

Validation of Automated Docking Programs for Docking and Database Screening against RNA Drug Targets

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The increasing awareness of the essential role of RNA in controlling viral replication and in bacterial protein synthesis emphasizes the potential of ribonucleoproteins as targets for developing new antibacterial and antiviral drugs. RNA forms well defined three-dimensional structures with clefts and binding pockets reminiscent of the active sites of proteins. Furthermore, it precedes proteins in the translation pathway; inhibiting the function of a single RNA molecule would result in inhibition of multiple proteins. Thus, small molecules that bind RNA specifically would combine the advantages of antisense and RNAi strategies with the much more favorable medicinal chemistry of small-molecule therapeutics. The discovery of small-molecule inhibitors of RNA with attractive pharmacological potential would be facilitated if we had available effective computational tools of structure-based drug design. Here, we systematically test automated docking tools developed for proteins using existing three-dimensional structures of RNA–small molecule complexes. The results show that the native structures can generally be reproduced to within 2.5 Å more than 50–60% of the time. For more than half of the test complexes, the native ligand ranked among the top 10% compounds in a database-scoring test. Through this work, we provide parameters for the validated application of automated docking tools to the discovery of new inhibitors of RNA function.

Introduction

The explosion in the number of structures of proteins and their complexes and advances in computational methods over the past decade have allowed great progress to be made in the development of automatic docking methods. Such programs now provide an established set of tools to discover novel inhibitors for protein drug targets.^{1–11} Combined with high-throughput screening methods and with new ways to use NMR and X-ray crystallography in the early phases of drug discovery,^{12–17} these methods have resulted in the discovery of new lead compounds.^{6,18–21} An important aspect of the successful application of these programs has been the validation of docking tools and the establishment of tested parameters for their application.^{22–24} In sharp contrast, rational drug design methods directed at DNA and RNA have received much less attention. In significant part, this has been due to the paucity of 3D structures of small molecule–nucleic acid complexes. Until recently, we simply did not know enough about RNA recognition to apply rational methods to the discovery of new RNA-binding compounds. It is nonetheless likely that the application of computational docking methods will provide very valuable approaches to increase the likelihood of discovering druglike RNA-binding compounds. Because existing computational approaches and scoring functions have been developed for proteins, we do not know how well they work in the RNA environment. It is the goal of this work to evaluate the performance of automated docking tools against RNA.

RNA is an attractive target for infectious disease because of unique resistance patterns and mechanism of action of potential RNA-binding drugs. Because RNA is located upstream from proteins in the gene-expression pathway, blocking one RNA molecule would inhibit the function of multiple proteins by affecting their synthesis.^{25–28} RNA has long been a target of antimicrobial therapy; many existing antibiotics bind to the ribosome. Among them are the aminoglycosides,^{26,29–37} pharmaceutically more important drugs such as the macrolide erythromycin,³⁸ and a recent new class of antibiotics (oxazolidinones^{39,40}). While the potential of RNA as a drug target has been advocated for some time,^{25,26,28,33,41–45} progress has not been easy. Existing RNA-binding antibiotics do not provide attractive paradigms. Most are natural products; they tend to be highly positively charged, have multiple torsional bonds, and are often relatively large compounds. They generally violate “rules” that describe the features of successful drugs established empirically through the analysis of existing therapeutic compounds.⁴⁶ They also tend to be poorly specific; they target not only the bacterial ribosomal RNA but many other RNAs as well with comparable activity *in vitro*. Clearly, there is a need to discover new scaffolds and functional groups suitable for RNA recognition. While the high-throughput screening of proprietary and combinatorial libraries has provided insight into RNA recognition and a number of chemically attractive RNA ligands,^{47–51} it is necessary to explore chemical space more widely in search of compounds suitable for binding to RNA.

RNA forms well-defined tertiary structures with deep pockets and clefts lined by hydrogen-bonding groups reminiscent of the active site of protein enzymes.^{26,28,44}

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Table 1. Database of RNA–Ligand Complexes

PDB code	compd	technique	K_d (μ M)	ΔG (kcal/mol)	ref
Rigid and Aromatic					
1AM0	AMP aptamer	NMR	2.7	−7.59	108
1EHT	theophylline aptamer	NMR	0.4	−8.73	109
1F1T	malachite green aptamer	X-ray/2.8 Å	0.04	−10.09	110
1F27	biotin aptamer	X-ray/1.3 Å	6	−7.12	111
1FMN	FMN aptamer	NMR	0.5	−8.59	112
1LVJ	TAR-acetylpromazine	NMR	0.1	−9.55	47
Weak Binders					
1AJU	HIV-2 TAR argininamide	NMR	1000	−4.09	113
1KOC	arginine aptamer	NMR	60	−5.76	114
1KOD	citrulline aptamer	NMR	68	−5.68	114
Aminoglycosides					
1BYJ	gentamycin ribosome A-site	NMR	0.01	−10.91	115
1EI2	neomycin splice regulator	NMR	1	−8.18	58
1NEM	neomycin aptamer	NMR	0.1	−9.55	30
1PBR	paromomycin ribosome A-site	NMR	0.2	−9.14	116
1QD3	neomycin HIV 1 TAR	NMR	1	−8.18	117
1TOB	tobramycin aptamer	NMR	0.0009	−12.34	118
2TOB	tobramycin aptamer	NMR	0.0012	−12.17	32

Table 2. Data Set of Antibiotic Structures

PDB code		resoln (Å)	ref
1FJG	thermophilus 30S ribosomal subunit + streptomycin, spectinomycin, and paromomycin	3.0	119
1HNW	thermophilus 30S ribosomal subunit + tetracycline	3.4	120
1HNX	thermophilus 30S ribosomal subunit + pactamycin	3.4	120
1HNZ	thermophilus 30S ribosomal subunit + hygromycin B	3.3	120
1J7T	16S-rRNA A-site + paromomycin	2.5	121
1JZX	50S ribosomal subunit + clindamycin	3.1	38
1JZY	50S ribosomal subunit + erythromycin	3.5	38
1JZZ	50S ribosomal subunit + roxithromycin	3.8	38
1K01	50S ribosomal subunit + chloramphenicol	3.5	38
1K8A	50S ribosomal subunit of <i>Haloarcula marismortui</i> + carbomycin	3.0	122
1K9M	50S ribosomal subunit of <i>Haloarcula marismortui</i> + tylosin	3.00	122
1KD1	50S ribosomal subunit of <i>Haloarcula marismortui</i> + spiramycin	3.0	122
1LC4	16S-rRNA A-site + tobramycin	2.54	123
1M90	50S ribosomal subunit + sparsomycin + CCA-phe-caproic acid	2.8	124
1MWL	16S-rRNA A-site + geneticin	2.4	125
1NJM	50S large ribosomal subunit + sparsomycin	3.6	126
1NJN	50S large ribosomal subunit + sparsomycin	3.7	126
1NJO	50S large ribosomal subunit + accupromycin (ACCP)	3.7	126
1NWY	Large ribosomal subunit + azithromycin	3.3	127
1OND	50S ribosomal subunit + troleandomycin	3.4	128

It should therefore be possible to apply rational drug design methods to discover new small-molecule inhibitors of RNA and RNA–protein complexes. Following a very early study on perfect double helical RNA,⁵² an important proving ground has been represented by the HIV-1 TAR RNA.^{41,47,48,50} Other targets have been the ribosomal A-site⁵³ (the binding site for aminoglycosides and other antibiotics) and a functional subdomain of 23S rRNA involved in the GTPase activity of the ribosome.⁵⁴

The considerable recent progress in understanding RNA structure and folding, as well as recognition by other ligands,^{26,33,35,55–59} makes it now possible to apply structure-based design and docking methods to discover new RNA-binding compounds. Our long-term objective is to develop reliable methods to screen chemical databases to find druglike molecules that bind to RNA tightly and specifically and that retain attractive pharmacological characteristics. Here, we investigate whether automated docking methods successfully used in protein-based drug design can be applied to RNA as well. For this purpose, we have tested two docking tools (AutoDock⁶⁰ and Dock⁶¹) to establish whether such programs would provide an effective platform for RNA-targeted database screening.

