JOURNAL OF CHEMICAL INFORMATION AND MODELING

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DOCKTITE—A Highly Versatile Step-by-Step Workflow for Covalent Docking and Virtual Screening in the Molecular Operating Environment

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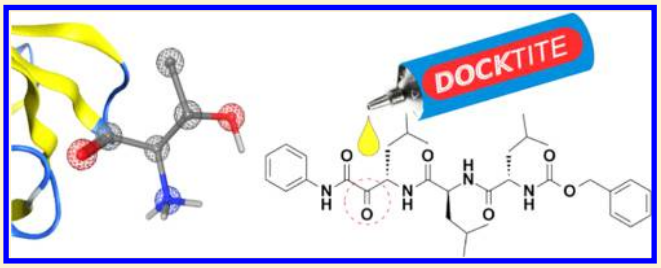
Supporting Information

ABSTRACT: The formation of a covalent bond with the target is essential for a number of successful drugs, yet tools for covalent docking without significant restrictions regarding warhead or receptor classes are rare and limited in use. In this work we present DOCKTITE, a highly versatile workflow for covalent docking in the Molecular Operating Environment (MOE) combining automated warhead screening, nucleophilic side chain attachment, pharmacophore-based docking, and a novel consensus scoring approach. The comprehensive validation study includes pose predictions of 35 protein/ligand complexes which resulted in a mean RMSD of 1.74 Å and a prediction rate of 71.4% with an RMSD below 2 Å, a virtual screening with an area under the curve (AUC) for the receiver operating characteristics (ROC) of 0.81, and a significant correlation between predicted and experimental binding affinities (ρ = 0.806, R^2 = 0.649, p < 0.005).



INTRODUCTION

Computational tools for protein/ligand docking have been developed predominantly for noncovalent modulators of a specific target protein.[1,2] However, the formation of a covalent bond is essential for a number of successful drugs, such as the first and second generation proteasome inhibitors bortezomib and carfilzomib, which are FDA-approved drugs for the treatment of multiple myeloma and mantle cell lymphoma.[3] Improved biochemical efficiency, selectivity, lower and less frequent dosing during patient treatment and improved pharmacodynamics are some of the potential benefits of covalent target inhibition.[4] In the scope of proteasome research, we were looking for suitable in silico methods to identify novel covalent inhibitors of this important threonine protease. Even though a number of popular docking programs employ covalent docking implementations, the structural versatility, accuracy, and automation needed for a large-scale docking-based virtual screening are rare. GOLD[5] for example uses a linker atom which is part of the ligand and the receptor. Schröder et al. expanded this method for virtual screenings by establishing an automated ligand modification script utilizing the software package CACTVS.[6] AutoDock[7] enables the user to choose between two methods for covalent docking. The grid-based method uses an additional grid map to fix the linker atom of the ligand, while the alternative method considers the ligand as a flexible side chain of the receptor. To our knowledge, an automated ligand preparation method has not been provided so far. The first systematic comparison of different covalent docking methods was reported by Ouyang et al.[8] Their method makes use of a dummy atom to ensure the covalent bond and explicitly scores its energy contribution using a Morse potential, which was parametrized for the two examined binding reactions. The most recent methods were published by Zhu et al. (CovDock) and London et al. (DOCKovalent).[9,10] In DOCKovalent, electrophilic warheads are identified by SMARTS patterns and the respective ligands are converted to their bound state prior to the covalent docking. The overall ligand preparation methodology makes use of five different programs, which might be discouraging to new users. To address a wide range of users, the authors provide an easier to use web-accessible version of their method, where predefined ligand libraries can be docked into individual receptor structures.[11] The docking itself is conducted using the software DOCK3.6 and a physics-based scoring function.[12] Zhu et al. use different tools of the software suite Schrödinger to mimic distinct stages of covalent inhibitor binding. The first step is a classical noncovalent docking with an alanine mutation of the nucleophilic side chain followed by an automated bond formation and a second docking step with the covalent bond in place. Their working hypothesis is that a covalently bound ligand has to adopt an energetically favorable unbound pose before bond formation occurs and that these unbound poses do not change dramatically during the reaction pathway, because

Received: November 12, 2014
Published: December 26, 2014

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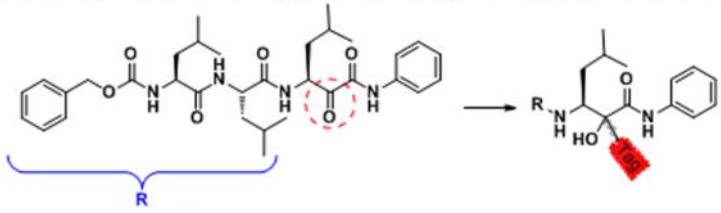
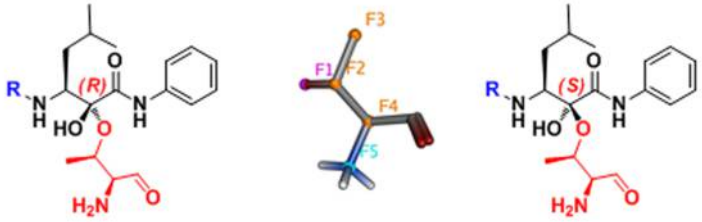
DOI: 10.1021/ci500681r
J. Chem. Inf. Model. 2015, 55, 398−406

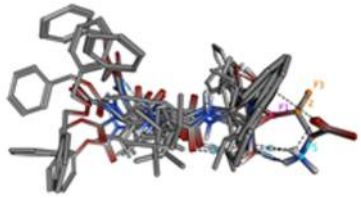
Step1: Warhead Screening. A database of ligands is searched for electrophilic warheads. Subsequently, the electrophilic atom is tagged and the ligand is converted to its bound shape:



Step 2: Side chain Attachment. The tagged ligand atom is now attached to the nucleophilic side chain of the receptor and, for prochiral warheads, stereoisomers of the chimeric molecules are generated. Additionally DOCKTITE generates an automatic pharmacophore (ph4) model for the attached residue and analyzes the active site automatically. This model is used for an exact positioning during the ph4-guided docking process.



