Autodock Report

Introduction

The protein studied here was the human p38 Mitogen-activated protein kinase molecule, which is an intracellular serine/threonine kinase, with the RCSB ID 1W82. The protein helps in the regulation of stress and inflammatory responses, cell differentiation, proliferation and apoptosis and has been implicated in the development and progression of many cancers as well. P38 MAP kinases are activated by dual-specific MAPK kinases, leading to downstream processes, such as phosphorylation of other intracellular proteins which are important for the post-transcriptional regulation of TNF-alpha and IL-1beta, important mediators in the progression of inflammatory diseases such as Inflammatory Bowel disease, Crohn's disease and psoriasis. The excessive activation of this protein hence, has some inflammatory effects. Thus, the p38 protein plays a major role in various pathological and physiological processes and is considered an important target for these diseases.

AutoDock is a widely used software tool for predicting the binding of ligands to proteins. It is a molecular docking program that helps to predict the binding of a ligand to a protein, by employing a search algorithm that explores all possible conformations of the ligand and protein receptor, and scores each of them based on factors such as steric hindrance, hydrogen bonding, and electrostatic interactions. AutoDock can also be used to predict the binding affinity of a ligand to a protein, which is a measure of how strongly the ligand binds to the protein receptor. AutoDock is a powerful tool for identifying potential binding sites for ligands on a protein, which can have important implications for drug discovery and design.

Structure of Protein and its Active Site

p38 alpha MAP kinase is a member of the mitogen-activated protein kinase (MAPK) family, and it plays an important role in cellular signaling pathways, such as inflammation, stress response, and cell differentiation. The structure of p38 alpha MAP kinase has been determined using X-ray crystallography.

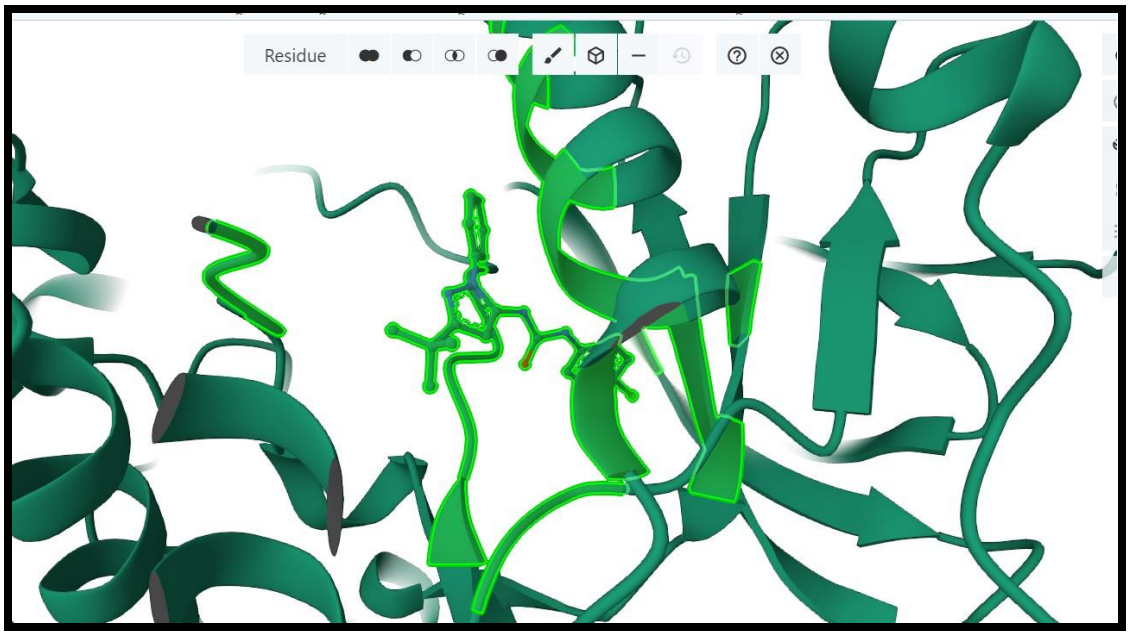
The p38 alpha MAP kinase protein is composed of 360 amino acid residues and has a molecular weight of about 41 kDa. It has a characteristic fold consisting of 13 alpha-helices and 10 beta-strands. The protein consists of two main lobes, the N terminal lobe and a larger C terminal

