Supplemental Material

CBE—Life Sciences Education

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Supplemental Materials

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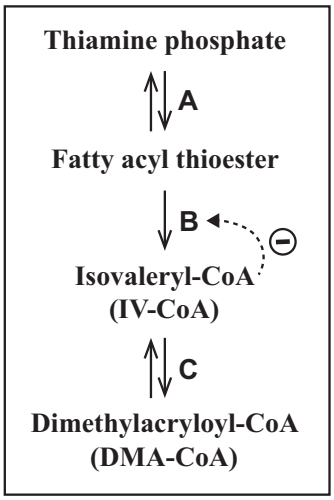
FIGURE S1. Complete problem set used in the study. Correct answers are available from the corresponding author upon request.

The metabolic pathway below shows the conversion of thiamine phosphate to dimethylacryloyl-CoA (DMA-CoA).



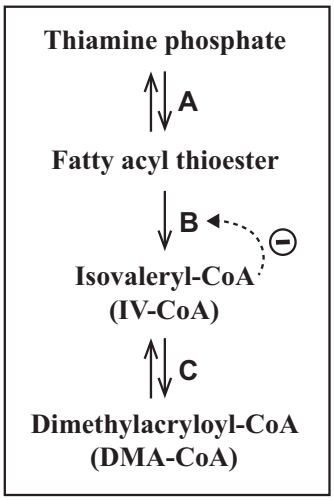
1. How many of each kind of molecule are shown in the figure?

	0	1	2	3	4	5	6	7	8	9	10
Enzymes	○	○	○	○	○	○	○	○	○	○	○
Metabolic compounds	○	○	○	○	○	○	○	○	○	○	○

2. What do the dashed arrow and circled bar represent?

	True	False
negative feedback or inhibition	○	○
positive feedback or activation	○	○
removal of IV-CoA	○	○
IV-CoA being added as a reactant	○	○
IV-CoA being recycled	○	○
reverse reaction	○	○
alternate pathway	○	○

The metabolic pathway below shows the conversion of thiamine phosphate to dimethylacryloyl-CoA (DMA-CoA).



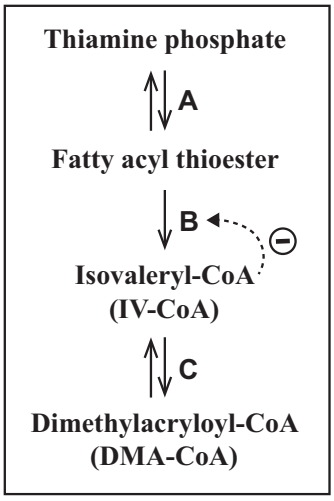
3. Do you predict that Enzyme A could convert fatty acyl thioester to thiamine phosphate?

- ○ Yes, enzymes are generally reversible.
- ○ No, enzymes are generally irreversible.

4. If enzyme C were inhibited, how might this eventually impact the activity of enzyme B?

- ○ The activity of enzyme B would not be affected.
- ○ The activity of enzyme B would increase.
- ○ The activity of enzyme B would decrease.

The metabolic pathway below shows the conversion of thiamine phosphate to dimethylacryloyl-CoA (DMA-CoA).



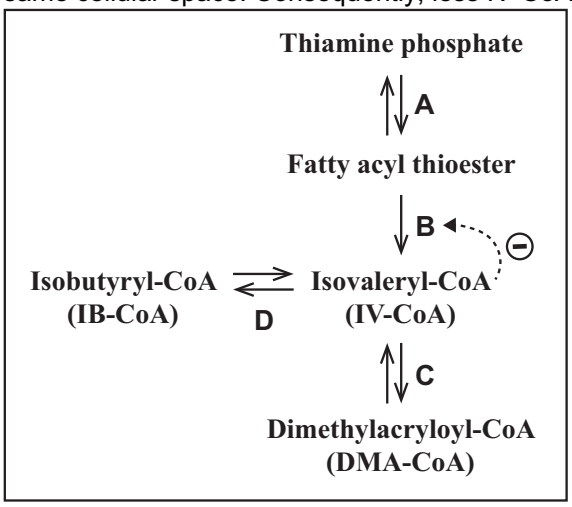
5. Assuming a constant concentration of all enzymes, which are fully functional, what controls how much DMA-CoA is made by this pathway?

