TOPICAL REVIEW — Soft matter and biological physics

Molecular dynamic simulation of the thermodynamic and kinetic properties of nucleotide base pair*

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A nucleotide base pair is the basic unit of RNA structures. Understanding the thermodynamic and kinetic properties of the closing and opening of a base pair is vital for quantitative understanding the biological functions of many RNA molecules. Due to the fast transition rate, it is difficult to directly observe opening and closing of single nucleic acid base pair in experiments. This review will provide a brief summary of the studies about the thermodynamic and kinetic properties of a base pair opening and closing by using molecular dynamic simulation methods.

Keywords: thermodynamics, kinetics, molecular dynamic simulation

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1. Introduction

RNA can carry out many biological functions, such as translation, transcription, conservation of genetic information, and regulation of gene expression.[1–4] To perform these biological functions, RNA adopts a variety of complex three-dimensional structures.[5–9] The functions of many RNAs are often kinetically controlled.[10–16] For instance, self-induced riboswitches can regulate gene expression by limiting the folding of functional structures to certain time windows.[15,16] The hok/sok system of plasmid R1[16] regulates the plasmid maintenance through mRNA conformation rearrangements into different functional forms. A nucleotide base pair is the basic unit of nucleic acids molecular structures. In the process of structural rearrangement, closing and opening of base pairs are fundamental events. So studying the thermodynamic and kinetic properties of opening and closing of a single base pair is crucial for further exploring and predicting the biological functions of RNA molecules.[17–20]

By assuming the nearest-neighbor interaction, the thermodynamic parameters of a base stacking have been experimentally determined by measuring the melting curves of a few duplexes with several base pairs through ultraviolet absorption.[21] The accuracy of the thermodynamic parameters of a base pair depends on the assemble of the duplexes and a great correction has been made since its first version.[21] The exchange of imino protons[22] and NMR[23–26] have been used to study the kinetic properties of single nucleotide base pair. However, due to the randomness of base pair folding, the complexity of overcoming the energy barrier,[27–29] as well as the limitations and effects of the experimental instrument,[30–32] it is very difficult to directly observe the detailed kinetic process and conformational changes of the base pair opening and closing in experiments. Molecular dynamic simulation, which can make up the experimental deficiencies, has been widely used to study the bio-molecules.[33–45] Recently, molecular dynamic simulation has been used to study the kinetic process of the DNA and RNA base pair opening and closing. Not only the transition states and pathways between open and closed states of the nucleotide base pair were identified, but also the thermodynamic parameters and the dynamic rates of a base pair opening and closing were obtained, which are in good agreement with those of the experiments. Here we will provide a brief overview of the studies about the thermodynamic and opening-closing kinetic properties of a nucleotide base pair.

2. The kinetic transition pathways of single base pair opening and closing

Using four parameters, namely the distance d_{16} and interaction energy E_{16} between the terminal base pair G6–C1, as well as the distance d_{12} and interaction energy E_{12} between the adjacent base G2–C1, Hagan *et al.*[39] have studied the kinetic pathways for DNA terminal single base pair G6–C1

$$\begin{pmatrix} G4 & C5 & G6 \\ | & | & | \\ C3 & G2 & C1 \end{pmatrix}$$

opening and closing through molecular dynamic simulation. The closing and opening of the base pair were defined by the four parameters. When the two interaction energy parameters were less than a certain value ($E_{12} < -4.0$ kcal/mol and

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$E_{16} < -12.0$ kcal/mol), the corresponding structures could be regarded as the closed state, and when the two distance parameters were within a certain range (12.5 Å $< d_{12} <$ 14.5 Å and 17.0 Å $< d_{16} <$ 2.0 Å), the corresponding structures could be regarded as the open state. Two qualitatively different pathways of the terminal base pair G6–C1 from the closed state to open state were identified through this method. One path was that the flipping base first opened its intramolecular hydrogen bonds of complementary base pair before it was unstacked, and the other path was that both sets of base stacking interaction between adjacent bases were ruptured simultaneously. The two kinetic pathways rejoined at the transition states, where the magnitudes of the interaction energies of E_{12} and E_{16} were comparable with the thermal energy.

