Electronic Supplementary Information (ESI) for Chemical Communications

Rapid and sensitive detection of HBV DNA based on nanopore sensor

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Experimental

Materials The wild-type α-HL protein was synthesized by in vitro by coupled transcription and translation (IVTT). All DNA samples, including probes and targets were purchased from AuGCT DNA-SYN Biotechnology Co., Ltd., (Beijing, China). Lipid 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). 25-μm thick Teflon film was obtained from Goodfellow Corp., (Malvern, PA, USA). The DNA stock solution (100 μM) were prepared in ultrapure water, heated at 95 ℃ for 30 min and cooling to room temperature. The electrolyte solution used in this study

was prepared in ultrapure water, which contains 1M KCl and 10 mM Tris-HCl with the pH = 8.5.

Table. S-1 Sequences used for HBV DNA detection in the nanopore study

Name	Sequences
Probe: double overhangs	5′-$(A)_{25}$-GAGGAGTTGGGGGAGGAGATT-$(A)_{25}$-3′
Probe: 5′overhang	5′-$(A)_{25}$-GAGGAGTTGGGGGAGGAGATT-3′
Probe: 3′overhang	5′-GAGGAGTTGGGGGAGGAGATT-$(A)_{25}$-3′
Target-fully matched	5′-AATCTCCTCCCCCAACTCCTC-3′
Target-one base mismatch	5′-AATCTCCTCCACCAACTCCTC-3′
Target-two base mismatch	5′-AATCTCCTCAACCAACTCCTC-3′

Note: the red colour characters represent the mismatched base position

Single-Channel Recording The buffer used for bilayer recording was 10 mM Tris-HCl containing 1M KCl (pH 8.5). Planar lipid bilayer membranes of DPhPC were formed using the method of Montal and Mueller[1] on a ~120 μm diameter aperture in teflon film which separates the cis and trans chambers (each 2.0 mL) of a teflon apparatus. Prior to formation of the bilayer, the aperture was pretreated with a 1:100 hexadecane/pentane mixture. Then the chamber on both sides were filled with 1 mL buffer solution to the level just below the aperture and 10 μL of lipid solution was transferred to each chamber. After 5 min, the pentane was evaporated and solvent-free lipid monolayer formed at the

solution-air interface. Then another 600 μL buffer solution was gradually added to each chamber until the buffer level in each chamber raised above the aperture. The formation of a bilayer was monitored by observing the increase in membrane capacitance of 8-10 fF μm^{-2}. The α-HL protein was added to the cis chamber, which was connected to "ground". The potential was applied at 150 mV from the cis chamber with two freshly prepared Ag/AgCl electrodes in 1.5 % agarose saturated with 3 M KCl. Once the successful insertion of a single α-HL pore occurred, the mixture of DNA probe (final concentration 250 nM) and DNA target (final concentration 250 nM) were added to the cis chamber. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon Instruments, Foster city, CA, USA). The signal was filtered with a low-pass Bessel filter set at 5 kHz and sampled at a frequency of 20 kHz with a Digidata 1440A A/D converter (Axon Instruments). All the recording were conducted at 25℃.

Data analysis Data were analyzed using pClamp 10.3 (Molecular Devices) and Origin 8.0 software. Events with current blockages larger than 70% and dwell time longer than 1 ms were analyzed as duplex unzipping events, which were well separated from translocation events of unbound strands in duration (< 0.5 ms). The mean dwell time (τ) for DNA polymers were obtained from the dwell time histograms by fitting the distributions to single exponential functions by the Levenberg-

Marquardt procedure. The predicted theoretical hybridzation free energy between the probe and DNA target is calculated by the DINAMelt Web Server.[2]

