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Evolutionary Origin and Conserved Structural Building Blocks of Riboswitches and Ribosomal RNAs: Riboswitches as Probable Target Sites for Aminoglycosides Interaction

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ABSTRACT

Purpose: Riboswitches, as noncoding RNA sequences, control gene expression through direct ligand binding. Sporadic reports on the structural relation of riboswitches with ribosomal RNAs (rRNA), raises an interest in possible similarity between riboswitches and rRNAs evolutionary origins. Since aminoglycoside antibiotics affect microbial cells through binding to functional sites of the bacterial rRNA, finding any conformational and functional relation between riboswitches/rRNAs is utmost important in both of medicinal and basic research.

Methods: Analysis of the riboswitches structures were carried out using bioinformatics and computational tools. The possible functional similarity of riboswitches with rRNAs was evaluated based on the affinity of paromomycin antibiotic (targeting "A site" of 16S rRNA) to riboswitches via docking method.

Results: There was high structural similarity between riboswitches and rRNAs, but not any particular sequence based similarity between them was found. The building blocks including "hairpin loop containing UUU", "peptidyl transferase center conserved hairpin A loop"," helix 45" and "S2 (G8) hairpin" as high identical rRNA motifs were detected in all kinds of riboswitches. Surprisingly, binding energies of paromomycin with different riboswitches are considerably better than the binding energy of paromomycin with "16S rRNA A site". Therefore the high affinity of paromomycin to bind riboswitches in comparison with rRNA "A site" suggests a new insight about riboswitches as possible targets for aminoglycoside antibiotics.

Conclusion: These findings are considered as a possible supporting evidence for evolutionary origin of riboswitches/rRNAs and also their role in the exertion of antibiotics effects to design new drugs based on the concomitant effects via rRNA/riboswitches.

Introduction

Today, it is evident that RNAs are not just intermediates between DNA and proteins. Their catalytic and regulating characteristics have been more verified since more than a decade ago. It has been revealed that there are RNA-based mechanisms which regulate gene expression in response to internal or external signals.[1-3] Accordingly, mRNA structure plays an essential role in this process and determines the fate of the mRNA.[4-7] As ribosome binds mRNA before transcription is completed, most regulatory regions are located within the 5' untranslated region (UTR) of mRNAs. These regulatory regions contain either *cis* acting binding sites or *trans*-acting regulators (non-coding RNAs).

Riboswitches, usually found within the 5'UTR of mRNAs, are *cis* acting RNA elements. They can adopt various conformations in response to environmental signals, including stalled ribosomes, uncharged tRNAs, elevated temperatures or small molecule ligands.[8] These metabolite sensors, which were identified a decade ago,[9] regulate the genes involved in the uptake and use of related metabolites without proteins interpretation.[1,9]

An ever-increasing number and variety of riboswitches are being identified in bacteria, as well as some

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eukaryotes. For example, as much as 2% of all *Bacillus subtilis* genes are regulated by riboswitches that bind to metabolites such as flavin mononucleotide (FMN), thiamin pyrophosphate (TPP), S-adenosylmethionine (SAM), lysine, and purines. Riboswitches generally consist of two parts: the aptamer region, a conserved sequence which binds the ligand, and the so-called expression platform, which regulates gene expression through alternative RNA structures that affect transcription or translation.[10,11] Upon binding of the ligand, the riboswitch changes the conformation which forms or disrupts transcriptional terminators or antiterminators, respectively. Therefore, in order to find out their mechanistic details, 2D and 3D structure of riboswitches' aptamers[12] and their binding characteristics[13] were extensively analyzed experimentally or computationally.[14-16] On the other hand, other possible interactions are suggested to introduce some molecules as new drugs which exert their effects via riboswitches.[17,18]

RNA structure is basically expressed at the sequence or primary structure level, the secondary and tertiary levels. Initially, RNA motifs were identified at the sequence level as generally existing short sequences in functional RNAs, such as transfer RNA (tRNA) or ribosomal RNA (rRNA).[19] Base-pairing or secondary structure constitutes both the canonically base-paired regions (helices) and non-paired regions (loops). Structural studies and comparative sequence analyses have suggested that biological RNAs are composed primarily of conserved structural building blocks or motifs[20] of secondary and tertiary structures. Forms and functions of RNAs in the biological systems which connected to their three-dimensional (3D) structures lead RNA molecules to perform specific roles. However, there are some similarities between various motifs in RNAs types with diverse functionalities.

Barrick and Breaker in 2007 detected some motifs in riboswitches, which are close in relation to rRNA structures.[21] In 2008, an artificial riboswitch for the aminoglycoside antibiotic, neomycin B, was engineered[22] which partially resembles the ribosomal A-site, the natural target for aminoglycoside antibiotics.[23] Based on the previous studies we aimed to investigate relation between rRNAs and riboswitches structures and their subsequent functions such as binding to specific antibiotics. In this path, in current study we attempted to survey structural similarity including primary, 2D, 3D and motifs as well as functional similarity among rRNAs and riboswitch elements through bioinformatics and computational tools.

Materials and Methods

Databases and Programs

Riboswitches data were collected from Rfam database[24] and structure information was acquied through PDB database (www.pdb.org). Sequences mostly gained from NCBI based on Rfam sequences information. Multiple sequence alignment was carried out via ClustalW implemented in Mega5 program. Needle (http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html) and Water (http://www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html) servers were applied in order to accomplish optimal pairwise global and local alignment, respectively.[25] Also, functional and structural alignments were done by means of SARA server (http://structure.biofold.org/sara/)[26] and R3D Align (http://rna.bgsu.edu/R3DAlign) servers,[27] respectively. Eventually, the interactions of an antibiotic with RNA structures were carried out via Autodock version 4.2 program.

Functional and structural riboswitch alignments

Ten riboswitch classes which have not only the most representatives in microorganisms,[28] but also have available PDB structures, were selected. Their PDB codes which represent preferably unbound state of riboswitches were extracted first from Rfam (http://rfam.sanger.ac.uk/) and then PDB (http://www.rcsb.org/pdb/home/home.do). They included TPP (PDB code: 2gdi), FMN (PDB code: 2yie), SAM-I (PDB code: 3iqn), lysine (PDB code: 3d0x), glycine (PDB code: 3ox0), purine (PDB code: 4fe5), c-di-GMP-I (PDB code: 3iwn), c-di-GMP-II (PDB code: 3q3z), preQ1(PDB code: 3fu2), THF (PDB code: 3suy) riboswitches. Each code was analyzed via SARA server (http://structure.biofold.org/sara/) to perform structure based function alignment. Default parameters are -7.00 for opening gap, -0.60 for extension gap and 3 consecutive vectors (4 atoms) for length of the Unit-Vector used to generate the comparison matrix. The results were sorted based on PSS (percentages of secondary structure identity) and top rRNA structures were selected and their sequences were acquired.

