Supporting Information For:

Single-Step Multivalent Capture Assay for Nucleic Acids Detection with Dual-Affinity Regulation Using Mutation Inhibition and Allosteric Activation

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EXPERIMENTAL SECTION

Materials and Reagents.

DNA oligonucleotides listed in Table S1 were ordered from Sangon Biotechnology Inc. (Shanghai, China). DNA Oligonucleotides were quantified using a UV-Vis absorption spectra (Shimadzu UV2550, Japan). Gelred, agarose, casein and reagents used to prepare different kinds of buffers (such as boric acid, EDTA) were purchased from Sangon Biotechnology Inc. Tris(2-carboxyethyl) phosphine hydrochioride (TCEP), 3,3',5,5'-tetramethylbenzidine (TMB, slow kinetic form), bovine serum albumin (BSA) and Fetal bovine serum (FBS) were purchased from Sigma-Aldrich. Streptavidin-HRP was purchased from Abcam (UK).

Native Gel Electrophoresis analysis.

Recognition hairpins with different binding affinities (mutation inhibition and allosteric activation) and signal hairpin diluted in SPSC buffer (50 mM PB, 1 M NaCl, pH 7.5) were heated separately to 95 °C for 5 min and then cool to 4 °C within 60 s by using an ABI Veriti96 thermal cycler (Thermofisher, USA). Then rH and sH were mixed together with the same concentration of target-24 (1 μM rH/sH and 0.2 μM target-24) in 50 μL 1× SPSC buffer. Hybridization chain reaction was carried out at room temperature. The 2% agarose gels contained 0.01 μL of gelred of gel volume and were prepared with 1× TBE buffer (40 mM of Tris-acetate, 25 mM of boric acid and 1 mM of EDTA) and were run at 150 V for 45 min and visualized under UV light (Gel Doc XR+, Bio-Rad, USA).

Fluorescence analysis

1 μM rH (rH-2v, rH-1v, rH, rH-1a, rH-2a) and 1 μM sH (FAM-sH-Dabcyl) were heated separately to

95°C for 5 min, allowed to cool to 4°C within 60 s. Time-dependent fluorescence monitoring was performed with a time interval of 1 s in a quartz cuvette on F7000 spectrofluorometer (50 nM rH/sH, 100 nM target-24).

Electrochemical analysis

Nucleic acids detection pretreatment.

5 μM rH (rH-i2-target-24, rH-i1-target-24, rH-0-target-24, rH-a1-target-24, or rH-a2-target-24) and 5 μM sH (Biotin-sH) were heated separately to 95°C for 5 min, allowed to cool to 4°C within 60 s, and then mixed together with different concentrations of target-24 (0, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM) in 100 μL 1× SPSC (contains 0.5% casein and 1% BSA) buffer for the final concentration of 100 nM. Multibranched and bidirectional hybridization chain reaction was performed out at room temperature. After 2 h, 0.25 μL of streptavidin−HRP (1 mg/mL) was added to bind with biotin on sH before incubated with TDN assembled electrode for further electrochemical measurements. For block samples, we treated mbHCR samples with 100 nM blocker to prohibit the hybridization of excess rH with TDN probes. Target-21 or target-18 detection pretreatment was carried out in similar procedures only replacing DNA target and recognition hairpin with different concentrations of target-21 or target-18 and rH- target-21 or rH -target-18.

Electrocatalytic bioassay for single-step nucleic acids detection.

Gold electrodes were cleaned following the reported protocols before incubated with tetrahedral DNA nanostructures (TDNs)[1]. Equimolar quantities of four DNA strands (1 μM A20, 1 μM SH-B20, 1 μM SH-C20 and 1 μM SH-D20) were mixed in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0, contains 3 mM TCEP), then heated to 95°C for 10 min and cooled to 4°C within 60 s. TDNs

were self-assembled during the cooling process. Next, gold electrodes were incubated with 3 μL of TDNs overnight at room temperature. The resulting electrodes were rinsed with PBS (10 mM PB, 137 mM NaCl, 2.5 mM KCl, 0.05% Tween 20, pH 7.4) and then incubated with mbHCR pretreatment solutions. Multivalent capture of mbHCR products can be finished in 30 minutes. All electrochemical experiments were carried out using an electrochemical work station modeled CHI 1040C with a three-electrode system including a reference electrode (Ag/AgCl, 3M KCl), a platinum counter electrode and a gold working electrode. Electrochemical signals were measured in slow kinetic TMB substrate at room temperature. Cyclic voltammetry signals were obtained from 0 to 0.7 V at a scan rate of 0.1 V/s. And amperometric signals were obtained at 0.1 V within 100 s.

