Electronic Supplementary Information

Label Free Fluorescence Turn-On Detection of Polynucleotide Kinase Activity with a Perylene Probe

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Experimental section

Materials

T4 polynucleotide kinase (10 units/μL), λ exonuclease (λ exo, 10 units/μL) were purchased from Fermentas (Canada). Adenosine triphosphate (ATP) was obtained from New England Biolabs (NEB, USA). Dithiothreitol (DTT) was bought from Beijing DingGuo Biotech. Co., Ltd (China). The DNA sequences were Ultra-PAGE purified and purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (China). The perylene probe (compound 1) was synthesized according to literature procedures and will be reported elsewhere.[s1] Other chemicals were all of analytical grade and obtained from commercial sources, and used without further purification.

The oligonucleotide sequences used in the current investigation are listed as follows: oligo-1: 5′-AAT CCA AGT CCG AAT CCG AAA CCT GCC AAA CAA ACC CAA T-3′; oligo-2: 5′-ATT GGG TTT GTT TGG CAG GTT TCG GAT TCG GAC TTG GAT T-3′; oligo-3 (5'-phosphorylated oligo-1): 5′-*P*-AAT CCA AGT CCG AAT CCG AAA CCT GCC AAA CAA ACC CAA T-3′.

Instrumentation

All stock and buffer solutions were prepared using water purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA). UV-Vis absorption spectra were obtained using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped

with a xenon flash lamp. Emission spectra were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA) with an excitation wavelength of 442 nm. Excitation and emission bandwidths were both of 4 nm. Quartz cuvettes with 10-mm path length and 2 mm window width were used for UV-vis and emission spectra measurements.

Assay procedures

100 nM oligo-1 was mixed with certain amounts of T4 polynucleotide kinase [total sample volume 10 μL, 50 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT, 100 μM spermidine, and 0.5 mM ATP]. The reaction was allowed to proceed at 37 ºC for 60 min. Samples were heated at 75 ºC for 15 min to inactivate the T4 PNK. 5 nM oligo-2 (1 μL) and 10 U/mL λ exo (1 μL) were added, and the enzymatic reaction proceeded for another 30 min. 487 μL H_2O and 1 μL compound 1 (10 μM) were added, and the emission spectra were recorded.

All perylene probe concentrations were those in the final 500 μL assay solution. Unless specified, all the enzyme, oligonucleotide, ATP, and ADP concentrations in the text and figures were those in the 10 μL (or 12 μL) assay solution.

Polyacrylamide gel electrophoresis

The assay samples were loaded onto 1.0 mm thick non-denaturing 20% polyacrylamide gel (acrylamide : bis-acrylamide = 29 : 1).[s2] The electrophoresis

buffer contained 1 × TBE (100 mM Tris-HCl, 83 mM boric acid, 1 mM EDTA, pH 8.0). After 3 h of electrophoresis (voltage: 80 V), the gel was visualized via silver staining (Figure S5).

PNK activity detection in A549 cell extracts

A549 cells were cultured in DMEM (HyClone, Thermo scientific) supplemented with 10% fetal calf serum (HyClone, Thermo scientific) in an incubator (5% CO_2, 37 ºC). Cell free extracts were prepared using a slightly modified procedure originally described by Tanaka et al.[s3] Exponentially growing cells were washed three times with ice cold 1 × PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2.0 mM KH_2PO_4) and resuspended in 10 μL buffer I (10 mM Tris-HCl, 200 mM KCl, pH 7.8). Equal volume of buffer II [10 mM Tris-HCl, (pH 7.8), 600 mM KCl, 2 mM EDTA, 40% glycerol, 0.2% NP40, 2 mM DTT, 0.5 mM PMSF protease inhibitor] was added. The sample mixture was shaken at 4 ºC for 1.5 h. Cell debris was removed by centrifugation at 16000 g for 10 min, and the supernatant was recovered. Diluted cell extracts were added to the assay solution (1%). The assay conditions were the same as those described for Figure 3.

