BNFO601: Introduction to Bioinformatics
Scenario: Metabolic Modeling (Fixing glycolysis.py)

Outline:
I. Classes and instances of classes
   II. Snake in Paradise, a problem with glycolysis.py
   III. The universal challenge to glycolysis

I. Classes and instances of classes
[I'll put something here if requested]

II. Snake in Paradise, a problem with glycolysis.py
By now you have run glycolysis.py (and if you haven’t, go back to the previous set of notes, and I’ll see you when you’ve finished with them!). You may be wondering… the program seems to work fine! What’s left for you to do? But examine the results you got (after modifying the program to keep blood glucose and pyruvate constant). It should seem very peculiar that fructose-bis-phosphate seems to rise forever. Pyruvate, the end product of glycolysis, also rises, but that might not be so surprising. The next highest metabolite is dihydroxyacetone phosphate. And that might give you pause.

SQ4. Consider these two metabolites -- fructose-bis-phosphate (FBP) and dihydroxyacetone phosphate (DHAP)
   a. Identify where in glycolysis they appear.
   b. Thinking of glycolysis as the flow of water through a hose with valves controlled by enzymes along the way, where is the obstruction that could cause the pooling of FBP and DHAP?
   c. If there is an obstruction at that point, what other metabolite would you expect to accumulate? Why don’t you see it on your graph?

We need to find a reason for this obstruction. One possibility is that the enzyme at which it occurs is just a very slow enzyme, unable to accommodate the flux of metabolites presented to it by upstream enzymes. Alternatively, the enzyme might require a high level of substrate for it to work effectively.

SQ5. Check out these possibilities
   a. Is the enzyme very slow? What parameter would indicate this? What is the value of this parameter?
   b. If the enzyme is not very slow, maybe it’s the next enzyme that’s the culprit. Check that out.
   c. Is the enzyme very fussy about substrate levels? What parameter would indicate this? Put another way, what do you call the level of substrate required to get half-maximal activity? What is the value of this parameter?
Not good. If the reason does not lie with an inability of the enzymes to keep up, then what’s left?

**SQ6. Consider the two reactions you (should have) checked out in SQ5**

a. **What are the substrates in each reaction?**

b. **Examine the concentrations of the substrates for the reactions, as listed in the Excel file you generated. If any of them get low and approach 0, then the velocity of the reaction will also approach 0. Does this happen?**

Whoops! There’s a substrate not listed in your output. How did that happen? Anyway, it’s time to rectify that situation

**SQ7. Modify glycolysis.py so that the missing metabolite is either printed out or written to the file you bring up into Excel. Either way, what is its value at the end of the run?**

We’ve discovered a big problem in glycolysis.py, one that must be solved for it even remotely model reality.

**III. The universal challenge to glycolysis**

Every organism that uses glycolysis, which is virtually every organism on earth, must somehow address a challenge built into the pathway. If you write glycolysis as a series of reactions that takes glucose to pyruvate, it will look like this:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{NAD}^+ \rightarrow 2 \text{Pyruvate} + 2 \text{ATP} + 2 \text{NADH} + 2 \text{H}^+
\]

Glucose comes somehow from the outside (from the blood, in the case of trypanosomes). Where does ADP and NAD\(^+\) come from? There is some new synthesis from adenosine in the first case and nicotinamide and adenosine in the second, but by far the greater amount of each is regenerated: ATP \(\rightarrow\) ADP + P\(_i\), and NADH + H\(^+\) \(\rightarrow\) NAD\(^+\). The regeneration of ADP is no problem. Many processes essential to life consume ATP and produce ADP, e.g., protein synthesis and active transport.

**SQ8. How do Eisenthal and Cornish-Bowden regenerate ADP in their model? Look at Table 1 and p.5501.**

NAD\(^+\) is another matter. Some NADH may be consumed (thereby regenerating NAD\(^+\)), for example in fatty acid synthesis, but there is a far greater need for ATP generated by glycolysis than for NADH. Every organism must solve the problem of how to oxidize NADH to NAD\(^+\), i.e. find a parking place for the two extra electrons on NADH. We solve the problem by oxidizing NADH to NAD\(^+\) through oxidative phosphorylation in the mitochondria, giving the electrons to
O₂ to form water. In muscle, under conditions of great exertion, there isn’t enough O₂ present, so an alternative route is used (see figure on previous page), using NADH to reduce (add electrons to) pyruvate, generating lactate and NAD⁺. Other organisms regenerate NAD⁺ by reducing pyruvate (through two steps) to ethanol, and there are other solutions. But something must be done to get NAD⁺ back!

SQ9. Examine Fig. 1 from Eisenthal and Cornish Bowden (1998). [Actually, I find their figure confusing with respect to NAD⁺/NADH, so I redrew it (see left). Note that red lines represent oxidized substrates and green lines reduced substrates. Note also that the lines cross for reaction 12, so NAD⁺ enters from the bottom.].

a. According to the figure, how do trypanosomes regenerate NAD⁺ in glycosomes?

b. What happens to the product of the reaction that regenerates NAD⁺ in glycosomes?

c. Are the reactions you identified represented in Eisenthal and Cornish-Bowden’s model (Table I)?

d. Are they represented in glycolysis.py?

SQ10. Modify glycolysis.py so that it incorporates the insights you have gained, regenerating NAD⁺. Run the program. What is the behavior of FDP now?

If you choose to add a reaction to your model of glycolysis, now and in the future, know why you did it. It's silly to think you're adding the reaction to make glycolysis "complete". What's complete? There are thousands of reactions that take place in a cell. Panel A to the right gives you one view of reactions related to glycolysis (click here for a better view).

Why not add the few dozen reactions shown in that image? Or if that's not enough, try Panel B, where you'll find several hundred reactions related at least distantly to glycolysis (here's a better view of them).

The point is, you need biological reasons to add a reaction to your model.