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Original research paper

In silico data mining of large-scale databases for the virtual screening of human interleukin-2 inhibitors

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Interleukin-2 (IL-2) is involved in the activation and differentiation of T-helper cells. Uncontrolled activated T cells play a key role in the pathophysiology by stimulating inflammation and autoimmune diseases like arthritis, psoriasis and Crohn's disease. T cells activation can be suppressed either by preventing IL-2 production or blocking the IL-2 interaction with its receptor. Hence, IL-2 is now emerging as a target for novel therapeutic approaches in several autoimmune disorders. This study was carried out to set up an effective virtual screening (VS) pipeline for IL-2. Four docking/scoring approaches (FRED, MOE, GOLD and Surflex-Dock) were compared in the re-docking process to test their performance in producing correct binding modes of IL-2 inhibitors. Surflex-Dock and FRED were the best in predicting the native pose in its top-ranking position. Shapegauss and CGO scoring functions identified the known inhibitors of IL-2 in top 1, 5 and 10 % of library and differentiated binders from non-binders efficiently with average *AUC* of > 0.9 and > 0.7, resp. The applied docking protocol served as a basis for the VS of a large database that will lead to the identification of more active compounds against IL-2.

Keywords: IL-2, virtual screening, FRED, MOE, GOLD, Surflex-Dock, ROC-curves

Interleukin-2 (IL-2) is a cytokine that is predominantly involved in the growth, activation, and differentiation of T-helper 1 (Th1) cells. Binding of IL-2 with its trimeric receptor (known as IL-2R) causes proliferation and clonal expansion of activated T-cells (1). The trimeric IL-2R is composed of α (IL-2Rα), β (IL-2Rβ) and γ (IL-2Rγ) subunits. Aberrant Th1 immune response leads to graft rejection. Moreover, several autoimmune disorders like inflammatory bowel disease (Crohn's disease and ulcerative colitis) and psoriasis are linked with the abnormal Th1 immune responses (2). An elevated level of IL-2 in serum is also seen in scleroderma, gastric and non-cell lung cancer, rheumatoid arthritis and several neoplastic diseases (3). Thus, IL-2 is a considerable therapeutic target for the treatment of these diseases. Current therapies target the IL-2 production/IL-2 signaling pathway to

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develop new immuno-modulators. Specific inhibition of IL-2 interaction with its receptor (IL-2Rα) by antibodies and general immuno-modulators inhibit the Th1 response without causing toxicity (4). However, antibodies have shown several drawbacks such as lack of oral bioavailability and high cost. Designing a specific inhibitor of IL-2 production that blocks direct interaction of IL-2 with IL-2Rα could offer a significant improvement in immunosuppressive therapy. Thus far, the discovery of small molecule inhibitors of IL-2 or other proteins that are involved in protein-protein interactions has been challenging (5).

Computational drug design approaches have been applied successfully in the development of new drugs at a lower cost and less screening time for different drug targets that are challenging (6). Pertaining to our interest in the computational analysis of immuno-modulators targeting IL-2 and other cytokines (7–13), we have conducted a comparative analysis of docking/scoring methods to develop the most effective virtual screening (VS) pipeline for the screening of large scale data against IL-2. Hence, four docking programs with different algorithms and scoring functions: FRED (14, 15), GOLD (16), Surflex-Dock (17) and MOE (18, 19) were extensively evaluated in this study. The selected programs were evaluated based on two criteria: the ability of docking programs to produce exactly similar conformations (pose or solution) of ligands to their native (experimentally characterized) conformation, their accuracy in the selection and identification of known active inhibitors implanted in the database of decoys (inactive compounds or compounds whose activities are not determined). The decoy set was selected from the ZINC database (20). Statistical analysis (enrichment factor and ROC-curves) was applied to VS results. The study resulted in the identification of an effective docking method that will be used for the multiple tasks of designing IL-2 inhibitors in the future.

EXPERIMENTAL

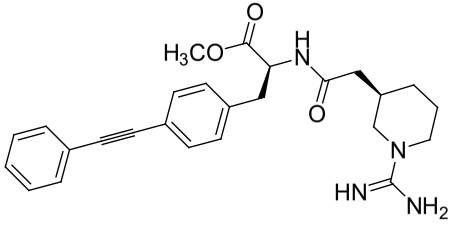
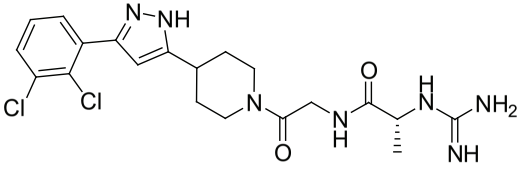
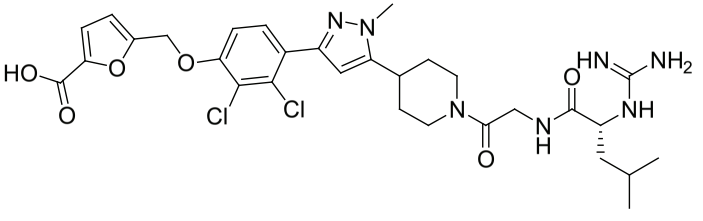
In this study, we have employed four docking programs, *i.e.*, MOE v2006.08 (Molecular Operating Environment) obtained from Chemical Computing Group ULC, Montreal, Canada (18), FRED (Fast Rigid Exhaustive Docking) taken from OpenEye Scientific Software Inc., Surflex-Dock (implemented in SYBYL v7.3) (21), and GOLD v3.2, (Genetic Optimization for Ligand Docking) obtained from Cambridge Crystallographic Data Center, University of Cambridge, UK (22).

The entire *in silico* experiments were performed on quad-core Intel 3.0 GHz Xeon Linux work station running under SUSE 11.0 operating system. Because docking speed is very important in the screening of huge datasets, computational (CPU) time of each docking method was recorded. FRED and Surflex-Dock took < 1 minute to dock one compound, whereas MOE and GOLD used 1–2 minutes for docking.

Selection and preparation of protein-ligand files for docking

Protein-ligand complexes were chosen for docking with special care. Flexibility in the protein structures was considered, and three human IL-2 X-ray structures in complex with ligands were downloaded from Protein Data Bank [PDB IDs: 1M48 (27), 1PW6 (28) and 1PY2 (28)] for the assessment of docking methods. The chemical structures of the co-crystallized ligands (so-called reference ligands) are shown in Table I. The protein-ligand complexes were selected based on the following criteria: protein structure should be of human origin,

Table I. Protein-ligand complexes used for docking purpose

PDB ID	Ligand	Chemical structure	IC_{50} (μmol L^{-1})	log *P* (o/w)	Resolution (Å)	RB
1M48	FRG		3	4.4	1.95	11
1PW6	FRB		6	2.3	2.60	10
1PY2	FRH		0.06	3.4	2.80	15

RB – number of rotatable bonds in the ligand (27, 28).

structure of protein should have good resolution (≤ 3.0 Å), protein-ligand interaction should be non-covalent and their experimental inhibitory concentrations (IC_{50}) must be reported.

For docking, protein and ligand's coordinate files were prepared by SYBYL7.3. All heteroatoms other than the ligand were removed. Water molecules within 3.0 Å of ligand were retained, the rest were removed from protein. On each protein, protons were added by the SYBYL biopolymer module. The co-crystallized ligands were extracted from their respective protein and saved as a reference structure for the comparison with their docked conformation and RMSD calculation. The atom and bond type of each ligand was rectified, protons were added to ligand molecules according to their protonation states and Gasteiger--Hückel charges were assigned. Finally, ligands coordinates were minimized by Tripos Force Field (1000 steepest descent steps) (29). The ionizable groups of ligands were protonated according to their physiological pH.

