# Electronic Supplementary Information

## Sensitive detection of T4 polynucleotide kinase activity based on β-cyclodextrin polymer enhanced fluorescence combined with exonuclease reaction

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# Experimental details

## 1. Chemicals and apparatus

T4 polynucleotide kinase (T4 PNK, 10000 unit/mL), λ exonuclease (λ exo, 5000 unit/mL) and Bst DNA polymerase (Bst pol, 8000 unit/mL) were purchased from New England Biolabs (NEB, UK). β-Cyclodextrin (β-CD) (97%), thrombin (from human plasma) and avidin (from egg white) were purchased from Sigma-Aldrich Co.. Bovine serum albumin (BSA) was purchased from Dingguo Biotechnology Co. Ltd. Lysozyme was purchased from Sangon Biotech. (Shanghai) Co., Ltd. Single-stranded DNA binding protein (SSB) was purchased from Promega Co., Ltd. Adenosine triphosphate (ATP), methylbenzene, epichlorhydrin and HCl were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai China). All other chemicals were of analytical grade. All stock and buffer solutions were prepared using Millipore water (18.2 MΩ·cm).

The oligonucleotides used in this work were ordered from TaKaRa Bio Inc. (Dalian, China). The sequences were listed as follows:

Oligonucleotide 1 (oligo 1), 5′-HO-GTACACAGACTCAGC (pyrene)-3′

Oligonucleotide 2 (oligo 2), 5′-HO-TTTTTGCTGAGTCTGTGTAC-3′.

Oligo 2 was partly complementary to oligo 1 with sticky end to prevent pyrene on the oligo 1 from entering the cavity of polyβ-CD. dsDNA-pyrene was the duplex DNA made by mixing of equimolar of oligo 1 and oligo 2. The ready of dsDNA-pyrene was carried out of hybridization between oligo 1 and oligo 2 (10 μM, respectively ) in Tris-HCl buffer (20 mM Tris-base, 300 mM NaCl, pH 8.0) for 1 h (48 $^{o}$C) and cooled to 25 $^{o}$C for at least 1 h before use. The obtained 10 μM dsDNA-

pyrene solution was stored at 4 $^{o}$C for further use.

All fluorescence measurements were carried out on a F7000 fluorometer (Hitachi, Japan) equipped with aqueous thermostat (Amersham) accurated to 0.1 $^{o}$C. Excitation and emission slits were all set for a 5.0 nm band-pass with a 700 V PMT voltage. The excitation wavelength was set at 345 nm and the emission spectra from 370 to 480 nm were collected with a 0.2∗1 $cm^2$ quartz cuvette containing 100 μL of solution.

## 2. Synthesis of β-cyclodextrin polymer (polyβ-CD).

The polyβ-CD was synthesized according to the literature[1]. Briefly, 10 g β-CD was dissolved in 15 mL 15% NaOH, and stirred for 2 h in 35 $^{o}$C and then 8.81 mmol methylbenzene was added with stirring and continued stirring for 2 h in 35 $^{o}$C. 8.81 mL epichlorhydrin was added drop by drop. After 3 h of stirring at 35 $^{o}$C, 8.81 mL epichlorhydrin was added to the mixture. The pH value of the flocculating constituent was adjusted to 7.0 with 6 M HCl. The mixture was dialyzed (MWCO 5000~8000) in water for 7 days. The dialysate in the dialysis tube was dried at -60·$^{o}$C under vacuum overnight. Thus the white water soluble polyβ-CD was obtained. The FTIR spectra (Fig. 1A) showed that most absorption bands of β-CD were still present in spectrum of polyβ-CD and the absorption bands of stretching vibration of C-O-C at 1070~1160 $cm^{-1}$ were broadened in the spectrum of polyβ-CD due to the cross-linking reaction of β-CD. The $^{1}$H NMR spectra also showed that most bands of β-CD at 4.0-3.4 ppm were broadened in the spectrum of polyβ-CD. The molecular weight of polyβ-CD (Mn ~94.4K) was measured by using gel permeation chromatography (GPC, waters-515).

