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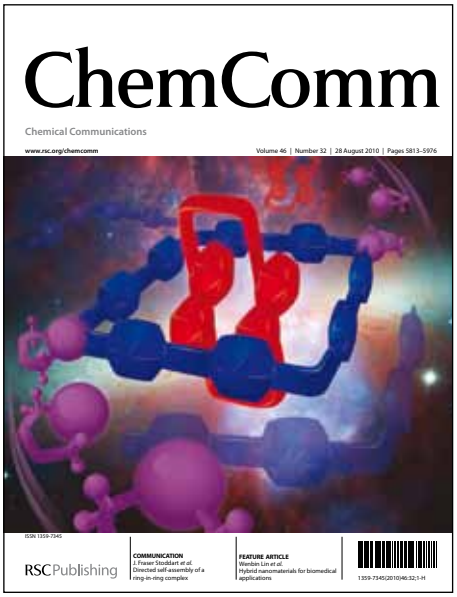

A FRET-based ratiometric fluorescent and colorimetric probe for the facile detection of organophosphonates nerve agent mimic DCP

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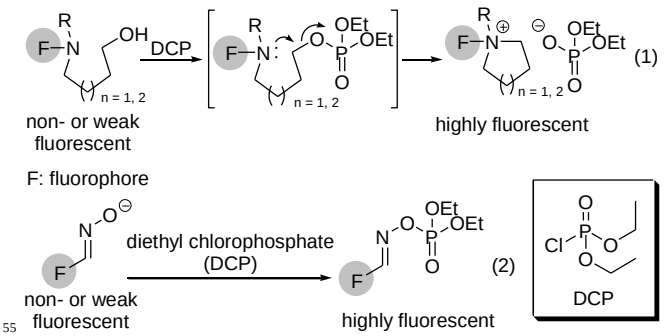
A FRET ratiometric fluorescent probe enabling a fast and highly sensitive response to OP nerve agent mimic DCP within 1 min and with as low as 0.17 ppm concentration detection limit has been developed. Moreover, the probe exhibits noticeable color change under UV and even with naked eyes. It is also demonstrated that it can detect both liquid and gas nerve agents.

The demand for facile detection of volatile organophosphonates (OP) nerve agents such as sarin (GB), soman (GD), and tabun (GA) has been driven by the growing concern of exposure to these chemical weapons on both the battlefield and through terrorist attacks.[1] These nerve gases, potent inhibitors of acetylcholinesterase (AChE) in the central nervous system,[2] have rapid, severe and even fatal effects on human and mammalian life if inhaled. In addition to the extreme toxicity, their ready production and easy operation render them attractive chemical weapons.

Although a variety of detection methods[3] have been developed for these nerve agents such as photoacoustics,[4] mass spectrometry,[5] capillary electropherograph, electrical sensors[6] and biosensors,[7] optical sensing (especially fluorescent detection) offers unrivalled merits of high sensitivity, low-cost (e.g., instrument), and operational simplicity. Furthermore, small molecule-derived fluorescent probes afford the advantages of easy production, low cost, high stability and easy storage. However, the development of such fluorescent probes is of formidable challenge. Only a handful of examples are available.[8,9] The widely used design principle takes advantage of the high electrophilicity of these OP nerve agents by reacting with a nucleophile embedded in a fluorophore to lead to the change of fluorescence properties. A nice approach is to utilize the reaction of nucleophilic hydroxyl group with OP nerve agents to form phosphate esters, which subsequent undergo an intramolecular *N*-alkylation reaction to generate fluorescent molecules (Scheme 1, eq. 1).[10] Pilato et al reported the first "off-on" fluorescent 1,2-enedithiolate complex probe.[10a] An improved organophosphate sensor which can work in an ambient condition and with faster response was reported by Swager and colleagues.[10b] Using a similar design strategy, Rebek and co-workers reported a photoinduced electron transfer (PET) fluorescence sensor with quick response and high sensitivity.[10c] Moreover, these pioneering studies have triggered intensive investigation with the design strategy.[10d-h] Anslyn et al employed a more nucleophilic oximate anion instead of hydroxyl group in a coumarin based PET sensor (Scheme 1, eq. 2).[11] Impressively, the reaction rate is enhanced by five orders of magnitude than those of hydroxyl-based sensors. The phosphorylation reactions of amine derived fluorescence molecules with OP nerve agents provide an alternative fertile approach.[12]



Scheme 1. General chemical reaction-based fluorescent deigsn strategies for the detection of OP nerve agents.

Despite these elegant studies, there is still significant room for improvement. In addition to quick response and high sensitivity, a truly practically useful fluorescence probe should display a ratiometric and colorimetric response. Such features can reduce the background interference and false positive results. Moreover, a probe enabling to analyze samples using easy-to-use devices is highly desired for the real time studies. A probe with color change by naked eyes is especially appealing. Finally, such a probe should be able to be employed for the analysis of both liquid and gaseous nerve agents.

