Enhanced Mimetic Enzyme Activity of Phosphorylated Porphyrin Nanocomposite Induced by Localized Surface Plasmon Resonance for Colorimetric Assay

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Plasmon-enhanced light harvesting has been of great interest to enhance the catalytic efficiency of some composites or hybrids. The enhanced peroxidase-like activity of phosphorylated iron(III) porphyrin (TPPFe(III))-based nanocomposite, induced by localized surface plasmon resonance for a colorimetric assay, was developed in this study. Firstly, a phosphate group modification strategy was adopted to synthesize water-soluble iron(III) porphyrin materials. Then, the as-synthesized TPPFe(III) was covalently attached to core-shell gold nanorods (GNRs), GNR@Au2S/AuAgS, to form TPPFe(III)-GNR@Au2S/AuAgS nanocomposite, which shows greatly enhanced peroxidase-like activity compared to TPPFe(III). A mechanism for the enhanced peroxidase-like activity of TPPFe(III)-GNR@Au2S/AuAgS was proposed, which results from a synergic effect of hot electrons excited by localized surface plasmon resonance and photogenerated electrons of the TPPFe(III), verified by experiments. Furthermore, a fast colorimetric assay for the detection of H2O2 and glucose was established based on the unique property of TPPFe(III)-GNR@Au2S/AuAgS. This colorimetric assay was applied to determine practical human serum samples; satisfactory results demonstrate this method has high accuracy. The present study would not only provide some insights into the mechanism of plasmon-activated enzyme-like reactions, but also offer new strategies for improving the catalytic activity of a mimetic enzyme.

Keywords Porphyrin, core-shell gold nanorods, hot electron, enzyme mimics, colorimetric assay

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Introduction

Natural enzymes can catalyze biological reactions with high specificity and high activity.1 However, the catalytic activity of the enzyme is usually affected by the ambient conditions, especially the high cost in preparing and purifying the enzyme, which greatly limits their practical applications.2 As an exciting branch of biomimetic chemistry, artificial enzyme mimics inspired by natural enzymes are clearly good alternatives.3–4 Developing artificial enzymes has received great interest recently in pharmaceutical processes, biosensors, and food industry due to the low cost of their preparation and high stability to environmental conditions.5–8

Numerous peroxidase enzymes, such as horseradish peroxidase (HRP) and some mimetic enzymes, are composed of ferriclorophyrin units.9 Porphyrins and their derivatives are functional molecules with large conjugated electron molecular structures that give them excellent performance in many biological systems.10,11 The porphyrin derivatives-based catalysis and biomimetic catalysis, especially those involved in catalytic redox reactions have always been the research focus.12 For example, porphyrins as light harvesting photosensitizers were used to enhance the catalytic ability of a variety of nanoscaled materials, such as Fe3O4,13 CuO,14 Co3O4,15 V2O5,16 gold nanoparticles (AuNPs),17 ceria nanoparticles,18 graphene oxide19 and carbon nanotubes.20 In contrast, there have been a few studies on the use of nanomaterials to enhance the catalytic activity of porphyrins.21 Generally, due to the lack of some specific structures or functional groups inherent to natural enzyme on the surface of the nanomaterial-based mimetic enzyme, the catalytic ability of a mimetic enzyme is less than that of a natural enzyme. Therefore, it is a challenge to develop nano-enzymes that are comparable to the catalytic activity of natural enzymes. Researchers have been working on the preparation of mimetic enzymes with high catalytic efficiency comparable to natural enzymes.22,23 Nanostructures with surface plasmon properties open up new opportunities for plasmon photocatalytic technology to develop mimetic enzyme nanoparticles with stable and high catalytic activity.24 Localized surface plasmon resonance (LSPR) is the result of interactions between a localized plasmon and incident light on the surface of nanoparticles, collecting and utilizing abundant low-energy photons to generate high-energy hot electrons,25,26 which have been used in a variety of applications, including sensors,27,28 nanomedicine,29 therapeutic diagnostics30 and plasmonic-enhanced chemical transformations,31 and solar cells.32 Previous studies have revealed that efficient surface plasmon excitation and decay induced by hot carriers generation might enhance the catalytic performance.33 Plasmon-enhanced light harvesting to strengthen the catalytic efficiency of some composites or hybrids inspired us to focus on...
this unique LSPR effect on enhancing the catalytic activity of phosphorylated iron(III) porphyrin (TPPFe(III)). In this study, a phosphate group modification strategy was firstly adopted to meet the solubility requirements of iron(III) porphyrin materials. The as-synthesized TPPFe(III) was covalently attached to core-shell gold nanorods (GNRs), GNR@Au2S/AuAgS, to form TPPFe(III)-GNR@Au2S/AuAgS nanocomposite. It was found that the catalytic activity of the TPPFe(III)-GNR@Au2S/AuAgS composite can be greatly enhanced compared to TPPFe(III) due to a synergistic effect of hot electrons excited by LSPR and photogenerated electrons of the TPPFe(III), Thereby, a hot-electrons-induced catalytic enhancement mechanism is proposed. A colorimetric assay for the detection of H2O2 and glucose was developed based on the peroxidase-like activity of TPPFe(III)-GNR@Au2S/AuAgS. This colorimetric assay was further applied to determine practical human serum samples; the satisfactory results demonstrate that this method is of high accuracy.

