Steady State Analysis

1.3 Steady State

The steady state is one of the most important states to consider in a dynamical model. It is the primary reference point from which to consider a model's behavior. At steady state, the concentrations of all molecular species are constant and there is a net flow of mass through the network. This is in contrast to systems at thermodynamic equilibrium, where, although concentrations are constant there is no net flow of mass across the systems boundaries. A convenient way to illustrate the steady state is graphically. Consider the simple model below:

$$X_o \xrightarrow{v_1} S_1 \xrightarrow{v_2} X_1$$

where X_o and X_1 are constant boundary species and S_1 is a floating species. For illustration purposes we will assume some very simple kinetics for each reaction, v_1 and v_2. Let us assume that each reaction is governed by simple first order mass-action kinetics,

$$v_1 = k_1 X_o$$
$$v_2 = k_2 S_1$$

where k_1 and k_2 are both reaction rate constants. In Figure 1.4 both reaction rates have been plotted as a function of the floating species concentration, S_1.

Note that the reaction rate for v_1 is a horizontal line because it is unaffected by changes in S_1 (no product inhibition). The second reaction, v_2 is shown as a straight line with slope, k_2. Notice that the lines intersect. The intersection marks the point when both rates, v_1 and v_2 are equal. This point marks the steady state concentration of S_1. By varying the value of k_2 we can observe the effect it has on the steady state, In this simple model it is straight forward to analytically determine S_1

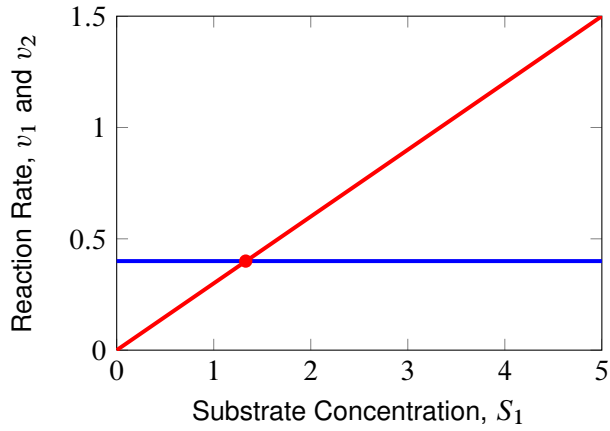

Figure 1.4: Plot of reaction rates versus concentration of S_1. The intersection of the two lines marks the steady state point where $v_1 = v_2$.

at the intersection point. The model for this system comprises a single differential equation:

$$\frac{dS_1}{dt} = k_1 X_o - k_2 S_1$$

At steady state, we set $dS_1/dt = 0$, from which we can solve for the steady state concentration of S_1 as:

$$S_1 = \frac{k_1 X_o}{k_2}$$

This solution tells us that the steady state concentration of S_1 is a function of all the parameters in the system. We can also determine the steady state rate, usually called the pathway flux and denoted by J, by inserting S_1 into one of the rate laws, for example into v_2:

$$J = k_2 \frac{k_1 X_o}{k_2} = k_1 X_o$$

This answer is identical to v_1 which is not surprising since in this model the pathway flux is completely determined by the first step and S_1 has no influence whatsoever on the flux. This simple example illustrates a rate limiting step in the pathway, that is one step, and one step only, that has complete control over the pathway flux.

A slightly more realistic model is the following:

$$X_o \xrightarrow{v_1} S_1 \xrightarrow{v_2} S_2 \xrightarrow{v_3} X_1$$

where the rate law for the first step is now reversible and is given by:

$$v_1 = k_1 X_o - k_2 S_1$$

The remaining steps are consider to be governed by simple irreversible mass-action rate laws, $v_2 = k_3 S_1$ and $v_3 = k_4 S_2$. The differential equations for this systems are:

$$\begin{aligned} \frac{dS_1}{dt} &= (k_1 X_o - k_2 S_1) - k_3 S_1 \\ \frac{dS_2}{dt} &= k_3 S_1 - k_4 S_2 \end{aligned}$$

The steady state solution for S_1 and S_2 can be obtained by setting both differential equations to zero to yield:

$$\begin{aligned} S_1 &= \frac{k_1 X_o}{k_2 + k_3} \\ S_2 &= \frac{k_3 k_1 X_o}{(k_2 + k_3) k_4} \end{aligned}$$

The steady state can be determined by inserting one of the solutions into the appropriate rate law, for convenience the easiest is to insert S_2 into v_3 to yield:

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

Once the first step is reversible we see that the steady state flux is a function of all the parameters except k_4 indicating that the first step is no longer the rate limiting step. The equation shows us that the ability to control the flux is shared between the first and second steps. Note that if we set $k_2 = 0$ then the solution reverts to the earlier simpler model.

We can also make all three steps reversible ($k_f S_i - k_r S_{i+1}$), so that the solution is given by:

$$\begin{aligned} S_1 &= \frac{X_o k_1(k_4 + k_5) + X_1 k_4 k_6}{k_3 k_5 + k_2(k_4 + k_5)} \\ S_2 &= \frac{X_1 k_6(k_2 + k_3) + X_o k_1 k_3}{k_3 k_5 + k_2(k_4 + k_5)} \end{aligned}$$

The last example illustrates the increase in complexity of deriving an analytical solution after only a modest increase in model size. In addition, once more complex rate laws as used, such as Hill equations or Michaelis-Menten type rate laws, the solutions become exceedingly difficult to derive. As a result, in most cases, steady states are computed numerically rather than analytically.

