Supplementary Material

Single-enzyme-driven cascade amplification integrating target recycling and DNA walker for highly sensitive nucleic acid detection

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Quantification of hDNA Loading on AuNPs

The surface-bound hDNA concentration on AuNPs was quantified using a fluorescence-based displacement method, following previously reported approaches for determining DNA loading on AuNPs.[1, 2] Briefly, a calibration curve was first established by measuring the fluorescence intensities of FAM-labeled hDNA at different concentrations (0.02-0.40 μM). Subsequently, β-mercaptoethanol (20 mM) was added to the hDNA@AuNPs solution to displace the surface-bound DNA via ligand exchange. After overnight incubation at room temperature, the released hDNA was collected by centrifugation, and its fluorescence intensity was measured with an excitation wavelength of 480 nm and an emission range of 500–600 nm. The amount of DNA released from the AuNP surface was then calculated according to the standard calibration curve.

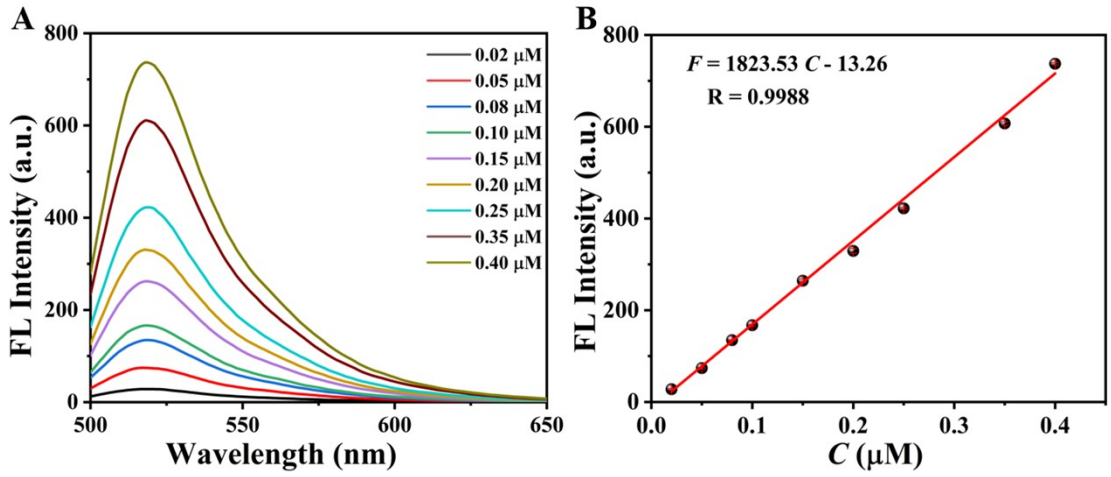

Fig. S1 (A) Fluorescence spectra of FAM-hDNA at different concentrations and the linear relationship between DNA concentration and fluorescence intensity at 520 nm.

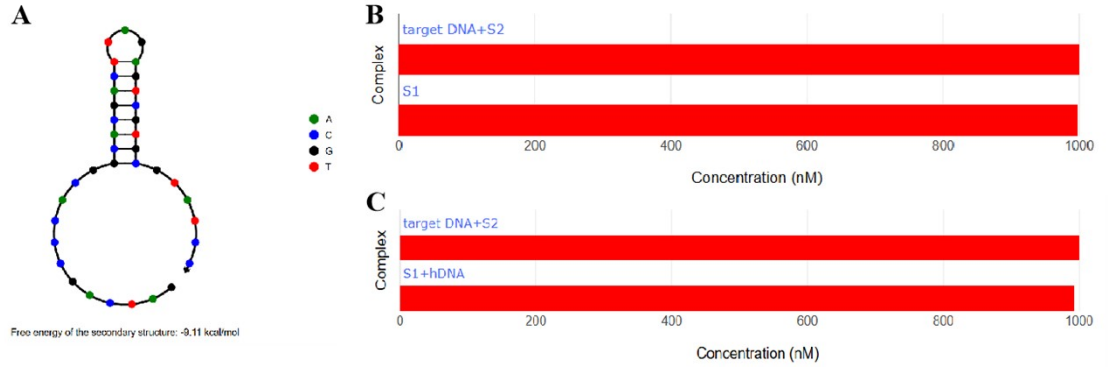

Fig. S2 Predicted analysis by NUPACK. (A) hDNA structure. (B) Hybridization between target DNA and S2. (C) Hybridization between hDNA and S1.

