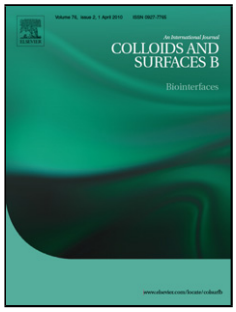
Accepted Manuscript



Title: Electrochemical nitric oxide biosensor based on amine-modified MoS_2/graphene oxide/myoglobin hybrid

Authors: Jinho Yoon, Taek Lee, Jae-Wook Shin, Joungpyo Lim, Mohsen Mohammadniaei, G. Bharate Bapurao, Jeong-Woo Choi

PII: S0927-7765(17)30548-9
DOI: http://dx.doi.org/10.1016/j.colsurfb.2017.08.033
Reference: COLSUB 8789

To appear in: *Colloids and Surfaces B: Biointerfaces*

Received date: 19-4-2017
Revised date: 28-7-2017
Accepted date: 17-8-2017

Please cite this article as: Jinho Yoon, Taek Lee, Jae-Wook Shin, Joungpyo Lim, Mohsen Mohammadniaei, G.Bharate Bapurao, Jeong-Woo Choi, Electrochemical nitric oxide biosensor based on amine-modified MoS2/graphene oxide/myoglobin hybrid, Colloids and Surfaces B: Biointerfaceshttp://dx.doi.org/10.1016/j.colsurfb.2017.08.033

Electrochemical nitric oxide biosensor based on amine-modified MoS_2/graphene oxide/myoglobin hybrid

Jinho Yoon[a], Taek Lee[b], Jae-Wook Shin[a], Joungpyo Lim[a], Mohsen Mohammadniaei[a], Bharate Bapurao G.[a] and Jeong-Woo Choi[a,*]

[a]*Department of Chemical & Biomolecular Engineering, Sogang University*

35 Baekbeom-Ro, Mapo-Gu, Seoul 04107, Republic of Korea

[b]*Department of Chemical Engineering, Kwangwoon University,*

Wolgye-dong, Nowon-gu, Seoul 01899, Republic of Korea

*To whom correspondence should be addressed to

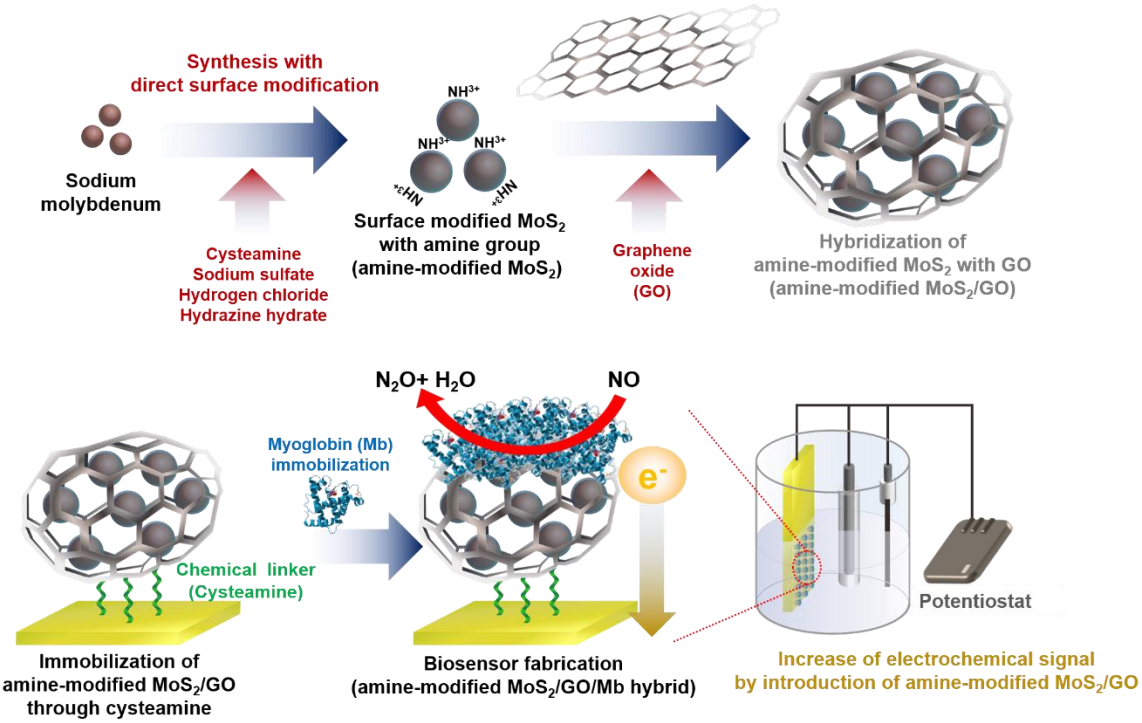
Prof. Jeong-Woo Choi

Department of Chemical & Biomolecular Engineering, Sogang University

Tel.: (+82)2-705-8480, Fax.: (+82)2-3273-0331

E-mail address: jwchoi@sogang.ac.kr (J.W. Choi).

Graphical abstract



Highlights

- ▶ The nitric oxide biosensor was developed by amine-modified MoS_2/GO/Mb hybrid.
- ▶ For the first time, synthesis of MoS_2 accompanying the amine-modification was done.
- ▶ The hybrid showed the increase of electrochemical signal and amperometric response.
- ▶ The hybrid showed high selectivity in nitric oxide detection.

Abstract

Nitric oxide (NO) is one of the most important molecules in living things due to its role as a signaling molecule in influencing pathological and physiological mechanisms including neurotransmission. In this study, the electrochemical biosensor based on the amine-modified molybdenum disulfide nanoparticles (MoS_2), graphene oxide (GO) and myoglobin (Mb) hybrid

material (amine-modified MoS_2/GO/Mb hybrid) is developed to achieve the accurate detection of NO with electrochemical signal improvement. For the first time, the synthesis of MoS_2 accompanying the amine-modification of the surface of MoS_2 is done to hybridize with GO efficiently through the short linkage. After the amine-modification of MoS_2, it is enclosed with GO directly (amine-modified MoS_2/GO). Then, Mb which can induce the reduction of NO is immobilized on the amine-modified MoS_2/GO to fabricate the amine-modified MoS_2/GO/Mb hybrid for NO detection. The prepared hybrid shows the signal improved redox properties relative to the result of the electrode prepared without hybrid. Furthermore, upon addition of NO, the electrode prepared with hybrid shows the improved amperometric response compared with that of the electrode without hybrid. This amine-modified MoS_2/GO/Mb hybrid can be used in the development of the biosensor platform accompanying the electrochemical signal improvement and accurate detection of target materials.

