JCIM JOURNAL OF CHEMICAL INFORMATION AND MODELING

pubs.acs.org/jcim Article

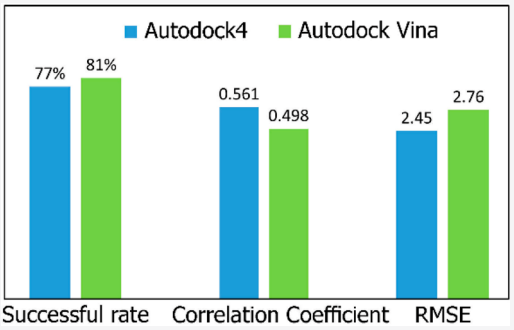
Autodock Vina Adopts More Accurate Binding Poses but Autodock4 Forms Better Binding Affinity

Nguyen Thanh Nguyen, Trung Hai Nguyen, T. Ngoc Han Pham, Nguyen Truong Huy, Mai Van Bay, Minh Quan Pham, Pham Cam Nam, Van V. Vu, and Son Tung Ngo*

Cite This: *J. Chem. Inf. Model.* 2020, 60, 204−211 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The binding pose and affinity between a ligand and enzyme are very important pieces of information for computer-aided drug design. In the initial stage of a drug discovery project, this information is often obtained by using molecular docking methods. Autodock4 and Autodock Vina are two commonly used open-source and free software tools to perform this task, and each has been cited more than 6000 times in the last ten years. It is of great interest to compare the success rate of the two docking software programs for a large and diverse set of protein−ligand complexes. In this study, we selected 800 protein−ligand complexes for which both PDB structures and experimental binding affinity are available. Docking calculations were performed for these complexes using both Autodock4 and Autodock Vina with different docking options related to computing resource consumption and accuracy. Our calculation results are in good agreement with a previous study that the Vina approach converges much faster than AD4 one. However, interestingly, AD4 shows a better performance than Vina over 21 considered targets, whereas the Vina protocol is better than the AD4 package for 10 other targets. There are 16 complexes for which both the AD4 and Vina protocols fail to produce a reasonable correlation with respected experiments so both are not suitable to use to estimate binding free energies for these cases. In addition, the best docking option for performing the AD4 approach is the *long* option. However, the *short* option is the best solution for carrying out Vina docking. The obtained results probably will be useful for future docking studies in deciding which program to use.



INTRODUCTION

Currently, computer-aided drug design (CADD) is often employed to predict top-lead potential compounds that can inhibit the activity of an enzyme.[1] This approach is widely used because it helps reduce significantly the cost and time to develop a pharmaceutical.[2] Ligand-binding affinity prediction is one of the most important parts of the CADD process.[1,3] In particular, the binding affinity of a large set of trial ligands to an enzyme is regularly estimated by using the molecular docking method.[4] A shortlist of these compounds would be then refined via a more computationally expensive binding free energy method such as the linear interaction energy,[5,6] molecular mechanism/Poisson−Boltzmann surface area,[7−9] or fast pulling of ligand[10,11] methods. The top-lead potential inhibitors will be finally validated through an accurate binding free energy approach such as the free energy perturbation,[12,13] thermodynamic integration,[14,15] and nonequilibrium molecular dynamics simulations.[16] Furthermore, calculations required higher accuracy and precision can be conducted via a combination of temperature/Hamiltonian replica exchange molecular dynamics (REMD) simulations and perturbation method.[17−20]

Autodock4 (AD4)[21] and Autodock Vina (Vina)[22] are free open-source packages that can rapidly determine the ligand-binding affinity. Both packages are widely used with approximately 6000 citations per package during the last ten years. AD4 was released in 2009, and Vina has been available since 2010. The AD4 scoring function is semiempirical involving a Coulomb potential term, a Lennard-Jones 12−6 potential term, desolvation associated with volume, and conformational entropy related to the number of rotational bonds.[21] AD4 was involved in the discovery of several potent inhibitors which bind to peptides, proteins, and genes.[23−25] On the other hand, the Vina scoring function is completely empirical and comprises Gaussian steric interactions, repulsion, hydrogen bonds, and hydrophobic and torsion terms.[22] Vina is designed with parallel computing capabilities and is very easy to use.[26] It was indicated that Vina is more accurate than AD4 in evaluating the ligand-binding affinity based on CASF-2013

Received: September 13, 2019
Published: December 30, 2019

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211

benchmark.[27] This explains why Vina has become more popular than AD4 over the past few years. Vina has been used not only to determine binding affinities of small molecules to biomolecular targets including peptides, proteins and genes[28−30] but also to predict binding poses of large substrates to protein targets due to its strong computing capabilities.[31,32]

Although Vina shows a very good performance in estimating the ligand-binding free energy for several targets including the *Hemagglutinin* of Influenza A virus,[33] *Cytochrome P450*,[34] and the *Amyloid beta (Aβ) 1−40* peptide systems,[35] etc. The package poorly estimates the ligand-binding affinity for some targets such as the *human glutaminyl cyclase*[36] and $A\beta_{1-42}$ peptide. Moreover, it is known that the geometrical structure of the $A\beta_{1-42}$ peptides is significantly different from the $A\beta_{1-40}$ peptides, although there are only two different residues.[37] The Vina docking energy changed significantly when ligands were docked to $A\beta_{1-40}$ compared to when they were docked to $A\beta_{1-42}$ peptide, although most of the ligands are docked to the middle of both peptides. This suggests that the docking programs are sensitive to the geometrical structure of the receptor's active site, although the sequences are the same. Furthermore, it implies that different scoring functions of AD4 and Vina may results in very different docking energies even for the same protein−ligand complex. It urges us to do this experiment, in which available inhibitors were docked to targets using both AD4 and Vina packages. Comparing the calculation results with available experimental results would be useful in guiding future studies to decide which packages are suitable for a particular receptor. In this study, we perform docking calculations for 800 ligand−protein complexes by using both AD4 and Vina with the different docking options, which is related to the computing resource consumption and accuracy of obtained results. It should be noted that the experimental structures of 800 complexes were reported on the protein data bank (PDB). The obtained results are in good agreement with the previous study that the Vina approach converges much faster than the AD4 one.[27] However, interestingly, AD4 showed better performance for all of metrics such as correlation coefficient, precision, and success rate over 21 receptors (the first subset). Vina performs better over the second subset which includes 10 receptors. The third subset includes 16 receptors for which both programs produced very poor results. The obtained results probably will be useful for future docking studies in deciding which program to use.

