Thermodynamic Competition for the Carboxysome Pore

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1 Competition for Entering the Carboxysome

We are interested in modeling the permeability of the pores on the carboxysome shell to CO_2 and HCO_3^-. Carboxysome pores tend to carry positive charge, and so it is likely that the abundant negatively charged metabolites in the cytosol can bind the pore and "plug it up," preventing CO_2 and HCO_3^- from entering. We decompose the permeability coefficient of these molecules into three multiplicative factors.

$$p = \hat{p} \times s \times o \tag{1}$$

where $\hat{p}$ is the permeability coefficient one would expect for a structure the size of a carboxysome, but without a shell (i.e. based on diffusional considerations), s is the reduction in permeability due to the fact that most of the shell is impassable (i.e. everywhere except the pores) and o is the fraction of pores that are "open."

1.1 The Effects of Shell Geometry and Pore Size

The surface area of the carboxysome shell can be calculated assuming a regular icosahedral geometry (i.e. 20 equilateral triangular sides). We will assume a carboxysome diameter of $100nm$ for simplicity. The edge length of a regular icosahedron equals $diameter/1.9$, giving $53nm$ for a $100nm$ carboxysome. The total surface area

$$SA = \frac{20\sqrt{3}}{4} \times (53nm)^2 = 2.4 \times 10^4 nm^2 \tag{2}$$

Though there is some variation in carboxysome diameter, we will proceed under the assumption that the carboxysome diameter is $100nm$, reflecting measurements from α-carboxysomes.

Pentameric proteins are thought to cap the 12 vertices of the icosahedral carboyxsome while hexamers form the faces of the shell. Based on the size of individual hexamers in crystal structures, we estimate roughly 40 hexamers per face. Therefore, there are $40 \times 20 + 12 = 812$ pores on the shell. If each has a diameter of 0.4 nm then there are $812 \times \pi \times 0.2^2 = 102nm^2$ of pore area on the shell, comprising about 0.4% of the carboxysome surface area. Note that

we are here ignoring carboxysome shell proteins with larger pores (e.g. Csos1D) because they are minor constituents of the shell.

We therefore expect a $\frac{1.0}{0.004} = 250$-fold reduction in diffusional flux simply due to occlusion of surface area. This calculation enables an upper bound estimate of the permeability of the carboxysome shell as follows. First we calculate a velocity on the assumption that the shell constitutes no barrier at all across the length of the pore ($2nm$), to arrive at $\hat{p}$ described above. Then we divide that velocity by 250 to arrive at $\hat{p} \times s$.

$$\hat{p} = \frac{D}{l} = \frac{10^{-}5cm^2/s}{2 \times 10^{-7}cm} = 50\frac{cm}{s} \tag{3}$$

$$\hat{p} \times s = \frac{50cm/s}{250} = 0.2\frac{cm}{s} \tag{4}$$

1.2 The Effect of Competition at the Pore

We will assume here that the pore has no affinity for CO_2, but that it can associate with HCO_3^- and some other negatively charged collection of competitors X. We will now calculate the fraction s from these binding energies, noting that s will differ for CO_2 and HCO_3^- because HCO_3^- can associate with the pore (based on its negative charge). We will begin with CO_2 and calculate $s = Pr(empty)$, the probability the pore is unoccupied.

1.3 Derivation for CO_2 Entry

Both HCO_3^- and CO_2 can plug the pore and prevent CO_2 entry. We will assume HCO_3^- binds the pore with an energy $E_{HCO_3^-} = -RT\ln(K_D^{HCO_3^-})$ and that the pore's interaction with X is potentially more favorable

$$E_X = E_{HCO_3^-} - \epsilon \tag{5}$$

with $\epsilon \geq 0\frac{kJ}{mol}$.