Keywords: Amine-modified MoS_2; Biosensor; Nitric oxide; Hybrid material; Graphene oxide; Myoglobin.

1. Introduction

Development of biomaterial-based biosensors has been received a subject of immense interest and decade-long efforts in biological research because of their medical and clinical applications [1]; [2]. The use of biomaterials in biosensors offers several advantages such as a fast response time and the elaborative selectivity in the detection of specific molecules. Thus, DNA and antibodies have been widely used to develop biosensors for detecting specific DNA sequences

and for monitoring antigens [3]; [4]; [5]; [6]. Among the various biomoelcules, metalloprotein have been found to be a suitable for fast and sensitive sensing of specific molecules because of their unique redox properties [7]; [8]; [9]. In certain electrochemical technique based on sensing specific molecules through metalloprotein, generation of electrochemical signals is simple and quick. Because of this unique advantage, various metalloprotein-based biosensors have been developed for the detection of specific molecules such as hydrogen peroxide and glucose [10]; [11].

Nitric oxide (NO) is one of the most important biological regulatory molecules. It is an important signaling molecule that can affect pathological and physiological mechanisms in living organisms such as neurotransmission and macrophage function [12]; [13]. As such, numerous researching groups have investigated the accurate measurement of NO [14]; [15].

However, electrochemical signals derived from biosensors reacting with specific molecules are too weak to be easily detected with conventional electrodes because of certain difficulties, namely, the random orientation of biomaterial on the electrode, slowness of the electron transfer reaction, and the drastic decrease in biomolecular activity. These limitations interrupt the accuracy of detecting target molecules, especially those at very low concentrations [16].

To overcome these limitations, new types of electrodes such as those based on carbon nanotubes and various nanomaterials have been introduced to develop biosensors with the amplified electrochemical signals and high sensitivity [17]; [18]. Graphene, one of the versatile materials, has been widely applied in the development of sensitive biosensors [19]; [20]. Also, metal dichalcogenide materials including bismuth selenide (Bi_2Se_3) and molybdenum disulfide (MoS_2) have recently gained much interest as advanced materials because of their properties

including direct bandgap, optical properties and conductance [21]; [22]; [23]; [24]. Furthermore, hybridization of metal dichalcogenides and graphene may lead to a synergetic effect, which arises from the retention of the effective carrier mobility, facilitation of electron transfer reaction, and enhancement of biocompatibility with biomaterials [25]; [26].

From this point of view, we previously developed the MoS_2 and GO hybrid material (MoS_2/GO) to fabricate the electrochemical hydrogen peroxide biosensor [27]. At that time, L-homocysteine thiolactone hydrochloride was introduced to amine-modification on the surface of MoS_2 for fabrication of the MoS_2/GO through the electrostatic bond [28]. However, L-homocysteine thiolactone hydrochloride had the complex pentose structure which may hindered the efficient electron transfer of the MoS_2/GO due to the capacitance role of the large and complex chemical linkage material [29]; [30]. To prevent this limitation of the past research and induce the efficient electron transfer of the MoS_2/GO via the short linker part, the synthesis of MoS_2 accompanying amine-modification (amine-modified MoS_2) was required to prevent the introduction of complex chemical linkage material.

For these reasons, in this study, hybrid composed of the amine-modified MoS_2, GO and myoglobin (Mb) was fabricated to develop a biosensor for NO detection with electrochemical signal improvement (Fig. 1). Moreover, for the first time, MoS_2 was synthesized accompanying amine-modification for effective hybridization with GO using the short linkage. To detect NO, Mb was used to detect NO by electrochemical reduction of NO. Mb is a metalloprotein that has the unique electrochemical properties due to the iron ion in its core [31]; [32]. Synthesis of the amine-modified MoS_2 was verified by transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS). Fabrication of the amine-modified MoS_2/GO was

confirmed by ultraviolet-visible (UV-vis) spectroscopy and TEM. Immobilization of the amine-modified MoS_2/GO/Mb hybrid on the electrode was verified by atomic force microscopy (AFM), scanning electron microscopy (SEM) and cyclic voltammetry (CV). Electrochemical property and sensing performance of the proposed amine-modified MoS_2/GO/Mb hybrid were investigated by CV and amperometric i-t technique upon NO addition.

2. Experimental details

2.1. Materials

Mb and phosphate buffered saline (PBS), as well as the chemical linkers cysteamine and 6-mercaptohexanoic acid (6-MHA), were obtained from Sigma-Aldrich (USA). PBS solution was used to prepare the Mb solution. The bare gold electrode (50 nm)/Cr (2 nm) on SiO_2 (5 mm) was fabricated by the National Nanofab Center (Korea). Sodium nitrite ($NaNO_2$) and sulfuric acid (H_2SO_4) for NO preparation were purchased from Daejung Chemical (Korea). GO was purchased from Graphene Supermarket (USA). Distilled water (DIW) was purified using a Milli-Q system (Millipore, USA). Sodium molybdate, hydrazine hydrate, hydrogen chloride (HCl) and sodium sulfide from Sigma-Aldrich (USA) were used to synthesize the amine-modified MoS_2. A TEM grid was obtained from Ted Pella Inc. (USA). Sodium bicarbonate ($NaHCO_3$) and L-ascorbic acid (AA) solutions (Sigma-Aldrich, USA) were used to verify the selectivity of fabricated hybrid.

2.2. Synthesis of the direct surface-modified MoS_2 and the amine-modified MoS_2/GO fabrication

To prepare the direct surface-modified MoS_2 with amine group, first of all, 0.75 g of sodium molybdate was dissolved in DIW (10 mL) for 30 min. Cysteamine (1.12 g) was added slowly for surface modification. After 30 min, a solution of sodium sulfide in DIW and hydrazine hydrate were sequentially introduced at 1 h intervals. HCl (2 M) was then added, and the resulting mixture was heated at 100 °C for at least 3 h until a color change was detected. After this process, the resultant brownish precipitate was collected on filter paper and washed with DIW and ethanol. After it was vacuum dried for 3 h at 70 °C, the amine-modified MoS_2 was obtained. Prepared amine-modified MoS_2 was characterized by high-resolution TEM (HR-TEM) using JEOL JEM-3010 operated at 300 kV, EDS with mapping and zeta potential technique.

To fabricate the hybrid particle composed of prepared amine-modified MoS_2 and GO (amine-modified MoS_2/GO), GO was introduced to enclose the amine-modified MoS_2. GO could enclose the amine-modified MoS_2 through electrostatic interaction between amine modified surface of MoS_2 and carboxylated part of GO. The amine-modified MoS_2/GO volume ratio for the conjugation reaction between the amine-modified MoS_2 and GO at the same concentrations (0.1 mg/mL) was 10:1. The conditions for the amine-modified MoS_2/GO fabrication were 6 h conjugation time, 4 °C, and 400 rpm. The amine-modified MoS_2/GO was then characterized by HR-TEM and UV-vis spectroscopy using a Nanodrop 2000 UV-vis spectrophotometer (Thermo scientific, USA).

