

Accuracy Assessment of Protein-Based Docking Programs against RNA Targets

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Ribonucleic acid (RNA) molecules play central roles in a variety of biological processes and, hence, are attractive targets for therapeutic intervention. In recent years, molecular docking techniques have become one of the most popular and successful approaches in drug discovery; however, almost all docking programs are protein based. The adaptability of popular docking programs in RNA world has not been systematically evaluated. This paper describes the comprehensive evaluation of two widely used protein-based docking programs—GOLD and Glide—for their docking and virtual screening accuracies against RNA targets. Using multiple docking strategies, both GOLD 4.0 and Glide 5.0 successfully reproduced most binding modes of the 60 tested RNA complexes. Applying different docking/scoring combinations, significant enrichments from the simulated virtual and fragment screening experiments were achieved against tRNA decoding A site of 16S rRNA (rRNA A-site). Our study demonstrated that current protein-based docking programs can fulfill general docking tasks against RNA, and these programs are very helpful in RNA-based drug discovery and design.

INTRODUCTION

RNA molecules play central roles in a wide range of biological processes, such as the storage of genetic materials, propagation of genetic information, protein biosynthesis, and enzymatic activity. Therefore, RNA molecules are potential targets in drug discovery, especially in the treatment of infectious diseases.^{1,2} The most widely used compounds targeting RNA are antibiotics, such as aminoglycosides.³

The advantages of RNA as drug targets are obvious. At first, RNA is located at the upstream of a gene-expression pathway, so blocking one RNA molecule would inhibit functions of multiple proteins by affecting their syntheses. The development of drug resistance for a novel RNA-binding drug can be slower than that of protein targets.⁴ In addition, compared with DNA, RNA often produces unique three-dimensional (3D) pockets for specific binding of small ligands.⁵ Despite all of the advantages mentioned above, RNA has received less attention in structure-based drug design, partly due to the paucity of RNA-related 3D structures. Furthermore, it is due to the unique physiochemical properties of RNA, such as unusual flexibility, highly negative charge, and large flat binding pockets. With the increasing number of RNA structures in the Protein Data Bank (PDB),⁶ RNA-targeted drug design is now technically feasible.²

Since the first automated docking program DOCK was introduced by Kuntz in 1982, many docking methods and scoring functions have been reported to predict ligand–protein interactions.^{7–9} They have been successfully used in lead discovery and optimization.^{10–14} Until now, many evaluations have been carried out on these methods for docking and virtual screening accuracy against various targets.^{15–20}

However, almost all these docking methods were developed on and for proteins. Their adaptability against RNA targets hence deserves exploration. The first attempt was done by Kuntz in 1997, using DOCK 3.5 to identify new RNA binding ligands.²¹ Later on, James and co-workers developed two different empirical free energy functions for RNA. With the empirical functions, they reported two successful virtual screening procedures against HIV-1 TAR RNA with DOCK and ICM.^{22,23} In 2004, Morley and Afshar developed a fast empirical function for scoring RNA–ligand interactions and implemented it in RiboDock.²⁴ A successful virtual screening work against rRNA A-site (tRNA decoding A site of 16S rRNA) was done with this program.²⁵ In the same year, Varani et al. validated DOCK and AutoDock for docking and database screening against RNA drug targets.²⁶ After that, Moitessier et al. developed a unique approach based on AutoDock for docking aminoglycosides to a rRNA A-site, with consideration of RNA flexibility and the first hydration shell.²⁷

More recently, several docking programs and scoring functions were developed particularly for RNA. Based on the modified DOCK suite, Kuntz et al. successfully applied multiple techniques, such as rescoring docked conformations with generalized Born/surface area (GB/SA) or Poisson–Boltzmann/SA (PB/SA) techniques, to recreate experimental binding poses.²⁸ James et al. developed a flexible docking method called MORDOR, which was validated with a test set of 57 RNA–ligand complexes and generated a success rate of 74% within a heavy atom root-mean-square deviation (rmsd) of 2.5 Å.²⁹ With MORDOR and NMR techniques, James' group discovered some interesting ligands bound to human telomerase RNA.³⁰ Zhao et al.³¹ and Pfeffer et al.³² reported two knowledge-based scoring functions to predict RNA–ligand interactions, which could generate fair correlations between experimental and computational values.

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Table 1. List of PDB IDs for 60 RNA–Ligand Complexes in the Test Set^a

target categories	PDB ID
rRNA A-site ^b	1J7T, 1LC4, 1MWL, 1O9M, 1YRJ, 1ZZ5, 2BE0, 2BEE, 2ESI, 2ESJ, 2ET3, 2ET4, 2ET8, 2ET5, 2F4U, 2F4T, 2G5Q, 1BYJ , 1PBR , 2OE5, 2OE8, 1FYP
HIV-1 DIS ^c	2FCX, 2FCZ, 2FD0, 3C44, 1XPF
TAR RNA ^d	1ARJ , 1LVJ , 1QD3 , 1UTS , 1UUD , 1UUI
riboswitch	1F27, 1U8D, 2G9C, 2B57, 1Y26, 2CKY, 2GDI, 2GIS
aptamer	1FIT, 1NTA, 1NTB, 1AM0 , 1EHT , 1FMN , 1KOC , 1KOD , 1NBK , 1O15 , 1Q8N , 1RAW , 1TOB , 2AU4 , 2TOB , 1NEM
tau exon 10 ^e	1E12
ribozyme	1YKV
mismatched duplex ^f	1UFU

^a The PDB IDs in bold are structures determined by NMR spectroscopy and others were determined using X-ray crystallography.

^b Ribosomal decoding region of the aminoacyl-tRNA acceptor (rRNA A-site).

^c The dimerization initiation site of HIV-1.

^d Trans-activation response element from HIV-1. ^e The tau exon 10 splicing regulatory element RNA. ^f RNA duplex with two UU-bulges.

Though above-mentioned efforts have been made, the adaptability of some popular docking programs to RNA targets has not been systematically evaluated. It would be helpful if existing programs could be adopted in the RNA world. In this work, we made a comprehensive evaluation on two docking programs, GOLD³³ and Glide,³⁴ which have been widely recognized in the field of protein–ligand docking. Our research focused in three major areas: (1) the reproduction of experimental binding poses, (2) the identification of cognate ligands against various RNA structures, and (3) the performance of docking programs on simulated virtual and fragment screening. The primary goal of this study is not to compare the performance of different programs but to evaluate the usefulness of docking programs under different experimental conditions. Our goal is to assess if these docking programs could conveniently predict RNA–ligand interaction modes and to identify new RNA-binding ligands from different compound collections.

MATERIALS AND METHODS

Data Set Preparation for Cognate Ligand Docking. A total of 60 RNA–ligand complexes, including 37 crystal and 23 NMR structures, were collected to test the performance of docking programs. Diverse RNA targets were selected, and these ligands covered a wide range of molecular weights, rotatable bonds, and formal charges. Most of these complexes had been previously used to evaluate other docking programs against RNA targets.^{24,26–29,32} A list of PDB codes and details of the RNA complexes were given in Table 1.

Preparation of RNA Targets for “Dry” Docking. Coordinates for each RNA complex in our data set were retrieved from PDB. The original PDB files were prepared using the Protein Preparation Wizard in Maestro 8.5.³⁵ In general, water molecules, counterions, and all others not related with ligand binding were removed from the complex. Exceptions were given to 2CKY and 2GDI, in which the key Mg²⁺ ions in the ligand binding site were preserved. For structures with multiple binding sites, the site bound with the lowest B-factor

ligand was selected. For structures containing NMR ensembles, clustering analysis was performed, and five conformers were then selected (see the Clustering of NMR Ensembles Section). Bond orders and formal charges were assigned for each heterogroup, and hydrogens were added to the whole complex system. When necessary, atom and bond types of the complex were manually corrected. To optimize the hydrogen-bond networks, polar hydrogens were sampled. In order to alleviate physically untenable steric clashes in the binding site, an all-atom restrained minimization of the RNA–ligand complex was carried out by the maximum rmsd of 0.3 Å. Prepared structures were saved as mae and pdb files. The mae files were used for Glide docking, and the pdb files were further converted to mol2 files in SYBYL 7.0³⁶ for GOLD docking.

