SUPPLEMENTARY INFORMATION

DNA substrates for testing helicase activity

The DNA hairpin used in the single molecule experiments was synthesized as explained elsewhere [1]. It consists of a 1239 bp hairpin with a 4 nt loop, a 76 nt 5'-biotinylated ssDNA tail and a 146 bp 3'-digoxigenin labeled dsDNA tail (Fig.1 (a)). This DNA construct (named FH) was used to carry out experiments with the E. Coli RecQ. For the T4 gp41 experiments, a half-hairpin (HH) substrate was created with a complementary 50-mer oligonucleotide used to reduce the length of the hairpin and increase the length of the 5' ssDNA tail (Fig.1 (b)). Initially, the 50-mer oligonucleotide was introduced into the chamber at a high concentration, 1μM. Next, a high force ($\sim$16 pN) was applied to denaturate the hairpin allowing the contact between the oligonucleotide and the complementary sequence in the hairpin. When the force was relaxed, the hairpin partially reannealed up to the position where the oligonucleotide was bound. This protocol generated a DNA substrate with $\sim$600 bp hairpin, and long 5' and 3' ssDNA tails of $\sim$600 nucleotides. The HH substrate was used in order to optimize the gp41 helicase loading conditions [1].

The mechanical stability of the hairpin was characterized by measuring the extension of the substrate as a function of the pulling force along a force-cycle in which the force is first increased and then relaxed. The force-extension curve for the FH substrate in T4 buffer is shown in Fig. 1 (c). As the force was increased, the hairpin remained annealed at a constant extension until the force reached $\sim$16 pN and the extension abruptly increased due to the mechanical unfolding of the hairpin. As the force was decreased below 15 pN, the hairpin reannealed.

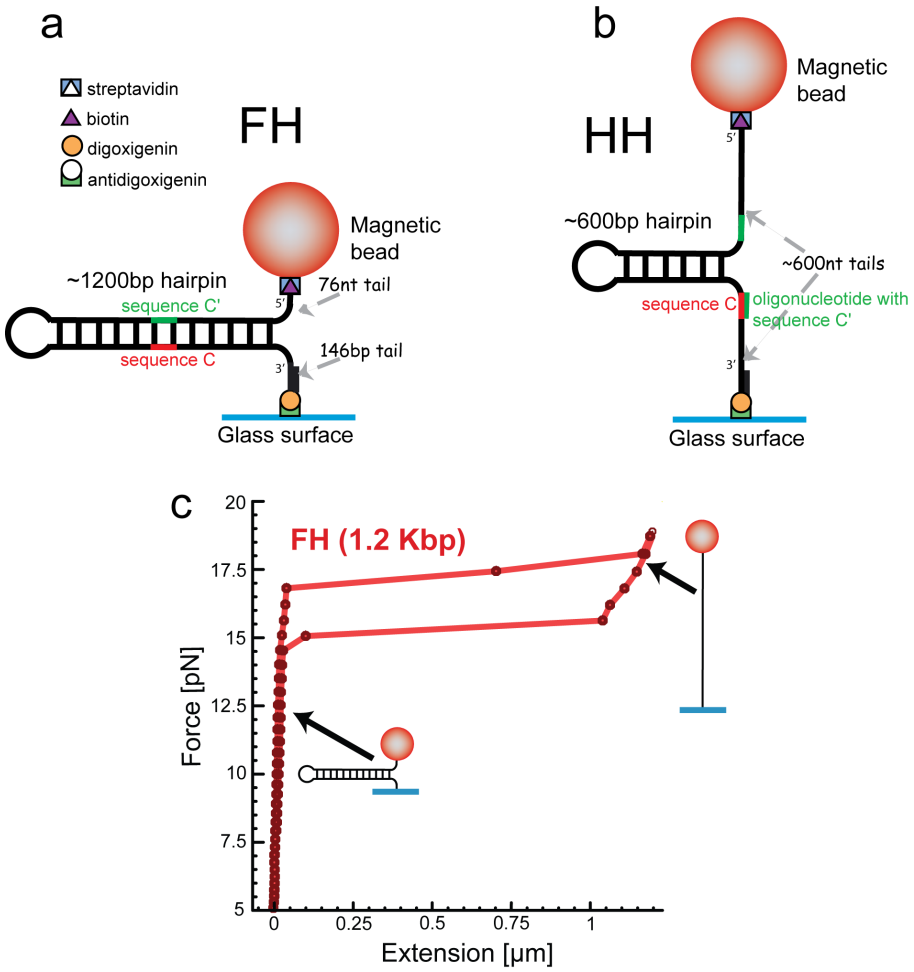

Figure 1: (a) Schematic representation of the DNA hairpin substrate (FH) used in RecQ experiments consisting of a ∼1.2 Kbp hairpin with short 5'-biotinylated ssDNA tail and 3'-digoxigenin labeled dsDNA tail. (b) Schematic representation of the half-hairpin substrate (HH) used in gp41 experiments and created with a complementary 50-mer oligonucleotide (shown in green). (c) Force extension curve of the HH construct in the T4 buffer and 29 C.

