Supplementary information

Polyarginine-functionalized AP site probes for mechanistic analysis of uracil-DNA glycosylase via nanopore-based single-molecule sensing

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Contents

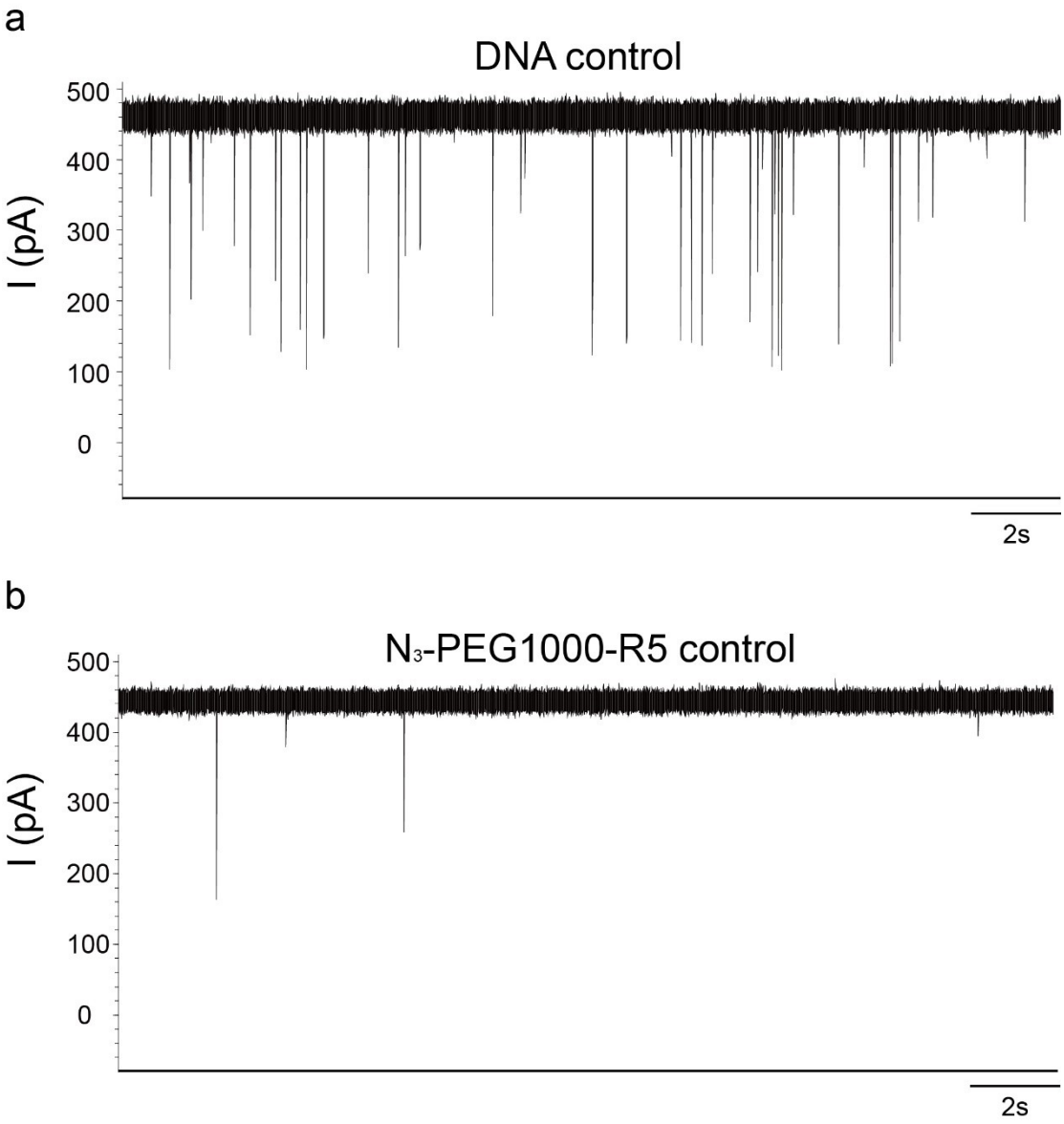
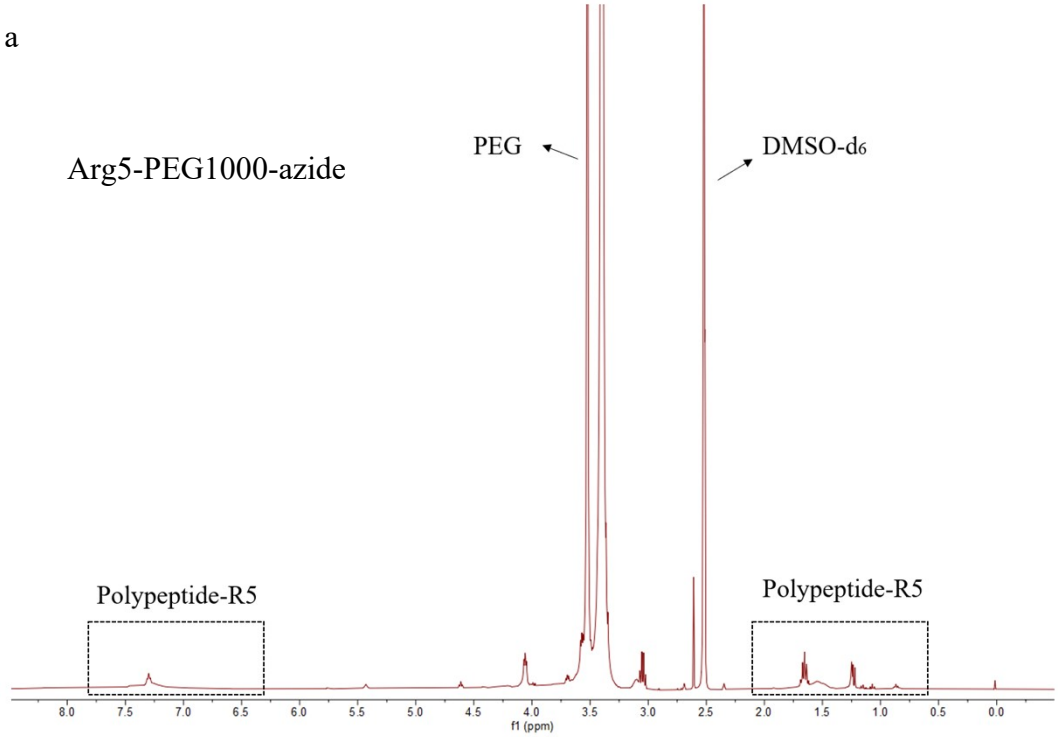
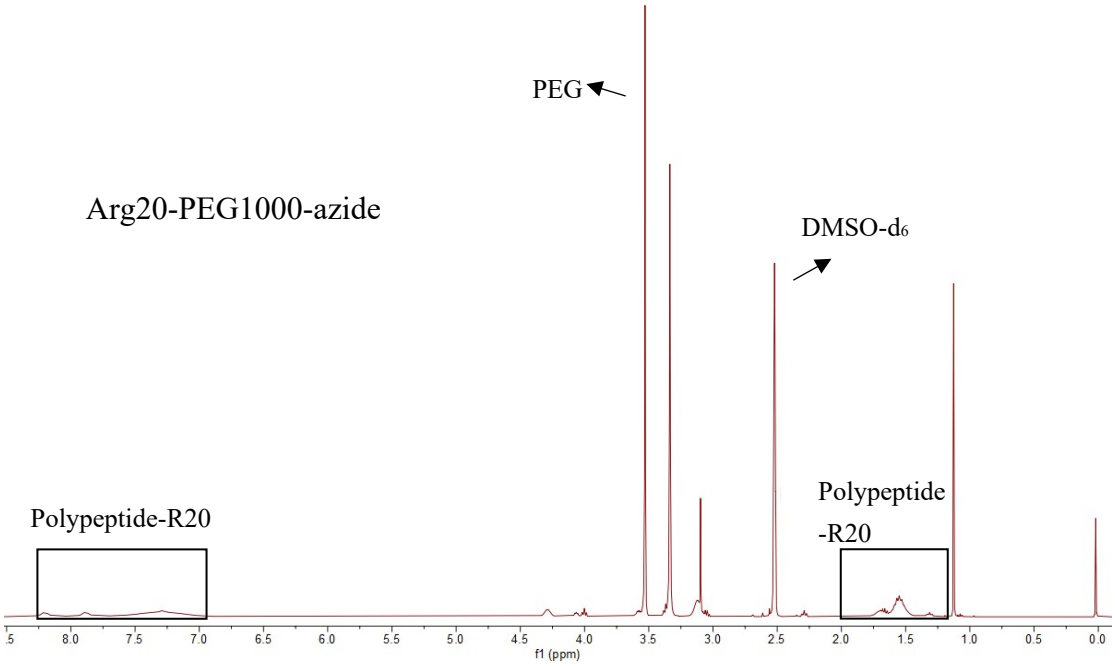


