Supporting Information for

Selenium Stimulates Cadmium Detoxification in *Caenorhabditis elegans* through Thiols-mediated Nanoparticles Formation and Secretion

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This supporting information contains 19-page document, including 3-page supporting materials and methods, 1 table, 14 figures and this cover page.

SI Materials and Methods

Analysis of Cd transformation efficiency. *C. elegans* in the Se & Cd group were collected, washed and homogenized into 10 mL liquid. To determine the total Cd amount in the *C. elegans* body, 500 μL of the homogenate were used for analysis by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The rest of homogenate was used for nanoparticles purifications. The purified nanoparticles were diluted to 5 mL by deionized water for Cd concentration determination by ICP-AES. The transformation efficiency from Cd cations to Cd nanoparticles was estimated by comparing the Cd in nanoparticles to the total Cd amount. The Cd transformation efficiencies in the exposure medium with *E. coli* OP50 and no *C. elegans* (group "no *C. elegans*"), where the nanoparticles were extracted and purified by the same procedures above, were also analyzed by ICP-AES.

Detection of bacterial activity. *C. elegans* were separated from *E. coli* OP50 bacteria by centrifuging at 400 g for 2 min. The supernatant (containing bacteria) was further centrifuged at 6,000 rpm for 5 min to collect the bacteria. The bacteria samples were further washed three times with 1×PBS (phosphate buffer salsine). After staining with diamidino-2-phenylindole (DAPI) and propidium iodide (PI) for 30 min at room temperature, the bacteria were washed three times again with 10 mM PBS and subjected to observation by epifluorescence microscope (BX51, Olympus Co., Japan).

Survival fraction detection of *C. elegans* under 3-fold higher Cd exposure. *C. elegans* were exposed to a culturing medium with 3-fold higher Cd concentration

than the normal case (375 μM $CdCl_2$ was added at 0 h and 48 h respectively, no Se was supplemented). Such a high Cd exposure is supposed to raise the intracellular Cd accumulation to a similar level as the co-exposure group. At different time points, the numbers of live and dead nematodes were counted using a dissecting microscope.

Laser confocal Raman microspectroscopy. *Raman spectra of the concentrated and freeze-dried CdSe containing nanoparticles in culture media*. The culture medium of Se & Cd group after 144 h exposure was completely separated from *C. elegans* and OP50 by centrifuging at 8,000 g for 5 min. However, the amount of CdSe containing nanoparticles in the medium was very limited and could not be directly detected by Raman spectroscopy. Thus, the supernatant was concentrated using 30-kDa tubular ultrafiltration membrane (MWCO-10000, Millipore Inc., USA) and freeze-dried prior to Raman analysis. The Raman spectroscope was equipped with a 532 nm laser.

Effect of extraction and purification process on CdSe containing nanoparticles. *C. elegans* were exposed to 200 μM Na_2SeO_3-only (the group named Se) or 250 μM $CdCl_2$-only (the group named Cd) respectively, while the group named Se & Cd was dosed with both 200 μM Na_2SeO_3 and 250 μM $CdCl_2$. All the groups were homogenized immediately followed by the same nanoparticles purification procedure as described in the manuscript, and the samples were freeze-dried prior to Raman analysis.

Effect of laser irradiation on the formation and stability of CdSe containing nanoparticles. Effects of laser irradiation on CdSe containing nanoparticles formation

were examined using 532 nm laser equipped Raman microspectroscopy. Specifically, 200 μM Na_2SeO_3 and 250 μM $CdCl_2$ were added into the *C. elegans* culture system and laser irradiation (10 mW powered) was implemented for 5 min. The Raman spectra were measured once every minute. The effects of laser irradiation on the Raman peak of bioformed nanoparticles were explored by applying 100 mW powered laser irradiation for 5 min.

GSH quantification. The amount of GSH and GSSG (oxidized GSH) of *C. elegans* in different test groups were detected using the GSH and GSSG Detection Assay Kit (Beyotime Biotechnology Co., China). The total protein content of each sample was determined by BCA Protein Assay Kit (Beyotime Biotechnology Co., China) and was used as a common denominator of the individual samples.

Effects of selenomethionine on Cd detoxification. *C. elegans* were exposed to 200 μM selenomethionine (Sangon Biotech Co., China) for 24-h incubation. Then 375 μM $CdCl_2$ was dosed (recorded as 0 h), followed by 48-h further incubation and dosing the same amount of $CdCl_2$ again. The numbers of live and dead nematodes as well as the fluorescence microscopic images of *C. elegans* were obtained following the same methods described above.