**Experimental**

**Reagents and chemicals**

Glutathione (GSH), hydrogen peroxide (H2O2), 3,3,5,5-tetramethylbenzidine (TMB), anhydrous ethanol, bovine serum albumin (BSA), chloroauric acid (HAuCl4·3H2O), chitosan, glucose, glucose oxidase, ethylcarbodiimide hydrochloride (EDC), albumin (BSA), tetramethylbenzidine (TMB), anhydrous ethanol, bovine serum albumin (BSA), and mercaptopyrrolic acid were purchased from Aladdin Industrial Corporation (Shanghai, China). Other chemicals were of analytical reagent grade and received from the commercial source without treatment.

**Preparation of GNRs and core-shell nanorods GNR@Au2S/AuAgS**

The preparation of GNRs was carried out according to previous methods. A seed solution was firstly prepared by the reaction of HAuCl4 and NaBH4: 100 L of ice-cold 0.01 M NAuCl4 and NaBH4 with various concentrations were added to 1.5 mL of 0.012 M HAuCl4 containing 0.1 M CTAB solution to form seed solution displaying a brownish-yellow color. The resulting seed solution was kept at room temperature for at least 2 h prior to preparing the GNRs. To grow gold nanorods, 1.0 mL of 0.01 M and AgNO3 1.5 mL of 0.02 M HAuCl4 were added to 30 mL of 0.1 M CTAB with continuous stirring, and then 0.8 mL of 0.08 M ascorbic acid was added to this mixture solution. At last, 70 L of the seed solution was added to the growth solution, stirred for 1 min, and then allowed to stand for at least 2 h.

Core-shell nanorods GNRs were synthesized by previous protocol. Briefly, a Na2S2O3 solution was added to a GNRs suspension, prepared as above, to a final concentration of 15 mM. The mixed solution was reacted at room temperature for about 20 min to produce core-shell nanorods, GNR@Au2S/AuAgS, separated by centrifugation. Finally, the resultant core-shell GNRs were stored by re-dispersing them in water.

**Characterization of TPPFe(III) nanocomposite**

The attachment of TPPFe(III) with GNR@Au2S/AuAgS was achieved as follows: 1 mL of a 0.1 M GSH solution was added into 5 mL of the GNR@Au2S/AuAgS suspension for at least 18 h under room temperature. Functionalized GNR@Au2S/AuAgS were then collected by centrifugation at 4000 after 2 h.

**Results and Discussion**

**Synthesis of phosphorylated iron(III) porphyrin TPPFe(III)**

A phosphate group modification strategy was firstly adopted to meet the solubility requirements of TPPFe(III) materials.

**Enhance catalytic activity by light illumination**

A 300-W xenon lamp (Solar-350, Beijing NBET Technology Co., Ltd., Beijing, China) was employed to act as the light source, which was equipped with a glass filter to filter out light below 400 nm. The light intensity incident on the sample solution was at ~0.1 W/cm², measured by an FZ-A optical Radiometer (Photoelectric Instrument Factory of Beijing Norman University, Beijing, China). The test samples in centrifuge tubes or micro-wells would be illuminated by a xenon lamp.

A colorimetric analysis for H2O2 was performed as follows. Firstly, 100 μL of TPPFe(III)-GNR@Au2S/AuAgS, 100 μL of 5 mM TMB, and 100 μL of H2O2 with various concentrations were added to 700 μL of 0.1 M NaAc buffer (pH 6.0). Secondly, the solution was incubated at room temperature for 15 min and the solution was used further in absorption experiments.

