



# A facile homogeneous electrochemical biosensing strategy based on displacement reaction for intracellular and extracellular hydrogen peroxide detection



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## ABSTRACT

Traditional electrochemical hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assays all indispensably require the immobilization of enzyme or nanomaterials on electrode surface. These complex electrode surface modifications are laborious and time-consuming. To overcome such limitations, we developed here a facile homogeneous electrochemical biosensor based on displacement reaction for detection of intracellular and extracellular H<sub>2</sub>O<sub>2</sub> produced by living cells. Methylene blue-labeled short single-stranded DNA strand (DNA-MB) was ingeniously designed as the reporter, which was adsorbed on CeO<sub>2</sub> nanoparticles to result in low electrochemical signal. In the presence of H<sub>2</sub>O<sub>2</sub>, DNA-MB was released from CeO<sub>2</sub> nanoparticles, leading to the significant enhancement of current signal. Therefore, a simple homogeneous electrochemical H<sub>2</sub>O<sub>2</sub> assay is successfully achieved. This strategy also displays excellent anti-interference capability and reproducibility for H<sub>2</sub>O<sub>2</sub> determination. More importantly, this method was capable of conveniently realizing intracellular and extracellular H<sub>2</sub>O<sub>2</sub> assay. Thus, with the excellent merits of simplicity, rapidness, good repeatability, high specific and sensitivity, the proposed strategy has great potential to be applied in exploring the roles of H<sub>2</sub>O<sub>2</sub> in physiological and pathological processes.

## 1. Introduction

Reactive oxygen species (ROS) are important biochemical mediators in cellular physiology and pathology (Finkel, 2005; Li et al., 2016a). In particular, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is an important representative of ROS molecules in living organisms (Rhee, 2006; Weinstein et al., 2014). Emerging evidences have demonstrated that maintaining H<sub>2</sub>O<sub>2</sub> concentration at normal level was critical in biological events such as cell growth and signal transduction, while disruption of its physiologic homeostasis would result in many serious diseases (Lundgren et al., 2018; Lippert et al., 2011). Meanwhile, the long lifetime of H<sub>2</sub>O<sub>2</sub> makes it be able to diffuse into cellular compartments as well as extracellular environment. Once diffused out of the cell membrane, extracellular H<sub>2</sub>O<sub>2</sub> impacts cell migration, cellular communications and so on. The intracellular concentration of H<sub>2</sub>O<sub>2</sub> was at the level of 0.05–0.7 μM and the extracellular H<sub>2</sub>O<sub>2</sub> concentration was about 10-fold higher than the intracellular level under normal conditions, while the local intracellular and extracellular concentrations of H<sub>2</sub>O<sub>2</sub> will be increased under pathological conditions (Antunes and Cadenas, 2001; Boveris et al.,

2002). Therefore, it is of great significance to develop a simple, sensitive and selective method to measure both intracellular and extracellular H<sub>2</sub>O<sub>2</sub> concentrations, which will be favorable to explore the roles of H<sub>2</sub>O<sub>2</sub> in occurrence and evolution of diseases.

Until now, many techniques have been explored for the detection of H<sub>2</sub>O<sub>2</sub>, including fluorescence, chemiluminescence, and electrochemical methods, and so on (Chen et al., 2014; Quintino et al., 2005; Xu et al., 2013; Lebiga et al., 2015; Yoon et al., 2017). Among these methods, the electrochemical H<sub>2</sub>O<sub>2</sub> assays have attracted much attention due to the outstanding advantages such as simple instrumentation, easy miniaturization, and convenient operation procedures (Liu et al., 2013; Shi et al., 2014; Li et al., 2014). Over the past decade, a variety of the enzyme-based methods have been developed for H<sub>2</sub>O<sub>2</sub> determination (Liu et al., 2017; Chen et al., 2016; Kafi et al., 2008; Kuposova et al., 2015). Nevertheless, the intrinsic shortcomings associated with enzyme-based detection methods in terms of high cost, environmental instability and denaturation inevitably limit their practical applications. Therefore, to overcome these limitations, non-enzymatic electrochemical H<sub>2</sub>O<sub>2</sub> sensors have drawn particular attention. Since direct

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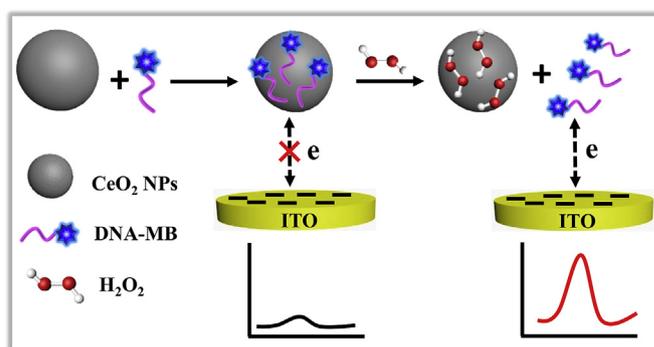
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detection of  $\text{H}_2\text{O}_2$  at solid electrodes usually requires a relatively high overpotential, great efforts have been made to utilize nanomaterials as effective electrocatalysts for developing nonenzymatic electrochemical  $\text{H}_2\text{O}_2$  sensors (Wang et al., 2013, 2015; Li et al., 2015b, 2016b; Sun et al., 2016, 2017; Bai et al., 2016; Wu et al., 2012; Ju and Chen, 2015; Dou et al., 2018). These methods have witnessed promising progresses, but some drawbacks still exist, including expensive raw materials, harsh reaction conditions and complex nanomaterials preparation processes. More importantly, these reported traditional electrochemical sensing methods for  $\text{H}_2\text{O}_2$  assay all indispensably required the immobilization of enzyme or nanomaterials on electrode surface, which were laborious and time consuming. Meanwhile, these tedious electrode modification processes also impaired the reproducibility of the biosensors. As a consequence, it is greatly desirable to develop facile and easier-to-use immobilization-free electrochemical approaches for  $\text{H}_2\text{O}_2$  detection.

