

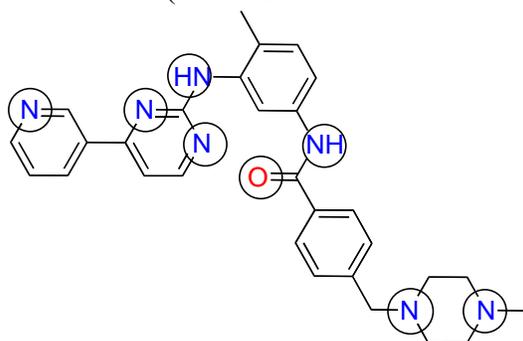
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5.36 Biochemistry Laboratory
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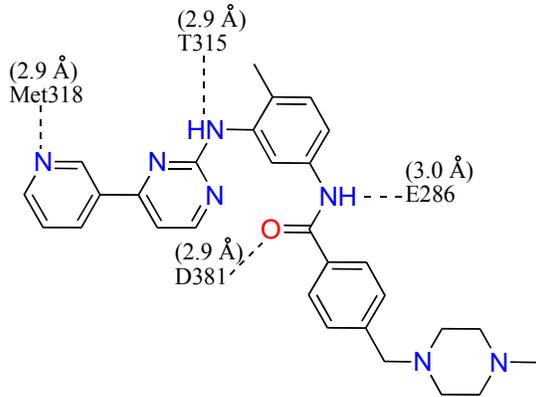
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SESSION 15 Answer Key

- 1) What type of secondary structure is most prominent in Abl? **alpha helix**
- 2) What type of secondary structure is most prominent in the N-lobe (residues 225-350) of Abl? **beta sheet (ok to also include that there is an alpha helix)**
- 3) What type of secondary structure is most prominent in the C-lobe (residues 354-498) of Abl? **alpha helix**
- 4) The protein contains a five-stranded β -sheet. Is this sheet located at the C or N terminus of the kinase domain? **N-terminus**
- 5) What residues comprise this beta-sheet? For this answer do not include the helix that is between the strands, so you should list two ranges. (For example "S229-G250 and L340-A350" would be in the correct form- although this is *not* the correct answer). It may be helpful to zoom in on your structure to answer this question. **I(242)-L(266) and L(301)-E(316)**
- 6) Where are most of the hydrophobic residues located, on the inside or the outside of the protein? (You may want to switch back and forth from viewing the sticks to the cartoon form to get a better view of this.) **on the inside**
- 7) Where are most of the polar residues located? **on the outside**
- 8) Does this make sense in terms of protein folding? Explain. **Yes. The residues on the outside of a protein will interact with water molecules, so it makes sense for them to be polar. The hydrophobic molecules are both shielded from the water and able to interact with each other on the inside of the protein.**
- 9) When bound to Gleevec, is the A loop of the Abl kinase domain in the open (extended) or closed conformation? Very briefly explain your answer. **Closed. The activation loop is blocking the substrate binding site and the Asp residue of the DFG motif is pointing away from the ATP binding site.**
- 10) When bound to Gleevec, is Abl kinase domain in the active or inactive form. Very briefly explain your answer. Inactive. **The A loop is closed in the inactive conformation.**
- 11) What groups on the structure of Gleevec above could participate in hydrogen bonds? (Draw Gleevec and indicate the groups by circling).



- 12) Looking at the Abl structure, name the residues that appear to hydrogen bond to Gleevec and give the distances between the atoms. Draw these on your Gleevec structure. *Hint: there are 4 possible hydrogen bonds within 3.0 Å.*



13) Name two other interactions that may be stabilizing the inhibitor in the active site.

Van der Waals and hydrophobic interactions

For questions 14 through 18, consider the most common mutation site found in cases of Gleevec-resistant CML, site 315.

- 14) What amino acid is at this site, and what sort of interaction does this particular residue have with the inhibitor? **Thr residue. H-bond.**
- 15) What might you predict would happen if this residue were mutated to Asn or Ile? **H-bond would not be possible and there would be steric interference with Gleevec binding.**
- 16) What if this residue were mutated to Ala? **H-bond not possible. No steric effect.**
- 17) Is residue 315 interacting with Dasatinib? If so, describe the interaction. **Yes. H-bond.**
- 18) Would you expect Dasatinib to effectively inhibit an Abl mutant with residue 315 mutated to an Ile? Very briefly explain why or why not. **No. H-bond would break and would sterically interfere with binding (although not as extremely as for Gleevec)**
- 19) When bound to Dasatinib, is the A loop of the Abl kinase domain in the open (extended) or closed conformation? Very briefly explain your answer. Open. **The activation loop away from the substrate binding site and the Asp residue of the DFG motif is pointing into the ATP binding site.**
- 20) How does the location of residue 393 differ in the two structures? **In the Gleevec-bound structure, Tyr393 is in the substrate binding pocket, and in the Dasatinib-bound structure, Tyr 393 is moved far away from the substrate binding pocket.**
- 21) Very briefly compare the binding orientation of Gleevec and Dasatinib. Many answers are acceptable for this. **For example, students could draw the parts of the two molecules that overlap and the parts that are pointing in opposite directions. Alternatively, they could write that Gleevec binds deep into a hydrophobic pocket, while Dasatinib binds more similarly to ATP (they would need to look at their Lecture 4 notes to see this).**
- 22) The H396P mutation in Abl destabilizes the inactive conformation of the kinase. Based on the Gleevec-bound and Dasatinib-bound structures of wt Abl, explain how your laboratory results from the kinase activity assays with wt and H396P Abl are consistent (or not consistent) with these structures. (If you have not yet completed

Session 14, predict what you would expect to see in the H396P assays based structural evidence. Compare this to your actual results after completing your assays.) **The structures show that Gleevec binds the inactive form, and Dasatinib binds the active form. This is consistent with the kinase assays, which show that the wt Abl is inhibited by both Gleevec and Dasatinib (consistent with the fact that the wt can take on both the active and inactive forms), while H396P is only inhibited by Dasatinib (which makes sense because the inactive form is destabilized in this mutant).**