Supporting Information

Title: Unzipping of double-stranded DNA in engineered α-hemolysin pores

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Methods

Materials and Reagents. Lipid 1,2-diphytanoylphosphatidylcholine was obtained from Avanti Polar Lipids (Alabaster, AL). Teflon film was purchased from Goodfellow (Malvern, PA). dsDNA samples (HPLC purification, Table S1) were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). All the dsDNA polymers were dissolved in HPLC-grade water (ChromAR, Mallinckrodt Baker). The concentrations of the stock solutions were 4 mM for each of the DNA samples.

Preparation of the wild-type and mutant α-hemolysin (αHL) proteins. The production of the wild-type and mutant αHL protein pores has been described elsewhere.[s1] Briefly, the mutant αHL M113F, M113E, and M113K genes were constructed by site-directed mutagenesis. Then, the wild-type and mutant monomers were first synthesized by coupled *in vitro* transcription and translation (IVTT) using the *E. coli* T7 S30 Extract System for Circular DNA from Promega (Madison, WI). Subsequently, they were assembled into homoheptamers by adding rabbit red cell membranes and incubating for 1 h. The heptamers were purified by SDS-polyacrylamide gel electrophoresis and stored in aliquots at -80 °C.

Single channel recording. A bilayer of 1,2-diphytanoylphosphatidylcholine was formed on an aperture (150 μm) in a Teflon septum (25 μm thick) that divided a planar bilayer chamber into two compartments, *cis* and *trans*. The formation of the bilayer was achieved by using the Montal-Mueller method.[s2] The experiments were carried out at 22 ± 1 °C under symmetrical buffer conditions with a 2.0 mL 1 M NaCl solution buffered with 10 mM Tris-

HCl (pH 7.5). Both the αHL proteins (with the final concentration of 0.2-2.0ng·mL^{-1}) and the dsDNA samples (with the final concentration of 1 μM) were added to the *cis* chamber compartment, which was connected to "ground". The applied potential was +200 mV unless otherwise noted. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon instruments, Foster City, CA). They were low-pass filtered with an external four-pole Bessel filter at 30 kHz and sampled at 125 kHz by a computer equipped with a Digidata 1440 A/D converter (Molecular Devices). At least three separate experiments were carried out for each dsDNA sample.

Data Analysis. Data were analyzed with QuB (www.qub.buffalo.edu), Python scripts (which was kindly provided by Electronic Biosciences, San Diego, CA), and Origin 6.0 (Microcal, Northampton, MA) software. Conductance values were obtained from the amplitude histograms after the peaks were fit to Gaussian functions. Mean residence time (τ_{off}) values of the short-lived events were obtained from the dwell time histograms by fitting the distributions to Gaussian functions, while those of the long-lived events were obtained by fitting the dwell time distributions to single exponential functions by the Levenberg-Marquardt procedure.

Table S1. dsDNA polymers used in this study.

dsDNA name	Sequence	Base pair
$d(A)_{20}$•$d(T)_{20}$	5’-AAAAAAAAAAAAAAAAAAAA-3’ 3’-TTTTTTTTTTTTTTTTTTTT-5’	20
$d(A)_{30}$•$d(T)_{30}$	5’-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3’ 3’-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-5’	30
$d(A)_{40}$•$d(T)_{40}$	5‘-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3’ 3’-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-5’	40
$d(GC)_{10}$•$d(GC)_{10}$	5’-GCGCGCGCGCGCGCGCGCGC-3’ 3’-CGCGCGCGCGCGCGCGCGCG-5’	20
$d(CTAG)_5$•$d(CTAG)_5$	5’-CTAGCTAGCTAGCTAGCTAG-3’ 3’-GATCGATCGATCGATCGATC-5’	20

