Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information For:

Biosensor based on multifunctional allostery for dynamic analysis of circulating tumor DNA

EXPERIMENTAL SECTION

Reagents. The DNA oligonucleotides listed in Table S1 were synthesized, modified and purified by Sangon Biotechnology Inc. (Shanghai, China). Gel red, agarose, casein and reagents used to prepare different buffers (such as boric acid, EDTA, etc.) were also purchased from Sangon Biotechnology Inc. tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 3,3',5,5'-tetramethylbenzidine (TMB, slow kinetic form), bovine serum albumin (BSA) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (USA). Streptavidin-HRP was obtained from Abcam (UK).

Buffers. TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0) was used to synthesize the tetrahedral DNA nanostructure. The electrode was rinsed with 0.1 M phosphate buffer (10 PB, 137 mM NaCl, 2.5 mM KCl, 0.05% Tween 20, pH 7.4). The hybridization chain reaction buffer 1× SPSC contained 50 mM sodium phosphate, 1 M NaCl, pH 7.5. The final 1×TBE buffer (40 mM Tris-acetate, 25 mM boric acid and 1 mM EDTA) was used for agarose gel electrophoresis (AGE). Stocking buffer (10 mM sodium phosphate pH 7.4, 0.1 M NaCl, 1% casein) was used to dilute streptavidin-HRP.

Native Gel Electrophoresis. The rH, sH and cH samples in SPSC were heated at 95°C for 5 min and then cooled to 4°C within 60 s using an ABI Veriti96 thermal cycler (Thermo Fisher Scientific, USA). The 2% agarose gels containing 0.01 μL of gel red were prepared with 1× TBE buffer, run at 150 V for 40 min, and visualized under UV light (Gel Doc XR+; Bio-Rad, USA).

Fluorescence Analysis. Samples comprising 100 μL of 1 μM rH (rH/inhibitor, rH, rH/activator), 1 μM sH (FAM-sH-dabcyl or FAM-sH'-dabcyl), or 1 μM cH, were individually incubated at 95°C for 5 min, allowed to cool to 4°C within 60 s, and then mixed together with different concentrations of the target (Exon-5: 0-10 μM) in 100 μL 1× SPSC buffer at a final concentration of 100 nM. DNA

nanowire assembly was carried out in 600 μL tubes for fluorescence analysis or on the surface of an Au chip for fluorescence imaging. The traditional molecular beacon fluorescence assay (FAM-rH-exon-5-dabcyl with or without cooperator) was carried out analogously. The fluorescence spectra were recorded at room temperature in a quartz cuvette on a F7000 spectrofluorometer (Hitachi, Japan). Time-dependent fluorescence monitoring was performed with a time interval of 1 s. The excitation wavelength was 492 nm and the emission wavelength was 516 nm. Fluorescence intensity with or without target DNA on the gold surface was directly imaged under a microscope (Axioskop2, Carl Zeiss Shanghai Co., Ltd).

Electrocatalytic Analysis. Samples comprising 100 μL of 5 μM rH (rH/inhibitor, rH, rH/activator), 5 μM sH (biotin-sH), or 5 μM cH were individually incubated at 95°C for 5 min, allowed to cool to 4°C within 60 s, and then mixed together with different concentrations of the target (ctDNA: 0-10 μM) in 100 μL 1× SPSC buffer (containing 0.5% casein and 1% BSA) for a final concentration of 100 nM. The hybridization chain reaction was carried out at room temperature. After 2 h, 0.25 μL of streptavidin−HRP (1 mg mL^{-1}) was added to bind with the biotin conjugated to sH before incubation with the FNA-assembled electrode for further electrochemical measurements. Gold electrodes were incubated with 3 μL of FNA overnight at room temperature. The resulting electrodes were rinsed with phosphate buffer and then incubated with the different DNA nanowire pretreatment solutions. Multivalent capture of DNA nanowire products could be completed in 30 minutes. The electrochemical measurements were conducted using slow kinetic TMB substrate at room temperature. ATP detection was performed analogously by replacing the DNA target and recognition probe with ATP and an ATP aptamer.

