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Structure and Thermodynamics of Drug-RNA Aptamer Interactions

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Abstract: This mini-review will provide an overview on the recent studies of structure and thermodynamics of RNA aptamers that target drug molecules. These aptamers are studied to provide insight into RNA drug interactions. This interaction is important due to the many roles RNA plays in cell biology.

Key Words: Ribonucleic acid, aptamer, thermodynamics, isothermal titration calorimetry, RNA structure.

RNA IN BIOLOGY AND MEDICINE

Ribonucleic acids (RNA) participate in many cellular processes including protein translation, signalling, and regulation [1, 2]. The first function of RNA identified was the transfer of information from deoxyribonucleic acid (DNA) to proteins. The RNA was termed messenger RNA or mRNA [3]. In this model DNA is responsible for information storage and proteins are responsible for catalytic function [4, 5], with RNA simply serving as a carbon copy of DNA. The discovery that RNA was capable of catalyzing RNA cleavage changed the perception of RNA's function [6, 7]. RNA sequences that are capable of catalytic activity are called ribozymes. This discovery led to the proposal of the RNA world hypothesis which suggests that early life went through a stage where RNA was responsible for information storage and catalytic function [8].

RNA in translation

The discovery of mRNA was followed by the identification of ribosomal RNA (rRNA) [9] and transfer RNA (tRNA) [10]. rRNA interacts with specific proteins to form the ribosome [11] which is responsible for translation of mRNA code to protein sequence. It achieves this through interactions with tRNA and mRNA [12]. tRNA assists the transcription of codons by acting as a translator [13]. The ribosome brings together mRNA, based on complementary interaction of mRNA codon and tRNA anti-codon [14]. This is followed by the peptidyl transferase reaction which is catalyzed by the ribosome. [15]. The discovery that the RNA component of the ribosome is responsible for the catalytic activity further emphasizes the central role that RNA plays in biochemistry and supports the idea of an RNA world at the origin of life. This function of RNA showed that large proteins required RNA to be synthesized and were possible after RNA developed this ability [16].

RNA in regulation

In the past 20 years more classes of RNA with diverse functions have been identified; these include small interfering RNA (siRNA) [17], micro RNA (miRNA) [1], small nucleolar RNA (snoRNA) [18], piwi interacting RNA (piRNA) [19], long non-coding RNA (lncRNA) [20] and riboswitches [21]. In addition to these, RNA has also been observed recently to function as an extracellular signalling molecule [22]. These RNAs functions in conjunction with protein molecules to achieve gene regulation. siRNA and miRNA are short sequences of RNA that form double stranded strands with target RNAs via Watson Crick base pairing. These double stranded RNAs are then targeted by cellular degradation mechanisms [23]. The difference between siRNA and miRNA lies in the role they play in cellular biochemistry. siRNA are largely regarded as defense against exogenous RNA while miRNA is responsible for regulation of endogenous genes [24]. snoRNAs identify RNA for modification and guide proteins to the modification sites. These modifications, including methylation and pseudouridylation, are important for proper folding in rRNA and tRNA [25]. piRNA are a testis specific class of RNA. They participate in gene silencing similar to miRNA and siRNA, and have been found to target sequences that are prone to relocating themselves within the genome also known as transposons [26]. With the exception of mRNA the above mentioned RNA are non-coding RNA (ncRNA), meaning that they have function other than providing the code for translation. Long ncRNA are ncRNA that are longer than 200 nucleotides. lncRNA can be processed to make siRNA or miRNA but are also functional as regulators in long form [20]. Riboswitches are sequences of RNA that bind to a specific small molecule target. The binding event causes a change in structure that regulates transcription of a neighbouring gene [27].

RNA therapeutics

The diverse and central roles that RNA plays in cells make it an appealing target for molecular therapeutic and diagnostic applications. RNA can be a candidate for a potential drug molecule itself due to the variety of molecules it can interact with. Using systematic evolution of ligands by exponential enrichment (SELEX) [28, 29] it is possible to select for an RNA sequence that targets a specific molecule or cell type. RNA sequences with a high affinity for a specific target are referred to as aptamers [28]. Even though this term was initially only applied to RNAs that were selected in vitro, it has since been expanded to include naturally occurring aptamers. These natural aptamers include riboswitches which are not only able to bind to a specific target but also play a role in gene regulation [27].

Currently there are several aptamers in phase I and II clinical trials [30]. These include aptamers for treatment of coronary artery bypass [31], macular degeneration [32] and acute myeologenous leukemia [33]. Macugen is an aptamer based drug that targets vascular endothelial growth factor (VEGF) currently available for treatment of macular degeneration [34]. This aptamer is conjugated to a polyethylene glycol (PEG) to increase its lifetime in vivo. PEG increases

the lifetime of biomolecules in vivo by changing the biomolecules hydrophobicity and electrostatic binding properties [35]. PEGylation turns the main disadvantages of RNA drugs, the low lifetime due to degradation, into an advantage by allowing tailoring of the lifetime of the drug based on size of PEG. It should be mentioned that therapeutics developed with siRNA [36] and ribozymes [37] have also shown some promise and several clinical trials are in progress. However, a detailed discussion of these systems is beyond the scope of this review.