Methods

Database Construction. We have tested the performance of AutoDock and Dock on a data set of 16 selected ligand–RNA structures derived from the PDB for which experimental binding constants are also available (Table 1). Approximately half of them are NMR structures of RNA aptamers; only two are crystallographic structures. Ribosomal complexes were not included in this test set because binding constants are not available. We retained these structures as an independent validation set (Table 2). The complexes cover several logs in affinity (ΔG varies from −4.09 to −12.34 kcal/mol) and are diverse in structure. The ligands range from small, very flexible aliphatic molecules with 12 heavy atoms to large flexible and cyclic molecules (30–40 heavy atoms, 3–4 rings) and rigid aromatic molecules with and without flexible side chains (13–30 atoms, 2 and 3 rings).

Molecule Preparation. The program MOE (Chemical Computing Group Inc., Montreal; <http://www.chemcomp.com>) was used to separate the ligand from the RNA, to model missing residues, and to remove water molecules and counterions. For the NMR structures we used the first one in the set unless a different

structure was specified as the minimum energy structure in the PDB file. Hydrogen atoms were added to the X-ray structures using standard geometrical parameters as implemented in MOE. Partial charges for the RNA were added according to Kollman '94.⁶² For the ligands, we used PEOE (partial equalization of orbital electronegativities) charges.^{63,64} It should be noted that the AutoDock scoring function was calibrated using these two same sets of charges for the receptor and the ligands.⁶⁰ We evaluated other charges for the RNA (CFF91, Charmm and Gasteiger), but the aforementioned combination gave the best results in correlating ΔG_{exp} with ΔG_{pred} (data not shown) and was therefore used throughout this study.

Electrostatics and Solvation. Force field scoring, as used in Dock, tends to overestimate electrostatic energy.⁶⁵ To reduce the overall negative charge on the backbone of the RNA (there are no counterions present in any of the NMR structures), we increased the charge on the phosphorus atom from 1.166 to 2.166 to simulate the presence of a sodium counterion. The advantage of this is that the charge on the phosphorus is reduced. James and colleagues similarly scaled the negative charge on the phosphate groups to 20–30%.^{66,67} Solvation parameters for AutoDock were derived from similar atom types in amino acids (in Supporting Information). In addition, nitrogen atoms accepting and not accepting hydrogen bonds were separately defined for the grid map calculation. A 12-6 Lennard-Jones potential was used for nitrogen-carrying groups that do not form hydrogen bonds, while a 12-10 potential was used for hydrogen-bond-forming nitrogens.

Dock. Version 4.0.1 of the Dock suite of programs is still regarded as state of the art^{68,69} and was used in this study. Connolly surfaces^{70,71} were constructed using MS (no. 429 in QCPE, Indiana University). Dock uses a parameter (dotlim in the INSPH file) to facilitate sphere construction for wide active sites (such as the DNA or RNA major grooves); it was set to -1 . The binding site was represented in each case through 20–100 overlapping spheres constructed with sphgen.⁷² The three-dimensional grid was then calculated with a spacing of 0.3 Å, centered in the active sites and extended by 10 Å in each direction. Energy scoring grids were obtained by using the all-atom model and a distance-dependent dielectric function with a 10 Å cutoff and a dielectric factor of 4. A bump filter was used with an overlap of 0.75. For rigid docking, we used automated matching with a maximum of 5000 orientations; zero bumps were allowed. For rigid database docking, we used manual matching⁶¹ with a maximum of 5000 orientations, a distance tolerance of 0.5 Å, and a bump filter with zero bumps allowed. For flexible docking of single compounds, we used no anchor search, clash overlap of 0.5 Å, and a conformation cutoff factor of 5. For flexible database docking, we used anchor search with torsion drive and 50 configurations per cycle, a flexible bond maximum of 20, and a distance tolerance of 0.5 Å.

AutoDock. AutoDock, version 3.05, was used in this study. The grid for energy evaluation was set in the center of gravity of the ligand with dimensions of 60 points \times 60 points \times 60 points and spacing of 0.375 Å. Initial translation, quaternion, and torsion steps of 2.0

Å, 50.0°, and 50.0°, respectively, were chosen with a reduction factor of 1 per cycle. Standard Lamarckian genetic algorithm parameters were used. We used a united atom representation for the ligand, which gave a better correlation of predicted and experimental binding energies when compared to the all-atom representation (data not shown). The united atom representation also speeds up the search. Each docking simulation consisted of 100 independent docking runs. For database docking, self-written shell scripts docked each ligand consecutively into the active site and extracted the ranking and scoring parameters from the docking log file.

Docking Accuracy and Reliability. An effective docking program must be reliable and accurate (multiple docking runs generate the target or a very similar structure), must be able to rank compounds accurately, and must identify putative ligands from random compound sets to minimize the number of false positives. It is also useful for the program to provide at least qualitative estimates of the binding constant. To establish reliability parameters, 100 independent docking runs were performed with both programs. We define docking accuracy as how well the native pose of the ligand is reproduced by the combination of algorithm and scoring function in multiple independent runs. An arbitrary value of a 2.5 Å rmsd from the experimental structure was chosen to separate successful and unsuccessful docking poses. Cutoff values between 1.0 and 3.0 Å root-mean-square deviation (rmsd) between docked and X-ray pose have been used in past studies to define successfully docked poses.^{2,65,73,74} While a value of 2 Å is most often used,^{23,24,75,76} we increased the cutoff to 2.5 Å because most test complexes are NMR structures and are therefore less precise and accurate. In testing how well the experimental binding constant is reproduced by AutoDock, we used the epdb function to evaluate the energy of the native structures in the active site of the RNA. For each NMR-derived coordinate set, 10 independent structures were extracted from the NMR ensemble to estimate how the coordinate error affects the energy evaluations.

Database Docking. A primary characteristic of an effective database-docking tool is the ability to rank cognate ligands highly while at the same time ranking nonbinding ligands unfavorably. This is crucial to minimize the number of false positives. We conducted two tests. In the cross-docking experiment, every ligand of the test set was docked against each of the RNA present in the test set. This test is highly demanding. The native ligand should ideally to be ranked as the highest compound among other RNA binding ligands. In the second test, we spiked a database of 49 drugs randomly chosen from the comprehensive medicinal chemistry database, CMC (MDL, San Leandro) with each RNA-binding ligand. This test represents a realistic if limited in size simulation of a real database docking experiment. For Dock, all ligands were saved in a single multi-mol2 file that was used as the ligand input file. Since AutoDock does not provide a routine for database docking, each ligand was docked separately into the receptor and the results were then collected in a single file.