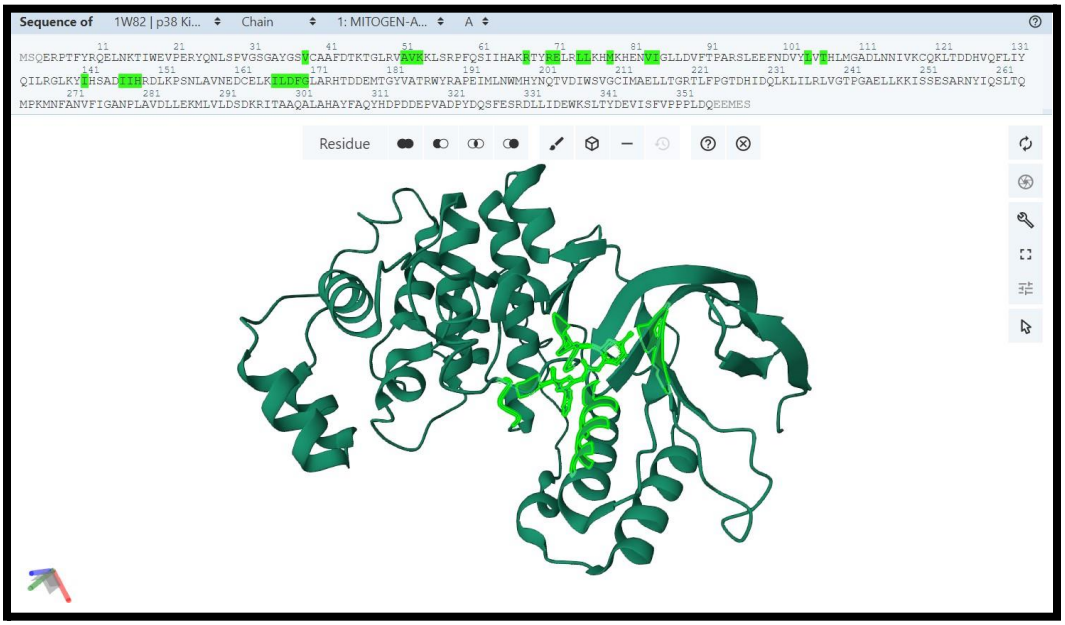
lobe. The N terminal domain is composed of mostly beta-sheets, while the C terminal is made of mostly alpha helices. The N-terminal domain contains the activation loop and contains a short alpha-helix and a loop that protrudes out from the rest of the protein. It is important for regulating the activity of the protein, while the C-terminal domain contains the ATP-binding site.

The protein is normally activated by bis-phosphorylation of the Threonine(Thr-180) residue and the Tyrosine residue(Tyr-182) present in the activation loop. However, inhibitors for this protein don't usually bind to this activation loop. They compete with ATP to bind to an ATP-binding pocket nearby, by the formation of bonds with residues in the ATP-binding site. However, this type of inhibitor is not very specific, and may bind to other protein kinases, which have their own ATP-binding pocket as well. To help increase the specificity of ligands which bind to this protein, alternate conformations of this protein should be considered in the process of drug design.

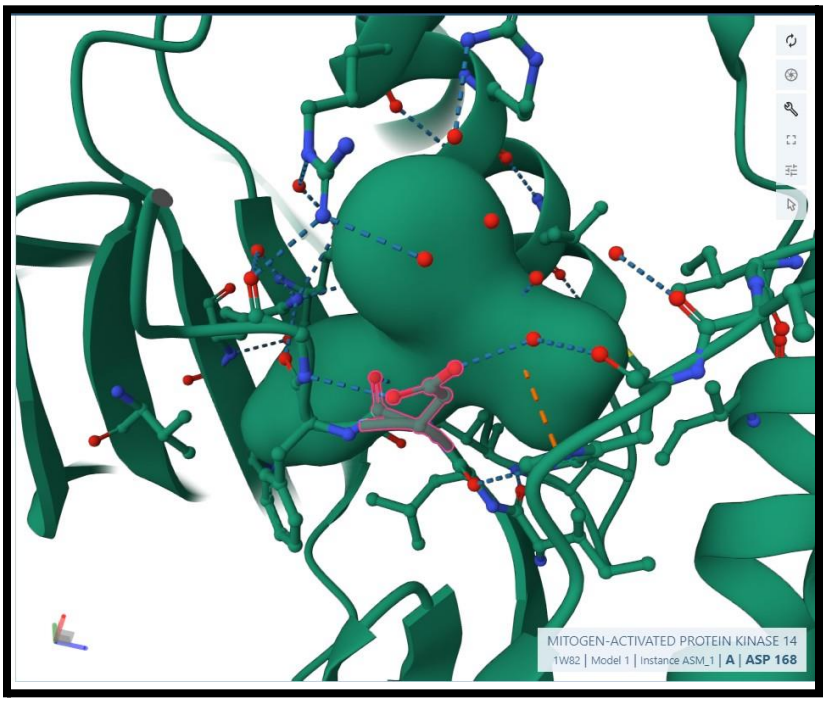
The given protein is shown in complex with a small molecule inhibitor, N-[(3Z)-5-TERT-BUTYL-2-PHENYL-1,2-DIHYDRO-3H-PYRAZOL-3-YLIDENE]-N'-(4-CHLOROPHENYL)UREA or L10. This ligand is bound to the ATP binding site via Hydrogen bonds with the residues THR-106, His-107, Leu-108 and Met-109. It also interacts with the Asp-168 and Asn-155 residues. Studies have shown that a conformational change in a mobile part of the activation loop, consisting of the residues Asp-168, Phe-169 and Gly-170 results in the formation of a polar channel between Asp-168 and Glu-71 present in the ATP binding site to another allosteric site. These residues, and the channel as well, forms crucial bonds between the ligand and the protein, and has been found to improve the efficiency and selectivity of the ligand.

