

# pH determines the energetic efficiency of the cyanobacterial CO<sub>2</sub> concentrating mechanism: Mathematical Supplement

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## Abstract

## 1 Equations when RuBisCO is saturated

The analytic solution for the CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> concentration in the carboxysome when RuBisCO is saturated is:

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

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

where,

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

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

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

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

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

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 CO<sub>2</sub>,  $k_c^C$ , and HCO<sub>3</sub><sup>-</sup>,  $k_c^H$ , independently, (2) substituted the pH dependent equilibrium constant for the carbonic anhydrase

reaction,  $K_{eq}(ph) = \frac{V_{ca}K_{ba}}{V_{ba}K_{ca}}$ , (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.

## 2 Analysis of membrane permeability effects

### 2.1 Cell permeabilty compared to diffusive velocities

Examining equation (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 (3-7) we note that the terms  $((\alpha + k_m^C)G^C + \frac{D}{R_b^2})$  appears repeatedly. We use the following argument:

$$(\alpha + k_m^C)G^C \geq (\alpha + k_m^C)/R_c \gg D/R_b^2, \quad (8)$$

$$\text{if } (\alpha + k_m^C) \gg DR_c/R_b^2 \quad (9)$$

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

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

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

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

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

## 2.2 Membrane permeability to CO<sub>2</sub> 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:

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

Examining equation (15), note that the only appearance of the membrane permeability to CO<sub>2</sub> is now in the denominator which we can rewrite as  $k_m^{\text{eff}}(G^C K_{eq} + \frac{G^H}{k_m^C}) + \left(k_m^{\text{eff}}G^H + \frac{D}{R_b^2}\right)$ . 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 CO<sub>2</sub> 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 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 CO<sub>2</sub> it would begin to effect the CO<sub>2</sub> concentration.

Thus far we have made a series of observations about the size of terms compared to the membrane permeability to CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> concen-

tration at the cell membrane:

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

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:

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

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

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

We have now shown that all the terms in  $H_{cyto}$  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.

### 2.3 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  $C_{out}$  term in equation(15) is negligible for the regimes we study. We will revisit  $\text{CO}_2$  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  $\text{HCO}_3^-$  is significant ( $j_c > k_m^{eff}(pH_{out})$ ) we arrive at

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

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

### 3 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  $\text{CO}_2$  will only matter if  $\frac{D}{R_c^2 k_c^C} > \frac{1}{R_c}$ . In other words the carboxysome permeability to  $\text{CO}_2$ ,  $k_c^C$ , begins to effectively trap  $\text{CO}_2$  in the carboxysome when  $k_c^C < \frac{D}{R_c} \approx 2 \text{ cm/s}$  for our base case of a 100 nm carboxysome ( $R_c = 50 \text{ nm}$ ). 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  $k_c^C < \frac{D}{R_c}$ .

#### 3.0.1 Different carboxysome permeability for $\text{HCO}_3^-$

An existing hypothesis in the CCM literature is that the carboxysome has differential permeability and is more permeable to  $\text{HCO}_3^-$  and less permeable to  $\text{CO}_2$ . Intuitively this would allow more  $\text{HCO}_3^-$  into the carboxysome and trap more  $\text{CO}_2$ , thereby accumulating more inorganic carbon in the form of  $\text{CO}_2$ . We use our model to test whether differential carboxysome permeability enables higher carboxysomal  $\text{CO}_2$  concentration for the same level of  $\text{HCO}_3^-$  transport. In the following figure we show the  $k_c$  vs  $j_c$  phase space where we have plotted the carboxysome permeability to  $\text{CO}_2$ ,  $k_c^C$ , on the y-axis. We plot different ratios (1, 10, 100, 1000) between  $k_c^C$  and the carboxysome permeability to  $\text{HCO}_3^-$ ,  $k_c^H = \text{ratio} \times k_c^C$ .

Examining Figure XX, we see that making the carboxysome more permeable to  $\text{HCO}_3^-$  does not improve the function of the CCM as drastically as one might assume. The "turn on" of  $\text{CO}_2$  accumulation with decreasing permeability is unaffected by changes to  $k_c^H$ , and depends only on the permeability to  $\text{CO}_2$ ,  $k_c^C$ . The "turn off" of accumulation for lower carboxysome permeabilities is greatly affected by the permeability of the carboxysome to  $\text{HCO}_3^-$ ,  $k_c^H$ . 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  $\text{CO}_2$  is trapped, but  $\text{HCO}_3^-$  enters readily. As we moved to lower permeabilities  $\text{CO}_2$  begins to be trapped, but there is a window where  $\text{HCO}_3^-$  still enters enough to supply the system. Eventually the carboxysome begins to restrict  $\text{HCO}_3^-$  entry. If the 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.

## 4 Effect of membrane permeability to $H_2CO_3$

The sensitivity of the cost to our assumption for the value of the membrane permeability to  $H_2CO_3$  can be determined from the equation derived previously. If we are in a regime where  $CO_2$  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.

## 5 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.

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.