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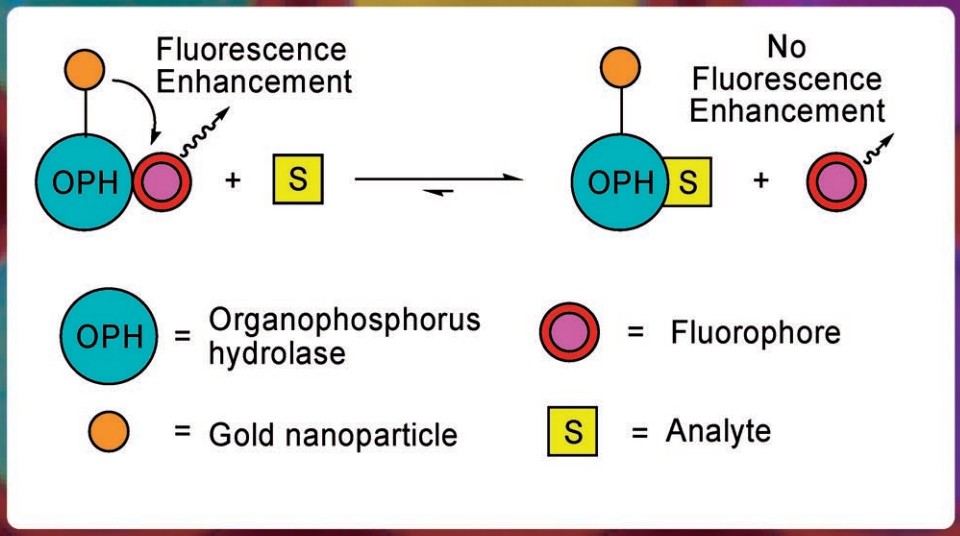

Fluorescent Sensors for the Detection of Chemical Warfare Agents

Mark Burnworth, Stuart J. Rowan,* and Christoph Weder*[a]

Abstract: Along with biological and nuclear threats, chemical warfare agents are some of the most feared weapons of mass destruction. Compared to nuclear weapons they are relatively easy to access and deploy, which makes them in some aspects a greater threat to national and global security. A particularly hazardous class of chemical warfare agents are the nerve agents. Their rapid and severe effects on human health originate in their ability to block the function of acetylcholinesterase, an enzyme that is vital to the central nervous system. This article outlines recent activities regarding the development of molecular sensors that can visualize the presence of nerve agents (and related pesticides) through changes of their fluorescence properties. Three different sensing principles are discussed: enzyme-based sensors, chemically reactive sensors, and supramolecular sensors. Typical examples are presented for each class and different fluorescent sensors for the detection of chemical warfare agents are summarized and compared.

Keywords: fluorescence · nerve agents · organophosphorus compounds · pesticides · sensors

Introduction

Since their early utilization in World War I, many different types of chemical warfare agents (CWAs) have been developed in laboratories around the world. Along with biological and nuclear threats, these weapons have become some of the most feared and least predictable mass destruction agents on the battlefield and in the hands of terrorists. A United Nations report from 1969 defines CWAs as "…chemical substances, whether gaseous, liquid, or solid, which might be employed because of their direct toxic effects on man, animals, and plants…". Some common representatives of the major classes of CWAs are shown in Figure 1. These include nerve agents (such as Sarin and Soman), vesicants (blister or mustard agents), blood agents (e.g. hydrogen cyanide), and pulmonary agents (choking agents such as phosgene). Another class of important chemical warfare agents includes natural toxins, such as the protein Ricin and the alkaloid Saxitoxin.

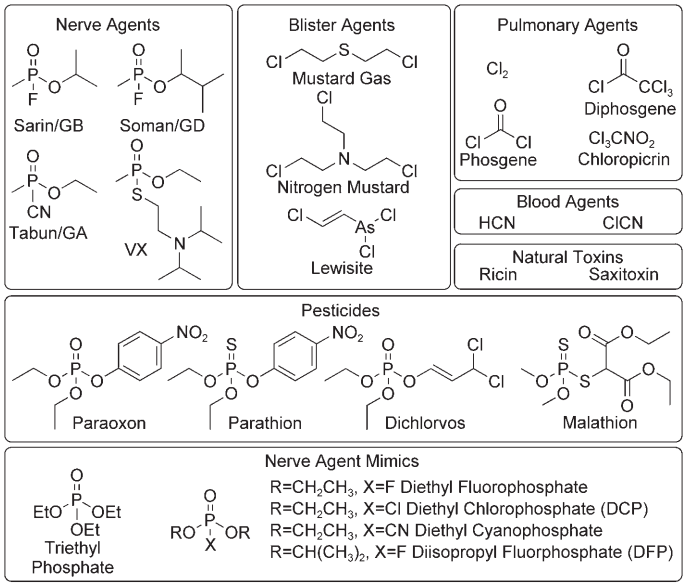

Figure 1. Major classes of chemical warfare agents, pesticides, and nerve agent mimics and chemical structures of common representatives.

