

Supplemental

Section S.2 Supplemental Figures

Section S.2 Supplemental Tables

Section S.3 Supplemental Discussion

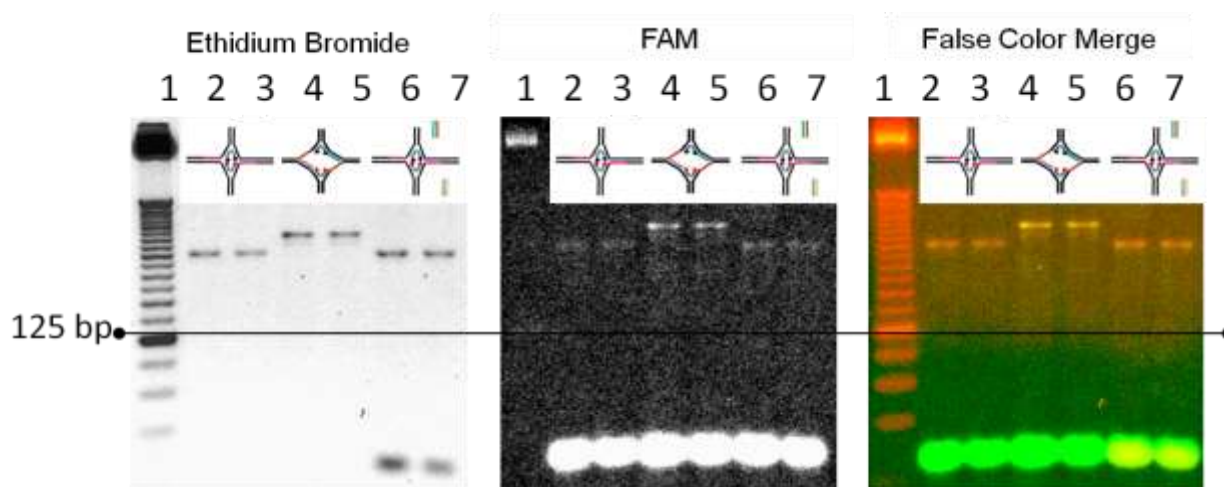
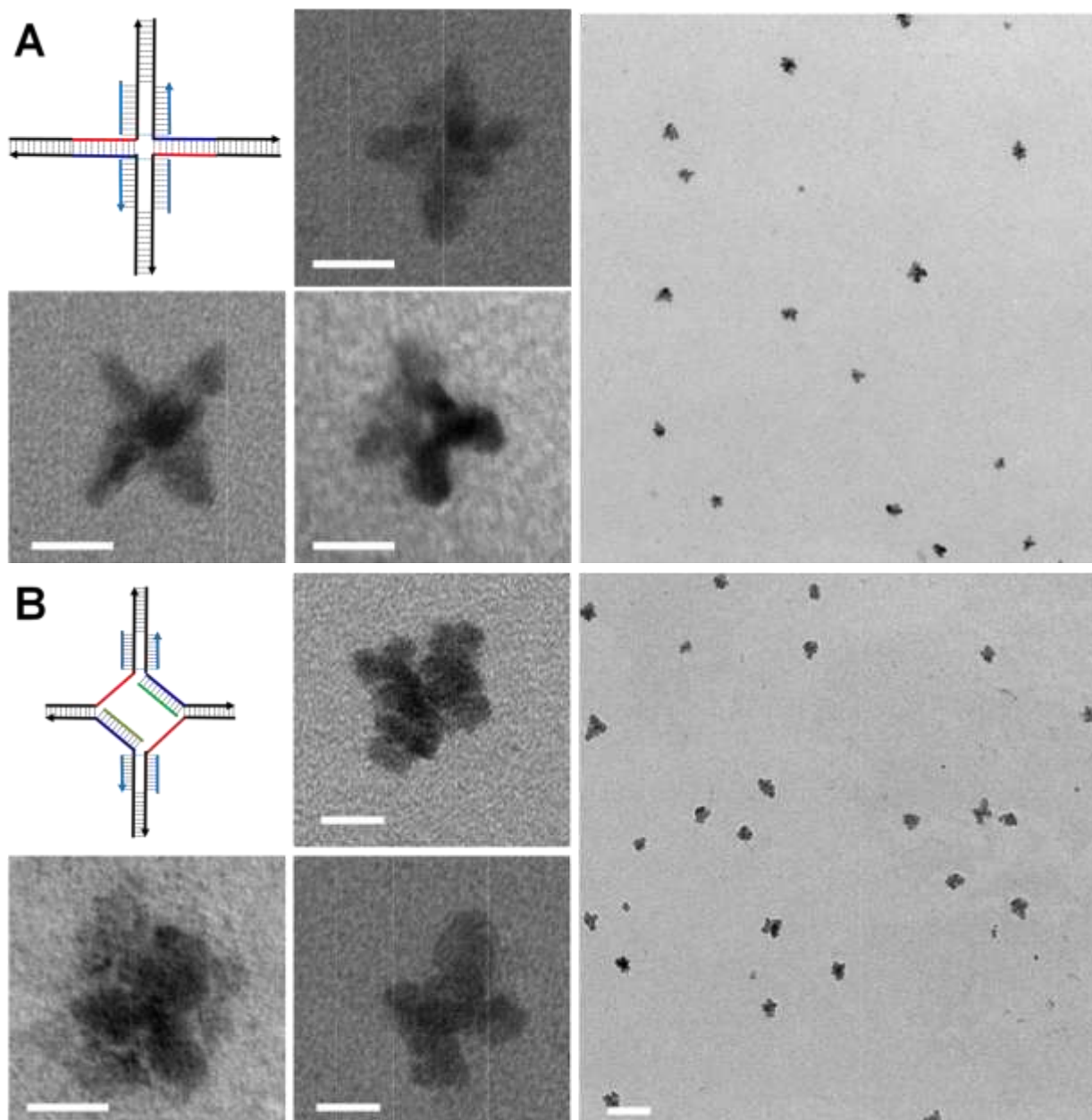


