

## Introduction to Quantitative Control and Regulation

### Symbol Glossary

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$X_i$	Boundary species (fixed external condition)
$S_i$	Floating molecular species, state variable
$v_i$	Reaction rate for reaction $i$
$J$	Pathway flux
$\delta x$	A small change in a quantity $x$

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### A Simple Example

Consider the simplest possible pathway:



The two species,  $X_o$  and  $X_1$ , are boundary species, that is they are external and fixed by the observer. Each reaction is catalyzed by an enzyme at a rate  $v_1$  and  $v_2$  by enzymes  $E_1$  and  $E_2$  respectively. The species  $S$  is a state variable of the system (eg a metabolite), that can change depending on the enzyme activities, kinetic constants and boundary species concentrations. To give a concrete example, let us assume that the reaction rates,  $v_1$  and  $v_2$  are given by two simple mass-action rate laws, one reversible and other irreversible:

$$\begin{aligned}v_1 &= E_1(k_1X_o - k_2S) \\v_2 &= k_3S\end{aligned}$$

The rate of change of  $S$  is given by:

$$\frac{dS}{dt} = v_1 - v_2$$

and at steady state  $v_1 = v_2$ . Given this condition, the steady state concentration of  $S$  can be easily shown to be:

$$S = \frac{E_1k_1X_o}{E_1k_2 + k_3}$$

For reason that will come clearer later, the rate law for  $v_1$  is also multiplied by the concentration of an enzyme  $E_1$  that is assumed to catalyze this reaction.

The steady state flux,  $J$ , through the pathway can be computed from the rate of  $v_2 = k_3S$  and by substitution yields:

$$J = k_3 \frac{E_1 k_1 X_o}{E_1 k_2 + k_3}$$

Note that for a linear chain pathway, any rate can be used to compute the flux since all steps carry the same flux. One immediate observation is that the flux,  $J$ , and steady state concentration,  $S$ , is a function of all kinetic constants in the pathway.

For most systems the algebraic analysis just described is not possible because the mathematics can become either too difficult or no closed algebraic solution exists. Instead we can use a graphical method to understand the dynamics of our simple two step pathway.

In general, the following relation will be true at steady state:

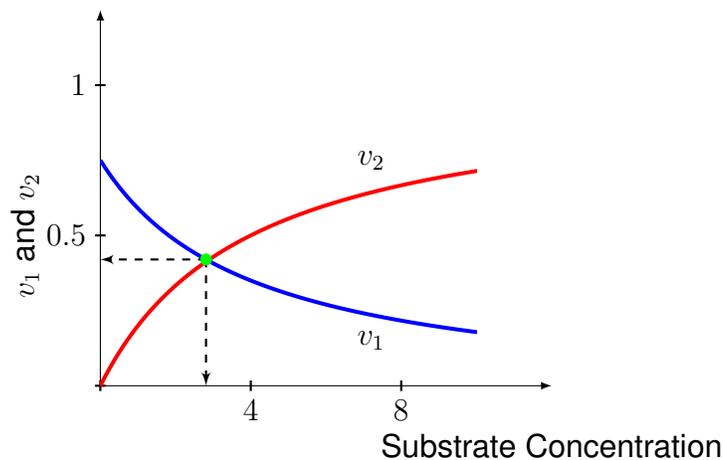
$$v_1(X_o, S, E_1, k_1, \dots) - v_2(S, X_1, E_2, k_2, \dots) = 0$$

where the rates are expressed as functions of their influencing factors. There is a simple graphical technique we can use to study how the enzyme activities,  $E_1$  and  $E_2$  control the steady state concentration  $S$ , and the steady state flux,  $J$  through the pathway. In this system, the steady state flux,  $J$  will be numerically equal to the reaction rates  $v_1$  and  $v_2$ ,

