Supporting Information for

An Investigation of Solid-state Nanopore on Label-free Metal Ion Signalling via the Transition of RNA-Cleavage DNAzyme and Hybridization Chain Reaction

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1. Supporting Figures and Tables

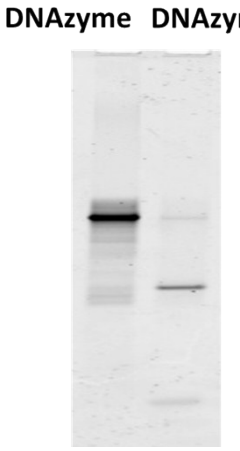

Fig.S1. **Gel verification of the DNAzyme activity.** 8% polyacrylamide gel electrophoresis that verifies the cleavage of the 1 μM DNAzyme without and with 50 μM Pb^{2+}. The smear bends above and under the main bend for DNAzyme (left) might probably come from the impurity during the chemical synthesis.

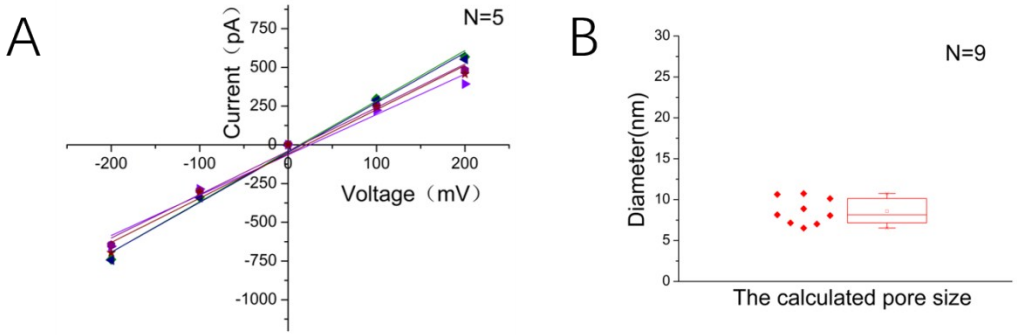

Fig.S2. **CGN set-up reproducibility.**
(A) *I-V* characteristics of conical glass nanopores in 0.1 M KCl (N=5) and corresponding calculated nanopore diameters (B). The reproducibility of nanopore combined with the calculation method as described below.

We could use the electrochemical measurement and estimate the nanopore diameter according to the classical equation (1).[1-3] We chose the current data between -100mV and 100mV because in this interval the linear and rectification ratio is close to 0. The calculated pore size (8.6 nm ±1.5 nm) is in good agreement with TEM image (8 nm ± 1.4 nm) according to the measurements using six randomly selected pores.

$$a = \frac{1}{\pi \kappa R tan\theta/2} \quad (1)$$

Where R is the measured pipette resistance, κ is the specific resistance of the electrolyte used (κ= 1.2 S/m in 0.1 M KCl), θ is the cone angle (θ=12 ° in Figure S1b) and a is the radius of the nanopore at the tip of the nanopipette.