Figure S1: Fluorescence Gel Electrophoresis: Lane 1: 25 bp Ladder, Lanes 2 & 3: Closed DNA 4WJ, Lane 4 & 5: Open DNA 4WJ, Lanes 6 & 7: Closed DNA 4WJ with excess closing strands. Fluorescent gel electrophoresis demonstrate thermodynamic stability of DNA 4WJ by itself Lanes (2 & 3), with opening strands (Lanes 4 & 5), with opening and excess closing strands (Lanes 6 & 7). The bands below the 50 bp markers were the duplexed waste product from the closing strands binding with the opening strands.

Figure S2: TEM



Uranyl acetate staining of inosine containing DNA four-way junctions without fluorophores in the **A**) closed and **B**) open conformation examined under TEM. A model of each conformation is shown next to zoomed in images of each state. A wide field image of each conformation is provided on the far right. Scale bar: 20 nm.

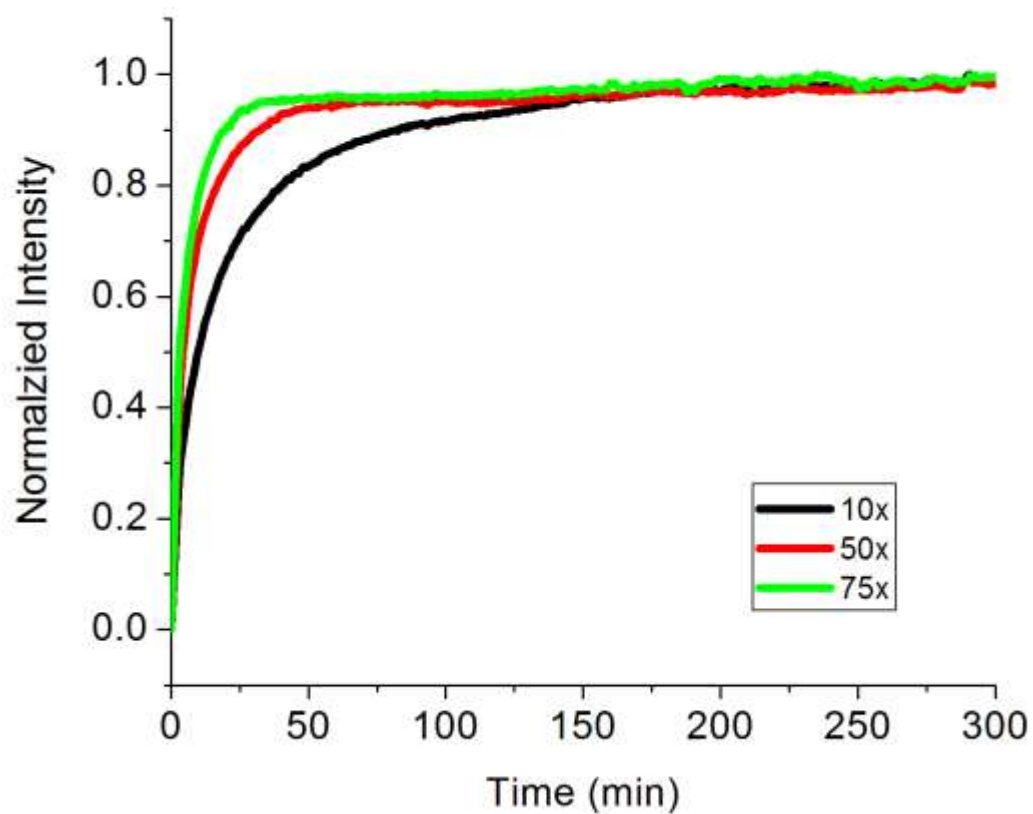


Figure S3: Different amounts of opening strands were mixed with the same amount of dual spring actuator and monitored simultaneously. It was discovered that full opening within the experiment's observation time was achieved with an amount of opening strands 10x concentration. $T = 37^{\circ}\text{C}$.