The nerve agents represent one of the most important and lethal classes of chemical warfare agents. Their rapid and severe effects on human and animal health stem from their ability to block the action of acetylcholinesterase (AChE), a critical central nervous system enzyme that is re-

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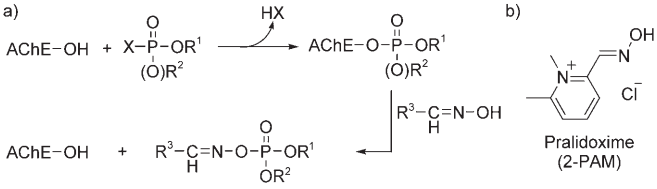
sponsible for the breakdown of the neurotransmitter acetylcholine. Virtually all nerve agents are organophosphates (OPs) and have the ability to react with the esteratic site of the enzyme leading to a loss of enzyme function (Scheme 1).



Scheme 1. a) Inhibition scheme of acetylcholinesterase (AChE) by organophosphates[1] and reactivation by an oxime and b) structure of the enzyme reactivating agent Pralidoxime chloride (pyridine-2-aldoxime methyl chloride, 2-PAM).

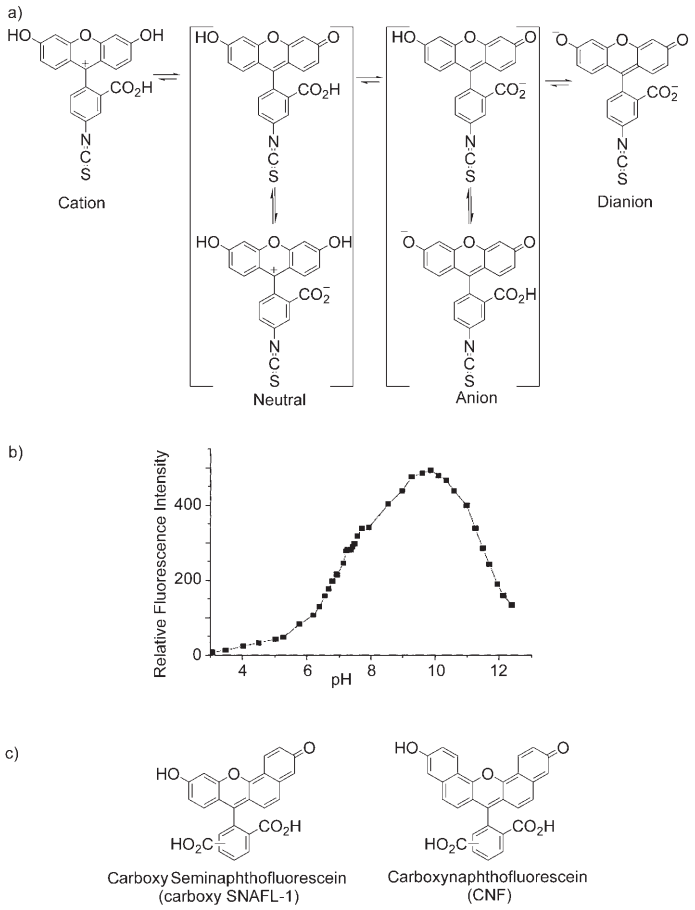
Compared to the normal acylated intermediate complex, the phosphorylated enzyme is very stable and breaks down only very slowly, leading to a loss of enzyme function.[1] This results in a build up of acetylcholine in the body, which can lead to organ failure and eventual death. The enzyme function can be restored with compounds that can dephosphorylate the enzyme and as a consequence such compounds can be used as antidotes. Examples of such reactivating agents include oximes, such as Pralidoxime chloride (Scheme 1).[1] OP nerve agents are generally stable, easy to disperse, and highly toxic. They can be absorbed through the skin, by ingestion, or by respiration and are generally classified into two groups, G-agents and V-agents. The G-series is older (developed in the World War II era) and encompasses compounds that are generally volatile, for example Tabun, Sarin, and Soman. The more modern V-series (developed in the 1950s) consists of nerve agents that are typically less volatile oils, such as VX. The V-agents generally degrade slower than G-agents, which along with their reduced volatility makes them more persistent. Furthermore, V-agents tend to be more toxic than representatives of the G-series (e.g. VX is about ten times more toxic than Sarin) and, as a consequence of their physicochemical properties, are primarily, although not exclusively, absorbed through the skin. Organophosphorus nerve agents were used in the Iraq–Iran war in the 1980s and as recently as 1995, in the Aum Shinrikyo terrorist attacks on the Tokyo subway system. In addition to CWAs, several organophosphorus derivatives, for example, paraoxon, parathion, dichlorvos, and malathion, have found commercial use as pesticides (Figure 1).[2] Their chemical structures closely resemble those of the above-discussed nerve agents, and hence, the development of detection systems for these compounds follows similar design principles.

