Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information For:

Stem-loop clutch probes for sequence specific dsDNA analysis with improved single-mismatch selectivity

EXPERIMENTAL SECTION

Materials and Reagents.

DNA oligonucleotides listed in Table 1 were synthesized, modified and purified by Sangon Biotechnology Inc. (Shanghai, China). Gelred, agarose and reagents used to prepare different kinds of buffers (tris, boric acid, EDTA and so on) were also purchased from Sangon Biotechnology Inc. Fetal bovine serum (FBS) were obtained from Sigma-Aldrich.

Buffers used in this experiment: Hybridization chain reaction buffer, SPSC, 50 mM PB, 1 M NaCl, pH 7.5. Agarose gel electrophoresis analysis buffer, TBE, 40 mM of Tris-acetate, 25 mM of boric acid and 1 mM of EDTA.

Instrument.

DNA Oligonucleotides were quantified using a UV-Vis absorption spectra (Shimadzu UV2550, Japan). Fluorescence intensity was measured at room temperature using a F7000 spectrofluorometer (Hitachi, Japan). ABI Veriti96 thermal cycler (Thermofisher, USA) was used to denature probes. Agarose gels were visualized under UV transilluminator (Gel Doc XR+, Bio-Rad, USA).

Native Gel Electrophoresis analysis.

The mixture of SLCPs (or LCPs) ,dsDNA and rH in SPSC was heated to 95 °C for 5 min and cooled to 80°C for 30 min by using a ABI Veriti96 thermal cycler. The denaturation sample was then slowly cooled to 4°C within about 10 min. Other probes in SPSC were separately heated to 95 °C for 5 min and then cool to 4°C within 60 s. Hybridization chain reaction was carried out at 25°C within 1 h. The 2% agarose gels contained 0.01 μL of gelred of gel volume were run at 150 V for

45 min and visualized under UV light.

dsDNA analysis in buffer using fluorescence biosensor based on SLCPs.

The mixture of 0.2 μM rH (rH-i2, rH-i1, rH, rH-a1, or rH-a2), 0.2 μM SLCPs and different concentrations of dsDNA (such as 200 nM, 20 nM and so on) in SPSC was heated to 95 °C for 5 min and cooled to 80°C for 30 min. The denaturation sample was then slowly cooled to 4°C within about 10 min. 0.2 μM sH (FAM-sH-Dabcyl) in SPSC was heated to 95°C for 5 min, allowed to cool to 4°C within 60 s. Above two solution were then mixed together (1:1) obtaining the final concentrations of 0.1 μM rH (rH-i2, rH-i1, rH, rH-a1, or rH-a2), 0.1μM sH, 0.1 μM SLCPs and different concentrations of dsDNA. bHCR was carried out in a 600 μL tube for fluorescence spectra measurements. The fluorescence spectra were recorded at environment temperature in a quartz cuvette on F7000 spectrofluorometer. The excitation wavelength was fixed at 492 nm and the emission wavelengths were in the range from 500 to 600 nm with both excitation and emission bandwidths of 5 nm under a PMT voltage of 700V.

dsDNA analysis in serum using fluorescence biosensor based on SLCPs.

The procedures of dsDNA analysis in 10% serum and 90% SPSC was similar to the dsDNA detection processes performed in buffer. The denatured serum proteins should be removed through centrifugation before fluorescence intensity measurement. The fluorescence spectra were also recorded at environment temperature in a quartz cuvette on F7000 spectrofluorometer.

Table S1 DNA sequences used in this experiment.

