MODELLING CELLULAR PROCESSES IN SPACE AND TIME
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A PRIMER ON DETERMINISTIC MODELS OF BIOCHEMICAL REACTIONS

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Contents

1. Preliminaries

This course introduces ordinary differential equations as a mathematical method to study regulation of gene expression by transcription regulators and in modelling the dynamics of metabolic networks.

Margin notes: 'Mpower' indicate insights that can only be achieved through mathematical modelling. 'Asmptn' indicate biological assumptions made in order to device a mathematical model. 'Ex' indicate a relvant exercise; see section 8 for the exercises.

2. Motivation for using mathematical models

"Mathematics provides a way to test experimental hypotheses".

Mathematics can be viewed as a tool to derive logical consequences from propositions; if a mathematical reasoning from a given hypothesis leads to a prediction that is not true, then the hypothesis is not true.

The power of mathematics comes from:

(1) The length of the chains of reasoning that rigorous logic and mathematical arguments make possible;
(2) The conclusions that can be reached, often revealing nonintuitive conclusions.

If this is not enough to convince you that mathematics can contribute in the study of biological systems, you may be interested in knowing what Darwin thought:

"...I have deeply regretted that I did not proceed far enough at least to understand something of the great leading principles of mathematics, for men thus endowed seem to have an extra sense."

The life and letters of Charles Darwin - day 13 of 188.

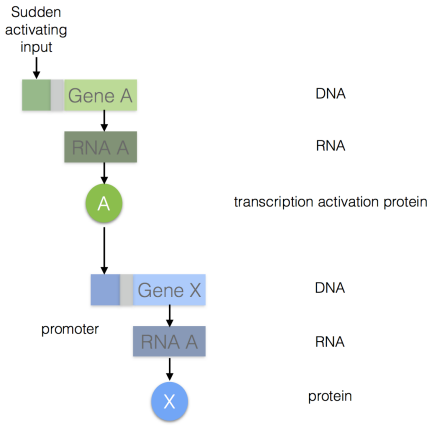
In this course we will focus on the principles of mathematics that teach us about the **dynamics** of molecular interactions. We will illustrate these principles using the regulation of gene expression by transcription regulators. The same principles apply also to the post-translational regulatory systems that govern cell signalling, cell-cycle control, and metabolic networks, amongst others.

3. Example 1: Regulatory networks of gene transcription depend on molecular interactions

To meaningfully asses the biological impact of any interaction in the cell, we need to know how molecules interact (concentrations, affinities, kinetic behaviours) and **how behaviours of the molecules change over time, i.e. the dynamics of the species**.

We begin with the simple system illustrated in figure 1: A gene encoding the activator gene $geneA$ will produce as its product protein A, via an RNA intermediate. Protein A will then bind to the regulatory promoter of gene $geneX$, p_X, to form the complex $A : p_X$. Once it is formed, it stimulates the production of an RNA transcript that is subsequently translated to produce protein X.

FIGURE 1. Some of the biochemical steps in the activation of gene expression by a transcription activator.



We are interested in knowing when transcription is induced. Therefore we will concentrate on understanding the interaction between protein A and the promoter region p_X. Specifically, we will investigate the amount of bound promoter complex $A : p_X$.

Note that complex $A : p_X$ can dissociate, and this is illustrated with the following notation:

$$A + p_X \rightleftharpoons A : p_X \tag{1}$$

Recall that the formation of the complex $A : p_X$ (between two binding partners A and p_x) depends on the **rate constant** k_{on}, which describes how many *productive* collisions occur per unit time, per protein, at a given concentration of the substrates. Similarly, there is a dissociation constant k_{off}. The rate of complex $A : p_x$ formation equals the product of the rate constant and the concentrations of the substrates. Similarly, the complex dissociation occurs at a rate k_{off} multiplied by the concentration of the complex. These are called the **rates of the reactions**. So we have:

$$\text{rate of complex formation} = k_{on}[A][p_X]$$
$$\text{rate of complex dissociation} = k_{off}[A : p_X]$$

