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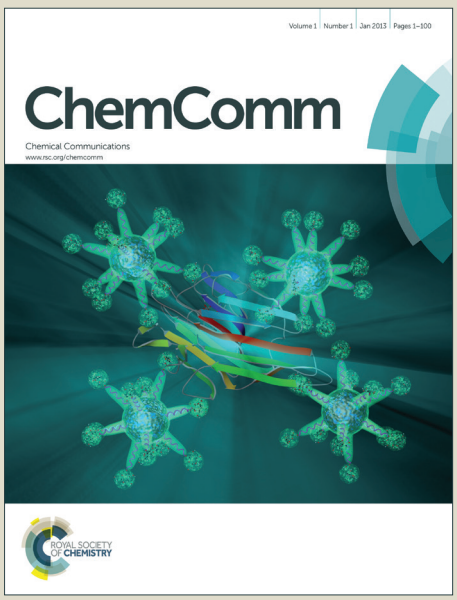

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Water-Dispersible Silicon Dots as Peroxidase Mimetics for High-Sensitive Colorimetric Detection of Glucose

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We demonstrate that photoluminescence Si-dots exhibit intrinsic peroxidase-like activity, and could catalyze the oxidization of 3,3',5,5'-tetramethylbenzidine (TMB) by H_2O_2 to produce a color reaction. This strategy could be used to detect glucose with high sensitivity and selectivity.

Semiconductor quantum dots (QDs) have enormous potential application due to their excellent optical property. For instance, Li reported glucose biosensor based on nanocomposite films of CdTe QDs and glucose oxidase (GOx).[1] Bahshi and colleagues reported the sensing of glucose by the MB^+-functionalized CdSe/ZnS QDs.[2] Cao demonstrated that a simply assembled complex consisting of CdTe QDs and GOx can be used to sensitively determine glucose based on its effective fluorescence quenching by H_2O_2.[3] However, heavy metal ion-containing nanoparticles may suffer from intrinsic limitations such as potential toxicity, intrinsic blinking and chemical instability.[4] Therefore, it is important to develop excellent nanomaterials for fabricating stable biosensors with good biocompatibility.

As one of the inert, nontoxic, abundant and low-cost nanomaterials, silicon nanomaterials are used in sensors and corrosion protection due to their attractive advantages including excellent optical, electronic, mechanical properties and surface tailorability[5]. Compared to heavy metal ions-containing quantum dots, Si-dots are biocompatible, inexpensive and chemical instability. In the past years, many reports were focused on the synthesis of Si-dots by physical, physicochemical, chemical, and electrochemical etching of bulk Si.[4, 6] And the reported Si-dots always have been modified by grafting a water-soluble materiel on the particle surface[4] and the modified Si-dots may be excellent candidates for biological imaging.[7] Recently, our research group reported a new way to obtain label-free Si-dots with highly Si-dots with highly label-free Si-dots with highly and applied it for fabricating several biosensors.[4, 8] Based on unique optical properties, the glucose[4] and pesticides[8] biosensors were developed. In order to expand the application of Si-dots, it is extremely important to explore the unknown prosperities and develop the new application of Si-dots.

In the present work, we demonstrated Si-dots have intrinsic peroxidase mimetics catalytic ability for the first time. Based on the peroxidase mimetics of Si-dots, and selective catalytic oxidation of glucose by glucose oxidase, [9] glucose colorimetric analysis with naked eyes was carried out, which is shown in Fig. 1. This new type of biosensor does not require complex modification and enzyme immobilization. This offers a simple, sensitive and selective colorimetric method for glucose determination in serum.

The Si-dots were successfully synthesized by the phosphomolybdic acid (POM) -assisted electrochemical etching of bulk Si (see detailed synthesis in the Supporting Information). The morphology and optical properties of the as-prepared Si-dots were characterized and the results are shown in Fig. S1. The TEM (Fig. S1A) image and the histogram of particles size distribution (inset) indicate that the Si-dots were mostly appeared as spherical dots with high monodispersity in diameters of 4~25 nm. The average size was about 12 nm in diameter. ED (Fig. S1B) and HRTEM (Fig. S1C) patterns of Si-dots indicate these nanoparticles exhibited single crystalline structures.