**Steady-state kinetic assays of TPPFe(III)-GNR@Au2S/AuAgS**

Steady-state kinetic assays were carried out in a reaction system using TPPFe(III)-GNR@Au2S/AuAgS in the reaction buffer (0.01 M NaAc buffer) in the presence of H2O2 at room temperature. A kinetic analysis of these TPPFe(III)-GNR@Au2S/AuAgS irradiated by Xenon lamp and in ambient light in the room with H2O2 as the substrate was performed. Catalytic parameters were obtained by Michaelis-Menten equation: v = Vmax × [S]/Km + [S], where Vmax is the maximal reaction velocity, V represents the initial velocity, [S] is the concentration of the substrate, and Km corresponds to the Michaelis constant. The apparent kinetic parameters were determined by fitting the absorbance data according to the Michaelis-Menten equation.
The materials modified by phosphate groups not only have perfect solubility, but also had good acid-base adaptability required in the current investigation. The synthetic procedures are outlined in Scheme 1. The free-base porphyrin containing four meso-bromophenyl groups was obtained according to Adler-Longo condensation methods.37 A modification of the C-P bond to bromophenyl components was based on the classic Sonogashira coupling condition.38 Then, an insertion of iron(III) ion into soluble porphyrin above was performed to give target material I after the hydrolysis of aryl phosphate ester.39 The synthesized phosphorylated iron(III) porphyrin (TPPFe(III)), characterized by ESI MS and UV-Vis, is presented in Fig. 1.

Characterization of TPPFe(III)-GNR@Au₅S/AuAg₅ nanocomposite

GNRs and GNR@Au₅S/AuAg₅ core-shell nanostructures were prepared according to previous reports.34–36,40 The as-synthesized GNRs displayed transverse band at 521 nm and the longitudinal LSPR peak at a longer wavelength of 776 nm. Core-shell nanorods were produced by the reaction of Na₂S₂O₃ and GNRs in an aqueous solution, along with a significant red-shift of transverse and longitudinal peaks, resulting the formation of core-shell nanostructure, GNR@Au₅S/AuAg₅. Particularly, the high refractive index medium of Au₅S/AuAg₅ may facilitate a large degree red shift of a longitudinal peak up to one hundred nanometers, controlled by the thickness of the shell, as shown in Fig. 2a. The size and morphology of the GNRs and the corresponding GNR@Au₅S/AuAg₅ were characterized by transmission electron microscopy (TEM), respectively. Figure 2b presents a typical TEM image of the as-prepared GNRs with an average length of 39 nm and a width of 10 nm. It can be seen from Fig. 2c that two different phases exist in the core-shell nanostructure, indicating the formation of GNR@Au₅S/AuAg₅.

To obtain a nanocomposite consisting of TPPFe(III) and GNR@Au₅S/AuAg₅, the TPPFe(III) would be covalent to GNR@Au₅S/AuAg₅ by phosphamide to form a TPPFe(III)-GNR@Au₅S/AuAg₅ nanocomposite.41 Firstly, the functionalization of GNR@Au₅S/AuAg₅ was implemented by making use of the affinity reaction between thiol compounds and Au₅S/AuAg₅. The Au₅S/AuAg₅-coated nanorods can react easily with –SH group of the GSH to form a self-assembled membrane (SAM) on them to attach recognition agents. In this study, SAM of GSH was formed to covalently link biomolecules via –PO(OH)₂ terminus groups and –NH₂. TPPFe(III) and GNR@Au₅S/AuAg₅ have distinct absorption spectra, which may provide a possibility to monitor whether the TPPFe(III) covalently attach to GNR@Au₅S/AuAg₅ or not. The characteristic absorption peak at 416 nm, ascribed to TPPFe(III), was measured from the produced TPPFe(III)-GNR@Au₅S/AuAg₅ after the reaction of TPPFe(III) and GNR@Au₅S/AuAg₅, as shown in Fig. 2a, indicating the successfully covalent attachment of TPPFe(III) on GNR@Au₅S/AuAg₅ activated by N-hydroxysuccinimide (NHS) and ethylcarbodiimide hydrochloride (EDC). Figure 2d displays a high-resolution TEM image of GNR@Au₅S/AuAg₅.

Scheme 1 Schematic presentation of the synthesis of TPPFe(III).

