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Computational analyses of JAK1 kinase domain: Subtle changes in the catalytic cleft influence inhibitor specificity

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ABSTRACT

The Janus kinases (JAKs) are a family of intracellular non-receptor tyrosine kinases which transmit signals by phosphorylation of downstream substrates. A myriad of cytokines can trigger the JAK-STAT pathway which influences immune response, embryonic development, and cellular transformation. Here, we built a comparative model for Jak1 based on the crystal structure of Jak2 (PDB code:2B7A) and Jak3 (PDB code: 1YVJ) using the InsightII package. 3D-Profile and stereochemical analysis further verified the validity of the proposed structure. Adenosine 5′-triphosphate (ATP) was then docked into its catalytic cleft. Although the shape of Jak1 kinase cleft is fairly similar to that of Jak3, we observed minute changes in the key residues of the binding interface which influenced the docking of a specific Jak3 inhibitor, WHI-P131. Superimposition of the interface residues suggested that substitution of Asp 99 (Jak3) into Glu 101 (Jak1) generated steric hindrance and a Tyr 91 to Phe 93 switch altered the shape of catalytic cleft which collectively prohibited the inhibitor binding. Furthermore, in-silico mutagenesis of these two residues back to Asp and Tyr enabled Jak1 to accommodate WHI-P131. These results may provide clues for the design and optimization of selective kinase inhibitors.

The Janus kinases (JAKs) are a family of receptor-associated tyrosine kinases and play important roles in numerous cytokine and growth factor signaling pathways [1]. JAKs constitutively bind to cytoplasmic region of cytokine receptors in the absence of stimulation. Following ligand interaction with its cognate receptor, signal transducers, and activator of transcription (STATs) were phosphorylated by members of Jak family. Activated STATs then dimerize and translocate into the nucleus and promote specific transcription programs [2–4]. In mammals, the JAK family has four members, Jak1, Jak2, Jak3, and Tyk2 each with over 1000 amino acids. These kinases are composed of an N-terminal receptor binding domain (also called FERM domain), a SH2-like domain, a pseudokinase domain important for regulating kinase activity and a carboxyl kinase domain. The functions of JAKs have been extensively studied. Jak1 is involved in IL-2R family, IL-4R family, gp130 receptor family and class II receptor family; Jak2 null mice exhibited embryonic lethality due to failure of erythropoiesis but it is also involved in IL-3, IFN-γ, and GM-CSF signaling [5]. The expression of Jak3 is confined in hematopoietic cells. It binds to common receptor gamma chain γC which associates with IL-2R, IL-4R, IL-7 etc. and regulate lymphoid development [6]. Jak3 has been shown to be highly expressed in most cases of acute lymphoblastic leukemia (ALL) and Jak3 specific inhibitors have been designed for treatment of leukemia [7], immediate hypersensitivity reactions [8] and transplant rejection [9].

Protein phosphorylation is the most common type of post-transcriptional regulation and influences a wide range of biological activities. The design of potent inhibitors for specific kinases has been proved to be an effective solution for various kinds of diseases [10]. Although a plethora of gene and protein sequences have been obtained owing to advances in molecular biology, we still lack accurate structures of proteins for drug design and detailed functional analyses.

Jak1 is a pleiotropic kinase involved in type I and II IFN signaling, IL-7 signaling etc. The absence of Jak1 leads to impaired T cell and B cell production and profound defect in response to IFNs [5]. An accurate 3D model could facilitate understanding in its substrate specificity and kinase–receptor interaction. Here, a comparative modeling method is used to provide a high-quality Jak1 structure of kinase domain (KD) based on the X-ray diffraction structures of Jak2 (2B7A) and Jak3 (1YVJ). The binding mode of ATP with Jak1 KD was investigated by protein–ligand docking simulations. Comparison between Jak1 and Jak3 catalytic core revealed key residues responsible for specificity of WHI-P131, a Jak3 selective inhibitor. These results provide important information on the characteristics of Jak1 KD and offer a starting point for further drug development and biochemical analysis.

