



5,10,15,20-tetrakis (4-carboxyl phenyl) porphyrin–functionalized urchin-like CuCo_2O_4 as an excellent artificial nanozyme for determination of dopamine

Yanlei He¹ · Ning Li¹ · Xiangwei Liu¹ · Wei Chen¹ · Xixi Zhu¹ · Qingyun Liu¹

Received: 24 January 2021 / Accepted: 2 April 2021

© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2021

Abstract

Urchin-like peroxidase mimics 5,10,15,20-tetrakis (4-carboxyl phenyl) porphyrin–functionalized CuCo_2O_4 nanospheres (Por- CuCo_2O_4) has been fabricated as an excellent visual biosensor. X-ray diffractometry (XRD), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) have been employed to characterize the composition, morphologies, and elemental analysis of the as-synthesized Por- CuCo_2O_4 . The catalytic activity of Por- CuCo_2O_4 was evaluated by the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) with the aid of H_2O_2 , which exhibited a visual blue change with an absorption maximum at 652 nm for only 10 s. The peroxidase-like behaviors of Por- CuCo_2O_4 conformed to the Michaelis-Menten equation. Electrochemistry, radical scavenger, and fluorescence probe experiments verified that electron transfer, $\bullet\text{O}_2^-$ radicals, and holes (h^+) are the important factors during the catalytic oxidation of TMB. Based on the inhibition of dopamine (DA) on TMB oxidation, the Por- CuCo_2O_4 -based colorimetric biosensor has been successfully constructed for sensitive determination of DA with a detection limit (LOD) of 0.94 μM . In addition, colorimetry was validated to detect DA in serum samples with high sensitivity and good selectivity.

Keywords Biosensors · Por- CuCo_2O_4 · Peroxidase mimic · Dopamine

Introduction

Dopamine (DA) acts as a neurotransmitter in the central nervous system, impacting physiological and neurological activities [1]. The abnormal levels of DA are able to trigger various neurological diseases, such as schizophrenia [2], cardiovascular disease [3], and Alzheimer's disease [4]. Fortunately, the relative pathological function can release a certain concentration of small biological molecules including H_2O_2 , glucose, and DA. Thus, we can detect these small biological molecules as metabolic parameters for promising disease diagnosis. Accordingly, accurate quantitative determination of DA is vitally essential for clinical analysis and early diagnosis of relative diseases.

Nevertheless, the lack of electrical activity, chromophores, and fluorophores makes the detection of DA become particularly difficult. In order to efficiently detect DA, various biosensors such as electrochemistry [5], chemiluminescence, colorimetry [6], and fluorescence [7] have been developed. Among these methods, the colorimetric method has been focused on due to the unique advantages of sensitive reaction, simple preparation, intuitive, good selectivity, and low requirements on the instrument [8]. Accordingly, it is necessary to establish a novel material for DA and apply it conveniently in practice.

Nanozymes, a class of simulated enzymes with unique properties of nanomaterials, have attracted more and more attention due to their advantages in economy, stability, and catalytic activity over natural enzymes. Recently, various nanozymes including metal oxides [9], metal sulfides [10], and precious metal nanoparticles [11] have been verified to possess good peroxidase activity. Compared with single-metal nanomaterials, bimetallic nanomaterials show excellent catalytic performance. Chen et al. developed CeCoO_3 bimetallic nanomaterials and successfully applied them to sensitive detection of glutathione. Although many nanozymes have

✉ Qingyun Liu
qyliu@sdust.edu.cn

¹ College of Chemical and Biological Engineering, Shandong University of Science and Technology, Qingdao 266590, People's Republic of China

been explored and developed, their catalytic mechanism, sensitivity, and selectivity are still important challenges in research. Among strategies of developing the catalytic activity of nanoenzymes, organic molecules with large conjugate macrocycles have been considered attractive ones to modify some semiconductor nanomaterials [12]. Porphyrins (Por) are such ones. Porphyrins are usually used as photosensitizers to enhance the performance of some inorganic nanomaterials applied in the solar cells [13], photodynamic therapy [14], and catalysis [15], etc. Moreover, some merits including good biocompatibility and strong coordination with metals make porphyrins become ideal candidates to functionalize inorganic nanoenzymes and develop catalytic activity, accordingly [16]. Therefore, considering an interesting spinel cobaltite $\text{Por-CuCo}_2\text{O}_4$ as an excellent peroxidase, a fast colorimetric sensing platform for DA can come true.

Herein, we prepared 5,10,15,20-tetrakis (4-carboxyl phenyl) porphyrin (Fig. S1)—functionalized urchin-like CuCo_2O_4 nanocomposites (Scheme 1), which were verified to possess an excellent peroxidase-like activity with the aid of TMB accompanied by a color change in the process of the catalytic reaction. The catalytic performance of $\text{Por-CuCo}_2\text{O}_4$ peroxidases conforms to the Michaelis-Menten equation. Notably, the response time of blue color (oxTMB) is 10 s, a very short time, which is very important to construct a fast colorimetric sensor to realize real-time detection. Electrochemistry, radical scavenger, and fluorescence probe experiments indicate that electron transfer, $\cdot\text{O}_2^-$ radicals, and holes (h^+) are the important factors during the catalytic oxidation of TMB. In addition, based on $\text{Por-CuCo}_2\text{O}_4$ peroxidases, a fast colorimetric sensing platform for H_2O_2 and DA was developed with high sensitivity and good selectivity.

Experiment and methods

Reagents

Copper chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), hydrogen peroxide (H_2O_2 , 30 wt.%), cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$),

and dopamine hydrochloride were commercially provided by Aladdin Biochemical Technology Co., Ltd. 3,3',5,5'-tetramethylbenzidine dihydrochloride ($\text{TMB} \cdot 2\text{HCl}$) was procured from Macklin (Shanghai, China). Ethylene diamine tetraacetic acid disodium salt (EDTA), urea, Na^+ (NaCl), K^+ (KCl), Mg^{2+} (MgSO_4), p-benzoquinone (PBQ), sucrose, lactose, fructose, D-histidine, L-arginine, D-serine, and isopropyl alcohol (IPA) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The synthesis of 5,10,15,20-tetrakis (4-carboxyl phenyl) porphyrin (H_2TCCP) was referred to by an earlier report [17].

Characterization

The products were analyzed by a powder X-ray diffractometry (XRD) instrument ($\text{Cu-K}\alpha$, $\lambda = 1.54178 \text{ \AA}$) and a scanning electron microscopy instrument (APREO, American) operated at an accelerating voltage of 2 kV and is equipped with an energy-dispersive X-ray spectroscopy (EDX) instrument. The valence state of the elements in $\text{Por-CuCo}_2\text{O}_4$ was analyzed by an X-ray photoelectron spectroscopy (XPS) instrument (Thermo ESCALAB 250 Xi). The specific surface areas and pore size distribution of nanoparticles were analyzed by the Brunauer-Emmett-Teller surface areas (BET) and the Barrett-Joiner-Halenda (BJH) process with a Micromeritics ASAP 2460 analyzer. Fluorometric data (FL) and the UV-vis absorption spectrum data were obtained on a Hitachi F-4600 spectrofluorophotometer (Japan) and the TU 1810 spectrophotometer (Puxi, China), respectively. The electrochemical performance is characterized by CHI 760E Chen-Hua electrochemical workstation.

Synthesis of urchin-like $\text{Por-CuCo}_2\text{O}_4$

Firstly, urchin-like CuCo_2O_4 microspheres were prepared by the hydrothermal method [18]. After that, the urchin-like $\text{Por-CuCo}_2\text{O}_4$ microspheres were prepared by a two-step method. The formation process of urchin-like $\text{Por-CuCo}_2\text{O}_4$ nanospheres is shown in Scheme 1. The detailed preparation was presented in supporting information.