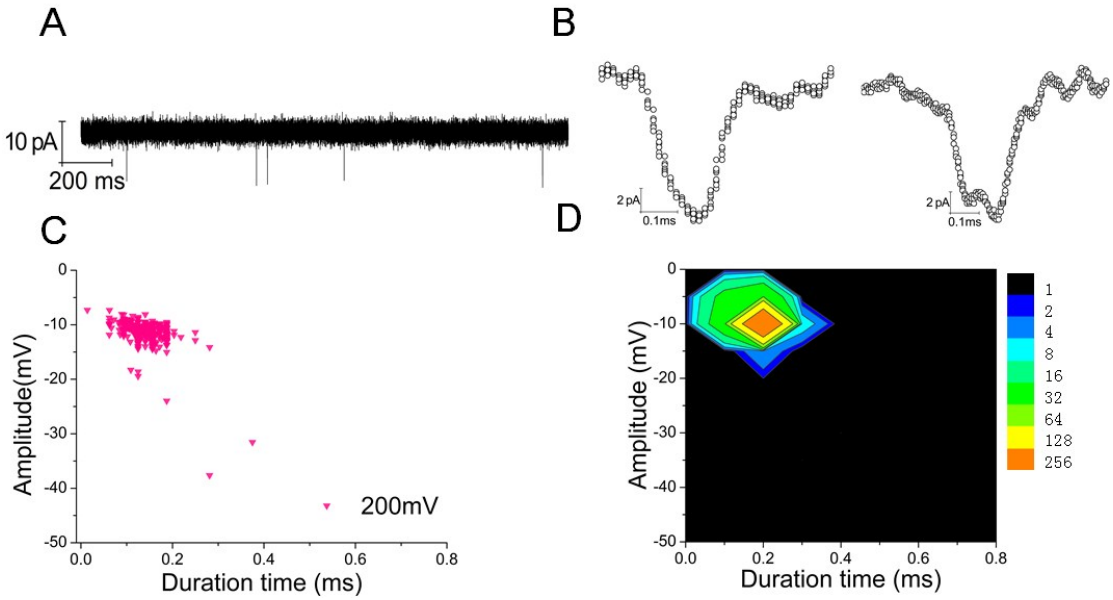

Fig.S3. **CGN performance verification with the 5 kb dsDNA marker.**
(A) Current trace of the DNA marker**.** (B) Examples of single current drop of the DNA marker. (C) Scatter plot of the DNA marker. at 200 mV and corresponding event density plot (D).

