Sensing Chemical Warfare Agent Simulants via Photonic Crystals of the *Morpho didius* Butterfly

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**Supporting Information**

**ABSTRACT:** The rapid and portable detection of trace chemical warfare agents (CWAs) remains a challenge for the international security and monitoring community. This work reports the first use of natural photonic crystals (*PhCs*) as vapor sensors for CWA simulants. Dimethyl methylphosphonate, a nerve agent simulant, and dichloropentane, a mustard gas simulant, were successfully detected at the parts per million level by processing visible light reflected from the PhC inherent to the wing scales of the *Morpho didius* butterfly. Additionally, modeling of this natural system suggested several parameters for enhancing the sensitivity of a synthetic PhC toward CWA simulants, including materials selection, structure, and spacing of the PhC, and partial functionalization of the PhC toward the analyte of interest. Collectively, this study provides strategies for designing a sensitive, selective, rapid, and affordable means for CWA detection.

**INTRODUCTION**

The homeland defense and battlespace operation communities require portable and reliable sensors for chemical warfare agent (CWA) detection. The inherent toxicity of these organophosphates necessitates identification at the parts per trillion level and, to date, only expensive and nonportable instrumentation, such as mass spectrometry and nuclear magnetic resonance spectroscopy, have shown clear discrimination at the requisite sensitivity. However, a variety of other methods to detect CWA and CWA simulants have shown promise, such as colorimetric, piezoelectric, and electrochemical techniques.

These methods, though, are typically unsuitable for long-term field use owing to one or more challenges, such as inadequate sensitivity and/or selectivity, sensor poisoning, cost, robustness, or portability. Recent studies into both natural and synthetic photonic crystals (*PhCs*) have shown that these systems are sensitive to vapors with similar chemical and physical properties, offering a new means to rapidly identify and quantify trace analytes. *PhCs* rely on the principle of light interference when moving through periodic structures of varied refractive index and dielectric constant, resulting in photonic band gaps and structural color. Small changes in either the refractive index or the distance between layers of the periodic structure of the PhC can lead to significant changes in the wavelength of reflected light—the basis of sensing via PhCs. Sensing in the visible light spectrum requires periodicity of the layers in the nanometer range. Although three-dimensional PhCs at the nanometer scale have been fabricated, nature of PhCs currently show a significantly higher sensitivity toward vapors because of their complex periodicity, as well as the polarity gradient found within the natural PhC architecture. A recent work has shown that the light reflected from the scales of the *Morpho* butterfly can be used to distinguish between vapors of similar polarity and refractive index. The polarity gradient within the ridges and lamella of the scales led to aggregation of the vapor in certain regions, altering the dielectric properties of the PhC in a manner unique to each vapor analyzed and detectable at the parts per million level.

Despite the promise of PhCs as vapor sensors, few studies of CWA simulants and PhCs are found in the literature and are largely confined to photonic hydrogel sensors in the aqueous phase. Walker et al. pioneered the use of photonic hydrogel sensors by using polymerized crystalline colloidal arrays in conjunction with an enzyme chosen to swell or shrink the PhC periodically in the presence of a CWA simulant. Concentrations of parathion and methyl parathion were detected at femtomolar levels by processing reflected light from the photonic hydrogel sensor.

This work examines the viability of using a natural PhC and visible light reflectance to detect and discriminate between CWA simulant vapors and other common vapors. Wing scales of the *Morpho didius* butterfly, a well-characterized natural...
PhCs,\(^{40}\) were exposed to water, methanol (MeOH), and ethanol (EtOH), as well as the CWA simulants 1,3-dichloropropene (DCP, mustard gas simulant) and dimethyl methylphosphonate (DMMP, nerve agent simulant). Reflectance spectra in the presence of each vapor were collected at several concentrations, and the entire data set was processed using principal component analysis (PCA). Further, the reflectance spectra for each vapor were modeled by generating a periodic nanoarchitecture similar to that of the natural wing scale upon exposure to each vapor. Collectively, the results of this work are expected to provide the first baseline data set for natural PhCs as CWA simulant vapor sensors and suggest avenues for designing synthetic CWA simulant vapor sensors that outperform sensors based on natural systems.

**RESULTS AND DISCUSSION**

The nanostructure of the *Morpho* butterfly has been well-characterized in other studies.\(^{32,34,37,38,43}\) In brief, Figure 1 shows that wing scales are comprised of parallel ridges normal to the plane of the wing. A cross section of the wing shows that each ridge in turn contains a tapered, tree-like structure with seven lamellae extending on each side of the center of the ridge. Lamellae are roughly 70–80 nm thick with widths ranging from 200 to 400 nm; the vertical spacing between lamella is on the order of 150 nm, and the spacing between ridges is approximately 800 nm. Hence, the wing scales are periodic on the nanometer scale, giving rise to photonic gaps in the visible light region. Unlike most synthetic PhCs, studies have shown that a polarity gradient exists within the ridges of the wing scales, with higher polarities at the top of the ridges. This polarity gradient is the key to sensing via reflected light from the *Morpho* butterflies, as the location of the analyte within the structure also contributes to the wavelengths of the light reflected rather than refractive index alone.\(^{31,37}\) However, the sensitivity and selectivity of this natural system to CWA simulants had not been previously determined.

The sensitivity of the *M. didius* PhCs toward CWA simulants was studied by exposing the wing to a variety of analyte vapors, with the reflectance (\(R\)) of each analyte measured individually at three different concentrations. As shown in Figure 2, a manual system was used to vary the fraction of the analyte vapor in the nitrogen carrier gas, and the reflectance spectra were collected via fiber optics. The differential reflectance (\(\Delta R\)) of DCP, DMMP, EtOH, MeOH, and water at each concentration relative to pure nitrogen is shown in Figure 3a–e. Raw reflectance data for all experimental runs can be found in the Supporting Information (Figure S1). Traces for each analyte are unique and vary in intensity with concentration, suggesting that the system is both selective and responsive to the vapors tested, including the CWA simulants. However, as evidenced by the smaller \(\Delta R\) values relative to EtOH, MeOH, and water, the sensitivity of this system toward the CWA simulants is challenged by the relatively low saturated vapor pressures of DCP (2750 ppm) and DMMP (1575 ppm).\(^{44,45}\) Thus, the limits of detection toward these CWA simulants in this study are on the order of parts per million. Considering that the nerve agent VX is lethal at concentrations of 0.3 ppm,\(^{31}\) this presents a challenge to use natural PhCs for CWA detection. However, as outlined below and in the literature, several avenues exist for increasing sensitivity via a synthetic mimic, including careful selection of PhC refractive index, targeted functionalization of certain regions within a PhC nanoarchitecture, and optimized PhC periodicity, among others.\(^{14}\) It is important to note that sensing nerve agents using an optimized, synthetic PhC is expected to provide significant advantages over current technologies in terms of sample acquisition and sensor portability, cost, and size. These advantages should enable the deployment of robust, real-time passive sensors and foster the integration of miniaturized sensors for rapid and remote forensic analysis.

To further examine the selectivity of the *M. didius* toward the CWA simulants, \(\Delta R\) data for all analytes and concentrations were processed collectively using PCA. PCA is a commonly used multivariate analysis technique that reduces the dimensionality of a data set by determining the principle

