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Electronic Supplementary Information

Direct observation of spatial configuration and structural stability of locked Y-shaped DNA structure

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Experimental details

Materials

All chemicals were used of analytical grade, if not otherwise stated were purchased from Sigma-Aldrich and used without further purification. The HPLC purified labelled and unlabeled oligos were purchased from IDT, USA and used as received without any further purification (Table S1). Annealing of the oligos was performed by mixing equimolar amounts of appropriate pairs of ssDNA. A buffer solution used for annealing, containing 10 mM Tris-HCl, (pH-7.5) 100 mM NaCl and 10 mM Mg^{2+} (buffer A) and heated at 92°C for 4 minutes and gradually cooled down to room temperature and stored at -20^0 C for experimental purpose. Same composition of buffer as buffer A is used to carry out all the experiments. The sequences of the oligos are designed in such a way that B1, B4 and B1, B5 would result 8-7-8 and 6 -11-6 composition respectively (i.e. LY1). The short oligo BBIO, having biotin tagged at the 5' end is designed to help in surface immobilization of the dsDNAs to carry out the smFRET experiments, when annealed at the 3' overhang of B1. The very short oligos 'BN2, BN3' and 'BN4, BN5' are the sets of complementary of individual single strands of 8-7-8 and 6 -11-6 composition respectively (i.e. LY3). Similarly 'BN7, BN8' and 'BN9, BN10' are another sets of continuous complementary of two half single strand from the centre and extending towards the two Y arms of both composition (i.e. LY4).

Table S1 The sequence of the oligo nucleotides with modification used in this study.

Name of the oligo	Sequence and Modification (5' to 3')
B1	CGGGGCAAGTAT(**Cy3**)AAGTAGGCGGGACACACACAC
B4	CCCGCCTAGAAT(**Cy5**)ATGTTGCCCCG
B5	CCCGCCATGAAT(**Cy5**)ATGAAGCCCCG
BBIO	**Biotin**-AAAAAGTGTGTGTGT
BN2	CTTATAC
BN3	CATATTC
BN4	TACTTATACTT
BN5	TTCATATTCAT
BN7	TTCCTTA
BN8	TACCATA
BN9	TTCATTACTTA
BN10	TACTTTTCATA

Fluorescence measurement with temperature variation

Individual sets of dsDNA (i.e. LY1, LY2, LY3 or LY4) of both compositions having concentration 50 nM in buffer A has been used for steady state fluorescence measurements, varying the temperature using Varian Cary Eclipse fluorescence spectrophotometer. Cy3 (donor) & Cy5 (acceptor) were exited at 530 nm and 635 nm respectively and emissions were recorded accordingly using slit width of 5 nm.

Single Molecule FRET Measurements

For single molecule experiments, a predrilled quartz microscope slides (25 mm x 75 mm) and cover slips (24 mm x 32 mm) were plasma cleaned and PEG/biotin-PEG (100:1) (MW 5,000, Laysan Bio, USA) coated onto it following a standard protocol. Micro-fluidic sample chambers were constructed by sandwiching cover slip with both sided tape and the edges were sealed with epoxy. 0.2 mg/ml streptavidin was injected into the sample chamber and incubated for 6-7 minutes before flushing with buffer A. The annealed constructs having Cy3, Cy5 and biotin attached were diluted to ~ 50 pM and ~ 50 µl of diluted sample was introduced into sample chamber. After incubation for 6-7 minutes, the unbound DNA molecules were washed out using buffer A. To improved dye stability and to avoid blinking, oxygen scavenging system consist of protocatechuic acid/protocatechuate-3,4-dioxygenase has been used with buffer A containing of 2 mM trolox.

We have used a home built prism-based total internal reflection (PTIR) based smFRET instrument. In brief the set up is constructed inverted fluorescence microscope (Olympus IX 71). Fluorescence from Cy3 and Cy5 were collected using water immersion objective (60X appo, 1.2 NA, Olympus). A solid state 532 nm diode laser (Laser Quantum, U.K.) was used to excite Cy3, and then the fluorescence signals from both Cy3 and Cy5 were recorded simultaneously. Donor and acceptor signals were spatially separated using a 550−630 nm dichroic mirror (640 DCXR, Chroma) after passing through a long-pass filter (550 nm, Chroma). Individual signal were projected onto one-half of the active area of an electron-multiplied charge-coupled-device camera (EMCCD, Ixon3+ 897, Andor Technologies, South Windsor, CT) with frame integration time of 30 ms. Data acquisition softwares used were generous gifts either from Tae-Hee Lee (Pensylvania State University, USA) or T. J. Ha (University of Illinois, USA), developed using Visual C++ (Microsoft,

WA). Each of the image frames contain an average of ~300 Cy3/Cy5 single-molecule features, which were analyzed using codes written in the IDL based programming language. All experiments were done at room temperature unless otherwise specified.