In response to the serious threats to national and global security that result from the comparatively easy access to nerve agents, intense research efforts have been directed over the years to develop sensitive and selective schemes for the detection of such organophosphorus derivatives.[3] Several different approaches have been used to detect organophosphorus compounds, including but not limited to: potentiometric methods,[4] colorimetric methods,[5] surface acoustic wave spectroscopy,[6] gas chromatography/mass spectrometry,[7] and interferometry.[8] One of the most convenient and simplest means of chemical detection is generating an optical event, such as a change in absorption or fluorescence intensity/color.[9] This review focuses primarily on the detection of CWAs, in particular nerve agents, with fluorescent detector molecules. Three different sensing principles are discussed: enzyme-based sensors, chemically reactive sensors, and supramolecular sensors. Typical examples are presented for each class and different fluorescent sensors for the detection of chemical warfare agents are summarized and compared.

Enzyme-Based Sensors

Enzyme-based fluorescent sensors for the detection of nerve agents can be categorized into two general classes on the basis of the enzyme employed—acetylcholinesterase (AChE) or organophosphorus hydrolase (OPH).

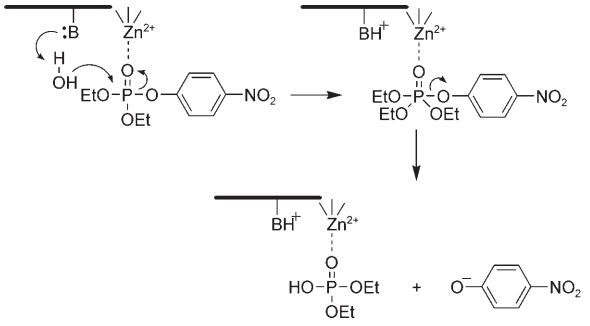
The AChE-based sensors rely on the fact that hydrolysis of acetylcholine by AChE produces a proton per substrate molecule, resulting in an increase of the acidity. This acidity increase can be measured in solution with the help of a pH-sensitive fluorescent dye. Rogers et al.[10] utilized this approach by labeling AChE with the pH-sensitive fluorescein isothiocyanate (FITC, Scheme 2a) and immobilizing the enzyme-dye adduct on a quartz fiber attached to a fluorescence spectrometer. In the absence of organophosphorus inhibitors, the labeled AChE was able to hydrolyze acetylcholine that was "fed" to the sensor, resulting in a pH reduction. This pH drop, in turn, lead to a reduction of the FITC fluorescence intensity (Scheme 2b), due to the interruption of the fluorophore's conjugation upon protonation (Scheme 2a).[11] If this bio-sensor was first exposed to the CWA mimic diisopropylfluorophosphate (DFP, Figure 1) and subsequently to acetylcholine, 90% of the enzyme activity was lost, as quantified by a less pronounced reduction of fluorescence intensity. The enzyme activity could be restored by treating the biosensor with Pralidoxime chloride (2-PAM, Scheme 1b), which removes the bound phosphorus from the enzyme and thus "resets" the sensor. However, if DFP and acetylcholine were added at the same time to the enzyme, only slight inhibition was observed, perhaps on account of competitive binding. This biosensor was capable of detecting the organophosphorus pesticide paraoxon in the nM range when it was exposed to a solution that contained this analyte for ten minutes. It was, however, unable to detect the pesticides malathion or parathion, even at mM concentrations, indicating some selectivity of the sensor for different organophosphorus compounds. A sensor for OP pesticides conceived by Jin et al.[12] also relied on the inhibition of AChE in the presence of a pH-dependent fluorescent dye. In this system the proton resulting from the hydrolysis of the acetylcholine resulted in an increase of the fluorescence intensity of 2-butyl-6-(4-methylpiperazin-1-yl)benzo-



Scheme 2. a) Structure of fluorescein isothiocyanate (FITC) at different pH, b) its relative fluorescence intensity at selected pH values,[11] and c) the structures of the fluorescein derivatives carboxy seminaphthofluorescein (carboxy SNAFL-1) and carboxynaphthofluorescein (CNF).

[*de*]isoquinolin-1,3-dione); the change of the fluorescence peak intensity was correlated with the organophosphorus concentration. A flow injection analysis setup allowed for detection of the organophosphorus derivative paraoxon in the 10^{-8} M range after an incubation time of ten minutes.

A second family of enzymatic sensors utilizes organophosphorus hydrolase (OPH) in conjunction with a pH-responsive fluorescent dye. This enzyme works differently from AChE (which, as discussed above, covalently binds to organophosphates and loses its activity in the process) in that it catalytically hydrolyses the organophosphate. As a result, sensors involving OPH allow for a more direct measurement of organophosphate molecules, instead of measuring enzyme inhibition. OPH is a dimeric metalloenzyme from the native soil bacteria *Pseudomonas diminuta*. The enzyme can be readily produced by using standard biochemical techniques from *Escherichia coli*.[13] OPH is widely used as a CWA biosensor, since its catalytic site is able to hydrolyze a wide range of OP compounds that contain P–O, P–F, P–S, or P–CN bonds (Scheme 3).[14] The stoichiometric hydrolysis produces two protons (again monitored with a pH-sensitive fluorescent dye), which can be directly correlated to the amount of OP substrate present.



