Supporting Materials for

Direct Measurements of Torque During Holliday Junction Migration

Scott Forth, Christopher Deufel, Smita S. Patel, Michelle D. Wang

Contents:

Supporting Methods
- Single molecule Holliday junction construct with full homology
- Single molecule Holliday junction construct with single base heterology
- Migration of Holliday junction with an angular optical trap

Supporting Discussion
- Derivation of the torque-force relation of a Holliday junction
- Estimation of the single base heterology energy barrier
- Discussion about migration in the presence of magnesium

Supporting Figures
- Figure S1. Cartoon illustrating the Holliday junction constructs
- Figure S2. Demonstration of Holliday junction migration
- Figure S3. Holliday junction migration in the presence of magnesium

Supporting References

Supporting Methods

Single molecule Holliday junction construct with full homology

The single molecule Holliday junction DNA template was constructed using the following steps.

First, two 1.1 kbp palindromic arms were generated via PCR from plasmid pCD1, a modified version of plasmid pRL574 (Supporting Fig. S1). Both arms were amplified from the same sequence with identical lower primers; the upper primer sequences were identical, but in one case the upper primer contained six biotinylated "T" bases and in the other, six digoxygenin "T" bases. The products were then restriction digested with BsaI to generate four bp overhangs with sequence 5'-TTAA.

The preformed Holliday junction with twenty base-pairs of sequence heterology and hairpins at the ends was generated by annealing Hol4 with Hol3-hp and Hol2 with Hol1-hp in annealing buffer (10 mM Tris pH 8.0, 50 mM NaCl, 1 mM EDTA). The single stranded DNA oligos were mixed at equimolar ratios (10 μM final concentration), heated to 94ºC, and slowly cooled to 22ºC over a one hour period.

Sequences of preformed arm segment oligos:

Hol1-hp
5'-
TGGTGAAGGAAACTTCACCATTTGATTACGAGATATCGATGCATGCGAATTCGAGCTCC
Hol2
5'-AATTGGAGCTCGAATTCGCATGCATCGATATAATACTTGAGG
Hol3-hp
5'-
GGATCGAAGGAAACTTCGATCCCCTCAAGTATTATATCGATGCATGCGAATTCGAGCTCC
Hol4
5'-AATTGGAGCTCGAATTCGCATGCATCGATATCTCGTAATCAAA

Next, the Hol3hp-4 (Hol1hp-2) products were ligated to the biotin (dig) long palindromic segments at the four bp overhang end for 2 hours at 16ºC.

Finally, the Bio-34 and Dig-12 were mixed and ligated for 2 hours at 16ºC, resulting in the construct shown in Supporting Fig. S1 (left).

Single molecule Holliday junction construct with single base heterology

The preparation of the construct with a single base heterology was identical to the fully homologous sequence, except for a single base alteration (highlighted in red in Supporting Fig. S1, right) in the preformed junction arms. The following oligo sequences were used to generate this structure:

Sequences of preformed arm segment oligos for the single base heterology construct:

Hol1-hp-1bp het
5'-
TGGTGAAGGAAACTTCACCATTTGATTACGAGATATCGATGCATGCGAATTCGAGCT
C**T**
Hol2-1bp het
5'-AATT**A**GAGCTCGAATTCGCATGCATCGATATAATACTTGAGG
Hol3-hp
5'-
GGATCGAAGGAAACTTCGATCCCCTCAAGTATTATATCGATGCATGCGAATTCGAG
CTCC
Hol4
5'-AATTGGAGCTCGAATTCGCATGCATCGATATCTCGTAATCAAA

The bases in bold represent the alteration made to the oligos to create the mis-pairing upon winding.

The annealing and subsequent ligation steps were identical to those detailed above; the resulting construct is shown in Supporting Fig. S1 (right).

Migration of a Holliday junction with an angular optical trap

The angular optical trap has been described previously (*S2, S3*). In brief, the DNA construct was rotationally constrained at one end to a microscope coverslip and at the other end to a nanofabricated quartz cylinder (with a diameter of ~600 nm and height of ~1 μm). The cylinder was trapped in all six lateral and angular dimensions by a linearly polarized optical trap. Twist was added to the molecule by rotation of the cylinder via rotation of the trapping polarization.

