Figure S1. A description of how YOYO intensity spikes were found along the DNA backbone. In Step 1, a T-test was used to search the intensity profile in 3 pixel bins. If the intensity in the previous 3 pixel bin was similar to the subsequent 3 pixel bin, then they were grouped together and the next 3 pixel bin tested. If they were different, the two binned regions were kept separate. After the T-test grouped the raw intensity profile into regions of similar intensity, the raw intensity values were averaged within each grouped region, generating a plot as shown in step 2. This dampened the noise of the raw data substantially allowing a hierarchical clustering algorithm to search for significant variations in the binned average intensity values. A plot in step 3 was generated flagging the position of the event in the molecule.
Figure S2. (a) A simple example for how the relative intensity was calculated. The area under the intensity profile for the significant YOYO fluctuation was calculated and compared to a region in the molecule of similar size that was deemed normal. (b) How the relative position was calculated. The location of the YOYO event was measured from the leading edge of the molecule and divided by the total length of the molecule.
Figure S3. The process for aligning molecules with YOYO intensity events to the reference. After the YOYO region was identified in the molecule, the distance between the flanking labels was measured in pixels and converted to basepairs. The molecule was then split into two pseudo-molecules, cutting out the suspect region. Each pseudo-molecule was then aligned to the reference separately and alignments with a p-value less than or equal to $10^{-4}$ were kept for further analysis. After the two pseudo-molecules were aligned with a p-value threshold less than or equal to $10^{-4}$, the distance between the flanking labels was measured on the reference and compared to what was the experimental distance. The difference between these values corresponds to the size of the knot/backfold.