Supplementary Materials for

DNA-based Detection of Mercury(II) Ions through Characteristic Current Signals in Nanopore with High Sensitivity and Selectivity

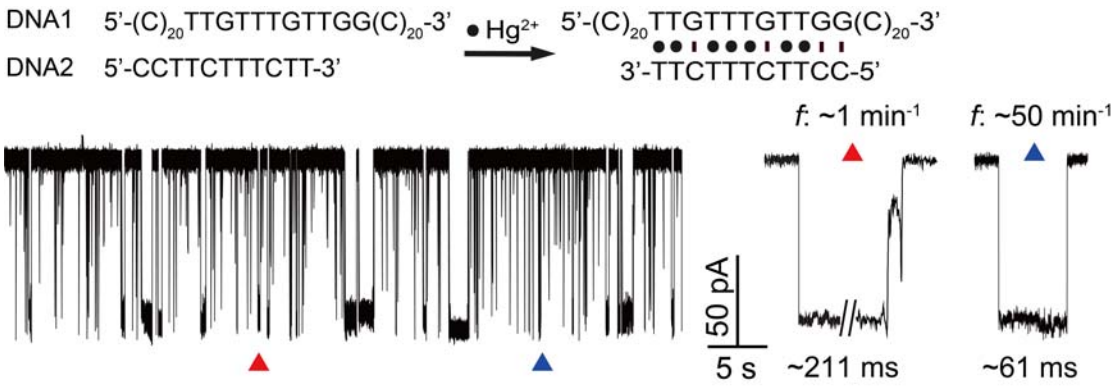

Fig. S1. A representative current trace of the translocation of probe DNA1/DNA2 (1 μM final concentration for each DNA fragment) through αHL in the presence of 100 μM Hg^{2+}. Events were dominated by single-level prolonged blocks (blue triangle, frequency: ~50 min^{-1}), and three-level signals only occasionally occurred (red triangle, frequency: ~1 min^{-1}). All traces were recorded at +120 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. DNA probe and Hg^{2+} were preincubated at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

