Supplementary Information

Displacement chemistry-based nanopore analysis of nucleic acids in complicated matrix

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Experimental Section

Materials and reagents. The nucleic acid release probe (sequence: 5'-CCCCTATCACGATTAGCATTAAAAAAAAAA-3') was synthesized by Integrated DNA Technologies (Coralville, IA), while both the capture probe (sequence: 5'-TCACGATTAGCCCCCCCCCC-(3'-Biotin)-3') and the target lung cancer biomarker microRNA (sequence: 5'-UUAAUGCUAAUCGUGAUAGGGG-3') were purchased from the Midland Certified Reagent Company (Midland, TX). All the nucleic acid solutions were prepared with DNase, RNase free water (Sigma-Aldrich) with their stock solutions at 1 mM each, and were kept at -20° immediately before and after use. Streptavidin modified magnetic beads (4mg/mL), rabbit whole blood (containing alsevers solution), and fetal bovine serum were obtained from New England BioLabs, Inc. (Ipswich, MA), HemoStat Laboratories (Dixon, CA), and Fisher Scientific (Suwanee, GA), respectively. Rabbit whole blood was kept at 4° C, while serum is stored at -20° C before and after use. Unless stated otherwise, all the other chemicals and reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO), and prepared with HPLC-grade water (ChromeAR, Mallinchkrodt Baker).

Planar bilayer experiments. Lipid bilayer was formed using 1,2-diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids, Alabaster, AL) according to the Montal-Mueller method[S1]. The insertion of α-hemolysin protein ion channel (0.01-0.1 ng/mL) into the bilayer membrane was achieved using a procedure as described in our previous reports[S2]. A pair of Ag/AgCl electrodes was employed to connect the *cis* (grounded) and *trans* compartments of the nanopore sensing chamber. Each chamber compartment contained an electrolyte buffer solution of 1 M KCl and 10 mM Tris-HCl (pH 8.0). The transmenbrane ionic current of the single α-hemolysin nanopore channel was recorded using a patch-clamp amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA) under an applied potential bias of +160 mV, unless stated otherwise. The current recording was filtered with a four-pore low-pass Bessel filter at 5 kHz, and digitized with a 1440A converter (Molecular Device) at a sampling frequency of 50 kHz. All the signatures of current modulations (events) were analyzed using Clampfit 10.5 software (Molecular Device).

Blood and serum sample analysis. The procedure for nanopore analysis of blood and serum samples was consisted of three main steps. The first step involved the coupling of biotin-modified ssDNA (i.e., capture probe) to the streptavidin-modified magnetic beads. Briefly, 250 μL of 4 mg/mL streptavidin-modified magnetic beads was washed with 100 μL washing/binding buffer (0.5 M NaCl, 20 mM Tris-HCl, 1 mM EDTA, pH 7.5). After applying a magnet to the beads and removing the supernatant, 250 nM biotin-modified ssDNA was added to the magnetic beads to form capture probe-beads complexes. The coupling reaction was held at room temperature for 10 min with occasional agitation. Then, the magnet was applied to remove the supernatant, followed by washing the capture probe-beads complex

three times with the washing/binding buffer. Next, 20 μL of rabbit blood or fetal bovine serum spiked with a certain concentration of the target microRNA (ranging from 50 to 200 nM) and 400 μL of the washing/binding buffer were added to the prepared capture probe-beads complex. After 10-min incubation with occasional agitation, magnet was applied to remove the supernatant. Then, the produced capture probe-microRNA-beads complex was washed with 100 μL washing/binding buffer, followed by applying the magnet to remove the supernatant. In the third step, 250 nM of replacement probe was added to the capture probe-microRNA-beads complex. After 15 min incubation (with stirring) and applying the magnet to the beads, the supernatant (containing release probe-microRNA duplex) was collected for nanopore analysis. It should be noted that, to improve the efficiency of hybridization, nucleic acid molecules were heated to 95 °C and then cooled in an ice bath for 3 minutes before the hybridization reaction.