2.3. Immobilization of the amine-modified MoS_2/GO/Mb hybrid on the electrode for biosensor fabrication

To immobilize the amine-modified MoS_2/GO/Mb hybrid on the gold electrode, the bare gold electrode was cleaned with piranha solution (H_2SO_4/H_2O_2, 8:2 volume ratio). After a 3 min treatment with the piranha solution, the electrode was washed with ethanol and DIW, and then completely dried with N_2 gas. Cysteamine in the form of a 10 μM solution in DIW was immobilized on the piranha-treated electrode for 3 h at 4 °C. Immobilization of cysteamine was proceeded by self-assembly through gold-thiol interaction and the amine group of cysteamine was on the upper side. Then, the amine-modified MoS_2/GO was immobilized for 3 h at 4 °C on the electrode with immobilized cysteamine. This step occurred through electrostatic bonding between the amine groups of cysteamine and the carboxylated surface of the amine-modified MoS_2/GO. After the amine-modified MoS_2/GO layer formation on the gold electrode, Mb was immobilized on the amine-modified MoS_2/GO layer for 3 h at 4 °C by electrostatic bonding to form the amine-modified MoS_2/GO/Mb hybrid on the electrode. To confirm the electrochemical signal improvement of the amine-modified MoS_2/GO/Mb hybrid, Mb immobilized electrode was fabricated for comparison. To fabricate the only Mb immobilized electrode, cysteamine was immobilized on the piranha-treated electrode for 3 h at 4 °C. Then, Mb was immobilized on the electrode for 3 h at 4 °C by electrostatic bonding.

2.4. Surface investigation of the amine-modified MoS_2/GO immobilized electrode and Mb immobilization on the amine-modified MoS_2/GO

The surface of the amine-modified MoS_2/GO electrode was investigated by AFM using Nanoscope/Multimode (Digital Instruments, USA). The bare gold electrode, as well as the electrodes with immobilized amine-modified MoS_2, and the electrode with immobilized GO,

were characterized for comparison. The operating parameters for AFM were 0.9 internal gain, 0.999 Hz scan rate, 1.9 proportional gain, 1.3 nA current set-point, and scan size of 500 nm. By SEM (Auriga, Carl Zeiss, Germany), the surface of the amine-modified MoS_2/GO immobilized electrode was also examined. CV was performed to confirm the immobilization of Mb on the amine-modified MoS_2/GO layer for fabrication of the amine-modified MoS_2/GO/Mb hybrid. Because the unique redox property of Mb could be detected with the formation of the amine-modified MoS_2/GO/Mb hybrid on the electrode. From this point of view, the amine-modified MoS_2/GO/Mb hybrid immobilized electrode and the amine-modified MoS_2/GO immobilized electrode were compared by CV to verify the immobilization of Mb for hybrid fabrication.

2.5. Electrochemical investigation and sensing performance measurement of the fabricated amine-modified MoS_2/GO/Mb hybrid

Electrochemical investigation of the fabricated amine-modified MoS_2/GO/Mb hybrid was performed using electrochemical techniques (CHI-660A; CH Instruments, Inc., USA) to determine any electrochemical signal improvement. For this investigation, we used a three-electrode system consisting of the fabricated electrode as the working electrode, a silver/silver chloride (Ag/AgCl) double-junction electrode as the reference electrode, and a platinum (Pt) wire electrode as the counter electrode. The electrochemical buffer solution (PBS solution) was used for electrochemical measurement. Parameters applied for CV were the scan rate of 50 mV/s, the quiet time of 2 s, the sampling interval of 1 mV/s, and 5×10^{-7} (A/V) sensitivity. The applied voltage range for CV was from 600 mV to -200 mV. Confirmation of the performance in

NO detection was done by the amperometric i-t curve technique. The electrochemical reduction reaction of NO may depicted as follows:

$Mb(Fe^{2+}) + NO \rightarrow Mb(Fe^{2+})NO$, $V_{oxidation} = 0.196$ V

$2Mb(Fe^{2+})NO + 2H^{+} + 2e \rightarrow 2Mb(Fe^{2+}) + N_2O + H_2O$, $V_{reduction} = 0.241$ V

Parameters for the amperometric i-t curve were -0.3 V, 0.1 s, and 5×10^{-7} (A/V) for initial potential, sampling interval and sensitivity, respectively. The selectivity of the fabricated hybrid was investigated by addition of NO with the other materials including $NaNO_2$, AA, $NaHCO_3$.

3. Results and Discussion

3.1. Confirmation of amine-modified MoS_2 synthesis

The synthesis of the amine-modified MoS_2 was verified by HR-TEM and EDS accompanying mapping. Fig. 2a showed the results of the HR-TEM investigation of the amine-modified MoS_2. In the HR-TEM image, the amine-modified MoS_2 have a 20 nm average diameter; the magnified image showed the discrete synthesized particle. The amine-modified MoS_2 showed the tendency for aggregation which property was induced the formation of the suitable form for encapsulation by GO easily. To verify the amine modification, we performed the EDS and EDS mapping. Fig. 2b showed the EDS mapping results of the amine-modified MoS_2. In EDS mapping images, molybdenum (Mo), sulfide (S) and nitrogen (N) of amine group were apparently displayed following the located amine-modified MoS_2. The EDS result in Fig. S1 (In supplementary materials) showed the apparent peaks of Mo, S and N. The Mo and S were constituents for MoS_2, and small amount of the nitrogen was the component for amine group located on the surface. The EDS result of pure MoS_2 didn't have the nitrogen peak (not presented). The amount of Mo, S,

and N were 27.06 %, 58.16 %, and 14.78 %, respectively. Thus, the Mo/S ratio was approximately 1:2, which was equal to the ratio for the composition of MoS_2; the relatively small amount of N was due to its limitation to the surface.