Scheme S1 Synthesis of polyβ-CD

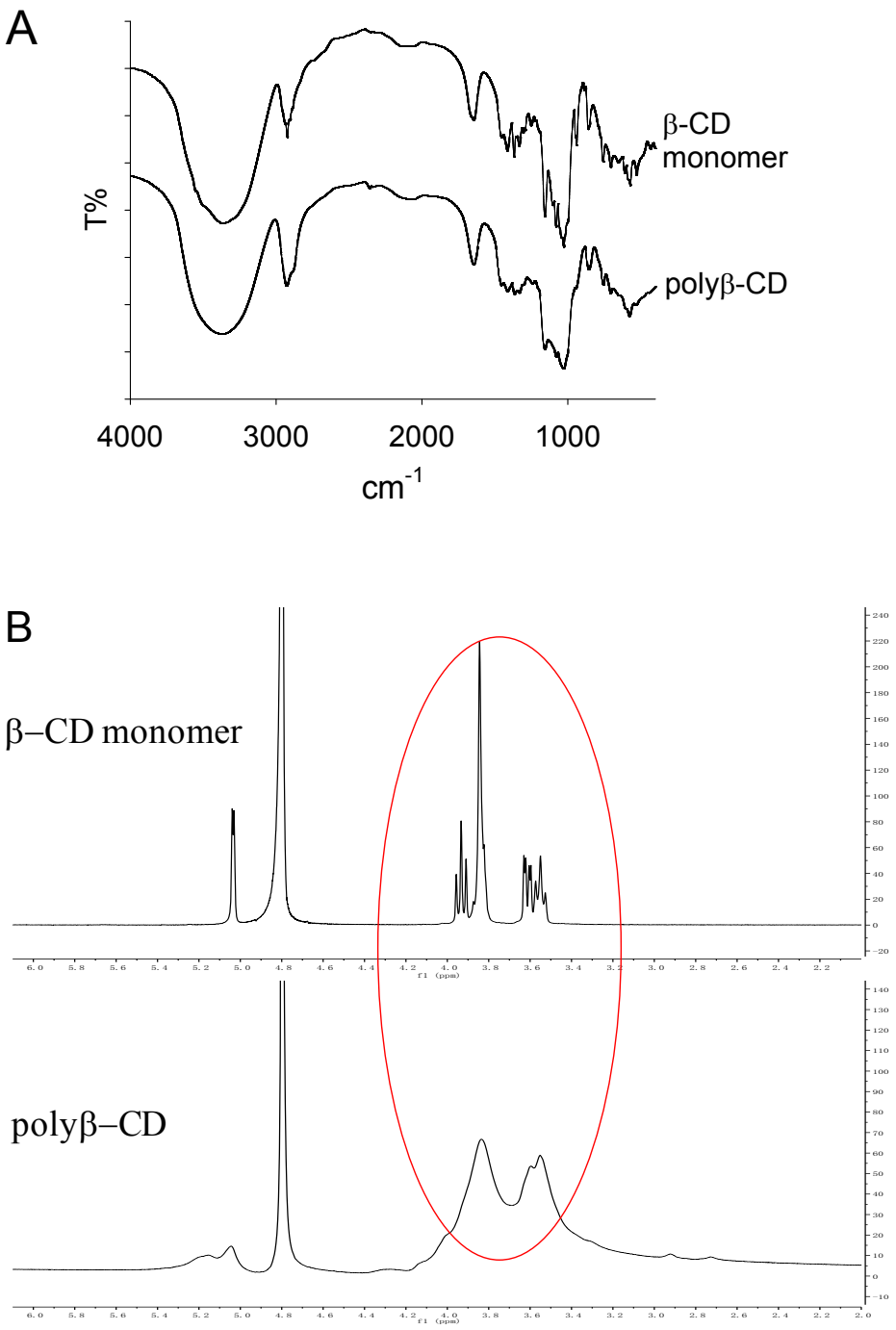


**Fig. S1** (A) FTIR spectra of β-CD monomer and polyβ-CD. (B) $^{1}$H NMR spectra of β-CD monomer and polyβ-CD.

## 3. T4 PNK activity detection using polyβ-CD

In a typical T4 PNK activity assay, 300 nM dsDNA-pyrene, 1 mM ATP, 100 unit/mL λ exo, 1.5 mg/mL polyβ-CD and a certain amount of T4 PNK activity were put into 100 μL reaction buffer (70 mM Tris-base, 10 mM $MgCl_2$, pH = 8.0). After incubation at 37 $^oC$ for 30 min, the fluorescence of the mixture was measured with the excitation wavelength of 345 nm and emission from 370 nm to 480 nm at 25 $^oC$.

In real-time monitoring the activity of T4 PNK, 100 μl mixture of 300 nM dsDNA-pyrene, 1 mM ATP and 1.5 mg/mL polyβ-CD was added into a quartz cuvette, which was then placed in the fluorometer and incubated at 37 $^oC$ for several minutes. Then 100 unit/mL λ exo and 10 unit/mL T4 PNK was added into the mixture and continued incubating at 37 $^oC$. The fluorescence of the system versus time was recorded by the fluorometer with the excitation wavelength of 345 nm and emission of 380 nm. The control experiment was the same as the above procedure, except for the addition of T4 PNK.

**Table S1** Comparison of our proposed strategy with reported T4 PNK activity detection method

| Method | Tool | Detection Limit | Ref |
|---|---|---|---|
| $^{32}$p-labeling | Radical isotope $^{32}$P-labeling polyacrylamide gel electrophoresis | 0.17 unit/mL | S2 |
| electrochemical | Ferrocene-functionalized SWCNT electrochemical signal amplification (our group) | 0.01 unit/mL | S3 |
| Fluorescence | Bimolecular beacons based real-time monitoring of phosphorylation (our group) | 0.002 unit/mL | S4 |
| Fluorescence | Grapheme oxide based fluorescence quenching probe | 0.05 unit/mL | S5 |
| Fluorescence | GGG-triplet based fluorescence quenching probe | 0.04 unit/mL | S6 |
| Fluorescence | β-Cyclodextrin polymer fluorescence enhancement strategy | 0.02 unit/mL | This work |

