Supplementary Information

Ratio fluorescence analysis of T4 polynucleotide kinase activity based on the formation of a graphene quantum dot-copper nanocluster nanohybrid

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Chemicals and characterizations

Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), citric acid ($C_6H_8O_7 \cdot H_2O$), ammonia ($NH_3 \cdot H_2O$, 25.0-28.0%), 1-ethyl-3-[3-di-methylaminopropyl] carbodiimide hydrochloride (EDC) and sodium ascorbate were purchased from Aladdin Reagent Co., Ltd. T4 polynucleotide kinase (T4 PNK), 5× T4 PNK reaction buffer (700 mM trizma hydrochloride (Tris-HCl), 100 mM magnesium chloride ($MgCl_2$), 50 mM dithiothreitol (DTT), pH = 7.6), lambda exonuclease (λ exo), 10× λ exo reaction buffer (670 mM glycine-potassium hydroxide (KOH), 25 mM $MgCl_2$, 0.1% (v/v) triton X-100, pH = 9.4), adenosine triphosphate (ATP) and pyrophosphatase (PPase) were bought from Sangon Biotech Co., Ltd. Tyrosinase, pepsin, lysozyme, urease and thrombin were obtained from Sigma-Aldrich Corporation. The 5′-amino-capped DNA strand 1 (DNA 1): 5'-NH_2-ATATATATATATACGGCAATTAATTAATTA-3', and DNA strand 2 (DNA 2): 5'-TAATTAATTAATTGCCGTATATATATATAT-3' were synthesized and purified by Sangon Biotech Co., Ltd.

Fluorescence measurements were conducted by a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a xenon lamp. Transmission electron microscopy (TEM) and high resolution TEM (HRTEM) were performed by the Hitachi H-800 electron microscope. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 400 Fourier transform infrared spectrometer. Dynamic light scattering (DLS) measurement were performed by a Zetasizer Nano ZS90 analyzer at 25 ℃. Electrochemical impedance spectroscopy (EIS) was conducted on the CHI 660B electrochemical workstation in 2.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ with 0.1 M KCl as the supporting electrolyte and a glassy carbon electrode (GCE) was used.

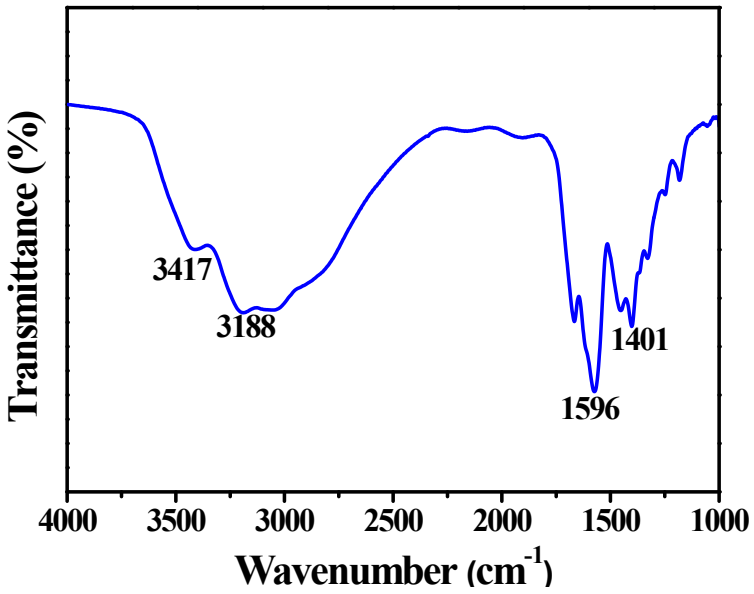

Fig. S1 FT-IR spectra of GQDs.

