Electronic Supplementary Information

Fluorescent Graphitic Carbon Nitride Nanosheet Biosensor for Highly Sensitive, Label-Free Detection of Alkaline Phosphatase

Mei-Hao Xiang, Jin-Wen Liu, Na Li, Hao Tang*, Ru-Qin Yu and Jian-Hui Jiang*

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P. R. China

* Corresponding authors. E-mail: haotang@hnu.edu.cn; jianhuijiang@hnu.edu.cn Tel.: 86-731-88664085; Fax: 86-731-88821848.

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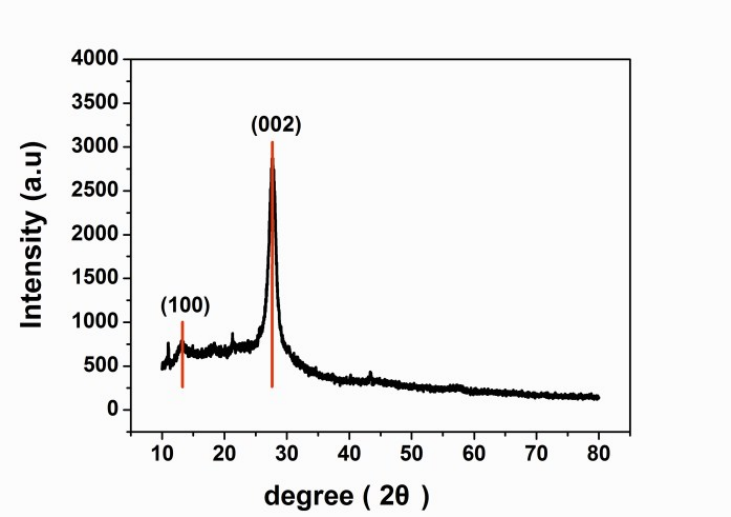

Fig. S1. The typical powder XRD pattern of the as-prepared ultrathin g-C_3N_4 nanosheets.

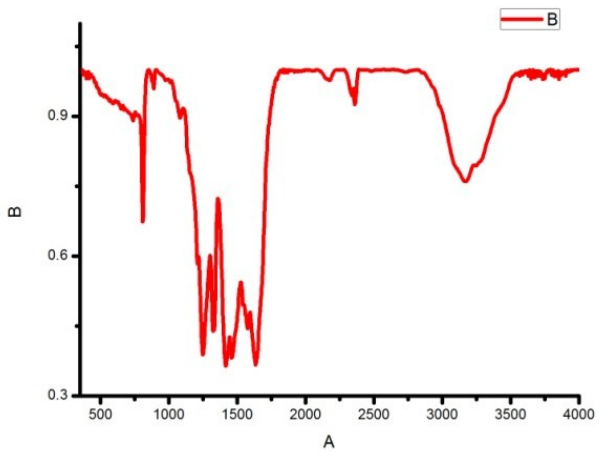

Fig. S2. FT-IR spectrum of the g-C_3N_4 nanosheets. The peak at 811 cm^{-1} was attributed to the vibration of the triazine ring. The peaks around 1000 and 1800 cm^{-1} represented the stretching modes of CN heterocycles (C−N(−C)−C and C−NH−C). The peaks between 3000 and 3600 cm^{-1} corresponded to N−H and O−H stretching.

