A Complementary Silicon Quantum Dot-Enzyme Platform for Selective Detection of Nitroaromatics Compounds

*Leanne A. Milburn, Christopher Jay T. Robidillo †, Rochelin Dalangin ††, and Jonathan G.C. Veinot**

Department of Chemistry, University of Alberta, Edmonton, T6G 2E9, Alberta, Canada

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To address the issue of poor selectivity in nanotechnology-driven, portable nitroaromatics sensors, we have coupled a ratiometric fluorescence sensor based on silicon quantum dots and fluorescent proteins with a colorimetric, enzyme-based sensor. Together, the sensors allow differentiation of nitroaromatic compounds – specifically, distinguishing acetylcholinergic nerve agents from the explosive compounds explored herein. The combined system can detect 2,4,6-trinitrotoluene, 2,4-dinitrotoluene and 4-nitrophenol with micromolar detection limits and affords subsequent differentiation from the nitro-containing nerve agent paraoxon. This demonstrates the advantage of merging elements of materials chemistry and biochemistry to devise customized sensors which can accurately identify hazardous chemical species.

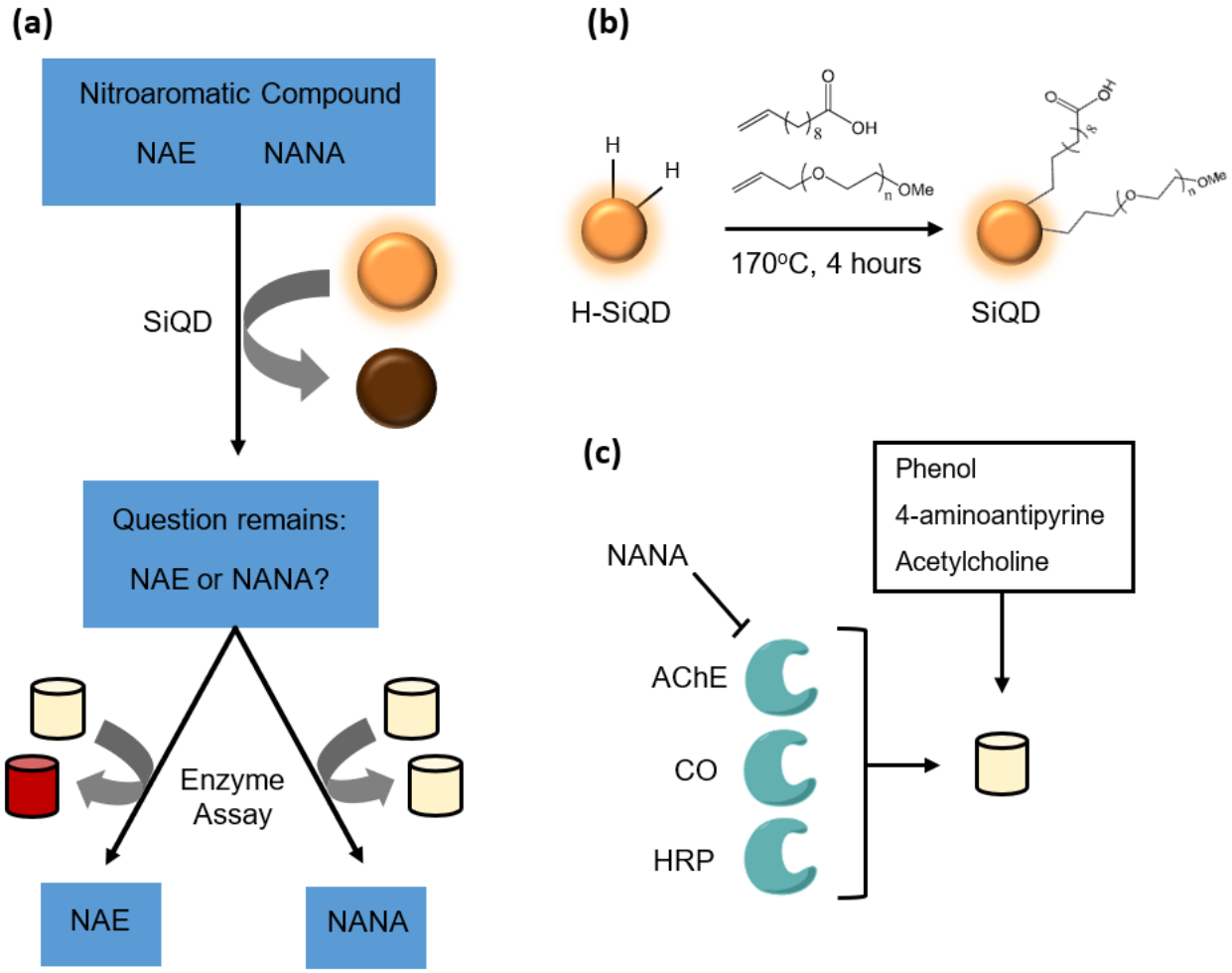
Nitroaromatic compounds (NACs) are molecules comprised of at least one aromatic ring bearing at least one nitro-group (-NO_2). They can be categorized into several classes of hazardous materials: many NACs are high energy materials (or explosives) — examples include 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) —others, like fenitrothion, parathion (PT) and paraoxon (PX) show adverse biological effects and act as nerve agents.[1,2] Beyond the obvious societal threats posed by explosives and nerve agents, these and other NACs are widespread pollutants and can be environmental hazards. For example, NACs can be carcinogens, cytotoxins, endocrine disruptors and/or reproductive toxins for a variety of organisms.[3] Complicating matters, NACs are generally resistant to degradation and accumulate in the environment which consequently increases the likelihood of harm to local populations.[3]

