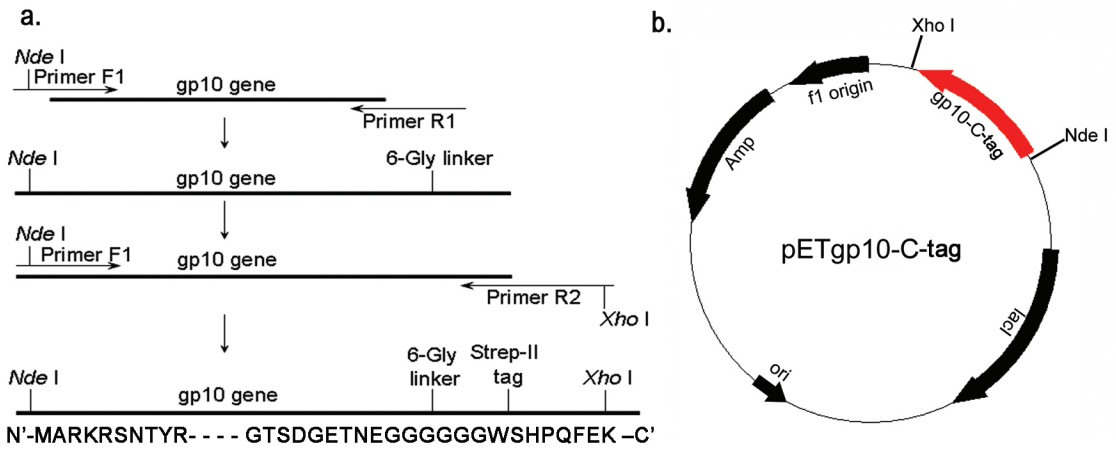
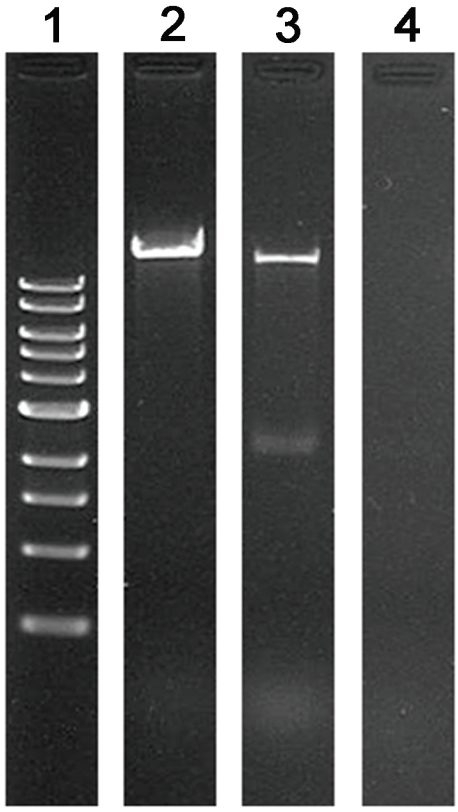


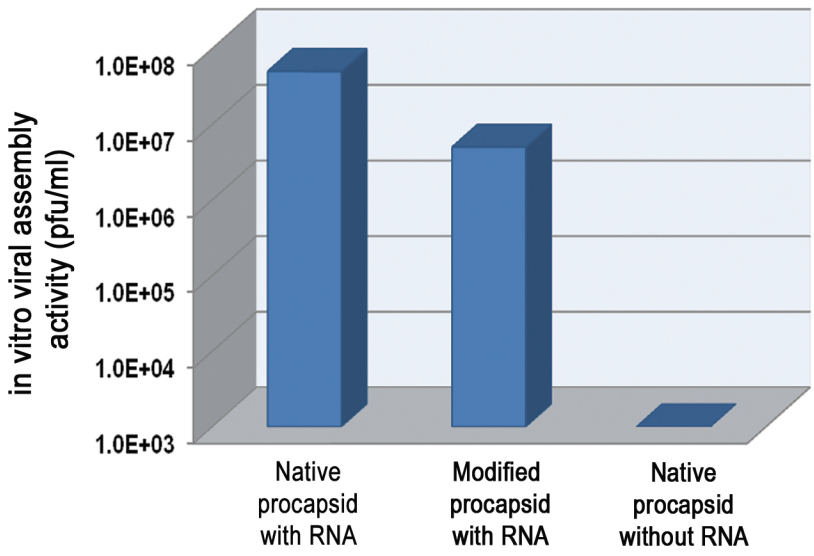
c.

