ACS chemical biology

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The Biophysics of RNA

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Published online July 20, 2007

10.1021/cb7001363 CCC: $37.00

In 1987, we both entered graduate school at the University of Rochester and began to study the biophysical chemistry of RNA. At that time, many things were poorly understood about RNA. There were a total of three X-ray structures of RNA (and no NMR structures); only two types of ribozymes were known; and the ribosome seemed like a mysterious blob that somehow used RNA as a scaffold to orchestrate the >50 ribosomal proteins. RNA structure seemed static and 2D because nearly all the presentations showed only the secondary structure determined from comparative sequence analysis. Molecular dynamics simulations were done *in vacuo* only on small RNA domains for subnanosecond trajectories. Of course, it was these myriad open questions that also attracted many of us to this field.

The Physical Chemistry Division met March 25–29, 2007, in Chicago, IL, at the American Chemical Society's 233rd National Meeting and Exposition. During the meeting, a four-day symposium on RNA biophysics was held, which was organized by Joseph D. Puglisi and James R. Williamson. The symposium touched on a number of outstanding and timely topics on the biophysics of RNA, including RNA structure, folding, and dynamics. The meeting showed that nothing short of a revolution in our understanding of RNA structure and function has occurred and that many of the questions asked 20 years ago are now being answered. Advances in RNA biophysics are having impacts on numerous other fields, including gene regulation, virology, and microbiology, to name a few.

The meeting was organized into six sessions, beginning Sunday morning with RNA structure. Advances in RNA structure have probably had the single greatest impact in the field in the past 10 years. Structures of numerous catalytic RNAs and the ribosome are among the many examples, and the Protein Data Bank now has >400 RNA structures. Adrian Ferré D'Amaré and Robert Batey presented structures of riboswitches, which are RNAs that bind small molecules through an aptameric domain and affect gene expression through an expression domain. The *glmS* riboswitch, which is activated by glucosamine-6-phosphate (GlcN6P), is unusual in that its expression domain is a novel ribozyme fold (*1*). Ferré D'Amaré presented pre- and post-cleavage structures of the *glmS* ribozyme-riboswitch, which folds into a double-pseudoknot topology (*2*). He presented evidence that the GlcN6P cofactor does not work through allostery but instead appears to be an active participant in chemistry through involvement of an amine group. Batey, who has solved several ligand-bound riboswitch crystal structures, addressed the issue of the structure of the free form of the RNA and communication between aptameric and expression domains (*3–5*). Through crystal structures, as well as a variety of biochemical techniques, including isothermal titration calorimetry, structure mapping with selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) chemistry, and fluorescence with stacking-sensitive 2-aminopurine, he provided evidence that only the internal binding pocket of the aptameric domain is unstructured in the ab-

sence of ligand; the global structure is largely unchanged. One theme for achieving tight overall binding was the importance of avoiding mispairing in the free state.

Whereas the riboswitch RNAs tend to be smaller in size (<200 nt), many functional RNAs are much larger. Alfonso Mondragón and Joe Piccirilli addressed structures and methodologies of crystallizing such RNAs. Mondragón and colleagues have solved several structures of the catalytic and specificity domains of RNase P RNA (*6*–*8*), which is a true multiple-turnover ribozyme, is responsible for 5′-end processing transfer RNAs (tRNAs), and was one of the first ribozymes discovered. At the meeting, a structure of the entire ~300-nt RNase P was presented (*8*). Communication between domains, clustering of conserved residues, and the basis for pseudoknot formation were found. Future studies are expected to unveil positioning of tRNA substrate and the catalytic mechanism of this ribozyme.

One of the themes of the meeting was the increasing development of methods for examining RNA structure at low-to-medium resolution, notably small-angle X-ray scattering (SAXS), cryo-electron microscopy (cryo-EM), chemical probing, and fluorescence methods. SAXS was presented by several different speakers with clever applications for dissecting the mechanism of RNA folding pathways, sometimes as snapshots of stepwise processes (Tao Pan) and sometimes by monitoring the folding in real time by SAXS analysis at different positions along a microfluidic channel (Lois Pollack). Eric Kool showed us that organic chemistry can provide synthetic base analogues that can be powerful tools in elucidating the mechanism of sequence recognition in RNA interference (*9*). Charles Hoogstraten presented a memorable "tai chi" demonstration of RNA domain docking dynamics in which the differences among all-or-none, stepwise, and concerted mechanisms were discussed and related to calorimetric and CD evidence. Peter Qin presented electron paramagnetic resonance methods that measure nanometer distances in nucleic acids by using sequence-independent nitroxide probes (*10*). Joachim Frank presented beautiful cryo-EM reconstructions of the ribosome in different functional states and proposed a model for translocation that involves two modes of movement: ratcheting of the subunits and swiveling of the head of the small subunit, thereby accounting for the necessary movement of the ribosome by one full codon during each cycle (*11*). Stunning, long-timescale molecular-dynamics simulations of the ribosome in complete atomic detail with solvation of the binding of tRNA to the A-site were presented by Kevin Sanbonmatsu. A remarkable series of dance steps were revealed in which the ribosome and tRNA mutually change conformation.