Table S1. DNA sequences used in this study

	DNA Sequence (5'-3')
rH-Exon-5 (rH)	TTAACCCACGCCGAATCCTAGACTGGCAACCAGCCCTGTCTGTAGGAACAAAGTA GTCTAGGATTCGGCGTGCCAATT
rH-Exon-6	TTAACCCACGCCGAATCCTAGACTGACCTCAGGCGACCCTGGGCAACCCAAAGTA GTCTAGGATTCGGCGTGCCAATT
rH-Exon-7	TTAACCCACGCCGAATCCTAGACTGTCTACAGTCAGAGCCAACCTAGGCAAAGTA GTCTAGGATTCGGCGTGCCAATT
rH-Exon-8	TTAACCCACGCCGAATCCTAGACTTTATGCCTTCAGAAGTCTAGGATTCAAAGTAG TCTAGGATTCGGCGTGCCAATT
Biotin-sH (sH)	Biotin-AGTCTAGGATTCGGCGTGGGTTAATCCTTCCTCTTCCGACACGACCTTCACGCCGA ATCCTAGACTACTTTG
FAM-sH-Dabcyl (F-sH-Q)	FAM-AGTCTAGGATTCGGCGTGGGTTAATCCTTCCTCTTCCGACACGACCTTCACGCCGA ATCCTAGACT/dabcyl/ACTTTG
FAM-sH'-Dabcyl (F-sH'-Q)	FAM-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACT/dabcyl/ACTTTG
cH	CTCTTCCGACACGACCTTGTATCAGTAAGGTCGTGTCGGAAGAGGAAGGA
Inhibitor	AATTGGTTGGTTAA
Activator 1	AATTGGCACGCC
Activator 2	ACAGACAGGG
Exon-5	TTCCTACAGACAGGGCTGGTTGTC
Exon-6	GGTTGCCCAGGGTCGCCTGAGGTC
Exon-7	CCTAGGTTGGCTCTGACTGTAGAC
Exon-8	AATCCTAGACTTCTGAAGGCATAA
Exon-5-M1	TTCCTACAGACACGGCTGGTTGTC
Exon-5-M2	TTCCTAGAGACAGGGCTGGTTGTC
Exon-5-M3	ATCCTACAGACAGGGCTGGTTGTC
Exon-5-M4	TTCCTACAGACAGGGCTGGTTGTC
Exon-5-M5	TTCCTACAGACAGCGGCTGGTTGTC
5'termination	AGTCTAGGATTCGGCGTGGGTTAA
3'termination	CACGCCGAATCCTAGACTACTTTG
5'termination-M1	AGTCTAGGATTCGCCGTGGGTTAA
5'termination-M2	AGTCTACGATTCGGCGTGGGTTAA
5'termination-M3	AGTCTAGGATTCGGCGTGGGTTTA
FAM-rH-Exon-5-Dabcyl (traditional molecular beacon assay)	TTAACCCACGCCGAAT/FAMCCTAGACTGGCAACCAGCCCTGTCTGTAGGAACAA AGTAGTCTAGGAT/dabcyl/TCGGCGTGCCAATT
rH-ATP	TTAACCCACGCCGAATCCTAGACTTGGGGGAGTATTGCGGAGGAACAAAGTAGT CTAGGATTCGGCGTGCCAATT
ATP aptamer	ACCTGGGGGAGTATTGCGGAGGAAGGT

C-ATP	TTCCTCCGCAATACTCCCCCA
A20	ACTGATACAAGGTCGTGTCGTTTTTTTTTTCTCAACTGCCTGGTGATACGAGGATG GGCATGCTCTTCCCGACGGTATTGGACCCTCGCATG
SH-B20	SH-CGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCGG GTGATAAA
SH-C20	SH-CGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCC AATACCG
SH-D20	SH-CGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCA TAGTAG
FNAs-20	Framework nucleic acids were 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.

