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Thermodynamics and kinetics of rna tertiary structure formation in the junctionless hairpin ribozyme

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Abstract

The hairpin ribozyme consists of two RNA internal loops that interact to form the catalytically active structure. This docking transition is a rare example of intermolecular formation of RNA tertiary structure without coupling to helix annealing. We have used temperature-dependent surface plasmon resonance (SPR) to characterize the thermodynamics and kinetics of RNA tertiary structure formation for the junctionless form of the ribozyme, in which loops A and B reside on separate molecules. We find docking to be strongly enthalpy-driven and to be accompanied by substantial activation barriers for association and dissociation, consistent with the structural reorganization of both internal loops upon complex formation. Comparisons with the parallel analysis of a ribozyme variant carrying a 2'-O-methyl modification at the self-cleavage site and with published data in other systems reveal a surprising diversity of thermodynamic signatures, emphasizing the delicate balance of contributions to the free energy of formation of RNA tertiary structure.

Abstract

Keywords

Binding Thermodynamics; RNA Catalysis; RNA-RNA Interactions; RNA Tertiary Structure; Surface Plasmon Resonance

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INTRODUCTION

More than two decades of increasingly comprehensive structural-biology studies have revealed that RNA can take on a wide variety of intricate three-dimensional structures that rival globular proteins in size and complexity.^{1–6} Nevertheless, the fundamental understanding of the energetics, thermodynamics, and driving forces of RNA tertiary-structure formation is substantially less advanced than that for polypeptides. In proteins, secondary structure units are rarely stable outside of the context of a folded domain with a buried hydrophobic core that provides the central driving force for folding. RNA tertiary structure, by contrast, is generally constructed from interactions between independently-existing modules of secondary structure (double helices and related structures), with most of the energetically-favorable nucleotide base stacking interactions forming at the secondary structure level.^{9;10} The association of helices into higher-order structures has long been observed to depend on multivalent cations,¹¹ and clusters of metal ions are found at the physical core of some RNA structures.¹⁵ Although these cations can be crucial to overcoming the unfavorable contribution of closely-juxtaposed phosphate groups, the dehydration and coordination of multivalent cations appear to provide a positive driving force for tertiary structure formation in only a minority of cases.¹⁸ A general answer to the question of the source of the favorable free energy change for RNA tertiary folding has remained elusive.

A great deal of thermodynamic data on RNA helix annealing and secondary structure formation is available.^{10;19;20} By contrast, the dataset for enthalpic and entropic contributions to the association of secondary structure elements into higher-order structures is extremely limited. One difficulty in the experimental analysis of RNA tertiary structure is the relative rarity of cases in which intermolecular association between RNA molecules involves the formation of tertiary structure in isolation from strong contributions of secondary structure annealing.^{8;13;14;17;21} The more common experimental situation of tertiary structure formation upon addition of multivalent metal ions to a unimolecular system is difficult to analyze thermodynamically since the enthalpy and entropy changes of the RNA structural transition generally cannot be isolated from those of the RNA-metal ion interactions.

The hairpin ribozyme is a paradigmatic member of the small, self-cleaving class of catalytic RNAs.^{22–24} The ribozyme consists of two RNA internal loops, termed loop A and loop B, that interact (dock) in minor groove-minor groove fashion to form an integrated active structure that cleaves a specific phosphodiester bond within loop A (Figure 1). Multivalent metal ions are required to drive the docking transition, but appear not to directly participate in reaction chemistry, as full activity and indistinguishable limiting k_{cat} values are observed when using the physiological ligand Mg^{2+} and substitution-inert hexamine cobalt $\text{Co}(\text{NH}_3)_6^{3+}$.^{25–27} Mg^{2+} and $\text{Co}(\text{NH}_3)_6^{3+}$ also have very similar effects on reaction kinetics upon phosphorothioate substitution or pH variation and on the formation of the docked complex.^{25;27–29} The reaction mechanism is thus understood to be essentially identical when supported by a variety of multivalent or even monovalent cations,³⁰ although some subtle differences in docked structure are observed.^{31;32} The hairpin has been studied intensively by a wide variety of experimental and calculational methods.^{16;23;24;33–44} NMR

structural models of the isolated loops have been determined, as have numerous crystal structures of the docked form.^{7;45–53} The docking of ribozyme loops A and B is accompanied by dramatic structural rearrangements of both loops (Figure 2). Since docking brings the helices distal from the junction into close proximity, fluorescence resonance energy transfer (FRET) is an effective assay for docking in unimolecular formats, and bulk, time-resolved, and single-molecule FRET studies have played a major role in structure-function studies of the ribozyme.^{54–62}

The docked complex of the hairpin is an intricate and tightly-integrated structure held together by multiple interactions (Figure 2). Notably, the formation of the docked complex is a purely tertiary structure transition that is not accompanied by any strand-strand annealing or helix formation processes. The hairpin is commonly studied in the naturally-occurring four-way junction form (Figure 1) or in a two-way junction (hinged) construct, but is also active in a junctionless form with loops A and B on separate molecules and docking taking place in intermolecular fashion.^{28;63} Our group reported a combined circular dichroism (CD), surface plasmon resonance (SPR), and ribozyme-kinetic analysis of the intermolecular docking transition for single loop A and loop B species at 25 °C, including a comparison of the $\text{Co}(\text{NH}_3)_6^{3+}$ requirements for docking and catalysis.¹⁶ Notably, using SPR, we observed submicromolar loop-loop binding but unusually slow on- and off-rates (see Table 1, line 1 below),¹⁶ consistent with the extensive structural changes necessary for the conversion of each loop structure to the docked conformation. Despite extensive efforts in our laboratory, the slow kinetics of docking have prevented the unambiguous analysis of the thermodynamics of the interaction using calorimetric techniques.