To demonstrate that the trap was capable of mechanically migrating the junction over a wide range along the DNA sequence, a tether was held at a constant force (2 pN) and first overwound to form positively supercoiled plectonemic DNA. Negative turns, in the sense of DNA unwinding, were then added over an extensive range (up to −100 turns) to demonstrate that the junction was capable of smoothly migrating over many hundreds of bases (Supporting Fig. S2). In addition, the slope of the extension change during migration per turn added was measured and consistent with the expected extension versus force relation (Supporting Fig. S2, inset). These results were found to be in good agreement with a previous report (*S4*).

In all experiments described in the main text, twenty turns were added to the molecule at a rate of 4 turns/s, first in the sense of DNA unwinding, then immediately in the opposite direction to return the migrated junction back to its original position. Force was held constant via a computer controlled servo loop which modulated the position of the coverslip mounted on a piezoelectric stage. Data were taken at 2 kHz and anti-alias filtered at 1 kHz. The extension data were filtered using a sliding window filter of width 25 ms, while the torque data were filtered with a sliding window of width 2 seconds. All experiments were performed in Phosphate Buffered Saline (pH 7.4), with $[Na^{+}]$ = 150 mM. A Holliday junction is known to adopt an "X-stacked" structure in the presence of Mg^{++} ions, preventing junction migration. To eliminate this stacking effect and encourage the active migration of the junction, no Mg^{++} was used in the experimental buffer. A fuller discussion and examples of migration data taken in the presence of Mg^{++} are presented below.

Supporting Discussion

Derivation of the torque-force relation of a Holliday junction

Consider a DNA molecule containing a Holliday junction with a fully homologous sequence which is held under a fixed tension *F* and a fixed angular orientation of one end relative to the other end θ, with a branch point located at base *n* from one end. The free energy for such a system can be written as a sum of a term related to DNA linear elasticity and a term related to DNA torsional elasticity:

$$\Delta G(n;F,\theta) = -2n\int_0^F z_{\mathrm{bp}}(F')dF' + 2n\int_0^{\frac{\theta}{2n}-\frac{2\pi}{10.4}} \tau(\varphi_{\mathrm{bp}})d\varphi_{\mathrm{bp}}$$

where $\tau = \alpha_{\mathrm{bp}}\varphi_{\mathrm{bp}} = \alpha_{\mathrm{bp}}\left(\frac{\theta}{2n} - \frac{2\pi}{10.4}\right)$. α_{bp} is the torsional stiffness for each basepair of dsDNA and φ_{bp} is the angular deviation from the rest angle for each basepair of the dsDNA.

To determine *n* at thermodynamic equilibrium, the free energy is minimized with respect to the branch location *n*:

$$\frac{\partial}{\partial n}\Delta G(n;F,\theta) = 0$$

$$-\int_0^F z_{\mathrm{bp}}(F')dF' + \frac{1}{2}\alpha_{\mathrm{bp}}\left(\frac{\theta}{2n} - \frac{2\pi}{10.4}\right)^2 - \alpha_{\mathrm{bp}}\left(\frac{\theta}{2n} - \frac{2\pi}{10.4}\right)\frac{\theta}{2n} = 0$$

from which the torque τ can be found to be

$$\tau = -\frac{2\pi}{10.4}\alpha_{\mathrm{bp}} + \frac{2\pi}{10.4}\alpha_{\mathrm{bp}}\sqrt{1 - \frac{2\alpha_{\mathrm{bp}}\int_0^F z_{\mathrm{bp}}(F')dF'}{\left(\frac{2\pi}{10.4}\alpha_{\mathrm{bp}}\right)^2}}$$

Over the range of forces used in these experiments, the second term under the square root is <<1. Hence, the torque required to migrate the junction may be simplified as:

$$\tau = -\frac{\alpha_{bp}\int_0^F z_{bp}(F')dF'}{\frac{2\pi}{10.4}\alpha_{bp}} = -\frac{10.4}{2\pi}\int_0^F z_{bp}(F')dF'.$$

Interestingly, a similar torque-force relation was derived based on phase transition theory by Marko (*S5*).

