Supplementary Information

Processive DNA Unwinding by RecBCD Helicase in the Absence of Canonical Motor Translocation

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Figure S1. Chemical structures of single stranded DNA with reversed polarity linkages in the phosphate backbone. A 5'-5' linkage (left) and a 3'-3' linkage (right).





Figure S2. Biotin Streptavidin at the duplex DNA ends blocks DNA unwinding by RecBCD.

A. Control experiment testing ability of RecBCD to unwind dsDNA with streptavidin blocks on both ends. Stopped-flow experiments were performed by pre-incubating RecBCD (15 nM) with DNA XVI (20 nM) and mixing with ATP (5 mM) and heparin (8 mg/mL) (blue) or no ATP (gray) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously.
B. Experiments were conducted as in panel A, with DNA Series V (L=5 bp).
C. Experiments were conducted as in panel A, with DNA Series V (L=20 bp).
D. Experiments were conducted as in panel A, with DNA Series V (L=40 bp).
E. Experiments were conducted as in panel A, with DNA Series VI (L=80 bp) Cy3 time

courses (green) and Cy5 time courses (red) are shown.

F. Experiments were conducted as in panel A, with DNA XXV at 200 μ M, 40 μ M, 20 μ M, and no ATP.



Figure S3. RecBCD unwinding of normal DNA.

A. Stopped-flow time experiments were performed by mixing pre-incubated RecBCD (15 nM) and DNA Series VIII (20 nM) with ATP (5 mM) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously. Cy3 fluorescence time course are shown for L=20 bp (blue), L=40 bp (red), and L=60 bp (green).

B. Lag times for RecBCD unwinding of normal DNA (VIII) increase linearly with duplex length, L, (5mM ATP).



Figure S4. RecBCD remains at RP linkages in the absence of a heparin trap.

A. Experiments were conducted as in Fig. 1B, with RecBCD (18.75 nM) pre-incubated with DNA XX (25 nM) and XXI (25 nM).

B. Experiments were conducted as in panel **A**, except with no heparin trap.



Figure S5 (A-F)



Figure S5 (G)

Figure S5. ssDNA translocation and DNA unwinding properties of RecB^{Δ Arm}CD and RecB^{Δ Nuc}CD.

A. Stopped-flow time experiments were performed by mixing pre-incubated RecBCD (15 nM) (pink) or RecB^{Δ Arm}CD (blue) (15 nM) and DNA Series VIII (L=20 bp) (20 nM) with ATP (5 mM) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously.

B. RecB^{Δ Arm}CD binds with high affinity (stoichiometrically) to DNA ends possessing 3'dT6/5'-dT6 tails (DNA XXII). Increase in Cy3 fluorescence was monitored upon titration of Cy3-labeled DNA (20 nM, 40 nM DNA ends) with RecB^{Δ Arm}CD in Buffer M (30 mM NaCl), 25°C. Cy3 fluorescence was excited at 515 nm, emissions were collected at 570 nm.

C. Stopped-flow time experiments monitoring 5' to 3' ssDNA translocation of RecB^{Δ Arm}CD. RecB^{Δ Arm}CD (18.75 nM) pre-incubated with DNA series XXIII (25 nM) (with (dT_L) extensions) in Buffer M (250 mM NaCl) in a 1:10 volumetric ratio with ATP (various concentrations) and heparin (8 mg/mL) in buffer M (8 mM NaCl) yielding the final concentrations listed above and in the figure, and a final NaCl concentration of 30 mM at 25°C. Cy3 fluorescence time courses are shown for L=15 nt (blue), L=35 nt (pink), L=51 nt (orange), and L=75 nt (green).

D. Lag times from the experiments in panel **C** increase linearly with L yielding a rate of 2058±42 nt/sec for 5'-3' translocation of RecB^{Δ Arm}CD along ssDNA at (5 mM ATP) RecB^{Δ Arm}CD.

