

Ultrasmall Pt Nanoclusters as Robust Peroxidase Mimics for Colorimetric Detection of Glucose in Human Serum

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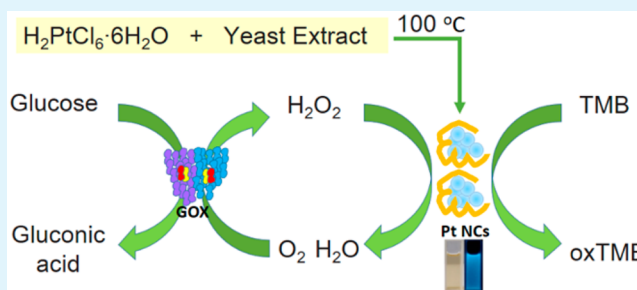
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Supporting Information

ABSTRACT: In this work, a new type of ultrasmall Pt nanoclusters (Pt NCs) was prepared via a facile one-pot approach by using yeast extract as the reductant and stabilizer. Besides their excellent water solubility, these yeast extract-stabilized Pt NCs also possess attractive peroxidase mimicking property. They can efficiently catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the coexistence of hydrogen peroxide (H_2O_2). Catalytic mechanism analysis suggested that the peroxidase mimicking activity of these Pt NCs might originate from their characteristic of accelerating electron transfer between TMB and H_2O_2 , and their enzymatic kinetics followed typical Michaelis–Menten theory. On the basis of these findings, we developed a new highly sensitive colorimetric method for glucose detection, and the limit of detection was calculated as low as $0.28\ \mu M$ ($S/N = 3$). Further application of the present system for glucose detection in human serum has been successfully demonstrated, suggesting its promising utilization as robust peroxidase mimics in the clinical diagnosis, pharmaceutical, and environmental chemistry fields.

KEYWORDS: Pt nanoclusters, peroxidase mimics, H_2O_2 , glucose, biosensor



1. INTRODUCTION

Enzyme is a kind of important biological catalyst that has been practically used in food processing, chemical industry, medicine, and biochemistry fields. Natural enzymes are usually composed of proteins and RNA molecules; thus, they inevitably suffer from some intrinsic drawbacks, such as expensive costs in extraction and separation, rigorous catalysis reaction condition, and weak stability due to protein denaturation.^{1,2} As a result, widespread application of these conventional natural enzymes is largely restricted.³ In the past two decades, a lot of effort has been made to the development of novel mimetic enzymes as the alternative to natural enzymes.^{4–8} Among them, nanomaterial-based mimic enzymes have attracted intensive attention, which mainly because their large surface-to-volume ratio is advantageous for achieving highly efficient catalytic activity.^{9–11} Several nanomaterials showing excellent artificial enzymatic activity have been reported, such as Au nanoclusters,¹² Pt nanotubes,¹³ Fe_3O_4 nanoparticles,¹⁴ WS_2 nanosheets,¹⁵ graphene oxide,¹⁶ and carbon dots.¹⁷ Compared with natural enzymes, these nanomaterial-based enzyme mimetics exhibit many distinct advantages, including less consumption, better stability, and higher tunability in catalytic activities, thus showing great potential in applications like bioassays and

clinical diagnosis.^{10,18–20} Nevertheless, with increasing enzyme-related technological sophistication, the desire to further explore more superior enzyme mimetic systems still exists, and the relevant progress is also important for the development of biomimetic chemistry.

Platinum (Pt) is one of the most common noble metal elements that are widely used in chemical industries. At bulk scales, Pt element is chemically inert, but it can serve as an important catalyst at nanoscale.^{21–23} Until now, several Pt nanomaterials have been found to possess enzyme mimetic activities including superoxide dismutase, catalase, oxidase, and peroxidase.^{24–30} However, utilization of such enzyme-like activities of these Pt nanomaterials for practical biological application, for example, biosensing, remains largely unexplored, which is mainly because of the challenges in controlling the size and structures of Pt nanomaterials to achieve superior enzymatic activities.^{31–35} In recent years, ultrasmall Pt nanoclusters (Pt NCs) have received increasing attention. With many favorable features including small size, unprece-

