Name:_____

1. (99 pts.) An important outcome for this course is gaining the ability to navigate scientific literature geared towards metabolism. In their recent paper, Colinet and Renault explored how fruit flies respond to CO_2 anesthesia. They state:

> *"In the present study, we used GC/MS-based metabolic fingerprinting to assess the effects of CO_2 anesthesia on Drosophila melanogaster females. We analyzed metabolic trajectories in order to understand (i) the degree to which the metabolome is altered by an acute CO_2 exposure and (ii) the pattern of homeostatic response during short- and long-term recovery".*
>
> H. Colinet and D. Renault. (2012) *Biol. Lett.* **8:**1050

In this study, several metabolites were followed to investigate metabolic flux as a whole within the fly. Identify the nutrient type (i.e. carbohydrate, lipid, or amino acid) and the metabolic pathway or pathways (i.e. glycolysis, gluconeogenesis, TCA, ETC, etc.) in which each metabolite participates. If we didn't study a particular molecule leave it blank.

a) Alanine_____

b) Citrate_____

c) Ethanolamine_____

d) Fructose_____

e) Fumarate_____

f) Glucono-δ-lactone_____

g) Glucose_____

h) Glucose-6-phosphate_____

i) Glutamate_____

j) Glycerate_____

k) Glycerol_____

l) Glycine_____

m) Inositol_____

n) Isoleucine_____

o) Leucine_____

Name:_____

p) Lysine_____

q) Malate_____

r) Ornithine_____

s) Phenylalanine_____

t) Phosphate_____

u) Proline_____

v) Putrescine_____

w) Ribose_____

x) Serine_____

y) Sorbitol_____

z) Succinate_____

aa) Sucrose_____

bb) Threonine_____

cc) Trehalose_____

dd) Tryptophan_____

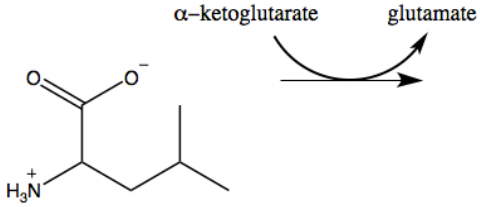
ee) Uric Acid_____

ff) Valine_____

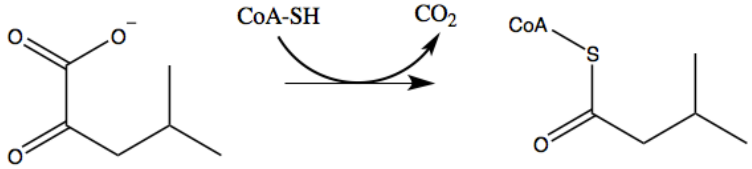
gg) Xylitol_____

Name:_____

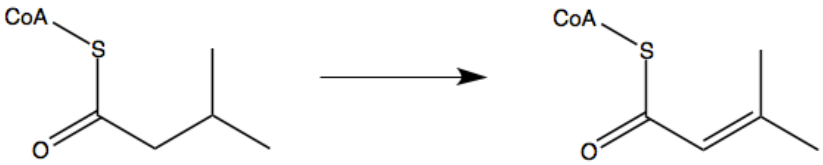
2. (6 pts. Each = 36 pts. total) Leucine Breakdown



a) Which enzyme likely catalyzes the first step? What cofactor or cofactors likely participate in this reaction?

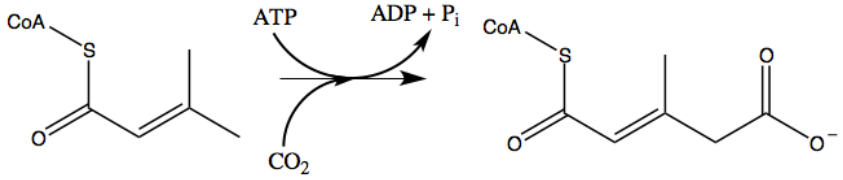


b) Which reaction or reactions that we studied in class are similar to the second step? What cofactor or cofactors likely participate in this reaction?

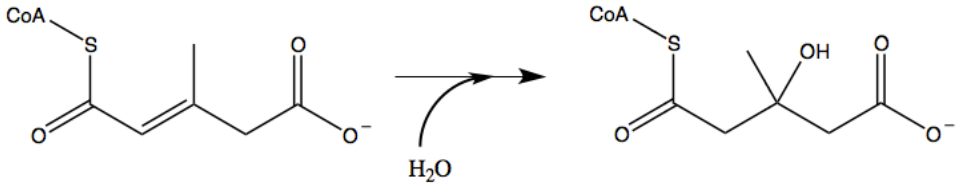


c) Which reaction or reactions that we studied in class are similar to the third step? What cofactor or cofactors likely participate in this reaction?

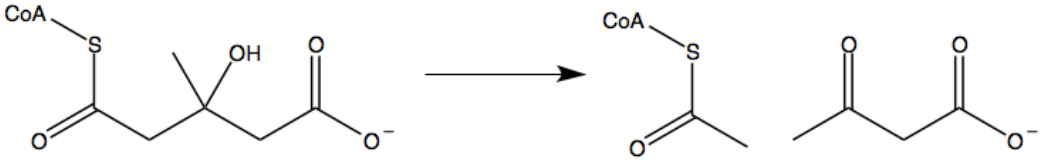
Name:_____



d) Which reaction or reactions that we studied in class are similar to the forth step? What cofactor or cofactors likely participate in this reaction?



e) Which reaction or reactions that we studied in class are similar to the fifth step?



f) What enzyme catalyzes the sixth and final reaction of leucine breakdown?

Name:_____

3. (15 pts) Solution/Dilution Calculations

a) In the transaminase lab, the GPT substrate contained 200 μM α-ketoglutarate and 20 mM alanine. Starting with jars of α-ketoglutarate and alanine powder, describe how to prepare 500 mL of GPT substrate.

b) From the lab protocol:

Assaying for Activity

Place 125 uL of the desired substrate solution (either GOT or GPT) in small glass test tubes: (i) test each tissue for both GOT and GPT activity and (ii) prepare one tube as a blank for GOT and for GPT activity. Pipet 25 uL of the clarified tissue solution into the two test assays for each tissue. Pipet 25 uL of phosphate buffer into each blank control tube. Mix each tube by inversion.

Incubate assays for 60 minutes at 37 °C. At the end of each incubation time, mix in 125 uL of 2,4-dinitrophenylhydrazine, and incubate at room temperature for 20 minutes. Mix in 2.5 mL of 0.4 N NaOH to each test tube, and allow to set at room temperature for 10 minutes. Read absorbance at 505 nm.

What is the final concentration of α-ketoglutarate and alanine in the blank controls where no transaminase reaction should occur?