33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

On modeling peptidomimetics in complex with the SH2 domain of Stat3

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Abstract— **Signal transducer and activator of transcription 3 (Stat3) plays a role in human cancers. One of the main approaches towards inhibiting its activity is the development of phosphopetides or peptidomimetics that competitively bind to the SH2 domain of Stat3. This work reports, to the best of our knowledge, the first computational molecular docking study to model all of the 142 peptidomimetics that mimic the Stat3 inhibitory pTyr-X-X-Glu motif. We used the docking programs AUTODOCK and VINA to model SH2 domain-peptidomimetic complexes and estimate their binding affinities. We obtained better screening accuracy using AUTODOCK which ranked the most potent inhibitor as second highest. Experimental binding energy values and scores from docking programs correlated poorly, confirming the limitations of many current docking programs when dealing with ligands that have a large number of rotatable bonds. Nevertheless, for close to 65% of peptidomimetics, the structures of complexes computed by AUTODOCK are in agreement with current understanding of the structures. Modeling of the SH2 domain-peptidomimetic complexes is essential to better understand and design drug compounds for curing cancer. Our study is an important first step forward towards that goal.**

I. INTRODUCTION

According to the World Health Organization, cancer is the leading cause of human death worldwide [1]. Design of drugs for curing human cancers is, thus, a major goal for the medicinal research community as well as the pharmaceutical companies. Signal transducer and activator of transcription 3 (Stat3) is a target for drug design as it is constitutively activated in human cancers such as breast cancer, lung cancer, multiple myeloma, leukemia and others [2], [3]. Upon extracellular signaling, Stat3 (inside the cell) is recruited to interleukin-6 or growth factor receptors via its SH2 domain where it is phosphorylated on Tyr705. The phosphorylated Tyr705 (pTyr705) then interacts with the SH2 domain of another Stat3 leading to dimer formation. Subsequently the dimer is translocated to the nucleus, resulting in the expression of cancer-associated genes. Blocking the SH2 domain to prevent the dimerization is an attractive strategy for designing drugs that inhibit Stat3 activity [4].

Computational molecular docking plays an important role in the drug discovery process. It is widely accepted that docking computations provide invaluable understanding of the interactions between the target protein and putative drug candidates (ligands) [5]. Medicinal chemists use this information to design new drug compounds or refine compounds in their existing drug discovery pipeline. A typical molecular docking program computes the preferred pose of the ligand when it binds to the target protein to form a stable protein-ligand complex. It also computes a score that estimates the binding affinity, i.e., a measure of how well the ligand binds to the target. The binding affinity estimates help identify the more potent putative drug compounds out of large sets of ligands. There are a plethora of commercial and non-commercial docking programs available, each of which differ mainly on the strategy used for exploring the poses of the ligand and the scoring function. Some of the major programs are DOCK [6], ICM [7], GOLD [8], FlexX [9], AUTODOCK [10], SURFLEX [11], and VINA [12].

In this paper, we describe a computational molecular docking study to model SH2 domain-peptidomimetic complexes and estimate binding affinities so that we can identify the most potent ligand. We have chosen 142 phosphopeptide analogs that mimic Stat3 inhibitory pTyr-X-X-Glu motif. This choice is due to two reasons: (1) the 142 peptidomimetics form a complete set of compounds that mimic a particular motif, and (2) we have access to a laboratory that is working on synthesizing such compounds. To our knowledge, there is no known experimental (X-ray Crystallography or Nuclear Magnetic Resonance) structure available for any of the SH2 domain-peptidomimetic complexes. Our docking study employed AUTODOCK [10] and VINA [12], two of the most popular non-commercial programs.

II. METHODS

Our molecular docking study was performed on the SH2 domain of Stat3 and a set of 142 peptidomimetics using AUTODOCK (version 4.2) and VINA (version 1.1.1). A typical docking study requires three computational steps before running the docking program: (1) preparation of the receptor, (2) preparation of the ligand, and (3) setup of the parameters of the docking program(s). The following subsections describe these three steps in detail.