Fig. S1 Control experiments with unmodified DNA1 and N3-PEG1000-R5. (a) Representative 20-s current-time (I-t) traces of 0.3 μM DNA1 acquisition at +140 mV. (b) Corresponding traces for 0.1 mg/mL N3-PEG1000-R5 under identical voltage conditions. All experiments were conducted in buffer containing 3 M KCl and 10 mM Tris-HCl (pH 8.0).

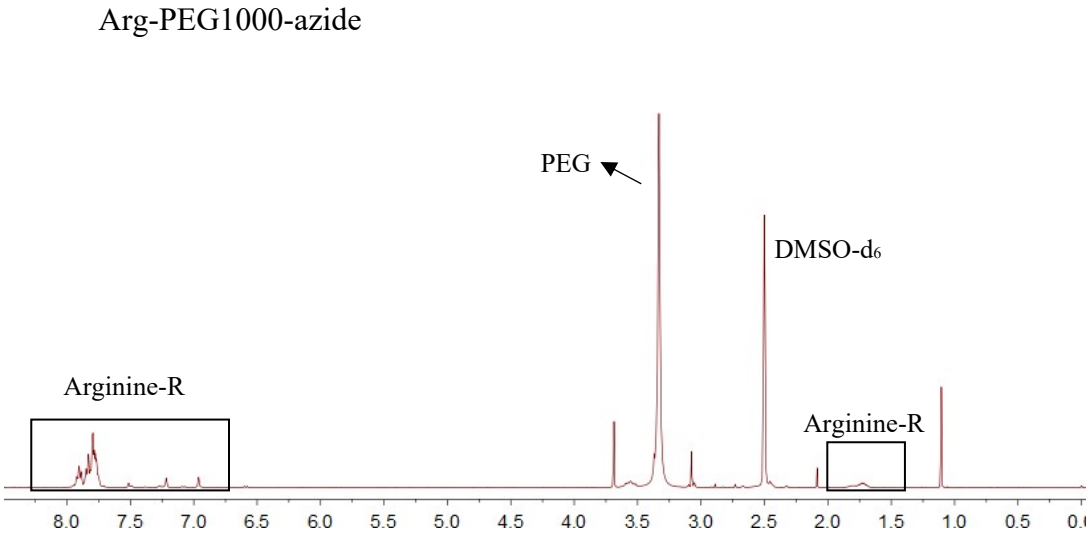
a


Arg5-PEG1000-azide
PEG
DMSO-d6
Polypeptide-R5
Polypeptide-R5
f1 (ppm)

b


Arg20-PEG1000-azide
PEG
DMSO-d6
Polypeptide-R20
Polypeptide
-R20
f1 (ppm)

