Lab 10 - Exploring Protein Drug interaction through Protein Structure Analysis

Chronic myeloid leukemia is associated with uncontrolled growth in the number of granulocytes in the blood, most often resulting from translocation between chromosomes 9 and 22. The translocation creates a fusion of the Bcr (breakpoint cluster region) gene and ABL tyrosine kinase gene. Tyrosine kinases function by transferring a phosphate from adenosine tri-phosphate (ATP) to specific amino acids on substrate proteins. This transfer, "phosphorylation", often acts as an on/off switch either activating or shutting off the activity of its substrate. ABL is an integral part of a cell-cycle signaling pathway and thus, normally, its expression is tightly regulated. The cap-SH3 domain of ABL is important to its negative regulation, but the fusion of ABL to the 5' region of Bcr results in the deletion of the cap-SH3 domain and puts the expression of the ABL-fusion under the control of the constitutively active (always on) Bcr promoter.

Figure 1. Protein Domains of Brc, ABL, and Bcr-ABL Fusions

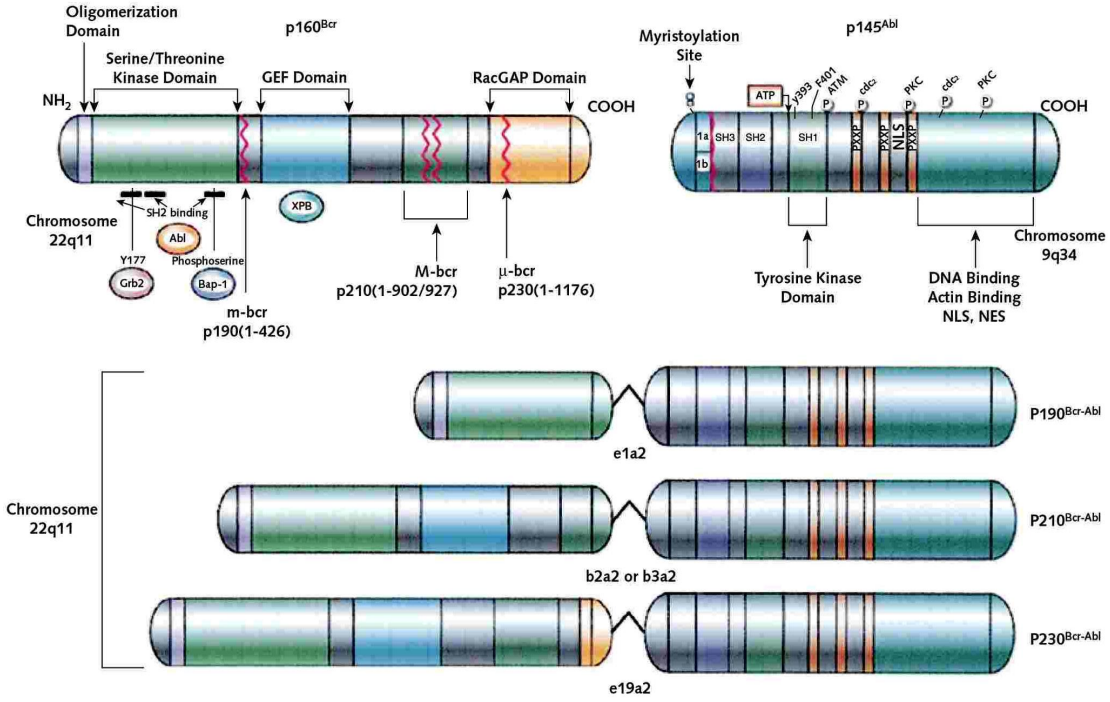

Figure from R. Kurzock, H.M. Kantarjian, B.J. Druker and M. Talpaz. (2003) Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Annals of Internal Medicine, 138(10):819-830.

The Bcr-ABL fusion takes on distinct active and inactive conformations. The small molecule Imatinib binds to and stabilizes the inactive conformation. Imatinib has been a very successful treatment of chronic myeloid leukemia as 90% of early stage patients go into remission after 18 months of treatment with Imatinib with only minor side-effects.