Research into the nature of the interactions between target and drug molecule has helped to improve protein targeting molecules. For example, in the case of protein kinase inhibitors structural work revealed non catalytic sites that could be targeted [38]. Investigation into the structure of VEGF and aptamer binding has revealed the interaction with the VEGF heparin binding domain [39]. More recently, the same approach is being taken with RNA targeting small molecules by using aptamer models to understand nature of interactions between RNA and small molecules.

This review attempts to provide an overview of the structural and thermodynamic information currently available for RNA aptamer interactions with proteins, RNA and small molecules. These studies form a crucial foundation for applying medicinal chemistry approaches to novel targets involving RNA.

LARGE BIOMOLECULE RNA APTAMER INTERACTIONS

Much effort has been directed at developing aptamers that target the human immunodeficiency virus (HIV) by specifically targeting the HIV regulator of virion expression (Rev) peptide, trans-activation response element (TAR) HIV RNAs or the HIV trans activator of transcription (Tat) peptide [40 – 42].

HIV Rev peptide

The HIV Rev peptide binds to the envelope gene at the Rev response element site. It is responsible for transporting mRNA that encodes viral proteins to the cytoplasm [43 – 44]. The studies of the structures of HIV rev peptide in complex with RNA aptamers demonstrate how RNA can influence the structure of its ligand. Depending on the RNA sequence the peptide binds in either a α-helical or elongated conformation [42, 46]. This knowledge is important if the peptide RNA complex is to be targeted by inhibitors.

HIV Tat

The HIV Tat peptide binds to the TAR HIV RNA and is necessary for transcription initiation, a vital part of the viral life cycle [47]. The HIV Tat aptamer complex was initially studied by using the simplest analog of Tat, an argininamide molecule [41]. Argininamide was used as an analog of Tat since the peptide is arginine rich and an arginine residue was implicated in binding to the TAR region [48]. This structure revealed the aptamer was binding two argininamide molecules suggesting that it interacts with two arginine residues on the peptide [41]. Inhibition of binding of Tat to TAR was observed when Tat is captured by aptamer, demonstrating that the aptamer is binding either at or near the TAR binding site [49].

TAR-HIV RNA

The interaction of the aptamer selected against TAR-HIV RNA reveals a kissing complex where non canonical RNA-RNA interactions along with stacking interactions create a stable intermolecular structure (Figure 1) [50]. These types of complexes are important to the viral life cycle. For example, the HIV-1 genomic RNA dimerization initiation site (DIS) kissing complex formed by two DIS elements triggers viral RNA dimerization [51]. It has been shown that the hydrogen bonding network within the kissing complex along with intermolecular stacking interactions is responsible for molecular recognition [40].