The field of computational RNA structure prediction was discussed by several speakers, and it is apparent that reliable predictions of RNA 3D structure (John SantaLucia, Jr., and Neocles Leontis) and dynamics (Sanbonmatsu, Xiaohua Zhang, and Devarajan Thirumalai) are on the near-term horizon. In fact, the wealth of experimental and predicted structures is creating a new problem of how to manage all of the data so that researchers with varying levels of expertise can access and mine the known RNA structural, biochemical, and dynamic databases. Leontis introduced the RNA Ontology Consortium, whose mission is to build an RNA Ontology that will work with the Gene and Sequence Ontologies to provide semantic standards for the integration of complex, heterogeneous data on RNA 3D structure, function, and sequence (*12*). The aim is to promote greater human understanding, as well as software interoperability.

Within the cell, the structure and behavior of RNAs are highly dependent upon the proteins to which they are bound, and these proteins are key determinants of their biophysical milieu. Andrew Feig and John Marino both presented work on the nature of kissing interactions and the way in which these structures resolve into strand-displaced states (*13*). Such reactions are found in the biology of noncoding RNAs and in virology, often facilitated by RNA binding proteins such as Hfq and nucleocapsid zinc finger protein 7. Structural analysis plays a critical role in understanding how proteins influence RNA behavior. On the other hand, Gabriele Varani showed how a combination of screening and rational redesign of ligands could be used to inhibit transactivator of transcription (Tat) protein binding to the *trans*-acting responsive element (TAR) element of HIV. Elizabeth Theil presented her crystallographic studies on the iron-responsive elements (IRE)/IRE-binding protein system, showing how protein contacts at very long ranges influence RNA structure to facilitate their regulatory roles. Overall, the session showed that the RNA–protein complexes are quite fluid. Although a single structure is often quite illuminating, scientists need a collection of biochemical, biophysical, and structural tools to look at the progression between observed states in order to understand the biological behavior.

Catalytic RNAs, or ribozymes, were discovered ~25 years ago. One of the current themes in the field is the understanding of mechanisms for chemical catalysis. Ribozymes appear in a variety of sizes and can either occur naturally or be selected *in vitro*. Philip Bevilacqua, Marty Fedor, and David Lilley presented their latest findings on small, naturally occurring ribozymes. Bevilacqua presented evidence that the pK_a of catalytic base in the hepatitis delta virus ribozyme shifts to 7; this makes it histidine-like and an ideal candidate for proton transfer (*14*). Fedor presented work on the mechanism of the hairpin ribozyme and showed the importance of nucleobases rather than metal ions in the catalytic mechanism (*15, 16*). These functional studies complement recent crystal structures on the hairpin ribozyme and showed the particular importance of a guanosine and an adenosine in the mechanism. In addition, new ap-

proaches for assessing the p*K* of catalytic guanosines by using 8-azaguanine were presented (*17*). This provides a promising approach for determining the presence and contribution of p*K* shifting of guanines to RNA catalysis.

Results in the above two talks were discussed in light of several high-resolution crystal structures. Lilley, on the other hand, presented evidence that another small ribozyme without a high-resolution structure, the Varkud satellite (VS) ribozyme, has a mechanism similar to that of the hairpin and also involves catalytic guanosine and adenosine residues (*18*). Lilley concludes that the hairpin and VS ribozymes have converged on similar mechanistic solutions to specifically cleave the phosphodiester backbone. Michael Harris presented functional studies on RNase P that included insights into the roles of metal ions in the mechanism. Harris and coworkers have studied heavy atom isotope effects and Raman difference spectroscopy to associate metal ions with nucleophilic activation in the catalytic mechanism (*19*). Calculations are also beginning to play a greater role in elucidating ribozyme catalysis. Zhang, from Tom Bruice's lab, presented computational studies on the mechanism of a selected ribozyme that catalyzes a Diels–Alder reaction. The studies suggest that the ribozyme catalyzes the reaction largely from positioning of the reactants in productive conformations and angles (*20*).