3.2. Confirmation of the fabricated amine-modified MoS_2/GO

Fabrication of the amine-modified MoS_2/GO was confirmed by HR-TEM and UV-vis spectroscopy. Fig. 2c-d showed TEM images of GO and the amine-modified MoS_2/GO. The size of GO was around 700 nm (Fig. 2c). The average size range of GO was from 1000 nm to 250 nm (data not shown). After reacting with the amine-modified MoS_2, GO encircled the numerous small amine-modified MoS_2. Fig. 2d showed the fabricated amine-modified MoS_2/GO. Also, Fig. 2e showed the UV-vis spectra of the amine-modified MoS_2 and GO. UV peak of GO was located at around 250 nm, and UV peaks of the amine-modified MoS_2 were found around 200 nm and 220 nm. Due to the encapsulation of the amine-modified MoS_2 by GO, the UV peaks of the amine-modified MoS_2/GO were located at around 200, 220 and 240 nm, respectively, which were similar to those of the amine-modified MoS_2 and GO. Also, EDS mapping investigation was performed (Fig. S3). In EDS mapping image of the amine-modified MoS_2/GO, Mo, S, N of the amine-modified MoS_2 and C, O of GO were detected following the located amine-modified MoS_2/GO. Furthermore, zeta potential investigation was done for the verification of the amine-modified MoS_2 in Fig. S2.

3.3. Verification of the amine-modified/MoS_2/GO/Mb hybrid immobilization on the electrode

Immobilization of the amine-modified MoS_2/GO/Mb hybrid on the gold electrode for biosensor preparation was confirmed by AFM, SEM and CV. Fig. 3a-d displayed AFM images and vertical investigation results of bare gold, amine-modified MoS_2, GO and amine-modified MoS_2/GO on the gold electrode. The AFM image of the bare gold (Fig. 3a) presented a regular pattern on the surface.

The AFM image of the amine-modified MoS_2 immobilized on the gold electrode (Fig. 3b) revealed immobilized particles with sizes of around 15 nm. The AFM result for GO immobilized on the gold electrode showed particles with diameter of around 260 nm (Fig. 3c). The amine-modified MoS_2/GO-immobilized gold electrode showed the apparent immobilized amine-modified MoS_2/GO with diameters of 200 nm size (Fig. 3d). The encapsulation of the amine-modified MoS_2 by GO therefore induced a decrease in the overall diameter and an increase in height of the hybrid (Fig. 3d). To confirm the immobilization of the amine-modified MoS_2/GO on the electrode more accurately, a vertical investigation was done by AFM. The vertical investigated results of the amine-modified MoS_2/GO on the gold electrode compared with the heights of bare gold, amine-modified MoS_2, GO on the gold electrode were shown in Fig. 3. The height of the bare gold, amine-modified MoS_2 and GO were 2.66, 5.69 and 17.47 nm, respectively. Compared to these results, the height of the amine-modified MoS_2/GO presented the 22.68 nm which was induced from the encapsulation of the amine-modified MoS_2 by GO. Also, by this encapsulating effect, the overall diameter of the amine-modified MoS_2 by/GO was finely decreased as presented in Fig. 3d.

Fig. 4a-b and Fig. S4 showed SEM results for the bare gold electrode and for the amine-modified MoS_2/GO-immobilized gold electrode. The SEM result for the amine-modified MoS_2/GO-immobilized gold electrode exhibited the immobilized amine-modified MoS_2/GO which showed around 500 nm size which is slightly larger than the diameter calculated from the AFM result. However, the wide size range of GO affected the overall size of the immobilized amine-modified MoS_2/GO.

Fig. 4c showed the cyclic voltammogram of the amine-modified MoS_2/GO-immobilized gold electrode and the amine-modified MoS_2/GO/Mb hybrid immobilized gold electrode. The cyclic voltammogram of the amine-modified MoS_2/GO-immobilized electrode showed the wide shape up and down compared to the bare gold electrode due to the capping effect of the introduced amine-modified MoS_2. In the case of the amine-modified MoS_2/GO/Mb hybrid immobilized electrode, upon immobilization of Mb on the amine-modified MoS_2/GO layer in the gold electrode, the unique redox peaks of Mb was detected which confirmed the immobilization of Mb on the electrode for the amine-modified MoS_2/GO/Mb hybrid formation.

3.4. Electrochemical properties of the amine-modified MoS_2/GO/Mb hybrid

Fig. 5 displayed the CV results for bare gold electrode, Mb and the amine-modified MoS_2/GO/Mb hybrid. The cyclic voltammogram of the amine-modified MoS_2/GO/Mb hybrid showed the improved electrochemical results with 3.47 μA at reduction peak current and -3.59 μA at oxidation peak current compared to the results of the Mb prepared without the amine-modified MoS_2/GO/Mb hybrid (1.08 μA at reduction peak current and -1.13 μA at oxidation peak) (Fig. 5a). Electrochemical signal improvement of the amine-modified MoS_2/GO/Mb

hybrid may be induced by the combination of the amine-modified MoS_2 and GO for the retention of the effective carrier mobility with facilitation of electron transfer property between the Mb and the gold electrode. Also, by introduction of the amine-modified MoS_2 and GO combination, the enlargement of the immobilizable surface area for Mb accompanying the biocompatibility of GO may affect the electrochemical signal improvement. Furthermore, reproducibility of the prepared amine-modified MoS_2/GO/Mb hybrid was investigated by CV. As shown in Fig. 5b, absolute redox peak current values of the amine-modified MoS_2/GO/Mb hybrid exhibited the reproducibility with 3.44 μA at reduction peak current and -3.58 μA at oxidation peak averagely. Error bars in Fig. 5b showed the standard deviation for five repetitive measurements. These results showed that the redox peak currents of the amine-modified MoS_2/GO/Mb hybrid were much higher than the average values of the MoS_2/GO/Mb hybrid prepared without amine-modification previously reported by us as shown in Fig. 5c. This result was induced by the introduction of short linkage through the direct surface modification of MoS_2 with amine group which affected the increment of the electrochemical signal by the replacement of complex chemical linker possessing the aromatic structure, such as L-homocysteine thiolactone hydrochloride, used in the previous research. Also, stability of this hybrid was investigated for 50 cycles as shown in Fig. S5. This hybrid showed the similar cyclic voltammogram for 50 cycles which verified the stability of the amine-modified MoS_2/GO/Mb hybrid.

