Supporting Information for

Photocatalysts encapsulation and amidation-mediated cell anchoring for advancing photosynthetic biohybrids

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Experimental

Synthesis of $CdS@SiO_2$ spheres

$CdS@SiO_2$ core-shell spheres were synthesized in reverse microemulsion method. Three reverse microemulsions containing nonylphenol ethoxylate-7 (NP-7), *n*-butanol, cyclohexane and aqueous phase were prepared with the weight ratios of 2:1:4:1, where the aqueous phase was 0.2 M $Cd(NO_3)_2$ solution, 0.2 M Na_2S solution and ammonia solution, respectively. For a typical synthesis of the reverse microemulsions, NP-7 and *n*-butanol were slowly added into cyclohexane under vigorous stirring. After complete dissolution of the three reagents, the aqueous phase ($Cd(NO_3)_2$ solution, Na_2S solution, or ammonia solution) was added dropwise upon stirring, and the clear reverse microemulsion was obtained. For the synthesis of $CdS@SiO_2$ spheres, 5 mL of $Cd(NO_3)_2$ microemulsion were added dropwise to 5 mL of Na_2S microemulsion under vigorous stirring, and the mixture was aged for 2 h. 10 mL of ammonia microemulsion and 1 mL of tetraethoxysilane (TEOS) were then added dropwise to the above mixture under vigorous stirring, followed by further aging for 24 h. Finally, 5 mL of acetone was added in the mixture, and the resulting $CdS@SiO_2$ product was separated by centrifugation, washed three times each with ultrapure water and absolute ethanol, and dried in a vacuum oven at 60 °C for 12 h. CdS nanoparticles without SiO_2 shell were also prepared under the same condition except that ammonia microemulsion and TEOS were omitted.

Surface-protected etching and surface functionalization of $CdS@SiO_2$ spheres

Surface-protected etching of the SiO_2 shell was applied to prepare mesoporous $CdS@SiO_2$ spheres. 100 mg of $CdS@SiO_2$ nanospheres and 100 mg of poly(vinylpyrrolidone) (PVP) were dispersed in 20 mL of ultrapure water and sonicated for 10 min. The mixture was then heated up to 60 °C under stirring. 1 mL of ammonia solution was added dropwise after 1 h, and maintain at 60 °C under stirring for an additional 30 min. The product was separated by centrifugation, washed three times each with ultrapure water and absolute ethanol, and dried in a vacuum oven at 60 °C for 12 h.

For the surface functionalization of mesoporous CdS@SiO_2 spheres with carboxyl group, 2 mL of (3-aminopropyl)triethoxysilane (APTES) and 1.0 g of succinic anhydride were dissolved in 20 mL of N,N-dimethylformamide (DMF). Mesoporous CdS@SiO_2 spheres were dispersed in the mixture by sonication for 10 min, and stirred at room temperature for 5 h. The product was separated by centrifugation, washed three times each with ultrapure water and absolute ethanol, and dried in a vacuum oven at 60 °C for 12 h.

Media and solutions

Luria-Bertani (LB) medium: 5 g L^{-1} of yeast extract, 10 g L^{-1} of tryptone, 10 g L^{-1} of NaCl, and pH of 7.0.

LB solid medium: 5 g L^{-1} of yeast extract, 10 g L^{-1} of tryptone, 10 g L^{-1} of NaCl, 15 g L^{-1} agar, and pH of 7.0.

LB+MOPS medium: 5 g L^{-1} of yeast extract, 10 g L^{-1} of tryptone, 10 g L^{-1} of NaCl, 20 mM of 3-(N-morpholino)propanesulfonic acid (MOPS), and pH of 7.0.

Phosphate buffer saline (PBS): 0.24 g L^{-1} of KH_2PO_4, 3.6 g L^{-1} of $Na_2HPO_4 \cdot 12H_2O$, 0.2 g L^{-1} of KCl, 8.0 L^{-1} of NaCl, and pH of 7.4.

Tris buffer: 1.21 g L^{-1} of tris(hydroxymethyl)aminomethane (Tris), and pH of 7.4.

Trace element solution for ATYP, UPM, and NFM media: 0.25 g L^{-1} of EDTA, 0.5 g L^{-1} of $FeSO_4$, 24.8 mg L^{-1} of $Co(NO_3)_2$, 17.7 mg L^{-1} of $Na_2B_4O_7$, 24.8 mg L^{-1} of $CuSO_4$, 154 mg L^{-1} of $MnSO_4$, 1.095 g L^{-1} of $ZnSO_4$, and pH of 6.5.

Vitamin Solution for ATYP medium: 0.5 mg L^{-1} of boric acid, 0.01 mg L^{-1} of Na_2SeO_3, 0.01 g L^{-1} of Na_2MoO_4, 0.01 mg L^{-1} of biotin, 0.035 mg L^{-1} of nicotinic acid, 0.01 mg L^{-1} of calcium pantothenate, 0.03 mg L^{-1} of thiamine hydrochloride, 0.005 mg L^{-1} of vitamin B12, 0.01 mg L^{-1} of pyridoxine hydrochloride, and 0.02 mg L^{-1} of aminobenzoic acid.

ATYP medium: 1 g L^{-1} of KH_2PO_4, 0.1 g L^{-1} of $CaCl_2$, 3 g L^{-1} of $NaHCO_3$, 0.5 g L^{-1} of $MgCl_2$, 1 g L^{-1} of NH_4Cl, 1 g L^{-1} of NaCl, 1 g L^{-1} of sodium acetate, 1 g L^{-1} of succinic acid, 0.5 g L^{-1} of yeast extract, 0.5 g L^{-1} of tryptone, 1 mL L^{-1} of trace element solution, 1 mL L^{-1} of vitamin solution, and pH of 6.8.

UPM medium: 16 g L^{-1} of $Na_2HPO_4 \cdot 12H_2O$, 6.8 g L^{-1} of KH_2PO_4, 4 g L^{-1} of CH_3COONa, 1 g L^{-1} of yeast extract, 0.002 g L^{-1} of $FeSO_4$, 0.0002 g L^{-1} of $(NH_4)_2MoO_4$, 0.58 g L^{-1} of $MgSO_4$, 0.067 g L^{-1} of $CaCl_2$, 1 mL L^{-1} of trace element solution, and pH to 6.8.

NFM medium: 16 g L^{-1} of $Na_2HPO_4 \cdot 12H_2O$, 6.8 g L^{-1} of KH_2PO_4, 0.58 g L^{-1} of $MgSO_4$, and 0.067 g L^{-1} of $CaCl_2$, 1 mL L^{-1} of trace element solution, and pH to 6.8.

Ferrous sulfate solution for DSMZ medium: 1 g L^{-1} of $FeSO_4$, and 0.05 M H_2SO_4.

Trace element solution for DSMZ medium: 1.5 g L^{-1} of $FeCl_2$, 10 mL L^{-1} of 25% HCl, 70 mg L^{-1} of $ZnCl_2$, 100 mg L^{-1} of $MnCl_2$, 6 mg L^{-1} of boric acid, 190 mg L^{-1} of $CoCl_2$, 2 mg L^{-1} of $CuCl_2$, 24 mg L^{-1} of $NiCl_2$, and 36 mg L^{-1} of Na_2MoO_4.

Vitamin solution for DSMZ medium: 20 mg L^{-1} of biotin, 20 mg L^{-1} of folic acid, 100 mg L^{-1} of pyridoxine hydrochloride, 50 mg L^{-1} of thiamine hydrochloride, 50 mg L^{-1} of riboflavin, 50 mg L^{-1} of nicotinic acid, 50 mg L^{-1} of calcium pantothenate, 1 mg L^{-1} of vitamin B12, 50 mg L^{-1} of p-aminobenzoic acid, and 50 mg L^{-1} of α-lipoic acid.

DSMZ Medium: 0.35 g L^{-1} of K_2HPO_4, 0.23 g L^{-1} of KH_2PO_4, 0.5 g L^{-1} of NH_4Cl, 0.5 g L^{-1} of $MgSO_4$, 0.25 g L^{-1} of $CaCl_2$, 2.25 g L^{-1} of NaCl, 2 g L^{-1} of yeast extract, 2 mL L^{-1} of ferrous sulfate solution, 1 mL

L^{-1} of trace element solution, 2 g L^{-1} of $NaHCO_3$, 0.3 g L^{-1} of L-cysteine, 1 mL L^{-1} of vitamin solution, 20 mL L^{-1} of 50% methanol solution, and pH of 6.5.

Saline solution (0.9%): 9 g L^{-1} of NaCl.

Culture of *Shewanella oneidensis* MR-1

Shewanella oneidensis MR-1 was cultured in 100 mL of LB+MOPS medium under shaking (180 rpm, 30 °C) until the optical density (OD_{600}) reached of 0.6-0.8. A fresh sterilized solution containing L-cysteine (10 mM) sodium fumarate (25 mM) and sodium lactate (18 mM) was added to the cultured bacterial solution, and further incubated anaerobically under shaking (180 rpm, 30 °C) for 20 hours to accumulate hydrogenase.

Culture of *Rhodopseudomonas palustris*

Rhodopseudomonas palustris was anaerobically incubated in ATYP medium with shaking (180 rpm, 30°C) under 50 W red light illumination. 1 mL of the bacterial culture was transferred into 100 mL of UPM medium, and incubated under shaking (180 rpm, 30 °C) until $OD_{600} = 0.35$.

Culture and starvation of *Methanosarcina barkeri*

Methanosarcina barkeri was anaerobically incubated in DSMZ medium at 37 °C. The culture was then centrifugally washed three times with saline solution in an anaerobic workstation, resuspended in saline, and anaerobically incubated at 37°C for 7 days.

Construction of the CdS@SiO_2 and *Shewanella oneidensis* MR-1 hybrid system

The biohybrid system based on *Shewanella oneidensis* MR-1 and CdS@SiO_2 was constructed via amidation-based covalent coupling. 45 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 25 mg of N-hydroxysuccinimide (NHS) were dissolved in 20 mL of PBS buffer. 10 mg of etched and functionalized CdS@SiO_2 was dispersed and activated in the buffer under continuous stirring at room temperature for 20 min. The product was collected by centrifugation, and washed three times with PBS.

The cultured *Shewanella oneidensis* MR-1 was collected by centrifugation, and washed three times with PBS in an anaerobic workstation. The cells were redispersed in PBS with an OD_{600} of 1. The activated CdS@SiO_2 was added to the bacterial suspension at a concentration of 1 g L^{-1}. The mixture was subsequently incubated under shaking (180 rpm, 30 °C) for 90 minutes. The CdS@SiO_2 and *Shewanella oneidensis* MR-1 hybrid system was denoted as "CdS@SiO_2/*SW*".

In addition, "CdS/*SW*" was prepared by hybridizing CdS nanoparticles without SiO_2 shell and *Shewanella oneidensis* MR-1, and "CdS@SiO_2/*SW**" was prepared by hybridizing CdS@SiO_2 (without surface functionalization of carboxyl group) and *Shewanella oneidensis* MR-1.

Construction of the CdS@SiO_2 and *Methanosarcina barkeri* hybrid system

The cultured *Methanosarcina barkeri* strain was washed three times with PBS buffer in an anaerobic workstation and resuspended to an OD_{600} of 1. The activated CdS@SiO_2 was added to the bacterial suspension at a concentration of 1 g L^{-1}. The mixture was subsequently incubated under shaking (180 rpm, 37 °C) for 90 minutes. The CdS@SiO_2 and *Methanosarcina barkeri* hybrid system was denoted as "CdS@SiO_2/*Mb*". In addition, "CdS@SiO_2/*Mb**" was prepared by hybridizing *Methanosarcina barkeri* and CdS@SiO_2 without surface functionalization of carboxyl group.

Construction of the CdS@SiO_2 and *Rhodopseudomonas palustris* hybrid system

The cultured *Rhodopseudomonas palustris* strain was washed three times with PBS buffer in an anaerobic workstation and resuspended to an OD_{600} of 1. The activated CdS@SiO_2 was added to the bacterial suspension at a concentration of 1 g L^{-1}. The mixture was subsequently incubated under shaking (180 rpm, 30 °C) for 90 minutes. The CdS@SiO_2 and *Rhodopseudomonas palustris* hybrid system was denoted as "CdS@SiO_2/*Rp*".

Characterization

The biohybrids were fixed with 2.5% glutaraldehyde for 8-10 h, and dehydrated in 30%, 50%, 70%, 80%, 90%, and 100% ethanol sequentially, with each lasting 2 h. X-ray power diffraction (XRD) was performed on a Bruker D8 Advance powder diffractometer using Cu Kα radiation (λ = 1.5406 Å). Morphological analyses were conducted via scanning electron microscopy (SEM, Regulus-8100, Hitachi, Japan), and transmission electron microscopy (TEM, HT-7800, Hitachi, Japan). Energy-dispersive X-ray spectroscopy (EDS) was performed using the same SEM system (Regulus-8100, Hitachi, Japan). High-resolution transmission electron microscopy was performed on a TF-G20 (Thermo Fisher Scientific) microscope. X-ray photoelectron spectroscopy (XPS) measurements were carried out with a Shimadzu AXIS-Ultra DLD system employing monochromatic Al Kα X-ray radiation. UV-visible (UV-vis) diffuse reflectance spectra were obtained using a Shimadzu UV-2600 spectrophotometer.

Quantitative analysis of bacterial viability

Bacterial viability was assessed using the LIVE/DEAD® BacLignt^{TM} Bacterial Viability Kit (Thermo Fisher Scientific) in combination with fluorescence microscope (MF-31, Maiying, Guangzhou, China).

At designated time intervals during the 72-hour reaction, aliquots of the bacterial suspensions were collected and stained according to the kit protocol. The stained samples were immediately observed and imaged using a fluorescence microscope. Semi-automated counting of the live cells (stained green by SYTO 9) and dead cells (stained red by propidium iodide) was performed using ImageJ software. Cell viability was then calculated as the ratio of live cells to the total number of cells. The quantitative cell viability kinetics were obtained from the time-dependent cell viability.

Photocatalytic gas production analysis

10 mL of the CdS@SiO_2 and *Shewanella oneidensis* MR-1 hybrid suspension (containing

approximately 10 mg of CdS@SiO_2) was washed three times with Tris buffer (10 mM, pH = 7.4), then resuspended in 10 mL of a freshly prepared 50 mM Na_2SO_3 solution (in Tris buffer) with the bacterial concentration adjusted to OD_{600} = 1. When indicated, riboflavin was added to a final concentration of 50 mg L^{-1}. The suspension was transferred into a 20 mL nitrogen-filled sealed vial. The vial was then placed under LED (TC-COB-305, Langdisi, Zhongshan, China) irradiation under shaking (180 rpm, 30 °C) for photocatalytic hydrogen evolution reaction.

10 mL of the CdS@SiO_2 and *Methanosarcina barkeri* hybrid suspension (containing approximately 10 mg of CdS@SiO_2) was washed three times with Tris buffer, then resuspended in 10 mL of a freshly prepared 10 mM of $NaHCO_3$, 10 mM of Na_2SO_3 and 10 mM of L-cysteine solution (in Tris buffer) with the bacterial concentration adjusted to OD_{600} = 1. The suspension was transferred into a 20 mL nitrogen-filled sealed vial. The vial was then placed under LED irradiation or 250 W xenon lamp under shaking (180 rpm, 37 °C) for photocatalytic methane production analysis.

10 mL of the CdS@SiO_2 and *Rhodopseudomonas palustris* hybrid suspension (containing approximately 10 mg of CdS@SiO_2) was washed three times with NFM buffer, then resuspended in 10 mL of a freshly prepared solution containing 10 mM of $NaHCO_3$ and either 10 mM Na_2SO_3 solution or 10 mM triethanolamine solution (in NFM buffer) with the bacterial concentration adjusted to OD_{600} = 1. The suspension was transferred into a 20 mL nitrogen-filled sealed vial. The vial was then placed under irradiation under shaking (180 rpm, 30 °C) for photocatalytic methane production analysis.

All reactions were performed under a top-illumination setup. The distance between the light source and the sample vial was fixed to maintain a constant optical power density of 3.20 mW/cm^2 at the top surface of the bacterial suspension. For gas analysis, 1 mL of headspace gas was extracted per sampling using a syringe. H_2 production was quantified by gas chromatography (GC7900, Tianmei, Shanghai, China) equipped with a 5 Å molecular sieve column and a thermal conductivity detector (TCD). CH_4 was measured using the same GC model with a flame ionization detector (FID) and high-purity nitrogen as the carrier gas. All experiments were performed in triplicate, and results are reported as mean values with standard deviations.

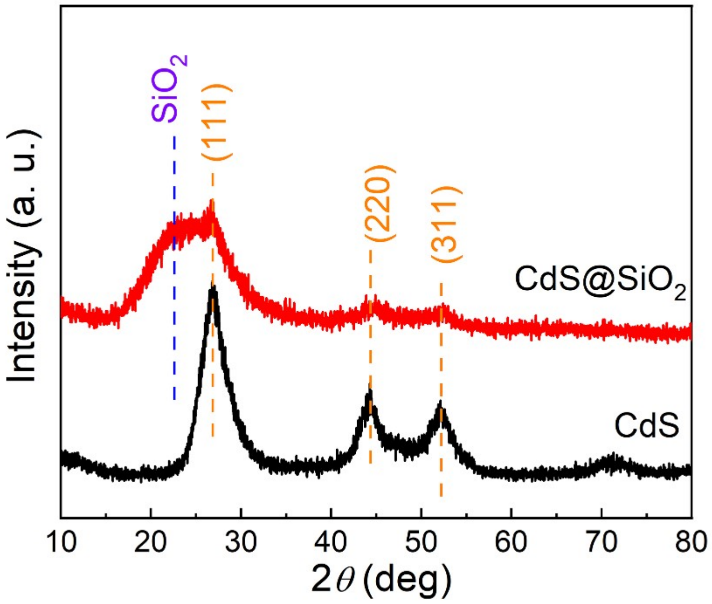


Fig. S1 XRD patterns of CdS nanoparticles and $CdS@SiO_2$ spheres.

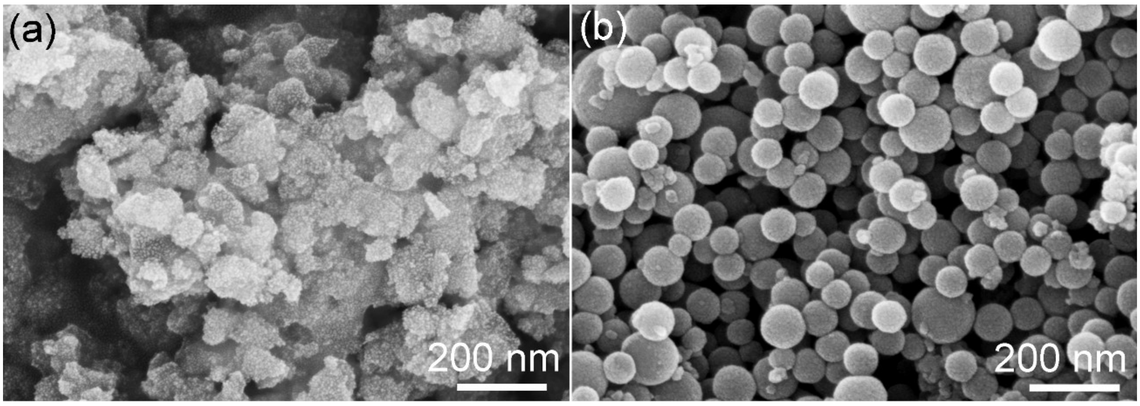
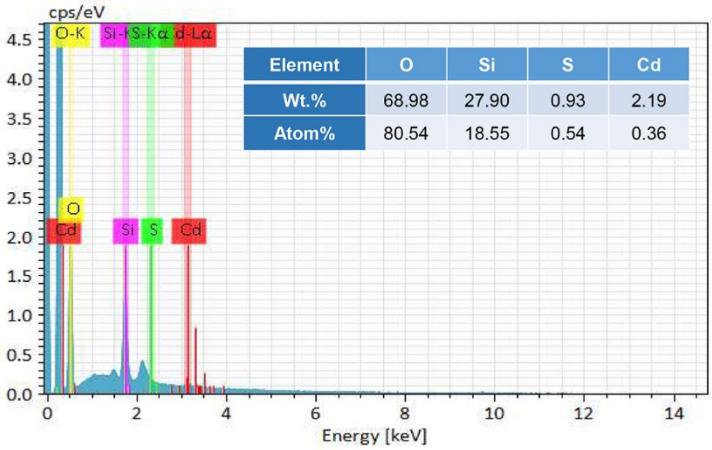


Fig. S2 SEM images of (a) CdS nanoparticles, and (b) $CdS@SiO_2$ spheres.



Element	O	Si	S	Cd
Wt.%	68.98	27.90	0.93	2.19
Atom%	80.54	18.55	0.54	0.36

Fig. S3 EDS analysis of CdS@SiO_2.

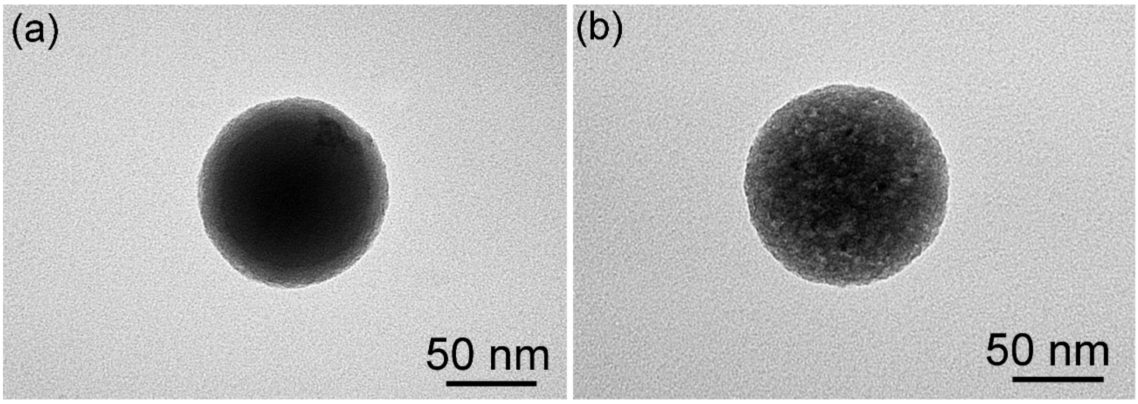


Fig. S4 TEM images of CdS@SiO_2 sphere (a) before and (b) after the etching and subsequent surface functionalization.

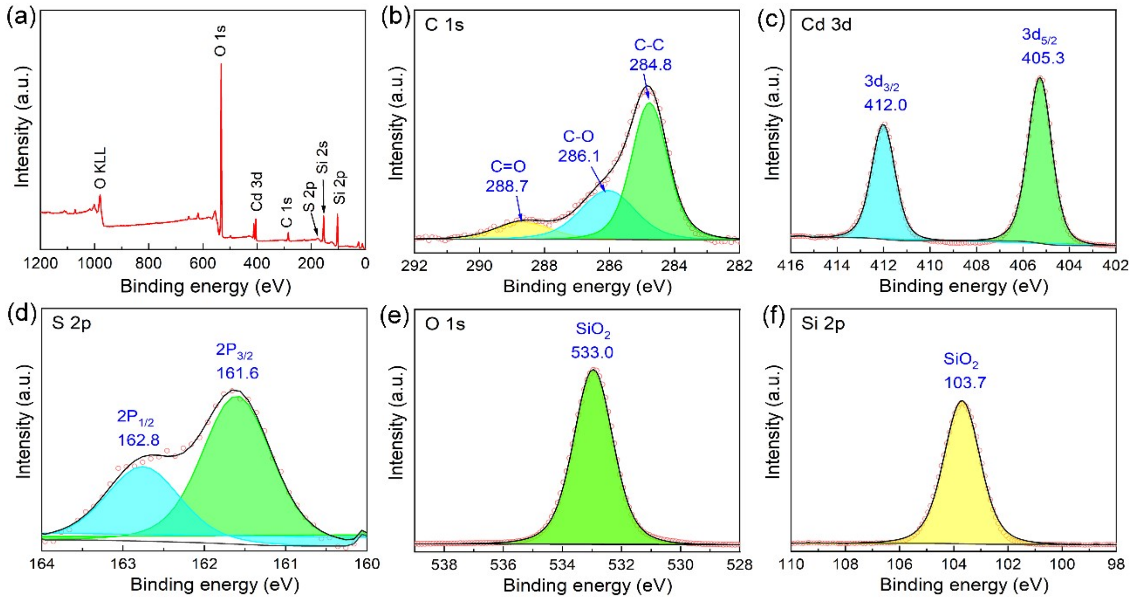


Fig. S5 XPS spectra of CdS@SiO$_2$: (a) survey, (b) C 1s, (c) Cd 3d, (d) S 2p, (e) O 1s, and (f) Si 2p.

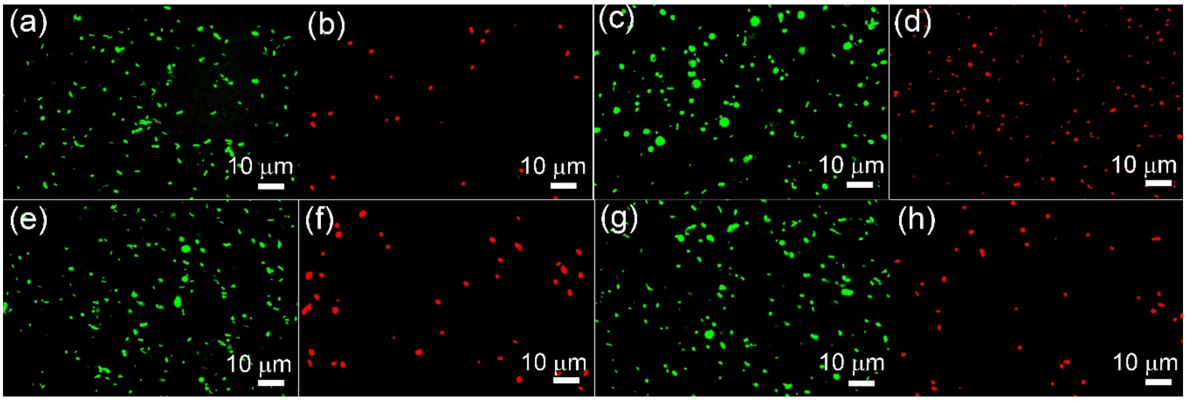


Fig. S6 Live/dead fluorescence staining images after 3 days of photocatalysis using Na$_2$SO$_3$ as the sacrificial agent for: (a,b) *SW*, (c,d) CdS/*SW*, (e,f) CdS@SiO$_2$/*SW*, and (g,h) CdS@SiO$_2$/*SW**.

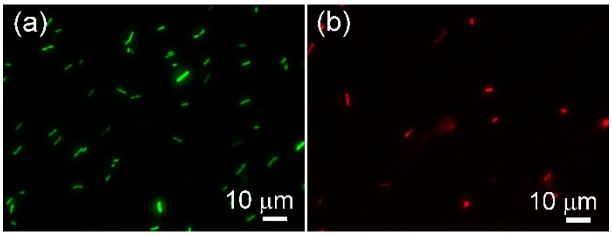


Fig. S7 Live/dead fluorescence staining images of CdS@SiO$_2$/*SW* control sample after dark incubation for 3 days.

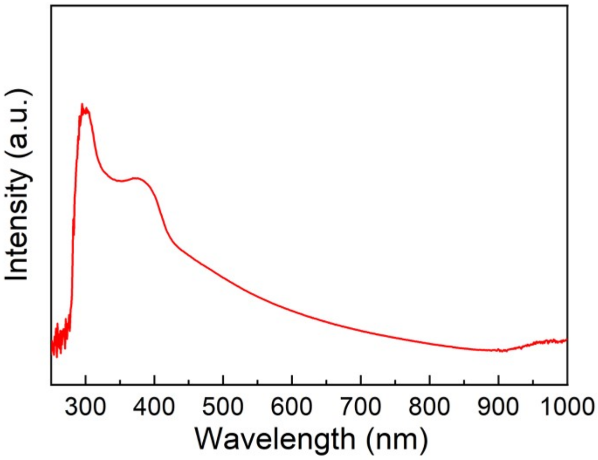


Fig. S8 UV-visible absorption spectrum of CdS@SiO$_2$/*Rp* biohybrid.