For this exercise we'll look explore the interactions of Imatinib sensitive and resistant ABL kinase domains with Imatinib and other ATP analogs through their 3D structures.

Your write-up for this week will consist of answers to the questions in the handout and copies of all the files you generate during the lab.

Technical Notes:
For this exercise use Internet Explorer as your web browser, not Chrome. You may be prompted to install or update Java – go ahead and do this.

Part 1
At the Protein Data Bank ("PDB", www.rcsb.org/pdb), explore the records of ABL1 kinase domain structures featured in the 2007 paper:
Cowan-Jacob SW, Fendrich G, Floersheimer A, Furet P, Liebetanz J, Rummel G, Rheinberger P, Centeleghe M, Fabbro D, Manley PW. (2007) Structural biology contributions to the discovery of drugs to treat chronic myelogenous leukemia. Acta Crystallographica. Section D, Biological Crystallography. 63(Pt 1):80-93.

Q1. What are the PDB accession numbers for the structures in the Cowan-Jacob paper? (there are 5)

Q2. Where does Imatinib bind relative to most of the alpha-helices and most of the beta-sheets?

Q3. For each structure, what percent of the secondary structure is helical, what percent is in beta sheets?

Part 2:
On the PDB website, use the "Compare Structures" tool to compare 1IEP.A and 1M52.A. First, compare the sequences using blast2seq and at least one other method.

Q4: Do the sequence alignment methods agree? If not, why might they differ? Are you convinced that the protein structures represent the same version of the domain, why or why not?

Next, compare the structures of the two records by selecting "external server: TopMatch". Be sure to also visualize the two ligands. Rotate the molecule so the differences between the structures is clear. Save two pictures, one picture with each ligand. Be sure to note which ligand is in which picture and which structure was used as the query and which was used as the target.

Q5: What is the biggest difference between the two structures? What is this region called? What is its function?

Measure the longest distance between the edges of the feature which differ between the two structures.

Q6: What is this distance in nm?

Right click on the image, select “Console Show”. Double click on one side of the non-aligning region; something such as [Gly]383:A.CA/2.1#1234 19.031 50.579 21.163. Do the same thing on the other end of the region. By comparing the first numbers after the residue you can identify the length of the region.

Q7: What is the length in amino acid positions of the non-aligning region?

Part 3:
Next, we’ll examine more closely how the ligand associates with the ABL kinase domain. Change the display so that only the structure of 1IEP.A and its ligand are visible. Rotate the image and identify the points on the protein structure that are closest to the ligand. Measure these distances by double clicking the protein position then double clicking on the closest point on the ligand. A black dotted line and measurement should now appear on the structure. Additionally the measurement and the amino acid involved will appear in the Jmol Script Console.

Q8: Build a list of the amino acid positions closely associated with Imatinib. Note the amino acids, their positions and chemical properties.

Next, highlight the positions associated with Imatinib resistance.
The mutations M244V, G250E, Y253F/H, E255K/V, T315I, M351T, and F359V are estimated to account for most occurrences of Imatinib resistance.

In the Jmol Script Editor, type:
select atomno=list of positions separated by commas; color [255, 0, 255];

Then click “Run”. The positions you listed should now be highlighted in bright pink.

Q9: Compare the positions on your list from Q8 to the known resistance conveying mutations. Do any appear on both lists?

Measure the distances of the resistance loci and the closest point on the Imatinib molecule.

Q10: Where are the resistance positions located relative to the Imatinib ligand?

Q11: Are the resistance loci closer or farther or the same distance to the ligand as the positions you identified in Q8. Take several pictures showing the measured distances from Q8 and the highlighted resistance mutations.

Q12: Briefly summarize your findings. Consider, the position of the known resistance mutations and hypothesize as to their function in inhibiting the fusion kinase. Based on

the structure, identify other locations which may interact with a bound ligand and could be targets of further research.

Congratulations on surviving the first offering of the bioinformatics lab!!! Any suggestions, comments, input on exercises you liked/didn't like/would have liked to have done – would be greatly appreciated!