Scheme 3. Mechanism for the hydrolysis of paraoxon by OPH.[14]

To create OPH-based CWA sensors, Rogers et al.[15] labeled the enzyme with FITC and coated this complex onto poly(methyl methacrylate) beads to create a biosensor. The fluorescence signal from this biosensor was monitored in a buffer solution. Upon addition of organophosphorus analytes, such as paraoxon, a decrease in pH in the local environment of the biosensor occurred and a decrease of FITC's fluorescence intensity was observed. The decrease of the fluorescence intensity could be directly related to the concentration of analyte. The detection limit for paraoxon, defined in the study as three times the standard deviation of experiments with the neat buffer solution, was in the μM regime. Cao et al.[16] labeled OPH with FITC and deposited this material onto silanized quartz slides in the form of Langmuir–Blodgett (LB) films to create organized monolayers of the enzyme-based sensors. These ultra-thin films reduce the problems associated with thicker films, which include the longer times required for the substrate to diffuse into the thicker film and the tendency of enzymes on the surface of the thick film to act as barriers for the analyte, thus reducing access to the enzymes within the film. Cao et al. demonstrate that the enzyme-based sensor LB films show enhanced sensitivity, detecting the analyte at nM concentrations. Orbulescu et al. further demonstrated that covalently immobilizing fluorescently labeled OPH on a silanized quartz substrate results in an increase of the enzyme stability, while a detection limit in the nM concentration regime can be maintained.[17]

Another method to access biosensors that incorporate OPH in combination with a fluorescent dye was developed by Russell et al.[18] In this study OPH was covalently functionalized with the pH-dependent fluorescent label carboxy seminaphthofluorescein (SNAFL-1, Scheme 2c) and with a poly(ethylene glycol) (PEG) acrylate derivative. The acrylated fluorescent enzyme was photopolymerized in the presence of PEG diacrylate, trimethylolpropane triacrylate, and/or tetraacrylated PEG to yield microspheres of a sensor-containing, lightly cross-linked polymer that formed hydrogels upon immersion in water. This system has several specific advantages over the above-discussed FITC-labeled OPH biosensors, namely, that SNAFL-1 is a "self-referencing" flu-

orescent dye. It features a broad fluorescence band that stretches from 500 to 700 nm. The fluorescence intensity of SNAFL at 620 nm is unchanged upon exposure to the OP analyte, while the intensity around 550 nm experiences a reduction. Thus, monitoring the ratio of the emission intensities at 620 and 550 nm allows one to generate a "referenced" signal that limits problems with effects such as photobleaching, concentration changes, and sensor noise. It is also claimed that PEG hydrogels further provides a protective environment for the enzyme; thus potentially inhibiting degradation and fouling. The OPH/SNAFL/PEG sensors investigated allowed the detection of paraoxon in aqueous solution, in concentrations as low as 10^{-7} M, with an estimated detection limit (defined as a signal to noise ratio of three) of 10^{-8} M.

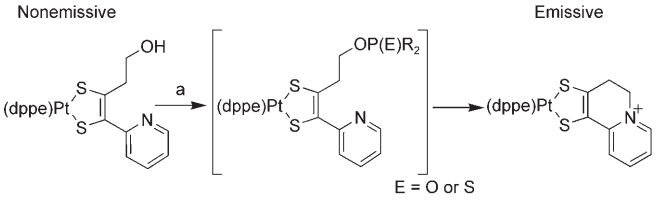
Sensory systems that rely on OPH/fluorescent dye systems have the potential to be used in portable electronic devices. Viveros et al.[19] reported an OPH biosensor in which the fluorescent dye carboxynaphthofluorescein (CNF, Scheme 2c) was covalently bound to the enzyme. The complex was reported to operate well in combination with a fiber-optic biosensor assay. The fluorescence intensity of CNF decreased with decreasing pH, and through correlation of the fluorescence intensity with the concentration of the OP, a detection limit of 10^{-8} M was claimed.

The OPH-containing sensors offer distinct advantages over AChE-based systems: 1) AChE can be inhibited by a wide range of other toxic compounds, leading to false positive results; 2) the inhibition of AChE is generally irreversible and requires enzyme re-activation steps (e.g. addition of Pralidoxime); 3) AChE sensors need to be "fed" with acetylcholine; and 4) generally, AChE sensors suffer from a rather slow response time. The OPH-containing sensors, in turn, directly measure the amount of analyte present through its hydrolysis. Furthermore, the enzyme's response towards different types of analytes is more specific. Both the AChE- and OPH-based biosensors described above rely on visualizing pH changes through pH-responsive fluorophores and optimized buffer solutions must be used for both systems; exposure to acidic and basic analytes is problematic as they directly influence the sensors' response and hinder accurate response. In addition, the lifetime of these sensors is limited by the degradation of the enzyme.

Chemically Reactive Sensors

Chemically reactive sensors are compounds which alter specific properties (e.g., optical) upon reaction with an analyte of interest. The general reaction mechanism exploited by chemically reactive sensors for the detection of OP CWAs mimics the chemical framework of the AChE inhibition by CWAs, namely nucleophilic attack of the sensor molecule on the electrophilic OP analyte. Upon reaction with the OP, the reactive sensor molecules are converted into phosphate esters. This process can lead, either directly or through subsequent reactions that are enabled by the activating nature of the phosphate ester formation, to a change of the sensor molecule's fluorescence characteristics.