3.5. Sensing performance of the amine-modified MoS_2/GO/Mb hybrid for NO detection

Fig. 6 showed the amperometric sensing performance of the fabricated amine-modified MoS_2/GO/Mb hybrid by addition of NO. The amperometric response of this hybrid by

successive addition of 18 nM NO was displayed in Fig. 6a. PBS solution (3 mL) was used as the electrolyte. As the parameter for the initial potential, -0.3 V was chosen to achieve the complete reduction state of Mb for NO detecting catalytic reaction. The reduction current of the amine-modified MoS_2/GO/Mb hybrid increased steeply and, with over 10 additions of NO, attained a stable state. To confirm the electrochemical signal improvement for the amperometric response, Mb-immobilized electrode was used to detect NO for the comparison. Fig. 6b showed the compared amperometric response data of the amine-modified MoS_2/GO/Mb hybrid and Mb. As similar to the result of CV in Fig. 5, the amperometric response of the amine-modified MoS_2/GO/Mb hybrid for NO detection showed the precipitous response compared to the result of Mb due to the introduction of the amine-modified MoS_2 and GO for fast electron transfer catalytic reaction. Fig. 6c showed the efficient selective sensing ability of the amine-modified MoS_2/GO/Mb hybrid by addition of NO with $NaNO_2$, AA, $NaHCO_3$ continuously. The amine-modified MoS_2/GO/Mb hybrid showed the amperometric response just with added NO and did not respond to the added $NaNO_2$, AA, $NaHCO_3$. Also, additional selective NO detecting property of the amine-modified MoS_2/GO/Mb hybrid was investigated by addition of NO with hydrogen chloride and dihydrogen sulfate existing gas type in nature (Fig. S6a). NO solutions at concentration of 1, 1.8, 3.6, 9, 18 and 36 nM were prepared to investigate the detection limit of the amine-modified MoS_2/GO/Mb hybrid. Fig. 6d and Fig. S6b revealed the amperometric response upon addition of NO at different concentrations. The detection limit of the amine-modified MoS_2/GO/Mb hybrid was 3.6 nM. NO solutions at 1 nM and 1.8 nM produced only noise, but the amine-modified MoS_2/GO/Mb hybrid showed the amperometric response curve upon addition of 3.6 nM NO solution. This results showed that the detection limit was more responsive and comparable to NO detection with simple preparation steps by self-assembly

compared to the other electrodes prepared for NO detection including the metalloprotein-used electrode [33]; [34]; [35]; [36].

4. Conclusions

In this study, the electrochemical biosensor based on the amine-modified MoS_2/GO/Mb hybrid was fabricated for NO detection with the electrochemical signal improvement. To achieve the improved signals, for the first time, MoS_2 was synthesized accompanying the amine-modification directly and hybridized with GO through the short linkage. Then, by immobilization of Mb on the amine-modified MoS_2/GO, proposed amine-modified MoS_2/GO/Mb hybrid was fabricated on the electrode. Synthesis of the amine-modified MoS_2 and the amine-modified MoS_2/GO were confirmed by TEM, EDS and UV-vis. Immobilization of the amine-modified MoS_2/GO on the electrode was confirmed by AFM and SEM. Immobilization of Mb on the amine-modified MoS_2/GO was confirmed by CV. From the electrochemical investigation, the amine-modified MoS_2/GO/Mb hybrid showed the improved electrochemical signal as 3.47 μA at the reduction potential peak and -3.59 μA at the oxidation potential peak which were much higher than redox signals of Mb. Furthermore, redox peak currents of fabricated hybrid was higher than results of the MoS_2/GO/Mb prepared using complex connecting material due to the direct amine-modification during MoS_2 synthesis by the introduction of short linkage for the effective electron transfer and fast amperometric response reaction. The amperometric NO sensing performance of this hybrid showed the improved amperometric response compared to the response of Mb prepared without the amine-modified MoS_2 and GO. Also, this hybrid-based biosensor showed the efficient selective detection of NO

by addition with the other materials accompanying the 3.6 nM of detection limit. In conclusion, the proposed amine-modified MoS_2/GO/Mb hybrid-based biosensor can be applied to fabricate the biosensor platform for the development of highly sensitive biosensor with improved electrochemical signal.

Acknowledgments

This research was supported by the Leading Foreign Research Institute Recruitment Program, through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT and Future Planning (MSIP) (2013K1A4A3055268) and by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (no. 2014R1A2A1A10051725) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education(2016R1A6A1A03012845).

References

[1] L. Gonzalez-Macia, A. Morrin, M.R. Smyth and A.J. Killard, Analyst, 135 (2010) 845.

[2] J. Kopeček and J. Yang, Angew. Chemie.-Int. Ed., 51 (2012) 7396.

[3] S. Liu, J. Liu, X. Han, Y. Cui and W. Wang, Biosens. Bioelectron., 25 (2010) 1640.

[4] J.H. Jung, D.S. Cheon, F. Liu, K.B. Lee and T.S. Seo, Angew. Chemie.-Int. Ed., 49 (2010) 5708.

[5] A.A. Ensafi, M. Sohrabi, M. Jafari-Asl and B. Rezaei, Appl. Surf. Sci., 356 (2015) 301.

[6] M. Chen, C. Hou, D. Huo, M. Yang and H. Fa, Appl. Surf. Sci., 364 (2016) 703.

[7] X. Zuo, S. He, D. Li, C. Peng, Q. Huang, S. Song and C. Fan, Langmuir 26 (2010) 1936.

[8] N.-H. Kwon, M.A. Rahman, M.-S. Won and Y.-B. Shim, Anal. Chem. 78 (2006) 52.

[9] T. Lee, S.-U. Kim, J. Min and J.-W. Choi, Adv. Mater., 22 (2010) 510.

[10] A.K. Yagati, T. Lee, J. Min and J.-W. Choi, Colloids Surf. B-Biointerfaces, 92 (2012) 161.

[11] F. Molaabasi, S. Hosseinkhani, A.A. Moosavi-movahedi and M. Shamsipur, RSC Adv. 5 (2015) 33123.

[12] V. Calabrese, C. Mancuso, M. Calvani, E. Rizzarelli, D.A. Butterfield and A.M.G. Stella, Nat. Rev. Neurosci., 8 (2007) 766.

[13] U. Förstermann and W.C. Sessa, Eur. Heart J., 33 (2012) 829.

[14] W. Li, X. Geng, Y. Guo, J. Rong, Y. Gong, L. Wu, X. Zhang, P. Li, J. Xu, G. Cheng, M. Sun and L. Liu, ACS Nano, 9 (2011) 6955.

[15] M.H. Lim, B.A. Wong, W.H. Pitcock, D. Mokshagundam, M.-H. Baik and S.J. Lippard, J. Am. Chem. Soc., 128 (2006) 14364.

[16] L. Thudi, L.S. Jasti, Y. Swarnalatha, N.W. Fadnavis, K. Mulani, S. Deokar and S. Ponrathnam, Colloids Surf. B-Biointerfaces, 90 (2012) 184.

[17] G.A. Rivas, M.D. Rubianes, M.C. Rodríguez, N.F. Ferreyra, G.L. Luque, M.L. Pedano, S.A. Miscoria and C. Parrado, Talanta, 74 (2007) 291.

[18] Z. Liu, J. Wang, D. Xie and G. Chen, Small, 4 (2008) 462.

[19] M. Pumera, Chem. Soc. Rev., 39 (2010) 4146.

[20] M.T. Tajabadi, M. Sookhakian, E. Zalnezhad, G.H. Yoon, A.M.S. Hamouda, M. Azarang, W.J. Basirun and Y. Alias, Appl. Surf. Sci., 386 (2016) 418.