The potential risks noted above highlight the importance of developing cost-effective, sensitive, selective and accurate sensors that differentiate between the different classes of NACs. While modern analytical instrumentation can identify molecular structures with precision, most are costly, lab-based systems that require extensive, specialized training to operate.[4] Small, portable sensors have emerged as an important research area because they can offer rapid, inexpensive, 'in the field' NAC detection.[5] Fluorescence-based, on/off sensors are attractive options that rely on energy (or electron) transfer between the active sensing media and the target analyte that results in a detectable modulation of the fluorescence intensity.[6] The electron-withdrawing nature of pendant NO_2 groups their aromatic structural units make NACs electron acceptors (i.e., oxidizing agents) that can predictably quench the photoemission from luminophores. Early work involving silicon nanomaterial based photoluminescence sensors focused on porous silicon.[7] Sailor *et al.* characterized a vapor-phase porous silicon sensor for TNT, DNT and nitrobenzene which demonstrated semi-reversible quenching.[8] Subsequently,

Germanenko *et al.* demonstrated NAC quenching of isolated, unfunctionalized silicon nanocrystals, or silicon quantum dots (SiQDs), prepare using laser ablation.[9] Drawing on this and other foundational work involving porous silicon and SiQDs, we demonstrated that functionalized, organic-soluble SiQDs obtained from thermally-induced disproportionation of hydrogen silsesquioxane (HSQ) provided convenient probes for detecting nitroaromatic explosives (NAEs) and that water-soluble SiQDs prepared similarly could detect nitroaromatic nerve agents (NANAs; PX and PT); in both cases the luminescent SiQDs probe were interfaced with standard filter paper to provide a convenient strip sensor platform.[2,10] In the case of the water-soluble SiQDs, we further extended our study by combining the SiQD PL response with the persistent (i.e., non-quenched) luminescence of fluorescent proteins (FPs) to afford a sensitive, ratiometric sensor.[2]

