Supporting Information *for*

Fabrication of Stable and Luminescent Copper Nanocluster -Based AIE Particles and Their Application in β-Galactosidase Activity Assay

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detection limit is estimated to be 0.9 U/L.

12. **Figure S12.** (A) Luminescence spectra of the mixture containing CuNC AIE particles and NPGal (200.0 μM) at varying β-galactosidase levels from 0.0 to 336.0 U/L. (B) Luminescence intensity of CuNC AIE particles vs β-galactosidase activity. The detection limit is estimated to be 1.1 U/L.
13. **Figure S13.** Specificity test of β-Gal assay using standard assay solution in the presence of different interferents.
14. **Table S1.** Comparison of luminescent β-galactosidase assay in detection limit and linear scope using different concentration of substrates.
15. **Table S2.** Comparison of luminescent β-Gal assays in analytical performance between our assay and previously reported assays.
16. **Table S3.** Data for standard addition experiments of β-Gal assay using human serum as the matrix.

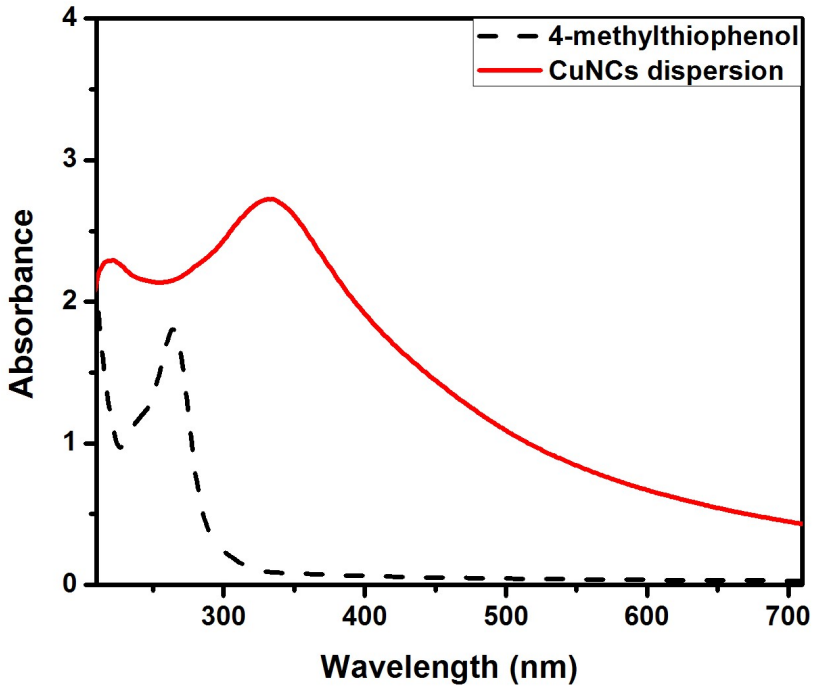

Figure S1. UV-visible spectra of CuNCs dispersion and 4-methylthiophenol.

