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ARTICLE

MoS₂ Nanosheets-Au Nanorods Hybrids for Highly Sensitive Amperometric Detection of H₂O₂ in Living Cells

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MoS₂ nanosheets-Au nanorods (MoS₂-Au) hybrids were utilized to immobilize catalase (CAT) to construct a sensitive hydrogen peroxide (H₂O₂) electrochemical biosensor for the reliable determination of H₂O₂ released from living cells. The fabricated biosensor was characterized by transmission electron microscopy (TEM), UV-vis spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and Raman spectroscopy, it is observed that MoS₂-Au hybrid provides excellent matrixes for the adsorption of CAT and the entrapped CAT maintains its native structure and bioactivity. The results of direct electrochemical measurements indicate that on the CAT/MoS₂-Au modified electrode, CAT exhibits a surface controlled and a fast electron transfer process towards H₂O₂ reduction. The MoS₂-Au hybrid has a large surface area and provides a biocompatible microenvironment for accelerating direct electron transfer between the enzyme and the electrode. The detection limit of the constructed H₂O₂ biosensor reaches 1×10⁻⁷ M (signal-to-noise=3) with a wide linear range from 5×10⁻⁷ M to 2×10⁻⁴ M and a high sensitivity of 187.4 mA•M⁻¹•cm⁻². The fabricated H₂O₂ biosensor also exhibits excellent selectivity, good reproducibility and long-time stability. Furthermore, the biosensor was used to perform real-time monitoring of H₂O₂ released from SP2/O cells, indicating the MoS₂-Au hybrid is an attractive material for application in the efficient immobilization of biomolecules and construction of high-performance biosensors.

Introduction

Reactive oxygen species (ROS), acting as an important role in the cell metabolism process, is the marker of progressive neurodegenerative disease and cancer, such as Alzheimer disease, Parkinson's disease and etc.¹⁻³ H₂O₂, which can diffuse through cell membranes freely, is a typical representative of ROS in living organisms.^{4,5} Keeping the amount of H₂O₂ at a proper level is important for intracellular signaling pathways of normal cells.^{6,7} In order to have a better understanding of the biological effects for H₂O₂, it is of great importance to determine H₂O₂ level in biological environment, especially in cellular environment.

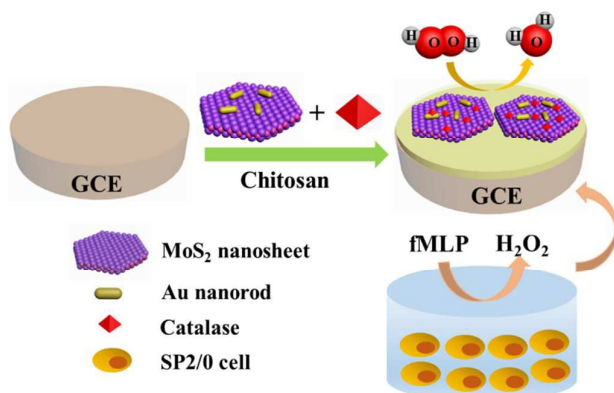
Until now, various methods such as fluorescence,⁸⁻¹⁰ chemiluminescence^{11,12} and electrochemical methods¹³⁻¹⁷ have been employed for H₂O₂ detection. Among these methods, the electrochemical technique have considered to be an efficient method owing to its low detection limit, high selectivity, low simple apparatus and low cost. Many nonenzymatic electrochemical sensors have been developed for real-time monitoring of H₂O₂ from living cells in recent years.¹⁸⁻²² However, the electrochemical methods based on enzyme-immobilized electrodes have captured great attention owing

to their intrinsic advantages, such as the high selectivity and low detection limit due to the high specific activity of enzyme toward H₂O₂ reduction.^{13,14,17} Unfortunately, owing to the redox center of enzyme is deeply entrapped in protein, it is difficult to realize direct electron transfer (DET) from redox center of enzyme to bare electrodes.^{23,24} Thus electron transfer mediator is required to promote the DET between the redox center of the enzyme and the electrode. In the past decades, various nanomaterials have been used as an electron mediator to enhance DET of redox enzyme on the electrode surface.²⁴⁻²⁶ Furthermore, the amount of enzyme that can be effectively immobilized on an electrode is determined by the protein adsorption ability of nanomaterials,²⁷ thus affecting the concentration of electroactive enzyme on the electrode surface and the performance of the biosensor. Protein adsorption ability of nanomaterials is affected by its size,²⁸ surface morphology^{29,30} and surface chemistry.^{31,32} Therefore, one of the major challenges is to find the desirable nanomaterials to accelerate the DET between the redox center of the enzyme and the electrode, increase the amount of enzyme adsorption, and retain enzyme activity.

