Computational Biochemistry
Laboration

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laboration 1

1 Create a model of an enzyme

You will in this laboration model a part of one really ancient biochemical pathway: the glycolysis, which is of greatest importance for living cells and converts glucose to pyruvate, which is then further degraded, yielding ATP, which has a high $\Delta_f G^\circ$ and is ubiquitous as a coupled reactant in energy-demanding reactions. In the first exercise you will model one of the enzymes in the pathway, phosphofructokinase (PFK), which catalyses the reaction from fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (FBP), while consuming ATP and yielding ADP. This reaction has a ΔG° of about -3.5 kcal/mol. This means that we can approximate the reaction as being irreversible.

- ► Q: Why is that? Calculate the equilibrium constant of the reaction. 1 kcal = 4.1868 kJ, R = 8.314 J/mol K (molar gas constant). Both ATP and F6P are generally present in greater concentrations in the cell than ADP and FBP, respectively.

As noted above, the enzyme has ATP and ADP as substrate and product respectively, but we'll ignore this and assume these species as being constant! Please use equation (33) in part 1 of the study notes. Note that since we assume that the reaction is irreversible, you do not need to include the terms that have to do with backward flow from product to substrate. This means that the π and $\frac{\Gamma}{K'}$ terms can be neglected (just pretend that they equal zero). However, the ξ term should not be neglected – this term has to do with the modulation of the enzyme in the forward (substrate to product) reaction!

Here are the facts you need to know:

- ◇ The enzyme is half activated when F6P is about 8mM and no FBP is present.

- ◇ The enzyme is activated by its product, FBP, which binds to the enzyme with a dissociation constant of 3μM.
- ◇ When adding excess FBP, the enzyme is half activated at only 0.8mM F6P! (*hint*: let the modifier concentration approach infinity. Then solve equation (33) for α assuming that the enzyme is half activated at 0.8mM F6P. Remember to neglect the π and $\frac{\Gamma}{K'}$ parameters in the equation since we assume that the reaction is irreversible.)
- ◇ The enzyme exhibits a sigmoid (s-shaped) concentration-activity curve, with a Hill coefficient of 2.5.
- ◇ The limiting rate is about $100\mu\text{mol} \cdot \text{g}_{\text{dw}}^{-1} \cdot \text{min}^{-1}$, where g_{dw} stands for gram dry weight of living tissue, that is with all water removed. Let's convert this to the standard chemical unit $\text{M} \cdot \text{s}^{-1}$. Since the rate per second must be $\frac{1}{60}$ of the rate per minute, we can first write the rate as $100 \cdot 10^{-6}\text{mol} \cdot \text{g}_{\text{dw}}^{-1} \cdot \frac{1}{60}\text{s}^{-1}$. Since M is mole per liter, we now have to convert the g_{dw} term to liters. For this we will use the fact that water constitutes about 75% of a living cell. This means that the dry weight constitutes the remainder, about 25%, so one gram of dry weight corresponds to three grams of water, which (using that the density of water is about 1 g/cm^3) is approximately 3 cm^3 or $3 \cdot 10^{-3}$ l. Thus, our expression for the limiting rate becomes $\frac{100 \cdot 10^{-6}}{60 \cdot 3 \cdot 10^{-3}}\text{mol} \cdot \text{l}^{-1}\text{s}^{-1} = \frac{100}{180 \cdot 10^{3}}\text{M} \cdot \text{s}^{-1}$. This value can now be plugged in at the appropriate place in the equation!

Study equation (33) and find out how to use the above information to infer the parameters in the equation.

- ▶ Please implement the equation as a matlab function, which takes F6P concentration as input parameter and returns the reaction rate j.
- ▶ Then plot the concentration-activity profile for the enzyme, for some different concentrations of FBP (including large excess) and make sure that the model behaves as is described above.

2 Watch the enzyme in action

Now it's time to see how this enzyme behaves when given an influx and an enzyme catalysed efflux. Assume that F6P is created with an influx of $0.6\mu\text{M} \cdot \text{s}^{-1}$. Also, FBP is removed through the enzyme catalysed reaction where FBP is split into dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP). This is catalysed by the enzyme aldolase. Assume that this reaction is irreversible, too (our little model won't include DHAP and GAP), and has a limiting rate of 60 $\mu\text{M} \cdot \text{s}^{-1}$. Aldolase operates with a hyperbolic concentration-activity curve (the Hill coefficient is equal to one) and has a Michaelis constant towards FBP of about 10μM. Start out from equation (1) in part 2 of the study notes and design and perform a simulation of the system!

- ▶ Start with the stoichiometry matrix and then implement the reaction fluxes. Write a function that computes the concentration derivatives.
- ▶ Then solve the small system of differential equations numerically.

It's convenient to use matlab's function `ode15s` for this purpouse. The syntax is

```
[T, Y] = ode15s('functionname', [0 finish], bv, options);
```

where `functionname` refers to a function file that in your case implements the system described as (1) in part 2 of the study notes, `finish` stands for the time in seconds when you abort the simulation and `options` is set with the line

```
options = odeset('AbsTol', 1e-12);
```

Finally, `bv` is a vector of initial values; you can use F6P = 1mM, FBP = 1μM. The solution will appear in the vector of time points `T` and the matrix of solution points `Y` with each column representing one variable (you have two variables in your system). Run the simulations at least 2000 seconds. Note that your function "functionname" must return a *column* vector. Type `help ode15s` for further information.

- ▶ Plot the concentration of F6P and FBP versus time. What does the behaviour mean thermodynamically?
- ▶ Increase the influx to $6\mu\mathrm{M}\cdot\mathrm{s}^{-1}$. Again, plot the concentration of F6P and FBP versus time. You should observe quite a different behaviour. What do you think this mean thermodynamically?

Good luck!