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Fluorescent Quantum Dots with Boronic Acid Substituted Viologens To Sense Glucose in Aqueous Solution**

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Since their development in the 1980s, fluorescent quantum-dot semiconductor nanoparticles have increasingly replaced traditional organic fluorophores in applications such as biomolecule tagging, tissue imaging, and ion sensing.[1–6] Interest in fluorescent quantum dots (QDs) derives from their broad absorption, narrow emission, intense brightness, and good photostability relative to organic dyes.[7] Surprisingly, despite the large and diverse set of fluorescence-based sensing systems for glucose,[8–11] no methods for glucose detection that utilize inherently fluorescent QDs have been reported.[12] Previously, we demonstrated a very general two-component glucose-sensing system in which glucose modulates the ability of a boronic acid substituted viologen quencher/receptor to quench the fluorescence of anionic organic dyes.[13–15] Signal modulation occurs when glucose binds to the boronic acid receptor moiety, which at pH 7.4 exists in its trigonal neutral form in the absence of glucose.[16] Formation of the more-acidic glucose boronate ester shifts the acid–base equilibrium of the boronic acid towards its anionic tetrahedral "-ate" form. These electronic and/or steric changes, which have been confirmed with ^{11}B NMR spectroscopy, cause a decrease in the quenching interaction between viologen and fluorophore and result in an increase in fluorescence. This two-component approach to glucose sensing allows considerable flexibility in choosing the quencher/receptor and fluorophore components depending on the particular requirements of the sensing application. For example, fluorophore components may be selected to provide any one of a range of desired excitation or emission wavelengths, whereas a particular quencher/receptor may be chosen for reasons of its monosaccharide-binding selectivity. Herein we show that some of the advantages of QDs can be realized in our two-component system to sense changes in glucose concentration in aqueous solution. The putative mechanism for glucose sensing with quantum dots and

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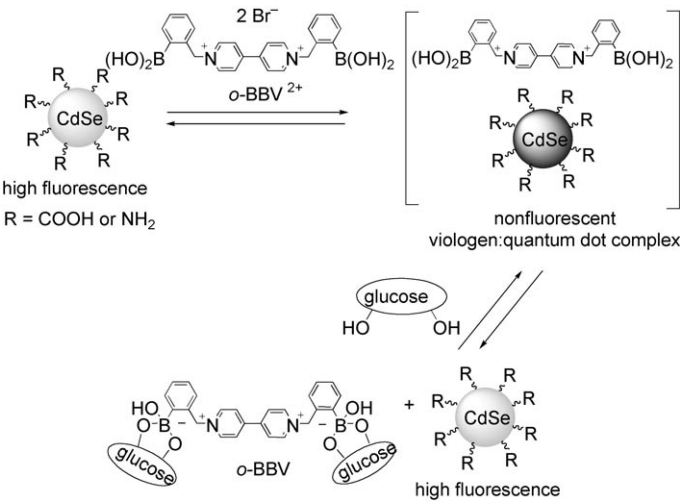
boronic acid substituted viologen quenchers/receptors is shown in Scheme 1.



Scheme 1. Putative mechanism for glucose sensing with fluorescent QDs.

Typically, fluorescent QDs are constructed from inorganic semiconductor core materials such as CdTe and CdSe, coated with an insulating shell material such as ZnS, and further treated to provide the desired surface chemistry. For the preparation of water-soluble core–shell QDs, surface functionalization with phosphonate, carboxy, or amine groups is often employed. The particular surface chemistry allows the QDs to bind to molecules of interest such as proteins and also determines their solubility, aggregation behavior, and sensitivity to quenching processes. Several groups have observed quenching of QD fluorescence with methyl viologen (MV^{2+}).[1,17–19] The process is believed to occur through excited-state electron transfer from the QD to the viologen, thus resulting in reduction of the viologen to $MV^{\bullet+}$. Our previous studies showed that viologens were extremely efficient in statically quenching the fluorescence of many organic dyes through complex formation with the fluorophore.[13,14] We reasoned that the fluorescence of core–shell QDs that bear polar surface groups such as carboxy and amine groups might be similarly quenched through complex formation with our boronic acid substituted viologen quenchers.

To test our hypothesis, we examined two sets of commercially available core–shell CdSe QDs coated with ZnS, which were identically prepared except for their surface functionalization; one set was prepared with carboxy groups on the surface, the other with amine groups.[20] Both sets had a fairly narrow fluorescence emission centered at 604 nm. We found that these QDs indeed functioned in our system in a manner similar to that of organic dyes: they showed a decrease in fluorescence upon the introduction of viologen quencher. To our delight, we also found a robust fluorescence recovery when glucose was added to the quenched QD solutions (Figure 1).