MoS₂, as a kind of two-dimensional layered materials, has been widely applied in the fields of energy conversion and storage due to its catalytic performance toward hydrogen evolution reaction (HER)³³⁻³⁵ and oxygen reduction reaction (ORR).³⁶ Recently, it also has attracted great attention in electrochemical biosensing attributed to its fast heterogeneous electron-transfer rate at the edge sites of MoS₂ nanosheet, large specific surface area and

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Scheme 1 Schematic of the catalase/MoS₂-Au/chitosan modified GCE used for detecting H₂O₂ released from cells stimulated with fMLP.

good biocompatibility.^{37–39} With a structure similar to graphene, MoS₂ is expected to be employed as a powerful atomic-scale scaffold for nanoparticles to form hybrid materials which can display novel and improved properties. The decoration of MoS₂ nanosheets with metal nanoparticles can supply larger electrochemically active surface area, thus effectively promote the electron transfer between electrode and analytes.^{33,40,41} Au nanoparticles and Au nanorods were decorated on MoS₂ nanosheet to improve its catalytic activity in photocatalytic water splitting and HER.^{33,40} However, so far, the research on the application of those hybrid materials in electrochemical biosensing is rarely reported.

In this work, H₂O₂ electrochemical biosensor was constructed based on MoS₂ nanosheets-Au nanorods hybrids to measure the release of H₂O₂ from living cells (Scheme 1). MoS₂-Au hybrid which combining the advantages of MoS₂ and Au nanorods were prepared to immobilize CAT on the glassy carbon electrode (GCE). Unsaturated sulfur atoms on the edge of MoS₂ nanosheets control the decoration of nanoparticles without aggregation. FT-IR, UV-vis and Raman spectra demonstrated the immobilized CAT retained its native structure and bioactivity. Amperometric responses show that the biosensor gives high sensitivity, selectivity and stability towards H₂O₂ detection. The trace concentration of H₂O₂ released by SP2/0 cells can be determined by the constructed biosensor in our work, which makes the MoS₂-Au hybrid as a promising platform in direct real-time biosensing.

Experimental Section

Reagents and Apparatus

Catalase from bovine liver, chloroauric acid (HAuCl₄·4H₂O) and fMLP (N-formylmethionyl-leucyl-phenylalanine) were bought from Sigma. Molybdenum disulfide (MoS₂) was bought from JC NANO, Inc. (China). Phosphate buffer solution (PBS, 0.1 M, pH 7.0) comprising NaH₂PO₄ and Na₂HPO₄ was used as the supporting electrolyte. 1×PBS (pH 7.4) including KH₂PO₄ (1.76 mM), Na₂HPO₄ (10.14 mM), KCl (2.68 mM) and NaCl (136.75 mM) was utilized to wash and disperse SP2/0 cells. All other

chemical reagents were bought from Sinopharm Chemical Reagent Shanghai Co., Ltd.

AuNRs synthesis

Au nanorods (AuNRs) were synthesized using the silver-assisted seed-mediated growth procedure.^{42,43} Typically, 0.25 mL HAuCl₄ (0.01 M) was added into 9.75 mL cetyltrimethyl ammonium bromide (CTAB, 0.1 M) with gentle mixing to prepare the seed solution. Then 0.2 mL ice-cold NaBH₄ solution (0.03 M) which was freshly prepared was added into the solution, following the mixture was rapid stirred for 2 min. The seed solution was placed at room temperature. 2 h later, HAuCl₄ (2.5 mL, 0.01 M), AgNO₃ (0.45 mL, 0.01 M), HCl (1 mL, 1 M) and ascorbic acid (0.4 mL, 0.1 M) were successively added into CTAB (40 mL, 0.1 M). Finally, 350 μL seed was added into above solution. Then it was slowly stirred for 30 s and kept still overnight to grow Au nanorods. The products were collected and centrifugated at 12000 rpm for 20 min. Finally the products were rinsed with double distilled water at least three times to wash excess unbound CTAB molecules and were redispersed in 2.5 mL of double distilled water for use. The extinction coefficient of AuNRs was assumed to be $4.6 \times 10^9 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 785 nm.⁴⁴ The concentration was measured to be $4.8 \times 10^{-9} \text{ M}$ using UV-Vis spectra.

Preparation of MoS₂-Au Hybrid

The MoS₂-Au hybrid was prepared by mixing the MoS₂ (2 mg/mL) and Au NRs solutions with a volume ratio of 3:1. Then, the mixture was sonicated for 1 h.

Fabrication of Different Modified Electrodes

MoS₂-Au hybrid solution was mixed with chitosan solution (0.5%, w/v) with a volume ratio of 2:1. After the mixture was sonicated for 20 min to form a homogeneous dispersion. 50 μL catalase solution (10 mg/mL) was added into 50 μL MoS₂-Au/chitosan suspension. After mixing for 1 min, it was stored at 4°C overnight for adsorption of enzyme onto MoS₂-Au hybrid. GCE (diameter 3 mm) was polished with 0.3 μm Al₂O₃ slurry, later cleaned by brief ultrasonication with ethanol and water respectively. Then a 5 μL of the above mixture was added onto the GCE surface and dried in 4°C overnight as a working electrode.

