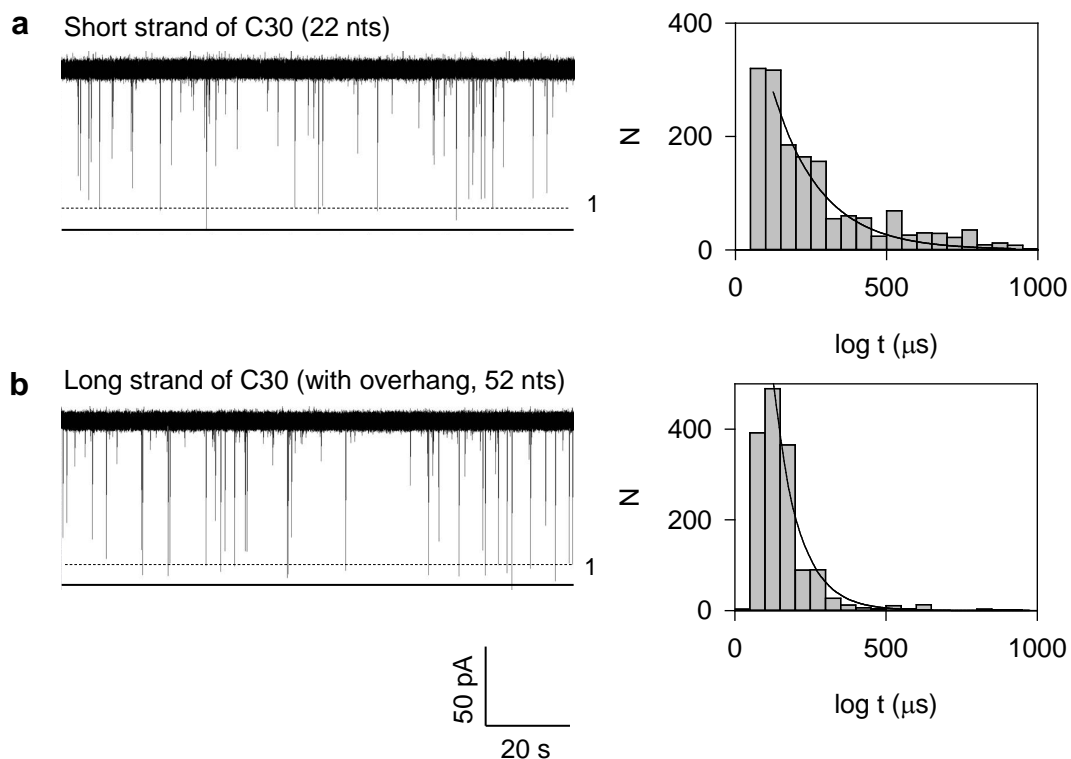


**Probing molecular pathways for DNA orientational trapping, unzipping  
and translocation in the nanopore by using a tunable overhang sensor**