Results

In seeking to validate automated docking tools for use with RNA, we selected two programs that use different algorithms and two different approaches to evaluate the interaction energy. Dock^{77–80} uses a shape-based matching algorithm and a force-field based scoring function derived from Amber.^{62,81,82} The interaction energy comprises van der Waals and electrostatic interactions but lacks explicit hydrogen bonding, solvation, and hydrophobicity, as well as entropic terms describing solvation and freezing of rotational degrees of freedom. This approach is inherently unable to provide absolute estimates of binding constant and can only rank compounds. AutoDock⁸³ uses a genetic algorithm for global search (Lamarckian genetic algorithm) and a local search algorithm.⁸⁴ The program uses a semiempirical energy function calibrated on 30 protein–ligand complexes.⁸⁵ It comprises van der Waals, hydrogen bond, electrostatics, torsional terms, and a solvation term based on a sigmoidal distance-dependent dielectric function.^{86,87} Unfortunately, other programs that use different algorithms are at present unsuitable for working with nucleic acids. FlexX, for example, lacks atom types definition for nucleotides.^{88–90}

The binding free energy of RNA–ligand complexes can be qualitatively reproduced by a semiempirical scoring function. We first evaluated how well the inhibition constants of RNA–ligand complexes can be predicted qualitatively through the semiempirical approach implemented in AutoDock. Although the database of 16 complexes available at the present time is small and the available K_D range not vast, the small molecules are diverse in structure. We expected that the experimental results would be reproduced only after recalibration of the weight of different components of the scoring function. However, when measured and predicted binding constants are compared, we obtain a best-fit line (excluding the two outliers) with a slope very close to ideal (1) and intercept close to the origin (Figure 1). Because many of the structures in the database are NMR-derived, multiple copies of the same coordinate set are generally available. By calculating the predicted free energy of binding for the same complex using different coordinate sets, we were able to evaluate how the uncertainty on the coordinates affects the free energy prediction. When considering the experimental uncertainty both in the measured (error bars in Figure 1a, left) and predicted (dots in Figure 1b, right) binding energies, and the very limited data set of structures (only 16), we concluded that recalibration of the scoring function would be unwarranted. The data of Figure 1 suggest that the AutoDock scoring function appears to reproduce existing binding constants for the RNA–small molecule constant semiquantitatively. A larger data set of structures will be required to improve the model and provide a more accurate description of the binding energy of RNA–small molecule complexes.

The outlier structures (1NEM and 1F1T, encircled in Figure 1) provide interesting insight into the limitations of the scoring function. Nitrogen atoms on the RNA bases are treated by AutoDock as hydrogen bond acceptors and are represented by a spherical potential around the atom center,⁹¹ leading to a region of unrealistically favorable interaction energy both above and

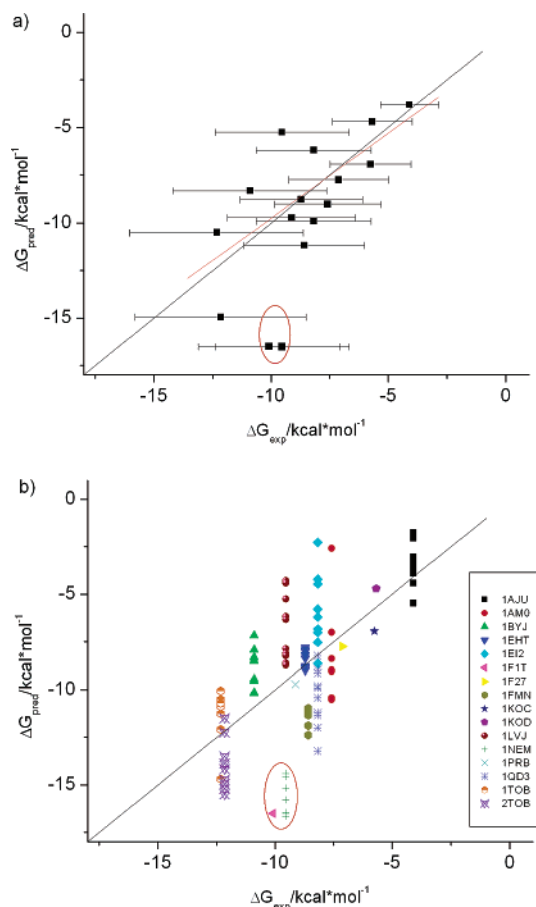


Figure 1. Predicted vs experimental free energy of binding for the 16 training complexes as calculated by AutoDock. (a) Predicted vs experimental binding energies. The error bars represent either reported uncertainties on measured K_D (whenever available) or a 20% uncertainty (whenever experimental error estimates were not reported). A line of best fit (shown in red, excluding the circled outliers) has a standard deviation of 2.12 kcal/mol. (b) Uncertainties in the experimental coordinates affect the prediction of the free energy of binding. For each complex, the binding free energy was calculated independently and plotted for the 10 best structures enclosed in the NMR-derived coordinate set.

below the plane of the base (Figure 2). Thus, the lack of directionality for hydrogen bond acceptors appears to be a limitation of the simple model used to derive energy estimates.

Given the highly charged nature of RNA molecules, we also examined whether different charge sets would improve the predictive value of the semiempirical potential. Different combinations of partial charges for the receptor and the ligands were independently evaluated. We found that the combination of Kollman94⁶² charges for the receptor and PEOE^{63,64} charges for the ligand gave the best results based on the correlation of predicted and experimental free energy of binding (data not shown). This result is probably attributable to the fact that AutoDock was calibrated using this exact combination of partial charges.⁶⁰

Reliability of the Docking Algorithms and Scoring Functions. A strongly performing docking program should be able to reproduce the target structure reliably and accurately. To examine this feature, we evaluated how many independent poses cluster to within a preset deviation from the experimentally determined target

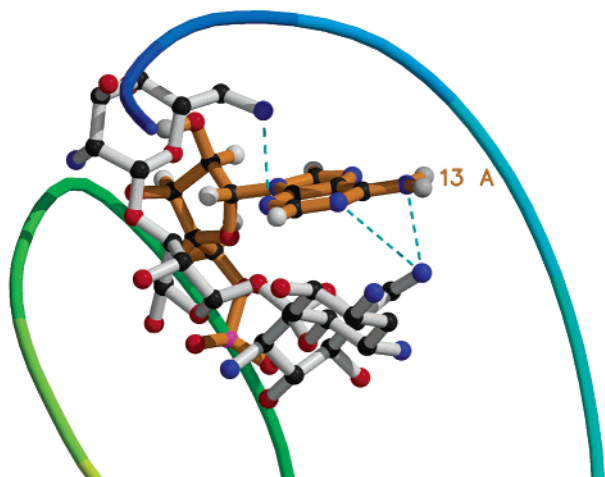


Figure 2. Close-up of 1NEM highlighting out-of-plane electrostatic interactions between neomycin amines and adenosine base nitrogens. Contacts with base nitrogen atoms above and below the base plane are identified with cyan lines. The RNA backbone is represented by the tube, and residue A13 of the RNA and the ligand are rendered in ball-and-stick representation. Ligand hydrogen atoms have been omitted for clarity. The figure was produced with MolScript¹²⁹ and rendered with Raster3D.^{130–132}

structures. An example of the performance of AutoDock on different sets of ligands is provided in Figure 3, which shows superposition of multiple independently generated docking poses for three complexes. Quantitative estimates are shown in Figure 4, where we report the “successful docking rate”: the number of structures in 100 independent docking runs that cluster within 2.5 Å of the target structure.

