Supporting Information

Indicator displacement assay for cholesterol electrochemical sensing using calix[6]arene functionalized graphene-modified electrode

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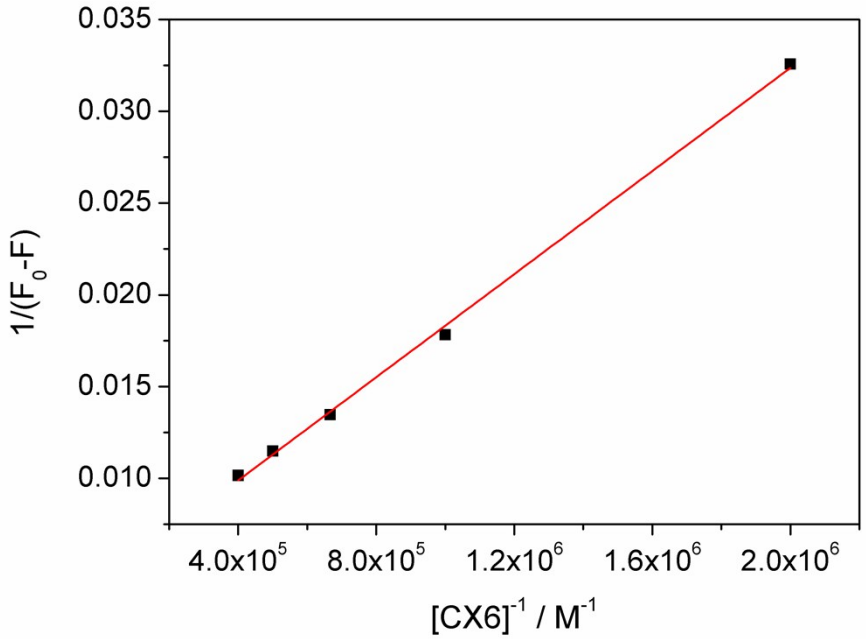

Fig. S1. Plot of $1/(F_0-F)$ versus 1/[CX6].

Fig. S2. Photographs of Gra and CX6-Gra aqueous dispersions (1.0 mg mL^{-1}).

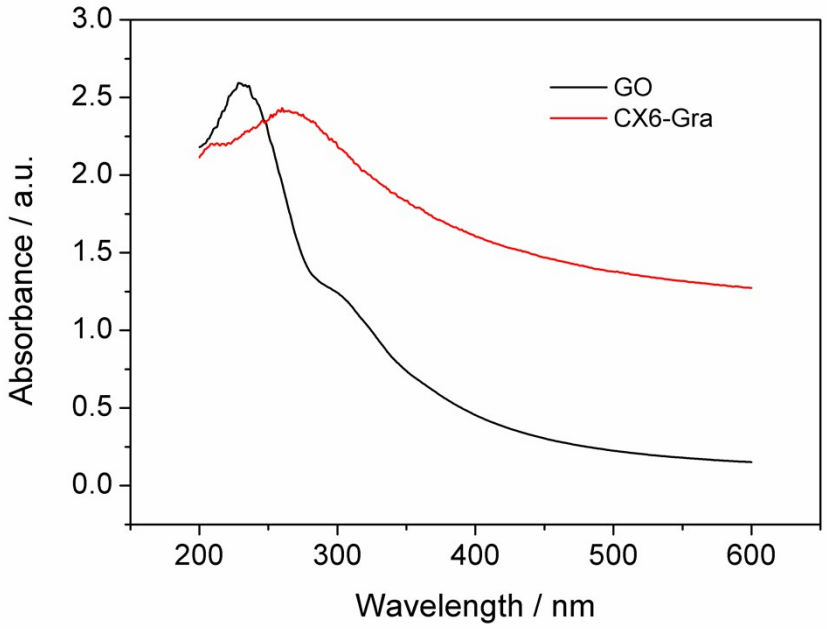

Fig. S3. UV–vis absorption spectra of GO and Gra.