Docking methods

MOE 2009. – Initially, the MMFF94 force field (30, 31) was applied to minimize the protein structure until the RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$. The active site was defined by the co-crystallized ligand. MOE possesses different docking algorithms including alpha PMI, alpha triangle, proxy triangle and triangle matcher, and four scoring functions: ASE, alpha HB, affinity dG, and London dG. Each docking method of MOE was applied in combination with each scoring function of MOE. Thus, sixteen docking-scoring methods were used in MOE (19) to recognize the most suitable method for IL-2. Finally, thirty docked poses from each combination were saved for further study.

Surflex-dock. – Surflex-dock implies hammerhead incremental construction (HIC) as docking engine (26) and empirical scoring function that includes hydrophobic and charged complementarity terms. HIC basically creates a pseudo-binding site which is known as protomol (17). Ligand is fragmented and fragments are aligned over protomol in order to generate poses that maximize molecular complementarity with the binding site. By HIC and a crossover, distinct poses are combined to generate a full molecule from aligned fragments which are scored by scoring function (17).

Docking is started with the generation of Protomol which depends on Proto_bloat and Proto_thresh parameters that define the active site. Proto_bloat describes how far from a potential ligand the site should extend, while Proto_thresh describes how deep into the protein the atomic probes used to define the protomol can penetrate. The default parameters for threshold and bloat are 0.50 and 0 Å, resp. By default, 30 orientations of each ligand were retained.

GOLD 3.2. – GOLD applies genetic algorithm (16) that offers full and partial conformational flexibility to ligand and protein, resp. The scoring function of GOLD includes the terms of hydrogen-bonding, vdW, and intra-molecular energies. vdW interactions of protein--ligand complex are described by 8–4 potential while ligand steric energies are described by 12–6 potential. For docking, active site was created around 10 Å of ligand. Docking parameters were set as 10 genetic algorithm (GA) run, 100,000 maximum number of operations and population size of 50 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, resp. The distance between fitting points and hydrogen donors was 4.0 Å. The non-bonded vdW energies cut-off was 2.5 Å. During docking, four scoring

functions: GoldScore, Astex Potential (ASP), ChemScore (GOLD_CS), and ChemPLP (22) were tested in separate runs. Thirty docked poses were saved for each ligand.

FRED 2.2.5. – FRED (14, 15) applies rigid docking by using exhaustive searching and has different scoring functions. An ensemble of rigid body poses is generated for each compound within the binding site, and these poses are passed against the negative image of active site. Poses clashing with this 'bump map' are eliminated. During docking, Gaussian shape function is used to score and rank those docked poses that are retained after shape fitting. FRED uses a prepared receptor file and a multi-conformer library of ligands which is generated by OMEGA (32). MMFF94s force field was applied with the dielectric constant of 4.0. FRED receptor 2.2.5 was used to prepare the receptor files by a shape-based site detection algorithm. The contours were created on the bound ligand that limits the search space to the volume enclosed by the bound ligand. Docked conformation of ligands was scored by nine scoring functions, *i.e.*, Chemgauss 2 (CG2), Chemgauss 3 (CG3) (15), ChemScore (FRED_CS) (23), Chemical Gaussian overlay (CGO), Chemical Gaussian Tanimoto (CGT) (15), Piecewise Linear Potential (PLP) (24), ScreenScore (SS) (25), Shapegauss (SG), and OEChemScore (OECS). For each ligand thirty docked poses were saved by default.

Virtual screening. – For virtual screening (VS), a set of 9.0 million drug-like compounds was selected from ZINC database (20). FILTER (34, 35) was used for further filtration of library according to Lipinski rule of five (33). 1,594,931 (approx. 1.5 million) compounds passed the filter criteria, among them 10,000 compounds were selected for VS. For enrichment purpose, 91 known inhibitors of IL-2 were selected from literature review (36–40). Their 3D-coordinates were generated by MOE. The molecular structures of known actives are presented in Table II. The known active inhibitors were seeded into the library of 10,000 decoys, and a set 10,091 molecules was imported into MOE database. The geometry of each compound in library was optimization by protonate-3D command of MOE.

Analysis measures. – By re-docking tests, we checked the ability of each docking method to predict accurate binding mode of reference ligands. By enrichment studies, scoring functions were scrutinized in the identification of known inhibitors among decoys.

Cognate ligand docking accuracy. – Root mean square deviation (RMSD) is usually calculated between the docked and un-docked conformation of cognate ligand to assess the predictive ability of docking algorithm. In this study, the following criteria were used: first ranked (top) solution with RMSD ≤ 2.0 Å was termed as 'good', top ranked conformation with RMSD 2.0–3.0 Å was regarded as 'fair', top ranked orientation with RMSD > 3.0 Å, or an inverted or incorrect position, was termed as 'inaccurate'.

Virtual screening accuracy. – By using enrichment factor (EF) and ROC curves, the effectiveness of scoring functions was assessed. EF is widely used metric for the comparison of VS results; it was calculated by *Eq. 1*. Scoring functions which correctly ranked 50 % of known inhibitors in top 1, 5 and 10 % of docked library, were considered successful.

$$\text{Enrichment factor} = \frac{\text{HITS sampled} / \text{HITS total}}{N \text{ sampled} / N \text{ total}} \qquad (1)$$

N_{total} – total number of compounds in the database, $N_{sampled}$ – number of ligands in the docked database to be examined, $HITS_{total}$ – total number of active compounds, $HITS_{sampled}$ – number of active inhibitors found in top $N_{sampled}$ ligands of docked library.

Percentual enrichment factor (%EF) for each scoring function was calculated by *Eq. 2*:

$$\text{Enrichment factor}\,(\%) = \frac{\text{Enrichment factor}}{\text{Ideal enrichment factor}} \times 100 \qquad (2)$$

Receiver operating characteristic curves (ROCs). – Structure-based VS and molecular modeling methods are routinely evaluated by ROC curves (41). It effectively differentiates between two populations, so it is suited for evaluating VS performance. Discrimination between active and decoy compounds are important issue. ROC curve describes the trade-off between sensitivity (detection of true positives by model) and specificity (ability of model to avoid false negatives and positives). Enrichment is quantified by calculating area under the curve (*AUC*). If the value of *AUC* is ≥ 0.9, the scoring function was considered as excellent, while the value of *AUC* ≤ 0.6 depicts least or no enrichment.

RESULTS AND DISCUSSION

Virtual screening is incomplete without molecular docking; especially when a 3D-structure of drug target is available docking is most commonly applied VS tool (6). Docking is based on two critical steps. In the first step, docking algorithms perform conformational sampling of small molecules to place them in the 3D-binding site of receptor/proteins. In the second step, scoring functions calculate the binding affinity of each conformation of docked ligand within the binding site. The binding affinities are estimated in terms of scores or binding free energy. Scoring functions must differentiate correct binding states of ligands with the non-native docked conformation during docking and should identify known potential inhibitors (active molecules) within the library of inactive compounds with reasonable accuracy and speed (42).