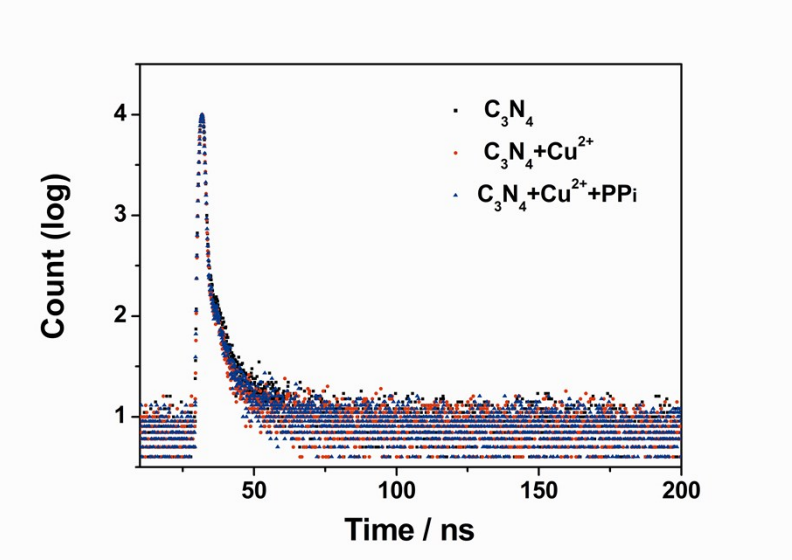

Fig. S3. Fluorescence lifetime decay curves of g-C_3N_4 (black), and g-C_3N_4 + Cu^{2+} (red) and g-C_3N_4 + Cu^{2+} + PPi (blue), respectively

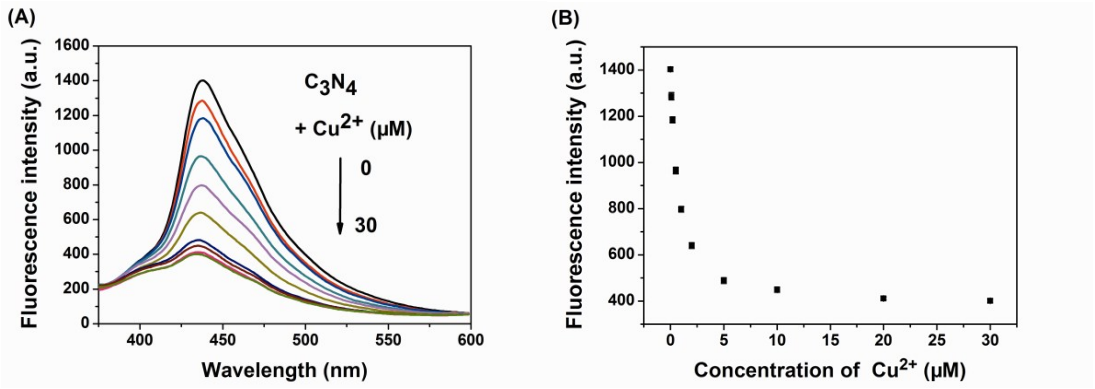

Fig. S4. The optimized concentration of Cu^{2+}. (A) Fluorescence emission spectra of the g-C_3N_4 with addition of different Cu^{2+} concentrations increasing from 0 to 30 μM (top to bottom). (B) The fluorescence intensity for g-C_3N_4 versus the Cu^{2+} concentrations. Measurements were performed by using g-C_3N_4 solution (final concentration of 5 μg mL^{-1}) in 10 mM HEPES buffer (pH 7.4, 5 mM $MgCl_2$) at 37 °C. Excitation: 310 nm and emission: 440 nm.

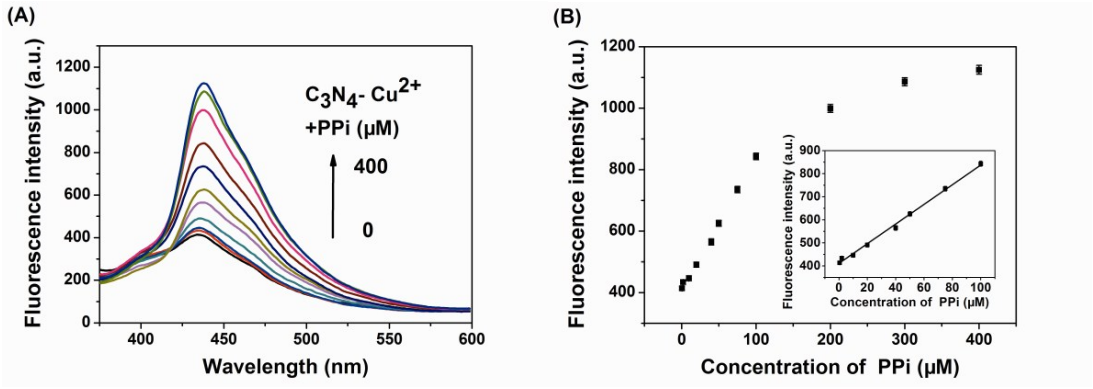

Fig. S5. The optimized concentration of PPi. (A)Fluorescence emission spectra of the g-C_3N_4-Cu^{2+} complexes with addition of different PPi concentrations increasing from 0 to 400 μM (bottom to top). (B) The fluorescence intensity for g-C_3N_4-Cu^{2+} versus the PPi concentrations. Inset: expanded linear region. Measurements were performed by using g-C_3N_4 solution (5 μg mL^{-1}) in 10 mM HEPES buffer (pH 7.4) containing Cu^{2+} (20 μM), and Mg^{2+} (5 mM) at 37 °C. Excitation: 310 nm and emission: 440 nm.

