Electronic supplementary information

Fine and bidirectional regulation of toehold-mediated DNA strand displacement by a wedge-like DNA tool

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Longjie Li,[1, 2, ‡] Wenkai Zhang,[1, 3, ‡] Xiaofeng Tang,[1] Zejian Li,[1] Yuzhou Wu,[2, *] and Xianjin Xiao[1, 3, *]

1 Institute of Reproductive Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. E-mail: xiaoxianjin@hust.edu.cn

2 Hubei Key Laboratory of Bioinorganic Chemistry and Materia Medica, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China. E-mail: wuyuzhou@hust.edu.cn

3 Department of Obstetrics and Gynaecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

‡ These authors contributed equally.

Materials and Methods

Materials and Instrumentation

ThermoPol reaction buffer were all purchased from New England Biolabs (MA, USA). The DNA sequences were synthesized from Sangon Co. (Shanghai, China) and purified by HPLC. All the DNA sequences were examined by NUPACK to limit secondary structures. Fluorescence measurements were performed on a Biotek Synergy HTX microplate reader (BioTek Instruments, Inc., VT, USA). DNA concentration measurements were performed on NanoDrop 2000(Thermo Fisher Scientific, USA).

Buffer Conditions

DNA oligonucleotides were stored in TE buffer. All DNA oligonucleotides were stored at 4°C.

Annealing[1]

240nM FAM labelled strand and 240nM BHQ labelled stand were first added. 4.8 µL of 10× ThermoPol Buffer was subsequently added and the total volume was brought up to 45 µL by deionized water (Tiangen Biotech, Beijing). The mixture was then heated and annealed with a designed procedure (85 °C for 60 s, 55 °C for 60 s, 37 °C for 5min).

Spectrofluorimetry[1-2]

We labelled the fluorophore FAM and the quencher BHQ1 at the end of target strand and template strand, respectively. The extent of the strand displacement reaction could be monitored by measuring increase of the fluorescence. 45ul annealed mixture were added into an ELISA plate. 240nM regulation strand were subsequently added (the concentration would change in different part of experiment) and the total volume was brought up to 50 µL by deionized water. The solution was then kept at 37 °C for 10 min and put into the plate reader to detect the fluorescence intensity under a detection temperature of 37 °C. Fluorescence was recorded with excitation/emission wavelengths of 485 nm/525 nm. Fluorescence intensity was measured every 60s. The overall detection time was 3600s.