References

[s1] B. Wang , C. Yu, *Angew. Chem. Int. Ed.*, **2010**, *49*, 1485.

[s2] B. J. Bassam, G. C. Anollés, P. M. Gresshoff, *Anal. Biochem.*, **1991**, *196*, 80.

[s3] M. Tanaka, J. Lai, W. Herr, *Cell*, **1992**, *66*, 755.

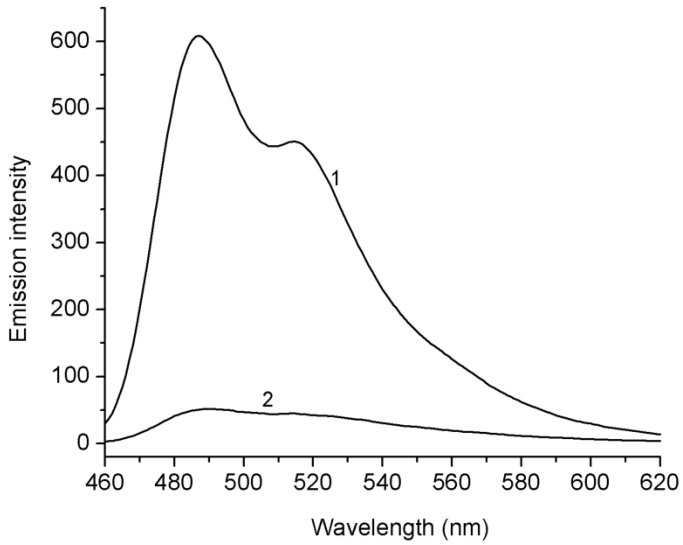

Figure S1. Curve 1: 100 nM oligo-3 + 5 nM oligo-2 + 10 U/mL λ exo. **Curve 2**: 100 nM oligo-1 was used instead of oligo-3. Compound 1, 20 nM.

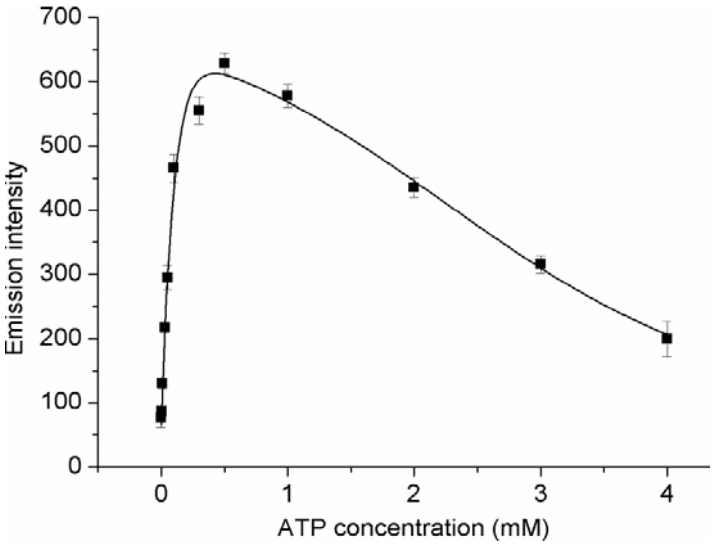

Figure S2-A. Compound 1 emission intensity changes at 488 nm upon the addition of ATP in different concentrations. Assay conditions: 100 nM oligo-1, 1.6 U/mL PNK, 5 nM oligo-2, different concentrations of ATP, and 10 U/mL λ exo.

