

# RNA secondary structures: from *ab initio* prediction to better compression, and back

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## Abstract

In this paper, we use the biological domain knowledge incorporated into stochastic models for *ab initio* RNA secondary-structure prediction to improve the state of the art in joint compression of RNA sequence and structure data (Liu et al., BMC Bioinformatics, 2008). Moreover, we show that, conversely, compression ratio can serve as a cheap and robust proxy for comparing the prediction quality of different stochastic models, which may help guide the search for better RNA structure prediction models.

Our results build on expert stochastic context-free grammar models of RNA secondary structures (Dowell & Eddy, BMC Bioinformatics, 2004; Nebel & Scheid, Theory in Biosciences, 2011) combined with different (static and adaptive) models for rule probabilities and arithmetic coding. We provide a prototype implementation and an extensive empirical evaluation, where we illustrate how grammar features and probability models affect compression ratios.

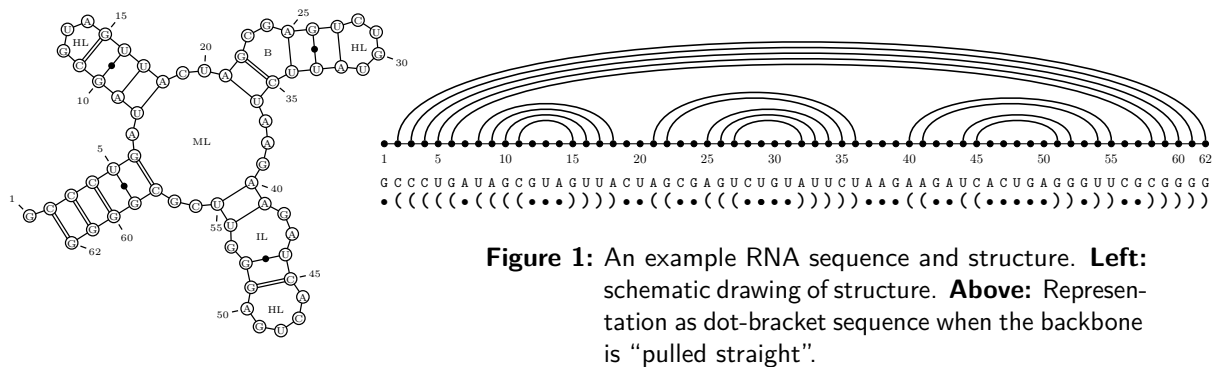
## 1 Introduction

In this article, we explore the interplay and potential symbiosis between data compression and probabilistic methods for predicting the folding structure of (non-coding) RNA molecules. Ribonucleic acid (RNA) is a bio-polymer that serves various roles in the coding, decoding, expression and regulation of genes in cells. An RNA molecule consists of a chain of *nucleotides* each having a *base* attached to it (either adenine (A), cytosine (C), guanine (G), or uracil (U)); this string of bases forms the *sequence* of the molecule. Unlike the related DNA, RNA is usually single-stranded and forms spatial structures by folding onto itself (similar to proteins), with complementary bases forming a stabilizing hydrogen bond. The set of (indices of the) bases that form such pairs is the *secondary structure* of the molecule; it can be encoded by the dot-bracket notation, (see Figure 1; a formal definition is given in Section 2).

The secondary structure is instrumental for the biological function of non-coding RNA molecules and of great interest to biologists. Much research has hence been devoted to computationally *predict* the secondary structure from a known RNA sequence (*ab initio* RNA secondary-structure prediction) [4, 9, 28], including human swarm intelligence [16], and it remains an active research area [24, 7, 25]. We explore areas around RNA secondary structures where innovations in compression methods are central for further progress.

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**Figure 1:** An example RNA sequence and structure. **Left:** schematic drawing of structure. **Above:** Representation as dot-bracket sequence when the backbone is “pulled straight”.

**Better RNA Compression.** Our first goal is to use the domain knowledge on RNA foldings incorporated into secondary-structure prediction models for improved methods for the joint compression of the sequence and secondary structure of RNA sequences. With biological databases ever increasing, compressed representations become desirable. In the case of databases for non-coding RNA sequences with known secondary structures, the data volume has long remained manageable, but growth is now accelerating: For example, *RNA Central* [2] now aggregates over 25 million trusted secondary structures 8 years after its first release; 1.8 million of these come from the *rfam* database [12], collected over its 20 years of existence.