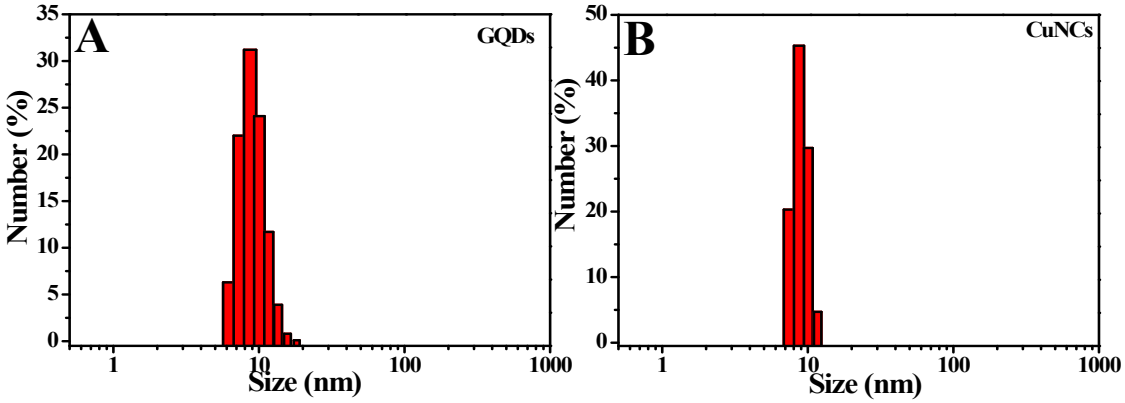

Fig. S2 Dynamic light scattering analysis of GQDs (A) and CuNCs (B).

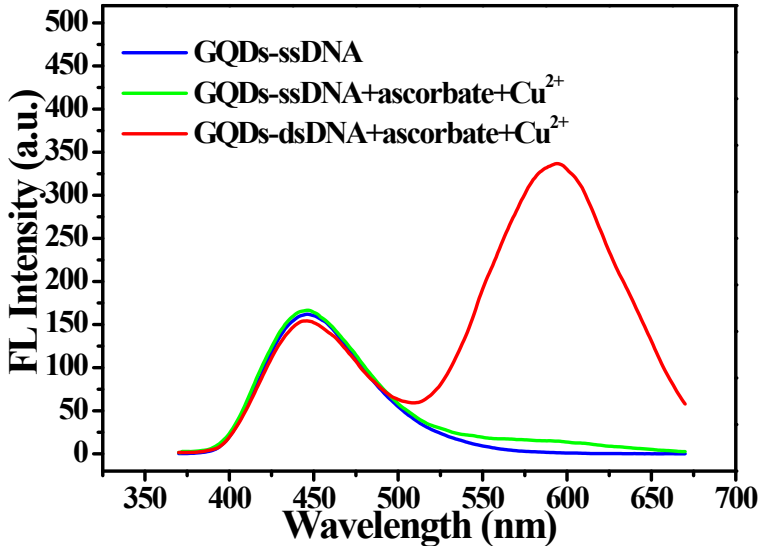

Fig. S3 Fluorescence emission spectra of GQDs-ssDNA, GQDs-ssDNA/ascorbate/Cu^{2+} and GQDs-dsDNA/ascorbate/Cu^{2+} system.

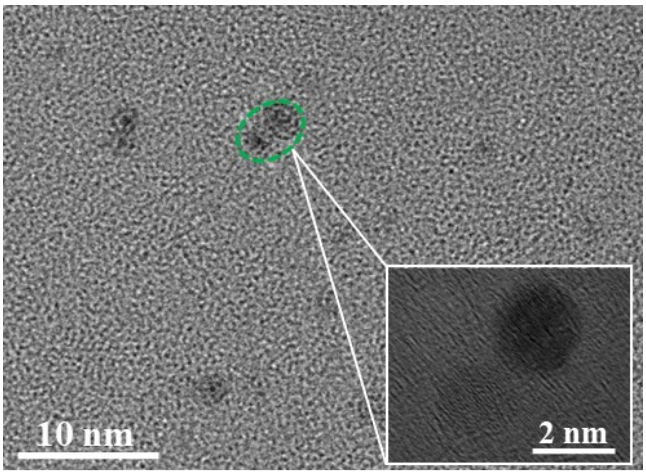

Fig. S4 TEM images of GQDs-CuNCs nanohybrid.