More recently, homogeneous electrochemical biosensing strategies have been intelligently explored, which open a new way for the development of immobilization-free electrochemical sensors (Luo et al., 2008). In a typical homogeneous electrochemistry biosensor, the analyte recognition is performed in solution phase instead of on the electrode surface and the quantitative determination of analyte is performed by detecting the changes of current of electroactive substance labeled DNA to the electrode surface. Therefore, compared with conventional immobilization-based electrochemical approaches, homogeneous electroanalytical technique possesses noticeable advantages, such as avoiding the tedious and time-consuming modification processes, which effectively improves the reliability and reproducibility of homogeneous electrochemical biosensors. In virtue of these remarkable advantages, numerous homogeneous electrochemical systems have been proposed for successful detection of all sorts of targets, such as metal ions, DNA, microRNA, small biological molecules, and so on (Xuan et al., 2013, 2015; Miranda-Castro et al., 2012; Zhuang et al., 2014; Hou et al., 2015; Liu et al., 2015b; Zhang et al., 2015; Tan et al., 2015; Ge et al., 2016; Li et al., 2015a). For instance, Hsing and co-workers achieved sensitive detection of DNA and mercury ion by utilizing homogeneous electrochemical strategies (Luo et al., 2008; Xuan et al., 2015; Miranda-Castro et al., 2012). Our group also reported highly sensitive homogeneous electrochemical biosensing methods for analysis of carcinoembryonic antigen, transcription factor, microRNA and human telomerase (Hou et al., 2015; Liu et al., 2015b; Zhang et al., 2015; Ge et al., 2016; Li et al., 2015a).

Herein, inspired by the aforementioned works, we propose a simple and reliable immobilization-free electrochemical biosensor for “signal-on” detection of intracellular and extracellular  $\text{H}_2\text{O}_2$  based on the displacement reaction. It has been reported that the  $\text{CeO}_2$  nanoparticles ( $\text{CeO}_2$  NPs) displayed different coordination affinities toward single-stranded DNA (ssDNA) and  $\text{H}_2\text{O}_2$  (Gao et al., 2016; Liu et al., 2015a). The ssDNA could easily adsorb on the surface of  $\text{CeO}_2$  NPs and thus forming the  $\text{CeO}_2$ -DNA nanocomposite. Because  $\text{H}_2\text{O}_2$  possesses much stronger coordination effect toward  $\text{CeO}_2$  NPs than ssDNA, the adsorbed ssDNA thus would be released from the nanocomposite in the presence of  $\text{H}_2\text{O}_2$ . In this strategy, by utilizing the diffusivity difference of ssDNA and  $\text{CeO}_2$ -DNA complex toward the negatively charged indium tin oxide (ITO) electrode, a simple immobilization-free electrochemical method for sensing of  $\text{H}_2\text{O}_2$  was readily developed. As shown in Scheme 1, a methylene blue-labeled short ssDNA strand with 5 bases (denoted as DNA-MB) was reasonably designed and used as the reporter. After adding  $\text{CeO}_2$  NPs into DNA-MB solution,  $\text{CeO}_2$ -DNA-MB nanocomposite was formed, which hardly be in close proximity to the negatively charged ITO electrode surface because of its low diffusivity, thus a small current signal was observed. In the presence of  $\text{H}_2\text{O}_2$ , it readily displaced the adsorbed DNA-MB from  $\text{CeO}_2$  NPs, thus a large amount of free DNA-MB molecules were released in the solution. Due to its small size and less negative charges, free DNA-MB was able to easily access the surface of the electrode, producing much enhanced



**Scheme 1.** Principle of the immobilization-free electrochemical strategy for  $\text{H}_2\text{O}_2$  assay.

electrochemical signal. Therefore, the as-proposed homogeneous electrochemical method, with excellent merits of simplicity, rapidness and high sensitivity, was successfully applied in detecting intracellular and extracellular  $\text{H}_2\text{O}_2$  generated by living cells. As far as we know, it is first reported homogeneous electrochemical biosensor for the analysis of  $\text{H}_2\text{O}_2$  until now.

## 2. Experimental section

### 2.1. Reagents and materials

Cerium (III) nitrate ( $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ , 99.99%) was purchased from Shandong Xiya Chemical Industry Co. Ltd. Dopamine, ethanol, tris (hydroxymethyl)aminomethane (Tris), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydrochloric acid (HCl), glucose, sucrose, lactose, sodium chloride (NaCl) and magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ascorbic acid, uric acid, histidine, glutamate, lysine, serine, glutathione, cysteine, HRP and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich (Shanghai, China). The human breast cancer cell line MCF-7 was purchased from Procell Life Science & Technology Co., Ltd (Wuhan, China). All reagents are of analytical grade and used without further purification. All aqueous solutions were prepared using ultrapure water obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). Methylene blue-modified oligonucleotide was synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), with the following sequence: 5'-methylene blue-AAAAA-3'.

### 2.2. Apparatus and instrumentation

Differential pulse voltammetric (DPV) measurements were measured on an Autolab electrochemical workstation (Metrohm, Netherlands) using a conventional three-electrode cell: an ITO working electrode, an Ag/AgCl reference electrode and a platinum wire counter electrode. Transmission electron microscopy (TEM) was carried out on a HT7700 microscope (Hitachi, Japan) operated at 100 kV. Absorption spectra were recorded on a pharماسpec UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). X-ray diffraction (XRD) analysis was performed with D8 ADVANCE (Bruker AXS) X-ray diffractometer. Zeta potential was measured on dynamic light scattering on the Zetasizer Nano ZEN3690 (Malvern Instruments Ltd., Malvern, UK). The scanning electron microscopy (SEM) and elemental analysis were performed on S-4800 (Hitachi, Japan) with an accelerating voltage of 15 kV.