E. RecB^{Δ Arm}CD shows little evidence for secondary translocase activity. Stopped-flow experiments monitoring Cy3 fluorescence performed by pre-incubating RecBCD or

RecB^{Δ Arm}CD (18.75 nM) with DNA IV (25 nM) in Buffer M (250 mM NaCl) in a 1:10 volumetric ratio with ATP (5 mM) and heparin (8 mg/mL) in buffer M (8 mM NaCl) yielding the final concentrations listed above and a final NaCl concentration of 30 mM at 25°C. RecBCD (blue), RecB^{Δ Arm}CD (red), or RecBCD in the absence of ATP (green). **F.** ATP dependence of rates of normal DNA unwinding by RecBCD (blue) and RecB^{Δ Nuc}CD (orange). Experiments were performed as described in Fig. 5C, at the indicated ATP concentrations.

G. RecB^{1080A}CD and RecBC, but not RecB^{ΔArm}CD or RecB^{ΔNuc}CD show DNA strand separation of RP DNA in a standard DNA helicase assay. DNA XXIV (25 nM), helicase (20 nM), and ATP (1 mM) were mixed in Buffer M (30 mM NaCl) at 22°C, and the reaction was stopped with EDTA after 3 minutes. Bound protein was then removed by adding SDS (2.5%). Each reaction was loaded onto a non-denaturing 10% PAGE TBE gel. Control reactions lacked ATP (third lane) or ATP and enzyme (first and second lane). DNA in the second lanes were heated at 95 degrees for 3 minutes and then cooled before loading. The fourth lane contained DNA, ATP, and a variant of the RecBCD helicase as indicated.