The obvious chemical similarities of NAEs and NANAs (Figure S1) make differentiating these hazardous chemical classes non-trivial as well as an important outstanding challenge. Typical approaches toward distinguishing these chemically similar molecules involve creating detection arrays comprised of complementary sensing species that are subsequently tested against the same analyte; this approach provides a 'fingerprint' used to identify specific analytes.[11-13] Such fingerprinting systems are often complicated and require computer processing infrastructure to evaluate output data. Herein, we demonstrate a sensing platform that differentiates NAEs from NANAs using straightforward visible inspection (Scheme 1a). To achieve this this important advance we have exploited the complementary virtues of materials chemistry and biochemistry and expanded the scope of our water-soluble SiQD/FP sensor (see SiQD surface tailoring in Scheme 1b) by demonstrating NAE sensitivity. Subsequently, to distinguish NAEs from NANAs, we constructed a literature-inspired enzymatic acetylcholine detection assay that exploits the Trinder reaction to generate a readily detected red quinoneimine dye.[14] Enzymes are highly

specialized protein catalysts that exploit hydrogen-bonding as well as hydrophobic interactions at their active sites to engage targets with exceptionally high selectivity. In the past, enzymes have been combined and/or interfaced with nanomaterials to afford detection of



Scheme 1. a) Flow chart of the two-fold sensing platform presented in this work to differentiate nitroaromatic explosives (NAEs) and nerve agents (NANAs). b) Surface-functionalization of SiQDs for water-solubility. c) Assembly of the enzymatic assay involving acetylcholinesterase (AChE), choline oxidase (CO) and horseradish peroxidase (HRP). Presence of a NANA inhibits activity of AChE, preventing dye development.

microbes,[15-17] glucose,[18,19] and nerve agents;[2, 20-22] to our knowledge, no examples have demonstrated the capacity to differentiate closely related analytes like the noted subclasses of NACs (e.g., NAEs and NANAs). For our application, acetylcholinesterase was the enzyme of interest because it is inhibited by NANAs (Scheme 1c). This allowed us to devise an enzyme-

based sensor for use alongside our SiQD/FP sensor– the combination of these two sensing motifs provides clear differentiation between NAEs and NANAs.

The functionalized SiQDs that serve as the primary NAC detector in the presented sensing motif were prepared using established literature procedures developed in our labs.[23] A solid obtained from HSQ thermal treatment comprising an amorphous silica matrix and nanocrystalline silicon inclusions was mechanically ground and etched with alcoholic hydrofluoric acid to provide freestanding hydride-terminated SiQDs (H-SiQDs). Because detection of environmental contamination is commonly performed by evaluating water samples and water is also a preferred solvent in public settings (e.g., airport security), we chose to employ water-soluble SiQDs in the presented sensor.

To impart appropriate aqueous compatibility, H-SiQDs were functionalized with poly(ethylene oxide) methyl ether and 10-undecenoic acid using thermally-induced hydrosilylation (Scheme 1b).[24] Straightforward pairing of the functionalized SiQDs with an appropriate FP in aqueous buffered solution provided the ratiometric sensor. For the presented study, the FPs of choice were determined to be mAmetrine or mCerulean because their photoluminescence does not overlap with that of the SiQDs (Figure S2). The interaction of the SiQDs with NAC analytes results in quenching the SiQD luminescence while the fluorescence of the FPs is protected by their established barrel structure and remains unaffected.[2] Evaluation of the SiQD/FP combinations in

the presence of dilute NAE solutions (i.e., TNT, DNT and 4-nitrophenol (NP)) was performed using a Molecular Devices SpectraMaxi3x photoluminescence spectrometer (Figure 1).