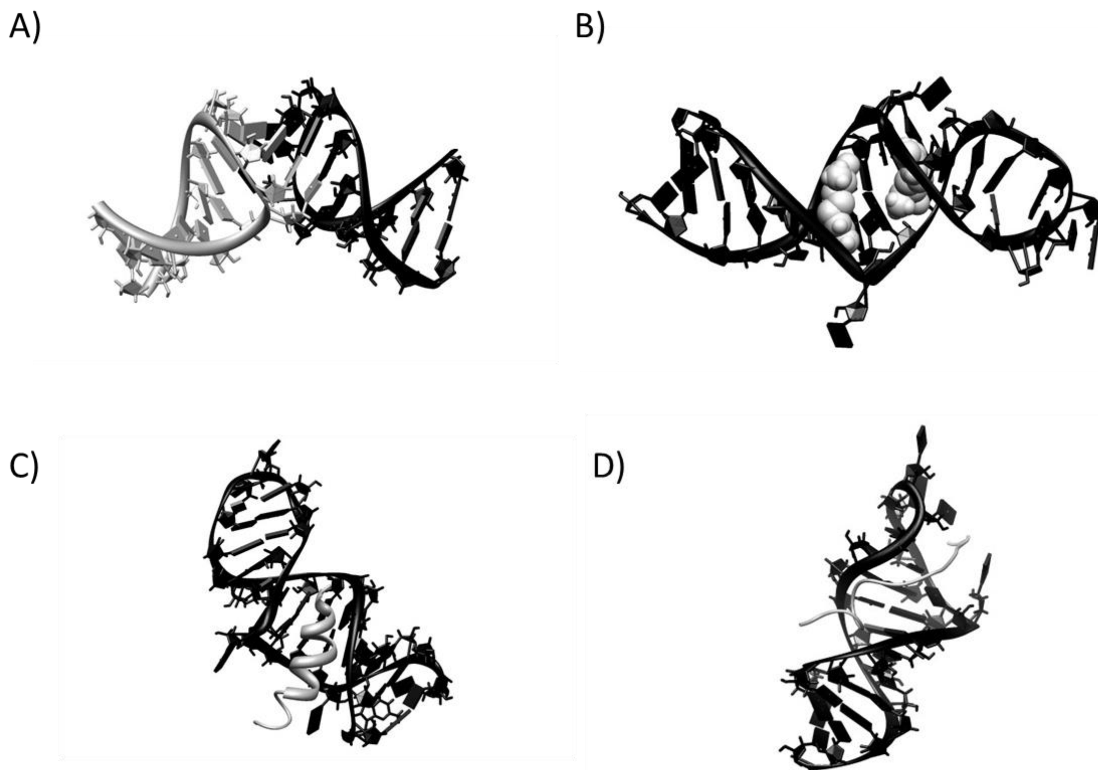

Figure 1. HIV related RNA aptamers. Aptamers in black, Target molecules in grey. A) TAR – TAR aptamer complex, PDB ID 2RN1 [50], showing the “kissing” interaction. B) HIV Tat aptamer in complex with two argininamide molecules, PDB ID 1NBK [41], showing the RNA wrapped around the molecules. C) HIV Rev peptide aptamer complex, PDB ID 1ULL [46], showing the peptide in the groove of the RNA. D) HIV Rev peptide aptamer complex, PDB 484D [42], showing the extended peptide bound to aptamer. Figure was generated using UCSF Chimera [52].

MS2 coat protein

Another virus that has been targeted for aptamer development is bacteriophage MS2. This virus has been used as a model system to study RNA-protein interactions [53, 43]. The MS2 coat protein is responsible for recognition of a stem loop within the genome and binding to this loop is part of a process that initiates encapsidation [55]. Structural investigations of this aptamer and MS2 coat protein revealed that the aptamer binds to the protein in a similar manner to other stem loop RNA’s. This binding interaction involves the phosphodiester backbone of the RNA. Phosphates of the backbone undergo subtle rearrangements that lead to different affinities when mutations were studied. The effect of rearrangement of the phosphate backbone changes the hydrogen bonding network, Horn et al. suggest that predicting these changes will lead to better *de novo* design of RNA binding ligands [56].

RNA polymerase

Viral RNA interactions are not the only RNA interactions of interest that have been addressed by aptamer development. Other targets include RNA polymerases and initiation factors. RNA polymerases, the enzymes responsible for transcription of DNA to RNA can be inhibited by binding of RNA molecules [57, 58]. Structural work on the RNA aptamer bound to RNA polymerase II (Figure 2A) highlighted a possible mechanism for inhibition. The aptamer binding blocks the initiation center so template DNA cannot reach the catalytic center. B2 RNA is a ncRNA that supresses mRNA transcription by inhibiting RNA polymerase II in response to heat shock [59]. Competitive binding of B2 RNA and RNA polymerase II RNA aptamer to RNA polymerase II was investigated by Kettenberger et al. The absence of B2 RNA binding revealed by these

experiments suggests that B2 RNA may bind in the same way as the aptamers [60]. The 6S RNA also binds to RNA polymerase II. Based on the RNA aptamer that binds in A-form within the RNA polymerase II cleft it was suggested that this maybe how 6S RNA binds [58]. This observation expands the understanding of how the RNA polymerase II cleft interacts with RNA, in addition to accommodating B-form DNA templates [60].