Step 3: Pharmacophore-guided Docking. The ph4 placement method is responsible for an exact positioning of the nucleophilic side chain during the docking step.



Step 4: Side chain Cleavage & Pose Rescoring. Estimating the final docking scores leads to best results with free ligands, disconnected from their attached side chain. After the fully automated cleavage step, the rescoring is realized by a newly developed consensus scoring approach using MOE-internal and the external knowledge-based scoring function DSX.

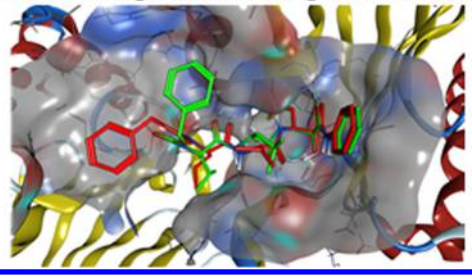


Figure 1. Overview of the DOCKTITE workflow.

conformational sampling is done solely prior the noncovalent docking step. This method works well in most cases but has predictable problems with ligands that change their conformation as a result of target binding, for example due to a distortion of conjugated systems, ring openings, or elimination of leaving groups, leading to limited warhead versatility of CovDock.

In this work we present DOCKTITE, the implementation of a highly versatile covalent docking workflow, capable of virtually screen large databases of potential lead structures, within the Molecular Operating Environment (MOE) via SVL-scripts.[13] The unique step-by-step method allows the user to keep control over each step of a docking project and to tailor it individually. The process is guided by an easy-to-use graphical user interface, so DOCKTITE addresses beginners and advanced users equally. The warhead screening step includes browsing a ligand database for user-defined covalent warheads which are afterward tagged and converted into a bound state. After connecting the potential ligands to the covalently bound residue of the receptor, taking additionally generated stereoisomers into account, a pharmacophore-based docking with optional force field refinement is conducted. Subsequently, the chimeric poses are cleaved and rescored by an innovative consensus scoring approach using MOE-internal empirical

DOI: 10.1021/ci500681r
J. Chem. Inf. Model. 2015, 55, 398−406

scoring functions and the external knowledge-based scoring function DSX.[14] To demonstrate the versatility and accuracy of DOCKTITE, we extensively challenged it to identify near-native binding modes of a large set of ligands with various electrophilic warheads, including aldehydes, boronates, vinyl sulfones, vinyl amides, α-halo ketones, α-keto amides, β-lactones, phosphonofluoridates, benzoxazinones or isocoumarins, and on a variety of proteases and kinases bearing serine, cysteine, or threonine residues as nucleophilic attachment points. In addition to the validation of docking power, we demonstrate that DOCKTITE is an extremely fast and useful tool for large-scale virtual screening purposes, able to differentiate binders from nonbinders and to rank active compounds regarding their experimentally determined binding affinity values in a congeneric series of ligands.

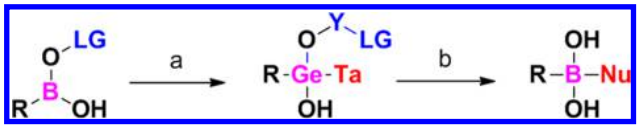
MATERIALS AND METHODS

Workflow. A fast and user-friendly method for covalent docking without restrictions upon warhead or receptor classes is presented, being able to deal with large-scale virtual screening tasks. Furthermore, advanced users may modify our open source program to suit their demands. MOE is predestined for this purpose, as the source codes of its applications are natively open source and written in the rather easy to learn *Scientific Vector Language* (SVL) that was exclusively developed for MOE.[15] The overall workflow is shown in Figure 1.

Step 1: Warhead Screening. In the first step, a database of ligands is screened for predefined electrophilic warheads. Subsequently, the electrophilic atom is tagged and the ligand is converted to its bound shape. All ligand manipulations are done fully automatic by a modified version of MOE's *MedChem Transformations* (MCT), an application initially used to discover novel chemical structures by applying a set of transformation rules to existing ligands. A major advantage of this method is the intuitive way to customize binding reactions. Even for simple warheads, the use of line notations like SMIRKS can be very confusing for the user while in DOCKTITE the reaction is simply drawn in ChemDraw or freeware 2D-Drawers that support the standard reaction format RXN.[16,17] SVL-based modifications were necessary to use a database of ligands instead of a single ligand as the input, whereas the overall algorithm of MCT was left untouched. It consists of a substructure search to match the *query* (left-hand side) of each transformation rule to the input ligands and a replacement of the matched atoms by those of the *result* (right-hand side), while each individual transformation rule indicates whether query atoms or bonds are copied rather than replaced. The tagging is realized by the introduction of a unique atom that has to be absent in all input structures and mimics the nucleophilic atom of the covalent receptor attachment point (see Scheme 1). Tantalum (Ta) is used for temporary tagging, as this element is usually not present in drug-like ligand sets. Furthermore, two additional tagging elements are used in the same manner: yttrium (Y) to define leaving groups and germanium (Ge) to mimic tetravalent boron atoms which are natively not allowed in MCT. Even though all three tagging elements are very uncommon in drug development, they can easily be substituted by the user in the unlikely case of interfering with the ligand set.

