Electronic Supplementary Information

Use of β-cyclodextrin-tethered cationic polymer based fluorescence enhancement of pyrene and hybridization chain reaction for enzyme-free amplified detection of DNA

Chunxia Song,[a,b] Bingjie Li,[a] Xiaohai Yang,[a]* Kemin Wang,[a]* Qing Wang,[a] Jianbo Liu,[a] Jin Huang[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]*School of Science, Anhui Agricultural University, Hefei 230036, China*

**To whom correspondence should be addressed. Tel: 86-731-88821566; Fax: 86-731-88821566; E-mail:*

kmwang@hnu.edu.cn, yangxiaohai@hnu.edu.cn

Experimental details

1. Sequences of DNA used

Table S1 Sequences of DNA used

Types	Sequences of DNA
H1	5′-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCG
H2	5′-pyrene-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTA
Target DNA	5′-AGTCTAGGATTCGGCGTGGGTTAA-3′
Mismatched DNA	5′-AGTCTAGGATTC*A*GCGTGGGTTAA-3′
Deleted DNA	5′-AGTCTAGGATTC_GCGTGGGTTAA-3′
Inserted DNA	5′-AGTCTAGGATTC*T*GGCGTGGGTTAA-3′

The underlined parts in Mismatched DNA, Deleted DNA and Inserted DNA were the mismatched, deleted and inserted parts respectively.

2. Synthesis and properties of β-cyclodextrin-tethered cationic polymer (cationic polyβ-CD)

The cationic polyβ-CD was synthesized according to the literature[1] and the scheme was shown in Fig. S1. Briefly, 0.2 mL of 10% poly(allylamine) hydrochloride (PAH) was diluted by 1 mL water and small portions of 0.17 g CDOTs were added while stirring at 75 °C. When the solution turned clear again the next portion was added. After addition of the last portion the solution was stirred for 12 h at 75 °C. Finally, the reaction mixture solution was diluted 10 times and then was dialyzed (MWCO 5000~8000) in water for 7 days. The dialysate in the dialysis tube was dried at -60 °C under vacuum overnight. Thus the white water soluble cationic polyβ-CD was obtained.

The FTIR spectra (Fig. S2) showed that most absorption bands of CDOTs were still exist in spectrum of cationic polyβ-CD and the absorption bands of stretching vibration of C-O-C at 1070~1160 cm^{-1} were broadened in the spectrum of cationic polyβ-CD due to the cross-linking reaction of cationic β-CD. The ^1H NMR spectra (Fig.S3) showed that the bands of β-CD at 3.4-4.0 ppm and the CH_2 bands of PAH at 1.0-2.5 ppm were coexited in the spectrum of polyβ-CD. FTIR and ^1H NMR measurement proved the successful synthesis of cationic polyβ-CD.

We also measured zeta potential of cationic polyβ-CD and electroneutral polyβ-CD, and proved that the zeta potential of the cationic polyβ-CD was +29.2 mV (Fig. S4 A) and for electroneutral polyβ-CD was +0.0373 mV (Fig. S4 B).

