Review

Journal of Molecular Recognition

Received: 12 May 2009, Revised: 16 September 2009, Accepted: 17 September 2009, Published online in Wiley InterScience: 2009

(www.interscience.wiley.com) DOI:10.1002/jmr.1000

Molecular recognition of RNA: challenges for modelling interactions and plasticity

Simone Fulle[a] and Holger Gohlke[a,b]*

There is growing interest in molecular recognition processes of RNA because of RNA's widespread involvement in biological processes. Computational approaches are increasingly used for analysing and predicting binding to RNA, fuelled by encouraging progress in developing simulation, free energy and docking methods for nucleic acids. These developments take into account challenges regarding the energetics of RNA–ligand binding, RNA plasticity, and the presence of water molecules and ions in the binding interface. Accordingly, we will detail advances in force field and scoring function development for molecular dynamics (MD) simulations, free energy computations and docking calculations of nucleic acid complexes. Furthermore, we present methods that can detect moving parts within RNA structures based on graph-theoretical approaches or normal mode analysis (NMA). As an example of the successful use of these developments, we will discuss recent structure-based drug design approaches that focus on the bacterial ribosomal A-site RNA as a drug target. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: docking; molecular dynamics simulations; continuum solvent; MM/PBSA; elastic network model; A-site; ribosome

INTRODUCTION

Targeting RNA structures with small molecules has great potential for drug design due to the key role of RNA in gene replication and expression (Hermann and Westhof, 1998; Gallego and Varani, 2001; Zacharias, 2003). The primary source of our understanding of molecular recognition modes of RNA to various ligands is high-resolution structures of RNA–ligand complexes. 300 of which are currently available in the Protein Data Base (PDB). They mostly comprise ribosomal RNA bound to clinically relevant antibiotics such as macrolides, aminoglycosides or oxazolidinones (Schluenzen *et al*., 2001; Hansen *et al*., 2002; Vicens and Westhof, 2003; Pyetan *et al*., 2007; Ippolito *et al*., 2008; Wilson *et al*., 2008) and aptamer-, riboswitch-, ribozyme- and tRNA–ligand complexes (Westhof *et al*., 1988; Blount and Breaker, 2006; Schwalbe *et al*., 2007; Suess and Weigand, 2008). Comprehensive overviews of RNA targets and different types of known RNA binders have been summarized in references (Carlson *et al*., 2003; Das and Frankel, 2003; Hermann, 2003; Thomas and Hergenrother, 2008). These high-resolution structures reveal that RNA contains well-defined binding sites that enable specific recognition of small molecules. The bound structures furthermore provide crucial insights regarding binding modes and mechanisms. This creates the possibility for rational structure-based drug design (SBDD) techniques to suggest chemical modifications, e.g. of antibiotic molecules in order to achieve higher binding affinity and selectivity (Shandrick *et al*., 2004; Murray *et al*., 2006; Ippolito *et al*., 2008; Skripkin *et al*., 2008).

Targeting RNA by small molecules is more challenging than targeting proteins. To the best of our knowledge, there are still no clinically relevant drugs available that target RNA structures and have been developed by structure-based drug design approaches. At least two challenges from a theoretical/computational point of view account for this.

First, the highly charged character of RNA structures results in both a strong solvation and the association of ionic molecules (Auffinger and Westhof, 2000; Auffinger and Hashem, 2007). Especially divalent ions, such as Mg^{2+}, are known to affect the conformational mobility and average conformation of RNAs (Al-Hashimi *et al*., 2003; Auffinger *et al*., 2003; Draper, 2004), and, therefore, also recognition processes (Misra and Draper, 2002). Long-resident water molecules and ions present at a small molecule/RNA interface can determine binding specificity. Both, water molecules and ions, should thus be considered in ligand docking approaches (Wallace and Schroeder, 2000). During the binding process, water molecules and (counter) ions can be displaced from the RNA structure. The rearrangement of the hydration shell will lead to an entropic contribution to the complex formation (Foloppe *et al*., 2006) that needs to be treated rigorously. Finally, in view of the highly charged nature of RNA, accurate and efficient modelling of long-range electrostatic interactions is still a major topic of research.

A second challenge arises from the highly flexible nature of RNA molecules. The structures can undergo large but controlled conformational changes to achieve their diverse functional roles comprising conformational switches in regulation processes and during RNA synthesis itself (Micura and Hobartner, 2003;

* *Correspondence to: H. Gohlke, Universitätsstr. 1, 40225 Düsseldorf, Germany. E-mail: gohlke@uni-duesseldorf.de*

a *S. Fulle, H. Gohlke*
Department of Biological Sciences, Molecular Bioinformatics Group, Goethe-University, Frankfurt, Germany

b *H. Gohlke*
Department of Mathematics and Natural Sciences, Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University, Düsseldorf, Germany

Al-Hashimi, 2005; Getz *et al*., 2007). Comprehensive investigations of RNA structures are complicated by the diversity of signals that can trigger such conformational changes (Ippolito and Steitz, 1998; Al-Hashimi *et al*., 2003; Casiano-Negroni *et al*., 2007; Ferner *et al*., 2008) and the variation of motional modes that span a large range of amplitudes and timescales (Puglisi *et al*., 1992; Fourmy *et al*., 1998; Nifosi *et al*., 2000; Al-Hashimi, 2005; Al-Hashimi and Walter, 2008). Depending on the respective RNA–ligand or RNA–protein complex, binding specificity is driven by a conformational selection or induced fit mechanism (Leulliot and Varani, 2001; Al-Hashimi, 2005). A precise knowledge of what can move, and how, is thus important to understand molecular recognition processes. Such information is also required to improve current docking algorithms (Ahmed *et al*., 2007). This includes considering energetic penalties associated with conformational capture and adoption (Hermann, 2002).

In recent years there has been encouraging progress in parameterizing simulation and docking methods for nucleic acids, taking into account the above challenges regarding energetics of RNA–ligand binding, RNA plasticity and the presence of bridging water molecules and ions. The present review will detail advances in force field and scoring function development for nucleic acids and in the application of free energy calculations and docking approaches. Furthermore, it will present methods that can detect moving parts within RNA structures based on normal mode analyses or graph-theoretical approaches. Finally, examples of SBDD approaches targeting the ribosomal A-site will be given to demonstrate the present scope and limitations of computational methods for this field.

MICROSCOPIC MODELS FOR DESCRIBING THE ENERGETICS OF MOLECULAR RECOGNITION INVOLVING RNA

Molecular dynamics (MD) simulations at the classical mechanical level are at present the most appropriate way to obtain detailed information about the complex processes of molecular recognition involving RNA. Conversely, characterizing the binding of small molecules, peptides, or proteins to RNA provides an excellent test of simulation methods. This is particularly true if not only structural or dynamic characteristics of RNA–ligand complex formation are considered, but if a comparison also involves the energetics of binding. As many excellent reviews of the field of MD simulations of nucleic acids in general are available (Giudice and Lavery, 2002; Orozco *et al*., 2003; Cheatham, 2004; McDowell *et al*., 2007; MacKerell and Nilsson, 2008; Orozco *et al*., 2008; Hashem and Auffinger, 2009), we will primarily focus on methodological developments involving MD simulations that allow investigating the energetics of RNA–ligand binding. Subsequently, we will address the field of scoring functions for docking applications involving RNA, which has seen a particularly lively development over the past years.

Recent force field and simulation method developments

While MD simulations of nucleic acids initially lagged behind protein simulations (MacKerell and Nilsson, 2008), introducing an explicit representation of solvent (Jorgensen *et al*., 1983) and rigorously treating long-range electrostatic forces (Cheatham *et al*., 1995) together with balanced molecular mechanical force fields (Cheatham, 2004) allow for stable simulations of nucleic acids on nanosecond to microsecond time scales nowadays (Perez *et al*., 2007a). Of the available force fields, those from the CHARMM and AMBER communities are the most popular ones for nucleic acid simulations (Orozco *et al*., 2003). Current versions (parm99 (Cheatham *et al*., 1999), CHARMM-27 (Foloppe and MacKerell, 2000; Mackerell *et al*., 2004)) provide accurate representations of standard DNA and RNA structures for trajectory lengths of $\sim$10 ns. More extended trajectory lengths on the order of μs revealed errors hidden in short trajectories of DNA generated with parm99, however, and required a revision of the force field, termed parmbsc0 (Perez *et al*., 2007a; Perez *et al*., 2007b). While simulations of canonical RNA duplexes with these recent force field versions appear to be straightforward (Cheatham, 2004; Perez *et al*., 2007b; Orozco *et al*., 2008), more work is needed to check their validity for non-canonical structural elements of RNA such as loops, hairpins and bulges, which play a dominant role of sites for binding (McDowell *et al*., 2007).

Regarding molecular recognition, intermolecular interactions to nucleobases are important, as they determine the specificity of binding. It is encouraging in this respect that nucleobase–nucleobase interactions, including stacked and hydrogen-bonded complexes, are described surprisingly well by the force field variants of the AMBER community, as revealed by comparison to high-level quantum mechanical electronic structure calculations (Sponer *et al*., 2005; Sponer *et al*., 2006). This finding also holds if modified nucleobases, e.g. fluorotoluenes and non-standard purines and pyrimidines, participate in the interaction and/or if nucleobase–water interactions or nucleobase hydration free energies are probed (Garcia *et al*., 1999). A good balance of intermolecular forces is most crucial when it comes to determining relative free energies of stability of RNA duplexes that differ only marginally with respect to the base composition. Reassuringly, when investigating the determinants of the unexpected stability of RNA fluorobenzene self pairs by means of thermodynamic integration calculations using the parm94 force field (Cornell *et al*., 1995), we were able to reproduce experimental relative free energies of stability to < 0.4 kcal mol^{-1} deviation, with a correlation between experimental and computed values of $R^2 = 0.97$ (Kopitz *et al*., 2008).

