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Supporting Information for

Catalytic metal-nucleotides coordinative cytoskeleton on algae cell towards photosynthetic hydrogen production under air

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Methods

Characterization. UV-vis spectra measurements were conducted on a PerkinElmer spectrophotometer (Lambda 750S, USA). Scanning electron microscopy (SEM) images were acquired on a SU8000 with the samples sputter-coated with 10 nm platinum. Transmission electron microscopy (TEM) images were performed on a JEM-1400, using filament at 120kV in bright field mode. Confocal scanning laser microscopy (CSLM) characterization were conducted on a Leica SP8 confocal laser scanning microscope attached to a Leica DMI 6000 inverted epifluorescence microscope. Oscillator was employed by a VORTEX instrument (IKA, GER). Atomic force microscope (AFM) were performed on a 3D manipulation force microscope. The dissolved oxygen content was detected using a dissolved oxygen meter (METTLER TOLEDO, F4-Field). X-ray photoelectron spectroscopy (XPS) measurements were operated using a PHI ESCA 5700 with Al Kα (1486.6 eV). The maximum quantum yield of PSII (F_v/F_m) was measured using chlorophyll fluorometer (Yaxin, 1161 G, Beijing, China). Assimilated solutions were conducted using pipettors (GILSON, FRA).

Cell culture conditions. *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* cells were cultured in TAP medium containing 2×10^{-2} M Tris, 7×10^{-3} M NH_4Cl, 8.3×10^{-4} M $MgSO_4\cdot7H_2O$, 4.5×10^{-4} M $CaCl_2\cdot7H_2O$, 1.65×10^{-3} M K_2HPO_4, 1.05×10^{-3} M KH_2PO_4, 1 mL/L Hunter's trace elements and 1 mL/L glacial acetic acid. The medium pH was adjusted to 7.0. The cells were cultured in 0.5 L of TAP medium at 25 ^oC with the alternative daytime (12 h, 40 $\mu E\cdot m^{-2}\cdot s^{-1}$) and night (12 h). Optical density at 750 nm (OD_{750}) was measured using UV-visible spectroscopy to determine the cell number. The chlorophyll concentration was determined spectrophotometrically in 95 % (v/v) ethanol.

Construction of metal-nucleotides cytoskeleton on individual algal cell. Typically, 20 mL of *Chlorella* cells (OD_{750} = 2.0) were thoroughly washed using aqueous NaCl (0.01 M) solutions and DI water, and then collected by centrifugation (4000 *g*). The cells were dispersed in 1 mL of DI water containing 10 mg/mL of guanosine 5'-monophosphate (GMP) disodium salt hydrate with a constant stirring rate of 500 rpm, followed by the gradual dropping of 200 μL of 3 mg/mL aqueous ferric chloride solution. After kept stirring for 10 min at room temperature, the cells were washed at least three times by centrifugation (4000 *g*) so as to obtain Fe-GMP-coated algal cells.

Zeta potential measurements. Native and Fe-GMP-coated algal cells were thoroughly washed and dispersed in 10 mM PBS buffer (pH = 7.0) with the cell densities being adjusted at about 4.5×10^6 cells/mL. Then the zeta potentials were tesed by Zetasizer Nano (Malvern, UK).

Photosynthetic hydrogen production. A fixed volume of the algal cells containing *ca*. 10 mg/L of chlorophyll was transferred into a 100 mL grinding triangle glass bottle with a tightly sealed suction connector (90 mL of head air space and 10 mL of culture medium with contained algal cells). Then, 200 mg of sodium ascorbate was added into the culture medium, followed by its complete dissolving via shaking the bottles by hand. Afterwards, the bottles were incubated with continuous shaking under a light intensity of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. At predetermined time intervals, the measurements of hydrogen production in the headspace were monitored using the Hydrogen Detector (AP-B-H_2-F; capacity, 20000 ppm; resolution ratio, 1 ppm). The hydrogen production rate was calculated based on the content of chlorophyll associated with the algal cells in the culture medium.

Viability test of cells. fluorescein diacetate (FDA) was dissolved in acetone with the concentration of 5 mg/mL, and then 5 μL of it was extracted into centrifuge tube containing 1 mL of native and Fe-GMP-coated aglal cells. After incubated at room temperature in dark environment for 30 min, the cells were thoroughly washed using DI water for at least three times. Afterwards, the sample was observed at a confocal fluorescence microscope.

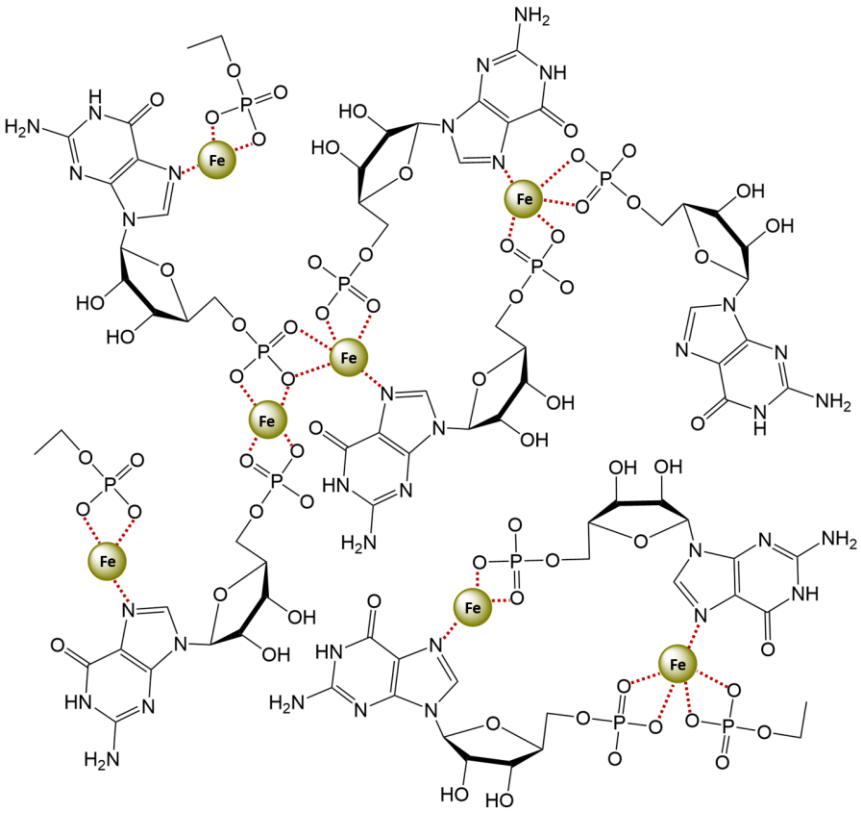

Figure S1. Schematic illustration showing the self-assembly between Fe and GMP. Fe was coordinated by the nucleobase and phosphate groups of GMP.

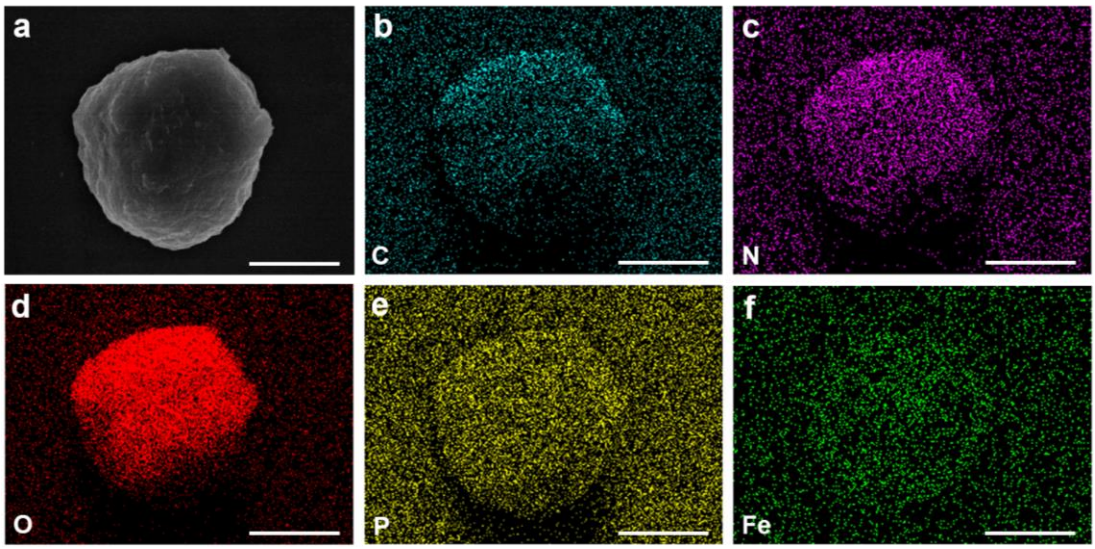

Figure S2. (a) SEM image of single Fe-GMP-coated *Chlorella* cell. (b-f) EDS elemental mappings of C (b), N (c), O (d), P (e) and Fe (f). Scale bars, 3 μm. P and Fe elements are homogeneously distributed on the cell surface, indicating the successful coating of Fe-GMP nanoparticles.

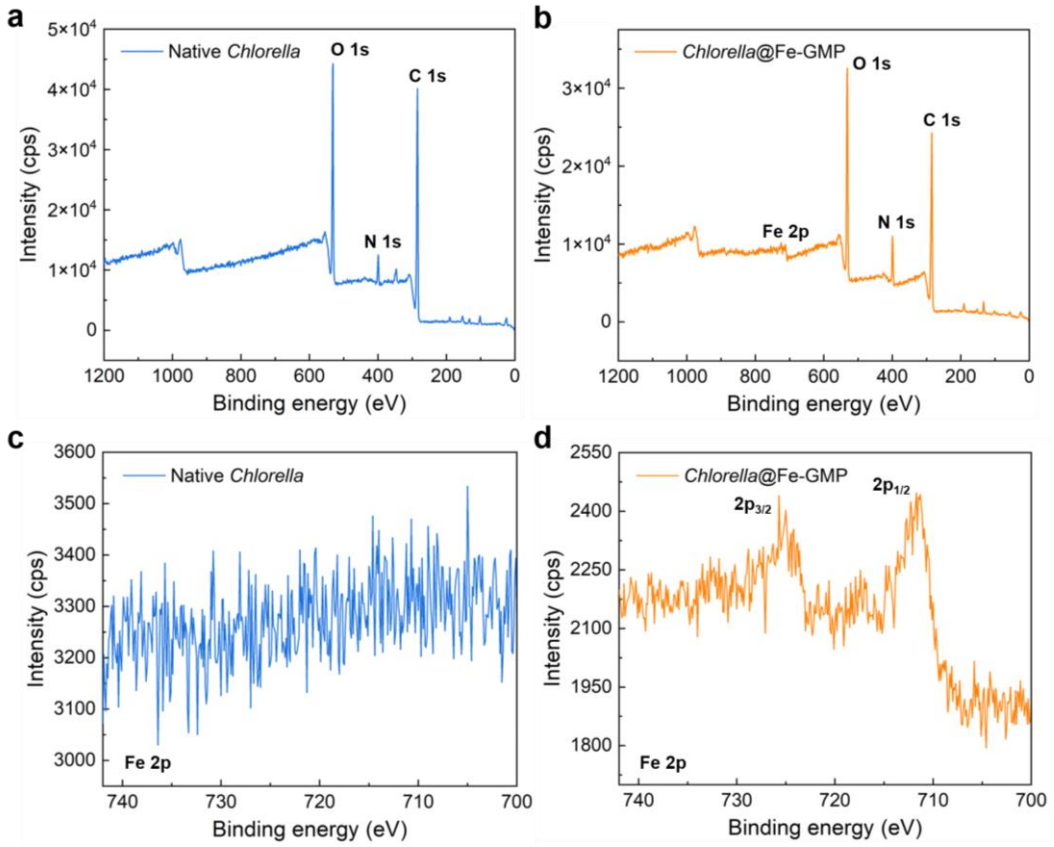

Figure S3. (a-b) XPS spectra of the native cell (a), Fe-GMP-coated (b) *Chlorella* cell. (c-d) High resolution of Fe 2p spectra of the native (c), Fe-GMP-coated (d) *Chlorella* cell. Peaks for Fe element are observed in the spectra of Fe-GMP-coated algal cells rather than native cells, indicating the successful coating of Fe-GMP nanoparticles.

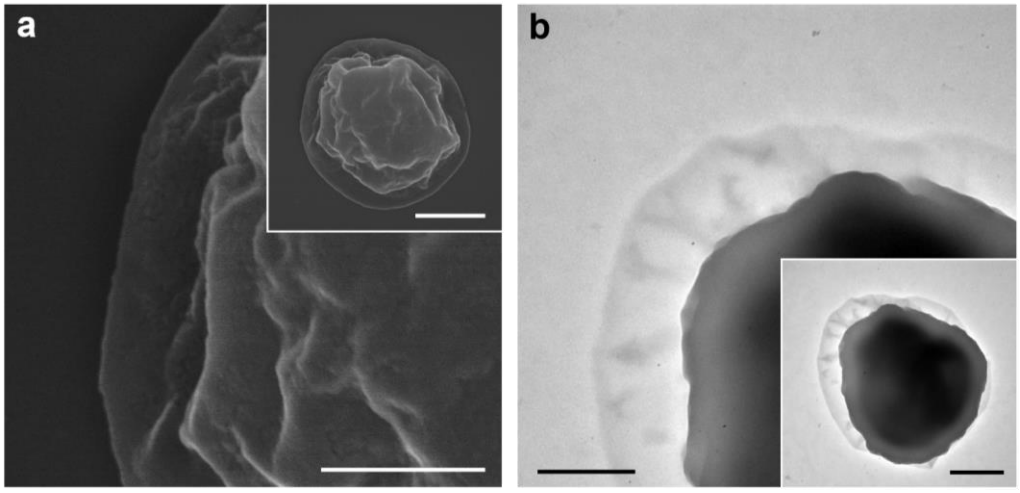

Figure S4. (a-b) SEM (a) and TEM (b) images of the localized region and whole cell (inset) of the native *Chlorella* cell. Scale bars, 500 nm and 1 µm (insets). It is observed that the cell wall of native algal cell is smooth and clean.

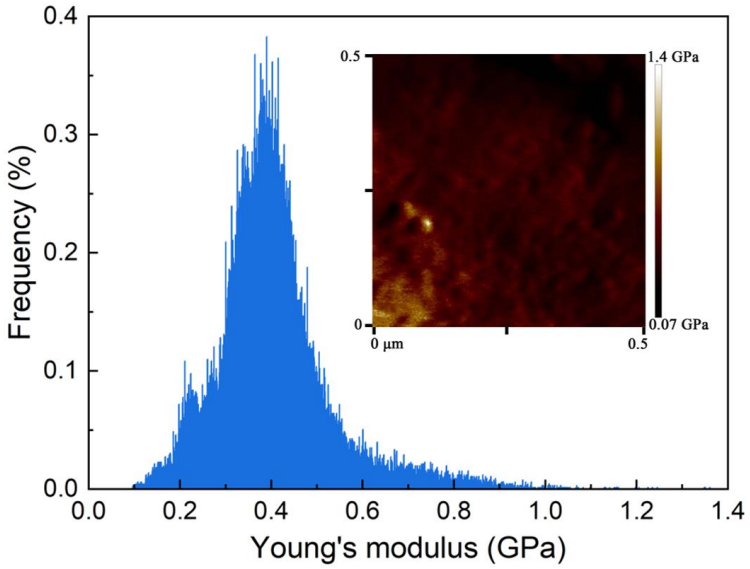

Figure S5. Histograms of Young's modulus of the native *Chlorella* cell with the corresponding value-distribution image (inset). The Young's modulus for native cells (*ca*. 0.4 Gpa) is obviously lower than that for Fe-GMP-coated cells (*ca*. 0.86 Gpa), which indicates the enhancement of surface mechanical performance by Fe-GMP nanoparticles.

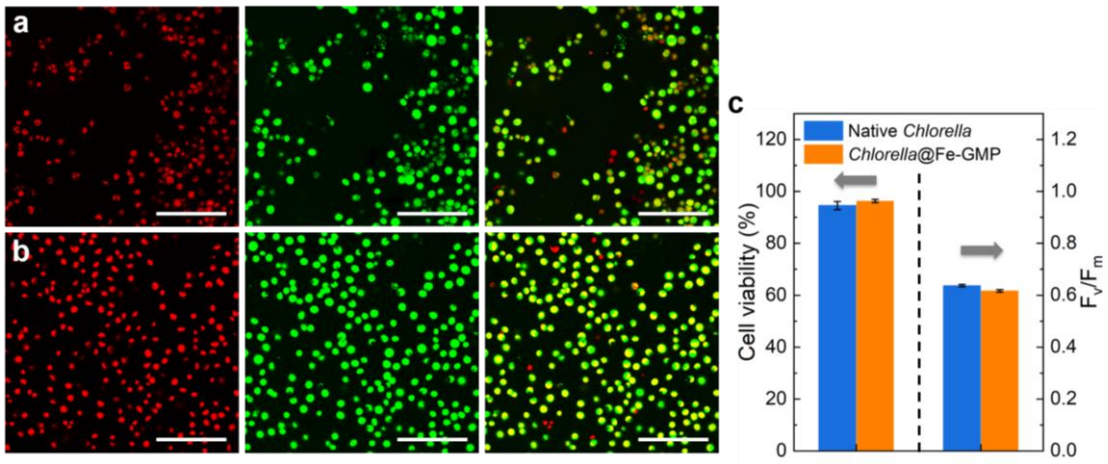

Figure S6. (a-b) Confocal microscopy fluorescence images of native (a) and Fe-GMP-coated (b) *Chlorella* cells. Red fluorescence is from the intracellular chlorophyll, green fluorescence is from the hydrolysis of FDA in viable cells, and yellow fluorescence is from the overlay of the red and green fluorescence. Scale bars, 40 μm. (c) Cell viability and F_v/F_m measurements of native and Fe-GMP-coated *Chlorella* cells. The result indicates that Fe-GMP nanoparticles impose negligible negative impact on cellular viability and the capability of absorbing and utilizing light.

Figure S7. Photograph showing the pure Fe-GMP nanoparticles dispersed in DI water.

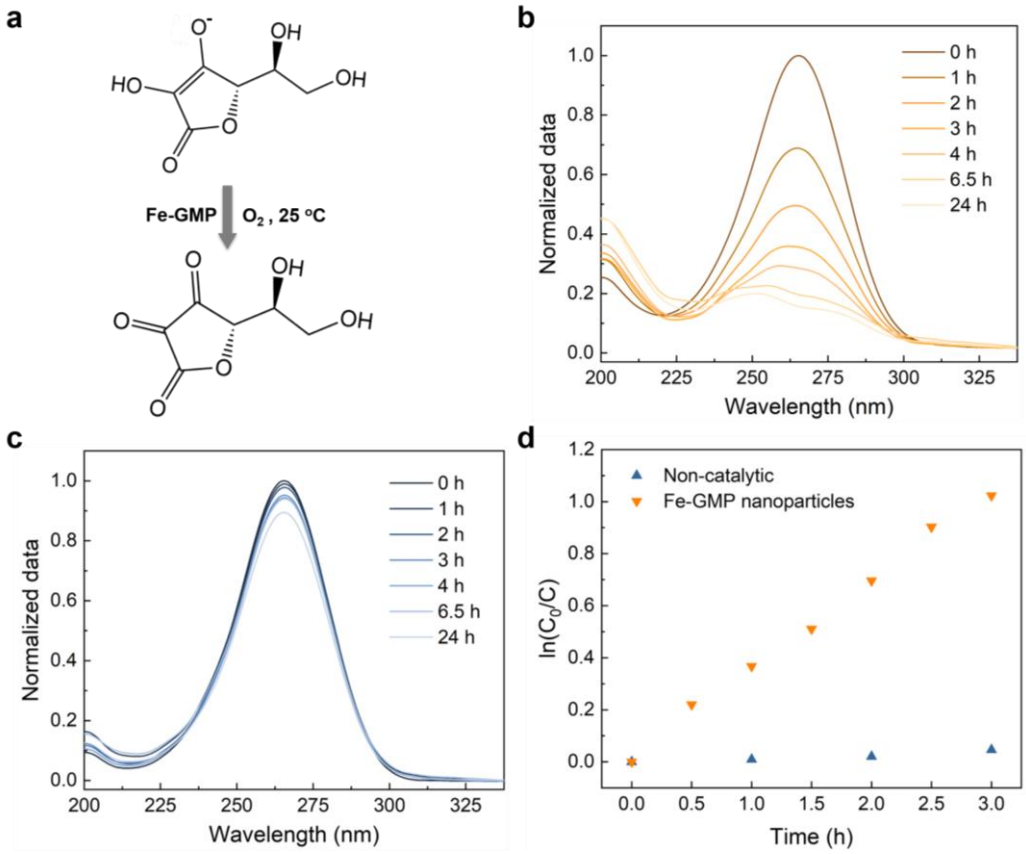

Figure S8. (a) Schematic illustration showing the oxidation reaction of ascorbate catalyzed by Fe-GMP nanoparticles at room temperature. (b-c) Time-dependent measurements of UV-vis spectra of ascorbate without catalysis (b) or catalyzed by Fe-GMP nanoparticles (c). (d) Plots showing the first-order kinetics of ascorbate oxidation without catalysis and catalyzed by Fe-GMP nanoparticles. Ascorbate, 0.5 mg/mL; Fe-GMP nanoparticles, 0.64 mg/mL.

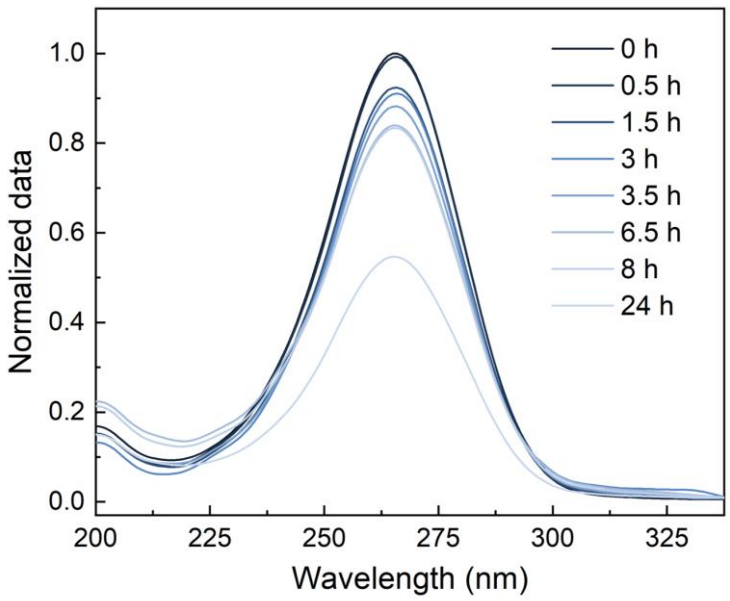

Figure S9. Time-dependent measurements of UV-vis spectra of ascorbate in the presence of native *Chlorella* cells. Ascorbate, 0.5 mg/mL; cell density, $1.27*10^7$ cells/mL.

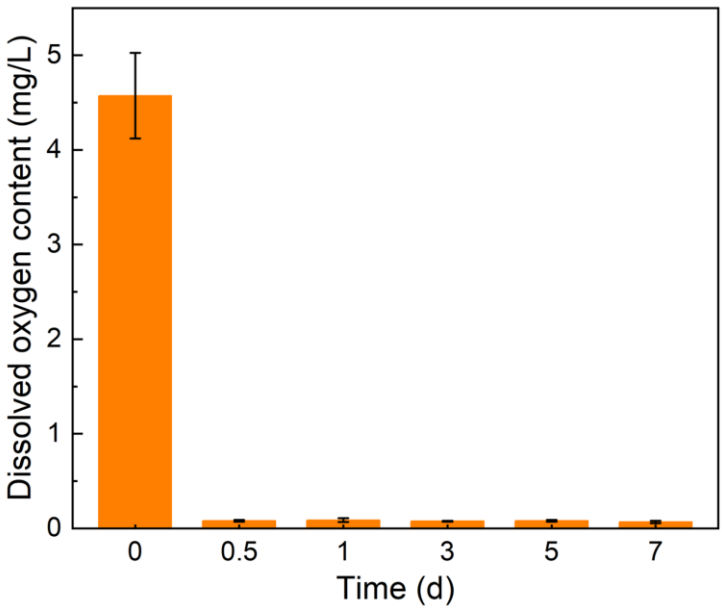

Figure S10. Time-dependent measurements of the dissolved oxygen concentration for Fe-GMP-coated *Chlorella* cells dispersed in ascorbate-containing TAP culture medium. Samples are in sealed vials and exposed to daylight with an intensity of 100 µE m^{-2} s^{-1}. The hypoxic condition is efficiently fabricated within 12 h.

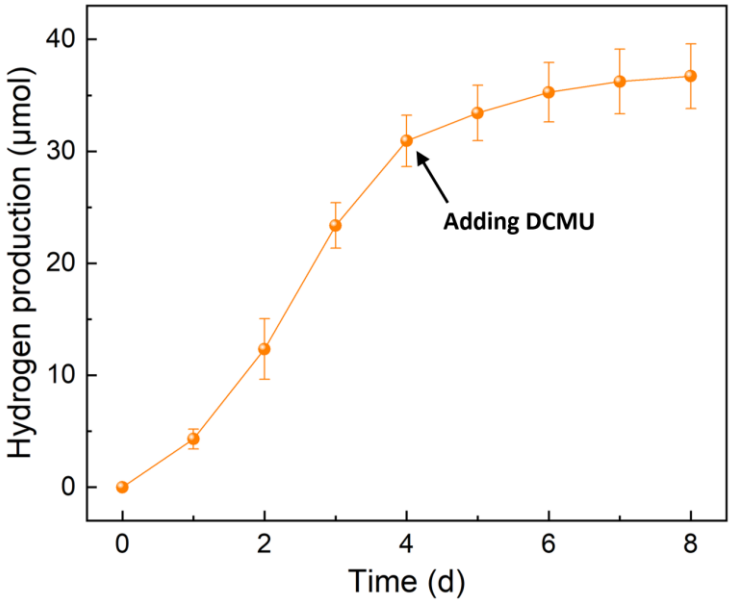

Figure S11. Time-dependent measurements of hydrogen production for the Fe-GMP-coated *Chlorella* cells under the addition of DCMU. Samples are in sealed vials and exposed to daylight with an intensity of 100 μE m^{-2} s^{-1}. The addition of DCMU greatly inhibits the hydrogen evolution, which should suggest that the electron source for hydrogenase-mediated hydrogen production mainly originates from PSII.

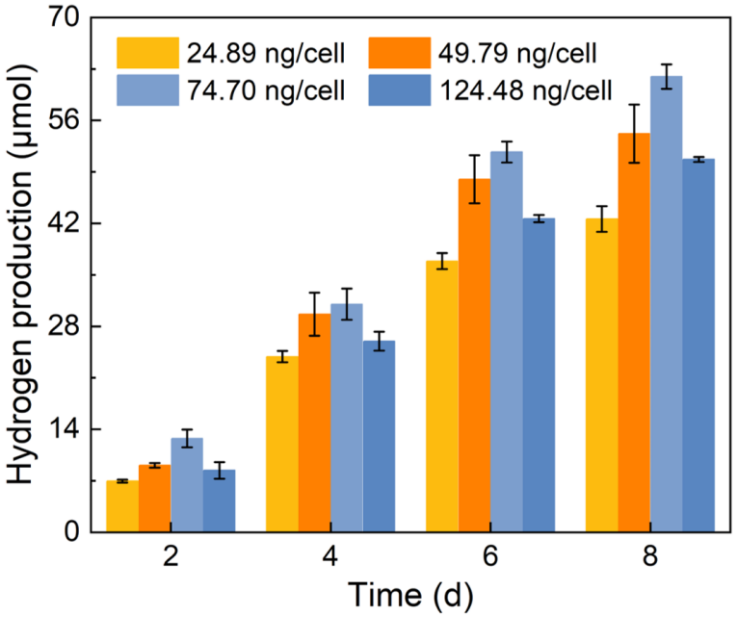

Figure S12. Time-dependent measurements of hydrogen production for the *Chlorella* cells coated with different levels of Fe-GMP nanoparticles when dispersed in ascorbate-containing TAP culture medium. Increasing the coating level of Fe-GMP nanoparticles from 24.89 to 74.70 ng/cell promotes the hydrogen production of *Chlorella* cells. However, when the coating level is up to 124.48 ng/cell, the hydrogen production decreases, which should suggest that the excess of Fe-GMP nanoparticles may in turn inhibit the hydrogen production process.

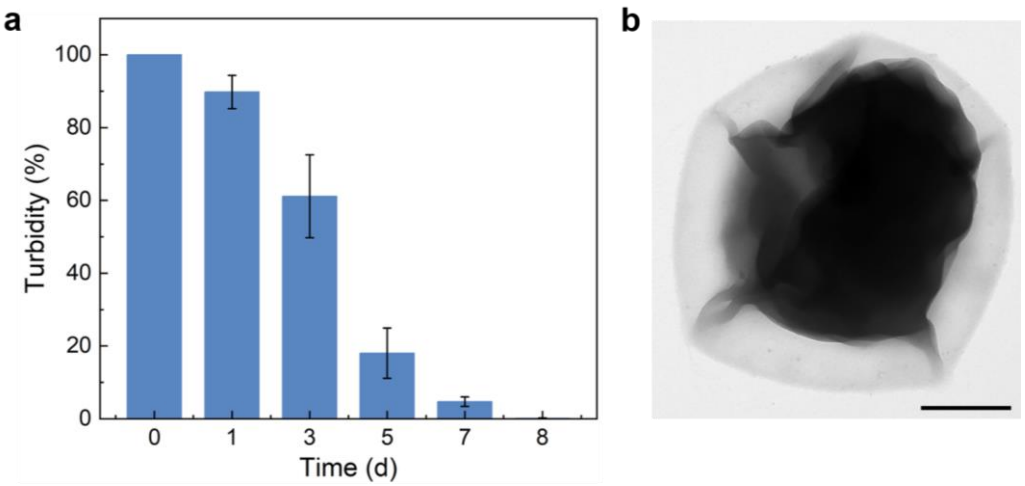

Figure S13. (a) Time-dependent measurements of turbidity for pure Fe-GMP nanoparticles dispersed in ascorbate-containing TAP culture medium. (b) TEM image of the Fe-GMP-coated *Chlorella* cell dispersed in ascorbate-containing TAP culture medium for 8 days. Scale bar, 500 nm. Ascorbate, 20 mg/mL; Fe-GMP nanoparticles, 0.64 mg/mL; Fe-GMP nanoparticles per cell, 49.79 ng/cell.

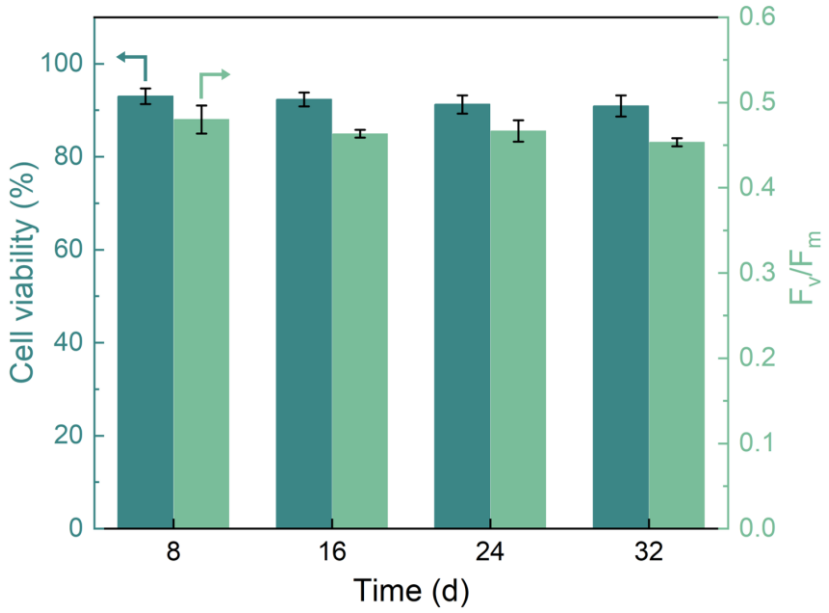

Figure S14. Time-dependent measurements of cell viability and F_v/F_m for the Fe-GMP-coated *Chlorella* cells cultivated in ascorbate-containing culture medium. At the determined time points, cells are recoated with new Fe-GMP nanoparticles and the culture solutions are also refreshed. Based on the results, cell viability as well as necessary PSII activity is well maintained for over 1 month.

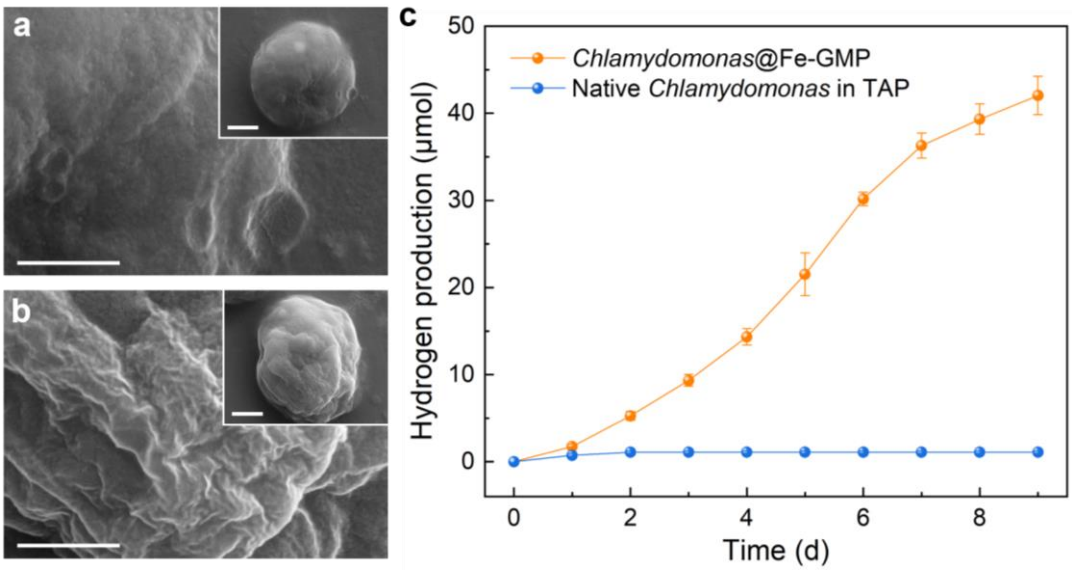

Figure S15. (a-b) SEM images of the localized region and whole cell (inset) of the native (a) and Fe-GMP-coated (b) *Chlamydomonas reinhardtii* cell. Scale bars, 1 μm and 2 μm (insets). (c) Time-dependent measurements of hydrogen production from Fe-GMP-coated *Chlamydomonas* cells dispersed in ascorbate-containing TAP culture medium and native cells in normal TAP culture medium. Samples are in sealed vials and exposed to daylight with an intensity of 100 μE m^{-2} s^{-1}. Fe-GMP nanoparticles could be also coated onto the *Chlamydomonas* cell, and allow the efficient hydrogen production under daylight in air.