

Proposing the Promiscuous Protein Structures in JNK1 and JNK3 for Virtual Screening in Pursuit of Potential Leads

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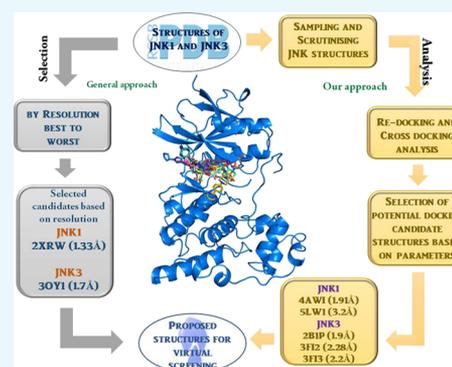


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ABSTRACT: Over the past decade, the available crystal structures have almost doubled in Protein Data Bank (PDB) providing the research community with a series of similar crystal structures to choose from for future docking studies. With the steady growth in the number of high-resolution three-dimensional protein structures, ligand docking-based virtual screening of chemical libraries to a receptor plays a critical role in the drug discovery process by identifying new drug candidates. Thus, identifying potential candidates among all the available structures in a database for docking studies is of utmost importance. Our work examined whether one could use the resolution of a number of known structures, without considering other parameters, to choose a good experimental structure for various docking studies to find more useful drug leads. We expected that a good experimental structure for docking studies to be the one that gave favorable docking with the largest number of ligands among the experimental structures to be selected. We chose three protein test systems for our study, all belonging to the family of MAPK: (1) JNK1, (2) JNK2, and (3) JNK3. On analysis of the results, the best resolution structures showed significant variations from the expected values in their result, whereas the poor resolution structures proved to be better candidates for docking studies.



1. INTRODUCTION

Drug discovery and drug research have contributed more to the progress in the field of medicine than any other scientific factors. Even a single disease can have many options for a drug.¹ As the pharmaceutical industry evolves, the need to find an easier way to access new drug compounds becomes a necessity.² Finding a new chemical entity and its structural scaffold is the essence of drug designing. Virtual screening (VS) emerged as one of the answers to this quest with numerous methodological protocols available in screening databases for the lead compounds, which fill this chasm in drug discovery.³ It is a high-throughput screening of millions of compound databases in the hope of finding a unique compound or a drug that can replace an existing drug or that can shed light on diseases with no drugs to treat them until now. In the virtual screening process, a target protein in a complex with a bound ligand represents a conformation of the target optimally adapted to accommodate that particular ligand. There are various screening algorithms and schemes that test and select among thousands of ligands based on their compatibility toward the target molecule of a particular disease.⁴ The efficiency of any virtual screening technique lies in its effective reciprocity between the computational technique and the experimental research that paves a way for proper screening of potential leads. For any drug discovery, after high-throughput screening (HTS), the small-molecule

hits undergo transformation into active compounds with selective binding behavior, which leads to identification of promising lead compounds with drug-like activities through limited optimization.^{5–7}

Out of the structure-based virtual screening and ligand-based virtual screening, molecular docking falls under the first category that brings and binds the two molecular structures together in a preferred conformation. Being the most widely used method in modeling three-dimensional structures, molecular docking consists of two steps: (i) searching the conformational space of the ligand that binds to target molecules and (ii) using a scoring function to evaluate these ligands.⁶ Thus, molecular-docking-based virtual screening essentially identifies chemically diverse hits when the three-dimensional structure of the target is available.

The recent years have seen an explosion in the number of available structures of biological targets in databases, thus making it difficult to surf through them all and choose a single target structure for docking studies. The success of the docking-based virtual screening is sensitive to the choice of the

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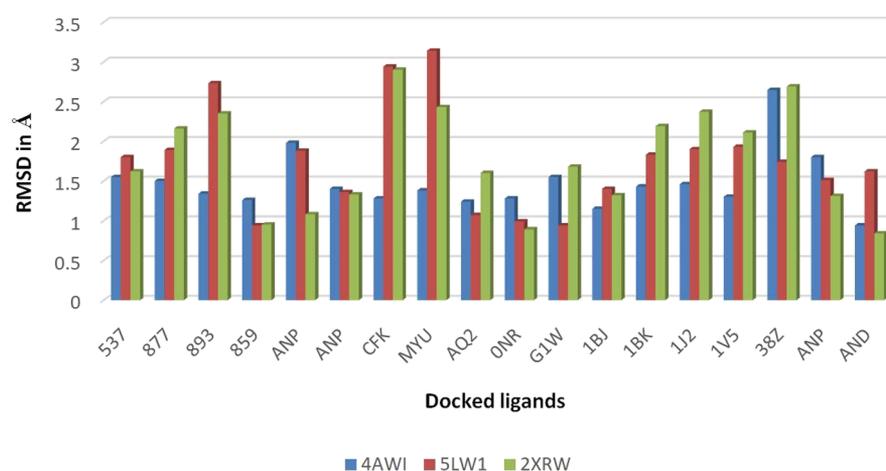


Figure 1. Comparison of cross docking results of JNK1 structures: 5LW1, 4AWI, and 2XRW.

3D structure of the target.⁸ Resolution essentially refers to the amount of information obtained from a crystal in a protein crystallography experiment, and it is a measure of the level of detail present in the diffraction pattern and the level of detail that will be seen when the electron density map is calculated. Best resolution structures are highly ordered, and it is easy to see every atom in the electron density map, whereas poorer resolution structures show only the basic contours of the protein chain. Selecting a best resolution structure among the available structures in a database is the common procedure followed in docking studies making resolution one of the important parameters in selection of target structures. A reliable resolution value for small-molecule docking is below 1.2 Å, which is when the atomic resolution is achieved. However, resolutions below 1.5 Å coinciding with the mean length of the covalent bond are rarely achieved, and most structures available have resolutions of 1.5 to 2.5 Å.⁹ Most software and algorithms yield a good result for structures below 2.2 Å, thus making it the threshold for selection.¹⁰

The significant future prospects of JNKs acting as targets for new drug discoveries triggered it to be taken as the test case subject. c-Jun N-terminal kinases (JNKs) are members of the MAPK family of protein kinases that influence gene transcription based on their ability to phosphorylate and activate multiple transcription factors like c-Jun.^{11–13}

JNKs encoded by the *JUN* gene consist of 10 isoforms derived from three genes, namely, JNK1 (4 isoforms), JNK2 (4 isoforms), and JNK3 (2 isoforms).¹⁴ The improved understanding of JNK regulation and derived functions strongly suggests that the time has come to seriously consider the development and clinical application of JNK inhibitors that may be used as insulin sensitizers and to treat rheumatoid arthritis.¹⁵ In PDB, there are 32 structures of JNK1 with 22 different ligands, 2 structures of JNK2 with 2 different ligands, and 51 structures of JNK3 with 48 ligands. In order to carry out an unbiased study, JNK2 was neglected due to its negligible number of structures.