In the current work, we determined the temperature dependence of rate and equilibrium constants for $\text{Co}(\text{NH}_3)_6^{3+}$ -driven intermolecular docking and undocking in the hairpin ribozyme using SPR, allowing the extraction of thermodynamic parameters and activation barriers for the process. Intriguing differences from previously-reported thermodynamic studies in unimolecular constructs were observed, with the current results presumed to reflect the isolated contributions of tertiary structure formation at the loop-loop interface. Comparisons of these results with a ribozyme variant carrying a 2'-*O*-methyl modification at the self-cleavage site emphasize the delicate balance of contributions to the free energy of formation of RNA tertiary structure.

MATERIALS AND METHODS

Experiments were performed with separate constructs for loop A and loop B, each incorporating a GNRA tetraloop distant from the docking interface for structural stabilization (Figure S1). Loop A molecules were synthesized by Dharmacon Inc. (Lafayette, CO) and contained either an unmodified or a 2'-*O*-methyl modified nucleotide at the fifth position (A-1) along with a 5'-biotin tag. Wildtype and A38C mutant loop B molecules were prepared by *in vitro* transcription and purified using a gel filtration column.⁶⁴ SPR experiments were performed on a Reichert SR7000DC two-channel instrument with loop A immobilized on the optical chip using an avidin-biotin linkage. The SPR running buffer was 20 mM HEPES pH 7.5 containing 20 μM EDTA and 250 μM $\text{Co}(\text{NH}_3)_6^{3+}$. This concentration of $\text{Co}(\text{NH}_3)_6^{3+}$ is saturating for docking in our constructs.¹⁶

The reference channel in all cases contained loop A carrying a point mutation that prevents docking (G+1A). Reported curves are the difference between sample and reference channels and thus reflect only specific, sequence-dependent ribozyme docking events.^{16;39} Data were obtained at a series of loop B concentrations and fit to a global single-site binding model incorporating bulk refractive index shift. Representative SPR data are shown in Figure 3. Detailed experimental procedures are given in Supporting Information.

RESULTS

In the hairpin and other self-cleaving ribozymes, structural and biophysical studies are often performed with a 2'-deoxy or 2'-*O*-methyl modification of the nucleophilic ribose at the cleavage site in order to inhibit the self-cleavage reaction. Our earlier work in the hairpin system incorporated a 2'-*O*-methyl at nucleotide A-1 in loop A for this purpose.¹⁶ Some data in the hairpin, however, have hinted that this modification may interfere with the detailed mechanism of loop-loop docking.^{50;65–67} In order to probe the precise thermodynamic effects of this modification in the junctionless construct, we investigated other possibilities for inhibiting ribozyme cleavage. Specifically, we performed assays with inactivating mutations present at loop B residue A38, which is understood to play a key general-acid/general-base role in the ribozyme chemistry.^{24;37;51;66;68} Long-term room temperature incubations of wild-type loop A with A38 loop B mutants at concentrations equal to or higher than those used here led to negligible observed cleavage of loop A (Minako Sumita and C.G.H., data not shown). In the presence of the 2'-*O*-methyl modification, very similar docking parameters for wild-type and A38C loop B were obtained (Table 1), consistent with the lack of participation of this residue in energetically critical docking interactions (Figure 2).⁵¹ Comparable results were obtained in initial experiments with A38U (data not shown). We therefore proceeded to assay docking between unmodified (all-RNA), cleavable loop A and A38C loop B (Table 1). Interestingly, at 25 °C, an approximately threefold increase in affinity upon removal of the cleavage-site modification was observed. This increased affinity arose entirely from a decrease in the docking off-rate, consistent with potential effects of the *O*-methyl group on the formation of native interactions in the docked state.^{65–67} The Walter group observed an even more significant off-rate-dominated increase in docking upon removal of the *O*-methyl group at the cleavage site using single-molecule kinetic “fingerprinting” studies in two-way junction constructs.⁶⁵ The current data, obtained using more direct methods in a very different form of the ribozyme, complement these results and suggest that the effect is general. For the unmodified species, the K_d reported here of (114 ± 33) nM, $\Delta G^\circ_{\text{dock}} = (-9.47 \pm 0.17)$ kcal mol⁻¹ at 298 K, shows an affinity similar to or tighter than other thermodynamically-analyzed RNA systems (see below), although much more stable RNA tertiary interfaces in systems such as the P5abc domain of the group I intron have been reported.⁶⁹