Preparation of RNA Targets for “Wet” Docking. In general, the process of preparation was similar to that mentioned above. The only difference was in the treatment of crystal waters. At first, all water molecules beyond 3.0 Å from any ligand atom were removed. Then hydrogen-bond assignment tool of Protein Preparation Wizard was used to sample water orientations and to optimize hydrogen-bond interactions between ligands and RNA. Only those water molecules which can form hydrogen bonds between ligands and the RNA were reserved. Finally, 25 of the 37 crystal structures were found containing water molecules in the binding sites.

Clustering of NMR Ensembles. In this study, 15 of the 23 NMR-derived structures were stored as an ensemble of conformers. Clustering analysis was performed on each ensemble with XCluster.³⁷ At first, RNA bases within 5 Å from the cognate ligand were selected as clustering objects. Then, five clusters were formed for each NMR ensemble based on the calculation of distance matrix. Finally, the representative conformer was chosen from each cluster, which led to five representative conformers for each ensemble.

For docking with NMR ensembles, a ligand was sequentially docked back into the five conformers of the corresponding target, and only the best scoring pose against each conformer was retained. The docking pose closest to the corresponding experimental one was considered as a success, and its corresponding target conformer was used for the docking with multiple ligand conformations as input.

Preparation of Ligands. The experimental conformation of each ligand was extracted from the corresponding complex structure. After hydrogen atoms were added, the 3D conformation of the ligand was rebuilt using LigPrep 2.2³⁸ at a pH of 7.0. For those ligands belonging to aminoglycosides, they were protonated based on experimental pK_a values.^{27,39} These rebuilt ligands were used for GOLD and Glide docking in single conformations.

An ensemble of conformations for each rebuilt ligand was also generated for the use of multiple conformation docking. The conformational space of each ligand was searched using MacroModel 9.6,⁴⁰ starting with the rebuilt conformation. The mixed torsional/low-mode sampling search method was used, and the calculations were performed with OPLS-2005 force field in a water solvent model. The energy window for saving structures was set to 5.02 kcal/mol. The rmsd cutoff value was set at 0.5 Å to avoid retrieving redundant conformations. When each search was finished, 10 representative ligand conformations were retained by the ligand

heavy-atom rmsd analysis of XCluster. In this way, low-energy and relatively diverse ligand conformation ensembles were generated.

Docking Programs and Running Parameters. GOLD 4.0 and Glide 5.0, the latest versions of the programs when this work was ongoing, were used in the docking and scoring studies.

GOLD 4.0. GOLD utilizes a genetic algorithm (GA) to create putative poses for a single ligand.⁴¹ The program could consider side chain flexibility and local backbone movement during docking. A switch for water molecules (on, off, and toggle) was also integrated into the suite.⁴²

Hermes Visualizer⁴³ was used to configure the input files for GOLD docking. For each prepared complex, the cognate ligand was removed first, and the binding pocket was defined by the cognate ligand pose. Here, the RNA bases within 10 Å of the cognate ligand were selected to scale the docking space. No soft potentials were applied to van der Waals radii of the RNA. “Allow early termination” in the “fitness and search option” was turned off to search larger ligand conformation spaces. Because of many highly flexible ligands involved in this study, 150% search efficiency in the “automatic GA settings” (ligand-dependent) was used, and 10 GA runs were performed. The native GoldScore function was used to evaluate the docking poses. For structures with metal ions, the metal coordination geometries were automatically matched by GOLD.

Glide 5.0. The Glide algorithm⁴⁴ utilizes precomputed grids to represent the shape and the properties of receptor sites. A series of hierarchical filters were used to quickly and effectively sample the possible conformation space of the docked ligand.

During the receptor grid generation, the cognate ligand was removed first from the complex, and the binding region was defined by the cognate ligand pose. Here, the binding region was defined by a 10 × 10 × 10 Å box centered on the mass center of the bound ligand. No van der Waals radius scaling factors and constraints were applied to the RNA. In ligand docking, the standard precision mode of GlideScore was selected as the scoring function, and the option of “Dock flexibly” was enabled. The top 10 docking poses were energy minimized. Default settings were used for all the remaining parameters.

Rescoring. Rescoring was implemented in the identification of cognate ligands, simulated virtual screening, and fragment screening experiments. For this purpose, the rescoring tools in Glide and GOLD were used. To rescore the poses from GOLD docking, the “Refine (do not dock)” option in “Ligand Docking” of Glide was enabled. Likewise, the poses from Glide docking were rescored using the rescoring tool in GOLD. Note that, it is essential to perform local optimization of docking solutions, so that optimized docking scores can be achieved.

Identification of Cognate Ligands. A powerful docking program should not only accurately reproduce the experimental binding modes of ligands but also effectively recognize cognate ligands from many others. Ideally, a RNA ligand should be ranked as high as possible against its cognate RNA. For this test, 40 different RNA binders were first taken from the 60 test complexes, to consist of a competitive RNA ligand database. Ligands in the database were then docked into a given RNA target by tested

programs, which meant that the cognate ligand of the given RNA target had to compete with the other 39 noncognate ligands. The docking settings of two programs were retained, as mentioned above. Names of the RNA ligands involved in this test were listed in Table S4 of the Supporting Information.

Simulated Virtual Screening. One of the rRNA A-site structures, PDB code 1J7T, was selected for simulated virtual screening. The active and decoy compounds were docked into the rRNA A-site structure with GOLD and Glide programs, according to the procedures described in the Cognate Ligand Docking Section.

Construction of Decoy Library. A total of 56 compounds, which were confirmed as binders of the rRNA A-site, were collected from literature.^{25,45–53} These compounds were then seeded into two different decoy databases, a charged decoy (selected from ZINC database)⁵⁴ and a clustered decoy database (selected from a large database including Maybridge, Specs, and Open NCI databases). The charged decoy database consisted of 2000 positively charged compounds which have similar charge distributions to the active ones. To build the clustered decoy database, the actives were first divided into 10 clusters according to their property descriptors. Then the decoys, which had similar properties with actives in each cluster, were selected from the large database to form the clustered decoy database. During the construction of the decoy databases, diversity analysis was also conducted to eliminate redundant structures. The construction of the decoy databases was performed using the “Library Analysis” tools in Discovery Studio 2.1.⁵⁵

Evaluation of Docking Screening. The receiver operating characteristic (ROC)^{56,57} curve was used to evaluate the performance of tested programs on simulated virtual screening. Because of many advantages of the ROC curve compared to that of the classical enrichment curve,⁵⁶ it is widely used in the evaluation of modeling methods. One advantage of this metric is to allow an intuitively visual comparison among different virtual screening workflows. More importantly, some performance indicators can be derived from the ROC curve for precisely assessing the performance of virtual screening, such as the area under ROC curve (AUC), the ROC enrichment,⁵⁸ and the “model exhaustion point”.⁵⁹ The AUC value measures the overall classification capacity of a virtual screening method between active and decoy compounds, independent of the selected cutoff value. For ideal distributions of active and decoy compounds an AUC value is set as 1, and a value of 0.5 signifies random selection. ROC enrichments from different cut off values can be used to describe “early recognition” of a virtual screening workflow. It is worth emphasizing that the ROC enrichment differs from the classical enrichment factor by referring to the fraction of decoy compounds rather than all compounds.⁶⁰ The “model exhaustion point” is a specific point on the ROC curve where the slope of the curve starts to become substantially lower than the diagonal line. This point means that a virtual screening method has just reached its capability to classify actives more efficiently than a random selection.⁵⁹ Therefore, it is used to determine the most appropriate threshold which divides the final list into two categories, testing and discarding compounds.

