



# Plasmonic Cu<sub>2-x</sub>Se Mediated Colorimetric/Photothermal Dual-Readout Detection of Glutathione

Guojuan Yan <sup>1,2</sup>, Huanhuan Ni <sup>2</sup>, Xiaoxiao Li <sup>2</sup>, Xiaolan Qi <sup>1</sup>, Xi Yang <sup>3,\*</sup> and Hongyan Zou <sup>2,\*</sup>

<sup>1</sup> Key Laboratory of Endemic and Ethnic Diseases, Ministry of Education, Guizhou Medical University, Guiyang 550004, China; ygj792989689@email.swu.edu.cn (G.Y.); xiaolan76@163.com (X.Q.)

<sup>2</sup> College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China; nhh1998@email.swu.edu.cn (H.N.); lxx314@email.swu.edu.cn (X.L.)

<sup>3</sup> Department of Basic Medical Science, Guiyang Healthcare Vocational University, Guiyang 550081, China

\* Correspondence: m1890850220@163.com or yangxi@gyhvu.edu.cn (X.Y.); zhy2013@swu.edu.cn (H.Z.)

## Experimental Procedures

### Calculation of the molar extinction coefficient

The molar extinction coefficient of Cu<sub>2-x</sub>Se NPs were calculated from the Beer-Lambert law by linearly fitting the absorbances for various concentrations of nanoparticles,

$$A(\lambda) = \epsilon LC \quad (S1)$$

Where  $A$  is the absorbance at a wavelength  $\lambda$ ,  $\epsilon$  is the molar extinction coefficient,  $L$  is the pathlength (1 cm), and  $C$  is the concentration of nanoparticles (mole L<sup>-1</sup>). The extinction coefficient was determined by plotting the slope of each linear fit against wavelength. The  $C$  (mole L<sup>-1</sup>) of Cu<sub>2-x</sub>Se is calculated using the following:

$$C = \frac{C_{wt}}{M_{NP}} = \frac{C_{wt}}{\frac{4}{3} \pi R^3 \rho_{Cu_{2-x}Se} N_A} \quad (S2)$$

$C_{wt}$  is the weight concentration of the nanoparticles (g L<sup>-1</sup>),  $M_{NP}$  is the molar weight of the nanoparticles (g mol<sup>-1</sup>),  $R$  is the average radius of the nanoparticles (21.3 nm),  $\rho$  is the density of the Cu<sub>2-x</sub>Se (5.8 g cm<sup>-3</sup>),  $N_A$  is Avogadro's constant (6.022\*10<sup>23</sup> mol<sup>-1</sup>). Above all, the molar extinction coefficient is calculated by :

$$\epsilon = \frac{A(\lambda)}{LC} = \frac{A(\lambda) \frac{4}{3} \pi R^3 \rho_{Cu_{2-x}Se} N_A}{LC_{wt}} \quad (S3)$$

### Photothermal activity and photothermal conversion efficiency

In order to evaluate the photothermal performance of Cu<sub>2-x</sub>Se NPs, 200  $\mu$ L Cu<sub>2-x</sub>Se solution with various concentrations (0, 50, 100, 200, 300, 400  $\mu$ g/mL) was exposed to 1064 nm laser (1.0 W/cm<sup>2</sup>, 5min), and the temperature variation were detected by the thermometer (LIHUAJIN, T1320), the infrared thermal images were recorded every 30 s with infrared thermal imager (Fluke Ti400, USA).

The photothermal conversion efficiency ( $\eta$ ) of Cu<sub>2-x</sub>Se was calculated according to equation (2)-(4):

$$\eta = \frac{mc(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{1064}})\tau_s} \quad (S4)$$