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**Figure 1.** *Morpho didius* butterfly (a), *M. didius* cover and ground scales (b), ground scale ridges (c), ridges showing lamella and microribs (d), and ridge cross section showing lamella outcropping (e).
components that capture the variance within that data. Two- and three-dimensional PCA score plots of the data for all vapors are shown in Figure 3f,g, with data points indicating an increase in concentration from left to right. Similar to other studies of the light reflected from the Morpho butterfly upon exposure to EtOH, MeOH, and water, this work showed good analyte discrimination (e.g., distinct PCA curves), despite similar concentrations and refractive indices. Additionally, the data also indicate good selectivity toward the CWA simulants DCP and DMMP, despite their low vapor concentrations. Although PCA score plots of previous studies of the Morpho sulkowskyi indicated a trend with solvent polarity, the trend was not clearly observed for this study of the M. didius. As mentioned previously, the Morpho butterfly scales contain a polarity gradient moving up the lamella of the ridges, suggesting that analyte vapors aggregate within certain regions of the scale based on their polarity which significantly contributes to spectral differences. Studies indicated that removing the polarity gradient within the wing resulted in trends that matched the refractive index, suggesting that analyte vapors aggregate within certain regions of the scale based on their polarity which significantly contributes to spectral differences.

Figure 4 demonstrates how three parameters—vapor concentration, vapor refractive index, and vapor location within the PhC—affected the light reflected from the PhC model. As shown in Figure 4a, when the refractive index and the location of the analyte within the PhC are held constant, but concentration is increased, a slight red shift, as well as a peak intensity increase, is evident near 500 and 600 nm. For Figure 4b, the concentration and the analyte location within the PhC are held constant, whereas the refractive index is varied. Of note, for
analytes with very similar refractive indices (e.g., water and MeOH), the model yields nearly identical traces (MeOH trace obscures the water trace), a result not observed experimentally, likely because of the polarity gradient of the natural system and aggregation of analytes in different regions. Figure 4b also illustrates the complex result when the refractive index is varied, as no clear trend is shown in terms of peak intensity or peak shift. For example, EtOH has the highest peak intensity but an intermediate refractive index relative to the other vapors for the model studied. This highlights the interdependent relationships between PhC structure and spacing, PhC refractive index and dielectric constant, and analyte refractive index, suggesting that these parameters can be tailored to increase the sensitivity toward an analyte of particular interest.

Similar to other studies, the model data also suggest that the location of a vapor within a PhC plays an important, even critical, role in the selectivity of natural PhC systems, especially when considering the combined effects of other parameters, such as concentration and refractive index. Figure 4c shows the reflectance data when the location of the analyte within the PhC is varied, whereas the concentration and the refractive index are held constant. Relative to the model with uniform coverage, a blue shift occurred, likely from a decrease in the overall refractive index of the PhC system. Significantly, differences in the reflectance spectra were observed at most wavelengths for the analyte concentrated in the top, middle, or bottom lamella. A three-dimensional PCA score plot of the modeled ΔR data for all five vapors is provided in Figure S7. Generally, the data are grouped on the basis of refractive index, concentration, and analyte location within the PhC structure, with similar values of these variables generating similarities in the traces. A two-dimensional PCA score plot of the first two PCs is provided in Figure S8 and highlights the refractive index and concentration effects.

Of particular interest in this work, the simulated reflectance data for all five analytes concentrated at each of the three locations within the model PhC demonstrate the variation caused by vapor concentration within certain regions of the model (Figure 4d). A greater variation between analytes was seen in the middle and bottom layers, relative to the top layer, indicating more pronounced spectral distinction between analytes in those layers. This increase in spectral distinction is attributed to changes in spacing between lamellae. For the model used here, a greater vertical separation between lamellae exists in the middle and bottom layers relative to the top layer (Figure S1), suggesting that the increase in vertical separation between lamellae drove the spectral variance. This result is further confirmed by careful examination of work by Potyrailo et al. Although not specifically mentioned by the authors, their modeling data also show increased spectral distinction with increased vertical separation between lamellae. However, in contrast to the work presented here, their model had a greater lamellar separation in the top layer and a corresponding increase in the spectral variance in the top layer over the middle and bottom layers. Coupled with their result that a tapered lamellar structure was not important for vapor selectivity, these studies highlight the relevance of lamellar spacing in spectral distinction. Collectively, these results show that targeted functionalization of regions with optimized periodic

Figure 4. Model reflectance spectra versus wavelength for (a) varied vapor concentration simulated as a film thickness d, (b) varied vapor refractive index, and (c) varied vapor location within the lamella. The two-dimensional PCA score plot from Figure S7 of the model ΔR data (d) highlighting the reflectance variance owing to vapor location within the lamella. Insets show the modeled distribution of vapors within the lamella.
dimensions could lead to increased sensitivity toward a trace analyte.

