Supplementary Information

A single-molecule FRET-based dynamic DNA sensor

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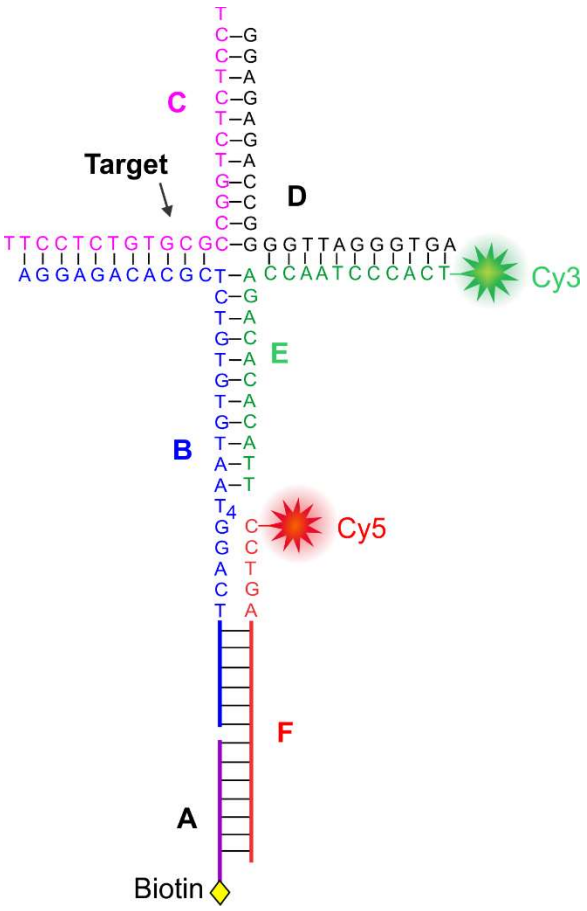
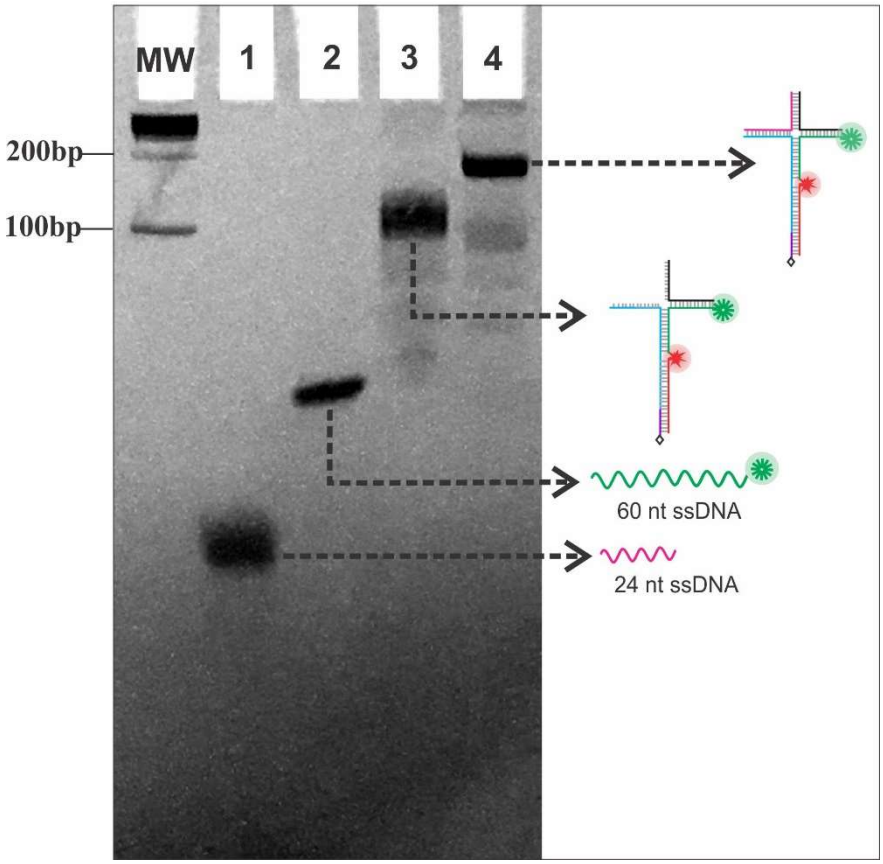
Supplementary Table S1. DNA oligonucleotides used in this study.