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Strand I-1	3'- THT THI GGI ACC GAG GAC TCG ATC GAC GTC THI
Strand I-2	S'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TT-3'
Strand II-1	3'- TIT TIT GGT ACC GAG GAC TCG ATC GAC GTC T -5'-5'- TIT TIT TIT TIT TIT TIT TIT TIT TIT TI
Strand II-2	5'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T -3'-3'- TTT TTT TTT TTT TTT TTT TTT T-5'
Strand III-1	5'-T TIT TIT TIT CCA TGG CTC CTG AGC TAG CTG CAG TIT TIT TIT TIT TIT TIT TIT TIT TIT TI
Strand III-2	3'-TTT TTT GAT ACC GAG GAC TCG ATC GAC GTC TTT TTT TTT TTT TTT TTT TT_5'
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DNA Series V	
L=1 Strand 1	3'-GGT C-5'-5'-G Cy3 C TCC TGA GCT AGC TGC AGT AGC CTA TGC TCC AG-3'
L=1 Strand 2	5'-CCA G-3'-3'-C Cy5 G AGG ACT CGA TCG ACG TCA TCG GAT ACG AGG TC-biotin-5'
L=3 Strand 1	3'-GGT C-5'-5'-GCT Cy3 CCT GAG CTA GCT GCA GTA GCC TAT GCT CCA G-3'
L=3 Strand 2	5'-CCA G-3'-3'-CGA Cy5 GGA CTC GAT CGA CGT CAT CGG ATA CGA GGT C-biotin-5'
1=5 Strand 1	31-66T C-51-51-6CT C 003 T61 6CT 16C
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L=40 Strand 2	5'-CCA G-3'-3'-CG AGG ACT CGA TCG AGG TCA TCG GAT ACC AGG TCG AGT GG Cyb AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-blotin-5'
DNA Series VI	
Series VI Template	5'-CCA G-3'-3'-C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AAG TAG CGC AAC CGT GAC GAG ACT GCA CTC TCT GCC-biotin-5'
L=20n Strand 1	3'-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG Cy3 T-3'
1=20n Strand 2	5'-CV5 AG CCT ATG GTC CAG CTC ACC TTC ATC GCG TTG GCA CTG CTC TGA CGT GAG AGA CGG-biotin-3'
L=40n Strand 1	21-001 C-51-51-0 CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC T Cv3 T-31
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L=80n Strand 1	3'-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CTC ATG GTC CAG CTC ACC TTC ATC GCG TTG GCA CTG CAC TGA CGT GAG AGA CGT TCT Cy3 T-3'
L=80n Strand 2	5'-Cy5 GG AGC ACT CGC TCC GG-biotin-3'
DNA Series VIII	
Series VII Template	5'-CCA GCG AGG ACT CGA TCG AGA TCA ACG GAT ACC AGG TCG AAT GGA GGT AGC GCA ACC GTG ACG TAA CTG CAC TCT CTG CC-biotin-3'
L=20n Strand 1	3'-GGT CGC TCC TGA GCT AGC TCT AGT-Cv3-5'
1=20n Strand 2	3'-T CV5 GC CTA IGG TCC AGC TTA CCT CCA ICG CGT IGG CAC IGC ATI GAC GIG AGA GAC GG-5'
L=40n Strand 1	
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Strand IX-1	3'-TTT TTT-5'-5'-GCT CCT GAG CTA GCT GCA GT Cy3 AGC CTA TGC TCC AGC TCG GTC-3'-3'-TTT TTT-5'
Strand IX-2	5'-TTT TTT-3'-3'-CGA GGA CTC GAT CGA CGT CA Cy5 TCG GAT ACG AGG TCG AGC CAG-5'-5'-TTT TTT-3'
Strand X-1	3 '-T-CY3 TT TTT GCT CCT GAG CTA GCT CCA GTA GCT ATG GTC CAG CTC ACC TCC ATC GG TTG GCA CTG CAC TGA CGT GAG AGA CGG-5'