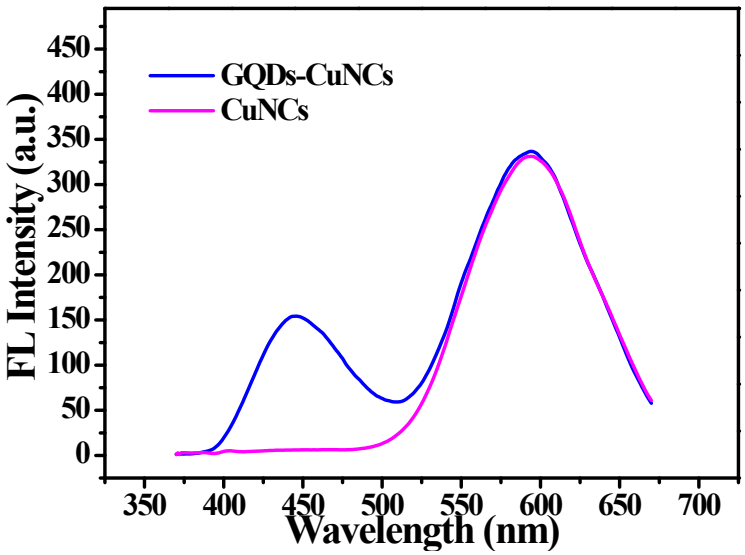

Fig. S5 Fluorescence emission spectra of GQDs-CuNCs nanohybrid and CuNCs.

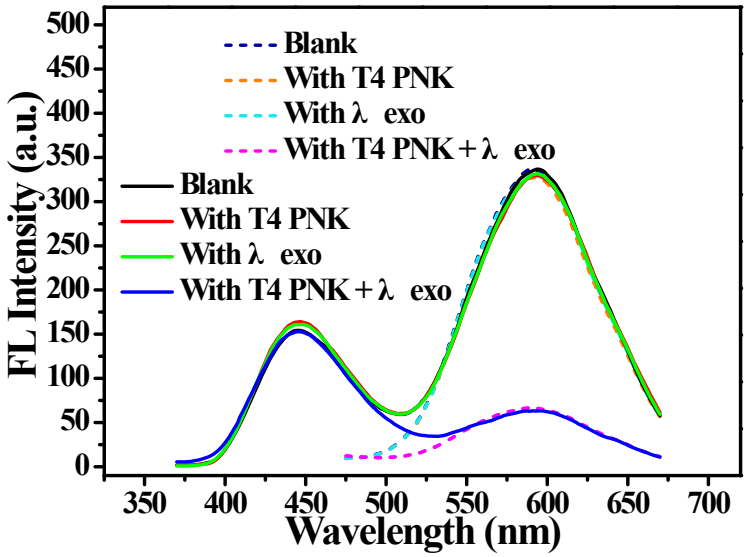

Fig. S6 Fluorescence emission spectra of the GQDs-dsDNA/ascorbate/Cu^{2+} (solid line) and dsDNA/ascorbate/Cu^{2+} (dash line) reaction system treated with T4 PNK, λ exo and T4 PNK/λ exo.

