

## Precision Medicine: Cancer and Beyond

2016 Whitehead Institute Spring Lecture Series for High School Students

### Using bioinformatics to advance precision medicine

<http://jura.wi.mit.edu/bio/education/HS2016/>

#### Exercise 2: Examining the protein structure of the Bcr-Abl fusion protein

In this exercise we'll examine how an amino acid change at a single residue may lead to serious consequences. In this case, the change affects the binding of a drug to the protein. We'll look at a protein (Bcr-Abl tyrosine kinase), and the interaction with a drug, an inhibitor (PHA-739358). This protein is a result of BCR-ABL fusion gene (two genes that are abnormally merged) that causes chronic myeloid leukemia (CML). The mutation, T315I, a tyrosine (T) to isoleucine (I) at position 315, affects the binding of a drug (Gleevec) given to treat CML. As a result, someone who has this particular mutation may not benefit from this drug and would need a different one.

1. Go to the NCBI's Molecular Modeling Database (MMDB) at <http://www.ncbi.nlm.nih.gov/Structure/MMDB/mmdb.shtml> and search using our structure's PDB ID: 2V7A
2. The search should bring you to the page <http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=59785>. Multiple software tools can be used to view protein structures. We're going to use Cn3D (abbreviation for "see in 3-D"), which is already installed on the BaRC laptops. Under "View or Save 3D Structure", click on the "View Structure" button. Open the file using "Cn3D".
  - You should see two windows: i) protein 3D structure and ii) Sequence/Alignment Viewer
  - Enlarge the two windows to make more use of your display/screen.
3. Get a feel for the structure by rotating (using your mouse) and also zoom in/out (View → Zoom In or Zoom out; you can use the shortcut as well)
  - Can you identify the drug that is bound to this protein?
  - You'll also see a grey sphere, can you identify what this represents? [Hint: This is listed in the bottom of the NCBI MMDB page you visited to download the structure.]
4. The default rendering is "worms" and in purple color. Try changing the rendering (Style → Rendering Shortcuts), and color (Style → Coloring Shortcuts).
5. The second window has the amino acid sequence (in single-letter abbreviations) that makes-up this protein. Highlight certain residues and see where they are in the protein 3D structure.

Examine how the amino acid sequence, which can be considered one-dimensional, is quite different from the actual 3D protein structure.

- The mutation of interest, **T315I**, is at the position described as “PDB 315”. In the typical Bcr-Abl, this position is the amino acid threonine (T), but in this mutated variant, the amino acid is isoleucine (I). Identity this position on the sequence and check if it’s indeed an isoleucine (I). We’ll highlight this residue. First we want to see the side chains of the amino acids, not just the backbone ([Style](#) → [Rendering Shortcuts](#) → [Toggle Sidechains](#)). Select residue 315 (should be highlighted in yellow), can you see the residue on the 3D structure? You may have to rotate the protein.
- We’ll now highlight this residue differently than default, go to annotate ([Style](#) → [Annotate](#) → [New](#)) and give a name (“mutation”) and description (“T315I mutation”). Click on “[Edit Style](#)”, in “[Protein backbone](#)”, choose “[Space Fill](#)” and change [Color Scheme](#) to “[User Selection](#)”. Choose a color of your choice under “[User Color](#)”, and select [Apply](#). Do the same for “[Protein sidechains](#)” and choose a different color but for “[Rendering](#)” use “[Ball and Stick](#)”. Select [Done](#) for [Style Options](#) once complete, and click on OK in [Edit Annotation](#).  
[Note: Your selection will be still in yellow since the residue “I” was selected, simply click anywhere on the protein sequence to un-highlight and get the selected coloring.] Zoom into the residue and see how close it is to the drug.
- Precision medicine application: Patients with chronic myelogenous leukemia (CML) are often treated with a drug that binds inside a “pocket” of the Bcr-Abl fusion protein (right next to amino acid 315) and reduces the effect of this oncoprotein. But if amino acid 315 is mutated from threonine to isoleucine, the side chain of the amino acid sticks out more into the drug-binding pocket. The drug no longer binds very well so the patient often develops resistance to the drug, and the doctor needs to try another drug.