Results from the modeling suggest several strategies to increase the sensitivity of a synthetic PhC toward CWA simulants. For example, the refractive index difference between the analyte vapor and the PhC structure passed through a maximum in the model studied. Thus, selecting a PhC material with a higher refractive index than chitin should increase the sensitivity toward CWA simulants. Additionally, the lethality of CWA agents is typically expressed in terms of exposure concentration per unit time (e.g., 10 mg·min·m⁻³ for VX). Thus, the functionalization of a PhC surface with a material that binds the CWA of interest (e.g., acetylcholnesterase for VX) could concentrate that analyte with increasing exposure time, resulting in a detectable signal over time that still falls below the lethal exposure dosage. Modeling also showed that spectral differences could be enhanced by altering the periodic spacing of the lamella. Thus, methods for generating polarity gradients or functionalization of the synthetic PhC mimic should focus on concentrating the analyte vapor in regions of optimized lamellar periodicity. When combined with other known methods to enhance the PhC sensitivity via operating in stimulated emission regimes, synthesizing higher numbers of lamellae, optimizing ridge spacing, and considering the PhC extinction coefficient, the detection of CWA simulants should be possible in the parts per billion range or better.

**CONCLUSIONS**

In summary, this work highlights the first report of sensing CWA simulant vapors via light reflected from a natural PhC. The system was selective toward different simulant vapors and was able to distinguish between analytes of similar polarity and refractive index. Sensitivity toward the analytes was in the parts per million range, although modeling suggested that the sensitivity of a synthetic mimic toward CWA simulants could be improved by the careful selection of PhC refractive index, targeted functionalization of certain regions within a PhC nanoarchitecture, and optimized PhC periodicity. These results are expected to serve as a baseline for future studies of PhCs as CWA simulant sensors and guide improvements in sensitivity toward these analytes.

**MATERIALS AND METHODS**

**Materials.** Butterflies (*M. didius*) were purchased from Butterfly Utopia and used as received. DMMP (97%, D169102) and DCP (99%, D69602) were purchased from Sigma-Aldrich. EtOH (ACS grade, 111000190) and MeOH (ACS grade, 339000000) were purchased from Pharmco-AAPER. Ultrapure water (Milli-Q gradient A-10, Milli-Q, 18.2 Ω·m·cm, <5 ppb organic impurities) and ultrahigh purity nitrogen (Airgas, UHP300) were used for all experiments.

**Reflectance Measurements.** A halogen light source (Ocean Optics, HL-2000) and a spectrophotometer (Ocean Optics, HR2000+) equipped with a fiber optic probe (Ocean Optics, QR400-7-UV–vis) were used for reflectance measurements. The reflectance probe was positioned normal to the butterfly surface, generating an illuminated area about 2 mm in diameter. In vapor measurements, the differential reflectance spectra (ΔR) were measured relative to nitrogen. Vapor concentrations were varied using a manual bubbler system to alter the fraction of vapor in the carrier gas so that the partial pressure of the analyte vapor was between 0.15P₀ and 0.50P₀, where P₀ is the saturated vapor pressure at 20 °C. The total gas flow rate for all measurements was 400 mL·min⁻¹. Each spectrum was collected after 5 min of exposure to the vapor and processed using binomial smoothing. PCA was performed on the raw spectra after mean-centering the data.

