Systems analysis of the CO2 concentrating mechanism in cyanobacteria
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Abstract Cyanobacteria are photosynthetic bacteria with a unique CO₂ concentrating mechanism (CCM), enhancing carbon fixation. Understanding the CCM requires a systems level perspective of how molecular components work together to enhance CO₂ fixation. We present a mathematical model of the cyanobacterial CCM, giving the parameter regime (expression levels, catalytic rates, permeability of carboxysome shell) for efficient carbon fixation. Efficiency requires saturating the RuBisCO reaction, staying below saturation for carbonic anhydrase, and avoiding wasteful oxygenation reactions. We find selectivity at the carboxysome shell is not necessary; there is an optimal non-specific carboxysome shell permeability. We compare the efficacy of facilitated CO₂ uptake, CO₂ scavenging, and HCO₃⁻ transport with varying external pH. At the optimal carboxysome permeability, contributions from CO₂ scavenging at the cell membrane are small. We examine the cumulative benefits of CCM spatial organization strategies: enzyme co-localization and compartmentalization.

Introduction Intracellular compartments are critical for directing and protecting biochemical reactions. One of the simplest and most striking known examples of compartmentalization are the carboxysomes (Cannon et al., 2001; Yeates et al., 2008) of cyanobacteria and other autotrophic proteobacteria (Savage et al., 2010; Rosgaard et al., 2012). These small, 100–200 nm compartments separate the principal reaction of the Calvin cycle, the RuBisCO catalyzed fixation of carbon dioxide (CO₂) into 3-phosphoglycerate, from the rest of the cell (Cannon et al., 1991). CO₂ and oxygen (O₂) competitively bind as substrates of RuBisCO, and the reaction with O₂ produces phosphoglycolate, a waste product which must be recycled by the cell (Jordan and Ogren, 1981; Tcherkez et al., 2006; Savir et al., 2010). To maximize carboxylation and minimize oxygenation, the carboxysome is believed to act as a diffusion barrier to CO₂ (Reinhold et al., 1989; Dou et al., 2008). There is much interest in the design and function of such compartments and whether they can be used to enhance carbon fixation in other organisms such as plants or to improve reaction rates in other metabolic systems (Papapostolou and Howorka, 2009; Agapakis et al., 2012; Ducat and Silver, 2012; Frank et al., 2013). Increased efficiency of biochemical reactions will lead to better yield in bioengineered bacterial systems, creating new possibilities for production of high-value products such as biofuels. Enhancing carbon fixation in plants or other organisms could lead to increased carbon sequestration, or crop yield.

The concentrating mechanism in cyanobacteria relies on the interaction of a number of well characterized components, as shown in Figure 1, transferring inorganic carbon from outside the cell into cytosol and carboxysomes (Allen, 1984; Kaplan and Reinhold, 1999; Badger and Price, 2003; Price et al., 2007). Due to this mechanism, inorganic carbon concentration is elevated well above 200–300 μM, the CO₂ concentration required for saturating the RuBisCO. Additionally a high CO₂ concentration increases the ratio of CO₂ to O₂ so that carboxylation dominates over oxygenation. Concentrations of 20–40 mM inorganic carbon, up to 4000-fold higher than external levels, have been observed (Sultemeyer et al., 1995; Price et al., 1998; Kaplan and Reinhold, 1999; Woodger et al.,
The bilipid outer and cell membranes are highly permeable to small uncharged molecules such as CO$_2$ (Gutknecht et al., 1977; Misner et al., 2008), so instead the cell primarily accumulates the charged and less membrane soluble bicarbonate (HCO$_3^-$) (Volokita et al., 1984; Price and Badger, 1989). Active transporters, both constitutive and inducible, bring HCO$_3^-$ into the cell (Omata et al., 1999; Price et al., 2004, 2008), and mechanisms exist to actively convert CO$_2$ to HCO$_3^-$ at the thylakoid and cell membrane (Shibata et al., 2001; Maeda et al., 2002; Price et al., 2008). Once it passively diffuses into the carboxysome, HCO$_3^-$ is rapidly brought into equilibrium with CO$_2$ by the enzyme carbonic anhydrase, resulting in the production of CO$_2$ near RuBisCO. Carbonic anhydrase is known to be localized on the interior side of the carboxysome shell (Cannon et al., 1991; Cot et al., 2008; Long et al., 2008; Yeates et al., 2008). The carboxysome shell must be permeable enough to allow HCO$_3^-$ and 3-phosphoglycerate to readily diffuse in and out. The function of this system and its ability to concentrate inorganic carbon depends on the interplay between these various molecular components. Without a model, flux measurements cannot determine the components relative roles in enhancing the CO$_2$ concentration in the carboxysome. To date, it has not been possible to directly measure the partitioning of the internal carbon concentration in the cytosol and carboxysomes. We wish to characterize the distribution of internal carbon. Visualizations of the location of the carboxysomes with fluorescent microscopy in S. elongatus PCC7942 demonstrated that the carboxysomes are evenly spaced along the centerline of the cell, (Savage et al., 2010), raising the question of how spatial organization, beyond simple partitioning, changes the efficacy of the system.
The goal of this study is to further develop a mathematical model of the CCM (Reinhold et al., 1989, 1991; Fridlyand et al., 1996) that uses recent experimental progress on the CCM to untangle the relative roles of the different molecular components, and predict the region of parameter space where efficient carbon fixation occurs. We are considering conditions where CO$_2$ is limiting (15 μM external inorganic carbon) and, for the moment, ignore other biological pressures. In this context, efficient carbon fixation requires two conditions: First, the CO$_2$ concentration must be high enough that RuBisCO is saturated, and the competitive reaction with O$_2$ is negligible. We emphasize that for the oxygenation reaction to be negligible the CO$_2$ concentration should be higher than needed to merely saturate RuBisCO. Secondly, the carbonic anhydrase within the carboxysome must be unsaturated, so that extra energy isn’t wasted transporting unused HCO$_3^-$ into the cell.

