

1 Control Theory

1.1 What is elasticity?

Metabolic Control Analysis [1] is a mathematical framework that is widely used to dynamic systems such as metabolic networks. In particular, it defines local properties called elasticities that can be used to quantify the control of some variables, such as fluxes, depend on other features of the system. The *scaled elasticity* is the infinitesimal response of a single flux (v) to one of the parameters (a), using a partial derivative of the log-scaled functions:

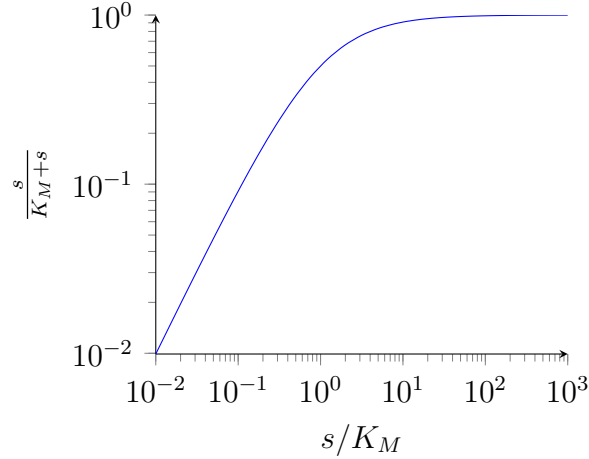
$$\epsilon_a^v \equiv \frac{\partial \ln(v)}{\partial \ln(a)} = \frac{\partial v}{\partial a} \frac{a}{v} \quad (1)$$

1.2 Single-substrate Control

Consider an enzyme-catalyzed reaction, described by a one-substrate irreversible Michaelis-Menten kinetic rate law:

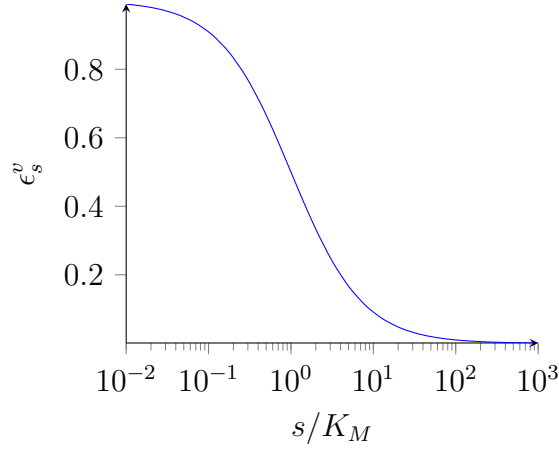
$$v = V^+ \frac{s}{K_M + s} \quad (2)$$

where s is the substrate concentrations (in units of molar), and K_M is the Michaelis-Menten coefficient (also in molar).



The scaled elasticities for s and i are given by:

$$\begin{aligned} \epsilon_s^v &= \frac{\partial v}{\partial s} \frac{s}{v} = V^+ \frac{K_M + s - s}{(K_M + s)^2} \frac{s}{v} \\ &= \frac{K_M}{(K_M + s)^2} (K_M + s) = \frac{K_M}{K_M + s} = 1 - \frac{s}{K_M + s} \end{aligned} \quad (3)$$



As shown in [2], this formula for substrate elasticity can be generalized to reversible Michaelis-Menten reactions, where the saturation term is separated from the thermodynamic term:

$$v = V^+ \cdot \kappa \cdot \gamma \quad (4)$$

$$\kappa \equiv \frac{s/K_S}{1 + s/K_S + p/K_P} \quad (5)$$

$$\gamma \equiv 1 - \frac{p/s}{K'_{eq}} \quad (6)$$

where p is the product concentration, K_S and K_P are the Michaelis-Menten constants for the substrate and product, and K'_{eq} is the apparent equilibrium constant. In this case, the elasticity of the substrate is:

$$\epsilon_s^v = \gamma^{-1} - \kappa \quad (7)$$

which converges to equation 3 as $p \rightarrow 0$.

1.3 Regulatory Effectors

Although previous publications have derived elasticities associated with small-molecule effectors for different types of rate laws [3, 4], the relationship between the elasticity and the relative activity of the enzyme has not been discussed. Here, we will demonstrate that in almost all cases, there is a direct trade-off between the two, namely that a regulator must decrease the activity of the enzyme in order to have a non-zero elasticity.

Without loss of generality, we will keep the separable form of the rate law

$$v = V^+ \cdot \kappa \cdot \gamma \cdot \theta(x)$$

where we add a multiplicative term $\theta(x)$ that will represent the decrease of activity due to the small-molecule regulation (x). As long as $\theta(x)$ is the only term affected by x , i.e. $\frac{\partial \kappa}{\partial x} = \frac{\partial \gamma}{\partial x} = 0$, the exact forms of κ and γ are irrelevant.