To investigate the detailed thermodynamics and kinetics of docking, we performed SPR assays using unmodified 2'-OH loop A and A38C loop B in duplicate at five temperatures varying from 20 °C to 50 °C. Individual SPR experiments at 20 °C and 40 °C are shown in Figure S2 and full results are reported in Table S1 and plotted as a function of temperature in Figure S3. A van't Hoff plot of the data was linear within the precision of the data (Figure 4) and yielded thermodynamic parameters of $\Delta H^\circ = (-11.2 \pm 2.4)$ kcal mol⁻¹ and $\Delta S^\circ = (-5.7$

± 4.8) cal mol⁻¹ K⁻¹. Thus, docking of these species is strongly enthalpy-driven, with the entropy change small and unfavorable ($-T \Delta S^\circ = +1.7$ kcal mol⁻¹ at 298 K).

By comparison, time-resolved FRET measurements of unimolecular hairpin folding with deconvolution of annealing contributions by the Millar group also found enthalpy-driven docking, with ΔH° varying from -4.1 kcal mol⁻¹ to -9.7 kcal mol⁻¹ depending on the nature of the junction.⁵⁷ The FRET measurements were performed in the presence of interdomain junctions with a 2'-deoxy group at the cleavage site and in the presence of subsaturating (1 mM) Mg²⁺, making quantitative comparisons to our results difficult. Nevertheless, these workers noted a substantial curvature of van't Hoff plots, requiring ΔC_p° values between -510 cal mol⁻¹ K⁻¹ and -950 cal mol⁻¹ K⁻¹ to fit the data. Attempts to fit our data to equations incorporating a ΔC_p° parameter did not result in a statistically significant improvement in the fit (see Supporting Material). We therefore do not report a heat capacity change for junctionless docking, and tentatively attribute the ΔC_p° contributions observed in the van't Hoff analysis of unimolecular systems to the contributions of conformational changes in the junction itself. In the most straightforward interpretation, a lack of measurable ΔC_p° in the junctionless system would indicate little to no net burial or exposure of nonpolar surface at the loop-loop interface upon domain docking.^{70;71} Heat capacity changes for binding reactions can be detected with far greater sensitivity using temperature-dependent isothermal titration calorimetry (ITC).⁷² Unfortunately, despite extensive efforts, we have been unable to detect interpretable ITC signatures for hairpin docking due to the slow kinetics of the system, and therefore cannot rule out a small to moderate heat capacity change upon docking.³⁹

To investigate the effects of active-site modification more fully, we performed parallel temperature-dependent SPR experiments on the 2'-*O*-methyl (A-1) species interacting with A38C loop B. Significant thermodynamic differences from the unmodified species were observed, with the K_d values converging at the upper end of the temperature range assayed (50 °C) (Table S1). A van't Hoff plot was again linear within the precision of the data (Figure 4) and resulted in thermodynamic values of $\Delta H^\circ = (-3.40 \pm 0.51)$ kcal mol⁻¹ and $\Delta S^\circ = (18.1 \pm 1.7)$ cal mol⁻¹ K⁻¹. Incorporation of ΔC_p° into the model did not result in a statistically significant improvement in the fit (Supporting Material). The cleavage-site modification, therefore, decreased the favorable enthalpy change upon docking by nearly 8 kcal/mol, with the consequent decrease in affinity partially offset by the conversion of the entropy change from slightly unfavorable to significantly favorable ($-T \Delta S^\circ = -5.4 \pm 0.5$ kcal mol⁻¹ at 298 K). The most parsimonious explanation for these observations is the model that the *O*-methyl group interferes with the formation of the fully stabilized network of interactions in the docked state, decreasing both the favorable enthalpy of binding and the loss of configurational entropy associated with the descent to a highly-stabilized minimum in the folding landscape. Differential solvation effects on ΔS° are difficult to rule out, but we consider this possibility less likely due to the lack of an observable ΔC_p for docking of either species. This model is consistent with crystallographic results and molecular-dynamics simulations that have found local but significant structural differences between 2'-*O*-methyl and 2'-OH species at A-1.^{50;66;67} The localization of the affinity affects to the off-rate may be consistent with observed distortions of sugar puckers and other local structural features of the docked complex induced by this modification,^{50;66;67} or may more directly reflect the

early nature of the transition state for docking demonstrated using Φ -value analysis of single-molecule data.⁷³ It should be noted, however, that at higher temperatures the differences between the off-rates for the two species diminish whereas the on-rates diverge somewhat (Table S1; Figure 4), potentially complicating this interpretation.

The measurement of docking affinities with SPR also allows the determination of forward and reverse activation barriers via an Arrhenius plot of $\ln(k_{\text{on}})$ or $\ln(k_{\text{off}})$. Such an analysis for the docking of unmodified (2'-OH at A-1) loop A to A38C loop B is shown in Figure 5A. For this interaction, the dependence of rate constants on temperature was significant (Table S1) and highly linear Arrhenius plots were observed. Extracted slopes yielded activation energies E_a of (11.7 ± 2.2) kcal mol⁻¹ for association and (23.2 ± 1.7) kcal mol⁻¹ for dissociation. These relatively large activation barriers imply a significant degree of structural disruption along the docking pathway, consistent with the significant differences in structure between the free and bound forms of each loop. Using time-resolved FRET techniques on a two-way junction construct, Burke and coworkers⁵⁴ reported a docking activation energy of 28.8 kcal/mol, substantially in excess of that observed here. This higher barrier presumably corresponds to the necessity for disruption of structure in the area of the junction, potentially including coaxial stacking in the construct used for ensemble FRET.