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Strand X-1 Strand X-2 Strand XI-1 Strand XI-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-2 Strand XIV-1 Strand XIV-1	3 '-T-CY3 TI TIT GCT CGT GAG CTA GGT CCA GTA GGT AGG GTC GAG GTC GAC TCC AGC GG TIG GCA CTG CAC TGA CGT GAG AGA CGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA GGT CAT CGA TAC CAG GTC GAG TGG AGG TGG GG GAG GTG GCC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T CY3 TI TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CGA GTG GAG GTG GAG GTG AGG GGA GGG TGG GCA CTG GCA CTG CAC TGA CGT GAG AGA CGG-3' 5'-T TIT TIT TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CGA TAC CAG GTC GAG GTG AGG GGA GGG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-5' 3'-TIT TIT G CY5 CTC CTG AGC TAG CTC GAG TAC CGA TAC CAG GTC GAC GTG AGG TGG CGC AAC CGT GAC GTG CAC TC CTC GCC -biotin-5' 3'-TIT TIT G CY5 CTC CTG AGC TAG GTC ATC GGA TAC CAG GTC CAG CTG CAC TCC ATC GCG TG GCA CTG CAC TC CY3 GA CGT GAA CGG-5' 5'-T TIT TIT TIT TT C GAG GAC TCG ATC GGA GTC ATC GGA TAC CAG GTC GAC TGC ACC TCC ATC GCG TG GCC TAC CGC TG CAC TC CTG CCC-biotin-3' 3'-TIT TIT 5'5'-G CY5 CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAC CTC ACC TCC ATC GCG TG GCA CTG CAC TC CTG CAC GGA GGA GGA CGG-3' 5'-T TIT TIT 5'5'-G CY5 CTC CTG AGC TAG CTC AGG GTA GC CAG CGG AGC CGA CGC GGA CGG GGA CGC GGA CGC GGA GGG AGG CGG-3' 5'-TIT TIT TT CGA GAC TCG ATC GGA TAC CAG GTC ATC GGA TGC CAC TCC ATC GCG TG GCA CTG CAC TC CTG CCC-biotin-3' 3'-TIT TIT TT AC CAA TUCA AGT TCC AGT AGC CTA TGG GCC CAC CTC CAC CGC GTG GCC GGA CTG CAC TC CTG GCC-biotin-3' 3'-TIT TIT TAT ACA GAT UCA AGT TCC AGT ACC CAT GG CGA CTC CAC CGC GCA ACC GTG ACC GTG AGC GGA AGA CGG -5' 5'-T TIT TIT TAT ACA GAT UCA AGT TCC AGT ACC CAT GG CTC ACC CCA CC CGC TG GCC TG CAC TCG ACC GTG AGC GCC AAC CGT GAC GTG ACC GTG AGC GCA CTC TCT GCC-biotin-3' 5'-T TIT TIT TAT ACA GAT UCA AGT TCA AGG TCA TCG GAT ACC AGG TGG GGA AGC GGCA ACC GTG ACC GTG ACC GTG ACC GC CAC TCT CTG CC-biotin-3'
Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XII-2 Strand XII-2 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-1 Strand XIV-1 Strand XIV-2 Strand XV-1	3 '-T-Cy3 TI TIT GCT CCT GAG CTA GCT CCA GTA GCT AGG GTC AGC TCG ACC GG TGG GCT GCC TCC GC GTG GCA CTG CAC TGA CGA GGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA TAC CGA TAC CAG TC GAG TGG AGG TGG AGG TAG CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T Cy3 TI TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCAG GTC GAG TGG CGA GTG AGC GTG ACC GGT GCA CTG CAC TGA CGT GAG AGA CGG-3' 5'-T TIT TIT TIT GC Q5 CTC GTG AGC TAG CTC CAG TAG CCAG GTC GAG GTC GAG GTG AGG TGG AGG TGG CGA CTG GAC GTG ACT GCA CTC TCT GCC -biotin-5' 3'-TI TIT TIT G CQ5 CTC GTG AGC TAG GTC ATG GGA TAC CAG GTC GAG GTG AGG GTG AGG GTG ACC GGT GAC GTG ACT GCA CTC TCT GCC -biotin-5' 3'-TI TIT TIT G CQ5 CTC GTG AGC TAG GTC ATG GGA TAC CAG GTC GAG GTG AGG GTG AGC GGA CTG GAC TG CAC T CY3 GA CGT GAG AGA CGG-5' 5'-T TIT TIT T C GAG GAC TCG ATG GAG GTC ATG GGA TAC CAG GTC GAC GTC ACC CGT GGC CGG ACC GGA CGC GTG ACT GCC Cbiotin-3' 3'-TIT TIT TT C GAG GAC TCG ATG GAG GTC ATG GGA CGC GGA GTG GAG GTG AGG GTG ACC GGT GAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TIT TIT TIT TAT ACA GAT UCA AGT TCC AGT AGC CTG GGA GTC AGG GTG AGG GTA GGC AAC CGT GAC GTG ACT GGA GTG AGA GGG -5' 5'-T TIT TIT TTT ACT CAA GAT UCA AGT TCC AGT AGC CTG GCA GTC GAG TGG AGG GTA CGT GCA CTG CAC GTG AGA GGA GGG -5' 3'-GGAG-5'-5'-GCT CCT GAG CTA GCT GCA GTQ AGC CTG GGA GT G GCA GTC GCA CTG GTG GAC GTG ACT GGA GGC -5' 3'-GGAG-5'-5'-GCT CCT GAG CTA GCT GCA GT Q3 AGC CTA TCC CCA GT GG GCA 3'-GACC-5'
Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XII-2 Strand XII-2 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XIV-1 Strand XV-1 Strand XV-1 Strand XV-1	3 '-T CAYS IT TIT GCT CCT GAG CTA GCT CCA GTA GCT AIG GTC CAG CTC ACC TCA GC THE GCA CTG CAC TGA CGT GAG AGA CGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA TAC CGA TAC CAG TC GAG GTC GAG TGG AGG TAG CGG AGC GTG ACC GTG ACT CTC TCG CC -biotin-3' 3'-T CAYS IT TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCAG GTC GAG GTC GAG TGG CGC TAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T TIT TIT TIT GCA GGA CTC GTG AGC TAG CTC CAG TAG CGA GTG GAG GTC GAG GTG GAG GTG GAG GTG GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-TT TIT TIT G CAYS CTC CTG AGC TAG CTC CAG TAG CGA TAC CAG GTC GAG CTC ACC TCC ATC GCG TTG GCA CTG CAC TCA CG GAG GAG CGG-3' 3'-TT TIT TIT G CAYS CTC CTG AGC TAG GTC CAG TAG CGA TAC CAG GTC GAG GTG AGG GTG GAG GTG GAC GTG GAC GTG ACT GCA CTC TCT GCC -biotin-5' 3'-TT TIT TIT G CAYS CTC CTG AGC TAG GTC CAG TAG CGA TAC CAG GTC GAG GTG AGG GTA CCGG TAG CGC TG CAC TC CAC TC GCC-biotin-3' 3'-TT TIT TIT C GAG GAC TCG AIC GAG GTC ATC GGA TAC CAG GTC CAC CTC ACC GCG TIG GCA CTG CAC TC CTC GCC-biotin-3' 3'-TT TIT TIT TT C GAG GAC TCG AIC GAG GTC ATC GGA TAC CAG GTC AGC TCA CC TC ACC GTG GAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TT TIT TIT TIT C GAG GAC TCG AIC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TT TIT TIT TAT ACA GAT UCA AGT TCC AGT AGC CTA TGG TCC AGC TCA CC CC ACC CGT GG CAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TTT TIT TAT ACT GT CTA AGT TCA AGG TCA TCG GAT ACC CAG TCG AGT GA GTG AGC GCA ACC GTG ACC GTG ACC GTG AGA GAC GG -5' 5'-T TIT TIT TIT ATA TGT CTA AGT TCA AGG TCA TCG GAT ACC CAG TCG AGT GA GTG AGC GCA ACC GTG ACC GTG ACC GTG CAC TCT CT GC C-biotin-3' 3'-GTAC-5'-5'-GCT CAG CT CAG TAC CAG TCG AT TCG GAT ACC AGG TCG AGT GA GTG AGC GCA ACC GTG ACC GTG ACC GTG CAC TCT CT GC C-biotin-3' 3'-GTAC-5'-5'-GCT CAG CT CAG CT ACG GT ACC CAG GTG AGG TGG AGC GAC ACC GTG ACC GTG ACC GTG CAC TCT CT GC C-biotin-3' 5'-CCTC-3'-3'-CCAG AGG CT CGA GT CGA TACC CAG GTG GA GTG GA GCG CAG-5'-5'-CTGG-3'
Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-2 Strand XIV-1 Strand XV-1 Strand XV-2 Strand XV-2 Strand XV-1	3 '-T-Cy3 TI TIT GCT CGT GAG CTA GCT CCA GTA GCT AGG GTC AGC TCA CC GCT CAC TCG GT GGC CAC TGA CGT GAG AGA CGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA GGT CAT CGA TAC CAG GTC GAG TGG AGG TAG CGG AAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T Cy3 TI TIT-3'-5'-5 G CTC CTG AGC TAG CTC CAG TAG CGA GTG GAG GTC GAG GTG AGG TAG CGC TAG CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T TTT TIT TTT TTT G Cy5 CTC CTG AGC TAG CTC