Peroxidase-like activity of TPPFe(III)-GNR@Au₅S/AuAg₅ nanocomposite

To investigate the peroxidase-like activity of the TPPFe(III)-GNR@Au₅S/AuAg₅ nanocomposite, the catalytic oxidation of the peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H₂O₂ was examined, along with the occurrence of blue color, as displayed in Fig. 4a, in which there is a maximum absorbance at 652 nm (Fig. 4b) due to the generation of oxidized TMB (Ox-TMB) transformed from TMB.42 The TPPFe(III)-GNR@Au₅S/AuAg₅ nanocomposite can rapidly catalyze the oxidation of TMB in the presence of H₂O₂, implying that the TPPFe(III)-GNR@Au₅S/AuAg₅ exhibits intrinsic
peroxidase-like activity. As a control, the mixture of GNR@Au2S/AuAgS and TPPFe(III) as well as TPPFe(III) at the same concentration were also evaluated, respectively. Similarly, they also displayed a light-blue color compared to the TPPFe(III)-GNR@Au2S/AuAgS composite, suggesting that the catalytic activity of TPPFe(III)-GNR@Au2S/AuAgS composite is much stronger than that of TPPFe(III) alone, or the mixture of TPPFe(III) and GNR@Au2S/AuAgS. Meanwhile, additional control experiments using TMB and H2O2 in the absence of the TPPFe(III)-GNR@Au2S/AuAgS composite or TPPFe(III) show no obvious color change, suggesting that the mimetic enzyme (TPPFe(III) or TPPFe(III)-GNR@Au2S/AuAgS) is necessary for the color reaction. The peroxidase-like activities of mimetic enzymes toward the oxidation of TMB by H2O2 were investigated while varying the pH from 1 to 14. The catalytic relative activity of the above mimetic enzymes is dependent on the pH, and the effects of pH on the catalytic activity of mimetic enzymes toward TMB oxidation are shown in Fig. 4c. At the optimal pH value (pH 6), a comparison of the absorption spectra among the three different systems depicted in Fig. 4d illustrates that the catalytic activity of TPPFe(III)-GNR@Au2S/AuAgS is larger than 13-times that of TPPFe(III), and the mixture of TPPFe(III) and GNR@Au2S/AuAgS is higher 7-times than TPPFe(III), revealing that the TPPFe(III)-GNR@Au2S/AuAgS exhibits enhanced peroxidase-like catalytic activity under identical conditions.

Enhance catalytic activity by LSPR

Experiments were conducted to investigate the catalytic activity of TPPFe(III)-GNR@Au2S/AuAgS by utilizing powerful light. A xenon lamp including a wide range of wavelengths within Vis-NIR light was used as the excitation light source to provide possible light coupling to the LSPR of TPPFe(III)-GNR@Au2S/AuAgS. Two identical solutions containing TPPFe(III)-GNR@Au2S/AuAgS, H2O2, and TMB were irradiated by a xenon lamp and ambient light, respectively, as shown in Fig. 5a. A blue-color was quickly observed when the tested solution was irradiated by the xenon lamp within 5 min; at the same time, only a light blue-color appeared in another solution illuminated with the ambient light. This implies that an enhanced enzyme-like catalytic performance of TPPFe(III)-GNR@Au2S/AuAgS was a result of light
stimulation. Two additional control experiments were introduced to explore the possible reasons for this promoted catalytic performance as follows: (1) TPPFe(III)-GNR@Au2S/AuAgS and H2O2 + TMB and (2) H2O2 + TMB. In the system (1) in which the xenon lamp was turned on and off, no distinguishable color change was observed, and there was almost no significant difference in the absorption spectrum when the xenon lamp was turned on and off, indicating that the light irradiation had no effect on the system. For system (2) (H2O2 + TMB), the light irradiation had no significant effect on the reaction, which means that H2O2 has a very weak oxidizing ability for TMB in the absence of TPPFe(III)-GNR@Au2S/AuAgS catalyst.
These results demonstrate that the enhancement of the catalytic performance does come from the peroxidase-like property of TPPFe(III)-GNR@Au2S/AuAgS.

