Docking of Aminoglycosides to Hydrated and Flexible RNA

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Although much effort has been devoted to the development of programs suited for the docking of ligands to proteins, much less progress has been achieved in the nucleic acid field. We have developed a unique approach for docking aminoglycosides to RNA considering the flexibility of these macromolecules using conformational ensembles and accounting for the role of the first hydration shell. This concept, successfully implemented in AutoDock, relies on the computation of the intermolecular interaction energy that accounts for the presence of dynamically bound water molecules to the RNA. As an application, a set of 11 aminoglycosides was docked with an average root-mean-square deviation (RMSD) of 1.41 Å to be compared with an average RMSD of 3.25 Å when the original AutoDock protocol was used.

Introduction

The aminoglycoside antibiotics have been in clinical use for over half a century.[1,2] Although the most widely used members of this important class are still the originally discovered fermentation products such as gentamicin, tobramycin, and neomycin (Figure 1), considerable efforts have been dedicated over the years to chemically modify these molecules in an attempt to overcome enzymatic deactivation and to target resistant strains.[3−5] Amikacin, the 6-(4-amino-2-*R*-hydroxy *N*-butyroyl) analogue of kanamycin, is the result of such an effort.[6]

The mode of action of aminoglycosides has been known for some time.[4,7,8] Their bactericidal properties originate at the ribosomal level where protein biosynthesis is inhibited. Our understanding of this process has been dramatically enhanced in recent years as a result of structural studies delineating the interactions of aminoglycosides such as paromomycin, tobramycin, and gentamicin in the decoding A-site of the 16S subunit of ribosomal RNA.[9] The first indication of the bioactive conformation of paromomycin in solution was deduced from NMR studies by Puglisi and co-workers.[10] X-ray structures were next provided by Vicens and Westhof in their study of the paromomycin−RNA complexes.[11] Other X-ray structural studies[12,13] contributed further insight in the design and synthesis of analogues.[14−22] The majority of these analogues involved the attachment of side chains or entities bearing a variety of basic groups in an effort to find new electrostatic interactions with phosphate groups of the RNA backbone. A new paradigm in analogue design was more recently introduced with the discovery that the attachment of hydrophobic tethers leads to improved antibacterial activity.[23]

Despite significant advances in X-ray crystallographic[24] and mass spectrometry[25] structural analysis of aminoglycoside/RNA subunit complexes, computer-aided approaches are less well developed to study the relationship between structures and function in this field. Although many programs have been developed for the docking/scoring of ligands to proteins,[26] comparatively little is known in the nucleic acid field. The first documented attempts relied on using docking methods initially developed for proteins. For instance, Kuntz used the DOCK program[27,28] to identify RNA binders.[29] This same program has also been successfully used in combination with ICM.[30] Similarly, Karplus and Leclerc proposed the use of the MCSS method, which took advantage of the nucleic acid parameters developed in the CHARMm force field.[31] Studies were also carried out using a combination of docking methods and molecular dynamics (MD) simulations.[32,33] However, automated docking methods specifically parametrized for docking to RNA appeared only recently with Morley and Afshar reporting an empirical scoring function for docking ligands to RNA implemented in RiboDock[34] and Kuntz and co-workers[35] using a modified DOCK scoring function to accommodate RNA binders. This same year, Varani and co-workers[36] reported a scoring function for protein/RNA interactions. Detering and Varani have also reported a comparative study on DOCK and AutoDock docking programs for their ability to dock compounds to RNA.[37] They found that the experimentally observed binding modes were poorly reproduced [around 50% success rate for AutoDock with a root-mean-square deviation (RMSD) < 2.5 Å as a success criterion] when using the original parameters developed for protein binders. In a similar comparative study, we have also found that AutoDock and DOCK were not accurate tools in the docking of aminoglycoside antibiotics to RNA.

In fact, flexibility of RNA/aminoglycoside complexes and the presence of bridging water molecules are main issues that are not yet addressed in the available docking methods for nucleic acids.[38] Superposition of a set of crystallographic structures revealed that the same ribosomal RNA oligomer can adjust the fine structure of its conformation to the bound aminoglycoside. The crystal structures also indicated that some water molecules participate to some key drug/RNA interactions. MD simulations have further shown that the first hydration shell of RNA is highly structured.[39−42] In addition to accounting for the RNA flexibility and solvation, the protonation state of the aminoglycosides has to be computed with accuracy since a lower pK_a (5.74−7.07) for one of the several amino groups has been reported.[43,44]

Combining our collective interests in the development of computational methods for the docking of small molecules to

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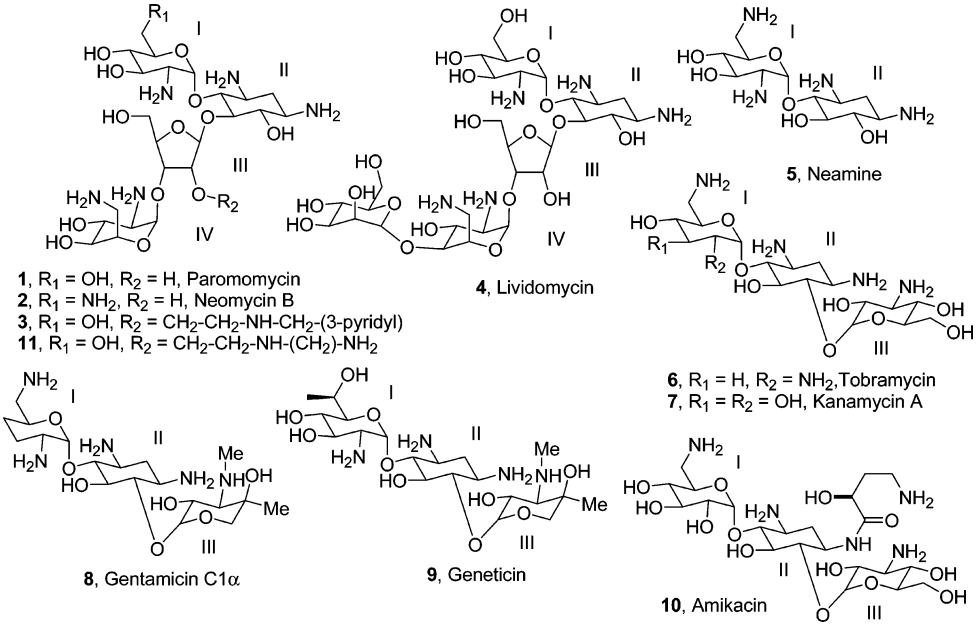

Figure 1. Selected aminoglycoside structures.

flexible macromolecules,[45,46] in the design and synthesis of antibiotics,[16,19,23] and in the crystallization of RNA/antibiotics complexes,[11−13,23] we set out to explore advanced methods for docking aminoglycosides to RNA. Herein we report our efforts to improve the accuracy of the AutoDock protocol in predicting the correct binding mode of aminoglycosides by considering the flexibility of the macromolecules and the role of the first hydration shell. This study includes the development of a specific potential for dynamically bound or displaceable water molecules, the docking to virtually mobile RNA, and the modification of the Autogrid code to compute RNA grids. After briefly summarizing the experimental data available at the outset of this study, we describe our initial efforts to dock aminoglycosides to rigid or flexible RNA, which gave modestly successful results. We next describe a novel approach to model key interacting water molecules. Implementation in AutoDock and application of this new concept improved the accuracy. Docking to flexible and solvated RNA was ultimately found to be highly accurate for docking the selected aminoglycosides to ribosomal A-site RNA and will be discussed in a third section.

