Supporting Information for the Article

An electrochemiluminescence biosensor for sensitive and selective detection of Hg^{2+} based on π-π interaction between nucleotide and ferrocene-graphene nanosheets

Bangrong Zhuo,†[a] Yuqin Li,†[b] An Zhang,[a] Fushen Lu,[a] Yaowen Chen[c] and Wenhua Gao*[ac]

[a] Department of Chemistry, Shantou University, Shantou, Guangdong 515063, P. R. China. E-mail: whgao@stu.edu.cn; Fax: +86-22-82903941; Tel: +86-22-86502774

[b] Department of Pharmacy, Taishan Medicine College, Taian, Shandong 271016, P. R. China.

[c] Analysis & Testing Center, Shantou University, Shantou, Guangdong 515063, P. R. China.

† Bangrong Zhuo and Yuqin Li contributed equally to this work.

Preparation of Ferrocence-graphene nanosheets (Fc-GNs)

Graphene Oxide (GO) was prepared from graphite flake based on the modified Hummers method. Firstly, GO (100 ml, 0.5 $mg \cdot mL^{-1}$) was intermittently dispersed in N, N'-Dicyclohexyl-carbodiimide (DCC, 25 mg) and Ethylenediamine (ED, 25 mL) followed by ultrasonication for 1 h, and the brown and homogeneous mixture was stirred and heated to 70 °C for 24 h. After that, the resulting black and homogeneous converted grapheme sheets, which were chemically aminated and functionalized, were centrifuged and washed with absolute tetrahydrofuran and ethanol, and then dried under vacuum 40 °C to get the aminated grapheme (GON).

45 mg of GON was dispersed in 15 mL doubly distilled water with ultrasonication for 10 min, and then was added to the ferrocene-carboxaldehyde (FcCHO) ethanol solution (30 mL, 7 $mg \cdot mL^{-1}$) with stirring at room temperature. After 3 h, 600mg Sodium borohydride ($NaBH_4$) was added to the mixture, and left to stir for 3 h. Then residual $NaBH_4$ was added to the above solution with stirring at 85 °C. After 12 h, the Fc-GNs nanocomposite was centrifuged, and washed with ethanol and water several times to remove the dissociative FcCHO or some physically adsorbed FcCHO from the surface of the graphene. The graphene without any modification was prepared by the reduction of $NaBH_4$ at 85 °C for 12 h.

Result and discussion

Fig. S1 FTIR transmittance spectra of GO (a), GON (b) and Fc-GNs (c).

The results from FTIR revealed that the characteristic band of the carboxyl group in GO (Fig. S1a) appeared at *ca.* 1702.57 cm^{-1} (C=O stretching) and the stretching of O-H at 3412.02 cm^{-1}. The C-O-C (*v* (epoxy symmetrical ring deformation vibration)) appeared at 1233.67 cm^{-1}. As shown in Fig. S1b, the stretches at 3178.23 cm^{-1}, 1538.11 cm^{-1} and 648.66 cm^{-1} corresponded to NH_2 (*v* (stretching vibration)), NH (*v* (plane deformation vibration, scissoring and swing)), the C=O of –CON appeared at 1642.07 cm^{-1} (*v* (stretching vibration)) and C=N at 1342.36 cm^{-1}. Under the given reaction conditions, the ED grafted to GO by -COOH and C-O-C, which was confirmed by our FTIR analysis. After Fc-GNs was reduced, the C=O stretching of -CON at *ca.* 1647.45 cm^{-1}, the asymmetric stretching vibrational of x-CON (x = C or Fc) at *ca.* 1042.39 cm^{-1} and the Ring-Titt of torsional vibration of Fc at *ca.* 445.90 cm^{-1} (Fig. S1c) confirm that Fc-GNs can be achieved by using the chemical approach.

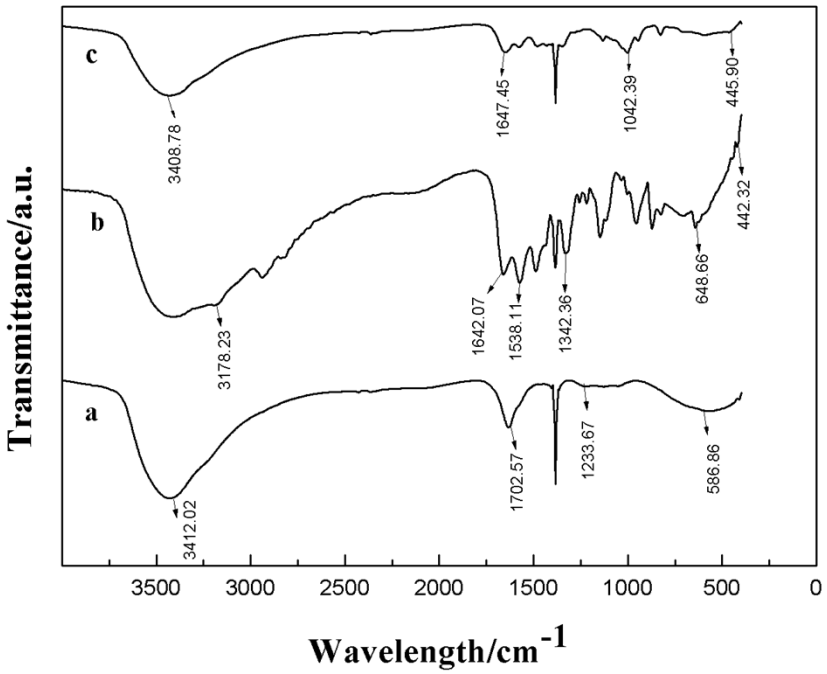

Fig. S1

Fig. S2 AFM and TEM images of GO (A, C) and Fc-GNs (B, D).