Apparatus and Electrochemical Tests

The morphology of nanomaterials was observed on TEM (JEM-2100) and field-emission scanning electron microscopy (FESEM, Supra 55, Zeiss). Fourier transform infrared spectra (FT-IR) were obtained with a Tensor 27 spectrometer (Bruker Co.). UV-vis absorption spectra were collected with a UV2550 spectrometer (SHIMADZU). Raman spectra were collected with a Confocal Raman spectrometer (In Via, Renishaw Co.).

Electrochemical tests were carried out by a CHI660D electrochemical workstation (CHI Co., Shanghai) in a three-electrode arrangement, equipped with a GCE as the working electrode, a platinum wire counter electrode and a saturated calomel electrode (SCE).

Cell Culture

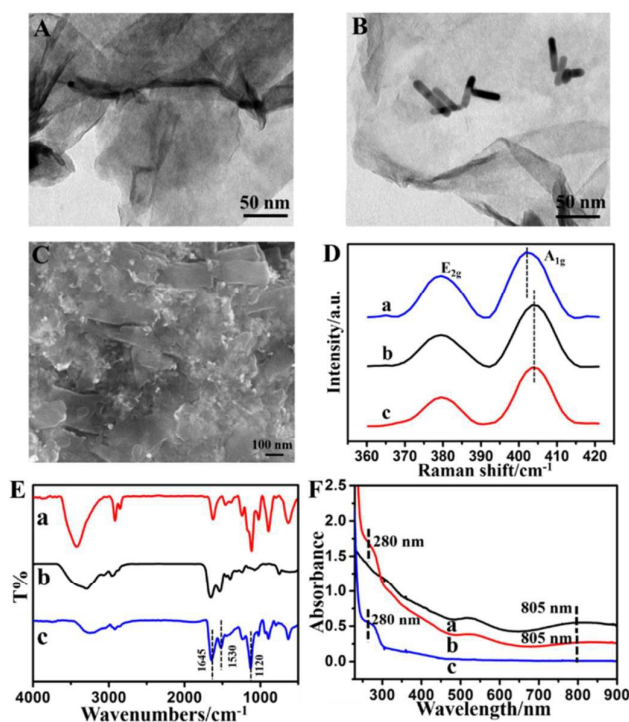


Fig. 1 (A), (B) are the TEM images of MoS₂ nanosheets and MoS₂-Au hybrid. (C) is the SEM image of CAT/MoS₂-Au/chitosan. (D) Raman spectra of MoS₂ (a), MoS₂-Au hybrid (b) and CAT/MoS₂-Au/chitosan (c). (E) FT-IR spectra of MoS₂-Au (a), catalase (b) and CAT/MoS₂-Au (c). (F) UV-vis absorption spectra of MoS₂-Au (a), CAT/MoS₂-Au (b) and catalase (c).

SP2/0 (mouse myeloma) cells were obtained from college of veterinary medicine, Yangzhou University, and were cultivated in DMEM medium (Gibco) supplemented with 15% fetal bovine serum (Gibco), 100 mg/mL streptomycin (Sigma) and 100 U/mL penicillin (Sigma) with 5% CO₂ at 37°C.

Electrochemical Determination of H₂O₂ Released by Living Cells

SP2/0 cells were centrifugated at 1500 rpm for 5 min to separate from DMEM medium. Afterwards, cells were washed with 1×PBS (pH= 7.4) for three times and dispersed in 4 mL 1×PBS. The number of cells was counted by a cell counter. 5 μM FMLP was injected into about 8.0×10⁷ cells in 4 mL 1× PBS, which can stimulate cells to generate H₂O₂ endogenously.¹⁶ The amperometric current response of H₂O₂ released by SP2/0 cells was recorded at catalase/MoS₂-Au/chitosan modified GCE. The experiments were carried out in the 37°C water bath.

Results and Discussion

Characterization of MoS₂, MoS₂-Au Hybrid and CAT/MoS₂-Au/chitosan

The TEM image in Fig. 1A shows the sheet structure of MoS₂. Fig. 1B depicts AuNRs are randomly dispersed on the surface of MoS₂ nanosheets. It indicates the AuNRs are successfully assembled on MoS₂ nanosheets to form MoS₂-Au hybrid,

which should be attributed to the electrostatic interactions of MoS₂ and AuNRs. The Au loading in MoS₂-Au hybrid is calculated as 11.48 atom % (Fig. S1). Chitosan which has good biocompatibility and excellent capability for film formation was used for dispersing MoS₂-Au hybrid. Fig. 1C shows the SEM image of CAT/MoS₂-Au/chitosan, aggregates of the loaded enzymes are distributed continuously and uniformly, indicating the enzyme molecules are successfully immobilized in the MoS₂-Au/chitosan nanocomposite film. The effect of assembled Au nanorods on the electronic properties of MoS₂ is revealed by Raman spectroscopy (Fig. 1D). E_{2g} and A_{1g} peaks represent the in plane (2 S atoms in opposite direction to the Mo atom) and out of plane (S atoms in opposite directions) vibrations for MoS₂ respectively.⁴⁵ The interaction between Au nanorods and MoS₂ affects the out of plane vibration of S atoms, thus leading to the A_{1g} mode blue shift by 2.43 cm⁻¹.⁴⁶

