Journal Name

ARTICLE

Supporting Information

The Release and Detection of Copper Ions from Ultrasmall Theranostic $Cu_{2-x}Se$ Nanoparticles

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Supplementary Figures

Figure S1. TEM image, XRD, UV-Vis-NIR spectra, hydrodynamic size, concentration dependent absorbance at 980 nm, and TGA curves of $Cu_{2-x}Se$ NPs.

Figure S2. XPS spectra of copper element in $Cu_{2-x}Se$ NPs oxidized by air at 37 °C and collected at day 0, day 5, day 10, day 15, and day 25, demonstrating the gradual increase of Cu^{2+}/Cu^{+} ratio.

Figure S3. TEM images and corresponding particle size distributions of $Cu_{2-x}Se$ NPs collected at various days during the oxidation.

Figure S4. Liquid Chromatography-mass spectrometry (LCMS) analysis of probe NCM.

Figure S5. The ^{1}H NMR spectrum of probe NCM.

Figure S6. The ^{13}C NMR spectrum of probe NCM.

Figure S7. The UV-Vis-NIR spectra of $Cu_{2-x}Se$ NPs after stirred with probe NCM or NCM-1, in comparison with that of unmodified $Cu_{2-x}Se$ NPs.

Figure S8. Mean fluorescence intensity of macrophages analyzed by flow cytometry, after they were cultured with the $Cu_{2-x}Se$-NCM NPs for different time.

Figure S9. Cu contents in feces and urine of BALB/c female mice after they were intravenously injected with $Cu_{2-x}Se$ NPs (dose: 10 mg/kg).

Figure S10. (a) The fluorescence images of major organs collected at different time points after the mice were treated with pure NCM and $Cu_{2-x}Se$-NCM NPs with the same NCM concentration. (b) Relative fluorescence intensity of liver at different time points.

Figure S11. Liquid Chromatography-mass spectrometry (LCMS) analysis report of NCM-1. (a) LC result from Evaporative Light Scattering Detector (ELSD). (b) mass spectrum of the peak at 2.334 min.

Figure S12. The ^{1}H NMR spectrum of compound NCM-1.

Figure S13. The ^{13}C NMR spectrum of compound NCM-1.

Table S1. Compositions of nanoparticles determined by inductively coupled plasma mass spectrometry (ICP-MS).

Table S2. Pharmacokinetic parameters for $Cu_{2-x}Se$ NPs after intravenous injection into female BALB/c mice.[a]

Synthesis of NCM.

NCM was synthesized according to previous publications. [1-6]

1-ethyl-2,3,3-trimethyl-3H-indol-1-ium iodide (1). A mixture of 2,3,3-trimethyl-3H-indole (2.55 g, 16 mmol) and ethyl iodide (4.68 g, 48 mmol) was stirred at 85 °C in 38 mL acetonitrile for 48 h. After cooling to room temperature, the solvent was evaporated then washed with cold acetone to give compounds 1 as pink crystals (4.12 g, 81.7%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78 – 7.68 (m, 1H), 7.61 – 7.52 (m, 3H), 4.76 (q, *J* = 7.5 Hz, 2H), 3.14 (s, 3H), 1.64 (s, 6H), 1.62 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 195.41, 141.68, 140.58, 130.16, 129.58, 123.36, 115.31, 77.24, 77.03, 76.82, 54.64, 45.44, 23.09, 16.86, 13.52.

(E)-1-ethyl-3,3-dimethyl-2-(3-(phenylamino)prop-1-en-1-yl)-3H-indol-1-ium iodide (2). A mixture of N,N'-bis-phenylformamidine (1050.6 mg, 5 mmol), compound 1 (1575.3 mg, 5 mmol) and triethyl orthoformate (820 μL, 5 mmol) in ethanol (3 mL) was heated under reflux for 2 h. Then the solution gradually turned into deep red color. The mixture was cooled to room temperature and then frozen by ice, the product was isolated by filtration under reduced pressure, recrystallized by acetone and ether to give compound 2 as yellow crystals (1.3 g, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (d, *J* = 12.4 Hz, 1H), 7.49 – 7.46 (m, 2H), 7.46 – 7.42 (m, 2H), 7.42 – 7.39 (m, 1H), 7.30 (dd, *J* = 13.3, 5.8 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 4.17 (dd, *J* = 14.5, 7.2 Hz, 2H), 1.70 (s, 5H), 1.51 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.48, 150.68, 141.20, 140.38, 138.45, 129.95, 129.03, 126.72, 125.87, 122.25, 117.96, 110.90, 91.76, 77.21, 77.00, 76.79, 49.24, 40.15, 29.03, 12.36.

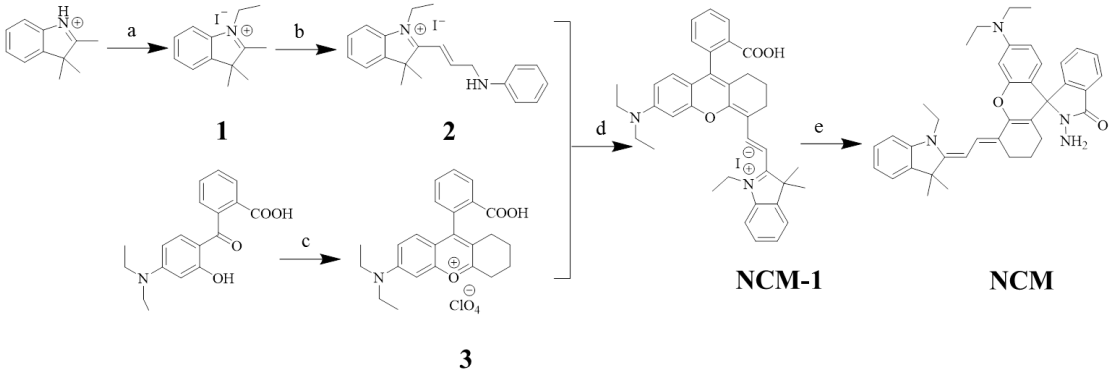
9-(2-carboxyphenyl)-6-(diethylamino)-1,2,3,4-tetrahydroxanthylium perchlorate (3). Cyclohexanone (6.6 mL, 63.7 mmol) was added dropwise into a concentrated H_2SO_4 solution (70 mL) under 0 °C. Then 2-hydroxy-4-(N,N-diethylamino)-(2-carboxy) benzophenone (10 g, 32 mmol) was added in portions with vigorous stirring. The reaction

