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Evaluating Protein-Protein Docking Web Servers

Proteins are involved in many cellular processes such as signal transduction, enzyme catalysis and gene expression. Proteins often carry out their functions through interactions with other proteins to form multi-protein complexes. Learning more about protein interactors, substrates or inhibitors can provide insight into the protein's endogenous function. Some approaches currently used to understand protein interactions are using the yeast two-hybrid system or tandem-affinity-purification mass spectroscopy, but these methods are limited in revealing how the proteins may interact. Even though many protein crystal structures are available in the Protein Data Bank (PDB), there is only a small population of solved structures for protein-protein complexes since the dynamics of complex formation complicates crystallization (Moreira et al., 2010).

In the past two decades, theoretical algorithms have been designed to predict the three-dimensional structure of protein-protein and protein-ligand complexes by a procedure called docking. The goal of protein docking is to assemble two separate protein components into a biologically relevant complex structure, ideally close to the native structure. The ability to accurately predict protein complexes will also open the potential for rational drug design and protein engineering.

In this paper, I will address how using computational techniques to predict protein-protein docking will further advance our understanding of molecular mechanisms. There are many computational servers available for predicting protein docking and I will be evaluating five protein docking web servers: PIPER, GRAMM-X, 3D Garden, SmoothDock and PatchDock. I

will also perform my own assessment of these programs in their ability to predict docking between calcineurin (protein phosphatase 2B) and its inhibitor FKBP12-FK506 complex and compare top ranked models against the solved structure.

All of these web servers are freely available with no requirement to have an account. These programs require a solved three-dimensional structure of the unbound proteins of interest in PDB format. The input source can be either a PDB ID or uploading a PDB file. These various programs require the input proteins to be designated as the "receptor" which would be the larger-sized docking partner and a "ligand" which would be the smaller-sized docking partner.

PatchDock: http://bioinfo3d.cs.tau.ac.il/PatchDock/index.html

SmoothDock: http://structure.pitt.edu/servers/smoothdock/

GRAMM-X: http://vakser.bioinformatics.ku.edu/resources/gramm/grammx/

PIPER: http://cluspro.bu.edu/home.php

3D-Garden: http://www.sbg.bio.ic.ac.uk/~3dgarden/

Most of these programs follow a four-step phase to predict protein-protein docking (Fig. 1). The differences of the approaches used by each program at each phase define their ability to predict protein docking models.

First Stage of Docking: Rigid Body or Simplified Search

Protein docking is based on complementarity between interacting proteins which could be geometric, electrostatic, hydrophobic, or all three. To create different docking models, one protein is fixed in space while the second protein is rotated and translated around the stationary protein. Performing a truly comprehensive global search with the receptor and ligand would require a large investment of computational power which is not practical with current resources.

As a result most protein docking prediction programs start with rigid body searches or by geometric matching (Smith and Sternberg, 2002). Most rigid body searches involve simplifying the protein structure as a rigid body representation on a 3D Cartesian grid then a docking involves searching for degrees of overlap between the pairs of grids (Vajda and Kozakov, 2009). One widely used technique to perform the global search is with a Fast Fourier Transform (FFT)-based method. FFT-based method of searching allows the evaluation of many docked conformations on a grid using a correlation-type scoring function.

SmoothDock, GRAMM-X, and PIPER use FFT-based approach during their initial docking step. SmoothDock uses the FFT-based program DOT to form 20,000 receptor-ligand complexes which are ranked by surface complementarity (Camacho and Gatchell, 2003). GRAMM-X uses FFT for shape complementarity and a softened Lennard-Jones potential function to model conformational changes that take place during protein-protein binding (Tovchigrechko and Vakser, 2006). PIPER is ClusPro version 2 which uses pairwise structure-based potentials to improve the FFT-based method during the initial rigid body docking step. It is an improvement to ClusPro version 1 because it provides 1000 of the lowest energy conformations instead of 2000 conformations that did not factor energy scoring (Kozakov et al., 2006).

Geometric matching involves local shape feature matching to dock proteins via shape complementarity which is an advantage over the rigid body searches because it avoids exhaustive orientation searches (Vajda and Kozakov, 2009). PatchDock and 3D-Garden both uses geometric matching for their initial docking step. PatchDock's algorithm involves first creating a molecular surface of the protein and then looking for geometric patches containing 'hot spot' residues. The hot spot residues represent residues that are likely to interact with other

proteins. Then the surface patches from one protein are matched to surface patches on the other (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). 3D-Garden uses an algorithm that generates the molecular surfaces using a Marching Cubes technique which represents the surface as facets. With the facets, an average of 340,000 models is formed by rigid transformation of the smaller interactor to the larger protein (Lesk and Sternberg, 2008).

Second Stage of Docking: Selecting the Regions of Interest

The first stage of docking will generate between a few hundred to a thousand docked complexes which will be filtered to select the best conformations judge by their scoring function (Vajda and Kozakov, 2009). PatchDock will perform steric clashes tests to remove conformations that result in steric clashes between the receptor and ligands (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). SmoothDock calculates the desolvation and electrostatic binding affinity between receptor and ligand and selects the 500 best desolvation energy and 1500 best electrostatic energy complexes (Camacho and Gatchell, 2003). GRAMM-X reranks their models in three steps: local minimization with soft van der Waals interaction, clustering of predictions within the same local minima, and rescoring with the target function combining Lennard-Jones (Tovchigrechko and Vakser, 2005). At the filtering step for PIPER, it retains structures with the lowest value of the desolvation free energy and with the lowest value of electrostatic energy (Kozakov et al., 2006). 3D-Garden filters for acceptable rigid models by scoring the complex with an all-atom modified Lennard-Jones potential with explicit hydrogens but no electrostatic terms and the top 5,000 scoring models move on to the refinement stage (Lesk and Sternberg, 2008).

Third stage of docking: refinement of docked structures.

At this stage, models can be refined by incorporating flexibility into the protein side-chains and possibly the backbone as well (Vajda and Kozakov, 2009). One problem at this stage is that the number of combinations of rotamers to test for each side-chain or backbone would be too large to sample. PatchDock provides some flexibility by adding hinge-bending regions using the FlexDock program (Moreira et al., 2010). 3D-Garden's refinement stage composes of searching for clashing side chains and resolving it through conformational exploration (Lesk and Sternberg, 2008). SmoothDock, GRAMM-X and PIPER do not perform any refinements in their docked structures. One note to add is that SmoothDock does account for side-chain flexibility through short molecular dynamics simulations during the initial searching stage (Camacho and Gatchell, 2003).