Dock reproduces the native structure to within 2.5 Å for complexes of rigid aromatic ligands but performs poorly with weak-binding ligands (1AJU, 1KOC, 1KOD) and with aminoglycosides (Figure 4a). The three weak-binding complexes (argininamide-TAR 1AJU, arginine aptamer 1KOC, and citrulline-aptamer 1KOD) are also very flexible ligands. The poor performance of the algorithm is understandable given the relatively weak binding constant and flexible nature of the ligands. Concerning 1AJU, it has also been shown that multiple binding sites on TAR exist.⁵¹ For aminoglycosides, only in some cases (paromomycin 1PBR and tobramycin I aptamer 1TOB) can the target structure be reproduced successfully. Complexes where hydrophobic interactions predominate (for example, complexes where intercalation is observed such as 1AM0, 1EHT, 1F1T, 1FMN) were instead docked successfully more than 50% of the time. Dock has been reported to perform better with hydrophobic binding sites and to be less effective with hydrophilic target structures.²² In a test conducted with herpes simple virus thymidine kinase, a receptor with a wide binding site and high accessibility to water (not unlike RNA), Dock was unable to find the correct pose for 3 out of 10 known ligands. It was also able to identify only 1 out of 10 known ligands from a database of 990 molecules (among the top 5% scorers).²² An additional limitation is of a technical nature. Dock generates an image of the active site through a set of spheres; the quality of the constructed spheres is critical to its success. The major groove of RNA is much more open and wide than a “classical” active site pocket, making sphere construction less rigorous.

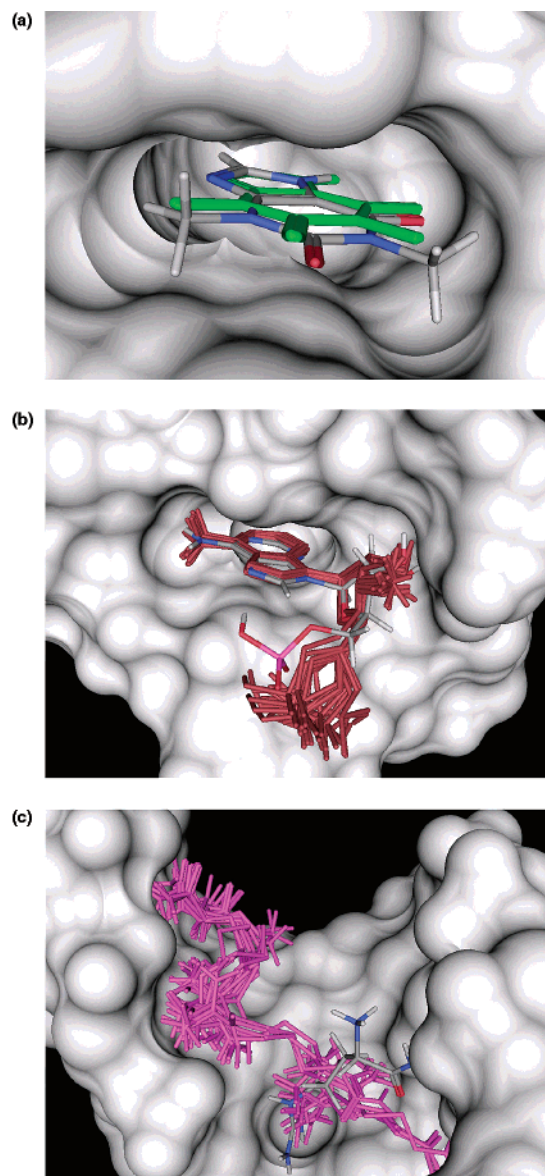


Figure 3. Superposition of representative structures from multiple independent docking runs (AutoDock): (a) 1EHT; (b) 1AM0; (c) 1AJU. Native ligands are in atom color. For 1AM0 and 1EHT, all docking runs cluster within 2.5 Å of the target structure. For argininamide (1AJU, a major groove binder with low affinity), the ligand is found to cover a wide section of the binding pocket. Clustering of the ligand in preferred locations is nonetheless observed even in this least successful example.

AutoDock reproduces the conformation observed in the target complex (Figure 4b) much more often than Dock. More than 50% of the docked structures lie within the cutoff value for most ligands, the exceptions being again two of the weak binders (1AJU, 1KOD) and some aminoglycosides (1EI2, 1QD3, 1PBR), the same structures that performed worse with Dock.

A complementary way to evaluate the reliability of docking programs is to plot the results of each independent docking run after they have been ordered for increasing deviation from the target (Figure 5). Ideally, the line should be knee-shaped, with a majority of docked structures falling below the rmsd cutoff value. This pattern is seen for AutoDock in a majority of cases (the most successful being 1EHT, 1F1T, 1TOB, 2TOB, 1BYJ, 1F27, 1AM0, 1KOC, 1NEM, and 1FMN). For

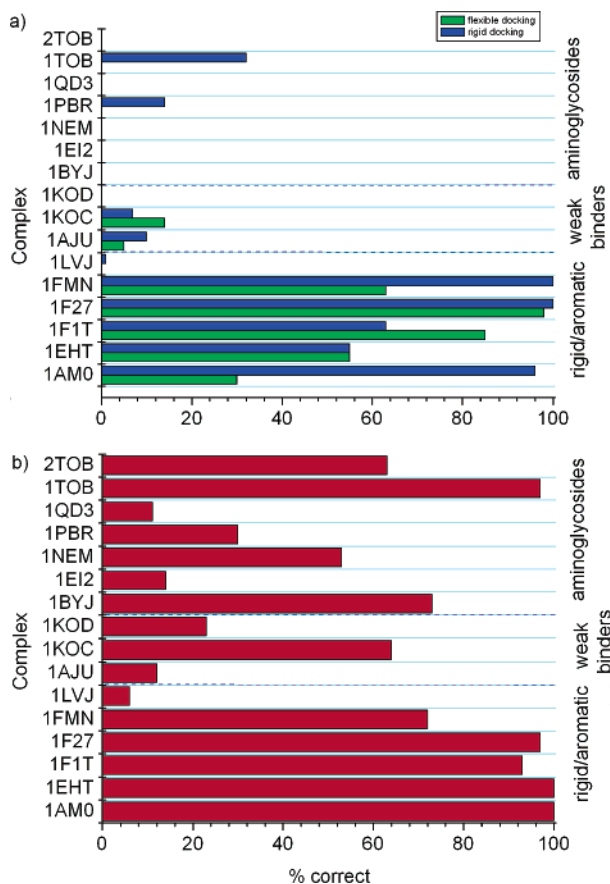


Figure 4. Reproducibility of the target structures in multiple independent docking runs. For each test structure, the ligand was independently docked 100 times into the active site and the resulting poses were compared to the experimentally determined native structure. Structures that cluster within 2.5 Å rmsd of the test structure were regarded as successfully docked. (a) Dock: blue bars show the result for rigid body docking, while green represents flexible ligand docking. (b) AutoDock.

Dock, only a few test structures (1EHT, 1F1T, 1F27, and 1FMN) yield rmsd-ordered curves comparable to those observed with AutoDock.

Docking and Ranking Accuracy. We next evaluated whether structures that cluster the closest to the target are also ranked most favorably. In a strongly performing docking program, poses that reproduce the experimental structure the best would also correspond to the best scoring docking runs. Ideally, one would like to observe a plot in which the structures closest to the target cluster closely in space and are ranked among the best scoring poses, thus generating a sector-shaped plot.

