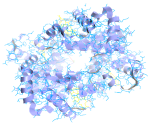

StarBiochem

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Aquaporin Exercise

Background

In this exercise, you will use StarBiochem, a protein 3-D viewer, to explore the structure of the protein aquaporin. Aquaporin is a water channel, found in cell membranes, which regulate the flow of water. In this exercise we will explore how aquaporin's specificity for water is defined by its structure.

Getting started with StarBiochem

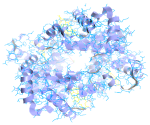
- To get to StarBiochem, please navigate to: **http://web.mit.edu/star/biochem**.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- In the top menu under **File** click on **Open/Import** and select "3D9S" and click **Open**.

You are now viewing the structure aquaporin (3D9S), with each bond in the protein drawn as a line.

Practice changing the viewpoint of this protein in the view window:

	Mac	PC
TO ROTATE	**click** and **drag** the mouse	**left-click** and **drag** the mouse
TO MOVE UP/DOWN RIGHT/LEFT	**apple-click** and **drag** the mouse	**right-click** and **drag** the mouse
TO ZOOM	**option-click** and **drag** the mouse	**Alt-left-click** and **drag** the mouse

Take a moment to look at the structure of aquaporin (3D9S) from various angles in this "bonds only" view. Before proceeding to answer the questions, review the basic structures and terms on the next page which you can refer to during this exercise.



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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.

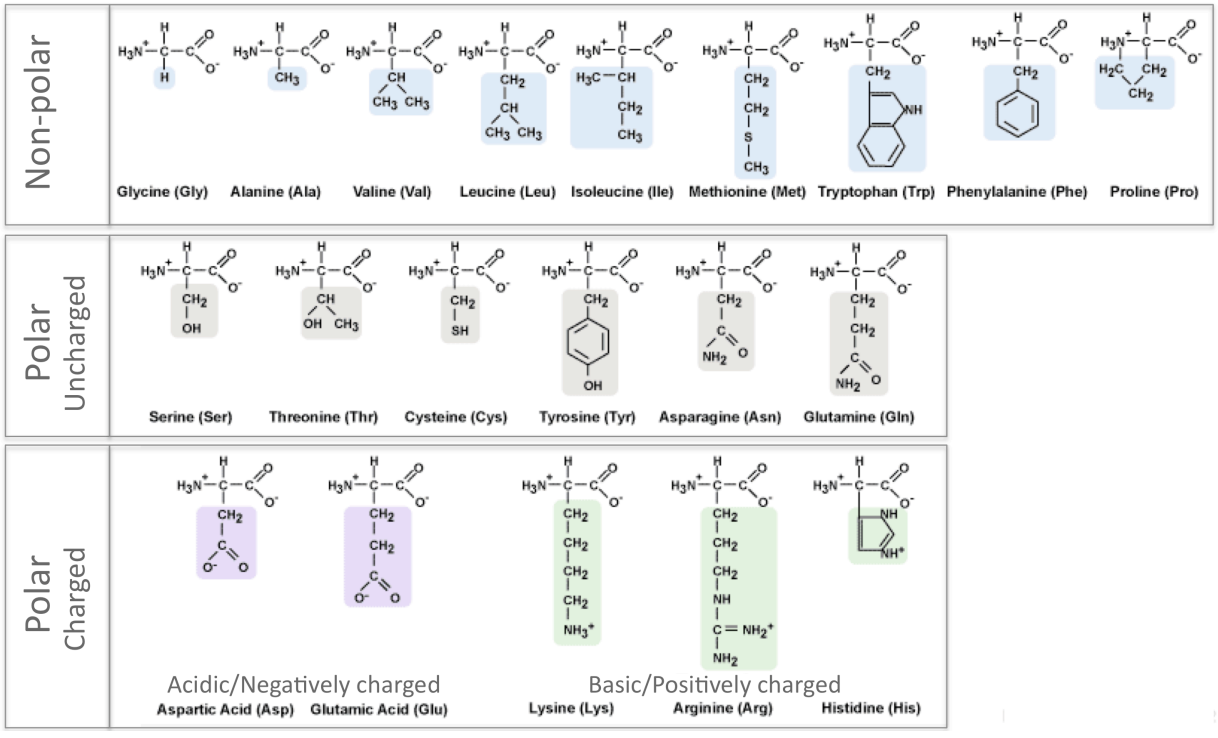
Secondary structure
Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

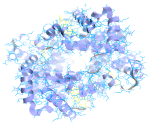
Tertiary structure
Describes the entire folded shape of a whole protein chain.

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.





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Protein Structure Questions

1 ***How many water channels does aquaporin (3D9S) have?***

- To get a more realistic view of how the protein looks when the space each atom occupies is visually represented, click on **View Controls**.
- Click on **Draw** and **Fill Space** within the **Atom** section.

Answer

2 The aquaporin channel is composed of multiple aquaporin monomers (amino acid chains) that interact with one another to form the structure that you see.

a) ***How many monomers/chains do you see?***

- On the top menu click on **View** and choose **Reset Molecule**.
- To help distinguish between the different monomeric subunits/chains that make up the aquaporin channel, click on **Structure**.
- Within **Structure,** click on **Quaternary**
- Click on the box next to **Chain**.