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Supplementary Table

Sequence of different DNA strands

Name	Sequence
Template strand	BHQ1-GCATAAGAAATCTACCGAACCCCTATTCCTACCACCAAATACCCAC
Target strand	TCGGTAGATTTCTTATGC-FAM
Invading strand-18 (IS18)	GTGGGTATTTGGTGGTAGCCTTTTGCTATCGGTAGATTTCTTATGC
20(PW)	GAATAGGGGTTAGCAAAAGG
19-1(PW) *(this wedge is used in Fig. S2)*	AATAGGGGTTAGCAAAAGG
19-2(PW) *(this wedge is used in Fig. 2, Fig 3c and Fig. S3)*	GAATAGGGGTTAGCAAAAG
18(PW)	AATAGGGGTTAGCAAAAG
17-1(PW) *(this wedge is used in Fig. S2)*	ATAGGGGTTAGCAAAAG
17-2(PW) *(this wedge is used in Fig. 2)*	AATAGGGGTTAGCAAAA
16(PW)	ATAGGGGTTAGCAAAA
20(NW)	TAGCAAAAGGGAATAGGGGT
19-1(NW) *(this wedge is used in Fig. S2)*	TAGCAAAAGGGAATAGGGG
19-2(NW) *(this wedge is used in Fig. 2)*	AGCAAAAGGGAATAGGGGT
18(NW)	AGCAAAAGGGAATAGGGG
17-1(NW) *(this wedge is used in Fig. S2)*	AGCAAAAGGGAATAGGG
17-2(NW) *(this wedge is used in Fig. 2 and Fig. S3)*	GCAAAAGGGAATAGGGG
16(NW)	GCAAAAGGGAATAGGG
(Second DNA circuit) Template strand	GCATAAGAAATCTACCGATCCCCTATTCAACACTGATCCTACCACCAAATACCCACCTTGACTT
(Second DNA circuit) Invading strand	AAGTCAAGGTGGGTATTTGGTGGTAGTGCGATTCACACTTTTGCTATCGGTAGATTTCTTATGC
PWor-1	GTGTTGAATAGGGGATAGCAAAAGTGTGAATCGCA
PWor-2 (input Z)	GATCAGTGTTGAATAGGGGATAGCAAAAGTGTGAA
input X	GATCAGTGTTGAATAGGGGAATCAGGAAGCATACATCAGG
input Y	CCTGATGTATGCTTCCTGATTAGCAAAAGTGTGAATCGCA
(First DNA circuit) Invading strand-10	TTGGTGGTAGTGCGATTCACACTTTTGCTATCGGTAGATTTCTTATGC
(First DNA circuit) template strand	TGCGATTCACACTTTTGCTATCCCCTATTTCATACGTACCTGACTCGATGACCTGACCA
(First DNA circuit) PWand-1	ATCGAGTCAGGTACGTATGAGGGAGCCATGTATGATCTAC
(First DNA circuit) PWand-2	GTAGATCATACATGGCTCCCTTCCTATGGCTTCTTCTCAG
Invading strand-6 (IS6)	TGGTAGCCTTTTGCTATCGGTAGATTTCTTATGC
Invading strand-8 (IS8)	GGTGGTAGCCTTTTGCTATCGGTAGATTTCTTATGC
Invading strand-10 (IS10)	TTGGTGGTAGCCTTTTGCTATCGGTAGATTTCTTATGC
Invading strand-12 (IS12)	ATTTGGTGGTAGCCTTTTGCTATCGGTAGATTTCTTATGC
(Second DNA circuit) invading strand-8 (SIS8)	GTCAGGTCCTGAGAAGAAGCCATAGGAAAATAGGGGATAGCAAAAGTGTGAATCGCA
(Second DNA circuit) invading strand-7 (SIS7)	TCAGGTCCTGAGAAGAAGCCATAGGAAAATAGGGGATAGCAAAAGTGTGAATCGCA
(Second DNA circuit) invading strand-6 (SIS6)	CAGGTCCTGAGAAGAAGCCATAGGAAAATAGGGGATAGCAAAAGTGTGAATCGCA