Table S1 Primer Sequences for qRT-PCR.

***C. elegans* Gene**		**Primer sequences (5'-3')**	**Product size (bp)**
act-1	forward	ATCGTCCTCGACTCTGGAGATG	101
	reverse	TCACGTCCAGCCAAGTCAAG	
mtl-1	forward	ATGGCTTGCAAGTGTGACTG	216
	reverse	AGCAGTTCCCTGGTGTTGAT	
mtl-2	forward	TGCAACACCGGAACTAAAGA	153
	reverse	TTAATGAGCAGCCTGAGCAC	
gcs-1	forward	ACGTCCCGATATTCAAGG	195
	reverse	CGTACAACCATCTGGCTTC	
gss-1	forward	GGAAGGATGCACCTGAGCTT	117
	reverse	CGTAGAGGAATGGGGTGTCG	
gsr-1	forward	GGACCACGCTGATTACGGAT	203
	reverse	TTCCACGATACTTCGCTCCG	
pcs-1	forward	TCTGAATGCGTTGGAAGT	324
	reverse	TGATAGGCGGCAAGTGGT	

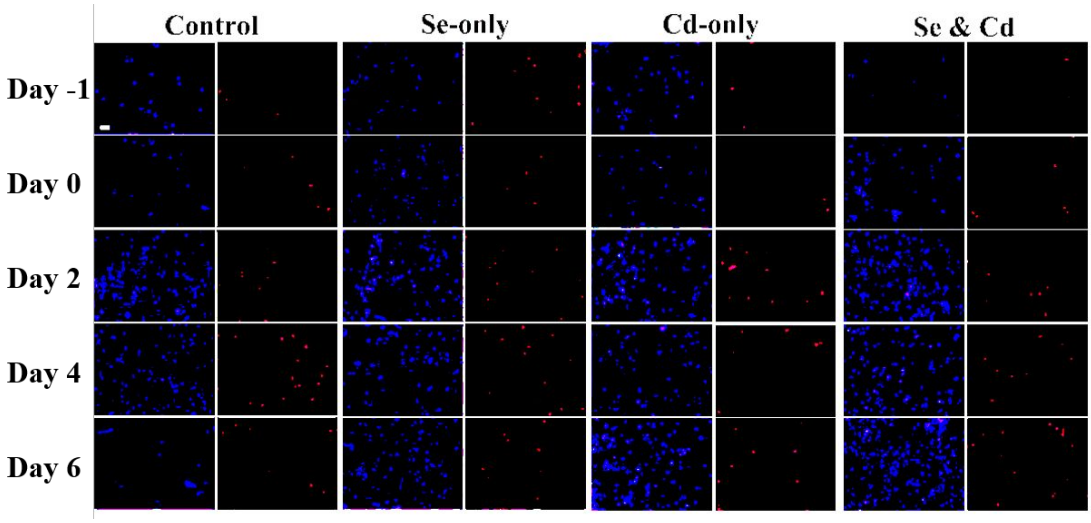

Figure S1 The activities of bacteria after exposure to Se (marked as Day -1) and Cd (from Day 0 to 6). The scale bar (white, 10 μm) in the upper-left is applicable to all the images.

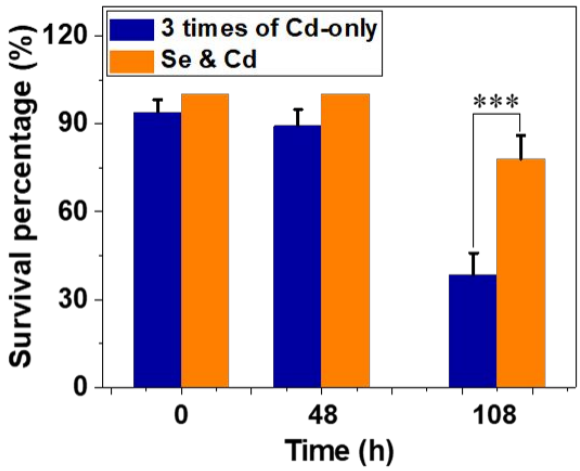

Figure S2 Survival percentages of *C. elegans* exposed to 3-fold higher concentrations of Cd (375 μM at 0 h and 48 h) and under normal exposure to Se and Cd (125 μM at 0 h and 48 h). Error bars represent the standard error for triplicate samples. The mark *** indicates $p < 0.001$.