Estimation of the single base heterology energy barrier

We employed a simple implementation of the dynamic torque spectroscopy method (DTS), which is analogous to the dynamic force spectroscopy method (DFS), outlined in (*S7*). The basic idea behind both DTS and DFS is that a bond will disrupt given sufficient time, though the rupture can be greatly facilitated by the application of an external torque (or force), tilting the energy landscape and lowering the activation barrier. By applying the torque at a linear rate (that is, $\frac{d\tau}{dt} = \text{constant}$), the rupture torque, τ^*, should increase linearly with the natural log of the loading rate. This relationship is expressed by:

$$\tau^* = \frac{k_B T}{\alpha}\left[\ln\left(\frac{d\tau}{dt}\right) - \ln\left(\frac{k_B T k_D(\tau_{eq})}{\alpha}\right)\right]$$

where α is the distance (in radians) between the state prior to disruption and the activation barrier peak, $k_B T$ is the thermal energy, and $k_D(\tau_{eq})$ is the rate constant for heterology disruption. Thus, by winding the Holliday junction through the heterologous base and measuring the mean torque at which the base will rupture and smooth migration continues, this relationship can be extracted.

Figure 2c shows the results of winding at 1, 2, and 4 turns/s (or, 5.2, 10.4, and 20.8 bp/s, respectively). After converting the junction point migration rate to an applied torque rate, a linear fit yields a slope and intercept, from which the two parameters, α and $k_D(\tau_{eq})$ can be extracted. From the fit to the data, we found $\alpha = 1.5(\pm 0.1) rad$ and $k_D(\tau_{eq}) = 0.31(\pm 0.06) s^{-1}$.

To extract the activation barrier height E_b relative to that of the homologous sequence, we use the well-known relation:

$$k_D(\tau_{eq}) = k_0 e^{-\frac{E_b}{k_B T}}$$

The prefactor, k_0, we take to be between 10^2-10^3 s^{-1}, as it is known that the stepping time for spontaneous branch migration in conditions similar to our own (150 mM NaCl, 23º C) were found to be in the 1-10 ms range, as measured previously in an elegant series of experiments (*S8)*.

With the measured parameters and considering the possible range available for values of k_0, we determine $E_b = 7(\pm 1) k_B T$. This value is in good agreement with the notion that the primary source for the activation barrier is the torsionally driven base-pairing disruption of the G-C and A-T bonds, which are known to have a ΔG of ~3.5 and ~2 $k_B T$ respectively.

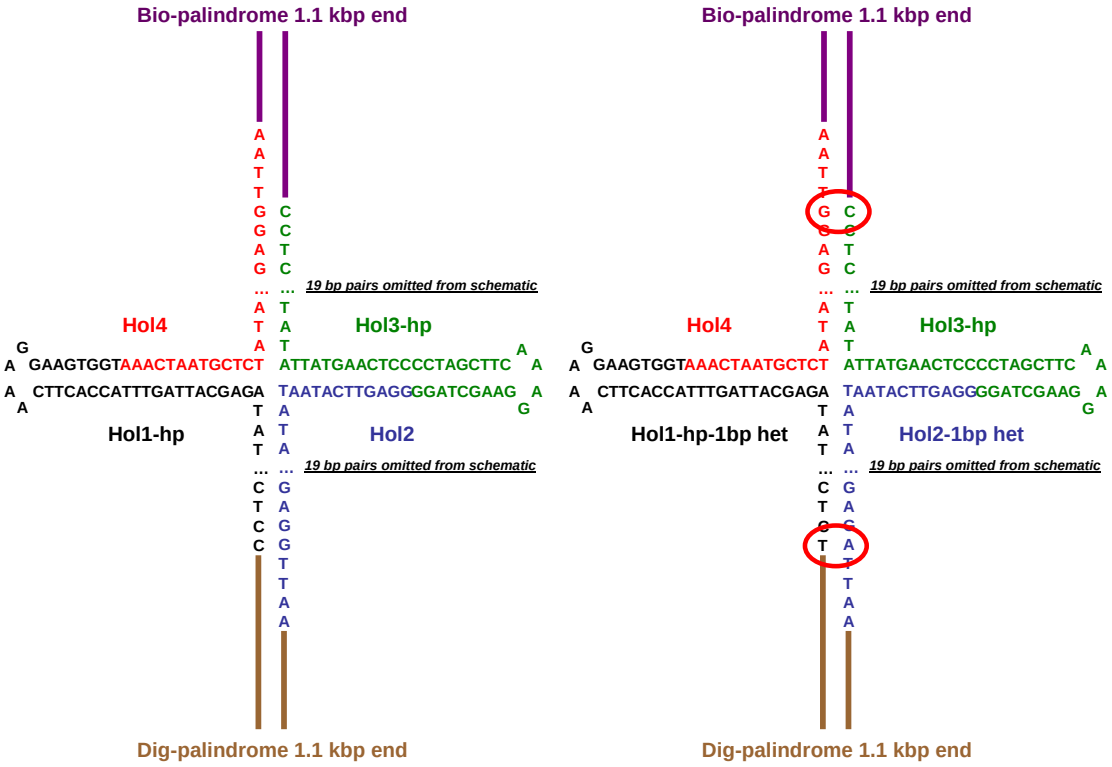
Discussion about migration in the presence of magnesium