2. RESULTS

2.1. Redocking and Cross Docking of JNK1 and JNK3.

Out of the 10 apo structures and 22 complex structures of JNK1, 2XRW had the best resolution of 1.33 Å making it the widely used structure compared to the rest of the structures in PDB (until 2019). 2G01, 2GMX, and 3VUD were less

preferred structures because of their poorer resolution (3.5 Å). We have scrutinized all structures of JNK1, and they had the DFG-IN conformation where the Asp169 of the DFG is able to coordinate magnesium. It also had an activation loop consisting of amino acid residues 169–190 in JNK1, which was present in 8 structures, absent in 14 structures, and disconnected due to missing residues in 10 structures.

The redocking of all the ligands of JNK1 to their corresponding apo structures with the RMSD value (in Å) and binding energies (in kcal/mol) is shown in Supporting Information S1. The 20 ligands of JNK1 that were docked with the 32 structures showed the docking efficiency of each structure by showing its degree of acceptance toward various ligands. The cross docking ability of each structure of JNK1 and the mean RMSD value in Å for each structure and each ligand are analyzed in Supporting Information S2. Though the 32 structures belonged to the same kinase, that is, JNK1, and had almost similar structures, they showed significantly diverse docking results with each ligand, and very little similarity was observed. The cross docking RMSD value for JNK1 was in the range of 0.41 (4QTD docked with MYU) to 7.41 Å (6F5E docked with 1J2), and this significant variation in docking paved a way for deeper analysis to understand the reason behind it. We have calculated the number of docked poses below an RMSD of 2 Å^{9,10} for all structures of JNK1 and JNK3 to evaluate their docking performances and provide a statistical basis for our study. The mean RMSD of the 18 ligands was around 2 Å (2.10 Å), which also justifies the threshold RMSD (2 Å) taken for our study. In our cross docking analysis, 5LW1 and 4AWI had less RMSD values than 2XRW in the case of 8 and 12 ligands, respectively (Figure 1). On average, the RMSD of the best resolution structure 2XRW was greater than 2 Å in 10 of the 20 ligands, whereas in the case of 4AWI, 19 of the 20 ligands had RMSD less than 2 Å and in the case of 5LW1, 17 of 20 ligands had RMSD less than 2 Å, thus making them more compatible for docking studies.

Similarly, the JNK3 structures deposited in PDB consist of 51 structures (until 2019) having 47 different ligands, out of which only four were apo structures. Among them, 3OY1 had the best resolution with 1.7 Å and 3CGF, 3CGO, and 4H36 having resolutions of 3 Å each. The DFG motif occupying positions 207–209 was in DFG-IN conformation for all the structures where the magnesium was steered to its position by the Asp207 of the motif. Redocking of ligands with their

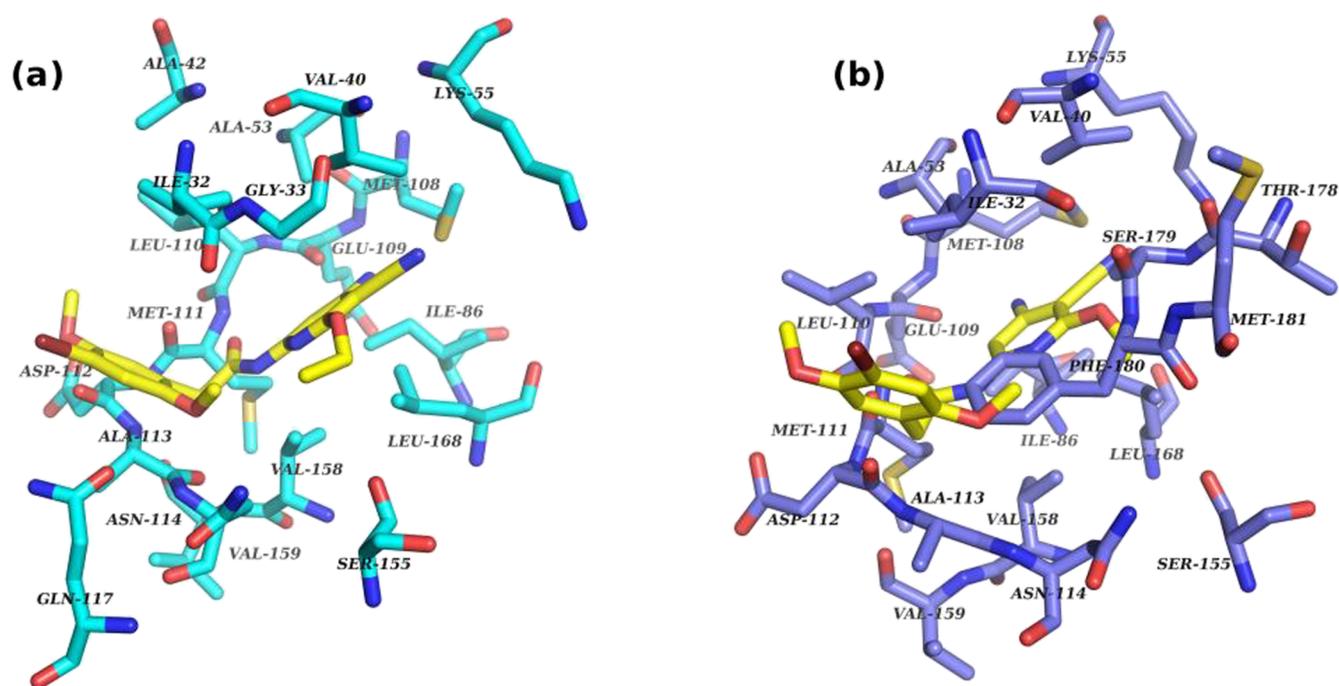


Figure 2. (a) Ligand G1W (yellow) is docked into the active site of 4AWI (cyan) where residues around 5 Å are shown. (b) The ligand G1W (yellow) is docked into the active site of 6F5E (purple) where residues around 5 Å are shown.

corresponding JNK3 structures posed a certain difficulty due to the various sizes and flexibility of ligands, but most of the structures with the exemption of three (3V6R, 3V6S, and 4Z9L) gave considerable redocking results as shown in Supporting Information S3. The RMSD in cross docking of JNK3 structures was in the range of 0.17 (cross docking between 1PMU and 9HP) to 10.3 Å (cross docking between 4W4X and 738), and these significant results are shown in Supporting Information S4. We also calculated the mean RMSD in each case of the JNK3 structures and their corresponding ligands docking efficiency in cross docking calculations.

