RNA as a drug target: chemical, modelling, and evolutionary tools

Thomas Hermann and Eric Westhof

Dramatic technical progress in RNA synthesis and structure determination has allowed several difficulties inherent to the preparation, handling and structural analysis of RNA to be overcome, and this has led to a wealth of information about RNA structure and its relationship with biological function. It is now fully recognized that RNA molecules intervene at all stages of cell life, not only because of key sequence motifs but also because of intricate three-dimensional folds. This realization has promoted RNA to a potential therapeutic target. As in protein motifs recognizing nucleic acids, groups of the molecule interacting with RNA contribute to specific binding through defined hydrogen bonds and van der Waals docking, while other parts contribute to the driving force of binding via less specific electrostatic interactions accompanied by water and ion displacement.

Addresses
Institut de Biologie Moléculaire et Cellulaire du CNRS, UPR 9002, 15 rue René Descartes, F-67084 Strasbourg, France
Correspondence: Eric Westhof; e-mail: westhof@ibmc.u-straibg.fr

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Abbreviations

HDV	hepatitis delta virus
RRE	Rev-response element
TAR	*trans*-activating region

Introduction

Because of its key and versatile roles in biological processes, such as protein synthesis, mRNA splicing, transcriptional regulation and retroviral replication, RNA is a prime target for natural compounds as well as for chemically designed drugs. Indeed, the binding of molecules to a specific RNA target might influence the biological activity of the RNA by preventing the binding of the biologically relevant macromolecule (either protein or RNA), by inhibiting RNA catalysis, or by forcing an alternative conformation on the RNA. The three-dimensional folding of RNA gives rise to complex structures allowing the highly specific binding of effector molecules. As with proteins, the structural features of the potential RNA targets that determine the selective interaction with drugs depend on the source organism. Compared to DNA, RNA displays a greater structural diversity and lacks repair mechanisms, a fact that enhances the impact of therapeutics directed at RNA.

Recent advances in RNA synthesis, *in vitro* evolution (systematic evolution of ligands by exponential enrichment [SELEX]) [1] and high-resolution NMR structure determination, together with improved methods for the combinatorial synthesis of therapeutic agents [2,3•,4,5•] have opened the road for drug discovery in the field of RNA-targeted effectors [6,7•,8]. Here, we will review the recent progress made in the study of the interaction of small molecules with functional RNA by means of chemical, modelling and evolutionary tools. More extensive reviews, especially on antibiotic and metal complex binding, were recently written by Wallis and Schroeder [9] and by Chow and Bogdan [10]. Among the numerous artificial RNA molecules (aptamers) that have been selected by *in vitro* evolution for specific binding to a target molecule, we will focus on RNA aptamers that specifically bind antibiotics. The recent work on all kinds of aptamers has been summarized in several excellent reviews [11–13]. Due to its therapeutical importance, we will discuss separately in the last section recent efforts in targeting RNA components of the replication process of HIV.

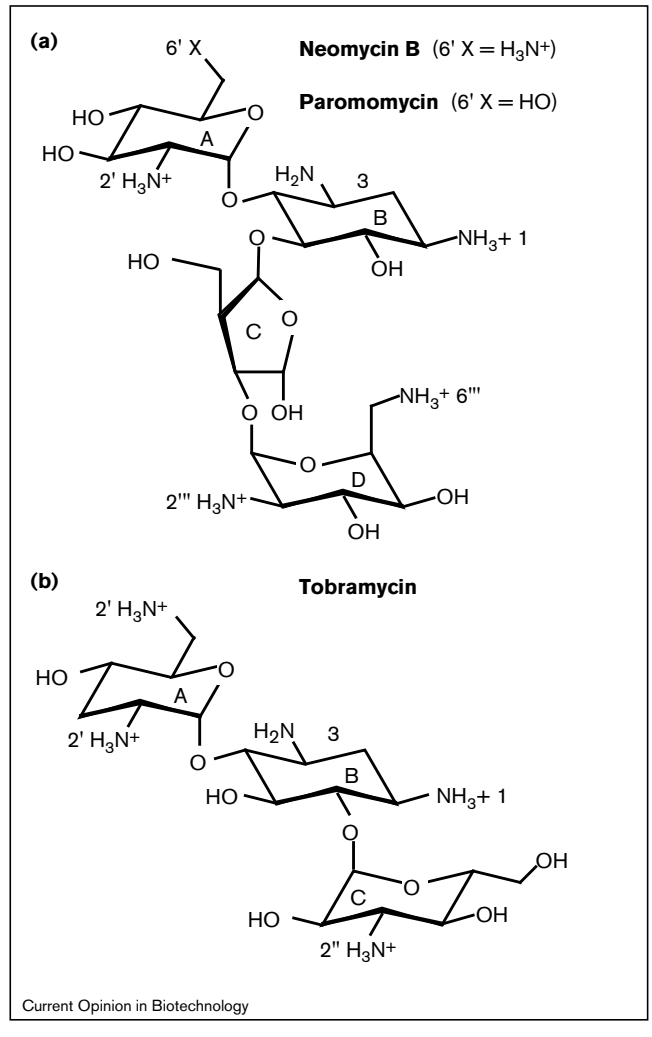
Targeting ribosomal RNA

The 16S RNA component of the prokaryotic ribosome was the first RNA to be identified as a target for small molecule effectors [14]. Binding of a variety of different antibiotics, such as aminoglycosides (Figure 1), to functional sites of the bacterial ribosomal RNA leads to miscoding during the translation process. Mutational and chemical probing experiments [15•,16], along with fluorescence measurements [17], on the interaction of aminoglycosides with model oligonucleotides that mimic the antibiotic target in the A site of the 16S rRNA decoding region (an asymmetric internal loop of three and four unpaired bases) have revealed structural features important for the specific interaction of aminoglycosides with RNA. The structure of a 27 nucleotide A-site model RNA complexed with the aminoglycoside paromomycin (Figure 1) has been determined by NMR spectroscopy [18••], showing that the antibiotic binds to the loop region in the deep groove of the RNA within a pocket opened by a single bulged adenine and an A–A base pair. Amino and hydroxyl substituents of rings A and B, conserved among aminoglycosides that bind to the A site, are required for specific interactions of paromomycin with the ribosomal RNA, while rings C and D, relatively disordered in the NMR structure [18••], contribute unspecifically to the complex formation by additional electrostatic and van der Waals interactions. The principle of providing a moiety that conveys binding specificity by recognizing internal bulges or asymmetric loops and another moiety that supports binding strength by non-specific interactions is frequently exploited by RNA-binding molecules, as we

