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Visible-Light-Induced Effects of Au Nanoparticle on Laccase Catalytic Activity

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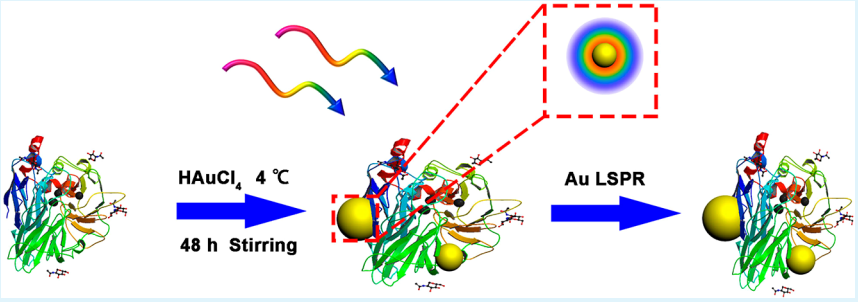
Supporting Information



ABSTRACT: A deep understanding of the interaction between the nanoparticle and enzyme is important for biocatalyst design. Here, we report the *in situ* synthesis of laccase−Au NP (laccase−Au) hybrids and its catalytic activity modulation by visible light. In the present hybrid system, the activity of laccase was significantly improved (increased by 91.2% vs free laccase) by Au NPs. With a short time visible light illumination (λ > 420 nm, within 3 min), the activity of laccase−Au hybrids decreased by 8.1% (vs laccase−Au hybrid without light), which can be restored to its initial one when the illumination is removed. However, after a long time illumination (λ > 420 nm, over 10 min), the catalytic activity of laccase−Au hybrids consecutively decreases and is not reversible even after removing the illumination. Our experiments also suggested that the local surface plasma resonance effect of Au NPs causes the structure change of laccase and local high temperature near the Au NPs. Those changes eventually affect the transportation of electrons in laccase, which further results in the declined activity of laccase.

KEYWORDS: *Au nanoparticle, laccase, hybrid catalyst, visible-light illumination, catalytic activity*

1. INTRODUCTION

Combining nanoparticle with enzyme has opened a new avenue in enzyme engineering and permitted the construction of enzyme−nanomaterial complexes with improved activities and stabilities.[1−5] Laccase, as multicopper oxidases, contains four copper ions that distribute into three sites (T1, T2, and T3 sites, defined according to spectroscopic properties),[6,7] in which electrons from the reducing substrate are extracted by the mononuclear T1 site and then are transferred to the trinuclear T2/T3 center (where molecular oxygen is reduced).[8] Up to now, it is a relative clear enzymatic reaction system and also has been paid much attention for enhancing the activity and stability. Some inorganic nanostructures (Au NPs, silver NPs, graphene oxide, graphene, Cu salts, etc.) have been tried to combine with laccase to construct the enzyme based hybrid catalyst.[9−13] Kahawong et al. demonstrated that enzymatic reaction of horseradish peroxidase (HRP) inserted into semiconducting iron-doped titanate (FT) layers can be controlled by ultraviolet (UV) light irradiation.[14] In addition, Au nanoparticles (Au NPs) have good biocompatibility, easy surface functionalization, and surface-plasmon-derived optical properties, which make Au NPs a promising candidate for biocatalysis application.[15−19] Although there are some qualitative and quantitative studies on gold-based biocatalysts,[20,21] very little is known about the exact subsequent impact of Au NPs on enzymes, especially in the condition of illumination which is important for biocatalyst design and also cannot be neglected in a further practical application. In this field, there is an urgent need for the deep understanding of the interaction between the Au NPs and enzymes.

Here, we describe the synthesis of novel laccase−Au hybrids, where Au NPs were generated *in situ* from an aqueous $HAuCl_4$ solution. In the present hybrid catalyst system, the laccase acts as both a reducing agent (for Au NPs formation) and a stabilizing and/or supporting agent (avoiding Au NPs aggregation), and the activity of laccase is improved significantly by Au NPs (increased by 91.2% vs free laccase). With a short time visible light illumination (λ > 420 nm, within 3 min), the activity of laccase−Au hybrids decreases by 8.1% (vs laccase−Au hybrid without light), but it can be restored to