3.1. **At steady state.**

Let us consider the **steady state** of this system. The steady state is the moment when chemical equilibrium is attained; there is no net change in free energy to drive the reaction in either direction and, as a result, *association rate* $=$ *dissociation rate*. Therefore, an **equilibrium constant**[1], K, can be defined to be the ratio of the association and dissociation constants, at equilibrium:

$$\textit{association rate} = \textit{dissociation rate} \Rightarrow k_{on}[A][p_X] = k_{off}[A : p_X] \Rightarrow \frac{[A : p_X]}{[A][p_X]} = \frac{k_{on}}{k_{off}} = K$$

Therefore, the amount of bound promoter complex $[A : p_X]$ *at equilibrium* is $K[A][p_X]$; that is:

$$[A : p_X] = K[A][p_X]$$

[1]The equilibrium constant measures the binding strength between molecules and it is larger, the greater the binding strength. Half of the binding sites are occupied by the ligands when the ligand's concentration reaches a value of 1/K.

Mpower Now, let's consider what happens when the concentration of A increases by, say, a factor of 10. Intuitively from equation (1), we know that $A : p_X$ should increase too, but we can not determine the amount of the increase without additional information (the concentrations of the components and the affinity of the binding interaction. In general, we know the total concentrations of the reactants, and not the concentrations of the bound and unbound species (which are $[A]$ and $[p_X]$). Since in a cell there are typically many copies of A, but only one or two copies of p_X (i.e. one gene
Asmptn of X per haploid genome), as far as A is concerned, it is safe to assume that $[A : p_X]$ is negligible, relative to the total amount of A, A^T. So we preserve the notation A to denote the total amount of A. For p_X, the total concentration of p_X is $[p_X^T] = [p_X] + [A : p_X]$. We can rewrite the amount of bound promoter in terms of the total concentration of p_X:

$$[A : p_X] = K[A]([p_X^T] - [A : p_X])$$

$$\Rightarrow [A : p_X] = \frac{K[A]}{1 + K[A]}[p_X^T]$$

Mpower We are now ready to determine the effect of incrementing the concentration of A on the number of complexes $[A : p_X]$ at steady state: Suppose $K = 10^8 M^{-1}$, $[A] = 10^{-9} M$, $[p_X^T] = 10^{-10} M$ (under the assumption that there is one copy of the gene in a haploid cell, e.g. with a volume of $2 \times 10^{-14} L$). Then, by $[A : p_X] = \frac{K[A]}{1+K[A]}[p_X^T]$, a 10-fold increase of $[A]$ from $10^{-9} M$ to $10^{-8} M$, increases $[A : p_X]$ 5.5-fold, from 0.09×10^{-10} to 0.5×10^{-10}. In other words, a 10-fold increase in $[A]$ induces a 5.5-fold increase in transcription of X.

```
*In MATLAB, in a new script file*

%% effect of increasing A, on steady state
K = 10^8;
A1 = 10^(-9);
A2 = 10^(-8);
pX_T = 10^(-10);
ApX1 = ((K*A1)/(1+K*A1))*pX_T
% =9.0909e-12
ApX2 = ((K*A2)/(1+K*A2))*pX_T
% = 5.0000e-11
```

To assess the biological impact of a change in transcription activator levels, it is also important to determine the fraction of the target gene promoter that is bound by the activator, since this is directly proportional to the activity of the gene's promoter. So rearrange the above equation:

$$[A : p_X] = \frac{K[A]}{1 + K[A]}[p_X^T]$$

$$\Rightarrow \frac{[A : p_X]}{[p_X^T]} = \frac{K[A]}{1 + K[A]}$$

Mpower This shows that when $[A] = 1/K$, the promoter p_X has a 50% chance of being occupied and when $[A] > 1/K$ the bound fraction is almost equal to 1, so p_X is almost fully occupied and transcription is maximal.

3.2. **Differential equations help understand transient behaviour.** We have looked at the dependancy of regulatory systems on molecular interaction, at steady state. Equation $[A:p_X] = \frac{K[A]}{1+K[A]}[p_X^T]$ tells us that when $[A]$ is changed, $[A:p_X]$ at steady state also changes. **However, this change is not immediate.** The behaviour of a regulatory system, over time provides some of the most important and basic insights. Understanding the behaviour of a system over time is the central theme of 'dynamical systems' and for which calculus was invented. The general problem is: given the rates of change of a set of variables that characterise the system at any instant, how can I compute future states?