Sequences alignment

All rRNA sequences which have high similarity with a definite riboswitch sequence were aligned applying ClustalW[29] implemented in Mega5[30] with the default parameters for multiple alignment stages as 15 and 6.66 for gap opening and gap extension penalties, respectively. If necessary, minor adjustments were manually made to the alignments. Also, global and local pairwise alignments of same rRNAs with the riboswitches sequences were conducted through Needle and Water server programs. All the parameters were set by default as 10 and 0.5 for gap opening and gap extension penalties, respectively.

Motifs categorization

The category (more than 50 percent secondary structure identity) for each type of riboswitches was used to organize similar rRNA motifs. Afterward, motifs were sorted out based on the number of similar riboswitches types. Also, average of PSS (percentages of secondary

structure identity) level for each type of motifs was determined. In addition, according to results, types of riboswitches are organized based on each kind of motif they could possess.

Docking

Preparation of the macromolecules

All crystal structures of 10 riboswitches (PDB IDs: 2gdi, 2yie, 3iqn, 3d0x, 3ox0, 4fe5, 3iwn, 3q3z, 3fu2, 3suy) and 16S-rRNA A-site (PDB ID: 1j7t) were selected. Water and ligand molecules were eliminated by the software program ViewerPro Version 5.0. Also non-polar hydrogens and Gasteiger charges were added during the preparation of the macromolecule input file using the AutoDockTools package.

Preparation of the ligand

Complex between paromomycin and the 16S-rRNA A-site (PDB code: 1j7t) was used to provide the three-dimensional (3D) structure of paromomycin by removing other atoms using ViewerPro Version 5. Gasteiger charges were added to the obtained structure of the ligand and the rotatable bonds were set to 9 by using AutoDock Tools.

Preparation of the grid files

The complex crystal structure of paromomycin antibiotic and 16S-rRNA A-site motif (PDB code: 1j7t) was selected as a control sample for assessments of the results. In order to find out the similar and conserved building blocks of the riboswitches with the rRNA, the 3D structure of 16S-rRNA A-site and 10 different riboswitches were structurally aligned via R3D Align. The aligned part of each riboswitch with "A site" motif of 16S rRNAs was considered to generate Grid maps. The interaction of the antibiotic ligand (paromomycin) and 16S-rRNA A-site as well as the homologue parts in different riboswitches were analyzed. Grid maps were generated by AutoGrid 4.2 based on the superimposed area of each riboswitch with 16S-rRNA A-site. The numbers of points in the grid boxes were 60×56×80; 84×86×82; 72×86×86; 76×68×100; 100×100×100; 98×88×88; 108×80×90; 76×98×100; 100×74×86; 98×74×88; 72×86×86 for 1j7t, 3iwn, 3q3z, 3ox0, 3d0x, 3fu2, 4fe5, 3iqn, 3suy, 2gdi and 2yie, respectively, with a grid spacing of 0.375A°.

Preparation of the docking files

Molecular docking was carried out by the molecular docking software, AutoDock Version 4.2 based on the Lamarckian genetic algorithm.[31] For each complex, 100 independent docking runs were conducted containing a population of 150 randomly positioned individuals. The maximum number of energy-evaluation retries and generations were 2500000 and 27000, respectively. Also, crossover rate of 0.8 and a mutation rate of 0.02 were set up. The docking results were clustered on the results of docking by using a root mean square (RMS) tolerance of 2.0 A°. During docking, macromolecules were set rigid, whereas all the torsional bonds of ligands were set free. The docking results were clustered according to a root-mean-square deviation (RMSD) tolerance of 0.2 nm.

The structure of 16S-rRNA A-site (PDB code: 1j7t) was taken as the control for docking. First, the ligand (paromomycin) was removed from the complex. Then, the ligand-free structure of 16S-rRNA A-site was docked with ligand for 100 independent runs. At last, binding energies obtained from docking of riboswitches with paromomycin were compared to control binding energy.

Statistical Analyses

Where needed, results were evaluated by excel (version 2007) and SPSS (version 16). Statistical analyses were performed using one-way analysis of variance (ANOVA). Statistical assessment of difference between mean values was performed by least significance difference (LSD) test at $p<0.05$ using SPSS (16 version) software.

Results and Discussion

Structural riboswitch alignments

Recent interest in non-coding RNA transcripts has culminated in a rapid increase of deposited RNA structures in the PDB database. However, functional classification and characterization of the RNA structure have not completely been addressed. There are many bioinformatics tools to investigate 2D and 3D structural alignments of DNA and RNA structures.[32-34] SARA (Structure Alignment of Ribonucleic Acids) web server (http://sgu.bioinfo.cipf.es/services/SARA)[26] is a promising program for aligning RNA 3D structures via PDB files based on unit-vector root mean square (URMS). Herein, PDB codes of a total of 10 riboswitch types were analyzed by SARA server and all rRNA PDB codes were collected. For all PDB codes in each group of data achieved from SARA server, primary, secondary and tertiary similarity percentages were calculated. Also they were sorted by average natural logarithm of PID (Percentage of sequence identity), PSS (percentages of secondary structure identity) and PSI (percentages of tertiary structure identity). For instance, among similar rRNA PDB codes for lysine riboswitch, the highest 2D similarity belongs to "helix 45" (PDB code: 1wts-chain A) which shares 28.6, 100 and 92.9 % primary, secondary and tertiary identity with this type of riboswitch, respectively. According to Figure 1, there is a correlation between tertiary structure identity (based on PSI) and secondary structure identity (based on PSS) with approximate coefficient of determination (R^2) of 0.8. As a result, the observed correlation between the similarity of 2D and 3D structures demonstrate that both 2D and 3D structures could be utilized for similarity studies in current study. Besides, those concerned with RNA-ligand interactions, generally give greater weight to secondary structure similarity, as ligand binding sites

typically consist of a single type of secondary structure.[35] As a result and due to interaction analysis in the subsequent stages of this study, secondary structure could be more useful and debatable in our study. Hence, in the following steps, sorting in order of PSS (based on secondary structure identity) was performed.

