Optical detection of glucose by CdS quantum dots immobilized in smart microgels†

Weitai Wu, Ting Zhou, Jing Shen and Shuiqin Zhou*

Received (in Cambridge, UK) 14th April 2009, Accepted 21st May 2009
First published as an Advance Article on the web 11th June 2009
DOI: 10.1039/b907348e

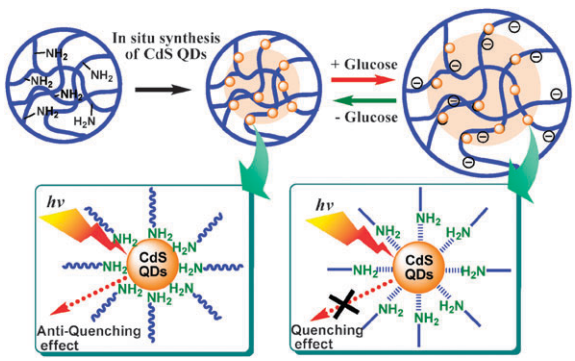
Reversible fluorescence quenching and anti-quenching of CdS quantum dots immobilized in boronic acid-based microgels can be used for the optical detection of glucose.

The *in situ* detection of saccharides, including D-glucose and D-fructose, has been of great interest for a long time due to the importance of saccharides in medical diagnostics and bioprocessing. Although enzyme-based detection approaches are highly selective and effective, many difficulties remain. For example, reliable continuous, non-invasive, or minimally invasive glucose monitoring systems are still a major challenge for optimal glycemic control in diabetic patients.[1–3] This has led to considerable research interest in developing synthetic chemical ligands for continuous monitoring of saccharides. The best ligands identified for binding glucose in aqueous media are boronic acids.[4] These boronic acid-based ligands have been coupled with fluorescent moieties[1,5] or polymers[6–9] for continuous glucose sensing. The selectivity and significant reduction of lactate interference of the boronic acid-based glucose sensors have been investigated.[8] Novel triply-responsive boronic acid block copolymers have been recently developed for potential applications as self-regulated drug delivery systems and sensors for sugars and glycoproteins.[10] On the other hand, there is a growing interest in using fluorescent quantum dots (QDs) as optical labels for biosensing events due to their size-controlled fluorescence properties, high quantum yield, and stability against photobleaching.[11–13] Singaram's group was the first one to utilize fluorescent QDs for glucose sensing.[14a] The fluorescence of CdTe/ZnS QDs can be quenched after their complexation with a viologen quencher. The binding of glucose to the boronic acid-substituted quencher weakens the quencher–QD interactions and induces a robust fluorescence recovery. Tang *et al.*[14b] conjugated CdTe QDs with concanavalin A (Con A) that can be combined to the β-cyclodextrin-modified fluorescence quenchers. The binding of glucose with ConA breaks the quencher–QD interactions, leading to fluorescence recovery. Willner *et al.*[15] prepared H_2O_2-sensitive CdSe/ZnS QDs that can be further linked with glucose oxidase (GOx). The enzyme GOx catalyzes the oxidation of D-glucose and produces H_2O_2, resulting in the quenching of fluorescence of the QDs. However, these systems require very complicated functionalizations of both QDs and quenchers and are only good for homogeneous detection.

Department of Chemistry and The Center for Engineered Polymeric Materials, College of Staten Island of The City University of New York, 2800 Victory BLVD, Staten Island, New York 10314, USA. E-mail: zhoush@mail.csi.cuny.edu; Fax: +1 718-982-3910
† Electronic supplementary information (ESI) available: Experimental procedures. See DOI: 10.1039/b907348e