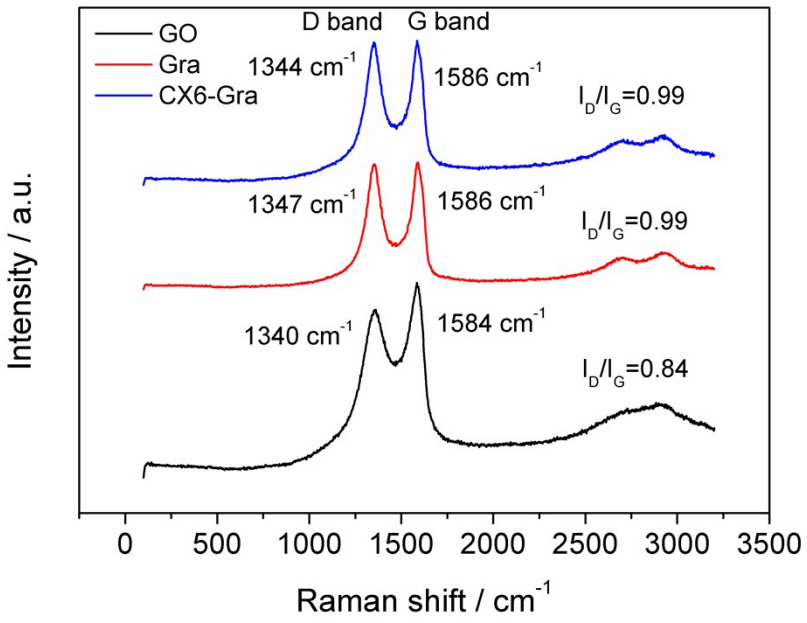

Fig. S4. Raman spectra of GO, Gra, CX6-Gra.

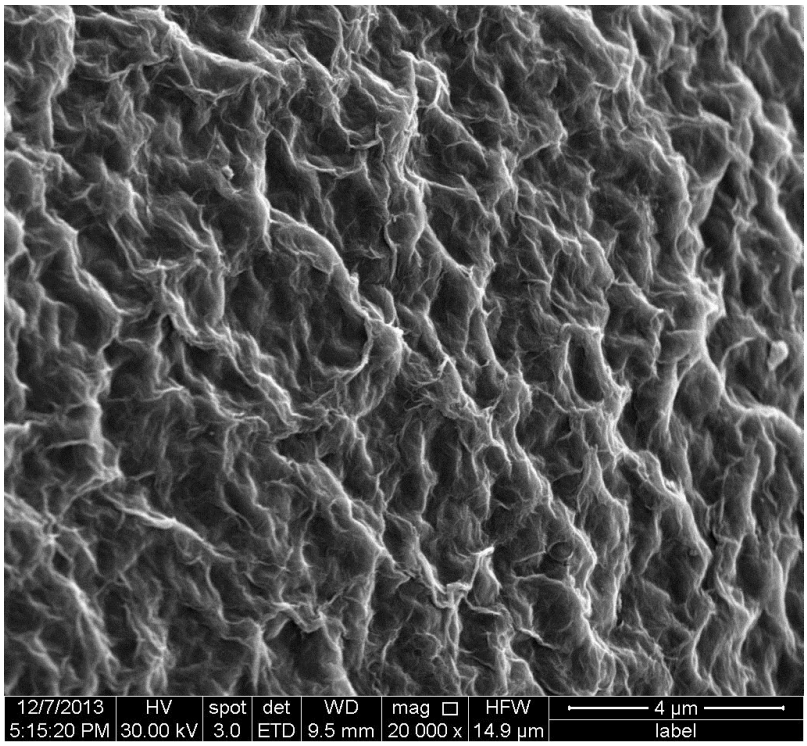

Fig. S5. SEM image of CX6-Gra.

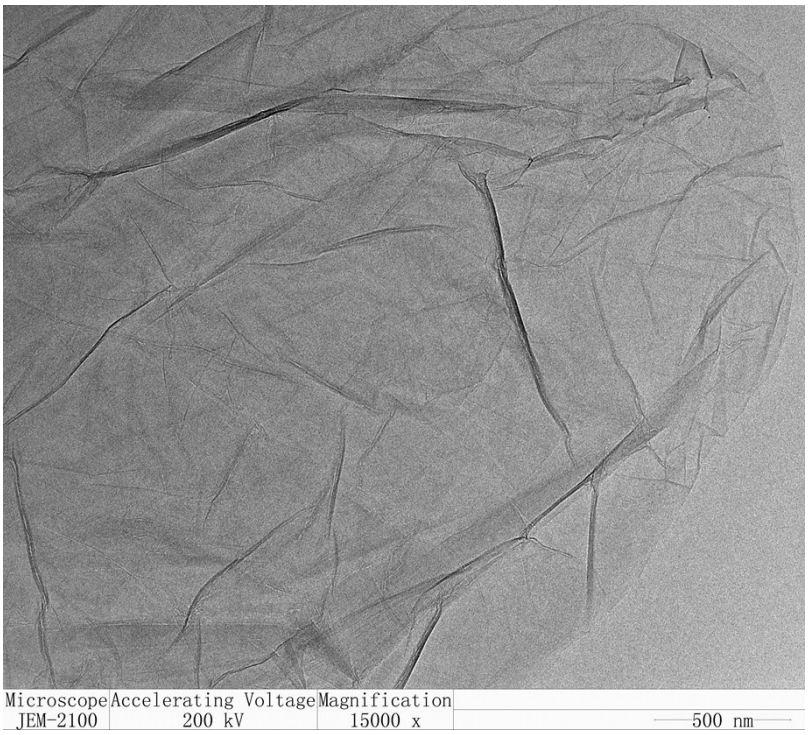

Fig. S6. TEM image of CX6-Gra.