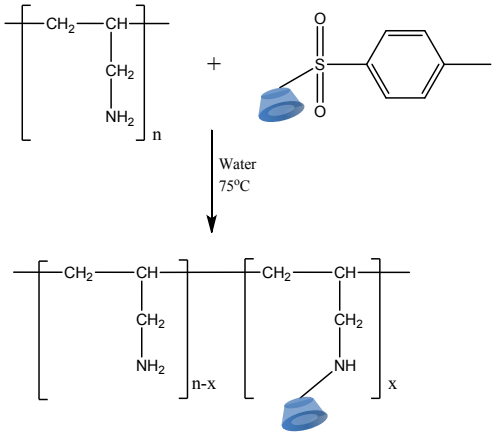

Fig. S1 Synthesis of cationic polyβ-CD

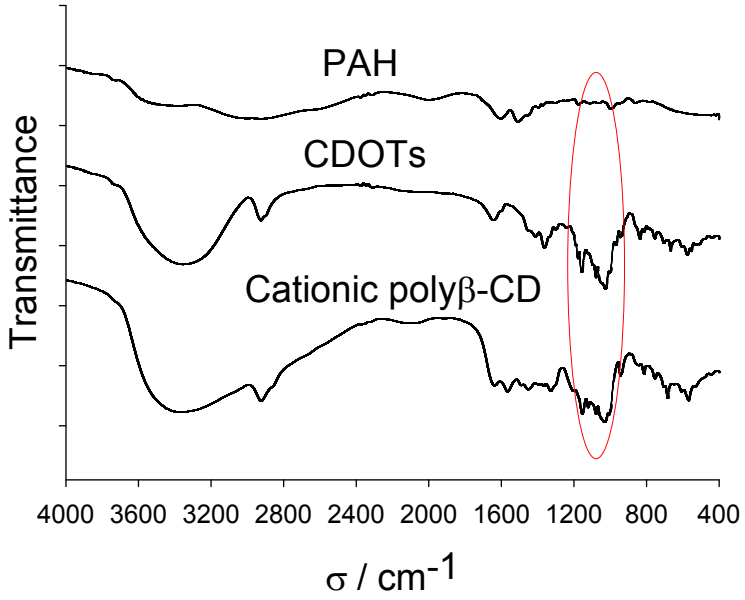

Fig. S2 FT-IR spectra of PAH, CDOTs and β-cyclodextrin-tethered cationic polymer (cationic polyβ-CD)

