Supplementary Information

Ultrasensitive analysis of microRNAs with gold nanoparticles-decorated molybdenum disulfide nanohybrids-based multilayer nanoprobes

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Reagents and apparatus

Molybdenum (IV) sulfide powder (<2 μm, 99%), gold (III) tetrachloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99%) and 6-mercapto-1-hexanol (MCH, 97%) were purchased from Sigma-Aldrich (USA). Potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$, ≥99.5%), potassium hexacyanoferrate (II) trihydrate ($K_4Fe(CN)_6 \cdot 3H_2O$, ≥99.5%), potassium chloride (KCl, ≥99.5%), Boric acid (≥99.5%), tris (hydroxymethyl) aminomethane (Tris, 99.0%) and sodium chloride (NaCl, ≥99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All solutions were prepared with Milli-Q water from a Milli-pore system. The buffer used in this work were listed as follows: DNA immobilization buffer: 0.5×TBE (44.5 mM Tris, 1 mM EDTA, 45 mM boric acid). Washing buffer: 10 mM Tris-HCl (pH 7.4). Electrochemical detection buffer: 10 mM Tris-HCl (pH 7.4) containing 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$ and 0.1 M KCl. The DNA and miRNA sequences used in the study were purchased from TaKaRa (Dalian, China, Table S2).

Construction of MoS_2-AuNPs-based single-layer nanoprobes (SLNPs)

MoS_2 nanosheet, MoS_2-AuNPs nanocomposite and SLNPs were prepared following previous literatures.[1,2] Briefly, 20 μL DNA3 solution (10 μM) was added to 180 μL (10 μM) MoS_2-AuNPs suspension and incubated overnight at 25 °C with gentle shaking (molar ratio 1:1). Then, the mixture was centrifuged twice (5000 rpm, 30 min) and re-dispersed in 200 μL 0.5×TBE to obtain DNA3/MoS_2-AuNPs (SLNPs) dispersion. Finally, the obtained SLNPs was purified and stored in 0.5×TBE at 4 ºC.

Construction of MoS_2-AuNPs-based multilayer nanoprobes (MLNPs)

Nanoprobes DNA1/MoS_2-AuNPs (probe 1) and DNA2/MoS_2-AuNPs (probe 2) were prepared with the same procedure. After purification, probe 1 and probe 2 (molar ratio 1:1) were incubated at 37 °C overnight to form multilayer nanoprobes. The product was centrifuged twice (5000 rpm, 15 min) to retain a multilayer structure. After purification, 20 μL DNA3 solution (10 μM) was added to block the remaining active sites of MLNPs and capture the target miRNA-21. Finally, the product was purified and re-dispersed in 0.5×TBE for further use at 4 ºC.

Preparation of electrochemical biosensors

5 μL DNA4 (1 μM) solution was dropped onto the cleaned gold electrode for 16 h at room temperature to obtain DNA4/Au electrode. After removing unbound DNA4 by Tris-HCl buffer, MCH was assembled on the surface of DNA4/Au to block the unspecific adsorption of miRNA-21.

Finally, the MCH/DNA4/Au was used to detect different concentrations of miRNA-21 in the presence of as-prepared MoS_2-based nanoprobes.