Studies were carried out to show that the poor docking performances of certain structures may have been effectively due to the orientation of few residues that almost blocked their active sites like a lid and reduced their sizes. We also grouped structurally similar ligands, and their cross docking performances were analyzed in both JNK1 and JNK3 to know if the structural similarity of the ligands influenced the docking studies. This was essential to check the contribution of the co-crystallized ligands to the docking performances of the JNK structures and to assess the importance of this contribution, thereby contributing to a full-fledged study. In JNK1 and JNK3, ligands with the highest and lowest mean RMSDs that were also structurally similar were grouped together to analyze their docking performances.

3. DISCUSSION

Virtual screening protocols with their ability to swiftly navigate through databases to finalize a lead compound in the drug discovery process have made a significant contribution in the field of computational biology. Thus, various research activities are being carried out related to virtual screening to further make the drug discovery process more quick and efficient. The curiosity that led us to choose JNKs as our test case is the plethora of studies where JNKs are used as targets for new drug

discoveries. In addition to the emerging role of JNK in insulin resistance and improving insulin sensitivity, JNK inhibitors may have the added bonus of reducing obesity, which is one of the most common diseases found among humans.¹⁶ Thus, docking studies of JNKs are significant, currently due to its various contributions toward drug discovery. The docking study carried out in our test case with JNK1 revealed that structures 3PZE and 4L7F having resolutions of 2 and 1.95 Å, respectively, had the best results with minimal RMSD values of 0.56 and 0.61 Å during redocking, respectively. Considering the general approach toward docking studies, the structure with the best resolution would be taken, and in our case, structures 2XRW, 4QTD, and 3ELJ are those possible candidates with resolutions of 1.33, 1.5, and 1.8 Å, respectively. However, profound analysis of cross docking results (Supporting Information S2) of JNK1 revealed that 4AWI (resolution of 1.91 Å) and 5LW1 (resolution of 3.2 Å) had the scope of being more potential candidates for docking analysis having compatibility with 19 out of 20 ligands (RMSD < 2 Å) in the case of 4AWI and 17 out of 20 ligands (RMSD < 2 Å) in the case of 5LW1. Meanwhile, we found that only eight ligands had RMSD less than 2 Å in the case of the best resolution structure 2XRW (Figure 1). This contradicted the preconceived idea that only the best resolution structures could be potential candidates leading to a more equivocal approach in docking analysis. Likewise, the cross docking of JNK1 structures 4HYU (2.31 Å) having compatibility with 17 ligands; 4E73 (2.27 Å) and 4L7F (1.95 Å) with 16 ligands; 4G1W (2.45 Å) and 4YR8 (2.4 Å) with 15 ligands; 2GMX (3.5 Å), 2XS0 (2.6 Å), and 4UX9 (2.34 Å) with 14 ligands was performed.

The most noncompatibility was observed with the structure of 6F5E having a resolution of 2.7 Å, which had an RMSD range of 1.99 to 7.41 Å. 6F5E had conformation of residues Ser179, Phe180, and Met181 protruding into the active site, thereby reducing its size and inhibiting docking of various

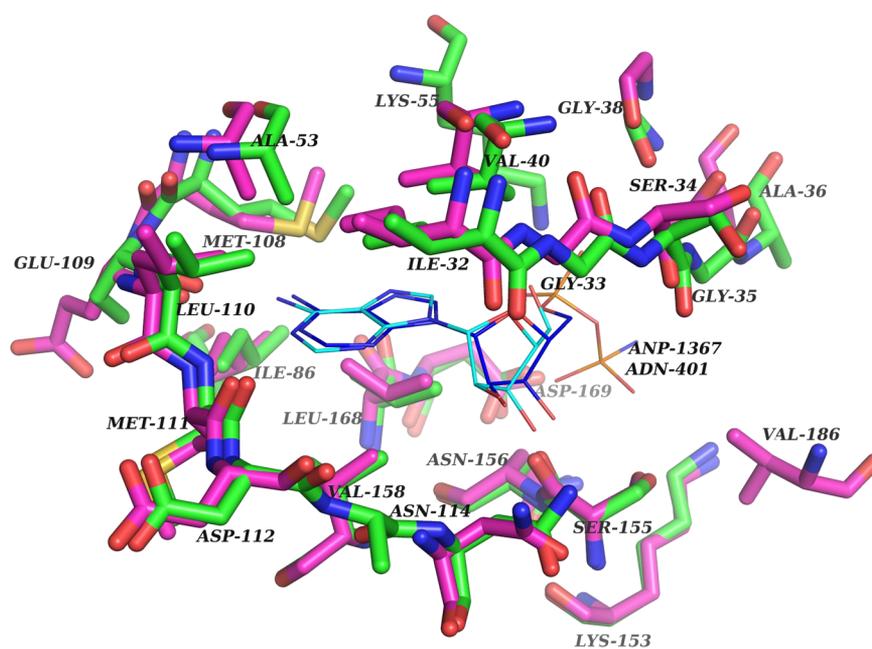


Figure 3. Superimposition of active sites of 2XRW (magenta) and 5LW1 (green). The residues within 5 Å of the active sites of 2XRW and 5LW1 are similar. Also, Lys55 of 5LW1 and Val186 of 2XRW are included and are not present in other structures due to the phosphate group of ligand ANP (blue) occupying a larger area in 2XRW. ADN is displayed in cyan.