The existing state of the catalase molecules loaded in the MoS₂-Au hybrid was characterized by FT-IR. Fig. S2 displays the FT-IR spectra of MoS₂ and AuNRs. FT-IR spectra of MoS₂-Au hybrid, CAT and CAT/MoS₂-Au are showed in Fig. 1E. Two characteristic peaks at 1634 and 1554 cm⁻¹ were found in the spectra of pure catalase, which are assigned to amide I and II bands of enzyme molecules (curve b). While the FT-IR spectrum of CAT/MoS₂-Au (curve c) also exhibits two characteristic absorption peaks at 1645 (amide I band) and 1530 (amide II band) cm⁻¹, demonstrating that catalase molecules are successfully immobilized in the MoS₂-Au hybrid matrix. UV-vis spectroscopy is an efficient means to investigate the possible conformational changes of enzyme in MoS₂-Au hybrid. Fig. S3 displays the UV-vis spectra of MoS₂, AuNRs and MoS₂-Au hybrid. MoS₂ nanosheets exhibit a clear near-UV absorption peak. The surface plasmon resonance (SPR) longitudinal band at 784 nm and transversal band at 520 nm are observed obviously in the UV-vis spectrum of AuNRs. While the spectrum of MoS₂-Au hybrid displays the similar characteristic peaks of MoS₂ nanosheets and a red-shift of SPR longitudinal band at 805 nm (Fig. 1F), which might be due to the electronic interactions between MoS₂ nanosheets and AuNRs. As seen in Fig. 1F, for the spectrum of native catalase (curve c), an absorption peak at about 280 nm is displayed. It is ascribed to the benzene ring conjugated double bonds of aromatic amino acids. The similar absorption peak is also existed in CAT/MoS₂-Au (curve b), indicating that the catalase immobilized in MoS₂-Au hybrid retains its native structure.

Direct Electrochemistry Performance of CAT/MoS₂-Au/Chitosan GCE

Cyclic voltammetry (CVs) study was conducted to investigate the direct electrochemical behavior of catalase immobilized in the MoS₂-Au hybrid. Fig. 2A displays CVs of MoS₂-Au/chitosan/GCE, CAT/chitosan/GCE, CAT/MoS₂/chitosan/GCE, CAT/Au/chitosan/GCE and CAT/MoS₂-Au/chitosan/GCE in 0.1 M PBS (pH 7.0) at the scan rate of 100 mV·s⁻¹. There is no redox peak appeared at MoS₂-Au/chitosan electrode (curve a), indicating electrodes cannot undergo redox reactions in the absence of catalase. However, CAT/chitosan/GCE exhibits a pair of obvious and well-defined redox peaks (curve b). The