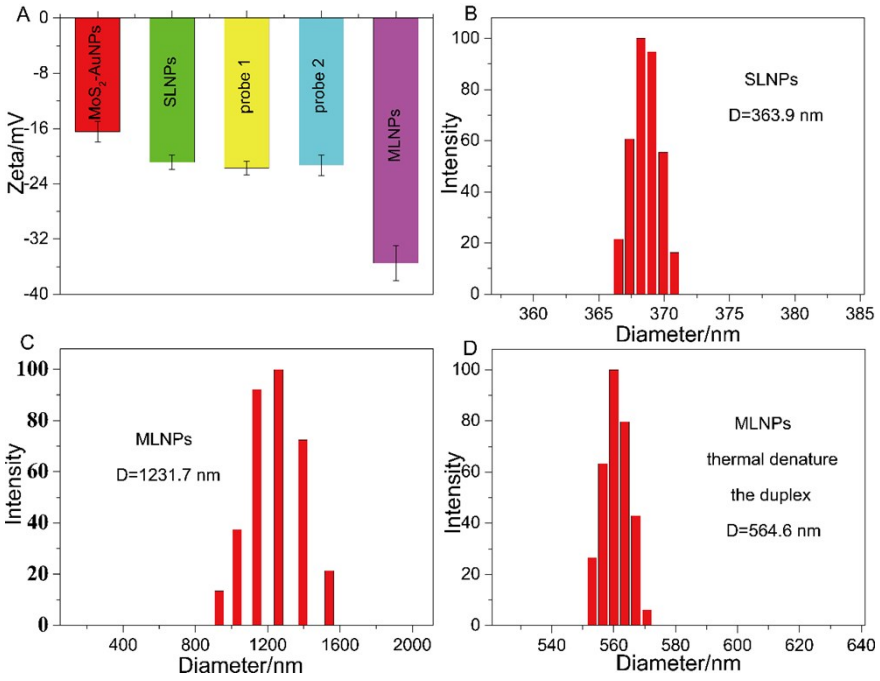

Fig. S1 ζ-potential values of (A) MoS_2-AuNPs, SLNPs, DNA1/MoS_2-AuNPs (probe 1), DNA2/MoS_2-AuNPs (probe 2) and MLNPs. (B-D) Size distribution of SLNPs, MLNPs and thermal denature of MLNPs (90 °C, 30 min), respectively.

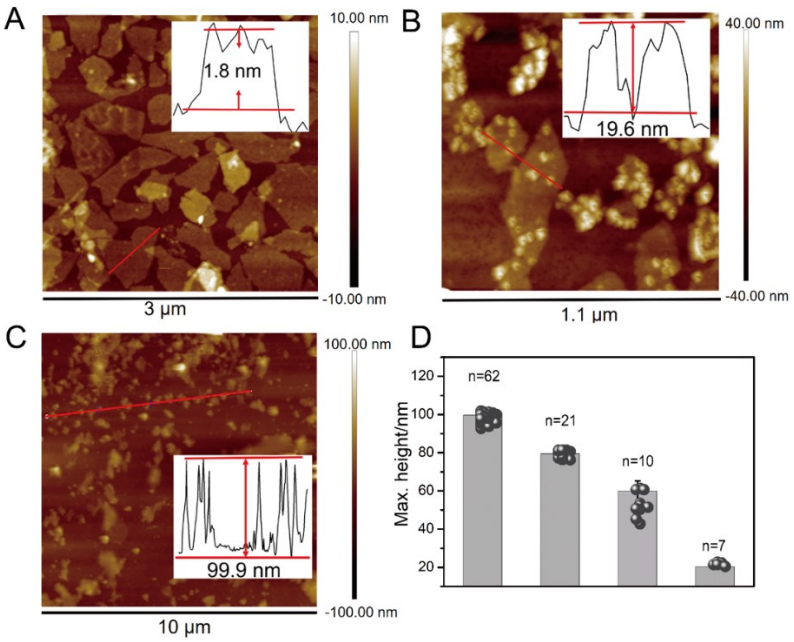

Fig. S2 AFM images of (A) MoS_2, (B) MoS_2-AuNPs and (C) MLNPs. (D) Statistical histogram of MLNPs' height. N in Figure 2D represent the statistical numbers of MLNPs.