Scheme 1 Schematic illustration for the formation process of urchin-like $\text{Por-CuCo}_2\text{O}_4$ nanospheres

After calcination, 3 mg H₂TCPP was dissolved in NaOH alkaline water (pH = 9). After that, 60 mg of CuCo₂O₄ sample was added to the above solution, which was ultrasonically dissolved and transferred to a Teflon-lined autoclave with 110 °C hydrothermal for 60 min. After the sample was collected by centrifugation and further purified, Por-CuCo₂O₄ composite has been successfully synthesized.

Peroxidase-like activity of Por-CuCo₂O₄ and the DA detection

The experiment was conducted in acetic acid buffer containing Por-CuCo₂O₄ (0.015 mg mL⁻¹), H₂O₂ (25 mM), and TMB (0.1 mM). The visible absorption spectra were collected after the system reacted for 90 s. Kinetic experiments were carried out by measuring the absorbance at 652 nm in acetate buffer containing Por-CuCo₂O₄, H₂O₂, and various concentrations of TMB at room temperature. Similarly, as a control experiment, other conditions remain unchanged as described above, by changing the H₂O₂ concentration and fixing the TMB concentration. The apparent kinetic parameters were calculated by the following equation: $1/v = (K_m/V_{max}) \times (1/[S]) + 1/V_{max}$, where v , $[S]$, V_{max} , and K_m stand for initial velocity, the substrate concentration, the maximal velocity, and the Michaelis constant, respectively.

The DA detection is described in detail as follows: freshly prepared various concentrations of DA (10–700 μM) were added into the reaction mixture containing the buffer solution acetate (pH 4.0), Por-CuCo₂O₄ (0.015 mg mL⁻¹), H₂O₂ (25 mM), and TMB (0.1 mM). The entire reaction mixture was incubated for 3 min, and the absorbance of various DA concentrations was recorded.

Test of active species

A standard three-electrode system (Por-CuCo₂O₄-modified glassy carbon electrode, the platinum wire electrode, and the saturated calomel electrode) was used in the electrochemical experiment. In the cyclic voltammetry experiment, the current and potential responses were recorded before and after the addition of H₂O₂ to form a control group. In the amperometric testing experiment, the electrodes of uncoated and coated materials were subjected to a control test, and the electrochemical response was collected after adding the H₂O₂ (1 M) to react for 60 s. H₂O₂ (1 M) was added to PBS every 50 s, and the electrochemical response of the solution was recorded.

The capture experiments were conducted by selecting EDTA, IPA, and PBQ to capture holes (h⁺), hydroxyl radicals (•OH), and superoxide radicals (•O₂⁻), respectively. Specifically, different scavengers (200 μL) and Por-CuCo₂O₄ (100 μL) were injected into 2 mL of H₂O₂-TMB system to form a mixture. The absorbance at 652 nm was

recorded after the above mixture reacted for 90 s at ambient temperature.

Fluorescent experiments were implemented by selecting terephthalic acid (TA) to capture •OH to form dihydroxyterephthalic acid (HOTA) with strong fluorescence. Specifically, freshly prepared various samples contain 5 mM TA, 25 mM H₂O₂, and Por-CuCo₂O₄ at a concentration of 0.1–0.8 mg mL⁻¹ and acetate buffer at pH 4. Fluorescence spectra were recorded after the above mixtures were reacted for 30 min at the optimal temperature.

Detection of DA

The human serum samples of two volunteers were provided by the Affiliated Hospital of Qingdao University, China. To avoid the interference of coexisting substances, the resulting serum sample was diluted 80 times with PBS, which diluted DA to a certain concentration, and then added into the system containing H₂O₂-TMB. After 3 min of reaction, the absorbance of the reaction system at 652 nm was recorded.

Results and discussion

Characterization of Por-CuCo₂O₄ spinel microspheres

The phase of such interesting hierarchical microspheres was studied by XRD (Fig. 1a). The diffraction peaks at 19.07°, 31.36°, 36.96°, 38.96°, 45.07°, 56.03°, 59.60°, 65.70°, and 77.55° are indexed to the crystal plane of (111), (220), (311), (222), (400), (422), (511), (440), and (533) of cubic CuCo₂O₄ phase (JCPDS card no. 01-1155) [19]. Carefully, one diffraction peak at 35.55° is attributed to monoclinic CuO (JPCDS card no. 05-0661) [20]. Therefore, the main composition of nanocomposites is CuCo₂O₄, accompanied by trace amounts of CuO [21], because CuCo₂O₄ has poor thermal stability and is easy to produce CuO [22]. Notably, the XRD peaks of Por-CuCo₂O₄ are almost consistent with that of CuCo₂O₄, suggesting that the introduction of porphyrin has no effect on the lattice structure of the composites, due to a small quantity of porphyrin in the composites.

XPS is used to further study the composition and oxidation states of Por-CuCo₂O₄, shown in Fig. 1b–f. The survey spectra in Fig. 1b display the composition of Cu, Co, O, and N elements in CuCo₂O₄ and Por-CuCo₂O₄, respectively. In the Cu 2p spectra (Fig. 1c), two major spin-orbit doublets with binding energy at approximately 935.1 and 933.7 eV are ascribed to Cu²⁺ and Cu⁺ [23]. The Gaussian fitting curves show that the Co 2p spectra (Fig. 1d) are composed of Co 2p_{3/2} and Co 2p_{1/2} peaks at 935.1 and 933.7 eV with two satellite peaks located at approximately 941.2 and 943.8 eV [24]. As seen from Fig. 1e, the O1s spectrum can be fitted to three Gauss peaks at 532.7 eV, 531.3 eV, and 529.8 eV, which are

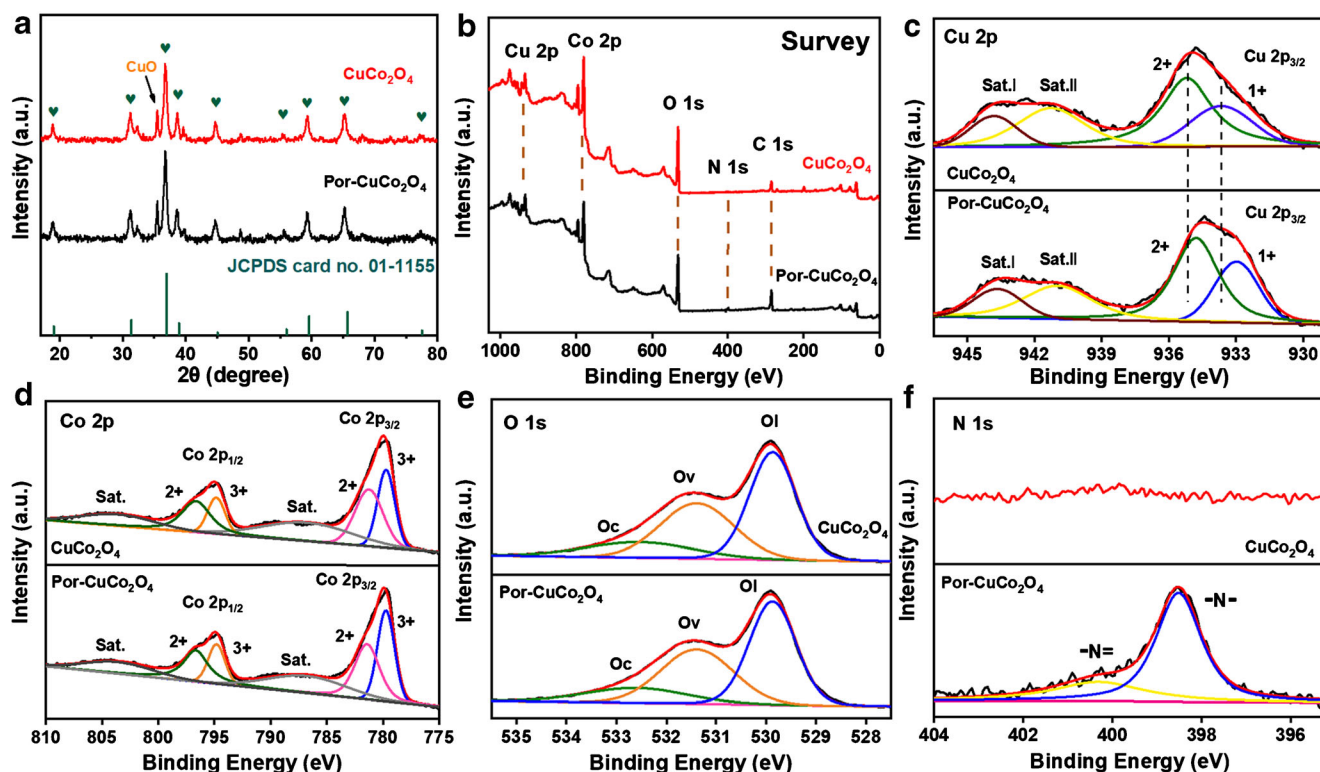


Fig. 1 XRD (a) and XPS spectra data of the as-prepared CuCo_2O_4 and $\text{Por-CuCo}_2\text{O}_4$ nanocomposites: Survey scan spectra (b), Cu 2p (c), Co 2p (d), O 1s (e), and N 1s (f), respectively