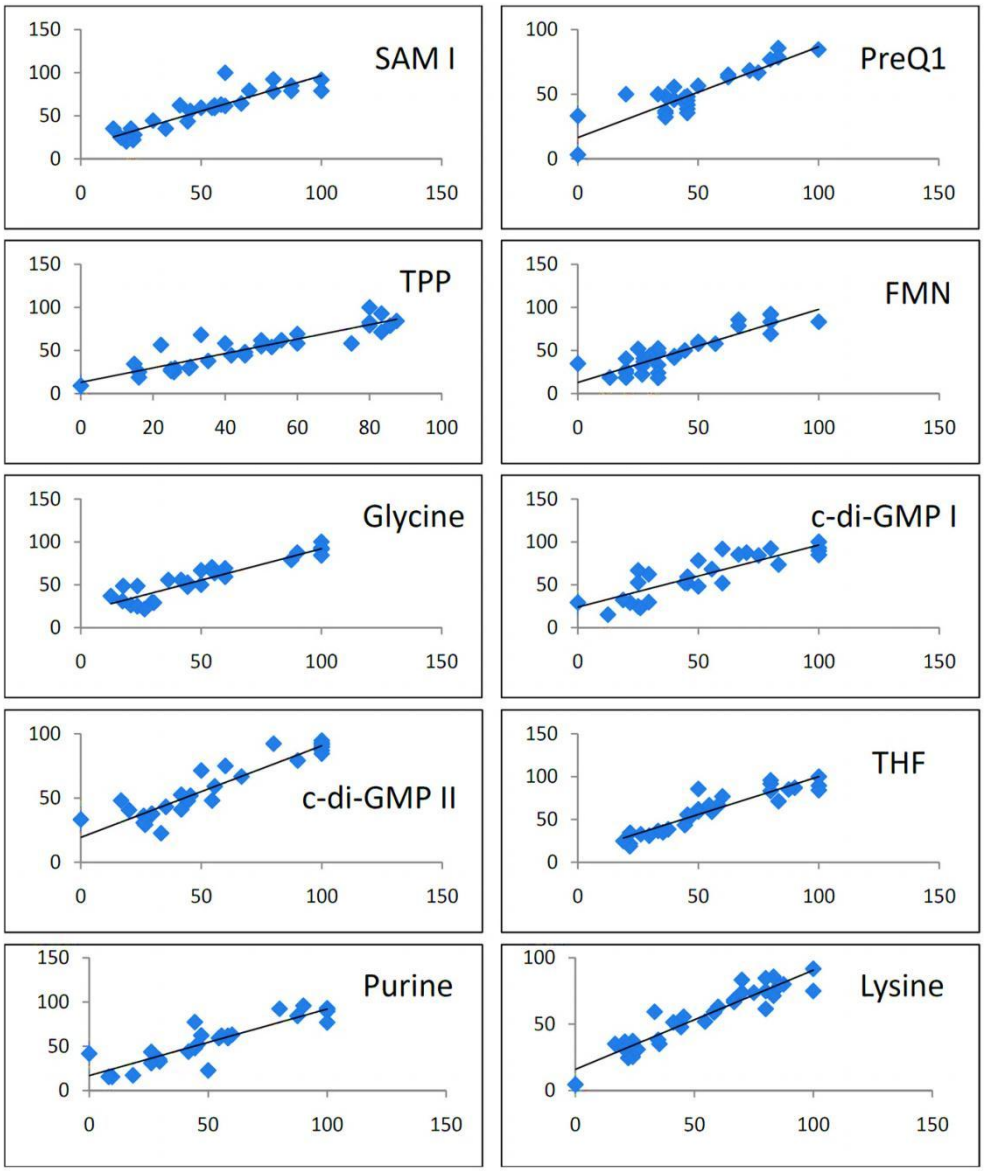

Figure 1. Correlation of secondary structure identity and 3D structure of rRNAs with the mentioned riboswitches. Vertical and horizontal axes are percentage of secondary structure similarity (PSS) and tertiary structure identity (PSI) of rRNAs with the associated riboswitches, respectively. There is high correlation between PSS and PSI of rRNAs with 10 different types of riboswitches (R^2 ~ 0.8).

Motifs categorization

The Structural Classification of RNA (SCOR) is a database designed to provide a comprehensive perspective and understanding of RNA motif structures, functions, tertiary interactions and their relationships (http://scor.berkeley.edu/). It is an inclusive, manually created source of RNA structural motifs which applies automated tools and literature descriptions to assist in the classification of RNA secondary and tertiary structure motifs such as Kink turns, S-turns, GNRA loops.[36,37]

All of the rRNA motifs having more than 50 percent secondary structure identity (PSS) with similar riboswitches were exploited to categorize in similar groups. In addition, averages of PSS for each type of motifs were calculated. Figure 2 illustrates rRNA motifs against the number of similar riboswitches. As it is shown these rRNA motifs are in common with different kinds of riboswitches structures. Also, there is a remarkable high amount of secondary structure identity between shown rRNA motifs and riboswitches. Table 1 shows the rRNA motifs/ riboswitches correlation with more details. Accordingly, glycine and THF riboswitches are similar to the most number of detected rRNA motifs (18 motifs). However, apart from “18S rRNA A site” and "helix 21", all of the mentioned ribosomal RNA motifs are similar among different 10 types of riboswitches. In this issue, 4

motifs including hairpin loop containing UUU, peptidyl transferase center conserved hairpin A loop, helix 45 and S2 (G8) hairpin are common in all kinds of riboswitches (see Table 1 and Figure 2). It means these motifs are highly similar among different types of riboswitches. Already, Winkler *et al.* figured out that the GA motif is a highly conserved structure in both TPP and SAM riboswitches and 23S rRNAs. In addition, it was observed that UA_handle motif , is common within both ribosomal RNAs and riboswitches.[38] Hence, our findings revealed that not only the mentioned GA and UA_handle motifs but also several other important and highly conserved rRNA motifs such as hairpin UUU, A loop, helix 45 and S2 (G8) hairpin and GNRA tetraloop are found in riboswitches with high similarity. GNRA motif provides tertiary contacts which are important for group I introns, hammerhead ribozymes, and the ribosome.[39-41] It is believed that GNRA has high selectivity and specificity in binding to different kinds of compounds;[42-44] a common characteristics for riboswitches. GNRA tetaraloop is a common motif in some riboswitches including glycine, purine, FMN, THF, c-di-GMP I (see Figure 2 and Table 1). The structural similarity of this motif with rRNAs is more than 75 percent. Consequently, the findings proposed that the common motifs in riboswitches structures should have common functional properties in similar rRNAs such as binding ligand molecules. The ligand binding characteristics of 10 riboswitches and RNAs which share similar motifs with them were considered as well (see section 3.3). Considering these findings, common evolutionary aspects of riboswitches and rRNAs are confirmed. As a result the resemblance of rRNA building blocks and riboswitches domains may be resulted either from connecting evolution or the dependent byproducts of historical events such as local segment duplication and recombination mechanisms that cause elevation of structural complexity of natural functional molecules.