Last stage of docking: final model selection.

This last stage is important because an ideal scoring function should be able to recognize a favorable native protein interface while discriminating from non-native contacts. PatchDock will rank their final models by a scoring function depending on the geometric fit and atomic desolvation energy. In addition a root mean square deviation (RMSD) clustering is done to remove redundant conformations (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). SmoothDock further filtered the complexes to retain 25 clusters with the highest number of neighbors then top ranked complexes from these clusters are scored based on lowest free energy estimates (Camacho and Gatchell, 2003). GRAMM-X displays their top scoring models based on soft Lennard-Jones potential, evolutionary conservation of predicted interface, statistical residue-residue preference, volume of the minimum, empirical binding free energy and atomic contact

energy (Tovchigrechko and Vakser, 2005; Tovchigrechko and Vakser, 2006). PIPER ranks their models based on cluster size which is based on the number of complexes that have the largest number of neighbors within a certain fixed cluster radius of ≤ 10 Å C_α RMSD (Kozakov et al., 2006). 3D-Garden will identify the best conformations by a score which is a weighted sum of Lennard-Jones and electrostatic terms (Lesk and Sternberg, 2008).

Protein Docking Accuracy and Validity

The true test to the accuracy of protein-protein docking prediction program is to compare the predicted models to a training set of proteins with their complexed structure determined. CAPRI (Critical Assessment of Protein-Protein Interactions) is an open community experiment that evaluates the potential of these programs through blind predictions (Janin, 2010; Janin et al., 2003; Méndez et al., 2003; Méndez et al., 2005). CAPRI evaluates the quality of the prediction by superimposing the prediction on the native structure and the RMSD is calculated for the whole molecule (L_{rms}) and for the residues of the native interface (I_{rms}). Biological relevance is determined by calculating the fraction of correctly identified residue-residue contacts (f_{nat}). CAPRI's ranking criteria for high, medium, acceptable and incorrect predictions are shown in figure 3 (Méndez et al., 2003).

SmoothDock participated in CAPRI Round 1and was able to show their program consistently ranked the correct model first with the highest confidence (Fig. 4, Camacho and Gatchell, 2003). GRAMM-X participated in CAPRI Round 5, one representative model shows Target 18 aligned against the native structure and the model was evaluated as 'acceptable' according to the number of predicted native residue contacts (Fig. 5, Tovchigrechko and Vakser, 2005; Tovchigrechko and Vakser, 2006). PIPER participated in rounds 1-11 of CAPRI with 26

evaluated targets and their model prediction gave 4 high, 5 medium and 12 acceptable models (Vajda and Kozakov, 2009).

Another method of testing the quality of the predictions is to compare predictions against established set of proteins. PatchDock determined the quality of their model prediction through comparison with their protein test set which contains 25 protein-protein cases. Figure 2 shows the best RMSD (0.87) between the native complex and their rank 2 predicted model (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005). 3D-Garden did not perform well on the test set of proteins or with the CAPRI targets as shown by their primary prediction being mostly incorrect (Fig. 6, Lesk and Sternberg, 2008).

Personal Evaluation of Programs

I will also personally evaluate these web servers on their ability to predict the docking interface between calcineurin and the immunosuppressant complex FKBP12-FK506. Calcineurin (CN) is a calcium/calmodulin-sensitive protein phosphatase that is important during the immune response (Aramburu et al., 2001). Calcineurin is a heterodimer composed of a catalytic subunit A (CNA) and regulatory subunit B (CNB). During the immune response, T cell activation involves a rise in intracellular calcium activates calcineurin activity which dephosphorylates the transcription factor NFAT to allow its translocation to the nucleus to regulate gene expression (Griffith et al., 1995; Shibasaki et al., 2002). FK506 is an immunosuppressant drug that is widely used to suppress the immune system to prevent organ rejection during transplantation. FK506 acts as an immunosuppressant drug through complexing with the cytosolic protein FKBP12 and inhibiting calcineurin's activity during the immune response (Griffith et al., 1995).

The crystal structure for human calcineurin has been crystallized at 2.1 Å resolution (Fig. 7) and human FKBP12 complexed with FK506 has also been crystallized at 1.7 Å resolution (Fig. 8) (Kissinger et al., 1995; Wilson et al., 1995). I will use these two unbound proteins as my input to see which web servers will best predict the docking by comparing the models to the crystallized calcineurin and FKBP12-FK506 complex at 3.1 Å resolution (Fig. 9, Griffith et al., 1995). One small note to make is that the calcineurin and FKBP12-FK506 is from bovine, but this will not be a problem for comparing the predicted docking of human calcineurin with human FKBP12-FK506 to the bovine native complex because these proteins are very highly conserved (Fig. 10). The FKBP12-FK506 complex binds to calcineurin at the base of the calcineurin B binding domain and forms contacts with CNB and the phosphatase domain of CNA (Griffith et al., 1995).

As most programs require identifying the receptor and ligand proteins, I will use calcineurin as the receptor and FKBP12-FK506 as the ligand. I will present top ranking models from each program and display the models alone and aligned in PyMOL to the native structure for visual comparison between the two structures.

PatchDock allows you to identify the complex type: enzyme/inhibitor; antibody/antigen; or protein/small ligand. I specified an enzyme and inhibitor complex which restricts the search space to cavities of the enzyme molecule. Experimental evidence has shown that site-directed mutagenesis of FKBP12 on residue Asp-37, Arg-42, His-87 and Ile-90 were important for binding to calcineurin (Griffith et al., 1995). I was able to incorporate experimental data by uploaded a text file listing these important residues. The output is an email when the job is completed with a link to access the models in PDB format. The resulting top ranked is shown in Figure 11. Big advantages are the fast turnaround time for jobs (10 minutes for my job) and this

was the only program that kept the FK506 ligand associated with FKBP12 in the structural prediction.

SmoothDock was the simplest interface to use because there were no options to customize docking such as chain specification, complex type or specifying important residues. The program required an uploaded PDB file and the output was supposed to be emailed after completion to the user, but upon submission of my two unbound proteins I have not received any updates or results from the web server after several attempts. I will conclude that the web server does not currently work and will not be available for my evaluation.