Pathway Stability

A topic we will consider in more detail in a later section is the stability of the steady state solution. Consider the simple two step model described earlier, the differential equation describing this model was given by, $dS_1/dt = k_1 X_o - k_2 S_1$. If the system is at steady state, we make a small positive perturbation to the steady state concentration of S_1, δS_1 and ask what is the rate of change of S_1 as a result of this perturbation, that is what is the value of:

$$k_1 X_o - k_2(S_1 + \delta S_1)$$

If we insert the solution for S_1 into the above equation we are left with:

$-k_2 \delta S_1$

In other words the rate of change of S_1 as a result of a positive perturbation is negative, that is, the system attempts to restore itself back to the original steady state. Systems with this kind of behavior are termed **stable**. If the rate of change in S_1 had been positive instead of negative however, the perturbation would have continued to diverge away from the original steady state and the system would them be considered **unstable**. The concept of stability and instability will be considered in more detail in a later section.

Computing the Steady State

In those (many) cases were we cannot derive an analytical solution for the steady state we must revert to numerical methods. There are at least two methods that can be used here. The simplest approach is to run a time course simulation for a sufficiently long time so that eventually the time course trajectories reach the steady state. This method works so long as the steady state is stable, it cannot be used to locate unstable steady states because such trajectories diverge. In addition, the method can sometimes be very slow to converge depending on the kinetics of the model. As a result, many simulation packages will provide an alternative method for computing the steady state that depends on the differential equation when they are set to zero. That is solving equations of the form

$$f(x) = 0$$

All numerical methods for computing solutions to equation 1.3 start with an initial estimate for the solution. The methods are then applied iteratively until the estimate converges to the solution. One of

the most well known methods for solving 1.3 is called the Newton-Raphson method. It can be easily explained using a geometric argument, Figure fig:NewtonRasphon. Suppose x_1 is the initial guess for the solution to 1.3. The method begins by estimating the slope of equation 1.3 at the value x_1, that is df/dx. A line is then drawn from the point x_1, $f(x_1)$, with slope df/dx until it intersects the x axis. The intersection, x_2, becomes the next guess for the method. This procedure is repeated until x_i is sufficiently close to the solution. From the geometry shown in Figure 1.5 one can write down the analytical equivalent of this procedure as:

$$\frac{\partial f}{\partial x_k} = \frac{f(x_k)}{x_k - x_{x+1}}$$

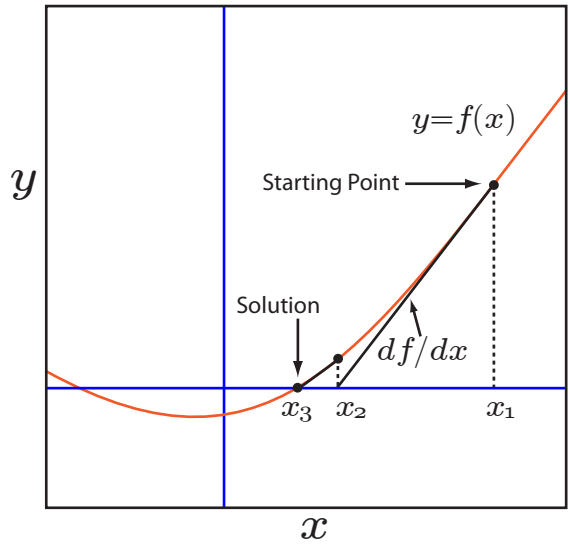

Figure 1.5: The geometry of Newton-Raphson's method

or by rearrangement:

$$x_{k+1} = x_k - \frac{f(x_k)}{\partial f/\partial x_k}$$

Iteration	Estimate
0	15
1	8.33333
2	5.666
3	5.0392
4	5.0001525
5	5.0

Table 1.1: Newton method used to compute the square root of 25

In this form we see the iterative nature of the algorithm.

Before the advent of electronic calculators that had a specific square root button, calculator users would exploit the Newton method to estimate square roots. For example, the square root of a number, a is the solution to the equation:

$$x^2 - a = 0$$

If we applying the Newton formula to this equation we obtain

$$x_{k+1} = \frac{1}{2}\left(x_k + \frac{a}{x_k}\right)$$

The table 1.1 shows a sample calculation using this equation to compute the square root of 25. Note that only a few iterations are required for convergence.

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The Newton-Raphson iteration can be terminated when the relative error is less than a certain threshold (say, 1%). The relative error is given by

$$\epsilon = \frac{x_{i+1} - x_i}{x_{i+1}} \times 100\%$$

The procedure can be made to stop in i-th step if $|f(x_i)| < \epsilon_f$ for a given ϵ_f.

The procedure should also be stopped if the number of iterations reaches a given maximal number of iterations; in this case the procedure can be restarted with a different initial approximation.

The Newton method can be easily extended to systems of equations so that we can write the Newton method in matrix form:

$$\boldsymbol{x}_{k+1} = \boldsymbol{x}_k - \left[\frac{\partial \boldsymbol{f}(\boldsymbol{x})}{\partial \boldsymbol{x}}\right]^{-1} \boldsymbol{f}(\boldsymbol{x}_k)$$

Newton Algorithm

1. Initialize the values of the concentrations of the molecules species to some initial guess.
2. Compute the values for $\boldsymbol{f}(\boldsymbol{x})$, that is the left-hand side of the differential equations $(\boldsymbol{dS}/dt)$.
3. Calculate the matrix of derivatives, $\partial \boldsymbol{f}/\partial \boldsymbol{x}$ that is $d(\boldsymbol{dS}/dt)/\boldsymbol{dS}$, at the current estimate for $\boldsymbol{x}$.
4. Compute the inverse of the matrix $\partial \boldsymbol{f}/\partial \boldsymbol{x}$
5. Using the information calculated so far, compute the next guess $\boldsymbol{x}_{k+1}$
6. Compute the new value of $\boldsymbol{f}(\boldsymbol{x})$ at $\boldsymbol{x}_{k+1}$. If the value is less than some error tolerance then assume the solution has been reached, else return to step 3, using $\boldsymbol{x}_{k+1}$ as the new starting point.