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Materials and methods

Homology modeling, molecular simulations and docking studies were performed on a SGI Tezro workstation using InsightII 2000 software package. The reference sequence of Jak1 (NP_002218) was obtained from the National center for biotechnology information (NCBI). The consistent valence forcefield (CVFF) and the Discover 3 simulation module were used for energy minimization and flexible docking. We searched Jak1 homologous entries in the protein data bank (www.rcsb.org) by PSI-BLAST (www.ncbi.nlm.nih.gov/blast/). The PSI-BLAST alignment was further refined by using *structural alignment* in the *Homology* module. This alignment was used for comparative modeling implemented in the *Modeler* module which generates structures by applying spatial restraints and molecular dynamics refinement [11]. After the initial coordinate was optimized by loop refinement, molecular mechanics minimization (steepest descent and conjugate gradient minimizations until the model reached 0.01 kcal/mol Å convergence) was also employed to remove unreasonable regions. The quality of the model was assessed by *Procheck*, *profile-3D* and *Prostat*.

Substrate docking was performed using the *Affinity* module in the InsightII package. The structures of ATP and WHI-P131 were generated and automatically optimized in the *builder* module. Substrate or inhibitors were placed into the catalytic groove; residues within 5 Å of the small molecule were defined as flexible. We first generated 50 structures with Monte Carlo search, the non-bonded method is Quartic VDW (van der waals) with coulombic interactions off and VDW scaled down to 0.1. Simulated annealing was subsequently used to further refine the docking results using cell multipole method, VDW and coulombic scales were 0.1 and finally brought to 1, a brief minimization step was used in the end of the run. All other settings were set to default. Ten complexes were generated in each docking run according to the energy ranking. These poses were evaluated and selected based on the interaction energy, hydrogen bonds, RMSD of the ligand and existing knowledge about the kinase fold. The interaction energy between each residue and inhibitor was measured by using the *evaluate-intermolecular* command in the *Affinity* module with 100 Å cutoff.

Results

A homology model for Jak1 kinase domain

To find homologous proteins in the protein data bank, PSI-BLAST [12], a profile–sequence comparison method was used. Jak2 (2B7A) and Jak3 (1YVJ) were found to be 52.5% and 56.1% identical to Jak1 KD, respectively. The localization of the gaps was further optimized (Fig. 1) after structural alignment. We used *Modeler* to generate homology structures and optimized them by a brief energy minimization in order to remove energy strains in the system.

The proposed Jak1 KD structures were evaluated by the *Prostat* and *Profile-3D* module in the InsightII suite and the Procheck program [13]. The final model was selected based on scores in these evaluations. Prostat checks bond lengths and bond angles for deviation from experimental data. Ramachandran plots were generated by Procheck which examines the stereochemical property of the model. As shown in Table 1, our Jak1 KD model satisfied the requirement of these criteria with 91.4% of the Φ–ψ angles in the favored area and none in the disallowed regions. Only one bond length was over 3-fold deviation from average length. Profile-3D employs a scoring method to evaluate the validity of the fold according to compatibility of sequence into the environment of the residue. Almost all the residues in the scoring plot are above zero which suggested a reasonable folding (Fig. 3). The profile-3D score is close to the expectation score as shown in Table 1 and comparable to those of Jak2 and Jak3.

The structure of Jak1 KD retained the key features of protein kinases [14,15] with a N-terminal lobe composed mainly of β-sheets and a C-terminal lobe of α-helices. These two lobes form a catalytic cleft which catalyzes ATP into ADP and transfer the γ-phosphate to tyrosine residues of substrate proteins. The backbone RMSDs between Jak1–Jak2 and Jak1–Jak3 are 1.127 Å and 0.967 Å, respectively, which show close resemblance to these two homologs.

Computational docking of ATP into Jak1 catalytic pocket

To investigate the detailed interaction mode of ATP, the natural substrate, with Jak1, we used a flexible docking approach which allows the interface residues and the ligand to move in the conformation search. The interface residues include: Leu16-Gly19, Val24, Ala41, and Glu91-Leu94 in β-sheets of the N-lobe, Val73, and Ser98 in turns, Arg142-Asp156 in β-sheet of the C-lobe. After several runs of Monte Carlo search and simulated annealing, we obtained an interaction conformation as shown in Fig. 2. The interaction energy is −138.44 kcal/mol which is composed of −40.81 kcal/mol Vdw energy and −97.63 kcal/mol electrostatic energy. The adenosine moiety is masked by the hydrophobic cleft and the phosphate extends toward the solvent and forms the majority of the hydrogen bonds. As shown in Table 2 and Fig. 2B, the three phosphates forms five hydrogen bonds with Glu18 and Arg142; the amino group in purine base forms a hydrogen bond with side-chain carboxyl group of Glu92 and the hydroxyl group in the ribose forms a hydrogen bond with side-chain carboxyl group of Asp156.