**4. Optimization of experimental conditions**

Some factors, such as the reaction time, concentration of dsDNA-pyrene and ATP, the activity of λ exo, and the pH of the system 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 T4 PNK activity assay. The fluorescence increasing factor used here and other place in this work was $(F-F_0)/F_0$, where F and $F_0$ were the fluorescence intensities of the detection system with and without T4 PNK respectively.

The reaction time was a crucial parameter for the T4 PNK-catalyzed phosphorylation and the coupled λ exo cleavage process. An excess of reaction time would make the fluorescence of blank enhanced as λ exo would also hydrolyze dsDNA-pyrene with 5′-hydroxyl slowly. As shown in Fig. 2, fluorescence intensity in the presence of T4 PNK enhanced gradually with the enhancement of the reaction time and then reached more than 90% of the maximum after 30 min. In addition, $(F-F_0)/F_0$ was maximum when the activity of λ exo was 100 unit/mL (Fig. S2(A)). Thus, the reaction time and the activity of λ exo were chosen to be 30 min and 100 unit/mL, respectively.

Since dsDNA-pyrene acted as substrate of enzyme, the concentration of dsDNA-pyrene was also a crucial parameter for the T4 PNK-catalyzed phosphorylation and the coupled λ exo digestion process. As shown in Fig. S2(B), $(F-F_0)/F_0$ achieved maximum when the concentration of dsDNA-pyrene was 300 nM, which was selected for further studies.

It is known that the phosphate group at the 5′-DNA end was offered by ATP during the phosphorylation process, and the absence of ATP would block the DNA phosphorylation. As shown in Fig. S2(C), $(F-F_0)/F_0$ reached its maximum at the ATP concentration of 1 mM, and then decreased with the further enhancement of ATP concentration. This inhibition effect is a consequence of the competitive binding between ATP and dsDNA-pyrene to T4 PNK, whose binding sites for DNA were partially blocked by ATP. Thus, 1 mM ATP was chosen for further study.