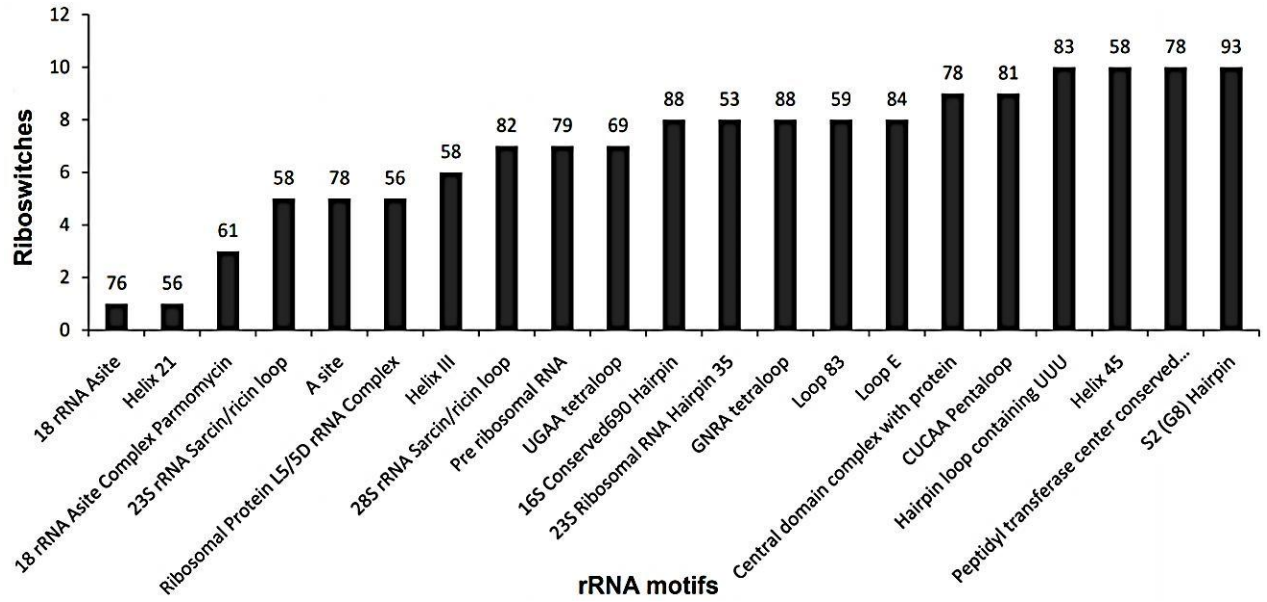

Figure 2. Correlation of ribosomal RNA motifs with 10 different types of riboswitches. Vertical axis demonstrates the number of similar riboswitches types which share at least 50% secondary structure identity (PSS>50%) with the shown rRNA motifs (average of PSS for each motif is shown above on the relative column). Please note that 4 motifs including "Hairpin loop containing UUU", "Helix 45", "Peptidyl transferase center conserved hairpin A loop", and "S2 (G8) Hairpin" are similar with all 10 types of riboswitches having 83, 58, 78, 93% PSS, respectively.

Sequences alignment

Multiple sequence alignment is a way of arranging the sequences of DNA, RNA, or protein molecules to similar regions that may be a consequence of functional, structural, or evolutionary origin. Multiple alignments are often used in identifying conserved sequences across a group of sequences hypothesized to be evolutionarily related. Herein, multiple sequence alignment of the riboswitches and similar rRNAs with more than 50 percent secondary structure identity (PSS, discussed above) was carried out. Although, all of the sequences were structurally similar to each other (PSS>50%) there were not any particular sequence similarity results using different programs (ClustalW2 and M-Coffee, data not shown). In order to do the alignment more precisely, pairwise alignment of each rRNA with the riboswitches was accomplished by Needle and Water servers as global and local alignment tools, respectively. Figure 3 illustrates the results achieved for each pairwise global alignment between every rRNAs and associated riboswitches. Each one of 10 riboswitches sequences was aligned with related rRNAs and an average for every kind of riboswitches was represented in Mean ± SEM (Standard Error of the

Mean). As it was shown, global identity percentage is considerably lower than 25% identity. More importantly, based on local alignment there is no unique region in rRNA sequences aligned with the similar riboswitches (Table S1). As a result, no common assembly of nucleotides was recognized in the alignments by different programs. Despite this fact that aptamer domains of riboswitches are highly conserved sequences,[1,9] no particular conserved element was observed in their similar rRNAs. In agreement with our findings, it is commonly established that functionally important RNA sequences could be less conserved than their structures[45] where maintaining the structure is more important than maintaining the sequence.[46] For instance, a study on human and mouse genome sequences suggested that there are corresponding non-coding RNA sequences regions between human and mouse with common RNA structures which are not alignable in primary sequence.[46] Therefore, in spite of the structurally similarity of riboswitches and rRNAs, no particular conserved primary sequence element observed in their similar rRNAs using pairwise/multiple alignment approach.

Table 1. Types of riboswitches which share more than 50% secondary structure identity with ribosomal RNA motifs. The type of riboswitch which include a motif is checked under its name.

Motif*	Riboswitch									
	Glycine	Lysine	Purine	THF	FMN	TPP	preQ1	SAM	c-di-GMP I	c-di-MP II
16S Conserved 690 Hairpin	✓	✓	-	✓	✓	✓	✓	-	✓	✓
18 rRNA A site	✓	-	-	-	-	-	-	-	-	-
18 rRNA A site Complex Parmomycin	✓	-	-	-	-	-	-	✓	✓	-
23S rRNA Sarcin/ricin loop	✓	✓	✓	✓	-	-	-	-	✓	-
23S Ribosomal RNA Hairpin 35	✓	✓	✓	✓	-	✓	-	✓	✓	✓
28S rRNA Sarcin/ricin loop	✓	✓	✓	✓	-	✓	-	✓	✓	-
A site	✓	✓	-	✓	-	-	-	✓	-	✓
Central domain complex with protein	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
CUCAA Pentaloop	✓	✓	✓	✓		✓	✓	✓	✓	✓
GNRA tetraloop	✓	-	✓	✓	✓	✓	-	✓	✓	✓
Hairpin loop containing UUU	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Helix 21	-	-	-	-	-	✓	-	-	-	-
Helix 45	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Helix III	✓	-	✓	✓	-	-	-	✓	✓	✓
Loop 83	✓	✓	✓	✓	✓	-	-	✓	✓	✓
Loop E	-	-	✓	✓	✓	✓	✓	✓	✓	✓
Peptidyl transferase center conserved hairpin A loop	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pre ribosomal RNA	✓	✓	✓	✓	-	✓	-	✓	-	✓
Ribosomal Protein L5/5D rRNA Complex	✓	✓	-	✓	-	✓	-	-	✓	-
S2 (G8) Hairpin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
UGAA tetraloop	✓	-	✓	✓	✓	✓	✓	✓	-	-
16S Conserved 690 Hairpin	✓	✓	-	✓	✓	✓	✓	-	✓	✓
Total number	18	14	15	18	10	15	9	16	16	14

*The name of each motif is extracted from SCOR (Structural Classification of RNA, http://scor.berkeley.edu/).