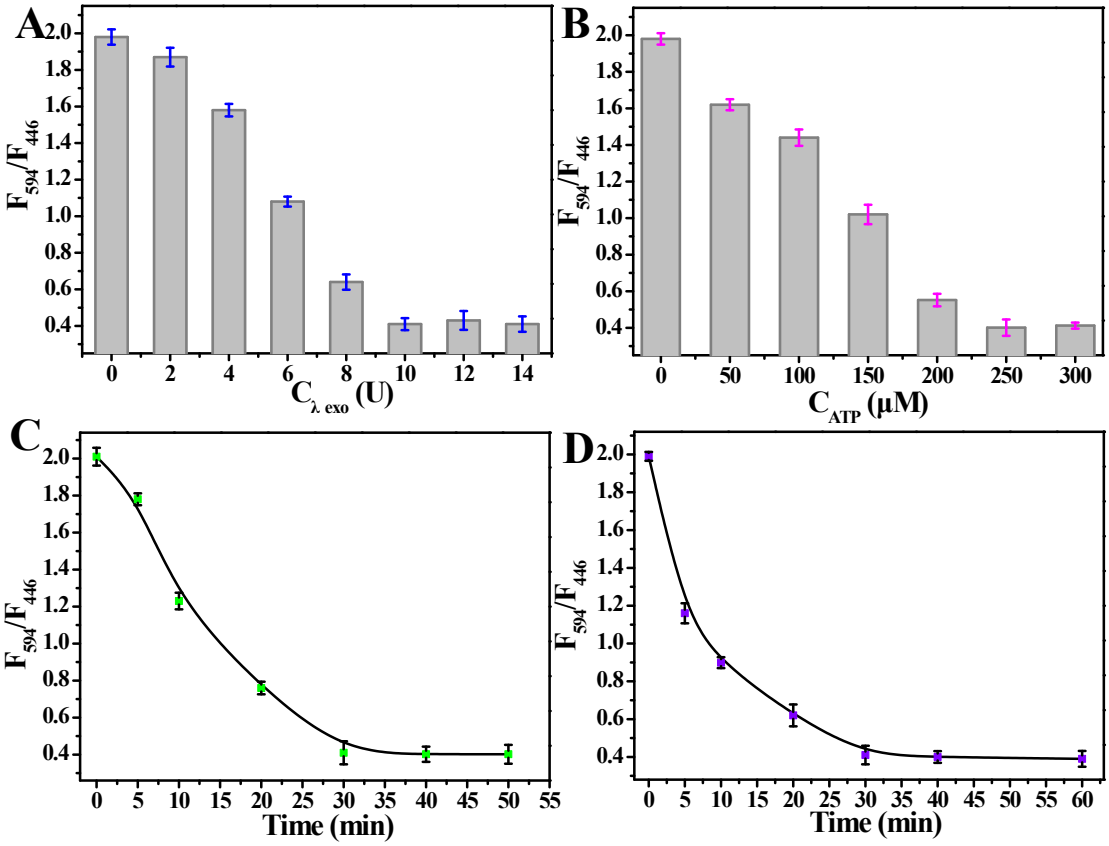

Fig. S7 Effect of λ exo (A), ATP concentration (B), T4 PNK phosphorylation time (C), λ exo cleavage reaction time (D) on the fluorescence intensity ratio F_{594}/F_{446}.

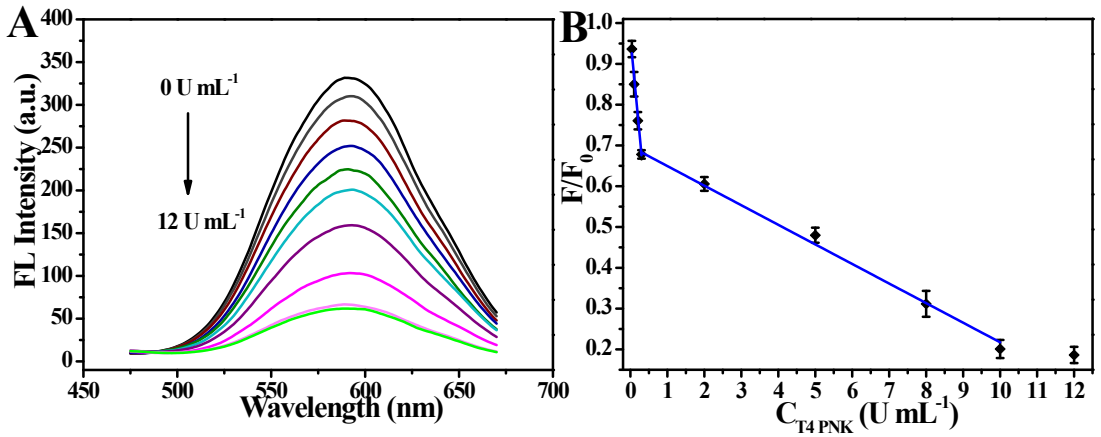

Fig. S8 (A) Fluorescence emission spectra of dsDNA/T4 PNK/λ exo/ascorbate/Cu^{2+} reaction system with various concentrations T4 PNK. (B) The linearity of the fluorescence intensity ratio (F/F_0) versus T4 PNK concentration. F and F_0 were the FL intensity of dsDNA/T4 PNK/λ exo/ascorbate/Cu^{2+} system in the presence and absence of T4 PNK, respectively.

