Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting information

Scalable DNA recognition circuits based on DNA strand displacement

Fang Wang [a], Beiyu Shi [b], Ying Chen [b], Xiaolong Shi [b], Zheng Kou [b, *], Xiaoli Qiang [a, *]

a School of Computer Science and Cyber Engineering, GuangZhou University, Guangzhou 510006, China
b Institute of Computing Science and Technology, Guangzhou University, Guangzhou 510006, China

Methods

Materials

The DNA samples in this study were synthesized by Sangon Bioengineering (Shanghai) Company in China. Unmodified DNA strands were purified by the HAP approach, and modified DNA strands were purified by high performance liquid chromatography (HPLC). Unmodified oligonucleotides were dissolved in 1× TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA2Na, pH equilibrated to 8.0), while modified oligonucleotides were dissolved in deionized water, both with 12.5 mM Mg^{2+} added, and stored at 4°C. Concentrations of all DNA samples were determined using NanoPhotometerr N120 (Implen Inc., Westlake Village, CA, USA), taking absorbance at an absorption wavelength of 260 nm.

Design of DNA sequence

Sequences of all DNA strands in the experiments were designed by NUPACK (https://nupack.org), then manually modified to prevent complex instability as well as self-hybridization, in order to reduce the interference of unrelated structural domains.

Production of molecular logic gates

All DNA logic gates were generated by mixing corresponding single strands with the same concentration in 1× TAE buffer with 12.5 Mg2+. The mixed ssDNA was then annealed to form DNA complexes. The annealing program was set to heat at 95◦ C for 2 min, followed by a cooling down at -1◦ C/min quarters until 4◦ C for 1.5 h.

Simulation experiment

The molecular circuit was simulated by Visual DSD [21] with the concentration at least 100 nM. The results show that the output of the simulated signals is as expected, i.e., the proposed molecular circuit is feasible.

Fluorescence dynamics experiments

The input strands were mixed with the synthesized logic gate molecules on ice, and the system was replenished to 20 uL using 1× TAE (12.5 mM Mg2+). The reaction was carried out at 25°C for the appropriate time duration, and real-time fluorescence intensity was measured using a Quant Studio Model 3 Real-Time Fluorescence Quantitative PCR System equipped with a 96-well fluorescence plate reader (Thermo Fisher, USA).

Data processing

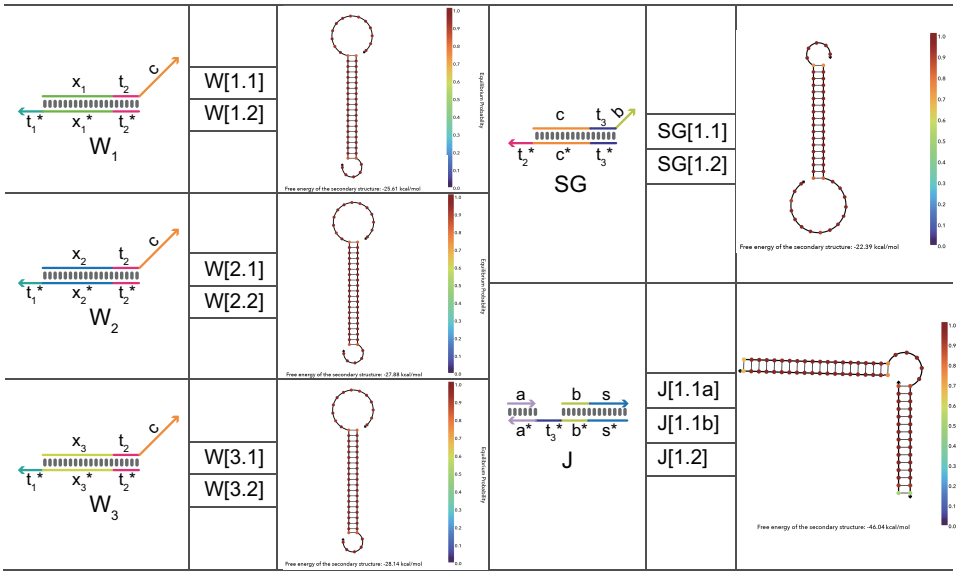

Fig. S1 MFE structure of molecular logic gates in recognition circuits. The result showed the state of each molecular logic gate after equilibration of the system as a minimum free energy (MFE) visualization graph, and the low free energy of the secondary structure indicated its stability.