The kinetics of a peroxidase-like reaction catalyzed by TPPFe(III)-GNR@Au2S/AuAgS was investigated by monitoring the changes of absorbance at different reaction times at room temperature (Fig. 5b). The absorbance of Ox-TMB that arises from the reaction of H2O2 and TMB catalyzed by TPPFe(III)-GNR@Au2S/AuAgS increases much faster with the xenon lamp than in the ambient light. The illumination-enhanced catalytic performance of TPPFe(III)-GNR@Au2S/AuAgS is more readily understood by using the absorption intensity as a function of the reaction time (Fig. 4c). At a fixed TMB concentration (1.8 mM), the enzyme-like reaction kinetics might be further monitored by varying the H2O2 concentration (from 1 – 60 mM). The typical enzyme reaction Michaelis–Menten curves (Fig. 5d) were obtained, and its reaction rate increased linearly at low substrate concentrations. The apparent Michaelis–Menten constant ($K_m$) and maximum reaction rate ($V_m$) can be obtained from the Lineweaver–Burk double-reciprocal plot of the Michaelis–Menten equation.43 The results listed in Fig. 5e clearly show that a much faster catalytic rate and lower $K_m$ value are achieved with the xenon lamp as compared to the ambient light. A lower $K_m$ value indicates higher affinity of the enzyme for the substrate. Thus, illumination increases the enzyme-like activity of TPPFe(III)-GNR@Au2S/AuAgS.

Mechanism of catalysis

The peroxidase-like activity of TPPFe(III)-GNR@Au2S/AuAgS composite depends on the ability to generate ·OH radical in the system of TPPFe(III)-GNR@Au2S/AuAgS and H2O2. To test this hypothesis, fluorescent probe terephthalic acid was used to evaluate the -OH radical, in which terephthalic acid could easily react with the -OH radical to produce highly fluorescent 2-hydroxyterephthalic acid. The fluorescence spectra with an excitation wavelength of 315 nm were collected. Figure 6a presents the fluorescence spectra of the 2-hydroxyterephthalic acid produced; the fluorescence intensity is proportional to the amount of ·OH radicals generated. This confirms that OH· was produced in the presence of H2O2 and TPPFe(III)-GNR@Au2S/AuAgS composite.

An investigation on the reaction rate and light intensity was performed; H2O2 and TMB in the presence of TPPFe(III)-GNR@Au2S/AuAgS and TPPFe(III) were carried out by irradiation of xenon lamp, ambient light in room and in dark, respectively, as shown in Fig. 6b. A blue color was quickly observed when the solution was irradiated by a xenon lamp; at the same time only a light blue color appeared in the ambient light, though almost no color change was found in the third solution in dark. This implies that the enzyme-like catalytic performance of TPPFe(III)-GNR@Au2S/AuAgS was affected by light stimulation. As a comparison, TPPFe(III) was conducted in a similar experiment at the same concentration. It is also observed that the catalytic activity of the TPPFe(III) is enhanced after irradiated by a xenon lamp, but is much lower than TPPFe(III)-GNR@Au2S/AuAgS. This phenomenon is a result of photogenerated electrons improving the catalytic activity by light-harvesting TPPFe(III). However, more conjugated electrons are generated from TPPFe(III)-GNR@Au2S/AuAgS compared to TPPFe(III); thus, it can be reasonably explained that the TPPFe(III)-GNR@Au2S/AuAgS has a more enhanced catalytic activity than TPPFe(III) molecules.

Based on the above results and discussion, we propose a possible mechanism on the enhanced catalytic activity of TPPFe(III)-GNR@Au2S/AuAgS, which results from a synergic effect of hot electrons excited by LSPR and a photochemically generated electron of the TPPFe(III), as shown in Fig. 6c. It is well known that noble-metal nanoparticles generate a large amount of hot carriers when excited by LSPR.44 Under light illumination, hot electrons would be generated due to the LSPR
of TPPFe(III) and GNR@Au2S/AuAgS, the TPPFe(III) only peroxidase-like property, the TMB can be transformed to Ox-TMB. That different catalytic activities can be reasonably explained by covalently anchored on the GNR@Au2S/AuAgS. Meanwhile, TPPFe(III) can absorb the light to photochemically generate electrons. In the nanostructure of TPPFe(III)-GNR@Au2S/AuAgS, hot electrons and holes would be generated upon LSPR excitation. Due to energy matching, the hot electrons are first transferred to TPPFe(III), then together with photogenerated electrons of TPPFe(III) inject into the molecular orbitals of H2O2, which activate the adsorbed H2O2 molecule into a transition state to produce ·OH radical. The produced ·OH radical is a very reactive species would fast oxidize TMB to form Ox-TMB.