MATERIALS AND METHODS

Structure and Parameter of Complexes. Three-dimensional structures of 800 complexes were obtained from the PDB, which were reported in the Supporting Information (SI file). The rigid receptors and flexible ligands were parametrized via AutodockTools 1.5.6.[21] The parametrized systems were recorded in the PDBQT file. In particular, both receptor and ligand were presented using a united atom model, which involves the polar hydrogen atoms.[38] Atomic charges were estimated through the Gasteiger−Marsili method.[39,40]

Molecular Docking via the Vina Package. Molecular docking using the Vina package was carried out with the global searching exhaustiveness of 8, 56, and 400, which corresponds to *short*, *medium*, and *long* options, respectively. The maximum energy difference between the worst and best docking modes was set to 7 kcal/mol. The grid center of Vina docking was selected as the center of mass of the ligand, which was obtained using the experimental pose. The grid size was set to 20 Å × 20 Å × 20 Å, which is large enough to cover the entire target active site. The best docking mode is the largest ligand-binding affinity. Furthermore, there are 371 200 ligand poses that are generated by the Vina approach, but 48 000 ligand poses were only recorded for evaluating the performance of the Vina approach.

Molecular Docking via the AD4 Package. AD4 docking calculations were performed with the same grid center as Vina ones. The grid size was chosen as 60 × 60 × 60 with the spacing of 0.333 Å, the AD4 grid size is thus of 20 Å × 20 Å × 20 Å. The grid was generated using Autogrid4. The genetic algorithm (GA) run was selected as 50 with the population size and the number of generations are 300 and 27 000, respectively. The GA number of evaluations was chosen as 250 000, 2 500 000, and 25 000 000, which correspond to *short*, *medium*, and *long* options, respectively. The best docking model is the conformational cluster with the lowest binding free energy. Furthermore, there are 120 000 ligand poses that are generated by the AD4 approach. The ligand-binding affinity is the mean binding free energy of the whole conformations located in the cluster.

Structural Analysis. The hydrogen bond (HB) and hydrophobic (HP) contacts between a ligand to a receptor were estimated using the protein−ligand interaction profiler (PLIP)[41] approach. The root-mean-square deviation (RMSD) between two structures was computed via GROMACS tools.[42] The computed error was estimated via 1000 rounds of bootstrapping calculations.[43]

RESULTS AND DISCUSSION

Convergence of Docking Approaches. The computational ligand-binding affinities of 800 compounds to 47 receptors were evaluated by using both docking packages with various options. Interestingly, the AD4 approach gives a larger correlation coefficient (R) with respected experiments than that given by the Vina approach (cf. Figure 1). Moreover,

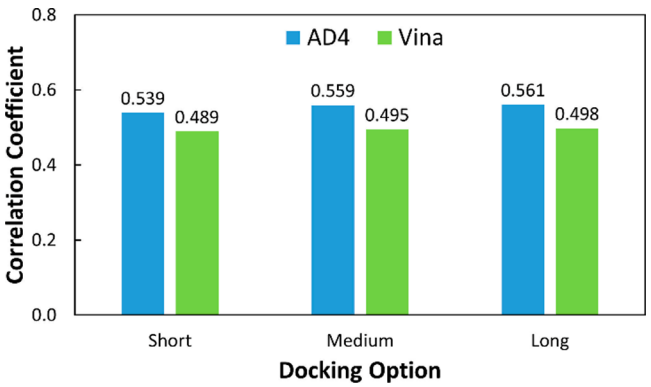


Figure 1. Correlation coefficients between computational and experimental ligand-binding free energies. The computational binding affinity was estimated via the molecular docking approaches including Autodock4 (AD4)[21] and Autodock Vina (Vina)[22] protocols.

the accuracy of the obtained results is dependent on the applied molecular docking options involving *short*, *medium*, and *long* settings, which is associated with the required computing resource. In particular, the AD4 accuracy is significantly enhanced when the docking options change from *short* to *medium* settings with the corresponding

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211

correlation coefficients are of R_{AD4}^{short} = 0.539 ± 0.024 and R_{AD4}^{medium} = 0.559 ± 0.023, respectively. Furthermore, it should be noted that the computing time was significantly increased ∼7 times when the docking option *medium* was selected instead of the *short* option. Consequently, the molecular docking using the *long* option was also carried out. The molecular docking calculations with the long option are tremendously costly and require at least 7 times longer computing time than using the *medium* option. However, the accuracy of molecular docking using the *long* option slightly increased compared to medium option with the correlation of R_{AD4}^{long} = 0.561 ± 0.023, although the mean of docking energy was significantly increased as mentioned below.

