Cell-Permeable Probes

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Glucose-Mediated Assembly of Phenylboronic Acid Modified CdTe/ZnTe/ZnS Quantum Dots for Intracellular Glucose Probing**

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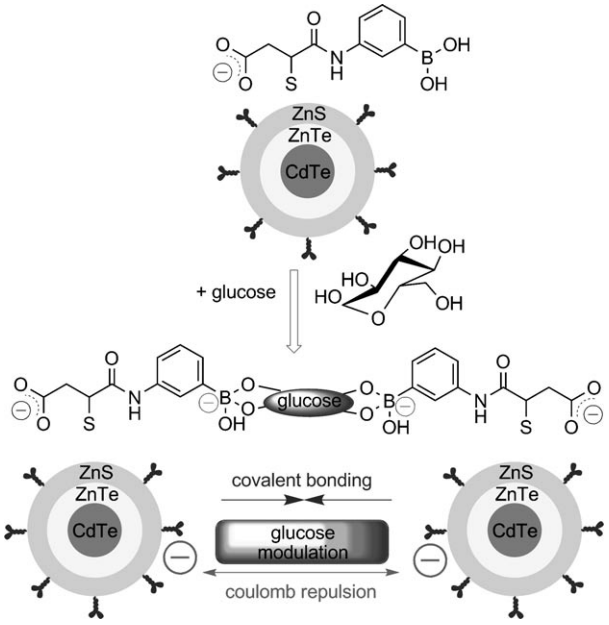
Molecularly mediated assemblies of nanoparticles open prospects for the combination of nanostructure-tuning capabilities with unique electrical, optical, and magnetic properties.[1] Quantum dots (QDs) have attracted increasing interest as fluorescent probes owing to their photostability, continuous absorption spectra, and efficient and tunable emission.[2] Different approaches to the assembly of QDs to explore their collective optical properties have been reported.[3] Mediation of the assembly of QDs by a small molecular analyte, such as glucose, may yield a facile means of fabricating QD arrays with tunable optical properties in response to concentration changes of the analyte, even in a living system. However, such approaches remain largely unexplored. Changes in the glucose level within cells are indicative of many cellular processes.[4–6] For example, glucose plays an important role in both energy metabolism and biosynthesis in the cells of most mammalians[5] and could thus potentially be used in cancer therapy to restrict cancer-cell growth.[6] Yet how cells sense and adapt to changes in the glucose level is not well understood. Although numerous fluorescent glucose probes have been developed from QDs, only a small number are potentially practical for intracellular imaging.[7]

On the basis of the observation by Yoon and Czarnik that the fluorescence of a fluorophore changed upon the reaction of glucose with a boronic acid group attached to the fluorophore,[7a] Singaram and co-workers were the first to develop fluorescent QDs for glucose sensing.[7b] Their approach involved reversible competition between glucose and CdSe QDs for a boronic acid functionalized organic quencher. An analogous method was reported by Tang et al., who used fluorescence resonance energy transfer (FRET) between CdTe QDs conjugated to concanavalin A (Con A) and gold nanoparticles modified by thiolated β-cyclodextrins.[7c] Willner and co-workers prepared H_2O_2-sensitive CdSe/ZnS QDs that could be linked to glucose oxidase.[7d] They also used FRET between CdSe/ZnS QDs and a dye-modified galactose–dopamine conjugate to develop a competitive assay for the optical detection of glucose.[7e] Although benefits of the use of QD-based glucose probes are already apparent, these systems were applied for homogeneous detection, and the potential of their use on a cellular level has not yet been investigated. Experiments on the selectivity of QD-based glucose probes are also quite limited.[7c]

Herein, we demonstrate that a simple and reliable glucose probe (Scheme 1) can be designed on the basis of the glucose-mediated assembly of phenylboronic acid (PBA) modified



Scheme 1. Glucose-mediated assembly of PBA-modified CdTe/ZnTe/ZnS CSS QDs. The fluorescence of the QDs depends on the assembly induced by the interactions of the analyte with the molecular-recognition groups anchored on the surface of the QDs.

CdTe/ZnTe/ZnS core/shell/shell (CSS) QDs (PBA-QDs). The PBA-functionalized groups attached to the QDs serve as linkers. Boronic acids reversibly form stable boronate complexes with *cis* diols; thus, PBA-modified probes are good for continuous glucose sensing.[8] Our probe shows high sensitivity and selectivity for glucose over the non-glucose metabolic end product L-lacate and common metal ions. The glucose-dependent assembly of QDs with a properly controlled degree of PBA functionalization could form the basis of a general method for the development of ratiometric sensors, which could be used to probe glucose in living cells through

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the monitoring of changes in the emission intensity, wavelength, or collection efficiency of QDs.