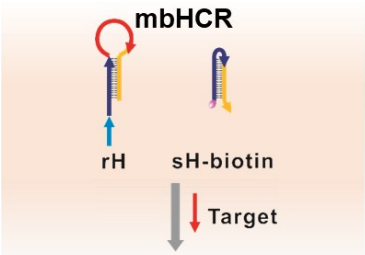
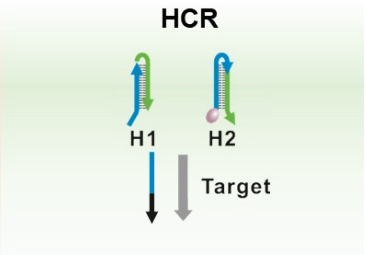
Table S1 DNA sequences used in this experiment.

	DNA Sequence (5'-3')
rH-Target-24(4-6)	CTGGATCGGTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTACGGG TACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24(6-6) (rH)	CACTGGATCGGTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTACG GGTACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24(8-6)	GTCACTGGATCGGTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTA CGGGTACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24-variant 1 (rH-v1)	CACTGGATCGGTTTAACCCACGCCGAATCCTAGACTACTCTCGGATTATGAAGATT ACGGGTACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24-variant 2 (rH-v2)	CACTGGATCGGTTTAACCCACGCCGAATCCTAGACTACTTTGCTCGGATTATGAAG ATTACGGGTACAAAGTAGTCTAGGATTCGGCGTG
Activator 1	GGCGTGGGTTAA
Activator 2	TTCGGCGTGGGTTAA
sH-Biotin (sH)	Biotin-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG
FAM-sH-Dabcyl (F-sH-Q)	FAM-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACT/Dabcyl/ACTTTG
rH-Target-18	CACTGGATCGGTTTAACCCACGCCGAATCCTAGACTCTGTAGGAAGAGGAAGGAC AAAGTAGTCTAGGATTCGGCGTG
rH-Target-21	CACTGGATCGGTTTAACCCACGCCGAATCCTAGACTACCCTGGGCAACCAGCCCTG TCAAAGTAGTCTAGGATTCGGCGTG
Target-18	TCCTTCCTCTTCCTACAG
Target-21	ACAGGGCTGGTTGCCCAGGGT
Target-24	TACCCGTAATCTTCATAATCCGAG
Target-18-M1	TCCTTCCTCATCCTACAG
Target-18-M2	TCCTTCCTCTTCCTACAC
Target-21-M1	ACAGGGCTGCTTGCCCAGGGT
Target-21-M2	ACAGGGCTGGTTGCCCACGGT
Target-24-M1	TACCCCTAATCTTCATAATCCGAG
Target-24-M2	TACCCGTAATCTTCATAATCCCAG
Target-24-M3	TACCCGTAATCTACATAATCCGAG
Target-24-M4	GACCCGTAATCTTCATAATCCGAG
Target-24-M5	TACCCGTAATCTTCATAA~~T~~CCGAG
rH-Target-24(4-8)	GGATCGGTTTTTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTACG GGTACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24(4-10)	CTGGATCGGTTTTTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATTAC GGGTACAAAGTAGTCTAGGATTCGGCGTG
rH-Target-24(4-12)	CACTGGATCGGTTTTTTTAACCCACGCCGAATCCTAGACTCTCGGATTATGAAGATT ACGGGTACAAAGTAGTCTAGGATTCGGCGTG
Blocker4	GGTTAAACCG
Blocker6	GGTTAAACCGAT
Blocker8	GGTTAAACCGATCC

5‘termination	AGTCTAGGATTCGGCGTGGGTTAA
3‘termination	CACGCCGAATCCTAGACTACTTTG
A20	ACCGATCCAGTGACTTTTTTTTTTCTCAACTGCCTGGTGATACGAGGATGGGCATGC TCTTCCCGACGGTATTGGACCCTCGCATG
SH-B20	SH-CGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCGGG TGATAAA
SH-C20	SH-CGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCA ATACCG
SH-D20	SH-CGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCA TAGTAG
TDN-20	Tetrahedral DNA nanostructure was assembled by using A20, SH-B20, SH-C20 and SH-D20

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

Table S2 Detection technologies and their analysis performance parameters.

Protocol		
Dynamic detection range	Negative and positive control 10 fM-10 μM	1 fM-10 nM
Detection process and time	Single step 2.5h	Multiple steps 4h 15min
Detection limit	Negative and positive control 10 fM-5 nM	1 fM
Probe universality	rH ✘ sH √	H1 ✘ H2 ✘
	Our work	Ref. 2