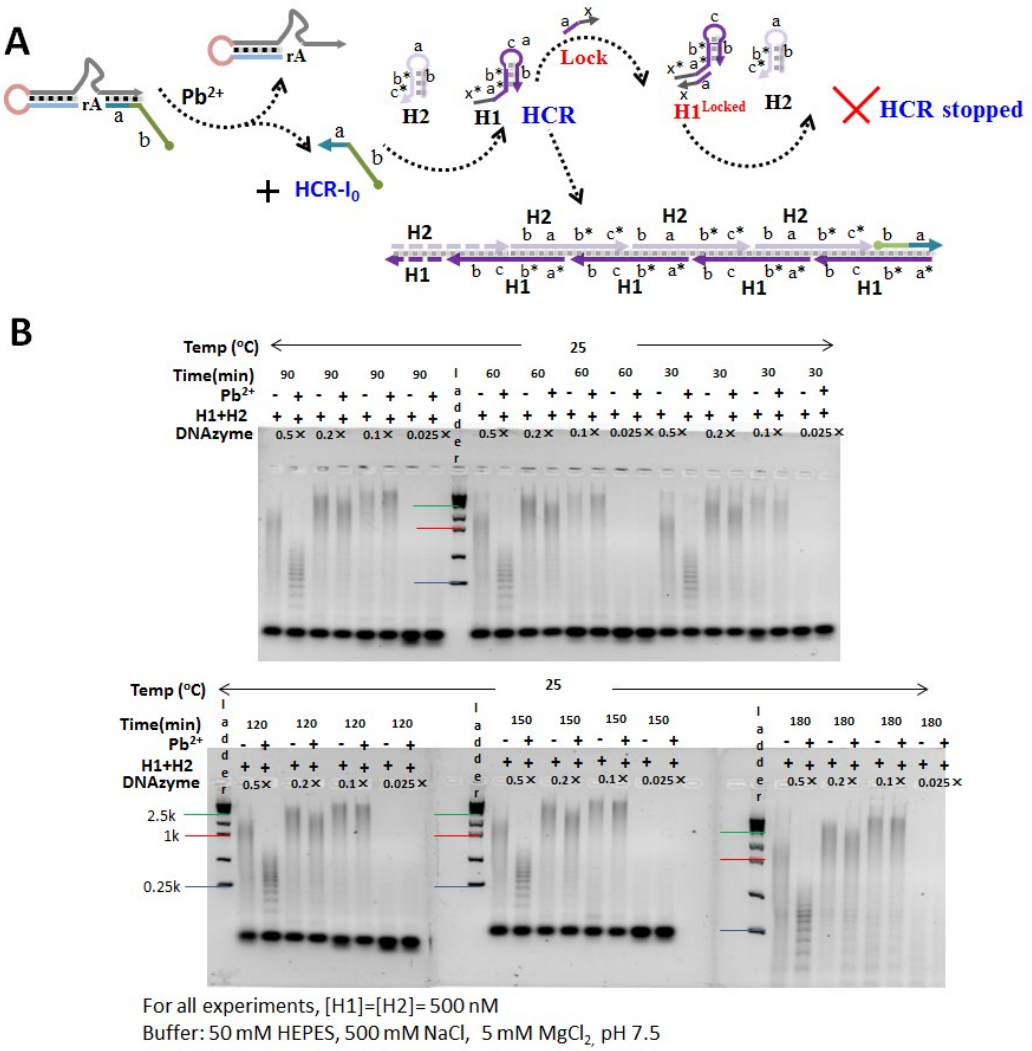

Fig.S4. Optimization of concentration ratio between HCR H1(H2) and DNAzyme GR-5. (A) The scheme of HCR reaction being stopped on the moment the Lock sequence is added. Note here domain x* of H1 is not presented in Fig.1B and Fig.3B for the easier understanding of the detection pathway. On the moment Lock sequence is added, it would bind H1 and then inhibit further HCR extension reaction. Therefore, the HCR reaction time could be controlled via the addition of Lock. (B) Gel optimization of concentration ratio between HCR H1(H2) and GR-5. Here "Time" and "Temp" means HCR reaction time and temperature, respectively**.** It could be observed when the HCR reaction time is longer than 30 min, the signal and background gets the best deviation when the concentration of GR-5 is half (0.5X) of that of H1(H2). Here the gels for 30 min (0.5 h), 90 min (1.5 h), and 150 min (2.5 h) are the same ones as presented in Fig. 3A.

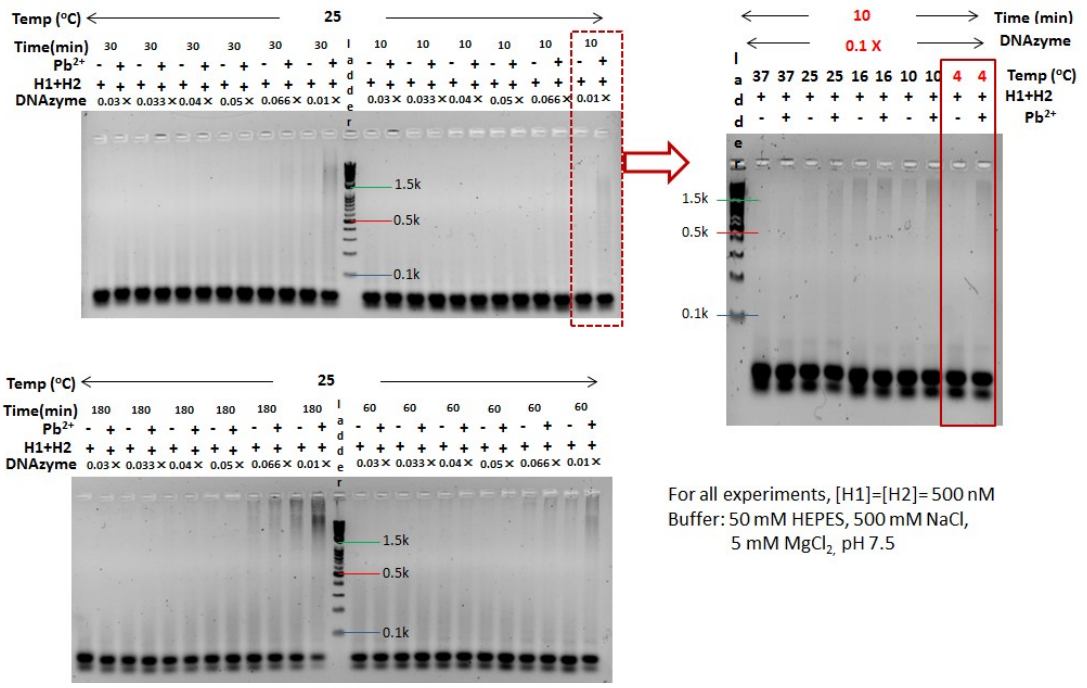

Fig.S5. **Gel electrophoresis shows the "on" signal pathway.** Plenty optimizations are carried out to search for an "on" signal in presence of Pb^{2+}, including HCR reaction temperature, reaction time, and the concentration ratio between HCR H1(H2) and DNAzyme GR-5. Finally, the bends in red box show that at 4 °C, the HCR reaction with Pb^{2+} finally produced longer and much more concatamers than that without Pb^{2+}. It means an "on" signal is achieved. But it should be noted that the HCR reaction has to be terminated exactly after proceeding for 10 min. And the concentration ratio between GR-5 and H1(H2) should be strictly controlled to be 1:10. With such a strict condition control, the reproducibility was decreased and the signal-to-background deviation was still unsatisfied.

