

# Parameter Sensitivity

Niall Mangan

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## Abstract

## 1 Parameter Sensitivity

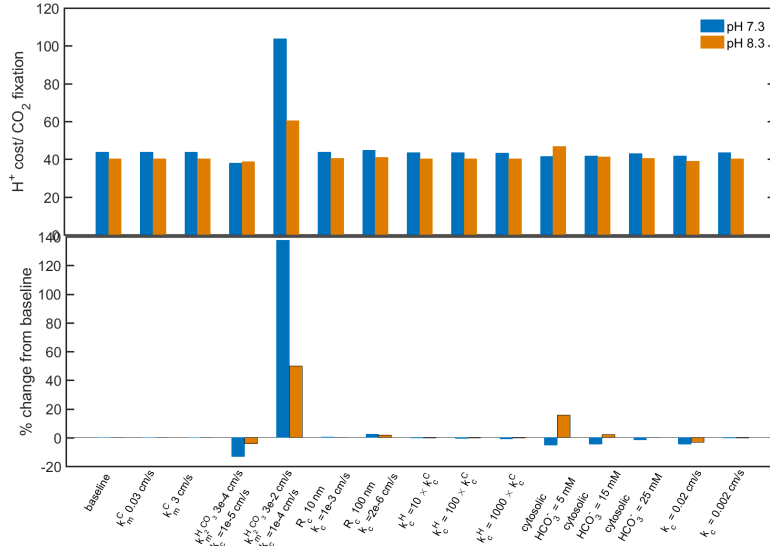


Figure 1: Cost and relative change from baseline for varying different parameters. For some parameters the carboxysome permeability is also changed to remain at optimal carboxysome permeability. Otherwise the main effect would be the shift away from optimum permeability.

We can completely analyze the behavior of the CCM system in terms of multiple parameters through analysis of the analytic solutions. However, to give intuition for which parameters are important near our current parameter regime we provide a sensitivity analysis of the cost of the CCM at cytosolic pH 7.3, the

measured value in the light, and pH 8.3, the measured value in the dark. We find that for all the parameters tested the total cost of the CCM is always higher at pH 7.3 than pH 8.3. However the change from baseline was not necessarily the same at pH 7.3 vs 8.3.

The sensitivity analysis also found that order of magnitude changes in the cell membrane permeability to  $\text{CO}_2$  and increasing the permeability of the carboxysome to  $\text{HCO}_3^-$  by a ratio of 10, 100, or 1000 compared to the permeability to  $\text{CO}_2$  have  $< 1\%$  change from baseline. Changing the target inorganic carbon pool (cytosolic  $\text{HCO}_3^-$ ) from 30 to 5, 15 and 25 mM has between 5 and 20 % change at pH 7.3 a  $< 2\%$  change from the pH = 8.3 baseline.

Changing the membrane permeability to  $\text{HCO}_3^-$  has a much more dramatic effect on the cost, even if we re-optimize the permeability for each case. Increasing the permeability of the carboxysome to all inorganic carbon also has a very large effect. Finally we test the sensitivity of our analysis to a 2-fold decrease and increase in the cost of each  $\text{HCO}_3^-$  transport.

Next we use the analytic solutions for the CCM system when RuBisCO is saturated to further understand the parameter dependence.

## 2 Equation when RuBisCO is saturated

The analytic solution for the  $\text{CO}_2$  and  $\text{HCO}_3^-$  concentration in the carboxysome when RuBisCO is saturated is:

$$C_{\text{carboxysome}} = \frac{N}{M} - \frac{R_c^3 V_{\text{max}} P}{3MD} \quad (1)$$

$$H_{\text{carboxysome}} = K_{eq}(pH)C_{\text{carboxysome}} \quad (2)$$

where,

$$N = (j_c + k_m^{\text{eff}}(pH_{\text{out}}))H_{\text{out}}((k_m^C + \alpha)G^C + \frac{D}{R_b^2}) + k_m^C C_{\text{out}}(k_m^{\text{eff}}G^H + \alpha G^C + \frac{D}{R_b^2}) \quad (3)$$

$$M = K_{eq} * k_m^{\text{eff}} \left( (\alpha + k_m^C)G^C + \frac{D}{R_b^2} \right) + k_m^C \left( k_m^{\text{eff}}G^H + \frac{D}{R_b^2} \right) + \alpha k_m^{\text{eff}}G^H \quad (4)$$

$$P = ((\alpha + k_m^C)G^C + \frac{D}{R_b^2})(k_m^{\text{eff}}G^H + \frac{D}{R_b^2}) \quad (5)$$