In contrast to the nucleobases, the sugar-phosphate backbone of nucleic acids is more difficult to model using the approximation of fixed atomic charges. First, the backbone has multiple conformational degrees of freedom. It cannot be expected that the electrostatic potential of different conformers can be fit equally well by just one set of atomic charges (Cieplak *et al*., 1995). Second, the fixed charge force fields do not account for the highly polarizable nature of the phosphodiester moiety. This difficulty is expected to be overcome by a new generation of polarizable force fields (Ponder and Case, 2003; Warshel *et al*., 2007). One such force field was recently tested in a simulation of B-DNA (Babin *et al*., 2006). While the sampled DNA structure was close to the experimental one, more work will be needed to reveal the advantage of polarizable force fields, in particular for the description of the influence of solvent and ions surrounding nucleic acids and the interactions involving partners with a high charge density.

The deficiency of fixed charge force fields to accurately describe interactions between nucleic acids and divalent ions should be noted, as Mg^{2+} ions may mediate the binding process of ligands (Schluenzen *et al*., 2001; Schluenzen *et al*., 2003). At present, force fields are biased towards direct binding of Mg^{2+} to a solute. In contrast, binding mediated by first-shell water

molecules, as anticipated in the case of ribosome-antibiotic complexes (Schluenzen *et al*., 2001; Schluenzen *et al*., 2003), is less well-described due to the lack of accounting for the polarization of these waters by the central ion (Sponer *et al*., 1998; Réblová *et al*., 2006; McDowell *et al*., 2007).

The highly charged nature of nucleic acids and their usually non-globular structure requires a careful treatment of the surrounding solvent and ion environment *per se*. In the case of molecular recognition involving nucleic acids, the importance of considering stabilizing effects of both mobile and specifically bound water has been emphasized for simulations involving DNA–ligand (Spackova *et al*., 2003) and RNA–ligand (Gouda *et al*., 2003) complexes. Thus, using an explicit solvent (and ion) representation has many advantages, but it is also very time consuming. As an efficient alternative, implicit solvation representations based on Poisson–Boltzmann (PB) (Warwicker and Watson, 1982) and Generalized Born (GB) (Still *et al*., 1990) approaches have been developed, which are expected to reproduce both electrostatic solute–solvent interactions and the screening effect of solvent on intra- or inter-solute interactions (Orozco and Luque, 2000). Together with a surface area-dependent term for non-polar contributions, the continuum models allow determining solvation free energies, which are required for describing the energetics of RNA–ligand binding within the framework of endpoint free energy methods (see below).

In an endeavour to use continuum electrostatic calculations on the Poisson level for determining solvation free energies for naturally occurring nucleic acids, Banavali and Roux (2002) reported a set of optimal Born atomic radii for small model compounds constituting the building blocks of nucleic acids. With these, the Poisson calculations not only provided excellent agreement with solvation free energies determined with explicit solvent, but accurately represented the free energy associated with base pairing of normal and mismatched base pairs, too. Although a continuum model undoubtedly has its limits, these results suggest that the approach can be used for studying RNA–ligand and RNA-protein complexes. Regarding GB/SA, one needs to consider that GB approximates the solution of the Poisson equation and that the experience in applying GB/SA calculations to RNA is still limited. However, when calculating absolute free energies of hydration for more than 500 neutral and charged compounds, Rizzo *et al.* (2006) found that PB/SA and GB/SA results were overall highly correlated. In fact, different partial charge models were found to influence the results much more than the type of continuum method that was used to compute hydration free energies, suggesting that GB/SA may be used as a more efficient alternative to PB/SA.

Free energy methods for binding to RNA

Rather than applying continuum solvent models directly for MD simulations of nucleic acid systems, a more widespread alternative is to, first, generate a conformational ensemble of structures by explicit solvent MD simulations and, second, post-process these snapshots with continuum solvent methods for averaging solvation free energies. Combined with molecular mechanics (MM) energies and entropy contributions, a free energy for the investigated state is computed. From this, free energy differences for describing energetic changes related to structural re-organization or binding free energies in the case of complex formation can be obtained. The prototype of these approaches is termed MM/PBSA and has been introduced initially for studying the stability of DNA and RNA helices (Srinivasan *et al*., 1998).

Regarding molecular recognition involving RNA, MM/PBSA was first applied to investigate the specificity of binding between the mammalian spliceosomal protein U1A and a hairpin RNA (Reyes and Kollman, 2000b). Relative binding free energies between mutant and wildtype obtained by applying methods of computational mutagenesis on protein and RNA residues verified mutations that abolished or improved binding. This suggested that the method could be used as a tool for investigating and predicting the effects of site-specific mutagenesis. When applied to the binding of different Tat peptides to TAR-RNA, however, disrupting interactions to phosphate oxygens were found to have a tendency to be more unfavourable than experimental measurements (Reyes *et al*., 2001). Whether this effect is due to the computational mutagenesis being performed by trajectory scanning, shortcomings in the continuum solvent representation, or even the use of a non-polarizable force field for snapshot generation has not been resolved.

In a subsequent study, MM/PBSA was used to determine the energetic contribution to binding of U1A-RNA due to the structural re-organization of the binding partners (Reyes and Kollman, 2000a). While experimentally difficult, if not impossible, to obtain, reliable estimates of the free energy costs of structural reorganization are needed for a complete description of binding processes. Here, as expected, the unbound states of U1A and the RNA were found to be more favourable compared to the bound ones by $\sim$10 kcal mol^{-1} per binding partner. Recently, Beveridge and coworkers again studied the U1A-RNA system, this time using MM/GBSA to investigate the origin of experimental binding free energy differences between wildtype and F56 U1A mutants (Kormos *et al*., 2007). The binding free energies were then decomposed into contributions from structural subcomponents, following an approach originally introduced to investigate protein–protein interactions (Gohlke *et al*., 2003). Notably, the study revealed that binding free energy differences originate from throughout the entire protein. Being able to decipher the determinants of binding on a sub-structural level is of great value when it comes to interpreting and improving binding affinities and specificities.

Regarding small molecule-RNA binding, aminoglycoside binding to the ribosomal A-site (Murray *et al*., 2006; Meroueh and Mobashery, 2007) and binding of theophylline and its analogues to an RNA aptamer have been investigated by MM/PBSA (Gouda *et al*., 2003). As for the latter, the MM/PBSA method was able to obtain a qualitatively good agreement with the experimental rank order of binding ($R^2 = 0.61$). While this result shows that MM/PBSA can be used as a fast method to rank binding affinities of ligands targeting RNA, thermodynamic integration calculations (Gouda *et al*., 2003) and absolute binding free energy calculations using non-equilibrium work values (Tanida *et al*., 2007) performed on the same data set resulted in much better correlations ($R^2 = 0.97$). Interestingly, the inadequate treatment of differences in the first-shell hydration of the ligands was identified as the source for the reduced accuracy in the MM-PBSA method. Accordingly, when treating some of the water molecules as part of the receptor in an *ad hoc* approach, more consistent free energies were obtained, in agreement with similar findings for drug–DNA interactions (Spackova *et al*., 2003). In our own experience, the use of a hybrid solvation model instead of PB also resulted in a better correlation of computed binding free

energies with the experimental ones in the case of protein–peptide binding (A. Metz, H. Gohlke, unpublished), again advocating for including the effects of explicit water.

Docking and scoring approaches to predict RNA–ligand interactions

The steep increase in functional and structural knowledge of RNA molecules calls for rational SBDD approaches that lead to the development of novel antibacterial and antiviral drugs. For these SBDD to be successful, accurate and efficient docking and scoring methods have to be developed and evaluated. While the past 25 years have seen great progress in the development of automatic docking tools to predict protein–ligand interactions (Gohlke and Klebe, 2002; Sousa *et al*., 2006), much less has been achieved in terms of efficiently and accurately modelling RNA–ligand interactions. The current approaches can be divided into three different classes.

First, computationally intense methods combine docking methods and MD simulations. These approaches strive to build models for a small number of RNA–ligand complexes, but are too time-consuming for large-scale screening applications (Srinivasan *et al*., 1996; Leclerc and Cedergren, 1998; Hermann and Westhof, 1999; Mu and Stock, 2006). Second, methods originally developed for protein-based drug design are subsequently applied to RNA. For example Kuntz and coworkers used the DOCK program to identify small molecules with binding specificity to the RNA double helix (Chen *et al*., 1997; Kang *et al*., 2004). Likewise, Leclerc and Karplus identified favourable RNA binding sites by the MCSS method, thereby making use of nucleic acid parameters from the CHARMM force field (Leclerc and Karplus, 1999). In a virtual screening study, James and coworkers successfully identified acetylpromazine as a lead compound that binds to TAR RNA by a rigid DOCK screen and subsequent flexible docking with ICM (Lind *et al*., 2002). Both methods are well-known for predicting protein–ligand complexes. However, to finally rank the compounds, an RNA-specific regression-based scoring function needed to be developed based on very limited structural and energetic knowledge of only 13 RNA–ligand complexes. In a related validation study, Detering and Varani generally concluded that it is possible to use the docking tools DOCK and AutoDock to increase the likelihood of discovering molecules in databases that bind to RNA (Detering and Varani, 2004). However, DOCK was successful only in the case of rigid aromatic ligands, whereas it performed poorly with weak-binding ligands and with aminoglycosides. Similarly, AutoDock failed on the complexes for which DOCK performed poorly as well. Very likely, this indicates a misbalance between charged and non-polar/aromatic interactions in both protein-based scoring functions. Consequently, the highest likelihood of identifying RNA-binding ligands from database screens was achieved by a successive application of DOCK and AutoDock. Thus, although both studies provide encouraging examples of the current scope of RNA-based virtual screening, they also point to shortcomings in the description of the energetic determinants of RNA–ligand binding.