The need for space-efficient representations of joint RNA sequence and secondary structure databases has been identified by Liu et al. in 2008 [17]. Their algorithm *RNACompress*, based on a stochastic context-free grammar (SCFG, defined below), has been recognized as an early application of ideas from grammar-based compression in the data-compression community [19, 13]. As we demonstrate in this article, substantially better compression ratios can be achieved than Liu et al. report; interestingly, by carefully extending their very method to a general framework of SCFG-based compression. Improvements are then realized by applying this framework on tried and tested grammars from the RNA secondary structure *prediction* literature [3, 22] (as well as further, orthogonal refinements).

Apart from the practical utility of less space, compression methods are of direct interest in bioinformatics as a way to upper bound the Kolmogorov complexity [14] of a dataset, and hence its inherent information content [8]. For example in the context of RNA sequences, one can ask how much additional information is contained in the secondary structure of the RNA when the sequence is known.

**Compression as a proxy for predictive power.** Our second and main goal is to test our hypothesis that for comparing probabilistic models for RNA secondary structures, *compression ratio can serve as proxy for prediction quality in RNA secondary-structure prediction*. Advances in next-generation sequencing allows determining the sequence of many molecules at scale, whereas secondary structures need to be determined by much more expensive techniques like X-ray crystallography [28]. A much cheaper and faster alternative is to computationally *predict* the structure from a known sequence. The state-of-the-art approaches either build on a chemical model of the molecules and try to identify a structure with minimal free energy or use a machine-learning approach. Both can formally be described by stochastic context-free grammars (see Section 2).

RNA secondary-structure prediction plays a vital role in studying the biological function of RNA molecules and for designing artificial RNA sequences, and so numerous software packages implement different algorithms for this task. Comparing their prediction quality is a delicate undertaking, because no definitive similarity metric is known to judge how close the predicted secondary structure is from an experimentally determined one [20]. Indeed, the method of

choice in the literature to compare structure prediction is solely based on individual base pairs [20, 3, 23, 22]: One compares the *sensitivity* and *positive predictive value (PPV)* of different approaches (defined in Section 2).

We will use the *compressed size* (in bits per base) of the reference structure under the trained stochastic model as a more direct means to compare how well different models capture RNA folding behavior. This compressed size effectively reflects the *log-likelihood* of the reference structure and hence has a natural interpretation as the information content that model assigns to the RNA structure.

This has several advantages over sensitivity/PPV: (a) It directly evaluates the quality of the *model*, separating it from the method to produce a (single) predicted secondary structure. There are different options to predict a structure; one can use the most likely structure, or a consensus structure containing the most likely individual pairs, or return a sample of several nearly optimal structures. No choice clearly dominates the others, but they affect the sensitivity and PPV scores. (b) Log-likelihood is a single natural metric derived from first principles of information theory; it does not need trade-offs or further parameters.

**Contributions.** Our contributions are as follows. First, we improve the compression ratio achieved for joint RNA sequence and structure data by 45% over the state of the art, Liu et al.’s RNACompress [17]; compared to the general-purpose compressor paq8l (<http://mattmahoney.net/dc/#paq>), we see a 70% improvement. The improvement over RNACompress is the combined result of several refinements, but a 30% reduction in compressed size is observed when keeping everything but the used SCFG constant. This clearly shows the relevance of the grammar and the validity of our approach to employ structure-prediction grammars. The proposal and implementation of the more sophisticated grammars (such as the one based on [22]) is hence a useful contribution. Second, we demonstrate that compression ratio can be used as a robust predictor of how well a grammar will perform for *ab initio* secondary-structure prediction. To our knowledge, this is the first such attempt to identify suitable probabilistic models for RNA structure prediction that is not based on comparing predicted structures to a benchmark dataset. Finally, we reproduce and confirm the computational study of [3] with an independent implementation and additional modifications to their grammars.