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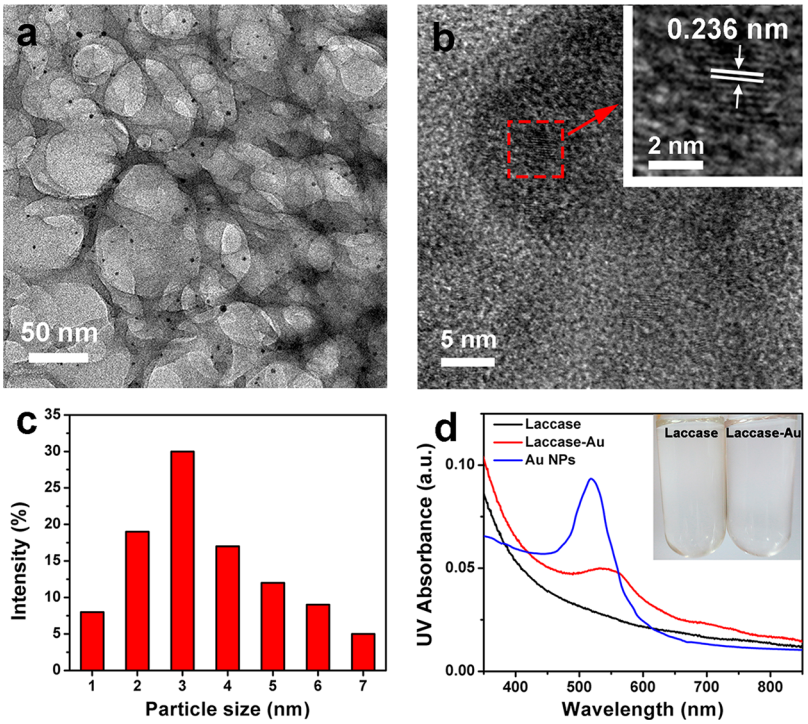

Figure 1. (a) TEM image; (b) HRTEM of laccase–Au hybrids; (c) Au nanoparticle size distribution based on TEM characterization shown in part a; (d) UV–vis absorbance spectrum of laccase (black trace), laccase–Au hybrids (red trace), and Au NPs (blue trace). Digital photographs (inset) of laccase and laccase–Au hybrids.

its initial activity when the illumination is removed. However, with long time illumination (λ > 420 nm, over 10 min), the catalytic activity of laccase–Au hybrids consecutively decreases and is not reversible even after removing the light. A series of control experiments suggested that the green light (corresponding to the local surface plasmon resonance (LSPR) of Au NPs) has the greatest impact on the activity of laccase–Au hybrids. On the basis of all experimental results, we proposed that the LSPR effect of Au NPs causes the structure change of laccase and local high temperature near the Au NPs. Those changes eventually affect the transportation of electrons in laccase, which further results in the declined activity of laccase.

2. EXPERIMENTAL SECTION

2.1. Instruments and Materials. The transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images were obtained using a FEI/Philips Tecnai G2 F20 TWIN transmission electron microscope. Room temperature UV–vis absorption was recorded using a Lambda 750 (Perkin Elmer) spectrophotometer in the wavelength range 300–800 nm. In the experiment, the light source is a xenon lamp, and its power is 300 W. The circular dichroism spectroscopy (CD) was measured with a model 410 circular dichroism spectrometer. Dynamic light scattering (DLS) measurements were carried out on a ZEN3690 instrument (Malvern, U.K.). X-ray photoelectron spectroscopy (XPS) was performed on a KRATOS Axis ultra-DLD X-ray photoelectron spectrometer with a monochromatized Mg Kα X-ray ($h\nu$ = 1283.3 eV). Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The laccase (≥5.43 U/g) was purchased from USA Sigma-Aldrich. The 2,2-azinobis(3-ethylbenzothiazolin-6-sulfnic acid) diammonium salt (ABTS) was purchased from Adamas-bate. All chemicals were purchased from Sigma-Aldrich. Unless specified otherwise, the detection buffer was PB buffer (pH 6.0). Milli-Q ultrapure water (Millipore, ≥ 18 MΩ·cm^{-1}) was used throughout.

2.2. Synthesis of Laccase–Au NPs Hybrids. Phosphate butter (PB) (pH 6.0) was prepared in advance. First, 5 mg of laccase powder was dissolved in the required amount in 40 mL of phosphate buffer (pH 6.0) and the solution was equally divided into two parts. Then, a specific volume of aqueous $HAuCl_4$ (0.5 mM) solution was injected into one part, while the volume of PB buffer (pH 6.0) injected into the other one was kept the same as that of $HAuCl_4$ (0.5 mM) solution. Different volumes (1.0, 1.5, 1.7, 2.0, and 3.0 mL) of $HAuCl_4$ (0.5 mM) were added to control the number of Au NPs in order to improve the specific activity of laccase. Finally, the solution was kept with stirring at 4 °C for 12, 24, 36, 48, and 60 h.