Using atomistic pulling simulations and reweighting scheme, Colizzi *et al.*[45] have studied the forming and melting of four different terminal complementary base pairs G–C

$$\begin{pmatrix} 5'-\mathrm{CC}\cdots & 5'-\mathrm{GG}\cdots & 5'-\mathrm{CG}\cdots & \text{and} & 5'-\mathrm{GC}\cdots \\ 3'-\mathrm{GG}\cdots & 3'-\mathrm{CC}\cdots & 3'-\mathrm{GC}\cdots & & 3'-\mathrm{CG}\cdots \end{pmatrix}$$

in RNA duplexes. The free-energy profile was reconstructed as a function of the number of water molecules surrounding the unbinding base, which could be used to distinguish the configuration from the other and to properly collect the corresponding weights. By fitting the free-energy profiles with the combination of two quadratic functions, the free energy difference between the open and closed states was calculated. According to a systematic step-by-step approach, two different opening paths from the closed state to open state were identified. Each path could be divided into two steps. First, the complementary base pair was partially opened by the unpaired base of the 5-terminal or 3-terminal; second, the remaining dangling base at the other terminal was then unstacked, and the terminal base pair was completely opened. The free energy changes from the closed state to the relatively stable intermediates and from the intermediates to the open state in each path were attained.

Recently, Xu *et al.*[42] applied a mixed method, which integrated molecular dynamic simulation, kinetic Monte Carlo simulation and master equation methods, to study the kinetic mechanism of RNA single base pair formation. The molecular dynamic simulation method was used to get the trajectory including many structures. All the conformations were assigned to 50 clusters according to the structural closeness by the RMSD (Root Mean Square Deviation), as well as three order parameters: the distance d_{12} between the geometric centers of all the heavy atoms in the G1 and G2 bases and the nonbonded interaction energies E_{12} and E_{16} between the sequentially neighboring nucleotides G1 and G2 and between the pairing nucleotides G1 and C6, respectively. Based on the behavior of the time-dependent population for each cluster, the 50 clusters were classified as four states: unfolded state, intermediate state, trapped state and folded state. At last, the overall folding kinetics could be described by the four-state kinetics scheme.

3. Thermodynamics parameters, transition states, and kinetic transition rates of a single base pair opening and closing

Since it usually takes a long time to open or close the RNA base pair at room temperature,[46] the simulation near its melting temperature of the base pair can quickly make the base pair reach the two-state equilibration between open and closed states. The melting temperature of the AU base pair can be estimated as: $T_m = \Delta H_{\mathrm{stack}}/\Delta S_{\mathrm{stack}} = 359 \pm 40$ K, where $\Delta H_{\mathrm{stack}} = -6.82 \pm 0.72$ kcal/mol and $\Delta S_{\mathrm{stack}} = -19 \pm 2.5$ eu are the enthalpy and entropy changes of the terminal base-stacking

$$\begin{pmatrix} 5'-\mathrm{AA}\cdots \\ 3'-\mathrm{UU}\cdots \end{pmatrix}$$

from the nearest-neighbor model.[47] The thermodynamic and kinetic properties of RNA terminal AU[48] base pair were attained through molecular dynamic simulation at different temperatures: 390 K, 400 K, 410 K, 420 K, and 430 K, nearing the melting temperature of the AU base pair. The initial structure of a 12-nucleotides oligonucleotide 5′-(AAGCGCAAGCUU)-3′, which includes a tetraloop and four base pairs, was obtained from its crystal structure (PDB ID:1ZIH). To specifically study the thermodynamic parameters and kinetic mechanism of the opening and closing of the terminal AU base pair, all other nucleotides except the two terminal nucleotides 5′-A and 3′-U of the RNA hairpin were fixed by using an additional force.

