Supplementary Figures

Chlorosis as a developmental program in cyanobacteria: The proteomic fundament of survival and awakening

Supplementary Figure 1:

Global phosphoproteome study

Experiment		time point	label	labeling efficiency*	total evidences
Replicate 1	Labeling 1	T0	light	75.3%	2784
		T24	intermediate	79.8%	3064
		T55	heavy	98.0%	3980
	Labeling 2	T2	light	76.5%	2739
		T8	intermediate	82.4%	3319
		T55	heavy	see above	see above
Replicate 2	Labeling 1	T0	light	94.1%	3133
		T24	intermediate	95.4%	4395
		T55	heavy	97.6%	3458
	Labeling 2	T2	light	95.5%	3161
		T8	intermediate	96.6%	3360
		T55	heavy	see above	see above

*: based on all detected evidences with intensities above zero

Proteomic study of CpcD mutants (compare Text Figure 7)

condition	mutant	label	labeling efficiency*	total evidences
growth	$CpcD^{Ala}$	light	95.5%	7246
growth	WT	intermediate	96.7%	7338
growth	$CpcD^{Asp}$	heavy	97.6%	7299
chlorosis	$CpcD^{Ala}$	light	94.7%	8003
chlorosis	WT	intermediate	96.8%	8208
chlorosis	$CpcD^{Asp}$	heavy	97.6%	7790
resuscitation	$CpcD^{Ala}$	light	95.7%	7300
resuscitation	WT	intermediate	97.5%	7457
resuscitation	$CpcD^{Asp}$	heavy	97.8%	6417

*: based on all detected evidences with intensities above zero

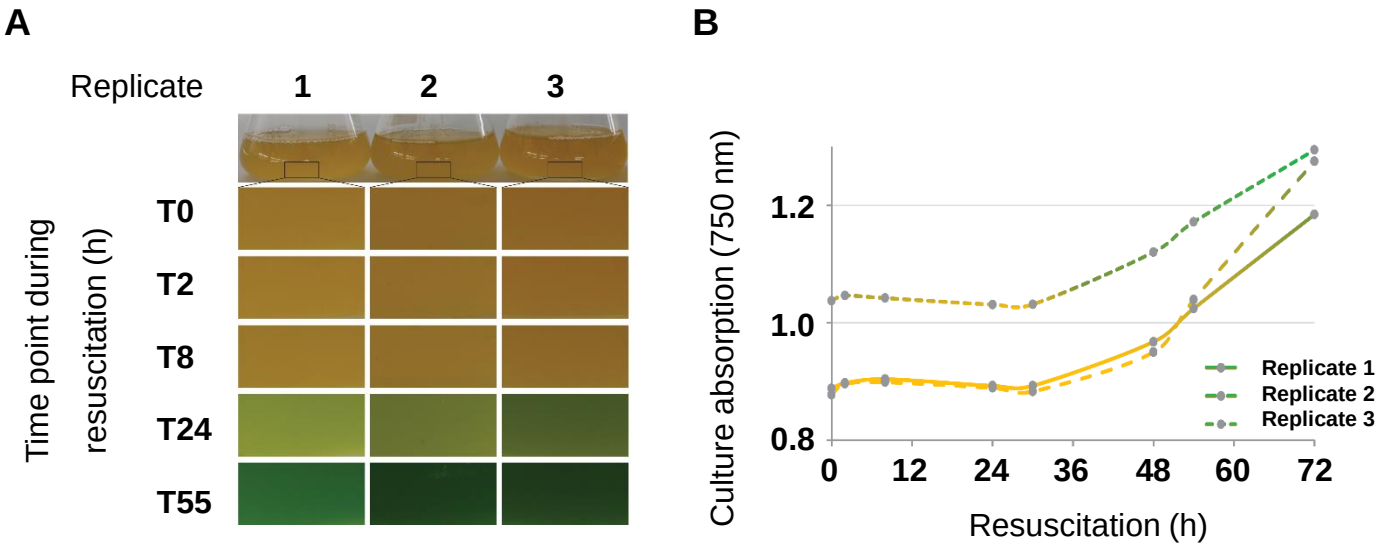
Supplementary Figure 1: Analysis of dimethyl-labeling efficiencies. The ratio (in percent) of labeled to unlabeled peptide spectrum matches (evidences) were measured in pilot MS-measurements and are indicated per sample, together with the total number of detected evidences.