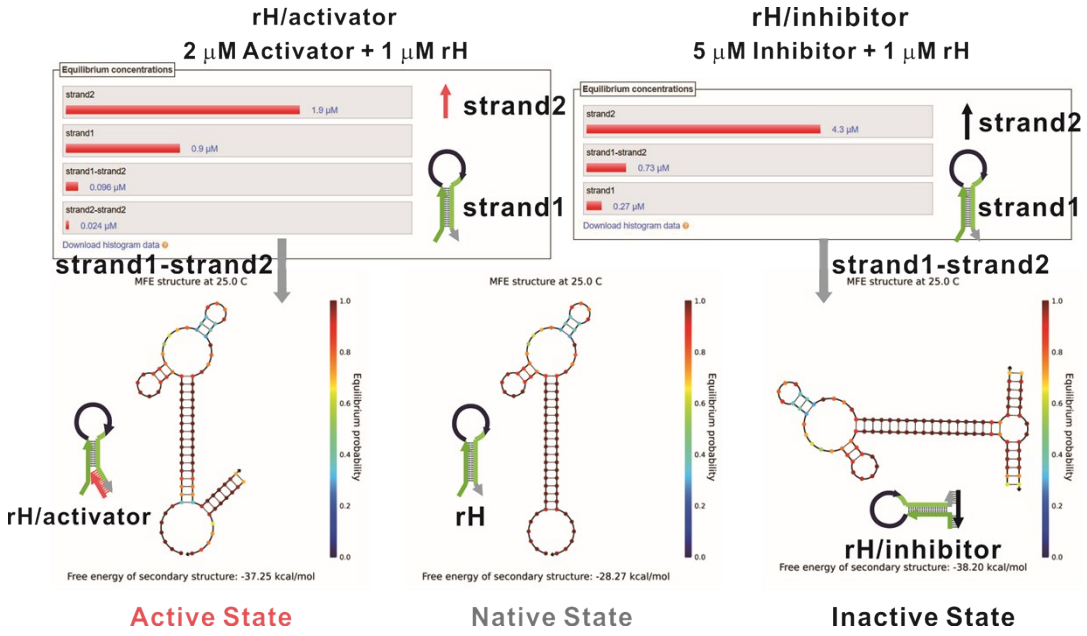

Figure S1. (a) The equilibrium concentrations and equilibrium probabilities of rH/activator, rH and rH/inhibitor were estimated via NUPACK at 25 °C. (Web for reference: http://www.nupack.org/).

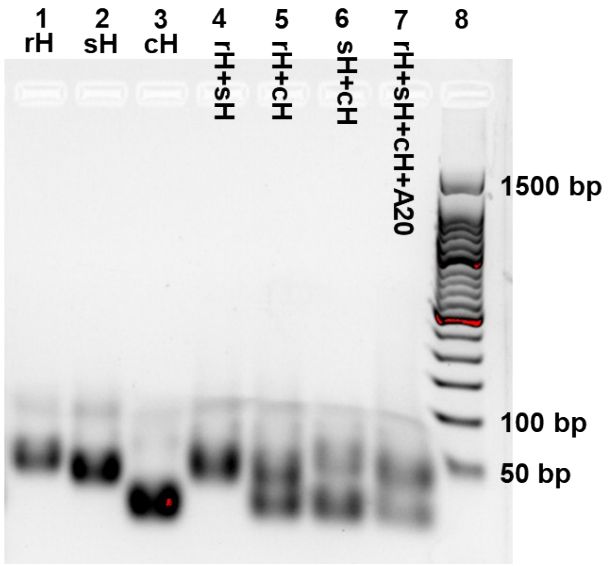

Figure S2. Lane 1, 2.5 µM rH; Lanes 2, 2.5 µM sH; Lane 3, 2.5µM cH; Lane 4, 1.5 µM rH/sH; Lane 5, 1.5 µM rH/cH; Lane 6, 1.5 µM sH/cH. Lane 7, 1 µM rH/sH/cH and 0.2µM A20; Lane 8, 50bp DNA ladder markers.