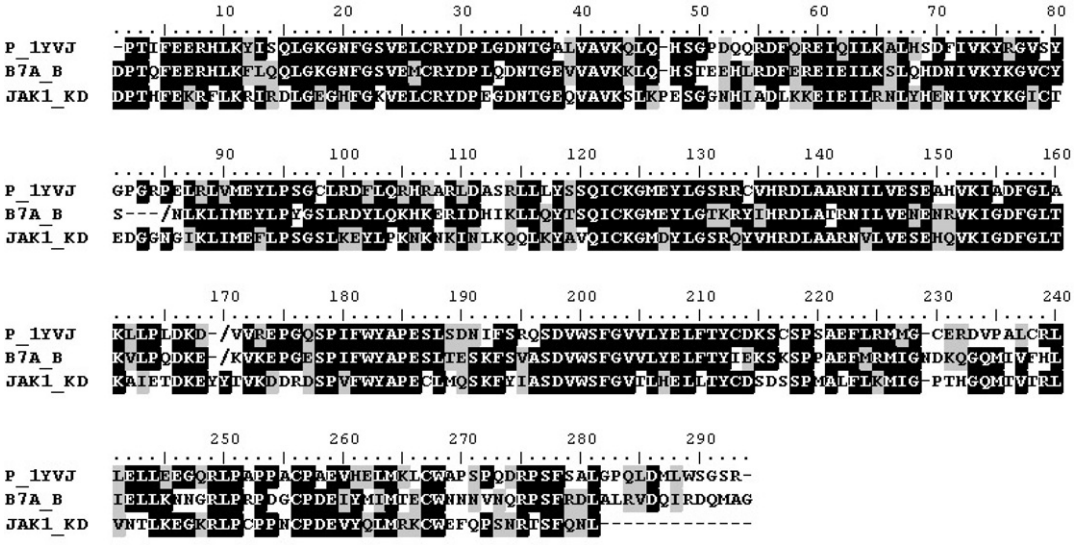

Fig. 1. Sequence alignment of Jak1 KD with Jak2 (B7A_B) and Jak3 (P_1YVJ).

Table 1
Dihedral angles, bond length and profile-3D scores of Jak1 compared with template structures

Target protein	Jak2 (2B7A)	Jak3 (1YVJ)	Jak1
% Φ–ψ Angles in favored region	91.2%	85.4%	91.4%
% Φ–ψ Angles in additional allowed region	8.2%	13.0%	7.0%
% Φ–ψ Angles in generously allowed region	0.4%	0.8%	1.6%
% Φ–ψ Angles in disallowed region	0.4%	0.8%	0
Number of bond lengths with >3-fold deviation	0	0	1
Profile-3D score (score/expected)	141.96/ 130.55	130.99/ 131.01	117.74/ 127.34

Table 2
Hydrogen bonds between ATP and Jak1

Donor	Acceptor	Distance (Å)	Angle (°)
Glu18:NH	ATP:β-phosphate:O11	2.46	151.18
ATP:γ-phosphate:H47	Glu18:OE1	1.53	167.31
ATP:β-phosphate:H45	Glu18:O	1.48	173.55
ATP:γ-phosphate:H46	Glu18:O	1.58	160.88
ATP:purine NH	Glu92:O	2.14	157.97
Arg142:HE	ATP:α-phosphate:O10	2.11	164.27
Arg142:HH22	ATP:β-phosphate:O13	2.05	142.24
ATP:ribose:OH	Asp156:OD2	1.38	174.64

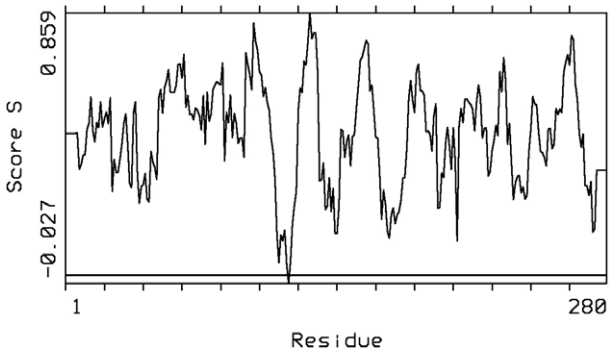

Fig. 3. Profile-3D plot of Jak1 KD model.