Primers	Sequence (5´-3´)
F1	CGCAGCTGGCATATGGCACGTAAACGCAGTAAC
R1	GGATGACTCCAACCTCCTCCACCACCTCCCTCATTTGTTTCACCGT
R2	ATAATGTTCTCGAGCTACTTTTCGAACTGCGGATGACTCCAACCTC

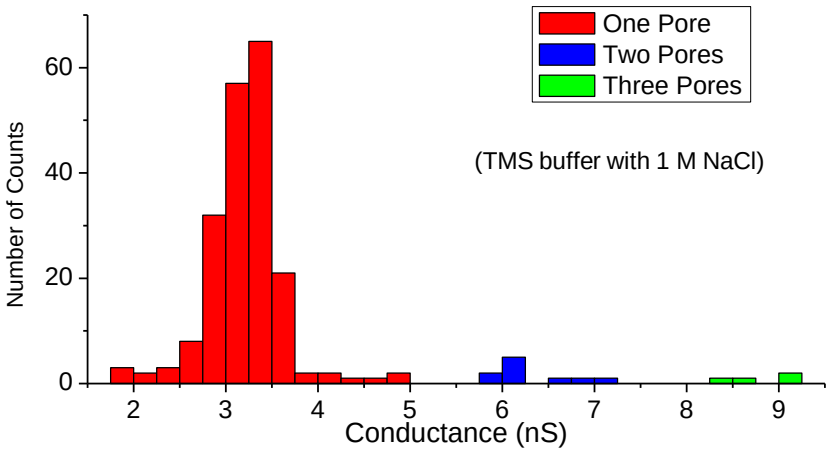
Supplementary Fig. 1. Example of plasmid construction for over-expression of one of the connectors with C-terminal modification by two-step PCR. a, The 6-Gly linker was attached to the 3'end of GP10 gene in the first PCR by a primer pair F1-R1. In a second PCR, an eight-amino acid was attached downstream of the Gly linker using primer pair F1-R2, which contained NdeI and XhoI restriction site, respectively. b, The second PCR product was digested with both NdeI and XhoI, and ligated into the NdeI/XhoI sites of the vector pET-21a(+). c, Sequence of primers.



Supplementary Fig. 2. DNA packaging activity of procapsid containing the reengineered connector. Lane 1, 1 kb DNA ladder; Lane 2, normal procapsid; Lane 3, procapsid with reengineered connector; Lane 4, negative control, DNA packaging without ATP.

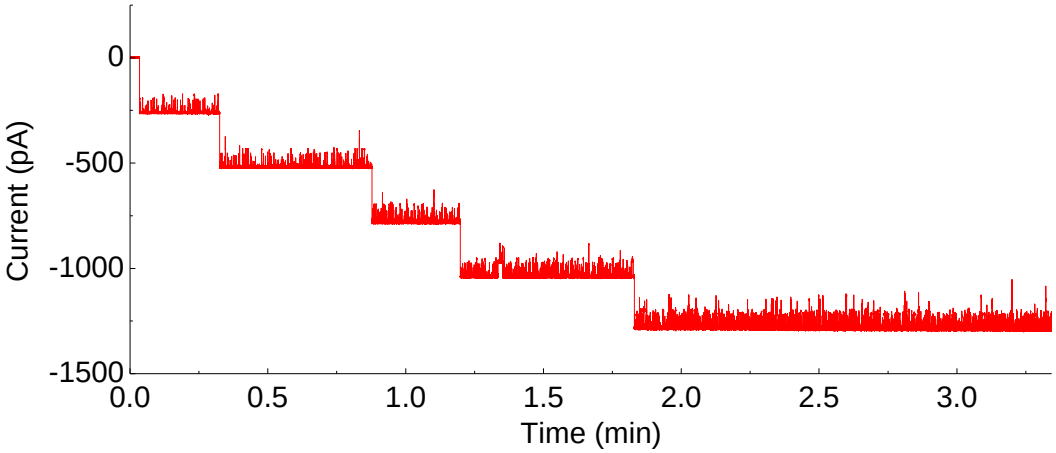


Supplementary Fig. 3. Phi29 virion assembly activity of procapsid containing the reengineered connector. In vitro viral assembly activity of the reengineered procapsid was compared to that of native procapsid in the presence of pRNA.



Supplementary Figure 4. A histogram of sizes of the conductance steps caused by connector insertions. The data was obtained from a total of 213 insertions in 40 individual experiments.