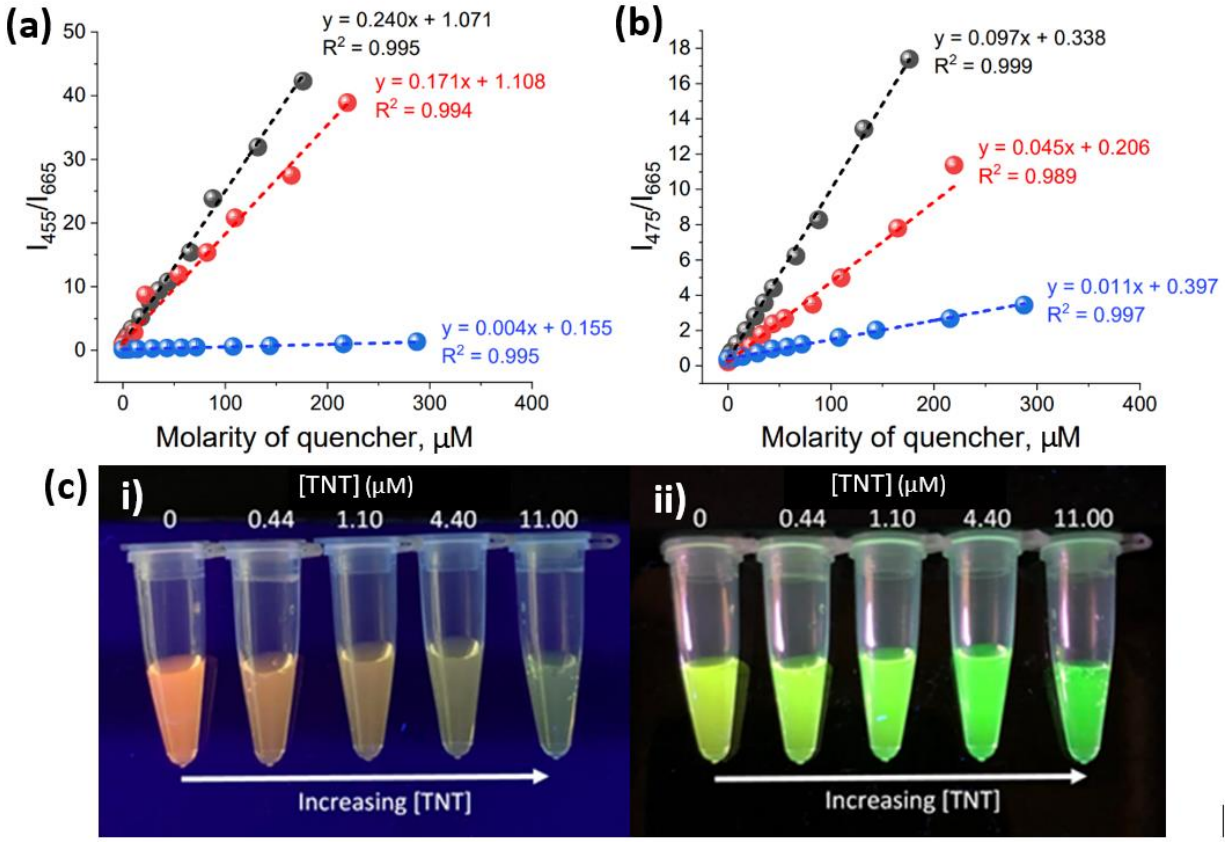


Figure 1. Representative photoluminescence quenching titration plots showing the dependence of the ratio of the photoluminescence intensities (i.e., I_{FP}/I_{SiQD}) upon excitation at 365 nm. Analyte titrations were performed with TNT (black), DNT (red), and NP (blue) for two ratiometric sensor FP/SiQD combinations: (a) mAmetrine ($\lambda_{PL,max}$ = 525 nm) and (b) mCerulean ($\lambda_{PL,max}$ = 475 nm). (c) Photographs of the photoluminescence quenching response of the mAmetrine/SiQD sensor to TNT exposure, using (i) an excitation source that selectively excites SiQDs, and (ii) a broadband UV source that excites the mAmetrine and SiQD luminophores.