Secondary structures present in the protein





The above images show the ligand L10, along with the protein residues it is interacting with.



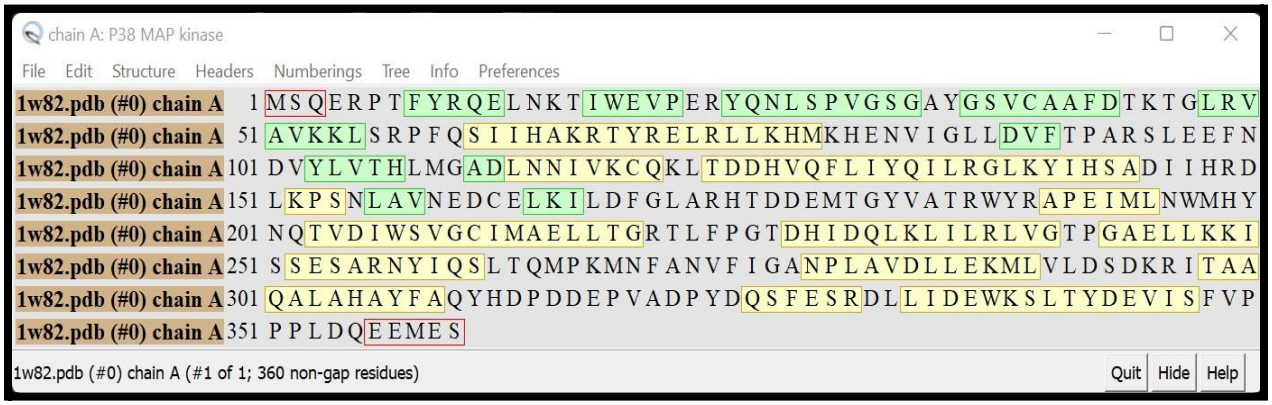
This image shows L10(Gaussian surface representation) along with the bonds it has with the residues around it. We can see that it is forming a bond with the residue Asp-168 in this image, as mentioned before.

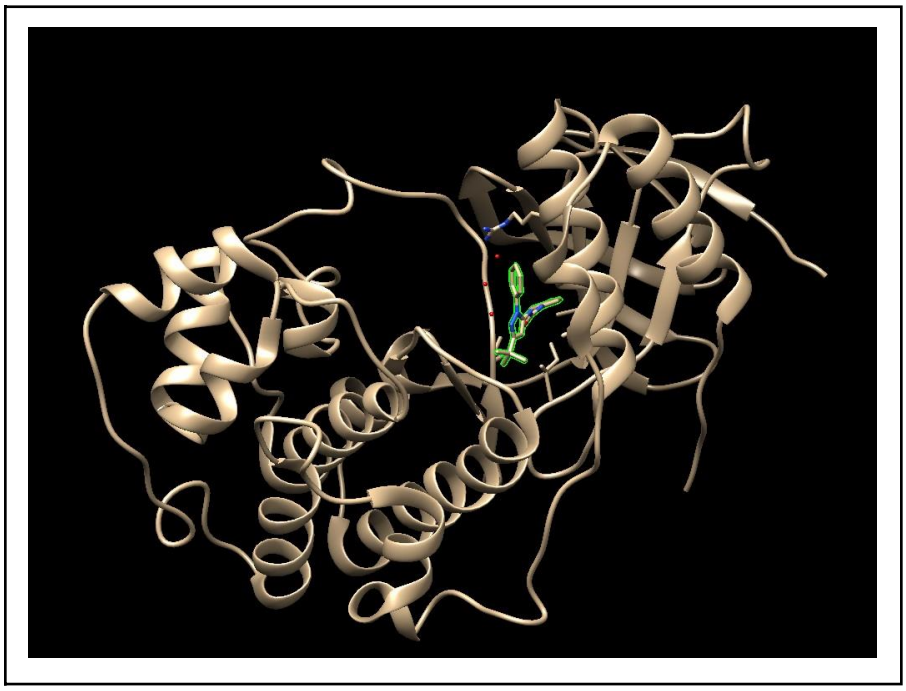
Autodocking

In this report, the structure of the protein 1W82 was analyzed using software such as Chimera, and Autodocking was performed to find which Zinc ligand can bind optimally to its active site, thus possibly providing an alternative to the ligand it was originally in complex with. A library of Zinc compounds were used and the binding affinities of each of these ligands to the protein was analyzed to find the one with the best binding affinity.