In addition, although the Vina approach is less accurate than AD4, an advantage of Vina is that it can run in parallel with multithreading. This results in the faster estimation of binding free energies and docking poses when multicore CPUs become available. Furthermore, calculations with Vina converged faster than with AD4.[27] Indeed, the correlation coefficient between computational and experimental binding free energy changes a tiny (<1%) over the different docking options. The obtained correlation coefficients are R_{Vina}^{short} = 0.489 ± 0.027, R_{Vina}^{medium} = 0.495 ± 0.027, and R_{Vina}^{long} = 0.498 ± 0.026 (cf. Figure 1) corresponding to the applied molecular docking options of *short*, *medium*, and *long* settings, respectively. Furthermore, the root-mean-square error (RMSE) slightly decreases when increasing the searching exhaustiveness option (cf. Figure 2).

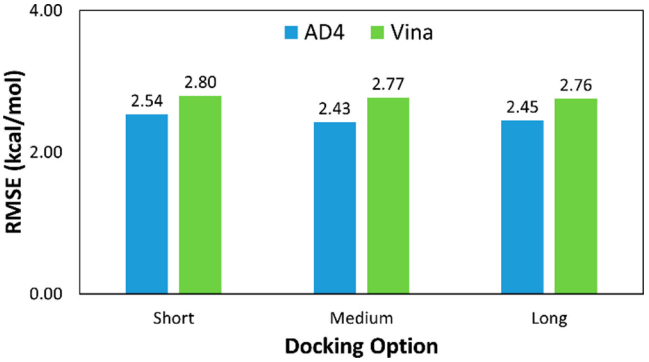


Figure 2. Obtained RMSE of docking results with the appreciated experiments. The docking results are obtained via AD4[21] and Vina[22] protocols.

This implies that, although increasing the searching exhaustiveness from short to long leads to significant increase in computational cost, it does not result in a significant improvement in accuracy and precision.

Estimated Ligand-Binding Free Energy. As mentioned above, the correlation coefficient between calculated and experimental values obtained by AD4 technique (R_{AD4}^{long} = 0.561 ± 0.023) is significantly larger than that obtained by Vina approach (R_{Vina}^{long} = 0.498 ± 0.026) as shown in Figure 1. Moreover, the RMSE of AD4 docking energies with respect to experimental values ($RMSE_{AD4}^{long}$ = 2.45 ± 0.006 kcal/mol) is obviously smaller than that of Vina docking energies ($RMSE_{Vina}^{long}$ = 2.76 ± 0.007 kcal/mol) as shown in Figure 2. The errors of correlation coefficients and RMSE were evaluated via 1000 rounds of bootstrapping estimation.[43] Overall, the AD4 approach is more accurate and precise than the Vina ones since it gives a better correlation and a smaller RMSE with respect to experiment.

The mean of the obtained binding free energies of ligands to receptors is shown in Table 1. Similar to the analysis of the correlation between experimental results and calculated values above, the binding free energies given by Vina approach for various docking options are almost unchanged. In particular, although the required computing times for various docking options are very different, the mean value of docking energies given by Vina protocol are almost the same (cf. Table 1). However, the obtained ligand-binding free energy using Vina approach is significantly different in comparison with the experiments. The average of docking energy given by the Vina approach (ΔG_{Vina}^{long} = −7.80 ± 0.06 kcal/mol) is larger than the experimental value (ΔG_{EXP} = −9.21 ± 0.09 kcal/mol) by an amount of ca. 1.41 kcal/mol (cf. Table 1). Moreover, the computed error by Vina approach, the standard error of the mean, is significantly smaller than that by both AD4 approach and the experiment. The obtained results imply that, on average, the Vina calculations converge faster than the AD4 ones.[27]

In addition, the mean magnitude of ligand-binding free energy estimated by AD4 approach kept increasing upon changing the docking option from *short* → *medium* → *long* settings. The increasing magnitude of docking energy is in good agreement with the increase in the correlation coefficient as mentioned above. Furthermore, the mean magnitude of docking energy is within the error bar of the experimental value when the docking option has selected as *long* option (cf. Table 1). This result suggests that the *long* option is the best solution for AD4 approach in the prediction of the ligand-binding free energy. In contrast, as mentioned above, the correlation coefficients between estimated and experimental metrics using Vina technique are unchanged upon increasing the exhaustiveness values as shown in Figure 1. The RMSE of Vina docking energies is also unchanged over the modification of docking options as shown in Figure 2. Moreover, the required computational resources are very low when the *short* option was selected. Therefore, the *short* option is the best choice in order to evaluate the binding free energy of a ligand to a receptor via Vina approach.

In addition, in order to clarify the physical insight into the docking results, the PLIP[41] approach was employed to analyze the docked conformation in comparison to experimental native poses. On average over 800 complexes, a ligand was found to form 4.06 ± 0.09 HB and 3.70 ± 0.09 HP contacts to a receptor in experiments. It should be noted that the calculated

Table 1. Computational Binding Free Energies in Comparison with Experiments[a]

no.	approach	ΔG^{short}	ΔG^{medium}	ΔG^{long}	ΔG_{EXP}
1	AD4	−8.35 ± 0.08	−8.93 ± 0.08	−9.14 ± 0.09	−9.21 ± 0.09
2	Vina	−7.75 ± 0.06	−7.78 ± 0.06	−7.80 ± 0.06	

[a]The computational binding free energy was obtained by Autodock4 and Autodock Vina. The error is standard error of the mean. The units are kilocalories per mole.