Supplementary Figure 2:

Fraction	Duration (min)	Flow (nL min^{-1})	Gradient (% HPLC solvent B)
1	73	200	10-22
	3	200	22-50
	3	200	50-90
	8	500	90 const.
2	6	200	10-14
	65	200	14-24
	2	200	24-29
	3	200	29-50
	3	500	50-90
	8	500	90 const.
3	24	200	10-19
	44	200	19-29
	5	200	29-33
	3	200	33-50
	3	500	50-90
	8	500	90 const.
4	60	200	10-27
	6	200	27-33
	7	200	33-50
	6	500	50-90
	8	500	90 const.
5	5	200	10-13
	56:30	200	13-28
	4	200	28-33
	7:30	200	33-50
	6	500	50-90
	8	500	90 const.
6	06:30	200	10-14
	55	200	14-28
	4	200	28-33
	7:30	200	33-50
	6	500	50-90
	8	500	90 const.
7	3	200	10-14
	59	200	14-30
	2	200	30-33
	9	200	33-50
	6	500	50-90
	8	500	90 const.
8 & 9	3	200	10-14
	57:30	200	14-27
	5	200	27-33
	7:30	200	33-50
	6	500	50-90
	8	500	90 const.

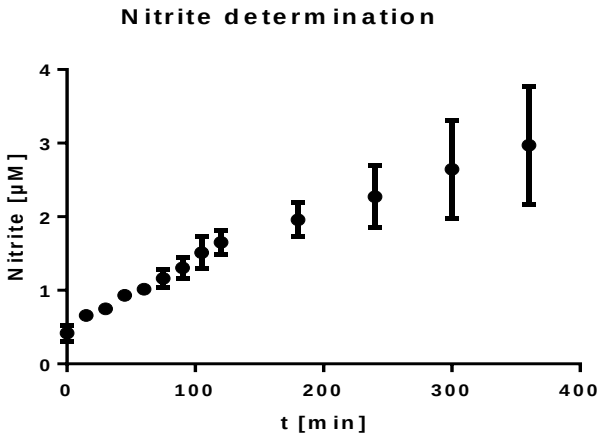
Supplementary Figure 2: Protein fraction specific nanoHPLC gradients for samples from high pH fractionation. Step-wise linear nanoHPLC elution gradients are indicated for each peptide fraction (1-9), with indication of duration, flow rate and percentage of HPLC solvent B (80% v/v acetonitrile in 0.5% v/v formic acid (aq.)) relative to HPLC solvent A (0.5% v/v formic acid in water).

Supplementary Figure 3:



Supplementary Figure 3: Visualization of the resuscitation process in *Synechocystis sp*. (A) The resuscitation process was induced in three independent batch cultures in the chlorotic state (21 days nitrogen starvation at time point T0) by addition of nitrate (final concentration 10 mM). **(B)** Absorption measurements at 750 nm of re-greening cultures from panel (A) were performed at indicated time points (grey circles).