The sensitivity of both QD sets to fluorescence quenching by the boronic acid substituted viologen *o*-BBV^{2+} was

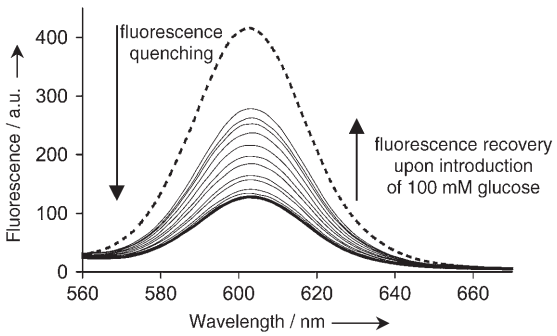


Figure 1. Characteristic fluorescence response upon introduction of quencher followed by glucose to amine-functionalized QD solution (5×10^{-8} M) at pH 7.4. Final quencher/QD (*o*-BBV^{2+}/NH_2 QD) ratio for this data = 50:1, final glucose concentration = 100 mM. The dashed line indicates unquenched fluorescence, the bold line indicates fluorescence after introduction of quencher.

determined in an aqueous solution at pH 7.4 (Figure 2). We found that the fluorescence of both the carboxy- and amine-substituted QDs were sensitive to quenching by *o*-BBV^{2+},

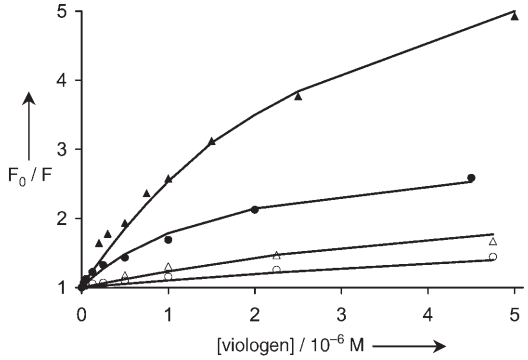


Figure 2. Stern–Volmer plot showing the quenching of the fluorescence of amine- and carboxy-substituted QDs (5×10^{-8} M) at pH 7.4 by *o*-BBV^{2+} and BV^{2+}. ▲ COOH QDs with *o*-BBV^{2+}, ● NH_2 QDs with *o*-BBV^{2+}, △ COOH QDs with BV^{2+}, ○ NH_2 QDs with BV^{2+}.

with the carboxy-substituted QDs showing a stronger sensitivity to quenching than the amine-substituted dots. Quenching experiments conducted at various temperatures showed that quenching was more efficient at lower temperatures, thus indicating a static quenching mechanism that involves complex formation between QD and viologen quencher.[21] We found that the fluorescence of both sets of QDs was also similarly quenched by simple unsubstituted benzyl viologen (BV^{2+}), though to a lesser degree than with *o*-BBV^{2+}, which suggests that the boronic acids may play some role in the quenching process. Control quenching experiments with 3-nitrophenylboronic acid showed that, on its own, the boronic acid produces insignificant quenching (less than 2% of that observed with *o*-BBV^{2+}).[21] Significantly, although we were unable to determine the degree of ionization of the surface groups, the carboxy-substituted dots are expected to exist primarily in their anionic form at pH 7.4, whereas the amine dots are most likely to be neutral. We speculate that the

enhanced sensitivity of the carboxy-substituted QDs is due to electrostatic attraction between the cationic viologen quencher and the anionic surface groups on the QD.

Surprisingly, we observed uncharacteristic profiles for our quenching studies compared with previous observations in which Stern–Volmer analysis gave linear or superlinear plots. All our plots in the study of QD fluorescence quenching showed negative deviations from linearity. Such downward deviations were observed by several other groups studying fluorescence quenching of core–shell QDs.[22] Such a profile may indicate the presence of multiple fluorescence pathways, some of which are less efficiently quenched than others, or it may reflect limited accessibility of the quencher to the surface. Regardless of these differences in quenching behavior, both sets of QDs examined in this study successfully detected changes in glucose concentration. As expected, the QDs quenched with BV^{2+}, which does not contain boronic acids, showed no fluorescence recovery when glucose was added.

Previous studies showed that the choice of an appropriate quencher/fluorophore ratio was critical for a strong and linear signal response across the physiological range of glucose concentrations ($\approx$2.5–20 mM).[13,14] When experimenting with several different quencher/QD ratios, we observed generally the same behavior as with traditional organic dyes, in which higher ratios tended to give larger, more-linear fluorescence signals in response to the introduction of glucose (Figure 3).