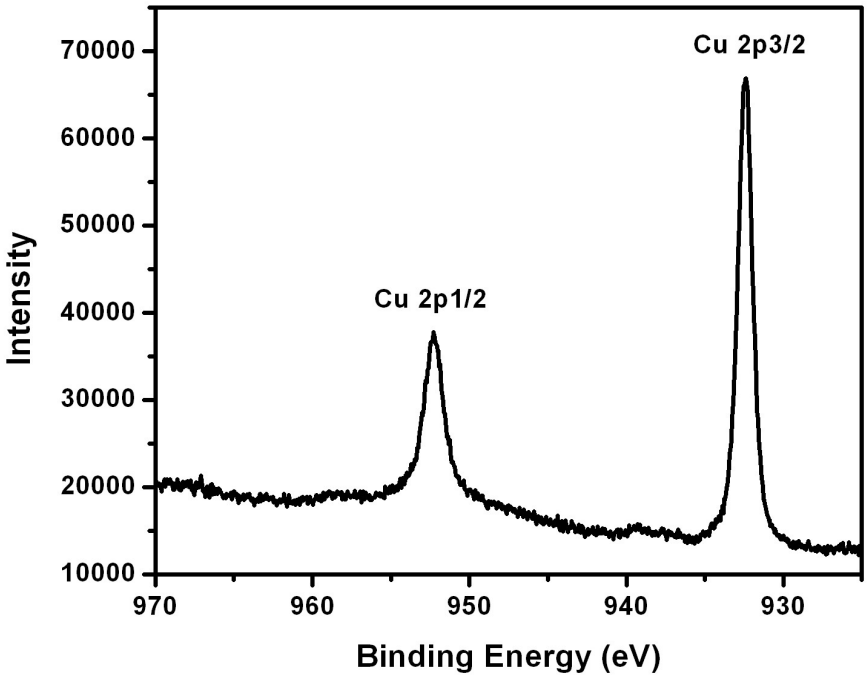

Figure S2. High-resolution X-ray photoelectron spectrum of Cu 2p electrons in CuNCs.

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 242.5	Peak 1:	254.5	100.0	75.41
PdI: 0.147	Peak 2:	0.000	0.0	0.000
Intercept: 0.962	Peak 3:	0.000	0.0	0.000
Result quality : Good				

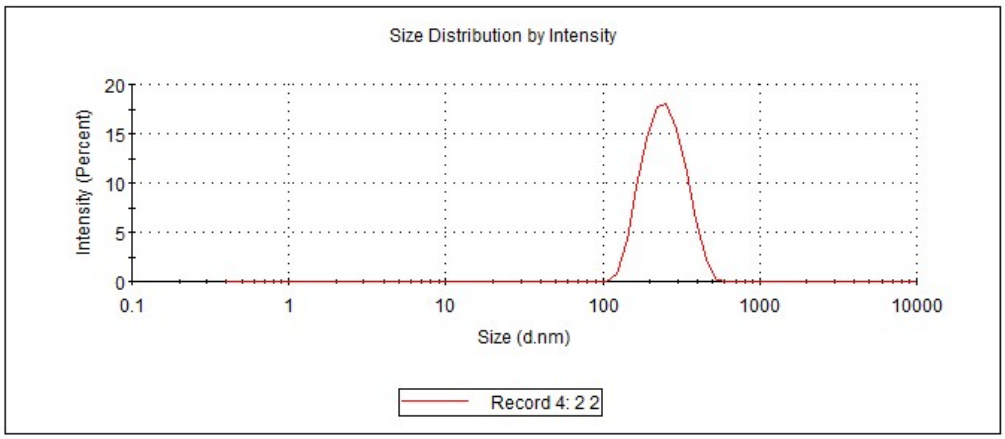

Figure S3. Dynamic light scattering size distribution of CuNC AIE particles (0.08 mg/mL) in aqueous solution.

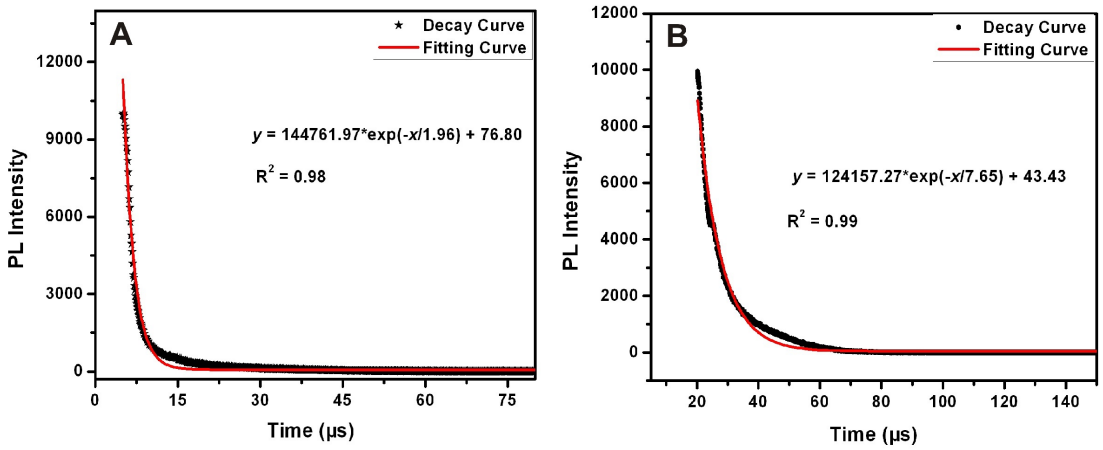

Figure S4. (A) Time-resolved decay curve of CuNC AIE particles (0.08 mg/mL) in water. (B) Time-resolved decay curve of the solid powder of CuNC AIE particles.