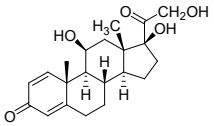
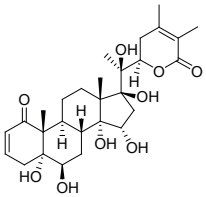
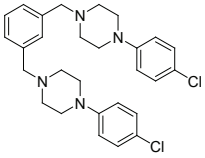
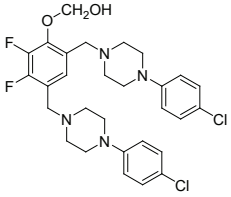
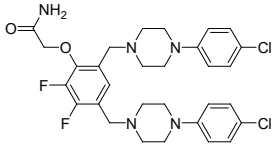
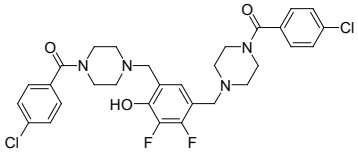
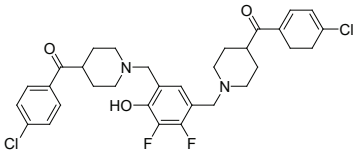
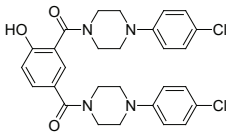
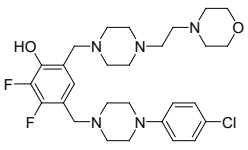
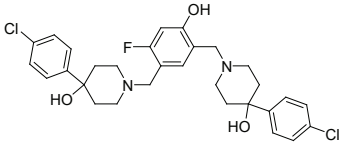
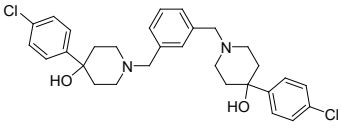
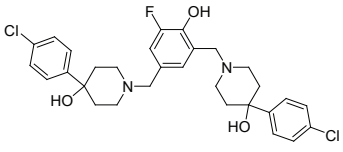
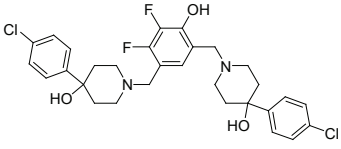
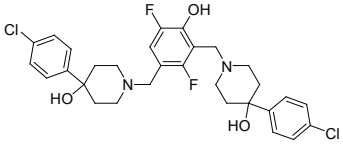
Several studies have been conducted to evaluate docking/scoring methods that primarily focused on pose prediction of ligands and VS. For selected drug targets, pose prediction accuracy has been successfully achieved. However, the scoring functions need more improvement to successfully predict the true binding affinity of a ligand for its receptor. Additionally, all the docking methods occasionally manifest false negative and false positive results. Therefore, it is challenging to improve the strength of docking methods in view of structural and energetic prediction.

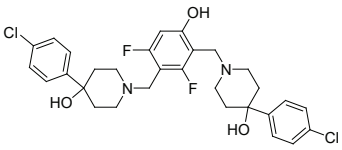
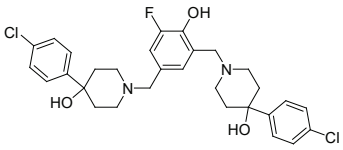
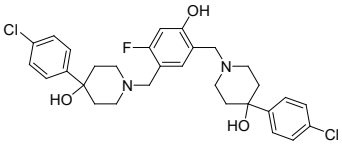
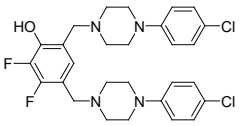
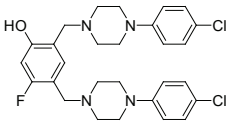
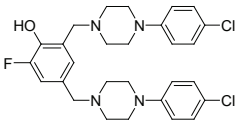
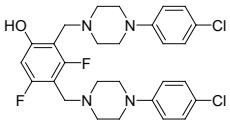
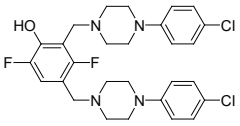
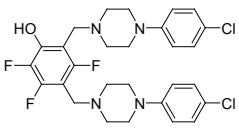
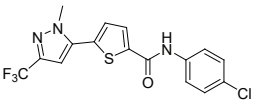
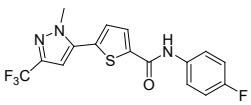
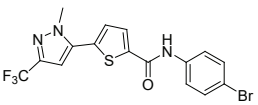
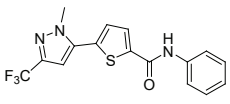
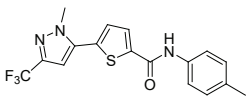
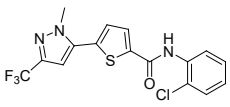
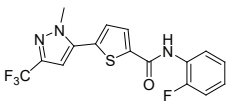
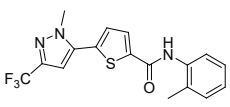
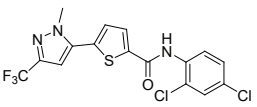
Furthermore, it is difficult to generalize a particular docking method for all the drug targets because a particular docking set up may work better for certain drug targets or compounds classes than for others. Therefore, it is highly recommended to validate a docking method before conducting VS against specific target of interest. This study was conducted in order to establish effective and appropriate VS protocol for the data mining of IL-2 inhibitors.

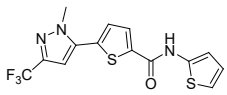
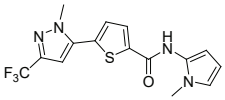
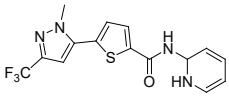
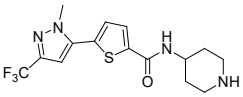
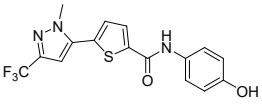
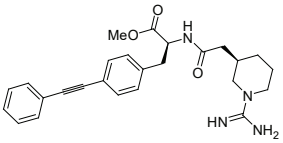
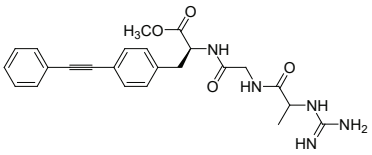
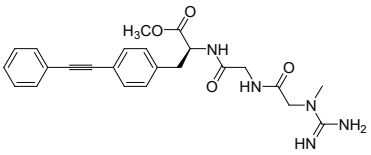
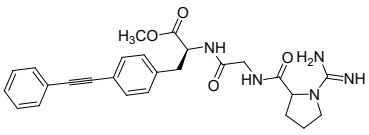
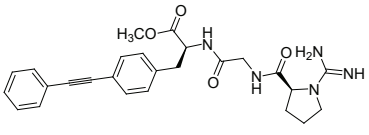
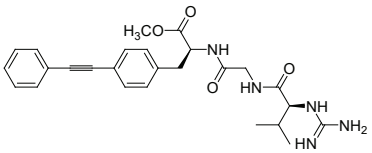
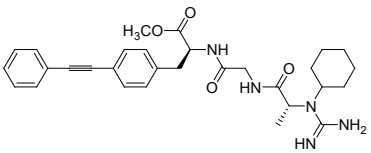
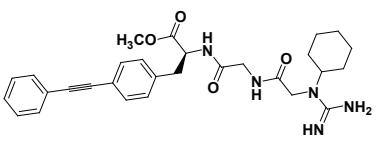
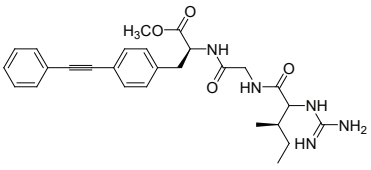
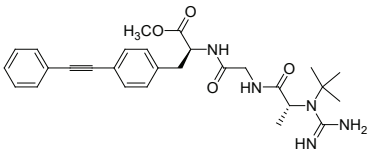
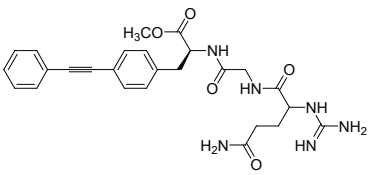
Validation of docking programs

