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Multifunctional Janus Hematite-Silica Nanoparticles: Mimicking Peroxidase-Like Activity and Sensitive Colorimetric Detection of Glucose

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ABSTRACT

The design and engineering of multifunctional nanostructure with multiple components and synergistic properties are in urgent demand for variety of acceptable biosensing platform, enabling to fulfilling multiple tasks in a single nanosystem. Herein, we report the using an asymmetric hematite-silica hybrid of Janus γ-Fe_2O_3/SiO_2 nanoparticles (JFSNs) as multifunctional biosensing platform for sensitive colorimetric detection of H_2O_2 and glucose. It was demonstrated that the JFSNs exhibit an intrinsic peroxidase-like catalytic activity. Compared with natural enzyme, the JFSNs nanoenzymes could be used over a wider range of pH and temperatures and were more stable over time. Importantly, besides its excellent catalytic activity, the asymmetric properties of the Janus nanoparticle enable to form the multiple functional utilities for various biosensing applications, including the ease of surface modification without deactivation of catalytic activity and recoverable use by a magnetic separation. Thus, we utilized the JFSNs with glucose oxidase (GOx) immobilization for glucose-sensitive colorimetric detection, which exhibited both catalytic activity of glucose oxidase and peroxidase with high selectivity and acceptable reproducibility. Combinng this two analysis system into Janus particles, an all-in-one and reusable sensor for blood glucose was formed and has the capability for determination of glucose in complex samples such as serum. These results suggest that such Janus nanosystems have potential to construct robust nano-architecture with multi-functionalities for various biosensing applications.

KEYWORDS. Hematite-silica, Janus nanoparticle, Colorimetric biosensor, Hydrogen peroxide, Glucose

INTRODUCTION

The thrust in (bio)sensor research moves towards systematic assembling of multiple components into multifunctional utility, enabling to simultaneously and rapidly detect a large number of analytes with integrated capabilities of separation and concentration, signal transducing and quantification.[1] To incorporate the multiple functionalities into sensing system, much efforts have been devoted to understanding and fine-tuning the multi-component nanostructures, including the material composition and interface engineering.[2] Conceptionally, various existing nanosystems (*e.g.* TiO_2,[2] CeO_2,[3] Au,[4] Fe_3O_4[5] and SiO_2[6]) can be structurally and chemically tailored to combine the complex properties and functionalities.[7] For example, the non-centrosymmetric coupling of Au and Fe_3O_4 nanoparticles has been reported to potentially generate extremely strong magnetic and enhanced catalytic activity, and can be potentially applied as an optical reporter and a magnetic handle for bioassays.[8] However, the current controllable synthesis of these multifunctional nanostructures still faces tremendous challenges and problems still exist in their biosensing application. One of the major limitations is that the interaction interface of different components may restrict partial properties of each component. For instance, as for the core/shell structures, additional coatings and bioconjugation will dramatically decrease their special properties, such as degradation of catalytic activity from the core component. Another significant limitation for the commonly used nanosystems with symmetrical geometry is their limited surface available for attaching multiple components, which are unfavorable structural and chemical arrangements of these functional components. Therefore, the design and assembly of multiple functional nanostructure with multi-components and synergistic properties are urgently desired for variety of acceptable biosensing platform, which is expected to best utilize the intrinsic properties of the whole nanosystem.

Recently, "Janus"-type nanoparticles, named after the double-faced Roman God Janus, have emerged as a new division of multifunctional nanocomposite.[9] Such asymmetric architectures are composed of two incompatible hemispheres with different chemistry, polarity, or other physicochemical properties on opposite sides.[10,11] Although Janus nanoparticles combine individual components together, the intrinsic optical, magnetic, and electronic properties of each component are not often altered, interfered or completely lost,[12] thereby exhibiting improved physical/chemical properties[13] and great potential in numerous applications.[14] This makes them a unique category of materials in contrast to other particle. Specifically, the ease of functional modification on the distinct surface, which is the key step for the utilization of nanosystems in the field of biosensor and other bionanotechnology, renders Janus nanoparticles truly "multifunctional entities". To date, a variety of Janus nanoparticles have been reported to synthesize for applications in (bio)sensing,[14] magnetic fluorescent display or imaging,[15] and drug delivery.[16] However, only a few studies explored the potential of these Janus nanoparticles for multifunctional biosensing applications that simultaneous achieve separation and concentration, signal transducing and rapid and sensitive detection.

On the other hand, the development of nanobiosensor for sensitive and rapid glucose monitoring is in great demand because of the subjects with diabetes mellitus, which represents one of the largest health concerns of the 21st century with worldwide prevalence.[17,18] Among all these analytical techniques, colorimetric biosensing method has received considerable attention owing to their simplicity, improved sensitivity and high selectivity. Moreover, through this method, changes can be read out directly by the naked eye. No expensive or sophisticated instrumentation is required and it may be applied in field analysis and clinical and point-of-care (POC) diagnosis.[19] In the past few years, it has been found that some enzyme-like nanomaterials, such as Fe_3O_4 and Au nanoparticle, can catalyze the reaction of the peroxidase substrate to produce color change,[20-23] which affords an important tool for glucose colorimetric biosensing. Moreover, these enzyme-like nanomaterials, show several

advantages over natural enzymes, such as controlled synthesis in low cost, tunability in catalytic activities, as well as high stability against stringent conditions, thus being used in various aspects.[24-30] Particularly, magnetic nanoparticles (MNPs) have been considered as one of the most important nanozymes because of their unique peroxidase-like activity, magnetic, non-toxic and highly biocompatible properties and potential applications in biological separation,[31] drug delivery,[32] and biological catalysis.[33] Unfortunately, there is still challenging problem for the use of peroxidase-like MNPs nanozyme in glucose colorimetric detection, because the surface modification (e.g. glucose oxidase) and additional coatings (e.g. SiO_2) can decrease the peroxidase activity dramatically.[20] Thus, novel surface structure and bioconjugation techniques are required to maximize the performance of nanozymes. Moreover, to offer multiple functions in response to the glucose, exploring or developing multifunctional enzyme mimetics with high sensitivity, catalytic activity, reusability, stability and low toxicity still requires further efforts.