Molecule Bound	Typical Concentration	Association Energy	K_D
None	N/A	0	N/A
CO_2	$10\mu M$	0	1
HCO_3^-	$10mM$	$E_{HCO_3^-}$	$\exp\left(\frac{E_{HCO_3^-}}{RT}\right)$
X	$100mM$	$E_X = E_{HCO_3^-} - \epsilon$	$\exp\left(\frac{E_{HCO_3^-} - \epsilon}{RT}\right)$

Consider the binding of HCO_3^- for example.

$$K_D^{HCO_3^-} = \frac{[P^{free}][HCO_3^{-free}]}{[P \cdot HCO_3^-]} \tag{6}$$

$$[P \cdot HCO_3^-] = \frac{[P^{free}][HCO_3^{-free}]}{K_D^{HCO_3^-}} \tag{7}$$

We are interested in calculating $Pr(empty) = \frac{[P^{free}]}{[P^{total}]}$, i.e. the degree to which competition reduces number of unoccupied pores and, therefore, the probability of CO_2 entry.

$$[P^{total}] = [P^{free}] + [P \cdot HCO_3^-] + [P \cdot X] \tag{8}$$

$$= [P^{free}] + \frac{[P^{free}][HCO_3^{-free}]}{K_D^{HCO_3^-}} + \frac{[P^{free}][X^{free}]}{K_D^X} \tag{9}$$

$$= [P^{free}] \left(1 + \frac{[HCO_3^{-free}]}{K_D^{HCO_3^-}} + \frac{[X^{free}]}{K_D^X}\right) \tag{10}$$

Finally,

$$Pr(empty) = \frac{[P^{free}]}{[P^{total}]} \tag{11}$$

$$= \frac{1}{\left(1 + \frac{[HCO_3^{-free}]}{K_D^{HCO_3^-}} + \frac{[X^{free}]}{K_D^X}\right)} \tag{12}$$

1.4 Simple Case: All Competitors are Equal

For the moment, let's assume that $E_{HCO_3^-} = E_X$ and so their K_D values are equal. We can then simplify

$$Pr(empty) = \frac{1}{\left(1 + \frac{[\tilde{X}^{free}]}{K_D^X}\right)} \tag{13}$$

Where $[\tilde{X}^{free}] = [X^{free}] + [HCO_3^{-free}]$. If we then assume that the $[\tilde{X}] \gg [P]$ then we can simplify further. Note that this is justified because there are typically < 20 carboxysomes per cell but the competitor (e.g. HCO_3^-, glutamate, 3-phosphoglycerate) typically have concentrations $> 10mM$ in the cytosol.

$$Pr(free) = \frac{1}{1 + \frac{[\tilde{X}]}{K_D^X}} = \frac{K_D^X}{K_D^X + [\tilde{X}]} = \frac{\exp\left(\frac{E_X}{RT}\right)}{\exp\left(\frac{E_X}{RT}\right) + [X]} \tag{14}$$

The competitor concentration $10mM \leq [X] \leq 200mM$ as

- HCO_3^- is a competitor and has a measured concentration $\geq 10mM$ in the cyanobacterial cytosol
- the maximum concentration of competitor cannot exceed the total concentration of all metabolites ($\approx 200mM$).

1.5 Derivation for HCO_3^- Entry

Considering the case of HCO_3^- entry, we must roll back our above assumption that HCO_3^- and X are in the same pool. As bicarbonate carries negative charge and can (presumably) interact with the pore, the definition of an empty pore is somewhat different in the case of HCO_3^- than it was for CO_2. Since an HCO_3^--bound pore can transit HCO_3^-, we now we define an empty pore as one not bound by X, rather than one that is entirely empty. We also "roll-back" the assumption that HCO_3^- and X have the same pore association energies - i.e. ϵ may be greater than 0.