Fragment Screening. An RNA-directed fragment library⁶¹ and a fragment-like library were used to evaluate the

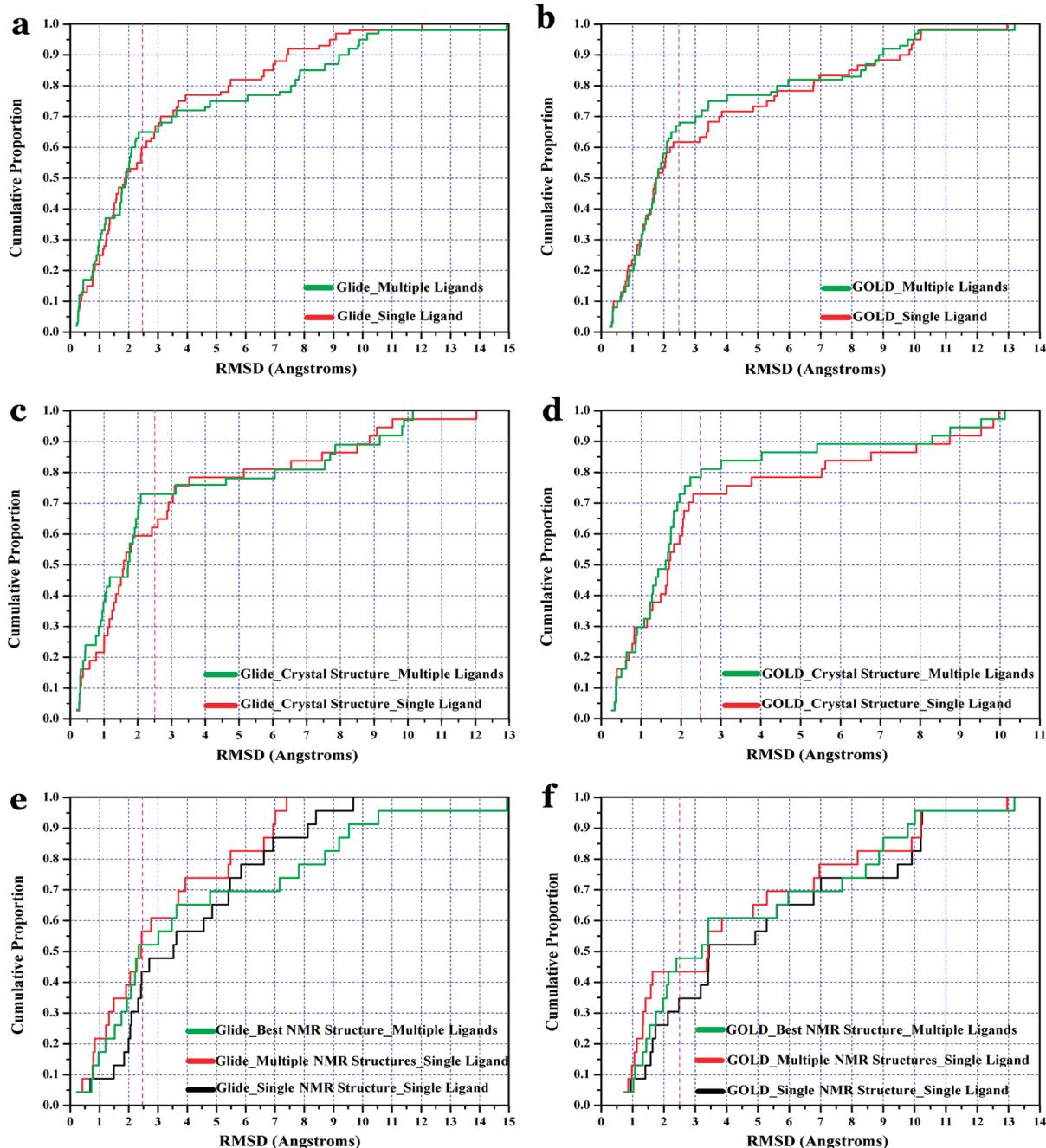


Figure 1. Cumulative distribution plots for cognate ligand docking of 60 RNA–ligand complexes using Glide and GOLD (a, c, e for Glide docking and b, d, f for GOLD docking). (a and b) all the 60 complexes; (c and d) 37 X-ray determined structures; and (e and f) 23 NMR-derived structures.

capability of two docking programs on fragment screening. The RNA-directed fragment library was composed of 109 RNA binder-like compounds, which had been verified as nonbinders of rRNA A-site by the NMR technique. The fragment-like library consisted of 1000 diverse fragment-like molecules, which were selected according to the physical properties of active fragment molecules from the fragment-like subset of ZINC.⁵⁴ The selection of decoy fragment molecules was also performed using the “Library Analysis” tools in Discovery Studio 2.1. Nine true rRNA A-site fragment molecules^{61,62} were seeded into two decoy libraries. Again, the rRNA A-site structure, PDB code 1J7T, was used as the target structure. The structure preparation and docking parameter settings were treated in the same way as those in the Simulated Virtual Screening Section.

RESULTS

Cognate Ligand Docking. The ability of a docking program to rebuild a ligand pose close to that resolved in an experimental complex is often a critical determinant of the program’s effectiveness for structure-based drug design. So, at first, the abilities of GOLD and Glide to reproduce experimental binding modes were evaluated through a well-designed complex data set. Each ligand was docked back into its cognate binding site, and an rmsd value of 2.5 Å from the experimental pose was set as the threshold to tell correct or incorrect docking poses according to previous RNA docking studies.^{26,29} Here, only the top-ranked poses identified by the native scoring functions of the tested programs were considered, so that we could maintain

simplicity in selection of docking poses and result analysis. Considering the natures of RNA–ligand systems, e.g. the high complexity of RNA ligands and the importance of water molecules in RNA–ligand recognition, a combination of strategies were used to test the ability of the docking programs on reproducing experimental binding modes. For ligands, both single and multiple conformations were considered, while for RNA targets, not only single conformations but also crystal water molecules in the X-ray determined structures or multiple conformers in the NMR determined structures were employed.

Docking to “Dry” RNA. The statistical results of cognate ligand docking against 60 RNA–ligand complexes in the absence of water molecules were illustrated in Figure 1a and b. For the docking with single ligand conformation as input, both Glide and GOLD performed well on the test complexes, correctly reproducing 36 and 37 experimental binding modes and leading to success rates of 60 and 62%, respectively (Figure 1a and b, red lines). When multiple ligand conformations were used as input, both Glide and GOLD correctly reproduced more experimental poses, leading to the increased success rates of 65 and 68%, respectively (Figure 1a and b, green lines).

In order to investigate if the success rates were dependent on the structural sources of the targets (crystallography or NMR), curves were drawn for docking success rates against crystal and NMR structures, separately (Figure 1c and d for those against crystal structures and Figure 1e and f for those against NMR structures). The results demonstrated that docking success rates of tested programs against NMR structures were lower than those against crystal structures in spite of docking strategies. For example, with single ligand conformations as input, GOLD correctly docked 73% ligands back to their cognate crystal RNA structures (Figure 1d, the red line) but only 43% NMR derived binding modes were reproduced by GOLD (Figure 1f, the red line). In contrast, with the same ligand conformation as input, the docking performance of Glide was less dependent on the structural sources. As shown in the red lines of Figure 1c and e, Glide correctly reproduced 62% crystal complex binding modes and 57% NMR modes. When multiple ligand conformations were used as input, the docking success rates of both programs were significantly increased for crystal structures, Glide from 62 to 73% (Figure 1c) and GOLD from 73 to 81% (Figure 1d). However, similar improvement was not observed against NMR-determined complexes.