In the present report, we described a multifunctional hybrid Janus nanoparticles that are designed for colorimetric detection of glucose, which are a new kind of multifunctional nanoenzyme with peroxidase-like activity, and explored their biosensing application of H_2O_2 and glucose detection, as schematically illustrated in **Scheme 1**. According to our previous report,[34] we designed and synthesized a unique spherical Janus nanoparticles composed of γ-Fe_2O_3 and SiO_2. Interestingly, we here found that the resulted Janus hematite nanoparticles exhibit excellent peroxidase-like activity as well as magnetism functions for separation and concentration.[35] Moreover, the existence of SiO_2 in Janus nanoparticle facilitate the surface modification and bioconjugation of the particles.[36] Importantly, due to their asymmetric structure, such Janus nanoparticles will not suffer from inertness towards bioconjugation and render multiple functions. Therefore, we immobilized glucose oxidase (GOx) onto Janus nanoparticle to prepare bioactive magnetic peroxidase-like nanozymes for glucose colorimetric sensing. It was demonstrated that the GOx-attached Janus nanoparticles have

both glucose oxidase-like and peroxidase-like activity, resulting in fast and sensitive detection of glucose in one system. Meanwhile, due to the magnetism nature, the Janus nanoparticles-based sensing platform permits easy sample separation/concentration, recycle and reusing,[37] and also allows in the multiple detection glucose in the complex sample such as serum.

EXPERIMENTAL SECTION

Chemicals and Materials: Horseradish peroxidase (HRP, 25000 IU/mg), glucose oxidase (GOx, from Aspergillus niger), 30% H_2O_2, 3, 3', 5, 5'-Tetramethylbenzidine (TMB) were obtained from Sigma-Aldrich (Beijing, China). Glucose, maltose, R-lactose and D-fructose were purchased from Beijing Chemical Reagent Factory (Beijing, China). All other chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared with Milli-Q water (>18.3MΩ cm). The Janus JFSNs were synthesized by a one-step flame-assisted spray pyrolysis (FASP) approach using an enclosed reactor according to previous methods.[34]

JFSNs Modification with GOx: The *JFSNs* (40 mg) were firstly introduced into a 20 mL isopropanol solution (2 mg mL^{-1}). The resultant suspension was ultra-sonicated for 30min, then 100 μL of (3-aminopropyl) triethoxysilane was added to the solution. After reaction under stirring for 12 h, the Janus magnetic nanoparticles was separated by magnetic force, and then washed by ethanol and distilled water for several times. Finally, it was re-dispersed in water (16 mL) and stored at 4°C, being referred to as amine-JFSNs solution. Then, 12 mL of the resulted amine-JFSNs solution was treated with 4 mL of 2 % glutaraldehyde in the 4 mL PBS solution (pH 7.4) at 25 °C for 2 h. After washing by distilled water, the resulted nanoparticles were dispersed in 2 mL PBS buffer solution (pH 6.0) containing 400 μL of 1.0 mg mL^{-1} GO_X. The mixture was incubated for reaction under shaking for 12 h at 25 °C. After removing the unbound enzyme by the separation and washing process, the GOx-immobilized γ- Fe_2O_3/SiO_2 Janus NP, termed as GOx-JFSNs, were obtained.

JFSNs Catalytic Study: The catalytic activities of JFSNs for oxidation of TMB in the presence of H_2O_2 were investigated as following procedures: JFSNs (60 μg/mL) was incubated with 100 μL potassium citrate buffer (0.1 M, pH 3.7) solution containing 510 mM TMB (freshly prepared) and 1.59 M H_2O_2. After the mixed solution was incubated at 30 ^oC for 10 min, the photographs and UV spectra of the mixtures were taken. The catalytic activity of HRP was also tested by incubation with 2 ng/mL HRP, 816 μM TMB and 8.8 mM H_2O_2.

To analysis the reaction kinetic, steady-state kinetic assays of JFSNs towards TMB oxidation were carried out with varied concentration of the substrate TMB or H_2O_2 at room temperature. Unless otherwise stated, the concentrations of TMB and H_2O_2, were used as the same as the above used. The absorbance variation of the reaction solution was monitored in time-scan mode at 652 nm.[38] The kinetic parameters of the catalytic reaction were determined based on the Michaelis–Menten function $v = V_{max}[S]/ (K_m + [S])$, where v is the initial velocity of the reaction, is the maximal rate of reaction, [S] is the substrate concentration, and K_m is the Michaelis–Menten constant which is equivalent to the substrate concentration at which the rate of conversion is half of V_{max} and denotes the affinity of the enzyme.[39] The V_{max} was calculated into molar change from UV absorbance based on the equation of $A=\varepsilon lc$ (A is the absorbance, ε is the absorbance coefficient, l is the path length and c is the molar concentration) with $\varepsilon= 3.9 \times 10^4$ M^{-1} cm^{-1} and $l = 10$ mm.[40]

To study influence effect of the reaction buffer pH and on the relative activity of JFSNs and HRP, 0.1 M potassium citrate buffer solutions from pH 1.0 to 12.0 were used. And, to examine the influence of incubation temperature on the relative activity of JFSNs and HRP, catalytic reactions were incubated from 30 to 60 °C under the identical conditions. To investigate the stability of peroxidase-like activity, JFSNs and HRP were stored at room temperature, and the catalytic activity was measured every 24 h in consecutive 7 days.