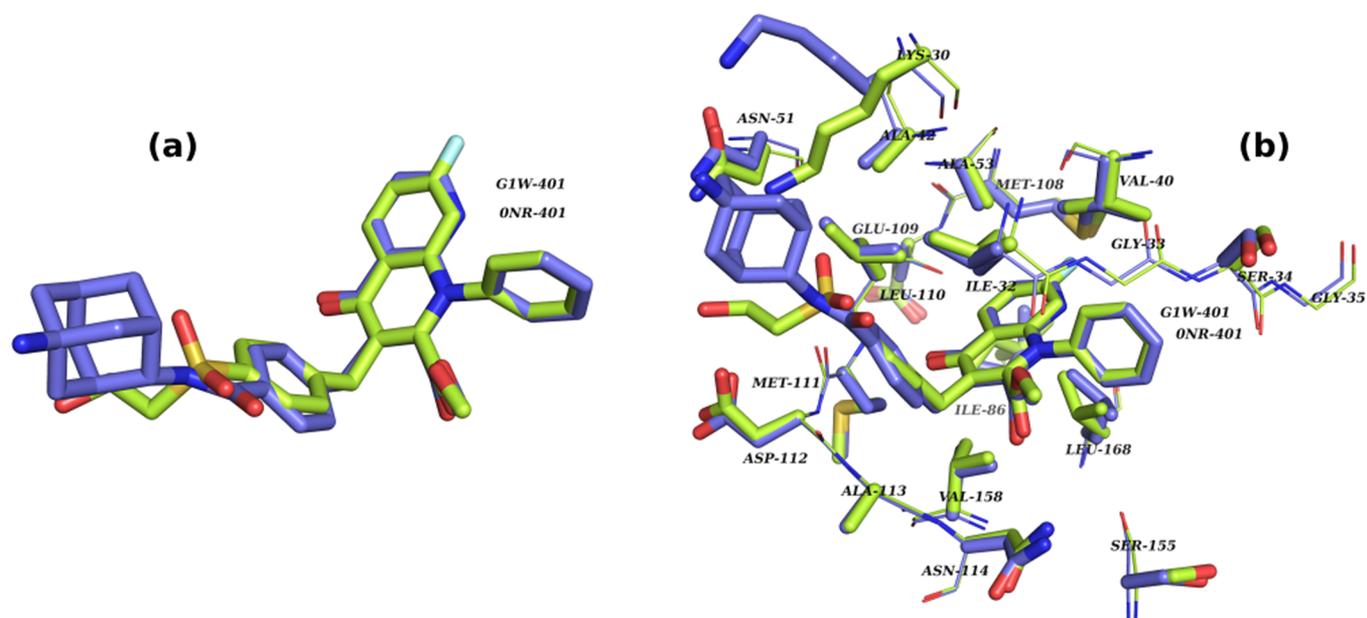


Figure 4. (a,b) Superimposition of structurally similar ligands of JNK1 with the lowest mean RMSD: with G1W of 4G1W (green) and ONR of 4E73 (purple).

ligands to it. Figure 2a,b shows the active sites of a well docked structure 4AWI and poorly docked structure 6F5E. It is clear that the residues around ligand 877 in 4AWI allow easy docking, whereas the conformations of the residues around ligand 877 in 6F5E restrict docking by protruding into the docking site.

The mean RMSD was calculated for the docking of each single ligand with the 32 structures to see how far the cross docked RMSD deviated from the mean RMSD in the case of each ligand. Ligand G1W showed the least mean RMSD of 1.472 Å followed by ligand ONR, which had a slightly higher mean RMSD of 1.475 Å. The highest mean RMSD was seen

with cross docking of ligands 1BK (mean RMSD of 2.653 Å), 1J2 (mean RMSD of 2.775 Å), and 1V5 (mean RMSD of 2.738 Å). The best resolution structure 2XRW (1.33 Å) exceeded mean RMSD when docked with six of the 18 ligands, while structures 5LW1 (resolution of 3.2 Å) and 4AWI (resolution of 1.91 Å) exceeded mean RMSD when docked with five and two ligands out of the 18 ligands, respectively, which are highlighted in Supporting Information S2.

The flexibility of the target protein is what allows it to adopt a different conformation in the presence of a chemically diverse ligand paving a way conducive to a new drug lead. In previous docking studies, apo structures and structures with larger

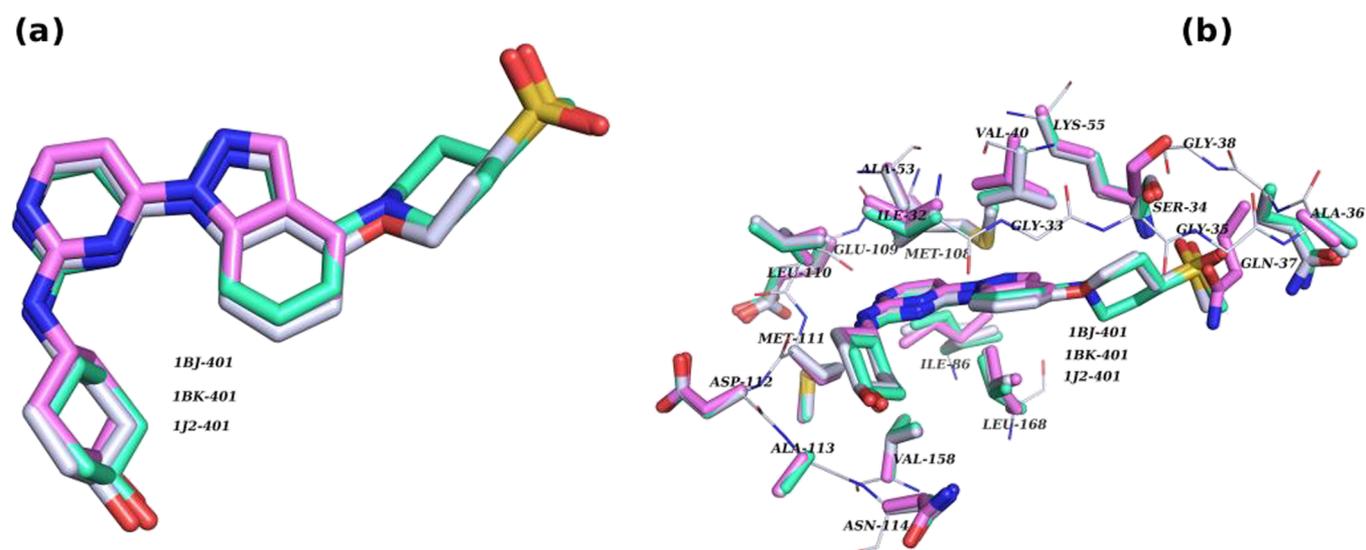


Figure 5. (a,b) Superimposition of structurally similar ligands of JNK1 with the highest mean RMSD: 1BJ of 4HYS (pink), 1BK of 4HYU (gray), and 1J2 of 4IZY (cyan).

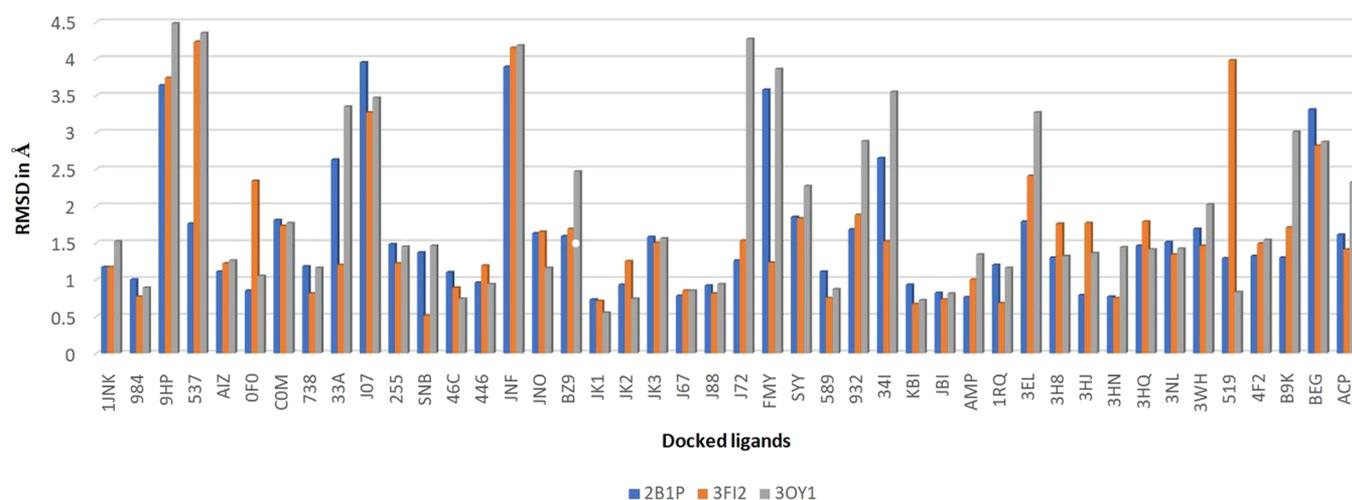


Figure 6. Comparison of cross docking results of JNK3 structures: 2B1P, 3FI2, and 3OY1.