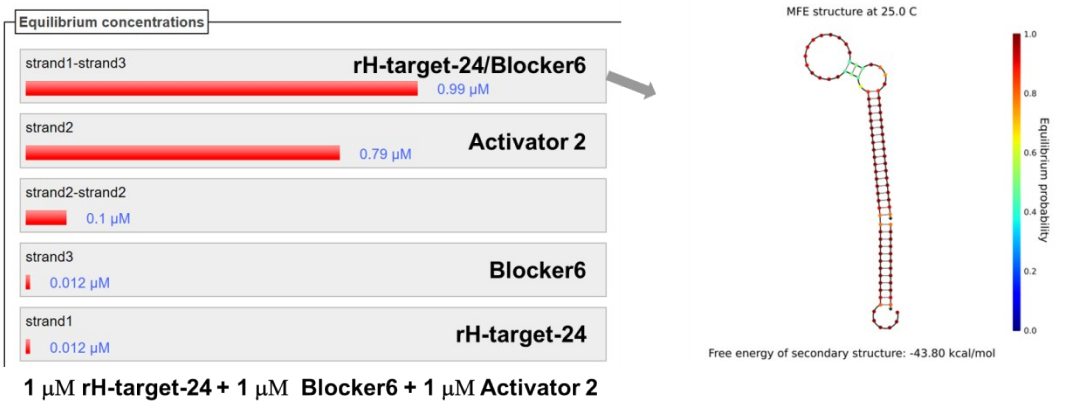


Fig. S1 The hybridization cases in the present of 1 μM rH (target-24), 1 μM activater 2 and 1 μM blocker6 are estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

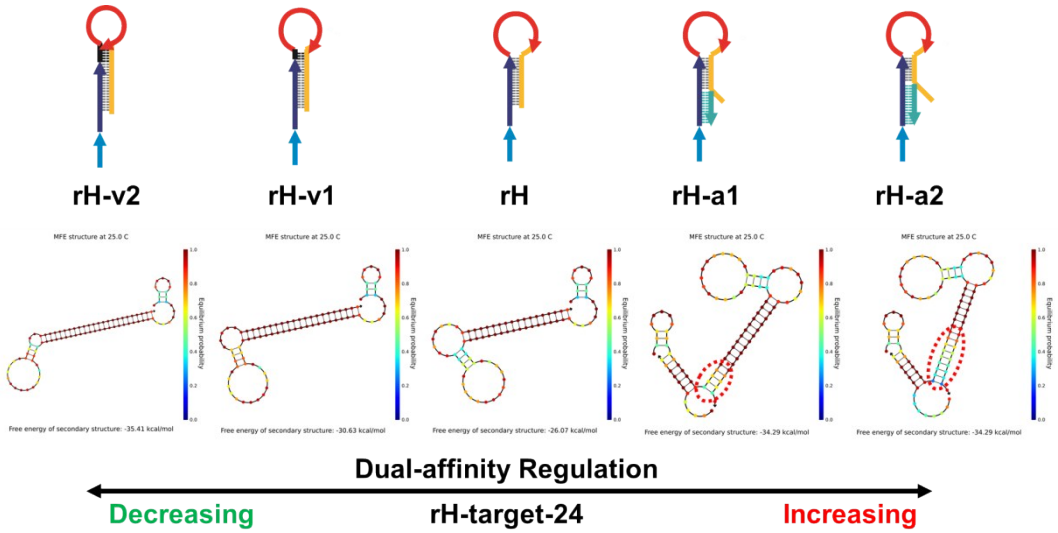


Fig. S2 The free energies and equilibrium probability of rH-Target24 with mutation inhibition and allosteric activation are estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

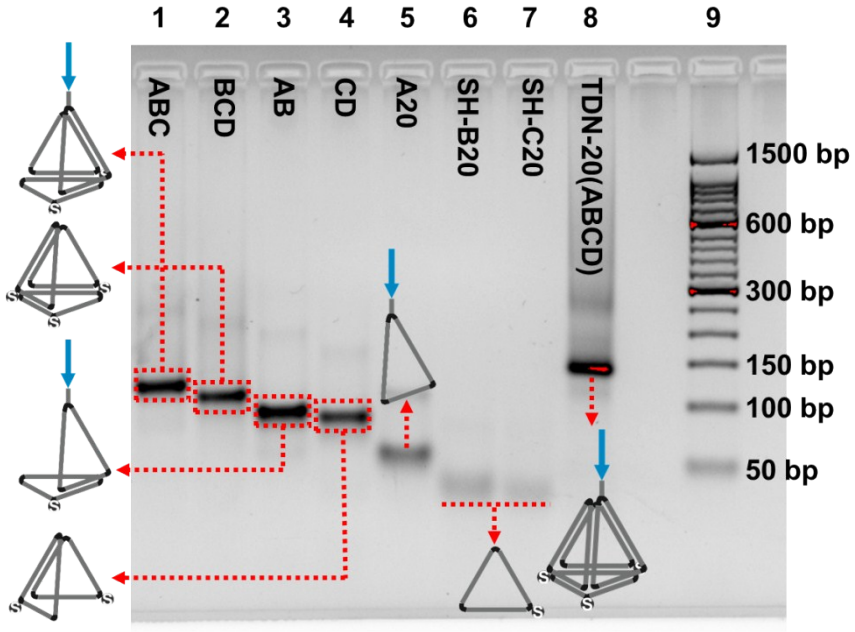


Fig. S3 AGE analysis validated the successfully self-assembly of TDN. Lane 1, 1 μM A20 + 1μM SH-B20 + 1 μM SH-C20; Lane 2, 1 μM SH-B20 + 1μM SH-C20 + 1 μM D; Lane 3, 1 μM A20 + 1μM SH-B20; Lane 4, 1μM SH-C20 + 1 μM D; Lane 5, 1 μM A20; Lane 6, 1 μM SH-B20; Lane 7, SH-C20; Lane 8, 1 μM A20 + 1μM SH-B20 + 1 μM SH-C20+ 1 μM SH-D20; Lane 9, 50bp DNA ladder markers.