will see later for other examples of RNA–drug interactions. The specificity of aminoglycoside binding to the 16S rRNA is highlighted by the fact that aminoglycoside antibiotics act preferentially on prokaryotic organisms due to a much higher affinity of prokaryotic 16S rRNA for these antibiotics compared to eukaryotic ribosomal RNA. The NMR analysis of the A-site RNA–paromomycin complex [18••] has revealed the structural basis for the difference: firstly, a disruption of the A–A base pair in eukaryotes, which have a G in place of one of the adenines; and, secondly, a mispairing at positions 1409–1491, which provide the floor of the antibiotic binding pocket in the prokaryotic A site. In eukaryotes, a G–A base pair equivalent to the A–A pair in prokaryotes cannot be formed due to geometrical reasons and, thus, the RNA deep groove is not opened, preventing aminoglycoside binding to the eukaryotic A site [18••]. Finally, the NMR structure of the A site RNA–paromomycin complex has uncovered the molecular basis of resistance development against specific aminoglycosides by methylation of nucleotides within the 16S rRNA that border the aminoglycoside binding pocket [18••].

It has been speculated that aminoglycosides, exerting their antibiotic effects by inducing miscoding during the translation process, may stabilize a high affinity conformation of rRNA for the aminoacyl-tRNA for which proofreading is impeded [18••]. Likewise, it has been suggested that binding of the pyrrolidine antibiotic anisomycin to 28S rRNA induces a conformational change in ribosomal RNA [19•], a system where the action of the antibiotic on the ribosomal RNA activates a stress-activated protein kinase and, thus, giving a response similar to that induced by the enzyme ribotoxins α-sarcin and ricin. The example of anisomycin nicely illustrates how a low-molecular-weight effector targeted at a specific binding site in RNA is able to trigger a complex process such as the activation of a protein kinase.

Figure 1



Structures of some aminoglycoside antibiotics discussed in the text. **(a)** Neomycin B and paromomycin, which belong to the 4,5-disubstituted deoxystreptamine class. **(b)** Tobramycin, which belongs to the 4,6-disubstituted deoxystreptamines.

Targeting catalytic RNAs

As well as being among compounds that bind to the 16S rRNA, the aminoglycoside antibiotics also act as inhibitors of catalytic RNAs (reviewed in [20]), such as the group I intron [21], the hammerhead ribozyme [22], and the ribozymes from hepatitis delta virus (HDV) [23]. Group I introns are attractive targets for therapeutics because they are interspersed in key genes of several pathogenic microorganisms, while they are not found in the human genome [21,24,25]. Self-splicing of group I intron RNA is inhibited by aminoglycosides non-competitively with respect to the binding of the guanosine cofactor, indicating that these antibiotics do not interfere with the G-binding site [26]. In contrast, the macrocyclic peptide antibiotics of the tuberactinomycin family, for example, viomycin, compete with the guanosine cofactor for the G-binding site within helix P7, part of a pseudoknot which is located in the conserved core of the intron [27]. Docking of both aminoglycosides and tuberactinomycins to a 3D model of group I introns [28] has been performed to construct model complexes useful in rationalizing the differences in inhibition behaviour between these antibiotics [27,29]. Another class of compounds that affect the self-splicing of group I introns is L-arginine and its derivatives, which competitively inhibit the cleavage step but stimulate the following ligation reaction [25]. Recently, a ribozyme system has been developed from group I introns and was used to rapidly identify small molecule inhibitors targeting catalytic introns in high-throughput screening assays [24,30•].

The influence of antibiotics on another large catalytic RNA, M1 RNA, the RNA component of RNase P, is not well documented. In contrast to group I introns and small ribozymes, M1 RNA is not inhibited by aminoglycoside antibiotics but only by puromycin, a nucleoside derivative [31]. The fact that M1 RNA is

catalytically active *in vitro* only under high magnesium concentration certainly contributes to the absence of inhibition by known cationic inhibitors of catalytic RNAs. Similarly, there is no report of compounds inhibiting the hairpin ribozyme.

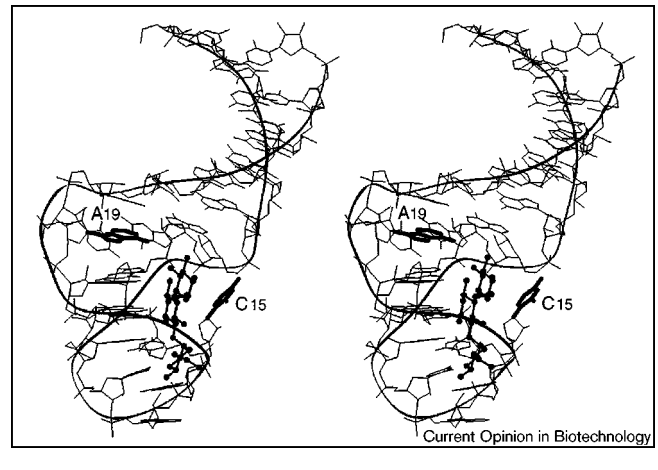
Of the small ribozymes that are inhibited by aminoglycosides, the catalytic RNAs from HDV, which are crucial in viral replication, might be a target for drugs due to the role of HDV as a co-infecting agent in patients suffering from hepatitis B [23]. The hammerhead ribozyme is an important model system for the study of the interaction of inhibitors with functional RNA, since the 3D structure of this ribozyme is available from crystal structure analysis [32,33]. The inhibition of both HDV and hammerhead ribozymes by aminoglycosides is a process governed by electrostatic competition of the cationic aminoglycosides with magnesium ions required for catalysis [23,34,35•,36]. Molecular dynamic simulations of aminoglycosides, the hammerhead RNA, and modelled complexes thereof, revealed a surprising structurally-based complementarity between the charged ammonium groups of the aminoglycosides and the magnesium ion binding sites in the hammerhead [37•]. In a variety of modelled aminoglycoside–RNA complexes, specific contacts between metal binding sites of the RNA and ammonium groups of rings A and B of the aminoglycosides are accompanied by varying additional, unspecific interactions which may contribute to the binding affinity, as in the case of aminoglycoside binding to the A site of 16S rRNA. It was suggested [37•] that the covalently linked ammonium groups of the aminoglycosides are able to complement in space the negative electrostatic potential created by a three-dimensional RNA fold, normally occupied by magnesium ions. Aminoglycoside-derived sugars could, thus, constitute a set of yardstick synthons for rational and combinatorial synthesis of drugs targeted at biologically relevant RNA folds.