	DNA Sequence (5'-3')
FAM-sH-Dabcyl (sH)	FAM-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACT/dabcyl/ACTTTG
rH-dsDNA-18	TTAACCCACGCCGAATCCTAGACTGTTCCAGCTGATGGCGTACAAAGTAGTCTAGGATTCGGCGTGCCAATT
rH-dsDNA-21	TTAACCCACGCCGAATCCTAGACTGGCGTTCCAGCTGATGGCGTACAAAGTAGTCTAGGATTCGGCGTGCCAATT
rH-dsDNA-24 (rH)	TTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTACGGGTACAAAGTAGTCTAGGATTCGGCGTGCCAATT
rH-dsDNA-24-variant-1 (rH-v1)	TTAACCCACGCCGAATCCTAGACTACTCTCGGATTATGAAGATTACGGGTACAAAGTAGTCTAGGATTCGGCGTGCCAATT
rH-dsDNA-24- variant-2 (rH-v2)	TTAACCCACGCCGAATCCTAGACTACTTTGCTCGGATTATGAAGATTACGGGTACAAAGTAGTCTAGGATTCGGCGTGCCAATT
Activator-1	AATTGGCACGCC
Activator-2	AATTGGCACGCCGAA
T-ssDNA-18	TACGCCATCAGCTGGAAC
T-ssDNA-21	TACGCCATCAGCTGGAACGCC
T-ssDNA-24	TACCCGTAATCTTCATAATCCGAG
C-ssDNA-18	GTTCCAGCTGATGGCGTA
C-ssDNA-21	GGCGTTCCAGCTGATGGCGTA
C-ssDNA-24	CTCGGATTATGAAGATTACGGGTA
LCP-24-10-1 (LCP10.1)	ATAATCCGAG
LCP-24-10-2 (LCP10.2)	TACCCGTAAT
SLCP-24-8-1 (SLCP8.1)	AATCCGAGGGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACC
SLCP-24-8-2 (SLCP8.2)	GGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACCTACCCGTA
SLCP-24-10-1 (SLCP10.1)	ATAATCCGAGGGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACC
SLCP-24-10-2 (SLCP10.2)	GGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACCTACCCGTAAT
SLCP-24-12-1 (SLCP12.1)	TCATAATCCGAGGGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACC
SLCP-24-12-2 (SLCP12.2)	GGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACCTACCCGTAATCT
SLCP-21-10-1	CTGGAACGCCGGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACC
SLCP-21-10-2	GGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACCTACGCCATCA

SLCP-18-9-1	AGCTGGAACGGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACC
SLCP-18-9-2	GGTTTGGCTTTCTTGTTAGGCAACCACCCAGGGTGTCGTAACAAGAAAGCCAAACCTACGCCATC
T-ssDNA-24-M1	CACCCGTAATCTTCATAATCCGAG
T-ssDNA-24-M2	TACCCGAAATCTTCATAATCCGAG
T-ssDNA-24-M3	TACCCGTAATCTTCATATTCCGAG
T-ssDNA-24-M4	TACCCGTAATCTTCATAATCCCAG
C-ssDNA-24-M1	CTCGGATTATGAAGATTACGGGTG
C-ssDNA-24-M2	CTCGGATTATGAAGATTTCGGGTA
C-ssDNA-24-M3	CTCGGAATATGAAGATTACGGGTA
C-ssDNA-24-M4	CTGGGATTATGAAGATTACGGGTA

In the hairpin sequences, loops are underlined in red and sticky ends are underlined in black.