	True	False
concentration of thiamine phosphate	○	○
concentration of fatty acyl thioester	○	○
concentration of IV-CoA	○	○
inhibition by IV-CoA	○	○
activation by IV-CoA	○	○
binding of IV-CoA to enzyme B	○	○

6. Under what conditions would it be possible for enzyme C to convert DMA-CoA into IV-CoA?

	True	False
the ratio of DMA-CoA to IV-CoA > the ratio at equilibrium	○	○
the conversion of DMA-CoA into IV-CoA has a negative delta G value	○	○
the reaction is coupled to another favorable reaction	○	○

As shown below, isovaleryl-CoA (IV-CoA) can also be consumed by another pathway that occurs in the same cellular space. Consequently, less IV-CoA is available for DMA-CoA production.

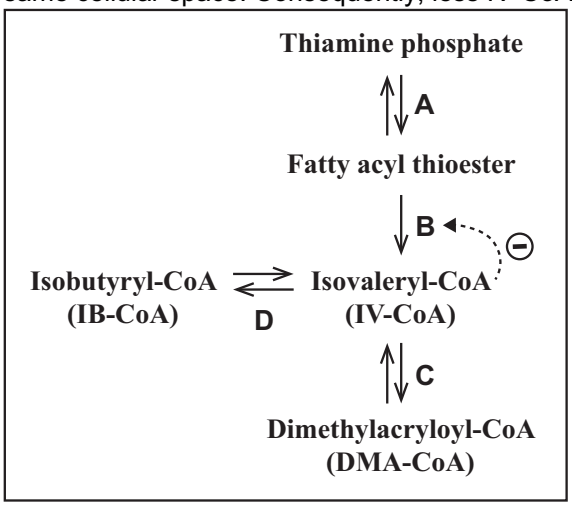


7a. Assume that both branches of the pathway are in place, and there are no other changes. Do you predict that having a branch point in this pathway will affect flux through the pathway as a whole, including both branches, as opposed to when only the main branch was in place?

- ❍ Yes, flux will be affected.
- ❍ No, flux will not be affected.

7b. Provide a scientific explanation to support your prediction.

As shown below, isovaleryl-CoA (IV-CoA) can also be consumed by another pathway that occurs in the same cellular space. Consequently, less IV-CoA is available for DMA-CoA production.

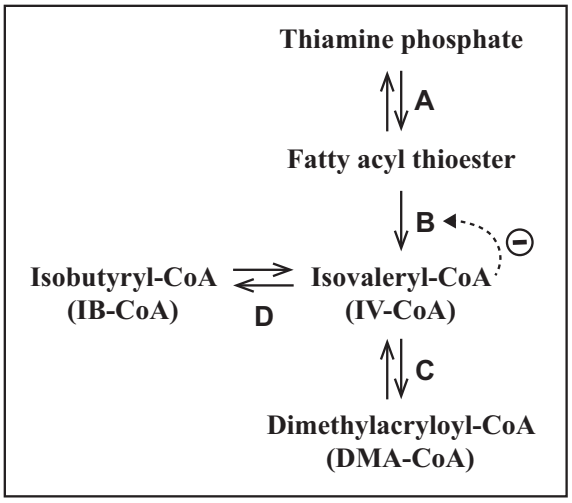


8a. Assume that both branches of the pathway are in place, and the only other change is that IV-CoA can no longer bind to enzyme B. Do you predict that this will affect flux through the pathway as a whole, including both branches, as opposed to when only the main branch was in place?

- ❍ Yes, flux will be affected.
- ❍ No, flux will not be affected.

8b. Provide a scientific explanation to support your prediction.

As shown below, isovaleryl-CoA (IV-CoA) can also be consumed by another pathway that occurs in the same cellular space. Consequently, less IV-CoA is available for DMA-CoA production.



9a. Assume that both branches of the pathway are in place, and the only other change is that enzyme C doesn't work anymore. Do you predict that this will affect flux through the pathway as a whole, including both branches, as opposed to when only the main branch was in place?

- ❍ Yes, flux will be affected.
- ❍ No, flux will not be affected.

9b. Provide a scientific explanation to support your prediction.

FIGURE S2. Coding process for qualitative content analysis. The *a priori* codebook was based on prior research and was refined to provide a final codebook of twelve codes which are presented in Tables 2 and 3 of the main paper. This figure illustrates the process. The original codebook contained the *a priori* code "Misinterpret the dotted feedback arrow." In round 2 coding, we revised this code to "The compound is 'reverting to a previous state'" in order to represent the dataset with greater precision. During a review of coding, the code name was revised yet again to indicate multiple ideas present in the dataset. In the final round of analysis, we split this code into two distinct knowledge elements students expressed about the dashed arrow and circled bar: recycling and reversion.

