Electronic Supporting Information

Label-free fluorescence light-up detection of T4 polynucleotide kinase activity using split-to-intact G-quadruplex strategy by ligation-triggered and toehold-mediated strand displacement release

Experimental section

Materials. T4 DNA ligase (5 Weiss U/μL), T4 polynucleotide kinase (10U/μL) were purchased from Thermo Fischer Scientific Co., Ltd. (Beijing, China). All the enzymes were stored at -20℃. Oligonucleotides (Table S1,) were synthesized by Sangon Biotechnoloy Co. Ltd. (Shanghai, China).NMM was acquired from J&K Scientific Ltd. (Beijing, China). All other reagents were purchased from Sigma Aldrich (St. Louis, MO). The water used was purified by Millipore Milli-Q (18MΩ/cm).

Stock solution of oligonucleotides (40μM) were prepared by Milli-Q water. The stock solution of NMM (3.2mM) was prepared in dimethyl sulfoxide (DMSO), stored in darkness at -20° C. Before use, the oligonucleotides, NMM and all enzymes solutions were diluted with water. The concentration of oligonucleotides were measured and qualified on a Biopotometer (Eppendorf, Germany) by absorbance at 260 nm. The concentration of NMM was measured on a Lambda 25 spectrophotometer (PE, USA) by absorbance at 379nm assuming an extinction coefficient of 1.45×10^5/M•cm. [1-3] Circular dichroism spectra were collected on a Chirascan^{TM}-plus CD Spectrometer

Table 1. Oligonucleotide sequences used in this study

	Sequence
S_1OH	5'-GTG GGT AGG
S_2OH	5'-GCG GGT TGG
$H_{12\text{-}22}$	5'-CTTAATTGAATTGGTACCCGCCCTACC
$cH_{12\text{-}22}$	5'-GGCGGGTACCAATTCAATTAAG
$H_{12\text{-}26}$	5'-CTTTTAATTGAATTGGTACCCGCCCTACC
$cH_{12\text{-}26}$	5'-GGCGGGTACCAATTCAATTAAAAG
PS2.M	5'-GTG GGT AGG GCG GGTTGG

Detection of T4 PNK activity

For measuring T4 PNK activity, 50μL of 1×T4 PNK Reaction Buffer(50mM Tris-HCl, 10mM $MgCl_2$, 5mM DTT, 1mM spermidine, pH 7.6) with 1.0mM ATP and the indicated concentrations of T4 PNK were added to the solution contain S_2OH (1 μM). The mixture was heated to 37° C for 30 min to allow the T4 PNK catalyzed phosphorylation to get S_2P. Then the mixture was heated to 75° C for 10min to inactivate T4 PNK, and cooled to room temperature for T4 DNA ligase step. Immediately S_1OH (1.0μM) and $H_{12\text{-}22}$ (1.5μM) were added into the solution above with S_2P. Then the solutions were heated to 95° C for 5min, and cooled to room temperature. 5μL of 10×T4 DNA ligase Reaction Buffer(400mM Tris-HCl, 100mM $MgCl_2$, 100mM DTT, 5mM ATP, pH 7.8) with 2.0 U/mL T4 DNA ligase was added. The mixture was heated to 22° C for 120 min to allow T4 DNA ligase to catalyze the connection of S_1 and S_2-P and the solution was heated to 75° C for

10min to inactivate T4 DNA ligase, and cooled to room temperature for next step.

