Supporting Information

Automated Study on Kinetics and Biosensing of Glow-Type Luminescence Reaction via Digital Microfluidic-Chemiluminescence

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1. Reagents and Apparatus

Luminol, L-Cysteine (Cys) and glucose oxidase (GOD) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). PBS powder was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Copper sulphate (II) pentahydrate, cobalt chloride hexahydrate and glucose was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-Histidine (His) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Superoxide dismutase (SOD) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Teflon AF was obtained from Dupont (China). Fluorinert FC-40 was obtained from 3M (USA). All the other reagents were analytical grade and used directly without further treatments. PBS (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4) was used for the dilution of Cu/Cys and H_2O_2. Ultrapure water was utilized throughout the experiments.

The CL spectrum was measured with a Fluoromax-4 spectrofluorometer (Horiba, USA). The contrast CL kinetics curve was recorded using commercial IFFL-D CL analyser (Xi'an Ruimai Electronic Sci. Tech. Co., Ltd., China). Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) images were taken by a SU8220 field emission scanning electron microscopy (Hitachi, Japan). Transmission electron microscopy (TEM) image was carried out using a Tecnai G2 F20 electron microscope (FEI, America). Fourier transform infrared (FT-IR) spectra were measured on a Tensor Spectrophotometer (Bruker, Germany) in the 500-4000 cm^{-1} region. X-ray photoelectron spectra (XPS) were performed using an XPS spectrometer (AXIS ULTRA, Kratos Analytical Ltd., U.K.). Electron paramagnetic resonance (EPR) spectra were recorded by JES FA200 spectrometer (JEOL Ltd., Tokyo, Japan). A D8 Advance diffractometer (Bruker, Germany) was employed to record X-ray diffraction (XRD) patterns.

2. Repeatability of 11 parallel CL measurements

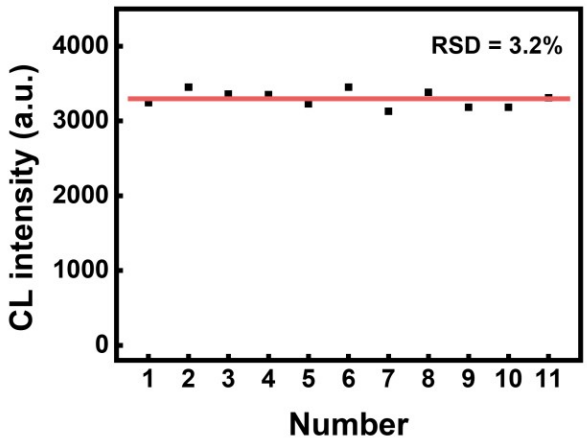

Figure S1. 11 parallel CL measurements.

3. Areas uniformity of asymmetrical splitting

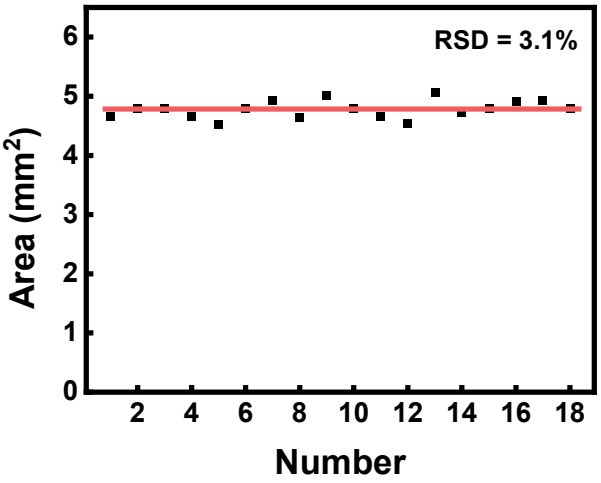

Figure S2. Areas uniformity of 18 droplets obtained from asymmetrical splitting.

4. Normalized kinetics curves of glow- and flash-type CL reactions

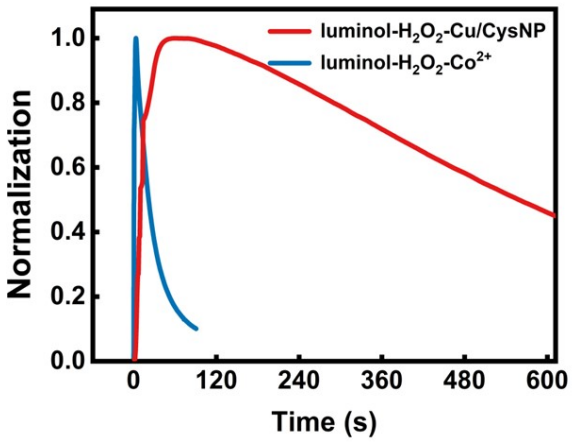

Figure S3. Normalized CL kinetics curves of luminol-H_2O_2-Cu/CysNP and luminol-H_2O_2-Co^{2+}

