Supplemental Material

CBE—Life Sciences Education

Villafañe *et al*.

SUPPLEMENTAL MATERIAL 1: DESCRIPTION OF INSTRUCTIONAL CONTEXT

Description of Instruction:

The instrument was administered as part of the final exam in an introductory biochemistry course (*Biochemistry: Introduction to Structure, Enzymes, and Metabolism*). Class periods are mainly lecture-based, augmented with Socratic questioning and clicker questions. The clicker questions are used to facilitate class discussion and some think-pair-share exercises. Guided inquiry activities are used occasionally. Students in this course did not use a common text, but instead were given suggested textbook resources.

The metabolism portion of the course is the last three to four weeks of a ten week quarter. In the sixth week we spend three class periods on an introduction to metabolism (see learning objectives below). Much of this section relates to elucidating the order of intermediates in metabolic pathways from experimental data and includes numerous worked examples. Worked examples are followed by extensive discussion aimed at developing a conceptual understanding of metabolic pathways and how they can be elucidated from data. There is a discussion section worksheet focused on this process: how the experiments work, what the results tell us, and what we can conclude from them. The discussion sections are collaborative group work led by the TA and facilitated by undergraduate learning assistants.

There are weekly study questions that are previous exam questions (see examples below). These are a resource for the students and are not monitored or graded in any way. These problems are based on experiments using inactivating mutations to a pathway enzyme or isotopic labeling.

The glycolysis section (see learning objectives below) starts with a guided inquiry activity, which focuses on the source of energy to make ATP. This usually takes two class periods. Then there are about one and a half class periods focused on the regulation of glycolysis. This is from the perspective of why would a pathway need to be active or inactive and how is activity regulated. This and the regulation of TCA cycle and ETC builds on previous studies of ligand binding and allostery in the context of hemoglobin and competitive vs non-competitive inhibitors in enzyme kinetics.

Student learning objectives related to metabolism:

Introduction to Metabolism Objectives

- Distinguish between catabolism and anabolism.
- Identify features common to metabolic pathways and locate these features within a given pathway.
- Be able to elucidate the order of metabolic pathways from experimental data.
- Define the term high energy compound in chemical terms.
- Explain why and how metabolic pathways are regulated/controlled.
- Distinguish between kinetic control and thermodynamic control of a reaction.

Glycolysis Objectives

- Distinguish between ΔG and $\Delta G^{o'}$
- Explain where the energy to make ATP in glycolysis comes from.
- Identify the chemical importance of reactions in glycolysis

- Identify the points of regulation in glycolysis and correlate activators and inhibitors of the reactions with the flow of metabolism.
- Characterize far from equilibrium (sometimes called “irreversible” reactions) and near equilibrium reactions, know which reactions in glycolysis are each.
- Describe the purpose of gluconeogenesis
- Explain glycolysis and gluconeogenesis as an example of opposing pathways.
- Distinguish between aerobic and anaerobic glycolysis in terms of overall reaction and the importance of NAD+ in the anaerobic process.

TCA Cycle Objectives

- For each step of the TCA cycle, Know:
  - What we get out of it.
  - Thermodynamics
  - Be able to follow where the carbons of Glucose end up in the CAC, or where each carbon in the CAC originated from.
- Explain how CAC intermediates are replenished.
- Know which steps of the CAC are regulated
- Understand the regulatory mechanisms in the CAC, and how they are applied at each step

Oxidative Phosphorylation Objectives

- Know how substrates for oxidative phosphorylation get into the mitochondria.
- Understand the chemiosmotic hypothesis and explain the evidence for it.
- Describe the effects of uncouplers on intact mitochondria.
- Distinguish between coupled and uncoupled mitochondria.
- Describe the components of the Electron Transport Chain
- Generalize the requirements for proton transport coupled to electron transfers.
- Discuss the current model of ATP synthesis.
- Explain how the regulation of the central metabolic pathway is coordinated
- Describe the advantages of regulatory cascades.
- Distinguish between hormonal versus metabolic control.

