Supplementary information for:

Nucleobase recognition at alkaline pH and apparent p*K*a of single DNA bases immobilised within a biological nanopore.

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Experimental procedures

Protein preparation.

Homo- and hetero-heptameric αHL proteins were prepared *in vitro* as described in detail.[1] In short, proteins were produced by expression in an *E. coli* in vitro transcription and translation (IVTT) system and assembled into heptamers on rabbit blood cell membranes. The heptamers were run in a 5% SDS-polyacrylamide gel and the region of the dried gel containing αHL heptamers was cut out, rehydrated and crushed in 10 mM Tris.HCl, pH 8.0, containing 100 μM EDTA. Aliquots of the purified proteins were stored at -80°C.

Single channel recordings and data analysis.

Electrical recordings were carried out with planar lipid bilayers as described.[2] In short, a bilayer of 1,2-diphytanoylphosphatidyl choline (Avanti Polar Lipids,

Alabaster, AL) was formed on an aperture of ~100 μm diameter in a Teflon film separating the cis and trans compartments. Both compartments were filled with 0.5 mL of 3 M KCl, 10mM Tris.HCl, 10 mM CAPS, 10mM potassium phosphate containing 100 μM EDTA. Gel-purified NN-M113R αHL heptamers were added to the cis chamber (ground) to obtain single channels. The pHs of the solutions were then adjusted by small addition of 1M solutions of KOH or HCl in 3M KCl directly to the cis and trans chambers. At the same time, 3' biotinylated 40-mer single-stranded DNA nucleotides [(5'-$C_{30}XC_8$-3'), where X was G, T, C or A] were incubated with a two-fold excess of streptavidin (per active site) for at least five minutes. Afterwards, the DNA-biotin:strepatavidin complexes were added to the cis chamber at final concentrations between 0.5 and 1.5 μM. The pH of the solution in both chambers was measured before and after the collection of the single-channel data. Single-channel recordings were obtained by using a patch clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA) connected to Ag/AgCl electrodes. For data acquisition and analysis we used Clampex 10.1 and Clampfit 10.1, respectively (Axon Instruments). All recordings were collected using a 1 kHz low pass filter at sampling frequencies of 5 kHz with a computer equipped with a Digidata 1440A digitizer. Data were collected by applying an Episodic Stimulation protocol in which the applied bias was automatically changed in two steps (Fig. s2a). In the first step a bias of +120 mV was applied for 850 ms to capture the DNA-biotin:streptavidin complex within the nanopore. In the second step the bias was reverse (-140mV for 50 ms) to eject the complex from the pore (Fig. S2). The protocol was repeated hundreds of times at each pH values to collect the data. The recorded current traces were analyzed by

using the “single channel search” option in the Clampfit software package (Molecular Devices). The main amplitude of the events corresponding to the open pore current were plotted as histogram and Gaussian fits to the events histogram were used to determine I_O. The main amplitude of the events corresponding to the blocked pore current were divided by I_O and multiplied by 100, and also plotted as histograms. Gaussian fits to the events histogram were used to determine I_{RES} (%). The pH dependence of the ΔI_{RES} values were fitted with the function

$$\Delta I_{RES\,(obs)} = \Delta I_{RES\,(AH)} + \frac{\Delta I_{RES\,(A-)} - \Delta I_{RES\,(AH)}}{1+10^{n(pKa-pH)}} \qquad \text{(S1)}$$

where n is the Hill slope. The errors on the p*K*a values were calculated by fitting all individually collected data points to eq. S1 and are expressed as standard errors (OriginLab Corporation, MA,USA).