Answer

b) ***How many amino acids make up each chain?***

- To look at individual amino acids, switch from **Quaternary** structure to **Primary** structure.

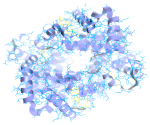
Answer

c) ***What is the last amino acid in each subunit?*** Spell the full name of the amino acid. Use the reference amino acid structure table.

Answer

d) Glance at the primary sequence of each monomer/protein chain. ***Are the protein chains within aquaporin likely to be identical or different?***

Answer



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3 Individual amino acids fold into different local structures called secondary structures (helices, sheets and coils). Please list which of these secondary structures are present in aquaporin (3D9S). ***Which secondary structure is predominant in aquaporin (3D9S)?***

- Within **Structure**, click on **Secondary**.
- Explore the different types of secondary structure found in aquaporin by either clicking on each one individually: **Helices**, **Sheets** & **Coils**.

Answer

4 Let's explore one of the secondary structures found in aquaporin (3D9S).

- Click on **View** and choose **Reset Molecule**.
- Go to **Secondary** structure.
- Click on **Track Selection** and within **Helix Selection** click on helix 5.

a) ***In what direction do the amino acid side chains point to, inside or outside the helix?***

- Close **Structure** and click on **View Controls**.
- Move the **Unselected** transparency slider to the left until the rest of the protein falls into the background or disappears.
- Move the **Sidechain** transparency slider to the left to determine the location of the side chains.

Answer

b) ***Estimate how many amino acids constitute a single helical turn.***

- Move the **Sidechain** transparency slider to the right and the **Backbone** slider to the left to obscure the backbone and highlight the side chains.

Answer

5 Now we will explore the relationship between aquaporin's environment and its structure.

a) ***What kind(s) of environment do you expect aquaporin to encounter (polar, nonpolar)? And where?*** Explain your reasoning.

Hint: consider the function of aquaporin and where it resides within the cell.

Answer

b) Examine the location of both the polar and nonpolar amino acids in aquaporin. ***Where do you find most of the nonpolar amino acids within aquaporin (3D9S)? Where do you find most of the polar amino acids within aquaporin (3D9S)?*** Be specific. ***Are the locations of the polar and nonpolar amino acids within aquaporin (3D9S) as you would have predicted based on part (a)?*** Explain your answer.

- Click on **View** and choose **Reset Molecule**.
- Within **Structure** select **Tertiary**.
- Click on **Non-polar**. This will highlight all the nonpolar residues within aquaporin.
- To improve your visualization of the protein click on **View Controls**. Within the **Atom** box click on **Draw** and **Fill Space**.
- To visualize the location of polar amino acids, repeat this series of steps by opening **Structure**, selecting **Tertiary**, and then clicking on **Polar**.

Answer

Structure -> Function Questions

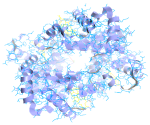
Now explore how the structure of aquaporin specifies its function. To do so, we will look at the crystal structure of a single aquaporin monomer (1H6I).

- In the top menu under **File** click on **Open/Import**.
- Click on "1H6I" and click on **Open**.

Take a minute to look at the structure of a single aquaporin (1H6I) monomer and then answer the questions below.

6 Aquaporin is an extremely selective channel protein, only allowing H_2O molecules to transverse the membrane and excluding protons (in the form of H_3O^+) from entering or exiting the cell. ***Please describe two possible mechanisms that would allow aquaporin to distinguish between H_2O and not H_3O^+.***
Hint: think of how adding a single proton might change the properties of H_2O or think of the differences between H_2O and H_3O^+.

Answer



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7 Helix #4 and #8 within aquaporin (16HI) have been found to contain essential amino acids essential for aquaporin's function. ***Given the location of the amino acid side chains within these two helices and with respect to the molecule, predict which amino acids could play a role in differentiating between H_2O and H_3O^+.*** Note: you do not need to look at the independent amino acids from each helix to respond to this question. Answer this question in global terms (no specific mention of amino acids is necessary).

- Under **Secondary** structure click on **Track Selection**
- Under **Helix Selection** click on helix 4 and 8.
- Close **Structure** and open **View Controls**.
- Within **Atoms** click on **Draw** to view all the atoms within the molecule.

Answer

8 Amino acid Arg 195 has been found to be critical for aquaporin's (16HI) ability to differentiate between H_2O and H_3O^+. ***Propose a hypothesis for how Arg 195 could help aquaporin (16HI) differentiate between H_2O and H_3O^+. How could you test this hypothesis?***

Hint: reference the amino acids structures at the end of this worksheet to help answer this question.

- Click on **View** and choose **Reset Molecule**.
- Within **Primary** click on **Structure**.
- Click on amino acid Arg 195.
- Close **Structure** and open **View Controls**.
- Within **Atoms**, click on **Draw** to view the atoms for this amino acid.
- Move the **Unselected** transparency slider to the left until the rest of the protein falls into the background but is still visible. Note the location of the side chain with respect to the molecule.

Answer