Int. J. Electrochem. Sci., 10 **(2015)** 1199 - 1207

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A Sensitive Amperometric Sensor for the Determination of Dopamine at Graphene and Bismuth Nanocomposite Film Modified Electrode

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Received: 13 October 2014 / *Accepted:* 19 November 2014 / *Published:* 16 December 2014

A sensitive amperometric sensor has been developed for the detection of dopamine (DA) at graphene nanosheets (GNS) decorated bismuth nanoparticles (Bi) composite film modified electrode. GNS-Bi nanocomposite has been prepared by simple solution based method and its successful formation was confirmed by scanning electron microcopy and Energy-dispersive X-ray spectroscopy studies. GNS-Bi nanocomposite film modified glassy carbon electrode (GCE) was fabricated and employed for the electrocatalysis of dopamine (DA). At GNS-Bi/GCE, well-defined redox couple corresponding to the redox reaction of DA was observed at the formal potential of + 0.195 V with highly enhanced peak currents. An amperometric sensor has been fabricated based on the GNS-Bi/GCE, which exhibited excellent electrocatalytic ability to the determination of DA. The amperometric sensor presents outstanding electrocatalytic parameters such as wide linear range (1 μM to 30 μM), high sensitivity (5.304 $\mu A \mu M^{-1} cm^{-2}$) and low detection limit (0.35 μM) . Real sample analysis of the sensor has been addressed in pharmaceutical sample which presents appreciable recovery results. The main advantages of sensor are very simple electrode fabrication procedures, roughed and stable, fast in sensing and highly reproducible sensor for DA. In addition, the sensor is exhibited high stability and acceptable repeatability and reproducibility results.

Keywords: Graphene, bismuth nanoparticles, dopamine, electrochemical sensor, electrocatalysis, amperometry.

1. INTRODUCTION

Dopamine (DA) is one of the important neurotransmitter; DA transports signal from central nervous system to brain and significantly involved in the mammalian central nervous systems and

therefore its presence is highly essential to perform the biological functions [1-4]. On the other hand, its concentration is important; irregular concentration of DA resulting in disorders such as Parkinson's disease and schizophrenia [5-7]. Hence, finding a suitable sensing platform for the sensitive determination of DA is of great significance in the biological diagnoses [8-11]. Electrochemical methods are most preferable methods for the determination of DA over other analytical methods due to their simplicity, selectivity, sensitivity and portability [12-14]. However, bare electrodes cannot be utilized for the sensitive detection of DA; they suffer from severe drawbacks such as large overpotential, electrode surface fouling related issues and interferences [15]. Therefore, numerous specifically designed chemically modified electrodes have been prepared and employed for the efficient determination of DA [1, 8-11, 13, 15-18]. Despite the reported literatures, finding a new electrochemical sensing platform is needed due to the importance of DA in biological functions. Recently, graphene nanosheets (GNS), a monolayered two dimensional carbon nanomaterial has grown to be hottest nanomaterial attributed to its interesting physicochemical properties such as large surface area, high conductivity and mechanical strength [7, 19-24]. Graphene oxide (GO), an oxygenated derivative of graphene is the ideal precursor for the preparation of GNS-metal nanoparticles composites owing to its unique advantageous such as, inexpensive and simple production from graphite, easy processing in aqueous dispersions and available sites for the functionalization [25, 26]. Recently, GNS and GO based nanomaterials with various metal nanoparticles have find massive electrochemical applications in sensor, biosensor and energy device applications [11, 27]. Bismuth nanoparticle (Bi) is a semi-metal known for its wide applications in pharmaceutical and metallurgical additives [28-31]. Due to its interesting electrocatalytic properties, Bi finds considerable applications in fabrication of electrochemical sensors [29]. However, Bi alone is not stable on the bare electrode surface and therefore it requires a suitable support such as GNS to stabilize it [32, 33].

The major aim of this present work is to develop a simple and sensitive modified electrode for the efficient determination of DA. We have prepared GNS-Bi nanocomposite by easily adoptable solution based reduction method. Preparation of the nanocomposite and fabrication of the modified electrode are involving simple and fast steps, highly reproducible and stable. The modified electrode possesses excellent electrocatalytic ability to DA. The developed amperometric sensor exhibited excellent electroanalytical parameters such as wide linear range, high sensitivity and low detection limit.