Additional Data

Table S1. Residual current blockades values (I_{RES}) for the DNA-blocked NN-M113R pores as a function of pH. I_O is the open pore current. The DNA nucleotides immobilised by streptavidin complexation used were (5'-$C_{30}XC_8$-3'-biotin), where X was G, T, C or A. Errors are shown as standard errors.

pH	I_O (pA)	I_{RES}^G (%)	I_{RES}^T (%)	I_{RES}^A (%)	I_{RES}^C (%)
7.1±0.0 (n=3)	356±1	12.63±0.08	13.10±0.02	13.92±0.04	14.60±0.05
8.0±0.1 (n=5)	359±2	12.42±0.004	12.94±0.04	13.77±0.03	14.42±0.05
9.2±0.0 (n=4)	362±4	12.53±0.08	13.09±0.06	13.69±0.04	14.39±0.06
10.5±0.1 (n=5)	358±4	12.49±0.10	13.18±0.08	13.47±0.04	14.16±0.07
11.0±0.1 (n=3)	358±3	12.41±0.06	13.18±0.10	13.43±0.07	14.10±0.07
11.6±0.1 (n=4)	344±3	12.24±0.09	13.17±0.09	13.77±0.14	14.21±0.10

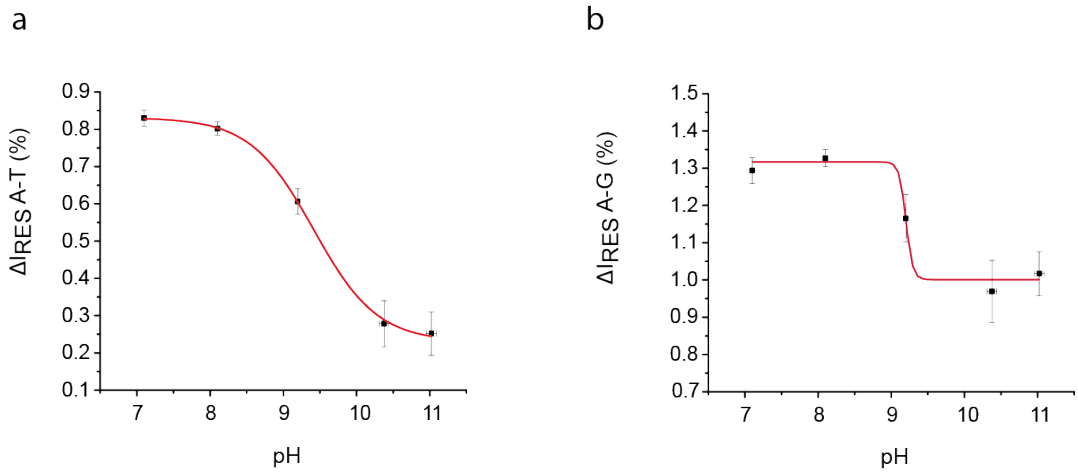

Fig. S1 Apparent p*K*a of a single nucleobases immobilised in the M113R-NN nanopore. a and b) pH dependences of the differences in I_{RES} between a poly dC oligonucleotide containing a single A substituted at position 9 and a poly dC oligonucleotide containing T and G substitutions at position 9, respectively. The midpoint of the transition of the graphs in a and b were 9.5±0.1and 9.2±0.2, respectively. Errors are shown as standard errors.

