Reporting Transient Molecular Events by DNA Strand Displacement

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Supporting Information

Material and Methods

Oligonucleotides. All oligonucleotides were purchased from IDT, Inc. and purified by 20% denaturing PAGE. DNA sequences:

H strand (Red): 5'-*ggaaggagag*TTTT*ctctccttcc*TAATCGCACTGCCGTCATAG-3';

S strand (Green): 5'-CTATGACGGCAGTGCGATTA-3';

T strand (Blue): 5'-CTATGACGGCAGTGCGATTA*ccttcctctc*-3'.

Formation of DNA complexes (H-S or H-T)**:** Equal molar DNA strands (H + S or H + T) were mixed in TAE/Mg^{2+} buffer [40 mM Tris base, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, adjusted to pH 8.0] and cooled from 95 ℃ to 4℃ over 3 hours.

Native PAGE. Native PAGE containing 4% polyacrylamide (19:1 acrylamide/bisacrylamide) was run on a FB-VE10-1 electrophoresis unit (FisherBiotech) at 4 °C (90 V, constant voltage). TAE/Mg^{2+} buffer (pH 8) was used both as the running buffer and the buffer in the gel. After electrophoresis, the gels were stained with Stains-All (Sigma) and scanned with an HP scanner (Scanjet 4070 Photosmart).

Triplex-induced strand displacement at different pH: After the assembly of the initial DNA duplex (H-S), T strand was added to the mixture, followed by adjusting the solution pH to a desired value using 1.0 M HCl. The resulted solution was incubated under 22 °C for indicated duration, and then loaded onto the native PAGE (pH 8.0), which terminated triplex-induced strand displacement.

Quantification in timecourse experiment. The intensities of the bands corresponding to H, S, T, H-S, and H-T were measured with ImageJ [C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nature*

Methods **2012**, *9*, 671.]. Abundance was calculated in relative percentage according to the band intensities. Such a semiquantification method produces a reliable trend.