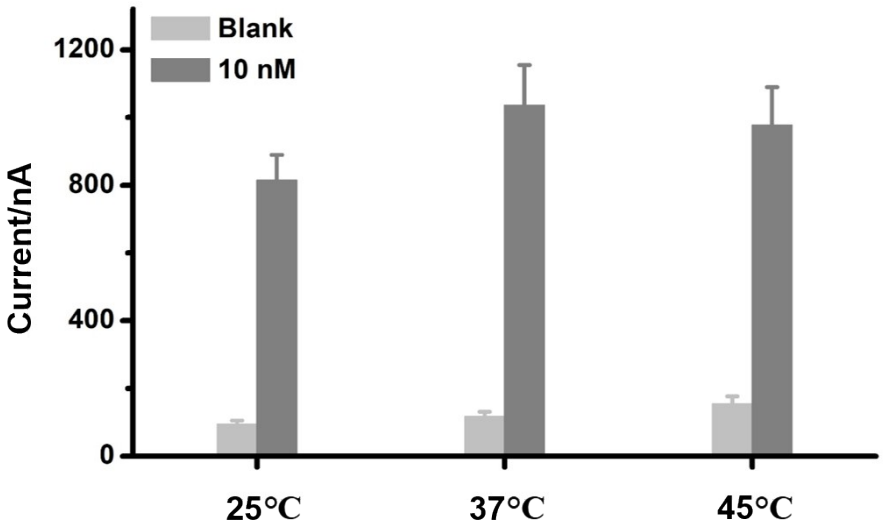


Fig. S4 The incubation temperature was investigated for the gap strategy.

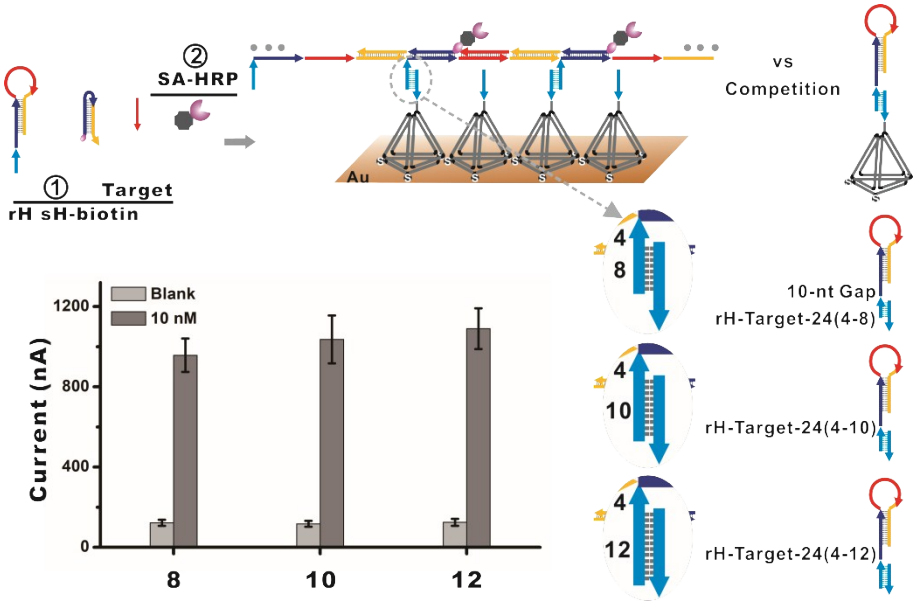


Fig. S5 Multivalent hybridization efficiency of extension sequence in rH with TDN probe is higher than monomer hybridization. The monomer hybridization is destabilized because a 10-nt gap is reserved between the capture TDN probe and rH stem. The length of single-stranded extension in rH was investigated.