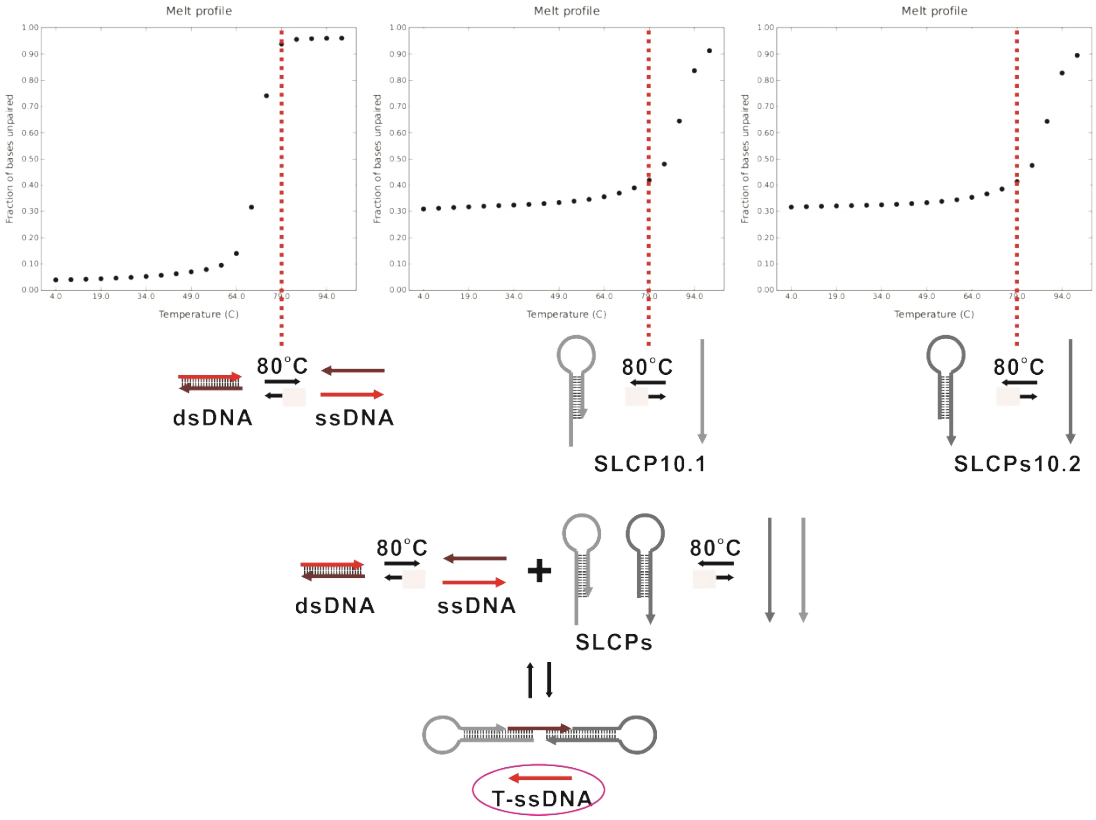

Fig. S1 The melt profiles of dsDNA and stem-loop clutch probes are estimated via NUPACK. (Web for reference: http://www.nupack.org/).