When the rank of each independent pose is plotted versus the rmsd from the native structure (Figure 1 in Supporting Information), Dock generally provides a poor correlation between ranking and docking except for 1F27, 1FMN, and 1F1T (where most poses cluster at low rmsd from the target). In contrast, the rigid aromatic compounds (1AM0, 1EHT, 1F1T, 1F27) and 1TOB can be docked by AutoDock to within a very narrow rmsd range near the target. Satisfactory behavior is observed for 1FMN and 1NEM and, less clearly, for 1PBR, 1QD3, 2TOB, and 1BYJ. The sector shape of the plot shows the clustering of low-rmsd docking runs among the higher-ranking compounds, while high-rmsd

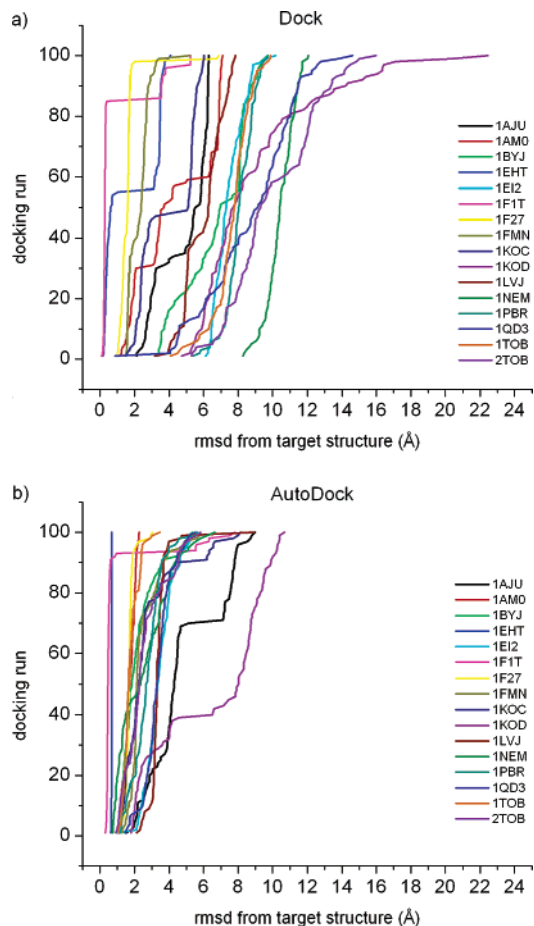


Figure 5. The rmsd from the target structure for independent docking runs. The figure reports the rmsd-ordered deviation from the target of successively docked structures for Dock (a) and AutoDock (b). For most test structures, many more poses cluster near the target structure when AutoDock is used. Each "docking run" represents an independent docking experiment.

compounds generally tend to rank worse. No correlation between ranking and scoring is observed instead for the weak binding compounds 1AJU and 1KOD, for 1LVJ (a rigid compound), and for 1EI2 (an aminoglycoside). For 1EI2, for example, the pose closest to the target (1.1 Å rmsd) ranks 16/100, while the best scoring structure is 4.73 Å away from the target. For 1KOD the closest-docked structure (1.78 Å rmsd from the native) ranks 44, while the best scoring structure is 10.28 Å away from the target.

A rigorous way to identify test structures with strong correlation between ranking and rmsd is provided by Spearman's rank correlation coefficient.⁹² Most test sets are positively correlated (as desired) (Table 3), but generally the correlation is weak, indicating the inability of the programs to favorably rank structures that cluster close to the target for these complexes.

Database Docking and Cross-Docking Tests. The first and most stringent test of the performance of the docking algorithms in a database search was the cross-docking experiment (Figure 6a). In this test, each of the 16 ligands competes with the other 15 to rank first for its cognate receptor. This test is very demanding because the programs have to sort the native ligand from a set of RNA binding molecules that generally share common RNA-binding characteristics (such as favorable electrostatics). Furthermore, the aminoglyco-

Table 3. Spearman's Rank Correlation Coefficient Calculated for the Results from 100 Docking Experiments for Each Complex in the Test Set (See Also Figure 1 in Supporting Information)

complex	rank/rmsd correlation coefficient for	
	Dock	AutoDock
Rigid and Aromatic		
1AM0	0.039 53	0.371 28
1EHT	0.356 82	-0.199 32
1F1T	0.196 62	0.258 22
1F27	-0.222 82	-0.256 91
1FMN	0.267 10	0.522 31
1LVJ	0.112 97	0.512 99
Weak Binders		
1AJU	-0.140 32	-0.205 67
1KOC	0.095 48	0.178 35
1KOD	0.608 99	-0.519 27
Aminoglycosides		
1BYJ	0.069 55	0.740 39
1EI2	-0.090 92	-0.124 55
1NEM	0.050 80	0.935 05
1PBR	0.408 17	0.671 41
1QD3	-0.117 40	0.456 42
1TOB	-0.032 96	-0.105 49
2TOB	0.063 09	0.729 89

sides are known to bind multiple RNA targets equally well and with activity significantly stronger than reported for the weak binding compounds in the data set. They should be expected to score favorably even against noncognate targets and to outrank weak-binding compounds such as arginineamide.

AutoDock positions the correct ligand near the top (rank 1 or 2) in 7 out of 16 cases. As expected, the algorithm has difficulties finding the correct ligand for the weak binders 1AJU, 1KOC, and 1KOD, but it performs surprisingly well for most aminoglycosides. Except for 1EI2 and 1QD3 (both neomycin complexes), all aminoglycoside complexes are correctly ranked as the top or second-best ligand. Dock found the cognate ligand in the top 2 positions in 4 out of 16 cases using flexible docking (bonds are allowed to rotate according to a rotamer library) and 9 out of 16 times using rigid docking (the conformation of the ligand is maintained and only its orientation is probed on the surface of the receptor). Because the orientation is predetermined and correct, it is easier for the ligand to find its cognate site; thus, executing this test in the "rigid" mode over-represents the success rate of the algorithm. None of the aminoglycosides were positioned satisfactorily in flexible docking, while three (1EI2, 1TOB, and 1PBR) were positioned correctly using rigid docking (data not shown). As in other tests, Dock produced better results for ligands with hydrophobic ring systems.

In the database-docking test (Figure 6b), we simulated the process of screening a database. A small test group of 49 drugs taken from the CMC (Comprehensive Medicinal Chemicals, MDL; San Leandro, CA) was spiked with each of the 16 native ligands one at a time. The algorithms were asked to dock the native ligand and each of the 49 noncognate molecules and to compare the respective ranks. An arbitrary 10% cutoff value (rank 1–5) was used to provide intuitive guidance. In the flexible docking test, 10 out of 16 native ligands are identified among the five best ranking compounds by both Dock and AutoDock. AutoDock ranked the entire set of the aminoglycosides among the best-ranking

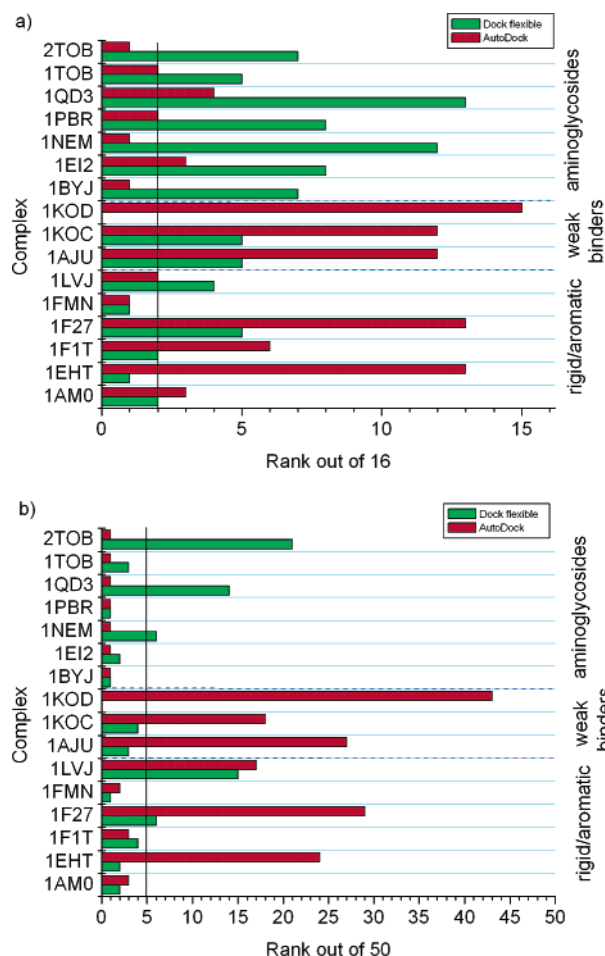


Figure 6. Cross-docking and database docking tests. (a) A small database containing all the ligands in the test set was constructed and screened against each of the target structures contained in the RNA–ligand database of Table 1. (b) In the database docking test, the programs are asked to recognize the native ligand in a test database of 49 non-RNA binding compounds. In both tests, AutoDock (red) performs better with aminoglycosides while Dock (green) is most effective with rigid aromatic compounds. The vertical lines represent the top 10% cutoff line.

ligands, but the weak binders were problematic (as expected) and so were several rigid aromatic compounds (surprisingly). Neomycin (1QD3) is well ranked by AutoDock but not by Dock. This is likely to be due to a partially closed binding site with one base partly enclosing the ligand, requiring a more sophisticated search method (as implemented in AutoDock) to position the ligand correctly.