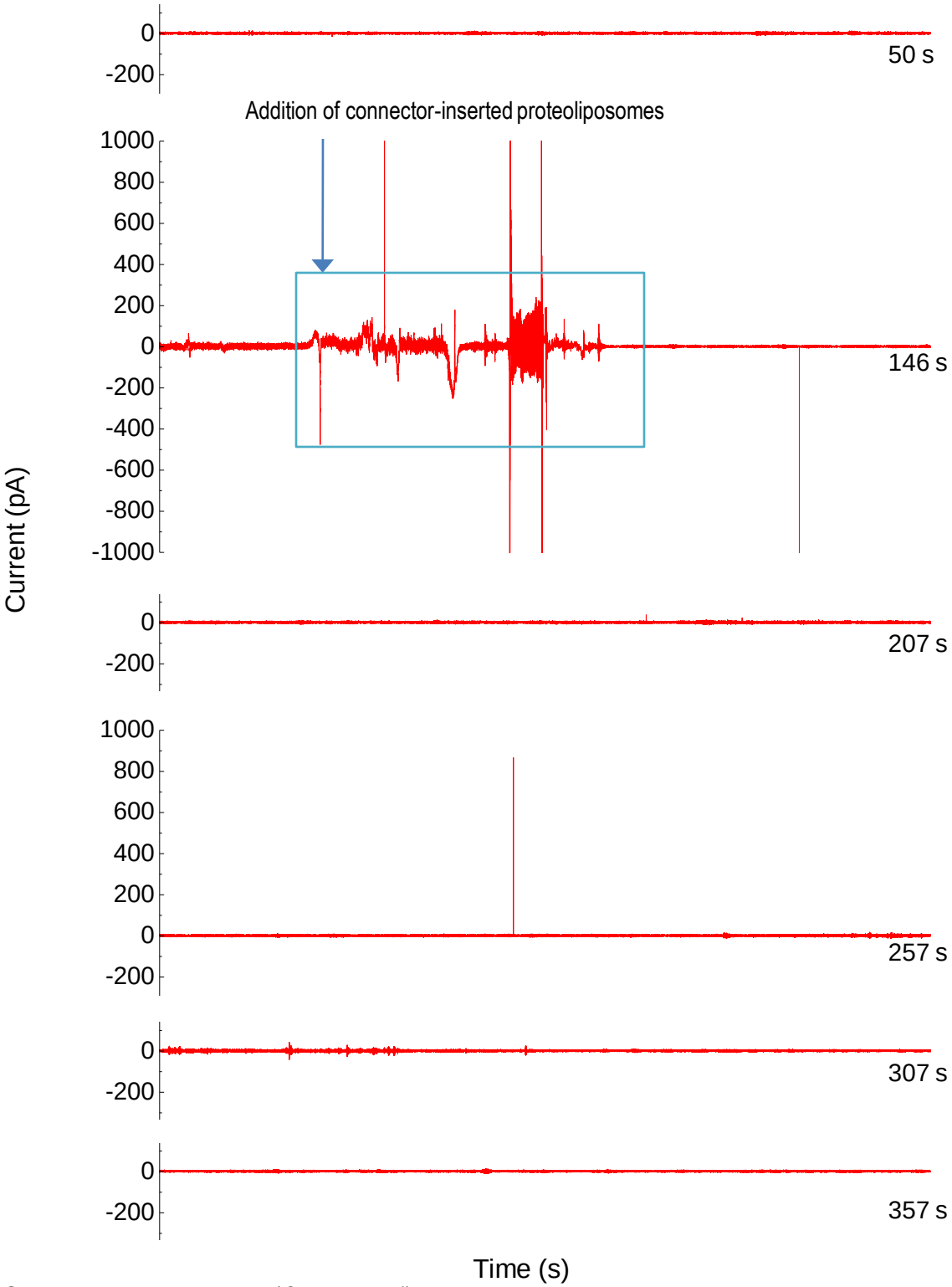
a. Current trace to record translocation of DNA pre-mixed in buffer