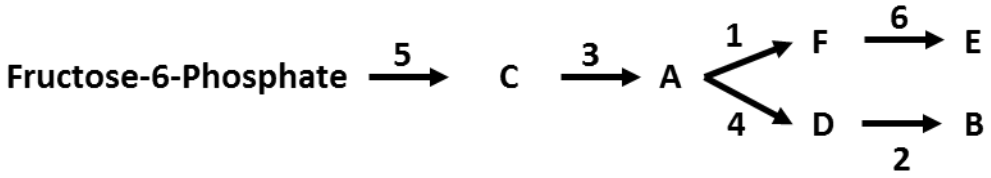
Sample Study Questions:

1. Compounds B and G are the products of a biosynthetic pathway, including intermediates A through H, that branches after intermediate A. B is an essential metabolite, whereas G is not. You have 7 individual strains containing a mutation in an enzyme involved in the overall pathway and assess them for their ability to grow on each metabolite. The data collected is presented in the table below. (a + means the strain with a mutation in that enzyme is capable of growing when fed the metabolite at the top of the column)

	A	B	C	D	E	F	G	H
1	+	+				+		
2		+						
3	**Not Auxotrophic**							
4	+	+	+		+	+		
5		+				+		
6	**Not Auxotrophic**							
7	+	+	+			+		

a. Indicate the possible orders of the metabolites and the enzymes in the pathway.
b. Given that there are 2 intermediates and 2 enzymes whose place in the pathway you cannot determine. How could you distinguish between these possibilities?
c. Which enzymes in this pathway would you expect to be regulated?

2. The pathway below is an anabolic pathway, whose two end products are both essential. You have strains containing mutations in all the enzymes (1,2,3,4, 5, and 6) known to be involved in the pathway. You grow each strain with the addition of each intermediate, and in some combination.



a). Predict the results you would expect in the table below. A + would mean that when a strain containing a mutation in the enzyme on the left is grown in the presence of the metabolite(s) at the top of the column the strain is able to grow.

	A	B	C	D	E	F	D & E
1							
2							
3							
4							
5							
6							

b). If you performed an accumulation experiment (growing strains containing mutations in the enzymes and observing which intermediates built up), what would your results be?

Fill in the following table:

	A	B	C	D	E	F
1						
2						
3						
4						
5						
6						

c). Which steps would you expect to be regulated?

d). For one of the steps in part b. Indicate which step you are choosing:

i. what compounds would you expect to activate this step?

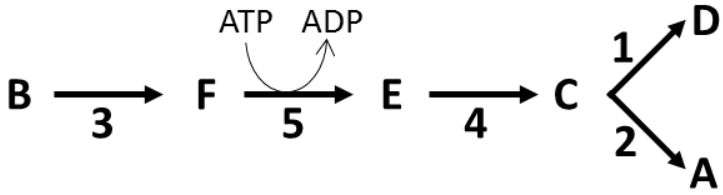
ii. What compounds would you expect to inhibit this step?

e). Fructose-6-Phosphate can be synthesized from fructose. This pathway ends up being activated by fructose but not by glucose. What advantage would this provide?

f). Briefly discuss how an enzyme binding site could distinguish between glucose and fructose.

3. Below is a non-essential biosynthetic pathway that you are studying.

A). Circle the Enzyme(s) for the step(s) that you would predict to be irreversible.



B). You have strains containing mutations in each enzyme in the pathway. **Based on your answer to part A**, predict the results of an experiment in which you analyzed each strain for accumulation of intermediates in the pathway. A + would mean that when you have a mutation in the enzyme on the left you detect accumulation of the intermediate at the top of the column.

