Supplementary Data

For

A novel electrochemical Aflatoxin B1 immunosensor based on gold nanoparticles decorated porous graphene nanoribbon and Ag nanocubes incorporated MoS_2 nanosheets

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2.8. Sample preparation

In this study, electrochemical AFB1 immunosensor was applied to wheat samples to present the application of biosensor. After pulverizing of wheat samples with a grinde, 1:2 mL methanol was added to 0.5 mg wheat sample in a 2.0 mL plastic centrifuge tube under strong stirring. After that, the centrifugation at 10000 rpm was performed for 20 minutes. The upper clear layer solution was diluted with 0.1 M PBS, pH 7.0 for analysis. For the experiments of recovery, five different wheat samples were prepared (sample1, sample2, sample3, sample4 and sample5). The contents of solutions were listed below:

(1): 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

(2): 0.100 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

(3): 0.100 + 0.200 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

(4): 0.100 + 0.400 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

(5): 0.100 + 0.600 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

The standard AFB2, AFG1, AFG2, OTA, DON, ZEN and BSA solutions (1.00 pg mL^{-1}) were firstly added into the diluted wheat samples with clear layer (sample1). After that, 0.100, 0.300, 0.500 and 0.700 pg mL^{-1} standard AFB1 solutions were added into the solutions one by one, respectively (sample2, sample3, sample4 and sample5). Then, the voltammograms were recorded in the potential range from +0.2 V to +0.6 V by electrochemical AFB1 immunosensor.

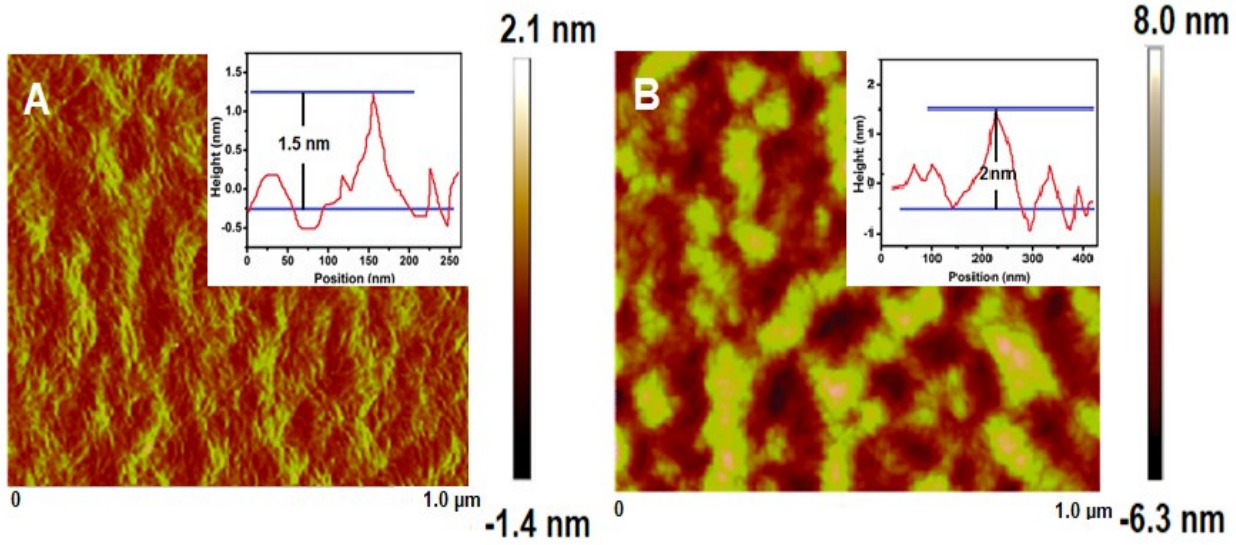

Fig. S1. AFM measurements of (A) 1T-MoS_2 and (B) 2H-MoS_2. Insets are the height profiles of 1T-MoS_2 and 2H-MoS_2 nanosheets

