Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

An Aptamer-Assisted Nanopore Strategy with a Salt Gradient for Direct Protein Sensing

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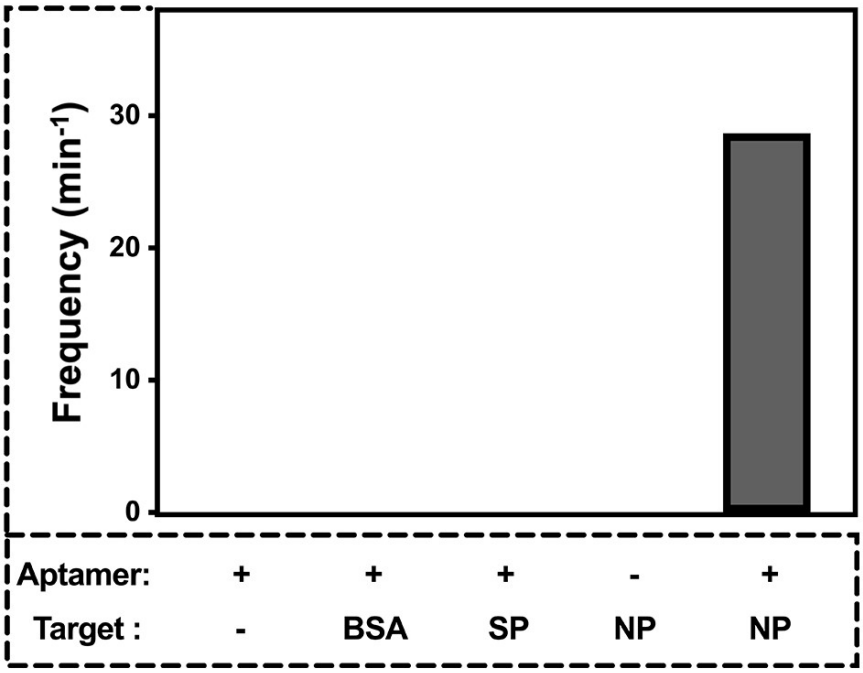
Results and Discussion

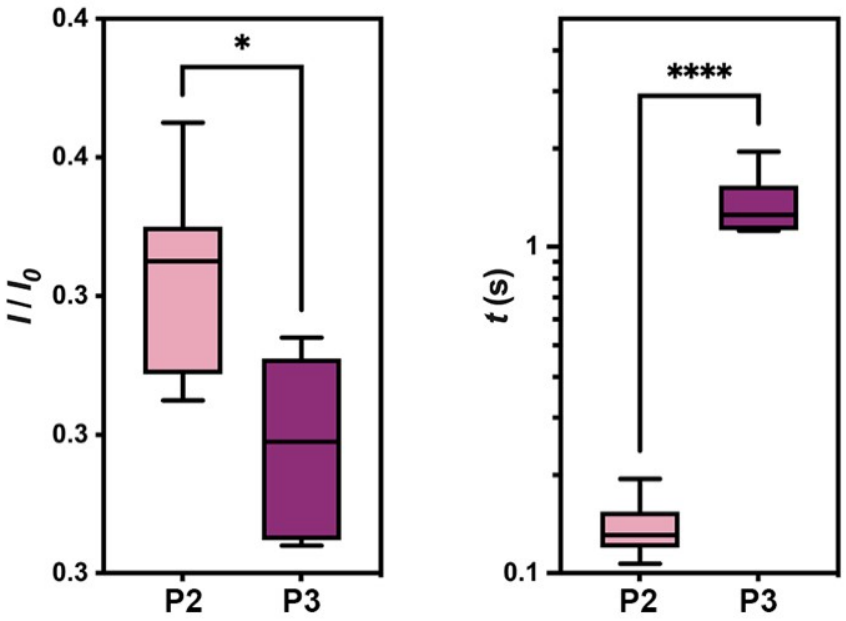
S1. Aptamer binding specificity

Figure S1. A series of control tests (from left to right) were conducted: (I) Aptamer with no target proteins; (II) Aptamer with target protein replaced of Bovine Serum Albumin (BSA); (III) Aptamer with target protein replaced of SARS-CoV-2 Spike Glycoprotein (SP); (IV) N protein with no aptamer added. The histograms show the capture frequencies (min^{-1}) calculated in each test, none of the control tests can induce the characteristic current blockades except the simultaneous presence of both NP and A48-$A_{10}C_{20}$ aptamer. Data were acquired in asymmetric salt conditions (2 M/0.15 M *trans/cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2) at 120 mV.