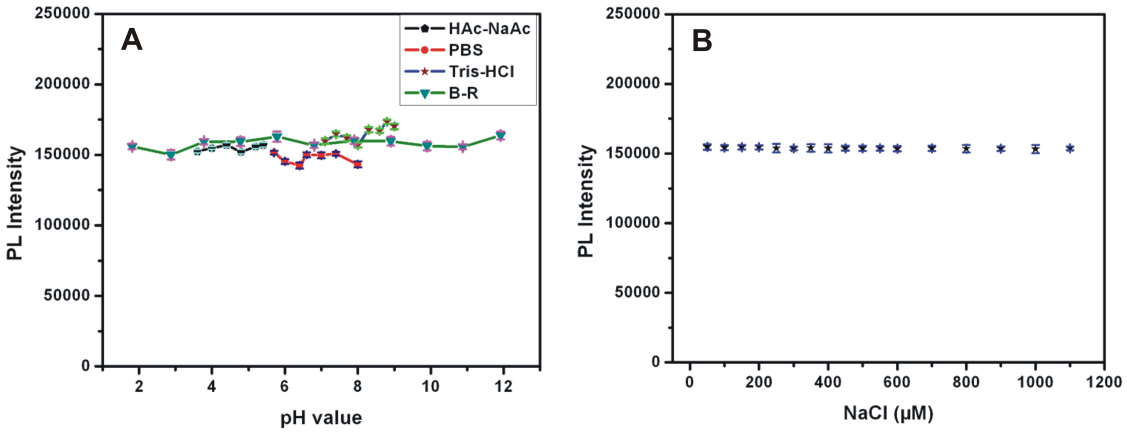

Figure S5. (A) The luminescence intensity of CuNC AIE particles (0.08 mg/mL) in various buffers at the appropriate pH values. (B) Effect of ionic strengths regulated by different concentration of NaCl in the range from 0.0 to 1.1 mM.

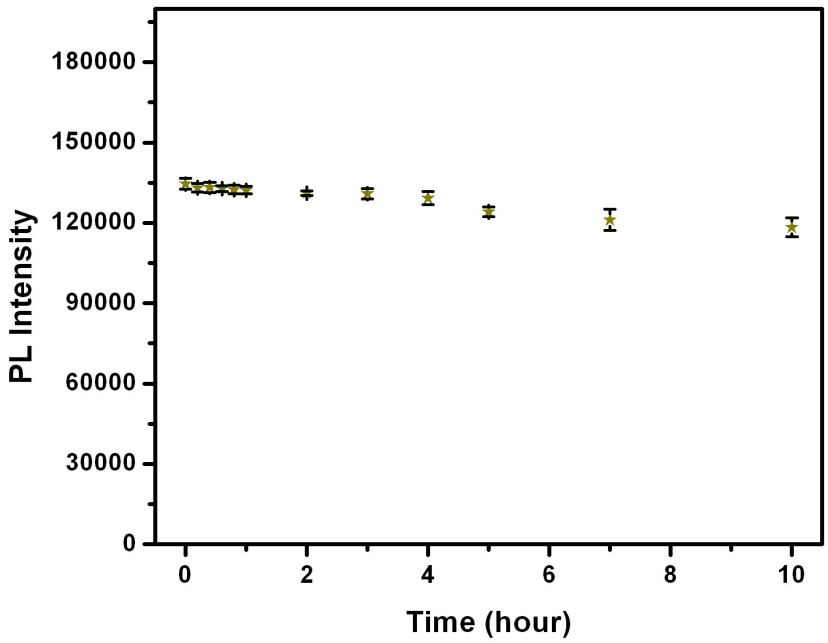

Figure S6. Change in luminescence of CuNC AIE particles (0.08 mg/mL) versus standing time from 0 to 10 h under a UV lamp.