1. Preparation of the Protein and Ligand Library:

The sequence was analyzed using Chimera, to check if it was complete, and that there were no gaps in the sequence. Gaps in the sequence would provide errors in the result or otherwise unreliable results.

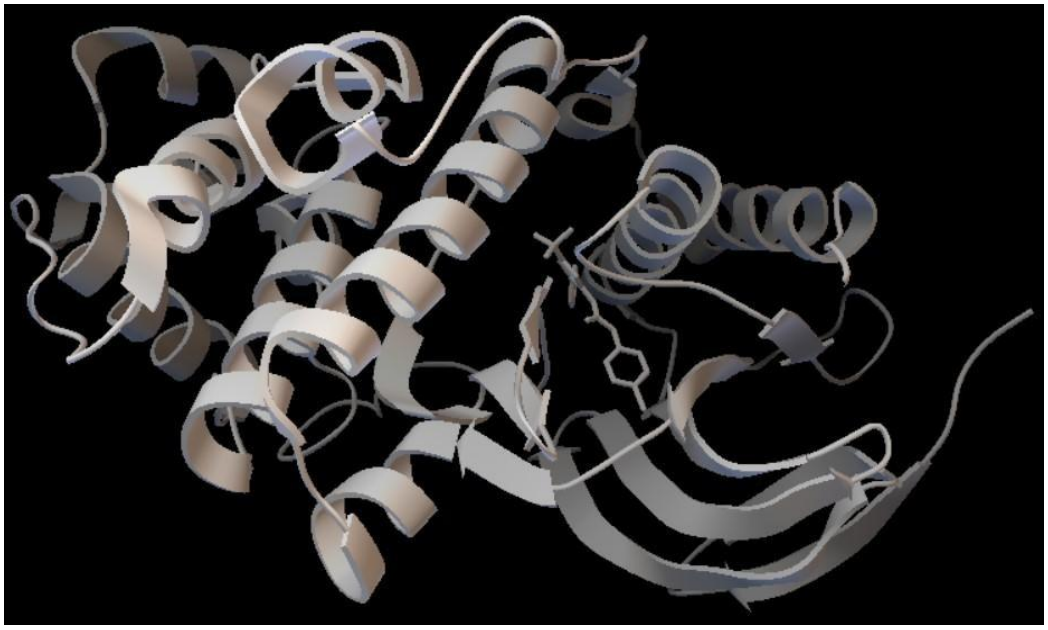




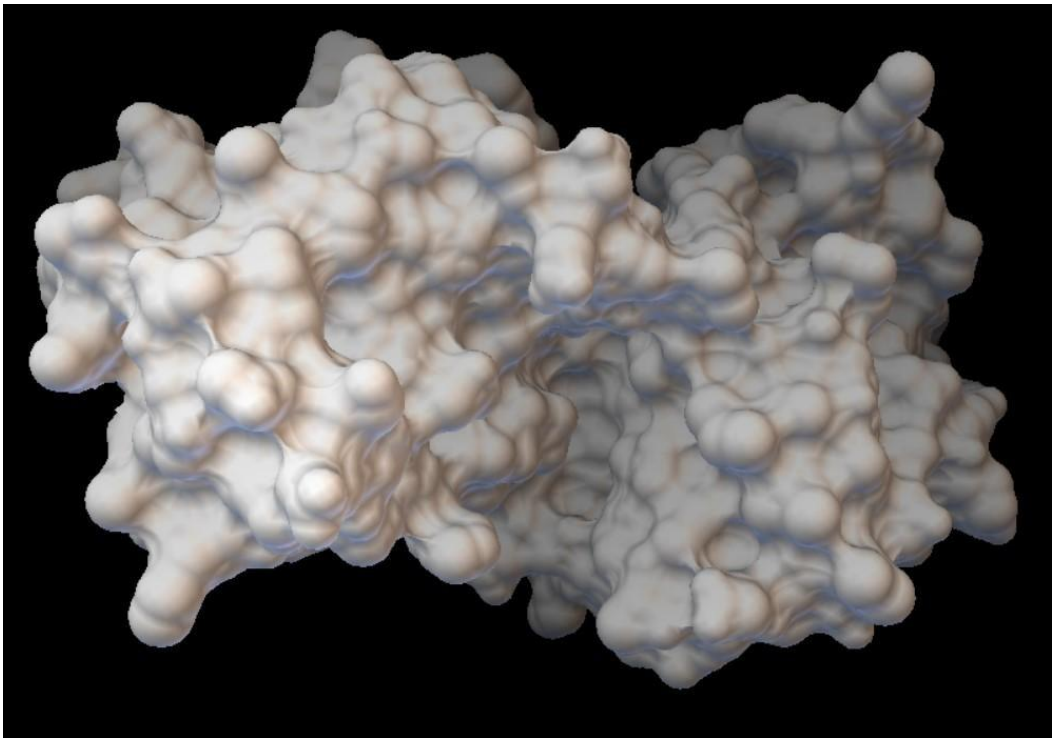
The above images show us that the protein was complete, and we could use it for Autodocking. The ligand to be deleted is selected in the image.