Consensus Scoring. Consensus scoring (i.e., combining different scoring functions to rank compounds) can drastically enhance the outcome of database screening by reducing the number of false positives.^{22,75,93,94} Consensus scoring can be executed through "rank-by-rank" (the score is the average rank of each compound calculated from each participating scoring function), "rank-by-number" (each scoring function contributes to the score with its individual predicted value, and the consensus score is simply the resulting average), and "rank-by-vote" (if a compound is ranked among the top $X\%$ of the database, it gets a vote from that scoring function; the final score is the number of votes from all participating scoring functions). Rank-by-number is not appropriate given the completely different nature of the

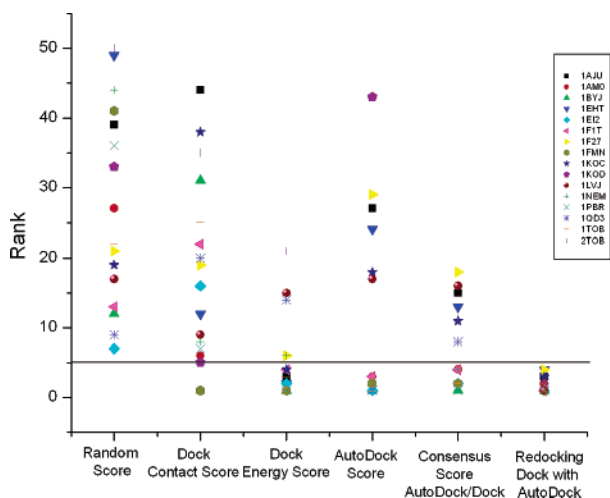


Figure 7. Consensus scoring for database docking. The figure shows the rank (the position out of 50 compounds after scoring) of each native ligand from our test set in the test database as generated at random (leftmost column, random score) and by individual scoring functions: (contact) Dock contact score; (energy score) Dock energy score; (AutoDock score) AutoDock semiempirical free energy score. The result of combining AutoDock and Dock energy scoring in a consensus score based on the rank-by-rank approach is shown in column five (consensus score AutoDock/Dock). The rightmost column shows the result of using AutoDock to rescore compounds that were prescreened with Dock (redocking Dock with AutoDock).

two scoring functions (force field versus empirical) while rank-by-vote is regarded as less reliable and is not recommended.⁹⁴ Therefore, we used “rank-by-rank”.

We wished to estimate which combination of scoring functions and docking algorithms is likely to provide the smallest number of false positives. If we were to pick compounds at random without any knowledge of their suitability for the target, there would be no “enrichment” of cognate ligands among the top ranking compounds (Figure 7, column 1). The combination of scoring and docking that provides the highest enrichment rate (the largest number of compounds known to bind among the top ranking compounds, i.e., the smallest number of false positives) would maximize the effectiveness of the protocol by placing the largest number of cognate ligands among those to be physically screened (the top ranking compounds, Figure 7). Because we do not have even a single example of several known inhibitors targeting the same receptor, it was not possible to generate a meaningful test through this approach. Instead, we generated a larger number of results by combining the results of the 16 database docking experiments against all 16 target RNAs (16 independent libraries), each containing the 49 known drugs and 1 of the ligands from our test case. For example, gentamycin was included in the “gentamycin” library with the 49 known drugs and this library was then docked against the RNA from the gentamycin–RNA complex. This procedure was repeated for each of the 16 complexes, and the results were evaluated together.

When compounds are picked at random, there is obviously no enrichment (Figure 7, column 1, random score). Some enrichment of native ligands among the top scoring compounds (a line is arbitrarily drawn for the top five scores to guide the eye) can be observed in the Dock contact score (second column from the left)

compared with the random result, but the spread is still reminiscent of a random distribution and the improvement is not significant. Contact scoring favors nonpolar interactions,⁹⁵ while RNA and most of our ligands are obviously charged. Energy scoring results in much better enrichment (column 3, Dock energy score); compounds known to bind the target are generally found within the best 20–30% scoring compounds in the database with the exception of 1LVJ, 1QD3, 2TOB (the last two are aminoglycosides). A similar pattern emerges when compounds are ranked on the basis of the AutoDock score (column 4), but the results are not as encouraging as with Dock. Combining the Dock energy score (column 3) with AutoDock (column 4) does not significantly improve the enrichment rate (column 5, consensus score AutoDock/Dock) in comparison to Dock alone. The highest level of enrichment is observed instead when compounds are docked first using Dock and then redocked by using AutoDock (column 6, redocking Dock with AutoDock). In this approach, we redocked the top five scoring compounds using the AutoDock scoring function and algorithm. If the native ligand was not in the top five, we redocked all compounds that ranked better than the native ligand. For two cases (1EHT and 1EI2) only, the ligand’s rank did not improve, and in two other cases it remained the same (1AJU and 1AM0). In all other cases, the ranking of the native ligand was improved.

Docking of Ribosome-Binding Antibiotics. The antibiotic structures (Table 2) were docked using AutoDock to evaluate whether docking could be executed on this well-validated drug target as well. Because this data set comprises X-ray structures of different resolution (2.4–3.8 Å), this test also provides us an opportunity to evaluate the effect of the quality of the structure on docking. In docking ribosome-binding compounds, the binding site was defined to encompass a radius of 30 Å around the ligand binding site, and each ligand was docked 100 times to evaluate how well docking poses could be reproduced and ranked. The results for two successful examples (accupuromycin and geneticin) are shown in Figure 8, where the top-ranking poses are superimposed on the X-ray structure (rmsd values are 1.92 and 1.13 Å, respectively). When the ribosome–ligand complexes were repeatedly docked to evaluate how reliably the program could identify the correct docking pose, however, the results were less satisfactory compared to the 16 compounds in the original data set (Figure 9). Only two compounds (geneticin (1MWL) and tobramycin (1LC4)) docked near the correct pose more than 50% of the time. For six compounds (paromomycin 1J7T, hygromycin B 1HNZ, accupuromycin 1NJO, spiramycin 1KD1, carbomycin 1K8A, and paromomycin 1FJG), the success rate was 30–40%. For all remaining compounds (streptomycin and spectinomycin 1FJG, tetracycline 1HNW, pactamycin 1HNX, clydamycin 1JZX, erythromycin 1JZY, chloramphenicol 1K01, tylosin 1K9M, azithromycin 1NWY, and sparsomycin 1M90) a pose close to the target structure was found less than 30% of the time. For sparsomycin (1NJN, 1NJM) and troleandomycin (1OND), we could not find a single docking solution within 2.5 Å of the target. In the three cases where the ligand was most reliably docked into its native binding

