

Improving Protein-Ligand Docking Results with High-Throughput Molecular Dynamics Simulations

Hugo Guterres and Wonpil Im*



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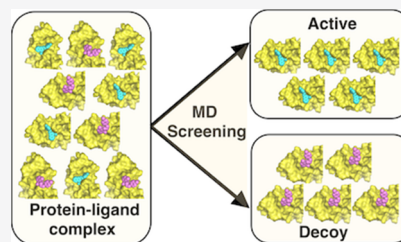


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ABSTRACT: Structure-based virtual screening relies on classical scoring functions that often fail to reliably discriminate binders from nonbinders. In this work, we present a high-throughput protein-ligand complex molecular dynamics (MD) simulation that uses the output from AutoDock Vina to improve docking results in distinguishing active from decoy ligands in a directory of useful decoy-enhanced (DUD-E) dataset. MD trajectories are processed by evaluating ligand-binding stability using root-mean-square deviations. We select 56 protein targets (of 7 different protein classes) and 560 ligands (280 actives, 280 decoys) and show 22% improvement in ROC AUC (area under the curve, receiver operating characteristics curve), from an initial value of 0.68 (AutoDock Vina) to a final value of 0.83. The MD simulation demonstrates a robust performance across all seven different protein classes. In addition, some predicted ligand-binding modes are moderately refined during MD simulations. These results systematically validate the reliability of a physics-based approach to evaluate protein-ligand binding interactions.



INTRODUCTION

Structure-based virtual screening (SBVS) is an important early-phase step in drug discovery workflow that is widely used in the development of new therapeutic agents.^{1,2} Molecular docking is a method of choice for SBVS to predict the binding mode and the binding energy of a small molecule compound to the binding site of a target protein. The increasing availability of high-resolution three-dimensional structures of holo target proteins and rapid advances in computational techniques have contributed to many successful results of SBVS in the past 2 decades. Most recently, Lyu and colleagues ran ultra-large library docking and discovered new chemotypes for AmpC β -lactamase and the D4 dopamine receptor.³ A few of the previous success cases include STX-0119 for lymphoma, novel histamine H4 receptor ligands, and novel Pim-1 kinase inhibitors.^{4–6} In practice, SBVS is attractive because it is capable of quickly screening large compound libraries. In addition, SBVS has been shown to reasonably reproduce experimental binding modes using standard docking programs: for example, AutoDock Vina with 78% success rate in self-docking (i.e., docking of a native ligand back to its receptor protein crystal structure).⁷ Despite this, a major limitation to docking methodology is still the scoring function that aims to approximate the binding affinity and discriminate binders from nonbinders.⁸

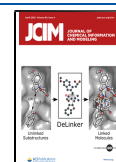
Over the past few years, numerous approaches have been developed to improve the scoring function in SBVS. The most notable ones are machine-learning-based methods that show significant improvements in screening for real binders from a given library of compounds. Recently, water and ligand stability were incorporated into machine-learning scoring functions that successfully boosted AutoDock Vina docking

results.⁹ An enhanced performance in scoring function using machine learning was persuasively reported by Wojcikowski and colleagues using a database of useful decoys—enhanced (DUD-E) dataset.^{10,11} More recently, Yasuo and Sekijima added interaction-energy-based learning to further improve scoring functions tested on the same DUD-E benchmark set.¹² Another notable case was shown by Pereira and colleagues using deep neural network that achieved significant improvements on docking results.¹³ While various types of machine-learning methods have shown success cases in improving the scoring function of SBVS, this approach is still relatively new and actively growing.¹⁴ A few problems persist in these machine-learning-based methods, including judicious choice to obtain a balance between model overfitting to a training set and keeping it generalizable.¹⁵

Physics-based molecular dynamics (MD) simulation is a powerful methodology in biophysical research that provides invaluable structural and dynamics information of protein-ligand interactions at the atomic level. The D.E. Shaw research lab showed a long and unbiased MD simulation that captured the process of a small molecule recognizing and binding to a protein target.¹⁶ The binding site was correctly identified by the ligand and the binding mode was essentially identical to the experimentally determined crystal structure. The MD

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approach effectively includes entropy effects and the role of water molecules during the binding process, unlike machine-learning-based methods. MD has also been sparingly used before and is continually being used today to evaluate and improve SBVS docking results.¹⁷ We had previously shown that short equilibration of MD simulations could better filter near-native pose prediction as compared to a docking scoring function for MDM2 and MDMX inhibitors.¹⁸ Analyzing the ligand stability, we showed that correctly predicted binding modes from docking results tend to be more stable than incorrectly bound ligands during MD simulations. Similarly, Cavalli and colleagues showed that short MD simulations were able to discriminate stable near-native poses from decoy poses of propidium on HuAChE (human acetylcholinesterase).¹⁹ MD was also used by Park and colleagues to demonstrate the differences in binding site flexibility between CDK4 and CDK2 that explained selective binding of CDK4 inhibitors.²⁰ Ogrizek and colleagues used MD to obtain various snapshots for docking that resulted in better discrimination between active from inactive compounds.²¹ Recently, Radom and colleagues showed that for protein-protein docking complexes, MD simulations successfully discriminated correct models from decoy models.²² They revealed that correct models were kinetically stable, whereas decoys were unstable and detached from each other during MD simulations. Nevertheless, relative to docking techniques, MD simulations are still more computationally intensive, challenging, and expensive. These limitations often discourage drug discovery researchers to actively incorporate MD simulations into their workflows. However, recent advances in computational power, decreasing cost, and user-friendly tools have alleviated most of the limitations associated with MD simulations.²³ A few examples include the use of graphics processing units (GPUs) to accelerate MD simulations and web-based graphical user interface (GUI) tools to facilitate system setup.^{24–26}