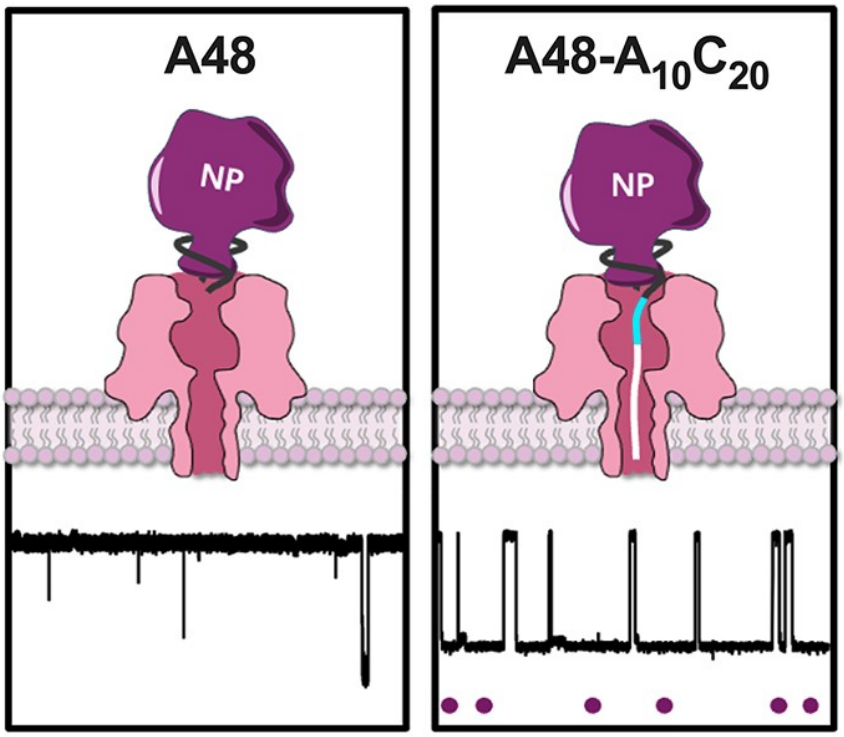
S2. Statistical difference between the current levels of P2 and P3

Figure S2. Left: The average I/I_o corresponding to P2 and P3 are 0.342 ± 0.014 and 0.318 ± 0.012 respectively (n = 6). Right: The average duration t of P2 and P3 are 0.138 ± 0.03 s and 1.351 ± 0.318 s respectively (n = 6). The standard t-tests results show a significant difference ($p < 0.05$) exists between the two phases both in space and time. Data were acquired in asymmetric salt conditions (2 M/0.15 M *trans*/*cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2) at 120 mV.



