

- A. Review all pre-lab questions, reports and post-lab questions.
- B. Work the sample questions. Remember that all possible types of questions may not be represented.

**Enzyme Kinetics:**

Given information:  $A = abc$      $b = 1.08 \text{ cm}$      $a = 10,300 \text{ M}^{-1}\text{cm}^{-1}$  (for benzoquinone under assay conditions)

Sample assay w/ 50 mM Catechol

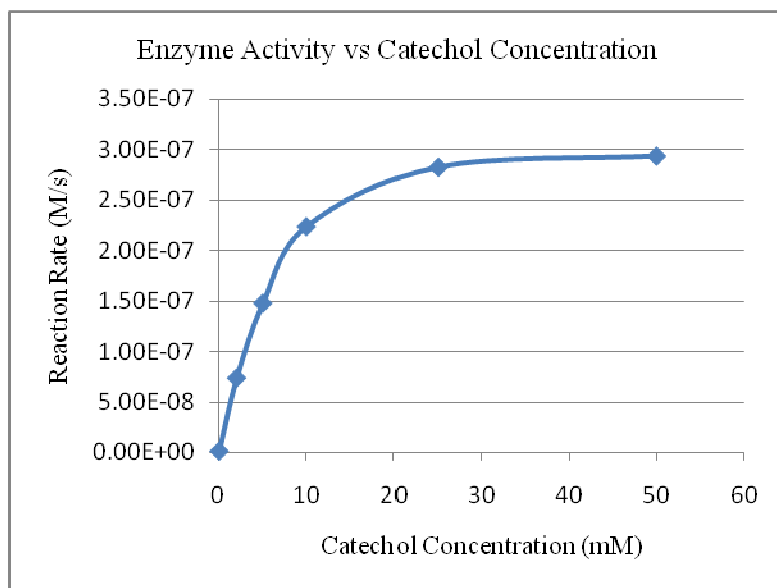
Time (s)	$A_{420}$
15	0.077
30	0.126
60	0.235
90	0.327
120	0.424

Summary Table

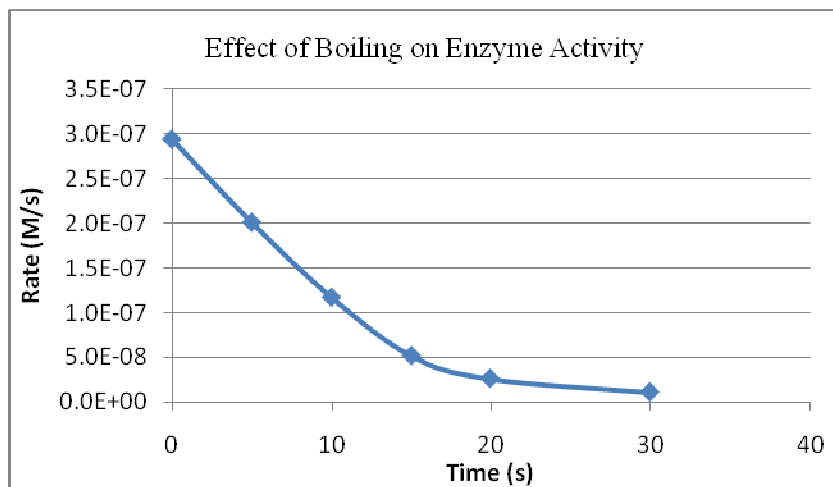
	Sample	Rate (M/s)
1	catechol, 10 mM	2.24E-07
2	catechol, 50 mM	2.94E-07
3	catechol, 50 mM, no enzyme	8.06E-09
4	catechol, 50 mM, boiled (20 s) enz.	2.90E-08
5	catechol, 50 mM, inhibitor	1.07E-08

1. Using the 30 s and 90 s data from the 50 mM catechol assay, calculate the approximate rate or  $\Delta[\text{benzoquinone}]/\Delta t$ . Express your rate in [benzoquinone]/sec.

