Supporting Information

Kinetics of Proximity-Induced Intramolecular DNA Strand Displacement

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Table S1. DNA sequences and modifications. Colors are assigned to each DNA sequence according to schemes in Figures 1-4, S1, S2, and S4.

DNA name	Sequences
O	5'-ATA GAT CCT CAT AGC GAG ACC TAG CAA-3'
T (12 nt)	5'-biotin-TTT TTT TTT TTT TTT TTG CTA GGT CTC-3'
T (14 nt)	5'-biotin-TTT TTT TTT TTT TTT TTG CTA GGT CTC GC-3'
T (16 nt)	5'-biotin-TTT TTT TTT TTT TTT TTG CTA GGT CTC GCT A-3'
C (12 nt)	5'-GAG ACC TAG CAA TTT TTT TTT TTT TTT-biotin-3'
C (14 nt)	5'-GC GAG ACC TAG CAA TTT TTT TTT TTT TTT-biotin-3'
C (16 nt)	5'-T AGC GAG ACC TAG CAA TTT TTT TTT TTT TTT-biotin-3'
C-H4	5'-GAG ACC TAG C**AAAA** TTTT TTT TTT TTT-biotin-3'
C-H6	5'-GAG ACC TAG C**AAAAAA** TT TTT TTT TTT-biotin-3'
C'	5'-GAG ACC TAG CAA TCAGTG-3'
D2	5'-GA TTG CTA GGT CTC-3'
D4	5'-CTGA TTG CTA GGT CTC-3'
D6	5'-CACTGA TTG CTA GGT CTC-3'
F	5'-FAM-ATA GAT CCT CAT AGC GAG AC-3'
Q	5'-TTG CTA GGT CTC GCT ATG AGG ATC TAT-Dacyl-3'
F'	5'-FAM-GAG ACC-TTT TTT TTT TTT TTT-biotin-3'
Q'	5'-biotin-TTT TTT TTT TTT TTT-GGT CTC-Dacyl-3'
Apt-T10	5'-TACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-TTT TTT TTTT TTG CTA GGT CTC-3'
Apt-T15	5'-TACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-TTT TTT TTT TTT TTT TTG CTA GGT CTC-3'
Apt-T24	5'-TACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-TTT TTT TTT TTT TTT TTT TTT TTT TTG CTA GGT CTC-3'
Apt-C10	5'-GAG ACC TAG CAA TTT TTTT TTT-T ACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-3'
Apt-C15	5'-GAG ACC TAG CAA TTT TTT TTT TTT TTT-T ACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-3'
Apt-C24	5'-GAG ACC TAG CAA TTT TTT TTT TTT TTT TTT TTT TTT-T ACT CAG GGC ACT GCA AGC AAT TGT GGT CCC AAT GGG CTG AGTA-3'

Monitor the Kinetics of Streptavidin-Biotin Interaction Using Fluorescence Quenching Assay

For a typical fluorescence quenching assay to measure streptavidin-biotin interaction, the reaction mixture contains 10 nM FAM-biotin dual labeled DNA probe (**F'**), 10 nM quencher-biotin dual labeled DNA probe (**Q'**), 50 nM ROX reference dye, 1 μM polyT oligo, varying concentrations of streptavidin, and TE-Mg buffer. Fluorescence was measured immediately after adding the mixture to the well of microplate and kept measuring every 1.5 min for the first 30 min and then every 5 min for another 20 min using a multi-mode microplate reader (DX880, Beckman Coulter) with both excitation/emission at 485/515 nm for the displacement beacon and excitation/emission at 535/595 nm for ROX as a reference dye. The relative fluorescent intensity was achieved by normalizing all fluorescence signals against the fluorescence signal obtained for the blank ([streptavidin] = 0 nM) at time point t = 0 min.