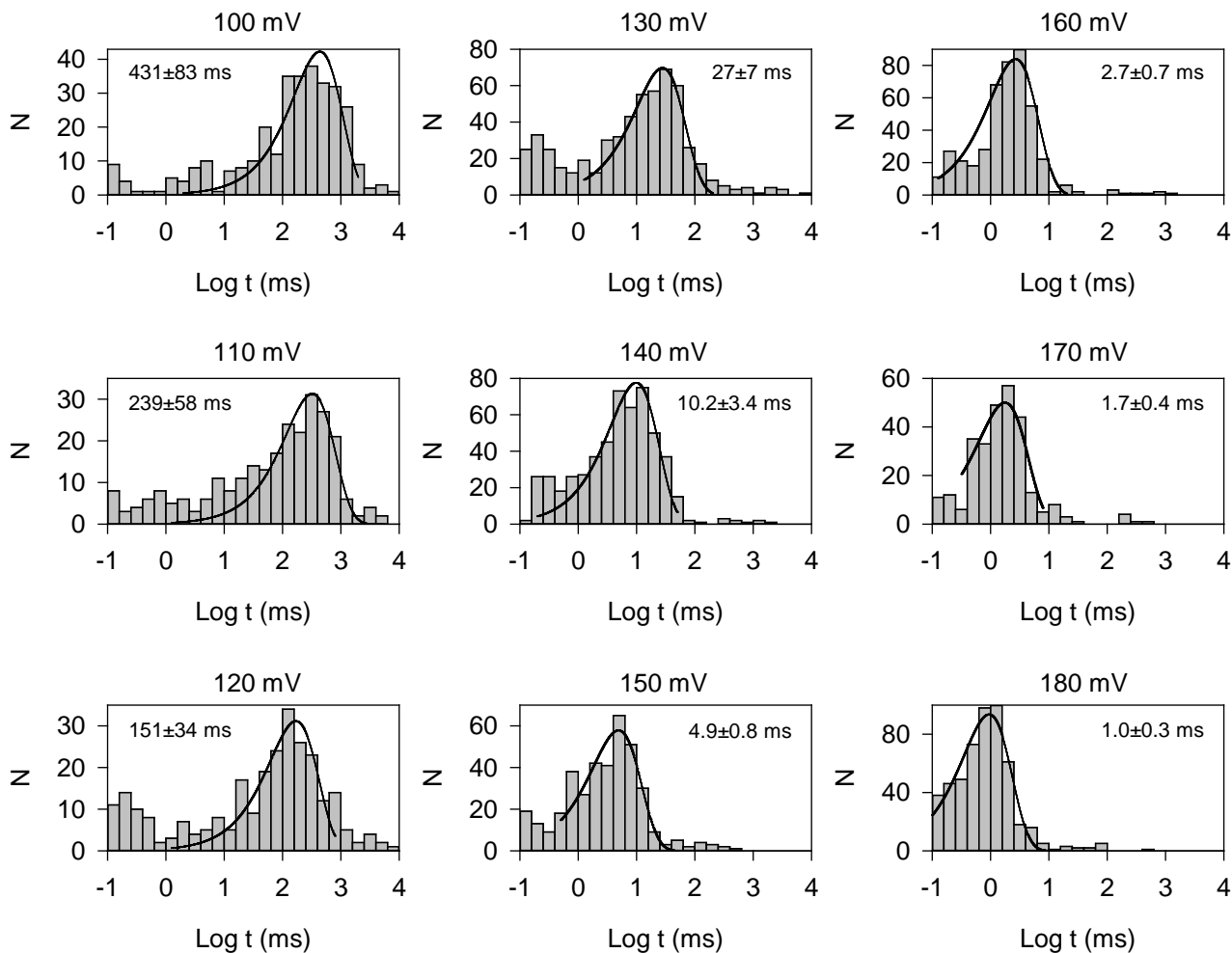
Yong Wang, Tian Kai, Lehr L. Hunter, Brandon Ritzo, Li-Qun Gu

**Supplementary Information**

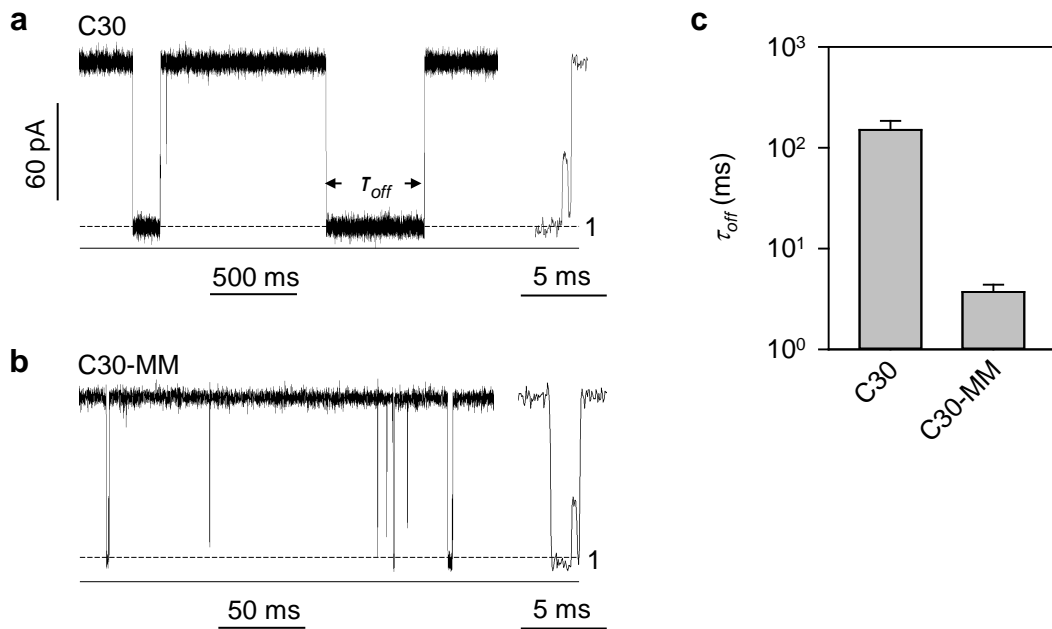
**S1. Unzipping time, dehybridization energy and Eyring rate equation.** The inverse of the unzipping time, or unzipping block duration,  $\tau_{off}$ , is the dehybridization rate  $k_{off}$ , i.e.  $k_{off} = 1/\tau_{off}$ . According to the Eyring rate equation  $k_{off} = \frac{k_B T}{h} \exp(-\Delta G/RT)$ , the energy for dsDNA dehybridization  $\Delta G$  can be calculated, where parameters and their definitions are,  $k_B$ , Boltzmann's constant, T, temperature (K),  $h$ , Planck's constant, R, Molar gas constant.