Montecarlo simulations of the BJ's model

Betterton and Julicher (BJ) proposed a framework to describe NA unwinding by helicases [2]. The model describes the helicase motion coupled to the thermally activated opening and closing of NA base-pairs (see Fig. 2 (a)). The dynamics of the helicase/NA fork is governed by the master equation for the probability P_j that the helicase is j bases away from the NA fork ($j > 0$) [2]:

$$\frac{dP_j}{dt} = -(\alpha_j + \beta_j + k_j^+ + k_j^-)P_j + k_{j-n}^- P_{j-n} + \alpha_{j-1}P_{j-1} + k_{j+n}^+ P_{j+n} + \beta_{j+1}P_{j+1}. \qquad (1)$$

where k^+ (k^-) and β (α) are respectively the ssNA translocation forward (backward) rate and the NA base-pair opening (closing) rate, and n is the helicase step size. We performed Montecarlo simulations of such model using the rates given in Eqs. 2 and 3 in the main text with a one-step helicase/NA fork interaction potential (Fig. 2 (b)) characterized by an amplitude $G_{\rm int}$ and a range m. Figure 2 (c) shows the results from the simulations for the ratio between the unwinding and translocation rates, $V_{\rm un}/V_{\rm trans}$, as a function of the unzipping force (upper panels) and the NA stability (lower panels) when changing the parameters of the model. In all simulations, the base-pair attempt frequency (Eq. 2 main text) was chosen to be $k_0 = 10^6 {\rm s}^{-1}$ and the helicase forward translocation rate k^+ was fixed to 300bp/s. The parameter g, that defines how the base-pair kinetics is affected by the interaction potential (Eq. 2 and 3 in the main text), was chosen to be $g = 0$.