Although the Newton method is seductively simple, it requires the initial guess to be sufficiently close to the solution in order for it to converge. As a result the unmodified Newton method is rarely used in practice for computing the steady state of biochemical models. One common variant, called the Damped Newton method is sometimes used, Gepasi and SCAMP use the Damped Newton method for computing the steady state. This method....

A further strategy that is frequently used to compute the steady state is to first use a short time course simulation to bring the initial estimate closer to the steady state. The assumption here is that the steady state is stable. The final point computed in the time course is used to seed a Newton like method, if the Newton method fails to converge then a second time course simulation is carried out. This can be repeated as many times as desired. If there is a suspicion that the steady state is unstable, one can also run a time course simulation backwards in time. In general there is no sure way of computing the steady state automatically and sometimes human intervention is required to supply good initial estimates.

In the last ten years more refined Newton like methods have been devised and one that is highly recommended is NLEQ2. This is used by both Jarnac and PySCeS for computing the steady state. The stiff solver suite sundials also incorporates an equation solver, however experience has shown that is it not as good as NLEQ2.

1.4 Stability and Robustness

Biological organisms are continually subjected to perturbations. These perturbations can originate from external influences such as changes in temperature, light or the availability of nutrients. Perturbations can also arise internally due to the stochastic nature of molecular events or by genetic variation. One of the most remarkable and characteristic properties of living systems is their ability to resist such perturbations and maintain very steady internal conditions. For example the human body can maintain a constant core temperature of 36.8°C $\pm$0.7 even though external temperatures may vary widely. The ability of a biological system to maintain a steady internal environment is called **homeostasis**, a phrase introduced by Claude Bernard almost 150 years ago. Modern authors also refer to this behavior as **robustness**.

Much of the remainder of this chapter will be devoted to the homeostatic properties of biochemical pathways and what mechanisms are

employed to improve homeostasis. Before we continue that discussion we should first discuss another important property of the steady state, its stability.

A biochemical pathway is dynamically stable at steady state if small perturbations in the floating species concentrations relax back to the original state.

Figure 1.6 illustrates the results from a simulation of a simple two step biochemical pathway with one floating species, S_1. A perturbation is made to the concentration of S_1 at a certain time by adding 0.25 units of S_1. The system is now allowed to evolve further. If the system is stable, the perturbation will relax back to the original steady state, as it does in this simulation. This system is therefore stable.

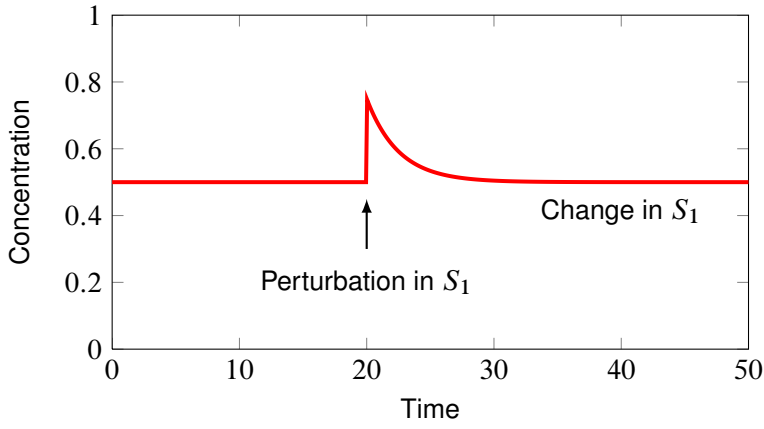
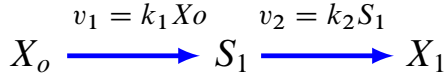


Figure 1.6: Stability of a simple biochemical pathway at steady state. The steady state concentration of the species S_1 is 0.5. A perturbation is made to S_1 by adding an additional 0.25 units of S_1 at time $= 20$. The system is deemed stable because the perturbation relaxes back to the original steady state.

Let us assume that the two step pathway has the following form:



The differential equation for the single floating species, S_1, is given by

$$\frac{dS_1}{dt} = k_1 Xo - k_2 S_1 \tag{1.5}$$

with a steady state solution $S_1 = k_1 Xo/k_2$. The question we wish to ask here is whether the steady state is stable or not?