Supplementary Figures

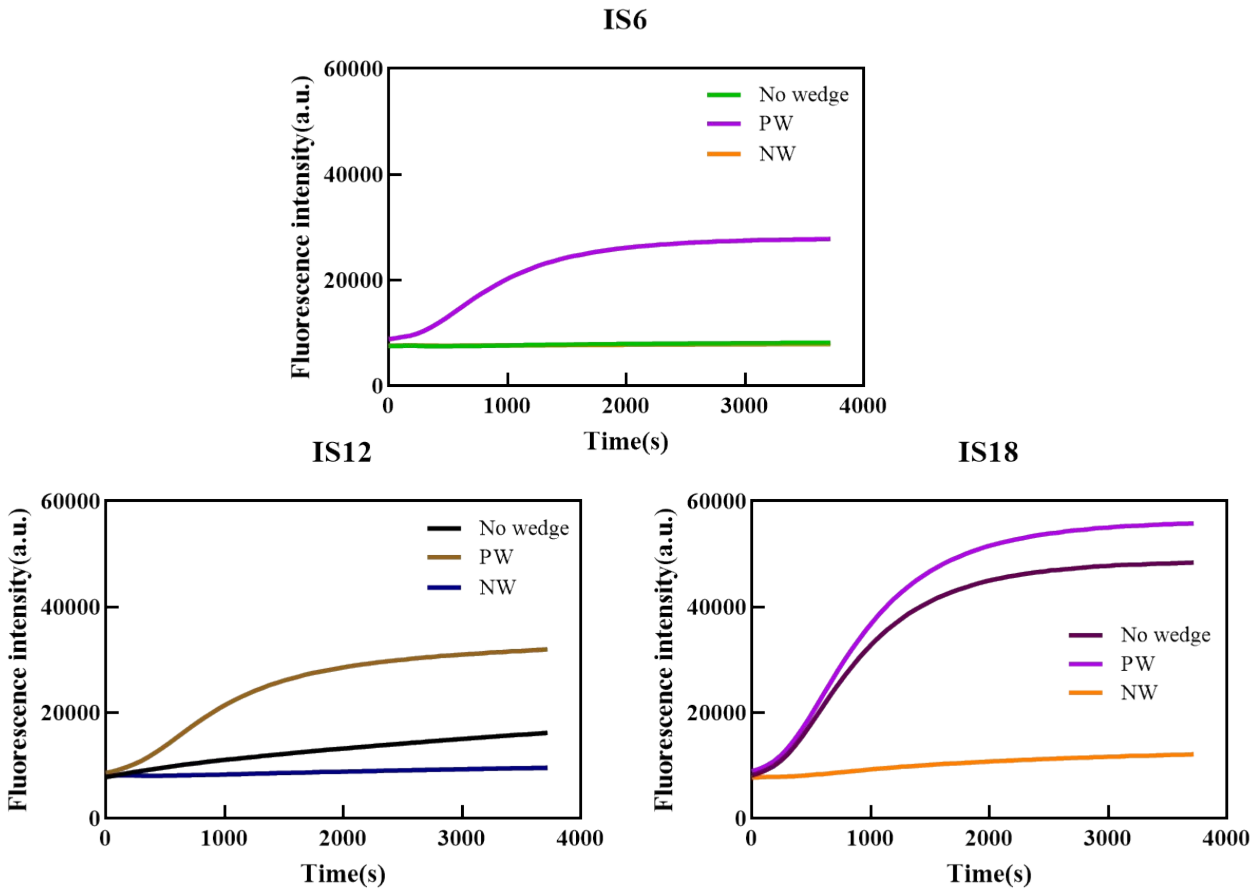

Figure S1. The effect of the length of toehold of invading strand on the DNA strand displacement reaction. IS6 represents the invading strand, of which the length of toehold is 6nt. IS12 represents the invading strand, of which the length of toehold is 12nt. IS18 represents the invading strand, of which the length of toehold is 18nt.

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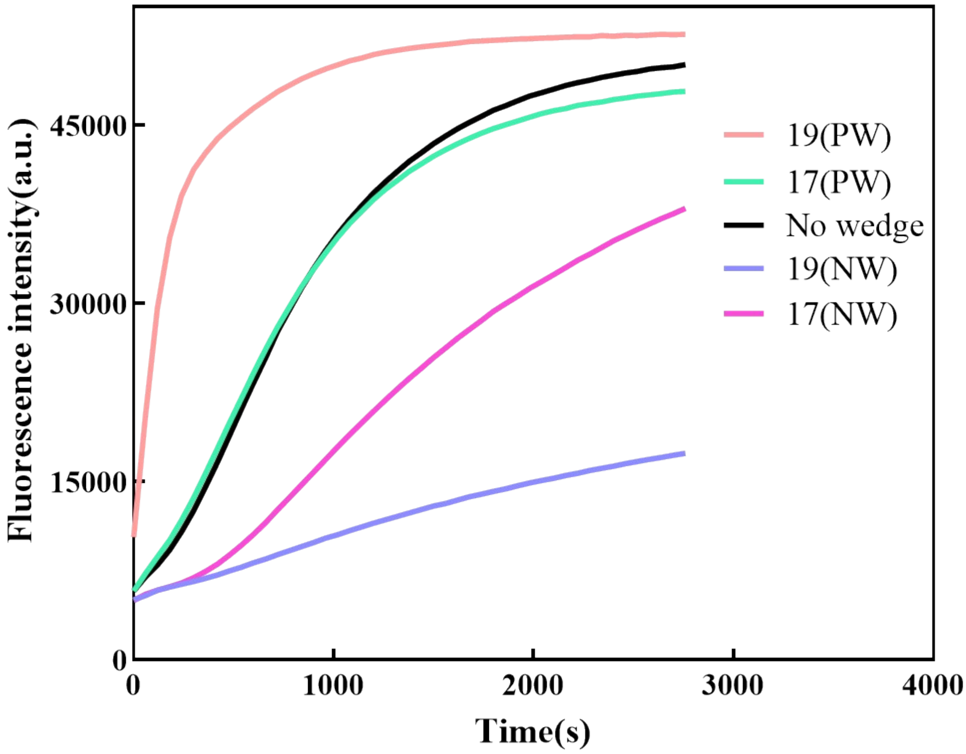