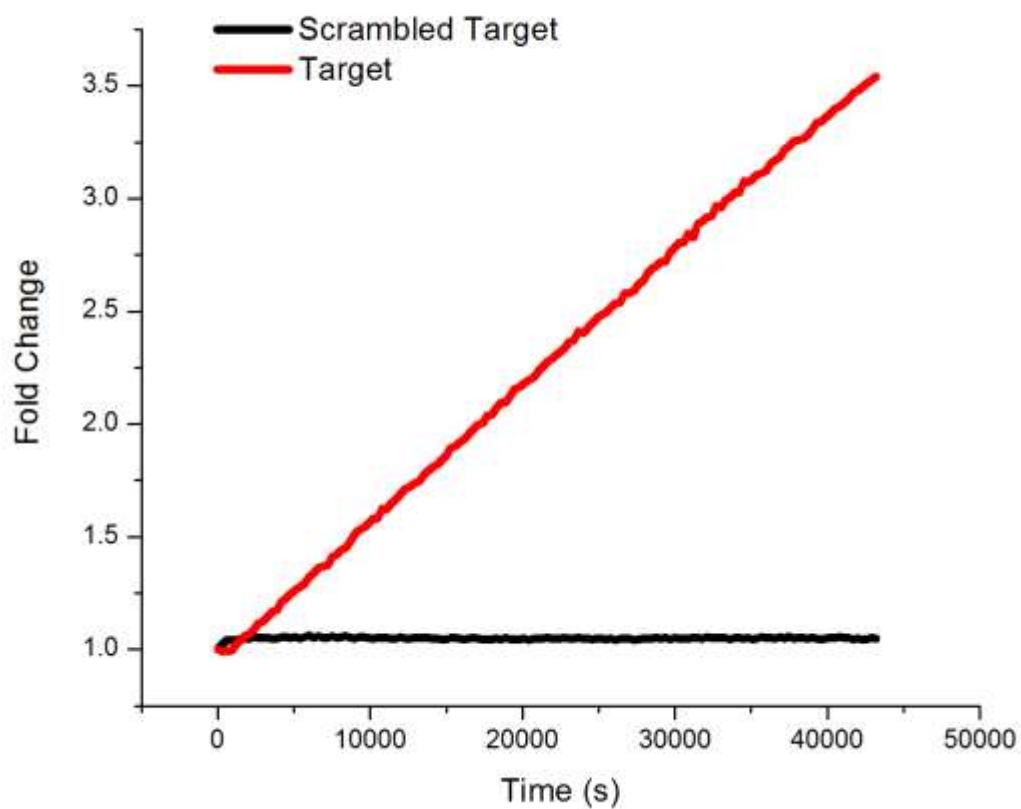


Figure S4: Target vs. Scrambled. A test to check for strand specific cutting. A scrambled target was prepared and mixed in with the target strand to check what would be cut. This figure shows that no scrambled target was cut by the DNAzyme and only the target strand was cut by the DNAzyme.

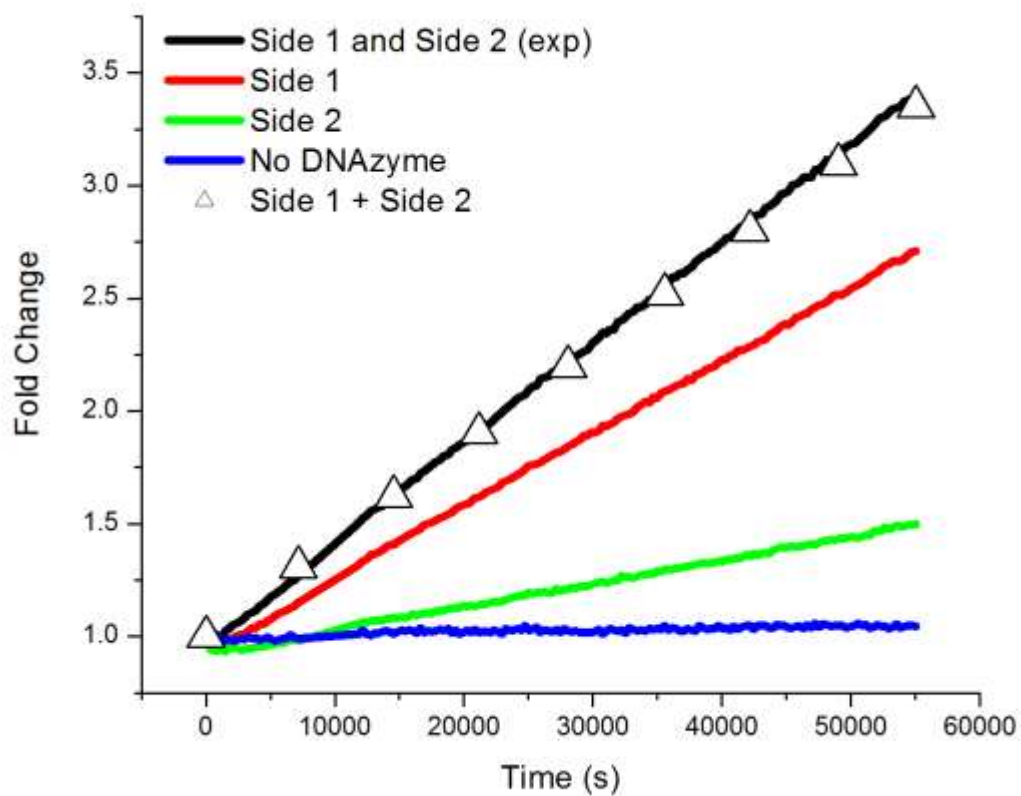


Figure S5: E6 Side 1 vs. Side 2. Four-way junctions with E6-type DNAzymes annealed into both sides (black), side 1 (red), or side 2 (green). 4WJ DNAzymes were prepared at a concentration of 0.5 μM and 1 μL of it was added into 50 μL of target strands (0.6 μM) in triplicate at 25°C. Addition of side 1 and side 2 data points (Δ) reveal that two sides' cutting rates were linearly superimposable.