Given that RNA has numerous functions in the cell, it becomes of interest to understand how it folds. It is no small feat for polyanions to fold into the compact structures needed for function. The uptake of metal ions, often Mg^{2+}, is critical to the process, and this can occur both early and late in the overall mechanism. Dan Herschlag, Michael Brenowitz, and Lois Pollack provided insight into how metal ions drive RNA folding, with an emphasis on the *Tetrahymena* ribozyme, and Kathleen Hall presented evidence for a Mg^{2+}-dependent conformational change in a putative new riboswitch. Herschlag and coworkers have applied atomic emission spectroscopy with inductively coupled plasma to give a direct count of the number of metal ions taken up during RNA folding (*21*). They are dissecting the mechanism of metal ion contributions by studies on model systems with helical docking and refining physical models for ion condensation, including the nonlinear Poisson–Boltzmann model. RNA tertiary structure can be conveniently mapped by using hydroxyl radical ions and has been time-resolved with synchrotron radiation. Brenowitz and colleagues have recently developed a convenient method for time-resolved mapping with hydroxyl radicals in the laboratory by using common reagents and a rapid mixer (*22*). This methodology was used to gather and analyze large amounts of data with a clustering approach. Computational analysis of the data provided a mechanism in which the *Tetrahymena* ribozyme folds through three parallel channels, each having its own intermediate, on its way to the native state (*23, 24*). This level of complexity has been seen with proteins as well. Pollack and coworkers used time-resolved SAXS and microfluidics with continuous flow mixing to look at the earliest events in RNA folding (500 μs timescale) (*25, 26*). They found that divalent ions first induce rapid compaction of the RNA without the formation of detectable tertiary contacts. Thus, a hierarchy appears to exist for attaining a complete tertiary structure, with collapse happening in a distinct early step.

The theme of complexity in RNA folding was echoed in Sarah Woodson's talk on the folding of 16S ribosomal RNA. The folding landscape for full assembly with proteins appears rugged, with many pathways to the final structure. Thus, many similarities appear to exist in the folding of RNA, proteins, and RNA–protein complexes. Woodson and coworkers also presented studies on the cooperativity of interactions in the collapse into compact intermediates (*27*). A variety of biophysical measurements gave identical thermodynamics, supporting a two-state model for the cooperative folding transition. A point mutant destabilized *all* tertiary contacts, also consistent with cooperativity in this process. Apparently, RNA can fold cooperatively at and between tertiary and secondary structural levels in the hierarchy of RNA folding.

Generally speaking, the RNA secondary structure folds first and the tertiary structure folds later. Much also remains to be understood about secondary structure formation. Thirumalai presented calculations on force-induced unfolding and force-quench refolding of RNA hairpins (*28, 29*). A coarse-grained off-lattice model was used for simulations and theoretical analysis. The position of the transition state was shown to be a function of the stretching force and loading rate. Such considerations are important for understanding how RNA folding depends on the conditions under which the studies are taken.

The structures of intermediates in RNA folding received attention from Pan and colleagues (*30*). This is a tricky problem, because folding intermediates, which are partially unfolded, are not generally amenable to crystallography. First, equilibrium folding methods were used to establish conditions under which a folding intermediate accumulates in the specificity domain of RNase P. Then, a battery of experimental techniques, including structure mapping, mutagenesis, and cryo-EM, together with computational modeling, was used, and this led to a structural model of the intermediate. This work nicely complemented that of Mondragón in the structural studies and showed that intermediates can involve not only secondary structure but also short-range native tertiary interactions.

The last session of the symposium focused on joining the structure and dynamics of RNA structures. Sam Butcher presented his NMR studies on the spliceosome, a notoriously dynamic and difficult system

