

Structure and Docking: BRAF Kinase Domain

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Problem

The BRAF gene has been identified as important in many cancers. Mutations in the kinase domain of BRAF have been implicated in melanoma and other cancers. I aim to evaluate the docking of BRAF inhibitor drugs using various combinations of BRAF mutations, specific drugs, and ligand docking methods. If there is no structure for a relevant mutation, protein structure prediction will be employed. Insight into the conformation of receptor/drug complexes will help future drug design efforts.

Background

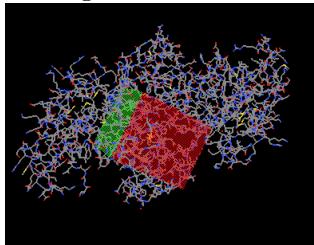
BRAF encodes the BRAF protein, which is a member of the Raf kinase protein family. The protein regulates the MAPK (mitogen-activated protein kinases) pathway (also known as the ERK pathway) which regulates cell growth. The conformations of the BRAF protein activate or deactivate the MAPK pathway. Mutations in BRAF which have been identified in melanoma tumor cell lines activate this pathway and lead to uninhibited cell division and growth. Specifically, the BRAF-V600E mutant (valine replaced by glutamic acid at residue 600) is common in melanoma tumors and encourages an active conformation of BRAF. BRAF inhibitors, such as vemurafenib, have been developed as treatment; they work by preferentially binding to BRAF-V600E mutants in the ATP pocket and interrupting the MAPK pathway. However, these inhibitors have a paradoxical activation effect on wild-type BRAF where the binding of the drugs encourages the active conformation, which activates the MAPK pathway to increase cell growth in tumors. Newer drug design efforts have focused on finding ligands which inhibit BRAF-V600E but do not induce the paradoxical activation of wild-type BRAF.

X-ray crystallography has been used to find structures of complexes of BRAF-V600E in a dimer and with various drugs bound. It is thought that the angle of a certain alpha-helix is changed which induces the activation of the pathway. However, the mutant BRAF-V600K has not had its structure analyzed via crystallography.

Methods

To learn more about the pose of new drugs being analyzed as well as verafimib, AutoDock Vina was used to conduct ligand docking. BRAF-V600E was docked with PX7904 (a new drug which experimentally doesn't cause the paradoxical activation in wild-type BRAF) and with Vemurafenib (an approved BRAF inhibitor with the paradoxical activation).

The receptor used was the crystal structure of the BRAF-V600E mutant from a complex with PX7904, which is a new drug. The receptor was prepared with AutoDock Tools. Nonpolar hydrogens were added to the receptor structure. Docking with an entirely rigid receptor was performed. Then, docking with four flexible residues was performed: ARG509, LYS507, GLU600, and PHE595. These residues were chosen due to their proximity to the binding site and to the alpha helix which is thought to be moved in the active conformation. The PX7904 was prepared with AutoDock Tools. All hydrogens were added to the ligand. The torsional angles of PX7904 were randomized before docking. Vemurafenib was downloaded from the ZINC ligand database. Vemurafenib was prepared with AutoDock Tools, and all hydrogens were added. The torsional angles of Vemurafenib were randomized before docking. The search space was chosen based on the known binding pocket and enlarged when flexible residues were used.



Search space in
AutoDock Tools

Unfortunately, Autodock Vina is limited to 32 total flexible angles when performing ligand docking.

Phyre2 was used to generate a protein structure of the BRAF-V600K mutant, but the structure did not match what was expected from the crystal structure to be confident enough to perform docking.

Results

The table below shows the main results of the ligand docking.