5. Additional Characterization of Cu/CysNP

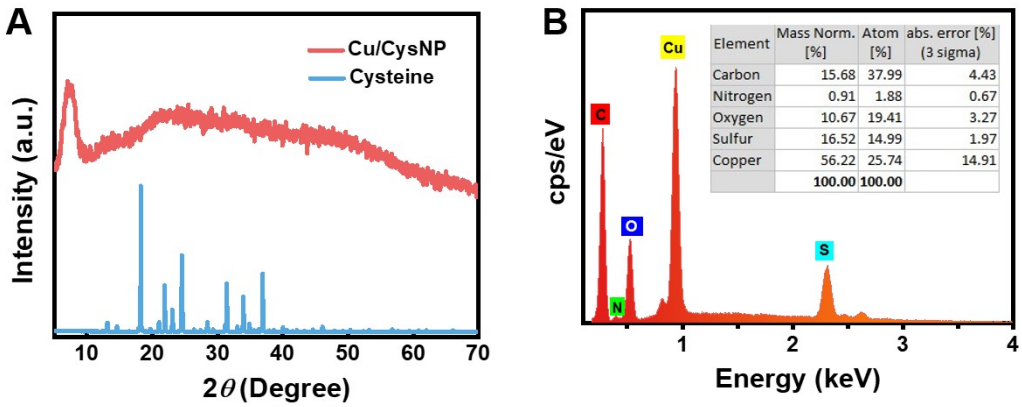

Figure S4. (A) XRD patterns of the Cu-CysNP and cysteine. (B) EDS line scanning and the corresponding atomic fraction percent of Cu/CysNP.

6. UV-vis Spectra of TMB

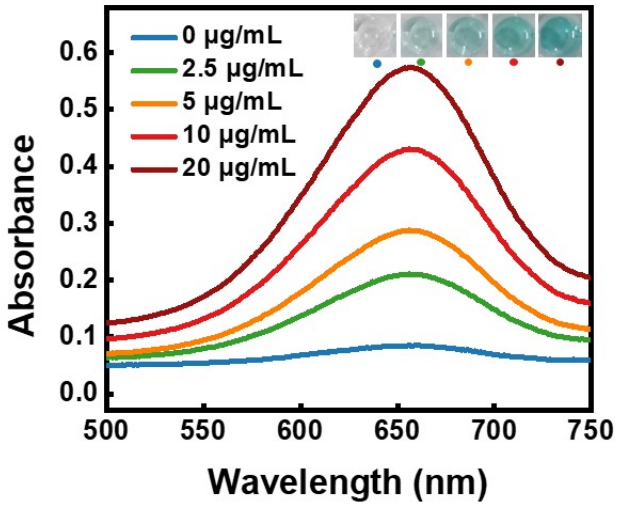

Figure S5. UV-vis Spectra of TMB-H_2O_2 solution in the presence of Cu/CysNP with different concentrations in NaAc-HAc buffer (50 mM, pH 4.5). The concentrations of TMB and H_2O_2 are 1 mM and 2.5 mM, respectively.

7. Optimization of reaction conditions

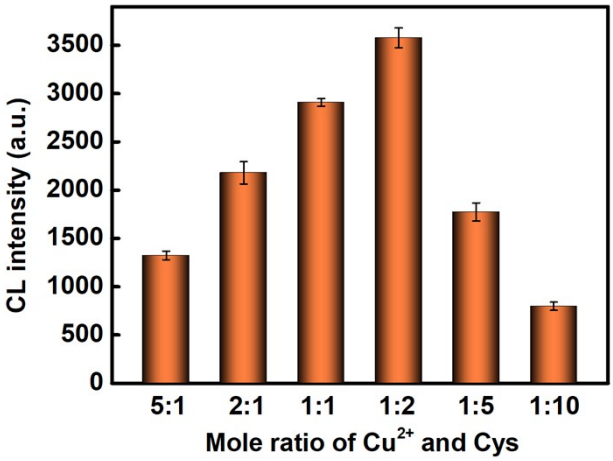

Figure S6. CL intensity of luminol-H_2O_2 catalyzed by different Cu/CysNP. Experimental conditions: 0.1 mM luminol, 0.3 mM H_2O_2, 4 mM NaOH, 5 μg/mL Cu/CysNP.