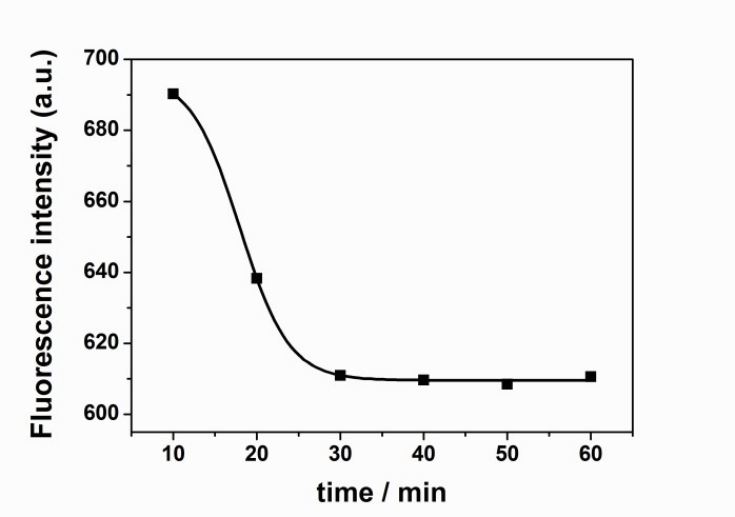

Fig. S6. The optimization of the reaction time of ALP and PPi. Measurements were performed by using g-C_3N_4 solution (5 μg mL^{-1}) in 10 mM HEPES buffer (pH 7.4) containing Cu^{2+} (20 μM), PPi (100 μM), ALP (2000 U/L) and Mg^{2+}(5 mM) at 37 °C.

Table S1. Fluorescence lifetimes obtained with biexponential fitting of the fluorescence decay curves of g-C_3N_4, g-C_3N_4-Cu^{2+} complex and the mixture of g-C_3N_4 + Cu^{2+} + PPi.

Samples	τ_1/ns(%)	τ_2/ns(%)
g-C_3N_4	3.30 (5.75)	13.22 (94.25)
g-C_3N_4 + Cu^{2+}	3.16 (3.62)	12.64 (96.38)
g-C_3N_4 + Cu^{2+} + PPi	3.10 (4.12)	12.42 (95.88)

Table S2. Comparison of analytical performance of some assays for ALP detection.

Analysis methods	Detection limit	Linear range	*Ref.*
Fmoc-K(FITC)FFYp	0.06 U/mL	0– 2.8 U/mL	*S1*
Coumarin@Tb-GMP ICPs	10 U/L	25 – 200 U/L	*S2*
ESIPT / AIE	0.15 U/L	0 –150 U/L	*S3*
CdTe QDs	10 U/L	0 – 800 U/L	*S4*
Tb-GMP	0.001 U/L	0.001 – 200 U/L	*S5*
CQDs-Ce^{3+}/ATP	1.4 U/L	4.6 – 383.3 U/L	*S6*
DNA-CuNPs	0.3 U/L	0.3 – 7.5 U/L	*S7*
AuNP-ATP/Pb^{2+}	0.1 U/L	0.2 – 20 U/L	*S8*
ECL/CdSe NPs	2 U/L	2 –25 U/L	*S9*
Electrochemstry	0.1 U/mL	1 – 20 U/mL	*S10*
C3N4 /Cu2+ /PPi	0.08 U/L	0.10– 1000 U/L	*This work*

References

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Table S3. Recovery experiments of ALP in human serum samples.

Samples	Added ALP (U/L)	Detected ALP [a] (U/L)	Recovery (%)
1	0.10	0.101±0. 005	101
2	1.00	0.932±0. 007	93.2
3	10.00	9.15±0. 06	91.5
4	100.00	94.4±0. 5	94.4

[a]Average of three determinations ± standard deviation