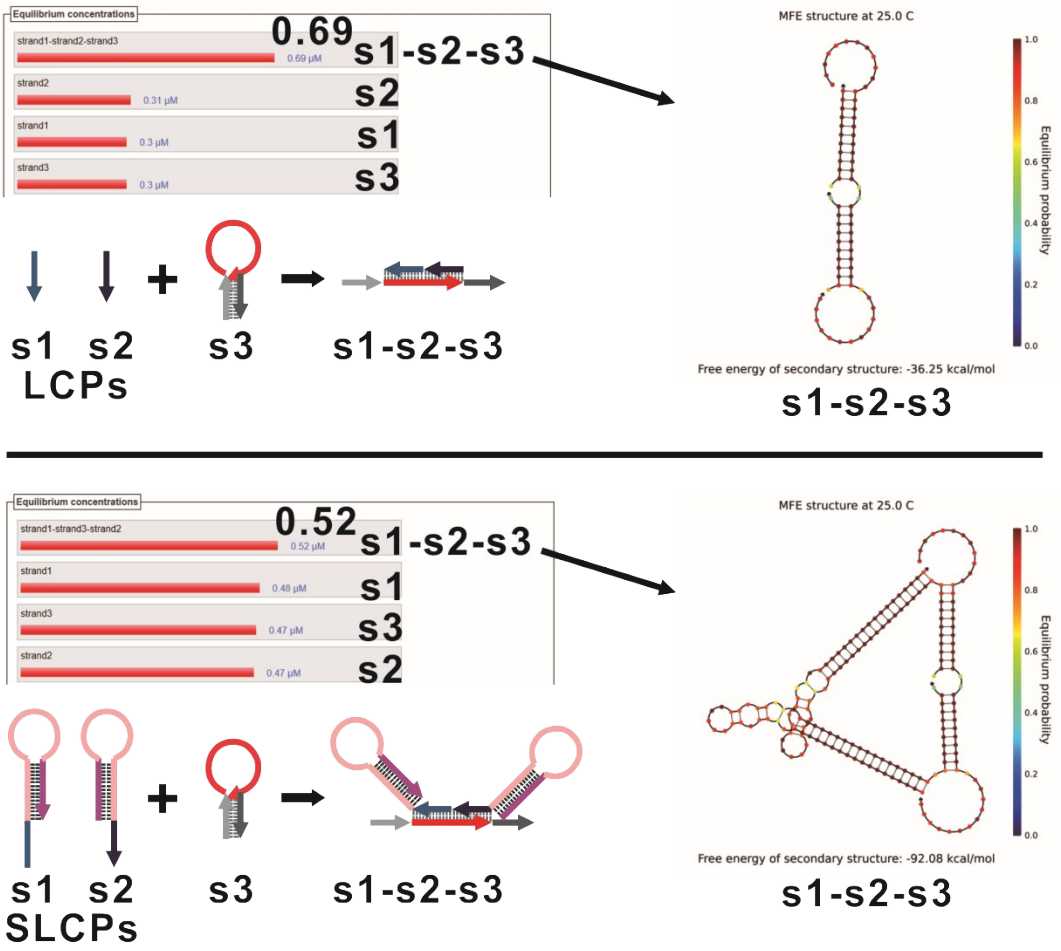

Fig. S2 The equilibrium concentrations of re-designed rH hybridize with SLCPs and LCPs are estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

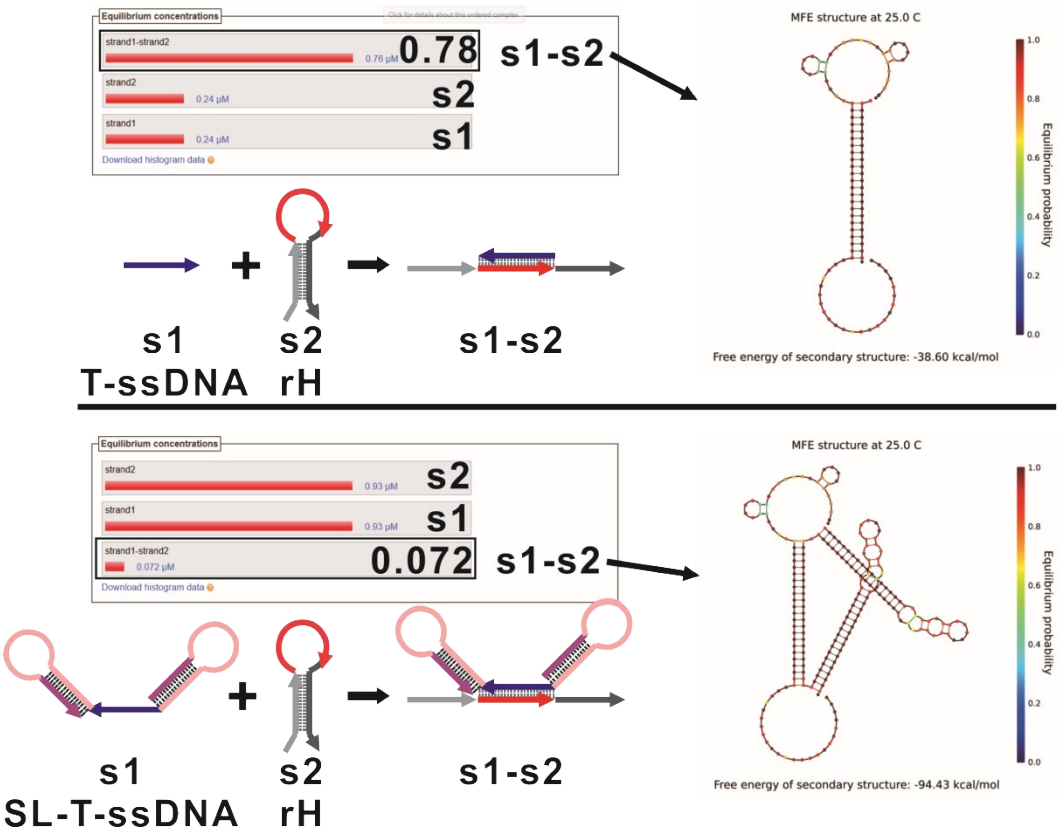

Fig. S3 The equilibrium concentrations of origin rH hybridize with T-ss-DNA and stem-loop T-ss-DNA (SL-T-ssDNA) are estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