Colorimetric Detection of H_2O_2: H_2O_2 was tested as follows: 0.625 μL of TMB (81.6 mM), 0.2 mg as-prepared JFSNs and 10 μL of H_2O_2 with various concentrations were added into 90

μL of potassium citrate buffer (0.1 M, pH 3.7). Then, the mixed solution was measured by UV-vis absorption spectroscopy.

Glucose Detection: 50 μL of GOx-JFSNs solution and 50 μL of glucose with different concentrations in PBS (pH7.0) were incubated at 37 ℃ for one hour. Then, 1.25 μL of 81.6 mM TMB and 100 μL potassium citrate buffer (pH 3.7) were added into the above 100 μL reaction solution. After incubation for 15 min, GOx-JFSNs were removed from the reaction solution by an external magnetic field. Subsequently, the final reaction solution was used for adsorption spectroscopy measurement. We also used fetal bovine serum as the model to verify the capability of detection in complex samples. Glucose was measured in the serum-containing PBS solution (pH 7.0).

Characterizations: High-resolution transmission electron microscopy (HRTEM: JEM- 2010, operated at 200 kV) and low-resolution transmission electron microscopy (TEM: H-800) were used to characterize the morphology microstructure. X-Ray diffraction (XRD, Rigaku D/max 2550VB/PC diffractometer with Cu Kα radiation) and energy dispersive X-ray spectroscopy (EDS) on JEM-2010 were employed to obtain chemical composition and structure information of flame made NPs. The absorption and reaction kinetics were measured on the UV-vis spectrometry (Thermofisher Evolution 201).

RESULTS AND DISCUSSION

In this study, the asymmetric hematite-silica nanoparticles of Janus γ-Fe_2O_3/SiO_2 nanoparticles, donated as JFSNs, were synthesized using a one-step flame-assisted spray pyrolysis approach according to our previous report[34]. As shown in the transmission electron microscopy (TEM) images (**Figure 1a and 1b**), the obtain nanoparticles have a typical asymmetric "Janus-type" morphology with ~20 - 120 nm in diameters. Each particle is composed of two distinct hemispheres and an obvious interface can be viewed between them. The high resolution TEM imaging (**Figure 1b**) also revealed that the inter planar spacing of

the lattice fringes of the dark hemisphere is about 0.252 nm, which is in good agreement with the interpalnar distance of γ-Fe_2O_3 phase.[41] In contrast, the other light hemisphere presented the amorphous nature of silica oxide. Furthermore, the constituents and crystal phases of as-prepared nanoparticles were confirmed by Energy Dispersive Spectroscopy (EDS) and X-ray diffraction (XRD). According to EDS and XRD results, the as-prepared nanoparticles showed the typical γ-Fe_2O_3 and SiO_2 signals, suggesting the successful synthesis of JFSNs nanohybrids (Figure S1a and Figure S1b, Supporting Information). In addition, the as-prepared nanoparticles can be well dispersed in aqueous solution (**Figure 1c, left**) and remain stable more than 12 hours without noticing any aggreation (verified by the UV-vis spectra, no detectable change occurred overnight). We also tested the magnetic response of the obtained JFSNs. When the JFSNs suspension solution was exposed to the external magnetic field, it was observed that the brown nanoparticles could be quickly attracted by an external magnet (**Figure 1c**). Meanwhile, the room-temperature magnetization result proved the JFSNs have a good magnetic response with a saturated magnetization at 22.5 emu g^{-1} (Figure S1c, Supporting Information). This magnetic nature of JFSNs suggests their potential applications in Magnetic Resonance Imaging (MRI), magnetic targeting, and hyperthermia. All together, these results demonstrated that the successful synthesis of the JFSNs.

To investigate the peroxidase-like activity of JFSNs, the catalysis of the chromogenic peroxidase substrates 3, 3, 5, 5-tetramethylbenzidine (TMB) was tested in the presence of H_2O_2. As shown in Figure 1d, the as-prepared JFSNs produced a blue color in the presence of H_2O_2 and TMB, indicating that the nanoparticles can catalyze the oxidation of TMB. The corresponding absorption spectra were shown in **Figure 1d**, in which the absorbance peak localized at 652 nm originates from the oxidation of TMB [38]. The possible reaction mechanism involves two cascade steps as investigated elsewhere[40]: First, H_2O_2 was first adsorbed onto the surface of JFSNs and the O–O bond was broken into ˙OH; Second, TMB was oxidized by ·OH to form a blue colored product. Additional control experiments using

TMB in the absence of JFSNs or H_2O_2 show no appearance of the oxidation peak of the TMB, indicating both the components are required for the reaction, as also the case of horseradish peroxidase (HRP). Moreover, it is found that the TMB oxidation catalyzed by the γ-Fe_2O_3/SiO_2 is dependent on the concentration of JFSNs (Figure S2, Supporting Information). Also, TEM imaging illustrated that the morphology and phase of the γ- Fe_2O_3/SiO_2 nanoparticles after the peroxidase reaction remained unchanged (Figure S3, Supporting Information). All these results confirm that the JFSNs exhibits an intrinsic peroxidase-like activity.