c



d

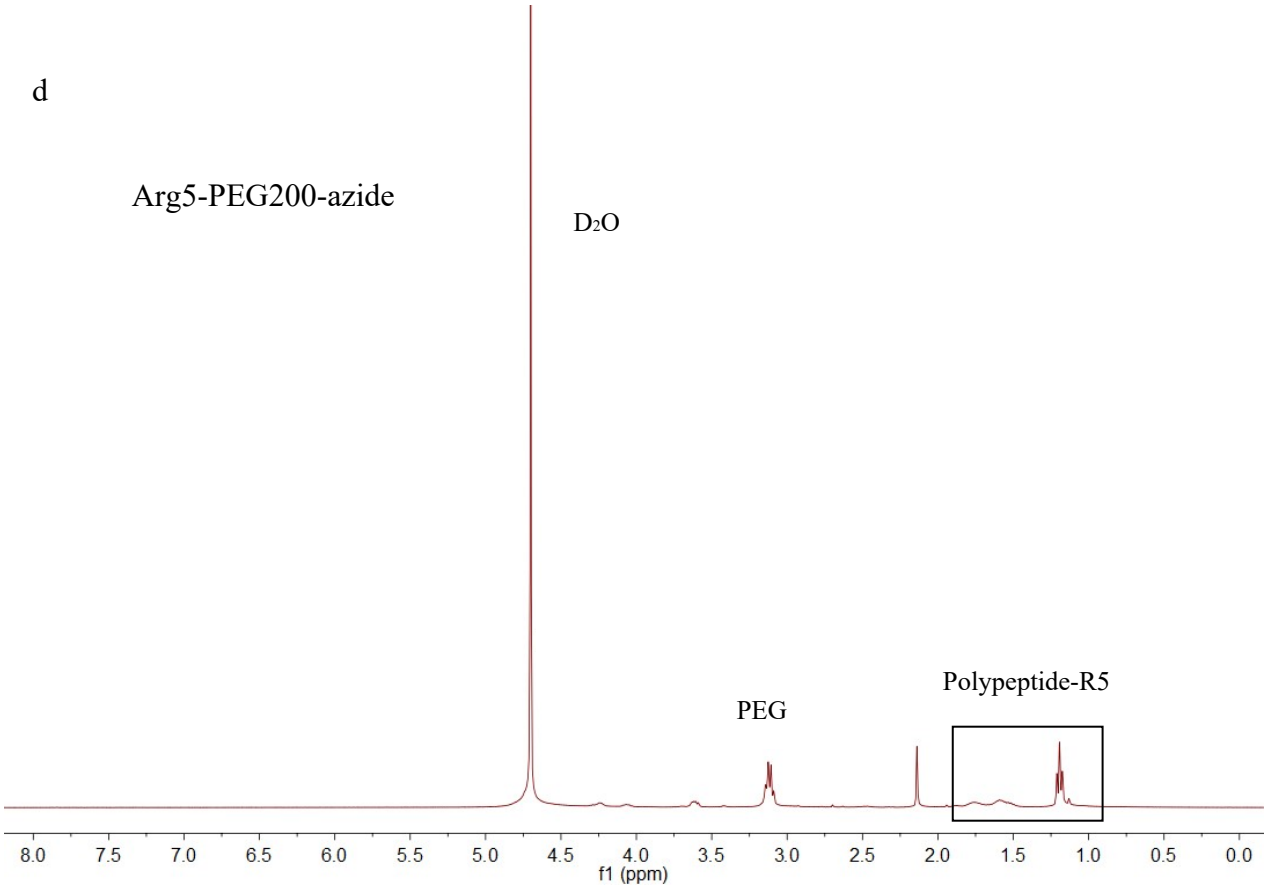


Fig. S2 ^{1}H-NMR spectra of Arg5-PEG1000-azide (a), Arg20-PEG1000-azide (b), Arg-PEG1000-azide (c), Arg5-PEG200-azide (d).

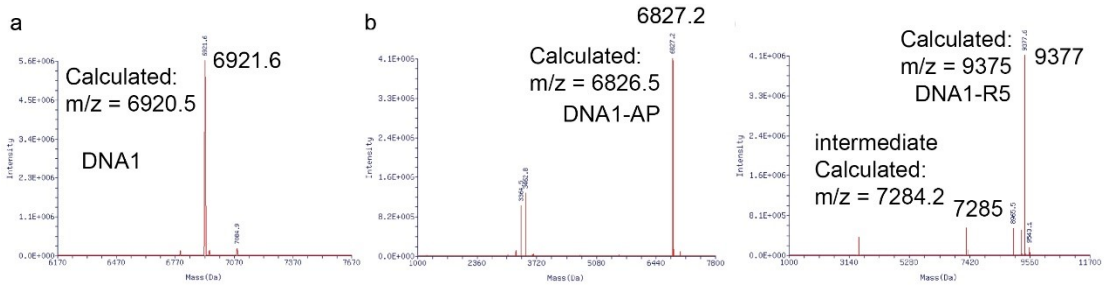


Fig. S3 Mass spectra of DNA1 (a), DNA1-AP (b), DNA1-R5 (c).