Examination of the system performance with varying expression levels of HCO$_3^-$ transporters, carboxysome permeability, and conversion from CO$_2$ to HCO$_3^-$, reveals a parameter window where these conditions are simultaneously satisfied. We comment on the relation of this window to measured carbon pools, carbon fixation rates, and HCO$_3^-$ transporters. We find that the HCO$_3^-$ concentration in the cytosol is constant across the cell, set by the HCO$_3^-$ transport and leakage rates, and depends very little on the carboxysome permeability. The carboxysome permeability does, however, set how the CO$_2$ is partitioned between the carboxysome and cytosol. At optimal carboxysome permeability, HCO$_3^-$ diffusion into the carboxysome is fast enough to supply inorganic carbon for fixation, but the rate of CO$_2$ leakage out of the carboxysome is low. We explore the fluxes from CO$_2$ facilitated uptake and scavenging with varying ratios of external CO$_2$ and HCO$_3^-$. Finally we discuss the proportion the carbon concentration comes from different methods of spatial organization such as co-localization, encapsulation, and spatial location of carboxysomes. Concentration of carbonic anhydrase increases...
the maximum rate of reaction for carbonic anhydrase per volume, causing carbonic anhydrase to saturate at a higher level of \( \text{HCO}_3^- \), and achieve an order of magnitude higher local \( \text{CO}_2 \) concentrations. Encapsulation of the reactions in an optimally permeable carboxysome shell achieves another order of magnitude of \( \text{CO}_2 \) concentration.

**Reaction diffusion model**

We present our mathematical model, which captures all aspects of the CCM as described above. This model is an expansion of previously developed models (Reinhold et al., 1989; 1991; Fridlyand et al., 1996). Our three dimensional model of the CCM solves for both the \( \text{HCO}_3^- \) and \( \text{CO}_2 \) concentration throughout the a spherical cell. We solve this model numerically and analytically at steady state for three different spatial organizations of carbonic anhydrase and RuBisCO in the cell (Figure 6): enzymes distributed evenly throughout the cell, enzymes localized to the center of the cell but not encapsulated (as they would be on a scaffold), enzymes encapsulated in a carboxysome. We compare the effects of these scenarios in the discussion section, and for now consider a spherical cell of radius \( R_c = 0.5 \mu \text{m} \) with a single spherical carboxysome of radius \( R_s = 50 \text{ nm} \) containing RuBisCO and carbonic anhydrase. Numerical computations are carried out with finite difference methods in MATLAB. The details of analytic solutions are given in the *Supplementary file 1*.

We include the effects of 3D diffusion, active transport and leakage through the cell membrane, and reactions with carbonic anhydrase and RuBisCO. In the carboxysome (\( r < R_s \)), the equations governing the \( \text{HCO}_3^- \) and \( \text{CO}_2 \), here \( H \) and \( C \) respectively, are

\[
\frac{\partial C}{\partial t} = D \nabla^2 C + R_{CA} - R_{Rub}
\]

where here \( D \) is the diffusion constant, and \( R_{ca} \) is the carbonic anhydrase reaction, and \( R_{rub} \) is the RuBisCO reaction. The carbonic anhydrase reactions follows reversible Michaelis–Menten kinetics (Kaplan and Reinhold, 1999; Price et al., 2007),

\[
R_{CA} (H, C) = \frac{V_{ca} K_{ca} H - V_{ca} K_{ba} C}{K_{ba} K_{ca} + K_{ba} H + K_{ca} C}
\]

where \( V_{ca} \) and \( V_{ba} \) are hydration and dehydration rates, proportional to the local carbonic anhydrase concentration. \( K_{ca} \) and \( K_{ba} \) are the concentration at which hydration and dehydration are half maximum. The RuBisCO reaction follows Michaelis–Menten kinetics with competitive binding with \( \text{O}_2 \),

\[
R_{Rub} = \frac{V_{rub} C (C + K_n)}{K_{rub} + \text{O}_2(-C)}
\]

where \( K_n = K_{n} (1+ O/K_m) \). Here \( V_{max} \) is the maximum rate of carbon fixation and \( K_m \) is the Michaelis–Menten constant for \( \text{O}_2 \) with the RuBisCO and \( K_m \) is the half maximum concentration with no \( \text{O}_2 \) present. RuBisCO also requires ribulose-1,5-bisphosphate, the substrate which \( \text{CO}_2 \) reacts with to produce 3-phosphoglycerate. Under \( \text{CO}_2 \) limiting conditions it has been shown that there is sufficient ribulose-1,5-bisphosphate to saturate all RuBisCO active sites, and the reaction rates are independent of ribulose-1,5-bisphosphate concentrations (Mayo et al., 1989; Whitehead et al., 2014).

In the cytosol there is no carbonic anhydrase or RuBisCO activity, so \( R_{ca} = 0 \) and \( R_{rub} = 0 \), and there is only diffusion of \( \text{CO}_2 \) and \( \text{HCO}_3^- \). We do not include the natural, but slow, interconversion of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) in the cytosol. This assumption is a good one given that the \( \text{HCO}_3^- \) concentration is known to be held out of equilibrium in the cell (Volokita et al., 1984; Price and Badger, 1989). In agreement with this experimental observation, we find that all the other processes effecting the concentration of \( \text{HCO}_3^- \) in the cytosol happen much faster than the natural interconversion.

Boundary conditions proscribe the inorganic carbon fluxes into the cell and the diffusion across the carboxysome boundary. We treat the inorganic carbon fluxes at cell and thylakoid membranes together. At this cell boundary, there is passive leakage of both \( \text{CO}_2 \) and \( \text{HCO}_3^- \): the velocity of \( \text{CO}_2 \) across the cell membrane, \( k_C^\text{\text{O}_2} \), is about 1000-fold higher than that of \( \text{HCO}_3^- \), \( k_C^\text{\text{HCO}_3^-} \) due to the lower permeability of the membrane to charged molecules. To account for active import of \( \text{HCO}_3^- \), we combine the total \( \text{HCO}_3^- \) flux, \( j_m \), from all \( \text{HCO}_3^- \) transporters. These transporters include BCT1 (encoded by cpm), which is thought to be powered by ATP, and BicA and SbtA which are thought to be
symporters between HCO$_3^-$ and Na$^+$, driven by the highly controlled electrochemical gradient for Na$^+$ (Omata et al., 1999; Price et al., 2004, 2008). Additionally, there are two complexes NDH-1$_3$ and NDH-1$_4$ responsible for converting CO$_2$ to HCO$_3^-$. This conversion is thought to either decrease CO$_2$, creating a gradient across the membranes and ‘facilitating uptake’ of CO$_2$, or ‘scavenge’ CO$_2$ which has escaped from the carboxysome. These are localized to the thylakoid and possibly the plasma membrane. They have been linked to the photosynthetic linear and cyclic electron transport chain (Shibata et al., 2001; Maeda et al., 2002; Price et al., 2008). It has been proposed that electron transport drives the formation of local alkaline pockets where CO$_2$ more rapidly converts to HCO$_3^-$. We simply describe the conversion with a maximal reaction rate $\alpha$, and concentration of half maximal activity of $K_a$. Combining these effects, the boundary condition setting diffusive flux of HCO$_3^-$ and CO$_2$ at the cell membrane is