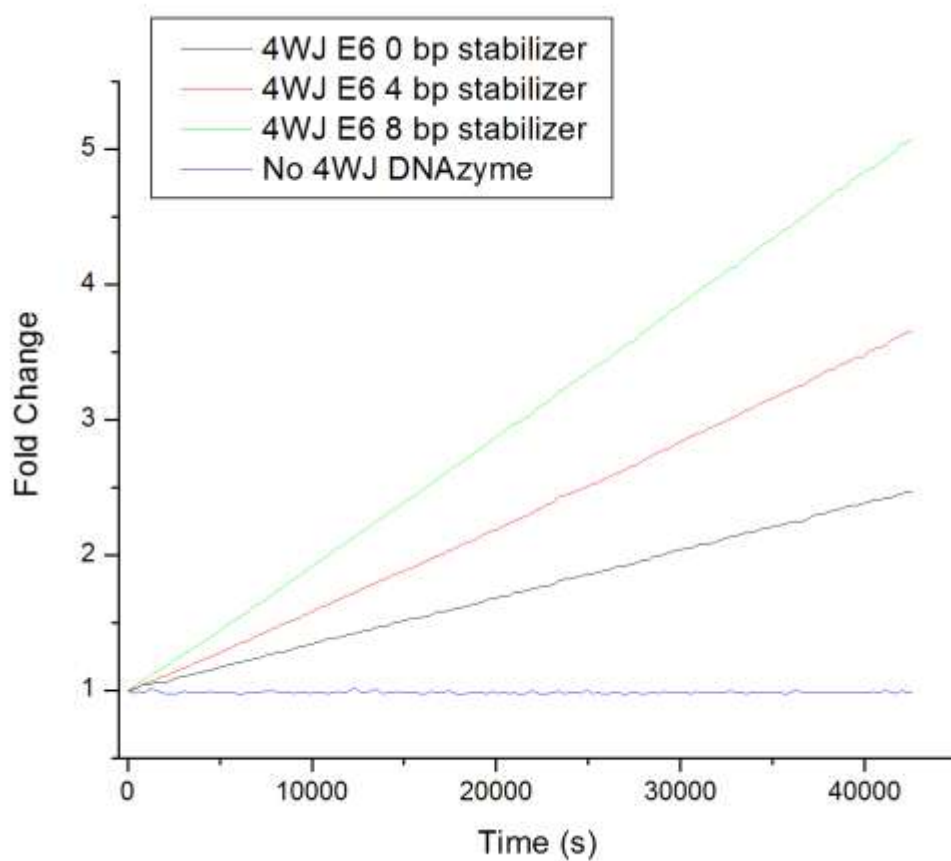


Figure S6: Comparison of 4WJ DNzyme Stabilizer Length. Comparison between 4WJ DNazymes with different length stabilizer section lengths between the 2 parts of the DNzyme when the 4WJ is closed. With a reporter strand concentration of 0.8 μM , the length of the stabilizer strand positively correlates with the rise in cutting velocity.