1.3.1 Activation

First, consider a cooperative [5, 6] activator with Hill coefficient h and activation coefficient K_A :

$$\theta = \frac{a^h}{K_A^h + a^h} . \quad (8)$$

The elasticity with respect to the activator concentration x will thus be:

$$\begin{aligned} \epsilon_a^v &= \frac{\partial v}{\partial a} \frac{a}{v} = V^+ \kappa \gamma \frac{h a^{h-1} (K_A^h + a^h) - h a^{h-1} a^h}{(K_A^h + a^h)^2} \frac{a}{v} \\ &= h \frac{K_A^h}{K_A^h + a^h} = h(1 - \theta) \end{aligned} \quad (9)$$

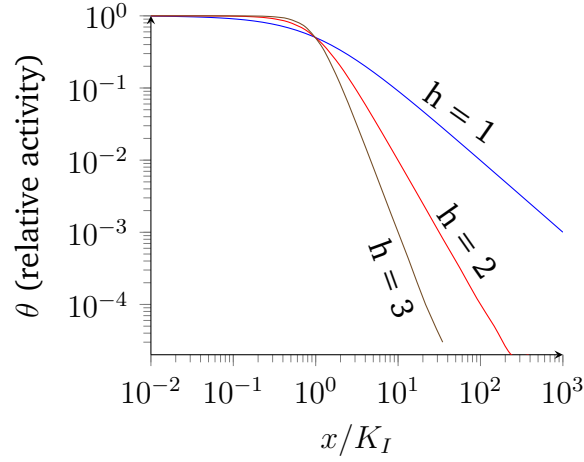
[to make this even more general, we can also mention the case of an activator that increases an already existing activity: instead of θ , we get $\alpha + (1 - \alpha)\theta$ with a constant relative baseline activity α . In this case, the elasticity formula remains the same, except for a constant prefactor; the same logic can also be applied to inhibitors.]

1.3.2 Pure non-Competitive Inhibition

First, consider a non-competitive inhibitor with Hill coefficient h and inhibition coefficient K_I :

$$\theta = 1 - \frac{x^h}{K_I^h + x^h} = \frac{K_I^h}{K_I^h + x^h} \quad (10)$$

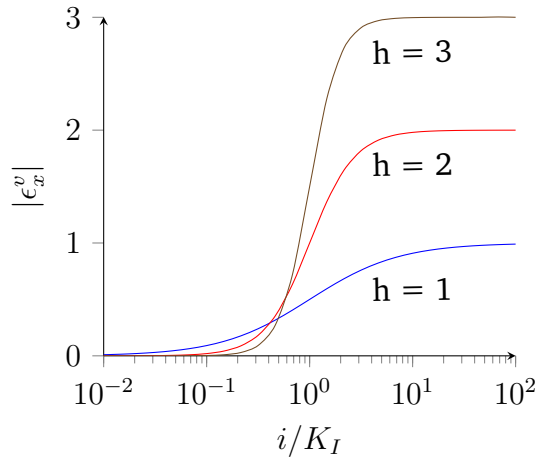
The following plot shows the response of θ to the concentration x in log-log scale.



In this case, the elasticity with regards to the inhibitor concentration would be:

$$\begin{aligned}\epsilon_x^v &= \frac{\partial v}{\partial x} \cdot \frac{x}{v} = V^+ \kappa \gamma \frac{-h x^{h-1}}{(K_I^h + x^h)^2} \frac{x}{v} \\ &= -\frac{h x^h (K_I^h + x^h)}{(K_I^h + x^h)^2} = -h \frac{x^h}{K_I^h + x^h} = -h(1 - \theta) .\end{aligned}\quad (11)$$

Plotting the elasticity as a function of x , we see that it is a monotonically decreasing negative function:



This means that substrates have the most control ($\epsilon_s^v \rightarrow 1$) when they are much below saturation ($s \ll K_M$) while inhibitors have the most control ($\epsilon_x^v \rightarrow -h$) when they are saturated ($i \gg K_I$).

1.3.3 Competitive Inhibition

Competitive inhibition is a one case, where a separable kinetic rate law is not sufficient since $\frac{\partial \kappa}{\partial x} \neq 0$. To analyze this case, we pick a simple one-substrate irreversible reaction, where the inhibitor affects the K_M according to the following formula:

$$v = V^+ \frac{s}{K_M \left(1 + \frac{x^h}{K_I^h}\right) + s} \quad (12)$$

In this case, we can define an *effective* inhibition constants K_{IC} , that will allow us to rewrite this rate law in a form identical to non-competitive inhibition:

$$\begin{aligned} K_{IC} &\equiv K_I \sqrt[1]{\frac{K_M + s}{K_M}} \\ v &= V^+ \frac{s}{K_M \left(1 + \frac{x^h}{K_I^h}\right) + s} = V^+ \frac{s}{K_M \left(1 + \frac{x^h (K_M + s)}{K_I^h K_M}\right) + s} = \\ &= V^+ \frac{s}{K_M + s + \frac{x^h (K_M + s)}{K_{IC}^h}} = V^+ \frac{s}{K_M + s} \cdot \frac{1}{1 + \frac{x^h}{K_{IC}^h}} \end{aligned} \quad (13)$$

so we can see here that in this case $\theta = \frac{K_{IC}^h}{K_{IC}^h + x^h}$, exactly like in the case of non-competitive inhibition. Of course, the difference here is that K_{IC} is not a binding constant but rather a function of s , K_M , and K_I . Nevertheless, we can use the same formula for the elasticity (since K_{IC} is a constant with regards to x):

$$\epsilon_x^v = \frac{\partial v}{\partial x} \frac{x}{v} = -h(1 - \theta) \quad (14)$$

1.3.4 Uncompetitive Inhibition

Uncompetitive inhibition is another case where substrate and inhibitor saturations are entangled:

$$v = V^+ \frac{s}{K_M + s \left(1 + \frac{x^h}{K_I^h}\right)} \quad (15)$$

However, the same procedure as in competitive inhibition can also be used here, by defining an effective constant K_{IU} , that will allow us to rewrite

this rate law in a form identical to non-competitive inhibition:

$$\begin{aligned}
K_{IU} &\equiv K_I \sqrt[h]{\frac{K_M + s}{s}} \\
v &= V^+ \frac{s}{K_M + s \left(1 + \frac{x^h}{K_I^h}\right)} = V^+ \frac{s}{K_M + s \left(1 + \frac{x^h (K_M + s)}{K_{IU}^h s}\right)} = \\
&= V^+ \frac{s}{K_M + s + \frac{x^h (K_M + s)}{K_{IU}^h}} = V^+ \frac{s}{K_M + s} \cdot \frac{1}{1 + \frac{x^h}{K_{IU}^h}} \quad (16)
\end{aligned}$$

which shows that just like in the case of competitive inhibition, $\theta = \frac{K_{IU}^h}{K_{IU}^h + x^h}$, and the formula for elasticity remains

$$\epsilon_x^v = -h(1 - \theta). \quad (17)$$

1.3.5 Generalized Inhibition Model

A general formula for reversible reactions with non-cooperative competitive, uncompetitive, mixed and pure non-competitive inhibition is given in Chapter 5 of Sauro [7]:

$$v = \frac{\frac{V_m}{K_S} \left(s - \frac{p}{K_{eq}}\right) \left(1 + b \frac{x}{aK_I}\right)}{\left(1 + \frac{s}{K_S} + \frac{p}{K_P}\right) + \frac{x}{K_I} \left(1 + \frac{s}{aK_S} + \frac{p}{aK_P}\right)}. \quad (18)$$

The parameter b represents the amount of inhibition, i.e. the activity of the enzyme when the inhibitor is bound to it. If we assume there is no activity in the bound state, also known as **complete inhibition**, then we can set $b = 0$. Then, depending on the value of a , the above formula converges to the different cases discussed earlier. For example, if $a \rightarrow \infty$, then this rate law is equivalent to competitive inhibition. When $a = 1$ it is equivalent to pure non-competitive inhibition. Finally, if $a \rightarrow 0$ (more precisely, when $K_I \gg aK_I$), it becomes uncompetitive inhibition.

The elasticity we get for this general form is:

$$\epsilon_x^v = \frac{b \frac{x}{aK_I}}{1 + b \frac{x}{aK_I}} - \frac{\frac{x}{K_I} \left(1 + \frac{s}{aK_S} + \frac{p}{aK_P}\right)}{\left(1 + \frac{s}{K_S} + \frac{p}{K_P}\right) + \frac{x}{K_I} \left(1 + \frac{s}{aK_S} + \frac{p}{aK_P}\right)} \quad (19)$$

as with previous cases, we can define an effective K_E :

$$K_{E1} \equiv K_I \frac{a}{b} \quad (20)$$

$$K_{E2} \equiv K_I \frac{1 + \frac{s}{K_S} + \frac{p}{K_P}}{1 + \frac{s}{aK_S} + \frac{p}{aK_P}} \quad (21)$$

and therefore that we get:

$$\begin{aligned}
v &= \frac{\frac{V_m}{K_S} \left(s - \frac{p}{K_{eq}} \right)}{\left(1 + \frac{s}{K_S} + \frac{p}{K_P} \right)} \cdot \frac{1 + \frac{x}{K_{E1}}}{1 + \frac{x}{K_{E2}}} \\
\epsilon_x^v &= \frac{x}{K_{E1} + x} - \frac{x}{K_{E2} + x}
\end{aligned} \tag{22}$$

As expected, in all cases of complete inhibition ($b \rightarrow 0$ which means that $K_{E1} \rightarrow \infty$), we reach exactly the same conclusion as in the extreme cases, namely:

$$\begin{aligned}
\theta &= \frac{1 + \frac{x}{K_{E1}}}{1 + \frac{x}{K_{E2}}} \rightarrow \frac{K_{E2}}{K_{E2} + x} \\
\epsilon_x^v &\rightarrow -(1 - \theta)
\end{aligned} \tag{23}$$

For incomplete inhibition, however, there is a more complex relationship between relative activity and inhibition.