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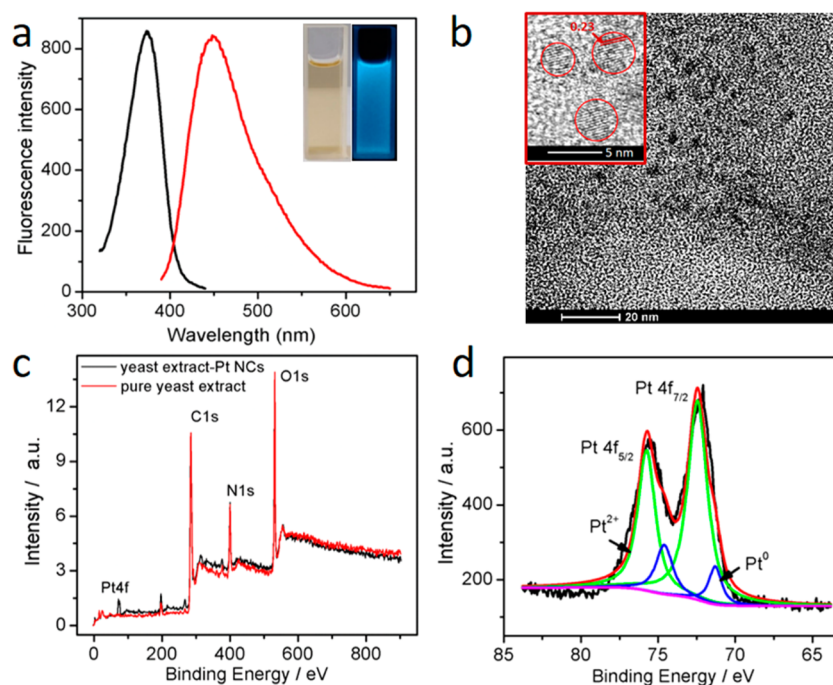


Figure 1. (a) Fluorescence spectra of Pt NCs solution. (black) Excitation spectra. (red) Emission spectra. (inset) Left photograph is Pt NC solution under visible light; right photograph is Pt NC solution under 365 nm UV light irradiation. (b) Representative TEM image of Pt NCs. (inset) HRTEM image of several individual Pt NC as indicated by the red circle. (c) The wide-scan survey XPS spectra of pure yeast extract (red) and Pt NCs (black). (d) XPS spectra of Pt 4f in Pt NCs.

dented catalytic performance, and good biocompatibility compared with other reported nanostructures, Pt NCs are considered to be more attractive for bioanalytical purposes.^{36–38} In this work, we synthesized a new kind of ultrasmall Pt NCs with yeast extract as both reductant and stabilizer. The obtained Pt NCs possess good water solubility and intense fluorescence property. More interestingly, they can efficiently catalyze the reaction of peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) with H_2O_2 , indicating a superior peroxidase mimetic activity. Meanwhile, the catalytic activity of Pt NCs shows a strong dependence on the concentration of H_2O_2 , based on which a highly sensitive colorimetric sensor for H_2O_2 and glucose determination was established by employing Pt NCs as robust peroxidase mimetics. This system also exhibits good reproducibility and high selectivity for glucose determination, suggesting its great potential for biocatalysis and bioassays in the future.

2. EXPERIMENTAL SECTION

2.1. Materials and Apparatus. Yeast extract (LP0021) was purchased from Oxoid (U.K.). Glucose oxidase (GOD from *Aspergillus niger*) was bought from Sigma-Aldrich. $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$, H_2O_2 , 3,3',5,5'-tetramethylbenzidine (TMB), glucose, and other salts were commercially available and used without further purification. All aqueous solution was prepared by deionized water.

An RF-5301 spectro-photometer (Shimadzu, Japan) was used to take the fluorescence emission spectra. A UV-2550 spectro-photometer (Shimadzu, Japan) was used to measure the UV/vis spectra. Tecnai G2 F20 instrument (FEI, USA) was employed to take transmission electron microscope (TEM) images of Pt NCs. For X-ray photoelectron spectroscopy (XPS) measurement, the Pt NC aqueous solution was first freeze-dried into powder and then determined on an Axis Ultra X-ray photoelectron spectrometer (Kratos, U.K.). ZetaPALS instrument (Brookhaven, USA) was used to measure the zeta-potential of Pt NCs in aqueous solution at room temperature.

CHI 842D workstation (Chenhua, China) was used to take on the electrochemical experiments.

