Supporting Information for

A high efficient electrochemiluminescence resonance energy transfer system in one nanostructure: its application for ultrasensitive detection of microRNA in cancer cells

Zhaoyang Li, Zongfan Lin, Xiaoyu Wu, Haotian Chen, Yaqin Chai*, Ruo Yuan*

Key Laboratory of Luminescent and Real-Time Analytical Chemistry, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P.R. China

* Corresponding author. Tel.: +86-23-68252277; Fax: +86-23-68253172.

E-mail address: yqchai@swu.edu.cn; yuanruo@swu.edu.cn

Contents of the Supporting Information:

Optical Spectrum Characterization of the ECL-RET between $Ru(dcbpy)_3^{2+}$ and QDs. To further confirm that the energy transfer can occur between QDs and $Ru(dcbpy)_3^{2+}$, fluorescence spectrums (FL spectrum) of QDs and $Ru(dcbpy)_3^{2+}$ were also studied. As can be observed in the Figure S1, the maximum emission wavelength of pure $Ru(dcbpy)_3^{2+}$ was at 604 nm (Figure S1A) and the maximum emission wavelength of pure QDs was at 630 nm (Figure S1B). As shown in Figure S1C, the FL spectrum of composite of QDs-$Ru(dcbpy)_3^{2+}$ was measured in the excitation wavelength of $Ru(dcbpy)_3^{2+}$ at 550 nm and there were two FL emission spectrums that could be observed which respectively corresponding to QDs and $Ru(dcbpy)_3^{2+}$. The results proved that the fluorescence resonance energy transfer were occurred between QDs and $Ru(dcbpy)_3^{2+}$, which further illustrated the occurrence of ECL-RET between QDs and $Ru(dcbpy)_3^{2+}$.

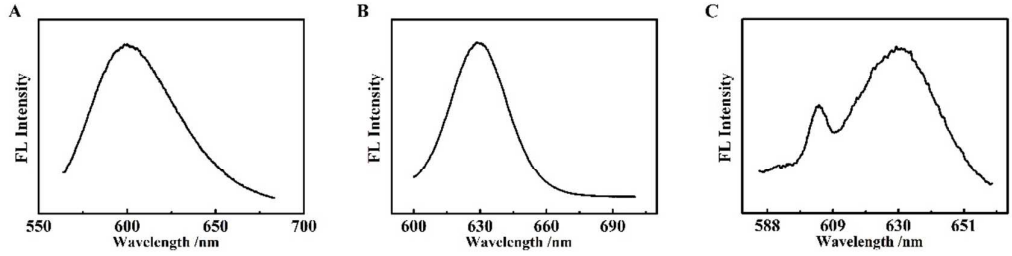
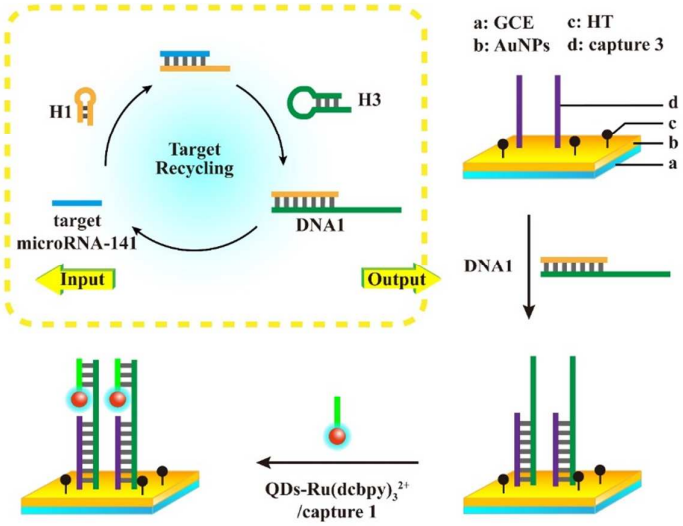


Figure S1. FL spectra of (A) $Ru(dcbpy)_3^{2+}$ with maximum excitation wavelength at 550 nm; (B) QDs with maximum excitation wavelength at 370 nm ; and (C) QDs-$Ru(dcbpy)_3^{2+}$ composite with maximum excitation wavelength at 550 nm.

Comparision of Different Amplification Strategies Based MiRNA Biosensor. To represent the merit of dual amplification including target recycling and double-output conversion strategies, the control experiments of target recycling strategy and single-output conversion strategy were performed. As shown in Scheme

S1, the determination of the target miRNA was performed with single amplification of target recycling. Specifically, the hairpin DNA 1 (H1) was opened by target miRNA-141, obtaining dsDNA with some exposed bases and then hairpin DNA 3 (H3) replaced miRNA-141 to achieve the DNA 1. Afterwards, the DNA 1 was modified on the surface of the electrode by hybridizing with capture 3, and QDs-Ru(dcbpy)$_3^{2+}$/capture 1 was introduced onto the proposed GCE in order to hybridize with capture 3. In order to assess the performance of the target recycling based biosensor, different concentrations of target miRNA-141 was detected. As can be seen from Figure S2A and Figure S2B, ECL response was found to be logarithmically to the concentration of miRNA with the range from 20 fM to 2nM and the detection limit of the proposed biosensor was 6.7 fM (S/N = 3).