For this simple pathway it is easy to show that a disturbance in the species, S_1, is stable. At steady state, $dS_1/dt = 0$, thus by making a small disturbance, δS_1 in S_1 we can compute the effect this has on the rate of change of δS_1 (from 1.5) to be:

$$\frac{d(\delta S_1)}{dt} = -k_2 \delta S_1 \tag{1.6}$$

This shows that after the initial disturbance, the disturbance itself declines exponentially to zero, in other words the system returns to the original steady state and the system is therefore stable. By dividing both sides by δS_1 and taking the limit to infinitesimal changes, $\partial(dS_1/dt)/\partial S_1$ is equal to $-k_2$. The stability of this simple system can therefore be determined by inspecting the sign of $\partial(dS_1/dt)/\partial S_1$. For larger systems the stability of a system can be determined by looking at all the terms $\partial(dS_i/dt)/\partial S_i$ which are given collectively by the expression:

$$\frac{d(d\boldsymbol{s}/dt)}{d\boldsymbol{s}} = \boldsymbol{J} \tag{1.7}$$

where $\boldsymbol{J}$ is called the Jacobian matrix containing elements of the form $\partial(dS_i/dt)/\partial S_i$. Equation 1.6 can be generalized to:

$$\frac{d(\delta \boldsymbol{s})}{dt} = \boldsymbol{J}\delta \boldsymbol{s} \tag{1.8}$$

where $\boldsymbol{J}$ is given by

$$\begin{bmatrix} \frac{\partial S_1/dt}{\partial S_1} & \cdots & \frac{\partial S_1/dt}{\partial S_m} \\ \vdots & \ddots & \vdots \\ \frac{\partial S_m/dt}{\partial S_1} & \cdots & \frac{\partial S_m/dt}{\partial S_m} \end{bmatrix}$$

Equation 1.8 is an example of an unforced linear differential equation and has the general structure:

$$\frac{d\boldsymbol{x}}{dt} = \mathbf{A}\boldsymbol{x}$$

Analytical solutions to such equations are well known and take the form:

$$x_j(t) = \sum_{k=1}^{n} \beta_{jk} e^{\lambda_k t}$$

That is the solutions to an unforced linear differential equations involves the sum of exponentials. The exponents of the exponentials are given by the eigenvalues of the matrix, $\mathbf{A}$. If the eigenvalues are negative then the exponents decay (stable) whereas if they are positive then the exponents grow (unstable). We can therefore easily determine the stability properties of a given model by computing the eigenvalues of the Jacobian matrix and looking for any positive eigenvalues. Note that the elements of the Jacobian matrix will often be a function of the species levels, it is therefore important that the Jacobian be evaluated at the steady state of interest.

There are many software packages that will compute the eigenvalues of a matrix and there are a small number packages that can compute the Jacobian directly from the biochemical model. For example, the script below is taken from Jarnac, it defines the simple model, initializes the model initial values, computes the steady state and then prints out the eigenvalues of the Jacobian matrix. For a simple one variable

model, the Jacobian matrix only has a single entry and the eigenvalue corresponds to that entry.

```
p = defn model
     $Xo -> S1; k1*Xo;
      S1 -> $X1; k2*S1;
end;

// Set up the model initial conditions
p.Xo = 1; p.X1 = 0;
p.k1 = 0.2; p.k2 = 0.3;

// Evaluation the steady state
p.ss.eval;
// print the eigenvalues of the model Jacobian
println eigenvalues (p.Jac);
```

phase plots

Different kinds of perturbations: step, ramp, impulse, sinusoidal, exponential

1.5 Small Perturbation Analysis

Given that deriving analytical solutions to the model equations is next to impossible except in simple or special cases, the one approach that can be used is small perturbation analysis. This technique involves making small changes around a steady state and observing the response. Small changes, strictly speaking infinitesimal changes, ensure that only the linear components of a system are stimulated.

1.6 Control Coefficients

At steady state, a reaction network will sustain a steady rate called the *flux*, often denoted by the symbol, J. The flux describes the rate of

mass transfer through the pathway. In a linear chain of reactions, the steady state flux has the same value at every reaction. In a branched pathway, the flux divides at the branch points. The flux through a pathway can be influenced by a number of external factors, these include factors such as enzyme activities, rate constants and boundary species. Thus, changing the gene expression that codes for an enzyme in a metabolic pathway will have some influence on the steady state flux through the pathway. The amount by which the flux changes is expressed by the flux control coefficient.

$$C_{E_i}^{J} = \frac{dJ}{dE_i}\frac{E_i}{J} = \frac{d\ln J}{d\ln E_i} \approx J\%/E_i\%$$

In the expression above, J is the flux through the pathway and E_i the enzyme activity of the i^{th} step. The flux control coefficient measures the fractional change in flux brought about by a given fractional change in enzyme activity. Note that the coefficient is defined for small changes.

1.7 Summation Theorem

Flux control coefficients are a useful measure to judge the degree to which a particular step influences the steady state flux. Even more interesting is that there a numerous relationships between the various coefficients. Of interest here is the summation theorem.

Consider the simple two step pathway:

$$X_o \xrightarrow{v_1} S \xrightarrow{v_2} X_1$$

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

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

where the rates are expressed as functions of their influencing factors. We will assume that each rate is a function of an enzyme concentration factor, E_i, a rate constant, k_i and a substrate and product.

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.

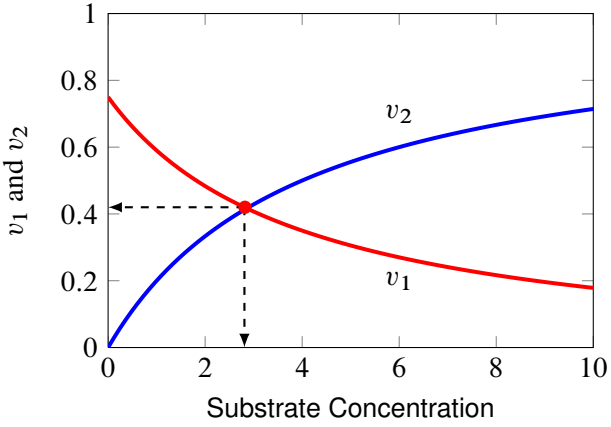


Figure 1.7: Plot of v_1 and v_2 versus the concentration of S for a simple two step pathway. The intersection of the two curve marks the point when $v_1 = v_2$, that is steady state. A perpendicular dropped from this point gives the steady state concentration of S