- The protein model was then opened using Autodock and the water molecules were removed.
- The ligand was selected so as to visualize the active site and the potential binding site for the ligands in the compound library.
- It was then deleted so as to leave the active site free for other ligands to bind to in the Autodocking process.
- Polar hydrogens were added, and missing atoms were checked for and repaired.
- The prepared protein was then saved as a .pdbqt file for later use.
- The ligand library was converted into .pdb format as well.

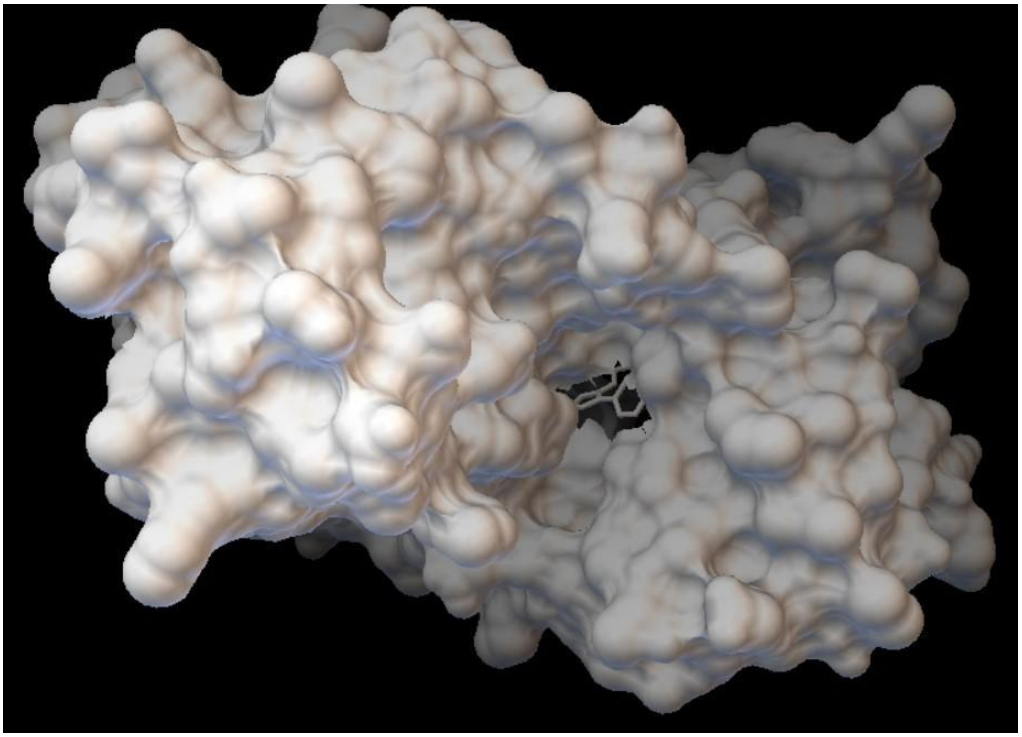
Protein Structure Analysis:



Protein and Ball and Stick Representation of Ligand L10



Molecular Surface Representation of Protein



Molecular Surface Representation with Binding Pocket of Ligand Visible

2. Autodocking Process:

- The prepared protein was opened using Autodock Vina and a grid box was created over the active site. This grid box is where the docking will be focussed on, and thus having it smaller and more focussed on the active site will prevent checking for any unwanted bonds with lower affinity in sites present towards the edges of the protein.
- The coordinates of the grid box were saved in a configuration file for later use.
- Docking of the compounds in the library was done using perl, through the command prompt.

