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Ratiometric Fluorescent Sensor for 2,4,6-Trinitrotoluene Designed Based on Energy Transfer between Size-different Quantum Dots

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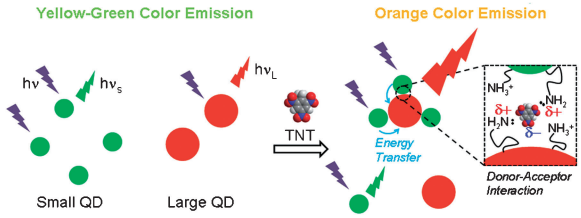
Aggregation of two different sizes of semiconductor quantum dots is successfully applied to a novel ratiometric fluorescent sensor, in which Förster type energy transfer between quantum dots is induced by the interaction with 2,4,6-trinitrotoluene (TNT) as a binder of quantum dots. The detection limit for TNT achieved is 5 pM, and the system shows relatively high sensitivity against TNT in comparison to 2,4-dinitrotoluene and 2-nitrotoluene at 5 pM–500 nM range.

Nanoparticles designed for semiconductors and metals are gathering a great deal of interests because of their fascinating nature characterized by quantum confinement of electrons or surface plasmon oscillation.[1] In particular, assemblies of nanoparticles are expected to be novel versatile tools in various fields including sensors, optoelectronics, and catalysts.[2] As a unique sensing application, Mirkin et al. employed aggregation of gold nanoparticles featuring a change in the color from surface plasmon resonance and succeeded in detection of hybridization with DNA.[3] However, aggregation of semiconductor quantum dots (QDs) has scarcely been applied for detecting analytes.

QDs are promising nanomaterials with characteristic emission depending on the particle size; the smaller the size is, the shorter the wavelength emission becomes. Recently, a few fundamental research papers about energy transfer between QDs appeared, which demonstrated that when the sizes are different, the energy of excited small QDs is transmitted to large QDs with lower exciton energy.[4] It thus occurred to us that aggregation-induced energy transfer between size-different QDs can be utilized for detection of organic analytes that feature π–π, donor–acceptor, and hydrogen-bonding interactions. Another interesting characteristic of QDs is that size-different QDs can be excited concurrently at a single wavelength band expanding from ultraviolet to visible wavelengths. These advantages will enable us to apply QDs to a ratiometric fluorescent detection method that utilizes the fluorescence intensity ratio of two emission bands. Conventional detection methods using a single wavelength emission band are prone to cause errors of detected signals, especially in weak signal response. In the ratiometric sensing, on the other hand, emission spectra are changed by added analytes and allow us to achieve not only accuracy enhancement but also visually detectable response.[5]

As a detection target we chose an explosive compound. Detecting explosives like 2,4,6-trinitrotoluene (TNT) is an important public safety issue. In particular, sensitive detection of trace analytes is of great important. So far, several TNT sensors have been fabricated on the basis of fluorescence, colorimetric, and electrochemical techniques.[6,7] As examples of fluorescence systems, small molecule fluorophores,[6] conjugated polymers,[6,8] and semiconductor quantum dots (QDs)[9] have been used as reporter materials, but examples using QDs are limited either to fluorescence quenching or recovery sensors or to Förster resonance energy-transfer (FRET) sensors employing organic dye molecules or gold nanoparticles.[10,11] We considered, therefore, that a new type of a fluorescence sensor system using FRET between QDs would be designed by utilizing the mechanism if TNT can work as a trigger for the aggregation of two different kinds of QDs. Recently, for design of a TNT sensor, Mao et al. utilized a change in the gold nanoparticle aggregation mode induced by the donor–acceptor interaction between TNT and amino groups of ligands deposited on gold nanoparticles.[12] It occurred to us that a change in the QD aggregation mode by the donor–acceptor interaction, which can induce a shift of the emission maximum leading to ratiometric sensing, should work better than gold nanoparticles (Scheme 1). Advantages of employment of QDs are shown as follows: First, QDs possess high stability relative to conventional organic dyes. This point is very important for sensor application to accomplish accuracy and reproducibility. Second, molecular recognition sites on QDs can be introduced simply by ligand modification or ligand exchange reactions. Third, the specific emission character depending on sizes, figures, and materials facilitates fine optimization of the sensors by their combinations in response to needed performance.



Scheme 1. Schematic diagram of this research concept; small QDs and large QDs emit green and red color fluorescence, respectively, resulting in yellow-green color emission from the mixtures. TNT binds QDs through the donor–acceptor interaction between TNT and amino groups of the ligands on QDs. The aggregation of QDs allows energy transfer from small QDs to large QDs, resulting in orange color emission from the mixtures.