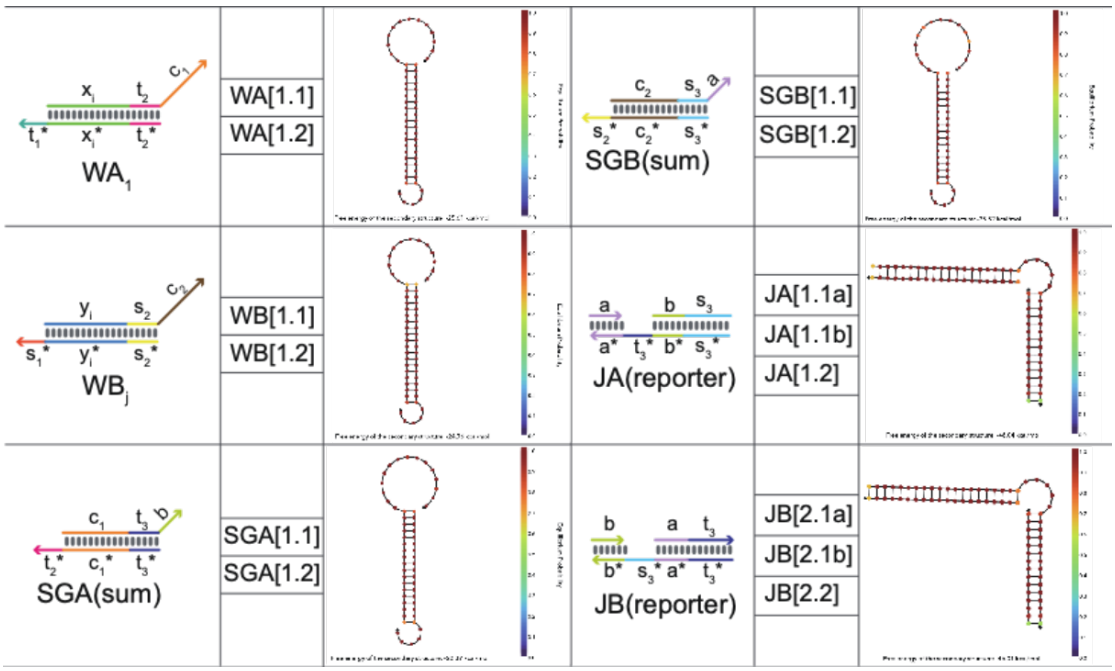

Fig.S2 MFE structure of molecular logic gates in molecular comparator.

Table S1 DNA sequences for molecular recognition circuits

Name	Sequence
A [1]	CATCTCAAACACTCTATTCA
A [2]	CATCTCAATCCACACTTCCA
A [3]	CATCTCAAACCCAACTCACA
W [1.1]	CAAACACTCTATTCATCTCCCACACTATAATTC
W [1.2]	GG AGATGAATAGAGTGTTTGAGA TG
W [2.1]	CAATCCACACTTCCATCTCC CACACTATAATTC
W [2.2]	GG AGA TGGAAGTGTGGATTGAGATG
W [3.1]	CAAACCCAACTCACATCTCCCACACTATAATTC
W [3.2]	GG AGATGTGAGTTGGGTTTGAGATG
E [1]	CAAACACTCTATTCATCTCC
E [2]	CAATCCACACTTCCATCTCC
E [3]	CAAACCCAACTCACATCTCC
SG [1.1]	GGAGT GAATTATAGTGTG GGAGA
SG [1.2]	CACACTATAATTCACTCCCACCACCAAACTTCC
J [1.1a]	CAACATATCAATTCC
J [1.1b]	CACCACCAAACTTCCCATCC
J [1.2]	GGATGGGAAGTTTGGTGGTGGGAGTGGAATTGATATGTTG

Table 2 DNA sequences for molecular comparator

Name	Sequence
A	CATCTCAAACACTCTATTCA
B	CCAATCTCTCACTCTAATCA
WA [1.1]	CAAACACTCTATTCATCTCCCACACTATAATTC
WA [1.2]	GGAGATGAATAGAGTGTTTGAGATG
WB [1.1]	CTCTCACTCTAATCACTATCCCTCACACTCTCA
WB [1.2]	GATAGTGATTAGAGTGAGAG ATTGG
SGA [1.1]	CACACTATAATTCACTCCCACCACCAAACTTCC
SGA [1.2]	GGAGTGAATTATAGTGTGGGAGA
SGB [1.1]	CCTCACACTCTCACATCCCAACATATCAATTCC
SGB [1.2]	GGATGTGAGAGTGTGAGGGATAG
JA [1.1a]	CAACATATCAATTCC
JA [1.1b]	CACCACCAAACTTCCCATCC
JA [1.2]	GGATGGGAAGTTTGGTGGTGGGAGTGGAATTGATATGTTG
JB [2.1a]	CACCACCAAACTTCC
JB [2.1b]	CAACATATCAATTCCACTCC
JB [2.2]	GGAGTGGAATTGATATGTTGGGATGGGAAGTTTGGTGGTG