Electronic Supplementary Information

DNA Nanomachine Based Regenerated Sensing Platform: a Novel Electrochemiluminescence Resonance Energy Transfer Strategy for Ultra-high Sensitive Detection of MicroRNA from Cancer Cells

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Table 1. *The oligonucleotide sequences applied in the proposed work.*

Name	Sequence (5′ to 3′)
A	TGCCTTGTAAGAATCAAATGCTTCGGAT
B	SH-GGTCGCTCTTACGGCATTCGACGACGGCTCGGAGAAGAGAT
C	ATCAGACTGATGTTGAACTCATATCCGAAGCATTCCAGGT-SH
DNA H1	GCATCCATCAGACTGATGTTGAACTCACTATARGGAAGAGATGTAGCTTATCAGACTGATGTTGATCAACATCAGTCTGATAGCTA
DNA H2	TAGCTTATCAGACTGATGTTGAACTCACTATGTCTATGCGTTTCATATATTCTCCGAGCCGGTCGAAATAGTGAGTTCAACATC

	AGTCTGATGGATGC
DNA H3	ATCAGACTGATGTTGAACTCACTATATCTCTTCTCCGAGCCG GTGCGAAATAGTGAGTTCAACATCAGTCTGATAAGCTA
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
miRNA-141	UAA CAC UGU CUG GUA AAG AUGG
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
One-base mismatched miRNA-21	UCG CUU AUC AGA CUG AUG UUG A

The buffer and detection solution used in this work.

TAE/Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, pH 8.0) was used to dilute DNA sequences in this work. RNA hybridization buffer (Tris-HCl containing 0.2 mM NaCl, 10 mM $MgCl_2$ and 1 mM EDTA, pH 8.0) was introduced to dilute miRNA-21. Thiolated DNA strand used in the paper was activated with 10 mM TCEP and stored at 4 °C for 2 h before introducing to the sensing platform. Phosphate buffered solution (PBS, pH 7.4) was employed for the preparation of AF488. CdSe@ZnS QDs were diluted by boric acid buffer (pH 8.4). Finally, Ferricyanide solutions ($Fe(CN)_6^{3/4-}$, 5 mM, pH 7.4) which were employed for CV measurements were achieved by dissolving potassium ferricyanide and potassium ferrocyanide with PBS buffer (pH 7.4). All the hairpin hybridizations were heated to 95 °C for 2 min before using and cool down to room temperature over 1h. Double distilled water was employed throughout.

Analytical apparatus and measurements.

Cyclic voltammetric (CV) and ECL detections were performed with a CHI 660E

electrochemistry workstation (Shanghai CH Instruments, China) and a MPI-E ECL analyzer (Xi'an Remax Electronic Science & Technology Co), respectively. The measurements were carried out with a three-electrode system with glassy carbon electrode (GCE) as the working electrode, platinum wire as the counter electrode and Ag/AgCl (sat. KCl) as the reference electrode. Additionally, the voltage of the photomultiplier tube (PTM) was set at 800 V, and the potential scanning ranged from -2 to 0 V.

The fluorescence response of AF488 and QDs

As shown in Fig. S1, In order to further understand the mechanism of the proposed ERET system, the fluorescence spectrum of AF488, QDs and the composite of AF488 and QDs were carried out to further confirm the luminescent properties for the clarification. As showed in the following figure A and B, the luminescent emission wavelength of AF488 was measured to be about 515 nm and the luminescent emission of QDs was about to be 630 nm. Importantly, the composite of AF488 and QDs exhibited a dual-emission at 515 nm and 630 nm with the excited wavelength of 496 nm. 496 nm was the excited wavelength of donor-AF488, while the composite of AF488 and QDs presented a dual-emission spectrum at the excitation of 496 nm, demonstrating the energy was transferred from AF488 to QDs successfully. The obtained result was accordance with the ECL investigation, which suggested the mechanism of the proposed ERET system comprehensively.

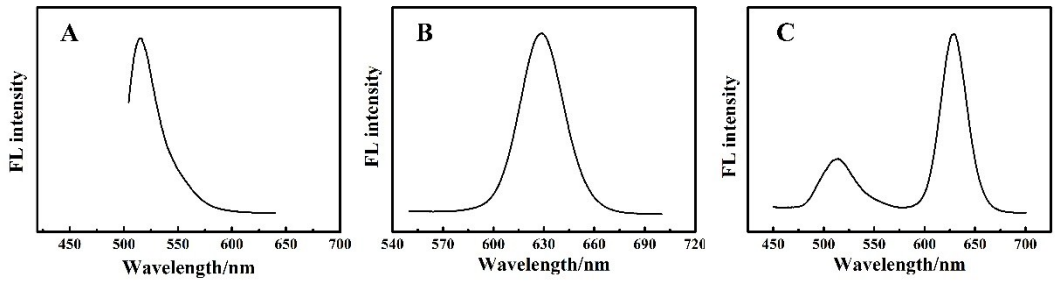

Fig. S1 The fluorescence response of AF488 (A), QDs (B) and the composite of AF488 and QDs (C).

