Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Dual target- recycling amplification strategy for sensitive detection of microRNAs based on duplex-specific nuclease and catalytic hairpin assembly

Nan Hao, Pan-Pan Dai, Tao Yu, Jing-Juan Xu* and Hong-Yuan Chen*

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

Probe DNA	NH_2-$(CH_2)_6$-TTTTCAACATCAGTCTGATAAGCTATTTGATCCCATTCCCATTGATGCCTCT-FITC
Hairpin DNA 1(H1)	HS-TTTAGAGGCATCAATGGGAATGGGATCATGCCTCTAACCTAGCGATCCCATTCCCATTG
Hairpin DNA 2(H2)	GTTATTAATGTGTGATGTATGGGATCGCTAGGTTAGAGGCATGATCCCATTCCCATACATGCCTCTAACCTAGC
Capture DNA	ACATCACACATTAATAACTTTTTT-$(CH2)_6$-NH_2
G-quadruplex	TGGGTAGGGCGGGTTGGGTTT-$(CH2)_6$-NH_2
miR-21	UAGCUUAUCAGACUGAUGUUGA
SM-21	UAGCUUAUCAGAAUGAUGUUGA
miR-15	UAGCAGCACAUAAUGGUUUGUG
miR-16	UAGCAGCACGUAAAUAUUGGCG

miR-141	UAACACUGUCUGGUAAAGAUGG

EXPERIMENTAL SECTION

Reagents: Imidazole, N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), dithiothreitol (DTT), diethypyrocarbonate (DEPC) were obtained from Sigma-Aldrich (St. Louis, USA). Magnetic beads were purchased from Najingtech (Hangzhou, China). Duplex-specific nuclease (DSN) was obtained from Evrogen (Russia). 0.1 M Tris−HCl buffer was employed for preparation of ECL detection solutions. Tris−HCl buffer (0.1 M) containing 0.1 M NaCl (pH 7.4) was employed for preparation of DNA stock solutions. 0.1 M PBS (pH 7.4) buffer containing K_2HPO_4 and KH_2PO_4 was used to wash working electrodes after each modification and reaction step. All other reagents were of analytical grade and used as received. All the water used in the work was RNase-free. DSN buffer contained 50 mM Tris−HCl, 5 mM $MgCl_2$, 1 mM DTT. DSN stop solution contained 10 mM EDTA. Labelled oligonucleotides were ordered from Sangon Biotech Co. Ltd. (Shanghai, China), RNAs were ordered from Invitrogen (Carlsbad, USA). Their sequences from 5'-3' are listed as follows.

Apparatus.

Fluorescent detections were performed on a F7000 fluorescence spectrophotometer (HITACH, Japan). The ECL measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'An Remax Electronic Science &Technology Co. Ltd., Xi'An, China) at room temperature with a standard three-electrode configuration including glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The voltage of the PMT was set at 800 V. The detection was performed in 0.1 M Tris−HCl buffer (pH 7.4) containing 5 mM H_2O_2 .

Preparation of CdS NCs, CdS NCs modified GCE and Hairpin DNA Modified CdS NCs/GCE.

CdS NCs and CdS NCs modified GCEs were prepared according to our previous work. Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL of ultrapure water and heated to 70 °C under stirring; then the mixture was injected into a freshly prepared solution of Na_2S (0.5960 g) in 30 mL of ultrapure water. Instantly, orange-yellow solution was obtained. The solution was held at 70 °C for 3 h with continuous refluxing and stirring. The final reaction precipitates were centrifuged and washed thoroughly with absolute ethanol and ultrapure water two times. Then, the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. After that, 10 μL of CdS solution was drop-cast on the pretreated GCE and then air-dried at room temperature to get the CdS film modified GCE electrode. Hairpin DNA (0.5 μM) was heated to 95℃ for 5 min and then allowed to cool to room temperature before use. Solution of Hairpin DNA 1(50 μL) was pretreated by 2 μL

TCEP, then the CdS NCs modified GCE was immersed in the solution for 24 h at 4ºC. Then 2 wt% BSA solution was used at 4 ºC for 1 h to block the non-specific active binding sites. The obtained electrode was rinsed with tris-HCl buffer to remove the unspecified adsorption.

Preparation of Modified Magnetic beads

500 μL carboxyl-modified magnetic bead suspension (5mg/mL) reacted with 10 mM EDC and 20 mM NHS in 5 mL PBS solution for 1 h to obtain the activated magnetic beads. Then DNA probe and magnetic beads were mixed together and reacted for 12 h to obtain the DNA-magnetic beads, which were washed three times with PBS buffer. Then this solution of bioconjugates was incubated in a buffer solution (pH 7.4, composed of 25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO), with 0.2 mM hemin for 1 h to form DNAzyme-MBs.