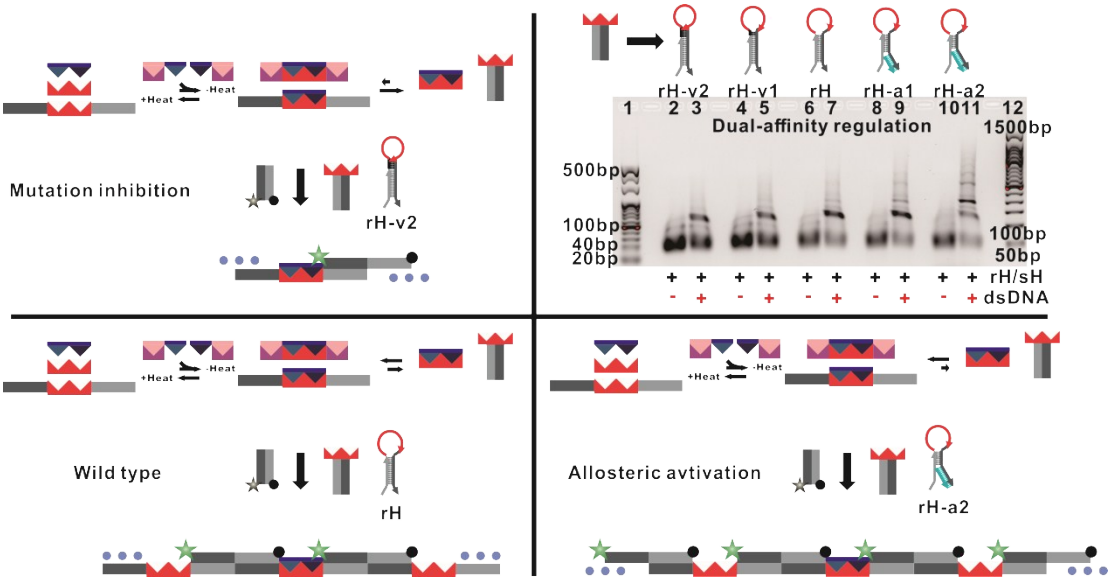

Fig. S4 The target binding affinity and hybridization chain reaction activity of recognition hairpin can be tuned by using distal-site mutation inhibition and allosteric activation. AGE analysis demonstrated the varied bidirectional hybridization chain reaction efficiencies. Lane 1, 20bp DNA ladder markers; Lane 2, 1 μM rH-v2/sH + 0.2 μM SLCPs; Lane 3, 1 μM rH-v2/sH + 0.2 μM dsDNA + 0.2 μM SLCPs; Lane 4, 1 μM rH-v1/sH + 0.2 μM SLCPs; Lane 5, 1 μM rH-v1/sH + 0.2 μM dsDNA + 0.2 μM SLCPs; Lane 6, 1 μM rH/sH + 0.2 μM SLCPs; Lane 7, 1 μM rH/sH + 0.2 μM dsDNA + 0.2 μM SLCPs; Lane 8, 1 μM rH-a1/sH + 0.2 μM SLCPs; Lane 9, 1 μM rH-a1/sH + 0.2 μM dsDNA + 0.2 μM SLCPs; Lane 10, 1 μM rH-a2/sH + 0.2 μM SLCPs; Lane 11, 1 μM rH-a2/sH + 0.2 μM dsDNA + 0.2 μM SLCPs; Lane 12, 50bp DNA ladder markers.

