Supporting Information

A Universal Electrochemical Biosensor Using Nick-HCR Nanostructure as Molecular Gate of Nanochannel for Detecting Chromium (III) ions and MicroRNA

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CONTENTS

1.Polyacrylamide Gel Electrophoresis

Because the cutting product of Ce13d DNAzyme was single-stranded nucleic acid with low molecular weight, we applied denaturing PAGE (dPAGE) electrophoresis to inspect the production of single-stranded nucleic acid. Firstly, prepare the gel solution as the following component (30 ml gel solution for 2 gels with 11-wells and 1.5 mm thicknesses) (Table S1).

Table S1. The configuration component of 20% dPAGE gel

Component	Volume/mL
40% acrylamide-methacrylamide monomer	15
10× TBE buffer	3
Nuclease-free water	1.5
The urea	14.4 g
Total volume	30

Secondly, the water bath heats mixing, then uses the syringe and the filter membrane to filter solution in the small beaker. Thirdly, adding 30 uLTEMED and 300 uL10% ammonium sulfate (AP) to the Beaker. Finally, pipet the gel solution into the gap between the glass plates of gel casting, and insert the 10-well combs at once. Let the gels stand at 37℃ for at least 1 h to ensure a complete polymerization. 1×TBE solution was used as the running buffer. The gel was run 2-3 h at 120 V until the loading buffer 2 cm away from the bottom. 10,000×SYBR Gold nucleic acid dye was used for gel stain at room temperate in dark approximately 10 min. Finally, the gel was washed twice and taken image.

2. The DNA sequences used in the paper

Table S2. Sequences used in the fabrication of nanochannel biosensor

Name	Sequence (5'- 3')
Sequences used in the assembly of nick-HCR	
Initiator DNA	CTGAGCTTCGGATTCTGTTTGGCC
dH1	GGCCAAACAGAATCCGAAGCTCAGAAAAAAAAAAAAAAAAC CCTGCTGAGCTTCGGATTCTGT
dH2	CTGAGCTTCGGATTCTGTTTGGCCGGGGGGGGGGGGGGGACA GAATCCGAAGCTCAGCAGGGT
The sequence list of the dispelling probes	
Y1	ATTCTGTCCCCCCCCCCCCCCGGCCAAA
Y2	GCTTCGGATTCTGTCCCCCCCCCCCCCCCGGCCAAACAGAATC
Y3	CTGCTGAGCTTCGGATTCTGTCCCCCCCCCCCCCCCGGCCAAA CAGAATCCGAAGCT
The sequence of cDNAzyme and the sequences for EXPAR	
Ce13d DNAzyme-Substrate Strand	ATCGGTCACGAGTCACTATrAGGAAGATGGCGAAAAAAA AAAAAA
Ce13d DNAzyme - Catalytic Strand	TTTTTTTTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTT ACTCGTTATAGTGACTCGTGAC
X	GGAAGATGGCGAAAAAAAAAAAA
X'-Y'	GATTCTGTTTGGCCGGGGGGGGGGGGGGGACAGAATCCGAA GCAACTGACTCGGTTTTTTTTTTTTCGCCATCTTCCAA
Y'-Y'	GATTCTGTTTGGCCGGGGGGGGGGGGGGGACAGAATCCGAA GCAACAGACTCTGATTCTGTTTGGCCGGGGGGGGGGGGGGGA CAGAATCCGAAGC