mixture was stirred at 90 °C for 1.5 h then cooled down, and poured into ice (500 g). 70% $HClO_4$ (7 mL) was slowly added into the solution. The precipitate was filtered and washed with cold water (100 mL), and recrystallized by ethyl acetate to give compounds 3 as yellow/orange crystals (7.63 g, 52.70%).^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.76 (t, *J* = 6.4 Hz, 1H), 7.68 (t, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.1 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.05 (d, *J* = 8.8 Hz, 1H), 6.87 (s, 1H), 3.61 (d, *J* = 6.2 Hz, 4H), 3.18 (m, 1H), 3.09 (m, 1H), 2.32 (m, 2H), 1.79 (m, 2H), 1.33 (s, 6H).

NCM-1 (4). Compound 2 (95.1 mg, 0.22 mmol), compound 3 (99.8 mg, 0.21 mmol) and NaOAC (1.23 g, 15 mmol) were added into freshly distilled acetic anhydride respectively, and the solution was stirred at 50 °C for 30 min. The solution was evaporated under reduced pressure to give crude product, which was purified by silica gel chromatography and by using pure ethyl acetate to CH_2Cl_2 : EtOH (20:1) as an eluent to afford the product. The compound 4 was isolated as a green solid (86.8 mg, 59.2%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.51 (d, *J* = 13.8 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz 1H), 7.50 (t, *J* = 7.2 Hz 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.30 (t, *J* = 7.2 Hz 1H), 7.16 (t, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 6.6 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.52 (s, 1H), 5.98 (d, *J* = 12.6 Hz, 1H), 4.08 (d, *J* = 6.0 Hz, 2H), 3.61 (dd, *J* = 13.8, 7.2 Hz, 2H), 3.44 (d, *J* = 6.6 Hz, 2H), 2.57 (s, 2H), 2.19 (s, 2H), 1.71 (s, 6H), 138 (t, *J* = 6.6 Hz 2H), 1.17 (t, *J* = 6.6 Hz 6H), 1.10 (t, *J* = 6.6 Hz, 1H).^{13}C NMR (151 MHz, $CDCl_3$) δ = 172.10, 168.57, 163.14, 155.83, 152.24, 142.01, 141.77, 140.88, 136.02, 133.30, 131.71, 129.37, 129.31, 128.73, 128.21, 125.10, 122.43, 115.63, 112.27, 110.38, 98.75, 95.82, 58.36, 49.28, 45.25, 39.46, 29.65, 28.46, 26.56, 24.20, 20.42, 18.09, 12.45, 12.14. ESIMS(+): calculated for $C_{38}H_{42}N_4O_2$ $[M]^+$ m/z 573.31; found 573.25.

NCM (5). To a solution of compound 4 (172.0 mg, 0.3 mmol) in 1,2-dichloromethane (10 mL), PyBOP (156.1 mg, 0.3 mmol) and hydrazine hydrate (150.2 mg, 3 mmol) were added in a 25 mL Schlenk tube, the mixture was stirred under an N_2 atmosphere at room temperature for 3 h and the solvent was evaporated under reduced pressure. The residue was purified by a silica gel column using ethyl petroleum: acetate ether (v/v, 10:1) to afford compound 5 as a yellow solid (88.7 mg, 56.7%).^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.47 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.17 (dd, *J* = 4.8, 6.6 Hz, 1H), 6.85 (t, *J* = 7.2 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 1H), 6.36 (m, 1H), 6.35 (m, 1H) 6.29 (m, 1H), 5.44 (d, *J* = 12.6 Hz, 1H), 5.29 (s, 1H), 3.68 (dd, *J* = 7.2, 14.4, Hz, 3H), 3.36(dd, *J* = 6.6, 13.8Hz, 4H), 2.6 (m, 2H), 2.51 (m, 2H), 1.72 (s, 3H), 1.71 (s, 3H), 1.25 (dd, *J* = 12.6, 5.4 Hz, 5H),

1.18 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.27, 156.22, 153.23, 149.58, 148.75, 148.55, 144.44, 138.98, 132.26, 130.77, 128.18, 127.69, 127.67, 123.42, 122.95, 121.56, 119.80, 119.68, 119.20, 108.36, 105.62, 104.03, 102.53, 97.92, 91.53, 77.23, 77.02, 76.81, 67.82, 45.53, 44.44, 44.36, 36.70, 30.05, 29.68, 28.33, 28.24, 25.31, 22.97, 22.27, 12.55, 10.96. ESIMS(+): calculated for $C_{38}H_{42}N_4O_2$ $[M + H]^+$ m/z 586.33; found 587.35.



Scheme S1. (a) ethyl iodide, acetonitrile, 85 °C, yield 81.7%, (b) N,N'-bis-phenylformamidine, triethyl orthoformate, ethanol reflux yield 78%, (c) i. Cyclohexanone concentrated H_2SO_4, 0 °C. ii. $HClO_4$ yield 52.70%, (d) NaOAC, acetic anhydride, 50 °C, yield 59.2%, (e) PyBOP, hydrazine hydrate, 1,2-dichloromethane, room temperature, yield 56.7%.