ligands proved to be efficient candidates compared to structures with smaller ligands,¹⁷ which may have been due to the size of the active site cavity. Similarly, in this case, apo structures or structures having larger ligands bound to them in their native state showed better results in docking. To further prove this, the active sites of 2XRW (best resolution structure) and 5LW1 (one of the structures with the best docking results) that were superimposed to see if any of the residues protruded into the active site that made these two structurally similar structures show varying docking results. The active sites of 2XRW and 5LW1 were similar except for Lys55 of 5LW1 and Val186 of 2XRW, which is not present in other structures due to the phosphate group of ligand ANP occupying a larger area in 2XRW (Figure 3).

One cannot come to a solid conclusion without discussing the role of the co-crystallized ligand whose study might provide a closure to the question of the influence of the co-crystallized ligand on docking performance. Hence, the structurally similar ligands of JNK1 were grouped together. In the case of ligands G1W and 0NR, which had the least mean RMSDs (Supporting Information S2) and also structurally very similar (Figure 4a),

redocking RMSDs were 0.81 and 1.12 Å when docked with their corresponding apo structures 4G1W and 4E73, respectively. Ligand G1W when cross docked with 4E73 showed an RMSD of 1.27 Å, which was less than the mean RMSD of 1.472 Å, whereas ligand 0NR when crossed docked with 4G1W had an RMSD of 2.21 Å and also exceeded the mean RMSD of 1.475 Å (Figure 4b). When grouping structurally similar ligands in the other end of the gradient, that is, grouping ligands with the highest mean RMSD, similar results were observed. In the case of ligands 1BJ, 1BK, and 1J2 that had the highest mean RMSDs (Supporting Information S2) and were also structurally similar (Figure 5a,b), the redocking RMSDs were found to be 0.82, 0.84, and 0.7 Å, respectively. In contrast to the common expectation, these ligands showed cross docking RMSDs that were poles apart even though they were structurally analogous. When 1BJ was docked with 4HYU and 4IZY, the cross docking RMSD was the same (0.61 Å), which was lower than their corresponding mean RMSD. Ligand 1BK when docked with 4IZY had an RMSD of 0.79 Å, and ligand 1J2 when docked with 4HYU had an RMSD of 0.64 Å. These docking RMSDs were much below

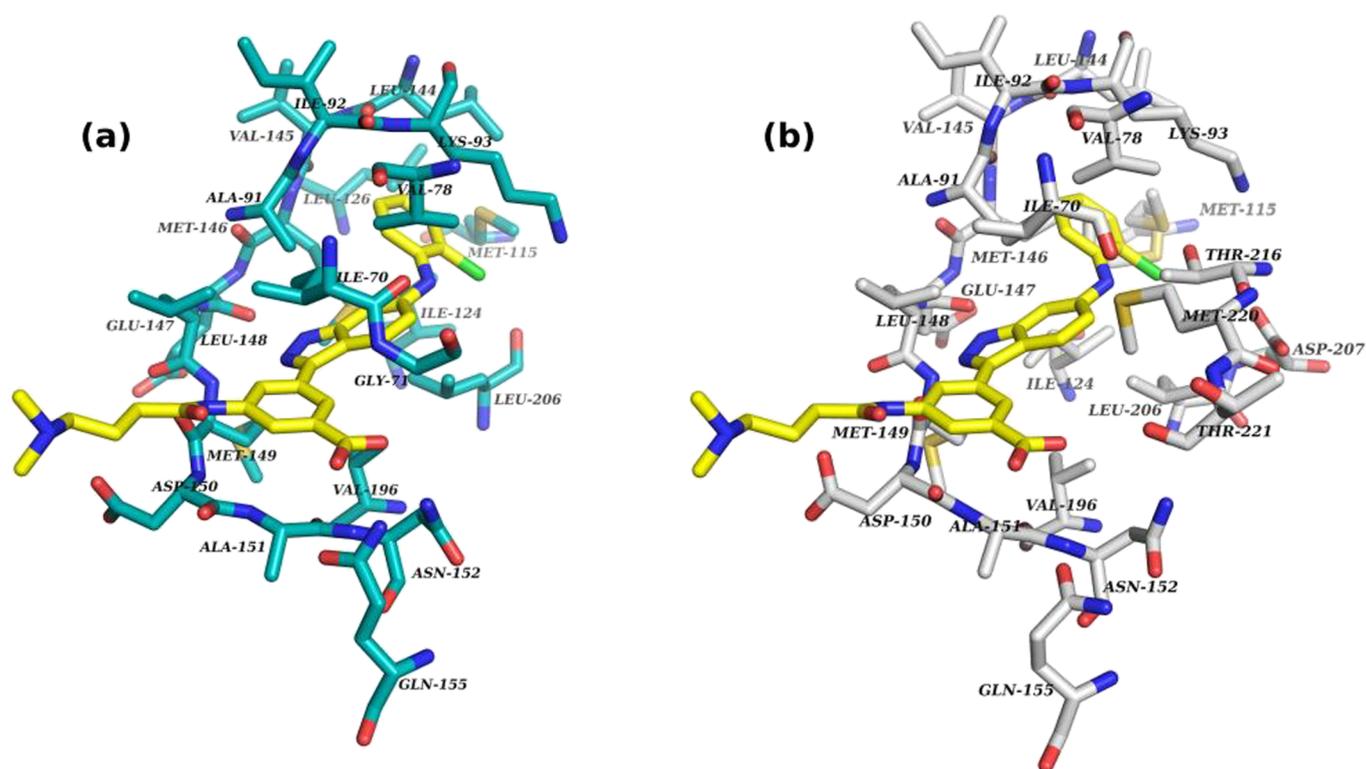


Figure 7. (a) Ligand AIZ (yellow) is docked into the active site of 2B1P (cyan) where residues around 5 Å are shown. (b) The ligand AIZ (yellow) is docked into the active site of 4H3B (white) where residues around 5 Å are shown.