We synthesized high-quality CdTe/ZnTe/ZnS CSS QDs capped with mercaptosuccinic acid (MSA) in an aqueous medium (see the Supporting Information for experimental details). Highly luminescent CdTe/ZnTe CS QDs were recently synthesized in an aqueous medium.[2d] If the ZnS shell was grown directly on the surface of CdTe QDs, serious strain would be generated between CdTe and ZnS owing to their large lattice mismatch (16.5%). Such strain can lead to low photoluminescent quantum yields (PLQYs) and broad size distributions.[9] To overcome this problem, we introduced a ZnTe shell, with a band-gap energy and a lattice constant between those of CdTe and ZnS, as a buffer layer between the CdTe core and the ZnS shell (see SI2 in the Supporting Information). As highly luminescent CdTe/CdS CS QDs have been prepared successfully despite the relatively large lattice mismatch (10.0%) between CdTe and CdS,[9c] the ZnS shell was expected to exhibit well-defined epitaxial growth on the surface of the ZnTe shell owing to the similar lattice mismatch (10.7%). Both UV/Vis and photoluminescence (PL) spectra of the synthesized MSA-capped CdTe/ZnTe/ZnS QDs indicated the desired CSS structure (see sections SI3 and SI4 in the Supporting Information). This double-shell structure enables a stepwise change in lattice spacing from the emitting CdTe core to the protecting ZnS shell and thus reduces the strain within the QD.[9b] Moreover, charge carriers are effectively confined within the core region and separated from the surface as a result of the adequately offset band-gap energies (ca. 2 eV) between the core and the double-shell region. This CSS structure reduces nonradiative surface defects and improves the PLQY (ca. 72%).[9b,10] PBA was covalently linked to MSA-capped CdTe/ZnTe/ZnS CSS QDs through EDC coupling (see section SI5 in the Supporting Information). We expected that the boronic acids anchored on the surface of our CSS QDs would be able to form stable boronate complexes with glucose.

Dynamic light scattering (DLS) was used to monitor the glucose-mediated assembly of PBA-QDs on the basis of the hydrodynamic diameter (D_h). Figure 1a shows a representative set of size-evolution data for the assembly of PBA-QDs in the presence of D-glucose (20.0 mM). Upon the addition of glucose to the solution containing QDs, the average D_h value increased gradually with reaction time until an equilibrium state was reached.

Interference with a glucose sensor by the non-glucose constituents of blood should be minimal. The concentration of pyruvate, galactose, and fructose in blood is below 0.1 mM;[11] thus, it is unlikely that these compounds will interfere significantly with the glucose reading. However, the concentration of L-lactate, a key metabolite that can bind to boronic acid derivatives, is usually 0.36–0.75 mM in blood at rest, but can rise to over 20 mM during intense exertion.[12] To examine the impact of L-lactate on our PBA-QD glucose probe, we also monitored the size evolution of PBA-QDs in the presence of lactate (20 mM). DLS indicated a larger apparent rate constant with lactate (first-order, $k = 1.27 \times 10^{-2}\ \mathrm{min}^{-1}$; see section SI6 in the Supporting Information) than that found with glucose ($8.59 \times 10^{-3}\ \mathrm{min}^{-1}$), possibly

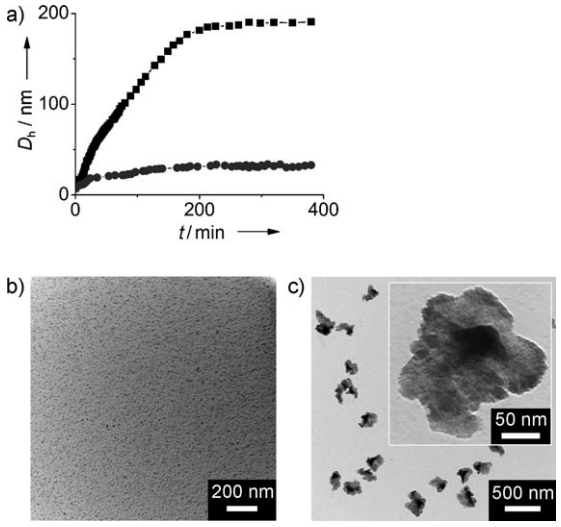

Figure 1. a) Time dependence of the mean hydrodynamic diameter for the assembly of PBA-QDs in the presence of glucose (20.0 mM, ■) and lactate (20.0 mM, ●). b) TEM image of PBA-QDs in the absence of glucose. c) TEM image of PBA-QDs in the presence of glucose (20.0 mM). Small spots can be distinguished clearly in the enlarged image of the structured assemblies (inset).