Analytical Procedure

200 μL mixture solution (containing various concentrations of target miRNA, 0.4 U DSN, 5 mM $MgCl_2$, 1 mM DTT in 50 mM Tris-HCl, pH 8.0) was allowed to stand for 1 h at 50 ° C. Then 100 μL DSN stop solution was added. Fluorescence measurements were conducted after magnetic separation. Modified electrodes were immersed into this solution with 100 nM H2 at 37 ºC for 1 h. Then magnetic beads modified with capture DNA and G-quadruplex were added and fully reacted.

Optimization of the working conditions of DSN Enzyme

We optimized the amount of DSN enzyme and incubation time. The enzyme amplification experiment was performed in DSN buffer with the presence of 1 nM miR-21.

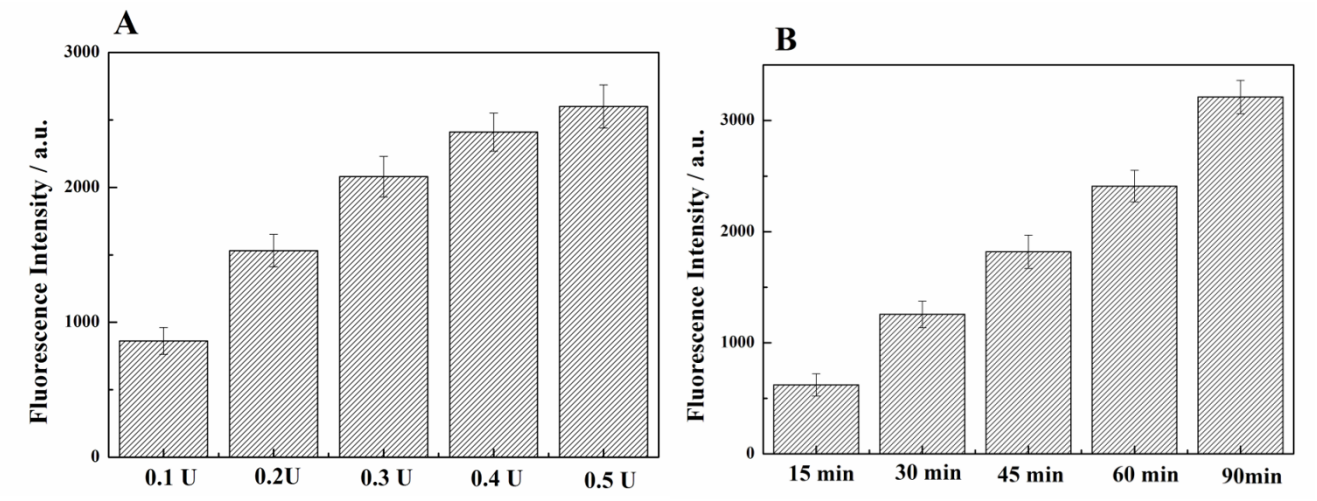

Figure S1. Optimization of the working conditions of DSN Enzyme. A : Effect of the amounts of DSN on fluorescence intensity. B: Effect of reaction time on fluorescence intensity.

Specificity of this ECL miRNA sensor

We tested the specificity of this sensor with miR-21, single-base mismatched miR-21 (SM-21),miR-15, miR-16, miR-141 (each for 10 pM).

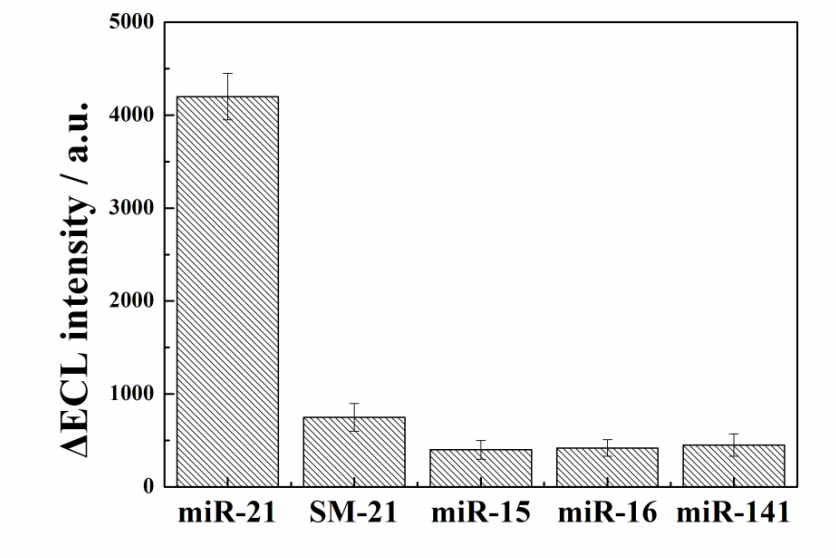

Figure S2. Specificity of this ECL miRNA sensor for miR-21