$$\tau_s = -\frac{t}{\ln(\theta)} \quad (S5)$$

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (S6)$$

$$Q_{dis} = \frac{mc(T_{max\ water} - T_{surr})}{\tau_{s\ water}} \quad (S7)$$

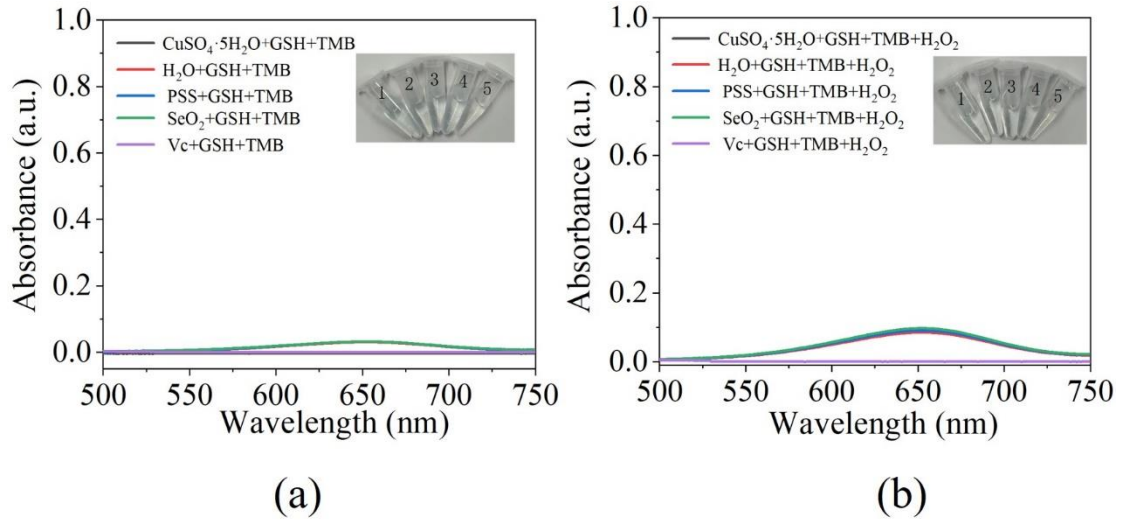
where the  $m$  and  $c$  were the mass (0.2 g) and heat capacity (4.2 J/g) of the water,  $T_{max}$  was the maximum equilibrium temperature of Cu<sub>2-x</sub>Se (55.3 °C),  $T_{surr}$  (23.0 °C) and  $T$  represent the environment and solution temperature,  $I$  was the laser power (0.32 W),  $A_{1064}$  was the absorbance of Cu<sub>2-x</sub>Se solution at 1064 nm as 0.710,  $\tau_s$  was the sample system time constant (232.15),  $Q_{dis}$  represents the heat loss due to absorption by the container,  $T_{max}$  and  $\tau_s$  of water was 35 °C and 220.46.

### Peroxidase-like activity

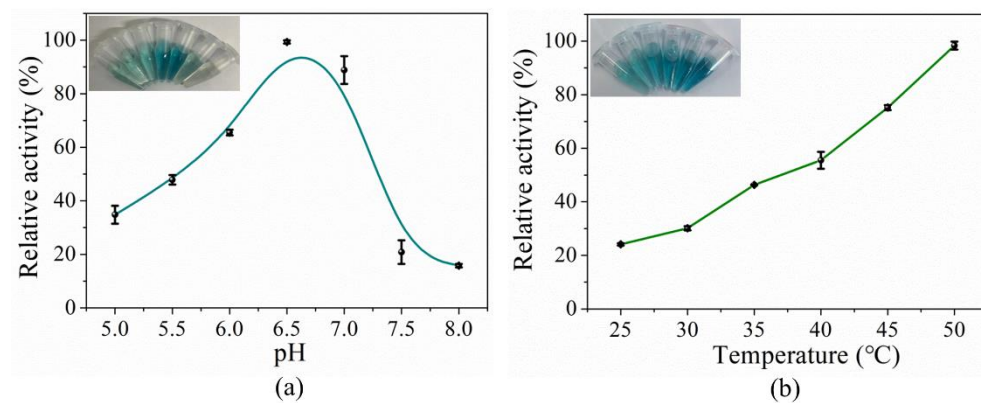
The Peroxidase-like activity assays of Cu<sub>2-x</sub>Se NPs were performed using TMB and H<sub>2</sub>O<sub>2</sub> as substrates. 25 µg/mL Cu<sub>2-x</sub>Se, 7.5 mM H<sub>2</sub>O<sub>2</sub> and different concentrations of TMB (0.75-3.0 mM) were added to PBS solution at pH 6.5, and the change of its absorbance (650 nm) in 60 min was recorded. Similarly, changed the concentration of H<sub>2</sub>O<sub>2</sub> (2.5-7.5 mM) and TMB (1.5 mM), and recorded the change of their absorbance (650 nm) under the same conditions. Enzyme kinetic parameters were calculated by Michaelis-Menten equation:

$$v_0 = \frac{v_{max} [S]}{K_m + [S]} \quad (S8)$$

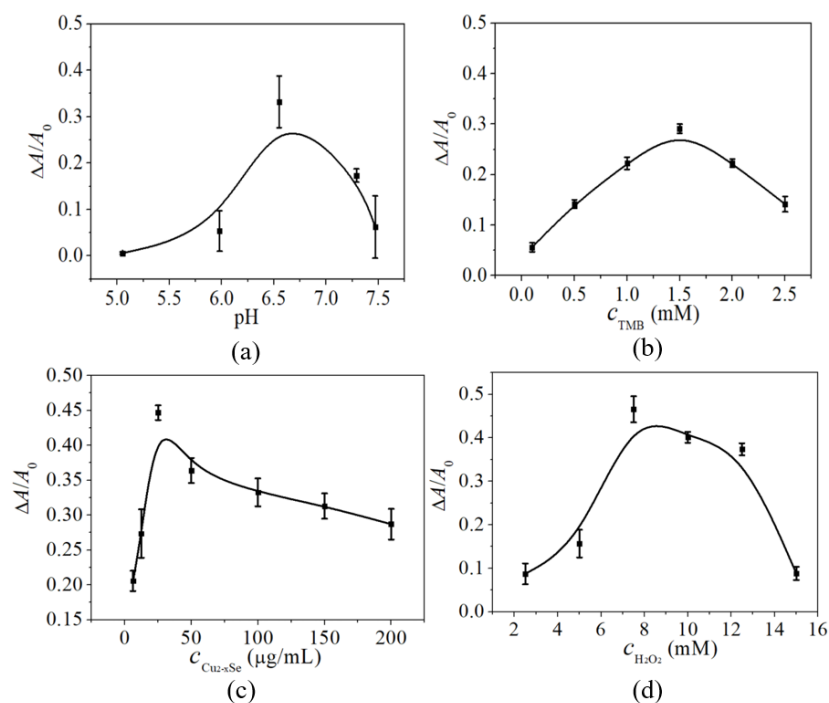
In this equation,  $v_0$  is the initial reaction rate of substrate conversion,  $V_{max}$  is the maximum reaction rate of substrate conversion,  $[S]$  is the substrate concentration, and  $K_m$  is the Michaelis-Menten constant.



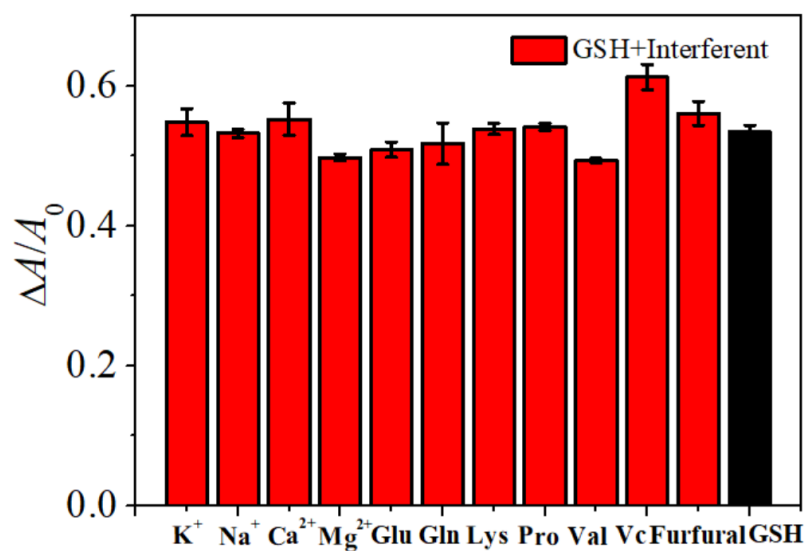
**Figure S1.** (a) The individual components used in the synthesis with glutathione to oxidize the TMB; (b) The individual components used in the synthesis with glutathione to oxidize the TMB with H<sub>2</sub>O<sub>2</sub>.



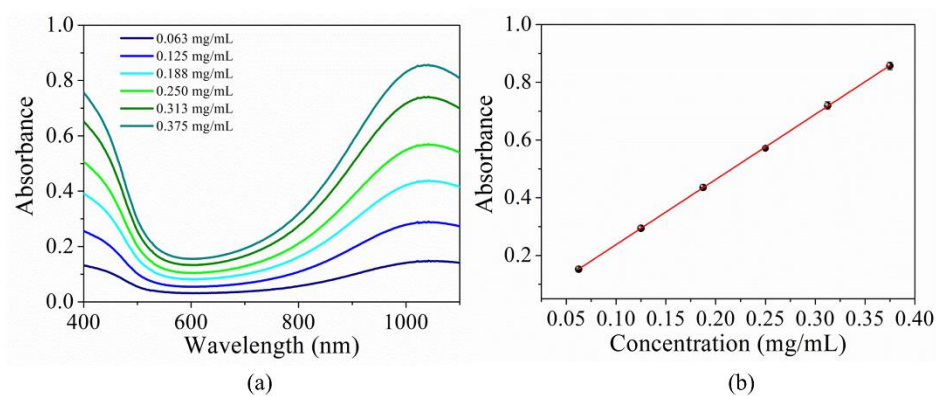
**Figure S2.** (a) The relative activity of  $\text{Cu}_{2-x}\text{Se}$  at different pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and (b) the relative activity of  $\text{Cu}_{2-x}\text{Se}$  at different temperatures (25, 30, 35, 40, 45 and 50  $^{\circ}\text{C}$ ).