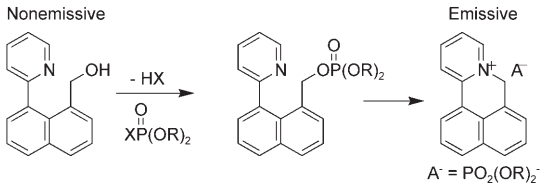
The first fluorescent reactive chemosensors for the detection of organophosphates were reported by Pilato et al.[20] A non-emissive platinum 1,2-enedithiolate complex with an appended primary alcohol was used as the chemosensor (Scheme 4). Upon exposure to an electrophilic OP analyte



Scheme 4. Mechanism of the chemically reactive sensor developed by Pilato and co-workers.[20] dppe = 1,3-bis(diphenylphosphoino)ethane. a) Triazole and one of the following: $(O)P(OEt)_2X$ in which X = F, Cl, CN; $(O)P(OPh)_2Cl$; $(S)P(OEt)_2Cl$; $(O)P(Me)_2Cl$; $(S)P(OEt)_2SPh$; or $(O)P(OC_6H_4p\text{-}NO_2)_2$.

and an activating agent (triazole) in dichloromethane, the alcohol is converted into the phosphate ester. This intermediate then undergoes a rapid intramolecular ring-closing reaction that is accompanied by the loss of a phosphate monoanion and affords a fluorescent cyclic product. The rate-determining step of this reaction is the nucleophilic attack of the sensor on the OP analyte. When immobilized in plasticized cellulose acetate/triethylcitrate films, chemosensors based on platinum 1,2-enedithiolate complexes were able to detect $PO(OEt)_2X$, (X = Cl, F, and CN) vapor in a nitrogen atmosphere. The fastest response time reported for such sensors was 15 s. The main limitation of this particular series of sensor molecules appears to be the fact that the fluorescence of the cyclic platinum complexes is quenched by oxygen, which, of course, stifles the use of these sensors in ambient conditions.

Utilizing a similar concept, Swager and co-workers[21] developed an organophosphate sensor that can be used in an ambient atmosphere. In this case a naphthalene scaffold (Scheme 5) effectively replaces the platinum complex uti-

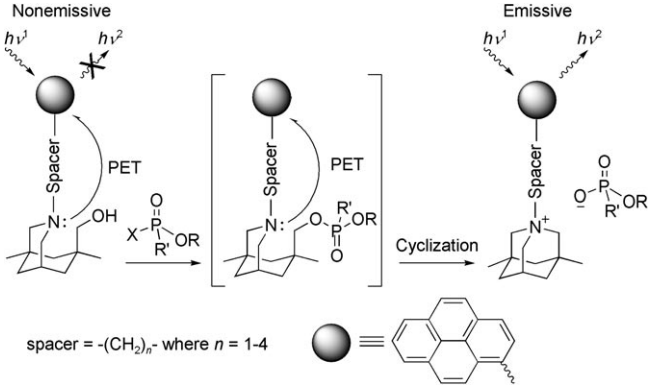


Scheme 5. The chemically reactive sensor developed by Swager and co-workers.[21]

lized by Pilato and co-workers. As in Pilato's system, the mechanism involves the reaction of the alcohol group of the sensor molecule with the analyte to yield a phosphate ester intermediate that is converted into a fluorescent cyclic prod-

uct. The naphthylpyridyl sensor molecule studied by Swager was found to react with the analyte under pseudo-first-order kinetics in CH_2Cl_2 with an observed rate constant (k_{obs}) > 0.024 s^{-1}. Chemosensors produced by impregnating a cellulose acetate film with this sensor molecule showed a complete response to 10 ppm diisopropylfluorophosphate (DFP) vapor within five minutes.

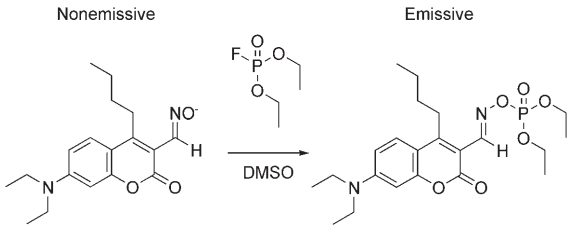
Rebek and co-workers recently reported[22] a reactive chemical sensor that utilizes a similar reaction mechanism as the sensor molecules designed by Pilato and Swager, but which relies on the suppression of a photoinduced electron transfer (PET) process to trigger a fluorescent signal (Scheme 6).[23] In this case, as in the previous examples, a