In this study, we present a high-throughput protein-ligand complex MD simulation method that integrates the functionality of the CHARMM-GUI (<http://www.charm-gui.org>) web server to improve docking results in discriminating small molecule binders from nonbinders.^{27,28} Using AutoDock Vina, we obtain docked protein-ligand complex structures and process them using a python script that uses CHARMM-GUI to automate the production of the MD simulation system and input files for each structure in a high-throughput manner.⁷ Postprocessing of MD data is simply based on the ligand root-mean-square deviation (rmsd) (calculating ligand-rmsd relative to the initial docking structure by aligning the protein structure but not the ligand) to assess ligand stability and distinguish small molecule binders from nonbinders. We evaluate our method on 56 target proteins of seven different protein classes selected from a DUD-E dataset based on their crystal structure resolution.¹¹ For each target, we randomly select 10 compounds, five of which are active and the other five are decoys. Our results show consistently better AUC ROC (area under the curve, receiver operating characteristics curve) compared to docking results. Overall, we achieve AUC of 0.83 compared to 0.68 (AutoDock Vina). In addition, we show that the MD simulation can relax and slightly improve the overall binding mode predicted from AutoDock Vina in some cases. These results indicate a promising high-throughput MD simulation protocol that can be integrated with docking from SBVS to improve its scoring function and binding mode results.

METHODS

We conducted all-atom MD simulations in explicit solvents on 56 protein targets from a DUD-E dataset.¹¹ The DUD-E dataset consists of 102 diverse protein targets and most of them have pharmacological precedence. This dataset has been frequently used as a benchmark to test proposed docking/scoring methodologies.^{10,12,13} In this study, we only included protein targets that have crystal structure resolutions of 2 Å or better and excluded transmembrane protein targets. It reduced our targets to 56 proteins, which still maintain the diversity of protein classes from the original dataset, including seven protein categories with 14 kinases, 10 proteases, 6 nuclear receptors, 2 ion channels (nontransmembrane domains), 2 cytochrome P450, 18 other enzymes, and 4 miscellaneous proteins. For each protein target, we randomly selected five active compounds and five decoy compounds to compare the ability of the docking program and MD simulation in discriminating active from decoy compounds. The native ligand for each target protein was also used as a control for this experiment. The complete list of protein targets and selected ligands are listed in Table S1. In addition, we calculated Tanimoto coefficients of the active compounds (Table S2) and the decoy compounds (Table S3). Tanimoto coefficients were calculated using the F2 fingerprint option on Open Babel 2.4.1.²⁹ Tanimoto score distributions in Figure S1 show that the majority of compounds used in this study are not similar to one another.

The workflow of our method is presented in Figure S2 and further described in the following paragraphs. We chose AutoDock Vina as our docking program, because it has been shown to be one of the best performing docking programs that is freely available to researchers in academia.^{7,30} AutoDock Vina provides a scoring function that relies on knowledge-based potentials and available empirical information to rank ligands docked to protein structures. Protein receptors and ligands were prepared using default criteria from AutoDock Vina.⁷ Receptors were treated as rigid, whereas the ligands were flexible. The search space for docking of ligands was determined based on the coordinate of the native ligand from the crystal structure. A cubic box search space with 22.5 Å edge in each direction was added for ligand docking (i.e., flexible ligand and rigid receptor). For the purpose of running a smooth high-throughput workflow, we only selected the top scoring docked output for each protein-ligand complex.

Protein-ligand complex structures were processed using a python script (script S1) to automate browser actions for the production of MD system and input files using CHARMM-GUI.^{27,28} This script was inspired by a script that utilizes the Solution Builder module for building systems that contain various combinatorial ligand structures from the CHARMM-GUI Ligand Reader and Modeler module.³¹ Topology and parameter files for the ligands were generated using the latest CGenFF via CHARMM-GUI.^{32,33} Protein-ligand structures were solvated in a cubic box using TIP3P water models that extended 10 Å from the protein.³⁴ The systems were neutralized using K⁺ and Cl[−] ions. Periodic boundary conditions were applied to the systems in the NPT (constant particle number, pressure, and temperature) ensemble using Langevin thermostat.³⁵ The particle-mesh Ewald method was used for electrostatic interactions.³⁶ The force-based switching function was used to truncate nonbonded interactions over 10 and 12 Å.³⁷ Hydrogen atoms were constrained using the

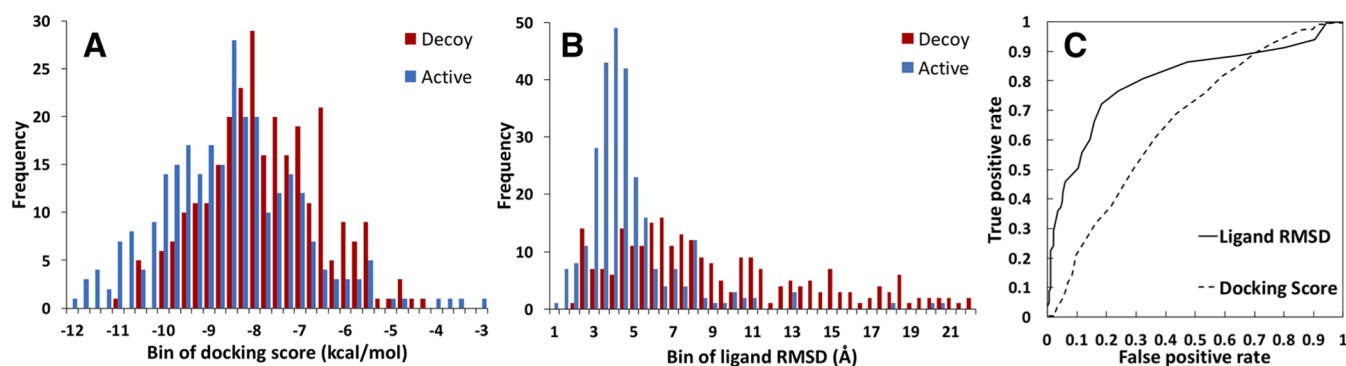


Figure 1. Histogram distributions of (A) docking scores and (B) ligand rmsd for 560 protein-ligand complex structures. Proteins bound to decoy compounds are shown in red and active compounds are shown in blue. (C) ROC plots comparing docking scores with ligand rmsds from MD simulations. The AUC values are 0.832 (ligand rmsd) and 0.683 (docking score).