Results and Discussion

Available Experimental Data. On the basis of the experimentally measured pK_a,[44] we considered the fully protonated aminoglycosides and planned to dock them to RNA, starting with the set of available crystal structures of aminoglycoside/RNA complexes.[9,11−13,47] The crystallized complexes contain two A-site motifs instead of a single one. Thus, as illustrated in Figure 2, each half of the system represents an aminoglycoside/RNA complex; hence, two slightly different complexes (two different conformations) were available for each aminoglycoside. When two RNA structures for the same aminoglycoside were available, the one with the highest number of crystallized water molecules, regardless of the B-factors, near the bound molecule was selected. Table 1 lists 11 available structures of RNA bound to different aminoglycosides.[11−13,23,47] Of these, **1**−**9** were included in the training set, while **10** and **11** were used as a validation set.

Initial Attempts To Dock to "Dry" RNA with AutoDock 3.0. To assess the influence of the RNA conformation on the docking accuracy, the nine aminoglycosides of the training set

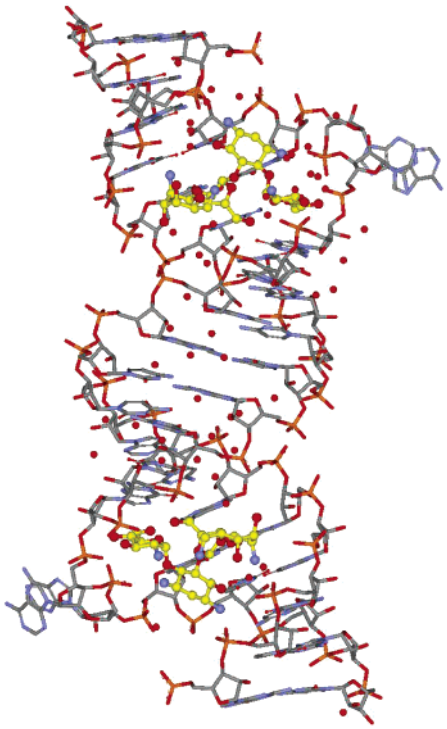

Figure 2. Crystal structure of paromomycin complexed to an oligonucleotide containing two A-sites (PDB code: 1J7T). The red spheres represent the water molecules determined by X-ray crystallography.

(**1**−**9**) were each docked into the nine RNA crystal structures **a**−**i**. The docking accuracy is illustrated in Figure 3. For the following discussion, the level of success was arbitrarily assigned to docking runs with an RMSD < 2.5 Å as proposed by Varani and co-workers.[40] This criterion is appropriate when investigating software ability to dock properly.[48] In contrast to Varani's study, we considered only the top ranking binding mode. In the following studies, 25 runs were carried out with sufficiently large populations and number of generations to ensure convergence. For the smallest aminoglycosides (**5**−**9**), more than half of the 25 poses were assigned RMSDs within 0.5 Å of each other. For the larger four-ring compounds, the

Table 1. Structures of Aminoglycoside−A-Site RNA Complexes[11−13,23,47] Used for the Docking Study

entry	aminoglycoside	RNA structure[a]	no. of water molecules[b]	resolution (Å)	PDB code
1	paromomycin, **1**	**a**	8	2.54	1J7T
2	neomycin, **2**	**b**	7	2.4	2ET4
3	aminopyridine derivative **3**	**c**	8	2.6	2BEE
4	lividomycin, **4**	**d**	15	2.2	2ESJ
5	neamine, **5**	**e**	2	2.5	2ET8
6	tobramycin, **6**	**f**	5	2.4	2LC4
7	kanamycin A, **7**	**g**	0	3.0	2ES1
8	gentamicin, **8**	**h**	0	2.8	2ET3
9	geneticin, **9**	**i**	3	2.3	1MWL
10	amikacin, **10**	**j**	4	2.7	not available[c]
11	paromomycin derivative, **11**	**k**	12	2.6	2BEO

[a] Crystal RNA conformation adopted when bound to the corresponding aminoglycoside (e.g., RNA adopts conformation **c** when bound to aminoglycoside **3**). [b] Within 3 Å from the aminoglycoside. [c] The structure is to be submitted to the PDB.

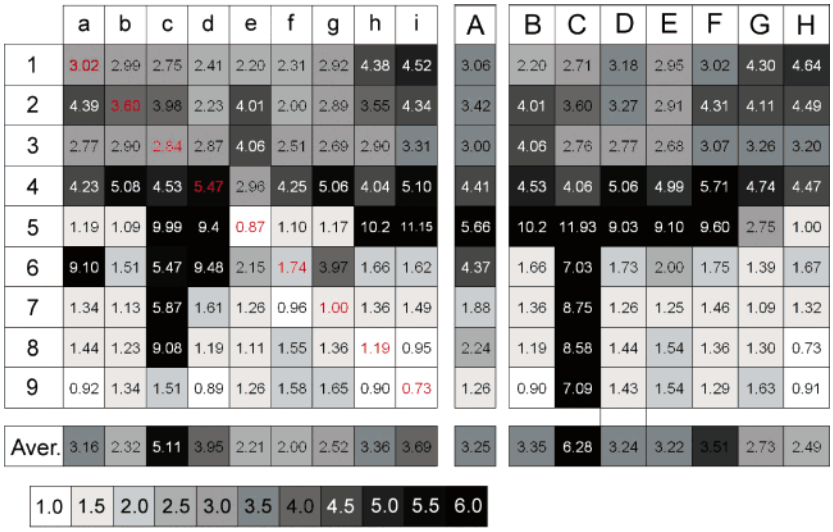

Figure 3. Docking accuracy expressed as RMSD in Å. **a**−**i** denotes the RNA conformations as defined in Table 1, **1**−**9** denotes the docked aminoglycosides as defined in Figure 1. Column A shows the average cross-docking RMSD for each aminoglycoside **1**−**9**. B: Docking to multiple conformations. C: Docking to conformational ensemble, initial attempts. D: Docking to conformational ensemble using the new "scaled" electrostatic interactions. E: Docking to conformational ensemble using the new "scaled" electrostatic interactions and the nonsmoothed scoring function (see text). F: Docking to an averaged structure. G: Docking to a soft average structure. H: Docking to a soft average structure using the nonsmoothed scoring function.

top 5 is often homogeneously populated by similar poses. However, for the largest aminoglycoside **4**, reaching the convergence is more problematic. When using large populations and more than 1000 generations, the top five poses are often diverse with RMSDs varying as much as 2.0 Å. The reproducibility of the docking was therefore assessed by running another two sets of 25 runs. In 74% of the cases, the top poses deviation did not exceed 1.0 Å. Considering the time needed for each set of runs (from 8 to 9 h on R16000 processors), we decided not to increase more the number of generations or the population size and to use the described parameters for the whole study. However, to ensure that the convergence was reached when using the later optimized conditions (considering solvation and flexibility, vide infra), 50 runs were carried out.