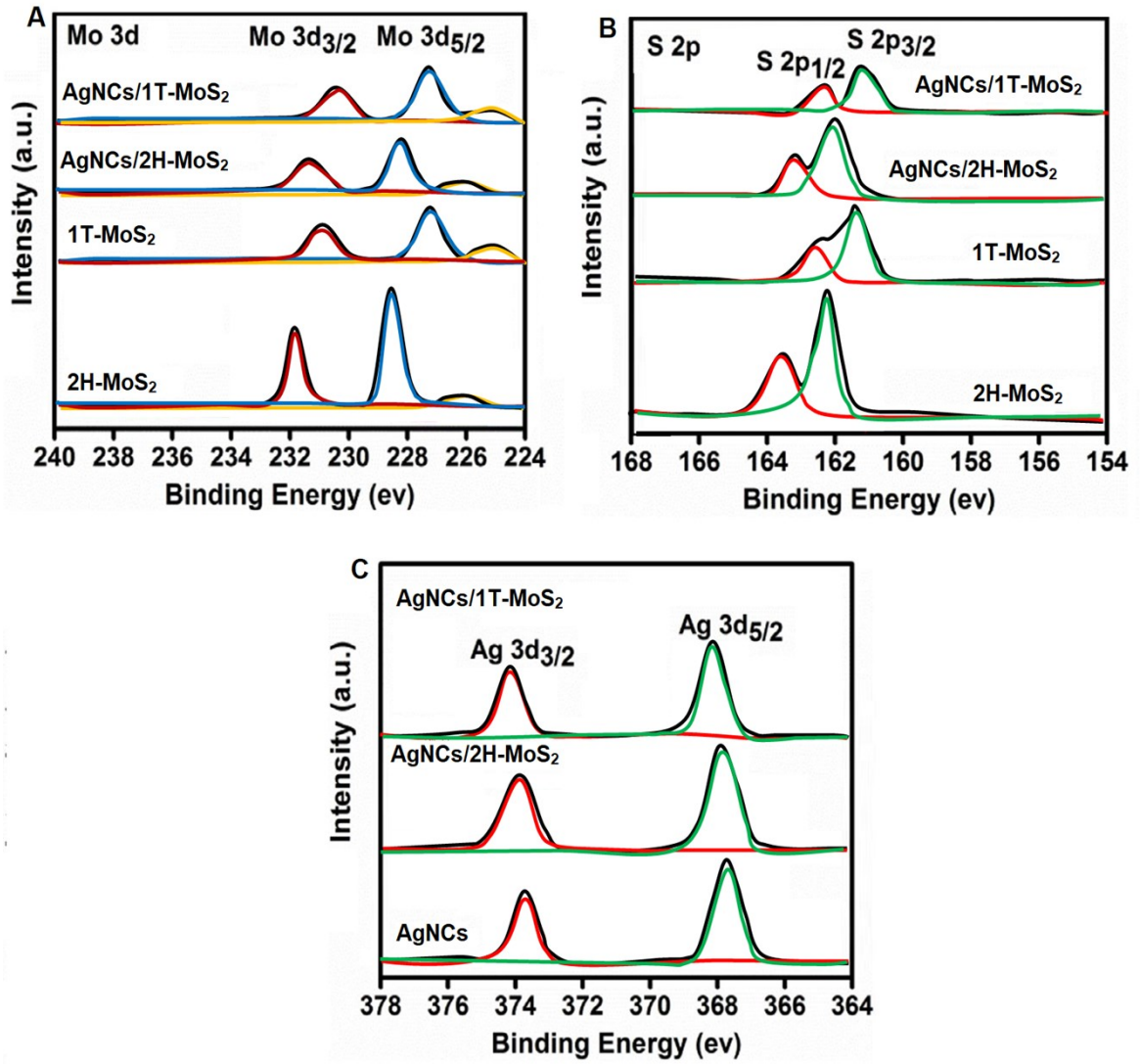

Fig. S2. XPS spectra of (A) Mo3d and (B) S2p and for 1T-MoS_2, 2H-MoS_2, AgNCs/1T-MoS_2 and AgNCs/2H-MoS_2, (C) Ag3d for AgNCs, AgNCs/1T-MoS_2 and AgNCs/2H-MoS_2

