Supporting Information

Signal on-off strategy based on digestion of DNA cube assisted by CRISPR-Cas12a system for ultrasensitive HBV detection in solid-state nanopores

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Oligonucleotides

Table S1 Oligonucleotides used in this study.

Name	Sequences (5'- 3')
A	GGCAACTTTGATCCCTCGGTTTAGCGCCGGCCTTTTC TCCCACACTTTCACG
B	GGGAAATTTCGTGGTAGGTTTTGTTGCCCGTGTTTCT ACGATTACTTTGGTC
C	GGACATTTTCGAGACAGCATTTTTTCCCGACCTTTGC GGATTGTATTTTAGG
D	GGCGCTTTTGACCTTCTGCTTTATGTCCCCTATTTCTT AATGACTTTTGGCC

E	GGGAGATTTAGTCATTAAGTTTTACAATCCGCTTTGTAATCGTAGTTTGTGT
F	GGGATCTTTACCTACCACGTTTTGCTGTCTCGTTTGCAGAAGGTCTTTCCGA
crRNA	UAAUUUCUACUAAGUGUAGAU**UCAUCUUCCUCUGCAUCCUG**
Tetrahedron- A	GCCTGGAGATACATGCACATTACGGCTTTCCCTATTAGAAGGTCTCAGGTGCGCGTTTCGGTAAGTAGACGGGACCAGTTCGCC
Tetrahedron- B	CGCGCACCTGAGACCTTCTAATAGGGTTTGCGACAGTCGTTCAACTAGAATGCCCTTTGGGCTGTTCCGGGTGTGGCTCGTCGG
Tetrahedron- C	GGCCGAGGACTCCTGCTCCGCTGCGGTTTGGCGAACTGGTCCCGTCTACTTACCGTTTCCGACGAGCCACACCCGGAACAGCCC
Tetrahedron- D	GCCGTAATGTGCATGTATCTCCAGGCTTTCCGCAGCGGAGCAGGAGTCCTCGGCCTTTGGGCATTCTAGTTGAACGACTGTCGC
Forward primer	TCGTGTTACAGGCGGGGTTT
Reverse primer	TGGCTCAGTTTACTAGTGCC

Table S2 Detailed compositions of NEBuffer 2.1, NEBuffer r2.1, TAEMg buffer and test buffer.

Buffer	**Compositions**
NEBuffer 2.1 (1X)	50 mM NaCl 10 mM Tris-HCl

	10 mM $MgCl_2$ 100 µg/ml BSA pH 7.9@25°C
NEBuffer r2.1 (1X)	50 mM NaCl 10 mM Tris-HCl 10 mM $MgCl_2$ 100 µg/ml Recombinant albumin pH 7.9@25°C
TAEMg buffer (1X)	2.5 mM Mg(OAc)2·4H2O 4.5 mM Tris 0.2 mM EDTA pH 8.0@25°C
Test buffer (1X)	1 M KCl 10 mM Tris 0.1 mM EDTA pH 8.0@25°C

Supplementary Figures

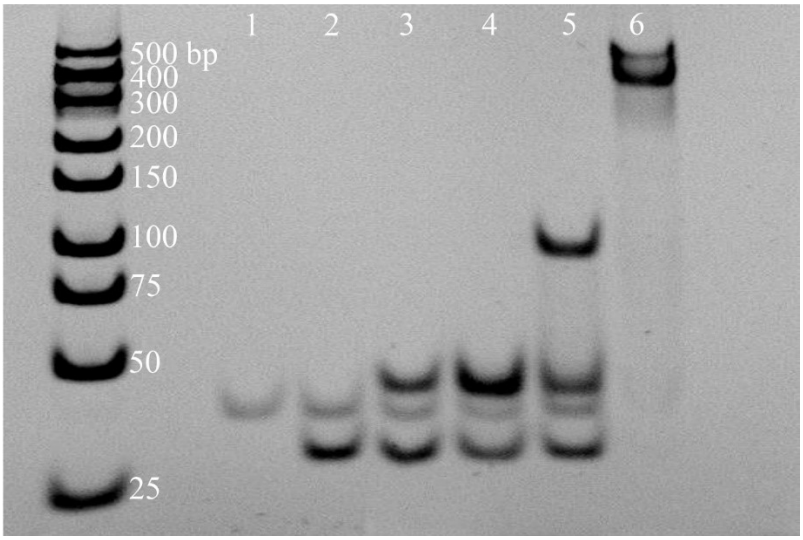

Fig. S1 Native-PAGE (8%) analysis of the self-assembled DNA cube. The complete DNA cube composed with six strands (lane 6) showed the lowest mobility compared with other hybridization products composed with one to five strands (lane 1-5, 1 μM for each product). This result indicates that only with all six elements, the DNA cube will be correctly synthesis with the formation reaching the max mass and spatial complexity.