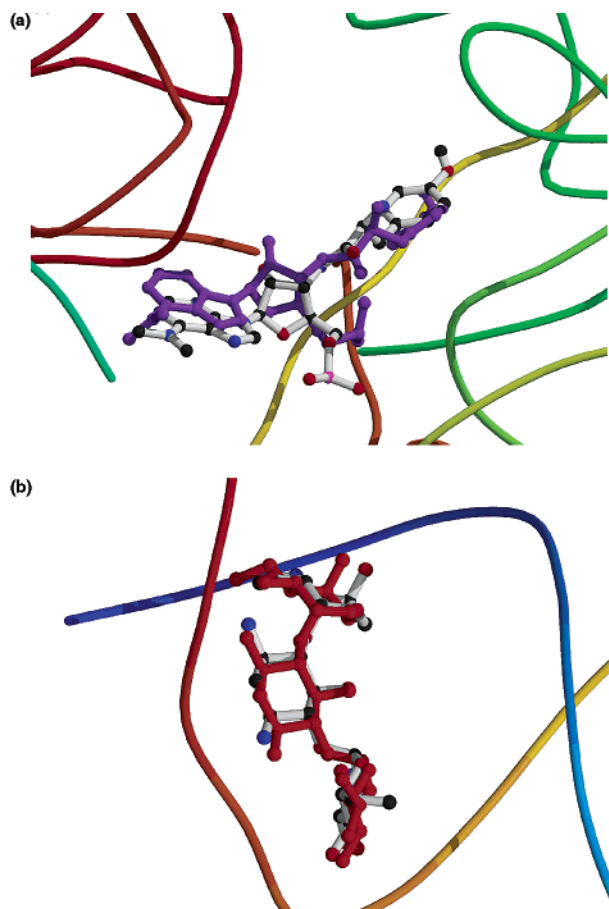


Figure 8. Ribosome docking test: (a) accupuromycin docked against the 50S ribosomal subunit D site (1NJ0), where the residue A2581 was omitted for clarity; (b) geneticin docked against the eubacterial ribosomal RNA A-site (1MWL). Native ligands are given in atom color. The figures were produced with MolScript¹²⁹ and rendered with Raster3D.^{130–132}

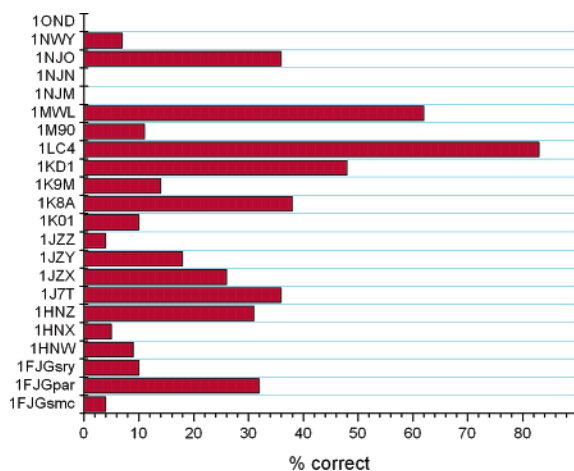


Figure 9. Reproducibility of automated docking poses for the ribosome complexes. For each structure (see Table 2), the ligand was independently docked 100 times into the active site and the resulting poses were compared to the experimentally determined structures. Poses that cluster within 2.5 Å of the target structure were considered successfully docked (see also Figure 4).

site, the ligands were aminoglycosides (geneticin 1MWL, tobramycin 1LC4, and spyracycin 1KD1). Surprisingly, the six ligands that exhibit stacking interactions between the ligand and the ribosome (e.g., sparsomycin

1M90) had low success rates of 9, 10, 11, 0, 0, and 36% (1HNW, 1K01, 1M90, 1NJM, 1NJN, and 1NJO, respectively).

We attempted to correlate these results with properties of the ligand or of the binding site, but the only significant correlation was with the crystallographic resolution. Structures of higher quality were docked more reliably (on average) than lower resolution structures (data not shown). It is to be remarked that in the ribosome test set, molecules are generally larger and more flexible than in the test database of Figure 1. For example, tylosin (1K9M) with 64 heavy atoms could only be ranked accurately about 14% of the time (Figure 9). Troleandomycin (1OND) has 57 heavy atoms and could never be docked successfully in our test (below 2.5 Å rmsd). In contrast, accupuromycin (1NJ0), a molecule with 37 heavy atoms, was docked 36 times out of 100 times within 2.5 Å of the target structure. We believe that the difficulty of docking algorithms to find correct solutions for large molecules with many flexible bonds may be responsible for the less satisfactory performance of the programs with the ribosome set of structures.

Discussion

Before database-screening experiments can be executed on RNA targets of pharmaceutical interest, it is important to establish how reliable and accurate docking tools are when used with RNA. James and co-workers have recently described the very successful discovery of a new class of ligands directed against HIV-1 TAR RNA from database screening.^{47,48,50} However, no attempt has so far been made, as far as we are aware, to analyze whether docking tools developed for database docking against protein receptors provide effective ligand docking and screening approaches for RNA as well. We chose two algorithms and scoring functions (Dock and AutoDock) that are widely available in the academic community and that provide a diverse approach to docking and scoring. While AutoDock uses a semiempirical scoring function and genetic programming,⁶⁰ Dock uses a simpler ranking score derived from the Amber force field and a shape-matching docking algorithm.⁶¹ Dock handles large databases rapidly and handily, while AutoDock is designed for use with one ligand at a time. Additional functionalities (specifically solvation and simulation of electrostatics using the generalized Born approach or the Poisson–Boltzmann equation) have recently been implemented in Dock 5. However, the β version of Dock 5 was released while these studies were already well underway and these improvements were not evaluated here. The results presented here concern two different, easily available docking programs that could be readily adapted to work with nucleic acids. Other attractive software packages (e.g., FlexX^{88–90}) and scoring functions (e.g., Chemscore⁹⁷ and Drugscore^{98,99}) lack at the moment the capability handling nucleic acids.

We were surprised to observe that the empirical scoring function implemented within AutoDock approximates at least in a qualitative sense the energy of binding of RNA–ligand complexes even if the training set used to optimize the parameters of the scoring function is composed of protein–drug complexes^{60,85,96}

(Figure 1). We expected to have to reparametrize the scoring function. When the uncertainties on the binding constant, the small size of the training set, and especially the limited precision of the coordinate set (most complexes are NMR structures, Figure 1b) are considered, it is questionable whether the existing data warrant an improvement of the free energy model.

We considered testing other scoring functions developed over the past several years, for example, ChemScore,⁹⁷ DrugScore,^{98,99} or XScore.^{24,100} However, none could be applied to RNA as currently implemented. Furthermore, all represent other examples of semiempirical scoring functions (as used in AutoDock) trained on different sets of protein–ligand complexes. It is very likely that a genuinely superior semiempirical model could be generated by training a semiempirical scoring function on an RNA-only data set and by introducing new terms to describe stacking and cation– π interactions (which are known to be especially important in RNA– and DNA–ligand recognition).^{101–103} This remains an important goal for future studies, but more experimental information will have to become available before it can be conducted effectively. It would also be highly valuable to test the performance of scoring functions that are neither force field derived (like Dock) nor semiempirical, for example, knowledge-based potentials. If this were to be done, scoring functions of genuinely different physical origin would become available, allowing consensus scoring to be conducted more effectively than currently possible.