A. Receptor preparation

The three-dimensional structure of Stat3 was obtained from the Protein Data Bank (PDB ID 1BG1). The structure contains residues 136 to 716 of Stat3, half a DNA duplex, and 127 water molecules per asymmetric unit [13]. For preparing the receptor, we used residues 586 to 688 that form the SH2 domain (Fig. 1). The water molecules and the DNA duplex were ignored. The receptor was subsequently prepared using a python script called *prepare_receptor.py* from the MGLTools package (version 1.5.4) [14]. The script adds hydrogen atoms in the receptor structure and then adds

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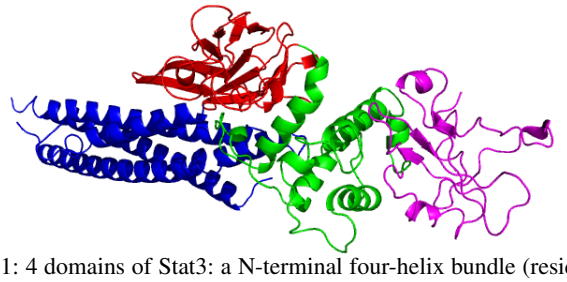


Fig. 1: 4 domains of Stat3: a N-terminal four-helix bundle (residues 138-320, blue), an eight-stranded β-barrel (residues 321-465, red), an α-helical connector domain (residues 466-585, green), and a SH2 domain (residues 586 to 688, magenta).

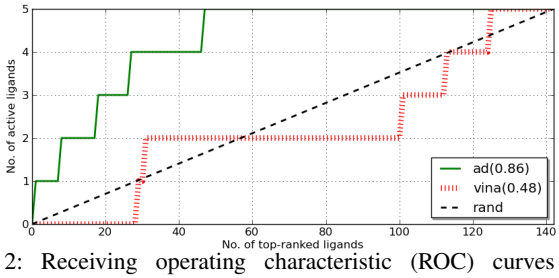


Fig. 2: Receiving operating characteristic (ROC) curves for AUTODOCK (green) and VINA (red). The dashed line represents ROC curve when ligands are randomly ranked. The area under curve (AUC) values are displayed in the legend.

gastegier charges to all the atoms. All non-polar hydrogens are removed and the charge of each removed hydrogen is added to the carbon to which it is bonded.

B. Ligand preparation

The ligands for the docking study were obtained from a comprehensive literature survey ([15], [16], [17], [18], [19]). Along with the 2-D (chemical) representations of the phosphopeptide analogs, binding affinity (IC_{50}) values, obtained from fluorescence polarization experiments, were provided. The molecule builder of Maestro software (version 9.1) [20] was used for generating the 3-D structures from the 2-D representations. The obtained 3-D structures of the ligands were energy minimized in vacuum using the *Clean Up Geometry* module of Maestro. Each ligand is subsequently prepared using a python script called *prepare_ligand4.py* from the MGLTools package. Hydrogen atoms were treated in a fashion similar to that explained in Section II-A. The script also identifies non-amide rotational bonds in the ligand. The ligands in our docking study have on an average 15 rotational bonds, which is substantially larger than the number of rotational bonds in small molecules generally used for docking studies. As a consequence, this study is a prime example of issues that arise due to large numbers of rotational bonds in ligands. It provides a thorough analysis of the docking results when current docking programs are applied to a problem involving large ligands. Thus, this study sets a benchmark for the development of new docking programs that attempt to model SH2-domain peptidomimetic complexes and, in general, address current limitations of docking ligands with large number of rotational bonds.

