

Supplementary information for:

Efficient modification of λ -DNA substrates for single-molecule studies

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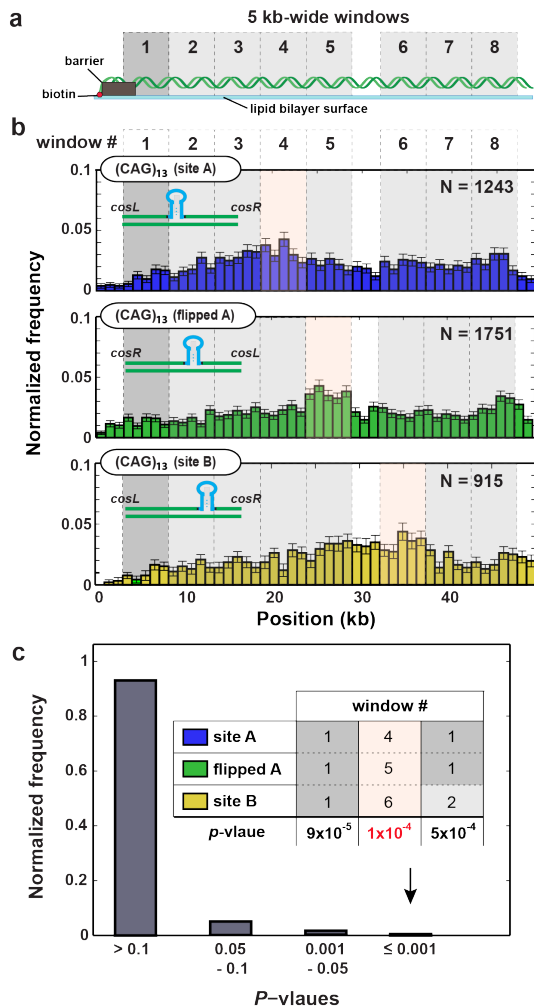


Figure S1. yRFC has a mild preference for loading yPCNA at (CAG)₁₃ repeats relative to homoduplex dsDNA. (a) To determine whether the mild yPCNA-yRFC(ATP γ S) enrichment at the (CAG)₁₃ was statistically significant, the DNA substrate was divided into eight 5 kb-wide windows (dashed lines). The first window (dark gray box) is partially obstructed by the Cr barriers. Windows containing the (CAG)₁₃ repeat are shown in pink. (b) Normalized binding distribution of yPCNA-yRFC(ATP γ S) complexes on three DNA substrates containing a (CAG)₁₃. The binding histograms were also divided into 5 kb-wide windows (dashed lines). The window partially obscured by the Cr barrier is marked in dark gray, homoduplex DNA is gray, and the target-containing window is pink. A two-tailed t-test was used to compare the groupings of three windows—including the three (CAG)₁₃-containing window—against all homoduplex DNA windows. (c) A normalized histogram of all two-tailed t-tests comparing the mean yPCNA occupancy of all three-window combinations relative to the mean yPCNA occupancy in all windows containing homoduplex DNA. Over 97% of the tests showed no significance (*p*-value > 0.05). The highest *p*-values are shown in the inset and include the (CAG)₁₃-containing sites, as well as the partially obstructed first window. This is because the Cr barrier causes the first window to underestimate yPCNA binding. All *p*-values in the *p*=0.001-0.05 range were from window groupings that included two of three (CAG)₁₃ repeat windows or window #1.

