Supplementary material for the manuscript:

The redox-sensitive R-loop of the carbon control protein SbtB contributes to the regulation of the cyanobacterial CCM

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Table S1: Primer list

Primers/ amplification	Sequence (5'→3')	Note/ Refs.
Mutant generation		
Insertion of AfeI restriction site at the end of *sbtB* coding sequence	**PR1_SbtB_fw:** GGATACACGGTAATGAATACC	[13]
	PR2_SbtB_AfeI-STOP_rv: CCTCAGGTCATTAAGCGCTGAAAGTATGCCCATAAAGTACTTC	[13]
Mutants verification	**2059:** GTTATTTTGTCGTCAAAC	[13]
	2092: GGATCCCCAATAGTTTATGGTC	[13]
	#PR6_SbtB_pUC19_seq_rv: CTGCCCTGCTGCGTAACATCGTTGC	This study
Recombinant proteins		
C-terminal StrepII-tagged *Sc*SbtB (*slr1513*); (pASK-IBA3_*Sc*SbtB-strep plasmid)	1256_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG GCTAAACCAGCGAACAAGCTCG	[11]
	1257_Rv: AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTACAGCCCT CAGGGCCACAGAAAG	[11]
C-terminal StrepII-tagged *Sc*SbtB-Δ104 (pASK-IBA3_*Sc*SbtB-Δ104)	1256_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG	[13]
	1663_Rv_SbtB delta 104: CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGAAAGTATGCCCATAAAGTACTTCTGC	[13]
C-terminal StrepII-tagged *Sc*SbtB-Δ109 (pASK-IBA3_*Sc*SbtB-Δ109)	1256: Slr1513_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG	This study
	1661: Rv_SbtB W/o C 110: AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCCCTCAGGGCCACAGAAAG	This study
C-terminal StrepII-tagged *Sc*SbtB-C105S+C110S (pASK-IBA3_*Sc*SbtB-C105S+C110S)	1256_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG GCTAAACCAGCGAACAAGCTCG	[13]
	1761_Rv_SbtB-C105+110S CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCTGCCCTCAGGGCCGCTGAAAGTATGCCCATAAAGTACTTCTGC	[13]
C-terminal StrepII-tagged *Sc*SbtB-C105A+C110A (pASK-IBA3_*Sc*SbtB-C105A+C110A)	1256_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATG GCTAAACCAGCGAACAAGCTCG	[13]
	1759_Rv_SbtB-C105+110A CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTTGCGCCCTCAGGGCCTGCGAAAGTATGCCCATAAAGTACTTCTGC	[13]
N-terminal StrepII-tagged *Sc*SbtB-WT (pASK-IBA5_*Sc*SbtB-WT)	1530: FW:_SbtB-Native- N ST: GAGCCACCCGCAGTTCGAAAAAGGCGCCGACGACGACGACAAGATGGCTAAACCAGCGAACAAGCTC	This study
	1531: Rv_SbtB-Native_N ST: CGACCTCGAGGGATCCCCGGGTACCGAGCTCGAATTCGGGACCTTAACAGCCCTCAGGGCCACAG	This study
C-terminal StrepII-tagged *Sc*SbtB-C105S (pASK-IBA3_*Sc*SbtB-C105S)	1256: Slr1513_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG	This study
	1760: RV_SbtB C-105-S: CAAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTACAGCCCTCAGGGCCGCTGAAAGTATGCCCATAAAGTACTTCTGC	This study
C-terminal StrepII-tagged *Sc*SbtB-C110S (pASK-IBA3_*Sc*SbtB-C110S)	1256: Slr1513_Fw: GTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAAACCAGCGAACAAGCTCG	This study
	1662: Rv_SbtB-C110S: AAGCTTATTATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGCTGCCCTCAGGGCCACAGAAAG	This study