to study at atomic resolution (*31, 32*). He focused on a stem-loop in U2 RNA, which is hypermodified with 2′-methoxy substitutions in humans and leads to unusual thermodynamic stability. It is intriguing that such stability may arise from a specific pattern of methylated nucleosides. Insight into how binding of the protein Prp24 remodels U6 RNA secondary structure to drive further spliceosome assembly was also discussed. The use of NMR relaxation studies to probe RNA dynamics was presented by Gary Dobny. Solid-state and solution NMR were shown to provide complementary information about sugar and base dynamics over a very wide timescale range (*33*). He used the Tat–TAR complex from HIV to measure complex formation. RNA conformational changes in the solid state were consistent with those in solution. He further obtained long-range dynamic information by using ^{31}P–^{19}F rotational-echo double-resonance methodologies and showed that the RNA structure undergoes large deformations upon protein binding. Taekjip Ha provided insight into the dynamics of a number of systems, including Holliday junctions, the hairpin ribozyme, and the ribosome (*34, 35*). In collaboration with Lilley, Ha and his group dissected details of the pH dependence and folding mechanism of the hairpin ribozyme at the single-molecule level. These data support acid–base chemistry by ribozyme nucleobases. An additional contribution at the single-molecule level was the work of Xiaowei Zhuang and coworkers on the assembly of telomerase, the complex of RNA and proteins that adds DNA to the ends of linear chromosomes. In collaboration with Kathleen Collins, Zhuang *et al.* used single-molecule FRET to show that this RNA–protein complex assembles in two steps in which the p65 protein directs folding of the RNA to allow binding of the catalytic subunit, telomerase reverse transcriptase (*36*).

One theme to RNA folding is the presence of multiple conformations of the RNA. It is therefore important to have various approaches to prepare the system in specific ways and to initiate and measure folding between states. Harald Schwalbe presented his studies on the folding of bistable RNAs or those that have two folds with similar stabilities (*37, 38*). He was able to photoprotect the RNAs and block base pairing within a given state. Photolysis was conducted in the NMR tube to initiate the folding process and monitor the kinetics of folding. This technique promises to be applicable to a variety of systems, including riboswitches. The last speaker was Yitzhak Tor, who discussed method development for exploring RNA recognition and dynamics. He has applied a number of fluorescent bases to elucidating RNA dynamics, including 2-aminopurine in hammerhead catalysis (*39*) and, more recently, pyrimidines modified with furan on the 5-position, which can be incorporated during *in vitro* transcription (*40*). These RNAs make good A-site model systems for antibiotic binding. Such affinity labeling techniques may find widespread application in the field of RNA folding and dynamics.

The past 20 years have provided an exhilarating series of advances in our understanding of the biophysics of RNA function. However, some old questions remain unanswered, and many new questions have emerged. The detailed mechanisms by which RNA is able to fold into its active conformation is still an unanswered question. We still do not fully understand the mechanism of catalysis of ribozymes. Where does the energy come from to stabilize the transition state? Do ribozymes work in the same way as protein enzymes, or are some fundamentally new ideas needed, particularly to account for the dense ionic character of RNA? It seems that we have more evidence supporting the RNA world hypothesis, yet a self-replicating RNA has not been discovered or engineered. Methods for studying RNA structure and dynamics will certainly continue toward higher structural and temporal resolution. The explosion in the discovery of noncoding RNAs will provide many new systems for biophysical studies. It will be interesting to see how our understanding of RNA biophysics allows us not only to explain biological function but also to invent artificial catalysts, materials, and new therapeutic methods. It is apparent that there is much to keep us gainfully employed for the next 20 years.

Acknowledgment: We thank Andrew Feig for contributing to the RNA–protein section of this article. We also thank the speakers for many helpful comments and suggestions.