Receptor	Ligand	Flexible Residues	Binding Affinity of best pose
BRAF-V600E	PX7904	None	-10.5 kcal/mol
BRAF-V600E	PX7904	ARG509, LYS507, GLU600, PHE595	-8.8 kcal/mol
BRAF-V600E	Vemurafenib	None	-11.0 kcal/mol
BRAF-V600E	Vemurafenib	ARG509, LYS507, GLU600, PHE595	-10.2 kcal/mol

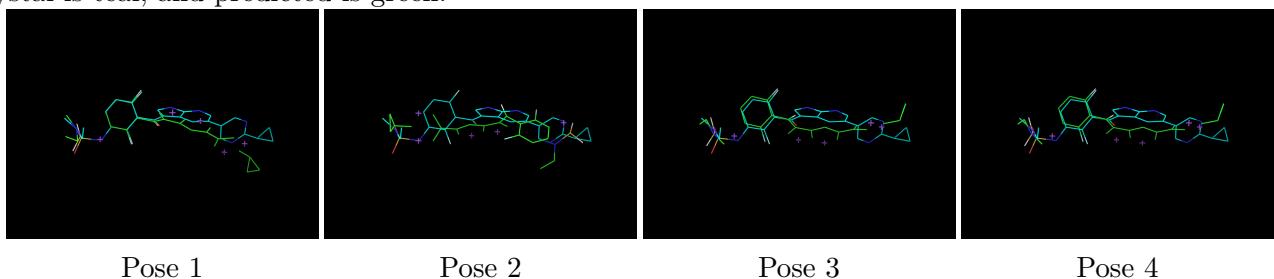
PX7904

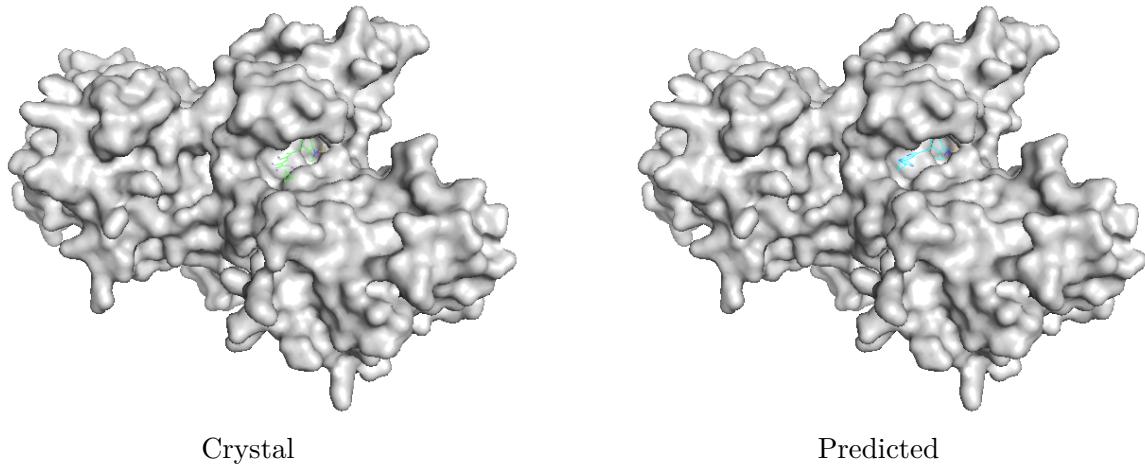
PX7904 was previously shown to not cause the paradoxical activation of wild-type BRAF. I wanted to see if docking could give any insight. Below are more detailed results for docking

PX7904 with BRAF-V600E

State	Affinity (kcal/mol)	Dist from best RMSD l.b.	" u.b.
1	-10.5	0.000	0.000
2	-9.6	4.181	10.706
3	-9.1	2.063	3.211
4	-8.3	3.993	10.984

Crystal is teal, and predicted is green.



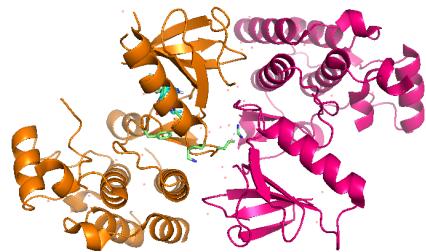


The highest scoring pose is close to the crystal structure, but not exactly the same as the crystal structure. The RMSD is 5.716 when using 36 atoms. The discrepancy could be due to the scoring function being coarse or that the complex behaves differently in crystal.