2.3. Synthesis of Au NPs. We made 3–4 nm Au NPs (as shown in Figure S1) by adding 0.6 mL of ice-cooled $NaBH_4$ solution (10 mM) into a 10 mL aqueous solution containing $HAuCl_4$ (0.25 mM) and dodecyltrimethylammonium bromide (100 mM), generating a brownish solution. The Au NP suspension and the products were collected by centrifugation (14 500 rpm, 1 h) and then washed with water twice.[22] Au NPs were evenly dispersed into 5 mL of PB (pH 6.0) solution. A 1.7 mL portion of Au NP solution (in synthesis of those Au NPs, 0.85 μmol of $HAuCl_4$ was consumed) was injected into 20 mL of laccase (0.25 mg) solution and the mixture was stirred for 2 h in a 4 °C water bath.

2.4. Activity Assays. For all experiments, the tests were repeated at least three times. The oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was tested as a model reaction to evaluate the catalytic performance of laccase, laccase–Au hybrids, and laccase + Au mixture. The intensity of the UV–vis absorption peak (at about 427 nm), which is resulting from the oxidation of ABTS, is proportional to the activity of biocatalyst. Specifically, the laccase solution (0.125 mg L^{-1}) and substrate solution (0.5 mM) were mixed quickly, and the absorbance of the oxidized product was recorded at 427 nm. The catalytic temperature was 30 °C.

2.5. Native-Polyacrylamide Gel Electrophoresis (Native-PAGE). Native-polyacrylamide gel electrophoresis (native-PAGE) was performed according to the method of Laemmli.[23] Protein solutions were mixed at a 1:1 (v/v) ratio with the native-PAGE sample buffer (1 mL of Tris–HCl (0.1 M) at pH 6.0, 0.1 mg of albutest, 7 mL of ultrapure water, and 1 mL of glycerol (87%)). Mixtures of laccase–

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Au hybrids and the free laccase were loaded on the gel made of 4% stacking and 12% separating gels, respectively, and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini-Protean II Cell apparatus (Atto Co., Beijing, China). After electrophoresis, the gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 45% methanol and 10% acetic acid and distained with 10% methanol and acid 10% acetic acid mixture solution.

3. RESULTS AND DISCUSSION

3.1. Characterization of Laccase−Au Hybrids. In typical experiments, laccase−Au hybrids were synthesized by using an *in situ* growth method where $HAuCl_4$ solution (0.5 mM, 1.7 mL) was injected into laccase (0.125 mg L^{-1}) PB buffer (pH 6.0) solution (20 mL) and stirred for 48 h at 4 °C. The morphology and the distribution of Au NPs were investigated using transmission electron microscopy (TEM)and high-resolution TEM (HRTEM) images. Figure 1a reveals that the formed hybrids were composed of an aggregate with a mesoporous amorphous superstructure (laccase) containing Au NPs dispersed into an organic matrix. The TEM image of laccase−Au hybrids (Figure 1a) shows that most of the Au NPs with a size of 3 ± 2 nm (for the size distribution, see Figure 1c) are evenly distributed within the laccase. The HRTEM image of the Au NPs shows that the lattice spacing was approximately 0.236 nm, of which the face was dominated by the (111) facets (Figure 1b). Figure 1d shows UV−vis absorption spectra of free laccase (black trace), laccase−Au hybrids (red trace), and Au NPs (blue trace). The UV−vis absorption spectrum of free laccase does not have a typical absorption peak in the range 400−800 nm. The bare Au NPs exhibit an obvious sharper peak centered at around 530 nm which derives from the LSPR of Au NPs.[24] Compared with Au NPs and free laccase, the UV−vis absorption spectra of laccase−Au hybrids display typical absorbance in visible regions at about 550 nm (see the photo image of laccase−Au hybrids in the inset of Figure 1d). Typically, Au NPs of about 3 nm show one major plasmonic absorption peak at 520−530 nm. Here, the Au plasma band red-shifted to a longer wavelength with a broad peak exhibited at about 550 nm that probably is attributed to the electron transferred from Au NPs to laccase.[25]

One of the interesting features of laccase−Au hybrids was that the average size of Au NPs entrapped in laccase was almost not changed after mixing up $HAuCl_4$ (0.85 μmol) with laccase solution for 48 h (Figure S3, black trace). In contrast, the average size of Au NPs without laccase continuously increases (Figure S3, red trace). It indicates that the size of Au NPs in hybrids is confined by laccases. The HRTEM images also evidence that. When a high voltage electron beam was shot on the laccase−Au hybrids, for a very short time, the layers of polypeptides gradually disappeared, which resulted in the appearance of Au NPs. Therefore, Au lattices can be observed from vagueness to sharpness without modifying any parameters of TEM (see Figure S2). The above results provide evidence of hybrid formation and indicate that most of the Au NPs were generated and confined by laccases rather than in solution.