N-terminal His_6-tagged TrxA (*slr0623*); (pET15b_TrxA-His_6 plasmid)	pET15b_slr0623_fw: CAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCTCGAGATGAGTGCTACCCCTCAAGTTTC	[13]
	pET15b_slr0623_rev: CCCTCAAGACCCGTTTAGAGGCCCCAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAAC	[13]

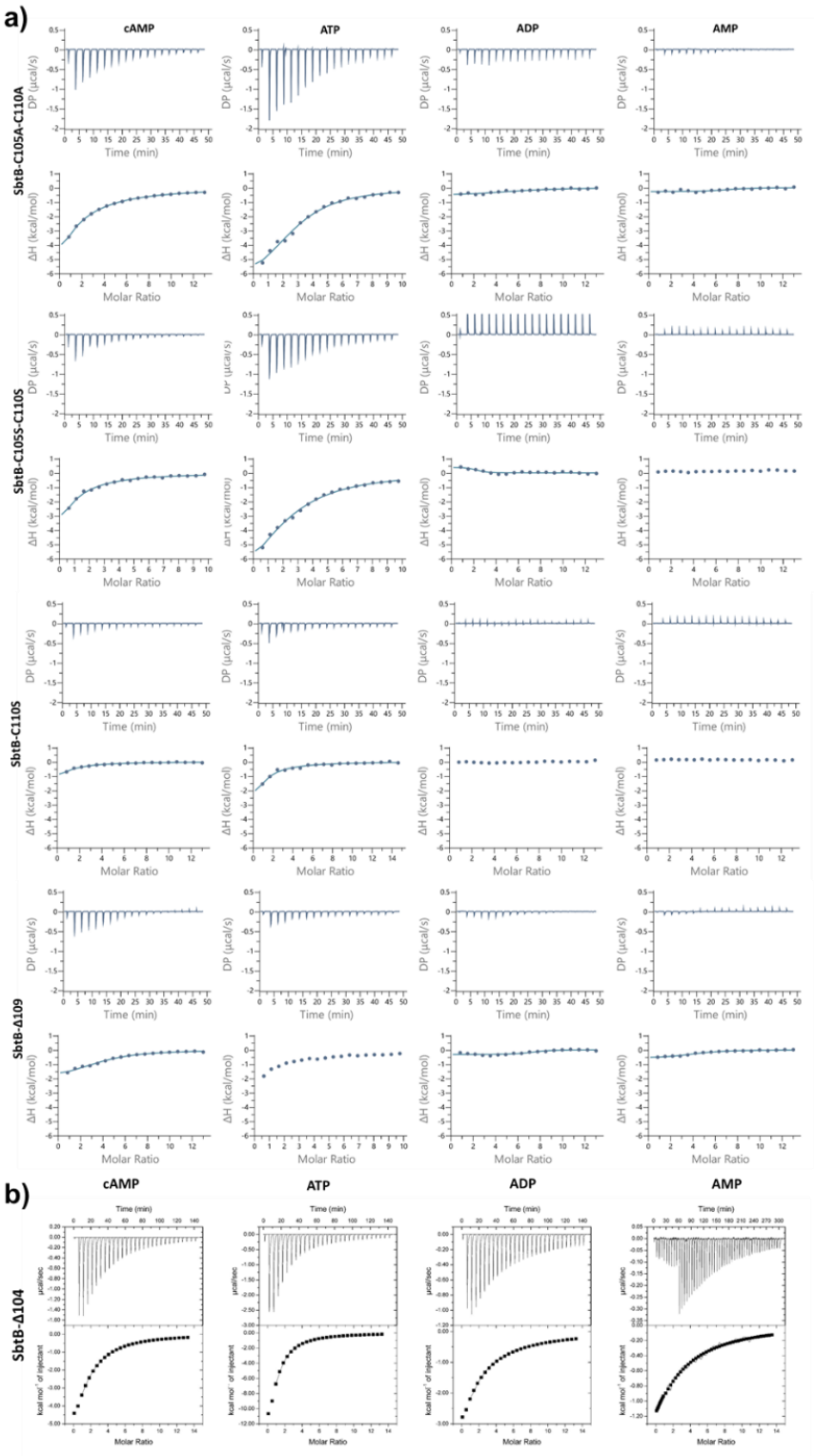


Figure S1: Isothermal titration calorimetry (ITC) analysis of ligand binding properties of the SbtB R-loop variants. a) Upper panels show the raw ITC data in the form of the heat produced during the titration of wild-type and site-specific variant SbtB (trimeric concentration), respectively, with