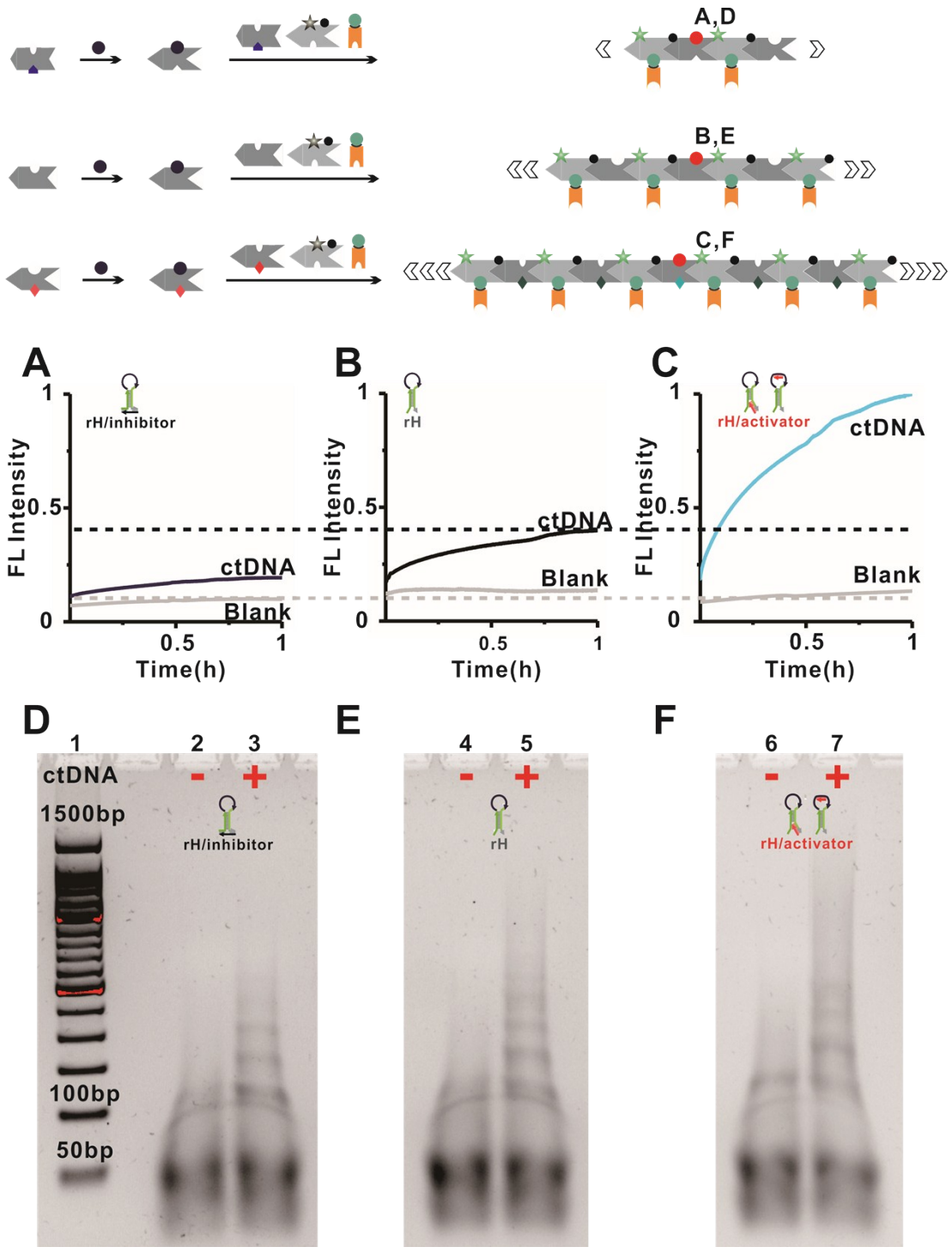

Figure S3. A-C) Kinetics of versatile DNA nanowire assembly with mFA regulation. 0 and 100 nM Exon-5 were introduced into the system at t≈0. Fluorescence intensity of each sample was monitored within 30 s of mixing by pipetting. Here, [rH] = [sH] = [cH] = 50 nM. The control trace and reaction trace show the DNA nanowire assembly induced with 0 and 100 nM ctDNA, respectively. The kinetics of DNA nanowire

assembly was accelerated by adding the activator (C) or hindered by adding the inhibitor (A). D-F) Agarose gel electrophoresis of bidirectional and multi-branched DNA nanowire assembly. Lane 1, 50bp DNA ladder markers; Lanes 2-3, 1 μM rH+5 μM inhibitor + 1 μM sH + 1 μM cH + 0 or 0.2 μM Exon-5; Lanes 4-5, 1 μM rH + 1 μM sH + 1 μM cH + 0 or 0.2 μM Exon-5; Lanes 6-7, 1 μM rH+2 μM activator + 1 μM sH + 1 μM cH + 0 or 0.2 μM Exon-5; Lane 8, 50bp DNA ladder markers.