2.2. Preparation of Pt NCs. In a typical synthesis, 4 mL of 20 mM $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ aqueous solution was added to a yeast extract aqueous solution (16 mL, 200 mg). Then the resulting mixture was refluxed for another 12 h under vigorous stirring at 100 °C. The light yellow reaction solution gradually changed to brown color. After that, the crude products were first purified by centrifugation to remove the precipitate from the supernatant (15 000 rpm, 20 min). Another purification step to remove the unreacted small molecule components was performed by ultrafiltration (3000 Da pore size Amicon ultracentrifugal filter units) with a cycle at 4000 g for 30 min. After the purification, Pt NCs were stored at 4 °C in the dark and used for the following experiment. The final concentration was calculated to be ~4 mM based on Pt atoms and the weight-to-volume concentration was ~15 mg/mL.

2.3. Steady-State Kinetic Assays. The reaction kinetic measurements were performed by using 100 μL of Pt NCs with 500 μM TMB as substrate, or 10 mM H_2O_2 . The reaction solution was diluted to 3 mL by 0.1 M, pH 5.0 phosphate-buffered saline (PBS) solution. Each absorbance was recorded at 652 nm in scanning kinetics mode with a 100 s interval at temperature 30 °C. The enzymatic parameters, V_{max} (the maximal reaction rate) and K_{m} (the Michaelis–Menten constant), can be obtained from the Lineweaver–Burk plot.¹⁴

2.4. Detection H_2O_2 and Glucose. For H_2O_2 standard curve measurement, 50 μL of TMB (30 mM), 100 μL of Pt NCs (15 mg/mL), and 100 μL of H_2O_2 with different concentrations were successively added into 2.75 mL of PBS (0.1 M, pH 5.0) solution. The mixture solution was then incubated in a thermostat at 30 °C for 20 min before the spectral measurements. For the glucose calibration, first 50 μL of different concentrations of D-glucose and 50 μL of 1 mg/mL glucose oxidase (GOD) were mixed in 200 μL of PBS (0.1 mM, pH 7.4) before incubation at 37 °C for 10 min. The glucose reaction solution was added to another mixture containing 50 μL of TMB (30 mM), 100 μL of Pt NCs (15 mg/mL), and 2.55 mL of PBS (0.1 M, pH 5.0). After it was incubated at 30 °C for another 20 min, the mixed solution was then applied to the spectral measurement. For evaluation of glucose assays in real biological samples, the serum samples from

healthy adults (provided by local hospital) was first centrifuged (12 000 rpm, 10 min) to remove possible large aggregates in human serum. The supernatant was diluted 150 times and measured with the same detection steps as mentioned above.

3. RESULTS AND DISCUSSION

3.1. Characterization of Pt NCs. The Pt NCs were synthesized in aqueous solution by a simple reflux reaction. Herein, yeast extract was selected as the template for NC synthesis, because it contains many biomolecules, such as amino acids and proteins, which provide abundant active sites that can efficiently bind and reduce metal ions.^{39,40} The aqueous solution of Pt NCs shows beige color and exhibits a bright blue emission under the irradiation of a UV lamp (365 nm). The emission spectrum of prepared Pt NCs shows a maximum at 448 nm upon excitation at 370 nm (Figure 1). In contrast, neither pure yeast extract nor H_2PtCl_6 solution exhibited the same fluorescence under the equal experimental conditions, indicating that our observed blue emission indeed originates from Pt NCs. The fluorescence quantum yield of Pt NCs was calculated to be 8.4% (referred as quinine sulfate in alcohol), which is beyond the value of most reported Pt NCs.^{37,38,41} Transmission electron microscopy (TEM; Figure 1b) revealed that as-prepared Pt NCs were mostly spherical in shape, and the average size was 3.0 ± 0.3 nm. The crystal lattice fringes of Pt NCs was 0.23 nm (high-resolution transmission electron microscopy (HRTEM), inset in Figure 1b), which corresponds to the (111) lattice plane of Pt crystals.²⁸ XPS was then performed to investigate the valence states of key elements in our as-prepared Pt NCs. As shown in Figure 1c, the peaks located at 73.2 eV in wide-scan survey of XPS correspond to Pt 4f, revealing the presence of Pt in our product. In the high-resolution Pt 4f_{7/2} region, the binding energy of Pt 4f_{7/2} can be deconstructed into two components: Pt⁰ and Pt²⁺, with binding energies placed at 72.7 and 71.4 eV, respectively (Figure 1d). These results indicated that the Pt⁴⁺ element in H_2PtCl_6 was completely reduced in the NCs and further supported the successful formation of yeast extract stabilized fluorescent Pt NCs.²⁹