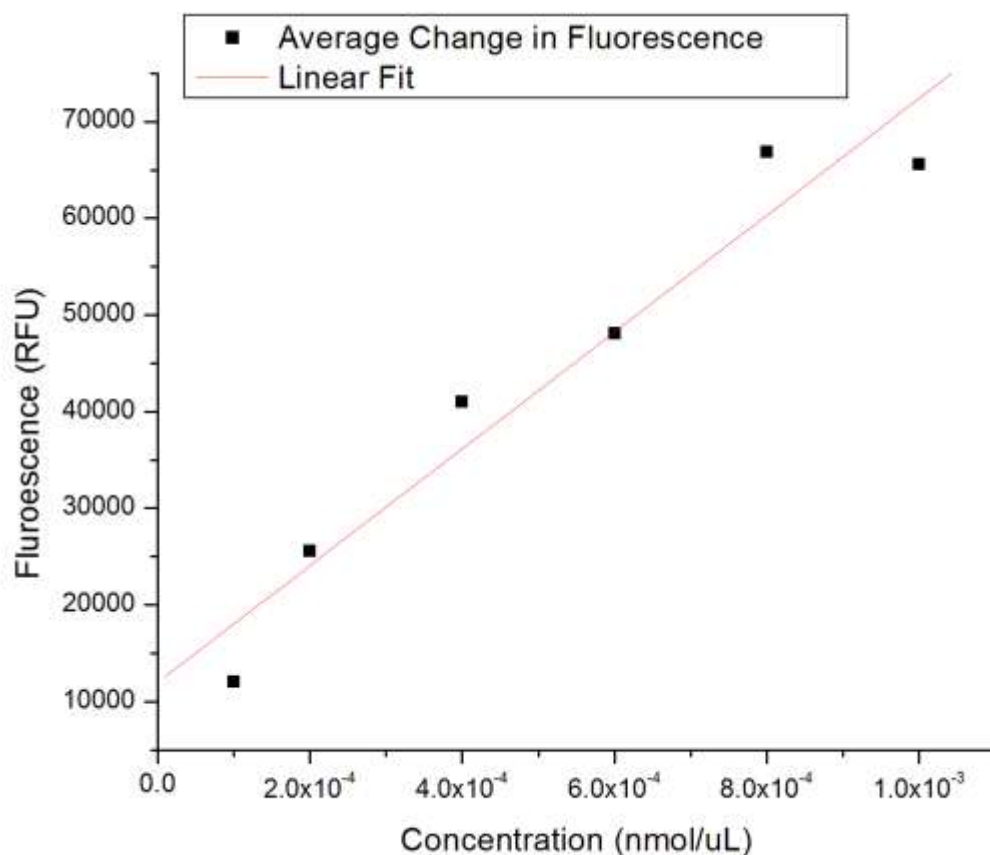


Figure S7: Fluorescence Conversion. For Michaelis-Menten kinetics, fluorescent units needed to be converted into estimates of concentration. Fluorescent units were converted into concentration by taking the difference between the averaged maximum and minimum fluorescent units at each concentration and plotting it against said concentration. The conversion factor, A , was obtained by finding the slope of a linear fit of this plot with units of fluorescent units per molar. Thus velocities measured were converted by the following equation.

$$V_c = V_F/A$$

V_c is velocity in concentration per unit time, V_F is the velocity in RFU per unit time, and A is the conversion factor.

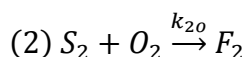
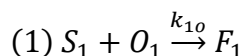
Table S1: List of all nucleotides used in experimentation. Notation: FAM- 6-carboxyfluorescein, IAbFQSp - Iowa Black® FQ quencher, TEX615- Texas Red, IAbRQSp – Iowa Black® RQ quencher, rA – Adenosine (RNA base), TET – tetrachlorofluorescein, IAbFQSp – Iowa Black® FQ quencher

Name	Sequence	Nt
N1	CGC AAT CCT ACC ACC ATC CAA ACT CTC AGA ATC GCA CAC AAC ACC CAA CCA CAA CAA ACC AAA CCA ACT ATA CC GCA AAC TCT AACT ATA CC	92
W1	GGT ATA GTT AGA GTT TGC GGT ATA GT TII TTT IIT TTI TTI TII TTI IIT CCA TCA TAA ATT CCC ATC CTT C TT CGT CCA TCC CTA CCC TTA	92
N2	TAA GGG TAG GGA TGG ACG AA A TTA GTA GAG AGA GAA TAA TGA CAC AAC ACA ACC AAA CAA CAC ACC CGT CGA CTT CCT AAA TCC AAA ATC AG	92
W2	CTG ATT TTG GAT TTA GGA AGT CGA CG IIT ITI TTI TTT IIT TIT ITT ITI AGA GTT TAT GAG CGA GGT AGA TTG GAT GGT GGT AGG ATT GCG	92
N1/W2	/FAM/ - TT GTG TGC GAT TCT GAG AAA ATC TAC CTC GCT CAT AAA CTC T-/FAM/	42
N2/W1	/IAbFQSp/TCA TTA TTC TCT CTC TAC TAA TTG GAA GGA TGG GAA TTT ATG ATG G/IAbFQSp/	46
E6 Target	/TEX615/ GAC GAG TrAGG AGC AGT /IAbRQSp/	16