Calculation of MicroRNA Recovery

The percent microRNA recovery from the blood sample was calculated by dividing the frequency of the release probe-microRNA duplex events obtained after treatment of the blood sample according to the displacement chemical reaction procedure by the frequency of the release probe-microRNA hybridization mixture in the $(M113F)_7$ αHL protein pore. The release probe-microRNA hybridization mixture was prepared in such a way that the release probe and microRNA were first incubated at 95 °C for 3 minutes, then cooled to room temperature to allow for the formation of double stranded DNA/RNA complex. The same concentrations of single-stranded microRNA and release probe were used in the blood sample analysis and release probe-microRNA hybridization control experiments. Single-channel recordings were performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0.

Determination of the Efficiency of the Capture Probe

The percent microRNA loss during the process of utilizing the capture probe to collect the target microRNA was obtained by dividing the event frequency of the free (unhybridized) single-stranded microRNA molecules in the supernatant after applying the magnet to the hybridization mixture of the capture probe and microRNA by the event frequency of the single-stranded MicroRNA standard solution in the $(M113F)_7$ αHL protein pore. The same concentrations of single-stranded microRNA solutions were used in these experiments. Single-channel recordings were performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0.

Supporting References

S1 Montal, M.; Mueller, P. Proc. Natl. Acad. Sci. U.S.A. 1972, **69**, 3561-3566.

S2 Chen, X.; Wang, L.; Roozbahani, G. M.; Zhang, Y.; Xiang, J.; Guan, X. Electrophoresis 2018, **39**, 2410-2416.

