

## Phenylalanine hydroxylase & PKU Disorder Exercise

### Learning Objectives

In this exercise, you will use StarBiochem, a protein 3D viewer, to explore:

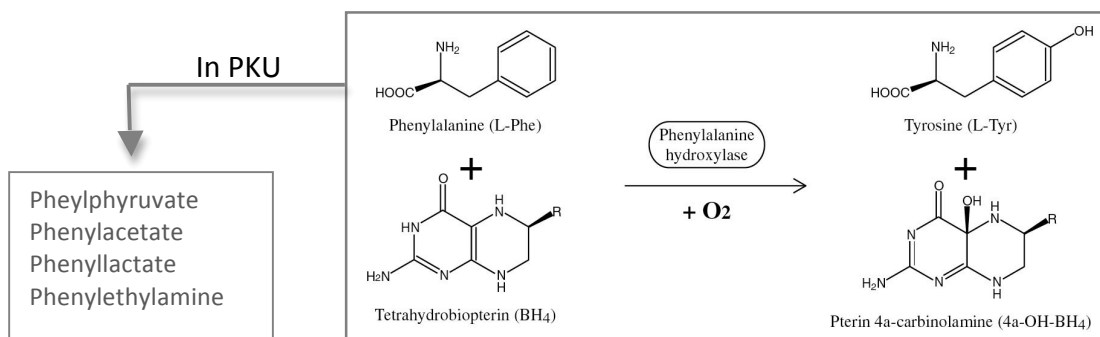
- the structure of the enzyme phenylalanine hydroxylase (PAH)
- the relationship between the structure of a mutated form of PAH and the function of this mutated protein in patients with phenylketonuria (PKU), a genetically inherited disease

### Background

Phenylketonuria (PKU) is a genetic disorder that is characterized by the inability of the body to utilize the essential amino acid, phenylalanine. PKU occurs in approximately 1 in 10,000 newborns in the US and 1 in every 50 individuals in the US is a carrier of PKU. This disorder is equally frequent in males and females.

“Classic PKU” is caused by the complete or nearly complete deficiency of functional phenylalanine (PAH). This enzyme is present both in prokaryotes and eukaryotes. Mammalian PAH occurs as a homodimer in its resting state but as a homotetramer in its functionally active form. Each monomer is composed of three domains: an N- terminus regulatory domain, a central catalytic domain, and a short C- terminus tetramerization domain that plays a role in interlocking the monomers to make a functionally active tetramer.

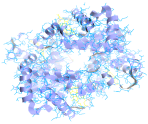
PAH is abundant in the liver. In normal healthy individuals, this enzyme converts phenylalanine to tyrosine, as shown in the figure below. These amino acids then move from the liver to the bloodstream and become available to cells for making proteins. However, in PKU patients who lack functional PAH, phenylalanine accumulates in the blood and tissues, where it is converted into toxic products. These toxic products result in severe mental retardation during development, as well as seizures and behavioral disorders.



### Getting started with StarBiochem

- To get to StarBiochem, please navigate to: <http://web.mit.edu/star/biochem>.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- In the top menu under **Samples** click on **Select from Samples**. Select “Phenylalanine hydroxylase partial – H. sapiens (2PAH)” and click **Open**.

Take a moment to look at the structure of PAH (2PAH) from various angles. Before proceeding to answer the questions, you can review the basic structures and terms on the last page, which you can refer to during this exercise.



### Protein Structure Questions - Level 1

**1** How many amino acids are likely present in the PAH structure: 2PAH?

Answer

**2** How many monomers/chains do you see in the current view of PAH (2PAH)?

Answer

**3** Glance at the primary sequence of each monomer/protein chain. **Are the protein/polypeptide chains within PAH (2PAH) likely to be identical or different?** Answer Yes/No and provide a brief explanation for the choice that you made.

Answer

**4** Does the current view of PAH (2PAH) represent the active form of this enzyme? Answer Yes /No and explain your answer.

Answer

### Protein Structure Questions - Level 2

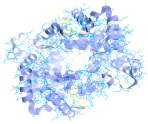
**5** Individual amino acids in a monomer fold into different local secondary structures.