### 2.3. ITO electrode pretreatment

Before electrochemical detection, the ITO electrode was sequentially sonicated in ethanol and ultrapure water for 30 min. Then, it was

soaked in 1 mM NaOH solution for 5 h, followed by sonication in ultrapure water for 10 min. Finally, a negatively charged working electrode surface was achieved.

#### 2.4. Synthesis of CeO<sub>2</sub> NPs

CeO<sub>2</sub> NPs were synthesized using a reported method with some modifications (Mai et al., 2005). Briefly, 5 mL of Ce(NO<sub>3</sub>)<sub>3</sub> solution (57 mM) was added dropwise into 35 mL of NaOH solution (80 mM) under vigorous stirring. After 30 min, the obtained white slurry mixture solution was added into an autoclave and kept at 180 °C for 24 h. After that, the as-prepared nanoparticles were washed by ultrapure water and ethanol for several times, respectively, and dried at 60 °C overnight to afford CeO<sub>2</sub> powders.

#### 2.5. Homogeneous electrochemical H<sub>2</sub>O<sub>2</sub> assay

The H<sub>2</sub>O<sub>2</sub> assay was performed in 50 µL of reaction buffer (10 mM Tris-HCl, 50 mM NaCl, pH 7.4) containing 1 µM DNA-MB, CeO<sub>2</sub> NPs (100 µg/mL), and H<sub>2</sub>O<sub>2</sub> with various concentrations. The experiment without adding H<sub>2</sub>O<sub>2</sub> was used as the control group. To investigate the selectivity of the as-proposed biosensor toward H<sub>2</sub>O<sub>2</sub>, glucose, sucrose, lactose, ascorbic acid, uric acid, histidine, glutamate, lysine, serine, glutathione and cysteine were examined. The measuring process was the same as described above but using these substances instead of H<sub>2</sub>O<sub>2</sub>.

#### 2.6. Cell culture

The human breast cancer cell line MCF-7 was cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 °C in a humidified environment containing 5% CO<sub>2</sub>.

#### 2.7. Detection of intracellular and extracellular H<sub>2</sub>O<sub>2</sub>

To detect intracellular H<sub>2</sub>O<sub>2</sub>, the PMA stimulated cell extracts was prepared. The non-treated MCF-7 cells were divided into three groups, each with the concentration of  $5.0 \times 10^6$  cells mL<sup>-1</sup> counted by a hemocytometer. The first group was not treated with PMA and used as the control group. The other two groups were treated with PMA (1 µg/mL) for 0.5 h. Subsequently, all the three groups of cells were suspended in 1 mL buffer solution (10 mM Tris-HCl, 50 mM NaCl, pH 7.4), respectively, and disrupted for 10 min using ultrasonic cell disruptor. Next, one group of PMA stimulated cell extract was incubated with HRP (300 U/mL) for 0.5 h. Then, the broken cell suspension of all three groups were centrifuged and the supernatant was collected. Finally, electrochemical measurements were carried out on the supernatant after diluted 10 times.

To further detect extracellular H<sub>2</sub>O<sub>2</sub> released from living cells, the MCF-7 cells were plated on Petri dish for 24.0 h. After the cells ( $5 \times 10^6$ ) were washed three times with PBS, PMA with different concentrations in buffer solution (0.1, 0.5 and 1.0 µg/mL) were added to stimulate the cells for 0.5 h. The cells with no PMA stimulation were used as the control. Subsequently, the culture solution of all PMA treated groups were collected. Finally, electrochemical measurements were carried out on the culture solution after diluted 10 times.

### 3. Results and discussion

#### 3.1. Characterization of CeO<sub>2</sub>-DNA nanocomposite

We synthesized the CeO<sub>2</sub> NPs by a facile previously reported hydrothermal method (Mai et al., 2005). TEM and SEM images showed that the diameter of the as-prepared CeO<sub>2</sub> NPs was about  $20.0 \pm 2.6$  nm (Fig. 1a and b). The maximum absorption of the CeO<sub>2</sub> NPs was observed at approximately 290 nm from the UV-vis absorption spectra (Fig. S1). Moreover, XRD spectrum (Fig. S2) indicated that

the CeO<sub>2</sub> NPs have typical fluorite cubic structure. Then, the CeO<sub>2</sub>-DNA nanocomposite was easily prepared through directly mixing the CeO<sub>2</sub> NPs and ssDNA solution, because ssDNA could adsorb on the surface of the CeO<sub>2</sub> NPs by the coordination effect. As shown in Fig. 1c, the Ce, O, N and P elements were obviously observed for CeO<sub>2</sub>-DNA nanocomposite. Zeta-potential tests were also performed to investigate whether the ssDNA has been assembled on the surface of CeO<sub>2</sub> NPs. As illustrated in Fig. 1d, the zeta potential value of the CeO<sub>2</sub> NPs was  $+1.3 \pm 0.2$  and shifted to  $-12.5 \pm 1.7$  after adsorption of ssDNA. All these results demonstrated that the ssDNA has been successfully assembled on the surface of CeO<sub>2</sub> NPs.