Figure S2. The effect of the length of positive wedge (19-1, 17-1) or negative wedge (19-1, 17-1) on DNA strand displacement reaction.

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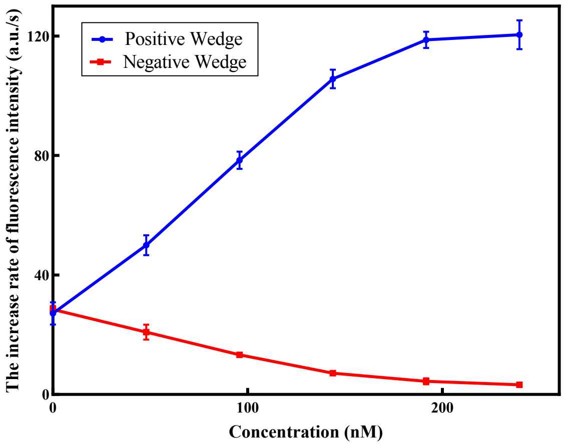

Figure S3. The increase rates of fluorescence intensity of DNA strand displacement reactions using different concentrations of positive wedge or negative wedge.

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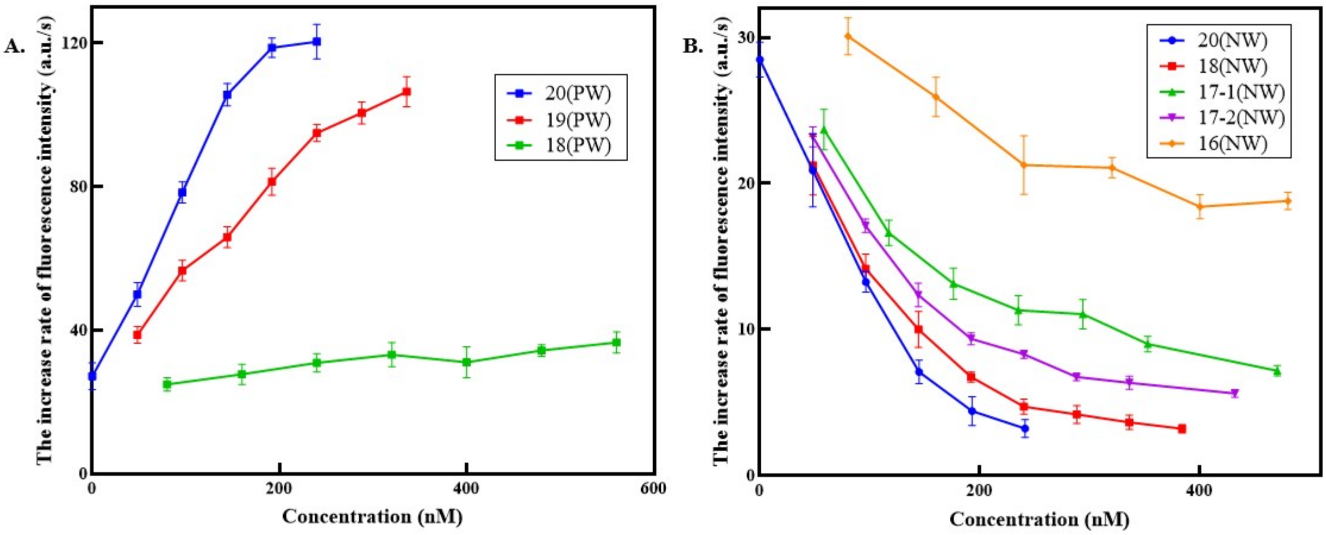

Figure S4. the increase rates of fluorescence intensity of DNA strand displacement reaction regulated by different concentration and length of wedges.