Up to now we have implemented 21 of the most common warhead classes known for covalent inhibitors in DOCKTITE, including binding mechanisms featuring rearrangements or leaving groups.[3,18] It is important to note that this set is easily expandable on demand (see Figure 2 for warheads used in this work and the Supporting Information for a complete list of implemented warheads).

Scheme 1. Tagging Elements Used in DOCKTITE[a]



[a](**a**) Tagging elements are placed in step 1: germanium (Ge) to mimic a tetravalent boron (B) atom (purple), tantalum (Ta) to tag the electrophilic atom of the warhead (red), and yttrium (Y) to separate a leaving group from the rest of the molecule (blue). (**b**) Tagging elements serve as signals in step 2 and are replaced: germanium is replaced by a tetravalent boron atom (purple), tantalum is replaced by the nucleophilic side chain of the receptor (red), and the leaving group is deleted (blue).

Step 2: Side Chain Attachment. This is the key step of DOCKTITE, as it includes a variety of modifications and preparations for the final covalent docking. The residue bearing the nucleophilic side chain of the prepared receptor is disconnected from the rest of the protein and fixed in its native position by ph4 constraints. For each individual heavy atom of the residue a ph4 feature is created with a standard size of 0.4 Å. Concurrently the active site is identified and analyzed by means of size and shape to generate an additional pharmacophore feature, requiring any ligand heavy atom, 1.5 Å away from the nucleophilic attachment point projected in the direction of the active site to guide the later pharmacophore-based docking (see Figure 3A). An important advantage of DOCKTITE is that additional information on essential interactions or other known constraints can be easily implemented by user-defined pharmacophore features in this step (see Figure 3B).

The tags, applied in step 1, serve as signals for ligand modifications. After deletion of a possible leaving group and converting a possible Ge-tag to boron, the nucleophilic residue is connected to the Ta-tagged electrophilic attachment point of the ligand. Then, the resulting chimeric molecule is energy-minimized using the force field loaded in MOE. If the warhead of the ligand is prochiral, stereoisomers are generated and treated as individual ligands in all following steps.

Step 3: Pharmacophore-Guided Docking. The final docking step is conducted using MOE's *DOCK* application which can be divided into the following subroutines: (1) generation of conformations, (2) placement, (3) scoring, (4) pose refinement, and (5) rescoring. Conformations are generated by applying a collection of preferred torsion angles to rotatable bonds. A systematic search is carried out covering all combinations of angles on a grid, if this will result in less than 5000 conformers. Otherwise a stochastic sampling of conformations is conducted. The pharmacophore placement method is responsible for an exact positioning of the nucleophilic residue and guides the docking and refinement step. For pharmacophore queries with high complexity, like those obtained in DOCKTITE, the pharmacophore search engine of MOE is used to orient the ligand conformations in the active site. The generated poses are ranked with the empirical scoring function *Affinity dG* which estimates enthalpic contributions to the free energy of binding by a linear function consisting of terms taking hydrogen bonds, ionic interactions, metal ligations, hydrophobic interactions, and

DOI: 10.1021/ci500681r
J. Chem. Inf. Model. 2015, 55, 398−406

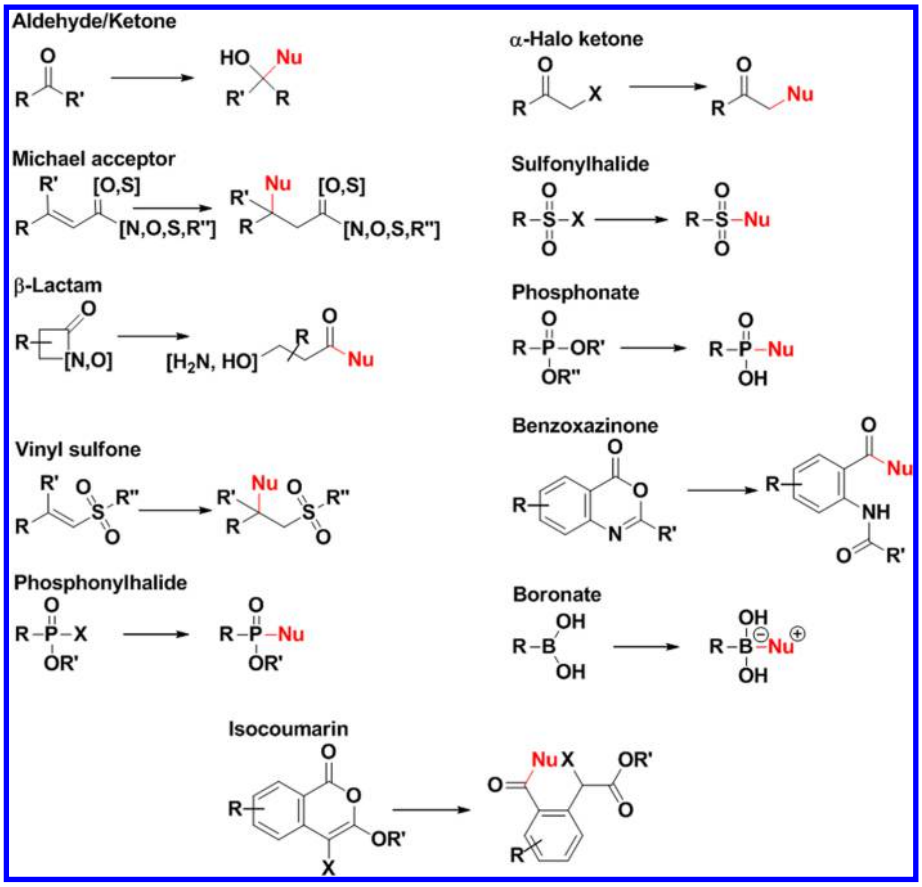


Figure 2. Selection of electrophilic warheads implemented in DOCKTITE (see the Supporting Information for a complete list). Nu: nucleophilic attachment point of the receptor. R, R′, R″: any substituent with terminal carbon. X: any halogene. Note: no stereoisomers and not all possible substitutions are shown for reasons of clarity. The covalent bond is shown in red.