PIPER took about a day to complete, but it displayed the most comprehensive models because it took into account different coefficients into the model building process such as balanced, electrostatic-favored, hydrophobic-favored and Van der Waals+ Electrostatic favored. PIPER offered advance options which included identifying attraction and repulsion residues, removal of unstructured terminal residues from the receptor or ligand, option to create receptors as multimers, and an antibody mode that sets the antibody as a receptor and the antigen as a ligand. Results are retrieved through the web server and jobs are stored for 4 months before deleted. Results can be downloaded as PDB files. Figure 12 represents the second ranking model since I discard the top ranking model because FKBP12-FK506 was docked at a very distinct location on CNA.

GRAMM-X offers the option of using interface residue constraints by identifying the potential residues of interaction on the receptor or ligand. This option allows the user to incorporate experimental data such as from mutagenesis studies about which residues are likely to be present on the receptor-ligand interface or set the threshold on how rigorous the global set of predictions has to be filtered. GRAMM-X was simple to use and the output was an email to a

link to retrieve the PDB files. The top ranked model is shown in Figure 13. A disadvantage of this program is no steric clash test for their predicted models.

The 3D-Garden web server was the most unintuitive program that I reviewed because the options available were difficult to know how to modify such as "# azimuthal steps for each facet pair (1-)" and "marching cubes grid spacing." I used the default conditions on the web server to predict docking between calcineurin (biomolecule 1) and FKBP12-FK506 (biomolecule 2). The output is received as an email attachment, one attachment for the scoring and ensemble generation and another attachment when the refinement process is completed. I present the best refined model out of the top ten models I received in Figure 14. A disadvantage about the program is that it took the longest to complete the job.

To evaluate the quality of the protein docking prediction, I first attempted to calculate the root-mean square deviation (RMSD) of the alignment between the model and native structure using PyMOL but the calculated RMSD did not correlate with my initial qualitative assessment by eye. For example, the 3D-Garden model was the worst model, but the rmsd calculations did not predict it to be the worst. Further research into the "align" rmsd calculation reveals that outlying residues that did not align were discarded, so this was not an ideal method to evaluate the predictions.

In my second attempt to evaluate the model prediction, I looked at the orientation of the key residues on FKBP12 that were important for binding to calcineurin (N37, R42, H87, and I90) and determined its neighboring residues on calcineurin within 5Å (CNA: Y341, P344, M347, W352, P355; CNB: N122, L123, K124, Q127). In a similar method to CAPRI in calculating the fraction of correct residue-residue contacts, I will rank the quality of the models based on the number of matching neighboring residues on calcineurin. The neighboring residues

determined for each model are presented in Table 1. PIPER ranked 1st because it was able to identify the neighboring residues CNA: W352, 355 and CNB: N122. GRAMM-X ranked 2nd because it there were two matching neighboring residues CNA: W352 and P355. PatchDock is ranked 3rd because even though there were not any matching neighboring residues, the key residues in FKBP12 were found to be close to other residues in CNB. Finally, 3D-Garden was ranked 4th and last because the FKBP12-FK506 complex docked at a very distinct site from the native site. The key residues in FKBP12 also did not have any neighbors on calcineurin within 5Å.

Conclusion:

I have presented a table representing the key features of the different protein docking servers and how they ranked based on my observations (Table 1). With my assessment of these protein docking servers in predicting the calcineurin and FKBP12-FK506 complex, I recommend the use of PIPER, GRAMM-X and PatchDock. The important of learning how two proteins dock together is for understanding how molecular mechanisms can occur between the proteins. The Cyert Lab is interested in understanding all of the substrates that interact with calcineurin and how and where they dock on calcineurin to get dephosphorylated at the active site. Docking then represents a physical measure of how likely a protein interactor is to being a substrate through its docking on calcineurin. There are characterized docking motifs used by calcineurin substrates, so one approach would be to screen potential substrates for these docking motifs. In addition, as more computational programs are developed to accurately predict protein docking, we can use protein docking programs as another method to screen for potential substrates. For example, we may use these protein docking web servers to predict if a protein interactor is suggested to be a

substrate by observing if it docks on calcineurin in such a way that allows dephosphorylation at the active site.

Figure 1: Computational steps in multistage docking (Vajda and Kozakov, 2009).

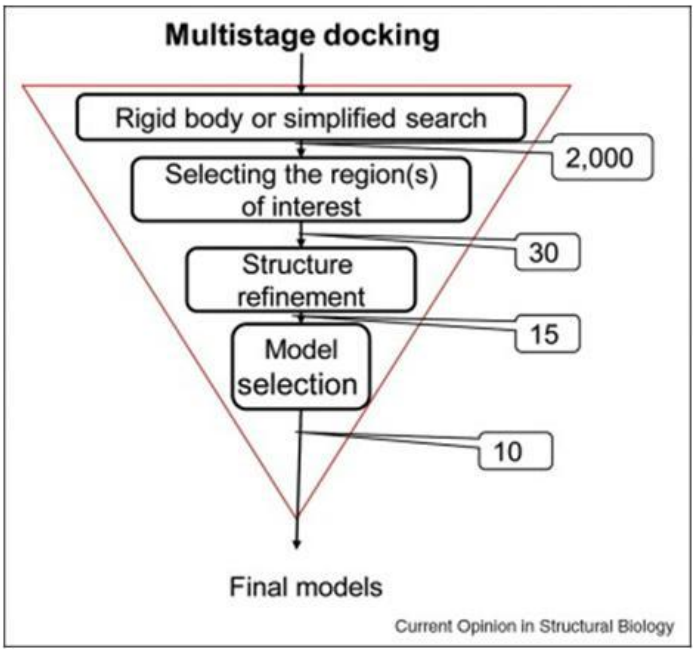
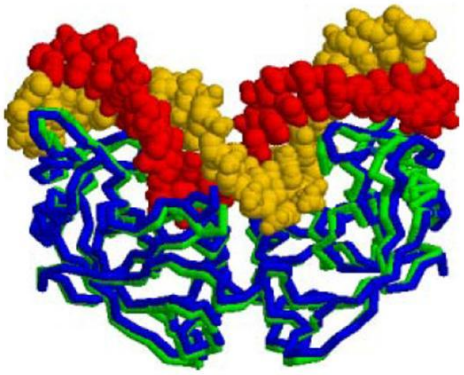


Figure 2: PatchDock Protein-DNA docking: unbound-bound case (PDB codes 1A73, 1EVX). Best RMSD obtained 0.87, rank 2. The DNA is shown in spacefill. PatchDock model is superimposed on the native complex is shown in backbone representation (Fig. 4B, Duhovny et al., 2002).



(b) Protein–DNA

Figure 3: CAPRI ranking criteria (Méndez et al., 2003).