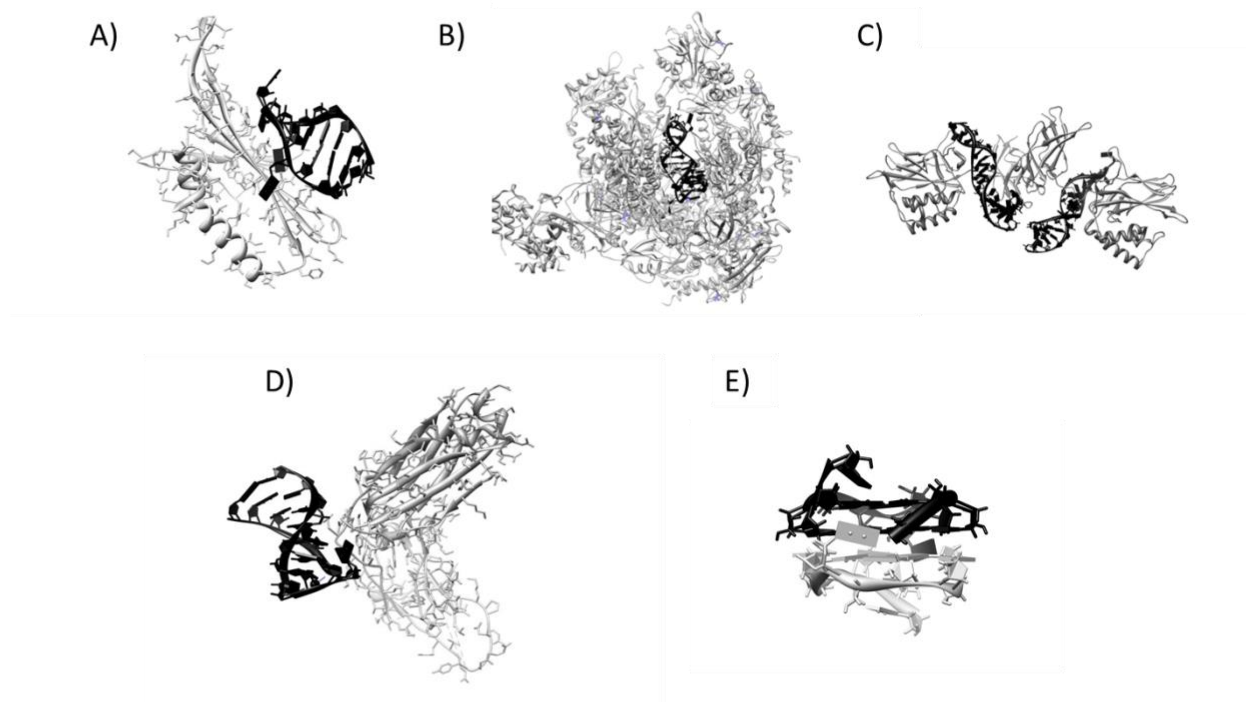

Figure 2. RNA aptamer (black) bound to protein targets (grey) A) MS2 coat protein, PDB ID 1U1Y [56], showing the loop interaction between protein and RNA. B) RNA polymerase II, PDB ID 2B63 [60], showing the aptamers buried within the polymerase. C) NF-κb homodimer, PDB ID 1OOA [61], showing the RNA helix interacting with protein. D) Human IgG, PDB ID 3AGV [70], showing interaction between RNA and specific site on protein (Tyr 333). E) Prion protein aptamer, PDB ID 2RQJ [66], this structure lacks the target protein, however the quadruple structure shown here is key to the interaction with protein, in this case both black and grey represent RNA. Figure was generated using UCSF Chimera [52].

Transcription and Initiation factors

Transcription factors are proteins that bind to the DNA sequences regulating transcription. The NF-κb factor is involved in activating genes and has been the target of therapeutic inhibition of cancer cells [62]. In the case of the mammalian transcription factor NF-κb binding, the RNA aptamer mimics DNA structure (Figure 2B). The pre-bound structure of the RNA resembles B-form DNA. The RNA upon binding to NF-κb adapts to the presence of the protein [63]. Initiation factors are proteins that bind to the ribosome during translation. The mammalian initiation factor 4A binding interaction with its RNA aptamer were studied by obtaining structures of individual RNA aptamer loops and evaluating the interactions with the initiation factor. These studies suggest that binding involves the interaction of the aptamer's AUCGCA loop with the initiation factor [64]. Future structural work may reveal how these aptamers could interfere with the different factors and affect protein expression.

Prion protein

RNA aptamers have also been used to study protein targets whose primary function does not involve interaction with RNA, such as prion proteins and immunoglobulins. Prion proteins are infectious particles responsible for diseases like Creutzfeldt Jacob disease and spongiform encephalopathy [65]. The RNA aptamer for bovine prion utilizes the electrostatic interaction between its phosphate

backbone and lysine clusters, along with stacking interactions between its GGGG quadruplex and a tryptophan residue to achieve binding [66]. Prion protein has been found to bind to amyloid–β oligomers leading to synaptic dysfunction. This aptamer has been proposed for development as a therapeutic agent for Alzheimer's disease, since the prion protein plays a role in impairment of synaptic plasticity [67]. The prion aptamer has already been shown to reduce the formation of prion protein in infected cells, most likely by preventing prion protein from interacting with native proteins [68].

Human immunoglobulin G

Human immunoglobulin G (IgG) is a protein responsible for identifying pathogens [69]. The structure of human IgG bound to its aptamer reveals the interactions that make the RNA specific for this particular immunoglobulin. The interactions involve stacking of bases with Tyr373; unlike other protein RNA interactions the binding is not driven by the interaction of negatively charged phosphate and positively charged surface of protein [70]. This is significant because it has expanded the type of ligand that can be considered a target for aptamer development.

DRUG/SMALL MOLECULE RNA APTAMER INTERACTIONS

Neomycin

he antibiotics that target rRNA have been investigated further by the development of aptamers that bind them with high affinity and specificity. Neomycin B is an aminoglycoside antibiotic that inhibits a variety of RNA-mediated biological functions [71]. The structure-function relationship of Neomycin B and other aminoglycosides has been studied in detail with respect to the ribosome. Two binding sites have been identified, one on helix 44 of the small ribosomal unit and the other on helix 69 of the large ribosomal subunit. In the small ribosomal subunit, neomycin binds within a pocket formed by an A:A base pair and an unpaired A. These residues are responsible for increasing affinity to cognate tRNA–mRNA pairings. With the antibiotic bound the ribosome is immobilized into a high affinity configuration that recognizes both cognate and non-cognate tRNA-mRNA complexes thereby leading to miscoding [72]. In the large ribosomal subunit it binds within helix 69 with binding stabilized by contacts including a CAAUAC loop and G1906. This helix is swung away from an interface of subunit association by the ribosome recycling factor (RRF). Binding of the antibiotic restores interactions destabilized by the RRF and inhibits ribosome recycling [73]. The structure of Neomycin B in complex with HIV-1 DIS (previous mentioned) has since been crystallized. This binding interaction is similar to that involved in the small ribosomal unit [74 – 76]. These structures reveal that the mode of interaction between RNA and Neomycin B can vary. The binding of Neomycin B to its aptamer has been investigated to obtain a better understanding of aminoglycoside-RNA interactions, Figure 3 shows a few of the different structures of Neomycin B RNA binding pockets.