According to the simulated trajectories, the time-dependent RMSD of the two terminal nucleotides 5′-A and 3′-U relative to the initial structure,

$$\mathrm{RMSD} = \left\{ \sum_{i}^{N_{\mathrm{atom}}} \left[\left(x_i^t - x_i^0\right)^2 + \left(y_i^t - y_i^0\right)^2 + \left(z_i^t - z_i^0\right)^2 \right] / N \right\}^{1/2},$$

where i represents the i-th atom, N_{atom} the total number of atoms, $\left(x_i^0, y_i^0, z_i^0\right)$ and $\left(x_i^t, y_i^t, z_i^t\right)$ represent the coordinates of the atom i at time zero and t, respectively, and the torsional dihedral angles ζ (C3′(j) − O3′(j) − P$(j+1)$ − O5′$(j+1)$,[49] where j represents the j-th nucleotide in the polynucleotide chain) on the backbone of the terminal base pair AU were obtained. These values clearly showed a two-state distribution, as shown in Fig. 1. Therefore, according to the distribution of RMSD and torsional angles, we divided the simulation structures into closed state (cs), open state (os), and transition state

(ts). When the RMSD value was less than 2.0 Å, at which the corresponding torsional angle ζ was always between $-50°$ and $-100°$, the conformation was treated as the closed state. On the contrary, when the RMSD value was larger than 2.0 Å and the corresponding torsional angle ζ was always between $50°$ and $100°$, the conformation was considered to be the open state (see Figs. 2(a) and 2(b)). When RMSD values were greater than 2.0 Å, but the torsional angle ζ were still between $-50°$ and $-100°$, then such conformations were regarded as the transition state (see Figs. 2(a) and 2(b)). The trajectories of transition paths showed that all the transitions from the closed states to open states or from the open states to closed states go through the transition states. Namely, in the process base pair from the closed states to open states, the bases of terminal nucleotides first flipped out from the closed states with the increased RMSD, and then the torsional angles of backbone changed from the range of $(-50°)$–$(-100°)$ to the range of $(50°)$–$(100°)$, however, in the process of base pair from the open states to closed states, the torsional angles of backbone first changed from the range of $(50°)$–$(100°)$ to the range of $(-50°)$–$(-100°)$, and then the flipped bases of terminal nucleotides returned to the closed states with the reduced RMSD. According to the location of the transition state appeared, the transition state could be classified into two types: ctc and oto, where ctc referred to the transition state that transited from the closed state and then back to the closed state, and oto referred to the transition state that transited from the open state and then back to the open state (see Fig. 2(c)).

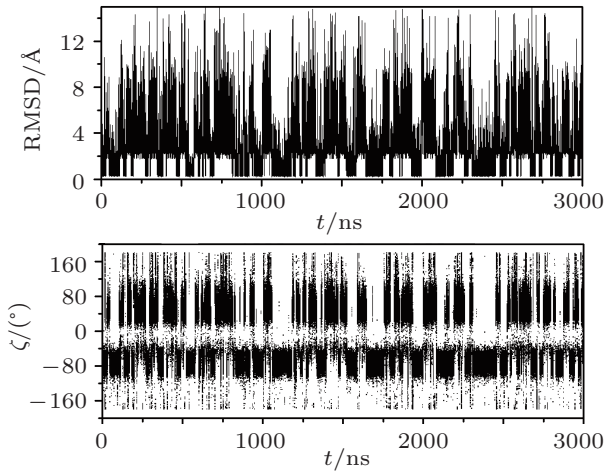


Fig. 1. The RMSD values and torsional angles ζ during the whole simulation times ($0 \sim 3000$ ns) at 400 K.