For the docking of 2'-*O*-methyl modified loop A to A38C loop B, by contrast, significant nonlinearities in the Arrhenius plots were observed (Figure 5B). Multiple mechanisms can lead to a breakdown of Arrhenius-linear behavior, including a finite heat capacity of activation, a breakdown of the two-state assumption along with a change in rate-limiting step with temperature, and fluctuations among multiple conformers of a binding partner.⁷⁴ The current data are insufficient to distinguish among the various possibilities for the docking of the 2'-*O*-methyl modified hairpin. In the absence of a well-established model for the source of the curvature, further quantitative analysis of this dataset was not attempted. Nevertheless, the combination of nonlinear Arrhenius plots with the changes in thermodynamic parameters described above indicates a very substantial qualitative alteration in the energy landscape for docking of the *O*-methylated species. This shift arises from the addition of a single methyl group, likely accompanied by a shift in sugar pucker at A-1^{50;66} and possibly the loss of a single or bifurcated hydrogen bond to the 2'-OH group.⁶⁷ These changes are well beyond what might be expected based on a relatively small 0.65 kcal mol⁻¹ decrease in ΔG° for the overall process (cf. Table 1) and emphasize the value of a detailed thermodynamic and kinetic analysis of macromolecular recognition.

The Arrhenius plots in Figure 5 contrast sharply with a temperature-dependent time-resolved FRET analysis of metal-initiated docking in two-way and four-way junction forms of the ribozyme, in which very weak temperature dependence of docking and undocking rate constants was observed, implying small enthalpic and large entropic barriers to docking⁵⁸. Although there are several differences between the trFRET data and the current work, including the use of a 2'-deoxy modification at the active site and potential contributions from ion-titration effects in the trFRET, we hypothesize that the major source of the difference in the observed barriers is the presence of the interdomain junction in the trFRET work. The SPR results shown here report directly and solely on the energy landscape of the loop-loop tertiary interaction itself.

DISCUSSION

The numerous crystal structures of the docked form of the hairpin ribozyme show substantial rearrangements within both internal loops compared to the NMR structures of the loops in isolation.^{7;45;46;48–53} This conformational-change requirement has a drastic effect on association and dissociation kinetics in both uni- and bimolecular formats. For the highest-affinity combination of loops tested here, an affinity of approximately 100 nM K_d is accomplished despite an on-rate several orders of magnitude below the diffusion limit via extremely slow dissociation of the complex once formed. Under our conditions the half-life of the complex is 87 minutes at 25 °C (Table 1; cf. Figure S2). We have hypothesized a “double conformational capture” mechanism for docking, in which both loops undergo fluctuations to states resembling their bound conformations and only collisions in which both loops are in their minor, docking-competent conformations are productive for binding.^{16;43} Recent unconstrained molecular dynamics simulations of isolated loop A support the sampling of many important features of the docked state in low-population conformers.⁴³ The apparent lack of a large C_p° for docking is consistent with a relatively well-structured (albeit heterogeneous) unbound form, and the number and strength of interactions that must be broken before the stably docked structure can be formed is emphasized by the magnitude of the activation energies reported here. It should be noted, however, that in the two-way-junction form of the ribozyme the rate constant for docking decreases slightly as the concentration of urea is increased from zero to 3.5 M, arguing against the loss of stacked structure in the undocked state comprising the major contribution to the free-energy barrier to docking.⁷³

Our data do not demonstrate a C_p° term (i.e., temperature-dependent H°) for the interaction, but the limitations of the van't Hoff analysis mean that we cannot rule out a small to moderate C_p° contribution. For systems with faster kinetics, ITC measurements as a function of temperature allow more sensitive analysis of heat capacity changes.^{8;72} For the junctionless hairpin ribozyme, however, ITC analysis of docking has not been possible due to the slow kinetics of the system, despite considerable effort (Kristine R. Julien and C.G.H., unpublished results). In certain energetic regimes, unrecognized C_p° contributions can lead to inaccuracies in measured thermodynamic parameters, implying some necessary caution in the quantitative analysis of the current results.^{75;76} The magnitude of $(\ln K_{eq})/(1/T)$ in the accessible temperature range for the native sequence and the strength of its attenuation upon *O*-methylation (Figure 4), however, support the robustness of the conclusions drawn in this paper even given the possibility of a moderate heat-capacity change for the junctionless hairpin system.