SHAKE algorithm.³⁸ We selected to use a CHARMM36m force field for all of the simulations.³⁹ Equilibration and production runs were carried out using OpenMM program.⁴⁰ Our systems were minimized for 5000 steps using the steepest descent method followed by 1 ns equilibration with an NVT (constant particle number, volume, and temperature) setting. During equilibration, the ligands were positionally restrained, whereas the proteins were allowed to relax. Restraints were removed for subsequent production runs that were carried out at 303.5 K and 1 bar with an integration time step of 2 fs. For each protein-ligand complex, we ran 3×100 ns production runs, each started with the same initial structure but using different initial velocity random seeds. To evaluate the binding stability of a ligand to its receptor protein, for each snapshot, we first aligned the protein structure only and calculated the ligand rmsd to capture rmsd from the ligand's translation and rotation during the simulation. The proteins were superimposed based on their entire heavy atom coordinates using CHARMM. For each protein-ligand complex, we averaged the ligand rmsd throughout the simulation time (100 ns) and obtained a single ligand rmsd value.

We used PBEQ Solver (Poisson–Boltzmann equation solver) in CHARMM-GUI to calculate and visualize electrostatic potential surface for proteins.^{27,41,42} Subsequently, we overlaid the native ligand-binding mode from each crystal structure as well as decoy ligands to visually assess their binding modes and interactions.

In order to evaluate the conformational changes that the active ligands underwent during the MD simulation, we performed binding pose comparisons with available crystal structures. As we had randomly chosen ligands for this study without considering the availability of their experimental structures, we had to conduct another set of simulations that include protein-ligand complexes with crystal structures. We chose one protein from each of the seven different protein classes and randomly selected five active ligands for each target structure. However, for cytochrome P450, we could only find crystal structures with three different ligands. The complete list of the seven protein targets and their ligand crystal structures are listed in Table S4. We obtained additional 33 ligands and docked these ligands using AutoDock Vina to the seven selected protein targets using the same protocol mentioned above. After docking, we calculated ligand rmsd relative to available crystal structures (Table S4), by aligning protein structures only and calculating their ligand rmsd (crystal structure against predicted docking pose). After MD

simulations, we obtained the last MD trajectory snapshot (a single protein-ligand complex structure) and calculated the ligand rmsd relative to the crystal structure (crystal structure against MD result), by superimposing the protein structures only and calculating their ligand rmsd.

Our simulations were conducted using 1 RTX 2080TI GPU and 1 core processing unit (CPU). The simulation time for the smallest system with 107 residues (23,310 atoms total) was 11.9 h for 100 ns. The biggest system with 540 residues (57,164 atoms) required 26.6 h for 100 ns (Table S5). The MD simulation's speed is orders of magnitude slower than computational docking, where AutoDock Vina docked a molecule to a target within 2 min using four CPUs.

RESULTS AND DISCUSSION

Short MD Simulations Effectively Distinguish Decoys from Active Binders. Docking results from randomly selected 280 active compounds and 280 decoy compounds on 56 target protein (10 compounds per target) show an ROC AUC of 0.683 in its ability to distinguish active from decoy compounds using the AutoDock Vina scoring function (Figure 1). The histogram distribution of active and decoy compounds across the docking scores shows that both have a roughly unimodal distribution. There is a lot of overlap between active and decoy docking scores, where both have similar peaks at -8.5 and -8 kcal/mol, respectively (Figure 1A). As a result, it is difficult to discriminate active from decoy compounds just based on their docking scores. The ROC plot suggests an optimal cutoff (to minimize the false-positive while maximizing the true-positive rate) at a docking score of -8 kcal/mol. At this cutoff, the true-positive rate (of correctly identifying active compounds with <-8 kcal/mol) is 61%, whereas the false-positive rate (of incorrectly identifying decoy compounds as active with <-8 kcal/mol) is 36% (Figure 1C).

The best complex model (with the highest docking score from Vina) for each protein-ligand pair is used as a starting structure for a 100 ns MD simulation. In order to set up this process in a high-throughput manner, only the best docking output is used. MD data are processed by calculating the ligand rmsd (from the starting structure) to evaluate binding stability. The ROC AUC result from ligand rmsd is 0.832 (Figure 1). The histogram shows that active compounds have a unimodal distribution centering at 4 Å, whereas decoy compounds have a skewed-right distribution, showing a lot of ligands leaving the binding site during MD simulations (Figure 1B). It is clear that the majority of decoy ligands can be distinguished from active

Table 1. Summary of AUC ROC from Docking Results Using AutoDock Vina and Ligand rmsd Using MD for a DUD-E Dataset^a

protein class	total ligands			AUC			
	total proteins	actives	decoys	docking	100-ns MD	50-ns MD	10-ns MD
total	56	280	280	0.683	0.832	0.840	0.807
kinase	14	70	70	0.812	0.806	0.836	0.800
protease	10	50	50	0.626	0.824	0.822	0.805
other enzymes	18	90	90	0.665	0.834	0.826	0.801
nuclear receptor	6	30	30	0.622	0.783	0.778	0.804
ion channel	2	10	10	0.950	0.970	0.980	0.910
cytochrome P450	2	10	10	0.550	0.890	0.900	0.835
miscellaneous	4	20	20	0.482	0.910	0.963	0.803

^aImproved cases of AUC values using MD simulations are highlighted in bold.

