Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Material

1. UV-Vis spectroscopy

The migration or disappearance of UV-Vis absorption spectrophotometry Soret absorption band of heme may provide structure information about denaturation or conformational changes of heme proteins. Fig. S1 showed UV-Vis spectra of Mb (curve a, the typical Soret absorption band at 410.4 nm) and TiO_2@CNFs/Mb composite (curve b, the Soret absorption band at 410.6 nm), which was formed on the surface of a glass slide. The results indicated that Mb retained its essential feature of native structure after mixing with TiO_2@CNF due to its good biocompatibility.

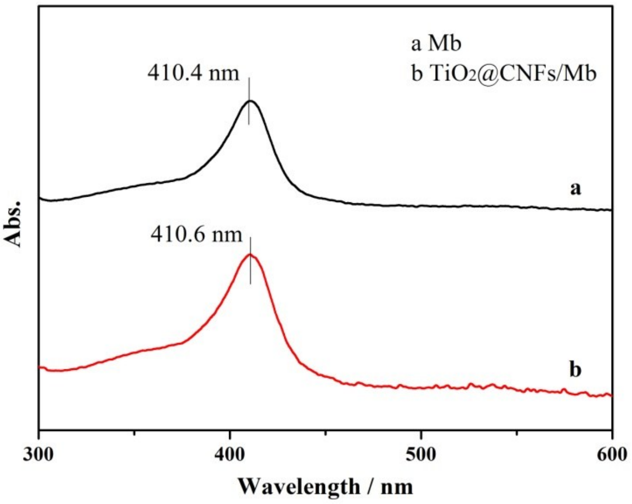

Fig. S1 UV-Vis absorption spectra of Mb and TiO_2@CNF/Mb.

2. FT-IR spectroscopy

FT-IR spectroscopy including the amide I band (1700~1600 cm^{-1}, C=O stretching vibrations of the peptide linkage) and the amide II band (1620~1500 cm^{-1}, a combination of N=H in-plane bending and C=N stretching vibrations) showed in Fig. S2. The changes of the intensity or shape of infrared bands such as diminishing or disappearing would illustrate the conformational transitions of Mb natural structure. The amide I and II bands of Mb appeared at 1650 and 1539 cm^{-1} (curve a), and that of Mb with TiO_2@CNF nanocomposite located at 1649 and 1537 cm^{-1} (curve b), which indicated that Mb molecule retained its natural conformation after mixing with

TiO_2@CNF nanocomposite. The minor shift of amide I and II band may be caused by hydrogen bond or electrostatic attraction that presented between Mb and oxygenal group of TiO_2@CNF nanocomposite.

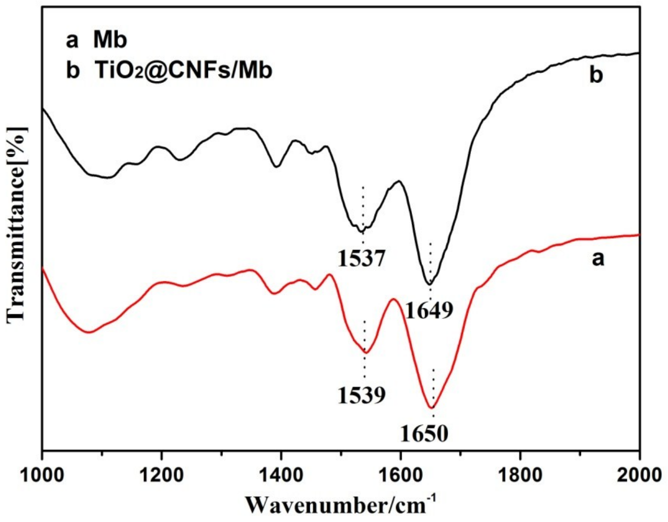

Fig. S2 FT-IR spectra of Mb and TiO_2@CNF/Mb.