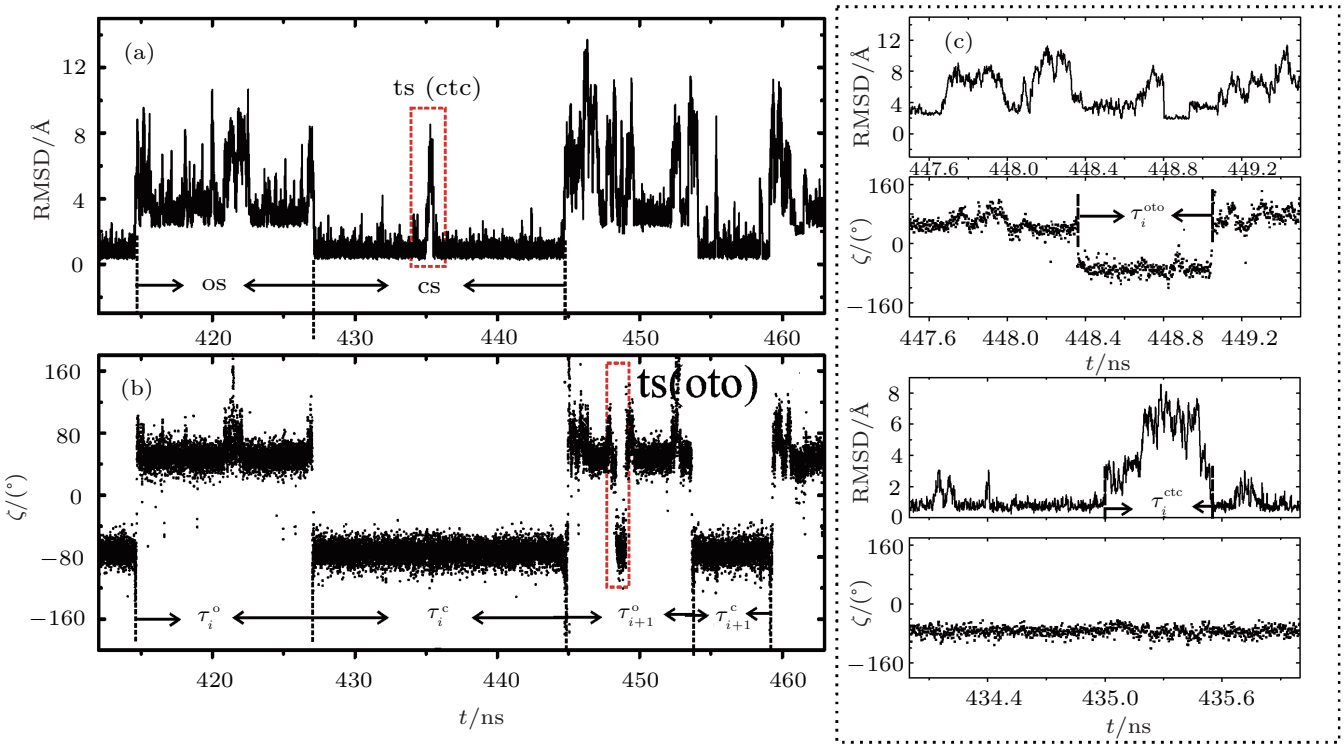


Fig. 2. (color online) (a) The RMSD values among the simulation times of 412 ns∼463 ns at 410 K. (b) The torsion angles ζ for the simulation times of 412 ns∼463 ns at 410 K. (c) The RMSD and torsion angles ζ near the transition states.

As compared to the open state and closed state, the probabilities of the transition state were very small. According to the temperature dependence population distribution of the closed state and open state, the enthalpy change ΔH and the entropy change ΔS of the terminal AU base pair opening and closing could be attained as $\Delta H - T\Delta S = -k_B T \ln(p_o/p_c)$ (see Fig. 3), where p_o and p_c are the occupied population of the open states and closed states, respectively; k_B is the Boltzmann constant; T is the absolute temperature. The results showed that thermodynamic parameters were in great agreement with the nearest-

neighbor model.[47]

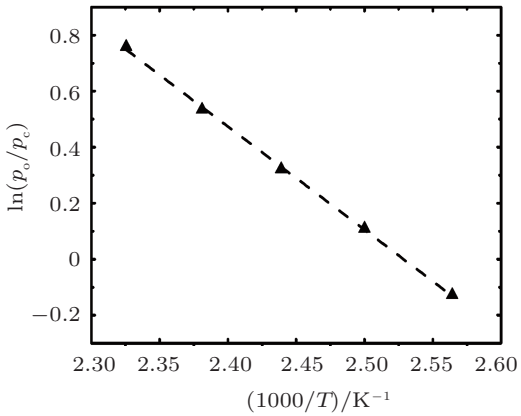


Fig. 3. The temperature dependence of $\ln(p_o/p_c)$. Symbol is the simulation results and line is the linear fit.

The average lifetime of each state could be calculated as $\tau_{ave} = \sum_{i=1}^{N} \tau_i/N$, where τ_{ave} is the average lifetime, N is the corresponding total number of times the molecules reside in the closed state or open state or transition state, and τ_i is the i-th lifetime of the conformation in the corresponding state. The average lifetime of the closed state and open state showed different temperature-dependent behaviors. The average lifetime of the closed state showed the strong temperature-dependent behavior, while that of the open state demonstrated the weak temperature dependence behavior, as shown in Fig. 4(a). Compared to the open state and closed states, the average lifetimes of transition states ctc and oto were very short and also exhibited the weak temperature dependence behavior, as shown in Table 1.