By evaluating the PL quenching of the SiQD/FP mixtures, we determined the SiQD PL decreases linearly with increasing TNT, DNT and NP concentrations. We also tracked quenching ratiometrically by evaluating the ratio of the SiQD and FP emission (Figures 1 a, b). As expected, the magnitude of the quenching response qualitatively correlates with the number of nitro ($-NO_2$) functional groups bonded the aromatic ring of the analyte. These results were independent of the

FP used (Figure 1a,b). Using the linear plots and a standard IUPAC equation (Equation S2), the detection limits (L_D) for the NAE were determined to be 1.9, 3.7 and 10.6 μM for TNT, DNT, and NP, respectively, depending on the SiQD/FP pairing (Table S1). The present L_D values are modest when compared with those achieved using other fluorescence quenching systems;[25] this performance has previously been attributed to limited interaction between the SiQD surface and the NAE analyte and its optimization is the subject of ongoing investigation.[26]

Having demonstrated that the detection limit of NAEs was consistent with that previously reported for NANAs using the same SiQD/FP system (4.9 μM for PX),[2] we sought to design and incorporate the NANA-specific enzymatic assay to provide a secondary test to differentiate unknown nitroaromatics. Constructing the acetylcholine detection assay (See Supporting Information for details) in solution verified that PX inhibited AChE activity and, thus, the formation of the red quinoneimine dye. In contrast, TNT did not influence AChE activity and the red quinoneimine dye formed. Clearly, visual detection of the appearance of the characteristic red color of the quinoneimine dye provides differentiation of TNT from PX and by extension NAEs from NANAs. To gain insight into the rapidity with which NAEs and NANA can be detected and differentiated, quinoneimine dye development was temporally tracked in the presence of the PX and TNT (Figure 2). We note that the AChE population utilized in the present study can be saturated at an analyte concentration of 5 μM so this concentration was used for all subsequent trials (Figure 2a). In solution phase, the development time necessary to visually distinguish 5 μM PX samples from the control (PX 0 μM) is approximately 5 minutes. For context, this response time is approximately twice as fast as most recent reported colorimetric detection systems.[27-29] Of important note, and as expected, TNT did not show statistically significant inhibition (Figure 2b).