3.2. Catalytic Activities of Laccase−Au Hybrids. The oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was tested as a model reaction to evaluate the catalytic performance of laccase, laccase−Au hybrids, and laccase and Au NPs mixture. Figure 2a shows the UV−vis absorbance spectra of ABTS at initial concentration (black trace) and ABTS added laccase (red trace), laccase and Au NPs (laccase + Au) mixture (blue trace), and laccase−Au hybrids

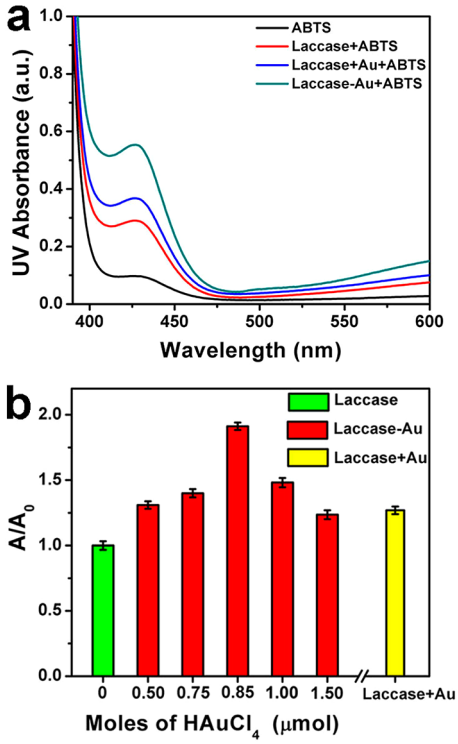

Figure 2. (a) The UV−vis absorbance spectra of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) at initial concentration (black trace) and ABTS after adding laccase (red trace), laccase + Au mixture (blue trace), and laccase−Au hybrids (green trace) and reacting for 3 min in a water bath at 30 °C. (b) The histogram of enzyme activity vs the amount of $HAuCl_4$ (green and red traces) and the enzyme activity of laccase and Au NP mixture (yellow trace).

(green trace) after 3 min in a water bath at 30 °C. The intensity of the absorption peak at about 427 nm (which is due to the oxidation of ABTS) is proportional to the activity of biocatalyst[26,27] and the reaction time (see Figure S4). Therefore, the following equation can be used to get the relative activity of hybrids

$$R = \frac{A_h - A_i}{A_l - A_i} \quad (1)$$

where A_i is the initial ABTS absorbance (at 427 nm), A_l is the absorbance (at 427 nm) after adding laccase, A_h is the absorbance (at 427 nm) after adding laccase−Au hybrids or the laccase + Au mixture, and R is the abbreviation of relative activity. To guarantee the accuracy, A_i is deducted from A_h and A_l during the calculation and the activity is calculated on the basis of the relative absorbance rather than on the basis of the absolute absorbance. The conditions were kept the same in all reactions and test processes. Relative activity is a measure used to compare the activity of different biocatalysts. The effect of Au NPs on oxidation of ABTS has been ruled out, as shown in Figure S5. It can be observed from Figure 2a that the peak intensity dramatically increases after adding biocatalysts.

In the following experiments, we investigated how adding different moles of $HAuCl_4$ influenced the catalytic activity of laccase in the laccase−Au hybrids. Au NPs of laccase−Au hybrids were generated *in situ* from an aqueous $HAuCl_4$ solution. The laccase acted as a reducing agent for Au NPs formation and also a stabilizing and supporting agent (avoiding Au NPs aggregation) at the same time. In our system, the size