Dispelling probe Y	GCTTCGGATTCTGTCCCCCCCCCCCCCCCGGCCAAACAGAATC
The sequence of miRNA and the sequence for EXPAR	
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
X''-Y'	GATTCTGTTTGGCCGGGGGGGGGGGGGGGACAGAATCCGAA GCAACTGACTCGGAACTATACAACCTACTACCTCAAA
Y'-Y'	GATTCTGTTTGGCCGGGGGGGGGGGGGGGACAGAATCCGAA GCAACAGACTCGGGATTCTGTTTGGCCGGGGGGGGGGGGGGG GACAGAATCCGAAGCAA
Let-7d	AGAGGUAGUAGGUUGCAUAGUU
Let-7f	UGAGGUAGUAGAUUGUAUAGUU
Let-7i	UGAGGUAGUAGUUUGUAUAGUU
Mir-122	UGGAGUGUGACAAUGGUGUUUG
Mir-200b	UAAUACUGCCUGGUAAUGAUGA
Mir-141	UAACACUGUCUGGUAAAGAUGG
Mir-21	UAGCUUAUCAGACUGAUGUUGA

Note: The yellow highlighted part of the substrate chain is the addition sequence, the green highlighted part of the substrate chain is the cutting site of endonuclease, and the red highlighted part "**-GACTC-**" of the substrate chain is the chromium (III) ion cutting recognition site. The design of sequence was assisted by IDT website. Initiator DNA is the capture probe when it immobilized on the surface of nanochannel.

3. The dispelling probe Y mediated disassembly

Three dispelling probes (Y1, Y2, Y3) which partially complementary with Hairpin 2 (Figure S1) to disassemble the nHCR nanostructure. The complementary condition is shown below:

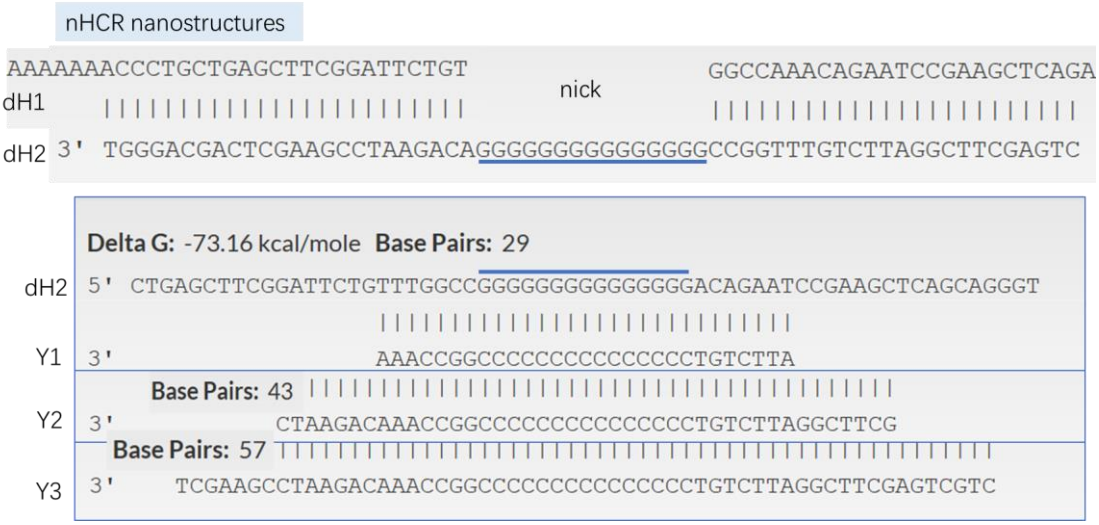

Figure S1. The complementary region of dH1 & dH2 and H2 & Y1, Y2, Y3.