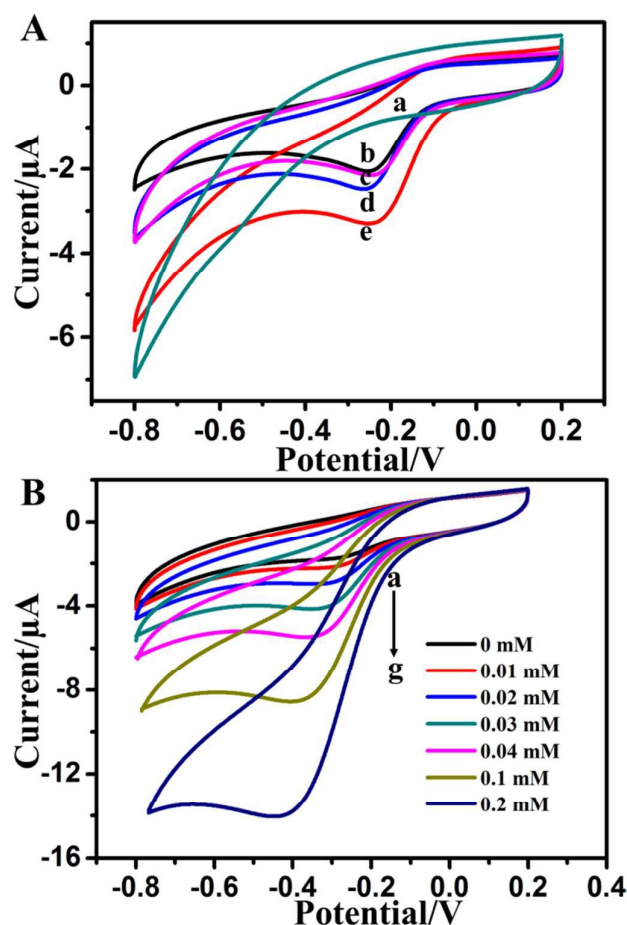


Fig. 2 (A) CVs of the MoS₂-Au/chitosan/GCE (curve a), CAT/chitosan/GCE (curve b), CAT/MoS₂/chitosan/GCE (curve c), CAT/Au/chitosan/GCE (curve d), CAT/MoS₂-Au/chitosan/GCE (curve e) in the N₂-saturated 0.1 M PBS (pH 7.0) at the scan rate of 100 mV•s⁻¹. (B) CVs of the CAT/MoS₂-Au/chitosan/GCE in the N₂-saturated 0.1 M PBS in the absence and presence of H₂O₂, where H₂O₂ concentrations are 0, 0.01, 0.02, 0.03, 0.04, 0.1, 0.2 mM. Scan rate: 100 mV•s⁻¹.

cathodic peak potential is -0.26 mV vs. SCE, which is consistent with the reported values for other nanomaterial modified electrodes.^{26,47} The peaks might be due to the redox reaction of the Fe (III)/Fe (IV) redox couple of the heme group in catalase. CAT/MoS₂/chitosan/GCE (curve c), CAT/Au/chitosan/GCE (curve d) and CAT/MoS₂-Au/chitosan/GCE (curve e) show a pair of more distinct redox peaks than CAT/chitosan/GCE. And CAT/MoS₂-Au/chitosan/GCE offers the strongest peak current among these modified electrodes. This suggests that MoS₂-Au hybrid has enhanced direct electron transfer from redox-active centers of catalase to electrode surface, thus facilitating direct electrochemistry of catalase. It is possible the electronic interactions between MoS₂ nanosheets and AuNRs that favored the direct electron transfer between the active centers of catalase and the electrode.

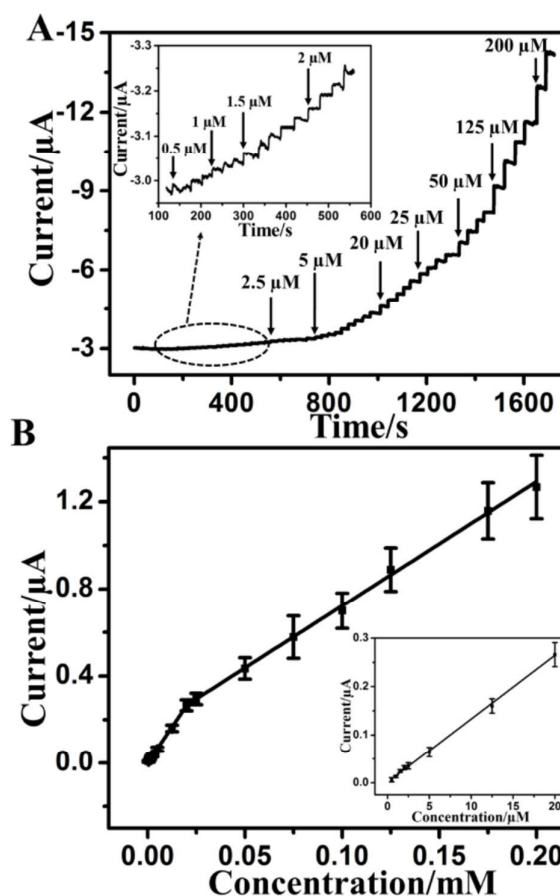


Fig. 3 (A) Amperometric response of CAT/MoS₂-Au/chitosan/GCE in the N₂-saturated 0.1 M PBS at -0.55 V with successive addition of H₂O₂. Inset: i - t curve shows the response of modified electrode to H₂O₂ with successive addition of 0.5, 1.0, 1.5 and 2.0 μ M H₂O₂. (B) Corresponding calibration curve of current versus H₂O₂ concentrations ranging from 0.5 to 200 μ M. Inset: Calibration curve of current versus H₂O₂ concentrations ranging from 0.5 to 20 μ M. Error bars are the standard error of the mean ($n = 5$ electrodes).