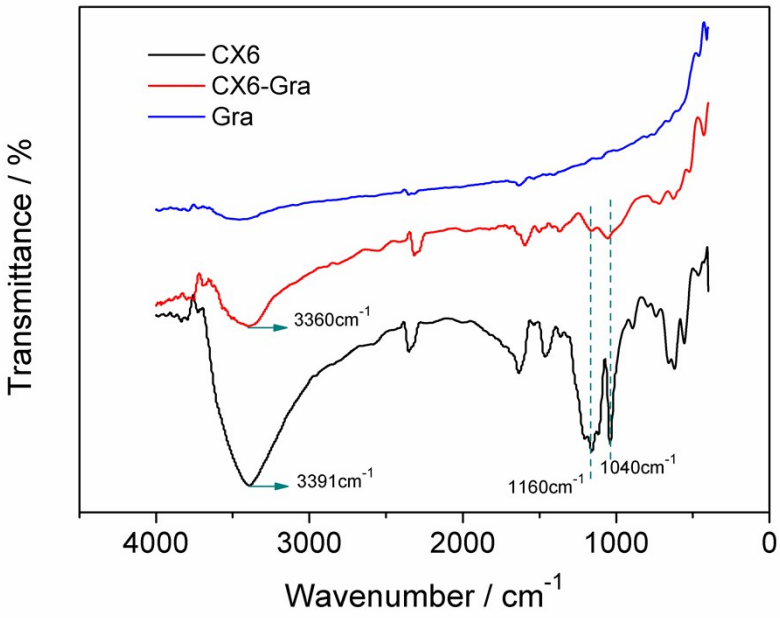

Fig. S7. FTIR spectra of CX6, CX6-Gra, and Gra.

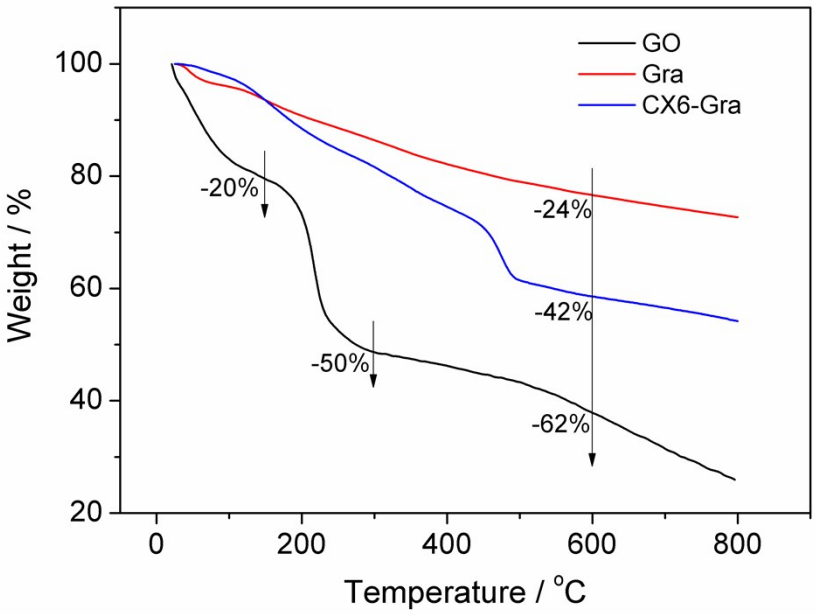

Fig. S8. TGA curves of GO, Gra, and CX6-Gra.