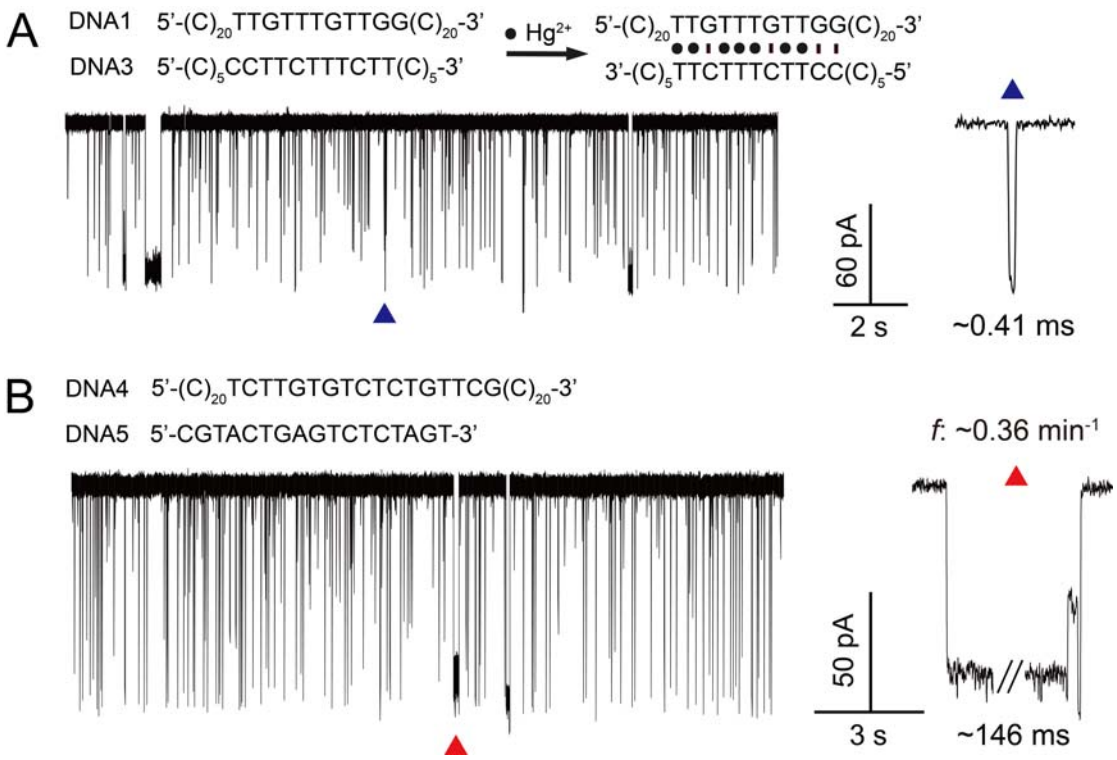

Fig. S2. (A) A representative current trace of the translocation of probe DNA1/DNA3 (1 μM final concentration for each DNA fragment) through αHL in the presence of Hg^{2+} (3 μM final concentration). DNA3 had an additional 5-cytosine tail at the 3' and 5' end as compared with DNA2. No three-level signals were observed when using this modification. Expanded view of a typical ssDNA translocation event was given on the right panel. (B) A representative current trace of the translocation of probe DNA4/DNA5 (1 μM final concentration for each DNA fragment) through αHL in the absence of Hg^{2+}. DNA4/DNA5 contained seventeen base pairings including six T-T mismatches. Signature events (red triangle) were observed in control group, at a frequency of ~0.36 min^{-1}. All traces were recorded at +120 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. DNA1/DNA3 and DNA4/DNA5 were preincubated with or without Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

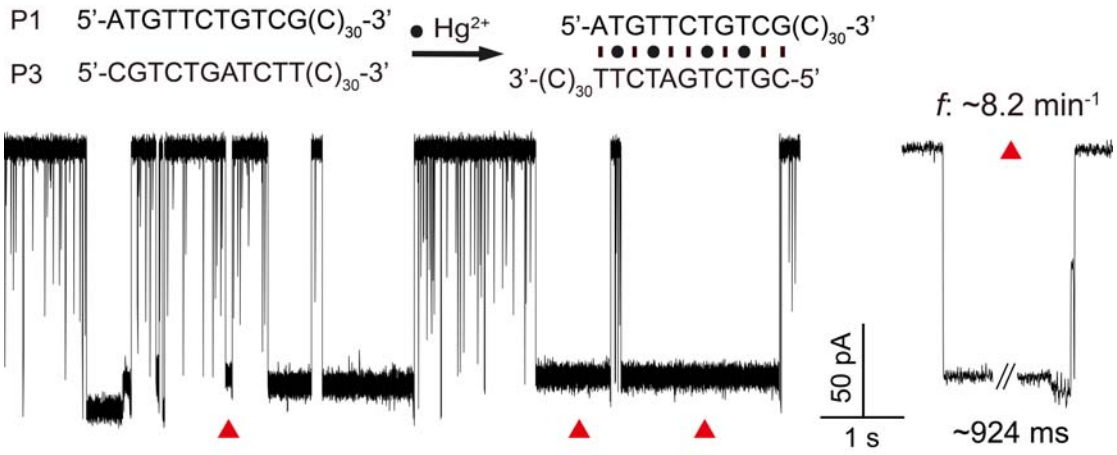

Fig. S3. (A) A representative current trace of the translocation of probe P1/P3 (1 μM final concentration for each DNA fragment) through αHL in the presence of Hg^{2+} (3 μM final concentration). P1/P3 contained seven Watson-Crick base pairs and four T-T mismatches; upon Hg^{2+} exposure, about 8.2 three-level signals were observed per minute (red triangle, expanded view on the right panel), which was comparable to the value obtained in P1/P2 which had a frequency of *ca*. 8.9 min^{-1}. Yet, a large number of ssDNA translocation events also appeared when using this probe, indicating that only part of the P1/P3 probe formed complexes with Hg^{2+}. Traces were recorded at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P1/P3 was preincubated with Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