The QDs used were purchased from Invitrogen Co: "Qdot® ITK™ amino (PEG) quantum dots, CdSe/ZnS," whose ligands are covalently attached poly(ethylene glycol)s (PEG) linking to amino groups at the terminal ends (Scheme S1).[19] The emission maxima of two QDs were 520 nm (green emission) and 605 nm (red emission), ascribable to the emission maxima from small

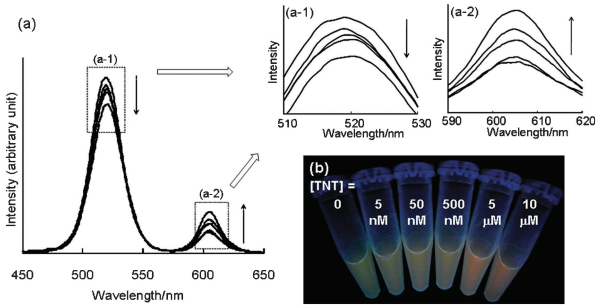


Figure 1. (a) Typical fluorescence spectra of QDs mixture without or with TNT; [TNT] = 0–5 × 10^{-6} M; (a-1) and (a-2) are magnified regions around two peaks. (b) Fluorescent photograph of QDs mixtures with TNT; [small QD] = 4 × 10^{-9} M, [large QD] = 6 × 10^{-10} M, λ_{ex} = 254 nm, 10 mM phosphate buffer (pH 8) containing 2.5 vol % methanol and 2.5 vol % acetonitrile.[19]

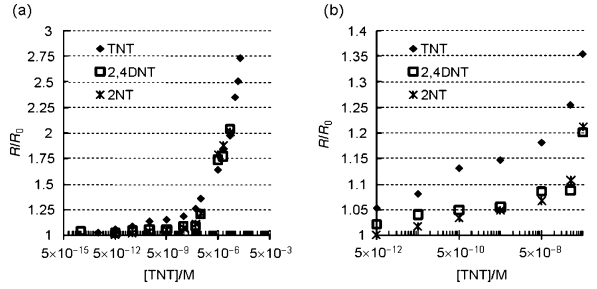


Figure 2. (a) Relative intensity ratio of 605 and 525 nm emission as a function of added TNT; R/R_0 is represented by $(I_{605}/I_{520})/(I_{0\,605}/I_{0\,520})$, where $I_{0\,605}/I_{0\,520}$ is evaluated from QDs mixture itself. (b) Magnified range from 5 × 10^{-12} to 5 × 10^{-6} M of TNT; [small QD] = 4 × 10^{-9} M, [large QD] = 6 × 10^{-10} M, λ_{ex} = 254 nm, 10 mM phosphate buffer (pH 8) containing 2.5 vol % methanol and 2.5 vol % acetonitrile.

and large QDs, respectively. For the FRET system, one of important factors is the spectral overlapping between emission band of the donor and absorption band of the acceptor. In our system also, the overlap appeared as shown in Figure S1.[19]

Figure 1a compares fluorescence spectra of QDs mixtures before and after adding TNT. The QDs mixture itself showed emission peaks at 520 and 605 nm that were emitted from small QD and large QD, respectively. Upon adding TNT, the emission intensity changes that accompanied the decrease in 520 nm and the increase in 605 nm were observed. As shown in Figure 1b, one can clearly confirm that the fluorescence colors were changed from yellow-green to orange depending on the added TNT concentrations. Although the spectral change is apparently small, it is very reproducible and detectable even with the naked eye.

Figure 2 shows the intensity ratio of 605 and 520 nm emission as a function of added TNT; herein, R/R_0 is represented by $(I_{605}/I_{520})/(I_{0\,605}/I_{0\,520})$, where $I_{0\,605}/I_{0\,520}$ is evaluated from the QDs mixture itself. Upon the increase in added TNT, the value of R/R_0 was raised, according to the enhanced intensity at 605 nm and the quenched intensity at 520 nm. This finding supports the view that QD aggregates are formed, where energy transfer between two different sizes of QDs is taking place in response to added TNT. The detection limit of this system is 5 pM based on a 5% change of the signal as a detectable standard.[9b,13] The ratiometric sensing employed here facilitates amplification and reliability of detection signals by using the intensity ratio of two emission bands with modest changes, therefore, we have succeeded in sensitive and reliable detection for a small amount of TNT. In the presence of TNT above 10 μM, the emission intensities of QDs were decreased in comparison to those of the QDs mixture itself. It is known that the CT complex formed between TNT and amine can work as a quencher of Mn^{2+}-doped ZnS nanocrystals.[9b] In the present system also, a large amount of CT complexes would play a similar role under the concentrated condition although the quenching effect for QDs was not conspicuous at lower concentration of TNT around nanomolar level.

