

Electronic Supplementary Information

Label-free impedimetric sensing platform for microRNA-21 based on ZrO₂-reduced graphene oxide nanohybrids coupling with catalytic hairpin assembly amplification

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1. Experimental section

1.1 Cell culture: MCF-7 (human breast cancer cell lines) and 293T (human embryonic kidney cell lines) cell lines were purchased from ATCC (Manassas, VA). All cells were cultured in RPMI 1640 medium (Gibco, Grand Island, NY) containing 10% FBS and 5% penicillin-streptomycin at 37 °C in a 5% CO₂ humid atmosphere. After the cell concentration reached 80%, the next cell experiments could be done. Cell density was determined using a hemocytometer prior to each experiment.

1.2 miRNA-21 extracts from cancer cells: The MCF-7/293T cells were grown to around 80% confluency for 24 h before the experiment. Total RNAs of MCF-7/293T cells were extracted with TRIzol reagent (Thermo, shanghai, China) according to the manufacturer's protocol. Then, the cleared lysate was flash frozen, and stored at -80 °C before use. The stored solution was further consecutively diluted with deionized water in order to obtain the proper solution for detection (our proposed methods).

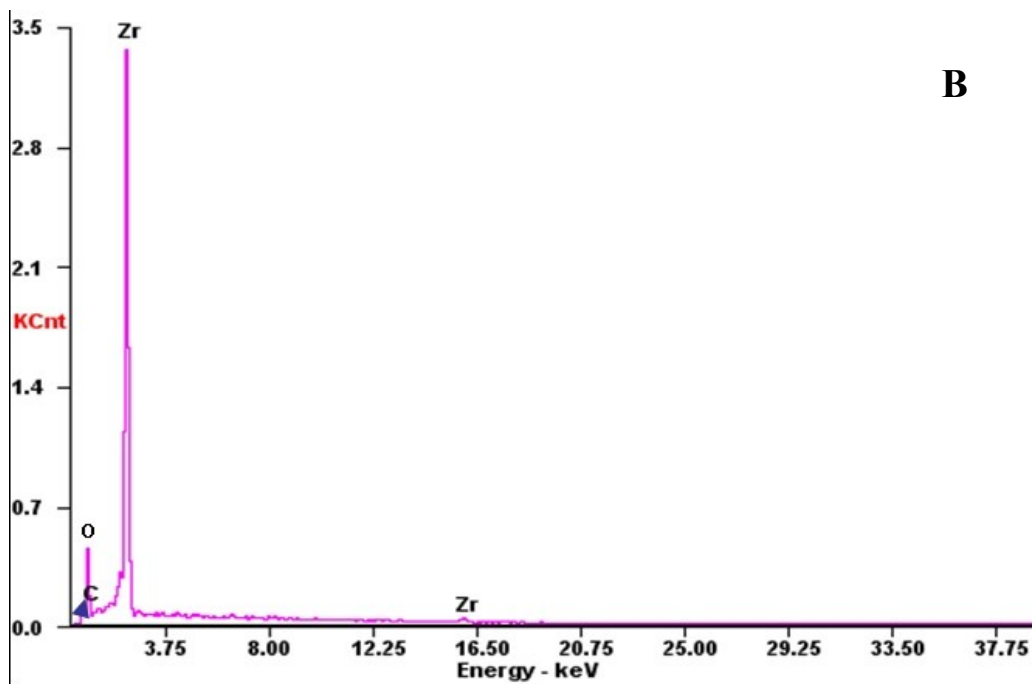
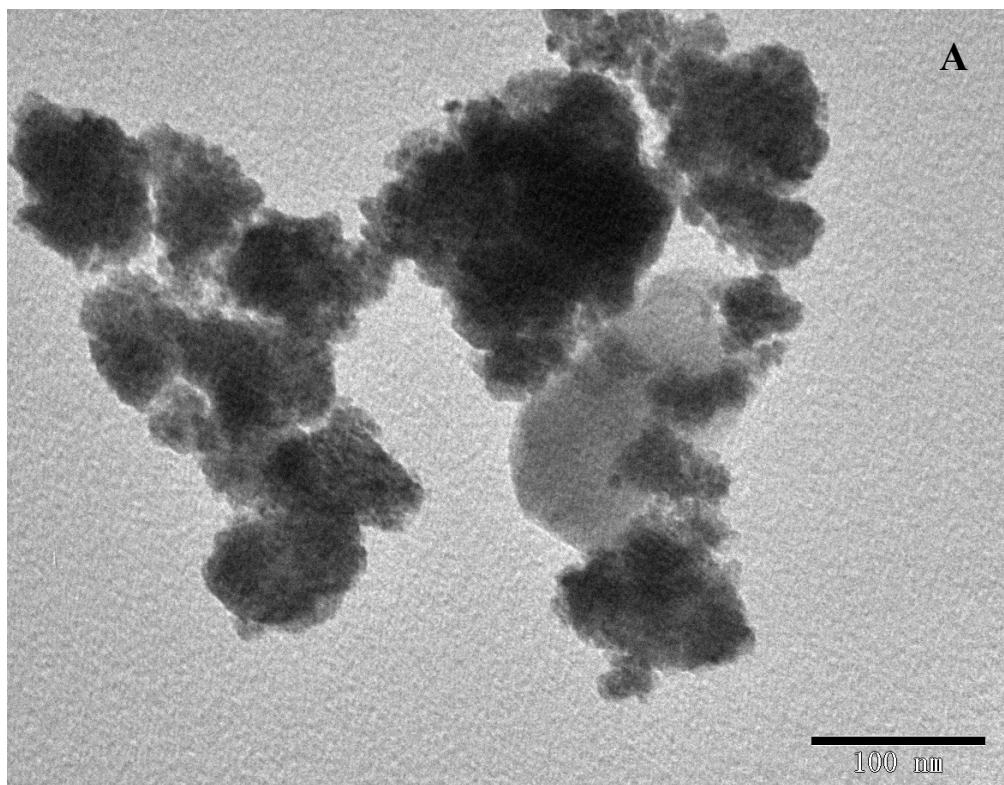


Figure S1. TEM images (A) and EDX (B) of ZrO_2 -RGO.