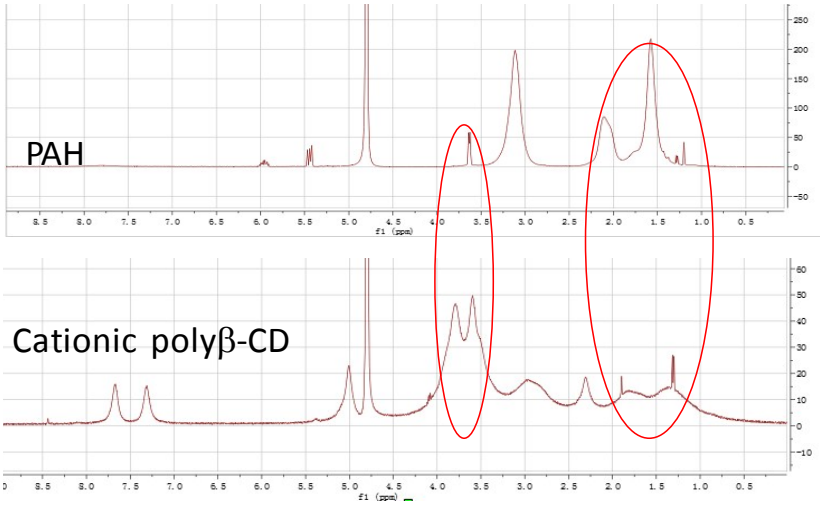

Fig. S3 ^{1}H NMR spectra of PAH and cationic polyβ-CD

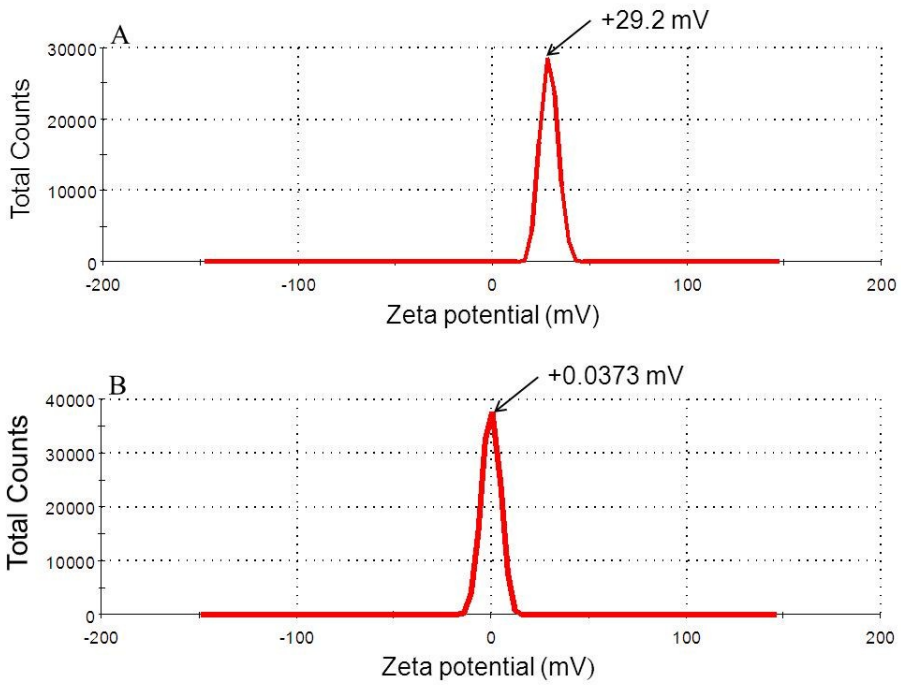

Fig. S4 Zeta potential of (A) cationic polyβ-CD and (B) electroneutral polyβ-CD

3. Synthesis and properties of β-cyclodextrin-tethered negatively-charged polymer (negatively-charged polyβ-CD).

The negatively-charged polyβ-CD was synthesized according to the literature[2] and the scheme was shown in Fig. S5. Briefly, 0.81 g (11.3 mmol of COOH groups) of polyacrylic acid (PAA) of 240 K was dissolved in 30 mL of N-methyl-2-pyrrolidone (NMP) at 60 °C for 24 h. Then, 3.5 g (3.3 mmol) of monoamino-β-cyclodextrin (dissolved in 60 g of NMP) and 0.10 g (0.48 mmol) of dicyclohexylcarbodiimide (DCC, dissolved in 2.0 g of NMP) were introduced into the PAA solution under vigorous stirring. After a reaction for 48 h at 60 °C, the system was cooled to room temperature, followed by addition of 35 mL of 40 wt % NaOH aqueous solution to precipitate the polymer. The precipitate was washed twice with 15 mL of hot NMP (60 °C) and then with 20 mL of methanol at room temperature. After filtration under vacuum, the solid product was dissolved in 12.5 mL of deionized water and precipitated in 100 mL of methanol (twice).

Finally, the product was dissolved into 20 mL of deionized water and dialyzed (MWCO 5000~8000) against deionized water until the conductivity of water outside the tube remained constant. The final dry product was obtained by freeze-drying.

The FTIR spectra (Fig. S6) showed that most absorption bands of CDOTs were still exist in spectrum of negatively-charged polyβ-CD, which proved the successful synthesis of negatively-charged polyβ-CD. We also measured zeta potential of negatively-charged polyβ-CD, and proved that the zeta potential of the cationic polyβ-CD was -50.3 mV (Fig. S7).