For investigating the TNT-sensing sensitivity, 2,4-dinitrotoluene (2,4DNT) and 2-nitrotoluene (2NT) were subjected to this system. In a concentration range 5 pM to 500 nM, TNT showed larger signal changes than 2,4DNT and 2NT, from which one can confirm the superior response of TNT in this system over 2,4DNT and 2NT. For high concentration of these nitrobenzene-based acceptors (>500 nM), remarkable differences were not detected among the acceptors. Presumably, a large amount of acceptors may induce similar aggregation of QDs, as indicated from nonlinear drastic increments of the signals under the above condition. As detection limits, 500 pM and 5 nM were needed for 2,4DNT and 2NT, respectively to accomplish 5% changes. Therefore, this system can work more sensitively and selectively for TNT, resulting from its higher electron-accepting character over others.

In this system, TNT will work as a binder of QDs through the donor–acceptor interaction between TNT and amino groups of QD ligands. Zhang et al. have reported that CT complex formation between TNT and amino groups shows a broad absorption band in a visible region (ca. from 300 to 700 nm).[9b,14] Figure S2a shows UV–vis absorption spectra of the QDs mixture and that mixed with TNT.[19] By addition of TNT, a new absorption band appeared in the 300–700 nm region, as clearly recognized from the differential spectrum in Figure S2b.[19] One can confirm that the donor–acceptor interaction does exist between TNT and amino groups on the ligands of QDs and does facilitate the assembly formation.

To further confirm the binding role of TNT, transmission electron microscope (TEM) observations were conducted (Figure 3). On the QDs mixture itself, two different sizes of QDs were observed discretely (Figure 3a). Small QDs were spherical particles whose diameter was around 4 nm, whereas large ones were ellipsoidal particles whose diameters were about 9 nm in the major axis and 4 nm in the semiminor axis. In the case of QDs mixture with TNT, on the other hand, QDs were observed as the aggregated state and as the individually dispersed state (Figure 3b). We could confirm from the TEM image that not only the aggregate consisting of the different size QDs but also that consisting of the same size QDs is formed in the presence of TNT. We consider that the former aggregate must be the origin of the fluorescence intensity change through the energy transfer. In the FRET system, it is known that the fluorescence lifetime change is observed for both donors and acceptors; the fluorescence lifetime of a donor is shortened and that of an acceptor is delayed, accompanying the decreased or the increased fluorescence intensity, respectively.[15] The fluorescence lifetime of QDs was obtained as a single average

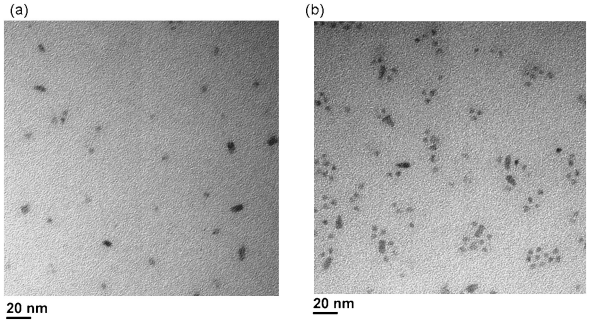


Figure 3. TEM images of QDs mixtures (a) without or (b) with TNT; [small QD] = 4×10^{-9} M, [large QD] = 6×10^{-10} M, and [TNT] = 5×10^{-6} M.

lifetime from the first-order fitting analysis of time-resolved fluorescence spectra (Figure S3).[19] For the QDs mixture itself, the fluorescence lifetimes of small QD and large QD were 22.2 and 16.9 ns, respectively (Table S1).[19] By addition of 5 μM TNT, on the other hand, the lifetimes were changed into 21.8 and 17.5 ns. As shown in Figure 1a, the fluorescence intensities of small QD and large QD showed the decrease or the increase by adding TNT. From these results, one can conclude that the changes in the fluorescence lifetime and intensity indicate the appearance of FRET between two different sizes of QDs.

In conclusion, we have succeeded in fabricating a novel TNT-sensing system that employs FRET between size-different QDs. The FRET is induced by aggregation of two different sizes of QDs through a donor–acceptor interaction between a target molecule TNT and amino groups of QD ligands. Moreover, this system features ratiometric sensing, providing high sensitivity and a unique fluorescence color change. The conventional approaches of QD-based sensors basically utilized a change in the fluorescence intensity at a single wavelength and in particular, FRET systems were scarcely utilized in combination with QDs. In fact, biosensing using FRET between QDs was limited to only two reports from Kotov group and Nie group.[16,17] On the other hand, only one example for chemical sensing was reported by Chou group, targeting potassium ion with a detection limit of 10^{-6} M.[18] In this research, we have succeeded for the first time in demonstrating the promising feature of a ratiometric fluorescent technique for chemosensing of 10^{-12} M level explosive organic compounds by using the FRET system between QDs. What we have to take into consideration to achieve further selectivity and sensitivity are the design of a molecular recognition based switch for aggregation or disaggregation of QDs and the ligand design of QD that facilitates effective FRET by shortening the separation distance between donor QD and acceptor QD. In our system, appropriate tuning by employing different sizes and kinds of QDs and design of molecular recognition sites depending on purposes have been accomplished. We believe, therefore, that this finding will develop various future applications in sensing and imaging fields utilizing QDs.

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