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

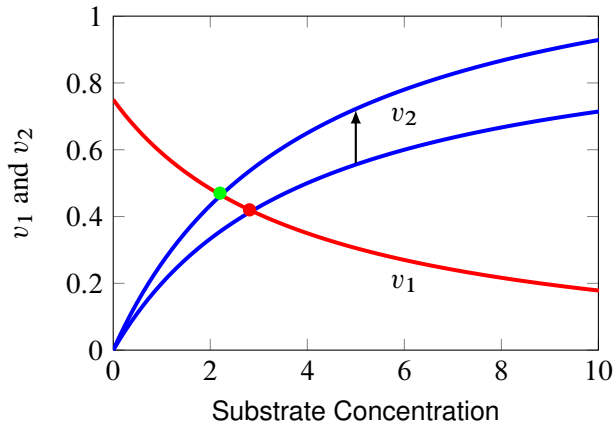


Figure 1.8: v_1 has been increased by 30% by increasing the enzyme activity on v_1. This results in a displacement of the steady state curve to the right, leading to an increase in the steady state concentration of S.

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 says 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_1 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

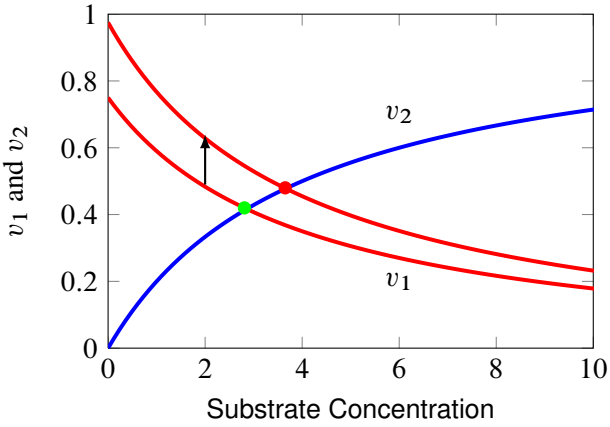


Figure 1.9: v_2 has been increased by 30% by increasing the enzyme activity on v_2. This results in a displacement of the steady state curve to the left, leading to a decrease in the steady state concentration of S.

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 fraction so that the intersection point can only move vertically. This experiment highlights an important result, when all enzyme activities are increased by the same fraction, the flux increases by that same fraction but the species or metabolite levels remain **unchanged**. We can summarize this with the following statement:

If all E_i are increased by a factor α 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

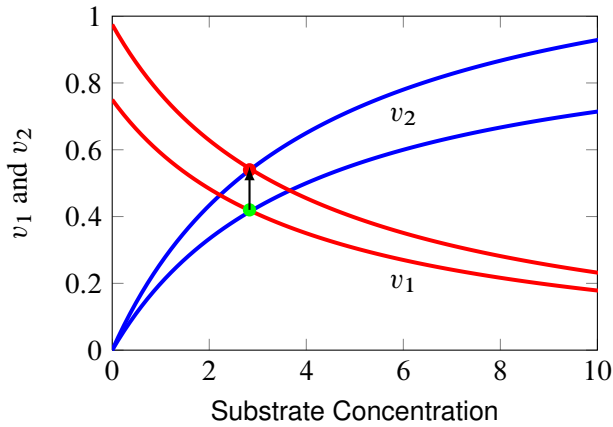


Figure 1.10: In this experiment, both v_1 and v_2 are increased by 30 %. Because both rates are increased by the same amount, the rate of change of S does not change. This means that there is no resulting change to the steady state concentration of S. The net flux through the pathway has however increased by 30 %.

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 the same 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 $v_i \propto E_i$).

The can expression the control coefficients in the following form:

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

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

These simple relations allow us to compute the change in flux or concentration given a change in an enzyme activities. 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:

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

Taking the total derivative of J:

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

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

$$\frac{\delta J}{J} = C^J_{E_1}\frac{\delta E_1}{E_1} + C^J_{E_2}\frac{\delta E_2}{E_2} + \cdots$$

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:

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

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

However, we know from the thought experiments that $\delta S = 0$ and the change in flux must equation 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:

$$\alpha = C^J_{E_1}\alpha + C^J_{E_2}\alpha$$
$$0 = C^J_{E_1}\alpha + C^S_{E_2}\alpha$$

from which we conclude:

$$1 = C^J_{E_1} + C^J_{E_2}$$
$$0 = C^J_{E_1} + C^S_{E_2}$$

These summations (or theorems) are in fact general and apply to any pathway so long as $v_i \propto E_i$:

Control Coefficient Summation Theorem

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

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

In both relationships, n, is the number of reaction steps in the pathway. The flux summation theorem suggests that there is a finite amount of 'control' (or sensitivity) and that control is shared between all steps. In addition, it states that if one step were to gain control then one or more other steps must lose control.

To summarize, these theorems suggest the following:

1) Control is shared throughout a pathway.

2) If one step gains control, one of more other steps must loose control.

3) Control coefficients are system properties, they can only be computed or measured in the intact system.

It is easy to confirm the summation theorems for the linear pathways studies in the previous section. For example, the flux control coefficient for the ith step was given by:

$$C_i^J = \frac{1/k_i \prod_{j=1}^{n} q_j}{\sum_{j=1}^{n} 1/k_j \prod_{k=j}^{n} q_k}$$

Summing this over all steps gives a value of one.