attributed to chemisorbed oxygen, the oxygen vacancy, and the lattice oxygen [25], respectively. The existence of oxygen vacancy is in favor of the improvement of the catalytic activity of $\text{Por-CuCo}_2\text{O}_4$. Figure 1f shows the comparison spectra of N 1s in CuCo_2O_4 and $\text{Por-CuCo}_2\text{O}_4$. Compared with that of CuCo_2O_4 , N 1s in $\text{Por-CuCo}_2\text{O}_4$ has peaks at bond energy of 398.5 eV and 400.4 eV, corresponding to the -N- bond and the -N= bond of the porphyrin ring [17], indicating that the porphyrin was successfully introduced.

Morphologies of CuCo_2O_4 and $\text{Por-CuCo}_2\text{O}_4$ were analyzed by SEM and TEM, respectively. As displayed in Fig. 2a and b, CuCo_2O_4 and $\text{Por-CuCo}_2\text{O}_4$ all exhibit urchin-like microsphere structure with 5–10 μm in size, which are composed of needle-like nanorods. Carefully, TEM images (Fig. 2c and d) display that needle-like nanorods are further composed of a lot of nanoparticles. From the HRTEM image of $\text{Por-CuCo}_2\text{O}_4$ (Fig. 2e), the lattice fringes of 0.456 nm, 0.284 nm, and 0.243 nm are in accordance with the crystalline planes of (111), (220), and (311) of CuCo_2O_4 in XRD data, respectively. Subsequently, the EDX element mapping (Fig. 2f) shows that Cu, Co, O, and N elements uniformly distribute $\text{Por-CuCo}_2\text{O}_4$ composite, indicating the successful preparation of $\text{Por-CuCo}_2\text{O}_4$. Additionally, the calculated BET specific surface area (Fig. S2a) of $\text{Por-CuCo}_2\text{O}_4$ composite material is $45.57 \text{ m}^2 \text{ g}^{-1}$, which is much larger than pure CuCo_2O_4 ($36.56 \text{ m}^2 \text{ g}^{-1}$). In addition, the Barrett-Joiner-Halenda (BJH) model calculates that the $\text{Por-CuCo}_2\text{O}_4$ pore

size distribution is about 2 nm and 6.9 nm in diameter (Fig. S2b). It can be seen that the introduction of porphyrin further increases the specific surface area of $\text{Por-CuCo}_2\text{O}_4$ and provides more active sites.

Peroxidase-like activity

In order to validate the peroxidase-like activity of $\text{Por-CuCo}_2\text{O}_4$, six reaction systems were devised as control experiments using TMB as the chromogenic substrate, which can be oxidized to generate blue oxTMB with a distinct absorption at 652 nm. As seen from curves 3c–3f in Fig. 3, four systems hardly have absorption in the absence of H_2O_2 or composites (CuCo_2O_4 , $\text{Por-CuCo}_2\text{O}_4$), suggesting that either individual H_2O_2 or nanocomposite cannot trigger the oxidation reaction of TMB. Interestingly, systems a and b (curves 4a and 4b) have stronger absorption intensity accompanied by a marked color change (illustration), indicating that both CuCo_2O_4 and $\text{Por-CuCo}_2\text{O}_4$ possess the peroxidase-like activity. Moreover, the absorption intensity of $\text{Por-CuCo}_2\text{O}_4$ (system a) is more than twice that of CuCo_2O_4 (system b), which indicates that $\text{Por-CuCo}_2\text{O}_4$ has a stronger peroxidase-like activity. Therefore, the simultaneous existence of $\text{Por-CuCo}_2\text{O}_4$ peroxidases and H_2O_2 is the basic condition for the rapid oxidation of TMB. The reason why $\text{Por-CuCo}_2\text{O}_4$ has more excellent peroxidase-like activity is ascribed to a lot of oxygen vacancies verified by XPS as well as the synergetic effect between

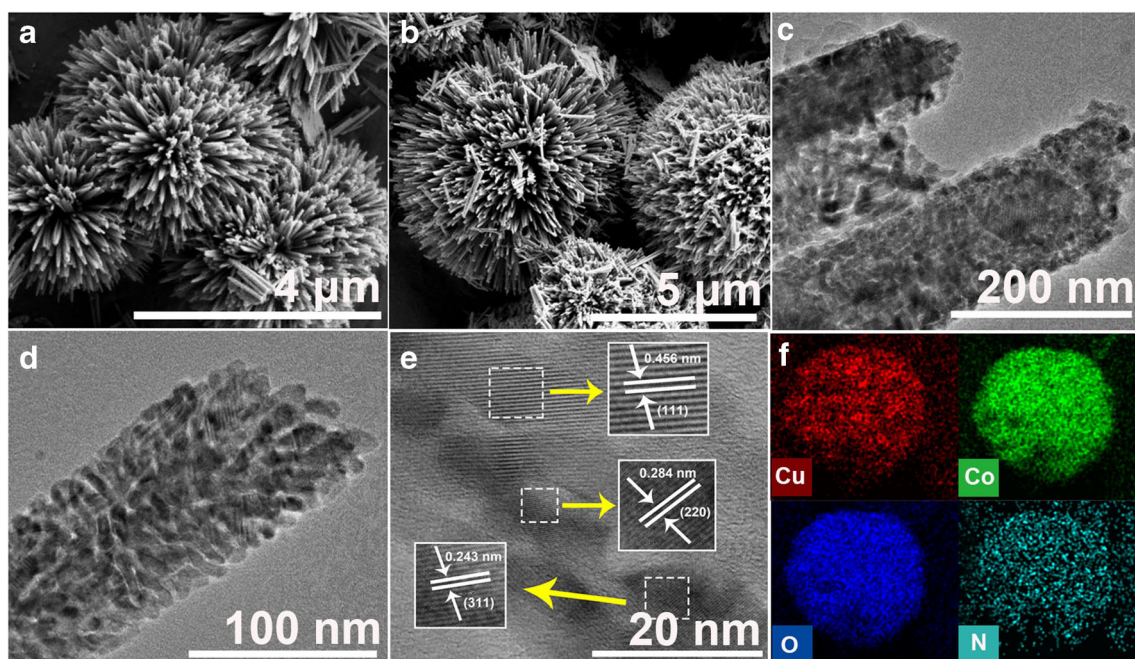


Fig. 2 SEM images of CuCo_2O_4 (a) and $\text{Por-CuCo}_2\text{O}_4$ (b), respectively. TEM images of CuCo_2O_4 (c) and $\text{Por-CuCo}_2\text{O}_4$ (d, e). EDX mapping images (f) of elements of $\text{Por-CuCo}_2\text{O}_4$: Cu, Co, O, and N

CuCo_2O_4 and H_2TCPP molecules, which is further explained in the catalytic section, subsequently. The according color of different reaction systems can be quickly distinguished in 90 s, shown in the inset of Fig. 3. As we know, the catalytic activity of artificial peroxidase and natural peroxidase is influenced by pH and temperature. Figure S3 shows that $\text{pH} = 4$ and 45°C are the best conditions, which will be applied to subsequent experiments.