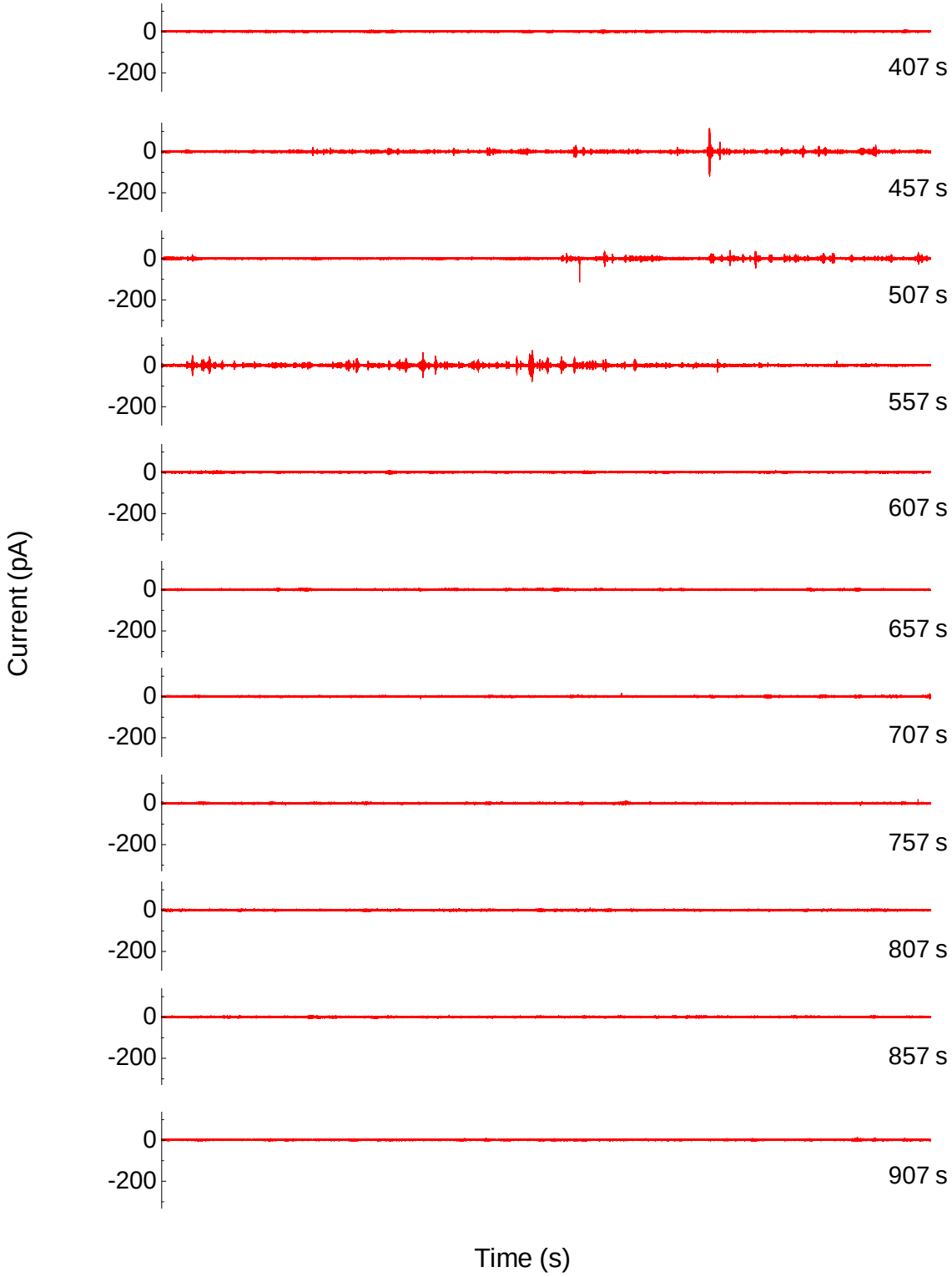
Supplementary Figure 5. A continuous current trace showing connector insertions and DNA translocation under -75 mV. a, The case in which 4 μM of a 35-bp DNA was pre-mixed in buffer (Method 2 in Supplementary Materials and Methods section); b, The case in which 4 μM of a 35-bp DNA was added after the insertion of connector into the bilayer lipid membrane (Method 1 in Supplementary Materials and Methods section).

b. Current traces continuously recording addition of connector and DNA

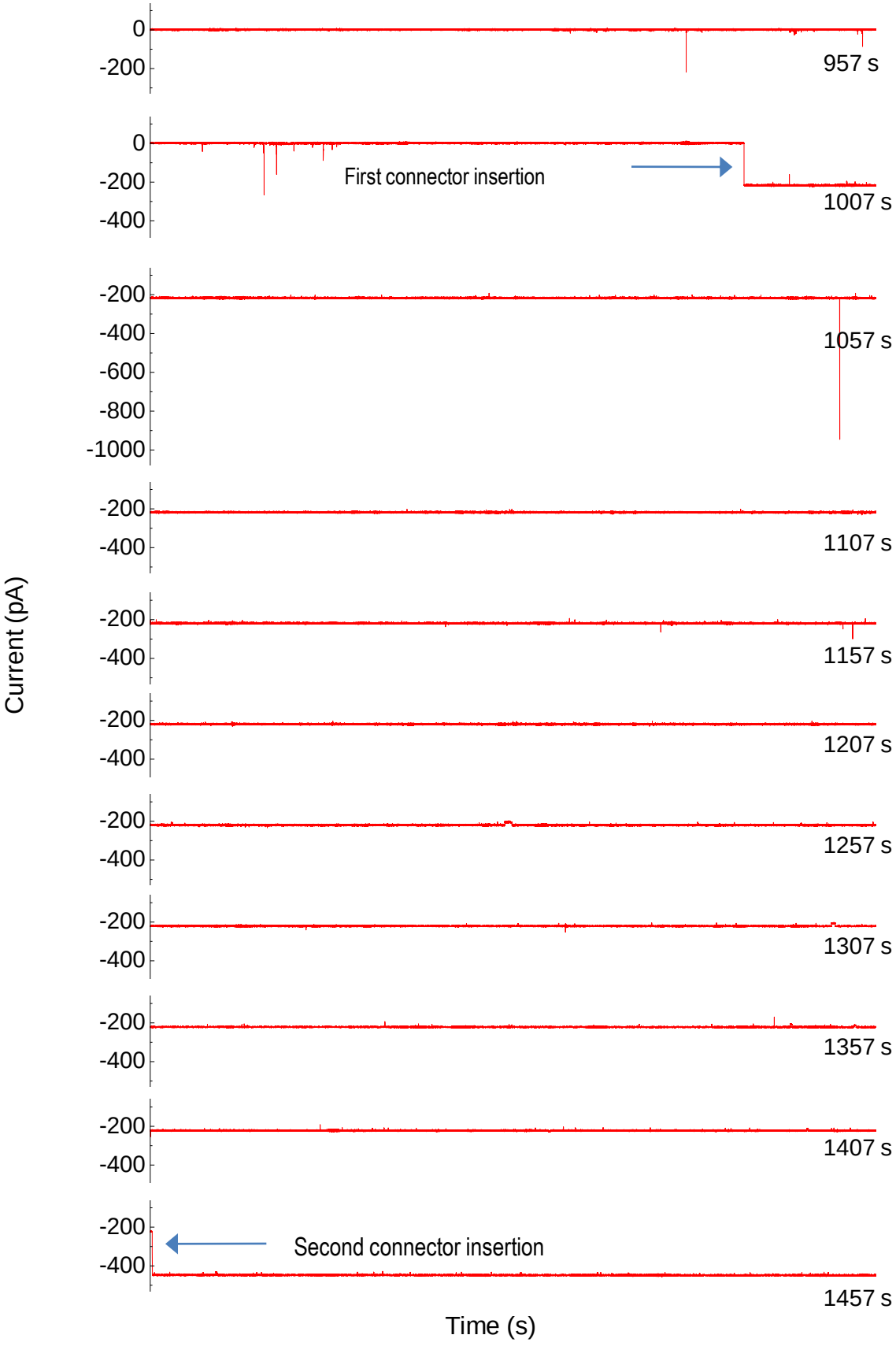
(Each trace was recorded in 50 second increments)