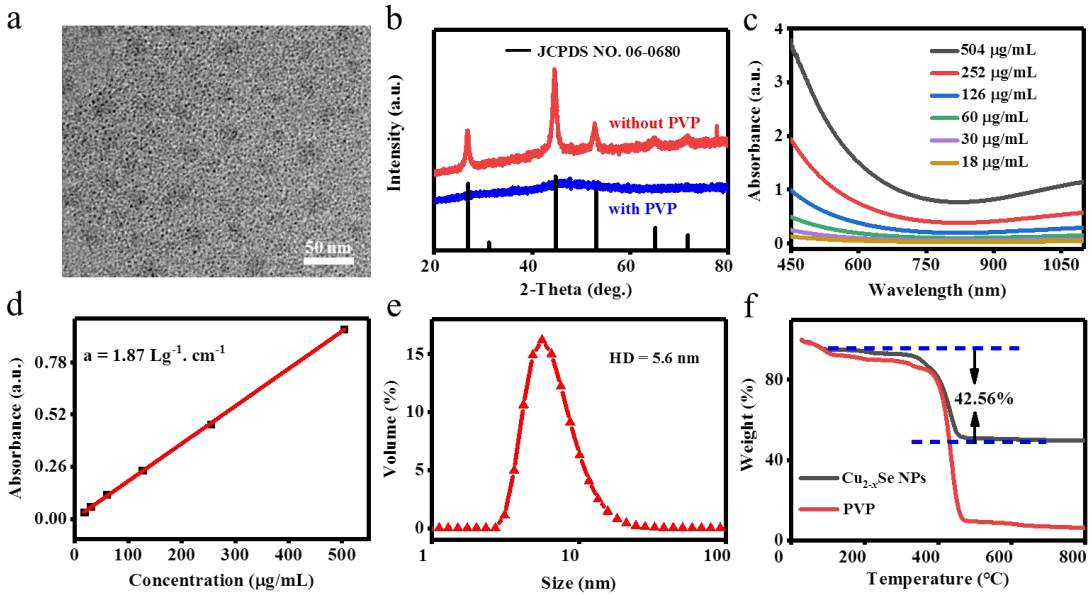


Figure S1. Characterization of as prepared $Cu_{2-x}Se$ NPs: (a) TEM image of $Cu_{2-x}Se$ NPs, (b) XRD patterns of $Cu_{2-x}Se$ prepared with and without PVP in comparison with the standard peaks of cubic berzelianite (JCPDS card no. 06-0680), (c) The UV-Vis-NIR spectra of $Cu_{2-x}Se$ NPs solution with different concentrations, (d) concentration dependent absorbance of $Cu_{2-x}Se$ NPs at 980 nm, (e) hydrodynamic size of $Cu_{2-x}Se$ NPs, (f) TGA curves of PVP functionalized $Cu_{2-x}Se$ NPs and PVP under nitrogen atmosphere.

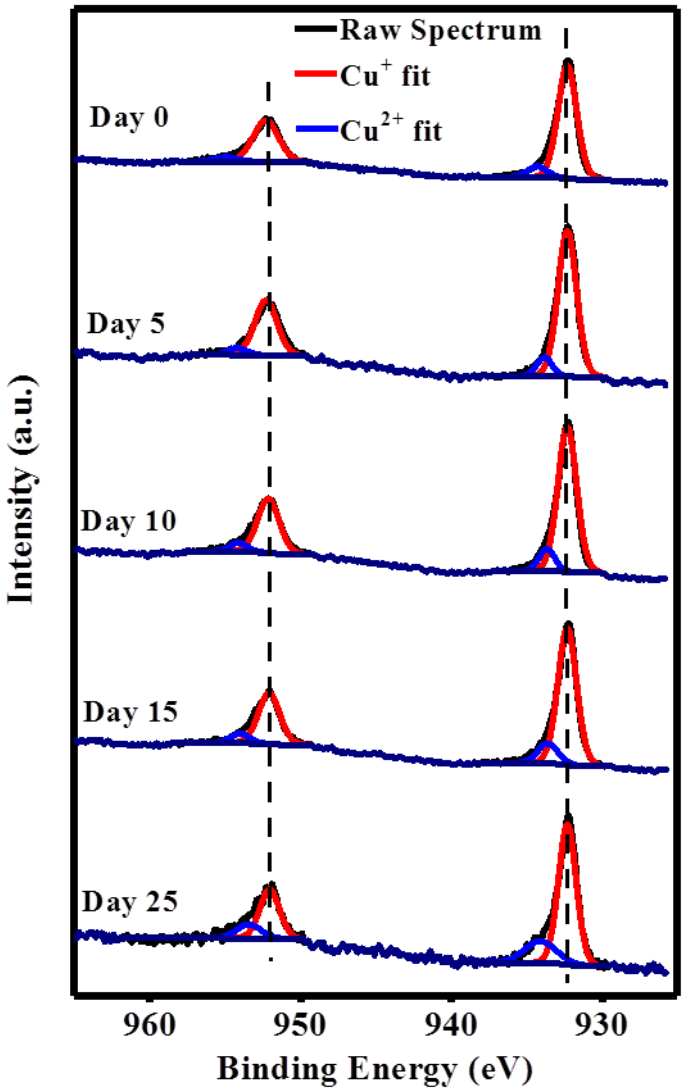


Figure S2. XPS Spectra of copper element in $Cu_{2-x}Se$ NPs oxidized by air at 37 °C and collected at day 0, day 5, day 10, day 15, and day 25, demonstrating the gradual increase of Cu^{2+}/Cu^{+} ratio.