Subtle changes in Jak1 catalytic pocket render it unaffected by WHI-P131

Although Jak1 shares high percentage of homology with other Jak family members, differences still exist in the binding region which is critical for the design of specific kinase inhibitors. Extensive investigations have been undertaken to select specific inhibitors for Jak3 because of its strict hematopoietic expression and potential application in leukemia and immunosuppressive therapy [16,17]. WHI-P131 [4-(49-hydroxyphenyl)-amino-6,7-dimethoxyquinazoline] is among the earliest Jak3 inhibitors and showed a modest potency (IC50 78 μM) without significant effect on Jak1 (Fig. 4A) [7].

Our docking simulations confirmed that WHI-P131 could fit into Jak3 catalytic groove (Fig. 4A). Two hydrogen bonds were formed between the inhibitor hydroxyl group and Asp 154, the key residue in the "DFG motif" which is critical for enzymatic activity [14]. However, no hydrogen bond was observed between the ring nitrogen and the main-chain NH of Leu 92 as described [7]. We calculated the contribution of key residues in the binding pocket to the interaction energy with WHI-P131 (Table 3) and found that Asp 99, a key residue in the first helix in the C-lobe, provides a high electrostatic energy by interacting with two methyl ether groups.

To answer why WHI-P131 is specific to Jak3, we attempted to dock this molecule into Jak1 but failed to obtain the desired conformation in repeated runs. Thus, we made a scrutiny into these two catalytic regions. By superimposing the binding pockets of the Jak3-inhibitor complex and Jak1 and measuring the interaction energy between Jak1 and WHI-P131, we observed that it is the high Vdw repulsion energy (421.13 kcal/mol) that keeps WHI-P131 away (Fig. 4C). Analysis of energy contribution of each residue revealed that two residues constitute the major force of repulsion. The Asp 99 of Jak3 is replaced by Glu 101 in Jak1, the longer side-chain length generated huge steric hindrance (421.56 kcal/mol Vdw repulsion); in addition, Tyr 91 in Jak3 becomes Phe 93 in Jak1, the hydrophobic nature of Phenylalanine causes the backbone to move inward which decreases the size of the pocket and caused 30.94 kcal/mol Vdw repulsion energy.

To test the validity of our hypothesis, we made a double mutant of Jak1 in which Glu 101 and Phe 93 are mutated to Asp and Tyr by employing the *build mutant* function of *Modeler*. Indeed, the mutated Jak1 can accommodate WHI-P131 in a pose similar to that in Jak3 (Fig. 4C and D) although the two rings of WHI-P131 was

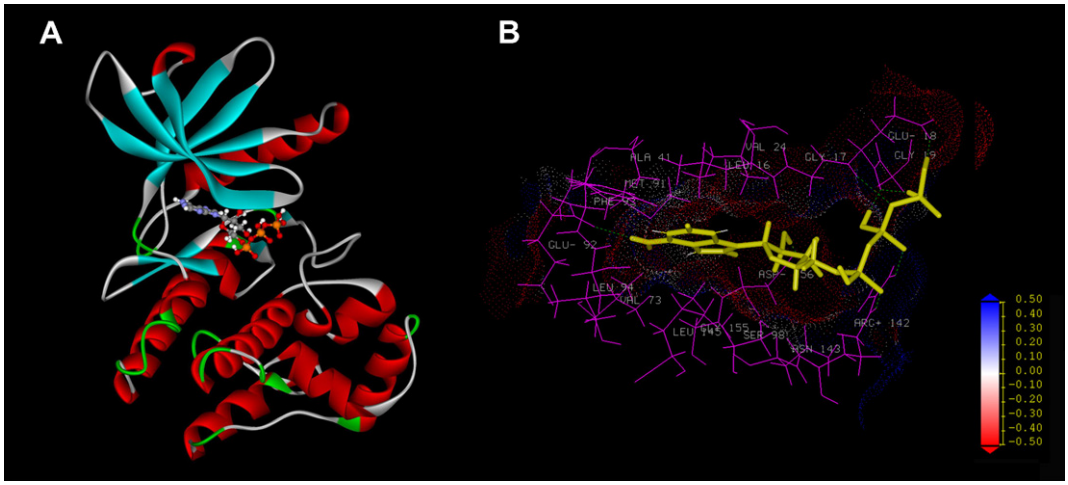

Fig. 2. The overall structure of Jak1 KD and ATP binding mode. (A) A solid ribbon presentation of Jak1 structure with ATP. (B) The detailed binding and hydrogen bonds of ATP with catalytic groove. The electrostatic potential around catalytic groove was generated by *Delphi* in the insightII package and plotted in colored dot surface; the detailed hydrogen bonds were depicted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