Third, applications are newly developed for scoring RNA–ligand interactions. In an attempt to overcome the above-mentioned shortcomings, Morley and Afshar (2004) developed a new regression-based scoring function that is RNA-specific ('RiboDock'). As in the case of the regression-based function by James and coworkers (Filikov *et al*., 2000), however, a limited training and validation set of only 10 RNA–ligand complexes was employed to parameterize the function. Similarly, Barbault *et al*. (2006) parameterized a specific free energy function for automated docking against RNA targets using a back-propagating neural network, but only eight RNA-drug complexes. Hence, the general applicability and predictive power of these functions remains elusive.

The above considerations provided the incentive for us to develop, for the first time, a knowledge-based scoring function to predict RNA–ligand interactions (Pfeffer and Gohlke, 2007). Based on the formalism of the DrugScore approach (Gohlke *et al*., 2000), distance-dependent pair potentials were derived from 670 crystallographically determined nucleic acid–ligand and -protein complexes. These potentials display quantitative differences compared to those of DrugScore (Gohlke *et al*., 2000) and DrugScore^{CSD} (Velec *et al*., 2005). When used as an objective function for docking 31 RNA–ligand complexes, DrugScore^{RNA} generates 'good' binding geometries (*rmsd* < 2 Å, Figure 1) in 42% of all cases on the first scoring rank. Encouragingly, good docking results were also obtained for a subset of 20 NMR structures not contained in the knowledge-base to derive the potentials. When applied for predicting binding affinities, a fair correlation between experimental and computed values was found for 15 diverse RNA–ligand complexes ($R^2 = 0.37$). This accuracy suffices to distinguish weak from strong binders, as is required in virtual screening applications.

In a later approach, Zhao *et al*. (2008) adapted the atom type definitions of PMF score and re-derived the scoring function using 97 RNA–ligand complexes. When applied to the same 15 RNA–ligand complexes as above, a rank ordering between computed and experimental binding free energies with $R^2 = 0.66$ was found. This result was attributed to the introduced base-specific atom-typing scheme for nucleic acids. The atom-typing scheme also considers water molecules embedded in the binding site region. While this may also contribute to the improved predictive power for binding energies

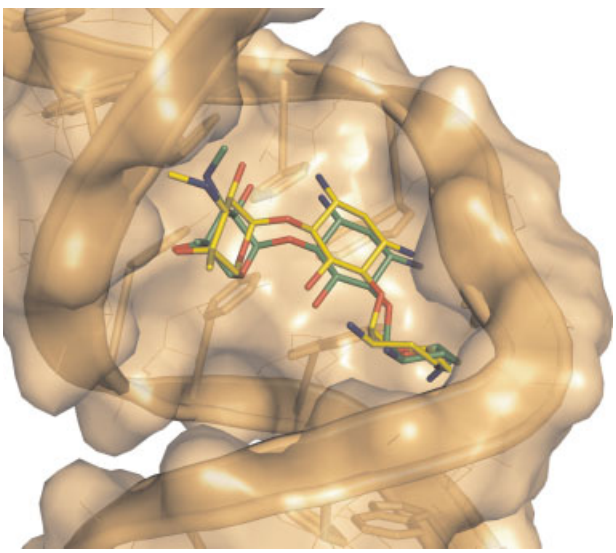

Figure 1. Ribosomal A-site/gentamicin complex (PDB code 1BYJ (Yoshizawa *et al*., 1998)). The experimental ligand pose is given in yellow, the docking solution ranked first by DrugScore^{RNA} is depicted in green (*rmsd* to the native structure: 1.99 Å).

of structurally known RNA–ligand complexes, it is questionable how the influence of water molecules will be treated for complexes whose experimental structure is not known.

Guilbert and James (2008) recently introduced an interesting docking approach in which a root mean square deviation-driven energy minimization allows for the mutual conformational adaptation of flexible ligands and flexible targets. When tested on 57 RNA–ligand complexes, a success rate of 'good' binding geometries of 65% was found. When applied to 28 complexes in common with those of the DrugScore^{RNA} study, however, this rate dropped to 53%. This finding clearly illustrates the data set dependence of such results. Furthermore, it is not clear to what extent the preparation of the docking data contributed to the success. Ligands were 'stripped' from the receptor and, after minimization *in vacuo*, only randomly rotated twice. No randomization of torsion angles was reported. In the first step of the docking protocol, the ligand was then placed on 'hot spots' of the receptor, essentially resembling a docking of a rigid ligand into a rigid receptor. As the ligand had not been purposefully distorted with respect to its bound conformation in the preparation step, it may thus not come as a surprise that good starting structures for the subsequent energy minimization step were generated that way.

Within the solvation module of DOCK (sDOCK), a GB/SA model based on Still's formulation had been implemented in an effort to incorporate solvation scoring in virtual screening of databases for protein targets (Zou *et al*., 1999). By separately scaling van der Waals and charge–charge interactions and the surface area-dependent non-polar contribution to solvation free energy, Kuntz and coworkers then tried to improve DOCK's performance with nucleic acid receptors (Kang *et al*., 2004). Fitting to the experimental values, binding free energies of a training set of 47 DNA–ligand complexes had an average error around 2 kcal mol^{-1} and $R^2 = 0.53$. Convincingly, a similar correlation coefficient was found when this function was tested against a data set of 11 RNA aptamer–ligand complexes. Likewise, the trained GB/SA model was also able to discriminate strong ligands from poor ones for a series of RNA aptamers. Interestingly, when the GB/SA approach was used for rescoring previously generated docking poses within a receptor in a vacuum, no improvement in the success rate compared to standard GridScore scoring was found (Lang *et al*., 2009). Only when receptors embedded into explicit waters and counterions were used, did the success rate of 'good' binding geometries become 42%. The authors interpreted this in that explicit waters and counterions are critical for properly modelling the energy landscape of RNA targets. An alternative interpretation grounded in the preparation of the solvated receptor structures may be more likely: counterions and water molecules were added to each receptor with the ligand present. After removal of excess water, the ligands were removed as well, and the solvated, rigid receptors were used in docking. This considerably restricts the configurational search space of the ligand and, hence, should be expected to improve the docking success.

PROSPECTS FOR MODELLING RNA FLEXIBILITY AND MOBILITY IN MOLECULAR RECOGNITION

The flexible nature of RNA calls for docking approaches that consider target conformational changes upon ligand binding. Analogous to protein–ligand docking (Ahmed *et al*., 2007), three major classes of approaches are conceivable. First, plasticity can be implicitly considered applying a soft-docking strategy with attenuated repulsive forces between target and ligand, but the range of possible movements that can be covered this way is rather limited. Second, only shifts of a few nucleotides are modelled, which assumes a rigid RNA backbone. This seems appropriate, e.g. in the case of the ribosomal A-site where only minor conformational changes occur during binding at the backbone level with some bases flipping their position (Tor, 2006). Third, large-scale conformational changes including backbone motions are taken into account. This approach is necessary to deal with gross conformational changes, e.g. observed upon binding to HIV-1 TAR RNA (Puglisi *et al*., 1992; Nifosi *et al*., 2000). So far, only two approaches that fall into this class and are fast enough to allow for flexible-RNA virtual screening have been introduced: the method by Guilbert and James (2008) mentioned above and the method by Moitessier *et al*. (2006) described in more detail below, which combines multiple RNA conformations at the level of interaction grids. Other strategies well known in the field of protein–ligand docking, including parallel docking into multiple target conformations (Cavasotto and Abagyan, 2004; Cavasotto *et al*., 2005; Huang and Zou, 2007; Nabuurs *et al*., 2007), modelling target motions in reduced coordinates (Bahar and Rader, 2005; Tozzini, 2005), or deforming interaction grids according to target movements (Kazemi *et al*., 2009), have not yet been described for RNA. All of these approaches require either one or multiple RNA structures close to bound conformations or knowledge about how a given RNA can move.

What are the prospects to provide such information by computational means? MD simulations are still too computationally expensive to investigate RNA structures on a routine basis for simulation times beyond hundred(s) of nanosecond range. More efficient alternatives based on graph theoretical or harmonic analysis techniques have been developed during the last decade, which allow for simulations of biomolecules within some hours or days, exploring large scale motions occurring over long timescales, and/or investigating biomolecules as large as the ribosome (Tama *et al*., 2003; Wang *et al*., 2004; Trylska *et al*., 2005; Kurkcuoglu *et al*., 2008; Fulle and Gohlke, 2009a). Concerning molecular recognition involving proteins, these methods have proven valuable to analyse *a posteriori* or even predict relevant conformational changes of the target upon ligand binding (Tama and Sanejouand, 2001; Temiz and Bahar, 2002; Zavodszky *et al*., 2004; Ahmed and Gohlke, 2006). In the case of RNA, we are not aware of any such study. Hence, below we briefly summarize a few selected applications of these methods to isolated RNA molecules and discuss their potential use in the case of molecular recognition.

Predicting RNA mobility using normal mode analysis and elastic network models

Normal mode analysis (NMA) provides an analytical solution to the equations of motions of a biomacromolecule (Brooks and Karplus, 1983; Go *et al*., 1983). Only a few of the lowest energy vibrational modes are usually considered, which give a clear representation of global and collective motions of the biomolecule (Bomble and Case, 2008). Due to the harmonic approximation, however, the description of motion is limited to movements that occur in the vicinity of a structure located at

an energy minimum. Likewise, local and/or non-linear movements are less well represented. At present, a number of mode analysis approaches are available, each showing a different compromise between computational cost and the level of detail in the calculation.

All-atom NMA was applied to the hammerhead ribozyme and a guanine riboswitch structure (Van Wynsberghe and Cui, 2005). Computed atomic fluctuations and the conformational space accessed matched very well with results obtained by a computationally much more demanding MD simulation. A systematic study of the conformational deformability of double-stranded RNA oligonucleotides was undertaken by Zacharias and Sklenar (2000) using an all-atom harmonic mode analysis technique. Calculated atomic fluctuations did not match the magnitude of experimental B-factors, but at least showed a good agreement with respect to trends in the movements. In comparison with MD simulations, a significant overlap between the subspaces spanned by the calculated soft harmonic modes and MD-derived principal components was found (Zacharias, 2000). Considering that RNA molecules can transiently sample bound conformations already in the free ensemble, e.g. as experimentally shown for TAR-RNA (Al-Hashimi *et al*., 2002; Al-Hashimi, 2005; Zhang *et al*., 2006; Zhang *et al*., 2007), harmonic analysis methods could thus be used for calculating an RNA conformational ensemble against which small molecules are screened in the computer.

