Supporting Information

Recognizing a Single Base in an Individual DNA Strand: A Step Toward Nanopore Sequencing☆☆

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Methods: DNA oligonucleotides were purchased from Qiagen (Valencia, CA), purified by PAGE and desalted. Stock solutions (100 μM) were prepared in ddH2O, heated at 95 °C, and annealed for 20 min by 0.1 °C/sec cooling to room temperature.

Lipid bilayers were formed from 1,2-diphytanoyl-sn-glycero-3-phosphocholine in a Teflon planar bilayer apparatus (M. Montal, P. Mueller, *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 3561). Preformed α-HL heptamers were incorporated into the lipid bilayer. In this configuration the stem domain spans the entire bilayer while the cap remains on the side of addition, the cis side. After the incorporation of a single pore, the ss-DNA hairpin under study was added to the cis chamber at final concentration of 0.6-2 μM. Measurements were done in KCl 500 mM, MOPS 5 mM, pH=7.5. The cis chamber was at virtual ground, and a positive potential refers to a higher potential in the trans chamber. DNA threading and capture were triggered by applying +140 mV positive bias. For each capture event (detected by a pronounce decrease in the observed current) the voltage was raised to +170 mV and a current voltage trace was recorded in the range of +170 to 0 mV in steps of 10 mV to lower potential each 2 sec long. In most cases, deblocking occurred at low positive biases. When strands remained in the pore at the end of a measurement a small negative bias was used to eject the strand from the pore.

To capture another DNA strand, +170 mV potential was reapplied.

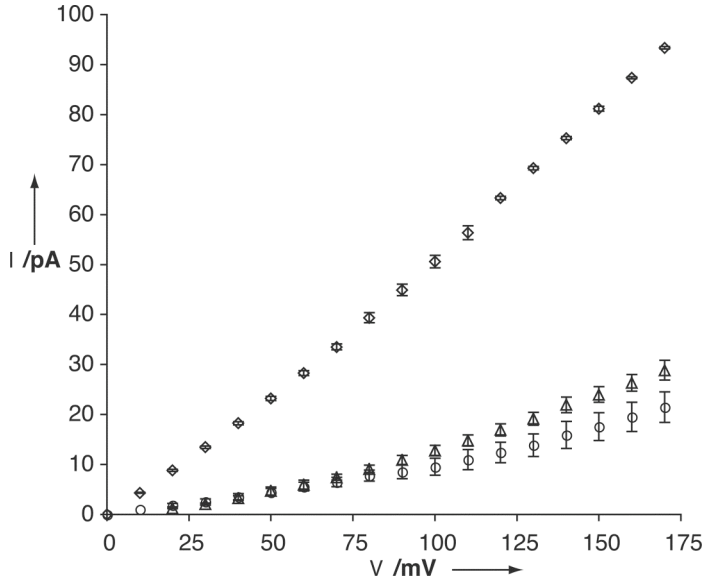

Figure S1: Averaged current-voltage relations of free and ss-DNA hairpin threaded α-HL pores. The current traces were recorded in symmetric 500 mM KCl, 5 mM MOPS, pH 7.5 by sweeping the potential in the range of +170 to 0 mV in steps of 10 mV each for 2 sec. The signal was passed through 5 kHz Bessel filter and sampled at 200 μs. Data were acquired by averaging over each voltage ramp, for several measurements (n=5-19) for the free channel (diamond), and for the channel threaded with **1** (circle) and **2** (triangle). Error bars represent standard deviations between different experiments. Only the conductance traces in which the DNA strand were still threaded in the channel were used in the analyses.