[21] D. Sarkar, W. Liu, X. Xie, A.C. Anselmo, S. Mitragotri and K. Banerjee, ACS Nano, 8 (2014) 3992.

[22] M.Z. Hossain, S.L. Rumyantsev, K.M.F. Shahil, D. Teweldebrhan, M. Shur and A.A. Balandin, ACS Nano, 5 (2011) 2657.

[23] S.A. Han, R. Bhatia and S.-W. Kim, Nano Convergence, 2 (2015) 17.

[24] R. Lv, J.A. Robinson, R.E. Schaak, D. Sun, Y. Sun, T.E. Mallouk and M. Terrones, Acc. Chem. Res., 48 (2015) 56.

[25] J. Kailashiya, N. Singh, S.K. Singh, V. Agrawal and D. Dash, Biosens. Bioelectron., 65 (2015) 274.

[26] K. Roy, M. Padmanabhan, S. Goswami, T.P. Sai, G. Ramalingam, S. Raghavan and A. Ghosh, Nat. Nanotechnol., 8 (2013) 826.

[27] J. Yoon, T. Lee, B. Bapurao G., J. Jo, B.-K. Oh and J.-W. Choi, Biosens. Bioelectron., 93 (2017) 14.

[28] W. Osim, A. Stojanovic, J. Akbarzadeh, H. Peterlik and W.H. Binder, Chem. Commun., 49 (2013) 9311.

[29] Y.-H. Chung, T. Lee, J. Min and J.-W. Choi, Colloid Surf. B-Biointerfaces, 87 (2011) 36.

[30] Z.Q. Feng, S. Imabayashi, T. Kakiuchi and K. Niki, J. Chem. Soc., Faraday Trans., 93 (1997) 1367.

[31] A.B. Moghaddam, M.R. Ganjali, R. Dinarvand, S. Ahadi and A.A. Saboury, Biophys. Chem., 134 (2008) 25.

[32] T.D.F. Paulo, I.C.N. Diogenes and H.D. Abruna, Langmuir, 27 (2011) 2052.

[33] J. Elliott, J. Duay, O. Simoska, J.B. Shear and K.J. Stevenson, Anal. Chem., 89 (2016) 1267.

[34] F.X. Hu, J.L. Xie, S.J. Bao, L. Yu and C.M. Li, Biosens. Bioelectron., 70 (2015) 310.

[35] N. Yusoff, A. Pandikumar, A.R. Marlinda, N.M. Huang and H.N. Lim, Anal. Methods, 7 (2015) 3537.

[36] M.-Q. Xu, J.-F. Wu and G.-C. Zhao, Sensors, 13 (2013) 7492.

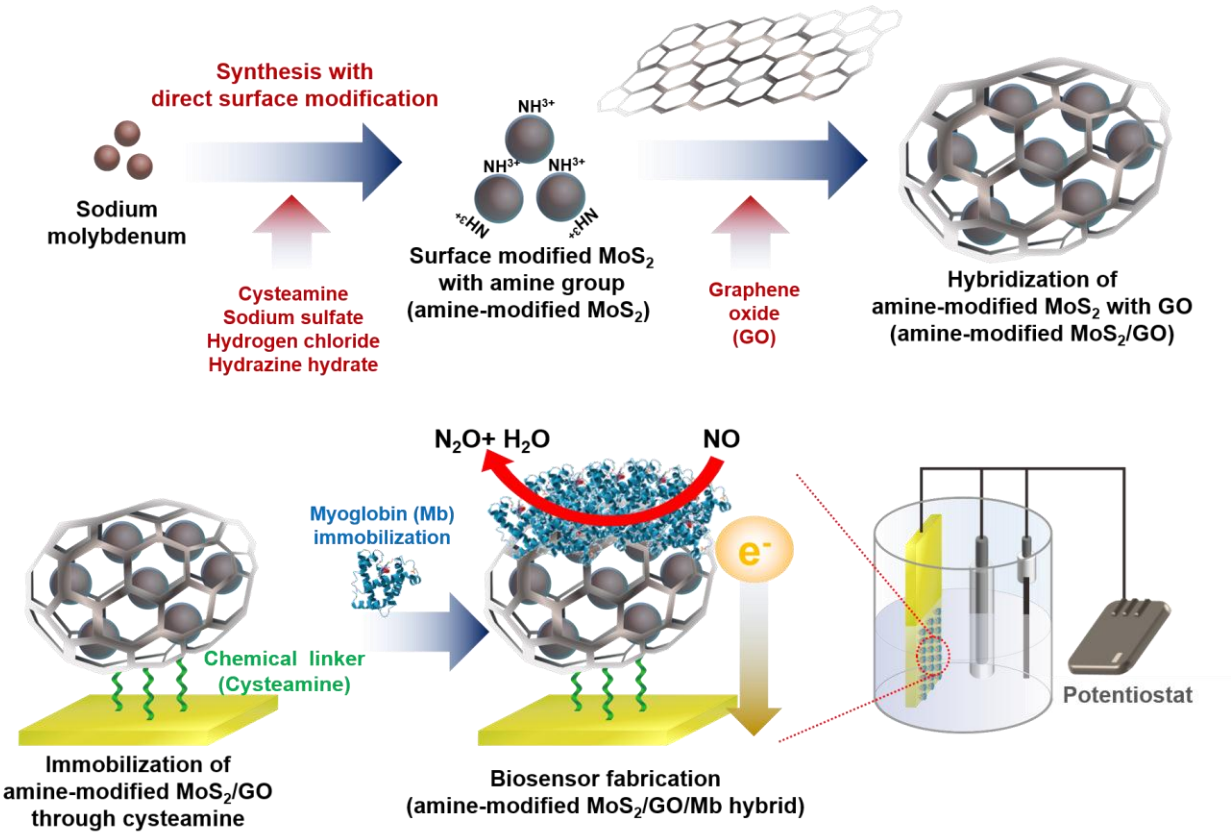


Fig. 1. Schematic image of the amine-modified MoS_2/GO/Mb hybrid fabrication and electrochemical biosensor preparation for NO detection.