Among coarse-grained network models, the Gaussian Network Model (Bahar *et al*., 1997) (GNM) and Anisotropic Network Model (ANM) are the most widely used (Atilgan *et al*., 2001; Eyal *et al*., 2006). Both methods create a network of Hookean springs from a given input structure. In the case of RNA, only phosphorous atoms or phosphorous atoms in combination with sugar C4′ and base C2 atoms are usually chosen as vertices in the network. GNM characterizes the *magnitudes* of thermal fluctuations but cannot predict the directions of molecular motions. ANM determines quantitatively the *directions* of the fluctuation vectors but performs worse in describing the magnitude of thermal fluctuations (Wang *et al*., 2004; Zheng, 2008).

A comprehensive evaluation of GNM was performed by Bahar and coworkers on a data set of 45 oligonucleotide–protein complexes and 19 oligonucleotide structures (Yang *et al*., 2006b). It turned out that GNM could produce good fluctuation predictions compared with experiment. Hence, GNM allows deriving information where conformational changes will be most pronounced. Furthermore, fluctuations calculated by GNM have already been shown to be useful for estimating entropic contributions to the change of free energy for the bulge regions of the Bovine Immunodeficiency Virus (BIV) and HIV-1 TAR-RNA structures (Lustig *et al*., 1998). These results are encouraging in view of applications in SBDD approaches.

In a study by Van Wynsberghe and Cui (2005), the performance of mode analyses of different resolutions was tested on the hammerhead ribozyme and a guanine riboswitch structure. Results from detailed quasiharmonic analyses of MD trajectories were used as a benchmark. Classical all-atom NMA as well as block NMA (Durand *et al*., 1994; Tama *et al*., 2000; Li and Cui, 2002) both matched very well the magnitudes of residue fluctuations and the directions of low frequency modes from the quasiharmonic analyses. In contrast, ANM neither reproduced adequately the qualitative trends of residue fluctuations nor the directions of the lowest-frequency modes for the Hammerhead ribozyme. In the case of the riboswitch structure, ANM did not predict the direction of structural mobility well either. Yet, the (relative) magnitudes of atomic fluctuations were described well in this case. The authors concluded that ANM, although successful for many globular proteins, may not be best suited for more loosely packed systems such as RNA structures.

Predicting RNA flexibility and mobility using concepts from graph theory

Precise knowledge about *what* can move may already be helpful when it comes to modelling conformational changes upon ligand binding. Constraint counting on a topological network representation of an RNA structure provides this information very efficiently by decomposing the molecule into rigid and flexible parts. Notably, rigidity and flexibility information is provided at various structural levels. While flexibility characteristics at the bond level are instructive for analysing binding site regions, flexibility characteristics of larger regions can be related to potential global conformational changes and provide hints about movements of structural parts such as rigid bodies (Fulle and Gohlke, 2009b).

For the analysis, a 3D structure of the molecule is modelled as a topological network in which vertices represent atoms and edges represent important forces: covalent bonds, strong hydrogen bonds and salt bridges, hydrophobic interactions as well as angular constraints (Gohlke and Thorpe, 2006). Remarkably, constraint counting on a network of a molecule of several thousands of atoms just takes a few seconds so that the approach is also efficiently applicable to large macromolecules (Hespenheide *et al*., 2004; Wang *et al*., 2004; Fulle and Gohlke, 2009a).

Recently, we adapted the approach to RNA structures by developing a new topological network representation for these molecules (Figure 2) (Fulle and Gohlke, 2008). The adaptation was necessary because different non-covalent forces determine the structural stability of proteins and RNA structures. The new parameters were shown to prevent the RNA network representation from being overly rigid and allow for determining subtle differences in RNA flexibility/rigidity.

The decomposition into rigid and flexible regions says nothing about the direction and amplitude of existing motions. However, it can be used as input for naturally coarse-grained simulations (Wells *et al*., 2005; Ahmed and Gohlke, 2006; Gohlke and Thorpe, 2006) that explore the molecule's mobility. Accordingly, the constrained geometrical simulation approach FRODA (Wells *et al*., 2005) has been applied to RNA. Atomic fluctuations calculated from FRODA-generated conformational ensembles of coarse-grained RNA structures are in good agreement with conformational variabilities obtained from NMR-derived ensembles (Figure 3) (Fulle and Gohlke, 2008). Again, simulations of this type may be helpful for generating a conformational ensemble of the target that can be exploited in subsequent SBDD.

STRUCTURE-BASED DRUG DESIGN APPROACHES TARGETING THE RIBOSOMAL A-SITE

The available high-resolution structures of antibiotics bound to ribosomal subunits provide crucial insights regarding binding sites, binding modes and mechanisms of action (Foloppe *et al*., 2004; Sutcliffe, 2005; Butler and Buss, 2006; Franceschi and Duffy, 2006). Such information creates possibilities for rational SBDD

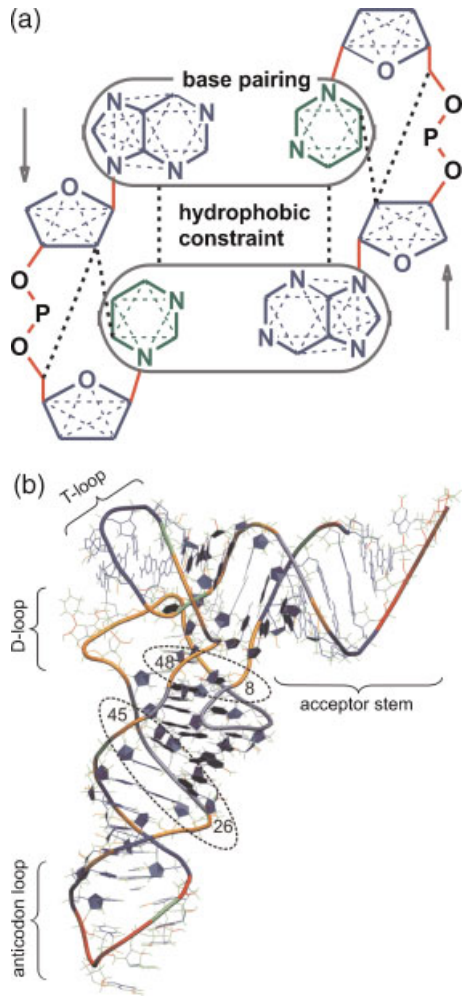

Figure 2. (a) Topological network representation of a canonical A-form RNA. Constraints between nearest neighbours are indicated by straight lines, constraints between next nearest neighbours (angle constraints) by dashed lines. For reasons of clarity, angle constraints are only indicated in the sugar and base scaffolds, and hydrogen bonds between bases are omitted. Hydrophobic constraints are indicated by black dashed lines. Flexible hinges are shown in red, minimally rigid regions in green and overconstrained regions (which contain redundant constraints) in blue. (b) Colour-coded representation of flexibility indices of the $tRNA^{ASP}$ structure (PDB code 2TRA (Westhof *et al.*, 1988)) as obtained by flexibility analysis using an RNA parametrization (Fulle and Gohlke, 2008). Over-constrained regions are indicated by bluish colours, rigid regions are represented in green colour and flexible regions are shown in reddish colours. The colouring of the backbone is according to the flexibility indices of the phosphorus atoms. The core region of the structure (nucleotides whose B-values < 15 Å^2) is highlighted with filled sugar and base scaffolds. The identified hinge regions of the structure (8 and 48, 26 and 45) are designated by dashed circles.

techniques to suggest chemical modifications in order to achieve higher binding affinity and selectivity or even to produce new classes of anti-bacterial drugs (Shandrick *et al.*, 2004; Murray *et al.*, 2006; Ippolito *et al.*, 2008; Skripkin *et al.*, 2008). Although clinically relevant antibiotics designed by SBDD approaches are not available so far, it is encouraging to note that currently two compounds designed by SBDD as new antibacterial drugs targeting the ribosome are in phase II clinical trials

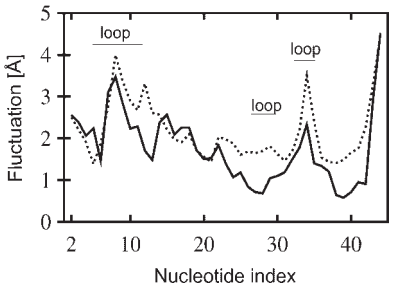

Figure 3. Mobility information of backbone phosphorous atoms predicted by FRODA simulations for the RNA structure 1A60 (Kolk *et al.*, 1998) (*straight line*). For comparison, conformational variabilities as measured in NMR are shown (*dotted line*).

(www.rib-x.com). In contrast to protein targets, the ribosome structure does not contain a single well-defined binding pocket. Instead, various classes of antibiotics bind to adjacent or overlapping regions. As a consequence it may be misleading to use ligand-based approaches in order to identify a common set of molecular features for screening approaches (Franceschi and Duffy, 2006). Rather, it appears more promising to use high resolution crystal structures for screening or constructing potential ligands based on the identified binding site.