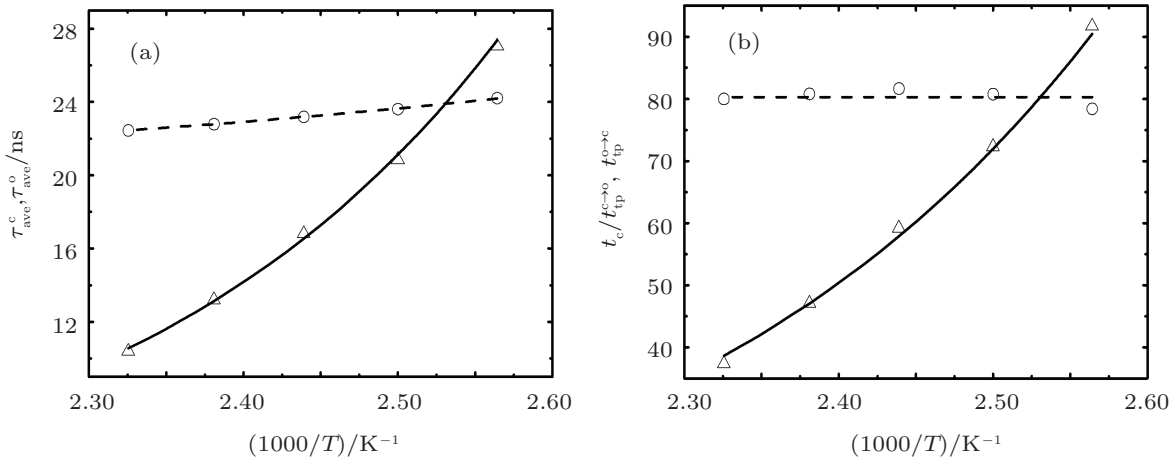


Fig. 4. (a) Temperature dependence of the average lifetime of the closed state (hollow triangle) and the open state (hollow circle). Symbol is the simulated results. Lines are fitted results with Eqs. (1) and (2). (b) Temperature dependence of the ratios of $t_c/t^{c\to o}_{tp}$ (hollow triangle) and $t_o/t^{o\to c}_{tp}$ (hollow circle). Symbol is the simulated results. Lines are fitted results with Eqs. (1)–(4).

Table 1. The average lifetime τ_{ave} (in unit ns), the occupied probability p, and the total number N of occurrences of conformations at closed, open, and transition states at different temperatures (in unit K) during the 3000-ns simulation time.

Temperature/K	Open state (os)			Closed state (cs)			Transition state (oto)			Transition state (ctc)		
T	τ^o_{ave}	N_o	p_o	τ^c_{ave}	N_c	p_c	τ^{oto}_{ave}	N_{oto}	p_{oto}	τ^{ctc}_{ave}	N_{ctc}	p_{ctc}
390	24.207	58	0.468	27.051	59	0.532	0.295	79	0.0078	0.309	212	0.0218
400	23.612	67	0.527	20.853	68	0.473	0.288	104	0.0100	0.292	304	0.0296
410	23.187	75	0.580	16.813	75	0.420	0.284	153	0.0145	0.284	391	0.0370
420	22.783	83	0.630	13.202	84	0.370	0.280	185	0.0173	0.282	335	0.0315
430	22.451	91	0.681	10.402	92	0.319	0.278	271	0.0251	0.281	363	0.0340

For the opening-closing two-state transition, the closing rate k_+ and the opening rate k_- of the base pair could be calculated from the average lifetime of the open and closed states as $k_+ = 1/\tau^o_{ave}$, $k_- = 1/\tau^c_{ave}$, where τ^o_{ave} and τ^c_{ave} are the average lifetime of the open and closed states, respectively. The transition rates from the transition state to the closed state ($k_{t\to c}$) and to the open state ($k_{t\to o}$) could be calculated according to the average lifetime of the transition state ctc (τ^{ctc}_{ave}) and oto (τ^{oto}_{ave}) based on the following formula, $k_{t\to c} = 1/\tau^{ctc}_{ave}$ and $k_{t\to o} = 1/\tau^{oto}_{ave}$. The transition path time from the closed state to the open state $t^{c\to o}_{tp} = \tau^{oto}_{ave} = 1/k_{t\to o}$, and from the open state to the closed state $t^{o\to c}_{tp} = \tau^{ctc}_{ave} = 1/k_{t\to c}$, were both weakly temperature-dependent behavior.