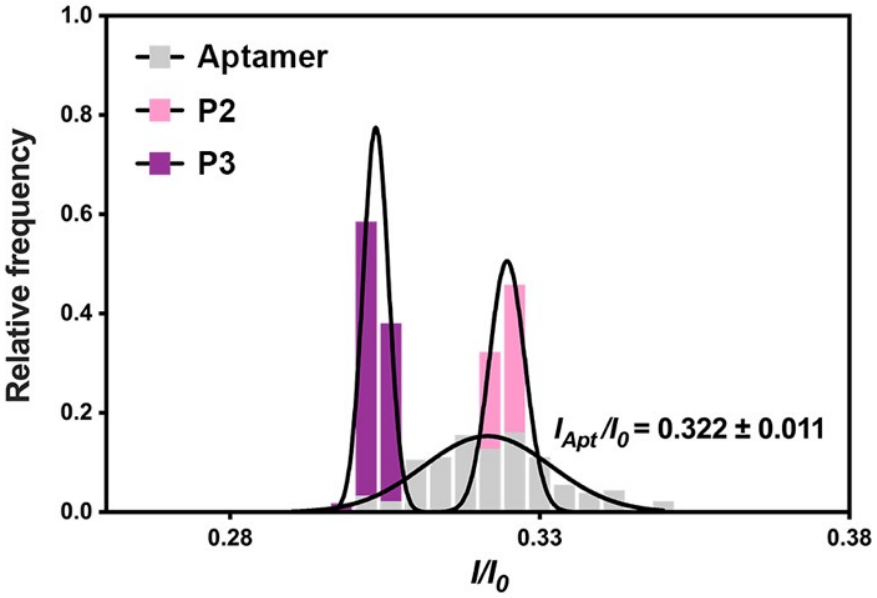
S3. Effects of different tail lengths on producing the characteristic current blockades

Figure S3. Top: The molecular model depicts the complexes of different tail lengths, which are formed by A48 and A48-$A_{10}C_{20}$ with NP respectively, interacting with α-HL nanopore. Bottom: A representative current trace (30 s) of the complex-nanopore interaction. The purple circles indicate the distinctive two-level current blockades. Data were acquired in asymmetric salt conditions (2 M/0.15 M *trans*/*cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2) at 120 mV.



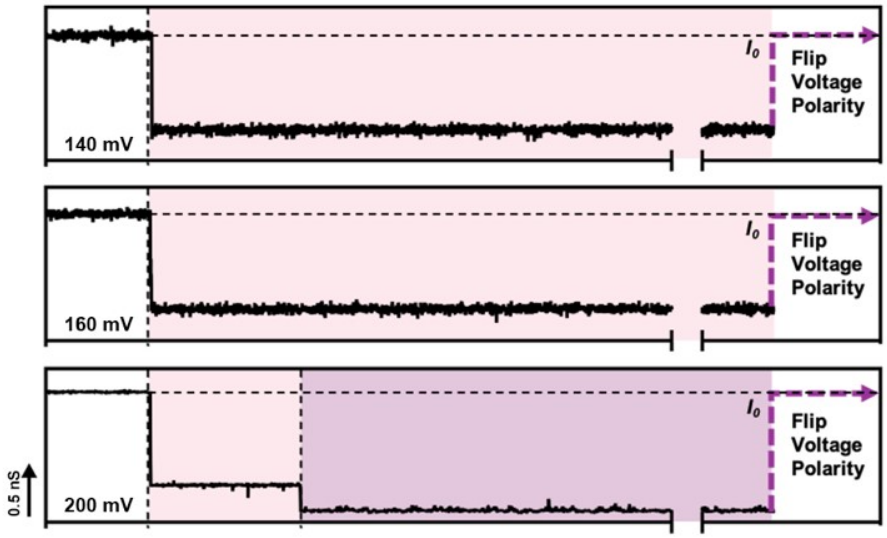
S4. Statistical results of the current levels of free DNA aptamer and complex-nanopore

Figure S4. Histogram shows the frequency counts of current blockades in a single experiment, fitting with Gaussian distribution in solid lines. The mean normalized current blockade of free DNA aptamer is 0.322 ± 0.011, which is approximately the same as the I_{P2} (0.325 ± 0.002) and much higher than I_{P3} (0.304 ± 0.001). Data were acquired in asymmetric salt conditions (2 M/0.15 M *trans*/*cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2) at 120 mV.