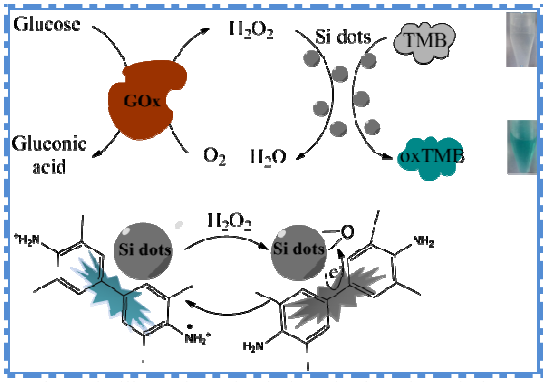

Fig. 1 Schematic illustration of colorimetric detection of glucose using glucose oxidase (GOx) and Si-dots.

To confirm the hydrogen terminated surface (H-Si), optical properties of Si-dots, FTIR, UV-Vis and fluorescence spectra (FL) measurements were performed. The FTIR spectrum (Fig. S1D) shows strong stretching vibration of Si-H bonds at around 900 cm^{-1} and the stretching vibration of coupled H-Si-Si-H bonds at 2100 cm^{-1}.[4] The UV-vis absorption (red) and photoluminescence (black) spectra of Si-dots in aqueous solution are presented in Fig. S1E. The absorbance about 290 nm is due to Si-dots.[10] It is reported that the PL properties (e.g., wavelength) of H-terminated Si-dots are sensitively dependent on the dot size.[11] The emission spectrum of the Si-dots solution has a narrow band ranged from 400 to 500 nm, which further illustrates that the size of the Si-dots is uniform.[11] The PL quantum yield of the as prepared Si-dots was up to ~9.4% according to the Williams method, which is the same as literature.[4]

All of the results show that the Si-dots with hydrogen terminated surface were successfully synthesized by the phosphomolybdic acid (POM)-assisted electrochemical etching of bulk Si. As shown in Figs. S2A and B, the PL intensity of Si-

dots was not affected by the temperature and pH, suggesting that the Si-dots were stable when they were incubated at a wide range of pH (2−9) and temperatures (20−90 °C) for 2 h.

To prove the feasibility of the strategy, the catalytic oxidation of peroxidase substrate TMB was tested in the presence of Si-dots and H_2O_2. Fig. 2 shows the UV–vis absorption spectra for different test solutions. Fig. 2 (inset) and Fig. S1E show that both Si-dots and TMB didn't have absorption at 370 nm and 652 nm. That to say, the absorption peaks at 370 nm and 652 nm reflect the existence of oxidative product of TMB. Compared with the absorption spectrum of the mixture of Si-dots and TMB solution (curve c), weak absorption at 370 nm and 652 nm were observed in the case of TMB and H_2O_2 (curve b), which indicated that the weak oxidation of TMB with H_2O_2 occurred. Much stronger absorbance was observed in the present of Si-dots in TMB and H_2O_2 solution (curve a), indicating that the reaction of TMB with H_2O_2 was greatly accelerated by

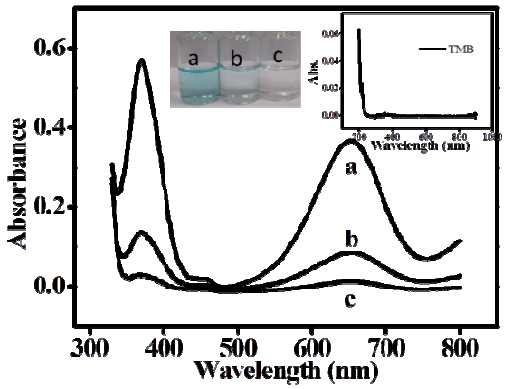

Fig. 2. The effect of the Si-dots in reaction systems. (a) TMB, Si-dots and H_2O_2, (b) TMB, and H_2O_2, (c) Si-dots and TMB. Reaction conditions: solutions were incubated in 0.2 mM NaAc/HAc buffer (pH 4.0) at 30 °C for 10 mins. Inset shows the color change of different samples.