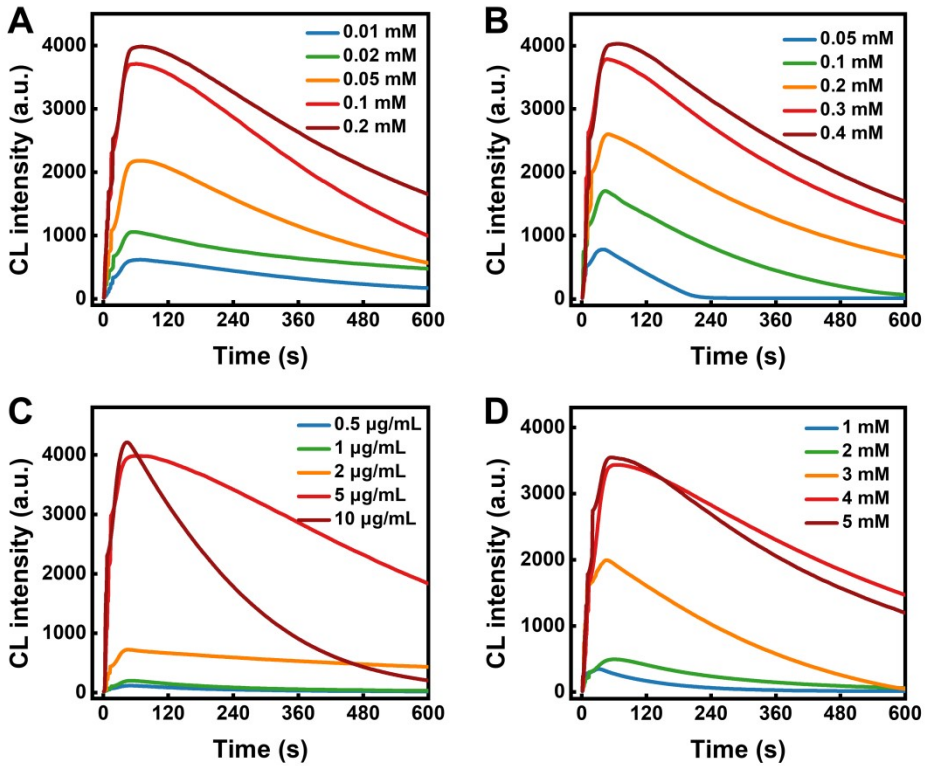

Figure S7. Effect of (A) luminol, (B) H_2O_2, (C) Cu/CysNP and (D) NaOH concentrations on the CL intensity of the luminol-H_2O_2-Cu/CysNP reaction.

8. Linear relationship curve and selectivity result for detection of H_2O_2

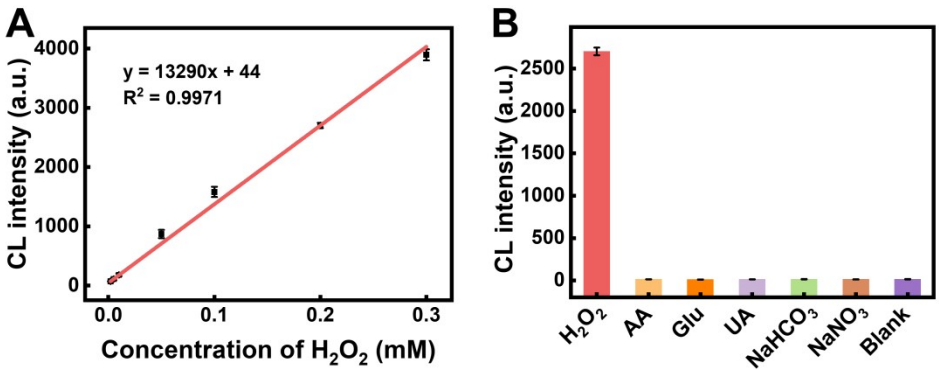

Figure S8. (A) Linear relationship between CL intensity and H_2O_2 concentration. (B) CL responses upon addition of 0.2 mM H_2O_2 and interferences (0.2 mM UA, 2 mM AA, Glu, $NaHCO_3$, and $NaNO_3$).

9. Comparison of the glucose detection performance

Table S1. Comparison of the glucose detection performance with different methods

Method	Sensing material	Linear range (μM)	Detection limit (μM)	Ref.
EC	Cu@C nanocubes	40–40,000	21.35	[1]
	GOD/PtNPs/SiC@C	-	41	[2]
FL	AgNC–GOx/Ag + -FP	50-5000	50	[3]
	APBA/QDs	100-2000	4.5	[4]
	Fe-Phen-CFs	0.5-200	0.19	[5]
CL	GOD/graphene oxide	50–5000	44	[6]
	GOD/g-CNOX	50-500	-	[7]
	GOD/Cu/CysNP	20-500	17	present work

10. Detection of glucose in diluted human serum samples

Table S2. Detection of glucose in diluted human serum samples

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
1	50.0	47.8	95.6	3.7
2	100.0	98.9	98.9	4.2
3	200.0	206.3	103.2	3.2

References

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