Supplementary Materials
Branch Migration Enzyme as a Brownian Ratchet

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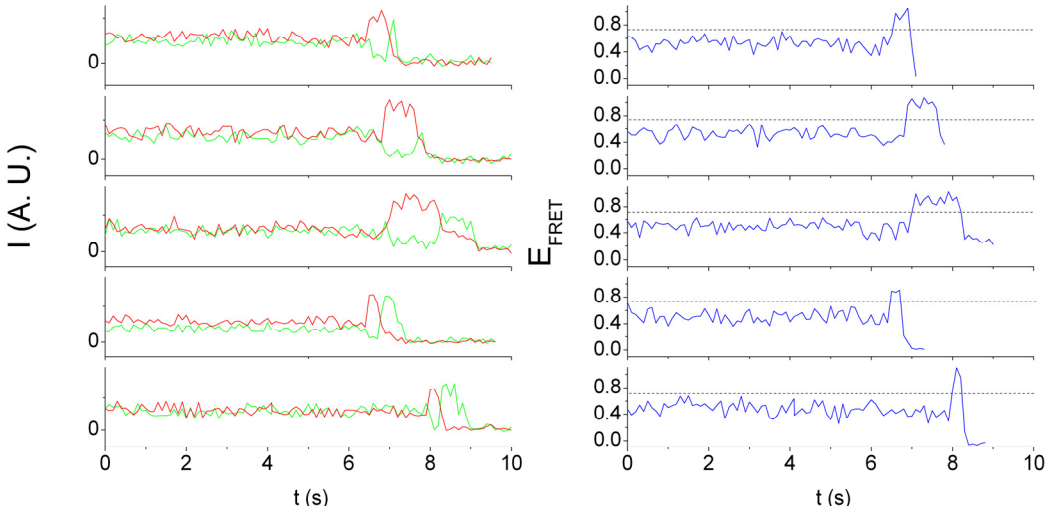

Figure 1. Representative time traces for individual Holliday junctions undergoing helicase catalyzed branch migration (see also main text Figure 1E). On the left: time traces for the intensity of the donor (Cy3-green) and acceptor (Cy5-red), on the right: the corresponding FRET efficiency time traces (blue). The dotted line on the righ curves shows the threshold value chosen to separate the course of the reaction in three different regions. (Main text figure 2C)

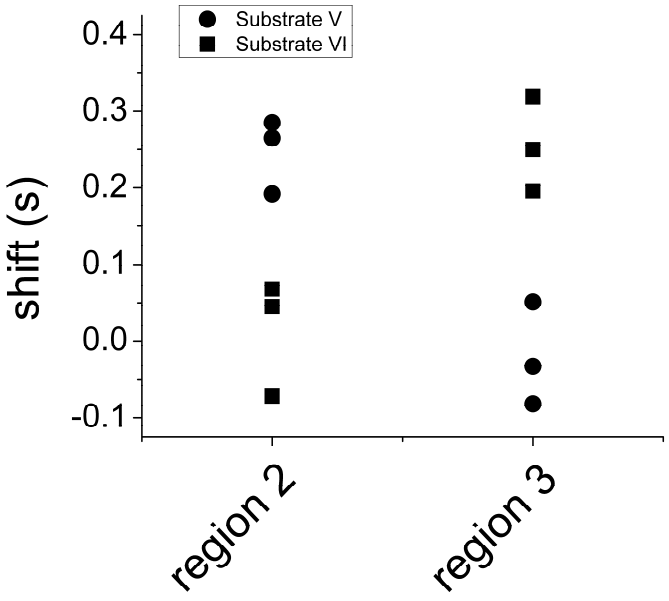

Figure 2. Determination of the shifts in the mean value of the time distributions (Figure 2C) for regions 2 and 3 for substrates V and VI (see Figure 3 for notation). Shifts were measured with respect to the mean values of the corresponding time distributions for substrate I. Points indicate shifts for a set of three independent experiments for each substrate. The shifts indicate that the presence of heterologies introduce delays that can be measured beyond the detection limit imposed by instrumentation and analyses.

Branch Migration Steps and FRET efficiency.

Determination of FRET efficiency values versus number of branch migration steps (Figure 5B) was performed by first determining the site to site distances for a Holliday junction in the open conformation (using a script developed in the VMD package (Humphrey et al., 1996)). The open conformation of the Holliday junction was found in a crystal structure of E. Coli RuvA-Holliday junction complex (Ariyoshi et al., 2000). We tested values of R_0 in the range 55-65 Å without any significant change in the qualitative dependence of FRET efficiency versus – number of branch migration steps. Because the Holliday junction found in the open conformation in the complex with RuvA show slight distortions the FRET efficiency curve is not completely symmetric with respect to branch migration steps at each side of the maximum FRET efficiency.

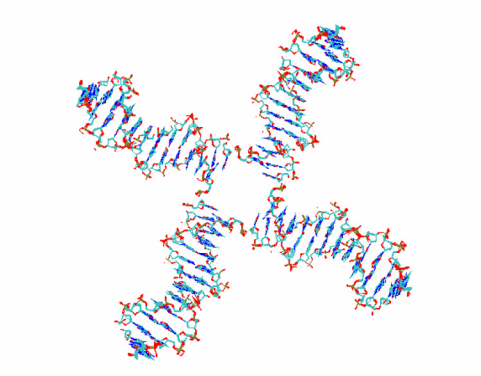

Figure 3 – Open conformation of the Holliday junction, in complex with RuvA (not shown). Image obtained using VMD (Humphrey et al., 1996).

Ariyoshi, M., Nishino, T., Iwasaki, H., Shinagawa, H. and Morikawa, K. (2000) Crystal structure of the holliday junction DNA in complex with a single RuvA tetramer. *Proc Natl Acad Sci U S A*, **97**, 8257-8262.

Humphrey, W., Dalke, A. and Schulten, K. (1996) VMD: visual molecular dynamics. *J Mol Graph*, **14**, 33-38, 27-38.