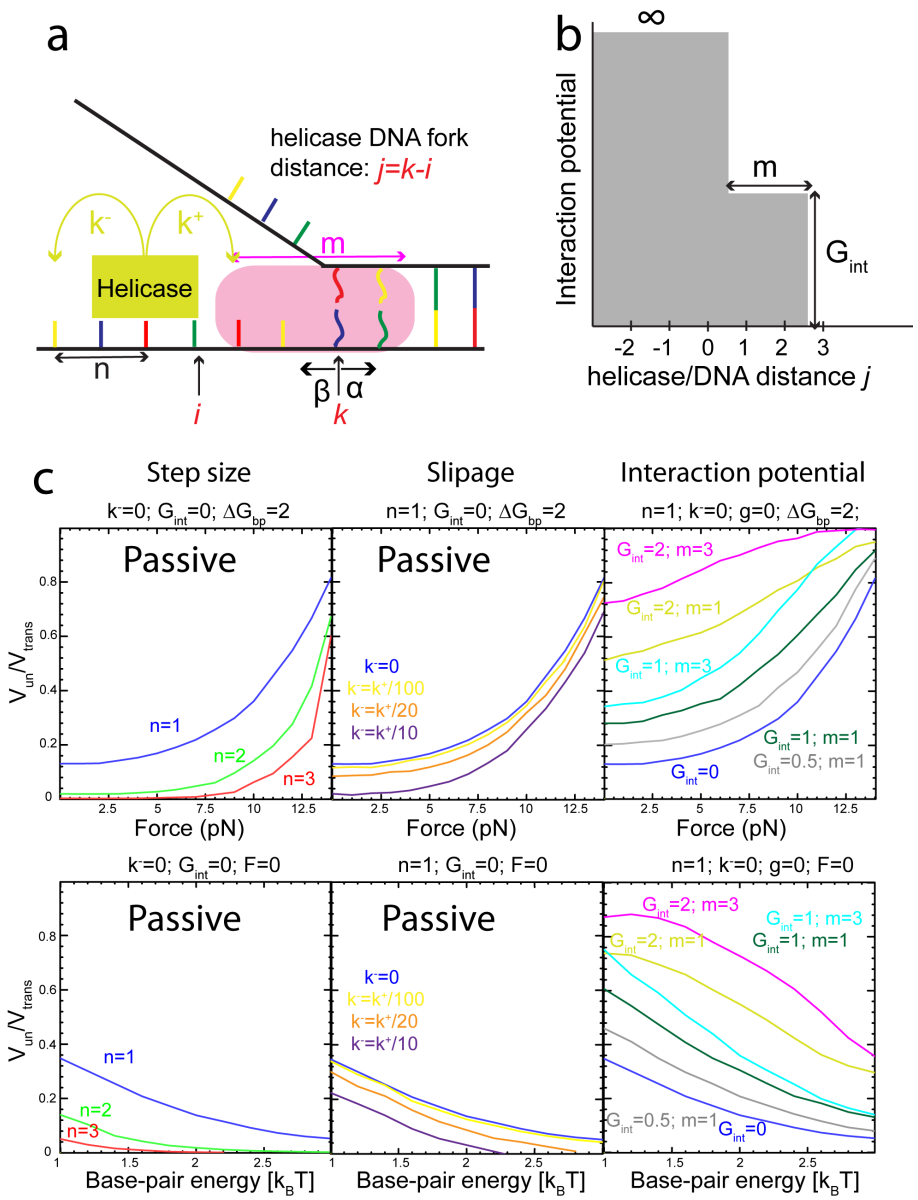

Figure 2: (a): Schematic figure showing the different parameters characterizing the BJ model: the ssDNA translocation forward k^+ and backward k^- rates; the helicase step size n; the base-pair opening β and closing α rates; and the range of the helicase/DNA fork interaction m. The position of the helicase and the DNA fork along the DNA lattice is represented by the indexes i and k respectively. The distance between both is given by the index $j = k - i$. (b) Schematic representation of the interaction potential used in the simulations. For $j < 1$ the interaction potential tends to infinity, meaning that the when the helicase is just at the DNA fork inhibits DNA closing. For $j > 0$ we considered a one step potential of amplitude G_{int} along a range of m base-pairs. (c) Ratio between the unwinding and translocation rates,$V_{\rm un}/V_{\rm trans}$, obtained from the Monte Carlo simulation of the BJ model as a function of the unzipping force (upper panels) and the DNA stability (lower panels) when changing the helicase step size n (left panels), the slippage trough k^- (central panels), and the helicase/DNA interaction potential throughout G_{int} and m (right panels). Note that left and central panels correspond to the case of a passive helicase.

Measurements of the ssDNA elasticity

A 20-mer oligonucleotide complementary to the loop region was used in order to prevent hairpin formation and measure the elasticity of the ssDNA. Initially, the 20-mer oligonucleotide was introduced to the chamber at high concentration (1μM) and the hairpin was mechanically denaturated by applying a ∼16 pN force, allowing the 20-mer oligonucleotide to hybridize in the loop region (Fig. 3 (a)). The hairpin conformation was thermodynamically stable below ∼15-16 pN; however, the kinetic barrier to hairpin reannealing generated by the presence of the oligonucleotide in the loop region was very large (several k_BT) above ∼2pN. Consequently the process of hairpin reformation was kinetically blocked, allowing us to measure the force-extension curve of the ssDNA above ∼2pN (Fig. 3 (b)). The base-pair free energy reduction due to the external force, ΔG_f, was then computed as the area under the ssDNA elasticity curve, $x_{\mathrm{ssDNA}}(f)$ (Fig. 3 (b) inset).

