Introduction to the Quantitative Control of Metabolism

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In this section of the course we will look at some of the basic principles by which metabolic networks operate and are controlled.

Control and **regulation** are two words that are frequently used in the literature in relation to cellular network studies. We don't have an adequate measure of what regulation is or in fact what it means. We do however have a quantitative definition of what we mean by control.

In metabolism, control is the ability of an outside agent to influence a system. The more influence an outside agent has the more control it has over the network. Regulation is the mechanism that allows or does not allow the influence to have control. To give two familiar examples. A driver of a car has control over the speed and direction by way of an active regulatory system that includes power steering and the automatic gear box. In contrast, a cruise control allows the speed of a car to be stable even though the vehicle may be forced to climb hills or speed up down hills. Sometimes regulation is passive and other times active. Active regulation is more interesting because it will usually involve special structures or network motifs to enable the regulation to occur.

1. A regulatory system that tracks is called a servo system.

2. A regulatory system that opposes control is called a homeostatic system.

Often systems are a combination of the two. For example, in order to do tracking, some elements of the system are being maintained constant, while in order to maintain hemostasis some elements of the system much be changing.

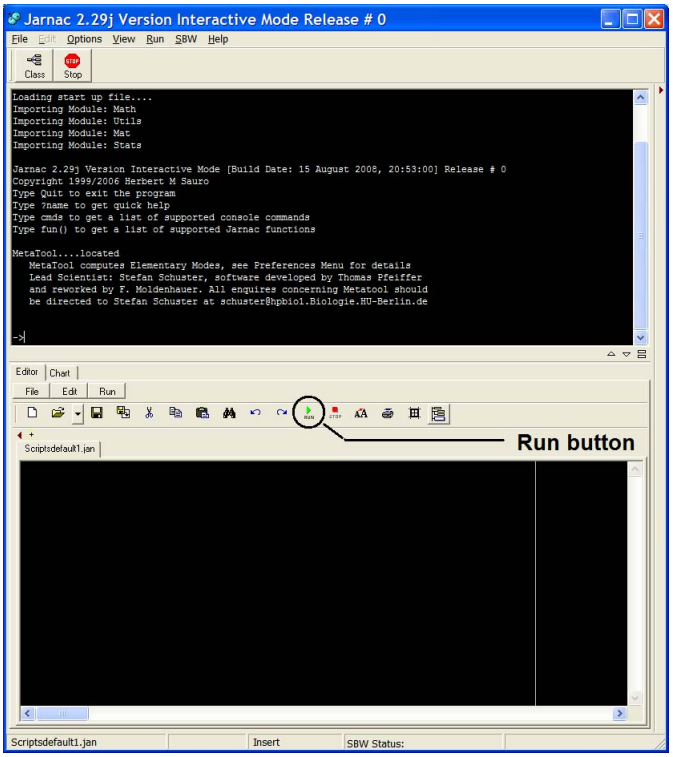
How can be quantify these ideas?

In cellular networks it is customary to partition a subsystem, such as glycolysis or oxidative phosphorylation and study it in isolation. Under these conditions it is possible to determine what the outside influences are and what the internal regulating mechanisms are. Thus if we take a simple metabolic pathway catalyzed by a series of enzymes, we can consider the enzyme activities under the control of an outside agent (gene expression or drug intervention), whereas the metabolite levels together with feedback

or feed-forward loops in the pathway are the regulating elements.

1. Computer Simulation Experiment: Simple First-Order Decay

We will be using Jarnac as the software tool to conduct metabolic simulation experiments.



Screenshot of Jarnac: Top panel is the console; Bottom panel is the editor

Jarnac has two windows, an upper console and a lower editor window. All models are entered into the lower window. Models can be controlled from the upper window.

In the lower window enter the following model:

```
p = defn model
     J1: S1 -> S2; k1*S1;
end;
```

```
p.S1 = 10;
p.S2 = 0;
p.k1 = 0.1;

m = p.sim.eval (0, 60, 100, [<p.time>, <p.S1>]);
graph (m);
```

To run this model click on the small green button in the center of the screen.