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211

error is the standard error of the mean. The correlation coefficient between AD4 number of HBs and the experimental values (R^{HB}_{AD4} = 0.716 ± 0.020) is significantly larger than that using Vina technique (R^{HB}_{Vina} = 0.678 ± 0.022). Moreover, the correlation coefficient of Vina number of HPs and the corresponding experimental values (R^{HP}_{Vina}= 0.758 ± 0.019) is larger than that via AD4 approach (R^{HP}_{AD4} = 0.733 ± 0.018). Furthermore, AD4 is more precise in estimating the HB between ligands and receptors ($RMSE^{HB}_{AD4}$ = 1.99 ± 0.06 contacts) than Vina is ($RMSE^{HB}_{Vina}$ = 2.03 ± 0.06 contacts). In contrast, Vina approach is better to predict the HP contact between ligands and receptors ($RMSE^{HP}_{Vina}$ = 2.13 ± 0.08 contacts) than AD4 one ($RMSE^{HP}_{AD4}$ = 2.44 ± 0.07 contacts). However, it is known that an HB contact is normally stronger than HP contact by ca. 10 times. Therefore, we may argue that it probably is the reason that the AD4 results are better than Vina approach in terms of correlation with experiment (Figure 1). The magnitude of Vina scoring function weight of hydrogen bonding term probably needs to increase to obtain a better result.

Predicted Ligand-Binding Pose. The predicted ligand-binding pose is also a critical metric beside the estimated ligand-binding free energy. Indeed, because both docking approaches have not considered the complexed dynamics, full influence of explicit solvent and limited-trial position of ligand, the accuracy of the predicted ligand-binding affinity is significantly reduced. The docking results were frequently validated via all-atom MD simulations.[35] The ligand-binding pose somehow emerges as an important factor since it associates with the accuracy of the free energy calculation using molecular dynamics simulations. Moreover, the metric is related to the successful-docking rate, in which the ligand-binding pose is considered as the successfully docked shape if their RMSD of atomic positions from the corresponding experimental structure is less than 2 Å. Interestingly, the obtained results are opposite to the estimation of the ligand-binding free energy that Vina approach is better than AD4 protocol in the determination of the ligand-binding pose. In particular, the mean value of RMSD between the experimental structures and the docked shapes by using AD4 approach is $RMSE_{AD4}$ = 1.42 ± 0.03 Å. This value differs significantly from the corresponding value provided by Vina approach, $RMSE_{Vina}$ = 1.30 ± 0.03 Å. It should be noted that the computed error is the standard error of the mean. Therefore, the successful-docking rate of the AD4 approach is 77 ± 1% which is smaller than that of the Vina scheme which is 81 ± 1%. The observation is very consistent with the previous work.[27] The picture is unchanged when the RMSD threshold for deciding whether a docking pose is considered successful was decreased to 1.5 and 1.0 Å. However, the successful-docking rates delivering by both docking tools are equal when the resolution is reached 0.5 Å (Figure 3). Overall, when considering to use which docking protocol, we need to consider the successful-docking rate also.

List of Systems Favoring the AD4 Approach. As mentioned in the Introduction, the different docking approaches probably give different accuracy for the different systems. The AD4 approach gives a better correlation coefficient with experiments in 21 complexes than that by the Vina approach. The obtained results are shown in Table 2. The correlation coefficient given by the AD4 approach R_{AD4} ranges from 0.508 to 0.904 corresponding to *thrombin* and *alpha-L-fucosidase systems*, respectively. The median is 0.699 over 21 systems. Meanwhile, the Vina approach gives the correlation coefficient R_{Vina} ranging from −0.073 to 0.808 which corresponds to the *neuraminidase* and *alpha-L-fucosidase* systems, respectively. The median is 0.467 only. In particular, there are six complexes having substantial difference between R_{AD4} and R_{Vina} (with deviations $\Delta R_{AD4\rightarrow Vina}$ > 0.3) including

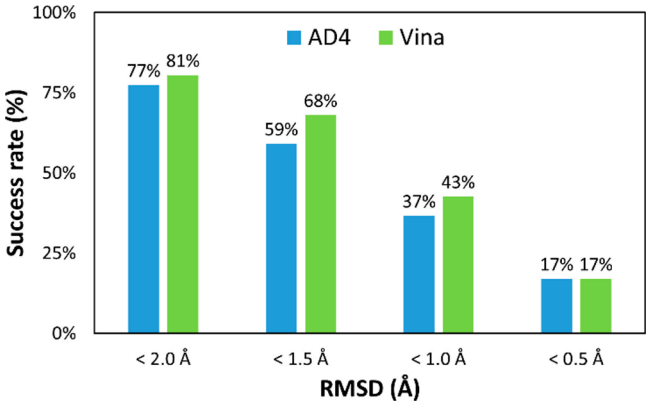


Figure 3. Successful-docking rates obtained via AD4 and Vina protocols upon various resolutions. The errors of successful-docking rates were predicted via 1000 rounds of bootstrapping calculations.[43]

Table 2. Correlation Coefficient between the Computational and Experimental Binding Free Energy for 21 Receptors Which Favor AD4[a]

no.	complexes	N_C	R_{AD4}	R_{Vina}	$\%_{AD4}$	$\%_{Vina}$
1	3-dehydroquinate dehydratase	12	0.782	0.743	100	83
2	alpha-L-fucosidase	7	0.904	0.808	71	86
3	beta-galactosidase	12	0.799	0.285	100	100
4	catechol *O*-methyltransferase	10	0.665	0.470	70	40
5	coagulation factor X	13	0.673	0.612	77	92
6	dipeptidyl-peptidase 4	19	0.571	0.269	89	79
7	glutamate receptor, ionotropic kainate 1	17	0.530	0.371	94	100
8	M1 family aminopeptidase	13	0.659	0.627	69	69
9	mitogen-activated protein kinase 1	9	0.661	0.439	78	67
10	neuraminidase	17	0.520	−0.073	100	100
11	phosphodiesterase 10A2	17	0.814	0.765	1oo	1oo
12	queuine tRNA-ribosyltransferase	18	0.776	0.573	89	94
13	serine/threonine-protein kinase Chk1	10	0.880	0.590	100	80
14	thrombin	86	0.508	0.181	73	78
15	tyrosine phosphatase 1B	20	0.540	0.116	55	50
16	tyrosine-protein kinase JAK1	11	0.882	0.583	100	100
17	tyrosine-protein kinase JAK2	14	0.706	0.528	79	86
18	urokinase-type plasminogen activator	45	0.739	0.273	80	89
19	beta-secretase 1	29	0.606	0.442	69	66
20	cell division protein kinase 2	7	0.777	0.697	100	100
21	cyclin dependent kinase 2	21	0.684	0.508	67	62

[a]The computational binding free energy was obtained via Autodock4 and Autodock Vina. Details of the results are in the Supporting Information. N_C is the number of evaluated complexes.