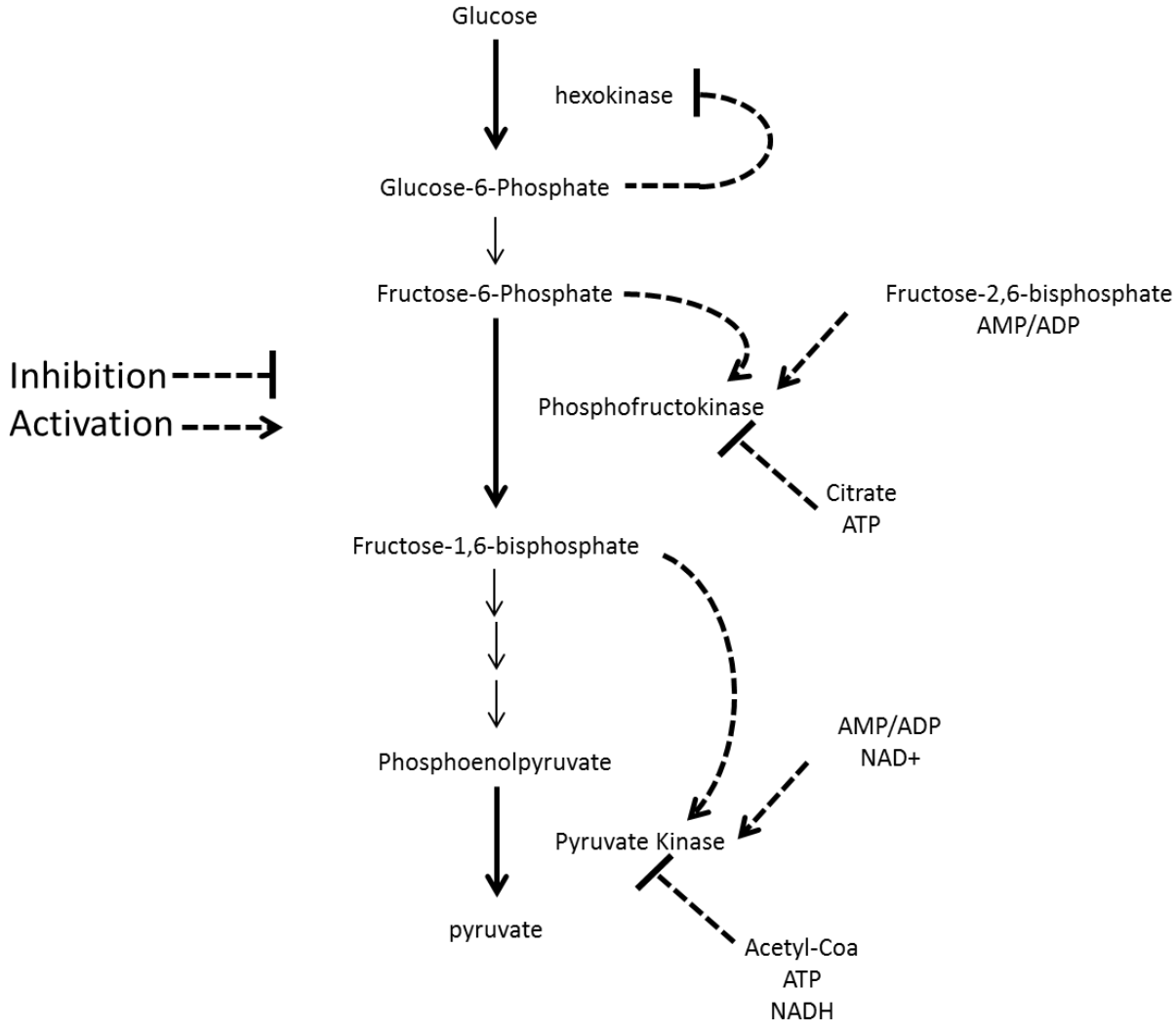
	A	B	C	D	E	F
1						
2						
3						
4						
5						