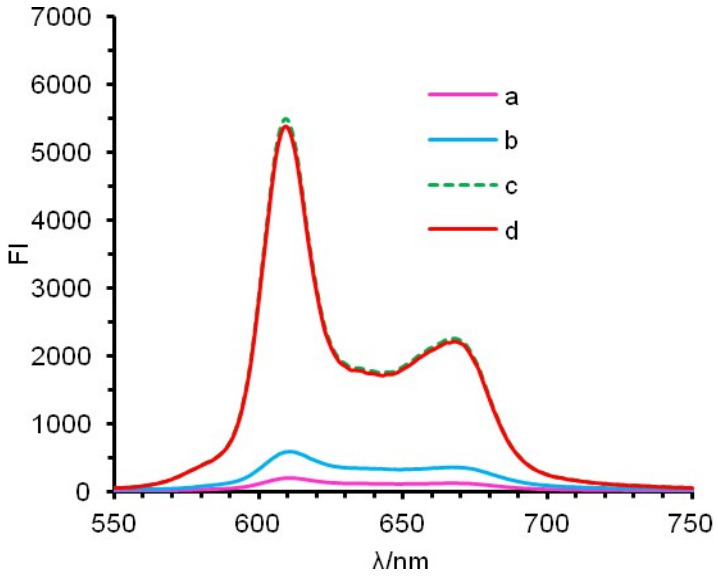

Fig. S1 Fluorescence emission spectra of the sensing system containing T4 PNK, T4 DNA ligase and NMM (a), S_1OH, S_2OH, $H_{12\text{-}22}$, $cH_{12\text{-}22}$, T4 DNA ligase and NMM (b) S_1OH, S_2P (S_2OH was phosphorylatd by T4 PNK, the same as below), $H_{12\text{-}22}$, $cH_{12\text{-}22}$, T4 DNA ligase and NMM (c)PS2.M and NMM (d). The concentrations were 1.0 μM for the oligonucleotides, 2.0 μM for NMM, 1 U/mL for T4 DNA ligase and 2 U/mL for T4 PNK.

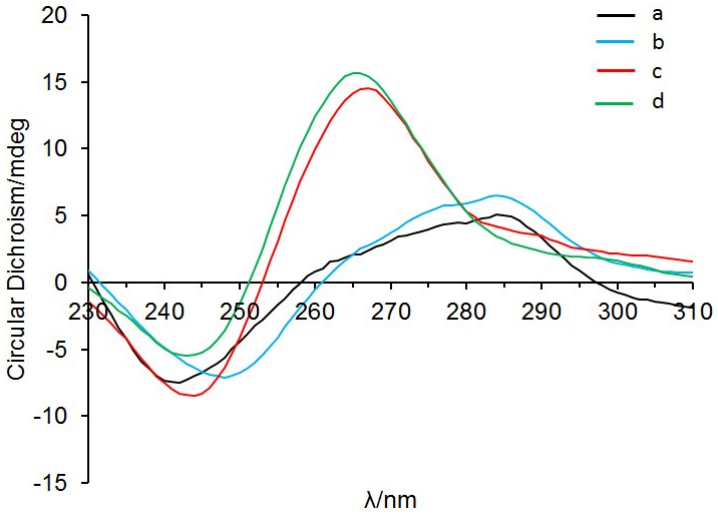

Fig.S2 Circular dichroism (CD) spectra of the sensing system containing S_1OH, S_2OH, $H_{12\text{-}22}$, $cH_{12\text{-}22}$, T4 DNA ligase (a), $H_{12\text{-}22}$ and $cH_{12\text{-}22}$, T4 PNK and T4 DNA ligase (b), S_1OH, S_2P, $H_{12\text{-}22}$, $cH_{12\text{-}22}$, T4 DNA ligase (c), PS2.M, T4 PNK and T4 DNA ligase (d). The concentrations were 5μM for the oligonucleotides, 1 U/mL for T4 DNA ligase and 2U/mL for T4 PNK.