Aptamers against antibiotics

While aptamers, obtained by *in vitro* selection from random sequence RNA pools, play no role as naturally occurring targets for drugs, study of their complexes with small molecules provides insight into the details of molecular recognition at the RNA level. Antibiotics that have been used in SELEX experiments to raise highly specific RNA aptamers include the aminoglycosides tobramycin (Figure 1) [38], kanamycin A, lividomycin [39] and neomycin B (Figure 1) [40,41], and the peptide antibiotic viomycin [42•]. All of the aminoglycoside aptamers characterized so far bind the drug in a hairpin structure, the stem region of which contains from one to several G–U wobble base pairs [39–41,43]. In contrast, the viomycin-binding aptamer forms a pseudoknot structure [42•], recurring in natural viomycin target sites such as 16S rRNA, the guanosine binding site in the P7 stem of group I intron (see above) and HDV ribozyme [23]. Few dominating high-affinity (nM) aptamer sequences were obtained when high-stringency conditions were imposed during the *in vitro* selection [38,40,41,42•], while a wide variety of low-affinity (mM) aptamers were isolated with less stringent selection procedures [38,39]. In contrast to the natural targets of aminoglycosides, such as 16S rRNA or the ribozymes, the binding of the antibiotics to aptamer RNAs is almost independent of the concentration of magnesium ions [40,41].

Recently, the solution structure of a tobramycin aptamer RNA [38] in complex with the drug has been analysed by NMR spectroscopy and molecular dynamic simulations [44••]. The 27 nucleotide RNA aptamer comprises a stem with a bulged adenosine adjacent to a six-nucleotide loop. Tobramycin (Figure 1) binds to a pocket formed by the deep groove of the aptamer, which is opened by the bulged A and a looped-out C residue (Figure 2). The antibiotic is almost encapsulated within the RNA fold and only the part of the aminoglycoside used for covalently linking the drug to the affinity column during the selection is accessible to solvent. Similarities between the hairpin loop of the tobramycin aptamer and a conserved hexanucleotide loop in ribosomal RNA have led to speculations that the binding site for tobramycin in the aptamer may represent a recurring scaffold for aminoglycoside binding sites in natural RNAs [44••].

Figure 2



Stereoview of the three-dimensional structure of an RNA aptamer complexed with the aminoglycoside antibiotic tobramycin, which has been determined by Jiang and co-workers using NMR and molecular dynamics techniques [44••]. The bulged A and the looped-out C bases that open the deep groove of the RNA forming the aminoglycoside binding pocket are enhanced. The antibiotic is shown in ball-and-stick representation. The drawing was made with INSIGHTII (Molecular Simulations Inc.) using coordinates from the Brookhaven Protein Data Bank file 1TOB.

Two lessons can be learned from the study of aminoglycoside aptamers. Firstly, many different RNA sequences exist that bind aminoglycoside antibiotics with

an affinity in the mM range, suggesting that natural low-molecular-weight effectors such as aminoglycosides, may have evolved to exploit molecular recognition of various RNA targets. Secondly, the natural targets of aminoglycosides, such as the ribosomal 16S RNA or the HIV RNA Rev responsive element (see below), generally have a lower affinity for the drugs than *in vitro*-selected aptamers; indicating that functional aspects other than binding strength, such as the ability to induce a conformational change in the RNA, play a role in the antibiotics potential as an effector compound. This observation and the lack of direct competition between aminoglycosides and magnesium ions for RNA binding in aptamers indicate that the mechanisms of molecular recognition might be different between artificially *in vitro* selected and known natural aminoglycoside targets. Therefore, drug design approaches, aiming at optimising aminoglycoside compounds for high-affinity binding at natural RNA targets and based on new combinations of aminoglycoside-derived substituted saccharide building blocks might be promising [37•].

Miscellaneous compounds targeting RNA

Besides aminoglycoside and peptide antibiotics, a variety of other small organic compounds known to bind nucleic acids have been systematically investigated to study their interaction with RNA [6,45,46]. The ethidium cation, used for a long time in analytical detection of nucleic acids, intercalates into double-stranded regions of RNA [6,45] with a preference for sites adjacent to bulged nucleotides [47]. Intercalation of ethidium into double-stranded RNA [47] and of phenanthroline complexes into stacked single-stranded RNA regions [48] was studied by molecular modeling approaches in order to rationalize the experimentally observed binding preferences. Of the compounds known to target DNA, the intercalating molecules, like fused aromatic systems, bind with similar affinity to RNA, while DNA groove-binding drugs do not generally bind to RNA or they may bind in a different way, an observation obviously due to the very different shapes and electrostatic potentials of the grooves in A form RNA and B form DNA duplexes [45,49]. For example, enediyne antibiotics, such as neocarzinostatin used in anticancer therapy, are known to target the minor groove of duplex DNA where they lead to oxidative cleavage of the nucleic acid, while binding of these drugs to RNA is bulge-specific [50–52]. There is even an example where a molecule that binds tightly to the major groove of duplex DNA, the cationic cyclophane CP66, locally melts RNA double-stranded helices, forcing a single adenine to flip-out from the duplex in order to become available for binding to the cavity inside the cycylophane [53,54]. Following on these findings, it has been speculated that cyclophanes could be used to design molecules that can cause base-flipping in double-stranded RNA as observed with DNA repair enzymes [53].

While both the shallow and the deep grooves of double-stranded RNA may serve as good drug-binding sites, no small molecules have yet been identified that have selective interactions for either of the RNA grooves, with the exception of a rhodium complex [55] and isoalloxazine derivatives that selectively target G–U wobble base pairs embedded within helices [56•,57]. Isoalloxazines, such as flavin mononucleotide, can cleave RNA molecules specifically at G–U base pairs via a photoinduced mechanism. Recently, a structural model has been derived [57] from experimental data which explains the recognition of G–U base pairs by showing that isoalloxazines exploit the shallow groove asymmetry displayed by those wobble base pairs.

