Unzipping Kinetics of Duplex DNA Containing Oxidized Lesions in an α-Hemolysin Nanopore

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Supporting Information

Contents **Page**

Kinetic equations for the Type I model

For a first-order reaction with a rate constant *k*,

$$A \xrightarrow{k} B$$

the reaction rates for *A* and *B* are:

$$\frac{d[A]}{dt} = -k[A]$$
$$\frac{d[B]}{dt} = k[A]$$

The solutions for the above two equations are

$$[A] = [A_0]e^{-kt}$$
$$[B] = [A_0](1 - e^{-kt})$$

where $[A_0]$ is the initial concentration of *A*. Taking the derivative of [*B*] and multiplying both sides by the time increment, Δt, yields

$$\frac{\frac{d[B]}{dt} \times \Delta t}{[A_0]} = ke^{-kt}\Delta t \,. \qquad \text{(SI1)}$$

The left side of eq SI1 is equal to the percentage of *B* generated in an increment Δt at time *t*, which corresponds to the counts/total (*C/T*) in eq 1 and Figure 3 of the main text.

Kinetic equations for the Type II model

For two sequential first-order reactions with rate constants k_1 and k_2

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

the reaction rates for *A* and *B* are:

$$\frac{d[A]}{dt} = -k_1[A]$$
$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$
$$\frac{d[C]}{dt} = -k_2[B]$$

The solutions are

$$[A] = [A_0]e^{-k_1 t}$$
$$[B] = [A_0]\frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t})$$
$$[C] = [A_0](1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1})$$

where $[A_0]$ is the initial concentration of *A*. Taking the derivative of [*C*] and multiplying both sides by Δt yields

$$\frac{\frac{d[C]}{dt} \times \Delta t}{[A_0]} = \frac{k_1 k_2}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \times \Delta t \quad . \qquad \text{(SI2)}$$

The left side of eq SI2 is equal to the percentage of *C* generated in an increment Δt at time *t*, and corresponds to the counts/total (*C/T*) in eq 2 and Figure 3 of the main text.

ESI-MS Characterization of Modified DNAs

Samples for mass spectrometry analysis were synthesized as described in the text. ESI-MS analysis was conducted on the 23-mer OG, Sp, and Gh strands (5'-TTTTGGAGCTGXTGGCGTAGGTT-3', X = OG, Sp, or Gh) for ease of analysis. The 65-mers used in the nanopore studies (5'-$(T)_{23}$-TTGGAGCTGXTGGCGTAGG-$(T)_{23}$-3', X = OG, Sp, or Gh) have the same sequence context for the oxidized lesion, and were oxidized under the same conditions. After synthesis and purification, 5 nmoles of each 23-mer sample were dialyzed against ddH_2O for 2 days while changing the water 3 times. On day three, dialysis was continued against a 3 mM NH_4OAc solution for another 2 days, while changing the solution 3 times. Next, the samples were lyophilized to dryness and resuspended in 30 µL of isopropanol and 30 µL of 1 mM NH_4OAc solution. ESI-MS analysis was conducted as previously described.[1] 23-mer OG calcd mass = 7188.7, exp mass = 7188.0; 23-mer Gh calcd mass = 7178.7, exp mass = 7177.6; 23-mer Sp calcd mass = 7204.7, exp mass = 7204.0.

1. Schibel, A.E.P.; An, N.; Jin, Q.; Fleming, A.M.; Burrows, C.J.; White, H.S. *J. Am. Chem. Soc*. **2010**, *132*, 17992-17995.

Figure SI 1. (A) Schematic illustration of the experimental setup. (B) Example current-time (*i-t*) trace obtained for the G:C duplex (sequence shown below) at -120 mV (*cis* vs. *trans*).