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211

beta-galactosidase, *dipeptidyl-peptidase 4*, *neuraminidase*, *thrombin*, *tyrosine phosphatase 1B*, and *urokinase-type plasminogen activator*, although the successful-docking rates of these complexes are almost equal. Therefore, the AD4 protocol is strongly recommended to apply to these receptors.

There are nine complexes having significant difference ($0.3 > \Delta R_{AD4 \to Vina} > 0.1$) such as *catechol O-methyltransferase*, *glutamate receptor*, *ionotropic kainate 1*, *mitogen-activated protein kinase 1*, *queuine tRNA-ribosyltransferase*, *serine/threonine-protein kinase Chk1*, *tyrosine-protein kinase JAK1*, *tyrosine-protein kinase JAK2*, *beta-secretase 1*, and *cyclin dependent kinase 2*. It should be noted that the successful-docking rates of these complexes change very little over various docking methods. Our results imply that AD4 should be employed to evaluate the binding affinities and poses of potential inhibitors targeting these systems. However, there are six complexes having insignificant difference ($\Delta R_{AD4 \to Vina} < 0.1$) such as *3-dehydroquinate dehydratase*, *alpha-L-fucosidase*, *coagulation factor X*, *M1 family aminopeptidase*, *phosphodiesterase 10A2*, and *cell division protein kinase 2*. The observation suggests that both AD4 and Vina approaches have similar results on these proteins, although the AD4 protocol is slightly better than the Vina one. More especially, the successful-docking rates of *alpha-L-fucosidase* and *coagulation factor X* given by Vina scheme are much larger than those by the AD4 approach. We may argue that the Vina approach should be used for searching potential inhibitors targeting two enzymes instead of employing the AD4 protocol.

List of Systems Favoring the Vina Approach. The Vina approach was found to be more accurate for 10 systems as shown in Table 3. In particular, the Vina approach produces a better correlation coefficient with experiments in 13 complexes than that by the AD4 approach. It should be noted that we have only discussed systems having an appropriate accuracy in this part ($\Delta R_{Vina} > 0.5$). Moreover, there are 10 systems having an appropriate docking-successful rate and correlation coefficient shown in Table 3, where the Vina approach is indicated as probably better than the AD4 scheme to evaluate the docking energy and pose of a ligand.

Table 3. Correlation Coefficient between the Computational and Experimental Binding Free Energy for 10 Receptors Which Favor Vina[a]

no.	complexes	N_C	R_{AD4}	R_{Vina}	$\%_{AD4}$	$\%_{Vina}$
1	AmpC beta-lactamase	13	0.653	0.654	54	54
2	beta-hexosaminidase	7	0.273	0.907	86	100
3	calmodulin-domain protein kinase 1	9	0.678	0.761	89	100
4	carboxypeptidase A	8	0.774	0.863	38	75
5	purine nucleoside phosphorylase	16	0.663	0.683	100	100
6	scytalone dehydratase	7	0.493	0.553	71	86
7	trypsin	57	0.587	0.675	93	93
8	beta-lactamase CTX-M-9a	6	0.871	0.938	100	83
9	alpha-mannosidase 2	6	0.617	0.734	100	83
10	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform	11	0.711	0.816	73	91

[a]The computational binding free energy was obtained via Autodock4 and Autodock Vina. Details of the results are in the Supporting Information.

For 10 systems reported in Table 3, the metric R_{Vina} obtained by Vina approach ranges from 0.553 to 0.938 corresponding to the *scytalone dehydratase* and *beta-lactamase CTX-M-9a* systems, respectively. The median is of 0.758 over 10 systems. While, the AD4 approach forms the correlation coefficient R_{Vina} ranging from 0.273 to 0.871 which corresponds to the *beta-hexosaminidase* and *beta-lactamase CTX-M-9a* systems, respectively. The median is only 0.632. Moreover, only the *beta-hexosaminidase* complex has a clearly substantial difference between R_{Vina} and R_{AD4} (with deviations $\Delta R_{Vina \to AD4} > 0.3$). The Vina approach also dominates over AD4 in the estimation of ligand binding affinity of *alpha-mannosidase 2* and *phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform* with the deviation $0.3 > \Delta R_{Vina \to AD4} > 0.1$. The seven remaining systems are insignificantly different with $\Delta R_{Vina \to AD4} < 0.1$. Furthermore, it should be noted that the Vina approach is much faster than the AD4 one in evaluating binding information on a protein and a ligand. It may be argued that the Vina approach should be used for searching potential inhibitors targeting these enzymes instead of employing the AD4 protocol.