REFERENCES

1. Winkler, W. C., Nahvi, A., Roth, A., Collins, J. A., and Breaker, R. R. (2004) Control of gene expression by a natural metabolite-responsive ribozyme, *Nature 428*, 281–286.
2. Klein, D. J., and Ferre-D'Amare, A. R. (2006) Structural basis of glmS ribozyme activation by glucosamine-6-phosphate, *Science 313*, 1752–1756.
3. Batey, R. T., Gilbert, S. D., and Montange, R. K. (2004) Structure of a natural guanine-responsive riboswitch complexed with the metabolite hypoxanthine, *Nature 432*, 411–415.
4. Gilbert, S. D., Stoddard, C. D., Wise, S. J., and Batey, R. T. (2006) Thermodynamic and kinetic characterization of ligand binding to the purine riboswitch aptamer domain, *J. Mol. Biol. 359*, 754–768.
5. Montange, R. K., and Batey, R. T. (2006) Structure of the *S*-adenosylmethionine riboswitch regulatory mRNA element, *Nature 441*, 1172–1175.
6. Krasilnikov, A. S., Yang, X., Pan, T., and Mondragon, A. (2003) Crystal structure of the specificity domain of ribonuclease P, *Nature 421*, 760–764.
7. Krasilnikov, A. S., Xiao, Y., Pan, T., and Mondragon, A. (2004) Basis for structural diversity in homologous RNAs, *Science 306*, 104–107.
8. Torres-Larios, A., Swinger, K. K., Krasilnikov, A. S., Pan, T., and Mondragon, A. (2005) Crystal structure of the RNA component of bacterial ribonuclease P, *Nature 437*, 584–587.
9. Somoza, A., Chelliserrykattil, J., and Kool, E. T. (2006) The roles of hydrogen bonding and sterics in RNA interference, *Angew. Chem., Int. Ed. 45*, 4994–4997.
10. Cai, Q., Kusnetzow, A. K., Hubbell, W. L., Haworth, I. S., Gacho, G. P., Van Eps, N., Hideg, K., Chambers, E. J., and Qin, P. Z. (2006) Site-directed spin labeling measurements of nanometer distances in nucleic acids using a sequence-independent nitroxide probe, *Nucleic Acids Res. 34*, 4722–4734.
11. Taylor, D. J., Nilsson, J., Merrill, A. R., Andersen, G. R., Nissen, P., and Frank, J. (2007) Structures of modified eEF2 80S ribosome complexes reveal the role of GTP hydrolysis in translocation, *EMBO J. 26*, 2421–2431.