As well as drugs that specifically bind to RNA, molecules have been investigated that can specifically cleave RNA, thus, mimicking naturally occurring ribonucleases. A wide variety of compounds is capable of cleaving RNA, ranging from complex molecules like bleomycin [58,59] to relatively simple structures like m-hydroxo-bridged dimers of La(III) ions [60] and we will not review them here but refer to the literature ([60–62] and references cited therein).

Targeting RNA components of HIV replication

HIV replication depends on the function of two regulatory proteins, Tat and Rev, stimulating transcription (Tat) and facilitating the nuclear export of unspliced and partially spliced viral mRNA (Rev). Both regulators act by specific targeting of internal loop structures on viral mRNA, namely Tat binding to *trans*-activating region (TAR) and Rev recognizing the Rev-response element (RRE) [63]. Selection procedures have been used on both RNA [64] and peptide [65] components of Tat–TAR and Rev–RRE in order to study features of the specific protein–RNA interaction in these complexes.

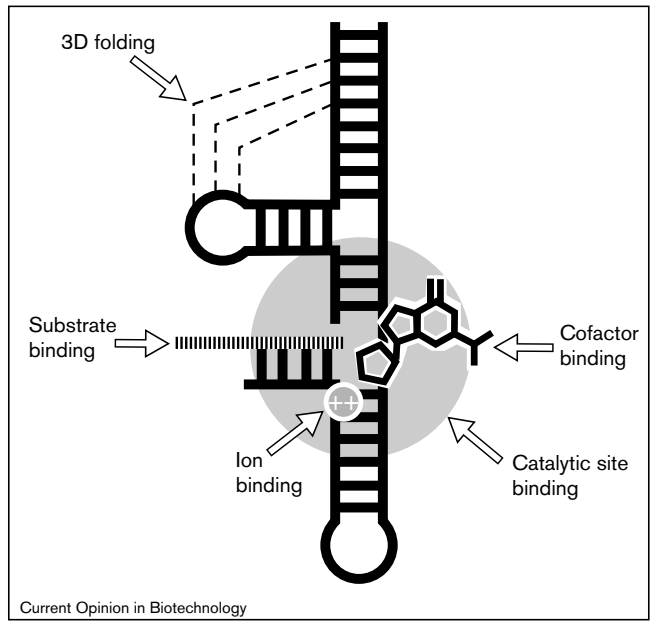
Both systems, Tat–TAR and Rev–RRE, are potential targets for anti-HIV therapeutics given their critical role in the HIV infection process and the fact that a large body of 3D structural information is now available from NMR studies on model complexes of HIV-1 Rev peptide–RRE RNA [66••,67], HIV-1 Rev peptide–RNA aptamer [68•], HIV-1 Tat peptide–TAR RNA [69], HIV-2 TAR RNA–argininamide [70•] and the HIV-related bovine immunodeficiency virus Tat peptide–TAR RNA [71,72]. In both Rev–RRE and Tat–TAR complexes, the protein binds to the RNA in the deep groove, which is widened either by two purine–purine base pairs and a bulged residue, in the cases of Rev–RRE and Rev–aptamer, or by a base triplet and a bulged residue, in the case of Tat–TAR. In both protein–RNA complexes, a conformational change in the RNA is observed such that the RNA structure becomes more ordered upon peptide binding. Aboul-Ela *et al.* [69] suggest that small molecules may be able to lock the flexible RNA structure into

a conformation that does not allow for binding of the cognate regulatory protein and, thus, inhibit viral growth. Similarly, results form circular dichroism and fluorescence measurements [73] suggest that two classes of inhibitors of the Rev–RRE interaction, namely aminoglycosides and diphenylfuranes, cause a significant conformational change in RRE upon binding to the RNA.

The specific interaction of a single arginine residue of the Tat peptide with the deep groove edge of guanine residues, observed in the Tat–TAR complex [72], is also responsible for high-affinity binding of the low-molecular-weight compound argininamide to TAR [69,70•]. Binding of argininamide stabilises the TAR RNA in a conformation from that of free TAR [69,70•] .Other small molecules that were investigated for their ability to bind to TAR RNA include classical intercalators such as ethidium [47,49] and a shape-selective rhodium–phenanthroline complex which specifically targets the widened deep groove of TAR where it competes for Tat peptide binding [74]. Combinatorial chemistry employing sequential optimization of residues has been used to select a hybrid peptoid/peptide oligomer of nine residues that is able to inhibit the Tat–TAR RNA complex formation *in vitro* at nanomolar concentrations [5•]. Recently, the Tat–TAR system has been used, similar to group I intron (see above), to conduct a mass screening for the discovery of HIV therapeutics that interfere with the binding of Tat protein to TAR RNA [75]. Aminoglycoside antibiotics inhibit the binding of Tat-derived peptides to TAR RNA, with neomycin B (Figure 1) being the most active compound [76], and block the binding of the Rev protein to its target site on the RRE RNA [77]. Investigations of *in vitro*-selected variants of RRE that bind to Rev protein in the presence of aminoglycosides have uncovered a non-canonical G–G base pair critical for the specific binding of both Rev protein and the drugs [78]. This purine–purine pair has been demonstrated to be required for the widening of the deep groove in RRE RNA which creates part of the Rev binding pocket [66••]. The interaction of aminoglycosides with the RRE RNA has been studied by a variety of methods, such as fluorescence measurements [17] and surface plasmon resonance (BIAcore) [79•]. Surface plasmon resonance is especially useful for the rapid screening of large libraries of RNA-binding compounds obtained by combinatorial chemistry. Again, aminoglycosides have been chosen to set up a combinatorial approach for the synthesis of novel inhibitors of the Rev–RRE interaction. Neamine, the core moiety of rings A and B (Figure 1) that is shared in common by all aminoglycosides discovered so far which act on functional RNAs, has been used as a basic building block in the synthesis of peptido aminoglycoside mimetics by combinatorial chemistry [3•].

Other compounds targeted at inhibiting the Rev–RRE interaction are polycationic RNA-binding agents [6,54], among them derivates of the diphenylfuran cation have been intensively studied [6,73,80]. Diphenylfuranes induce a conformational change in the RRE RNA after binding to the internal loop of the RNA by a threading intercalation mode [73].