The morphologies of GO (in water) and Fc-GNs (in water) nanocomposites were characterized by AFM and TEM. Fig. S2A, C images revealed that GO nanosheets were well exfoliated and prefect single-planar sheets, with a thickness of 0.85 nm. After reduction of Fc functionalized GO, spherical nanoparticles were formed on the graphene sheets (Fig. S2B, D). Observation under higher magnification revealed the well-dispersed Fc nanoparticles on the wrinkly graphene support. The exfoliated Fc-GNs with an average thickness of *ca.* 18 nm has been obtained in the work. The Fc nanoparticles were stable under the ultrasonic condition, and the resulting biosensor based on Fc nanoparticles exhibited conspicuous performance with long-term stability, which to some extent indicated that the Fc nanoparticles were immobilized on the

surface of graphene through stable bonding, not simple physical adsorption.

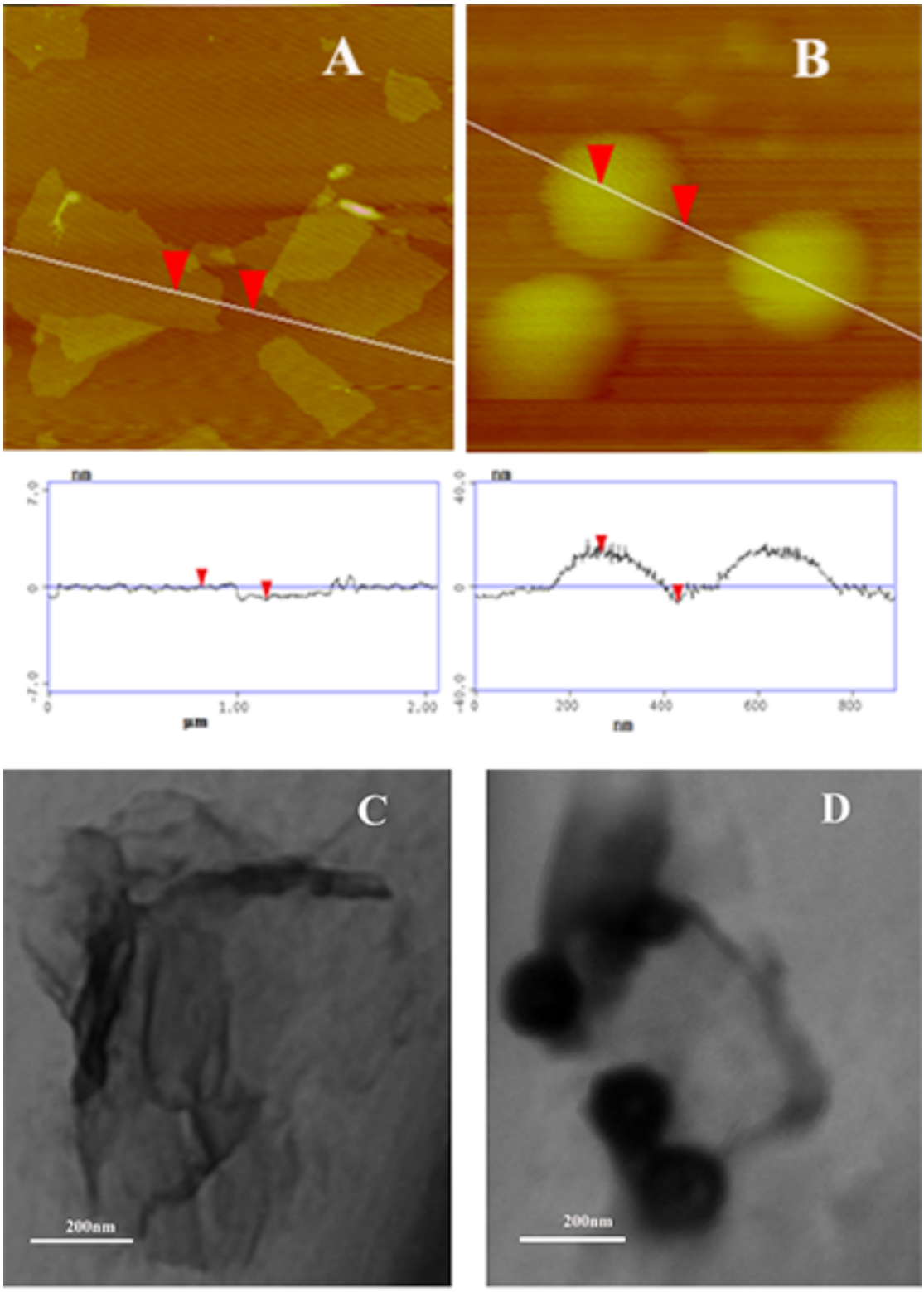

Fig. S2

Table S1 Determination the concentrations of Hg^{2+} in water samples.

Water sample	Added (nM)	Biosensing method (nM)	RSD (%)	Recovery (%)
Xinhua River 1	0	70.42	1.74	-
	100	168.73	1.35	98.31
Xinhua River 2	0	91.08	2.48	-
	100	192.97	2.94	101.89
Niutianyang bay 1	0	105.48	1.97	-
	100	203.26	2.31	97.78
Niutianyang bay 2	0	140.86	3.14	-
	100	241.15	2.86	100.29