Self-docking, which is defined as the docking of an aminoglycoside back to its cocrystallized RNA structure (e.g., **1** docked to **a**), was achieved with an average RMSD of 2.27 Å corresponding to a success rate of 56%. Only five aminoglycosides, namely, **5**−**9**, were docked back to their corresponding RNA structure with an RMSD below 2 Å. Cross-docking, which is defined as the docking of a small molecule to the conformations adopted by RNA when bound to other aminoglycosides (e.g., docking of **1** to the structures **b**−**i**), was even less successful with a 48% success rate and an average RMSD of 3.25 Å. The three-ring systems were found to be more accurately docked that the smaller compound **5** and larger compounds **1**-**4**. The large difference in accuracy between the self- and cross-docking studies clearly indicated the critical importance of the RNA conformation through an induced-fit effect. Indeed, when compared to each other, the nine RNA structures have RMSDs ranging from 0.5 to 1.6 Å. As a consequence, the calculated average RMSD of the docked poses compared to the observed binding modes range from 2.00 Å (eight compounds docked within 2.51 Å) when the RNA conformation **f** was used to 5.11 Å (one compound docked within 2.5 Å) when the conformation **c** was used. Thus, this docking method should not be used to accurately dock aminoglycosides to RNA and therefore is not a tool of choice for designing new potential ligands. To improve the accuracy of the method, we next considered the docking to flexible RNA docking alternatively to multiple conformations, to conformational ensembles, to an averaged structure or using a soft docking approach.

Docking to Flexible RNA − Multiple Conformations and Conformational Ensembles. The docking of small molecules to flexible proteins has been the subject of recent reviews.[49,50] For instance, one can use multiple conformations (parallel docking to each discrete conformation), although this process is time-consuming.[51,52] Methods that dock to a single average structure or an ensemble of structures have also been developed that include the use of soft van der Waals parameters on a single structure[53] and the use of combined grids.[54,55] Although the use of grids was initially restricted to rigid macromolecules, induced-

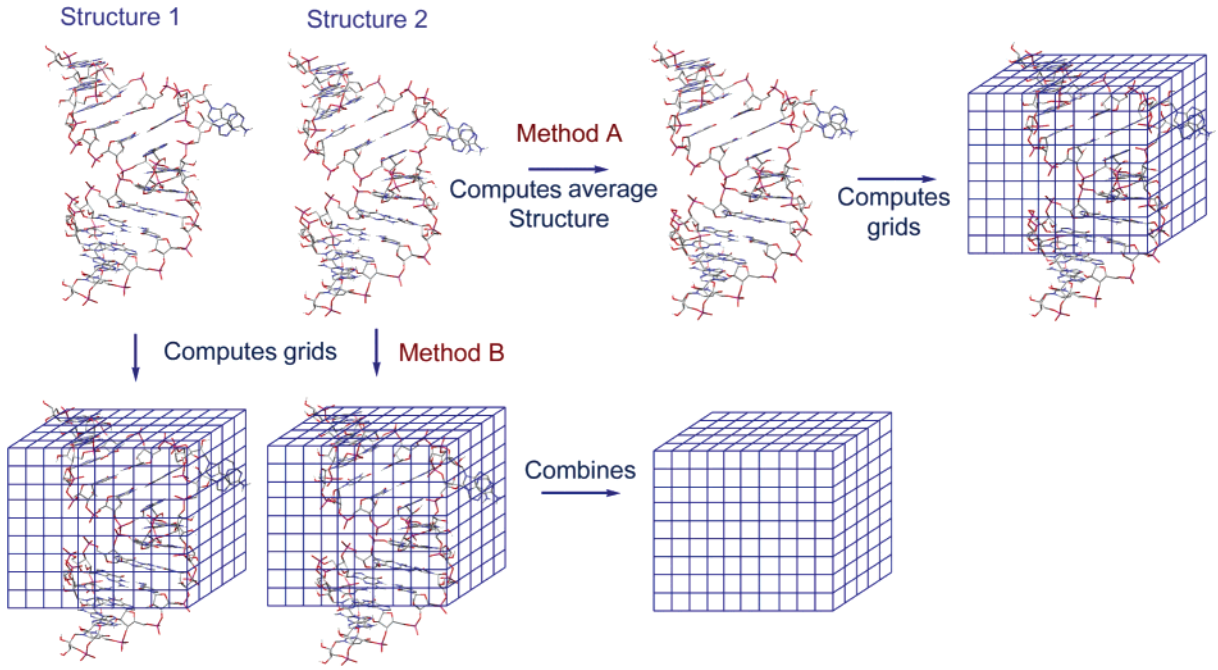

Figure 4. Methods A and B used to include flexibility of the oligonucleotide in the docking process.

fit docking has been achieved by combining the sets of grids computed for each rigid conformational structure into a single set of grids (conformational ensemble, method B, Figure 4).[54,55]

To account for RNA flexibility, we first selected the highest scoring of the nine runs previously carried out (docking to multiple RNA conformations **a**−**i**). When this approach was used, the success rate (56%) improved slightly (column B, Figure 3) relative to the previously measured cross-docking success rate (48%), although this increase is not significant.

We next tried to use the docking to conformational ensembles using AutoDock as proposed by Goodsell and co-workers.[55] In the present work, the combined van der Waals/hydrogen bond interaction grids were computed using the Boltzmann-weighting scheme initially developed for the use of AutoDock with flexible proteins.[55] This weighting scheme has been found to lead to more predictive grids than a simple averaging scheme.[55] Although the application of the Boltzmann-weighting scheme to the computation of van der Waals/hydrogen bonds grids has been described in great detail,[55] no mention has been made on the weighting scheme used for the electrostatic grids. In the present work, the use of Boltzmann's weights applied to the combined electrostatic grids led to inaccurate predictions (data not shown). We next constructed this electrostatic grid by averaging the values from the nine grids and again observed a poor predictive power (column C, Figure 3). To understand this loss of accuracy, we looked closer at the combined grids. The interaction energies of a probe atom with two different conformations (one at position 0, the other one at position −0.5) are illustrated in Figure 5a. When moving left, the probe atom first interacts strongly with one of the conformations and weakly with the other conformation then bumps to one conformation while interacting strongly with the second structure at smaller distances. As illustrated in Figure 5b, at small distances, the Boltzmann-weighting scheme reduces the large van der Waals repulsion while favoring highly attractive electrostatic interactions. As a result, the total interaction energy would show a deep well at very short distances. That artifact was reflected in the poor accuracy of the docking using such combined grids. To remove these artifactually favored positions in space, we developed a scaling scheme for the electrostatic grid that removes the high electrostatic interactions at small distances (Figure 5c). Each electrostatic map for each structure was recomputed using a protocol that computes the "scaled electrostatic interactions" based on the van der Waals repulsion (Figure 5c). The nine resulting scaled electrostatic grids were subsequently combined into an averaged grid. As illustrated in Figure 3 (column D), this last combination was not successful, showing average RMSDs above 3 Å and a low success rate (44%). However, accuracy similar to the ones previously observed has been restored. This indicates that the scaled electrostatic potential is more appropriate when combining grids.