Neomycin B binds to the aptamer within a pocket consisting of three consecutive GU base pairs and a flap formed by an adenine base [77]. This pocket is different from the aforementioned binding sites. Further investigation of this interaction showed that hydrogen bonding interactions between aptamer and ligand are the major mechanism responsible for binding [79]. A more recent study used NMR techniques to show that protonation of Neomycin B accompanies binding its cognate aptamer, demonstrating the ability of RNA to influence its ligand [80]. The thermodynamic differences in binding of these complexes and other RNA complexes will be discussed in the following section. Other antimicrobial agents have been used as targets for development of aptamers. These include tetracycline, tobramycin and streptomycin. Characterization of these complexes has illuminated different aspects of RNA small molecule interactions and most of these aptamers have also been used to develop biosensors for medical applications [80]. For example the tobramycin aptamer has been incorporated as part of an aptasensor that can detect tobramycin and other aminoglycosides in human serum [82].

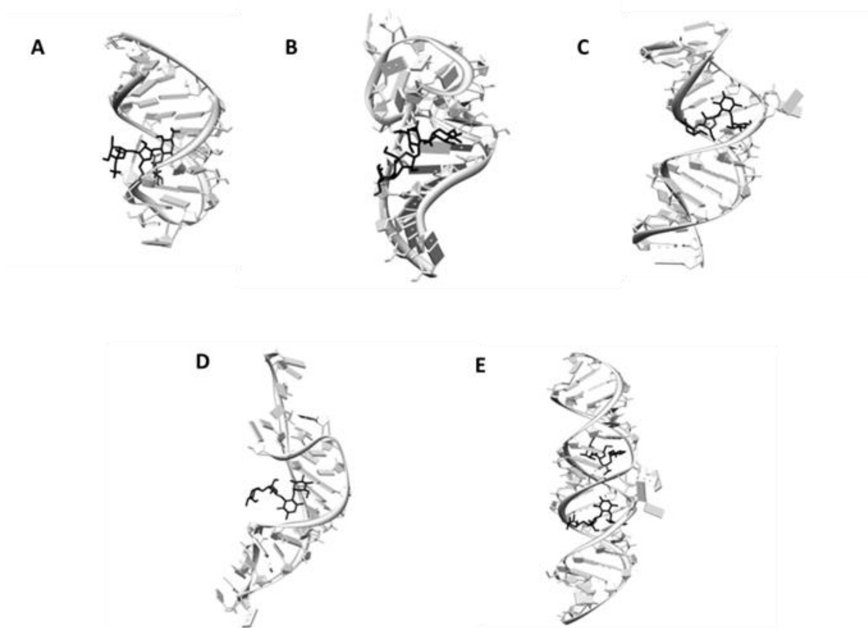

Figure 3. Neomycin B bound to RNA. A) Neomycin B aptamer, PDB ID 1NEM [77], B) HIV TAR, PDB ID 1QD3 [78], C) 30S ribosome Neomycin binding site, PDB ID 2QAN [73], D) 50S ribosome Neomycin B binding site, PDB ID 2QAO [74], E) HIV DIS complex, PDB ID 3C7R [75]. Neomycin B is coloured black. RNA is coloured grey. This figure shows the variety of different RNA sequences that can interact with Neomycin B. Figure was generated using UCSF Chimera [52].

Tetracycline

Tetracycline, tobramycin and streptomycin are antibiotics that like neomycin, target the bacterial ribosome. The tetracycline aptamer boasts a more complicated aptamer structure than usually obtained by SELEX against a small molecule [83]. The tetracycline aptamer consists of three helices, creating a binding site out of tertiary contacts between the helices. ITC studies of the tetracycline riboswitch revealed a two-step binding mechanism driven by favourable enthalpy. Tetracycline's interaction with the 30S ribosomal subunit is stabilized by hydrogen bonding interactions as opposed to the aptamer which has stacking interactions with two rings of tetracycline [84].

Tobramycin and Streptomycin

The structures of two aptamers selected for tobramycin have been solved [85, 86]. Both aptamer tobramycin complexes revealed that the antibiotic binds within a deep groove and has multiple hydrogen bonding contacts with the RNA. While both aptamers share the binding motif, one uses a base bulge to widen RNA major groove while the other has three mismatch pairs. Since both aptamers have comparable binding affinities this example reveals that changes to the binding site can be diverse. The streptomycin aptamer has also been studied by the same group that investigated the structure of tobramycin and neomycin aptamers [87]. This aptamer consists of S turns that create a cavity to encapsulate streptomycin.