3.2. Peroxidase Mimics Activity of Pt NCs. Herein, we first studied the catalytic oxidation abilities of Pt NCs toward peroxidase substrate TMB to evaluate whether they can be applied as peroxidase mimics. As displayed in Figure 2, in coexistence of H_2O_2 , it is found that Pt NCs could accelerate the oxidation reaction of TMB to produce an obvious blue color change (inset in Figure 2), with a maximum absorption

peak centered at 652 nm (curve C in Figure 2). In contrast, neither Pt NCs nor H_2O_2 with TMB system yields an absorption band at 652 nm (curves A and B in Figure 2), and the results indicate that both two components must be required and that the reaction of TMB with H_2O_2 was greatly accelerated by Pt NCs. Meanwhile, our control experiments showed that neither pure yeast extract solution nor yeast extract in acid environment after the identical reaction conditions display the similar peroxidase-like activity, demonstrating that the observed catalysis behaviors indeed originate from Pt NCs (Figure S1). To further characterize the peroxidase mimicking property of yeast extract-Pt NCs, we also investigated their reaction with other typical peroxidase substrates, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and *o*-phenylenediamine (OPD; Figure S2), which all confirmed that these Pt NCs can be considered as an efficient peroxidase mimic to imitate horseradish peroxidase (HRP).

The response of the catalytic activity of peroxidase mimetics was usually dependent on many surrounding conditions, such as the solution pH, temperature, and the concentration of TMB or H_2O_2 . As shown in Figure 3, compared with neutral and basic solutions, Pt NCs exhibit much higher catalytic activity in weakly acidic solutions, and they yield a maximum absorbance at 30 °C when varying the temperature from 20 to 80 °C. Under our experimental conditions, as well as considering the future physiological application, the optimal pH (pH 5.0) and temperature (30 °C) were chosen for subsequent experiments. For the substrate concentration-dependent activity, the results showed that the largest Pt NCs activity could be obtained by adding 500 μM TMB. Therefore, the concentration of TMB for the following experiments was set as 500 μM . In addition, no inhibition was found for the Pt NC-catalyzed reaction at a H_2O_2 concentration up to 600 μM , suggesting that Pt NCs also exhibit stable catalytic activity at high H_2O_2 concentration.

3.3. Kinetic and Mechanism Investigation of Pt NCs as Peroxidase Mimics. To obtain the steady-state kinetic parameters, we further studied the catalytic behavior of Pt NCs with H_2O_2 or TMB as substrates, based on enzyme kinetics theory and methods. As displayed in Figure 4, typical Michaelis–Menten curves could be observed with either TMB or H_2O_2 as the substrate. The enzymatic parameters K_m and $V_{\max}$ can be calculated from Lineweaver–Burk plots and were summarized in Table S1. As shown, compared with natural enzymes HRP, the K_m value of Pt NCs with both H_2O_2 and TMB are much lower, suggesting that Pt NCs possess higher affinity for two substrates than HRP.^{12,42}

To explore the possible mechanism of catalytic behavior from Pt NCs, their enzymatic activity in several different systems was then studied. First, we chose terephthalic acid/ H_2O_2 system to examine whether the peroxidase mimicking property of Pt NCs was related to the generation of the $\cdot\text{OH}$ radical. Herein, the terephthalic acid was employed as a fluorescence probe, which can yield a highly fluorescent product upon reacting with $\cdot\text{OH}$ (2-hydroxy terephthalic acid).^{43,44} As it turned out, the fluorescence intensity gradually decreased with increasing Pt NC concentration (Figure S3a,b), suggesting that Pt NCs could consume $\cdot\text{OH}$ radicals instead of generating them. Therefore, similar to the reported behavior of ferritin–platinum nanoparticles,²⁸ the nature of catalytic behavior of our Pt NCs was not attributed to the $\cdot\text{OH}$ radical generation.