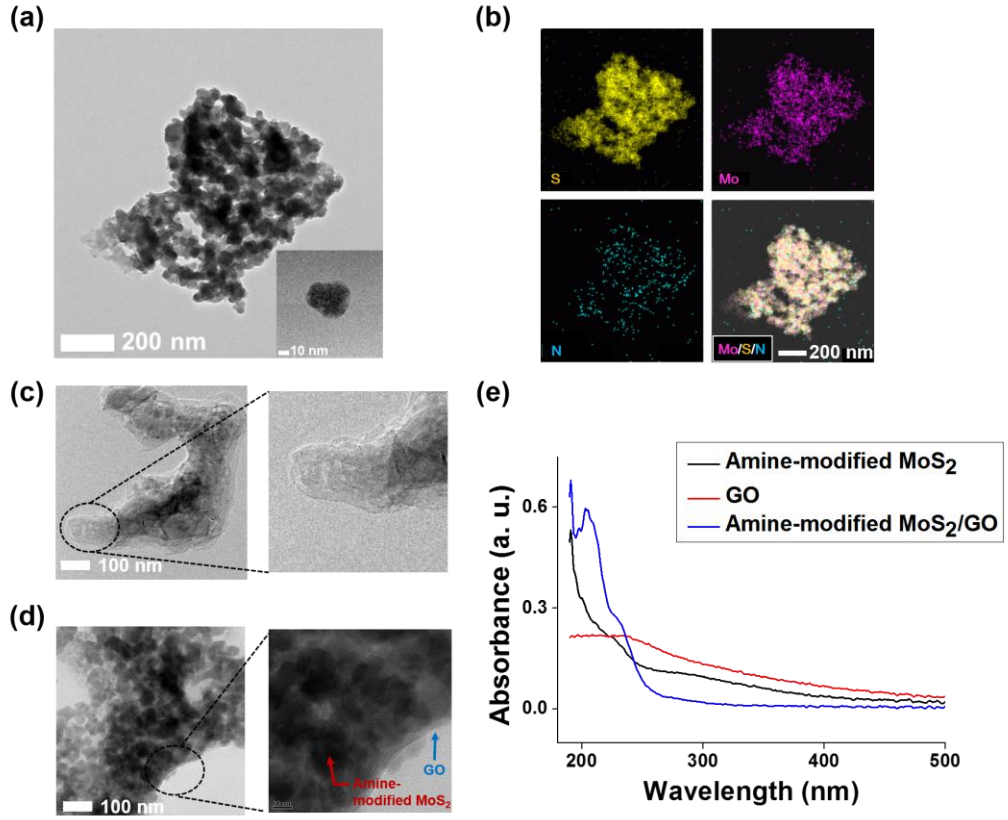


Fig. 2. (a) TEM images and (b) EDS mapping images of the amine-modified MoS_2, TEM images of (c) GO and (d) the amine-modified MoS_2/GO, (e) UV-vis spectra of the amine-modified MoS_2, GO and the amine-modified MoS_2/GO.

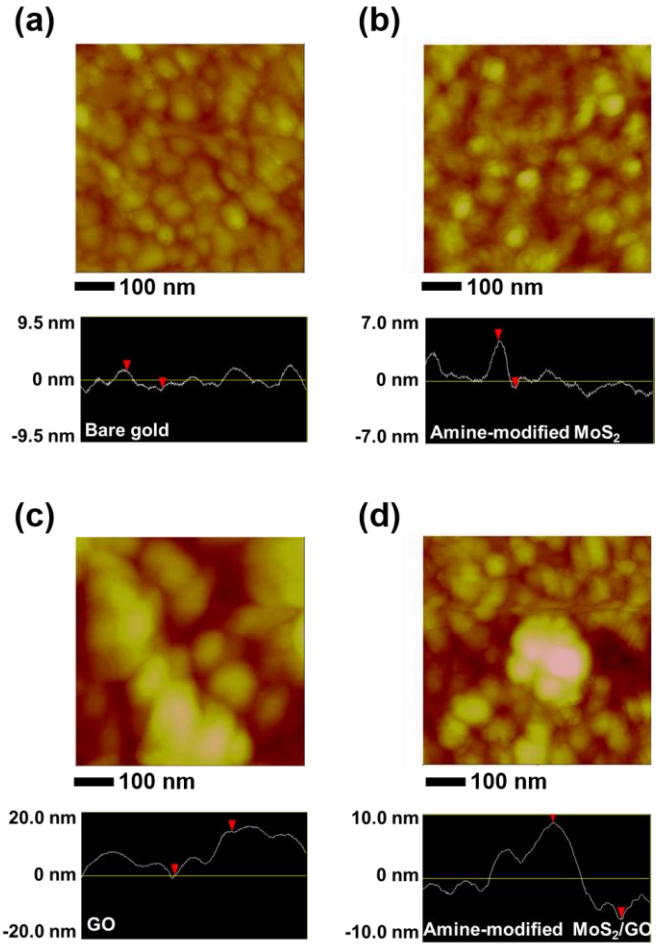


Fig. 3. AFM images and vertical investigation results of (a) bare gold, (b) the amine-modified MoS_2, (c) GO and (d) the amine-modified MoS_2/GO.

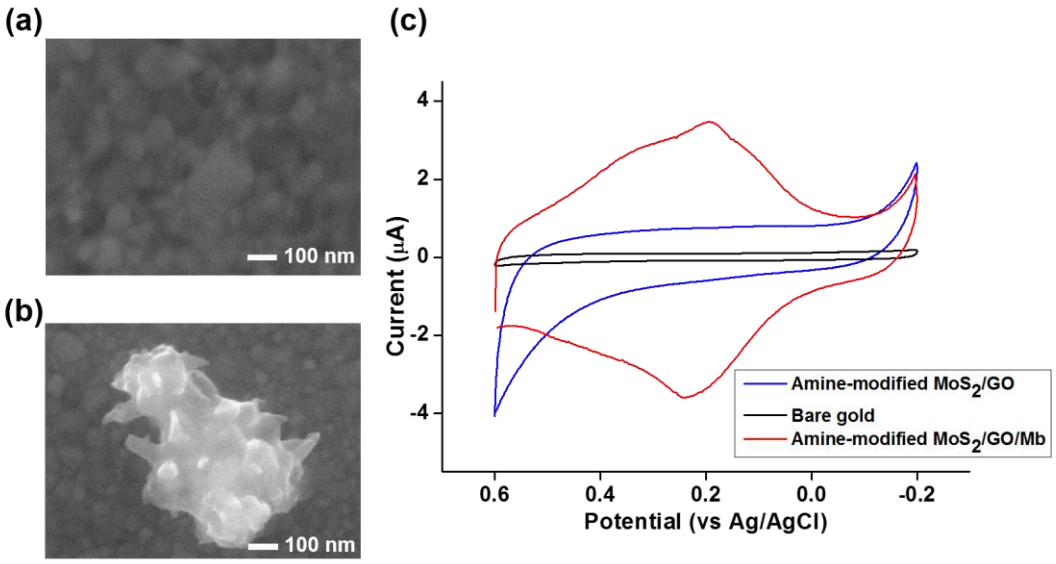
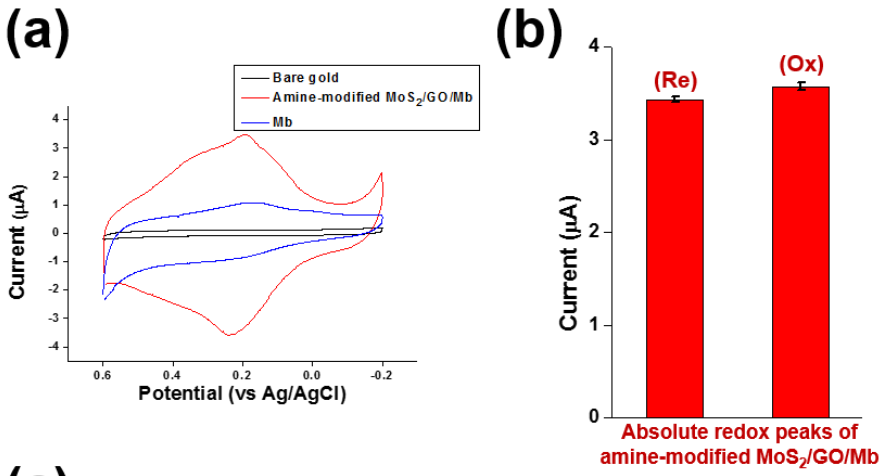