TPPFe(III)-GNR@Au2S/AuAgS, TPPFe(III), mixture of TPPFe(III) and GNR@Au2S/AuAgS shown in Fig. 4a exhibits that different catalytic activities can be reasonably explained by the above reaction mechanism. Because TPPFe(III) exhibits a peroxidase-like property, the TMB can be transformed to Ox-TMB in the presence of TPPFe(III) and H2O2, displaying blue color. As for TPPFe(III)-GNR@Au2S/AuAgS, the TPPFe(III) is covalently anchored on the GNR@Au2S/AuAgS, the hot electrons generated through LSPR would be efficiently transferred to TPPFe(III) to form conjugated electrons, which make the TPPFe(III) exhibit enhanced catalytic activity to facilitate the H2O2 molecule to form ·OH radical. In the mixture of TPPFe(III) and GNR@Au2S/AuAgS, the TPPFe(III) only simply mixed with GNR@Au2S/AuAgS. Some of the TPPFe(III) may beadsorbed on the surface of GNR@Au2S/AuAgS, enabling the hot electrons to inject to TPPFe(III); however, the rest of TPPFe(III) molecules cannot efficiently accept the hot electrons. Therefore, the catalytic activity of a mixture of TPPFe(III) and GNR@Au2S/AuAgS is between TPPFe(III) and TPPFe(III)-GNR@Au2S/AuAgS.

Based on the proposed mechanism, the addition of an electron donor reagent can lead to an efficient separation of hot electrons and holes. These holes can be scavenged by additional electron donors, and the effective scavenging of holes may reduce the probability of a recombination of hot electrons and holes, which facilitate the rapid transformation of H2O2 into ·OH radical, resulting in greatly increase of reaction rate. Here, ethanol acts as the electron donor reagent to investigate the effect of electron donor on the catalytic activity of peroxidase-like TPPFe(III)-GNR@Au2S/AuAgS. Because of the TMB solution using ethanol as a solvent, another water-soluble reagent, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), served as a substrate in place of TMB to display color. A comparison of the absorbance with and without ethanol upon continuous LSPR excitation, shown in Fig. 6d, elucidates the ethanol favors to increase the ABTS absorption intensity upon LSPR excitation. This figure also reveals that in both cases the absorption intensities increase with the light irradiation time, but the increasing magnitude of the reaction system with ethanol is obviously larger than the system without ethanol.

To verify the effect of LSPR on the catalytic activity of TPPFe(III)-GNR@Au2S/AuAgS, a typical reaction irradiated by monochromatic light to test a peroxidase-like property was carried out. Figure 7a shows the absorption spectrum of TPPFe(III)-GNR@Au2S/AuAgS, including 416 nm attributed to the characteristic peak of TPPFe(III), 573 and 916 nm ascribed to transverse and longitudinal peaks of GNR@Au2S/AuAgS, which might be selected to probe the light excitation. In the study, monochromatic light was provided by a fluorescence spectrometer, which may offer exited light in the range of 200 to 750 nm. Experiments were carried out by selecting the two absorption peak wavelengths of the absorption spectrum, 416, 573 nm, and another wavelength without significant absorption as a reference wavelength. The tested solution sample was inserted into the cuvette holder, and monochromatic light was offered by a fluorescence spectrometer, where the monochromatic light passed through a solution containing TMB, H2O2 and TPPFe(III)-GNR@Au2S/AuAgS. The appearance of a blue band irradiated by 416 and 573 nm (Fig. 7b) further confirmed two facts: firstly, the TPPFe(III) is a light-harvesting photosensitizer to form photoinduced electrons, which improves the catalytic activity; secondly, plasmon-enhanced light harvesting can strengthen the catalytic efficiency of TPPFe(III)-GNR@Au2S/AuAgS. Meanwhile, a negligible color variation upon irradiated by 719 nm elucidates the light of other wavelength hardly affect the catalytic performance. The results validate the proposed mechanism that results from a synergic effect of hot electrons excited by LSPR and photoinduced electrons of the TPPFe(III). In fact, a xenon lamp offering wide range of light within visible to NIR light may simultaneously excite transverse and longitudinal plasmonic peaks to generate more hot electrons and holes; thus, the catalytic activity was significantly enhanced compared to TPPFe(III). On the other hand, the color intensity was gradually deepened along with longer illumination (Fig. 7c). This interesting finding indicates that the catalytic enhancement effect is triggered by LSPR excitation, relying on the incident light. These results would seem to demonstrate that the LSPR plays a critic role in the enhanced catalytic activity of TPPFe(III) owing to a more efficient production of hot carriers with a stronger light intensity.

