Supporting Information

Versatile and highly sensitive homogeneous electrochemical strategy

based on split aptamer binding-induced DNA three-way junction and

exonuclease III-assisted target recycling

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Fig. S1. (A) The DPV peak current versus pH value of the buffer solution in the absence and presence of 50 nM ATP, respectively, and the signal-to-noise ratio (S/N) versus pH value. (B) The DPV peak current versus the reaction temperature in the absence and presence of 50 nM ATP, respectively, and the signal-to-noise ratio (S/N) versus the reaction temperature. (C) The DPV peak current versus the reaction time toward the detection of 50 nM ATP.



Fig. S2 The schematic illustration of the DNA three-way junction self-assembled by DNA1, DNA2 and MB-DNA in the presence of the target analyte (ATP).

Name	Sequence (from 5' to 3')
DNA1	5'– <u>AGACG</u> GG <i>CTT</i> C GGAGGAGG TATAGA–3'
DNA2	5'- CTGGGGGAG AAG <u>AAACCAA</u> TGGAGAG-3'
MB-DNA	5'-ATTGTGTATGTTGC <u>TTGGTTTCGTCT</u> -methylene blue-3'

Table S1 Sequences of the oligonucleotides used in the experiments ^a

^{*a*} In DNA1 and DNA2, the boldface letters represent the sequences of ATP split aptamer fragments. The italic letters in DNA1 and DNA2, the singly underlined letters in DNA1 and MB-DNA, and the doubly underlined letters in DNA2 and MB-DNA represent the sequences complementary to each other, respectively.

Method	Detection Limit	Strategy	Ref.
	(M)		
Homogeneous	1.0 × 10 ⁻¹⁰	Split aptamer binding-induced three-way junction and signal amplification by Exo III-assisted target recycing	This work
Homogeneous	1.0 × 10 ⁻⁹	Aptamer-based strategy with signal amplification by Exo III-assisted ATP recycling	1
Heterogeneous	1.0 × 10 ⁻⁸	Highly generalizable target-responsive electrochemical aptamer switch (TREAS)	2
Heterogeneous	1.0 × 10 ⁻⁸	Utilization of the aptamer complementary DNA oligonucleotides as probes for electrochemical sensing	3
Heterogeneous	1.0 × 10 ⁻⁶	An electrochemical sandwich assay based on split aptamers	4
Heterogeneous	1.0 × 10 ⁻¹⁰	Blank peak current-suppressed electrochemical aptameric sensing platform	5
Heterogeneous	3.0×10^{-10}	Microfluidic electrochemical aptamer- based sensor by constructing Au/Ag dual- metal array three-electrode on-chip	6
Heterogeneous	3.0 × 10 ⁻⁸	"Signal on" and one-spot simultaneous detection of multiple small molecular analytes based on electrochemically encoded barcode quantum dot tags	7

 Table S2 Comparison of analytical performance for ATP detection by our strategy and other

 electrochemical methods reported in literature

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