For those targets determined as NMR ensembles, previous studies usually selected only one conformer from the NMR ensemble as target; however, there was no guarantee that the selected conformer was the best for docking purpose. Here, to minimize the effect of different NMR conformers on docking quality, we clustered out five representative members from each NMR ensemble for the docking of a given target. Comparison was then performed to show the differences between single and multiple NMR conformers as docking targets. Figure 1e and f illustrated the differences of docking success rates against NMR structures, in which black curves denoted the success rates of single ligand docked into single NMR conformer, whereas the red lines described the success rates of single ligand docked into multiple NMR conformers. Obviously, when multiple NMR conformers were used as docking targets, the docking quality

of both programs was improved a lot. The success rates of Glide and GOLD were increased by 14 and 8%, respectively.

Some successful examples of “dry” docking were shown in Figure 2. Figure 2a and b illustrated two cases that single ligand conformation was docked into the crystallographic binding sites of *N*-pentylmaleimide (1YKV) and genenticin (1MWL) by both programs. The case of the docking strategy with multiple ligand conformations as input was demonstrated in Figure 2c (the ligand colored in yellow). The corresponding single ligand conformation docking resulted in an rmsd of 2.8 Å (Figure 2c, the one in pink) from the experimental binding mode. In Figure 2d, the advantage of docking with multiple NMR conformers was revealed. Glide could not successfully rebuild the experimental binding mode of complex 1FMN when the first structure in the NMR ensemble was used (Figure 2d, the pink one in the lower left corner), whereas a pose close to the experimental one was generated with multiple NMR conformers as target (Figure 2d, the pink ligand bound with RNA). The rmsd values of “dry” docking were listed in the Supporting Information (Table S1 and S2).

Docking to “Wet” RNA. It is well-established that water molecules often play important roles in protein– and RNA–ligand recognition.^{27,63–65} Once water-mediated interactions are ignored during docking process, the docking pose might be misleading.

In order to assess the effects of bridging water molecules on docking, the binding modes of 25 hydrated RNA structures were reproduced using Glide. As seen in the cyan line of Figure 3, the quality of the docking results was improved significantly when water molecules were included. Compared to the corresponding “dry” docking with single ligand conformer as input, the success rate of “wet” docking was dramatically increased from 44 to 84%. As illustrated in Figure 4, both binding modes of lividomycin and neomycin (taken from 2ESJ and 2FCX, respectively) were reproduced well within 2.0 Å of rmsd in the presence of crystal waters, whereas they could not be rebuilt correctly in “dry” docking.

Identification of Cognate Ligands. Here two test sets, namely “GOLD docking” and “Glide docking” (see Figure 6a and b, respectively), were defined, and 29 common RNA structures were included in the two test sets (see Figure 5). The binding modes of the complexes in “GOLD docking” and “Glide docking” were successfully reproduced by GOLD and Glide in the Docking to “Dry” RNA Section with a single ligand conformation as input, respectively. This ensured that the correct binding mode of a specified cognate ligand against its corresponding RNA structure could be generated by the tested programs.

The results are shown in Figure 5. An arbitrary 10% cutoff value (ranking in top four) was used to provide an intuitive guidance. In general, Glide performed better than GOLD in identifying the cognate ligands with their native scoring functions. Glide positioned 13 cognate ligands among the top 4 out of 29 entries, compared with 10 cognate ligands ranked out of the top 4 by GOLD. Moreover, Glide performed better than GOLD in 19 out of the 29 cases (Figure 5, the entries in the green box). For four purine riboswitch ligands extracted from 1U8D, 1Y26, 2B57, and 2G9C, both GOLD and Glide could accurately reproduce their experimental binding modes within 0.5 Å of the rmsd.

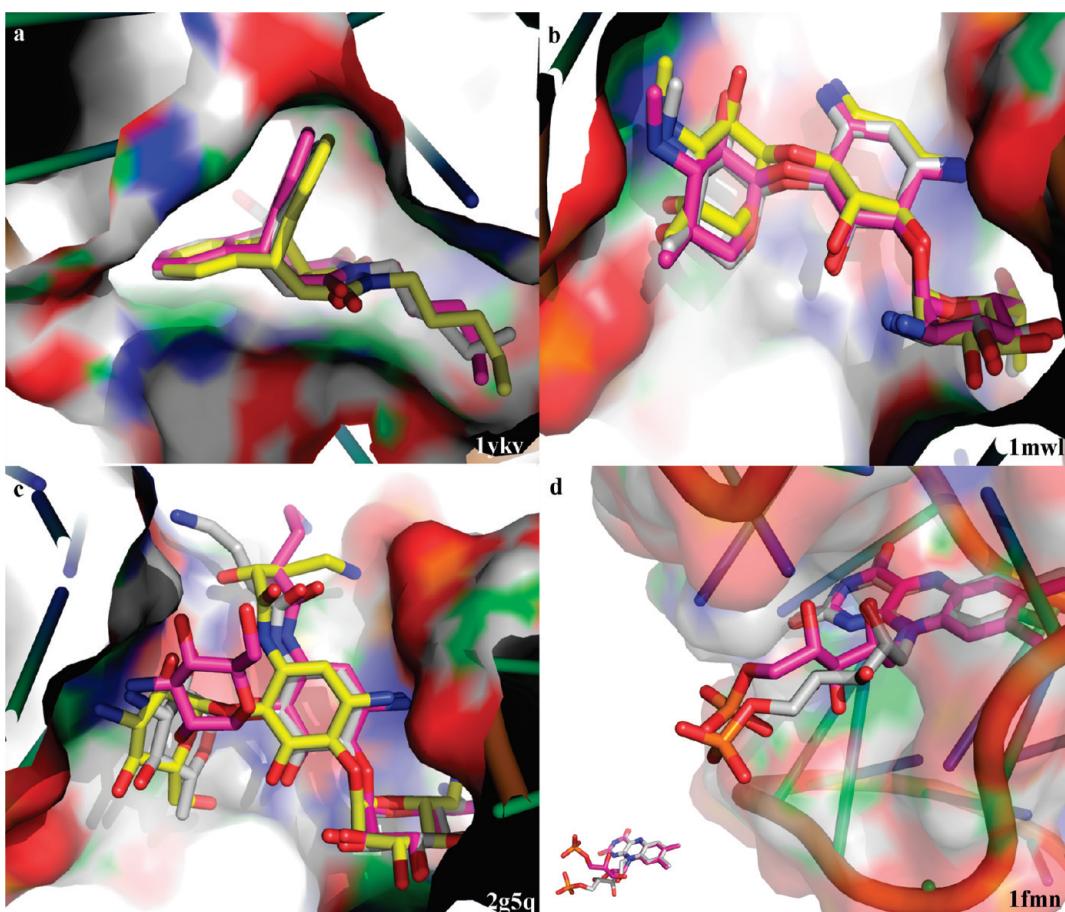


Figure 2. Examples of successful dockings in absence of crystal water molecules. The experimental pose was colored in gray, and PDB code for the RNA complex was put in the lower right corner. (a and b) The best scoring poses, with single ligand conformation as input, from GOLD and Glide were shown in yellow and pink, respectively. (c) The docking poses with single/multiple ligand conformation as input were colored in pink and yellow, respectively. (d) The best docking pose with multiple NMR conformers as target was shown together with the RNA structure, and the pose with the first conformer in the PDB file was shown in the lower left corner. This figure, together with Figure 4, was prepared with PyMol.⁴³

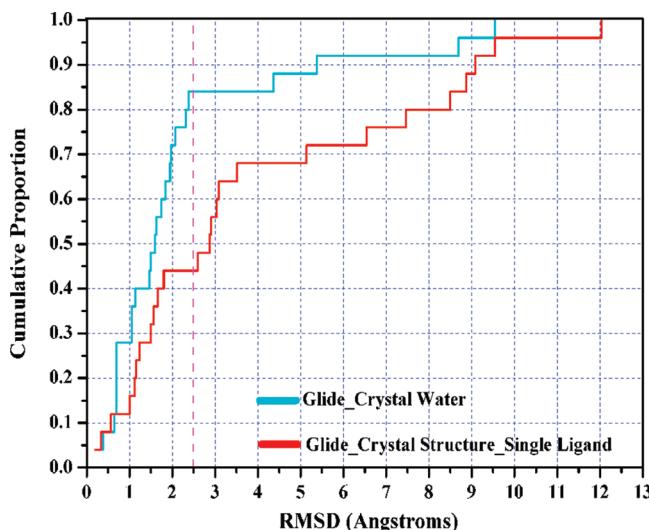


Figure 3. Comparison between “wet” and “dry” docking. Cumulative distribution plots for “wet” and “dry” docking were shown in cyan and red, separately.