$$ D \partial_{r} C = - \frac{\alpha C_{\text{cytosol}}}{K_a + C_{\text{cytosol}}} + k_m^C (C_{\text{out}} - C_{\text{cytosol}}) $$

$$ D \partial_{r} H = j_c H_{\text{out}} + \frac{\alpha C_{\text{cytosol}}}{K_a + C_{\text{cytosol}}} + k_m^H (H_{\text{out}} - H_{\text{cytosol}}) $$

where the subscript cytosol and out indicate we are taking the concentration immediately inside and outside the cell boundary respectively. The diffusion constant times partial derivatives with respect to the radial coordinate, $r$, define the diffusive flux at the membrane.

The carboxysome shell is composed of proteins with a radius $R_c = 50$ nm. As of yet, there have been no direct measurements of the carboxysome permeability to small molecules. Using the carboxysome geometry, we can calculate an upper bound for the diffusive velocity across the carboxysome shell, which is directly related to the carboxysome permeability. Crystal structures (Yeates et al., 2007, 2008; Cheng et al., 2008) show the surface has approximately $N_{\text{pores}} = 4800$ small pores with radius $r_{\text{pore}} = 0.35$ nm, and thickness $l = 1.8$ nm. If $k_v$ is the characteristic velocity that small molecules pass through the shell, these numbers imply the upper bound for diffusive transport $k_v < \frac{\pi r_{\text{pore}}^2 D}{4\pi R_c l} (N_{\text{pores}}) = 0.02$ cm$^2$/s. This calculation is done by taking the probability that a molecule will encounter a pore on the carboxysome shell [$N_{\text{pores}} \times \text{pore surface area} \over \text{carboxysome surface area}$] and multiplying it by the speed a small molecule will diffuse through the length of the pore ($D/l$). Since it does not take into account any charge effects, which would add an additional energy barrier, it is an upper bound. Although there has been much speculation that the positively charged pores might enhance diffusion of negatively charged HCO$_3^-$ (Cheng et al., 2008; Dou et al., 2008; Yeates et al., 2008), here we explore the simplest assumption, that both HCO$_3^-$ and CO$_2$ have the same permeability. Namely, the boundary conditions at the carboxysome shell are

$$ D \partial_{r} C = k_v (C_{\text{cytosol}} - C_{\text{carboxysome}}) $$

$$ D \partial_{r} H = k_v (H_{\text{cytosol}} - H_{\text{carboxysome}}). $$

We will vary $k_v$ (henceforth called carboxysome permeability, although it is a velocity) within our model and see that there is a range of $k_v$ where the CCM is effective even with $k_v$ identical for CO$_2$ and HCO$_3^-$. **Results**

**Analysis of model: finding functional parameter space**

Now that we have defined our model, we wish to find the range of parameters where efficient carbon fixation occurs. In what follows, we fix the enzymatic rates, cell membrane permeability, and diffusion constant as reported in the literature (Gutknecht et al., 1977; Jordan and Ogren, 1981; Heinhorst et al., 2006; Missner et al., 2008; Tables 1 and 2). Note that full analytic solutions are available in
We consider the efficacy of the CCM as a function of \( j_c \), the flux of \( \text{HCO}_3^- \) into the cell, \( k_c \), the carboxysome permeability, and the parameters \((\alpha, K_\alpha)\) governing the \( \text{CO}_2 \) facilitated uptake mechanism. Both \( \alpha \) and \( j_c \) can be regulated by the organism and vary depending on environmental conditions, whereas the carboxysome permeability, \( k_c \), is the parameter with the largest uncertainty and debate (Cannon et al., 2001; Cheng et al., 2008; Yeates et al., 2008).

For any given pair of \( k_c \) and \( j_c \), we ask whether the \( \text{CO}_2 \) concentrating mechanism is effective, using the criteria of saturating RuBisCO, reducing oxidation reactions, and not increasing the \( \text{HCO}_3^- \) concentration beyond carbonic anhydrase saturation. Our central result is presented in Figure 2, which shows the range of \( k_c \) and \( j_c \) where these conditions are met, assuming that there is no facilitated uptake, \( \alpha = 0 \). The blue shaded region shows where RuBisCO is unsaturated, and the red shaded region shows where carbonic anhydrase is saturated. There is a crescent shaped region between these regions, where the CCM is effective according to our criteria. In the white region oxygenation