C. Docking program setup

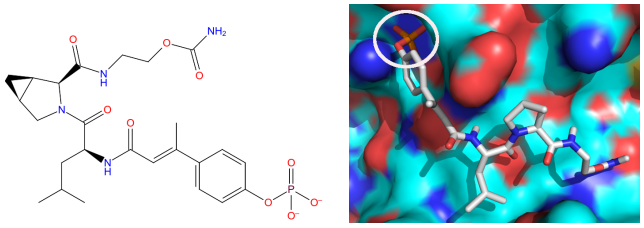
Both AUTODOCK and VINA use the same input format for receptor and ligand structures which are obtained from the preparation steps described above. All the parameters of VINA were set to their default values. In AUTODOCK, the number of energy evaluations to be performed (*ga_num_evals*) was set to 25 million and the number of docked poses to be computed (*ga_run*) was set to 50, which is standard practice. All other parameters were set to the default values. Both programs use a rectangular 3-dimensional grid for specifying the binding site of the receptor as well as for efficient evaluation of the scoring function. The grid is centered on coordinates $x = -5.22\mathring{A}, y = -1.37\mathring{A}, z = -0.43\mathring{A}$. The dimensions of the AUTODOCK grid are $54 \times 42 \times 54$ with a grid spacing of $0.375\mathring{A}$. The dimensions of the VINA grid are identical.

III. RESULTS

Each docking run with AUTODOCK produced 50 poses of the ligand while VINA produced 9 poses. Each pose is defined by the position and orientation of the ligand and its rotational bonds. We evaluated the results from the docking study in the following three ways: (1) screening accuracy, (2) comparison of the experimental binding affinities and docking program scores, and (3) structure analysis of the docked complex.

A. Screening accuracy

An important criterion for the success of docking programs is their screening accuracy. Though there are many other useful criteria, our goal in this study is not the evaluation of docking programs. Rather our aim is to model the SH2 domain-peptidomimetic complexes using popular docking programs and screen the most potent inhibitors. Area under the receiving operating characteristic curve is an important statistic to evaluate screening accuracy [21]. In general, given a set of known active and decoy ligands, the number of active ligands found among the top-n ranked ligands is plotted against n, where n is the size of the set. This plot is known as the receiver operating characteristic (ROC) curve and the area under the curve (AUC) is given as a fraction of the total plot area. Our docking study is not a typical study with active and decoy ligands. Therefore for computing AUC, we assume that the top 5 most potent (lowest IC_{50} values) inhibitors are the active ligands that are to be screened from the set of 142 ligands. Figure 2 shows the ROC curves obtained from docking of the ligands with AUTODOCK and VINA. AUTODOCK (AUC=0.86) performed much better than VINA (AUC=0.48) which performed worse than a screening process that randomly ranks ligands (AUC=0.50). AUTODOCK was able to find the 5 active ligands in the top 47 ligands ranked according to decreasing AUTODOCK scores. The best scoring pose obtained for each ligand was used for ranking. Interestingly, the most potent inhibitor with $IC_{50} = 39nm$ (Fig. 3(a)) was ranked second highest by AUTODOCK.



(a) (b)

Fig. 3: Most potent (lowest IC_{50} value) peptidomimetic: (a) 2-D representation and (b) 3-D structure of complex modeled by AUTODOCK. The SH2 domain is shown as a surface and the peptidomimetic is shown with sticks. The phosphate group (white circle) of the peptidomimetic fits in its known binding pocket.

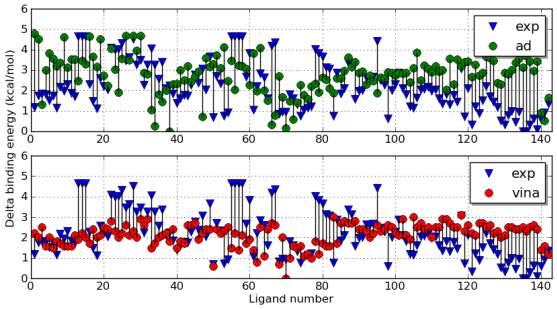


Fig. 4: Comparison of difference scores from AUTODOCK (green) and VINA (red), and difference binding energies (blue) from fluorescence polarization experiments.

