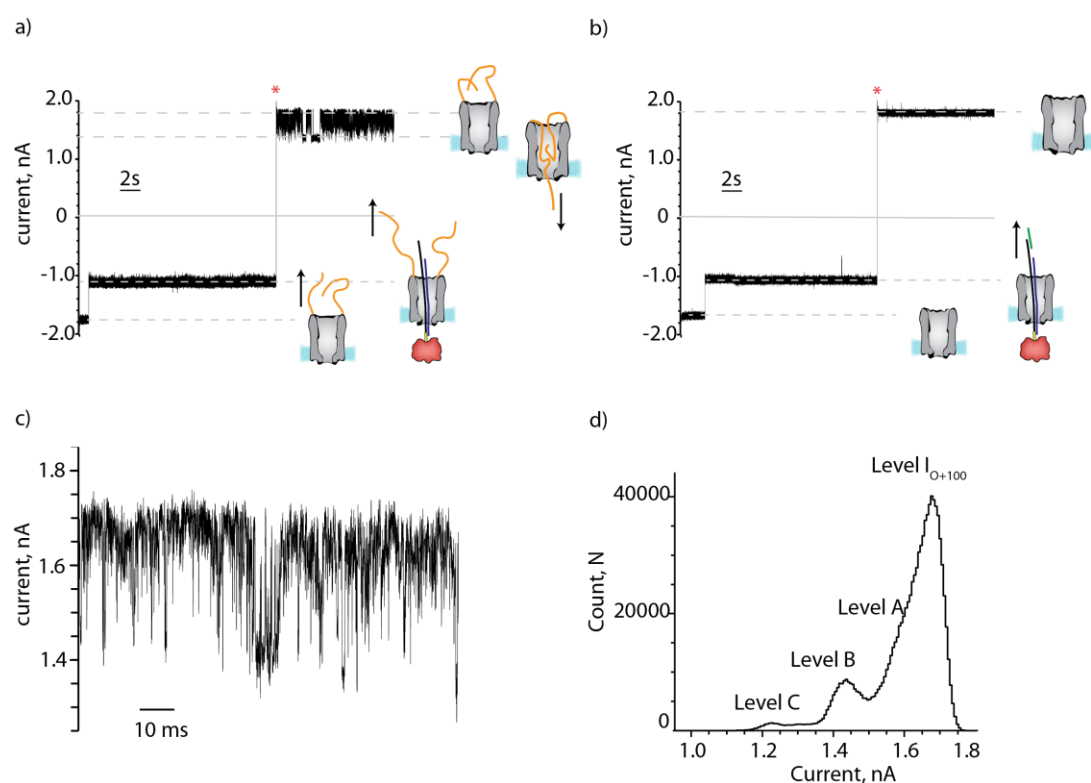
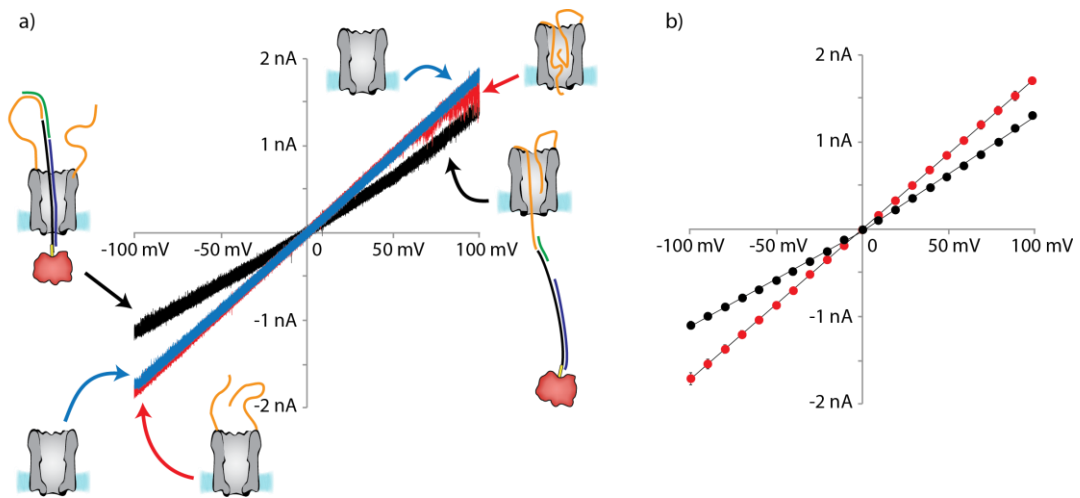