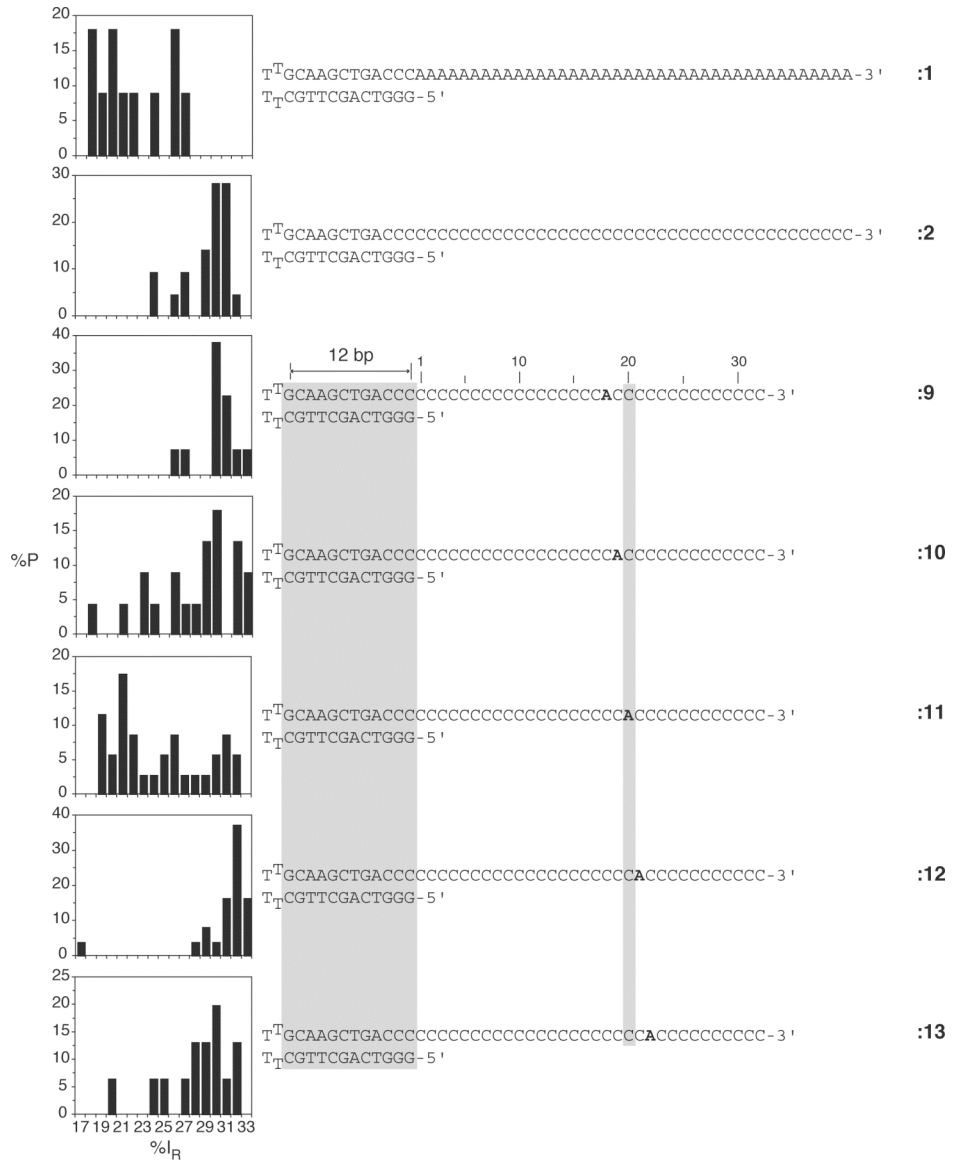

Figure S2: Percent probability of residual currents for single deoxyadenosine substituted poly d(C) DNA hairpins captured inside α-HL pore as a pseudorotaxane. Percent probability was calculated by the number of events (n) (measured at +170 mV) at a specific residual current (bins of 1%) divided by the total number of events recorded for a specific strand (n=11,21,22,32,22,14 for **1,2,9-13** respectively). Similar trends were observed throughout the +100 to +170 mV range.