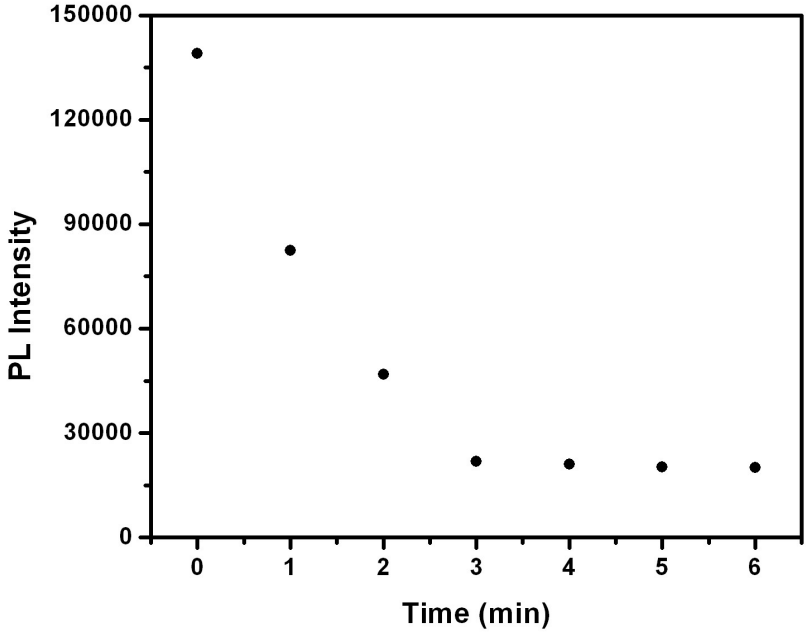

Figure S7. Luminescence quenching efficiency vs incubation time of CuNCs AIE particles (0.08 mg/mL) by 4-nitrophenol (100.0 μM).

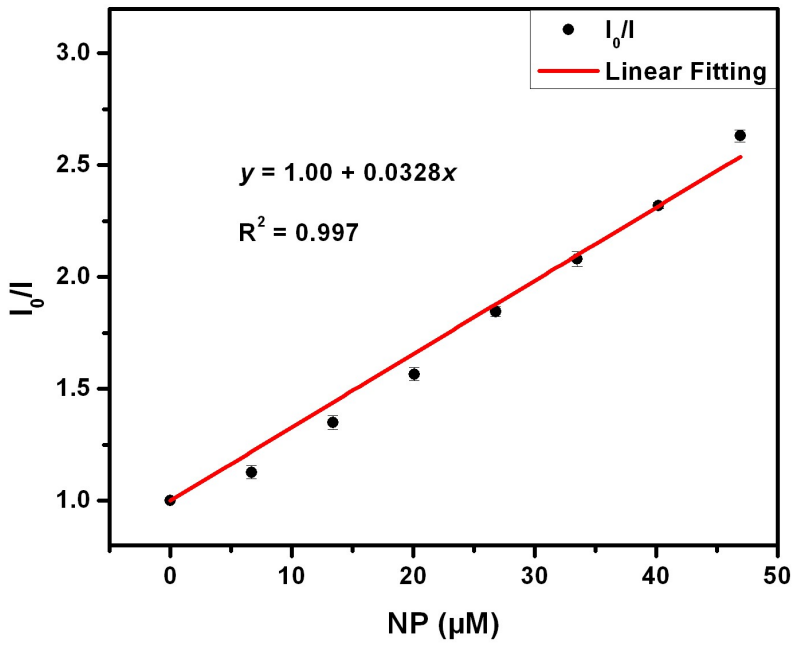

Figure S8. The value (I_0/I) vs the concentration of 4-nitrophenol in the range of 0.0 to 46.0 μM. Linear fitting curve accords to Stern-Volmer equation.

