BNFO601: Integrated Bioinformatics  
Scenario: Metabolic Modeling – Eisenthal & Cornish-Bowden

Outline:

I. Tour of Eisenthal & Cornish-Bowden (1998)
II. Overview of glycolysis.py
III. Output of glycolysis.py

I. Tour of of Eisenthal & Cornish-Bowden (1998)

What follows are questions that occurred to me as I read the article:


I.A. Introduction

SQ1. (Paragraph 1) In the first sentence I read "...designing molecules ab initio...". What do you think is meant by that? What is their motivation for bringing up the matter?

SQ2. (Paragraph 3) The authors are clearly irritated by a series of articles collected under the title "Intelligent Drug Design". To pursue this matter, we would need to look up some of those articles to see what they're about, but without going that far (unless you want to), what do you think's their beef? What, in their view, should those articles have done that they didn't do?

SQ3. (Paragraph 4) Why do they consider substrate analogs as the easiest to design? Why do they consider this a futile approach?

SQ4. (Paragraph 5) The authors describe two general strategies to affect the metabolism of an organism. What are ways in which they differ from one another?

SQ5. (Paragraph 5) They seem to like "uncompetitive inhibitors". What are they and why the preference?

SQ6. What do you perceive to be the purpose of the work reported in this article? (Note this to look back on later)

I.B. Experimental Model

Note: Ordinarily, I’d skip the Materials and Methods section of a research paper, going back to pertinent parts of it as needed as I go through the Results section. In this case, however, it is the methods that are of particular interest.

SQ7. (Paragraph 1) Why is it “obvious from inspection” that the sum [ATP] + [ADP] + [AMP] is a constant? Why is the same true with [NAD] + [NADH]?

SQ8. (Kinetics, paragraph 1) Where does Equation 2 come from? You don’t have to derive it (unless you want to), but describe the steps by which you could derive it and the basis for your belief.
SQ9. (Kinetics, paragraph 2) “...all rates... are expressed as dimensionless numbers...” through a trick. Take the $V_{\text{max}}$ for glucose transport in Table I and express redimensionalize it, expressing it with real units.

SQ10. (Kinetics, paragraph 3) Where does Equation 3 come from? It looks like a different species from Equation 2. Compare them carefully and determine how they really differ from one another.

SQ11. (Kinetics, paragraph 6) As the authors say, the equation looks excessively complex. We’re not going to learn where it came from without going to reference 19, which I’ll leave for another day. For now, let’s just check the authors claims about this equation.

11a. Simplify the equation for the condition that the product concentrations are zero, and compare the resulting equation to Equation 2, informed by Table 1.

11b. Simplify the equation for the condition that the reaction is at equilibrium ($\text{velocity} = 0$)

SQ12. How does Figure 1 relate to Table 1? Determine how you would use the information in Table 1 to populate the variables of Equation 2.

II. Using the model (overview of glycolysis.py)

Our goal is to capture the essential features of glycolysis in trypanosomes, using the model provided by Eisenthal and Cornish-Bowden, so that we can manipulate a model of the pathway to determine which step may be most effectively inhibited to lower ATP concentration. The basic tool of modeling we’re considering is the use of differential equations to compute the change in metabolite concentrations over a short time interval. The work cycle in the program should look something like this:

1. Initialize concentrations for each metabolite
2. Initialize constants for each reaction
3. Calculate the rate of change of each metabolite, given the current concentrations (each rate of change is analogous to a velocity)
4. Multiply each rate of change by a time increment, giving an increment for each metabolite (analogous to multiplying a velocity by a duration to give the distance traveled)
5. For each metabolite, add the increment to the original concentration to a new concentration (analogous to finding a new location by adding the distance traveled to the old location)
6. Accommodate those metabolites that are in equilibrium with each other
7. Repeat steps 3 through 6 as many times as you like

Now download glycolysis.py and glycolysis_subroutines.py (go to the main scenario page and scroll down to the list of programs).

SQ13. Identify segments of the program that accomplish each of the processes identified above.

This program is in some ways more complicated than any we’ve encountered thus far in the course, owing to the large number of constants and variables. On the other hand, those constants
and variables should seem pretty familiar. And the Main Program isn’t too complicated. Actually, from a computational point of view, this may be one of the simplest programs you've encountered in the course (once you get past the definitions of reactions and metabolites).

SQ14. In the program segment entitled “METABOLITES and VARIABLES” there is the line Glc = metabolite("glucose",0). What does this line do, and how does it do it?

SQ15. Identify the source of the constants in the program segment entitled “REATIONS”. For example, where does "106.2" in Rxn1 come from? How about "0.68" in Rxn5?

SQ16. Compare the two lines Rxn1 = reaction(...) and Rxn2 = reaction(...), noting that the corresponding lists in these two lines are of different sizes. How does the definition of the reaction class work its magic of assigning the constants to the right places?

SQ17. What is default? What kind of value does it contain? If you run a test program containing only the line of code that sets the value of default, it will fail. Why does it work within glycolysis.py?

SQ18. In the subroutine Calculate_dxdt, there is the following line:
   FruP2.dxdt = Rxn4.v - Rxn5.v
   What does FruP2.dxdt represent? Rxn4.v? Rxn5.v?
   Why is FruP2.dxdt defined as the sum of Rxn4.v and -Rxn5.v?

SQ19. How is Rxn4.v calculated?

III. Output of glycolysis.py

The time has arrived. Run the program (on my computer it takes about 20 sec). Other than displaying "DONE", what did it do?

SQ20. Examine the documentation of the program and the program itself to determine what of use the program actually produces.

SQ22. Examine the output in Excel (block out all the data and use a Scatter chart).

SQ23. Identify on the chart the metabolite that most conspicuously drops in concentration throughout the duration of the simulation and the metabolite that most conspicuously increases in concentration. Do these behaviors make intuitive sense?

It seems pretty absurd to think that a population of trypanosomes can suck up a significant amount of blood glucose in 0.3 seconds – that would be instant death the moment a trypanosome is injected by the tse-tse fly! Yet that is what this simulation seems to be saying, because the model doesn’t know enough to consider blood glucose to be a nearly inexhaustible pool. Let's help it out. There are several ways one could change the program to capture the idea that blood glucose remains constant. One way is to put in the total amount of glucose in the blood and the
total number of trypanosomes… too much work for now. Let’s just hard wire in the constancy of blood glucose.

**SQ24.** Change the subroutine `Calculate_dxdt` to ensure that the concentration of external glucose (external to the trypanosome) doesn’t change. (I chose this subroutine as the best place to make the change, because it seems to me that the subroutine should contain all our thinking about what affects metabolite concentrations.) Run the program with this change and display the results in Excel. Use this version as the starting point of further modifications to the program.

**SQ25.** What is the behavior now of the metabolites you identified in SQ23? Is there still a problem to be solved? If so, solve it.

**SQ26.** Which metabolites achieve, after an initial sorting out period, near steady state (a nearly constant concentration over time)? You may have to adjust the scale of the Y-axis to see the behavior of some of the metabolites.