Nevertheless, Glide could rank these cognate ligands in top four but GOLD could not.

In order to inspect if non-native scoring functions could improve the ranking results, rescoring operations were executed against the test sets. As shown in Figure 6a (the entries in the green box), eight unfavorable ranking results

from GOLD, i.e. 1U8D, 1Y26, 2B57, 2G9C, 1LVJ, 1Q8N, 2ESJ, and 2ET8, were improved significantly by GlideScore rescoring. Likewise, four ranking results from Glide, namely 1O9M, 1KOC, 1YKV, and 1MWL, were enhanced by GoldScore rescoring dramatically (Figure 6b, the entries in the green box).

In spite of original scoring or rescoring, both GlideScore and GoldScore performed poorly on some complexes containing aminoglycosides, such as 2ET5, 2ESI, and 1FYP, which might be caused by many similar aminoglycosides mixed in the RNA ligand database.

Simulated Virtual Screening. Two decoy databases with different property profiles were used in this test, and rescoring was also used to see if ranked results could be improved by non-native scoring functions.

Figure 7 illustrated the ROC curves for each database and docking/scoring combination, and Table 2 reported the AUC values deduced from these ROC curves and the ROC enrichments calculated from five early false positive rates. As shown in Table 2, all the AUC values were beyond 0.5, actually ranging from 0.73 to 0.96, which meant that all docking/scoring combinations performed better than random discriminations of actives and decoys. For either database, the AUC values derived from GlideScore were greater than those from GoldScore, no matter which docking engine was used. This result indicated that GlideScore possessed better

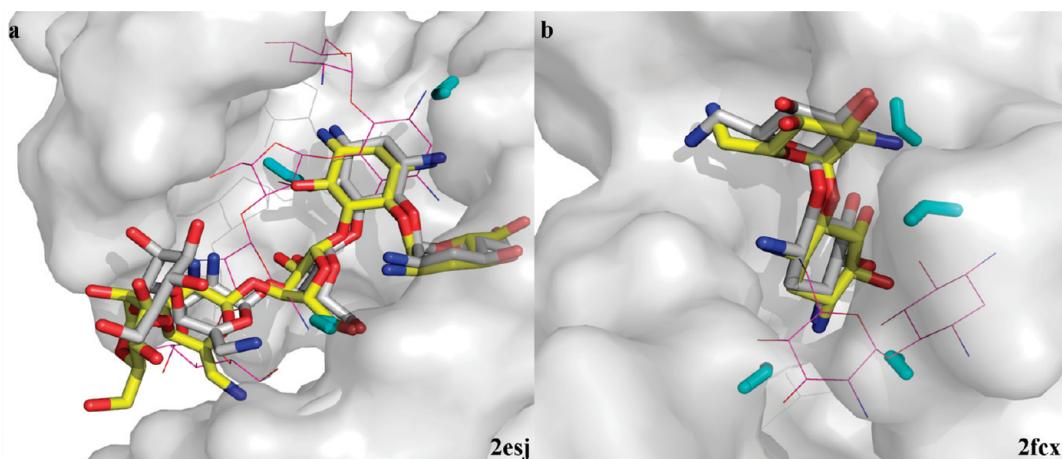


Figure 4. Examples of correct docking poses predicted by Glide in the presence of crystal water molecules. Experimental poses were colored by gray, and crystal water molecules are shown in spring green. The docking poses with and without crystal waters were shown in yellow sticks and purple lines, respectively. (a) X-ray structure of lividomycin bound to rRNA A-site. (b) X-ray structure of neomycin bound to HIV-1 DIS.

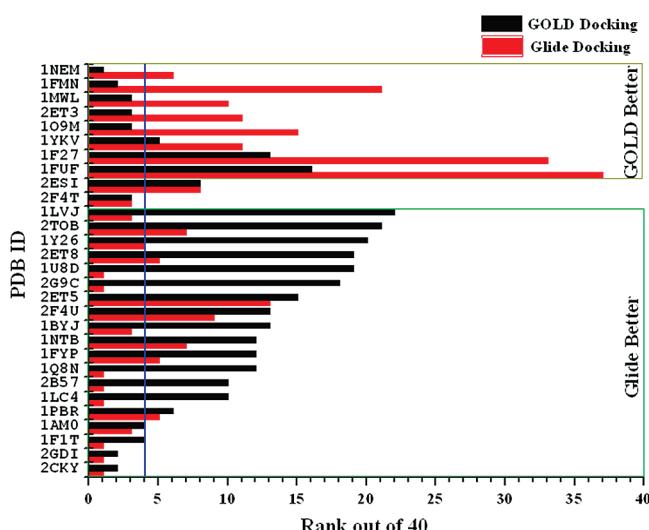


Figure 5. Comparison of the capability of GOLD and Glide to identify cognate ligands on 26 cases. Black bar represents the result of ranked order from GOLD docking, and red bar depicts the one from Glide docking. The vertical line presents the top four cutoff line.

ability than GoldScore to roughly differentiate between actives and decoys. After analyzing “early recognition” of actives according to ROC enrichments, we found that GlideScore often promised better early recovery of actives than GoldScore. However, in some cases, GoldScore produced better enrichments than GlideScore. For example, against the clustered decoy set GoldScore reported better “early” enrichments than GlideScore at five different negative rates when Glide was used to generate original docking poses.

Interestingly, the combination of GOLD/GlideScore gave the best AUC values and ROC enrichments in both decoy sets (Table 2, the data in bold). Actually, for the rRNA A-site complexes, GOLD docking gave better performance on reproducing experimental binding modes, while GlideScore tended to recognize more cognate ligands on the top portion in the identification of cognate ligands. So, the better pose generator plus the better scoring function promise the best performance of virtual screening.

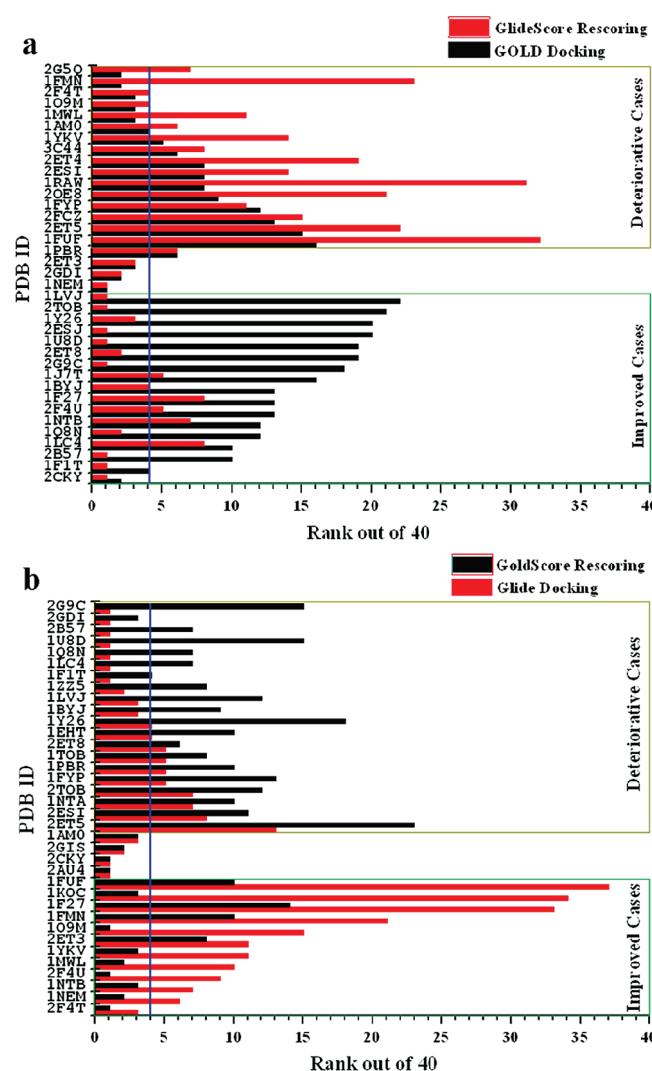


Figure 6. Identification of cognate ligands and rescoring. (a) Black bar represents the results of ranked order from original GOLD docking, and red bar indicates the one from rescoring the GOLD docking pose via GlideScore. (b) Red bar represents the result of ranked order from original Glide docking, and black bar indicates the one from rescoring the Glide docking pose via GoldScore.