Individual traces were chosen to construct single-molecule time-dependent trajectories for the donor–acceptor chromophore. The smFRET efficiency was calculated using relationship:

$$E_{fret} = \frac{I_A}{I_D + I_A} \ldots\ldots\ldots\ldots 1$$

Where E_{fret} is the FRET efficiency and I_D and I_A is intensity of Cy3 and Cy5 respectively. Hidden Markov model (HMM) algorithm was used to fit the FRET trajectories. FRET distance of different states was calculated using the following equation:

$$E = \frac{R_0^6}{R^6 + R_0^6} \ldots\ldots\ldots\ldots 2$$

Where, R_0 and R were the Förster distance and distance between the donor-acceptor respectively.

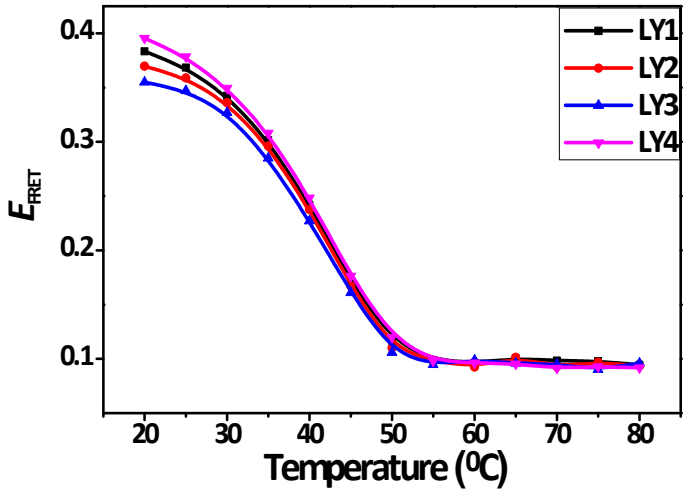

Fig. S1 Ensemble FRET measurements of 8-7-8 composition having four different system; LY1 (black), LY2 (red), LY3 (blue) and LY4 (magenta) respectively. E_{FRET} of 8-7-8 composition (0.3833 (LY1), 0.3696 (LY2), 0.3550 (LY3) and 0.3955 (LY4)) is higher than that of 6-11-6 composition (0.2225 (LY1), 0.2179 (LY2), 0.2130 (LY3) and 0.2276 (LY4)) of all systems and decreases with increasing temperature in both cases.

