

March 8, 2004

Name \_\_\_\_\_

ID number \_\_\_\_\_

## CH462/562 Exam 2

There are **4** short answer questions worth **6** points each; answer **3** of them.

There are **5** problems worth **24** points each:

If you are a CH462 student, you must answer **3** problems for full credit.

If you are a CH562 student, you must answer **4** problems for full credit.

You may answer one extra problem for extra credit:

For the problem on which you score the lowest, you will get  $2/3$  credit.

(If you are a CH462 student and attempt 5 problems, the lowest will be dropped, and the second-lowest will be counted for extra credit.)

The maximum amount of points (not including extra credit) is thus **90** (CH462) or **114** (CH562).

You are advised to look over the problems first before starting to work them. Time should not be a factor, if you are well prepared.

Multiple choice		/18
Problems:	1	/24
	2	/24
	3	/24
	4	/24
	5	/24
Total		

**No notes or books of any sort may be used during the exam.**

I have neither given nor received aid on this exam.

\_\_\_\_\_  
(signature)



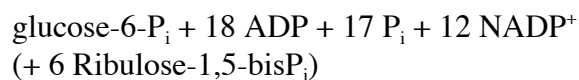
- 3) Show the reaction catalyzed by acetyl-CoA carboxylase to generate malonyl-CoA. Does the  $\text{CO}_2$  added to acetyl-CoA end up in the fatty acid product? If not, what is the point of doing the carboxylation reaction?
- 4) Show the mechanism used by ribonucleotide reductase.

# Problems

## 1) Carbohydrate synthesis

- a) Show the mechanism used by RuBisCO to carboxylate ribulose-1,5-bisP<sub>i</sub>:  
ribulose-1,5-bisP<sub>i</sub> + CO<sub>2</sub> + H<sub>2</sub>O → 2 phosphoglycerate

- b) Show the overall transformations used to create 1 hexose by the reductive pentose-phosphate pathway (Calvin cycle). It can be summarized thus:  
6 CO<sub>2</sub> + 18 ATP + 12 NADPH (+ 6 Ribulose-1,5-bisP<sub>i</sub>) →



(Note: not balanced for H<sup>+</sup> or H<sub>2</sub>O.)

Show each reaction and how many molecules go through it in order to make 1 glucose-6-P<sub>i</sub> from 6 CO<sub>2</sub>. Only structures are necessary; no names required. (If you give the names of intermediates instead of structures, you will receive only half credit.) Indicate any cofactors by the common abbreviations (*e.g.* ATP, *etc.*)



**2) Lipid synthesis**

a) Show the last “pseudo-cycle” for synthesis of palmitate:

from  $\text{CH}_3\text{-(CH}_2\text{)}_{12}\text{-(C=O)-S-ACP}$  to  $\text{CH}_3\text{-(CH}_2\text{)}_{14}\text{-CO}_2^-$

Be sure to include all the cofactors at each step. You do not need to try to draw out the entire enzyme and recreate the figure from the book. Just show each step. Names of enzymes or intermediates are not required.

- b) Show how the palmitates and a serine are attached to glycerol to make phosphatidylserine. (In this case, it is the lipid part that is activated by attachment to CDP, not the serine headgroup.)



d) Show how glutamate is converted to proline.

**4) Synthesis of pentoses, nucleotides, & amino acids**

a) The oxidative pentose-phosphate pathway is used to make NADPH and pentoses for anabolism. Show how it accomplishes the overall transformation of glucose-6-phosphate to ribose-5-phosphate.

b) Show how CTP is made, using as a starting point the ribose-5-phosphate (made above), Gln, Asp, bicarbonate, and whatever other cofactors are necessary.

- c) Show how newly synthesized nucleobases would be labeled, if you added these isotopically labeled amino acids or amino acid precursors to a cell that can synthesize amino acids and nucleotides *de novo* using the pathways you have learned. Draw the predicted products and indicate the labeled atoms (you do not need to include the sugar). Note: consider only molecules **one step away** from the added molecule.