**Related Work.** Liu et al. [17] proposed RNACompress in 2008; we discuss their methodology in detail in Section 3. Naganuma et al. [21] explore a related method of SCFG compression closer to grammar-based compression using straight-line programs. They create a stochastic grammar from the text to compress with a variation of the RePair heuristic [15]. For a broader context of grammar-based compression, see the recent survey of Kieffer and Yang [13]. Friemel [6] also targets the joint RNA compression problem, but using a different approach. He encodes RNA structures as labeled trees with each node representing a nucleotide and the branches representing the bonds; unpaired bases yield unary nodes. Friemel’s algorithm RNAContract contracts sequences of unary nodes (similar to compact tries) or a sequence of multiple nested brackets in the dot-bracket notation. After the node contraction the algorithm encodes the contracted node tree using Huffman coding.

**Outline.** The rest of this paper is structured as follows. Section 2 collects required concepts. Section 3 explains the grammar-based compression of RNA. Then we report on our two studies: Section 4 discusses the compression achieved with various grammars and Section 5 explores the connection between compressed size and prediction quality. We conclude in Section 6 with future

work. Further details, all datasets and code to produce the figures in this article are available online as supplementary material: <https://www.wild-inter.net/publications/onokpasa-wild-wong-2023>.

## 2 Preliminaries

**Dot-bracket notation.** An RNA sequence is a string of bases **A**, **C**, **G**, **U**. Stable hydrogen bonds are possible between **A** and **U** resp. **C** and **G** (the Watson-Crick pairs) and to a lesser extent also between **G** and **U**. RNA secondary structures<sup>1</sup> can be represented by the dot-bracket notation [10]: a well-nested string over  $\{\bullet, (, )\}$  where a base pair is denoted by matching parentheses  $()$  and an unpaired base by  $\bullet$ ; see Figure 1 for an example. We use “RNA” as an abbreviation for “a pair of an RNA sequence and its secondary structure”.

**SCFG.** Dot-bracket strings can be generated by a context-free grammar (CFG). A CFG is a tuple  $(N, T, R, S)$  where  $N$  and  $T$  are finite sets of *nonterminals* and *terminals*, respectively,  $R \subseteq N \times (N \cup T)^*$  is a finite set of *production rules*, and  $S \in N$  is the *start symbol*. A rule in  $R$  is written as  $A \rightarrow \alpha$ . A *stochastic context-free grammar* (SCFG) is a tuple  $G = (N, T, R, S, W)$  such that  $(N, T, R, S)$  is a CFG and  $W : R \rightarrow [0, 1]$  is a function satisfying  $\sum_{(A \rightarrow \alpha) \in R} W(A \rightarrow \alpha) = 1$  for all  $A \in N$ . For every  $A \in N$ ,  $W$  represents a probability distribution over the set of rules with left-hand side  $A$ .

**Earley Parser.** The Earley Parsing algorithm [5] is able to process any SCFG and efficiently determine whether a string belongs to the language of the grammar. We use the Earley parser implementations by [27, 29] when comparing various SCFGs since it does not require a rigid normal form for grammars.

**RNA secondary-structure prediction.** A stochastic context-free grammar can be used for RNA secondary-structure prediction where terminals correspond to bases and the leftmost derivation of an RNA sequence encodes a secondary structure of the sequence. The used SCFGs allow many different derivations (and hence secondary structures) for a given sequence and the rule probabilities induce a probability distribution over those. Using a classical machine-learning approach, the rule probabilities are chosen as maximum likelihood parameters w.r.t. a given training dataset (with known secondary structures). For predicting/infering the (unknown) secondary structure of a new RNA sequence, a probabilistic parser determines the maximum-likelihood derivation (Viterbi parse) of the RNA sequence in the SCFG, which encodes the most likely secondary structure (under the given probabilistic model).

We measure the quality of prediction by *sensitivity* and *positive predictive value (PPV)*: the fraction of correctly predicted base pairs among all pairs in the reference structure resp. all pairs in the predicted structure. More formally, let  $TP$ ,  $TN$ ,  $FP$ ,  $FN$  be the number of base pairs that are true positives, true negatives, false positives, and false negatives, respectively. Then Sensitivity =  $\frac{TP}{TP+FN}$  and PPV =  $\frac{TP}{TP+FP}$ .