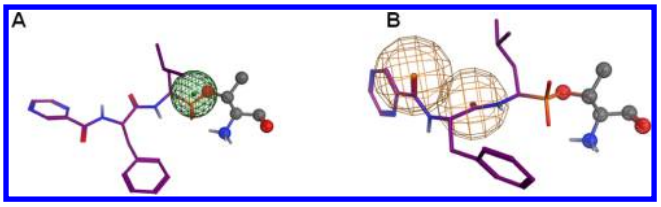


Figure 3. Pharmacophore models mapped on proteasome inhibitor bortezomib (PDB ID: 2f16): **A** automatic ph4 model used in pose prediction and **B** extended ph4 model with additional constraints used in virtual screening. Purple: ligand carbon atoms. Gray: nucleophilic residue carbon atoms. Ph4 features are represented as colored spheres: oxygen (red), nitrogen (blue), carbon (gray), any heavy atom (green), H-bond acceptor (orange).

unfavorable polar/hydrophobic contacts into account. The 100 best scored poses are further refined inside the active site either by energy minimization (in pose prediction, exhaustive virtual screening, and ranking of congeneric ligands) or by grid minimization (in fast virtual screening). For the refinement by energy minimization the *AMBER12:EHT* force field is used, which is parametrized for both proteins and small molecules.[19,20] Using force field refinement, a docking process of one ligand approximately takes 0.5−1 CPU hours on a medium-priced workstation to complete. For typical docking-based virtual screenings with thousands or even millions of ligands, this time scale is not applicable. For these purposes the poses are refined by grid minimization which reduces the CPU time to 10−20 s per ligand. The refined poses are again scored using the *Affinity dG* scoring function. It is noteworthy that, due to the modular nature of the DOCKTITE workflow, this step can be conducted in any modeling software being able to do a pharmacophore-based placement.

Step 4: Side Chain Cleavage and Pose Rescoring. The estimation of final docking scores leads to best results with free ligands, disconnected from their attached side chain, because small conformational alterations in the residue part of the chimeric molecule may have a huge impact of calculated binding affinities. Subsequent to the fully automated cleavage step a multiscoring approach, called consensus scoring, is used to (1) identify the near-native docking pose and (2) estimate the respective free energy of binding. The first task, which is a prerequisite for the second one, is done by the knowledge-based scoring function DSX. In contrast to their empirical counterparts, e.g. *Affinity dG*, knowledge-based scoring functions are not trained with binding affinities. These functions calculate the total score as a sum of statistical potentials, which are derived from a database of known protein/ligand complexes with solved 3D structures, in this case from the Protein Data Bank (PDB).[21] As the score of DSX reflects native binding geometries, predicting a near-native conformation is its key skill while it is not designed to calculate binding energies in particular. The second task is performed by the *Affinity dG* or *London dG* scoring function implemented in MOE, considering only the top pose according to DSX. This consensus scoring approach employed in DOCKTITE combines the superior binding mode predictivity of DSX with greater accuracy of energy estimation by empirical scoring

DOI: 10.1021/ci500681r
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Table 1. Pose Prediction Results for the 76 Complexes and Comparison with Previously Reported Covalent Docking Programs

	DOCKTITE	CovDock	DOCKovalent[d]	CovalentDock	Autodock	GOLD
Top pose[a]	**2.4 Å**	**1.8 Å**	**2.9 Å**	**3.4 Å**	**3.5 Å**	**4.0 Å**
<2 Å[b]	48.7%	63.2%	37.7%	n.a.[e]	n.a.	n.a.
<1 Å[c]	5.3%	31.6%	6.6%	n.a.	n.a.	n.a.
Best in top 10[a]	**1.7 Å**	**1.4 Å**	n.a.	**1.9 Å**	**2.5 Å**	**3.4 Å**
<2 Å[b]	73.7%	77.6%	n.a.	n.a.	n.a.	n.a.
<1 Å[c]	18.4%	50.0%	n.a.	n.a.	n.a.	n.a.

[a]All heavy atom RMSD values are calculated in angstroms from the unmodified X-ray structure. [b]Percentage of correctly predicted poses, when a threshold of 2 Å is defined. [c]Percentage of correctly predicted poses, when a threshold of 1 Å is defined. [d]Two ß-lactam and 13 Michael acceptor inhibitors were excluded from the validation of DOCKovalent.[10] [e]Data not available.

functions, the latter being rather useless when calculated for a "wrong" docking pose.