The most common strategy is to use **ordinary differential equations**, which, when describing biochemical reactions, have a simple premise:

the rate of change in the concentration of a molecular species Z, that is $\frac{d[Z]}{dt}$, is given by the balance of the rate of its appearance with that of its desappearance. *Asmptn*

In the case of the complex $A:p_X$:

rate of change in concentration of $A:p_X =$ rate of complex formation $-$ rate of complex dissociation

$$\frac{d[A:p_x]}{dt} = k_{on}[A][p_X] - k_{off}[A:p_X] \tag{2}$$

Again, at steady state $\frac{d[A:p_x]}{dt} = 0$, the concentration of $A:p_X$ does not change, so $k_{on}[A][p_X] = k_{off}[A:p_X]$.

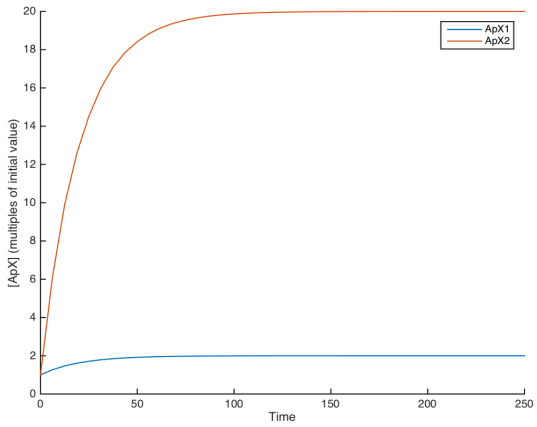
Calculation of all values of $[A:p_X]$ at all times (i.e. solving ODE (2)) allows the determination of the dynamics of $A:p_X$, including knowing the rate at which it reaches steady state. The simplest way to do so, is using **numerical integration**[2]. Reference [4] gives an introduction to the topic of numerical methods. For the rest of this course we will use computational tools for numerical integration such as MATLAB's *ode*45 function. Follow the instructions below to learn how to use MATLAB to obtain a numerical approximation to the solution of the ODE in equation (2). At the end, you should get something like figure 2.

```
*In MATLAB in a script file named reg_network.m file:*

function dApXdt = reg_network(t, ApX)
% parameters
A = 10^(-9); % M
pX =10^(-10); % M
k_on = 0.5*10^7; %sec-1 M-1
k_off = 0.5*10^(-1); %sec-1 M-1

% ODE
dApXdt = k_on*A*pX - k_off*ApX;
```

[2]This method only gives you a *numerical approximation* to the solution, at specific times. To get an exact solution, for all time, one can try solving the ODE using software such as Mathematica.

```
*in command window or new script file*

%% solve reg_network

        % for A = 10^(-9);
% initial concentration
ApX0 = 0.5e-10;

% time of simulation
tspan = [0 250];

% solve ODE(s)
[Tode, ApX1ode] = ode45(@reg_network, tspan, ApX0);


    % for A = 10^(-8);

% solve ODE(s)
[Tode, ApX2ode] = ode45(@reg_network, tspan, ApX0);


%% analyse

% plot original and increase of A in the same figure
figure
hold on
plot (Tode, ApX1ode);
plot (Tode, ApX2ode);
hold off
legend('ApX1', 'ApX2');
xlabel('Time');
ylabel('[ApX]');


% to facilitate analysis, calculate and plot in multiples of initial value
ApX1_num = ApX1ode/ApX1ode(1);
ApX2_num = ApX2ode/ApX2ode(1);

figure
hold on
plot (Tode, ApX1_num);
plot (Tode, ApX2_num);
hold off
legend('ApX1', 'ApX2');
xlabel('Time');
ylabel('[ApX] (multiples of initial value)');
```

FIGURE 2. Dynamics of the concentration of $A : p_X$. Blue line: for $[A] = 10^{-9}$. Red line: for $[A] = 10^{-8}$.



To understand the behaviour of the system, one of the questions one might want to answer is how long it takes $[A : p_X]$ to get to the steady state. The figure (orange line) looks like the steady state is reached asymptotically. To check it is useful to compare the times that it takes to get to 50, 90, 99 % of the steady state, which are about 16, 47 and 90, respectively. Ex1

Note that figure two has two plots, for two different concentrations of A.

