

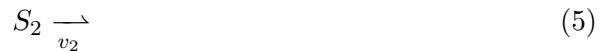
Home Assignment 1: Enzyme Dynamics

2017

Preparation: exercises in Chapters 1-2 of the exercise manual.

In the human cell, glycolysis is the first step of three in the fundamental glucose metabolic pathway. The glycolysis itself consists of ten different steps where glucose is converted to pyruvate, which is the input to the citric acid cycle (also known as Krebs cycle). In this assignment, we will study the dynamics of the third step of the glycolysis. It is considered to be the most rate-limiting step of the process but also an important source to possible oscillations in ATP (Adenosine triphosphate) and ADP (Adenosine diphosphate).

The third step of the glycolysis includes phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate as well as hydrolysis of ATP to ADP, catalyzed by the enzyme phosphofructokinase, PFK1. In the active state, PFK1 catalyzes the production of ADP from ATP as fructose 6-phosphate is phosphorylated. A simplified model of the enzyme reactions is given by the following stoichiometry where PFK1 is denoted by E , ATP is denoted by S_1 and ADP is denoted by S_2 .



In (1), the enzyme PFK1 (E) is activated or deactivated by binding or unbinding with γ number of molecules of ADP (S_2). Complex C_1 is the active form of PFK1. Constants $k_3 > 0$ and $k_{-3} > 0$ denote the rate of the reaction in either direction. Similar denotation is used in the remaining reactions. In (3), ATP (S_1) can bind with the activated form of the enzyme (C_1) to produce a complex C_2 . Complex C_2 can then produce complex C_1 and ADP (S_2), as given by (4). Furthermore, there is a constant supply rate of ATP (S_1) given by (2) while ADP (S_2) is removed at a rate proportional to its concentration in (5).

We will now analyze the dynamics of the enzyme reactions by the methods treated in Lectures 2-3 (Chapters 1-2 of the exercise manual). The analysis is divided into the following steps:

1. Consider the notation $s_1 = [S_1]$, $s_2 = [S_2]$, $e = [E]$, $x_1 = [C_1]$ and $x_2 = [C_2]$. The system of differential equations describing the overall dynamics of the substrates, enzyme and complex products in reactions (1)-(5), using the law of mass action, is given by

$$\frac{ds_1}{dt} = v_1 - k_1 s_1 x_1 + k_{-1} x_2 \quad (6)$$

$$\frac{ds_2}{dt} = -v_2 s_2 + k_2 x_2 + \gamma k_{-3} x_1 - \gamma k_3 e s_2^\gamma \quad (7)$$

$$\frac{dx_1}{dt} = k_3 e s_2^\gamma - (k_1 s_1 + k_{-3}) x_1 + (k_2 + k_{-1}) x_2 \quad (8)$$

$$\frac{dx_2}{dt} = k_1 s_1 x_1 - (k_{-1} + k_2) x_2 \quad (9)$$

$$\frac{de}{dt} = -k_3 e s_2^\gamma + k_{-3} x_1. \quad (10)$$

Given the differential equations of the complexes and the enzyme, i.e., equations (8-10) above, verify that

$$\frac{de}{dt} + \frac{dx_1}{dt} + \frac{dx_2}{dt} = 0.$$

If $e(0) = e_0 > 0$ while $x_1(0) = x_2(0) = 0$, what can be said about the sum $e(t) + x_1(t) + x_2(t)$ based on the observation above? Interpretation?

2. Now, you will simulate the behavior of the system for a given initial state. However, we will first rewrite and simplify the model further as our main interest is the dynamics of the substrates, ATP and ADP. Using the observation in the previous step, we can exclude equation (10) from our analysis by replacing e with $e_0 - x_1 - x_2$ in the remaining equations, i.e., equations (6-9). Furthermore, we can introduce the dimensionless concentrations

$$\begin{aligned} \sigma_1 &= \frac{k_1}{k_2 + k_{-1}} s_1, \\ \sigma_2 &= \left(\frac{k_3}{k_{-3}} \right)^{\frac{1}{\gamma}} s_2, \\ \xi_1 &= \frac{x_1}{e_0}, \\ \xi_2 &= \frac{x_2}{e_0}, \end{aligned}$$

and the new time scale

$$\tau = \frac{e_0 k_1 k_2}{k_2 + k_{-1}} t.$$

Moreover, we will apply steady-state assumptions for the dimensionless complex products ξ_1 and ξ_2 (i.e., $d\xi_i/dt = 0$) to derive the dimensionless substrate differential equations

$$\frac{d\sigma_1}{d\tau} = \nu - f(\sigma_1, \sigma_2), \quad (11)$$

$$\frac{d\sigma_2}{d\tau} = \alpha f(\sigma_1, \sigma_2) - \eta \sigma_2, \quad (12)$$

where

$$f(\sigma_1, \sigma_2) = \frac{\sigma_1 \sigma_2^\gamma}{\sigma_2^\gamma \sigma_1 + \sigma_2^\gamma + 1}, \quad (13)$$

$\nu = v_1/k_2e_0$, $\alpha = (k_2 + k_{-1})/k_1(k_3/k_{-3})^{-1/\gamma}$ and $\eta = v_2(k_2 + k_{-1})/k_1k_2e_0$.