different effector molecules; lower panels show the binding isotherms and the best-fit curves according to the one binding site models for trimeric SbtB. b) ITC analysis of ligand binding properties of the SbtB R-loop variant (Δ104).

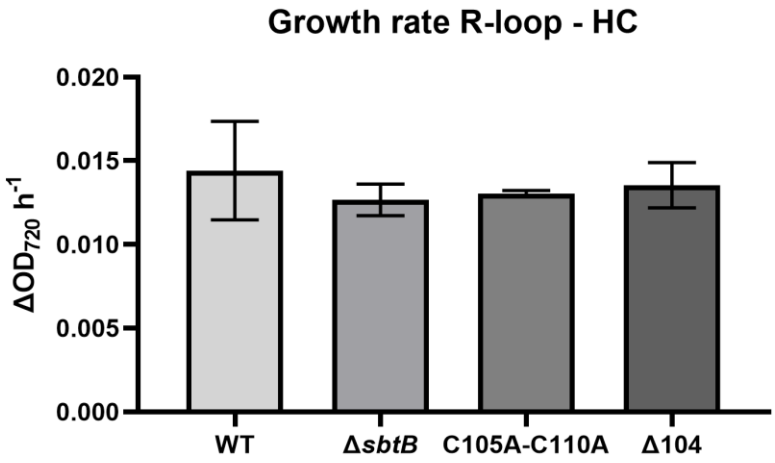


***Figure S2: Growth rate analysis of* Synechocystis *R-loop mutants under HC conditions*.** Growth of strains wild type (WT), Δ*sbtB*, C105A-C110A, and Δ104 was analyzed at high CO_2 (5%, HC) and continuous light in the Multicultivator MD1000. The growth rate (ΔOD_{720}/h) was determined during the exponential phase.