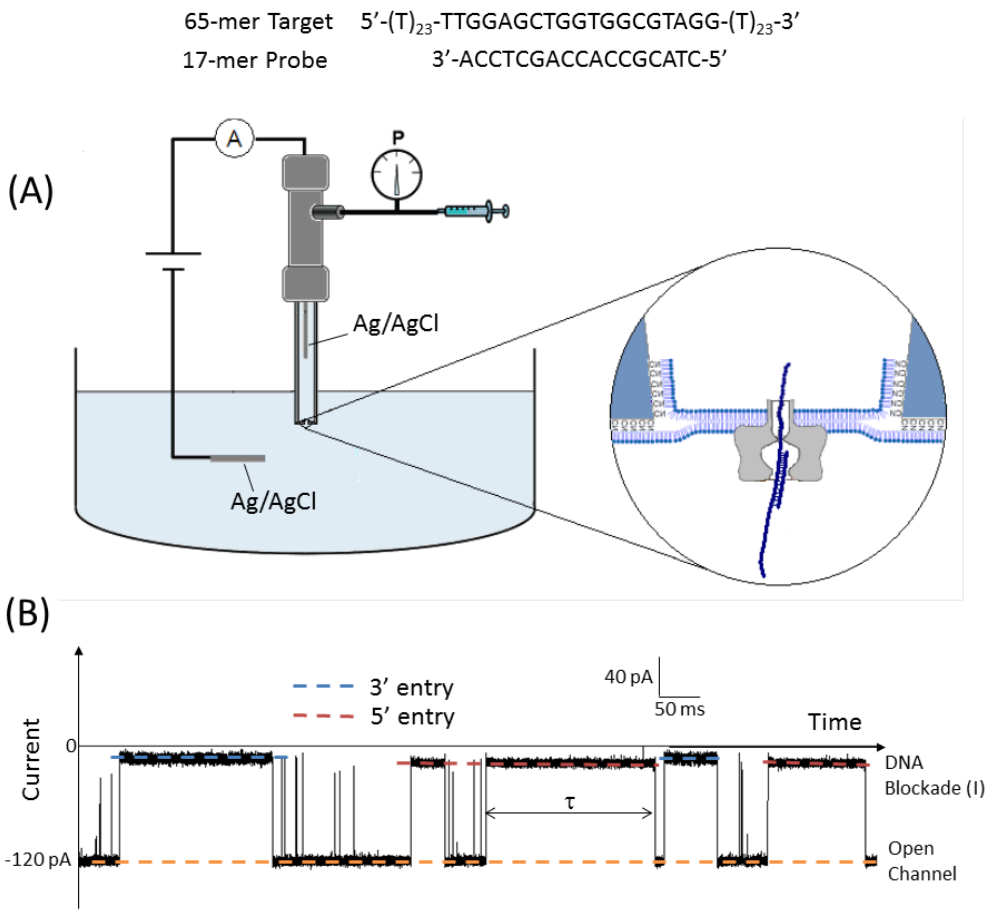


Figure SI 2. Histograms of blockage current (*I*) for the target-probe mixture with a single-sided overhang or double-sided overhangs, and event population density plots of *I* as a function of *t*. Due to the equilibrium between the complementary ssDNA and the duplex, solutions examined contain both ssDNAs and the duplex. In order to shift the equilibrium towards the duplex, a 5-fold excess of the short probe stands versus target strands was added to the solution. At -120 mV, the excess ssDNA probe strands generated a translocation population at times shorter than 0.5 ms, while the duplex underwent an unzipping process that was typically two or three orders of magnitude longer.

