

Introduction to the Quantitative Control of Metabolism

August, 2009: Herbert M Sauro

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

Two words are frequently when we talk about cellular network studies, **control** and **regulation**.

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 outside influence to have control. Sometimes regulation is passive and other times active. Active regulation may be the more interesting as it will usually involve special structures or network motifs to enable the regulation. An example of passive regulation is where an enzyme is near saturation and and change in the substrate concentration causes no change in the reaction rate.

An indicated before, some regulation opposes change while other types of regulation encourages change. A driver of a car has control over the speed and direction by way of an active regulatory system such as 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.

1. A regulatory system that tracks an external control signal is called a servo system (Car steering).
2. A regulatory system that opposes control is called a homeostatic system (Cruise control).

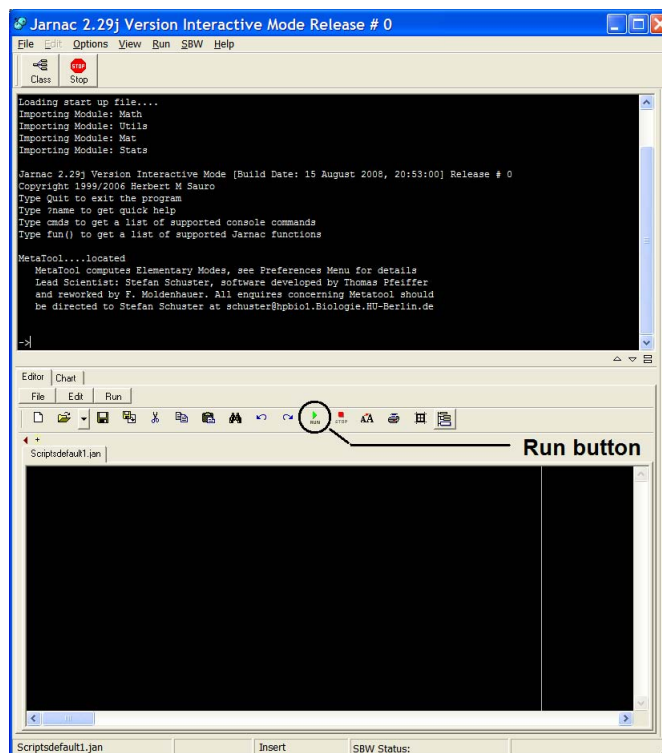
Often systems are a combination of the two. For example, in order to track some element of the system is being maintained constants, while in order to maintain homeostasis some elements of the system must be changing to compensate.

How can we 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 v_{m1} 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.

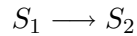
Carry out this experiment and fill in the following table:

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



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 i th 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 (K_{eq} 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?

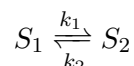
The second question is what happens to ρ if a reaction is at equilibrium?

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:



Since the forward rate will always be 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