2(a) Determine the maximum velocity of the enzyme from the graph. (b) At what catechol concentration was half-maximal activity obtained? Show the lines on graph that help in this determination.



3. From the summary data, determine the percent activity remaining in the sample having enzyme boiled for 20 sec compared to unboiled enzyme.



4. Using the data from the accompanying graph, approximately how long of a boiling time was required to reduce enzyme activity to 20% of its original value? Show the lines on graph that help in this determination.

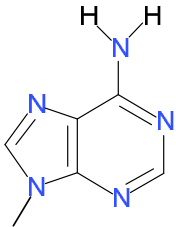
5. Did you perform an experiment that affected the activity of the active site of the enzyme? If so explain the experiment and the reason for the effects.

## DNA I and II

1. In a pedigree, what shape is used to represent a female?
2. What disease did Alexei of the Romanovs suffer from?
3. What type of protein malfunctions occur in this disease?
4. What are some areas of a skeleton that can be used to determine at what age the individual died?
5. Beside the nucleus, what other location in a human cell contains DNA? From whom does an individual receive this DNA?
6. Which people shown are not related to Phillip? Explain.

Comparing Bases Present in Particular Locations					
sample	location 1	location 2	location 3	location 4	location 5
Phillip	T	C	C	A	T
person 1	T	C	C	A	T
person 2	C	A	G	T	C
person 3	A	G	G	C	A
person 4	T	C	C	A	T

7. Is it likely that Anna Anderson was Anastasia of the Romanovs?
8. Indicate all possible hydrogen bonding acceptor and donor sites on the following nitrogenous base. Make sure you also show all non-bonding electron pairs.



9. What is the electron pair geometry \_\_\_\_\_ and hybridization \_\_\_\_\_ of the C's and N's in the nitrogenous bases? Are the bases planar or puckered? Are the sugars planar or puckered?

10. What base replaces T in RNA?

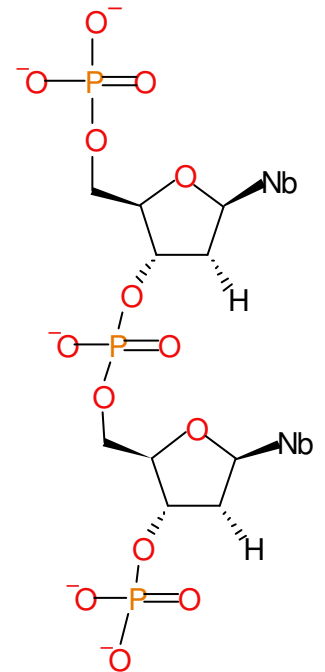
11. Is the 5' to 3' polarity of the following sugar-phosphate backbone (shown on the right) top to bottom or bottom to top? (Nb stands for nitrogenous base)

12. Under oxidizing conditions, C is converted to what base? When that converted base is replicated, what base is placed opposite it? If this mismatch is not reversed, what is the result?

13. What is the RNA sequence that would be made from the following DNA sequence?  
3' TACGAAGTGCCT 5'

14. Given a chart showing the genetic code, be able to translate the RNA into protein. (Indicate the amino terminal end.)

15. What aspect of the base structure of DNA determines the major and minor grooves?



**Enzyme Kinetics:**

Given information:  $A = abc$   $b = 1.08 \text{ cm}$   $a = 10,300 \text{ M}^{-1}\text{cm}^{-1}$  (for benzoquinone under assay conditions)

Sample assay w/ 50 mM Catechol

Time (s)	A <sub>420</sub>
15	0.077
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Summary Table

	Sample	Rate (M/s)
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5	catechol, 50 mM, inhibitor	1.07E-08