### Table 1. Parameter values chosen for main set of simulations, unless otherwise indicated

| Parameter   | Definition                                      | Value              | Reference                              |
|-------------|-------------------------------------------------|--------------------|----------------------------------------|
| \( H_{\text{out}} \) | concentration of bicarbonate outside the cell | 14 \( \mu \text{M} \)* | (Price et al., 2008)                    |
| \( C_{\text{out}} \) | concentration of carbon dioxide outside of cell | 0.14 \( \mu \text{M} \)* | (Price et al., 2008)                    |
| \( D \) | diffusion constant of small molecules, \( \text{CO}_2 \) and \( \text{HCO}_3^- \) | \( 10^{-5} \) \( \text{cm}^2 \text{s}^{-1} \) | (Fridlyand et al., 1996)                |
| \( k_m^c \) | permeability of cell membrane to \( \text{CO}_2 \) | 0.3 \( \text{cm} \text{s}^{-1} \) | (Gutknecht et al., 1977; Missner et al., 2008) |
| \( k_m^n \) | permeability of cell membrane to \( \text{CO}_2 \) | \( 3 \times 10^{-4} \) \( \text{cm} \text{s}^{-1} \) | (Gutknecht et al., 1977; Missner et al., 2008) |
| \( R_c \) | radius of carboxysome                          | 5 \( \times 10^{-6} \) cm | (Schmid et al., 2006; Cheng et al., 2008) |
| \( R_b \) | radius of bacteria                            | 5 \( \times 10^{-5} \) cm | (Savage et al., 2010)                  |
| \( j_c \) | \( \text{HCO}_3^- \) transport rate resulting in 30 mM cytosolic \( \text{HCO}_3^- \) pool | 0.7 \( \text{cm} \text{s}^{-1} \) | calculated                             |
| \( k_s \) | optimal carboxysome permeability               | \( 6 \times 10^{-1} \) \( \text{cm} \text{s}^{-1} \) | calculated                             |
| \( V_{\text{cell}} \) | cell volume                                    | 5.2 \( \times 10^{-10} \) \( \mu \text{L} \) | calculated                             |
| \( S_{\text{Acell}} \) | cell surface area                              | 3 \( \times 10^{-6} \) \( \text{cm}^2 \) | calculated                             |

*these parameters are varied in the text, but these values are used unless noted otherwise.

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### Table 2. Table comparing enzymatic rates (Sultemeyer et al., 1995; Woodger et al., 2005; Heinhorst et al., 2006)

| Enzyme reaction | active sites | \( k_{cut} \) [1] | \( V_{\text{max}} \) in cell [\( \mu \text{M} \text{s}^{-1} \)] | \( V_{\text{max}} \) in carboxysome [\( \mu \text{M} \text{s}^{-1} \)] | \( K_{1/2} \) [\( \mu \text{M} \)] |
|----------------|--------------|-------------------|---------------------------------|---------------------------------|-------------------|
| carbonic anhydrase hydration | 80 | \( 8 \times 10^4 \) | 1.1\( \times 10^4 \) | 2 \( \times 10^5 \) | 3.2 \( \times 10^5 \) |
| carbonic anhydrase dehydration | 80 | \( 4.6 \times 10^4 \) | 9.5\( \times 10^4 \) | 1.7 \( \times 10^5 \) | 9.3 \( \times 10^5 \) |
| RuBisCO carboxylation | 2160 | 26 | 103 | 1.7 \( \times 10^4 \) | 270 |

\( V_{\text{max}} \) in cell and carboxysome refer to the volumetric reaction rate assuming the enzymes are distributed through cellulose the entire cell or only carboxysome. \( V_{\text{max}} \) (\( V_{\text{max}} \) for carbonic anhydrase dehydration) is estimated by assuming \( K_{\alpha} = 5 \) and using parameters found in Heinhorst et al. (2006). \( V_{\alpha} \) is \( V_{\text{max}} \) for carbonic anhydrase hydration. Similarly, \( K_{\alpha} \) and \( K_{\beta} \) are \( K_{1/2} \) for dehydration and hydration respectively.

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reactions happen at a rate of greater than 1%. In the green shaded region oxygenation reactions occur at a rate of less than 1%. Within the white and green regions the CO$_2$ concentration in the carboxysome varies greatly.

The lines dividing the regions in Figure 2 are lines of constant carboxysomal CO$_2$ concentration in $j_c$ and $k_c$ parameter space. The dark blue line is where CO$_2$ = $K_m$, the CO$_2$ concentration for half maximum RuBisCO reactions. The light blue line indicates parameter values resulting in the CO$_2$ concentration (C$_{ppw}$) where rate of oxygenation reactions is 1% for O$_2$ concentration of 260 μM. Varying carboxysome permeability, $k_c$ values, require more or less HCO$_3^-$ transport, $j_c$, to achieve the same carboxysomal CO$_2$ concentration.

We can calculate an amplification factor for the C$_{ppw}$ line of constant carboxysomal CO$_2$ concentration as $A_C = \frac{C_{\text{carboxysome}}}{C_{\text{out}} + H_{\text{out}}} = 133$. Any combination of $j_c$ and $k_c$ which produce C = C$_{ppw}$ make 133 times more CO$_2$ available in the carboxysome than there is total inorganic carbon outside the cell. Generally, increasing HCO$_3^-$ transport, below the carbonic anhydrase saturation point results in higher CO$_2$ concentration in the carboxysome.

Figure 2. Phase space for HCO$_3^-$ transport, $j_c$, and carboxysome permeability $k_c$. Plotted are the parameter values at which the CO$_2$ concentration reaches some critical value. The left most line (dark blue) indicates for what values of $j_c$ and $k_c$, the CO$_2$ concentration in the carboxysome would half-saturate RuBisCO ($K_m$). The middle line (light blue) indicates the parameter values which would result in a CO$_2$ concentration where 99% of all RuBisCO reactions are carboxylation reactions and only 1% are oxygenation reactions when O$_2$ concentration is 260 μM. The right most (red) line indicates the parameter values which result in carbonic anhydrase saturating. Here $\alpha = 0$, so there is no CO$_2$ scavenging or facilitated uptake. The dotted line (grey) shows the $k_c$ and $j_c$ values, where the HCO$_3^-$ concentration in the cytosol is 30 mM. The HCO$_3^-$ concentration in the cytosol does not vary appreciably with $k_c$ in this parameter regime, and reaches 30 mM at $j_c = 0.8 \text{ cm}^{-1}$. All other parameters, such as reaction rates are held fixed and the value can be found in the Table 1 and 2.

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The following figure supplements are available for figure 2:

Figure supplement 1. Phase space for HCO$_3^-$ transport and carboxysome permeability.
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Figure supplement 2. Effect of CO2 scavenging or facilitated uptake on phase space for HCO$_3^-$ transport, $j_c$, and carboxysome permeability, $k_c$.
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Varying HCO$_3^-$ transport saturates enzymes

The basic physics of the phase diagram Figure 2 follows from examining how CO$_2$ and HCO$_3^-$ in the carboxysome change as $j_a$ is varied. Figure 3 shows the response to varying $j_a$ with $k_c = 6 \times 10^{-3}$ cm$^5$/s (the optimal value in Figure 4).

