pH determines the energetic efficiency of the cyanobacterial CO2 concentrating mechanism: mathematical supplement

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1 Equations when RuBisCO is saturated

The analytic solution for the CO_2 and HCO_3^- concentration in the carboxysome when RuBisCO is saturated is:

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

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

where,

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

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

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

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

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

The derivation of this equation can be found in the supplementary material of (Mangan & Brenner, eLife 2014). Here we have made a few modifications: (1) kept track of the carboxysome permeability to CO_2, k_c^C, and HCO_3^-, k_c^H, independently, (2) substituted the pH dependent equilibrium constant for the carbonic anhydrase reaction, $K_{eq}(ph) = \frac{V_{ca}K_{ba}}{V_{ba}K_{ca}}$, (3) written the $CO_2 \rightarrow HCO_3^-$ reaction with α as the linear reaction rate (in Mangan 2014 the linear rate was α/K_α), (4) we have replaced the membrane permeability to 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:

$$(\alpha + k_m^C)G^C \geq (\alpha + k_m^C)/R_c >> D/R_b^2, \tag{8}$$

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

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 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

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

Inserting this into equations (1-5) we get

$$C_{carboxysome} = \frac{(j_c + k_m^{eff}(pH_{out}))H_{out}(k_m^C + \alpha)G^C + k_m^C C_{out}(k_m^{eff}G^H + \alpha G^C + \frac{D}{R_b^2})}{K_{eq}k_m^{eff}(\alpha + k_m^C)G^C + k_m^C \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \alpha k_m^{eff}G^H}$$

$$- \frac{R_c^3 V_{max}(\alpha + k_m^C)G^C(k_m^{eff}G^H + \frac{D}{R_b^2})/(3D)}{K_{eq}k_m^{eff}(\alpha + k_m^C)G^C + k_m^C \left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \alpha k_m^{eff}G^H}. \tag{11}$$

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

$$C_{carboxysome} = \frac{(j_c + k_m^{eff}(pH_{out}))H_{out}G^C + \frac{k_m^C}{(k_m^C+\alpha)}C_{out}(k_m^{eff}G^H + \alpha G^C + \frac{D}{R_b^2})}{K_{eq}k_m^{eff}G^C + \frac{k_m^C}{(k_m^C+\alpha)}\left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{\alpha}{(k_m^C+\alpha)}k_m^{eff}G^H}$$

$$- \frac{R_c^3 V_{max}G^C(k_m^{eff}G^H + \frac{D}{R_b^2})/(3D)}{K_{eq}k_m^{eff}G^C + \frac{k_m^C}{(k_m^C+\alpha)}\left(k_m^{eff}G^H + \frac{D}{R_b^2}\right) + \frac{\alpha}{(k_m^C+\alpha)}k_m^{eff}G^H}. \tag{12}$$

We now want to examine the remaining terms in the membrane permeability to CO_2, k_m^C.

2.2 Membrane permeability to CO_2 has little effect.

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

$$\frac{k_m^C}{k_m^C + \alpha} \tag{13}$$

$$\frac{\alpha}{k_m^C + \alpha} \tag{14}$$

Therefore if $k_m^C > \alpha$ or $CO_2 \to HCO_3^-$ 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:

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

Examining equation (15), note that the only appearance of the membrane permeability to 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 CO_2 will always be smaller than or equal to the permeability to 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_2 it would begin to effect the CO_2 concentration.

Thus far we have made a series of observations about the size of terms compared to the membrane permeability to CO_2 and found that when $(\alpha + k_m^C) >> DR_c/R_b^2$, $k_m^C > \alpha$ and $k_m^C > \frac{G^H}{G^C K_{eq}} \approx 1/K_{eq}$ the CO_2 concentration in the carboxysome reduces to

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

We can make a similar argument taking the equation for the CO_2 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 CO_2 leakage term will be negligible since the cytosolic CO_2 concentration will be approximately equal to the external CO_2 concentration. The HCO_3^- transport required to sustain a given internal inorgainc carbon pool will then be:

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

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

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

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

$$(19)$$

We have now shown that all the terms in H_{cyto} are negligibly dependent on the membrane permeability to CO_2. Therefore, the HCO_3^- transport level require to satisfy equation (18) is independent of the membrane permeability to CO_2. This observation is consistent with the low flux of CO_2 leakage in main text Figure 2.

2.3 Without facilitated CO_2 uptake external CO_2 has little effect

Unless conversion from CO_2 to 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 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 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 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 CO_2, k_c^C, begins to effectively trap CO_2 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 $k_c^C < \frac{D}{R_c}$.

3.0.1   Different carboxysome peremability for HCO_3^-

An existing hypothesis in the CCM literature is that the carboxysome has differential permeability and is more permeable to HCO_3^- and less permeable to CO_2. Intuitively this would allow more HCO_3^- into the carboxysome and trap more CO_2, thereby accumulating more inorganic carbon in the form of CO_2. We use our model to test weather differential carboxysome permeability enables higher carboxysomal CO_2 concentration for the same level of HCO_3^- transport. In the Figure S12 we show the k_c vs j_c phase space where we have plotted the carboxysome permeability to 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 HCO_3^-, $k_c^H = \text{ratio} \times k_c^C$.

Examining Figure S12, we see that making the carboxysome more permeable to HCO_3^- does not improve the function of the CCM as drastically as on might assume. The "turn on" of CO_2 accumulation with decreasing permeability is unaffected by changes to k_c^H, and depends only on the permeability CO_2, k_c^C. The "turn off" of accumulation for lower carboxysome permeabilities is greatly effected by the permeability of the carboxysome to 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 CO_2 is trapped, but HCO_3^- enters readily. As we moved to lower permeabilities CO_2 begins to be trapped, but there is a window where HCO_3^- still enters enough to supply the system. Eventually the carboxysome begins to restrict HCO_3^- entry. If the carboxysome is more permeable to HCO_3^- than to CO_2 then the window where CO_2 trapping is effective without restricting HCO_3^- entry broadens. The width of this window (on the y-axis) will also depend strongly on how much of the CO_2 is being fixed.

The "turn off" of the optimum, caused by not allowing enough 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.

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

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 past on to the active HCO_3^- transport required to replenish the leaked inorganic carbon, and therefore onto the CCM cost. In Figure S14 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.