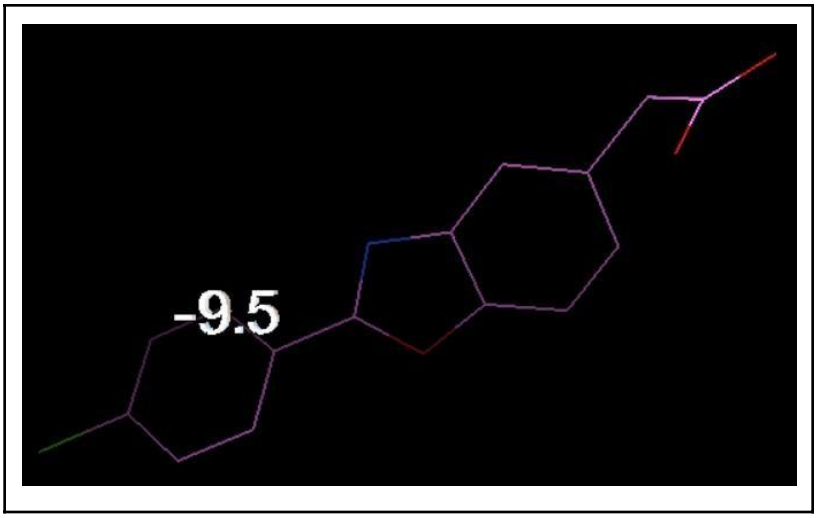
Results From Autodocking

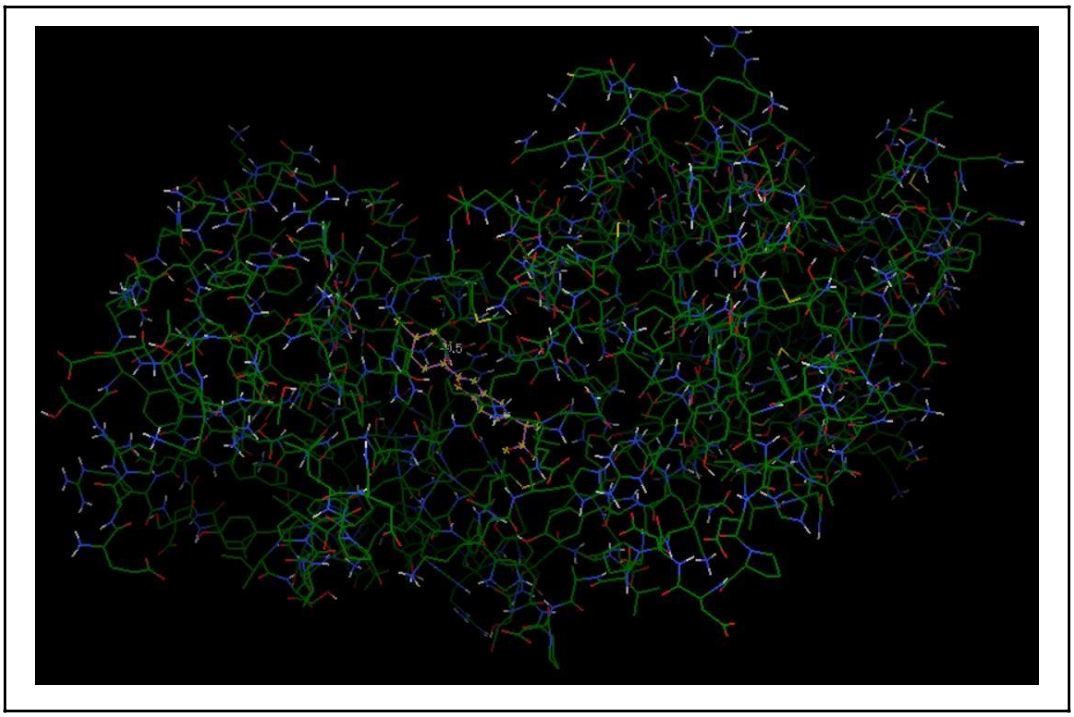
Autodocking of each of the zinc compounds in the library was done. The software attempted to dock the compounds to whichever site it could within the grid box, and checked for various orientations of the ligand as well. 10 orientations of each ligand, along with their binding affinities were printed in an output file. The more negative the binding affinity, the stronger the ligand-protein interaction and the better ligand-protein docking prediction. Ligands which were binding to the protein with an affinity of -7.5 kcal/mol or less were considered to have good binding affinity.

Ligand ‘ZINC00000070’ was found to have the most negative binding affinity, with a value of -9.5 kcal/mol and further analysis of where exactly in the protein it binds was done.