The steady-state kinetics of $\text{Por-CuCo}_2\text{O}_4$ was studied by keeping the TMB concentration constant while changing the H_2O_2 concentration (and vice versa). Figure 4a and c respectively show the relationship between the concentration of

H_2O_2 and TMB as substrates and the initial reaction rate. The values of K_m and $V_{\max}$ were calculated according to the Michaelis-Menten equation (Fig. 4b and d). In comparison with the affinity of HRP together with other artificial peroxidase, some related parameters are shown in Table S1. Compared with that of HRP and other artificial peroxidase ($\text{Cu}(\text{OH})_2$, $\text{Co}_3\text{O}_4@ \text{CeO}_2$, PtCNP s, $[\text{Cu}(\text{PDA})(\text{DMF})]$), our obtained $\text{Por-CuCo}_2\text{O}_4$ has the lower K_m value, indicating that $\text{Por-CuCo}_2\text{O}_4$ has a stronger affinity towards TMB, which is a favorable factor for catalytic enhancement of $\text{Por-CuCo}_2\text{O}_4$.

Colorimetric detection of small biological molecules and selective exploration

As an oxidant, H_2O_2 is not only used in industrial production but also plays a key role in biological processes. However, long-term excessive use of H_2O_2 can cause diseases such as low immunity, genetic mutations, arteriosclerosis, and diabetes [26]. Therefore, designing a fast colorimetric sensing platform for H_2O_2 is particularly important. Herein, based on $\text{Por-CuCo}_2\text{O}_4$ with higher peroxidase-like activity, a series of experiments (Fig. 5a) were designed and implemented by changing the concentration of H_2O_2 (0.1–10 mM). From the data, a very good linear relationship (inset) of absorbance and H_2O_2 concentration in the concentration range of 0.1–1.0 mM ($R^2 = 0.998$) was obtained. The detection limit (LOD) is calculated to be $90.26 \mu\text{M}$ ($\text{LOD} = 3 \text{ s/k}$), suggesting a good sensitivity of the constructed colorimetric sensor based on $\text{Por-CuCo}_2\text{O}_4$ to the TMB- H_2O_2 system.

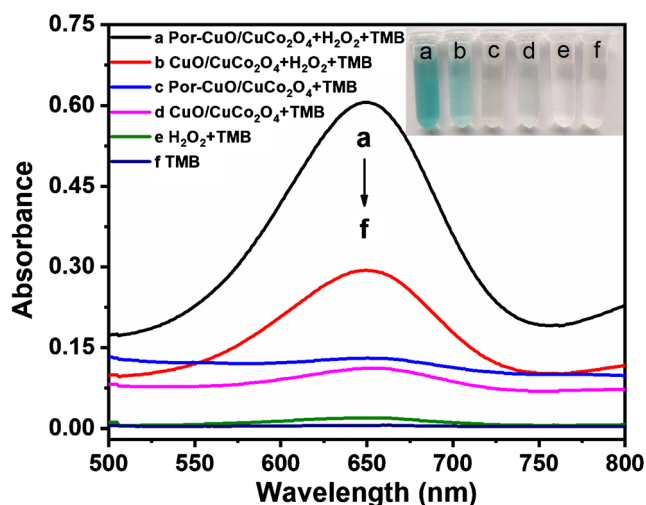


Fig. 3 UV-visible absorption spectra and corresponding photograph of color change of different reaction systems in 90 s

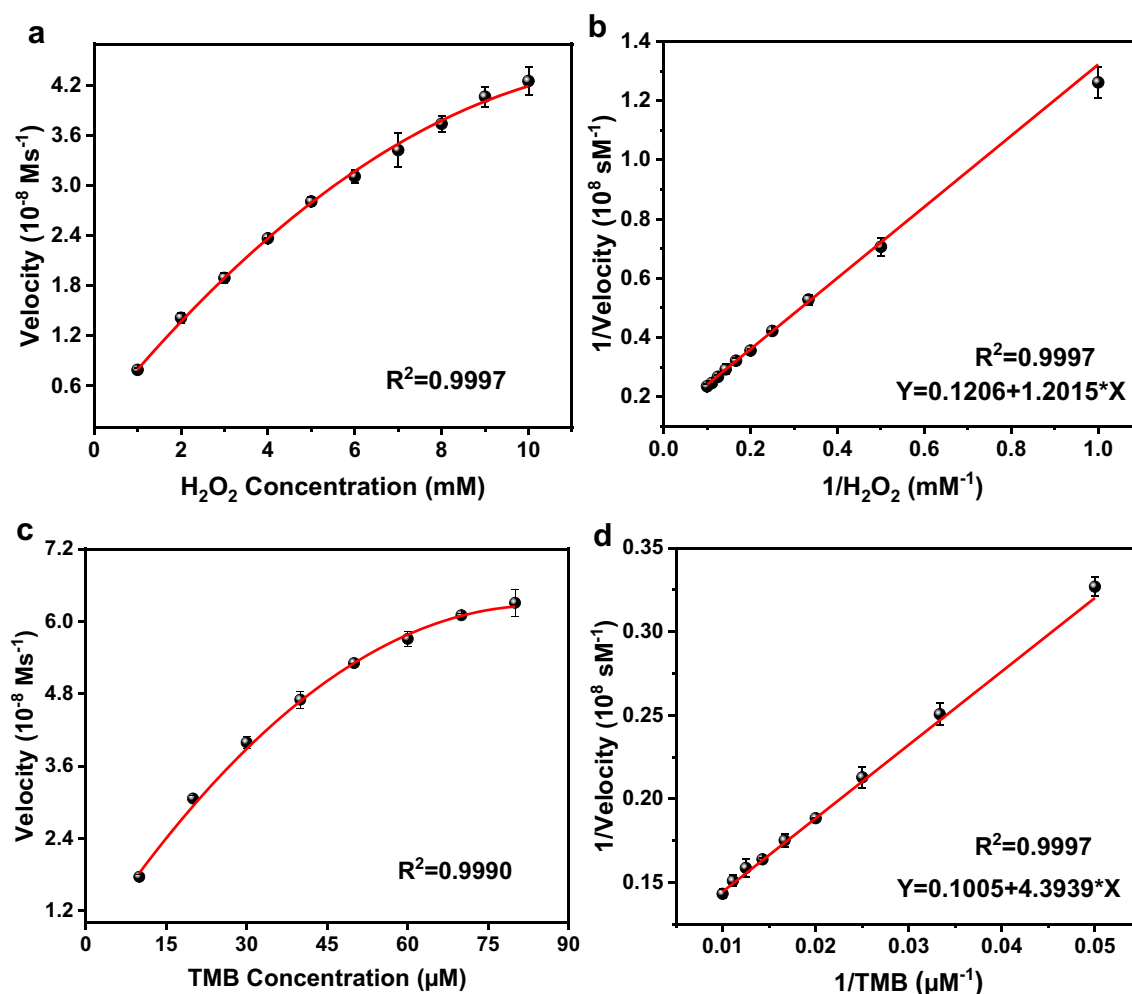


Fig. 4 Steady-state kinetic tests of Por-CuCo₂O₄ using Michaelis-Menten model. **a** The concentration of TMB was 100 μ M and varying the H₂O₂ concentration from 1 to 10 mM. **b** The corresponding double reciprocal curves for H₂O₂. **c** The concentration of H₂O₂ was 35 mM and

varying the TMB concentration from 10 to 100 μ M. **d** The corresponding double reciprocal curves for TMB. Error bars represent the standard deviation for three measurements. The data was collected at 652 nm

As a neurotransmitter, DA as a necessary substance for our body can regulate various physiological functions of the central nervous system. Therefore, a simple method for rapid determination of DA is meaningful. Therefore, a series of experiments were carried out by tuning DA concentration (10–700 μ M) under the optimal conditions (pH = 4 and 45 °C). Because phenol hydroxyl and amino groups are attached in the molecule structure of DA, DA molecules exhibit reducibility. Thus, in our designed sensing system (DA + Por-CuCo₂O₄ + H₂O₂ + TMB), DA molecules can prevent TMB from being oxidized by H₂O₂, resulting in a reduction of the oxTMB absorbance together with fading of blue color visually [27]. The response curve of DA concentration and absorbance difference value ΔA ($\Delta A = A_{\text{blank}} - A_{\text{DA}}$) has a good linear relationship at 10–100 μ M (inset of Fig. 5b). In addition, compared with that of DA measured by different methods (colorimetry, fluorimetry, and electrochemistry) in different catalyst systems (Co₃O₄@NiO, CeO₂, Pt/CoSn(OH)₆,

ZnO@Cys NPs, Pd@AMP-Cu, RGO-ZnO, CRGO-Au NCs, Ag/rGO) listed in Table S2, a lower LOD (0.94 μ M, S/N = 3) of the Por-CuCo₂O₄-based colorimetric sensor was found. As we know, electrochemistry method is known as high sensitivity. Expectably, our designed colorimetric sensing platform for DA was on a par with the electrochemistry method.