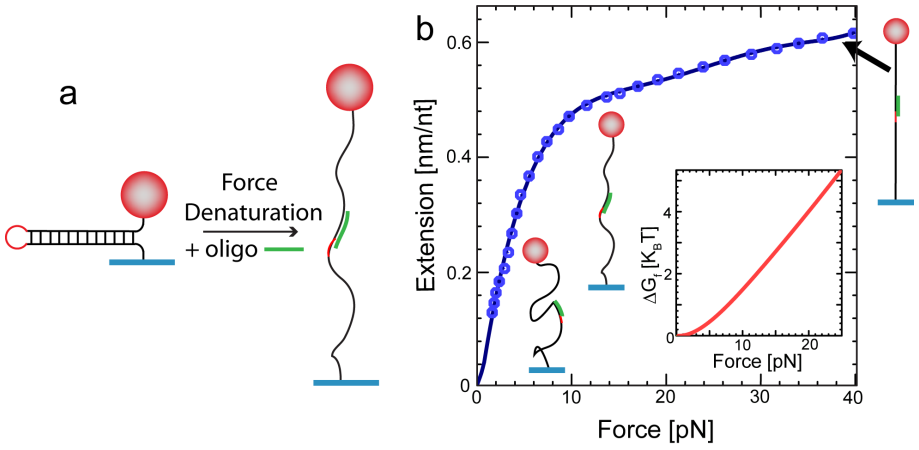

Figure 3: (a): Schematic representation of the experimental protocol to obtain the ssDNA substrate from the DNA hairpin. (b) Molecular extension in nm per nucleotide of ssDNA as a function of the force applied, $x_{\mathrm{ssDNA}}(f)$, measured in the T4 buffer and 29C. The experimental measured values are shown in light blue circles, whereas the continuous blue line corresponds to a polynomial fit to the data (6th order). The inset shows the free energy ΔG_f computed as the area under the extension curve $x_{\mathrm{ssDNA}}(f)$, $\Delta G_f = \int 2x_{\mathrm{ssDNA}}(f)df$.

Measurements of translocation rate

In order to measure the translocation rate, V_{trans}, of helicases two different approaches can be used. In the first one, the hairpin reannealing in the wake of the helicase after the enzyme has passed the apex is used to report the helicase translocation activity (left panels Fig4). On the second one, the translocation rate is deduced from an experiment in which the force is transiently increased (to a value F ∼17pN) to unfold the hairpin during an unwinding event (central panels Fig.4). For both helicases, the translocation rates estimated from the two different protocols described here are consistent (right panels), showing that the force does not affect the translocation activities of gp41 and RecQ. We have therefore considered a translocation rate independent on the force.

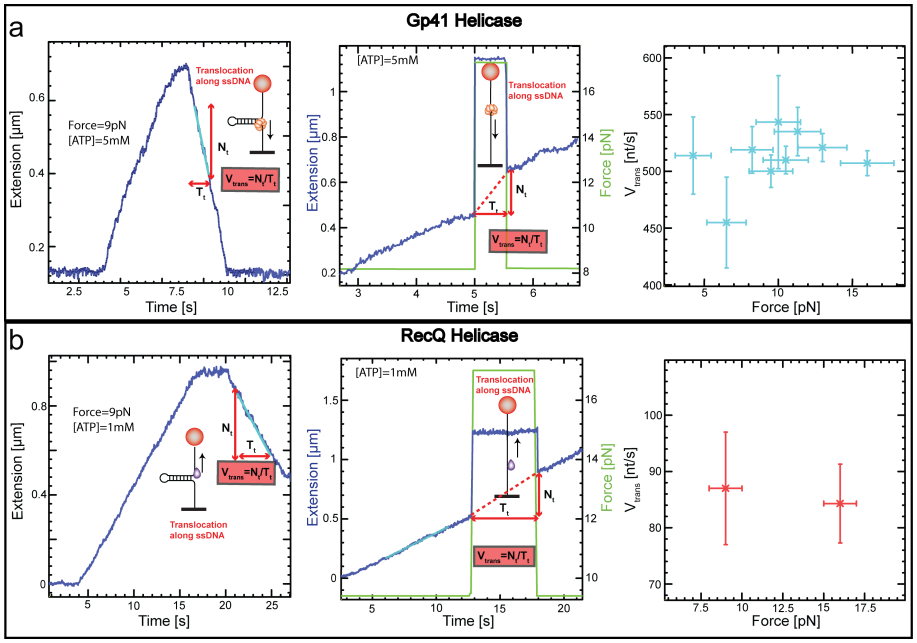

Figure 4: Left panels: Experimental traces corresponding to the gp41 helicase activity in the HH hairpin (a) and to the RecQ helicase activity in a FH hairpin (b). The traces show the unwinding phase (rising edge) and the rezipping phase (falling edge) along which the enzyme translocates on ssDNA while the hairpin reanneals in its wake. Central panels: Experimental traces showing gp41 (a) and RecQ (b) helicase activity along the FH hairpin in a experiment in which the force is transiently increased during unwinding at 17pN to measure the translocation activity on ssDNA. Molecular extension is shown in blue and the applied force in green. Right panels: Measured translocated rate at different forces for gp41 (a) and RecQ (b) obtained from the two different protocols shown in left and central panels.

References

[1] Manosas, M., Spiering, M.M, Zhuang, Z., Benkovic, S.J., and Croquette, V. (2009) Coupling DNA unwinding activity with primer synthesis in the bacteriophage T4 primosome *Nat. Chem. Biol.*, **5**, 904912.

[2] Betterton, M.D. and Julicher,F. (2005) Opening of nucleic-acid double strands by helicases: Active versus passive opening. *Phys. Rev. E*, **71**, 11904-11.