The results in this paper may be compared to a relatively small number of other RNA-RNA tertiary interactions that have been analyzed thermodynamically. An overview of the enthalpic and entropic contributions to ΔG° for a number of selected systems (Figure 6) reveals a surprising diversity of thermodynamic signatures, although the widely varying solution conditions and methodologies in these measurements require caution in quantitative comparison. The strongly enthalpically-driven docking observed for the unmodified hairpin ribozyme in this paper resembles that determined by isothermal titration calorimetry (ITC) for the GNRA tetraloop-tetraloop receptor interaction,⁸ which is accompanied by significant

conformational rearrangements and ordering of the receptor component. The latter system has also been the subject of extensive single-molecule characterization, with the same pattern of enthalpy-driven docking observed²¹ along with an entropy change that depends strongly on the concentration of Mg^{2+} used to drive the interaction.⁷⁷ Intriguingly, when the tetraloop-tetraloop receptor interaction was instead driven by high levels of monovalent cation, the thermodynamic signature observed depended strongly on the concentration of the facilitating ion.⁷⁸ A stable kissing-loop interaction critical to the structure of the *Neurospora* VS ribozyme was also found to be enthalpy-driven with an unfavorable entropy contribution.¹⁷ In contrast, van't Hoff analysis of biochemical data for the docking of the substrate-recognition helix into the group I intron¹³ and hammerhead ribozyme (HH16) core folding determined using ITC with subtraction of the contributions from flanking helices¹⁴ were both found to be strongly entropy-driven. Both of the latter systems also showed evidence for cold denaturation of RNA tertiary structure.^{13;79} Figure 6 also shows data for secondary-structure hybridization for a typical short self-complementary RNA oligomer for reference.¹⁹ Finally, we note that in the two cases discussed here for which ITC data were obtained at multiple temperatures, a substantial C_p° contribution was required to explain the data, $-0.24 \pm 0.05 \text{ kcal mol}^{-1} \text{ K}^{-1}$ in the case of the tetraloop-receptor interaction and $+0.5 \pm 0.1 \text{ kcal mol}^{-1} \text{ K}^{-1}$ for HH16 core folding.^{8;14} Although van't Hoff analysis is inherently less sensitive to C_p° terms than ITC, it is worth noting that linear plots were observed for P1 docking¹³ and for junctionless hairpin docking (this work).

Although the data in Figure 6 were obtained in a variety of solution conditions, constructs, and (for ITC) temperatures, and quantitative comparisons are therefore difficult, the general observation that RNA tertiary structure formation proceeds with a significant diversity of thermodynamic patterns is clear. The variability in thermodynamic mechanisms for RNA tertiary structure formation is further emphasized by our observation of a qualitative change in signature upon 2'-*O*-methylation of a single ribose group in loop A. Whether this diversity is accompanied by a qualitative shift in the nature of the driving force from one system to another is plausible but uncertain. It seems likely, for example, that the entropic contributions of diffusely bound cations that were invoked in the case of hammerhead core folding¹⁴ do not contribute substantially to G°_{dock} in the unmodified junctionless hairpin.

CONCLUSIONS

We have determined that, in the absence of active-site modifications, the docking transition in the junctionless hairpin ribozyme is strongly enthalpy-driven, slightly entropy-opposed, and not accompanied by a heat capacity change detectable using our van't Hoff analysis across a 30 °C temperature range. The transition also shows substantial activation barriers that emphasize the extensive rearrangements upon tertiary-structure formation found using structural biology. These results complement a variety of previous measurements performed in unimolecular constructs of the hairpin system by isolating the effects of the loop-loop interface. The thermodynamic signature of RNA tertiary structure formation appears to vary qualitatively from one system to another and even upon changes in solution conditions^{77;78} or relatively modest chemical modifications (this work). The alterations in thermodynamic parameters and the shape of the activation curve arising from the active-site 2'-*O*-methyl group particularly emphasize the unexpectedly significant potential effects from