## 3 RNA compression using stochastic context-free grammars

We now show how to jointly compress an RNA sequence and secondary structure using a SCFG  $G$ . This method has been used by Liu et al. [17] on a fixed grammar; we generalize it here to arbitrary grammars  $G$  and rule-probability models. The terminals of  $G$  are pairs of

<sup>1</sup>As is often done in the area, we do not consider structures with pseudoknots in this paper.



and final encoders (Huffman or arithmetic coding). We use an existing open-source Earley Parser implementation [27] for obtaining a parse tree (given a SCFG and an RNA with sequence and structure).<sup>3</sup> Apart from  $G_L$  from [17], we use the structure-prediction grammars from [3] and [22]. Since non-canonical bonds are regularly found in experimentally determined secondary structures, all our grammars come in two versions: one that only allows the Watson-Crick and “G-U wobble” pairs, and one that allows all 16 pairs. The difference for compression is small: while most RNA structures do contain non-canonical bonds, most contain only very few of them.

For the compression-quality study, we use the “friemel” dataset, consisting of 17 000 ribosomal RNAs from [1] where ambiguously sequenced bases, non-canonical base pairs and pseudoknots have been removed [6]. Information of each RNA in the given datasets is stored in a text file, using the dot-bracket notation. 24 contained empty hairpin loops; since 2 grammars from [3] exclude these, we replaced the innermost pair by two unpaired bases; for the evaluation, we exclude these 24 RNAs.

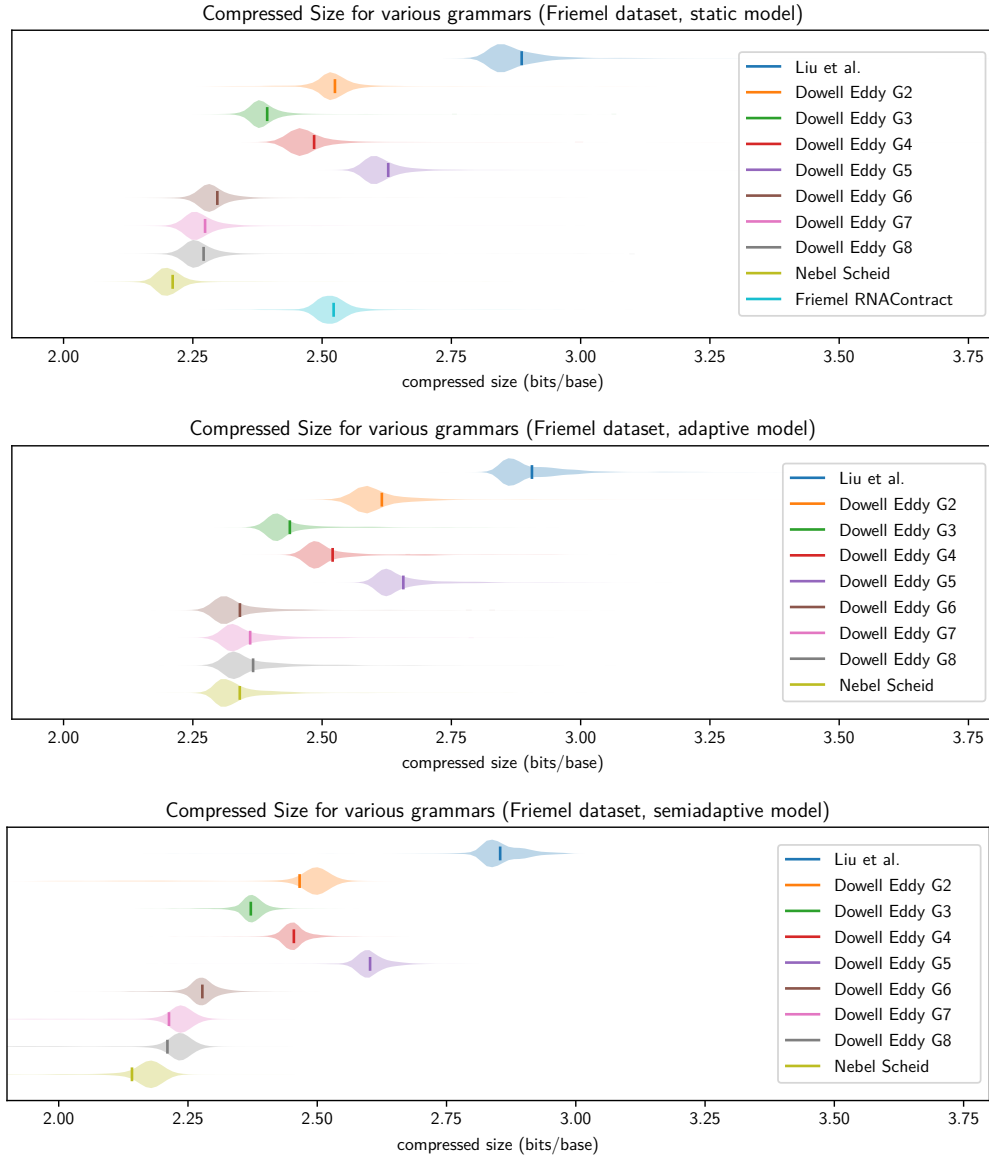
Figure 2 shows the compression quality of different grammars, normalized to the (average) number of bits per base in the RNA. It is striking that the current state-of-the-art method from the literature, Liu et al.’s RNACompress [17], performs much worse than all the structure-prediction grammars (for all rule-probability models), indicating that these grammars indeed incorporate effective domain knowledge about RNA structures. Also note that a simplistic encoding of the RNA sequence alone would use 2 bits/base; the most sophisticated grammars come very close to that for the joint encoding of sequence *and* structure: 2.21 bits/base on average for the grammar of Nebel and Scheid [22]. The large grammars  $G_2$ ,  $G_7$ , and  $G_8$  [3] (those with “stacking parameters”) and the huge grammar by Nebel and Scheid [22] perform overall best. But some much smaller grammars like  $G_6$  come very close, despite having a factor 10 fewer parameters. This shows that it is the structure of the grammar, not merely the number of parameters of the model, that improve compression of RNA secondary structures.