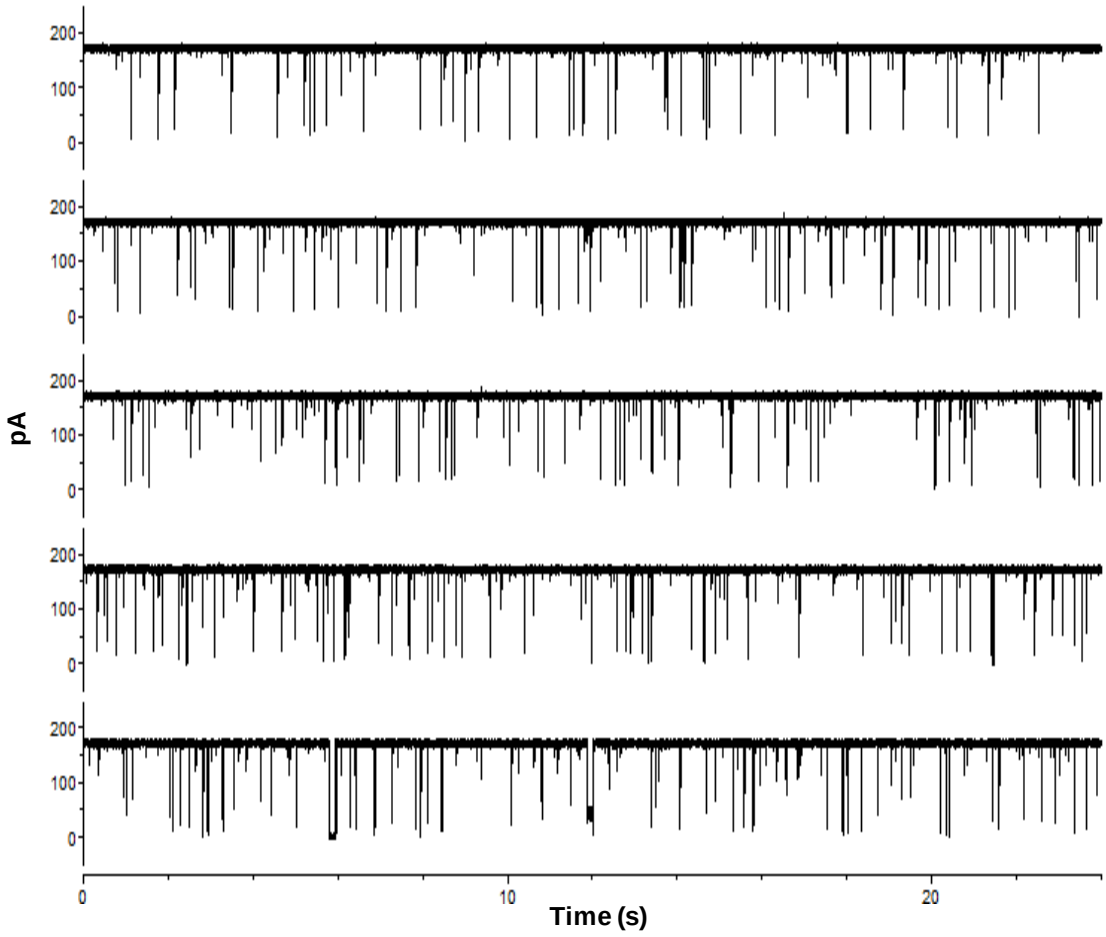

Figure S1. An uninterrupted 2-min single-channel recording trace segment of the target biomarker microRNA in the $(M113F)_7$ α-hemolysin pore. The experiment was performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentration of the microRNA used was 250 nM.

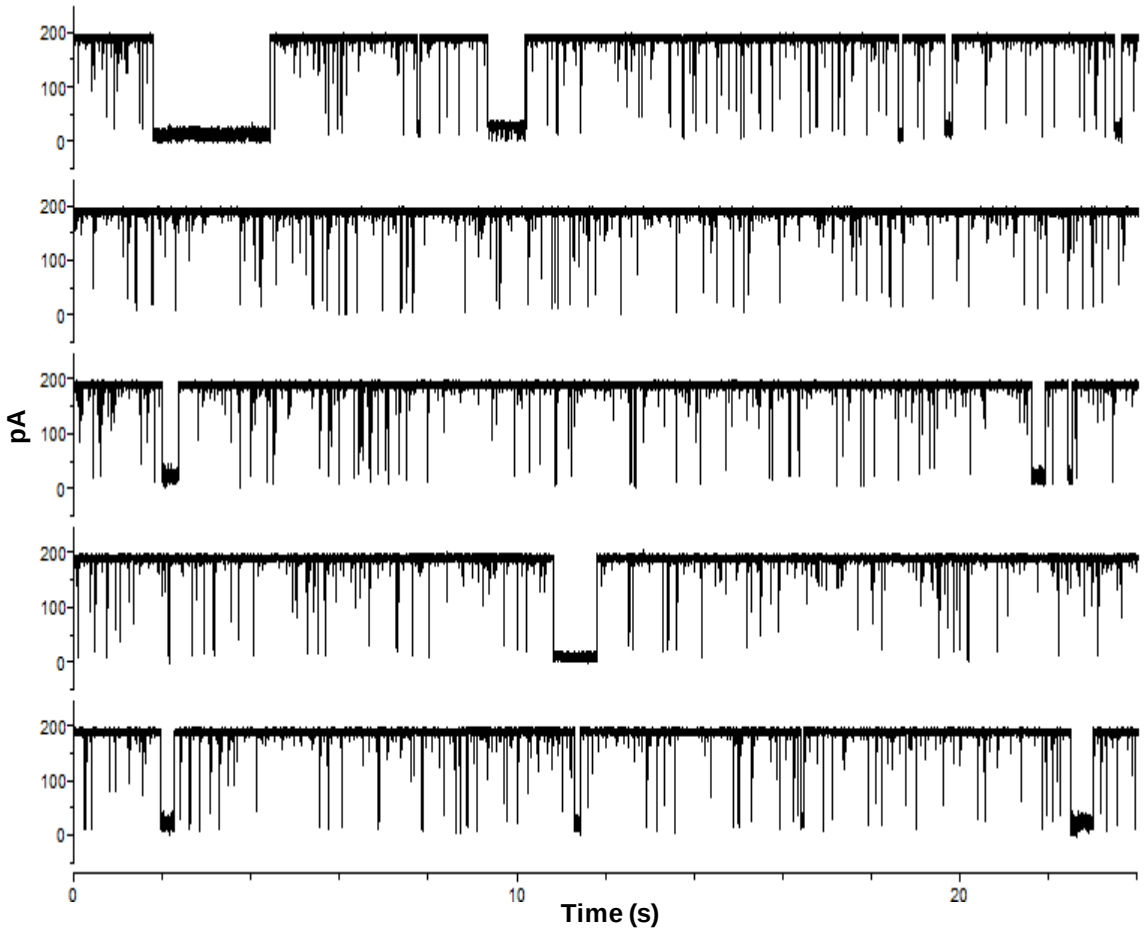

Figure S2. An uninterrupted 2-min single-channel recording trace segment of the hybridization mixture of capture probe and microRNA in the $(M113F)_7$ α-hemolysin pore. The experiment was performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of microRNA and capture probe were 250 nM each.