12. Leontis, N. B., Altman, R. B., Berman, H. M., Brenner, S. E., Brown, J. W., Engelke, D. R., Harvey, S. C., Holbrook, S. R., Jossinet, F., Lewis, S. E., Major, F., Mathews, D. H., Richardson, J. S., Williamson, J. R., and Westhof, E. (2006) The RNA Ontology Consortium: an open invitation to the RNA community, *RNA 12*, 533–541.
13. Zhao, C., and Marino, J. P. (2007) Synthesis of HIV-1 Ψ-site RNA sequences with site specific incorporation of the fluorescent base analog 2-aminopurine, *Tetrahedron 63*, 3575–3584.
14. Nakano, S., and Bevilacqua, P. C. (2007) Mechanistic characterization of the HDV genomic ribozyme: a mutant of the C41 motif provides insight into the positioning and thermodynamic linkage of metal ions and protons, *Biochemistry 46*, 3001–3012.
15. Kuzmin, Y. I., Da Costa, C. P., Cottrell, J. W., and Fedor, M. J. (2005) Role of an active site adenine in hairpin ribozyme catalysis, *J. Mol. Biol. 349*, 989–1010.
16. Cottrell, J. W., Kuzmin, Y. I., and Fedor, M. J. (2007) Functional analysis of hairpin ribozyme active site architecture, *J. Biol. Chem. 282*, 13498–13507.
17. DaCosta, C. P., Fedor, M. J., and Scott, L. G. (2007) 8-Azaguanine reporter of purine ionization states in structured RNAs, *J. Am. Chem. Soc. 129*, 3426–3432.
18. Wilson, T. J., McLeod, A. C., and Lilley, D. M. J. (2007) A guanine nucleobase important for catalysis by the VS ribozyme, *EMBO J. 26*, 2489–2500.
19. Cassano, A. G., Anderson, V. E., and Harris, M. E. (2004) Analysis of solvent nucleophile isotope effects: evidence for concerted mechanisms and nucleophilic activation by metal coordination in nonenzymatic and ribozyme-catalyzed phosphodiester hydrolysis, *Biochemistry 43*, 10547–10559.
20. Zhang, X., and Bruice, T. C. (2007) Diels–Alder ribozyme catalysis: a computational approach, *J. Am. Chem. Soc. 129*, 1001–1007.
21. Das, R., Travers, K. J., Bai, Y., and Herschlag, D. (2005) Determining the Mg^{2+} stoichiometry for folding an RNA metal ion core, *J. Am. Chem. Soc. 127*, 8272–8273.
22. Shcherbakova, I., Mitra, S., Beer, R. H., and Brenowitz, M. (2006) Fast Fenton footprinting: a laboratory-based method for the time-resolved analysis of DNA, RNA and proteins, *Nucleic Acids Res. 34*, e48.
23. Laederach, A., Shcherbakova, I., Liang, M. P., Brenowitz, M., and Altman, R. B. (2006) Local kinetic measures of macromolecular structure reveal partitioning among multiple parallel pathways from the earliest steps in the folding of a large RNA molecule, *J. Mol. Biol. 358*, 1179–1190.
24. Laederach, A., Shcherbakova, I., Jonikas, M. A., Altman, R. B., and Brenowitz, M. (2007) Distinct contribution of electrostatics, initial conformational ensemble, and macromolecular stability in RNA folding, *Proc. Natl. Acad. Sci. U.S.A. 104*, 7045–7050.
25. Russell, R., Millett, I. S., Tate, M. W., Kwok, L. W., Nakatani, B., Gruner, S. M., Mochrie, S. G., Pande, V., Doniach, S., Herschlag, D., and Pollack, L. (2002) Rapid compaction during RNA folding, *Proc. Natl. Acad. Sci. U.S.A. 99*, 4266–4271.
26. Kwok, L. W., Shcherbakova, I., Lamb, J. S., Park, H. Y., Andresen, K., Smith, H., Brenowitz, M., and Pollack, L. (2006) Concordant exploration of the kinetics of RNA folding from global and local perspectives, *J. Mol. Biol. 355*, 282–293.
27. Chauhan, S., Caliskan, G., Briber, R. M., Perez-Salas, U., Rangan, P., Thirumalai, D., and Woodson, S. A. (2005) RNA tertiary interactions mediate native collapse of a bacterial group I ribozyme, *J. Mol. Biol. 353*, 1199–1209.
28. Hyeon, C., and Thirumalai, D. (2006) Forced-unfolding and force-quench refolding of RNA hairpins, *Biophys. J. 90*, 3410–3427.
29. Hyeon, C., Dima, R. I., and Thirumalai, D. (2006) Pathways and kinetic barriers in mechanical unfolding of RNA and proteins, *Structure 14*, 1633–1645.
30. Baird, N. J., Westhof, E., Qin, H., Pan, T., and Sosnick, T. R. (2005) Structure of a folding intermediate reveals the interplay between core and peripheral elements in RNA folding, *J. Mol. Biol. 352*, 712–722.
31. Bae, E., Reiter, N. J., Bingman, C. A., Kwan, S. S., Lee, D., Phillips, G. N., Jr., Butcher, S. E., and Brow, D. A. (2007) Structure and interactions of the first three RNA recognition motifs of splicing factor prp24, *J. Mol. Biol. 367*, 1447–1458.
32. Sashital, D. G., Venditti, V., Angers, C. G., Cornilescu, G., and Butcher, S. E. (2007) Structure and thermodynamics of a conserved U2 snRNA domain from yeast and human, *RNA 13*, 328–338.
33. Olsen, G. L., Edwards, T. E., Deka, P., Varani, G., Sigurdsson, S. T., and Drobny, G. P. (2005) Monitoring tat peptide binding to TAR RNA by solid-state 31P-19F REDOR NMR, *Nucleic Acids Res. 33*, 3447–3454.
34. McKinney, S. A., Freeman, A. D., Lilley, D. M., and Ha, T. (2005) Observing spontaneous branch migration of Holliday junctions one step at a time, *Proc. Natl. Acad. Sci. U.S.A. 102*, 5715–5720.
35. Nahas, M. K., Wilson, T. J., Hohng, S., Jarvie, K., Lilley, D. M., and Ha, T. (2004) Observation of internal cleavage and ligation reactions of a ribozyme, *Nat. Struct. Mol. Biol. 11*, 1107–1113.
36. Stone, M. D., Mihalusova, M., O'Connor, C. M., Prathapam, R., Collins, K., and Zhuang, X. (2007) Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein, *Nature 446*, 458–461.
37. Wenter, P., Furtig, B., Hainard, A., Schwalbe, H., and Pitsch, S. (2005) Kinetics of photoinduced RNA refolding by real-time NMR spectroscopy, *Angew. Chem., Int. Ed. 44*, 2600–2603.
38. Fürtig, B., Buck, J., Manoharan, V., Bermel, W., Jäschke, A., Wenter, P., Pitsch, S., and Schwalbe, H. (2007) Time-resolved NMR studies of RNA folding, *Biopolymers 86*, 360–383.
39. Kirk, S. R., Luedtke, N. W., and Tor, Y. (2001) 2-Aminopurine as a real-time probe of enzymatic cleavage and inhibition of hammerhead ribozymes, *Bioorg. Med. Chem. 9*, 2295–2301.
40. Srivatsan, S. G., and Tor, Y. (2007) Fluorescent pyrimidine ribonucleotide: synthesis, enzymatic incorporation, and utilization, *J. Am. Chem. Soc. 129*, 2044–2053.