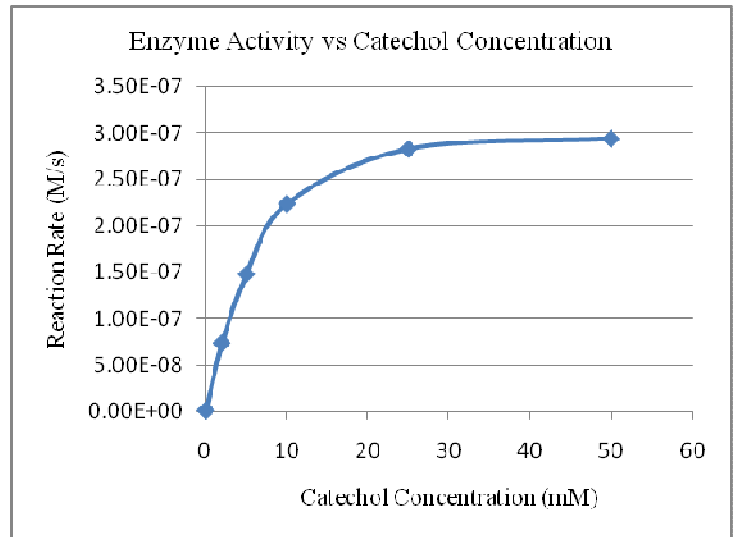
1. Using the 30 s and 90 s data from the 50 mM catechol assay, calculate the approximate rate or  $\Delta[\text{benzoquinone}]/\Delta t$ . Express your rate in [benzoquinone]/sec.

$$c = A/ab = 0.126 / (10,300 \times 1.08) = 1.13\text{E-}05$$

$$c = A/ab = 0.327 / (10,300 \times 1.08) = 2.94\text{E-}05$$

$$\text{Rate} = (2.94\text{E-}05 - 1.13\text{E-}05) / (90 - 30) = 1.81\text{E-}05 / 60 = 3.0\text{E-}07 \text{ M/s}$$

2(a) Determine the maximum velocity of the enzyme from the graph. (b) At what catechol concentration was half-maximal activity obtained? Show the lines on graph that help in this determination.

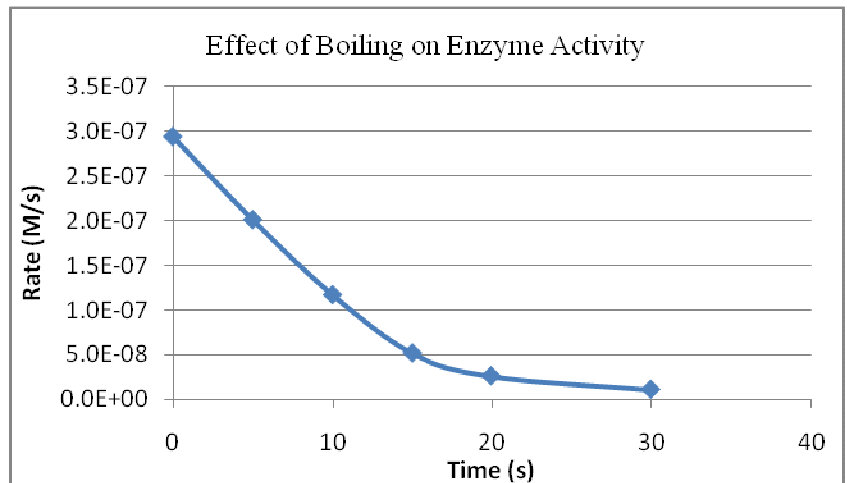


- a)  $\sim 2.94\text{E-}07 \text{ M/s}$  (Look at the fastest rate shown on the graph, up on the flattened area.)
- b) Divide the maximum value by 2, which is  $1.47\text{E-}07$ . Find that on the graph. Draw a horizontal line at that level until it hits the curve and then drop a vertical line down to the x-axis and read the catechol concentration at that point,  $\sim 5 \text{ mM}$  catechol. (Obviously I have not drawn the line, but a correct answer would have it drawn.)