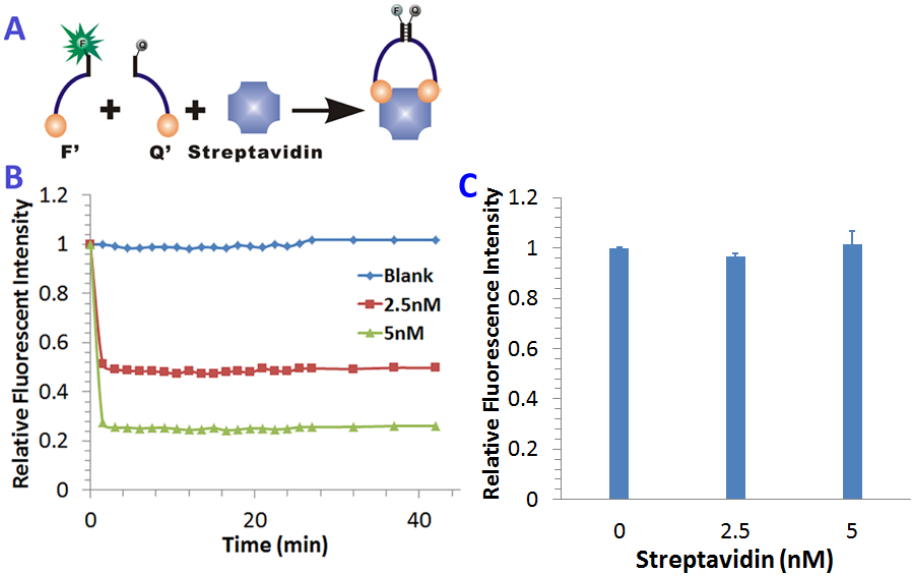

Figure S1. (**A**) Schematic illustrating the binding-induced fluorescence quenching assay to monitor the streptavidin-biotin interaction in real-time. DNA probes **F'** and **Q'** were designed to contain a biotin motif each and have a short complementary sequence of 5 nt. Therefore, in the absence of target streptavidin, **F'** and **Q'** could not hybridize due to the short complementary part, thus fluorescence signal from F' stayed on. In the presence of streptavidin, the binding of the two biotin molecules to the same streptavidin brought **F'** and **Q'** to close proximity. As a result, **F'Q'** DNA duplex was formed and fluorescence signal of F' was quenched. By monitoring the fluorescence attenuation in real-time, we were able to determine the kinetic profile of streptavidin-biotin binding. (**B**) Fluorescence attenuation as a function of time. No fluorescence attenuation was observed when only mixing probes **F'** and **Q'** in buffer (Blank). However, immediately when target streptavidin was mixed with **F'** and **Q'**, the fluorescence was quenched and saturated within 1 minute, confirming the fast binding kinetics between streptavidin and biotin. (**C**) Control experiment to exclude the potential influence of streptavidin by itself on the fluorescence of **F'**. The reaction mixture contained 20 nM **F'** and varying concentrations of streptavidin in buffer. The mixture was incubated at room temperature for 50 min and end point fluorescence was detected using a multimode microplate reader. No fluorescence attenuation was observed when mixing streptavidin with **F'**.

Monitor the Kinetics of Proximity-Induced Intramolecular DNA Strand Displacement

For a typical intramolecular DNA strand-displacement reaction, the reaction mixture contained 10 nM **OT**, 10 nM competing DNA (**C**), 20 nM **FQ**, 50 nM ROX reference dye, 1 μM polyT oligo, varying concentrations of streptavidin, and TE-Mg buffer. The reaction mixture was incubated at 25 ℃ for 150 min in a 96-well microplate. Fluorescence was measured immediately after adding the mixture to the well of microplate and kept measuring every 1.5 min for the first 30 min and then every 5 min for another 2 hours using a multi-mode microplate reader (DX880, Beckman Coulter) with both excitation/emission at 485/515 nm for the displacement beacon and excitation/emission at 535/595 nm for ROX as a reference dye. The measured fluorescent signal was normalized so that 1 n.u. of fluorescence corresponded to fluorescent signal generated by 1 nM **O**. This normalization was achieved using a positive control containing 10 nM **O**, 20 nM **FQ**, 1 μM polyT oligo, and 50 nM Rox in TE-Mg buffer, and a negative control containing identical reagents in positive control except that there was no **O** added .

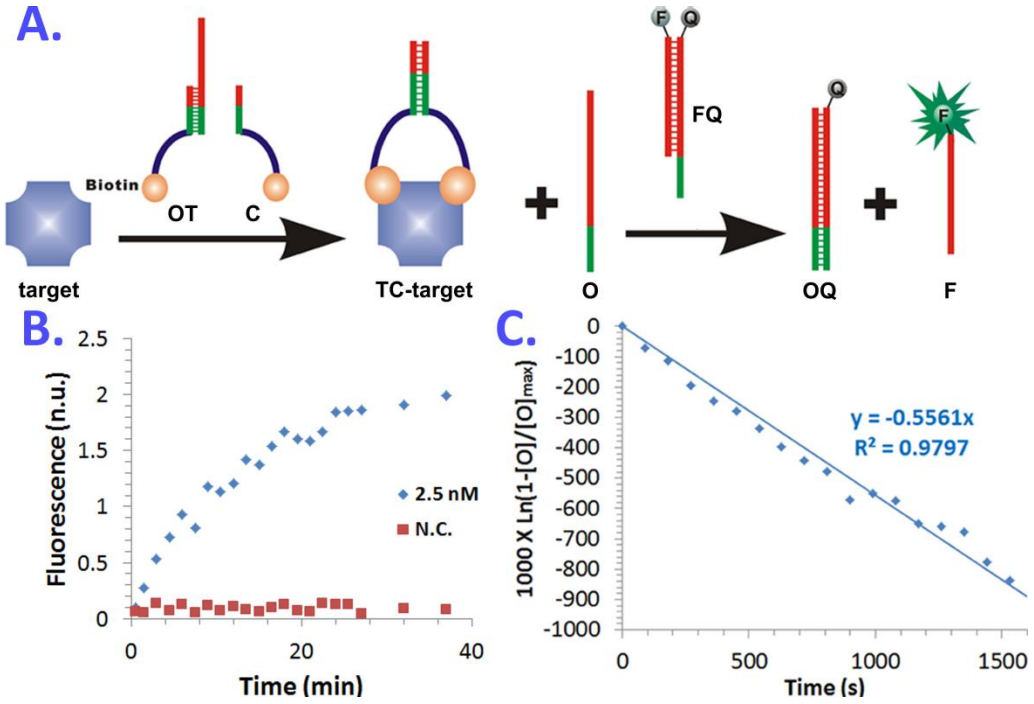

Figure S2. (A) Schematic illustration the strategy to monitor each PiDSD reaction in real-time by using the displacement beacon **FQ**. (B) Real-time monitoring PiDSD reactions in the presence of 2.5 nM streptavidin. The fluorescent intensity was normalized such that 1 normalized unit (n.u.) corresponds to 1 nM **O**. The sample (target) solution contained 2.5 nM streptavidin, 10 nM OT, 10 nM C, and 20 nM FQ in TE-Mg buffer. In the negative control (N. C.), all reagents were the same as in the sample, except that there was no streptavidin. **(B)** Determination of the reaction rate constant k_{obs} by plotting data calculated using equation **(5)** and a subsequent linear regression.