The PNK and λ exo coupled reaction is also sensitive to pH. In our system, the optimum pH of reaction buffers for these two enzymes are quite different. So the pH was optimized in our experiments. As shown in Fig S2(D), the optimal pH for the coupled enzyme catalyzed procedure was 8.0 in our work. Thus, pH 8.0 was chosen for further study.

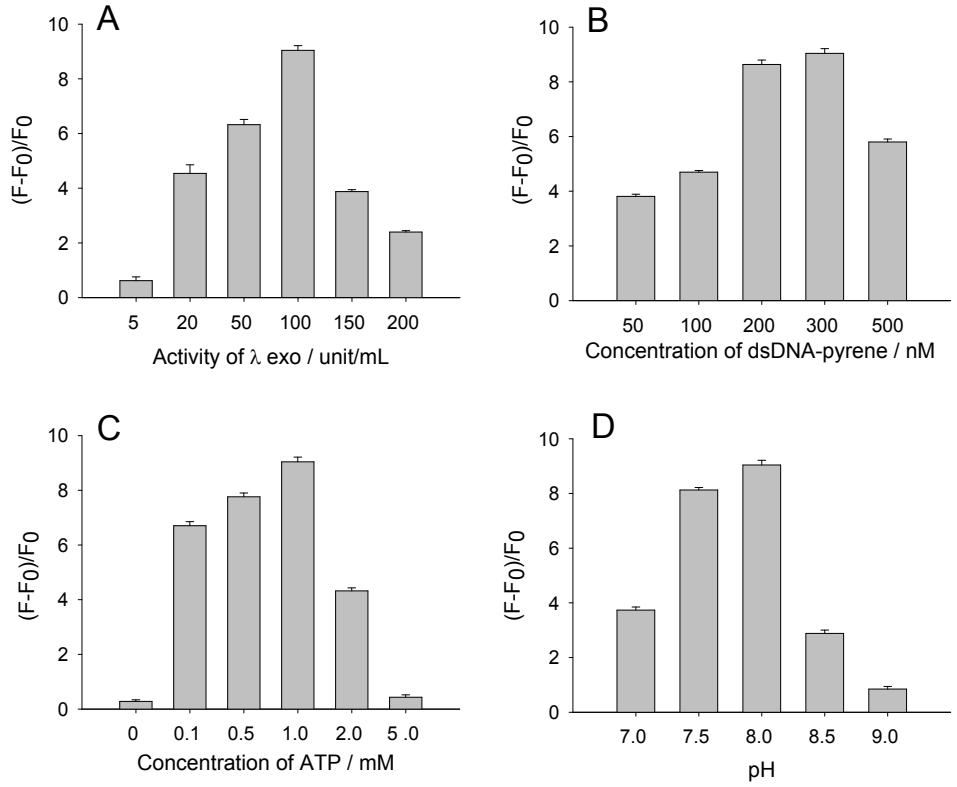


**Fig. S2** The effect of different condition for detection of T4 PNK activity using polyβ-CD based fluorescence strategy. (A) Different activity of λ exo, the concentration of dsDNA-pyrene and ATP were 300 nM and 1 mM, respectively, and pH of the system was 8.0; (B) Different concentration of dsDNA-pyrene, the activity of λ exo and concentration of ATP were 100 unit/mL and 1 mM, respectively and pH of the system was 8.0; (C) Different concentration of ATP, the concentration of dsDNA-pyrene and activity of λ exo was 300 nM and 100 unit/mL respectively and pH of the system was 8.0; (D) Different pH of the system, the concentration of dsDNA-pyrene and ATP were 300 nM and 1 mM, respectively, and the activity of λ exo was 100 unit/mL. The excitation/emission wavelength was set at 345 nm/380 nm. The reaction time was 30 min and the concentration of polyβ-CD was 1.5 mg/mL. The concentration of T4 PNK was 10 unit/mL. Error bars indicated the standard deviations of three experiments.

