Supporting Information

Graphene Oxide-Protected DNA Probes for Multiplex MicroRNA Analysis in Complex Biological Samples Based on Cyclic Enzymatic Amplification Method ‡

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Experimental Section

Materials

DNase I, HPLC-purified RNA, RNase inhibitor, and DEPC-treated water were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). The DNA probes were synthesized on a PolyGen Column 12 DNA synthesizer and the reagents were purchased from Glen Research (Sterling, VA, USA). All DNA/RNA sequences are listed in Tables S1. Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method.[1] Cell lysate from lung carcinoma cell line A549 and MCF-10A was obtained according to the manufacturer's protocol.

Fluorescence measurements

Fluorescence measurements were carried out on a RF-5301-PC Fluorescence Spectrophotometer (Shimadzu, Japan). For P7a, excitation and emission wavelengths were set at 560 and 582 nm, respectively, with a 5 nm bandwidth. The emission spectra were obtained by exciting the samples at 560 nm and scanning the emission from 570 to 650 nm in steps of 1 nm. For P7e and P7i, excitation and emission wavelengths were set at 490 and 520 nm, 643 and 670nm, respectively, with same a bandwidth of 5 nm. All experiments were conducted in 20mM Tris-HCl (pH 8.0) buffer containing 5 mM $MgCl_2$ and 50 mM NaCl. The amplified detection of target were performed in 200 μL solution consisting of 50 nM probe, 20 units of DNase I and varying concentrations of target miRNA at RT for 20 min.

Gel electrophoresis

A 20% navitve PAGE analysis of the products from the cyclic enzymatic amplification reaction was carried out in 1×TBE (89mM Tris base, 89mM Boric acid, 2 mM EDTA, pH 8.3) for about 2 hours. After Stains-All staining, gels were scanned.

Table S1. Sequences of oligonucleotides used in this study

Name	Sequence
P7a	**5' -AAC TAT ACA ACC TAC TAC CTC A -TMR -3'**
P7e	**5'- ACT ATA CAA CCT CCT ACC TCA - FAM -3'**
P7i	**5'- AAC AGC ACA AAC TAC TAC CTC A - Cy5 -3'**
P-mir21	**5'-T CAA CAT CAG TCT GAT AAG CTA -TMR -3'**
Let-7a	**5' - UGA GGU AGU AGG UUG UAU AGU U -3'**
Let-7e	**5'- UGA GGU AGG AGG UUG UAU AGU -3'**
Let- 7i	**5'- UGA GGU AGU AGU UUG UGC UGU U -3'**

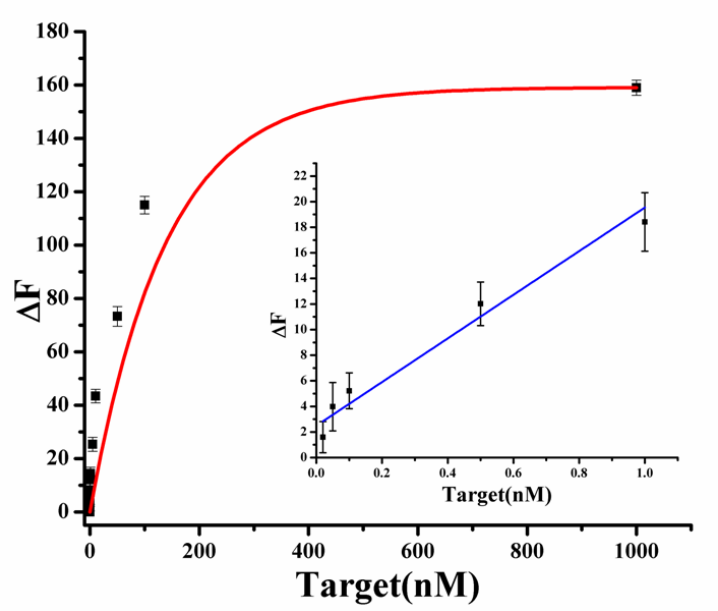

Figure S1. Change of fluorescence intensity as a function of target RNA concentration

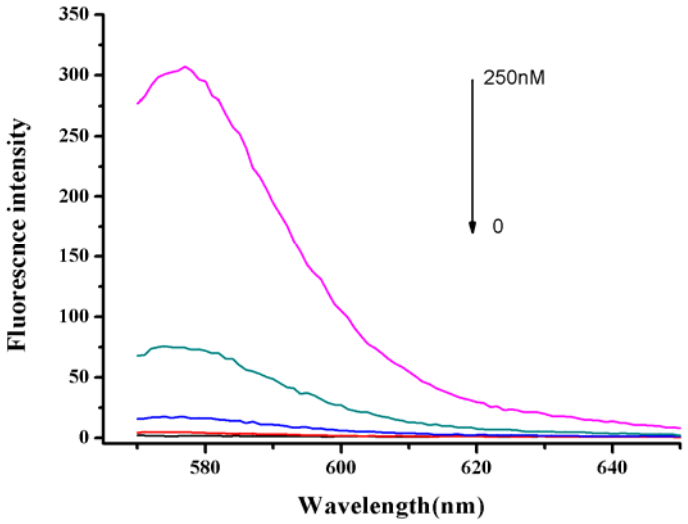

Figure S2. Detection of different concentrations of microRNA target in the absence of DNase I. Experiment was performed with 1×10^{-7} M probe and different concentrations of target. The curves from top to down contain target microRNA at the concentration of 2.5×10^{-7}, 5×10^{-8}, 1×10^{-8}, 5×10^{-9} and 0 M, respectively.

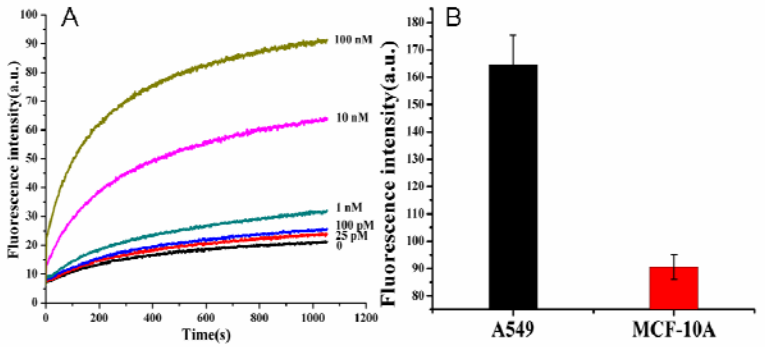

Figure S3. (A) Response of CEAM to different concentrations of target in cell media. (B) The expression profiles of mir-21 from A549 and MCF-10A.

References:

1. W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80,** 1339.