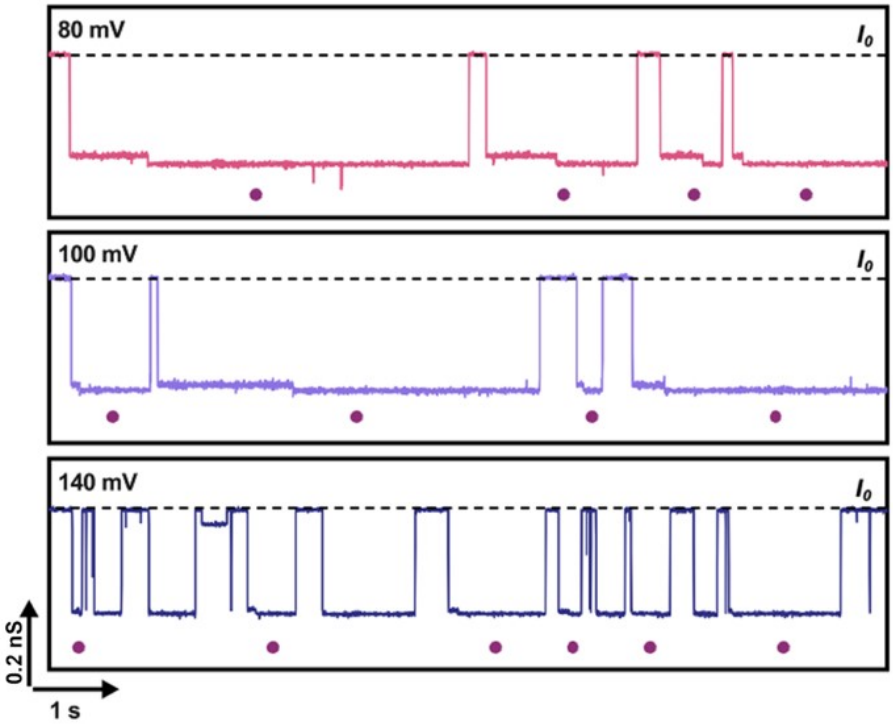
S5. Voltage dependence of the blockades of the complex in symmetric salt conditions

Figure S5. Top and middle: Representative conductance traces of the complex-nanopore interaction with the absence of a second level indicates insufficient electrophoretic force (140 mV and 160 mV). Bottom: A representative track of ion conductance with the presence of a second level indicates a large enough electrophoretic force (200 mV) to unfold the protein part of the complex. Data were acquired in symmetric salt conditions (1 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2) with different voltages.



S6. The characteristic blockades at different voltage biases

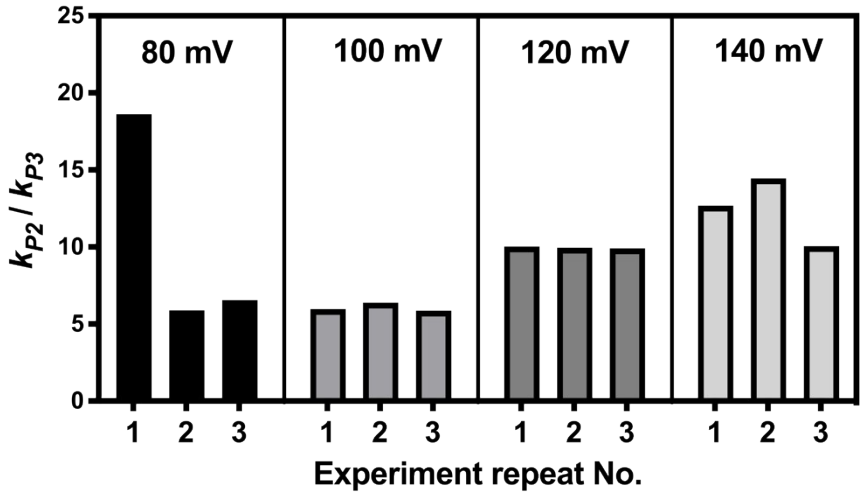
Figure S6. The plots show representative conductance traces at different voltage biases. The characteristic blockades are marked with purple circles at each test voltage. The data were acquired in asymmetric salt conditions (2 M/0.15 M *trans/cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2).