Other than high sensitivity, good selectivity is also necessary for colorimetric sensors. From Fig. 5c and d, absorbance difference value ΔA of two systems is the highest and hardly influenced in the presence of common interfering molecules (Suc, Fru, Lac Arg, Ser, His, Na⁺, K⁺, Mg²⁺, UA, ISO, Leu), even though their concentration is 10 times than that of H₂O₂ and DA, respectively. Nevertheless, this method is still a big challenge for distinguishing DA and materials with reducing substances. It is suggested that the colorimetric sensing platform has good selectivity for the determination of H₂O₂ and DA in the absence of reducing substances.

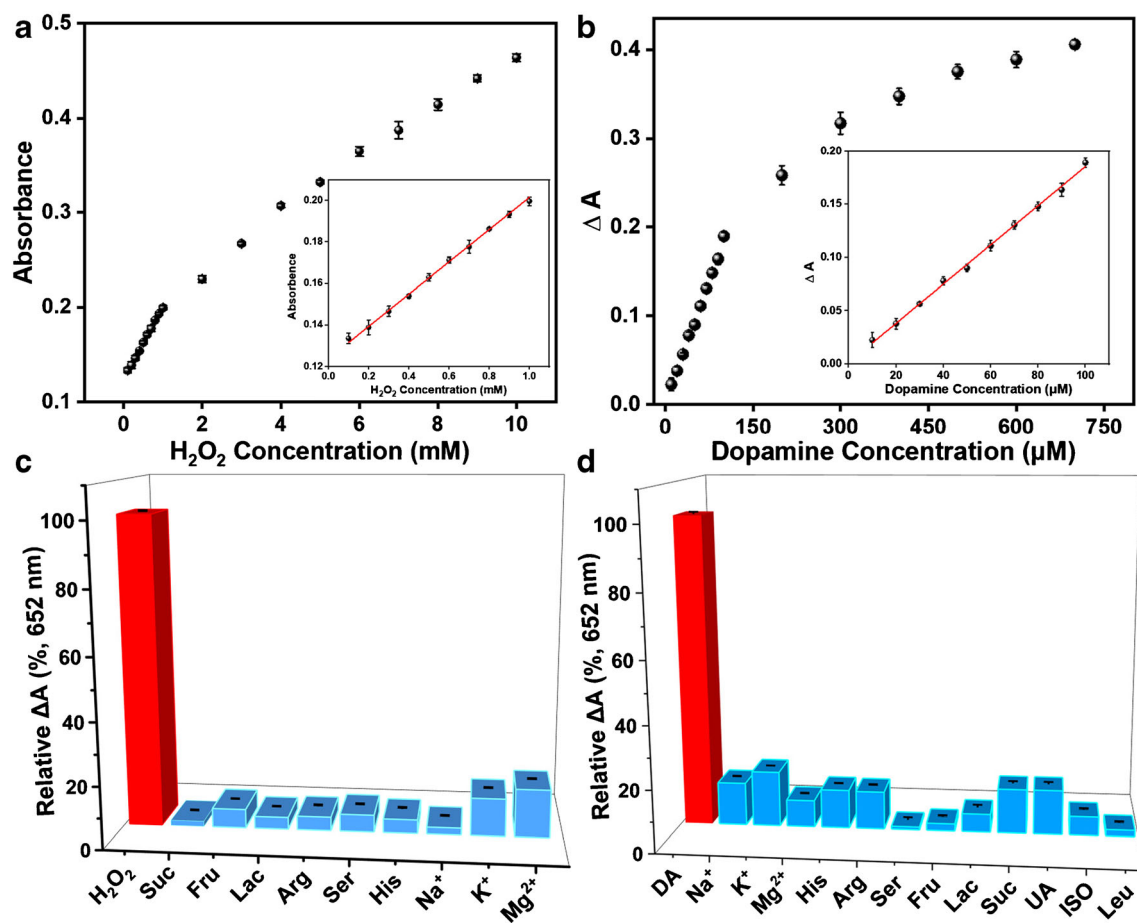


Fig. 5 Dose-response curve for H₂O₂ (a) and or DA (b) determination at 652 nm. **b** ΔA response of H₂O₂-TMB system towards different DA concentrations from 10 to 700 μ M; the inset is the linear fitting curve from 10 to 100 μ M. The variance of net absorbance (ΔA) in the presence of various substances. $\Delta A = A_0 - A$, where A_0 and A are the absorbance of

the Por-CuCo₂O₄ system at 652 nm in the presence and absence of DA. **c** The selectivity of the Por-CuCo₂O₄ towards H₂O₂ (0.1 mM) and interferents (1.0 mM). **d** The selectivity of the Por-CuCo₂O₄ towards DA (1.0 mM) and interferents (10 mM). Error bars denote standard deviations based on three measurements

Proposed mechanism

According to publications, the catalytic mechanism of artificial peroxidases is ascribed to two kinds as follows: one is from direct electron transfer between the substrates and reactants. The other is due to some active species during the catalytic reaction. Therefore, the possible catalytic mechanisms need to be verified one by one. Firstly, an electron transfer process was studied by electrochemical methods (cyclic voltammetry and amperometric methods). In the cyclic voltammetry experiment (Fig. 6a), the Por-CuCo₂O₄ modified electrode (control group) has no obvious current in the absence of H₂O₂. Nevertheless, after adding 1 M H₂O₂, a clear current response with an obvious redox peak at -0.45 eV was obtained, indicating that Por-CuCo₂O₄ has the ability to transfer electrons between the electrode surface (electron donor) and H₂O₂ (electron acceptor) [28]. At -0.45 eV, the amperometric experiment of Por-CuCo₂O₄ catalyzed bare GCE and Por-CuCo₂O₄ modified GCE (Por-CuCo₂O₄/GCE) is shown in Fig. 6b. Adding H₂O₂ every 50 s, the reduction current in

the Por-CuCo₂O₄/GCE electrode increases steadily with a high sensitivity. The data suggest that Por-CuCo₂O₄ peroxidases can speed up electron transfer.