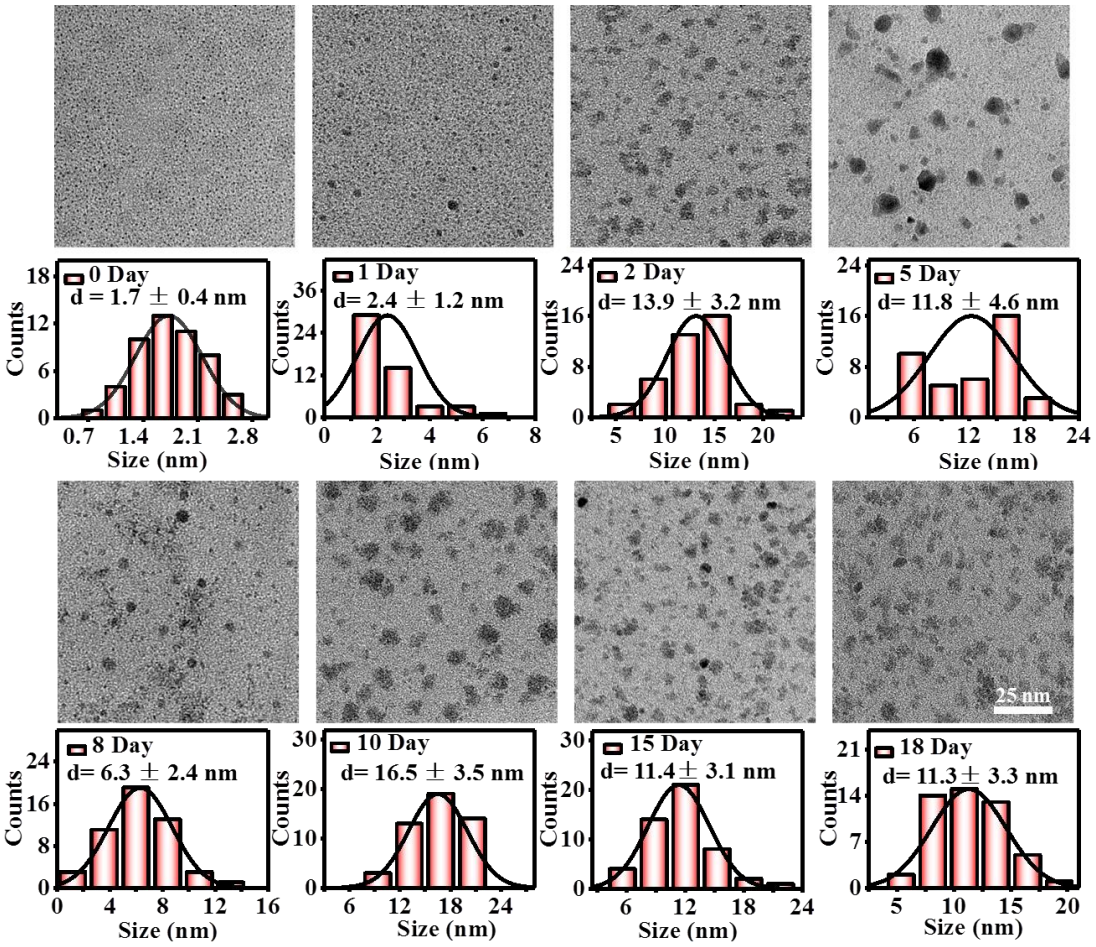


Figure S3. TEM images and corresponding particle size distributions of $Cu_{2-x}Se$ NPs collected at various days during the oxidation.

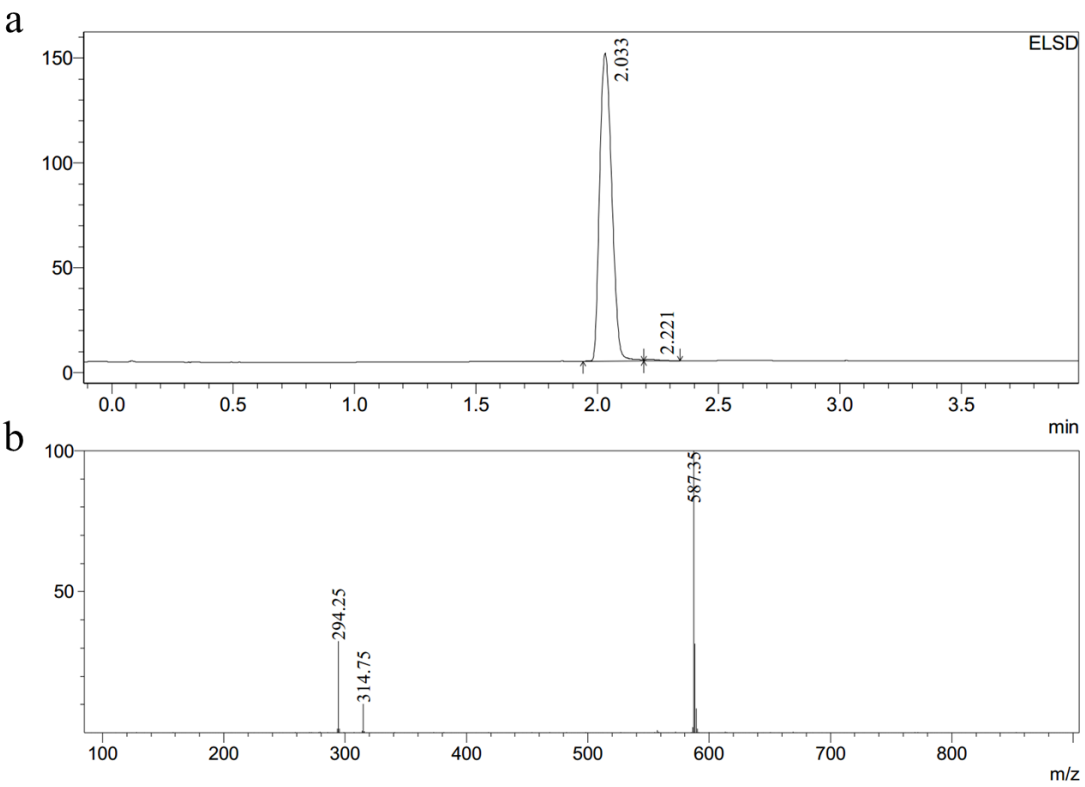


Figure S4. Liquid chromatography-mass spectrometry (LCMS) analysis of probe NCM. (a) LC result from Evaporative Light Scattering Detector (ELSD). (b) Mass spectrum of the peak at 2.033 min.