3. From the summary data, determine the percent activity remaining in the sample having enzyme boiled for 20 sec compared to unboiled enzyme.

$$2.90\text{E-}08 / 2.94\text{E-}07 \times 100 = 9.86 \%$$

4. Using the data from the accompanying graph, approximately how long of a boiling time was required to reduce enzyme activity to 20% of its original value? Show the lines on graph that help in this determination.



Take the unboiled sample activity,  $2.94\text{E-}07$  and find 20% of that value or  $5.88\text{E-}08$ . Locate that value on the y-axis and draw a horizontal line over to the graph line, then drop a vertical line to the x-axis and read the value. Should be  $\sim 14$  seconds.

5. Did you perform an experiment that affected the activity of the active site of the enzyme? If so explain the experiment and the reason for the effects.

**Yes. The experiments where the enzyme was boiled for different lengths of time affected the activity. The energy (heat) of boiling was sufficient to break the bonds that hold the secondary and tertiary structure together, for**

example the hydrogen bonds that hold together the alpha helices. As the helices unfold, the structure of the active site is no longer maintained and the activity of the enzyme is destroyed.

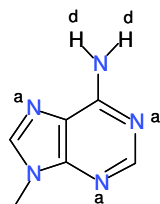
### DNA I and II

- In a pedigree, what shape is used to represent a female? **circle**
- What disease did Alexei of the Romanovs suffer from? **hemophilia**
- What type of protein malfunctions occur in this disease? **clotting factor**
- What are some areas of a skeleton that can be used to determine at what age the individual died? **Wisdom teeth, vertebrae, pelvic region.**
- Beside the nucleus, what other location in a human cell contains DNA? **In the mitochondria.**  
From whom does an individual receive this DNA? **From their mother.**
- Which people shown below are not related to Phillip? Explain.

Comparing Bases Present in Particular Locations					
sample	location 1	location 2	location 3	location 4	location 5
Phillip	T	C	C	A	T
person 1	T	C	C	A	T
person 2	C	A	G	T	C
person 3	A	G	G	C	A
person 4	T	C	C	A	T

**Person 2 and 3 are not related to Phillip. Their sequence differs from his at all 5 locations. It would be seriously unlikely to have people only few generations apart have mutations at so many locations in a short stretch of DNA.**

- Is it likely that Anna Anderson was Anastasia of the Romanovs? **No**
- Indicate all possible hydrogen bonding acceptor and donor sites on the following nitrogenous base. Make sure you also show all non-bonding electron pairs.



**The donor and acceptor site are indicated. The three N acceptor sites should also have attached non-bonding pairs.**

- What is the electron pair geometry **trigonal planar** and hybridization **sp<sup>2</sup>** of the C's and N's in the nitrogenous bases? Are the bases planar or puckered? **Planar.** Are the sugars planar or puckered? **Puckered.**
- What base replaces T in RNA? **U**
- Is the 5' to 3' polarity of the following sugar-phosphate backbone (shown on the right) top to bottom or bottom to top? (Nb stands for nitrogenous base)

**Polarity is from top to bottom. Note the addition of the 5' and 3' labels of the carbons in the sugars.**

- Under oxidizing conditions, C is converted to what base? **U.** When that converted base is replicated, what base is placed opposite it? **A** If this mismatch is not reversed, what is the result? **A mutation.**

- What is the RNA sequence that would be made from the following DNA sequence?

DNA 3' TAC GAA GTG CCT 5'  
RNA 5' AUG CUU CAC GGA 3'

- Given a chart showing the genetic code, be able to translate the RNA into protein. (Indicate the amino terminal end.)

**Amino terminal-met-leu-his-gly-carboxyl terminal**

- What aspect of the base structure of DNA determines the major and minor grooves.

**The major and minor grooves are determined by the location of the attachment on each base pair to the sugar of sugar-phosphate backbone. The attachment sites are not directly across from each other, thereby creating a larger angle between the attachment sites on one side of the DNA and a smaller angle on the other side.**