2. Simulating Two Steps

Let us now change the model and include an additional step:

```
p = defn model
     J1: S1 -> S2; k1*S1;
     J2: S2 -> S3; k2*S2;
end;

p.S1 = 10;
p.S2 = 0;
p.k1 = 0.1;
p.k2 = 0.2;

m = p.sim.eval (0, 60, 100, [<p.time>, <p.S1>, <p.S2>, <p.S3>]);
graph (m);
```

3. More realistic kinetics

In reality most kinetics are not irreversible. To make the last model more realistic we will make each step reversible:

```
p = defn model
     J1: S1 -> S2; k11*S1 - k12*S2;
     J2: S2 -> S3; k21*S2 - k22*S3;
end;

p.S1 = 10;
p.S2 = 0;
p.k11 = 0.1; p.k12 = 0.08;
p.k21 = 0.2; p.k22 = 0.06;

m = p.sim.eval (0, 60, 100, [<p.time>, <p.S1>, <p.S2>, <p.S3>]);
graph (m);
```

What happens to the concentrations in this model?

What happens to the net reaction rates? We can output the reaction rates by specifying the reaction name in the output list:

```
p = defn model
     J1: S1 -> S2; k11*S1 - k12*S2;
     J2: S2 -> S3; k21*S2 - k22*S3;
end;

p.S1 = 10;
p.S2 = 0;
p.k11 = 0.1; p.k12 = 0.08;
p.k21 = 0.2; p.k22 = 0.06;

m = p.sim.eval (0, 60, 100, [<p.time>, <p.S1>, <p.S2>, <p.S3>, <p.J1>, <p.J2>]);
graph (m);
```

What happens to the reaction rates?

4. Simulating an Open System

The previous examples illustrate models that are **closed**, that is no mass enters or leaves the system. To simulate an **open system** we must fix the boundaries. This is done easily by adding a $ symbol in front of each fixed species.

In the model below the previous model was been modified by adding two more reactions, one from a fixed source (Xo) and another to a fixed sink (X1).

```
p = defn model
     J1: $Xo -> S1;  k0*Xo;
     J2:  S1 -> S2;  k1*S1;
     J3:  S2 -> S3;  k2*S2;
     J4:  S3 -> $X1; k3*S3;
end;

p.Xo = 10;
p.S1 = 0;
p.S2 = 0;
p.S3 = 0;
p.X1 = 0;

p.k0 = 0.05;
```

```
p.k1 = 0.1;
p.k2 = 0.2;
p.k3 = 0.34;

m = p.sim.eval (0, 60, 100, [<p.time>, <p.S1>, <p.S2>, <p.S3>, <p.J1>, <p.J2>]);
graph (m);
```

Explain what is happening to the metabolite concentrations and reaction rates as the simulation proceeds?

5. Adding enzyme kinetics

We will now modify the model so that each reaction is governed by a reversible Michaelis-Menten rate law.

```
p = defn model
     J1: $Xo -> S1;  (Vm1/Km1)*(Xo - S1/Keq1)/(1 + Xo/Km1 + S1/Km2);
     J2:  S1 -> S2;  (Vm2/Km3)*(S1 - S2/Keq2)/(1 + S1/Km3 + S2/Km4);
     J3:  S2 -> S3;  (Vm3/Km4)*(S2 - S3/Keq3)/(1 + S2/Km5 + S3/Km6);
     J4: S3 -> $X1;  (Vm4/Km7)*(S3 - X1/Keq4)/(1 + S3/Km7 + X1/Km8);
end;

p.Xo = 10;
p.S1 = 0;
p.S2 = 0;
p.S3 = 0;
p.X1 = 0;

p.Vm1 = 1.5; p.Vm2 = 2.9; p.Vm3 = 10.5; p.Vm4 = 7.3;
p.Km1 = 1; p.Km2 = 1;
p.Km3 = 0.2; p.Km4 = 3.4;
p.Km5 = 0.9; p.Km6 = 1.2;
p.Km7 = 6.7; p.Km8 = 1.1;

p.Keq1 = 3.2;
p.Keq2 = 2.1;
p.Keq3 = 14.6;
p.Keq4 = 5.3;

m = p.sim.eval (0, 140, 200, [<p.time>, <p.S1>, <p.S2>, <p.S3>, <p.J1>, <p.J2>]);
graph (m);
p.ss.eval;

println p.J1;
```

There is a quick way to compute the steady state of a model:

```
p.ss.eval;
```

We can also print out variable values using the `println` command.

6. Perturbation experiments

What kinds of experiments can we do with the model in experiment 5? One thing we can do is investigate how much each enzyme controls the level of the steady state flux. To do this try the following:

1. Compute the steady state and record the steady state flux.
2. Increase the enzyme activity of E_1 by 5%, i.e change `Vm1` by 5%
3. Recompute the steady state flux and compute the % change in flux
4. Compute the ratio of J% to E%
5. Repeat for each enzyme step in the pathway.