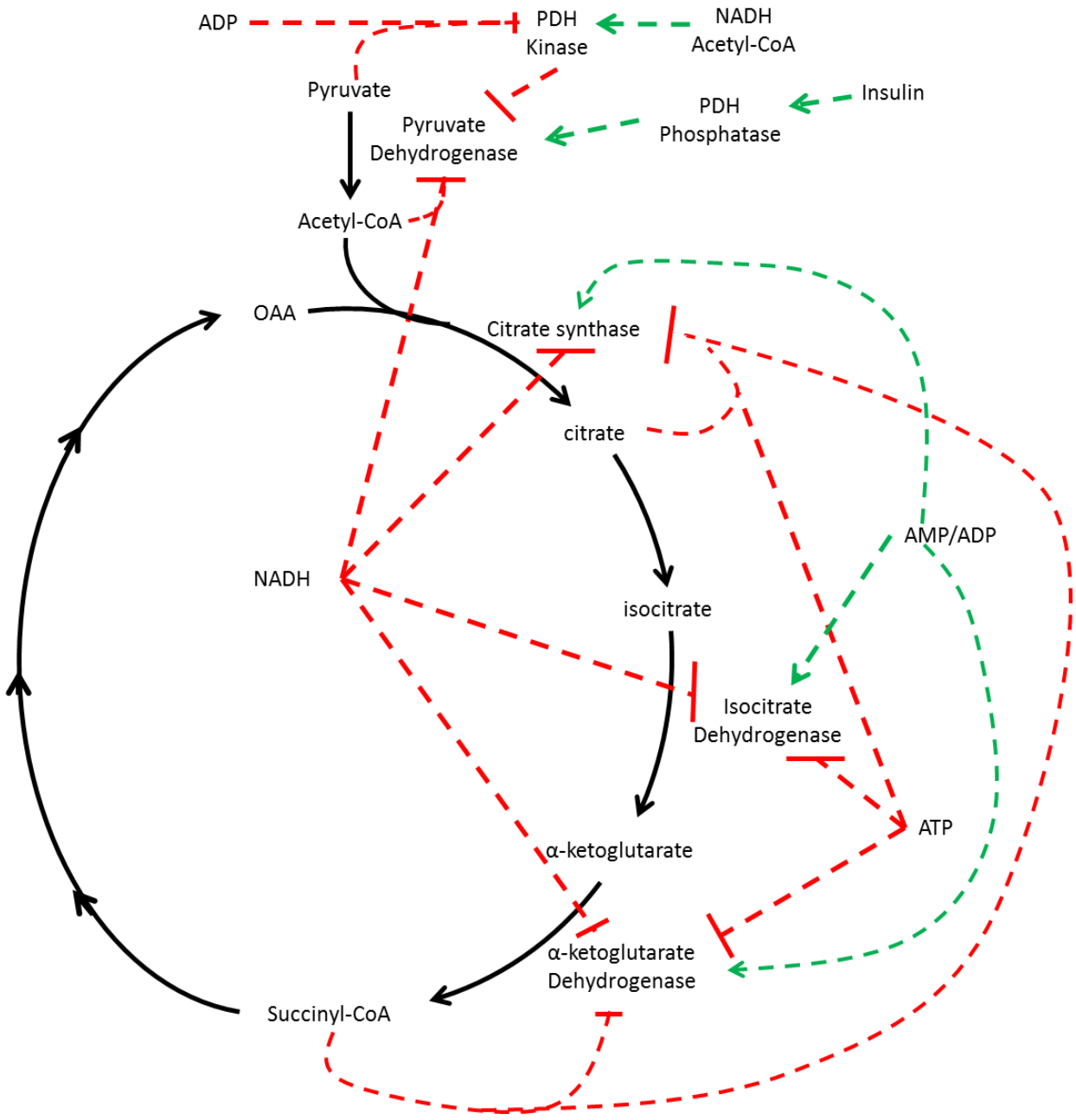
C). Would you be able to elucidate the entire pathway with just the data from that table? Briefly explain your reasoning.

D). Describe a different experiment that would be more effective for determining the overall pathway. Briefly explain how results would allow you to determine the pathway.

E). How would you expect the regulated enzymes in this pathway to be regulated by energy charge?

