CC/NUMBER 6
FEBRUARY 10, 1986

This Week's Citation Classic®

Tinoco I, Jr., Borer P N, Dengler B, Levine M D, Uhlenbeck O C, Crothers D M & Gralla J. Improved estimation of secondary structure in ribonucleic acids. *Nature—New Biol.* 246:40-1, 1973.
[Dept. Chem., Univ. California, Berkeley, CA; Biochem. Dept., Univ. Illinois, Urbana, IL; and Dept. Chem., Yale Univ., New Haven, CT]

A simple method is presented for assessing which base-paired structures an RNA molecule will form in solution. Rules are given to calculate the free energy of each structure. The structures with the lowest free energy are the most probable. [The *SCI*® indicates that this paper has been cited in over 720 publications since 1973.]

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September 25, 1985

As soon as the first sequence of an RNA molecule was determined,[1] possible double-stranded structures were proposed. The structures were drawn by pairing adenine (A) with uracil (U) and guanine (G) with cytosine (C), but there was no way of choosing among the possible structures. It was clear that the base-paired, double-stranded regions would stabilize the molecules, but what about the unpaired bases left in hairpin loops, bulges, and internal loops?

In 1969 Olke Uhlenbeck came to work in my lab in Berkeley and decided to synthesize some RNA hairpin loops. A hairpin loop in RNA is a loop of unpaired bases closed by a stem of base pairs. He challenged me and the graduate students in the laboratory to predict the "melting temperatures" of the loops. (The melting temperature in this context is the temperature at which there are equal amounts of single strands and hairpin loops in equilibrium.) The challenge led to predictions (mostly wrong), but, more importantly, it led to a general procedure to predict folded structures (secondary structure) in RNA.[2]

The procedure involves (1) forming all possible Watson-Crick base pairs in the RNA, (2) summing the favorable free energies of the double-strand regions with the unfavorable free energies of the loops and bulges, and (3) finding the structure with the lowest free energy. I was told that the biochemists and molecular biologists would be confused by having the most negative free energy give the most favorable structure and would in any case be repelled by thermodynamics. Therefore, the recipe for predicting secondary structure was written in terms of stability numbers (positive numbers meant stable structures, negative numbers meant unstable structures).

In the next two years, our laboratory continued synthesizing and studying model RNA molecules. Independently, Donald Crothers and his colleagues at Yale studied similar RNA molecules. Unfortunately, our conclusions did not agree. We were each certain that the other group was wrong. The two groups finally met in neutral territory at a conference on transfer RNA structure at Princeton. We decided that maybe we were both right and jointly wrote this most-cited paper on *improved* estimation of secondary structure. By this time, our opinion of the biochemists and molecular biologists had improved so we gave the rules in terms of free energies, not stability numbers.

The recipe presented in the two pages of the article is a very simple one to use. Any folded structure proposed for a messenger RNA, ribosomal RNA, or viral RNA can be assigned a free energy to assess its stability. I am told that referees usually insist that each published structure be given an authorized free energy calculated with our procedure. This has undoubtedly increased the number of citations. As thermodynamic values for other RNA and DNA model molecules are obtained,[3,4] the power of prediction of RNA and DNA structures should increase, and the citations to this paper will decrease.

1. **Holley R W, Apgar J, Everett G A, Madison J T, Marquisee M, Merrill S H, Penswick J R & Zamir A.** Structure of ribonucleic acid. *Science* 147:1462-5, 1965. (Cited 720 times.)
2. **Tinoco I, Jr., Uhlenbeck O C & Levine M D.** Estimation of secondary structure in ribonucleic acids. *Nature* 230:362-7, 1971. (Cited 240 times.)
3. **Freier S M, Petersheim M, Hickey D R & Turner D H.** Thermodynamic studies of RNA stability. *J. Biomol. Struct. Dyn.* 1:1229-41, 1984.
4. **Aboul-ela F, Koh D, Tinoco I, Jr. & Martin F H.** Base-base mismatches. Thermodynamics of double helix formation for $dCA_3XA_3G + dCT_3YT_3G$ (X, Y=A,C,G,T). *Nucl. Acid. Res.* 13:4811-24, 1985.