TABLE II. Criteria for Ranking the CAPRI Predictions

Rank	f_{nat}	L_rms	or I_rms
High	≥0.5	≤ 1.0	or ≤ 1.0
Medium	≥0.3	1.0 < x ≤ 5.0	or 1.0 < x ≤ 2.0
Acceptable	≥0.1	5.0 < x ≤ 10.0	or 2.0 < x ≤4.0
Incorrect	<0.1		

Figure 4: SmoothDock Performance on CAPRI (Camacho and Gatchell, 2003).

Table I. Comparison of CAPRI Predictions

			Our Submissions			Community Submissions			
ID	Receptor	Ligand	Rank[a]	RMSD (Å)	Correct contacts	Best RMSD[b] (top/all)	Correct contacts[c] (top/all)	Rankings[d]
1	1JB1	1SPH	1	9.5	11/52	9.5	12/52	1st/2nd
			2	11.5	8/52	7.5	17/52	3rd/3rd
2	1QHD	Bound Fab	1	74.75	0/52	6.3	20/52	Lower 50%
			2	37.01	2/52	2.3	50/52	in all cases
3	2VIU	Fab HC63	1	57.16	0/63	---[e]	6/63	Lower 50%
						4.6	45/63	in all cases
4	1PIF	Ig VH Domain 1	1	54.53	0/58	---	0/58	n/a[f]
			4	38.07	0/58	---	1/58	
5	1PIF	Ig VH Domain 2	1	35.92	1/64	---	1/64	---/1st
			2[g]	26.59	0/64			
			3	32.39	7/64	---	10/64	---/2nd
6	1PIF	Ig VH Domain 3	1	2.42	54/65	2.42	54/65	1st/1st
			4	6.64	34/65	0.7	60/65	6th/3rd
7	1BEC	1B1Z	1	2.62	29/37	2.62	31/37	1st/3rd
			2	8.36	20/37	2.62	31/37	1st/3rd

[a]Number of the model predicted by the *SmoothDock* algorithm. A value of one indicates that the model was our best *a priori* prediction for that target. [b]The best RMSD of all top submissions, i.e., models submitted 1st by each of their respective predictors, and the best RMSD of all submissions regardless of the order in which they were submitted. [c]The highest number of correct contacts predicted for all top submissions and for all submissions regardless of the order in which they were submitted. [d]Rankings of our best predictions relative to the top submissions (top row), and to all of the predicted complexes (bottom row). Values are shown for RMSD and correct contacts. [e]RMSDs were not calculated if no near-native complexes were predicted. [f]No good models were found for this complex. [g]Our second submission for this target correctly identified 22/29 and 13/25 of the ligand and receptor binding site residues.

Figure 5: GRAMM-X prediction for CAPRI Target 18 (TAXI xylanase inhibitor and *Aspergillus niger* xylanase; 1.8 Å rmsd prediction accuracy for the ligand interface area. The correct and predicted structures are shown in different colors (Fig.1, Tovchigrechko and Vakser, 2006).

Figure 6: 3D-Garden performance (Lesk and Sternberg, 2008).

Table 2. Profile of modelling performance for 3D-Garden

Dataset	Incorrect	Acceptable	Medium	High
Test (45):				
Quality of primary prediction	29	10	6	0
Best quality in preferred 10	16	8	19	2
Rigid-body test	22	8	5	0
(35)	9	6	18	2
Medium difficulty test	4	1	1	0
(6)	4	1	1	0
Difficult test	3	0	1	0
(4)	3	0	1	0
Enzyme-Inhibitor test	8	2	2	0
(12)	3	4	4	1
Antibody-Antigen test	4	4	3	0
(11)	2	0	8	1
CAPRI targets	17	5	2	1
(25)	9	9	3	4

All models belong to exactly one model quality category.

Figure 7: Receptor Input: Calcineurin heterodimer (PDB ID: 1AUI). Magenta-CNA; White-CNB; 2 orange spheres represent metal ions at the phosphatase site.

Figure 8: Ligand Input: FKBP12-FK506 complex (PDB ID: 1FKJ). Red-FKBP12; Pink-FK506

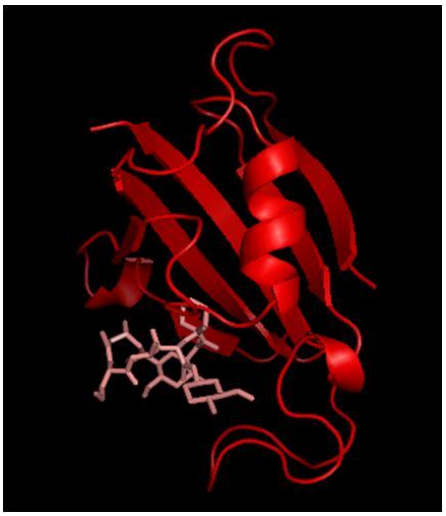


Figure 9: Crystallized structure of CN and FKBP12-FK506: (PDB ID: 1TCO). Green-CNA; White-CNB; Red-FKBP12-FK506; FKBP12 residues (N37, R42, H87, and I90) labeled.