Sample Pathway Diagrams Used During Instruction:




ADP
PDH
Kinase
NADH
Acetyl-CoA
Pyruvate
Pyruvate
Dehydrogenase
PDH
Phosphatase
Insulin
Acetyl-CoA
OAA
Citrate synthase
citrate
AMP/ADP
NADH
isocitrate
Isocitrate
Dehydrogenase
ATP
α-ketoglutarate
α-ketoglutarate
Dehydrogenase
Succinyl-CoA

SUPPLEMENTAL MATERIAL 2: PHASE 1 DATA ANALYSIS

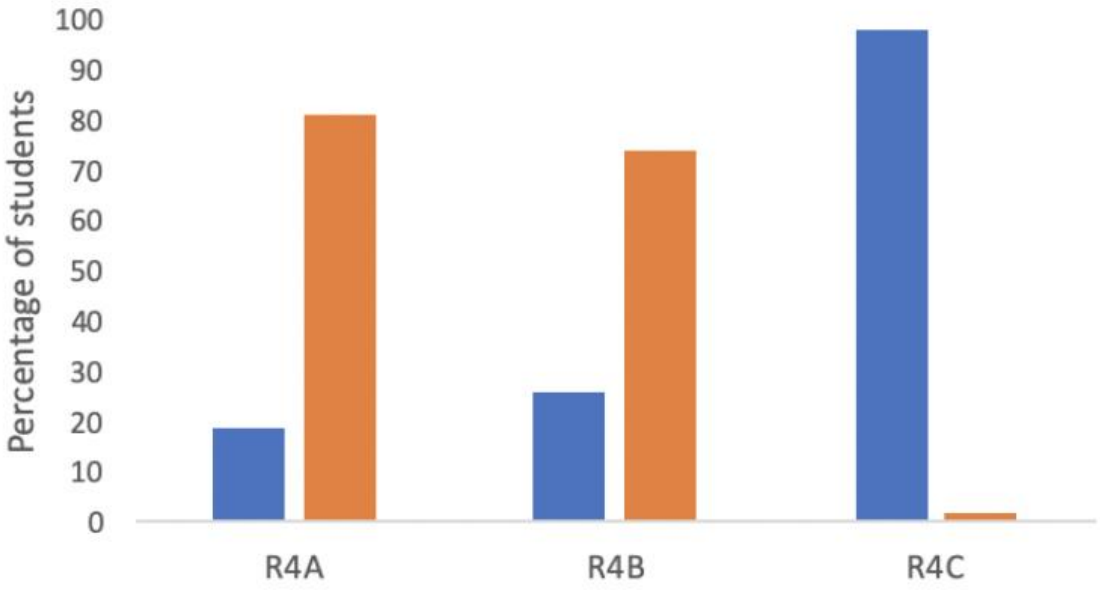


FIGURE S1. Students' constructed responses for Q4 were scored by two different raters using the rubric. Data from both raters were pooled for a total of 248 responses scored. The rubric directed raters to assess three different aspects of student responses shown as R4A (increase rate of delta production), R4B (foxtrot is an inhibitor of X_2 activity), and R4C (loss of allosteric binding leads to increased rate of delta production). Raters assigned a score of 0 for each statement that was absent (blue bars) and a score of 1 for each statement that was present (orange bars).

