Electronic Supplementary Material (ESI) for Analytical Methods.


Supporting information

# A DNA polymerase-powered self-propelled DNA walking strategy for one-step, amplified and dual-signal electrochemical target detection

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**Table S1.** DNA sequences used in this work

| **Name** | **Sequences (from 5' to 3')** |
|---|---|
| HP1 | SH-TTTTTCCTGACTTCCAACGCCTCGACAGATCACTTTGTGG AAAATCTCTAGCAGTCTTGGTGATCTGTCGAGTAT |
| HP2 | SH-TTTTTTGTTCAGCTCGATCGGGAAAAGGAGCCGTGGATAC TCGACTAGTTTCTCCCTCCACGGCTCCTTTTCCACA |
| S1 | GCGTTGGAAGTCAGG-MB |
| S2 | CGATCGAGCTGAACA-Fc |
| Target (HIV DNA fragment) | ACTGCTAGAGATTTTCCACAT |
| 1M (One-base mismatched) | ACTCCTAGAGATTTTCCACAT |
| 3M (Three-base mismatched) | ACTCCTAGTGAATTTCCACAT |
| NC (Non-complementary) | TATTGCATGCTACCTGACTGA |

Underline letter in 1M and 3M sequences indicate the mismatched bases.

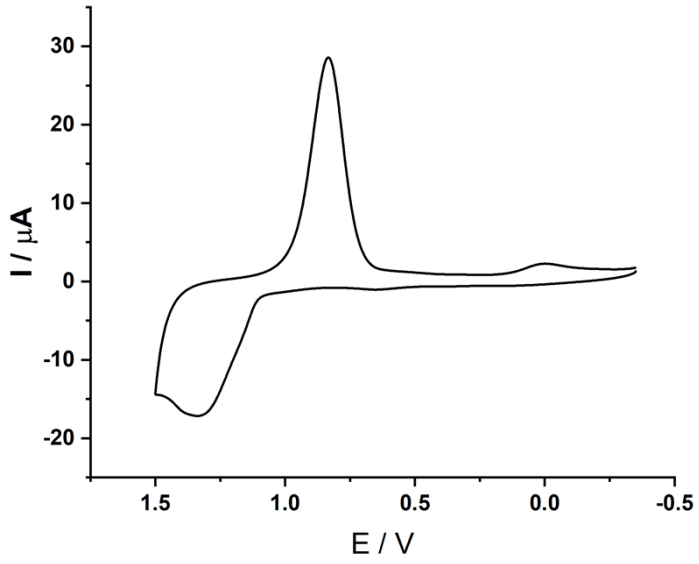


**Figure S1.** Cyclic voltammetric scan for the bare electrode in 0.05 M $H_2SO_4$ solution between -0.35V and 1.5V at a scan rate of 100 mV/s.

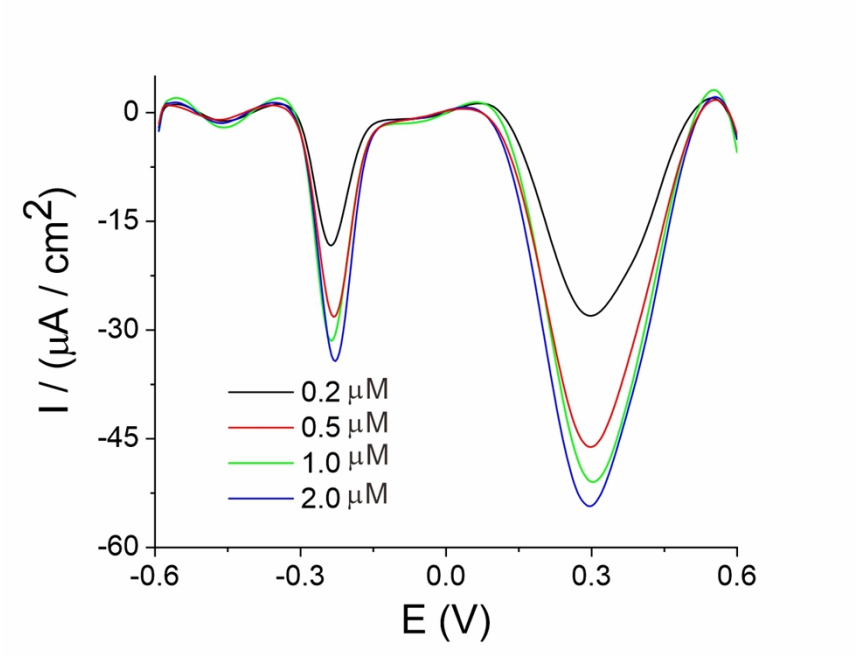


**Figure S2**. The SWV responses of the biosensors obtained at different immobilization concentrations of HP1/S1 and HP2/S2. The HP1/S1 and HP2/S2 were used with the same concentration.