$$G^C = \frac{D}{R_c^2 k_c^C} + \frac{1}{R_c} - \frac{1}{R_b} \quad (6)$$

$$G^H = \frac{D}{R_c^2 k_c^H} + \frac{1}{R_c} - \frac{1}{R_b} \quad (7)$$

The derivation of this equation can be found in the supplementary material of (Mangan 2014). Here we have made a few modifications: (1) kept track of the carboxysome permeability to  $\text{CO}_2$ ,  $k_c^C$ , and  $\text{HCO}_3^-$ ,  $k_c^H$ , independently, (2)

substituted the pH dependent equilibrium constant for the carbonic anhydrase reaction,  $K_{eq}(ph) = \frac{V_{ca}K_{pa}}{V_{ba}K_{ca}}$ , (3) written the  $\text{CO}_2 \rightarrow \text{HCO}_3^-$  reaction with  $\alpha$  as the linear reaction rate (in Mangan 2014 the linear rate was  $\alpha/K_\alpha$ ), (4) we have replaced the membrane permeability to  $\text{HCO}_3^-$  with the effective membrane permeability to the bicarbonate pool, and designated when is dependent on the external pH,  $k_m^{eff}(pH_{out})$ . This term only appears once in equation 3 for  $N$ . For all other  $k_m^{eff} = k_m^{eff}(pH_{in})$  values it is dependent on the pH inside the cell, so we have dropped indicating the pH dependence to simplify the formulas.

### 3 Detailed analysis on the effect of cell membrane permeability on the CCM

#### 3.1 Cell membrane permeability compared to diffusive velocities

Examining equations (6) we note that for large carboxysome permeability  $1/R_c$  will be the dominant term, and for smaller carboxysome permeability values the first term will be larger and dominate. Therefore  $G^C \geq 1/R_c$ . Studying the equations we note that the terms  $((\alpha + k_m^C)G^C + \frac{D}{R_b^2})$  appears repeatedly. We use the following argument:

$$\begin{aligned} (\alpha + k_m^C)G^C &\geq (\alpha + k_m^C)/R_c \gg D/R_b^2, \\ \text{if } (\alpha + k_m^C) &\gg DR_c/R_b^2 \end{aligned} \quad (8)$$

For even a small 20 nm diameter ( $R_c = 10^{-6}$  cm) carboxysome this will hold as  $k_m^C \approx 0.3$  cm/s and  $DR_c/R_b^2 = 4 \times 10^{-3}$  cm/s from the values in Table S1. So the membrane permeability to  $\text{CO}_2$  could be an order of magnitude too high in our model and this would still be a reasonable assumption. Therefore we will substitute

$$(\alpha + k_m^C)G^C + D/R_b^2 \approx (\alpha + k_m^C)G^C. \quad (10)$$

Inserting this into equations (1-5) we get

$$\begin{aligned} C_{carboxysome} = & \frac{(j_c + k_m^{eff}(pH_{out}))H_{out}(k_m^C + \alpha)G^C + k_m^C C_{out}(k_m^{eff}G^H + \alpha G^C + \frac{D}{R_b^2})}{K_{eq}k_m^{eff}(\alpha + k_m^C)G^C + k_m^C \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \alpha k_m^{eff}G^H} \\ & - \frac{R_c^3 V_{max}(\alpha + k_m^C)G^C(k_m^{eff}G^H + \frac{D}{R_b^2})/(3D)}{K_{eq}k_m^{eff}(\alpha + k_m^C)G^C + k_m^C \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \alpha k_m^{eff}G^H}. \end{aligned} \quad (11)$$

We can divide through by  $(k_m^C + \alpha)$  to obtain:

$$C_{carboxysome} = \frac{(j_c + k_m^{eff}(pH_{out}))H_{out}G^C + \frac{k_m^C}{(k_m^C + \alpha)}C_{out}(k_m^{eff}G^H + \alpha G^C + \frac{D}{R_b^2})}{K_{eq}k_m^{eff}G^C + \frac{k_m^C}{(k_m^C + \alpha)}\left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{\alpha}{(k_m^C + \alpha)}k_m^{eff}G^H} - \frac{R_c^3V_{max}G^C(k_m^{eff}G^H + \frac{D}{R_b^2})/(3D)}{K_{eq}k_m^{eff}G^C + \frac{k_m^C}{(k_m^C + \alpha)}\left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{\alpha}{(k_m^C + \alpha)}k_m^{eff}G^H}. \quad (12)$$

We now want to examine the remaining terms in the membrane permeability to  $\text{CO}_2$ ,  $k_m^C$ .

