Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A signal-on electrochemical DNA biosensor based on exonuclease III-assisted recycling amplification

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Feasibility validation

In order to verify the feasibility of the sensor using Exo III-assisted signal amplification, we evaluated the conformations and molecular hybridization of pDNA and cDNA oligonucleotides using the NUPACK web server. The evaluation result showed that pDNA forms a stable hairpin structure in solution and the free energy of the secondary structure (E_{free}) was -9.92 kcal/mol (Fig.S1A). pDNA hybridize with tDNA to form a more stable double-stranded structure with free energy (E_{free}) was -22.02 kcal/mol, while Exo III could cleave its blunt 3 ' terminus (Fig. S1B). However, pDNA and cDNA cannot hybridize because they have the same number of base pairings. Alternatively, the remaining DNA and cDNA hybridize to form a stable double-stranded structure with free energy (E_{free}) was -13.52 kcal/mol (Fig. S1C), which can facilitate successful anchoring of methylene blue at the electrode.

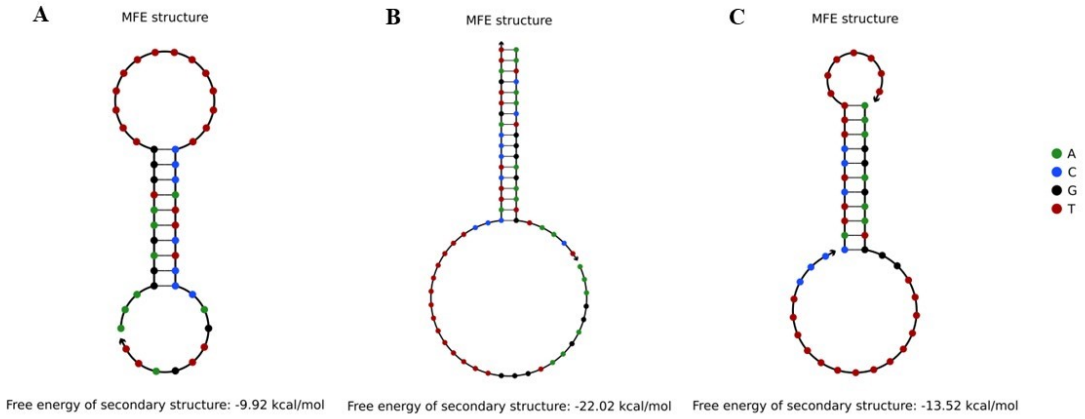

Fig. S1. The conformation and secondary structure free energy of oligonucleotide sequences were evaluated by NUPACK analysis：(A) pDNA; (B) pDNA+tDNA; (C) rDNA+ cDNA.

Polyacrylamide Gel Electrophoresis

Electrophoresis experiment was performed by referencing the standard protocol to prove the target DNA -triggered digestion of probe DNA by Exo-III. PAGE (12%) in the 1 × TBE buffer for 80 min under a voltage of 100 V was used to test different samples. The results showed that the Exo III was able to specifically cleave the target/probe DNA duplex with 3′ blunt end.

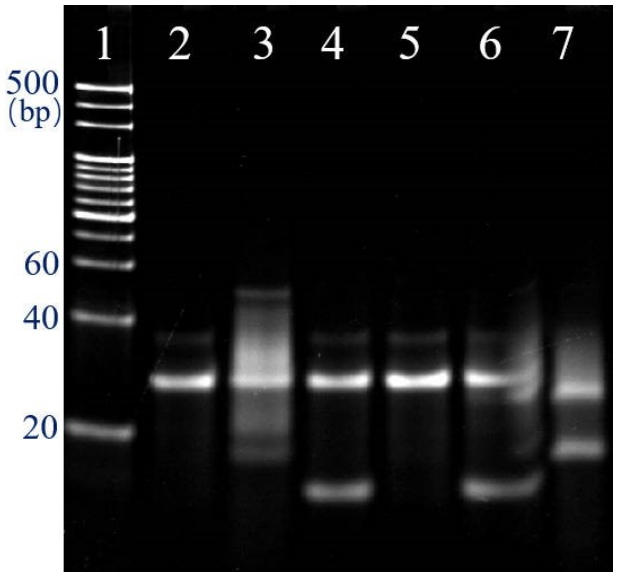

Fig. S2. The image of polyacrylamide gel electrophoresis of Exo III-assisted signal amplification. lane 1: 20 bp DNA ladder; lane 2: pDNA; lane 3: pDNA + tDNA; lane 4: pDNA + cDNA; lane 5: pDNA + Exo; lane 6: pDNA + cDNA + Exo III; lane 7: pDNA + tDNA + Exo III; Gel Red nucleic acid stain used in the experiment.

Table S1. Comparison of different methods for specificity

Amplification strategy	Signal change ratio of mismatched DNA to T-DNA		Reference
	Sm-DNA (%)	Tm-DNA (%)	
Exo III T-AuNSs	45	-	1
Exo III and HCR	60	20	2
Exo III	69	33	3
eMB-DNA	49	26	4
AuNPs/g-C3N4@rGO biotin-streptavidin	33	20	5
CDs and GO	41	-	6
AuNPs	62	51	7
Exo III and AuNPs	43	10	This work

Exo-III: Exonuclease III; HCR: hybridization chain reaction; eMB-DNA: electrochemical molecular beacon-based DNA; AuNPs: gold nanoparticles; g-C3N4:

graphitic carbon nitride; rGO: reduced graphene oxide; T-AuNSs: triangular Au nanosheets; CDs: carbon dots; GO: graphene oxide;

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