Supplemental Text S1. Model Sensitivity Analysis

The effects of varying model parameters on the model goodness of fit (sum of squared errors, SSE) and estimated carboxysome mass transfer coefficient (f_{c-x}) was assessed. Parameter variability was set based on standard deviations or standard errors in parameters or, if these were unavailable, were set to 50% of the base model value (Supplemental Table S1). First the model was rerun varying each of the parameters singly. The model only showed substantial sensitivity (defined as >±5% change in SSE) to variation in pH_c, k_{cat-R}, K_{m-B}, and V_{max-B}, and the optimized parameter f_{c-x} stayed within a fairly narrow range (1.5 - 5.0 x10^{-15} cm^3 s^{-1}). Since K_{m-B} and V_{max-B} are derived from the same data the model attempts to fit, the sensitivity of the model to these parameters was expected and is not very relevant to understanding model performance. As can be seen in Table S2 varying these parameters in either direction from the base model value decreases model performance. Decreasing pH_c, the internal pH, improves the model fit because higher CO_2 values are achieved in the carboxysome at low internal C_i concentrations. However, to keep CO_2 effluxes consistent with the data, the carboxysome CO_2 mass transfer coefficient (f_{c-x}) must be reduced to compensate for the increased CO_2 concentrations. Similarly increasing pH_c, worsens the model fit since lower carboxysome CO_2 concentrations are achieved and f_{c-x} increases so that CO_2 effluxes remain constant. Decreasing k_{cat-R}, the RubisCO maximal turnover rate, decreases model performance because it reduces RubisCO capacity (k_{cat-R} * m_R), which as described in the main text is critical to model performance. On the other hand increasing k_{cat-R} and so RubisCO capacity improves the model, showing that the model would actually fit the data better with even more excess RubisCO capacity. We tested for potential interactions between variability in pH_c and k_{cat-R} and found that the model behaved in a predictable manner. Increasing pH and decreasing k_{cat-R} decreased model performance to a much greater extent than changing either alone (Table S3), whereas decreasing pH and increasing k_{cat-R} significantly improved the model fit. The other combinations of these parameters essentially canceled each other out.

Supplemental Table S1. Model Parameters and Variability. When available parameter error estimates (standard deviations, SD, or standard errors, SE) were used to establish variability in parameters for the model sensitivity analysis, but when not available variability was typically set to 50% of the base value. Units for each parameter are given in Table 3.

parameter	base value	error estimate	estimated variability	notes
pH_c	7.35	0.13	--	SD between values reported by Falkner et al. (1976), Kallas and Dahlquist (1981), and Belkin et al. (1987).
k_{cf}	3×10^{-2}	--	1.5×10^{-2}	50% of base parameter value
k_{xf}	1000	--	500	50% of base parameter value
m_R	6.6×10^{-21}	0.56×10^{-21}	--	from SD of measurements
$K_{m\text{-}R}$	263	21	--	SD between our value and Roberts et al. (2012) value
$k_{cat\text{-}R}$	10.6	3.3	--	SD among bacterial RubisCOs in Tcherkez et al. (2006)
$K_{m\text{-}B}$	82	15	--	SE of model fit to Bup data; Table 1
$V_{max\text{-}B}$	2.5×10^{-20}	0.12×10^{-20}		SE of model fit to Bup data; Table 1
N_x	6	--	3	50% of base parameter value
$f_{c\text{-}c}$	1×10^{-8}	--	0.5×10^{-8}	50% of base parameter value
$f_{b\text{-}x}$	6×10^{-10}	--	3×10^{-10}	50% of base parameter value

Supplemental Table S2. Effect of varying model parameters on the optimized carboxysome mass transfer coefficient ($f_{c\text{-}x}$) and the model fit (% change in sum of squared error (SSE) from base model SSE). A positive change in SSE indicates a worse fit of the model to the data, while a negative change in SSE indicates and improved fit.