Functional properties of riboswitches-rRNAs motifs

Ligand binding is one of the main functions of RNAs for structural stabilization, as well as producing signals. RNA ligand binding is important for ribozymes, riboswitches and splicing functions, along with mediating RNA-protein and RNA-RNA intermolecular interactions.[47] The first ribosomal RNA identified as a small molecule target was 16S RNA component of the prokaryotic ribosome.[48] Various antibiotics, such as aminoglycosides, affect bacterial cells through binding to functional sites of the bacterial rRNA which leads to miscoding during the translation process. Since similarity between riboswitches and different rRNA motifs were revealed in previous parts of current study, a question was raised that "could binding characteristics of these structures be similar too?" Therefore, the binding affinity of riboswitches for paromomycin as a functional characteristic of these structures was evaluated for a common motif in riboswitches-rRNA structures using molecular docking approach via Autodock 4.2. Paromomycin is a member of aminoglycosides antibiotics family that has high functional affinity for "A site" motif of 16S rRNAs.[49-51] The aligned part of each riboswitch with "A site" motif of 16S rRNAs (1j7t) was considered to evaluate the possibility of functional antibiotic binding affinity. Table 2 illustrated the binding

energy of docked ligand with riboswitches and 16S rRNA structures. According to the docking results, except for c-di-GMP I riboswitch, there is a high functional affinity of paromomycin to the riboswitches in comparison with 16S rRNA. Statistical analysis showed that apart from c-di-GMP I, *p* value for all kinds of riboswitches relative to "16S rRNA A site" is less than 0.01. As a result, there is a remarkable significant upshift was occurred in binding energy of different riboswitches types with paromomycin. The range of appropriate binding energies for riboswitches is from -13 to -22 kcal/mol whereas it is -11.7 kcal/mol for "16S rRNA A site" (see Table 2). Seven riboswitches including lysine, THF, SAM, c-di-GMP II, purine, glycine and TPP riboswitches have 1.5-~2 times higher affinity for paromomycin than "A site" motif. Among them, lysine, THF, SAM and c-di-GMP II have more than 50% of secondary structure similarity with "A site" motif (Table 1). However, despite having less similarity of purine and TPP riboswitches with "A site" motif, they have also considerable low binding energy with paromomycin. But only c-di-GMP I riboswitch showed completely different functional behavior of quite not suitable interaction with the desired ligand. It could be possibly due to nucleotide types in defined binding site which cause weak electrostatic interaction with paromomycin. According to Table 2, maximum and minimum binding energy of each type of riboswitches demonstrates that the most involved intermolecular energy (van der Waals, H bonding, desolvation and electrostatic energy) in mentioned interactions is electrostatic energy.

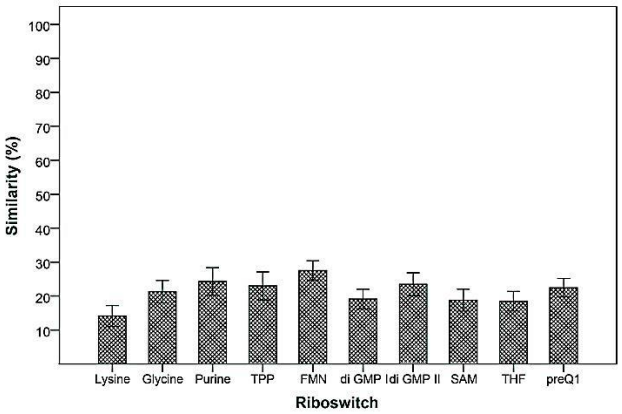

Figure 3. Average of global pairwise alignment similarity percentage of rRNA sequences with structurally-based similar riboswitches via Needle program [25]. The sequences of all structurally similar rRNAs (PSS>50%.) were aligned with related riboswitch and the similarity percentages were represented in Mean ± SEM for all types of riboswitches (it should be noted that the average number of rRNAs for the analysis were more than 30 strings for each type of riboswitches). Global identity percentages in the range of 14% to 26% denote no sequence correlation between riboswitch and structurally similar rRNAs (For complete data see Table S1).

Table 2. Binding energy of paromomycin interactions with different types of riboswitches and "16S rRNA A site" as receptors.
Binding energy of each interaction is divided to van der Waals energy, hydrogen bonding energy, desolvation energy and electrostatic energy.

Receptors*	Mean binding energy ± SD (kcal/mol)	Max binding energy(kcal/mol)			Min binding energy(kcal/mol)		
		vdW + Hbond + desolv Energy	Electrostatic Energy	Total binding energy	vdW + Hbond + desolv Energy	Electrostatic Energy	Total binding energy
Lysine (3d0x)	-22.75 ± 1.12	-6.87	-20.91	-25.1	-5.5	-17.4	-20.22
THF (3suy)	-20.29 ± 1.47	-5.5	-19.99	-22.8	-3.35	-16.49	-17.15
SAM (3iqn)	-19.05 ± 0.77	-8.32	-15.6	-21.24	-3.66	-16.66	-17.64
c-di-GMP II (3q3z)	-19.3295 ± 0.61	-5.62	-17.96	-20.9	-3.95	-16.48	-17.75
Purine (4fe5)	-19.17 ± 0.62	-6.02	-17.25	-20.59	-4.81	-15.58	-17.7
Glycine (3ox0)	-18.97 ± 0.90	-6.04	-17.48	-20.83	-2.52	-17.06	-16.89
TPP (2gdi)	-18.52 ± 0.94	-6.32	-16.92	-20.56	-3.1	-15.88	-16.3
preQ (3fu2)	-15.12 ± 1.64	-5.67	-15.33	-18.32	-3.39	-11.47	-12.17
FMN (2yie)	-13.07 ± 0.93	-5.16	-12.74	-15.21	-3.68	-9.65	-10.65
c-di-GMP I (3iwn)	0.53 ± 0.76	-7.04	2.89	-25.1	-3.47	2.98	2.2
A site (1j7t)**	-11.75 ± 0.79	-5.29	-10.93	-13.54	-3.54	-9.23	-10.08

*Receptors indicated riboswitches with their PDB codes. ** "A site" refers to "16S rRNA A site" set as a control.