When $j_a$ is low, the ratio of CO$_2$ and HCO$_3^-$ is constant, set by the chemical equilibrium at a given pH. In this case the rate of the carbonic anhydrase reaction is much faster than diffusion within the carboxysome, so that $V_{ba}K_{ca}H = V_{ca}K_{ba}C$. Unlike the uncatalyzed interconversion of CO$_2$ and HCO$_3^-$ in the cytosol, carbonic anhydrase brings the concentrations in the carboxysome to equilibrium very quickly. The chemical equilibrium is $K_{eq} = \frac{H/C}{K_{ba}V_{ca}}(K_{ca}V_{ba}) = 5$, for pH around 7 (DeVoe and Kistiakowsky, 1961), so that HCO$_3^->CO_2$ in the carboxysome. Increased pH would increase $K_{eq}$ and the proportion of HCO$_3^-$, while decreased pH would decrease $K_{eq}$ and the proportion of HCO$_3^-$. Such variations do not substantially effect the subsequent discussion and mechanisms, although they will change the absolute values of CO$_2$ concentration in the carboxysome.

The $K_c$ dashed line in Figure 3 shows the CO$_2$ level above which RuBisCO reaction is saturated: this gives the RuBisCO saturated (blue) boundary in Figure 2. We have similarly marked the concentration $C_{99\%}$ where there is a 1% oxygen reaction error rate with a dash-dotted line.

At higher levels, the CO$_2$ concentration no longer increases with increasing $j_a$, because the carbonic anhydrase is saturated. The saturated regime occurs in Figure 3 when $H_{carboxysome}>K_{ba}$ so that increasing $H_{carboxysome}$ (controlled directly by $j_a$) no longer increases the rate of production of $C_{carboxysome}$. This transition from unsaturated to saturated carbonic anhydrase defines the line for the carbonic anhydrase saturated region in Figure 2.

**Carboxysome permeability has optimal value**

For each line of constant concentration in Figure 2 there is an optimal permeability value, where the least HCO$_3^-$ transport is required to achieve the same CO$_2$ concentration. The optimal permeability value shifts downward with increasing CO$_2$ concentration (compare light and dark blue curves). For $C_{99\%}$ the optimal permeability is $k_c = 6 \times 10^{-3}$ cm$^5$/s, below the calculated upper bound: $k_c < 0.02$ cm$^5$/s obtained above from the carboxysome structure. To further understand the effect of permeability, we examine the CO$_2$ concentration in the carboxysome for varying carboxysome permeabilities and a fixed HCO$_3^-$ transport rate in Figure 4. Figure 4A, shows that there is a broad range of $k_c$ where the CCM has maximal efficacy. Figure 4 shows the distribution of inorganic carbon throughout the cell when the permeability is low (B), optimal (C), and high (D). At high permeability, the CO$_2$ produced in the carboxysome rapidly leaks out of the carboxysome, and the CO$_2$ concentration in the cytosol, shown in Figure 4D, is relatively high. When the carboxysome permeability decreases to near the optimal value, Figure 4D, the carboxysome traps CO$_2$, and the cytosolic levels are lower, decreasing leakage out of the cell. This transition occurs when diffusion across the cell (and carboxysome) takes a shorter time than diffusion through the carboxysome shell; or the CO$_2$ in the carboxysome is effectively partitioned from the CO$_2$ in the cell.
If the carboxysome permeability is below optimal, diffusion of $\text{HCO}_3^-$ into the carboxysome cannot keep up with consumption from RuBisCO. The existence of an optima requires RuBisCO consumption to be low enough that there is a $k_c$ where the cytosol and carboxysome are partitioned, but $\text{HCO}_3^-$ diffusion in can keep up. When such an optima exists, the carboxysome permeability can improve the $\text{CO}_2$ concentration in the carboxysome without any special selectivity between $\text{HCO}_3^-$ and $\text{CO}_2$.

**Discussion**

**Are the fluxes and concentrations reasonable?**

While we have solved our model to describe a vast parameters space it is instructive to compare the fluxes and concentrations we find within our optimal parameter space (the green region in *Figure 2*) to actual numbers. At low external inorganic carbon conditions, internal inorganic carbon pools due to CCM activity are regularly measured as high as $C_i = 30\text{mM}$. The inorganic carbon is predominantly in the form of $\text{HCO}_3^-$, and measurements do not distinguish between the cytosol and carboxysome
In our model, we find that the cytosolic HCO$_3^-$ concentration is 30 mM when $j_c = 0.7 \text{ cm}^{-1} \text{s}$. over a wide range of the carboxysome permeability (indicated as the dashed grey line in Figure 2).

From Figure 4 we can also see that the cytosolic HCO$_3^-$ concentration is the dominate form of inorganic carbon in the cell at $j_c = 0.7 \text{ cm}^{-1} \text{s}$. We examine the fate of the HCO$_3^-$ transported into the cell in terms of the HCO$_3^-$ leaking out, CO$_2$ leaking out, CO$_2$ fixation or carboxylation, and O$_2$ fixation or oxygenation (Table 3).

For cells grown under low inorganic carbon conditions net HCO$_3^-$ fluxes (transport–leakage) are measured $10^5 \text{ pmol (mgChl s)}^{-1}$, with CO$_2$ net flux being slightly lower but the same order of magnitude (Badger et al., 1994; Whitehead et al., 2014). High external inorganic carbon conditions produce slightly higher net HCO$_3^-$ rates (Tchernov et al., 1997). Assuming chlorophyll per cell volume of around $10^{-11} \text{ mgChl cell}^{-1}$ for cells of our size we can convert this into a flux of $10^{-6} \text{ pmol (cell s)}^{-1}$ (Keren et al., 2002, 2004; Whitehead et al., 2014). The net HCO$_3^-$ flux for our model cell is $10^{-5} \text{ pmol (cells s)}^{-1}$, so we are about an order of magnitude too high. If we choose a HCO$_3^-$ transport value one order of magnitude smaller, we will get net fluxes of the same order of magnitude as the measurements at the cost of slightly lower carboxylation rates and higher oxygenation rates (Table 4). This would also mean a lower internal HCO$_3^-$ pool. Alternatively, the same internal HCO$_3^-$ could be reached at a lower flux rate, if the external HCO$_3^-$ is higher. We have chosen a dramatically low external inorganic carbon concentration, where the CCM is known to be up-regulated (Price et al., 2008). The general results we present hold until the internal and external concentration are of the same order of magnitude, at which point the CCM is no longer necessary. Since the majority of the HCO$_3^-$ transport is balanced by HCO$_3^-$ leakage, we can find the transport rate needed to sustain a particular internal concentration by the simple formula: $j_c = k_{H^-}^m (H_{out} - H_{cytosol}(R_b))/H_{out}$.