We also investigated the electrocatalytic behavior of Pt NCs toward the electrochemical reduction of H_2O_2 . As shown in

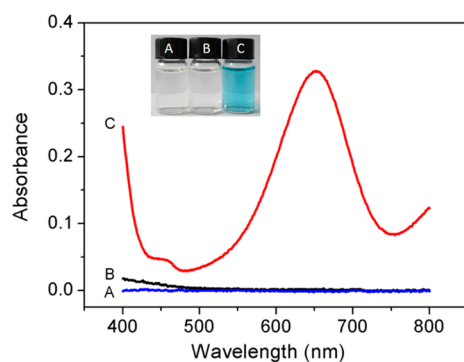


Figure 2. Typical absorption spectra of the different solutions: (A) TMB+Pt NCs, (B) TMB+ H_2O_2 , (C) TMB+ H_2O_2 +Pt NCs. (inset) The color of corresponding solutions.

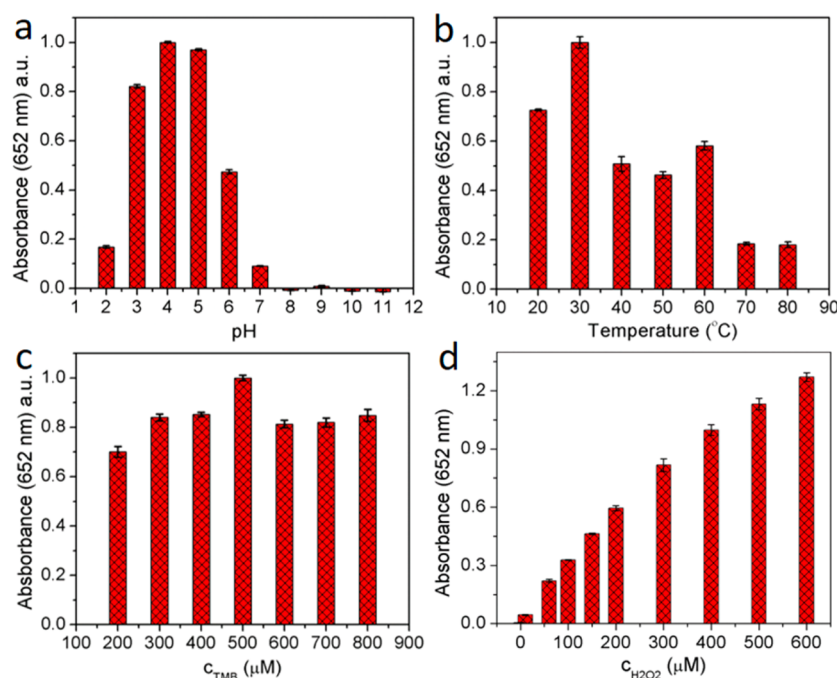


Figure 3. Effect of different surrounding conditions on the peroxidase mimetics property of Pt NCs: (a) pH; (b) temperature; (c) TMB concentration; (d) H_2O_2 concentration.

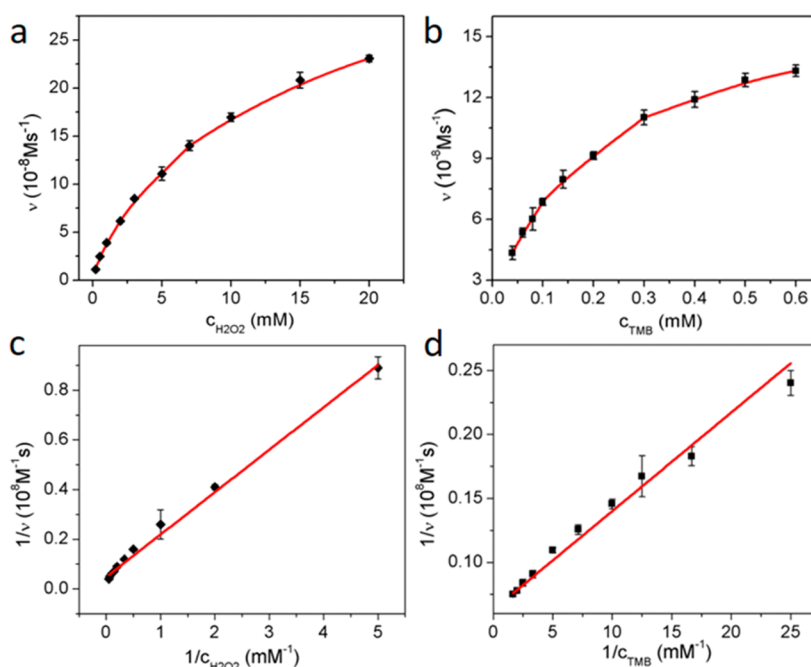


Figure 4. Steady-state kinetic assay of Pt NCs: (a) 500 μM TMB with different concentrations of H_2O_2 . (b) 10 mM H_2O_2 with different concentrations of TMB. (c, d) Double-reciprocal plots of (a, b), respectively.