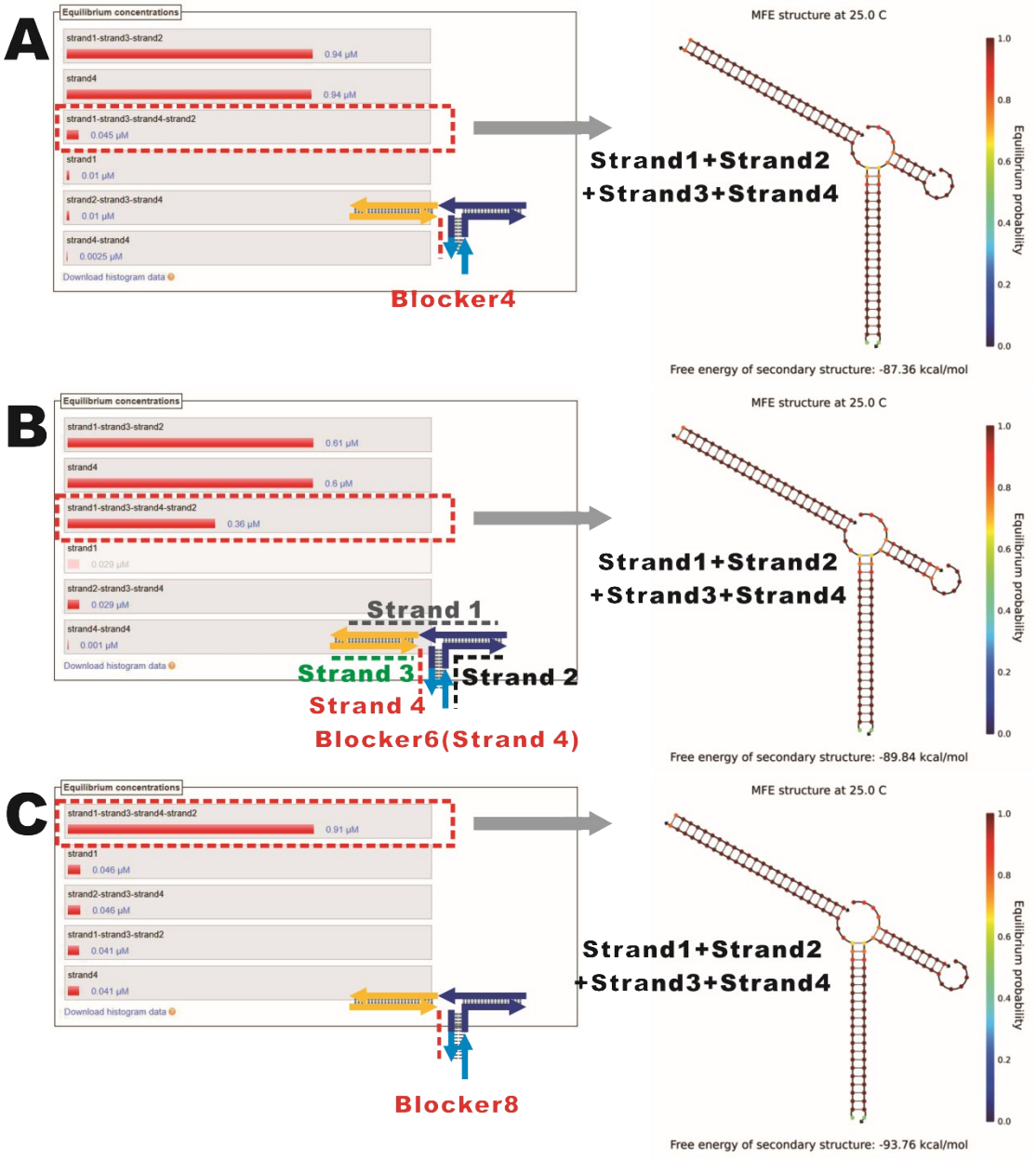


Fig. S6 The hybridization cases in the present of 1 μM sH (strand1), 1 μM partial sequence of rH (strand2, strand3) and 1 μM blocker (stand4, A: blocker4 B: blocker6 C: blocker8) were estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