owing to the flexible orientation of the adjacent hydroxy groups in lactate and its small size.[12c] However, we found that the lactate-induced assembly of the PBA-QDs was nearly negligible in comparison with glucose-induced assembly. This different reactivity can be explained by the structural difference between glucose and lactate molecules. Although both glucose and lactate can complex with boronic acids, each lactate molecule can only provide one pair of *cis* hydroxy groups. The binding of lactate to the PBA-QDs can form negatively charged boronate groups and slightly increase the capping-shell thickness; however, lactate cannot bridge the PBA-QDs to form an interparticle assembly. In contrast, each glucose molecule has two pairs of *cis* diols that can covalently bridge the PBA-QDs to form a large assembly (Scheme 1). This unique glucose-induced assembly of the PBA-QDs essentially leads to a high selectivity of the probe for glucose over lactate. Furthermore, the negative charges on the boronate complexes and the unfunctionalized carboxylate groups enable excellent water stability of the assembled QDs at pH 7.4.

To examine the morphology of the glucose-mediated assembly of the PBA-QDs, TEM images were taken of the assembled particles formed at different glucose concentrations (Figure 1b,c; see section SI7 in the Supporting Information). The TEM images are representative of the characteristic particle sizes and shapes on the entire TEM grid for all the samples. An increase in glucose concentration led to an increase in the particle size of the PBA-QD assemblies. A close view of the morphology of the assemblies revealed many smaller dots of a size consistent with that of PBA-QDs without the addition of glucose and thus suggested a close packing of the PBA-QDs. The glucose-induced assembly of the PBA-QDs was well reproducible from batch to batch.

The glucose-induced close packing of the PBA-QDs significantly affects the PL properties of the resultant assemblies. Figure 2a shows the PL spectra of the PBA-QDs as a function of glucose concentration. Two effects were observed when the glucose concentration was increased: a) a quenching of the PL intensity of both low- and high-energy emissions; and b) a significant red shift of the high-energy emission associated with the 2D systems (shell, ca. 585 nm), but barely any shift of the low-energy emission associated with the 0D system (core, ca. 640 nm). At pH 7.4, glucose can bind about 30% of the total PBA groups to form charged boronate complexes;[13] in this way, Coulomb repulsion is introduced between the negatively charged boron atoms and thus between the bonded QDs. On the other hand, because the glucose is covalently bound to the PBA groups anchored on the QDs, these QDs act as cross-linking points. Although electrostatic repulsion between these QDs would favor their existence as individual entities, the "cross-linked" structure offers the retractive force to restrain the disassembly of the aggregate. The counterbalance of the repulsive and retractive forces introduces an elastic tension in the bonds that could stretch the interface of the thiol-modified QDs to create surface states that can quench the PL.[8d] A similar phenomenon was reported by Wuister et al., namely, that short stabilizer molecules (2-mercaptoethanolamine) can propagate the exogenous strain to the surface of CdTe QDs to create surface quenching states.[14] Consequently, when the glucose concentration was increased to 20.0 mM, a PL quenching of the PBA-QDs as high as 67% and a red-shift of about 83.5 meV (24 nm) were observed. The energy difference is taken as an indication of cluster–cluster interaction in a closely packed layer of QDs.[2e]

