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Turn-on detection of pesticides *via* reversible fluorescence enhancement of conjugated polymer nanoparticles and thin films†

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Reported herein is the significant fluorescence enhancement of conjugated polymer nanoparticles in the presence of aromatic organochlorine pesticides. This pesticide-mediated fluorescence enhancement leads to reversible pesticide detection systems with high sensitivity (as low as 5 μM), as well as significant generality and straightforward reversibility.

The widespread use of pesticides has been highly effective in increasing the harvested yields of many crops worldwide through eliminating the threat of common pests, but their use has also been of concern due to their known and suspected toxicity to humans and other species, as well as their long term environmental persistence.[1] One class of pesticides that is of continuing concern is organochlorine pesticides (OCPs), the most common of which is dichlorodiphenyltrichloroethane (DDT), sold commercially as a mixture of the *para*, *para*- (compound **1**, Fig. 1) and *ortho*, *para*- (compound **4**) isomers.[2] Dichlorodiphenyldichloroethane (DDD, compound **2**) and dichlorodiphenyldichloroethylene (DDE, compound **3**) are some of the metabolites of DDT, also with known toxicities.[3]

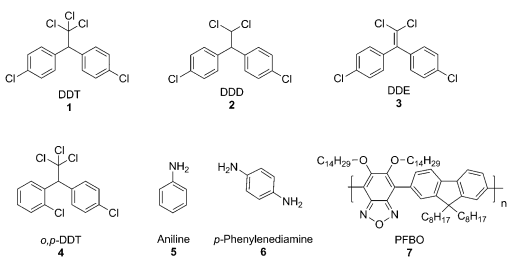

Fig. 1 Pesticides (**1–4**), control analytes **5–6**, and conjugated polymer **7**.

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† Electronic supplementary information (ESI) available: Syntheses of polymers **7** and **9**, experimental details for fluorescence experiments, dynamic light scattering experiments, nanoparticle and thin film fabrications. See DOI: 10.1039/c6nj00690f

Techniques for the detection of organic pesticides generally rely on chromatography followed by mass spectrometry.[4] These methods offer good sensitivity and resolving power, but suffer from the high cost of operation and tedious and time-consuming sample preparations,[5] which limits the ability to conduct high throughput assays. Newer techniques for pesticide detection include molecularly imprinted polymer systems,[6] nanoparticle-based immunoassays,[7] and gold nanoparticle-based Raman spectroscopy.[8] A variety of fluorescence-based methods for pesticide detection have also been reported,[9] although in many cases these methods require derivatization steps,[10] chromatographic purification,[11] and/or are substantially limited in terms of the range of pesticides that can be detected.[12]

One method of detection that has shown a lot of promise in the detection of multiple classes of analytes with extremely high sensitivity and selectivity is the use of conjugated fluorescent polymer sensors.[13] Typically, detection efficiencies are optimal in polymer aggregates such as thin films[14] or conjugated nanoparticles,[15] which enable inter-polymer as well as intra-polymer exciton migration.[16] Formation of conjugated polymer-derived nanoparticles can occur through a variety of methods,[17] including reprecipitation,[18] in which the hydrophobic polymer collapses upon its introduction into aqueous solution, resulting in the formation of well-defined spherical nanoparticles.

Reported herein is the detection of DDT and its metabolites (compounds **1–4**) in aqueous solutions *via* the fluorescence enhancement of nanoparticles derived from conjugated organic polymers. These particles were fabricated *via* the reprecipitation of 2,1,3-benzooxadiazole-*alt*-fluorene (PFBO, polymer **7**), synthesized following literature-reported procedures.[19] This polymer was fully characterized by spectroscopic techniques, with a M_n = 3.8 × 10^3 g mol^{-1} and M_w = 7.3 × 10^3 g mol^{-1}. The polymer-derived nanoparticles were characterized by dynamic light scattering experiments, with an average particle diameter of 139 nm (see ESI† for details).

