

Supporting Information

A Label-Free Bioluminescent Sensor for Real-Time Monitoring Polynucleotide Kinase Activity

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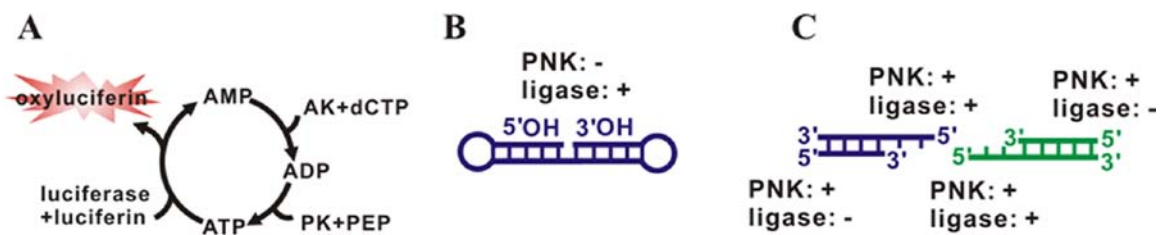


Figure S1. Detection of AMP by the bioluminescence readout (A), and detection of PNK with different probe designs: intramolecular ligation of hairpin probes (B) and intermolecular ligation of dsDNA hybrids probes (C). The labels “+” and “-” indicate the strong and low enzyme activity toward the specific terminals of different DNA substrates, respectively.

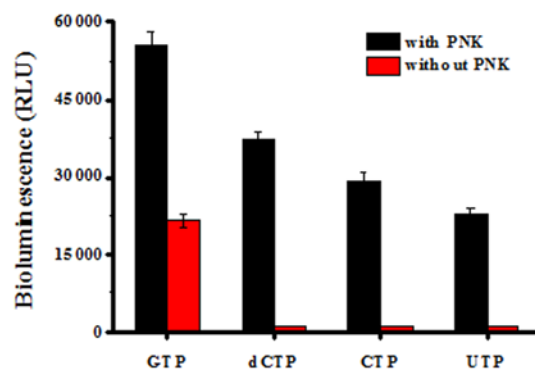


Figure S2. Influence of phosphate donor upon the bioluminescence signal. Red and black columns represent the measured bioluminescence signal in the absence and in the presence of PNK, respectively. Error bars show the standard deviation of three experiments.

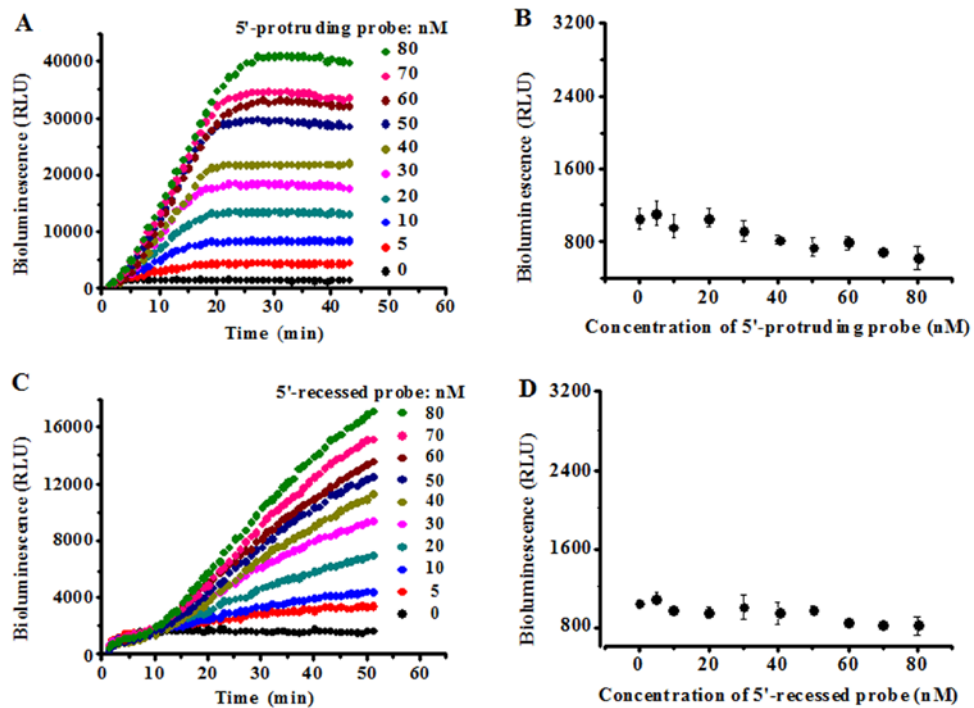


Figure S3. Variance of bioluminescence signal in response to the DNA probes with 5'- protruding (A, B) and 5'- recessed (C, D) in the presence (A, C) and in the absence (B, D) of PNK, respectively. Error bars show the standard deviation of three experiments.