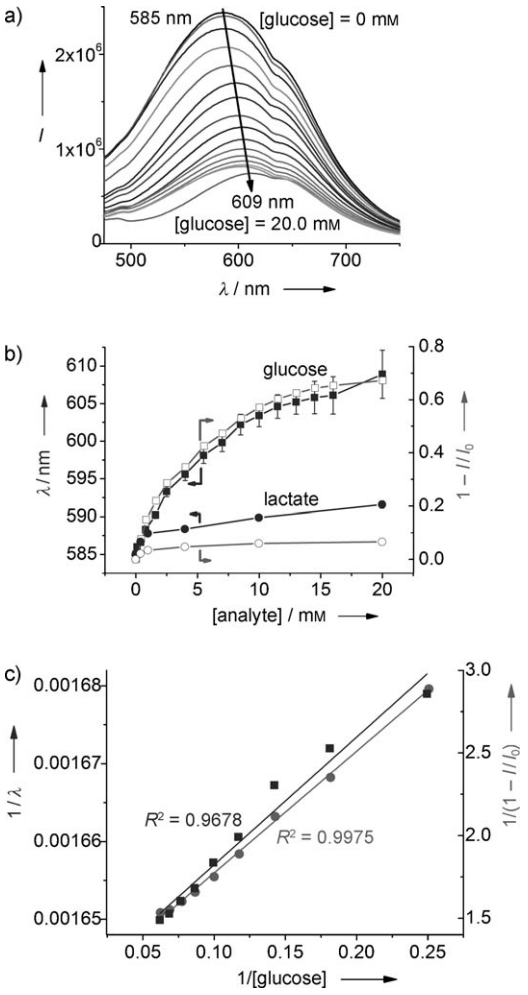

Figure 2. a) Characteristic PL response of the PBA-QDs as a function of the D-glucose concentration. b) Evolution of the emission position and the quenched PL as a function of the concentration of glucose (■) at 609 nm and lactate (●) at 586 nm. c) Linear plot showing the glucose-responsive PL properties in the reciprocal space.

Interestingly, the glucose-induced PL quenching and red shift of the 2D emission as a function of glucose concentration follow the same trend (Figure 2b), which also mirrors that of the glucose-induced size evolution of the assemblies as observed by TEM. This observed relationship serves as further confirmation that the PBA-QDs are bound together by the glucose molecules during assembly. The PBA-QD probes are sensitive to glucose concentrations in the typical physiologically important range of 0.4–20.0 mM. The linear plot shown in Figure 2c gives glucose-dependent PL properties in a more orthogonal fashion. The detection limit estimated from the color change is 0.3 mM; it is reduced to as low as 50 μM when estimated from the intensity change. The glucose sensitivity of the PBA-QD probe can be further tuned through the control of the amount of PBA groups anchored on the QDs. Glucose-induced PL quenching increases as the number of PBA groups on the QDs is increased (see section SI8 in the Supporting Information). Thus, our approach is suitable for the quantitative detection of glucose in biological samples.

To further examine the potential interference of lactate, lactate-dependent PL quenching was also investigated for comparison. Only a slight quenching of 7% and a red shift of 23.8 meV were observed at a lactate concentration of 20.0 mM; these quantities were decreased remarkably to only 2% and 8.1 meV at a lactate concentration of 1.0 mM (Figure 2b). The relative error for lactate is within the range of 10% at normal blood levels. The PBA-QD probe clearly shows excellent sensitivity and selectivity for glucose over lactate.

We also checked the influence of metal ions. Although QD ion sensors have been demonstrated, the QDs protected by a ZnS shell in this study should have no significant response to metal ions in blood.[2e,f] The mechanism of the fluorescence quenching of QDs by metal ions is most probably related to the transfer of electrons from the photoexcited QDs to the cation bound at the surface and the formation of new nonradiative surface channels for electron annihilation, which effectively competes with radiative electron–hole recombination within the QDs.[2g] The relative error in the glucose concentration (0.4–20.0 mM) in the presence of common metal ions (1.0 mM) found in biological systems, such as K^+ (−1.83%), Na^+ (−0.52%), Mg^{2+} (−2.31%), Al^{3+} (−0.35%), Zn^{2+} (+0.06%), Fe^{3+} (+4.03%), and Cu^{2+} (+1.32%), is within the range of

±4.03%. The observed different quenching behaviors of metal ions with the PBA-QDs most likely result from the different electronic structures and redox potentials of the metal ions, as well as the different ion strengths involved in the electrostatic attractions between the metal ions and the boronic acid groups or carboxyl groups. Thus, the present glucose probe should be free from significant interference by the non-glucose constituents of blood.

The reaction of glucose with PBA-QDs is unique, since the formation of the covalent bonds between the boronic acid groups and *cis* diols is reversible. As glucose was removed from the bathing medium of the PBA-QD probe by dialysis against very frequently changed water, the boronate ester started to dissociate, and disassembly proceeded to form small QDs, which could readily permeate across the dialysis membrane. At the end of the experiment, nearly no QDs remained inside the dialysis tube, as revealed by an extreme decrease in fluorescence. This good reversibility will be an important feature for future in situ studies in biological systems.