The AutoDock scoring function smoothes the ligand/macromolecule interactions and uses wider potential energy wells.[56] This smoothed potential energy accounts in part for the flexibility of the macromolecules as do soft van der Waals potentials by reducing the repulsion contribution.[52] The use of both the smoothed potential energy and any other virtual flexibility would be redundant. The docking to conformational ensembles should therefore preferentially be applied to nonsmoothed grids. Thus, new sets of grids were computed for each structure **a**−**i** without this smoothing effect and combined into a set of grids. This approach was more successful, with seven aminoglycosides (**1**−**3**, **6**−**9**) docked within 3.0 Å from the observed binding modes (column E) but with four aminoglycosides (**6**−**9**) within 2.5 Å. Surprisingly, failures are observed for the smallest (**5**) and the largest compounds (**4**) of the set and for aminoglycosides from the two main families: 4,5-disubstituted (**4**) and 4,6-disubstituted neamine (**6**). These failures cannot therefore be attributed to a specific type of aminoglycoside structure. Although encouraging, the obtained accuracy is still not good enough to be used for the design of new binders leading us to consider other methods for docking to flexible RNA.

Docking to Flexible RNA − Single Structure and Soft Docking. We have next investigated the use of a single average structure computed from the nine conformational structures (Figure 4, method A). This structure was constructed by averaging the Cartesian coordinates. To evaluate the flexibility of the RNA structure, we computed the deviation between each of the nine structures and the average structure and found

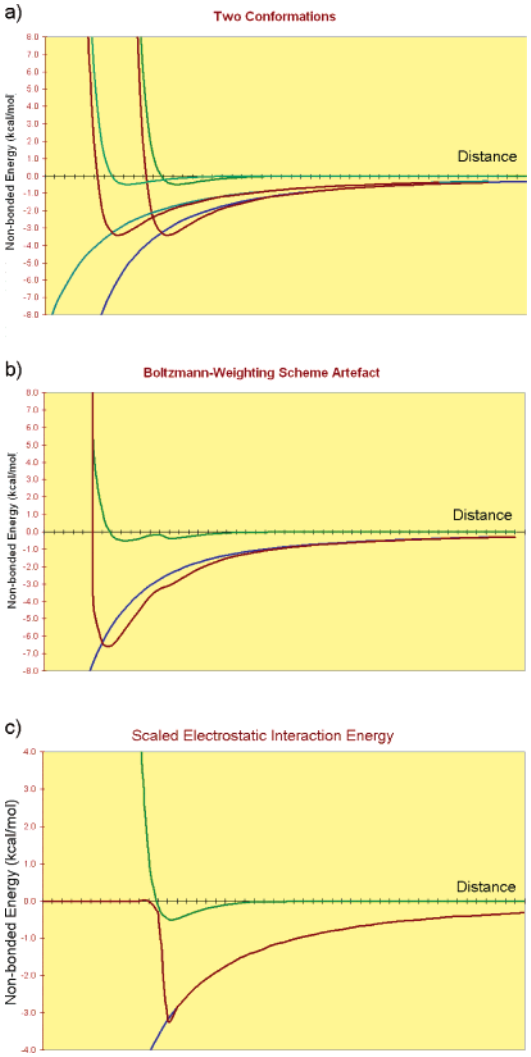

Figure 5. (a) Electrostatic (blue), van der Waals (green), and total (red) interaction energy for two different conformations (different positions); (b) Boltzmann weighting scheme applied to these two conformations; (c) scaled electrostatic potential for one structure. van der Waals potential (green), electrostatic potential (blue), and scaled electrostatic potential (red). The smoothing effect included in the AutoDock scoring function is not shown on this graph.

RMSDs ranging from 0.5 to 1.0 Å for eight of the nine structures and an RMSD of 1.7 Å with structure **e**. We then prepared a standard set of grids for this new representative conformation. Docking to this single average structure did not improve the AutoDock accuracy, showing an average RMSD of 3.51 Å and a success rate of 44% (column F, Figure 3). Soft van der Waals parameters have also been considered and applied to this average structure. Such a set of parameters (Lennard-Jones 9−6 in place of Lennard-Jones 12−6) tends to reduce the steric clashes and models the fit of the macromolecule by softening the repulsive term of the Lennard-Jones potential. When applied to the docking of aminoglycosides to RNA, this approach improved slightly the accuracy (column G, Figure 3). Soft van der Waals parameters have also been used with a standard (nonsmoothed, see above) Lennard-Jones potential, affording an increase in accuracy (column H, Figure 3). When applied to the averaged structure, the nonsmoothed potential, in combination with soft van der Waals parameters, allowed for the docking of five aminoglycosides (**5**−**9**) with RMSDs below 1.70 Å from the observed binding modes. The collected data also indicate that docking to conformational ensembles (combining grids) or to softened average structures (soft van der Waals potential) is more accurate when a standard Lennard-Jones potential is used in place of the smoothed potential used in the original AutoDock scoring function.

Thus, the docking of the set of nine aminoglycosides to rigid RNA was achieved with an average RMSD of 3.25 Å and a 49% success rate while the docking to flexible RNA was achieved with an average RMSD of 2.49 Å and a 55% success rate. This encouraging result prompted us to further investigate the use of AutoDock for docking aminoglycosides to RNA. We next turned our attention to the role of the bridging water molecules observed in many aminoglycoside/RNA complexes.

Modeling Bridging Water Molecules − A New Concept. It is well-established that water molecules play key roles in ligand binding to protein or RNA. Although attempts have been made to account for these water molecules in protein/ligand complexes,[57−59] they have not been considered in the docking of small molecules to nucleic acids. A common practice is to dock the ligand alternatively to the protein including a single water molecule or to the water-free protein. However, this approach can be practically used for proteins containing up to two water molecules but cannot be exploited in the present study where four to five water molecules can simultaneously be involved in the binding of aminoglycosides. Placing discrete water molecule while docking ligands has also been envisaged.[57,58] Crystallographic water molecules (Figure 2) are found in most of the aminoglycoside/RNA crystal structures and will now be considered in the docking process. To complete the solvation shell of the RNA strands, quick MD simulations in explicit water were first carried out. Each crystallographic water molecule was restrained to its position and the RNA heavy atoms were fixed during the simulations. The final nine structures were energy-minimized and used in the subsequent docking study. These quick simulations aimed to remove close contacts between crystallographic water molecules and additional water molecules and by no means to study the first solvation shell in details. Indeed, the first shell is mostly constituted of crystallographic molecules or molecules added by analogy with water molecules observed in the other eight crystallographic structures. Longer MD simulations could also be used to further improve the positioning of water molecules around the RNA strand.

