

In the second half of the lab, we will be examining the effects of mutations upon the function of fumarase. To help us prepare for this, read the paper “Mutations of fumarase that distinguish between the active site and a nearby dicarboxylic acid binding site,” by Weaver T, Lees M, Banaszak L., Protein Sci. 1997 Apr;6(4):834-42. For the exam, you will use the information from that paper and the Proteopedia page, [https://proteopedia.org/wiki/index.php/Fumarase\\_2](https://proteopedia.org/wiki/index.php/Fumarase_2), to choose what amino acid mutation in the active site you would like to test. You may NOT make a mutation that was included in that paper.

Your answer should clearly describe:

- the debate about the two possible locations of the active site, and how the true active site was determined (including the mutations made and the data used)
- the mechanism of the addition of water across the double bond of fumarate, as supported by your laboratory data
- a proposed role for the amino acid you have selected and why the proposed mutation is a reasonable one to make (your hypothesis should be supported by material from the first two parts of your answer).

You MAY use your laboratory manual, laboratory notebook, and class notes in addition to the materials described above. You MAY consult with the instructor if you have questions; you may NOT talk with any other people about this assignment. As always, the Gentleman’s rule and expectations for academic honesty are in full effect. Put things in your own words (preferable) or use quotes and cite your sources.

(N.B. The students determined fumarase’s mechanism of addition of water across the double bond of fumarate as described in “An NMR study of the stereochemistry of the fumarase-catalyzed hydration of fumaric acid” by Julie A. Olsen and Robert J. Olsen, J Chem Educ, 1991, 68, 5, 436.)