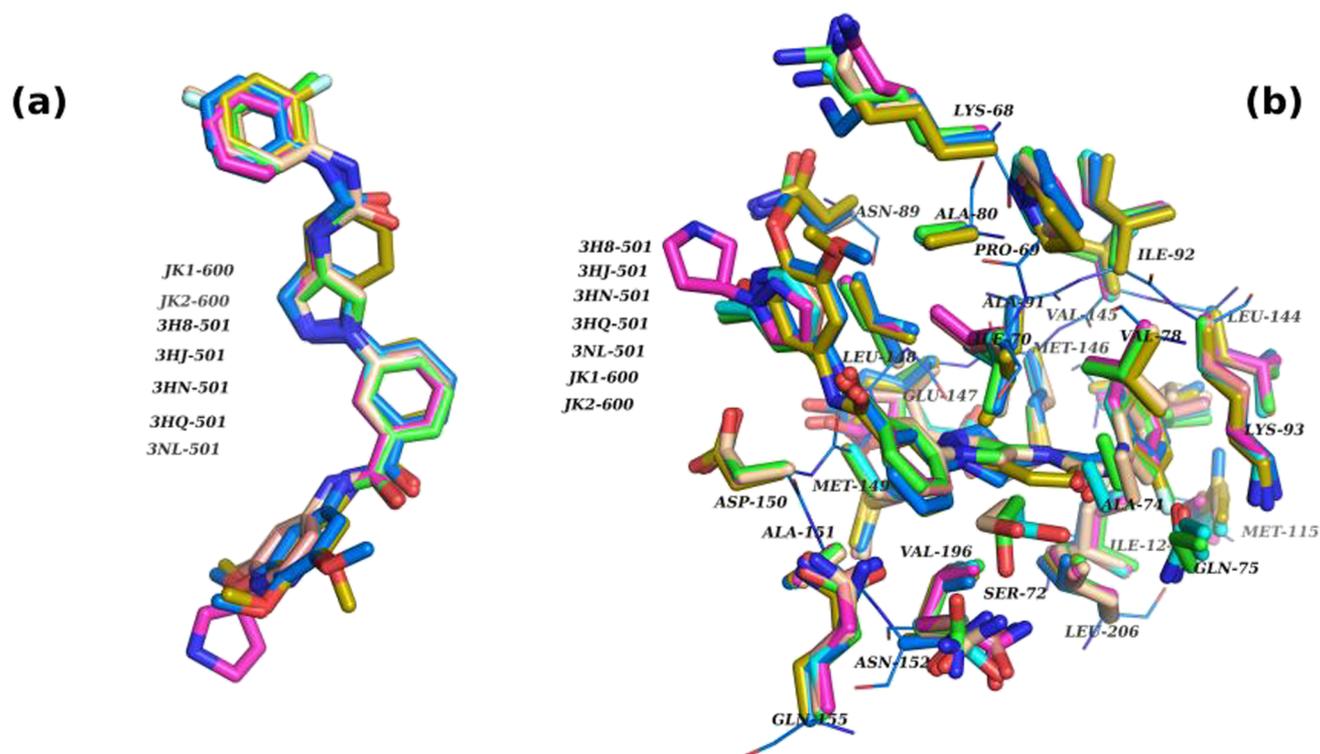


Figure 8. (a,b) Superimposition of structurally similar ligands of JNK3: JK1 of 3FI2 (blue), JK2 of 3FI3 (pale yellow), 3H8 of 4W4V (cyan), 3HJ of 4W4W (pink), 3HN of 4W4X (green), 3HQ of 4W4Y (wheat), and 3NL of 4WHZ (magenta).

the mean RMSD values. This was not the case with ligand 1BK when docked with 4HYS with an RMSD of 1.75 Å and ligand 1J2 when docked with 4HYS with an RMSD of 2.19 Å, thus exceeding the mean RMSD in each case. These results provide

imperative evidence that ligand similarity does not affect the docking studies.

In the case of JNK3, the results of all the redocking and cross docking showed a favorable outcome toward our study

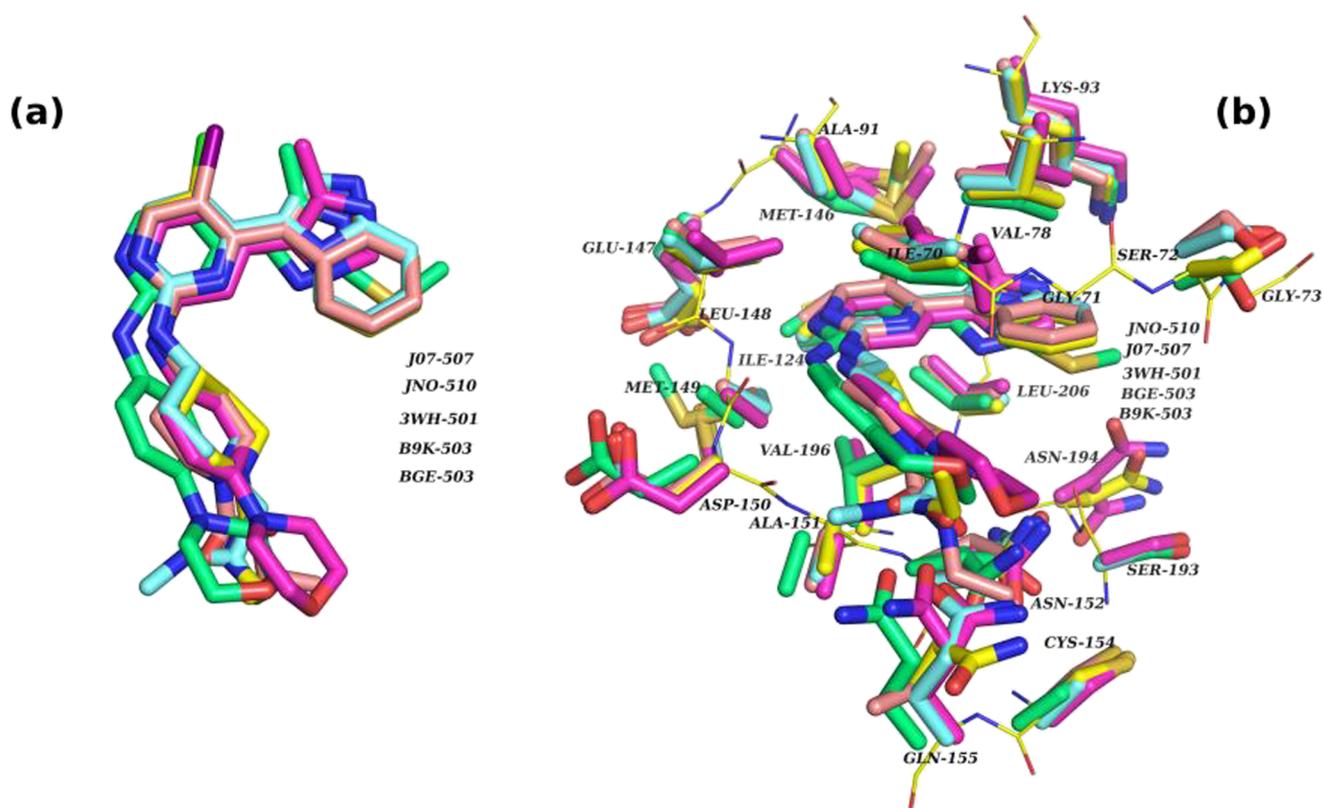


Figure 9. (a,b) Superimposition of structurally similar ligands of JNK3: J07 of 2P33 (yellow), JNO of 3CGO (cyan), 3WH of 4X21 (pink), B9K of 6EKD (lime green), and BGE of 6EMH (magenta).