Secondly, to study the possible active species produced in the process of Por-CuCo₂O₄ catalytic reaction, various capture experiments were implemented. Based on the previous results and our constructed colorimetric sensing system, three active species, namely, holes (h^+), superoxide radicals ($\bullet O_2^-$), and hydroxyl radicals ($\bullet OH$), are produced, and the catalytic system may exist during the catalytic reaction. The active species including h^+ , $\bullet O_2^-$, and $\bullet OH$ can be captured by trapping agents PBQ, EDTA, and IPA, respectively. If three active species are produced during the catalytic reaction, the absorbance of the catalytic system will be decreased by addition of the trapping agents. Obviously, as seen from Fig. 6c, $\bullet O_2^-$ and h^+ play an important role while $\bullet OH$ is not detected by the trapping agent method. In order to further determine whether the catalytic oxidation of TMB by Por-CuCo₂O₄ nanospheres is based on the hydroxyl ($\bullet OH$) mechanism, terephthalic acid (TA) was selected as the trapping agent. It is known that the

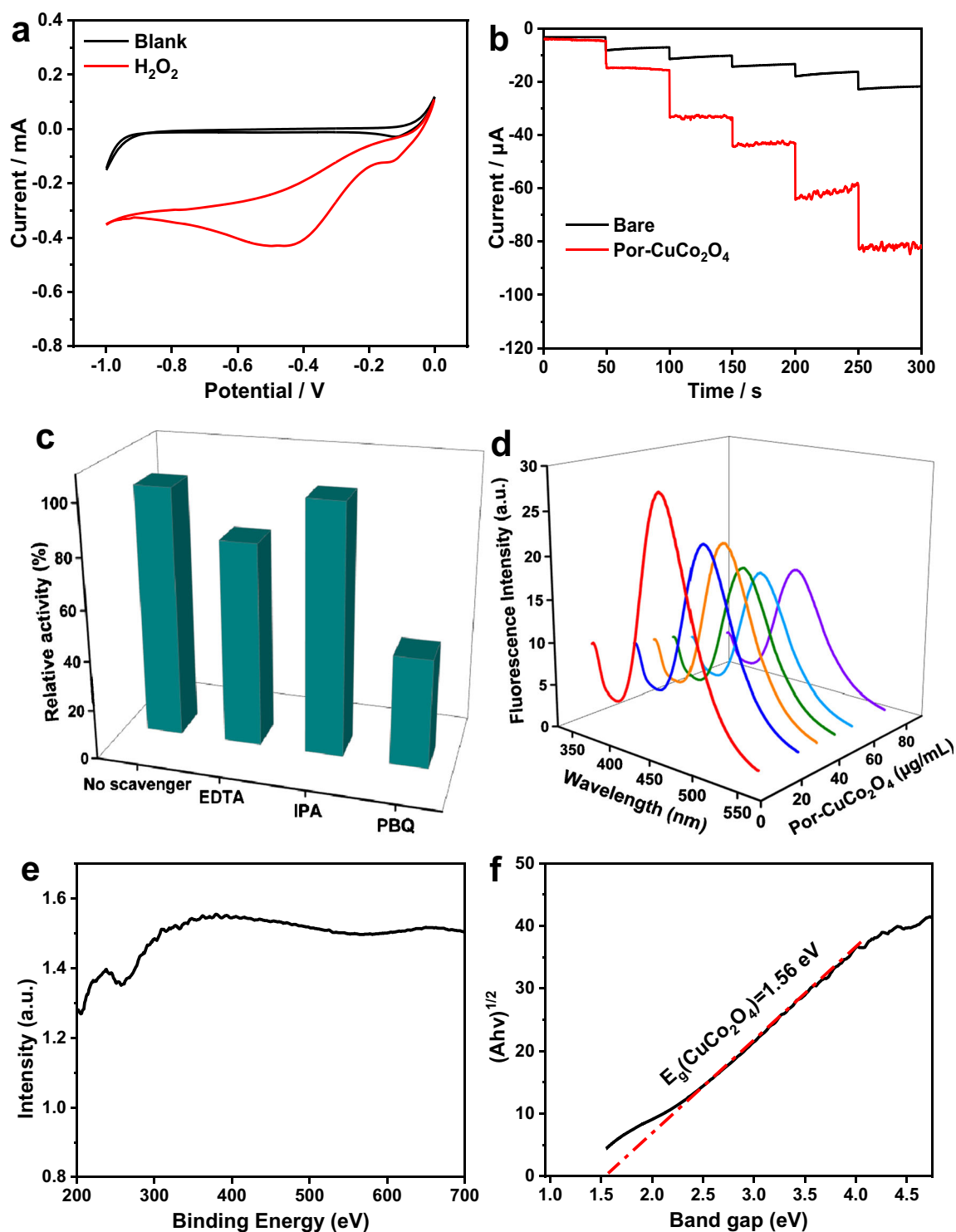
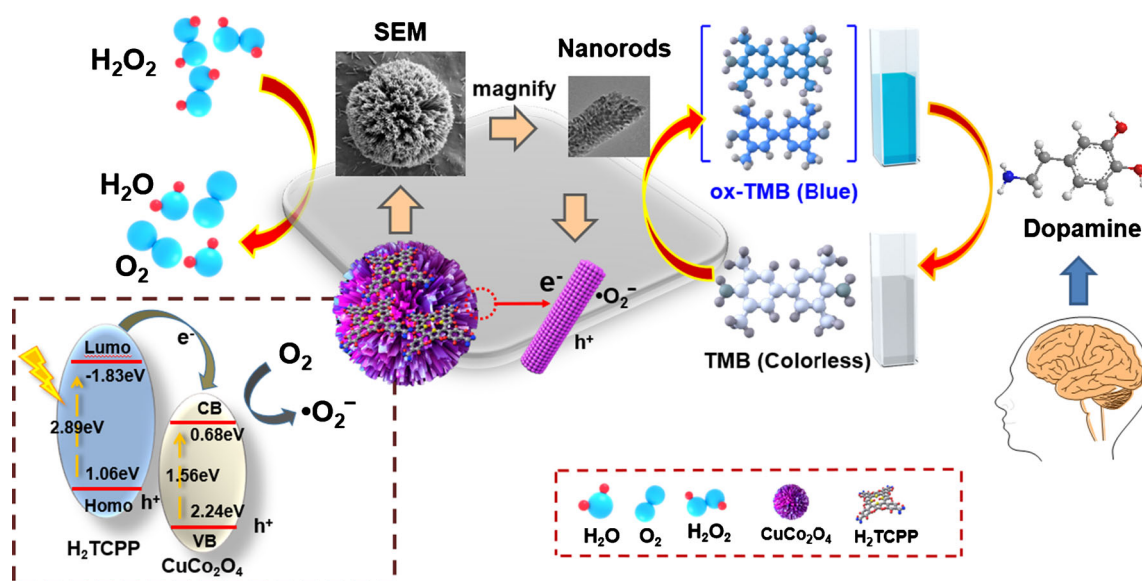


Fig. 6 **a** Cyclic voltammetry of Por-CuCo₂O₄/GCE in 40 mL PBS in the absence (blank) and in the presence (red) of 1 M H₂O₂ (scan rate: 0.05 V/s). **b** The typical steady-state current response of the Por-CuCo₂O₄/GCE to the successive addition of H₂O₂ (1 M) every 50 s into the PBS under stirring at the applied potential of -0.45 V. **c** Effects of various active

scavengers during the catalysis of TMB with the aid of Por-CuCo₂O₄. **d** Fluorescence intensity varies with the concentration of Por-CuCo₂O₄ at different wavelengths. **e** The UV-vis DRS spectra and **f** Tauc plots of CuCo₂O₄

resulting $\cdot\text{OH}$ reacts with TA, a non-fluorescent molecule, to form highly fluorescent hydroxy terephthalate acid (HTA), which shows an emission maximum at 432 nm when excited

at 315 nm [29]. Figure 6d shows the relationship of the fluorescence intensity and Por-CuCo₂O₄ concentration. As seen from the figure, the fluorescence intensity is decreased with



Scheme 2 Schematic illustration of Por-CuCo₂O₄ as a peroxidase mimic for H₂O₂ and dopamine detection

increasing of Por-CuCo₂O₄ concentration (10–80 µg/mL), further suggesting that •OH does not exist during the catalytic reaction. The fluorescence result is consistent with the capture experiment. Therefore, based on the results, the catalytic mechanism of Por-CuCo₂O₄ is not only from the direct electron transfer between reactants but also from two active species (•O₂[−] and h⁺) produced in the course of the catalytic reaction.