Figure 4 shows RMSD (Root Mean Square Deviation) against binding energy for all kinds of riboswitches and "16S rRNA A site". Accordingly, the steadiness of all graphs showed that most conformations in studied structures have similar behavior to interact with receptors. Consequently, it verified the docking results and similar condition of docking in all of the riboswitches and 16S rRNA A site. Figure 5 illustrates the schematic interaction of paromomycin with "A site" and the riboswitches types. As paromomycin and "A site" interaction, the ligand binds to minor groove of riboswitches too. However, this kind of binding is not observed in c-di-GMP I riboswitch which may be the reason for not suitable interaction. Apart from c-di-GMP I riboswitch, paromomycin is covered in all types of riboswitches

which may reduce the access of the molecule with around environment.

These findings support a report which introduced an engineered riboswitch for the aminoglycoside antibiotic neomycin B.[22] The resulting neomycin B responsive RNA-element partially resembles the ribosomal A-site, the natural target for aminoglycoside antibiotics.[23] Furthermore, recently Jia *et al.* discovered an aminoglycoside-binding riboswitch that is related to induction of aminoglycosides antibiotic resistance.[52]

The targeting of RNA with small molecules is the complementary or even basic of targeting of proteins. Through this phenomenon, riboswitches demonstrate regulatory mechanisms in which proteins do not take part. Furthermore, the importance of RNA-binding small molecules such as antibiotics is undeniable. All clinically approved drugs which exert their effects by binding to RNA are totally recognized as rRNA-targeting molecules.[35] Accordingly, it could be discussed that some of resulted motifs in this study could be alternative binding sites for antibiotics and related small molecules in riboswitches. As though, if further studies on other motifs and antibiotics verify these findings, there is another mechanism for antibiotics effects or resistance apart from rRNA binding signaling in bacteria. It means these kinds of small molecules may bind to same motifs in riboswitches to generate their impact leading to bacteria's death or growth repression. However, this suggestion needs more computational and experimental confirming findings.

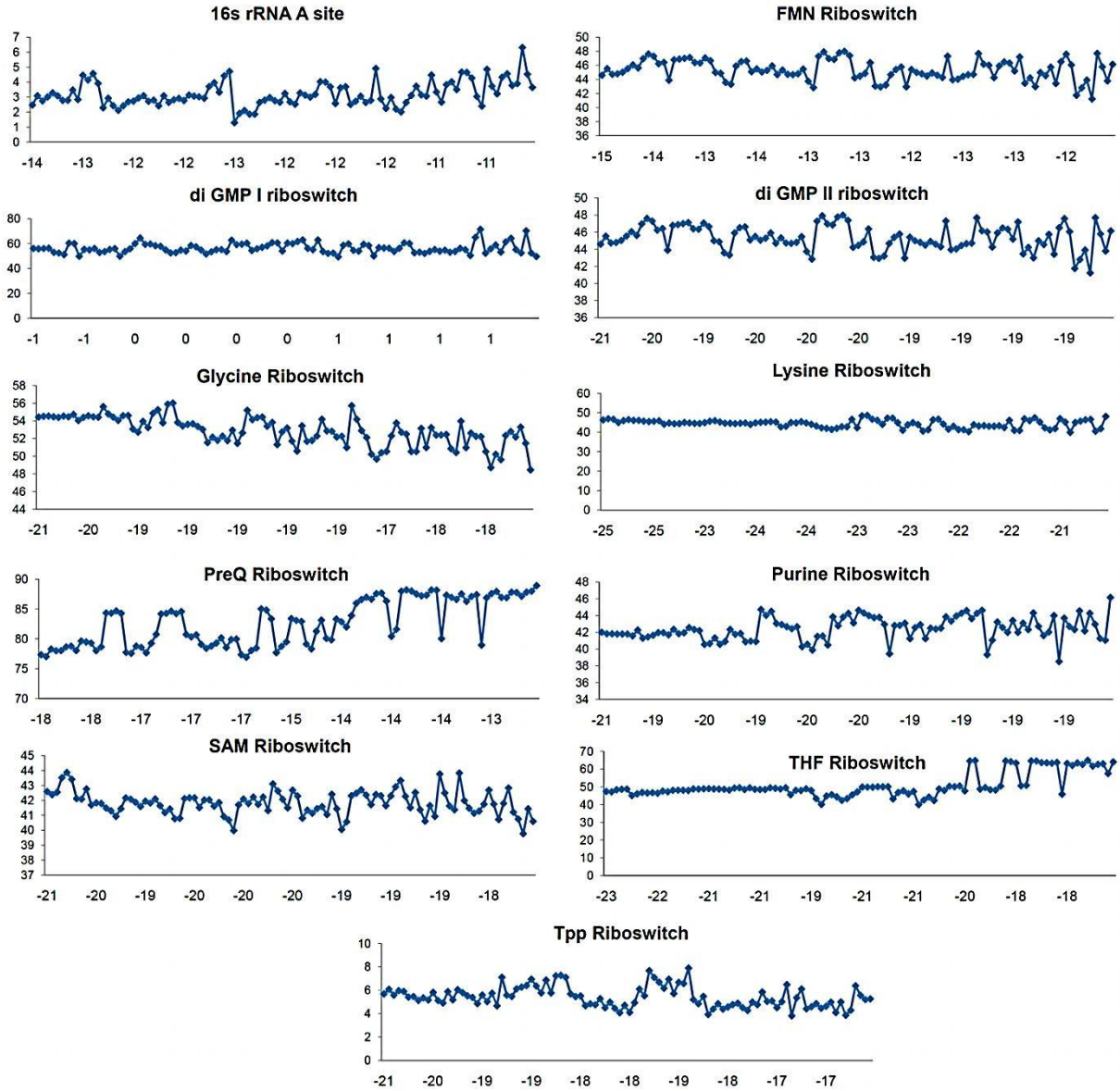

Figure 4. RMSD vs. binding energy for 10 types of riboswitches and "16S rRNA A site" based on Autodock results. Vertical and horizontal axes represented RMSD and binding energy of each docked conformations.