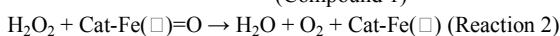
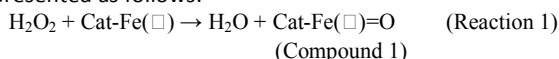
Fig. S4 (A) shows the CVs of CAT/MoS₂-Au/chitosan/GCE at different scan rates. The peak currents changes linearly with scan rates range from 25-500 mV•s⁻¹, demonstrating that the redox reaction of catalase at MoS₂-Au modified GCE is a surface controlled redox process. According to the Laviron equation, the electron transfer rate constant (k_s) could be evaluated by measuring the variation of peak potential with scan rate.⁴⁸ The electron transfer rate constant is calculated to be 1.07 s⁻¹. The surface coverage Γ of catalase molecule can be calculated from the equation $\Gamma = Q/nFA$, where Q is the charge consumed in the reaction, n is the number of electrons transferred, F is the Faraday constant, A is the effective surface area of the modified electrode. The surface coverage Γ of catalase was estimated to be 3.61×10^{-10} mol•cm⁻² using the charge integration of cathodic peak in CV at 100 mV•s⁻¹, indicating an approximate monolayer of catalase immobilized on the MoS₂-Au hybrid modified GCE. The value is comparable

to $1.53 \times 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$ at catalase/NiO/MWCNTs/GCE,⁴⁸ $3.33 \times 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$ at catalase/AuNPs/graphene-NH₂/GCE,⁴⁹ $1.64 \times 10^{-10} \text{ mol} \cdot \text{cm}^{-2}$ at catalase/SWCNTs/GCE⁵⁰ reported previously. The large surface coverage may be attributed to the two-dimensional sheet structure of MoS₂ nanomaterial, thus giving rise to the large surface area of the modified electrode.

The influence of pH value on the peak potentials of CAT/MoS₂-Au/chitosan/GCE was also discussed by CVs. As shown in Fig. S4 (B), both the cathodic and anodic peak potentials shifted negatively with increasing pH values. The redox potentials of CAT/MoS₂-Au/chitosan/GCE exhibit a linear relationship vs. the solution pH in the range of 5.0–8.5 with a slope of 43.5 mV/pH. This value is close to the theoretical value of 58 mV/pH (20°C), indicating one-electron and single-proton joining in this electron transfer process.⁵¹

Electrocatalytic Reduction of H₂O₂ at CAT/MoS₂-Au/chitosan GCE

Owing to the high electron transfer rate of enzyme at the CAT/MoS₂-Au/chitosan/GCE. And it is well known that proteins contain heme group such as HRP, cytochrome c, myoglobin and catalase can electrocatalytic reduction of H₂O₂.⁵² Thus the CVs studies were conducted to testify the electrocatalytic reduction activity of CAT/MoS₂-Au/chitosan/GCE towards H₂O₂. Fig. 2B shows the CVs of CAT/MoS₂-Au/chitosan/GCE in 0.1 M PBS (pH=7.0) when in the absence (curve a) and presence of H₂O₂ (curve b-g). Larger cathodic peak currents appear when in the presence of H₂O₂, indicating the catalase shows excellent electrocatalytic activity for the reduction of H₂O₂. It is also found that when the concentration of H₂O₂ increased, the cathodic current response increased correspondingly. The reaction mechanism could be represented as follows:⁵³



Firstly, electron transfer from enzyme to the substrate to form compound 1. Then compound 1 rapidly react with another H₂O₂ to generate H₂O and O₂. Reaction 1 is the rate-limiting step, thus playing an important role in electron transfer process.

Real-time amperometric study was carried out to explore the detection sensitivity on the CAT/MoS₂-Au/chitosan modified electrode. Fig. S5 displays the influences of pH value and applied potential on the amperometric response of CAT/MoS₂-Au/chitosan GCE towards H₂O₂ determination. The optimal pH value and applied potential are chosen as pH 7.0 and -0.55 V. Fig. S6 shows the amperometric response for the successive injection of different

Table 1. Comparison of different electrochemical measurements for H₂O₂ detection. amounts of H₂O₂ at -0.55 V for different electrodes. It is observed that CAT/MoS₂-Au/chitosan/GCE exhibits enhanced amperometric response for H₂O₂ detection compared with CAT/Au/chitosan/GCE, demonstrating the superiority of CAT/MoS₂-Au/chitosan/GCE for electrocatalytic reduction of H₂O₂. Fig. 3A shows the amperometric response of CAT/MoS₂-Au/chitosan modified electrode for the

Electrode materials	Linear range (μM)	Detection limit (μM)	ref
ND-NS(HRP)	1000-45000	59	15
HRP/Ag@C/ITO	0.5-140	0.02	55
HRP-Au-chitosan-clay	39-3100	3.1	56
HRP/TiO ₂ /ITO	1-780	0.5	57
Fe ₃ O ₄ -Au-HRP/GS-Nafion/SPCE	20-2500	12	17
Hb/CS@Fe ₃ O ₄ /Au	2.3-9600	1.1	58
HRP/BSA/SPCNTS	5-100	0.85	59
GNP-TNT/HIL/HRP	15-750	2.2	60
HRP/gold nanoseeds-TiO ₂	41-630	5.9	61
Catalase/MoS ₂ -Au/chitosan/GCE	0.5-200	0.1	this work