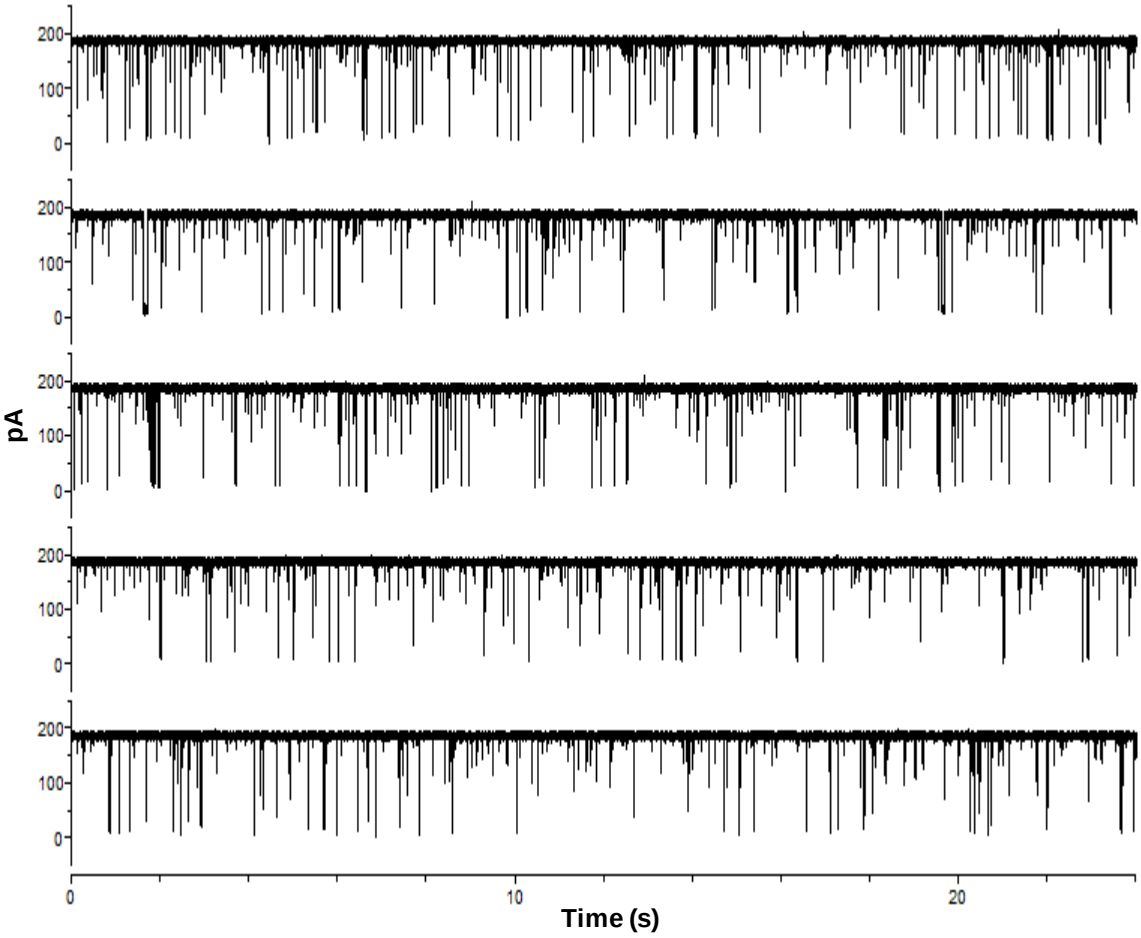

Figure S3. An uninterrupted 2-min single-channel recording trace segment of the hybridization mixture of release probe and microRNA in the $(M113F)_7$ α-hemolysin pore. The experiment was performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of microRNA and release probe were 250 nM each.