Ligand Preparation. In general, all ligands are used in their prereacted form. Manual structure modifications are done with the *MOE Builder* if necessary; otherwise, they are directly used from their source database. Hydrogens are added and charges assigned using the potential setup interface. Low-energy conformations of ligands are prepared via energy minimization using the *MMFF94x* force field and a distance-dependent dielectric for solvation treatment.[22]

Receptor Preparation. The X-ray structures are prepared with the *LigX* interface of MOE. *LigX* combines several structure preparation steps including deletion of incidental water molecules, *Protonate 3D*, assigning ASN/GLN/HIS-"Flips", geometry optimization, and energy minimization (RMSD gradient = 0.1 kcal/mol) of the prepared receptor. The *Protonate 3D* application assigns protonation states from a discrete collection of states by optimizing the titration free energy of all titratable groups in the context of an all-atom model of a macromolecular structure (including ligands and solvent).[23] The Generalized Born/Volume Integral Electrostatics model is used for long-range interactions and solvation effects.[24] For energy minimization the *AMBER12:EHT* force field is used.[19,20]

Pharmacophore Preparation. In case of unknown essential interactions between the ligand and the active site, the automatic ph4 model can be used or extended with an additional *AtomQ* feature (which stands for any heavy atom) placed on a dummy atom that is generated with the *MOE SiteFinder* application. In virtual screening and ranking of congeneric ligands, a more precise ph4 model with additional essential features is used, which represents the typical state of knowledge during a drug development process (see Figures 3B and 6B).

Validation Sets. The validation of DOCKTITE was subdivided into three core disciplines exceptionally important for drug design: prediction of near-native binding modes, fast and accurate virtual screening, and ranking of ligands in a congeneric series regarding their experimentally determined binding affinities. Where possible, a comparison of our method with previously published protocols was conducted using the same data sets. However, a new data set for virtual screening was used and the previously reported validation set of CovDock was discarded.[9] We partly disagree with the compound selection, as nothing was known about the inhibition potency of the decoy compounds. The prediction of near-native binding modes was tested using two data sets. The first set was published by Ouyang et al. and contains 76 covalent complexes, subdivided in 13 Michael acceptor and 63 ß-lactam inhibitors.[8] The advantage of this data set is that five previously published methods for covalent docking were validated against it: GOLD, Autodock, CovalentDock, CovDock, and DOCKovalent.[8−10] Nevertheless, warhead and receptor variability in this set of compounds is not representative for today's repertoire of covalent inhibitors. Hence, the versatility of DOCKTITE was validated against a broad data set containing 35 complexes, spanning a majority of known covalent warheads used in drug discovery today. The database is partly derived from the work of Zhu et al. and extended by 11 warhead classes. To our knowledge, most of them have never been explored in a systematic covalent docking study before.[9] To validate the applicability of DOCKTITE in virtual screenings, we used a set of 49 known covalent 20S proteasome inhibitors derived from the public database BindingDB.[25−27] The search for 20S proteasome inhibitors (ß5-subunit) resulted in 63 nonduplicate entries with published K_i or IC_{50} values. This data set was subdivided into 7 active ($K_i/IC_{50} \leq 500$ nM) and 42 inactive compounds ($K_i/IC_{50} \geq 10$ μM), which are further annotated as actives and inactives, respectively (see the Supporting Information for a complete list).[28−33] The validation of DOCKTITE's scoring power was conducted by ranking a congeneric series of 10 c-Src kinase inhibitors with an acrylamide (Michael acceptor) warhead according to their experimentally determined apparent binding affinities (pIC_{50}).[34]

RESULTS AND DISCUSSION

Prediction of Native Binding Modes. Reliability in pose prediction is a prerequisite for other tasks like virtual screening or ranking of congeneric ligands and a focus of the DOCKTITE evaluation. The heavy atom RMSD between a predicted pose and the pose observed in the unmodified crystal structure was used as a metric for prediction accuracy. To compare DOCKTITE with previously reported methods for covalent docking, we validated it against the same 76 complexes used by Zhu et al. and Ouyang et al. as well (see Table 1 and the Supporting Information).[8,9] The pose prediction accuracy (top pose) of DOCKTITE turned out to be superior to those of CovalentDock, AutoDock, and GOLD with an RMSD benefit of at least 1 Å. However, we were unable to outperform the results of CovDock. This special test set consists only of two kinds of covalent ligands (Michael acceptors and ß-lactams), and their binding modes seem to follow the assumption made by Zhu et al., that a ligand keeps its conformation after covalent binding, almost perfectly. The main focus during the development of DOCKTITE was its versatility with respect to electrophilic warhead and receptor classes. This versatility was challenged with an additional validation set of 35 known covalent complexes, partly derived from the work of Zhu et al. and expanded with ligands of 11 warhead classes.[9]

DOI: 10.1021/ci500681r
J. Chem. Inf. Model. 2015, 55, 398−406

Table 2. Redocking of 35 Covalent Complexes, Bearing 14 Distinct Electrophilic Warheads