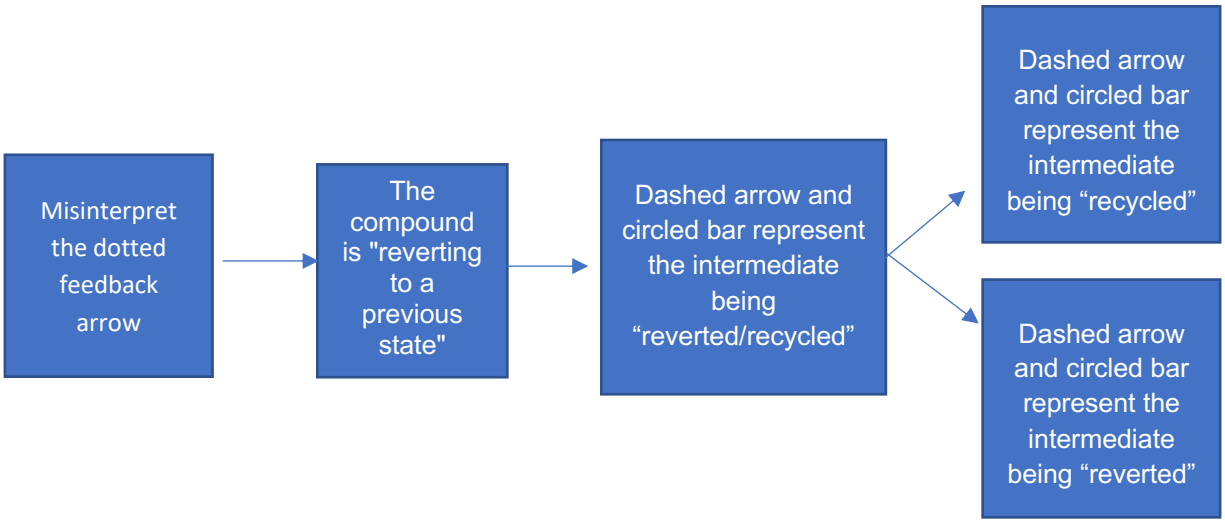
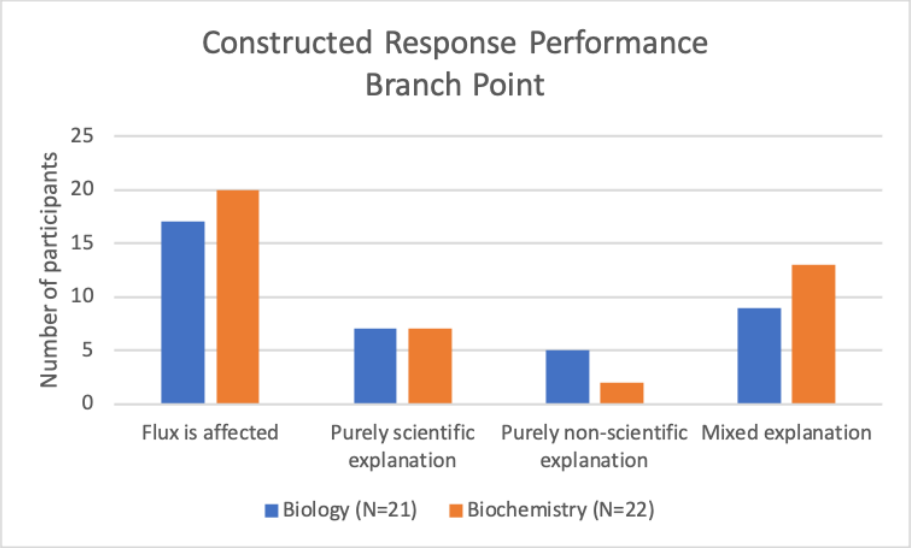
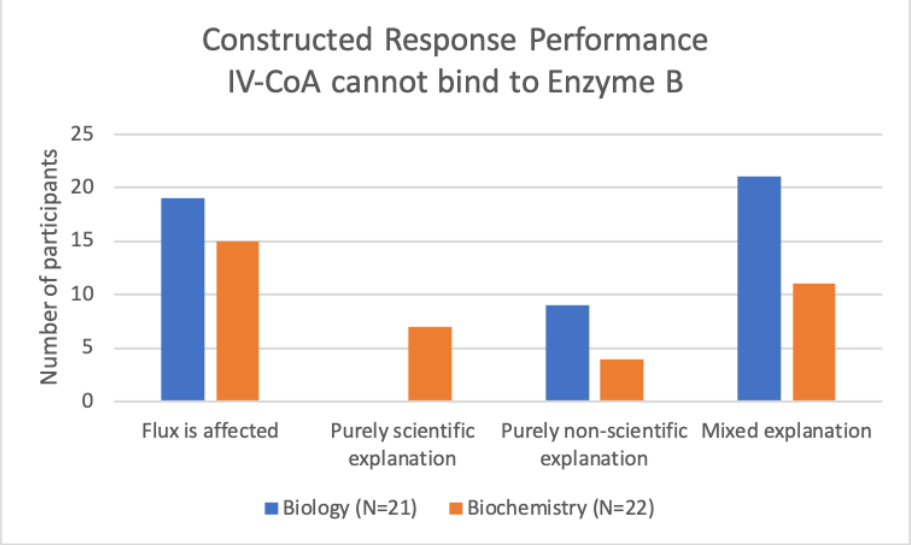