Given parameter-values $\nu = 0.0285$, $\alpha = 1.0$, $\eta = 0.1$ and $\gamma = 2$ and initial values of $(\sigma_1, \sigma_2)_{\tau=0} = (0.3, 0.3)$, simulate the system in Matlab for 1000 time steps by filling in the missing code in the file `enzymeskeleton.m` provided on the course home page. Use the `function handle` `@(t,x)f(t,x)` as well as the `ode45` command. Produce the plots in Fig. 1 by running the script. The dynamics is clearly oscillatory. Glycolytic oscillations have been observed *in vitro* in human cell extracts and in yeast cells, and is hypothesized to play a key role in, e.g., pulsatile pancreatic insulin secretion. Explain what happens with the concentrations as time progress.

3. Set the initial values to $(\sigma_1, \sigma_2)_{\tau=0} = 3 \cdot (0.3, 0.3)$. Describe what happens with the concentrations as time progress. Compare to the simulation made in Step 2.
4. The system in Eq. 11 - 12 is nonlinear as can be seen in Eq. 13. Consider the linear system

$$\dot{x} = \begin{bmatrix} -1 & 0.5 \\ -3 & 0.5 \end{bmatrix} x.$$

Simulate the system for $t \in (0, 25)$ and initial values $x_0 = (0.3, 0., 3)$ and $x_0 = 3 \cdot (0.3, 0, 3)$. Plot x_1 and x_2 as a function of time. How does the behavior of the linear system change when the initial value is changed? Compare with the non linear system studied previously.

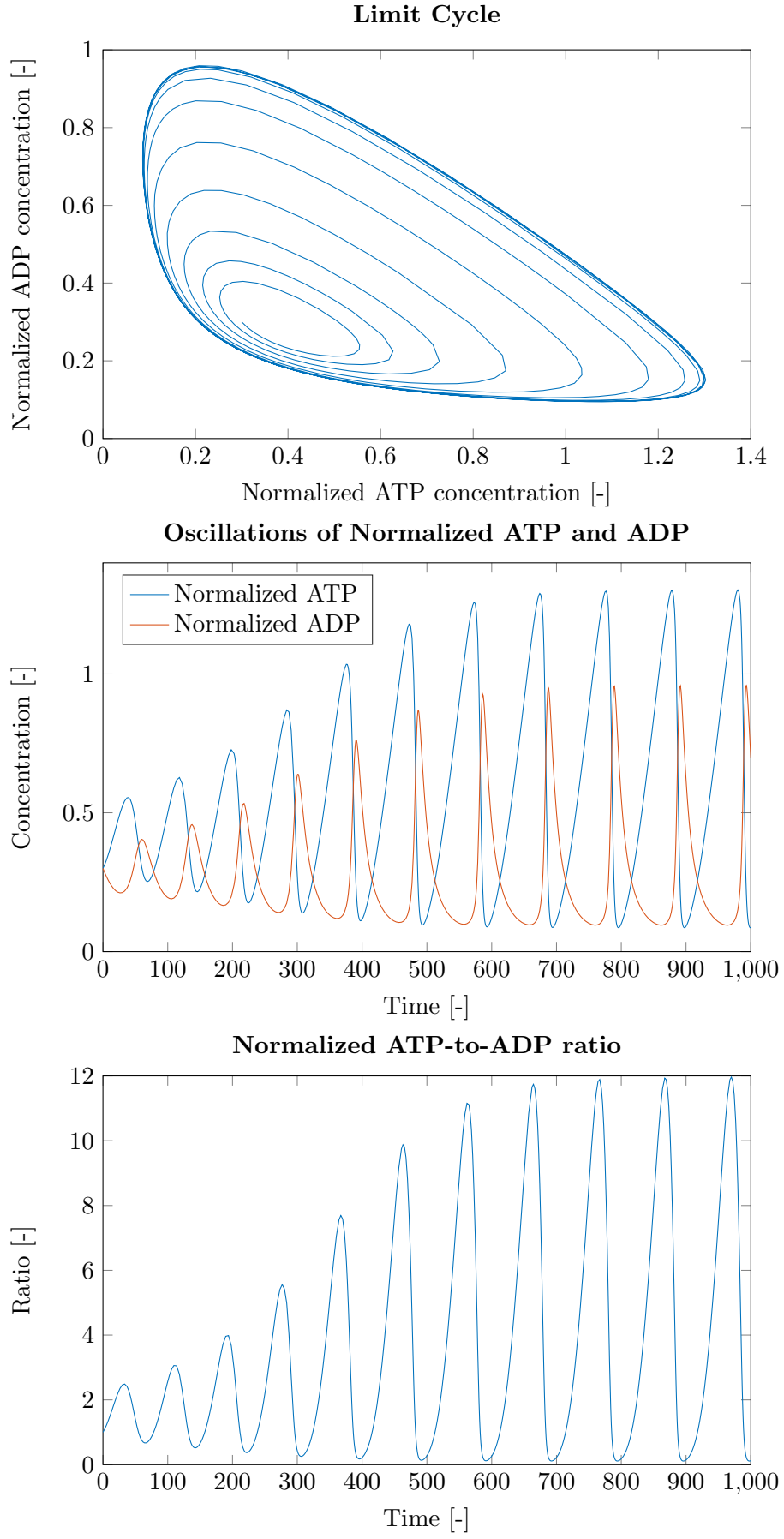


Figure 1 Simulation plots of the normalized ATP and ADP dynamics.