While we can compare the net fluxes, we have not found direct experimental evidence for the absolute HCO$_3^-$ uptake rate. To determine whether this HCO$_3^-$ transport rate is reasonable we perform a back of the envelope calculation. Our simulated cell has a flux of $2 \times 10^7 \text{ molecules/s}$.

Assuming the rate of transport per transporter of $3 \text{ molecules/s}$ and our cell’s surface area this

| Table 3. Fate of carbon brought into the cell for $j_c = 0.7 \text{ cm/s}$ and $k_c = 6 \times 10^{-3} \text{ cm/s}$ |
|-------------------------------------------------|-----------------|---------|
| formula                                      | pmol (cell s) | % of flux |
| HCO$_3^-$ transport                           | $j_c H_{out}$  | $3.3 \times 10^{-4}$ |
| HCO$_3^-$ leakage                             | $k_{H^-}^n (H_{out} - H_{cytosol}(R_b))$ | $3.2 \times 10^{-4}$ | 96.6 |
| CO$_2$ leakage                                | $k_c^c (C_{out} - C_{cytosol}(R_b))$ | $10^{-5}$ | 3.1 |
| carboxylation                                 | $V_{max} C$    | $9 \times 10^{-7}$ | 0.3 |
| oxygenation                                   | $V_{max}^O C$  | $3 \times 10^{-9}$ | 0.001 |

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requires about \(10^3\) transporters \(\mu m^2\). This is actually not that far off from the number of ATP synthase complexes on the thylakoid membrane in spinach, \(700\) complexes \(\mu m^2\) (Miller and Staehelin, 1979), although it is still quite high.

According to our calculation only around 1% of the inorganic carbon transported into the cell is fixed into 3-phosphoglycerate. The conclusion that about 99% of inorganic carbon transported into the cell is lost through leakage challenges the assumption that the 3 ATP and 2 NADPH used during the Calvin-Benson-Bassham cycle is the dominant energy expenditure. If it holds true, then cyanobacteria invest much more energy in inorganic carbon uptake than was previously understood. Even in this highly CO\(_2\)-concentrating regime, \(5 \times 10^4\) 2-phosphoglycolate are produced per second. Cyanobacteria have been shown to have multiple pathways for recycling 2-phosphoglycolate (Hackenberg et al., 2009). Our system fixes CO\(_2\) at a rate of 0.14 pg/hr. Given the volume of our cell, and the fact that between 115–300 fg/\(\mu m^3\) of carbon are needed to produce a new cyanobacterial cell (Mahlmann et al., 2008) we need between 0.1 and 0.3 picograms of carbon per cell. At the higher flux rate (Table 3) this means that a cell could replicate every 1–2 hr, so faster than cyanobacteria replicate. The lower flux rate (Table 4) would produce fix enough CO\(_2\) for the cell to replicate every 8 to 21 hr, which is similar to the division times of cyanobacteria.

### Concentration profiles of CO\(_2\) and HCO\(_3^-\) across the cell

At \(j_c = 0.7\) cm/s, varying the carboxysome permeability changes how the available inorganic carbon is partitioned between the carboxysome and cytosol, thereby setting the carboxysomal CO\(_2\) concentration as shown in Figure 4. Strikingly, the HCO\(_3^-\) concentration is constant across the cytosol. This is because the cell membranes have low permeability to HCO\(_3^-\); thus, the rate of escape is slow and HCO\(_3^-\) equilibrates across the cell. A consequence of this flat HCO\(_3^-\) profile is that the carboxysome experiences the same HCO\(_3^-\) concentration, independent of its position in the cell. This means the incoming inorganic carbon source for the carboxysome system is invariant with the position of the carboxysome in the cell.

In contrast, there is a gradient in CO\(_2\) concentration across the cell when the carboxysome permeability is at or above the optimum (Figure 4C, D). The cell membrane is more permeable to CO\(_2\). The gradient means that the CO\(_2\) leakage out of the cell affects the CO\(_2\) leakage out of the carboxysome. Moving the carboxysome close to the cell membrane increases the leakage rate of CO\(_2\) out of the carboxysome. Notably, in *S. elongatus* the carboxysomes are located along the center line of the cell, away from the cell membranes (Savage et al., 2010). The spatial profiles of HCO\(_3^-\) and CO\(_2\) give no hint as to why the carboxysomes are spaced apart from one another. Since the gradient in HCO\(_3^-\) is flat, there is no competition between the carboxysomes for HCO\(_3^-\) (the main incoming source of

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**Table 4. Fate of carbon brought into the cell for \(j_c = 0.07\) cm/s and \(k_c = 6 \times 10^{-3}\) cm/s**

| formula | pmol (cell s) | % of flux |
|---------|---------------|-----------|
| HCO\(_3^-\) transport | \(j_{H\text{out}}\) | 3.3 \(\times 10^{-5}\) |
| HCO\(_3^-\) leakage | \(k_c^\text{H\text{out}} (H_{\text{out}} - H_{\text{cytosol}} (R_b))\) | 3.2 \(\times 10^{-5}\) | 95.3 |
| CO\(_2\) leakage | \(k_c^\text{C\text{out}} (C_{\text{out}} - C_{\text{cytosol}} (R_b))\) | 9 \(\times 10^{-7}\) | 2.8 |
| carboxylation | \(V_{\text{fix,C}} \frac{C + K_m^{\text{1+O,C}}}{C + K_m^{\text{1+O,C}}}\) | 6 \(\times 10^{-7}\) | 1.7 |
| oxygenation | \(V_{\text{fix,O}} \frac{C}{C + K_m^{\text{1+C,O}}}\) | 2 \(\times 10^{-8}\) | 0.07 |

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inorganic carbon). In fact, since the local concentration of CO$_2$ is higher near a carboxysome, nearby carboxysomes would “feed” each other CO$_2$. As has been shown, such clumping would reduce the probability of distributing carboxysomes equitably to daughter cells, possibly countering any benefit (Savage et al., 2010).