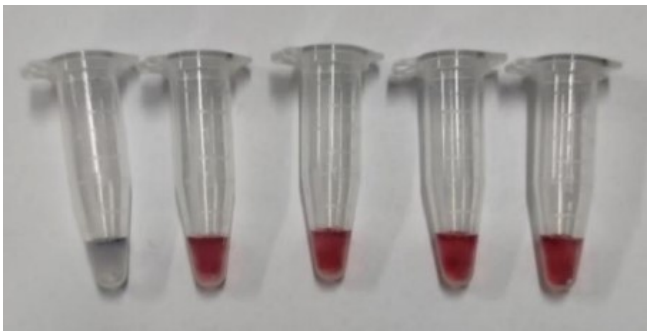

Fig. S3 Solution under different hDNA/AuNPs ratio, from left to right: 50:1, 100:1, 150:1, 200:1, 250:1.

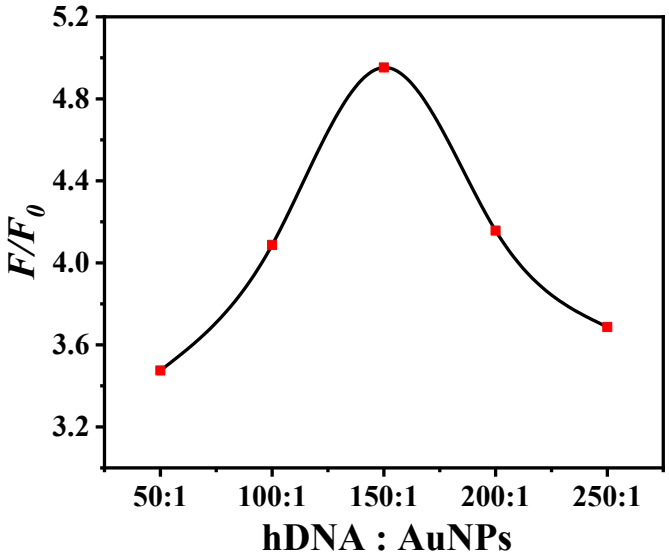

Fig. S4 F/F_0 under different hDNA/AuNPs ratio.

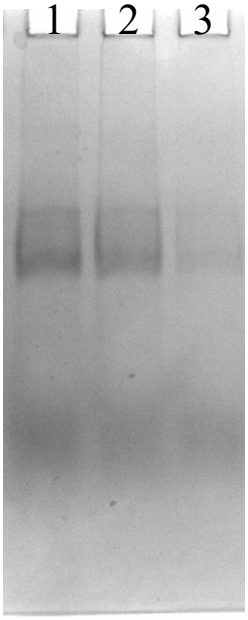

Fig. S5 Electrophoresis analysis of different reaction systems. Lane 1: S1 + S2 + hDNA; lane 2: S1 + S2 + hDNA + Exo III. Lane 3: S1 + S2 + hDNA + Exo III + target DNA.

Table S1. DNA sequences used in this work

Name	Sequences(5′→3′)
target DNA	GGACTGGATACGCACGACCTAGTTTTT
hDNA	SH-GATCAGCCCACGGCACGACTTAGAGTCGTGCGTATCC-FAM
S1	GGATACGCACGA CTCTAG
S2	CTAGGTCGTGCGTATCCAGTCC
Mis-1	GGACTGGATACCCACGACCTAGTTTTT
Mis-2	GGACAGGATACCCACGACCTAGTTTTT
Mis-3	GGACAGGATACCCACGACCTTGTTTTT
non-cDNA	AAGTCTAGGATTCGGCGTGGGTTAAAAAAAAAAAA

Table S2. Comparison of this method with reported nucleic acid detection strategies.

Signal amplification strategy	LOD	Ref.
DNA SPR biosensor	1.5 nM	[3]
Multifunction-integrated molecular beacon	0.5 nM	[4]
Graphene-based molecular beacon	0.29 nM	[5]
Intermolecular G-quadruplex structure	2.0 nM	[6]
Polymerase and endonuclease	1.0 nM	[7]
Polymerization-mediated strand displacement amplification	80 pM	[8]
T7 exonuclease and graphene oxide	386 pM	[9]
Silver nanoclusters and Exo III	0.25 nM	[10]
Exo III-powered target tecycling and DNA walker	48.8 pM	This work

References

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