Scheme S1. The construction of miRNA biosensor based on target recycling strategy.

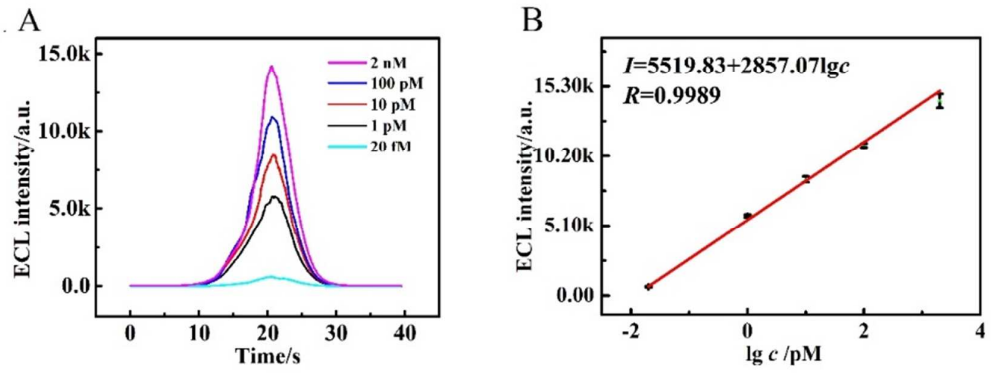
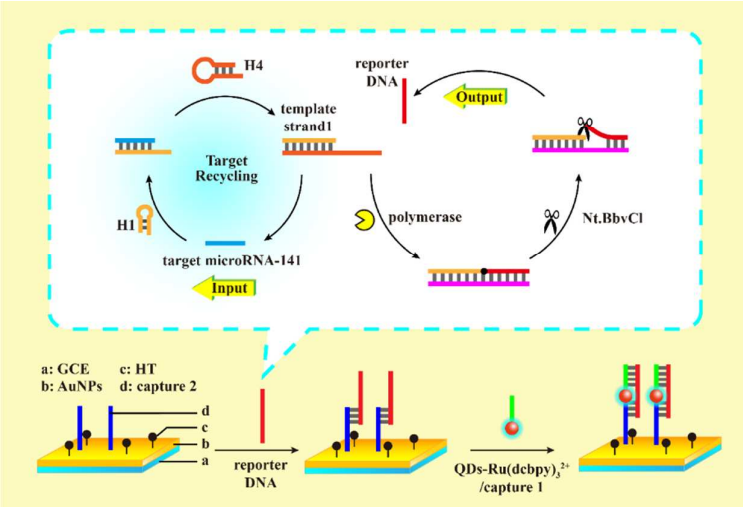


Figure S2. (A) Typical ECL response of the target recycling based miRNA biosensor introduced with different concentrations of miRNA-141 recorded in 0.05 M $S_2O_8^{2-}$; (B) Calibration curve for the various logarithm concentrations of miRNA-141.

Furthermore, the single-output conversion strategy based miRNA biosensor was also constructed to demonstrate the outstanding performance of the dual amplification in the proposed strategy. As displayed in Scheme S2, after the target recycling, the single-output conversion strategy was applied to realize the analysis of target miRNA. The template strand was polymerized to generate a DNA duplex with only one specific cleavage site, achieving reporter DNA with the recognition of nicking endonuclease Nt.BbvCI. Subsequently, the reporter DNA and QDs-Ru(dcbpy)$_3^{2+}$/capture 1 were introduced to the referred sensing platform to hybridize the capture 2 on the surface of the electrode. From the Figure S3A and Figure S3B, the single-output conversion strategy provided a detection range for the detection of miRNA-141 from 5 fM to 1 nM and the detection limit of single-output conversion strategy was 1.7 fM (S/N = 3), which was higher than double-output conversion strategy.



Scheme S2. The construction of miRNA biosensor with single-output conversion strategy.

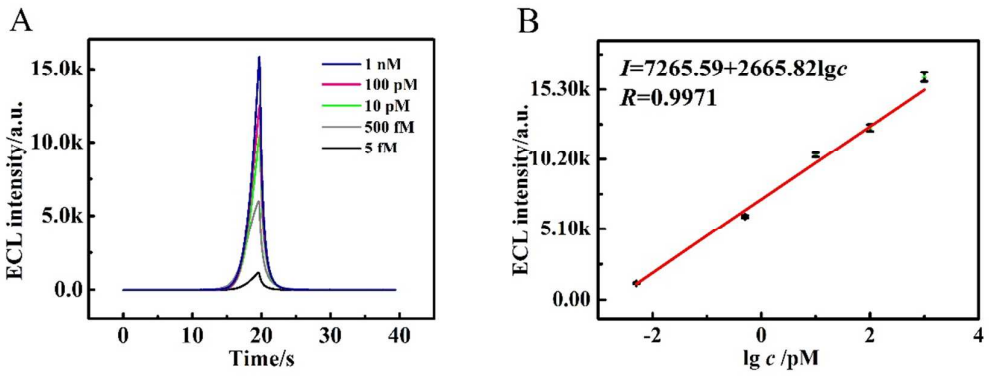


Figure S3. (A) Typical ECL response of the single-output conversion strategy miRNA biosensor introduced with different concentrations of miRNA-141 recorded in 0.05 M $S_2O_8^{2-}$. (B) Calibration curve for the various logarithm concentrations of miRNA-141.

