Electronic Supplementary Information (ESI)

A Multiple Amplification Strategy for Nucleic Acid detection Based on Host-guest Interaction between β-Cyclodextrin Polymer and Pyrene

Xiaochen Guo[a], Pei Liu[b], Xiaohai Yang[a,*], Kemin Wang[a,*], Qing Wang[a], Qiuping Guo[a], Jin Huang[a], Jianbo Liu[a], Chunxia Song[a], Wenshan Li[a]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha 410082, China.

[b] Hunan Province Environmental Monitoring Centre, Changsha 410014, China.

*To whom correspondence should be addressed:

Phone: +86-731-88821566.

Fax: +86-731-88821566.

E-mail: kmwang@hnu.edu.cn, yangxiaohai@hnu.edu.cn.

Table S1. All the sequences are listed as below (from 5′ to 3′):

Name	**Sequence (5′-3′)**
template	TACTAAAGTACGTGTGCGACTGATGGTTTACCTCAGCTC AACATCAGTCTGATA AGCTA
cDNA	TGAGGTAAACCATCAGTCGCACACGTACTTTAGTA
pyrene-labeled probe	AGTACGT(pyrene)GTGCGACTGATGGTT
target DNA	TAGCTTATCAGACTGATGTTGA
1MT-DNA	TAGCTTATCAGACTGATGTT*C*A
2MT-DNA	TAGCTTATCAGACTGATGT*AC*A
Random DNA	N_{22}
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
1MT-RNA	UAGCUUAUCAG*U*CUGAUGUUGA
3MT-RNA	U*C*GCUUAUC*G*GACUGAU*C*UUGA
Random RNA	AAUAUAUCUGCUGAGGAUCAGA

Nucleotide mismatches were marked in red and indicated as italic letters.

Analytical data for the pyrene-labeled probe

As shown in Fig. S1 A, the m/z difference between before labeling and after labeling of DNA probe is about the molecular weight of pyrene. It means that pyrene was successfully labeled on the DNA. In addition, the purity is rather high after purification from the high-performance liquid chromatography characteristic spectrum for the pyrene-labeled probe as shown in Fig. S1 B. (Note: All the analytical data for the pyrene-labeled probe were provided by TaKaRa Bio Inc.).