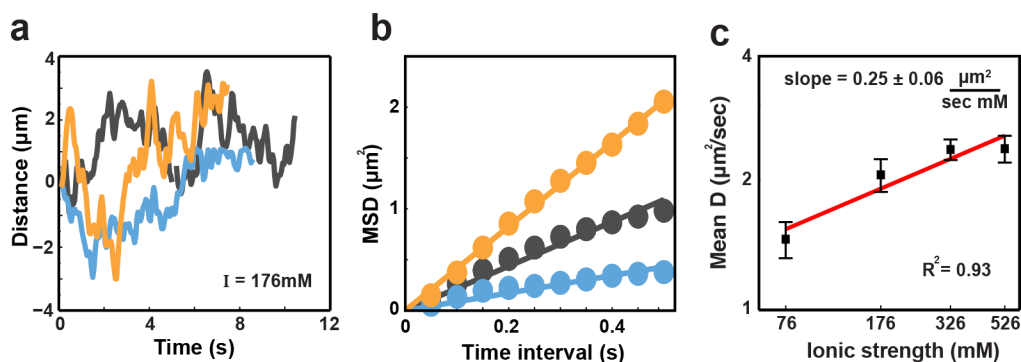


Figure S2. Characterization of yPCNA diffusion on homoduplex λ -DNA. (a) Representative single-molecule traces of the position of individual yPCNA molecules (shown in three different colors) on double-tethered DNA curtains at a total ionic strength $I = 176$ mM. (b) Mean squared displacement (MSD) of each of the molecules in (a). The MSDs are fit to a line, and the slopes are used to calculate the one-dimensional diffusion coefficients (solid lines). (c) Mean yPCNA diffusion coefficients as a function of the ionic strengths (error bars: S.E.M; to $N = 30, 29, 29, 31$ for 76 mM, 176 mM, 326 mM, and 525 mM ionic strengths, respectively). The red line indicates a linear fit through the data with a slope of $0.25 \pm 0.06 \mu\text{m}^2 (\text{sec mM})^{-1}$. The error in the slope represents the standard error of the fit. These results are consistent with a single-molecule study that looked at hPCNA diffusion on homoduplex DNA¹. Diffusion of both hPCNA and yPCNA was weakly dependent on the ionic strength ($0.33 \pm 0.04 \mu\text{m}^2 \text{sec}^{-1} \text{mM}^{-1}$ and $0.25 \pm 0.06 \mu\text{m}^2 \text{sec}^{-1} \text{mM}^{-1}$ for hPCNA and yPCNA, respectively).

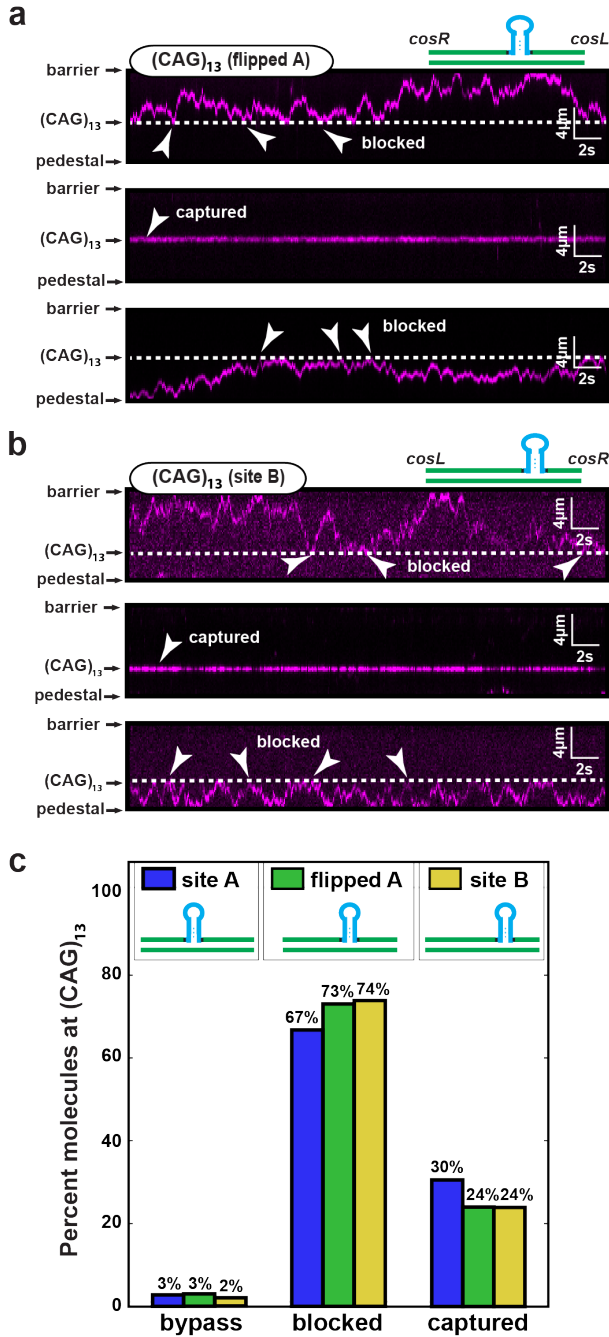


Figure S3. yPCNA diffusion on DNA substrates containing (CAG)₁₃ repeats. (a) Representative kymographs of diffusing yPCNA on DNA having (CAG)₁₃ at flipped site A or at (b) site B. Dashed lines indicate the position of a (CAG)₁₃ repeat. Arrows: yPCNA is blocked by the (CAG)₁₃ structure. (c) Percentage of molecules showing either bypass, blocked, or captured behavior at (CAG)₁₃ inserts. At least 35 DNA molecules were analyzed and classified into each of three categories (N=36, 62, and 46 for site A (from Fig. 5d), flipped site A, and site B). A chi-squared test shows that the distribution of yPCNA behaviors at each of the three (CAG)₁₃-containing DNAs is statistically indistinguishable (p-value = 0.95).

Supplemental DNA Sequences

Complete DNA sequences for each of the six plasmids in Figure 1 are also available via Benchling: <https://benchling.com/ifinkelstein/>

Legend:

bold: homology for λ -DNA

orange: LacO

blue: Kanamycin resistance cassette

green: three BspQI sites

restriction sites: NcoI is double-underlined and NotI site is single-underlined.

