## **Supplementary Material**

Single molecule measurements of DNA helicase activity with magnetic tweezers and T-testbased step-finding analysis

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## Figure S1



Supplementary Figure 1. The effects of window size, *n*, on T-test based step finding with simulated stepping traces. **A.** T-test step finding was performed on a simulated stepping trace (black solid line) with three different window sizes (n = 10, 20, and 30 points) and  $\alpha = 0.005$  (99% confidence for two tail T-test). The step trace corresponds to a stochastic simulation trace (200 points per second) as described in the main text, without added noise. In the absence of noise, the step fitting performs better with a smaller window size and displays less distortion of the fitted steps. **B.** Comparison between the step trace and the fits from T-test step finding. The errors in the fit, represented by  $\chi^2$ , increase with increasing *n*.



Supplementary Figure 2. The effects of window size, *n* on T-test based step finding with simulated noisy stepping traces. **A.** T-test step finding was performed on simulated stepping traces (200 points per second) with Gaussian noise ( $\sigma = 5$  bp) (black solid line) with three different window sizes (n = 10, 20, and 30 points) and  $\alpha = 0.005$  (99% confidence for two tail T-test). Due to the noise in the stepping trace, more T-step values for fitting with n = 10 are within the acceptance level of the null hypothesis test, i.e., no significant difference between two means from two windows, resulting in missed steps. **B.** Comparison between the step trace and the fits from T-test-based step finding. **C**. The error in the fit,

represented by the  $\chi^2$  value, is minimum at n = 20 indicating the optimal window size for this particular set of step-size (5 bp), step-rate (10 s<sup>-1</sup>), and noise (5 bp).