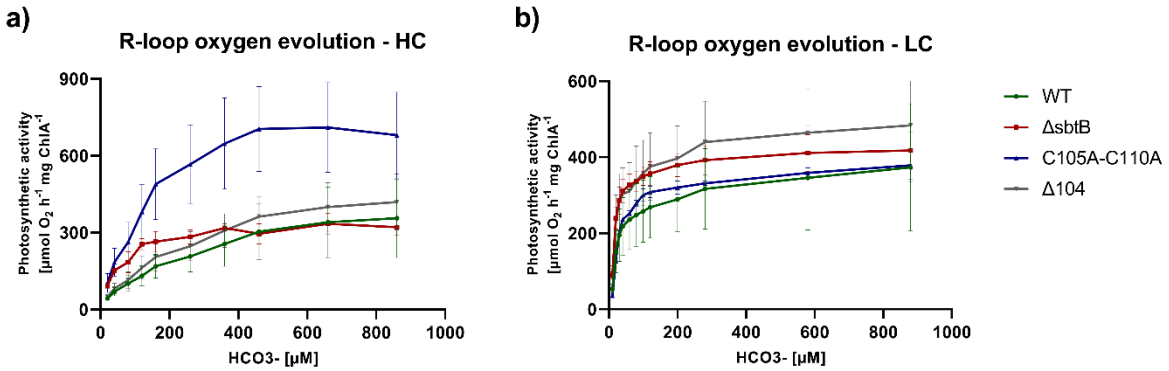
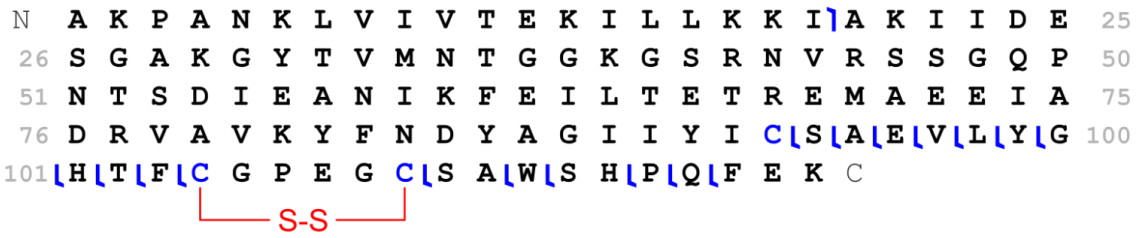
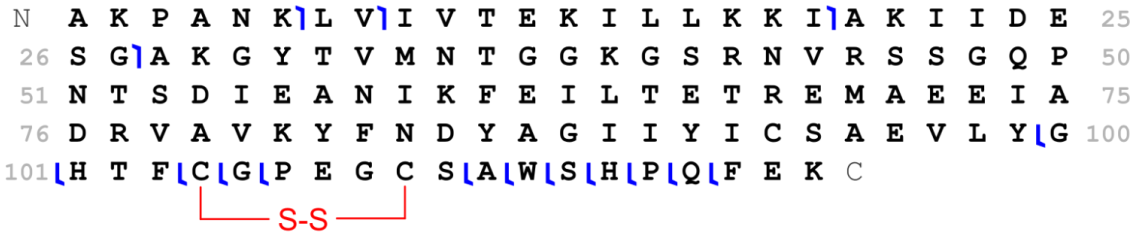


***Figure S3: Bicarbonate-dependent oxygen evolution analysis of* Synechocystis *R-loop mutants*.** Bicarbonate-dependent oxygen production via photosynthesis was measured for strains wild type (WT), Δ*sbtB*, C105A-C110A, and Δ104 that were acclimated to either high CO_2 (5%, HC) (**a**) or ambient CO_2 (0.04%, LC) (**b**) conditions.

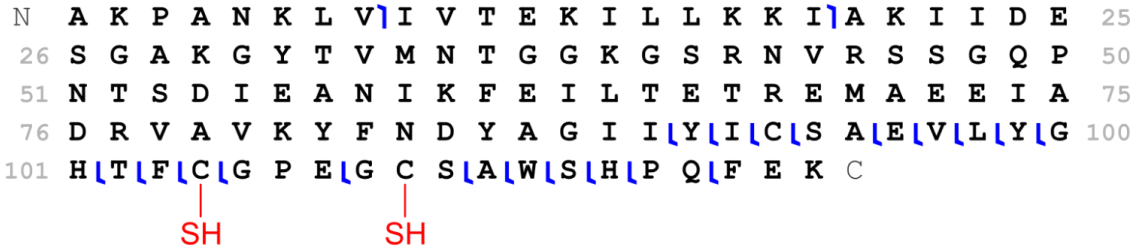
Sample A): SbtB C-terminal tagged (oxidized)
Experimental Monoisotopic Mass: 13063.55



Sample B.1): SbtB C-terminal tagged (DTT reduced)
Experimental Monoisotopic Mass: 13063.57



Sample B.2): SbtB C-terminal tagged (DTT reduced)
Experimental Monoisotopic Mass: 13065.56



Sample C): SbtB C-terminal tagged (DTT-TrxA-reduced)
Experimental Monoisotopic Mass: 13065.59