2. EXPERIMENTAL

2.1 Apparatus and Reagents

Electrochemical measurements were carried out using CHI 611A work station in a conventional three electrode cell with modified GCE as a working electrode (area 0.071 cm^2), saturated Ag|AgCl (saturated KCl) as a reference electrode and Pt wire as a counter electrode. All the electrochemical experiments were carried out at ambient temperature. Amperometric measurements were performed with analytical rotator AFMSRX (PINE instruments, USA) and rotating disc glassy carbon electrode (RDE, area 0.21 cm^2). Scanning electron microscope (SEM) and Energy-dispersive

X-ray (EDX) spectra were carried out using Hitachi S-3000 H scanning electron microscope and HORIBA EMAX X-ACT respectively.

Bismuth (III) nitrate pentahydrate, $Bi(NO_3)_2$. $5H_2O$ was purchased from Wako pure chemical industries, Ltd. Graphite (powder, <20 μm) and all other chemicals were purchased from Sigma-Aldrich. All the chemicals used were of analytical grade and used without further purification. 0.1 M of phosphate buffer solution (PBS) was prepared using Na_2HPO_4 and NaH_2PO_4. Double distilled water with conductivity ≥18 MΩ was used for all the experiments. The commercial sample of dopamine hydrochloride injection (easy dopa) has been purchased from O-Smart Company, Taiwan (1.6 mg mL^{-1}, 8.44 mM) and diluted to the required concentrations in PBS (pH 7).

2.2 Preparation of GNS-Bi nanocomposite film modified electrode

Graphite oxide was prepared through Hummer's Method as reported elsewhere [34]. It was dispersed in DMF (1 mg mL^{-1}) and exfoliated to graphene oxide (GO) via ultrasonication for 2 h. The as-obtained homogenous dispersion of GO was collected. $Bi(NO_3)_2$. $5H_2O$ was added to the as-prepared 50 ml dispersion of GO (w/w: 2:3) and ultrasonicated for 1 h [32]. Afterwards, 0.5 ml of hydrazine monohydrate (32.1 mmol) was added and refluxed at 160°C for 24 h under nitrogen atmosphere. Upon completion of the reaction, the product was isolated, washed with copious amount of water and ethanol. The obtained GNS-Bi nanocomposite was dried overnight at 60°C and redispersed in DMF (0.5 mg mL^{-1}). GCE surface was polished with 0.05 μm alumina slurry using Buehler polishing kit, cleaned and dried. Then, 5 μl dispersion of GNS-Bi nanocomposite was drop casted onto the pre-cleaned GCE, dried at ambient conditions and used for experiments. As a control, GNS/GCE was also prepared.

3. RESULTS AND DISCUSSION

3.1. Characterization of GNS-Bi nanocomposite

The SEM image of GNS shows typical wrinkled sheet like morphology of graphene sheets (Figure 1A). EDX profile of GNS is exhibited with the signals of C and O with wt. % of 96.17 and 5.83 respectively (Fig. 1B). The SEM image of GNS-Bi nanocomposite (Fig. 1C) presents high level distribution and decoration of Bi nanoparticles at both faces of GNS. The nanoparticle size varies between 50 nm and 150 nm. The thickness of the GNS varies between 1 and 3 nm. EDX profile of GNS-Bi nanocomposite (Fig. 1D) has exhibited the signals of C, O and Bi with wt. % of 52.05, 7.27 and 40.68 respectively.