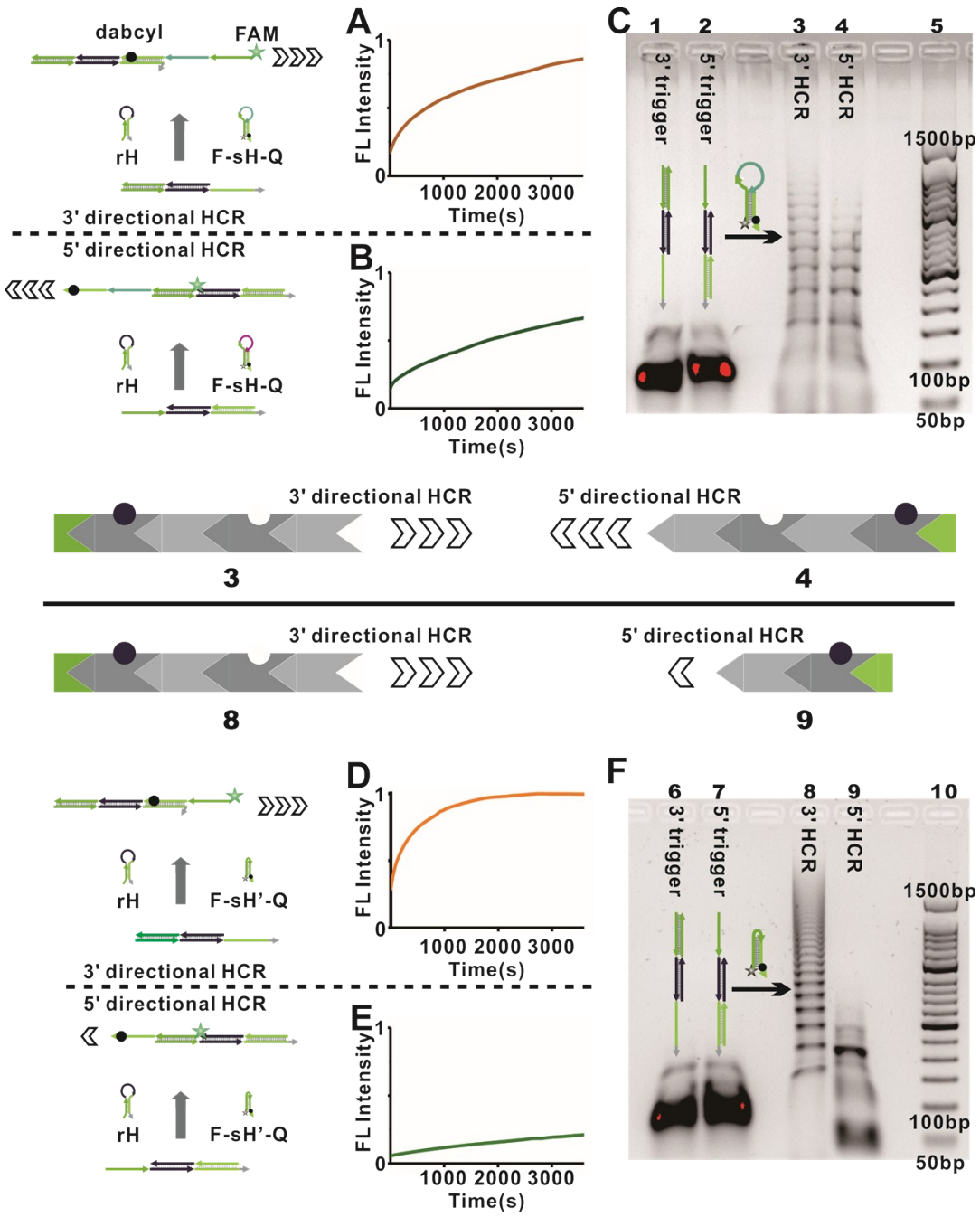

Figure S4. Same amounts of 3' trigger (A) and 5' trigger (B) were added into the system. Here, [rH] = [F-sH-Q] = 50 nM. Increasing traces of fluorescence indicated the kinetics of DNA nanowire assembly along 3' direction or 5' direction. C) Analysis by AGE of the DNA nanowire assembly along the 3' direction or 5' direction. Lanes 1, 3 μM 3' trigger (3 μM rH + 3 μM Exon-5 + 3 μM 5'termination); Lane s2, 3 μM 5' trigger (3 μM rH + 3 μM Exon-5 + 3 μM 3'termination); Lane 3, 3' HCR (1 μM rH + 1

μM F-sH-Q + 1 μM cH + 0.2 μM 3'trigger); Lanes 4, 5' HCR (1 μM rH + 1 μM F-sH-Q + 1 μM cH + 0.2 μM 5'trigger); Lane 5, 50bp DNA ladder marker. Same amounts of 3'trigger (D) and 5' trigger (E) were added into the system. Here, [rH] = [F-sH'-Q] = 50 nM. Increasing traces of fluorescence indicate the kinetics of DNA nanowire assembly along the 3' direction or 5' direction. (F) Analysis by AGE of the DNA nanowire assembly along the 3' direction or 5' direction. Lane 6, 3 μM 3' trigger (3 μM rH + 3 μM Exon-5 + 3 μM 5' termination); Lane 7, 3 μM 5' trigger (3 μM rH + 3 μM Exon-5 + 3 μM 3' termination); Lane 8, 3' HCR (1 μM rH + 1 μM F-sH'-Q + 1 μM cH + 0.2 μM 3'trigger); Lane 9, 5' HCR (1 μM rH + 1 μM F-sH'-Q + 1 μM cH + 0.2 μM 5'trigger); Lane 10, 50bp DNA ladder markers.

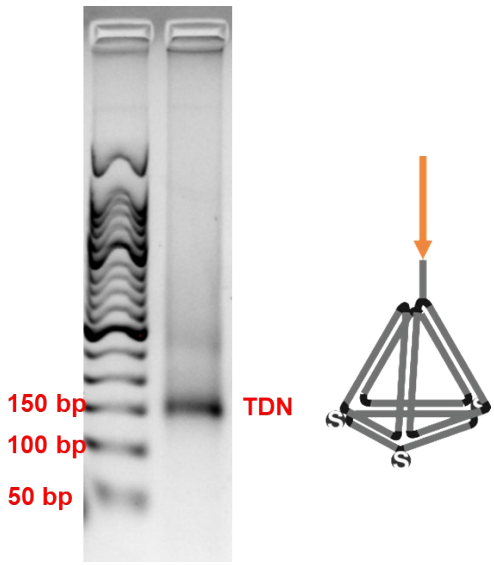

Figure S5. AGE analysis validates the formation of FNAs.