**Figure S3.** The changes of the  $\Delta A/A_0$  with (a) different pH (5.0, 6.0, 6.5, 7.4 and 7.5) (b) different concentrations of TMB (0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mM) (c) different concentrations of  $\text{Cu}_{2-x}\text{Se}$  (6.25, 12.5, 25, 50, 100, 150 and 200  $\mu\text{g/mL}$ ) (d) different concentrations of  $\text{H}_2\text{O}_2$  (2.5, 5, 7.5, 10, 12.5 and 15 mM).



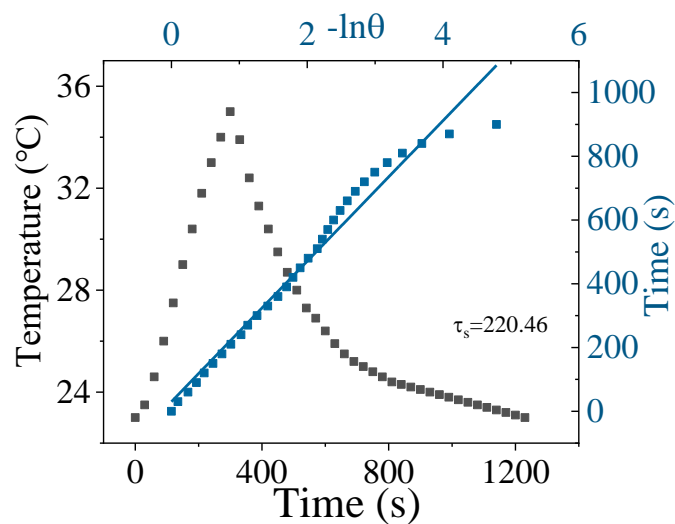
**Figure S4.** Selectivity of the sensing system for the detection of GSH.



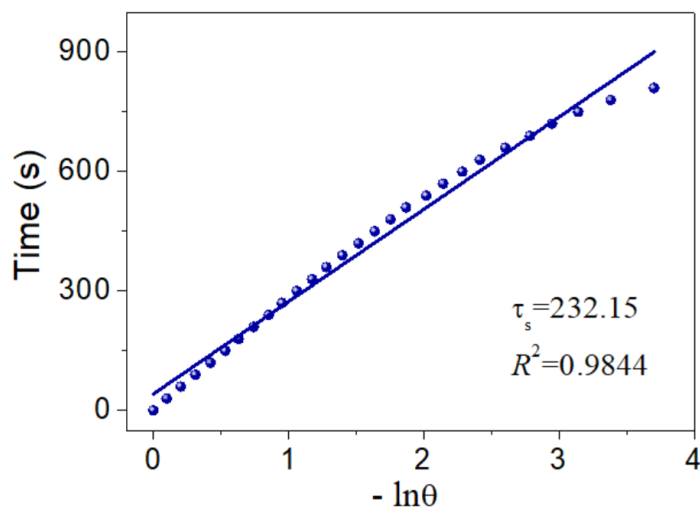
**Figure S5.** (a) UV-Vis absorbance spectra of Cu<sub>2-x</sub>Se measured at different concentration (b) Plots of absorbance vs concentration for Cu<sub>2-x</sub>Se at specific wavelength (1058 nm) with linear regression curve.