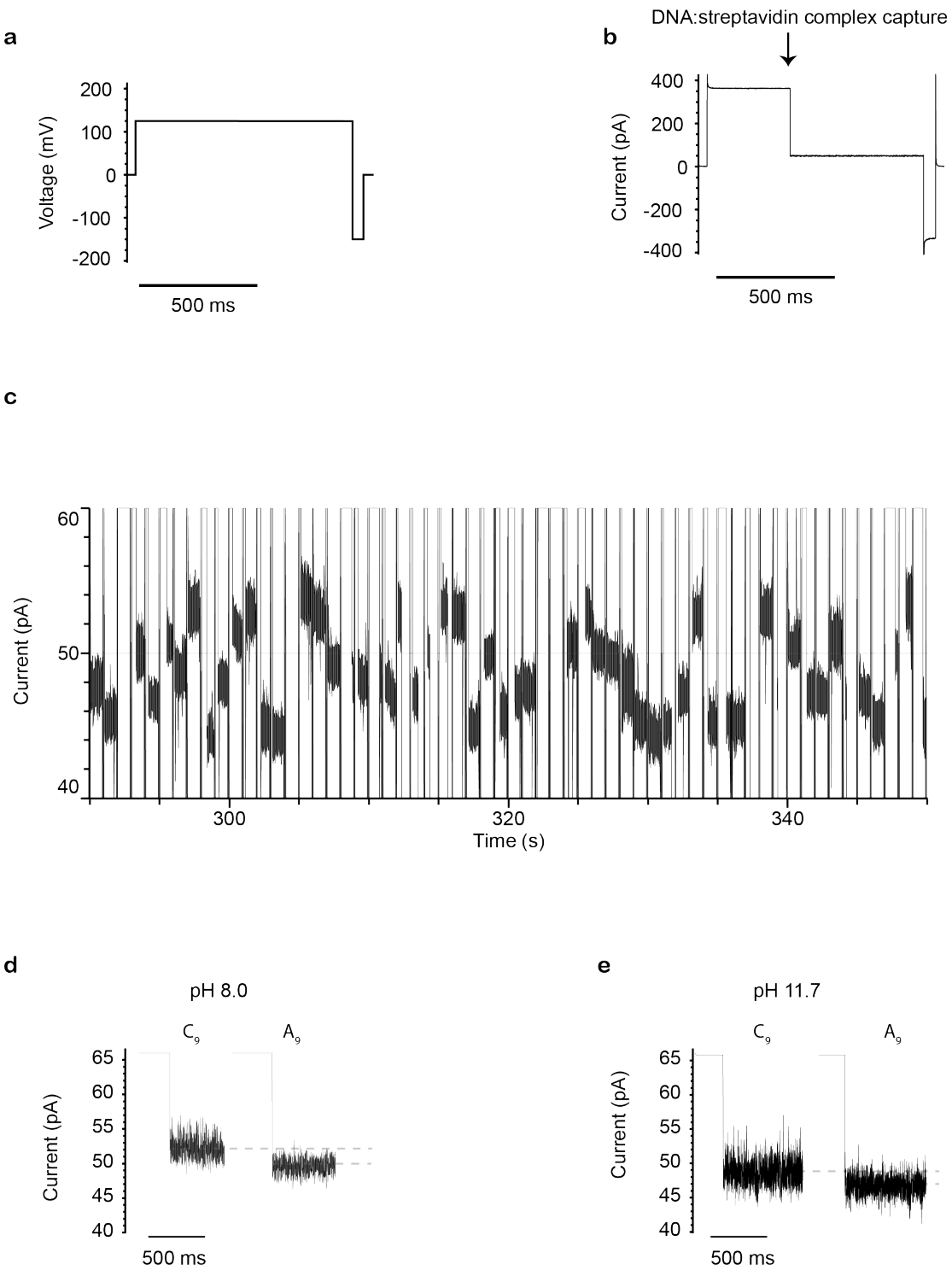

Fig. S2 Examples of typical voltage protocol and current recordings for the 2N-M113R pores when blocked by the DNA-biotin:streptavidin complex. a) Individual sweep of the episodic stimulation protocol used in this study. A positive potential bias of +120 mV is applied to the system for 850 ms before reversing the potential to -140mV for 50 ms. The protocol is repeated

numerous times. b) Typical current trace recorded when applying the episodic stimulation protocol described in a. Under the positive applied potential, a DNA molecule is driven into the pore and becomes immobilized (through a terminal biotin:streptavidin complex), and the current level is reduced. When the potential bias is reversed the DNA molecule is ejected. c) Representative current trace showing 60 consecutive sweeps (typically 200 sweeps were recorded in each run) for poly(dC) oligonucleotides containing a single A,T,C or G at position 9 at pH 8.0. d and e) Typical current traces for poly(dC) and poly(dC) oligonucleotides containing a single A at position 9 at pH 8.0 (c) and 11.7 (d). The traces were collected at sampling frequencies of 5 kHz using a 1 kHz low pass filter. For the sake of clarity, traces in c were filtered digitally using a 200 Hz cut-off low pass Gaussian filter (Clampfit 10.1, Axon Instruments).

References

1. S. Cheley, O. Braha, X. Lu, S. Conlan and H. Bayley, *Protein Sci*, 1999, 8, 1257-1267.
2. G. Maglia, A. J. Heron, D. Stoddart, D. Japrung and H. Bayley, *Methods in Enzymology, Vol 475: Single Molecule Tools, Pt B*, 2010, 474, 591-623.