List of Unfavorable Systems for Both the AD4 and Vina Approaches. Table 4 lists 16 complexes that failed to estimate ligand binding affinity or binding pose via both AD4 and Vina approaches. In particular, three systems including *dehydrosqualene synthase*, *endothiapepsin*, and *N-terminal human maltase-glucoamylase* form poor correlation coefficients and successful-docking rates. Moreover, four complexes including *macrophage metalloelastase*, *stromelysin-1*, *cathepsin K*, and *thermolysin* adopt appropriate correlation coefficients RAD4/Vina > 0.5, but the successful-docking rates are smaller than 50%. Therefore, we have to report that both docking protocols failed when considering the binding affinity of ligands to these

Table 4. Correlation Coefficient between the Computational and Experimental Binding Free Energy for 16 Receptors Which Do Not Favor Either AD4 and Vina[a]

no.	complexes	N_C	R_{AD4}	R_{Vina}	$\%_{AD4}$	$\%_{Vina}$
1	carbonic anhydrase 2	56	0.234	0.11	84	86
2	cathepsin K	8	0.595	0.673	25	38
3	dehydrosqualene synthase	7	−0.054	0.231	29	43
4	endothiapepsin	9	0.407	0.364	11	11
5	estrogen receptor	14	0.388	0.484	100	100
6	factor XA	17	−0.151	−0.183	100	100
7	glycogen phosphorylase	14	0.353	0.214	100	93
8	Hsp90	27	0.263	0.235	63	89
9	macrophage metalloelastase	14	0.931	0.833	21	43
10	NS3 protease, NS4A protein	10	0.432	0.419	70	70
11	N-terminal human maltase-glucoamylase	7	0.137	0.454	43	57
12	penicilin amidohydrolase	7	0.299	0.148	70	70
13	ribonuclease A	27	0.081	0.212	81	89
14	stromelysin-1	8	0.629	0.607	15	7
15	thermolysin	15	0.319	0.501	33	47
16	thymidylate kinase	13	0.425	0.212	62	92

[a]The computational binding free energy was obtained via Autodock4 and Autodock Vina. Details of the results are in the Supporting Information.

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211

enzymes. On the contrary, nine remaining systems adopt poor correlation coefficients, although they form very high docking-successful rates ($\%_{AD4/Vina}$ > 70% 0.3) including *carbonic anhydrase 2*, *estrogen receptor*, *factor XA*, *glycogen phosphorylase*, *Hsp90*, *NS3 protease-NS4A protein*, *penicilin amidohydrolase*, *ribonuclease A*, and *thymidylate kinase*. Clearly, both AD4 and Vina approaches are unable to estimate accurately the ligand-binding affinity targeting the nine systems, but the docking protocols could be employed to evaluate the ligand-binding pose. The ligand-binding affinity would then be clarified by using further study such as molecular dynamics simulations. Overall, the list may lead future studies to use docking protocols accordingly.

CONCLUSIONS

The AD4 and Vina approaches are two popular docking programs widely used to predict protein−ligand binding affinities and poses. Their relative strengths and weaknesses are useful information for the docking community. In this study, we have performed a comparative investigation to benchmark the two programs in terms of their accuracy, precision, and convergence of binding affinity and pose prediction for 800 complexes including 800 ligands and 47 receptors. For each program, we considered three running options, including *short*, *medium*, and *long*, which correspond to increasing computation time and, hopefully, to increasing accuracy. Overall, AD4 gives higher accuracy and precision of the calculated binding energies with respect to experiment than Vina does. However, Vina calculations converge faster than those of AD4 because when changing the docking option from *short* to *medium* to *long*, the correlation coefficient of AD4 binding energies increases quite significantly whereas that of Vina is almost unchanged. The two programs were also benchmarked for their ability to reproduce experimental binding poses. In this regard, we have seen the opposite trend in which Vina is better at predicting the native poses than AD4 with the success rate of 81% versus 77%. Furthermore, the noncovalent bonding analyses indicate that the number of HBs between a ligand and a receptor obtained via the AD4 approach correlates more with experiments that found with Vina. The increase in the magnitude of Vina scoring function weight of the hydrogen bonding term probably enhances the performance of the Vina protocol.

Since no program is better than the other for all systems, we have tried to provide a useful lesson learned from this benchmarking study in terms of which system should be run with which program. To this aim, we have classified the total of 47 receptors used in this study into 3 subsets. The first subset includes 21 receptors for which AD4 showed better performance for the correlation coefficient, precision, and success rate. The second subset includes 10 receptors for which Vina performs better. The third subset includes 16 receptors for which both programs produced very poor results. We expect that the lesson we learned here will be useful for future docking studies in deciding which program to use.

In addition, the best docking option for performing the AD4 approach is the *long* option. However, the *short* option with exhaustiveness = 8 is the best solution for carrying out Vina docking. It is one of the critical factors when considering which approach should be used due to the different required computing resources.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jcim.9b00778.

List of complexes and details of computational results (PDF)

AUTHOR INFORMATION

Corresponding Author

Son Tung Ngo − *Ton Duc Thang University, Ho Chi Minh City, Vietnam;* orcid.org/0000-0003-1034-1768; Email: ngosontung@tdtu.edu.vn

Other Authors

Nguyen Thanh Nguyen − *Ho Chi Minh City University of Science, Ho Chi Minh City, Vietnam*

Trung Hai Nguyen − *Ton Duc Thang University, Ho Chi Minh City, Vietnam;* orcid.org/0000-0003-1848-3963

T. Ngoc Han Pham − *Ton Duc Thang University, Ho Chi Minh City, Vietnam*

Nguyen Truong Huy − *Ton Duc Thang University, Ho Chi Minh City, Vietnam*

Mai Van Bay − *The University of Da Nang, University of Science and Technology, Da Nang City, Vietnam*

Minh Quan Pham − *Vietnam Academy of Science and Technology, Hanoi, Vietnam;* orcid.org/0000-0001-6922-1627

Pham Cam Nam − *The University of Da Nang, University of Science and Technology, Da Nang City, Vietnam;* orcid.org/0000-0002-7257-544X

Van V. Vu − *Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam;* orcid.org/0000-0003-0009-6703

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jcim.9b00778

Author Contributions

All authors designed the studies, collected and analyzed data, and wrote the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Vietnam National Foundation for Science & Technology Development (NAFOSTED) grant no. 104.99-2019.57.