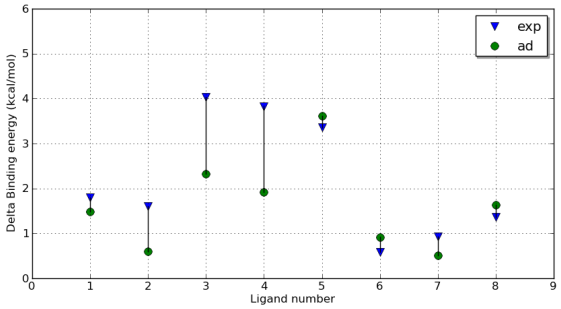


Fig. 5: Comparison of difference scores from AUTODOCK (green)and difference binding energies (blue) from fluorescence polarization experiments. Only the ligands that have 10 or fewer rotational bonds were considered. Comparison of scores from VINA results in a similar figure.

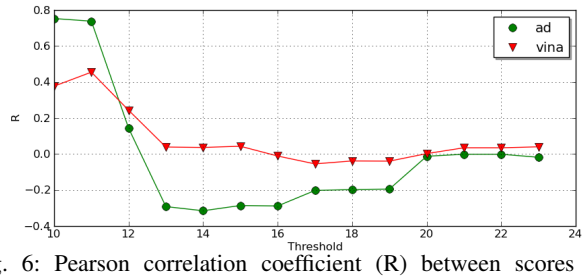


Fig. 6: Pearson correlation coefficient (R) between scores and binding energies is plotted against threshold on the number of rotatable bonds. The scores were obtained from AUTODOCK (green) and VINA (red).

B. Binding affinity comparison

Binding affinity gives a measure of the thermodynamic stability of a SH2 domain-peptidomimetic complex. We collected binding affinity (IC_{50}) values for the 142 peptidomimetics from the literature survey (section II-B). The IC_{50} value gives the concentration of the peptidomimetic that is required to competitively inhibit the binding of FAM-Ala-pTyr-Leu-Pro-Gln-Thr-Val-NH_2 (FAM=5-carboxyfluorescein) to Stat3 by 50% [15]. Since scoring functions of AUTODOCK and VINA give binding affinity estimates in terms of energy values (in kcal/mol), we convert IC_{50} values to energy values using the following equation:

$$\Delta G = RTln(IC_{50}/1.066) \quad (1)$$

where, $R = 1.986 \times 10^{-3} kcal K^{-1} mol^{-1}$, $T = 298K$, and IC_{50} is in nanomolar.

Figure 4 compares scores of the lowest scoring poses from the docking programs and binding energy values from (1). It is assumed that in general there is an offset between scores from the docking programs and binding energy values. To eliminate the offset, we compared difference scores with difference energies. Each score/energy was subtracted by the lowest score/energy value among all ligands. The comparison shows that scores and binding energies do not correlate well. This is not surprising because, as mentioned in section II-B, the ligands have on an average 15 rotational bonds. It is generally understood that docking programs are more accurate when docking smaller ligands ($\leq$ 10 rotational bonds) which is reflected in Figure 5. To investigate this further, we plotted (see Figure 6) the variation of Pearson correlation coefficient (R) between the scores and the binding energies with increasing threshold on the number of rotatable bonds. At each threshold value, only ligands that have number of rotatable bonds less than or equal to the value were considered for computing R. The plot shows high correlation for the smaller ligands and poor correlation for the larger ones.

C. Structure analysis

There is very little knowledge regarding the structures of SH2 domain-peptidomimetic complexes and modeling these structures is one of our main goals. From crystallography data and the relatively few modeling studies on a couple of peptidomimetics ([13], [4], [17]), it is clear that the phosphate group in the peptidomimetics sits in the *binding pocket* formed by residues Lys591, Arg609, Ser611, Glu612, and Ser613. For the most potent inhibitor, that is indeed the case (Fig. 3(b)). We computed the Euclidean distance (D) of the phosphorus atom in the phosphate group of the lowest scoring pose of each ligand from coordinates $x = -8.42\mathring{A}, y = 4.50\mathring{A}, z = -6.09\mathring{A}$. These coordinates correspond to the location of the phosphorus atom in the above *binding pocket* as proposed in [4]. For a given threshold (D_{thresh}), we calculated the fraction of the total number of ligands that have $D <= D_{thresh}$. Figure 7 plots the fraction against varying values of the distance threshold. The plot shows that poses generated by AUTODOCK are much better than the poses generated by VINA when evaluating

