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BCR-ABL and Gleevac Exercise

Learning Objectives

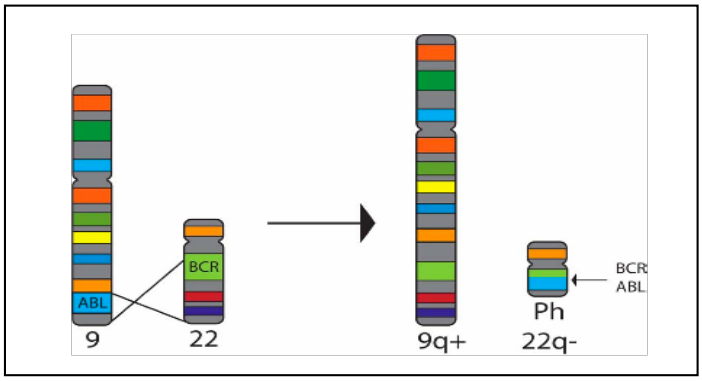
In this exercise, you will use StarBiochem, a protein 3D-viewer, to explore:

- the interaction of the ABL tyrosine kinase with Gleevac, a small molecule ABL inhibitor
- point mutations in the BCR-ABL protein that may confer drug resistance to Gleevac

Background

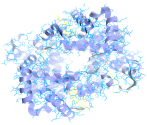
The *ABL* proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response.

Chronic myelogenous leukemia (CML) is caused by a reciprocal translocation between chromosomes 9 (containing the ABL gene) and chromosome 22 (containing the BCR gene). This reciprocal translocation results in a longer chromosome 9, a shorter chromosome 22 (called Philadelphia chromosome) and the fusion of the BCR and ABL genes. Fusion of the BCR and ABL genes in hematopoietic stem cells leads to CML. In wild type hematopoeitic cells the ABL and BCR genes produce two separate proteins that are only activated when their signal transduction pathways are turned on. In contrast, the fused BCR-ABL gene encodes a single protein that is active irrespective of the presence of activating signals. Constitutively activation of the BCR-ABL tyrosine kinase leads to uncontrolled proliferation, reduced differentiation, and enhanced survival of hematopoietic stem cells. These effects are leading causes of many hematological malignancies.



Nagar, B et al cancer Research 2002, 62, 4236-4243

A form of the BCR-ABL fusion protein, **p210**, is expressed in 95% of patients with CML and 25-30% of patients with acute lymphoblastic leukemia (ALL). Another form of this protein, **p190,** is expressed in 33% of patients with ALL.



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Imatinib mesylate (Gleevac, STI571), a drug that targets the tyrosine kinase activity of the BCR-ABL fusion protein, is an effective treatment for CML patients. It binds to the kinase domain of the BCR-ABL protein and disables the kinase by preventing ATP from binding to the active site. Gleevac may not work in advanced stage CML, because cancer cells become more genetically unstable and can develop point mutations which interfere with Gleevac binding. These point mutations are the major mechanism for the development of drug resistance in CML patients and demonstrate the need to develop new drugs that can avoid resistance.

Getting started with StarBiochem

To help you learn how to use the program, a tutorial is available under StarBiochem User Guide.

- 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 you if you trust the certificate.
- In the top menu under File click on **Open/Import**, select "1fpu" and click **Open**.

You are now viewing the structure of a Gleevac variant bound to the **ATP binding domain** of the ABL protein, with each bond in the protein drawn as a line ("bonds only view").

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 ABL (1FPU) bound to the Gleevac variant from various angles in this "bond only" view.

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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.