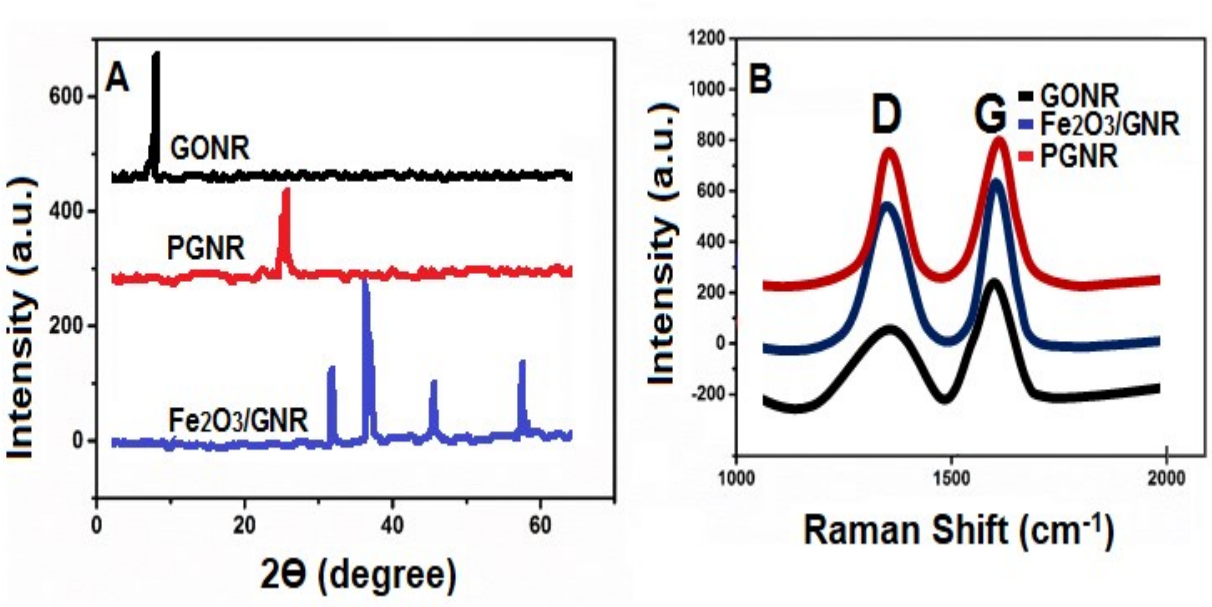

Fig. S3. (A) XRD patterns and (B) Raman spectra of GONR, Fe_2O_3/GNR, PGNR

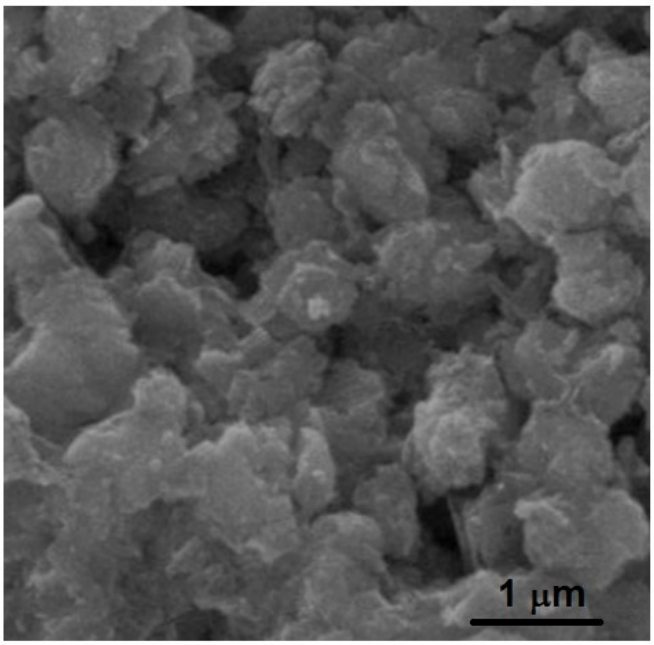

Fig. S4. SEM image of the resulted immunosensor (AgNCs/1T-MoS_2/anti-AFB1-Ab_2/AFB1/BSA/anti-AFB1-Ab_1/AuNPs/PGNR/GCE)