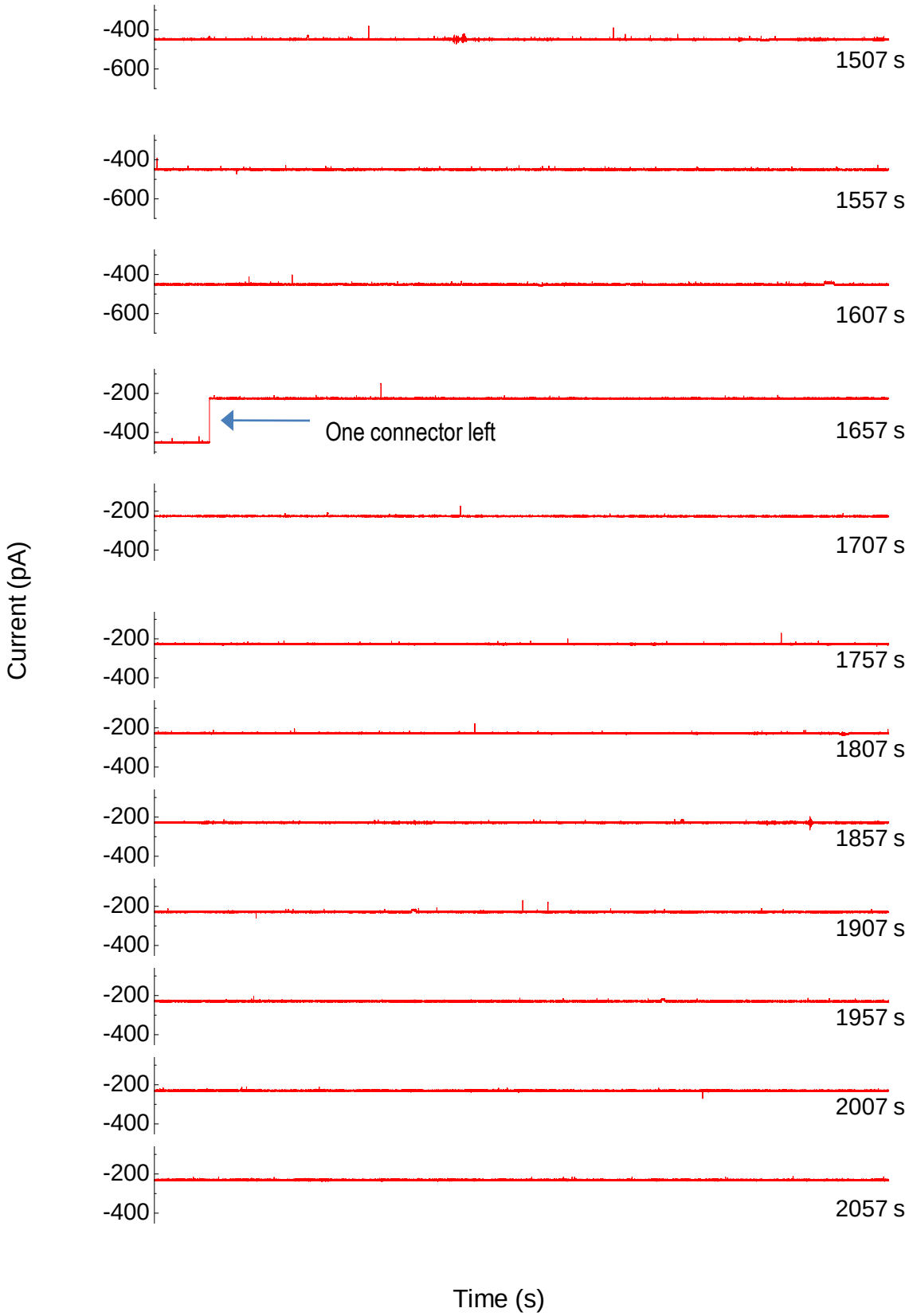
Supplementary Figure 5 (*Continued. 1*)



