

Biochemistry I, CH4110
Pet Enzyme Project 2
Kinetics and Mechanism

This assignment is intended to familiarize you with published, peer-reviewed scientific literature, therefore I will accept information gleaned from the Web **ONLY** if it appeared in an electronic journal, properly referenced (except for question 1). You will need to become familiar with literature searches. I recommend using the electronic databases “PubMed” (via Entrez) or “Web of Science” for your searches. Both of these are accessible through the library web page.

Literature is a code word for published research articles in journals. A **primary source** is the research article that ‘proves’ a particular point, that is, **provides the experimental evidence** that is consistent with the point. These articles will have pictures, tables, graphs, data! A **review article** is one which summarizes the state of a particular field of research and usually points out new findings in the context of older findings. These do not provide experimental evidence and thus **are not** primary references, though they are excellent places to find references to the original research articles. They may have tables and graphs, but again, these tend to be compilations of data published IN OTHER PLACES.

For each piece of information you are asked to find, I want to see references to the primary literature. For this assignment, the references can be listed at the end of each question. **References must include all authors, title of article, journal name, journal volume, year published and page numbers.** Example:

Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D., and Shinozaki, K. 1997. Role of *Arabidopsis* MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *Plant Cell* **9**: 1859-1868

You must also hand in a copy of the front page of every article used in your answers.

1. What reaction does your enzyme catalyze? If there is more than one, list the primary one. You can find this information back on the pdb pages. Go to ‘biology and chemistry’; there you will find some helpful terminology. At the bottom, click on the EC number. It will send you to a page that will give you the reaction. If this page does not appear, simply try one of the databases for the same information; try BRENDA. No reference is necessary for this information. I want the equivalent of $A + B \rightarrow C + D$. Please **draw complete chemical structures** of all products and all reactants – you may have to find these somewhere else, but you will not receive full credit unless I see atoms and bonds. The enzyme, not being a substrate or a product, should not appear, except perhaps as a name, above the arrow. (If your enzyme catalyzes cleavage or formation of cell walls or proteins or some other polymeric species, draw the site of the chemistry with **at least one full unit** of the polymer depicted chemically.)

2. Find in the research literature at least one kinetic parameter for your enzyme, such as K_M , k_{cat} , turnover number or the inhibition constant of an inhibitor (K_I). (Useful key words to try in your searches are *ligand*, *affinity*, *kinetics*.) Please give complete reference(s) of the paper(s) in which you found it. Note the reaction conditions used to arrive at this constant. Why is it so important to be aware of the reaction conditions that were used to determine the kinetic parameter?



3. Find the reaction mechanism or as much information about the reaction mechanism as you can. For instance, which amino acid residues are involved? Is the electron movement known or theorized? Give complete references for all the primary sources from which you obtained this information. If you can't find a proposed mechanism for your specific enzyme, find one for a closely related enzyme (the same activity from a close evolutionary relative). If there is no published electron pushing diagram, **hypothesize one given the information that you do have**. It needs to be chemically feasible. None of the enzymes on the list have chemistries that are beyond what you should be capable of. You CAN do this. I want something like what is in the book for the mechanism of RNase A, postulated electron movement, potential participation of amino acids of the enzyme.

4. Using the information that you have obtained, decide which of the catalytic strategies (Chapter 11 in the text) your enzyme uses. Justify your choices. (That means, make sure that you describe here, what is diagrammed in #3 above, and vice versa.)

Some of you may have difficulty in finding this material. Since all of these proteins have been crystallized, there should be a wealth of information about each of them. However, some of the information asked for **may not yet be known** about your enzyme. If you don't seem to be able to find the necessary information, you may hand in a copy of your literature search (all key words used and the results obtained with each), demonstrating that you did an adequate job of looking for the material required. If you find a listing for an article that sounds like it would have the right information, but the journal is not available, list the article and that it was not available. Our library has the journals that most frequently carry this information, but it certainly does not contain all of them. Make sure you check the electronic journal listings, we have a number electronically that we do not carry on paper. Many journals that WPI lacks can be found in the UMass Medical School library, if someone in the group can make the trip. You can check from the library web pages.

Let me re-emphasize the importance of good writing. I will again grade for content **and form**. **The remaining 10 pts are for English and style**. The clearer you are, the better your grade will be. Be sure to use your OWN words. If you use more than about 3 words in a row from a source you need to use quotation marks and cite the source. Dropping one word from a sentence does not make it original writing. Again there is a possibility for bonus points.

As before, print out the last page of this document, fill in your group members last names and your enzyme name and include it as the final page of your report.

Don't forget to hand in your peer evaluations with your report! Missing evaluations will result in point deductions for that member. You may submit these individually or as a group if you are able to reach a consensus.



Pet Enzyme Project II Grading Sheet (30 possible points)
Grading Sheet for Group members:

With the enzyme: _____

(6 points each section)

- _____ Enzyme chemical reaction – complete substrate/product pairs with references.

- _____ Kinetic parameter with references. Reaction conditions and explanation for importance of conditions in reaction.

- _____ Reaction mechanism with references. Validation for any substitutions. Valid chemical mechanisms with all important molecular structures.

- _____ Chapter 11 strategies used by enzyme. Discussion and justification for choices.

- _____ Style, form and proper English grammar ☺