The next step was to select the water molecules that will be considered in the following docking studies. Detailed MD simulations could allow the selection of water molecule with the longer half-life time at specific locations. However, some water molecules may have higher density only when the RNA strand is bound to a specific aminoglycoside. For this proof-of-concept, we chose a simpler approach. One could have considered the B-factors as a criterion of selection; however, these factors are directly related to the bound aminoglycosides and should significantly differ with any other designed binders. With the main goal of this work being to identify a docking method for further drug design, we decided to restrict our selection to water molecules that can potentially bridge a small molecule to the RNA and retained any water molecule within 2.5 Å of any RNA atom. Despite the fact that sometimes water molecules interact with both the RNA and the small molecule, keeping explicit water molecule in the docking process might be problematic. In fact, large molecules might need to displace them for an optimal binding. To tackle this problem, we had to consider the inclusion of "displaceable" water molecules. The proposed approach made use of a combination of grids corresponding to solvated and dry RNA.

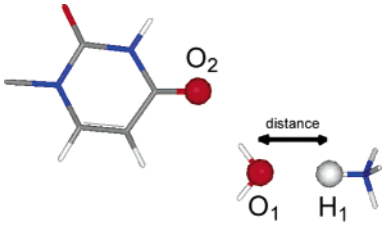

Figure 6. The three-atom system used to illustrate the weighting scheme.

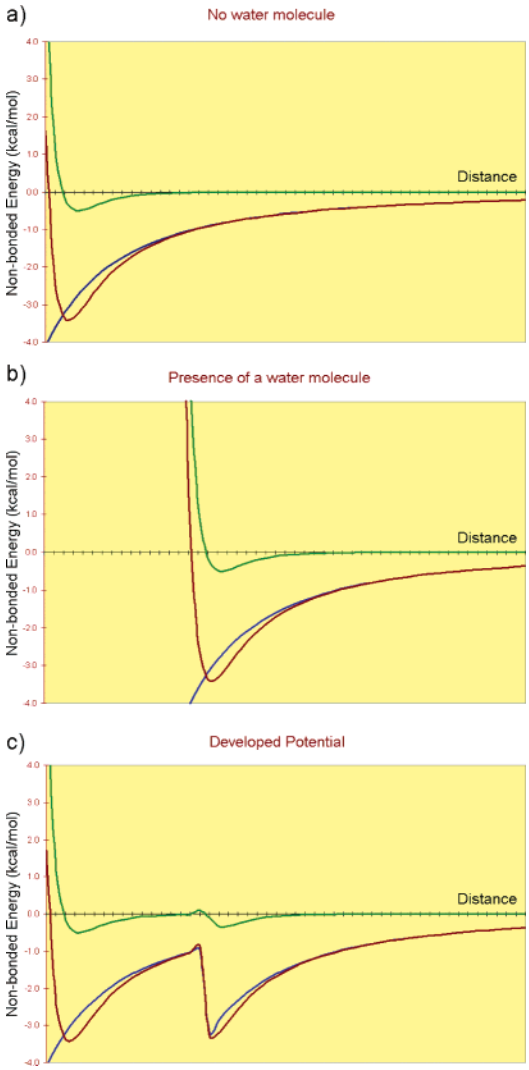

Figure 7. Interaction energies as a function of the distance of the ammonium hydrogen (H_1) and the purine base oxygen (O_1). van der Waals (green), electrostatic (blue), and total nonbonded energy (dotted red line) when the water molecule is considered (a), removed (b) and when both situations are considered (c).

To illustrate the method, we selected a three-atom system shown in Figure 6 (O_1, O_2, and H_1). In a real binding process, two configurations can be adopted. The ammonium ion can either displace the water molecule and interact with the RNA molecule (Figure 7a) or interact with the water molecule (Figure 7b). When water molecules are considered, no ligand atom is tolerated at the same position (interpenetration) and a wall is therefore observed at a van der Waals distance from the water oxygen atom (Figure 7b). When no water molecules are considered, the potential interaction with the bridging molecule is lacking and the ammonium nitrogen atom of the aminoglycoside interacts with the base (Figure 7a). The wall is observed at a van der Waals distance from the base oxygen atom O_2. With these first two potentials, the water molecule cannot be displaced. To model the two situations in one set of grids (Figure 7c), we developed a new weighting scheme where the scaled-electrostatic grid described previously was used. The potential energy well due to favorable interaction with water molecules is observed and at a shorter distance the interaction with the base is computed with the water molecule being displaced. As a consequence, both the interaction and the displacement of the water molecule are considered simultaneously.

Although we expect this approach to increase the accuracy of the docking process, shortcomings were identified. First, an artifact can appear if two atoms are positioned at these two positions. However, the close proximity of these two wells prevents the presence of two positively charged groups at these two positions. Second, although it is known that some bound water molecules have higher free energies of binding compared to others,[60] the shape of the curve will be similar for any water molecule. As a consequence, the described scoring may overestimate some interactions with water molecules.

The use of these "displaceable" water molecules in the docking process has next been investigated. We will use the word pseudo-solvation in the following sections to describe this type of water model, each water molecule being described as a "displaceable" water molecule.

Docking to Pseudo-Solvated RNA. To evaluate the reliability of the developed potential energy function, each aminoglycoside **1**−**9** was docked to each structure **a**−**i** with explicit water molecules or with the developed two-well interaction energy. The binding energy and the RMSD, compared to the observed binding mode, were evaluated. This set of calculations is summarized in Figure 8.

The observed increased accuracy in respect to the docking to dry RNA highlighted the role that water plays in the docking process. For any aminoglycoside, the predicted binding mode was correct when the proper solvated structure was used (self-docking). The positions of the exploited water molecules were defined by first optimizing each complex in the presence of a drop of water then by selecting a layer of water molecules retained as a part of the binding site for this docking study. Since the water molecules are now part of the binding site, they reduce the size and shape of the binding pocket fitting the aminoglycoside. Thus, back docking the aminoglycoside into this "cushion" of water is expected to be highly accurate. The self-docking is obviously biased, and when other solvated structures were used (cross-docking), the docking was less accurate. For instance, the flexible arm of aminoglycoside **3** was not positioned as observed when **3** was docked to structures **a**,**b** and **d**−**i**. It is worth noting that the side chain of **3** shows large B-factors revealing its flexibility. Predicting the exact location of this chain is therefore expected to be challenging. The region of space where this arm was observed contains at least one water molecule in each structure but structure **c** thus precluding the correct positioning of this arm in the other eight structures **a**,**b** and **d**−**i**. As a result, the average RMSD for the cross-docking of **3** was 3.12 Å in solvated RNA, and 3.00 Å in dry RNA. Although the presence of explicit water molecules significantly increased the accuracy of the docking relative to the docking to dry RNA, these water molecules would not allow for proper docking of larger molecules and are therefore not useful for designing new structures. The use of "displaceable" water molecules would address this issue.