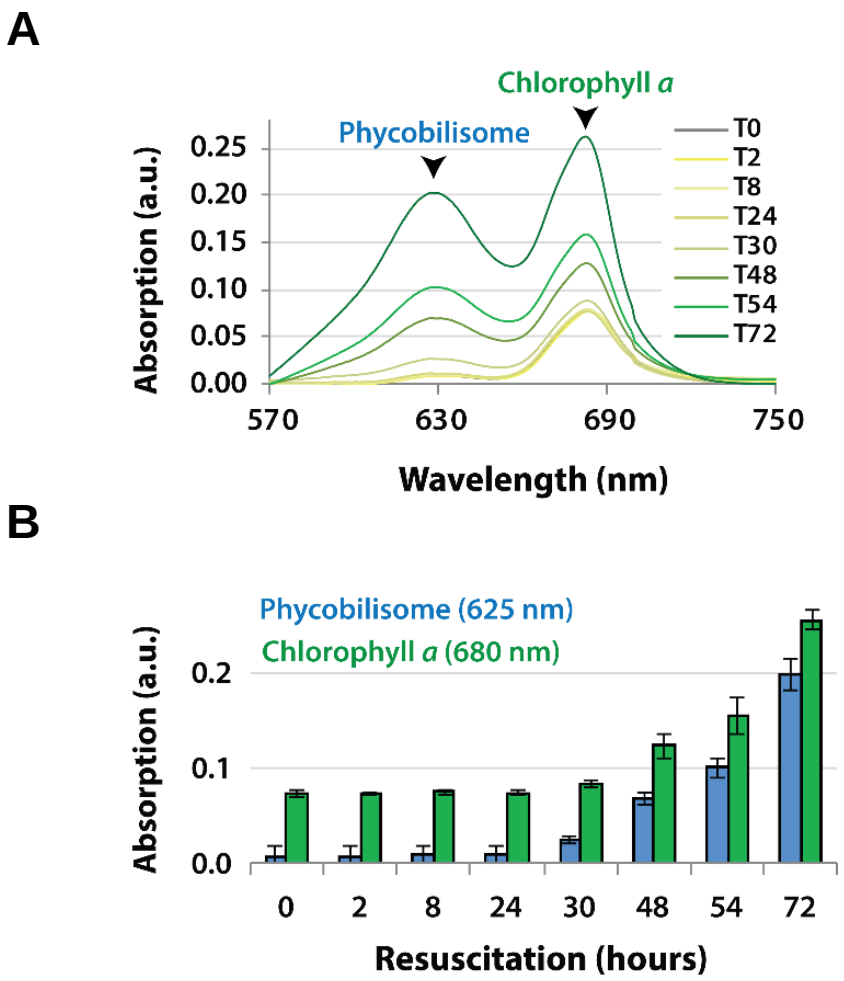
Supplementary Figure 4:



Supplementary Figure 4: Determination of nitrite concentrations in the medium during early resuscitation. Displayed nitrite concentrations (in µM) were measured in the culture medium during the resuscitation process (in min) according to the method described by Snell and Snell (1949). Error bars indicate the standard error of mean., n=3.

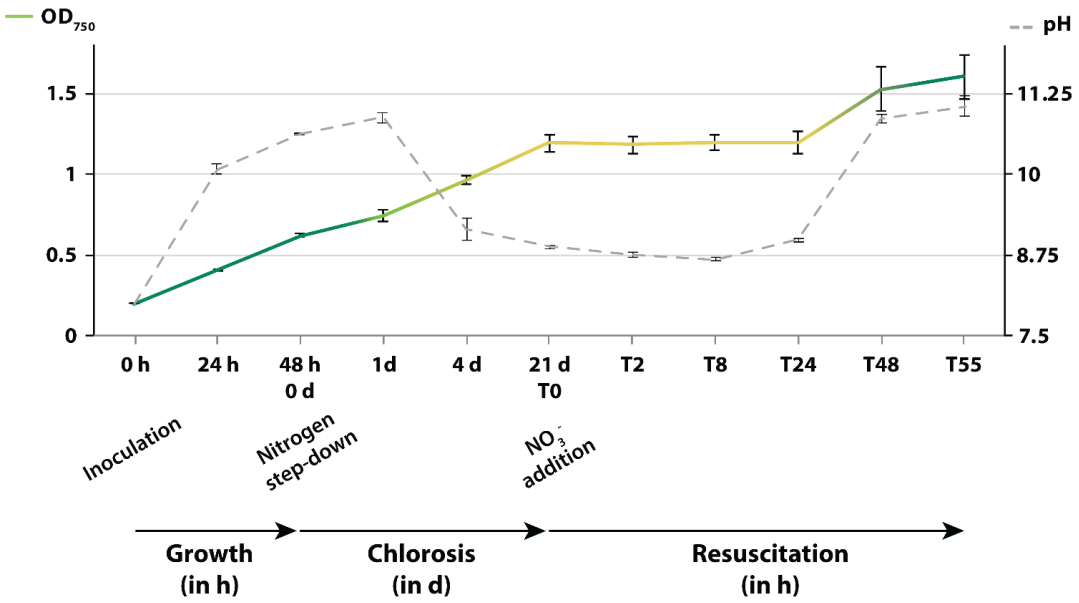
Reference: (**Snell, F.D., and Snell, C.T.** (1949). Colorimetric Methods of Analysis, F.D. Snell and C.T. Snell, eds (Van Nostrand Co.), pp. 802–807-802–807.