Figure 3



RNA as a drug target. Small molecule effectors targeted at RNA can interfere with RNA biological activity via different mechanisms. The RNA 3D folding may be disrupted or locked in an altered conformation, a situation occurring in the HIV-1 RRE system, the conformation of which is changed after binding of aminoglycosides or diphenylfurans that hinder the binding of the Rev protein [69,73]. Binding of other molecules required for the biological action of the RNA, such as substrates, proteins or cofactors, may be impeded by drugs as it was shown for the tuberactinomycins (viomycin) which compete with the guanosine cofactor for the G-binding site in group I introns [27]. The catalytic power of ribozymes may be altered by small molecules inhibiting catalytic activity. It has been recently suggested [37•] that aminoglycoside antibiotics inhibit the hammerhead ribozyme by displacing the catalytically active magnesium ions.

Conclusions

Inhibition of RNA biological activity can occur in various ways (Figure 3), by preventing binding of another macromolecule (protein or RNA), by distorting the RNA active conformation, by competitive binding for a cofactor binding site, or by displacing catalytically important ions, such as magnesium ions. The interaction of effector molecules with RNA displays specific characteristics for the two principal 'surfaces' of RNA, the deep and the shallow groove.

The deep groove of RNA (equivalent to the major groove of DNA) is lined by negatively charged phosphate groups, while the shallow groove (equivalent to the minor groove of DNA) forms a large and slightly concave surface of rather hydrophobic character. Binding on the deep groove

side requires distortion and widening of the regular RNA helix deep groove [81]. This is achieved with great versatility by non-Watson-Crick base pairs and bulging bases. Such three-dimensional pockets present, therefore, a vast specificity potential. On the other hand, binding on the shallow groove side would require large and flat aromatic ring systems with substituents for hydrogen bonding to hydroxyl and phosphate groups. The example of the specific binding of rhodium complexes [55] and isoalloxazines [57] to G–U pairs from the shallow groove side indicates that there might be additional possibilities for specific drug binding to the shallow groove, in particular, especially non-Watson-Crick base pairs.

Given the wealth of structural information currently emerging in the RNA field [82], structure-based drug design [37•,83] will become an important tool in the search for effectors targeted at RNA. Dramatic improvements in theoretical methods for modelling and simulation of nucleic acid systems ([84,85] and reviewed in [86]) will pave the way for computer-aided rational drug design, now routinely used for protein targets.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of oustanding interest

1. Ellington AD: **Using *in vitro* nucleic acid selections for conventional drug design.** *Drug Dev Res* 1994, **33**:102-115.

2. Ellman J, Stoddard B, Wells J: **Combinatorial thinking in chemistry and biology.** *Proc Natl Acad Sci USA* 1997, **94**:2779-2782.

3. • Park WKC, Auer M, Jaksche H, Wong CH: **Rapid combinatorial synthesis of aminoglycoside antibiotic mimetics: use of a polyethylene glycol-linked amine and a neamine-derived aldehyde in multiple component condensation as a strategy for the discovery of new inhibitors of the HIV RNA Rev responsive element.** *J Am Chem Soc* 1996, **118**:10150-10155.
In this straightforward study, combinatorial chemistry was used to synthesize a number of aminoglycoside mimetics based on neamine, the central building block conserved among aminoglycoside antibiotics which are active against functional RNAs. The activity of the newly synthesized compounds was tested for their capacity to inhibit the binding of the HIV regulatory Rev protein to its target RNA.

4. Tong G, Nielsen J: **A convergent solid-phase synthesis of actinomycin analogues – towards implementation of double-combinatorial chemistry.** *Bioorg Med Chem* 1996, **4**:693-698.

5. • Hamy F, Felder ER, Heizmann G, Lazdins J, Aboul-Ela F, Varani G, Karn J, Klimkait T: **An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication.** *Proc Natl Acad Sci USA* 1997, **94**:3548-3553.
This study describes comprehensively the search for a Tat–TAR inhibitor by outlining several stages of the investigation, such as the drug synthesis by combinatorial chemistry, NMR-based molecular modelling studies on the drug–RNA complex and, finally, *in vivo* inhibition assays. Starting from pools of a partially randomized peptoid/peptide, consecutive rounds of binding selection were used to identify step by step the residues that result in a molecule which binds to TAR RNA and inhibits Tat binding *in vitro* at nanomolar concentrations.

6. Wilson WD, Ratmeyer L, Zhao M, Ding D, McConnaughie AW, Kumar A, Boykin DW: **Design and analysis of RNA structure-specific agents as potential antivirals.** *J Mol Recogn* 1996, **9**:187-196.

7. • Hecht SM: **Strategies for RNA recognition.** *Bioorg Med Chem* 1997, **5**:1001-1248.
This timely symposium-in-print contains 25 articles especially devoted to RNA recognition by low-molecular weight ligands giving a bird's eye view of the diverse activities in present day research.

8. Pearson ND, Prescott CD: **RNA as a drug target.** *Chem Biol* 1997, **4**:409-414.

9. Wallis MG, Schroeder R: **The binding of antibiotics to RNA.** *Prog Biophys Molec Biol* 1998, in press.

10. Chow CS, Bogdan FM: **A structural basis for RNA–ligand interactions.** *Chem Rev* 1997, **97**:1489-1513.

11. Uphoff KW, Bell SD, Ellington AD: ***In vitro* selection of aptamers: the dearth of pure reason.** *Curr Opin Struct Biol* 1996, **6**:281-288.

12. Feigon J, Dieckmann T, Smith FW: **Aptamer structures from A to Z.** *Chem Biol* 1996, **3**:611-617.

13. Patel DJ, Suri AK, Jiang F, Jiang L, Fan P, Kumar RA, Nonin S: **Structure, recognition and adaptive binding in RNA aptamer complexes.** *J Mol Biol* 1997, **272**:645-664.

14. Moazed S, Noller HF: **Interaction of antibiotics with functional sites in 16S ribosomal RNA.** *Nature* 1987, **327**:389-394.

15. • Recht MI, Fourmy D, Blanchard SC, Dahlquist KD, Puglisi JD: **RNA sequence determinants for aminoglycoside binding to an A-site rRNA model oligonucleotide.** *J Mol Biol* 1996, **262**:421-436.
A thorough biochemical analysis using mutational and chemical probing techniques to study the interaction of aminoglycosides with RNA oligos which are minimal models of the decoding region of 16S rRNA. Sequence differences between prokaryotic and eukaryotic 16S rRNA are uncovered that are responsible for the higher sensitivity of prokaryotes against low antibiotic concentrations.