Surprisingly, to date, no computational analyses of molecular recognition properties of the ribosomal peptidyl-transferase centre or the macrolide binding site within the ribosomal exit tunnel have been reported, although both regions are important binding regions for clinically relevant antibiotics. Rather, the majority of computational SBDD approaches that have been described over the last 3 years have focused on the ribosomal decoding region of the aminoacyl-tRNA acceptor site (A-site). The A-site is a small internal loop within the 16S rRNA of the 30S subunit, which ensures fidelity during protein synthesis by monitoring base pairing between the mRNA codon and tRNA anticodon (Ogle *et al.*, 2001). A major component to distinguish between cognate and non-cognate tRNA-mRNA hybrids is two highly conserved adenine bases A1492 and A1493 (*E. coli* numbering) (Figure 4) (Foloppe *et al.*, 2004). Binding of aminoglycoside antibiotics (Vicens and Westhof, 2003) decreases the selection fidelity by stabilizing a bulge-out conformation of A1492 and A1493 that closely resembles the conformation for near cognate complexes during mRNA decoding (Carter *et al.*, 2000; Ogle *et al.*, 2001).

Pharmacophore approach

Foloppe *et al.* (2006) reported that the pharmacophoric features in the ribosomal A-site resemble those of protein kinase inhibitors, as deduced from a superpositioning of the hydrogen-bond donor and acceptor from the backbone kinase motif of protein kinase A with the Watson-Crick face of A1408. Based on this finding, they proposed that compounds designed against kinases may also be interesting candidates as A-site ligands. To illustrate their hypothesis, a kinase-directed library of 3500 drug-like compounds was docked to the A-site. Indeed, the determined pharmacophore model, which includes a ring stacking on G1491 in addition to the hydrogen-bond donor and acceptor interaction located at A1408, was reproduced during the docking runs (Foloppe *et al.*, 2006).

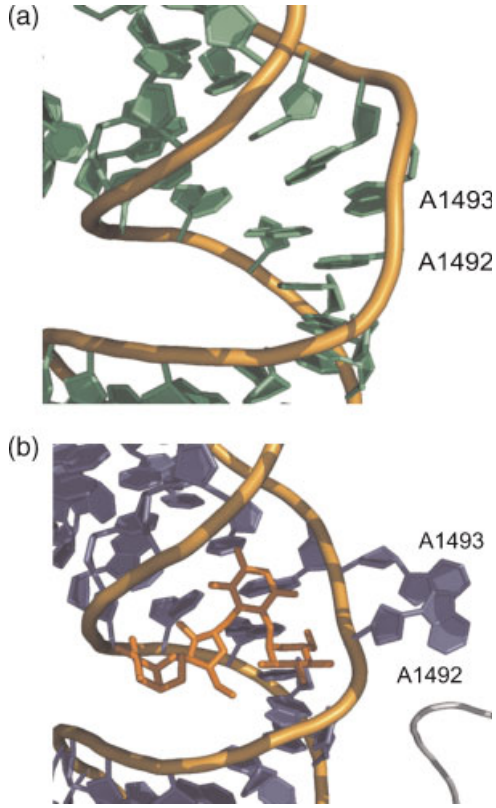

Figure 4. Different conformations of A1992 and A1993 in the ribosomal A-site. (a) In the native 30S structure (PDB code 1FKA (Schluenzen *et al*., 2000)) A1992 and A1993 are stacked inside a loop; (b) upon binding of the aminoglycoside antibiotic paromomycin (PDB code 1IBK (Ogle *et al*., 2001)) A1992 and A1993 are flipped out so that codon–anticodon recognition in mRNA-tRNA hybrids can be sensed.

Calculation of binding free energies of aminoglycosidic antibiotics in complex with the A-site

A series of studies by Mobashery and coworkers nicely demonstrates a hierarchical combination of computational analysis, X-ray crystallography, and experimental assays in order to design novel antibiotics that bind to the bacterial A-site. Knowing that neamine is a minimal structural motif required for binding, seven designer antibiotics binding to the A-site with dissociation constants in the lower micromolar range were found by screening 273 000 compounds (Haddad *et al*., 2002). X-ray structures of some of these novel antibiotics bound to A-site models revealed the underlying molecular interactions (Russell *et al*., 2003; Murray *et al*., 2006). The structures finally allowed a combination of MD simulations with the free energy calculation method MM-PBSA (Murray *et al*., 2006). The computations revealed that the designed compounds provide additional stability to the bases A1492 and A1493 in their extrahelical conformation when compared to neamine. The computed binding free energies (ΔG^{calc}: designed compound 3: -7.7 ± 1.6 kcal/mol; neamine -6.8 ± 1.4 kcal/mol) were in good agreement with experimental values (-7.4 kcal/mol; -6.5 kcal/mol), which indicates that MM-PBSA can be suggested to probe ligand binding to the ribosomal A-site. The free energy contributions showed that van der Waals and hydrophobic components are the driving forces for the enhanced binding of the designer compound compared to the parental neamine (Murray *et al*., 2006). The enhanced interactions likely result from a high degree of complementarity between the new, partly aliphatic arms of the ligand and the A-site pocket.

Another hierarchical screening strategy has been described by Foloppe *et al.* (2004). Docking of about one million compounds with RiboDock against a crystal structure of the bacterial A-site yielded 129 potential ligands, of which about one quarter showed affinity for an A-site RNA oligonucleotide in a FRET-based assay. For selected compounds, NMR experiments confirmed the binding. Based on intermolecular NOE, a tentative binding mode was finally suggested for the most potent compound.

A good correlation with experimental affinities of aminoglycosides binding to the A-site was also obtained by the group of McCammon by using a continuum electrostatics method (Ma *et al*., 2002; Yang *et al*., 2006a). To obtain total binding free energies, they calculated electrostatic properties by solving the linearized Poisson–Boltzmann equation combined with a surface area-dependent non-polar term and contributions from conformational changes (Yang *et al*., 2006a). Despite many approximations, the approach reaches a fair correlation of $R^2 = 0.71$ on the tested aminoglycosides. Strikingly, if aminoglycosides of only one family were considered, an even better fit of $R^2 > 0.9$ was achieved. As the calculated free energies revealed the correct trend in binding energies, the approach should be applicable to identify modified aminoglycosides with increased binding affinity in reasonable time. The authors further noted that the method works well for cases where the electrostatic contribution dominates the binding affinity or where changes in the studied ligand structures result in changes of its net charges (Yang *et al*., 2006a). However, for more subtle changes such as substituting a methyl group for a hydrogen atom, more accurate methods such as free energy perturbation calculations are necessary.

Incorporating water-mediated contacts and RNA flexibility

A series of crystallographic studies revealed the presence of an organized network of water-mediated contacts that complements the network of RNA-aminoglycosides hydrogen bonds (Vaiana *et al*., 2006). The first attempt to include bridging water molecules of the A-site into a docking approach was performed by Moitessier *et al.* (2006). A short MD simulation in explicit solvent was applied first to supplement missing water molecules in the solvation shell of the A-site. Potential bridging water molecules were considered to be located within a distance of 2.5 Å of any RNA atoms. Since water molecules may be displaced during the binding process, a displacement possibility was included in the interaction energy function (Moitessier *et al*., 2006).

In addition, the authors investigated several strategies to include the flexibility of the ribosomal A-site into the docking approach. Including both the dynamically bound water molecules by a pseudo-solvation scheme and the intrinsic RNA flexibility by combining interaction grids of different RNA conformations and using soft van der Waals parameters resulted in correct docking solutions for eight out of nine aminoglycosides. Including the first hydration shell, therefore, had a larger impact on the improvement than including the flexibility of the RNA receptor. As a reason, only minor conformational changes occur during binding at the backbone level with some bases flipping their position (Tor, 2006).

CONCLUSION

Our understanding of molecular recognition involving RNA has advanced significantly in recent years, fuelled by an intricate interplay between theoretical and experimental work. With respect to the former, important steps were the development of balanced force fields for nucleic acids, endpoint free energy methods based on a continuum representation of the solvent, scoring functions to describe RNA–ligand interactions, and the adaptation of elastic and constraint network models to describe flexibility and mobility of RNA structures.

These developments are beginning to be integrated into methods for structure-based design of RNA ligands. However, several problems still remain unsolved, such as an *ab initio* prediction of solvent and ion binding sites for complex structures, an efficient modelling of RNA conformational changes upon binding, and consideration of contributions to the binding free energy arising from structural reorganization and entropic changes. As such, methods developed for RNA as a target by and large face similar problems as approaches developed for the protein field. Thus, one might favourably expect that novel developments in either field will fertilize the other one.

Acknowledgements

This work was supported by the Goethe-University, Frankfurt, Heinrich-Heine-University, Düsseldorf, and the DFG (SFB 579, 'RNA–ligand interactions'). SF acknowledges financial support from the Hessian Science Program, the Frankfurt International Graduate School for Science (FIGSS) and the Otto-Stern-School in Frankfurt. The authors are grateful to Daniel Cashman for critically reading the manuscript.

REFERENCES

Ahmed A, Gohlke H. 2006. Multiscale modeling of macromolecular conformational changes combining concepts from rigidity and elastic network theory. *Proteins* **63**: 1038–1051.

Ahmed A, Kazemi S, Gohlke H. 2007. Protein flexibility and mobility in structure-based drug design. *Front. Drug Des. Discov.* **3**: 455–476.

Al-Hashimi HM. 2005. Dynamics-based amplification of RNA function and its characterization by using NMR spectroscopy. *ChemBioChem* **6**: 1506–1519.

Al-Hashimi HM, Gosser Y, Gorin A, Hu W, Majumdar A, Patel DJ. 2002. Concerted motions in HIV-1 TAR RNA may allow access to bound state conformations: RNA dynamics from NMR residual dipolar couplings. *J. Mol. Biol.* **315**: 95–102.

Al-Hashimi HM, Pitt SW, Majumdar A, Xu W, Patel DJ. 2003. Mg2+-induced variations in the conformation and dynamics of HIV-1 TAR RNA probed using NMR residual dipolar couplings. *J. Mol. Biol.* **329**: 867–873.

Al-Hashimi H, Walter N. 2008. RNA dynamics: it is about time. *Curr. Opin. Struct. Biol.* **18**: 321–329.

Atilgan AR, Durell SR, Jernigan RL, Demirel MC, Keskin O, Bahar I. 2001. Anisotropy of fluctuation dynamics of proteins with an elastic network model. *Biophys. J.* **80**: 505–515.