Supplementary Figure 5:



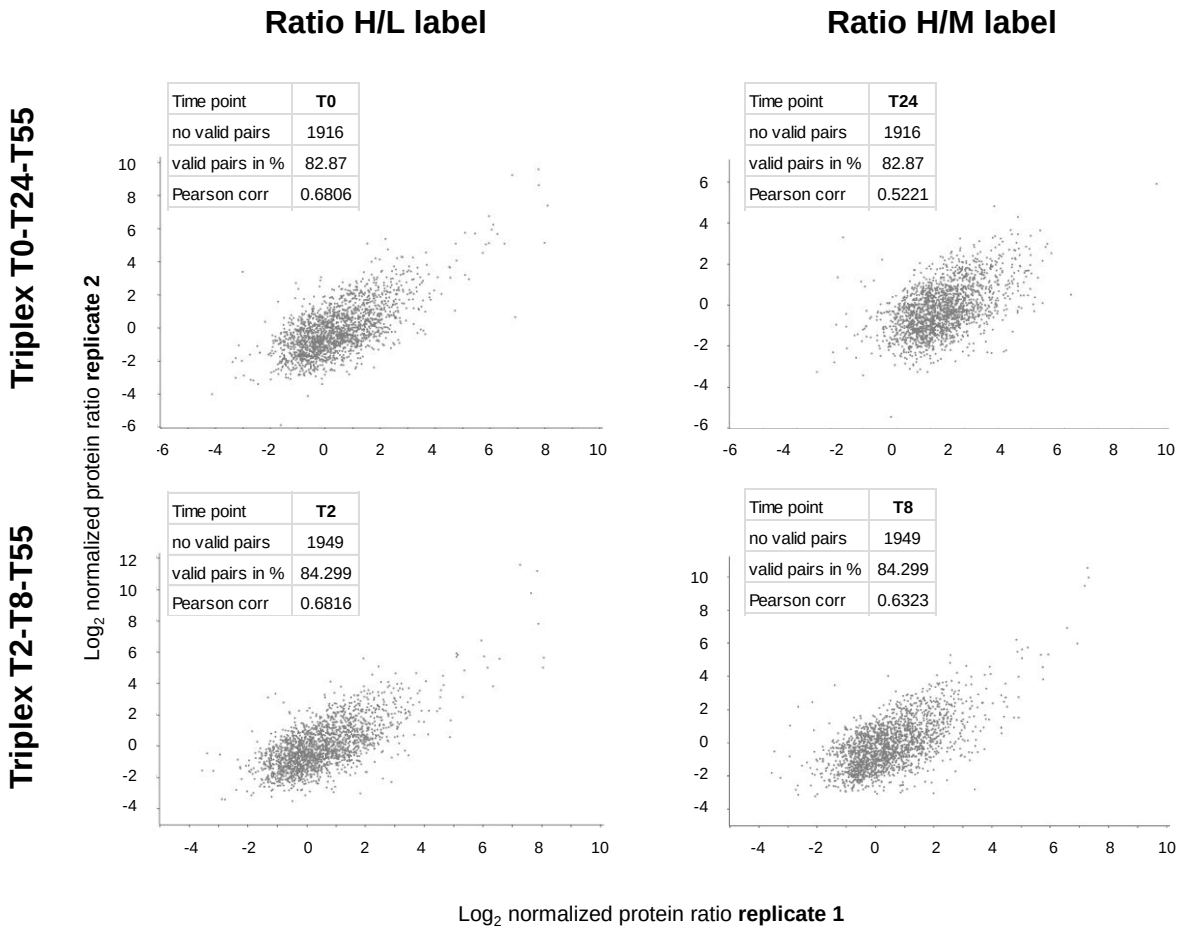
Supplementary Figure 5: Pigment absorption during the resuscitation process in *Synechocystis sp*. (A) Absorption spectra of photosynthetic pigments during resuscitation. Displayed curves represent an average from three independent batch cultures (see Supplementary Figure 3). **(B)** Absorption peaks of phycobilisomes (625 nm) and chlorophyll *a* (680 nm) extracted from spectra in panel (A). Error bars indicate the standard error of mean., n=3.