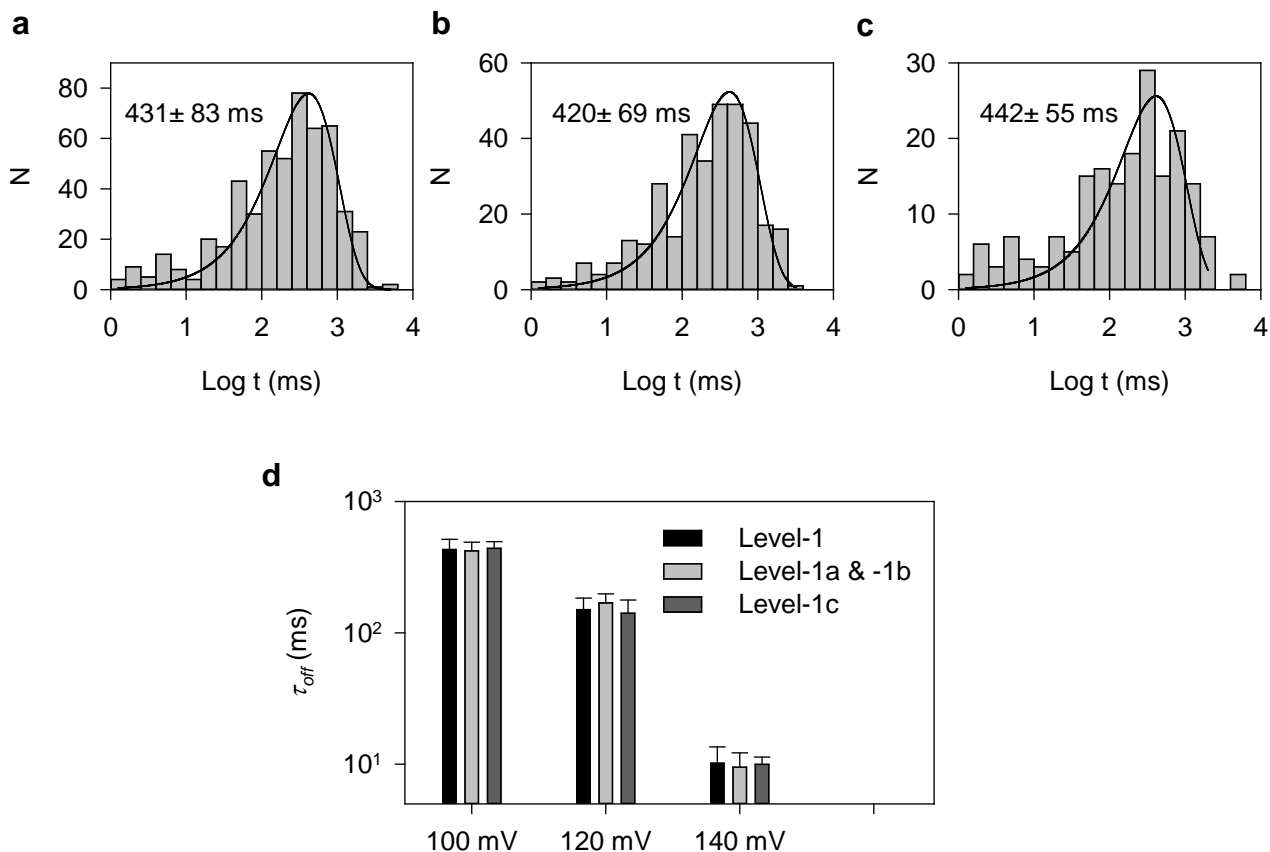
**Figure S1.** Current traces (left) and duration histograms (right) for translocation of each strand of C30 through the  $\alpha$ -hemolysin pore. The current was measured at 100 mV in 1 M KCl buffered with 10 mM Tris (pH7.5) in the presence of 100 nM of DNA in cis solution. Duration histograms were fitted to an exponential probability density function to give the time constant  $\tau_{off}$ . **a**, Translocation of the 22-nt short strand of C30.  $\tau_{off}=160 \mu\text{s}$ ; **b**, Translocation of the 52-nt long strand that contains a poly(dC)<sub>30</sub> overhang.  $T_{off}=83 \mu\text{s}$ . Sequences of both strands are given in Table 1.



