

Supporting information DOI:

**Alleviation of High Light-induced Photoinhibition in
Cyanobacteria by Artificially Conferred Biosilica Shells**

Wei Xiong, Zhou Yang, Halei Zhai, Guangchuan Wang, Xurong Xu,* Weimin Ma,*
and Ruikang Tang*

Experimental Section

Cell Cultures: *Synechocystis* sp. strain PCC 6803 cells were cultured at 30°C in BG-11 medium[1], buffered with Tris-HCl (5 mM, pH8.0) bubbled with 2% (v/v) CO₂ in air, and subjected to continuous illumination by fluorescent lamps (40 μEm⁻²s⁻¹). For encapsulation studies, cells cultured for 3 days (*A*₇₃₀=0.6-0.8) that showed the highest photosynthetic activity[2] were harvested by centrifugation (5,000 ×g for 5 min) at 25°C. After encapsulation, cells were recovered under growth conditions for 2 h.

Biomimetic silica encapsulation: Aqueous NaCl solution (0.02M) was used for preparing poly(diallyldimethylammonium chloride) (PDADMAC, Mw: 100000–200000, 20 wt% in H₂O, Aldrich) and sodium polystyrene sulfonate (PSS, average Mw: ca. 70000, powder, Aldrich) solutions. The final concentration of the solutions was 1mg mL⁻¹. The cells were alternately immersed in the PDADMAC solution and the PSS solution for 15 min for each step. After the LbL process, multilayer-coated cells were placed in the 10 mM silicic acid solution, which had been independently prepared by adding HCl solution (0.1 mM) into phosphate buffer saline solution (100 mM, pH 7.4) of sodium silicate (Na₂SiO₃, 10 mM) at room temperature until the solution was set to pH 7.5. After 30 min, the substrate was removed and washed with 0.02M aqueous NaCl solution.

Characterization: SEM and TEM studies were conducted with S-4800 (HITACHI, Japan) and JEM-1230 (JEOL, Japan), respectively. The cells were washed using 0.02M NaCl solution and distilled water. They were lyophilized with Vertris 12SL (Vertris, USA). Biological TEM was performed using JEM-1230 (JEOL, Japan). The

specimens were fixed with gluteraldehyde, OsO_4 , and $\text{K}_2\text{Cr}_2\text{O}_7$, and were dehydrated in ethanol/acetone. They were embedded in Epon 812/Araldite M resin. Thin sections (8010 nm) were cut by using a Reichert ultratome (Zeiss, Germany) and were stained with uranyl acetate and lead citrate. Fourier transform infrared (FTIR) spectra were collected on a NICOLET iS10 spectrometer (Thermo SCIENTIFIC, USA) by using a KBr wafer technique in order to study the composition of the samples. The TGA curves were obtained with SDT Q600 (TA, USA) in air and heated from room temperature to 800 °C at a scan rate of 10 °C/min.

Oxygen Evolution Activity: The activities of photosystem II (PSII) and photosynthetic O_2 production in native cyanobacteria and cyanobacteria@ SiO_2 cells were measured at 30°C by monitoring the evolution of O_2 with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, UK). The activity of PSII was measured in the presence of 2 mM 1,4-benzoquinone (BQ), as an artificial electron acceptor. Oxygen production by photosynthesis was measured in the presence of 10 mM NaHCO_3 . The intensity of light used for the measurements of O_2 evolution activity was 800 $\mu\text{Em}^{-2}\text{s}^{-1}$.

Chlorophyll Fluorescence Analysis: The yields of chlorophyll (Chl) fluorescence at steady-state of electron transport were measured at room temperature by using a Dual-PAM-100 monitoring system (Walz, Effeltrich, Germany) equipped with an ED-101US/MD unit.[3] Minimal fluorescence at open PSII centers in the dark-adapted state (F_o) was excited by a weak measuring light (650 nm) at a PFD of 0.05 to 0.15 $\mu\text{Em}^{-2}\text{s}^{-1}$. A saturating pulse of red light (600 ms, 10,000 $\mu\text{Em}^{-2}\text{s}^{-1}$) was applied to determine the maximal fluorescence at closed PSII centers in the dark-adapted state (F_m) and during actinic light (AL) illumination (F_m'). The steady state fluorescence level (F_s) was recorded during AL illumination (200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Maximal quantum yield of PSII (F_v/F_m) was evaluated as $(F_m - F_o)/F_m$. [4] The effective quantum yield of PSII (Φ_{PSII}) was evaluated as $(F_m' - F_s')/F_m'$. [5]

Respiratory Oxygen Consumption: The respiration in native cyanobacteria and cyanobacteria@ SiO_2 cells were measured at 30°C by monitoring the consumption of

O₂ with a Clark-type oxygen electrode (Hansatech Instruments, Kings Lynn, UK).

UltraViolet-Visible (UV-Vis) Spectra: The UV-vis spectra were obtained with a SHIMADZU UV-3150 spectrometer in the diffuse reflectance mode and a PGENERAL T6-New Century spectrometer in the transmission mode. The absorption spectra were determined in an aqueous dispersion; the scattering spectra and the transmittance spectra were determined in a powder state.

References:

1. M. M. Allen, *J. Phycol.* **1968**, 4, 1-4.
2. W. Ma, H. Mi, *Physiol. Plant* **2005**, 125, 135-140.
3. a) U. Schreiber, U. Schliwa, W. Bilger, *Photosynth Res* **1986**, 10, 51-62; b) W. Ma, L. Wei, Q. Wang, *Sci China Life Sci* **2008**, 51, 1082-1087.
4. M. Kitajima, W. L. Butler, *Biochim Biophys Acta* **1975**, 376, 105-115.
5. B. Genty, J. M. Briantais, N. R. Baker, *Biochim Biophys Acta* **1989**, 990, 87-92.

Additional Figures

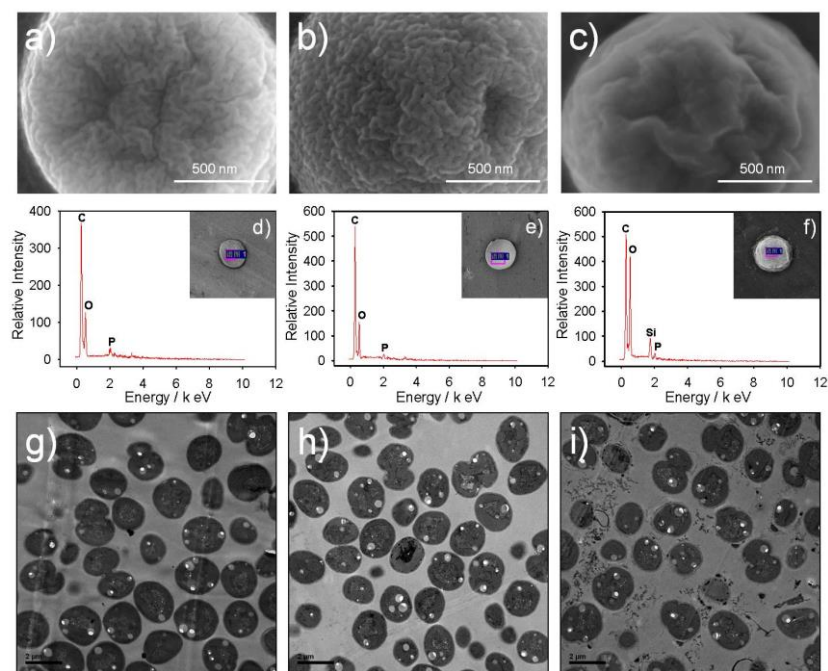


Fig. S1. High-magnification SEM micrographs of a) native cyanobacteria, b) PDADMALC/PSS-multilayer-coated cyanobacteria, and c) cyanobacteria@SiO₂. EDX of d) native cyanobacteria, e) PDADMALC/PSS-multilayer-coated cyanobacteria, and f)

cyanobacteria@SiO₂. TEM micrograph of the microtome-sliced g) native cyanobacteria, h) PDADMAC/PSS-multilayer-coated cyanobacteria, and i) cyanobacteria@SiO₂.

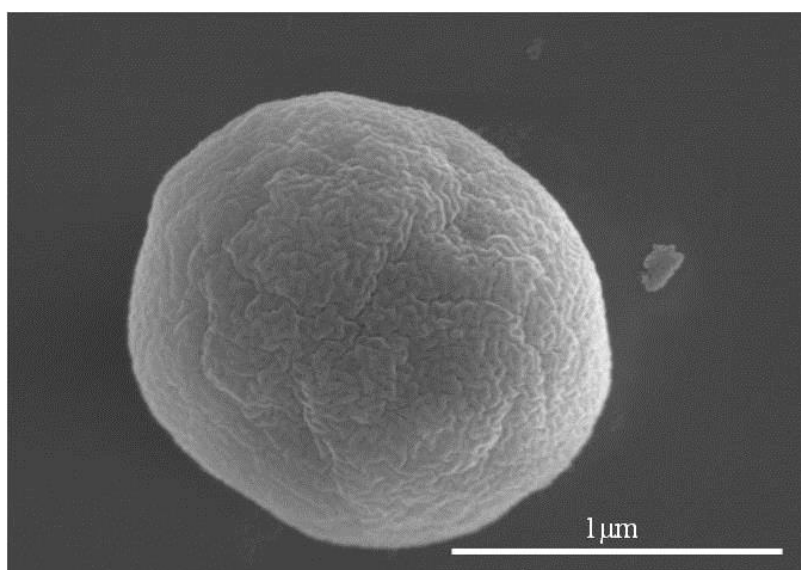


Fig. S2. SEM micrograph of native cyanobacteria treated by silicification solution.

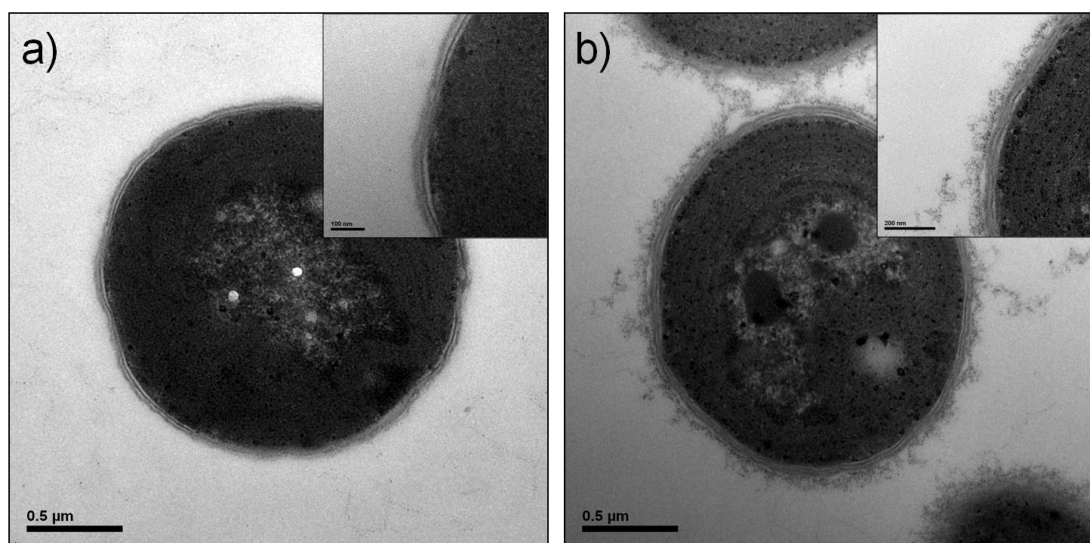


Fig. S3. TEM of microtome-sliced cyanobacteria@SiO₂ with different thickness of biosilica shell. a) Cyanobacteria@(PDADMAC/PSS)₃-PDADMAC@SiO₂, biosilica shell thickness ≤ 10 nm; b) Cyanobacteria@(PDADMAC/PSS)₉-PDADMAC@SiO₂, biosilica shell thickness ≈ 100 nm.

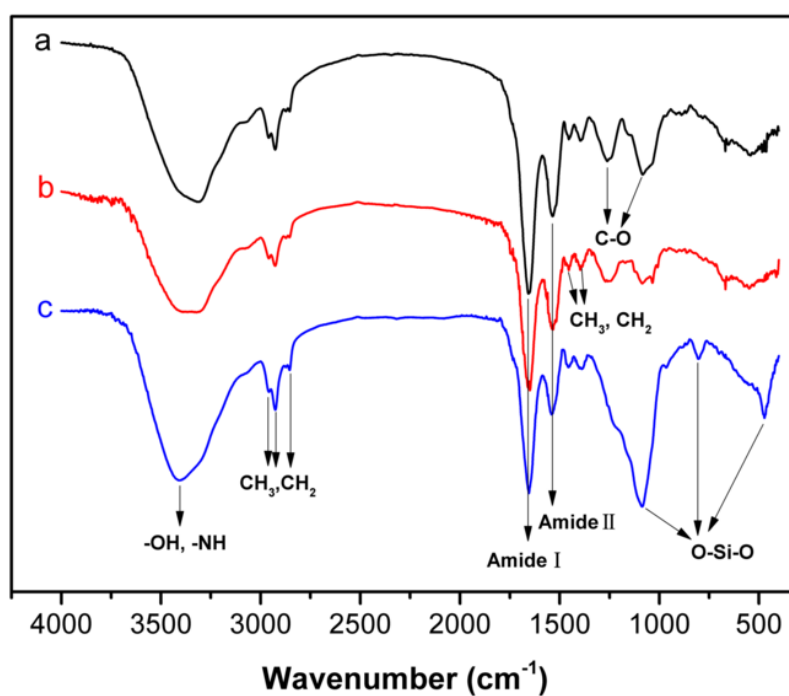


Fig. S4. FTIR spectra of the products obtained in different steps. Curve a: dried native cyanobacteria cells; curve b: PDADMAC/PSS-multilayer-coated cyanobacteria cells; and curve c: dried cyanobacteria@SiO₂.

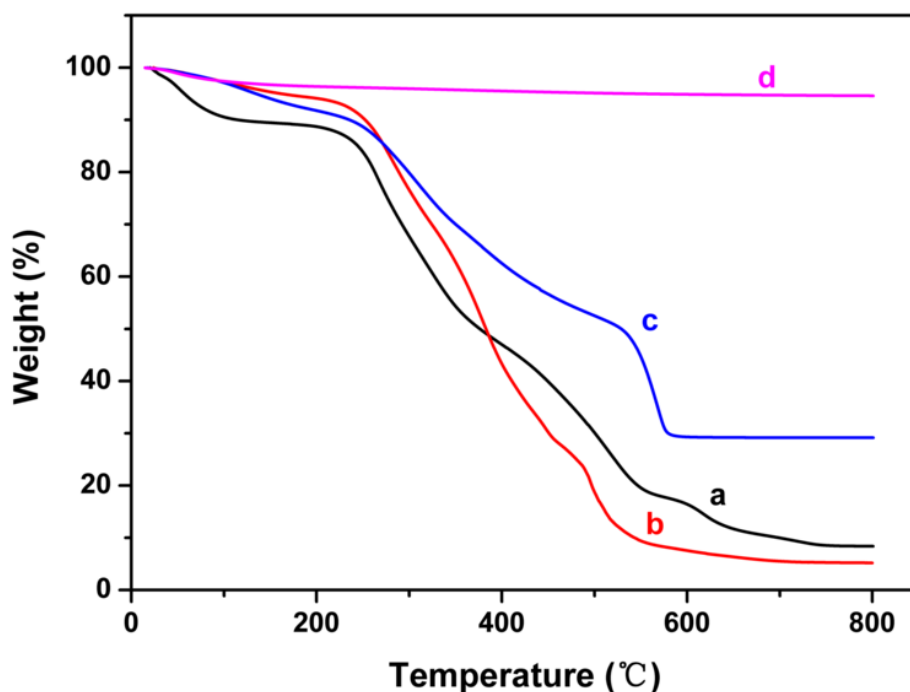


Fig. S5. TGA curves of a) dried native cyanobacteria cells, b) PDADMAC/PSS-multilayer-coated cyanobacteria cells, c) dried cyanobacteria@SiO₂, d) dried silica nanoparticle powders.

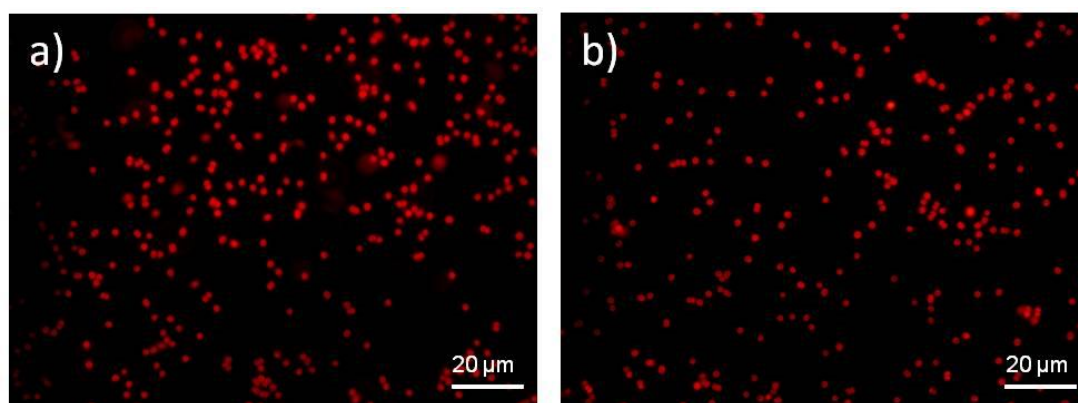


Fig. S6. Epifluorescence microscopy image of a) native cyanobacteria and b) cyanobacteria@SiO₂.

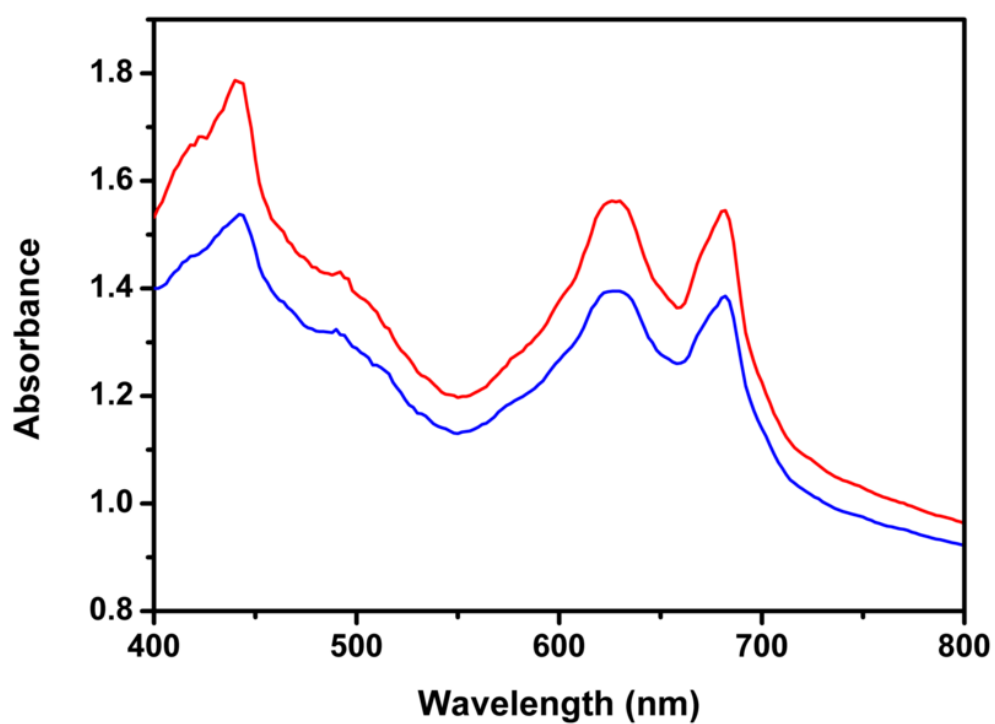


Fig. S7. Visible range spectra of native cyanobacteria (red curve) and cyanobacteria@SiO₂ (blue curve) cells.

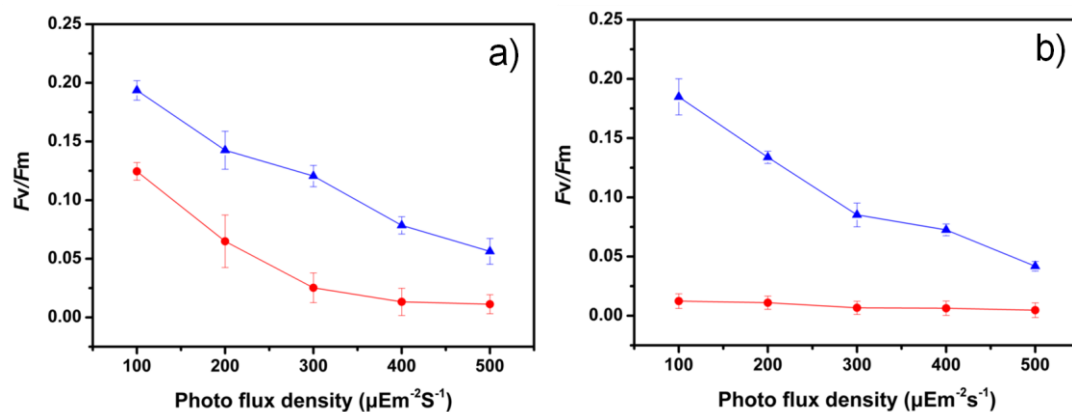


Fig. S8. Comparison of maximum quantum yield of PSII (F_v/F_m) in native cyanobacteria and cyanobacteria@SiO₂ cells.(n=3) a) Performance for 4h of exposure; b) Performance for 6h of exposure. The red line indicates native cyanobacteria, the blue line indicates cyanobacteria@SiO₂.

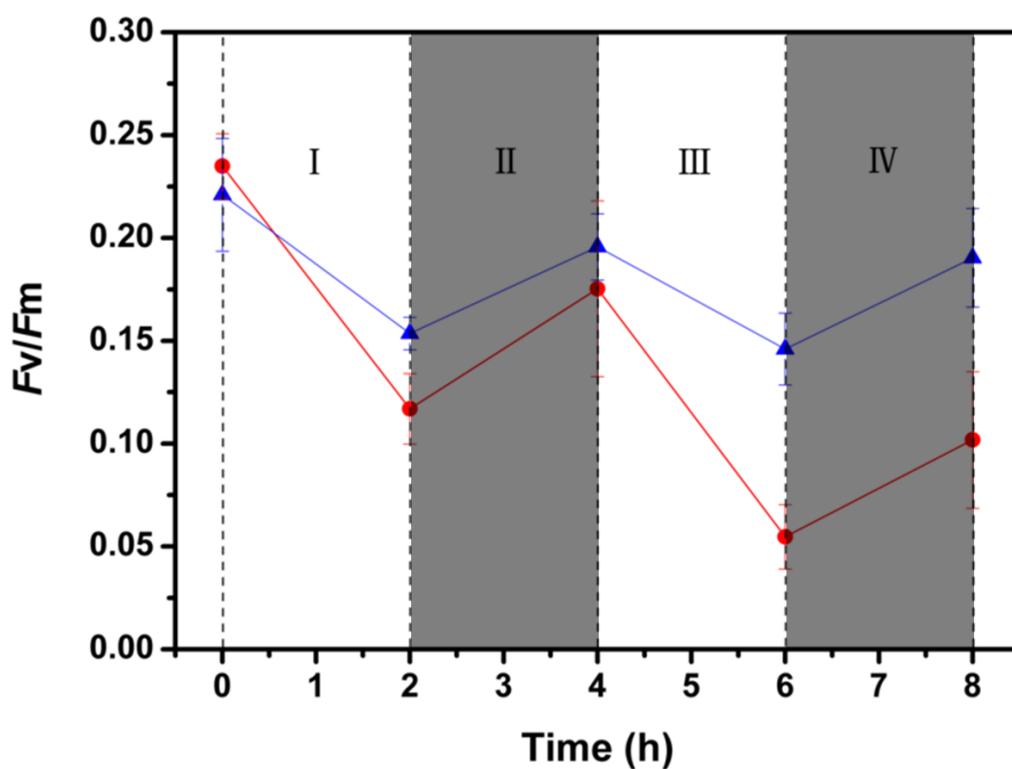


Fig. S9. Comparison of maximum quantum yield of PSII (F_v/F_m) in native cyanobacteria and cyanobacteria@SiO₂ cells under alternating light-dark diurnal cycle.(n=3) The time course was divided into four phases: I and III — light treatment, II and IV — dark treatment. The intensity of light is $300 \mu\text{Em}^{-2}\text{s}^{-1}$. The red line indicates native cyanobacteria, the blue line indicates cyanobacteria@SiO₂.

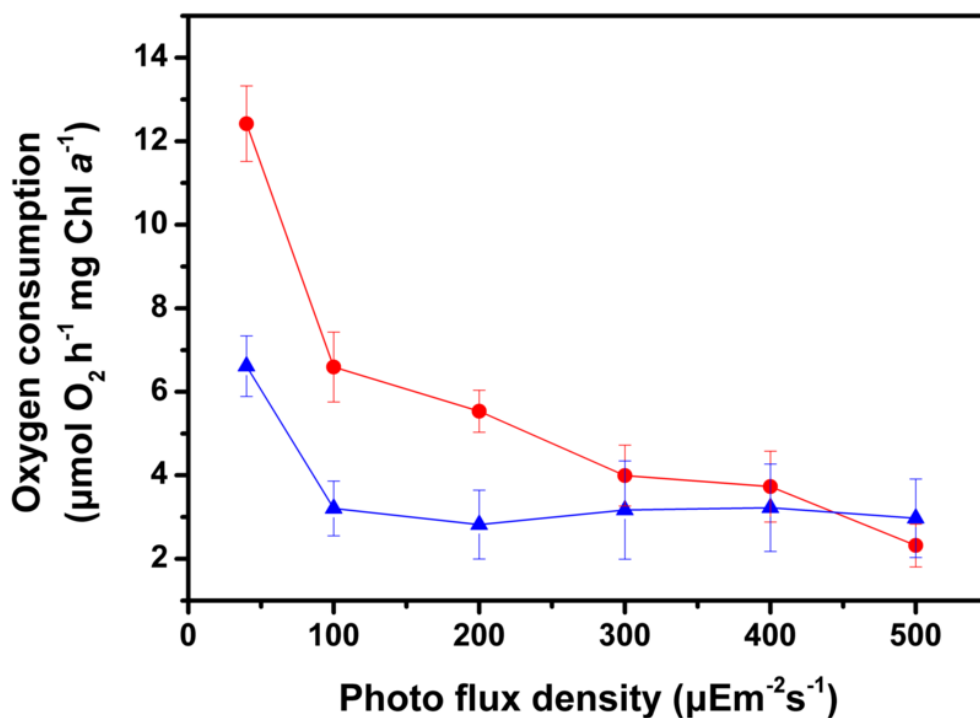


Fig. S10. Comparison of respiratory oxygen consumption in native cyanobacteria and cyanobacteria@SiO₂ cells.(n=3) The red line indicates native cyanobacteria, the blue line indicates cyanobacteria@SiO₂.

Table S1. The effect of thickness of biosilica shell on the performance of alleviating high light-induced photoinhibition. (n=3)

Thickness of biosilica shell (nm)	Fv/Fm under growth light conditions	Fv/Fm after light treatment under 500 μE m ⁻² s ⁻¹ for 2h	Photosynthetic conservation rate
0	0.235 ± 0.032	0.017 ± 0.005	7.2%
10	0.228 ± 0.021	0.033 ± 0.006	14.5%
30	0.221 ± 0.018	0.115 ± 0.008	52.0%
100	0.215 ± 0.026	0.039 ± 0.005	18.1%

Annotations: Photosynthetic conservation rate was calculated as (Fv/Fm after light treatment under 500 μE m⁻²s⁻¹ for 2h)/(Fv/Fm under growth light conditions) ×100%.