Electronic Supplementary Information

Amplified fluorescence detection of T4 polynucleotide kinase activity based on coupled Exonuclease III reaction and graphene oxide platform

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**Experimental section**

**Reagents and apparatus**

ExoIII (100 U μL⁻¹), T4 PNK (10 U μL⁻¹), T4 DNA Ligase (400 U μL⁻¹), Dam (8 U μL⁻¹) were purchased from New England Biolabs Ltd. (Beijing). ALP (EC 3.1.3.1) was purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Bovine serum albumin (BSA), human serum albumin (HSA), were purchased from Dingguo Biotech. Co. (Beijing, China). Adenosine diphosphate (ADP) and ammonium sulfate ((NH₄)₂SO₄) were purchased from Sigma Co., Ltd. (St. Louis, MO, USA). Ethylenediaminetetraacetic acid sodium (EDTA) and disodium phosphate (Na₂HPO₄) were purchased from Shanghai Chemical Reagents (Shanghai). GO was bought from Leadernano Co. Ltd. (Jining, China) and used without further purification. The buffers for enzyme reactions involved in this work were T4 DNA ligase buffer (66 mM Tris-HCl, 6.6 mM MgCl₂, 10 mM DDT, 100 mM ATP, pH 7.6), the detection buffers contained 100 mM NaCl and 25 mM HEPES (pH 7.4). All solutions were prepared with Milli-Q water (resistance >18 Ω·cm) from a Millipore system. DNA oligonucleotides used in this work were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai), and their sequences are shown as follows:

- P1: 5′-PO₄-TAC AAG CG-3′
- P2: 5′-AGT CGA GGA GAT-3′
- P3: 5′/-FAM/-CCT CGA CTC GCT TGTA-3′
- P4: 5′-TAC AAG CG-3′
- P5: 5′-PO₄-AGT CGA GGA GAT-3′

All fluorescence measurements were carried out on an F-4600 spectrometer (Hitachi, Japan). The instrument settings were chosen as follows: λex=494 nm (slit 5 nm), λem=518 nm (slit 5 nm), PMT detector voltage=950 V.

**Optimization of GO concentration**

To investigate the effect of GO concentration for fluorescence quenching, different concentrations of GO were mixed with 50 nM P3 in 80 μL HEPES buffer (100 mM NaCl, 25 mM HEPES, pH 7.4) for 15 min at 37 °C, then the fluorescence changes were recorded.
Amplified assay of T4 PNK activity

To investigate the activity of T4 PNK, samples were first prepared with 200 nM P1, 200 nM P2 and 200 nM P3, T4 PNK at various concentrations and T4 DNA ligase (20 U) was added into 19 μL of 1 × T4 DNA ligase buffer (66 mM Tris-HCl, 6.6 mM MgCl₂, 10 mM DDT, 100 mM ATP, pH 7.6) for 30 min at 37 °C. Then, 1 μL ExoIII (2 U μL⁻¹) was introduced into the mixture immediately for 30 min at 37 °C. Finally, the reaction mixture was added of 8 μL GO (200 μg mL⁻¹) and 52 μL detection buffer (100 mM NaCl, 25 mM HEPES, pH 7.4) to keep the final volume reach 80 μL, the solutions were incubated at 37 °C for 15 min prior to fluorescence measurement.

Detection of the inhibition of T4 PNK

In the inhibition assay, ADP (0 ~ 10 mM), (NH₄)₂SO₄ (0 ~ 60 mM), Na₂HPO₄ (0 ~ 60 mM) and EDTA (0 ~ 60 mM) were selected as four kinds of inhibitors to investigate the effects of inhibitors on the PNK-catalyzed phosphorylation process. The experiments were performed in the typical reaction buffer which contained 200 nM P1, 200 nM P2 and 200 nM P3, 0.5 U mL⁻¹ T4 PNK and T4 DNA ligase 1 U μL⁻¹. The reaction and measurement procedures were same to the above activity assays.

T4 PNK activity detection in diluted cell extracts

A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U mL⁻¹ penicillin, 100 μg mL⁻¹ streptomycin at 37 °C under 5% humidified CO₂. Cell extracts were obtained from 1 ×10⁶ cells which prepared according to the previous reports.¹² For the T4 PNK activity detection in cell extracts, 1% (v/v) cell extracts was added in the reaction buffer with all the other conditions the same as the description in the aforementioned experiment in pure buffer solution. All experiments were performed in compliance with the relevant laws and institutional guidelines of the Qufu Normal University hospital.

Supplementary Figures

**Fig. S1** Different temperature effects on the fluorescence responses of the sensing system.

(a) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase (1 U μL⁻¹) + T4 PNK (0.2 U mL⁻¹) + ExoIII (0.1 U μL⁻¹) react at 37 °C;
(b) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase (1U μL⁻¹) + ExoIII (0.1 U μL⁻¹) react at 37 °C;
(c) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase (1 U μL⁻¹) + T4 PNK (0.2 U mL⁻¹) + ExoIII (0.1 U μL⁻¹) react at 37 °C;
(d) P1 (50 nM) + P2 (50 nM) + P3 (50 nM) + T4 DNA ligase (1 U μL⁻¹) + ExoIII (0.1 U μL⁻¹) react at 37 °C.
Fig. S2 Fluorescence intensities of 50 nM P3 in the presence of different concentrations of GO. Error bars were estimated from three replicate measurements.
Fig. S3 The effect of the (A) T4 DNA ligase concentration and (B) ExoIII concentration on the fluorescence responses of the sensing system. The concentration of T4 PNK was 0.2 U mL$^{-1}$. The descriptors $F$ and $F_0$ are the fluorescence intensity in the presence and absence of target, respectively. Error bars were estimated from three replicate measurements.
Fig. S4 (A) Fluorescence spectra responses of the sensing system in the presence of different concentrations of T4 PNK in diluted cell extracts (1%). (B) The relationship of the fluorescence enhancement with the T4 PNK concentration in diluted cell extracts (1%). Error bars were estimated from three replicate measurements.
**Table S1** Comparison of different fluorescent systems for PNK activity detection.

<table>
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<th>System</th>
<th>Linear Range</th>
<th>Detection Limit</th>
<th>Ref.</th>
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**References**