Si-dots. Accordingly, a noticeable color change of the solution also was observed in the present of Si-dots in the oxidation system of TMB and H_2O_2 (see inset in Fig. 2). To further characterize the peroxidase-like activity of the Si-dots, several typical peroxidase substrates, such as 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS) and o-phenylenediamine (OPD) were used as replacement of TMB. (Fig. S3). All of the results confirmed that the as-prepared Si-dots exhibited an intrinsic peroxidase-like activity which was similar to HRP (eqn. 1).

$$H_2O_2 + TMB \xrightarrow{Si-dots} H_2O + OxidizedTMB \quad (1)$$

It is well known that the enzymatic activity is dependent upon the substrate and the reaction conditions. The catalytic activity of Si-dots may be dependent on pH, temperature and H_2O_2 concentration. As shown in Fig. S4, the UV-vis absorbance at 652 nm increased with pH and temperature in the range of pH 3.0-4.0 and temperature 25-40 °C, then declined at higher pH and temperature. The experimental phenomena implied that the Si-dots reached highest catalytic activity at the vicinity of pH 4.0 and 40 °C. The effect of the H_2O_2 concentration on catalytic activity of Si-dots was also investigated. The intensity of absorption at 652 nm sharply increase with increasing in H_2O_2 concentration from 10 mM to 200 mM and then it gradually slows down when the concentration beyond 300 mM. It is suggested that the interaction of H_2O_2 with TMB catalyzed by Si-dots reached equilibrium within 300 mM. Hence, the catalytic activity was the best in 300 mM H_2O_2 acidic solutions (pH 4.0) at 40 °C. From Fig. S4，a linear relationship in range of 1 to 200 mM (R^2=0.956) was obtained, which implied that the Si-dots catalytic system could be used in analytical system involved H_2O_2.

To investigate the kinetic mechanism of the peroxidase-like activity of Si-dots, apparent steady-state kinetic parameters for the peroxidase-like color reaction was determined by changing the concentration of TMB and H_2O_2 in this system, respectively. Kinetic experiments were carried out using 10 μL of Si-dots in 0.5 mL of 0.2 M NaAc/HAc buffer solution (pH 4.0) containing 320 μM TMB as the substrate and the H_2O_2 concentration was 20 mM. Method for calculation of initial reaction rate is using Beer–Lambert Law $c = \frac{A}{\varepsilon b}$. Absorbance data were back-calculated to concentrations by using a molar absorption coefficient ε of 39000 M^{-1} cm^{-1} for TMB-derived oxidation products.[12] Apparent steady-state reaction rates at different concentrations of the substrate were obtained by calculating the slopes of the absorbance change with time. In this work, typical Michaelis−Menten curves were obtained with both TMB and H_2O_2 by monitoring the absorbance change at 652 nm for 5 mins (Fig. S5A and B, Supporting Information).[13] Michaelis−Menten constant (K_m) and maximum initial velocity (V_{max}) were obtained by Lineweaver−Burk plots of the double reciprocal of the Michaelis-Menten equation,

$$\frac{1}{v} = Km / V_{\max}(\frac{1}{[S]} + \frac{1}{K_m}) \quad (2)$$

Where ν is the initial velocity, V_{max} is the maximal reaction velocity, [S] is the concentration of the substrate and K_m is the Michaelis constant. The results were shown in Fig. S5 and summarized in Table 1. The smaller the value of K_m, the stronger the affinity between the enzyme and the substrate and the more efficient is the catalyst. The apparent K_m value for the Si-dots with H_2O_2 as the substrate was lower than that of HRP (Table 1), suggesting that the Si-dots had higher affinity to H_2O_2 than HRP. The apparent K_m value of the Si-dots with TMB was larger than that of HRP, suggesting that the Si-dots had a lower affinity for TMB than that of HRP.

Table 1. Comparison of Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}) of the Oxidation Reaction Catalyzed by the Si-dots and Reported C-Dots and HRP

Catalyst	Substance	K_m/mM	V_{max} /10^{-8}M*s^{-1}
HRP[34]	TMB	0.434	10
	H_2O_2	3.702	8.71
Si-dots	TMB	1.502	14.72
	H_2O_2	0.065	5.65

To further investigate the catalysis mechanism of the Si-dots in the system, we measured their activity under standard reaction condition by varying concentrations of TMB at a fixed concentration of H_2O_2 or *vice versa*. The double reciprocal plots of the initial velocities against the concentrations of one substrate were obtained over a range of concentrations of the second substrate (Fig. S5C and D). The lines were parallel, and it was

the characteristic of a ping-pong mechanism,[14] which was also observed in HRP-based systems. The experimental facts indicated that Si-dots bind and react with the first substrate, then release the first product before reacting with the second substrate, which is similar to HRP and some other nano-materials.[12]

A possible catalytic mechanism of Si-dots is presented in Fig. 1. First, It is well known that the as-prepared hydrogen terminated Si-dots were catalytically inactive.[4] Therefore, H-Si-dots were partially oxidized into core-shell structure by H_2O_2, on which H_2O_2 molecules adsorbed, and H_2O_2 was decomposed into active oxygen species with oxene characteristics.[15] They became electrophilic and are prone to oxidize TMB to TMB_{OX} (Fig. 3). We measured the amperometric responses of the Si-dots modified glassy carbon electrode (Si-dots/GCE). The reduction current increased sharply to reach a steady-state value at Si-dots/GCE with the addition of H_2O_2 (Fig. S6), which clearly demonstrated that the Si-dots exhibited electrocatalytic activity to H_2O_2 reduction, which may promote electron transfer between electronic acceptor (H_2O_2) and electronic donator (the underlying electrode).