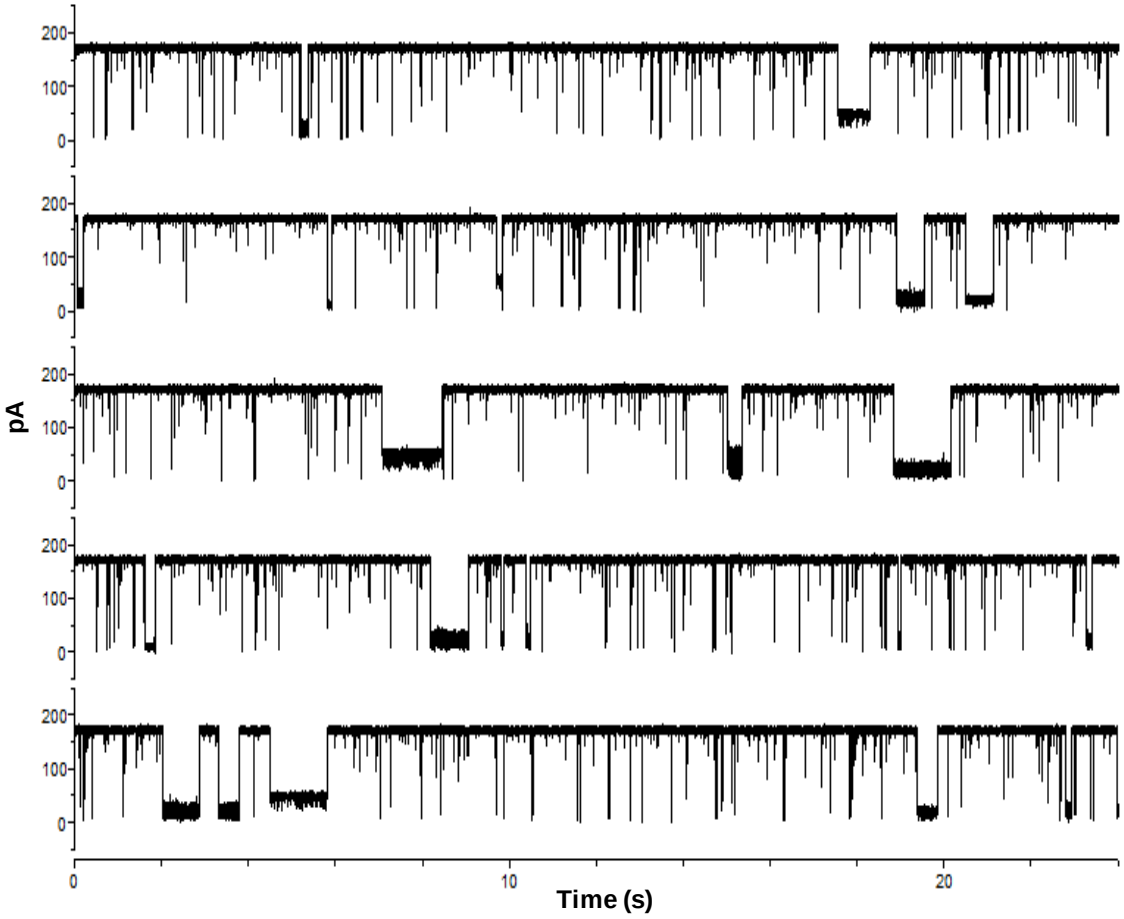

Figure S4. An uninterrupted 2-min single-channel recording trace segment of the hybridization mixture of capture probe, release probe and microRNA in the $(M113F)_7$ α-hemolysin pore. The experiment was performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of microRNA, capture probe, and release probe were 200 nM, 250 nM, and 250 nM, respectively.

