Supporting information
Signal-on electrochemical Y or junction probe detection of nucleic acid

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Experimental Section

Materials

All unlabeled DNA oligonucleotides were synthesized in house whereas labeled oligonucleotides were purchased from Biosearch Technology. Restriction endonucleases were purchased from Frementas Inc. USA. 6-Mercaptohexanol and Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. All chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ·cm) was used.

Au Electrode surface cleaning and modification

Au electrodes (2mm in diameter, CH Instrument, Inc.) were cleaned before DNA self-assembly. The cleaning procedures were done according to published protocol (Nature Protocols, 2007, *2*, 2875-2879). 200 μM Probe A (1μL) was mixed with 10 mM TCEP (2 μL) at room temperature in the dark for 45 min. The mixture was then diluted with PBS buffer (10 mM phosphate-buffered saline pH 7.4 with 1M NaCl and 1mM Mg^{2+}) to a final Probe A concentration of 200 nM. Each electrode was immersed with 100 μL of 200 nM stock Probe A in PBS for 30 min. After rinsing with deionized water, the electrodes were incubated in 2 mM 6-Mercaptohexanol solution for 3-5 h at room temperature in the dark. The electrodes were rinsed for 3 min using deionized water to remove any remaining 6-Mercaptohexanol solution. The electrodes were then soaked in PBS for 5 min before any electrochemical measurements.

Square wave voltammetry measurements (SWV) of signal-off JP and different restriction endonucleases screening

All electrochemical measurements were performed with a CHI 660 electrochemical workstation (CH Instrument, Inc,). The normal three-electrode system consisted of Au working electrode, platinum wire counter electrode, and 3M NaCl saturated Ag/AgCl reference electrode. SWV was carried out in the 10 mM PBS (pH 7.4) from -0.1V to -0.4V with 0.001V interval, 60Hz frequency and 0.025 amplitude. Reaction condition: 1μL of restriction endonuclease (stock solution from company), 1 μL of 20 μM Probe B, 1 μL of 2 μM template, 10 μL of 10 x restriction endonuclease buffer, 87 μL of deionized water. Reaction time and temperature were 4 h and 31 □C respectively. Final concentrations: 200 nM Probe B, 20 nM template.

Alternating current voltammetry measurements (ACV) of signal-on JP

System set up was the same as the SWV measurements. ACV was performed in the 10 mM PBS (pH 7.4) from -0.1V to -0.45V with 0.01 V interval, 50Hz frequency, 0.025 amplitude, 1 sec sample period.

Table S1 Sequences of oligonucleotides used in this study.

Oligonucleotide	Sequences (5'->3')
Probe A1	5'-SH-C_6H_{12}-TTTTCCACCGCCAATATTTTT*GATC*TGTGG(MB)-3'
Probe A2	5'-SH-C_6H_{12}-TTTTCCT(MB)CCGCCAATATTTTT*GATC*TGAGG-3'
Probe B (helper probe)	5'TG(S)*GATC*GGAAAACC(S)*GATC*CA(S)*GATC*ATATACGTGCTGCTA-3'
Full match template	5'-TAGCAGCACGTA-AATATTGGCG-3'
Mismatch A template	5'-TAGCAGCACGTA-AATAATGGCG-3'
Mismatch G template	5'-TAGCAGCACGTA-AATAGTGGCG-3'
Mismatch C template	5'-TAGCAGCACGTA-AATACTGGCG-3'

The locations of mismatched bases are colored blue.
Endonuclease cognate recognition site are highlighted in italic and underlined.
(S) denotes phosphorthioate linkage.