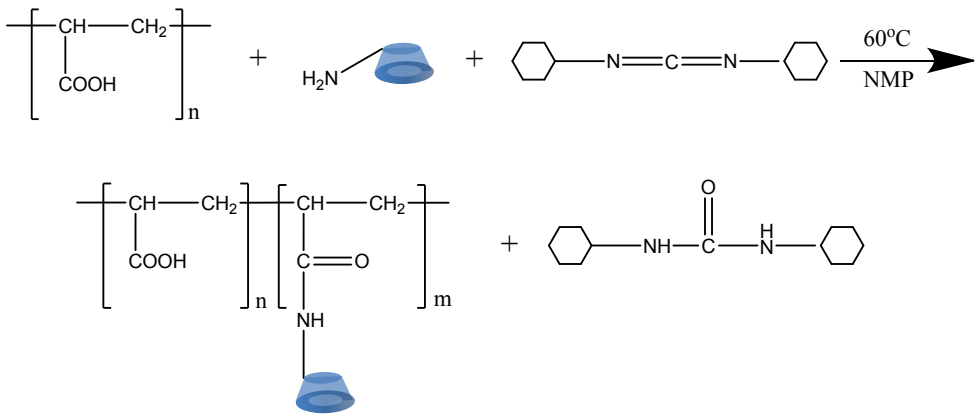

Fig. S5 Synthesis of negatively-charged polyβ-CD

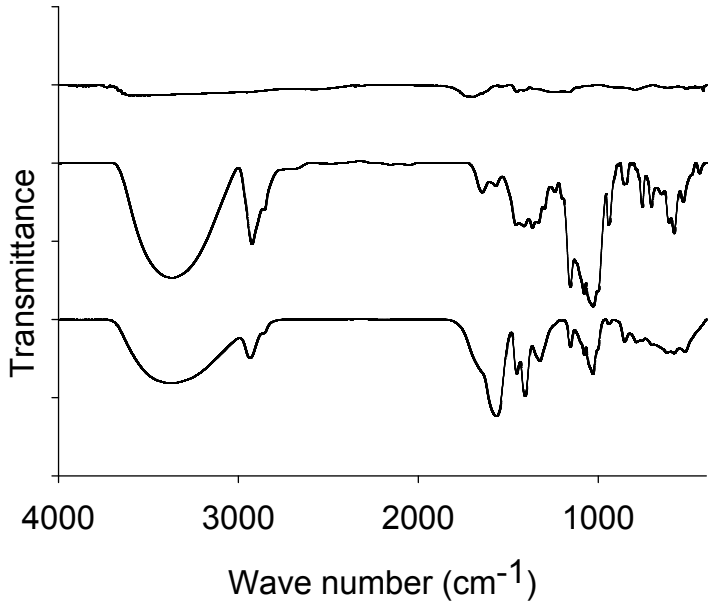

Fig. S6 FT-IR spectra of PAA, monoamino-β-CD and negatively-charged polyβ-CD

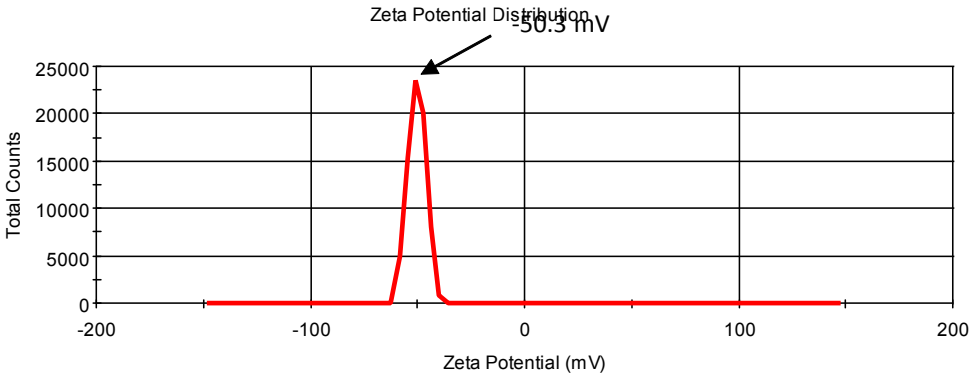

Fig. S7 Zeta potential of negatively-charged polyβ-CD

4. Optimization of experimental conditions

Some factors, such as the concentration of cationic polyβ-CD, NaCl, H1/H2 and the reaction time of HCR process, would affect the performance of the detection method, thus the effects of these factors were investigated in order to obtain high effective analysis performance for target DNA assay. $(F_0\text{-}F)/F_0$ was used here and other place in this work, where F_0 and F were the fluorescence intensities of the detection system without and with target DNA respectively.

The concentration of cationic polyβ-CD was a crucial parameter for target DNA detection. Adding appropriate amount of cationic polyβ-CD can gain favorable fluorescence enhancement, which is conducive to produce obvious response signals. Excess cationic polyβ-CD may cause strong background signals, so the concentration of cationic polyβ-CD was optimized. As shown in Fig. S8, $(F_0\text{-}F)/F_0$ achieved maximum when the concentration of cationic polyβ-CD was 0.5 mg/mL, which was selected for further studies.