## 5 Compression ratio vs. prediction quality

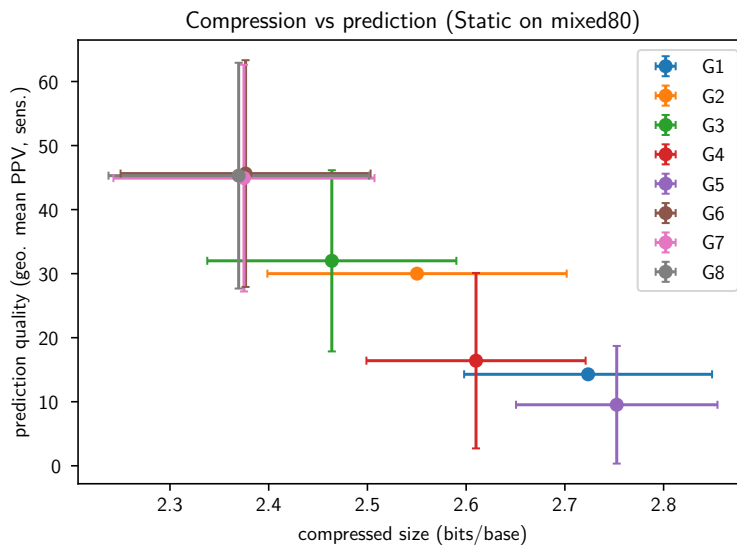
We have seen that the choice of the grammar heavily influences the compression quality of our generic joint RNA compressor. In this section, we take a closer look at this grammar dependence from the perspective of both compression and secondary-structure prediction. For that, we reproduced the classic study of Dowell and Eddy [3] comparing several hand-crafted SCFG for their ability to correctly infer RNA secondary structures given only the RNA sequence as input. Due to the bugs from [27], we here used the probabilistic Earley parser from [29]. We use the original datasets from [3] (available at <http://eddylab.org/software/conus/>): The “benchmark” dataset was used in [3] to compare the prediction quality of SCFGs, whose rule probabilities have been trained on their “mixed80” dataset; see [3] for further details. Both datasets contain many non-canonical bonds and 8 RNAs contain empty hairpin loops; we again eliminated the latter. Mixed80 contains numerous ambiguous bases; these were randomly replaced with a compatible base.

Figure 3 shows the results of comparing for each grammar how well it compresses the benchmark dataset of RNAs and how well it predicts secondary structures of this set (using the setup and parameters as in [3]). Taking into account the variability across different RNAs within the dataset, a clear and strong negative correlation is visible between compressed size and prediction quality; in particular, there is a clearly distinct cluster of grammars that

<sup>3</sup>This parser has been reported to yield incorrect results for certain inputs; for the compression experiment, we could confirm that it works correctly on all our inputs and grammars.