Supplementary Figure 6:



Supplementary Figure 6: Growth curve of *Synechocystis* sp. cultures for phosphoproteomic experiments. The optical density (at 750 nm; solid line; color gradient following culture appearance) and the culture medium pH (dotted line) were monitored during growth, chlorosis and resuscitation (not in scale) in 2.5 L batch cultures, bubbled with ambient air. Error bars indicate the standard error of mean., n=3.

Supplementary Figure 7:



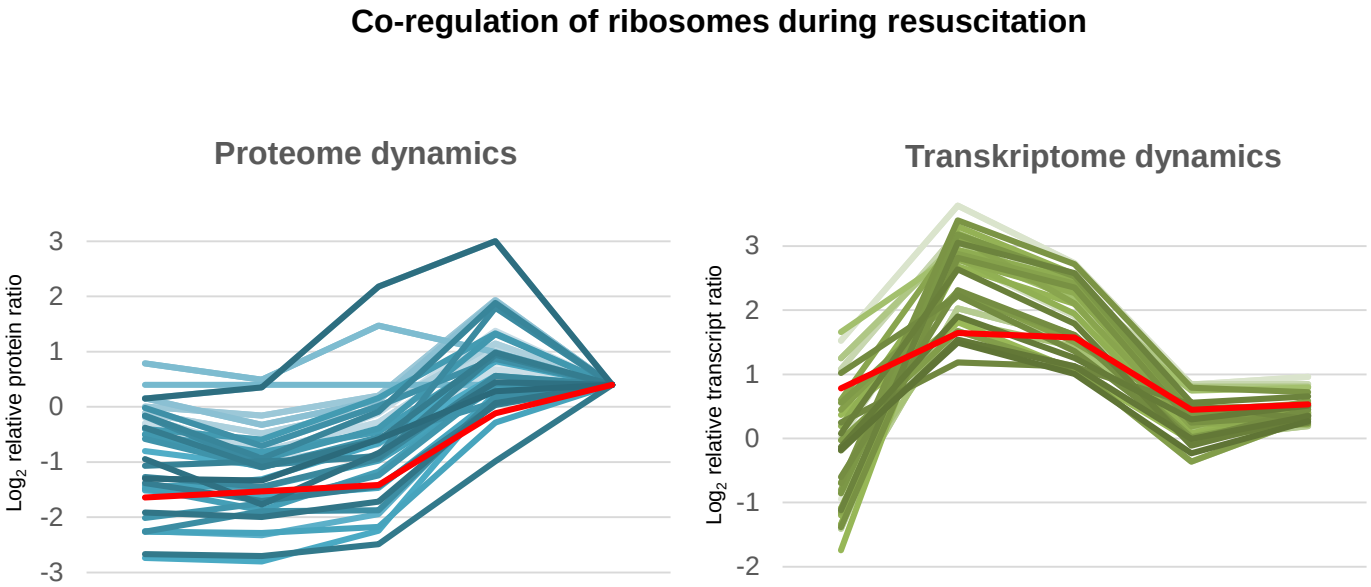
Supplementary Figure 7: Correlation of protein ratios between replicates. The detected protein ratios (in log_2 scale) relative to the reference point T55 are indicated in scatter plots and number of corresponding (paired) proteins in both replicates (number of valid pairs) and their percentage (valid pairs in %) compared to the total of identified proteins in the dataset are stated together with the calculated Pearson correlation coefficient between paired protein ratios. Dimethyl-labels were: light (L) for T0 (Triplex labeling of T0-T24-T55) and T2 (Triplex labeling of T2-T8-T55); intermediate (M) for T8 (in T2-T8-T55) and T24 (in T0-T24-T55); heavy (H) for T55 (inT0-T24-T55 and T2-T8-T55).