Figure S3c, upon addition of 1.0 mM H_2O_2 , no obvious current was found for bare GCE, while the reduction current increases significantly for Pt NC-modified GCE. The result suggested that Pt NCs possess an electrocatalytic ability to the reduction of H_2O_2 through promoting electron transfer between the GCE and H_2O_2 . Thus, it is very likely that the nature of peroxidase mimicking of our Pt NC was attributed to their ability of facilitating electron transfer between TMB and H_2O_2 .^{16,18} The control experiment showed that the Pt NCs are more efficient in acidic conditions, and the pK_a of Pt NCs was calculated to be

6.4 based on the relationship between fluorescence intensity and pH. Therefore, in the optimum pH condition, as-prepared Pt NCs are negatively charged (zeta potential in pH 5.0 was -10.2 mV), which can adsorb positively charged TMB on their surfaces via electrostatic interactions. As a result, the electron density of Pt NCs is greatly increased, which can simultaneously further accelerate the reaction rate of TMB oxidation by H_2O_2 .¹⁸

3.4. Colorimetric Detection of H_2O_2 and Glucose with Pt NCs. On the basis of the above optimum assay conditions,

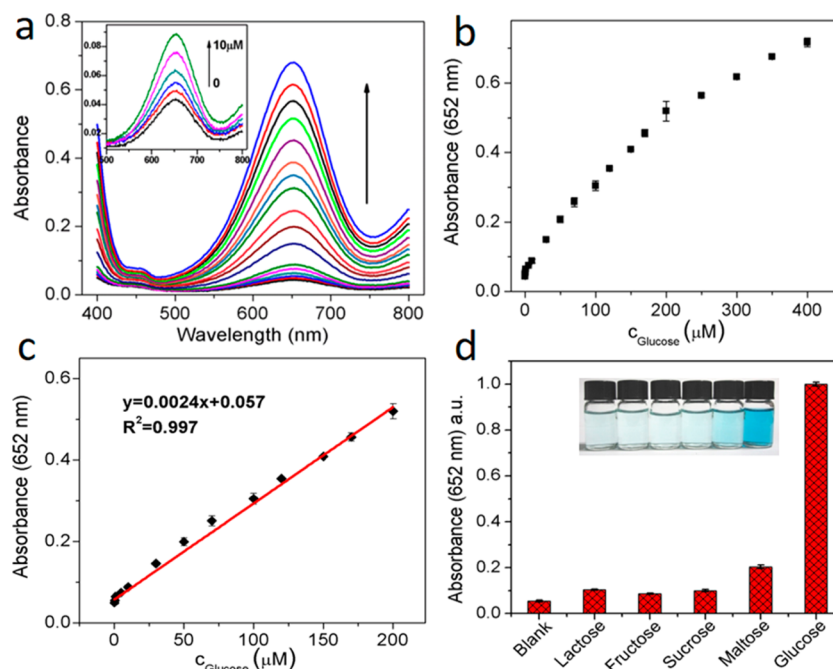


Figure 5. (a) The absorption spectra of Pt NCs and TMB system upon adding various concentrations of glucose (0–400 μM , from bottom to top). (inset) Spectral change within 0–10 μM glucose concentration range. (b) Calibration plots of the absorbance vs the glucose concentration under the optimum conditions. (c) The linear regression to plots of the absorbance with the glucose concentration. (d) The absorbance at 652 nm of the system with different carbohydrates (2 mM), while the concentration of glucose was 0.2 mM. (inset) Color change with the corresponding substances (from left to right: blank, lactose, fructose, sucrose, maltose, and glucose).

we evaluated the analytical performance of our H_2O_2 sensing system. As shown in Figure S4, the absorbance of the oxidized TMB at 652 nm sharply increased with raising H_2O_2 concentration from 0 to 200 μM , after which it gradually slowed. The Stern–Volmer plot shows a good linear relationship ($R^2 = 0.994$) with a range from 0 to 200 μM . The limit of detection, defined as a signal-to-noise ratio (S/N) of 3, was calculated to be 0.46 μM . The relative standard deviation (RSD) of seven repeated measurements of 100 μM H_2O_2 was 1.42%, illustrating a highly reproducible response of our system for H_2O_2 detection.