Effect of transmembrane voltage on the tranlocation time of the hybrid

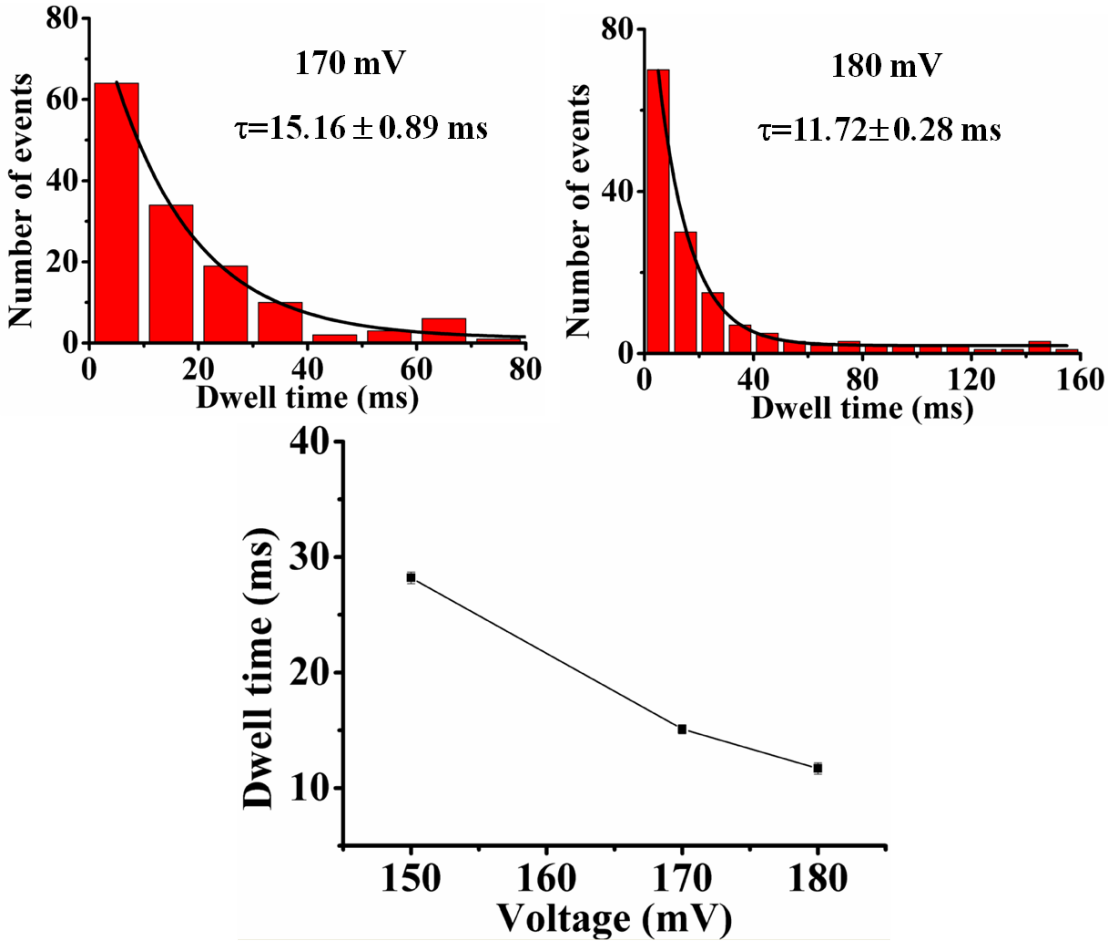

Fig. S1 Effect of the transmembrane voltage on the mean tranlocation time of the probe/target hybrid through the pore. The experiments were performed with the αHL protein nanopore in solution containing 1M KCl buffered with 10 mM Tris (pH 8.5), the applied potential was +150 mV. Concentration of probe and target both were 250 nM. Three separate experiments were performed, and the mean value is plotted.