Monitor the Kinetics of Intermolecular DNA Strand exchange

For a typical intermolecular DNA strand exchange, the reaction mixture contained 10 nM **OT**, 20 nM **FQ**, 50 nM ROX reference dye, 1 μM polyT oligo, varying concentrations of competing DNA **C**, and TE-Mg buffer. The reaction mixture was incubated at 25 ℃ for 150 min in a 96-well microplate. Fluorescence was measured immediately after adding the mixture to the well of microplate and kept measuring every 1.5 min for the first 30 min and then every 5 min for another 2 hours using a multi-mode microplate reader (DX880, Beckman Coulter) with both excitation/emission at 485/515 nm for the displacement beacon and excitation/emission at 535/595 nm for ROX as a reference dye. The measured fluorescent signal was normalized so that 1 n.u. of fluorescence corresponded to fluorescent signal generated by 1 nM **O**. This normalization was achieved using a positive control containing 10 nM **O**, 20 nM **FQ**, 1 μM polyT oligo, and 50 nM Rox in TE-Mg buffer, and a negative control containing identical reagents in positive control except that there was no **O** added. Reaction rate constants were estimated by plotting the value of $\boldsymbol{ln(1-[O]_t/[O]_{max})}$ at each time point against the reaction time *t*, and a least-square linear regression was used to determine the observed rate constant k_{obs}. Linear fittings were performed using OriginPro 9 and confidence intervals were determined using the York method.

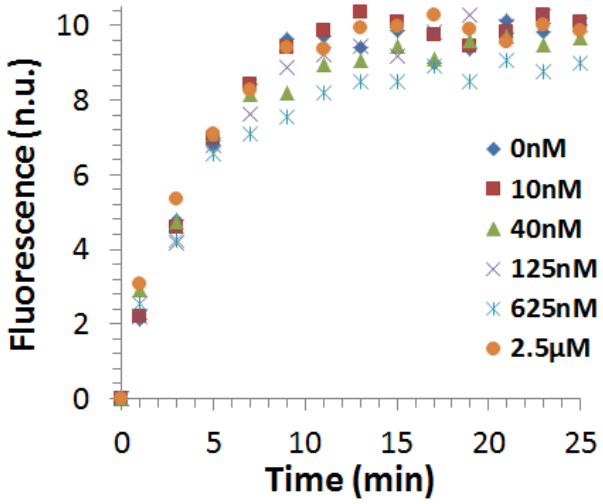

Figure S3. Effect of varying concentrations of **C** on the toehold-mediated DNA strand displacement between **O** and **FQ**. The reaction solution contained 10 nM **O**, 20 nM **FQ**, and varying concentrations of **C12** from 0 nM to 2.5 µM in TE-Mg buffer.