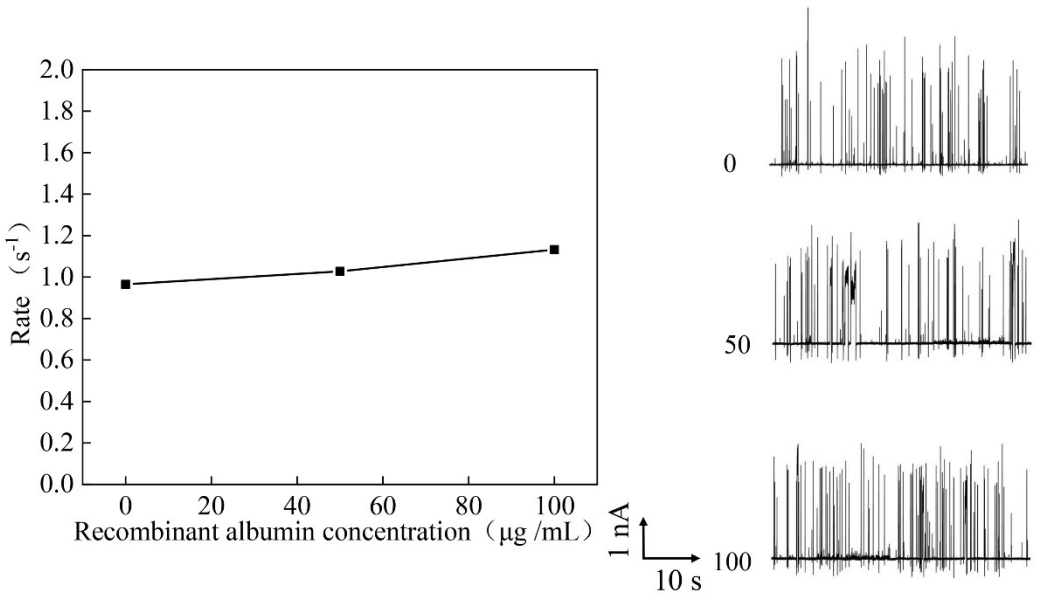

Fig. S2 Performance of the event rate of the DNA cube when buffer contained different concentration of recombinant albumin. In all concentration, DNA cube showed good translocation signal. When the concentration of recombinant albumin increases in the reaction buffer, the event rate of cube slightly increases.

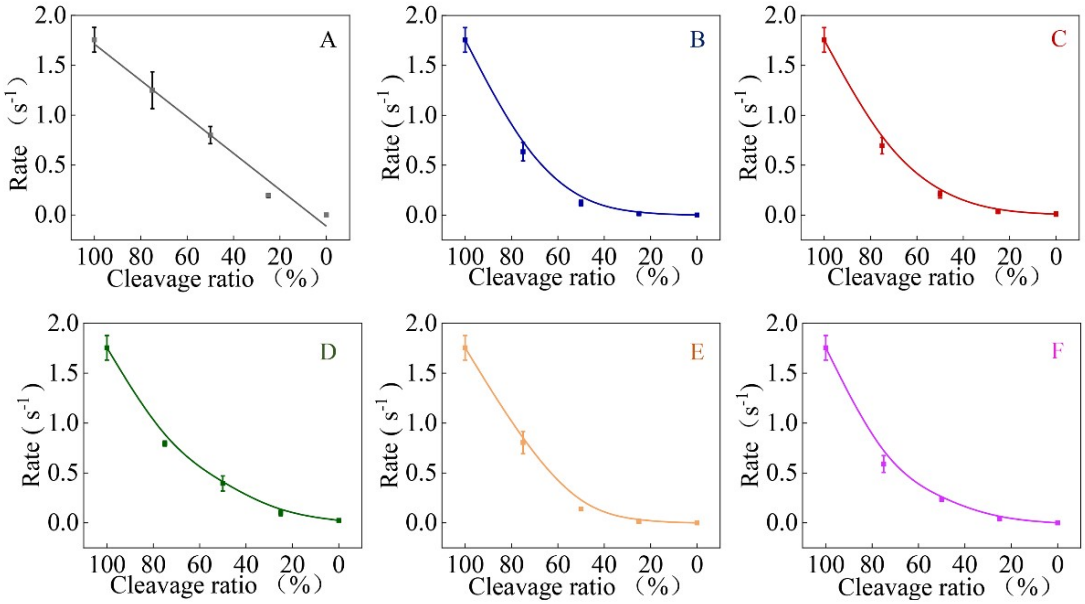

Fig. S3 The event rate change when elements A, B, C, D, E, or F were cleaved. No matter which element was cleaved, the event rate dropped with the increase of the

cleavage ratio. However, the event rate when element A was cleaved decreased linearly, while decreased exponentially when other elements were cleaved, which may be due to more starting and ending points (3) when complementary pairing with other elements.