CAG TAG CGA GTG GAG GTG GAG GTG GAG GTG GAC GTG GAC GTG GAC GTG GAC GTG GAG AGA CGG-3' 3'-TTT TIT G Cy5 CTC CTG AGC TAG CTC GAG TAG CCT ATG GTC CAG CTC GAG TGG AGG TAG CGC AAC CGT GAC GTG GAC GTG GAC GGA GAG CGG-5' 5'-T TIT TIT TTT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-5' 3'-TTT TIT TTT TT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG CAAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TTT TIT TTT TTT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAC GTC ACC GCC ATC GCC ACC GTG ACC GTG GAC GGA GAG AGG-3' 5'-T TTT TTT TTT TTT C GAG GAC TCC AGT GAG GTC ATC GGA TAC CAG TCC ACC CATC GCC ATC GCC ACC GTG ACC GTG GAC GGA GGC -5' 5'-T TTT TTT TTT TTT ATA AGA TUCA AGT TCC AGT GGA TAC CAG GTC GAC TCA C CY3 CT CCA TCG GCC TAG CGT GAC GTG ACG GTG AGG GGA GGC -5' 5'-T TTT TTT TTT ATA TGT CTA AGT TCC AGT AGC CTA TGG TCC AGC TGC AGC GTC ACC GGA ACC GTG ACC GTG ACC GTG AGA GAC GG -5' 5'-T TTT TTT TTT ATA TGT CTA AGT TCC AGT GCG GAT ACC AGG TCG AGC GA ACC GT GAC GCA ACC GTG ACC GTG CAC TCT CTG CC-biotin-3' 3'-GGAG-5'-5'-GCTC CT GAG CTA GCT GCA GT CAG GTC AGG GGA AGC GGA CGC GCA ACC GTG ACC GTG ACC GTG CCA TCT CTG CC-biotin-3' 3'-GGAG-5'-5'-GCTC CT GAG CTA CC GA CT CAG CTC CAG CTC CAG CTG CAG CTG C-3'-3'-GACCC-5' 5'-T TTT TTT TTT ATA AGT TCA AGT TCA AGC TAG CTG CAG GTG AGG GAG CGC CACC CTG CGG CTG CAC TCT CTG GCA CTC CY3 T-3'
Strand X-1 Strand X-2 Strand XI-1 Strand XI-2 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-2 Strand XII-2 Strand XIV-1 Strand XIV-1 Strand XV-1 Strand XV-1 Strand XV-1 Strand XV-1 Strand XV-1	3 '-T-Cy3 TI TIT GCT CCT GAG CTA GCT CCA GTA GCT AIG GTC CAG CTC ACC TCA GG TIG GCA CTG CAC TGA CGT GAG AGA GCGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA TAC CGA TAC CAG TC GAG TGC GAG TGG AGG TAG CGA ACC GT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T Cy3 TI TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCAG GTC GAG TGG CG GAG GTG GCG GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T TIT TIT TIT GA GGA CTC GAT GGA CTC GAG TGA CCGA GTG GAG GTG GAG GTG GAG GTG GCC GAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-TTT TIT G Cy5 CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAC GTC GC CTC CC TCC ACT GCG GTG GCA CTG GAC GTG GAG GGA GGG-5' 3'-TIT TIT GT CGA GGA CTCG ATG GAG GTC ATG GGA TAC CAG GTC GAG TGG AGG TGG GCC AAC CGT GAC GTG ACT GCA CTC GCC -biotin-3' 3'-TITT TIT G Cy5 CTC CTG AGC TAG GTC CAG TAG GCC ATG GGA TGG GAG TGG AGG TGG GCC ACC CGT GAC GTG GCA TG CGC TG GCC GAG GGA 3'-TITT TITT G Cy5 CTC CTG AGC TGC GAG GTC ATG GGC CAG GTC ACC TCC ATC GCG TTG GCA CTG CACT GCA GTG GAG AGA CGG-3' 5'-T TITT TIT TT C GAG GAC TCG ATG CTC GAG TAC GGA TAC CAG GTC AGC TCC ATC GCG TTG GCA CTG CACT GCA CTC TCT GCC-biotin-3' 3'-TITT TITT TTT TT C GAG GAC TCG ATG GAG GTC ATG GGA TAC CAG GTC AGC GTC ACC CTG ACC GTG ACT GGA CTG CACT GC CG-3' 5'-T TITT TIT TTT TAT ACT GTA AGT TCC AGT AGC CTA TGG GT CAG CTC ACC TCC ATC GCG T GG CAT GC CT GCA CTG CACT GG CG -5' 5'-TITT TITT TTAT TAT GT CTA AGT TCC AGT AGC CTA TGG TCC AGC TGG GT GG CAT GC GTG GAC TGG CAT GC CTG CACT GG CG CACC CTG CACT GGA CTG CACT GC CGG CC
Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XII-2 Strand XII-2 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XIV-1 Strand XIV-1 Strand XV-1	3 '-T-Cy3 TI TIT GCT CCT GAG CTA GCT CCA GTA GCT ATG GTC CAG CTC ACC CGG TG GCA CGG GTA GCA CGT GAC CGA CGC GAG CGG-5' 5'-T TIT TIT TIT GCA GGA CTC GAT CGA TAC CGA TAC CAG TC GAG TGG AGG TGG AGG TAG CGG ACC GTG ACC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T Cy3 TI TIT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CGA GTC GAG GTC GAG TGC AGG TGG AGC GTG ACC GTG ACC TCT CTC GCC -biotin-3' 3'-T TIT TIT G Cy5 CTC CTG AGC TAG CTC CAG TAG CTA AGC GTC AGC GTG GAG GTG AGG TGG AGC GTG ACC GTG ACC GTG GAG AGA CGG-3' 3'-TI TIT TIT G Cy5 CTC CTG AGC TAG CTC CAG TAG CTA ACC GGA TAC CAG GTC GAC CTC ACC GCG TTG GCA CTG CAC TC GCA CTG CGC -biotin-5' 3'-TI TIT TIT G Cy5 CTC CTG AGC TAG CTC CAG TAG CTA AGC GTC GAC GTC GAC GTG ACC GTG GAC GTG ACC GGA GAG CGG-5' 5'-T TIT TIT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAC GTC ACC CCC TC GCC TTG GCA CTG CAC TC TCT GCC-biotin-3' 3'-TIT TIT-5'5'-6 Cy5 CTC CTG AGC TAG GTC ATC GGA TAC CAG GTC GAC GTG AGC GGA GTG GAC GTG ACC GGA GAC GGG-5' 5'-T TIT TIT TTT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAC TCA CC CC ACC GGG TG GAC GTG ACT GCA CTC TCT GCC-biotin-3' 3'-TIT TIT TAT ACA GAT UCA AGT TCC AGT AGC CTA GG TCC AGC TGG GTG GAG GTG AGC GTG ACC GTG AGC GTG AGC GGA GGC G-5' 5'-T TIT TIT TAT ACA GAT UCA AGT TCC AGG GAT ACC AGG GTG GAG TGG AGG TGG AGC GTC GC GTG GAC GTG ACC GTG AGC GGA GGC G-5' 5'-TIT TIT TIT ATA ACA GAT UCA AGT TCC AGG GAT ACC AGG GTG GAG TGG GGA GG TAG CGC TACC GTG GAC GTG ACC GTG AGC GGA GGC G-5' 5'-TIT TIT TIT TAT ACA GAT UCA AGT TCC AGG GAT ACC AGG GTG GGA GG TGG GGA GC GC G-5' 5'-CCTC-3'-3'-CGA GGA CTC GAT CGA CGT CA CY5 TOG GAT ACC AGG TGG AGC GGA GCC CA-5'-3'-GACC-5' 5'-CCTC-3'-3'-CGA GGA CTC GAT CGA CGT CA CY5 TOG GAT ACG AGG TGG AGC GGA GCC CA-5'-5'-CCTGG-3' 5'-TIT TIT TIT GG TACC GAG GAC CGG AGC CGG TTI TIT TIT TIT TIT TIT TIT TIT TIT T
Strand X-1 Strand X-1 Strand XI-2 Strand XI-2 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-1 Strand XII-2 Strand XIV-1 Strand XIV-1 Strand XV-1	3 '-T-Cy3 IT ITT GCT CCT GAG CTA GCT CCA GTA GCT AGG GTC GAG GTC GAC GCG GGT GGC GAG GGG GAC GGC CAC GGA CGG GAG GGG GAG GGG GG
Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-1 Strand XII-2 Strand XIV-1 Strand XIV-2 Strand XV-1 Strand XV-2 Strand XV-1 Strand XV-1 Strand XV-1 Strand XV-2 Strand XV-1 Strand XVI-1 Strand XVI-2 Strand XVI-1 Strand XVI-2	3 '-T-Cy3 IT ITT GCT CCT GAG CTA GCT CCA GTA GCT ATG GTC CAG CTC ACC CG GT TG GCA CTG GAC GTG ACT GAA GAG CGG-5' 5'-T TITT ITT TTT GCA GGA CTC GAT CGA GGT CAT CGA TAC CAG GTC GAG GTG AGG TGA CGG AAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T Cy3 IT ITT-3'-5'-6 CTC CTG AGC TAC GCA TAC CAG TG CGAG GTC GAC GTG AGG TAC CAG CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3' 3'-T TTT TTT TTT GA GGA