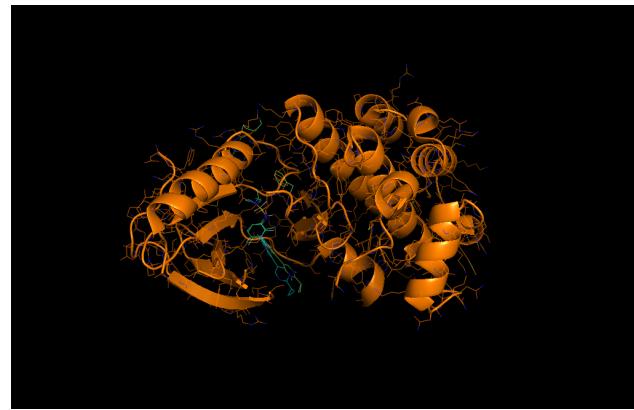
Below are results for the flexible residue docking:

State	Affinity (kcal/mol)	Dist from best RMSD l.b.	" u.b.
1	-8.8	0.000	0.000
2	-8.6	2.092	2.792
3	-8.2	2.490	6.683
4	-8.2	2.610	6.187
5	-8.1	3.503	5.187
6	-8.0	2.186	3.227
7	-7.7	2.800	6.139
8	-7.6	3.690	8.171
9	-7.5	2.715	3.824

Surprisingly, the highest scoring pose does not match the crystal structure. The pose that most closely matches the crystal structure is pose 8. Below are two views of the docking results when selecting flexible residues in the receptor protein. As is shown in the rendering of the pose, the residues do move from their original position.

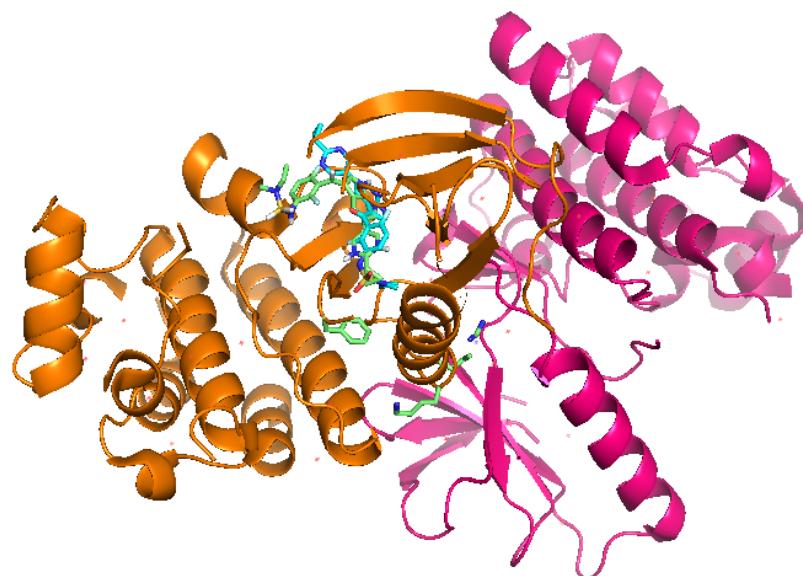


Pose 8, Flexible complex



Pose 8, Flexible

The pose with the highest binding affinity is visualized below. The crystal ligand structure is teal, and the predicted ligand structure and the flexible residues are green:



Pose 1, Flexible

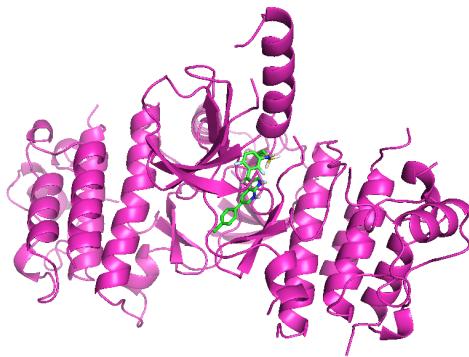
Possibly, PX7904 does bind in such a position some amount of the time, but the binding affinity difference between the poses is not dramatic. Had the results for the all-rigid receptor been closer to the crystal structure, I would have had more confidence in the flexible structure pose. However, it is a good sign that the known binding pose was identified.