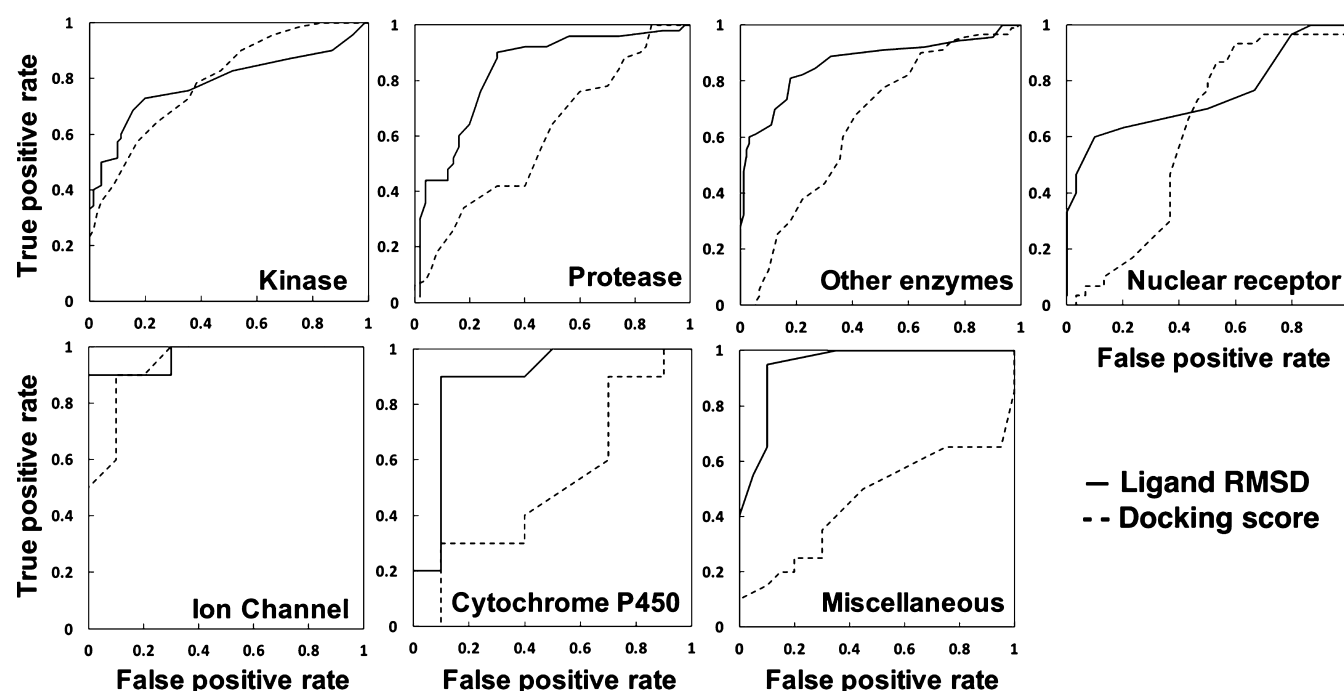


Figure 2. ROC plots comparing docking scores with ligand rmsds from MD simulations for 14 protein kinases, 10 proteases, 18 other enzymes, 6 nuclear receptors, 2 ion channels, 2 cytochrome P450 proteins, and 4 miscellaneous proteins.

ligands based on the distribution of their ligand rmsds. The optimal ROC plot cutoff is at an rmsd of 5.5 Å, where the true-positive rate is 79% with a false-positive rate of 24%. AUC improvement from AutoDock Vina scoring function (AUC of 0.683) to the MD simulation ligand rmsd (AUC of 0.832) is 22%. Using a deep convolution neural network, Pereira and colleagues performed their method on eight randomly chosen protein targets from the DUD-E dataset. Their overall result showed the same AUC from AutoDock Vina as ours (0.68), and their method improved that to 0.77, which amounted to 13% improvement.¹³

The early stage of our workflow, which is directly using the output binding mode from docking, is similar to any other machine-learning-based methods to better score protein-ligand model complex structures.^{10,12,13,22} The limitation of this approach is that incorrect binding modes from docking for active compounds have the potential to deteriorate the ability of MD simulation to correctly identify active compounds. Our results show that a few active ligands are detached from the binding site during the simulation, suggesting that they might have been docked incorrectly at the binding site (Figure 1B).

This problem has also been mentioned in machine-learning-based methods. For example, Pereira and colleagues reported that some poor quality docking output binding modes negatively affected the performance of their method to improve docking results.¹³

Ligand rmsd Scoring from MD Shows Robust Performance across Different Protein Classes. In total, we have 56 target proteins from seven protein classes that include 14 kinases, 10 proteases, 6 nuclear receptors, 2 ion channels, 2 cytochrome P450, 18 other enzymes, and 4 miscellaneous proteins. Table 1 summarizes the AUC for each protein class and Figure 2 shows their ROC plots. Through MD simulations and ligand rmsd evaluation, we observe a similar distribution of ROC AUC values (around an average value of 0.832) across different protein classes. All but one of the protein classes have AUC values of 0.8 and above. The exception is the nuclear receptor protein class that has an average AUC of 0.783. One possible explanation is that nuclear receptor proteins have binding pockets that are closed and buried. Deeply buried (active or decoy) ligands in closed binding pockets tend to stay in the binding sites throughout