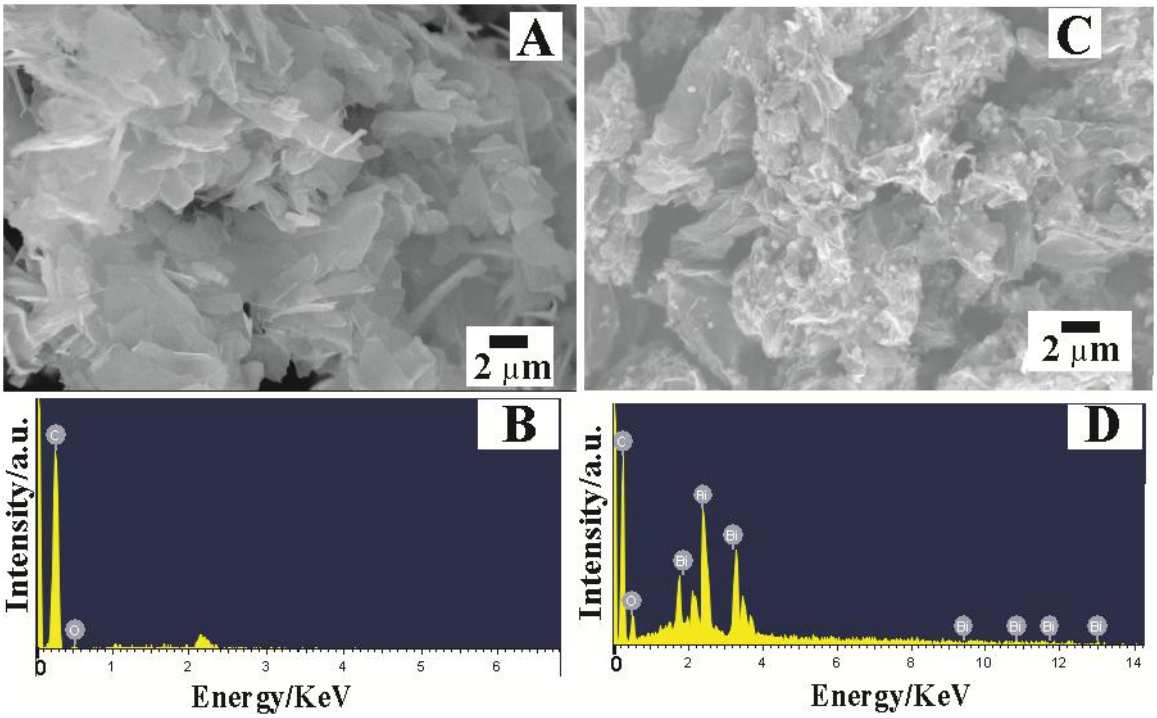

Figure 1. SEM images of GNS (A) and GNS-Bi nanocomposite (C). EDX profiles of GNS (B) and GNS-Bi nanocomposite (D).

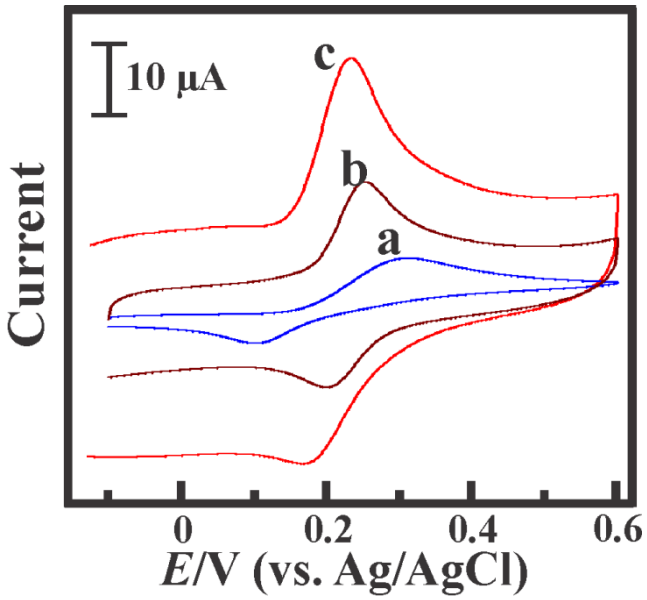

Figure 2. (A) CVs obtained at bare (a), GNS (b) and GNS-Bi (c) films modified GCEs in PBS (pH 7) containing 0.1 mM DA at the scan rate of 50 mVs^{-1}.

3.2 Electrocatalysis of DA

Figure 2 shows the cyclic voltammograms (CVs) obtained at unmodified (a), GNS (b) and GNS-Bi (c) films modified GCEs in PBS (pH 7) containing 0.1 mM DA at the scan rate of 50 mVs^{-1}.

Electrochemical parameters of the electrocatalysis of DA such as, anodic peak current (I_{pa}) and cathodic peak current (I_{pc}), formal potential ($E^{o\prime}$) and peak potential separation value (ΔE_p) at various modified electrodes are listed in Table 1. The electrocatalytic ability of these modified electrodes to the redox reaction of DA are in the order of GNS-Bi/GCE>GNS/GCE>unmodified GCE.