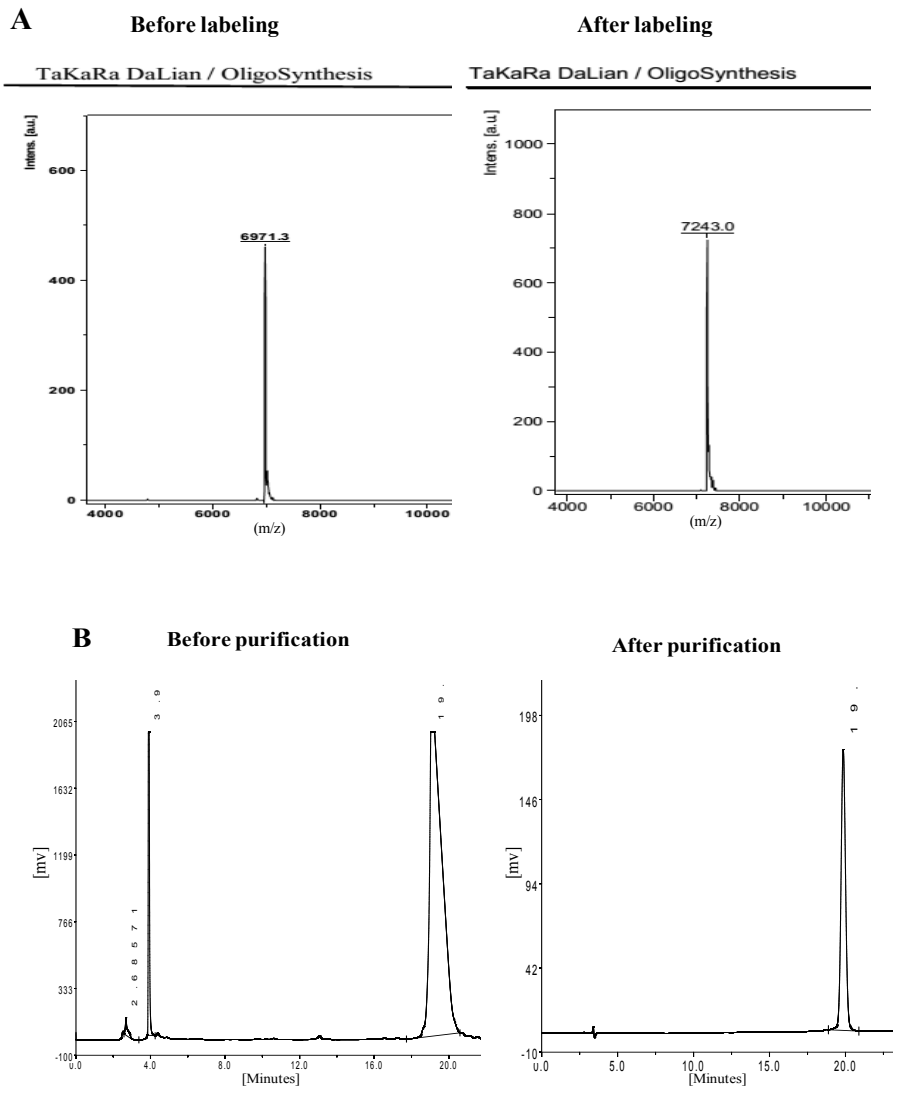

Fig. S1 (A) Mass spectra for the pyrene-labeled probe. (B) HPLC characteristic spectra for the pyrene-labeled probe.

Characterization of β-CDP

As shown in Fig. S2 A, the FTIR spectra showed that most absorption bands of β-CD were still present in spectrum of β-CDP. Due to the cross-linking reaction of β-CD, the absorption bands of stretching vibration of C-O-C at 1070~1160 cm^{-1} were broadened as shown in the spectrum of β-CDP. The ^{1}H NMR spectra showed that most bands of β-CD at 4.0-3.4 ppm were broadened in the spectrum of β-CDP (Fig. S2 B).

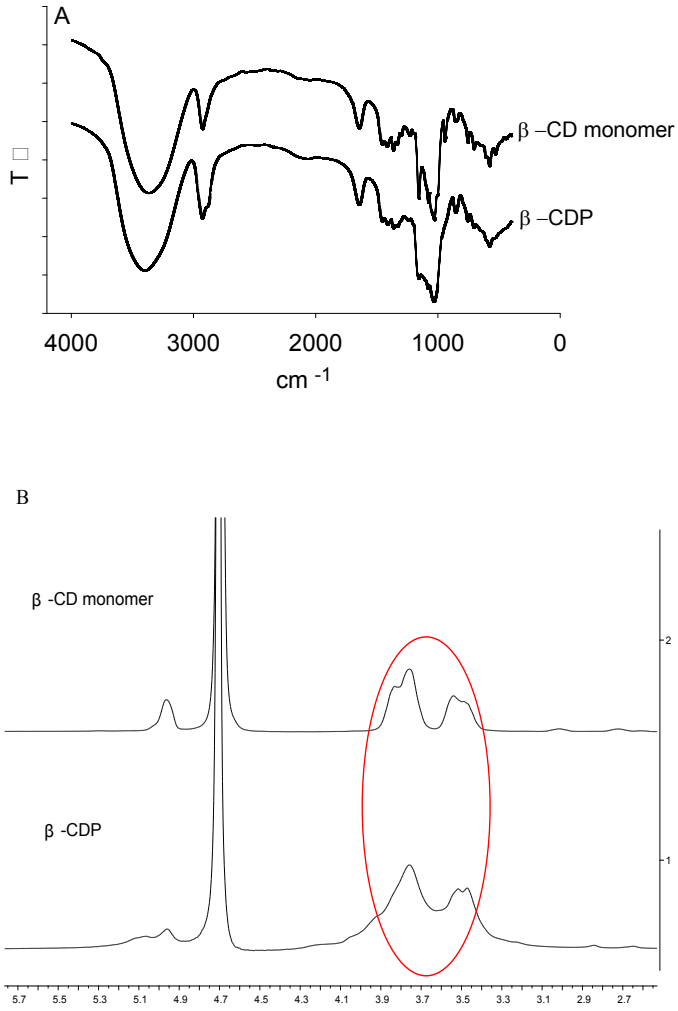

Fig. S2 (A) FTIR spectra of β-CD monomer and β-CDP. (B) ^{1}H NMR spectra of β-CD monomer and β-CDP.