To better understand the mechanism of the peroxidase-like catalytic activity of JFSNs, we analyzed the steady-state kinetics for TMB oxidation reaction compared to horseradish peroxidase (HRP) at neutral pH. The kinetic data were obtained by varying one substrate concentration while keeping the other substrate concentration constant. Under the optimum conditions (details seen in Supporting Information), a series of initial reaction rates were calculated and applied to the double reciprocal of the Michaelis–Menten equation[39]. From the Lineweaver–Burk plot, the key enzyme kinetic parameters such as maximum initial velocity (V_{max}) and Michaelis–Menten constant (K_m) were obtained in **Table 1.** It is observed that the oxidation reaction catalyzed by the JFSNs (**Figure 2a, 2b**) and HRP (**Figure 2c, 2d**) follows the typical Michaelis–Menten model towards both substrates, TMB and H_2O_2. So we can use the Michaelis–Menten model to evaluate the catalysis of JFSNs and HRP, The V_{max} value is a direct measure of the enzymatic catalytic activity. K_m is identified as an indicator of enzyme affinity to substrates. A low K_m represents a high affinity and vice versa.[42] The reaction rate obtained from JFSNs had a significantly increase at low TMB concentration (< 0.612 mM) and reach to V_{max} when TMB exceeds 0.612 mM. In contrast, the reaction from HRP is much slower. The V_{max} of JFSNs is nearly twice that of the HRP when TMB was used as substrate. Whereas, the apparent K_m value of JFSNs with substrate H_2O_2 was significantly higher than that for HRP, in agreement with the observation that a higher H_2O_2 concentration was

required to achieve maximal activity for JFSNs. The apparent K_m value of JFSNs with TMB as the substrate was lower than that of HRP, suggesting JFSNs has a higher affinity for TMB. These results indicate that the JFSNs exhibits peroxidase-like activity towards typical peroxidase substrates. This may be due to the existence of more "active sites" on the nanosized surface of the Janus particles compared to HRP, which has only one iron ion at the active center. It is worthwhile to point out that, as JFSNs -based catalysis is a complicated reaction with multiple active sites for TMB oxidation and possible be able to support multiple reactions simultaneously, the classic Michaelis–Menten equation cannot precisely describe this reaction. The other reactions are, nevertheless, at a much smaller level than JFSNs -based catalysis. However, this equation is still employed as it provides a simple measurement for comparison of kinetics.[21]

It is known that the catalytic activity of natural enzyme or nanoenzyme is dependent on pH and temperature.[43] Accordance with the previous reports,[27] HRP was largely inhibited after incubation at either lower or higher pH or higher temperature. As a kind of inorganic nanomaterial, JFSNs is expected to function in wider pH and temperature range than HRP. To prove this, varied reaction conditions with a range values of pH (1 - 12) or temperature (1 - 12) were used to investigate the catalytic activity of JFSNs. For comparison, catalytic activity of natural enzymes HRP was tested under same environments. As shown the pH-dependent response in **Figure 3a**, the catalytic activity of the JFSNs remained stable over a wider pH range (pH 2-5). In contrast, HRP was largely inhibited when pH either lower than 4 or higher than 5. On other hand, as shown in **Figure 3b**, the catalytic activity of JFSNs was less sensitive to temperature as HPR did., The JFSNs preserves a high activity between 30 °C ~ 55°C, while the activity of HRP dramatically decreased when the temperature exceeded 30 °C. These results indicated that the catalytic activity of the JFSNs remained stable over a wide temperature and pH range. Especially, the JFSNs can properly operate at a near neutral pH, which would open various potential applications of this catalytic system in biological systems.

Furthermore, the storage stability, or the ability of enzyme to maintain activity over a long period of time, is an important aspect to be considered in the practical applications. To evaluate the catalytic activity stability of the JFSNs with long-term storage, the same batch of JFSNs nanoparticles was measured with the same analytical operation within 7 days. HRP were chose as the comparison enzyme, which was stored at room temperature when not in use. As shown in **Figure 3c**, the peroxidase-like activity of JFSNs remained intact in 7 days, exhibiting good stability. In contrast, the activity of HRP decreased significantly, which is consistent with that HRP have low stability and is of easy deactivation in surrounding environments. Thus, JFSNs, as HRP mimics, behave insensitively to ambient conditions and keep excellent catalytic activity.

To sum up above, the JFSNs Janus nanopaticles exhibit high and stable peroxidase-like activity over a wide rage of pH and temperature toward H_2O_2. Accordingly, direct quantitative colorimetric detection of H_2O_2 based on such peroxidase-like activity of JFSNs can be acheived. In the presence of TMB (0.625 M TMB) and different concentraton of H_2O_2, the JFSNs could catalyze a color reaction that could be monitoried either by the naked eyes or by the absorbance change at 652 nm. Figure S4a (in Supporting Information) showed absorbance changes in presence of different concentrations of H_2O_2. A color change of the reaction solution could be noticed when H_2O_2 concentration was above 200 μM (Fig S4b, inset) indicating oxidation of TMB. A linear range from 1 ~ 100 μM and limit of detection (LOD) of 10.6 nM can be achieved (LOD = 3σ/k, where σ is the standard deviation of the blank sample and k is the slope of the analytical calibration). To our best knowledge, it is one of the most sensitive one for H_2O_2 sensor, which is 1~100 fold more sensitive than the previous colorimetric detection.[20, 38]

As the SiO_2 on the other side of JFSNs could be used for surface modification and bioconjugation, thus rendering JFSNs a mutifunctional sensing platform. Here we chose to immobilize glucose oxidase (GOx) on the surfae of JFSNs (GOx-JFSNs) for glucose sensing.