Table 1. Electrochemical parameters for the redox reaction of DA at various modified electrodes

Electrode	E_{pa} (V)	E_{pc} (V)	$E^{o\prime}$/V	ΔE_p/V	I_{pa}/μA	I_{pc}/μA
Unmodified GCE	0.30	0.10	0.20	0.20	6	-3
GNS/GCE	0.26	0.20	0.225	0.06	15	-6
GNS-Bi/GCE	0.21	0.185	0.195	0.025	27	-16

Among the above-mentioned modified electrodes, GNS-Bi/GCE has exhibited maximum electrocatalytic ability, while unmodified GCE has exhibited poor electrocatalytic ability. Obviously, the large ΔE_p value and high overpotential observed at the bare GCE and GNS/GCE revealed the occurrence of sluggish electron transfer kinetic process for DA at these electrodes. However, well-defined quasi-reversible redox couple with highly enhanced peak currents has been observed for GNS-Bi/GCE. Low ΔE_p (25 mV) and high peak currents observed at GNS-Bi/GCE indicates the fast electron transfer kinetics and promising electrocatalytic ability of the modified electrode towards electrocatalysis of DA. The anodic and cathodic peaks are attributed to the oxidation of DA to *o*-dopaminequinone and reduction of *o*-dopaminequinone to DA respectively [7]. Compared with GNS-Bi/GCE, GNS/GCE is exhibited poor electrocatalytic ability which indicating that Bi nanoparticles play significant role in enhancing electrocatalytic ability of the nanocomposite. The large surface area, high porosity, high electrical conductivity and good synergy between GNS and Bi are the chief factors associated with the outstanding electrocatalytic ability of the GNS-Bi nanocomposite.

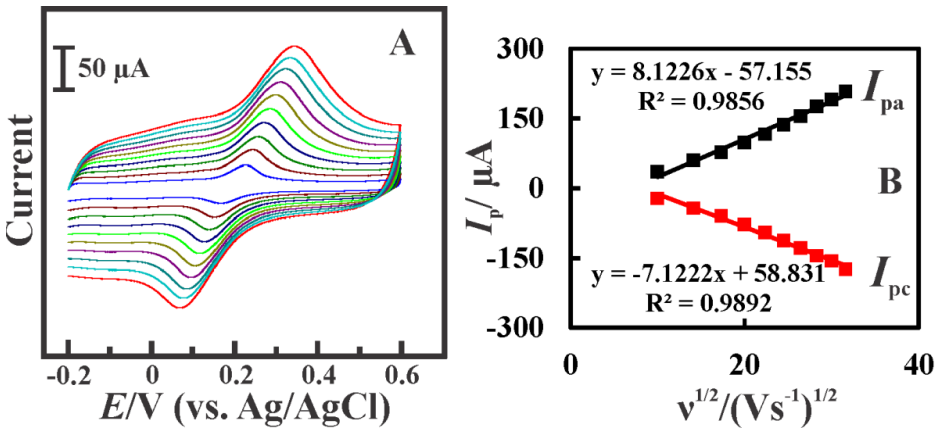

Figure 3. (A) CVs obtained at GNS-Bi/GCE in PBS (pH 7) containing 0.1 mM DA at different scan rates from 0.1 Vs^{-1} to 1 Vs^{-1}. (B) Plot of $v^{1/2}$ vs. I_p.

3.3 Different scan rate studies

The effect of scan rate (v) towards redox reaction of DA at GNS-Bi/GCE was investigated in PBS (pH 7) containing 0.1 mM of DA (Figure 3A). The scan rate (v) was varied from 0.1 to 1 Vs^{-1}. Both the anodic and cathodic peak currents are increases upon increase in the scan rate. In addition, I_{pa} and I_{pc} are shifted to positive and negative potential sides upon scan rate increases. A plot between square root of scan rate ($v^{1/2}$) versus I_{pa} and I_{pc} has exhibited linear relationship indicating that the redox behavior of DA at the GNS-Bi/GCE is controlled by diffusion (Figure 3B). The corresponding linear regression equation can be expressed as: I_{pa} (μA) = 8.123 $v^{1/2}$ $(Vs^{-1})^{1/2}$ – 57.15, R^2 = 0.986 and I_{pc} (μA) = -7.122 $v^{1/2}$ $(Vs^{-1})^{1/2}$ + 58.83, R^2 = 0.989.