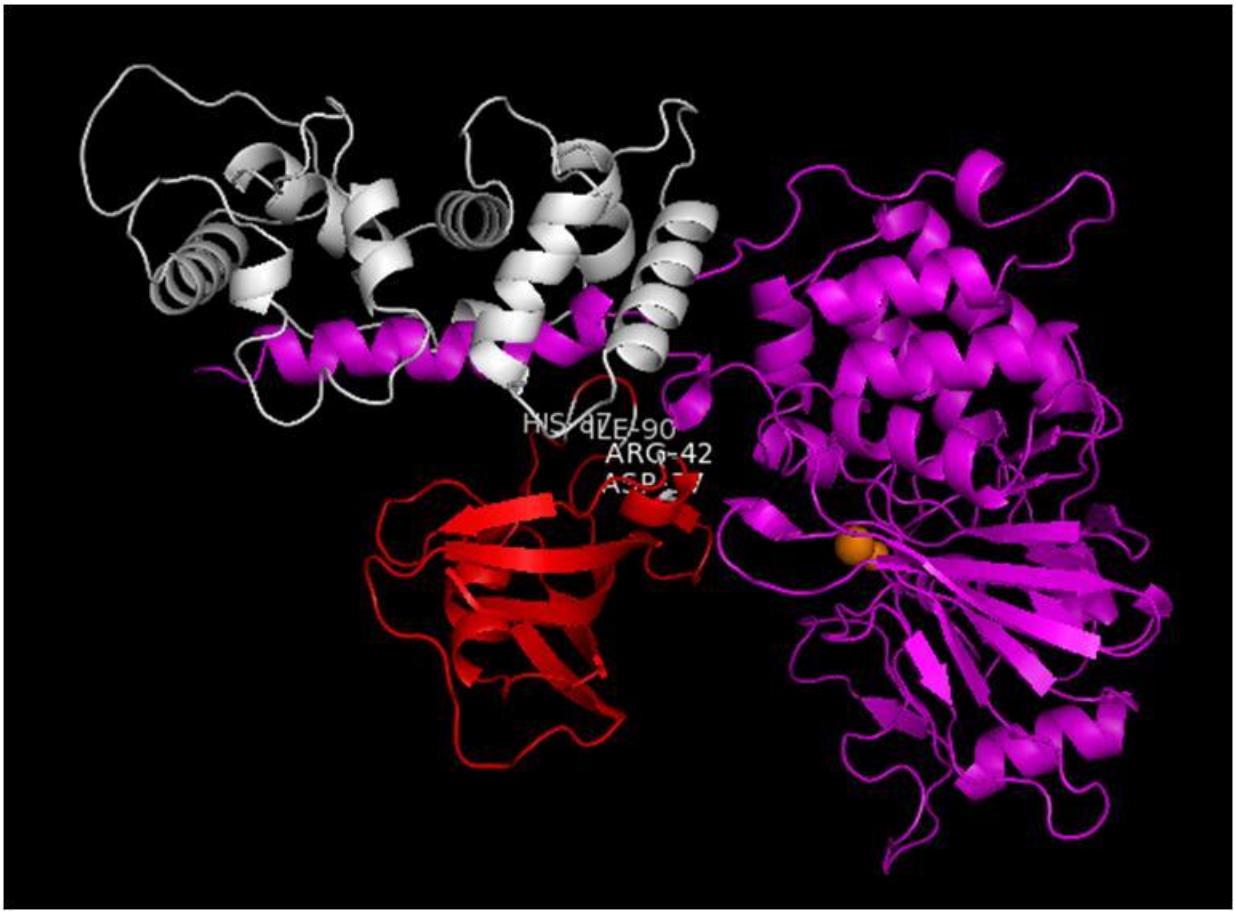


Figure 10A: Sequence alignment between human FKBP12 (FKB1A_HUMAN) and bovine FKBP12 (FKB1A_BOVIN).

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1    MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVG   70  P62942   FKB1A_HUMAN
1    MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFVLGKQEVIRGWEEGVAQMSVG   70  P18203   FKB1A_BOVIN
     **************************************************:*******************

71   QRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE  108  P62942   FKB1A_HUMAN
71   QRAKLTISPDYAYGATGHPGIIPPNATLIFDVELLKLE  108  P18203   FKB1A_BOVIN
     ************************:***:*********
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Figure 10B: Sequence alignment for human calcineurin subunit A (PP2BA_HUMAN) and bovine calcineurin subunit A (PP2B_BOVIN).

Figure 10C: Sequence alignment for human calcineurin subunit B (CANB1_HUMAN) and bovine calcineurin subunit B (CANB1_BOVIN).

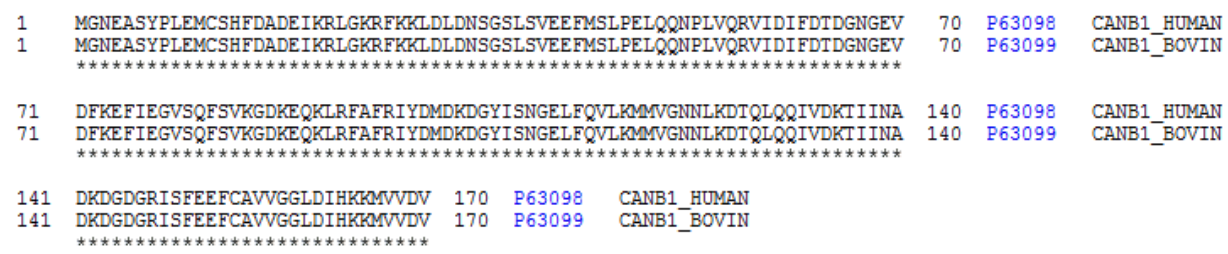

Figure 11A: PatchDock top ranked model. Green-CNA; White-CNB; Cyan-FKBP12-FK506; FKBP12 residues (N37, R42, H87, and I90) labeled.

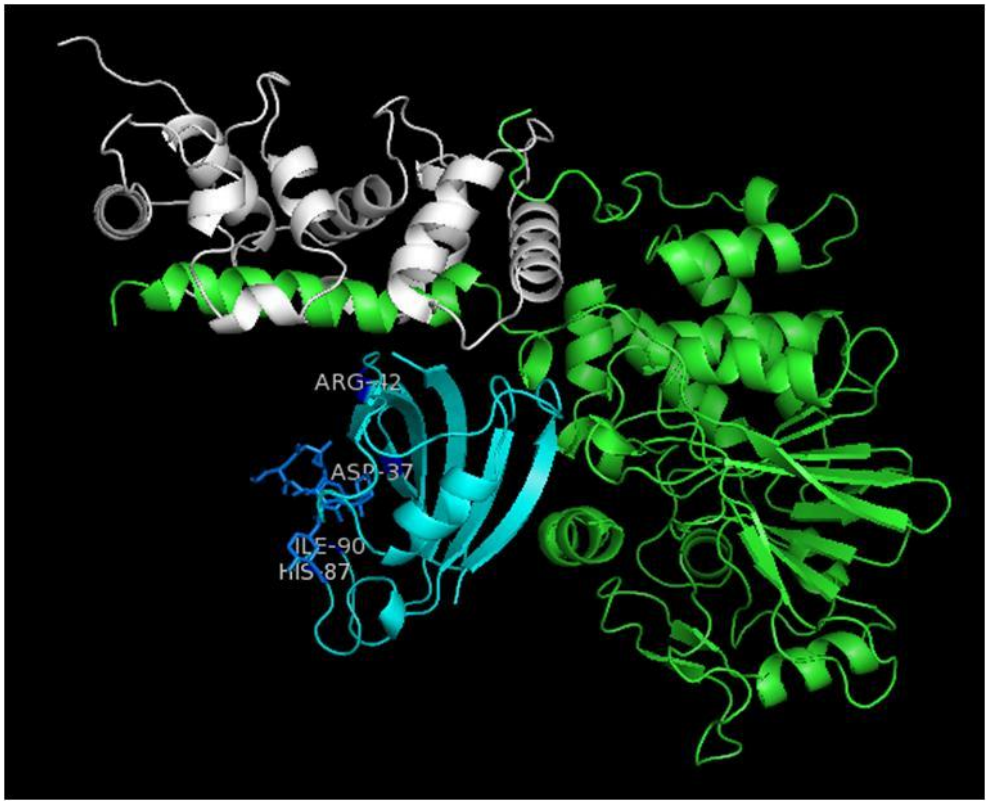

Figure 11B: PatchDock model alignment with native structure. CNB structure is not shown.