According to the UV-vis diffuse reflectance spectra and Tauc plots (Fig. 6e and f), the forbidden bandwidth (E_g) of the CuCo₂O₄ is determined to be 1.56 eV [30]. Through the following Mulliken electronegativity theory formula ($E_0 = 4.5$ eV, X is the geometric mean of the absolute electronegativity of the constituent atoms in CuCo₂O₄), the valence band position (VB) and conduction band (CB) position of CuCo₂O₄ are located at 2.24 and 0.68 eV, respectively [31]. The position of the conduction band valence band of H₂TCPP refers to previous publications [32].

$$E_{VB} = X - E_0 + 0.5E_g$$

$$E_{CB} = E_{VB} - E_g$$

Based on the mentioned information, a catalytic mechanism of Por-CuCo₂O₄ is proposed (Scheme 2). As we know, porphyrin molecules have absorption in the visible region and are usually considered photosensitizers. Thus, the electrons on HOMO can jump into LUMO of the porphyrin under visible light, resulting in the production of h⁺. At the same time, the unstable electrons on LUMO of porphyrin are further rapidly transferred to the CB of CuCo₂O₄. TMB adsorbed on the surface of Por-CuCo₂O₄ due to the accumulation effect provides the lone electron pair in the amino group to Por-CuCo₂O₄, increasing its electron density and mobility [33]. After that, the electron-rich Por-CuCo₂O₄ center can make electrons be transferred to H₂O₂, thereby reducing it to oxygen [34]. Furthermore, the transferred electrons reduced O₂ into •O₂[−] radicals, due to the a lot of oxygen vacancies that existed in Por-CuCo₂O₄. In addition, due to the quantum size effect, the large specific surface area, and small crystal size, Por-CuCo₂O₄ composed of a lot of smaller nanoparticles can provide much more catalytic active sites, thereby binding substrates with smaller steric hindrance and stronger redox

Table 1 Results of dopamine detection in human serum samples

Sample	Detected (µM)	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%) (n=3)
Sample 1	ND	35	33.82±0.36	96.64	0.91
	ND	55	51.94±0.54	94.44	1.03
	ND	75	67.70±1.09	90.27	1.59
Sample 2	ND	20	21.87±0.91	109.34	3.67
	ND	40	35.45±0.90	88.64	2.29
	ND	60	56.29±2.17	93.81	3.43

Note: Serums are obtained from the Affiliated Hospital of Medical College Qingdao University, China

capacity [33]. In addition, based on the reducing property of DA (which can fade blue oxTMB), sensitive and selective detection of DA can come true.

Determination of DA in serum

Before determination, the DA is dissolved into the serum to make a standard solution and then diluted to different concentrations within the linear range. The accuracy of this method can be measured by the recovery and relative standard deviation (RSD) between the determined concentration and the standard concentration [35]. As displayed in Table 1, the recovery rate and RSD are in the range of 88.64–109.34% and 0.91–3.67%, respectively. Therefore, the recoveries are acceptable, and the Por-CuCo₂O₄-based colorimetric sensing platform can be used to determine DA in real samples, which has a good application prospect in biological monitoring.

Conclusions

A novel urchin-like Por-CuCo₂O₄ with enhanced peroxidase-like activity was successfully synthesized by a simple hydrothermal method. Due to the synergistic effect between H₂TCPP molecules and CuCo₂O₄ under visible light, a large amount of active free radical species ($\bullet\text{O}_2^-$ and h^+) was produced and improved to the catalytic activity of Por-CuCo₂O₄. Even in a wide temperature range (35–55 °C), the relative activity of Por-CuCo₂O₄ is still higher than 90%. The TMB + Por-CuCo₂O₄ colorimetric sensing platform has high selectivity and good sensitivity and can accurately determine H₂O₂ and DA, and its detection limits are 90.26 μM and 0.94 μM , respectively. In addition, the determination of DA in serum has achieved satisfactory results. We believe that this work will help the design of other artificial mimic with enzyme activity and be used for real-time monitoring of certain key biomolecules related to H₂O₂ in the fields of medicine and food.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00604-021-04819-9>.

Funding This work was financially supported by SNFC (21971152, 21805168, and 51904175) and the Natural Science Foundation of Shandong Province (ZR2018MB002, ZR2018MEE003, ZR2018BB046, and ZR2018PEE006).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

- Rostami S, Mehdinia A, Niroumand R, Jabbari A (2020) Enhanced LSPR performance of graphene nanoribbons-silver nanoparticles hybrid as a colorimetric sensor for sequential detection of dopamine and glutathione. *Anal Chim Acta* 1120:11–23. <https://doi.org/10.1016/j.aca.2020.04.060>
- Wang Y, Yang L, Liu Y, Zhao Q, Ding F, Zou P, Rao H, Wang X (2018) Colorimetric determination of dopamine by exploiting the enhanced oxidase mimicking activity of hierarchical NiCo₂S₄-rGO composites. *Mikrochim Acta* 185(10):496. <https://doi.org/10.1007/s00604-018-3035-8>
- Liu H, Ding YN, Bian B, Li L, Li R, Zhang X, Liu Z, Zhang X, Fan G, Liu Q (2019) Rapid colorimetric determination of dopamine based on the inhibition of the peroxidase mimicking activity of platinum loaded CoSn(OH)₆ nanocubes. *Mikrochim Acta* 186(12):755. <https://doi.org/10.1007/s00604-019-3940-5>
- Belujon P, Grace AA (2017) Dopamine system dysregulation in major depressive disorders. *Int J Neuropsychopharmacol* 20(12):1036–1046. <https://doi.org/10.1093/ijnp/pyx056>
- Qing X, Wang Y, Zhang Y, Ding X, Zhong W, Wang D, Wang W, Liu Q, Liu K, Li M, Lu Z (2019) Wearable fiber-based organic electrochemical transistors as a platform for highly sensitive dopamine monitoring. *ACS Appl Mater Interfaces* 11(14):13105–13113. <https://doi.org/10.1021/acsami.9b00115>
- Wen D, Liu W, Herrmann AK, Haubold D, Holzschuh M, Simon F, Eychmüller A (2016) Simple and sensitive colorimetric detection of dopamine based on assembly of cyclodextrin-modified Au nanoparticles. *Small* 12(18):2439–2442. <https://doi.org/10.1002/sml.201503874>
- Zhao J, Zhao L, Lan C, Zhao S (2016) Graphene quantum dots as effective probes for label-free fluorescence detection of dopamine. *Sensors Actuators B Chem* 223:246–251. <https://doi.org/10.1016/j.snb.2015.09.105>
- Ding Y, Yang B, Liu H, Liu Z, Zhang X, Zheng X, Liu Q (2018) FePt-Au ternary metallic nanoparticles with the enhanced peroxidase-like activity for ultrafast colorimetric detection of H₂O₂. *Sensors Actuators B Chem* 259:775–783. <https://doi.org/10.1016/j.snb.2017.12.115>
- Zhang L, Zhong H, Zhang H, Ding C (2021) A multifunctional nano system based on DNA and CeO₂ for intracellular imaging of miRNA and enhancing photodynamic therapy. *Talanta* 221:121554. <https://doi.org/10.1016/j.talanta.2020.121554>
- Jiao Y, Li J, Xiang J, Chen Z (2020) Tungsten disulfide nanosheets-based colorimetric assay for glucose sensing. *Spectrochim Acta A Mol Biomol Spectrosc* 242:118706. <https://doi.org/10.1016/j.saa.2020.118706>
- Zeng G, Duan M, Xu Y, Ge F, Wang W (2020) Platinum (II)-doped graphitic carbon nitride with enhanced peroxidase-like activity for detection of glucose and H₂O₂. *Spectrochim Acta A Mol Biomol Spectrosc* 241:118649. <https://doi.org/10.1016/j.saa.2020.118649>
- Guo Y, Deng L, Li J, Guo S, Wang E, Dong S (2011) Hemin/graphene hybrid nanosheets with intrinsic peroxidase-like activity for label-free colorimetric detection of single-nucleotide polymorphism. *ACS Nano* 5(2):1282–1290
- Yella A, Lee HW, Tsao HN, Yi C, Chandiran AK, Nazeeruddin MK, Diau EWG, Yeh CY, Zakeeruddin SM, Grätzel M (2011) Porphyrin-sensitized solar cells with cobalt (II/III)-based redox electrolyte exceed 12 percent efficiency. *Science* 334(6056):629–634. <https://doi.org/10.1126/science.1209688>
- Alemayehu AB, Day NU, Mani T, Rudine AB, Thomas KE, Gederaas OA, Vinogradov SA, Wamser CC, Ghosh A (2016) Gold Tris (carboxyphenyl) corroles as multifunctional materials: room temperature near-IR phosphorescence and applications to photodynamic therapy and dye-sensitized solar cells. *ACS Appl*