3. EIS results

Electrochemical impedance spectroscopy (EIS) is often used to monitor the assembly process. The semicircle diameter of Nyquist plot at high frequencies reflects the electron transfer resistance (R_{et}), which controls the electron transfer kinetics of the redox probe $[Fe(CN)_6]^{3-/4-}$ at the electrode interface. Fig. S3 displayed the EIS observed upon the changes of surface-modified process with the frequencies swept from 10^4 to 0.1 Hz (initial potential as 0.202 V and amplitude as 0.005 V). The R_{et} value of Nafion/CILE was recorded as 35.50 Ω (curve a), when TiO_2@CNF nanocomposite was modified on the surface of the electrode, the impedance value of Nafion/TiO_2@CNF/CILE (curve b) was decreased to 23.57 Ω. Further modified by Mb, R_{et} of Nafion/Mb/CILE (curve c) and Nafion/Mb/TiO_2@CNF/CILE (curve d) increased to 72.37 Ω and 46.86 Ω respectively, indicating the presence of Mb on the electrode surface hindered the electron transfer and resulted in an increase of impedance. Because CNF is carbon nanomaterial with high conductivity and the doped TiO_2 is a semiconductor, therefore the TiO_2@CNF also exhibited excellent conductivity with the interfacial resistance decreased correspondingly.

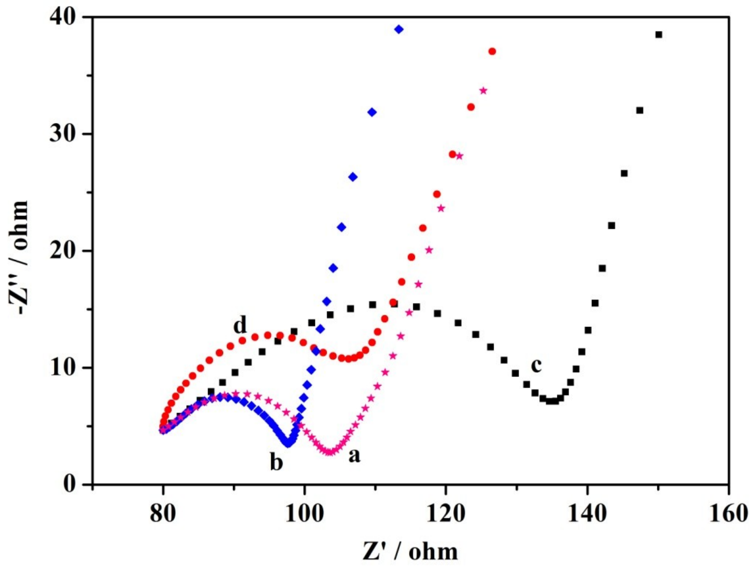

Fig. S3 EIS of Nafion/CILE (curve a), Nafion/TiO_2@CNF/CILE (curve b), Nafion/Mb/CILE (curve c) and Nafion/Mb/TiO_2@CNF/CILE (curve d) in the presence of 10.0 mmol·L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol·L^{-1} KCl with the frequencies swept from 10^4 to 0.1 Hz.

4. Electrocatalysis

For comparison the control experiments to the electroreduction of these targets were performed with other modified electrodes including Nafion/CILE, Nafion/TiO_2@CNF/CILE and Nafion/Mb/CILE and the results were shown in Fig. S4. As for electrocatalytic reduction of 0 and 10 mmol·L^{-1} TCA with different electrodes, on Nafion/CILE (Fig. S4A, curve a and b) and Nafion/TiO_2@CNF/CILE (Fig. S4B, curve a and b), electrocatalysis could not be observed with obvious reduction peak. On Nafion/Mb/CILE (Fig. S4C, curve b) the electrocatalysis could be observed at -0.5 V for 10 mmol·L^{-1} TCA with response that was smaller than Nafion/Mb/TiO_2@CNF/CILE (Fig. S4C, curve d), which could be ascribed to the presence of Mb on the electrode surface. Mb exhibits peroxidase activity and Mb modified electrode can catalyze the reduction of specific analytes. TiO_2@CNF on the electrode surface acts as fast electron transfer bridge for the DET process of Mb. Similar phenomenon could be found for the electrode reaction with $NaNO_2$ (Fig. S4D, E, F) and H_2O_2 (Fig. S4G, H, I) on different electrodes. Therefore the presence of TiO_2@CNF on the electrode played important roles in the acceleration of direct electron transfer of Mb.

