Supporting Information for

From semiconductor diversity to mechanistic specificity: S-doped graphitic carbon nitride reprogramming metabolic pathways for bioplastic production

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Experimental methods

Preparation of CdS:

1. Gradually add 10 mL of TEOA dropwise into 10 mL of $CdCl_2$ solution (0.2 M) and stir until the mixture becomes homogeneous;
2. Add the above solution dropwise into 40 mL of NaOH solution (5 M) and stir for 10 min;
3. Add 10 mL of Na_2S solution (0.2 M) dropwise and continue stirring for 2 h;
4. Transfer the reaction mixture into a Teflon-lined autoclave and heat at 200 °C for 12 h in a constant-temperature oven;
5. After the reaction, allow the mixture to cool to room temperature, filter it under vacuum, and wash the precipitate with ultrapure water and anhydrous ethanol, followed by an additional thorough wash with ultrapure water;
6. Dry the precipitate at 60 °C overnight in a constant-temperature oven, and grind thoroughly to obtain bright yellow CdS nanorods.

Preparation of Co_3O_4:

The preparation begins with the synthesis of ZIF-67. $Co(NO_3)_2 \cdot 6H_2O$ (4.192 g) was dispersed in 80 mL of methanol and labeled as solution A. Separately, 2-methylimidazole (2-MeIm, 3.164 g) was dispersed in 80 mL of methanol and labeled as solution B. Solution B was then added dropwise to solution A and stirred at room temperature for 2 h. Afterward, the mixture was left to stand in the dark for 24 h. The precipitate was collected by centrifugation, washed three times with ethanol, and dried to obtain a purple ZIF-67 powder. The ZIF-67 polyhedra were then calcined in air at 350 °C for 2 h (heating rate: 5 °C min^{-1}) to produce porous Co_3O_4 polyhedra.

Preparation of Cu-MOF:

Copper nitrate hexahydrate ($Cu(NO_3)_2 \cdot 6H_2O$, 4.8 mmol) was added to 180 mL of methanol and designated as solution A. 2-Methylimidazole (54.6 mmol) was added to 140 mL of methanol and designated as solution B. Both solutions were stirred for 10 min. Solution A was then added dropwise into solution B, followed by stirring for 30 min. The mixture was left to stand in the dark for 24 h to obtain the Cu-MOF.

The composition of nitrogen-limited basal medium：

The nitrogen-limited (N-limited) medium contained NH_4Cl (1 g L^{-1}), $NaH_2PO_4 \cdot 2H_2O$ (5.2 g L^{-1}), $Na_2HPO_4 \cdot 12H_2O$ (11.6 g L^{-1}), K_2SO_4 (0.45 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.8 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.08 g L^{-1}), and 1 mL L^{-1} of a trace element solution. The trace element solution consisted of HCl (0.1 M), $FeSO_4 \cdot 7H_2O$ (15 g L^{-1}), $MnSO_4 \cdot H_2O$ (2.4 g L^{-1}), $ZnSO_4 \cdot 7H_2O$ (2.4 g L^{-1}), and $CuSO_4 \cdot 5H_2O$ (0.48 g L^{-1}). The medium was sterilized at 121 °C for 20 min, and fructose was used as the carbon source for heterotrophic cultivation.

Detailed procedures for heterotrophic cultivation:

(1) *C.N* was transferred from a cryovial into 50 mL of TSB medium and incubated at 30 °C on a rotary shaker at 200 rpm for 12 h to reach the logarithmic growth phase;

(2) The activated culture was inoculated (5%, v/v) into 50 mL of nitrogen-limited basal medium supplemented with fructose and TEOA in a 250 mL Erlenmeyer flask. Cultivation was performed at 30 °C and 200 rpm using a magnetic stirrer inside a constant-temperature incubator. LED strips with a wavelength of 400 ± 5 nm and a light intensity of 3.07 ± 0.14 mW cm^{-2} were used as the illumination source. The cultures were maintained for 120 h under optimized conditions, and samples were periodically collected to determine cell dry weight (DCW) and PHB production.

Detailed procedures autotrophic cultivation:

(1) *C.N* was first inoculated from a cryovial into 50 mL of TSB medium and incubated at 30 °C at 200 rpm for 12 h (logarithmic phase);

(2) The activated culture was inoculated (5%, v/v) into 50 mL of nitrogen-limited basal medium in a 125 mL serum anaerobic bottle. Cultures were incubated at 30 °C and 200 rpm using a magnetic stirrer until reaching $OD_{600} = 0.5$. The gas atmosphere in the serum bottle was controlled at $H_2:O_2:CO_2 = 7:1:2$. Every 12 h, gas was replenished from a mixed-gas bag using a syringe to pressurize the headspace to atmospheric pressure 60 ± 5 kPa.

(3) The culture from step (2) was inoculated (5%, v/v) into fresh nitrogen-limited medium in serum bottles and grown again to $OD_{600} = 0.5$;

(4) The culture was subcultured once more under the same conditions (5% inoculum) and grown to $OD_{600} = 0.5$;

(5) The resulting culture was transferred (5%, v/v) into fresh nitrogen-limited medium in serum bottles and incubated in the dark for 12 h. Intermittent illumination (400 ± 5 nm LED, 3.07 ± 0.14 mW cm^{-2}) was then applied. Cultures were maintained for 120 h under controlled conditions, and samples were periodically collected to monitor gas consumption as well as PHB and biomass accumulation.

Results and discussion

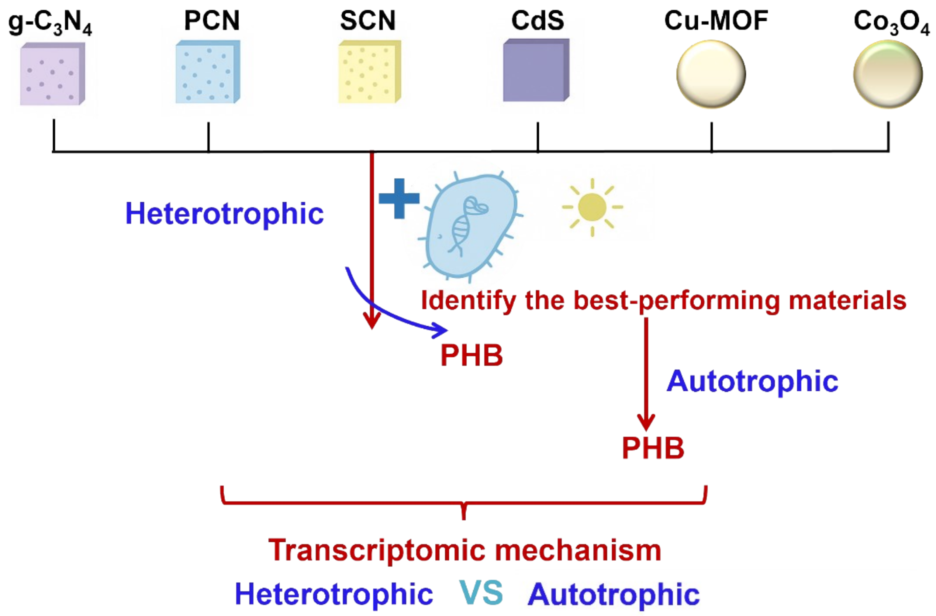

Figure S1. Schematic workflow of this study.

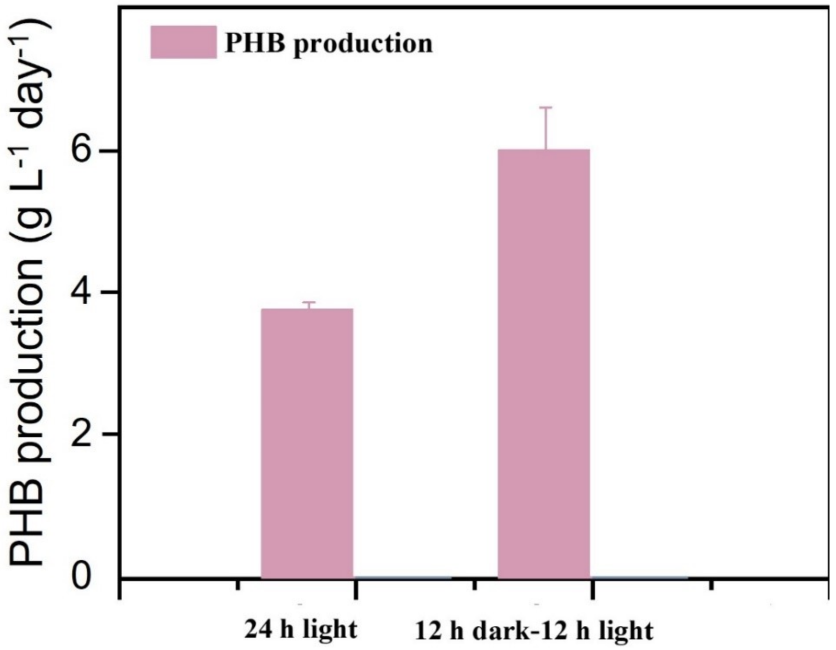

Figure S2. The impact of different light conditions on PHB synthesis in H-*C.N*@SCN-light at 48 h.

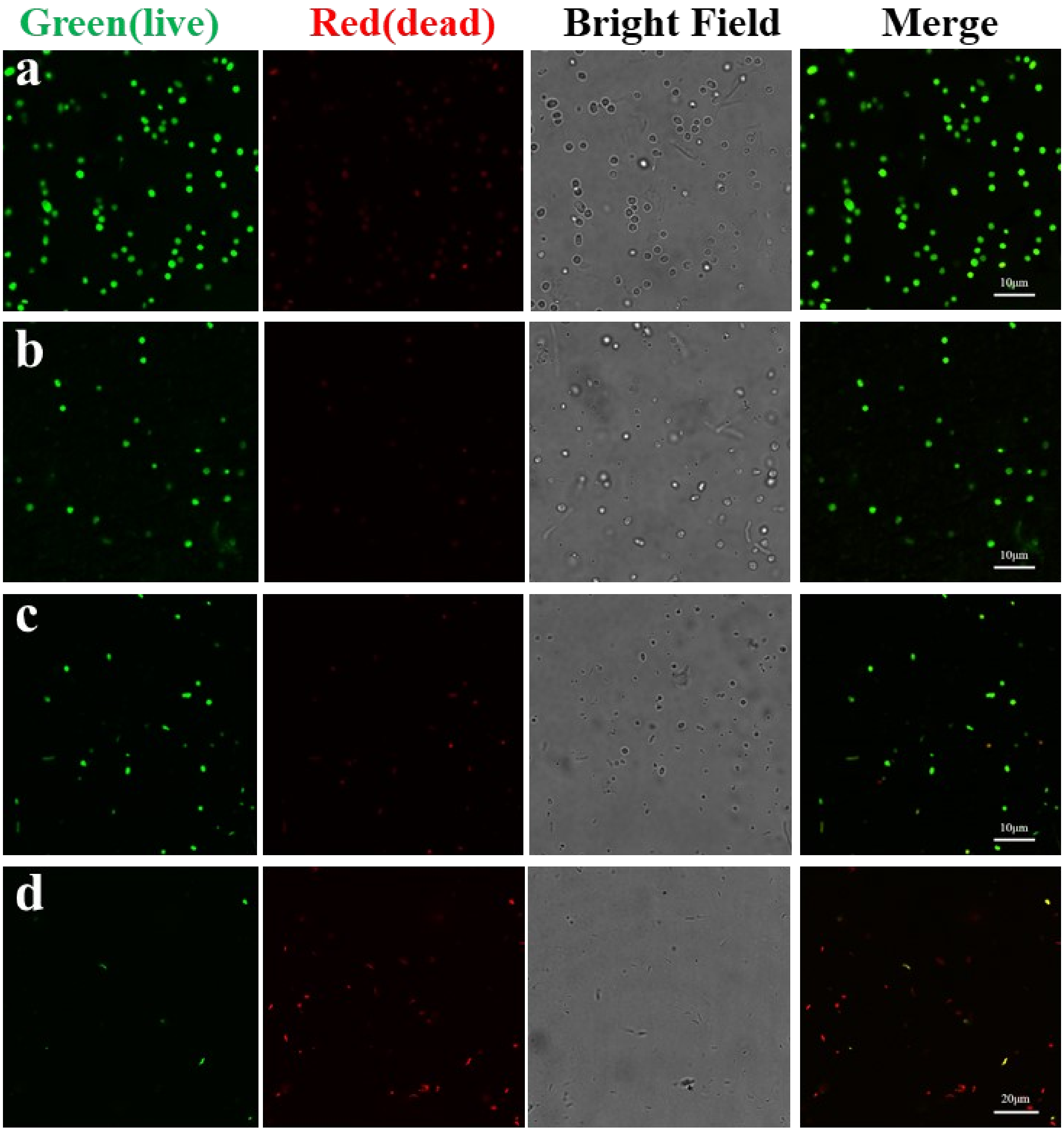

Figure S3. CLSM images of different biohybrid systems: (a) control group of H-*C.N*-dark, (b) H-*C.N*-@SCN-light, (c) H-*C.N*-@Cu-MOF-light, and (d) H-*C.N*-@Co_3O_4-light.

Table S1 Summary of semiconductor properties and heterotrophic PHB production performance of the constructed *C.N*–semiconductor biohybrid systems

Semiconductors	Specific surface area (m^2 g^{-1})	Pore diameter (nm)	Band gap (eV)	Electrochemical response	PHB production rate-48 h (g L^{-1} day^{-1})
g-C_3N_4	74.8	47.2	2.80 eV	Decreased the SCD	2.05 ± 0.02
PCN	59.6	21.8	2.74 eV	Decreased the SCD	2.28 ± 0.005
SCN	51.7	67.9	1.90 eV	Increased the SCD	3.12 ± 0.13
CdS	89.6	11.2	2.40 eV	Increased the SCD	1.21 ± 0.08
Cu-MOF	112.4	74.6	2.91 eV	Increased the SCD	0.01 ±0.0001
Co_3O_4	113.3	6.7	2.16 eV	Increased the SCD	0.26 ± 0.08

Note: SCD denotes the system current density.

Calculations of Band-Gap Energy:

Based on the absorption spectra of the materials, the wavelength at which significant light absorption begins—referred to as the absorption edge—can be used to estimate the excitation wavelength. The corresponding photon energy (i.e., the direct band gap) can then be calculated using Equation 1:

$$E = \frac{hc}{\lambda} \# (1)$$

where:

E is the photon energy (eV)

h is Planck's constant (6.626×10^{-34} J·s)

c is the speed of light (3.0×10^{8} $m \cdot s^{-1}$)

λ is the wavelength (m)

Using the absorption-edge wavelengths obtained from the spectra, the direct band gaps of g-C_3N_4, PCN, and SCN were estimated under the assumption that all three are direct band-gap semiconductors. Their absorption edges at 443.15 nm, 452.86 nm, and 695.75 nm correspond to direct band-gap energies of 2.80 eV, 2.74 eV, and 1.79 eV, respectively.

The Tauc plot is a commonly used method to estimate the optical band gap of semiconductor materials. Depending on the type of band gap, different equations are used.

For direct band-gap semiconductors, Equation 2 is applied:

$$(\alpha h\nu)^2 = A(h\nu - E_g)\# \ (2)$$

For indirect band-gap semiconductors, Equation 3 is used:

$$(\alpha h\nu)^{\frac{1}{2}} = A(h\nu - E_g)\# \ (3)$$

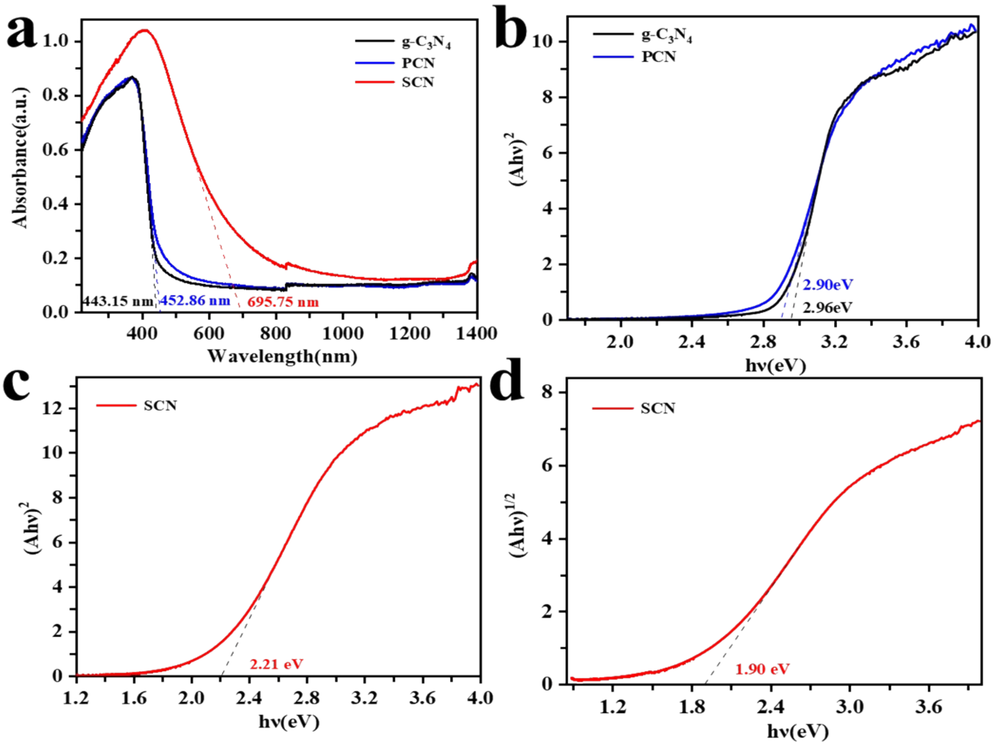

Figure S4. (a) UV-Vis DRS of g-C_3N_4, PCN and SCN; (b) direct bandgap of g-C_3N_4 and PCN; (c) direct bandgap of SCN; (d) indirect bandgap of SCN.

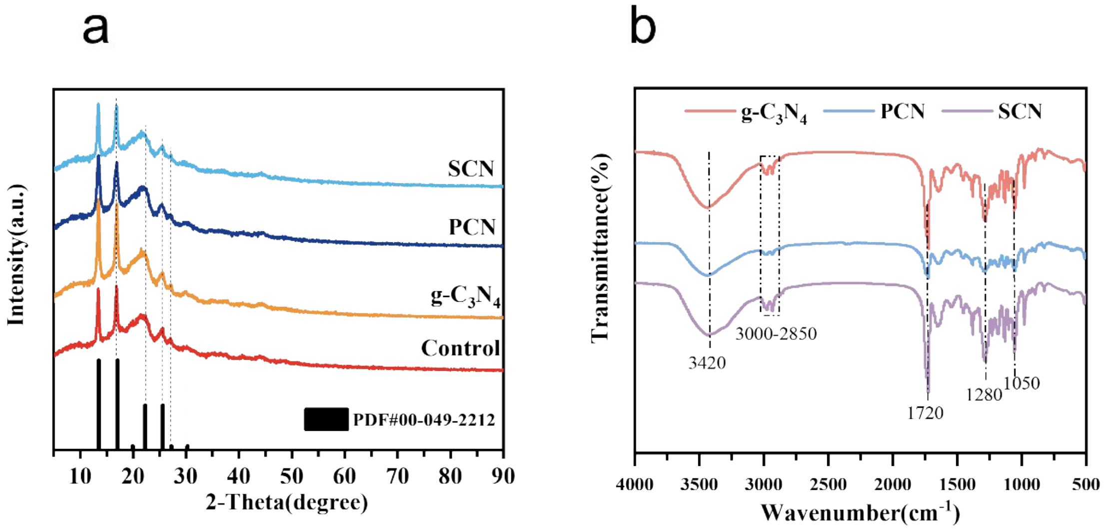

Figure S5. (a) XRD patterns of the H-*C.N*-@g-C_3N_4-light, H-*C.N*-@SCN-light and H-*C.N*-@PCN-light groups and the control group of H-C.N-dark. (b) FTIR spectra of the H-*C.N*-@g-C_3N_4-light, H-*C.N*-@SCN-light and H-*C.N*-@PCN-light groups.

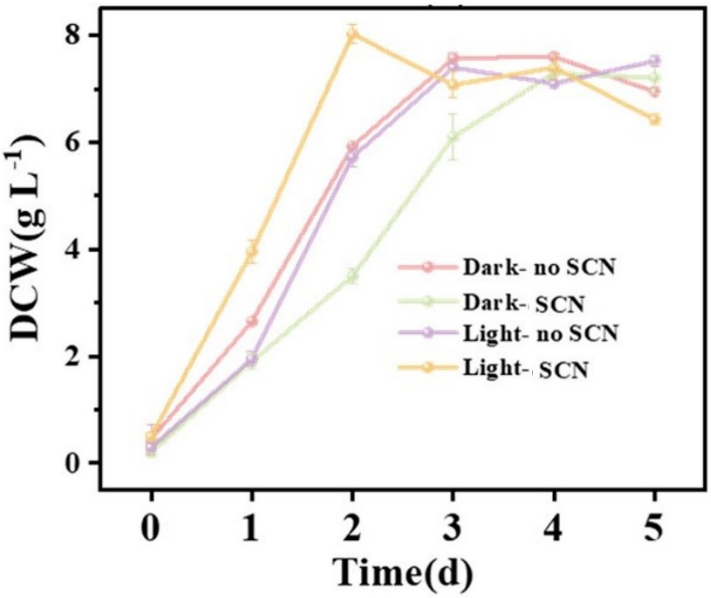

Figure S6. DCW changes as function of time in the A-*C.N*@SCN-light group.

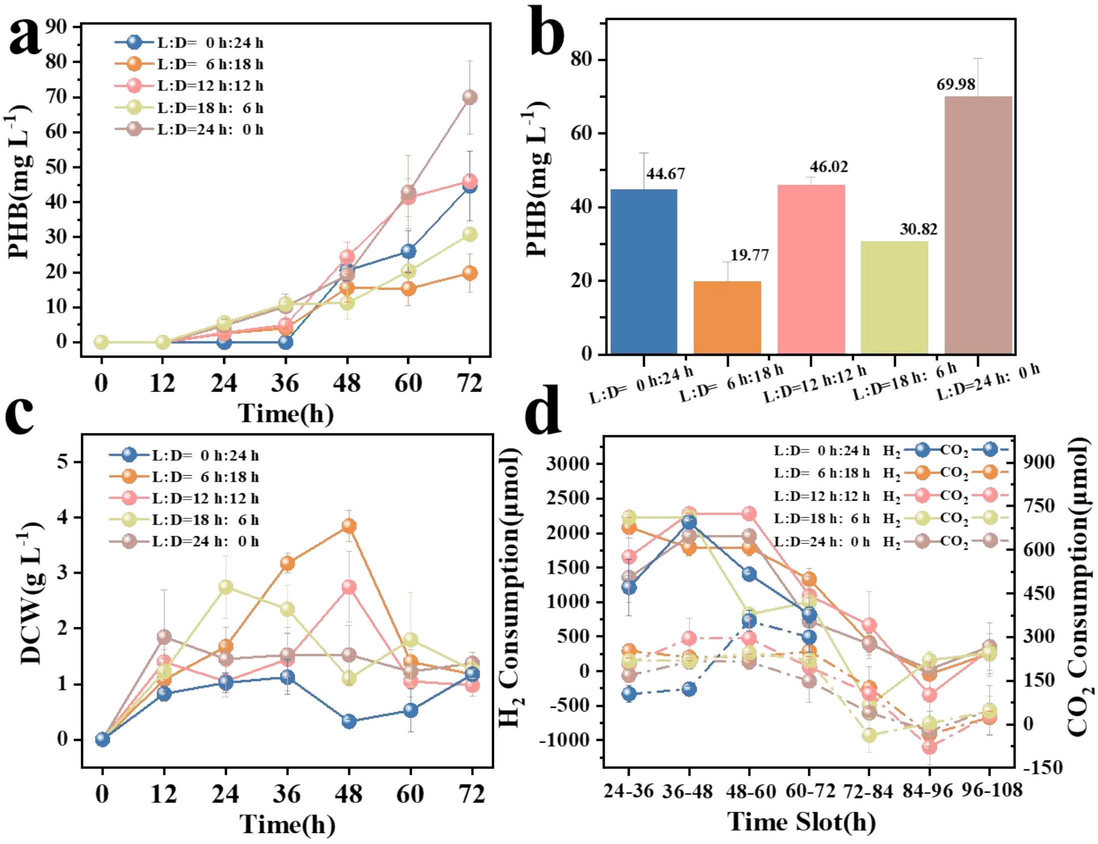

Figure S7. The impact of different light conditions on PHB synthesis in the autotrophic biohybrid systems. (a) PHB changes; (b) maximum accumulation and production rate of PHB; (c) DCW changes; (d) changes in gas consumption.

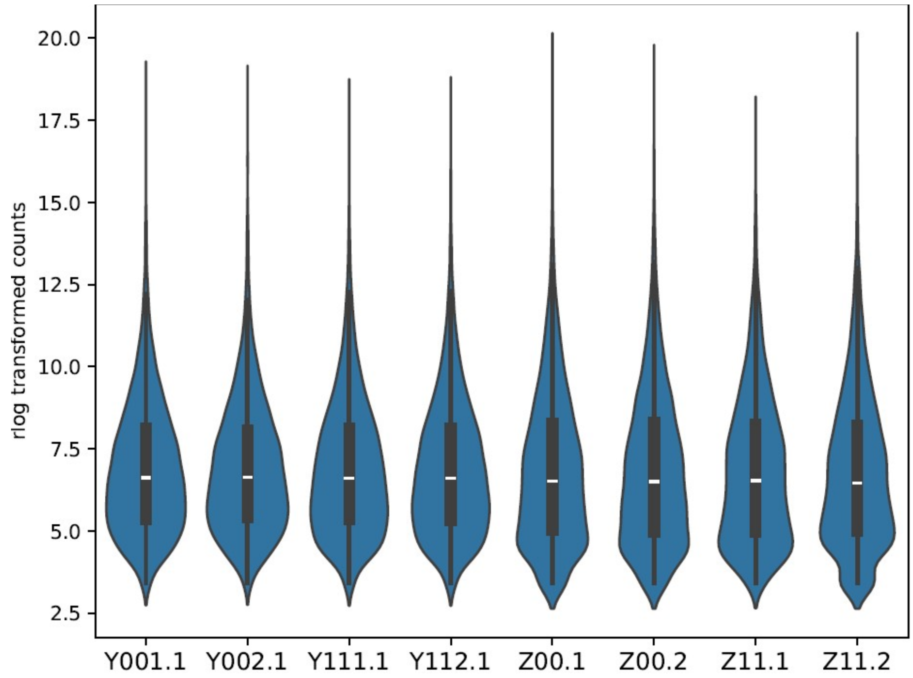

Figure S8 Distribution of gene expression levels in the constructed biohybrid system under heterotrophic (H-*C.N*@SCN-light) and autotrophic (A-*C.N*@SCN-light) conditions: violin plot.

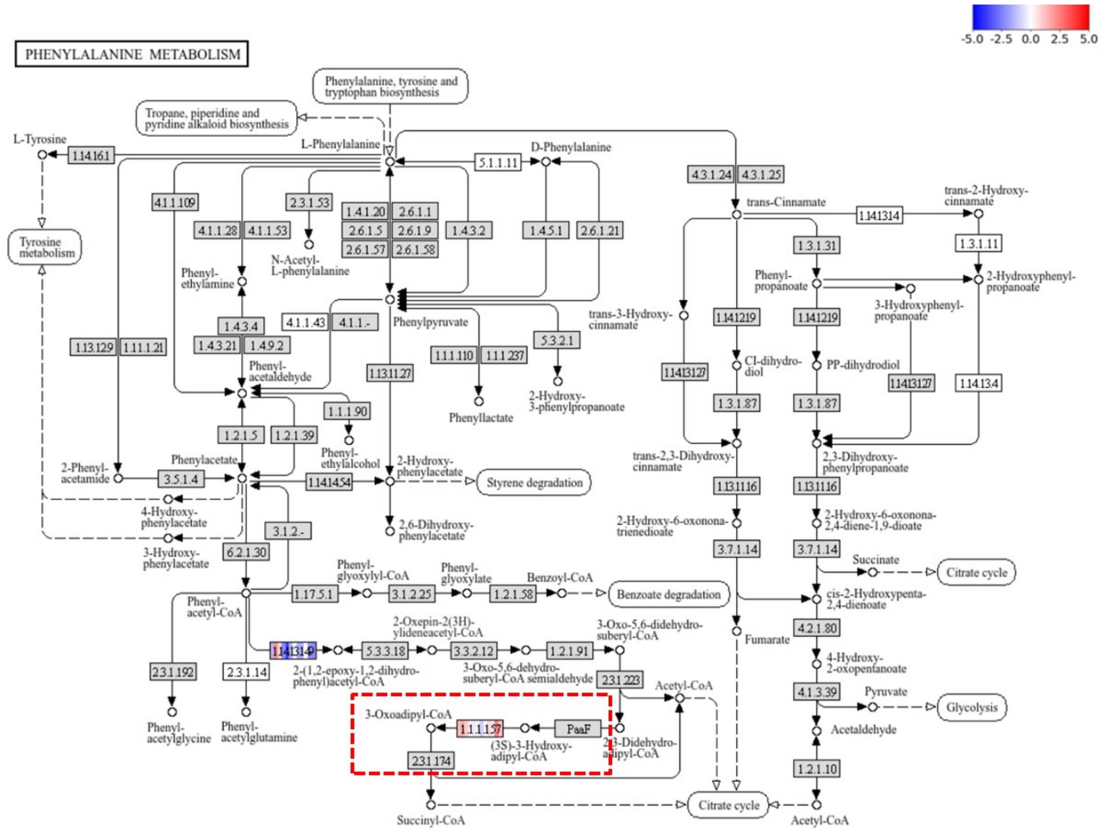

Figure S9. Phenylalanine metabolism pathway in the H-*C.N*@SCN-light group and the control group of H-*C.N*-dark.

Quantum efficiency (QE) calculation for the autotrophic system

Here we report an apparent quantum efficiency based on incident photons.[1,2] The quantum efficiency (QE) was calculated on an incident-photon basis as the ratio between the amount of PHB produced (expressed in moles of PHB repeating units) and the amount of incident photons during the illuminated cultivation:

$$\mathrm{QE} = n_{\mathrm{PHB}} / n_{\mathrm{photon}} \tag{4}$$

The incident radiant energy was calculated from the measured irradiance I, effective illuminated area A, and illumination time t:

$$E_{\mathrm{light}} = \mathrm{I} \times \mathrm{A} \times \mathrm{t} \tag{5}$$

The energy of a photon at wavelength $\lambda$was calculated as:

$$E_{\mathrm{photon}} = (\mathrm{h} \times \mathrm{c}) / \lambda \tag{6}$$

The incident photon amount (in mol photons) was obtained as:

$$n_{\mathrm{photon}} = \mathrm{Elight} / (E_{\mathrm{photon}} \times N_{\mathrm{A}}) \tag{7}$$

The irradiation conditions were $I = 3.07\ \mathrm{mW\ cm^{-2}} = 0.00307\ \mathrm{W\ cm^{-2}}$, $\lambda = 400$ nm, and $t = 48\ \mathrm{h} = 172800$ s. Physical constants were $h = 6.626 \times 10^{-34}$ J·s, $c = 3.00 \times 10^{8}\ \mathrm{m \cdot s^{-1}}$, and $N_A = 6.022 \times 10^{23}$ mol⁻¹. A was estimated to be ~15 cm^2 (effective illuminated area) based on the illuminated region of the reactor surface.

Photon energy. Using $\lambda = 400$ nm $= 4.00 \times 10^{-7}$ m:

$$E_{\text{photon}} = (6.626 \times 10^{-34} \times 3.00 \times 10^{8}) / (4.00 \times 10^{-7}) = 4.97 \times 10^{-19} \text{ J photon}^{-1}$$

Incident radiant energy:

$$E_{\text{light}} = 0.00307 \times 10 \times 172800 = 7957.44 \text{ J}$$

Incident photons (mol):

$$n_{\text{photon}} = 5304.96 / [(4.97 \times 10^{-19}) \times (6.022 \times 10^{23})] = 2.66 \times 10^{-2} \text{ mol photons}$$

PHB produced (autotrophic). The PHB production and working volume were $C_{PHB} = 116.24$ mg L^{-1} and V = 0.25 L. Therefore:

$$m_{PHB} = C_{PHB} \cdot V = 116.24 \times 0.25 = 29.06 \text{ mg} = 0.02906 \text{ g}$$

$$n_{PHB} = m_{PHB} / M_{\text{repeat}} = 0.02906 / 86.09 = 3.38 \times 10^{-4} \text{ mol}$$

$$QE = (3.38 \times 10^{-4}) / (2.66 \times 10^{-2}) = 1.90 \times 10^{-2} = 1.27 \text{ \%}$$