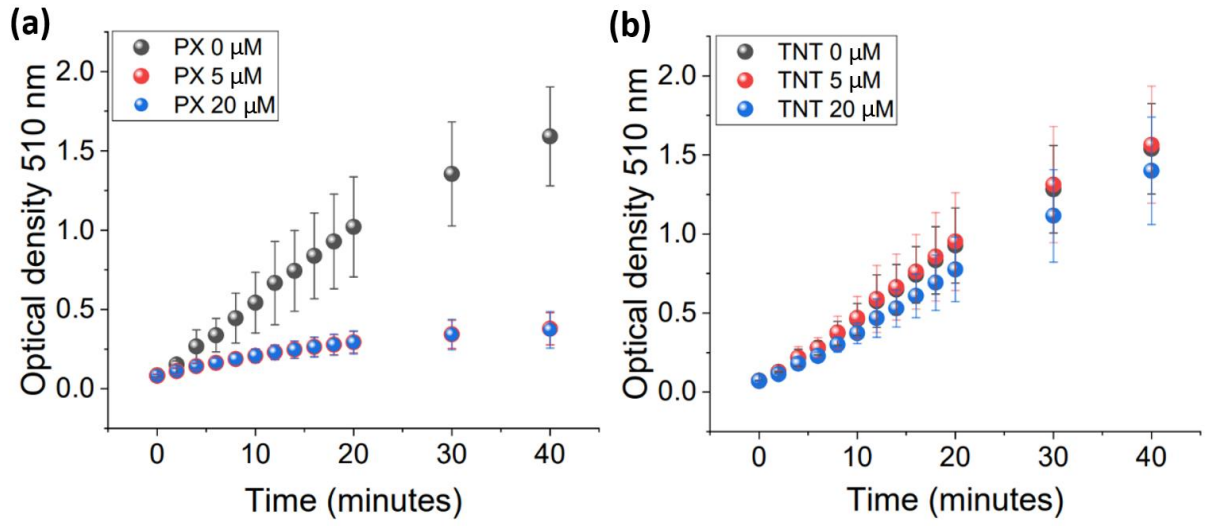


Figure 2. Solution-phase AChE inhibition trials for (a) PX and (b) TNT analytes. Inhibition is evidenced by an impairment of quinoneimine dye formation ($\lambda_{abs,max}$ = 510 nm), as monitored over time. Measurements were performed in triplicate, plotting the average and standard deviation.

Recognizing the well documented instability of enzymes toward ambient conditions,[30] we chose to address this potential limitation by immobilizing the present enzymes in an agarose gel. Our data indicate that the performance of the AChE detection assay in an agarose gel is near-identical to its performance in solution. DNT and NP behaved similarly to TNT, having no effect on the quinoneimine dye development (Figure 3). Importantly, the absorption spectrum after gel development highlights the selectivity of our approach and confirms that the increase in the absorption at 510 nm can be solely attributed to the formation of the quinoneimine dye (Figure 3c). The only notable difference from the solution-based assay, that we attribute to the increased viscosity of the gel medium, is gel-encapsulation slowed the dye development and extended the time necessary to visually differentiate PX and TNT to approximately 10 minutes. Furthermore, the added stability provided by the gel scaffold increases the longevity and portability of the assay and is expected to facilitate effective field deployment.