3.4 Linear Sweep Voltammetry

Figure 4A presents the linear sweep voltammograms (LSV) obtained at GNS-Bi/GCE in the absence (curve a) and presence of DA (curves b to u; each addition of 10 μM) in PBS (pH 7). Upon addition of 10 μM DA into the PBS solution, an obvious anodic peak was observed responsive for the oxidation of DA as explained in the previous section. The anodic peak current was linearly increases upon further additions of DA from 10 to 100 μM indicating the efficient electrocatalytic ability of the GNS-Bi/GCE without any fouling effect at the electrode surface. A plot of I_{pa} and I_{pc} versus concentration of DA has exhibited linear relationship (Figure 4B). The linear concentration range is observed between 10 and 100 μM with a sensitivity of 0.590 μA/μM.

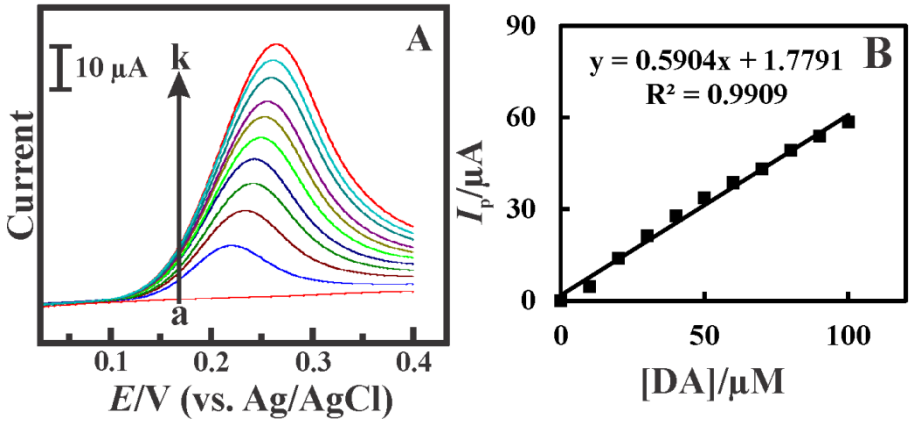

Figure 4. (A) LSVs obtained at GNS-Bi/GCE in the absence (a) and presence of DA from 10 μM to 100 μM (curves *b* to *k*; each 10 μM) in PBS (pH 7) at the scan rate 50 mVs^{-1}. (B) Plot of I_{pa} vs. [DA].

3.5 Amperometric determination of DA

Figure 5A presents the amperogram obtained at GNS-Bi/GCE film modified rotating disc GCE upon sequential injection of 1 μM DA into deoxygenated PBS (pH 7) at regular interval of 50s. The

rotation speed of the electrodes was hold at 1500 RPM, while the applied potential (E_{app}) of the electrode was hold at + 0.20 V. For every addition, rapid and stable responses are observed. The amperometric response current reaches 95% its steady-state current within 4s of the injection which indicating the fast analysis. A plot between the concentration of DA versus peak current exhibited linear relationship and the linear range was obtained between 1 µM and 30 µM (Fig. 5B). The respective linear regression equation expressed as I_p/µA =1.114 [DA]/µAnM^{-1} + 2.452; R^2=0.998. Sensitivity of the sensor is calculated to be 5.304 µAµM^{-1} cm^{-2}. The limit of detection (LOD) of the sensor was calculated to be 0.35 µM. Thus, the modified electrode possesses excellent electrocatalytic parameters to the detection of DA in terms of wide linear ranges, high sensitivity and low LOD.

