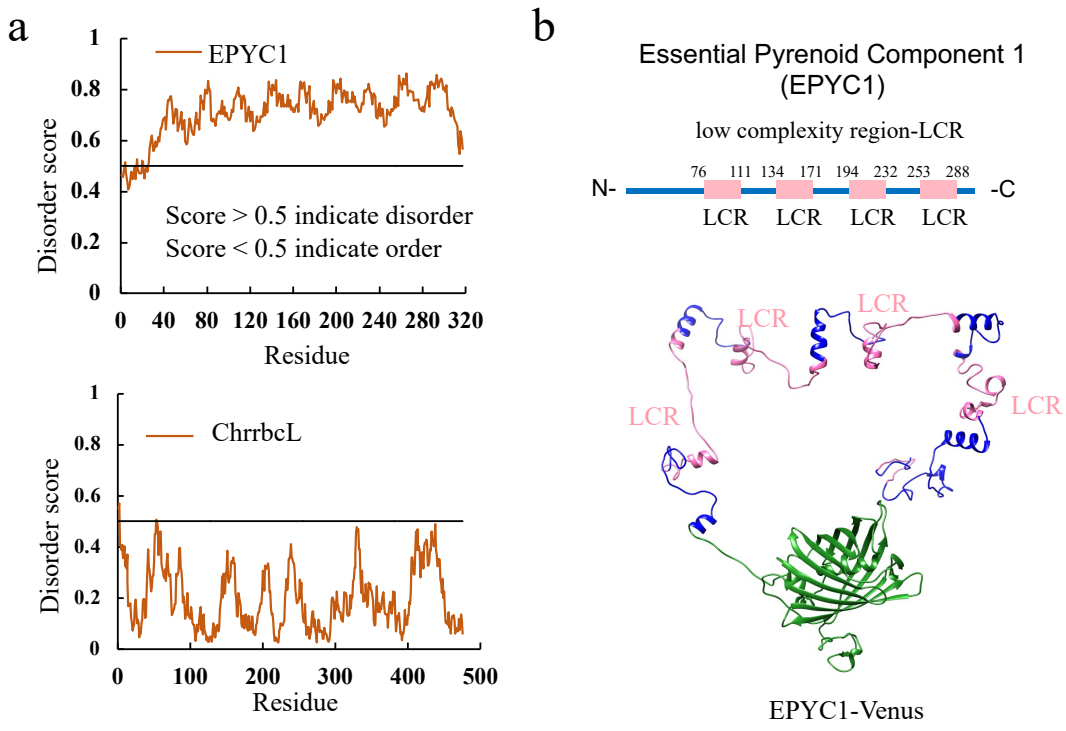
Supporting Information

Spatially-defined intracellular biohybrid for light-driven CO_2 conversion

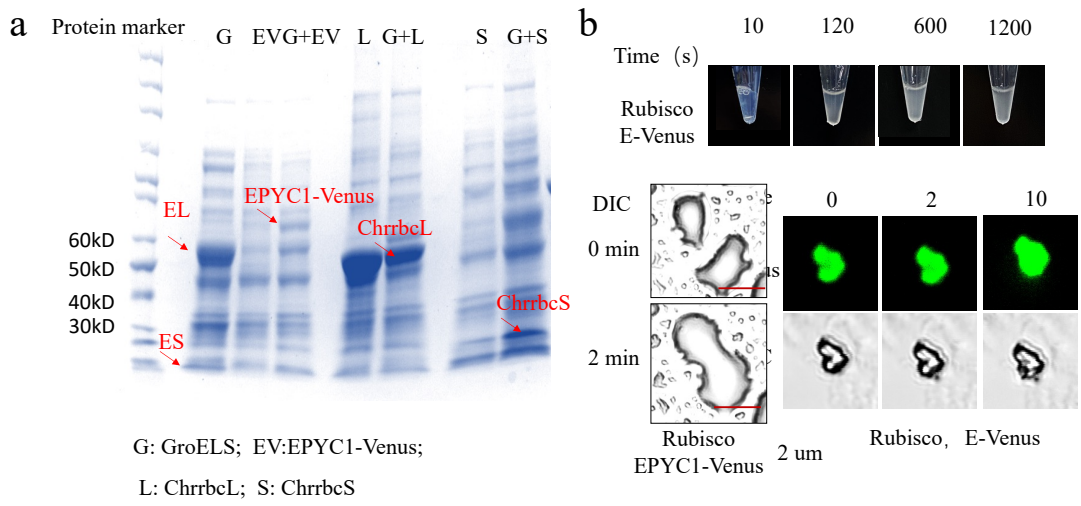
Jing Li[1#], Jicong Zhang[1#], Zhangfeng Yu[1], Xinyi Yuan[1], Xinyu Wang[1], Zehao Zhang[2], Hongyu Chen[3], Junfeng Shen[1], Yi Liu[1], Zhiyong Zhang[2], Liming Wang[2], Xihan Chen[3], Chao Zhong[1,*]

[1]Key Laboratory of Quantitative Synthetic Biology, Center for Materials Synthetic Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China
[2]Cas Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[3]Shenzhen Key Laboratory of Intelligent Robotics and Flexible Manufacturing Systems, SUSTech Energy Institute for Carbon Neutrality, Department of Mechanical and Energy Engineering, Southern University of Science and Technology, Shenzhen 518055, China.

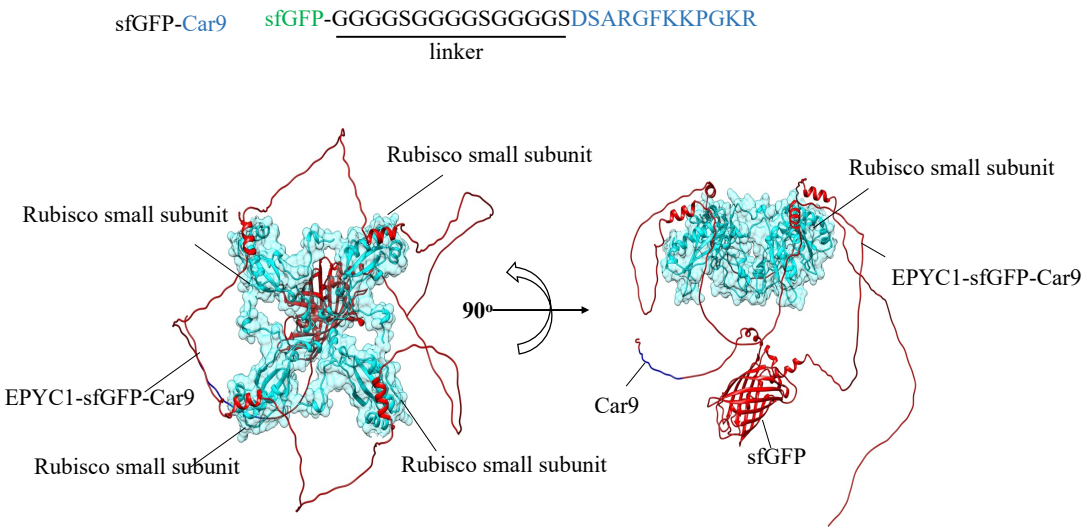
*E-mail address: chao.zhong@siat.ac.cn



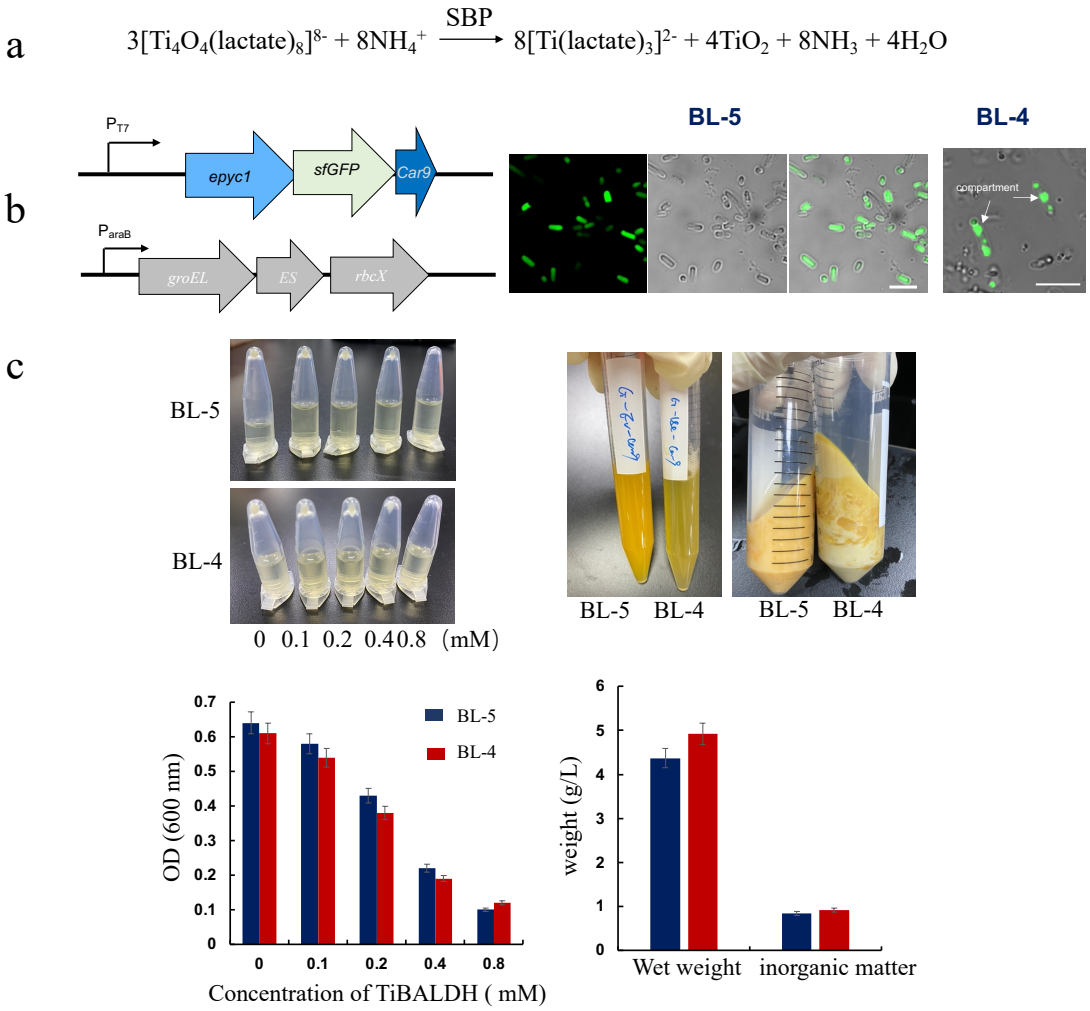
Supplementary Figure 1. (a) Disordered index analysis of EPYC1 and ChrrbcL, (b) protein structure analysis of EPYC1-venus fused protein.



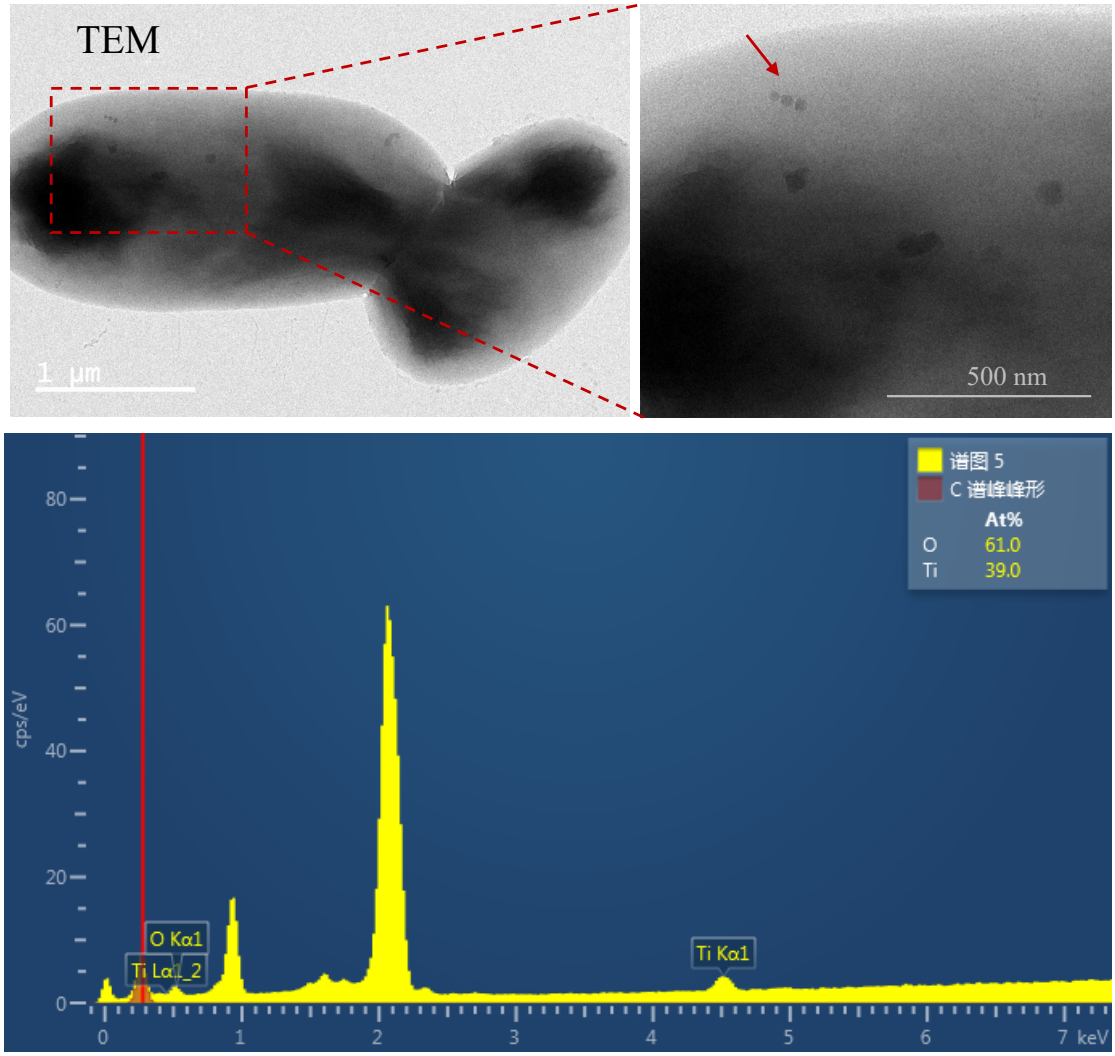
Supplementary Figure 2. Droplet formation through mixing purified protein together *in vitro*.



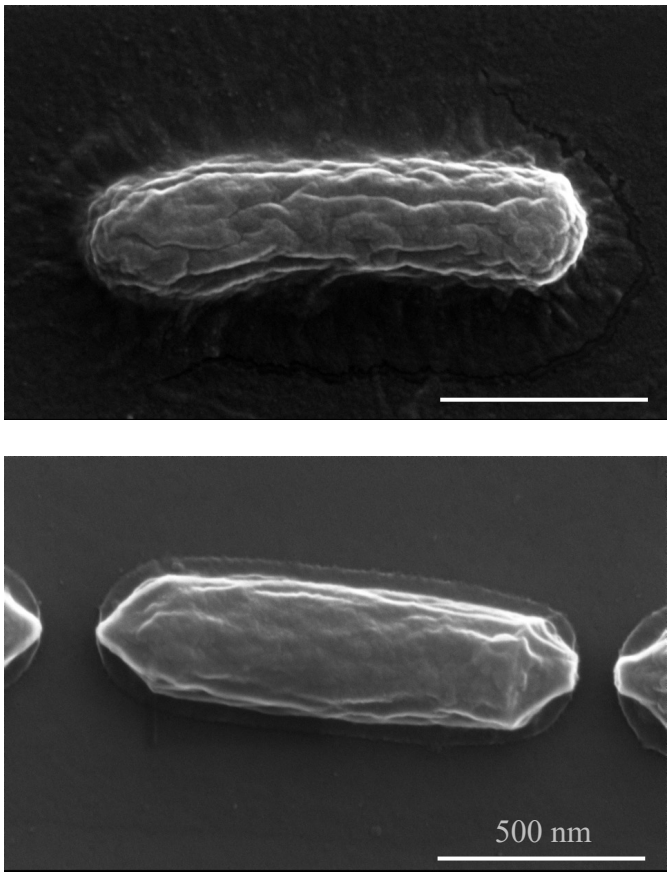
Supplementary Figure 3. Protein structure prediction of complex of Rubisco and EPYC1-sfGFP-car9.



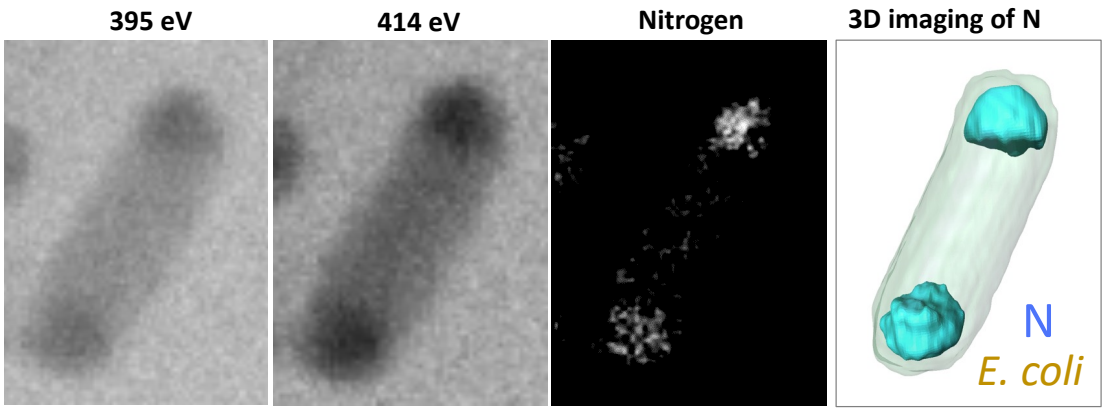
Supplementary Figure 4. Cytotoxicity and Mineralization of TiBALDH in *E. coli.*



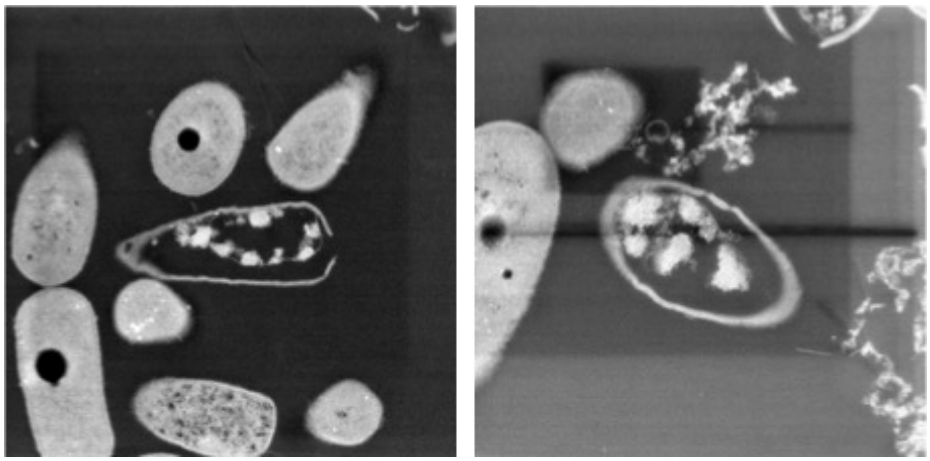
Supplementary Figure 5. Transmission Electron Microscope (TEM) and Energy-dispersive X-ray spectroscopy (EDS) mapping of BL-4.



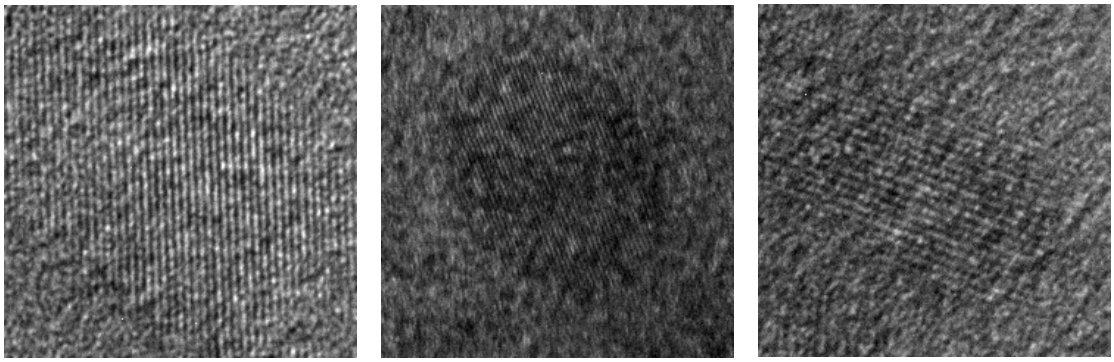
Supplementary Figure 6. Scan Electron Microscope (SEM) analysis of mineralized BL-4.



Supplementary Figure 7. Spatial distribution of N element from Rubisco proteins (blue) in *E. coli* (green) as measured by 3D tomographic TXM images.

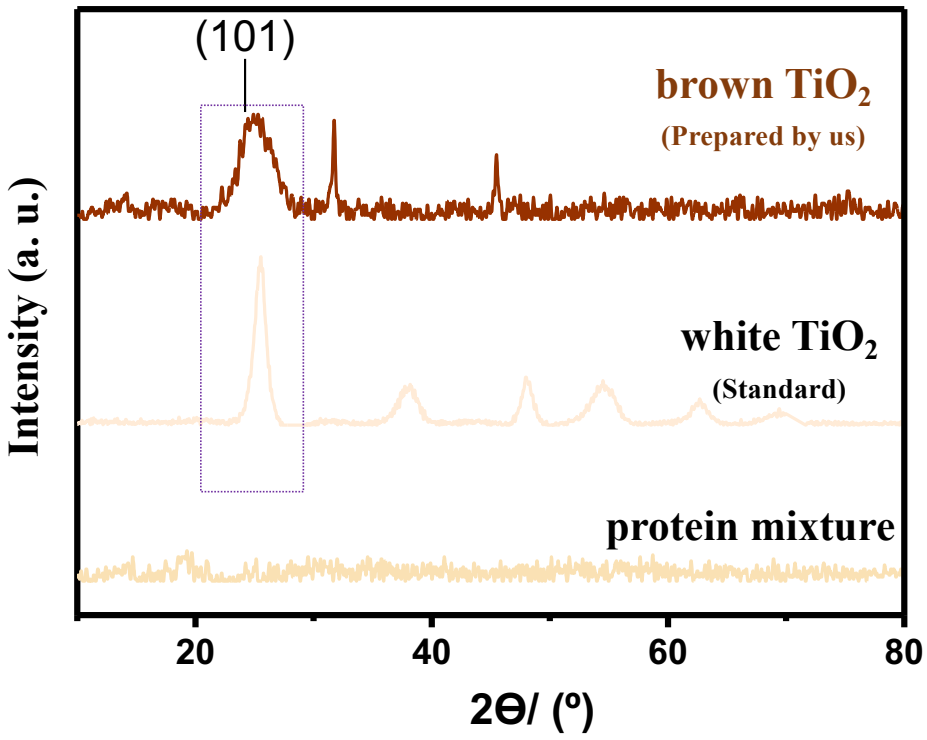


Supplementary Figure 8. High Angle Annular Dark Field (HAADF) analysis of mineralized BL-4 ultrathin sections.

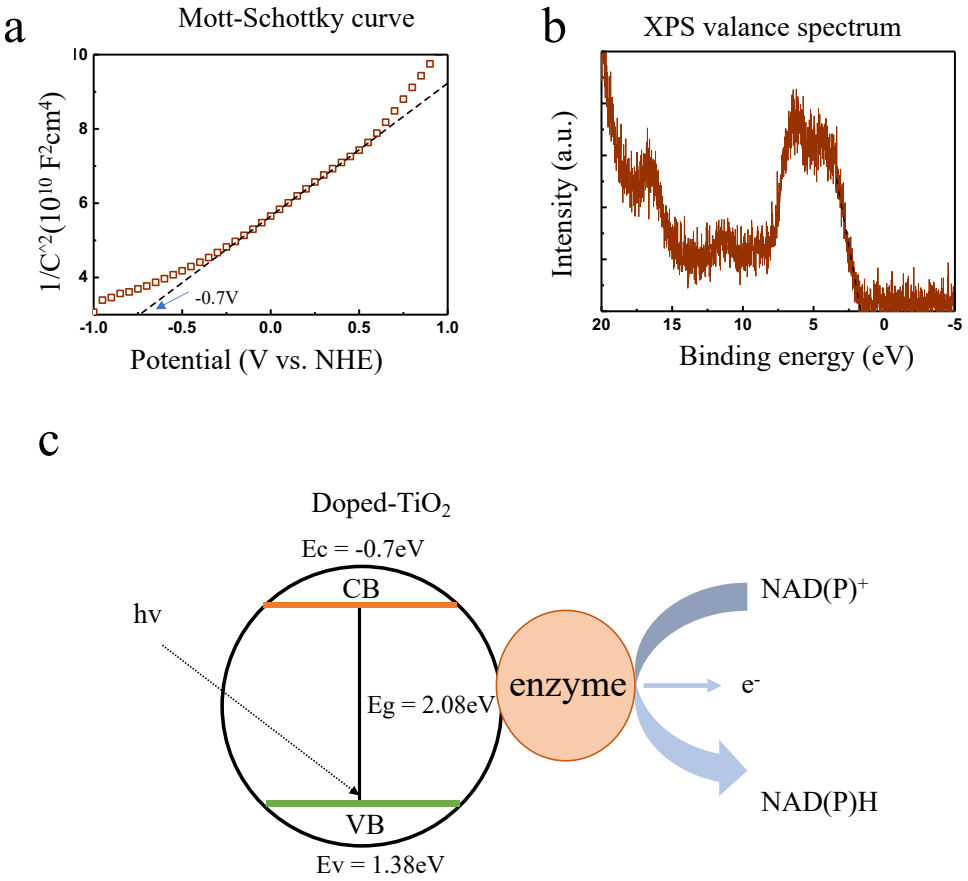


d (111) =0.284nm d(121)=0.29nm d (101) =0.365nm

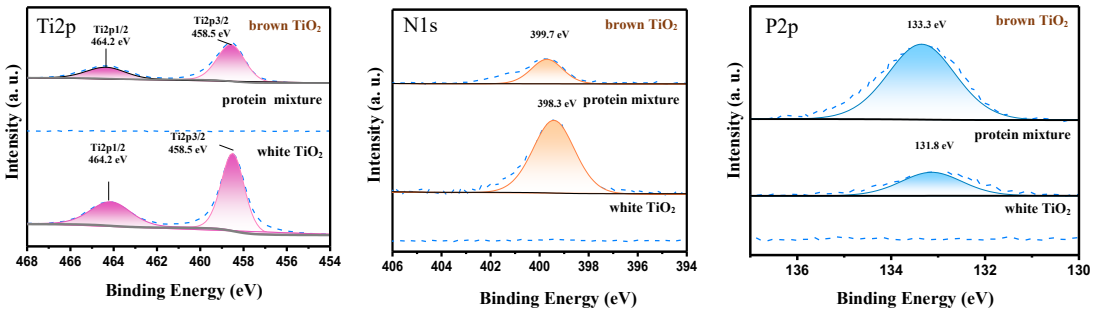
Supplementary Figure 9. Details of (101), (112) and (121) lattice planes of doped TiO_2 from BL-4 ultrathin section.



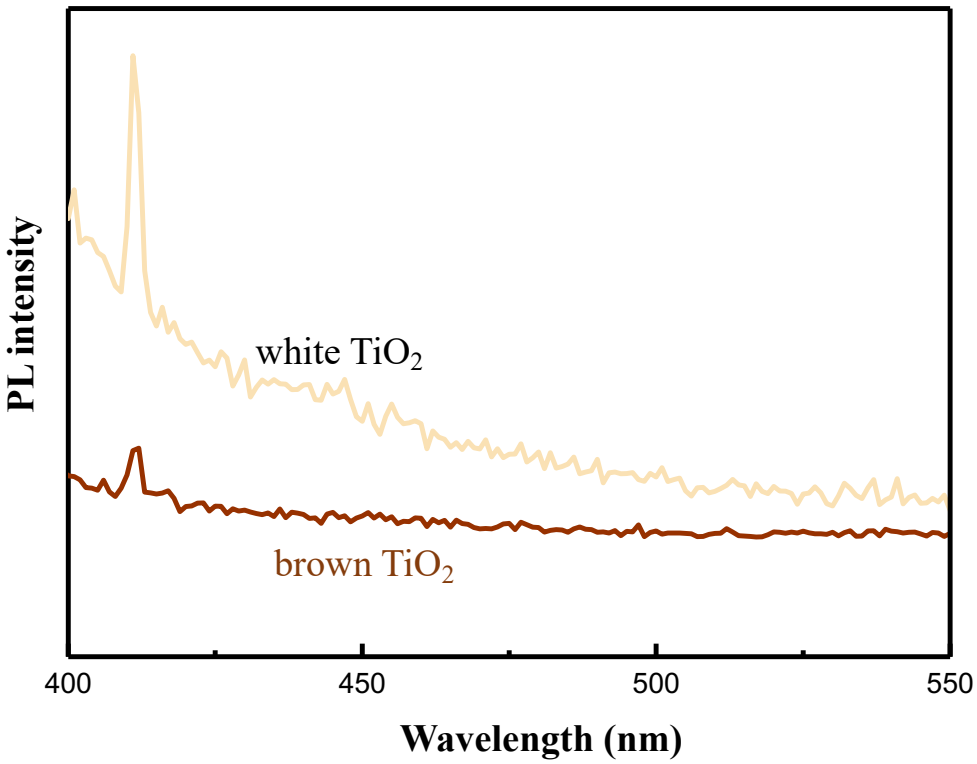
Supplementary Figure 10. X-ray power diffraction (XRD) of TiO_2 prepared by us, standard TiO_2 and protein mixture. Three color powers images in circles referred to brown TiO_2 prepared by us, white TiO_2 (standard) and protein mixture.



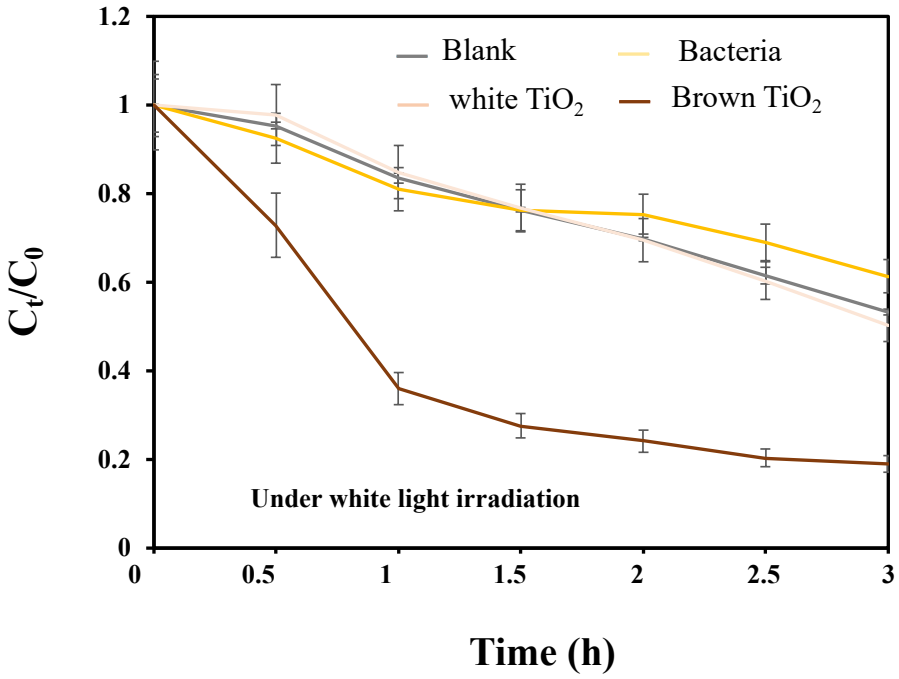
Supplementary Figure 11. Mott-Schottky curve(a), XPS valance spectrum (b) and band structure (c) of brown or doped TiO_2.



Supplementary Figure 12. High-resolution XPS spectra of Ti 2p, N1s and P2p were recorded from the pristine surface layer of the brown and white TiO2, protein mixture.



Supplementary Figure 13. Photoluminescence emission spectra (excitation/emission bandwidth=5 nm) at an excitation wavelength of 360 nm of white and brown TiO_2.



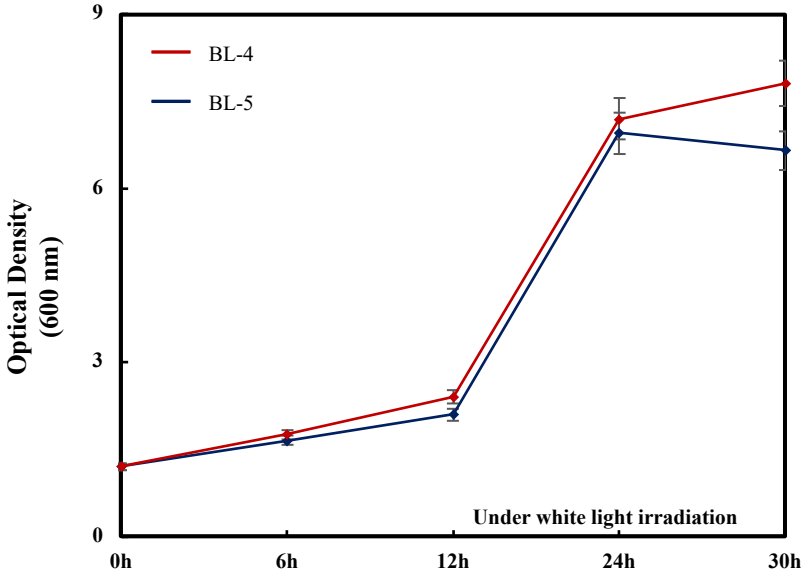
Supplementary Figure 14. Photocatalytic degradation of methylene blue (MB) under white light irradiation as the normalized concentration change versus irradiation time for different samples. In detail, C_t is the concentration of MB at the irradiation time t and C_0 is the initial concentration.

Model	ExpDec1
Equation	y = A1*exp(-x/t1) + y0
Plot	Les BacTiO2
y0	0.12816 ± 0.01261
A1	0.75428 ± 0.02719
t1	6.87032 ± 0.57905
Reduced Chi-Sqr	0.00384
R-Square (COD)	0.93764
Adj. R-Square	0.9358

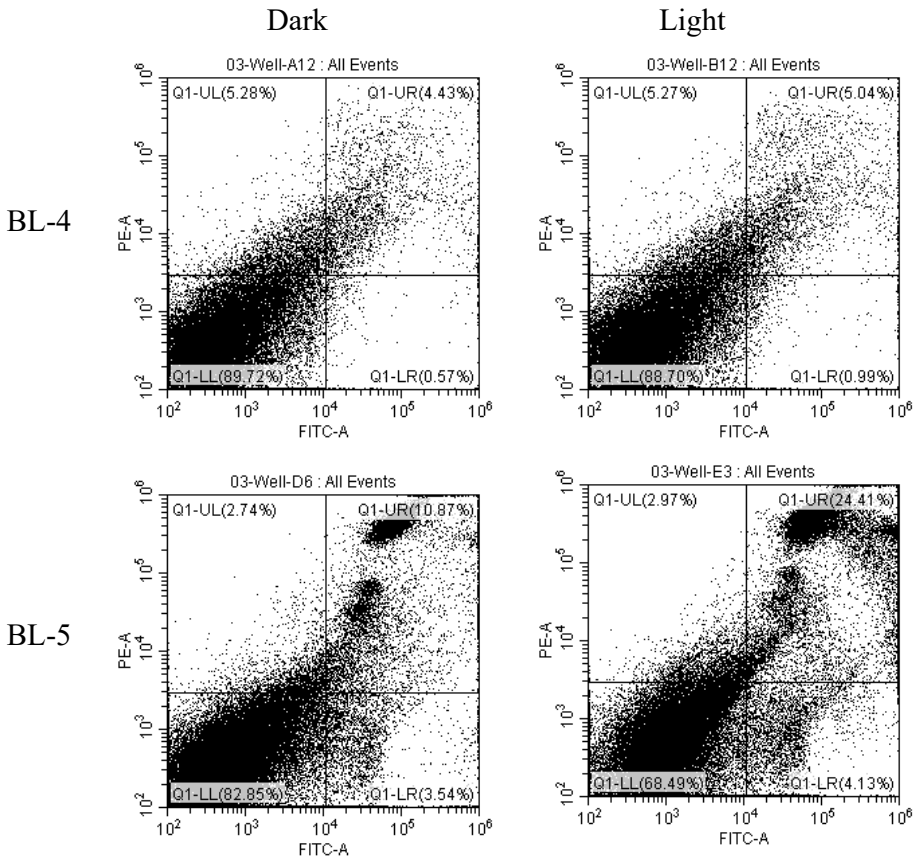
Model	ExpDec1
Equation	y = A1*exp(-x/t1) + y0
Plot	Ev BacTiOB2
y0	0.20326 ± 0.00988
A1	0.59666 ± 0.01804
t1	8.48124 ± 0.64563
Reduced Chi-Sqr	0.00205
R-Square (COD)	0.95004
Adj. R-Square	0.94858

Model	ExpDec1
Equation	y = A1*exp(-x/t1) + y0
Plot	TiO2
y0	0.12795 ± 0.0689
A1	0.69425 ± 0.06651
t1	25.03727 ± 7.04829
Reduced Chi-Sqr	0.02629
R-Square (COD)	0.67125
Adj. R-Square	0.66158

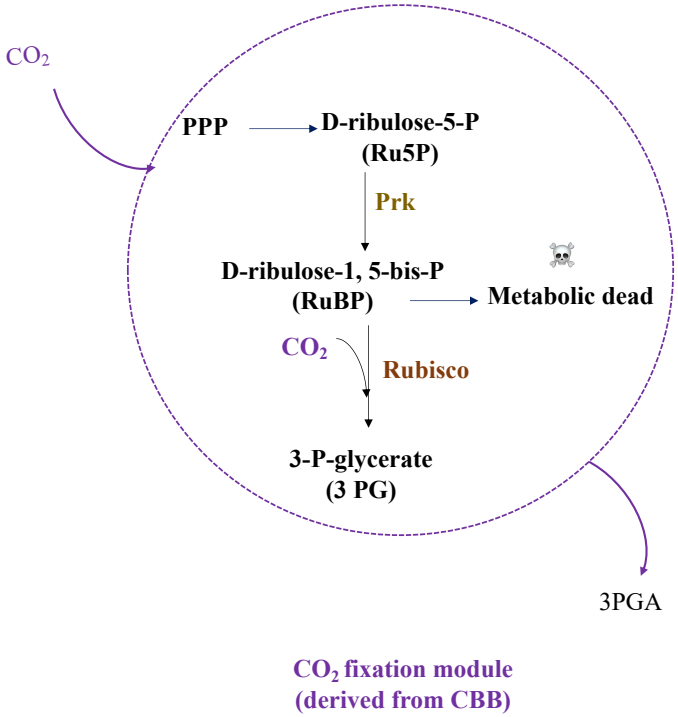
Supplementary Figure 15. The ExpDec1 fitting model and parameters of electron transfer time in BL-4 (Les BacTiO_2), BL-5 (Ev BacTiO_2) and doped TiO_2 powders (TiO_2).



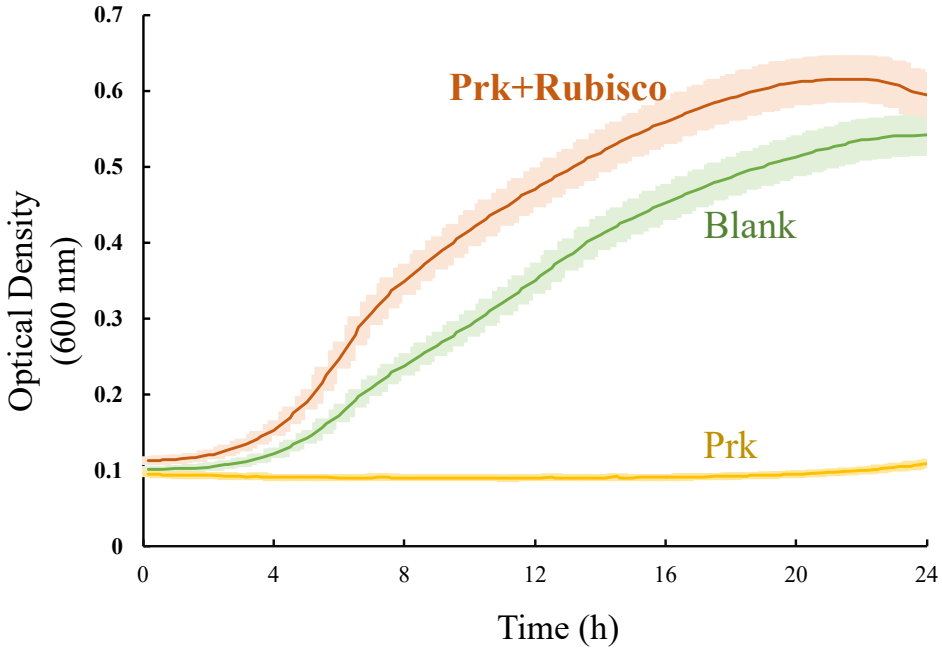
Supplementary Figure 16. bacteria density variation of strain BL-4 and BL-5 cultured in dark condition.



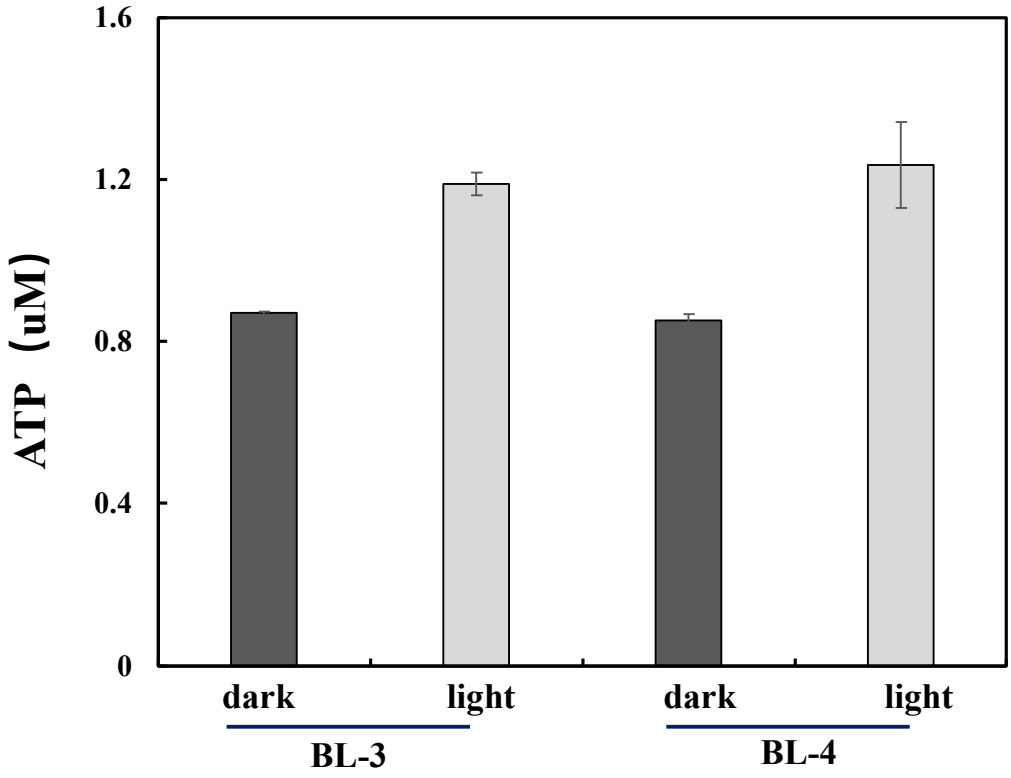
Supplementary Figure 17. Flow analysis of BL-4 and BL-5 cultured for 72h in dark and light condition.



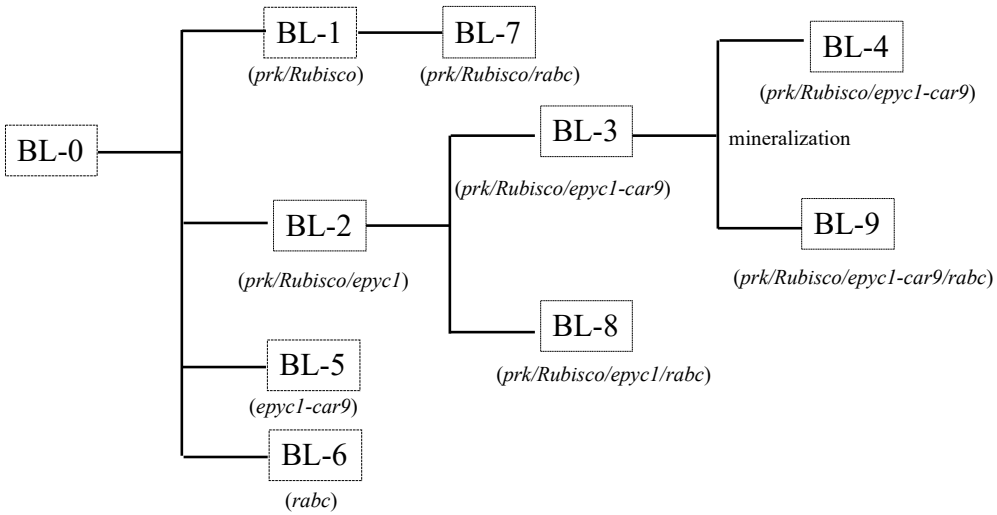
Supplementary Figure 18. The carbon fixation pathway was built on the CBB pathway from *C. reinhardtii.*



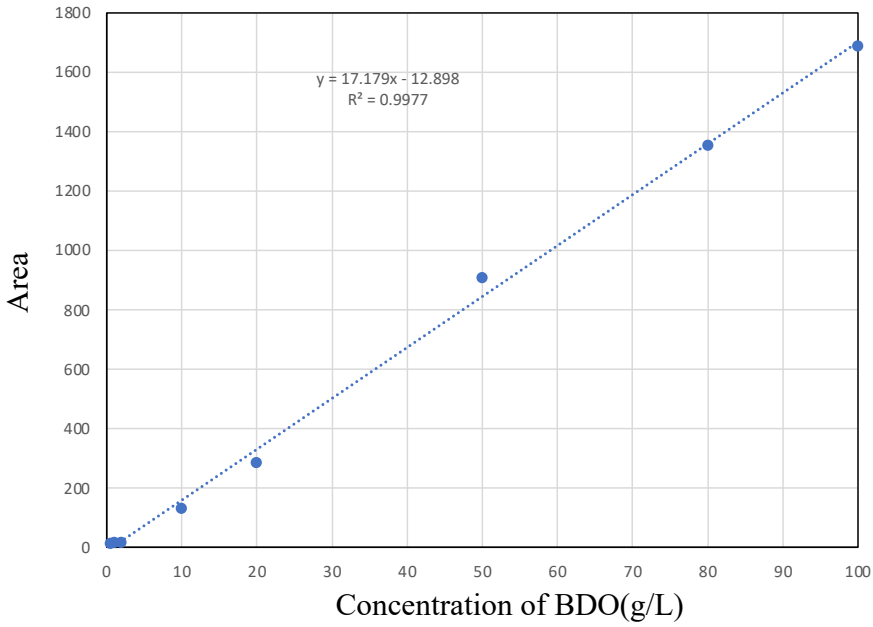
Supplementary Figure 19. Growth curves of strains with protein prk and prk+Rubisco.



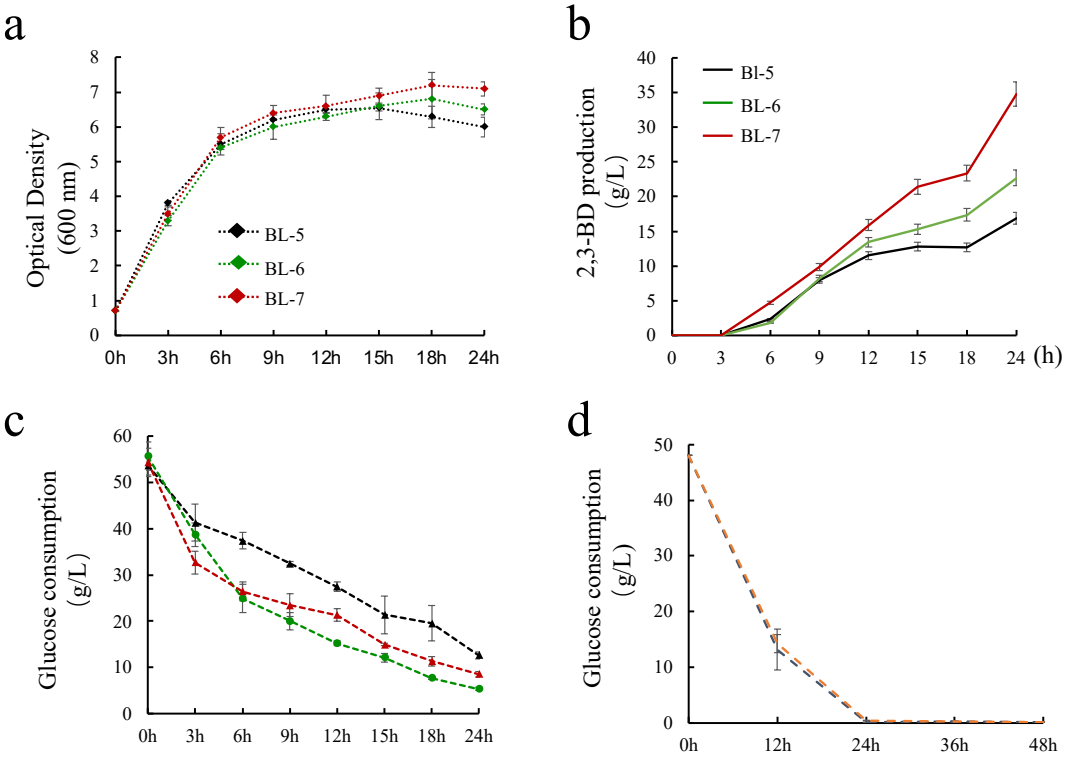
Supplementary Figure 20. ATP detection in nonmineralized and mineralized BL-3 and BL-4 under light and dark.



Supplementary Figure 21. Flowchart of *E. coli* strains construction in our study.



Supplementary Figure 22. The standard curve of 2,3-BD detected by GC, which fitted by using linear function.



Supplementary Figure 23. Cell density (a), 2,3-BD production (b) and glucose consumption (c & d) of strain BL-5, BL-6 and BL-7.

Table S1. Construction of plasmid used in this study

Plasmid name	Description	Source
pBAD33-CmR-***groEl-groEs***	groELS gene cluster inserted in expressing region of the commercially available pBAD33 vector	This study
pBAD33-CmR-***groEl-groEs-rbcX***	groELS gene cluster and rbcX genes inserted in expressing region of the commercially available pBAD33 vector	This study
pBAD33-CmR-***prk-groEl-groEs-rbcX***	prk gene, groELS gene cluster and rbcX genes inserted in expressing region of the commercially available pBAD33 vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-venus***	Rubisco fused with Venus fluorescent protein gene inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***epyc1-venus***	EPYC1 fused with Venus fluorescent protein genes inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-venus***	Rubisco and EPYC1 fused with Venus fluorescent protein genes inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-sfgfp-car9***	Rubisco and EPYC1-Car9 genes inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-***rabc***	RABC gene cluster inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-rbc***	Rubisco and RABC gene cluster inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-rabc***	Rubisco, EPYC1 and RABC gene cluster inserted in expressing region of the commercially available pET22b vector	This study
pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-sfgfp-car9-rabc***	Rubisco, EPYC1-Car9 and RABC gene cluster inserted in expressing region of the commercially available pET22b vector	This study

Table S2. Information of strains used in this study

Strain name	Description	Antibiotic	Source
BL-1	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-venus*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-2	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-venus*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-3	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-car9*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-4 (mineralized BL-3)	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-car9*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-5	Co-transforming two plasmid pET-22b-AmpR-pT7-***epyc1-car9*** and pBAD33-CmR-***groEl-groEs*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-6	Transforming pET-22b-AmpR-***rabc*** into BL21(DE3) commercial strain.	Amp	This study
BL-7	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-rabc*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-8	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-rabc*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study
BL-9	Co-transforming two plasmids pET-22b-AmpR-pT7-***rbcL-rbcS-epyc1-car9-rabc*** and pBAD33-CmR-***prk-groEl-groEs-rbcX*** into BL21(DE3) commercial strain.	Cm and Amp	This study

Supplementary table 3. Primers used in this study

Vector	Primer name	Sequences（5'---->3'）
pET-22b-AmpR-pT7-rbcL-rbcS-epyc1-venus；pET-22b-AmpR-pT7-rbcL-rbcS-venus；pET-22b-AmpR-pT7-rbcL-rbcS-epyc1-sfgfp-car9；pET-22b-AmpR-pT7-epyc1-sfgfp-car9	rbcL F	AAGGAGATATACATATGGTTCCGCAGACCGAAAC CAAAGCG
	pET22b R	TCTGCGGAACCATATGTATATCTCCTTCTTAAAGT TAAACAAAAT
	chrS F	AACTGGGATCCTAACTGCAGATAACCCAAATAAT GTTTTAAAATTTTAAAAATAATGTAGGAGGAAAA ATTATGGCAGCAGTTATTGCAAAATC
	chrL R	CTGCCATAATTTTTCCTCCTACATTATTTTTAAAA TTTTAAAACATTATTTGGGTTATCTGCAGTTAGGA TCCCAGTTTATCGATGGTATCG
	pET22b F	AAGATCAGTTTAACTGCAGATAACCCAAATAATG TTTTAAAATTTTAAAAATAATGTAGGAGGAAAAA TTATGGCGACCATTAGCAGCAT
	chr S R	TCGCCATAATTTTTCCTCCTACATTATTTTTAAAA TTTTAAAACATTATTTGGGTTATCTGCAGTTAAAC TGATCTTTTATTTGCCG
	V-His F	ATGATGATAAATAACACCACCACCACCACCACTG AGAT
	His-V R	TGGTGGTGGTGGTGTTATTTATCATCATCGTCTTT GTAGTC
	E-sfGFP Fw2	CAAAGGCCTGAGAAAGGGAGAAGAACTT
	sf-E Rv2	TTCTCCCTTTCTCAGGCCTTTGCGCCAAT
	Car-His Fw2	TGGAAAGAGACACCACCACCACCACCACTG
	his-Car Rv2	TGGTGGTGGTGTCTCTTTCCAGGCTTCTTA
	pET22b-EPYC-Vennus_rev	TGGTCGCCATATGTATATCTCCTTCTTAAAGTTAA AC
	EPYC1-VENUS_fwd	AGATATACATATGGCGACCATTAGCAGC
pBAD33-CmR-groEl-groEs-rbcX; pBAD33-CmR-groEl-groEs-rbcX; pBAD33-CmR-prk-groEl-groEs-rbcX	BAD-prk Fw	ATTCGAGCTCATGGCGTTCACCATGCG
	prk-BAD Rv	TGAACGCCATGAGCTCGAATTCGCTAGC
	rbs-ES Fw	TCTAGAGAAAGAGGGGACAAACTAGATGATGAA TATTCGTCCATTGCATG
	rbs-prk Rv	CATCTAGTTTGTCCCCTCTTTCTCTAGATTAAACC GGAACAACATCTT
	p-rbcX Fw	AGACTAGATGATGAGCCTGTCTGC
	rbcX-p Rv	CAGGCTCATCATCTAGTCTGTCCCT
	rbcX-BAD Fw	CCCGCGTGTTTGAGGTACCCGGGGATCCTCT
	BAD-rbcX Rv	TCCCCGGGTACCTCAAACACGCGGGGACGC
pET-22b-AmpR-rabc；	ter-RABC Fw	ACTATATCCGGATTTTAGTTGAACACCATCC
	RABC-ter Rv	TGTTCAACTAAAATCCGGATATAGTTCCTCC

pET-22b-AmpR-pT7-rbcL-rbcS-rbc； pET-22b-AmpR-pT7-rbcL-rbcS-epyc1-rabc； pET-22b-AmpR-pT7-rbcL-rbcS-epyc1-sfgfp-car9-rabc	f1 ori Fw	GCGAGGAGTAGAGAGAAGGCCATCCTGACGGAT GGCCTTTTTGGCGAATGGGACGCGCCCTGTA
	RABC Rv	GTCCCATTCGCCAAAAAGGCCATCCGTCAGGATG GCCTTCTCTCTACTCCTCGCTTATCATCG
	rbcL F	AAGGAGATATACATATGGTTCCGCAGACCGAAAC CAAAGCG
	PET22b R	TCTGCGGAACCATATGTATATCTCCTTCTTAAAGT TAAACAAAAT
	pET22b-ChrrbcS_fwd	AAGATCAGTTCACCACCACCACCACCAC
	ChrrbcS_rev	GGTGGTGGTGAACTGATCTTTTATTTGCCGG
	Car-His Fw2	TGGAAAGAGACACCACCACCACCACCACTG
	his-Car Rv2	TGGTGGTGGTGTCTCTTTCCAGGCTTCTTA