Holliday junction migration in vivo must occur in the presence of divalent ions, such as magnesium (Mg^{++}), which is required for ATP-dependent enzymatic activity. The kinetics of Holliday junction folding has been well studied in the presence of Mg^{++}, and it has been shown that the dominant effect is to promote the stacking of the four junction arms into an "X-stacked" structure (*S9*). This structure is not free to migrate, and can therefore be considered off the major migration pathway. Migration will only proceed once the X-stacked structure has unfolded and the open configuration is recovered (*S10*).

To determine how these effects behaved with our experimental assay, a fully homologous sequence was held at a constant force of 2 pN, and twenty turns in the negative direction (same sense as DNA unwinding) were rapidly added over the course of one second. The rotation was then stopped, and the junction was allowed to reach its new equilibrium extension. In the absence of Mg^{++}, the junction was, within our instrument resolution, always in equilibrium; that is, the extension always corresponding with an equilibrium prediction for the location of the junction at a given turn number (Figure S3 – black curve).

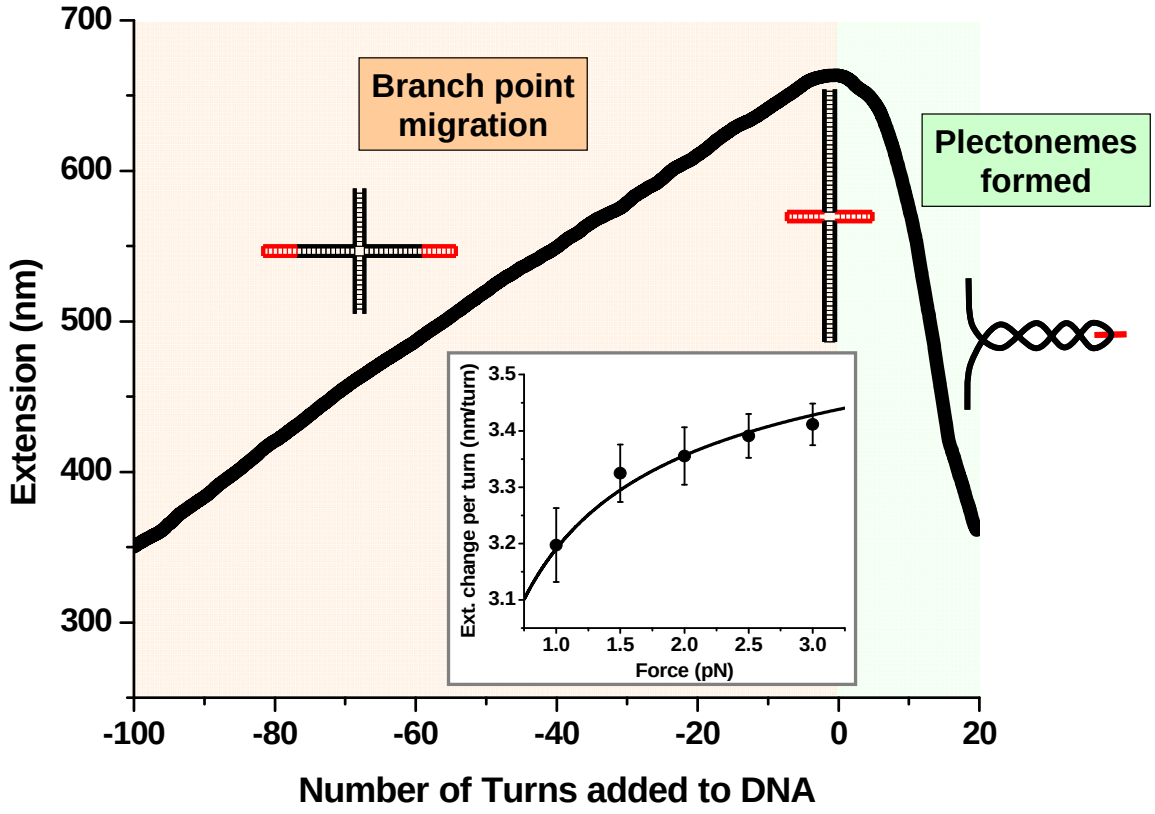