Carry out this experiment and fill in the following table:

E%	J%	Ratio
	Total:	

In the last entry, total up the ratios and record the value.

The ratio J% to E% is called the **flux control coefficient** (C_E^J) and indicates how much control a particular enzyme has over the flux. What determines the individual values of C_E^J?

We can get some idea from the disequilibrium ratio. Given the following reaction

$$S_1 \longrightarrow S_2$$

The equilibrium constant is given by:

$$K_{eq} = \frac{S_2}{S_1}$$

The mass-action ratio, Γ is given by:

$$\Gamma = \frac{S_2}{S_1}$$

Γ is the ratio of metabolites at steady state. The disequilibrium ratio, ρ, is then given by:

$$\rho = \frac{\Gamma}{K_{eq}}$$

If the step is near equilibrium then $\rho \simeq 1$, if the step is away from equilibrium then $\rho \ll 1$.

$$C_1^J : C_2^J : C_3^J : C_4^J = (1-\rho_1) : \rho_1(1-\rho_2) : \rho_1\rho_2(1-\rho_3) : \rho_1\rho_2\rho_3(1-\rho_4)$$

or for an arbitrary length pathway, the n^{th} term is equal to:

$$\left(\prod_{i=2}^{n-1} \rho_i\right)(1-\rho_n)$$

Consider the following question. If the ith reaction is irreversible what will be its disequilibrium value? Hint: What would the value for the equilibrium constant be? Answer on next page.

The disequilibrium ratio for an irreversible step is ZERO (Keq is infinity, no reactants at equilibrium). What effect will this have on the distribution of control? For example, assume the second step is irreversible, $\rho = 0$. This means that all terms from the third onwards are zero ($\rho_1\rho_2(1 - \rho_3)$. This means that there is not control beyond the irreversible step? What is the mechanistic explanation for this?

The second question is what happens to ρ is a reaction is at equilibrium?

Give an answer to the above, what effect does this have on the distribution of control?

Finally, it is also possible to show that the disequilibrium ratio is equal to the ratio of the reverse rate and forward rate:

$$\rho = \frac{v_r}{v_f}$$

Show that the above relation is true for the simple reversible reaction:

$$S_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S_2$$

Since the forward rate will always to greater than the reverse rate for a positive net rate, the disequilibrium ratio will always be less than one.

$$\rho \leq 1$$

Since ρ is always less than one, the tendency is for most of the flux sensitivity to be at the front of the pathway since downstream steps have more and more multipliers of ρ values that are less than one.

Computing control coefficients by computer. A quick way to compute the control coefficients is to use the commands:

```
println p.cc (<p.J1>, p.Vm1);
println p.cc (<p.J1>, p.Vm2);
println p.cc (<p.J1>, p.Vm3);
println p.cc (<p.J1>, p.Vm4);
```

7. Effect of negative feedback: Part I

Experiments have shown that only a fraction of control (20%) is found near the front of a given pathway and most of the control is located at the end. In this section we will investigate why this is so.

Let us change the previous model by adding a negative feedback loop to the first step:

```
p = defn model
     J1: $Xo -> S1;  (Vm1/Km1)*(Xo - S1/Keq1)/(1 + Xo/Km1 + S1/Km2 + pow(S3,J0_h));
     J2:  S1 -> S2;  (Vm2/Km3)*(S1 - S2/Keq2)/(1 + S1/Km3 + S2/Km4);
     J3:  S2 -> S3;  (Vm3/Km4)*(S2 - S3/Keq3)/(1 + S2/Km5 + S3/Km6);
     J4: S3 -> $X1;  (Vm4/Km7)*(S3 - X1/Keq4)/(1 + S3/Km7 + X1/Km8);
end;

p.Xo = 10;
p.S1 = 0;
p.S2 = 0;
p.S3 = 0;
p.X1 = 0;
p.J0_h = 0;  // This controls the strength of the feedback

p.Vm1 = 1.5; p.Vm2 = 2.9; p.Vm3 = 10.5; p.Vm4 = 7.3;
p.Km1 = 1; p.Km2 = 1;
p.Km3 = 0.2; p.Km4 = 3.4;
p.Km5 = 0.9; p.Km6 = 1.2;
p.Km7 = 6.7; p.Km8 = 1.1;

p.Keq1 = 3.2;
p.Keq2 = 2.1;
p.Keq3 = 14.6;
p.Keq4 = 5.3;

m = p.sim.eval (0, 140, 200,
       [<p.time>, <p.S1>, <p.S2>, <p.S3>, <p.J1>, <p.J2>]);
graph (m);
p.ss.eval;
println p.cc (<p.J1>, p.Vm1);
println p.cc (<p.J1>, p.Vm2);
println p.cc (<p.J1>, p.Vm3);
println p.cc (<p.J1>, p.Vm4);
```

Your task is to investigate how the distribution of control changes as the strength of the feedback is increased. Explain what you find..