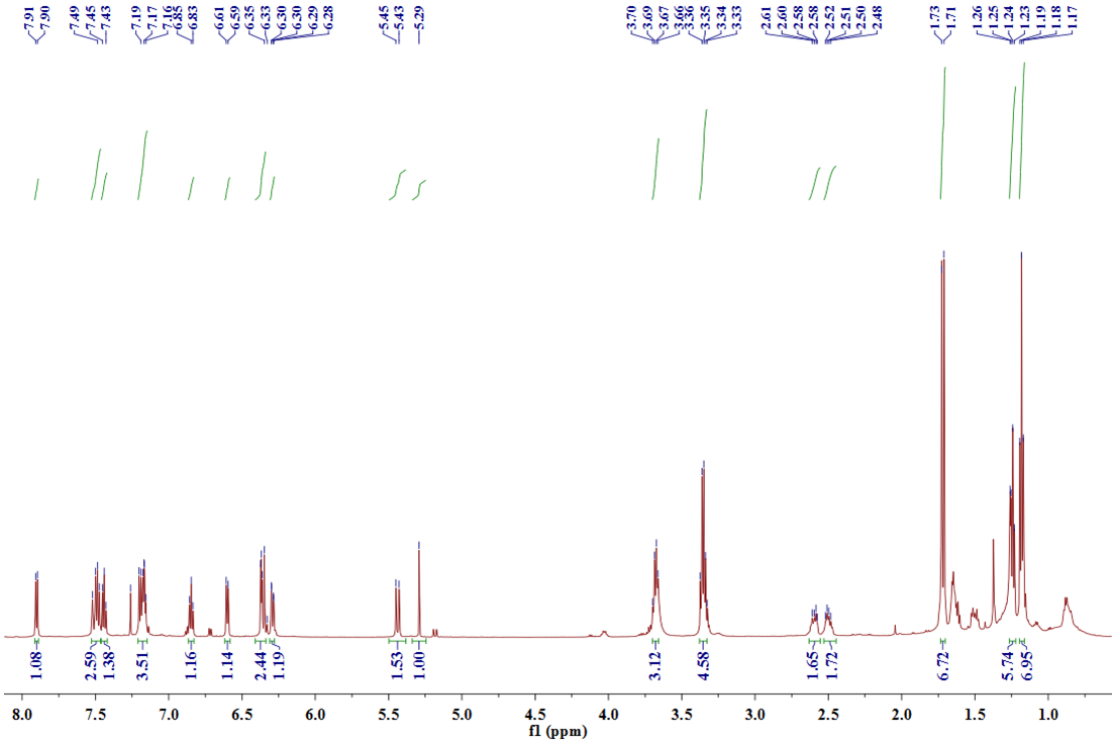


Figure S5. The ^{1}H NMR spectrum of probe NCM.

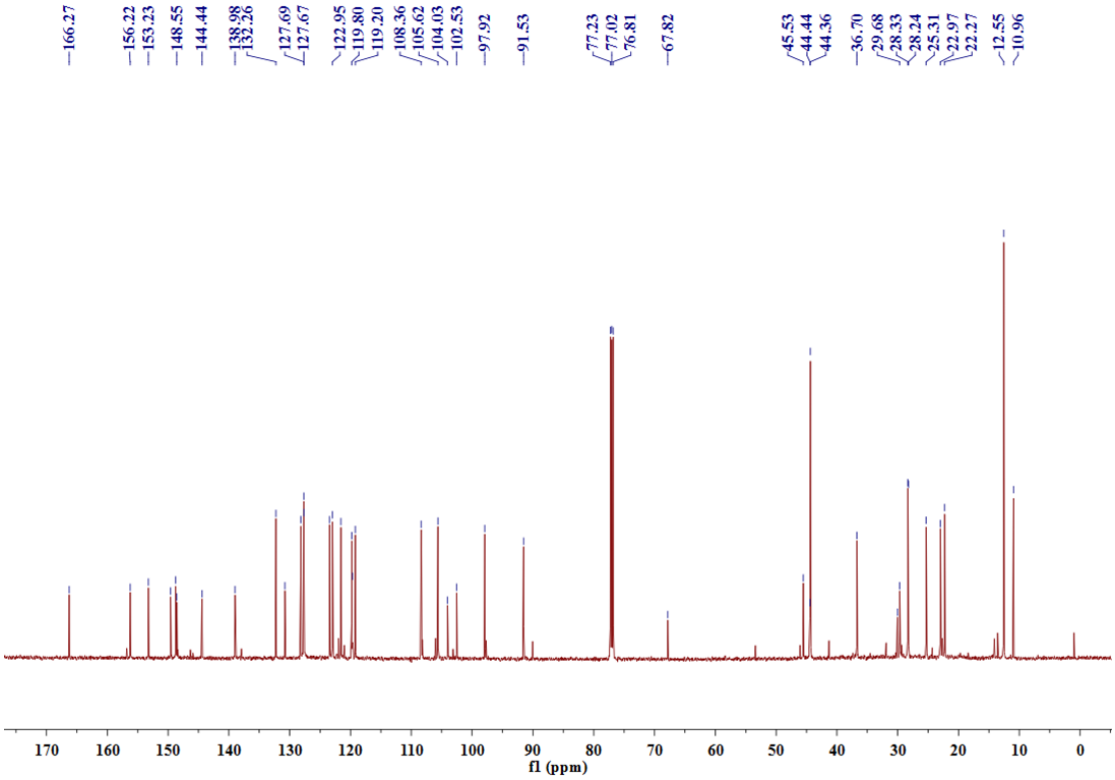


Figure S6. The ^{13}C NMR spectrum of probe NCM.