Toward this end, herein we wish to disclose the first FRET ratiometric fluorescent probe for the detection of OP-nerve agents.[13] The probe displays several notable features. A carboxylate driving the formation of highly reactive nucleophilic phenol anion is exploited for the first time. The handle can react with electrophilic OP nerve agents within fast response (within 1 min). A FRET strategy by combination of rhodamine and fluorescein (termed 'rhodol') structures is implemented by incorporation of coumarin as a donor in a ratiometric response manner. Furthermore, the probe exhibits noticeable color change under UV and even with naked eyes. Finally, it can detect both liquid and gas nerve agents.

We envisioned that the acidic phenol could be readily ionized to form more nucleophilic phenoxide ion, which might be a good nucleophile for quick conjugation with OP nerve agents. The phenoxide anion could be generated *in situ* from its precursor quinone (Scheme 3). This could be driven by the formation of lactone from an intramolecular carboxylate ion conjugate addition. The acylation of the formed phenoxide is trapped by an

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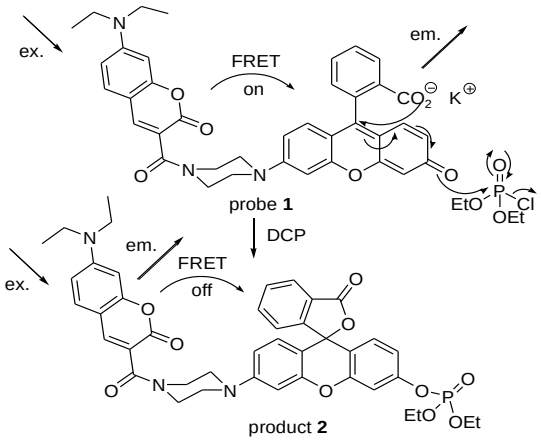
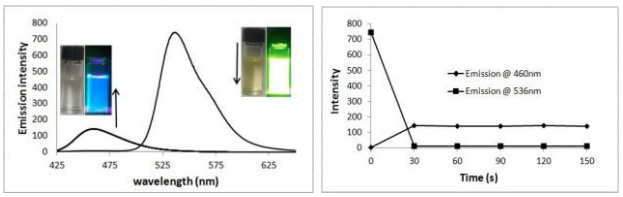
OP nerve agent. On the other hand, in the construction of a FRET sensor, we decided to choose commonly used coumarin as the fluorescent donor, which emission lies in the excite range of rhodamine/fluorescein with high efficiency.[14] Generally, the coumarin moiety is tethered though a rigid piperazine linker at the carboxylate position of fluoresecein/rhodamine. However, in our design, we proposed to attach the moiety to one of the amino group in rhodamine. As mentioned above, the reason for this is that the carboxylate moiety is essential for the formation of the lactone, which leads to the generation of FRET-based ratiometric response and nucleophilic phenoxide. Before reacting with an OP nerve agent, the FRET must be on and the carboxylate is in a free open state. Nevertheless, after the reaction, the free carboxylate is converted to a closed spirolactone form to induce ratiometric response. Furthermore, the new hybrid rhodol fluorophore can maintain the stable open form prior to reaction with OP nerve agents.

The designed probe **1** was synthesized in 6 steps with a total yield of 11% (see SI). With the probe in hand, we firstly tested its response to OP nerve agents. Due to its similar reactivity while much less toxicity, diethyl chlorophosphate (DCP, Scheme 1) has been generally used as the nerve agent surrogate for studies.[10] After addition of 100 μM DCP to 5 μM probe **1** in DMF, to our delight, an immediate color and fluorescent changes were observed (Figure 1). The largest emission wavelength was shifted from 536 nm (bright yellow) to 460 nm (blue), while the solution color was changed from yellow to colorless (Figure 1a). These shifts are attributed to the lactonization and phosphorylation of the phenoxide with DCP. The formation of the proposed product **2** was confirmed by mass spectrometer (M + H, 780.2659, see Figure S6 in SI). It is noted that the phenol moiety in fluoresceinamine chemosensor is in a neutral form,[12b] while in our case, the functionality is deprotonated. The reaction occurred within 30 s (Figure 1b). Meanwhile, obvious UV absorption change was observed (see Figure S2 in SI). The probe **1** exhibited the typical absorption peaks of both coumarin and a rhodol. Upon the treatment with DCP, the rhodol absorption disappeared indicating the formation of spirolactone ring. In the absence of DCP, the probe emission spectra remained the same (see Figure S3 in SI). Also to exclude the influence of a possible acid, the solution after treatment with DCP was further added K_2CO_3, and no observable change can be seen (see Figure S4 in SI). Moreover, even in the presence of an acid (e. g., HOAc), DCP is still capable of inducing significant fluorescence enhancement (see Figure S5 in SI). However, the carboxylic acid instead of carboxylate salt of **1** failed to induce fluorescence change because the weaker nucleophilicity of the COOH could not trigger the lactonization to form lactone and concurrently generate phenoxide. These studies demonstrated that the probe **1** displayed fast response to DCP. The changes can be observed by simple UV light or even naked eyes.