Understanding transient dynamics of biochemical reactions helps us determine the dependance of the dynamics inside the cell on parameters that are specific to the particular molecules involved. For example, the steady state equation indicates that doubling k_{on} and k_{off} does not affect $[A : p_X]$. Mpower The analysis done above and in exercise 1 shows the effect of changing $[A]$ 10-fold, on the dynamics of $[A : p_X]$; i.e. whether there is a new steady state and whether there is a change in the speed that it is attained.

4. Example 2: Rate of change of protein concentration

Again, using ODEs, the rate of change of concentration of protein X is determined by the balance of the rate of production of X, through expression of the gene X, and the protein's rate of degradation.

The **rate of production** is determined by the occupancy of the promoter of gene X by protein A. The binding and dissociation of a transcription regulator at a promoter generally occurs at a much faster time scale than transcription initiation. As a result, we can assume that this binding reaction Asmptn is at equilibrium on the time scale of transcription, and can thus use equation $\frac{[A:p_X]}{[p_X^T]} = \frac{K[A]}{1+K[A]}$ to calculate promoter occupancy by A. To calculate **transcription rate**, multiply occupancy of promoter by A by a *transcription rate constant* β, which represents the steps that lead to production of mRNA. If on average, each mRNA molecule produces m molecules of protein, then the **protein production rate** is calculated by multiplying the transcription rate by m. That is:

$$\text{transcription rate} = \beta \frac{K[A]}{1 + K[A]}$$

$$\text{protein production rate} = m\beta\frac{K[A]}{1+K[A]}$$

Degradation consists of active protein degradation and of dilution due to cell growth and results in an exponential decline in protein levels. The average time it takes for a protein to be degraded is defined as its **average lifetime** τ. The rate of degradation is calculated by dividing the concentration of the protein by the lifetime:

$$\text{degradation rate} = \frac{[X]}{\tau_X}$$

We are now ready to generate a differential equation that will help us determine the rate of change of protein X as a function of time:

$$\frac{d[X]}{dt} = \text{protein production rate} \; - \; \text{protein degradation rate}$$

$$\frac{d[X]}{dt} = m\beta\frac{K[A]}{1+K[A]} - \frac{[X]}{\tau_X} \tag{3}$$

Ex2 Using numerical methods, a numerical approximation to the solution of equation (3) can be mapped out and plotted.

Recall that at steady state, the concentration of X does not change. Rearranging the equation, we can calculate $[X]$ at steady state ($[X_{st}]$):

$$\frac{d[X]}{dt} = m\beta\frac{K[A]}{1+K[A]} - \frac{[X]}{\tau_X} = 0$$

$$[X_{st}] = m\beta\frac{K[A]}{1+K[A]}\tau_X$$

Mpower Note that the steady state concentration is directly proportional to the lifetime. In addition, it is clear that when protein A rises, protein X increases to a new steady state value. As discussed before,
Mpower this does not happen instantaneously. The solution to the ODE in (3) (shown below) indicates that the concentration of X over time is related to its steady state concentration. In addition, when transcription begins, the concentration of X rises to steady-state level at an exponential rate that is inversely related to its lifetime; that is, the faster X is degraded, the less time it takes to reach steady state and levels off.

$$[X](t) = [X_{st}](1 - e^{\frac{-t}{\tau_X}})$$

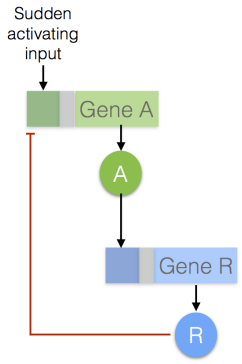
5. Example 3: Similar quantitative methods are used for transcription repressors and activators

The cell uses both positive control and shutting off genes to regulate expression of genes. Since repressor proteins bind to specific sites on target genes, the fraction of the gene binding sites that are occupied by the repressor are specified by the same equations used for the transcription activator. However, since it is when the DNA is free that the RNA polymerase can bind to the promoter region, the quantity of interest this time is the unbound fraction of sites on the target
Ex3 genes. Using this information, one can write the differential equation that describes the rate of change in concentration of X as a function of time, under the influence for transcription repressors.