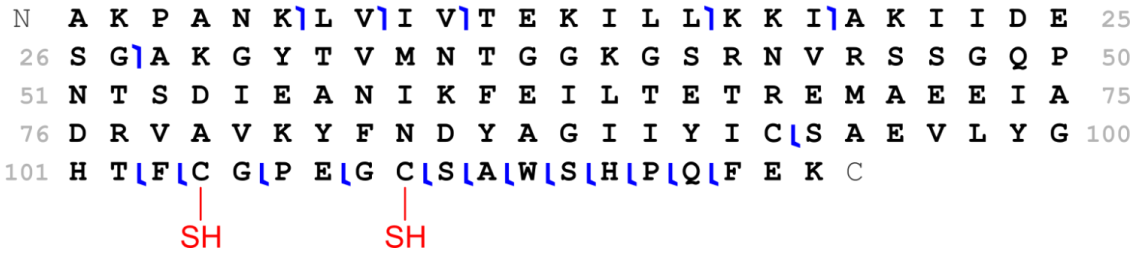


Figure S4: Peptide sequence of oxidized and reduced SbtB. Peptide fragment maps were generated with the MS/MS data shown in Fig. 5. **A-C** indicate the presence of a disulfide bond in (**Sample A**; black peaks in Figure 5A) and the absence of such bond in (**Sample C**; red peak in Figure 5 C). **Sample B** contained SbtB in both oxidized and reduced states.

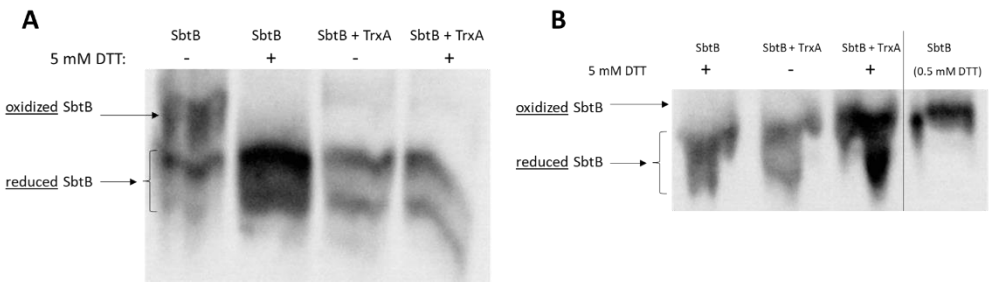


Figure S5: Effect of TrxA and/or DTT additions on reduction of SbtB, resolved on Urea-PAGE and detected using α-SbtB antibody. Urea-PAGE gel showing the reduction of SbtB by 5 mM DTT or equimolar TrxA concentrations (**A**), compared to 0.5 mM DTT (**B**).

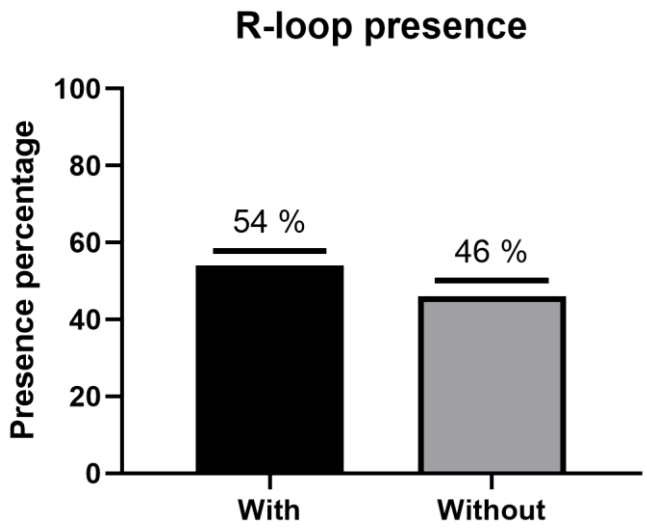


***Figure S6: Presence of the R-loop with the highly conserved cysteine residues among cyanobacterial SbtB homologs*.** R-loop presence percentage was calculated from a multiple sequence alignment of 460 SbtB sequences (sequences obtained from the NCBI data base in June 2023, alignment done with ClustalW).