parameter	base value	low value	$f_{c\text{-}x}$ fit	%SSE	high value	$f_{c\text{-}x}$ fit	%SSE
pH_c	7.35	7.22	1.5×10^{-15}	**-5.9%**	7.48	3.7×10^{-15}	**10.1%**
k_{cf}	3.0×10^{-2}	1.50×10^{-2}	2.9×10^{-15}	-0.3%	4.5×10^{-2}	2.0×10^{-15}	0.3%
k_{xf}	1000	500	2.5×10^{-15}	0.8%	1500	2.4×10^{-15}	-0.3%
m_R	6.60×10^{-21}	6.04×10^{-21}	2.4×10^{-15}	2.8%	7.16×10^{-21}	2.4×10^{-15}	-2.2%
$K_{m\text{-}R}$	263	242	2.4×10^{-15}	-2.0%	284	2.4×10^{-15}	2.0%
$k_{cat\text{-}R}$	10.6	7.3	2.4×10^{-15}	**16.2%**	13.9	2.5×10^{-15}	**-6.2%**
$K_{m\text{-}B}$	82	67	2.7×10^{-15}	**25.2%**	97	2.3×10^{-15}	**10.6%**
$V_{max\text{-}B}$	2.54×10^{-20}	2.42×10^{-20}	2.0×10^{-15}	**16.0%**	2.66×10^{-20}	2.9×10^{-15}	**16.0%**
N_x	6	3	5.0×10^{-15}	0.8%	9	1.6×10^{-15}	-0.3%
$f_{c\text{-}c}$	1.0×10^{-08}	0.5×10^{-08}	2.4×10^{-15}	0.0%	1.5×10^{-08}	2.4×10^{-15}	0.0%
$f_{b\text{-}x}$	6.0×10^{-10}	3.0×10^{-10}	2.4×10^{-15}	0.0%	9.0×10^{-10}	2.4×10^{-15}	0.0%

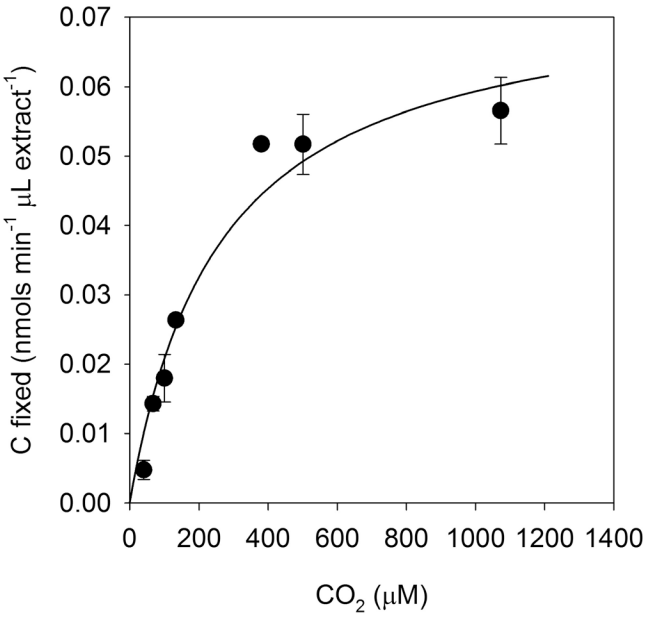
Supplemental Table S3. Interactions between model parameters.

pH_c	k_{cat-R}	f_{c-x} fit	%SSE
7.22	7.3	1.50E-15	**3.9%**
7.22	13.9	1.55E-15	**-9.2%**
7.48	7.3	3.44E-15	**44.5%**
7.48	13.9	3.68E-15	**0.0%**