#### 3.2. Feasibility investigation of the H<sub>2</sub>O<sub>2</sub> assay

Before performing the H<sub>2</sub>O<sub>2</sub> assay, the feasibility of the proposed strategy was evaluated by measuring DPV signals. As shown in Fig. 2A, when the system contained only DNA-MB and CeO<sub>2</sub> NPs, a small peak current of about 18 nA was observed (red line) due to the weak diffusivity of CeO<sub>2</sub>-DNA-MB nanocomposite towards the electrode. With the addition of H<sub>2</sub>O<sub>2</sub> into the system, the DPV signal exhibited a significant increase (black line). This is because H<sub>2</sub>O<sub>2</sub> can displace the adsorbed DNA-MB from CeO<sub>2</sub>-DNA-MB nanocomposite, thus generating multiple free DNA-MB reporters. Due to its small size and less negative charges, DNA-MB shows much larger diffusivity than that of CeO<sub>2</sub>-DNA-MB nanocomposite towards the negatively charged ITO electrode surface. Therefore, the generated DNA-MB was able to reach the electrode surface with much ease, resulting in big electrochemical signal. Moreover, the redox peak currents of MB also showed obvious increase in the presence of H<sub>2</sub>O<sub>2</sub> (Fig. S3). Taken together, these proof-of-concept tests shows the feasibility of using DNA-functionalized CeO<sub>2</sub> NPs for rapid and facile detection of H<sub>2</sub>O<sub>2</sub>.

#### 3.3. Optimization of experimental conditions

In order to get the best performance, some experimental parameters were carefully optimized. The initial amount of CeO<sub>2</sub> NPs is crucial to the analytical performance of the biosensor. If CeO<sub>2</sub> NPs are not enough, a high background signal will result, and excessive amount of CeO<sub>2</sub> NPs will affect the detection sensitivity. Thus, the impact of the CeO<sub>2</sub> NPs concentration on the current of DNA-MB was first evaluated. As shown in Fig. 2B, the peak currents of DNA-MB gradually reduced with increased amount of CeO<sub>2</sub> NPs. When the amount of CeO<sub>2</sub> NPs increased to 100.0 µg/mL, the DPV signal levelled off (Fig. 2C). This could be the result of the low diffusivity of CeO<sub>2</sub>-DNA-MB nanocomposite, making it hardly be in close proximity to the negatively charged ITO electrode surface. Therefore, the peak current of DNA-MB gradually reduced with the increase of CeO<sub>2</sub> concentration. Moreover, the peak current of the MB molecules almost not changed by directly mixing the MB molecules and CeO<sub>2</sub> NPs (Fig. S4), demonstrating that the reduced current of DNA-MB definitely contributed to its adsorption on CeO<sub>2</sub> NPs surface. Therefore, 100.0 µg/mL was used as the optimal concentration of CeO<sub>2</sub> NPs in the subsequent experiments. Next, a time-course experiment was carried out to study the speed of the reaction between CeO<sub>2</sub> NPs and DNA-MB. The concentrations of DNA-MB and CeO<sub>2</sub> NPs were set as 1.0 µM and 100.0 µg/mL, respectively. It should be noted that the DPV signal sharply decreased after CeO<sub>2</sub> NPs mixed with DNA-MB within 2 min (Fig. S5), and then reached a plateau, indicating a fast adsorption of DNA-MB on the surface of CeO<sub>2</sub> NPs. Moreover, the reaction time was also investigated to guarantee the complete reaction between H<sub>2</sub>O<sub>2</sub> and CeO<sub>2</sub>-DNA-MB nanocomposite. Fig. 2D showed that the DPV peak current change  $\Delta I_p$  ( $\Delta I_p = I_p - I_{p,0}$ , in which  $I_p$  and  $I_{p,0}$  are the peak currents in the presence and absence of H<sub>2</sub>O<sub>2</sub>, respectively) enhanced progressively with the increase of reaction time and reached a plateau after 10 min. Therefore, 10 min was selected as the optimal reaction time and used for the following study.