FIGURE S3. Participant performance on individual constructed response items. Participants were asked to predict the outcomes of three perturbations to the metabolic pathway (items 7-9 in Figure S1). Figure 2 in the main paper shows the average performance across all three problems. The figures below show student performance on each item. A) Item 7: Predict the impact of adding a branch point to the pathway; B) Item 8: Predict the impact if IV-CoA can no longer bind to enzyme B; C) Item 9 (next page): Predict the impact if enzyme C doesn't work anymore. The leftmost set of bars shows the number of participants who predicted that flux would be affected by the perturbation. The remaining three sets of bars show the number of students who provided explanations that were purely scientific, purely non-scientific or mixtures of both across all contexts. Blue bars correspond to biology participants (N=21) and orange to biochemistry participants (N=22).





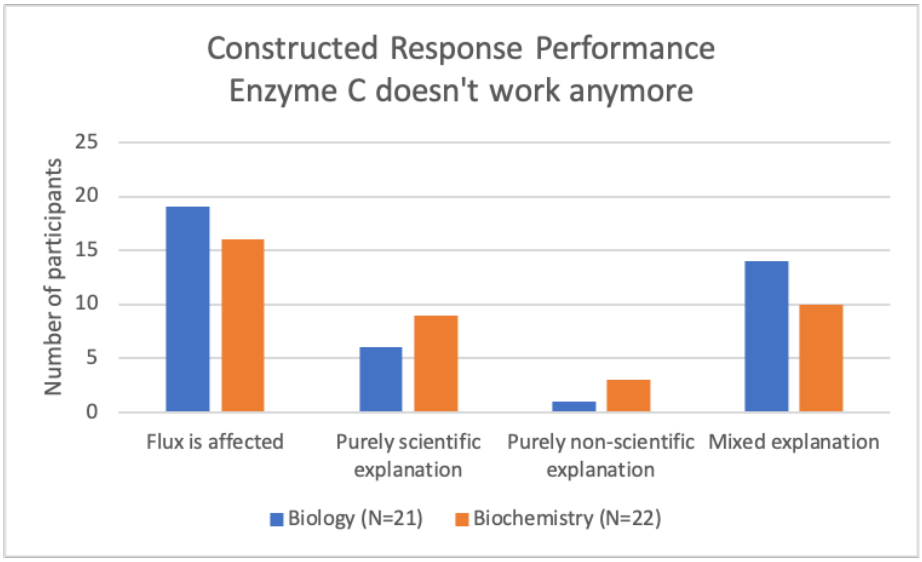

Constructed Response Performance
Enzyme C doesn't work anymore
Number of participants
25
20
15
10
5
0
Flux is affected
Purely scientific explanation
Purely non-scientific explanation
Mixed explanation
Biology (N=21)
Biochemistry (N=22)

FIGURE S4. Lesson designed to improve student learning about metabolism

The following activity was designed to address the results reported in this article. The activity provides scaffolding for students to refine their intuitions of the visual representations commonly used to depict metabolism, the principles of reaction reversibility, metabolic pathways as whole systems instead of individual reactions, and predictions of the dynamics of a pathway in response to changes in the cellular environment. A key for the activity is available from the corresponding author upon request.