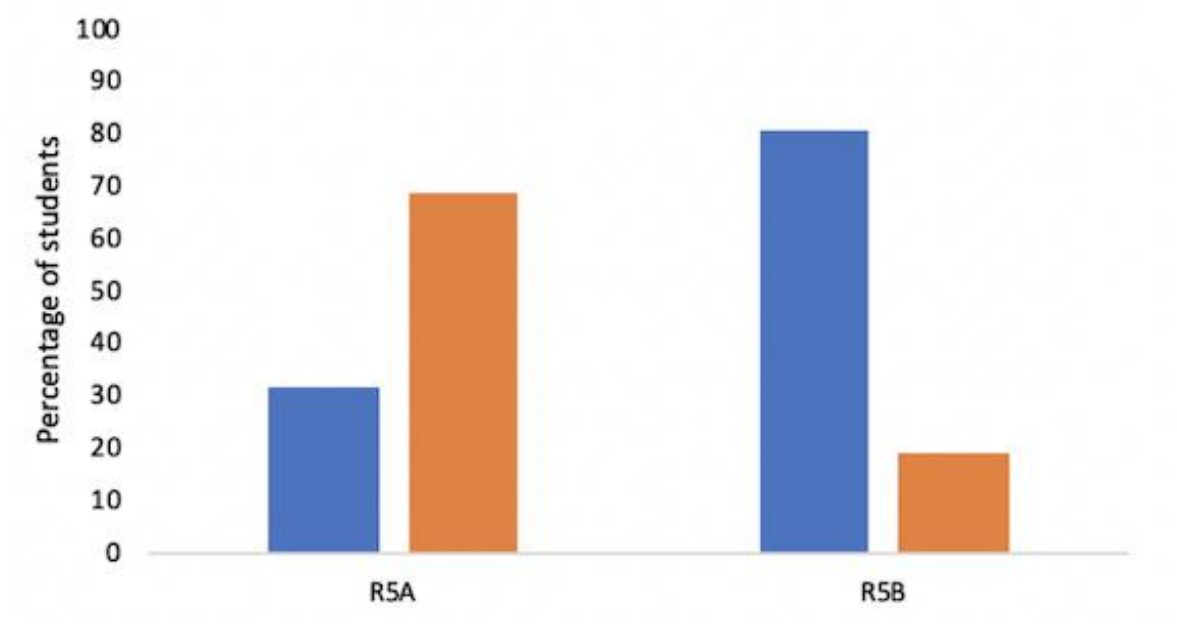


FIGURE S2. Students' constructed responses for Q5 were scored by two different raters using the rubric. Data from both raters were pooled for a total of 248 responses scored. The rubric directed raters to assess two different aspects of student responses shown as R5A (foxtrot production initially increases due to inhibition of Y) and R5B (over time there will be little change in foxtrot production). Raters assigned a score of 0 for each statement that was absent (blue bars) and a score of 1 for each statement that was present (orange bars).