More interestingly, the developed approach demonstrated a significant increased accuracy comparatively to the original

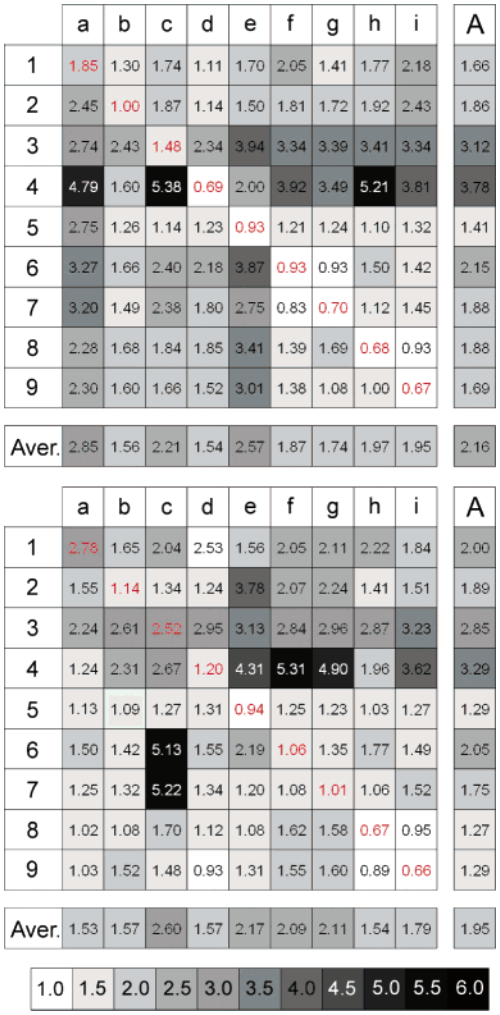

Figure 8. Self- and cross-docking using explicit (top) and "displaceable" (bottom) water molecules. Column A shows the average cross-docking RMSD for each aminoglycoside **1**−**9**.

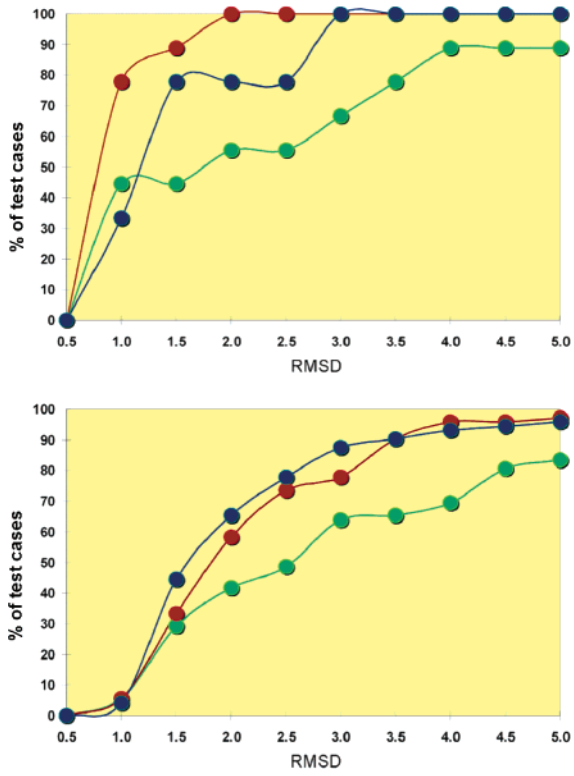

Figure 9. Accuracy of self-docking (top) and cross-docking (bottom). Color code: green: dry RNA, red: solvated RNA, blue: developed solvated RNA referred to as pseudo-solvated RNA.

AutoDock protocol. When considering the cross-docking (Figure 9), a 44% success rate was calculated for the developed approach at RMSDs below 1.5 Å to be compared with 33 and 29% with the solvated (explicit water) and dry RNA, respectively. A 65% success rate at RMSDs below 2.0 Å is to be compared with 58 and 42% with the solvated and dry RNA, respectively.

Thus, the docking of the nine selected aminoglycosides to rigid and dry RNA was achieved with an average RMSD of 3.25 Å and a 49% success rate, while the docking to rigid and pseudo-solvated RNA was achieved with an average RMSD of 1.95 Å and a 78% success rate. The synergetic effect of the developed potential accounting for displaceable water molecules and the RNA flexibility should now be investigated.

Docking to Flexible and Pseudo-Solvated RNA. As presently coded, AutoDock considers the water molecules as "disordered hydroxyl groups" and does not recognize the phosphate oxygen bonds. These groups are therefore not making proper directional hydrogen bonds. New atom types (P, phosphorus, W, water oxygen, and Y, water hydrogen) were incorporated in the Autogrid code and used for the study of docking to flexible and solvated RNA.

We illustrate the method used to investigate the flexibility of the pseudo-solvated RNA macromolecule in Figure 10 with two structures (the nine structures of the training set were used). Grids were first computed for each set of water molecules. These nine sets were next combined into a single one, which virtually modeled a heterogeneous continuum of water molecules with deep energy wells at positions of highly conserved water molecules. The Boltzmann weighting scheme was used to compute the van der Waals interaction grids while the electrostatic grids were first scaled (Figure 5) then combined. When combining the electrostatic grids, high weights must be attributed to highly conserved water molecules while the grid points around water molecules not strongly bound to RNA should have low electrostatic potentials. The Boltzmann scheme was therefore not appropriate for combining the electrostatic maps. In fact, a simple averaging scheme was found to be more appropriate. Finally, addition of the water grids to the grids of the flexible RNA (from either the average structure or the conformational ensemble) led to grids modeling flexible and solvated RNA.

The accuracy of the docking to a "flexible and pseudo-solvated" RNA is illustrated in Figure 11. The first column (column B, Figure 11) summarizes the RMSDs for the highest score among the nine docked complexes of each aminoglycoside (docking to multiple conformations). The RMSDs ranged from 0.67 Å (**8**) to 2.67 Å (**4**) with three aminoglycosides (**1**, **3**, **4**) being docked with RMSDs above 2.0 Å. A more interesting observation is the high accuracy of the docking to the pseudo-solvated average RNA structure (column C, average RMSD = 1.33 Å). This set of grids has been prepared by adding the grids computed for the water molecules to the grids previously developed for the average structure. With this set of grids eight (**1**, **2**, **4**−**9**) of the nine aminoglycosides were docked with RMSDs below 1.5 Å with the ninth (**3**) being docked with reasonable accuracy (RMSD of 2.52 Å). This last result validated the developed approach. Only the flexible side chain of compound **3** was not properly positioned. When the developed set of grids for the water molecules was prepared using the new atom types for the water molecules ("ordered