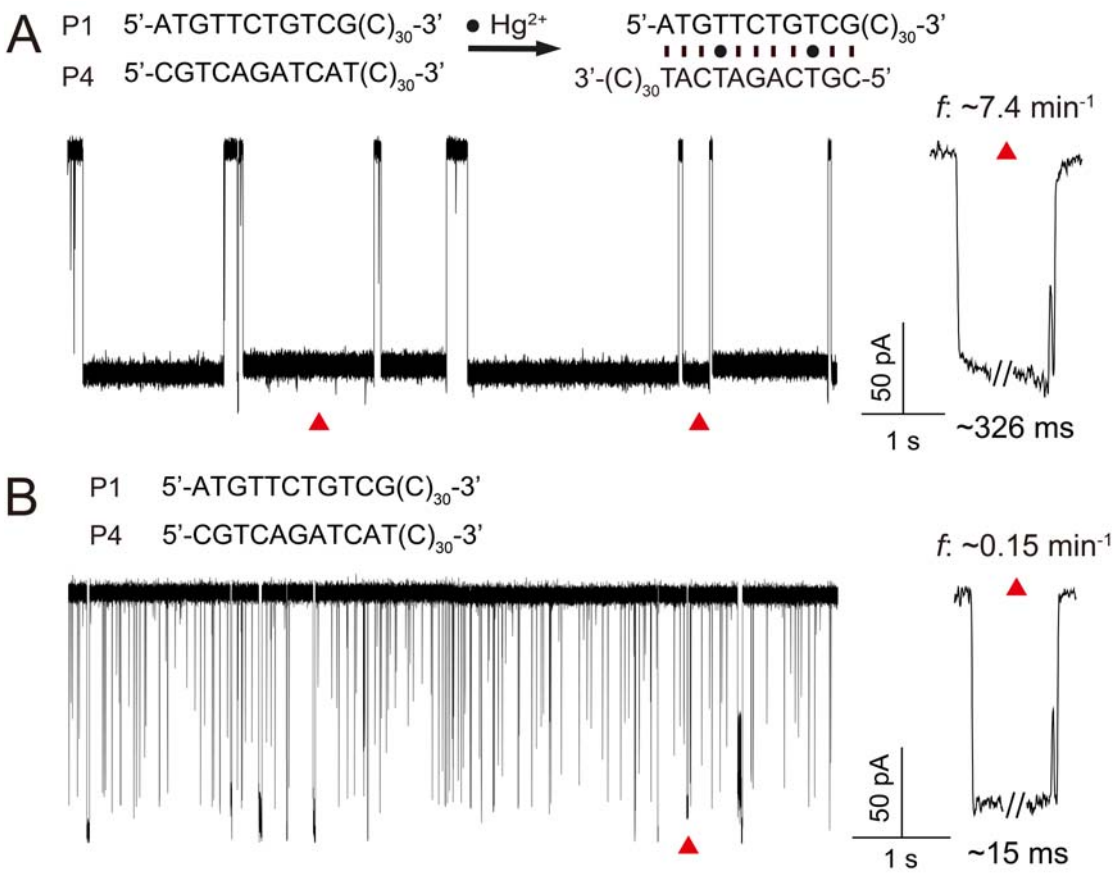

Fig. S4. Representative current traces of the translocation of probe P1/P4 (1 μM final concentration for each DNA fragment) through αHL in the presence (A) or absence (B) of 3 μM Hg^{2+}. P1/P4 contained nine Watson-Crick base pairs and two T-T mismatches. Though incubation of P1/P4 with Hg^{2+} would result in a signature occurrence rate of ~7.4 min^{-1} (A), occasional background interference was also observed, at a frequency of *ca.* 0.15 min^{-1} (B), limiting the application of this probe for highly sensitive detection of Hg^{2+}. Expanded view of typical signature events (red triangle) were given on the right panel. All traces were recorded at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P1/P4 was preincubated with or without Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