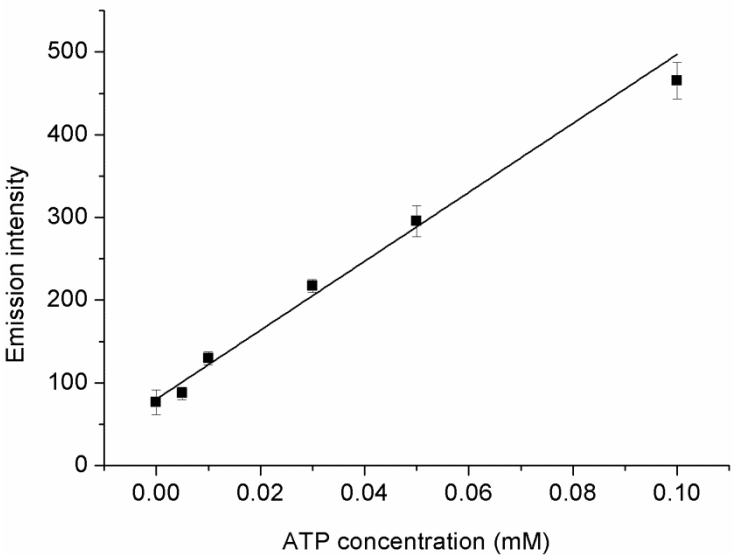

Figure S2-B. Expanded linear region of Figure S2-A.

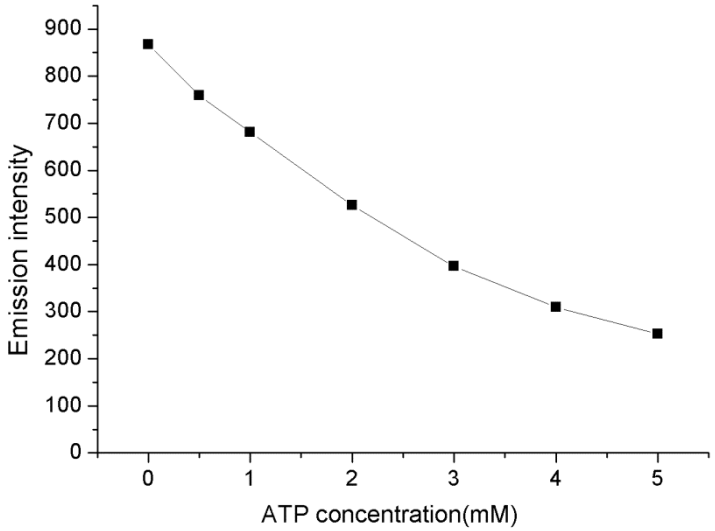

Figure S2-C. Compound 1 emission intensity changes at 488 nm upon the addition of ATP in different concentrations. One ATP molecule contains 4 negative charges. It could induce weak compound 1 aggregation. And the induced aggregation could possibly contribute to some decrease of the compound 1 monomer fluorescence at higher ATP concentrations in Figure S2-A.

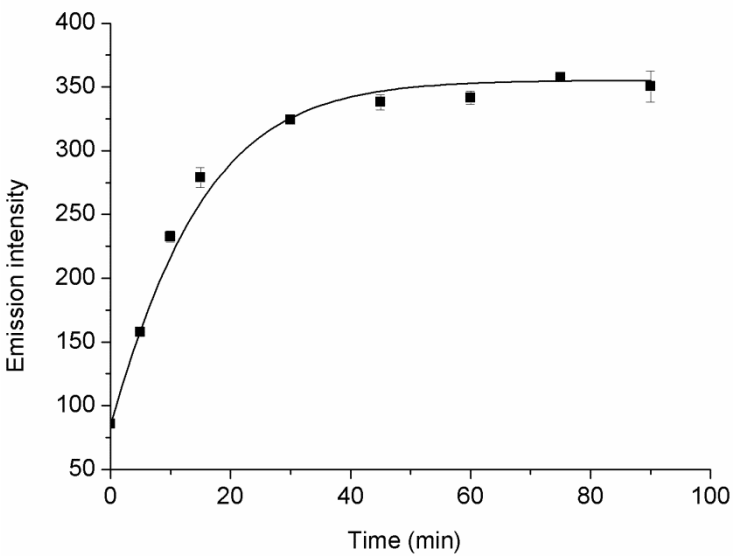

Figure S3. Compound 1 emission intensity changes at 488 nm as a function of the PNK phosphorylation reaction time. Assay conditions: 100 nM oligo-1, 0.32 U/mL PNK, 5 nM oligo-2, 0.5 mM ATP, and 10 U/mL λ exo.