Labeled molecule	Nucleobase
$\delta[^{15}\text{N}]\text{-glutamine}$ $\text{NH}_2\text{-CH-CO}_2\text{H}$   $\text{CH}_2$   $\text{CH}_2\text{-C=O}$   $^{15}\text{NH}_2$	cytosine
$\beta[^{13}\text{C}]\text{-oxaloacetate}$  $\text{O=C-CO}_2^-$   $^{13}\text{CH}_2\text{-CO}_2^-$	uracil
$\alpha[^{13}\text{C}]\text{-serine}$  $\text{NH}_2\text{-}^{13}\text{CH}_2\text{-CO}_2\text{H}$   $\text{CH}_2\text{-OH}$	adenine
$\delta[^{15}\text{N}]\text{-glutamine}$ $\text{NH}_2\text{-CH-CO}_2\text{H}$   $\text{CH}_2$   $\text{CH}_2\text{-C=O}$   $^{15}\text{NH}_2$	guanine

**5) Signal transduction**

- a) Imagine if you could tinker with any component of the signal transduction pathway involved in vision in vertebrates. In this problem, you will predict the effect of a change in the activity of a single component of the signal transduction pathway in vision. The output that you will measure will be the frequency of firing (i.e. action potentials) of the ganglion cells that receive the input from the illuminated rod cell via the bipolar cells. The rod cell secretes the neurotransmitter glutamate onto the bipolar cell as a function of its membrane potential – the more depolarized, the more it secretes; the more hyperpolarized, the less it secretes. As it turns out, there are 2 types of bipolar cells: ON bipolar cells have glutamate receptors that cause closure of cation channels, hyperpolarizing their membrane. They synapse with ON ganglion cells, which respond to reception of light by sending a train of action potential pulses to the brain. (OFF bipolar cells have ligand-gated  $\text{Na}^+$  channels that open upon binding glutamate and cause a depolarization. They synapse with OFF ganglion cells, which respond to a *drop* in light by sending a train of action potential pulses to the brain.)

In your experiment, you will expose the rod cells to a pre-determined illumination (same length and intensity every time) and measure the output of the ON ganglion cells to which they are connected. Then you will repeat this in each perturbed system. In each case, predict whether the (average) frequency of action potentials sent out by the ON ganglion in response to light will be higher or lower than the unperturbed system. Use an up or down arrow, if you wish. If you think your reasoning may not be obvious, write it down. This is *optional* – you not will be counted off, if your answer is right and you do not put it down, but you may get partial credit if your answer is wrong.

<b>Perturbation in rod cell</b>	<b>Prediction (and reasoning)</b>
Mutation in the transducin $\alpha$ subunit ( $G_{\alpha}$ ) that gives it a slower intrinsic GTPase activity	
Mutation in the cGMP phosphodiesterase inhibitory subunit that gives it lower affinity for $G_{\alpha}$ -GTP	
Addition of a competitive inhibitor of cGMP phosphodiesterase	
Mutation in the ligand-gated $\text{Na}^+/\text{Ca}^{2+}$ channel with higher affinity for cGMP	

Addition of a competitive inhibitor of guanylyl cyclase	
Mutation in the guanylyl cyclase with higher affinity for $\text{Ca}^{2+}$ at the inhibitory allosteric site	

- b) There are several different pathways used by ON bipolar cells to transmit the information from the rod cell to the ganglion cell. Here we will consider one of them. In it the glutamate receptor on the bipolar cell facing the synapse with the rod cell is a serpentine receptor that works with  $G_q$  to activate phospholipase C (PLC). Activated protein kinase C (PKC) phosphorylates a cation channel, which closes it. There is a phosphatase that removes the phosphate in a constitutive fashion. Like the rod cells, bipolar cells do not make action potentials (they do not even have voltage-gated  $\text{Na}^+$  channels); the rate of secretion of neurotransmitter to the ON ganglion cell is a function of membrane depolarization. Using this information, make some predictions about perturbation of the ON bipolar cell and how these would affect action potentials in the ON ganglion (as in part a).

<b>Perturbation in bipolar cell</b>	<b>Prediction (and reasoning)</b>
Mutation in glutamate receptor that gives it lower affinity for Glu	
Mutation in $G_q$ with faster GTPase activity	
Addition of a competitive inhibitor of PLC	
Mutation in the $\text{IP}_3$ -gated $\text{Ca}^{2+}$ channel on the ER that gives it higher affinity for $\text{IP}_3$	
Mutation of PKC with lower affinity for DAG	
Addition of a competitive inhibitor for the phosphatase	