Recombineering cassette for site A

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CGGGAATGATCCAGATTTTGGCTACCACCATGACTAACCGCCTTGGCGGTAAACAACCGAAGAATGCGACAC
TGACGGCGCTGGCAGGGCTTTCCACGGCGAAAAATAAATTACCGTATTTTGGCGAAAATGATGCCGCCAGC
CTGACTGAACTGACTCAGGTTGGCAGGGATATTCTGGCAAAAAATTCCGTTGCAGATGTTCTTGAATACCT
TGGGGCCGGTGAGAATTCGGCCTTTCCGGCAGGTGCGGAGCATTGCTACGGCGATTCTAGAAATTGTGAGC
GCTCACAATTCTAGTTGGAGAGCCACTGTTTCATTTAAATAAAGCTCTTCATGCATGCGGCCGCTCTTCCCA
TGGTGCATCGCTCTTCCGGGATTTAAATAGGTACCTATGGACAGCAAGCGAACCAGGAAATGCCAGCTGGGG
CGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGGCCCAAGGATCTGATGG
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CGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGCT
CTGATGCCGCCGCTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGT
GCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCTTCCTTGGCAGC
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CGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAGTTCGCCAGGC
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AGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACG
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GTTTTGAGTCTGCTGTTGATATTTCTAAAGTCGGTTTTTTTTTTCTTCGTTTTCTCTAACTATTTTCCATGAA
ATACATTTTTGATTATTATTTGAATCAATTCGAATACCTGAAGTCTTTTCATCTATAATTGGCATTGTATG
TATTGGTTTTATTGGAGTAGATGCTTGGCTTTTCTGAGCCATAGCTCTGATATCCAATGAAGCCATAGGCAT
TTGTTATTTTGGCTCTGTGAGCTGCATA
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Recombineering cassette for site B

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TTTAGTCTGGATAGCCATAAGTGTGGATCCATTCTTTGGGACTCCTGGCTGATTAAGTATGTCGATAAGG
CGTTTTCCATCCGTCACGTAATTTACGGGTGATTCTGTTCAAGTAAAGATTGGAAGGGCAGCCAGCAACAGG
CCACCCTGCAATGGCATAATTGCATGGTGTGCTCCTTATTTTATACATAACGAAAAACGCCTCGAGTAGTGCA
GCCCTAGAAATTGTGAGCGCTCACAATTCTAGACTCGATGACGCGGCCGCTATGGACAGCAAGCGAACCAGG
ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGGCCG
CAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGAA
CAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACA
GACAATCGGCTGCTCTGATGCCGCCGCTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGA
CCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGG
GTTCTTGGCGAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCC
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ACTCGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGA
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CCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCT
TCTGAGCTAGCA**AATTAATGTGCATCGATTATCAGCTATTGCCAGCGCCAGATATAAGCGATTTAAGCTAAG**
AAAACGCATTAAGATGCAAAACGATAAAGTGCATCAGTAATTCAAAACCTTACAGAAGAGCAATCTATGG
TTTTGTGCGCAGCCCTTAATGAAGGCAGGAAGTATGTGGTTACATCAAAACAATTCACATACATTAGTG

Recombineering cassette for site C

ACATCAAAGCAGTCTGTCTCAGTCTGCGTGAAGCCACCACCGCCTCCGGCGTGGATAATGCAGCCTCCCC
CGACTGGCAGACACCGCTGAACGGGATTATTTACCCTCAGAGAGAGGCTGATCACTATGCAAAAAACAAT
GGAAGGAACCCAGAAGTATATTAATGAGCAGTGCAGATAGAGTTGCCATATCGATGGCTCGAGTAGTGCA
GCC**CTAGAATTGTGAGCGCTCACAATTC**TAGACTCGATGACGCGGCCGCTATGGACAGCAAGCGAACC
ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCGC
CAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTTCGCATG**ATTGAA**
CAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACA
GACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTGAGCGCAGGGGGCGCCCGTTCTTTTTGTCAAGA
CCGACCTGTCCGGTGCCTGAATGAAGTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGC
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ACTGTTCCGAGGCTCAAGGCGCGCATGCCGACGCGGAGGATCTCGTCGTGACCCATGGCGATGCCTGCT
TGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGAC
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CCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCT
TCTGAGCTAGC**CTCTGGAAGCATT**CAGAGCAATTGAGGCAGCGTTGGTGAAGCACGATAATAATATGAAGG
ATTATTCCCTGGTGGTTGACTGATCACCATAACTGCTAATCATTCAAACCTATTAGTCTGTGACAGAGCCA
ACACGCAGTCTGTCACTGTGAGGAAAGTGGTAAAACGCAACTCAATTACTGCAATGCCCTCGTAATTAA

Nickase cassette

ATTTAAATAAAG**CTCTT**CATGCATGCGGCC**GCTCTTCC**ATGGTGCATCG**CTCTT**CGGGATTAAATA