Fig. 7 (a) Absorption spectrum of TPPFe(III)-GNR@Au2S/AuAgS, (b) photographs of TPPFe(III)-GNR@Au2S/AuAgS, H2O2 and TMB irradiated by different wavelengths of light (i: λ = 416, ii: λ = 573 and iii: λ = 719 nm), (c) photographs of TPPFe(III)-GNR@Au2S/AuAgS, H2O2 and TMB irradiated by monochromatic light (λ = 573) at different times (i = 20, ii = 30, iii = 40, iv = 50 min).
Development of colorimetric assay for detection of glucose

A colorimetric assay for the detection of H\textsubscript{2}O\textsubscript{2} was developed based on the peroxidase-like activity of TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS. In principle, the color change results from the produced ox-TMB correlated with TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS, H\textsubscript{2}O\textsubscript{2} and TMB, in which the color change may depend on the amount of H\textsubscript{2}O\textsubscript{2} under the optimal reaction conditions. To make full use of H\textsubscript{2}O\textsubscript{2}-based reactions and the color property of Ox-TMB as readout, a colorimetric assay for H\textsubscript{2}O\textsubscript{2} is fabricated. The insert picture in Fig. 8a displays a color tonality of Ox-TMB along with an increasing concentration of H\textsubscript{2}O\textsubscript{2}, the absorption intensity of Ox-TMB at 652 nm is proportional to the H\textsubscript{2}O\textsubscript{2} concentration, and the linear relationship between the absorbance and H\textsubscript{2}O\textsubscript{2} concentration is plotted in Fig. 8a. The color-variation-accompanying Ox-TMB offers a direct principle to determine the H\textsubscript{2}O\textsubscript{2} level. It is easy to extend the principle to the detection of other chemicals that can directly or indirectly change the level of H\textsubscript{2}O\textsubscript{2}. Since H\textsubscript{2}O\textsubscript{2} is one of the products of the glucose oxidase (GOx)-catalyzed reaction, this colorimetric method combined with GOx can be adopted to detect glucose, as shown in Fig. 8b. Thereby, a colorimetric determination of glucose can be realized using the TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS scheme shown in Fig. 8c. The linear relationship between the absorbance and glucose concentration (Fig. 8b) and the limit of detection (LOD, S/N = 3) was calculated to be 0.26 M, which manifests a quantitative analysis of the concentration of glucose is feasibility. This colorimetric assay was further applied to determine practical human serum samples. A 100-μL aliquot of a serum sample is added to the TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS and TMB in the presence of GOx. By using this method, the glucose concentration is determined from serum samples of five healthy volunteers; the results illustrated in Fig. 8d are satisfactory compared with the values obtained from a local hospital. Importantly, the technique is largely free from any complicated matrix effect caused by the preparation of human serum samples. In addition, this method induced by LSPR has a faster detection speed and higher sensitivity than the conventional method without LSPR, as summarized in Table S1 in Supporting Information.

Conclusions

In conclusion, we developed an enhanced peroxidase-like activity of TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS nanocomposite induced by LSPR for a colorimetric assay. A phosphate group modification strategy was adopted to synthesize water-soluble TPPFe(III). The as-synthesized TPPFe(III) was covalently anchored to core-shell gold nanorods to form a TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS composite, which exhibits significantly enhanced peroxidase-like activity compared to TPPFe(III). Moreover, the catalytic performance of TPPFe(III) was covalently anchored to core-shell gold nanorods to form a TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS composite, which exhibits significantly enhanced peroxidase-like activity compared to TPPFe(III). The proposed mechanism was also further validated by experiments, and the efficiency of the hot-electron injection can be further increased by scavenging the generated hot holes using an electron donor, like ethanol. The present study would not only provide some insights into the mechanism of plasmon-activated enzyme-like reactions, but also offer new strategies for improving the catalytic activity of enzyme mimics. Furthermore, a fast colorimetric assay for the detection of H\textsubscript{2}O\textsubscript{2} and glucose was established based on the unique property of TPPFe(III)-GNR@Au\textsubscript{2}S/AuAgS. This colorimetric assay was applied to determine practical human serum samples, and the satisfactory results demonstrate this method is of high accuracy.
References