Electronic Supplementary Information (ESI)

Graphene Oxide for Rapid MicroRNAs detection

Zhuoxuan Lu,[ab] Liming Zhang,*[ab] Yan Deng,[a] Song Li,[a] and Nongyue He*[ab]

Experimental section

Synthesis of GO

GO was prepared by Hammer's method[1] with minor modification followed by strong sonication and centrifugation to disperse and remove large GO layers. Briefly, native graphite flake (1 g) was mixed with concentrated H_2SO_4 (1.5 mL), $K_2S_2O_8$ (0.5 g, 1.85 mmol), and P_2O_5 (0.5 g, 3.52mmol), and then incubated at 80 °C for 6 h to preoxidize the graphite. The product was then dried in air at ambient temperature overnight, after washing with distilled water until neutral and filtering. This preoxidized graphite was then subjected to oxidation by Hammer's method. The preoxidized graphite powder (1 g) was placed in concentrated H_2SO_4 (23 mL) at 0 °C. $KMnO_4$ (3 g, 19.0mmol) was added gradually with stirring while keeping the temperature of the mixture below 20 °C. The mixture was then stirred at 35°C for 2 h, followed by the addition of distilled water (46 mL), and stirring was continued for 15 min. Distilled water (140 mL) was then added to terminate the reaction. Subsequently, 30% H_2O_2 (2 mL) was added and the color of the mixture changed to bright yellow. The mixture was centrifuged and washed with 10% HCl solution (250 mL) to remove residual metal ions. The precipitate was then washed with distilled water and centrifuged repeatedly until the solution became neutral. To exfoliate the oxidized graphite, the product was treated with an ultrasonic probe at 400 W for 30 min, followed by centrifuging at 13 000 rpm for 30 min. The exfoliated GO was obtained in the supernatant. The precipitate was exfoliated repeatedly. Finally, a total of 700 mL supernatant was

obtained. The product obtained was steadily dispersed in water and would not precipitate for several months. Atomic force microscopy (AFM) images were recorded using a Nanoscope IIIa multimode atomic force microscope (Veeco Instruments, USA) in tapping mode to simultaneously collect height and phase data. TEM images were obtained on a Tecnai G2 F20 STWIN transmission electron microscope.

Sequences of miRNA and DNA

Sequences of miRNAs including the mir-21, let-7a, let-7d and let-7f were selected from the Sanger Center miRbase (http://microrna.sanger.ac.uk/). The Synthetic miRNAs and FAM-labeled DNA probes were purchased from GenePharma (Shanghai, China) and Generay (Shanghai, China), respectively.

Fluorescence emission spectra of at different conditions

Fluorescence measurements were performed on a Hitachi 4600 fluorospectrophotometer (Hitachi Co. Ltd., Japan) to investigate the binding of probe under three conditions including: 1) FAM-labeled probe was prepared as 50 nm in 20 mm Tris-HCl buffer (pH 7.4, containing 100 mm NaCl, 5 mm KCl and 5 mm $MgCl_2$); 2) FAM-labeled probe (50 nm) was mixed with GO in 20 mm Tris-HCl buffer for 5 min; 3) FAM-labeled probe (50 nM) was mixed with GO in 20 mm Tris-HCl buffer for 5 min prior to the addition of target miRNA (mir-21). And then mir-21 (300 nM) was added and this mixture to hybridize for about 30 min at room temperature.

The selectivity and sensitivity of the sensing platform

The selectivity of the sensing platform described herein has been determined by

examining the fluorescence responses of P7f-GO toward miRNA let-7f and the single-base different miRNA let-7a in two concentrations of buffer. The experimental procedures include two steps: 1) FAM-labeled probe P7f (50 nM) was mixed with GO in 1×Tris-HCl buffer (20 mM) and 0.5×Tris-HCl buffer for 5 min prior to the addition of miRNAs; 2) miRNA let-7f and let-7a (300 nM) was added and hybridized for about 30 min at room temperature, respectively. All reactions were run in triplicate and all solutions were prepared in DEPC-treated deionized water (TaKaRa Biotechnology Co.Ltd.)

The sensitivity of the approach described herein has been determined by examining the fluorescence responses of P7d-GO toward let-7d. The experimental procedures include two steps: 1) FAM-labeled probe P7d (50 nM) was mixed with GO in 1× Tris-HCl buffer (20 mM) buffer for 5 min prior to the addition of the target miRNA; 2) different concentrations of miRNA (ranging from 50 to 400 nM) was added and hybridized for about 30 min at room temperature. All reactions were run in triplicate and all solutions were prepared in DEPC-treated deionized water (TaKaRa Biotechnology Co.Ltd.)

Reference:

1. W. S. Hummers and R. E. Offeman, *Journal of the American Chemical Society*, 1958, **80**, 1339-1339.

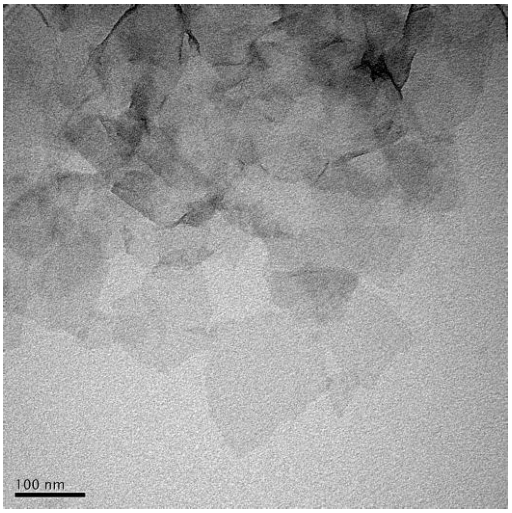

Figure S1. TEM image of as-prepared GO.

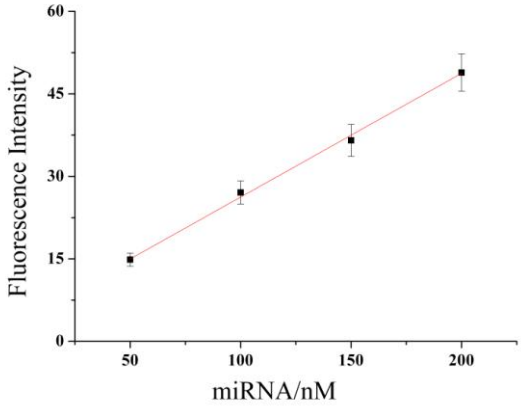

Figure S2 linear relationship between fluorescence intensity and concentration of target miRNA let-7d.