16. Miyaguchi H, Narita H, Sakamoto K, Yokoyama S: **An antibiotic-binding motif of an RNA fragment derived from the A-site-related region of *Escherichia coli* 16S rRNA.** *Nucleic Acids Res* 1996, **24**:3700-3706.

17. Wang Y, Hamasaki K, Rando RR: **Specificity of aminoglycoside binding to RNA constructs derived from the 16S rRNA decoding region and the HIV-RRE activator region.** *Biochemistry* 1997, **36**:768-779.

18. •• Fourmy D, Recht MI, Blanchard SC, Puglisi JD: **Structure of the A site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic.** *Science* 1996, **274**:1367-1371.
This paper describes the 3D solution structure, determined by NMR, of a complex between a model RNA derived from the A site of 16S rRNA and the aminoglycoside antibiotic paromomycin, the only complex between a non-aptamer RNA and a small effector molecule for which detailed structural information is available to date. The 3D model of the complex uncovers the structural basis for, firstly, the difference in sensitivity against low antibiotic concentrations between prokaryotic and eukaryotic 16S rRNA, and secondly, mechanisms of antibiotic resistance by site-specific methylation of the 16S rRNA in bacteria.

19. • Iordanov MS, Pribnow D, Magun JL, Dinh TH, Pearson JA, Chen SLY, Magun BE: **Ribotoxic stress response: activation of the stress-activated protein kinase JNK1 by inhibitors of the peptidyl transferase reaction and by sequence-specific RNA damage to the α-sarcin/ricin loop in the 28S rRNA.** *Mol Cell Biol* 1997, **17**:3373-3381.
An original analysis of an unprecedented effect of the antibiotic anisomycin which induces the activation of a protein kinase by specific binding to eukaryotic 28S rRNA at concentrations where the protein biosynthesis is not impeded. The authors demonstrate that the kinase-activating signal induced by anisomycin-binding to the RNA component of active ribosomes is comparable to the effect of ribotoxic enzymes that chemically modify the 28S rRNA despite the fact that the binding site for the antibiotic is different from the recognition site of these enzymes.

20. Schroeder R, von Ahsen U: **Interaction of aminoglycoside antibiotics with RNA.** *Nucleic Acids Mol Biol* 1996, **10**:53-74.

21. von Ahsen U, Davies J, Schroeder R: **Antibiotic inhibition of group I ribozyme function.** *Nature* 1991, **353**:368-370.

22. Stage TK, Hertel KJ, Uhlenbeck OC: **Inhibition of the hammerhead ribozyme by neomycin.** *RNA* 1995, **1**:95-101.

23. Rogers J, Chang AH, von Ahsen U, Schroeder R, Davies J: **Inhibition of the self-cleavage reaction of the human hepatitis**

delta virus ribozyme by antibiotics. *J Mol Biol* 1996, **259**:916-925.

24. Mei HY, Cui M, Sutton ST, Truong HN, Chung FZ, Czarnik AW: **Inhibition of self-splicing group I intron RNA: high-throughput screening assays.** *Nucleic Acids Res* 1996, **24**:5051-5053.

25. Liu Y, Leibowitz MJ: **Bidirectional effectors of a group I intron ribozyme.** *Nucleic Acids Res* 1995, **23**:1284-1291.

26. von Ahsen, Davies J, Schroeder R: **Non-competitive inhibition of group I intron RNA self-splicing by aminoglycoside antibiotics.** *J Mol Biol* 1992, **226**:935-941.

27. Wank H, Rogers J, Davies J, Schroeder R: **Peptide antibiotics of the tuberactinomycin family as inhibitors of group I intron splicing.** *J Mol Biol* 1994, **236**:1001-1010.

28. Michel F, Westhof E: **Modelling of the three-dimensional architecture of group I introns based on comparative sequence analysis.** *J Mol Biol* 1990, **216**:585-610.

29. Wank H, Schroeder R: **Antibiotics that interfere with RNA/RNA interactions.** In *Ribosomal RNA and Group I Introns.* Edited by Green R, Schroeder R. New York: Chapman and Hall; 1996:129-151.

30. • Mei HY, Cui M, Lemrow SM, Czarnik AW: **Discovery of selective, small-molecule inhibitors of RNA complexes. 2. Self-splicing group I intro ribozyme.** *Bioorg Med Chem* 1997, **5**:1185-1195.
Structurally diverse compounds inhibiting self-splicing group I introns that could not have been rationally predicted were isolated by the authors using a novel high-throughput screening approach (see Mei *et al.* 1996 [24]).

31. Vioque A: **Protein synthesis inhibitors and catalytic RNA: effect of puromycin on tRNA precursor processing by the RNA component of *Escherichia coli* RNase P.** *FEBS Lett* 1989, **246**:137-139.

32. Pley HW, Flaherty KM, McKay DB: **Three-dimensional structure of a hammerhead ribozyme.** *Nature* 1994, **372**:68-74.

33. Scott WG, Finch JT, Klug A: **The crystal structure of an all-RNA hammerhead ribozyme: a proposed mechanism for RNA catalytic cleavage.** *Cell* 1995, **81**:991-1002.

34. Clouet-d'Orval B, Stage TK, Uhlenbeck OC: **Neomycin inhibition of the hammerhead ribozyme involves ionic interactions.** *Biochemistry* 1995, **34**:11186-11190.

35. • Wang H, Tor Y: **Electrostatic interactions in RNA-amino -glycosides binding.** *J Am Chem Soc* 1997, **119**:8734-8735.
A first systematic analysis of the influence of substituents in aminoglycosides on inhibition of the hammerhead ribozyme. The authors demonstrate that the basicity of the amino substituents in the antibiotics, important for the interaction with the RNA, is modulated by neighboring hydroxyl groups. Both basicity and position of the amino substituents play a critical role in governing the eletrostatic interactions between aminoglycosides and RNA.

36. Wang H, Tor Y: **RNA-aminoglycoside interactions: the design, synthesis and RNA binding of "amino-aminoglycosides".** *Angew Chem* 1998, in press.

37. • Hermann T, Westhof E: **Aminoglycoside binding to the hammerhead ribozyme: a general model for the interaction of cationic antibiotics with RNA.** *J Mol Biol* 1998, in press.
A theoretical approach combining molecular modelling and dynamics simulations that puts forward the idea that high-affinity binding of aminoglycosides to RNA folds is due to a structural electrostatic complementarity between positively charged ammonium groups in the antibiotics and metal binding sites in the RNA. In the paradigm system of the hammerhead RNA, solution conformations of aminoglycosides can replace magnesium ions by engaging into interactions with the RNA virtually identical to those made by the metals in the crystal structure.