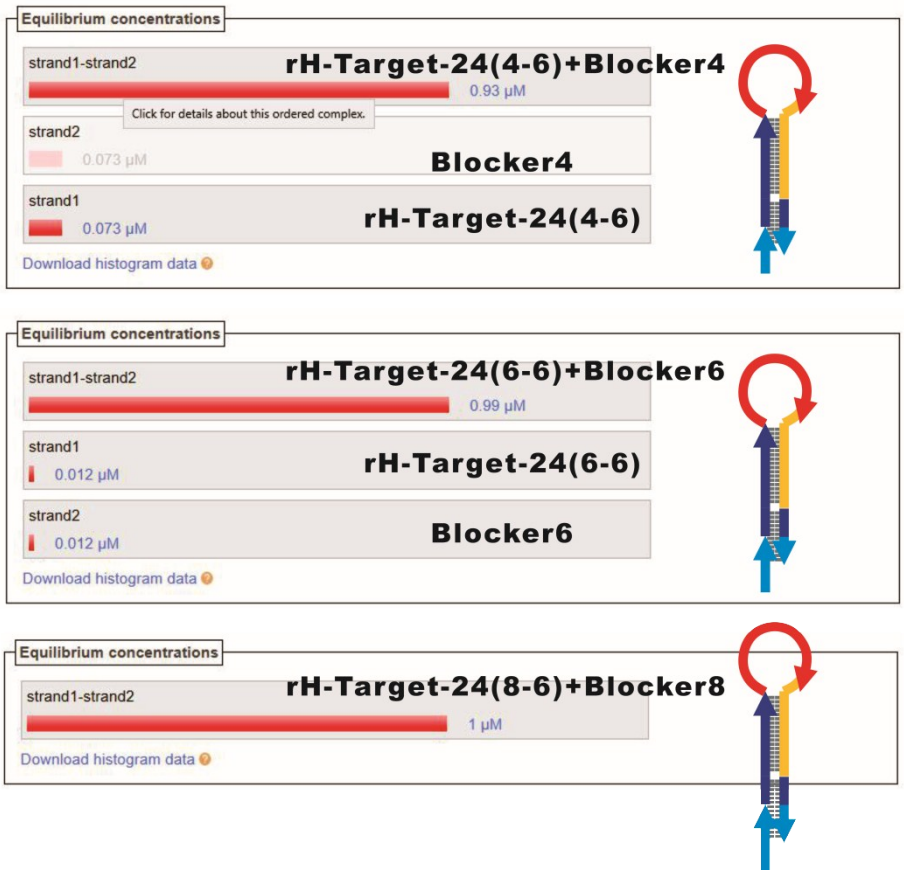


Fig. S7 The hybridization cases in the present of 1 μM rH and 1 μM blocker (stand4, A: blocker4 B: blocker6 C: blocker8) are estimated via NUPACK at 25°C. (Web for reference: http://www.nupack.org/).

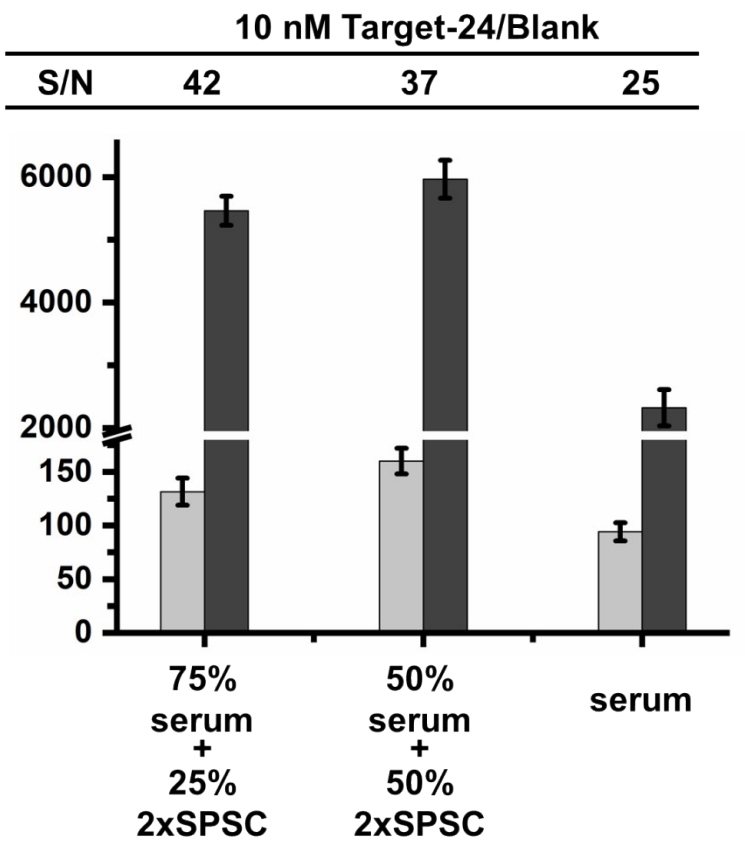


Fig. S8 The signal to noise ratio of 10 nM target-24 detected in diluted and undiluted serum.

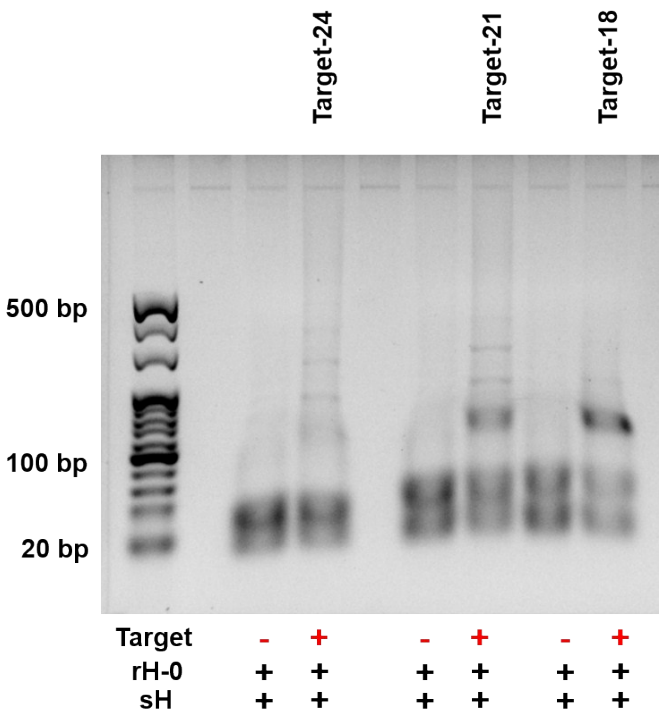


Fig. S9 Analysis by AGE of the dual-affinity controlled multibranched hybridization chain reaction triggered by target-24, target-21 and target 18 respectively.