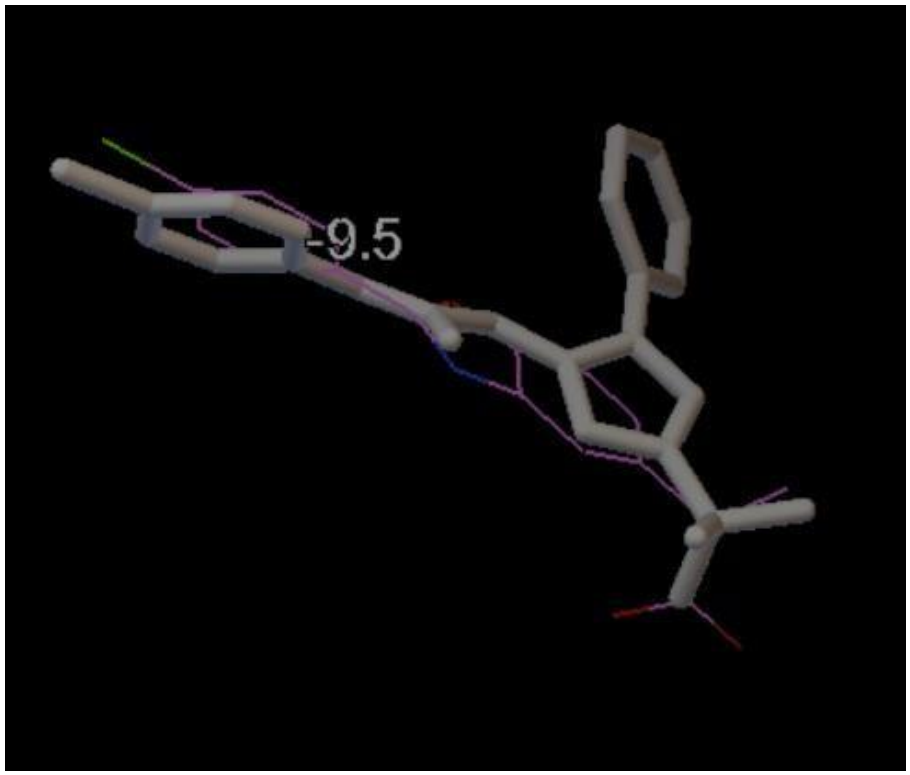
Analysis of Ligand Binding

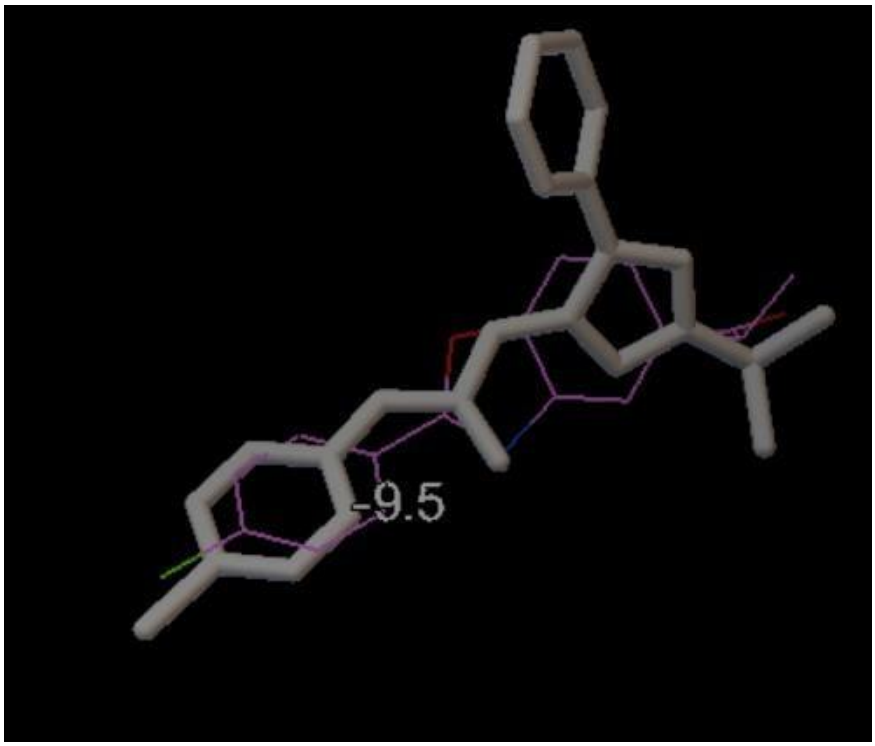
ZINC00000070 ligand was analyzed in comparison to the original ligand, to see where exactly it is bound, and to which residues.