1.3.6 The Monod-Changeux-Jacob model

Probably the most widespread model for cooperativity of substrate and effector binding was proposed by Monod et al. [6]. In this model the rate law is given by the following formula:

$$v = V^+ \cdot \frac{s}{K_M + s} \cdot \frac{\left(1 + \frac{s}{K_M} \right)^h \left(1 + \frac{a}{K_A} \right)^h \left(1 + \frac{x}{K_I} \right)^{-h}}{L + \left(1 + \frac{s}{K_M} \right)^h \left(1 + \frac{a}{K_A} \right)^h \left(1 + \frac{x}{K_I} \right)^{-h}} \tag{24}$$

where a is the concentration of an inhibitor, and K_M , K_A and K_I are the dissociation constants for the active form of the enzyme (for s , a and x respectively), n is the number of subunits, and L is the allosteric constant. As before, we can define the saturation term κ and the relative activity term θ (i.e. the decrease from maximal activity):

$$\begin{aligned}
\kappa &\equiv \frac{s}{K_M + s} \\
\theta &\equiv \frac{\left(1 + \frac{s}{K_M} \right)^h \left(1 + \frac{a}{K_A} \right)^h \left(1 + \frac{x}{K_I} \right)^{-h}}{L + \left(1 + \frac{s}{K_M} \right)^h \left(1 + \frac{a}{K_A} \right)^h \left(1 + \frac{x}{K_I} \right)^{-h}} \\
v &= V^+ \cdot \kappa \cdot \theta
\end{aligned} \tag{25}$$

In [3], the elasticity of the inhibitor was calculated for this rate law (denoted as *effector strength* or X_I in the original text):

$$\epsilon_x^v = \frac{\partial \ln v}{\partial \ln x} = \frac{\partial \ln \theta}{\partial \ln x} = -h \frac{x}{K_I + x} (1 - \theta) . \quad (26)$$

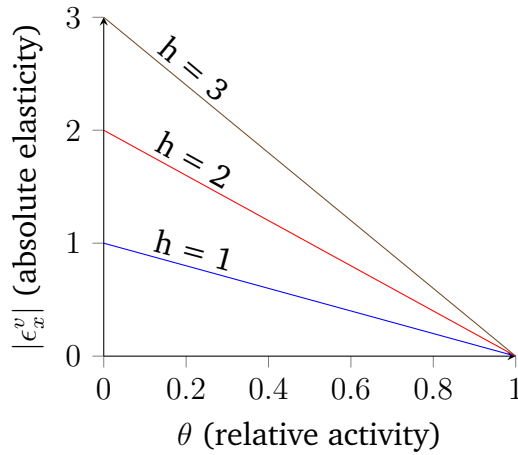
Although the final formulae for the elasticities in the MWC case are not identical to the other cases dealt with before, we can still reach a similar conclusion, where the absolute value of the elasticity is larger when the relative activity is smaller.

1.4 A Trade-off between Enzyme Cost and Flux Control

By taking the absolute value of the elasticity, we can compress the results for activators (Equation 9) and inhibitors (Equations 11, 13, 17, and 23, excluding the MWC model) into one formula:

$$|\epsilon_x^v| = h(1 - \theta) . \quad (27)$$

[this is basically Eq. D.23 from the modular rate law paper supplement, but written here in a much easier way] This relationship can be visualized in the following plot (for different values of h):



Since θ represents the fraction of active enzyme, we see here that there is direct trade-off between the activity of the enzyme and the elasticity. It should be noted, that evolution can easily adjust θ for an individual enzyme by changing the K_A or K_I values, even without changing the concentration of the small-molecule effector (assuming it has other crucial functions in the cell). Therefore, evolution needs to weigh between how much of the

enzyme is "wasted" by inhibition (or by inactivation), versus how much control it has on the flux. This can be viewed as a trade-off between the short-term goal of being able to adjust things quickly and the long-term goal of allocating resources efficiently in order to grow as fast as possible.

Another corollary of Equation 27 is that the control can be increased by changing the Hill coefficient (h). This could be a reason why mechanisms with very high cooperativity evolve for allosteric regulation [6], as it increases elasticity without the added cost of losing enzyme activity.

References

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