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distribution of Au NPs was different with incremental moles of $HAuCl_4$ added into enzyme solution, as shown in Figure S6. It can be obviously found that most of the Au NPs sizes are larger than 5 nm when adding more $HAuCl_4$ (>0.85 μmol) into laccase (0.125 mg L^{-1}) solution. Also, there is an optimum reaction time between $HAuCl_4$ and laccase after adding $HAuCl_4$ into laccase solution. As shown in Figure S7, the maximum activity of the laccase–Au hybrids is reached after 48 h of preincubation in a water bath at 4 °C and a prolonged preincubation time resulted in a decline in the activities of the laccase–Au hybrids. The following activities of laccase, laccase–Au hybrids, and laccase + Au mixture were tested after 48 h of preincubation. Figure 2b reveals the effect of different moles of $HAuCl_4$ (from 0.5 to 1.5 μmol) on the activity of laccase, showing that their activity is mole-dependent. The first column without adding $HAuCl_4$ is in contrast to experiment. On the basis of eq 1 and their intensity of the UV–vis absorption peak at 427 nm (Figure 2a), the relative activities of laccase–Au hybrids were acquired. In contrast, laccase–Au hybrids show the highest activity when the amount of $HAuCl_4$ is 0.85 μmol, which is 92.1% higher than that of free laccase. However, when the added amount of $HAuCl_4$ is more (or less) than 0.85 μmol, the relative activity of laccase–Au hybrids is also higher than that of free laccase (increasing by 30.9, 39.9, 48.1, and 23.5%, respectively, vs free laccase). These results reveal that the Au NPs can affect the activity of laccase. At the same time, we find that the relative activity of laccase + Au mixture increases by 26.8% vs free laccase (as shown in Figure 2b, yellow column). The relative activity of laccase–Au hybrids is much better than that of laccase + Au mixture, which suggested a strong interaction between Au NPs and laccase in the laccase–Au hybrids.

To further investigate the interaction between Au NPs and laccase in the laccase–Au hybrids, the laccase–Au hybrids were separated by native-PAGE (native polyacrylamide gel electrophoresis). The inset of Figure 3a shows that the Au NPs and laccase formed stable complexes. The catalytic activity of enzyme is regulated by the structure of the protein.[28] Thus, circular dichroism (CD) spectra were measured to evaluate the structural change of laccase induced by Au NPs. Figure 3a shows that the conformational changes of laccase were induced under the conditions of the presence of Au NPs. Compared to the native laccase, for laccase–Au hybrids, the decrease of the native peak at 217 nm and the native peak at 227 nm indicates that the β-sheet and β-turn content of laccase–Au hybrids lessen. In Figure 2b, comparing the relative activity of laccase–Au hybrids (increased by 91.2% vs free laccase) and that of the laccase + Au mixture (increased by 26.8%), it also indicates that Au NPs combined with laccases rather than generated in solution. All of these results demonstrate that Au NPs could greatly enhance the oxidase activity through unfolding of laccase by forming a complex with laccase.

To better understand the interaction between Au and laccase, the chemical states of Au NPs were tested by the XPS techniques. As shown in Figure S8a, it can be observed that the Au 4f7/2 peak was fitted by using two synthetic peaks positioned at BE = 83.8 and 84.7 eV and the Au 4f5/2 peak was fitted by using two synthetic peaks positioned at BE = 87.4 and 88.3 eV. For the Au 4f7/2 peak or Au 45/2 peak,[29] the first contribution is assigned to elemental gold (Au^0), while the second one is related to the stable state of Au^+.[30] This result indicates the presence of two different Au species that can be explained by the model of a metallic core covered with a

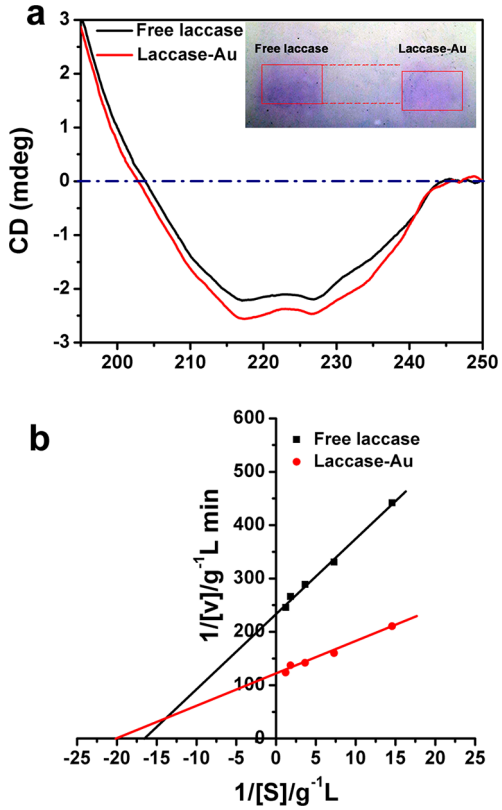

Figure 3. (a) Circular dichroism (CD) spectra of laccase (black trace) and laccase–Au hybrids (red trace) in PB buffer (pH 6.0). Digital photographs (inset), native-PAGE of the free laccase and laccase–Au hybrids (12% gels) under visible light. (b) Lineweaver–Burk plot analyses are shown for the enzymatic kinetics of laccase and laccase–Au hybrids. The concentrations of ABTS were 0.125, 0.25, 0.5, 1.0, and 1.5 mmol L^{-1}; the solvent was phosphate buffer (0.02 mol L^{-1}, pH 6.0); black and red curves represents laccase and laccase–Au hybrids, respectively.

positively charged shell. The positively charged shell of Au NPs in laccase–Au hybrids may result from the strong interaction between Au NPs and the amino acid residues (such as NH_2 and SH).[31] As shown in Figure S8b, the content ratio of Au^+ in the laccase + Au mixture remarkably decreases owing to the weak interaction between Au NPs and the amino acid residues. This difference may be the reason why the activity of the laccase–Au hybrids is much better than that of the laccase + Au mixture.