6. Example 4: Negative feedback loops are a powerful strategy in cell regulation

Most regulatory systems that govern cell behaviour have more than just a few components. Most, consist of multiple *modules* linked together to produce large circuits called *network motifs*. An example of such a network is a *negative feedback loop* (an example is illustrated in figure 3). In this loop, an input signal initiates transcription of gene A, which produces a transcription activator protein A. This activates gene R which produces a repressor protein R, which in turn binds to the promoter of gene A, thus inhibiting its expression.

Figure 3.



Intuitively, a negative feedback loop can be understood as being a mechanism by which to prevent high protein accumulation. But what else can we learn about such a system? The mathematical models of $[A]$ and $[R]$ in figure 3 are: Ex4

$$\frac{d[A]}{dt} = \frac{\beta_A m_A}{1 + K_R[R]} - \frac{[A]}{\tau_A} \tag{4}$$

$$\frac{d[R]}{dt} = \beta_R m_R \frac{KA[A]}{1 + K_A[A]} - \frac{[R]}{\tau_R} \tag{5}$$

and at steady state:

$$\frac{\beta_A m_A}{1 + K_R[R]} = \frac{[A]}{\tau_A} \tag{6}$$

$$\beta_R m_R \frac{KA[A]}{1 + K_A[A]} = \frac{[R]}{\tau_R} \tag{7}$$

These ODEs are said to be *coupled*, i.e., they must be solved together in order to describe the behaviour of either $[A]$ or $[R]$ over time.

As before, solutions to the equations (4) and (5) and analysis of the steady state equations (6) and (7) can reveal important aspects that can often escape intuition alone. Furthermore, varying the values of the different parameters (e.g. compound concentrations, rates of reactions, constant values, etc...) involved can reveal how the system is dependent on those parameters.

6.1. **Final remark on assumptions and chemical kinetic laws.** Above, we employed the famous **law of mass action** to derive 'standard' ODEs that are used to model transcription activators and repressors, the evolution of protein concentrations and negative feedback loops. In some instances, however, it might not be appropriate to assume the law of mass action, for example, when an enzyme is involved, or when there is cooperativity[3]. Appropriated laws for such systems would be **Michaelis-Menten** kinetics and the **Hill coefficient**, respectively. In order to choose appropriate assumptions, an intimate knowledge of the biology of the system is required.

7. So what does a 'deterministic' model mean any way?

Biological systems are complex processes consisting of many chemical reactions, that are potentially significantly **stochastic** (random). Using ODEs to model the time evolution of compounds that take part in a system of reactions ignores the variability and forces the behaviour of each component to be perfectly **predictable**, from the initial conditions of the system.

When there are only a few molecules that take part in a reaction, stochastic effects can become prominent and are manifested by occurrence of fluctuations in the time course of the reactants. When large numbers of molecules are present reactions usually proceed in a predictable manner because the fluctuations are averaged out. Deterministic behaviour can be seen as a limit of the stochastic behaviour when the number of molecules is high.

Reference [3] is an introduction to stochastic models of biological systems.

8. Exercises

Exercise 1. Use MATLAB to calculate the exact time it takes to reach 50, 90 and 99 % of the steady state. Calculate the difference in time it takes to reach each of these, with and without a 10-fold increase in $[A]$.

Exercise 2. Use MATLAB to numerically approximate a solution to the ODE describing the change in concentration of X with respect to time. Choose appropriate values for the parameters.

Exercise 3. Write out the exact equations for the bound fraction of sites on the target gene and for the unbound fraction of sites. Use these to write the differential equation for the protein production rate and the differential equation that describes the rate of change in concentration of X as a function of time, when repressor concentrations change. Deduce observations about the steady state of X, the effect of changing concentrations of R and how the change in concentration is related to its lifetime.

Exercise 4. Check that this model is correct.

