self-assembled multilayer **30** was composed of the trialkoxysilane modified wafer, gold nanoparticles, and L-cysteine. The trialkoxysilane modified wafer was used to adsorb gold nanoparticles to amplify the signal during the detection, and L-cysteine was used to exert the electrostatic interaction with the molecular probe. The product of the nucleophilic substitution between the OPs and perborates oxidized the nonfluorescent indole into the fluorescent indoxyl unit. Therefore, the emission at 525 nm upon the excitation at 493 nm was observed. As the stable and portable self-assembled multilayers are easy to be obtained, it will become one of the promising methods for the in-situ detection.



Scheme 3 The structure of probe **28** and the mechanism of detection.



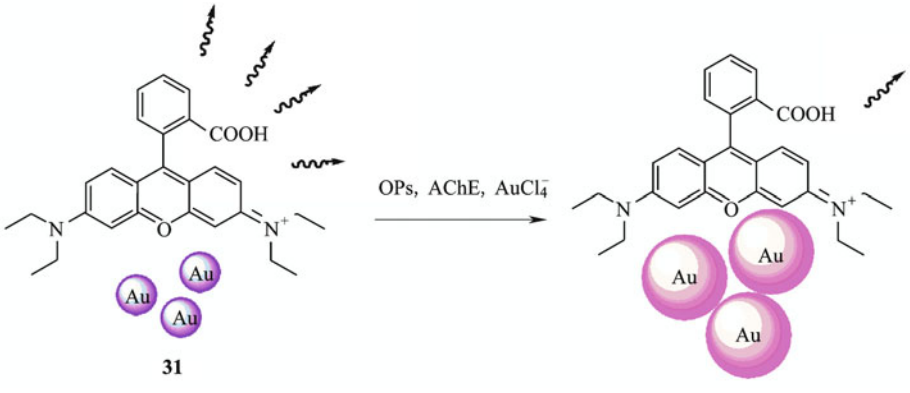
Scheme 4 The structure of probe **29** and the mechanism of detection of CWAs.



Scheme 5 The structure of probe **30** and the mechanism of detection.

Recently, a new mechanism with the system of Au NPs and fluorescent molecular probes **31** was developed for the detection of CWAs (Scheme 6) [31]. The absorption of Au NPs focuses at 515 to 530 nm, which depends on the size of the Au NPs. If the emission of fluorescent molecules (for example, fluorescein and rhodamine B) locates at the same wavelength range, the emission will be quenched by the process of fluorescence resonance energy transfer (FRET). Based on the reduction of the Au ions on the surface of the gold nanoparticles, the size of the Au NPs will be increased in order to modulate the absorption of Au NPs to match the emission of fluorescent molecules. As acetylthiocholine can react with acetylcholine esterase and produce thiocholine and then two thiocholine molecules react to produce a dithiocholine and one electron, the electrons reduce the Au ions to enlarge the Au NPs and then quench the emission of the fluorescent molecules. It provides us a novel approach to utilize FRET mechanism and Au NPs for the detection of CWAs.

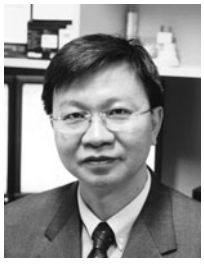
Cooperated with the gold nanoparticles and the ingenious designs, the novel strategies for the detection of CWAs have been developed. Not limited in the gold nanoparticles, many enlightening results were obtained depending upon the development of the advanced materials and technologies. Just as the result shown by Jiang and Xie [32], based on the acetylcholinesterase-acetylthiocholine enzyme catalytic reaction system, the thioglycolic acid capped CdS photoluminescent semiconductor nanocrystal without further modification provided a novel strategy for the high sensitive detection of CWAs. Furthermore, combined with the superiority of miniaturization and automation of the microfluidic devices, the detection of CWAs in blood was carried out by Nguyen et al. [33]. It will be a promising device that can be utilized for the in-situ detection, analysis, and treatment for the CWA disasters. Furthermore, through the combination with the microfluidic devices, the catalysis properties of the CWAs and their mimics in some unique reactions will still be our focus on the design of fluorescent molecular probes. Moreover, the recent result showed by Blum et al. revealed that applying the mechanism of enantioselectivity in the design of the fluorescent molecular probes for the detection of CWAs will also become a tendency [34].



Scheme 6 The structure of probe **31** and the mechanism of detection.

5 Conclusion and perspective

The serious threat of the chemical warfare agents and their mimics urges the development of novel methods for their sensitive and selective detection. In recent years, many fluorescent molecular probes with excellent fluorescent properties have been designed and synthesized for the detection. With the rational design, the minimum detection limit of CWAs has reached the ppb level. In addition, upon the modification of the fluorescent molecular probes, the selective detection of CWAs has been achieved. However, there are some issues yet to be solved. First, in order to achieve higher detection efficiencies, exploring the novel fluorescent molecular probes based on the PET and FRET processes should be continued, particularly for those fluorescent molecular probes which can execute the detection under the excitation with visible light. Second, aiming at higher detection selectivities, the subtle modification of the fluorescent molecular probes should attract special attention. Third, for easy operation and in-situ detection, solidification of the fluorescent molecular probes into a proto-type device should be carried out. Finally, novel techniques and materials, such as the microfluidic devices and nano materials, should be also assimilated to the detection.



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