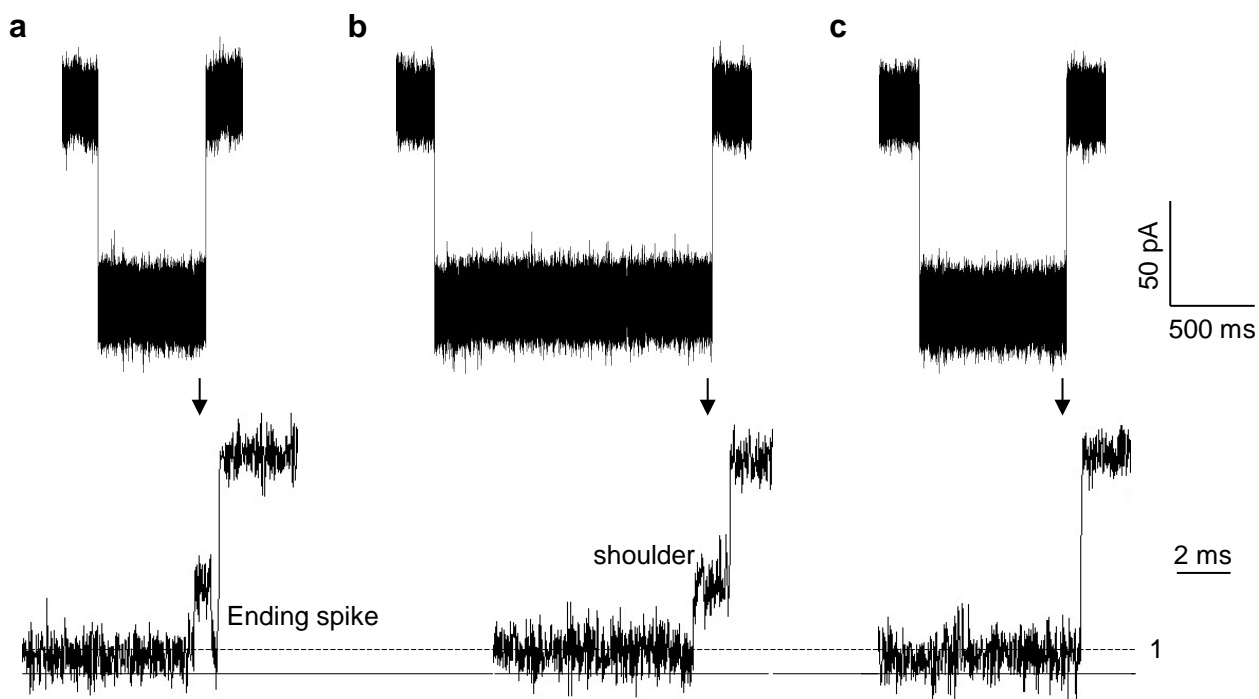
**Figure S2.** Logarithmic-binning duration histograms of Level-1 events for C30 at various voltages from +100 mV to +180 mV at a 10 mV increment. The histograms were fitted to a log-transformed exponential probability density function in pClamp software. The fitted  $\tau_{off}$  and its voltage-dependence are shown in Fig. 1c in the main text.