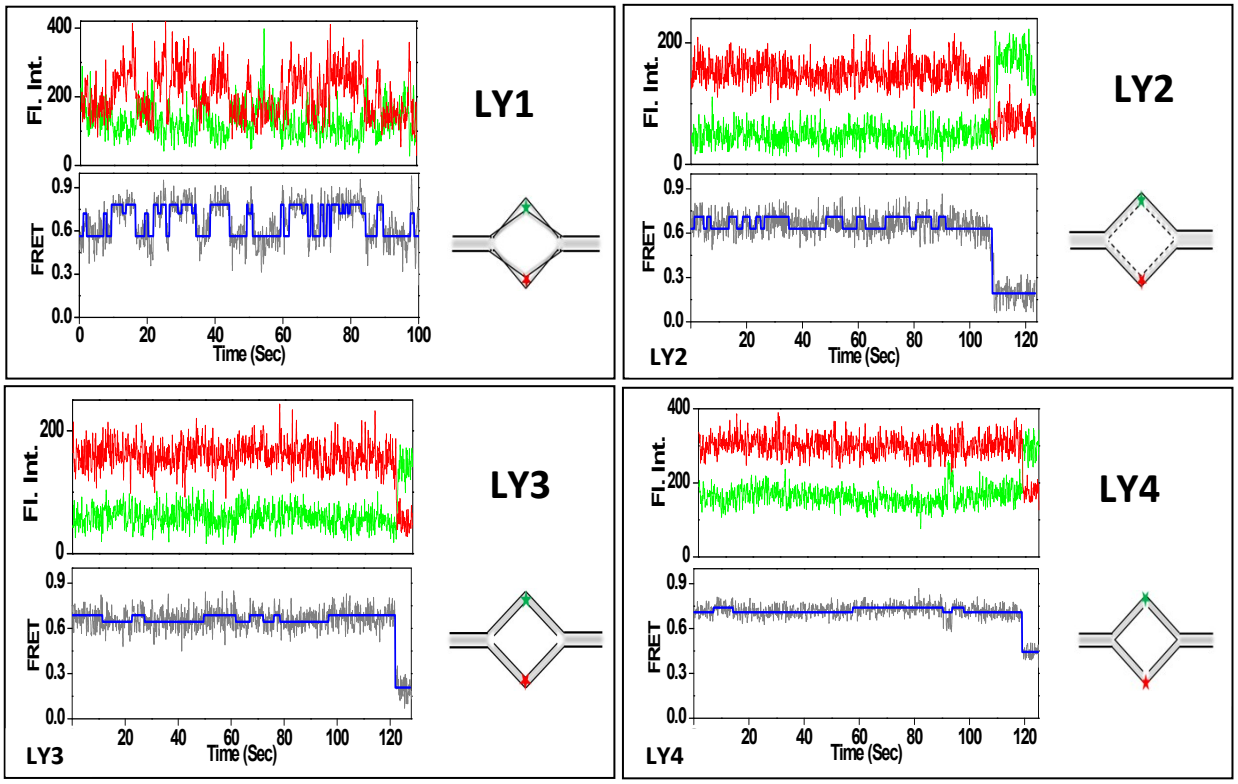

Fig. S2 Representative single molecule time traces 8-7-8 composition of four different systems internally labelled with Cy3 and Cy5 FRET pair. The red and green colure trajectories represent the acceptor and donor signal and there corresponding FRET shown in grey colour. Signal fluctuation indicates that the locked two Y arms were try to come closer and farthest apart to each other. The fluctuation is higher in case of LY1 and that fluctuation decreases upon addition of dNTP or complementary strands. Blue line is the fitted FRET obtained by HMM analysis. Schematic of the respective locked Y DNA has been presented at the right side of the traces.

Table S2 Single molecule FRET measurements 8-7-8 composition of different systems containing different FRET states. Corresponding distance was calculated taking R_0 of Cy3 and Cy5 is 5.4 nm.

Different systems	LY1			LY2		LY3		LY4	
FRET efficiency (E_{FRET})	0.56	0.72	0.78	0.63	0.71	0.64	0.68	0.70	0.73
Distance (nm)	5.19	4.61	4.37	4.94	4.65	4.91	4.76	4.69	4.58

Table S3 For 6-11-6 composition (the calculations are done assuming the nucleotide contribution for dsDNA and ssDNA to be 0.34 nm and 0.7 nm respectively.)

Probable 'BC' distance (nm)	LY3 (CP)	Angle	LY4 (CP)	Angle
(11 x 0.34) / 2 = 1.87	2.56 or 2.42	Not possible	2.40 or 2.31	Not possible
(10 x 0.34 +0.7) / 2 = 2.05	2.56 or 2.42	Not possible	2.40 or 2.31	Not possible
(9 x 0.34 +1.4) / 2 = 2.23	2.56 or 2.42	Not possible	2.40 or 2.31	Not possible
(8 x 0.34 +2.1) / 2 = 2.41	2.56 or 2.42	Not possible	2.40 or 2.31	170 or 147
(7 x 0.34 +2.8) / 2 = 2.59	2.56 or 2.42	162 or 138	2.40 or 2.31	136 or 126
(6 x 0.34 +3.5) / 2 = 2.77	2.56 or 2.42	135 or 122	2.40 or 2.31	120 or 113
(5 x 0.34 +4.2) / 2 = 2.95	2.56 or 2.42	120 or 110	2.40 or 2.31	109 or 103
(4 x 0.34 +4.9) / 2 = 3.13	2.56 or 2.42	110 or 101	2.40 or 2.31	100 or 95
(3 x 0.34 +5.6) / 2 = 3.31	2.56 or 2.42	101 or 94	2.40 or 2.31	93 or 88
(2 x 0.34 +6.3) / 2 = 3.49	2.56 or 2.42	94 or 88	2.40 or 2.31	87 or 83
(1 x 0.34 +7.0) / 2 = 3.67	2.56 or 2.42	88 or 82	2.40 or 2.31	82 or 78
(11 x 0.7) / 2 = 3.85	2.56 or 2.42	83 or78	2.40 or 2.31	77 or 74

Table S4 For 8-7-8 composition

Probable 'BC' distance (nm)	LY3 (CP)	Angle	LY4 (CP)	Angle
(11 x 0.34) / 2 = 1.87	2.46 or 2.38	Not possible	2.35 or 2.29	Not possible
(10 x 0.34 +0.7) / 2 = 2.05	2.46 or 2.38	Not possible	2.35 or 2.29	Not possible
(9 x 0.34 +1.4) / 2 = 2.23	2.46 or 2.38	Not possible	2.35 or 2.29	Not possible
(8 x 0.34 +2.1) / 2 = 2.41	2.46 or 2.38	-- or 162	2.35 or 2.29	154 or 144
(7 x 0.34 +2.8) / 2 = 2.59	2.46 or 2.38	144 or 134	2.35 or 2.29	130 or 124
(6 x 0.34 +3.5) / 2 = 2.77	2.46 or 2.38	125 or 118	2.35 or 2.29	116 or 111
(5 x 0.34 +4.2) / 2 = 2.95	2.46 or 2.38	113 or 108	2.35 or 2.29	106 or 102
(4 x 0.34 +4.9) / 2 = 3.13	2.46 or 2.38	104 or 99	2.35 or 2.29	97 or 94
(3 x 0.34 +5.6) / 2 = 3.31	2.46 or 2.38	96 or 92	2.35 or 2.29	90 or 88
(2 x 0.34 +6.3) / 2 = 3.49	2.46 or 2.38	90 or 86	2.35 or 2.29	85 or 82
(1 x 0.34 +7.0) / 2 = 3.67	2.46 or 2.38	84 or 81	2.35 or 2.29	80 or 77
(11 x 0.7) / 2 = 3.85	2.46 or 2.38	79 or76	2.35 or 2.29	75 or 73