The concentration in the carboxysome is basically constant, because the carboxysome is so small that diffusion across it takes very little time. A consequence of this is that the organization of the reactions in the carboxysome does not effect the CO$_2$ concentration in the carboxysome (Figure 3—figure supplement 1). Therefore, the localization of the carbonic anhydrase to the inner carboxysome shell seems to have no effect on the CCM. It has been suggested that diffusion in the carboxysome should be slower, since the carboxysome is packed with RuBisCO. One proposed consequence of slower diffusion in the carboxysome is that it could trap CO$_2$, making a low carboxysome permeability unnecessary. We have tested this hypothesis (Figure 2—figure supplement 1), and find that assuming the diffusion constant one would expect for small molecules in a 60% sucrose solution ($D_s = 10^{-7}$ cm$^2$/sec), does not reduce the optimal carboxysome permeability. However, for any carboxysome permeability a higher HCO$_3^−$ transport rate is needed to achieve the same carboxosomal CO$_2$ concentration. So, if the diffusion is slower in the carboxysome it does not aid the CCM. Even at this slower diffusion, the CO$_2$ concentration across the carboxysome is flat.

**Benefit of CO$_2$ to HCO$_3^−$ conversion: facilitated uptake or scavenging of CO$_2$**

We investigate the effect of CO$_2$ to HCO$_3^−$ conversion at the thylakoid and cell membranes (combined in the model). Increasing conversion, $\alpha > 0$, can facilitate uptake of CO$_2$ from outside the cell and scavenge CO$_2$ escaped from the carboxysome. Facilitated uptake results in saturating both carbonic anhydrase and RuBisCO at a lower level of HCO$_3^−$ transport. Scavenging broadens the range of carboxysome permeability which will effectively separate the inorganic carbon pools in the carboxysome and outside. Scavenging decreases the concentration of CO$_2$ in the cytosol, so a more permeable carboxysome can still result in a low leakage rate of inorganic carbon out of the cell (more of the inorganic carbon in the cytosol is in the form of HCO$_3^-$ which leaks out less readily). However, neither of these effects is particularly strong in our current range of reaction rates, and cell membrane permeability (Figure 2—figure supplement 2).

The relative effects of these two mechanisms depends on the external CO$_2$ and HCO$_3^−$ concentrations. In saltwater environments the pH is near 8 and HCO$_3^−$ is the predominant inorganic carbon source. While external pH is not explicitly treated in our model, we can account for changes to pH through the external inorganic carbon concentration. To be consistent with oceanic environment, thus far we have shown results for low external inorganic carbon concentrations of $[\text{CO}_2] = 0.1$ μM and $[\text{HCO}_3^-] = 14.9$ μM. The effect of facilitated uptake, under these assumptions, is very small. In freshwater or under conditions of ocean acidification, where the pH could fall to 6 or lower, there can be a much larger proportion of CO$_2$ (>50%). Figure 5 shows the absolute contribution of HCO$_3^−$ transport, facilitated CO$_2$ uptake, and CO$_2$ scavenging for varying proportions of external CO$_2$. Even though we assume the same velocity of facilitated uptake and HCO$_3^−$ transport ($j = \frac{\alpha}{K_s} \frac{1}{\text{cm}}$), facilitated uptake contributes less because it is limited by CO$_2$ diffusion across the membrane. At the same rates of transport the facilitated uptake mechanism only contributes more than active HCO$_3^−$ if the CO$_2$ concentration is greater than 80% of external inorganic carbon. This is consistent with observations that oceanic cyanobacteria such as Prochlorococcus only seem to possess gene homologs for HCO$_3^−$ transport systems, while other freshwater and estuarine cyanobacteria have gene homologs for both constitutive (NDH-1) and inducible (NDH-1) CO$_2$ uptake systems as well as inducible HCO$_3^−$ transport systems (BiC$A$, Sbt$A$, and BCT$1$) (Price, 2011).

Scavenging only contributes significantly to total incoming HCO$_3^−$ when the carboxysome permeability is higher than optimal, Figure 5, and does not contribute significantly below our calculated upper bound of $k_s < 0.02$ cm$^{-1}$ sec$^-1$. In these ranges for carboxysome permeability, there is very little CO$_2$ leaking out of the carboxysome into the cytosol, so there is very little CO$_2$ to scavenge, Figure 5. The effect of scavenging is dependent on the cell membrane permeability to CO$_2$ and HCO$_3^−$.

Given that scavenging has no obvious effect on HCO$_3^−$ concentrations, it is reasonable to wonder why this mechanism exists at all. One might assume that scavenging prevents leakage, but if the energy
Effects of pH

Cyanobacteria must regulate pH as almost all biochemical reactions are pH sensitive. We do not attempt to model this regulation or potential pH variation within the cell, however pH may be included implicitly in a couple ways. We have already explored the effect of varying external pH, and the effects of pH on carbonic anhydrase. Cytosolic pH would have little direct effect on the CO$_2$ and HCO$_3^-$ levels since the non-enzymatic interconversion is very slow as previously discussed. The effect of internal pH could also be explored by varying the reaction rate of RuBisCO, which is pH sensitive. Varying the reaction rate of RuBisCO greatly could change the range where a non-specific

Figure 5. Size of the HCO$_3^-$ flux in one cell from varying sources, as the proportion of CO$_2$ to HCO$_3^-$ outside the cell changes changes. We show results for three carboxysome permeabilities, $k_s$, and only the scavenging is effected. Total external inorganic carbon is 15 µM, $j_c = 1 \text{ cm}^3 \text{s}^{-1}$ and $\alpha = 1 \text{ cm}^3 \text{s}^{-1}$. When the carboxysome permeability is larger than optimal, $k_s = 1 \text{ cm}^3 \text{s}^{-1}$, scavenging can contribute more than facilitated uptake at low external CO$_2$ concentrations. However, when the carboxysome permeability at or below our geometric bound, $k_s \leq 0.02 \text{ cm}^3 \text{s}^{-1}$, scavenging is negligibly small. Unless there is very little HCO$_3^-$ in the environment, HCO$_3^-$ transport seems to be more efficient than CO$_2$ facilitated uptake.