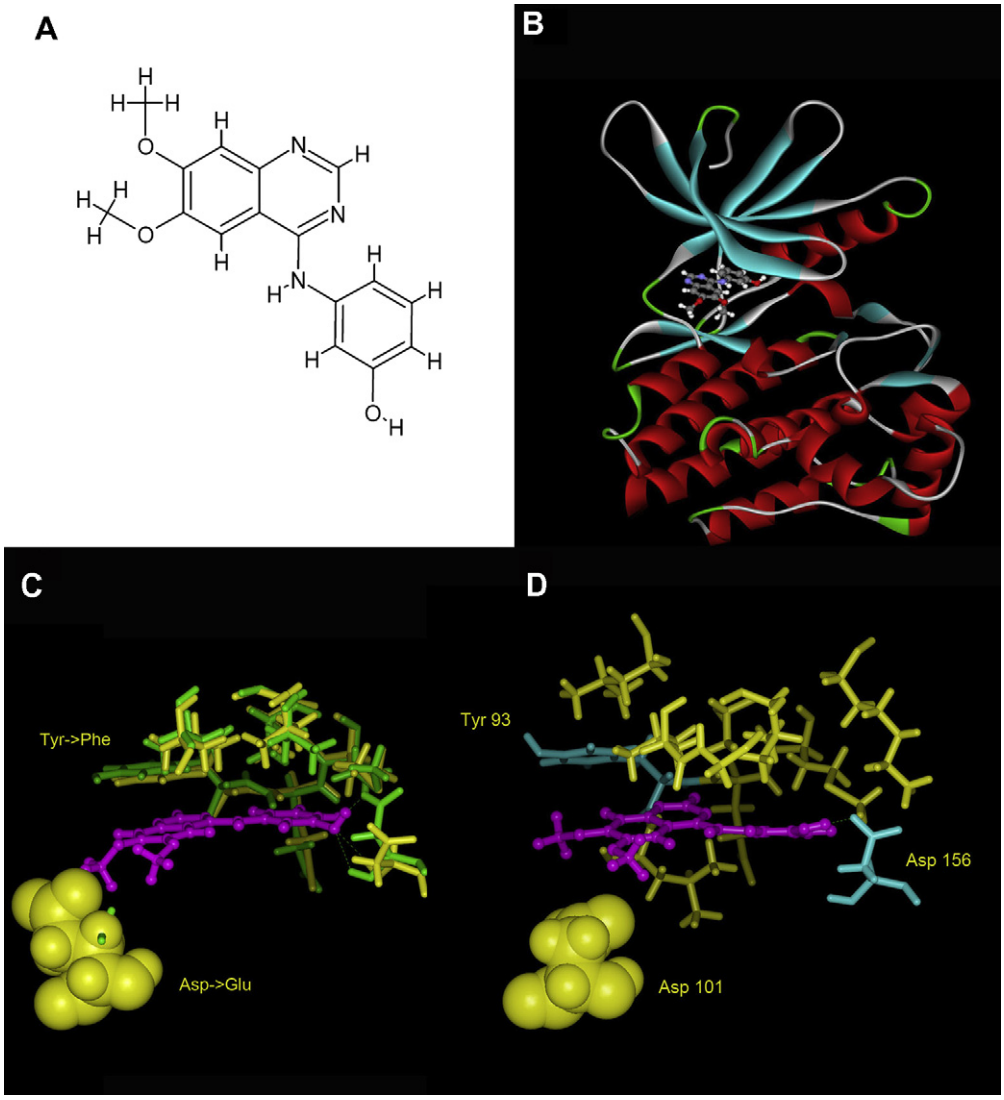

Fig. 4. Two residues in Jak1 catalytic pocket influence WHI-P131 specificity. (A) Chemical structure of WHI-P131. (B) The solid ribbon presentation of Jak3 with WHI-P131 rendered in ball-stick. (C) Superimposition of Jak1 and Jak3 catalytic groove with WHI-P131 in ball-stick. Jak1 residues were colored yellow and Jak3 residues were in green. Two key residue substitutions were marked. The Glu in Jak1 were rendered CPK to illustrate the steric hindrance. The hydrogen bonds were depicted in dash line. (D) Docking mode of mutated Jak1 with WHI-P131 in ball-stick. Mutated Tyr 93 and Asp 156 were colored cyan and mutated Asp 101 was rendered CPK. The hydrogen bonds were depicted in dash line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Energy contributions of key residues interacting with WHI-P131 in Jak3 binding pocket

Residue	E_{vdw} (kcal/mol)	E_{ele} (kcal/mol)	E_{total} (kcal/mol)
Asp 154	2.33	−16.99	−14.56
Asp 99	−1.57	−5.97	−7.54
Leu 15	−7.04	0.43	−6.61
Leu 143	−5.00	−0.28	−5.28
Val 23	−3.39	−0.13	−3.52
Tyr 91	−3.68	−0.42	−4.1
Leu 92	−3.15	−0.19	−3.34
Gly 95	−3.01	−0.24	−3.25
Cys 96	−2.85	−0.09	−2.94
Ala 40	−1.99	−0.47	−2.46
Met 89	−1.97	0.18	−1.79
Glu 90	−1.24	−0.67	−1.91
Pro 93	−1.52	−0.17	−1.69
Val 71	−1.47	−0.22	−1.69
Ala 153	−1.88	−1.21	−3.09
Total	−46.49	−19.06	−65.55

slightly distorted (dihedral angle of two planar groups 27.56°, RMSD 0.46 Å). The interaction energy of this complex is −74.89 kcal/mol (Vdw energy, −50.58, Electrostatic energy, −24.30). Thus, we conclude that these two residues are largely responsible for not interacting with WHI-P131.

Discussion

In this study, we constructed a comparative model for Jak1 kinase domain (KD) by using the *InsightII/Homology* module and further refined the coordinate by energy minimization using the *Discover 3* molecular simulation engine. The sterochemical property and folding quality was further assessed by *Prostat*, *Procheck*, and *Profile*-3D. We implemented a flexible docking method and created an interaction model for Jak1 KD and ATP which is compatible with existing literature [14].

More importantly, we analyzed the detailed mechanism by which the specificity of WHI-P131, a selective Jak3 inhibitor, is