An effective database-docking tool should identify the most favorable binding pose reliably and rank this mode of interaction highly. We addressed these features of Dock and AutoDock by repeatedly docking each of the complexes in the training set, thereby generating multiple (100) independent poses. AutoDock identifies the target structure to within 2.5 Å for all ligands except very weak binders (1AJU and 1KOD) and some aminoglycosides (1QD3, 1PBR, and 1EI2) (Figures 4b and 5b). In our tests, Dock was found to work best with nonpolar and small compounds perhaps because its force field based scoring function underemphasizes solvent effects and hydrogen-bonding interactions. These are more extensively treated in AutoDock, perhaps explaining the improved docking with aminoglycoside drugs, since these molecules form many NH and OH hydrogen bonds with nucleic acids. The performance with rigid aromatic compounds (which are most attractive for genuine drug discovery efforts) is particularly encouraging. Dock is also capable of docking these rigid compounds accurately and reliably into their cognate binding sites, but the performance with weak binders and aminoglycosides is very poor (Figures 4a and 5a). While these results may superficially indicate a substantial limitation of both sets of docking tools, a more careful consideration of the results is reassuring. It is not surprising that weak binding ligands are docked poorly, and the same is true for aminoglycosides; they are large, highly charged, and flexible molecules. Although aminoglycosides have provided a source of chemical inspiration for many studies aimed at discovering new RNA-binding ligands, these molecules are quite unlike most successful drugs and do not exhibit druglike properties.^{46,104–106}

The results for docking accuracy obtained in this study are comparable to those obtained with proteins. For example, in a study of 200 protein–small molecule complexes, Dock succeeded in 105/200 cases in docking the ligand within 2.0 Å of the target structure.¹⁰⁷ In another test aimed at evaluating the effect of ligand flexibility on docking accuracy, 6/12 ligands were docked within 2.0 Å of the experimental structure.⁶¹ AutoDock was compared with 10 other scoring functions with respect to docking accuracy. By use of the same cutoff rate used in the present study (2.5 Å), AutoDock had a success rate of 62%.²⁴ The docking accuracies obtained with RNA in the present study are comparable to those obtained with proteins. This result is the more pleasing when it is considered that algorithms and, especially, scoring functions were trained on proteins.

The ribosomal test set included higher-quality X-ray structures compared to the mostly NMR structures contained in the data set of Table 1, and we expected increased success. However, docking of ribosomal ligands was generally less successful compared to the test database results (Figure 9). Although there was a weak correlation between the quality of the X-ray structure and the success of the docking test, the generally larger and more flexible character of the ribosome-binding antibiotics probably led to the inferior performance. It is more difficult for the algorithm to find a correct solution for a larger molecule with many flexible bonds. In a genuine database screening experiment, molecules of the size and chemical complexity of the natural products that bind the ribosome will not be likely to be highly represented nor would they generally provide attractive leads for further studies.

The results of Figures 4 and 5 indicate that both Dock and AutoDock can identify the target structure in the large majority of cases to within 2.5 Å for the type of druglike rigid molecules that are of most interest in drug discovery. In a real database screening experiment, however, the target structure is unknown. Success in a realistic database screen relies on the ability of the program to rank poses that correspond to more favorable orientations of the ligand in the receptor as having better interaction energy than incorrect poses. Our results indicate that many compounds in the test database show a good correlation between scoring rank and accuracy of the pose when compounds are docked with AutoDock. A sector-shape plot is generated when scoring rank is correlated with deviation from the target structure for the “rigid aromatic” class of ligands and even for several aminoglycosides (1NEM, 1TOB, 1PBR, and 2TOB). In other words, favorably scoring poses tend to cluster nearer the target structure than higher-ranking poses. The performance of Dock is much less satisfactory; for a few cases only (1F1T, 1F27, and 1FMN), there is a good correlation between ranking and docking accuracy (see Supporting Information).

The tests of greatest importance for database docking are reported in Figure 6. First, we established how well the program can discriminate between several related ligands by asking the algorithm to identify the cognate compound in a test database containing all other RNA-binding ligands present in the data set. This test is very demanding, since most compounds in the data set tend to be basic molecules with some affinity for all RNA

molecules. Furthermore, aminoglycosides are known to bind a variety of RNA molecules with approximately micromolar affinity and there is every reason to expect that at least some of them (if not all) will bind some of the RNAs as well as their cognate ligand, particularly in the case of the weaker ligands 1AJU, 1KOC, and 1KOD. It is also well-known that larger compounds tend to be docked more favorably by many database docking programs.²² To our surprise (Figure 6a), Dock was more successful in identifying the cognate ligand among the top scoring compounds for the "rigid aromatic" class of ligands. Perhaps AutoDock overemphasizes electrostatic interactions, thereby allowing the basic aminoglycosides to outscore the cognate ligand. Alternatively or in addition, the genetic algorithm may allow aminoglycosides to find favorable docking poses, while the simpler approach used by Dock may not.

Figure 6b reports the simulation of a database docking experiment. We generated a test library by spiking a common set of 49 compounds with each of the 16 RNA-binding ligands and ranked the 50 compounds after docking them against the 16 target structures. Complexes were sorted as rigid aromatic compounds, weak binders, and aminoglycosides for ease of comparison. The almost opposite pattern of success for the two programs is noteworthy; AutoDock performs best with aminoglycosides, and Dock performs best with "rigid planar" compounds. As in other tests, Dock produced better results for ligands with hydrophobic ring systems. Its shape-based algorithm works better with hydrophobic pockets, while shallow and wide binding sites are problematic, as are large flexible molecules.

The final set of tests was aimed to assess which combination of docking and scoring would provide the highest level of enrichment, in other words, the highest likelihood of ranking the known RNA-binding compounds near the top of the database (Figure 7). This test essentially measures how useful in silico screening is in prefiltering large databases to identify potential ligands for the receptor of choice. The Dock energy score (column 2) locates a majority of ligands within the top 10% of the database and performs considerably better than AutoDock (column 4). The combination of the Dock and AutoDock score does not significantly improve the enrichment level (column 5). However, using AutoDock to redock and rerank the compounds identified by Dock as the top ligands (top 10%) leads to the highest level of enrichment (column 6).

The results reported in Figure 7 indicate that the highest likelihood of identifying RNA-binding ligands from database screens are obtained by using Dock to prefilter the initial library and then by redocking the top ranking compounds using AutoDock. This approach provides the highest likelihood of enriching RNA-binding molecules at or near the top of the ranking list. An analogous approach was demonstrated to work successfully in a genuine database screening experiment conducted with HIV-1 TAR RNA.^{47,48,50} The effectiveness of the Dock algorithm in handling large database and of the docking/scoring of rigid druglike molecules will likely identify a reduced library enriched with druglike RNA-binding molecules. These preselected molecules will then be rescreened using AutoDock to reduce false negatives by ranking molecules through an independ-

ent, empirical scoring function (Figure 1). This approach has several additional advantages. First is the speed of Dock. Second, the two algorithms and scoring functions appear to perform best on different classes of compounds. Furthermore, they take advantage of the superior ability of AutoDock in finding poses close to the target structure (Figure 4) and in correlating high-scoring poses with orientations with low rmsd from the target (see Figure 1, Supporting Information).

In summary, we have examined the validity of protocols to automatically dock large databases against RNA drug targets that optimally enriches the database for potential ligands. The results show that it is possible to use automated docking tools developed for proteins to increase the likelihood of discovering molecules that bind to RNA in chemical databases. The protocol we have tested uses docking tools that are widely available and take advantage of the different characteristics of the two algorithms to optimally use their respective strengths. While large and flexible molecules are clearly problematic, the protocol works best with rigid planar molecules that are of the greatest interest in realistic drug discovery efforts.

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Supporting Information Available: One figure with rank/rmsd correlation of docking results and one table with solvation parameters for nucleic acids. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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