According to the transition-state theory,[50–54] the average residence time in the closed state (t_c) and open state (t_o), the transition path time $t_{o\to c}$ and $t_{c\to o}$ could be expressed by the following equations

$$t_c = 1/k_- = \frac{2\pi}{\beta D^* \omega^* \omega_c} \exp(\beta \Delta G_c), \tag{1}$$

$$t_{\rm o} = 1/k_{+} = \frac{2\pi}{\beta D^{*}\omega^{*}\omega_{\rm o}} \exp(\beta \Delta G_{\rm o}), \quad (2)$$

$$t_{\rm tp}^{\rm o\to c} = \frac{1}{\beta D^{*}(\omega^{*})^{2}} \ln(2{\rm e}^{\gamma}\beta \Delta G_{\rm o}), \quad (3)$$

$$t_{\rm tp}^{\rm c\to o} = \frac{1}{\beta D^{*}(\omega^{*})^{2}} \ln(2{\rm e}^{\gamma}\beta \Delta G_{\rm c}), \quad (4)$$

where $\Delta G_{\rm c}$ and $\Delta G_{\rm o}$ are free energy barrier heights of the closed state and open state; (D^{*}) is the diffusion coefficient at the free energy barrier top; $(\omega^{*})^{2}$, $(\omega_{\rm c})^{2}$, and $(\omega_{\rm o})^{2}$ are the curvatures of the free energy surface at the barrier top, the closed state, and the open state, respectively. According to Eqs. (1)–(4), we found that $t_{\rm c}/t_{\rm tp}^{\rm c\to o}$ showed strong temperature-dependent behavior, while the $t_{\rm o}/t_{\rm tp}^{\rm o\to c}$ showed temperature-independent behavior, as shown in Fig. 4(b).

Assuming that $\omega^{*}/\omega_{\rm o}$ was temperature-independent as in protein folding,[54] the free energy barrier of closing the base pair from the open state should depend on temperature as $\Delta G_{\rm o} \propto T$. By fitting the above two curves, we found that the free energy barrier from the closed state to the open state corresponded to the enthalpy change, while the free energy barrier of the reverse transition corresponded to the entropy change. The temperature dependence of the residence time in the open state $t_{\rm o}$ came from the prefactor βD^{*} of Eq. (2), which demonstrated that the simple Einstein relationship $D^{*} = k_{\rm B}T/\eta$, where η is the viscosity of the solvent, was not applicable to base folding. Assuming that $D^{*} = D_0 \exp\left[-\left(\Delta E/k_{\rm B}T\right)^{2}\right]$ as for protein folding, in which $\left(\Delta E\right)^{2}$ is the local mean-squared fluctuation in energy and is a measurement of the underlying landscape roughness, ΔE was found to be $\sim k_{\rm B}T$. These results suggested that a one-dimensional free energy surface was sufficient to accurately describe the dynamics of base pair opening and closing, and the dynamics were Brownian.

4. Conclusion and perspectives

In conclusion, by simulating the opening-closing switch transition of RNA single base pair, we could not only qualitatively obtain the thermodynamic and kinetic properties of the base pair, such as the kinetic transition pathways, the transition states and the kinetic mechanisms of the base pair opening and closing, in which a one-dimensional free energy surface was sufficient to accurately describe the dynamics of RNA single base pair opening and closing, and the dynamics were Brownian. But also the quantitative thermodynamic parameters, such as the enthalpy and entropy changes, and the kinetic parameters, such as the transition rates and the transition path time, were in good agreements with the experimental results upon the base pair opening and closing. Although molecular dynamics simulation has its limits, such as the force constants and simulation times,[55] it would be a powerful tool to further study the thermodynamic and kinetic properties of base pair and base stacking interaction,[56,57] the next nearest-neighbor and the nearest-neighbor effects,[58] as well as pseudoknot,[59] and the ion effects,[60] which are still hard to experimentally determine, but are important to understand the biological relevance of RNA structures.

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