S7. The ratios of k_{P2} / k_{P3} at different voltages

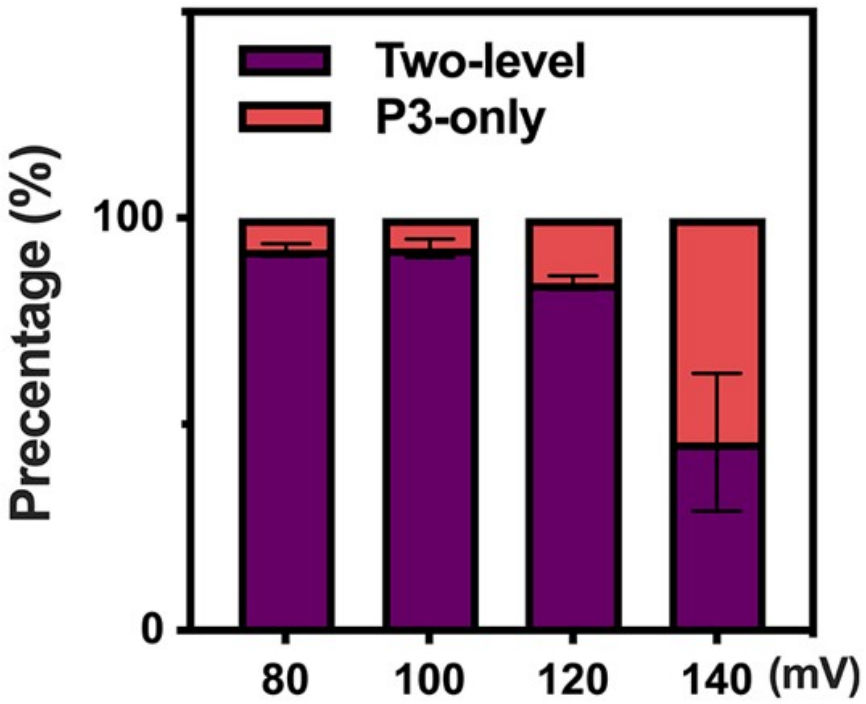
The ratios of k_{P2} / k_{P2} are 10.35 ± 7.17, 6.08 ± 0.28, 9.94 ± 0.07 and 12.39 ± 2.22 for the voltages of 80 mV, 100 mV, 120 mV and 140 mV respectively. The standard deviations of the ratios of 80 mV and 140 mV are much larger but are attributed to totally different causes. For 80 mV, the results are subjectively biased by our manual terminations of the endless states which are similar to those mentioned earlier and hence are excluded from the following discussion. For 140 mV, the results are affected by ionic fluctuations attributed to the change of complex structure or complex-nanopore interaction at high voltages. For 100 mV and 120 mV, the duration ratios possess nice consistency among three repeats, which implies a rather fixed interaction mechanism under a same electrophoretic force at the voltages range from 100 mV to 120 mV. However, the ratio increases as the voltage goes up from 100 mV to 140 mV, indicating that the increasing voltage shortens the duration of P2 in a larger scale. We attributed it to that the increasing electrophoretic force accelerates the aptamer threading (P2) and the protein unfolding (P3) differently.

Figure S7. The bar plot shows the ratios of k_{P2} / k_{P3} corresponding to the three experiment repeats at 80 mV, 100 mV, 120 mV and 140 mV respectively.



S8. The percentages of P3-only blockades at different voltages

Figure S8. The percentages of the two-level (purple bar) and P3-only (orange bar) current blockades at each test voltage are illustrated in a bar plot (n = 3). The portions of P3-only blockades are 7.6 ± 1.5 %, 7.3 ± 2.2 %, 15.6 ± 1.6 % and 54.3 ± 16.7 % corresponding to the voltages of 80 mV, 100 mV, 120 mV and 140 mV respectively. The data were acquired in asymmetric salt conditions (2 M/0.15 M *trans/cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2).



S9. Efficiency evaluation of the NP detection

Figure S9. Normalized frequency of the characteristic current blockades is calculated in a 10 min interval for 1 hour at 100 mV (n = 3). The result of a one-way ANOVA analysis shows no significant differences among all time intervals (p = 0.36 > 0.05). The data were acquired in asymmetric salt condition (2 M/0.15 M *trans/cis* NaCl, 10 mM Tris, 1 mM EDTA, pH 7.2).