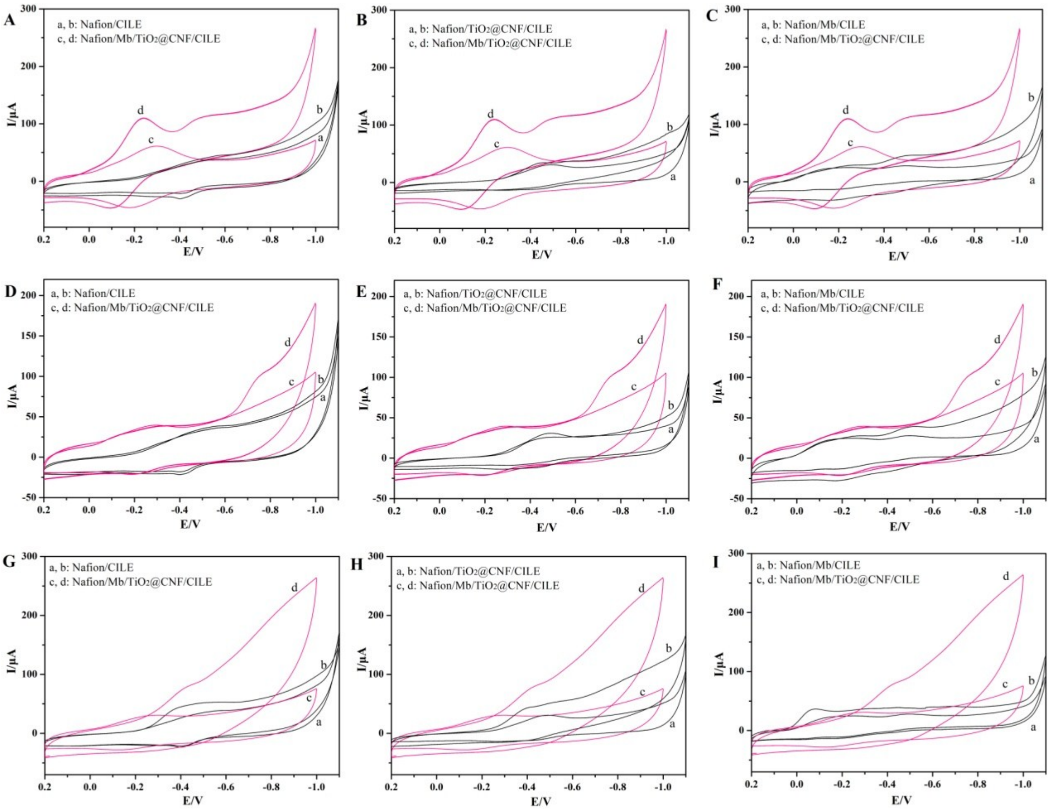

Fig. S4 Comparisons of modified electrodes for the detection of various targets. (A, B, C) CVs of different electrodes in pH 4.0 PBS with the presence of (a, c) 0 and (b, d) 10 mmol·L^{-1} TCA; (D, E, F) CVs of different electrodes in pH 4.0 PBS with the presence of (a, c) 0 and (b, d) 20 mmol·L^{-1} $NaNO_2$; (G, H, I) CVs of different electrodes in pH 4.0 PBS with the presence of (a, c) 0 and (b, d) 10 mmol·L^{-1} H_2O_2.

5. A comparison of the analytical parameters of different sensors for TCA, $NaNO_2$ and H_2O_2 detection

Table 1 Comparison of analytical performances of CILE/Mb/TiO_2@CNF/Nafion with other reported modified electrodes for TCA, $NaNO_2$ and H_2O_2 detection.

No.	Modified electrode	Analyte	Linear range (mmol·L^{-1})	Detection limit (mmol·L^{-1})	Reference
1	HA-HRP-CdS-IL/CILE	TCA	1.6~18.0	0.53	[1]
2	CTS-Mb-GR-IL/CILE	TCA	2.0~16.0	0.583	[2]

3	Nafion-Mb-SGO- GCE	$NaNO_2$	2.0~24.5	1.5	[3]
4	Nafion/Hb/B-GQDs/CILE	H_2O_2	4.0~24.0	1.3	[4]
5	Nafion/Mb/TiO_2@CNF/CILE	TCA	5.0~105.0	1.6	this work
		$NaNO_2$	1.0~70.0	0.3	
		H_2O_2	2.8~32.0	1.0	

* HA: hyaluronic acid; IL: ionic liquid; CILE: carbon ionic liquid electrode; CTS: chitosan; Mb: myoglobin; GR: graphene; GCE: glassy carbon electrode; Hb: hemoglobin; B-GQDs: boron-doped graphene quantum dots;

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