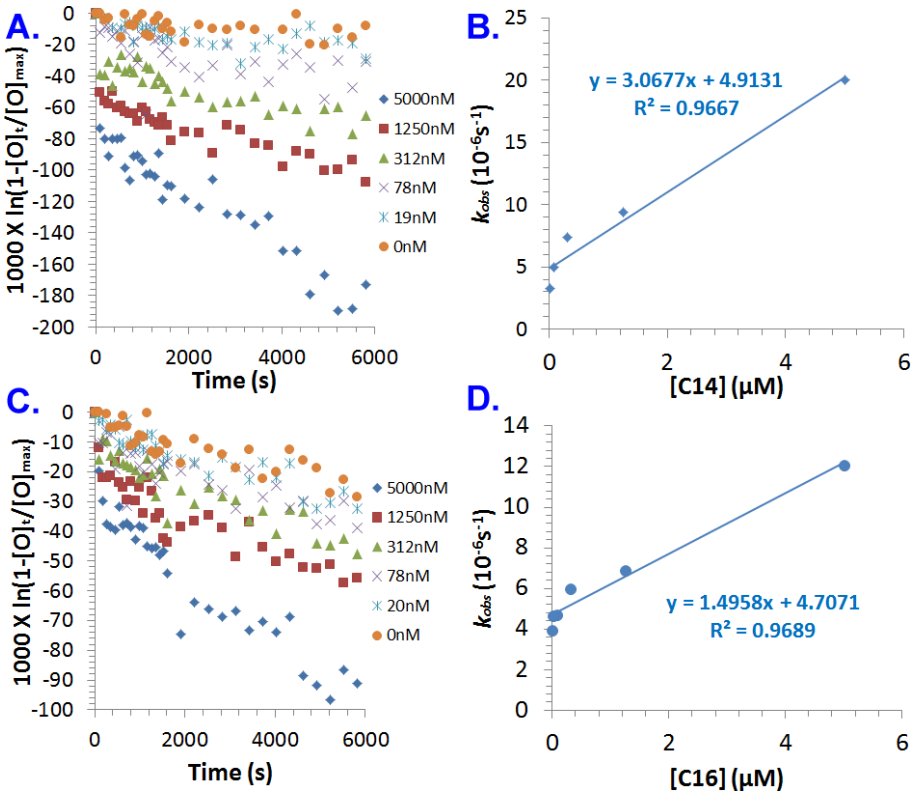

Figure S4. Monitoring the intermolecular strand exchange between **OT** and **C** using the displacement beacon **FQ**. (**A**) Determining the rate constant k_{obs} for **C14** under different concentrations of **C14** by plotting the data according to equation (**4**). (**B**) The value of the observed rate constant as a function of the concentrations of the competing DNA **C14**, providing the information on k_1 and k_2 for intermolecular strand displacement between **OT14** and **C14**; (**C**) Determining the rate constant k_{obs} for **C16** under different concentrations of **C16** by plotting the data according to equation (**4**). (**D**) The value of the observed rate constant as a function of the concentrations of the competing DNA **C16**, providing the information on k_1 and k_2 between **OT16** and **C16**.

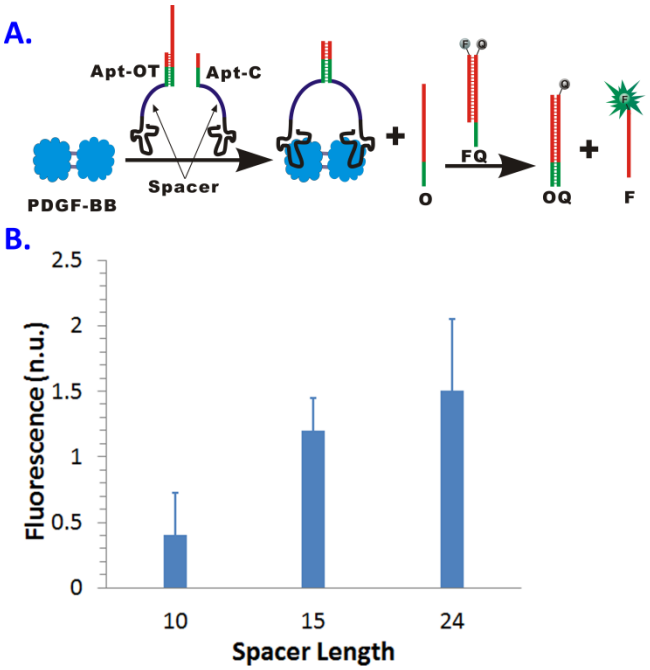

Figure S5. Effect of the spacer length on the kinetics of PiDSD. **(A)** Schematic illustration of a PiDSD that is triggered by the binding between PDGF-BB and its aptamers. Aptamers that are specific to PDGF-BB were conjugated to DNA probes OT (Apt-**OT**) and C (Apt-**C**) through a polydT spacer of varying lengths. In the presence of PDGF-BB, two aptamers binding to the same target assembles **OT** and **C** into close proximity, triggering the PiDSD between **OT** and **C** and the release of output DNA **O** that can be detected by the displacement beacon **FQ**. **(B)** The amount of the output DNA **O** generated by PDGF-BB through PiDSD as a function of spacer length. For each reaction, 20 nM Apt-**OT**, 20 nM Apt-**C**, 20 nM **FQ**, and 10 nM PDGF-BB were mixed in TE-Mg buffer. The fluorescence intensity was normalized such that 1 normalized unit (n.u.) corresponds to 1 nM **O**. When increasing in spacer length from 10 nt to 24 nt, increases in signal response were observed in the presence of 10 nM PDGF-BB over an incubation time of 30 min. This trend is the opposite of that was observed for the remote DNA toehold, suggesting that the flexibility to ensure sufficient PiDSD shall be a primary concern when designing DNA spacers for proximity assays and protein-responsive DNA devices. Each error bar represents a standard deviation from triplicate analyses.