Auffinger P, Bielecki L, Westhof E. 2003. The Mg2+ binding sites of the 5S rRNA loop E motif as investigated by molecular dynamics simulations. *Chem. Biol.* **10**: 551–561.

Auffinger P, Hashem Y. 2007. Nucleic acid solvation: from outside to insight. *Curr. Opin. Struct. Biol.* **17**: 325–333.

Auffinger P, Westhof E. 2000. Water and ion binding around RNA and DNA (C,G) oligomers. *J. Mol. Biol.* **300**: 1113–1131.

Babin V, Baucom J, Darden TA, Sagui C. 2006. Molecular dynamics simulations of DNA with polarizable force fields: convergence of an ideal B-DNA structure to the crystallographic structure. *J. Phys. Chem. B* **110**: 11571–11581.

Bahar I, Atilgan A, Erman B. 1997. Direct evaluation of thermal fluctuations in proteins using a single-parameter harmonic potential. *Fold. Des.* **2**: 173–181.

Bahar I, Rader AJ. 2005. Coarse-grained normal mode analysis in structural biology. *Curr. Opin. Struct. Biol.* **15**: 586–592.

Banavali NK, Roux B. 2002. Atomic radii for continuum electrostatics calculations on nucleic acids. *J. Phys. Chem. B* **106**: 11026–11035.

Barbault F, Zhang LR, Zhang LH, Fan BT. 2006. Parametrization of a specific free energy function for automated docking against RNA targets using neural networks. *Chemom. Intell. Lab. Syst.* **82**: 269–275.

Blount K, Breaker R. 2006. Riboswitches as antibacterial drug targets. *Nat. Biotechnol.* **24**: 1558–1564.

Bomble Y, Case D. 2008. Multiscale modeling of nucleic acids: insights into DNA flexibility. *Biopolymers* **89**: 722–731.

Brooks B, Karplus M. 1983. Harmonic dynamics of proteins: normal modes and fluctuations in bovine pancreatic trypsin inhibitor. *Proc. Natl Acad. Sci. U.S.A.* **80**: 6571–6575.

Butler MS, Buss AD. 2006. Natural products–the future scaffolds for novel antibiotics? *Biochem. Pharmacol.* **71**: 919–929.

Carlson C, Stephens O, Beal P. 2003. Recognition of double-stranded RNA by proteins and small molecules. *Biopolymers* **70**: 86–102.

Carter AP, Clemons WM, Brodersen DE, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V. 2000. Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* **407**: 340–348.

Casiano-Negroni A, Sun X, Al-Hashimi HM. 2007. Probing Na(+)-induced changes in the HIV-1 TAR conformational dynamics using NMR residual dipolar couplings: new insights into the role of counterions and electrostatic interactions in adaptive recognition. *Biochemistry* **46**: 6525–6535.

Cavasotto CN, Abagyan RA. 2004. Protein flexibility in ligand docking and virtual screening to protein kinases. *J. Mol. Biol.* **337**: 209–225.

Cavasotto CN, Kovacs JA, Abagyan RA. 2005. Representing receptor flexibility in ligand docking through relevant normal modes. *J. Am. Chem. Soc.* **127**: 9632–9640.

Cheatham TE. 2004. Simulation and modeling of nucleic acid structure, dynamics and interactions. *Curr. Opin. Struct. Biol.* **14**: 360–367.

Cheatham TE, Cieplak P, Kollman PA. 1999. A modified version of the Cornell *et al.* force field with improved sugar pucker phases and helical repeat. *J. Biomol. Struct. Dyn.* **16**: 845–862.

Cheatham TE, Miller JL, Fox T, Darden TA, Kollman PA. 1995. Molecular dynamics simulations on solvated biomolecular systems: the Particle Mesh Ewald method leads to stable trajectories of DNA, RNA, and proteins. *J. Amer. Chem. Soc.* **117**: 4193–4194.

Chen Q, Shafer RH, Kuntz ID. 1997. Structure-based discovery of ligands targeted to the RNA double helix. *Biochemistry* **36**: 11402–11407.

Cieplak P, Cornell WD, Bayly C, Kollman PA. 1995. Application of the multimolecule and multiconformational RESP methodology to biopolymers - charge derivation for DNA, RNA and proteins. *J. Comput. Chem.* **16**: 1357–1377.

Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. 1995. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* **117**: 5179–5197.

Das C, Frankel A. 2003. Sequence and structure space of RNA-binding peptides. *Biopolymers* **70**: 80–85.

Detering C, Varani G. 2004. Validation of automated docking programs for docking and database screening against RNA drug targets. *J. Med. Chem.* **47**: 4188–4201.

Draper DE. 2004. A guide to ions and RNA structure. *RNA* **3**: 335–343.

Durand P, Trinquier G, Sanejouand YH. 1994. New approach for determining low-frequency normal-modes in macromolecules. *Biopolymers* **34**: 759–771.

Eyal E, Yang L-W, Bahar I. 2006. Anisotropic network model: systematic evaluation and a new web interface. *Bioinformatics* **22**: 2619–2627.

Ferner J, Villa A, Duchardt E, Widjajakusuma E, Wöhnert J, Stock G, Schwalbe H. 2008. NMR and MD studies of the temperature-dependent dynamics of RNA YNMG-tetraloops. *Nucleic Acid. Res.* **36**: 1928–1940.

Filikov AV, Mohan V, Vickers TA, Griffey RH, Cook PD, Abagyan RA, James TL. 2000. Identification of ligands for RNA targets via structure-based virtual screening: HIV-1 TAR. *J. Comput. Aid. Mol. Des.* **14**: 593–610.

Foloppe N, Chen IJ, Davis B, Hold A, Morley D, Howes R. 2004. A structure-based strategy to identify new molecular scaffolds targeting the bacterial ribosomal A-site. *Bioorg. Med. Chem.* **12**: 935–947.

Foloppe N, MacKerell AD. 2000. All-atom empirical force field for nucleic acids: I. Parameter optimization based on small molecule and condensed phase macromolecular target data. *J. Comput. Chem.* **21**: 86–104.

Foloppe N, Matassova N, Aboul-Ela F. 2006. Towards the discovery of drug-like RNA ligands? *Drug Disc. Today* **11**: 1019–1027.

Fourmy D, Yoshizawa S, Puglisi JD. 1998. Paromomycin binding induces a local conformational change in the A-site of 16 S rRNA. *J. Mol. Biol.* **277**: 333–345.

Franceschi F, Duffy E. 2006. Structure-based drug design meets the ribosome. *Special Issue on Antibacterials* **71**: 1016–1025.

Fulle S, Gohlke H. 2008. Analysing the flexibility of RNA structures by constraint counting. *Biophys. J.* **94**: 4202–4219.

Fulle S, Gohlke H. 2009a. Statics of the ribosomal exit tunnel: implications for co-translational peptide folding, elongation regulation, and antibiotics binding. *J. Mol. Biol.* **387**: 502–517.

Fulle S, Gohlke H. 2009b. Constraint counting on RNA structures: linking flexibility and function. *Methods* **49**: 181–188.

Gallego J, Varani G. 2001. Targeting RNA with small-molecule drugs: therapeutic promise and chemical challenges. *Acc. Chem. Res.* **34**: 836–843.

Garcia RG, Ferrer E, Macias MJ, Eritja R, Orozco M. 1999. Theoretical calculations, synthesis and base pairing properties of oligonucleotides containing 8-amino-2 ′-deoxyadenosine. *Nucleic Acids Res.* **27**: 1991–1998.

Getz M, Sun X, Casiano-Negroni A, Zhang Q, Al-Hashimi HM. 2007. NMR studies of RNA dynamics and structural plasticity using NMR residual dipolar couplings. *Biopolymers* **86**: 384–402.

Giudice E, Lavery R. 2002. Simulations of nucleic acids and their complexes. *Acc. Chem. Res.* **35**: 350–357.

Go N, Noguti T, Nishikawa T. 1983. Dynamics of a small globular protein in terms of low-frequency vibrational modes. *Proc. Natl Acad. Sci. U.S.A.* **80**: 3696–3700.

Gohlke H, Hendlich M, Klebe G. 2000. Knowledge-based scoring function to predict protein-ligand interactions. *J. Mol. Biol.* **295**: 337–356.

Gohlke H, Klebe G. 2002. Approaches to the description and prediction of the binding affinity of small-molecule ligands to macromolecular receptors. *Angew. Chem. Int. Ed.* **41**: 2645–2676.

Gohlke H, Kiel C, Case DA. 2003. Insights into protein-protein binding by binding free energy calculation and free energy decomposition for the Ras-Raf and Ras-RalGDS complexes. *J. Mol. Biol.* **330**: 891–913.

Gohlke H, Thorpe MF. 2006. A natural coarse graining for simulating large biomolecular motion. *Biophys. J.* **91**: 2115–2120.

Gouda H, Kuntz ID, Case DA, Kollman PA. 2003. Free energy calculations for theophylline binding to an RNA aptamer: MM-PBSA and comparison of thermodynamic integration methods. *Biopolymers* **68**: 16–34.

Guilbert C, James T. 2008. Docking to RNA via root-mean-square-deviation-driven energy minimization with flexible ligands and flexible targets. *J. Chem. Inf. Mod.* **48**: 1257–1268.

Haddad J, Kotra LP, Llano-Sotelo B, Kim C, Azucena EF, Liu M, Vakulenko SB, Chow CS, Mobashery S. 2002. Design of novel antibiotics that bind to the ribosomal acyltransfer site. *J. Am. Chem. Soc.* **124**: 3229–3237.

Hansen J, Ippolito J, Ban N, Nissen P, Moore P, Steitz T. 2002. The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol. Cell* **10**: 117–128.

Hashem Y, Auffinger P. 2009. A short guide for molecular dynamics simulations of RNA systems. *Methods* **47**: 187–197.

Hermann T. 2002. Rational ligand design for RNA: the role of static structure and conformational flexibility in target recognition. *Biochimie* **84**: 869–875.