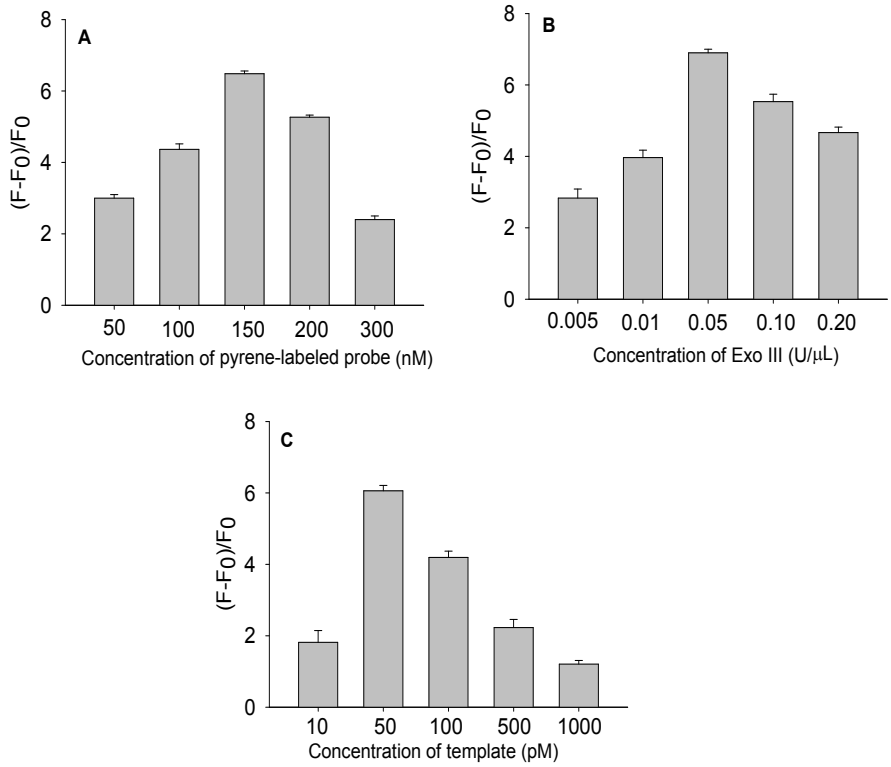

Fig. S3 The effect of different conditions for target DNA detection based on host-guest interaction between β-cyclodextrin polymer and pyrene. (A) Different concentrations of pyrene-labeled probe, the concentrations of target DNA, Exo III, template and β-CDP were 10 pM, 0.05 U/μL, 50 pM and 2 mg/mL respectively; (B) Different concentrations of Exo III, the concentrations of target DNA, pyrene-labeled probe, template and β-CDP were 10 pM, 150 nM, 50 pM and 2 mg/mL respectively; (C) Different concentrations of template, the concentrations of target DNA, pyrene-labeled probe, Exo III and β-CDP were 10 pM, 150 nM, 0.05 U/μL and 2 mg/mL respectively. The excitation/emission wavelength was set at 345 nm/380 nm. Error bars indicated the standard deviations of three experiments.

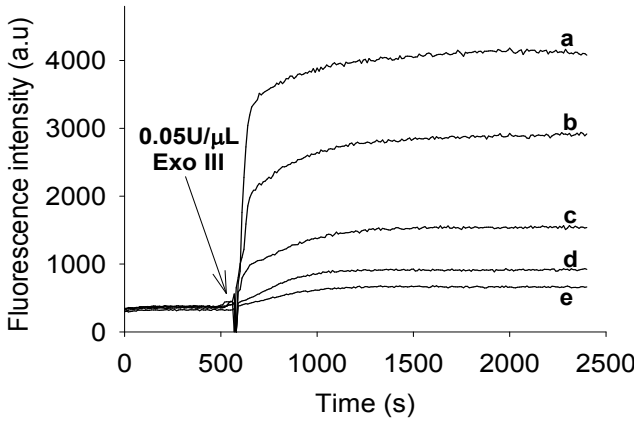

Fig. S4 Response of pyrene-labeled probe to various concentrations of SDA products. The curves from a to e contain target DNA with 5 pM, 1 pM, 0.15 pM, 0.1 pM and 0 pM, respectively. The fluorescence intensity of the system reached 95% of the maximum in 900 seconds.