The degree of fluorescence changes observed with the introduction of aromatic pesticides to the aqueous nanoparticle (or free polymer) solution was calculated according to eqn (1):

$$\text{Fluorescence modulation} = \text{PFBO}_{70\mu\text{M}}/\text{PFBO}_{0\mu\text{M}} \quad (1)$$

where $\text{PFBO}_{70\mu\text{M}}$ is the integrated polymer fluorescence in the presence of 70 μM analyte in acetonitrile, and $\text{PFBO}_{0\mu\text{M}}$ is the integrated polymer fluorescence in the presence of 0 μM analyte in acetonitrile. Little to no fluorescence interference from the pesticides themselves is expected due to the fact that these analytes show absorption and emission maxima primarily in the ultraviolet region of the UV-Vis spectra,[20] well removed from the absorption and emission of the donor–acceptor polymer (λ_{max} absorption: polymer = 413 nm; nanoparticles = 411 nm; λ_{max} emission: polymer = 507 nm; particles = 534 nm).[21] The concentration of 7 was varied (see ESI† for more details), and optimal fluorescence responses were obtained with a 1.25×10^{-3} mg mL^{-1} polymer solution.

Results of the fluorescence modification experiments are shown in Table 1 and Fig. 2, and key trends are discussed in further detail below.

Fluorescence enhancements of the PFBO nanoparticles were observed in the presence of compounds **1–4**. In contrast to the strong fluorescence responses observed in the case of the conjugated polymer-derived nanoparticles, the conjugated polymer itself displayed a marked insensitivity to the presence of any of the pesticides investigated (Table 1 and Fig. 3). The strong dependence of the PFBO fluorescence responses on its aggregation state indicates the necessity of inter-chain polymer communication to enable efficient fluorescence enhancement behaviors, a result that has been demonstrated previously in the literature for the detection of other analytes, although not for the detection of pesticides to date.[22] Additionally, the differential responses of compound **1** and compound **4** are particularly noteworthy as these compounds are structural isomers (with identical masses) and would be difficult to differentiate using standard mass-spectral techniques.

Table 1 Average% change fluorescence of PFBO **7** and particle size with added pesticide

Analyte	Fluorescence modulation particle[a]	Fluorescence modulation polymer[a]	Size[b] (nm)
1	2.47	1.02	220
2	1.17	1.03	164
3	3.48	0.96	190
4	3.08	1.01	205
5	1.02		
6	0.99		

[a] Fluorescence modulation calculated according to eqn (1); [PFBO] = 1.25×10^{-3} mg mL^{-1}. [b] Particle size with 70 μM analyte in acetonitrile as measured by dynamic light scattering experiments.

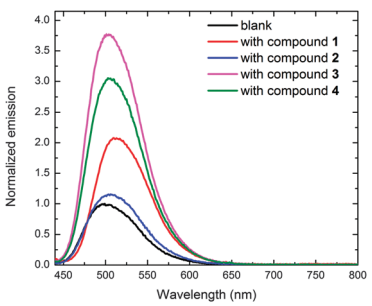

Fig. 2 Fluorescence changes of PFBO nanoparticles in the presence of pesticides **1–4**. [PFBO] = 1.25×10^{-3} mg mL^{-1}.

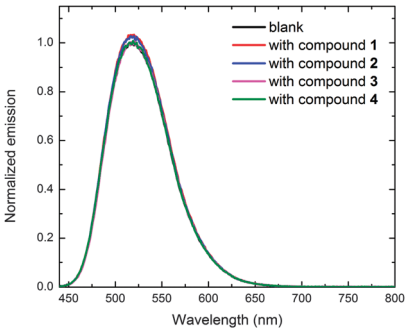

Fig. 3 Fluorescence changes of PFBO polymer in the presence of pesticides **1–4**. [PFBO] = 1.25×10^{-3} mg mL^{-1}.

This phenomenon was shown to be specific for organochlorine pesticides by measuring the fluorescence responses of the nanoparticles to aromatic compounds **5** and **6**, which have been found in a variety of food products.[23] Neither analyte was found to effect significant fluorescence changes (a fluorescence modulation value of 1.02 with 70 μM of analyte **5** in acetonitrile; a value of 0.99 with 70 μM of analyte **6** in acetonitrile). Substantially higher concentrations of the control analytes led to limited fluorescence decreases of the nanoparticle solution (Fig. 4), highlighting the selectivity of the fluorescence-based detection system.

The sensitivity of the fluorescence enhancement-based detection for analytes **1–4** is shown through the low limits of detection (Table 2),[8] which approach current levels of concern for these pesticides[24] and highlight the practicality of this fluorescence-based detection system. Other literature-reported detection systems for these compounds have also been reported, with somewhat more sensitive detection limits (8 μg L^{-1} for a custom-made C18 column;[20] 50 ppt for a molecularly imprinted polymer),[25] although many of these systems may have other operational disadvantages.