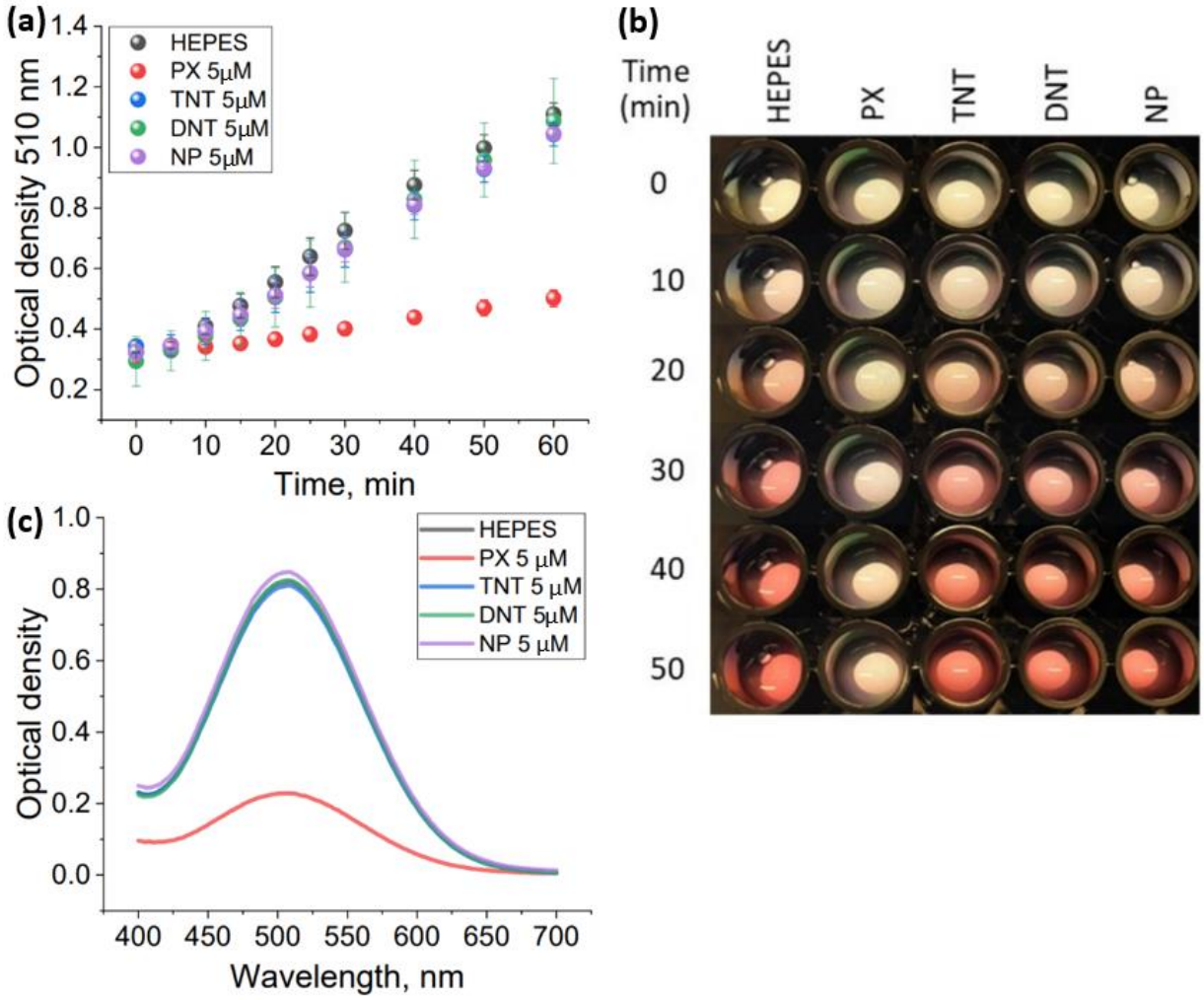


Figure 3. The gel-stabilized acetylcholine detection assay (a) expressed as the quinoneimine dye ($\lambda_{abs,max}$ = 510 nm) formed over time (n=3), (b) a visual representation of such, and (c) the absorption spectrum after 50 minutes of development. For triplicate results, the average and standard deviation were plotted.

To summarize, we have confirmed that our SiQD/FP luminescence-based sensor is sensitive to NAEs such as TNT, DNT and NP. Further, a secondary enzymatic assay can be used to obtain accurate and timely differentiation of hazardous nitroaromatic explosives and nerve agents. This proof-of-concept work demonstrate the versatility of combining SiQD luminescence sensors with enzymatic colorimetric sensors in the detection and differentiation of chemically similar analytes.

ASSOCIATED CONTENT

Supporting Information.

Silicon quantum dot synthesis and functionalization, experimental details for assembly and titration of silicon quantum dots and the enzymatic assay, detection limit calculations and values, silicon quantum dot and fluorescent protein photoluminescence spectra.

AUTHOR INFORMATION

Corresponding Author

*Jonathan G.C. Veinot – Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton, Alberta, T6G 2G2, Canada; orcid.org/0000-0001-7511-510X; Email: jveinot@ualberta.ca

Authors

Leanne Milburn – Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton, Alberta, T6G 2G2, Canada; orcid.org/0000-0001-8966-6375

Christopher Jay T. Robidillo – Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton, Alberta, T6G 2G2, CanadaDepartment of Physical Sciences and Mathematics, University of the Philippines Manila, P. Faura Street, Ermita, Manila 1000, Philippines; orcid.org/0000-0001-6492-5107

Rochelin Dalangin – Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton, Alberta, T6G 2G2, Canada; orcid.org/0000-0002-1914-507X

Present Addresses

† Department of Physical Sciences and Mathematics, University of the Philippines Manila, P. Faura Street, Ermita, Manila 1000, Philippines

†† Department of Biochemistry and Molecular Medicine, University of California, Davis, Davis, CA, 95616

Author Contributions

This work was conceived of by C.J.T.R and J.G.C.V. The described synthesis, sensor assembly, and testing were performed by L.A.M. under the guidance of C.J.T.R. and J.G.C.V. Fluorescent proteins were provided by R.D. The manuscript was written by L.A.M. and J.G.C.V. All authors have reviewed and approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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