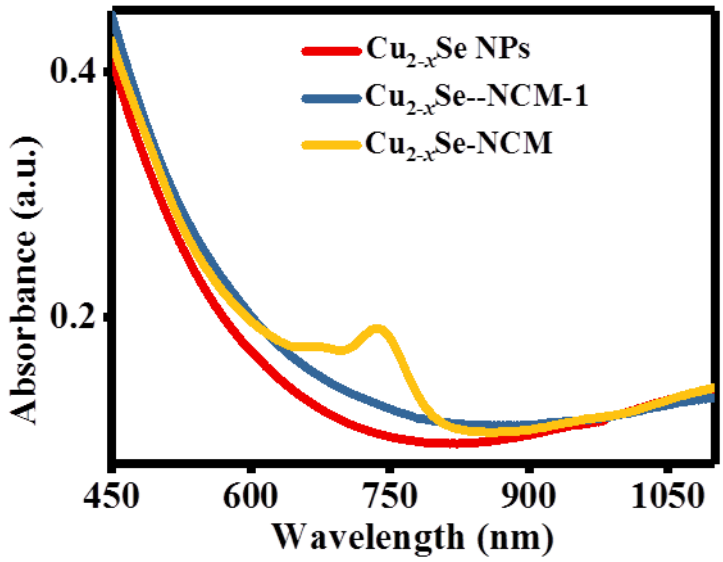


Figure S7. The UV-Vis-NIR spectra of $Cu_{2-x}Se$ NPs after stirred with probe NCM or NCM-1, in comparison with that of unmodified $Cu_{2-x}Se$ NPs.

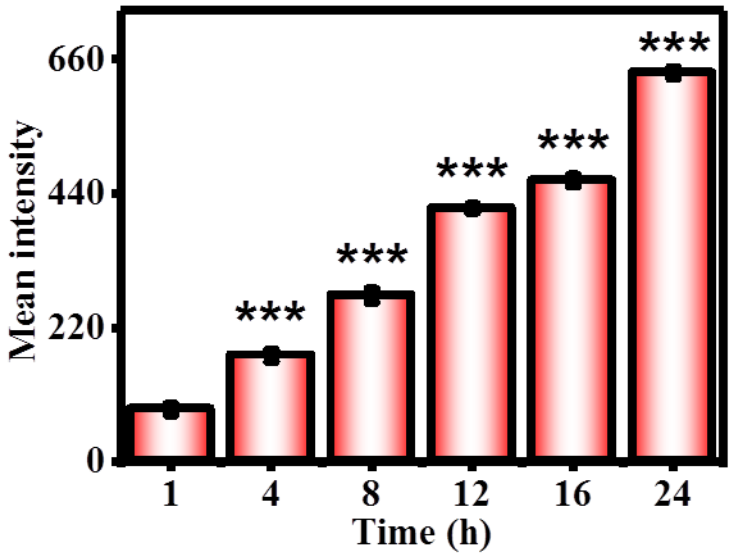


Figure S8. Mean fluorescence intensity of macrophages analyzed by flow cytometry, after cultured with the $Cu_{2-x}Se$-NCM NPs for different time (*p < 0.05, **p < 0.001, ***p < 0.0001).

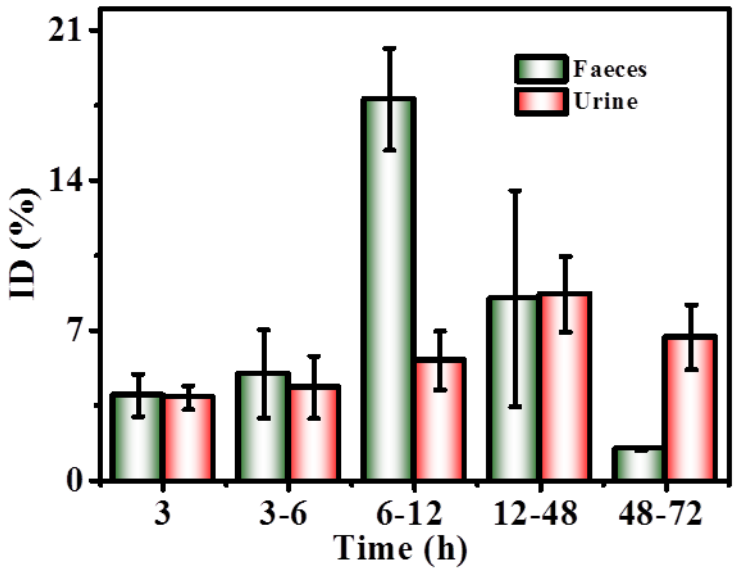


Figure S9. Cu contents in feces and urine of BALB/c female mice after they were intravenously injected with $Cu_{2-x}Se$ NPs (dose: 10 mg/kg).