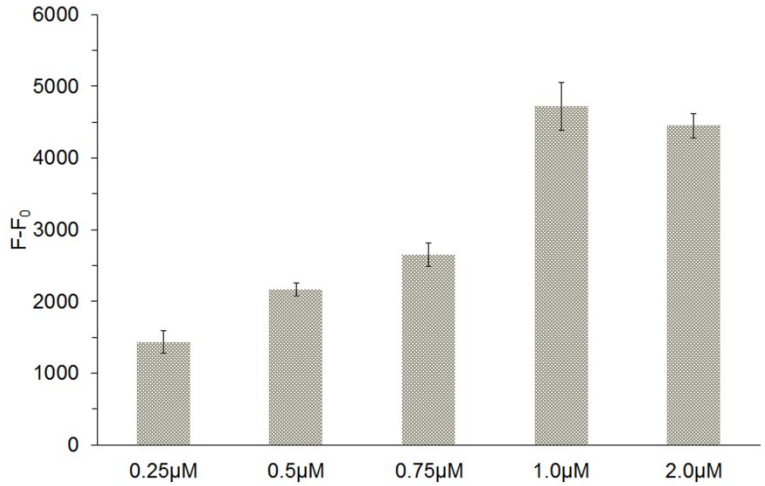

Fig.S3 Relative fluorescence intensity at 610nm of the system (T4 PNK concentration is 3.0 U/mL, T4 DNA Ligase concentration is 2.0U/mL, ATP concentration is 1.0mM, T4 PNK reaction time is 30min) at various concentrations of S_1OH, S_2OH and $H_{12\text{-}22}$, (0.25, 0.5, 0.75, 1.0 and 2.0μM) in aqueous buffered solution (20mM Tris, 10mM KCl, 20mM NaCl, 5mM $MgCl_2$, pH 7.4). It was observed that the fluorescence response of the system was highest at 1.0μM of oligonucleotides. A higher concentration of the oligonucleotides would result in a higher background signal of system.

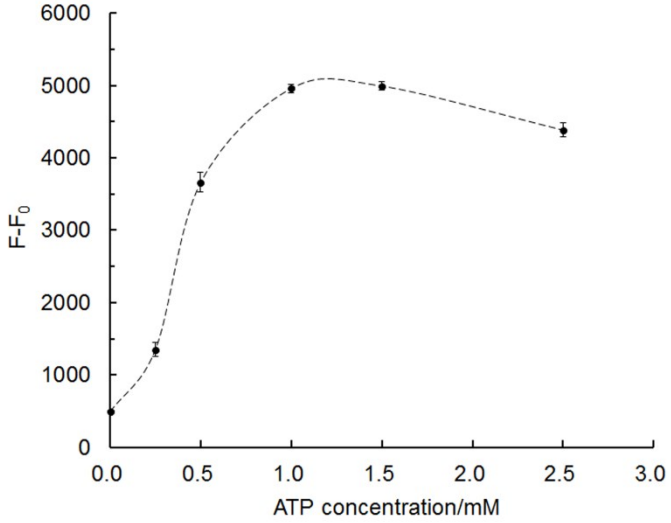

Fig.S4 Relative fluorescence intensity at 610nm of the system (T4 PNK concentration is 3.0 U/mL, T4 DNA Ligase concentration is 2.0U/mL, T4 PNK reaction time is 30min.) at various concentrations of ATP (0, 0.25, 0.5, 1.0, 1.5and 2.0mM) in aqueous buffered solution (20mM Tris, 10mM KCl, 20mM $NaCl_2$, 5mM $MgCl_2$, pH 7.4). The fluorescence response of the system was highest at 1.0mM of ATP. Higher concentrations of ATP resulted in a decreased enhancement of the system, which may be partially due to the blockage of the PNK binding site for DNA at higher concentration of ATP.

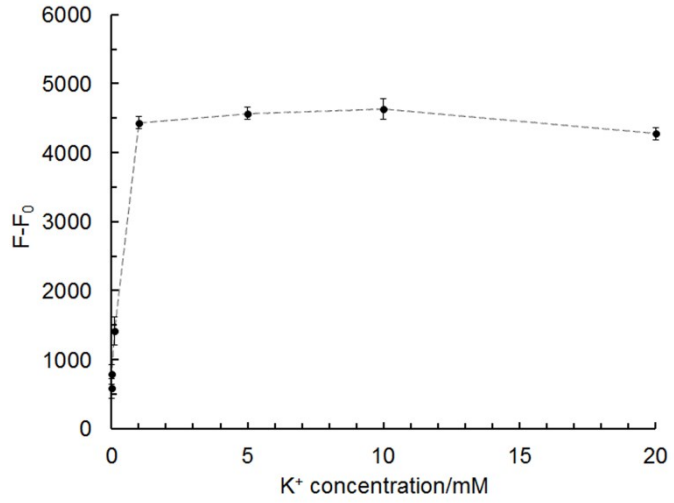

Fig.S5 Relative fluorescence intensity at 610nm of the system (T4 PNK concentration is 3.0 U/mL, T4 DNA Ligase concentration is 2.0U/mL, ATP concentration is 1.0mM, T4 PNK reaction time is 30min.) at various concentrations of KCl (0.005, 0.01, 0.1, 1.0, 5.0, 10.0 and 20.0mM)) in aqueous buffered solution (20mM Tris, 20mM NaCl, 5mM $MgCl_2$, pH 7.4). The fluorescence response of the system was highest at 10mM of K^+. Higher concentrations of KCl resulted in a saturation of the system, which may be partially due to the G-quadruplex formation from the split sequences at higher concentrations of K^+.