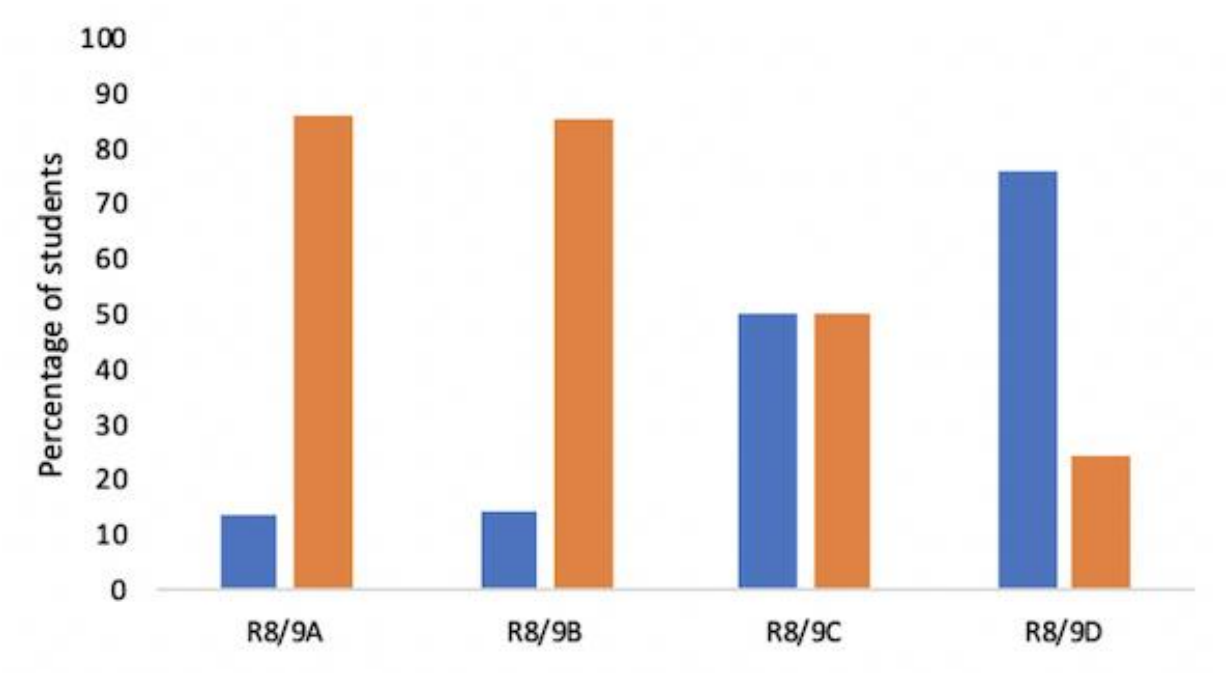


FIGURE S3. Students' constructed responses for Q8 and Q9 were scored by two different raters using the rubric. Only data for students choosing to mutate enzyme Y is depicted. Data from both raters were pooled for a total of 194 responses. The rubric directed raters to assess four different aspects of student responses shown as R8/9A (regulatory site of Y is mutated), R8/9B (mutation will relieve the inhibitory effect of echo on Y), R8/9C (the mutation will prevent echo from binding Y), and R8/9D (the mutation creates unregulated (high) production of echo). Raters assigned a score of 0 for each statement that was absent (blue bars) and a score of 1 for each statement that was present (orange bars).