In summary, the desired dual amplification including target recycling and double-output conversion strategies based biosensor have demonstrated a highly sensitive performance for detection of miRNA-141 in the range from 100 aM to 10 pM with a detection limit of 33 aM. Comparing with the above target recycling strategy and single-output conversion strategy based miRNA biosensor, the proposed

dual amplification based miRNA biosensor possessed a better performance for the analysis of miRNA-141.

Reagents and materials. Bst 2.0 DNA polymerase, nicking endonuclease Nt.BbvCI and the solution of deoxyribonucleoside triphosphate (dNTPs) mixture were purchased from New England Biolabs, Inc. (Beverly, MA, USA). Amino functionalized QDs were synthesized and purified by Wuhan Jiayuan Co. Ltd. (Wuhan, China). Tris (4,4'-dicarboxylicacid-2,2'-bipyridyl) ruthenium(II) dichloride ($Ru(dcbpy)_3^{2+}$) was purchased from Suna Tech Inc. (Suzhou, China). $HAuCl_4$ was obtained from Kangda Amino Acid Ltd. (Shanghai, China). $K_2S_2O_8$ was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). Hexanethiol (HT) was provided by Sigma (St. Louis, MO, USA). N-Hydroxysuccinimide (NHS) and N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide hydrochloride (EDC) were supplied from Shanghai Medpep Co. Ltd. (Shanghai, China). Phosphate-buffered solutions (PBS) (pH 7.4, 0.10 M) were compounded by 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. Double-distilled water was used throughout in this work. HPLC-purified miRNA was supplied from Takara Biotechnology Company Ltd. (Dalian, China). The DNA probes were synthesized and purified by Sangon, Inc. (Shanghai, China). Buffer to dilute miRNA and DNA probes was confected according the previous work[1]. The nucleotide sequences used in this work were shown as follows:

Table S1. The sequence information of DNA and RNA used in this study.

Name	Sequence

Hairpin probe	H1	5′-CCA TCT TTA CCA GAC AGT GTT ACA TTG GAT GCT CGG TCC TGA TAA CAC TGT CTG GTA-3′
	H2	5′-GGC ATT TCA TCG CTA CGT CAA TGG CTG AGG CAT TTC ATC GCT ACG TCA ATG GCT GAG GTA CCA GAC AGT GTT ATC AGG ACC GAG CAT CCA ATG TAA CAC TGT CTG GTA AAG CAT TGG ATG CTC GGT CCT GA-3′
	H3	5′-TCA GCC ATT GAT GGT CAT ACC GAC AGT GTT ATC AGG ACC GAG CAT CCA ATG TAA CAC TGT CTG GTA AAG CAT TGG ATG CTC GGT CCT GA-3'
	H4	5′-GGC ATT TCA TCG CTA CGT CAA TGG CTG AGG TAC CAG ACA GTG TTA TCA GGA CCG AGC ATC CAA TGT AAC ACT GTC TGG TAA AGC ATT GGA TGC TCG GTC CTG A-3'
Capture probe	capture 1	5′-COOH-TCA ATG GCT GA-3′
	capture 2	5′-SH-GGC ATT TCA TC-3′
	capture 3	5'-SH-TCA GGA CCG AGC ATC CAA TGC TTT ACC AGA CAG TGT TAC ATT GGA TGC TCG GTC CTG ATA ACA CTG TGG GTA-3'
MicroRNA-141		5′-UAA CAC UGU CUG GUA AAG AUG G-3′
MicroRNA-21		5′-UAG CUU AUC AGA CUG AUG UUG A-3′
MicroRNA-155		5′-UUA AUG CUA AUC GUG AUA GGG GU-3′

Apparatus. The ECL exploration was performed on a model MPI-E analyzer (Xi'An Re-max Electronic Science & Technology Co. Ltd., Xi'an, China) with a conventional three-electrode system used with an Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as auxiliary electrode, and a modified glassy carbon electrode (GCE, Φ = 4 mm) as the working electrode in the experiment, and the photomultiplier tube was set at 800 V with continuous potential scanning from -1.6 V to 0 V. Cyclic Voltammetry (CV) was performed with a CHI 660E electrochemical workstation (CH Instruments Inc., Shanghai, China) and the

electrochemical experiments were performed in a conventional three-electrode system: a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and the modified glassy carbon electrode (GCE, Φ= 4 mm) as working electrode. UV-vis absorption spectra was carried out on a UV-2450 UV-vis spectrophotometer (Shimadzu Inc., Tokyo, Japan).

REFERENCES

(1) Tian Q. Q.; Wang Y.; Deng R. J.; Lin L.; Liu Y.; Li J. H. *Nanoscale* **2015**, 7, 987-993.