**a)** What are the different secondary structures that you observe in PAH (2PAH)?

Answer

**b)** How many of each type of secondary structure are present in each monomer of PAH (2PAH)?

Answer



**6** Let's explore one of the secondary structures found in PAH (2PAH).

**a) In which direction do the amino acid side chains point: towards or away from the helix?**

Answer

**b) How many amino acids are likely present in each turn of a helix?**

*Hint: All the amino acids that make helix #32 are highlighted in the primary structure of 2PAH.*

Answer

### Protein Structure Questions - Level 3

**7** Explore the tertiary structure of PAH (2PAH) and its relationship with the environment.

**a) Where do you find most of the non-polar amino acids within PAH (2PAH)?** Your choices are: 'hidden within the inner part of the protein', 'away from the surrounding environment', or 'on the surface of the protein/exposed to the surrounding environment'.

Answer

**b) Where do you find most of the polar amino acids within PAH (2PAH)?** You have the same choices as stated in part (a).

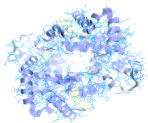
Answer

**c) Where do you find most of the charged amino acids within PAH (2PAH)?** You have the same choices as stated in part (a).

Answer

**d) What are the two classes of charged amino acids you observe?**

Answer



**e) If instead of PAH (2PAH) you were viewing the structure of a transmembrane protein, would your answer to parts (a) through (c) be the same or different? Explain.**

Answer

**8** Now let's explore certain amino acids within the structure of PAH (2PAH) and their potential relationship with other amino acids.

To answer all parts of this question, please take a moment to look at the chart of essential amino acids provided in this exercise. Please note the similarities and differences between all amino acids.

**a) What type of amino acid would you expect to be closed to an acidic amino acid? Circle the most appropriate option(s).**

Answer

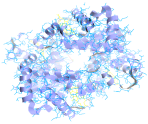
- i. Basic
- ii. Acidic
- iii. Either
- iv. Neither

**b) Provide an explanation for the options you did not select in part (a).**

Answer

**c) Give an example within the PAH structure (2PAH) to explain the option(s) that you have selected in part (a).**

Answer



## Structure -> Function -> Disease Questions

**9** Now let us explore how the structure of PAH specifies its function. For this purpose, we will look at the crystal structure of two PAH monomers: "1J8U" and "1TDW".

**a) Which of the two structures likely represents the functionally active form of a PAH?** Explain your choice.

Note: for simplicity you may assume that each of these monomers can bind to phenylalanine.

*Hint: Compare the molecules shown in yellow in the two protein structures. Refer to the reaction that is provided in the background section.*

Answer

**b) Identify the molecules that are bound to the functionally active form of PAH.**

Answer

**c) Now look at the PAH structure that you did not select above. Propose how this form of PAH may account for the development of PKU.**

Answer

### Keywords:

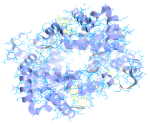
Essential amino acids, phenylketonuria, phenylalanine hydroxylase, prokaryotes, eukaryotes, seizures.

### Thought Questions

Answer only one of these three questions

**1** Why are PKU patients advised by their doctors to avoid diet soda?



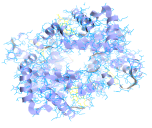


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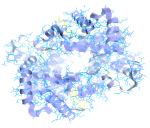
**2** Proper function of the nervous system requires a fine balance between the release, synthesis, uptake and degradation of different neurotransmitters. Dopamine and norepinephrine are common examples of neurotransmitters. Extrapolate why PKU patients may demonstrate neurologic abnormalities related to dopamine and norepinephrine.

**3** PKU patients are also known to have a low rate of aerobic respiration but a normal rate of glycolysis. Explain why.



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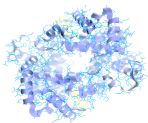
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## PROTEIN STRUCTURE BASICS

Each protein has the following three levels of protein structure:

### Primary structure

Lists the amino acids that make up a protein's sequence, but does not describe its shape.

### Secondary structure

Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

### Tertiary structure

Describes the entire folded shape of a whole protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. How these proteins interact and fold to form a larger protein complex is termed **Quaternary structure**.

## CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different 'R' groups, highlighted in various colors below.