Herein, we report a simple and reliable glucose sensing system based on the *in situ* immobilization of fluorescent CdS QDs in the interior of boronic acid based microgels for non-invasive continuous optical detection of saccharides. We demonstrate that the fluorescence of CdS QDs in the copolymer microgels of poly(*N*-isopropylacrylamide-acrylamide-phenylboronic acid) [p(NIPAM-AAm-PBA)] could be reversibly quenched and anti-quenched when the microgel undergoes swelling and deswelling in response to the glucose concentration change (Scheme 1). The hybrid materials with inorganic QDs embedded in smart microgels, combining the properties of both QDs and smart microgels, can offer the possibilities of external switching and manipulation for sensor devices. Microgels also offer several advantages over other polymer template systems: simple synthesis, easy functionalization, uniform size distribution, tunable dimension (tens of nm to a few μm), potential biocompatibility, and rapid response time.[16] The pAAm segments designed in the microgels are not only able to complex with the Cd^{2+} precursors and stabilize the produced CdS QDs,[17] but also greatly improve the stability of the resulting p(NIPAM-AAm-PBA)–CdS hybrid microgels. The optical nanoscale glucose "meter" reported here is likely to be highly useful in a wide range of applications in biology and biomedicine for research, diagnosis, or monitoring.

The preparation of p(NIPAM-AAm-PBA) microgels involves the first synthesis of monodispersed poly(*N*-isopropylacrylamide-acrylamide-acrylic acid) [p(NIPAM-AAm-AA)] microgels, followed by modification with glucose-sensing PBA moieties.[16a] With a similar monomer reactivity, the p(NIPAM-AAm-AA) microgels should have randomly well-distributed functional groups of AA and AAm through the copolymer chains. The p(NIPAM-AAm-AA) microgels



Scheme 1 Reversible fluorescence quenching and antiquenching of CdS QDs embedded in the interior of p(NIPAM-AAm-PBA) microgels in response to the change in glucose concentration.

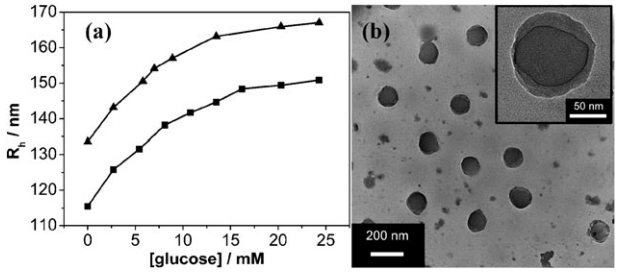


Fig. 1 (a) Average R_h of p(NIPAM-AAm-PBA) (■), and p(NIPAM-AAm-PBA)–CdS microgels (▲) as a function of glucose concentration, measured at 22.1 °C, pH = 8.8, and a scattering angle $\theta = 60°$. (b) TEM images of the p(NIPAM-AAm-PBA)–CdS hybrid microgels.

were functionalized with PBA through the coupling of 3-aminophenylboronic acid to the –COOH groups of the AA units under EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) catalysis. The complexation of PBA with 1,2-*cis*-diols such as glucose produces a thermodynamically favorable anionic boronate to form, resulting in an increase in the degree of ionization that builds up a Donnan potential causing the microgels to swell. Fig. 1a shows the glucose induced swelling curve of the p(NIPAM-AAm-PBA) microgels, in terms of the hydrodynamic radius (R_h) measured in a 0.005 M phosphate buffer solution (PBS) of pH = 8.8. When the glucose concentration is above 16 mM, the microgel network chains stretch to near maximum. This reversible volume phase transition allows us to change the interactions of the CdS QDs with the polymer ligands and the local surface environments of the QDs embedded in the microgels in response to glucose concentration change.

The p(NIPAM-AAm-PBA)–CdS hybrid microgels were synthesized through the *in situ* formation of CdS QDs in the interior of the microgels at pH ~ 5.9, at which the PBA groups were nearly uncharged. Our motivation is to use the amide groups in the AAm units to complex the Cd^{2+} ions into the microgels from the external precursor $Cd(ClO_4)_2$ solution and to protect the obtained CdS QDs from agglomeration or release. The TEM images of the p(NIPAM-AAm-PBA)–CdS hybrid microgels (Fig. 1b) clearly indicate a core–shell structure with a CdS QD rich core (higher contrast), which is very stable even at high glucose concentrations where the hybrid microgels swells (see Fig. S4 of ESI†). The hybrid microgels are still glucose-sensitive after the immobilization of CdS QDs (Fig. 1a), but become larger in size. This could be related to the relatively compact structure of the p(NIPAM-AAm-PBA) template microgels due to the hydrophobic PBA groups and the high cross-linking density. The *in situ* formed CdS QDs filled the relatively compact chain networks, and thus increased the total volume of the microgels. Importantly, the hydrophilic AAm segments prevented the hybrid microgels from aggregation in PBS even at the shrunk states for 2 months at room temperature.