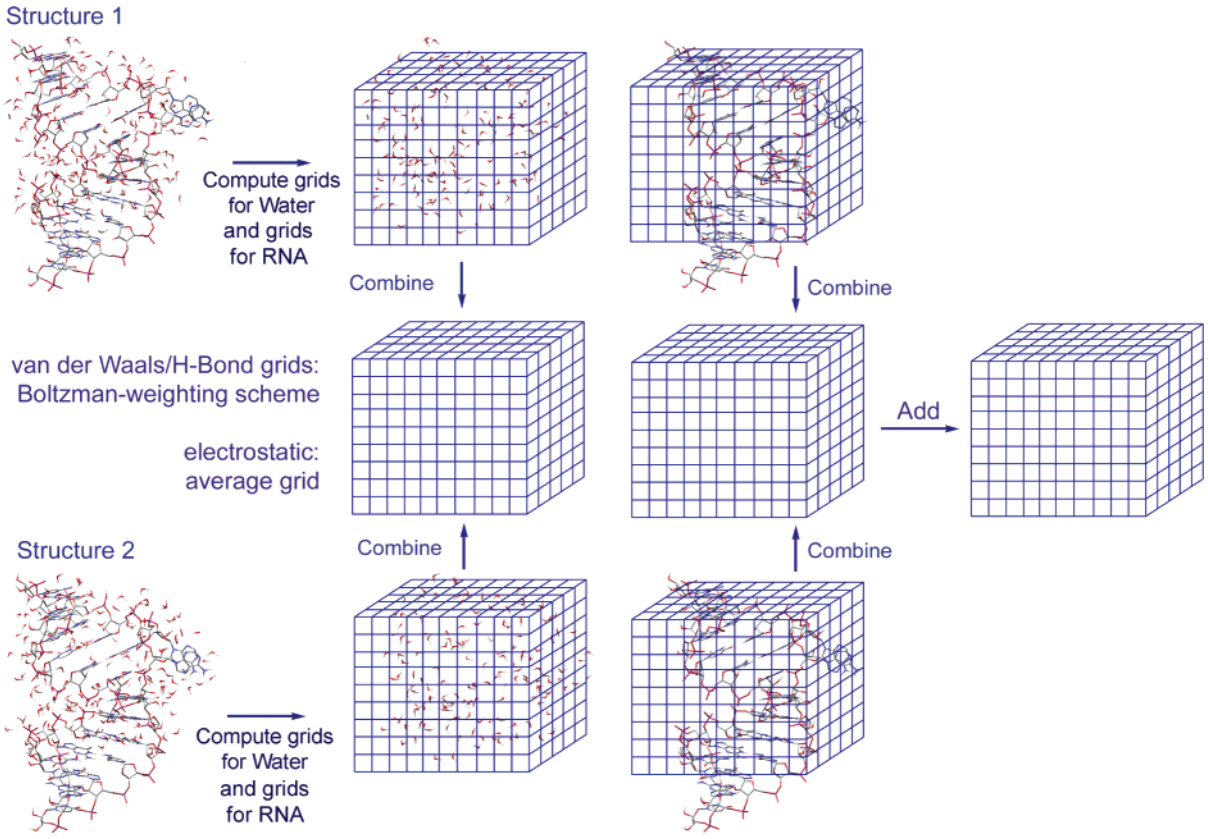

Figure 10. Schematized flexible and pseudo-solvated RNA.

	B	C	D	E	F	G
1	2.22	1.48	6.19	1.86	1.90	1.58
2	1.34	1.28	5.97	1.31	1.66	1.36
3	2.52	2.60	2.42	3.39	2.18	2.63
4	2.67	1.09	5.63	3.12	3.25	3.40
5	1.03	1.25	10.39	1.28	1.27	1.26
6	1.77	1.39	7.79	1.39	1.41	1.50
7	1.06	0.92	8.34	1.33	1.33	0.93
8	0.67	0.92	5.53	1.03	0.86	0.91
9	0.89	1.08	7.59	1.18	1.08	1.08
A	1.57	1.33	6.65	1.77	1.66	1.63

1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0

Figure 11. Accuracy of the developed docking approach.

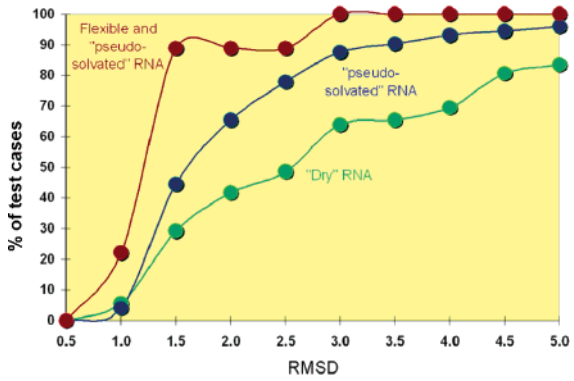

Figure 12. Accuracy of the developed approaches: docking to flexible and solvated RNA (red) cross-docking to rigid and solvated RNA (blue) compared to the cross-docking to rigid and dry RNA (green).

water molecules") making directional hydrogen bonds, the accuracy dramatically dropped (column D). It is not clear why the accuracy dropped while it was expected to increase. A better distribution of the water molecules through longer MD simulations may address this issue. Other successful attempts were carried out using soft van der Waals parameters with smoothed (column E) or nonsmoothed Lennard-Jones potential (column F). However, these sets of grids were much less successful in docking aminoglycoside **4** properly. The same observation was made when using the conformational ensemble of structures developed previously that was "soaked" using the set of water grids (column G). Interestingly, the combination of soft van der Waals parameters and smoothed potential was the least successful approach of the ones tested. As hypothesized earlier, the smoothed potential may act as does the soft van der Waals potential and these two soft repulsive van der Waals should not be used simultaneously.

When comparing the accuracy of the developed protocol to the initial docking study (Figure 12), one can observe a significant improvement in the accuracy. These data demonstrate that AutoDock appears as an accurate tool for docking aminoglycosides to RNA when both the flexibility and solvation are considered. The red curve in Figure 12 represents the accuracy of the docking to "pseudo-solvated" and flexible RNA compared to the accuracy of the docking to "pseudo-solvated" and rigid RNA (blue curve) and to the dry and rigid RNA (green curve). However, although the accuracy increased, the transferability of the method to other classes of molecule will have to be assessed. As illustrated in Figure 13, the docking of paromomycin was more accurate when the pseudo-solvated RNA structure was used. The least accurate positioning was