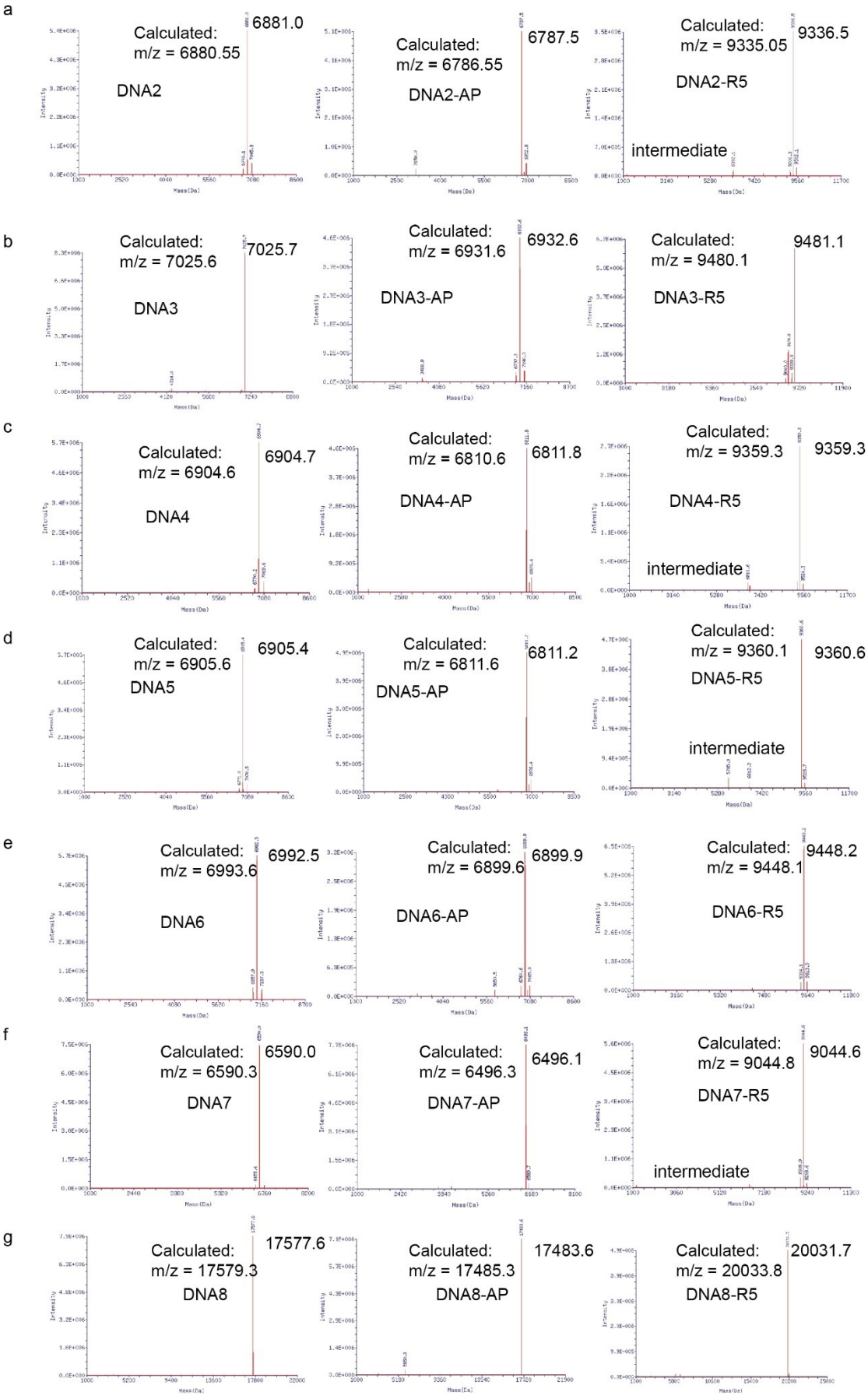

a
Calculated:
m/z = 6880.55
6881.0
DNA2
Calculated:
m/z = 6786.55
6787.5
DNA2-AP
Calculated:
m/z = 9335.05
9336.5
DNA2-R5
intermediate
b
Calculated:
m/z = 7025.6
7025.7
DNA3
Calculated:
m/z = 6931.6
6932.6
DNA3-AP
Calculated:
m/z = 9480.1
9481.1
DNA3-R5
c
Calculated:
m/z = 6904.6
6904.7
DNA4
Calculated:
m/z = 6810.6
6811.8
DNA4-AP
Calculated:
m/z = 9359.3
9359.3
DNA4-R5
intermediate
d
Calculated:
m/z = 6905.6
6905.4
DNA5
Calculated:
m/z = 6811.6
6811.2
DNA5-AP
Calculated:
m/z = 9360.1
9360.6
DNA5-R5
intermediate
e
Calculated:
m/z = 6993.6
6992.5
DNA6
Calculated:
m/z = 6899.6
6899.9
DNA6-AP
Calculated:
m/z = 9448.1
9448.2
DNA6-R5
f
Calculated:
m/z = 6590.3
6590.0
DNA7
Calculated:
m/z = 6496.3
6496.1
DNA7-AP
Calculated:
m/z = 9044.8
9044.6
DNA7-R5
intermediate
g
Calculated:
m/z = 17579.3
17577.6
DNA8
Calculated:
m/z = 17485.3
17483.6
DNA8-AP
Calculated:
m/z = 20033.8
20031.7
DNA8-R5

Fig. S4 Mass spectra of DNA2, DNA2-AP , DNA2-R5 (a), DNA3, DNA3-AP , DNA3-R5 (b), DNA4, DNA4-AP , DNA4-R5 (c), DNA5, DNA5-AP , DNA5-R5 (d), DNA6, DNA6-AP , DNA6-R5 (e), DNA7, DNA7-AP , DNA7-R5 (f), DNA8, DNA8-AP , DNA8-R5 (g).

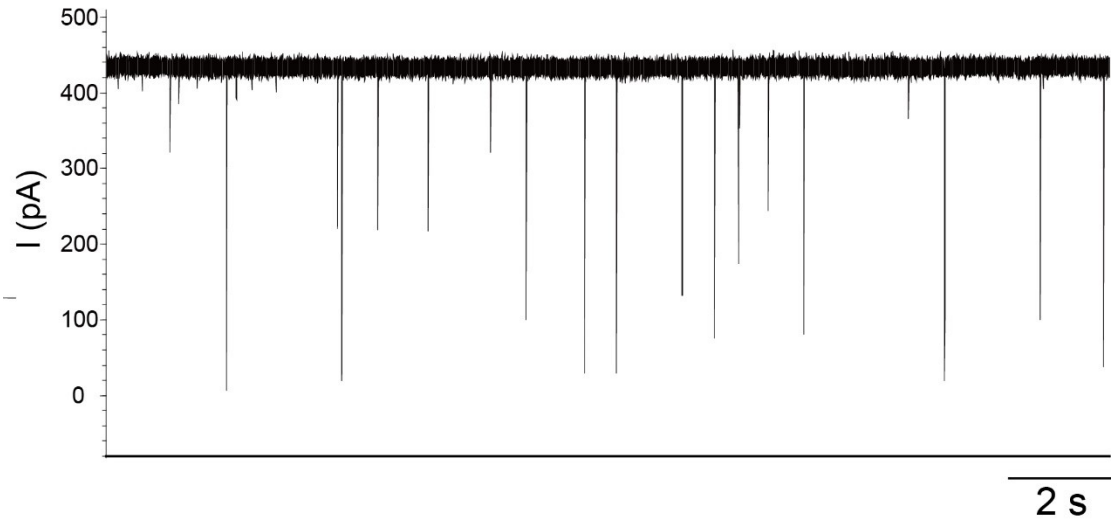


Fig. S5 20-s representative I-t traces of DNA1-R5 recording at pH 11.0 condition. Data were acquired in buffered solution containing 3 M KCl and 10 mM Tris (pH 11.0) under an applied potential of 140 mV.