This activity addresses fourteen learning objectives and is taught over two weeks of a four-credit course.

Metabolic Pathway Dynamics and Regulation

Learning Objectives

1. State the cause, symptoms, and treatment for Hereditary Orotic Aciduria
2. Explain what it means to say that a metabolic pathway is a system and why it is important to think of them as systems
3. Interpret visual representations of metabolic pathways
4. Define “flux” in a chemical pathway
5. Explain the following ways that enzymes are regulated: substrate-level control, feedback control, heteroallostery
6. Use the pyrimidine synthesis pathway to illustrate the fact that enzyme regulation allows metabolic pathways to be sensitive and responsive to an organism’s environment
7. Use the example of aspartate transcarbamoylase to describe the noncovalent interactions and structural changes that happen when allosteric molecules bind to allosteric enzymes
8. Note that metabolic pathways include near-equilibrium and far-from-equilibrium reactions.
9. Distinguish among standard, actual, and equilibrium conditions and ΔG values.
10. Use the following equation to calculate Q: $\Delta G = \Delta G^{\circ\prime} + RT \ln Q$.
11. Distinguish between Q and Keq.
12. Define steady state.
13. Provide examples of the fact that steady state levels of metabolic compounds are influenced by the regulation of numerous biological reactions.
14. For a given scenario, predict how changes in one metabolic pathway can influence flux through another pathway.

To this point in the semester we have studied the structure and function of macromolecules like proteins, considered how enzymes (i.e., one class of proteins) work to change the kinetics of chemical reactions. We have also considered that enzymes can be inhibited. We have only considered isolated chemical reactions. Now we will shift our thinking to systems of chemical reactions that we call metabolic pathways. Metabolic pathways show us how biological molecules connect to each other in complex networks and that a change in one component of the network can affect many other components.

1. **Read the case study of Orotic Aciduria posted to eLC.**

   A. What enzymes are deficient in Orotic Aciduria?

   B. What are the symptoms or Orotic Aciduria?

C. Why does orotic acid buildup in these patients?

D. What is the treatment and why does it work?

2. Use your textbook Figure 19.9, de Novo biosynthesis of pyrimidine, as a reference for this question. In this case, we are considering the pyrimidine biosynthesis pathway as an example that illustrates the importance and features of metabolic pathways. Work your way through the questions below to make sure you know how to "read" the pathway.

A. How many enzymes are in this pathway? How are they visually represented?

B. How many distinct substrates are in this pathway? Not all substrates are visually represented in the same way. What are the different ways the substrates are visually represented?

C. The reactions shown are all visually represented with unidirectional arrows. Should we assume that the reactions can only go in the forward direction? Why or why not? What additional information would we need to determine the favored direction of each reaction?

D. How many enzymes in the pathway are heteroallosterically regulated? What are the allosteric molecules and do they activate or inhibit the enzymes? How is heteroallostery visually represented?

E. Tying back to question 1c, state how problems with heteroallosteric regulation lead to orotic aciduria?

NOTE: Biochemists do not have good conventions for visually representing metabolic pathways. Enzymes, substrates, arrows, and heteroallostery are visualized in different ways across textbooks and even within the same textbook. Beware of this. Ask if you are unsure how to interpret the representation. Now let's try to understand more about the concepts that the visual representations attempt to convey.

3. **Explain how substrate-level control could influence pyrimidine synthesis.** Use step 4 (dihydroorotate → orotate) as your example for thinking about substrate-level control. *Note: In this scenario, assume the enzyme is functional.*

4. Feedback control and heteroallostery

A. Consider enzyme 2 in pyrimidine biosynthesis. This enzyme is aspartate transcarbamoylase (ATCase), and it catalyzes the conversion of aspartate + carbamoyl phosphate to carbamoyl aspartate. CTP is a heteroallosteric regulator of ACTase; CTP participates in feedback inhibition of ATCase. Explain what it means for CTP to be a feedback inhibitor and why cells benefit from feedback inhibition.

B. Summarize the mini-lecture you heard about the reasons that ATP is a heteroallosteric activator of ATCase.

C. Imagine a scenario in which CTP is inhibiting ACTase. How will this affect the concentrations of the following molecules?
- Aspartate -
- Bicarbonate -
- Carbamoyl aspartate -
- Orotate -
- Uridine monophosphate -
- CTP –

D. Use Figure 8.37 from your textbook to describe how CTP and ATP affect the structure and activity of ATCase.

E. Consider Figure 8.38 from your textbook. Noncovalent interactions are enabling ATP, CTP, UTP, and Mg^{2+} to interact with ATCase. Based on the structures of these heteroallosteric molecules, propose what types of ATCase R groups they may be interacting with. Name at least two types of R groups that may be involved.