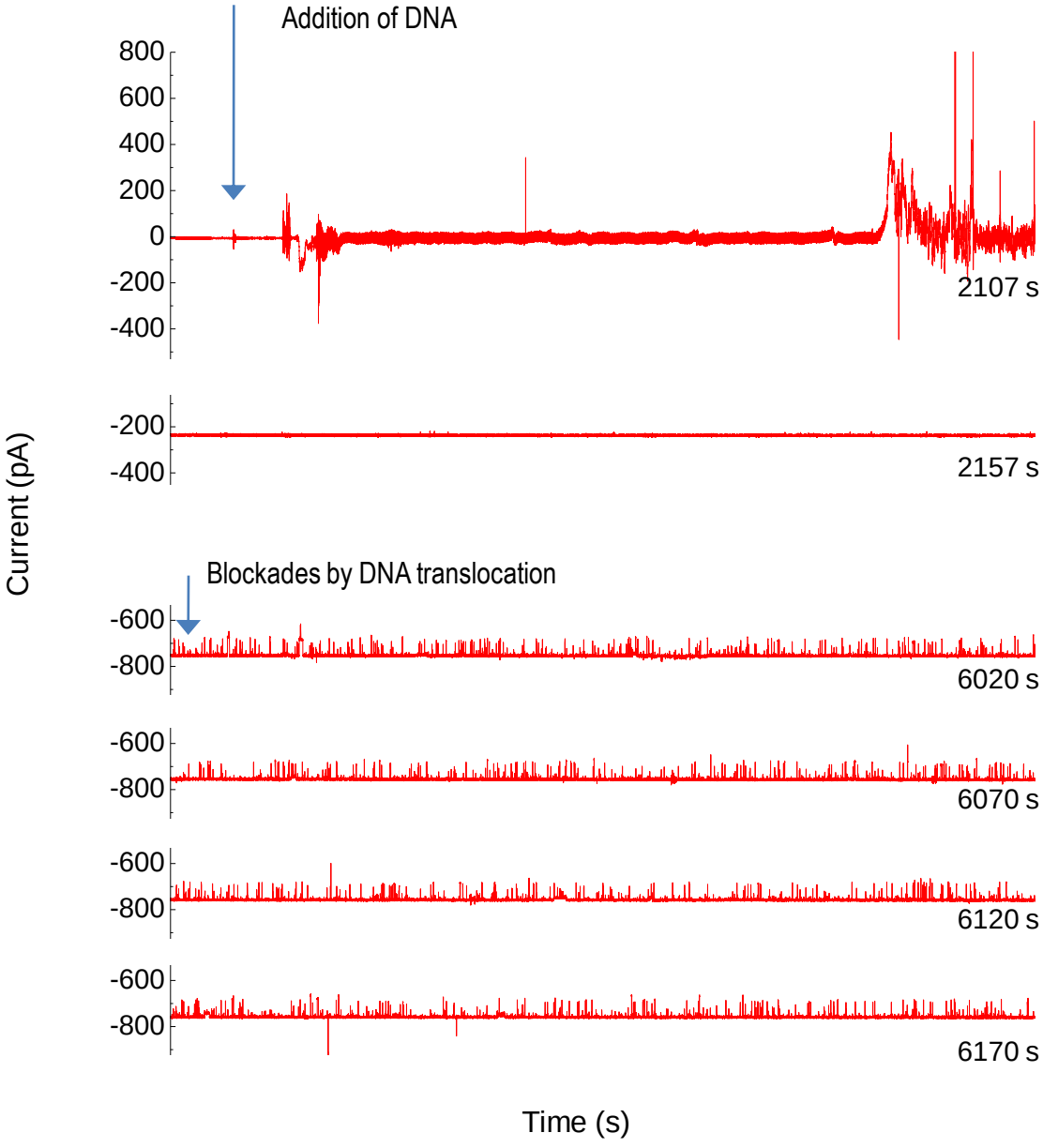
Supplementary Figure 5 (*Continued. 2*)