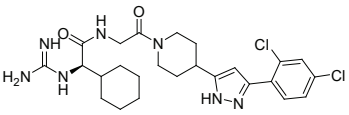
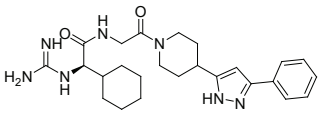
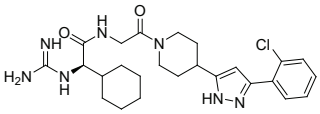
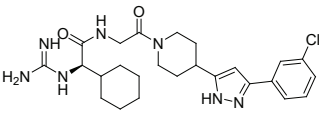
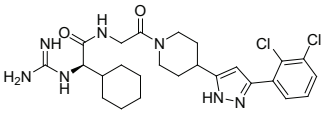
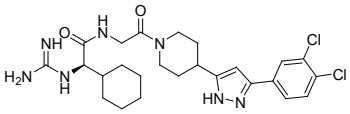
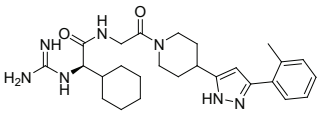
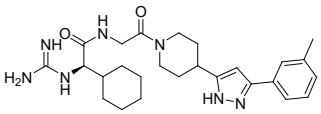
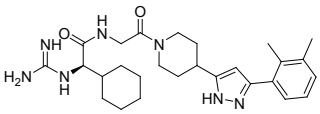
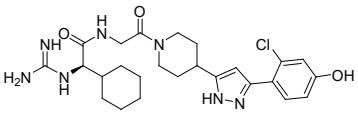
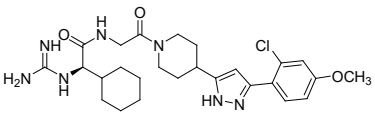
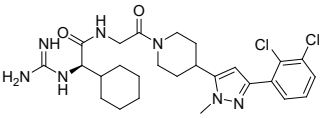
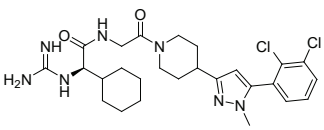
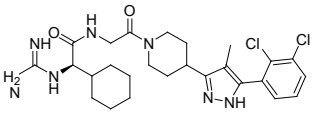
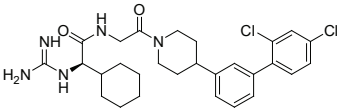
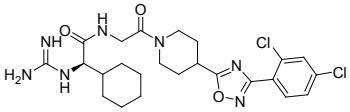
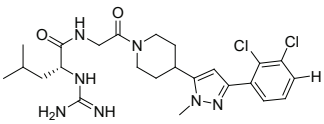
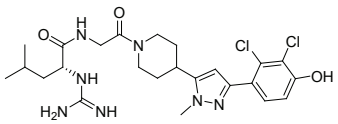
The success of VS depends on the accurate prediction of protein-ligand interactions (42, 43). For this purpose, a docking tool is required that generates suitable conformation of ligand within the protein binding site. The quality of interaction is governed by reliable energetic evaluation and the docking accuracy is validated by the RMSD values between crystal structures and predicted docked poses.

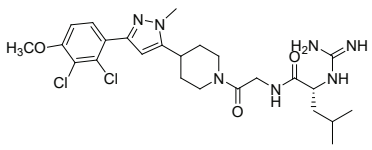
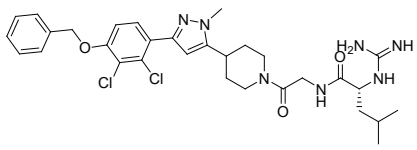
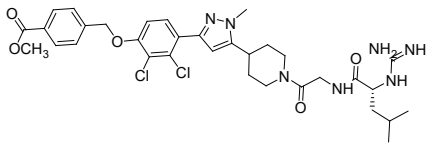
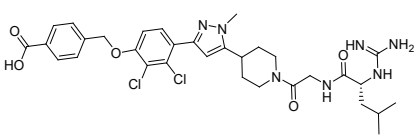
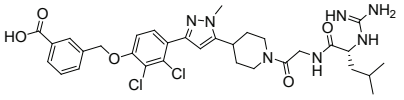
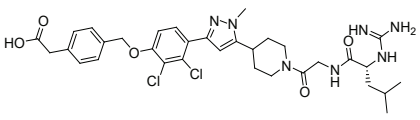
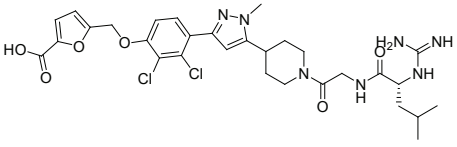
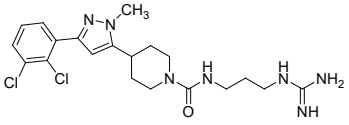
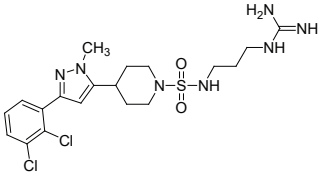
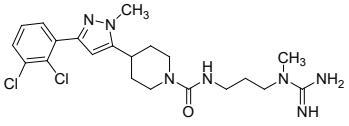
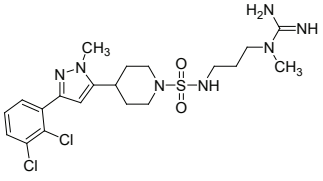
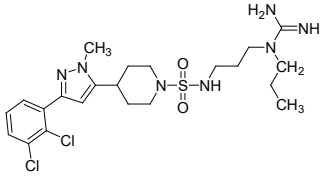
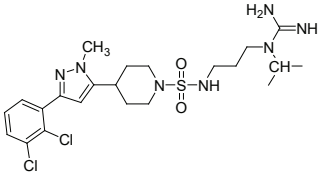
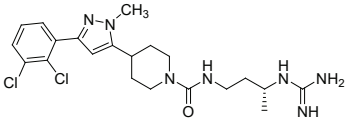
Table II. Chemical structures of known inhibitors

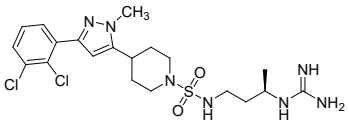
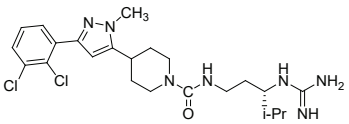
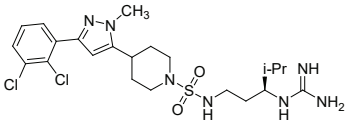
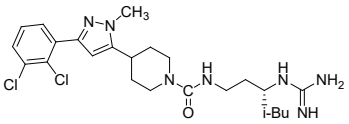
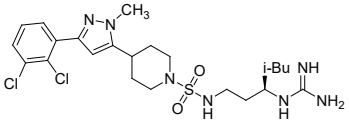
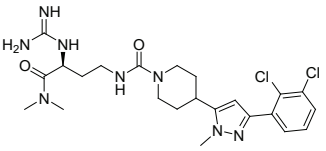
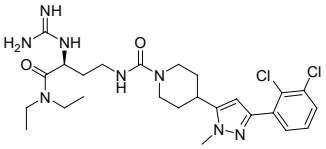
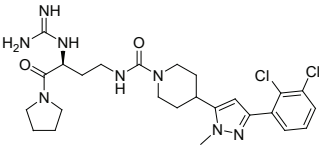
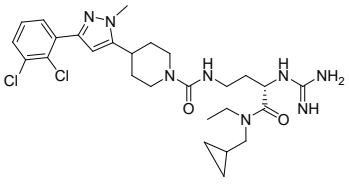
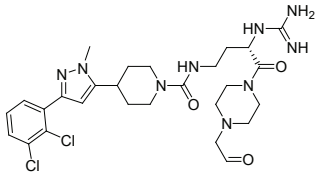
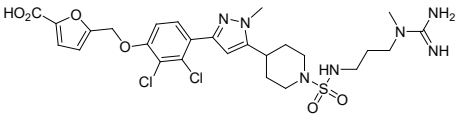
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5		6	
7		8	
9		10	
11		12	
13		14	

No.	Structure	No.	Structure
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17		18	
19		20	
21		22	
23		24	
25		26	
27		28	
29		30	
31		32	

No.	Structure	No.	Structure
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37		38	
39		40	
41		42	
43		44	
45		46	
47		48	

No.	Structure	No.	Structure
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51		52	
53		54	
55		56	
57		58	
59		60	
61		62	
63		64	
65		66	

No.	Structure	No.	Structure
67		68	
69		70	
71		72	
73		74	
75		76	
77		78	
79		80	

No.	Structure	No.	Structure
81		82	
83		84	
85		86	
87		88	
89		90	
91			

To scrutinize the docking accuracy, three IL-2 structures in complex with known inhibitors were retrieved, namely, 1M48, 1PW6 and 1PY2 with the cognate ligand FRG, FRB and FRH, resp. The IC_{50} values, resolution of X-ray structures, rotatable bonds and log *P* values of ligands are tabulated in Table I. The structures of selected known ligands are shown in Table II. The top ranked docked poses of each ligand are shown in Figs. 1–3, while their RMSDs are tabulated in Table III.