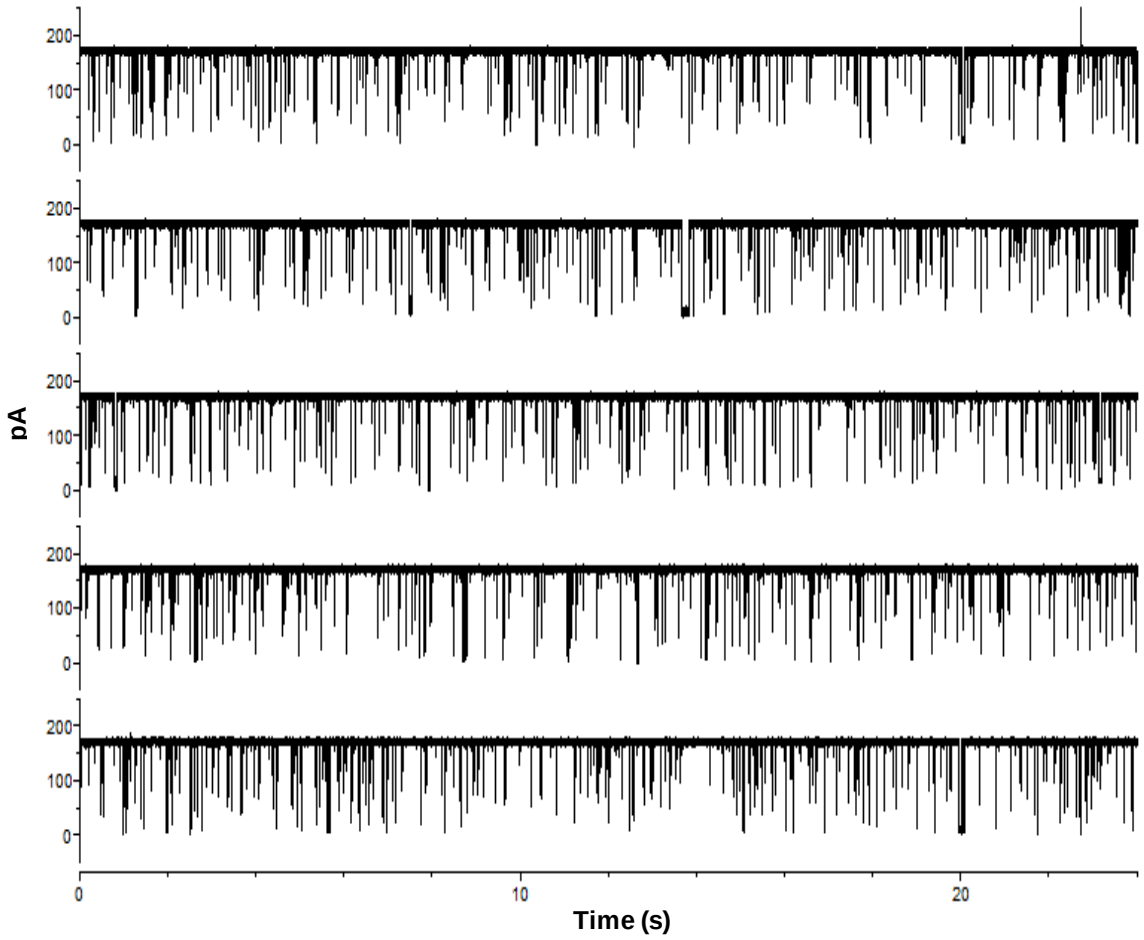

Figure S5. An uninterrupted 2-min single-channel recording trace segment of nanopore analysis of microRNA-containing whole blood. The experiment was performed at +160 mV with the $(M113F)_7$ α-hemolysin pore in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of microRNA, capture probe, and release probe were 250 nM each.