$$Pr(empty) = 1 - Pr(Xbound) = 1 - \frac{[P^{free}][X]}{K_D^X[P^{total}]} \tag{15}$$

$$= 1 - \frac{[X]}{K_D^X\left(1 + \frac{[HCO_3^{-free}]}{K_D^{HCO_3^-}} + \frac{[X^{free}]}{K_D^X}\right)} \tag{16}$$

$$= 1 - \frac{f}{\frac{K_D^X}{[HCO_3^-]} + f + \exp\left(-\epsilon/RT\right)} \tag{17}$$

where $f = \frac{[X]}{[HCO_3^-]}$ is the fold excess of competitor over HCO_3^-. Notice that this expression is independent of the absolute binding energy of HCO_3^-, $E_{HCO_3^-}$, and instead depends only on the absolute binding energy of the competitor X and the difference in binding energy between X and HCO_3^-, ϵ. So while we must set ϵ and $E_{HCO_3^-}$ to calculate $Pr(empty)$, we need not fix $E_{HCO_3^-}$ explicitly (although its value is implicitly set by the other two).

In 2 we assumed a $E_{HCO_3^-} = 2.5RT$ and calculated $Pr(free)$ for varying ϵ and $[X]$. Notice that this plot is essentially a rightward-shifted version of 1.

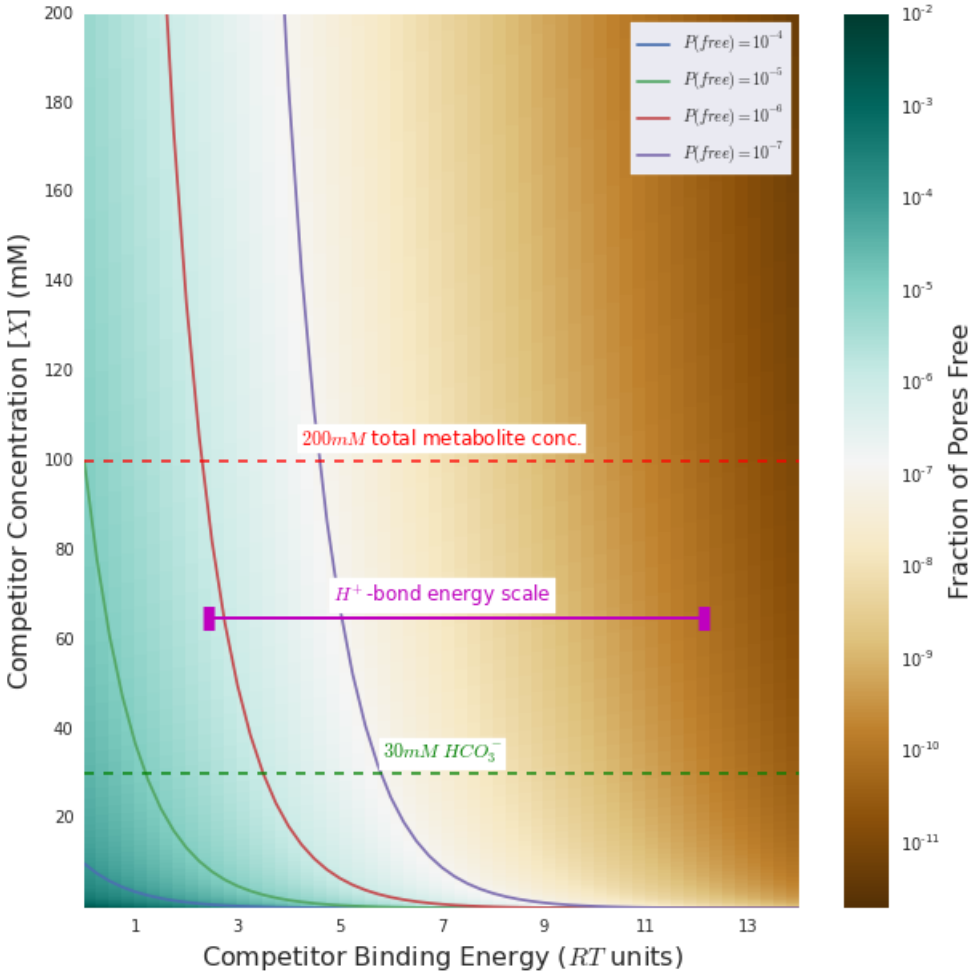

Figure 1: The effect of competition on the probability that the pore is free for CO_2 transit, i.e. is not bound of X or HCO_3^-. A $10^4 - 10^5$ fold reduction in CO_2 permeability is required to make the carboxysome 'CO_2-tight.' This is entirely feasible with reasonable binding energies and competitor concentrations, as is evident in the figure.

