Supporting information

Highly Sensitive Chiral Recognition of Amino Propanol in Serum with R-mandelic acid-Linked Calix[4]arene Modified Graphene

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1. Synthetic routes and procedures of FC4D:



In a 50 mL flask, calix[4]arene 2 was dissolved in ethanol (20 mL) under an argon atmosphere. Mandelic acid hydrazide (0.12 g, 0.51 mmol) was added. The solution was stirred for 30 min at ambient temperature and then catalytic amount of HOAc was added. The mixture was stirred for 10 h. After evaporation of the solvent, the crude product was dissolved in CH₂Cl₂, washed with brine for three times, and dried over with anhydrous Na₂SO₄. The residue was subject to column chromatography (silica gel, petroleum ether-ethyl acetate, 10 : 1), yielding Calix[4]arene R-mandelic acid derivatives 1 as a white powder (0.13 g, 65%). ¹H NMR (600 MHz, DMSO) δ: 8.27 (d, J = 57.6 Hz, 2H, HC=N), 8.10 (s, 1H, ArH), 7.95 (s, 1H, ArH), 7.82 (s, 1H, ArH), 7.34 (d, J = 7.4 Hz, 3H, ArH), 7.31 – 7.27 (m, 2H, ArH), 7.22 (d, J = 7.3 Hz, 1H, ArH), 7.07 – 6.88 (m, 4H, ArH), 5.10 (d, J = 31.5 Hz, 2H, CH2OH), 4.90–4.70 (m, 4H, OCH2), 4.23 (d, J = 12.8 Hz, 4H, ArCH2), 3.77 (s, 2H, OH), 3.53 (d, J = 12.5 Hz, 4H, ArCH2), 1.10 – 0.87 (m, 18H, tBu) ppm; ¹³C NMR (151 MHz, DMSO) δ: 184.76, 169.11, 155.74, 149.90, 147.68, 141.43, 132.90, 128.41, 127.53, 80.09, 79.23, 73.39, 63.57, 34.47, 31.39, 25.55 ppm; Calcd for m/z=964.4. found m/z= 963.2.

2. Preparation of Graphene Oxide

GO was prepared by oxidation of natural graphite powder (325 mesh, Tianjing) according to the method developed by Hummers and Offemann.^[18] Briefly, native graphite flake (2 g) was mixed with concentrated H_2SO_4 (12 mL), $K_2S_2O_8$ (4 g), and P_2O_5 (4 g), and then incubated at 80 °C to preoxidize the graphite. The resultant dark blue mixture was thermally isolated and allowed to cool to room temperature over a period of 6 h. The mixture was diluted to 150 mL and then filtrated. The product was then dried in air at ambient temperature overnight, after washing with distilled water until neutral. This preoxidized graphite was then subjected to oxidation by Hummer's method. The preoxidized graphite powder (1 g) was added to 46 mL of cold H_2SO_4 (0 °C), and 6 g of KMnO₄ was gradually added under stirring in an ice bath with stirring while keeping the temperature of the mixture below 20 °C. After 1 g of NaNO₃ was added to the mixture stirred for 15 min, and the resulting mixture was further stirred at 35 °C for 2 h and distilled water (100 mL). The reaction was ceased by the addition of a large amount of distilled water (280 mL) and 5 mL of 30% H₂O₂ was added, after which the colour of the mixture changed to bright yellow. The mixture was filtered and washed with 1:10 HCl aqueous solution (250 mL) to remove metal ions followed by washing with 200 mL of water to remove the acid. The resulting solid was dried in air and diluted to make a GO aqueous dispersion (0.5 mg/mL), which is stable for a period of years, was used to prepare exfoliated GO. The purified GO dispersion was sonicated for 1.5 h at 300W to exfoliate GO, and unexfoliated GO was removed by centrifugation (3000 rpm, 5min). Eventually, it was further purified dialysis for one

week to remove the remaining metal species. The GO solution was stable for a period of months and was used for film preparation.