The Michaelis constant (K_m) reflects the binding efficiency of the enzyme with the substrate. This constant was determined for each kind of enzyme using Lineweaver–Burk plot analysis.[32,33] To determine how the Au NPs affect the activity of laccase in the hybrids, Menten kinetics of the hybrid system was tested and analyzed, as shown in Figure 3b. In the Michaelis–Menten kinetics (details in the Supporting Information), the apparent values of K_m and V_{max} were then calculated from the slope and the intercept values, respectively. Figure 3b shows the Lineweaver–Burk plot analysis of free laccase and laccase–Au hybrids. A plot of $1/v$ versus $1/[S]$ gives a family of lines with different slopes and intersects in the X-axis. The K_m values of the free laccase and the laccase–Au hybrids are determined to be 59.93 and 43.70 mg L^{-1}, respectively. The K_m value of laccase–Au hybrids is lower than that of free laccase, indicating a higher affinity of laccase–Au hybrids toward ABTS. The change of laccase kinetics (K_m from

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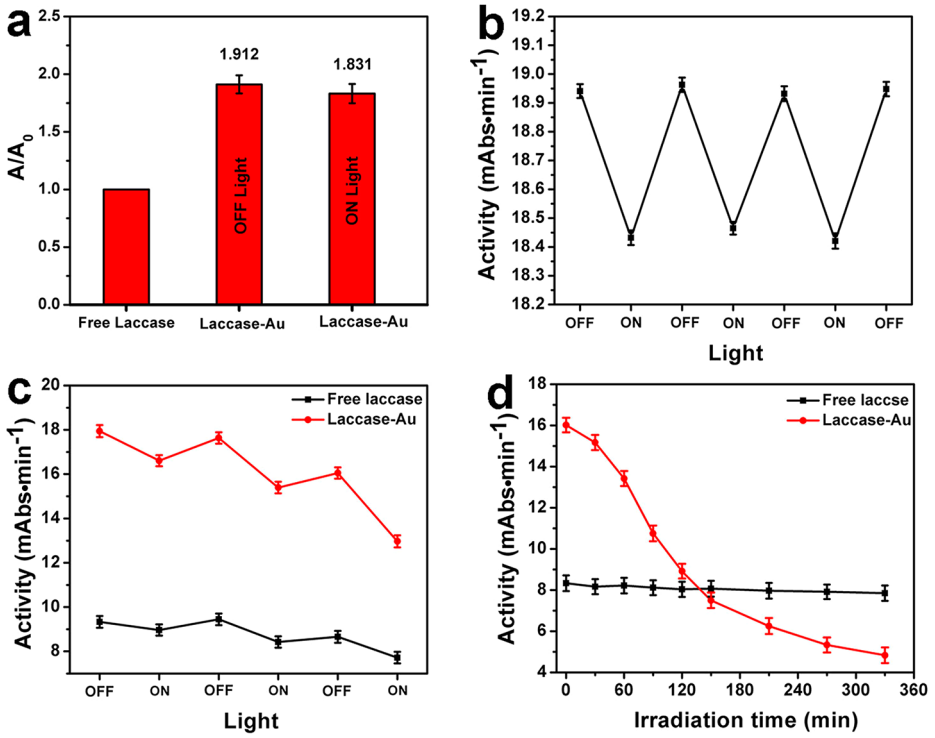

Figure 4. (a) The activity of laccase and the activity of laccase−Au hybrids under irradiation or not. (b) The activity of laccase−Au hybrids. The reaction time was 3 min under visible light illumination or without visible light illumination. (c) The activity of laccase (black trace) and laccase−Au hybrids (red trace). The reaction time was 10 min under visible light illumination or without visible light illumination. (d) The activity of laccase (black trace) and laccase−Au hybrids (red trace) under continuous irradiation.