Purine

Naturally occurring aptamers consist mainly of riboswitches, these sequences affect the secondary structure upon binding its ligand to allow for transcription of the following sequence. Similarity between an artificially selected aptamer and naturally occurring RNA can be seen in purine binding RNAs. The structure of the purine riboswitch has been obtained and reveals that the ligand is encapsulated by the RNA [88]. Kinetic studies of this binding interaction suggest that the ligand first engages in Watson-Crick base pairing with Y74 followed by a loop closure that traps the ligand in the binding pocket and subsequent formation of hydrogen bonds between ligand and RNA. Furthermore mutation studies of the purine riboswitch reveal the ability of RNA to form a bound-like structure through ligand mimicking. The term ligand mimicking is used to describe a receptor mimicking the presence of a ligand with a residue that has characteristics of the

ligand. The mutant purine riboswitch is inactive, with one of its nucleotides (G39) occupying the active site [89]. While the structure is not yet available other biochemical methods suggest that an artificially selected adenine aptamer has a similar dependence on hydrogen bonding versus stacking interactions [90]. The guanine aptamer has a consensus sequence similar to tobacco ring spot virus satellite RNA hairpin ribozyme [91]. The part of purine riboswitch sequence that matches closest to the guanine aptamer is involved in binding (Figure 4). The purine riboswitch family has been reviewed in more detail [92, 93].This family includes the purine riboswitch discussed above as well as preQ [93], cyclic-dGMP [95] and tetrahydrofolate [96] binding riboswitches.

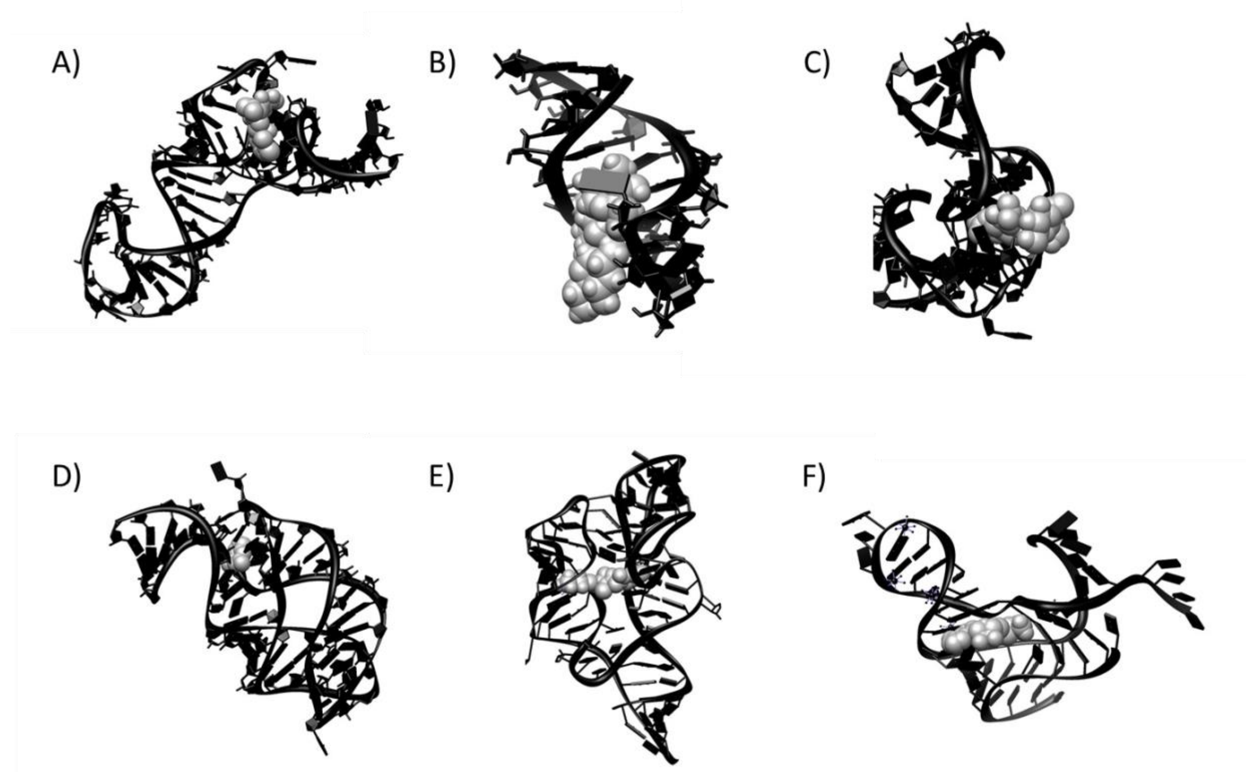

Figure 4. RNA aptamers (black) complexed with small molecules (grey). A) Tetracycline aptamer complex, PDB ID 3EGZ [84], showing three helix wrapping around target. B) Tobramycin aptamers complex, PDB ID 2TOB [85], showing target binding within deep groove. C) Streptomycin aptamers complex, PDB ID 1NTB [87], showing S turn wrapping target. D) Purine aptamer complex, PDB ID 2B57 [88], showing target buried within aptamer. E) TPP aptamer complex, PDB ID 3K0J [99], showing target interacting with two stems. F) AdoMet aptamer complex PDB ID 3NPQ [102], showing the target interaction with RNA. Figure was generated using UCSF Chimera [52].

Thiamine pyrophosphate

Thiamine pyrophosphate (TPP) is a cofactor that regulates expression of proteins that are involved in the biosynthesis and transport of thiamine [97, 98]. The regulation occurs via the TPP riboswitch. TPP binds to the riboswitch between two parallel stems [99]. Investigation into the folding on this riboswitch has shown a two-step binding process involving first ligand independent secondary structure formation followed by ligand dependent tertiary structure formation [100].