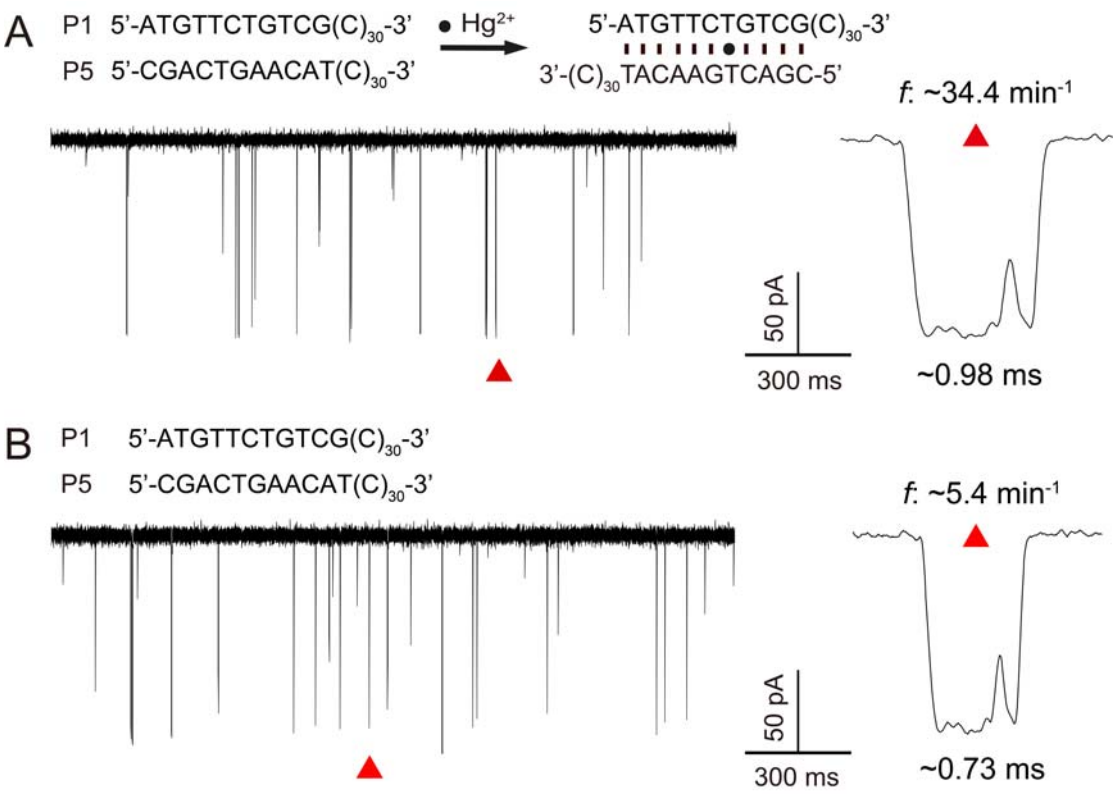

Fig. S5. Representative current traces of the translocation of probe P1/P5 (1 μM final concentration for each DNA fragment) through αHL in the presence (A) or absence (B) of 3 μM Hg^{2+}. P1/P5 contained ten Watson-Crick base pairs and one T-T mismatch. Detection of Hg^{2+} was significantly perturbed by the frequent background signals (~5.4 min^{-1}) that could hardly be separated with those obtained in the presence of Hg^{2+}. Expanded view of typical signature events (red triangle) were given on the right panel. All traces were recorded at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P1/P5 was preincubated with or without Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