1M48. – The known inhibitor 2-[3-methyl-4-(*N*-methyl-guanidino)-butyrylamino]-3-(4-phenylethynyl-phenyl)-propionic acid methyl ester (ligand ID: FRG) was taken from

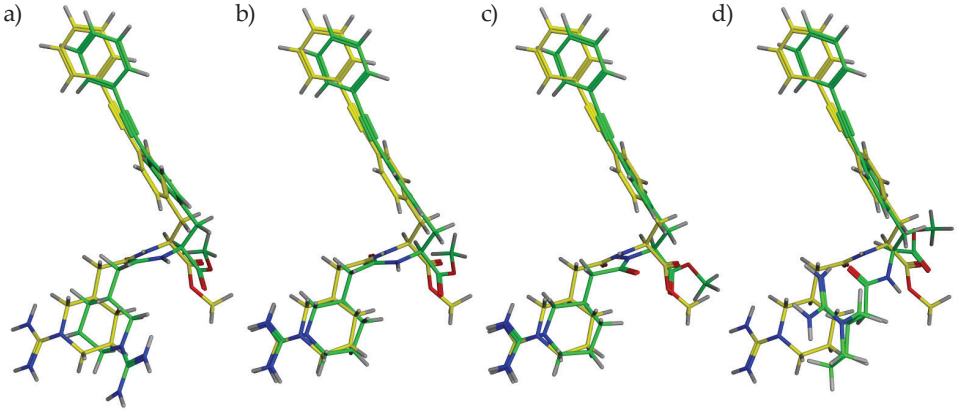


Fig. 1. Docked poses of 1M48, generated by: a) MOE (Molecular Operating Environment), b) GOLD (Genetic Optimization for Ligand Docking), c) FRED (Fast Rigid Exhaustive Docking) and d) Surflex Dock. The experimental and docked conformations are presented in yellow and green stick model, resp.

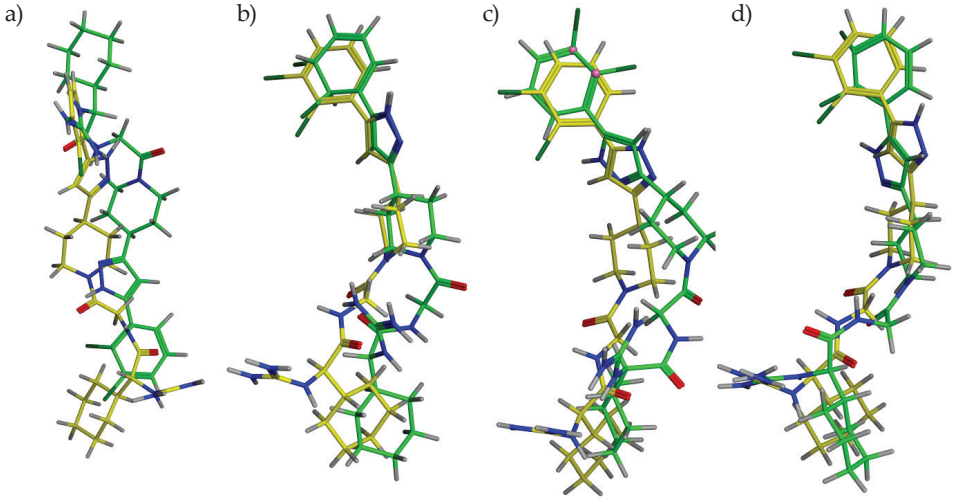


Fig. 2. Docked poses of 1PW6 generated by: a) MOE (Molecular Operating Environment), b) GOLD (Genetic Optimization for Ligand Docking), c) FRED (Fast Rigid Exhaustive Docking) and d) Surflex Dock. The experimental and docked conformations are presented in yellow and green stick model, resp.

protein 1M48 and re-docked into the receptor (IL-2R) binding site of IL-2 by all the docking methods used in this study (Table III). In MOE, TM and AT were able to generate good pose when used with LdG scoring function, while APMI and PT with LdG failed to generate good pose. APMI and PT predicted docked solution was in inverted position. TM with

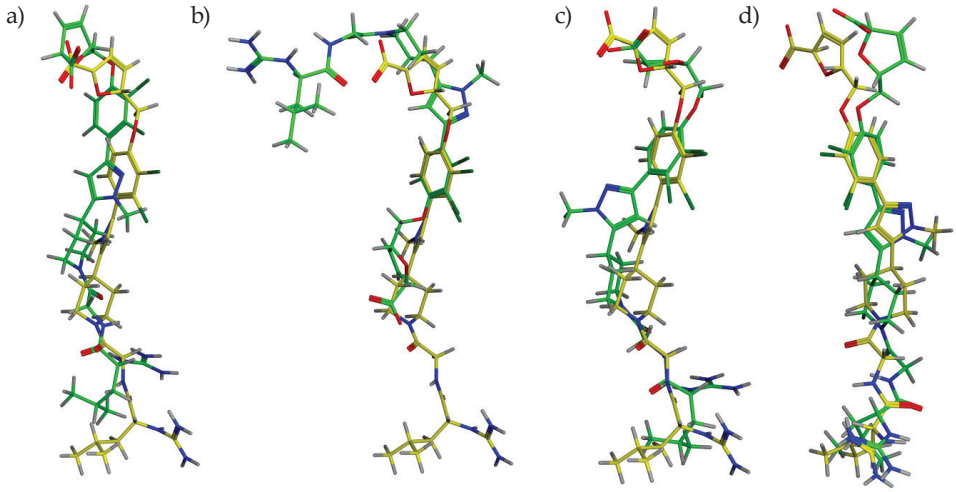


Fig. 3. Docked poses of 1PY2 generated by: a) MOE (Molecular Operating Environment), b) GOLD (Genetic Optimization for Ligand Docking), c) FRED (Fast Rigid Exhaustive Docking) and d) Surflex Dock. The experimental and docked conformations are presented in yellow and green stick model, resp.

ASE depicted good binding mode of this compound as compared to other MOE algorithms. APMI, AT and PT with ASE were unsuccessful to produce good pose. The docked conformation of the compound was inverted by 180 º. TM, AT, APMI, and PT showed poor performance when used with AdG and their predicted RMSDs were ≥ 10 Å. It was observed that each algorithm produced the conformation with < 2.0 Å within thirty poses, which was not ranked accurately in top position. It reflects that the docking program faced difficulty to correctly rank the correct pose in the top position. When applied with AHb, TM and PT showed top ranked best poses, while AT and APMI were unsuccessful. It indicates that the combination of TM and AT algorithms with LdG, and TM and PT algorithms with AHb, are able to generate good poses. The docked views of 1M48 generated by MOE, FRED, GOLD and Surflex-Dock are shown in Fig. 1.

Surflex-Dock performed well in the prediction of good pose in top ranked position (Fig. 1**)**. In GOLD, four scoring functions (ASP, ChemPLP GOLD_CS, and GOLD score) were used. Each scoring function of GOLD produced docking solution with RMSD < 1.2 Å (Table III) and showed excellent agreement with the crystal structure. In FRED, nine scoring functions (CG2, CG3, FRED_CS, OECS, SG, SS, CGO, CGT and PLP) were used that depicted good poses of ligand in the top ranked solution.