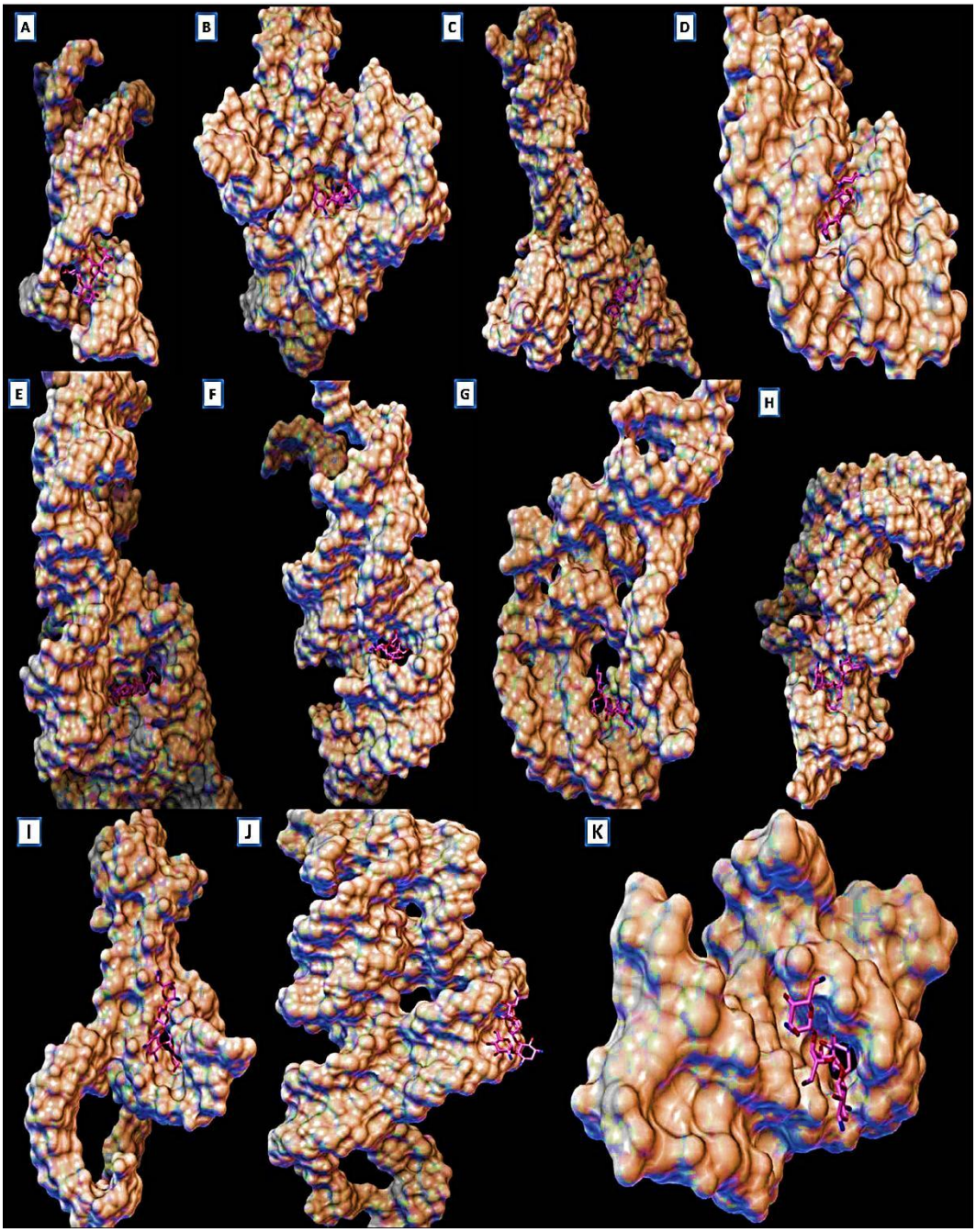

Figure 5. Docking conformations of paromomycin and RNAs. The ligand Paromomycin was shown stick-line and all of receptors were presented charge-space including (**A**) "16S rRNA A site" (1j7t), (**B**) SAM riboswitch (3iqn), (**C**) Glycine riboswitch (3ox0), (**D**) Purine riboswitch (4fe5), (**E**) Lysine riboswitch (3d0x), (**F**) THF riboswitch (3suy), (**G**) c-di-GMP II riboswitch (3q3z), (**H**) TPP riboswitch (2gdi), (**I**) FMN riboswitch (2yie), (**J**) c-di-GMP I riboswitch (3iwn), (**K**) preQ riboswitch (3fu2). The conformation with lowest binding energy was selected for each structure.

Conclusion

In this study, the relation between ribosomal RNAs and riboswitches in terms of structural and functional similarity was evaluated. Our findings indicated these two types of RNAs are structurally similar (secondary and tertiary based level) rather than in primary sequences. Accordingly, similar secondary structure motifs with high identity as Hairpin loop containing UUU, Peptidyl transferase center conserved hairpin A loop, Helix 45 and S2 (G8) Hairpin were detected between riboswitches and rRNAs. Consequently, investigation on the connection between binding sites of aminoglycosides in rRNAs and riboswitches using docking method revealed that riboswitches bind more tightly than "16S rRNA A site" to paromomycin. Considering other studies suggesting any kind of structural, functional or evolutionary similarity of ribosomal RNAs and riboswitches, these results could verify that these two apparent diverse types of RNAs show strong correspondence to each other.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Montange RK, Batey RT. Riboswitches: emerging themes in RNA structure and function. *Annu Rev Biophys* 2008;37:117-33.
2. Toledo-Arana A, Repoila F, Cossart P. Small noncoding RNAs controlling pathogenesis. *Curr Opin Microbiol* 2007;10(2):182-8.
3. Waters LS, Storz G. Regulatory RNAs in bacteria. *Cell* 2009;136(4):615-28.
4. Serganov A, Patel DJ. Amino acid recognition and gene regulation by riboswitches. *Biochim Biophys Acta* 2009;1789(9-10):592-611.
5. Serganov A. The long and the short of riboswitches. *Curr Opin Struct Biol* 2009;19(3):251-9.
6. Geissmann T, Marzi S, Romby P. The role of mRNA structure in translational control in bacteria. *RNA Biol* 2009;6(2):153-60.
7. Cruz JA, Westhof E. The dynamic landscapes of RNA architecture. *Cell* 2009;136(4):604-9.
8. Grundy FJ, Henkin TM. From ribosome to riboswitch: Control of gene expression in bacteria by RNA structural rearrangements. *Crit Rev Biochem Mol Biol* 2006;41(6):329-38.
9. Nahvi A, Sudarsan N, Ebert MS, Zou X, Brown KL, Breaker RR. Genetic control by a metabolite binding mRNA. *Chem Biol* 2002;9(9):1043.
10. Nudler E, Mironov AS. The riboswitch control of bacterial metabolism. *Trends Biochem Sci* 2004;29(1):11-7.
11. Mandal M, Breaker RR. Gene regulation by riboswitches. *Nat Rev Mol Cell Biol* 2004;5(6):451-63.
12. Petrone PM, Dewhurst J, Tommasi R, Whitehead L, Pomerantz AK. Atomic-scale characterization of conformational changes in the preQ(1) riboswitch aptamer upon ligand binding. *J Mol Graph Model* 2011;30:179-85.
13. Ling B, Wang Z, Zhang R, Meng X, Liu Y, Zhang C, et al. Theoretical studies on the interaction of modified pyrimidines and purines with purine riboswitch. *J Mol Graph Model* 2009;28(1):37-45.
14. Vicens Q, Mondragon E, Batey RT. Molecular sensing by the aptamer domain of the FMN riboswitch: A general model for ligand binding by conformational selection. *Nucleic Acids Res* 2011;39(19):8586-98.
15. Kelley JM, Hamelberg D. Atomistic basis for the on-off signaling mechanism in SAM-II riboswitch. *Nucleic Acids Res* 2010;38(4):1392-400.
16. Gong Z, Zhao Y, Chen C, Xiao Y. Computational study of unfolding and regulation mechanism of preQ1 riboswitches. *PLoS One* 2012;7(9):e45239.
17. Mulhbacher J, Brouillette E, Allard M, Fortier LC, Malouin F, Lafontaine DA. Novel riboswitch ligand analogs as selective inhibitors of guanine-related metabolic pathways. *PLoS Pathog* 2010;6(4):e1000865.
18. Daldrop P, Reyes FE, Robinson DA, Hammond CM, Lilley DM, Batey RT, et al. Novel ligands for a purine riboswitch discovered by RNA-ligand docking. *Chem Biol* 2011;18(3):324-35.
19. Woese CR, Winker S, Gutell RR. Architecture of ribosomal RNA: Constraints on the sequence of "tetra-loops". *Proc Natl Acad Sci U S A* 1990;87(21):8467-71.
20. Leontis NB, Westhof E. Analysis of RNA motifs. *Curr Opin Struct Biol* 2003;13(3):300-8.
21. Barrick JE, Breaker RR. The distributions, mechanisms, and structures of metabolite-binding riboswitches. *Genome Biol* 2007;8(11):R239.
22. Weigand JE, Sanchez M, Gunnesch EB, Zeiher S, Schroeder R, Suess B. Screening for engineered neomycin riboswitches that control translation initiation. *RNA* 2008;14(1):89-97.
23. Duchardt-Ferner E, Weigand JE, Ohlenschlager O, Schmidtke SR, Suess B, Wohnert J. Highly modular structure and ligand binding by conformational capture in a minimalistic riboswitch. *Angew Chem Int Ed Engl* 2010;49(35):6216-9.
24. Burge SW, Daub J, Eberhardt R, Tate J, Barquist L, Nawrocki EP, et al. Rfam 11.0: 10 years of RNA families. *Nucleic Acids Res* 2013;41(D1):D226-32.
25. Rice P, Longden I, Bleasby A. EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet* 2000;16(6):276-7.
26. Capriotti E, Marti-Renom MA. Sara: A server for function annotation of RNA structures. *Nucleic Acids Res* 2009;37(Web Server issue):W260-5.
27. Rahrig RR, Leontis NB, Zirbel CL. R3d align: Global pairwise alignment of RNA 3D structures using local superpositions. *Bioinformatics* 2010;26(21):2689-97.
28. Breaker RR. Riboswitches and the RNA world. *Cold Spring Harb Perspect Biol* 2012;4(2).
29. Thompson JD, Higgins DG, Gibson TJ. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22(22):4673-80.
30. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28(10):2731-9.
31. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J Comput Biol* 1998;19(14):1639-62.
32. Chang YF, Huang YL, Lu CL. SARSA: A web tool for structural alignment of RNA using a structural alphabet. *Nucleic Acids Res* 2008;36(Web Server issue):W19-24.