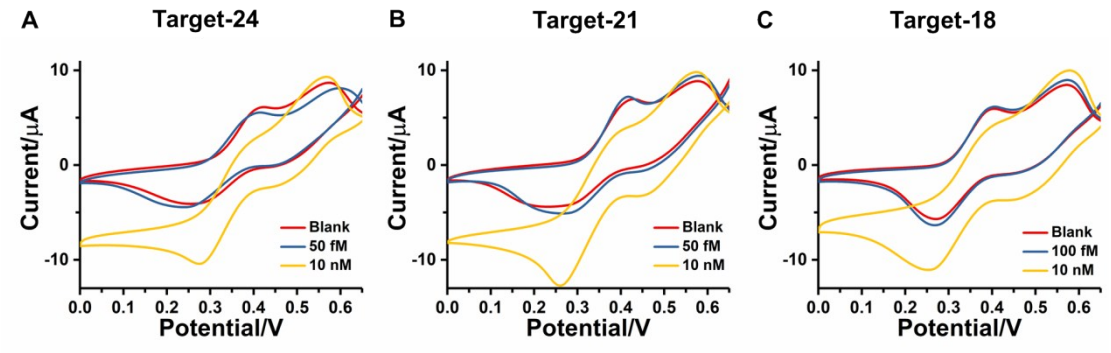


Fig. S10 Typical CV curves for the detection of different lengths of targets using single-step electrocatalytic assay in 75% serum. (A: target-24, B: target-21, C: target-18). The recognition probes were rH-a2-target-24, rH-a2-target-21 and rH-a2-target-18 respectively.

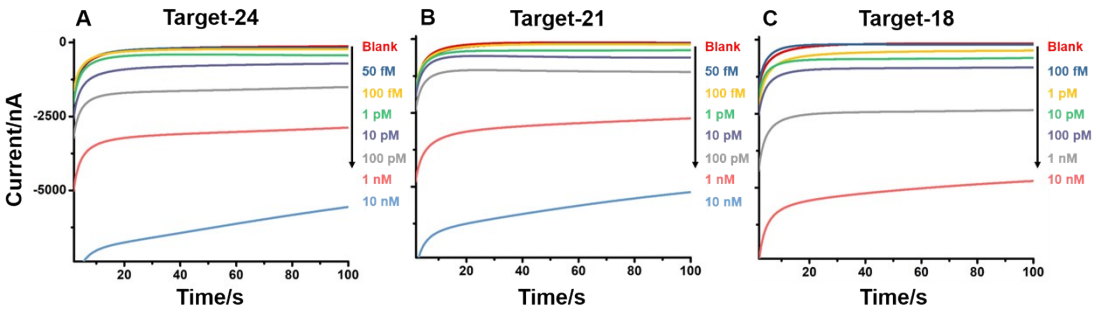


Fig. S11 Typical i-t curves for the detection of different length of targets using single-step electrocatalytic assay in 75% serum. (A: target-24, B: target-21, C: target-18). The recognition probes were rH-a2-target-24, rH-a2-target-21 and rH-a2-target-18 respectively.