To evaluate whether the surface modification would influence the catalytic activity of JFSNs, the peroxidase-like activities of JFSNs before and after modification were measured (i.e., 30 °C, 0.1 M pH 3.7 potassium citrate buffer). As shown in Figure S5 (in Supporting Information), hardly any difference could be observed after the modification steps, indicating the attachment of GOx on JFSNs exert little influence on the catalytic activity. Therefore, it is believed that JFSNs is an ideal multifunctional platform for biosensing applications. It is known that H_2O_2 is the main product of the GOx-catalyzed reaction, therefore, colorimetric quantitative test of glucose can also be realized using JFSNs instead of traditionally used HRP. Because of low stability of GOx in pH 3.7 buffer solution, the glucose detection was performed in two separated steps. GOx first catalyzes oxidation of the glucose to product gluconic acid and H_2O_2 in a pH 7.0 buffer solution. Subsequently, the resulted H_2O_2 is *in situ* reduced by JFSNs in the presence of TMB co-substrate at pH 3.7, generating a detectable signal. The detailed protocols are described in the Experimental sectionhich. **Figure 4** shows a typical glucose-concentration response absorption profile. By varying the glucose concentration, a linear response (0 ~ 20 μM) was observed with a detection limit of 3.2 μM glucose (inset b in Figure 4). Moreover, as the glucose concentration increased above 100μM, the blue color variation can also be observed directly by the naked eye (Fig. 4, inset a). Since the ranges for the serum glucose concentrations in healthy and diabetic individuals are 3~8 mM and 9~40 mM, respectively,[44] our biosensor with LOD of 3.2 μM should be sufficient for detection of glucose in blood normally.

Selectivity is another major concern one in analysis. As shown in **Figure 5**, the reaction is highly specific to glucose. Even the concentrations of control sample (fructose, lactose and maltose) were 5-fold higher concentration (5 mM) than glucose, the absorbance at 652 nm remained as low as the background. In contrast, for glucose, the resulting blue color can be distinguished directly by the naked eye. This indicates the high selectivity of the GOx-attached JFSNs for glucose analysis. To further demonstrate the sensing capability in the real

sample, we tested glucose in serum (fetal bovine serum as serum model), using a standard addition method. As shown in **Figure 6a**, we compared the catalytic response of GOx-attached JFSNs-based sensor to 50 μM glucose in different serum concentration. The results indicate that serum had scarce influence to the catalytic activity of the nanocomposite, suggesting that JFSNs sensing platform may allow for monitoring glucose in complex samples, such as the blood samples of diabetic patients.

Additionally, as its magnetic properties, JFSNs may offer the additional advantages for JFSNs-based biosensor of recylability and resusability. We investigated the possibility of GOx-JFSNs for reapeately used in glucose measurements. After each measurement, the JFSNs nanoparticles were magnetically separated, washed with a buffer solution and then reused for glucose testing. As shown in **Figure 6b**, only a slight decrease in the absorbance at 652nm was observed after five cycles, which might cause seldom loss of the GOx-JFSNs in the magnetic separatation procedsures, or slight degrading the activity during successive experiment. The above results highly suggest the reusability of the GOx-JFSNs.

CONCLUSIONS

In summary, we reported asymmetric hematite-silica nanocomposite of JFSNs as multifunctional peroxidase mimetics and investigated their applications for colorimetric biosensing. The obtained results indicate JFSNs exhibit intrinsic peroxidase-like activity, which is a higher and more stable over a wide range of pH and temperature values compared with natural enzymes HRP. Furthermore, the as-prepared JFSNs offer a multiple functions platform for biosensing, due to their unique asymmetric structure. Thus, we demonstrated that JFSNs can be used as a robust analytical platform for glucose colorimetric biosensing through bioconjugated enzyme GOx, providing sensitive and selective colorimetric assay for glucose. The results suggest the good reproducibility and long-term stability of the GOx-JFSNs, which make it a promising candidate for peroxidase and glucose oxidase mimics for bioassays and

medical diagnostics. Therefore, it is believed that the coupling Janus architecture can pave a way for the fabrication of the multiple functional nanocomposite with higher performance in biosensing, adsorption, catalysis and separation, etc.

ASSOCIATED CONTENT:

Supporting Information Available: Characterization of the as-synthesized JFSNs. The influence of concentrations of JFSNs on the catalytic reaction. TEM image of JFSNs after the catalytic reaction. Absorbance changes in presence of different concentrations of H2O2. Influence of modification of JFSNs on the catalytic activity. This information is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. C. L and X. J. L. contributed equally to this work.

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CAPTIONS

Scheme1. Schematic illustration of the peroxidase-like activity of JFSNs in catalysis of the TMB–H_2O_2 system and using GOx-JFSNs as biosensing platform for colorimetric detection of H_2O_2 and glucose. Pictures present the color change of TMB before and after the catalytic reaction.

Figure 1 (a) low-resolution and (b) high-resolution TEM images of JFSNs. (c) the photograph of JFSNs dispersed in water (left) and their response to the external magnetic field (right). (d) Typical absorption spectra of TMB–H_2O_2 mixed solution in the absence and presence of JFSNs: (I) TMB + JFSNs; (II) TMB + H_2O_2; and (III) TMB + H_2O_2+JFSNs. A photograph of the solutions is shown in the inset. [TMB]: 510 μM; [H_2O_2]: 1.59M; [JFSNs]: 0.06 mg/mL; reaction buffer: potassium citrate buffer (0.1 M, pH 3.7); incubation temperature: 30 °C; reaction time: 10 min.