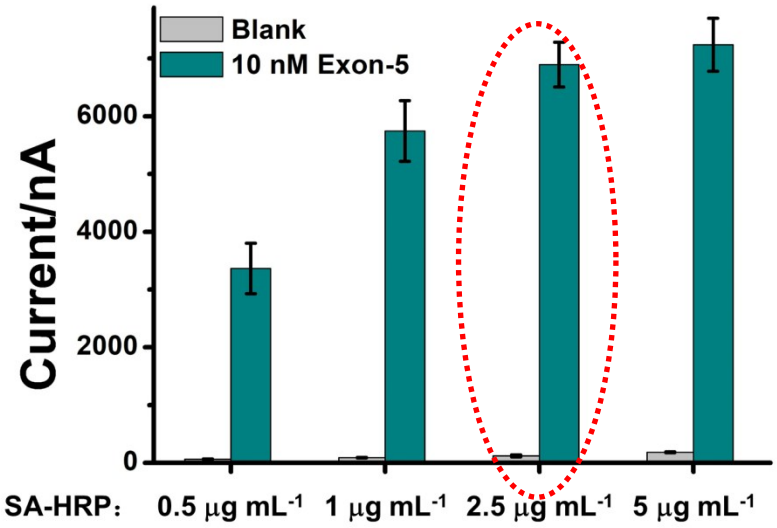

Figure S6. The concentration of SA-HRP pretreated with DNA nanowires for Exon-5 detection was optimized.

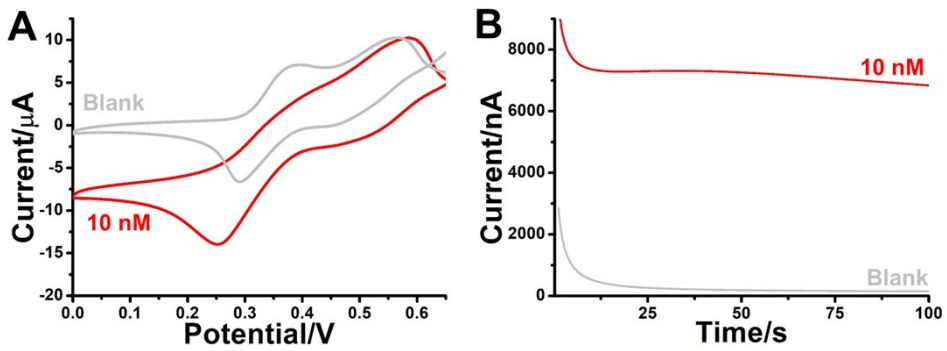

Figure S7. (a) We evaluated the biosensor with 10 nM Exon-5. We observed a significant increase of current in cyclic voltammetry compared with the control group, which indicated the occurrence of electrocatalytic reaction. (b) We used i-t curves to quantify the electrocatalytic signal, and obtained a current of ~6841 nA with 10 nM Exon-5, which was ~49 times higher than the current without the Exon-5 (~139.6 nA).