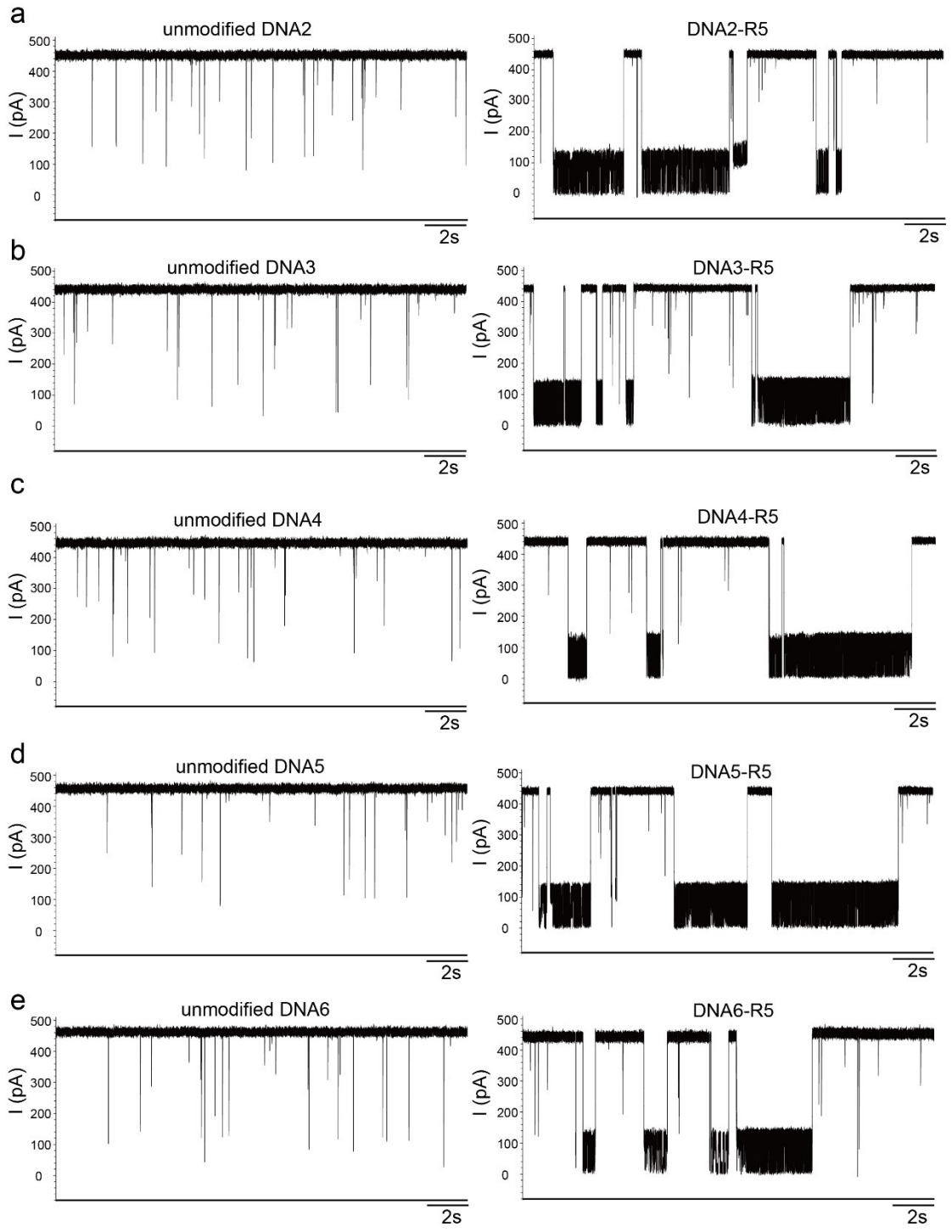


Fig. S6 Current traces of unmodified and R5-modified DNA constructs (DNA2-DNA6). (a) Comparative 20-s I-t traces for unmodified DNA2 and DNA2-R5 adduct. (b) Comparative 20-s I-t traces for unmodified DNA3 and DNA3-R5 adduct. (c) Comparative 20-s I-t traces for unmodified DNA4 and DNA4-R5 adduct. (d) Comparative 20-s I-t traces for unmodified DNA5 and DNA5-R5 adduct. (e) Comparative 20-s I-t traces for unmodified DNA6 and DNA6-R5 adduct. All

measurements were conducted in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV.

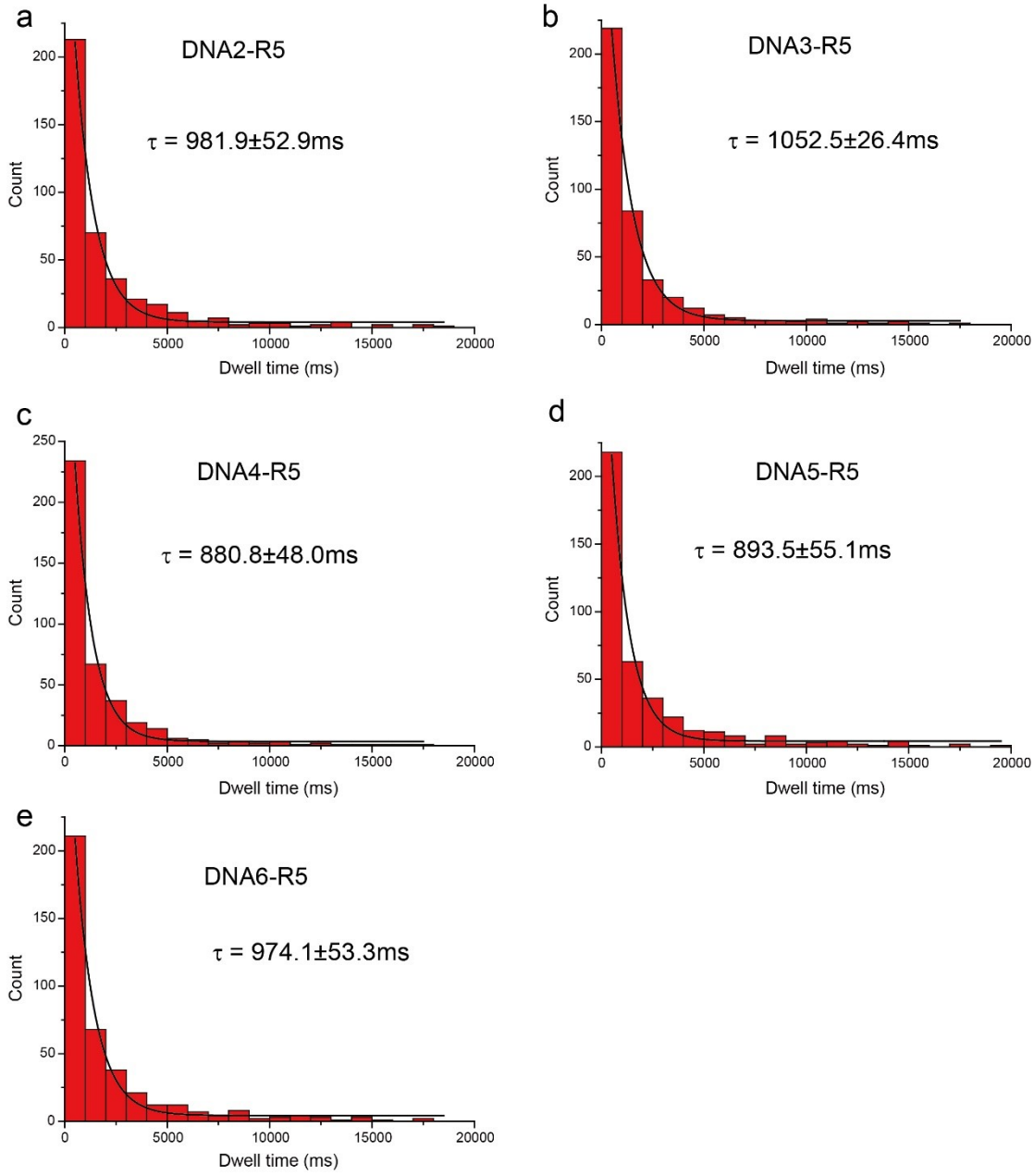


Fig. S7 Dwell time histograms for R5-modified DNA constructs (DNA2-R5 to DNA6-R5). (a) Dwell time distribution for DNA2-R5. (b) Dwell time distribution for DNA3-R5. (c) Dwell time distribution for DNA4-R5. (d) Dwell time distribution for DNA5-R5. (e) Dwell time distribution for DNA6-R5. Data represent n = 400 events collected from 10 independent nanopores. All distributions were fitted with exponential decay functions. Measurements were performed in buffered solution containing 3 M KCl and

10 mM Tris (pH 8.0) under an applied potential of 140 mV.