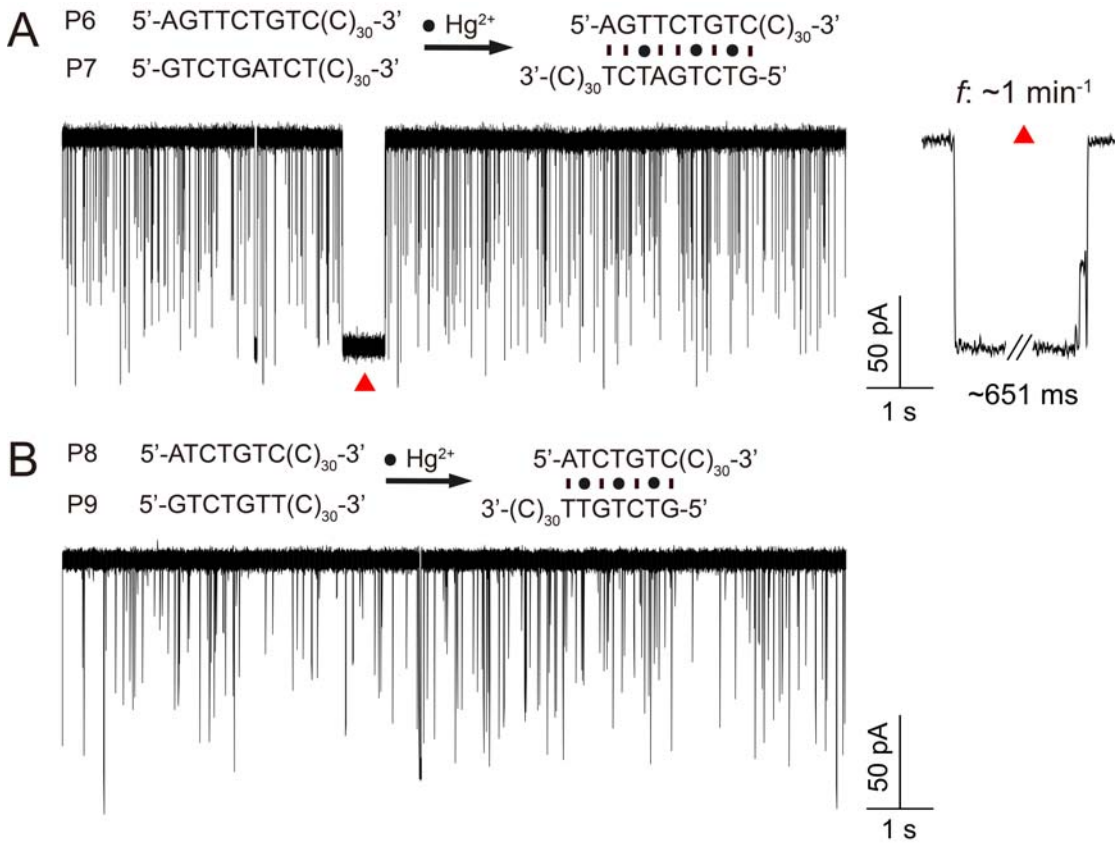

Fig. S6. Representative current traces of the translocation of probe P6/P7 (A) and P8/P9 (B) through αHL in the presence of 3 μM Hg^{2+}, respectively (final concentration of probe: 1 μM for each DNA fragment). P6/P7 contained six Watson-Crick base pairs and three T-T mismatches, while P8/P9 had four Watson-Crick base pairs and three T-T mismatches. Upon Hg^{2+} exposure, the desired three-level events significantly decreased (~1 min^{-1}, P6/P7) or even completely disappeared (P8/P9). All traces were recorded at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P6/P7 and P8/P9 were preincubated with Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

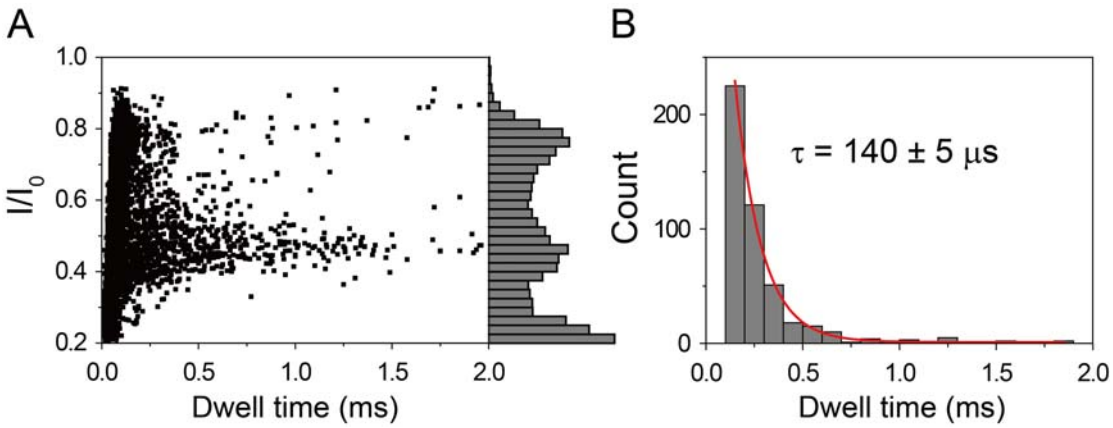

Fig. S7. (A) Scatter plot of the events caused by probe P1/P2 (1 μM final concentration for each DNA fragment) in the absence of Hg^{2+}. According to I/I_0 histogram, the translocation of single-stranded probe fragments gave an average current blockage of ~77%. (B) Dwell time histogram of P1/P2 translocation events ($I/I_0 > 0.7$). Data are fitted by a monoexponential function (solid line), giving a lifetime (τ) of 140 ± 5 μs. All traces were recorded at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P1/P2 was incubated without Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 6)