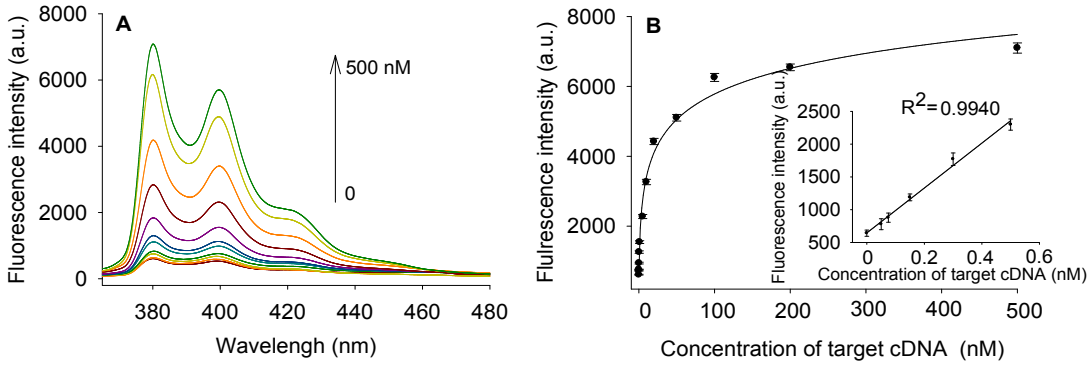

Fig. S5 (A) Fluorescence spectra of the CEA method based on host-guest interaction between β-CDP and pyrene over a range of target cDNA concentrations. (B) Plot of target cDNA *vs* fluorescence intensity. Inset is the calibration curve for concentrations of target cDNA from 0 to 0.5 nM. The concentrations of pyrene-labeled probe, Exo III and β-CDP were 150 nM, 0.05 U/μL and 2 mg/mL, respectively. Error bars indicated the standard deviations of three experiments. The detection limit of CEA method based on host-guest interaction between β-CDP and pyrene is 3 pM (three times the standard deviation of the blank solution).

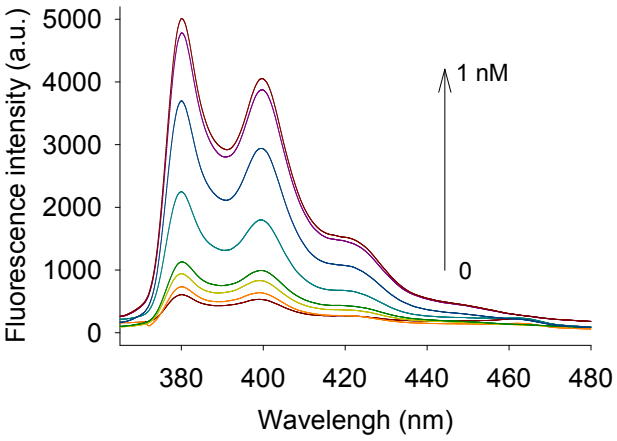

Fig. S6 Fluorescence spectra of the multiple amplification method based on host-guest interaction between β-CDP and pyrene over a range of target miRNA-21 concentrations. The concentrations of template, pyrene-labeled probe, polymerase, nicking enzyme, Exo III and β-CDP were 500 pM, 150 nM, 0.15 U/μL, 0.1 U/μL, 0.05 U/μL and 2 mg/mL, respectively.

Table S2. Comparison of the new method with other multiple amplification strategies.

Style	Detection limit	Output	Target	References
MB-symmetric split	1 pM	Colorimetric	Disease-related gene	S1
MB-SDA	176 fM	Fluorescence	DNA	S2
MB-Exo-CRA	0.1 pM	Colorimetric	DNA	S3
CHA-HCR-DNAzyme	5 pM	Fluorescence	DNA	S4
TR-RCA	0.1 pM	Fluorescence	DNA /microRNA	S5
RCA-AuNPs	70 fM	Colorimetric	SNP	S6
Exo-Cascade-DNAzyme	8 fM	Chemiluminescence	DNA	S7
MB-Endo-CESA	1 fM	Fluorescence	DNA	S8
EASA-AuNPs	15 pM	Colorimetric	DNA	S9
MB-SDA-GO	4 pM	Fluorescence	DNA	S10
CEA-GO	9 pM	Fluorescence	microRNA	S11
This method	41 fM/~10 pM	Fluorescence	DNA/microRNA	

MB=molecular beacon strategy, Exo=Exonuclease, Endo=Endonuclease, SDA=strand displacement amplification, TR-RCA=target sequence recycled rolling circle amplification, HCR=hybridization chain reaction, CHA=catalytic hairpin assembly, CESA=cascade enzymatic signal amplification, EASA=ExoIII assisted signal amplification, CEA=cyclic enzymatic amplification. AuNPs=gold nanoparticles, GO=graphene oxide.

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