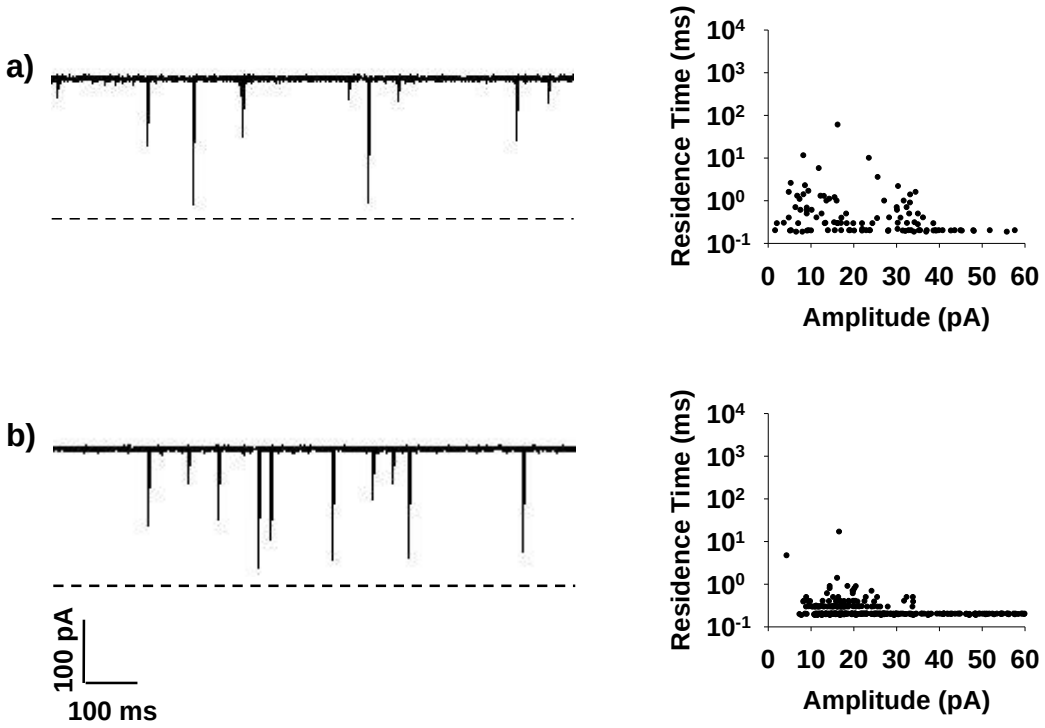

Figure S6. Translocation of ssDNA in the nanopore. (*Left*) Typical single-channel recording trace segments; (*Right*) the corresponding scatter plots of event residence time vs. residual current. (a) Capture probe; and (b) release probe. The experiments were performed at +160 mV with the $(M113F)_7$ αHL protein pore in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of capture probe and release probe were 500 nM each. Dashed lines represent the levels of zero current. Note that the capture probe used in this experiment was not immobilized on the streptavidin-coated magnetic beads.

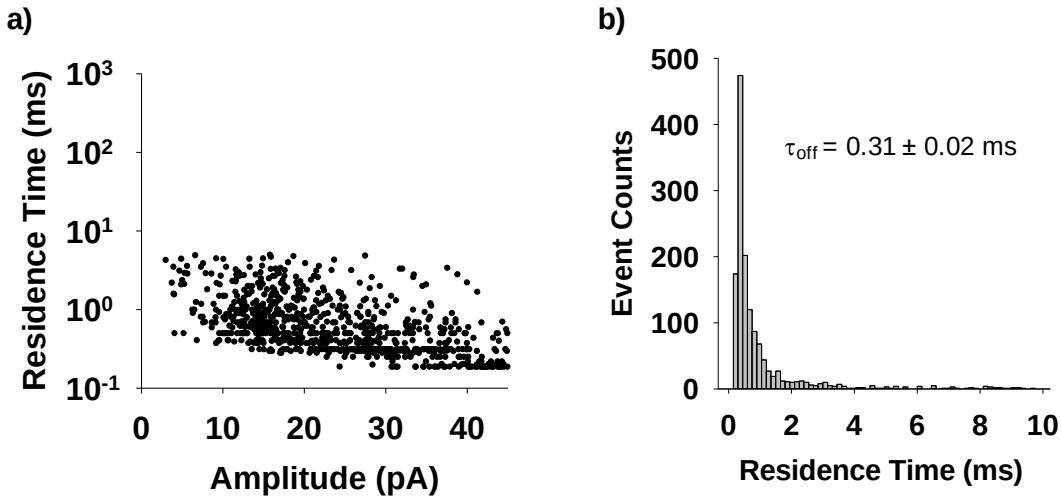

Figure S7. Characteristics of the short-lived events for the hybridization mixture of capture probe and microRNA in the $(M113F)_7$ α-hemolysin pore. (a) Scatter plot of event residence time vs. residual current; and (b) event residence time histogram. The experiment was performed at +160 mV in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. The concentrations of microRNA and capture probe were 250 nM each.

