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

Ruiping Wu\textsuperscript{a,b}, Zhentong Zhu\textsuperscript{a,c}, Xiaolong Xu\textsuperscript{a}, Chunmiao Yu\textsuperscript{a,b} and Bingling Li\textsuperscript{*a,b}

\textsuperscript{a} State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun, Jilin, 130022, P.R. China.
\textsuperscript{b} University of Science and Technology of China, Hefei, Anhui 230026, China.
\textsuperscript{c} University of Chinese Academy of Sciences, Beijing, 100049, China.
\* Email: binglingli@ciac.ac.cn. Phone: +86-431-85262008

Contents

Gel verification of the DNAzyme activity ........................................................................................................... Fig.S1
CGN set-up reproducibility .................................................................................................................................Fig.S2
CGN performance verification with the 5 kb dsDNA marker ........................................................................... Fig.S3
Optimization of concentration ratio between HCR H1(H2) and DNAzyme GR-5 ..........................................Fig.S4
Gel electrophoresis shows the “on” signal pathway ..........................................................................................Fig.S5
The current power spectral density of the nanopore used in Fig.4 ....................................................................Fig.S6
Oligonucleotides used in this paper ..................................................................................................................Table S1
Number of events in each scatter plot and histogram .........................................................................................Table S2
1. Supporting Figures and Tables

**DNAzyme** DNAzyme+Pb(II)

**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 Pb²⁺. 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.

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

Where R is the measured pipette resistance, \( \kappa \) is the specific resistance of the electrolyte used (\( \kappa = 1.2 \text{ S/m in 0.1 M KCl} \)), \( \theta \) is the cone angle (\( \theta = 12^\circ \) in Figure S1b) and \( a \) is the radius of the nanopore at the tip of the nanopipette.

S-2
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.

For all experiments, [H1]=[H2]= 500 mM
Buffer: 50 mM HEPES, 500 mM NaCl, 5 mM MgCl₂, pH 7.5
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.
Table S1. Oligonucleotides used in this paper. All labeled sequences were purified with high-pressure liquid chromatography.
Table S2. Number of events in each scatter plot and histogram.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Number of events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 4d</td>
<td>6</td>
</tr>
<tr>
<td>Figure 4e</td>
<td>883</td>
</tr>
<tr>
<td>Figure 4f</td>
<td>873</td>
</tr>
<tr>
<td>Figure 5b</td>
<td>1524</td>
</tr>
<tr>
<td>Figure 5c</td>
<td>61</td>
</tr>
<tr>
<td>Figure 5d</td>
<td>35</td>
</tr>
<tr>
<td>Figure 5e</td>
<td>30</td>
</tr>
<tr>
<td>Figure S2d</td>
<td>344</td>
</tr>
</tbody>
</table>

Reference:
