

Supplementary information

Solar-energy-facilitated CdS_xSe_{1-x} quantum dot bio-assembly in *Escherichia coli* and *Tetrahymena pyriformis*

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This supporting information contains 24-page document, including results, 2 - table, 18 - figures, references and this cover page.

Experimental Section

Fourier transform infrared (FT-IR) spectra detection

FT-IR spectra of the purified Bio-QDs assembled in *E.coli* were collected using Nicolet iN10 Fourier infrared microspectroscopy (Thermal Fisher Scientific Co., USA) at a wavenumber ranged from 600 to 4000 cm^{-1} with a resolution of 4.0 cm^{-1} .

Photocurrent detection

The prepared samples were loaded onto Ti sheet for photocurrent detection in 0.1 M Na_2SO_4 solution. The counter electrode was Pt wire and the reference electrode was Ag/AgCl (KCl, 3 M). A 500 W Xe arc lamp (PLS-SXE500, Trusttech Co., China) was applied as the light source and the detection was performed by an electrochemical workstation (CHI 750e, Chenhua Co., China). A bias voltage of 0.1 V was applied for amperometric i-t curve measurement. All of the measurements were repeated three times.

Viability test

$\text{CdS}_x\text{Se}_{1-x}$ Bio-QDs assembled in *E. coli* under light irradiation and in the dark were washed by 10 mM Tris-HCl. A sufficient amount of 4',6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) were added into *E. coli* and reacted at room temperature in the dark for 30 min. The resulting *E. coli* cells were washed by 10 mM Tris-HCl three times and detected by the SpectraMax M2 plate reader (Molecular Devices Co., USA) or observed by fluorescence microscopy. The detection Excitation/Emission wavelengths of DAPI were 358/461 nm, and the Excitation/Emission wavelengths of PI were 535/615 nm.¹ The amount of dead bacteria was quantified using the PI/DAPI intensities ratio.

Fluorescence lifetime imaging microscope observation

Wild type *Caenorhabditis elegans* (*C. elegans*, Bristol strain N2) were purchased from Caenorhabditis Genetics Center (Minneapolis, USA). They were cultured in nematode growth medium (2.5 g/L peptone, 3 g/L NaCl, 1 mM CaCl_2 , 1 mM MgSO_4 , 17 g/L agar, 5 mg/L ethanol and 25 mM KPO_4 buffer) with food source *E. coli* OP50 at 20 °C for 60 h.² The nematodes were harvested and transferred to a K medium and co-incubated with purified Bio-QDs assembled in *E. coli* JM109 for 8 h.³ *C. elegans* were washed by K medium for three times for fluorescence lifetime imaging microscope system (ISS Inc., US). The images were recorded with 488 nm excitation wavelength and 20 MHz repetition rate.

Results

Characterization of $\text{CdS}_x\text{Se}_{1-x}$ QDs assembled in the GSH system under UV light irradiation and dark conditions

The chemical information, crystal structures and morphological features of fluorescent material prepared in the UV irradiation group were revealed by Raman, X-ray diffraction (XRD) and HRTEM characterizations. The observed Raman signals contained two peaks at 196 cm^{-1} and 279 cm^{-1} , which were attributed to Cd-Se and Cd-S chemical bonds, respectively (Fig. S6A). The matter phases and crystal structures of purified fluorescent material were acquired by powder XRD, and the results matched $\text{CdS}_{0.54}\text{Se}_{0.46}$ (JCPDS No. 89-3682) crystalline material well (Fig. S6B). The above results prove the $\text{CdS}_x\text{Se}_{1-x}$ QDs formation in the GSH system with UV irradiation. In fact, $\text{CdS}_x\text{Se}_{1-x}$ QDs was also fabricated in the GSH system without photo assistance, as supported by Raman, X-ray diffraction (XRD) and HRTEM characterizations (Fig. S7).

Characterization of QDs assembled in *T. pyriformis* during mechanism study

As shown in Fig. S9A, the Raman peak at 203 cm^{-1} was assigned to Cd-Se longitudinal optical phonon-type Raman signals.⁴ Nanometer-sized crystalline materials were observed in HRTEM (Fig. S9B). The crystal lattice spacings highlighted in Fig. 9C corresponded to (200) and (110) planes of hexagonal $\text{CdS}_{0.42}\text{Se}_{0.58}$ (JCPDS No. 50-0720) and the angle between these two planes was 60° . Besides, the (110) planes of $\text{CdS}_{0.75}\text{Se}_{0.25}$ (JCPDS No. 49-1459), CdS (JCPDS No. 41-1049), and CdSe (JCPDS No. 08-0459) were also captured by HRTEM. In addition, the (110) and (103) planes of hexagonal $\text{CdS}_{0.33}\text{Se}_{0.67}$ (JCPDS No. 50-0721) were also observed through HRTEM. Together, the above results confirm $\text{CdS}_x\text{Se}_{1-x}$ QD formation in *T. pyriformis* under UV irradiation.

Table S1. Performance of the synthesized Bio-QDs reported in literature

Organism	Material	Optical properties	Method	Assembly time	Ref.
Engineered <i>E. coli</i>	CdS	Ex: 320 nm Em: 384 nm	Express SpPCS (PCs synthase), γ -glutamylcysteine synthetase	1 h	5
Engineered <i>E. coli</i>	CdS	Ex: 350 nm Em: 445-513 nm	Express peptide CDS 7	1.5 h	6
<i>E. coli</i>	CdS	-	Add 10 mM phosphate	-	7
<i>Acidithiobacillus thiooxidans</i> ATCC 19703	CdS	Ex: 370 nm Em: 470 - 550 nm	Optimization - Response Surface Methodology	24 h	8
Engineered <i>E. coli</i>	CdSe/CdTe	-	Express AtPCS (PCs synthase) and/or PpMT (MT) one-step	6-12 h	9
Engineered <i>E. coli</i>	CdTe	Em: 450 nm FWHM: 110 nm	Overexpress GSH synthesis genes gshA one-step	24 h	10
<i>E. coli</i>	CdSe	Em: 495-510 nm	Optimize the synthesis conditions	4 h	11
Engineered <i>S. cerevisiae</i>	CdSe	Ex: 365 nm Em: 525-635 nm	Overexpress GSH metabolism gene	-	12
Engineered <i>S. cerevisiae</i>	CdSe	Ex: 400 nm Em: 450–650 nm	Overexpress <i>MET6</i> gene (methionine synthase)	24 h	13
Engineered <i>S. cerevisiae</i>	CdSe	-	Overexpress <i>ADK1</i> gene (accumulation of ATP)	24 h	14
<i>Phanerochaete chrysosporium</i>	CdS	Em: 458 nm	Optimize the synthesis pH one-step	12 h	15
Earthworm	CdTe	Ex: 365 nm Em: 520 nm Lifetime: 4.54 ns	One-step	-	16
<i>E. coli</i>	Cd _x Se _{1-x}	Ex: 375 nm Em: 525 nm Lifetime: 24.8 ns	Sunlight irradiation	1 h	This work
<i>T. pyriformis</i>	Cd _x Se _{1-x}	Em: 525 nm	UV irradiation	24 min	This work

Table S2. Reaction conditions and results of *in vitro* photo-facilitated QDs synthesis

Reaction Conditions				Results	
QDs	Selenite	CdCl ₂	UV light	QDs production	Selenite reduction
<i>E. coli</i> -synthesized	+	+	+	✓	✓
<i>E. coli</i> -synthesized	+	-	+	NO	✓
<i>E. coli</i> -synthesized	-	+	+	NO	NO
-	+	+	+	NO	NO
GSH-mediated	+	+	+	✓	✓
GSH-mediated	+	-	+	NO	✓
GSH-mediated	-	+	+	NO	NO
-	+	+	+	NO	NO

+, added the materials; -, absent the materials.

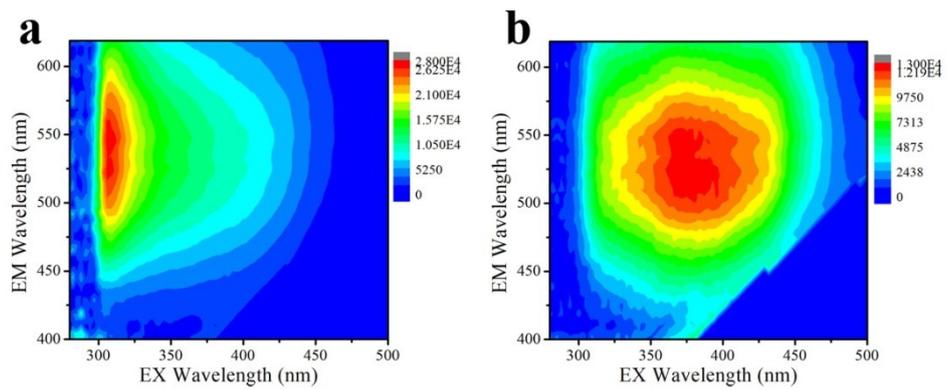


Fig. S1 EEM of purified fluorescence materials synthesized under dark (a) and sunlight irradiation (b) conditions in *E. coli*.

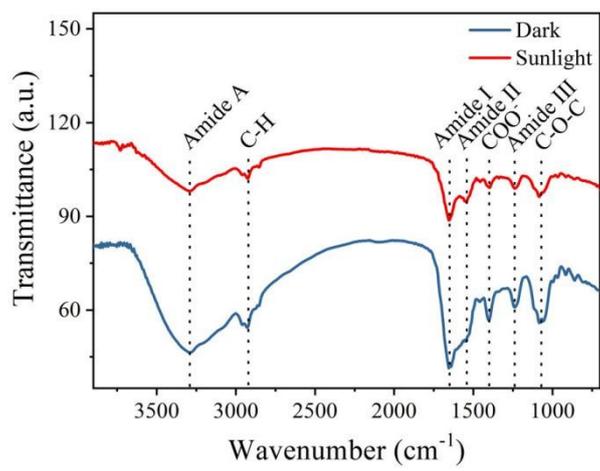


Fig. S2 FT-IR spectrum of the purified Bio-QDs assembled under sunlight irradiation and in the dark.

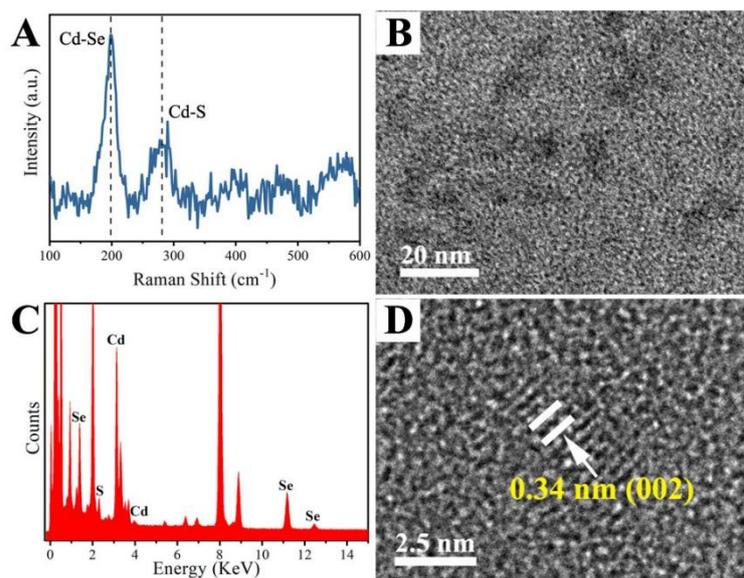


Fig. S3 Morphology and elemental composition of synthesized Bio-QDs assembled in *E. coli* under dark conditions. (a) In-situ Raman spectra of the as-prepared cells showing the characteristic signals of Cd-Se (200 cm⁻¹) and Cd-S (~280 cm⁻¹) bonds. (b) HRTEM and (c) The corresponding EDX spectrum confirming the existence of Cd, Se and S elements in purified Bio-QDs. (d) Lattice planes of the purified Bio-QDs.

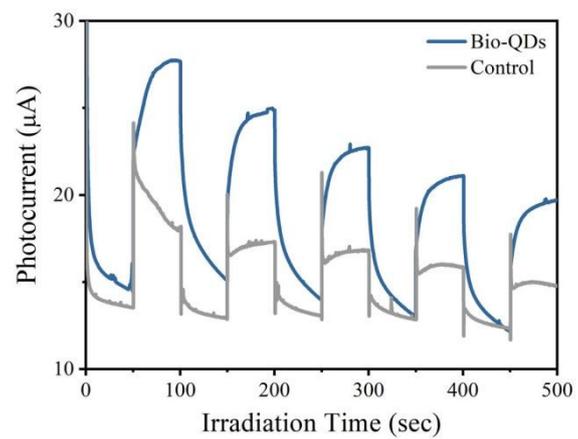


Fig. S4 Photocurrents of homogenated *E. coli* and the purified Bio-QDs assembled in *E. coli* in the dark.

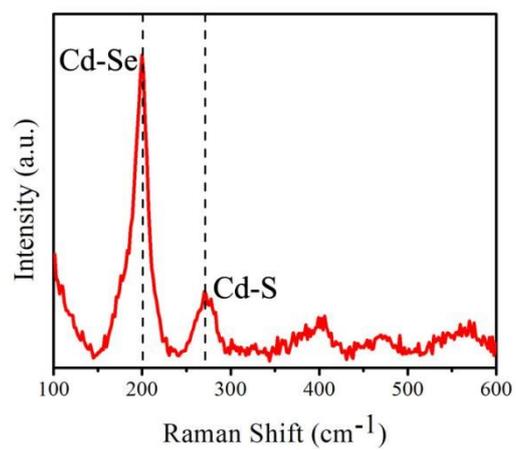


Fig. S5 In situ Raman spectrum of Bio-QDs assembled in *E. coli* under UV irradiation.

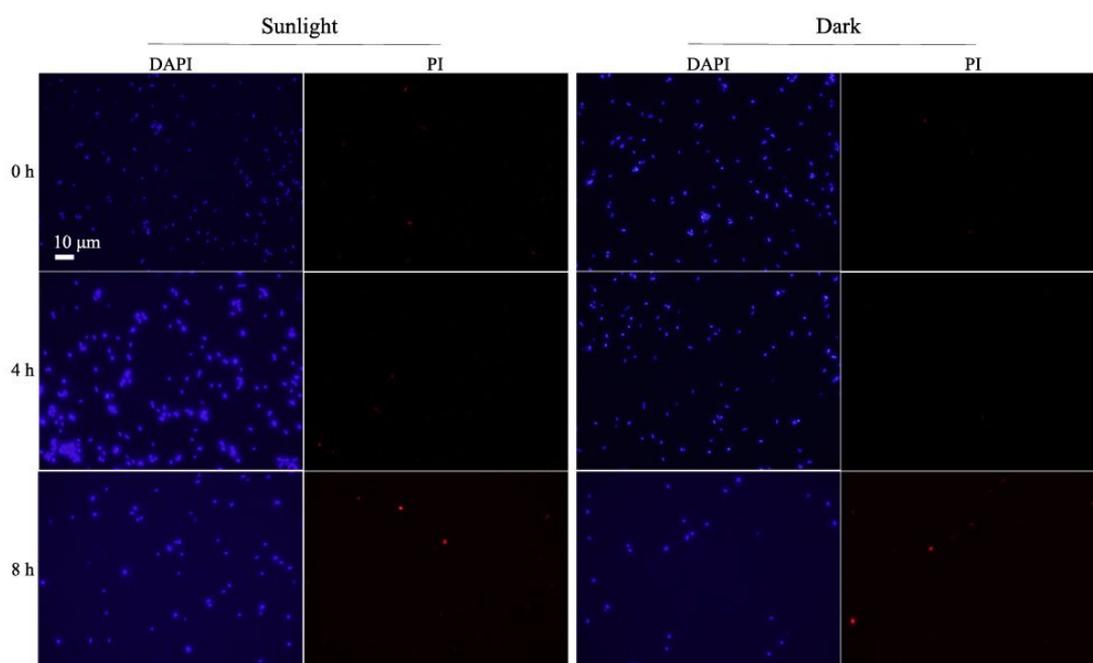


Fig. S6 Fluorescence images show the cell viabilities of *E. coli*, which assembled $\text{CdS}_x\text{Se}_{1-x}$ Bio-QDs for 0 h, 4 h and 8 h under sunlight irradiation in the dark. The blue signals stand for the alive cells and the red signals represent the dead cells.

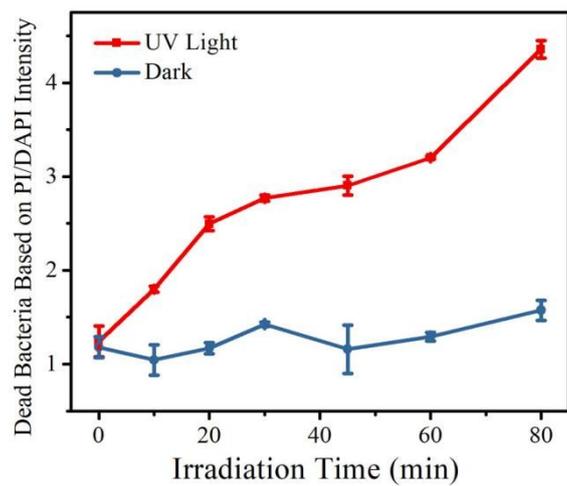


Fig. S7 Viability test of $\text{CdS}_x\text{Se}_{1-x}$ Bio-QDs assembled in *E. coli* under UV irradiation and dark conditions.

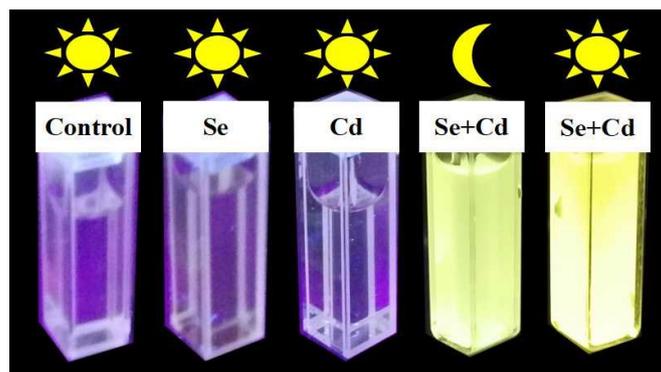


Fig. S8 Fluorescence pictures of controls and fluorescent materials assembled groups under UV irradiation in the GSH system.

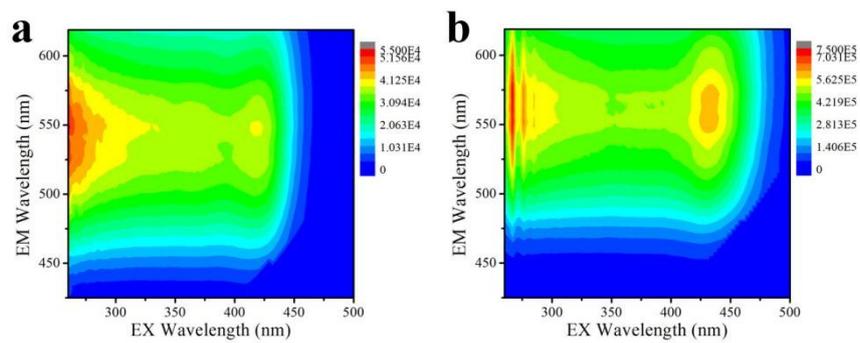


Fig. S9 EEM of fluorescence materials produced under dark (a) and UV irradiated (b) conditions in the GSH system.

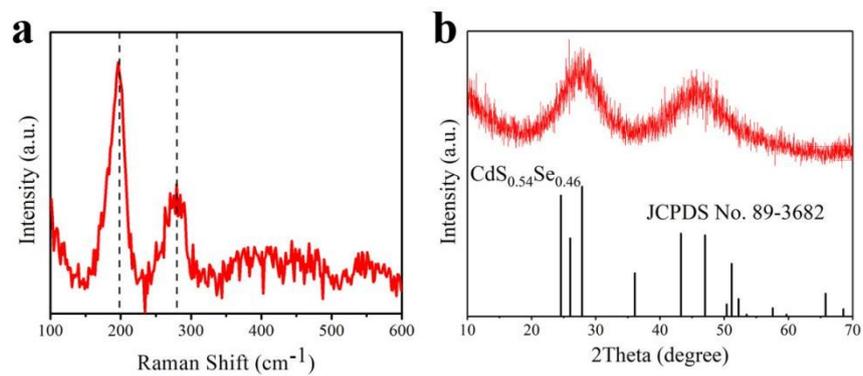


Fig. S10 Morphology of synthesized Bio-QDs assembled in the GSH system under UV light irradiation. (a) Raman spectrum showing the characteristic signals of Cd-Se (196 cm⁻¹) and Cd-S (279 cm⁻¹) bonds. (b) XRD patterns of purified Bio-QDs.

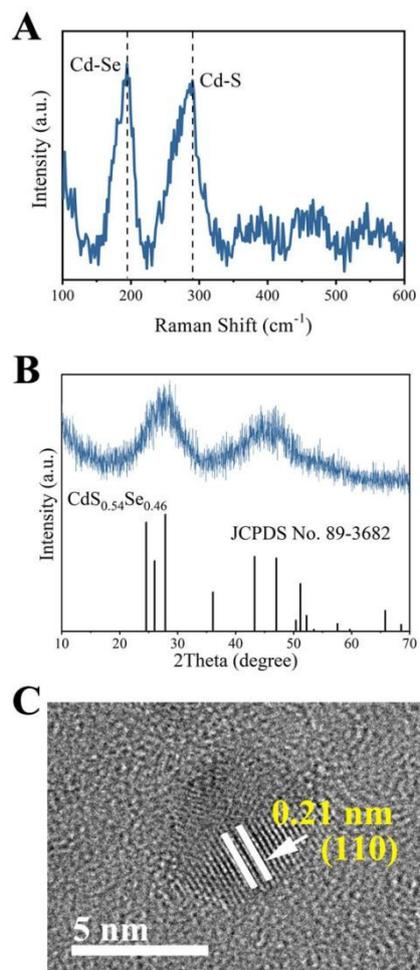


Fig. S11 Morphology of synthesized Bio-QDs assembled in the GSH system under dark conditions. (a) Raman spectrum showing the characteristic signals of Cd-Se (194 cm⁻¹) and Cd-S (290 cm⁻¹) bonds. (b) XRD patterns of purified Bio-QDs. (c) Lattice planes of assembled Bio-QDs.

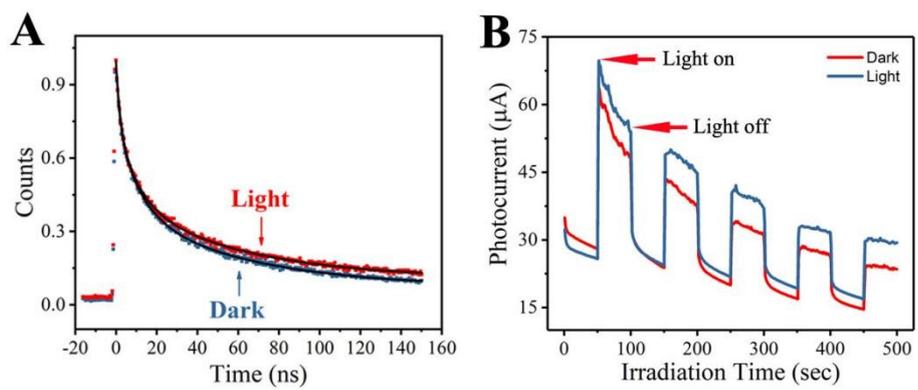


Fig. S12 Fluorescence lifetimes (a) and photocurrents (b) of Bio-QDs assembled in the GSH system under UV light irradiated and dark conditions.

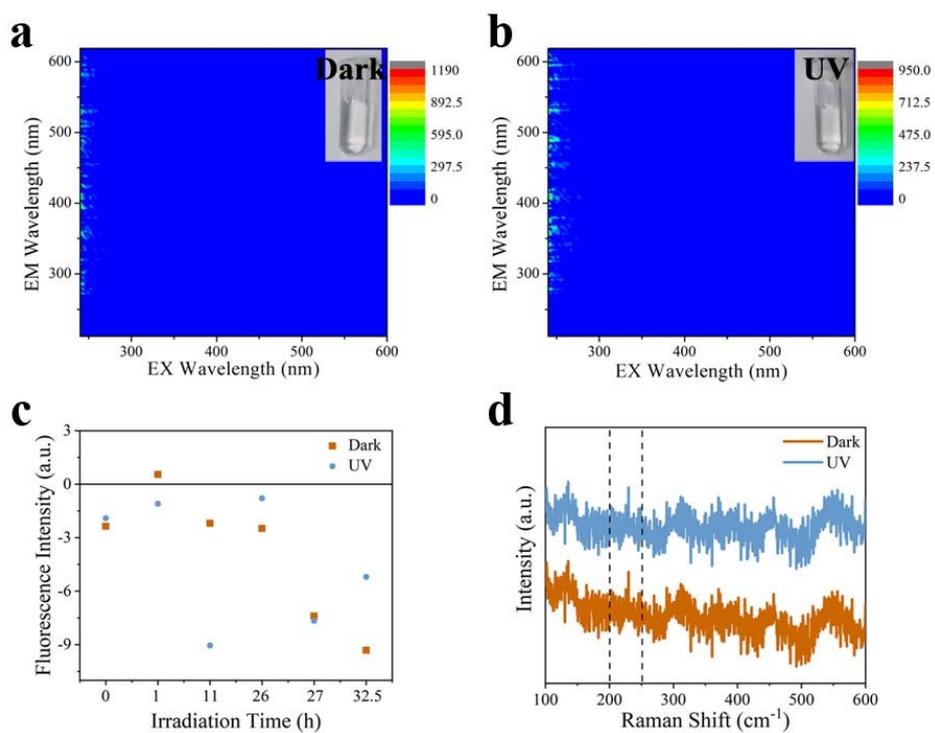


Fig. S13 *In vivo* characteristics of the samples collected from the systems with/without 32.5 h UV illumination. The systems contained 10 mM Tris-HCl buffer, 500 μM Na_2SeO_3 and 500 μM CdCl_2 . (a, b) Fluorescence EEM spectrum of the samples. Inserts images were collected under bright field. (c) Fluorescence intensities and (d) Raman spectra of the samples. Fluorescence intensities were generated at a 435 nm of excitation wavelength and 550 nm of emission wavelength.

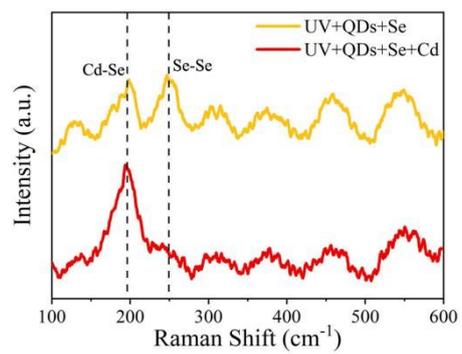


Fig. S14 Raman spectra of the samples collected from the systems with 32.5 h UV irradiation. The systems contained 10 mM Tris-HCl buffer, 500 μ M Na₂SeO₃, 500 μ M CdCl₂, 2.5% methanol and GSH-synthesized QDs.

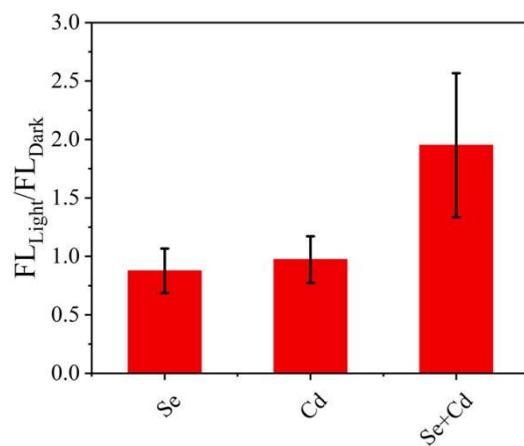


Fig. S15 Ratio of the fluorescence intensities of the UV light-irradiated and dark systems in samples with 32.5 h UV irradiation. The systems contained 10 mM Tris-HCl buffer, 2.5% methanol, GSH-synthesized QDs, and different combinations of precursors.

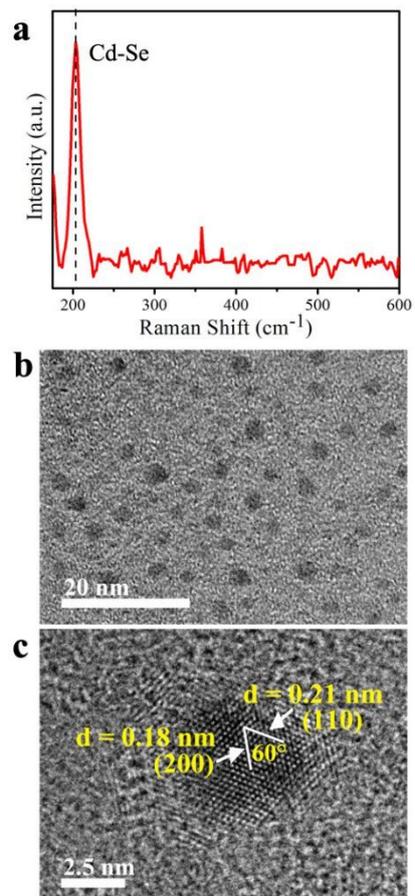


Fig. S16 Morphology of synthesized Bio-QDs assembled in *T. pyriformis* under UV light irradiation. (a) In-situ Raman spectrum of the as-prepared cells showing the characteristic signals of Cd-Se (203 cm⁻¹) bonds. HRTEM (b) and lattice planes (c) of the purified Bio-QDs.

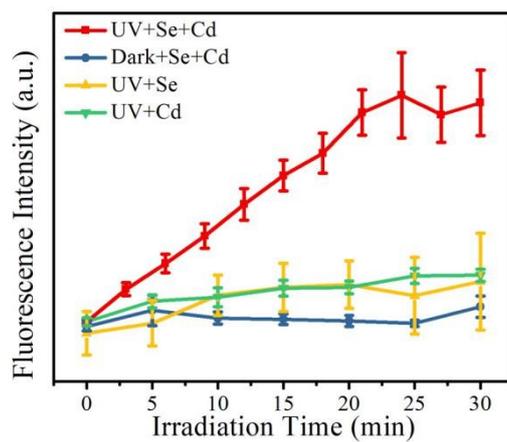


Fig. S17 Fluorescence intensities of Na_2SeO_3 - and CdCl_2 -treated *T. pyriformis* cells over time. The recorded fluorescence intensities were acquired using a 396 nm excitation wavelength and 525 nm emission wavelength.

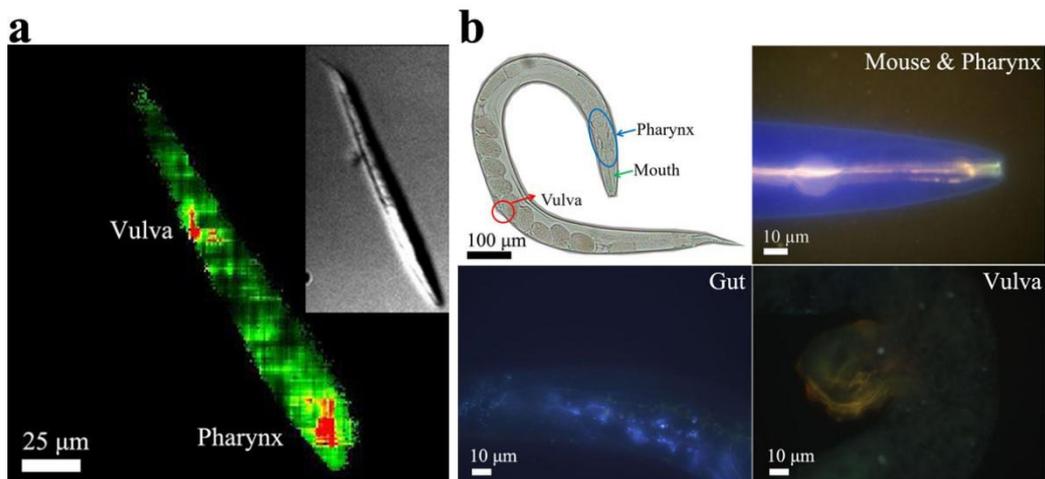


Fig. S18 (a) FLIM and corresponding bright field images of *C. elegans* after 8-h soaking in Bio-QDs medium. The red and green color represent the high and low amounts of longer fluorescence lifetime materials (Bio-QDs). (b) Bio-QDs location in *C. elegans* was recorded by fluorescence microscopy. Fluorescence images show that Bio-QDs became aggregated in pharynx and vulva and dispersed in gut. The blue fluorescence was attributed to the autofluorescence of *C. elegans*. The yellow fluorescence was assigned to Bio-QDs.

References

- 1 C. R. Carvalho, W. R. Clarindo, M. M. Praça, F. S. Araújo and N. Carels, *Plant Sci.*, 2008, **174**, 613-617.
- 2 C. J. Zhu, Y. Peng, Z.-H. Tong, L.-Y. Lu, Y.-H. Cui, H.-Q. Yu, *Chemosphere*, 2016, **157**, 65-70.
- 3 X. Wu, Z.-H. Tong, L.-L. Li, H.-Q. Yu, *Chemosphere*, 2013, **93**, 2399-2404.
- 4 A. Tu and P. D. Persans, *Appl. Phys. Lett.*, 1991, **58**, 1506-1508.
- 5 S. H. Kang, K. N. Bozhilov, N. V. Myung, A. Mulchandani and W. Chen, *Angew. Chem. Int. Edit.*, 2008, **47**, 5186-5189.
- 6 C. Mi, Y. Wang, J. Zhang, H. Huang, L. Xu, S. Wang, X. Fang, J. Fang, C. Mao and S. Xu, *J. Biotechnol.*, 2011, **153**, 125-132.
- 7 F. A. Venegas, L. A. Saona, J. P. Monras, N. Ordenes-Aenishanslins, M. F. Giordana, G. Ulloa, B. Collao, D. Bravod and J. M. Perez-Donoso, *RSC Adv.*, 2017, **7**, 40270-40278.
- 8 G. Ulloa, C. P. Quezada, M. Araneda, B. Escobar, E. Fuentes, S. A. Álvarez, M. Castro, N. Bruna, R. Espinoza-González, D. Bravo and J. M. Pérez-Donoso, *Front. Microbiol.*, 2018, **9**, 234.
- 9 T. J. Park, S. Y. Lee, N. S. Heo and T. S. Seo, *Angew. Chem. Int. Edit.*, 2010, **49**, 7019-7024.
- 10 J. P. Monras, V. Diaz, D. Bravo, R. A. Montes, T. G. Chasteen, I. O. Osorio-Roman, C. C. Vasquez and J. M. Perez-Donoso, *PLoS ONE*, 2012, **7**, e48657.
- 11 Z. Yan, J. Qian, Y. Gu, Y. Su, X. Ai and S Wu, *Mater. Res. Express*, 2014, **1**, 015401.
- 12 Y. Li, R. Cui, P. Zhang, B. B. Chen, Z. Q. Tian, L. Li, B. Hu, D. W. Pang and Z. X. Xie, *ACS Nano*, 2013, **7**, 2240-2248.
- 13 M. Shao, R. Zhang, C. Wang, B. Hu, D. Pang and Z. Xie, *Nano Res.*, **11**, 2498-2511.
- 14 R. Zhang, M. Shao, X. Han, C. Wang, Y. Li, B. Hu, D. Pang, Z. Xie, *Int. J. Nanomed.*, 2017, **12**, 3865-3879.
- 15 G. Chen, B. Yi, G. Zeng, Q. Niu, M. Yan, A. Chen, J. Du, J. Huang and Q. Zhang, *Colloid. Surface. B*, 2014, **117**, 199-205.
- 16 S. R. Stürzenbaum, M. Höckner, A. Panneerselvam, J. Levitt, J. S. Bouillard, S. Taniguchi, L. A. Dailey, R. A. Khanbeigi, E. V Rosca, M. Thanou, K. Suhling, A. V. Zayats and M. Green, *Nat. Nanotechnol.*, 2013, **8**, 57-60.