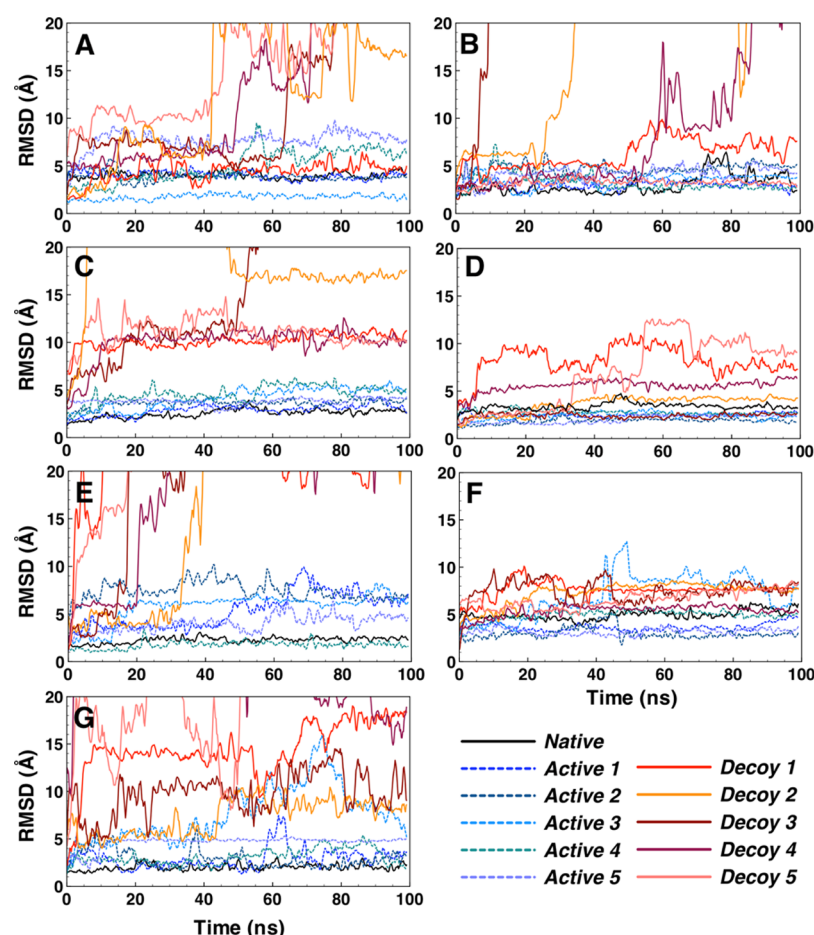


Figure 3. Stability of bound ligands evaluated using ligand rmsd for representative proteins from seven different protein classes. (A) Kinase: tyrosine protein kinase, PDB 2of2, (B) protease: blood coagulation factor VIIa, PDB 1w7x, (C) other enzyme: thymidine kinase, PDB 2b8t, (D) nuclear receptor: retinoid x receptor, PDB 1mv9, (E) ion channel: glutamate receptor, PDB 1vso, (F) cytochrome P450: human CP450 3A4, PDB 3nxu, and (G) miscellaneous: inhibitor of apoptosis, PDB 3hl5.

the simulations, making it more difficult to distinguish between active and decoy ligands. Nevertheless, ligand rmsd improves the overall AUC of nuclear receptor proteins from 0.622 (docking scoring) to 0.783 (Table 1, Figure 2).

The AutoDock Vina scoring function combines knowledge-based potentials with available empirical data, so it relies on a machine-learning predictive nature to discriminate active from decoy compounds.^{1,7} An apparent disadvantage to this approach is that the scoring function can be biased toward certain protein classes with the most available empirical data and/or most popular drug target classes. Our results show that docking outputs from AutoDock Vina tend to favor kinase (AUC of 0.812) and ion channels (AUC of 0.950) in terms of discriminating active from decoy compounds using its scoring function. Meanwhile, miscellaneous proteins (including fatty acid binding protein, heat shock protein, leukocyte adhesion glycoprotein, and inhibitor of apoptosis) have an average AUC of 0.482. This AUC value means that docking scoring function cannot discriminate active from decoy compounds for this set of proteins. Other enzymes, proteases, nuclear receptors, and cytochrome P450 have an average AUC of 0.665, 0.626, 0.622, and 0.550, respectively (Table 1, Figure 2). This problem of scoring functions being biased toward specific protein families has been acknowledged and discussed in a few previous reports.^{10,43,44} Schneider discussed that a specific scoring function calibrated for a data set is usually preferred, because of

their varying predictive accuracy across different protein families.⁴³ Recently, using AutoDock, Ramirez and Caballero showed that its scoring function favored B-Raf kinase over MAO-B and thrombin.⁴⁴ Taken together, having MD simulations as a step after docking to re-assess docked complexes using physics-based theoretical considerations appears to be a more objective and attractive approach. MD simulations incorporate the effects of solvation, flexibility of both protein target and ligand, and better evaluation of nonbonded interactions, which ultimately account for induced fit.