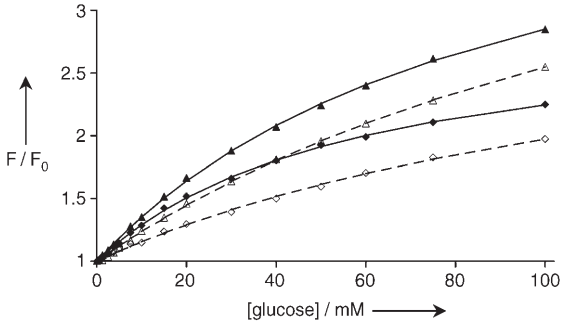


Figure 3. Glucose-response curves obtained from *o*-BBV^{2+}-fluorescence-quenched amine- and carboxy-substituted QDs (5×10^{-8} M) at pH 7.4. ▲ NH_2 QDs 1000:1, ◆ NH_2 QDs 50:1, △ COOH QDs 1000:1, ◇ COOH QDs 50:1.

We screened both sets of QDs for glucose response at quencher/QD ratios of 50:1, 200:1, 500:1, and 1000:1. For both amine- and carboxy-substituted QDs, we obtained optimal results with quencher/QD = 1000:1. While the fluorescence-signal response is modest in the physiological range, this application of QDs allows a large signal response and a considerable degree of recovery of the initial unquenched QD fluorescence after the introduction of glucose (100 mM) (Figure 3).

Studies are currently underway to examine further the use of QDs in two-component sensing systems for the detection of monosaccharides and other analytes.

Experimental Section

The fluorescent QDs (CdSe/ZnS Fort Orange COOH-functionalized QDs, CdSe/ZnS Fort Orange NH_2-functionalized QDs) were acquired from Evident Technologies as buffered aqueous stock solutions (7.76 nmols mL^{-1}). All data were analyzed with Solver (nonlinear least-squares curve fitting) in Microsoft Excel.

Fluorescence emission: All studies were carried out in buffer solution (pH 7.4) prepared with water purified by a Nanopure ultrafiltration system. The buffer solution (pH 7.4, ionic strength 0.1) was freshly prepared with KH_2PO_4 and Na_2HPO_4. The QD stock solutions were diluted in a quartz cuvette (path = 1 cm) with phosphate buffer (pH 7.4) to afford solutions (5×10^{-8} M) with a total volume of 2 mL. Fluorescence spectra were acquired with a Perkin–Elmer LS50-B luminescence spectrometer, except for temperature studies, which were conducted on a Varian Cary Eclipse fluorescence spectrophotometer. Studies were carried out at 22 °C without the exclusion of air, with the exception of temperature-dependent quenching experiments.[21] Excitation was performed with a slit width of 15 nm and a wavelength of 460 nm. The emission slit was set at 20 nm, and fluorescence emission was taken as the area under the emission curve from 560 to 670 nm, with peak emission for both sets of QDs at 604 nm. For fluorescence-titration experiments, the volume added did not exceed 3 % of the total, and the absorbance for all fluorescence measurements was below 0.1.

Fluorescence quenching: A quartz cuvette filled with QD solution (2 mL, 5×10^{-8} M in pH 7.4 buffer) was irradiated at 460 nm. The emission of the unquenched solution was obtained, then aliquots of *o*-BBV^{2+} quencher (0.001 M) were added, the solution was gently shaken for 60 s, and the new fluorescence was measured. Fluorescence intensity was taken as the area under the emission curve. Temperature-dependent quenching studies were conducted at 15, 25, and 65 °C with amine-functionalized QDs. Stern–Volmer constants were calculated by fitting the data to [Eq. (1)], where F_o is the initial unquenched fluorescence, F is the fluorescence in the presence of quencher, V is the dynamic quenching constant, K_S is the static quenching constant, [Q] is the quencher concentration.

$$\frac{F_o}{F} = \frac{V K_S [Q]}{1 + K_S [Q]} \quad (1)$$

Fluorescence glucose sensing: A quartz cuvette filled with QD solution (2 mL, 5×10^{-8} M in pH 7.4 buffer) was irradiated at 460 nm. The emission of the unquenched solution was obtained, then the solution was quenched with *o*-BBV^{2+}, and the new emission was measured. Aliquots of concentrated glucose solution (2.5 M, pH adjusted in buffer to 7.4) were then added, the mixture was gently shaken for 60 s, and the new fluorescence was measured. Fluorescence intensity was taken as the area under the emission curve. Apparent glucose-binding constants were calculated by fitting the data to the [Eq. (2)], where F_{calcd} is the calculated fluorescence intensity, F_{min} is the initial fluorescence intensity of the quenched dye, F_{max} is the calculated intensity at which the fluorescence increase reaches its maximum, K is the apparent binding constant, and [glucose] is the concentration of glucose.[23]

$$F_{calcd} = \frac{F_{min} + F_{max} K [\text{glucose}]}{1 + K [\text{glucose}]} \quad (2)$$

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