	input complex			predicted pose			best pose in top 10	
PDB	warhead	residue	R/S^a	$score^{DSX}$	$RMSD^b$	R/S^a	$score^{DSX}$	$RMSD^b$
2awz	acrylamide	Cys366	*R*	−65.88	2.45	*R*	−62.29	1.13
2ax0	acrylamide	Cys366	*R*	−85.06	1.00	*R*	−85.06	1.00
2ax1	acrylamide	Cys366	*R*	−65.47	1.68	*R*	−57.92	1.20
2e14	vinyl ketone	Cys166	*S*	−98.14	1.76	*R*	−98.14	1.76
2hwo	acrylamide	Cys345		−87.63	1.94		−77.17	1.37
2hwp	acrylamide	Cys345		−61.69	2.38		−61.69	2.38
2jiv	acrylamide	Cys797	*S*	−187.30	1.74	*R*	−183.41	1.59
2qlq	acrylamide	Cys345	*R*	−99.80	2.73	*R*	−89.07	1.13
3c9w	vinyl ketone	Cys164	*R*	−102.06	1.08	*R*	−99.38	1.07
3ika	acrylamide	Cys797		−23.00	4.46		−23.00	4.46
3lok	acrylamide	Cys345		−75.21	1.60		−69.29	0.78
3oyp	acrylamide	Cys159		−99.05	2.38		−88.98	2.35
3t9t	acrylamide	Cys442	*S*	−132.14	1.39	*R*	−128.62	0.65
2gvf	α-keto amide	Ser139	*S*	−188.85	1.49	*R*	−165.02	1.33
2obo	α-keto amide	Ser139	*S*	−121.07	0.69	*S*	−117.63	0.68
2oc1	α-keto amide	Ser139	*S*	−142.84	1.47	*R*	−142.84	1.47
2p59	α-keto amide	Ser1165	*S*	−146.83	1.36	*S*	−142.90	1.25
3lox	α-keto amide	Ser139	*S*	−104.97	2.17	*S*	−102.97	1.31
2oc7	α-keto amide	Ser139	*S*	−104.63	1.26	*S*	−89.36	1.05
2oc8	α-keto amide	Ser139	*S*	−118.59	1.23	*S*	−118.59	1.23
2oc0	α-keto amide	Ser139	*S*	−121.24	0.91	*S*	−116.24	0.83
3knx	α-keto amide	Ser139	*S*	−166.90	0.99	*S*	−158.63	0.96
1w3c	α-keto amide	Ser139	*S*	−85.03	2.15	*R*	−83.74	1.45
2f9u	α-keto amide	Ser139	*S*	−154.44	3.43	*S*	−154.44	3.43
1zcm	α-haloketone	Cys115		−70.53	4.00		−47.26	1.55
2g8e	aldehyde	Cys115	*R*	−88.81	0.93	*R*	−88.81	0.93
2f16	boronate	Thr1		−123.52	1.24		−123.52	1.24
4lqi	ß-lactone	Thr1		−58.49	1.61		−53.15	1.24
4int	vinyl sulfone	Thr1	*R*	−145.02	1.36	*S*	−145.02	1.36
3ubb	phosphono-fluoridate	Ser201	*R*	−83.33	2.46	*R*	−78.00	1.45
3zmh	ß-lactam	Ser201		−112.85	1.27		−106.04	1.09
8est	isocoumarin	Ser195		−49.08	1.56		−48.81	0.38
1inc	benzoxazinone	Ser195		−80.19	1.24		−77.25	1.02
1max	phosphonate	Ser195		−118.37	1.00		−118.37	1.00
1klt	sulfonyl halide	Ser195		−37.88	0.49		−37.88	0.48
				mean:	1.74			1.36
				<2 Å:	71.4%			88.6%
				<1 Å:	20.0%			28.6%
				R/S^c:		68.2%		

[a]Configuration of the electrophilic attachment point in the bound state for all prochiral ligands. [b]All heavy atom RMSD values are calculated in angstroms from the unmodified X-ray structures. [c]Percentage of correctly predicted configurations of electrophilic attachment points in bound states.

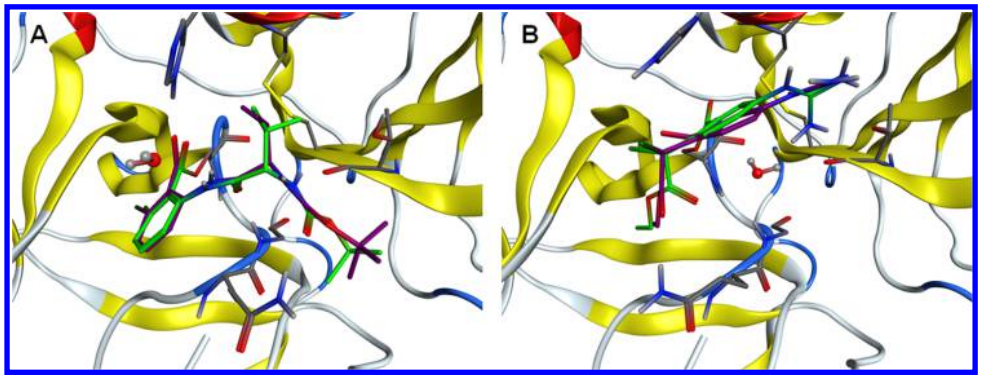

Figure 4. Successful binding mode prediction for ring opening reactions: **A** predicted binding mode of PDB-ID 1inc (benzoxazinone warhead, RMSD = 1.24 Å); **B** predicted binding mode of PDB-ID 8est (isocoumarin warhead, RMSD = 1.56 Å). Green: ligand carbon atoms as observed in the native X-ray structure. Purple: ligand carbon atoms of the predicted pose. Gray: receptor carbon atoms.

DOI: 10.1021/ci500681r
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The RMSDs of the top scoring poses vary from 0.49 to 4.46 Å with a mean deviation of 1.74 Å (see Table 2). Using an RMSD cutoff of 2 Å for correctly predicted poses, DOCKTITE was able to predict experimentally determined native binding modes for 71.4% of the test set. The correct configuration (*R* or *S*) of the electrophilic attachment point for prochiral warheads was predicted in 68.2% of all cases. To check whether pose generation or the scoring function is the weaker link in the chain, we expanded our criterion of success to the top 10 predicted poses which increased the prediction rate of native binding modes to 88.6% with a mean RMSD of 1.36 Å.