38. Wang Y, Rando RR: **Specific binding of aminoglycoside antibiotics to RNA.** *Chem Biol* 1995, **2**:281-290.

39. Lato SM, Boles AR, Ellington AD: ***In vitro* selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution.** *Chem Biol* 1995, **2**:291-303.

40. Wallis MG, von Ahsen U, Schroeder R, Famulok M: **A novel RNA motif for neomycin recognition.** *Chem Biol* 1995, **2**:543-552.

41. Famulok M, Hüttenhofer A: ***In vitro* analysis of neomycin binding RNAs with a mutagenized pool of variants of the 16S decoding region.** *Biochemistry* 1996, **35**:4265-4270.

42. • Wallis MG, Streicher B, Wank H, von Ahsen U, Clodi E, Wallace ST, Famulok M, Schroeder R: ***In vitro* selection of a viomycin-binding RNA pseudoknot.** *Chem Biol* 1997, **4**:357-366.
By comparing *in vitro*-selected viomycin aptamers with naturally occurring RNAs that bind the cyclic peptide antibiotic viomycin the authors could show that viomycin, needs a complex 3D RNA fold for binding.

43. Wang Y, Killian J, Hamasaki K, Rando RR: **RNA molecules that specifically and stochiometrically bind aminoglycoside antibiotics with high affinities.** *Biochemistry* 1996, **35**:12338-12346.

44. •• Jiang L, Suri AK, Fiala R, Patel DJ: **Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.** *Chem Biol* 1996, **4**:35-50.
The solution structure of an tobramycin aptamer RNA in complex with the drug, analysed by NMR spectroscopy, provides a first in-depth view of aminoglycoside-binding by RNA. Whereas at the resolution of this study it has not been possible to identify specific contacts between the aminoglycoside and the RNA, interesting features of RNA folding were uncovered showing how an almost channel-like pocket is created for the incorporation of a substrate molecule. Direct impact on the development of drugs comes from the fact that the studied tobramycin aptamer bears similarities to a conserved hexanucleotide loop in ribosomal RNA.

45. Wilson WD, Ratmeyer L, Zhao M, Strekowski L, Boykin D: **The search for structure-specific nucleic acid-interactive drugs: effects of compound structure on RNA versus DNA interaction strength.** *Biochemistry* 1993, **32**:4098-4104.

46. Neault JF, Tajmir-Riahi HA: **RNA-diethylstilbestrol interaction studied by Fourier transform infrared difference spectroscopy.** *J Biol Chem* 1997, **272**:8901-8904.

47. Yao S, Wilson WD: **A molecular mechanics investigation of RNA complexes. I. Ethidium intercalation in an HIV-1 TAR RNA sequence with an unpaired adenosine.** *J Biomol Struct Dynam* 1992, **10**:367-387.

48. Hermann T, Heumann H: **Determination of nucleotide distances in RNA by means of copper phenanthroline-generated hydroxyl radical cleavage pattern.** *RNA* 1995, **1**:1009-1017.

49. Bailly C, Colson P, Houssier C, Hamy F: **The binding mode of drugs to the TAR RNA of HIV-1 studied by electric linear dichroism.** *Nucleic Acids Res* 1996, **24**:1460-1464.

50. Battigello JMA, Cui M, Roshong S, Carter BJ: **Enediyne-mediated cleavage of RNA.** *Bioorg Med Chem* 1995, **3**:839-849.

51. Kappen LS, Xi Z, Goldberg IH: **Effect of ribonucleotide substitution on nucleic acid bulge recognition by neocarzinostatin.** *Bioorg Med Chem* 1997, **5**:1221-1227.

52. Sugiura Y, Totsuka R, Araki M, Okuno Y: **Selective cleavages of $tRNA^{Phe}$ with secondary and tertiary structures by enedyine antitumor antibiotics.** *Bioorg Med Chem* 1997, **5**:1229-1234.

53. Fernandez-Saiz M, Schneider HJ, Sartorius J, Wilson WD: **A cationic cyclophane that forms a base-pair open complex with RNA duplexes.** *J Am Chem Soc* 1996, **118**:4739-4745.

54. Li K, Fernandez-Saiz M, Rigl CT, Kumar A, Ragunathan KG, McConnaughie AW, Boykin DW, Schneider HJ, Wilson WD: **Design and analysis of molecular motifs for specific recognition of RNA.** *Bioorg Med Chem* 1997, **5**:1157-1172.

55. Chow CS, Barton JK: **Recognition of G-U mismatches by tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III).** *Biochemistry* 1992, **31**:5423-5429.

56. • Burgstaller P, Famulok M: **Flavin-dependent photocleavage of RNA at GU base pairs.** *J Am Chem Soc* 1997, **119**:1137-1138.
The authors present isoalloxazine derivatives that specifically recognize non-Watson-Crick base pairs within a helical context. The findings constitute the basis for a three-dimensional model of isoalloxazine binding to RNA presented by Burgstaller *et al.* 1997 [57].

57. Burgstaller P, Hermann T, Huber C, Westhof E, Famulok M: **Isoalloxazine derivatives promote photocleavage of natural RNAs at G·U base pairs embedded within helices.** *Nucleic Acids Res* 1997, **25**:4018-4027.

58. Keck MV, Hecht SM: **Sequence-specific hydrolysis of yeast $tRNA^{Phe}$ mediated by metal-free bleomycin.** *Biochemistry* 1995, **34**:12029-12037.

59. Holmes CE, Duff RJ, Vandermarel GA, Vanboom J, Hecht SM: **On the chemistry of RNA degradation by Fe-bleomycin.** *Bioorg Med Chem* 1997, **5**:1235-1248.

60. Hurst P, Takasaki BK, Chin J: **Rapid cleavage of RNA with a La(III) dimer.** *J Am Chem Soc* 1996, **118**:9982-9983.

61. Lorente A, Espinosa JF, Fernandez-Saiz M, Lehn JM, Wilson WD, Zhong YY: **Syntheses of imidazole-acridine conjugates as ribonuclease A mimetics.** *Tetrahedron Lett* 1996, **37**:4417-4420.

62. Perreault DM, Cabell LA, Anslyn EV: **Using guanidinium groups for the recognition of RNA and as catalysts for the hydrolysis of RNA.** *Bioorg Med Chem* 1997, **5**:1209-1220.