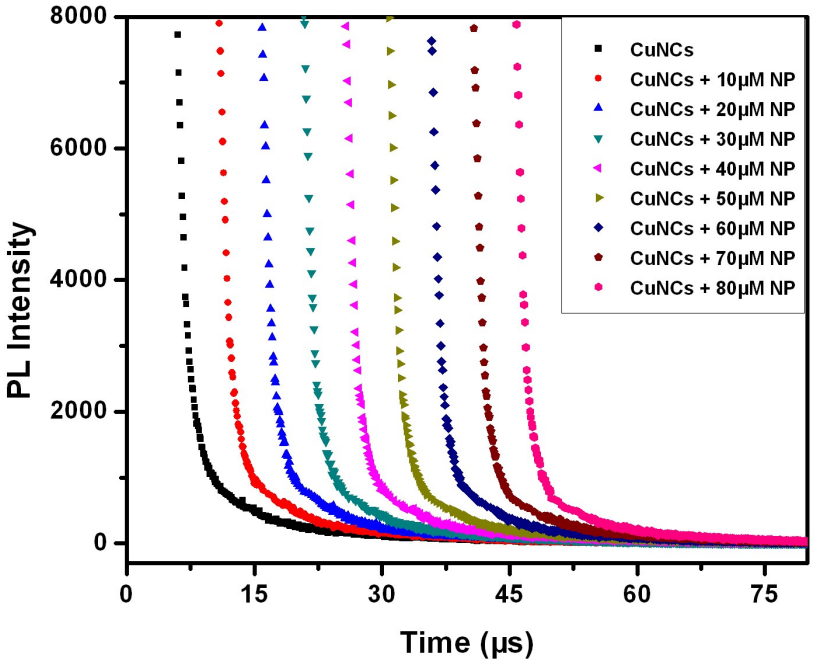

Figure S9. Time-resolved luminescence decay curves of CuNC AIE particles solution (pH 7.0) in the presence of different concentrations of 4-nitrophenol from 0.0 to 80.0 μM. The estimated lifetimes are almost identical to 1.96 μs.

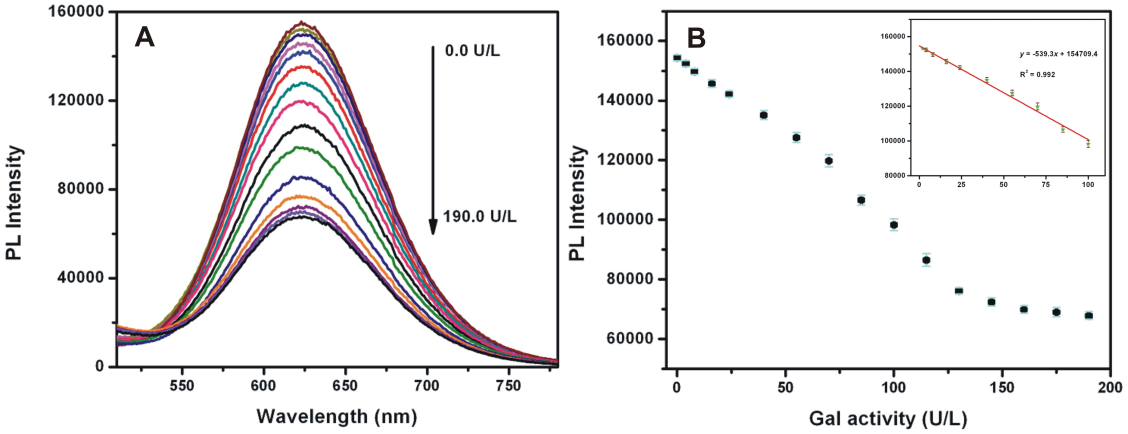

Figure S10. (A) Luminescence spectra of the mixture containing CuNC AIE particles (0.08 mg/mL) and NPGal (50.0 μM) at different β-galactosidase levels from 0.0 to 190.0 U/L. (B) Luminescence intensity of CuNC AIE particles vs β-galactosidase activity. The detection limit is estimated to be 0.9 U/L. All experiments were conducted at 37 °C and in PBS buffer solution (pH 7.0).