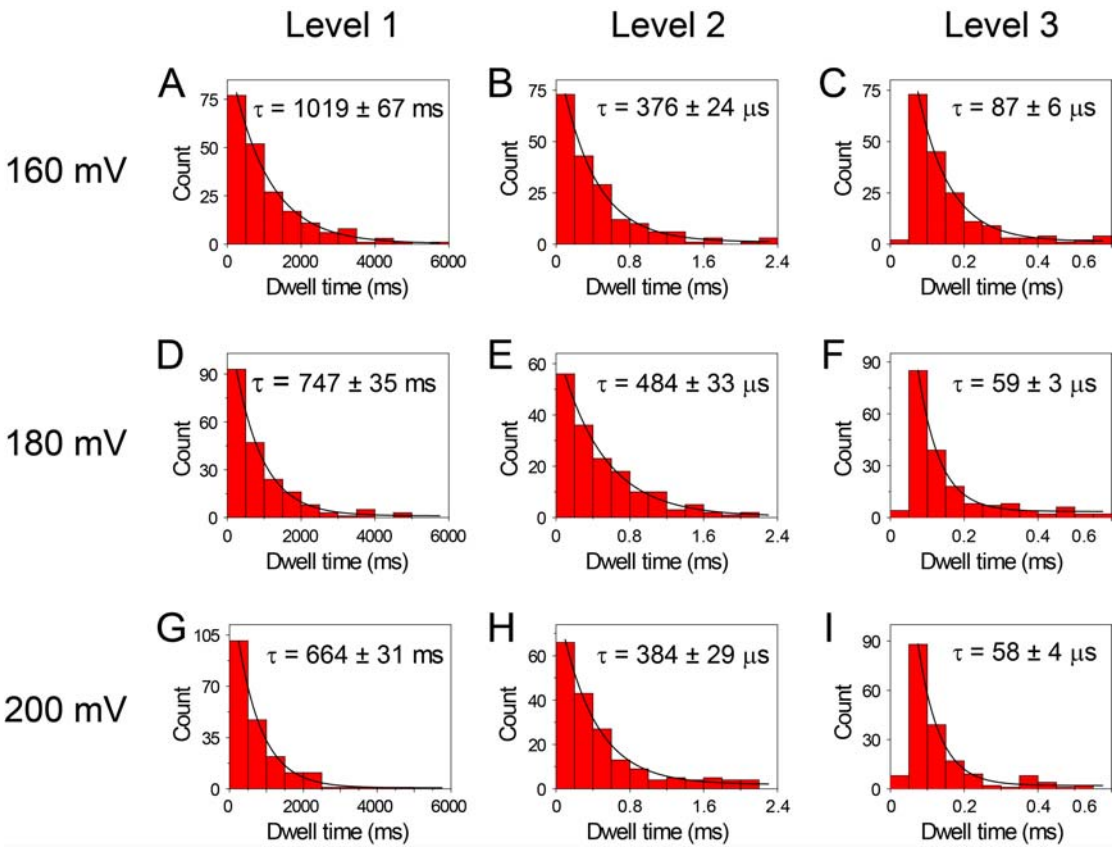

Fig. S8. Voltage-dependence of the durations of the three levels in Hg^{2+}-related signature events. All histograms were constructed based on at least 200 signature events. Data were fitted by a monoexponential decay function. Data were acquired at +160 mV, +180 mV, and +200 mV respectively in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. Probe P1/P2 (1 μM) and Hg^{2+} (3 μM) were preincubated at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3).

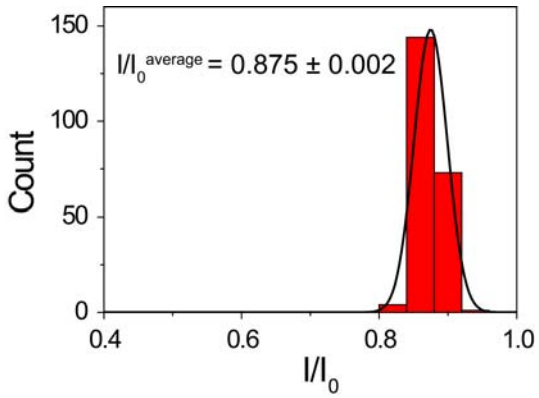

Fig. S9. I/I_0 histogram of the long-lived single-level blockades caused by P1/P2 (1 μM final concentration for each fragment) in the presence of 3 μM Hg^{2+}. The Gaussian fitting results indicated that the blockades had an average current blockage of 0.875 ± 0.002, which highly resembles the value of Level 1. The data were acquired at +140 mV in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. P1/P2 was incubated with Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 6)