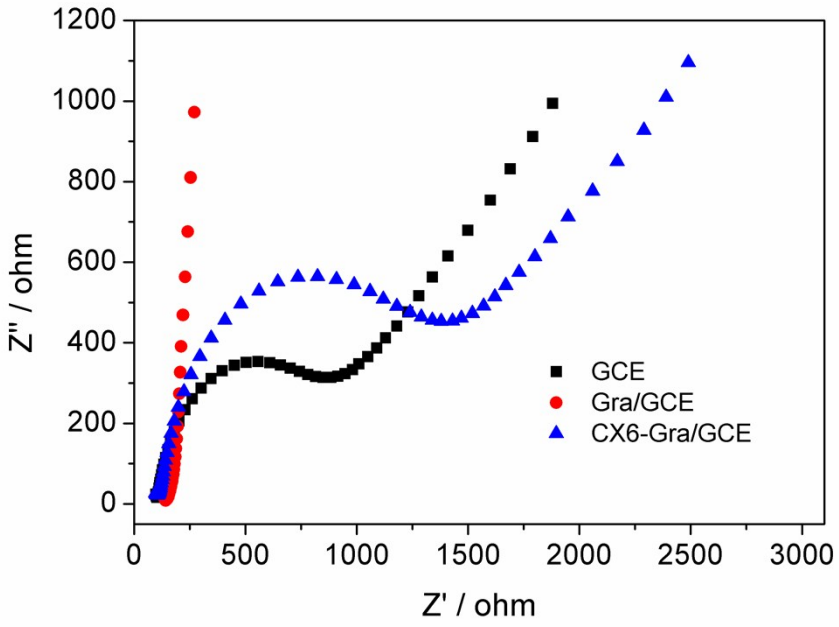

Fig. S9. Nyquist diagram of electrochemical impedance spectra of bare GCE, Gra/GCE, CX6-Gra/GCE using 2.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple (1:1) with 0.1 M KCl as supporting electrolyte.

Optimization of experimental conditions

Several control experiments were carried out to determine the optimum reaction conditions. The incubation time of the CX6-Gra/GCE in MB solution is one of the key parameters that will affect the response performance of the electrode. **Fig. S10** shows that the reduction peak current of the electrode increases with the increase of the incubation time and reaches a maximum at 30 min for MB incubation. Therefore, 30 min is selected as the optimum incubation time for the as-prepared electrode in MB solution. Similarly, the effect of the incubation time of the MB-bound CX6-Gra/GCE in cholesterol solution was also studied. **Fig. S11** shows that the reduction peak current of the electrode decreases with the increase of the incubation time up to 30 min and then level off thereafter for cholesterol incubation. Therefore, 30 min is

selected as the optimum incubation time for the as-prepared MB-bound CX6-Gra/GCE in cholesterol solution.

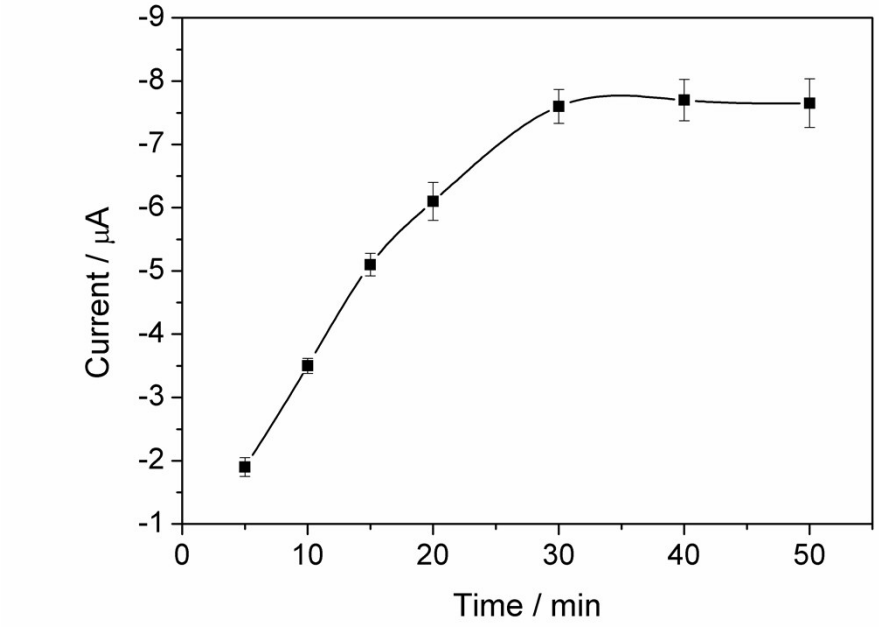

Fig. S10. Effects of incubation time on the DPV peak currents of the CX6-Gra/GCE in 0.1 M PBS (pH 7.0) after incubation in 100 μM MB solution.