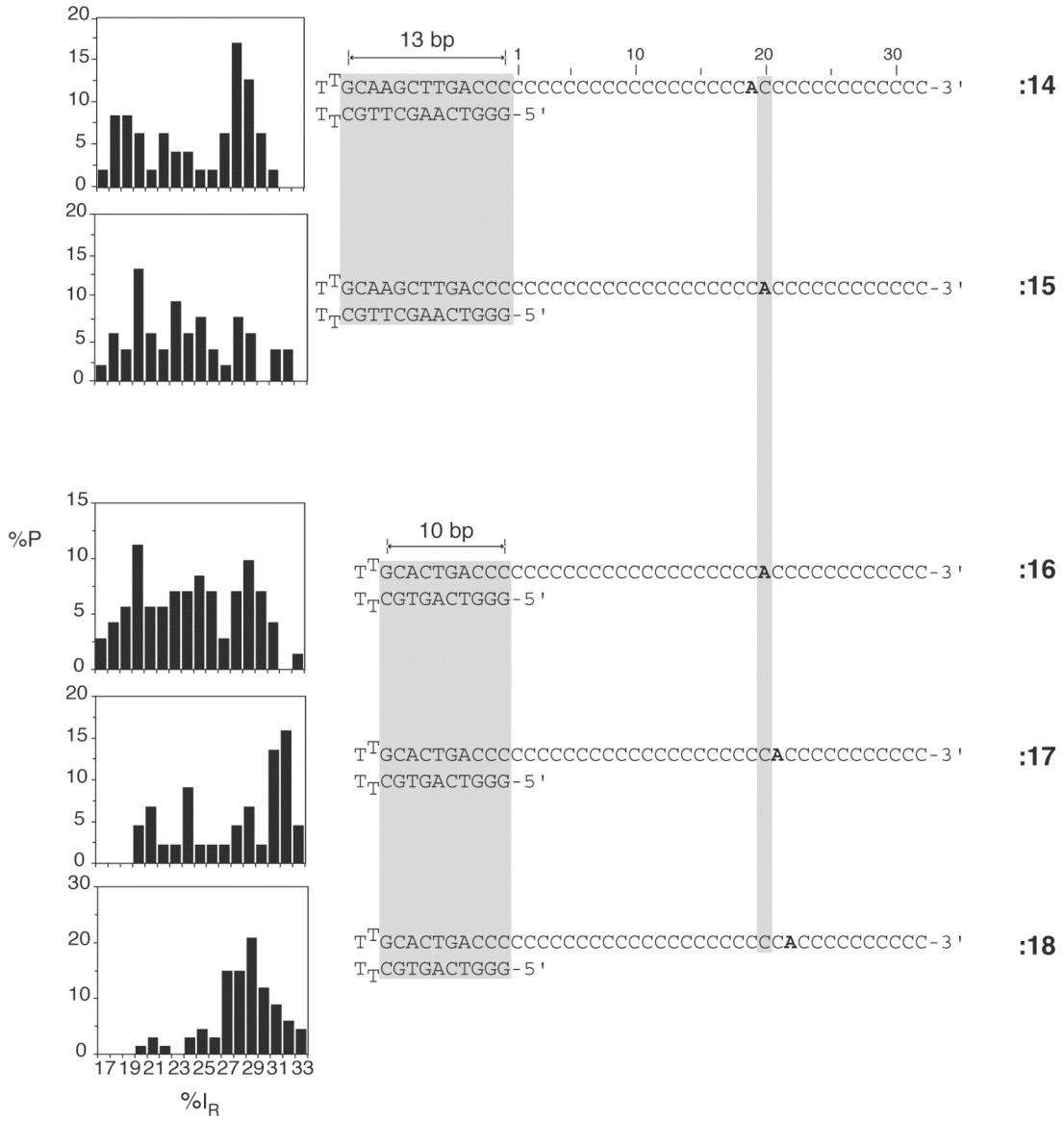

Figure S3: The effects of hairpin stem size on single nucleobase recognition. Percent probability of residual currents are shown for single deoxyadenosine substituted poly d(C) strands captured inside αHL pore as a pseudorotaxane (calculated as noted in Fig. S2. n=43,44,69,35,66 for **14**-**18** respectively).

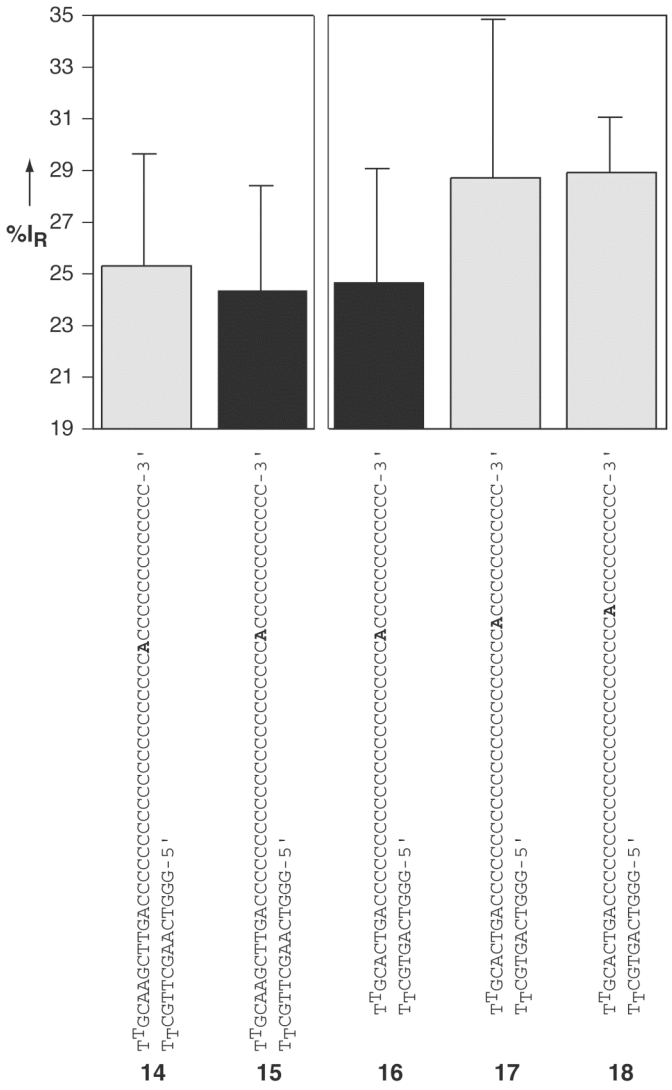

Figure S4: The effects of hairpin stem size on single nucleobase recognition. Averaged residual current for hairpin modified DNAs. The average residual currents were calculated from the data of Fig. S3. Error bars represent the standard deviations. The strands with higher probability for "A"/ "C"-type events (taken from Fig. 4) are shown in black and gray bars, respectively.