1PW6. – The ligand FRB is complexed in 1PW6 which possesses eleven rotatable bonds. Usually, docking methods fail to produce good solutions of highly flexible ligands. The re-docking performance of MOE was declined and the combination of APMI, PT and TM with LdG showed unsatisfactory results, and showed inverted conformation in top ranked position. It indicates that these methods could not handle ligands with > 10 rotatable bonds. AT generated docked pose in appropriate orientation, however, due to high flexibility, the compound was deviated from its X-ray conformation. As a result, RMSD

Table III. The docking performance of MOE, GOLD, FRED and Surflex-dock in the prediction of good conformation in a top-ranking position

	1M48		1PW6		1PY2	
	Score	RMSD	Score	RMSD	Score	RMSD
MOE						
TM_LdG	–10	1.3	–12	6.19	–12	2.87
APMI_LdG	–7	10.5	–6	3.21	2	9.40
AT_LdG	–10	1.26	–10	12.97	–10	9.61
PT_LdG	–10	11.60	–11	7.94	–13	5.11
TM_ASE	–8	2.04	–11	3.73	–10	13.19
APMI_ASE	–10	10.29	–12	11.47	–19	14.56
AT_ASE	–7	10.57	–11	11.24	–13.50	13.56
PT_ASE	–7	10.63	–9	11.66	–12.94	4.73
TM_AdG	–3	12.24	–3	11.28	–2	14.74
APMI_AdG	5	10.47	22	9.39	–60.93	9.40
AT_AdG	–3	11.47	–4	17.19	–3.14	15.74
PT_AdG	–4	12.24	–3	11.28	–5.94	7.07
TM_AHB	–100	1.3	–58	11.28	–97	5.71
APMI_AHB	–50	10.47	–86	2.81	–11.7	7.73
AT_AHB	–54	10.92	–68	10.92	–76.83	19.60
PT_AHB	–100	1.29	–89	11.73	–120.5	2.86
GOLD						
GOLD_Score	73	0.87	82	0.87	68	14.44
GOLD_CS	37	0.79	37	0.78	29	14.63
ASP	38	0.91	43	0.85	38	14.74
ChemPLP	86	1.12	90	0.80	87	14.97
FRED						
SG	–299	1.84	–362	2.3	–347	2.26
SS	–140	1.84	–153	2.3	–127	2.26
PLP	–64	1.84	–57	2.3	–47	2.26
OECS	–44	1.84	–34	2.3	–31	2.26
FRED_CS	–26	1.84	–14	2.3	–4.41	2.26
CG3	–38	1.84	–36	2.3	–16.06	2.26
CG2	–53	1.84	–50	2.3	–49.4	2.26
CGO	–46	1.84	–56	2.3	–48	2.26
CGT	–54	1.84	–59	2.3	–47	2.26
SurflexDock						
Hammerhead	5.80	1.32	5.54	1.46	8.62	0.67

AdG – affinity dG, AHB – alpha HB, APMI – alpha PMI, ASP – Astex Statistical Potential, AT – alpha triangle, CG2 – Chemgauss2, CG3 – Chemgauss3, FRED – Fast Rigid Exhaustive Docking, FRED_CS – ChemScore, GOLD – Genetic Optimization for Ligand Docking, GOLD_CS – ChemScore, LdG – London dG, OECS – OEChemScore, PLP – piecewise linear potential, PT – proxy triangle, SG – Shapegauss, SS – ScreenScore, TM – triangle matcher

Table IV. Percent enrichment factor (%EF) calculated for 11 scoring functions over three protein-ligand complexes

	FRED									GOLD	Surflex-Dock
	CGO	CGT	CG2	CG3	FRED_CS	OECS	SS	SG	PLP	Gold_ score	SD_ score
1M48	Enrichment factor (%)										
1 %	51.65	6.59	24.18	4.40	12.09	41.76	15.38	76.92	28.57	28.57	0.11
5 %	61.54	14.28	31.87	6.59	16.48	50.55	21.98	81.32	43.96	37.36	26.37
10 %	65.93	24.17	34.06	9.89	19.78	63.74	26.37	81.32	51.65	46.15	31.87
1PW6	Enrichment factor (%)										
1 %	72.53	38.46	42.86	1.10	17.58	37.36	4.39	71.43	10.99	5.49	3.30
5 %	73.63	54.94	59.34	2.20	20.88	50.55	27.47	76.92	27.47	17.58	17.58
10 %	73.63	64.84	64.83	3.30	24.17	58.24	36.26	76.92	40.66	25.27	32.53
1PY2	Enrichment factor (%)										
1 %	67.03	31.87	63.74	6.59	17.58	52.75	21.98	79.12	32.97	5.49	7.69
5 %	79.12	50.55	79.12	15.38	17.58	68.13	50.55	84.61	59.34	15.38	16.48
10 %	80.22	56.04	81.319	19.78	28.57	73.63	21.98	87.91	71.43	19.78	26.37

CGO – Chemical Gaussian Overlay, CGT – Chemical Gaussian Tanimoto, CG2 – Chemgauss2, CG3 – Chemgauss3, FRED – Fast Rigid Exhaustive Docking, GOLD – Genetic Optimization for Ligand Docking, OECS – OEChemScore, PLP – piecewise linear potential, SS – ScreenScore, SG – Shapegauss.

became > 3.0 Å. Similarly, ASE with TM produced top ranked conformation with RMSD < 4.0 Å. The re-docking results of all the algorithms with AdG were similar to the results obtained in the case of 1M48. Similarly, AHb in combination with TM, AT and AT did not produce significant results, while docking poses generated by APMI in combination with AHb fell into fair category (RMSD > 2.0 and ≤ 3.0 Å).

During re-docking, all the scoring functions of GOLD produced conformation of FRB with RMSD < 1.0 Å (Fig. 2), while Surflex-Dock generated the docked pose of FRB with RMSD ≤ 1.5 Å. Thus, we can assume that GOLD and SurflexDock are able to generate good conformation of highly flexible ligand (with ≤15 RBs). FRED also produced good orientation of FRB in its top ranked conformation (RMSD < 2.3 Å). These results suggest that GOLD, FRED and Surflex-Dock are appropriate for the surface binding protein like IL-2.

1PY2. – 1PY2 is co-crystallized with the most active inhibitor FRH (IC_{50} = 0.060 μmol L^{-1}) which possesses 15 RBs. The re-docking result of FRH is shown in Fig. 3. The re-docking results of MOE were the same as observed in 1M48 and 1PW6. When applied with LdG, APMI, AT and PT did not give significant results, however, TM depicted 'fair' conformation. The increase in the flexibility of ligand is the main reason of insignificant performance of MOE. TM also produced 'wrong' conformation when used with ASE. The performance of APMI, AT and PT remained the same and these scoring functions failed

Table V. AUC calculated for each scoring function

Scoring functions	1M48	1PW6	$_1PY_2$	Average
CGO	0.764	0.760	0.859	0.794
CGT	0.558	0.697	0.755	0.670
CG2	0.475	0.790	0.905	0.723
CG3	0.334	0.202	0.497	0.494
FRED_CS	0.374	0.480	0.549	0.468
OECS	0.762	0.754	0.845	0.787
SS	0.562	0.594	0.770	0.642
SG	0.936	0.878	0.952	0.922
PLP	0.766	0.622	0.836	0.741
GOLD score	0.786	0.653	0.597	0.679
Surflex score	0.666	0.567	0.546	0.593

CGO – Chemical Gaussian Overlay, CGT – Chemical Gaussian Tanimoto, CG2 – Chemgauss2, CG3 – Chemgauss3, FRED–CS – FREDChemScore, OECS – OEChemScore, PLP – piecewise linear potential, ROC–curve – receiver operating characteristics curve, SS – ScreenScore, SG – Shapegauss. Qualitative interpretation of the area under ROC curve is as follows: fail: 0.0–0.6, poor: >0.6–0.7, fair: >0.7–0.8, good: >0.8–0.9, excellent: >0.9–1.0.

to rank good docking pose as top ranked solution. AdG in combination with all the docking algorithms was unable to find good pose. The RMSD was in the range of 7.0 to 16.0 Å. When applied with AHb, APMI, AT and TM AdG failed to generate good pose, while PT was able to generate fair docked poses with RMSD 2.86 Å.