Fig. 2 shows the glucose-sensitive UV-Vis absorption properties of the p(NIPAM-AAm-PBA)–CdS hybrid microgels. A hump observed around 332 nm is assigned to the excitonic transition, which possibly results from the $1P_h$–$1P_e$ transition,[18] while the hump at 483 nm is possibly related to the $1S_h$–$1S_e$

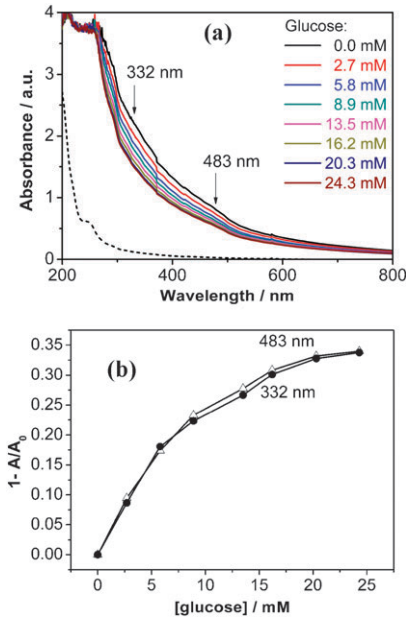


Fig. 2 (a) UV-vis absorption spectra and (b) the relative transmittance at 332 nm (●) and 483 nm (△), respectively, of the p(NIPAM-AAm-PBA)–CdS hybrid microgels at different glucose concentrations in PBS at pH = 8.8. A and A_0 represent the absorbance of the hybrid microgels in the presence and absence of glucose. The dashed line in (a) shows the absorption spectrum of the template p(NIPAM-AAm-PBA) microgels.

transition. The CdS QDs exhibit extremely strong quantum confinement in the microgels. Two effects were observed with a gradual increase in glucose concentration: (1) a decrease in absorbance at both low and long wavelengths; and (2) the gradual disappearance and featurelessness of the high-energy hump. Both effects are likely to arise from a decrease in the refractive index of the hybrid microgels during their swelling, which results in a decrease in the Rayleigh scattering due to a smaller refractive index contrast with the solvent, as well as a decrease in the local refractive index around the CdS QDs, leading to a weakening and featureless absorption. Interestingly, the glucose-induced increase in the transmittance of the p(NIPAM-AAm-PBA)–CdS hybrid microgels qualitatively reproduces the glucose-induced swelling curves (Fig. 2b).

The glucose-induced volume phase transition of the hybrid microgels could also significantly affect the photoluminescence (PL) properties of the CdS QDs embedded in the interior of the microgels. Fig. 3a–c shows the evolution of PL emissions, centred at 638 nm, of the p(NIPAM-AAm-PBA)-CdS hybrid microgels in the presence of D-glucose and D-fructose at different concentrations and pH values. It is clear that the PL of CdS QDs was gradually quenched when the microgels gradually swelled up in the presence of D-glucose or D-fructose. Fig. 3d summarizes the glucose and fructose sensitivity based on the fluorescence quenching of the CdS QDs embedded in the p(NIPAM-AAm-PBA) microgels. At pH = 7.4, the percentage of the charged boronate complexes produced with PBA was *ca.* 30% for D-glucose and *ca.* 80% for D-fructose,[19] thus the fluorescence quenching of the CdS QDs showed a stronger sensitivity to D-fructose over D-glucose. At pH = 8.8, the complexation between D-glucose and the PBA groups in