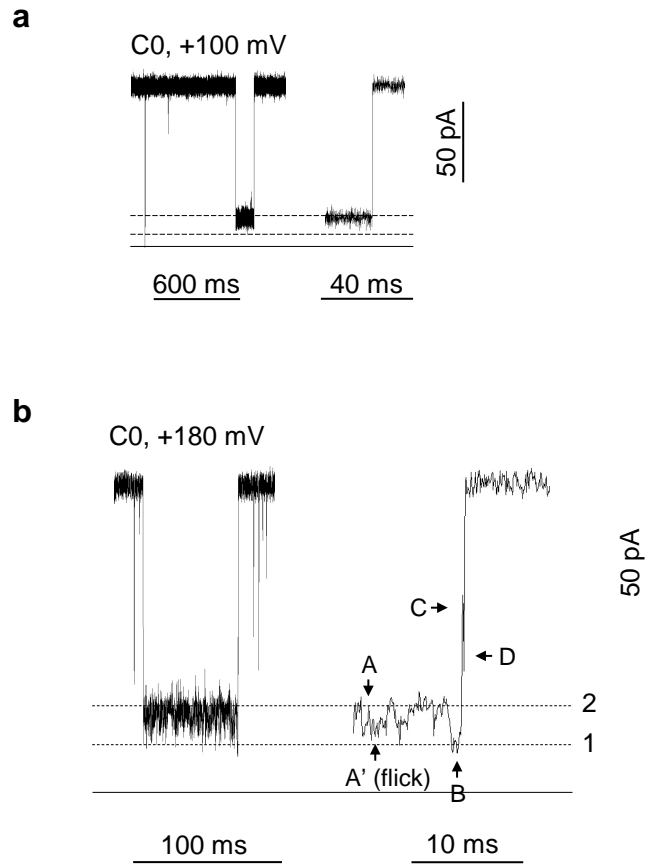
**Figure S3.** Characterization of Level-1 events for C30 and its variant C30-MM containing 2 mismatched base pairs. **a-b**, Current traces showing the Level-1a events (with ending spike) for C30 (a) and C30-MM (b) recorded at +120 mV; **c**, Duration of the Level-1 events ( $\tau_{off}$ ) for C30 and C30-MM. Two mismatched base pairs introduced in the duplex domain of C30 shorten  $\tau_{off}$  by almost 50-fold, suggesting that the voltage-driven unzipping occurs in the signature. Mismatched base-pairs greatly reduce the hybridization strength, resulting in much shorter event duration at the same voltage.



**Figure S4.** Duration of C30 signatures. **a-c**, Logarithmic-binning duration histograms for all Level-1 events (a), Level-1a & -1b events (b) and Level-1c (c) events at +100 mV. The histograms were fitted to a log-transformed exponential probability density function in pClamp software; **d**, Duration of all Level-1 events, Level-1a & -1b events and Level-1c events at +100 mV, +120 mV and +140 mV. This result indicates that the duration for different types of C30 Level-1 events are very similar and can be shortened in a similar manner as the voltage increases, suggesting that the same unzipping process occurs in all types of Level-1 events.