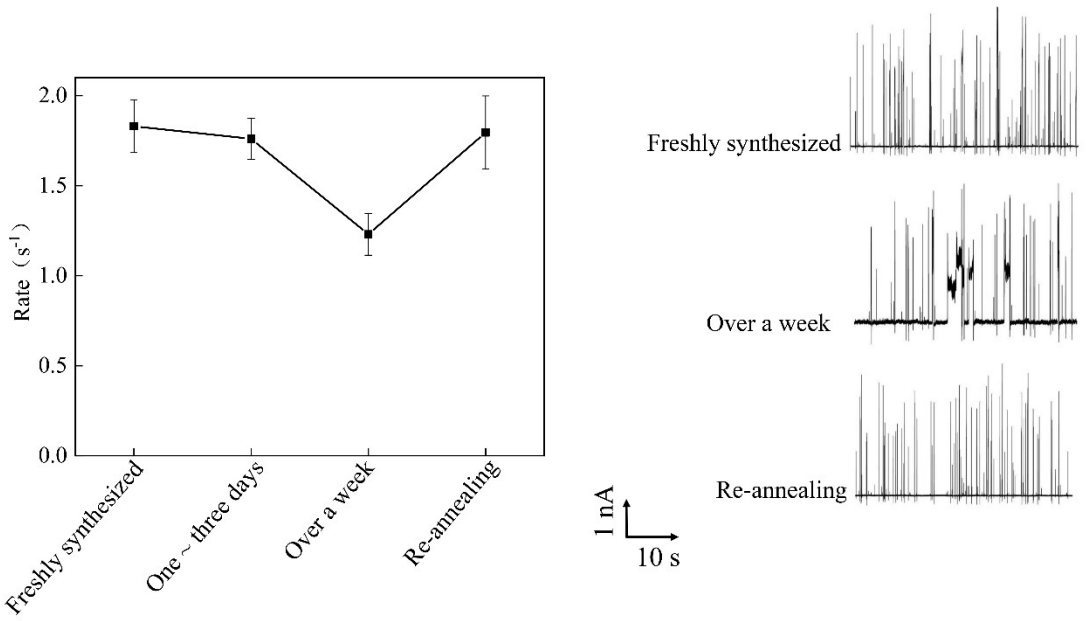

Fig. S4 The event rate change of DNA cube with time (stored at 4 ° C). The event rate of the DNA cube can maintain stable within a 1~3 day, and drop to about 65% when stored over a week. After re-annealing, the event rate can be recovered.

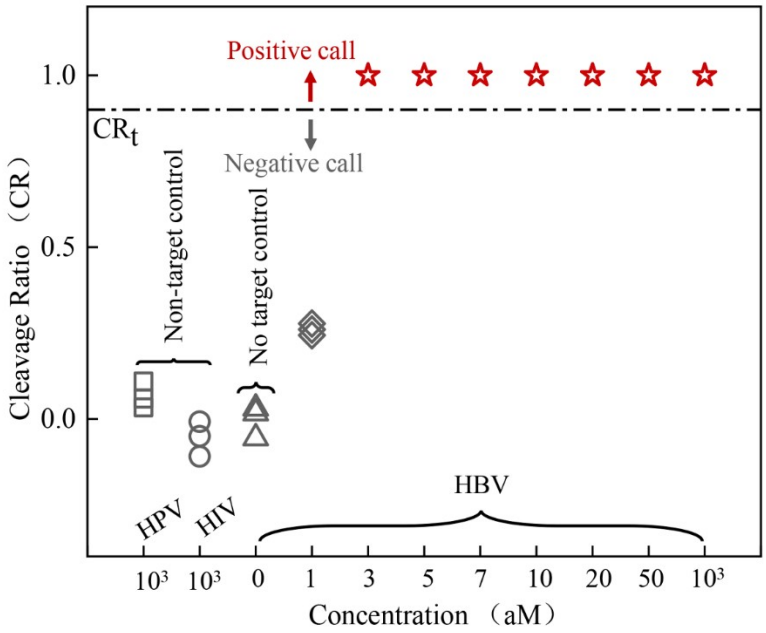

Fig. S5 Sensor performances in human serum. The cleavage ratio of HPV and HIV samples was about 0, indicating the sensor was inert to these interferents. For HBV target, the result was negative when the concentration of HBV target was below 3 aM, while when the concentration of HBV exceeded 3 aM, the results turned positive. When HBV is 1 aM, false-negative judgment occurs.