modifications used at the active site of the hairpin and numerous other systems to prevent catalytic RNA cleavage, and strengthen the case to interpret such studies with caution and to look for experimental alternatives whenever possible.^{50;80} Dissection of the factors controlling the apparently finely-balanced thermodynamic contributions to RNA tertiary structure stabilization across a variety of systems represents a promising area for further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Vicens Q, Cech TR. Trends Biochem Sci. 2006; 31:41–51. [PubMed: 16356725]
2. Hoogstraten CG, Sumita M. Biopolymers. 2007; 87:317–328. [PubMed: 17806104]
3. Toor N, Keating KS, Pyle AM. Curr Opin Struct Biol. 2009; 19:260–266. [PubMed: 19443210]
4. Wedekind JE. Met Ions Life Sci. 2011; 9:299–345. [PubMed: 22010277]
5. Pyle AM. Annu Rev Biophys. 2016; 45:183–205. [PubMed: 27391926]
6. Gebetsberger J, Micura R. Wiley Interdiscip Rev RNA. 2016
7. Rupert PB, Ferre-D'Amare AR. Nature. 2001; 410:780–786. [PubMed: 11298439]
8. Vander Meulen KA, Davis JH, Foster TR, Record MT Jr, Butcher SE. J Mol Biol. 2008; 384:702–717. [PubMed: 18845162]
9. Tinoco I Jr, Bustamante C. J Mol Biol. 1999; 293:271–281. [PubMed: 10550208]
10. Bloomfield, VA., Crothers, DM., Tinoco, I, Jr. Nucleic acids: Structures, properties, and functions. University Science Books; Sausalito, CA: 2000.
11. Leroy JL, Guéron M. Biopolymers. 1977; 16:2429–2446. [PubMed: 334280]
12. Leontis NB, Westhof E. RNA. 2001; 7:499–512. [PubMed: 11345429]
13. Narlikar GJ, Herschlag D. Nat Struct Biol. 1996; 3:701–710. [PubMed: 8756329]
14. Mikulecky PJ, Takach JC, Feig AL. Biochemistry. 2004; 43:5870–5881. [PubMed: 15134461]
15. Cate J, Hanna RL, Doudna JA. Nature Struct Biol. 1997; 4:553–558. [PubMed: 9228948]
16. Sumita M, White NA, Julien KR, Hoogstraten CG. RNA Biol. 2013; 10:425–435. [PubMed: 23324606]
17. Bouchard P, Legault P. RNA. 2014; 20:1451–1464. [PubMed: 25051972]
18. Draper DE, Grilley D, Soto AM. Annu Rev Biophys Biomol Struct. 2005; 34:221–43. 221–243. [PubMed: 15869389]
19. Xia T, SantaLucia J Jr, Burkard ME, Kierzek R, Schroeder SJ, Jiao X, Cox C, Turner DH. Biochemistry. 1998; 37:14719–14735. [PubMed: 9778347]
20. Rauzan B, McMichael E, Cave R, Sevcik LR, Ostrosky K, Whitman E, Stegemann R, Sinclair AL, Serra MJ, Deckert AA. Biochemistry. 2013; 52:765–772. [PubMed: 23356429]
21. Fiore JL, Kraemer B, Koberling F, Edmann R, Nesbitt DJ. Biochemistry. 2009; 48:2550–2558. [PubMed: 19186984]
22. Fedor MJ. Biochem Soc Trans. 2002; 30:1109–1115. [PubMed: 12440984]
23. Wilson TJ, Nahas M, Ha T, Lilley DM. Biochem Soc Trans. 2005; 33:461–465. [PubMed: 15916541]
24. Fedor MJ. Annu Rev Biophys. 2009; 38:271–299. [PubMed: 19416070]

25. Hampel A, Cowan JA. Chem Biol. 1997; 4:513–517. [PubMed: 9263639]
26. Young KJ, Gill F, Grasby JA. Nucleic Acids Res. 1997; 25:3760–3766. [PubMed: 9380495]
27. Nesbitt S, Hegg LA, Fedor MJ. Chem Biol. 1997; 4:619–630. [PubMed: 9281529]
28. Hampel KJ, Walter NG, Burke JM. Biochemistry. 1998; 37:14672–14682. [PubMed: 9778342]
29. Walter F, Murchie AI, Thomson JB, Lilley DM. Biochemistry. 1998; 37:14195–14203. [PubMed: 9760257]
30. Murray JB, Seyhan AA, Walter NG, Burke JM, Scott WG. Chem Biol. 1998; 5:587–595. [PubMed: 9818150]
31. Lambert D, Heckman JE, Burke JM. Biochemistry. 2006; 45:829–838. [PubMed: 16411759]
32. Kraemer-Chant CM, Heckman JE, Lambert D, Burke JM, Inorg J. Biochem. 2014; 131:87–98.
33. Walter NG, Burke JM. Curr Opin Chem Biol. 1998; 2:24–30. [PubMed: 9667918]
34. Ferre-D'Amare AR. Biopolymers. 2004; 73:71–78. [PubMed: 14691941]
35. Ditzler MA, Otyepka M, Sponer J, Walter NG. Acc Chem Res. 2010; 43:40–47. [PubMed: 19754142]
36. Mlynsky V, Banas P, Walter NG, Sponer J, Otyepka M. J Phys Chem B. 2011; 115:13911–13924. [PubMed: 22014231]
37. Kath-Schorr S, Wilson TJ, Li NS, Lu J, Piccirilli JA, Lilley DM. J Am Chem Soc. 2012; 134:16717–16724. [PubMed: 22958171]
38. Nivon LG, Shakhnovich EI. J Mol Biol. 2011; 411:1128–1144. [PubMed: 21740912]
39. Hoogstraten CG, Sumita M, White NA. Methods Enzymol. 2014; 549:407–432. [PubMed: 25432758]
40. Mlynsky V, Banas P, Sponer J, van der Kamp MW, Mulholland AJ, Otyepka M. J Chem Theory Comput. 2014; 10:1608–1622. [PubMed: 26580373]
41. Mlynsky V, Kuhrova P, Zgarbova M, Jurecka P, Walter NG, Otyepka M, Sponer J, Banas P. J Phys Chem B. 2015; 119:4220–4229. [PubMed: 25692537]
42. Heldenbrand H, Janowski PA, Giambasu G, Giese TJ, Wedekind JE, York DM. J Am Chem Soc. 2014; 136:7789–7792. [PubMed: 24842535]
43. Ochieng PO, White NA, Feig M, Hoogstraten CG. J Phys Chem B. 2016
44. Schuabb C, Kumar N, Pataria S, Marx D, Winter R. Nat Commun. 2017; 8:14661. [PubMed: 28358002]
45. Cai Z, Tinoco I Jr. Biochemistry. 1996; 35:6026–6036. [PubMed: 8634244]
46. Butcher SE, Allain FH, Feigon J. Nat Struct Biol. 1999; 6:212–216. [PubMed: 10074938]
47. Butcher SE, Allain FH, Feigon J. Biochemistry. 2000; 39:2174–2182. [PubMed: 10694382]
48. Rupert PB, Massey AP, Sigurdsson STh, Ferré-D'Amaré AR. Science. 2002; 298:1421–1424. [PubMed: 12376595]
49. Grum-Tokars V, Milovanovic M, Wedekind JE. Acta Crystallogr D Biol Crystallogr. 2003; 59:142–145. [PubMed: 12499551]
50. Salter J, Krucinska J, Alam S, Grum-Tokars V, Wedekind JE. Biochemistry. 2006; 45:686–700. [PubMed: 16411744]
51. MacElrevey C, Salter JD, Krucinska J, Wedekind JE. RNA. 2008; 14:1600–1616. [PubMed: 18596253]
52. Spitalé RC, Volpini R, Mungillo MV, Krucinska J, Cristalli G, Wedekind JE. Biochemistry. 2009; 48:7777–7779. [PubMed: 19634899]
53. Liberman JA, Guo M, Jenkins JL, Krucinska J, Chen Y, Carey PR, Wedekind JE. J Am Chem Soc. 2012; 134:16933–16936. [PubMed: 22989273]
54. Walter NG, Hampel KJ, Brown KM, Burke JM. EMBO J. 1998; 17:2378–2391. [PubMed: 9545249]
55. Wilson TJ, Zhao ZY, Maxwell K, Kontogiannis L, Lilley DM. Biochemistry. 2001; 40:2291–2302. [PubMed: 11329299]
56. Wilson TJ, Lilley DM. RNA. 2002; 8:587–600. [PubMed: 12022226]
57. Klostermeier D, Millar DP. Biochemistry. 2000; 39:12970–12978. [PubMed: 11041862]