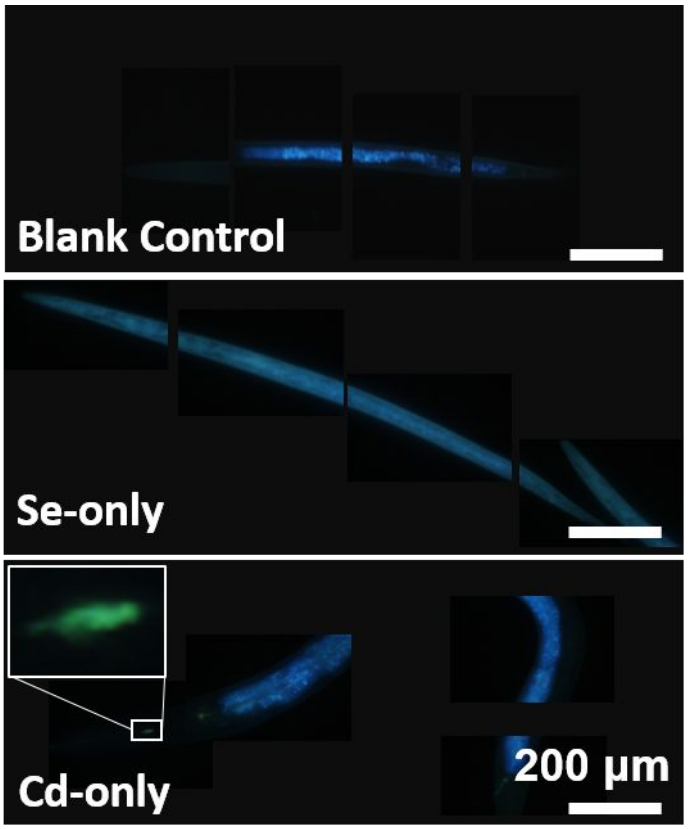

Figure S3 Fluorescence microscopic images of *C. elegans* in the blank control (without Cd nor Se), Se-only (adding Se alone), and Cd-only (adding Cd-alone) groups.

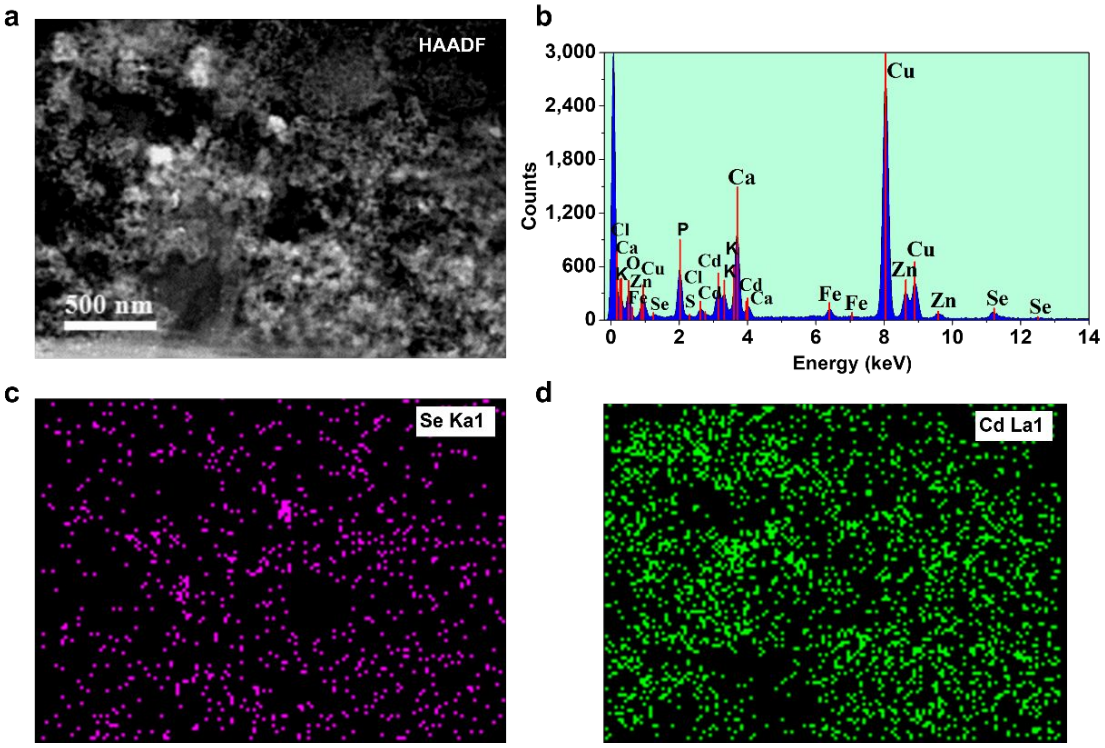

Figure S4 Morphologies and elemental distribution of the biogenic fluorophores in the disrupted nematode. Highangle Annular Dark Field image showing nanoparticles in the fracture position of nematode (a); the EDX pattern showing the elemental proportion in Chart a (b); and the corresponding elemental mapping of Se (c) and Cd (d).