Figure 2 Steady-state kinetic assays of the JFSNs and HRP. (a, b) The concentration of TMB was fixed (510 μM for JFSNs and 816 μM for HRP) and H_2O_2 concentration was varied. (c, d) The concentration of H_2O_2 was fixed (1.5 M for JFSNs and 8.8 mM for HRP) and TMB concentration was varied. Insets are the Line Weaver–Burk plots of the double reciprocal of the Michaelis–Menten equation

Figure 3. Comparison of the stability of JFSNs and HRP. Peroxidase activities of JFSNs and HRP were measured at (a) pH 1 ~ 12 or (b) 30 °C ~ 60 °C under standard conditions. (c) Long-term stability of JFSNs and HRP stored at room temperature. Peroxidase activities were measured at pH 3.7 and 30 °C under standard conditions. Standard conditions were as follows: experiments were carried out using 0.06 mg/mL JFSNs in 100 μL potassium citrate buffer with 510 μM TMB and 1.59 M H_2O_2 as a substrate; or 1 ng/mL HRP in 100 μL potassium citrate buffer with 816 μM TMB and 8.8 mM H_2O_2 as a substrate. The maximum point in each curve(a-c) was set as 100%.

Figure 4. The dose–response curves for glucose detection using JFSNs as peroxidase mimic Dependence of the absorbance at 652 nm on the concentration of glucose in the range from 0 to 1 mM. Insets: photograph (inset a) shows the production of colored products for different

concentrations of glucose, figure (inset b) shows the corresponding linear calibration plots. The error bars represent the standard deviation of the three measurements.

Figure 5. Determination of the selectivity of glucose detection with 1mM glucose, no saccharide, 5 mM fructose lactose, 5 mM maltose, and 5 mM lactose. Inset: photograph showing colorimetric responses of the system.

Figure 6. (a) The reusability of JFSNs after repeated cycles of glucose sensing using identical reaction conditions. (b) The catalytic activity of 50 μM glucose in buffer and in 10% to 50% serum (fetal bovine serum). The maximum point in each curve was set as 100%. Here, we used relative activity to present the catalytic activity.

Table 1. Comparison of the apparent Michaelis–Menten constant (K_m) and maximum reaction rate (V_{max}).of JFSNs and HRP Table

Scheme 1

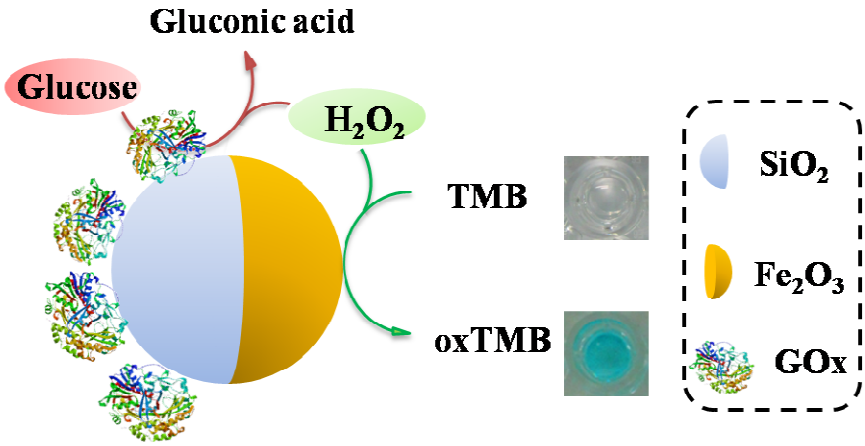

Figure 1

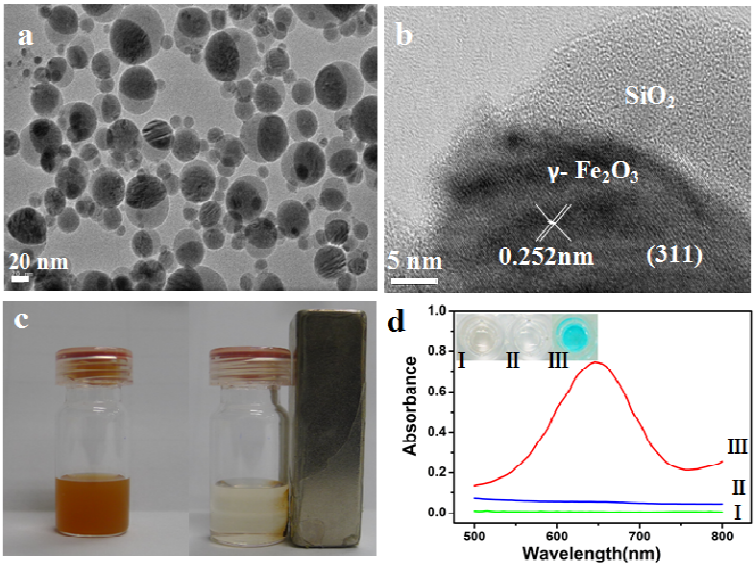
a
20 nm
b
SiO2
γ- Fe2O3
0.252nm
(311)
5 nm
c
d
I
II
III
Absorbance
Wavelength(nm)