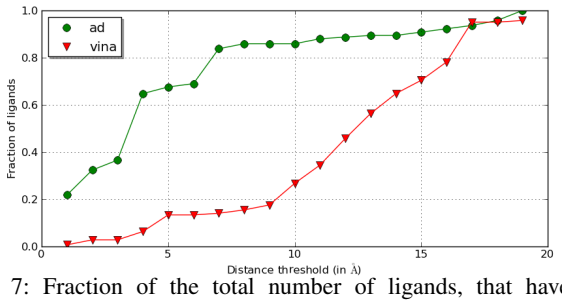


Fig. 7: Fraction of the total number of ligands, that have the phosphate group of the lowest scoring pose in or near the known binding pocket, is plotted against the distance threshold. The poses were obtained from AUTODOCK (green) and VINA (red).

the fit of the phosphate group to its known binding pocket. Interestingly, in approximately 65% of the ligands poses computed by AUTODOCK, the phosphate group of the lowest scoring pose fits within $4.0\mathring{A}$ of the binding pocket. This is an important outcome of our study as it shows that even with the current limitations of docking programs (Section III-B), we are able to model many SH2 domain-peptidomimetic complexes in agreement with the existing observations about the complexes.

IV. DISCUSSION

We described, to our best knowledge, the first comprehensive study on modeling the SH2 domain of Stat3 (a cancer target) in complex with a set of phosphopeptide mimics. We used the popular docking programs AUTODOCK and VINA for modeling the complexes. AUTODOCK computed more accurate ligand poses as compared to VINA. AUC values were higher for AUTODOCK and it found the most potent inhibitor in the second highest ranked ligand. The experimentally derived binding energy values did not correlate well with the scores from the docking program and this was due to the known limitations of docking programs in dealing with ligands with large number of rotational bonds. However, AUTODOCK computed reasonable binding poses, which were in agreement with the existing knowledge on the structure of SH2 domain-peptidomimetic complexes, for approximately 65% of ligands. To model the complexes more accurately, new docking programs will have to be developed that are capable of accurately docking ligands with large number of rotational bonds. New docking programs might have to account for the flexibility of the SH2 domain as the binding pocket is surrounded by loops. Limitations of docking programs notwithstanding, this study sets a good starting point for the modeling of SH2 domain-peptidomimetic complexes.

V. ACKNOWLEDGMENTS

This work has been supported in part by a training fellowship from the Keck Center Computational Cancer Biology Training Program of the Gulf Coast Consortia (CPRIT RP101489), The Texas Higher Education Cooordinating Board (NHARP 01907), The National Cancer Institute (RO1 CA096652), and The John and Ann Doerr Fund for Computational Biomedicine at Rice University.