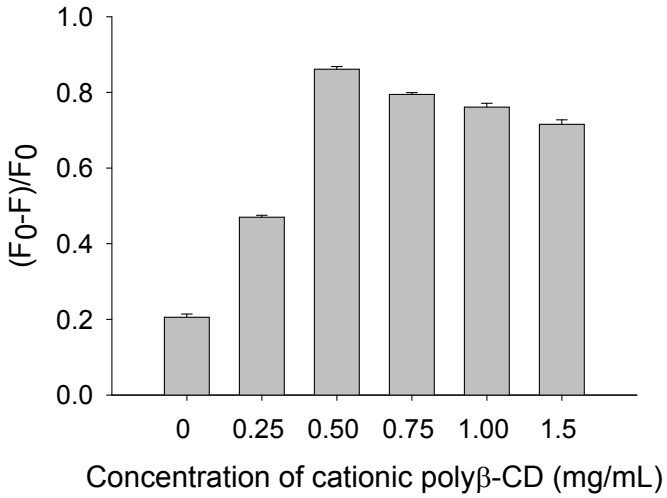

Fig. S8 The effect of different cationic polyβ-CD concentration for target DNA detection. The concentration of NaCl and H1/H2 was 300 nM and 100 nM respectively. The reaction time of HCR process was 60 min. The excitation/emission wavelength was set at 345 nm/380 nm. Error bars indicated the standard deviations of three experiments.

The concentration of NaCl was also a crucial parameter for target DNA detection. Since ion was indispensable in HCR process for shielding the negative charge on the hairpin probes, and it was import factor to adjust the interaction between positive cationic polyβ-CD and negative hairpin probes. As shown in Fig. S9, $(F_0\text{-}F)/F_0$ achieved maximum when the concentration of NaCl was 300 nM, which was selected for further studies.

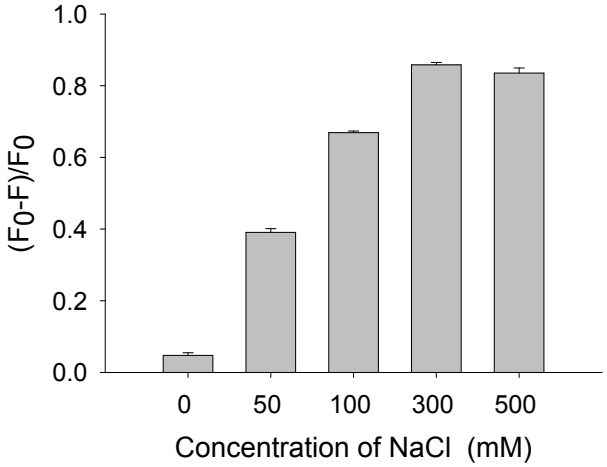

Fig. S9 The effect of different NaCl concentration for target DNA detection. The concentration of cationic polyβ-CD and H1/H2 was 0.5 mg/mL and 100 nM respectively. The reaction time of HCR process was 60 min. The excitation/emission wavelength was set at 345 nm/380 nm. Error bars indicated the standard deviations of three experiments.

The reaction time of HCR process and concentration of H1/H2 also influenced the performance of this method. The effect of reaction time of HCR process on $(F_0\text{-}F)/F_0$ was examined, and the results are shown in Fig. S10, $(F_0\text{-}F)/F_0$ increased with increasing reaction time of HCR process, and then almost kept constant after 60 min, which was selected as reaction time for further studies. The effect of concentration of H1/H2 from 20 nM to 500 nM was also studied. As shown in Fig. S11, $(F_0\text{-}F)/F_0$ achieved maximum when the concentration of each hairpin probes was 100 nM, which was selected for further studies.

