

Biochemistry Laboratory I CHEM 4401
Exploring Enzyme databases (10 pt)
Please bring your laptop computer to laboratory

This laboratory will introduce you to the use of online databases for finding an assortment of information on enzymes, their metabolic functions, and their roles in specific diseases. The key to finding such information is having the proper (systematic) name for an enzyme or, better yet, the *enzyme classification* (E.C.) number. The E.C. system assigns a numeric ID to an enzyme based on a number of factors including the class of reaction catalyzed, substrates involved and the specific functional groups that participate in the reaction. We will be finding the E.C. number for a mammalian analog of PPO. Polyphenoloxidase belongs to a class of enzymes that oxidize phenolic groups. In mammals, for instance, the enzyme that catalyzes the reactions we performed in lab is known as *phenylalanine hydroxylase* (PAH). Because much more data exists for mammalian forms of PAH than for plant forms of PPO, we will use this enzyme in our exercise today.

There are many databases on the web that contain a huge amount of information on specific enzymes. One of the best is ExPASy, which stands for the Expert Protein Analysis System. ExPASy is a clearinghouse of information, containing links to numerous databases containing data on reactions, substrates, inhibitors, kinetic values and other physical and chemical characteristics.

Instructions

1. The first thing we will do is find the Enzyme Commission (E.C.) number for our PPO analog, *phenylalanine hydroxylase*. Open Internet Explorer and Go to the ExPASy homepage (<http://us.expasy.org/>). Near the top of the page you will see a search box. Type in **phenylalanine hydroxylase**, set the search option to **PROSITE** and select **Go**. What is the E.C. number for phenylalanine hydroxylase? (1 pt)
2. Click on the E.C. link for phenylalanine hydroxylase. This will bring you to a **NiceZyme** page that contains some additional information. What reaction does phenylalanine hydroxylase catalyze? (1 pt)
3. What cofactor is listed for the enzyme? (1 pt)
4. Scroll down the web page to the **cross-references** section. A number of additional databases are listed here. Select the hyperlink to the **human** form of this enzyme in the **UniProtKB/Swiss-Prot** subsection. How is the human form of phenylalanine hydroxylase regulated? (1 pt)
5. Return to the **cross-references** section on the **NiceZyme** page. Select the **E.C.** link associated with the **BRENDA** subsection. Once at the **BRENDA** site, select *Homo sapiens* (human) for the **organism** and submit. What is the **systematic name** for this enzyme? (1 pt)

6. As you can see from the left-hand sidebar, the **BRENDA** site contains a lot of information for enzymes. What is the **K_m** (Michaelis constant) value for phenylalanine hydroxylase? Use the value for the enzyme isolated from liver, using L-phenylalanine as a substrate. (1 pt)

7. What is the **turnover** (K_{cat}) value for phenylalanine hydroxylase? Use the value from the “recombinant wild-type enzyme”. What does the K_{cat} number tell you? (1 pt).

8. Identify 3 inhibitors of phenylalanine hydroxylase. Draw one of their structures (see **2D-image**). (1 pt)

9. What is the optimum pH for phenylalanine hydroxylase activity? How about optimum temperature?(1 pt)

10. Are there any diseases associated with phenylalanine hydroxylase? Identify one and briefly describe the problem (you may have to follow a few links) (1 pt).