33. Dror O, Nussinov R, Wolfson H. Arts: Alignment of RNA tertiary structures. *Bioinformatics* 2005;21 (Suppl 2):ii47-53.
34. Ferre F, Ponty Y, Lorenz WA, Clote P. DIAL: A web server for the pairwise alignment of two RNA three-dimensional structures using nucleotide, dihedral angle and base-pairing similarities. *Nucleic Acids Res* 2007;35(Web Server issue):W659-68.
35. Thomas JR, Hergenrother PJ. Targeting RNA with small molecules. *Chem Rev* 2008;108(4):1171-224.
36. Klosterman PS, Hendrix DK, Tamura M, Holbrook SR, Brenner SE. Three-dimensional motifs from the SCOR, structural classification of RNA database: Extruded strands, base triples, tetraloops and U-turns. *Nucleic Acids Res* 2004;32(8):2342-52.
37. Tamura M, Hendrix DK, Klosterman PS, Schimmelman NR, Brenner SE, Holbrook SR. SCOR: Structural classification of RNA, version 2.0. *Nucleic Acids Res* 2004;32(Database issue):D182-4.
38. Jaeger L, Verzemnieks EJ, Geary C. The UA_handle: A versatile submotif in stable RNA architectures. *Nucleic Acids Res* 2009;37(1):215-30.
39. Nissen P, Ippolito JA, Ban N, Moore PB, Steitz TA. RNA tertiary interactions in the large ribosomal subunit: The A-minor motif. *Proc Natl Acad Sci U S A* 2001;98(9):4899-903.
40. Cate JH, Gooding AR, Podell E, Zhou K, Golden BL, Kundrot CE, et al. Crystal structure of a group I ribozyme domain: Principles of RNA packing. *Science* 1996;273(5282):1678-85.
41. 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(7):991-1002.
42. Yan Z, Baranger AM. Binding of an aminoacridine derivative to a GAAA RNA tetraloop. *Bioorg Med Chem Lett* 2004;14(23):5889-93.
43. Yan Z, Rao Ramisetty S, Bolton PH, Baranger AM. Selective recognition of RNA helices containing dangling ends by a quinoline derivative. *Chembiochem* 2007;8(14):1658-61.
44. Yan Z, Sikri S, Beveridge DL, Baranger AM. Identification of an aminoacridine derivative that binds to RNA tetraloops. *J Med Chem* 2007;50(17):4096-104.
45. Chursov A, Walter MC, Schmidt T, Mironov A, Shneider A, Frishman D. Sequence-structure relationships in yeast mRNAs. *Nucleic Acids Res* 2012;40(3):956-62.
46. Torarinsson E, Sawera M, Havgaard JH, Fredholm M, Gorodkin J. Thousands of corresponding human and mouse genomic regions unalignable in primary sequence contain common RNA structure. *Genome Res* 2006;16(7):885-9.
47. Hendrix DK, Brenner SE, Holbrook SR. RNA structural motifs: Building blocks of a modular biomolecule. *Q Rev Biophys* 2005;38(3):221-43.
48. Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* 1987;327(6121):389-94.
49. Francois B, Szychowski J, Adhikari SS, Pachamuthu K, Swayze EE, Griffey RH, et al. Antibacterial aminoglycosides with a modified mode of binding to the ribosomal-RNA decoding site. *Angew Chem Int Ed Engl* 2004;43(48):6735-8.
50. Vicens Q, Westhof E. Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. *Structure* 2001;9(8):647-58.
51. 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(5291):1367-71.
52. Jia X, Zhang J, Sun W, He W, Jiang H, Chen D, et al. Riboswitch control of aminoglycoside antibiotic resistance. *Cell* 2013;152(1-2):68-81.