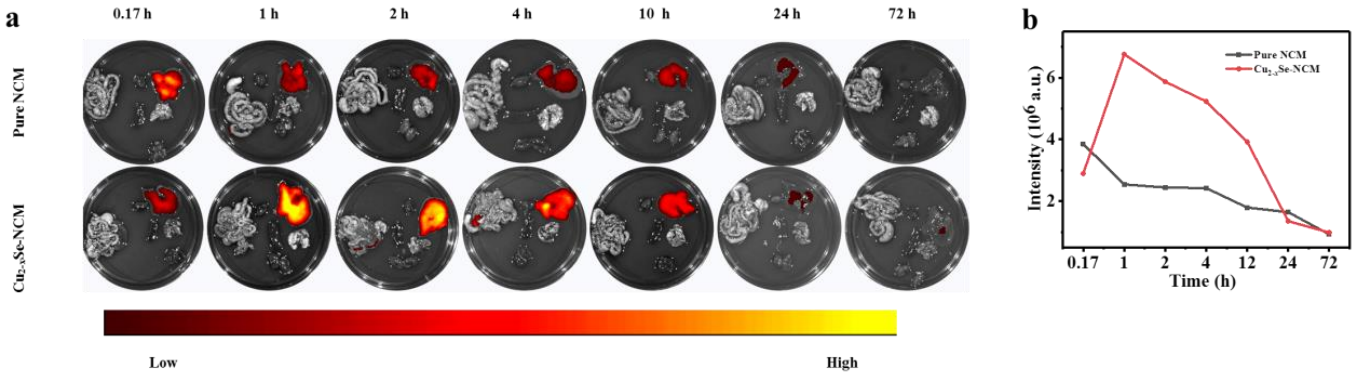


Figure S10. (a) The fluorescence images of major organs collected at different time points after the mice were treated with 100 μM pure NCM and $Cu_{2-x}Se$-NCM NPs with the same NCM concentration. (b) Liver relative fluorescence intensity at different time points.

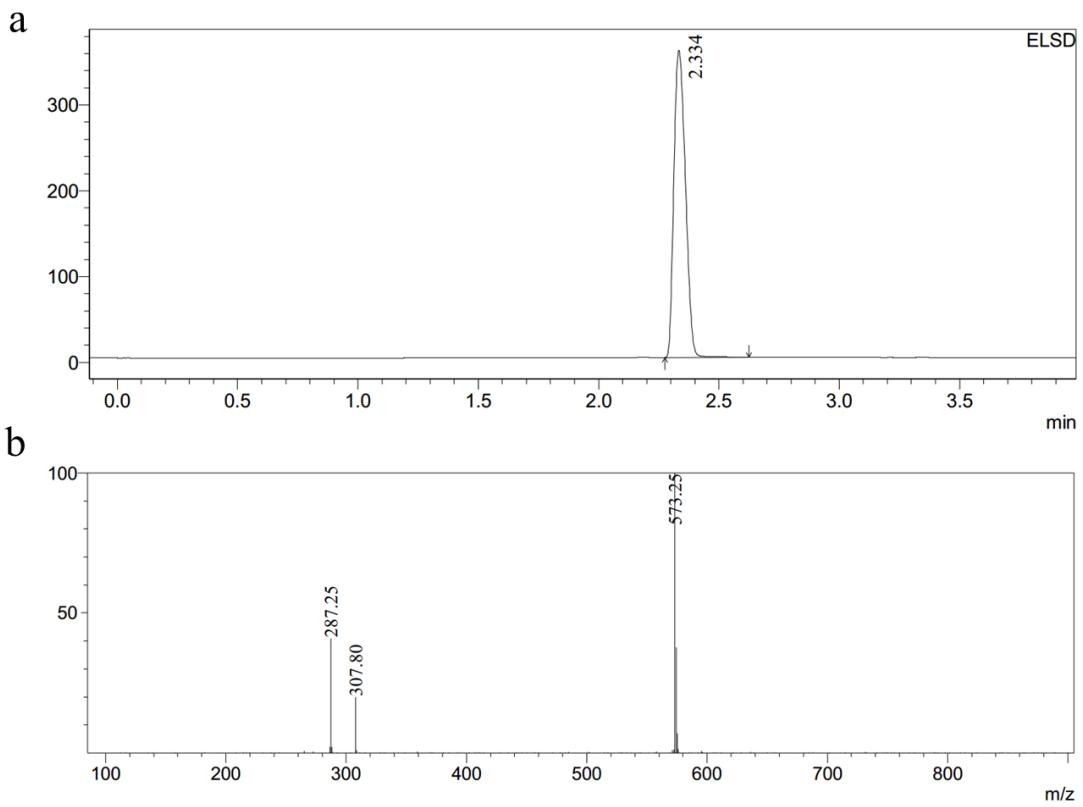


Figure S11. Liquid chromatography-mass spectrometry (LCMS) analysis of NCM-1. (a) LC result from Evaporative Light Scattering Detector (ELSD). (b) Mass spectrum of the peak of 2.334 min.