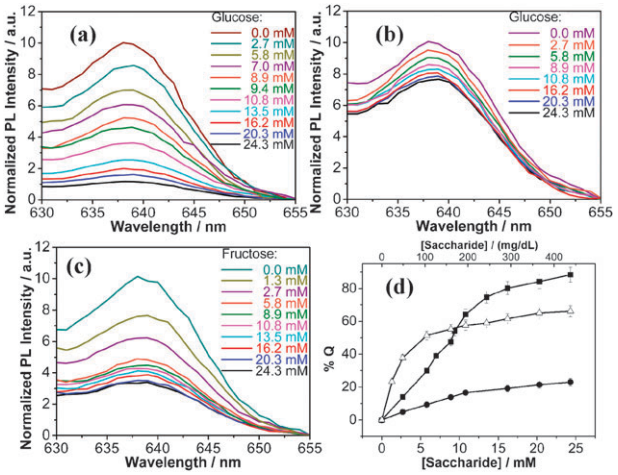


Fig. 3 Characteristic PL response of the p(NIPAM-AAm-PBA)–CdS hybrid microgels at 638 nm in the presence of saccharides: (a) D-glucose at pH = 8.8, (b) D-glucose at pH = 7.4, and (c) D-fructose at pH = 7.4. (d) Quenched PL at 638 nm as a function of the concentration of glucose (●: pH = 7.4, ■: pH = 8.8) and fructose (△: pH = 7.4), respectively. The excitation wavelength was 390 nm.

the microgels was almost complete, resulting in a high swelling degree of the microgels. Consequently, the fluorescence quenching of the CdS QDs can reach as high as 90%. The luminescence quenching of the QDs almost linearly increased with the glucose concentration from 0 to 13.5 mM, and then the rate of fluorescence quenching slowed down at higher glucose concentrations when the microgel network chains stretched to near maximum. It should be mentioned that glucose in the tested concentration range 0–50 mM had no effect on the fluorescence of the CdS QDs (see Fig. S2 in ESI†), which is consistent with the recent report that glucose by itself has no effect on the fluorescence of CdSe/ZnS QDs.[15]

We believe that the increase in the number of emission quenching centers located on the polymer–CdS QD interface provides the essential scenario for the PL quenching during the microgel swelling. At low glucose concentrations, the polymer is less soluble and the polymer chain networks have less elastic tension. In the swollen states at higher glucose concentrations, the polymer chain tends to dissolve, however, the cross-linkage of the polymer chains hinders the network expansion, creating elastic tensions localized at the cross-linking points. Because of the binding between the polymer ligands and the CdS QDs, the CdS QDs also act as cross-linking points, introducing an elastic tension in the bond that could stretch the polymer–CdS QD interface, creating interfacial states that quench the PL (Scheme 1). This phenomenon is similar to the temperature induced quenching of QDs,[20] where the freezing of the solvent (water) induces strain in the capping shell and the short stabilizer molecules (2-mercaptoethanolamine) propagate the strain to the surface of the nanocrystals, creating surface quenching states.

In summary, we demonstrate a new molecular recognition motif based on the fluorescence quenching of CdS QDs immobilized in glucose-sensitive microgels in the physiologically important glucose concentration range 1–25 mM, which shows promise for a continuous non-invasive *in vivo* glucose sensing system. We are currently using a new PBA analogue that can highly bind D-glucose at physiological pH to modify the microgels. We are also examining how the different cross-linking degrees and AAm/PBA contents of the microgels affect the size and content of the QDs as well as the sensing range of glucose concentration to provide a controllable glucose sensing range under physiological conditions.

We thank the financial support of this work from the US Agency for International Development under the US–Pakistan Science and Technology Cooperative Program (PGA-P280422).