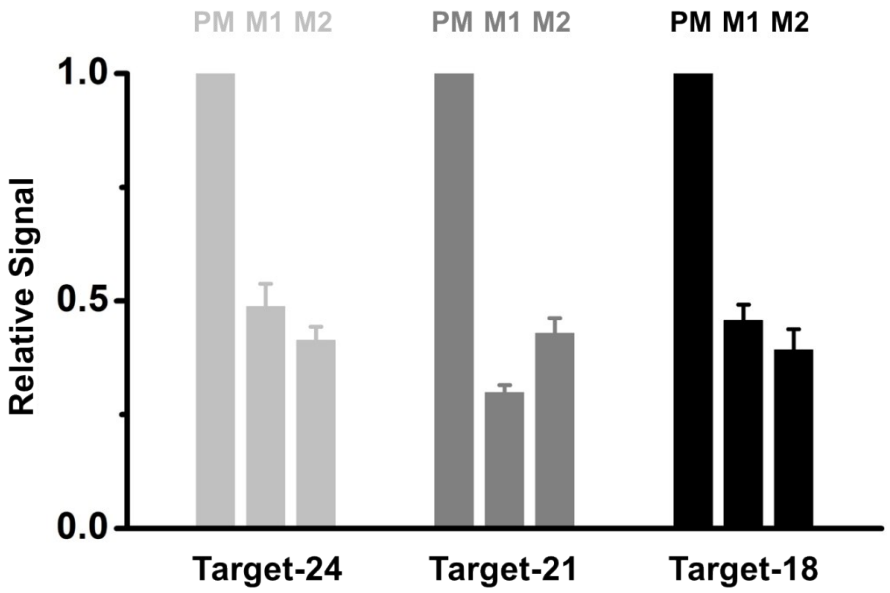


Fig. S12 Differentiates between perfectly matched and mismatched nucleic acids. The data shown were collected at 10 nM of target-24 (light gray), target-21 (gray) and target-18 (black) using rH-a2 as receptor.

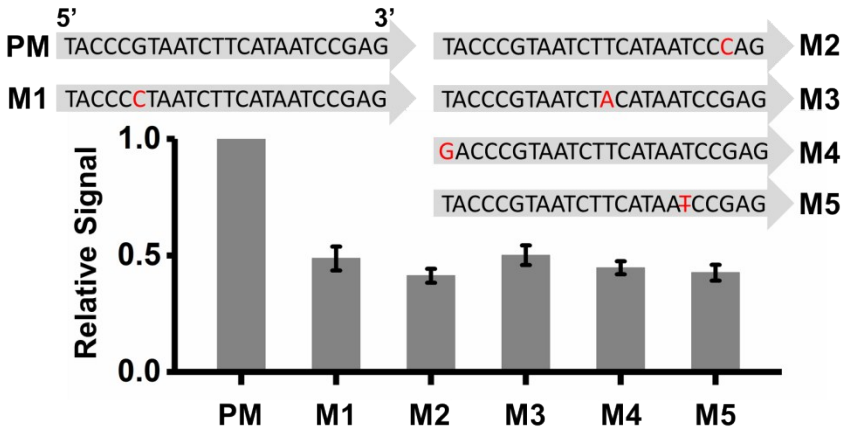


Fig. S13 Differentiates between perfectly matched and mismatched nucleic acids at five different positions.

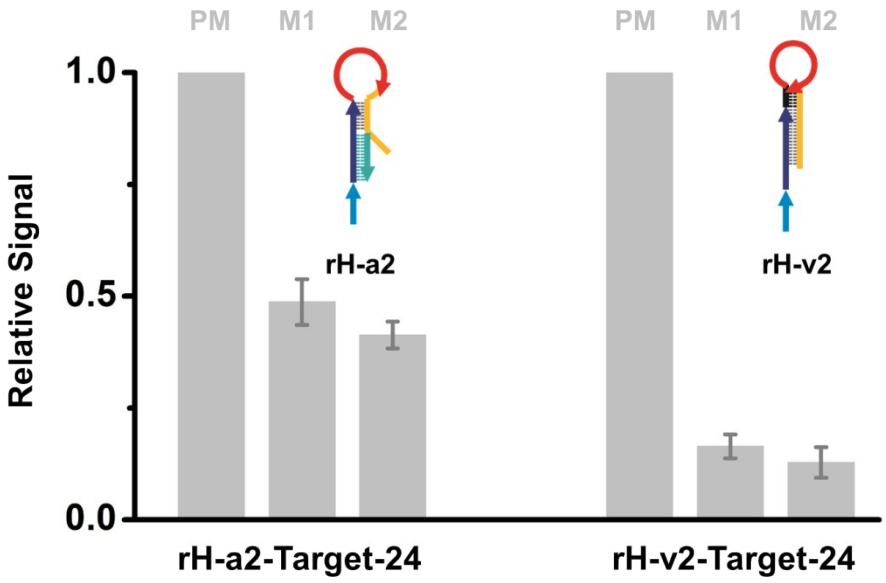


Fig. S14 Differentiates between perfectly matched and mismatched nucleic acids. The data shown were collected at 10 nM of target-24 using rH-a2 (left) and rH-v2 (right) as receptor respectively.

1. X. Q. Chen, G. B. Zhou, P. Song, J. J. Wang, J. M. Gao, J. X. Lu, C. H. Fan, X. L. Zuo, Ultrasensitive Electrochemical Detection of Prostate-Specific Antigen by Using Antibodies Anchored on a DNA Nanostructural Scaffold. *Anal. Chem.,* 2014**, 86**, 7337-7342.

2. Z. L. Ge, M. H. Lin, P. Wang, H. Pei, J. Yan, J. Y. Shi, Q. Huang, D. N. He, C. H. Fan, X. L. Zuo, Hybridization Chain Reaction Amplification of MicroRNA Detection with a Tetrahedral DNA Nanostructure-Based Electrochemical Biosensor. *Anal. Chem.* 2014, **86**, 2124-2130.