Shorter MD Simulation Runs Can Still Consistently Improve Docking Results. We observe that most ligands with incorrect binding modes and decoy compounds show signs of binding instability when evaluating their ligand rmsd over simulation time (Figure 3). In general, ligands that have to undergo major binding adjustments from their initial docked conformation end up leaving the binding site. During MD simulation, docked ligands change their binding interactions with the proteins to adjust to physics-based potential energy functions from MD force fields. Unfavorable electrostatics and van der Waals interactions result in unbinding of ligands from the proteins. We calculate the AUC ROC by only taking a shorter MD trajectory and compare with the AUC from the full 100 ns. Interestingly, shorter MD simulations, 10 ns (AUC of 0.807) and 50 ns

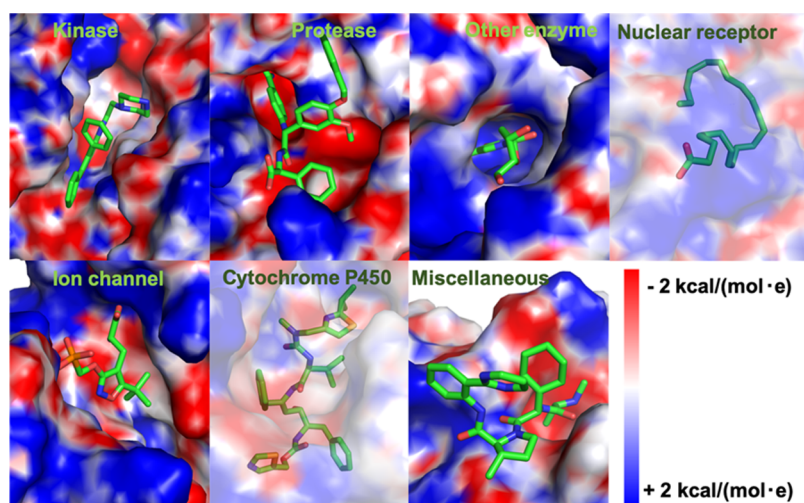


Figure 4. Surface representations showing electrostatic potentials of holo structures with their native ligand-binding modes for representative proteins from seven different classes. Kinase: tyrosine protein kinase, PDB 2of2. Protease: blood coagulation factor VIIa, PDB 1w7x. Other enzyme: thymidine kinase, PDB 2b8t. Nuclear receptor: retinoid x receptor, PDB 1mv9. Ion channel: glutamate receptor, PDB 1vso. Cytochrome P450: human CP450 3A4, PDB 3nxu. Miscellaneous: inhibitor of apoptosis, PDB 3hl5. A transparent surface is used to show ligands that are buried in nuclear receptor and cytochrome P450.

(AUC of 0.840), show similar ability to distinguish active from decoy compounds based on ligand rmsd (Table 1). It has been observed that short simulations can more effectively evaluate the stability of bound ligand than the scoring functions from docking.^{17–20} We previously showed that several hundreds of picoseconds simulations could evaluate ligand-binding stability in MDM2 better than the AutoDock Vina scoring function.¹⁸ It is encouraging to notice that shorter simulations can effectively improve docking results, because it can save resources, time, and be efficiently incorporated into a high-throughput workflow.

We use the DUD-E dataset, which provides decoy compounds that have similar physical properties to known active compounds, but are different in their 2D-topology.⁷ One of the advantages of using MD simulation is that the protein-ligand interactions are depicted as a function of simulation time. In Figure 3, ligand rmsds of representatives from each of the seven protein classes show clear differences in ligand-binding stabilities between active and decoys compounds. During the simulation, we observe that false/weak interactions between decoy ligands and protein targets tend to be short-lived, and some ligands detach from their binding sites. In contrast, strong interactions between active ligands (and native ligands self-docked back to its binding site) and their protein receptors stay stable throughout the simulation. For example, in the case of tyrosine protein kinase (PDB 2of2), a representative of the kinase class, ligand rmsds for four active compounds are stable, whereas all but one decoy compounds are detached from the protein during the simulation (Figure 3A). Binding instabilities are more pronounced as the simulation proceeds to 100 ns, where decoy ligands only start to drift away from the binding site at around 40 ns. Native ligand, furanopyridine 8 binds to tyrosine protein kinase (PDB 2of2) with its pyridine and piperidine rings filling the negative potential binding pocket (Figure 4).⁴⁵ Ligand decoy 5 that detaches from the binding site at 40 ns shows unstable binding with carbonyl group that fills the negative potential binding pocket (Figures 3A and S3).

Protease representative (PDB 1w7x) shows good results of discriminating active from decoy compounds based on their ligand rmsd. All five active ligands and the native ligand show stable binding throughout the simulation. However, only one of the decoy compounds is stable, three of them detach from the binding pocket, and one shows high fluctuations toward the end of the simulation (Figure 3B). The protease-binding site contains an evolutionary conserved S1 pocket that accommodates big ring structures from small molecules.⁴⁶ The native ligand contains a benzamidine that fits in the S1 pocket and forms a salt bridge with Asp189 at the base of the binding pocket (Figure 4).⁴⁷ This binding mode stabilizes the native ligand-binding throughout the simulation time. In contrast, ligand decoy 2 does not contain a moiety that binds at the S1 pocket (Figure S3), resulting in weaker binding affinity and unbinding in MD simulation.

Another enzyme class is represented by thymidine kinase (PDB 2b8t) that shows clear distinctions between active and decoy compounds binding stability. All five active compounds are stable, showing similar fluctuations to the native ligand. However, all five decoy compounds get displaced from the binding pocket within the first 10 ns of the simulation time (Figure 3C). Thymidine kinase has a specific lasso-like loop in its binding site that stabilizes the binding of thymidine moiety (Figure 4).⁴⁸ This binding specificity does not seem to tolerate decoy compounds with a different topology to bind at the binding pocket (Figure S3).

The ion channel protein class is represented by the ionotropic glutamate receptor (PDB 1vso) that demonstrates clear differences in binding stability of active and decoy compounds. All decoy compounds are detached from the binding site by 40 ns. Two of them leave in the first 5 ns (Figure 3E). In contrast, all active compounds stay in the binding site. However, most of the active compounds appear to undergo binding adjustments at the binding pocket with fluctuations in their ligand rmsd. We observe that the binding pocket has an open architecture that allows for more movements of the bound ligand. The binding site of the ligand-binding domain from glutamate receptor adopts a

clamshell-like conformation with S1 and S2 amino acid sequences that loosely sandwich a bound ligand (Figure 4).^{49,50} Ligand decoy 5 contains a big three-ring structure that ends up detaching from the binding site (Figure S3).