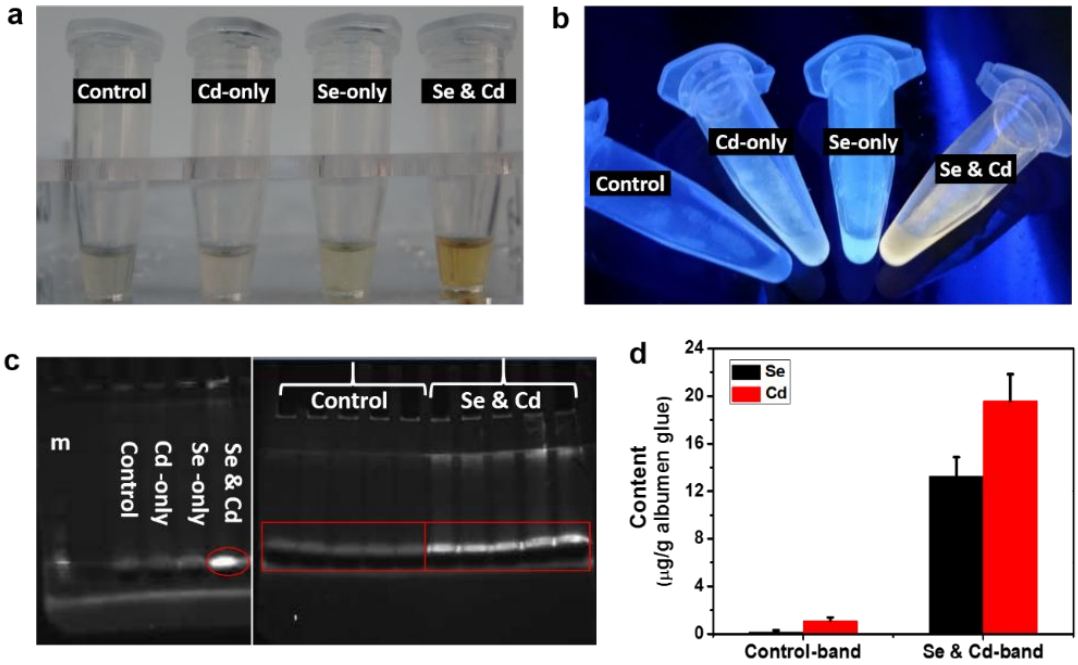

Figure S5 Properties and chemical contents of the *C. elegans* extracts. Images of the extracted samples under visual light (a) and under UV irradiation (365 nm) (b); the NPs fluorescence visualized under UV illumination after SDS-PAGE treatment of the extracted nanoparticles (c); Se and Cd contents of the fluorescent band in SDS-PAGE gel analyzed by ICP-AES (d). Error bars represent the standard errors for triplicate samples.

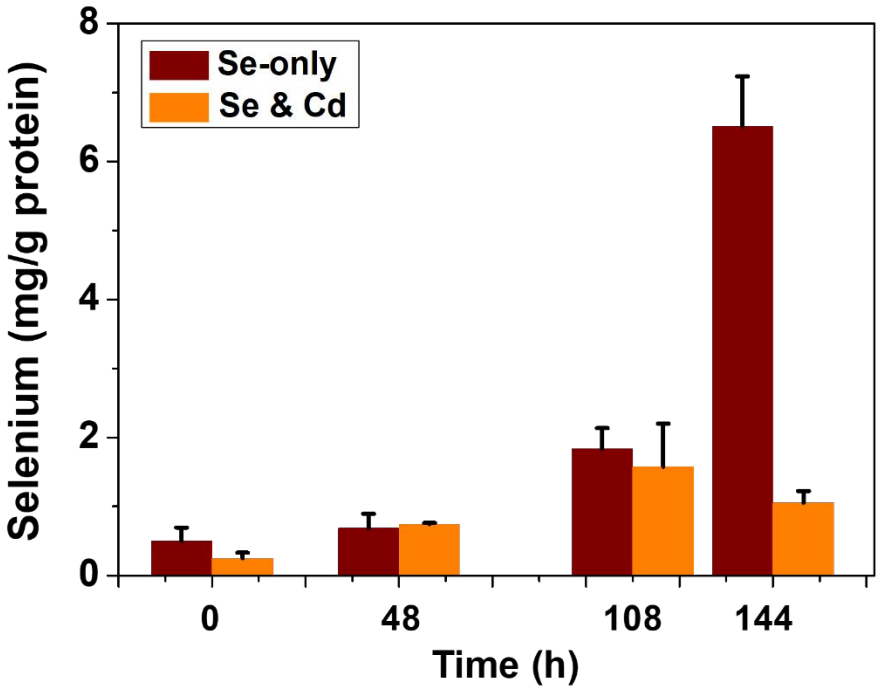

Figure S6 Profiles of element Se content in *C. elegans* over time. The accumulation of Se in the nematodes was quantified by ICP-AES. Error bars represent the standard errors for triplicate samples.

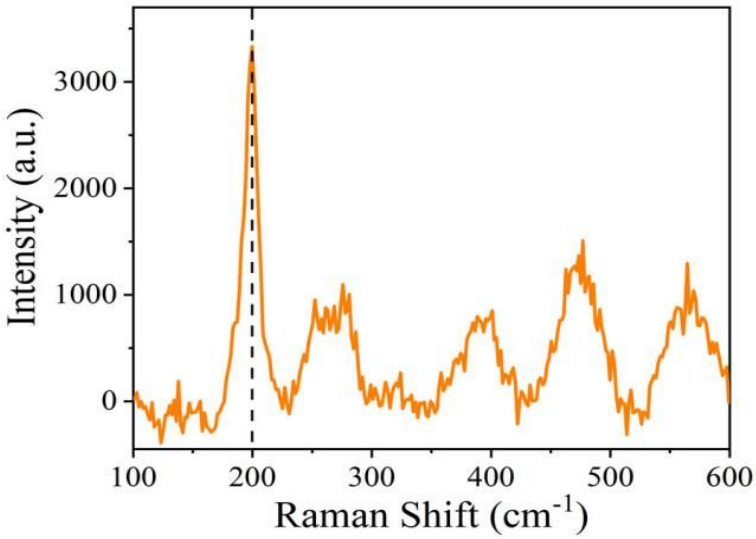

Figure S7 Raman spectra of the concentrated and freeze-dried CdSe containing nanoparticles in culture media. The laser excitation wavelength was 532 nm. The peak at 203 cm^{-1} matched that of CdSe-like substances.

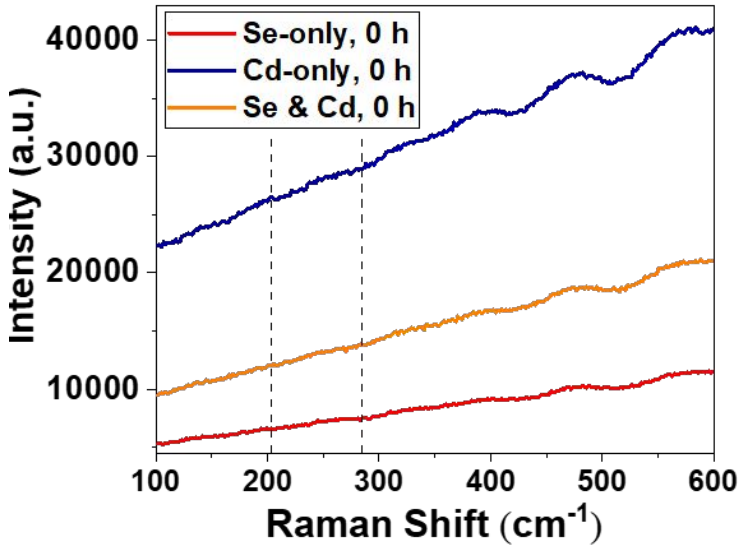

Figure S8 Raman spectra of different treated groups without culturing for 144 hours. The same extraction and purification procedure as the 144-h cultured group was applied. Neither Cd-Se nor Cd-S bonds were detected, indicating that CdSe/CdS nanoparticles could not be synthesized during the reported purification process.