CTC GAT GCA GTC ACT GGA TAC CAG GTC GAC GTC GAC GTG AGC GTG ACT GCA CTG GAC GTG AGA GGG-3' 3'-TTT TTT TTT G Cy5 CTC GAG GAC TCG ATC CAG TAG CCT ATG GTC CAC GTC GAC TCC ACT CCC TGAC GTG GAC GTG AGA GGA CGG-3' 3'-TTT TTT TTT G C GAG GAC TCG ATC GAG GTC ATG GGC CAC GCC GAG GTG GAG GTG GAC GTG GAC GTG GAC GTG GAC GGA GAC GGG-3' 3'-TTT TTT TTT G C GAG GAC TCG ATG CTC CAG TAG GCT ATG GTC CAG GTC GAC GTG GAG GTG GAC GTG GAC GTG GAC GGA GCG-3' 3'-TTT TTT TTT C GAG GAC TCG ATG GAG CTC ATG GGC CAG GTC ACC TCC ATC GCG TTG GCA CTG CAC T CY3 GA CGT GAG AGA CGG-3' 3'-TTT TTT TTT TTT C GAG GAC TCG ATG GAG GTC ATG GGC TAG GGT GAG GTG AGG TGG GGA TGC GCA TC CCT GC C-biotin-3' 3'-TTT TTT TTT TTT C GAG GAC CCA AGG TCC CAG TAG GCC TAG GTC AGC TCA C CY3 CT CCA TCG GGT GG CAT GCA CT GCA CT CT GCC-biotin-3' 3'-TTT TTT TTT TTT TTT TA ACT GTA AGT TCC AGT AGC CTA GG CT CA GC GTG GG TGG GGT GG CCT GCA CT GC CT GG CT GCA CT CT CT GC C-biotin-3' 3'-GGAG-5'-5'-GCT CT GAG CTA GCT GCA GT CY3 AGC CTA TGC TCC AGC TCG GCT GG CT GG CCT GG CT GG CT GG CT GC CT GG CC GAG GG CG CAT CC CY3 I-3' -GGAG-5'-5'-GCT CT GAG CTA GCT GCA GT C AY5 TCC GAT ACC AGC GT GAG GC GG-5'-5'-C'CGG-3' 3'-GGAG-5'-5'-GCT CT GAG GAC CTC AGC TAG CTA GC GTT TTT TTT TTT TTT TTT TTT TTT TTT
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Strand X-1 Strand X-1 Strand XI-1 Strand XI-2 Strand XI-2 Strand XII-2 Strand XII-1 Strand XII-2 Strand XII-1 Strand XII-1 Strand XII-2 Strand XII-1 Strand XIV-1 Strand XIV-1 Strand XV-1 Strand XV-1 Strand XV-1 Strand XVI-3 Strand XVII-3 Strand XVII-3 Strand XVII-1 Strand XVII-3 Strand XVII-3 Strand XVII-3 Strand XVII-3 Strand XX-1	3 '-T-Q3 TI TH GCT CCT GAG CTA GCT CA TAG CTA AGE AND CAGE CCA ACC TO ATC GGT THG GCA CTG CAC TGA CGT GAA GAG AGA CGG-5' 5'-T TH TH TH TO CAG GGA CTG GAL CGA GGT CAL CGG TAC CGA GGT CAGE CGA CGT GGA CTG GCA CTG CGA CTG CGC -biotin-3' 5'-T TG JTH TH TH CGA GGA CTG GAL CGA GGT CAL CGG TAC CGA GTG GAG GGA GG TAG CG ALC CGT GAC GGA CGG CGA CGT GGA CGG CGA CGG -3' 5'-T TH TH TH TH CGA GGA CTG GAL CGA GGT CAL CGGA TAC CAG GTC GAC TG CAC TGC CAC CGT GAC GTG GAC TG GAC CGA CGG C-3' 5'-T TH TH TH CGA GGA CTG GAL CGA GGA CAC CGGA TAC CAG GTC GAC TG CAC TGC CAC CGT GAC GTG GAC TG CAC CTG CGC -biotin-3' 3'-TH TH TH CGA GGA CTG ALC GAG GTC ALC GGA TAC CAG GTC GAC TGC ACC TGC ACC GGG TG GAC TG CAC CTG CAC CTG CGC TGG CGT GGA CTG GAC CGG GGA GGA CGG-3' 5'-T TH TH TH TH CGA GGA CTG ALC GAG GTC ALC GGA TAC CAG GTC GAC TGC CAC CGG GTG GGA CTG CAC CTG CAC CTG CGC TGG CGT GGA GGA GGA CGG-3' 5'-T TH TH TH TAL ACC GAL UCA AGT TCC AGT GGA TAC CAG GTC GAC CTG CAC TGC CGT GGA CTG CAC CTG CGC TGG CGT GGA GGA GGA GGA GGA GGA GGA GGA GGA G
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Supplementary Table S1:

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Table of oligonucleotide sequences annealed to construct substrates for experiments in this paper. Dyes are indicated by "Cy3" or "Cy5 in the sequence. Reverse polarity linkages are indicated by "-3'-3'-" or "-5'-5'-" in the sequence.