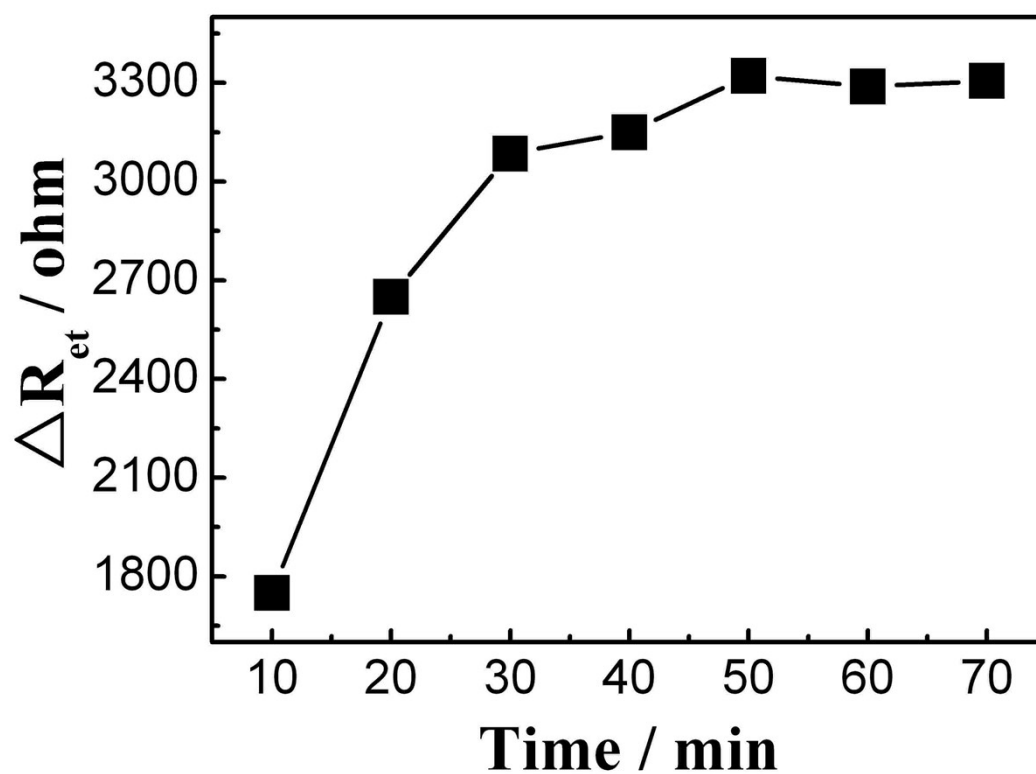


Figure S2. The influence of the hybridization time on ΔR_{et} .

Table S1. The sequences of DNA and RNA used in this work

| Names | | From 5' to 3' |
|---|----|---|
| Hairpin DNA | H1 | ATAAGCTATCTACACATGGTAGCTTATCAGACTCCATGTGT AGA - (CH ₂) ₆ -NH ₂ |
| | H2 | TCAACATCAGTCTGATAAGCTACCATGTGTAGATAGCTTAT CAGACTCCTAATGGTGTGGC |
| miRNA-21 | | UAGCUUAUCAGACUGAUGUUGA |
| miRNA-141 | | UAACACUGUCUGGUAAGAUGG |
| Three-base mismatched miRNA-21(t-miRNA-21) | | TAGCUUAUCAGCCUGAUGUTGA |

Table S2. Comparison of linear range and detection limit of the reported sensors

| Sensors | Technique | Linear range | Detection limit | Reference |
|---------------------------------------|-----------|-------------------|-----------------|-----------|
| ssDNA/AuNPs/PNR/GCE | DPV | 0.01 nM – 17 nM | 4.2 pM | 1 |
| PNA/poly(JUG-co-JUGA)/GCE | SWV | 10 nM – 100 nM | 10 nM | 2 |
| ssDNA/PICA/GCE | CV | 3.34 nM – 10.6 nM | 1.0 nM | 3 |
| ssDNA/ZrO ₂ /SWNTs/PDC/GCE | EIS | 10 pM – 1.0 μM | 1.38 pM | 4 |
| ssDNA/AuNPs/GO/GCE | DPV | 60 pM – 0.6 nM | 27pM | 5 |
| H1/PAA/ZrO ₂ -RGO/GCE | EIS | 10 fM – 0.1 nM | 4.3 fM | This work |

Table S3. Determination results of miRNA-21 in human serum

| Sample Num. | Added (pM) | Found (pM) | Recovery (%) |
|-------------|------------|------------|--------------|
| 1 | 0.5 | 0.48 | 96.0 |
| 2 | 2.0 | 1.94 | 97.0 |

Notes and references

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3. X.M. Li, J.P. Xia and S.S. Zhang, *Anal. Chim. Acta*, 2008, **622**, 104-110.
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