## 5. Investigation of the influence of proteins and enzymes

In order to investigate the influence of proteins and enzymes, 10 nM of proteins (Bovine serum albumin (BSA), avidin, DNA binding protein (SSB)) and enzymes (lysozyme, thrombin, Bst DNA polymerase (Bst pol)) were added to the buffer respectively. The detection procedure was the same as those described in the aforementioned experiment for T4 PNK activity detection in reaction buffer.

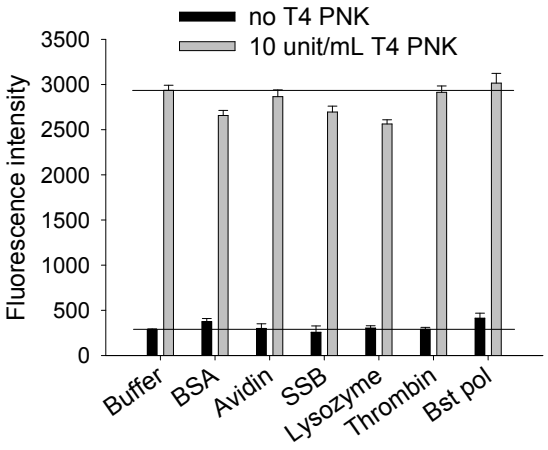


**Fig. S3** Fluorescence intensity of the system in the presence of proteins and enzymes. The concentration of BSA, avidin and SSB was 10 nM, and the activity of lysozyme, thrombin and Bst pol was 20 units/mL. The concentration of dsDNA-pyrene and ATP was 300 nM and 1 mM, the activity of λ exo was 100 unit/mL and pH of the system was 8.0. The excitation/emission wavelength was set at 345 nm/380 nm. The reaction time of the system was 30 min and the concentration of polyβ-CD was 1.5 mg/mL. The activity of T4 PNK was 10 unit/mL. Error bars indicated the standard deviations of three experiments.

**6. T4 PNK activity detection in cell extracts**

In this work, cell extracts was used as the complex fluid. Hela cells were cultured in RPMI 1640 medium supplemented with 15% fetal bovine serum (FBS) and 100 IU/mL penicillinstreptomycin and incubated at 37 °C in a humidified incubator containing 5% wt/vol $CO_2$. The cell density was determined using a hemocytometer. Cell extracts were prepared according to the previous reports[7] with slightly modification. A suspension of $5 \times 10^5$ cells was centrifuged at 1000 rpm for 3 min, then washed with tris-HCl buffer (70 mM tris-base, 10 mM $MgCl_2$, pH = 8.0) three times and at last suspended in tris-HCl buffer. Finally, the cells were disrupted by sonication for 30 min at 0 °C. The lysate was centrifuged at 18000 rpm for 20 min at 4 °C and the supernatant was used as cell extract in our work. Various activity of T4 PNK was added to the cell extract and then the mixture was filtered using 0.2 μM PES filter media (Whatman) to remove cell debris completely. And later the mixture was added to the reaction buffer solution (50%). The detection procedure was the same as those described in the aforementioned experiment for T4 PNK activity detection in reaction buffer.

**7. Application of our strategy for T4 PNK inhibitors screening.**

In the application of our strategy for T4 PNK inhibitors screening, ADP, $NaH_2PO_4$, and $(NH_4)_2SO_4$ were selected as T4 PNK inhibitors. 300 nM dsDNA-pyrene, 1 mM ATP, 100 unit/mL λ exo, 1.5 mg/mL polyβ-CD, 10 unit/mL T4 PNK and various amount of inhibitor were put into 100 μL reaction buffer (70 mM tris-base, 10 mM $MgCl_2$, pH = 8.0). After incubation at 37 $^oC$ for 30 min, the fluorescence of the mixture was measured with the excitation wavelength of 345 nm and emission from 370 nm to 480 nm at 25 $^oC$.

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