### 3.2 Membrane permeability to $\text{CO}_2$ has little effect.

There are two parameter groupings in equation (12) containing  $k_m^C$ :

$$\frac{k_m^C}{k_m^C + \alpha} \quad (13)$$

$$\frac{\alpha}{k_m^C + \alpha} \quad (14)$$

Therefore if  $k_m^C > \alpha$  or  $\text{CO}_2 \rightarrow \text{HCO}_3^-$  conversion is negligible the first term (13) reduces to 1, and the second reduces to  $1/k_m^C$ . We will return to the case where this conversion is not negligible later.

With these two simplifications we obtain:

$$C_{carboxysome} = \frac{(j_c + k_m^{eff}(pH_{out}))H_{out}G^C + C_{out}(k_m^{eff}G^H + \alpha G^C + \frac{D}{R_b^2})}{K_{eq}k_m^{eff}G^C + \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{1}{k_m^C}k_m^{eff}G^H} - \frac{R_c^3V_{max}G^C(k_m^{eff}G^H + \frac{D}{R_b^2})/(3D)}{K_{eq}k_m^{eff}G^C + \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{1}{k_m^C}k_m^{eff}G^H}. \quad (15)$$

Examining equation (15), note that the only appearance of the membrane permeability to  $\text{CO}_2$  is now in the denominator which we can rewrite as  $k_m^{eff}(G^C K_{eq} + \frac{G^H}{k_m^C}) + \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right)$ . Using this equation, we can write a strong bound on when the membrane permeability will effect the function of the CCM.

We find  $k_m^C$  has no significant effect when  $K_{eq}G^C > \frac{G^H}{k_m^C}$  or  $k_m^C > \frac{G^H}{G^C K_{eq}}$ . If we assume that the carboxysome permeability to  $\text{CO}_2$  will always be smaller than or equal to the permeability to  $\text{HCO}_3^-$  ( $k_c^C \geq k_c^H$ ) then  $G^H \geq G^C$  and  $\frac{G^H}{G^C} \leq 1$ , so  $k_m^C$  will be negligible as long as  $k_m^C > 1/K_{eq}$ . For  $\text{pH} > 6.6$ ,  $1/K_{eq} > 0.3$  and therefore the assumed value of  $k_m^C = 0.3$  will be negligible. However, if the cell operated in a lower pH regime and the membrane permeability was substantially lower to  $\text{CO}_2$  it would begin to effect the  $\text{CO}_2$  concentration.

Thus far we have made a series of observations about the size of terms compared to the membrane permeability to  $\text{CO}_2$  and found that when  $(\alpha +$

$k_m^C) \gg DR_c/R_b^2$ ,  $k_m^C > \alpha$  and  $k_m^C > \frac{G^H}{G^C K_{eq}} \approx 1/K_{eq}$  the  $\text{CO}_2$  concentration in the carboxysome reduces to

$$C_{\text{carboxysome}} = \frac{(j_c + k_m^{\text{eff}}(pH_{\text{out}}))H_{\text{out}}G^C + C_{\text{out}}(k_m^{\text{eff}}G^H + \alpha G^C + \frac{D}{R_b^2})}{k_m^{\text{eff}}(G^C K_{eq} + G^H) + \frac{D}{R_b^2}} - \frac{R_c^3 V_{\text{max}} G^C (k_m^{\text{eff}}G^H + \frac{D}{R_b^2})/(3D)}{k_m^{\text{eff}}(G^C K_{eq} + G^H) + \frac{D}{R_b^2}}. \quad (16)$$

We can make a similar argument taking the equation for the  $\text{CO}_2$  concentration at the cell membrane:

$$C_{\text{cytosol}}(r = R_b) = \frac{k_m^C C_{\text{out}} - (\alpha + k_m^C) C_{\text{carboxysome}}}{(\alpha + k_m^C) G^C + D/R_b^2} G^C + C_{\text{carboxysome}} \approx C_{\text{out}} \quad (17)$$

This means that the  $\text{CO}_2$  leakage term will be negligible since the cytosolic  $\text{CO}_2$  concentration will be approximately equal to the external  $\text{CO}_2$  concentration. The  $\text{HCO}_3^-$  transport required to sustain a given internal inorganic carbon pool will then be:

$$j_c H_{\text{out}} = \left( \frac{R_c^3}{3R_b^2} V_{\text{max}} - k_m^C (C_{\text{out}} - C_{\text{cytosol}}) - k_m^{\text{eff}} H_{\text{out}} + k_m^{\text{eff}} H_{\text{cytosol}} \right) = \left( \frac{R_c^3}{3R_b^2} V_{\text{max}} - k_m^{\text{eff}} H_{\text{out}} + k_m^{\text{eff}} H_{\text{cytosol}} \right) \quad (18)$$