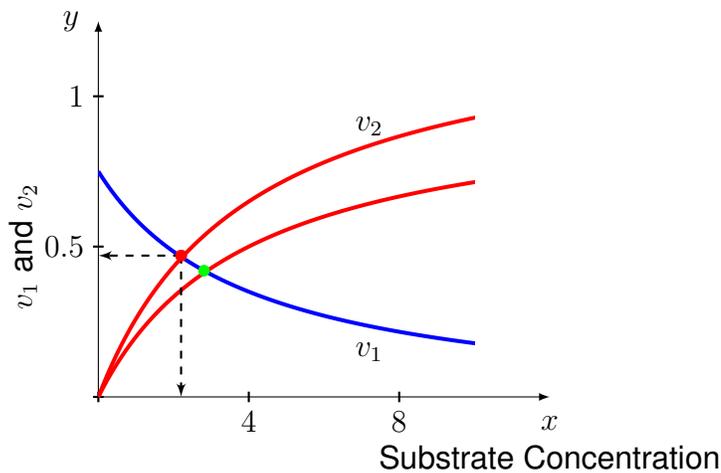
$$J = v_1 = v_2$$

It is important to recall that for many enzyme catalyzed reactions the rate,  $v$  is proportional to the concentration of enzyme,  $E$ ,  $v \propto E$ .

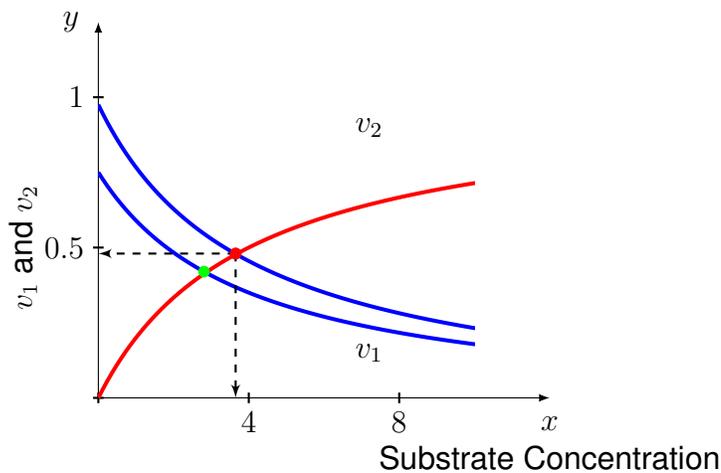
Let us plot both reaction rates,  $v_1$  and  $v_2$  against the substrate concentration  $S$ .



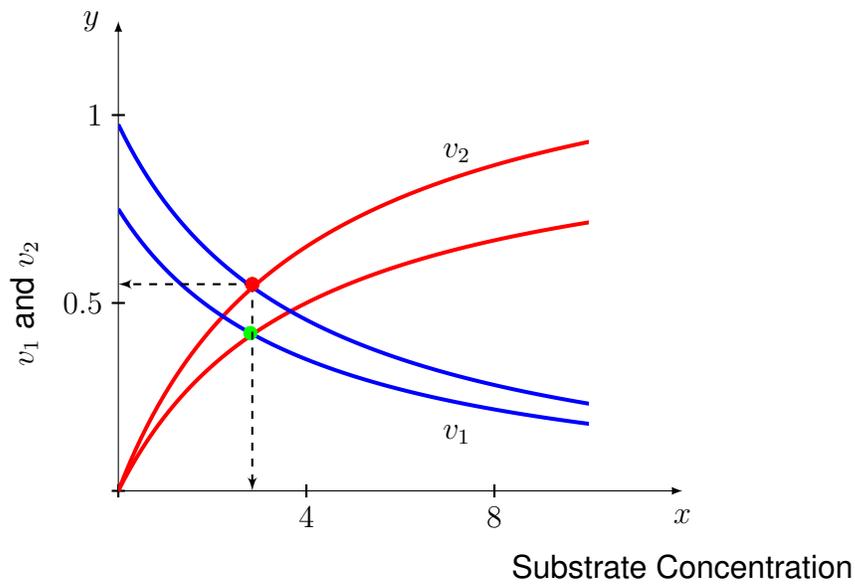
Note the response of  $v_1$  to changes in  $S$ .  $v_1$  falls as  $S$  increases due to product inhibition by  $S$ . The intersection point of the two curves marks the point when  $v_1 = v_2$ , that is the steady state. A line dropped perpendicular from the intersection point marks the steady state concentration of  $S$



Let us now increase the activity of  $E_2$  by 30% by adding more enzyme. Because the reaction rate is proportional to  $E_2$ , the curve is scaled upwards although its general shape stays the same. Note how the intersection point moves to the left, indicating that the steady state concentration of  $S$  **decreases** relative to the reference state. This is understandable because with a higher  $v_2$ , more  $S$  is consumed therefore  $S$  decreases.