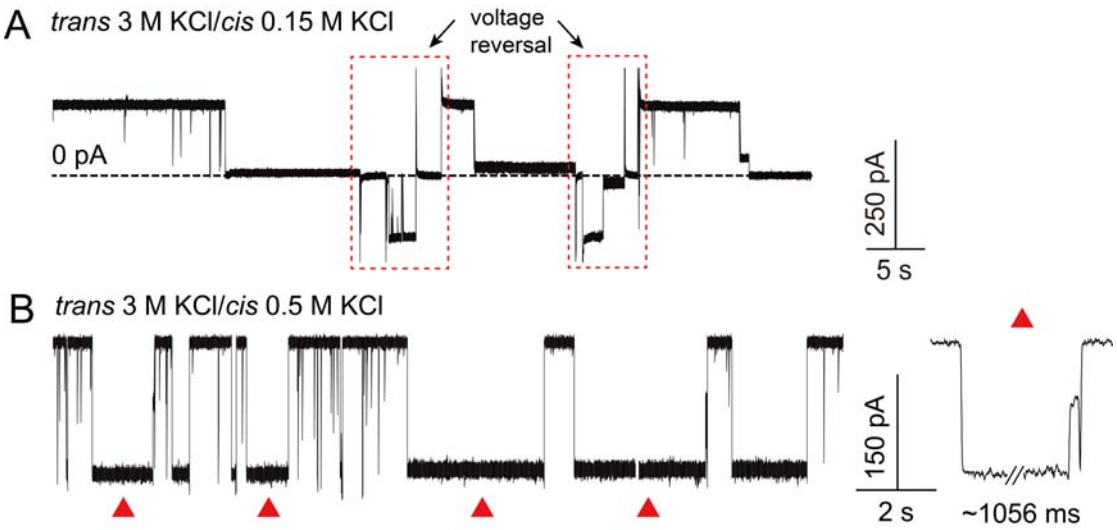

Fig. S10. (A) A representative current trace of the translocation of probe P1/P2 (1 nM final concentration for each DNA fragment) through αHL in 3 M *trans*/0.15 M *cis* KCl in the presence of 10 nM Hg^{2+}. Permanent blocking frequently occurred under this condition, which needed brief voltage reversal (dashed rectangle) to continue recording. (B) A representative current trace of the translocation of probe P1/P2 (10 nM final concentration for each fragment) through αHL in 3 M *trans*/0.5 M *cis* KCl in the presence of 100 nM Hg^{2+}. As was shown, pore cloggings were significantly reduced with lowered salt gradient; at the same time, characteristic current patterns (red triangle, expanded view on the right panel) were unaffected under such an asymmetrical condition. All traces were recorded at +140 mV. Probe P1/P2 was preincubated with Hg^{2+} at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 3)

Table S1. Voltage-dependence of the frequency of Hg^{2+}-related signature events. Data were acquired at +140 mV, +160 mV, +180 mV, and +200 mV respectively in the buffer of 1 M KCl and 10 mM Tris, pH 8.0. Probe P1/P2 (1 μM) and Hg^{2+} (3 μM) were preincubated at 15 ± 2 ºC overnight before measurement. (number of individual experiments n = 6 for 140 mV or 3 for other potentials).

Voltage (mV)	f_{Hg} (min^{-1})
140	8.9 ± 0.6
160	14.8 ± 0.9
180	26.6 ± 1.8
200	30.2 ± 1.3

Table S2. Frequency of the Hg^{2+}-related three-level signals (f_{Hg}) at different Hg^{2+} concentrations. Experiments were conducted in asymmetrical KCl solutions, 3 M *trans*/0.5 M *cis*, buffered with 10 mM Tris, pH 8.0 with the transmembrane potential held at +140 mV. DNA probe P1/P2 (10 nM final concentration for each DNA fragment) was preincubated with 0, 0.5, 1.0, 5, 10, 50, 100, and 250 nM of Hg^{2+} respectively at 15 ± 2 ºC overnight. (number of individual experiments n = 3)

Concentration of Hg^{2+} (nM)	f_{Hg} (min^{-1})
0	0 ± 0
0.5	0.13 ± 0.06
1	0.50 ± 0.10
5	2.05 ± 0.21
10	4.12 ± 0.35
50	5.51 ± 0.56
100	6.63 ± 0.51
250	6.44 ± 0.65