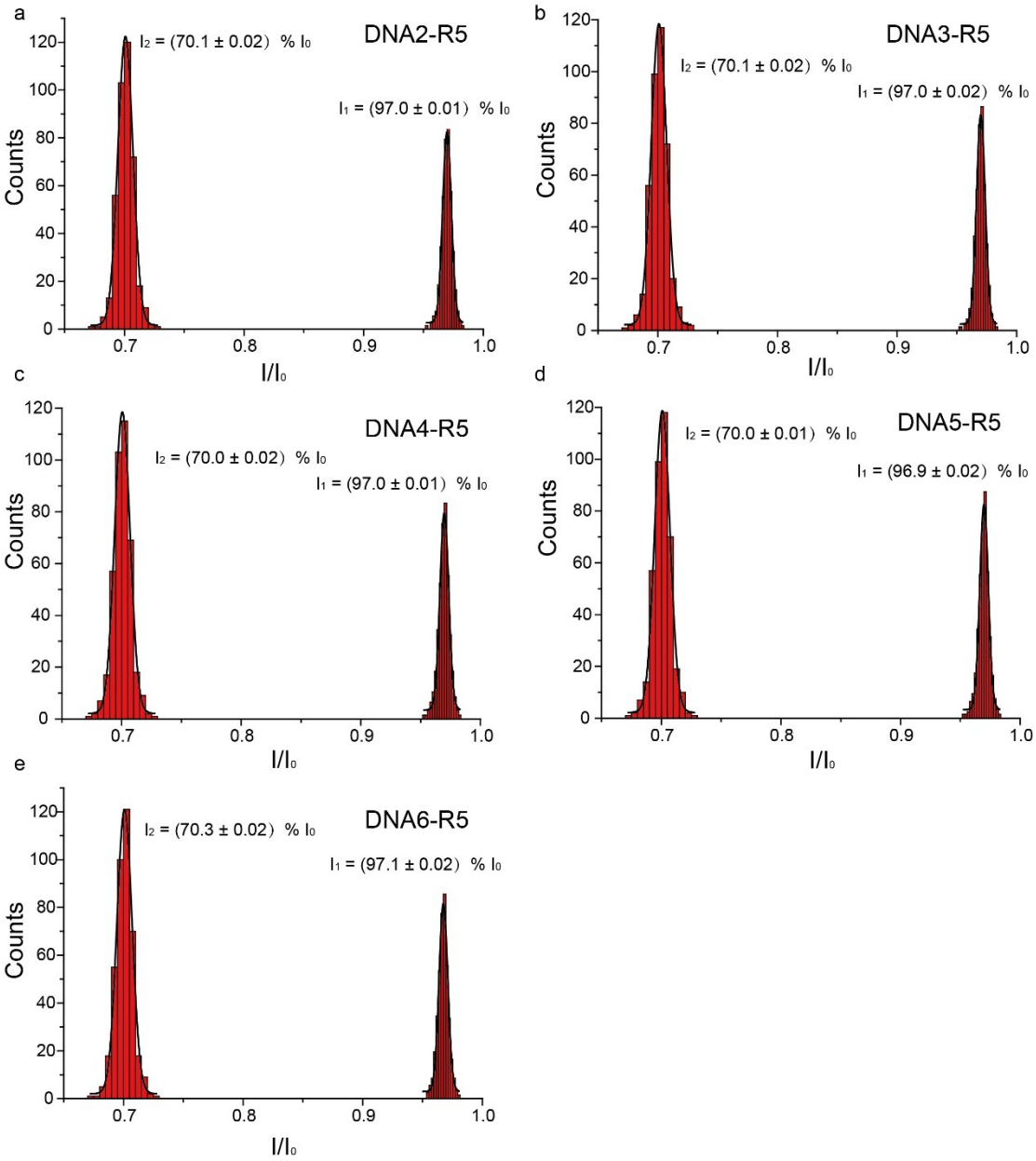


Fig. S8 Amplitude histograms for R5-modified DNA constructs (DNA2-R5 to DNA6-R5). (a) Amplitude distribution for DNA2-R5. (b) Amplitude distribution for DNA3-R5. (c) Amplitude distribution for DNA4-R5. (d) Amplitude distribution for DNA5-R5. (e) Amplitude distribution for DNA6-R5. Data represent n = 400 events collected from 10 independent nanopores. All distributions were fitted with Gaussian fitting equation. Measurements were performed in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV.

Table S1 Amplitude, dwell time and signal frequency for each sequence (DNA1-

DNA6)

DNA	Amplitude	Dwell time (ms)	Event frequency (min^{-1})
DNA1-R5	I_1 = (96.9 ± 0.01)%I_0 I_2 = (70.2 ± 0.01)%I_0	τ = 798.9 ± 33.8	10.6±0.1
DNA2-R5	I_1 = (97.0 ± 0.01)%I_0 I_2 = (70.1 ± 0.02)%I_0	τ = 981.9 ± 52.9	9.3±0.1
DNA3-R5	I_1 = (70.1 ± 0.02)%I_0 I_2 = (97.0 ± 0.02)%I_0	τ = 1052.5 ± 26.4	9.2±0.3
DNA4-R5	I_1 = (97.0 ± 0.01)%I_0 I_2 = (70.0 ± 0.02)%I_0	τ = 880.8 ± 48.0	10.0±0.2
DNA5-R5	I_1 = (96.9 ± 0.02)%I_0 I_2 = (70.0 ± 0.01)%I_0	τ = 893.5 ± 55.1	10.2±0.1
DNA6-R5	I_1 = (70.3 ± 0.02)%I_0 I_2 = (97.1 ± 0.02)%I_0	τ = 974.1 ± 53.3	9.8±0.3