Exercise 5. To see how the techniques learned can be generalised to investigate metabolic networks, we will use the model proposed in [2]. In this paper, oscillations in the glycolysis reaction system in figure 4 are studied. As part of their work, the authors measured oscillations within

[3]For example, when promoters contain multiple adjacent binding sites for the same transcription regulator and these regulators interact with each other regularly, forming various oligomers, these can be considered to cooperate in binding, which results in an increased DNA-binding affinity, which increases at higher concentrations of the transcription regulator.

glycolysis in an experimental system and then tried to model those in a simulated reaction network. They uploaded their model to the Biomodels Database, which we have copied here (in blue, below).

(i). Use this model (in blue) to simulate the system in MATLAB. Note that you need to use the information in blue to set up a model in MATLAB, as you learned above. You need to have a function file that defines the equations and a script file that approximates the ODEs with *ode45* and plots the results. Plot the results, for 600 units of time.

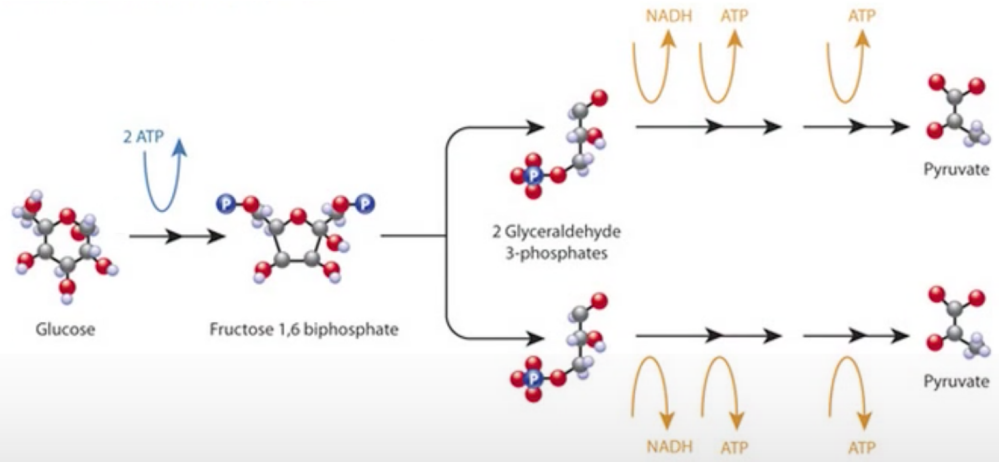
(ii). Inspect the curves and decide on similarities and differences in amplitudes, frequency of oscillation, how quickly the steady state value is reached, the shapes of the waveforms.

(iii). Inspect the curve for ATP. What do you see? What is happening at the steady state?

(iv). Generate a plot just with ATP and NADH. What can you say about them?

(v). Test your intuition: guess how the AT in the system will behave as glucose flow varies over the 11-fold range.