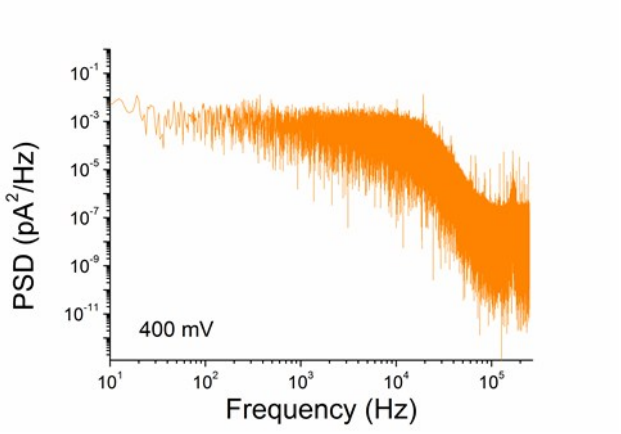

Fig.S6. The current power spectral density of the nanopore used in Fig.4. It is measured under 400 mV applied bias using a 100 kHz sampling rate and 10 kHz Bessel filter.

Name	Sequence 5'-3'	Label and notes
Sequences for DNAzyme-HCR reaction		
DNAzyme (GR-5)	[b''|b'|a|rA|…|a*|b'*} [ACTCTAGGATTCGGCC|CT|CACTAT|rA|GGAAGAGATGATGTCT GTTTTTTTACAGACATCATCATCTCTGAAGTAGCGCCGCCGT|AT AGTG|AG}	（b'+b''=b）
HCR-H1	[x*|a*|b*|c|b} [GTGGTG|ATAGTG|AGGGCCGAATCCTAGAGT|CAAAGT|ACTCT AGGATTCGGCCCT}	Domain x* was not presented in Figure 1B and 3B for the easier understanding of the detection pathway.
HCR-H2	[b|a|b*|c*} [ACTCTAGGATTCGGCCCT|CACTAT|AGGGCCGAATCCTAGAGT| ACTTTG}	
Lock	[a|x} [CACTAT|CACCAC}	
Sequences for real-time fluorescence kinetic reading of DNAzyme-HCR reaction		
DNAzyme	[b''|b'|a|rA|…|a*|b'*} [ACTCTAGGATTCGGCC|CT|CACTAT|rA|GGAAGAGATGATGTCT GTTTTTTTACAGACATCATCATCTCTGAAGTAGCGCCGCCGT|AT AGTG|AG}	
HCR-F-H1	[a*|b*|c|b} [ATAGTG|AGGGCCGAATCCTAGAGT|CAAAGT|ACTCTAGGATTC GGCCC**T-6-FAM**}	3' T with 6-FAM
HCR-Q-H2	[b|a|b*|c*} [ACTCTAGGATTCGGCCCT|CACTA**T-BHQ1**|AGGGCCGAATCCTAGAGT|ACTTTG }	T (in loop) with BHQ1
Sequences for real-time fluorescence kinetic reading of DNAzyme cleavage reaction		
DNAzyme (F-GR-5)	[b''|b'|a|rA|…|a*|b'*} [ACTCTAGGATTCGGCC|CT|CACTAT|rA|GGAAGAGATGATGTCT GTTTTTTTACAGACATCATCATCTCTGAAGTAGCGCCGCCGT|AT AGTG|AG}	3' with BHQ1 5' with 6-FAM

Table S1. **Oligonucleotides used in this paper.** All labeled sequences were purified with high-pressure liquid chromatography.

	Number of events
Figure 4d	6
Figure 4e	883
Figure 4f	873
Figure 5b	1524
Figure 5c	61
Figure 5d	35
Figure 5e	30
Figure S2d	344

Table S2. Number of events in each scatter plot and histogram.

Reference:

(1). Fu, Y.; Tokuhisa, H.; Baker, L. A. *Chem.Commun.* 2009, 4877.

(2). Z. Zhu, Y. Zhou, X. Xu, R. Wu, Y. Jin, B. Li, *Anal. Chem.*, 2018, **90**, 814-820.

(3). Z. Zhu, R. Wu, B. Li, *Chem. Sci.*, 2019, **10**, 1953-1961.