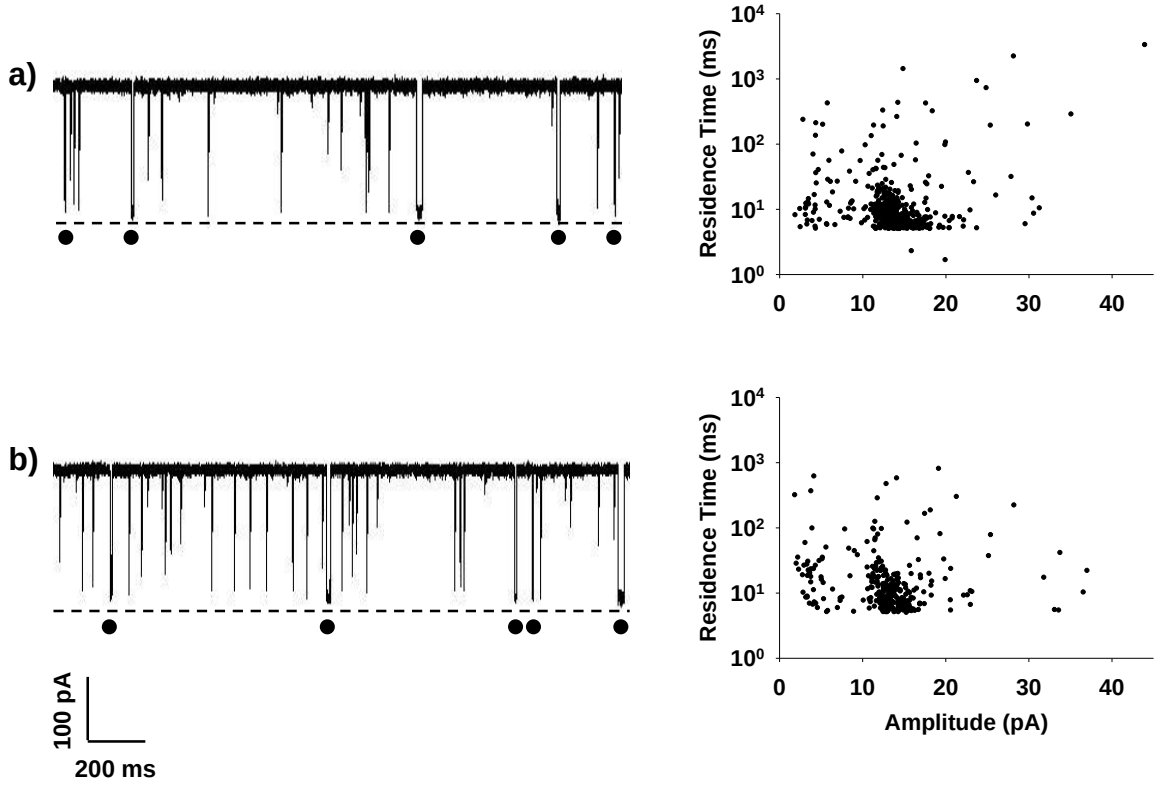

Figure S8. Nanopore detection of microRNA in the (a) absence and (b) presence of fetal bovine serum, showing that serum matrix didn't affect nanopore analysis. (*Left*) Typical single-channel recording trace segments; (*Right*) the corresponding scatter plots of event residence time vs. residual current. The experiments were performed at +160 mV with the $(M113F)_7$ αHL protein pore in 1 M KCl solution containing 10 mM Tris-HCl at pH 8.0. Dashed lines represent the levels of zero current. The characteristic release probe-microRNA duplex events were highlighted (black dot-marked). The microRNA samples (without/with serum) were analyzed according to the displacement chemistry-based nucleic acid detection principle and using the procedure as described in the experimental section. The concentrations of capture probe, microRNA, and release probe used in Fig. S8a were 250 nM, 200 nM, and 250 nM, respectively, while those in Fig. 8b were 500 nM, 200 nM, and 500 nM, respectively.