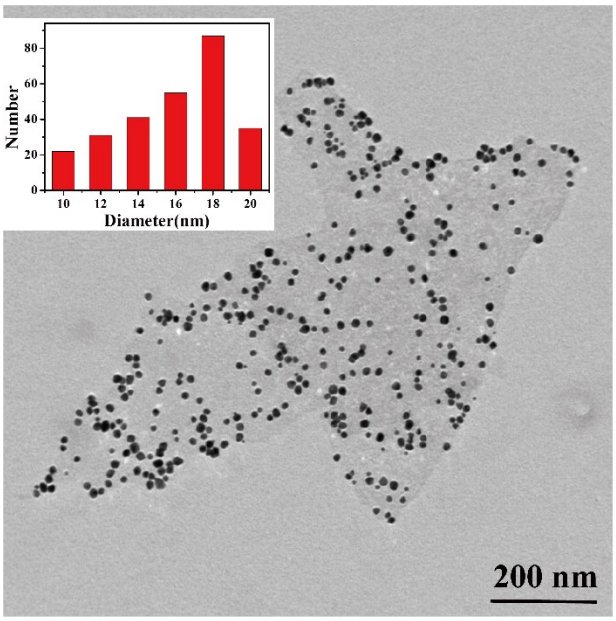

Fig. S3 TEM image of MoS_2-AuNPs nanocomposites. Inset: statistical diameter of surface-loaded gold nanoparticles on MoS_2 nanosheets.

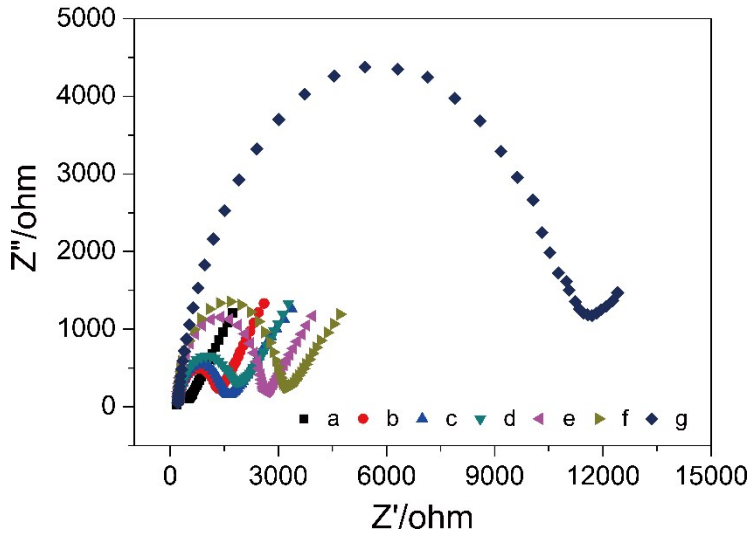

Fig. S4 EIS curves of (a) Au, (b) DNA4/Au, (c) MCH/DNA4/Au; (d) MLNPs incubated with MCH/DNA4/Au, (e) miRNA-21/MCH/DNA4/Au; (f) SNLPs/miRNA-21/MCH/DNA4/Au, (g) MLNPs/miRNA-21/MCH/DNA4/Au in 10 mM Tris-HCl buffer (pH 7.4) containing 5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4[Fe(CN)_6]$ and 0.1 M KCl.