Strand		
E6 Target scrambled	/5TET/ AGA GTA TrAGG GAT ATC/3IABkFQ/	16
N1 E6	ACT GCT C AGC GAT GTT GTG TGC GAT TCT GAG AGT T	35
W2 E6	ATC TAC CTC GCT CAT AAA CTC T CAC CCA TGT CTC GTC	37
N2 E6	ACT GCT C AGC GAT TCA TTA TTC TCT CTC TAC TAA T	35
W1 E6	GAAGG ATGGG AATTT ATGAT GG CAC CCA TGT CTC GTC	37
Open N1	TGG TTT GGT TTG TTG TGG TTG GGT TCA TAG	30
Close N1	CTA TGA ACC CAA CCA CAA CAA ACC AAA CCA	30
Open N2	GGT GTG TTG TTT GGT TGT GTT GTG GTC ATG	30
Close N2	CAT GAC CAC AAC ACA ACC AAA CAA CAC ACC	30

Supplemental 3: Curve Fitting:

To characterize the device different reaction schemes were used to describe the opening and closing of the device. The following reaction described the opening:



Where S_1 and S_2 represent the two closed sides of the 4WJ, O_1 and O_2 represent opening strands for the respective sides, and F is the normalized fluorescent signal representing an open side 1 or side 2 of the 4WJ. The fluorescence signal for the full device can be represented as an average of the normalized signal for side 1 and side 2.

$$(3) [F] = \frac{[F_1] + [F_2]}{2}$$

And the rate at which the fluorescent signal changes is thus given by:

$$(5) \frac{d[F_1]}{dt} = -\frac{d[S_1]}{dt} = k_{1o} [S_1][O_1]$$

$$(6) \frac{d[F_2]}{dt} = -\frac{d[S_2]}{dt} = k_{2o} [S_2][O_2]$$

The follow assumptions were made for both reactions:

$$(7) [S_1]_i = [S_2]_i = 1$$

$$(8) [S_1] = 1 - [F_1], \quad [S_2] = 1 - [F_2]$$

$$(9) [O_1] = [O_1]_i - [C_1]_i - [F_1], \quad [O_2] = [O_2]_i - [C_2]_i - [F_2]$$

The first assumption states that the maximum normalized fluorescent level during an experiment represents a completion of the reaction and thus represents the concentration of devices in solution. The second assumption is based on the one-to-one conversion of closed side 1 or 2 to fluorescent side 1 or 2. Likewise the concentration of opening strands at any particular time is the number of excess opening strands subtracted from the fluorescent open side 1 or side 2 strands. Nonlinear curve fitting of equations 5 and 6 can be used to find k_{10} and k_{20} :

$$(10) \frac{d[F_1]}{dt} = k_{10}(1 - [F_1])([O_1]_i - [C_1]_i - [F_1])$$

$$(11) \frac{d[F_2]}{dt} = k_{20}(1 - [F_2])([O_2]_i - [C_2]_i - [F_2])$$

Solving equations 5 and 6 respectively yields:

$$(12) \frac{[S_1]}{[O_1]} = \frac{[S_1]_i}{[O_1]_i} \exp(([S_1]_i - [O_1]_i)k_1 t)$$

$$(13) \frac{[S_2]}{[O_2]} = \frac{[S_2]_i}{[O_2]_i} \exp(([S_2]_i - [O_2]_i)k_2 t)$$

Substituting in the appropriate assumptions, analytical equations can be derived for F_1 and F_2 :