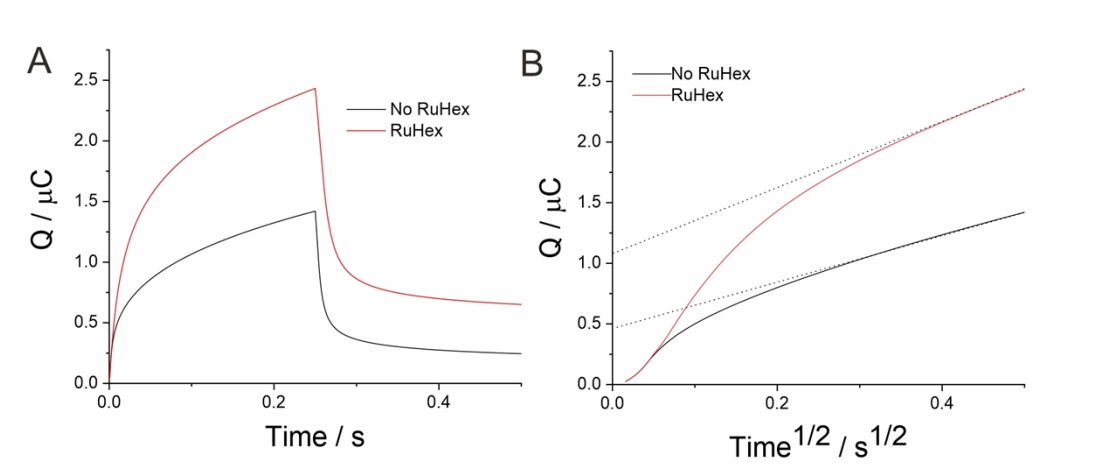


**Figure S3.** Chronocoulometric curves for HP1/S1 and HP2/S2 assembled electrode in 10 mM Tris-HCl buffer (pH 7.4) containing RuHex (50 μM) or not. Both the immobilization concentration of HP1/S1 and HP2/S2 were 0.5 μM. The relationship of charge versus time (A) and charge versus square root of time (B).

The surface density of the assembled probe was based on a typical chronocoulometric curves according to the reported method.[1]

The integrated current, or charge (Q), as a function of time (t) in a chronocoulometric experiment is given by the integrated Cottrell expression 1,

$$Q = \frac{2nFAD^{1/2}C}{\pi^{1/2}}t^{1/2} + Q_{dl} + nFA\Gamma_0 \qquad (1)$$

where n is the number of electrons per molecule for reduction, F is the Faraday constant (C/equiv), A is the electrode area ($cm^2$), D is the diffusion coefficient ($cm^2/s$), C is the bulk concentration (mol/ $cm^2$), $Q_{dl}$ is the capacitive charge (C), and $nFA\Gamma_o$ is the charge from the reduction of $\Gamma_o$ ($mol/cm^2$) of adsorbed redox marker. The term $\Gamma_o$ designates the surface excess and represents the amount of redox marker confined near the electrode surface.

The chronocoulometric intercept at t = 0 is then the sum of the double layer charging and the surface excess terms. The surface excess is determined from the difference in chronocoulometric intercepts for the identical potential step experiment in the presence and absence of redox marker. Then, the surface density of probe was calculated according to the equation 2.

$$\Gamma_{DNA} = \Gamma_0\left(\frac{Z}{m}\right)N_A \qquad (2)$$

where $\Gamma_{DNA}$ is the probe surface density in molecules/$cm^2$, m is the number of bases in the probe DNA, z is the charge of the redox molecule, and $N_A$ is Avogadro's number.

**Table S2.** Comparison of the detection performance of current biosensor with some reported methods

| Method | Linear range | Detection limit | Strategy | Ref. |
|---|---|---|---|---|
| Electrochemistry | 0.1 fM-10 pM | 31.6 aM | T7 exonuclease-assisted strand displacement amplification | [2] |
| Electrochemistry | 10 fM-1 nM | 3.62 fM | Hollow carbon sphere/polyaniline | [3] |
| Electrochemistry | 0.5 pM-80 pM | 0.12 fM | Triple-helix molecular switch | [4] |
| Electrochemistry | 50 fM-500 fM | 36 fM | Strand displacement and DNA walker | [5] |
| Electrochemistry | 5 fM-50 nM | 4.86 fM | Single-particle electrochemistry and DNA walker | [6] |
| Fluorescence | 50 pM-10 nM | 20 pM | exonuclease-assisted target recycling and perylene diimide quencher | [7] |
| Fluorescence | 0.8 nM-200 nM | 0.2 nM | Tripyridinyl ruII complex-encapsulated $SiO_2$@polydopamine | [8] |
| Colorimetry | 1 pM-75 nM | 0.14 pM | Associative toehold activation and catalyzed hairpin assembly | [9] |
| Colorimetry | 0.1 pM - 1500 nM | 0.042 pM | DNAzyme Hydrogel | [10] |
| Electrochemistry | 0.1 fM-0.1 pM (methylene blue）<br>0.1 fM-1 pM (ferrocene) | 0.1 fM | DNA polymerase-powered DNA walking | This work |

**Table S3.** Recovery experiments of the sensing system toward spiked target DNA in buffer and 2% diluted serum

| Samples | Added (fM) | Detected (fM) | Recovery |
|---|---|---|---|
| Buffer | 5 | 4.63 ± 0.25 (MB) | 92.6% |
| | | 5.31 ± 0.61 (Fc) | 106% |
| Serum | 5 | 5.49 ± 0.65 (MB) | 110% |
| | | 4.89 ± 0.73 (Fc) | 97.8% |
| Buffer | 50 | 52.4 ± 3.5 (MB) | 105% |
| | | 61 ± 5.3 (Fc) | 122% |
| Serum | 50 | 58.2 ± 2.72 (MB) | 116% |
| | | 44.6 ± 3.88 (Fc) | 89% |

The results were based on three repetitive experiments.

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