Now that we have studied some principles of metabolism using the pyrimidine biosynthesis pathway, let's look at another pathway that is more familiar to you, glycolysis.

5. **Near- and Far-From-Equilibrium Reactions.** Examine Figure 16.1 and Table 16.1 from your textbook. These figures describe glycolysis, which we will spend more time on in Unit 3. For now, we want to use glycolysis as an example of metabolic pathway control and steady state.

A. Name two glycolysis steps that are near equilibrium and two that are far from equilibrium. Explain which delta G value you used to make your decision and why.

B. Explain the relationship between the driving force of a reaction and the distance of that reaction from equilibrium.

C. Compare the near- and far-from-equilibrium steps of glycolysis. Are near- or far-from-equilibrium steps more likely to go in reverse? Explain.

D. Based on your answer to #4, do you think near- or far-from-equilibrium steps are more likely to be the control points in a metabolic pathway? Explain.

6. **Distinguish among the ΔG of reactions under various conditions.** We have established that cells tend to exert control over flux through a pathway at one or a few steps, not every step. We have also established that these steps tend to be far-from-equilibrium steps. The questions below help you distinguish the relationships among ΔGs values at standard, steady state, and equilibrium conditions. We will do this by considering step 2 in glycolysis: the interconversion of glucose-6-phosphate and fructose-6-phosphate.

	Concentration of Glucose-6-phosphate	Concentration of Fructose-6-phosphate	Mass Action Ratio ([F6P]/[G6P])	Free Energy
Standard conditions				**ΔG°'= 1.7 kJ/mol**
Steady state conditions				**ΔG = -2.5 kJ/mol**
Equilibrium conditions				**ΔG =**

   A. Under standard conditions, what is the concentration glucose-6-phosphate? Fructose-6-phosphate? The mass action ratio? Write these numbers in the table.

   B. Calculate the concentrations and mass action ratio for this reaction under steady state conditions. Use this equation: ΔG = ΔG°' + RT ln Q. *Note that you are solving for Q. R = 0.008314 kJ/(mol*K) ; T = 298K*

      i. What is the mass action ratio?
      ii. Assuming there is 1 mM of fructose-6-phosphate in the cell under steady state conditions, what would be the concentration of glucose-6-phosphate?
      iii. Write these numbers in the table.

   C. Equilibrium conditions:
      i. What is the ΔG at equilibrium?
      ii. Use the following equation to calculate the equilibrium concentration and mass action ratio at equilibrium: ΔG = ΔG°' + RT ln Q. Note that you are solving for Q again.

      iii. Under equilibrium conditions Q has a special name. What is it?

      iv. Write this information in the table.

      v. Is the interconversion of glucose-6-phosphate and fructose-6-phosphate near- or far-from-equilibrium? Is this step likely to be regulated? Explain.
      vi. ***For practice on your own,*** create and complete a similar table for step 1 of glycolysis: glucose + ATP → glucose-6-phosphate + ADP + H^+

7. **Maintenance of steady state.** Given our work so far, we know we need to consider the following to understand flux through a metabolic pathway:
   - What are the control points?
   - What are the near- and far-from-equilibrium steps?

- How do the concentrations of reactants and products compare among standard, steady state, and equilibrium conditions?
- Given all these considerations, let's think about steady state and how cells maintain it.

A. Explain steady state in your own words.

B. In this case, we have considered pyrimidine biosynthesis and glycolysis. These two pathways may seem disconnected, yet figure 13.17 from your textbook shows linkages between them. Consider three questions:

   i. Explain how glycolysis could influence steady state levels of the metabolic compounds in pyrimidine synthesis. Remember that the end product of glycolysis is pyruvate.
   ii. Imagine that pyrimidine biosynthesis slows down (e.g., through inhibition of ATCase). How might this impact flux through glycolysis? Explain.
   iii. Imagine a person who has just consumed a glucose-rich meal. As a result, flux through glycolysis increases. Could this impact flux through the pyrimidine biosynthesis pathway? Explain.
   iv. Given these three scenarios, generalize to describe how steady state levels of metabolic compounds are influenced by the regulation of numerous biological reactions.