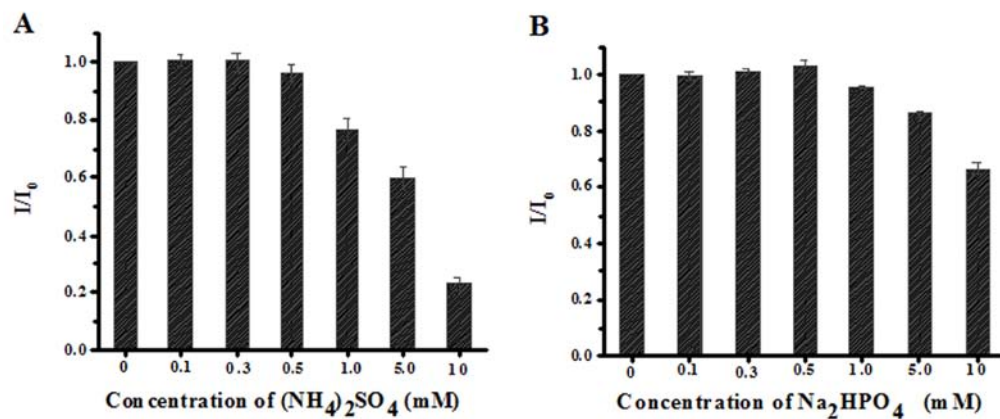


Figure S4. Influence of $(\text{NH}_4)_2\text{SO}_4$ and Na_2HPO_4 upon the cascade of enzymatic reactions including DNA ligation, AMP-to-ATP conversion and ATP detection. The DNA probes with 5'-phosphate group were used in this research. I and I_0 represent the bioluminescence signal in the presence and in the absence of inhibitors, respectively. The results indicate that these chemicals at high concentration (>0.5 mM) exhibit obvious inhibition effect on the subsequent enzymatic reactions including ligation, conversion and detection. Error bars show the standard deviation of three experiments.

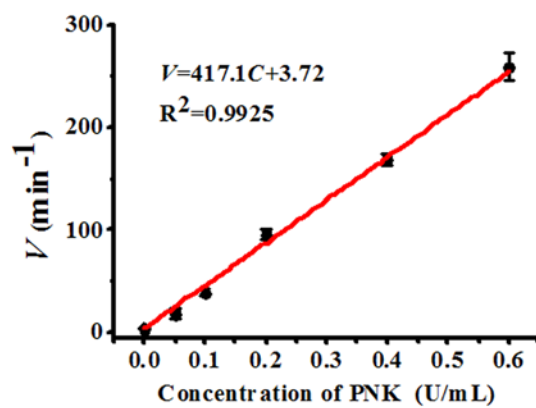


Figure S5. Construction of standard work curve by the addition of PNK into the nucleoprotein. The nucleoprotein is denatured by heating treatment. Error bars show the standard deviation of three experiments.

Table S1. Comparison of various methods for PNK assay.

strategy ^a	signal readout	label- free	real- time	assay time (h)	LOD ^b (U/mL)
cleavage ¹	recover the fluorescence of guanosine- quenched fluorescence label	-	+	0.06	0.04
cleavage ²	release 8-17 DNAzyme for catalyzing the cleavage of molecular beacon	-	-	1.3	5.0×10 ⁻³
cleavage ³	induce the quenching of fluorescence label by graphene oxide	-	-	0.5	0.05
cleavage ⁴	release DNA trigger for assembling the bimolecular beacons	-	-	3.5	1.0×10 ⁻³
cleavage ⁵	recover the fluorescence of perylene monomer	+	-	1.75	3.0×10 ⁻³
cleavage ⁶	prohibit the formation of fluorescent copper nanoparticles	+	-	1.6	0.49
cleavage ⁷	release G-quadruplex for generation of fluorescence signal	+	-	1.0	0.05
cleavage ⁸	release G-quadruplex for generation of colorimetric signal	+	-	1.3	0.06
cleavage ⁹	reduce current signal of nanochannel biosensor	-	-	1.5	0.01
cleavage ¹⁰	induce electrochemical response of alkaline phosphatase	-	-	2.0	0.01
biotinylation ¹¹	facilitate the counting of single nanoparticles by fluorescence	-	-	1.0	5.0×10 ⁻⁶
binding to	Ti ⁴⁺ -assisted affinity binding between	-	-	2.0	0.01

Ti ⁴⁺ ¹²	phosphorylated DNA and electrochemical labels				
binding to Ti ⁴⁺ ¹³	TiO ₂ nanotube-assisted affinity binding between phosphorylated DNA and electrochemical labels	-	-	11.0	3.0×10 ⁻³
ligation ¹⁴	induce the recovery of molecular beacon	-	+	0.18	2.0×10 ⁻³
ligation ¹⁵	induce the recovery of graphene oxide- quenched fluorescence label	-	-	0.9	1.0×10 ⁻³
ligation ¹⁶	trigger the recycling cleavage of molecular beacon	-	-	0.5	1.0×10 ⁻⁵
ligation ¹⁷	trigger replication of hemin DNAzyme for generation of chemiluminescence signal	+	-	5.0	2.2×10 ⁻⁴
ligation ¹⁸	trigger replication of hemin DNAzyme for generation of colorimetric signal	+	-	17.5	1.8×10 ⁻³
ligation ^c	release AMP for generation of bioluminescence signal	+	+	0.1	4.0×10 ⁻³

^a with the following DNA manipulation after PNK phosphorylation, ^b LOD, limit of detection, ^c this work.

Table S2. Analytical results for the detection of PNK activity in HEK293T cell extracts.

Sample	Added (U/mL)	Mean found (U/mL)	Mean recovery (%)	RSD (%) (n=3)
cell extracts	0	0.056		3.7
spiked PNK 1	0.2	0.264	104.0	3.4
spiked PNK 2	0.4	0.45	98.5	2.2
spiked PNK 3	0.6	0.637	96.8	0.8

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