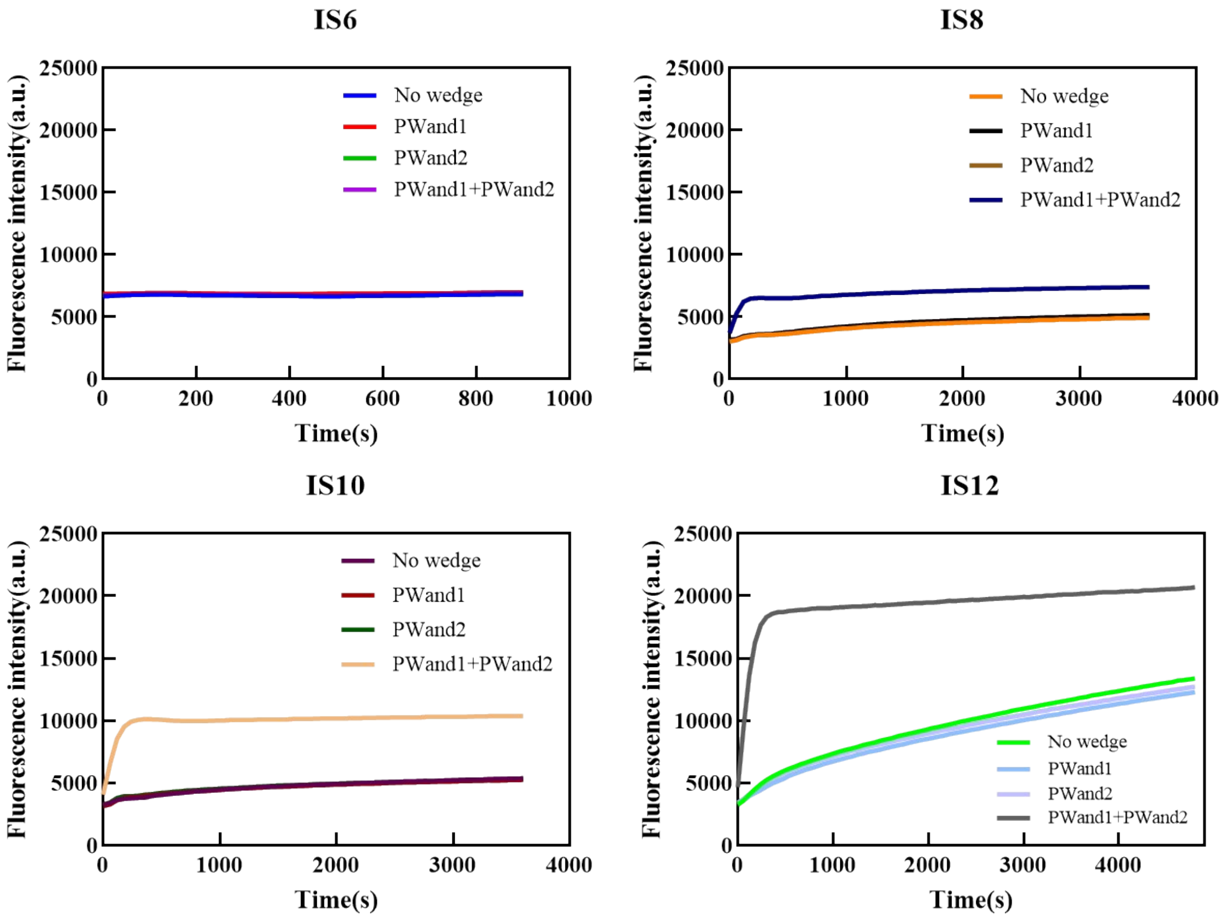

Figure S5. The effect of length of invading strand on the “AND” gate of the DNA circuit. IS6 represents the invading strand, of which the length of toehold is 6nt. IS8 represents the invading strand, of which the length of toehold is 8nt. IS10 represents the invading strand, of which the length of toehold is 10nt. IS12 represents the invading strand, of which the length of toehold is 12nt.