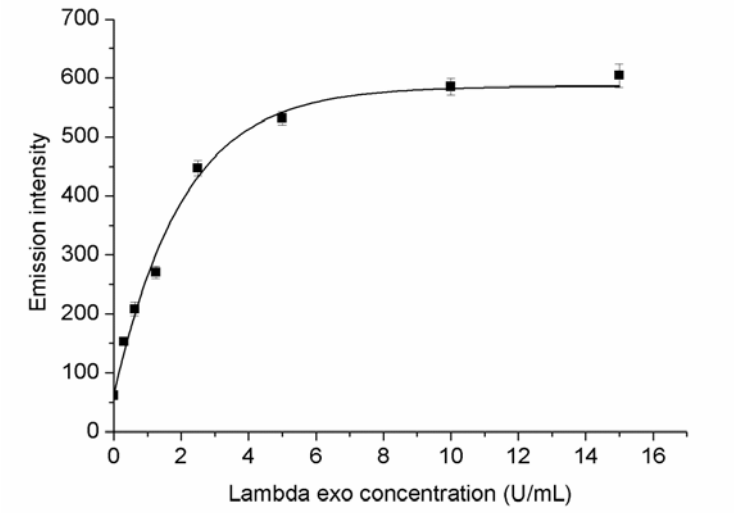

Figure S4. Compound 1 emission intensity changes at 488 nm upon the addition of λ exo in different concentrations. Assay conditions: 100 nM oligo-1, 0.32 U/mL PNK, 5 nM oligo-2, 0.5 mM ATP, reaction time: 30 min.

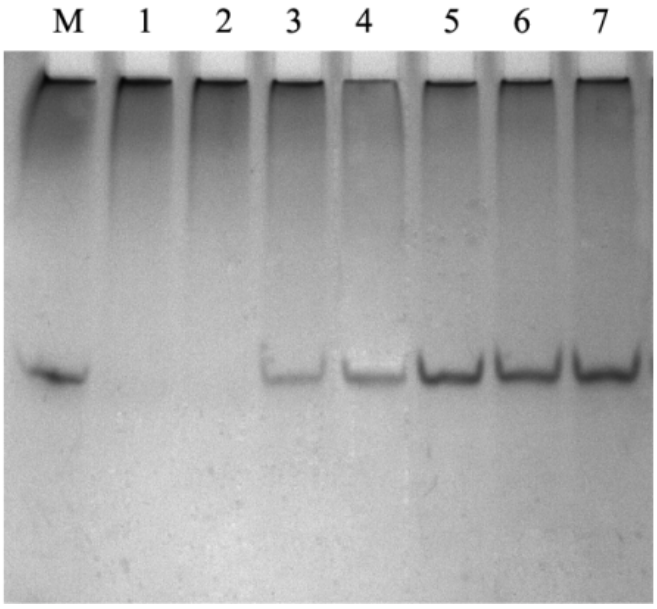

Figure S5. Polyacrylamide (20%) gel electrophoresis. Lane M (marker), oligo-1 only; lines 1-7, with decreased concentrations of PNK added (3.2, 1.6, 0.32, 0.16, 0.064, 0.032, and 0.016 U/mL PNK, respectively). Oligo-2 could not be observed because of its low concentration.