Two examples for inherently difficult covalent docking tasks are shown in Figure 4. The difficulties with benzoxazinone and isocoumarin warheads arise because of the ring opening reaction upon ligand binding as shown in Figure 2 and are even more obvious with warheads containing leaving groups (see the Supporting Information). However, in both cases DOCKTITE predicted near-native binding modes with RMSD values of 1.24 Å (benzoxazinone, Figure 4A) and 1.56 Å (isocoumarin, Figure 4B), respectively.

Virtual Screening. The ability to screen large databases for putative covalent inhibitors in a manageable time scale on a common desktop computer was one of the key issues during the development of DOCKTITE. As prediction accuracy and speed often relate inversely, the user has to choose a compromise optimized for a specific project. To give an idea of how such a compromise could look like, the virtual screening validation was conducted twice using an accurate but slow force field refinement step as well as a very fast grid minimization refinement. The data set used consists of 7 active and 42 inactive 20S proteasome inhibitors from 7 warhead classes (aldehydes, ketones, α-keto amides, epoxyketones, β-lactams, boronates, and vinyl sulfones; see the Supporting Information). All ligands were docked into the structure of the bortezomib/20S proteasome complex (PDB-ID 2f16, $RMSD_{Redocking}$ = 1.24 Å). That DOCKTITE is able to predict suitable binding modes for 20S proteasome inhibitors was additionally demonstrated in our pose prediction study using PDB-IDs: 4lqi and 4int (see Table 2).

The identification of active ligands within the library was realized using ligand efficiency (LE = *London dG* score/number of heavy atoms) values derived from the consensus scoring approach, mentioned previously.[35] As a metric for screening quality, the area under curve (AUC) for the receiver operating characteristics (ROC) was used. In a perfect virtual screening, all active ligands would be scored on top of all inactive ligands with an AUC of 1 (see Figure 5, ideal curve). With a random selection of ligands the ROC shows a diagonal line and an AUC of 0.5 (see Figure 5, random curve).

Using the exhaustive mode (force field refinement, Figure 5, blue curve), the AUC of the virtual screening validation was 0.81, which shows that DOCKTITE has a very high predictive power of active ligands in the validation set. With this method, a database of 1000 ligands would approximately need 500−1000 h to complete using a common desktop workstation, which is in the time scale of most covalent docking programs, e.g., CovDock (1−3 h per ligand).[9] In fast mode (grid refinement, Figure 5, red curve), the drop in predictivity is just marginal with an AUC of 0.79 but the gain in speed is enormous: a database of 1000 ligands now approximately needs only 3−5 h to complete. Furthermore, the early enrichment using the faster grid minimization refinement turned out to be superior to using the time-consuming forcefield refinement.

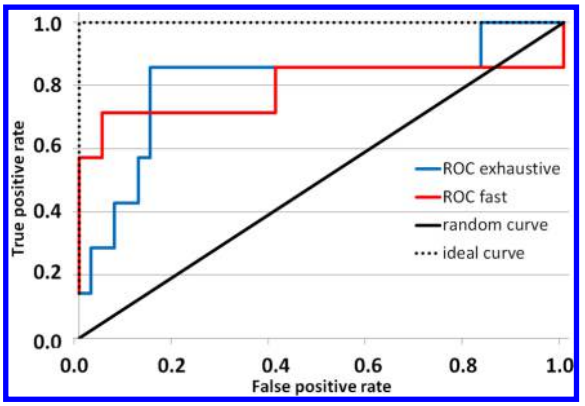

Figure 5. ROC for an exhaustive virtual screening, using force field refinement (blue curve, AUC = 0.81) and fast virtual screening, using grid minimization in the refinement step (red curve, AUC = 0.79).

It has to be noted, that a docking-based virtual screening of potential covalent inhibitors is an extraordinarily difficult task, because input databases generally consist of ligands bearing a variety of electrophilic warheads that differ in reactivity toward a nucleophilic receptor residue. Most scoring functions, also the ones used in DOCKTITE, do not score these reactivity patterns explicitly. As the reactivity of a specific electrophilic warhead is influenced by the rest of the molecule, such a scoring would require a time-consuming quantum mechanical calculation for each ligand, which is counterproductive to a fast virtual screening. Another problem is that IC_{50} or K_i values for irreversible or pseudoirreversible covalent inhibitors are time-dependent and therefore highly controlled by the experimental details.

Ranking of Congeneric Ligands. The validation of scoring power, or affinity prediction, of DOCKTITE was done with a congeneric series of 10 covalent c-Src kinase inhibitors, binding to Cys345 via a Michael acceptor mechanism.[34] The success critically depends on the scoring function used and is extremely error-prone due to simplifications of the underlying biophysics.[14] Adequate calculation of entropic and desolvation effects or protein flexibility would benefit from computationally expensive methods like energy perturbation or *MM-PBSA* calculations. Furthermore, many covalent inhibitors show an irreversible or pseudoirreversible binding mode, thus, the experimental determination of IC_{50} values takes place in a nonequilibrium state and the apparent results are time-dependent.

However, it is expected that in one congeneric series of ligands with IC_{50} values determined under identical experimental conditions, the obtained ligand efficiency scores (LE = *Affinity dG* score/number of heavy atoms) show a modest correlation with experimentally determined apparent binding affinities (pIC_{50}) and that DOCKTITE is of valuable use in the lead optimization process during drug development. In fact, the resulting calculated binding affinities displayed a significant correlation with the experimentally determined values resulting in a Pearson correlation coefficient of ρ = 0.806 (R^2 = 0.649, $p < 0.005$, Figure 6A). All ligands were docked into the X-ray structure of an acrylamide/c-Src kinase complex (PDB-ID: 2qlq, $RMSD_{Redocking}$ = 2.73 Å). Additional complexes for c-Src kinase inhibitors used in our pose prediction validation are PDB-IDs: 2hwo, 2hwp, and 3lok (see Table 2).