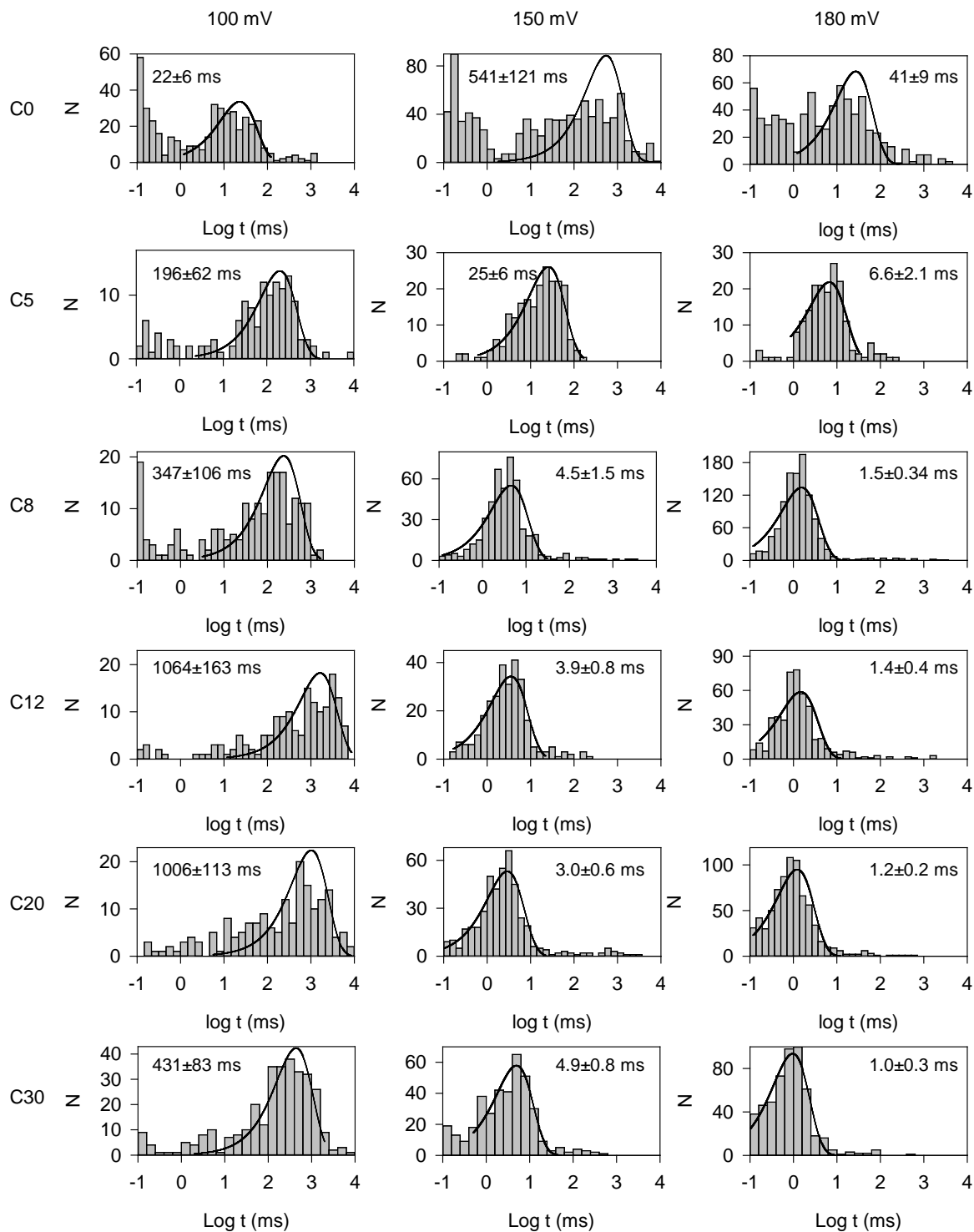


**Figure S5.** Observation of all types of Level-1 events for C30 at high filtering frequency and high data acquisition rate. **a**, Level-1a; **b**, Level-1b; and **c**, Level-1c. 1 M KCl, 10 mM Tris, pH7.5, +100 mV. These events were selected from current traces recorded at a filtering frequency of 10 kHz and acquired at a rate of 100 kHz. By comparison, the three types of Level-1 events shown in Fig. 2a-c in main text were recorded at 5 kHz frequency and data acquired at 20 kHz. This result indicates that the missing of the ending spike in terminals of Level-1b and Level-1c events are not caused by the data filtering, but correspond to different molecular processes (see main text).

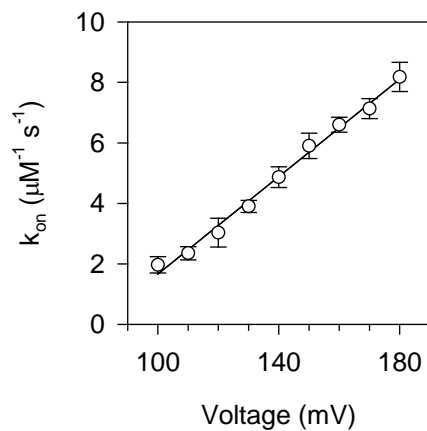


**Figure S6.** Representative C0 signatures at different voltages. C0 sequence is given in Table 1. **a**, +100 mV. Only Level-2 events without the ending spike at the terminal were observed, which correspond to the escaping of C0 back to cis solution; **b**, +180 mV. The A-A'-B-C-D current profile observed at high voltage illustrates the unzipping occurrence at the Level-2 event terminal.





**Figure S7.** Logarithmic-binning duration histograms of Level-1 events for C5 through C30 and Level-2 events for C0 at +100 mV, +150 mV and +180 mV. The histograms were fitted to a log-transformed exponential probability density function in pClamp. The fitted  $\tau_{off}$  and its dependence on the overhang base number are shown in Fig. 4c in the main text.



**Figure S8.** Voltage-dependent trapping rate ( $k_{on}$ ) of C30.  $k_{on}$  is the number of C30 signature events per unit C30 concentration per unit time. For long overhang such as C30, which contains a 30 cytosine overhang, its  $k_{on}$  is linearly correlated to the voltage, suggesting a diffusion-limited trapping step.