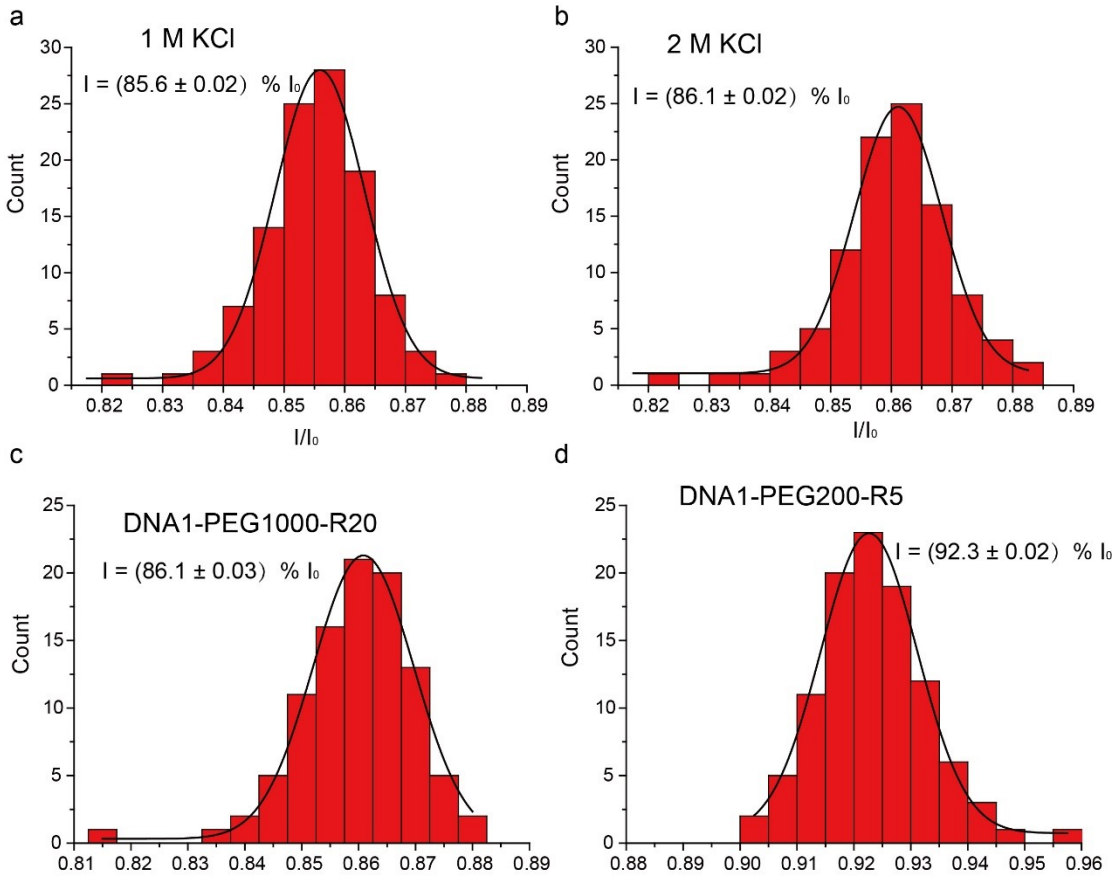


Fig. S9 Histogram of signal amplitudes based on each tested condition (1 M KCl, 2 M KCl, PEG1000-R20 connection, PEG200-R5 connection). (a) Histogram of signal amplitudes in 1 M KCl, 10mM Tris, pH 8.0. (b) Histogram of signal amplitudes in 2 M KCl, 10mM Tris, pH 8.0. (c) Histogram of signal amplitudes for DNA1-PEG1000-R20. (d) Histogram of signal amplitudes for DNA1-PEG200-R5. Data for both (c) and (d) were acquired in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV. (n = 100 independent events).

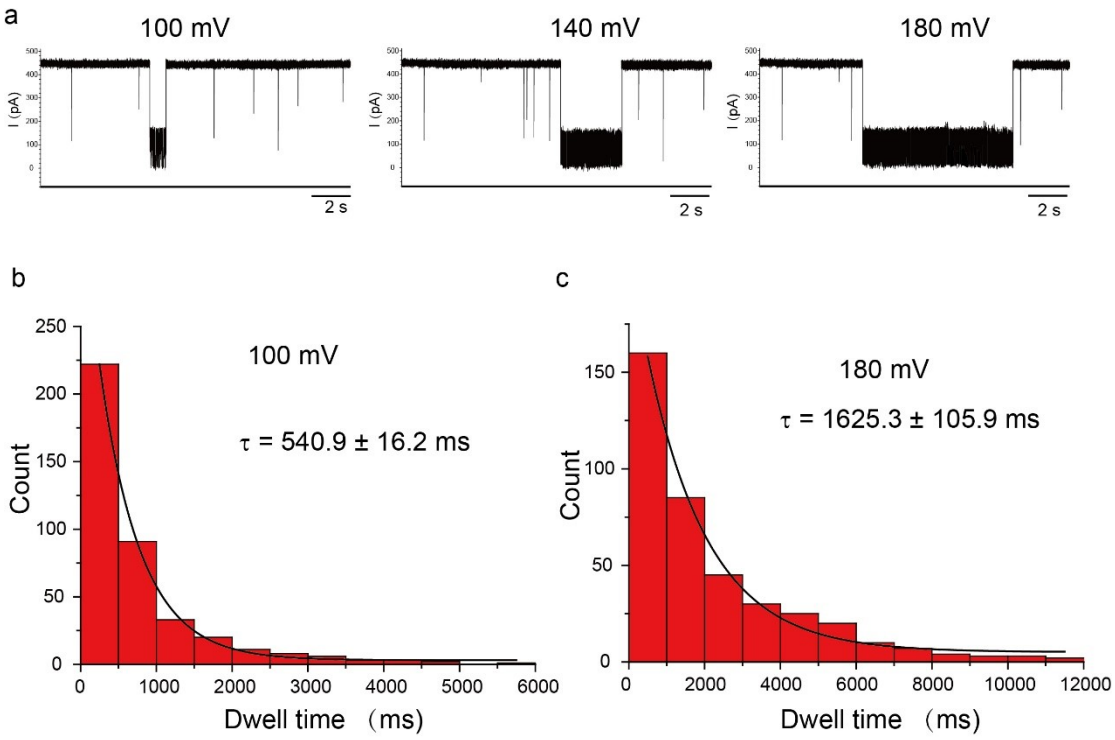


Fig. S10 Voltage experiment for DNA1-R5. (a) Typical oscillation signal patterns of 100 mV, 140 mV, 180 mV. (b) Dwell time distribution for DNA1-R5 at 100 mV. (c) Dwell time distribution for DNA1-R5 at 180 mV. All measurements were conducted in 3 M KCl, 10 mM Tris (pH 8.0). (n = 400 independent events).