Upon the addition of small amounts (0.1 and 0.2 mM) of $MgCl_2$, the junction became frequently stuck upon winding, indicating the presence of off-pathway "X-stacked" structures, which occurred stochastically and with variable pause durations. Representative traces are shown in red and blue in Figure S3. We determined that the effect of Mg^{++} was to reduce the time spent in the main migration pathway and the junction became stuck at random locations. Thus, interpretation of both the extension and torque data as they relate to the main migration pathway of study would be overly complicated. As such, the focus of the main text on migration through homologous and heterologous sequences required that Mg^{++} be excluded from the experimental buffers.

In vivo, the "open" junction, which is fully free to migrate, is maintained by the binding of protein complexes, such as the tetrameric RuvA complex in *E.Coli* (*S11*). Though motor complexes (such as the *E.Coli* junction-migrating enzyme RuvB) require ATP and therefore magnesium to function, the off-pathway stacking conformations are quenched by the binding of proteins such as RuvA. Thus, the direct torque measurements during migration presented in this work correspond directly to this physiologically relevant condition, despite being made in a buffer without Mg^{++}.



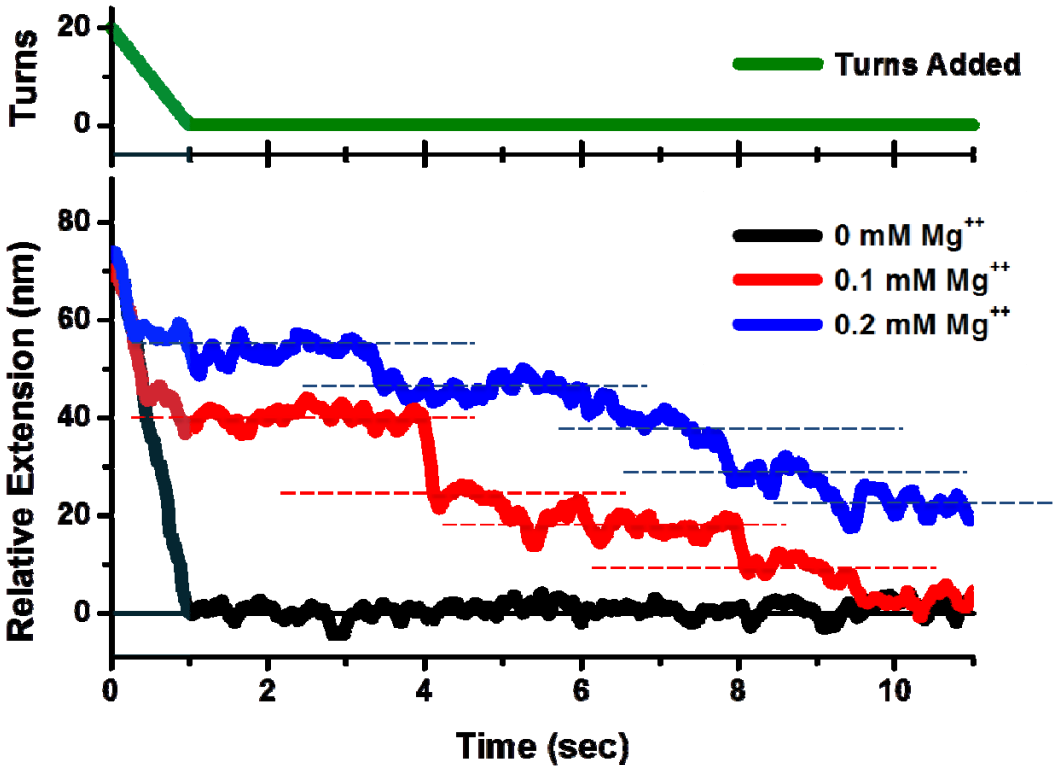
Supporting Figure S1. Cartoon illustrating the single molecule Holliday junction constructs: Fully homologous on left, Single Base Heterology on right).