The optimization of incubation time of Pb^{2+}

In order to make this study more convincing, we optimized the incubation time of Pb^{2+}. As shown in Fig. S2, the ECL intensity increased with the increasing incubation time of Pb^{2+} and reached a platform after the incubation time was longer than 30 min. So the optimal incubation time of Pb^{2+} incubation was 30 min.

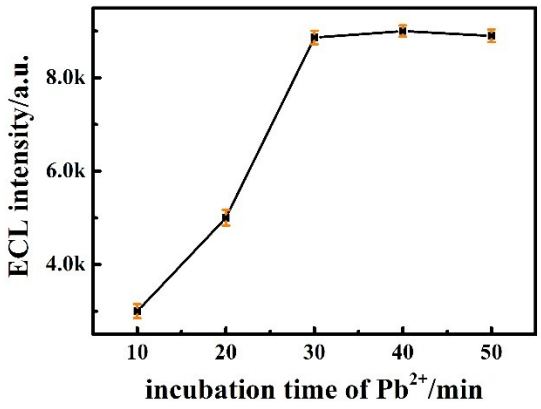

Fig. S2 The optimization of the Pb^{2+} incubation time of the proposed sensor.

Polyacrylamide gel electrophoresis (PAGE) characterization

In order to characterize the successful synthesis of the DNA tweezer, polyacrylamide gel electrophoresis (PAGE) was employed and the result was shown as follows. The distinct bands from lanes 1-3, respectively, correspond to the three assembly strands (DNA-A, DNA-B and DNA-C). As can be seen, the mixture of the

three assembly strands (lane 4) showed a band with lower mobility accompanied by the disappearance of the corresponding bands in lanes 1-3, indicating the successful formation of the DNA tweezer.

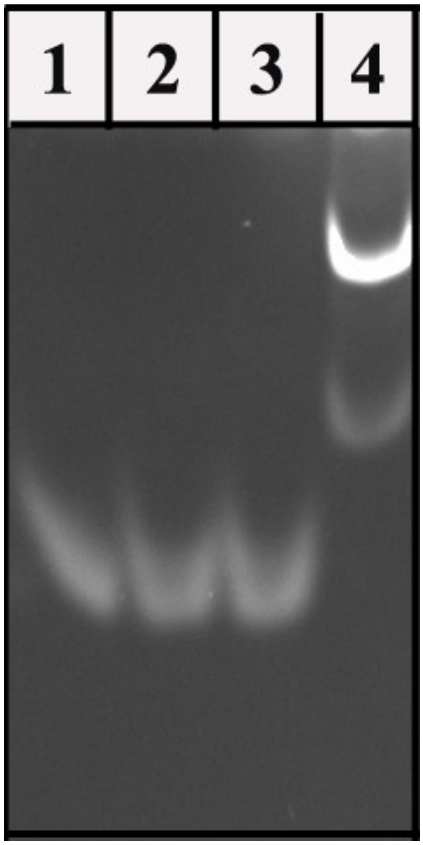

Fig. S3 PAGE (16%) analysis of assembly of the DNA tweezer. The concentration of the strands was 2μM respectively.

PAGE performance of the formation and cleaving of "Y" structure

As shown in Fig. S4, the formation of "Y" structure and production of reporter DNA were investigated by PAGE. Lane 1-3 represented hairpin structure of H1, H2 and H3, respectively. A band with low mobility was observed with the aid of target miRNA, suggesting the successful formation of "Y" structure. When the "Y" structure was added with Pb^{2+}, the band of lane 4 disappeared and the band of lane 1-3 showed

up, which demonstrated that the "Y" structure was cleaved by the DNAzyme with the generation of numerous reporter DNA.

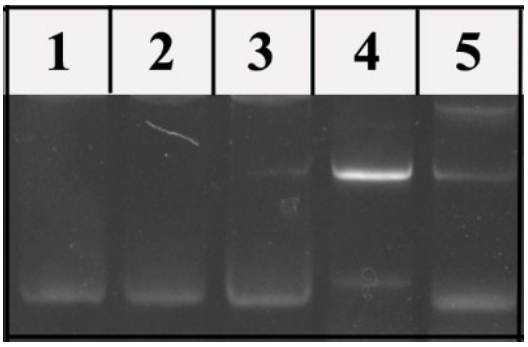

Fig. S4 PAGE (16%) analysis of the formation of "Y" structure and reporter DNA. Lane 1: H1; Lane 2: H2; Lane 3: H3; Lane 4: H1, H2, H3 and target miRNA; Lane 5: the "Y" structure was cleaved by Pb^{2+}. The concentration of the strands was 2μM respectively.