The next two classes of proteins, nuclear receptors and cytochrome P450, are represented by RXR retinoid receptor (PDB 1mv9) and CYP3A4 (PDB 3nxu), respectively. The binding pockets in both structures are closed and buried, which substantially limit the ability of bound ligands to escape (Figure 4). The ligand-binding site of a nuclear receptor is enclosed in a deep pocket that is formed by stable structures of the surrounding helices.⁵¹ In addition, ligand-binding has been shown to be largely stabilized by strong hydrophobic interactions and extensive hydrogen-bonding networks that closely contour the shape of specific native ligands. The binding pocket of cytochrome P450 is closed up, but of a significantly bigger volume as compared to a nuclear receptor.⁵² In general, bound ligands in these binding pockets show smaller and more stable ligand rmsd relative to other protein classes (Figure 3D,F). At the same time, they show that active ligands have a better ligand stability relative to decoy ligands, making it possible to discriminate the two. Ligand decoy 1 for a nuclear receptor and decoy 3 for cytochrome P450 appear to bind loosely at the binding site and show more fluctuations during the MD simulation (Figure S3).

The remaining proteins are classified into the miscellaneous class, which is represented by an inhibitor of apoptosis, IAP (PDB 3hl5). It is a relatively small protein (136 residues) with a flat and shallow binding site.⁵³ Its native ligand CS3 shows a hydrogen bond that forms between the terminal amine group of the ligand and Asp320 on the surface binding groove of IAP (Figure 4). At the same binding groove, ligand decoy 1 positions a hydroxyl terminal that results in unstable binding (Figure S3). The AutoDock Vina scoring function cannot discriminate active from decoy compounds for this protein (and others in this class), but the MD simulation can effectively discriminate binders from nonbinders for this protein (Table 1, Figure 3G). All decoy ligands are shown to escape the binding site, and all but one of the active ligands show stable binding throughout the simulation.

Short MD Simulations Show Limited Improvement of Ligand-Binding Modes for Active Compounds. Self-docking is the practice of computational docking of a native ligand back to its receptor protein crystal structure. Cross-docking is the practice of docking a ligand to a receptor protein that originally contains a different ligand. It is commonly accepted that most docking programs can produce good self-docking results across different datasets. A successful docking result generally passes the criterion of having ligand rmsd < 2 Å.⁵⁴ AutoDock Vina was shown to have 78% success rate in self-docking for 190 protein–ligand complex structures in the PDBbind dataset.⁷ Recently, we tested AutoDock Vina self-docking on 40 structures from the Astex list and showed an 82.5% success rate.⁵⁵ Here, a 79% success rate is obtained from self-docking of 56 DUD-E proteins. However, the success rate for cross-docking has been low because of the specific variations in the local shape of the binding site crystal structure that fits its native ligand.⁴⁴

Here, we explore the ability of MD simulations to refine the predicted ligand-binding modes from cross-docking results. Unfortunately, most of the active ligands from the DUD-E dataset do not have crystal structures that we can use as

references. To solve this, we collected active ligands with available crystal structures. Using the same workflow, we selected seven proteins, each one from the seven different protein classes (Table S4). For each target, we selected five ligands, except for CP450 that only has three ligands with crystal structures. For cross-docking, we extracted ligands from these crystal structures and performed docking of these ligands to seven target proteins from the DUD-E. The resulting ligand-binding poses are assessed by calculating their ligand rmsd from their crystal structure. Our results show that short MD simulations can moderately improve the predicted ligand-binding modes from docking (Figure 5). The average

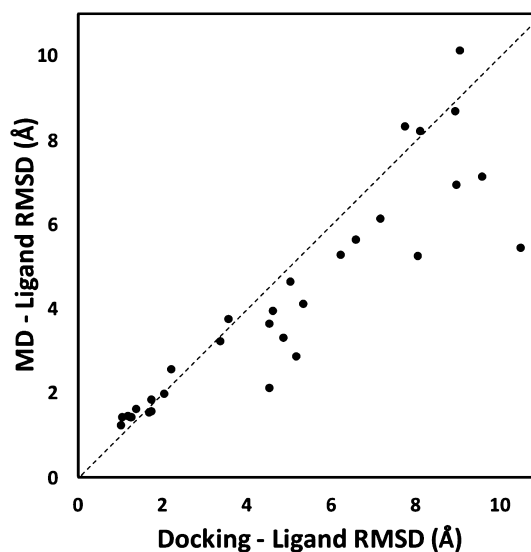


Figure 5. Ligand rmsd values comparing initial docking results with final conformations obtained from MD simulations against their crystal structures for 33 protein–ligand complexes. In many cases, MD simulations moderately improve the predicted ligand-binding modes from docking results. The average improvement is 0.85 Å.

improvement is 0.85 Å (from initial value of 5.21 to a final value of 4.36 Å). Predicted ligand-binding modes from docking that have <2 Å ligand rmsd relative to their crystal structures do not show significant changes in binding modes during MD simulations. Their average change is an increase of 0.08 Å from docking to MD simulation, suggesting that ligands with good initial binding modes tend to stay stable throughout MD simulation runs. Ligands with initial predicted binding modes between 2 and 5 Å relative to their crystal structures show moderate improvements of 0.69 Å on average. The rest of the ligands with >6 Å rmsd away from their crystal structures do not show any significant improvements that can bring them below 5 Å rmsd, suggesting that the changes in their ligand rmsd during MD simulations are random and not significant.