Different decoy sets had little impact on the screening results. Both Figure 7 and Table 2 demonstrated that the

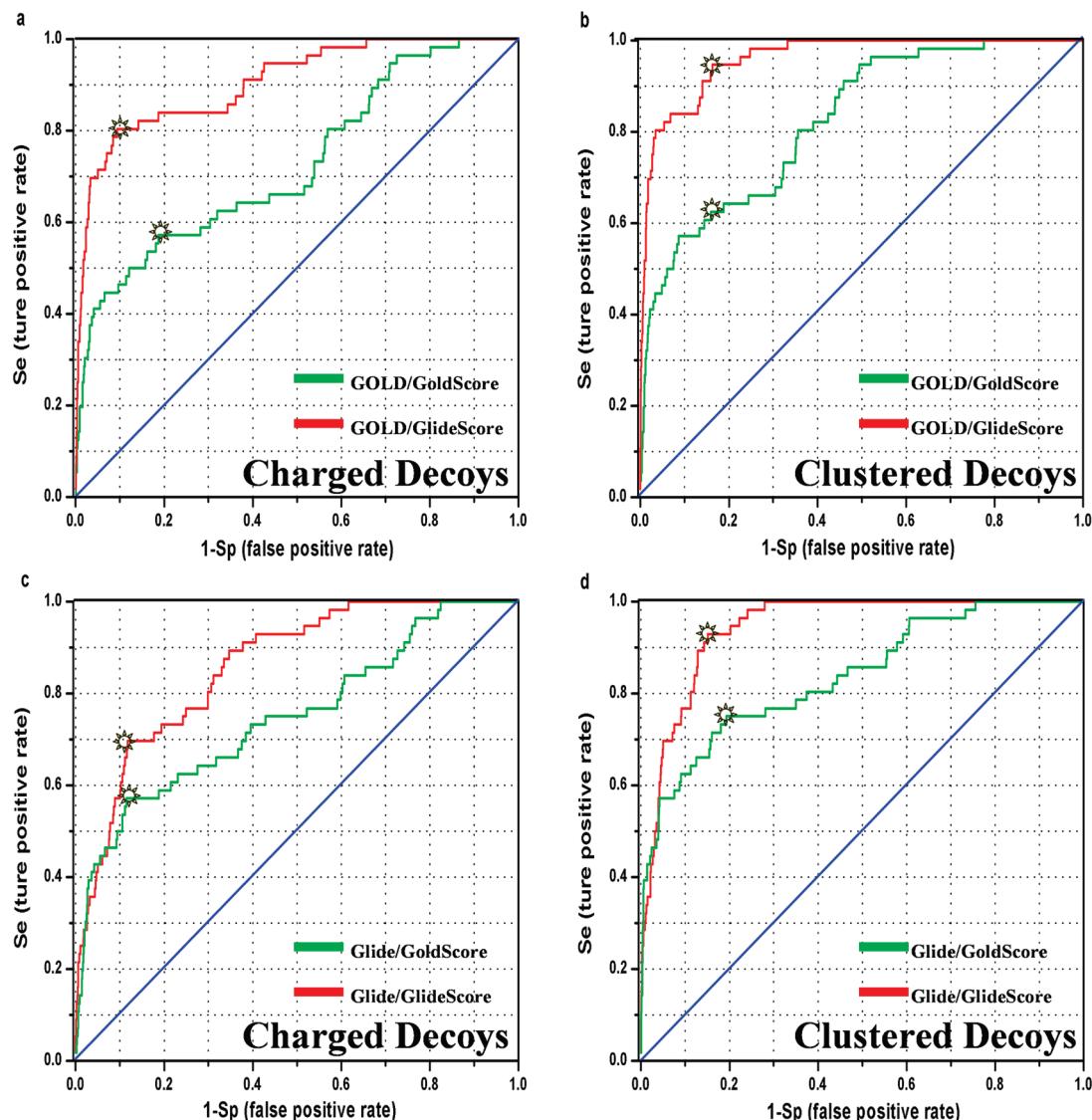


Figure 7. ROC plots of the screening performance of tested programs against rRNA A-site. The combinations of docking engines and scoring functions and the name of decoy sets were shown in the lower right corner. The sun-shaped points on the ROC curve indicated the “model exhaustion points”, and diagonal lines in blue indicated random performance.

Table 2. Statistical Results of Simulated Virtual Screening

program/scoring functon	decoy sets	ROC AUC	ROC enrichment ^a					
			0.5%	1.0%	2.0%	5.0%	10.0%	
GOLD/GoldScore	charged	0.73	25.0	19.6	14.3	8.2	4.6	
	clustered	0.82	28.6	26.8	19.6	9.3	5.7	
GOLD/GlideScore	charged	0.91	50.0	37.5	26.8	13.9	8.0	
	clustered	0.96	78.6	51.8	34.8	16.1	8.4	
Glide/GlideScore	charged	0.86	32.1	23.2	14.3	8.2	5.7	
	clustered	0.94	50.0	30.4	17.9	13.6	7.7	
Glide/GoldScore	charged	0.75	14.3	14.3	14.3	8.6	5.0	
	clustered	0.84	60.7	39.3	21.4	11.4	6.3	

^a ROC enrichment = $Hits_{sel}/Hits_{tot} \times NN_{tot}/NN$: $Hits_{sel}$ = the number of actives selected by the scoring function at a specific % level of negatives; $Hits_{tot}$ = the total number of actives seeded in the decoy database; NN_{tot} = the total number of negatives in the decoy database; and NN = the number of negatives selected by the scoring function at a specific % level of negatives.

charged decoy set was more challenging than the clustered one. Even so, reasonable screening performance could be observed from each docking/scoring combination against the

charged decoy set, especially for the GOLD/GlideScore combination.

Moderate hit rates determined by “model exhaustion points” could be reached with each docking/scoring combination (Table 3). The intersection approach of consensus scoring⁶⁶ was applied by combining the compound lists to further improve the hit rates. As a result, the consensus scoring method significantly increased the hit rates of active compounds (Table 3, the data in italic). Another method named “consensus docking”¹⁸ was also used to exclude false-positive compounds. However, the method was not effective because of its high expense of computational cost and limited hit improvement (Table 3, the data in bold).