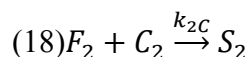
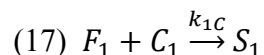
$$(14) [F_1] = \frac{[O_1]_i - ([O_1]_i - [C_1]_i) \exp((1 - [O_1]_i)k_{10} t)}{[O_1]_i - \exp((1 - [O_1]_i)k_{10} t)}$$

$$(15) [F_2] = \frac{[O_2]_i - ([O_2]_i - [C_2]_i) \exp((1 - [O_2]_i)k_{20} t)}{[O_2]_i - \exp((1 - [O_2]_i)k_{20} t)}$$

The average of these two signals represents the behavior of the full device opening.

$$(16) [F] = \frac{1}{2} \left(\frac{[O_1]_i - ([O_1]_i - [C_1]_i) \exp((1 - [O_1]_i)k_{10} t)}{[O_1]_i - \exp((1 - [O_1]_i)k_{10} t)} + \frac{[O_2]_i - ([O_2]_i - [C_2]_i) \exp((1 - [O_2]_i)k_{20} t)}{[O_2]_i - \exp((1 - [O_2]_i)k_{20} t)} \right)$$

The closing reaction is modeled as a reversible process following the below reaction equations:



where C_1 and C_2 represent the closing strands for their respective sides. The opening closing strands form a stable duplex O_1C_1 and O_2C_2 respectively that do not participate in the reaction. The full process can be also be modeled as the average of the normalized fluorescent closing of side 1 and side 2 like in equation 3. The kinetic equation for the closing reaction is given by

$$(19) \frac{d[F_1]}{dt} = -k_{+1c}[F_1][C_1]$$

$$(20) \frac{d[F_2]}{dt} = -k_{+2c}[F_2][C_2]$$

And the following assumptions were made for the closing part of the cycle:

$$(21) [S_1] = 1 - [F_1], [S_2] = 1 - [F_2]$$

$$(22) [C_1] = [C_1]_i - [O_1]_i - [F_1], [C_2] = [C_2]_i - [O_2]_i - [F_2]$$

The first assumption is as before; each 4WJ is composed of two sides which contribute to half the fluorescence. The second assumption is that the duplex waste product was created at the same rate and quickly and can be assumed to be concentration of opening strand in solution previously added subtracted by the amount of open 4WJ at a particular time. From these assumptions the following equation was used for fitting the closing reaction to find the reaction constants k_{1c}, k_{2c} .

$$(23) \frac{d[F_1]}{dt} = -k_{+1c}[F_1]([C_1]_i - [O_1]_i - [F_1])$$

$$(24) \frac{d[F_2]}{dt} = -k_{+2c}[F_2]([C_2]_i - [O_2]_i - [F_2])$$

The integrated forms of these rate equations are the following with the initial condition $F(t=0) = I$:

$$(25) [F_1] = \frac{([O_1]_i - [C_1]_i) \exp([O_1]_i - [C_1]_i) k_{1c} t}{[O_1]_i - [C_1]_i + 1 - \exp([O_1]_i - [C_1]_i) k_{1c} t}$$

$$(26) [F_2] = \frac{([O_2]_i - [C_2]_i) \exp([O_2]_i - [C_2]_i) k_{2c} t}{[O_2]_i - [C_2]_i + 1 - \exp([O_2]_i - [C_2]_i) k_{2c} t}$$

And thus the full device can be represented as:

$$(27) [F] = \frac{1}{2} \left(\frac{([O_1]_i - [C_1]_i) \exp([O_1]_i - [C_1]_i) k_{1c} t}{[O_1]_i - [C_1]_i + 1 - \exp([O_1]_i - [C_1]_i) k_{1c} t} + \frac{([O_2]_i - [C_2]_i) \exp([O_2]_i - [C_2]_i) k_{2c} t}{[O_2]_i - [C_2]_i + 1 - \exp([O_2]_i - [C_2]_i) k_{2c} t} \right)$$