Glucose can be oxidized to produce gluconic acid and H_2O_2 after the reaction with GOD and O_2 .⁴⁵ Thus, the sensitive H_2O_2 response of the present system made it possible to further establish a platform for detecting glucose, an important biological analyte. As shown in Figure 5a, with increasing the concentration of glucose up to 450 μM , the absorbance at 652 nm was found to raise gradually. A good linear relationship ($R^2 = 0.997$) was obtained in the concentration range of 0–200 μM (Figure 5c). The calculated limit of detection (LOD) of glucose was measured to be 0.28 μM at S/N = 3, which is comparable or even superior to those achieved by using other colorimetric methods,^{31,46–48} as we summarized in Table S2. Additionally, the color of the system exhibited perceivable blue when the glucose concentration was 1 μM (Figure S5), suggesting that our method can perceive as low as 1 μM glucose with the naked eyes.

To evaluate the selectivity of the proposed method for glucose detection, control experiments were performed by monitoring the absorbance change upon addition of various other carbohydrates. No significant interference was observed from fructose, lactose, sucrose, maltose, or mannose (Figure 5d), suggesting our system is highly specific for glucose detection. Seven replicate measurements of 100 μM glucose

yield a relative standard deviation of 1.41%, implying an excellent reproducibility. Moreover, we also investigated the robustness and the stability of Pt NCs at different pH and temperature surroundings (Figure S6a,b). The results showed that the catalytic behavior of Pt NCs did not change obviously after incubation for 2 h with varying pH (1 to 9) and temperature (0 to 80 $^\circ\text{C}$) conditions. The catalytic activity of Pt NCs could be maintained above 90% after one month (Figure S6c), suggesting an excellent stability during long-term storage. Furthermore, the fluorescence property of Pt NCs has no obvious change after catalyzing the oxidation of TMB with H_2O_2 (Figure S7). All the above results demonstrated that the catalytic ability of our Pt NCs provides a simple, sensitive, and specific strategy for glucose determination.

3.5. Glucose Detection in Human Serum. To explore the practical usability of our sensing system, we then determined the glucose concentration in human serum. Time-diluted concentrations (150) of original serum samples were adopted to guarantee the glucose content in samples was in the range of our established standard curve. As listed in Table 1, the results obtained by our approach were close to that measured with local hospital-used instrument (Bekman

Table 1. Detection of the Content of Glucose in Human Serum Sample

human serum sample	diluted 150 times (μM)	added (μM)	recovery (%)	RSD ($n = 3$) (%)	experimental result (mM)	glucose assay kit (mM)
1	25.80	50.00	97.8	1.97	3.96	3.87
2	30.33	50.00	101.5	4.04	4.53	4.55
3	33.00	50.00	96.1	3.81	5.19	4.95
4	44.07	50.00	96.7	3.13	6.86	6.61

AUS800). Furthermore, the recovery of this sensing system was in the 96.1–101.5% range, and relative standard deviation of three repeated measurements was within the range of 1.97–4.04%. All these results demonstrated an excellent precision and reliability of our proposed method for future practical application.

4. CONCLUSIONS

To summarize, we have developed a new colorimetric method for glucose detection by using yeast extract stabilized Pt NCs as peroxidase mimetics. The enzymatic kinetics of Pt NCs follow typical Michaelis–Menten theory and can facilitate the electron transfer between TMB and H_2O_2 . Compared with other nanomaterial-based colorimetric approaches, our method is more sensitive and robust, allowing the detection of glucose concentration within the range of 0–200 μM , with a limit of detection as low as 0.28 μM . More importantly, this method has also been successfully used for glucose determination in real serum samples, which suggests its promising application in clinical diagnosis and pharmaceutical and environmental chemistry fields.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b01616.

Additional absorption spectra and images of Pt NCs-based sensing system, the catalytic activity results of Pt NCs after incubation in different environment conditions, the comparison data of K_m and $V_{\max}$ of the oxidation reaction catalyzed by Pt NCs and reported HRP, the comparison data of this work with previous nanomaterials-based mimic enzymes for glucose detection (PDF)

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Notes

The authors declare no competing financial interest.

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