Scheme 6. The chemically reactive sensor developed by Rebek and co-workers.[22]

hydroxyl group reacts with the analyte to yield a reactive intermediate which undergoes an intramolecular reaction with a tertiary amine that is part of the sensor molecule. The system is designed so that the electron pair of the unreacted tertiary amine quenches the emission of a fluorescent moiety attached to the sensor compound by way of PET. As the chemosensor is exposed to the OP analyte (diethylchlorophosphate, DCP, was used in this study), an intramolecular cyclization reaction occurs. In the case of the sensor molecules designed by Rebek et al., a quaternary azaadamantane ammonium salt is formed. Upon loss of the nitrogen's lone electron pair, the PET is suppressed and the fluorophore (pyrene) is rendered fluorescent. A maximum increase of the fluorescence intensity of up to 22 times of the original signal was observed upon exposure of the sensor molecules to DCP. Interestingly, minor structural variations proved to have rather significant effects; maximum "contrast" was achieved if one methylene unit was used as the spacer between the nitrogen and the fluorophore, while a butylene spacer only displayed a 1.1-fold increase in fluorescence upon exposure to DCP. The sensor complexes investigated by Rebek and co-workers were deposited on a piece of filter paper. The simple solid-state sensors thus produced displayed a practically instantaneous (5 s) fluorescence upon exposure to vapor comprising 10 ppm of DCP. This sensor system offers the distinct advantage of being useful in conjunction with many existing fluorophores.

Besides the limitations that arise from the diffusion of the analyte to the sensing molecules (which can, as nicely demonstrated by the experiments of Cao et al.,[16] be minimized by maximizing the surface area of the sensory materials), the response time of the above-described chemically reactive systems primarily depends on the rate of the nucleophilic attack of the alcohol on the analyte. A sensor complex with a faster response time was developed by Anslyn and co-workers,[24] who replaced the hydroxyl group with the much more nucleophilic oximate anion. Their recent study demonstrated that the use of the oximate moiety as the nucleophile can significantly increase the response time of PET-based chemosensors that comprise coumarin as the fluorophore[25] (Scheme 7). The fluorescence of the anionic



Scheme 7. The chemically reactive sensor developed by Anslyn and co-workers.[25]

sensor molecules in DMSO is suppressed as a consequence of the lone pair of oximate anion by means of the PET mechanism. Upon addition of DFP, a pronounced fluorescence associated with the coumarin rapidly developed. The intensity increase followed pseudo-first-order reaction kinetics with a k_{obs} of 1410 s^{-1}. This rate constant is five orders of magnitude higher than those of hydroxyl-based sensors discussed above and nicely demonstrates the outstanding potential of chemically reactive sensory systems with respect to response time. The concept was also successfully applied to chromogenic sensory complexes, that is, nonfluorescent oximate derivatives that show a change in their UV/Vis absorption properties upon exposure to CWAs such as DCP and DFP.[24]

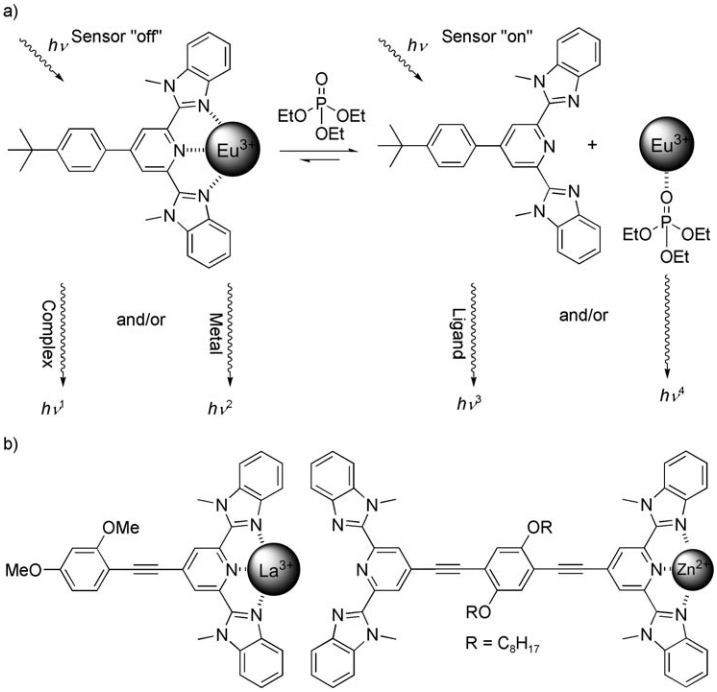
Supramolecular Sensors

Supramolecular sensors utilize noncovalent interactions to bind with an analyte to produce a detectable change of the sensor molecule's properties (e.g., optical).[26] It has been previously shown that lanthanide complexes, which exhibit characteristically narrow excitation and emission bands, intense fluorescence, and long excited-state lifetimes, are well suited to be used in chemical sensors.[27] For example Eu^{3+} complexes can exhibit intense luminescence in the presence of an appropriate UV-light-absorbing ligand through the so-called "antenna effect".[28] This process involves optical ab-

sorption by the ligand, followed by ligand-to-metal energy transfer, and results in metal-ion-based fluorescence. Their fluorescent properties combined with the fact that lanthanide ions are known to bind to organophosphates[29] makes them attractive candidates for the detection of nerve agents.