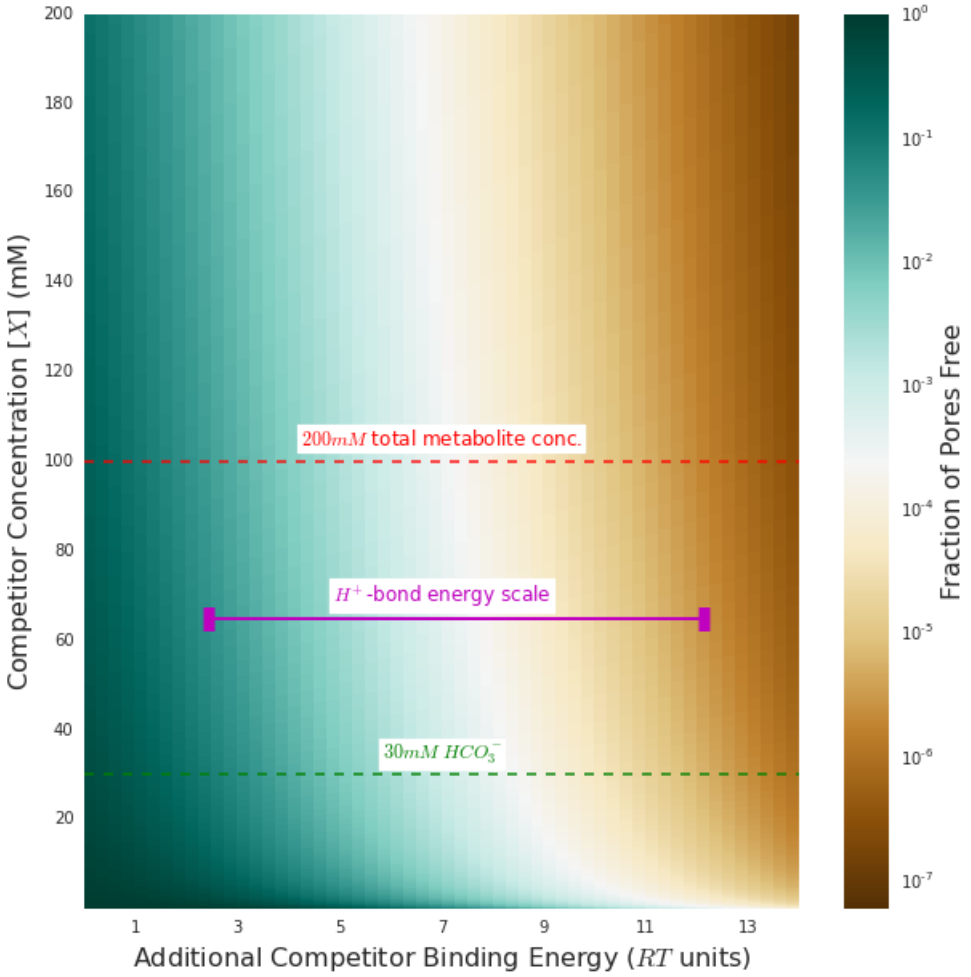

Figure 2: The effect of competition on the probability that the pore is free for HCO_3^- transit, i.e. is not bound of X. Since HCO_3^- has a high ($\approx 30mM$) concentration and can also bind the pore (unlike CO_2), competition from X has a much smaller effect on it.