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.

1.8 Connectivity Theorem

The connectivity theorem is an extremely important result in the theory of cellular networks. The theorem relates the control coefficients to the elasticities, that is it relates system wide properties to local properties.

$$\sum_{i=1}^{n} C_{E_i}^{J} \varepsilon_{S}^{v_i} = 0$$

$$\sum_{i=1}^{r} C_{E_i}^{S_m} \varepsilon_{S_k}^{v_i} = 0$$

$$\sum_{i=1}^{r} C_{E_i}^{S_k} \varepsilon_{S_k}^{v_i} = -1$$

In the derivation of the summation theorems, certain operations were performed on the pathway such that the flux changed value but the concentrations of the metabolites were unchanged, thus $dJ/J \neq 0$ and $dS/S = 0$.

The constraints on the flux and concentration variables in the summation theorems suggest another set of operations which could accomplish the opposite. That is can we perform one or more operations to the enzymes such that $dJ/J = 0$ and $dS/S \neq 0$. The short answer is that we can and such a set of operations which preserve the flux but

change the metabolite concentrations leads to another set of theorems, called the *connectivity theorems*.

Consider the following pathway fragment:

$$\xrightarrow{v_0} S_1 \xrightarrow{v_1} S_2 \xrightarrow{v_2} S_3 \xrightarrow{v_3}$$

Let us make a change to the rate through reaction 1 (v_1) by increasing the concentration of the enzyme catalysing reaction 1 (E_1). Let us assume we increase E_1 by an amount, δE_1. This will result in a change in the steady state of the pathway. The concentration of S_2, S_3, and the flux through the pathway will rise and the concentration of S_1 will decrease because it is upstream of the disturbance.

The condition we wish to impose is now to make a second change to the pathway so that we restore the flux back to what it was before either change was made. Since the flux has increased we need to decrease the flux and we can easily do this by decreasing one of the other enzyme activities. If we decrease the concentration of E_2 this will reduce the flux. Decreasing E_2 will also cause the concentration of S_2 to increase further. However, S_1 and S_3 will change in the opposite direction to the change they made when E_1 was modulated.

In fact when E_2 is changed sufficiently so that the flux is restored to its original value, the concentrations of S_1 and S_3 will also be restored to their original values and it is only S_2 that will be different. This is true because the flux through v_o is now the same as it was originally, and coupled to the fact that E_o has not been manipulated in any way must mean that the concentrations of S_1 and all metabolites upstream of S_1 must be the same as they were before the modulations were made. The same arguments apply to S_3.

We have thus accomplished the following: E_1 has been increased by δE_1, this results in a change δJ to the flux. We know decrease the concentration of E_2 such that the change in flux compared to its original value is zero. In the process, S_2 has changed by δS_2 and neither S_1 nor S_3 have changed. In fact *no other* metabolite in the entire metabolic system has changed other than S_2.

The ability to perform such a manipulation is quite general and even

if a particular metabolite had many rates coming in and many rates leaving we would still be able to perform the necessary manipulations on all the adjacent enzymes such that only that metabolite changed in concentration and the flux was unaltered.

Flux Connectivity Theorem

We can now write down two sets of equations which apply simultaneously to the pathway, a local equation and a system equation. The system equation will describe the effect of the enzyme changes on the flux. Since the net change in flux is zero and the fact that we only changed E_1 and E_2, we can write for the change in the system flux the following system equation:

$$\frac{dJ}{J} = 0 = C^J_{E_1}\frac{dE_1}{E_1} + C^J_{E_2}\frac{dE_2}{E_2}$$

To determine the local equations we concentrate on the what is happening at a particular reaction rate. For example, as a result of making changes to E_1 and E_2, the rate change v_1 is given by

$$0 = \frac{dv_1}{v_1} = \frac{dE_1}{E_1} + \varepsilon^{v_1}_{S_2}\frac{dS_2}{S_2}$$

and at v_2

$$0 = \frac{dv_2}{v_2} = \frac{dE_2}{E_2} + \varepsilon^{v_2}_{S_2}\frac{dS_2}{S_2}$$

Note that dE_1/E_1 will not equal dE_2/E_2 and that the changes in the rates were zero. The local equations can be rearranged so that:

$$0 = \frac{dE_1}{E_1} = -\varepsilon^{v_1}_{S_2}\frac{dS_2}{S_2} \tag{1.9}$$

$$0 = \frac{dE_2}{E_2} = -\varepsilon^{v_2}_{S_2}\frac{dS_2}{S_2} \tag{1.10}$$

We can now insert dE_1/E_1 and dE_2/E_2 from the local equations into the system equations and obtain:

$$0 = \frac{dJ}{J} = -\left(C_{E_1}^{J}\varepsilon_{S_2}^{v_1}\frac{dS_2}{S_2} + C_{E_2}^{J}\varepsilon_{S_2}^{v_2}\frac{dS_2}{S_2}\right)$$

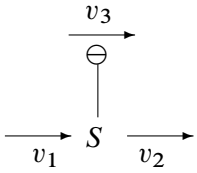
and therefore:

$$0 = \frac{dS_2}{S_2}\left(C_{E_1}^{J}\varepsilon_{S_2}^{v_1} + C_{E_2}^{J}\varepsilon_{S_2}^{v_2}\right)$$