Vemurafenib

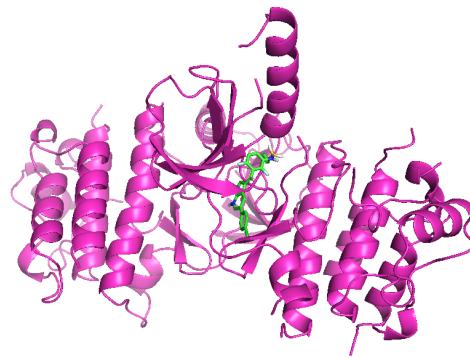
Vemurafenib with BRAF-V600E

Below is a table of results for docking Vemurafenib to a rigid receptor.

State	Affinity (kcal/mol)	Dist from best RMSD l.b.	" u.b.
1	-11.0	0.000	0.000
2	-10.6	2.274	2.801
3	-10.0	2.369	3.035
4	-8.8	1.532	1.994

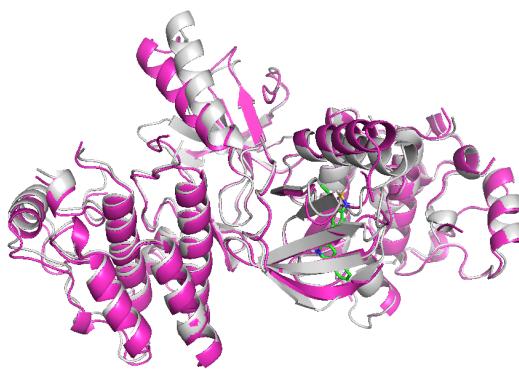


Pose 1, Rigid

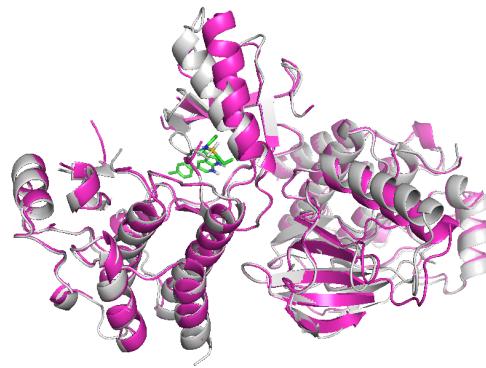


Pose 2, Rigid

The poses for Vemurafenib are similar to PX7904. The pose with the highest binding affinity (Pose 1) does not match the crystal structure; instead the second pose (Pose 2) more closely matches the crystal structure. The views below show the crystal structure of the receptor when bound to Vemurafenib. The angle between the alpha helices at the top of each of the visualizations of the complex show the difference between conformations.



Pose 2, Rigid



Pose 2, Alternate view

Below are the results for flexible residue docking:

State	Affinity (kcal/mol)	Dist from best RMSD l.b.	" u.b.
1	-10.2	0.000	0.000
2	-10.1	3.486	6.147
3	-9.8	4.143	7.271
4	-9.8	3.704	4.840
5	-9.4	3.563	4.664
6	-9.3	3.841	6.742
7	-9.1	3.743	4.861
8	-9.1	3.657	6.169
9	-9.0	3.908	6.418

Pose 4 matches the crystal structure the closest. Pose 1 is oriented in the binding pocket, but the molecule is bent.