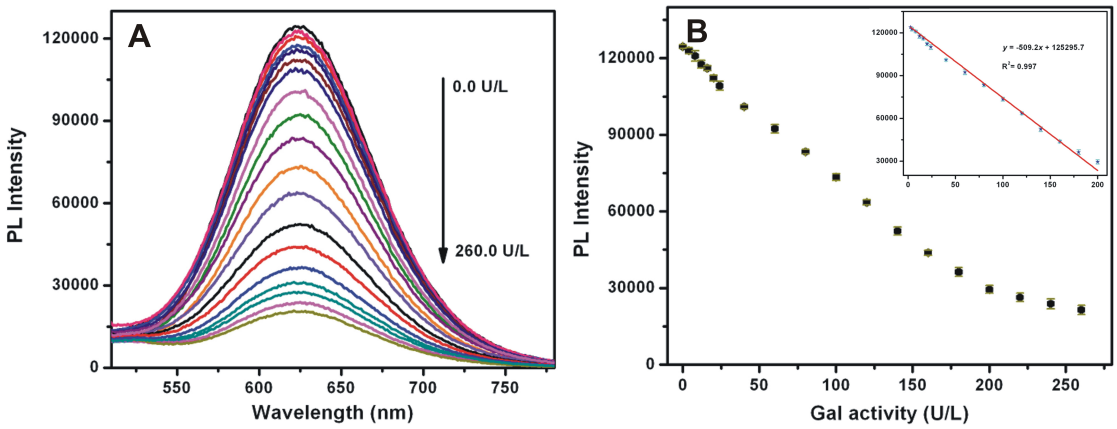

Figure S11. (A) Luminescence spectra of the mixture containing CuNC AIE particles (0.08 mg/mL) and NPGal (100.0 μM) at varying β-galactosidase levels from 0.0 to 260.0 U/L. (B) Luminescence intensity of CuNC AIE particles vs β-galactosidase activity. The detection limit is estimated to be 0.9 U/L. All experiments were conducted at 37 °C and in PBS buffer solution (pH 7.0).

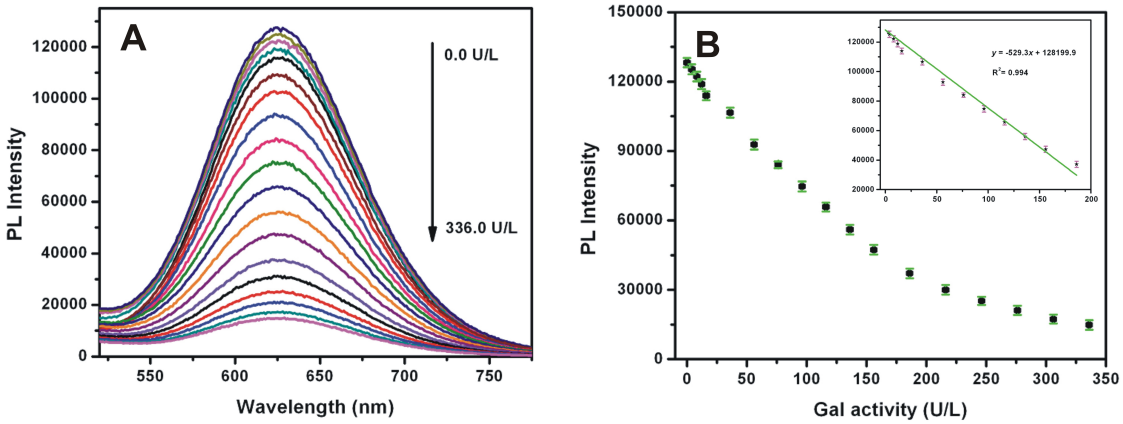

Figure S12. (A) Luminescence spectra of the mixture containing CuNC AIE particles (0.08 mg/mL) and NPGal (200.0 μM) at varying β-galactosidase levels from 0.0 to 336.0 U/L. (B) Luminescence intensity of CuNC AIE dots vs β-galactosidase activity. The detection limit is estimated to be 1.1 U/L. All experiments were conducted at 37 °C and in PBS buffer solution (pH 7.0).