**Scanning Electron Microscopy.** Butterfly wing samples were mounted to stubs using conducting carbon tape and sputtered with 5 nm of gold to limit the charge buildup. Scanning electron microscopy images were captured using an FEI Helios NanoLab 600 scanning electron microscope operated at 5.0 kV, providing image magnifications up to 30 000.

**Optical Modeling.** A 2D model based on the ridge and lamella nanostructure of the *M. didius* wing scales was generated in RSoft DiffractMod (Synopsys), which uses rigorous coupled wave analysis to simulate the diffraction of light moving through an infinite periodic structure. The complex refractive index of chitin with n = 1.56 and k = 0.06 was used for the structure. Various concentrations of the analyte vapor were simulated by coating the structure with a thin film of thickness d and analyte refractive index n. The incident light was normal to the structure, while the electric field was in-plane.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01680.

Reflectance data, model PhC schematic, and model reflectance data (PDF)

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Notes

The authors declare no competing financial interest.

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**REFERENCES**

(1) Noort, D.; Benschop, H. P.; Black, R. M. Biomonitoring of exposure to chemical warfare agents: a review. *Toxicol. Appl. Pharmacol.* 2002, 184, 116–126.

(2) Koskela, H. Use of NMR techniques for toxic organophosphorus compound profiling. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* 2010, 878, 1365–1381.

(3) Pavlov, V.; Xiao, Y.; Willner, I. Inhibition of the acetycholine esterase-stimulated growth of Au nanoparticles: nanotechnology-based sensing of nerve gases. *Nano Lett.* 2005, 5, 649–653.

(4) DeFrank, J. J.; White, W. E. Phosphofluoridates: biological activity and biodegradation. In *The Handbook of Environmental Chemistry*; Neilson, A. H., Ed.; Springer: Berlin, 2002; Vol. 3N, p 295.

(5) Costero, A. M.; Parra, M.; Gil, S.; Gotor, R.; Mancini, P. M. E.; Martínez-Máñez, R.; Sancenón, F.; Royo, S. Chromo-Fluorogenic Detection of Nerve-Agent Mimics Using Triggered Cyclization Reactions in Push—Pull Dyes. *Chem.—Asian J.* 2010, 5, 1573–1585.
(6) Walker, J. P.; Asher, S. A. Acetylcholinesterase-based organophosphate nerve agent sensing photonic crystal. Anal. Chem. 2005, 77, 1596–1600.

(7) Jenkins, A. L.; Uy, O. M.; Murray, G. M. Polymer based lanthanide luminescent sensors for the detection of nerve agents. Anal. Commun. 1997, 34, 221–224.

(8) Wild, A.; Winter, A.; Hager, M. D.; Schubert, U. S. Fluorometric, water-based sensors for the detection of nerve gas G mimics DMMP, DCP and DCNP. Chem. Commun. 2012, 48, 964–966.

(9) Dale, T. J.; Rebek, J. Fluorescent sensors for organophosphorus nerve agents. J. Am. Chem. Soc. 2006, 128, 4500–4501.

(10) Akthakul, A.; Maklakov, N.; White, J. Improved Vapor Sensitivity by Rationally Designing Fluorescent Turn-on Sensors. Anal. Chem. 2010, 82, 6487–6494.

(11) Jang, Y.-J.; Tsay, O. G.; Murale, D. P.; Jeong, J. A.; Segev, A.; Churchill, D. G. Novel and selective detection of Tabun mimics. Chem. Commun. 2014, 50, 7531–7534.

(12) Wu, C.-F.; Cha, H. J.; Valdes, J. J.; Bentley, W. E. GFP-visualized immobilized enzymes: Degradation of paraaxon via organophosphorus hydrolase in a packed column. Biotechnol. Bioeng. 2002, 77, 212–218.

(13) Malosse, L.; Buvat, P.; Aides, D.; Siove, A. Detection of degradation products of chemical warfare agents by highly porous molecularly imprinted microspheres. Analyst 2008, 133, 588–595.