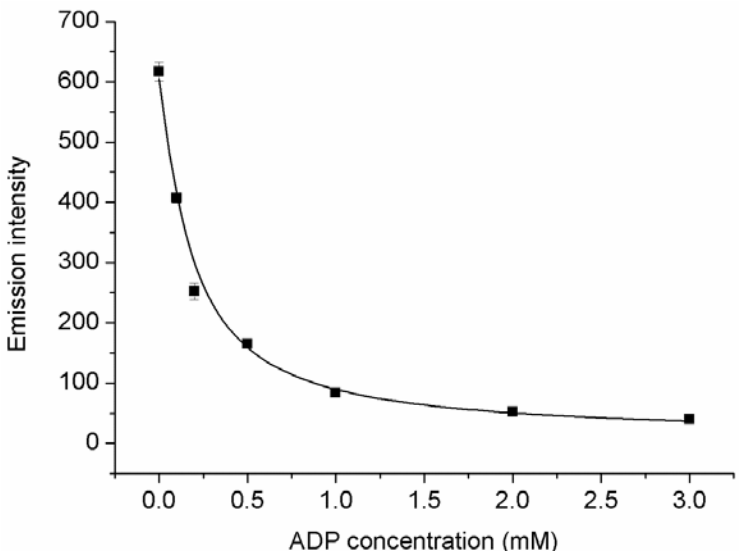

Figure S6-A. Compound 1 emission intensity changes at 488 nm upon the addition of ADP (a PNK inhibitor) in different concentrations. Experimental conditions: the same as those described for Figure 3.

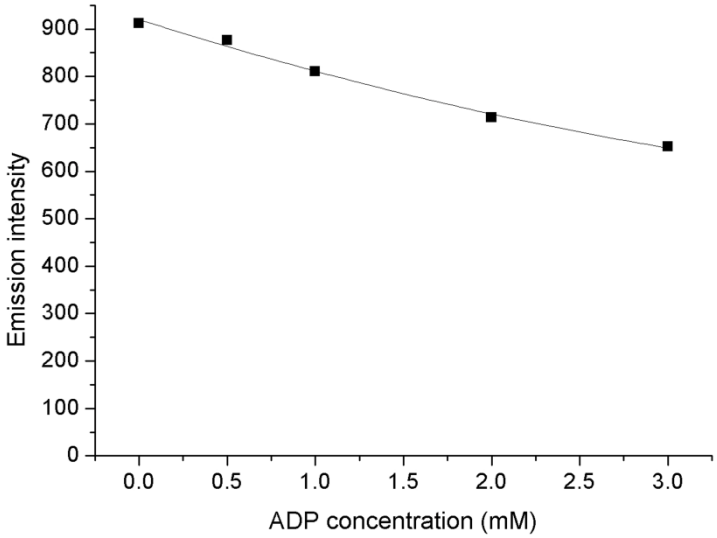

Figure S6-B. Compound 1 emission intensity changes at 488 nm upon the addition of ADP in different concentrations. ADP could induce weak compound 1 aggregation, and contribute to some decrease of the compound 1 monomer fluorescence in Figure S6-A.

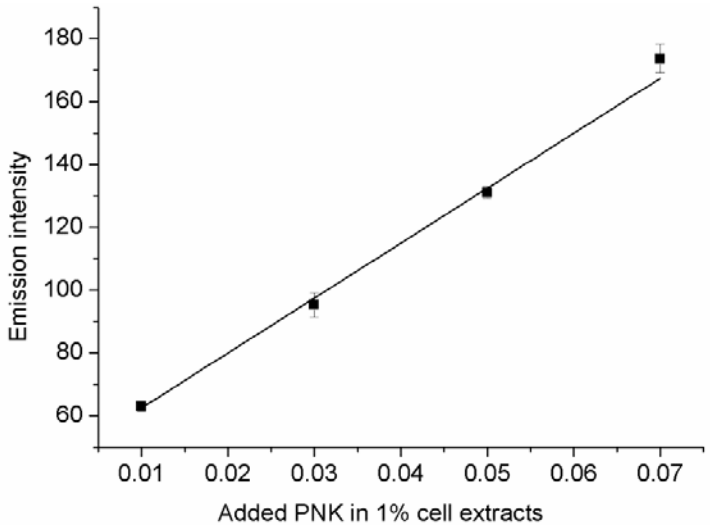

Figure S7. Different amounts of PNK were added to the assay mixture containing 1% A549 cell extracts, and the emission intensity of compound 1 at 488 nm was measured.

Table S1. PNK recovery in diluted cell extracts.[a]

PNK added (U/mL)	Recovery (%)
0.01	104.4
0.03	104.1
0.05	100.0
0.07	101.9

[a]Known amounts of PNK were added to the diluted cell extracts, their concentrations were determined, and the percentage of recovery was calculated.