REFERENCES

[1] [Online]. Available: http://www.who.int/mediacentre/factsheets/fs297/en/index.html
[2] D. E. Levy and J. E. Darnell, "Stats: transcriptional control and biological impact." *Nature reviews. Molecular cell biology*, vol. 3, no. 9, pp. 651–62, Sept. 2002.
[3] H. Yu and R. Jove, "The STATs of cancer–new molecular targets come of age." *Nature reviews. Cancer*, vol. 4, no. 2, pp. 97–105, Feb. 2004.
[4] J. S. Mcmurray, "Structural basis for the binding of high affinity phosphpeptides to Stat3," *Biopolymers*, vol. 90, no. 1, 2007.
[5] G. Schneider, "Virtual screening: an endless staircase?" *Nature reviews. Drug discovery*, vol. 9, no. 4, pp. 273–6, Apr. 2010.
[6] I. Kuntz, "A geometric approach to macromolecule-ligand interactions," *Journal of Molecular Biology*, vol. 161, no. 2, pp. 269–288, Oct. 1982.
[7] R. Abagyan, M. Totrov, and D. Kuznetsov, "ICM-A new method for protein modeling and design: Applications to docking and structure prediction from the distorted native conformation," *Journal of Computational Chemistry*, vol. 15, no. 5, pp. 488–506, May 1994.
[8] G. Jones, P. Willett, and R. C. Glen, "Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation." *Journal of molecular biology*, vol. 245, no. 1, pp. 43–53, Jan. 1995.
[9] M. Rarey, B. Kramer, T. Lengauer, and G. Klebe, "A fast flexible docking method using an incremental construction algorithm." *Journal of molecular biology*, vol. 261, no. 3, pp. 470–89, Aug. 1996.
[10] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, and A. J. Olson, "Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function," *Journal of Computational Chemistry*, vol. 19, no. 14, pp. 1639–1662, Nov. 1998.
[11] A. N. Jain, "Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine." *Journal of medicinal chemistry*, vol. 46, no. 4, pp. 499–511, Feb. 2003.
[12] O. Trott and A. J. Olson, "Software News and Update AutoDock Vina : Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading," *Journal of Computational Chemistry*, 2009.
[13] S. Becker, B. Groner, and C. W. Müller, "Three-dimensional structure of the Stat3β homodimer bound to DNA." *Nature*, vol. 394, no. 6689, pp. 145–51, July 1998.
[14] M. F. Sanner, "Python: a programming language for software integration and development." *Journal of molecular graphics & modelling*, vol. 17, no. 1, pp. 57–61, Feb. 1999.
[15] D. R. Coleman, Z. Ren, P. K. Mandal, A. G. Cameron, G. a. Dyer, S. Muranjan, M. Campbell, X. Chen, and J. S. McMurray, "Investigation of the binding determinants of phosphopeptides targeted to the SRC homology 2 domain of the signal transducer and activator of transcription 3. Development of a high-affinity peptide inhibitor." *Journal of medicinal chemistry*, vol. 48, no. 21, pp. 6661–70, Oct. 2005.
[16] D. Coleman, K. Kaluarachchi, Z. Ren, X. Chen, and J. McMurray, "Solid phase synthesis of phosphopeptides incorporating 2,2-dimethyloxazolidine pseudoproline analogs: Evidence for trans leu-pro peptide bonds in stat3 inhibitors," *International Journal of Peptide Research and Therapeutics*, vol. 14, pp. 1–9, 2008.
[17] P. K. Mandal, D. Limbrick, D. R. Coleman, G. a. Dyer, Z. Ren, J. S. Birtwistle, C. Xiong, X. Chen, J. M. Briggs, and J. S. McMurray, "Conformationally constrained peptidomimetic inhibitors of signal transducer and activator of transcription 3: Evaluation and molecular modeling." *Journal of medicinal chemistry*, vol. 52, no. 8, pp. 2429–42, Apr. 2009.
[18] P. K. Mandal, Z. Ren, X. Chen, C. Xiong, and J. S. McMurray, "Structure-affinity relationships of glutamine mimics incorporated into phosphopeptides targeted to the SH2 domain of signal transducer and activator of transcription 3." *Journal of medicinal chemistry*, vol. 52, no. 19, pp. 6126–41, Oct. 2009.
[19] P. K. Mandal, F. Gao, Z. Lu, Z. Ren, R. Ramesh, J. S. Birtwistle, K. Kaluarachchi, X. Chen, R. C. Bast Jr., and M. J. S. Liao, W S, "Potent and Selective Phosphopeptide Mimetic Prodrugs Targeted to the Src Homology 2 (SH2) Domain of Signal Transducer and Activator of Transcription 3," Manuscript submitted.
[20] [Online]. Available: http://www.schrodinger.com/products/14/12/
[21] M. W. Chang, C. Ayeni, S. Breuer, and B. E. Torbett, "Virtual screening for HIV protease inhibitors: a comparison of AutoDock 4 and Vina." *PloS one*, vol. 5, no. 8, pp. 1–9, Jan. 2010.