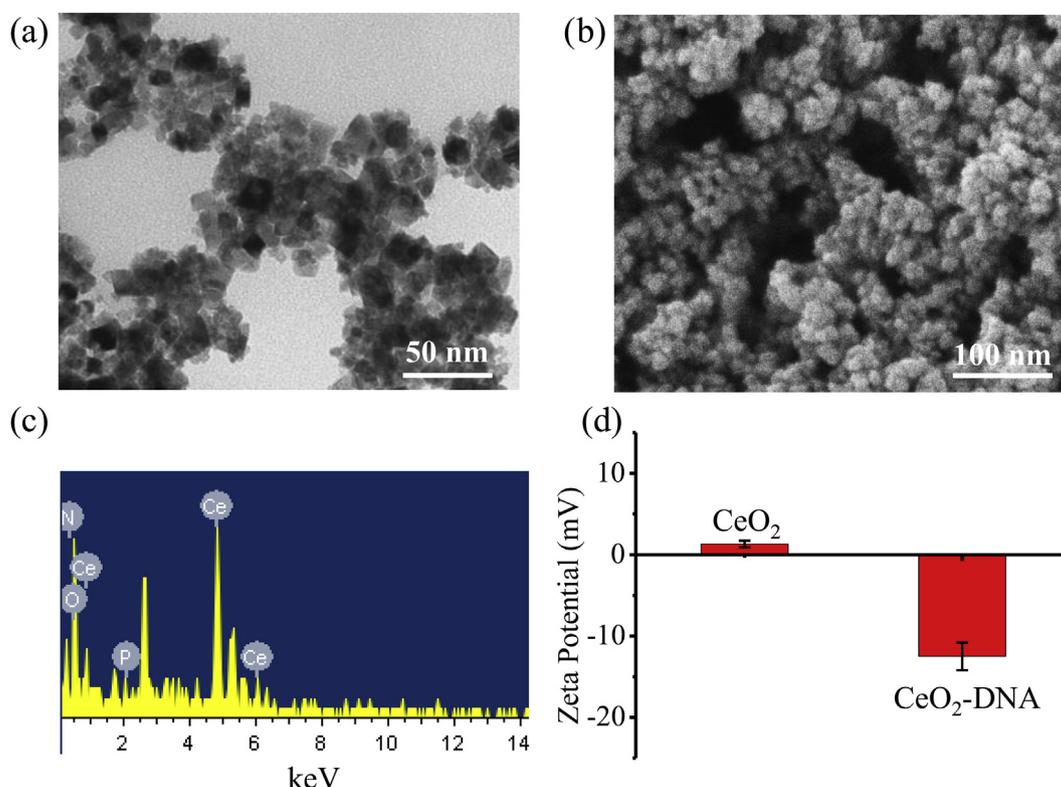


Fig. 1. Characterization of the CeO<sub>2</sub>-DNA nanocomposite. (a) TEM and (b) SEM images of the CeO<sub>2</sub> NPs. (c) EDX analysis elemental composition of CeO<sub>2</sub>-DNA nanocomposite. (d) Zeta potential of CeO<sub>2</sub> NPs and CeO<sub>2</sub>-DNA nanocomposite.

### 3.4. Homogenous electrochemical biosensing of H<sub>2</sub>O<sub>2</sub>

Under the optimized experimental conditions, the performance of the developed homogeneous electrochemical biosensor toward H<sub>2</sub>O<sub>2</sub> with different concentrations was investigated. Fig. 3A showed that the DPV signal gradually enhanced with the increase of the concentration of H<sub>2</sub>O<sub>2</sub>. The DPV peak current change  $\Delta I_p$  ( $\Delta I_p = I_p - I_{p,0}$ , in which  $I_p$  and  $I_{p,0}$  are the peak currents in the presence and absence of H<sub>2</sub>O<sub>2</sub>, respectively) gradually increased with the augment of H<sub>2</sub>O<sub>2</sub> concentration from 0.1 to 40.0  $\mu$ M (Fig. 3B). The enhanced current signals could be attributed to the fact that the increased amount of H<sub>2</sub>O<sub>2</sub> caused more DNA-MB released into the solution. Compared with the CeO<sub>2</sub>-DNA-MB nanocomposite, the DNA-MB are easier to reach the electrode surface due to the weak electrostatic repulsion, thus leading to increased current signal. A good linear relation between the  $\Delta I_p$  and the concentration of H<sub>2</sub>O<sub>2</sub> was clearly observed (inset of Fig. 3B). The detection limit toward H<sub>2</sub>O<sub>2</sub> was estimated to be 35 nM (S/N = 3). Furthermore, to test the repeatability of the homogeneous electrochemical approach, six replicate assays in the presence of 1.0  $\mu$ M and 10.0  $\mu$ M H<sub>2</sub>O<sub>2</sub> were performed, respectively. The results showed that the relative standard deviations (RSDs) were calculated to be 3.9% and 3.1%, respectively, demonstrating a satisfactory repeatability of the developed strategy for H<sub>2</sub>O<sub>2</sub> assay. Besides the repeatability, the reproducibility of the homogeneous electrochemical approach was also investigated with six different biosensors under the same experimental conditions. The results showed that RSD was estimated to be 3.6%, indicating the proposed method possessed acceptable reproducibility. The detection performance of the as-proposed homogeneous electrochemical biosensing system was also compared with some methods reported in literature (Table S1), implying an acceptable and competitive detection sensitivity. More significantly, compared to those traditional heterogeneous electrochemical H<sub>2</sub>O<sub>2</sub> assays, the as-proposed immobilization-free solution-phase electrochemical method avoids sophisticated immobilization procedures, making it cost-effective and

easy to carry out.

### 3.5. Selectivity of H<sub>2</sub>O<sub>2</sub> assay

Next, to investigate the selectivity of the developed homogeneous electrochemical biosensor for H<sub>2</sub>O<sub>2</sub> detection, a few interference species, including histidine, glutamate, lysine, serine, glutathione and cysteine, glucose, sucrose, lactose, ascorbic acid and uric acid with the same concentration were examined, respectively. Significantly raised  $\Delta I_p$  and peak currents were observed only in the presence of H<sub>2</sub>O<sub>2</sub> (Fig. 4 and Fig. S6), whereas no noticeable increase of  $\Delta I_p$  was observed in the presence of the above interference substances. These results definitely indicated that the developed homogeneous electrochemical strategy exhibited excellent selectivity for H<sub>2</sub>O<sub>2</sub> over other competing substances, which could be attributed to the strong coordination effect between H<sub>2</sub>O<sub>2</sub> and CeO<sub>2</sub> NPs. Taken together, these results demonstrated that the proposed biosensor is suitable for detecting H<sub>2</sub>O<sub>2</sub> in biological samples.

### 3.6. Detection of intracellular and extracellular H<sub>2</sub>O<sub>2</sub>

Aberrant generation of intracellular and extracellular H<sub>2</sub>O<sub>2</sub> is closely associated with multiple diseases. Determination of H<sub>2</sub>O<sub>2</sub> variation is crucial to the understanding of its roles in physiology and pathology. Thus, we first examined the performance of the biosensor for detecting intracellular H<sub>2</sub>O<sub>2</sub> by using PMA as the stimulant to elevate intracellular H<sub>2</sub>O<sub>2</sub> levels (Srikun et al., 2008). MCF-7 cell was chosen as the model cell line. As shown in Fig. 5A, a negligible enhancement of peak current was observed in cell lysate without PMA treatment (red line). However, an obvious enhancement of peak current was observed in PMA-stimulated cell lysate (blue line), indicating the dramatic increase of H<sub>2</sub>O<sub>2</sub> concentration. To confirm that the change of current signal is indeed the response of intracellular H<sub>2</sub>O<sub>2</sub>, HRP (an H<sub>2</sub>O<sub>2</sub> scavenger) was added into the PMA-stimulated cell lysate. Upon