58. Pljevaljcic G, Klostermeier D, Millar DP. *Biochemistry*. 2005; 44:4870–4876. [PubMed: 15779913]
59. Wilson TJ, Nahas M, Araki L, Harusawa S, Ha T, Lilley DM. *Blood Cells Mol Dis*. 2007; 38:8–14. [PubMed: 17150385]
60. Zhuang X, Kim H, Pereira MJ, Babcock HP, Walter NG, Chu S. *Science*. 2002; 296:1473–1476. [PubMed: 12029135]
61. Ditzler MA, Aleman EA, Rueda D, Walter NG. *Biopolymers*. 2007; 87:302–316. [PubMed: 17685395]
62. Paudel BP, Rueda D. *J Am Chem Soc*. 2014; 136:16700–16703. [PubMed: 25399908]
63. Butcher SE, Heckman JE, Burke JM. *J Biol Chem*. 1995; 270:29648–29651. [PubMed: 8530348]
64. McKenna SA, Kim I, Puglisi EV, Lindhout DA, Aitken CE, Marshall RA, Puglisi JD. *Nat Protoc*. 2007; 2:3270–3277. [PubMed: 18079727]
65. Liu S, Bokinsky G, Walter NG, Zhuang X. *Proc Natl Acad Sci USA*. 2007; 104:12634–12639. [PubMed: 17496145]
66. Ditzler MA, Sponer J, Walter NG. *RNA*. 2009; 15:560–575. [PubMed: 19223444]
67. Mlynsky V, Banas P, Hollas D, Reblova K, Walter NG, Sponer J, Otyepka M. *J Phys Chem B*. 2010; 114:6642–6652. [PubMed: 20420375]
68. Bevilacqua PC. *Biochemistry*. 2003; 42:2259–2265. [PubMed: 12600192]
69. Doherty EA, Herschlag D, Doudna JA. *Biochemistry*. 1999; 38:2982–2990. [PubMed: 10074350]
70. Spolar RS, Record MT Jr. *Science*. 1994; 263:777–784. [PubMed: 8303294]
71. Mikulecky PJ, Feig AL. *Biopolymers*. 2006; 82:38–58. [PubMed: 16429398]
72. Salim NN, Feig AL. *Methods*. 2009; 47:198–205. [PubMed: 18835447]
73. Bokinsky G, Rueda D, Misra VK, Rhodes MM, Gordus A, Babcock HP, Walter NG, Zhuang X. *Proc Natl Acad Sci USA*. 2003; 100:9302–9307. [PubMed: 12869691]
74. Case A, Stein RL. *Biochemistry*. 2003; 42:3335–3348. [PubMed: 12641466]
75. Naghibi H, Tamura A, Sturtevant JM. *Proc Natl Acad Sci USA*. 1995; 92:5597–5599. [PubMed: 7777555]
76. Chaires JB. *Biophys Chem*. 1997; 64:15–23. [PubMed: 9127935]
77. Fiore JL, Holmstrom ED, Nesbitt DJ. *Proc Natl Acad Sci USA*. 2012; 109:2902–2907. [PubMed: 22308376]
78. Holmstrom ED, Fiore JL, Nesbitt DJ. *Biochemistry*. 2012; 51:3732–3743. [PubMed: 22448852]
79. Mikulecky PJ, Feig AL. *Nucleic Acids Res*. 2004; 32:3967–3976. [PubMed: 15282329]
80. Chi YI, Martick M, Lares M, Kim R, Scott WG, Kim SH. *PLoS Biol*. 2008; 6:e234. [PubMed: 18834200]