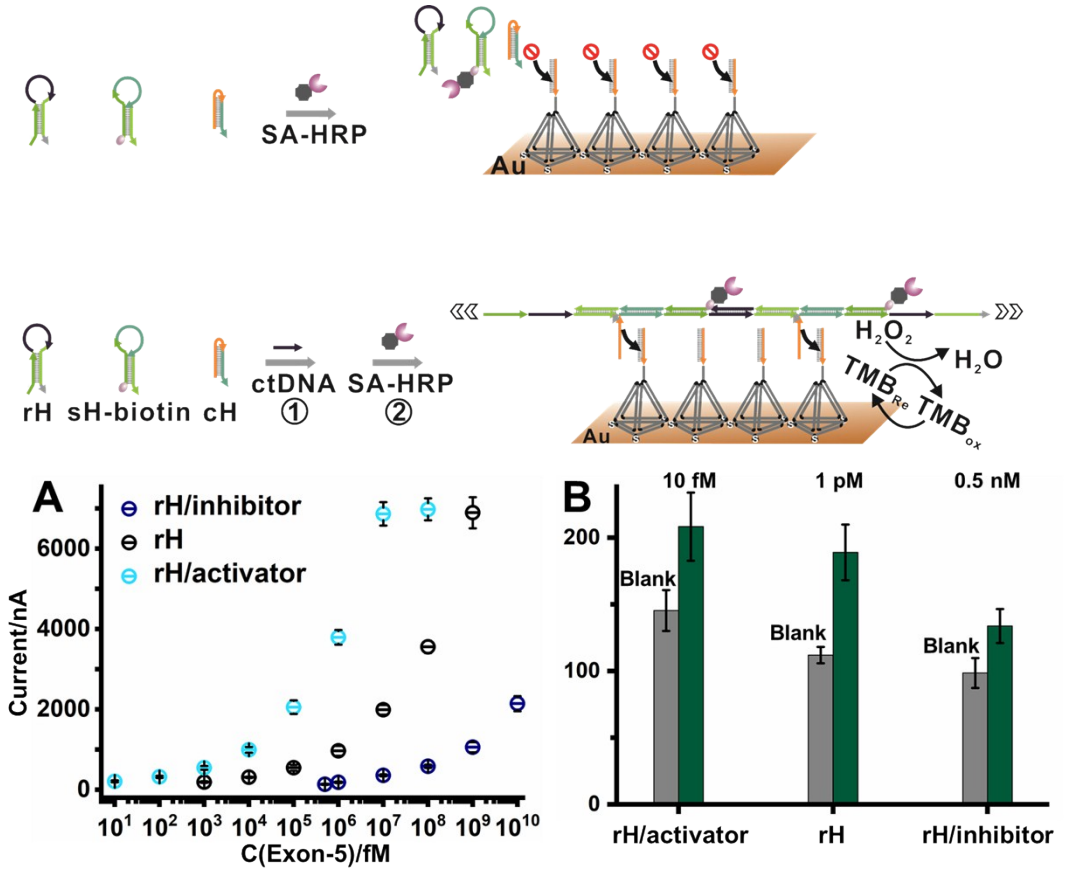

Figure S8. (A) Logarithmic plot of amperometric current versus Exon-5 concentration for the multifunctional allostery controlled electrocatalytic assay. (B) Electrocatalytic i-t signal of the background and the detection limits in (A).

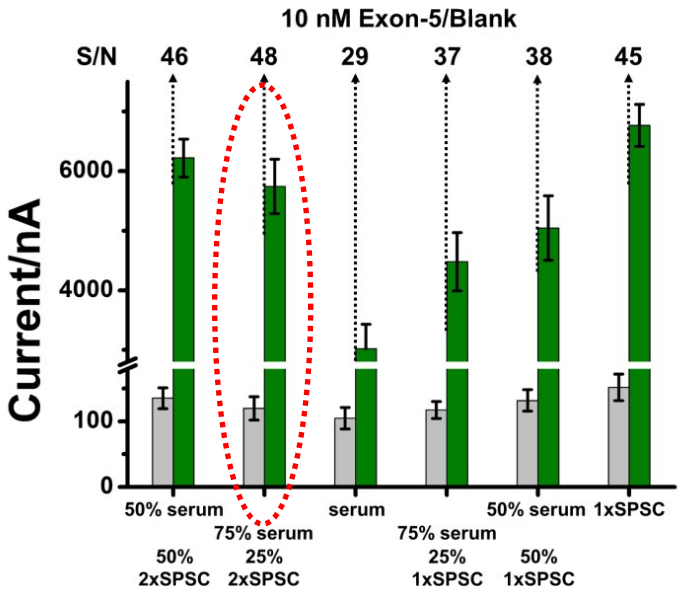

Figure S9. The signal-to-noise ratio of 10 nM Exon-5 detected in SPSC buffer, diluted and undiluted serum.