To further enhance the scoring power of DOCKTITE, the implementation of more precise scoring algorithms will be the

DOI: 10.1021/ci500681r
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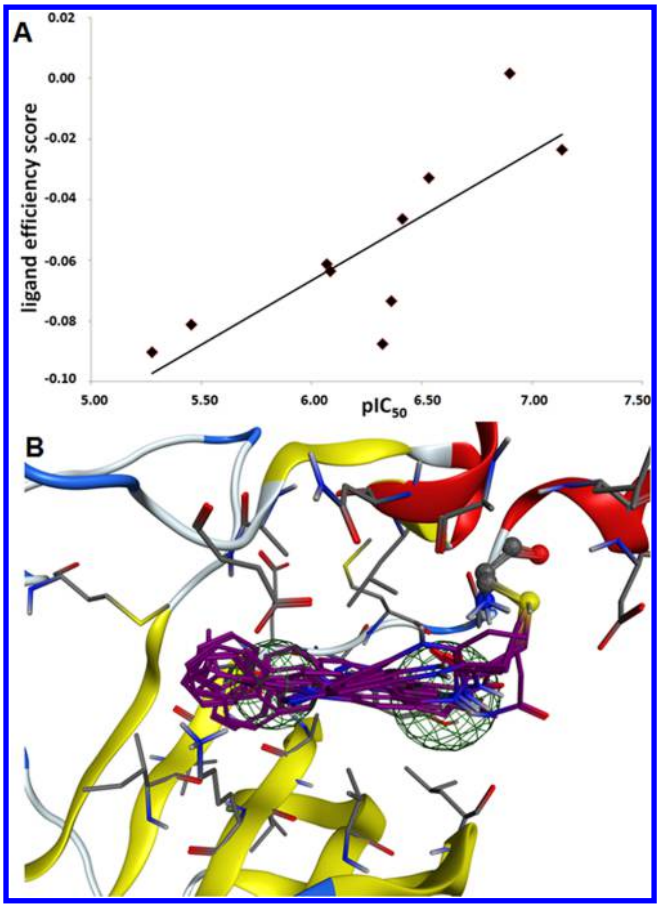

Figure 6. Ranking of congeneric ligands: **A** correlation plot of 10 c-Src kinase inhibitors docked in the X-ray structure of PDB-ID 2qlq (ρ = 0.806, R^2 = 0.649, $p < 0.005$); **B** obtained docking poses mapped on the utilized ph4 model. Purple: ligand carbon atoms. Gray: receptor carbon atoms. Ph4 features are represented as colored spheres: sulfur (yellow), oxygen (red), nitrogen (blue), carbon (gray), any heavy atom (green).

main focus in the future advancement and is under current investigation.

CONCLUSIONS

With DOCKTITE, we have developed a reliable, highly versatile, and user-friendly workflow for pose prediction and virtual screening of covalently bound ligands. Furthermore, it is possible to predict experimental binding affinities with good precision in convenient congeneric series of ligands. The warhead screening step is connected to the *MedChem Transformations* application and allows the user to implement any binding reaction in a very intuitive way. Twenty-one of the most common electrophilic warheads are already preinstalled. Because of the modular and open source nature of DOCKTITE, users may modify any step on their demands and may even outsource specific tasks to other software. The automated pharmacophore model is suitable to guide the docking simulation of any kind of ligand or receptor and can be refined as the knowledge of essential interactions increases during the drug development process. The novel consensus scoring approach used in DOCKTITE combines the strengths of the knowledge-based scoring function DSX and the empirical scoring functions implemented in MOE to give authentic information on near-native docking poses and their related binding affinities. In addition to our pose prediction validation this combined strength becomes apparent in the additional validations of virtual screening performance, where DOCKTITE was able to differentiate between binders and nonbinders very precisely and the validation of scoring power, where the linear correlation of predicted with experimental binding affinities was significant. These characteristics enable DOCKTITE to be a valuable aide at all stages of a modern drug development process and clearly extend the already excellent software environment of MOE.

Software Download. All DOCKTITE SVL scripts including the source codes and graphical user interfaces are available free of charge from the *SVL Exchange* Web site http://svl.chemcomp.com.

ASSOCIATED CONTENT

Supporting Information

Pose prediction results of 76 complexes used in the comparison with other docking programs (Table S1), complete list of implemented electrophilic warheads (Figure S1), structures of proteasome inhibitors used in virtual screening (Chart S1), and structures of c-Src kinase inhibitors used in ranking of congeneric ligands (Chart S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was supported by the Deutsche Forschungsgemeinschaft (DFG), Germany (GrK1657, projects 2E and 3C).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Guido Kirsten, Dr. Kristina Grabowski, Dr. Barbara Sander (Chemical Computing Group), Dr. Paul Czodrowski (Merck KGaA), Dennis Bensinger, and Dr. Constantin Voss (TU Darmstadt) for their help and valuable comments.

ABBREVIATIONS

AUC, area under the curve; CPU, central processing unit; DSX, drug score extended; FDA, Federal Food and Drug Administration; ID, identification number; LE, ligand efficiency; MCT, MedChem Transformations; MM-PBSA, Molecular Mechanics Poisson–Boltzmann Surface Area; MOE, Molecular Operating Environment; n.a., not available; ρ, Pearson correlation coefficient; ph4, pharmacophore; RMSD, root of the mean square distance; pIC_{50}, $-\log(IC_{50})$; ROC, Receiver Operating Characteristics; SVL, Scientific Vector Language

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