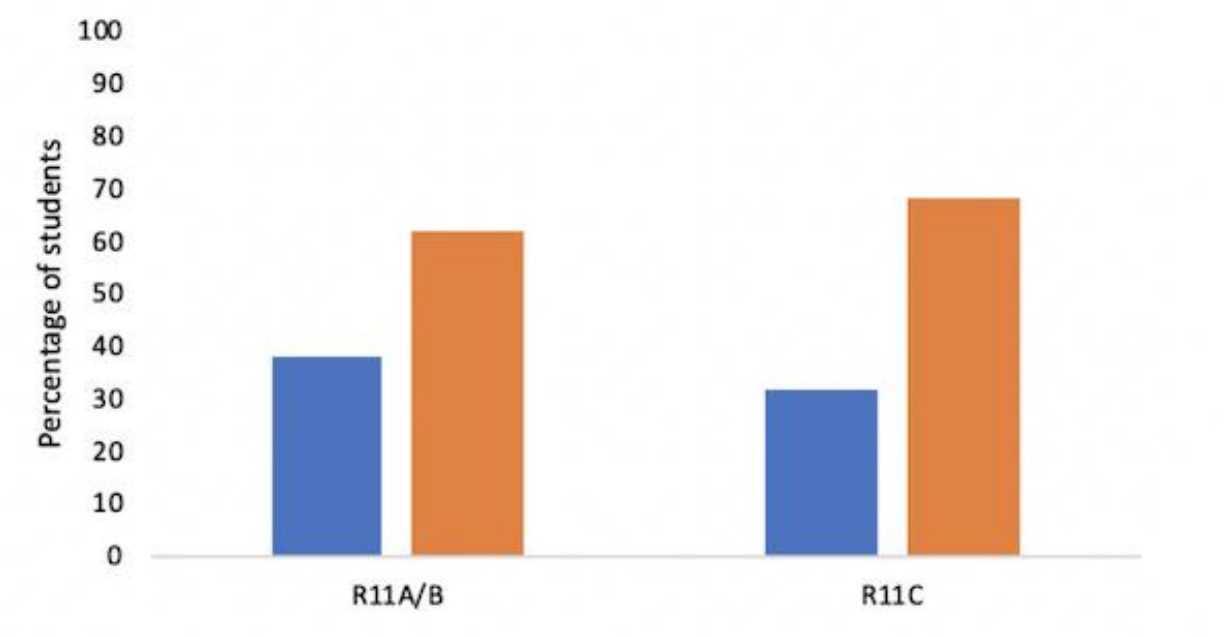


FIGURE S4. Students' constructed responses for Q11 were scored by two different raters using the rubric. Data from both raters were pooled for a total of 248 responses scored. Student responses that addressed the role of isoenzymes, R11A (isoenzymes allow for fine tuning of metabolites under fluctuating conditions) and R11B (isoenzymes are regulated differently under different conditions), were pooled due to their similarity. R11C (X1 and X2 have different regulatory properties) was also tracked. Raters assigned a score of 0 for each statement that was absent (blue bars) and a score of 1 for each statement that was present (orange bars).

SUPPLEMENTAL MATERIAL 3: CONFIDENCE QUESTION DATA ANALYSIS

Confidence questions were included throughout the assessment to evaluate students' confidence in responding to each of the four sections of questions. The confidence question asks students to rate their confidence in a five-point scale from extremely confident to not at all confident. There were four confidence questions, one in each block of questions described on Table 2. Figure S5 describes the percentage of students who are in each level of confidence for each question.

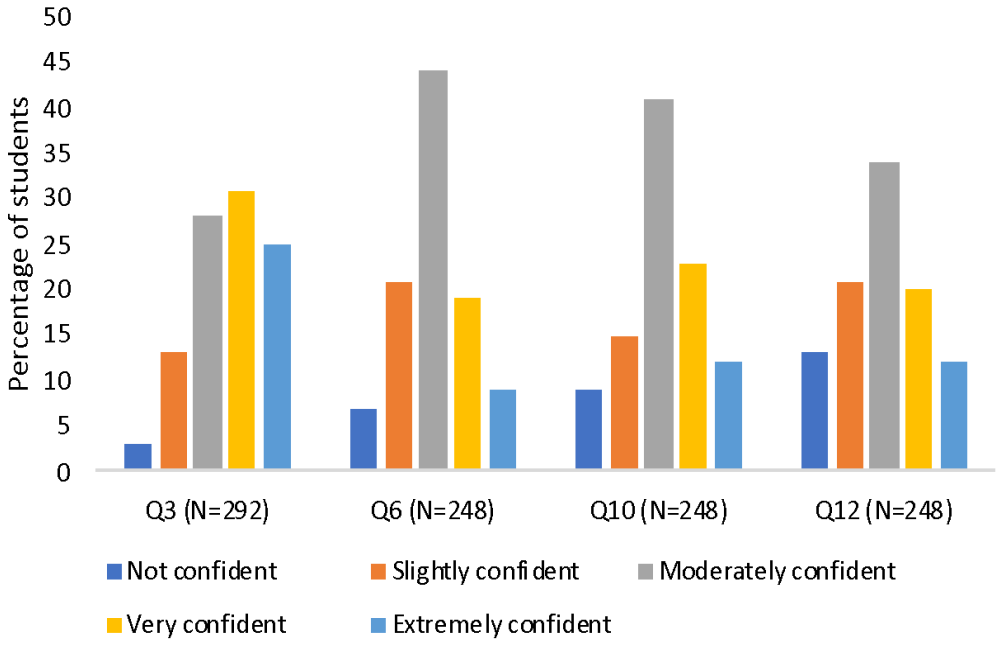


FIGURE S5. Students' responses to confidence questions for the different blocks of questions described in Table 2. Q3 corresponds to students' confidence after responding to Q1 and Q2 for all students in the sample set (N = 292). Q6 corresponds to students' confidence after responding to Q4 and Q5. Q10 corresponds to students' confidence after responding to Q7, Q8 and Q9. Q12 corresponds to students' confidence after responding to Q11. For Q6, Q10, and Q12 we only analyzed students' confidence for those students who answered Q1 and Q2 correctly (N = 248).