where poor resolution structures proved to be better candidates for docking. More than 1000 redocked and cross docked structures were categorized based on the docking result, and the mean RMSD was calculated to act as a threshold value to evaluate the docking of each structure of JNK3 (Supporting Information S4). 2B1P, 3FI2, and 3FI3 (having resolutions of 1.9, 2.2, and 2.28 Å, respectively) emerged as the best structures that were compatible with 36, 35, and 35 ligands by having RMSD values in the range of 0.73 to 1.81 Å in the case of 2B1P and the other two structures having RMSDs in the range of 0.5 to 8.99 Å and 0.51 to 9.81 Å, respectively (Figure 6). The supposedly best resolution structures like 3OY1 (1.7 Å), 4WHZ (1.79 Å), 6EMH (1.76 Å), and 6EQ9 (1.83 Å) showed an average of 60% acceptable RMSD value (less than 2 Å). The structures 3G9L (compatible with 32 ligands), 3TTJ (compatible with 32 ligands), 2EXC (compatible with 31 ligands), 4U79 (compatible with 31 ligands), 4W4W (compatible with 31 ligands), 4Z9L (compatible with 30 ligands), and 2WAJ (compatible with 30 ligands), which are usually not considered for docking studies due to their resolution being greater than 2 Å, showed nearly good results like the structures of 2B1P, 3FI2, and 3FI3. However, structures 4H3B and 4H36 had residues Thr216, Met220, and Thr221 in their active sites, visibly reducing the volume of the cavity and hence prohibiting the docking of many ligands into them. Thus, they showed docking with only one and four ligands of the total 44 ligands. Figure 7a,b shows the active sites of the best docked structure 2B1P and poorly docked structure 4H3B.

It is observed that the ligands 255 and J88 had the least mean RMSDs of 1.352 and 1.413 Å and ligands 3EL and FMY have the highest mean RMSDs of 3.382 and 3.275 Å,

respectively. The best resolution structure of 3OY1 (1.7 Å) in its cross docking results with 44 ligands deviated from the mean RMSD in 15 cases, whereas much poorer resolution structures like 3FI2, 3FI3, and 2B1P (having resolutions of 2.2, 2.28, and 1.9 Å, respectively) deviated from the mean RMSD only in 9, 7, and 11 out of the 44 cases, respectively. This was a crucial delegation that supported our case wherein the supposedly preferred structures like 3OY1 were surpassed by much poorer resolution structures such as 3FI2, 3FI3, and 2B1P.

The roles of the co-crystallized ligands of JNK3 were analyzed in depth to check if they contributed to the docking studies. The structurally similar ligands of JNK3 were grouped together like in the case of ligands JK1, JK2, 3H8, 3HJ, 3HN, 3HQ, and 3NL, which produced varying cross docking RMSD results though they were structurally alike (Figure 8a,b). Ligand JK1 exceeded its mean RMSD when docked with 4W4W, whereas ligand JK2 did not exceed its mean RMSD in all the six cross docking cases. Though the results of cross docking of ligands 3H8, 3HJ, 3HN, 3HQ, and 3NL varied significantly, they never exceeded the mean RMSD and were all below 2 Å (Supporting Information S4). The second group of structurally similar ligands of JNK3 includes J07, JNO, 3WH, B9K, and BGE (Figure 9a,b), which when redocked with their corresponding apo structures had RMSDs of 0.36, 1.4, 0.52, 1.69, and 1.36 Å, respectively. However, when these ligands were cross docked with the other ligands' apo structures, they showed intense variation in their cross docking RMSDs. Ligand J07, which when docked with 3CGO, 4X21, 6EMH, and 6EKD, had RMSDs of 1.77, 0.66, 4.17, and 2 Å, respectively. The ligand JNO when docked with 2P33, 4X21, 6EMH, and 6EKD had RMSDs of 1.02, 1.15, 1.36, and 1.26 Å,

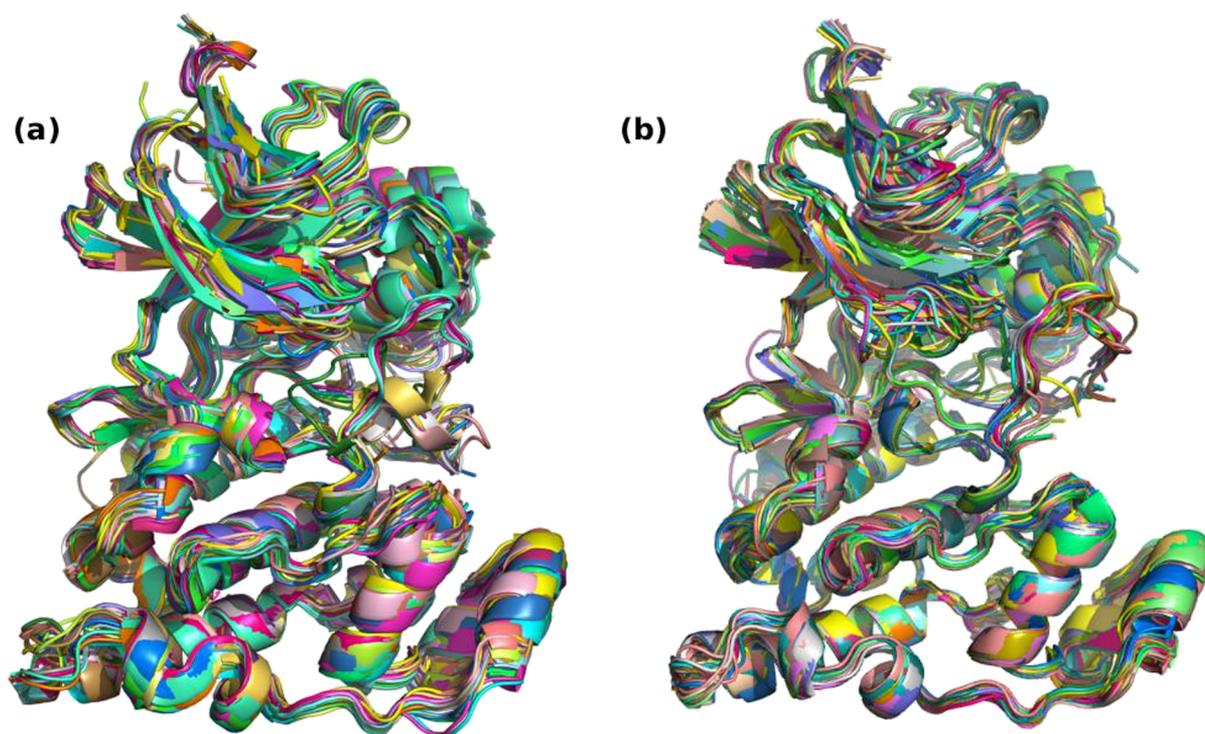


Figure 10. (a) 32 structures of JNK1 were superimposed onto reference structure 2XRW to set them all into same coordinates. (b) 51 structures of JNK3 were superimposed onto reference structure 2B1P to set them all into the same coordinates.