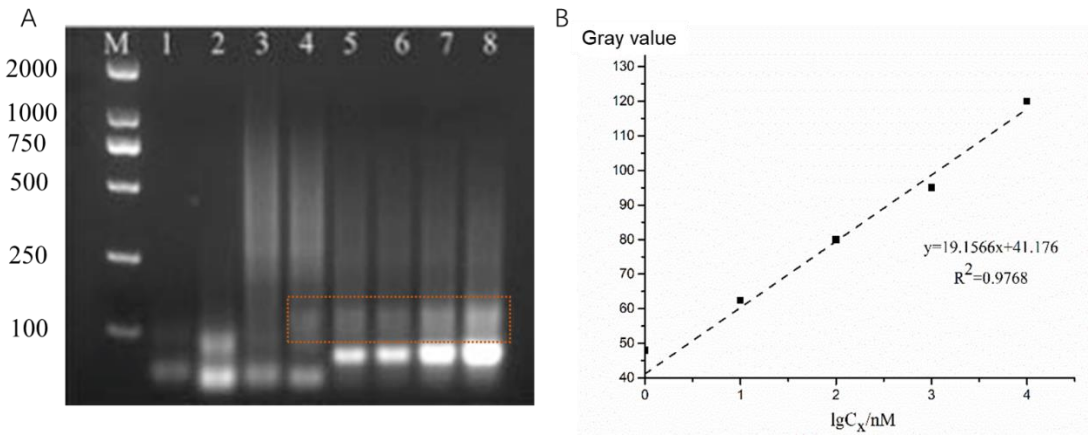

Figure S2. (A)The agarose gel electrophoresis for characterizing the disassembly of nHCR nanostructure. Experimental conditions: Lane1: 100 nM dH1;Lane 2: 100 nM dH2; Lane 3: 100 nM dH1+100 nM dH1+ 10 nM initiator DNA; Lane 4: 1nM dispelling Y2+nHCR product; Lane 5: 10 nM Y2+nHCR product ; Lane 6: 100 nM Y2+nHCR product; Lane7: 1 μM Y2+nHCR product; Lane 8: 10 μM Y2+nHCR product.(B) Calibration plot of the probe Y concentration with gray value of disassembly products. The dotted line represents the linear fitting of the data.

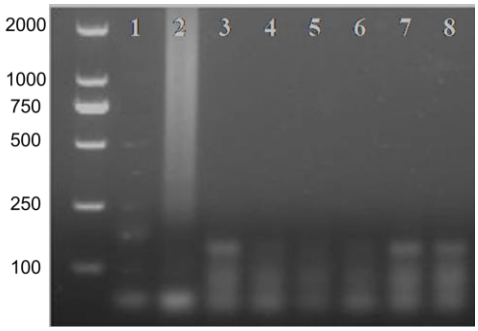

Figure S3 The optimization of disassembly time. Experimental conditions: (1) 100 nM dH1/dH2; (2)100 nM dH1/dH2, 10 nM initiator; (3) nHCR+EXPAR, 180 min; (4) nHCR+EXPAR, 150 min; (5) nHCR+EXPAR, 120 min; (6) nHCR+EXPAR, 90 min; (7) nHCR+EXPAR, 60 min; (8) nHCR+EXPAR, 30 min.