We can calculate  $H_{\text{carboxysome}} = K_{eq} C_{\text{carboxysome}}$  from equation (17), and is therefore also independent of  $k_m^C$ . In previous work we showed that

$$H_{\text{cytosol}} = \frac{(j_c + k_m^{\text{eff}}(pH_{\text{out}}))H_{\text{out}} + \frac{\alpha}{K_{eq}} C_{\text{cytosol}}(r = R_b) - k_m^{\text{eff}} H_{\text{carboxysome}}}{k_m^{\text{eff}} G^H + \frac{D}{R_b^2}} G^H \quad (19)$$

We have now shown that all the terms in  $H_{\text{cyto}}$  are negligibly dependent on the membrane permeability to  $\text{CO}_2$ . Therefore, the  $\text{HCO}_3^-$  transport level require to satisfy equation (18) is independent of the membrane permeability to  $\text{CO}_2$ . This observation is consistent with the low flux of  $\text{CO}_2$  leakage in Figure 2.

### 3.3 Without facilitated $\text{CO}_2$ uptake external $\text{CO}_2$ has little effect

Unless conversion from  $\text{CO}_2$  to  $\text{HCO}_3^-$  is large we note that the second  $C_{\text{out}}$  term in equation(15) is negligible for the regimes we study. We will revisit

CO<sub>2</sub> uptake and recycling later. Comparing this term against the first term in the numerator, again allows us to put a quantitative description on when this regime holds. Additionally we find that when the transport of HCO<sub>3</sub><sup>-</sup> is significant ( $j_c > k_m^{eff}(pH_{out})$ ) we arrive at

$$C_{carboxysome} = \frac{j_c H_{out} G^C - R_c^3 V_{max} G^C (k_m^{eff} G^H + \frac{D}{R_b^2}) / (3D)}{k_m^{eff} (G^C K_{eq} + G^H) + \frac{D}{R_b^2}} \quad (20)$$

$$H_{carboxysome} = K_{eq} C_{carboxysome} \quad (21)$$

## 4 Effect of Carboxysome permeability

Recalling the equation for  $G^C = \frac{D}{R_c^2 k_c^C} + \frac{1}{R_c} - \frac{1}{R_b}$ , we can see that the carboxysome permeability to CO<sub>2</sub> will only matter if  $\frac{D}{R_c^2 k_c^C} > \frac{1}{R_c}$ . In other words the carboxysome permeability to CO<sub>2</sub>,  $k_c^C$ , begins to effectively trap CO<sub>2</sub> in the carboxysome when  $k_c^C < \frac{D}{R_c} \approx 2$  cm/s for our base case of a 100 nm carboxysome ( $R_c = 50$  nm). Similarly  $G^H \approx \frac{D}{R_c^2 k_c^H}$  when  $k_c^H < \frac{D}{R_c}$ . As common thinking is that  $k_c^H \geq k_c^C$ ,  $k_c^H < \frac{D}{R_c}$  may not always hold when  $k_c^C < \frac{D}{R_c}$ .

### 4.0.1 Different carboxysome permeability for HCO<sub>3</sub><sup>-</sup>

An existing hypothesis in the CCM literature is that the carboxysome has differential permeability and is more permeable to HCO<sub>3</sub><sup>-</sup> and less permeable to CO<sub>2</sub>. Intuitively this would allow more HCO<sub>3</sub><sup>-</sup> into the carboxysome and trap more CO<sub>2</sub>, thereby accumulating more inorganic carbon in the form of CO<sub>2</sub>. We use our model to test whether differential carboxysome permeability enables higher carboxysomal CO<sub>2</sub> concentration for the same level of HCO<sub>3</sub><sup>-</sup> transport. In the following figure we show the  $k_c$  vs  $j_c$  phase space where we have plotted the carboxysome permeability to CO<sub>2</sub>,  $k_c^C$ , on the y-axis. We plot different ratios (1, 10, 100, 1000) between  $k_c^C$  and the carboxysome permeability to HCO<sub>3</sub><sup>-</sup>,  $k_c^H = \text{ratio} \times k_c^C$ .