3. Preparation of FC4D-G

GO (400 mg) was dispersed by ultrasonication in water/AcCN 1:1 (20 mL). NaN₃ (500 mg, 7.7 mmol) were added and the mixture was heated to reflux for one week under nitrogen (Warning: azide derivatives are potentially explosive! They must be handled with care). After cooling, CH_2Cl_2 (80 mL) was added and the solid was filtered and washed under ultrasonication many times with water and AcCN, and dried under vacuum for 5 h. FC4D-G was prepared by dispersing GO-N₃ (10 mg) by ultrasonication into a mixture of H₂O/DMSO (2:1). FC4D (10 mg, 3 mL), sodium ascorbate (4 mg), and CuSO₄ (1 mg) were added to the solution, which was then stirred at room temperature for 10 days. During the reaction, the mixture was sonicated several times. Afterwards, functionalized GO were treated by centrifugalization method and washed with water several times to remove the excess of CC4D. The obtained product was characterized by atomic force microscopy (AFM), static contact angle (CA), and Fourier-transform infrared (FTIR) spectrum.



Figure S1. The ¹H NMR spectrum of FC4D.



Figure S2. The ¹³C NMR spectrum of FC4D.



Figure S3. ¹H NMR spectra (DMSO, 600 MHz, 298 K) of (a) R/S-AP (15 mM), (b) FC4D and R-AP (15 mM each), (c) FC4D and S-AP (15 mM each), (d) FC4D (15 mM).



Figure S4. MALDI-TOF mass spectrum of FC4D.



Figure S5. MALDI-TOF mass spectrum of an equimolar mixture of FC4D and 1 • S-AP in DMSO solution.



Figure S6. The complex modes formed by FC4D with S-AP, which were calculated at b3lyp/6-31G(d) levels using Gaussian 03[1] package. Three hydrogen bonds were turned out in these FC4D and S-AP complex.



Figure S7. Direct ultra violet titration of FC4D (10⁻⁴ M) with R-AP A); and S-AP C); The associated titration curve, fitted according to a 1 : 1 binding stoichiometry, between FC4D and R-AP B); or S-AP D).



Figure S8. TEM images of the GO.



Figure S9. FT-IR spectra of the reduced graphene oxide (curve black), GO-N₃ (curve red), and FC4D-G (curve blue).

element	С	0	Ν
GO	51.22%	48.78%	
GO-N ₃	78.08%	18.99%	2.93%
FC4D-G	80.03%	16.42%	3.55%

Figure S10. Element content analysis of GO, GO- N_3 , and FC4D-G.



Figure S11. Raman spectra showing the D and G bands of GO-N₃, FC4D-G.



Figure. S12. Impedance spectroscopy of 5.0 m M Fe(CN) $6^{3-/4-}$ In 0.10 M KNO₃ aqueous solution on bare GE electrode, GO and FC4D-G electrode with the frequency ranging from 10 kHz to 1.0 kHz, scan rate: 0.10 v/s, indicating that FC4D successfully modified on G surface;



Figure S13. The hydrodynamic radius of A) GO, B) GO-N₃ C) FC4D-G confirmed by diffraction light scattering were 451.7 nm, 532.6 nm, 622.9 nm, respectively.



Figure S14. Parallel experiments for detection of R/S-AP (10⁻³ M) with FC4D-G modified GC.



Figure S15. Impedance spectroscopy of $Fe(CN)_6^{3-/4-}$ as redox probe in the presence of increasing concentrations of A) S-AP B) S-AP ($1.0 \times 10^{-10} - 1.0 \times 10^{-3}$ M); C) a plot of the Rct versus concentrations of R/S-AP ($1.0 \times 10-10 - 1.0 \times 10-3$ M), indicating that Rct value is good linear relationship with the increasing concentration of R/S-AP.



Figure S16. Impedance spectroscopy of $Fe(CN)_6^{3-/4-}$ as redox probe in the presence of increasing concentrations of R/S-AP A) (1.0×10^{-4} M), B) (1.0×10^{-5} M), C) (1.0×10^{-6} M), D) (1.0×10^{-7} M), E) (1.0×10^{-8} M), F) (1.0×10^{-9} M) in serum solution.