4. The Laser Scanning Confocal Microscopy characterization

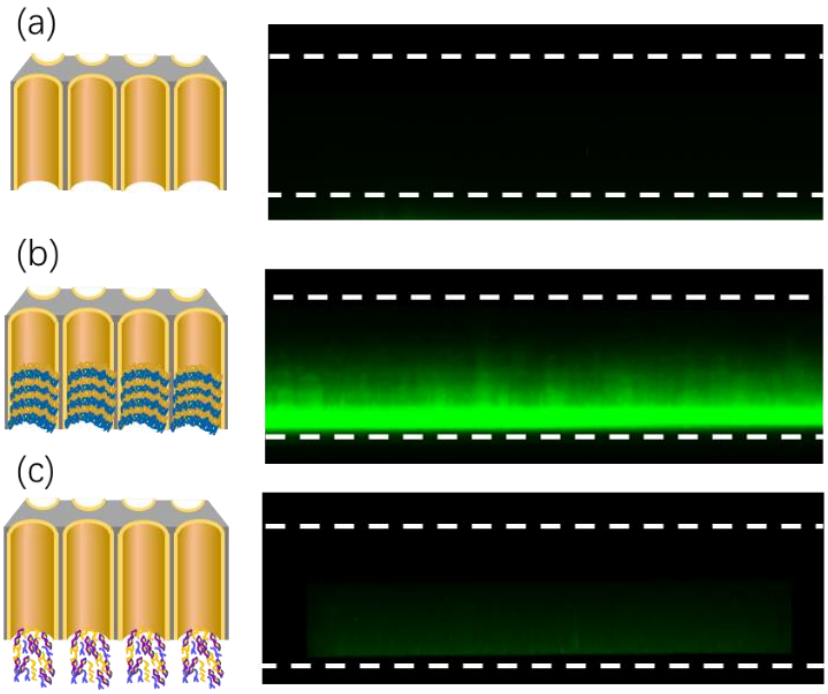

Figure S4. The LSCM characterization of DNA assembly and disassembly inside the nanochannels membrane (b) before and (c) after addition of dispelling sequence Y. (a) bare nanochannel without any modification.

5. Comparison with other nanochannel based biosensor

Table S3. Comparison of detection limit (LOD), linear range, target analytes and other properties of our method with other nanochannel-based biosensor.

Detection method	LOD	Target	Material	Linear range	Signal output	Ref.
Electrochemical biosensor [this paper]	1 pM/200 fM	*microRNA and* Cr^{3+}	*AAO Nanochannel & nick-HCR assembly*	1 pM-10 nM/ 200 fM-20 nM	On-Off ratio	this paper
stochastic nanopore sensor	40 nM	*Copper ions*	*α-hemolysin Protein nanopore*	Not provided	Event Frequency (s^{-1})	1
AAO nanochannel biosensor	10 pM	*Copper ions*	*AAO Nanochannel & HCR assembly*	Not provided	On-Off ratio	2
PAA nanochannel sensor	0.1 nM.	*DNA*	*morpholino–DNA functionalized* PAA membrane	Not provided	steady-state ionic current	3
Biomimetic logic gate sensor	0.1 nM	*Pb(II) ion*	*morpholino–DNA functionalized PAA membrane*	0.1 nM–5μM	steady-state ionic current	4
asymmetric nanochannel biosensor	0.82 ng/mL	*Cr (III) ion*	*Bare conical nanopore embedded in polymer membrane*	2.60–20.8 ng/mL	Surface coverage (theta)	5
PAA nanochannel sensor	7 cells	Telomerase Activity	*DNA functionalized PAA Nanochannels*	10−5000 cells	current drop ratio	6

We can see that our approach using nick-HCR assembly can detect both Non-nucleic acid target and nucleic acid target, simultaneously.

6. The reproducibility and stability

It should be mentioned that the AAO membrane used in this paper is a circular membrane with a diameter of 25 mm. During we constructed the nanochannel sensor, the big circular membrane was broken into small pieces to under batch processing, including cleaning, amination, glutaraldehyde functionalization, nucleic acid functionalization, nucleic acid assembly and other series of operations. The reproducibility was studied using 5 group of our proposed electrochemical biosensors which prepared at identified conditions using five different slices of PAO membranes on different days. A relative standard deviation (RSD) of 12.49 % for 10 pM Cr^{3+} was obtained (Table S4), indicating the satisfied inter-biosensor reproducibility of the resulting electrochemical biosensor. We further evaluate the accuracy of nanochannel sensors which was fabricated using five different pieces of PAO membranes. A relative standard deviation (RSD) of 9.46 % for 10 pM Cr^{3+} was obtained (Table S5), indicating the satisfied accuracy of the independently fabricated biosensor for practical application.

Table S4. The reproducibility of the inter-biosensor

Electrochemical biosensor	Ion current (A)	
Group 1	9.4E-08	
Group 2	8.53E-08	
Group 3	7.45E-08	STDEV(G1-G5) = 1.01463E-08
Group 4	8.43E-08	AVERAGE(G1-G5)=8.12176E-08
Group 5	6.7921E-08	R.S.D= 12.49 %

Ionic current was measured at 2V.

Table S5. The accuracy of the independently fabricated biosensor

Sensor	Ion current (A)	
1	9.23081E-08	
2	7.89841E-08	
3	7.9305E-08	STDEV(G1-G5) = 8.16967E-09
4	9.73636E-08	AVERAGE(G1-G5) = 8.63606E-08
5	8.38424E-08	R.S.D= 9.46 %

Ion current was measured at 2V.

