Supporting Information

Exonuclease III-propelled electrochemical sensor for highly efficient microRNA-155 detection

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Materials and methods

Materials and Reagents

Acrylamide, Tris(hydroxymethyl)aminomethane, 6-mercapto-1-hexanol (MCH) were obtained from Macklin (Shanghai, China). DEPC water, Rnase Inhibitor, Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 1×TE buffer，were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). N,N,N',N'-Tetramethylethylenediamine (TEMED), Methylene blue (MB) was obtained from Aladdin biochemical technology Co., Ltd. (Shanghai, China). Exo-III was purchased from New England Biolabs (NEB). All other reagents were of analytical grade. All DNAs, miRNAs and DNA Marker A (25-500 bp) were custom-synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequence of oligonucieotides were listed in Table S1. All the solutions were prepared by ultrapure water from Milli-Q water system with 18.25 MΩ.

Apparatus

Electrochemical measurements were performed on electrochemistry workstation (CHI660E，Shanghai Chenhua, China) with a conventional three-electrode system, a modified gold electrode (AuE) as working electrode, a platinum wire as counter electrode, a Ag/AgCl as reference electrode and. Polyacrylamide gel electrophoresis (PAGE) were realized using electrophoresis apparatus (BIO RAD). Gels imaging was performed using WD-9413B gel image system (Beijing, China).

Exonuclease III assisted double enzyme amplification reaction

Prior to the start of the experiment, all hairpin DNA were heated to 95 ℃, maintained for 5 min, and then slowly cooled to room temperature to ensure that the DNA formed a stable structure. For Exo-III enzymatic amplification reaction, at first, HP1, HP2, Rnase Inhibitor (2μL), and different concentration of miRNA-155 with Exo-III (2U/μL) were incubated separately in Tris buffer (prepared with DEPC water, including 10 mM Tris，0.1 M NaCl，0.01 M $MgCl_2 \cdot 6H_2O$, pH 7.4,) for 90 min at 37 ℃, then heated to 70 ℃ for 30 minutes to inactivate Exo-III enzyme. The reaction mixture stored at 4 ° C before use.

Native Polyacrylamide Gel Electrophoresis

Sterilized water 5.68 mL, 3.5 mL of acrylamide gel (30%), 200 μL of 50× TAE (Tris Acetate-EDTA buffer), 100 μL of 10% ammonium persulfate and 5 μL of TEMED were mixed well formation acrylamide gel (10%) and added to the gel glass plate and placed at 37 ℃ for 30min to solidify the acrylamide gel. Different samples (5 μL) were mixed separately with 1 μL of DNA loading buffer (6×), and transferred into acrylamide gel (10%). The electrophoresis process was running under 1× TAE (40 mM Tris, 20 mM HAc, 1 mM EDTA, pH 8.0) at a voltage of 100V for 1h, then the gel was placed in 4S Red Plus (3×) to stain for 30min, and finally imaged under gel imager for analysis.

Construction of electrochemical Sensor

Gold electrodes were polished with 0.3 μm and 0.05 μm alumina paste in turn. The electrodes were cleaned by ultrasonic in ultrapure water for three minutes, and then washed by anhydrous ethanol, ultrapure water in turn, dried with nitrogen gas. Finally, the electrode was cleaned in 0.5 M H_2SO_4 until a stable cyclic voltammetry curve was obtained. SH-HP (2 μM) and TCEP (40 μM) were mixed and incubated at room temperature for 1 h to remove the S-S bonds, and then the SH-HP 10 μL was droped on the electrodes overnight at 4℃, to formation the SH-HP/AuE. Thereafter,

10 μL MCH was droped on the AuE for 1h at room temperature to occupy additional sites. Next 10 μL of the reaction mixture was dropped on MCH/SH-HP/ AuE for 1 h. HP3 was mixed with HP4 and dropped on the electrode for 90 min, and methylene blue (MB) was dropped on the surface for 30 min to embed into the dsDNA. After each step of the above reactions, the electrode was washed with ultrapure water and dried. Differential pulse voltammetry (DPV) was measured in 10× PBS. Electrochemical impedance spectroscopy (EIS) experiments in 5 mM $Fe(CN)_6^{3-/4-}$ solutions containing 100 mM KCl, recorded at a stable open circuit potential (OCP) in the frequency range from 0.1 Hz to 100 kHz.

Electrochemical detection in real samples

In order to evaluate the detection effect in actual samples, we added different concentrations of miRNA-155 into in dilution solution of human serum samples that were obtained from Linyi People's Hospital and diluted 500-fold with DEPC treated water, and used it for electrochemical detection after full and uniform shaking. The experimental procedure was the same as above. Three groups of experiments were performed for each concentration to calculate the relative deviation.

The reproducibility and stability experiments are performed in the same way as the sensor property studies.

Table S1. All sequences used in experiment.

Name	Sequence (5'-3')
HP1	ATC ACG TCA GCG ACT GTG AAA AAAA CAC AGT CGC TGA ATT AGC ATT AA
HP2	TTAAT GCA CCA GC TAAA TCC GCT GCTGG TGC ATTAA CAC AGT CGC TGA
HP3	AGCGGATTTA GCTGGTGCATTAA CACCTTTGCC TTAATGCACCAGC
SH-HP	AGCGGATTTA GCTGGTGCATTAA CACCTTTGCC TTAATGCACCAGC

HP4	TTAAT GCA CCA GC TAAA TCC GCT GCT GGT GCA TTAA GGC AAA GGTG
miRNA-155	UUA AUG CUA AUC GUG AUA GGG GU
miRNA-141	UAA CAC UGU CUG GUA AAG AUG G
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A
Single-base matched miRNA 15（smiRNA-155）	UUA AUG CUA AUA GUG AUA GGG GU
Triplex-base matched miRNA 155（tmiRNA-155）	UUA AUA CUA AUA GUG AUC GGG GU

Table S2. Comparison of various biosensors for detection of target miRNA-155

Technique	Amplification strategy	Linear range	LOD	Ref.
DPV	THD/TSDR	5 fM - 100 pM	0.27 fM	1
ICP-MS	multicomponent nucleic acid enzymes (MNAzymes)/ lanthanide labeling	50 pM - 2nM	11 pM	2
DPV	3D DNA origami enzymatic amplification	100 pM - 1 μM	10 pM	3
DPV	GNR/GO	2.0fM - 8.0pM	0.6 fM	4
Fluorescence	CHA/AgNCs	200 pM - 20 nM	200 pM	5
PEC	$ZnIn_2S_4$/DSN	100 fM - 10 nM	33 fM	6

ECL	nitrogen dots/AuNRs	1 fM - 10 nM	0.33 fM	7
Fluorescence	MnO_2/DNAzyme	10 pM - 10 nM	1.6 pM	8
DPV	Tetrahedral DNA	1 pM – 100 μM	1 pM	9
Fluorescence	DNA-CuNC	50 pM - 10 nM	11 pM	10
Fluorescence	MWCNT/AuNCs	100 fM - 5 nM	33.4fM	11
Fluorescence	DNA polymerase /G-quadruplex/ThT	0.1 pM - 100 pM	35 fM	12
DPV	AuNPs@MoS_2	1 fM - 100 nM	0.32fM	13
SWV	AuNPs/GQDs/GO	1 fM - 1 nM	0.33fM	14
DPV	Exo III/HCR	0.1 fM - 0.1 nM	35 aM	this work

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