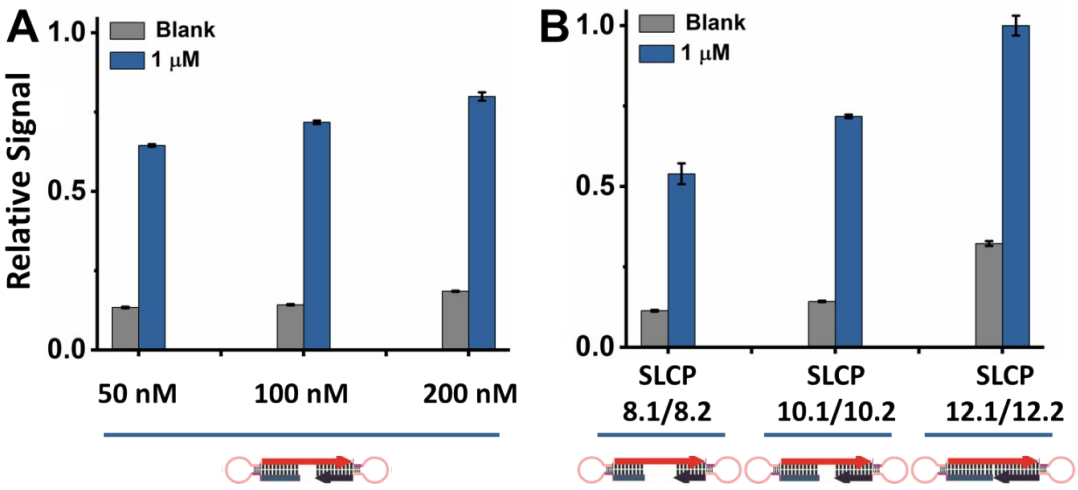

Fig. S5 Effects of SLCPs concentration on signal to noise ratio for dsDNA detection. (B) Effects of the length of clutch segment in SLCPs for dsDNA detection.

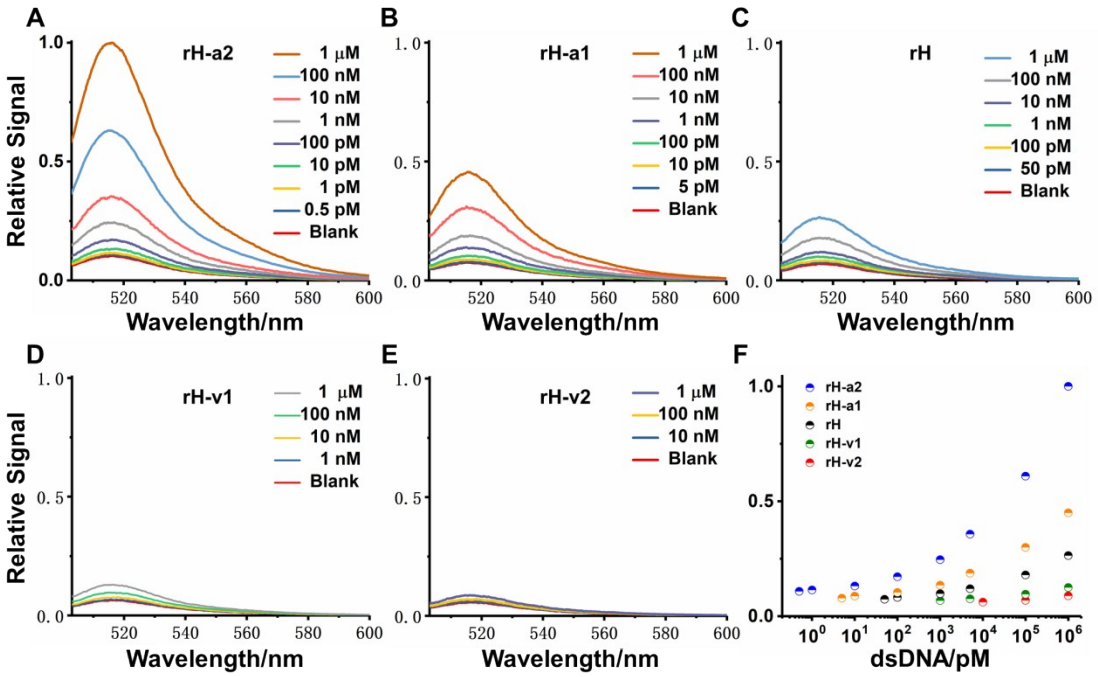

Fig. S6 (A-E) Fluorescence responses of designed biosensor to dsDNA of varying concentrations. Fluorescence responses of designed biosensor to the same dsDNA concentration can be tuned by using mutation inhibition and allosteric activation. (F) The plot of relative signal vs logarithm of dsDNA concentration using SLCPs with tunable dynamic range.