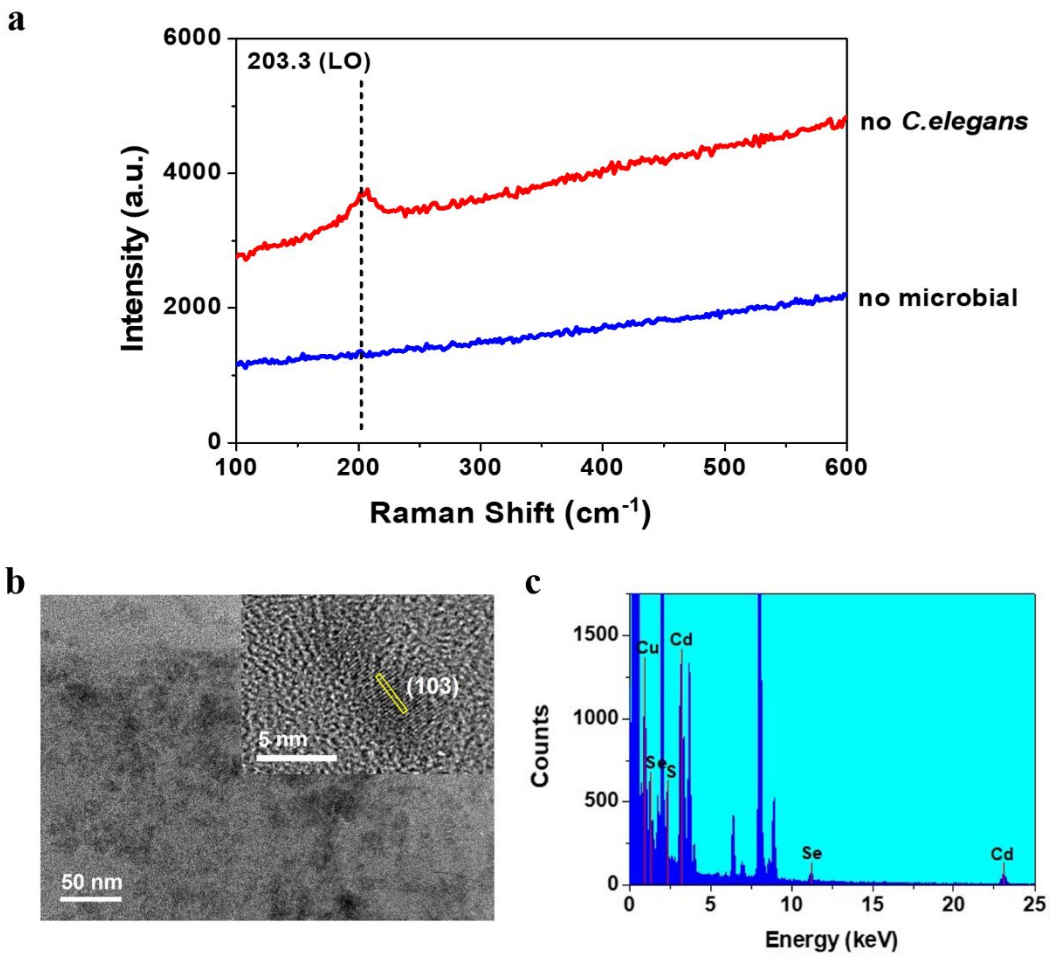

Figure S9 Raman spectra of two control groups under 532 nm laser excitation (a), and the HRTEM images (b) and EDX spectra (c) of the nanoparticles produced by *E. coli* OP50 in no *C. elegans* group. In the "no *C. elegans*", *E. coli* OP50 was added as the only microbe showing far less capacity of CdSe production than *C. elegans* with a very weak peak of CdSe-like substances (203 cm^{-1}) and the lattice spacing of $CdS_{0.33}Se_{0.67}$ (JCPDS 50-0721) formed, indicating that the CdSe formation was ascribed primarily to *C. elegans* and slightly to the reporter strain (i.e., *E. coli* OP50); "no microbial", the abiotic group.

