Supplementary Information

A Synthetic Protein Plug for Assembly of Bio-nano-hybrids with Direct Electron Transfer Powered Photobiocatalysis

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Materials and methods

1. Microbial Cultures and Media

Escherichia coli K12, *Shewanella oneidensis* MR-1, *Bacillus subtilis* DSMZ 10, *Staphylococcus aureus* B6, *Saccharomyces cerevisiae* P11, *Methanosarcina barkeri* DSM800 were used in this study. *E. coli*, *B. subtilis* and *S. aureus* were aerobically cultivated in Luria-Bertani medium (Table S2) at 37 ^{o}C.[1] *S. oneidensis* was grown in Luria-Bertani medium at 30 ^{o}C. *S. cerevisiae* was cultured in yeast extract peptone dextrose medium (Table S3) at 30 ^{o}C.[2] *M. barkeri* was cultivated anaerobically in modified DSM311b medium (Table S4) at 37 ^{o}C.[3]

2. Construction of Synthetic Protein Plugs on Microorganisms

To construct the synthetic protein plugs, diverse microbial cells were harvested by centrifugation, washed three times with physiological saline (0.9% NaCl), and resuspended in physiological saline (OD_{600}=1.0). The cell suspension was then incubated with 50 μM azidobutyric acid NHS ester (NHS-N_3) for 1 h with gentle agitation to chemically transform surface exposed lysine residues. After the reaction, the cells were washed three times with physiological saline to remove excess NHS-N_3, yielding the microbial chassis equipped with synthetic protein plugs.

3. Synthesis and Modification of Alkyne Capped Carbon Dots

Alkyne capped carbon dots (CD) were fabricated according to a previously reported method.[4] Briefly, citric acid was heated at 180 ^{o}C in air for 40 h to synthesis carboxylic acid capped carbon dots. Subsequently, the carboxylic acid surface groups were then modified using NH_2-PEG-alkyne via a carbodiimide-mediated amidation reaction to yield alkyne capped carbon dots (CD).[5]

4. "Plug-and-play" Assembly of Membrane Protein-targeted CD@SO PBHS

The membrane protein-targeted CD@*SO* PBHS were assembled via a versatile "plug-and-play" mode using the click chemistry. Briefly, prepared *S. oneidensis* cells (*SO*) equipped with synthetic protein plugs (OD_{600}=1.0) were resuspended in a reaction mixture containing 0.1 mg/mL CD nanoparticles, 50 μM copper sulfate, 500 μM BTTAA, and 5 mM sodium ascorbate.[6] The assembly process was conducted at room temperature for 1 h in the dark. The resulting CD@*SO* PBHS were collected and washed three times with normal saline to remove unbound nanoparticles.

For comparison, two control PBHS were prepared. The simple mixing PBHS (CD+*SO*) were prepared by directly mixing *S. oneidensis* cells (OD_{600}=1.0) with CD nanoparticles (0.1 mg/mL). Besides, the glycan-targeted PBHS (CD/*SO*) were assembled according to the established metabolic oligosaccharide engineering (MOE) strategy.[4] Specifically, *S. oneidensis* cells were cultured in Luria-Bertani medium supplemented with 2 mM GalNAz to incorporate azide groups into cell surface glycans. The resulting GalNAz-modified cells were then coupled with CD nanoparticles via the click chemistry to assemble the CD/*SO* PBHS.

5. Photocatalytic H_2 Production

Photocatalytic H_2 production assays were conducted to evaluate the photobiocatalytic activity of the membrane protein-targeted CD@*SO* PBHS. Firstly, cultured *S. oneidensis* cells were transferred to a sealed serum bottle containing anaerobic Luria-Bertani medium (supplemented with 18 mM sodium lactate, 10 mM cysteine, 25 mM fumaric acid, 1 mM $FeCl_3$, and 0.5 mM nickel ammonium sulfate), and incubated for 20 h at 30 ^{o}C to induce hydrogenase expression.[7] Subsequently, the cells underwent surface lysine transformation with NHS-N_3 to develop synthetic protein plugs, followed by the specific linkage of CD nanoparticles via the click chemistry. Finally, the resulting CD@*SO* PBHS were resuspended in an anaerobic photocatalytic reaction buffer (100 mM Tris-HCl, 10 mM sodium sulfite as a sacrificial electron donor, pH 7.0) to a final OD_{600} of 1.0. The photocatalytic reaction was irradiated using a 50 W LED lamp (emitting range: 400-780 nm). The amount of generated H_2 was quantified at periodic intervals using gas chromatography (7980, Techcomp, China). In parallel, bare CD nanoparticles, *S. oneidensis* cells and control PBHS were performed under the same condition. Data were collected from three biologically independent samples (n=3). The fold enhancement of H_2 production was calculated based on values rounded to one decimal place, and P values were determined by ordinary one-way ANOVA with Tukey's multiple comparisons test.

6. Characterizations

The microorganisms equipped with synthetic protein plugs were stained with AZDye 488 DBCO and imaged using confocal laser scanning microscopy (CLSM, TCS SP5, Leica, Germany). The morphological features of the CD nanoparticles and the assembled PBHS were investigated using transmission electron microscopy (TEM, HT7800, Hitachi, Japan). The surface functional groups of carbon dot nanoparticles were analyzed using a Fourier transform infrared (FTIR) spectrometer (ALPHA II, Bruker, Germany). The Fluorescence characteristics and cell viability of the assembled PBHS were evaluated using CLSM. The photoluminescence (PL) spectra were measured using a QuantaMaster^{Tm} 40 (Photo Technology International, Inc., USA) with an excitation wavelength of 337 nm. To confirm the binding localization of CD nanoparticles, membrane and intracellular protein fractions from both *S. oneidensis* and CD@*SO* PBHS were extracted using a protein extraction kit (PH1461, Phygene Life Sciences, China). The fluorescence emission spectra of the extracted protein fractions was recorded at 400 nm with a fluorospectrophotometer (F-320, Tianjin Gangdong Sci.&Tech. Co., Ltd., China). The mass of CD nanoparticles anchored onto the bacterial surface was determined based on the fluorescence intensity at 400 nm.

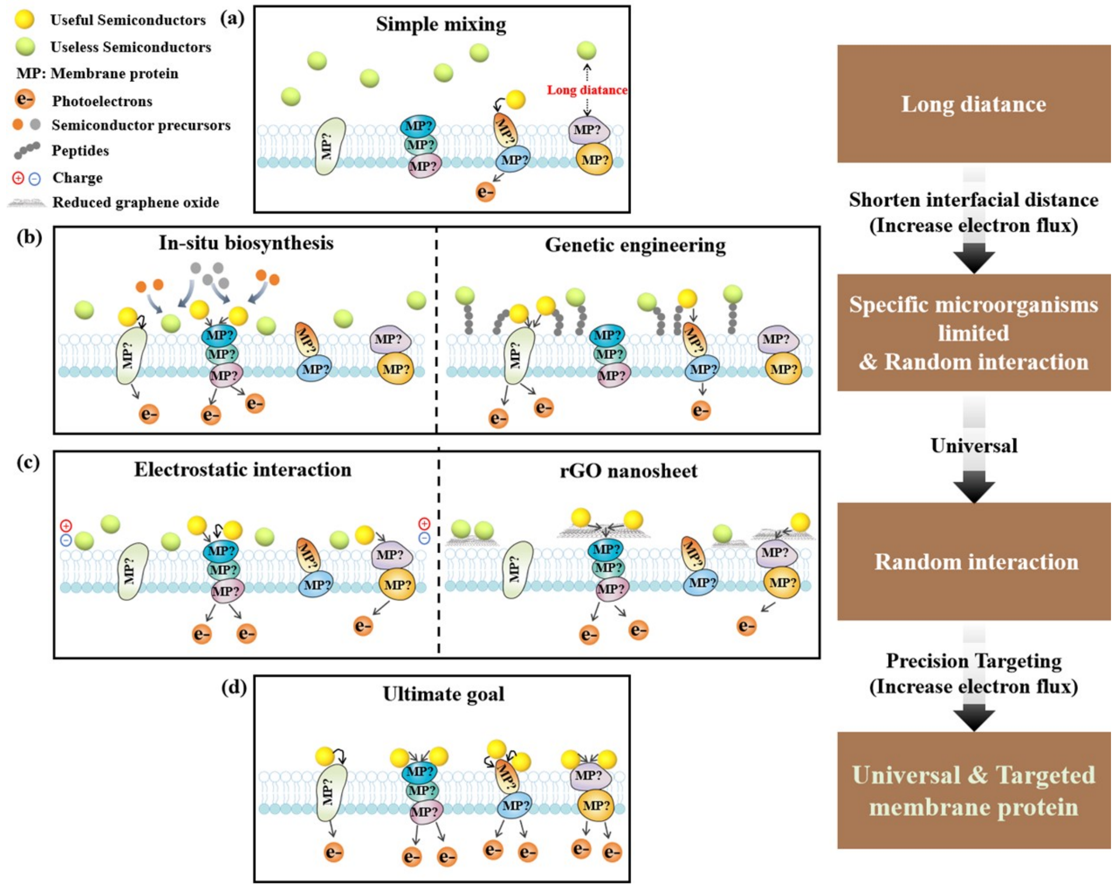

Figure S1. Charge separation and transfer in PBHS assembled with (a) simple mixing, (b) In-situ biosynthesis and genetic engineering, (c) Electrostatic interaction and rGO nanosheet, and (d) ideal PBHS proposed in this study

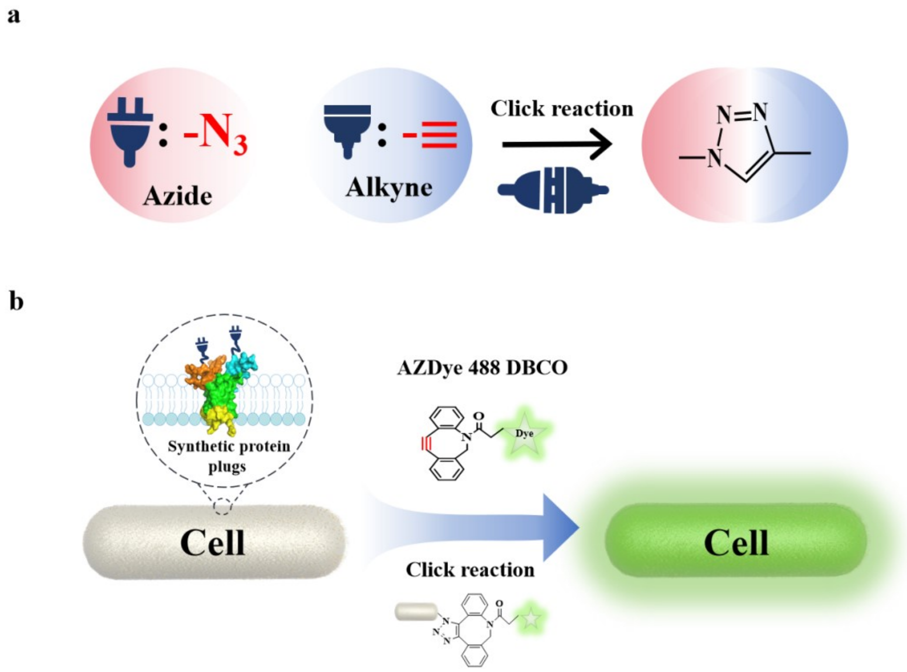

Figure S2. (a) Mechanism of click chemistry. (b) Schematic illustration of fluorescent probe AZDye 488 DBCO verifying the synthetic protein plugs

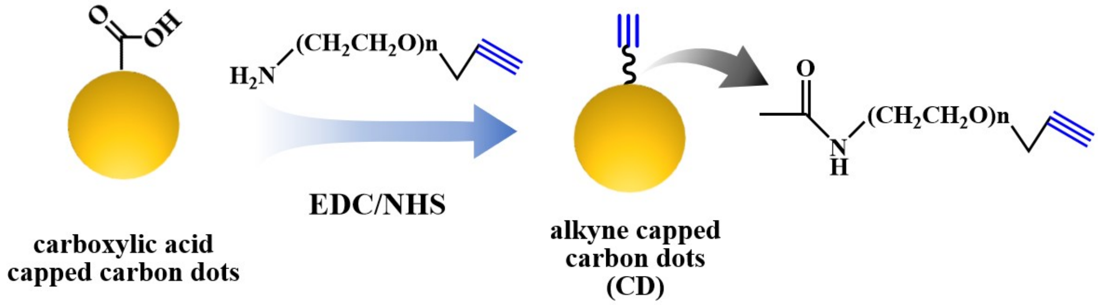

Figure S3. Schematic illustration for the fabrication of alkyne capped carbon dots (CD) from carboxylic acid capped carbon dots

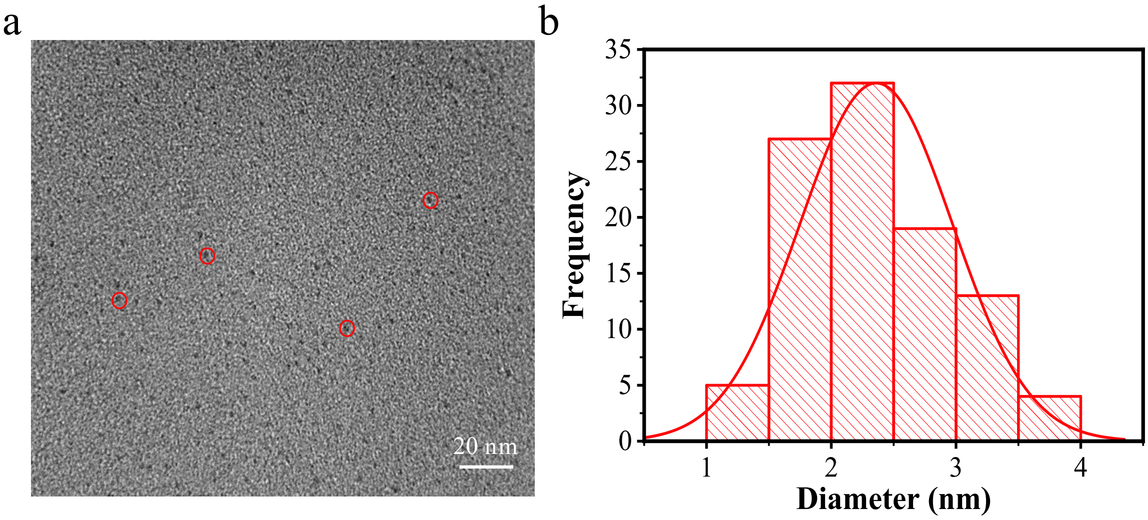

Figure S4. (a) TEM image of alkyne capped carbon dots (CD). (b) Particle size distribution map of CD nanoparticles

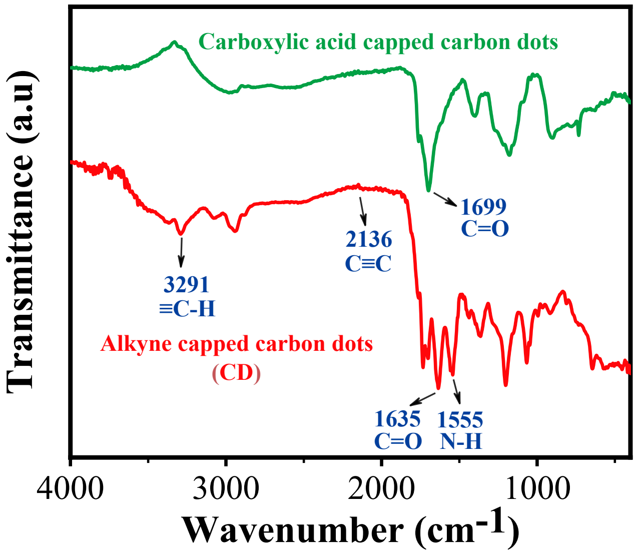

Figure S5. FTIR spectra of carboxylic acid capped carbon dots and alkyne capped carbon dots (CD)

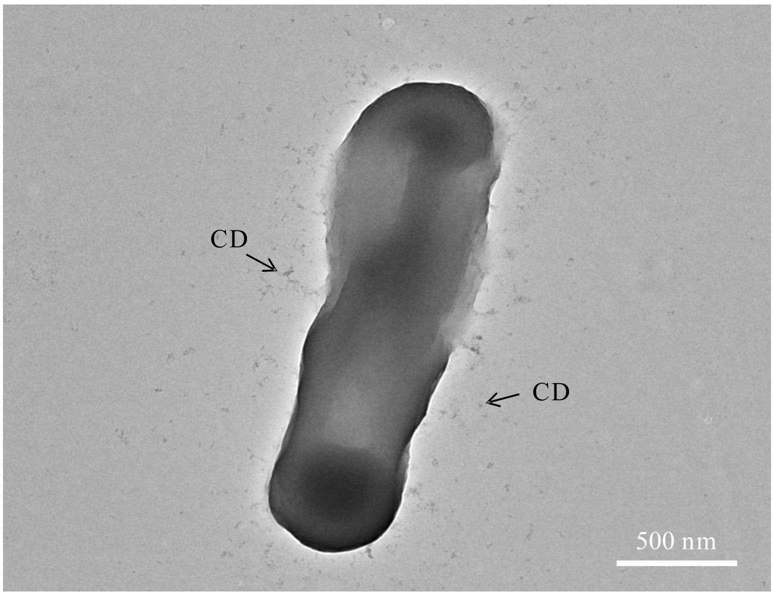

Figure S6. TEM image of simple mixing PBHS CD+*SO*

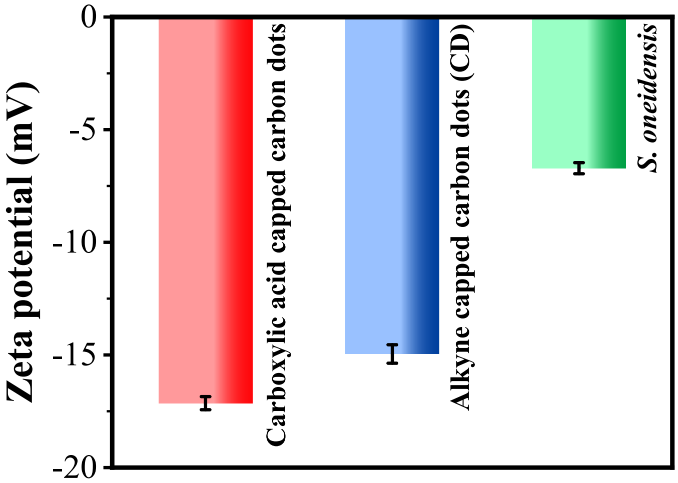

Figure S7. Zeta potential of carbon dot nanoparticles and *S. oneidensis* cells

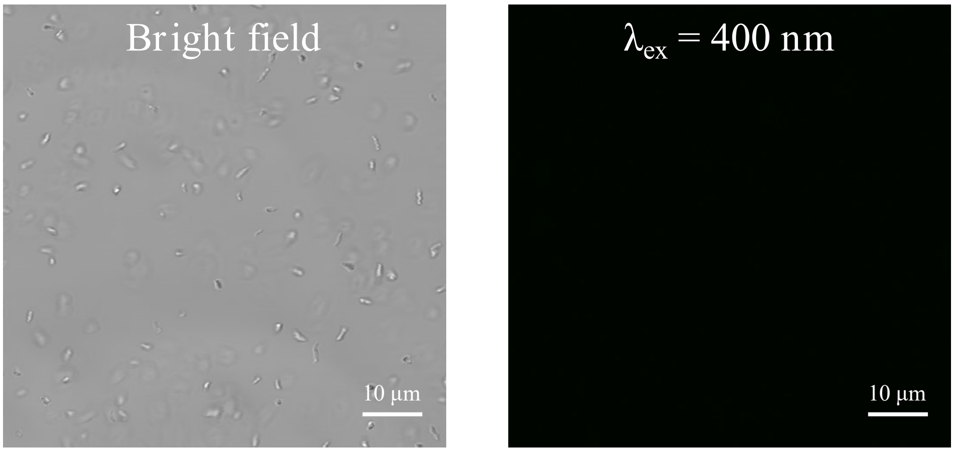

Figure S8. Fluorescence image of simple mixing CD+*SO* under 400 nm excitation after repeated washing

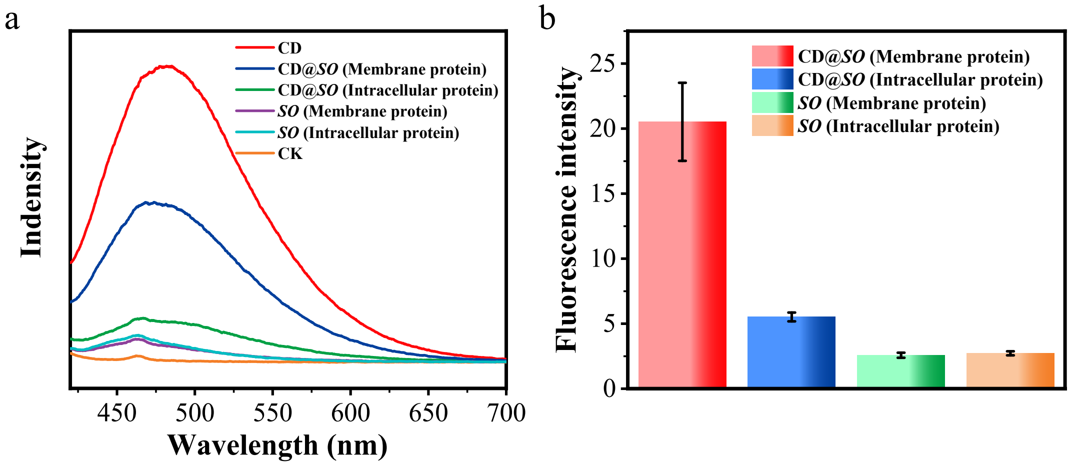

Figure S9. (a) Fluorescence emission spectra of CD nanoparticles, membrane/intracellular proteins from CD@*SO* PBHS and *S. oneidensis* cells (*SO*), and double distilled water (CK). (b) Fluorescence intensity of membrane/intracellular proteins from CD@*SO* PBHS and *SO* cells (λ_{ex} = 400 nm, λ_{em} = 480 nm, n = 3). All protein samples were normalized by total protein prior to measurement.

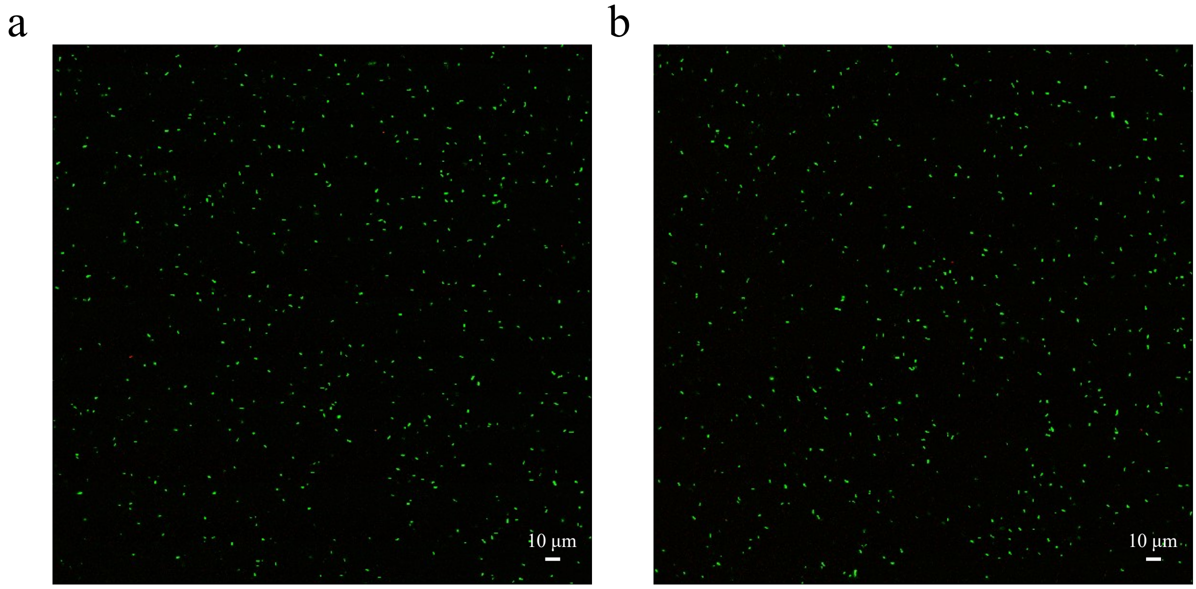

Figure S10. CLSM image of the CD@*SO* PBHS strained with LIVE/DEAD assay kit (green indicates the live cells, red indicates the dead cells). (a) Immediately after the biohybrid assembly. (b) After 24 h of continuous photocatalytic H_2 production reaction

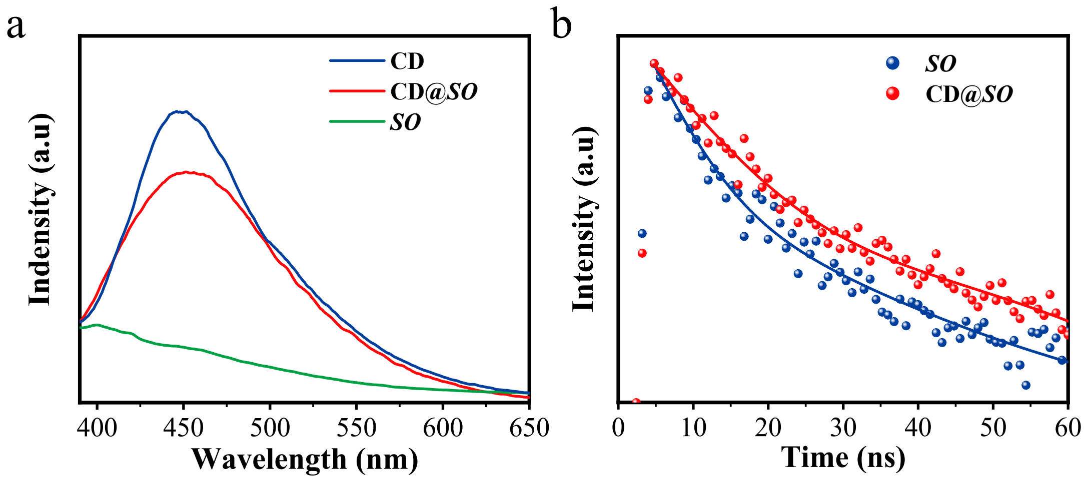

Figure S11. (a) Steady-state photoluminescence spectra of CD nanoparticles, *S. oneidensis* cells (*SO*) and CD@*SO* PBHS. (b) Time-resolved photoluminescence spectra of CD nanoparticles and CD@*SO*

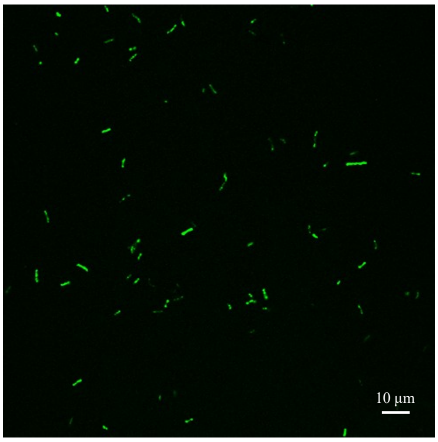

Figure S12. Fluorescence image of GalNAz labeled *S. oneidensis* cells stained with AZDye 488 DBCO

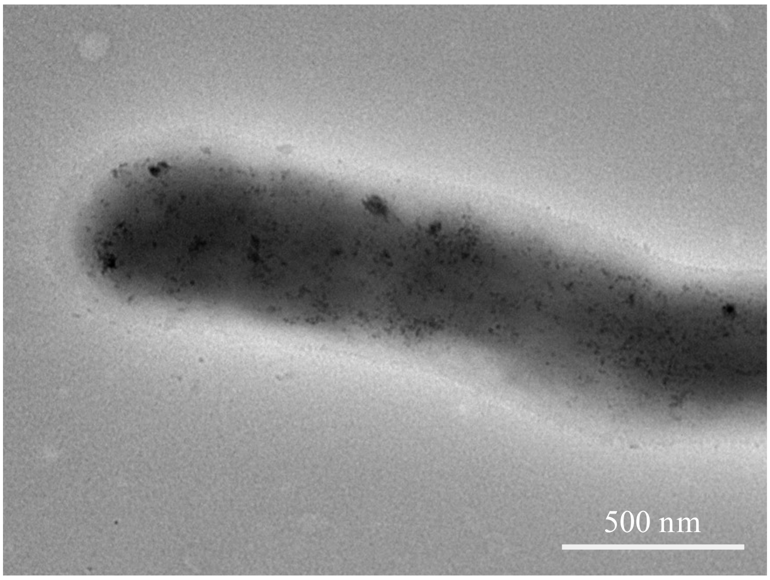

Figure S13. TEM image of CD/*SO* PBHS (glycan-targeted PBHS)

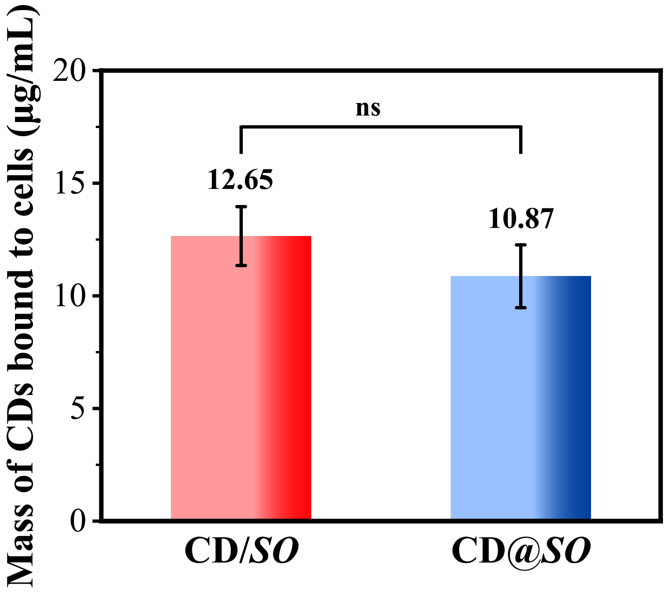

Figure S14. Mass of CDs bound to cells for CD/*SO* (glycan-targeted PBHS) and CD@*SO* (membrane protein-targeted PBHS). Both PBHS were assembled with 0.1 mg/mL (100 μg/mL) CDs and cells to an OD_{600} of 1.0. n = 3; ns, P > 0.05 (not significant)

Table S1. The details of PBHS (simple mixing CD+*SO*, glycan-targeted CD/*SO* and membrane protein-targeted CD@*SO*) used in the photocatalytic reaction

PBHS	CDs in the reaction (μg/mL)	Cells in the reaction (OD_{600})
CD+*SO*	102.39 ± 3.89	1.01 ± 0.07
CD/*SO*	12.65 ± 1.31	0.95 ± 0.04
CD@*SO*	10.87 ± 1.39	0.96 ± 0.02

Table S2. The composition of Luria-Bertani (LB) medium[1]

Reagent	Final concentration (g/L)
Tryptone	10
Yeast extract	5
NaCl	10
Adjust pH to 7.0	

Table S3. The composition of yeast extract peptone dextrose (YPD) medium[2]

Reagent	Final concentration (g/L)
Yeast extract	10
Tryptone	20
Glucose	20
Adjust pH to 7.0	

Table S4. The composition of modified DSM311b medium[3]

Reagent	Final concentration
$MgCl_2 \cdot 6H_2O$	0.4 g/L
$CaCl_2 \cdot 2H_2O$	0.1 g/L
NH_4Cl	0.1 g/L
KH_2PO_4	0.2 g/L
KCl	0.5 g/L
HEPES	7.16 g/L
$NaHCO_3$	2.52 g/L
$Na_2S \cdot 9H_2O$	0.24 g/L
NaAc	1.394 g/L
HCl	50 μL
$FeCl_2 \cdot 4H_2O$	2 mg/L
$ZnCl_2$	0.2 mg/L
$MnCl_2 \cdot 4H_2O$	0.1 mg/L
H_3BO_3	0.18 mg/L
$CoCl_2 \cdot 6H_2O$	50 μg/L
$CuCl_2 \cdot 2H_2O$	6 μg/L
$NiCl_2 \cdot 6H_2O$	72 μg/L
$Na_2MoO_4 \cdot 2H_2O$	108 μg/L
NaOH	0.5 mg/L
$Na_2SeO_3 \cdot 5H_2O$	3 μg/L
$Na_2WO_4 \cdot 2H_2O$	4 μg/L

4-aminobenzoic acid	40 μg/L

Continued

D(+)-biotin	10 μg/L
DL-a-lipoic acid	10 μg/L
Calcium-D(+)-panto-thenate	100 μg/L
Pyridoxine-HCl	100 μg/L
Folic acid	30 μg/L
Nicotinic acid	50 μg/L
Riboflavin	50 μg/L
Thiamin-HCl • $2H_2O$	10 μg/L
Vitamin B12	50 μg/L

REFERENCES

1. G. Bertani, *J. Bacteriol.*, 1951, **62**, 293–300.

2 R. Schiestl and U. Wintersberger, *Mol. Gen. Genet.*, 1982, **186**, 512 – 517.

3 J. Ye, J. Yu, Y. Zhang, M. Chen, X. Liu, S. Zhou and Z. He, *App. Catal. B. Environ.*, 2019, **257**, 117916.

4 T.-Y. Zhu, Y.-C. Zhao, C. Sha, S. Nawab, J.-Y. Liu and Y.-C. Yong, *Bioresour. Technol.*, 2025, **427**, 132410.

5 X. Hu, Y. Li, Y. Xu, Z. Gan, X. Zou, J. Shi, X. Huang, Z. Li and Y. Li, *Food Chem.*, 2021, **339**, 127775.

6 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, **41**, 2596–2599.

7 H. Shen, Y.-Z. Wang, G. Liu, L. Li, R. Xia, B. Luo, J. Wang, D. Suo, W. Shi and Y.-C. Yong, *ACS Catal.*, 2020, **10**, 13290–13295.