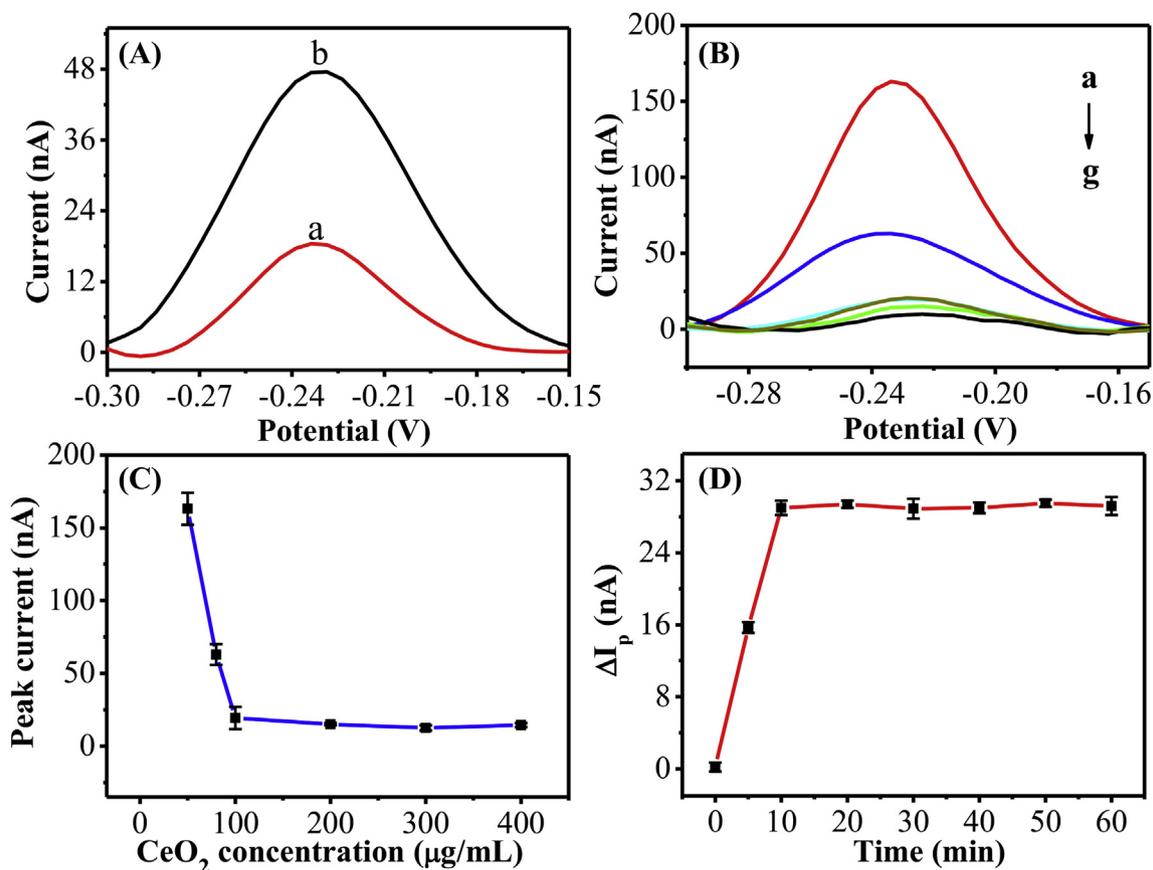


Fig. 2. (A) Differential pulse voltammograms under different conditions: (a) DNA-MB + CeO<sub>2</sub> NPs, (b) DNA-MB + CeO<sub>2</sub> NPs + H<sub>2</sub>O<sub>2</sub>. The concentrations of DNA-MB, H<sub>2</sub>O<sub>2</sub>, and CeO<sub>2</sub> NPs were 1.0 μM, 20.0 μM, and 100.0 μg/mL, respectively. (B) Differential pulse voltammograms of DNA-MB in the presence of CeO<sub>2</sub> NPs with different concentrations (from a to g: 50, 80 100, 200, 300, 400 μg/mL). (C) DPV peak currents of DNA-MB versus different concentrations of CeO<sub>2</sub> NPs. (D) The ΔI<sub>p</sub> versus H<sub>2</sub>O<sub>2</sub> at different times. ΔI<sub>p</sub> = I<sub>p</sub> - I<sub>p,0</sub>, in which I<sub>p,0</sub> is the DPV peak current in the absence of H<sub>2</sub>O<sub>2</sub>, and I<sub>p</sub> is the peak current in the presence H<sub>2</sub>O<sub>2</sub>.

addition of HRP, the current signal of the PMA-stimulated cell lysate was noticeably reduced (green line), suggesting the successful scavenging of H<sub>2</sub>O<sub>2</sub> by HRP. It is indicated that the evident the increased ΔI<sub>p</sub> was indeed attributed to H<sub>2</sub>O<sub>2</sub> produced from the PMA-stimulated cell lysate. The results of the ΔI<sub>p</sub> value were consistent with the DPVs experiments (Fig. 5B). Next, we determined the concentration of H<sub>2</sub>O<sub>2</sub> in PMA-treated cell. When the concentration of H<sub>2</sub>O<sub>2</sub> in the cell lysate was higher than 1.0 μM, the lysate was properly diluted before analyzing. The H<sub>2</sub>O<sub>2</sub> concentration in PMA-treated cell was calculated to about

3.8 fmol per cell. These results clearly proved that the proposed strategy could be used to evaluate the changes of the intracellular H<sub>2</sub>O<sub>2</sub> level. Moreover, the RSDs were estimated to be 3.8% and 4.2% by six successive assays and six different biosensors in the presence of PMA-treated cell lysate, indicating an acceptable repeatability and reproducibility for the as-proposed method for H<sub>2</sub>O<sub>2</sub> assay in real biological sample.