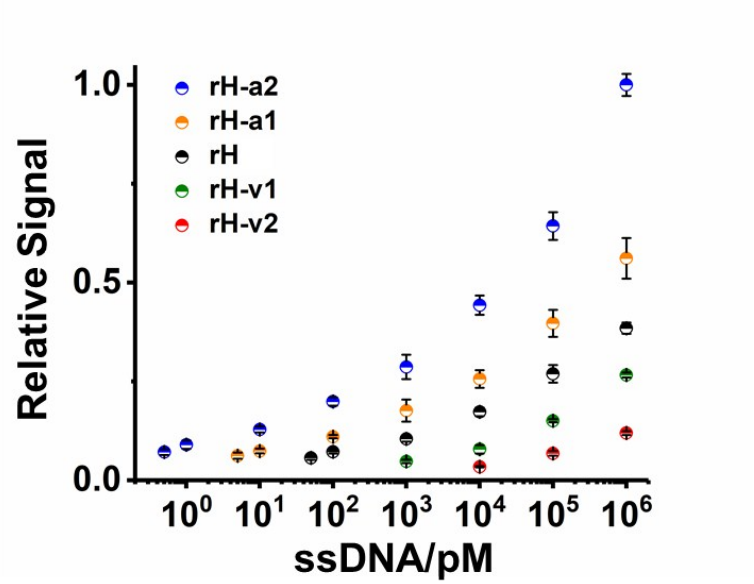

Fig. S7 The plot of relative signal vs logarithm of ssDNA concentration with tunable dynamic range.

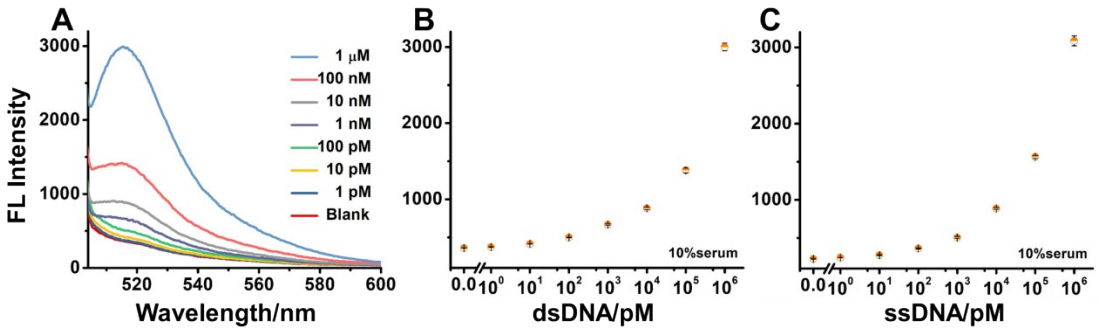

Fig. S8 (A, B) Fluorescence responses of designed biosensor to dsDNA of varying concentrations in 10% serum and 90% SPSC buffer. (C) Fluorescence responses of designed biosensor to ssDNA of varying concentrations in 10% serum and 90% SPSC buffer.