S-Adenosyl methionine

The S-Adenosyl methionine (AdoMet) riboswitch is important in the regulation of amino acid biosynthesis [101]. The structural study of the AdoMet bound aptamer revealed that the interaction between RNA and AdoMet primarily involves the

adenosine of AdoMet. Investigation of the interaction of other metabolites with the AdoMet riboswitch has suggested possible regulatory roles [102]. The AdoMet riboswitch is a potential target for antimicrobials due to its regulatory role in bacteria [103].

THERMODYNAMICS OF RNA-LIGAND INTERACTIONS

The binding of two molecules is generally discussed in terms of the free energy change (ΔG), the enthalpy change (ΔH), the entropy change (ΔS) and dissociation constant (K_d). The thermodynamic parameters involved in binding interactions are related to each other by the Gibbs Helmholz equation (Equation 1).

$$\Delta G = \Delta H - T\Delta S \qquad \text{(Equation 1)}$$

ΔG is related to the binding affinity by Equation 2, with R being the gas constant and T the absolute temperature.

$$\Delta G = RT \ln(1/K_d) \qquad \text{(Equation 2)}$$

It is apparent from these equations that binding is stronger, K_d lower, the more negative the value of ΔG is. From Equation 1 it can be gleaned that a negative ΔH will push the value of ΔG to a larger negative value. Likewise, a positive value for ΔS will push the value of ΔG to a larger negative value. A negative value of ΔH will be referred to as favourable ΔH and is associated with the increase of interactions between ligand and receptor, while a positive value of ΔH will be referred to as unfavourable ΔH and is associated with reducing number of interactions [104]. A negative value of ΔS will be referred to as unfavourable ΔS and is associated with a decrease in randomness (*e.g.* restricting movement) while a positive value of ΔS will be referred to as favourable ΔS and is associated with an increase in randomness (*e.g.* release of water molecules) [104].
Table 1 shows a summary of thermodynamic data for binding of the above mentioned RNA targets. The general trend is that binding is driven by favourable ΔH which is countered by unfavourable ΔS. The two outliers to this trend are the purine riboswitch and the DIS HIV kissing loop.

Table **1**: Summary of RNA Binding Thermodynamic Data.

Target	RNA	ΔH(kcal/mol)	ΔS(kcal/mol/K)	ΔG(kcal/mol)	K_d (nM)	References
Neomycin B	Aptamer	-21.6	-4.40E-02	-8.49	6.90E+02	[78]
Tetracycline	Aptamer	-22.9	-3.52E-02	-12.4	1.00E+00	[95]
Purine	Riboswitch	-40.3	-9.76E-02	-10.7	1.70E+01	[87]
AdoMet	Riboswitch	-24.0	-4.62E-02	-10.2	3.20E+01	[92]
Tyrosinamide	DNA Aptamer	-23.7	-5.39E-02	-7.64	2.44E+03	[96]
Malachite green	Aptamer	-25.9	-5.69E-02	-8.79	2.80E+02	[97]
Neomycin B	DIS kissing-loop	-9.4	2.70E-03	-10.2	3.40E+01	[73]
Neomycin B	Ribosomal A site	-16.2	-2.79E-02	-7.90	1.55E+03	[98]
TPP	Riboswitch	-58.1	-1.62E-02	-7.70	2.00E+02	[100]

The purine riboswitch has a higher amount of favourable enthalpy change while DIS HIV kissing loop has a lower ΔH. It is interesting to note that in both cases the variation of favourable ΔH is compensated by the inverse adjustment in unfavourable ΔS. The DIS HIV binding has a 20% greater amount of ΔG from charge related interactions than the small ribosomal binding site [74]. While the purine riboswitch has a significant amount of hydrogen bonding [88], this could explain the ΔH differences. These two examples suggest that artificially selected aptamer binding follows a different trend compared to the naturally occurring RNA small molecule interactions. However examining the AdoMet riboswitch the thermodynamic parameters are almost identical to the aptamers. This suggests that there is still too small a set of data to make generalizations about the thermodynamic trends. The similarity in energetics of binding is eclipsed by the specificity of each binding pocket created by differences in sequence and geometry of the binding pocket (Table 2).
An interesting trend is observed in the investigation of small molecule binding aptamers, binding involves

two stages [84, 105,100]. The first stage is an interaction independent of the target and the second stage is target dependent. The knowledge of these thermodynamic distinguishable steps should provide for clues to improve target recognition.

Table 2: RNA Sequences For Various Targets With Structural Features Identified To Showcase The Difference In Sequence And Structure.