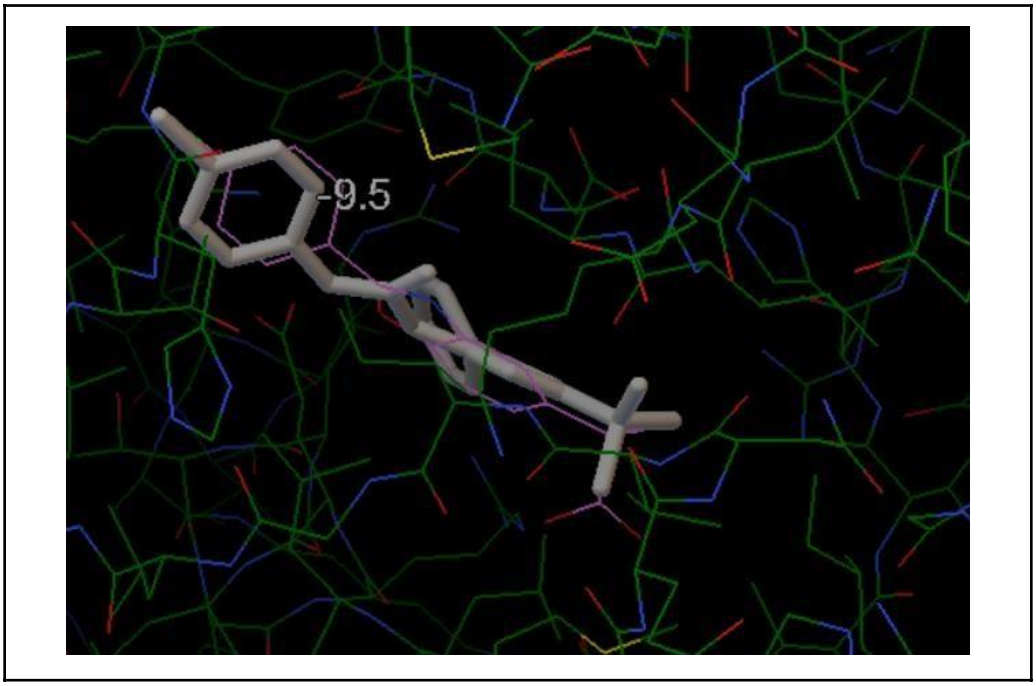


The above images show the structure of the ligand, and its binding site in the protein. We can see in the images below that this ligand and the original ligand are very similar in structure and orientation. The original ligand is shown as gray, while the new one is shown in thin purple lines.

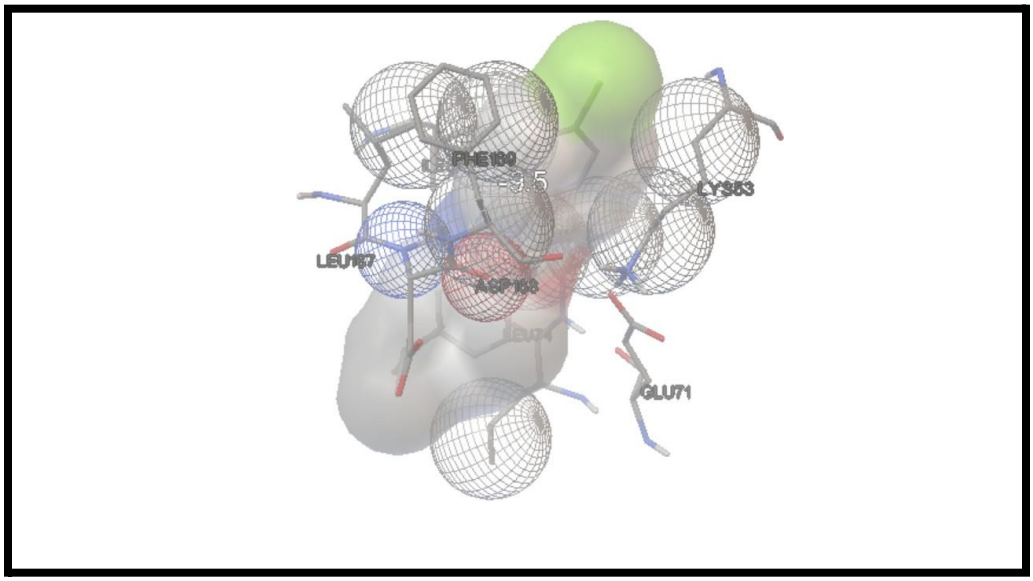

-9.5


-9.5

The following image shows L10 and ligand ZINC00000070 superimposed over each other and in relation to their binding on the protein. We can see that the zinc and the original L10 ligand both show similar binding orientations and bind to the same location in the protein. They both bind to the ATP binding site of the protein. Though they both may not bind to all the same residues, enough similarity is there to assume that the Zinc ligand can compete with L10 to bind at the same site.



The following image shows the interactions between the Zinc ligand and the residues around it. We can see that bonds are present between it and residues such as Asp-168, Phe-169 and Glu-71. Thus, it shows interactions similar to those between the L10 ligand and the protein.



From the above analysis, we can see that the ligand ‘ZINC00000070’ binds to much of the same residues as the original ligand. Since the original ligand was used in an anti-inflammatory drug, we can hypothesize that this ligand too can be used in such drugs to inhibit this protein, and prevent its overactivation.

References:

1. Gill AL, Frederickson M, Cleasby A, Woodhead SJ, Carr MG, Woodhead AJ, Walker MT, Congreve MS, Devine LA, Tisi D, O'Reilly M, Seavers LC, Davis DJ, Curry J, Anthony R, Padova A, Murray CW, Carr RA, Jhoti H. Identification of novel p38alpha MAP kinase inhibitors using fragment-based lead generation. J Med Chem. 2005 Jan 27;48(2):414-26. doi: 10.1021/jm049575n. PMID: 15658855.
2. Wang Z, Harkins PC, Ulevitch RJ, Han J, Cobb MH, Goldsmith EJ. The structure of mitogen-activated protein kinase p38 at 2.1-A resolution. Proc Natl Acad Sci U S A. 1997 Mar 18;94(6):2327-32. doi: 10.1073/pnas.94.6.2327. PMID: 9122194; PMCID: PMC20087.