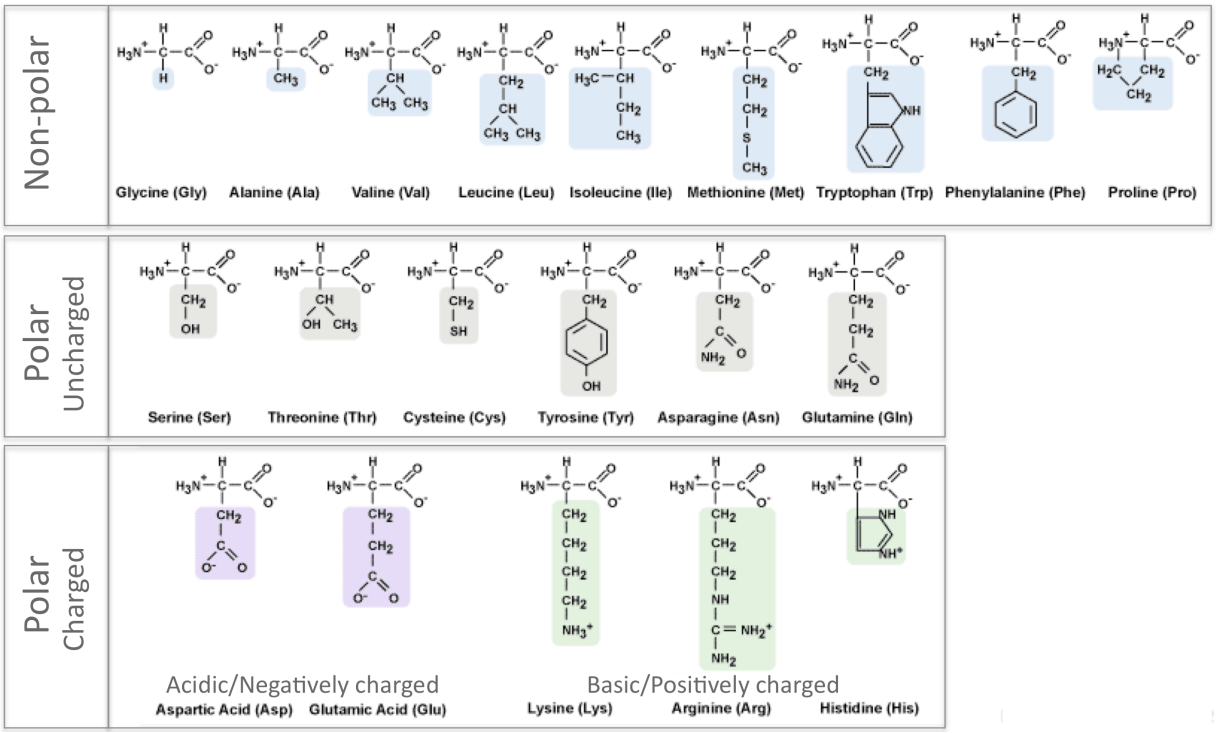
Secondary structure
Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

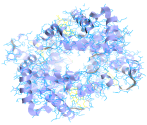
Tertiary structure
Describes the entire folded shape of a whole protein chain.

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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1 We will first look at the overall structure of ABL.

a) ***Would you classify the ABL protein as a homodimer or a heterodimer?***

- Click on **Structure** and select **Primary**. The amino acids of each polypeptide chain/monomer are highlighted by a specific color and can be distinguished from those of other polypeptide chains.
- Compare the sequence and number of amino acids between the two monomers.
- To distinguish between the different monomers that make up this protein, under **Structure** click on **Quaternary**.
- Click on **Chain**.

Answer

b) ***Does this structure represent the full length ABL protein****?* Answer Yes/No and explain your choice.

Answer

2 We will now study the interaction between Gleevac and ABL.

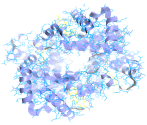
a) ***How many Gleevac molecules are bound to each ABL monomer in this structure?***
Hint: look at the molecules shown in yellow.

Answer

b) ***Draw the structure of the Gleevac molecule present in this structure.***

- Under **PDB tree**, click on **1fpu** and then click on "PRC_1" or "PRC_2".
- In the **View Controls** panel, set the **Unselected** transparency slider to "**0**".
- Click on **Draw** within the **Atoms** box to see what atoms are present. Each atom is color coded: Carbon is grey, Nitrogen is blue, Oxygen is red and Sulfur is orange in this exercise.