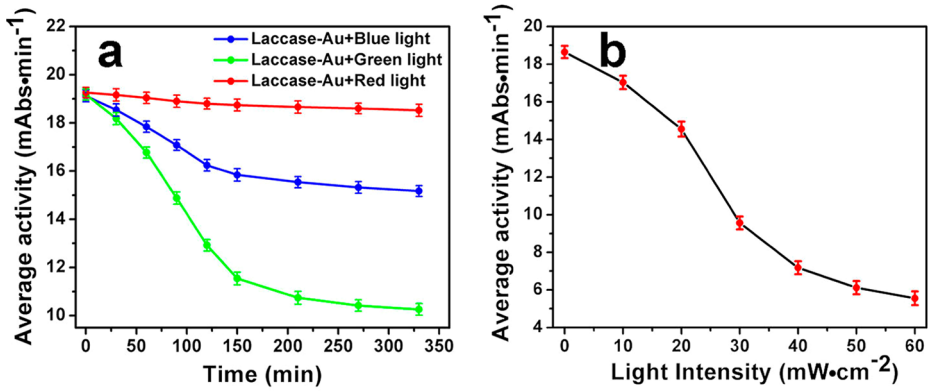

Figure 5. (a) Illumination wavelength dependence of the activity of laccase−Au hybrids as a catalyst by different lighting conditions: blue light (450−490 nm), green light (500−560 nm), red light (640−700 nm), using a xenon lamp (300 W, >420 nm) as a light source (applying a band-pass filter of $\lambda \pm 20$ nm for 470 nm and a band-pass filter of $\lambda \pm 30$ nm for 530 and 670 nm; all of the light irradiation intensities were kept at about 25 mW·cm^{-2}). (b) Light intensity dependence of laccase−Au hybrid catalytic activity, using a xenon lamp (300 W, >420 nm) as a light source (applying a band-pass filter of $\lambda \pm 30$ nm for 530); the activity of laccase−Au hybrids was tested after 150 min of irradiation for each intensity.

59.97 to 43.70 mg L^{-1}) in the laccase−Au hybrids can be attributed to the structural change, which promotes laccase disintegration of the substrate.

3.3. Visible-Light-Induced Tuning and Decline Effects of Au NPs on Laccase Activity. To explore the photocatalytic activity of the new hybrids, a series of experiments were carried out under different visible light illumination times (xenon lamp, 300 W, $\lambda \geq 420$ nm). Here, the average activity was needed to compare the activity of the enzyme under different illumination time activity. The average activity is defined as

$$\text{Average activity} = \frac{R}{t} \quad (2)$$

where R is the relative activity and it can be calculated by eq 1; t is the illumination time. The unit of the average activity is mAbs·min^{-1}. For a 3 or 10 min reaction, the average activity obtained is detailed in Figure S9. As shown in Figure 4, the photocatalytic performances of laccase−Au hybrids are illumination-time-dependent. As shown in Figure 4a and b, the relative activity of laccase−Au hybrids was tested after reacting with ABTS for 3 min in a water bath at 30 °C with illumination. Compared to that of laccase−Au hybrids (1.912 vs free laccase), the relative activity of laccase−Au hybrids

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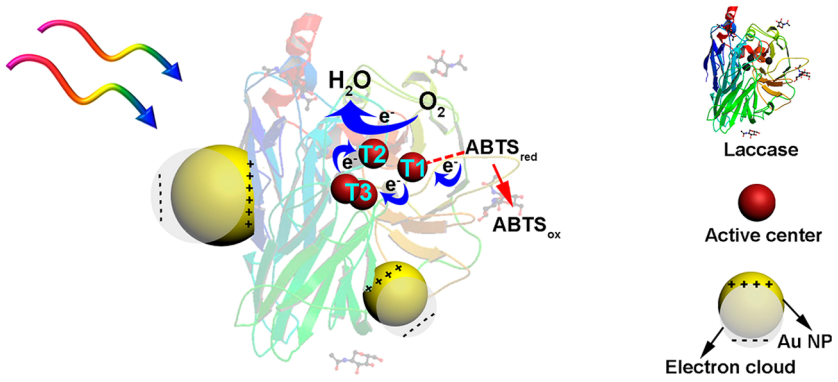

Figure 6. Schematic of the photocatalytic behavior of laccase−Au hybrids.

decreases to 1.831 after 3 min of illumination, while it is still much higher than that of free laccase (Figure 4a). Figure 4b shows the activity of laccase−Au hybrids with xenon lamp illuminating every 3 min. When the light resource is removed, the activity of laccase−Au hybrids could recover to its initial level. Hence, the activity of laccase−Au hybrids is reversible with short time illumination. Next, we tried to lengthen the illumination time to 10 min for the catalytic reaction. As shown in Figure 4c, the activity of laccase−Au hybrids declines after illumination and that can partly recover after removing the light resource. However, the activity recovered of laccase−Au hybrids is still lower than the initial activity of laccase−Au hybrids. Besides, the activity of laccase−Au hybrids keeps on decreasing when the light resource is added again. For 10 min-reaction, the overall trend of hybrids activity continuously declines that may result from LSPR of Au NPs or the local high temperature near the Au NPs during irradiation.