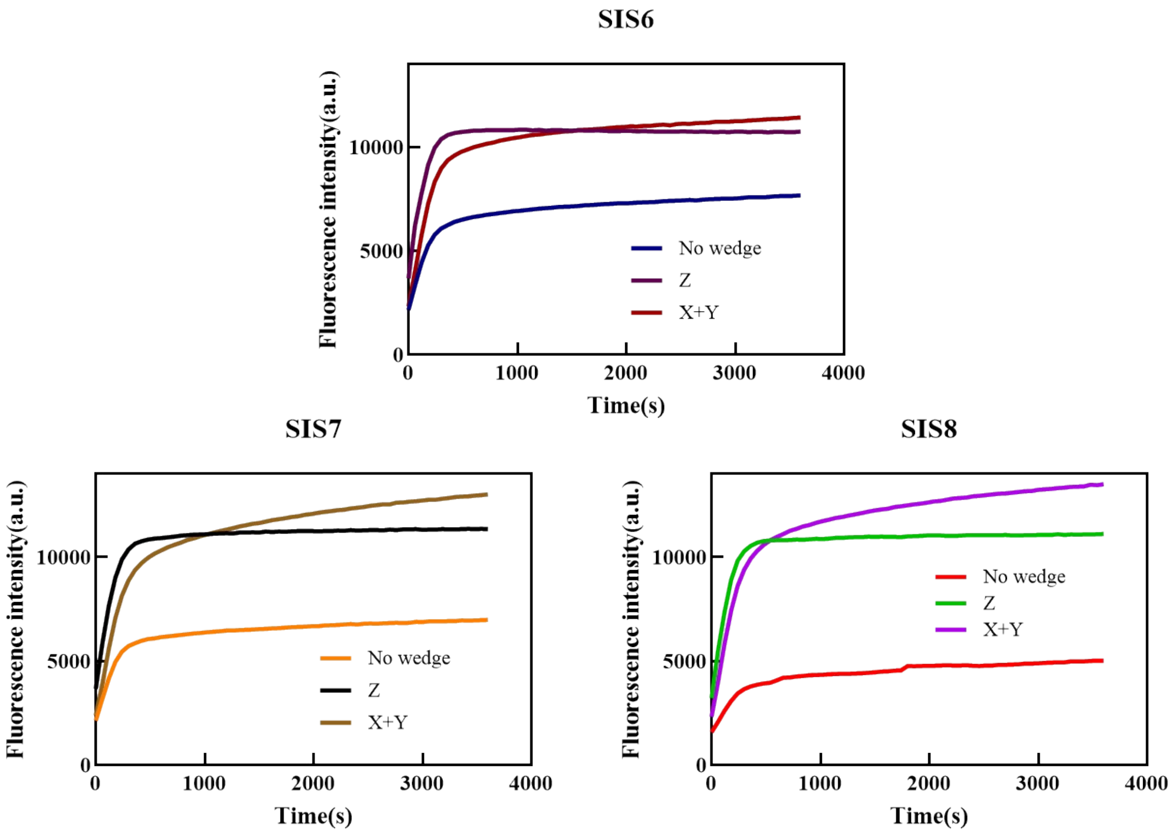

Figure S6. The effect of length of invading strand for the first "AND" gate on the secondary DNA circuit. IS6 represents the invading strand for the first "AND" gate, of which the length of toehold is 6nt. IS7 represents the invading strand for the first "AND" gate, of which the length of toehold is 7nt. IS8 represents the invading strand for the first "AND" gate, of which the length of toehold is 8nt.

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Supporting Information References

1 X. Chen, N. Liu, L. Liu, W. Chen, N. Chen, M. Lin, J. Xu, X. Zhou, H. Wang, M. Zhao and X. Xiao, *Nat Commun*, 2019, 10, 4675.
2 T. Wu, Y. Yang, W. Chen, J. Wang, Z. Yang, S. Wang, X. Xiao, M. Li and M. Zhao, *Nucleic Acids Res*, 2018, 46, 3119-3129.

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