Hermann T. 2003. Chemical and functional diversity of small molecule ligands for RNA. *Biopolymers* **70**: 4–18.

Hermann T, Westhof E. 1998. RNA as a drug target: chemical, modelling, and evolutionary tools. *Curr. Opin. Biotechnol.* **9**: 66–73.

Hermann T, Westhof E. 1999. Docking of cationic antibiotics to negatively charged pockets in RNA folds. *J. Med. Chem.* **42**: 1250–1261.

Hespenheide BM, Jacobs DJ, Thorpe MF. 2004. Structural rigidity in the capsid assembly of cowpea chlorotic mottle virus. *J. Phys.: Condens. Matter* **16**: 5055–5064.

Huang SY, Zou XQ. 2007. Ensemble docking of multiple protein structures: considering protein structural variations in molecular docking. *Proteins* **66**: 399–421.

Ippolito JA, Kanyo ZF, Wang D, Franceschi FJ, Moore PB, Steitz TA, Duffy EM. 2008. Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. *J. Med. Chem.* **51**: 3353–3356.

Ippolito JA, Steitz TA. 1998. A 1.3-A resolution crystal structure of the HIV-1 trans-activation response region RNA stem reveals a metal ion-dependent bulge conformation. *Proc. Natl Acad. Sci. U.S.A.* **95**: 9819–9824.

Jorgensen W, Chandrasekhar J, Madura J, Impey R, Klein M. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* **79**: 926–935.

Kang XS, Shafer RH, Kuntz ID. 2004. Calculation of ligand-nucleic acid binding free energies with the generalized-born model in DOCK. *Biopolymers* **73**: 192–204.

Kazemi S, Krüger DM, Sirockin F, Gohlke H. 2009. Elastic potential grids: accurate and efficient representation of intermolecular interactions for fully flexible docking. *ChemMedChem* **4**: 1264–1268.

Kolk MH, van der Graaf M, Wijmenga SS, Pleij CW, Heus HA, Hilbers CW. 1998. NMR structure of a classical pseudoknot: interplay of single- and double-stranded RNA. *Science* **280**: 434–438.

Kopitz H, Zivkovic A, Engels JW, Gohlke H. 2008. Determinants of the unexpected stability of RNA fluorobenzene self pairs. *ChemBioChem* **9**: 2619–2622.

Kormos BL, Benitex Y, Baranger AM, Beveridge DL. 2007. Affinity and specificity of protein U1A-RNA complex formation based on an additive component free energy model. *J. Mol. Biol.* **371**: 1405–1419.

Kurkcuoglu O, Kurkcuoglu Z, Doruker P, Jernigan RL. 2008. Collective dynamics of the ribosomal tunnel revealed by elastic network modeling. *Proteins* **75**: 837–845.

Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC, Thomas V, Rizzo RC, Case DA, James TL, Kuntz ID. 2009. DOC 6: combining techniques to model RNA-small molecul complexes. *RNA* **15**: 1219–1230.

Leclerc F, Cedergren R. 1998. Modeling RNA-ligand interactions: the Rev-binding element RNA-aminoglycoside complex. *J. Med. Chem.* **41**: 175–182.

Leclerc F, Karplus M. 1999. MCSS-based predictions of RNA binding sites. *Theor. Chem. Acc.* **101**: 131–137.

Leulliot N, Varani G. 2001. Current topics in RNA-protein recognition: control of specificity and biological function through induced fit and conformational capture. *Biochemistry* **40**: 7947–7956.

Li GH, Cui Q. 2002. A coarse-grained normal mode approach for macromolecules: an efficient implementation and application to Ca2+-ATPase. *Biophys. J.* **83**: 2457–2474.

Lind KE, Du Z, Fujinaga K, Peterlin BM, James TL. 2002. Structure-based computational database screening, *in vitro* assay, and NMR assessment of compounds that target TAR RNA. *Chem. Biol.* **9**: 185–193.

Lustig B, Bahar I, Jernigan RL. 1998. RNA bulge entropies in the unbound state correlate with peptide binding strengths for HIV-1 and BIV TAR RNA because of improved conformational access. *Nucleic Acids Res.* **26**: 5212–5217.

Ma C, Baker N, Joseph S, McCammon J. 2002. Binding of aminoglycoside antibiotics to the small ribosomal subunit: a continuum electrostatics investigation. *J. Am. Chem. Soc.* **124**: 1438–1442.

Mackerell AD, Feig M, Brooks CL. 2004. Extending the treatment of backbone energetics in protein force fields: limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. *J. Comput. Chem.* **25**: 1400–1415.

MacKerell AD, Nilsson L. 2008. Molecular dynamics simulations of nucleic acid-protein complexes. *Curr. Opin. Struct. Biol.* **18**: 194–199.

McDowell ES, Spacková Na, Sponer J, Walter N. 2007. Molecular dynamics simulations of RNA: an in silico single molecule approach. *Biopolymers* **85**: 169–184.