Fig. 4. SEM images of (a) bare gold and (b) the amine-modified MoS_2/GO on the electrode, (c) cyclic voltammograms of bare gold, the amine-modified MoS_2/GO and the amine-modified MoS_2/GO/Mb hybrid.



(c)

Name	Reduction peak current (μA)	Oxidation peak current (μA)	Reference
Amine-modified MoS_2/GO/Mb	3.44 ± 0.0320	- 3.58 ± 0.0452	This work
MoS_2/GO/Mb	1.94 ± 0.0283	- 1.86 ± 0.0194	Ref. [27]

Fig. 5. (a) Cyclic voltammograms of bare gold, Mb and the amine-modified MoS_2/GO/Mb hybrid, (b) Absolute redox peak current values of the amine-modified MoS_2/GO/Mb hybrid ((Re) and (Ox) meant the reduction current peak value and the oxidation current peak value, respectively), (c) Table for the redox peak current comparison of the amine-modified MoS_2/GO/Mb hybrid and the MoS_2/GO/Mb without direct amine-modification. Error bars show the standard deviation for five repetitive measurements.

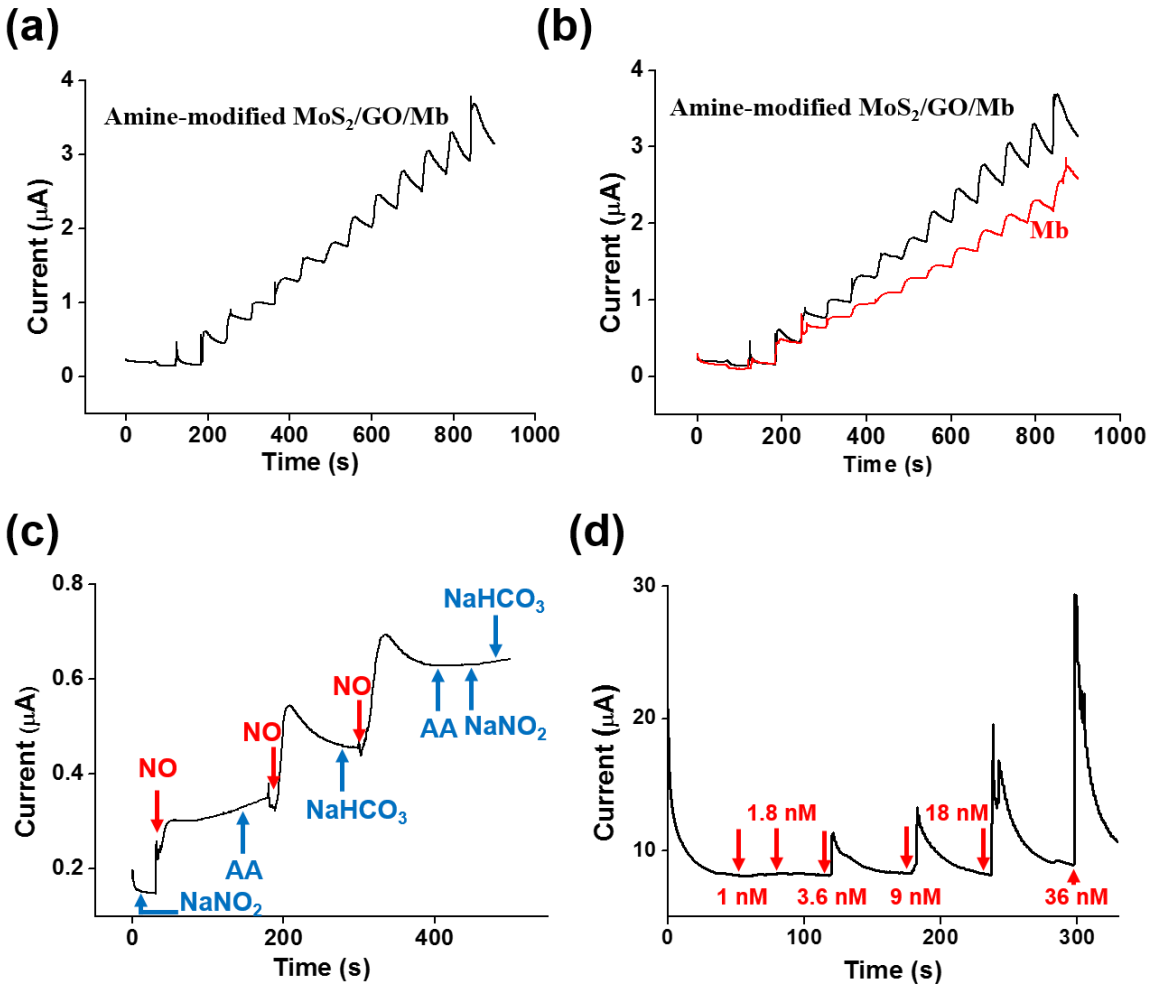


Fig. 6. Amperometric NO response results of (a) the amine-modified MoS_2/GO/Mb hybrid, (b) comparison of Mb and the amine-modified MoS_2/GO/Mb hybrid, (c) the amine-modified MoS_2/GO/Mb hybrid obtained with addition of 100 nM $NaNO_2$, 100 nM AA, 100 nM $NaHCO_3$ and 36 nM NO, (d) the amine-modified MoS_2/GO/Mb hybrid obtained with addition of 1 nM, 1.8 nM, 3.6 nM, 9 nM, 18 nM and 36 nM NO concentration.