Examining Figure XX, we see that making the carboxysome more permeable to HCO<sub>3</sub><sup>-</sup> does not improve the function of the CCM as drastically as one might assume. The "turn on" of CO<sub>2</sub> accumulation with decreasing permeability is unaffected by changes to  $k_c^H$ , and depends only on the permeability to CO<sub>2</sub>,  $k_c^C$ . The "turn off" of accumulation for lower carboxysome permeabilities is greatly affected by the permeability of the carboxysome to HCO<sub>3</sub><sup>-</sup>,  $k_c^H$ . These two effects are exactly what we previously discussed as defining the carboxysome permeability optimum.

As we start at the top of the y-axis and decrease the carboxysome permeability the following occurs: At high permeability not enough CO<sub>2</sub> is trapped, but HCO<sub>3</sub><sup>-</sup> enters readily. As we moved to lower permeabilities CO<sub>2</sub> begins to be trapped, but there is a window where HCO<sub>3</sub><sup>-</sup> still enters enough to supply the system. Eventually the carboxysome begins to restrict HCO<sub>3</sub><sup>-</sup> entry. If the

### Effect of increasing differential carboxysome permeability to $\text{HCO}_3^-$

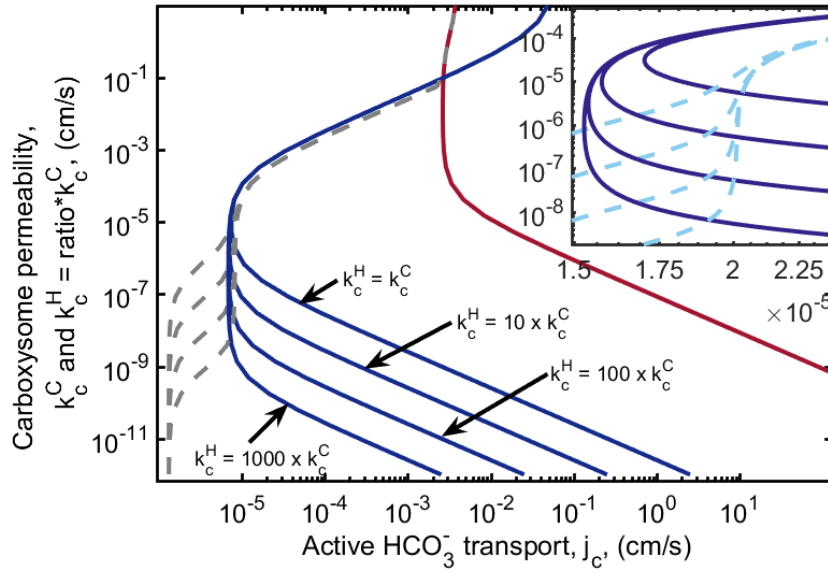


Figure 2: Increasing the carboxysome permeability to  $\text{HCO}_3^-$  relative to  $\text{CO}_2$  mostly effects the required transport rates for  $\text{CO}_2$  carboxysome permeabilities below the optimum. Inset shows that there is a small decrease in necessary flux near the optimum.

carboxysome is more permeable to  $\text{HCO}_3^-$  than to  $\text{CO}_2$  then the window where  $\text{CO}_2$  trapping is effective without restricting  $\text{HCO}_3^-$  entry broadens. The width of this window (on the y-axis) will also depend strongly on how much of the  $\text{CO}_2$  is being fixed.

The "turn off" of the optimum, caused by not allowing enough  $\text{HCO}_3^-$  into the carboxysome, does slightly increase the amount of transport required to saturate RuBisCO at the carboxysome optimum. The reduction in transport required, and therefore CCM cost is around 5% when going from a  $k_c^C$  to  $k_c^H$  ratio of 1 to 1000.