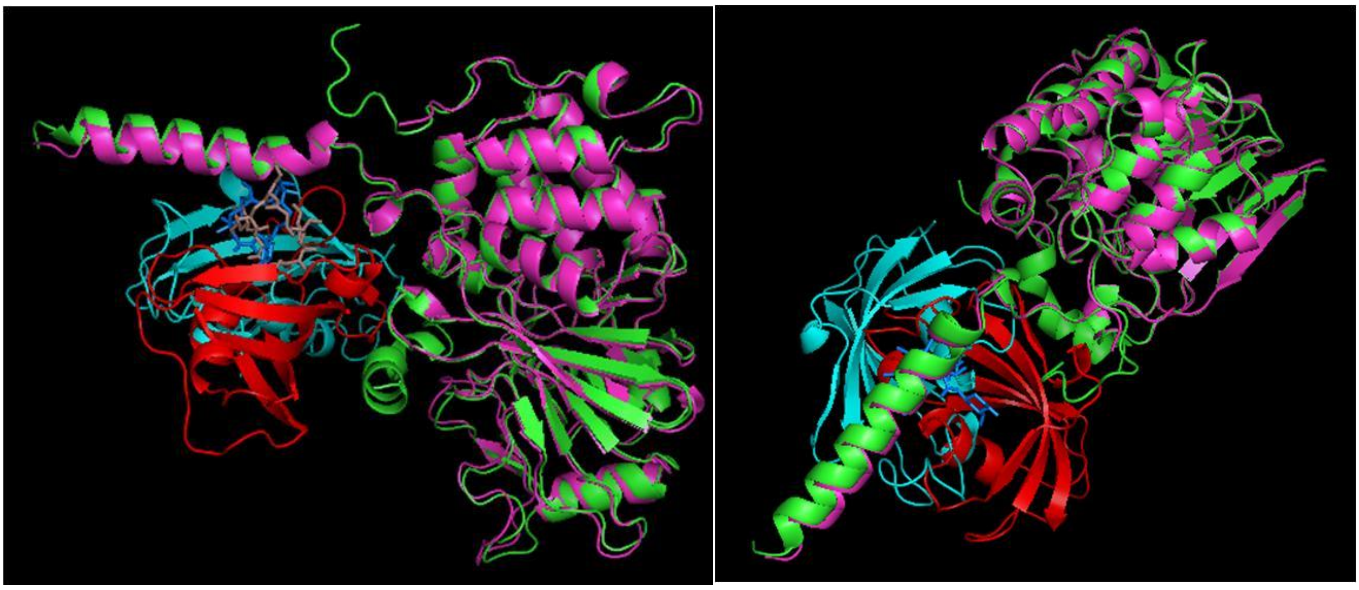

Figure 12A: 2nd ranked model from PIPER. Green-CNA; White-CNB; Cyan-FKBP12-FK506; FKBP12 residues (N37, R42, H87, and I90) labeled.

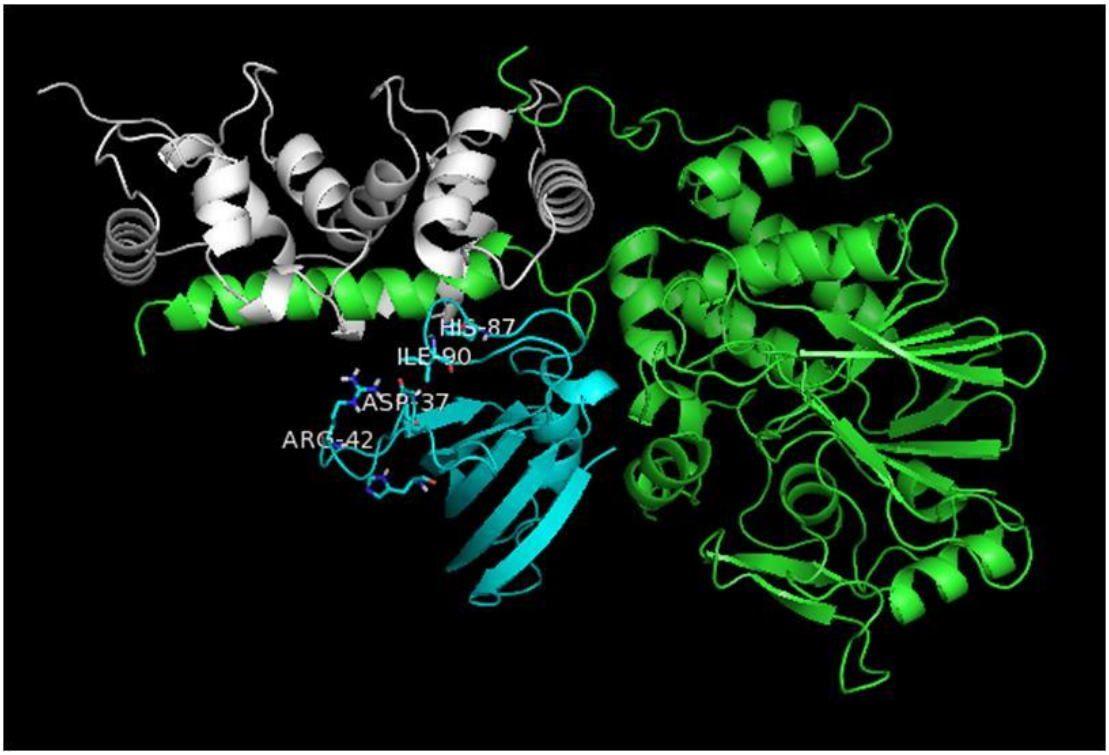

Figure 12B: PIPER model alignment with native structure. CNB structure is not shown.