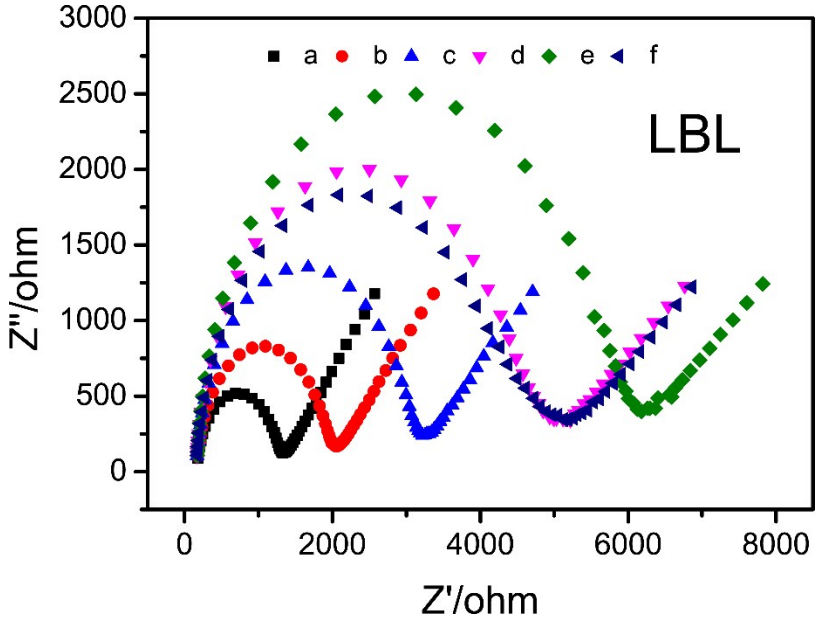

Fig. S5 EIS curves of (a) MCH/DNA4/Au, (b) miRNA-21/MCH/DNA4/Au, (c) 1-layer LBL probes/miRNA-21/MCH/DNA4/Au, (d) 2-layer LBL probes/miRNA-21/MCH/DNA4/Au, (e) 3-layer LBL probes/miRNA-21/MCH/DNA4/Au and (f) 4-layer LBL probes/miRNA-21/MCH/DNA4/Au.

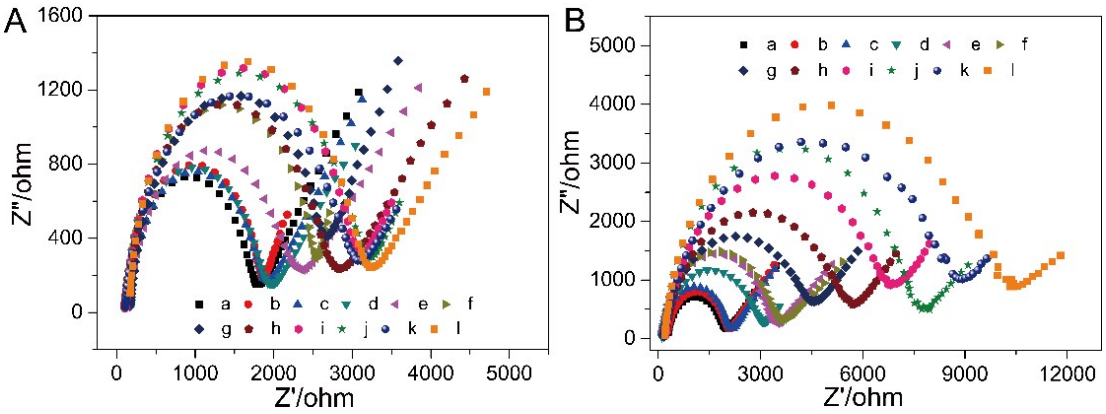

Fig. S6 (A) EIS curves of SLNPs-amplified electrochemical biosensor incubated with different concentrations of miRNA-21 (a→l, 0.01 fM, 0.1 fM, 1 fM, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, 1 μM). (B) EIS curves of MLNPs-amplified electrochemical biosensor incubated with different concentrations of miRNA-21 (a→l, 0.01 fM, 0.1 fM, 1 fM, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, 1 μM).