From the re-docking conducted on three protein-ligand complexes, it can be concluded that the re-docking performance of MOE is relatively less accurate as compared to other docking programs. Some of the docking algorithms resulted with good RMSD with particular scoring function, however, results were inconsistent. For instance, TM performed well with LdG and predicted good and fair conformations of 1M48 and 1PY2 but failed to reproduce good/fair pose of 1PW6. Similarly, PT returned with good and fair pose of 1M48 and 1PY2, resp., when used with AHb. It can be concluded that parameter optimization can lead to enhanced prediction ability. As observed previously, the top ranked docked pose of Surflex-Dock was in good agreement with the experimental data. Surflex-Dock returned with good poses of all three ligands used in this study. Among all the docking programs, the performance of Surflex-Dock was the best when generating the poses with RMSD < 1.5 Å.

GOLD failed to generate either good or fair pose of FRH when used with ASP, ChemPLP, GOLD_CS, and GOLD scoring function. Hence, the docking accuracy of these scoring functions drastically dropped and resulted in RMSD > 10 Å, reflecting that these scoring functions are unable to correctly dock highly flexible molecule.

The FRED predicted top ranked docked pose falls into fair category. The docked conformation was superimposed on the reference ligand, however, the orientation of the

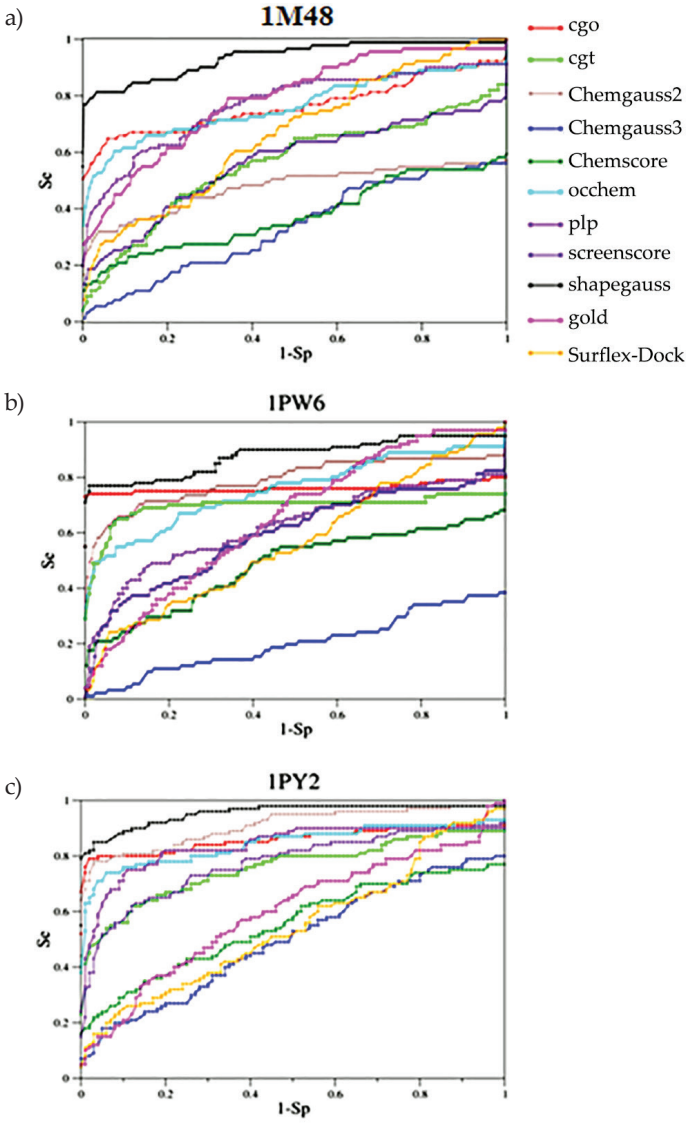


Fig. 4. ROC curve for: a) 1M48, b) 1PW6 and c) 1PY2 (CGO – Chemical Gaussian Overlay, CGT – Chemical Gaussian Tanimoto, GOLD – Genetic Optimization for Ligand Docking, PLP – piecewise linear potential, ROC–curve – receiver operating characteristics curve, Sp – specificity, Sc – selectivity).

flexible alkyl chain at terminal guanidinium moiety is tilted 80°, causing the increase in RMSD.

The re-docking experiments revealed that docking methods faced difficulty to handle highly flexible ligands because of the increased number of possible conformations due to

increased number of rotatable bonds, thus affecting docking accuracy. Hence, docking performance of Surflex-Dock, GOLD with GOLD score and FRED was encouraging, while MOE was neither able to generate good poses in thirty conformations nor, if generated, those conformations were placed in top ranked solutions.

Virtual screening (VS) accuracy. – In this step, only those docking/scoring methods were used that performed well in re-docking or pose selection step. Hence, Surflex-Dock, GOLD_score, and FRED were selected. To scrutinize VS accuracy, 10,000 compounds were selected from ZINC database and 91 known actives were embedded in 10,000 molecules. A set of 10,091 compounds was prepared and docked into each receptor (1M48, 1PW6 and 1PY2) by GOLD, Surflex-Dock and FRED. The ability of scoring methods to effectively differentiate between active ligands and decoys and to rank known inhibitors in top of screening list was assessed by enrichment factor (%EF) which was calculated for each scoring function in top 1, 5 and 10 % of the database screened. The purpose was to identify those scoring functions which have ability to correctly score at least 50 % of the active compounds at the top of their scoring order.

The optimal threshold for the VS accuracy was justified by the following criteria: if in top 1 % (top 100 compounds), in top 5 % (top 500 compounds) and in top 10 % (top 1000 compounds) all the 91 active compounds are successfully identified, then the ideal enrichment factor would be 100, 20, and 10, resp.

1M48. – The EF of 11 scoring functions on 1M48 is tabulated in Table IV. All scoring methods identified active compounds in the decoys set. CGO and SG showed 51 and 76 % for %EF, resp., in top 1% of the screened library, whereas CGT, CG3, and Surflex-Dock score identified only 6, 4 and 0.1 active compounds in top 1 % list, resp. FRED_CS and SS have similar performance, with 12 and 15 enrichment, resp. The performance of CG2, PLP and GOLD score was similar with %EF of 24, 28 and 28 %, resp. The performance of CG2 was better than its newer version CG3. The performance of OECS was also good in identifying the known actives, with 41 % enrichment. OECS achieved better enrichment as compared to FRED_CS. SG achieved better enrichment in top 5 % of the screened database. The %EF of SG and CGO was 81 and 61 %, resp. The performance of Surflex score improved drastically and 26 % active compounds were identified in the top 5 % of the screened database, while CGT, CG2, CG3, FRED_CS, SS and GOLD score showed 14, 31, 6, 16, 21 and 37 % enrichment, resp., in top 5 % of database. OECS and PLP placed 50 and 43 % active compounds, resp., in 5 % list. In 10 % of the screened database, CGO showed 65 % enrichment; CGT, CG2, FRED_CS, SS and Surflex score identified 24, 34, 19, 26 and 31 % active compounds, resp. The overall performance of CG3 was not encouraging; it identified only 9 % active compounds in the top 10 % of the screened database. The performance of GOLD score was improved with 46 % enrichment. PLP correctly identified 51 % active compounds, while OECS achieved 63 % enrichment in top 10 % of ranked library. CGO and OECS identified 50 % active compounds in top 5 % screened library.

$_1$PW$_6$. – In 1PW6, CGO and SG accurately identified 72 and 71 % active compounds in top 1 % of ranked database, resp., while other scoring functions showed < 50 % enrichment in this list. CG3, Surflex score, SS and GOLD score identified < 6 % known inhibitors in top 1 % library, while PLP and FRED_CS identified 10 and 17 % known actives in top 1 % of database screened, resp. OECS, CGT and CG2 showed 37, 38 and 42 % enrichment, resp.