## 5 Effect of membrane permeability to $\text{H}_2\text{CO}_3$

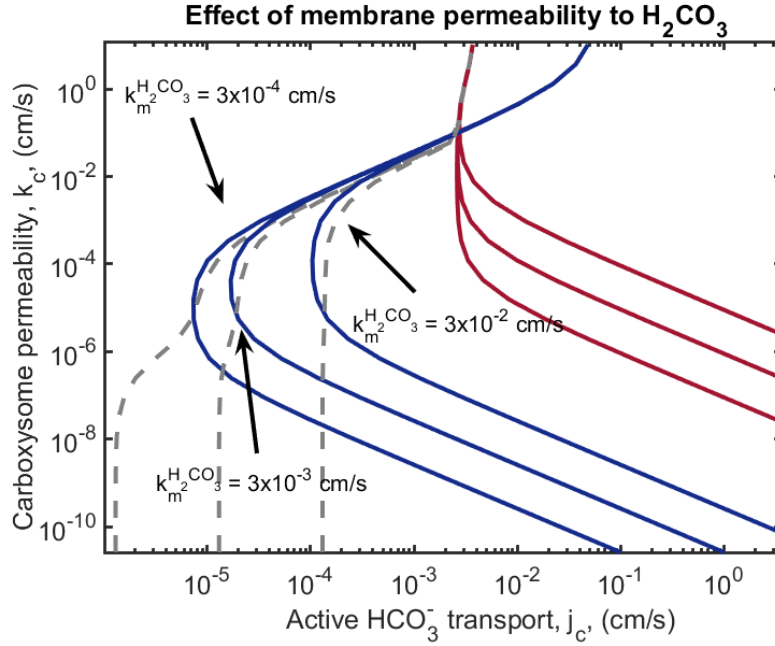


Figure 3: CCM functionality space for varying membrane permeability to  $\text{H}_2\text{CO}_3$ ,  $k_m^{\text{H}_2\text{CO}_3}$ . Blue lines indicate where RuBisCO is saturated. Red lines indicate where carbonic anhydrase is saturated. Grey dashed lines indicate where the  $\text{HCO}_3^-$  pool in the cytosol is 30mM. Each set of lines correspond to values  $k_m^{\text{H}_2\text{CO}_3} = 3 \times 10^{-4}, 3 \times 10^{-3}, 3 \times 10^{-2}$  from right to left.

The sensitivity of the cost to our assumption for the value of the membrane permeability to  $\text{H}_2\text{CO}_3$  can be determined from the equation derived previously. If we are in a regime where  $\text{CO}_2$  leakage is negligible, as is the regime presented



in the main paper, the second line holds.

$$\begin{aligned} j_c H_{out} &= \left( \frac{R_c^3}{3R_b^2} V_{max} - k_m^C (C_{out} - C_{cytosol}) - k_m^{eff} (pH_{out}) H_{out} + k_m^{eff} (pH_{in}) H_{cytosol} \right) \\ &= \left( \frac{R_c^3}{3R_b^2} V_{max} - k_m^{eff} (pH_{out}) H_{out} + k_m^{eff} (pH_{in}) H_{cytosol} \right) \quad (22) \end{aligned}$$

In this equation  $k_m^{eff} = k_m^{H_2CO_3} \times 10^{(pK_1 - pH)}$ . Therefore, the leakage of  $H_{total}$  out of the cell will depend linearly on what we assume for  $k_m^{H_2CO_3}$ . This linear dependence is passed on to the active  $HCO_3^-$  transport required to replenish the leaked inorganic carbon, and therefore onto the CCM cost. In Figure XX you can see this effect, where going from  $k_m^{H_2CO_3} = 3 \times 10^{-2}$  to  $k_m^{H_2CO_3} = 3 \times 10^{-3}$  (an order of magnitude change), decreases the active  $HCO_3^-$  transport needed by an order of magnitude. Decreasing to  $k_m^{H_2CO_3} = 3 \times 10^{-4}$  is a little less than an order of magnitude, indicating that the linear dependence breaks down and  $CO_2$  leakage would become important for that value. There is also an order of magnitude change in the optimal carboxysome permeability from  $10^{-4}$  to  $10^{-5}$  across the 2 order of magnitude change in  $k_m^{H_2CO_3}$  we are checking.

## 6 Selection and effect of cytosolic $HCO_3^-$ pool size

The  $HCO_3^-$  cytosolic pool we assume in our cost calculation has a large effect on the absolute values for the cost calculation. The dependence of  $HCO_3^-$  transport required to support a given internal cytosolic pool can also be seen in the equation in the previous section.

In Figure XX the active  $HCO_3^-$  transport and carboxysome permeability values required to achieve a particular cytosolic pool are shown. For the RuBisCO half-max values assumed in the main text  $K_m = 276 \mu M$  at internal pH 8, internal  $HCO_3^-$  cytosolic pools between 20 and 50 mM are required to saturate RuBisCO. For lower  $K_m$  values, lower cytosolic pools would be required. Additionally, as the  $K_m$  values are pH dependent, the internal pH of the cell will effect when saturation takes place.

It has recently been suggested that cytosolic  $HCO_3^-$  pools of around 5-10 mM can saturate RuBisCO. As was discussed in Whitehead et al., this is only possible with our given understanding of the CCM mechanism if the pH in the carboxysome is lower than the rest of the cell, or if the carbonic anhydrase does not act to bring  $CO_2$  and  $HCO_3^-$  into equilibrium. Either of these possibilities seems physically questionable given our current understanding of the diffusion rate of protons and the mechanism of carbonic anhydrase – its speed is considered linked to its lack of directionality.