An early example of a supramolecular fluorescent sensor for the detection of OP compounds was reported by Jenkins et al.[30] In their study a molecularly imprinted polymer (MIP)[31,32] was employed in combination with Eu^{3+} ions with the goal of sensing the hydrolysis product of Soman. The template component of this MIP sensor was [Eu-(dvmb)$_3$(pmp)(NO$_3$)$_2$] (dvmb = 3,5-methyl divinyl benzoate; pmp = pinacolyl methylphosphonate), a Eu^{3+} complex with three different ligands. The pmp ligand, which is structurally similar to the hydrolysis product of Soman, was employed as an imprinting ion with the goal of developing a Soman-selective sensor. The incorporation of the complex into a polystyrene matrix was achieved through the utilization of the polymerizable ligand dvmb. This molecule acts as a cross-linking agent for the polystyrene, which was formed in the presence of the template, and is also an "antenna" ligand for Eu^{3+}. The sensor complex further comprised nitrate ions to ensure a high coordination number. The [Eu(dvmb)$_3$-(pmp)(NO$_3$)$_2$] complexes were copolymerized with styrene (and divinylbenzene) in an azobisisobutyronitrile-initiated free-radical polymerization. After the reaction was partially complete, the pmp ligands were removed by solvent extraction and the resulting material was further cured on an optical fiber to create a MIP for pmp. In these sensor MIPs, the intensity of one of the Eu^{3+} emission bands (at ca. 609 nm) is sensitive (increases) to the binding of PMP to the Eu^{3+} ion. Thus when the sensor was exposed to pmp in aq. NaOH (i.e., a solution that mimicked the conditions that could be used in the field to ensure the hydrolysis of Soman before the analyte would be fed into the sensor) a significant increase of the Eu-based 609 nm fluorescence band was observed. The sensor showed a remarkable sensitivity—the detection limit was reported to be 660 ppq in a benchtop system and 7 ppt for a portable system, without showing false positives for chemically similar OP compounds, such as dichlorvos. However, the response time of this system was limited by the diffusion of the analyte into the MIP. The sensor response time, defined in the study as the time to reach 80% of the sensor's maximum response, was 30 min. A recent study reported MIPs that were imprinted with the chemical warfare agents soman, sarin, and VX, instead of their hydrolysis product pmp; successful detection of these agents in the ppt range in tap water was reported, with a response time of 15 min.[33] While this response time is about half of that of the pmp MIPs described above, it is still much slower than some of the other sensors that have been discussed.

A versatile approach for OP sensor systems that utilizes noncovalent metal-ligand interactions and is based on the competitive binding of metal ions was developed by Rowan and Weder (Scheme 8).[34] Several fluorescent ligands were developed based on the 2,6-bis(1′-methylbenzimidazolyl)pyridine (Mebip) motif. Upon complexation to either Zn^{2+}, La^{3+}, or Eu^{3+} ions, a pronounced change (wavelength shift and intensity decrease) of the ligand-based fluorescence (in the case of Zn^{2+} and La^{3+}), or a change from ligand-to-metal-based emission (in the case of Eu^{3+} in which the Mebip ligand acts as an antenna chromophore) was observed. If triethyl phosphate was added to solutions of the lanthanide-based sensor complexes, the fluorescence spectra experience a significant and instantaneous blue shift and an increase of the fluorescence intensity. This response is proportional to the amount of analyte and corresponds to the release of free ligand, as a consequence of the competitive binding of the phosphate to the lanthanide ion (Scheme 8). The lanthanide-based sensors were able to successfully detect triethylphosphate in the μM regime. Isosbestic points were observed in spectral series if the analyte concentration was gradually varied. This feature confirms the direct conversion between sensor complex and free ligand upon phosphate binding and also highlights the "self-referencing" capability of these sensors: if the increase of free-ligand emission intensity (or the decrease of metal–ligand-complex emission intensity) is expressed relative to the intensity at the isosbestic point, possible errors associated with concentration fluctuations, photobleaching, quenching effects, intensity variation of the light source, and so forth can be compensated. The sensor systems also displayed excellent selectivity as no response was observed when the lanthanide sensor complexes were exposed to a range of common chemicals compounds, such as ketones, esters, organic acids, ethers, alcohol, and water in concentrations similar to that



Scheme 8. a) Proposed displacement mechanism for the supramolecular sensors developed by Rowan and Weder[34] and b) other ligand/metal complexes used in this study.

of the OP analytes of interest. The lanthanide-based sensors were further able to distinguish between different OP compounds: in contrast to triethyl phosphate, the bulkier aromatic OP tri-*o*-tolyl phosphate did not elicit a response. The only other compound tested to which the lanthanide-based sensor complexes did provide a response was triethylamine. While the lack of complete specificity (which is common to all of the sensory molecules discussed in this review) offers the possibility of undesirable false positive readings, the selectivity can be enhanced through combination of several sensor molecules. For example, Mebip–Zn^{2+} sensor complexes display a different binding behavior than the Mebip–lanthanide complexes, resulting in a different response when exposed to the same range of possible analytes; more specifically, the Mebip–Zn^{2+} sensor complexes studied (Scheme 8b) do not respond to the presence of aliphatic phosphates, but do respond to amines. Thus, combining both the lanthanide- and zinc-based sensor complexes into a simple array allowed the selective determination of the aliphatic phosphate from a variety of compounds tested (Figure 2a). The judicious design of a small number of fluorescent ligands and the careful selection of metal/ligand combinations is the basis of a very simple, modular system that appears to allow the selective detection of aliphatic organophosphates with good sensitivity. By tailoring the nature of the metal–ligand interactions, it should be possible to further enhance the sensitivity of these systems on the one hand and tailor their selectivity towards different analytes on the other.