Supplementary Figure 8:

Timepoint	harvested volume (mL)	total protein (mg)	protein yield (µg mL^{-1})
T0	500	2.74	**5.47**
T2	500	2.59	**5.19**
T8	400	2.14	**5.35**
T24	400	2.73	**6.83**
T55	500	9.70	**19.41**

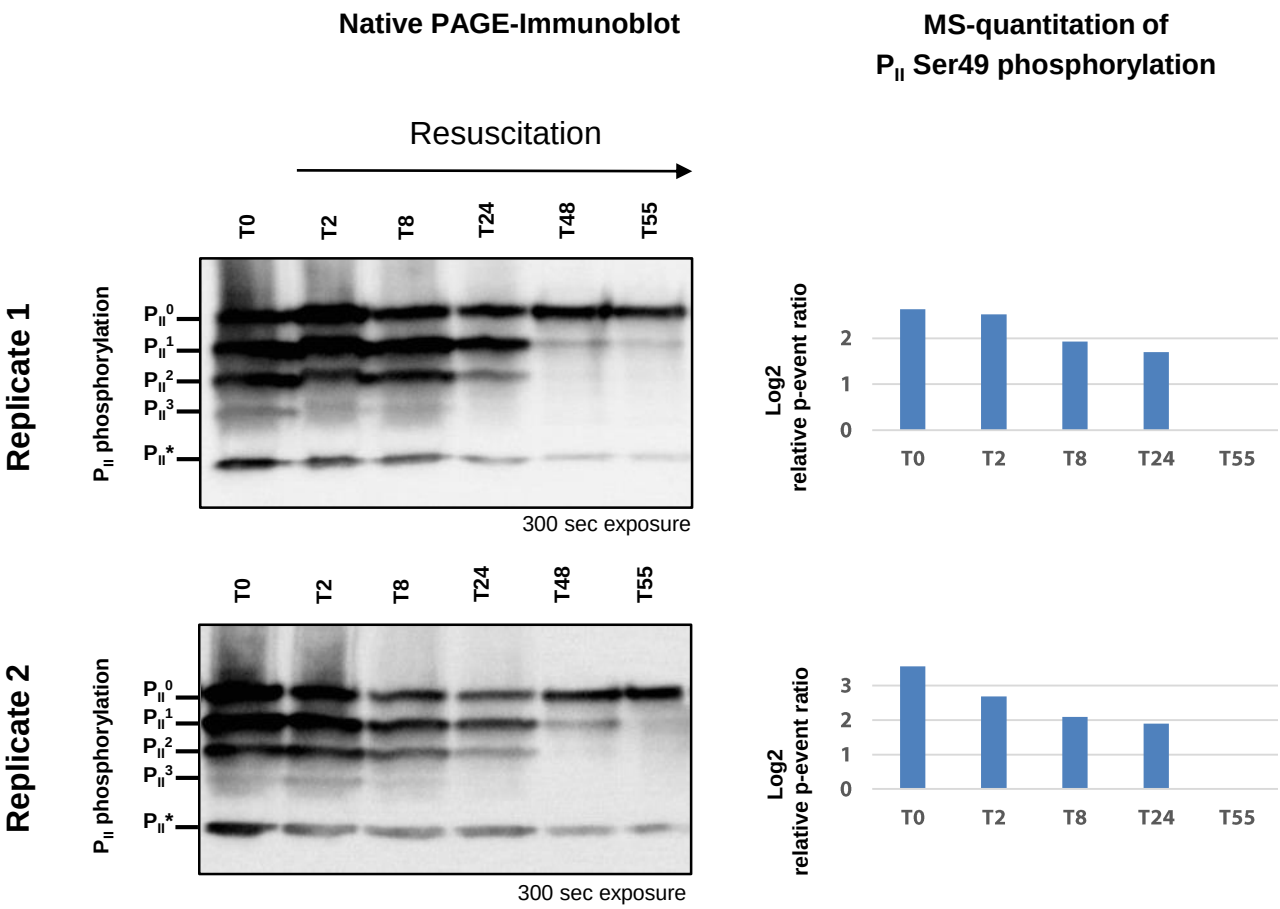
Supplementary Figure 8: Protein yields from 2.5 L batch cultures. Harvested culture volume per time point, the respective total protein yield (in mg) and relative protein yield (in µg protein mL^{-1} culture) are indicated (compare to Supplementary Figure 6).