Pose 1, Flex



Pose 2, Flex



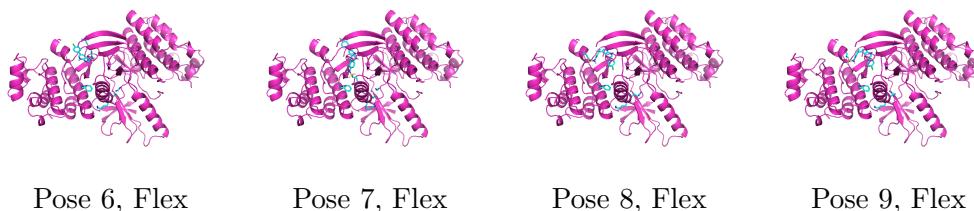
Pose 3, Flex



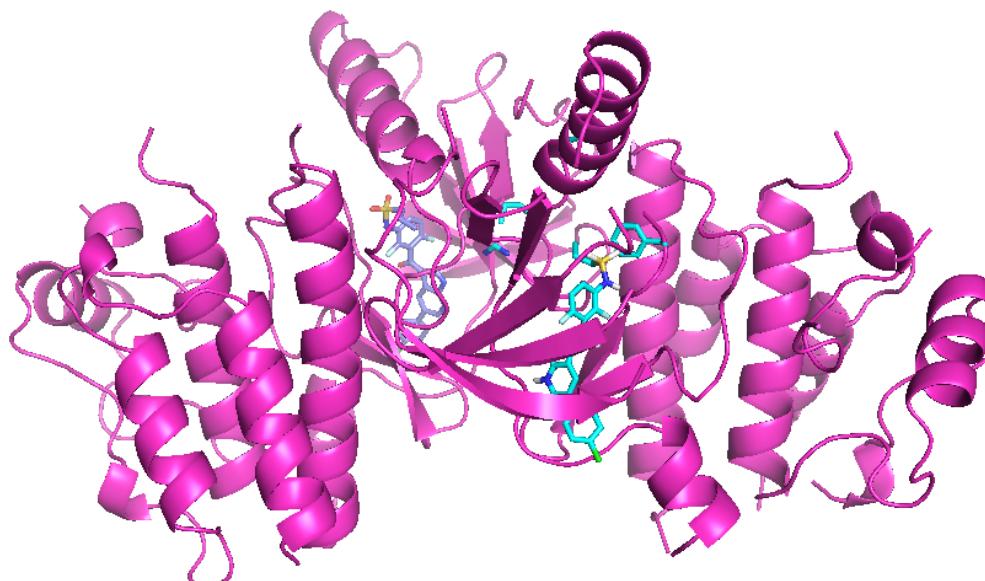
Pose 4, Flex



Pose 5, Flex



Below is the dimer with vemurafenib from crystal structure bound to the complex in purple, and the predicted docking in teal:

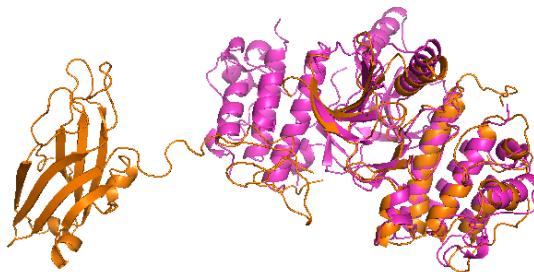


Pose 4, Flex

Overall, the results of the docking were interesting because they didn't match the crystal structure perfectly. I was disappointed by the limitations on the number of flexible torsional angles. The results would have been more interesting because we could have seen entire secondary structures move. Autodock Vina uses a united-atom scoring function (which means that the only heavy atoms are used and requires the user to specify a search space. I ad intended to use SwissDock to compare

my results, but unfortunately the web server has been down intermittently recently. Even so, I believe that a more fruitful approach would combine more heavy use of protein structure prediction along with ligand docking. The issue with the rigid receptor here is that we have information about where and how the ligand docks but are interested in how the protein conformations change. It is more challenging to interpret the Vina results, which are small changes in a small number of residues. A combined or iterative approach to ligand and receptor conformation/pose would be more interesting. One approach would be to perform molecular dynamics simulation, with the obvious downside of vastly increased computational time. However, with more resources, a MD simulation can elucidate the changes in conformation and what leads to the active conformations in the first place.

The project was started with the intention of using the BRAF-V600K molecule as well. V600K is another mutation that occurs with some frequency in melanoma tumors. However, my attempt to use Phyre2 for the structure in the absence of a crystal structure did not result in a cohesive structure.



Predicted BRAF-V600K (orange) and crystal BRAF-V600E (pink)

Part of the predicted structure aligns well with the BRAF-V600E structure. Unfortunately, the rest of the structure does not. From the fact that the BRAF-V600K mutant does function in these

cancer cells, my intuition was that the complex would increase dimerization and mostly maintain a similar conformation to the original protein. In the analysis section, you should include results of your experiments as well as an analysis of their quality.