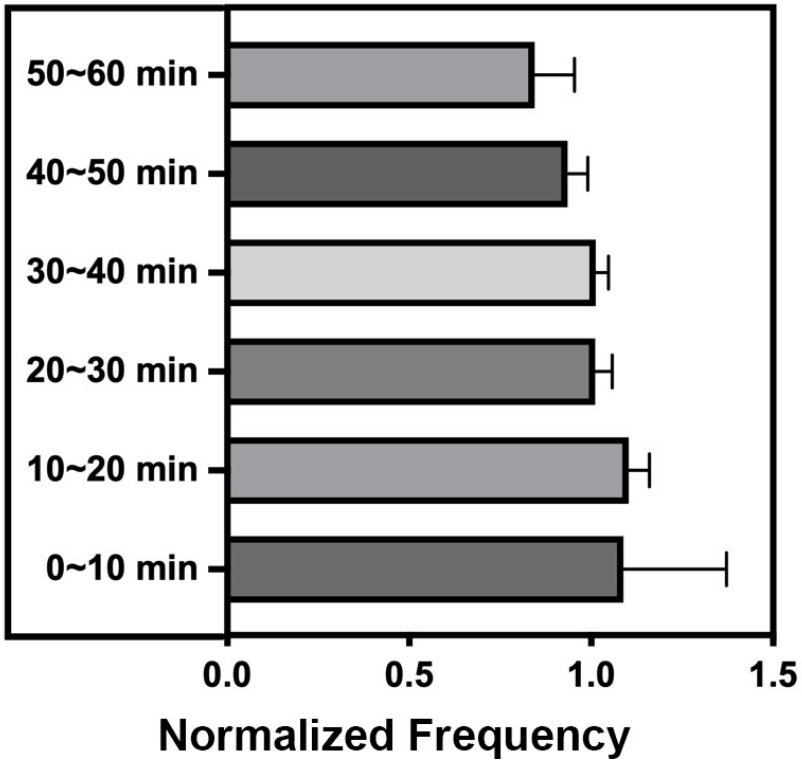


Table S1. The duration times corresponding to P1, P2 and P3 versus the lengths of tail.

Analytes	ΔV (mV)	τ_{p1} (s)	τ_{p2} (s)	τ_{p3} (s)
Np + A48	100	-	-	-
Np + A48-A_{10}	100	-	-	-
Np + A48-$A_{10}C_{10}$	100	12.93 ± 2.173	0.268 ± 0.069	1.947 ± 0.492
Np + A48-$A_{10}C_{20}$	100	3.510 ± 0.575	0.324 ± 0.091	1.927 ± 0.564

Table S2. The duration times corresponding to P1, P2 and P3 versus the applied voltages.

Analytes	ΔV (mV)	τ_{p1} (s)	τ_{p2} (s)	τ_{p3} (s)
Np + A48-$A_{10}C_{20}$	80	11.29 ± 3.789	0.470 ± 0.127	5.098 ± 4.203
Np + A48-$A_{10}C_{20}$	100	3.510 ± 0.575	0.324 ± 0.091	1.927 ± 0.564
Np + A48-$A_{10}C_{20}$	120	2.063 ± 0.515	0.119 ± 0.011	1.139 ± 0.027
Np + A48-$A_{10}C_{20}$	140	1.221 ± 0.178	0.044 ± 0.004	0.546 ± 0.080

Table S3. The duration times corresponding to P1 versus the Np concentrations.

Analytes	Np Concn. (pM)	ΔV (mV)	τ_{p1} (s)
Np + A48-$A_{10}C_{20}$	10000	120	2.283 ± 0.555
Np + A48-$A_{10}C_{20}$	5000	120	4.022 ± 1.116
Np + A48-$A_{10}C_{20}$	2000	120	9.918 ± 2.322
Np + A48-$A_{10}C_{20}$	1000	120	38.14 ± 16.43
Np + A48-$A_{10}C_{20}$	100	120	183.9 ± 90.42
Np + A48-$A_{10}C_{20}$	10	120	150.0 ± 300.0