REFERENCES

(1) Yu, W.; MacKerell, A. D. Computer-Aided Drug Design Methods. In *Antibiotics: Methods and Protocols*; Sass, P., Ed.; Springer New York: New York, 2017; pp 85−106.

(2) Marshall, G. R. Computer-Aided Drug Design. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 193−213.

(3) Nguyen, T. H.; Zhou, H.-X.; Minh, D. D. L. Using the fast fourier transform in binding free energy calculations. *J. Comput. Chem.* **2018**, *39* (11), 621−636.

(4) Gehlhaar, D. K.; Verkhivker, G.; Rejto, P. A.; Fogel, D. B.; Fogel, L. J.; Freer, S. T. Docking Conformationally Flexible Small Molecules into a Protein Binding Site through Evolutionary Programming. In *Proceedings of the Fourth International Conference on Evolutionary*

Programming, March 1−3, 1995; McDonnell, J., Reynolds, R., Fogel, D., Eds.; MIT Press: San Diego, 1995.
(5) Aqvist, J.; Medina, C.; Samuelsson, J.-E. A New Method for Predicting Binding Affinity in Computer-Aided Drug Design. *Protein Eng., Des. Sel.* **1994**, *7* (3), 385−391.
(6) Jones-Hertzog, D. K.; Jorgensen, W. L. Binding Affinities for Sulfonamide Inhibitors with Human Thrombin Using Monte Carlo Simulations with a Linear Response Method. *J. Med. Chem.* **1997**, *40* (10), 1539−1549.
(7) Kollman, P. A.; Massova, I.; Reyes, C.; Kuhn, B.; Huo, S.; Chong, L.; Lee, M.; Lee, T.; Duan, Y.; Wang, W.; Donini, O.; Cieplak, P.; Srinivasan, J.; Case, D. A.; Cheatham, T. E. Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Acc. Chem. Res.* **2000**, *33* (12), 889−897.
(8) Kuhn, B.; Kollman, P. A. Binding of a siverse set of ligands to avidin and streptavidin: an accurate quantitative prediction of their relative affinities by a combination of molecular mechanics and continuum solvent models. *J. Med. Chem.* **2000**, *43* (20), 3786−3791.
(9) Wang, W.; Kollman, P. A. Computational study of protein specificity: the molecular basis of HIV-1 protease drug resistance. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98* (26), 14937−14942.
(10) Ngo, S. T.; Hung, H. M.; Nguyen, M. T. Fast and Accurate Determination of the Relative Binding Affinities of Small Compounds to HIV-1 Protease using Non-Equilibrium Work. *J. Comput. Chem.* **2016**, *37* (31), 2734−2742.
(11) Ngo, S. T.; Nguyen, M. T.; Nguyen, M. T. Determination of the absolute binding free energies of HIV-1 protease inhibitors using non-equilibrium molecular dynamics simulations. *Chem. Phys. Lett.* **2017**, *676*, 12−17.
(12) Zwanzig, R. W. High-temperature equation of state by a perturbation method. I. Nonpolar gases. *J. Chem. Phys.* **1954**, *22* (8), 1420−1426.
(13) Beveridge, D. L.; DiCapua, F. M. Free energy via molecular simulation: applications to chemical and biomolecular systems. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18* (1), 431−492.
(14) Kirkwood, J. G. Statistical Mechanics of Fluid Mixtures. *J. Chem. Phys.* **1935**, *3* (5), 300−313.
(15) Kollman, P. Free energy calculations: applications to chemical and biochemical phenomena. *Chem. Rev.* **1993**, *93* (7), 2395−2417.
(16) Jarzynski, C. Equilibrium free-energy differences from non-equilibrium measurements: A master-equation approach. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **1997**, *56* (5), 5018−5035.
(17) Ngo, S. T.; Nguyen, T. H.; Tung, N. T.; Nam, P. C.; Vu, K. B.; Vu, V. V. Oversampling Free Energy Perturbation Simulation in Determination of the Ligand-Binding Free Energy. *J. Comput. Chem.* **2019**, DOI: 10.1002/jcc.26130.
(18) Jiang, W.; Roux, B. Free Energy Perturbation Hamiltonian Replica-Exchange Molecular Dynamics (FEP/H-REMD) for Absolute Ligand Binding Free Energy Calculations. *J. Chem. Theory Comput.* **2010**, *6* (9), 2559−2565.
(19) Meng, Y.; Sabri Dashti, D.; Roitberg, A. E. Computing Alchemical Free Energy Differences with Hamiltonian Replica Exchange Molecular Dynamics (H-REMD) Simulations. *J. Chem. Theory Comput.* **2011**, *7* (9), 2721−2727.
(20) Jiang, W.; Thirman, J.; Jo, S.; Roux, B. Reduced Free Energy Perturbation/Hamiltonian Replica Exchange Molecular Dynamics Method with Unbiased Alchemical Thermodynamic Axis. *J. Phys. Chem. B* **2018**, *122* (41), 9435−9442.
(21) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30* (16), 2785−2791.
(22) Trott, O.; Olson, A. J. Improving the speed and accuracy of docking with a new scoring function, efficient optimization, multithreading. *J. Comput. Chem.* **2009**, *31*, 455−461.