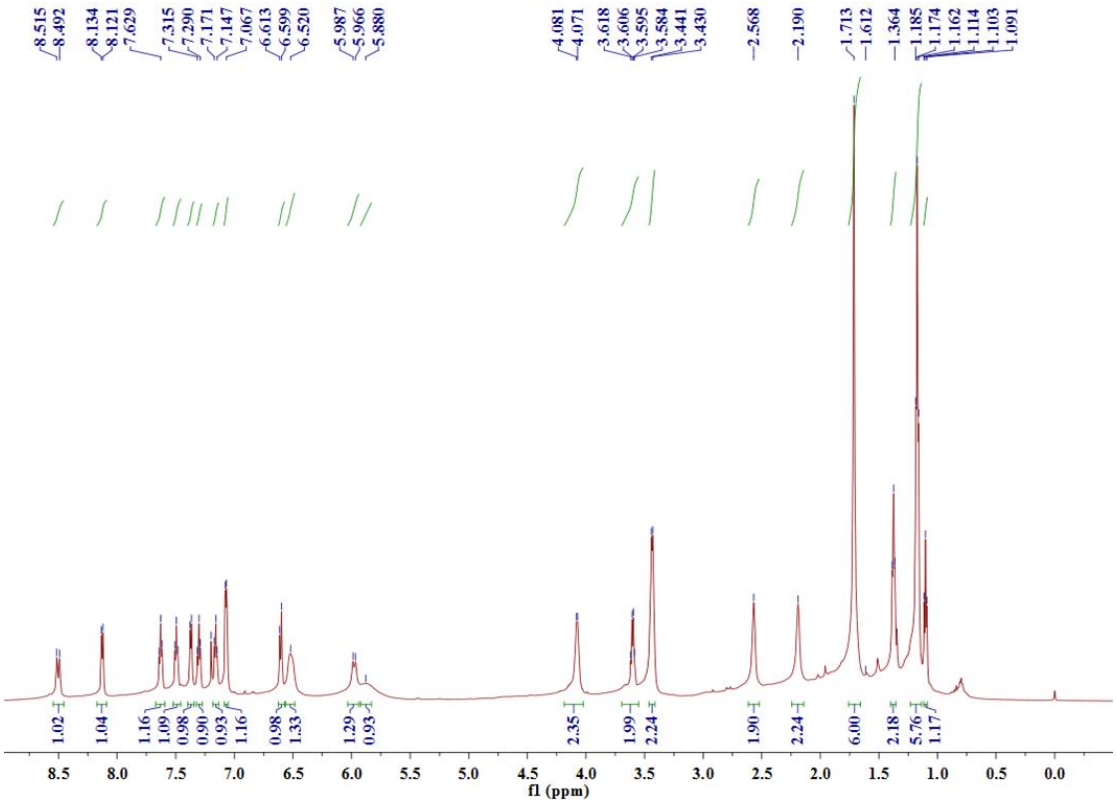


Figure S12. The ^{1}H NMR spectrum of compound NCM-1.

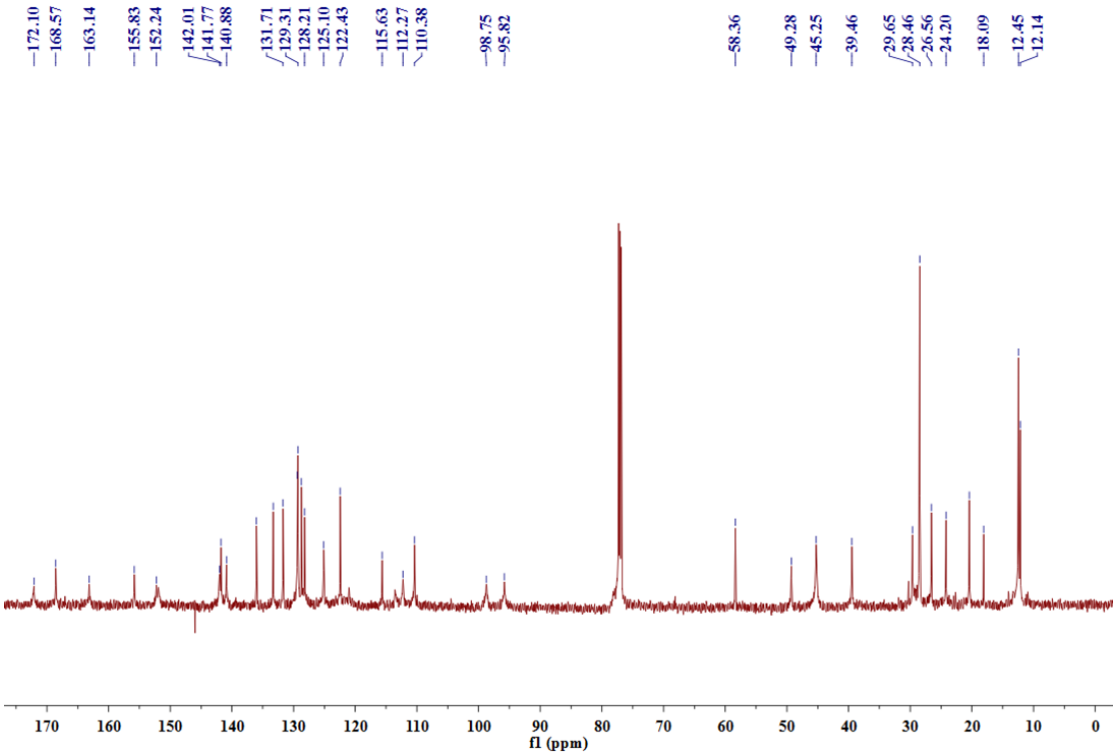


Figure S13. The ^{13}C NMR spectrum of compound NCM-1.

Table S1. Compositions of nanoparticles determined by inductively coupled plasma mass spectrometry (ICP-MS).

Element at%	In precursor	In product by ICP-MS
Cu	**2**	**1.97**
Se	**1**	**1**

Table S2. Pharmacokinetic parameters for $Cu_{2-x}Se$ NPs after intravenous injection into female BALB/C mice[a]

$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	AUC (%ID h/mL)	Vc (mL)	CL (mL/h)	MRT (h)
0.91 ± 0.32	10.13 ± 2.35	297.43 ± 14.33	1.65 ± 0.28	0.31 ± 0.02	12.33 ± 2.30

[a] Values are means ± standard deviations. Abbreviations: $t_{1/2}\alpha$, blood distribution half-life; $t_{1/2}\beta$, blood terminal elimination half-life; AUC, area under the blood activity time curve; Vc, volume of distribution in center compartment; CL, total body clearance; MRT, mean residence time.