Overall, most of the changes in ligand rmsds are small. Each protein–ligand complex was simulated for 100 ns, which is not enough to refine significantly incorrect binding poses (e.g., ligand rmsd > 5 Å). This is because ligand-binding modes in MD simulations can get trapped in a local minimum (in an energy landscape), which requires a significant amount of time to escape. In particular, for the cross-docking binding pose, two conformations need to change to allow for the correction of the binding pose, including the protein binding site and the ligand. Binding site structures of the same protein that binds different ligands can be significantly different because of an

induced fit.⁵⁶ In a rugged energy landscape with many available local minima, it can be quite difficult for a short MD simulation to correctly identify the global minimum. We expect that significantly longer simulations may result in better improvements, as suggested by previous MD runs by the DE Shaw group that successfully docked ligand dasatinib to its target Src kinase.¹⁶

Ligand-binding modes from docking show a significant majority at 64% with incorrect binding poses (>2 Å). At the same time, ROC AUC values are reasonably good from both Vina (0.68) and MD (0.83) methods to discriminate active from inactive compounds, suggesting that reproducing crystal-like binding poses is not necessary. In our simulations, we observe that most incorrect binding poses of active compounds contain some favorable interactions between the ligand and the binding site residues that are maintained during the simulations. Conversely, most decoy compounds lack favorable interactions and unbind during the simulations (Figure S3). In general, cross-docking results have been shown to be relatively poor compared to self-docking.^{44,56} Ramirez and Caballero recently showed that cross-docking of 30 ligands to three different targets (B-Raf, thrombin, and monoamine oxidase B) resulted in bad ligand poses with >3 Å rmsd relative to their crystal structures.⁴⁴ Our results suggest that severely incorrect predicted binding modes cannot be sufficiently refined during 100 ns MD simulation. However, some moderately incorrect binding modes can be refined with a short MD simulation. There are a few approaches that can be taken to avoid poor cross-docking results. One approach is to filter ligands based on their similarity to the bound ligand in the target structure (similarity selection) suggested by Sutherland and colleagues.⁵⁶ They found that cross-docking accuracy increased when ligands that are similar to the native ligand are used for docking. Another approach is to include side chain residue flexibility in docking experiments to allow for flexible receptor docking. Adding receptor flexibility has been shown to increase the docking success rate using AutoDockFR.⁵⁷ A different approach that considers receptor flexibility is ensemble docking, where an ensemble of representative structures is used to account for differences in binding site structures.⁵⁸ Ensemble docking has also been shown to improve cross-docking outputs.⁵⁹

CONCLUSIONS

It is widely accepted that standard scoring functions from molecular docking programs are still having difficulties in discriminating binders from nonbinders. Here, we propose a high-throughput protein-ligand complex MD simulation method to improve docking results and more accurately distinguish binders from nonbinders. MD simulation effectively incorporates the flexibility of both receptor and ligand, more accurately describes their interactions, and adds solvation effects, which more realistically depicts the protein-ligand binding process. Therefore, MD simulation is an attractive method that has been shown to improve docking results for a few protein targets.^{17–20,22,60} To the best of our knowledge, this is the first study using MD simulations that systematically use a large set of 56 diverse target proteins and 560 ligands from the DUD-E dataset. Our high-throughput method shows consistent improvements in discriminating active from decoy compounds compared to docking scores. Using 100 ns MD simulation as a step after AutoDock Vina docking produces an average AUC of 0.83 from its initial value of 0.68 using

docking score. MD results show robust performance across different protein classes without favoring a particular protein class as observed in docking results. Shorter MD simulations of 50 and 10 ns show similar improvements compared to running 100 ns simulations. Furthermore, we show that some of the ligand-binding modes of active compounds can be moderately refined during MD simulations.

We expect that our high-throughput protocol will make it easier to prepare simulation systems and inputs for virtual screening of protein-ligand complexes using CHARMM-GUI.^{27,28} Our results can serve as a benchmark that showcase the ability of physics-based MD simulations in helping to discriminate binders from nonbinders across a large number of 56 target proteins in the DUD-E. At the same time, a few limitations persist, including the accuracy of ligand force field parameters and computational cost in running virtual screening using MD simulations for thousands of ligands. As described in Introduction, many machine-learning-based methods have been applied to docking results to boost their scoring functions.^{10,12–14} Going forward, it appears that we can take advantage of our benchmark MD dataset for machine-learning training that can be applied to the rest of the ligand dataset in the DUD-E. For example, we can include receptor flexibility information from MD to discriminate binders from nonbinders. Various types of regularly occurring stable interactions throughout MD simulations can also be useful information for machine-learning. We expect that this next step will effectively alleviate limitations that we have with our current protocol.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jcim.0c00057>.

Methods; list of protein targets and ligands; Tanimoto scores between the active compounds; Tanimoto scores between the decoy compounds; list of ligands with crystal structures selected for cross-docking; MD simulation (GPU hours) for the smallest and biggest systems in this study; histogram distributions of Tanimoto scores from active compounds and decoy compounds; schematic workflow; surface representations with bound decoy ligands for representative proteins from seven different classes; and python script for high-throughput protein–ligand complex MD simulations using a CHARMM-GUI webserver (PDF)

AUTHOR INFORMATION

Corresponding Author

Wonpil Im – Departments of Biological Sciences, Chemistry, and Bioengineering, Lehigh University, Bethlehem, Pennsylvania 18015, United States; School of Computational Sciences, Korea Institute for Advanced Study, Seoul 02455, Republic of Korea; orcid.org/0000-0001-5642-6041; Phone: +1-610-758-4524; Email: wonpil@lehigh.edu; Fax: +1-610-758-4004

Author

Hugo Guterres – Departments of Biological Sciences, Chemistry, and Bioengineering, Lehigh University, Bethlehem, Pennsylvania 18015, United States

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.jcim.0c00057>

Notes

The authors declare no competing financial interest.

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