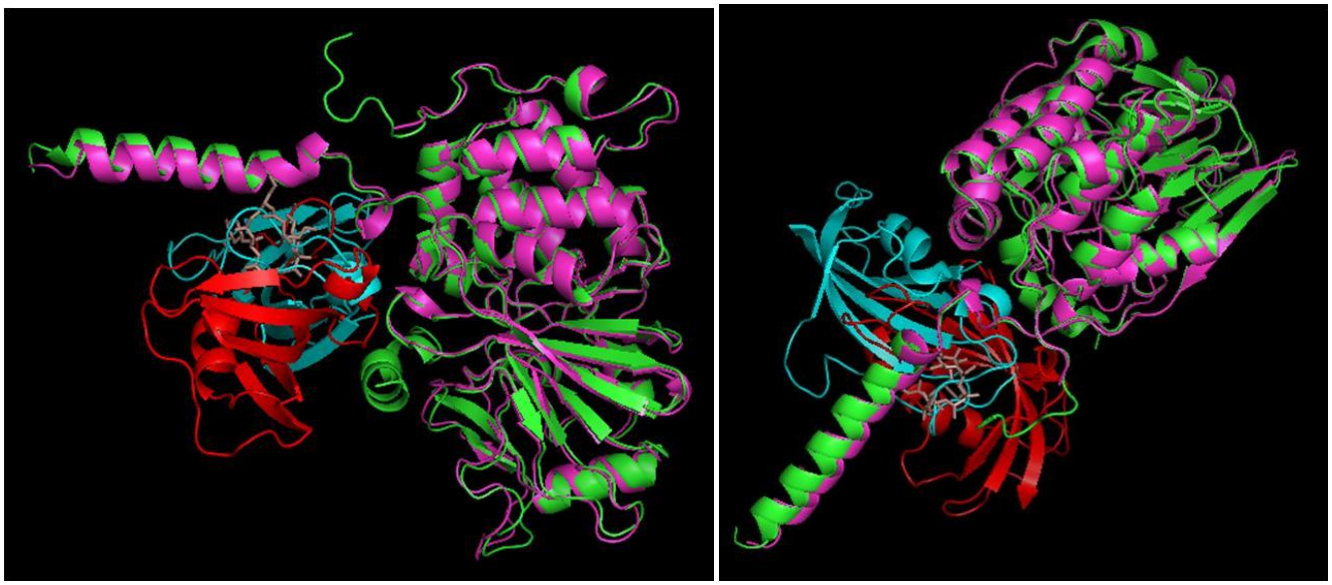

Figure 13A: GRAMM-X, model #1. Green-CNA; White-CNB; Cyan-FKBP12-FK506; FKBP12 residues (N37, R42, H87, and I90) labeled.

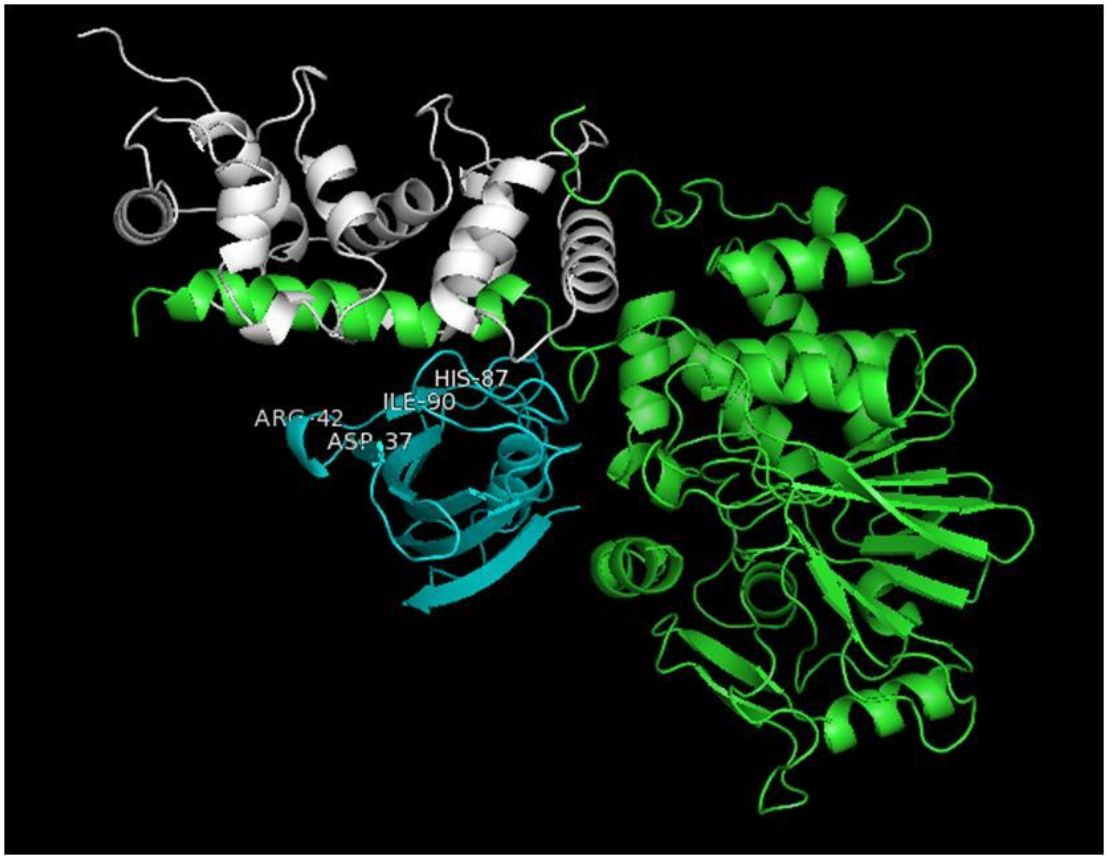

Figure 13B: GRAMM-X alignment to native structure. CNB structure is not shown.