established. We first evaluated the interaction conformation between Jak3 and WHI-P131. Our docking result partially agreed with a previous article [7], but some discrepancies were also found: (1) We did not found the hydrogen bond between nitrogen ring with Leu 92. (2) The two methyl ether groups are not completely solvent exposed, they can interact with Asp 99. The difference should largely be caused by the different template structures used. In that study, the authors could only use hematopoietic cell kinase (33% identity), fibroblast growth factor (34%) and insulin receptor kinase (32%) in which sequence similarity is just slightly above the twilight zone [18]. Indeed, modeled structures using relatively lower identity templates frequently contain inaccuracies [19]. In our study, due to the higher homology templates used and careful assessment of structure quality, we believe that a much more precise coordinate is generated.

By superimposition of two catalytic interfaces, we found that changes in two residues (Jak3 Asp99–Jak1 Glu101 and Jak3 Tyr91–Jak1 Phe93) are crucial for inhibitor specificity. In silico generation of Jak1 double mutant partially gained the binding activity with WHI-P131. It should be noted, however, that minute changes in the shape of the catalytic cleft can also be influenced by subtle changes of backbone residues that do not reside in the interface. Thus, changes in two residues of Jak1 should be necessary but possibly not sufficient for complete compatibility with WHI-P131.

In recent years, Jak3 has been increasingly recognized as an attractive target for cancer and post-transplantation immuno-suppression [4,7,9,20]. Although, multi-targeted inhibitor may be beneficial for malignant cancers [21], a high degree of specificity is generally required for most clinical needs. Thus, understanding the structural and energetic basis for inhibitor specificity is of crucial importance. Our work may be helpful for further study on Jak3 inhibitor design. Furthermore, with the high-quality Jak1 KD model, computational docking study on Jak1 substrate peptides (e.g. STAT1 Tyr701) become feasible which may provide new insights into mechanisms of specificity in target phosphorylation.

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