respectively. In both these cases, except for the docking between ligand J07 with 6EMH, the RMSD values did not exceed the mean RMSD values but still produced extensively varying results in docking. Similarly, ligand 3WH showed radical deviation from its mean RMSD of 3.113 Å when cross docked with structures 2P33, 3CGO, 6EMH, and 6EKD (having RMSD values of 5.53, 3.7, 3.89, and 1.91 Å, respectively). In contrast, ligands B9K and BGE did not exceed their mean RMSD values but still showed considerable variation in their cross docking RMSD values. This gives concrete evidence that ligand similarity does not affect the docking results in JNK3.

4. CONCLUSIONS

The success rate of docking of poor resolution structures in both JNK 1 and JNK3 was better than that of best resolution structures. Though all the structures of JNK1 and JNK3 had almost similar sequences and structures, they produced varied docking results. In the structures that produced poor docking results like 6F5E in JNK1 and 4H3B and 4H36 in JNK3, the orientation of few residues resulted in reduction in the size of the active site of the structure, thus potentially making the structure lose its binding affinity toward larger ligands. It was also proven that, in JNK1 and JNK3, the structural similarity of ligands did not influence the docking studies. Of all the available structures, choosing a structure of best resolution (below a threshold value of 2.2 Å) may lead to losing a potential candidate with higher docking capability. In the case of JNK1 and JNK3, it is proven that the threshold value does not hold good when it comes to docking studies and that resolution alone cannot be used as selection criteria for docking associated virtual screening studies. Our test case using JNKs is the first step to find a good experimental structure suitable for docking studies among the numerous

options available in various databases, and this work needs confirmation on other kinases and other protein systems.

5. COMPUTATIONAL METHODS

The docking procedure was carried out using AUTODOCK 4.2 using the Lamarckian genetic algorithm,^{18–20} and images were generated using PyMOL on a Linux environment.

5.1. Sampling and Scrutinizing the Data. The structures of JNK1 (32 structures) and JNK3 (51 structures) were downloaded from PDB and were sorted based on parameters like their resolution, ligand, number of protein chains, residue count, and author name (Supporting Information S5 and S6). With the help of the KLIFS database,²¹ the structures were also sorted based on the position of the DFG motif and its conformation (IN or OUT), activation loop, hinge region, gatekeeper residue, and the corresponding ligand's binding position (orthosteric or allosteric site) (Supporting Information S7 and S8). The DFG motif is also called the magnesium positioning loop because the Asp169 in JNK1 and Asp207 in JNK3 coordinate magnesium at the active site that occupies positions 169–171 of the activation loop in JNK1 and positions 207–209 in JNK3. The sequences of all the corresponding PDB structures of both JNKs were compared with the JNK1 sequence and JNK3 sequence from Uniprot (Uniprot ID - P45983 MK08_HUMAN and P53779 MK10_HUMAN) to find the missing residues in each case.

5.2. Preparing the Structures for Redocking and Cross Docking. Using PyMOL, all the 32 structures of JNK1 and 51 structures of JNK3 were superimposed onto a reference structure (2XRW for JNK1 and 3OY1 for JNK3) to set them all into the same coordinates (Figure 10a,b), and their ligands were extracted with the superimposed coordinates of the protein structures for the same purpose. In order to use every

ligand individually for docking, the complex structures were separated into their corresponding co-crystals and apo structures and saved separately.

5.3. Molecular Docking Protocol. The capability to correctly predict the ligand–protein interaction is fundamental to any reliable docking algorithm and the necessary starting point for any virtual screening protocol. AUTODOCK 4.2 performs automated computer simulated docking studies using grid-based energy evaluation in a docking technique, which was utilized for the study of binding modes of the potential ligands with the JNKs. Docking entails predicting the protein–ligand complex structure by sampling conformations of the ligand in the active site of the protein using Lamarckian genetic algorithm and is followed by scoring in order to rank the compounds. The docking parameters were set as default, and the numbers of GA runs were limited to 10. The co-crystals were redocked into their apo structures to validate using AUTODOCK 4.2;²² they were also cross docked into the remaining structures, and the results like binding energy, RMSD value, and mean RMSD were noted. To ease the protocol of docking multitudinous structures, a loop program was created using the shell script to generate the grid and dock log files (Supporting Information S9). The whole process of docking was fully automated using various programs developed in-house. This was done for 20 ligands of JNK1 and 47 ligands of JNK3. Also, their rotatable bonds were adjusted, and ligands of two structures of JNK1 (3O2M and 3VUM) were not considered as their ligands bound to the allosteric site, which poses a complication during docking.^{23,24} In JNK3, three structures (3V6R, 3V6S, and 4Z9L) were not included in the docking studies because their ligands were too flexible (no. of rotatable bonds was more) to be considered for docking as it could be a limitation in any docking protocol. The roles of co-crystallized ligands of JNK1 and JNK3 were analyzed by grouping together the structurally similar ligands and analyzing their docking performances with the various structures to check if the ligand study has an imperative contribution toward the docking studies.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.9b03458>.

- S1 Redocking analysis of JNK1 (XLSX)
- S2 Cross docking analysis on JNK1 with mean RMSD (in Å) (XLSX)
- S3 Redocking analysis of JNK3 (XLSX)
- S4 Cross docking analysis on JNK3 with mean RMSD (in Å) (XLSX)
- S5 Sampling JNK1 data (based on parameters like resolution, ligand, number of protein chains, residue count, and author name) (XLSX)
- S6 Sampling JNK3 data (based on parameters like resolution, ligand, number of protein chains, residue count, and author name) (XLSX)
- S7 Scrutinizing JNK1 data (based on the position of DFG motif and its conformation (IN or OUT), activation loop, hinge region, gatekeeper residue, and the corresponding ligand's binding position (orthosteric or allosteric site)) (XLSX)
- S8 Scrutinizing JNK3 data (based on the position of DFG motif and its conformation (IN or OUT),

activation loop, hinge region, gatekeeper residue, and the corresponding ligand's binding position (orthosteric or allosteric site)) (XLSX)

S9 Loop program created using shell script to generate the grid and dock log files (PDF)

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Notes

The authors declare no competing financial interest.

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