Question 2: Enzyme Kinetics

Enzymes are catalysts that help convert molecules that we will call substrates into other molecules that we will products. They themselves are not changed by the reaction. Within cells, enzymes are typically proteins. They can speed up biological reactions, sometimes by up to millions of times. They are also regulated by a very complex set of positive and negative feedback systems. Computational biologists are painstakingly mapping out this complex set of reactions. In this problem, we will model and simulate a simplified enzyme reaction.

An enzyme *E* converts the substrate *S* into the product *P* through a two-step process. First, *E* forms a complex with *S* to form an intermediate species *ES* in a reversible manner at the forward rate *k1* and reverse rate *k2*. The intermediate *ES* then breaks down into the product *P* at a rate *k3*, thereby releasing *E*. Schematically, we write

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_3}{\rightarrow} E + P$$

8.1. Using the law of mass action, write down four equations for the rate of changes of the four species, *E*, *S*, *ES*, and *P*.

8.2. Write a code to numerically solve these four equations using the fourth-order RungeKutta method. For this exercise, assume that the initial concentration of *E* is 1 μM, the initial concentration of *S* is 10 μM, and the initial concentrations of *ES* and *P* are both 0. The rate constants are: *k1*=100/μM/min, *k2*=600/min, *k3*=150/min.

8.3. We define the velocity, *V*, of the enzymatic reaction to be the rate of change of the product *P*. Plot the velocity *V* as a function of the concentration of the substrate *S*. You should find that, when the concentrations of *S* are small, the velocity *V* increases approximately linearly. At large concentrations of *S*, however, the velocity *V* saturates to a maximum value, *Vm*. Find this value *Vm* from your plot.

SOLUTION:

1.

$$\frac{d[S]}{dt} = k2 * [ES] - k1 * [E] * [S]$$

$$\frac{d[E]}{dt} = (k2 + k3) * [ES] - k1 * [E] * [S]$$

$$\frac{d[ES]}{dt} = k1 * [E] * [S] - (k3 + k2) * [ES]$$

$$\frac{d[P]}{dt} = k3 * [ES]$$

2. I'm sorry I couldn't write the code to solve these four equations using the fourth-order RungeKutta method, but I try to search for relevant information to transform the equations. (Reference: Meena, A. , Eswari, A. , & Rajendran, L. . (2010). Mathematical modelling of enzyme kinetics reaction mechanisms and analytical solutions of non-linear reaction equations. Journal of Mathematical Chemistry, 48(2), p.179-186.)

the concentration of the reactants `is denoted by lower case letters:

s=[S] e=[E] c=[ES] p=[P]

subject to the initial conditions:

$s(0) = s_0$=10 μM, $E(0) = e_0$=1μM, c(0) = 0, P(0) = 0

four equations can be reduced to only two equations for s and c:

$$\frac{ds}{dt} = (k1 * s + k2) * c - k1 * e0 * s$$

$$\frac{dc}{dt} = k1 * e0 * s - (k1 * s + \mathrm{k2} + \mathrm{k3}) * c$$

by introducing the following parameters:

$$\tau = \frac{k1 * e0 * t}{\varepsilon}, \quad u\ (\tau) = \frac{s(t)}{s0}, \quad v\ (\tau) = \frac{c(t)}{c0},$$

$$w(\tau) = \frac{p(t)}{e0}, \quad \lambda = \frac{k3}{k1 * s0}, \quad k4 = \frac{k2+k3}{k1 * s0}, \quad \varepsilon = \frac{e0}{s0}$$

substitute $e0 = 1\ \mu\mathrm{M}, s0 = 10\ \mu\mathrm{M}, k1 = 100/\mu\mathrm{M}/\mathrm{min}, k2 = 600/\mathrm{min}, k3 = 150/\mathrm{min}$ into the equations, the result is

$$\lambda = 0.15, \quad k4 = 0.75, \quad \varepsilon = 0.1$$

then, final equations:

$$\frac{du}{dt} = -\varepsilon * u + \varepsilon * (u + k4 - \lambda) * v$$

$$\frac{dv}{dt} = u - (u + k4) * v$$

$$\frac{dw}{dt} = \lambda * v$$

substitute λ、$k4$、ε into the equations， the result is

$$\frac{du}{dt} = -0.1u + 0.11(u + 0.6) * v$$

$$\frac{dv}{dt} = u - (u + 0.75) * v$$

$$\frac{dw}{dt} = 0.15v$$

u(0)=1, v(0)=0, w(0)=0

3. (I will use formulas to derive result)

Based on knowledge of enzyme kinetics, when the reaction is at steady state, the rate of ES generation is equal to the rate of ES decomposition. (Set [Et] as total enzyme concentration):

$$k1 * ([Et] - [ES]) * [S] = k2 * [ES] + k3 * [ES]$$

so,

$$[ES] = \frac{[Et] * [S]}{\frac{k2 + k3}{k1} + [S]}$$

$$km = \frac{k2 + k3}{k1}$$

then,

$$V = \frac{d[P]}{dt} = k3 * [ES] = k3 * \frac{[Et] * [S]}{km + [S]}$$

at large concentrations of S,

$$Vm = k3 * [Et]$$

then,

$$V = \frac{Vm * [S]}{km + [S]}$$

use double-reciprocal plot

$$\frac{1}{V} = \frac{km + [S]}{Vm * [S]} = \frac{km}{Vm} * \frac{1}{[S]} + \frac{1}{Vm}$$

So the line obtained by plotting $\frac{1}{V} - \frac{1}{[S]}$ with a vertical axis intercept of $\frac{1}{Vm}$