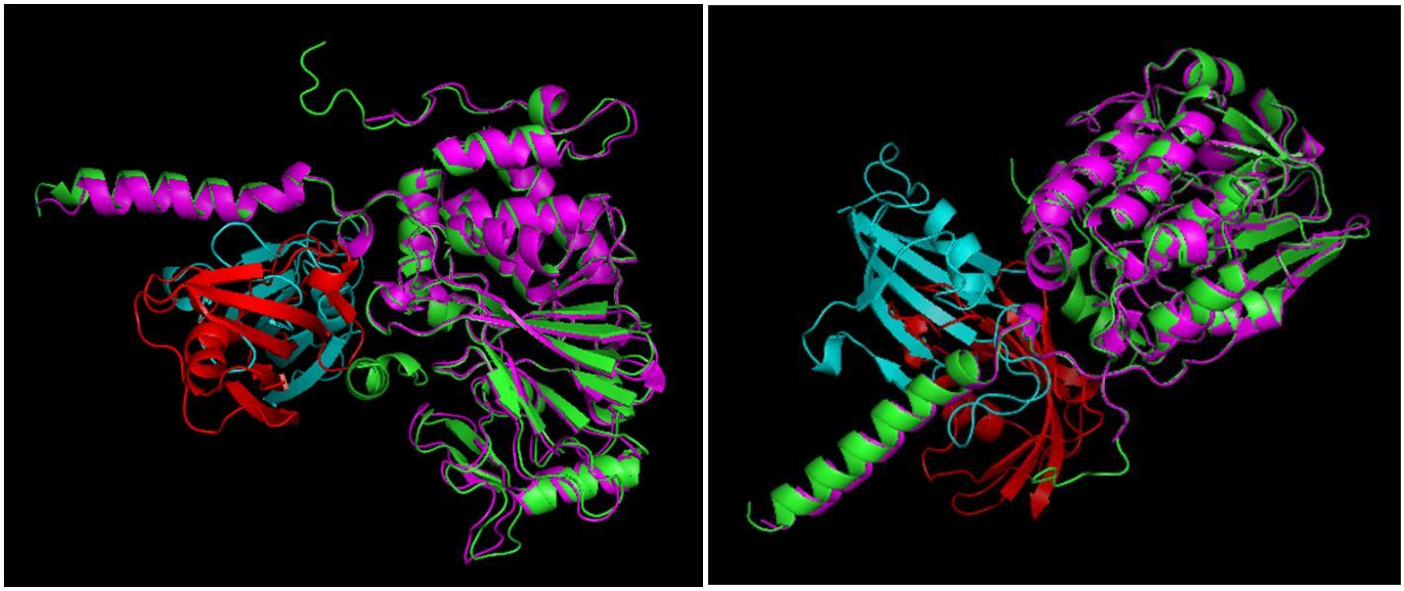

Figure 14: 3D-Garden top ranked model. Green-CNA; White-CNB; Cyan-FKBP12-FK506; FKBP12 residues (N37, R42, H87, and I90) labeled.

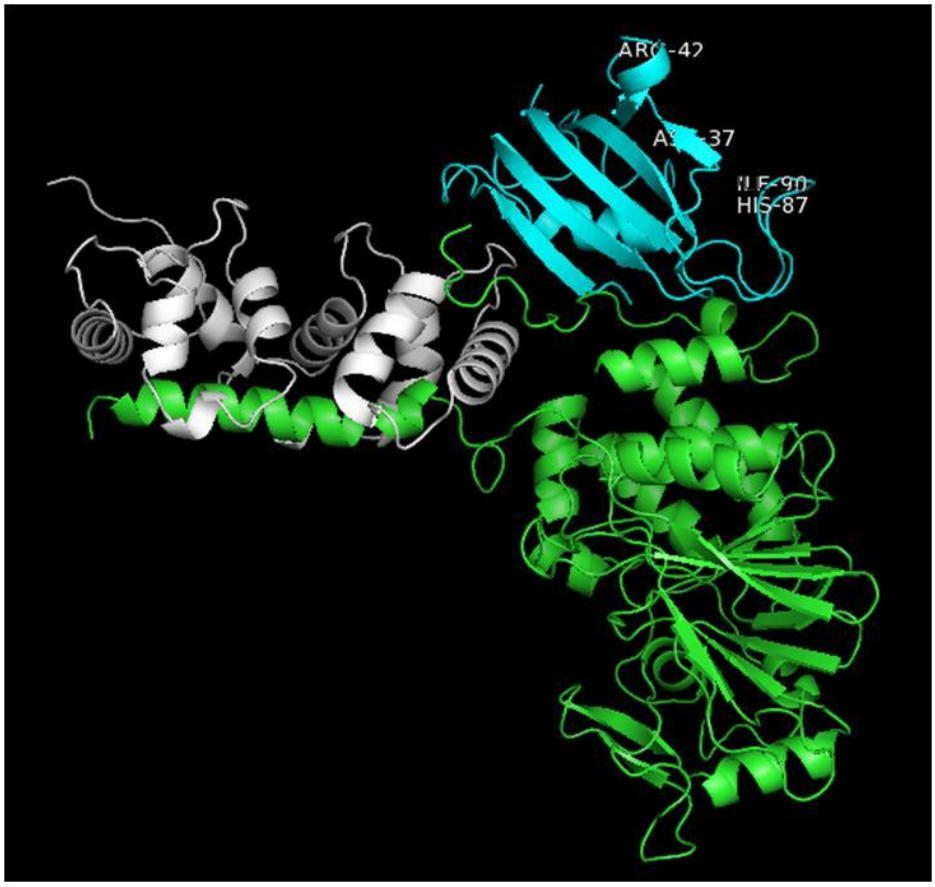

Table 1: Comparison of protein docking prediction web servers. Neighboring residues in calcineurin are determined in PyMOL by using FKBP12 residues (N37, R42, H87, I90) as reference points and finding residues in calcineurin within 5 Å. The native structure was shown to have neighboring residues: CNA: Y341, P344, M347, W352, P355; CNB: N122, L123, K124, Q127. Residues highlighted in yellow represent matching residues.

Program	Multimeric/ chain selection	Searching	Backbone flexibility	Advantages	Disadvantages	Rank	Neighboring residues (within 5 Å) in calcineurin
GRAMM-X	Both	FFT	No	-can specify receptor/ligand binding site	-complexes with significant backbone changes are difficult to predict	2	CNA: K164, W352, S353, P355, D359, K360
PatchDock	Both	Geometric hashing	Introduces hinges with FlexDock	-RMSD option -option to specify complex type -can specify receptor/ligand binding site -fast!	-Does not include side chain flexibility	3	CNB: L159, D160
PIPER	Both	FFT	No	-can specify attraction/repulsion residues -structure modification option -antibody mode	-no email update of job completion, required frequent checks to the web server	1	CNA: W352, S353, P355, F356, E359 CNB: N122, K164
SmoothDock	Multimeric only	FFT	No	-consistently ranked the correct model first	-web server does not currently work	N/A	N/A
3D-Garden	Both	Geometric hashing	No	-two separate files for ensemble generation and refinement	-options difficult to understand -most high ranking models failed in initial docking site	4	No near neighbors

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