Notes and references

1 *Glucose Sensing (Topics in Fluorescence Spectroscopy)*, ed. C. D. Geddes and J. R. Lakowicz, Springer, New York, 2006, vol. 11.
2 V. R. Kondepati and H. M. Heise, *Anal. Bioanal. Chem.*, 2007, **388**, 545.
3 A. Heller and B. Feldman, *Chem. Rev.*, 2008, **108**, 2482.
4 A. P. Davis and R. S. Wareham, *Angew. Chem., Int. Ed.*, 1999, **38**, 2979.
5 (*a*) W. Yang, H. He and D. G. Drueckhammer, *Angew. Chem., Int. Ed.*, 2001, **40**, 1714; (*b*) B. Peng and Y. Qin, *Anal. Chem.*, 2008, **80**, 6137.
6 E. Shoji and M. S. Freund, *J. Am. Chem. Soc.*, 2002, **124**, 12486.
7 (*a*) S. A. Asher, V. L. Alexeev, A. V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox and D. N. Finegold, *J. Am. Chem. Soc.*, 2003, **125**, 3322S. A. Asher, V. L. Alexeev, A. V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox and D. N. Finegold, *Anal. Chem.*, 2003, **75**, 2316; (*b*) M. M. W. Muscatello, L. E. Stunja and A. A. Asher, *Anal. Chem.*, 2009, **81**, DOI: 10.1021/ac900006x.
8 (*a*) S. Kabilan, A. J. Marshall, F. K. Sartain, M. C. Lee, A. Hussain, X. Yang, J. Blyth, N. Karangu, K. James, J. Zeng, D. Smith, A. Domschke and C. R. Lowe, *Biosens. Bioelectron.*, 2004, **20**, 1602; (*b*) X. Yang, M. C. Lee, F. Sartain, X. Pan and C. R. Lowe, *Chem.–Eur. J.*, 2006, **12**, 8491.
9 J. T. Suri, D. B. Cordes, F. E. Cappuccio, R. A. Wessling and B. Singaram, *Angew. Chem., Int. Ed.*, 2003, **42**, 5857.
10 D. Roy, J. N. Cambre and B. S. Sumerlin, *Chem. Commun.*, 2009, 2106.
11 (*a*) W. C. Chan and S. M. Nie, *Science*, 1998, **281**, 2016; (*b*) W. C. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han and S. M. Nie, *Curr. Opin. Biotechnol.*, 2002, **13**, 40.
12 I. L. Medintz, A. R. Clapp, F. M. Brunel, T. Tiefenbrunn, H. T. Uyeda, E. L. Chang, J. R. Deschamps, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2006, **5**, 581.
13 D. Bardelang, Md. B. Zaman, I. L. Moudrakovski, S. Pawsey, J. C. Margeson, D. Wang, X. Wu, J. A. Ripmeester, C. I. Ratcliffe and K. Yu, *Adv. Mater.*, 2008, **20**, 4517.
14 (*a*) D. B. Cordes, S. Gamsey and B. Singaram, *Angew. Chem., Int. Ed.*, 2006, **45**, 3829; (*b*) B. Tang, L. Cao, K. Xu, L. Zhou, J. Ge and L. Yu, *Chem.–Eur. J.*, 2008, **14**, 3637.
15 R. Gill, L. Bahshi, R. Freeman and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 1676.
16 (*a*) Y. Zhang, Y. Guan and S. Q. Zhou, *Biomacromolecules*, 2006, **7**, 3196Y. Zhang, Y. Guan and S. Q. Zhou, *Biomacromolecules*, 2007, **8**, 3842; (*b*) T. Hoare and R. Pelton, *Macromolecules*, 2007, **40**, 670.
17 (*a*) P. Yez-Sedeo and J. M. Pingarrn, *Anal. Bioanal. Chem.*, 2005, **382**, 884; (*b*) D. Mandal, H. Hosoi, U. Chatterjee and T. Tahara, *J. Chem. Phys.*, 2009, **130**, 034902.
18 (*a*) Y. Nosaka, *J. Phys. Chem.*, 1991, **95**, 5054; (*b*) H. Matsumoto, T. Sakata, H. Mori and H. Yoneyama, *J. Phys. Chem.*, 1996, **100**, 13781.
19 S. A. Baker, A. K. Chopra, B. W. Hatt and P. J. Somers, *Carbohydr. Res.*, 1973, **26**, 33.
20 S. R. Wuister, C. M. Donegá and A. Meijerink, *J. Am. Chem. Soc.*, 2004, **126**, 10397.