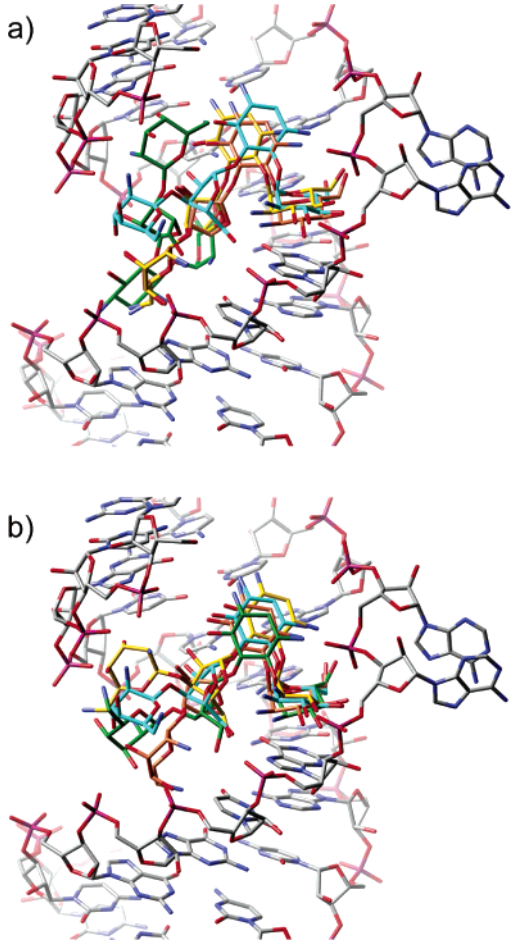

Figure 13. (a) Docking to "dry" RNA. (b) Docking to "pseudo-solvated" RNA. Paromomycin carbon code: blue: crystallographic structure, green: cross-docking to rigid RNA, yellow: self-docking to rigid RNA, pink: docking to flexible RNA.

observed for ring IV, which also exhibits the largest B-factors in the crystallographic structure.

Validation. For the purpose of validation, the two crystal structures of the testing set (RNA cocrystallized with **10** and **11**) were used. It is worth mentioning that the conformations (**j** and **k**, Table 1) adopted by the RNA structures when bound to **10** or **11** were not included in the averaged structure developed previously and used in this validation study. In addition, these two aminoglycosides feature a highly flexible side chain and predicting their correct binding modes was expected to be challenging. The data collected is presented as Supporting Information.

The conclusions drawn with the training set (**1**−**9**) were confirmed with the testing set (**10**-**11**). (1) The accuracy was highly dependent on the conformation used (**a**−**i**: RMSDs ranging from 1.83 to 5.86 Å). (2) Therefore, accounting for the flexibility (combined grids or soft van der Waals parameters) of the dry RNA improved the predictions (RMSDs ranging from 1.55 to 2.28 Å). (3) The use of the developed scoring function with dynamically bound water molecules also lead to increased accuracy. (4) The use of flexible and pseudo-solvated RNA led to the best accuracy (RMSDs of 1.47 Å for **10** and 2.05 Å for **11**).

More interestingly, the introduction of explicit water molecules decreased the accuracy comparatively to the docking to dry RNA. This observation strongly supports the need for dynamically bound water molecules. As previously hypothesized, larger aminoglycosides (introduction of side chains as in **10** and **11**) cannot be accurately docked if water molecules are explicitly included in the docking process.

Although these results appeared to validate the proposed approach, it should be stressed that the present study focused on a restricted class of RNA binders and that others issues may arise with other systems.

Conclusion

Although efforts have been dedicated to the improvement of scoring functions for docking compounds to nucleic acids,[31] this work represents the first attempts to account for the role of water molecules in the binding of compounds to RNA.

AutoDock was initially found to poorly predict the binding modes of aminoglycosides in the 16S RNA major groove showing an average RMSD of 3.25 Å for the cross-docking study and a success rate lower than 50%. Consideration of the flexibility of the RNA (average RMSD of 2.49 Å and 55% of success) or of the bridging water molecules (average RMSD of 1.95 Å and 78% of success) revealed the role of the mobility of the RNA structure and of key water molecules in the docking accuracy.

The flexibility was considered through the use of soft van der Waals parameters and the use of grids modeling conformational ensembles. Implementation of a new potential for virtually "displaceable" water molecules and of a protocol to combine water grids into a continuum grid allowed us to include key water molecules in the docking process. When the available AutoDock scoring approach was used a success rate of 48% and an average RMSD of 3.25 Å was measured for cross-docking. Instead, with the developed method, the nine aminoglycosides of the training set along with the two from the validation set were properly docked with an average RMSD of 1.41 Å.

In conclusion, this work supports the use of AutoDock with the proposed modifications as an accurate tool for docking aminoglycosides to ribosomal A-site RNA. Further studies using larger and more diverse testing sets are needed to assess the transferability to other RNA or DNA binders.

Experimental Section

General. The molecules were manipulated using InsightII version 2000[61] (Accelrys) and modeled using the InsightII/Discover package with AMBER94 as a force field (MD simulations). Structures for docking studies (AutoDock) were generated from Sybyl version 6.9.1 (Tripos Inc.).[62]

Relaxation by Molecular Dynamics Simulation. The Cartesian coordinates of the complexes were used as starting points. The hydrogen atoms were added and visually inspected. The set of complexes were superimposed, and the crystallographic molecules were compared. This superposition revealed that some positions are favored for crystallographic water molecules. When missing in the crystal structure, water molecules were added at each of these positions to each of the complexes. A water solvent layer (TIP3P) of 15 Å around each of the complexes was added and the complexes were allowed to relax following the procedure described below. The RNA, the aminoglycoside, and the crystallographic molecules were held fixed, the added crystallographic molecules and the added solvent water molecules being free to move. A preliminary minimization was performed to remove close atom contacts by 10 000 cycles of minimization using conjugate gradients using a dielectric constant of 1.00. The obtained complexes were next subjected to Newtonian molecular dynamics (MD) simulations with 500 steps of initialization followed by 5000 steps of simulation at constant temperature (300 K). During these simulations, steps of 1.0 fs were used. The simulations at 300 K were followed by 3000 steps of minimization using conjugate gradients with the heavy atoms of the RNA, the aminoglycoside, and the crystallographic

molecules being held fixed. The whole process was reiterated to lead to the models used for the subsequent docking studies.

Docking. The structures obtained through the relaxation process were next prepared for docking. For this purpose, either the water molecules were removed (for the dry systems) or kept if within 2.5 Å from any RNA atom. AutoDock[56] is a fully automated docking suite of programs, which employs a Lamarckian genetic algorithm (LGA) as a search engine and a LUDI-type scoring function. The grids were prepared using the autogrid facility provided with the AutoDock package. All the energy scoring grids have the same size ($60 \times 60 \times 60$ points, spacing 0.375 Å) and the same position in space. The RNA oligomers and ligands were charged according to AMBER[63,64] charges and Gasteiger-Marsilli charges,[65] respectively. Using the same approach as Detering and Varani, a charge of +1 was added to each phosphorus atom to neutralize the systems. Perl scripts were prepared to merge the grids into "hybrid" grids. 25 (smallest aminoglycosides into the dry RNA) to 50 runs (docking to flexible and/or solvated RNA, validation study) with a maximum of 5 000 000 energy evaluations were performed. The default parameters for the LGA and Sollis and Wet local search were used. In practice, when combining grids, a minimal weight is used to account for the conformations with high repulsive interactions. In the present study, minimal weights of 0.0001, 0.00001, or 0.000001 led to similar results.

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Supporting Information Available: Validation results of the RMSDs collected for the docking of compounds **10** and **11** to the nine RNA structures **a**−**i** (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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