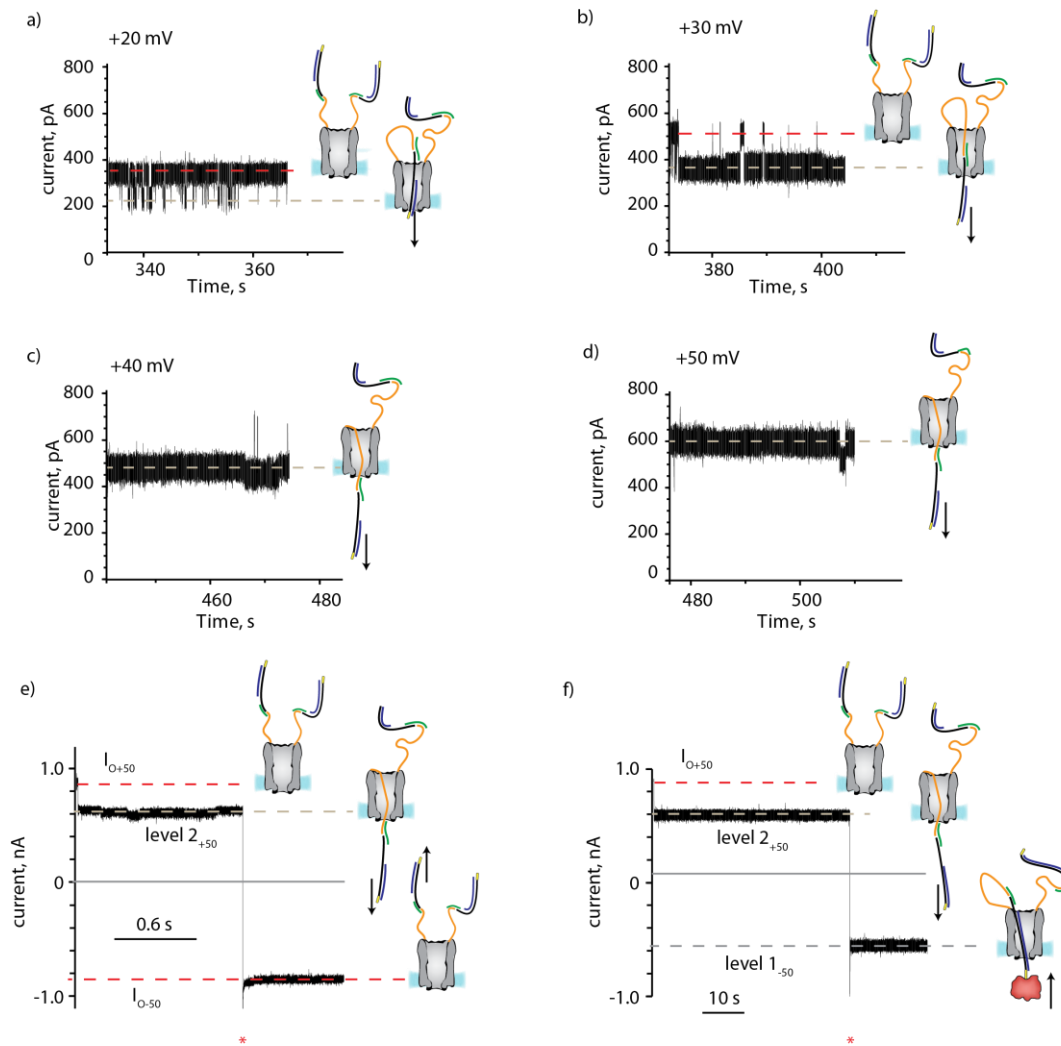
Supplementary Figures



Supplementary Figure S1. Control experiments showing that all components of Figure 2 are necessary to form a DNA rotaxane. a) The absence of the bridging sequence 4 does not allow a linkage to form between ClyA-2 and 3. After the complex is captured at -100 mV it is readily expelled from the pore at +100 mV (red asterisk) as shown by the typical current signature of an open pore current for ClyA-2 at +100 mV. b) The removal of 2 from the pore top (for example after cleavage with DTT) does not allow the 3•4 DNA hybrid to bind to the pore when captured at -100 mV. Upon reversing the potential to +100 mV (red asterisk) the open pore current is restored. c) Typical current recording for a ClyA-2 nanopore at +100 mV. d) All points histogram (5 pA bin size) for 20 seconds of ClyA-2 open pore currents. Level $I_{O+100} = 1.71 \pm 0.07$ nA, level A = 1.62 ± 0.12 nA, level B = 1.43 ± 0.05 nA and level C = 1.28 ± 0.06 nA. These values, determined from 12 experiments, presumably represent different arrangements of the DNA strands lodged within the lumen of the ClyA pore. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C. Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μ s (50 kHz) sampling rate.