The fact that there were many poses with similar binding affinities was interesting. This implies that the drugs could spend time in conformations other than the conformation measured in the crystal structure. Many of the poses showed the ligand not fully inserted into the binding pocket. The poses could represent portions of the ligand's journey into the binding pocket, though this method of ligand docking does not show the process of docking (unlike molecular dynamics simulation).

Further Work

Looking ahead, I would like to employ protein structure prediction methods to the BRAF-V600K mutant. I would also like to identify the docking poses of both Vemurafenib and PX7904 to the wild-type BRAF. Hopefully this will give more insight into the interactions that might cause the paradoxical activation of BRAF. I would also like to screen more drugs. Unfortunately, it took quite a while to create the structure files and get AutoDock Tools and AutoDock Vina running, but now that they are, the sky is the limit.

I would also like to try other docking tools, especially ones that allow more flexibility or freedom in the receptor's conformation. Ultimately I am interested in the complex of BRAF with these ligands and the effect on dimerization.

If such methods are unsuccessful, running molecular dynamics simulations of BRAF in complex with each drug would be my next step. I'm hopeful that, with increased computing power in the future, molecular dynamics simulations will be employed to examine these types of problems more frequently.

References

- Adams, J. L. et al. (2015). "Big opportunities for small molecules in immuno-oncology". In: *Nat Rev Drug Discov* 14.9, pp. 603–622.

- Bateman, A. et al. (2015). “UniProt: a hub for protein information”. In: *Nucleic Acids Res.* 43.Database issue, pp. D204–212.
- Berman, H. M. et al. (2000). “The Protein Data Bank”. In: *Nucleic Acids Res.* 28.1, pp. 235–242.
- Bollag, G. et al. (2010). “Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma”. In: *Nature* 467.7315, pp. 596–599.
- Holderfield, M., T. E. Nagel, and D. D. Stuart (2014). “Mechanism and consequences of RAF kinase activation by small-molecule inhibitors”. In: *Br. J. Cancer* 111.4, pp. 640–645.
- Kelley, L. A. et al. (2015). “The Phyre2 web portal for protein modeling, prediction and analysis”. In: *Nat Protoc* 10.6, pp. 845–858.
- Morris, G. M. et al. (2009). “AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility”. In: *J Comput Chem* 30.16, pp. 2785–2791.
- Schrödinger, LLC (2015a). “The AxPyMOL Molecular Graphics Plugin for Microsoft PowerPoint, Version 1.8”.
- (2015b). “The JyMOL Molecular Graphics Development Component, Version 1.8”.
- (2015c). “The PyMOL Molecular Graphics System, Version 1.8”.
- Thevakumaran, N. et al. (2015). “Crystal structure of a BRAF kinase domain monomer explains basis for allosteric regulation”. In: *Nat. Struct. Mol. Biol.* 22.1, pp. 37–43.
- Trott, Oleg and Arthur J. Olson (2010). “AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading”. In: *Journal of Computational Chemistry* 31.2, pp. 455–461. ISSN: 1096-987X. DOI: [10.1002/jcc.21334](https://doi.org/10.1002/jcc.21334). URL: <http://dx.doi.org/10.1002/jcc.21334>.
- Trudel, Stéphanie et al. (2014). “The clinical response to vemurafenib in a patient with a rare BRAF V600DK601del mutation-positive melanoma”. In: *BMC Cancer* 14.1, p. 727. ISSN: 1471-2407. DOI: [10.1186/1471-2407-14-727](https://doi.org/10.1186/1471-2407-14-727). URL: <http://dx.doi.org/10.1186/1471-2407-14-727>.
- Verkhivker, G. M. (2016). “Molecular dynamics simulations and modelling of the residue interaction networks in the BRAF kinase complexes with small molecule inhibitors: probing the allosteric effects of ligand-induced kinase dimerization and paradoxical activation”. In: *Mol. BioSyst.* 12 (10), pp. 3146–3165. DOI: [10.1039/C6MB00298F](https://doi.org/10.1039/C6MB00298F). URL: <http://dx.doi.org/10.1039/C6MB00298F>.

- Zhang, C. et al. (2015). “RAF inhibitors that evade paradoxical MAPK pathway activation”. In: *Nature* 526.7574, pp. 583–586.