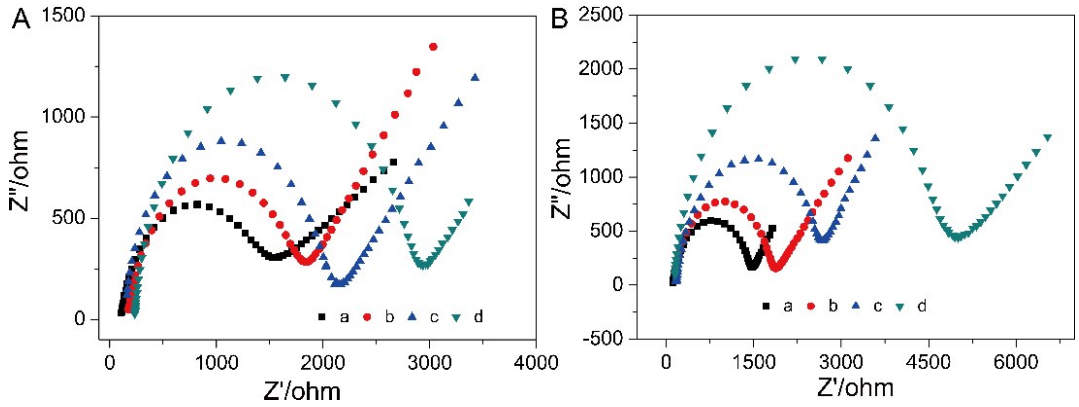

Fig. S7 (A) EIS curves of (a) MCH/DNA4/Au and the SLNPs-amplified electrochemical biosensor incubated with (b) miRNA-486, (c) SM-miRNA and (d) miRNA-21, respectively. (B) EIS curves of (a) MCH/DNA4/Au and the MLNPs-amplified electrochemical biosensor incubated with (b) miRNA-486, (c) SM-miRNA and (d) miRNA-21, respectively. The concentrations of all miRNAs were 100 pM.

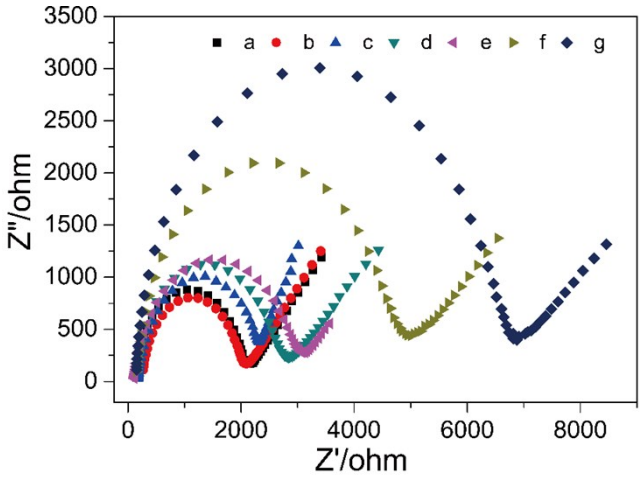

Fig. S8 EIS curves of miRNA-21 analysis in lysates of HeLa cells (b→g, 10, 10^2, 10^3, 10^4, 10^5, 10^6) and L-O2 cells (a, 10^5) by using MLNPs-amplified electrochemical biosensor.

Table S1. Analysis results of the MLNPs electrochemical biosensor for miRNA-21 detection in human serum samples (n=3).

Sample	Added (pM)	Found (pM)	Recovery (%)	RSD (%)
1	1	0.91, 1.35, 0.64	96.69	1.39
2	10	9.02, 10.12, 9.64	95.90	0.55
3	100	96.56, 108.34, 103.00	100.03	5.89

Table S2. The DNA and miRNA sequences used in the study.

Name	Sequence (5'-3')
DNA1	HS-$(CH_2)_6$-GAGGACATCGCTCATCTC
DNA2	GAGATGAGCGATGTCCTC-$(CH_2)_6$-HS
DNA3	TCTGATAAGCTATTTTT-$(CH_2)_6$-HS
DNA4	HS-$(CH_2)_6$-TTTTTTCAACATCAG
microRNA-21	UAGCUUAUCAGACUGAUGUUGA
single-base mismatch microRNA (SM-miRNA)	UAGCCUAUCAGACUGAUGUUGA
microRNA-486	UCCUGUACUGAGCUGCCCCGAG

References

1 S. Su, C. Zhang, L. Yuwen, J. Chao, X. Zuo, X. Liu, C. Song, C. Fan and L. Wang, *ACS Appl. Mater. Interfaces* 2014, **6**, 18735-18741.
2 S. Su, H. Sun, W. Cao, J. Chao, H. Peng, X. Zuo, L. Yuwen, C. Fan and L. Wang, *ACS Appl. Mater. Interfaces* 2016, **8**, 6826-6833.