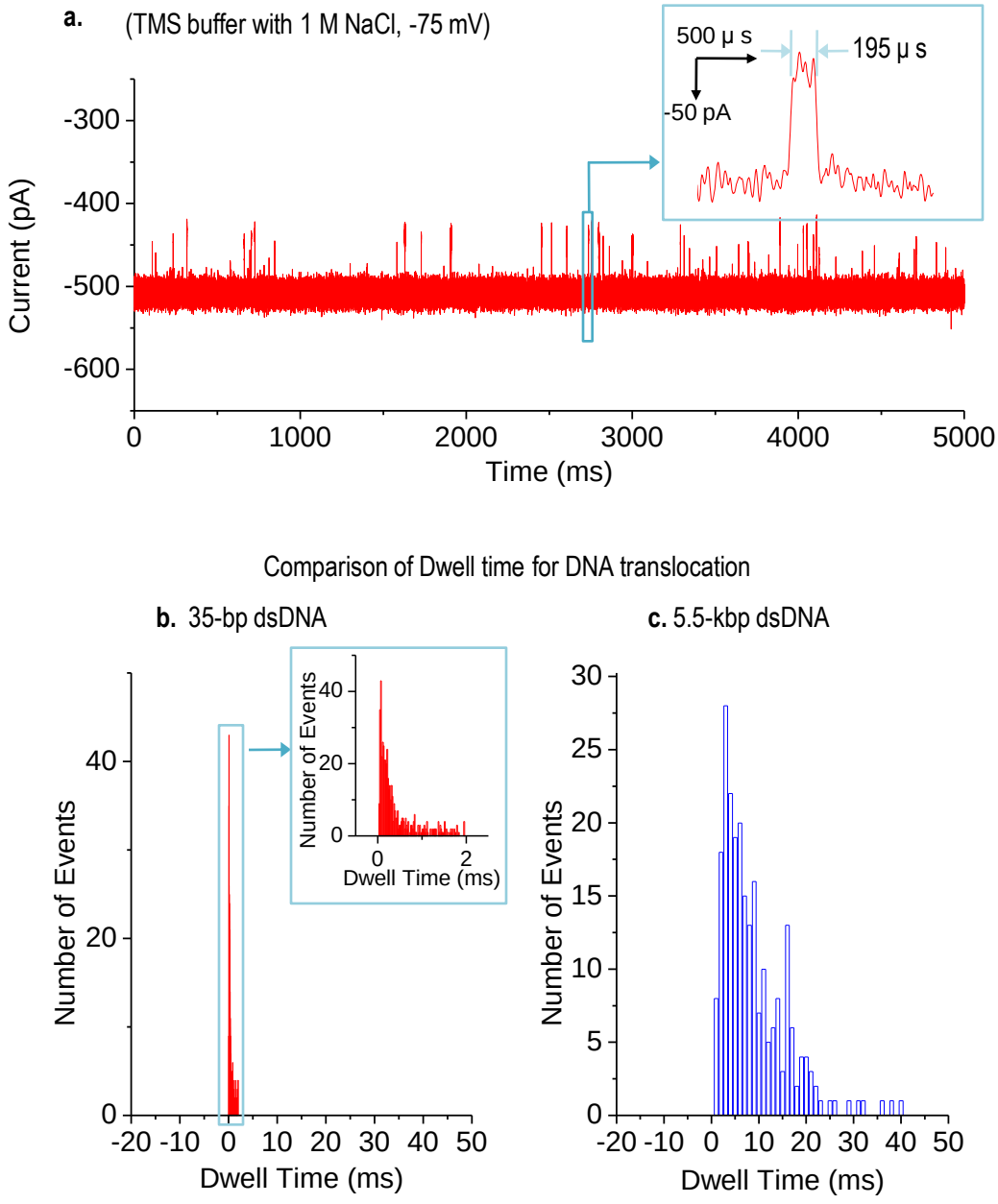
Supplementary Figure 5 (*Continued. 3*)



Supplementary Figure 5 (*Continued. 4*)

