

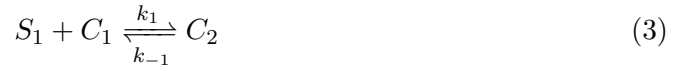
Home Assignment 1: Enzyme Dynamics

November 1, 2016

Preparation: Go through the exercises in Chapter 1, 2 and 3 of the exercise manual.

In the human cell, the 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 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 the ATP.

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 , ADP is denoted by S_2 and ATP is denoted by S_1 .



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 Chapter 1-3 of the exercise manual. The analysis is divided into the following five steps:

1. Draw a compartment representation of the enzyme reactions given in (1-5).
2. Write down the dynamics of the reaction in (3).
3. 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

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

What can be said about the sum $e_0 := e + x_1 + x_2$ based on this observation? Interpretation?

4. Now, we 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 step 3, we can exclude equation (10) from our analysis by replacing e with $e_0 - x_1 - x_2$ in the remaining equations, 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 quasi-steady-state assumptions ($d\xi_i/dt = 0$) for the normalized complex products ξ_1 and ξ_2 (compare to the derivation of Michaelis-Menten relationships) to derive the normalized 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 $\eta = v_2(k_2 + k_{-1})/k_1k_2e_0$. If you are interested, you can try to derive expressions (11) and (12) by yourself. The function in (13) is the quasi-steady-state solution $\xi_2 = f(\sigma_1, \sigma_2)$.

Given parameter-values $\nu = 0.0285$, $\alpha = 1.0$, $\eta = 0.1$ and $\gamma = 2$ and initial values of $\sigma_1 = \sigma_2 = 0.3$, simulate the system in Matlab for 1000 timesteps 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.

5. Linearize the dynamics around the (unique) stationary point $d\sigma_1/d\tau = 0$, $d\sigma_2/d\tau = 0$. Notice that this system does not have any input u or output y . Hence, you only need to construct the state matrix A of the linearized system. Is the linearized system stable around this point? Can you motivate your answer using the plots shown in Fig. 1?

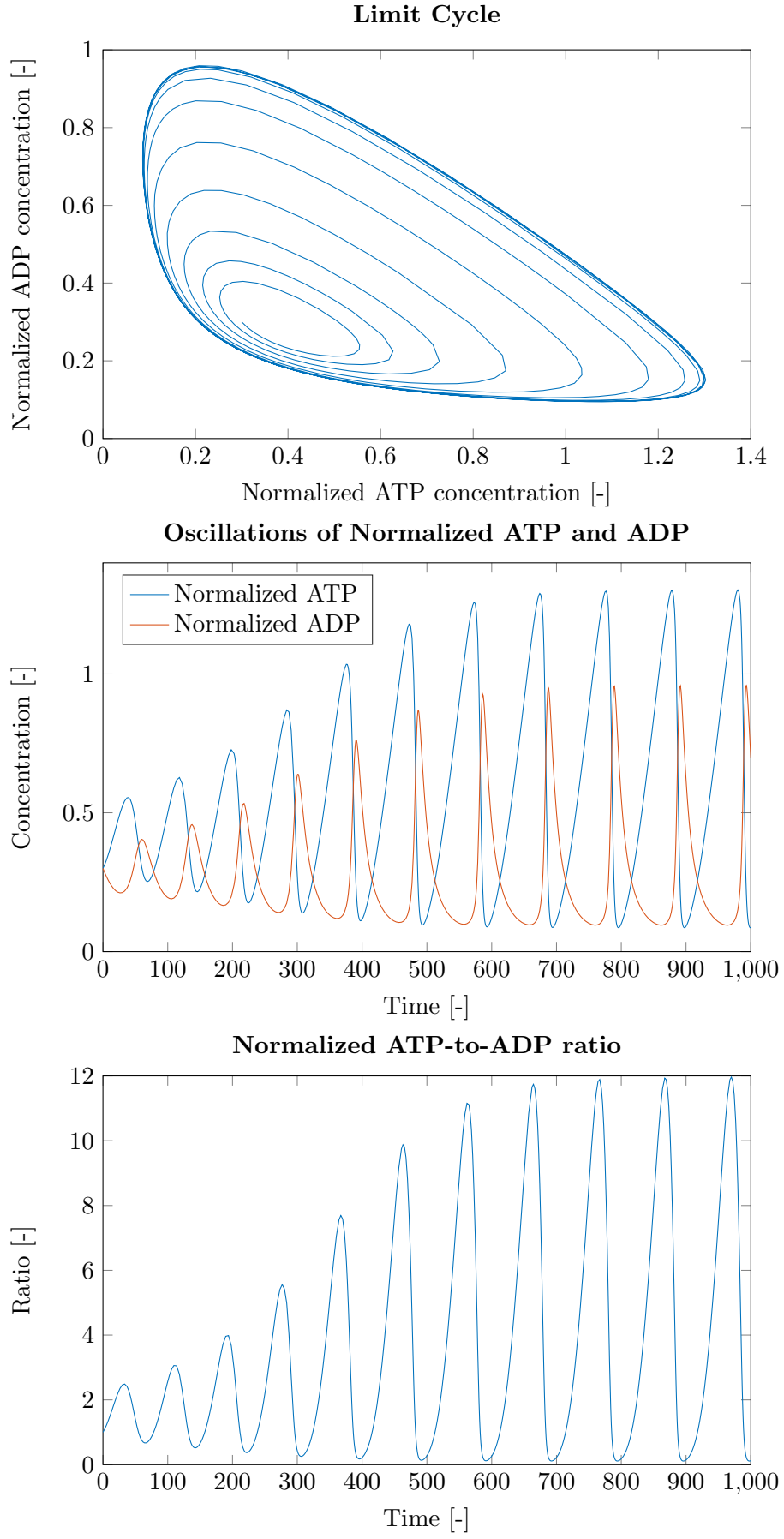


Figure 1 Simulation plots of the normalized ATP and ADP dynamics.