The stability of the Si-dots and robustness of peroxidase activity in wide pH and temperature ranges is crucial to extend their applications. Fig. S7 showed the catalytic activity of Si-dots has not change when they were incubated at a wide range of pH (2−9) and temperatures at 20−90 °C for 2 h, which makes them suitable for expand their applications in biomedicine and environmental fields.

The nature of peroxidase-like activities of the Si-dots nanostructures may originate from their catalytic ability to H_2O_2. According to the research mentioned above, H_2O_2 can oxide the TMB in the present of peroxidase. H_2O_2 as a product of catalytic oxidation of glucose, glucose could be detected based on the flowing reaction.

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{GOx} H_2O_2 + gluconic\ \text{acid} \qquad (3)$$

Considering the assay conditions, such as enzymatic factor, temperature, pH value and incubation time, have a significant effect on the detection of glucose, the experimental conditions, such as, pH incubation temperature and time were optimized (Fig. S8). The optimum temperature, time and pH were 35 °C, 30 min and 7, respectively. Under the optimized conditions, a linear relationship was obtained between absorbance and glucose concentration in the range of 0.17 to 200 μmol L^{-1} (R^2=0.987), with a detection limit of 0.05 μmol L^{-1} (Fig. 3). This detection method based on Si-dots gave a lower detection limit than the method using HRP and other nanoparticles as catalyst (Table S1).

To investigate the selectivity for glucose detection, the control experiments were taken using fructose, lactose, and maltose. The results were shown in Fig. S9. Even the concentration of the control samples was 10 times larger than glucose, no obvious response was observed, indicating that the colorimetric detection method can be used to detect the glucose selectively.

The excellent specificity and high sensitivity of the sensor suggested that the developed method might be directly applied for detecting glucose in real samples. Therefore, we detected glucose in diabetes serum sample provided by Hunan Normal University Hospital. Fig. S10 showed the UV−vis spectra and the experiment performed good colorimetric differentiation. According to the calibration curve, the concentration of glucose in blood was 10.2 mM (110.8 mg dL^{-1}). The blood glucose test is positive when the amount of glucose is more than 100 mg dL^{-1}. This colorimetric method is applicable to determine glucose in blood sample, suggesting that our system was successful in detection of glucose in real samples.

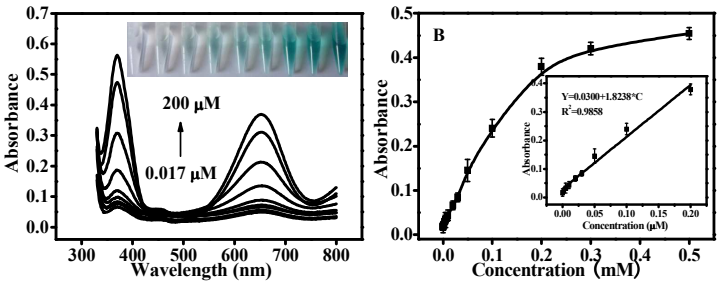

Fig. 3. (A) UV/vis spectra for the mixed solution of TMB, Si-dots and glucose incubation solution (pH 7.0 buffer, containing GOx) in 0.2 M NaAc/HAc buffer (pH 4.0) at 40°C. The TMB concentration was 320 μM and 10 μL Si-dots dispersion was used. Inset: the corresponding images of colored products. (B) Dependence of the absorbance at 652 nm on the concentration of glucose from 0.017 μM to 5 mM. Inset: the corresponding linear calibration plot.

In summary, we firstly report that the Si-dots possess intrinsic peroxidase-like activity. On the basis of research, we provide a simple, inexpensive, highly sensitive and selective colorimetric assay to detect serum glucose. As a novel nano-peroxidase mimetics, the Si-dots show several advantages over HRP, such as stability, dispersibility and high catalytic efficiency. Therefore, we expected that Si-dots have great potential applications in biotechnology.

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Notes and references

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