Meroueh SO, Mobashery S. 2007. Conformational transition in the aminoacyl t-RNA site of the bacterial ribosome both in the presence and absence of an aminoglycoside antibiotic. *Chem. Biol. Drug Des.* **69**: 291–297.
Micura R, Hobartner C. 2003. On secondary structure rearrangements and equilibria of small RNAs. *ChemBioChem* **4**: 984–990.
Misra VK, Draper DE. 2002. The linkage between magnesium binding and RNA folding. *J. Mol. Biol.* **317**: 507–521.
Moitessier N, Westhof E, Hanessian S. 2006. Docking of aminoglycosides to hydrated and flexible RNA. *J. Med. Chem.* **49**: 1023–1033.
Morley SD, Afshar M. 2004. Validation of an empirical RNA-ligand scoring function for fast flexible docking using RiboDock. *J. Comput. Aid. Mol. Des.* **18**: 189–208.
Mu Y, Stock G. 2006. Conformational dynamics of RNA-peptide binding: a molecular dynamics simulation study. *Biophys. J.* **90**: 391–399.
Murray J, Meroueh S, Russell R, Lentzen G, Haddad J, Mobashery S. 2006. Interactions of designer antibiotics and the bacterial ribosomal aminoacyl-tRNA site. *Chem. Biol.* **13**: 129–138.
Nabuurs SB, Wagener M, De Vlieg J. 2007. A flexible approach to induced fit docking. *J. Med. Chem.* **50**: 6507–6518.
Nifosi R, Reyes CM, Kollman PA. 2000. Molecular dynamics studies of the HIV-1 TAR and its complex with argininamide. *Nucleic Acids Res.* **28**: 4944–4955.
Ogle JM, Brodersen DE, Clemons WM, Tarry MJ, Carter AP, Ramakrishnan V. 2001. Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292**: 897–902.
Orozco M, Luque FJ., 2000. Theoretical methods for the description of the solvent effect in biomolecular systems. *Chem. Rev.* **100**: 4187–4225.
Orozco M, Noy A, Perez A. 2008. Recent advances in the study of nucleic acid flexibility by molecular dynamics. *Curr. Opin. Struct. Biol.* **18**: 185–193.
Orozco M, Perez A, Noy A, Luque FJ., 2003. Theoretical methods for the simulation of nucleic acids. *Chem. Soc. Rev.* **32**: 350–364.
Perez A, Luque FJ, Orozco M. 2007a. Dynamics of B-DNA on the microsecond time scale. *J. Am. Chem. Soc.* **129**: 14739–14745.
Perez A, Marchan I, Svozil D, Sponer J, Cheatham T, Laughton C, Orozco M. 2007b. Refinement of the AMBER force field for nucleic acids: improving the description of α/γ conformers. *Biophys. J.* **92**: 3817–3829.
Pfeffer P, Gohlke H. 2007. DrugScore^{RNA} - knowledge-based scoring function to predict RNA-ligand interactions. *J. Chem. Inf. Mod.* **47**: 1868–1876.
Ponder JW, Case DA. 2003. Force fields for protein simulations: *Adv. Protein Chem.* **66**: 27–85.
Puglisi JD, Tan R, Calnan BJ, Frankel AD, Williamson JR. 1992. Conformation of the TAR RNA-arginine complex by NMR spectroscopy. *Science* **257**: 76–80.
Pyetan E, Baram D, Auerbach-Nevo T, Yonath A. 2007. Chemical parameters influencing fine-tuning in the binding of macrolide antibiotics to the ribosomal tunnel. *Pure Appl. Chem.* **79**: 955–968.
Réblová K, Lankaš F, Rázga F, Krasovska MV, Koča J, Šponer J. 2006. Structure, dynamics, and elasticity of free 16S rRNA helix 44 studied by molecular dynamics simulations. *Biopolymers* **82**: 504–520.
Reyes CM, Kollman PA. 2000a. Structure and thermodynamics of RNA-protein binding: using molecular dynamics and free energy analyses to calculate the free energies of binding and conformational change. *J. Mol. Biol.* **297**: 1145–1158.
Reyes CM, Kollman PA. 2000b. Investigating the binding specificity of U1A-RNA by computational mutagenesis. *J. Mol. Biol.* **295**: 1–6.
Reyes CM, Nifosi R, Frankel AD, Kollman PA. 2001. Molecular dynamics and binding specificity analysis of the bovine immunodeficiency virus BIV Tat-TAR complex. *Biophys. J.* **80**: 2833–2842.
Rizzo RC, Aynechi T, Case DA, Kuntz ID. 2006. Estimation of absolute free energies of hydration using continuum methods: accuracy of partial, charge models and optimization of nonpolar contributions. *J. Chem. Theor. Comput.* **2**: 128–139.
Russell RJ, Murray JB, Lentzen G, Haddad J, Mobashery S. 2003. The complex of a designer antibiotic with a model aminoacyl site of the 30S ribosomal subunit revealed by X-ray crystallography. *J. Am. Chem. Soc.* **125**: 3410–3411.
Schluenzen F, Tocilj A, Zarivach R, Harms J, Gluehmann M, Janell D, Bashan A, Bartels H, Agmon I, Franceschi F, Yonath A. 2000. Structure of functionally activated small ribosomal subunit at 3.3 angstrom resolution. *Cell* **102**: 615–623.
Schluenzen F, Harms JM, Franceschi F, Hansen HA, Bartels H, Zarivach R, Yonath A. 2003. Structural basis for the antibiotic activity of ketolides and azalides. *Structure* **11**: 329–338.
Schluenzen F, Zarivach R, Harms J, Bashan A, Tocilj A, Albrecht R, Yonath A, Franceschi F. 2001. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* **413**: 814–821.
Schwalbe H, Buck J, Fürtig B, Noeske J, Wöhnert J. 2007. Structures of RNA switches: insight into molecular recognition and tertiary structure. *Angew. Chem. Int. Ed.* **46**: 1212–1219.
Shandrick S, Zhao Q, Han Q, Ayida BK, Takahashi M, Winters GC, Simonsen KB, Vourloumis D, Hermann T. 2004. Monitoring molecular recognition of the ribosomal decoding site. *Angew. Chem. Int. Ed.* **43**: 3177–3182.
Skripkin E, McConnell TS, Devito J, Lawrence L, Ippolito JA, Duffy EM, Sutcliffe J, Franceschi F. 2008. Rχ-01: a new family of oxazolidinones that overcomes ribosomal-based linezolid resistance. *Antimicrob. Agents Chemother.* **52**: 3550–3557.
Sousa SF, Fernandes PA, Ramos MJ. 2006. Protein-ligand docking: current status and future challenges. *Proteins* **65**: 15–26.
Spackova N, Cheatham TE, Ryjacek F, Lankas F, van Meervelt L, Hobza P, Sponer J. 2003. Molecular dynamics simulations and thermodynamics analysis of DNA-drug complexes. Minor groove binding between 4′ ;6-diamidino-2-phenylindole and DNA duplexes in solution. *J. Am. Chem. Soc.* **125**: 1759–1769.
Sponer J, Burda JV, Sabat M, Leszczynski J, Hobza P. 1998. Interaction between the guanine-cytosine Watson-Crick DNA base pair and hydrated group IIa (Mg2+, Ca2+, Sr2+, Ba2+) and group IIb (Zn2+, Cd2+, Hg2+) metal cations. *J. Phys. Chem. A* **102**: 5951–5957.
Sponer J, Jurecka P, Marchan I, Luque FJ, Orozco M, Hobza P. 2006. Nature of base stacking: reference quantum-chemical stacking energies in ten unique B-DNA base-pair steps. *Chemistry* **12**: 2854–2865.
Sponer JE, Spackova N, Leszczynski J, Sponer J. 2005. Principles of RNA base pairing: structures and energies of the trans Watson-Crick/sugar edge base pairs. *J. Phys. Chem. B* **109**: 11399–11410.
Srinivasan J, Cheatham TE, Cieplak P, Kollman PA, Case DA. 1998. Continuum solvent studies of the stability of DNA, RNA, and phosphoramidate - DNA helices. *J. Am. Chem. Soc.* **120**: 9401–9409.
Srinivasan J, Leclerc F, Xu W, Ellington AD, Cedergren R. 1996. A docking and modelling strategy for peptide-RNA complexes: applications to BIV Tat-TAR and HIV Rev-RBE. *Fold Des.* **1**: 463–472.
Still WC, Tempczyk A, Hawley RC, Hendrickson T. 1990. Semianalytical treatment of solvation for molecular mechanics and dynamics. *J. Am. Chem. Soc.* **112**: 6127–6129.
Suess B, Weigand JE. 2008. Engineered riboswitches: overview, problems and trends. *RNA Biology* **5**: 24–29.
Sutcliffe J. 2005. Improving on nature: antibiotics that target the ribosome. *Curr. Opin. Microbiol.* **8**: 534–542.
Tama F, Gadea FX, Marques O, Sanejouand YH. 2000. Building-block approach for determining low-frequency normal modes of macromolecules. *Proteins* **41**: 1–7.
Tama F, Sanejouand YH. 2001. Conformational change of proteins arising from normal mode calculations. *Protein Eng.* **14**: 1–6.
Tama F, Valle M, Frank J, Brooks CL. 2003. Dynamic reorganization of the functionally active ribosome explored by normal mode analysis and cryo-electron microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 9319–9323.
Tanida Y, Ito MS, Fujitani H. 2007. Calculation of absolute free energy of binding for theophylline and its analogs to RNA aptamer using nonequilibrium work values. *Chem. Phys.* **337**: 135–143.
Temiz NA, Bahar I. 2002. Inhibitor binding alters the directions of domain motions in HIV-1 reverse transcriptase. *Proteins* **49**: 61–70.
Thomas JR, Hergenrother PJ. 2008. Targeting RNA with small molecules. *Chem. Rev.* **108**: 1171–1224.
Tor Y. 2006. The ribosomal A-site as an inspiration for the design of RNA binders. *Biochimie* **88**: 1045–1051.
Tozzini V. 2005. Coarse-grained models for proteins. *Curr. Opin. Struct. Biol.* **15**: 144–150.
Trylska J, Tozzini V, McCammon JA. 2005. Exploring global motions and correlations in the ribosome. *Biophys. J.* **89**: 1455–1463.
Vaiana AC, Westhof E, Auffinger P. 2006. A molecular dynamics simulation study of an aminoglycoside/A-site RNA complex: conformational and hydration patterns. *Biochimie* **88**: 1061–1073.
Van Wynsberghe AW, Cui Q. 2005. Comparison of mode analyses at different resolutions applied to nucleic acid systems. *Biophys. J.* **89**: 2939–2949.

Velec HF, Gohlke H, Klebe G. 2005. DrugScore(CSD)-knowledge-based scoring function derived from small molecule crystal data with superior recognition rate of near-native ligand poses and better affinity prediction. *J. Med. Chem.* **48**: 6296–6303.
Vicens Q, Westhof E. 2003. RNA as a drug target: the case of aminoglycosides. *ChemBioChem* **4**: 1018–1023.
Wallace ST, Schroeder R. 2000. *In vitro* selection and characterization of RNAs with high affinity to antibiotics. *Methods Enzymol.* **318**: 214–229.
Wang Y, Rader AJ, Bahar I, Jernigan R. 2004. Global ribosome motions revealed with elastic network model. *J. Struct. Biol.* **147**: 302–314.
Warshel A, Kato M, Pisliakov AV. 2007. Polarizable force fields: history, test cases, and prospects. *J. Chem. Theor. Comput.* **3**: 2034–2045.
Warwicker J, Watson HC. 1982. Calculation of the electric-potential in the active-site cleft due to alpha-helix dipoles. *J. Mol. Biol.* **157**: 671–679.
Wells S, Menor S, Hespenheide B, Thorpe MF. 2005. Constrained geometric simulation of diffusive motion in proteins. *Phys. Biol.* **2**: 127–136.
Westhof E, Dumas P, Moras D. 1988. Restrained refinement of two crystalline forms of yeast aspartic acid and phenylalanine transfer RNA crystals. *Acta. Crystallogr. A* **44** (Pt 2): 112–123.
Wilson DN, Schluenzen F, Harms JM, Starosta AL, Connell SR, Fucini P. 2008. The oxazolidinone antibiotics perturb the ribosomal peptidyl-transferase center and effect tRNA positioning. *Proc. Natl Acad. Sci. U.S.A.* **105**: 13339–13344.
Yang G, Trylska J, Tor Y, McCammon JA. 2006a. Binding of aminoglycosidic antibiotics to the oligonucleotide A-site model and 30S ribosomal subunit: Poisson-Boltzmann model, thermal denaturation, and fluorescence studies. *J. Med. Chem.* **49**: 5478–5490.
Yang LW, Rader AJ, Liu X, Jursa CJ, Chen SC, Karimi HA, Bahar I. 2006b. oGNM: online computation of structural dynamics using the gaussian network model. *Nucleic Acids Res.* **34**: W24–W31.
Yoshizawa S, Fourmy D, Puglisi JD. 1998. Structural origins of gentamicin antibiotic action. *EMBO J.* **17**: 6437–6448.
Zacharias M. 2000. Comparison of molecular dynamics and harmonic mode calculations on RNA. *Biopolymers* **54**: 547–560.
Zacharias M. 2003. Perspectives of drug design that targets RNA. *Curr. Med. Chem.: Anti-Infect. Agents* **2**: 161–172.
Zacharias M, Sklenar H. 2000. Conformational deformability of RNA: a harmonic mode analysis. *Biophys. J.* **78**: 2528–2542.
Zavodszky MI, Ming L, Thorpe MF, Day AR, Kuhn LA. 2004. Modeling correlated main-chain motions in proteins for flexible molecular recognition. *Proteins* **57**: 243–261.
Zhang Q, Sun X, Watt ED. Al-Hashimi HM. 2006. Resolving the motional modes that code for RNA adaptation. *Science* **311**: 653–656.
Zhang Q, Stelzer A, Fisher C, Al-Hashimi H. 2007. Visualizing spatially correlated dynamics that directs RNA conformational transitions. *Nature* **450**: 1263–1267.
Zhao XY, Liu XF, Wang YY, Chen Z, Kang L, Zhang HL, Luo XM, Zhu WL, Chen KX, Li HL, Wang XC, Jiang HL. 2008. An improved PMF scoring function for universally predicting the interactions of a ligand with protein, DNA, and RNA. *J. Chem. Inf. Mod.* **48**: 1438–1447.
Zheng W. 2008. A unification of the elastic network model and the Gaussian network model for optimal description of protein conformational motions and fluctuations. *Biophys. J.* **94**: 3853–3857.
Zou XQ, Sun YX, Kuntz ID. 1999. Inclusion of solvation in ligand binding free energy calculations using the generalized-born model. *J. Am. Chem. Soc.* **121**: 8033–8043.