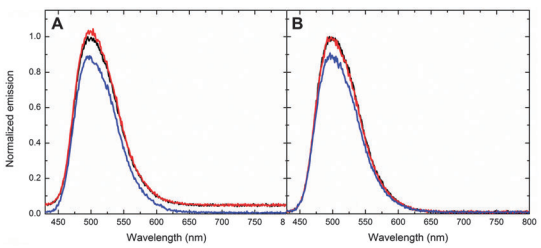

Fig. 4 Fluorescence changes of PFBO nanoparticle solutions in the presence of (A) analyte **5** and (B) analyte **6**. The black line represents emission in the presence of 0 μM analyte, the red line represents emission in the presence of 70 μM analyte, and the blue line represents emission in the presence of 1 mM analyte.

Table 2 Limits of detection for pesticides **1–4** and literature-reported levels of concern

Analyte	LOD (ppm)	Literature-reported levels of concern (ppm)
1	1.6	0.05–5[26]
2	33.8	0.05–5[26]
3	27.9	0.05–5[26]
4	26.2	0.05–5[26]

Literature precedent by Swager and co-workers demonstrated that fluorescent polymer systems underwent reversible fluorescence enhancements as a result of analyte-mediated reduction of the polymer chain,[27] an effect that was easily reversed by introduction of iodine vapor.[28] Although similar reversibility was observed in this system, with the fluorescence increases demonstrated by solutions of polymer **7**-derived nanoparticles in the presence of analyte **1** nearly completely reversed with the addition of iodine (Fig. 5A), compound **1** is highly unlikely to act as an effective reductant of the polymer chain.[29] Rather, the reversibility in our system is likely a result of the formation of reversible charge-transfer complexes between the conjugated polymer chain and iodine vapor, which is disrupted with the addition of aromatic organochlorine pesticides that are able to pi-stack efficiently with the conjugated polymer chain. Selectivity for compounds **1–4** compared to control analytes **5** and **6**, in turn, is likely due to the electron deficient nature of analytes **1–4** and the resultant electronic complementarity with the conjugated polymer. Other examples of iodine doping of conjugated polymer systems have also been reported,[30] although to the best of our knowledge, this phenomenon has not been used for reversible fluorescence-based detection to date. The fact that this fluorescence switching was reversible over several cycles (Fig. 5B) is highly significant for the development of practical fluorescence detection systems.

Oftentimes fluorescence enhancements of conjugated polymer-derived nanoparticles involve macroscopic changes in the particle architecture that translate into measurable fluorescence response changes;[31] however, in this case the addition of pesticides **1–4** effected little to no change in the average particle size and size distribution (Fig. 6).

An extension of this fluorescence-based detection to polymer **7**-derived thin films was conducted by fabricating fluorescent thin films from the spin casting of a polymer **7** solution in chloroform

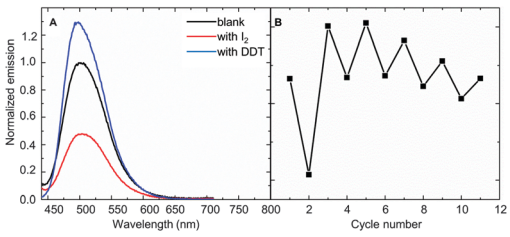

Fig. 5 (A) Illustration of reversibility of fluorescence changes of polymer **7**-derived nanoparticles (polymer treated with I_2 prior to addition of compound **1**). (B) Switching behavior of polymer **7**-derived nanoparticles with alternating additions of I_2 and compound **1** over 11 cycles.

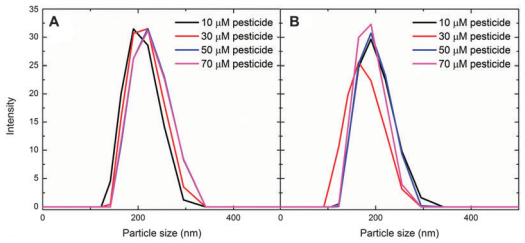

Fig. 6 Dynamic light scattering experiments of polymer **7**-derived nanoparticles with (A) pesticide **1** and (B) pesticide **2**, indicating no significant changes in particle size in the presence of the pesticides.

onto glass slides. These films were briefly exposed to the vapor from a solution of compound **1** in tetrahydrofuran. The measurable response of these films to compound **1** vapor (Fig. 7A) is remarkable considering the low vapor pressure of compound **1**,[32] and indicates high levels of sensitivity in these fluorescent polymer-derived detection systems. Moreover, control experiments indicated that the tetrahydrofuran itself had negligible effects on the photophysical properties of polymer **7**-derived thin films. These fluorescence changes were also reversible with exposure of the thin film to iodine vapor, leading to a nearly complete return to the initial thin film fluorescence state (1.27-fold increase followed by 1.20-fold decrease, Fig. 7).