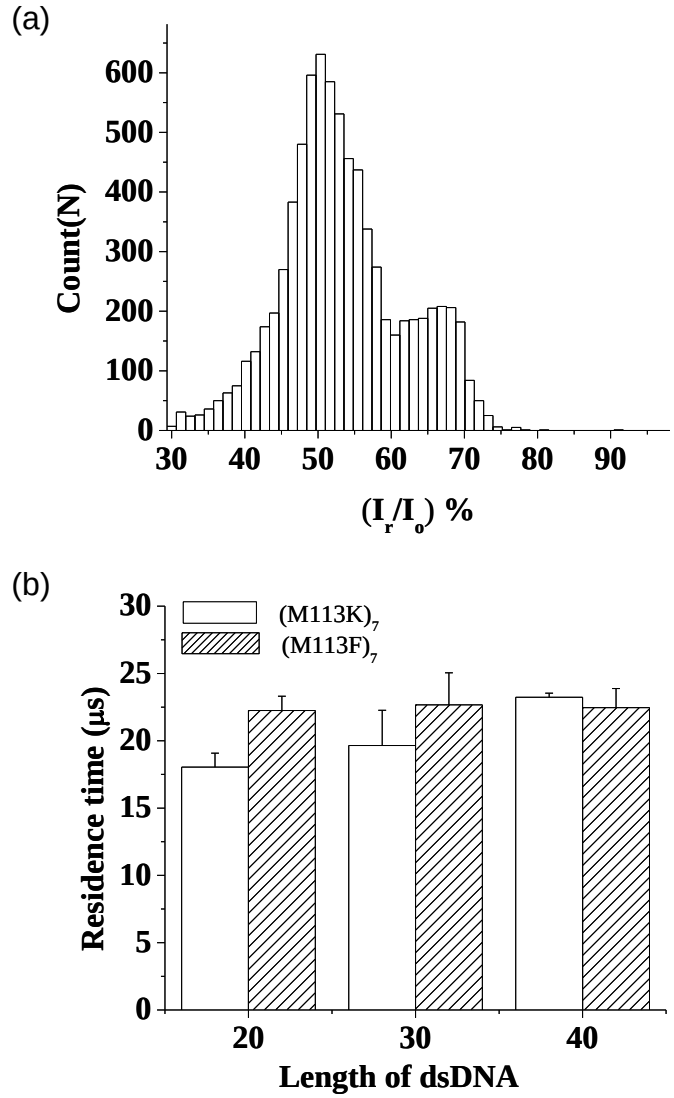

Figure S1. Characteristics of the current modulations having the blockage amplitudes less than 70% of the open channel current. (a) Typical amplitude histogram with $d(A)_{20}$•$d(T)_{20}$ in the $(M113F)_7$ pore; and (b) plot of the event mean residence time vs. the length of dsDNA. I_r/I_o in Fig. S1a is normalized blockage residual current, which was obtained by dividing the average blockage residual current of events by the average open channel current. The 20-mer, 30-mer, and 40-mer dsDNA samples in Fig. S1b were $d(A)_{20}$•$d(T)_{20}$, $d(A)_{30}$•$d(T)_{30}$, and $d(A)_{40}$•$d(T)_{40}$, respectively. The experiments were performed at +200 mV in a solution containing 1 M NaCl and 10 mM Tris-HCl (pH 7.5).

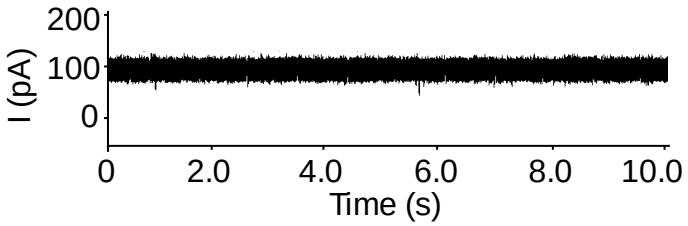

Figure S2. Representative single channel current recording of $d(A)_{20}$•$d(T)_{20}$ in the $(M113E)_7$ αHL pore. The experiment was performed at +200 mV in 1 M NaCl buffered with 10 mM Tris-HCl (pH 7.5) in the presence of 1.0 μM $d(A)_{20}$•$d(T)_{20}$.