- Mater Interfaces 8(29):18935–18942. <https://doi.org/10.1021/acsami.6b04269>
15. Azcarate I, Costentin C, Robert M, Saveant JM (2016) Through-space charge interaction substituent effects in molecular catalysis leading to the design of the most efficient catalyst of CO₂-to-CO electrochemical conversion. *J Am Chem Soc* 138(51):16639–16644. <https://doi.org/10.1021/jacs.6b07014>
 16. Zhu X, Li H, Zhang D, Chen W, Fu M, Nie S, Gao Y, Liu Q (2019) Novel “on–off” colorimetric sensor for glutathione based on peroxidase activity of Montmorillonite-loaded TiO₂ functionalized by porphyrin precisely controlled by visible light. *ACS Sustain Chem Eng* 7(21):18105–18113. <https://doi.org/10.1021/acssuschemeng.9b05146>
 17. He Y, Li N, Li W, Zhang X, Zhang X, Liu Z, Liu Q (2021) 5,10,15,20-tetrakis(4-carboxylphenyl) porphyrin functionalized NiCo₂S₄ yolk-shell nanospheres: excellent peroxidase-like activity, catalytic mechanism and fast cascade colorimetric biosensor for cholesterol. *Sensors Actuators B Chem* 326. <https://doi.org/10.1016/j.snb.2020.128850>
 18. Song Y, Zhao M, Li H, Wang X, Cheng Y, Ding L, Fan S, Chen S (2018) Facile preparation of urchin-like NiCo₂O₄ microspheres as oxidase mimetic for colorimetric assay of hydroquinone. *Sensors Actuators B Chem* 255:1927–1936. <https://doi.org/10.1016/j.snb.2017.08.204>
 19. Chen C, Liu L, Li Y, Li W, Zhou L, Lan Y, Li Y (2020) Insight into heterogeneous catalytic degradation of sulfamethazine by peroxy-monosulfate activated with CuCo₂O₄ derived from bimetallic oxalate. *Chem Eng J* 384. <https://doi.org/10.1016/j.cej.2019.123257>
 20. Qi Y, Mei Y, Li J, Yao T, Yang Y, Jia W, Tong X, Wu J, Xin B (2019) Highly efficient microwave-assisted Fenton degradation of metacycline using pine-needle-like CuCo₂O₄ nanocatalyst. *Chem Eng J* 373:1158–1167. <https://doi.org/10.1016/j.cej.2019.05.097>
 21. Goudarzi M, Salavati-Niasari M, Yazdian F, Amiri M (2019) Sonochemical assisted thermal decomposition method for green synthesis of CuCo₂O₄/CuO ceramic nanocomposite using *Dactylopius Coccus* for anti-tumor investigations. *J Alloys Compd* 788:944–953. <https://doi.org/10.1016/j.jallcom.2019.02.288>
 22. Nakhowong R, Chueachot R (2017) Synthesis and magnetic properties of copper cobaltite (CuCo₂O₄) fibers by electrospinning. *J Alloys Compd* 715:390–396. <https://doi.org/10.1016/j.jallcom.2017.04.323>
 23. Zhen S-y, Wu H-t, Wang Y, Li N, Chen H-s, Song W-l, Wang Z-h, Sun W, Sun K-n (2019) Metal–organic framework derived hollow porous CuO–CuCo₂O₄ dodecahedrons as a cathode catalyst for Li–O₂ batteries. *RSC Adv* 9(29):16288–16295. <https://doi.org/10.1039/c9ra02860a>
 24. Han X, Sheng H, Yu C, Walker TW, Huber GW, Qiu J, Jin S (2020) Electrocatalytic oxidation of glycerol to formic acid by CuCo₂O₄ spinel oxide nanostructure catalysts. *ACS Catal* 10(12):6741–6752. <https://doi.org/10.1021/acscatal.0c01498>
 25. Alali KT, Lu Z, Zhang H, Liu J, Liu Q, Li R, Aljebawi K, Wang J (2017) P–p heterojunction CuO/CuCo₂O₄ nanotubes synthesized via electrospinning technology for detecting n-propanol gas at room temperature. *Inorg Chem Front* 4(7):1219–1230. <https://doi.org/10.1039/c7qi00192d>
 26. Ma D, Yu J, Yin W, Zhang X, Mei L, Zu Y, An L, Gu Z (2018) Synthesis of surface-modification-oriented nanosized molybdenum disulfide with high peroxidase-like catalytic activity for H₂O₂ and cholesterol detection. *Chemistry* 24(59):15868–15878. <https://doi.org/10.1002/chem.201803040>
 27. Zhu Y, Yang Z, Chi M, Li M, Wang C, Lu X (2018) Synthesis of hierarchical Co₃O₄@NiO core-shell nanotubes with a synergistic catalytic activity for peroxidase mimicking and colorimetric detection of dopamine. *Talanta* 181:431–439. <https://doi.org/10.1016/j.talanta.2018.01.019>
 28. Mu J, Wang Y, Zhao M, Zhang L (2012) Intrinsic peroxidase-like activity and catalase-like activity of Co₃O₄ nanoparticles. *Chem Commun (Camb)* 48(19):2540–2542. <https://doi.org/10.1039/c2cc17013b>
 29. Hu AL, Liu YH, Deng HH, Hong GL, Liu AL, Lin XH, Xia XH, Chen W (2014) Fluorescent hydrogen peroxide sensor based on cupric oxide nanoparticles and its application for glucose and L-lactate detection. *Biosens Bioelectron* 61:374–378. <https://doi.org/10.1016/j.bios.2014.05.048>
 30. Xue W, Chang W, Hu X, Fan J, Bai X, Liu E (2020) Highly dispersed copper cobalt oxide nanoclusters decorated carbon nitride with efficient heterogeneous interfaces for enhanced H₂ evolution. *J Colloid Interface Sci* 576:203–216. <https://doi.org/10.1016/j.jcis.2020.04.111>
 31. Xu C, Jin C, Chang W, Hu X, Deng H, Liu E, Fan J (2019) Interfacially bonded CuCo₂O₄/TiO₂ nanosheet heterostructures for boosting photocatalytic H₂ production. *Catal Sci Technol* 9(18):4990–5000. <https://doi.org/10.1039/c9cy01209e>
 32. Yang H, Yin D, Gao L, Zhang X, Zhang X, Liu Q (2020) 5,10,15,20-tetrakis(4-carboxylphenyl) porphyrin modified nickel-cobalt layer double hydroxide nanosheets as enhanced photoelectrocatalysts for methanol oxidation under visible-light. *J Colloid Interface Sci* 561:881–889. <https://doi.org/10.1016/j.jcis.2019.11.071>
 33. Aneesh K, Rao vusa CS, Berchmans S (2017) Enhanced peroxidase-like activity of CuWO₄ nanoparticles for the detection of NADH and hydrogen peroxide. *Sensors Actuators B Chem* 253:723–730. <https://doi.org/10.1016/j.snb.2017.06.175>
 34. Song Y, Qu K, Zhao C, Ren J, Qu X (2010) Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. *Adv Mater* 22(19):2206–2210. <https://doi.org/10.1002/adma.200903783>
 35. Rostami S, Mehdiinia A, Jabbari A (2020) Intrinsic peroxidase-like activity of graphene nanoribbons for label-free colorimetric detection of dopamine. *Mater Sci Eng C Mater Biol Appl* 114:111034. <https://doi.org/10.1016/j.msec.2020.111034>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.