8. Effect of negative feedback: Part II

```
p = defn feedback

J0: $X0 -> S1; J0_VM1*(X0-S1/J0_Keq1)/(1+X0+S1+pow(S4,J0_h));
J1: S1 -> S2; (10*S1-2*S2)/(1+S1+S2);
J2: S2 -> S3; (10*S2-2*S3)/(1+S2+S3);
J3: S3 -> S4; (10*S3-2*S4)/(1+S3+S4);
J4: S4 -> $X1; J4_V4*S4/(J4_KS4+S4);
```

```
end;

p.X0 = 10;
p.X1 = 0;
p.S1 = 0;
p.S2 = 0;
p.S3 = 0;
p.S4 = 0;
p.J0_VM1 = 10;
p.J0_Keq1 = 10;
p.J0_h = 1;
p.J4_V4 = 2.5;
p.J4_KS4 = 0.5;

m = p.sim.eval (0, 100, 200, [<p.time>, <p.S4>]);
graph (m);
```

Run this model and investigate its time dependent properties as you increase the feedback strength.

1 Quantitative Theory

Useful reading material:

1. H Kacser, JA Burns, DA Fell - Biochemical Society Transactions, 1995 (Or original from 1973) (Use Google scholar and search for "control of flux", it will be the first match.

2. Anything written by David Fell (including his text book), Athel Cornish-Bowden and Jannie Hofmeyr

3. A recent paper by Piero Morandini, on plant metabolism but a very fun read. Rethinking metabolic control. Plant Science (2009), 176(4), 441-451

To make things simpler we will only consider properties at steady state.

Consider the simplest possible pathway:

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

We will assume that the two boundary species, X_o and X_1 are external and fixed by the observer. In addition each reaction is catalyzed by an enzyme

at a rate v_1 and v_2 respectively by enzymes E_1 and E_2. The species S is a state variable of the system and can change depending on the enzyme activities, kinetic constants and boundary species concentrations. At steady state the following relation will be true:

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

where the rates are expressed as functions of their influencing factors. There is a simple graphical technique we can used 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 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, $v \propto E$, if not, then there is probably some other parameter that is.

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

The above plot represents our reference state, in the plot we graph v_1 and v_2 against 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 to the intersection points marks the steady state concentration of S

Let us now change the activity of E_2 by 30%. Note that the curve for v_2 is scaled upwards, this in turns moves the intersection point to the left, indicating that the steady state concentration of S **decreases** relative to the reference state.

Let us now change the activity of E_1 by 30%. Note that the curve for v_1 is scaled upwards, this in turns 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. That is

$$\delta J > 0 \text{ but } \delta S = 0$$

This a very important result.

From these thought experiments we conclude that by increasing the activities of both enzymes by the **same fraction** we increase the flux through the pathway but do not change the concentration of the pathway species, S. This conclusion is in fact quite generate and no matter how complex the pathway, if we increase the activity of every step in the pathway by a given proportion, the same result will be obtained. 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

Let us consider another thought experiment. Assume the pathway is at steady state, let us now increase the activity of enzyme E_1 by a factor α, 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 δJ and the species concentration by δS. 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 what are called the control coefficient:

$$C^J_{E_i} = \frac{dJ}{dE_i}\frac{E_i}{J}$$

$$C^{S_j}_{E_i} = \frac{dS_j}{dE_i}\frac{E_i}{S_j}$$

Here we have generalized the definition 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 coefficients 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. 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.

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:

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

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

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:

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

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

We can do this because the changes are assumed to be small and therefore only the linear modes are stimulated.

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:

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

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

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 loose 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. In reality no one has measured a control coefficient of one, and in fact control is distributed throughout a pathway, sometimes there may be 'hot spots' where more control is concentrated but in general the notion of the rate-limiting step is a theoretical and experimental fiction.