successive injection of various amounts of H₂O₂ into N₂-saturated 0.1 M PBS (pH=7.0) solution at -0.55 V. After H₂O₂ is added, it takes 2.5 s to achieve the maximum steady-state current, indicating the fast response to H₂O₂. This fast response is caused through the rapidly absorption and activation of H₂O₂ on the surface of the CAT/MoS₂-Au/chitosan modified electrode. Fig. 3B shows the corresponding calibration plots of current vs concentration. The linear range for the H₂O₂ detection contains two segments: $5 \times 10^{-7} \text{ M} - 2 \times 10^{-5} \text{ M}$ (correlation coefficient $R^2 = 0.998$) and $2 \times 10^{-5} \text{ M} - 2 \times 10^{-4} \text{ M}$ ($R^2 = 0.998$). The sensitivity is $187.4 \text{ mA} \cdot \text{M}^{-1} \cdot \text{cm}^{-2}$ based on five parallel electrodes. The detection limit for the H₂O₂ sensor reaches 0.1 μM (ratio of signal-to-noise $S/N = 3$). Compared with other reported electrochemical sensors (Table 1), our sensor shows low detection limit and wide linear range, which indicates a bright future in practical applications.

The Michaelis-Menten constant (K_M) can reflect the reaction kinetics of the enzyme and substrate and characterize the affinity between enzyme and substrate. According to the Lineweaver-Burk equation:⁵⁴ $1/I_{ss} = (1/I_{max}) + (K_M/I_{max} C)$. I_{ss} is the steady-state current after the addition of substrate, I_{max} is the maximum current measured at saturated substrate solution and C is the bulk concentration of substrate. The apparent K_M value was estimated to be 0.1 mM , which is lower than the value for catalase reported by other modified electrodes,^{47,49,62} showing that the CAT/MoS₂-Au/chitosan modified electrode exhibits a high affinity for H₂O₂.

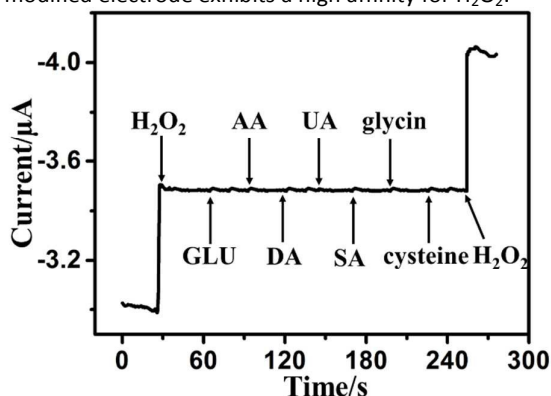


Fig. 4 Amperometric $i-t$ response of CAT/MoS₂-Au/chitosan/GCE in the N₂-saturated PBS (0.1 M, pH 7.0) at an applied potential of -0.55 V with successive addition of 80 μM

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H₂O₂, 0.1 mM GLU, 0.1 mM AA, 0.1 mM DA, 0.1 mM UA, 0.1 mM SA, 0.1 mM glycine, 0.1 mM L-cysteine and a second addition of 80 μ M H₂O₂.

Reproducibility, Stability and Interference Study of the H₂O₂ Biosensor

The reproducibility of the biosensor was tested by five measurements of the current response to 50 μ M H₂O₂ under the same condition for the same electrode. The relative standard deviation (RSD) of the current response to H₂O₂ sensing was less than 4.3%. Five parallel modified electrodes were used for detecting 50 μ M H₂O₂ under the same condition. The RSD was estimated as 3.5%. All above results indicate the biosensor has good reproducibility. To investigate the stability of the sensor, the current response to H₂O₂ for modified electrode was determined every two days for 2 weeks. The modified electrode was stored at 4°C when not use. The current response to H₂O₂ can retain 93% of its initial current after two weeks (Fig. S7), demonstrating the biosensor has good stability. The loss of the current response to H₂O₂ might be attributed to the reasons as follows: (1) the environmental instability of enzyme biomolecule. (2) the electroactive-species absorption on the surface of electrode, thus blocking contact of the CAT/MoS₂-Au/chitosan/GCE with H₂O₂ and disturbing the electron transfer between the electrode and solution.

The capability of distinguishing the target molecule from the interferences normally present in a complex biological environment is extremely important for a biosensor. Thus, we further discuss the impact of some electroactive species on the current response of CAT/MoS₂-Au/chitosan/GCE. Amperometric response of the modified electrode was tested by adding 80 μ M H₂O₂, 0.1 mM β -D(+) glucose (GLU), 0.1 mM ascorbic acid (AA), 0.1 mM dopamine (DA), 0.1 mM uric acid (UA), 0.1 mM salicylic acid (SA), 0.1 mM glycine, 0.1 mM L-cysteine and a second addition of 80 μ M H₂O₂ (Fig. 4). It indicates that GLU, AA, DA, UA, SA, glycine and L-cysteine generate little current response at the CAT/MoS₂-Au/chitosan/GCE under the applied potential, which means that the sensor has good selectivity.