63. Gait MJ, Karn J: **RNA recognition by the human immunodeficiency virus Tat and Rev proteins.** *Trends Biochem Sci* 1993, **18**:255-259.

64. Tao J, Frankel AD: **Arginine-binding RNAs resembling TAR identified by *in vitro* selection.** *Biochemistry* 1996, **35**:2229-2238.

65. Harada K, Martin SS, Frankel AD: **Selection of RNA-binding peptides *in vivo*.** *Nature* 1996, **380**:175-179.

66. •• Battiste JL, Mao H, Rao NS, Tan R, Muhandiral DR, Kay LE, Frankel AD, Williamson JR: **α helix-RNA major groove recognition in an HIV-1 Rev peptide-RRE RNA complex.** *Science* 1996, **273**:1547-1551.

The solution structure solved by NMR of a peptide derived from HIV-1 Rev protein bound to Rev responsive element RNA reveals many structural details of the binding pocket in RNA formed to accommodate the α-helical peptide which penetrates deeply into a widened deep groove. A G·G base pair that was recently found to be critical for both Rev and aminoglycoside binding (Werstuck *et al.* 1996 [78]) is shown to play a key role for opening up the deep groove.

67. Peterson RD, Feigon J: **Structural change in Rev responsive element RNA of HIV-1 on binding Rev peptide.** *J Mol Biol* 1996, **264**:863-877.

68. • Ye X, Gorin A, Ellington AD, Patel DJ: **Deep penetration of an α-helix into a widened RNA major groove in the HIV-1 Rev peptide-RNA aptamer complex.** *Nat Struct Biol* 1996, **3**:1026-1033.

NMR was used to determine the solution structure of an HIV-1 Rev-derived peptide bound to an RNA aptamer. The complex displays striking structural similarities to the Rev–Rev responsive element complex (Battiste *et al.* 1996 [66••]) in that both RNA targets, despite their different origin, bind the α-helical peptide in the deep groove, which is widened by non-canonical purine–purine base pairs.

69. Aboul-Ela F, Karn J, Varani G: **The structure of the human immunodeficiency virus type-1 TAR RNA reveals principles of RNA recognition by Tat protein.** *J Mol Biol* 1995, **253**:313-332.

70. • Brodsky AS, Williamson JR: **Solution structure of the HIV-2 TAR-argininamide complex.** *J Mol Biol* 1997, **267**:624-639.

NMR investigations on a complex of HIV-2 TAR RNA–argininamide suggest that the low-molecular-weight compound argininamide recognizes a set of structural elements in the RNA which also play a critical role in the interaction between an arginine residue of the Tat protein and TAT RNA.

71. Puglisi JD, Chen L, Blanchard S, Frankel AD: **Solution structure of a bovine immunodeficiency virus Tat-TAR peptide–RNA complex.** *Science* 1995, **270**:1200-1203.

72. Ye X, Kumar RA, Patel DJ: **Molecular recognition in the bovine immunodeficiency virus TAT peptide–TAR RNA complex.** *Chem Biol* 1995, **2**:827-840.

73. Ratmeyer L, Zapp ML, Green MR, Vinayak R, Kumar A, Boykin DW, Wilson WD: **Inhibition of HIV-1 Rev-RRE interaction by diphenylfuran derivatives.** *Biochemistry* 1996, **35**:13689-13696.

74. Lim AC, Barton JK: **Targeting the Tat-binding site of bovine immunodeficiency virus TAR RNA with a shape-selective rhodium complex.** *Bioorg Med Chem* 1997, **5**:1131-1136.

75. Mei HY, Mack DP, Galan AA, Halim NS, Heldsinger A, Loo JA, Moreland DW, Sanneslowery KA, Sharmeen L, Truong HN, Czarnik AW: **Discovery of selective, small-molecule inhibitors of RNA complexes. 1. The Tat protein–TAR RNA complexes required for HIV-1 transcription.** *Bioorg Med Chem* 1997, **5**:1173-1184.

76. Mei HY, Galan AA, Halim NS, Mack DP, Moreland DW, Sanders KB, Truong HN, Czarnik AW: **Inhibition of an HIV-1 Tat-derived peptide binding to TAR RNA by aminoglycoside antibiotics.** *Bioorg Med Chem Lett* 1995, **5**:2755-2760.

77. Zapp ML, Stern S, Green MR: **Small molecules that selectively block RNA binding of HIV-1 Rev protein inhibit Rev function and viral production.** *Cell* 1993, **74**:969-978.

78. Werstuck G, Zapp ML, Green MR: **A non-canonical base pair within the human immunodeficiency virus Rev-responsive element is involved in both Rev and small molecule recognition.** *Chem Biol* 1996, **3**:129-137.

79. • Hendrix M, Priestley ES, Joyce GF, Wong CH: **Direct observation of aminoglycoside-RNA interaction by surface plasmon resonance.** *J Am Chem Soc* 1997, **119**:3641-3648.

The surface plasmon resonance (SPR) technique has been used to quantitatively study the interaction of various aminoglycoside antibiotics with RNA constructs mimicking the HIV-1 Rev response element. The authors demonstrate the excellent performance of SPR for rapidly screening effector compounds for their RNA-binding capacity.

80. Zapp ML, Young DW, Kumar A, Singh R, Boykin DW, Wilson WD, Green MR: **Modulation of the Rev-RRE interaction by aromatic heterocyclic compounds.** *Bioorg Med Chem* 1997, **5**:1149-1155.

81. Weeks KM, Crothers DM: **Major groove accessibility of RNA.** *Science* 1993, **261**:1574-1577.

82. Uhlenbeck OC, Pardi A, Feigon J: **RNA structure comes of age.** *Cell* 1997, **90**:833-840.

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

84. Hermann T, Auffinger P, Scott WG, Westhof E: **Evidence for a hydroxide ion bridging two magnesium ions at the active site of the hammerhead ribozyme.** *Nucleic Acids Res* 1997, **25**:3421-3427.

85. Hermann T, Auffinger P, Westhof E: **Molecular dynamics investigations on the hammerhead ribozyme RNA.** *Eur Biophys J* 1998, in press.

86. Louise-May S, Auffinger P, Westhof E: **Calculations of nucleic acid conformations.** *Curr Opin Struct Biol* 1996, **6**:289-298.