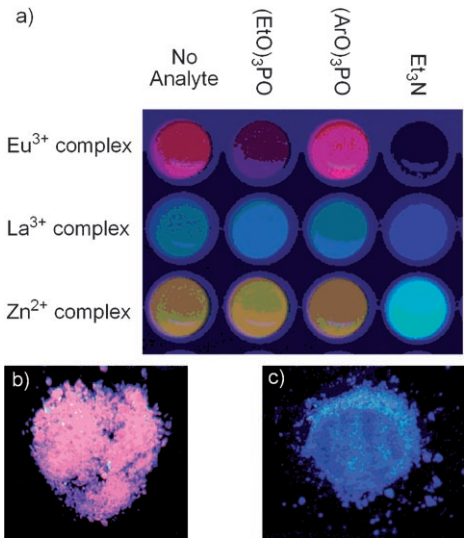
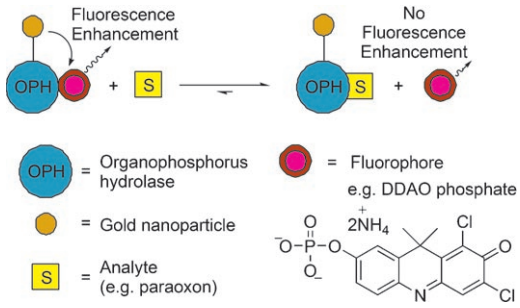


Figure 2. Pictures (excitation at 365 nm) of a) a sensor array illustrating the selective detection of $(EtO)_3PO$, $(ArO)_3PO$ {Ar = *o*-tolyl} and Et_3N by the metal ion complexes (25 μM in 9:1 $CHCl_3$:CH_3CN and [analyte] = 2.5 mM) shown in Scheme 8; b) hydrophobic silica particles coated with the Eu^{3+} complex shown in Scheme 8 and c) as in b) after exposure to $(EtO)_3PO$ vapor for 2 h at 60 °C.

First experiments demonstrated that such organometallic sensor complexes can be deposited onto a solid carrier and are able to detect both liquid and vapor aliphatic phosphates. Coating the Eu^{3+} complex shown in Scheme 8a onto hydrophobic silica particles produced a pink-fluorescent powder (Figure 2b). The fluorescence of the powder instantaneously changed to blue when exposed to liquid triethyl phosphate. Exposure to triethyl phosphate vapor also elicited a response (Figure 2c), albeit (on account of the low vapor pressure of the analyte) much slower.

Supramolecular displacement sensors have also been developed by using an enzyme as the binding motif. For example, Simonian et al.[35] recently reported the use of organophosphorus hydrolase (OPH) as the receptor for organophosphorus analytes. In this system a phosphate derivative of a fluorophore (e.g. diammonium 9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl, DDAO phosphate) was weakly bound to the enzyme, which in turn was covalently attached to gold nanoparticles (Scheme 9). This design leads to sensor



Scheme 9. Schematic of analyte displacement of a fluorophore (diammonium 9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl, DDAO phosphate) from OPH-gold complex, leading to a reduction in fluorescence.[35]

complexes in which the fluorescence intensity of the fluorophore is significantly enhanced through the strong local electric field of the gold nanoparticle. The fluorescence enhancement is a function of the distance from the fluorophore to the gold nanoparticle, and therefore a significant reduction of the fluorescence intensity is observed if the fluorophore is displaced from the enzyme binding site. This displacement occurs through competitive binding with an analyte (e.g. paraoxon) that has a higher binding affinity for the enzyme than the DDAO phosphate (Scheme 9). In the system studied, the sensor provided a linear response when exposed to paraoxon solutions with concentrations in the μM regime. This study nicely demonstrates that the concepts of enzyme-based and supramolecular sensory systems can be married, resulting in OP sensors that operate without pH-sensitive dyes, unlike the "classic" enzyme-based systems discussed in the first section of this review.

Conclusion

The continuous threat of exposure to chemical warfare agents on both the battlefield and through terrorist attacks has lead to a recent surge in the research aimed at the de-

tection of these highly toxic compounds. Focusing on fluorescent sensors developed to counter the threat of nerve agents, this review has emphasized only a small segment of this overall effort. As outlined herein, a wide variety of approaches are being studied, many of which are also applicable to transduction schemes involving effects other than fluorescence changes, for example, changes of optical absorption, electrical conductivity, and so forth. Each approach has certain advantages and drawbacks. Fluorescence sensing offers a number of benefits from the perspective of detecting chemical warfare agents, such as high sensitivity, large signal changes, and even on–off responses. One key aspect to this research is of course being able to use the systems outlined above in easy-to-use devices.[36] This is a field that is going to continue to grow over the next couple of years as more and more researchers (and funding agencies) commit resources to this issue. We hope that this review will help aid this growth by providing a summary of the current state-of-the-art of the field.

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