FIGURE 4. Glucose pathway to Pyruvate




```
%ODEs:
d(ATP)/dt = 1/compartment*(ReactionFlux1 - ReactionFlux16 - ReactionFlux17 +
ReactionFlux20 + ReactionFlux21 - ReactionFlux24);
d(ADP)/dt = 1/compartment*(ReactionFlux2 + ReactionFlux16 + ReactionFlux17 -
ReactionFlux20 - ReactionFlux21 + 2*ReactionFlux24);
d(AMP)/dt = 1/compartment*(-ReactionFlux14 - ReactionFlux24);
d(GLC)/dt = 1/compartment*(ReactionFlux5 - ReactionFlux16);
d(F6P)/dt = 1/compartment*(-ReactionFlux6 + ReactionFlux16 - ReactionFlux17 -
ReactionFlux25);
d(FBP)/dt = 1/compartment*(-ReactionFlux7 + ReactionFlux17 - ReactionFlux18);
d(GAP)/dt = 1/compartment*(-ReactionFlux8 + 2*ReactionFlux18 - ReactionFlux19);
d(NAD)/dt = 1/compartment*(ReactionFlux4 - ReactionFlux19 + ReactionFlux23);
d(NADH)/dt = 1/compartment*(ReactionFlux3 + ReactionFlux19 - ReactionFlux23);
d(DPG)/dt = 1/compartment*(-ReactionFlux9 + ReactionFlux19 - ReactionFlux20);
d(PEP)/dt = 1/compartment*(-ReactionFlux10 + ReactionFlux20 - ReactionFlux21);
d(PYR)/dt = 1/compartment*(-ReactionFlux11 + ReactionFlux21 - ReactionFlux22);
d(ACA)/dt = 1/compartment*(-ReactionFlux12 + ReactionFlux22 - ReactionFlux23);
d(EtOH)/dt = 1/compartment*(-ReactionFlux13 + ReactionFlux23);
d(P)/dt = 1/compartment*(-ReactionFlux15 + ReactionFlux25);
```

```
%Fluxes:
ReactionFlux1 = compartment*(3.5-ATP)*flow;
ReactionFlux2 = compartment*(1.1-ADP)*flow;
ReactionFlux3 = compartment*(0.24-NADH)*flow;
ReactionFlux4 = compartment*(4-NAD)*flow;
ReactionFlux5 = compartment*(50-GLC)*flow;
ReactionFlux6 = compartment*F6P*flow;
ReactionFlux7 = compartment*FBP*flow;
ReactionFlux8 = compartment*GAP*flow;
ReactionFlux9 = compartment*DPG*flow;
ReactionFlux10 = compartment*PEP*flow;
ReactionFlux11 = compartment*PYR*flow;
ReactionFlux12 = compartment*ACA*flow;
ReactionFlux13 = compartment*EtOH*flow;
ReactionFlux14 = compartment*AMP*flow;
ReactionFlux15 = compartment*P*flow;
ReactionFlux16 = compartment*(V1*ATP*GLC/((K1GLC+GLC)*(K1ATP+ATP)));
ReactionFlux17 = compartment*(V2*ATP*power(F6P,2)/((K2*(1+k2*
power(ATP/AMP,2))+power(F6P,2))*(K2ATP+ATP)));
ReactionFlux18 = compartment*(k3f*FBP-k3b*power(GAP,2));
ReactionFlux19 = compartment*(V4*NAD*GAP/((K4GAP+GAP)*(K4NAD+NAD)));
ReactionFlux20 = compartment*(k5f*DPG*ADP-k5b*PEP*ATP);
ReactionFlux21 = compartment*(V6*ADP*PEP/((K6PEP+PEP)*(K6ADP+ADP)));
ReactionFlux22 = compartment*(V7*PYR/(K7PYR+PYR));
ReactionFlux23 = compartment*(k8f*NADH*ACA-k8b*NAD*EtOH);
ReactionFlux24 = compartment*(k9f*AMP*ATP-k9b*power(ADP,2));
ReactionFlux25 = compartment*k10*F6P;

%Parameter Values:
V1 = 0.5;
K1GLC = 0.1;
K1ATP = 0.063;
V2 = 1.5;
K2 = 0.0016;
k2 = 0.017;
K2ATP = 0.01;
k3f = 1;
k3b = 50;
V4 = 10;
K4GAP = 1;
K4NAD = 1;
k5f = 1;
k5b = 0.5;
V6 = 10;
K6PEP = 0.2;
K6ADP = 0.3;
V7 = 2;
K7PYR = 0.3;
```

```
k8f = 1;
k8b = 0.000143;
k9f = 10;
k9b = 10;
k10 = 0.05;
flow = 0.011;
compartment = 1;

%Initial Conditions:
ATP = 4.4906;
ADP = 0.10837;
AMP = 0.0026115;
GLC = 0.011282;
F6P = 0.65939;
FBP = 0.0077014;
GAP = 0.0019092;
NAD = 3.6206;
NADH = 0.61612;
DPG = 0.29911;
PEP = 0.0021125;
PYR = 0.004227;
ACA = 0.073833;
EtOH = 0.33981;
P = 0;
```

9. References

[1] Alberts *et al.*, Molecular biology of the cell, Sixth Edition, GS, 2015

[2] K. Nielsen et al., Sustained oscillations in glycolysis: an experimental and theoretical study of chaotic and complex periodic behaviour and quenching of simple oscillations, *Biophysical Chemistry 72, 49-62, 1998*
and
https://www.youtube.com/watch?v=3cDSUuyC3bM

[3] Karin Sasaki, EMBL-CBM, A primer on stochastic models of biochemical reactions

[4] Karin Sasaki, EMBL-CBM, A primer on numerical methods - Euler's method