3.5. Optimization for electrochemical measurements

AgNCs/1T-MoS_2/anti-AFB1-Ab_2 concentration effect

AgNCs/1T-MoS_2/anti-AFB1-Ab_2 concentration has important effect on the developed immunosensor performance. The optimal and symmetrical peaks were observed up to 30.0 mg mL^{-1}. Especially, after 30.0 mg mL^{-1} AgNCs/1T-MoS_2/anti-AFB1-Ab_2, the optimal and symmetrical peaks remained constant. Because of this, the optimal concentration of AgNCs/1T-MoS_2/anti-AFB1-Ab_2 was selected as 30.0 mg mL^{-1} (Fig. S5A) (In the presence of 1.0 mM H_2O_2 in 0.1 M PBS, pH 7.0).

pH effect

Secondly, pH effect was investigated on immunosensor performance. The immunosensor response increased up to pH 7.0. Furthermore, highly acidic or alkaline medium damages the structures of immobilized proteins. Hence, optimal pH was selected to be pH 7.0 (close to physiological pH) (Fig. S5B) (In the presence of 1.0 mM H_2O_2).

H_2O_2 concentration effect

In this study, different H_2O_2 concentrations were tried for obtaining optimal immunosensor signals (Fig. S5C). When H_2O_2 concentration gradually increased to 1.0 mM, the peak current gradually increased. After 1.0 mM H_2O_2, peak current decreased inversely. Due to overdose of H_2O_2 catalyst causing the inhibition of catalytic reaction, the activity of the proteins was negatively affected. Thus, the optimal signals were obtained in 1.0 mM H_2O_2 in 0.1 M PBS (pH 7.0).

Immune reaction time effect

When incubation time increased from 5 min to 30 min, peak current responses increase rapidly. After 20 min, immunosensor signals (μA) slightly diminished. Thus, optimal immune reaction time was selected to be 20 min (Fig. S5D) (In the presence of 1.0 mM H_2O_2 in 0.1 M PBS, pH 7.0).