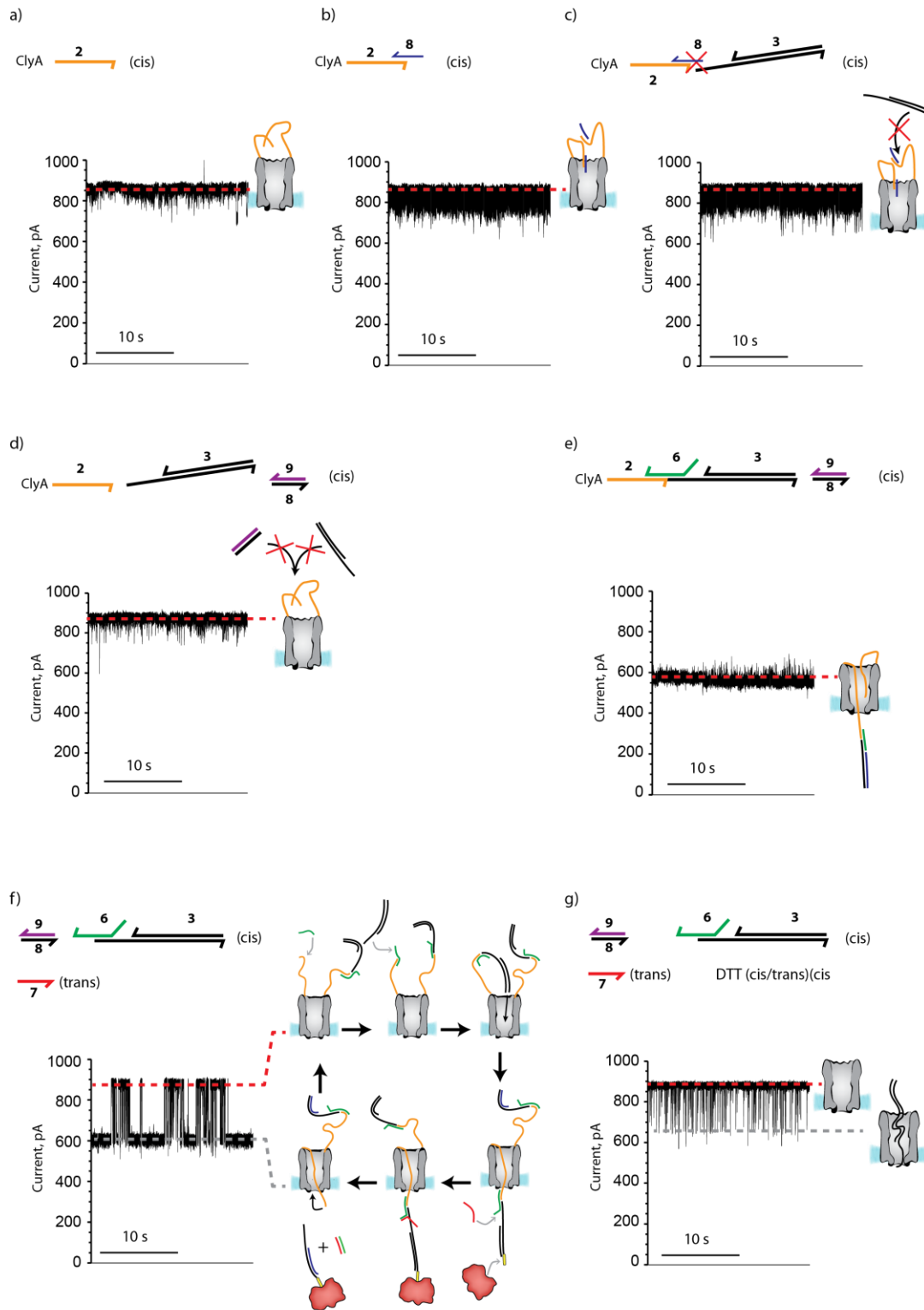


Supplementary Figure S2. Current versus voltage (IV) relationships for ClyA-2 nanopores a) A typical IV curve for ClyA-2 before (red line) and after (black line) rotaxane formation. The blue line indicates the same nanopore after the DNA molecules attached to the nanopore have been removed by the addition of DTT. The current recordings were measured by applying an automated protocol that ramped the voltage from -100 mV to +100 mV in 4 seconds. b) I-V curves calculated from the average of four experiments showing the steady-state (1 s) ClyA-2 open pore current levels (red spheres) and ClyA-2 open pore current levels in a rotaxane configuration (black spheres). The unitary conductance values of the nanopores as calculated from the slopes of the I-V curves were 17.1 nS for ClyA-2 at both positive and negative bias, 10.8 nS for the rotaxane at negative bias and 13.0 nS at positive bias. The rotaxanes were prepared as described in Figure 2. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C. Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μ s (50 kHz) sampling rate.