Figure 2

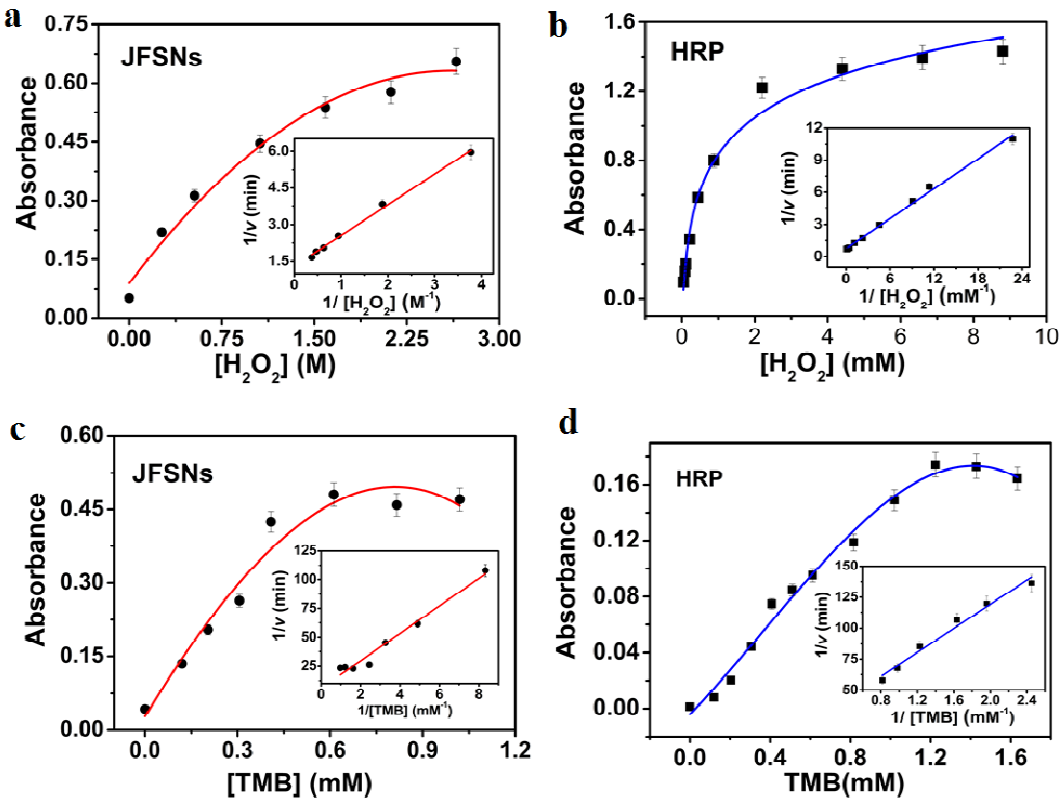
a
JFSNs
Absorbance
[H2O2] (M)
1/v (min)
1/ [H2O2] (M-1)
b
HRP
Absorbance
[H2O2] (mM)
1/v (min)
1/ [H2O2] (mM-1)
c
JFSNs
Absorbance
[TMB] (mM)
1/v (min)
1/[TMB] (mM-1)
d
HRP
Absorbance
TMB(mM)
1/v (min)
1/ [TMB] (mM-1)

Figure 3

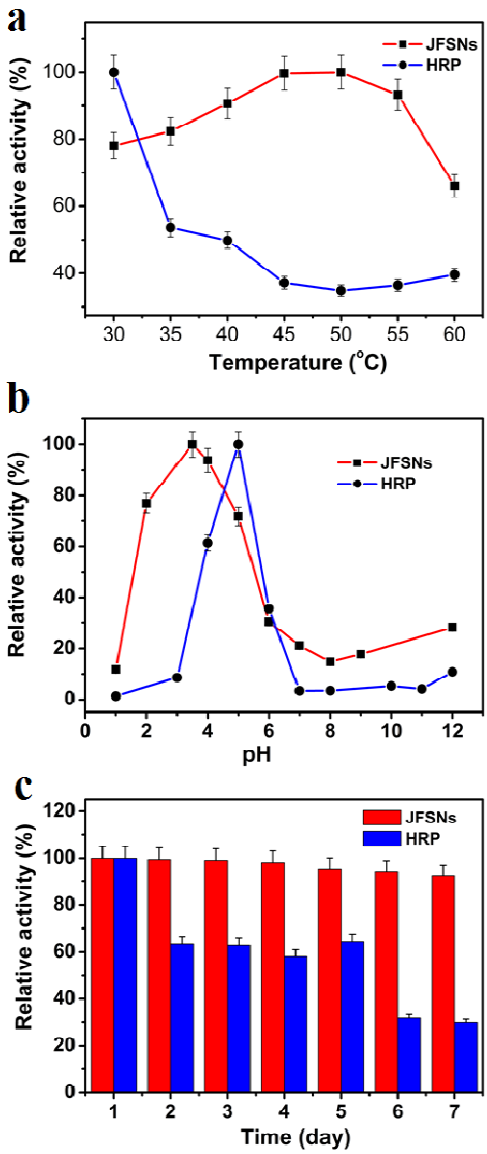
a
JFSNs
HRP
Relative activity (%)
100
80
60
40
30
35
40
45
50
55
60
Temperature (°C)
b
JFSNs
HRP
Relative activity (%)
100
80
60
40
20
0
0
2
4
6
8
10
12
pH
c
JFSNs
HRP
Relative activity (%)
120
100
80
60
40
20
0
1
2
3
4
5
6
7
Time (day)