Total cell extract preparation

Red blood cells (RB cells) were separated from fresh human blood by centrifuge. CCRF-CEM cells, HBL-100 cells, MCF-7 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS). All the cells were washed three times with PBS and 3 freeze/thaw circles (liquid nitrogen/37℃ water bath) were performed before centrifugation. The supernatants were collected for PNK measurement. Around one million cells were collected for the recovery rate assay. The extracts of cells were diluted 2times during the activity detection of PNK in human cells.

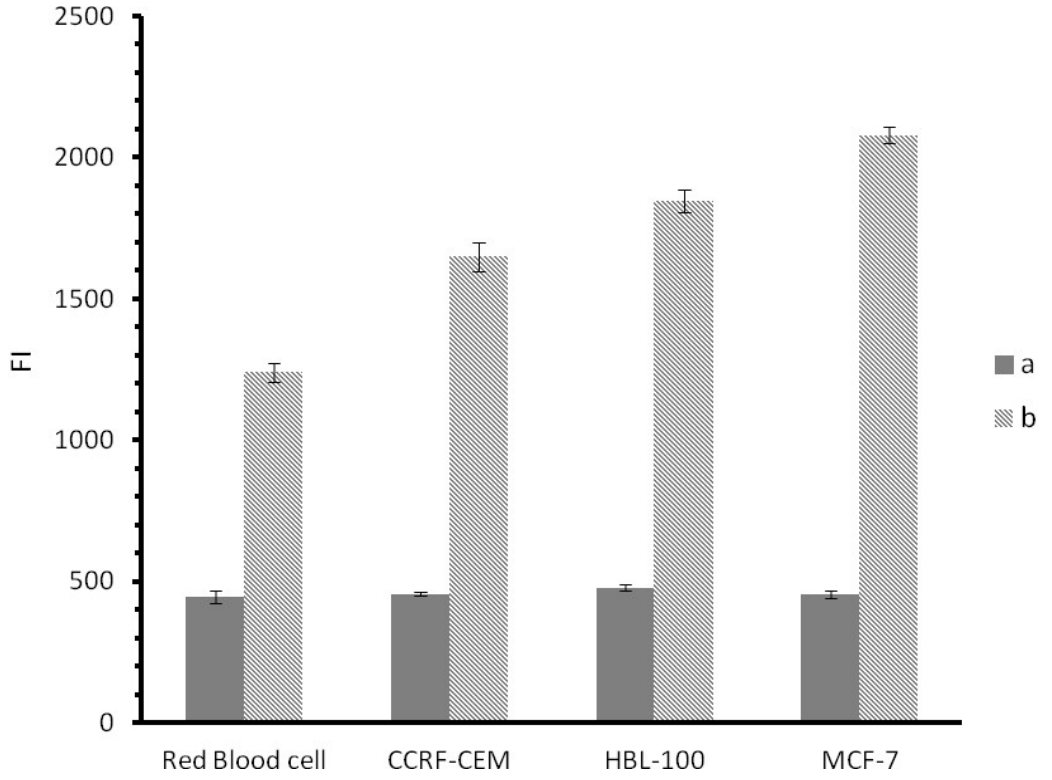

Fig.S6 Relative fluorescence intensity at 610nm of the system in NMM (a) and in T4 DNA ligase (2.0U/mL), ATP (1.0mM), oligonucleotides and NMM(b). It was observed that the fluorescence response was higher in presence of T4 DNA ligase and oligonucleotides than NMM only. It indicated the cell samples contain certain concentration of PNK originally.

1. D. Hu, F. Pu, Z. Huang, J. Ren and X. Qu, *Chemistry*, 2010, 16, 2605-2610.
2. D. Hu, J. Ren and X. Qu, *Chemical Science*, 2011, 2, 1356.
3. C. Zhao, L. Wu, J. Ren and X. Qu, *Chem Commun (Camb)*, 2011, 47, 5461-5463.