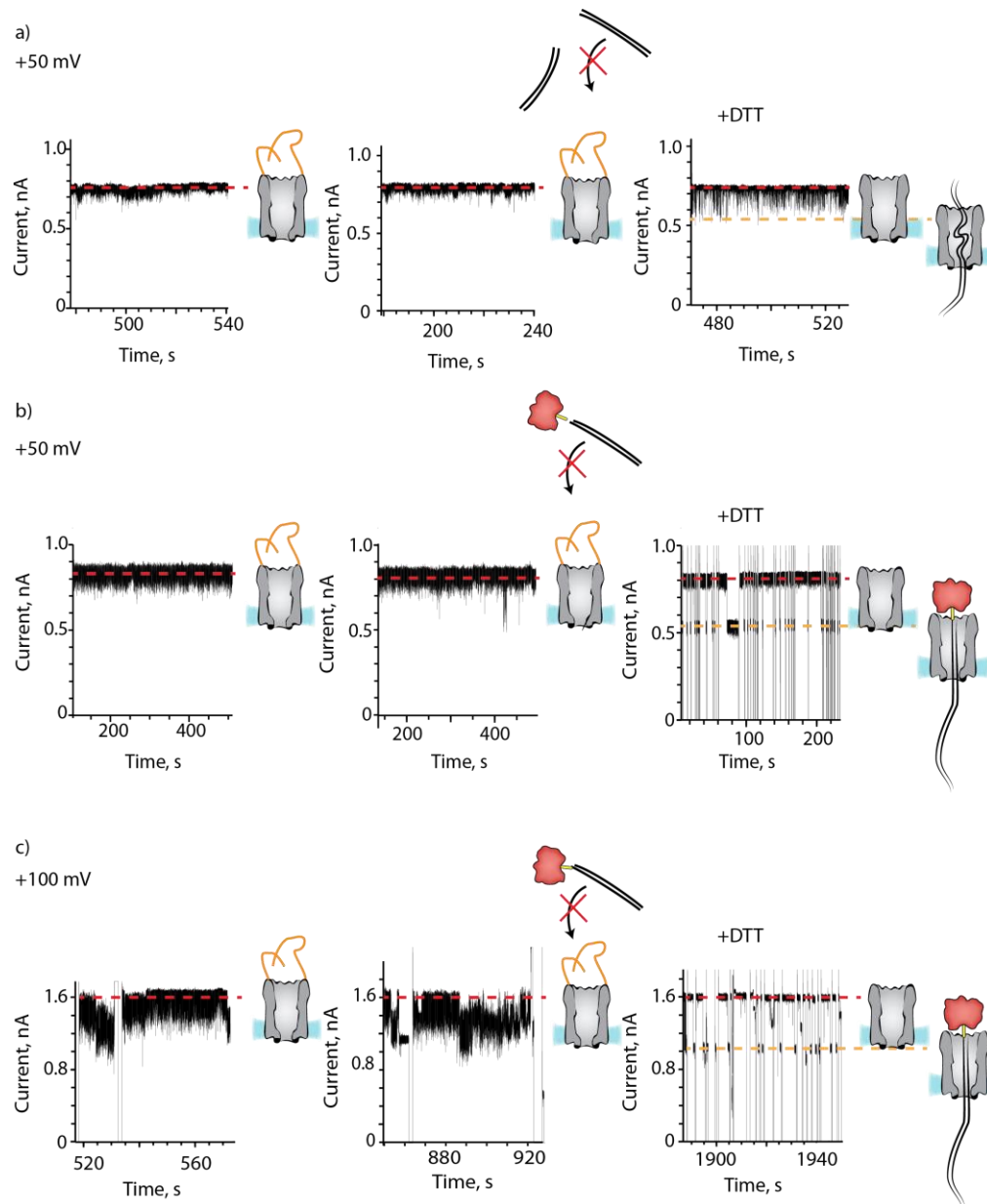
Supplementary Figure S3. *dsDNA current blockades to ClyA-2.* On the right of each current trace the cartoon represents the physical interpretation of the current recordings. a-d) Current recordings for ClyA-2 after hybridisation with a **3•6** (green and black lines, respectively) at different applied potentials. e) At +50 mV, upon hybridisation with **6** (green strand, 40 nM) and **2** (orange strand) the DNA duplex **3** (black strand, 0.3 μ M) is transported through the pore as shown by the drop in the ionic current from $I_{O+50} = 0.85 \pm 0.01$ nA, to a level 2₊₅₀ block ($I_{RES+50} = 0.70 \pm 0.02$). Reversal of the applied potential to -50 mV restored the open pore current ($I_{O-50} = -0.83 \pm 0.01$ nA). f) The subsequent addition of 0.3 μ M neutravidin (red) to the trans compartment locked the DNA thread within the pore as revealed after the reversal of the potential to -50 mV when a blocked pore level ($I_{RES-50} = 0.67 \pm 0.02$) was observed. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C.

Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μ s (50 kHz) sampling rate.



Supplementary Figure S4. Selective DNA capture by ClyA-2 pores at +50 mV. In all panels at the top is the depiction of the DNA molecules used in the experiment that are added in succession (from panel a to panel g). The DNA oligos were added to the *cis* compartment except for oligo 7 and neutravidin

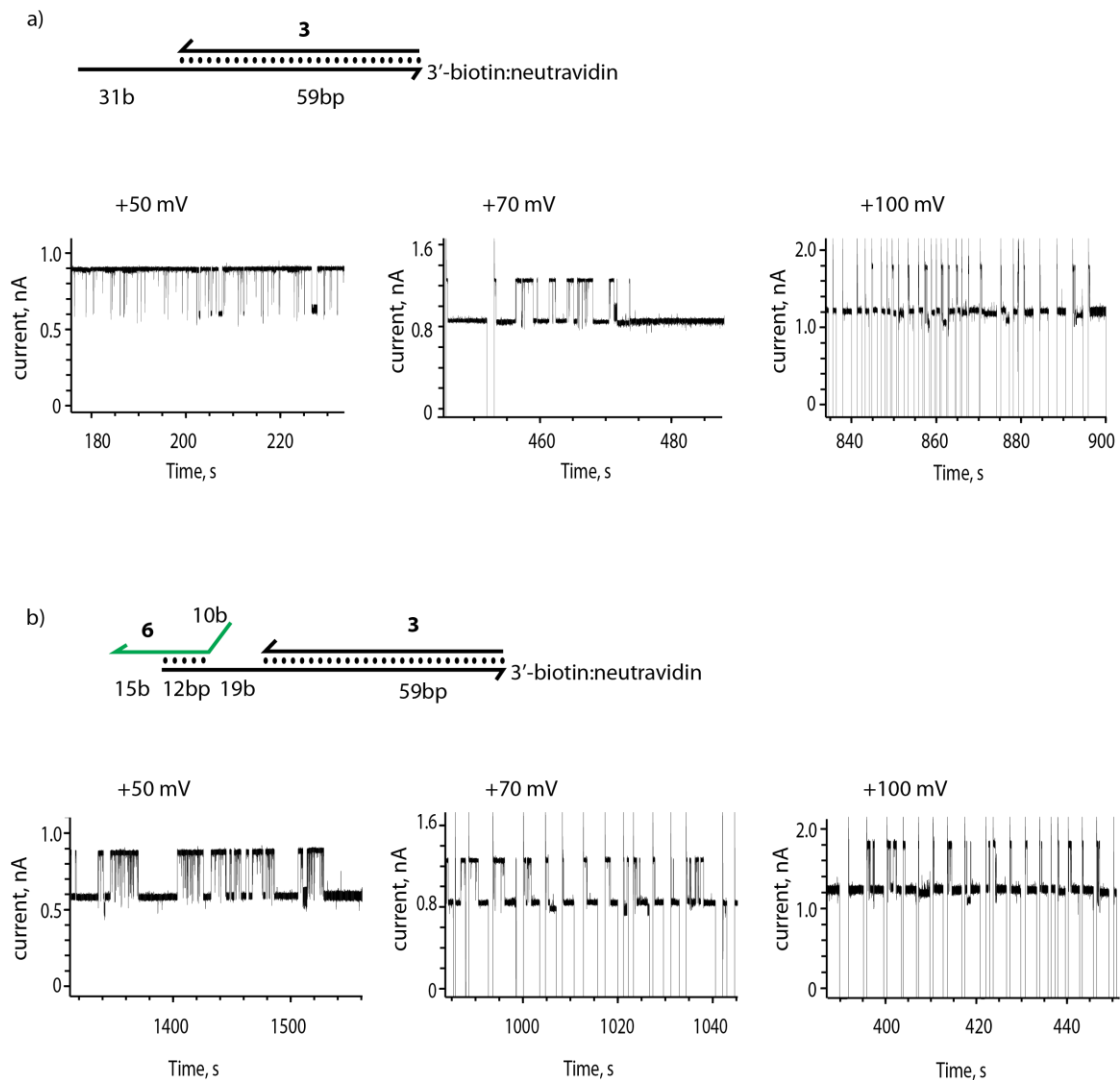
that were added to the *trans* compartment. DTT was added to both compartments. a) Open pore current of a ClyA-2 nanopore (oligo **2** is shown in orange). The transient ionic current blockades below the open pore current indicate the transient entry of the tethered ssDNA molecules in the lumen of the pore. b) Addition of oligo **8** (20 nM, blue, 5'-TTTTTTTTTTTTTCAGGTCATCCCTAAGTAACTCTGCA-3') to the *cis* compartment. The 15 nucleotides at the 3' end of oligo **8** hybridised with the last 15 nucleotides of **2** as shown by the more frequent ionic current blockades due to the entry of the dsDNA hybrid into the pore lumen. c) Addition of DNA duplex **3** (1 μ M, black) to the *cis* compartment. The 5' end of **8** contained a stretch of poly(dT) that could not bind to the 3' ssDNA stretch of **3**, which then could not enter the pore, as shown by no additional current blockades. d) Addition of oligo **9** (60 nM, purple, 5' -TGCAGAGTTACTTAGGGATGACCTGAAAAAAAAAAAAA-3') to the *cis* compartment. Oligo **9** was complementary to oligo **8** and detached it from ClyA-2 by a strand displacement reaction and restored the open pore current typical for ClyA-2 nanopores (panel a and Figure S4). e) Addition of oligo **6** (600 nM) to the *cis* compartment. Since oligo **6** was complementary to the ssDNA attached to ClyA-2 and the ssDNA overhang in the duplex **3**, a dsDNA became tethered to the top of ClyA, which was then captured by the pore, as shown by a permanent current blockade. f) Addition of oligo **7** (1 μ M, red) and neutravidin (0.3 μ M, tetramer) to the *trans* compartment. Oligo **7** promoted the release of the DNA duplex **3** to the *trans* compartment as shown by the cycling of the open and the blocked pore currents. g) Addition of DTT (20 mM) to both *cis* and *trans* compartments. After DTT was added, the DNA molecules were detached from the pore entrance and the dsDNA in solution could enter the ClyA pore, as shown by the transient current blockades. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C. Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μ s (50 kHz) sampling rate.