Supplementary Figure 9:



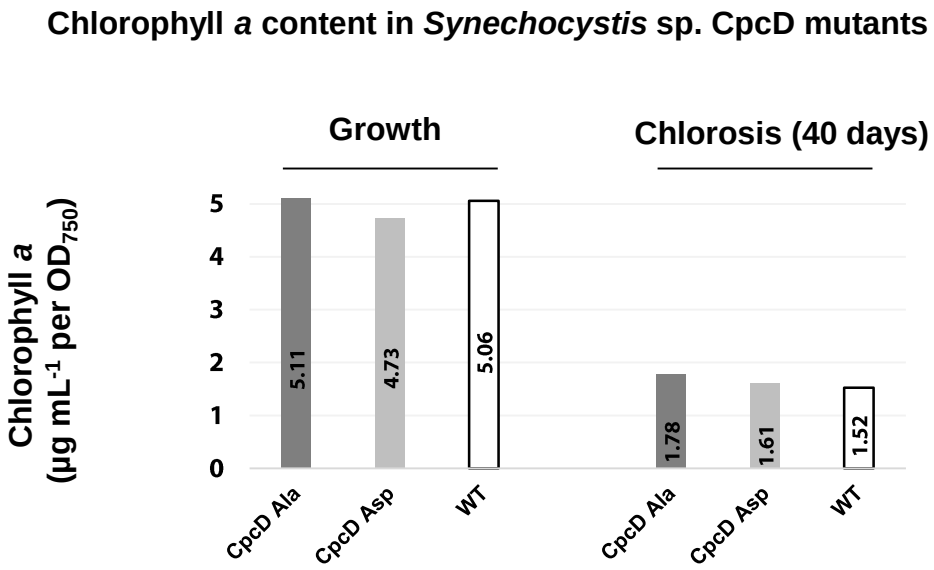
Supplementary Figure 9: Relative dynamics of ribosomal proteins and their corresponding transcripts during resuscitation. Protein ratios of 50 ribosomal subunits from the present study (left panel; in log_2 scale) and corresponding transcript ratios (right panel; from Klotz et al. (2016)) are displayed. The detected protein and transcript dynamics of the hypothetical protein Slr0923 are indicated by a red line, respectively.

Supplementary Figure 10:



Supplementary Figure 10: Coherence of P_{II} phosphorylation dynamics between replicates. The phosphorylation level of the homotrimeric P_{II} protein was analyzed during the resuscitation process in samples for phosphoproteome experiments by Native PAGE-Immunoblot and MS-based detection (MS ratios relative to T55 in $\log_2$ scale). Separated protein bands in native PAGE-Immunoblot analyses correspond to unphosphorylated (P_{II}^0), and single, double or triple phosphorylated (P_{II}^{1-3}) P_{II} trimers, respectively (compare to Spät et al. 2015). The protein bands indicated by * are only detectable after long-term chlorosis and potentially corresponds to further modification. Native PAGE-Immunoblot analyses were conducted as described in Spät et al. (2015) utilizing self-casted 8% native polyacrylamide gels as described in Forchhammer and Tandeau de Marsac (1994).

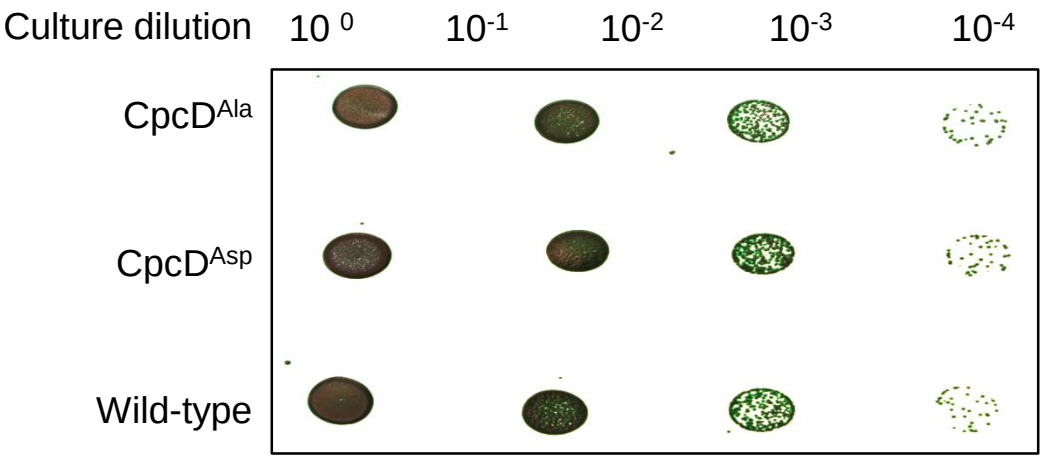
Supplementary Figure 11:



Supplementary Figure 11: Chlorophyll *a* content of *Synechocystis* sp. CpcD mutants and wild-type at vegetative and chlorotic conditions. Cellular chlorophyll *a* concentrations were determined according to Lichtenthaler (1987).

Reference: Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology 148, 350-382.

Supplementary Figure 12:



Supplementary Figure 12: Test of mutant viability. Dilution plating of 21 days. chlorotic *Synechocystis* sp. CpcD mutant and wild-type cultures (at OD_{750}=0.6, corresponding to undiluted plating) on nitrate supplemented BG11 agar. The resuscitation) after 7 days at standard cultivation conditions is shown (see Methods).

Supplementary Figure 13:

	Protein name	Cyanobase ID	Phycobilisome component	Nitrogen depletion study; Spät et al., 2015		Present study				
				Experiment 1	Experiment 2	Resuscitation				
				24 h -N/NO_3	24 h -N/NO_3	T0	T2	T8	T24	T55
Core components	ApcA	slr2067	Allophycocyanin alpha chain	**0.95**	**0.93**	0.024	0.025	0.031	0.232	1
	ApcB	slr1986	Allophycocyanin beta chain	**0.94**	**0.76**	0.022	0.023	0.033	0.407	1
	ApcC	ssr3383	Phycobilisome small core linker polypeptide	**0.95**	**0.67**	0.027	0.025	0.028	0.242	1
	ApcD	sll0928	Allophycocyanin subunit alpha-B	**0.94**	**0.79**	0.011	0.010	0.023	0.106	1
	ApcE	slr0335	Phycobilisome core-membrane linker polypeptide	**1.04**	**0.72**	0.047	0.037	0.052	0.360	1
	ApcF	slr1459	Allophycocyanin subunit beta	**0.97**	**0.91**	0.014	0.015	0.023	0.291	1
Antennae components	CpcA	sll1578	Phycocyanin alpha subunit	**0.55**	**0.32**	0.008	0.010	0.014	0.138	1
	CpcB	sll1577	Phycocyanin beta subunit	**0.56**	**0.34**	0.003	0.004	0.009	0.238	1
	CpcC1	sll1580	Phycobilisome 32.1 kDa linker polypeptide	**0.54**	**0.37**	0.003	0.004	0.006	0.153	1
	CpcC2	sll1579	Phycobilisome 32.1 kDa linker polypeptide	**0.15**	**0.07**	0.003	0.003	0.004	0.089	1
	CpcD	ssl3093	Phycobilisome small rod linker polypeptide	**0.88**	**0.83**	0.003	0.004	0.006	0.126	1
	CpcG1	slr2051	Phycobilisome rod-core linker polypeptide;	**0.91**	**1.01**	0.015	0.017	0.018	0.183	1
	CpcG2	sll1471	Phycobilisome rod-core linker polypeptide	**0.11**	**0.04**	0.028	0.029	0.029	0.442	1

Supplementary Figure 13: Dynamics of phycobiliproteins during chlorosis and resuscitation. Protein ratios of phycobilisome components during initial chlorosis (24 h nitrogen depletion relative to vegetative growth conditions) were retrieved from our previous study (Spät et al., 2015; with two experiments with slightly divergent cultivation conditions). The dynamics of corresponding proteins during resuscitation (detected in the present study) are indicated on the right panel.

Reference: Spät, P., Macek, B., and Forchhammer, K. (2015). Phosphoproteome of the cyanobacterium *Synechocystis* sp. PCC 6803 and its dynamics during nitrogen starvation. Frontiers in Microbiology 6.