In the next experiment, let us restore  $E_2$  back to its original level and instead increase the amount of  $E_1$  by 30%. Again, changing  $E$  simply scales the  $v_1$  curve but because of the negative curvature, the  $v_1$  curve shifts right. This moves the intersection point to the right, indicating that the steady state concentration of  $S$  **increases** relative to the reference state.



Let us now change the activity of both  $E_1$  and  $E_2$  by 30%. Note that the curve for  $v_1$  and  $v_2$  are both scaled upwards, this in turns moves the intersection point upwards but **doesn't** change the steady state concentration of  $S$ . This happens because both curves move vertically by the same amount so that the intersection point can only move vertically. This experiment highlights an important result, when all enzyme activities (or concentrations) are increased by the same fraction, the flux increases by that same fraction but the species or metabolite levels remain **unchanged**, that is:

If all  $E_i$  are increased by a factor  $\alpha$  then the steady state change in  $J$  and  $S_i$  is:

$$\delta J = \alpha J \quad \text{and} \quad \delta S = 0$$

From these thought experiments we can conclude that increasing the activities of both enzymes by the **same fraction** will increase the flux through the pathway but will not change the concentration of the pathway species,  $S$ . This conclusion is in fact quite general and no matter how complex the pathway. If we were to increase the activity of every step in the pathway by a given proportion, the concentration of **every** metabolite would remain unchanged but with a proportionate change in flux. Although we know that  $\delta S = 0$ , how much has the flux increased under these conditions?

Since  $\delta S = 0$ , the only change that could possibly effect the flux is the change in enzyme activity, since the enzyme activity has increased by a given proportion (30%), then the flux must also have increased by the same proportion since the rate is proportional to the enzyme activity. (i.e  $v_i \propto E_i$ ).

## Control Coefficients - How enzymes control pathways

Let us consider another thought experiment. Assume the pathway is at steady state, let us increase the activity of enzyme  $E_1$  by a factor  $\alpha$ , that is  $\delta E_1/E_1 = \alpha$ . The system will now move to a new steady state with a different flux and metabolite concentration. Let us assume that the flux has changed by  $\delta J$  and the species concentration by  $\delta S$ . We can measure the influence of enzyme  $E_1$  on the flux and concentration of  $S$  by the ratio:

$$\frac{\delta J}{\delta E_1}$$

$$\frac{\delta S}{\delta E_1}$$

However, these ratios depend on the size of the change and the units we choose. We can eliminate these problems by taking infinitesimal changes and scaling the ratio to remove units, when we do this we obtain the control coefficient:

$$C_{E_i}^J = \frac{dJ}{dE_i} \frac{E_i}{J}$$

$$C_{E_i}^{S_j} = \frac{dS_j}{dE_i} \frac{E_i}{S_j}$$

The above statement has been generalized for any enzyme step,  $E_i$  and flux,  $J$ , and any species,  $S_j$ . The scaling values are obtained from the reference state. The first coefficient is called the **flux control coefficient** and the second one the **concentration control coefficient**. The values are dimensionless and when measured give us some idea of how a particular enzyme controls either the flux or some particular species concentration. For a straight chain pathway one can show that the flux control coefficients ( $C_E^J$ ) are bounded between zero and one.

There are various ways to look at the above definitions, these are given below.

$$C_E^J = \frac{d \ln J}{d \ln E}$$

$$C_E^J = \frac{dJ}{J} / \frac{dE}{E}$$

$$C_E^J \approx \frac{\text{Percentage change in } J}{\text{Percentage change in } E}$$

The last definition is a useful but approximate way to interpret the control coefficients as a ratio of percentage changes.

## Computing Control Coefficients

There are different way to estimate the control coefficients, including experimentally, by simulation or analytically. Experimentally the control coefficients can be measured by changing the expression level for the particular enzyme and measuring the resulting new steady concentrations and fluxes. The control coefficients are then the ratio of the fractional changes:

$$C_{E_i}^J \approx \frac{J_n - J}{J} / \frac{E_n - E}{E}$$

where  $J_n$  is the new flux after the perturbation and  $E_n$  the perturbed enzyme concentration.

Control coefficients can also be measured by simulation, that is a computer simulation of a pathway is constructed and the enzyme perturbations carried out using the simulation.