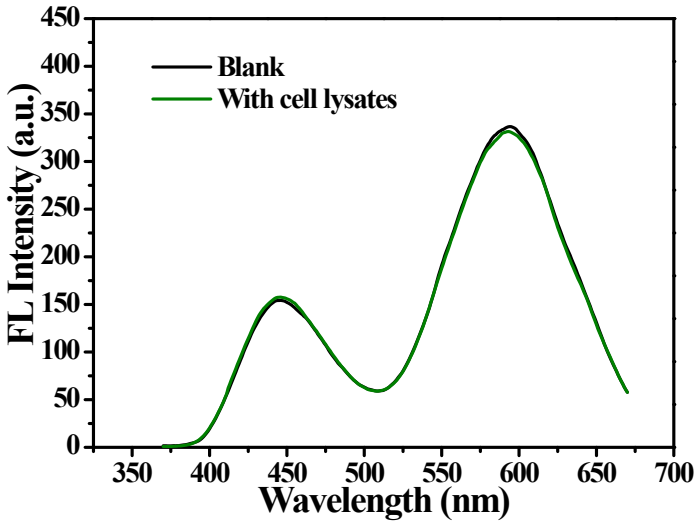

Fig. S9 Fluorescence emission spectra of GQDs-CuNCs and GQDs-CuNCs/cell lysates (10%) system.

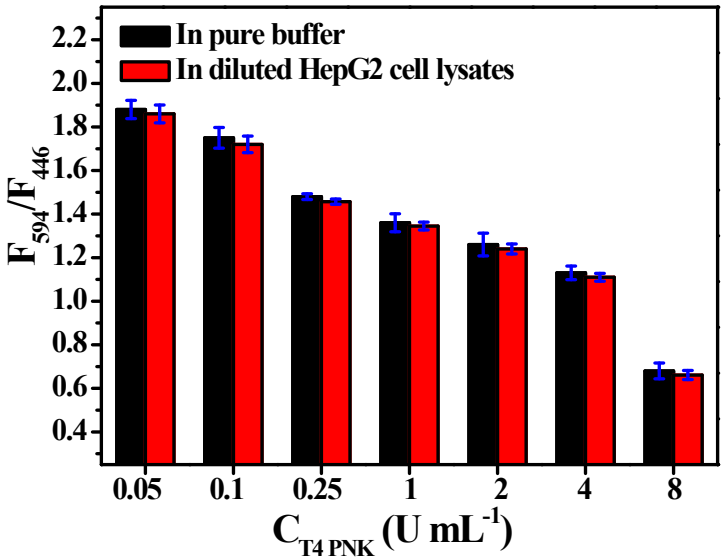

Fig. S10 Fluorescence response for detecting T4 PNK activity in pure buffer and diluted cell lysates (10%) respectively.

Table S1 Comparison of different fluorescence methods for T4 PNK detection.

Material	Linear range ($U\ mL^{-1}$)	LOD ($U\ mL^{-1}$)	References
Deoxyguanosines/FAM	0.005-10	0.0021	1
Graphene oxide/exonuclease III	0-0.2	0.003	2
Perylene	0-0.08	0.003	3
Allosteric aptamer probe	0-1	0.01	4
FAM/cobalt oxyhydroxide	0.01-1	0.01	5
PFP/SYBR Green I	0.001-5	0.001	6
FAM/polydopamine nanospheres	0.01-2.5	0.01	7
β-cyclodextrin polymer	0-0.25	0.02	8
GQDs-CuNCs	0.01-10	0.0037	This work

Notes and references

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