To better understand the influence of the visible light on the activity of laccase−Au hybrids, the experiment was carried out with a continuous illumination time of 5.5 h by xenon lamp (see Figure 4d). During illumination, the solutions of free laccase and laccase−Au hybrids were incubated in a cold water bath at 4 °C to eliminate the effect of heating which is due to the irradiation. The activity of laccase−Au hybrids dramatically decreases, and the declined rates of hybrid activity are sustained increasing. After illumination for more than 1.5 h, the declined rates of hybrid activity become slow. The activity of hybrids is lower than that of free laccase, while the illumination time is up to 2.5 h. In contrast, the activities of free laccase are essentially unchanged during the illumination time.

Subsequently, the photocatalytic activity of laccase−Au hybrids was further investigated with different monochromatic light. On the basis of the UV−vis absorbance spectra of laccase (black trace), laccase−Au hybrids (red trace), and Au NPs (blue trace) shown in Figure S10, the blue light, green light, and red light were chosen. Figure 5a shows that the greatest impact on the activity of hybrids is green light (500−560 nm), whose wavelengths include the surface plasma resonance zone of Au NPs (around 520 nm). To gain insight into the effects of green light, relative activity experiments with different light intensities were carried out. A significant reduction of laccase activity can be observed when light is irradiated on laccase−Au hybrids. As shown in Figure 5b, with increasing incident green light intensity in the range 0−60 $mW{\cdot}cm^{-2}$, the activity dramatically decreases, while beyond 40 $mW{\cdot}cm^{-2}$, there is a plateau which may originate from the saturation of electron field dispersed on the Au NPs surface as a result of its LSPR effect. Because of that, change of laccase activity is not obvious along with increasing the intensity of green light. These experiments further clarify the possible reasons for the declined activity of laccase−Au hybrids that may be attributed to the light-driven LSPR of Au NPs, which would result in the structure change of laccase and significantly alter the electronic structure of T1 and/or T2/T3 sites.

On the basis of the above results, we further proposed a mechanism for photocatalytic behavior of laccase−Au hybrids. As shown in Figure 6, laccase, as multicopper oxidases, contains four copper ions that distribute into three sites (T1, T2, and T3 sites). The catalytic mechanism of laccase consists of two parts: the T1 site, functioning as the primary electron acceptor, can extract electrons from the reducing substrate and then electrons are transferred to the trinuclear T2/T3 center where the reduction of molecular oxygen takes place. Because of the strong interaction between Au NPs and the amino acid residues (such as NH_2 and SH),[31] Au NPs are more likely to connect with laccase to form the laccase−Au hybrids. The insertion of Au NPs in laccase led to a looser structure of laccase that may help the T1 site to more easily extract electrons from ABTS. Thus, the activity of laccase−Au hybrids was increased by 91.2% (vs free laccase). Then, the LSPR of Au NPs results from incident light being scattered and absorbed at a resonant frequency due to the collective oscillation of conduction electrons.[34] The collective oscillation of conduction electrons in Au NPs has an effect on the structure of laccase, and the LSPR effect of Au NPs could produce heat and local high temperature near the Au NPs. Those changes eventually affect the electron transfer in intermolecular T1, T2, and T3 sits. On the basis of all the above, the activity of laccase−Au hybrids continually decreases and that was even lower than the activity of free laccase when the illumination time reached 2.5 h by xenon lamp.

4. CONCLUSION

We demonstrated a simple method for the preparation of laccase−Au hybrids with relatively high activity (increase by 91.2%). Under 3 min of illumination, the activity of laccase−Au hybrids decreases by 8.1% (vs laccase−Au hybrids without light), but it can be restored to its initial activity when the light resource was removed. The activity of laccase−Au hybrids is reversible with short time illumination. However, long irradiation has an irreversible effect on the activity of laccase−Au hybrids. Green light (500−560 nm) has the greatest impact on the activity of laccase−Au hybrids. The LSPR of Au NPs causes the structure change and local high

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temperature near the Au NPs. Those changes eventually affect the transportation of electrons in laccase, resulting in the activity decline.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b06472.

The detailed analysis of Michaelis−Menten kinetics; TEM image; UV−vis absorbance spectrum; XPS image (PDF)

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Notes

The authors declare no competing financial interest.

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