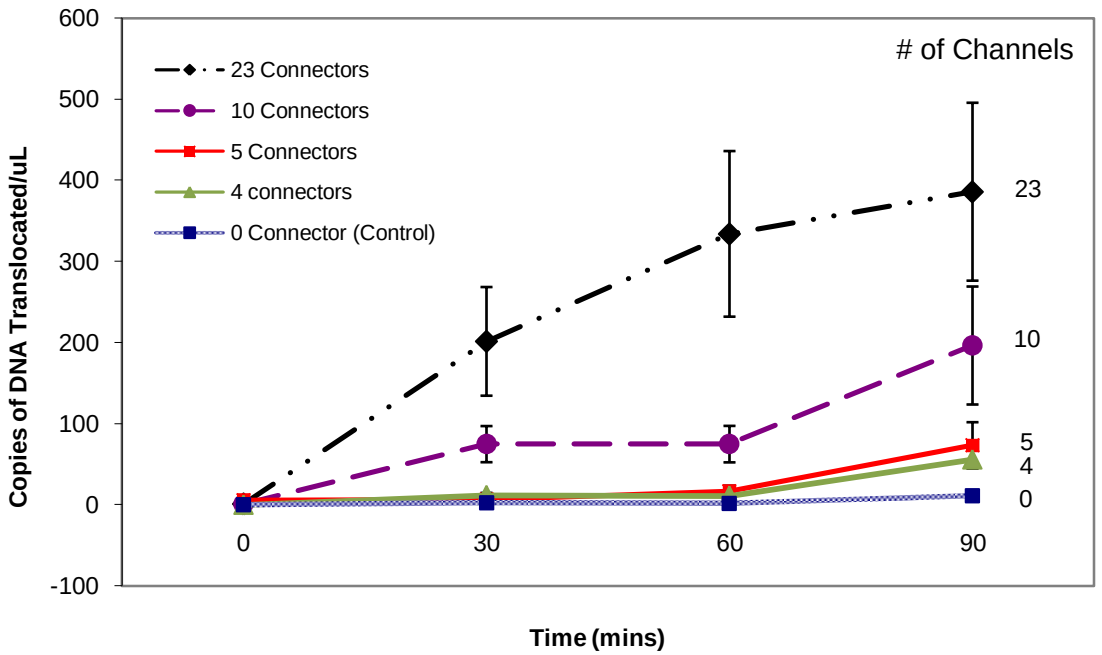


Supplementary Figure 5 (*Continued. 5*)

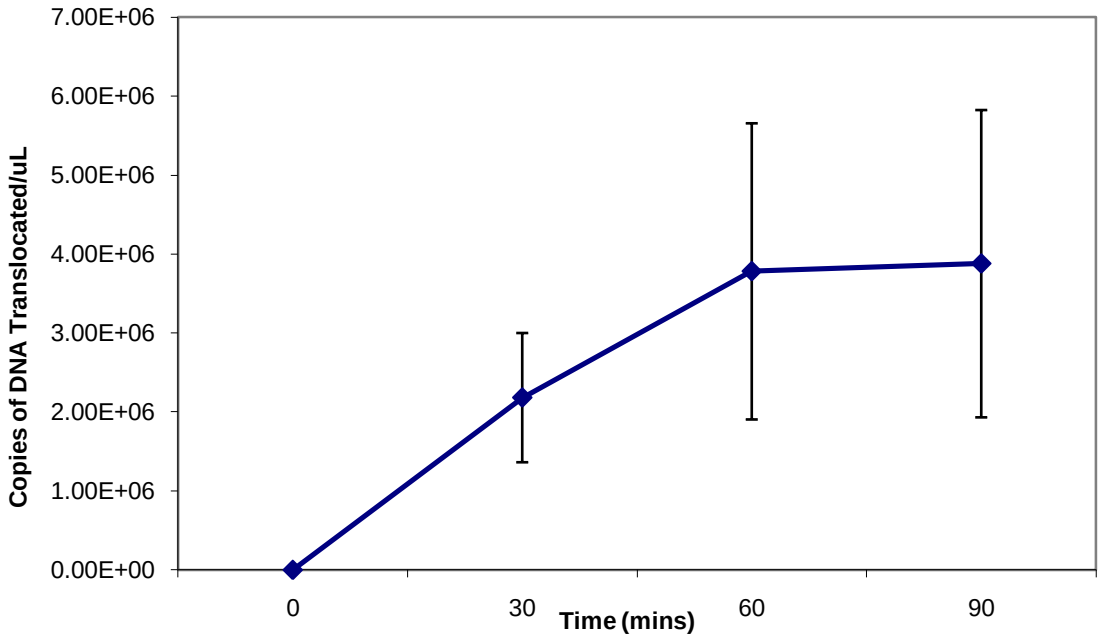


Supplementary Figure 6. Translocation of a 35-bp DNA through connector channels. a, A typical current trace recorded from the bilayer in the presence of 4 μM of DNA (low-pass filtered at a frequency of 10 kHz and acquired at sampling frequency of 200 kHz); b-c, Comparison of dwell time of channel blockades by the translocation of 35-bp dsDNA (b) and 5.5-kbp dsDNA (c). b-c, TMS buffer with 1 M NaCl, -75 mV.

a.



b.



Supplementary Figure 7. Quantitative PCR analysis of DNA translocation under various conditions. a, Q-PCR analysis of DNA translocating through different number of connectors. The error bars represent the measurement errors for each sample under identical Q-PCR conditions. b, Q-PCR analysis of DNA molecules when leaking occurred. The error bars represent the standard deviations of the mean from three independent experiments.