DNA molecules each containing a Holliday junction were created as described in the Materials and Methods section. The preformed arms (labeled Hol1-hp, Hol2, Hol3-hp, and Hol4 for the fully homologous construct (shown left) and Hol1-hp-1bp het, Hol2-1bp het, Hol3-hp, and Hol4 for the single base heterologous sequence (shown right)) contained regions of sequence heterology at the extremity, which prevented junction migration into the central twenty basepairs of the junction in the arms. This ensured that a Holliday junction in a DNA tether did not collapse prior to measurements and also allowed for easy identification of the end of the migratable region. Each end of the DNA tether was tagged with multiple linkages, either six biotin tags or six digoxygenin tags as shown. Multiple contact points with the cylinder and coverslip were required to maintain torsional constraint during the experiment. The construct is depicted above with the junction at the maximum migratable base. Applying negative twist (in the sense of DNA unwinding) would serve to extrude the arms and shorten the vertical trunk. Up to 1.1 kbp of DNA may be migrated as shown.



Supporting Figure S2. Demonstration of Holliday junction migration.

A DNA molecule was held at a constant force of 2 pN and overwound, creating positively supercoiled DNA which, after ~8 turns, buckled and formed a plectoneme (data not shown). The end of the molecule was then rotated in the negative sense up to −100 turns. Below 0 turns, a smooth and continuous migration of the junction was observed over many hundreds of base pairs, as evidenced by a nearly constant decrease in extension. This experiment was repeated at different forces. The inset shows the extension change per turn versus force (black points), which is in excellent agreement with a prediction (black line, DNA pitch = 3.65 nm) from the force-extension relation of dsDNA (*S6*). This result shows that there was a direct conversion of rotational motion into an extension change, indicating migration of the junction. These observations are in good agreement with similar experiments done using a magnetic tweezers instrument (*S4*).



Supporting Figure S3. Holliday junction migration in the presence of Mg^{++}.

A fully homologous DNA construct was held at 2pN. At time = 0 seconds, twenty turns in the negative direction (promoting shortening of the molecule) were applied at a rate of 20 Hz. At time = 1 seconds, the rotation was stopped and the junction was allowed to continue migration to its equilibrium position (plotted here as "0 nm" Relative Extension). In the absence of Mg^{++}, the junction was migrated smoothly and reached equilibrium immediately within the resolution of our technique. Upon the addition of Mg^{++}, the migration of the junction back to its equilibrium position after the rapid addition of torque shows frequent pauses, presumably due to formation of X-stacked structures in the junction arms, which inhibit smooth migration. The pause locations and durations were not reproducible, as expected, but the total time required for the junction to reach its equilibrium increased with increasing Mg^{++} concentration. It should be noted that such off-pathway migration inhibiting effects were seen at Mg^{++} concentrations as small as 0.1 mM, far below typical physiological levels. As a result of this data, experiments to determine the effect of torque on the main migration pathway were conducted in the absence of Mg^{++} for clarity of data taking and presentation. Dashed lines indicate regions of lengthier pausing.

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