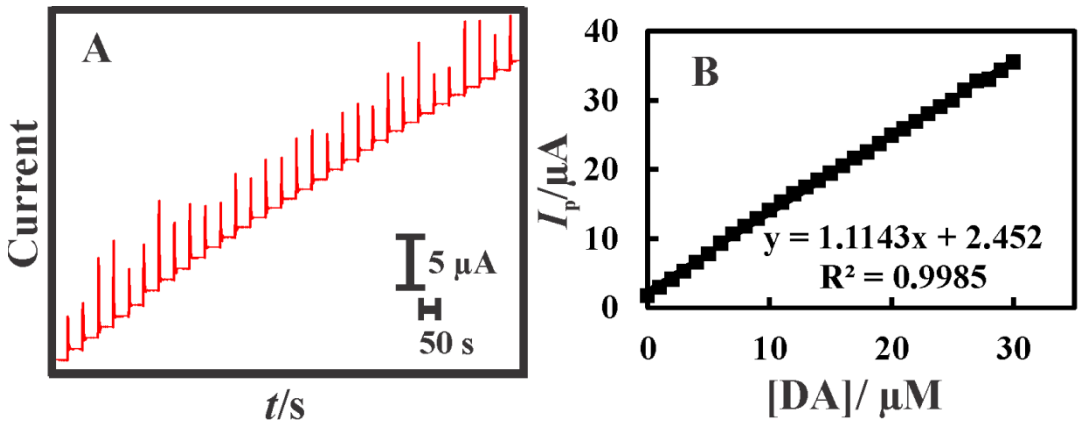

Figure 5. (A) Amperometric i-t response obtained at GNS-Bi/GCE upon each addition of 1 µM DA into continuously stirred PBS (pH 7) at the rotation speed of 1500 RPM. E_{app} = + 0.20 V. (B) Plot of [DA] vs. I_p.

3.6 Stability, repeatability and reproducibility studies

In order to determine storage stability of the sensor, the electrocatalytic response of the GNS-Bi/GCE towards 1 mM DA was monitored every day. The GNS-Bi/GCE electrode was kept stored in PBS (pH 7) at 4°C when not in use. During one month storage, the modified electrode has presented well defined catalytic response; 95.72% of its initial peak current has been retained in one month of its continuous use which revealing the acceptable storage stability of the sensor. Besides, stability of the GNS-Bi/GCE under hydrodynamic conditions is vital for amperometric sensing applications. Therefore, operational stability of the GNS-Bi/GCE has been investigated upon continuous rotation of the film modified electrode at the rotation speed of 1500 rpm in PBS (pH 7). Stable current response was observed for the addition of 1 µM of DA; Only 6.51% of the initial current was decreased even after continously rotated for 3000 s revealing the excellent operational stability of the modified electrode. Repeatability and reproducibility of the GNS-Bi/GCE sensor was evaluated in PBS (pH 7) containing 1 mM DA. The sensor showed good repeatability with relative standard deviation (R.S.D) of 1.83% for five repeatitive measurements carried out using single electrode. The sensor has shown

promising reproducibility of 2.27% for five independent measurments carried out in five different electrodes.

3.7 Real sample analysis

The practical feasibility of the sensor has been assessed in pharmaceutical samples. Commercially available dopamine hydrochloride injection sample was acquired which contains 8.44 mM. The concentration of the injection sample has been diluted to the final concentrations of 1 μM and 10 μM. The amperometric experiments were performed using GNS-Bi/GCE by following the optimized experimental conditions of lab samples analysis (Table 2). The appreciable found and recovery results obtained at the GNS-Bi/GCE revealing the promising practical feasibility of the GNS-Bi/GCE.

Table 2. Determination of DA in pharmaceutical samples using GNS-Bi/GCE.

Real Sample	Sample	Concentration samples (added)	Found	Recovery	RSD
Dopamine hydrochloride injection	1	1 μM	0.97 μM	98.2	2.5
	2	10 μM	9.84 μM	98.4	2.2

4. CONCLUSIONS

In summary, we described a sensitive amperometric sensor for the determination of DA at GNS-Bi/GCE. The GNS-Bi nanocomposite was prepared by simple solution based chemical reduction method and its successful was confirmed by SEM and EDX methods. GNS-Bi/GCE has exhibited excellent electrocatalytic ability towards electrocatalysis DA. The fabricated amperometric sensor presented excellent analytical parameters for the detection of DA such as wide linear range (1 μM – 30 μM), high sensitivity (5.304 $\mu A \mu M^{-1}$ cm^{-2}) and low LOD (0.35 μM). GNS-Bi/GCE has shown excellent stability, repeatability and reproducibility for the determination of DA. In addition, the sensor has shown promising practical feasibility which was assessed in pharmaceutical samples.

ACKNOWLEDGEMENT
This work was supported by the National Science Council and the Ministry of Education of Taiwan (Republic of China).

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