Target DNA translocation through the pore

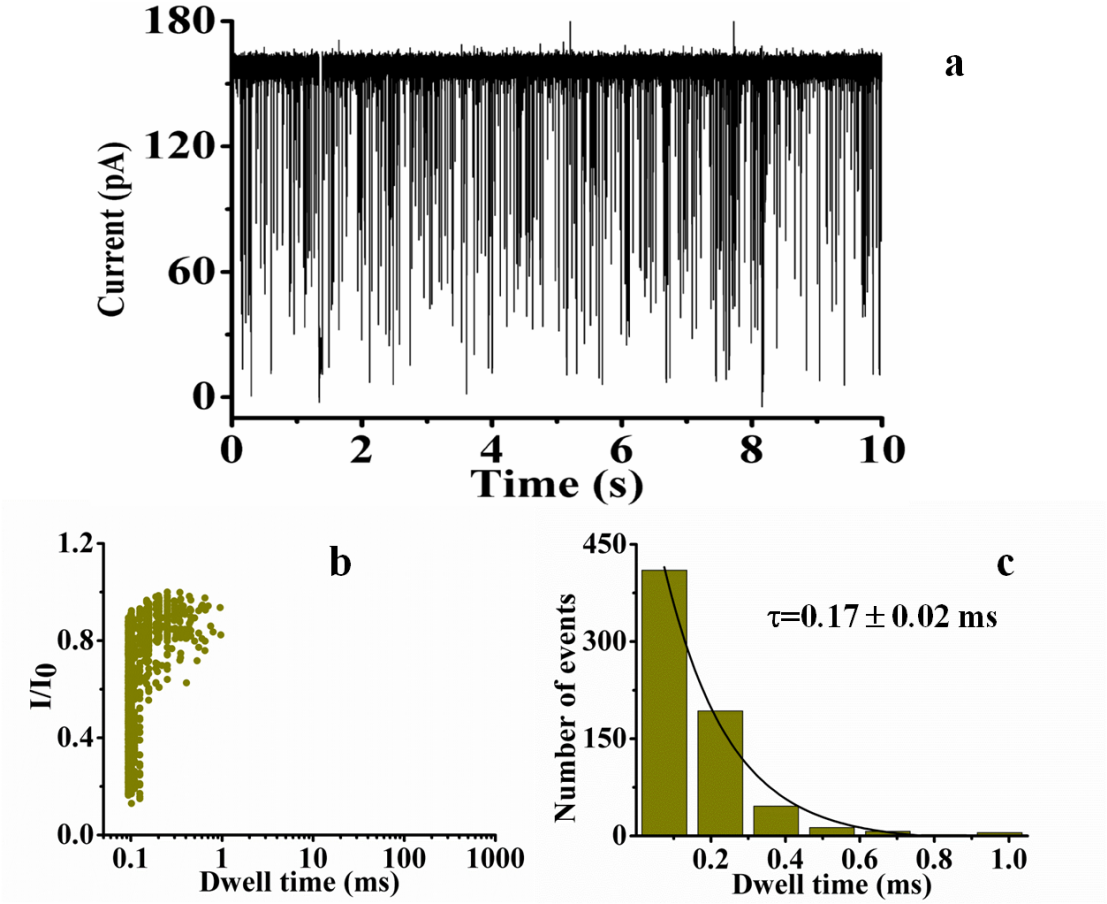

Fig. S2 Translocation of the HBV target DNA in the αHL protein nanopore in solution containing 1M KCl buffered with 10 mM Tris (pH 8.5), the applied potential was +150 mV. (a) Representative traces of the target DNA added from the cis side. (b) The scatter plot of events blockage amplitude vs dwell time. Each point represents a single translocating events. (c) The histogram of dwell time. I in Figure S2b is current blockage of translocation events, and I_0 is the open pore current. I/I_0 is normalized current blockage.