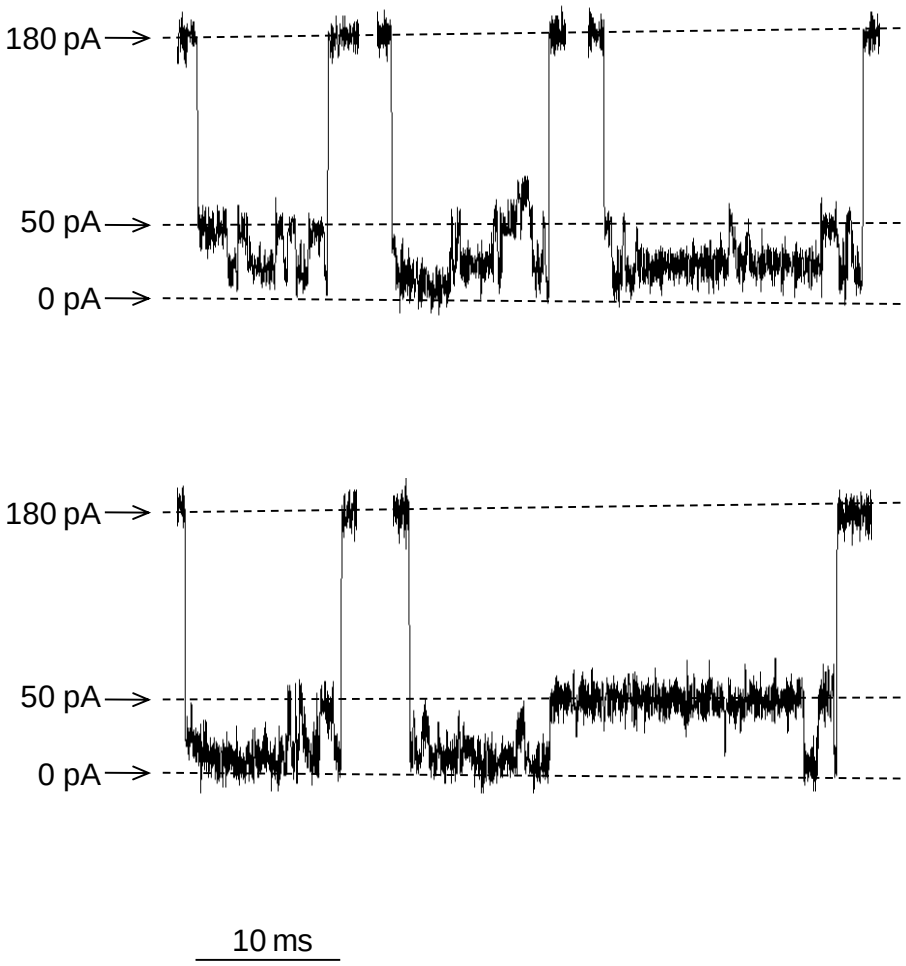

Figure S3. Selection of current modulations caused by the interaction of $d(CTAG)_5$•$d(CTAG)_5$ with the $(M113F)_7$ pore. Such events with a sequence of substate current modulations at different blockage levels were not observed in the wild-type αHL pore. The experiment was performed at +200 mV in 1 M NaCl buffered with 10 mM Tris-HCl (pH 7.5). The same phenomenon (events having substates) was also observed in the experiment with $d(CTAG)_5$•$d(CTAG)_5$ in the $(M113F)_7$ pore at +120 mV, in which DNA events showed more substate current modulations than those at +200 mV.

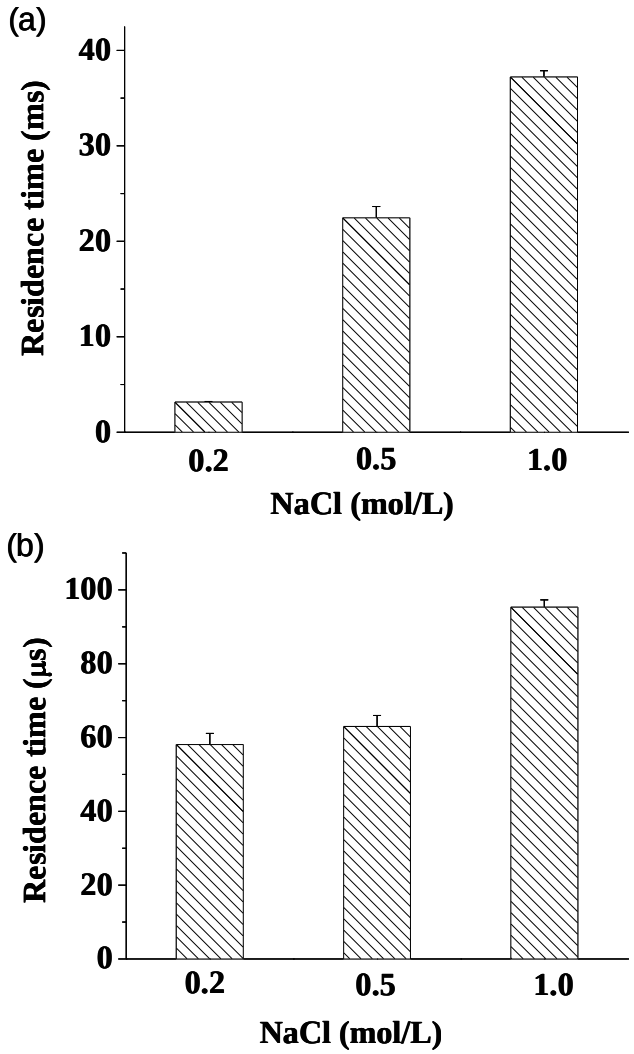

Figure S4. Salt effect on the event mean residence time: (a) long-lived events; and (b) short-lived events. The experiment was performed with 1.0 μM $d(A)_{40}$•$d(T)_{40}$ and the $(M113F)_7$ pore at +200 mV in 1 M NaCl buffered with 10 mM Tris-HCl (pH 7.5).

References

(s1) Zhao, Q.; Jayawardhana, D. A.; Wang, D.; Guan, X. Study of Peptide Transport through Engineered Protein Channels. *J. Phys. Chem. B* **2009**, *113*, 3572-3578.

(s2) Montal, M.; Mueller, P. Formation of Bimolecular Membranes from Lipid Monolayers and a Study of their Electrical Properties. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 3561-3566.