(23) Salveson, P. J.; Haerianardakani, S.; Thuy-Boun, A.; Yoo, S.; Kreutzer, A. G.; Demeler, B.; Nowick, J. S. Repurposing Triphenylmethane Dyes to Bind to Trimers Derived from Aβ. *J. Am. Chem. Soc.* **2018**, *140* (37), 11745−11754.
(24) Corre, S.; Tardif, N.; Mouchet, N.; Leclair, H. M.; Boussemart, L.; Gautron, A.; Bachelot, L.; Perrot, A.; Soshilov, A.; Rogiers, A.; Rambow, F.; Dumontet, E.; Tarte, K.; Bessede, A.; Guillemin, G. J.; Marine, J.-C.; Denison, M. S.; Gilot, D.; Galibert, M.-D. Sustained Activation of the Aryl Hydrocarbon Receptor Transcription Factor Promotes Resistance to BRAF-Inhibitors in Melanoma. *Nat. Commun.* **2018**, *9* (1), 4775.
(25) Almaqwashi, A. A.; Zhou, W.; Naufer, M. N.; Riddell, I. A.; Yilmaz, Ö. H.; Lippard, S. J.; Williams, M. C. DNA Intercalation Facilitates Efficient DNA-Targeted Covalent Binding of Phenanthriplatin. *J. Am. Chem. Soc.* **2019**, *141* (4), 1537−1545.
(26) Pozzi, C.; Di Pisa, F.; Benvenuti, M.; Mangani, S. The structure of the human glutaminyl cyclase−SEN177 complex indicates routes for developing new potent inhibitors as possible agents for the treatment of neurological disorders. *JBIC, J. Biol. Inorg. Chem.* **2018**, *23* (8), 1219−1226.
(27) Gaillard, T. Evaluation of AutoDock and AutoDock Vina on the CASF-2013 Benchmark. *J. Chem. Inf. Model.* **2018**, *58* (8), 1697−1706.
(28) Ngo, S. T.; Thu Phung, H. T.; Vu, K. B.; Vu, V. V. Atomistic Investigation of an Iowa Amyloid-β Trimer in Aqueous Solution. *RSC Adv.* **2018**, *8* (73), 41705−41712.
(29) Grither, W. R.; Longmore, G. D. Inhibition of tumor−microenvironment interaction and tumor invasion by small-molecule allosteric inhibitor of DDR2 extracellular domain. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (33), E7786.
(30) Noike, M.; Matsui, T.; Ooya, K.; Sasaki, I.; Ohtaki, S.; Hamano, Y.; Maruyama, C.; Ishikawa, J.; Satoh, Y.; Ito, H.; Morita, H.; Dairi, T. A peptide ligase and the ribosome cooperate to synthesize the peptide pheganomycin. *Nat. Chem. Biol.* **2015**, *11*, 71.
(31) Vu, V. V.; Hangasky, J. A.; Detomasi, T. C.; Henry, S. J. W.; Ngo, S. T.; Span, E. A.; Marletta, M. A. Substrate selectivity in starch polysaccharide monooxygenases. *J. Biol. Chem.* **2019**, *294*, 12157−12166.
(32) Caffalette, C. A.; Corey, R. A.; Sansom, M. S. P.; Stansfeld, P. J.; Zimmer, J. A lipid gating mechanism for the channel-forming O antigen ABC transporter. *Nat. Commun.* **2019**, *10* (1), 824.
(33) Perrier, A.; Eluard, M.; Petitjean, M.; Vanet, A. In Silico Design of New Inhibitors Against Hemagglutinin of Influenza. *J. Phys. Chem. B* **2019**, *123* (3), 582−592.
(34) Dennig, A.; Lulsdorf, N.; Liu, H. F.; Schwaneberg, U. Regioselective o-Hydroxylation of Monosubstituted Benzenes by P450 BM3. *Angew. Chem., Int. Ed.* **2013**, *52* (32), 8459−8462.
(35) Ngo, S. T.; Fang, S.-T.; Huang, S.-H.; Chou, C.-L.; Huy, P. D. Q.; Li, M. S.; Chen, Y.-C. Anti-Arrhythmic Medication Propafenone a Potential Drug for Alzheimer's Disease Inhibiting Aggregation of Aβ: *In Silico* and *In Vitro* Studies. *J. Chem. Inf. Model.* **2016**, *56* (7), 1344−1356.
(36) Tran, P.-T.; Hoang, V.-H.; Lee, J.; Hien, T. T. T.; Tung, N. T.; Ngo, S. T. In vitro and in silico determination of glutaminyl cyclase inhibitors. *RSC Adv.* **2019**, *9* (51), 29619−29627.
(37) Nguyen, P. H.; Campanera, J. M.; Ngo, S. T.; Loquet, A.; Derreumaux, P. Tetrameric Aβ40 and Aβ42 β-Barrel Structures by Extensive Atomistic Simulations. I. In a Bilayer Mimicking a Neuronal Membrane. *J. Phys. Chem. B* **2019**, *123*, 3643−3648.
(38) Forli, S.; Huey, R.; Pique, M. E.; Sanner, M. F.; Goodsell, D. S.; Olson, A. J. Computational protein−ligand docking and virtual drug screening with the AutoDock suite. *Nat. Protoc.* **2016**, *11*, 905.
(39) Gasteiger, J.; Marsili, M. New Model for Calculating Atomic Charges in Molecules. *Tetrahedron Lett.* **1978**, *19*, 3181.
(40) Gasteiger, J.; Marsili, M. Iterative Partial Equalization of Orbital Electronegativity—A Rapid Cccess to Atomic Charges. *Tetrahedron* **1980**, *36* (22), 3219−3228.
(41) Salentin, S.; Schreiber, S.; Haupt, V. J.; Adasme, M. F.; Schroeder, M. PLIP: fully automated protein−ligand interaction profiler. *Nucleic Acids Res.* **2015**, *43* (W1), W443−W447.

(42) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High Performance Molecular Simulations through Multi-Level Parallelism from Laptops to Supercomputers. *SoftwareX* **2015**, *1*−*2*, 19−25.
(43) Efron, B. Bootstrap Methods: Another Kook at the Jackknife. *Ann. Stat.* **1979**, *7*, 1−26.

https://dx.doi.org/10.1021/acs.jcim.9b00778
J. Chem. Inf. Model. 2020, 60, 204−211