Optimization of the probe

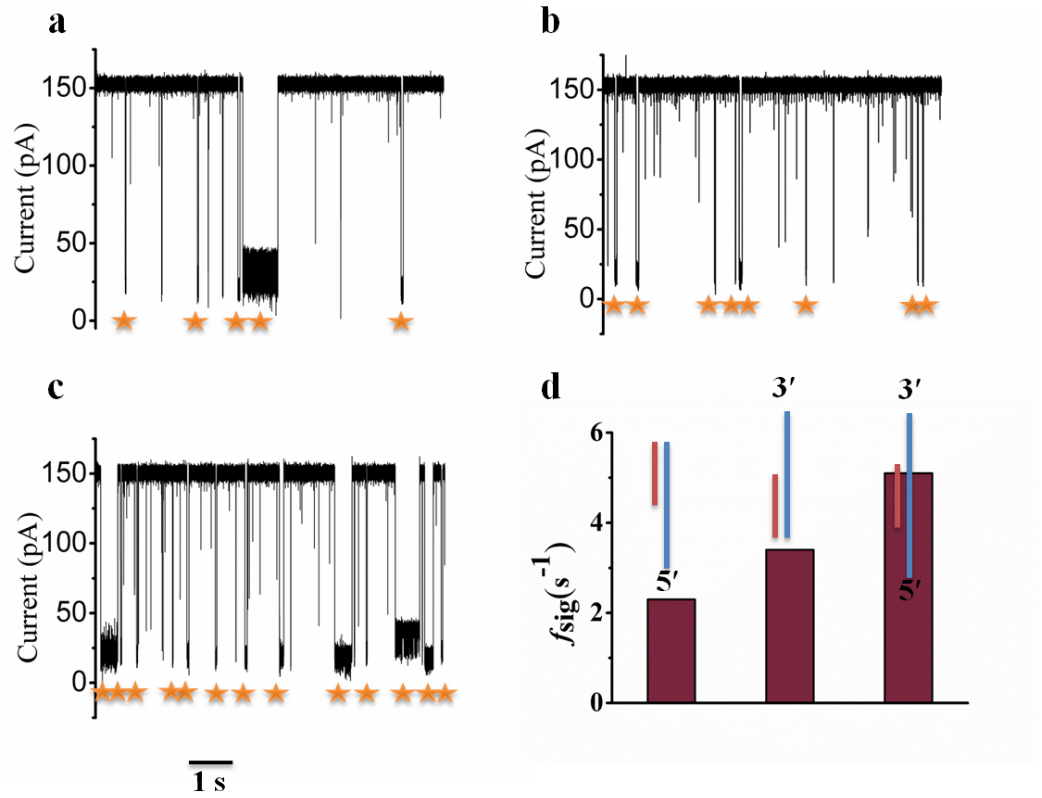

Fig. S3 Optimization of the probe for improving the α-HL nanopore sensors sensitivity. Representative current traces showing the frequencies of signature events for HBV target DNA hybridized to the probes with ploy$(dA)_{25}$ overhang at the 5′ end (a), at the 3′end (b) and at the both end (c). the ☆ at the bottom of current traces (a), (b) and (c) indicated the signature events. The experiments were performed respectively at +150 mV in the presence of 250 nM probe and 250 nM target in the *cis* solution. (d) The histogram of signature event frequency.

The serum sample experiments

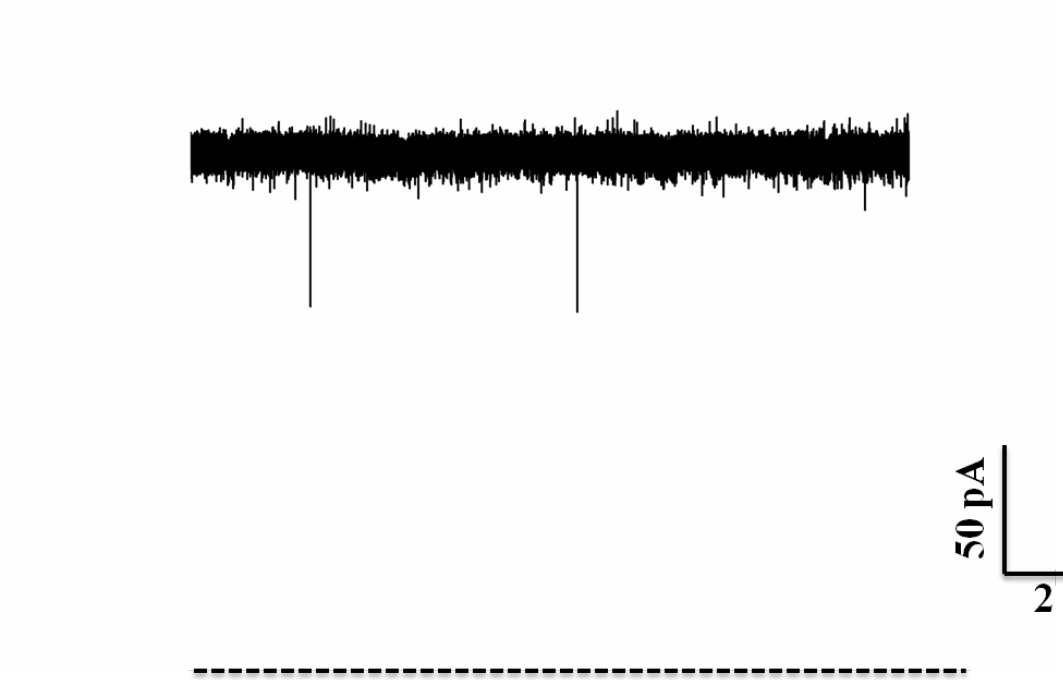

Fig. S4 Representive trace for the serum sample without the probe. The expreriments were performed with the αHL nanopore in solution containing 1M KCl buffered with 10 mM Tris (pH 8.5), the applied potential was +150 mV. Normal human serum were purchased from Shanghai Biosun Biotech. Inc (No 3H1000). The samples were centrifuged at 2000 rpm for 15 min. Then, the supernatants were collected. Added 10 μL of the supernatants to the cis chamber for single-channel recording.

Reference

1. M. Montal and P. Mueller, *Proc. Natl. Acad. Sci. U S A*, 1972, 69, 3561-3566.
2. N. R. Markham and M. Zuker, *Nucleic Acids Res.*, 2005, 33, W577-581.