Answer



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3 We will now analyze the secondary structure of ABL.

- Within the main menu go to **View**.
- Click on **Reset molecule**.
- Under **Structure**, click on **Secondary**.
- Explore the different secondary structures by individually clicking **Helices**, **Sheets** or **Coils**.
- Alternatively, click on **All Ribbons** within the **Show Ribbons** box to view all the secondary structures at the same time.

a) ***How many structures are present for each type of secondary structure?***

Answer

b) Carefully look at the region that brings the two monomers together. ***Which secondary structure(s) interact to bring the two monomers together?***

Answer

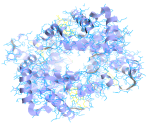
c) Carefully analyze the region of the protein that interacts with Gleevac. ***Which secondary structure(s) is present within this region of the protein?***

Answer

4 We will now create a stereoview of the drug binding pocket of ABL. Gleevac interacts with the sidechains of amino acids 271, 286, 290, 315, 318 and 381.

a) ***Identify these amino acids, draw their sidechains, and classify them.***

Amino acid #	**Name**	**Sidechain**	**Acidic, basic, polar, and/or nonpolar (state all that apply)**
#271			



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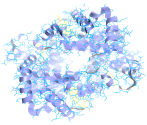
#286			
#290			
#315			
#318			
#381			

b) ***Sketch the drug binding pocket of ABL bound to Gleevac by drawing the chemical structure of Gleevac and the side chains of each of these interacting amino acids.*** Label the Gleevac molecule and each of the side chains involved.

- Under **Structure**, click on **Primary**.
- Highlight the amino acids state above for the first Abl monomer by individually clicking on them and simultaneously pressing **Control** and **Apple** key(Mac)/**right-click** (PC).
- Under **PDB Tree**, click on the **1fpu** file. Click on "PRC_1".
- In the **View Controls** panel, set the **Unselected** transparency slider to "**0**" while keeping the **Selected transparency slider** at "**1**".
- Click on **Draw** in the **Atoms** box to see what atoms are present. Each atom is color coded: Carbon is grey, Nitrogen is blue, Oxygen is red and Sulfur is orange in this structure.

Answer



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b) ***State the most likely mode of interaction between the side chains of each of these amino acids and the Gleevac molecule in this structure. Indicate the groups involved.*** Your choices are 'ionic', 'hydrogen', 'van der Waals forces', 'hydrophobic' or 'covalent'.

Answer

c) ***Which of these amino acids may be responsible for ABL's kinase activity?*** Explain your answer.
Hint: pay close attention to the side chains of the specified amino acids in part (a) while answering this question.

Answer

d) ***Based on what you have learnt from Question 4 and the information presented in the 'Background' section, propose a mechanism that explains how Gleevac inhibits ABL's kinase activity.***

Answer

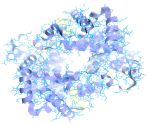
5 Specific point mutations in the BCR-ABL protein may confer resistance to Gleevac treatment. For each of the following point mutations, explain how each of these mutations might confer resistance to Gleevac treatment.

Answer

- Leu248Val

- Tyr253His

- Thr315Ile



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Keywords:

Kinase, point mutations, drug resistance, reciprocal translocation, chromosome, Philadelphia chromosome, hematopoetic stem cells, constitutively active protein, cell differentiation, cell proliferation, hematological malignancies, Gleevac, oncogenes, proto-oncogenes, tumor suppressor genes, and cell cycle.

Thought questions

1 "The origin and proliferation of a cancer cell is an excellent example of microevolution. " Elaborate and explain this statement.

2 The BCR-ABL protein has three different isoforms: p210, p190 and p230. Propose a theory to explain how these isoforms /variants of BCR-ABL can be produced.

3 Cell proliferation is regulated by the fine balance between the function of proto-oncogenes and tumor suppressor genes. Would you classify the wild type ABL gene as an oncogene or a tumor suppressor gene. Explain your answer.