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to bring a ‘new’ HCO$_3^-$ molecule from outside the cell is the same as the energy required to save an ‘old’ CO$_2$ molecule from leaking out, there is no obvious advantage of preventing the leakage. It is possible that since the scavenging mechanism is associated with the electron transport chain of the light reactions of photosynthesis scavenging can be ramped up more easily when there is excess light energy. If this were the case, a comparison of $j_c = 1 \text{ cm}^3 \text{s}^{-1}$ and $\alpha = 1 \text{ cm}^3 \text{s}^{-1}$ is deceiving and $\alpha$ could be much larger. It has been suggested that the cell uses this mechanism as a way to dissipate excess light energy (Tchernov et al., 1997, 2003).

Cellular organization

The most striking aspect of the CCM is the way that spatial organization is used to increase the efficacy of the reactions. Figure 6 compares the effect of different enzymatic reaction organizations. Concentrating carbonic anhydrase and RuBisCO to a small region in the center of the cell, on a scaffold for example, leads to an order of magnitude increase in the concentration of CO$_2$. Localizing the carbonic anhydrase to a small volume concentrates it, increasing the maximum reaction rate per volume, $V_a$, and $V_{ba}$. A larger $V_a$ increases the HCO$_3^-$ concentration at which carbonic anhydrase is saturated allowing the mechanism to take advantage of a larger HCO$_3^-$ flux, $j_c$. A small increase can be gained from encapsulating the enzymes in a permeable carboxysome shell and another order of magnitude is gained at the optimal permeability. At optimal carboxysome permeability, the CO$_2$ is effectively partitioned into the carboxysome and conversion can act only as facilitated uptake as shown in Figure 5.

Another advantage of localizing the enzymes in a small region at the center of the cell is separating carbonic anhydrase from the α (CO$_2$ to HCO$_3^-$) conversion mechanism, preventing a futile cycle. The futile cycle is most detrimental when the enzymes are distributed throughout the cytosol, and increases the oxygenation error rate by 10% (data not shown). Concentrating the enzymes away from the cell and thylakoid membranes, where conversion happens, removes this effect. With a carboxysome or scaffold the oxygenation rate is almost exactly the same with and without the α conversion mechanism. This is consistent with the previously shown detrimental effect of having active carbonic anhydrase free within the cytosol (Price and Badger, 1989). It would be impossible to keep the cytosol completely free from carbonic anhydrase enzyme, so there must be a way of activating it within the carboxysome only. Carbonic anhydrase is inactivated under reducing conditions (Peña et al., 2010). Recently it was shown that carboxysomes oxidize after assembly, providing a way to keep carbonic anhydrase inactive until fully enclosed in a carboxysome (Chen et al., 2013).
Carboxysome permeability can increase the concentration of CO$_2$ in the carboxysome. It would be unexpected that the RuBisCO rate be much faster than we assume, as we have assumed a rate on the high end. A lower RuBisCO rate would increase the range of effective carboxysome permeabilities. As previously mentioned it has been hypothesized that the CO$_2$ facilitated uptake mechanism functions by creating local alkaline pockets. Diffusion of hydrogen ions across the cell would be very fast, so it could be very difficult to maintain local alkalinity. Whether such pH gradients are possible, is certainly a subject of future interest.

**Conclusions**

We have described and analyzed a model for the CO$_2$ concentrating mechanism in cyanobacteria. There exists a broad range of HCO$_3^-$ transport and carboxysome permeability values which result in effective CO$_2$ concentration in the carboxysome. This effective concentration parameter space is defined by CO$_2$ levels high enough to saturate RuBisCO and produce a favorable ratio of carboxylation to oxygenation reactions, but not so high as to saturate carbonic anhydrase (after which increasing HCO$_3^-$ transport will not increase the CO$_2$ concentration). An optimal, non-specific carboxysome permeability exists, where HCO$_3^-$ diffusion into the carboxysome is not substantially inhibited, but CO$_2$ leakage is minimal. HCO$_3^-$ concentrations across the cell are flat and are predominately set by the transport rate in, and leakage out. We quantitatively compare the transport rates and concentrations we predict in our optimal parameter space, and find them to be in good agreement with experiment. We also comment on the effects of external pH on CO$_2$ versus HCO$_3^-$ uptake mechanisms. Finally we describe the cumulative benefits of co-localization, encapsulation, and optimal carboxysome permeability on the CCM.

Further comparison of this model to experimental flux measurements, especially to determine the quantitative contributions of different transporters under different physiological conditions would be very interesting. Current solutions are for steady state at constant external concentration, but most gas exchange measurements, by necessity, measure the fluxes as the inorganic carbon is depleted in the media. The model could be modified to solve the time dependent problem with varying external inorganic carbon. As of yet the carboxysome permeability has not been measured directly, and it would be quite interesting to see how close it is to our ‘optimal’ prediction.

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Additional files

Supplementary file
- Supplementary file 1. Mathematical derivation appendix. Mathematical derivations of analytic solutions for a spherical cell with reactions organized in a variety of ways. We present analytic solutions for the concentration of CO₂ and HCO₃⁻ a carboxysome located at the center of the cell. We derive analytic solutions assuming a number of different cases for the enzymatic rates in the carboxysome: RuBisCO reaction rate negligible with carbonic anhydrase saturated and unsaturated, RuBisCO reaction rate non-negligible with carbonic anhydrase unsaturated. Additionally we derive analytic solutions for the enzymatic reactions throughout the cell and localized to a scaffold without a carboxysome shell.

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