Having demonstrated the glucose-signaling ability of the PBA-QDs, we next tested the ability of the PBA-QDs to enter cells and detect intracellular glucose levels by using mouse melanoma B16F10 cells as a model. The small QDs (< 10 nm) can attain deep penetration into poorly permeable tumor cells.[15] B16F10 cells treated with PBA-QDs were highly luminescent, with fluorescence seen primarily in specific areas of the cytoplasm (Figure 3a).[16] A cross-sectional Z scan also confirmed the entry of the QDs into the cells (see section SI9 in the Supporting Information). The QDs within the cells produced a bright color, and the PL intensity was retained even after continuous irradiation for 30 min. This result confirmed the photostability of the QDs in cells.

Typically, glucose utilization by a cell depends on transport and metabolism. Most mammalian cells transport hexoses into or out of the cytosol, as mediated by a family of monosaccharide facilitators.[4] The B16F10 cells were grown in sugar-free Dulbecco modified Eagle medium (DMEM). When fed different amounts of glucose, the B16F10 cells labeled with the PBA-QDs could be simply optically differentiated by using appropriate filter systems in the confocal microscope. Some assembled particles may be too big to be taken up by the living cells automatically.[15] The fluorescence observed for repeatedly washed cells is due to QDs preloaded into the cells. Local spectral analysis of the overall staining of QDs in the cells indicated a difference in the PL signal (Figure 3). Without glucose feeding, the emission maximum appeared at about 590.2 nm. On the basis of the calibration curve in Figure 2c, a glucose concentration of approximately 1.6 mM was estimated for the intracellular region labeled with a yellow circle in Figure 3a. When the cells were fed with extracellular glucose at concentrations of 2.0 and 20.0 mM, the emission maximum shifted to approximately 596.7 and 609.8 nm, which correspond to glucose concentrations of about 4.0 and 15.9 mM, respectively, for the intracellular regions shown in Figure 3b,c. Thus, the engineered QDs have the potential to serve as a selective nanoprobe for specific diagnosis. The ability of the QDs to change color may provide a powerful tool for monitoring the complex changes that can occur within living systems at the single-cell level, through a proper functionalization of the QDs.

In summary, we have developed a new strategy for chemical and biological probing by tethering environmentally sensitive molecules to emissive water-soluble QDs. The resulting QDs can be used to detect and quantify a small-molecule analyte in living cells. The unique glucose-mediated assembly of PBA-QDs could be used to modulate the PL properties of QDs to enable a selective ratiometric response to glucose. The optical approach to glucose probes has considerable potential for modifications with other QDs and molecules to give a spectrum of probes for the detection of saccharides and other analytes at various concentrations in cells.

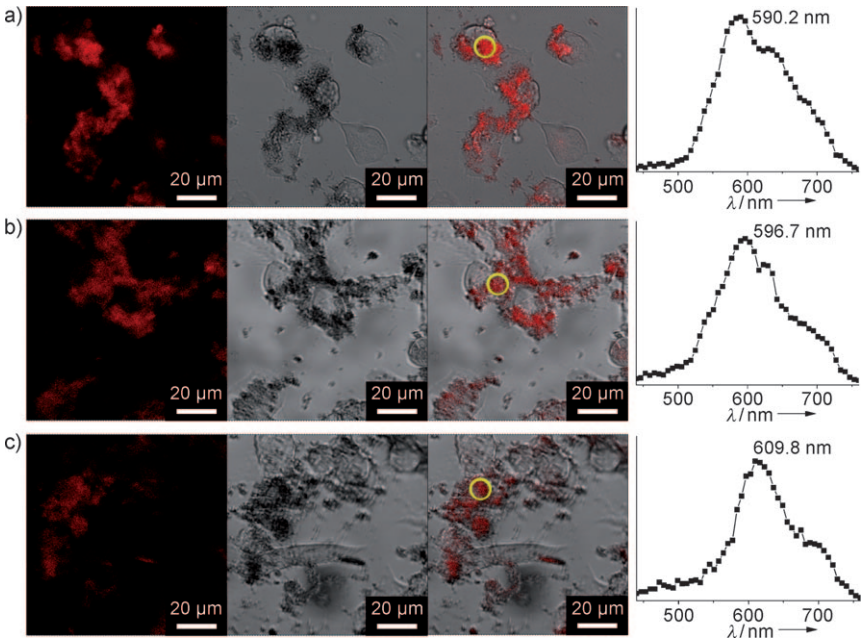

Figure 3. Scanning confocal fluorescence microscopy images (left), transmission microscopy images (center), and overlaid images (right) of mouse melanoma cells B16F10 incubated with PBA-QDs (5.0 μg mL^{-1}), with the additional feeding of glucose at concentrations of a) 0 mM, b) 2.0 mM, and c) 20.0 mM. The local PL spectra were obtained from the regions within the yellow circles.

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