**Figure 2:** Means (vertical bars) and distributions (shaded violin plot) of the normalized compressed size using various grammars on Friemel’s RNA dataset. All compressed sizes are shown as bits per base. **Top:** results using static rule probabilities, determined from the entire dataset. **Middle:** results using adaptive rule-probabilities model (LaPlace model). **Bottom:** semi-adaptive rule probabilities (ignoring space for storing rule probabilities).



**Figure 3:** Scatter plot of compression vs. prediction quality for the grammars from [3]. Each grammar is presented as one point with error bars. The  $x$ -axis shows the compressed size (in bits per base) for joint compression of RNA sequence and secondary structure, averaged over the benchmark dataset [3]. Horizontal error bars show one standard deviation of compressed size over the benchmark dataset. The  $y$ -axis shows the geometric mean of sensitivity and PPV (for each predicted RNA secondary structure, averaged over the benchmark dataset); error bars show one standard deviation. For the ambiguous grammars  $G_1$  and  $G_2$ , no vertical error bars are available (we did not reproduce predictions for these; the average is taken from [3]). Both compression and prediction use the same training dataset (mixed80 from [3]) to determine the parameters of the grammars; compression here uses the static model for rule probabilities.

simultaneously give the best compression and the best prediction. At least for the grammars from [3], this shows that one can use compressed size as a more rigidly defined and robust proxy for secondary-structure prediction quality.

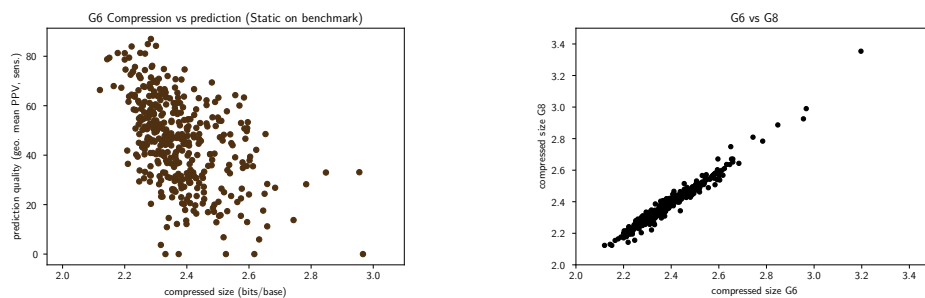
Figure 4 takes a closer look at the correlation on a per-RNA level. Even there, a correlation remains visible; in particular very accurately predicted structures are also well compressed. The right panel in Figure 4 shows that compressed size for different grammars is very strongly correlated; pictures for other grammar pairs are similar (excluding the poor performing  $G_1$ ,  $G_4$ , and  $G_5$ ). Note that despite the strong correlation at RNA level, there is a significant difference in the (mean) compression ratio between different grammars. This might indicate that there are intrinsically more and less “surprising” RNA secondary structures (knowing only the RNA sequence).

## 6 Conclusion

In this paper, we demonstrated how domain knowledge of RNA secondary structures encapsulated in stochastic context-free grammars for structure prediction can be used to obtain the best single-RNA compression ratios known for this type of data. Moreover, we showed promising first evidence for the utility of compression ability as a cheap and robust proxy for prediction quality for RNA secondary-structure prediction.

This work opens up several enticing avenues for future research. Using compression ability as simpler guide, we are working on an approach to discover new promising models for secondary-





**Figure 4:** Scatter plots with one point per RNA sequence in the benchmark dataset. **Left:** compressed size against prediction quality using  $G_6$ . **Right:** compressed size using  $G_6$  against compressed size using  $G_8$ . All compression methods use the static rule probabilities trained on mixed80.

structure prediction. It would be interesting to investigate whether the robust correlation between prediction quality and compressed size continues to hold for large grammars with many parameters; here prediction could suffer due to overfitting issues, whereas compression might continue see improvements from additional parameters. Since many natural RNA secondary structures contain “pseudoknots”, a principled approach for compressing such structures would be interesting. If the compression-prediction correlation can be demonstrated in this domain as well, the lack of reliably free-energy models for pseudoknotted RNA structures and the relative lack of high-fidelity training data would make compression ability of even greater value in the search for better predictions models.

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