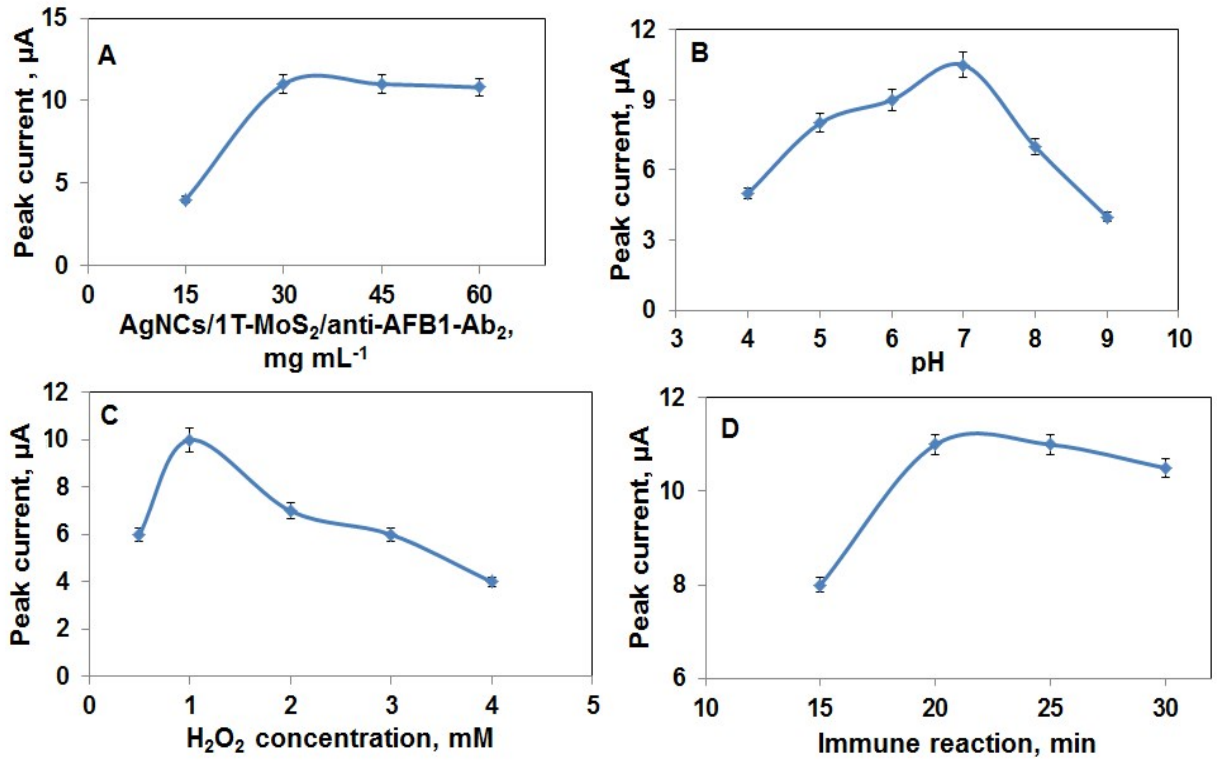

Fig. S5. Effect of (A) AgNCs/1T-MoS_2/anti-AFB1-Ab_2 concentration, (B) pH, (C) H_2O_2 concentration, (D) immune reaction time (Antigen AFB1 concentration: 0.100 pg mL^{-1}, frequency of 50 Hz, pulse amplitude of 20 mV, scan increment of 3 mV for DPV measurements) (n = 6)

3.7. Recovery

Table S1. The recovery of AFB1 in 1.0 mM H_2O_2 in pH 7.0, 0.1 M PBS (n=6)

Wheat sample	Added AFB1 (pg mL^{-1})	Found AFB1 (pg mL^{-1})	Recovery (%)
[a]Sample (1)	-	0.106 ± 0.003	-
[b]Sample (2)	0.100	0.205 ± 0.001	99.51 ± 0.04
[c]Sample (3)	0.300	0.406 ± 0.002	100.00 ± 0.07
[d]Sample (4)	0.500	0.605 ± 0.002	99.83 ± 0.04
[e]Sample (5)	0.700	0.807 ± 0.003	100.12 ± 0.03

[a]containing 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA
[b]containing 0.100 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA
[c]containing 0.100 + 0.200 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA
[d]containing 0.100 + 0.400 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA
[e]containing 0.100 + 0.600 pg mL^{-1} AFB1, 1.00 pg mL^{-1} AFB2, 1.00 pg mL^{-1} AFG1, 1.00 pg mL^{-1} AFG2, 1.00 pg mL^{-1} OTA, 1.00 pg mL^{-1} DON, 1.00 pg mL^{-1} ZEN and 1.00 pg mL^{-1} BSA

3.9. Precision and Accuracy

Table S2. Intra-day and inter-day precision and accuray results of AFB1 in 1.0 mM H_2O_2 in pH 7.0, 0.1 M PBS (n=6)

Added pg mL^{-1}	Intra-day			Inter-day		
	Found[a] (pg mL^{-1})	Precision[b] (%)	Accuracy[c] (%)	Found[a] (pg mL^{-1})	Precision[b] (%)	Accuracy[c] (%)
0.100	0.102 ± 0.0001	0.240	2.00	0.101 ± 0.0002	0.485	1.00
0.300	0.301 ± 0.0002	0.163	0.33	0.299 ± 0.0002	0.164	0.33
0.500	0.502 ± 0.0001	0.049	0.40	0.501 ± 0.0002	0.098	0.20

[a]Mean ± Standart Error, [b]Precision %: Relative Standart Deviation (RSD), [c]Bias %: [(found – added)/added]×100%