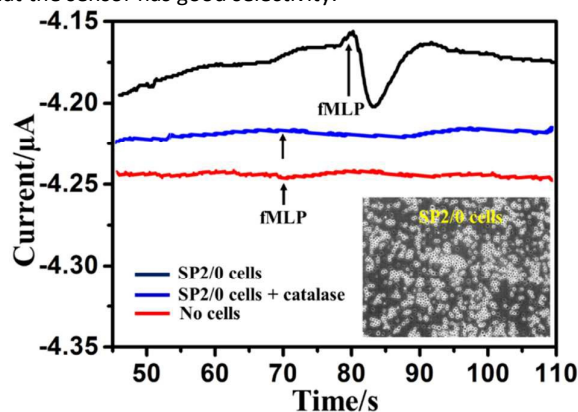


Fig. 5 Amperometric responses of the CAT/MoS₂-Au/chitosan/GCE for the reduction of H₂O₂ released from

about 8.0×10^7 SP2/O cells in 4 mL 1× PBS (pH 7.4) upon the addition of 5 μ M fMLP. The blue line is for the experiment without cells. The red line is for the experiment SP2/O cells and 300 U/mL catalases. Inset: the bright-field microscopy image of SP2/O cells.

Determination of H₂O₂ Released from Living Cells

The CAT/MoS₂-Au/chitosan/GCE was applied to perform real-time monitoring of H₂O₂ released from SP2/O cells. fMLP is selected as a stimulant for the cell to generate H₂O₂. Fig. 5 shows the amperometric responses of the CAT/MoS₂-Au/chitosan/GCE in the presence of about 8.0×10^7 SP2/O cells suspended in 4 mL 1× PBS (pH 7.4) upon the addition of 5 μ M fMLP at -0.55 V versus SCE. After the addition of fMLP, a significant increased current is observed at the CAT/MoS₂-Au/chitosan/GCE electrode. The current gradually decreases and reaches a plateau after 15 s. The maximum current change is around 44 nA corresponding to 3.26 μ M H₂O₂ in cell solution (calculate from the calibration curve in Fig. 3B). The H₂O₂ released from each cell is estimated about 160 amol, which is consistent with previously reported results.^{16,63,64,65} 1× PBS buffer solution without cells (blue line in Fig. 5) and 1× PBS buffer solution with cells and catalase (red line in Fig. 5) under the same conditions were set as controls. There is no current change observed, further suggesting that H₂O₂ is released from cells under the stimulation of fMLP. Fig. S8 is the corresponding current responses obtained from amperometric curves shown in Fig. 5. These results demonstrate the constructed biosensor is sensitive and reliable for intracellular H₂O₂ detection.

Conclusions

A sensitive H₂O₂ electrochemical biosensor was constructed by immobilize CAT on the MoS₂-Au hybrid modified GCE to monitor the H₂O₂ released from living cells. The results of FT-IR, UV-vis and Raman spectrum characterizations indicate that the immobilized CAT molecule retains its native structure and bioactivity. Direct electrochemical results show that on the MoS₂-Au hybrid electrode, CAT exhibits a surface controlled and a fast electron transfer process towards H₂O₂ reduction. All above results suggests that the MoS₂-Au hybrid has a large surface area for adsorption of enzyme and provides an excellent matrix for accelerating DET between enzyme and electrode. The constructed H₂O₂ biosensor shows high performance, such as a wide linear range from 5×10^{-7} M to 2×10^{-4} M, a high sensitivity ($187.4 \text{ mA} \cdot \text{M}^{-1} \cdot \text{cm}^{-2}$) and a low detection limit (1×10^{-7} M). Furthermore, the biosensor was applied to achieve real-time determination of a trace amount of H₂O₂ released from SP2/O cells, indicating the MoS₂-Au hybrid is an attractive material for application in the efficient immobilization of biomolecules and construction of high-performance biosensors.

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Notes and references

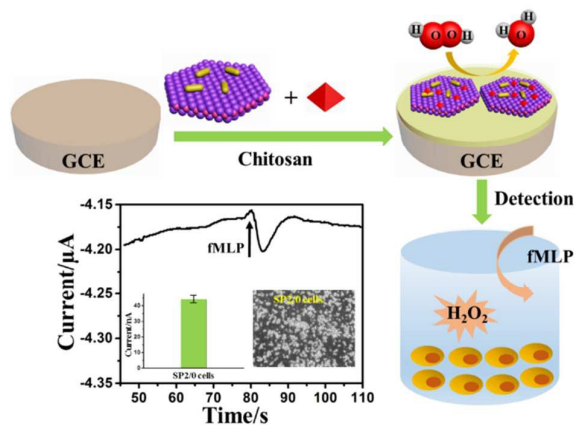
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Table of Contents Graphic



MoS₂-Au hybrids were utilized to construct a sensitive H₂O₂ electrochemical biosensor for determination of H₂O₂ released from living cells.