Since we know that dS_2/S_2 is not equal to zero then it must be true that

$$0 = C_{E_1}^{J}\varepsilon_{S_2}^{v_1} + C_{E_2}^{J}\varepsilon_{S_2}^{v_2}$$

This derivation is quite general and can be applied to a metabolite that interacts with any number if steps, In general the sum of the terms will equal the number of interactions a metabolite makes. For example, in the pathway fragment:



where S interacts with it production rate, v_1, a consumption rate, v_2, and an inhibitory interaction with v_3, then the connectivity may be written as

$$C_{E_1}^{J}\varepsilon_{S}^{v_1} + C_{E_2}^{J}\varepsilon_{S}^{v_2} + C_{E_3}^{J}\varepsilon_{S}^{v_3} = 0$$

$$0 = \sum_{i=1}^{r} C_{E_i}^{J}\varepsilon_{S}^{v_i}$$

Concentration Connectivity Theorem

To derive the flux connectivity theorem we had to use the system equation that was related to the flux. It is however possible to use a different set of systems equations, those with respect to the metabolite concentrations. In the case of the metabolites there will be two distinct systems equations. One of these will describe the effect that our modulations have on the common metabolite (S_2 in the example), and a second describing the effect on any other metabolite (S_1, S_3, etc.) in the pathway. Consider first the system equation involving the common metabolite; for the pathway under consideration this equation is given by:

$$\frac{dS_2}{S_2} = C_{E_1}^{S_2}\frac{dE_1}{E_1} + C_{E_2}^{S_2}\frac{dE_2}{E_2}$$

We must remember that the change in the common metabolite, dS_2/S_2, is non-zero. Therefore substituting in the local equations given previously leads to:

$$\frac{dS_2}{S_2} = -C_{E_1}^{S_2}\varepsilon_{S_2}^{v_1}\frac{dS_2}{S_2} - C_{E_2}^{S_2}\varepsilon_{S_2}^{v_2}\frac{dS_2}{S_2}$$

Since $dS_2/S_2 \neq 0$, we can cancel the term dS_2/S_2 and this leads to the first concentration connectivity theorem:

$$-1 = C_{E_1}^{S_2}\varepsilon_{S_2}^{v_1} + C_{E_2}^{S_2}\varepsilon_{S_2}^{v_2}$$

A second theorem can be derived by considering the effect of our modulations on a distant metabolite, for example S_3. In this case, the system equation now with respect to S_3, becomes:

$$0 = \frac{dS_3}{S_3} = C_{E_1}^{S_3}\frac{dE_1}{E_1} + C_{E_2}^{S_3}\frac{dE_2}{E_2}$$

Note that the equation equals zero because our operations ensure that metabolites other than the common metabolite do not change in concentration.

Substituting once again the local equations into the above system equation leads us to:

$$0 = \frac{dS_3}{S_3} = -C_{E_1}^{S_3}\varepsilon_{S_2}^{v_1}\frac{dS_2}{S_2} - C_{E_2}^{S_3}\varepsilon_{S_2}^{v_2}\frac{dS_2}{S_2}$$

or

$$0 = -\frac{dS_2}{S_2}\left(C_{E_1}^{S_3}\varepsilon_{S_2}^{v_1} + C_{E_2}^{S_3}\varepsilon_{S_2}^{v_2}\right)$$

However, we know that dS_2/S_2 is not zero, therefore it must be the case that:

$$0 = C_{E_1}^{S_3}\varepsilon_{S_2}^{v_1} + C_{E_2}^{S_3}\varepsilon_{S_2}^{v_2}$$

That completes the proof for the concentration connectivity theorems. As with the flux connectivity theorems, the concentration connectivity theorems can be generalized to any number of steps that a metabolite may interacts with.

To summarize, the connectivity theorems are:

Flux Connectivity Theorem with respect to a common metabolite, $\mathbf{S_k}$ where r is the number of interactions.

$$\boxed{\sum_{i=1}^{r} C_{E_i}^{J}\varepsilon_{S_k}^{v_i} = 0}$$

Concentration Connectivity Theorem with respect to the common metabolite $\mathbf{S_k}$ where r is the number of interactions.

$$\boxed{\sum_{i=1}^{r} C_{E_i}^{S_k}\varepsilon_{S_k}^{v_i} = -1}$$

Concentration Connectivity Theorem with respect to the common metabolite $\mathbf{S_k}$ and a distant metabolite, $\mathbf{S_m}$ where r is the number of interactions.

$$\sum_{i=1}^{r} C_{E_i}^{S_m} \varepsilon_{S_k}^{v_i} = 0$$

Interpretation The connectivity theorems are important for a number of reasons. The first and foremost is that the theorems link local effects in terms of the elasticities to global effects in terms of the control coefficients. Consider for example the following linear pathway.

$$\xrightarrow{v_1} S_1 \xrightarrow{v_2}$$

The flux connectivity can be written in the form:

$$\frac{C_{E_1}^{J}}{C_{E_2}^{J}} = -\frac{\varepsilon_{S_1}^{v_2}}{\varepsilon_{S_1}^{v_1}}$$

That is the ratio of two adjacent flux control coefficients is inversely proportional to the ratio of the corresponding elasticities. This confirms the result in the last chapter where it was seen that high flux control coefficients tend to be correlated with small elasticities of the enzyme and small flux control coefficients with large elasticities. This was easily explained in terms of changes to metabolites opposing changes in rates by metabolites moving in a direction opposite to the rate change. Since metabolites with high elasticities are able to oppose rate changes more effectively than small elasticities then it follows that large elasticities are associated with small flux control coefficients and *vice versa*. The classic example of this is the case of a reaction operating near equilibrium where the elasticities are very high relative to adjacent elasticities on neighboring enzymes. In such situations the flux control coefficients of near equilibrium enzymes are *likely* to be small. However, one must bear in mind that it is the

ratio of elasticities which is important and not their absolute values. Simply examining the elasticity of a single reaction may lead to incorrect conclusions. Even more so, one must also consider all the ratios of the elasticities along a pathway because even though one elasticity ratio may suggest a high or low flux control coefficient on a particular enzyme, it is a consideration of the other ratios coupled to the flux summation theorem that will give an absolute value to a particular flux control coefficient. Control coefficients are truly system wide properties. The examination of a single enzyme will not give an absolute indication of the ability of that enzyme to control the flux.