On the basis of the above interesting results, we next explored the feasibility of the as-proposed method for determination of extracellular

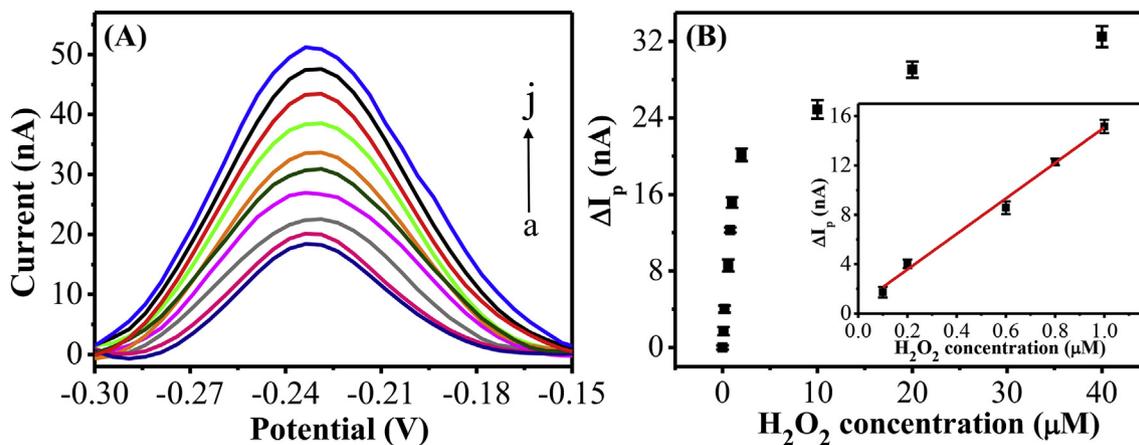
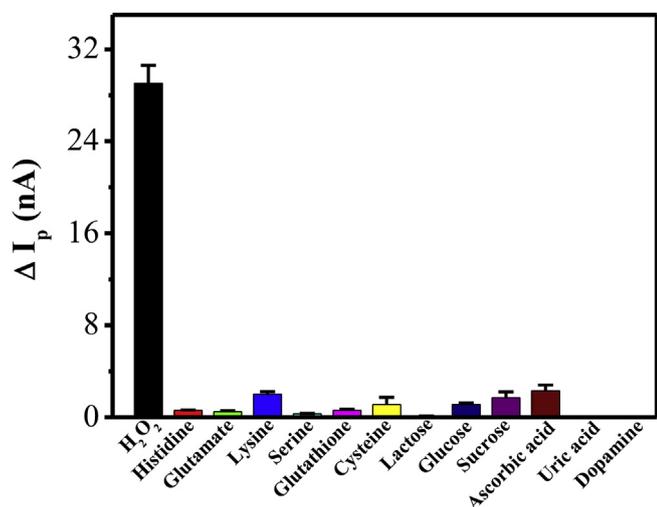
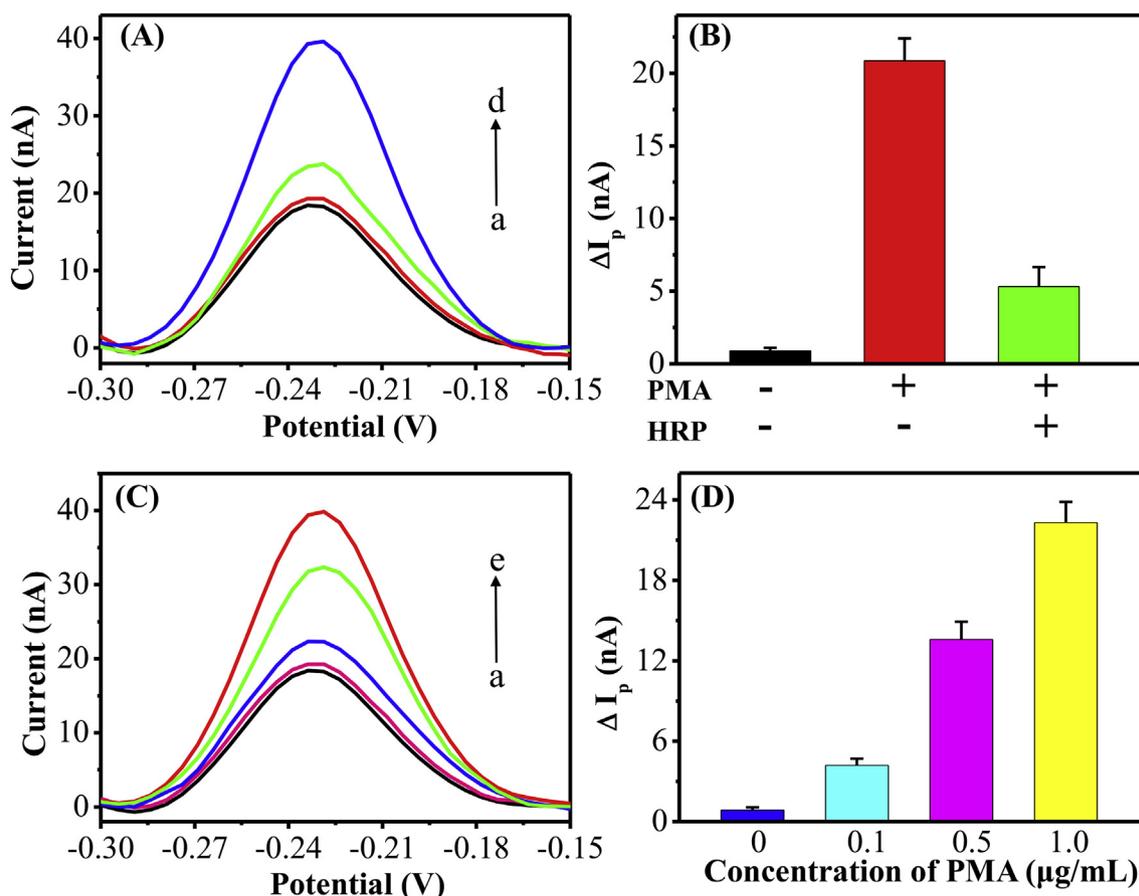