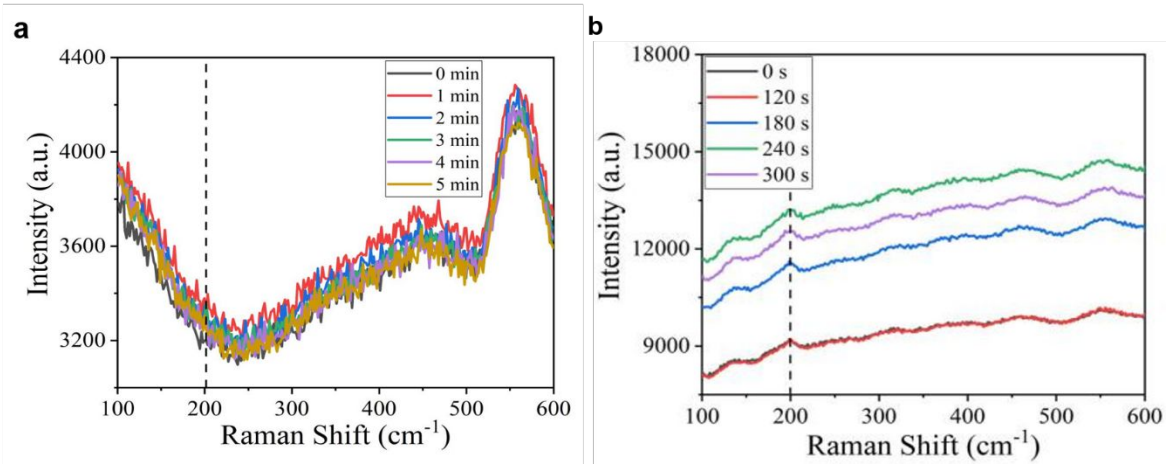

Figure S10 Raman spectra of *C. elegnas* which added 200 μM Se and 250 μM Cd precursors (a) for 5 min irradiation at 10 mW under 532 nm laser excitation; Raman spectra of the biosynthesized sample (b) for 5 min irradiation at 100 mW under 532 nm laser excitation.

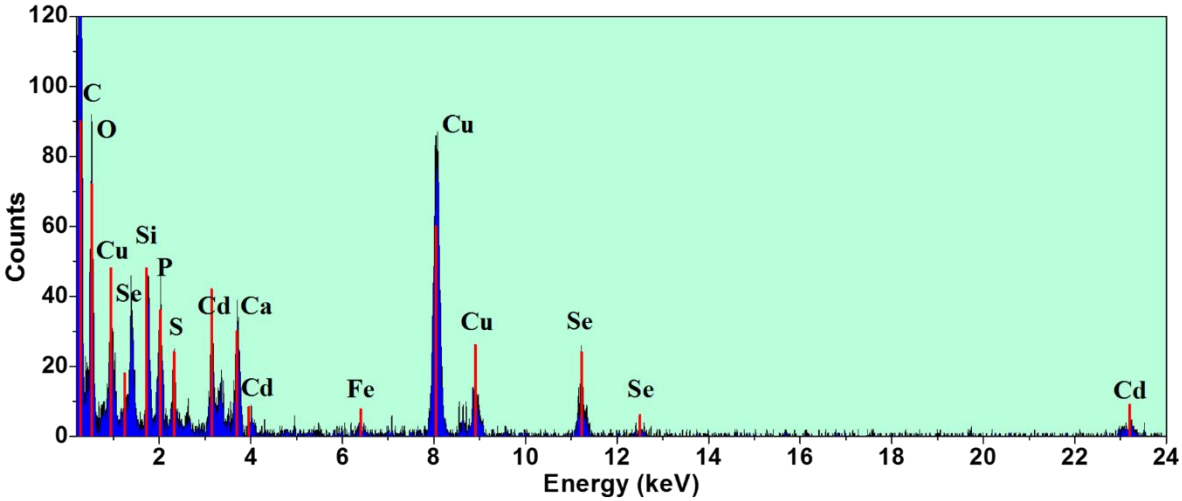

Figure S11 EDX spectra of the extracted and purified nanoparticles.