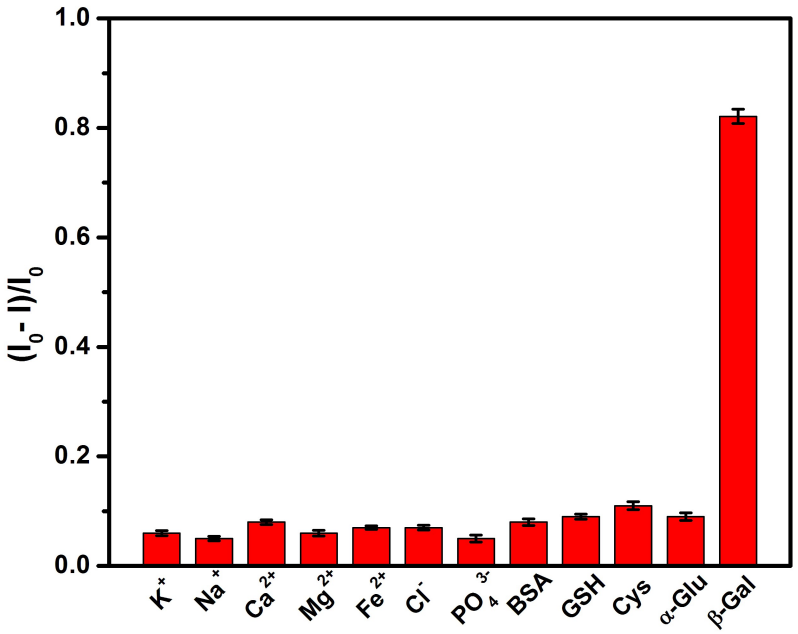

Figure S13. Specificity test of β-gal assay using standard assay solution in the presence of different interferents. The assay solution contains CuNC AIE particles (0.08 mg/mL) and NPGal (150.0 μM). The concentrations of inorganic ions, glutathione (GSH) and cysteine (Cys) are 1.0 mM, the amount of serum albumin is 4.0 mg/L, and the activity of α-glucosidase and β-galactosidase is 322.0 U/L respectively.

Table S1. Comparison of luminescent β-galactosidase assay in detection limit and linear scope using different concentration of substrates.

NPGal (μM)	Linear scope (U/L)	Detection limit (U/L)
50.0	2.1 - 100.0	0.9
100.0	2.7 - 200.0	0.9
150.0	2.5 - 212.0	0.9
200.0	2.9 - 186.0	1.1

Table S2. Comparison of luminescent β-Gal assays in analytical performance.

Luminogens	Linear scope	Detection limit	Refs
CMFβ-gal	<11.6 (μg/mL)	5.0 (nM)	24
AuNPs	< 15.0 (μM)	9.2 (nM)	26
Gold nanorods	0.1 -10.0 (nM)	0.13 (nM)	27
carbon quantum dots	1.9 - 70.0 (U/L)	0.6 (U/L)	28
salicylaldehyde azines	< 100 (U/L)	14.0 (U/L)	38
MHQ-Gal	5.0 (U/L)	100.0 - 3200.0 (U/L)	39
CuNC AIE particles	0.9 (U/L)	2.5 - 212.0 (U/L)	This work

Table S3. Data for standard addition experiments of β-Gal assay using human serum as the matrix.

Sample Number	Added β-Gal (U/L)	Measured β-Gal (U/L)	Recovery Ratio (%)	SD (n = 3)	RSD (n = 3, %)
1	10.0	10.2	102.0	0.58	0.57
2	20.0	20.3	101.5	0.77	0.76
3	30.0	30.8	102.7	0.51	0.50
4	40.0	41.5	103.8	1.37	1.32
5	50.0	51.7	103.4	0.95	0.92
6	60.0	60.7	101.2	0.06	0.06
7	70.0	71.4	102.0	0.76	0.75
8	80.0	81.3	101.6	0.26	0.25