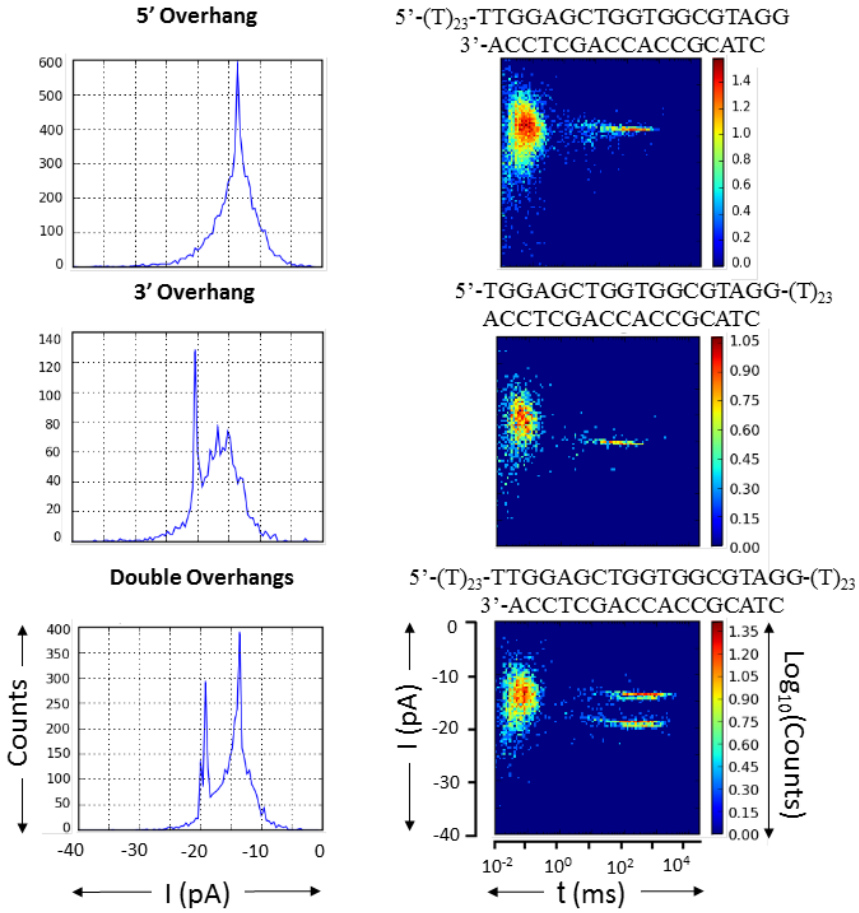
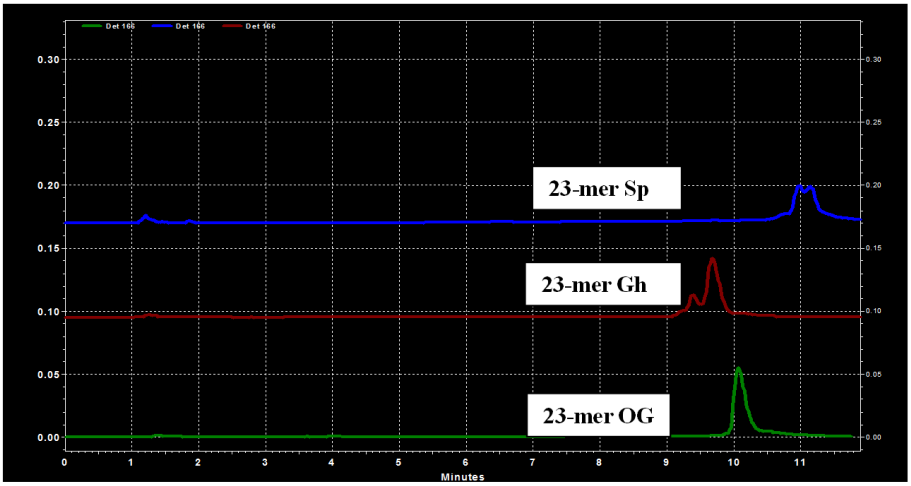


Figure SI 3. (A) HPLC traces for the 23-mer used in the T_m analysis and ESI-MS characterization. (B) HPLC traces for the 65-mer used in the nanopore measurements. Note that the lesions Sp and Gh are generated as a ~1:1 mixture of diastereomers that were not separated before use in these experiments.

A



B

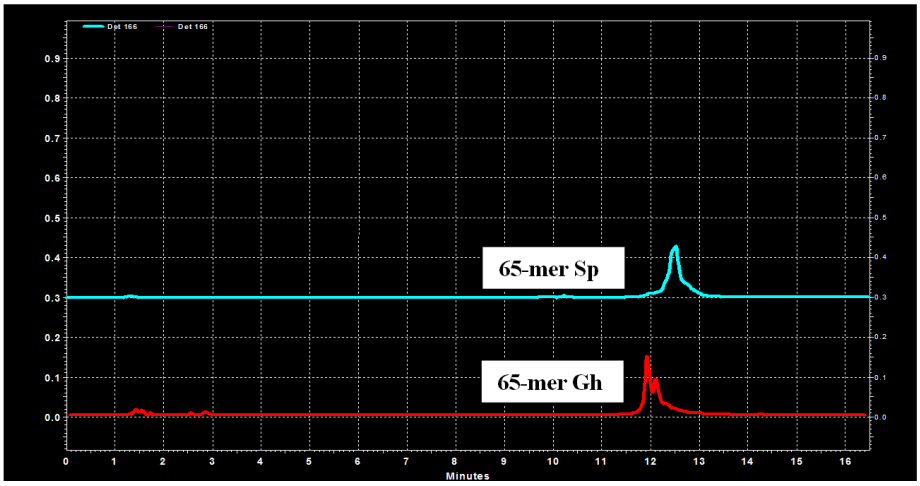


Figure SI 4. Histograms of unzipping time (*t*) for 3' and 5' entry at -140 mV for Gh:C duplexes formed with (top) the 17-mer probe; (middle) the 13-mer 5'-truncated probe; and (bottom) the 13-mer 3'-truncated probe. The red curves show the fits using the Type II model, eq SI 2. k_1 and k_2 values obtained from the fits are listed in Table 2 of the main text. Blockades that lasted longer than 0.5 ms were analyzed as DNA unzipping events and are plotted below, while the shorter blockades (< 0.5 ms) were identified as translocation events of unbound strands.