**Table S1.** Comparison of the kinetic constants of Cu<sub>2-x</sub>Se with other nanozymes and HRP.

Catalyst	Substrate	$K_m$ (mM)	$V_{max}$ (M S <sup>-1</sup> )	References
Cu <sub>2-x</sub> Se	H <sub>2</sub> O <sub>2</sub>	8.16	$2.67 \times 10^{-8}$	this work
	TMB	2.74	$1.10 \times 10^{-8}$	
HRP	H <sub>2</sub> O <sub>2</sub>	3.7	$8.7 \times 10^{-8}$	[1]
	TMB	0.4376	$1.0 \times 10^{-7}$	
Ag@Fe <sub>3</sub> O <sub>4</sub>	H <sub>2</sub> O <sub>2</sub>	75.2	$2.28 \times 10^{-8}$	[2]
	TMB	3.46	$2.288 \times 10^{-8}$	



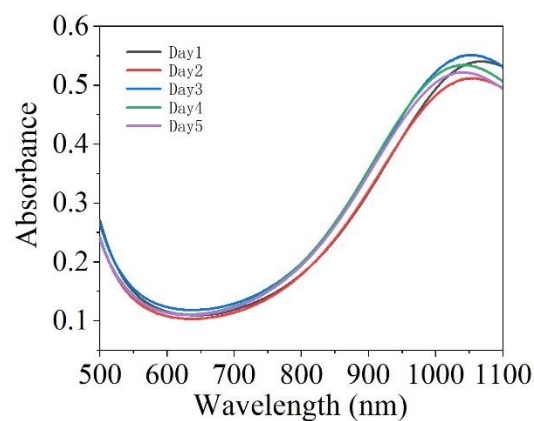
**Figure S6.** Temperature profile of H<sub>2</sub>O under laser irradiation (black line), and linear relationship between  $-\ln\theta$  and time in the cooling stage (blue line).



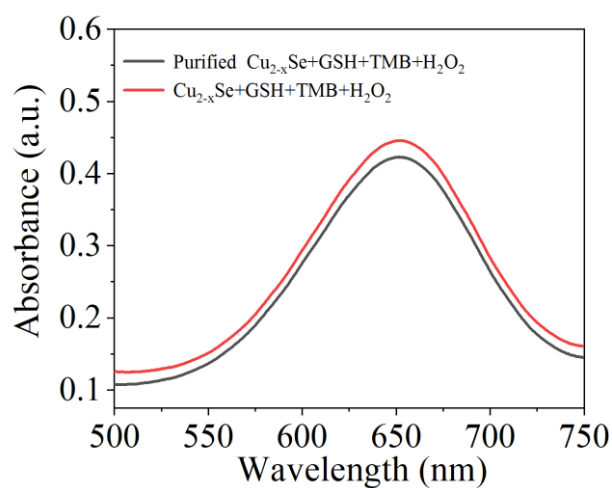
**Figure S7.** The linear relationship between  $-\ln\theta$  and time in the cooling stage of Cu<sub>2-x</sub>Se.

**Table 2.** Molar extinction coefficients (per mol of molecules or nanocrystals) of common photoabsorbers.

Photoabsorber	Dimension (nm)	Molar extinction coefficient (M <sup>-1</sup> cm <sup>-1</sup> )	Wavelength (nm)
Rhodamine 6G [3]	molecular	1.2 X 10 <sup>5</sup>	530
Malachite Green [4]	molecular	1.5 X 10 <sup>5</sup>	617
Carbon nanotubes [5]	$r=0.6$ $L=150$	7.9 X 10 <sup>6</sup>	808
Gold nanospheres [6]	$r=20$	$\sim 7.7 \times 10^9$	530



**Figure S8.** The absorbance of  $\text{Cu}_{2-x}\text{Se}$  in 5 days.



**Figure S9.** The absorbance of GSH pretreated  $\text{Cu}_{2-x}\text{Se-H}_2\text{O}_2\text{-TMB}$  and  $\text{Cu}_{2-x}\text{Se-H}_2\text{O}_2\text{-TMB}$  system.

## References

1. B. Jiang, D. Duan, L. Gao, et al. Standardized assays for determining the catalytic activity and kinetics of peroxidase-like nanozymes[J]. *Nat. Protoc.*, 2018, 13(7): 1506-1520.
2. J. Chen, Y. Liu, G. Zhu, et al.  $\text{Ag@Fe}_3\text{O}_4$  nanowire: fabrication, characterization and peroxidase-like activity[J]. *Crystal Research and Technology*, 2014, 49(5): 309-314.
3. Du, H.; Fuh, R. C. A.; Li, J. Z.; Corkan, L. A.; Lindsey, J. S. *Photochem. Photobiol.* 1998, 68, 141-142.
4. Yu, W. W.; Qu, L. H.; Guo, W. Z.; Peng, X. G. *Chem. Mater.* 2003, 15, 2854-2860.
5. Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. J. *Proc. Natl. Acad. Sci. U.S.A.* 2005, 102, 11600-11605..
6. Jain, P. K.; Lee, K. S.; El-Sayed, I. H.; El-Sayed, M. A. *J. Phys. Chem. B* 2006, 110, 7238-7248.