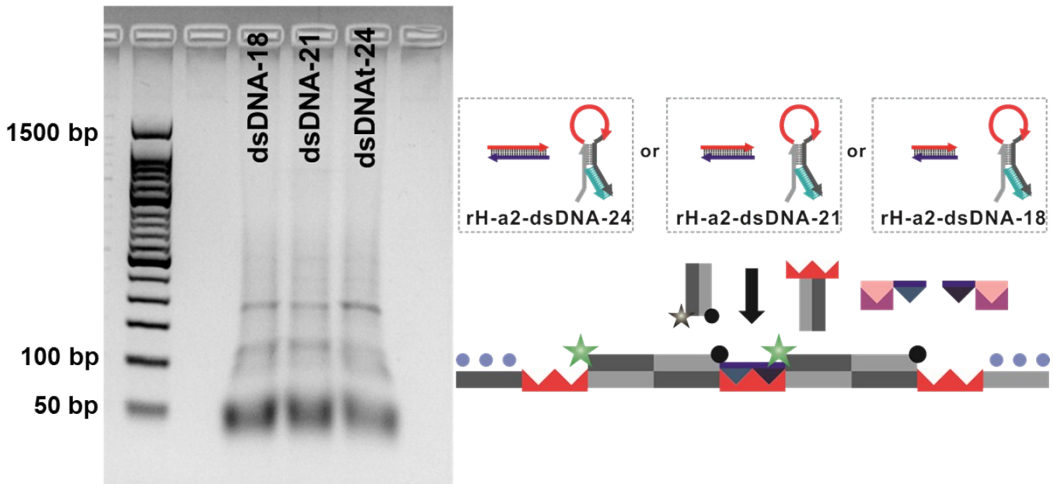

Fig. S9 Analysis by AGE of the bidirectional hybridization chain reaction triggered by dsDNA-24, dsDNA-21 and dsDNA 18 respectively.

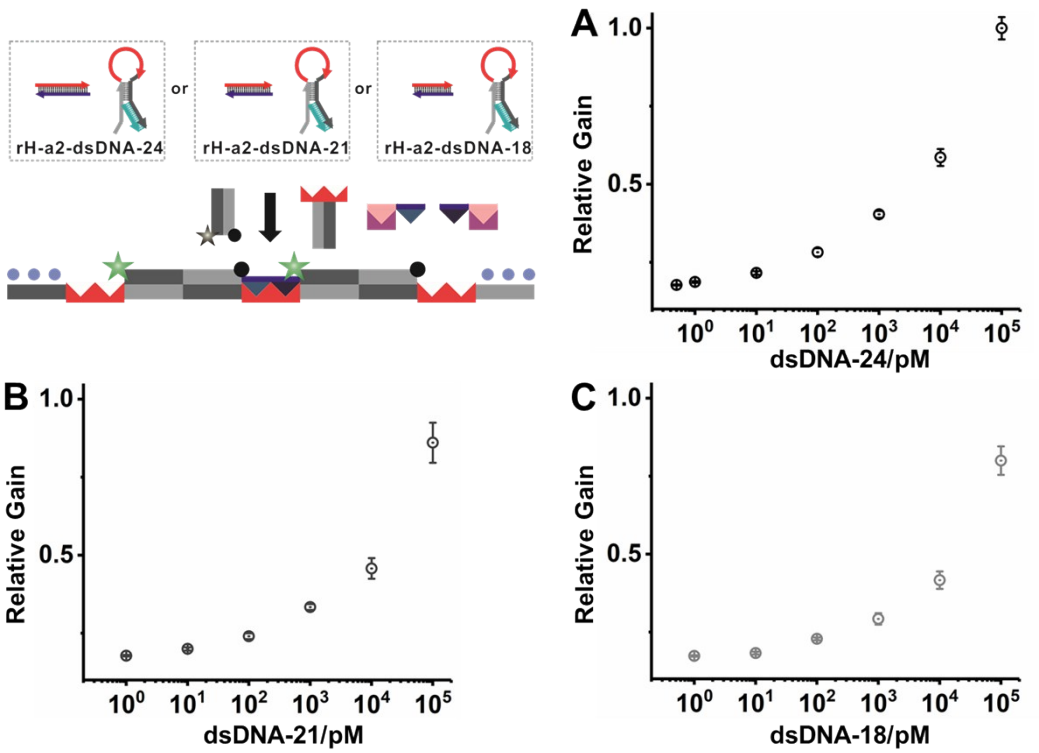

Fig. S10 Analysis of dsDNA of diverse lengths in ~~10% serum and 90%~~ 1× SPSC buffer. Logarithmic plot of fluorescence intensity versus dsDNA-24 (A) (black), dsDNA-21 (B) (gray) and dsDNA-18 (C) (light gray) concentrations using SLCPs strategy.