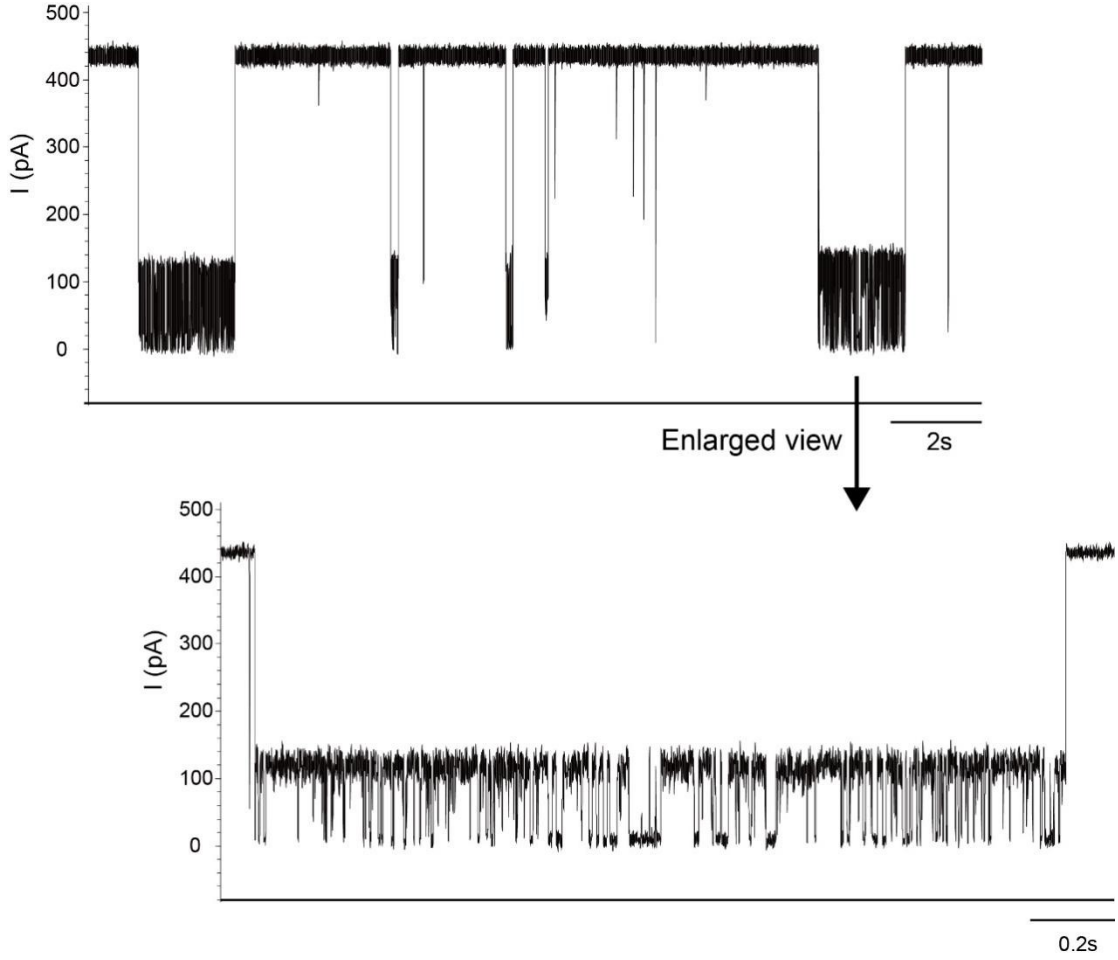


Fig. S11 Current traces of the DNA7-R5 adduct. Representative 20-s I-t trace recorded for DNA7-R5, with an enlarged view displayed below. Data were acquired in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV.

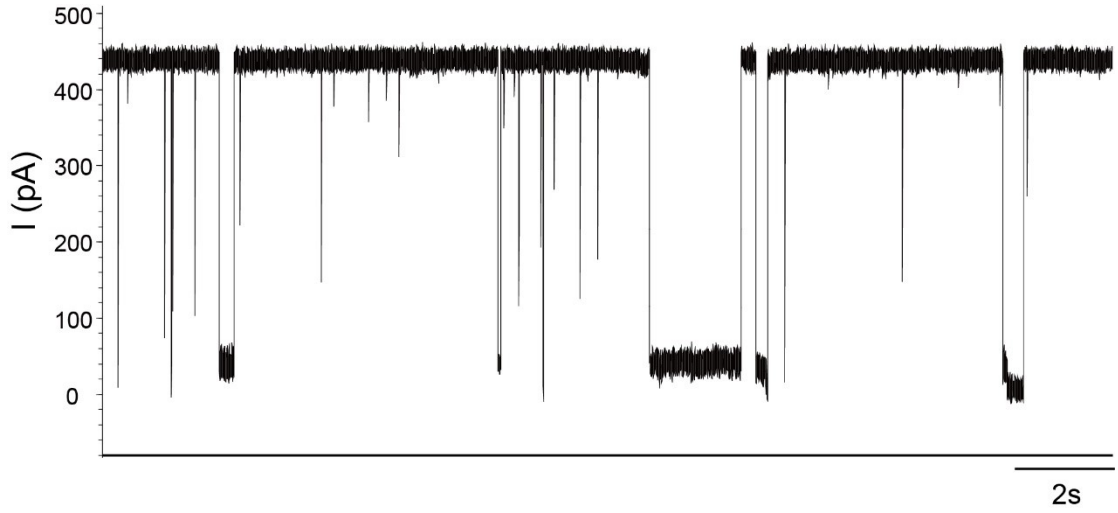


Fig. S12 Current traces of the DNA8-R5 adduct. Representative 20-s I-t trace recorded for DNA8-R5. Data were acquired in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV.

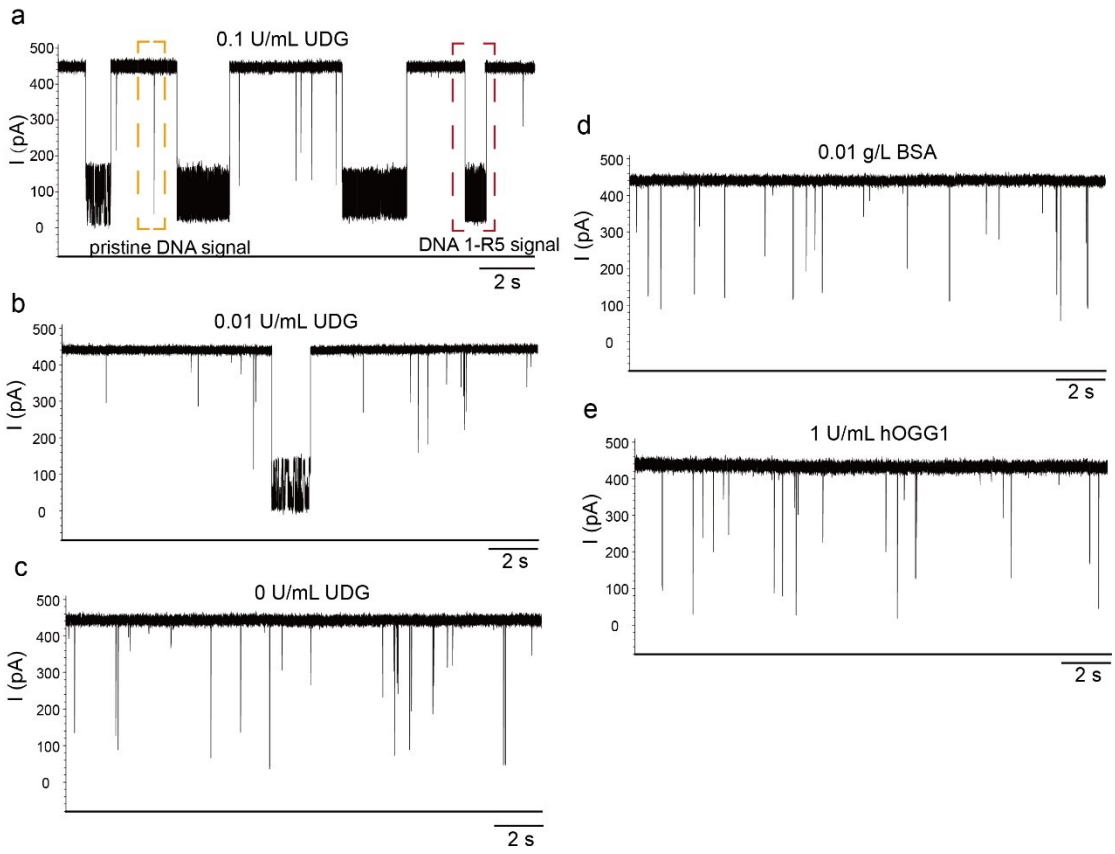


Fig. S13 Current traces of samples treated with different UDG concentrations (UDG: 0, 0.01, 0.1 U/mL) and the non-target controls. (a) Representative 20-s I-t trace for UDG (0.1 U/mL). (b) Representative 20-s I-t trace for UDG (0.01 U/mL). (c) Representative 20-s I-t trace for UDG (0 U/mL). (d) Representative 20-s I-t trace for BSA (0.01g/L). (e) Representative 20-s I-t trace for hOGG1 (1U/mL). Data were acquired in buffered solution containing 3 M KCl and 10 mM Tris (pH 8.0) under an applied potential of 140 mV. Pristine DNA signal and DNA-R5 signal were marked in the orange and red dashed rectangle.