Fig. 3. (A) The DPV responses of the proposed biosensor to different concentrations of H<sub>2</sub>O<sub>2</sub>: (a) 0, (b) 0.1, (c) 0.2, (d) 0.6, (e) 0.8, (f) 1.0, (g) 2.0, (h) 10, (i) 20, (j) 40 μM. (B) The ΔI<sub>p</sub> as a function of H<sub>2</sub>O<sub>2</sub> concentrations. Insert shows the calibration curve of the ΔI<sub>p</sub> versus H<sub>2</sub>O<sub>2</sub> concentrations from 0.1 μM to 1.0 μM. ΔI<sub>p</sub> = I<sub>p</sub> - I<sub>p,0</sub>, in which I<sub>p,0</sub> is the DPV peak current in the absence of H<sub>2</sub>O<sub>2</sub>, and I<sub>p</sub> is the peak current in the presence H<sub>2</sub>O<sub>2</sub>.



**Fig. 4.** Selectivity test of H<sub>2</sub>O<sub>2</sub> detection toward different interference species. All interference species concentrations were tested at 20.0 μM.  $\Delta I_p = I_p - I_{p,0}$ , in which  $I_p$  and  $I_{p,0}$  are the peak currents in the presence and absence of the interference species, respectively.



**Fig. 5.** (A) Differential pulse voltammograms corresponding to the analysis of intracellular H<sub>2</sub>O<sub>2</sub>: (a) blank, (b) the cell lysate without any treatment, (c) PMA-stimulated cell lysate + HRP, (d) PMA-stimulated cell lysate. The concentrations of PMA and HRP are 1.0 μg/mL and 300 U/mL, respectively. (B) The  $\Delta I_p$  in the presence of cell lysate under different conditions. (C) Differential pulse voltammograms corresponding to the analysis of extracellular H<sub>2</sub>O<sub>2</sub> released from MCF-7 cells: blank (a), culture medium without any treatment (b), culture medium collected from the cells treated with 0.1 μg/mL PMA (c), 0.5 μg/mL PMA (d) and 1.0 μg/mL PMA (e). (D) The  $\Delta I_p$  in the presence of culture medium under different conditions.  $\Delta I_p = I_p - I_{p,0}$ , in which  $I_{p,0}$  is the DPV peak current of the blank, and  $I_p$  is the DPV peak current of the detection system under different treatment. For the group without PMA and HRP,  $I_p$  was the DPV peak current of the detection system in the presence of cell lysate without any treatment. For the group without PMA,  $I_p$  was the DPV peak current of the detection system in the presence of culture medium without any treatment.

H<sub>2</sub>O<sub>2</sub> released from living cells. The MCF-7 cells were incubated with different concentrations of PMA to stimulate the cells releasing H<sub>2</sub>O<sub>2</sub>, and MCF-7 cells without PMA simulation were used as the control group. The current signal and  $\Delta I_p$  enhanced successively with the increase of PMA concentration (Fig. 5C and D), indicating that the amount of H<sub>2</sub>O<sub>2</sub> released from cells exhibited a PMA dose-dependent trend. In addition, for 0.1 μg/mL, 0.5 μg/mL and 1.0 μg/mL PMA-treated cell, the amounts of H<sub>2</sub>O<sub>2</sub> were estimated to be ~0.4 fmol, 1.8 fmol and 3.8 fmol generated per cell. To further confirm the selectivity of the as-proposed method for extracellular H<sub>2</sub>O<sub>2</sub> released from living cells, the PMA-treated cells were further incubated with HRP. Fig. S7 showed that the  $\Delta I_p$  decreased sharply after the HRP treatment. All these results evidently demonstrated that the developed homogeneous electrochemical strategy was capable of detecting intracellular and extracellular H<sub>2</sub>O<sub>2</sub>, and could be potentially applied in physiological and pathological researches.

#### 4. Conclusions

In summary, a facile homogeneous electrochemical biosensor was developed for H<sub>2</sub>O<sub>2</sub> assay based on displacement reaction. By making use of the diffusivity difference between DNA-MB and CeO<sub>2</sub>-DNA-MB nanocomposite toward the negatively charged ITO electrode, and the different coordination affinities of CeO<sub>2</sub> NPs toward ssDNA and H<sub>2</sub>O<sub>2</sub>, highly sensitive and selective detection of H<sub>2</sub>O<sub>2</sub> was successfully

achieved. Furthermore, this strategy has been efficiently used to detect intracellular and extracellular H<sub>2</sub>O<sub>2</sub>. More importantly, the as-proposed homogeneous electrochemical strategy avoided the complex modification and immobilization procedures, displaying the distinctive features of simplicity, excellent specificity and good repeatability. Therefore, we anticipate that the current approach can provide new opportunities for exploring the physiological effect of H<sub>2</sub>O<sub>2</sub> in various pathological conditions and diseases.

#### Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### CRediT authorship contribution statement

**Fengjuan Liu:** Conceptualization, Software, Supervision, Resources, Writing - review & editing. **Limin Yang:** Conceptualization, Data curation, Project administration, Software, Supervision, Resources, Writing - original draft. **Xuehan Yin:** Conceptualization, Data curation, Investigation, Methodology. **Xiaojuan Liu:** Conceptualization, Investigation, Methodology. **Lei Ge:** Conceptualization, Investigation, Methodology. **Feng Li:** Funding acquisition, Project administration, Resources.

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#### Appendix A. Supplementary data

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