Fragment Screening. Fragment-based drug discovery was increasingly utilized to identify new lead compounds⁶⁷ or to optimize existing leads for a given target.⁶⁸ Herein, the abilities of GOLD and Glide to identify potential RNA-binding fragments were evaluated through screening an RNA-directed fragment library and a fragment-like library against a rRNA A-site. The ROC curves were shown in Figure 8 for different docking/scoring combinations. Each

Table 3. Hit Rates Determined by “Model Exhaustion Points”

combinations ^a	hit rate ^b in charged decoys	hit rate in clustered decoys
Glide-GlideScore/GoldScore ^c	32.9 (28/87) ^d	30.0 (42/98)
GOLD-GoldScore/GlideScore ^e	24.8 (31/94)	29.2 (35/85)
GOLD docking/Glide docking ^f	25.7 (27/78)	15.8 (35/187)
Glide/GlideScore	14.2 (39/235)	14.7 (52/302)
Glide/GoldScore	12.2 (32/230)	9.8 (42/386)
GOLD/GoldScore	7.8 (32/375)	9.9 (35/318)
GOLD/GlideScore	19.4 (45/187)	14.0 (53/326)

^a Different docking engine/scoring function combinations. ^b Hit rate = the number of active in the hit list/the total number of compounds in the hit list × 100. ^c Consensus scoring with a single docking engine and double scoring functions. ^d (30/61): the number of actives/the number of nonactives. ^e Consensus scoring with a single docking engine and double scoring functions. ^f Consensus docking with two docking engines and their native scoring functions.

docking/rescoring combination showed prominent ability to discriminate actives from decoys over random selection, and moderate ‘early identification’ of active fragments was observed from the ROC curves. Different from simulated virtual screening, rescoring original docking poses did not improve the results. The tested docking programs with native scoring functions showed better identification ability than non-native scoring ones.

DISCUSSION

Factors Affecting Docking Accuracy. Recently, numerous research papers systematically explored effects of different factors on docking accuracy in protein fields, such as ligand input conformations,⁶⁹ ligand protonation states,⁷⁰ target conformations,⁷¹ and crystal water molecules.⁶⁴ In this work, we mainly explored three factors affecting docking accuracy against RNA targets, i.e., ligand input conformations, RNA target conformations, and crystal water molecules.

The results demonstrated that multiple ligand conformations as input significantly improved the docking accuracy compared to single ones, especially for large and flexible molecules like aminoglycosides. Because multiple ligand conformation input could increase the chance to search the conformational space globally, high-complexity RNA ligand could be correctly docked back to their cognate RNA targets in this way.

Structural sources of RNA targets might affect the docking accuracy, too. In this study, X-ray determined structures led to better results than NMR-derived structures. The same findings were reported in recent papers.^{28,29} Though multiple NMR conformers were considered as docking targets, which obviously improved the docking success rates, the results were still worse than those from crystal structures. The reason might be that the scoring functions of tested programs failed to accurately evaluate some specific RNA–ligand interactions determined by NMR, such as HIV-1 TAR RNA complexes.

In some cases, crystal water molecules may also affect the accuracy of RNA–ligand docking. Compared to the “dry” docking, “wet” docking was quite effective to rebuild experimental binding modes. Several reasons could account for this. At first, the presence of water molecules narrowed the space of the docking searching and sterically excluded

some docking modes that might mislead the scoring function to rank them higher. Second, bridging water molecules promised additional hydrogen-bond interactions, which greatly increased the probability of the correct docking. In addition, water molecules might partly shield strong electrostatic interactions between RNA and ligands. We also investigated the docking ability of GOLD in the presence of water molecules (data not shown). Unfortunately, preliminary tests showed most experimental ligand poses could not be correctly generated using the water switch embedded in the program. This was probably because the σ_P , representing the loss of rigid-body entropy, was optimized against a training set of protein–ligand complexes,⁴² rather than RNA–ligand complexes.

Analysis of Docking Failures. Analyzing the failed cases of the docking predictions from different docking strategies, we found some common features about RNA targets and ligands, which could provide some implications for RNA–ligand docking.

At first, when the same ligand was docked into different RNA targets, the docking performance could be quite different. For example, neomycin A could be correctly docked by GOLD and Glide against rRNA A-site (2ET8) but failed against HIV-1 DIS (dimerization initiation site, PDB code 2FCX). Actually, for five HIV-1 DIS complexes, GOLD only correctly reproduced two of them (2FCZ and 3C44) in “dry” docking, while Glide failed in all cases, even if multiple ligand conformations were considered in the docking. For such RNA targets, key water molecules were necessary for a reasonable docking accuracy.

Second, ligands located in solvent-exposed sites or specific identification moieties were difficult to dock correctly into the original sites by GOLD and Glide, such as HIV-1 TAR RNA binders including arginine, RBT203, RBT205, and RBT550 (taken from 1ARJ, 1UUD, 1UUI, and 1UTS, respectively). Similar docking performance on TAR RNA was also reported in recent papers.^{29,32} One of the reasons for the mis docking, we analyzed, was that no well-defined shape of the binding pocket could restrict the ligand orientations, especially for the solvent-exposed part of the ligand. In addition, some specific interactions, e.g., the interaction between A22/U23 of HIV-1 TAR RNA and the guanidine moiety of rat205 (1UUI),⁷² could not be described by the tested programs, which might be a scoring problem.

Hit Identification Capability of Docking Programs. Here three experiments were designed to validate the screening capabilities of the tested programs, including identification of cognate ligands, simulated virtual screening with two distinct decoy databases, and fragment screening against two fragment libraries.

Identification of cognate ligands of a specified RNA was regarded as a very demanding test because the programs should discriminate the cognate ligand from many others at first.²⁶ The results demonstrated that both programs could identify cognate ligands reasonably, and GlideScore possessed a global advantage compared to that of GoldScore. When rescoring the docking results, significant improvements were obtained in some cases.

In simulated virtual screening and fragment screening, rRNA A-site was selected as the target because of the availability of sufficient activities and small conformational changes in RNA upon binding of different molecules.^{73,74}