Target	Sequence	Notable Structural Features	PDB	Ref#
Neomycin B	GGACUGGGCGAGAAGUUUAGUCC	three consecutive GU base pairs	1NEM	[77]
Tetracycline	GGGCCUAAAACAUACCAGAGAUCGCCACCCGCGCUUUA AUCUGGAGAGGUGAAGAAUACGACCACCUAGGCUC	three helices	3EGZ	[84]
Tobramycin	GGGACUUGGUUUAGGUAAUGAGUCCC	UG,GU,GA,UU base pairs	2TOB	[85]
Streptomycin	GGAUCGCAUUUGGACUUCUGCC/CGGCACCACGGUCGG AUC	S turns	1NTB	[87]
Purine	GCGAGUAUAACCUCAAUAAUAUGGUUUGAGGGUGUCUA CCAGGAACCGUAAAAUCCUGACUACUCGC	Encapsulating ligand, base pairing with Y74	2B57	[88]
Guanine	GGCACGUGUAUUACCCUAGUGGUCGACGUGCC	structure not available	n/a	[89]
AdoMet	GGACGAGGAGCGCUGCAAGCGAGAGCCCGAAGCUCGU CCGUUCAAACGGCGCUCA	Psuedoknot	3NPQ	[102]
TPP	GCGACTCGGGGTGCCCTTCTGCGTGAAGGCTGAGAAATA CCCGTATCACCTGATCTGGATAATGCCAGCGTAGGGAAG TCGC	Two parallel stems	2HOJ	[99]
HIV Rev	GGUGUCUUGGAGUGCUGAUCGGACACC	UAU triple, hydrogen bonding with Arg 35 and Arg 39, elongated form	484D	[42]
HIV Rev	GGCUGGACUCGUACUUCGGUACUGGAGAAACAGCC	UAU triple, stacking of Trp45, helical form	1ULL	[46]
HIV TAR	GGCUGGUCCCAGACGACC	kissing complex	2RN1	[50]
HIV DIS	CUUGCUGAAGUGCACACGCAAG	kissing complex	3C7R	[75]
HIV Tat	GGGAGCUUGAUCCCGGAAACGGUCGAUCGCUCCC	two adjacent UAU triples	1NBK	[41]
MS2 coat protein	CCGGAGGUCACCACGGG	A10 makes three hydrogen bonds with target	1U1Y	[56]
RNA polymerase II	ACAGCACUGAUUGCGGUCGAGGUAGCUUGAUGG	A-form helix	2B63	[60]
Prion	GGAGGAGGAGGA	GGGG tetraplane	2RQJ	[66]
Human IgG	GGAGGUGCUCCGAAAGGAACUCCA	G7 stacking with Tyr373	3AGV	[70]
NF-κb factor	GAUACUUGAAACUGUAAGGUUGGCGUAUC	resembles B-form DNA	1OOA	[61]
Mammalian factor 4A	GGAGAUCGCACUCCACAUGUGAGUGAGGCCGAAACAUA GAUUCGAGAGGAGGCUCACA	AUCGCA loop	n/a	[64]

CONCLUSIONS

A common trend seen with the aptamers selected for larger biomolecules is that they mimic the type of interactions that already exist in organisms. This is seen clearly in the aptamer for TAR HIV RNA, which forms a kissing complex similar to that seen in HIV DIS [50, 51]. This phenomenon is implied by the work with RNA polymerase II aptamer and B2RNA competitive binding to RNA polymerase II [59].

In the case of aptamers for antibiotics the naturally occurring RNA that binds to the same targets has clear differences in binding pockets. This is a reflection of the evolution of RNA, since the naturally occurring RNA did not have a selection pressure driven purely by affinity to target, but rather by their function within the system (cell/virus). As

seen in the case of tobramycin with two aptamers that use different means to widen binding pocket [85, 86] and multiple ATP aptamers [106] it is possible to have variations of sequences that have strong binding affinity and nature has had multiple variations to explore other functions.

The closer relatives to artificially selected aptamers, riboswitches, share similarity, at least in the case of adenine. Another example of similarity in sequence between artificially selected aptamer and naturally occurring RNA is that of the ATP aptamer binding motif which was found in a bacterial virus [107]. The aptamers selected for small molecules do provide a window into the capacity for function inherent to RNA, and the roles it could have played in a prebiotic world.

The number of aptamers is increasing as methods to select them become more routine and even include *in silico* techniques [108]. 2',4'-Locked RNA aptamers are aptamers which include a 2'-O, 4'-C methylene link, which confers a C3' endo conformation. This change results in an entropic constraint that leads to increased affinity for their target [109]. This along with other developments in degradation resistant nucleotides [110] promises many applications for aptamers to come.

RNA binding usually involves adaptive interactions. This has been seen for binding to peptides and small molecules [42, 111 – 113]. This behavior makes it important to probe the conformational mobility and thermodynamics of binding so that the aptamer target molecule interaction can be fully characterized. This line of investigation can be supplemented by site-directed modification of the aptamer to change the spatial configuration or the energy of the binding interaction. For example the binding affinity of tRNA gln to glutaminyl-tRNA synthetase was improved by studying the related aptamer. The improvements did not involve the RNA protein contacts but instead were driven by tertiary structure stabilization of the RNA [114]. Another important reason for the study of structure and thermodynamics of RNA-ligand interactions is to understand the nature of RNA-induced modifications of the ligand itself. A good example of such behaviour is seen in the conformational changes induced in malachite green molecules binding to their cognate aptamer, and these changes are significant enough to change the optical properties of the dye molecule [115]. This type of binding interaction could expand modalities of drug interactions, such as aptamer-assisted pro-drug delivery.

Understanding the nature of the interactions between target and drug molecule has improved the rational design of protein targeting molecules [38]. The structure-function paradigm is being applied to rational design of biologically active therapeutic RNA. This effort is assisted by the use of aptamer models to understand nature of RNA-molecule interactions.

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