Supplementary Figure S5. Selective DNA threading through ClyA-2 pores.

a) Left, at +50 mV the ionic current through ClyA-2 nanopores showed fast and shallow current blockades, suggesting that the ssDNA molecules attached at the *cis* entrance of ClyA-2 transiently occupy the lumen of the pore. Middle, after dsDNA strand 1 (50 nM) was added to the *cis* compartment the current signals did not change, indicating that dsDNA did not enter ClyA-2. Right, 20 minutes after the addition of 20 mM DTT to the *cis* compartment the DNA molecules atop the ClyA pore were removed and the DNA could translocate through the pore. b) Same experiment as described in panel a but in the presence of 1 μ M of neutravidin in the *cis* compartment

resulting in pseudorotaxane formation as shown by the permanent ionic current blockades due to the threading of DNA through the pore. The open pore current was restored by reversing the potential to -100 mV and the stepping back to +100 mV. Spikes above and below the open pore current level represent capacitive transients that followed the reversing of the bias that was applied after each DNA threading event. c) Same as in panel b, but at +100 mV. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C. Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μ s (50 kHz) sampling rate.



Supplementary Figure S6. Voltage dependence of the interaction of a ssDNA-dsDNA hybrid construct with ClyA-CS pores. The DNA molecules used in each experiment are depicted above the current recordings. a) Current blockades of the DNA hybrid **3** (1.0 μM) in complex with neutravidin (0.3 μM) at +50 mV (left), +70 mV (middle) and +100 mV (right). b) current blockades of the DNA hybrid **3•6** in complex with neutravidin at +50 mV (left), +70 mV (middle) and +100 mV (right). After each DNA threading event, the open pore was regenerated by manual switching the potential to $-/+100$ mV. Spikes above and below the open pore current level represent capacitive transients. The electrical recordings were carried out in 2.5 M NaCl, 15 mM Tris.HCl, pH 7.5, at 22°C. Data were recorded by applying a 10 kHz low-pass Bessel filter and using a 20 μs (50 kHz) sampling rate.

Supplementary Tables

Supplementary Table S1. DNA molecules used in this work. **1** was prepared by PCR as described in Methods. **3** was formed by incubating **3a** with a 20% excess of **3b** and purified by affinity chromatography as described in Methods. The complementary sequences in the two DNA strands are shown in italics. The suffix bio indicates a biotin moiety.

Name		DNA sequence
1	1a	Bio- 5' TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACT TTAAGAAGGAGATATACATATGACGGGTATCTTTGCGGAACAGACGGTGGAAAGTTGTGAAAAG TGCGATTGAAACGGCTGACGGTGCCTGGACGTATAATAAATATCTGGATCAGGTCATCCC GTGGAAAACCTTTGACGAAACGATTAAAGAAGCTGAGCCGTTTCAAACAGGAATACAGTCAAGA AGCGTCCGTCCTGGTGGGCGATATCAAAGTGCTGCTGATG3'
	1b	5' CATCAGCAGCACTTTGATATCGCCAC C AGGACGGACGCTTCTTGACTGTATTCCCTGTTG AAACGGCTCAGTTCTTTAATCGTTTCGTCAAAGGTTTTCCACGGGATGACCTGATCCAGATAT TTATTATACAGGTCCAGCGCACCGTCAGCCGTTTCAATCGCACTTTTCACTACTCCACCGTC TGTTCCGCAAAGATACCCGTCATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTA GAGGGAAACCGTTGTGGTCTCCCTATAGTGAGTCGTATTA3'
2		5' TTTTTTTTTATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTACTTAG3'
3	3a	5' GGATGACCTGATCCAGATATTTATTATACAGGTCCAGCGCACCGTCAGCCCAATCGCACTT TTCACAAAAAGAGAGAGATCGATTACC3' -bio
	3b	5' GGTAATCGATCTCTCTCTCTTTTTTGTGAAAAGTGCGATTGGGCTGACGGTGCCTGGAC- 3'
4		5' AATAAATATCTGGATCAGGTCATCCCTAAGTAACTCTGCAC3'
5a		5' GGATGACCTGATCCAGATATTTATTATACAGGTCCAGCGCACCGTCAGCCCAATCGCACTT TTCACAAAAAGAGAGAGATCGATTACC3' bio
5b		5' GGTAATCGATCTCTCTCTCTTTTTTGTGAAAAGTGCGATTGGGCTGACGGTGCCTGGACCT GTATAAATAAATATCTGGATCAGGTCATCC3'
6		5' CGCTATATATCAGGTCATCCCTAAGTAACTCTGCA3'
7		5' TGCAGAGTTACTTAGGGATGACCTGATAATATAGGCG3'