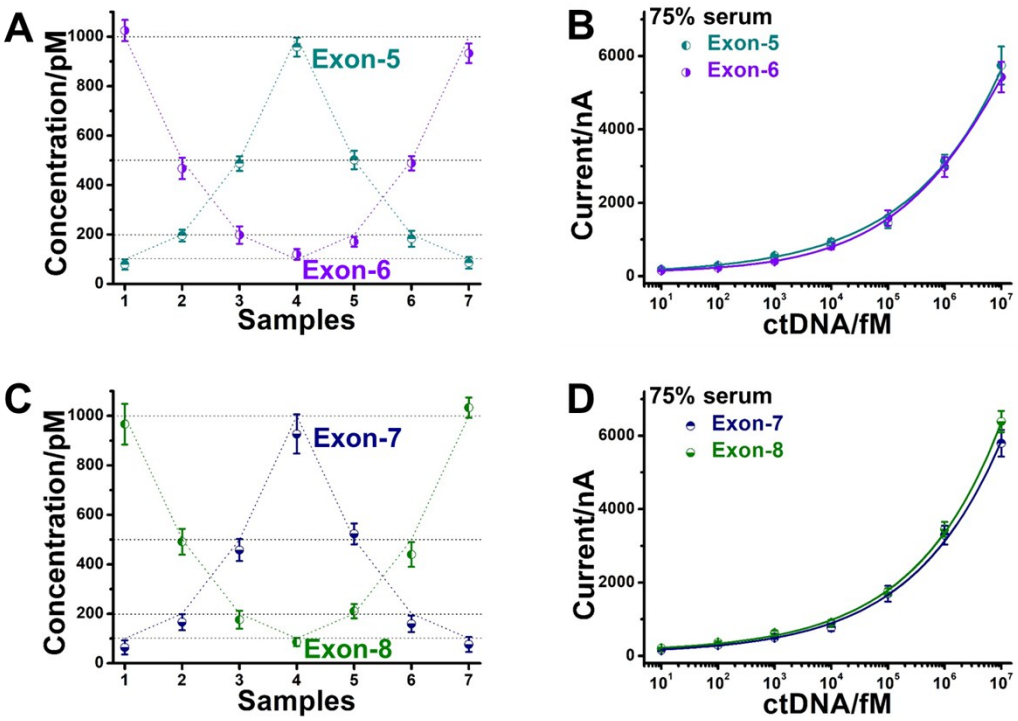

Figure S10. Detection of varying ctDNA concentrations in 75% serum and 25% of 2× SPSC buffer. (A) Seven samples (1 to 7) containing varying amounts of Exon-5 (dark cyan) and Exon-6 (violet) (dotted lines indicate the known concentrations) were measured. The results deviating from the dotted lines are due to experimental errors. (B) Logarithmic plot of amperometric current versus Exon-5 (dark cyan) and Exon-6 (violet) concentration for the electrocatalytic assay in 75% serum and 25% of 2× SPSC buffer. (C) Seven samples (1 to 7) containing varying amounts of Exon-7 (navy) and Exon-8 (green) were measured. (D) Logarithmic plot of amperometric current versus Exon-7 (navy) and Exon-8 (green) concentration for the electrocatalytic assay in 75% serum and 25% of 2× SPSC buffer.

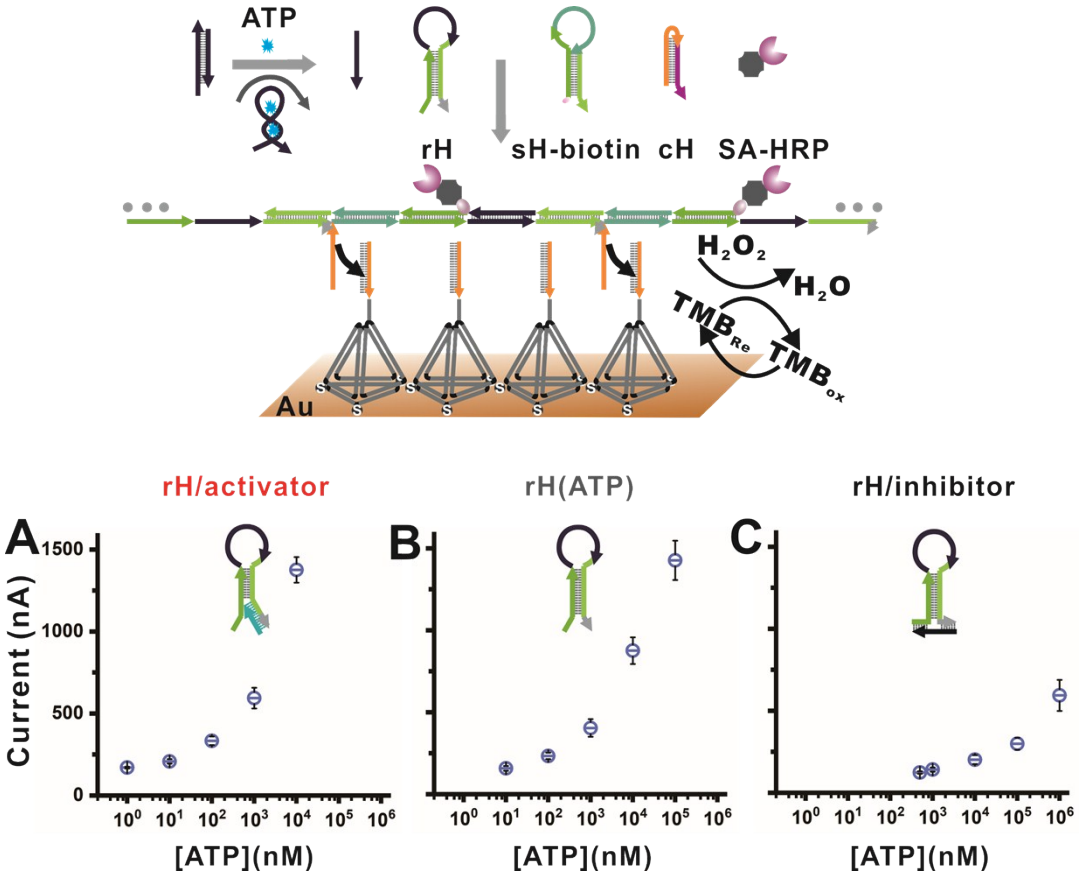

Figure S11. Schematic illustration of the dynamic electrocatalytic assay for ATP detection. The recognition probe is an ATP aptamer sequence hybridized with its complementary sequence with a teminal deletion of six bases. The dynamic range of the single-step electrocatalytic assay for ATP detection can be tuned using multi-functional allostery. The detection limits were 1 nM, 10 nM, and 500 nM for (A) rH-1a, (B) rH and (C) rH-3i, respectively.