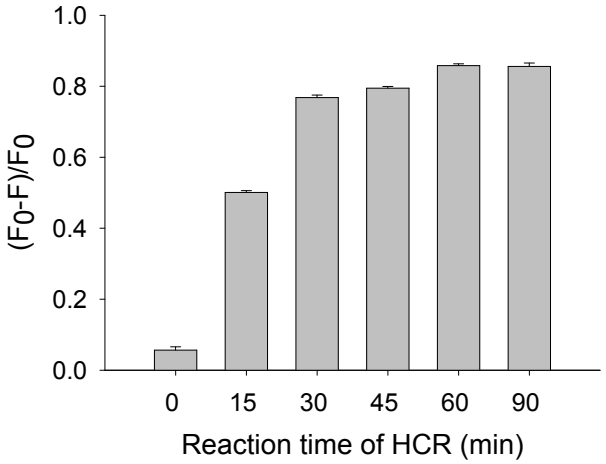

Fig. S10 The effect of HCR time for target DNA detection. The concentration of cationic polyβ-CD, NaCl and H1/H2 was 0.5 mg/mL, 300 mM and 100 nM respectively. The excitation/emission wavelength was set at 345 nm/380 nm. Error bars indicated the standard deviations of three experiments.

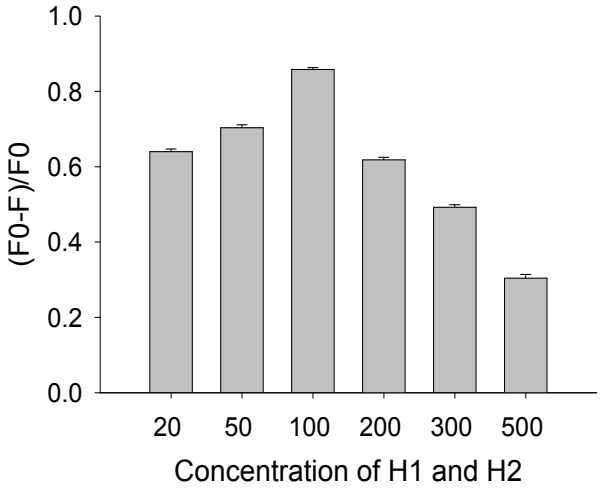

Fig. S11 The effect of H1/H2 concentration for target DNA detection. The concentration of cationic polyβ-CD and NaCl was 0.5 mg/mL and 300 mM respectively. The HCR process time was 60 min. The excitation/emission wavelength was set at 345 nm/380 nm. Error bars indicated the standard deviations of three experiments.

5. The fluorescence of H2 in the presence of poly(allylamine) hydrochloride (PAH)

As control, we also measured the fluorescence of pyrene attached on H2 after addition of PAH, and found the fluorescence was scarcely changed (Fig. S12), which proved that PAH could not obviously influence the fluorescence of pyrene attached on H2.

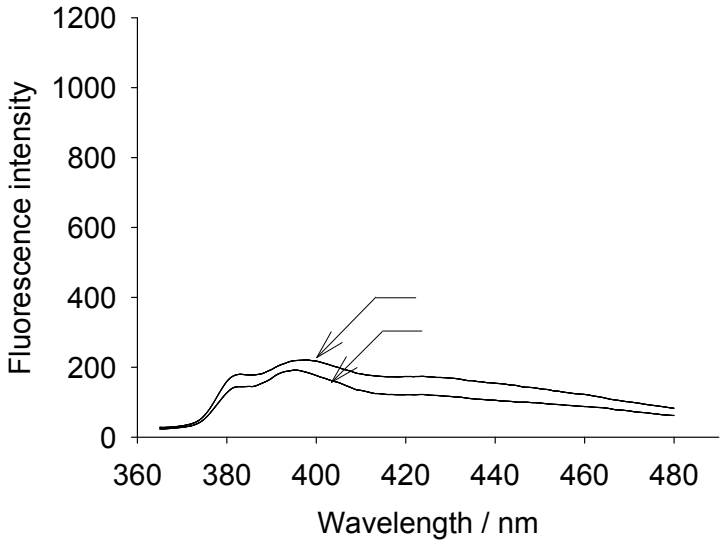

Fig. S12 The fluorescence spectra of pyrene-labelled H2 in the presence or absence of PAH. The concentration of H2 was 100 nM, and the concentration of PAH was 0.5 mg/mL.

S1. M. Hollas, M.-A. Chung and J. Adams, *J. Phys. Chem. B*, 1998, **102**, 2947-2953.
S2. X. Guo, A. A. Abdala, B. L. May, S. F. Lincoln, S. A. Khan and R. K. Prud'homme, *Macromolecules*, 2005, **38**, 3037-3040.