(14) Knappit, D.; Burnworth, M.; Rowan, S. J.; Wieder, C. Fluorescent Organometallic Sensors for the Detection of Chemical-Warfare-Agent Mimes. Angew. Chem., Int. Ed. 2006, 45, 5825–5829.

(15) Kanan, S. M.; Tripp, C. P. An infrared study of adsorbed organophosphonates on silica: a prefiltering strategy for the detection of nerve agents on metal oxide sensors. Langmuir 2001, 17, 2213–2218.

(16) Sohn, H.; Létant, S.; Sailor, M. J.; Trogler, W. C. Detection of fluorourophorчате chemical warfare agents by catalytic hydrolysis with a porous silicon interferometer. J. Am. Chem. Soc. 2000, 122, 5399–5400.

(17) Aderkule, A. S.; Pillay, J.; Ozonema, K. I. Electrocatalysis of 2-Diethylaminooctanethiol at Nickel Nanoparticle-Electrodecorated Single-Walled Carbon Nanotube Platform: An Adsorption-Controlled Electrode Process. Electroanalysis 2008, 20, 2587–2591.

(18) Kim, Y.; Lee, S.; Choi, H. H.; Noh, J. S.; Lee, W. Detection of a nerve agent simulant using single-walled carbon nanotube networks: dimethyl-methyl-phosphonate. Nanotechnology 2010, 21, 495801.

(19) Wang, Y.; Yang, Z.; Hou, Z.; Xu, D.; Wei, L.; Kong, E. S.-W.; Zhang, Y. Flexible gas sensors with assembled carbon nanotube thin films for DMMP vapor detection. Sens. Actuators, B 2010, 150, 708–714.

(20) Kwon, O. S.; Park, S. J.; Lee, J. S.; Park, E.; Kim, T.; Park, H.-W.; You, S. A.; Yoon, H.; Jang, J. Multidimensional Conducting Polymer Nanotubes for Ultra-sensitive Chemical Nerve Agent Sensing. Nano Lett. 2012, 12, 2797–2802.

(21) Kim, H. J.; Lee, J. H.; Lee, H.; Lee, J. H.; Jung, J. H.; Kim, J. S. A Mesoporous, Silica-Immobilized-Nanoparticle Colorimetric Chemosensor for the Detection of Nerve Agents. Adv. Funct. Mater. 2011, 21, 4035–4040.

(22) Yilmaz, M.; Senlik, E.; Biskin, E.; Yavuz, M. S.; Tamer, U.; Demirel, G. Combining 3-D plasmonic gold nanorod arrays with colloidal nanoparticles as a versatile concept for reliable, sensitive, and selective molecular detection by SERS. Phys. Chem. Chem. Phys. 2014, 16, 5563–5570.

(23) Liu, G.; Lin, Y. Electrochemical sensor for organophosphate pesticides and nerve agents using zirconia nanoparticles as selective sorbents. Anal. Chem. 2005, 77, 5894–5901.

(24) Scheide, E. P.; Guibault, G. G. Piezoelectric detectors for organophosphorus compounds and pesticides. Anal. Chem. 1972, 44, 1764–1768.

(25) Nieuwenhuizen, M. S.; Harteveld, J. L. N. A surface acoustic wave gas sensor for organophosphorus compounds. Sens. Actuators, B 1994, 19, 502–505.
(46) Abdi, H.; Williams, L. J. Principal component analysis. Wiley Interdiscip. Rev. Comput. Stat. 2010, 2, 433.

(47) Sidell, F. R. Nerve Agents. In Medical Aspects of Chemical Biological Warfare; Zajtchuk, R., Bellamy, R. F., Eds.; Office of the Surgeon General: Bethesda, 1997; p 141.

(48) Noh, H.; Yang, J.-K.; Liew, S. F.; Rooks, M. J.; Solomon, G. S.; Cao, H. Control of lasing in biomimetic structures with short-range order. Phys. Rev. Lett. 2011, 106, 183901.