1.9 Computing Control Equations

In previous sections we have seen how the control coefficients can give useful information on the robustness of a network to parameter changes. In addition we have seen that relationships exist between the control coefficients and the elasticities. In this section we will look at ways to express the control coefficients in terms of the elasticities of which there are a number. The simplest way to derive the control equations is to combine the summation and connectivity theorems. For example, a two step pathway such as:

$$X_o \xrightarrow{v_1} S \xrightarrow{v_2} X_1$$

There is one connectivity theorem for every species in a pathway so that in the above example there will only be one connectivity theorem centered around S:

$$C^J_{E_1}\varepsilon^{v_1}_S + C^J_{E_2}\varepsilon^{v_2}_S = 0$$

In addition, there will be a flux summation theorem:

$$C^J_{E_1} + C^J_{E_2} = 1$$

These two equations can be combined to give expressions that relate

the control coefficients in terms of the elasticities, thus:

$$C_{E_1}^J = \frac{\varepsilon_S^2}{\varepsilon_S^2 - \varepsilon_S^1}$$

$$C_{E_2}^J = -\frac{\varepsilon_S^1}{\varepsilon_S^2 - \varepsilon_S^1}$$

These equations, possibly the most important result of the theory, allow us to understand how system responses depend on local properties.

Using these equations we can look at some simple extreme behaviors. For example, let us assume that the first step is completely insensitive to its product, S, then $\varepsilon_S^1 = 0$. In this case, the control coefficients reduce to:

$$C_{v_1}^J = 1$$

$$C_{v_2}^J = 0$$

That is all the control (or sensitivity) is on the first step. This situation represents the classic rate-limiting step that is frequently mentioned in text books. The flux through the pathway is completely dependent on the first step. Under these conditions, no other step in the pathway can affect the flux. The effect is however dependent on the complete insensitivity of the first step to its product. Such a situation is likely to be rare in real pathways. In fact the classic rate limiting step has almost never been observed experimentally. Instead, a range of "limitingness" is observed, with some steps having more "limitingness" (control) than others. We can shift control off the first step by increasing the product inhibition.

For more complex pathways such as branches and moiety conserved cycles, additional theorems are required.

Hofmeyr matrix equation, scaling

Advanced: state variable approach.

Software ?

Examples.

1.10 Response Coefficients

Control coefficients measure the response of a pathway to changes in enzyme activities. What out the effect or external factors such as inhibitors, pharmaceutical drugs or boundary species? Such effects are measured by another coefficient called the response coefficient. The flux response coefficient is defined by:

$$R_X^J = \frac{dJ}{dX}\frac{X}{J}$$

and the concentration response coefficient by:

$$R_X^S = \frac{dS}{dX}\frac{X}{S}$$

The response coefficient measures how sensitive a pathway is to changes in external factors other than enzyme activities. What is the relationship of the response coefficients with respect to the control coefficients and elasticities?

Like many proofs in this theory we can carry out a thought experiment as follows. Consider the pathway fragment below:

$$X \xrightarrow{v_1} S \xrightarrow{v_2}$$

where X is the pathway boundary species. Let us increase the activity of v_1 by increasing the concentration of E_1. This will cause the steady state flux and concentration of S and in fact all downstream species to increase. Let us now decrease the concentration of X such that we restore the flux and steady state concentration of S back to its original value. From this thought experiment we can write the operations in terms of the local response equation and a system response equation as follows:

$$\frac{\delta v_1}{v_1} = \varepsilon_X^{v_1}\frac{\delta X}{X} + \varepsilon_{E_1}^{v_1}\frac{\delta E_1}{E_1} = 0$$

$$\frac{\delta J}{J} = R_X^J\frac{\delta X}{X} + C_{E_1}^J\frac{\delta E_1}{E_1} = 0$$

We can eliminate the $\delta E_1/E_1$ term in the system response equation by substituting the term from the local response equation and recalling that $\varepsilon_{E_1}^{v_1} = 1$ we can obtain:

$$0 = R_X^J \frac{\delta X}{X} - C_{E_1}^J \varepsilon_X^{v_1} \frac{\delta X}{X}$$

From this we conclude that:

$$R_X^J = C_{E_1}^J \varepsilon_X^{v_1}$$

This gives use the relationship we seek. It can be generalized for multiple external factors acting simultaneously but summing up individual responses:

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

Likewise the response of a species, S to an external factor is given by:

$$R_X^S = \sum_{i=1}^{n} C_{E_i}^S \varepsilon_X^{v_i}$$

The response coefficient carries an important message, which is that the response of some external factor, X, is a function of two things, the effect the factor has on the step it acts upon and the effect that the step itself has on changing the system. This means than an effective external factor, such as a pharmaceutical drug, must not only be able to bind and inhibit the enzyme being targeted, but the step itself must be able to transmit the effect to the rest of the pathway.