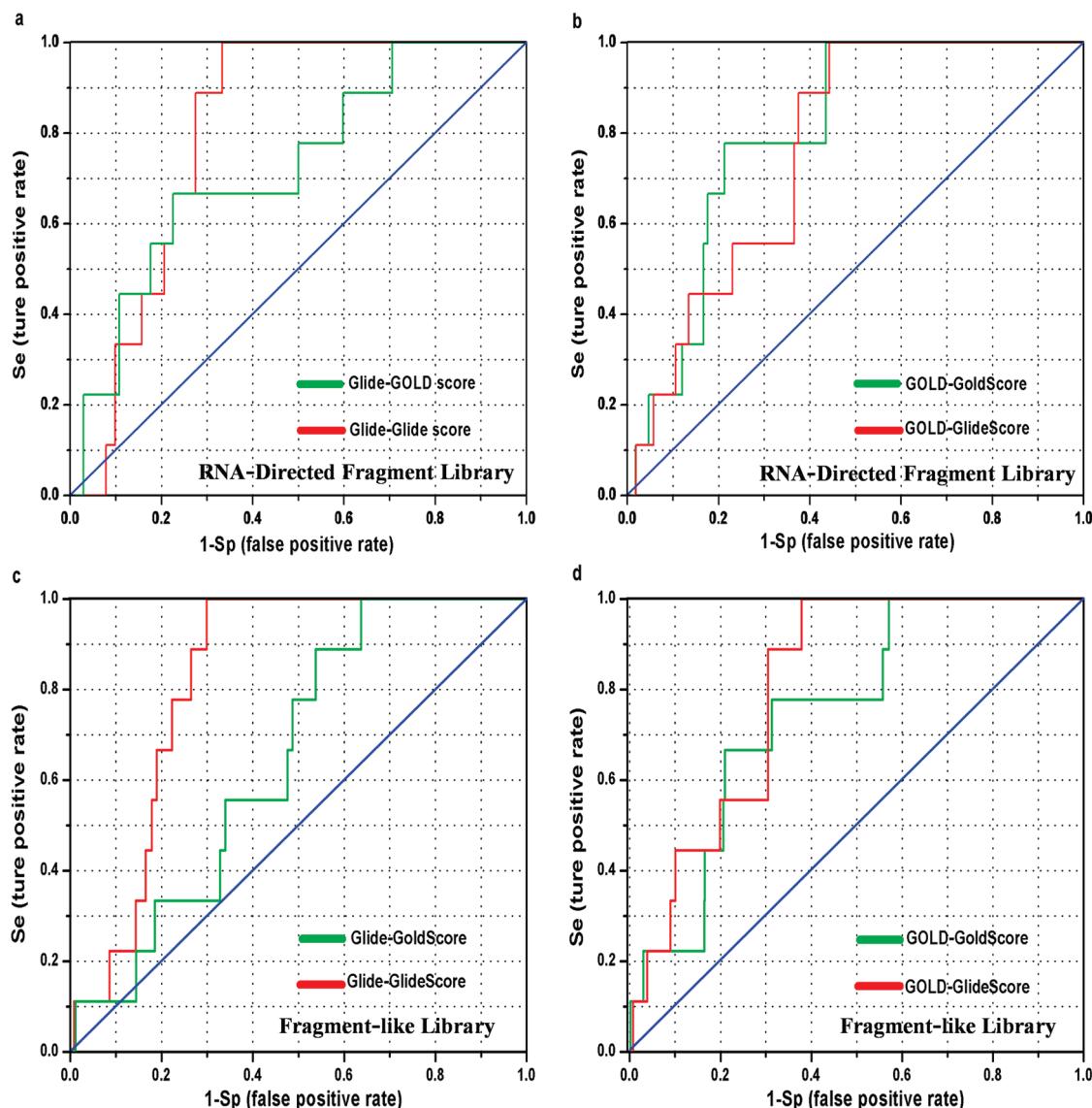


Figure 8. ROC plots of fragment screening performance of tested programs against rRNA A-site.

Though the GOLD/GlideScore combination provided the best performance in virtual screening, all other docking/scoring combinations in the test also led to good enrichments, which implied that either program could be independently used to find new RNA binders in virtual screening.

In fragment screening experiment, the results illustrated that both GOLD and Glide could effectively identify active fragment molecules targeting a rRNA A-site. Actually there were similar reports about docking accuracy of GOLD and Glide for fragment docking in the protein field.^{75,76} However, the adaptability in a protein field could not be simply shifted to a RNA field. Our test demonstrated that either program could be used in fragment-based drug design targeting RNA.

Comparison with Other RNA Docking Studies. Comparison of our results with other RNA docking studies was also performed. Three previous RNA–ligand docking studies, namely the studies with programs MORDOR,²⁹ RiboDock,²⁴ and DrugScore^{RNA},³² were compared with ours here, because they shared most of RNA complexes with ours. In these docking studies, the authors generally executed many independent docking runs per ligand to ensure that the validation of the scoring functions was not limited by sampling effects. In view of this situation, we only compared

our results from multiple ligand conformations as input with theirs. To facilitate the comparison, an rmsd cutoff value of 2.5 Å was set as the criterion of a successful docking, and only the best scoring pose was taken for comparison. As illustrated in Figure 9, the capabilities of GOLD and Glide to reproduce experimental poses were stronger than those of RiboDock and DrugScore^{RNA} but poorer than that of MORDOR.

To our knowledge, MORDOR is the most powerful RNA-specified docking program in reproducing RNA–ligand experimental poses so far, because it simultaneously considers the flexibilities of both the RNA target and its ligand during docking. Therefore, MORDOR performed better than GOLD and Glide on those RNA targets containing highly flexible ligands, such as rRNA A-site and HIV-1 DIS. However, considering the docking time, GOLD and Glide could be faster than MORDOR, especially for small molecules with less rotatable bonds, as they treated RNA rigidly. Therefore, GOLD and Glide could be more effective for virtual screening of a large compound library than MORDOR. In spite of the relatively low docking success rates in

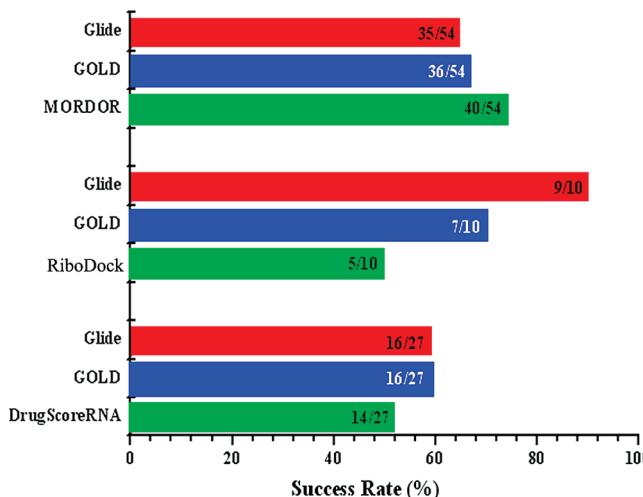


Figure 9. Success rate comparison of GOLD and Glide with MORDOR, RiboDock, and DrugScore^{RNA}. In each pair of comparison, the denominator is the number of common structures used in the comparison, and the numerator is the number of successful docking.

this comparison, RiboDock and DrugScore^{RNA} have their own advantages on some special RNA targets, such as HIV-1 TAR RNA.

Implications for RNA-Targeted Drug Discovery. In general, the molecular docking technique can be applied for three main purposes at different phases of drug discovery process,⁷⁷ i.e., to predict the binding mode of known active compound against a specific target, to identify new lead candidates in virtual screening workflows, and to predict binding affinities of related compounds from known active series.

In this study, we tested the capabilities of two protein-based docking programs to reproduce experimental binding modes against RNA targets and obtained acceptable docking accuracy, which provided us an opportunity to predict bioactive conformation of a ligand within the RNA binding pocket. Based on the RNA–ligand binding mode, further studies could be carried out, such as 3D quantitative structure–activity relationship (QSAR) analysis on a series of ligands, molecular dynamics simulation on RNA–ligand interactions.

In simulated virtual screening, reasonable enrichment was obtained by GOLD and Glide, which indicated that both programs could provide helpful information in the discovery of RNA-targeted ligands. A consensus ranking by combining GoldScore with GlideScore can significantly enhance the hit rate of new active RNA binders. Ligand-based data mining methods, such as support vector machines and Bayesian classifiers, can be implemented for the prescreening of a compound database before the uses of the tested docking programs. These methods are likely to further improve the hit rate of actives.

The capabilities of GOLD and Glide in fragment screening against RNA targets were also validated, which may be very helpful for side chain transformation or scaffold hopping during lead optimization. Actually, there was a report before in which several novel antibiotics were successfully designed by an *in silico* fragment screening method.⁶⁸

As for the prediction of binding affinities of a series of compounds, multiple ligand conformations should be used

as input so that more accurate docking poses and stable docking scores could be received, in particular for those highly flexible molecules. In fact, this goal was essentially beyond most current docking methods.⁷⁷ For that purpose, more sophisticated techniques can be implemented on the modeled complexes to estimate the binding free energy, e.g., molecular dynamics-based linear interaction energy and molecular mechanics-Poisson–Boltzmann surface area (MM-PBSA) methods.⁷⁸

CONCLUSIONS

In this paper, two protein-based docking programs, GOLD and Glide, were validated to be suitable for structure-based drug design and virtual screening against ribonucleic acid (RNA). When a test set of 60 RNA–ligand complexes was applied to assess the capability of the cognate ligand docking, both GOLD 4.0 and Glide 5.0 could reproduce experimental binding modes. Even for those highly flexible ligands, like aminoglycosides, the docking programs still performed well.

This work also presented how the crystal structure of the bacterial rRNA A-site was applied for structure-based virtual screening. By analysis of the receiver operating characteristic (ROC) curves, we explored the virtual screening capability of two docking programs. With the consensus scoring method, false-positive rates in simulated virtual screening were substantially reduced. The capability of tested programs on fragment screening was also validated against the rRNA A-site. Taking all the results into account, we believe that current protein-based docking programs can be well used in RNA-based drug discovery and design.

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Supporting Information Available: “Dry” docking results for 60 RNA–ligand complexes, comparison of docking results using different conformers in NMR ensembles, comparison between “wet” and “dry” docking results, RNA ligand names included in “identification of cognate ligands” test. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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