For simple systems, the control coefficients can also be computed analytically. For example, the flux,  $J$ , in the simple example shown at the start was given by:

$$J = k_3 \frac{E_1 k_1 X_o}{E_1 k_2 + k_3}$$

The flux control coefficient can be computed by taking the derivative and scaling. This can be done for both  $E_1$  and  $k_3$  in the second step to yield:

$$C_{E_1}^J = \frac{k_3}{E_1 k_2 + k_3}$$

$$C_{k_3}^J = \frac{E_1 k_2}{E_1 k_2 + k_3}$$

Note that the sum  $C_{E_1}^J + C_{k_3}^J = 1$ .

The concentration control coefficients can be similarly computed.

## Summation Theorems

The definitions given above can be rearranged and given the approximation:

$$\frac{\delta J}{J} = C_{E_i}^J \frac{\delta E_i}{E_i}$$

$$\frac{\delta S_j}{S_j} = C_{E_i}^{S_j} \frac{\delta E_i}{E_i}$$

These simple relations allow us to compute the change in flux given a change in an enzyme activity. If we perturb more than one enzyme activity, we can get the overall change by summing up the individual changes. In general, if we make changes to  $n$  reaction steps, then the overall change in flux and species concentrations is given by:

$$\frac{\delta J}{J} = \sum_{i=1}^n C_{E_i}^J \frac{\delta E_i}{E_i}$$

$$\frac{\delta S}{S} = \sum_{i=1}^n C_{E_i}^S \frac{\delta E_i}{E_i}$$

The above relationship can be justified by assuming that there exists a relationship between the flux,  $J$ , and enzyme concentrations, that is:

$$J = J(E_1, E_2, \dots)$$

Taking the total derivative of  $J$ :

$$dJ = \frac{\partial J}{\partial E_1} dE_1 + \frac{\partial J}{\partial E_2} dE_2 + \dots$$

Dividing both sides by  $J$  and dividing top and bottom of each term by the appropriate  $E_i$  leads to the relation

$$\frac{\delta J}{J} = C_{E_1}^J \frac{\delta E_1}{E_1} + C_{E_2}^J \frac{\delta E_2}{E_2} + \dots$$

The same reasoning applies to the species relationship.

For the two step pathway, let us repeat the thought experiment where we increased both enzyme activities at the same time. So long as we consider small changes, we can compute the overall change in flux or species concentration by simply adding the control coefficient terms, thus:

$$\frac{\delta J}{J} = C_{E_1}^J \frac{\delta E_1}{E_1} + C_{E_2}^J \frac{\delta E_2}{E_2}$$

$$\frac{\delta S}{S} = C_{E_1}^S \frac{\delta E_1}{E_1} + C_{E_2}^S \frac{\delta E_2}{E_2}$$

However, we know from the thought experiments that  $\delta S = 0$  and the change in flux must equal the fractional change in enzyme activity, that is  $\delta J/J = \delta E_1/E_1 = \delta E_2/E_2 = \alpha$

Rewriting the above equations as:

$$\alpha = C_{E_1}^J \alpha + C_{E_2}^J \alpha$$

$$0 = C_{E_1}^J \alpha + C_{E_2}^S \alpha$$

from which we conclude:

$$1 = C_{E_1}^J + C_{E_2}^J$$

$$0 = C_{E_1}^J + C_{E_2}^S$$

These summations (or theorems) are in fact general. For any pathway with any topology the following is true:

### Control Coefficient Summation Theorem

$$\sum_{i=1}^n C_{E_i}^J = 1$$

$$\sum_{i=1}^n C_{E_i}^{S_j} = 0$$

These theorems suggest the following:

- 1) Control is shared throughout a pathway.
- 2) If one step gains control, one of more other steps must lose control.
- 3) Control coefficients are system properties, they can only be computed or measured in the intact system.

## Rate-limiting Steps

In much of the literature and some contemporary textbooks, one will often find a brief discussion of an idea called the rate-limiting step. The literature is in general unclear about the meaning of this phrase but some interpret the rate-limiting step to be the single step in pathway which limits the flux. In terms of our control coefficients we can interpret the rate-limiting step as the step with a flux control coefficient of unity. This means, by the summation theorem, that all other steps (at least in a linear chain) must have flux control coefficients of zero. Such a situation is not likely to occur in a real system and experiments show in fact that control is shared amongst many steps and no one step can be designated the rate-limiting step.