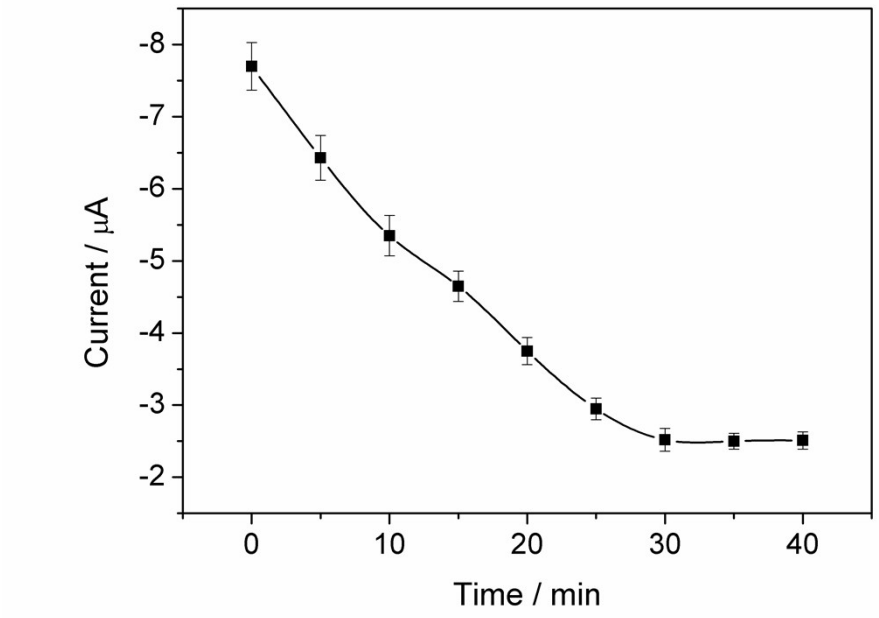

Fig. S11. Effects of incubation time on the DPV peak currents of the MB-bound CX6-Gra/GCE in 0.1 M PBS (pH 7.0) after incubation in 50 μM cholesterol solution.

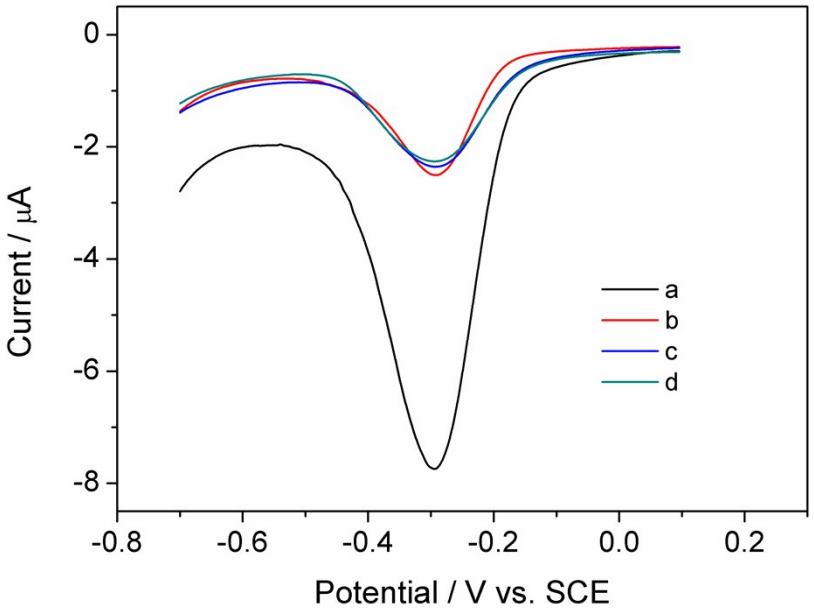

Fig. S12. Comparative studies of DPV for MB-bound Gra/GCE and MB-bound CX6-Gra/GCE before and after incubation in 50 μM cholesterol solution. MB-bound CX6-Gra/GCE before (a) and after (b) incubation in 50 μM cholesterol; MB-bound Gra/GCE before (c) and after (d) incubation in 50 μM cholesterol.

Table S1

The interaction energy between cholesterol and CX6 for 1:1 stoichiometry calculated by PM3 method.

System	Cluster rank	Number in cluster	ΔG (kcal/mol)
Cholesterol/CX6	1	17	–8.01
	2	2	–7.52
	3	6	–7.48
	4	12	–7.32
	5	4	–7.25
	6	3	–7.23

Reproducibility and stability of the sensor

Six equal MB-bound CX6-Gra/GCEs were used to evaluate the fabrication reproducibility of the present method for cholesterol detection. The six modified electrodes exhibited similar signals with a relative standard deviation of 3.7%, indicating satisfactory reproducibility. Additionally, a long-term stability experiment

was performed intermittently (every 5 days) and used to examine the stability of the MB-bound CX6-Gra/GCE. The constructed sensor was stored in a refrigerator at 4 °C when not in use. Initial responses of over 95.2% and 86.4% remained after storage for 15 and 30 days, respectively, indicating an acceptable stability of the MB-bound CX6-Gra/GCE.