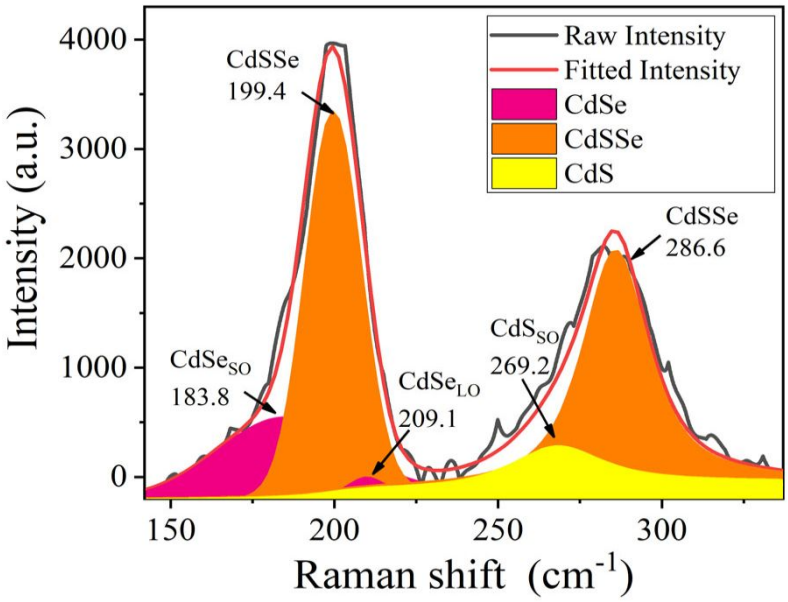

Figure S12 Fitted Raman spectra of purified nanoparticles after baseline drift correction from Figure 3a. The formed nanoparticles in *C. elegans* mainly consisted of Cd/Se/S alloy and minor amount of CdSe and CdS on the surface. The peak at 199.4 cm^{-1} belongs to CdSe alloy and the weak peak at 193.8 cm^{-1} belongs to surface optical (SO) phonon peak of CdSe, while the peak at 286.8 cm^{-1} belongs to CdS alloy and the peak at 269.2 cm^{-1} belongs to CdS_{SO}.

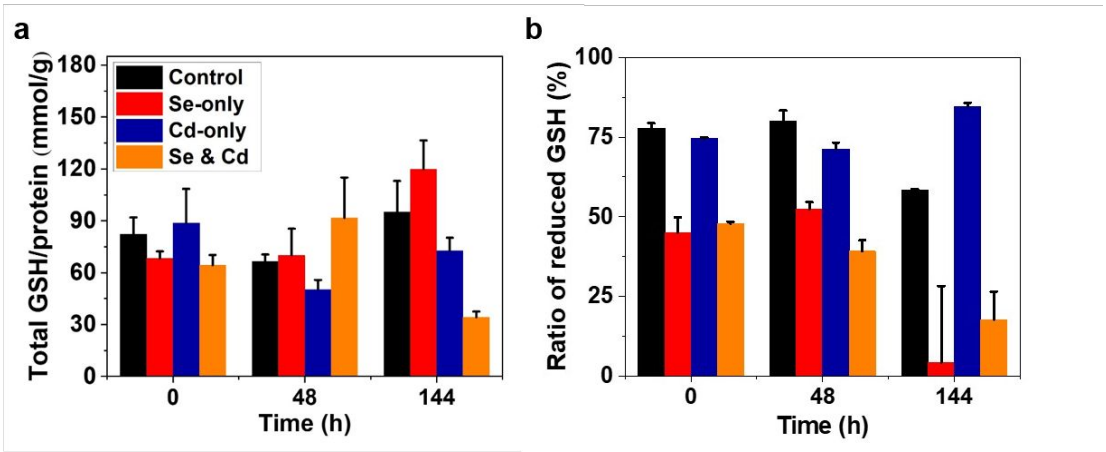

Figure S13 Total GSH contents (a) and the ratio of reduced GSH (b) in *C. elegans* over time. Error bars represent the standard errors for triplicate samples.

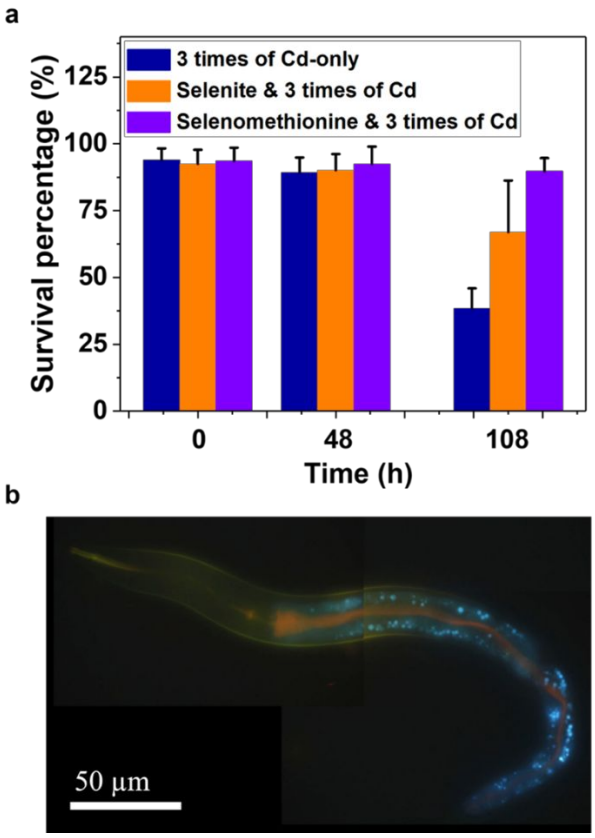

Figure S14 The survival percentages of *C. elegans* (a) treated by 375 μM Cd^{2+} at 0 h and 48 h and cultivated until 108 h under single- or co-exposure condition; Fluorescence microscopic image (b) of *C. elegans* after 108 h exposure to selenomethionine and Cd.