RESEARCH HIGHLIGHTS

- SPR studies of domain docking kinetics and thermodynamics in the hairpin ribozyme.
- The reaction is strongly enthalpy-driven in the native ribozyme.
- High activation barriers are consistent with structural rearrangements.
- O-methylation at the cleavage site significantly alters the reaction energetics.
- RNA tertiary structure formation displays a diversity of thermodynamic signatures.

Figure 1. Schematic of the cation-driven docking transition of the hairpin ribozyme in its naturally-occurring, four-way junction form. The arrow indicates the location of self-cleavage within the docked structure.

Figure 2.

Molecular nature of the loop A-loop B interface. The two loops interact in minor groove-minor groove fashion. The three major components of the interface are the G+1:C25 Watson-Crick interdomain base pair (red), a ribose zipper (green), and a complex pocket surrounding the extruded base U42 (blue).⁷ Open and solid pentagons represent C3'-endo and C2'-endo sugars, respectively; shaded nucleotide bases have *syn* glycosidic angles. Noncanonical base pair symbols are according to the convention of Leontis & Westhof.¹² Ribozyme self-cleavage occurs between A-1 and G+1 (red); the 2'-*O*-methyl modifications used in this work are sited at A-1, which supplies the nucleophilic group for the reaction of the active ribozyme. Redrawn in simplified form from Sumita *et al* (2013).¹⁶

Figure 3.

Illustrative SPR data acquired at 25 °C for unmodified loop A/A38C loop B. Concentrations of loop B in the mobile phase were 30 nM, 75 nM, 150 nM, 300 nM, 450 nM, 600 nM, and 750 nM (bottom to top). Red lines are a global fit to all data. Visible spikes at the end of the association phase arise from imperfect alignment of sample and reference channels and do not affect the global fits (see Supporting Material). Data were analyzed with Scrubber and the final figure was prepared in Igor Pro 6.0.

Figure 4. van't Hoff plots for docking of unmodified (solid circles) and 2'-O-methyl modified (open circles) loop A to A38C loop B. In this and subsequent plots, the error bars are displayed as the mean of the experimental standard deviations for the five temperature points on a given curve.

Figure 5. Arrhenius plots for the docking of (A), $2'$ -OH loop A to A38C loop B; (B), $2'$ -O-methyl loop A to A38C loop B. Upper plot is k_{on} and lower is k_{off} in both cases. A linear fit is shown in A; for B, the line is a spline curve meant only to guide the eye.

Figure 6.

Comparison of the thermodynamic contributions to the driving force for RNA tertiary structure formation in selected systems. Hairpin ribozyme unmodified and modified, this work; tetraloop-tetraloop receptor, ITC data at 40 °C in 2 mM Mg^{2+} in a construct containing two motifs (values plotted here are half of those observed);⁸ P1 helix docking, biochemical methods in 10 mM Mg^{2+} with helical contributions removed by difference;¹³ hammerhead (HH16) core folding, ITC data at 308 K (S° estimated at 298 K) in 10 mM Mg^{2+} and 100 mM Na^+ with helical contributions removed by difference;¹⁴ VS kissing-loop interaction, VS ribozyme preshifted stem-loop I/wildtype stem-loop V, ITC in 20 mM Mg^{2+} , 25 °C;¹⁷ annealing of r(CGCG)₂ provided for reference.¹⁹ Entropy parameters for P1 helix docking and hammerhead core folding may vary from other systems in contributions from translational/rotational entropy loss.

Table 1
Comparison of rate and dissociation constants for variants of the hairpin ribozyme at 298 K.

loop A	loop B	k_a , M ⁻¹ s ⁻¹	k_d , s ⁻¹	K_d , nM	G°_{dock} , kcal mol ⁻¹
2'-OCH ₃ ^a	wild-type	$(1.97 \pm 0.29) \times 10^3$	$(7.1 \pm 1.0) \times 10^{-4}$	372 ± 101	-8.76 ± 0.16
2'-OCH ₃	A38C	$(1.13 \pm 0.06) \times 10^3$	$(3.79 \pm 0.56) \times 10^{-4}$	338 ± 66	-8.82 ± 0.12
2'-OH	A38C	$(1.24 \pm 0.35) \times 10^3$	$(1.33 \pm 0.23) \times 10^{-4}$	114 ± 33	-9.47 ± 0.17

^aData from ref. 16