And to measure the reproducibility of the equipment (Keithley 2636B picoammeter/voltage source (Keithley Instruments)), a set of 5 successive ion current measurements for 10 pM Cr^{3+} using same electrochemical biosensor yield an RSD of 6.28 %, showing that the equipment was stable and the good intra-biosensor reproducibility. The detailed ion current was listed in table S6.

Table S6. The reproducibility of the intra-biosensor

Counts	Ion current (A)	
1	7.55178E-08	
2	6.47317E-08	
3	6.9724E-08	STDEV(G1-G5) = 4.40879E-09
4	7.37169E-08	|AVERAGE(G1-G5) | = 7.02494E-08
5	6.75569E-08	RSD= 6.28 %

Ion current was measured at 2V.

Table S7. The storage stability of the developed biosensor

Week	Ion current (A)	Signal decline ratio
Initial response	7.98301E-08	
First week	7.37169E-08	7.66 %
Second week	6.75569E-08	15.37 %
Third week	5.59026E-08	29.97 %
Fourth week	1.1001E-08	86.22 %

7. The regeneration of nanochannel sensor

We evaluated the regeneration of our proposed biosensors by starting the assembly and disassembly of nHCR nanostructures in the nanochannel in cycles. Firstly, we applied the dH1 and dH2 to initiate nHCR nanostructures inside nanochannel. After assembly of nick-nanostructure, nanochannel was immersed into 1 μM dispelling chain Y. The ionic current measurements showed that the added dispelling chain Y efficiently switched nanochannel from close- to open-state. We alternately modified the nanochannel with dH1 & dH2 and dispelling chain Y, the nanochannel showed a significantly reversible switch between low- and high-ionic current in the first three circles while showed a remarkable decline in the gating efficiency in the fourth cycles (Figure S4).

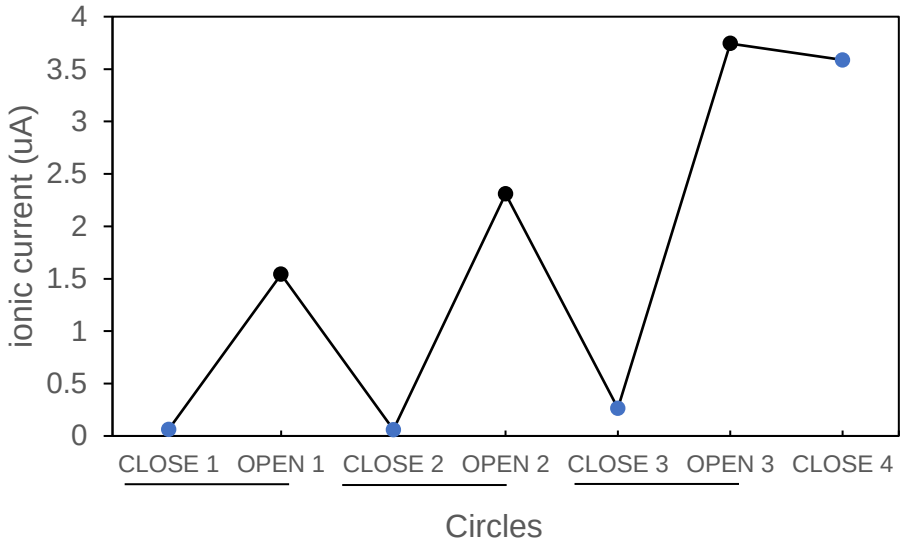

Figure S5. The regeneration of the nanofluidic gating device. The gating device can clyclicly regenerated by introducing dH1&dH2 and dispelling Y. The device can be reversibly switched between high- and low-conducting states at least in the first 3 cycles. In the following cycles, the devices show a remarkable decline in the gating efficiency.

Reference

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