Strand Name	**Sequence (**5′-3′)
Strand A- (Biotin labeled)	/biotin/-AC GCG CTG GGC TAC GTC TTG CTG GCC GCA T
Strand B	CTG TGC GGT ATT TCA CAC CGT TAG CTC AGG TTT TAA TGT GTG TCT CGC ACA GAG GA
Strand C (p53gene-T1)	TTC CTC TGT GCG CCG GTC TCT CCT
Strand D	GGA GAG ACC GGG GTT AGG GTG A
Strand E (Cy3 strand)	/5Cy3/TCA CCC TAA CCA GAC ACA CAT T
Strand F (Cy5 Strand)	/Cy5/CCT GAG CTA ACG GTG TGA AAT ACC GCA CAG ATG CGG CCA GCA AGA CGT AGC CCA GCG CGT
Strand C (Mut1)	TTC CTC TGT GCT CCG GTC TCT CCT
Strand C (Mut2)	TTC CTC TGT GCA CCG GTC TCT CCT
Strand C (Mut3)	TTC CTC TCT GCG GCG GTC TCT CCT
Modified sequence for better stability (added nucleotides are in bold) of the sensor	
Strand B′	CTG TGC GGT ATT TCA CAC CGT TAG CTC AGG TTT TAA TGT GTG TCT CGC ACA GAG GA**A**
Strand D′	GGA GAG ACC GGG GTT AGG GTG **CG**A
Strand E′	/5Cy3/T**CG** CAC CCT AAC CAG ACA CAC ATT **AAA A**



Supplementary Figure S1. The 4-way sensor design with corresponding strand names (A to F) listed in Table 1. Biotin, Cy3 and Cy5 labels as well as the target have been identified.

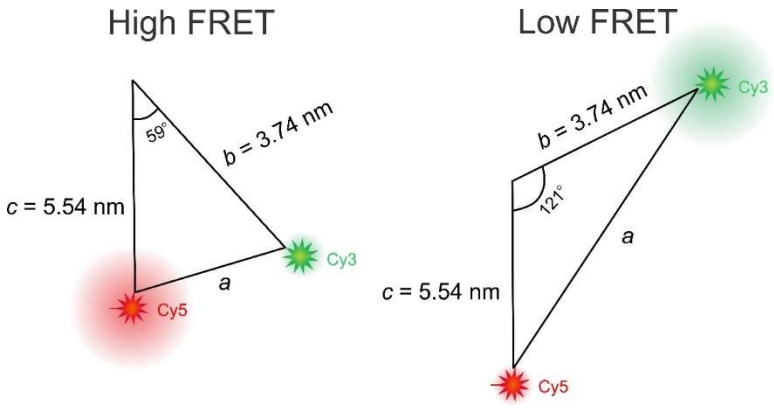


Supplementary Figure S2. Native gel characterization of the formation of the DNA sensor using a 12% polyacrylamide gel. MW: DNA molecular weight marker; Lane 1: 24 nt ssDNA; Lane 2: 60 nt ssDNA; Lane 3: sensor without the target, Lane 4: sensor with the target. The 24 nt ssDNA and 60 nt template strands were used as controls. Slower migration of the band in lane 4 compared to that of lane 3 confirms the successful assembly of the sensor in the presence of target. The gel was run for 90 minutes at 75 V and stained in ethidium bromide (EtBr) solution for 20 min before taking an image under UV-Vis transilluminator.