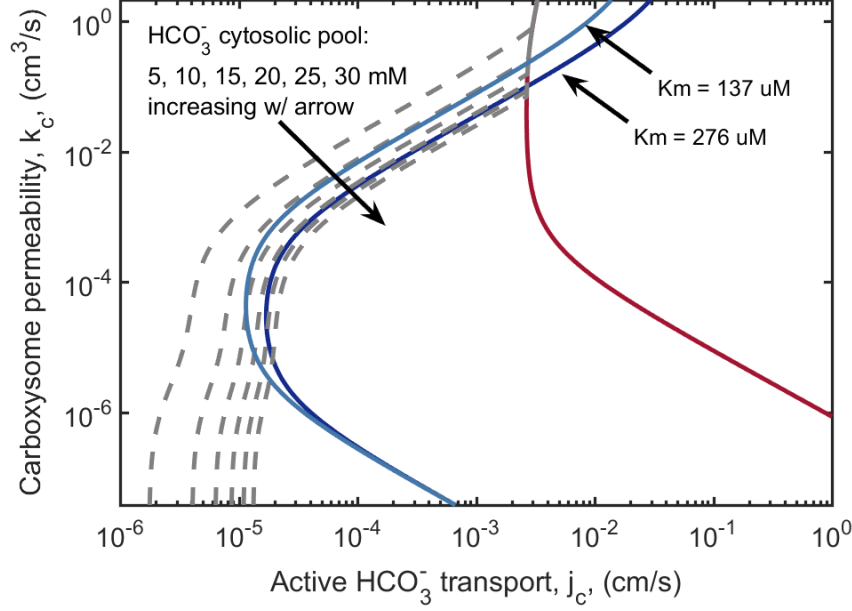


Figure 4: CCM functionality space showing carboxysome permeability and  $\text{HCO}_3^-$  transport rates resulting in varying cytosolic  $\text{HCO}_3^-$  pool. Grey dashed lines indicate where the  $\text{HCO}_3^-$  pool in the cytosol is 5, 10, 15, 20, 25, and 30 mM. Blue lines indicate where RuBisCO is saturated. Red lines indicate where carbonic anhydrase is saturated.

## 7 When can $\text{CO}_2$ scavenging have an effect

Next we examine for which parameter regimes  $\text{CO}_2 \rightarrow \text{HCO}_3^-$  conversion activity acts as facilitated uptake, resulting in a flux of  $\text{CO}_2$  into the cell, or scavenging, reducing the leakage of  $\text{CO}_2$  out of the cell. As written, the conversion activity does not discriminate between  $\text{CO}_2$  that has recently diffused into the cell and  $\text{CO}_2$  which has already been in the carboxysome, but leaked back out. In other words, we do not "trace" the history of each  $\text{CO}_2$  molecule, or even model each  $\text{CO}_2$  molecule explicitly as we treat concentrations. Therefore, we must think about the net  $\text{CO}_2$  flux at the cell membrane.

When there is a net flux of  $\text{CO}_2$  out of the cell, there is by definition, no facilitated uptake. If there is zero net flux of  $\text{CO}_2$  at the cell membrane, then the cell is scavenging 100% of the  $\text{CO}_2$  leaking out of the carboxysome. If there is net flux of  $\text{CO}_2$  into the cell, then not only is the conversion mechanism scavenging all  $\text{CO}_2$  leaking out of the cell, but also reducing the cytosolic  $\text{CO}_2$  concentration below the external concentration and facilitating uptake.

From Figure XX it is evident that increasing  $\text{CO}_2 \rightarrow \text{HCO}_3^-$  decreases the