Figure 4

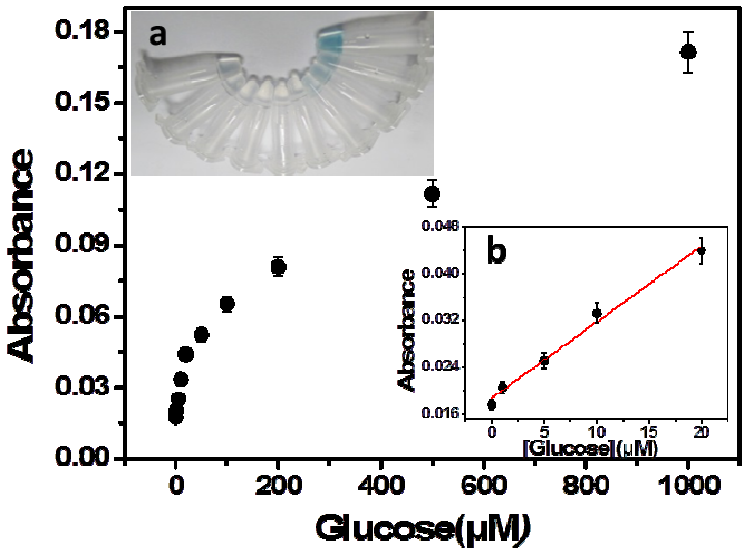
a
b
Absorbance
Glucose(μM)
0.00
0.03
0.06
0.09
0.12
0.15
0.18
0
200
400
600
800
1000
Absorbance
[Glucose](μM)
0.016
0.024
0.032
0.040
0.048
0
5
10
15
20

Figure 5

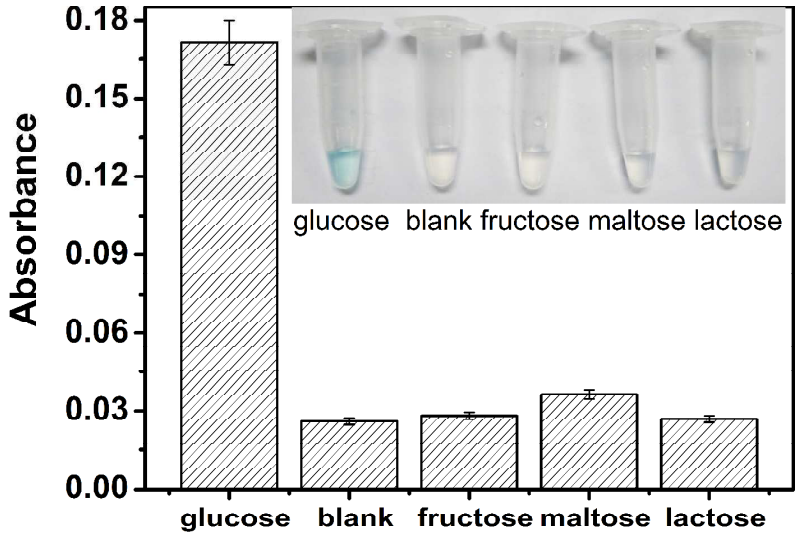
Absorbance
0.18
0.15
0.12
0.09
0.06
0.03
0.00
glucose blank fructose maltose lactose
glucose
blank
fructose
maltose
lactose

Figure 6.

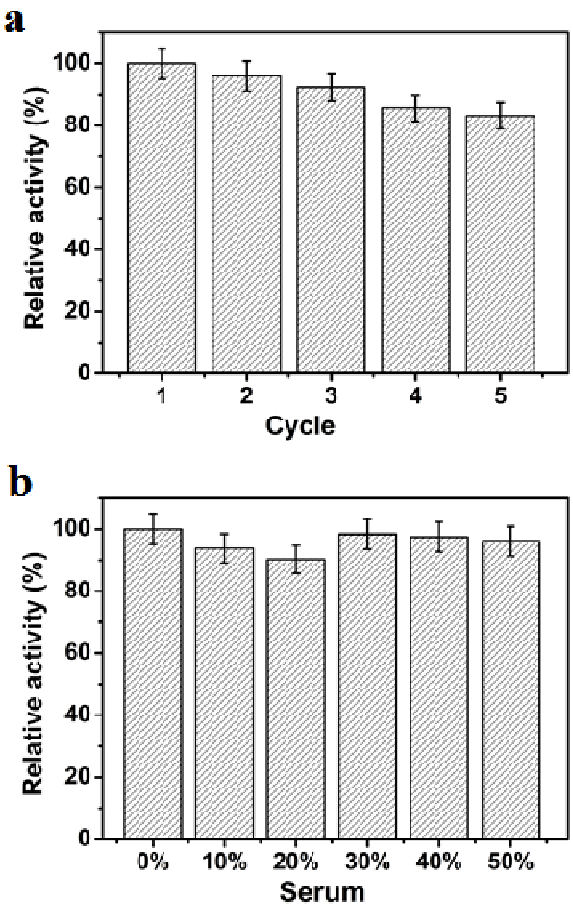
a
Relative activity (%)
0
20
40
60
80
100
1
2
3
4
5
Cycle
b
Relative activity (%)
0
20
40
60
80
100
0%
10%
20%
30%
40%
50%
Serum

Table 1.

Substrate	Catalyst	K_m (mM)	V_{max} (mM min^{-1})
TMB	JFSNs	3.05	0.25
TMB	HRP	5.90	0.11
H_2O_2	JFSNs	965.98	769.65
H_2O_2	HRP	0.63	1.35

Illustration for Table of Contents: