Supporting Information

Carbon Nanotubes Influence the Toxic Effects of Chloramphenicol and Tetracycline to Cyanobacterium *Synechocystis* sp. in Different Ways

Mingtao You[a], Xiuqi You[a], Jingrun Hu[a], Xi Yang[b], Weiling Sun[a,*]

[a] College of Environmental Sciences and Engineering, Peking University, State Environmental Protection Key Laboratory of All Material Fluxes in River Ecosystems, The Key Laboratory of Water and Sediment Sciences, Ministry of Education, International Joint Laboratory for Regional Pollution Control, Ministry of Education, Beijing 100871, China

[b] State Key Laboratory of Plateau Ecology and Agriculture, Qinghai University, Xining 810016, China

*** Corresponding authors:**

W.L. Sun

E-mail: wlsun@pku.edu.cn

Text S1 Proteomic sample preparation and analysis

Algae cells transferred to the mortar with liquid nitrogen precooling, and liquid nitrogen was added to fully grind to the powder. The samples of each group were respectively added with 4-fold volume cracking buffer (8 M urea, 1% Triton X-100, 10 mm dithiothreitol, 1% protease inhibitor) for ultrasonic cracking. Centrifugation of 20000 g at 4 °C for 10 min, taking the supernatant and adding trichloroacetic acid with final concentration of 20%, and standing at 4 °C for 2 h. At 4 °C, 12000 g was centrifuged for 3 min, the supernatant was discarded, and the precipitate was washed three times with precooled acetone. Finally, the precipitate was re-dissolved with 8 M urea, and the protein concentration was determined with BCA kit.

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration less than 2 M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion. The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 μm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, 23% to 35% in 8 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system.

The peptides were quantified using UPLC-tandem mass spectrometry (MS/MS) in Q ExactiveTM Plus (Thermo) coupled with a nanoelectrospray ion source. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0 s dynamic exclusion. Automatic gain control (AGC) was set at 5×10^4. Fixed first mass was set as 100 m/z.

The MS/MS data were processed using Maxquant search engine (v.1.5.2.8). Tandem mass spectra were searched against *Synechocystis* sp. strain PCC 6803 uniprot database concatenated with reverse decoy database. Trypsin/P was specified as cleavage enzyme allowing up to 4 missing cleavages. The mass tolerance for precursor ions was set as 20 ppm in First search and 5 ppm in Main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification and acetylation modification and oxidation on Met were specified as variable modifications. FDR was adjusted to < 1% and minimum score for modified peptides was set > 40.

Text S2

As shown in Fig. S9a, the concentrations of CAP did not change with time in different experimental groups. This indicates that no adsorption and photodegradation occurred for CAP. However, decreases in TC concentrations were observed in all experimental groups (Fig. S9b). The decrease of TC concentrations in CTR sample suggests the occurrence of TC photodegradation. In the group of CNTs, TC concentrations decreased most rapidly due to photodegradation and adsorption of TC by CNTs. Moreover, CNTs can produce ROS (Fig. S9c), which can also react with TC, so photocatalytic degradation occurred. In the group of Cya-CNTs, the reduction of TC induced by CNTs was weakened because algae consumed the ROS produced by CNTs (Fig. S9c) or inhibited the adsorption of TC through aggregation with CNTs (Fig. S5c). In the group of Cya, TC was only adsorbed or bioaccumulated by algae. Therefore, the decrease of TC in this group is only faster than the CTR group.

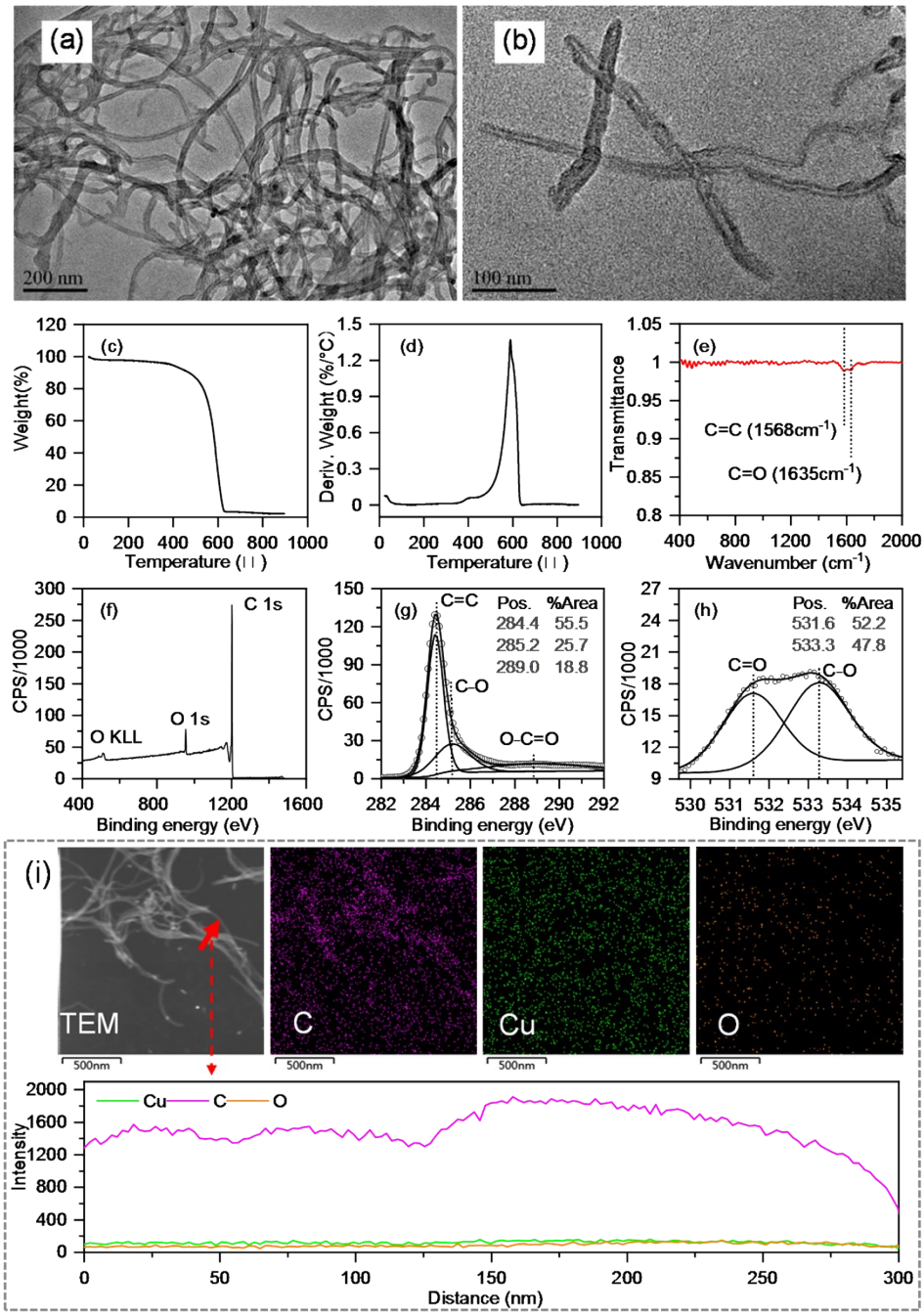


Fig. S1 TEM images (a,b), TGA curves (c,d), FTIR spectra (e), XPS spectra of survey (f), C 1s (g) and O1s (h), and TEM-EDS (i) of CNTs.

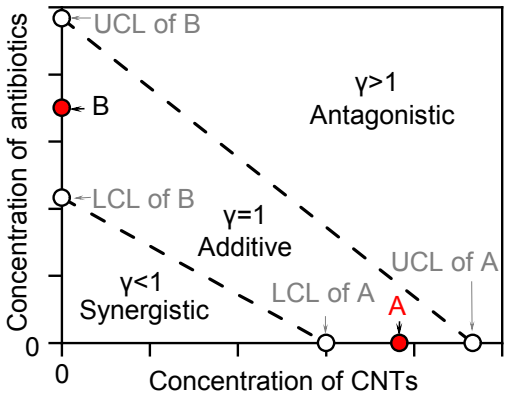


Fig. S2 Graphical representation of the combined toxic effects of two substances.

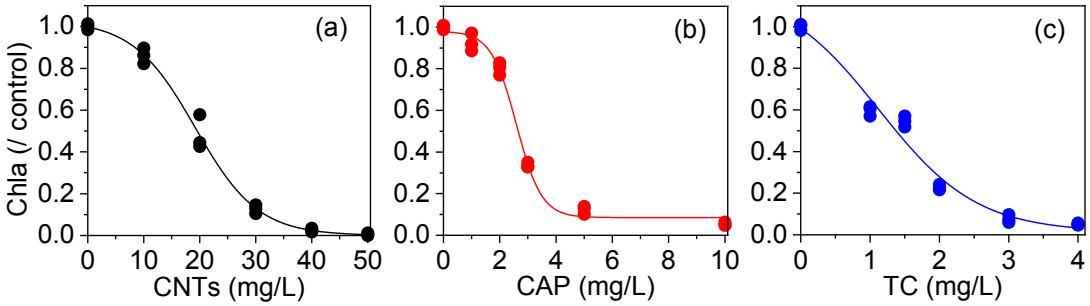


Fig. S3 Changes of chlorophyll a at various exposure concentrations of CNTs (a), CAP (b), and TC (c) (line is the dose response curve).

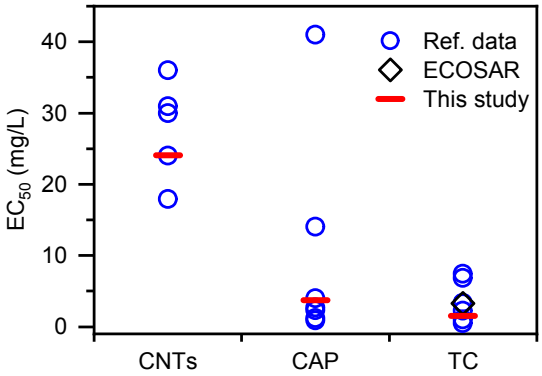


Fig. S4 Comparison of CNTs, CAP, and TC toxicity in the present and previous studies.

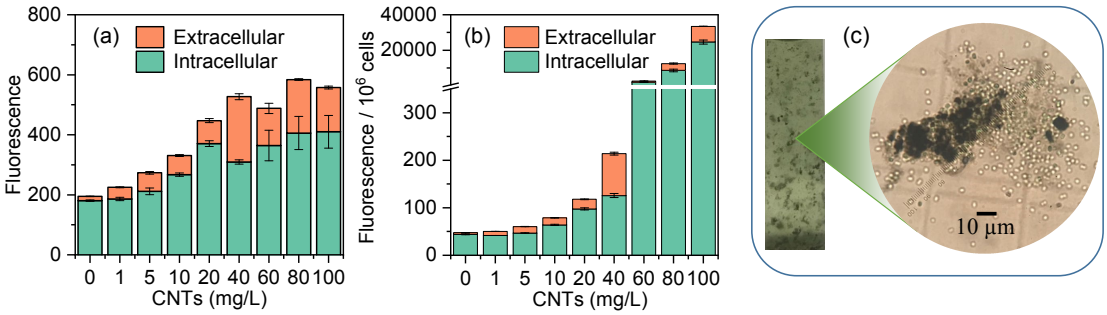


Fig. S5 The fluorescence intensity of intracellular and extracellular ROS at different CNTs concentrations (a) and the aggregation of CNTs and/or aggregation algal cells (b).

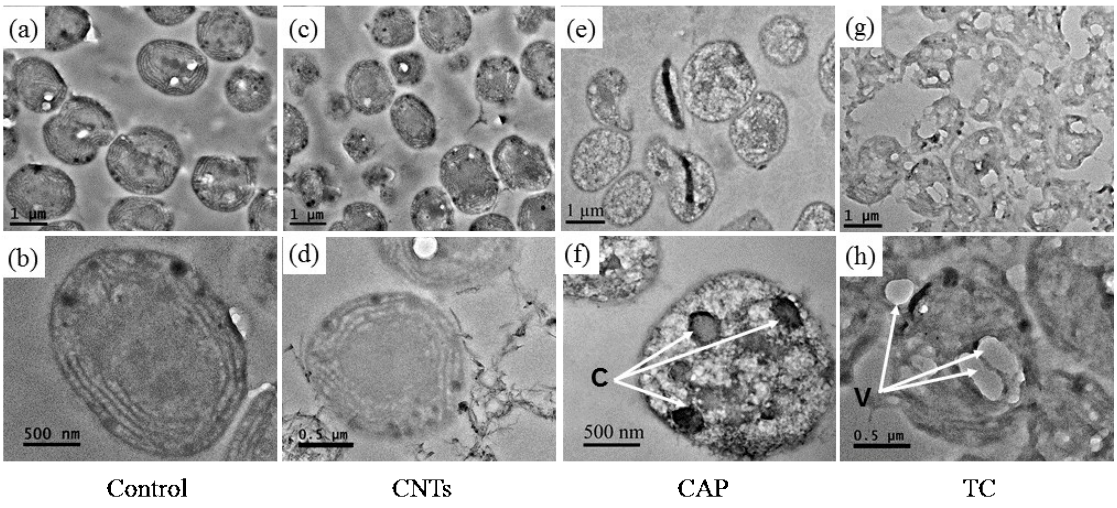


Fig. S6 TEM images of *Synechocystis* sp. PCC 6803 exposed to control (a and b), CNTs (c and d, 20 mg/L), CAP (e and f, 2 mg/L) and TC (g and h, 1.5 mg/L) for 96 h (C and V represent carboxysomes and vacuoles, respectively). First row: zoomed out images showing the cell integrity; Second row: zoomed in TEM images showing the cell structure of *Synechocystis*.

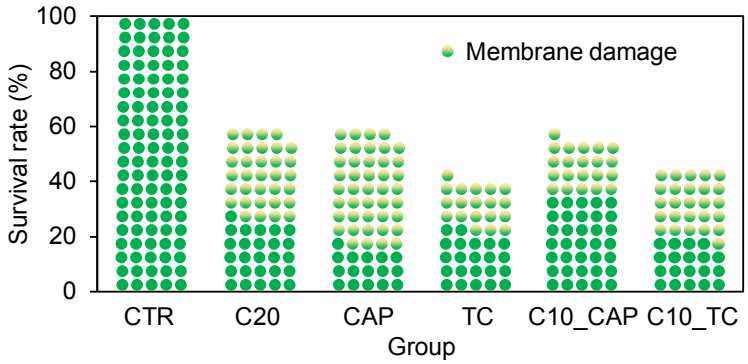


Fig. S7 Survival rate and cell membrane damage under different exposure modes (Ctr: control sample, C20: CNTs 20 mg/L, CAP: CAP 2 mg/L, TC: TC 1.5 mg/L, C10_CAP: CNTs 10 mg/L + CAP 2 mg/L, C10_TC: CNTs 10 mg/L + TC 1.5 mg/L).

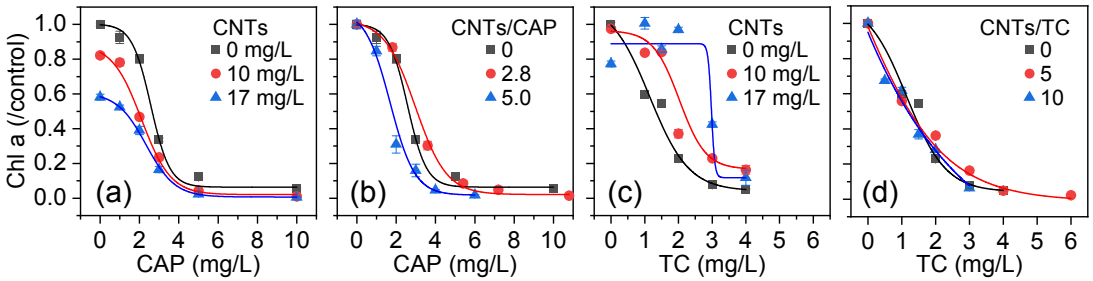


Fig. S8 Chlorophyll a of CNTs with CAP (a) or TC (b) to *Synechocystis* sp. PCC 6803.

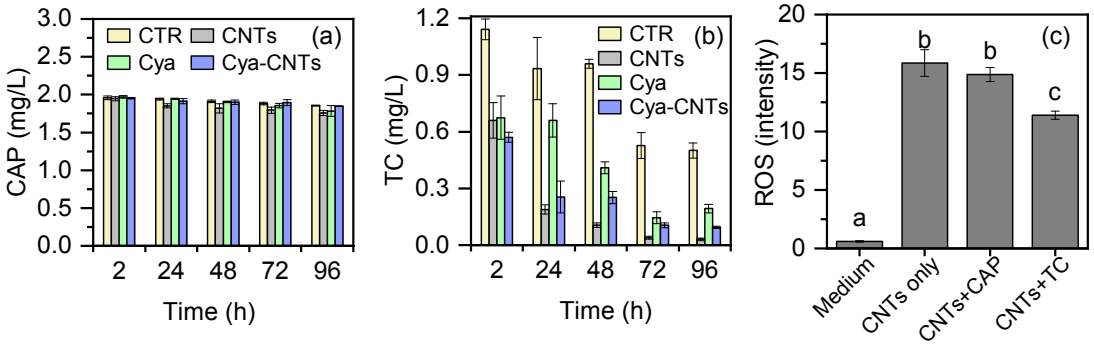


Fig. S9 Changes of CAP and TC concentrations during 96 h incubation (CTR: antibiotics only, 2 mg/L CAP or 1.5 mg/L TC; CNTs: 10 mg/L CNTs + 2 mg/L CAP or 10 mg/L CNTs + 1.5 mg/L; Cya: Cyanobacteria + 2 mg/L CAP or Cyanobacteria + 1.5 mg/L TC; Cya-CNTs: Cyanobacteria + CNTs 10 mg/L + 2 mg/L CAP or Cyanobacteria + CNTs 10 mg/L + 1.5 mg/L TC) and ROS contents in different exposure groups after 96 h incubation (Medium: BG-11 medium, CNTs only: 10 mg/L CNTs in BG-11 medium, CNTs+CAP: 10 mg/L CNTs and 2 mg/L CAP in BG-11 medium, CNTs+TC: 10 mg/L CNTs and 1.5 mg/L TC in BG-11 medium; Different letters suggest significant differences between different exposure groups, one-way ANOVA: $p < 0.05$).

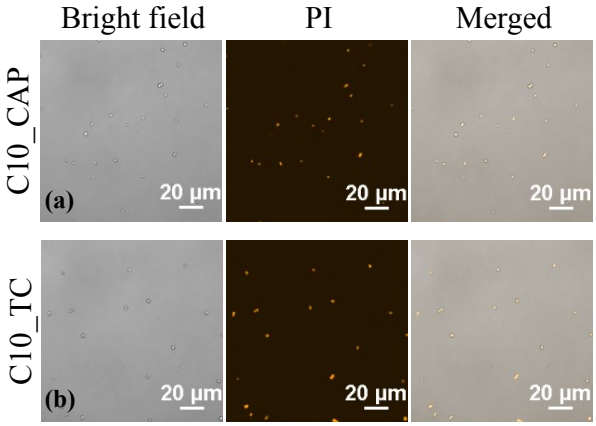
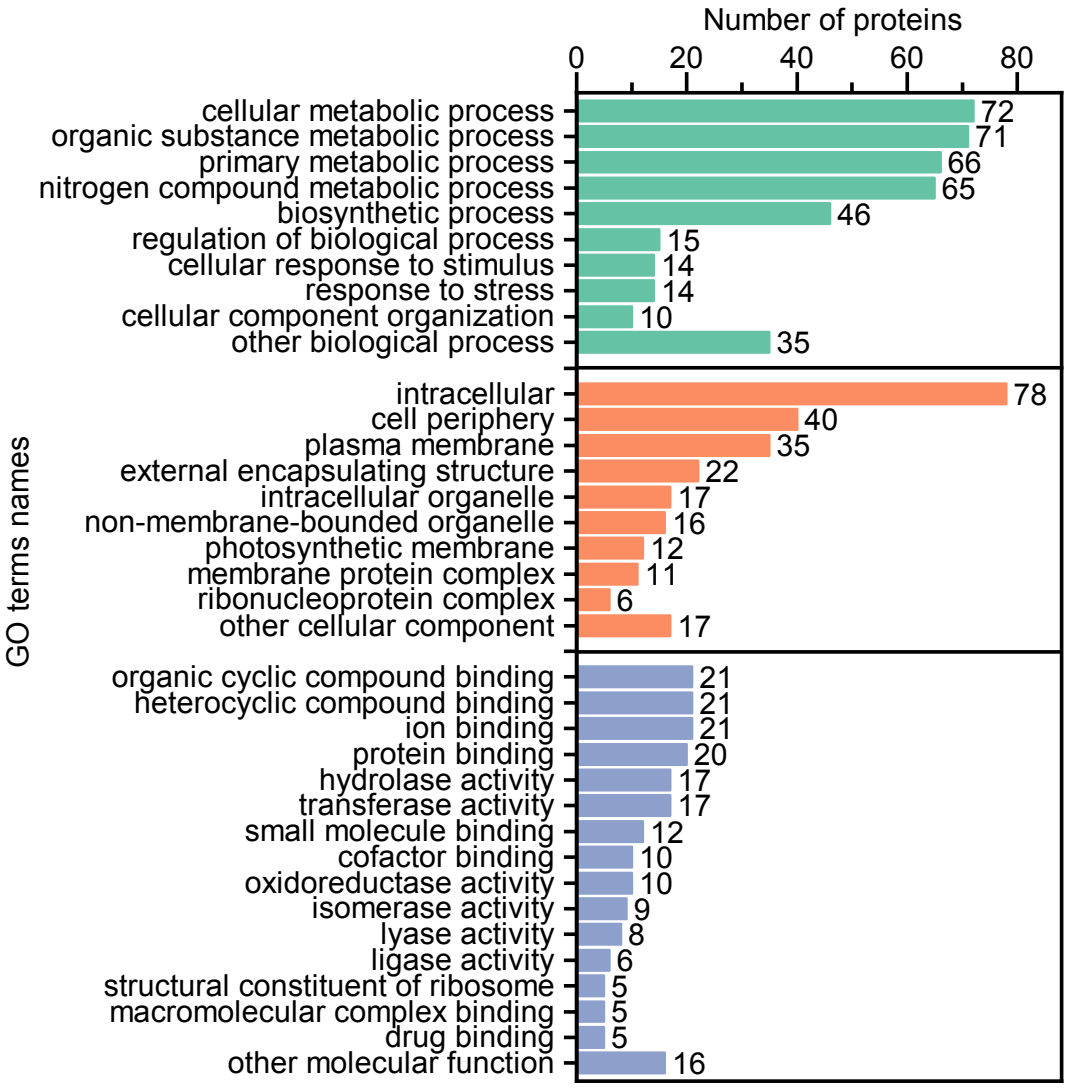
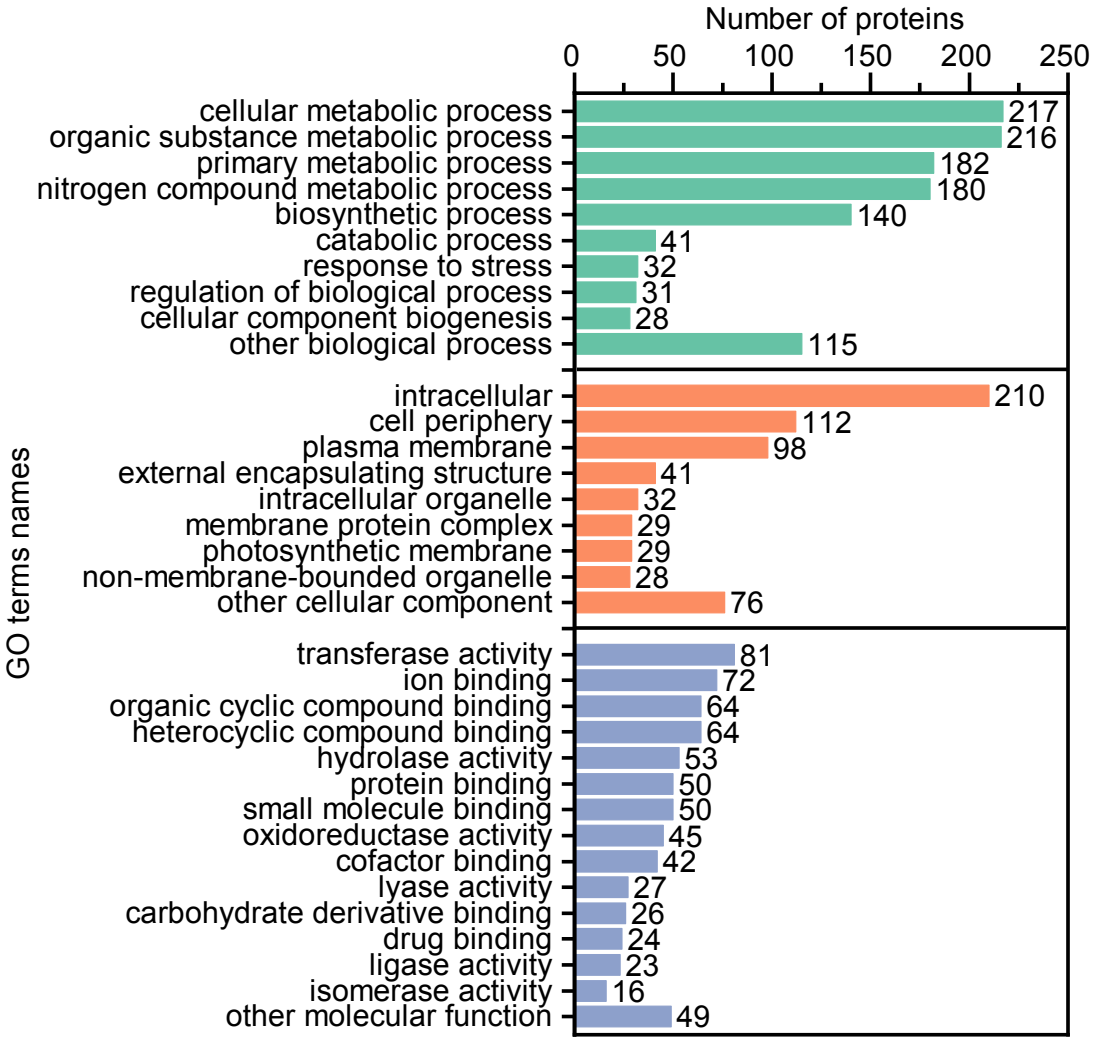


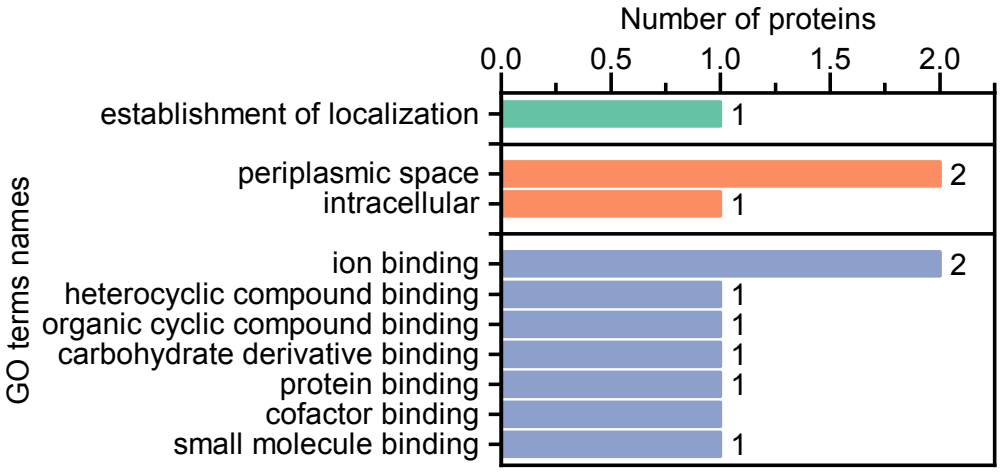
Fig. S10 The LCSM images of *Synechocystis* cells treated with 10 mg/L CNTs and 2 mg/L CAP (C10_CAP) or 10 mg/L CNTs and 1.5 mg/L TC (C10_TC) for 96 h and dyed with PI.



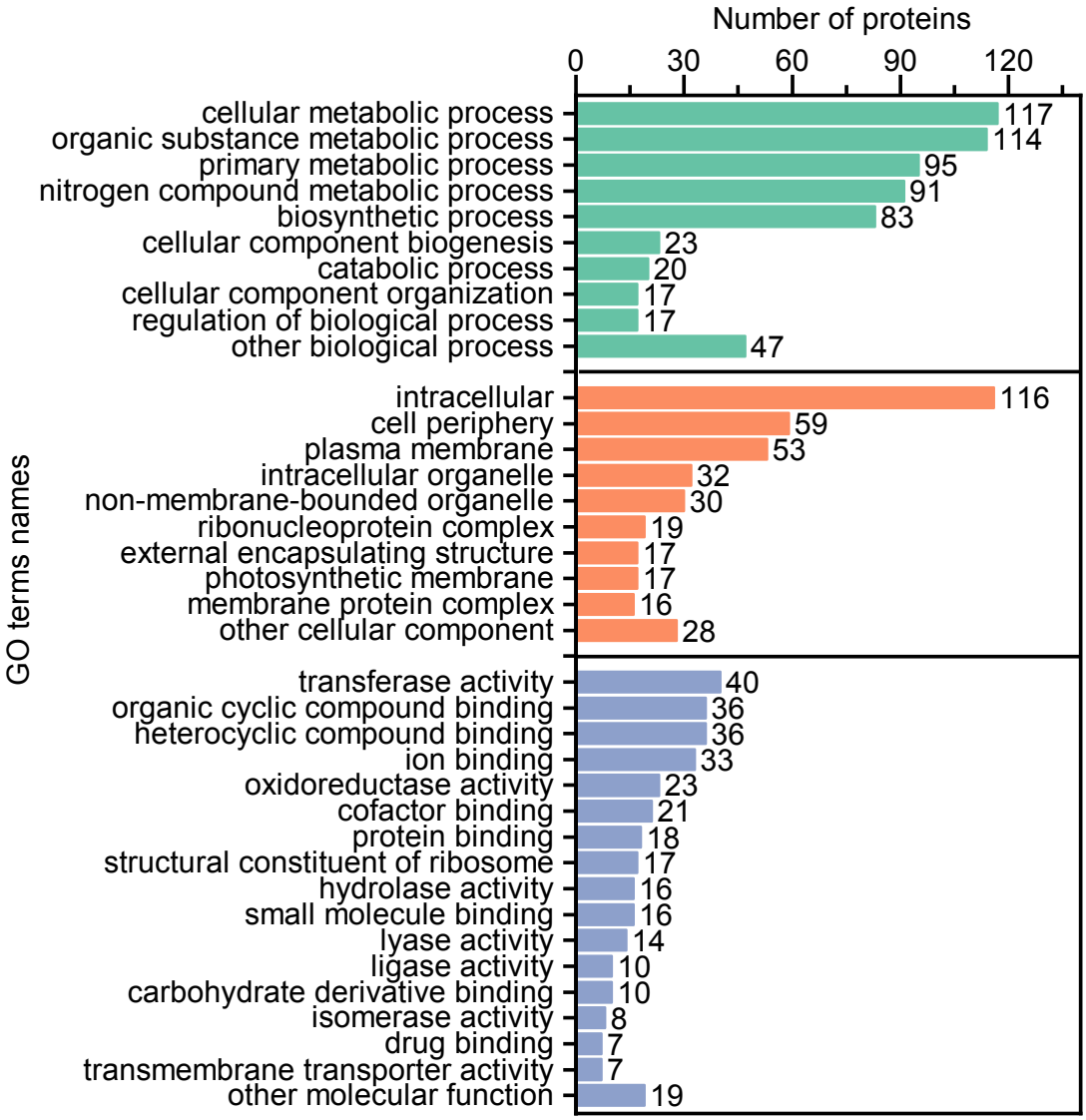
(a) C20, upregulated



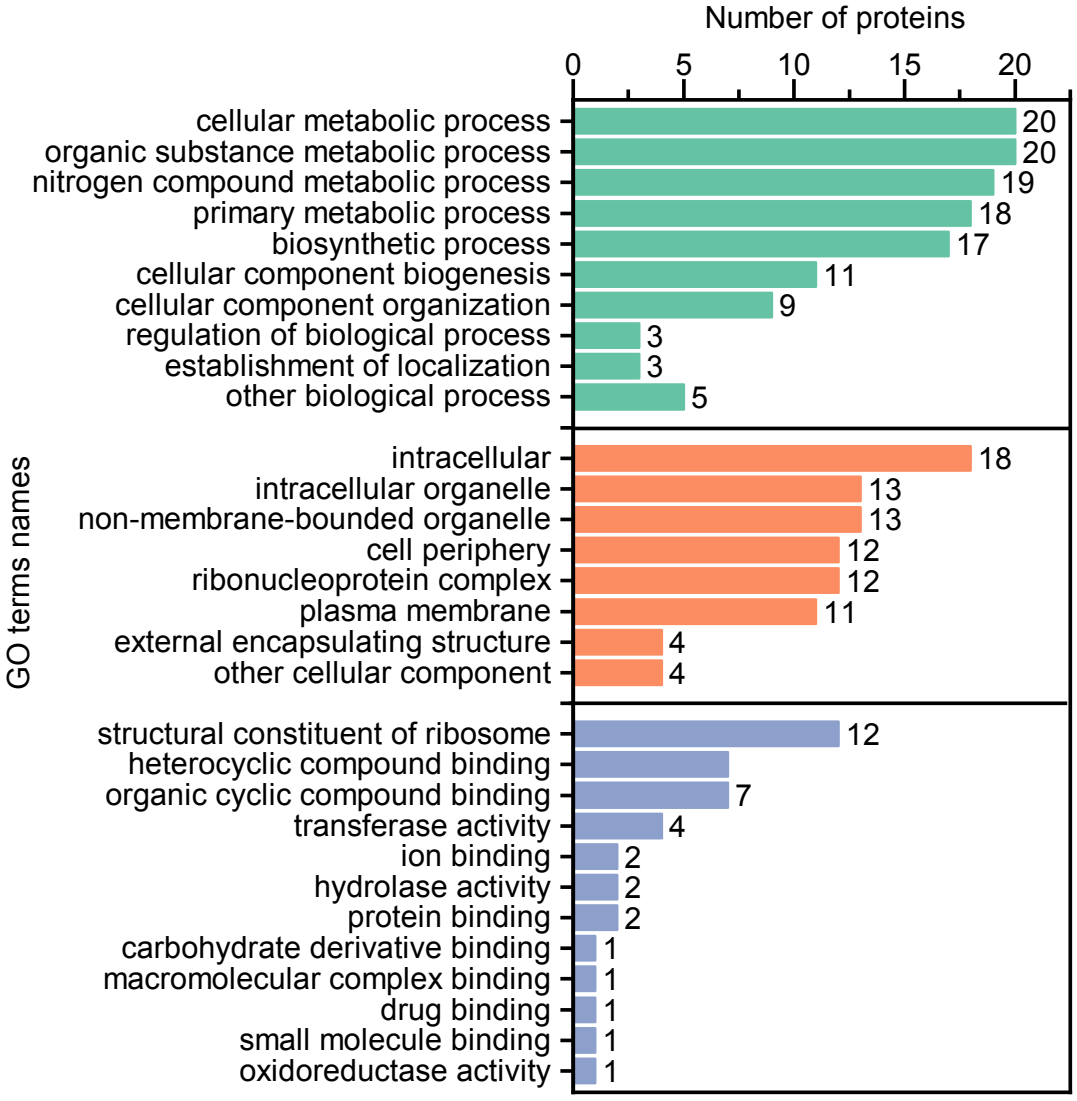
(b) CAP, downregulated



(c) TC, downregulated

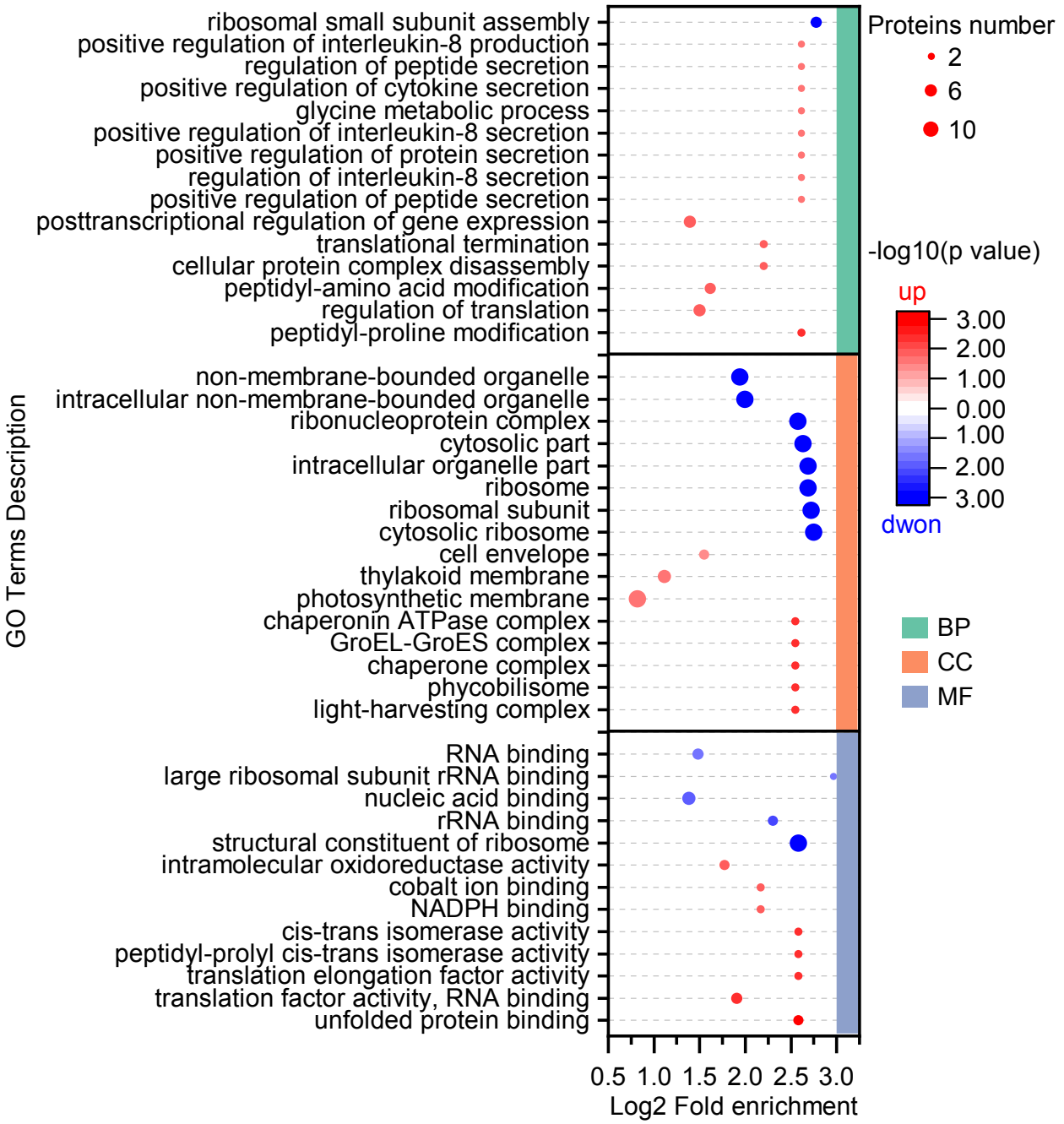


(d) C10_CAP, downregulated

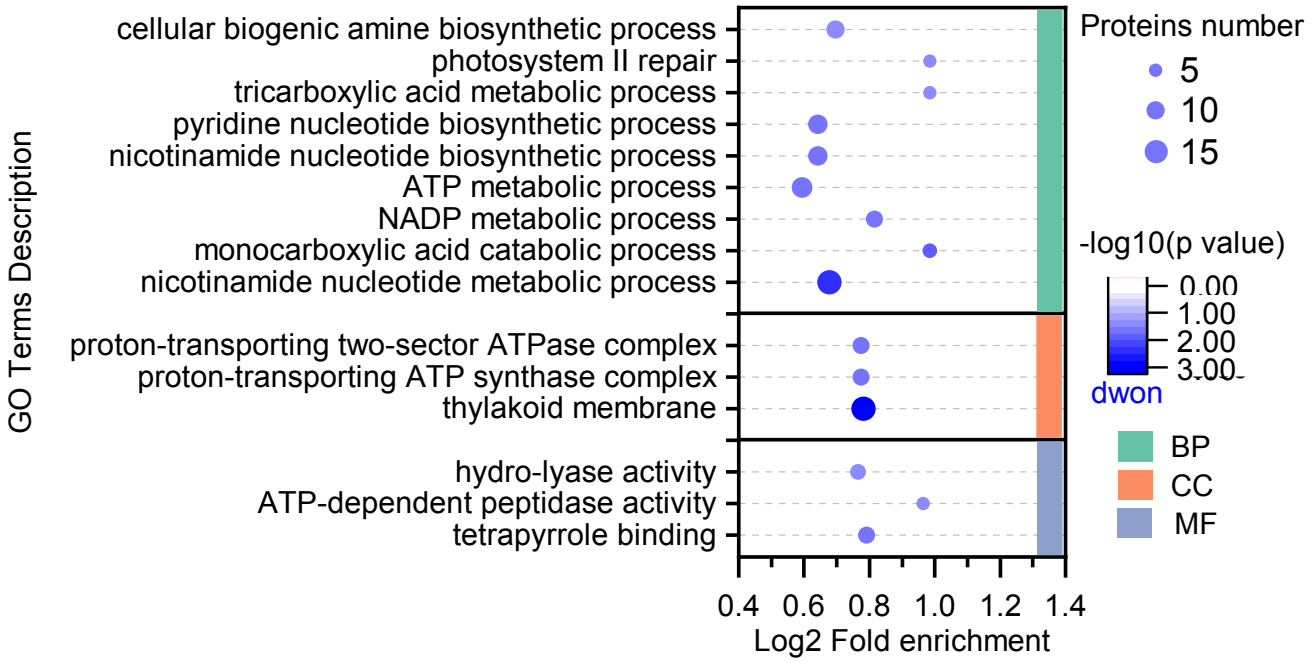


(e) C10_TC, downregulated

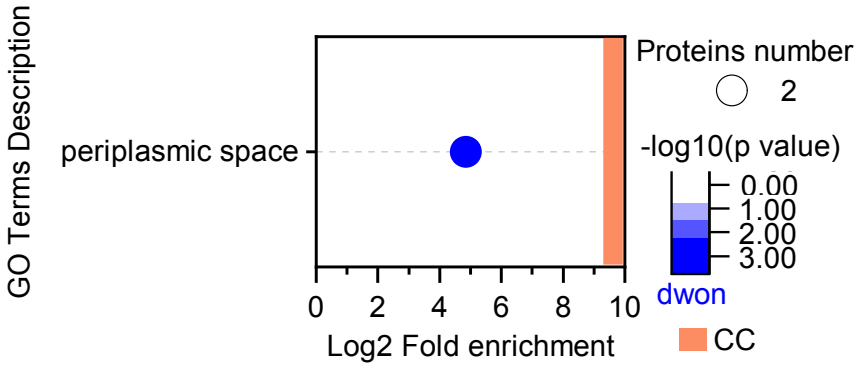
Fig. S11 GO function classification of DEPs (Biological process, Cellular component, Molecular function).


ribosomal small subunit assembly
positive regulation of interleukin-8 production
regulation of peptide secretion
positive regulation of cytokine secretion
glycine metabolic process
positive regulation of interleukin-8 secretion
positive regulation of protein secretion
regulation of interleukin-8 secretion
positive regulation of peptide secretion
posttranscriptional regulation of gene expression
translational termination
cellular protein complex disassembly
peptidyl-amino acid modification
regulation of translation
peptidyl-proline modification
non-membrane-bounded organelle
intracellular non-membrane-bounded organelle
ribonucleoprotein complex
cytosolic part
intracellular organelle part
ribosome
ribosomal subunit
cytosolic ribosome
cell envelope
thylakoid membrane
photosynthetic membrane
chaperonin ATPase complex
GroEL-GroES complex
chaperone complex
phycobilisome
light-harvesting complex
RNA binding
large ribosomal subunit rRNA binding
nucleic acid binding
rRNA binding
structural constituent of ribosome
intramolecular oxidoreductase activity
cobalt ion binding
NADPH binding
cis-trans isomerase activity
peptidyl-prolyl cis-trans isomerase activity
translation elongation factor activity
translation factor activity, RNA binding
unfolded protein binding
GO Terms Description
0.5 1.0 1.5 2.0 2.5 3.0
Log2 Fold enrichment
Proteins number
2
6
10
-log10(p value)
up
3.00
2.00
1.00
0.00
1.00
2.00
3.00
dwon
BP
CC
MF

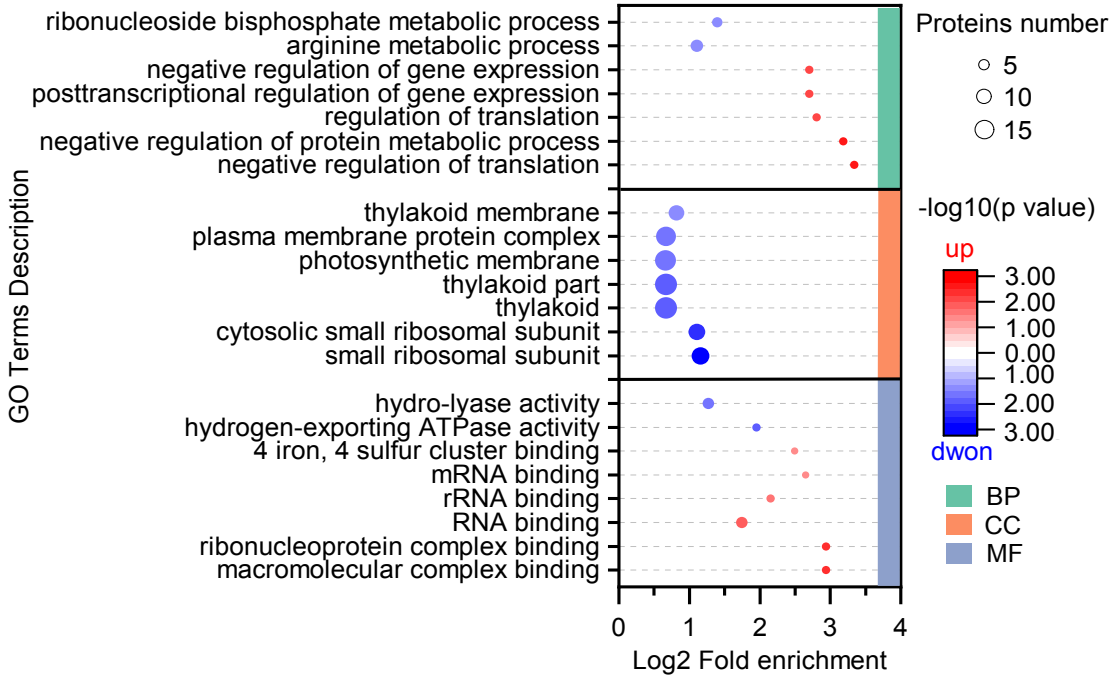
(a) C20


GO Terms Description
cellular biogenic amine biosynthetic process
photosystem II repair
tricarboxylic acid metabolic process
pyridine nucleotide biosynthetic process
nicotinamide nucleotide biosynthetic process
ATP metabolic process
NADP metabolic process
monocarboxylic acid catabolic process
nicotinamide nucleotide metabolic process
proton-transporting two-sector ATPase complex
proton-transporting ATP synthase complex
thylakoid membrane
hydro-lyase activity
ATP-dependent peptidase activity
tetrapyrrole binding
0.4 0.6 0.8 1.0 1.2 1.4
Log2 Fold enrichment
Proteins number
5
10
15
-log10(p value)
0.00
1.00
2.00
3.00
dwon
BP
CC
MF

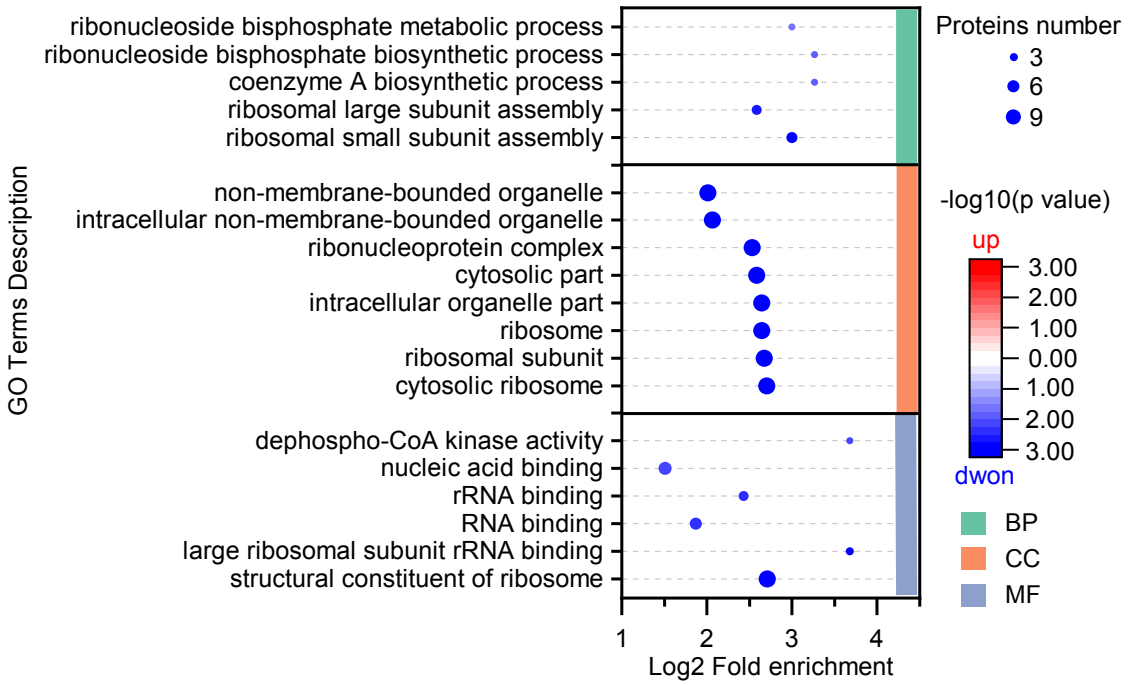
(b) CAP


GO Terms Description
periplasmic space
0 2 4 6 8 10
Log2 Fold enrichment
Proteins number
2
-log10(p value)
0.00
1.00
2.00
3.00
dwon
CC

(c) TC

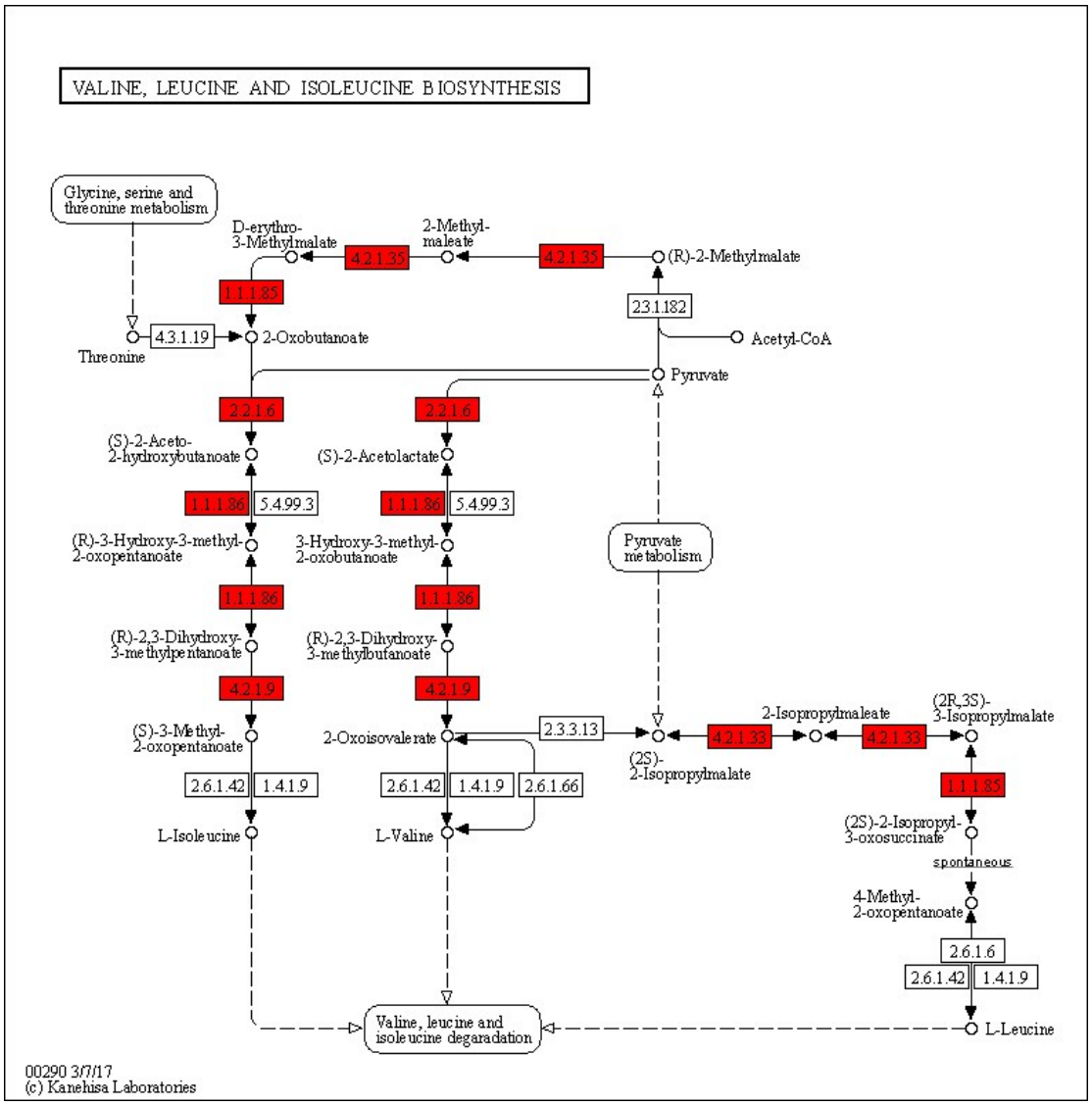


(d) C10_CAP

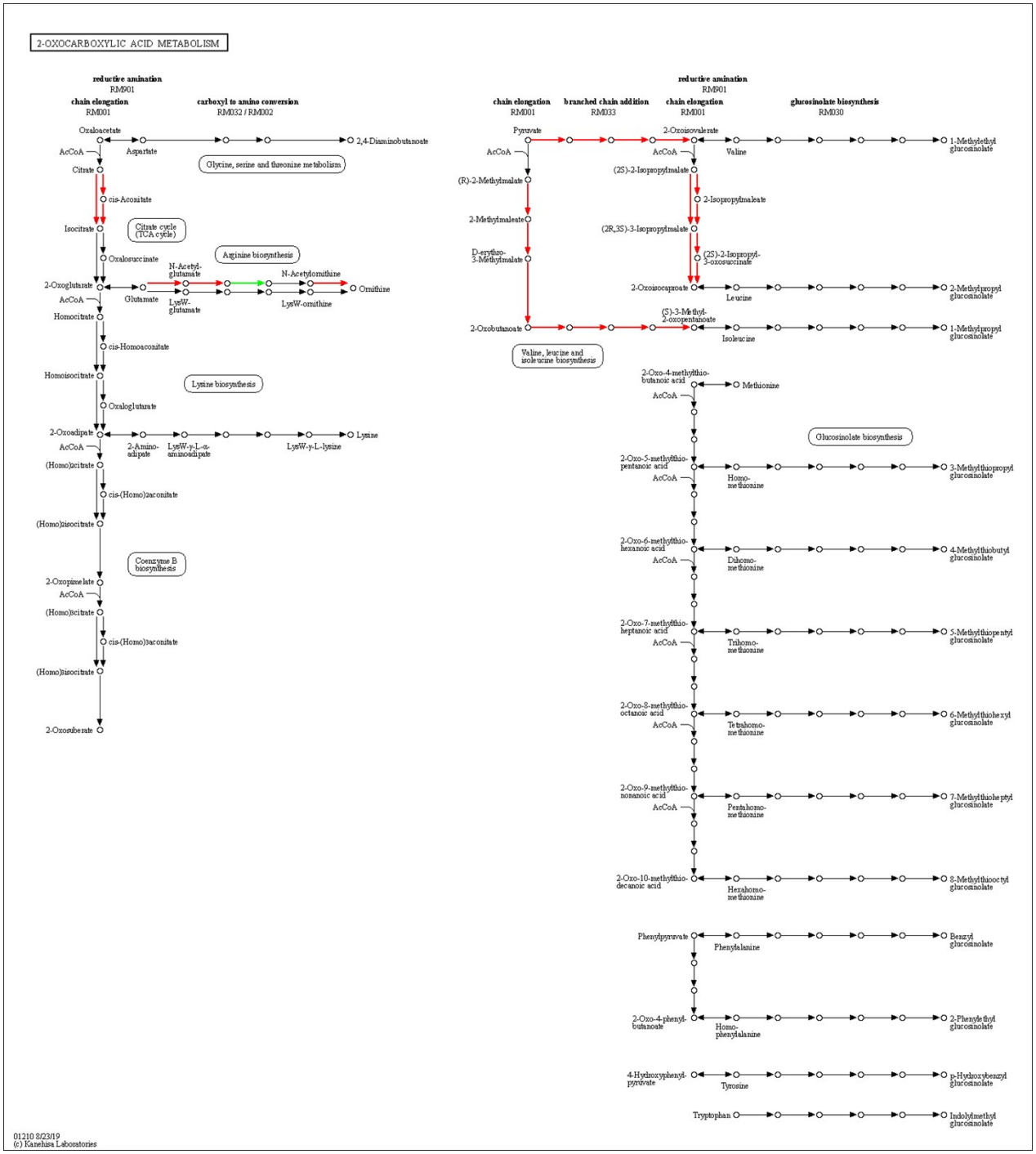


(e) C10_TC

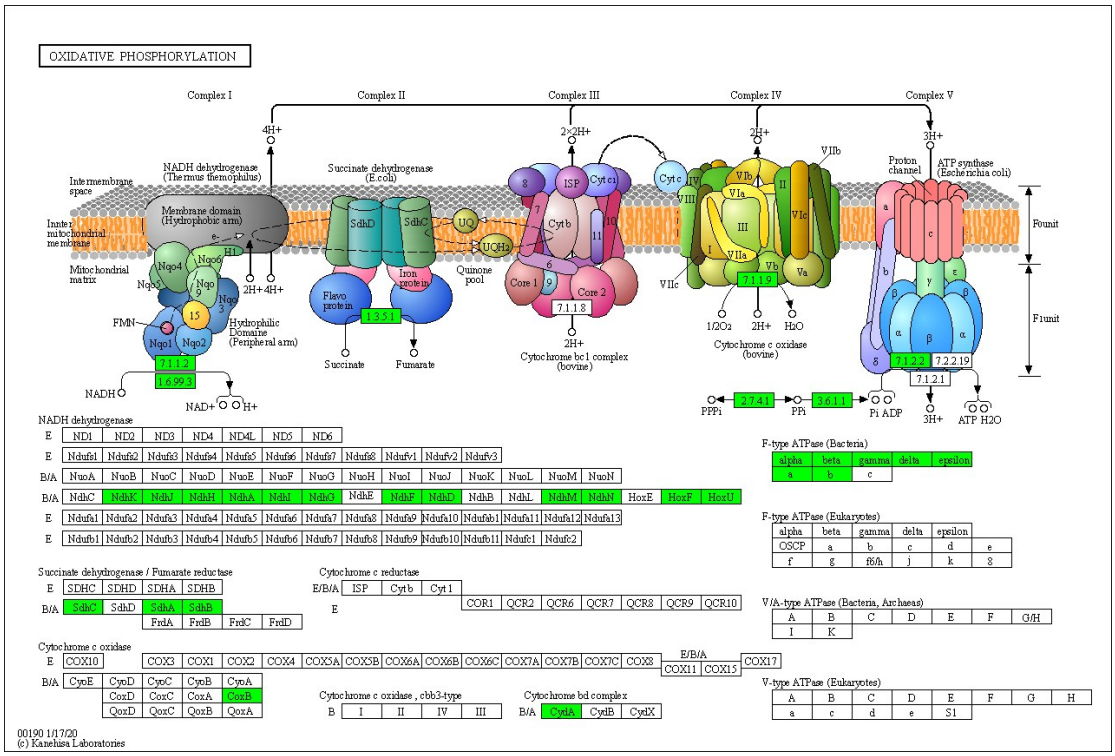
Fig. S12 GO functional enrichment of DEPs in different exposure groups.



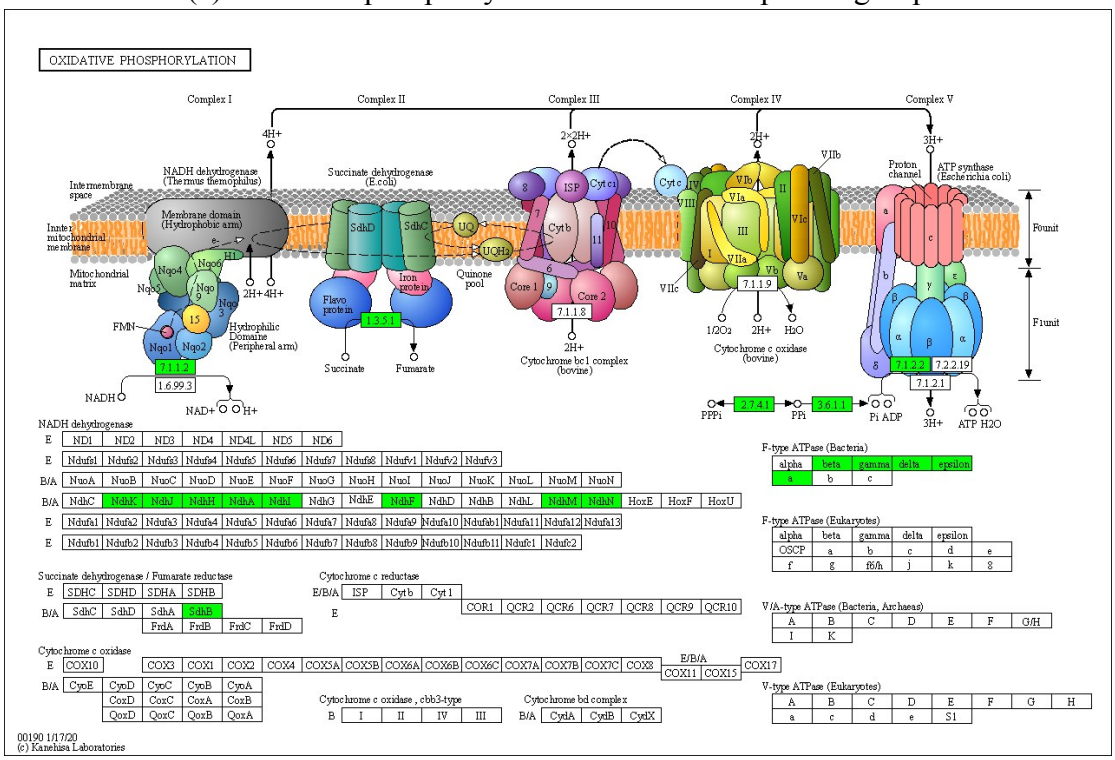
(a) Valine, leucine and isoleucine biosynthesis in the CNTs exposure group



(b) 2-oxocarboxylic acid metabolism in the CNTs exposure group



(c) Oxidative phosphorylation in the CAP exposure group



(d) Oxidative phosphorylation in the C10_CAP exposure group

Fig. S13 KEGG pathway analysis of DEPs in C20 (a and b), CAP (c) and C10_CAP (d) exposure groups, compared with control group (red arrow or red rectangle mean upregulation).

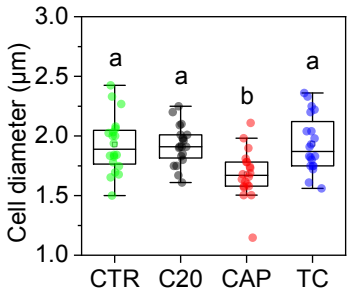


Fig. S14 Cell size of *Synechocystis* sp. in different exposure groups (Different letters suggest significant differences between different exposure groups, one-way ANOVA: $p < 0.05$).

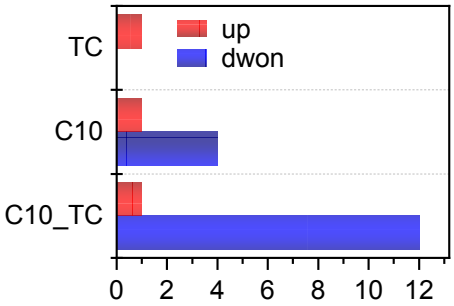


Fig. S15 DEPs related to ribosome induced by the exposure of C10, TC, and C10_TC.

Table S1 Components of BG11 algae medium.

Compound	Concentration (mg/L)
$NaNO_3$	1500
K_2HPO_4	50
$MgSO_4•7H_2O$	65
$CaCl_2•2H_2O$	36
Citric acid	6
Ferric ammonium citrate	6
$EDTANa_2$	1
Na_2CO_3	20
H_2BO_3	2.86
$MnCl_2•4H_2O$	1.86
$ZnSO_4•7H_2O$	0.22
$Na_2MoO_4•2H_2O$	0.39
$CuSO_4•5H_2O$	0.08
$Co(NO_3)_2•6H_2O$	0.05

Table S2 Seven scenarios designed for proteomic analysis.

Conditions	CNTs (mg/L)	CAP (mg/L)	TC (mg/L)
Ctr	0	0	0
C10	10	0	0
C20	20	0	0
CAP	0	2	0
TC	0	0	1.5
C10_CAP	10	2	0
C10_TC	10	0	1.5

Table S3 Fitting parameters in dose-response curves of CNTs and antibiotics for their single and binary exposure tests.

Toxicity of CNTs or antibiotics					
Pollutants		A1	A2	$LOG^{x_0}(EC_{50})$	p
CNTs		-0.3174	16.68	24.09	-0.0636
CAP		1.905	17.09	3.757	-0.3848
TC		0.4518	25.63	1.516	-1.1989
Toxicity of CNTs and antibiotics (Series 1)					
Antibiotics	CNTs (mg/L)	A1	A2	$LOG^{x_0}(EC_{50})$	p
CAP	10	0.3544	15.93	12.20	-0.5897
	17	-0.1503	16.10	18.25	-0.3327
TC	10	0.6822	24.98	11.69	-1.484
	17	-0.0960	21.97	19.36	-1.145
Toxicity of CNTs and antibiotics (Series 2)					
Antibiotics	CNTs/antibiotic	A1	A2	$LOG^{x_0}(EC_{50})$	p
CAP	2.8:1	4.974	32.83	10.07	-0.0855
	5.0:1	26.13	26.13	12.27	-0.0791
TC	10:1	-0.4404	27.12	12.19	-0.0851
	5.0:1	0.1334	30.40	8.056	-0.0930

Table S4 EC_{50} values in the previous studies.

Antibiotics	Algae	EC_{50} (mg/L)	Reference
TC	*Chlorella vulgaris*	7.73	Xu et al., 2019
TC	*Pseudokirckneriella subcapitata*	3.31 ± 0.96	González-Pleiter et al., 2013
TC	*Microcystis aeruginosa*	< 0.5	Yang et al., 2013
TC	*Pseudokirchneriella subcapitata*	2.25	Yang et al., 2008
CAP	*Chlorella pyrenoidosa*	14	Lai et al., 2009
	Isochrysis galbana	41	
	Tetraselmis chui	4	

Table S5 Detailed protein information in protein-protein interaction network of 13 DEPs related to ribosome in the C10_TC group.

Gene name	Regulated Type	Protein description
rplT	Up	50S ribosomal protein L20
rpsP	Down	30S ribosomal protein S16
rpsG	Down	30S ribosomal protein S7
rpmA	Down	50S ribosomal protein L27
rpsS	Down	30S ribosomal protein S19
rplC	Down	50S ribosomal protein L3
rpsN	Down	30S ribosomal protein S14
rplN	Down	50S ribosomal protein L14
rplI	Down	50S ribosomal protein L9
rpsI	Down	30S ribosomal protein S9
rplS	Down	50S ribosomal protein L19

References

M. González-Pleiter; S. Gonzalo, I. Rodea-Palomares, F. Leganés, R. Rosal, K. Boltes, E. Marco and F. Fernández-Piñas, Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: implications for environmental risk assessment, Water Res., 2013, **47**, 2050-2064.

H.T. Lai, J.H. Hou, C.I. Su and C.L. Chen, Effects of chloramphenicol, florfenicol, and thiamphenicol on growth of algae Chlorella pyrenoidosa, Isochrysis galbana, and Tetraselmis chui, Ecotox. Environ. Safe., 2009, **72**, 329-334.

D. Xu, Y. Xiao, H. Pan and Y. Mei, Toxic effects of tetracycline and its degradation products on freshwater green algae, Ecotox. Environ. Safe., 2019, **174**, 43-47.

L. H. Yang, G. G. Ying, H. C. Su, J. L. Stauber, M. S. Adams and M. T. Binet, Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga pseudokirchneriella subcapitata, Environ. Toxicol. Chem., 2008, **27**, 1201-1208.

W. Yang, Z. Tang, F. Zhou, W. Zhang and L. Song, Toxicity studies of tetracycline on Microcystis aeruginosa and Selenastrum capricornutum, Environ. Toxicol. Pharmacol, 2013, **35**, 320-324.