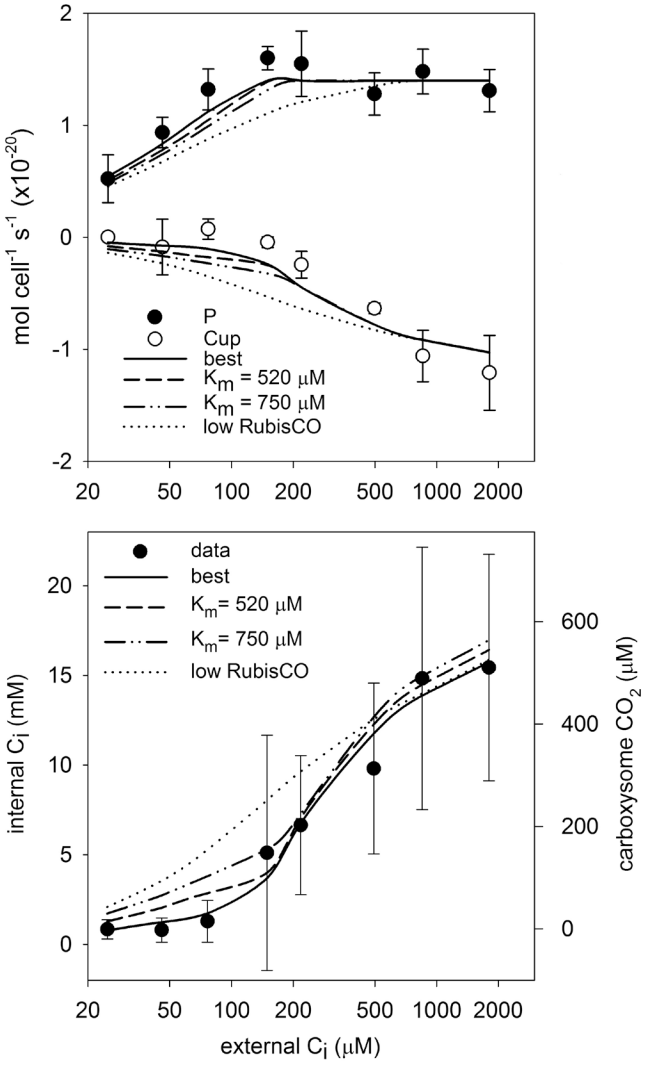
Supplemental Table S4. qPCR primers

Gene	Forward Primer	Reverse Primer
csoS1	ATGACCAAGGCAGCAGAAGT	ACCAAGTGCTGGCTCAACTT
csoS2	CTGCGGATCTACTCCAAAGC	GCTCATCGCCTGTTACGATT
csoS3	AGGGCCTTATATGCATGGTG	GAGCCAATCTTCCGTCAGAG
cbbL	GCTGAAGCCGTTAAACTTGC	GCAAACTCTGCACGTTCGTA
cbbS	CCCTTTCTTTGGGGAAAAAG	TAGCGTCCTTGGAAAACTGC
bicA1	GCGATGGTGCATTAGACAGA	GCTCCAAGAACTGCCAGAAC
bicA2	GTTGGACTTTATGGCGCATT	CCAATAGCAACAACCCCAGT
sbtA	CCCAGAAGCACGATCAAGTT	CGGGGAAAGTGGGATAATTT
rpoD	AAGGGGTTTTGGCGACTTTA	CCATTTCTGTTTGGATGTCTTAGC
gyrA	GTGCTTTTGGTCTTGGGAAA	TGTTGAGAGATTTTATCGGCATT

Supplemental Figure S1. Carbon fixation rates of crude Rubisco extract from *Prochlorococcus* MED4 measured at 20 °C (black circles, error bars are standard deviation of 2 technical replicates of extracts comprised of 3 pooled biological replicates). Half saturation constant for CO_2 was calculated from a Michaelis-Menten curve using a least-squares fit (line).



Supplemental Figure S2. Effect of varying RubisCO K_m on model fit to data.



Supplemental Figure S3. Sample data showing continued CO_2 concentrations above equilibrium after turning off the light and how CO_2 efflux is calculated from this data. The raw data is first smoothed by LOESS smoothing using cross-validation to select the bandwidth of the smoothing. The smoothed CO_2 data is then used to calculate the CO_2 efflux rate based on equation 1.