Supplementary Table S2. Estimated inter-dye distances (*R*) as well as estimated and experimentally determined FRET efficiencies (*E*) for different conformations of the sensor. It has been reported that the average inter-helix angles are approximately 59° and 121° for conformer 1 (*iso-I*) and conformer 2 (*iso-II*) of the HJ[1,2,3], which give the high-FRET and low-FRET states, respectively. Considering the parameters: 0.34 nm height per base pair in the dsDNA; ~0.45 nm contour length per nucleotide in the ssDNA; 11 bp arm E of the junction (3.74 nm), 11 bp + 4 nt arm F is (~5.54 nm), we determined the expected FRET efficiencies for the two conformational states of the sensor using Equation 1.

$$E_{\mathrm{FRET}} = \frac{1}{1+\left(\frac{R}{R_0}\right)^6} \quad \ldots\ldots\ldots\ldots\ldots\ldots \textit{(Eq. 1)}$$

where R_0 is the inter-dye distance at 50% FRET efficiency and is 5.4 nm for the Cy3/Cy5 pair.[4]



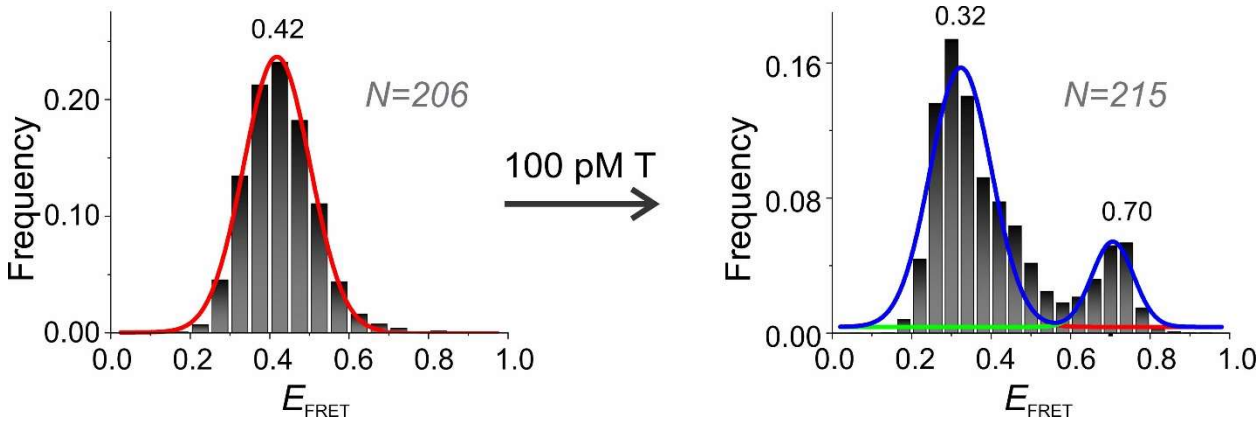
The inter-dye distance was calculated using the cosine rule for non-right angle triangle.[5]

$$a^2 = b^2 + c^2 - 2bc\,\cos(A) \quad \ldots\ldots\ldots\ldots\ldots\ldots \textit{(Eq. 2)}$$