Finally, the fluorescence responses of other conjugated polymers (Fig. 8) in the presence of 70 μM of compound **1** were measured, and the results are summarized in Table 3 and Fig. 9. These polymers were either commercially available (compounds **8**, **10**, and **11**) or easily synthesized using a synthetic procedure developed for the undergraduate teaching laboratory (compound **9**).[33] For most of the polymers, analogous fluorescence enhancements in the presence of compound **1** were observed, highlighting the general applicability of the pesticide-mediated fluorescence

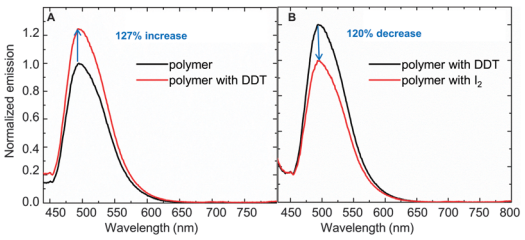

Fig. 7 Fluorescence changes of thin films of polymer **7** with exposure to vapors from compound **1**.

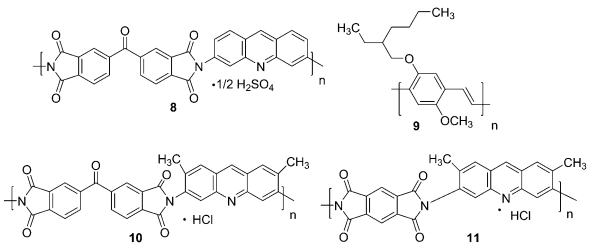

Fig. 8 Structures of other fluorescent conjugated polymers investigated.

Table 3 Average% change fluorescence of polymers **8–11** with 70 μM analyte **1**[a]

Polymer	Fluorescence modulation particle[a]	Fluorescence modulation polymer[a]
8	1.81	0.96
9	2.34	1.03
10	1.67	1.28
11	1.23	0.94

[a] Fluorescence modulation calculated according to eqn (1); [Particles] = 1.25×10^{-3} mg mL^{-1}; [Polymers] = 1.25×10^{-3} mg mL^{-1}.

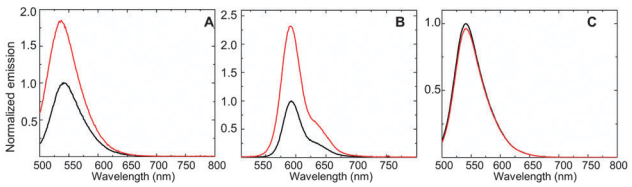

Fig. 9 Illustration of fluorescence emission of conjugated polymers in the presence of 70 μM of analyte **1** with: (A) polymer **8** in nanoparticles; (B) polymer **9** in nanoparticles; and (C) polymer **8** in free solution. The black line represents the fluorescence emission of the polymer in the presence of 0 μM analyte **1** and the red line represents the emission of the polymer in the presence of 70 μM analyte **1**.

enhancements. In all cases, the fluorescence enhancements of the nanoparticle solution were markedly higher than the enhancements observed in the presence of the free polymer, which confirms the importance of inter-polymer communication in enabling the highly sensitive fluorescence changes to occur.

In summary, reported herein is the substantial fluorescence enhancement of PFBO-derived nanoparticles and thin films in the presence of aromatic organochlorine pesticides, and marked class-specific fluorescence changes of PFBO-derived nanoparticles in the presence of a variety of other small molecule pesticides. These fluorescence responses have a number of notable features, including: (a) a requirement for polymer chain aggregation to enable efficient inter-polymer exciton migration; (b) high levels of reversibility through the introduction of iodine vapor; (c) a 'turn-on' rather than 'turn-off' fluorescence signal, which has the potential to lead to improved sensitivity in practical detection schemes; (d) low limits of detection, which approach practical levels of concern; and (e) general applicability for other fluorescent organic polymers, including both commercially available and easily synthesized polymers. Efforts towards developing practical turn-on detection systems for aromatic pesticides based on this research are currently in progress in our research laboratory, and results of these and other investigations will be reported in due course.

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