Next, we investigated the sensitivity of this probe with different concentrations of DCP in DMF. As shown in Figure 2, the response of this probe to DCP is concentration-dependant. When 80 μM DCP was used, the highest fluorescence change was obtained, and the intensity ratio change (F460/F536) reached a plateau with an enhancement of 1731 folds. According to the fluorescence increase at 460 nm or decrease at 536 nm, the FRET efficiency was calculated to be 96.0% - 98.6%. Meanwhile a clear



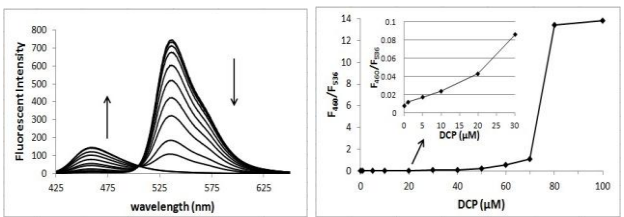
Scheme 2. Design of FRET-based ratiometric fluorescence probes for OP nerve agents.



(a) (b)

Figure 1. (a) To 5 μM probe **1** in DMF was added 100 μM DCP, the emission spectra was recorded every 30 s over 150 s. The inset picture showed the color change and the fluorescence change under a UV lamp. (b) The fluorescence intensities at 460 and 536 nm vary in a time range of 150 s. The excitation was set at 410 nm with slit/slit 5/1.5.

isoemission point was observed at 504 nm. Furthermore and notably, the probe can detect DCP at as low as 0.17 ppm with discernible emission ratio change. Besides DCP, other related phosphates were also tested and some of them can also induce fluorescence intensity enhancement (Figure S7 in SI).



(a) (b)

Figure 2. (a) To 5 μM probe **1** in DMF was added 0 - 100 μM DCP, the emission spectra was recorded in 1 min after addition of DCP, λ_{ex} = 410 nm. (b) Fluorescence intensity ratio changes (F460/F536) of the probe at 5.0 μM upon addition of various concentrations of DCP.

Having demonstrated that the probe **1** can response to a DCP solution with high sensitivity, we also carried out experiments to detect DCP in gaseous state. To make the detection experiments operationally simple and practical, pH paper was used. It was immersed in 0.1 mg/mL probe solution in MeOH in the presence of K_2CO_3. Then the paper was dried in air and the white filter paper was changed to slightly pink color (Figure 3a, top row). The probe-loaded filter paper was put across the top of a vial containing one drop of DCP for a defined exposure time. As

shown, an obvious color change under a UV hand lamp can be seen in 1 min (Figure 3b, bottom row), and an enhanced result can be obtained in a longer time (5 min) (Figure 3c, bottom row). Compared with the reported filter paper-based sensing method,[10c] the difference induced by DCP in this new filter paper based method is that it could be clearly observed by naked eyes (Figure 3c, top row). In control studies, we also tested the fresh filter paper without containing the probe **1** to DCP vapor, and no difference was observed (see Figure S8 in SI).

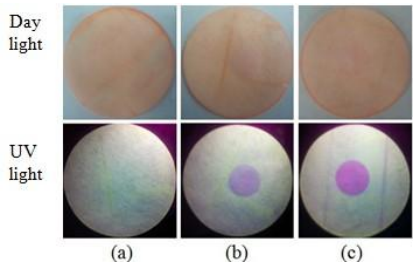


Figure 3. (a) Filter paper treated with 0.1 mg/mL probe solution in MeOH in the presence of K_2CO_3. (b) Filter paper exposed to DCP vapor for 1 min. (c) Filter paper exposed to DCP vapor for 5 min. Pictures at the bottom was irradiated at 365 nm with a UV lamp. Filter paper was used.

In conclusion, we have developed the first FRET-based ratiometric fluorescent probe for the detection of OP nerve agents. Notably, a new rhodol fluorophore has been designed and synthesized for the first time. Fast response, high sensitivity and obvious color change makes it very suitable for real time detection and field analysis. Furthermore it also enables to detect DCP vapor with a UV hand lamp or even naked eyes. These features render this probe a very promising tool to detect nerve agents in practical settings.

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Notes and bibliographic references

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† Electronic Supplementary Information (ESI) available: Experimental details and spectroscopic data for syntheiss of probe **1** and Figures S1-6 see DOI: 10.1039/b000000x/

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