Supporting Information for

Bi-directional DNA Walking Machine and Its Application in an Enzyme-Free Electrochemiluminescence Biosensor for Sensitive Detection of MicroRNAs

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Contents of the Supporting Information:

Table S1. DNA Sequences Used In This Study

name	sequences (5′-3′)
W1	GGA TAC TGA ACC TAG AGA GCA GA
W2	TCT GCT CTC TAG TTA ATA TCA GT-$(CH_2)_6$-NH_2
T1	CTG ATA ACT ACG AAA GGG AGT GGC TCG GA
T2	TCC GAG CCA CTC CCT GGA CAC CAT CTA AAC ACT TGT ATG GGA CG
T3	CGT GAT AGA AAC AAT GCA AAG ACA CGT TAC GTT TAG ATG GTG TCC
ssDNA	GTA ACG TGT CTT TGC TCC T-$(CH_2)_6$-NH_2
A2	GTT CAG TAT CCC GTC CCA TAC A
D1	TCA ACT CAG TCT GAT AAG CTA
A3	ACC CCT ATC ACG ATT AAT ATT AA
B1	GTA CTT ATC AGA CTG ACA TTA A
p53 gene	TCA TCA CAC TGG AAG ACT C
oral cancer (ORVOA 1) gene	CAG AAA AGA CTC TCG TTC TTT
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA-199a	ACA GUA GUC UGC ACA UUG GUU A
miRNA-182-5p	UUU GGC AAU GGU AGA ACU CAC ACU

The buffers involved in this work. DNA hybridization buffer (HB1): 10 mM Tris-HCl, 1.0 mM EDTA, 1.0 M NaCl (pH 7.0). MiRNA hybridization buffer (HB2):

10 mM Tris-HCl, 1.0 mM EDTA, 0.2 M NaCl, 10 mM $MgCl_2$ (pH 8.0), 0.1 M phosphate buffered solutions (PBS, pH 7.0, 8.0). PBS was employed as working buffer, containing 10 mM Na_2HPO_4, 10 mM NaH_2PO_4 and 2.0 mM $MgCl_2$. Ultrapure water (specific resistance of 18 MΩ·cm) obtained from ultrapure water systems controller (Lidi, Chongqin, China) was used throughout the study.

TEM characterization of CdS:Mn NCs and Au NPs. To prove the successful synthesis of CdS:Mn NCs and AuNPs, TEM was employed to characterize the nanomaterials. The prepared CdS:Mn NCs had a nearly spherical shape and average size of 5 nm (Figure S1A). Lattice fringes of CdS:Mn NCs could be clearly observed from HRTEM image (Figure S1B). The gingili-like dark spots of the particles were AuNPs whose average particle size was 5±1 nm (Figure S1C).

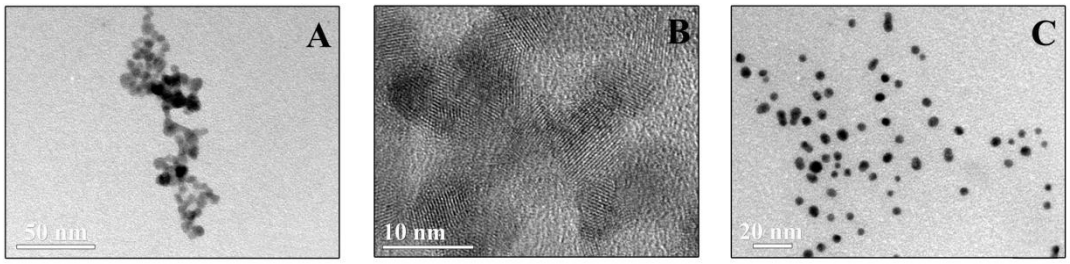

Figure S1. (A) TEM image of CdS:Mn NCs, (B) HRTEM image of CdS:Mn NCs, and (C) TEM image of AuNPs.

Optimization of the detection conditions. Experimental parameters containing the volume of CdS:Mn NCs and the concentration of the DNA track was optimized to achieve sensitive detection of miRNAs. From Figure S2A, the ECL intensity increased with increasing of the volume of CdS:Mn NCs and reached a relative stable value when the volume was greater than 30 μL. As a result, 30 μL was chosen as the optimal volume of CdS:Mn NCs in this work. Furthermore, the concentration of the

DNA track was also an important parameter for sensitive detection of miRNAs. In Figure S2B, with the increase of the concentration of DNA track, the ECL signal enhanced rapidly and then reached a constant value at 10 μM because the ECL peak was barely changed when the concentration of the DNA track was higher than 10 μM. Thus, the concentration of DNA track of 10 μM was the optimal choice in the proposed biosensor.

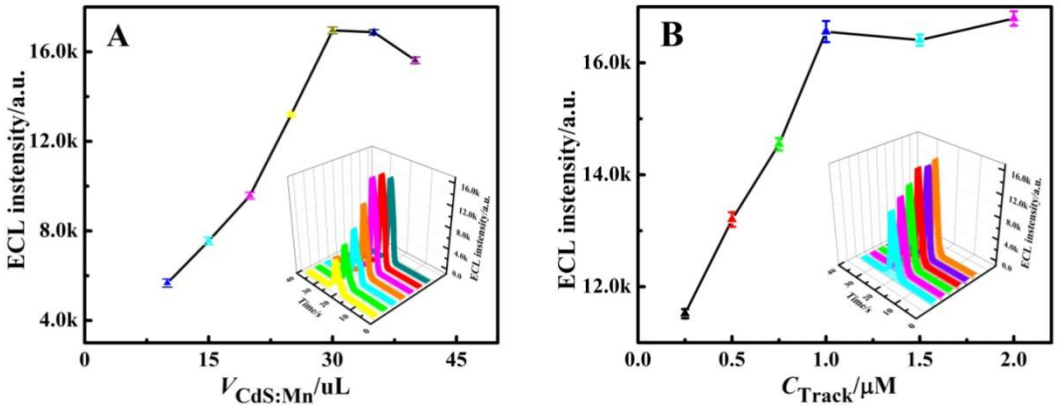

Figure S2. The optimization of (A) the volume of CdS:Mn NCs and (B) the concentration of the DNA track of the proposed biosensor. The ECL intensity of the biosensor was measured in 2 mL PBS (pH 8.0) with 0.05 M $S_2O_8^{2-}$. Error bars: standard deviation (SD), n = 3.

Reproducibility of the proposed biosensor. The reproducibility of the fabricated biosensor has been explored by determination of three different sensing electrodes modified under the same condition (Figure S3), and a relative standard deviation (RSD) of 3.83% was achieved. Moreover, different batches of obtained electrode were analyzed and the RSD value was 4.07%. These results demonstrated that the proposed biosensor presented a remarkable reproducibility.

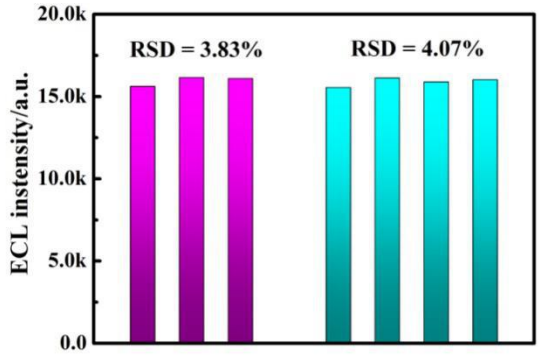

Figure S3. The reproducibility of the proposed biosensor.