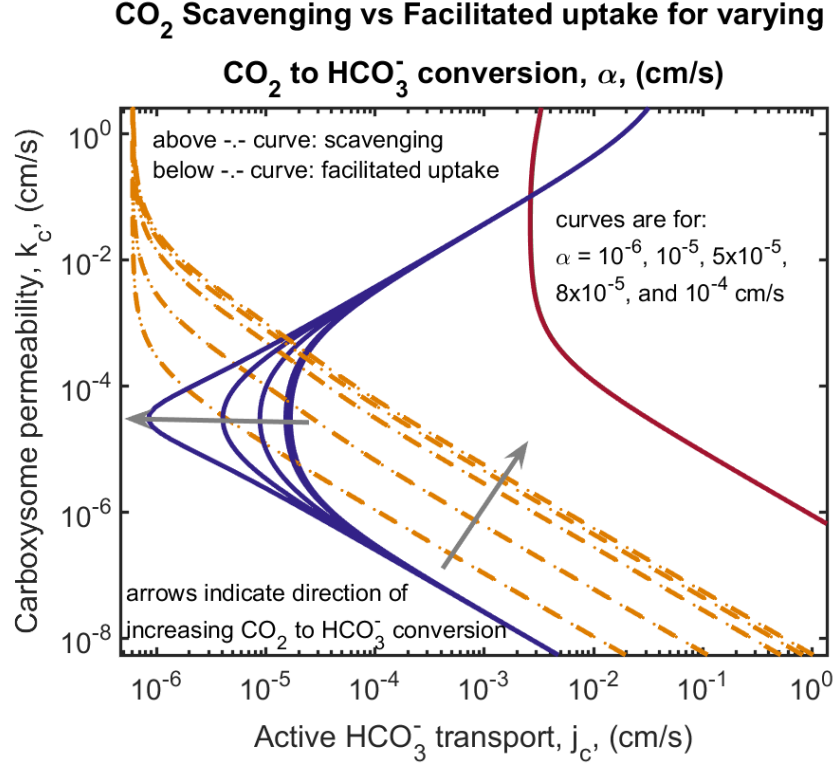


Figure 5: Effect of CO<sub>2</sub> → HCO<sub>3</sub><sup>-</sup> conversion on the active transport vs carboxysome permeability plot. RuBisCO is unsaturated to the left of the blue line and saturated to the right. Carbonic anhydrase is unsaturated to the left of the red line and saturated to the right. Orange dashed lines show where the CO<sub>2</sub> flux across the membrane is zero. Above/to the right of an orange dashed line there is net CO<sub>2</sub> leaking out of the cell, and the main function of the conversion is scavenging. Below and to the left of an orange line the net CO<sub>2</sub> flux at the cell membrane is into the cell, and conversion is both scavenging and facilitating CO<sub>2</sub> uptake. Multiple lines are for increasing CO<sub>2</sub> → HCO<sub>3</sub><sup>-</sup> conversion strength in the direction of the grey arrows.

need for active HCO<sub>3</sub><sup>-</sup> uptake at optimal carboxysome permeability. We can also determine when the conversion mechanism switches from being primarily a scavenging mechanism to also acting as facilitated uptake. The orange dashed lines show the active HCO<sub>3</sub><sup>-</sup> transport rate for each carboxysome permeability  $k_c$  value where the net CO<sub>2</sub> flux at the cell membrane is zero. Above this line the system is scavenging and below it the system is scavenging and causing facilitated uptake. We have plotted these curves for increasing CO<sub>2</sub> → HCO<sub>3</sub><sup>-</sup> conversion strengths.

For  $\alpha = 10^{-6}$  cm/s, the system is not facilitating uptake of  $\text{CO}_2$  for the optimal permeability. At this point the minimum active  $\text{HCO}_3^-$  transport rate required to saturate RuBiSCO is approximately the same as without the  $\text{CO}_2$  conversion mechanism. For higher  $\alpha$  the conversion system is scavenging at the optimal carboxysome permeability. In this case the system requires less  $\text{HCO}_3^-$  transport because  $\text{CO}_2$  conversion is acting as facilitated uptake, and contributing to the internal inorganic carbon pool. Note that the absolute flux from scavenging and facilitated uptake will vary drastically over this space.

In the main text we showed that the loss of  $\text{CO}_2$  from leakage out of the cell is about an order of magnitude smaller than  $\text{HCO}_3^-$  leakage without  $\text{CO}_2$  conversion. When we are at optimal carboxysome permeability, scavenging will have little effect, because there is just not very much  $\text{CO}_2$  in the cytosol to scavenge. At higher carboxysome permeabilities though, the scavenging mechanism could compensate and matter much more. Thus the relative importance of the  $\text{CO}_2$  conversion mechanism for scavenging vs facilitated uptake depends highly on the yet experimentally undetermined carboxysome permeability.

To identify when facilitated uptake starts, we can find for what  $\text{CO}_2$  concentration in the carboxysome the flux at the cell membrane is zero, or when  $C_{\text{cytosol}} = C_{\text{out}}$ .

$$\text{CO}_2 \text{ flux} = k_m^C (C_{\text{cytosol}}(R_b) - C_{\text{out}}). \quad (23)$$

We do not know enough about the  $\text{CO}_2 \rightarrow \text{HCO}_3^-$  mechanism to estimate a cost in the way we have done for  $\text{HCO}_3^-$  uptake. Therefore we cannot assess the effect of conversion on the cost.