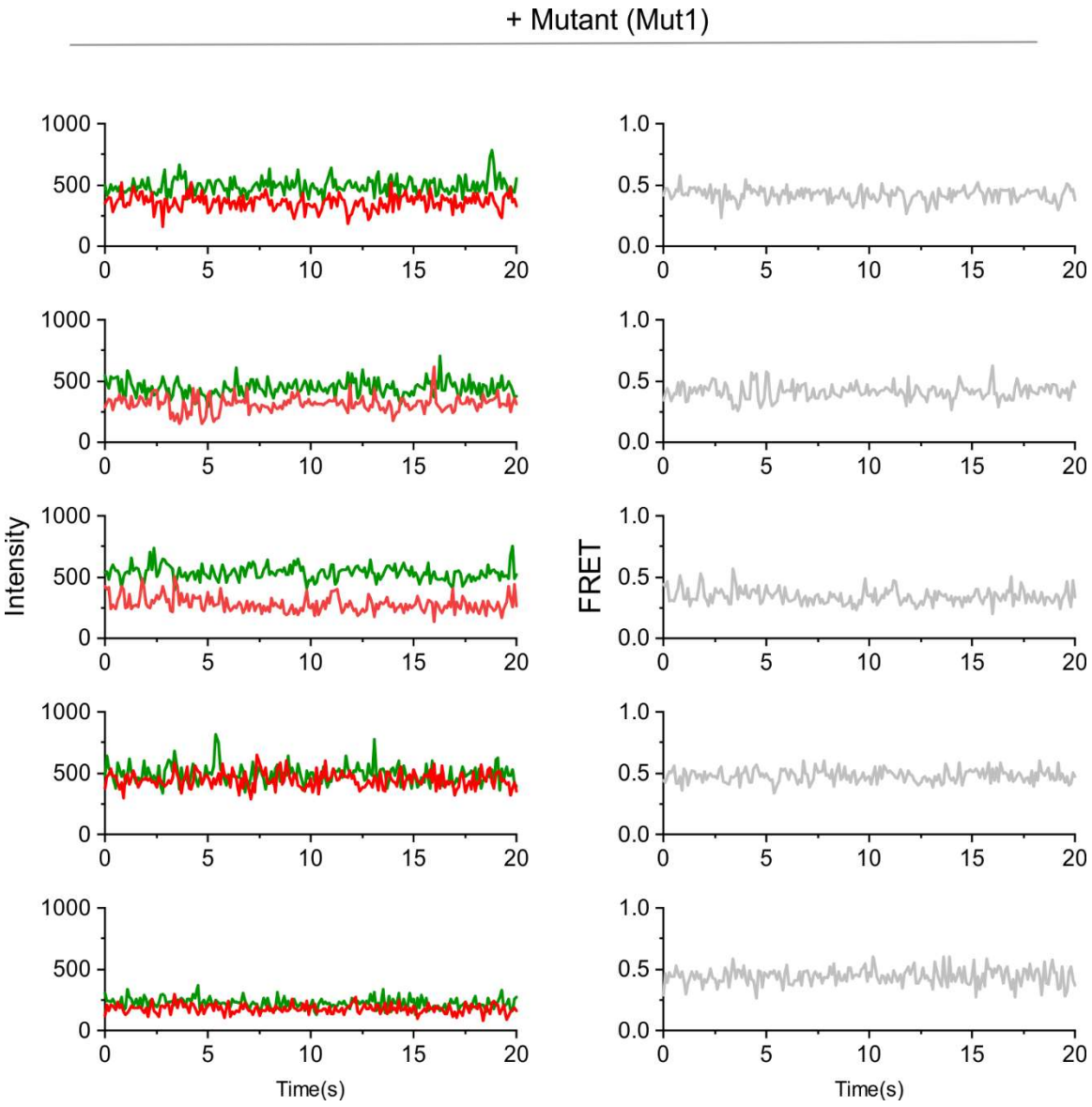
Where A = 59° and 121° for high FRET and low FRET states respectively and *a* is the inter-dye distance (*R*).

Note that the inter-dye distance (*R*) and estimated FRET efficiency (*E*) values are calculated without considering dye linkers and other local microenvironments.

Conformation	Estimated *R* and FRET Efficiency (*E*)	Experimental *R* and FRET Efficiency (*E*)
High-FRET state	R = 4.83 nm, E = 0.66	R = 4.69 nm, E = 0.70
Low-FRET state	R = 6.17 nm, E = 0.31	R = 6.12 nm, E = 0.32



Supplementary Figure S3. smFRET analysis of the sensor in the absence (left) and presence of 100 pM Target (right) in 1× TAE buffer (pH 7.4) and 100 mM Mg^{2+}. While relatively static traces were obtained in the absence of target, dynamic traces were obtained in the presence of target.



Supplementary Figure S4. Typical single molecule traces in the presence of mutant 1 (Mut1). Typical intensity-time (left) and corresponding FRET traces (right). Five representative molecules are shown. The molecules exhibited static fluorescence intensities of Cy3 and Cy5. A static FRET level of ~0.5 was observed in the absence of target DNA. All experiments were done at room temperature (23°C).

References

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