The performance of GOLD score and Surflex score was improved with 17 % enrichment in top 5 % of screened library. The performance of FRED_CS, SS and PLP was consistent as observed previously and only 20 to 27 % enrichment was observed in top 5 % of the list. However, CG3 identified only 2 % active compounds in 5 % screened library. In top 5 % of ranked library 50, 54 and 59 % enrichment were given by OECS, CGT and CG2, resp. In 10 % screened library, CGO and SG correctly ranked more than 70 % of active compounds. CGT and CG2 found >64 % active hits in top 10 % list. OECS and PLP achieved 58 and 40 % enrichment, resp. FRED_CS and GOLD score showed similar results with more than 20 % enrichment. SS identified only 36 % active hits. The performance of CG3 was poor as observed in case of M48. The results indicate that CGO and SG performed well, while CGT, CG2 and OECS were also good as compared to the rest of scoring functions.

1PY2. – On 1PY2, SG, CGO and CG2 successfully identified more than 60 % known inhibitors in top 1 % of the screened library. OECS showed more than 50 % enrichment. The performance of CG3, GOLD score and Surflex score was poor; each of them identified less than 8 % of actives. The scoring prediction of FRED_CS and SS was same with 17 and 21 % enrichment, resp. CGT and PLP identified >30 % active hits. In top 5 % of library, the enrichment of CGO and SG significantly exceeded > 70 %. CG2 and OECS achieved 79 and 68 % enrichment, resp. The performance of CGT, SS and PLP was similar; each of them showed > 50 % enrichment. The success rate of CG3, FRED_CS, GOLD score and Surflex score was < 20 % and did not reached the docking success threshold.

In 10 % of the screened library, CGO, CG2 and SG showed 80, 81 and 87 % enrichment, resp. The success rate of OECS and PLP ranged from 50 to over 70 %. CGT correctly identified 56 % active compounds, however, CG3 and GOLD score showed more than 20 % enrichment. FRED_CS, SS and Surflex score identified <30 % hits. It was observed that some of the scoring functions including CGO and OECS consistently returned with more than 50 % success rate in top 10 % list, while SG efficiently yielded > 75 % hit rate in this cut off. In contrast, CG2 exceeded the docking success rate compared to CG3 for each receptor. The docking success rate of CG2 on 1M48 was < 35 % while it drastically improved with > 60 % on 1PW6 and > 80 % hit rate on 1PY2. Similarly, CGT returned with enrichment < 30 % on 1M48, while the success rate came to around 60 % on 1PW6 and 1PY2. The performance of CG3, FRED_CS, SS, GOLD score and Surflex score was unsatisfactory. The performance of PLP was inconsistent, on 1M48 it showed 51 % hit rate, which decreased to 40 % on 1PW6. However, the success rate drastically improved from 40 to over 70 % on 1PY2 in 10 % screened list.

Binder/non-binder separation

In order to check the consistency of our applied scoring functions, docking results were also analyzed in terms of ROC curves. The *AUC* values are tabulated in Table V. Fig. 4 shows the ROC curves for all three protein complexes with each of the scoring functions studied herein.

1M48 – Fig. 4a shows that CG3, FRED_CS, CG2, CGT and SS produced unsatisfactory results and failed to separate binder from non-binders appropriately. The *AUC*s are shown in Table V. Surflex score produced poor results with an *AUC* of 0.666. The performance of CGO, OECS, PLP and GOLD score was fair with *AUC* 0.7–0.8. SG efficiently separated

binders from non-binders. The average *AUC* was 0.936. SG uses Gaussian function to represent the shapes of molecules. This led to the conclusion that shape based scoring function can be reasonable to find inhibitors for protein-protein interfaces.

1PW6. – The performance of CGO was the same as observed in the case of 1M48 which returned with fair results. CGT showed poor results with *AUC* of 0.697, while CGT failed to differentiate between known actives from decoys. The performance of CG2 was improved and it returned with fair results. The performance of CG3 was insignificant throughout the VS step. It was observed that CG2 outperformed CG3 during VS. The performance of FRED_CS, SS and Surflex score was unsatisfactory, and GOLD score and PLP showed poor result with calculated *AUC* of 0.6–0.66. OECS and CGT showed fair results. SG was outstanding in separating active compounds from non-binders with the calculated *AUC* 0.8–0.9. Fig. 4b represents the calculated ROC curve for all scoring functions applied on 1PW6.

1PY2. – The performance of CGO on 1PY2 was improved with *AUC* 0.859. CGT and SS also resulted with fair *AUC* (Fig. 4c). However, CG3, FRED_CS, GOLD score and Surflex score showed unsatisfactory results as compared to other scoring functions. OECS and PLP significantly differentiated between binders and non-binders and returned with good *AUC*, *i.e.*, 0.845 and 0.836, resp. The results show that SG consistently produced excellent results and efficiently identified known actives from a pool of decoys. Among the nine scoring functions of FRED, SG and CGO were sufficient enough for VS, while CG3 and FRED_CS proved to be unsuitable for VS of IL-2 inhibitors. In this study CG2 was found to be better than CG3. The VS performance of Surflex dock and GOLD was also unsatisfactory. The sequential use of ROCs led us to know that SG and CGO may be considered as a quick way to identify hits.

The average *AUC* was calculated to find out which scoring functions are appropriate for all three protein setups. SG outperformed all the scoring functions in discriminating binders and non-binders. The performance of CGO, OECS, PLP and CG2 was relatively fair, while CG3, FRED_CS and Surflex-dock failed to discriminate known active compounds from decoys. CGT and SS performed poorly with an average *AUC* of 0.6–0.7 (Table V). The results clearly show the effectiveness of SG in finding active compounds in the top of the screened library and efficiently differentiating binders from non-binders.

CONCLUSIONS

IL-2 is involved in protein-protein interactions (PPIs) and play major role in cell development. Finding small molecules that modulate PPIs continues to be a major challenge for drug discovery. With the aim to establish effective VS protocol for IL-2, the feasibility of FRED, GOLD, MOE and Surflex-Dock with multiple scoring functions was assessed. The pose prediction performance of Surflex-Dock was outstanding followed by FRED and GOLD with GOLD score. We identified that two scoring functions namely SG and CGO were the best in the selection of known inhibitors in top 1, 5 and 10 % of the screened library and in differentiating between binders and non-binders. In conclusion, a comprehensive assessment of widely used docking/scoring methods was conducted and an effective VS protocol was set up for the screening of IL-2 inhibitors, which will be used to identify novel IL-2 inhibitors.

Abbreviations, acronyms, symbols. – AHB – alpha HB, AdG – affinity dG, APMI – alpha PMI, ASP – Astex Statistical Potential, AT – alpha triangle, *AUC* – area under the curve, CADD – Computer Aided Drug Design, Chemgauss 2, CG3 – Chemgauss 3, CS – ChemScore, CGO – Chemical Gaussian Overlay, CGT – Chemical Gaussian Tanimoto, %EF – percentual enrichment factor, FRED – Fast Rigid Exhaustive Docking, GA – Genetic Algorithm, GOLD – Genetic Optimization for Ligand Docking, HIC – Hammerhead Incremental Construction, IL-2 – Interleukin-2, IL-2R – IL-2 receptor, IC_{50} – concentration for 50 % inhibition, LdG – London dG, MMFF94 – molecular mechanics force field 94, MOE – Molecular Operating Environment, OECS – OEChemScore, PDB – Protein Data Bank, PLP – piecewise linear potential, PMF – Potential of Mean Force, PPI – Protein-Protein Interactions, PT – proxy triangle, RB – number of rotatable bonds in ligand, RMSD – root mean square deviation, ROC–curve – receiver operating characteristics curve, Sc – selectivity, Sp – specificity, SS – ScreenScore, SG – Shapegauss, Th1 – T-helper 1, TM – triangle matcher, VS – virtual screening, ZINC – Zinc is not Commercial

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