# End-to-end RNA Secondary Structure Prediction using Deep Neural Network

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## 1 Introduction

Once believed to be an intermediate molecule that serves as a messenger between DNA and protein, RNA is now known to be involved in many aspects in gene regulation and expression. Unlike DNA which forms stable double helix (between two molecules), RNA is highly versatile and? a single RNA moldecule can fold onto itself by forming base pairs via hydrogen bonds, including Watson-Crick pairs A-U, G-C and non-canonical pairs such as G-U. These base pairs act as building blocks, from which local structure forms? global?

State-of-the-art RNA structure prediction algorithms, such as ViennaRNA**?** and Mfold**?**, are based on the fundamental property of base-pairing. Each type of base-pairing, as well as each type of local structure, has its own associated free energy that is measured experimentally. Total free energy is assumed to be the sum of all local free energy, and can be minimized efficiently using dynamic programming. Although people have worked out a large set of local structure types and their associated formulation for free energy calculation, there is no guarantee that it's either accurate or complete. There has been effort to fine tune the free energy parameters by training on experimentally solved structures**?**, but it's still limited by the known set of local structure types.

Moreover, over the last decade, combination of RNA structure probing and high throughput sequencing has enabled the measurement of genome-wide RNA structural at single nucleotide resolution in multiple organisms and cell types, which have only been combined? with dynamic programming using heuristics (ref?). Such data also exhibit a high level of noise and sometimes with missing data (ref, sequencing, DMS), which also calls for a new framework that can be trained end-to-end on different types of data.

TODO if we mention the above we'll need to talk about how to train on those data.

sequence alone

one sequence, many structures

## 2 Related Work

## 3 Method

### 3.1 Problem Formulation

For a sequence of length $L$, it is possible? to take on a distribution of strutures?. A specific RNA secondary structure can be represented by an upper triangular matrix (excluding the diagonal) of size $L \times L$ with binary values, where a value of $0$ at $(i, j)$ represents base pairing between sequence position $i$ and $j$, and 1 represents no base paring.

As an example, a short RNA sequence GUUGUGAAAU of length 10 (ID `CRW_00083` TODO ref to database) takes a structure that consists of a stem and a loop, as seen in Fig 1(a). This structure can be represented by the upper triangular $10 \times 10$ matrix with all 0's, except for positions $y_{1,10}, y_{2,9}$ and $y_{3,8}$,all being 1. This contiguous stretch of 1's corresponds to the stem formed by the three base pairs: G-U, U-A and U-A. (TODO define x and y first) (TODO cite FORNA)

We formulate the predictive task as a conditional generative process. Specifically, given an input sequence with arbitrary length $L$: $\boldsymbol{x} = x_1, x_2, \dots x_L$, we want to predict a distribution of structures conditioned on the sequence $P(\boldsymbol{Y} \mid \boldsymbol{x})$.

(move the following paragraph to the model section) We factorize the conditional distribution as follows:

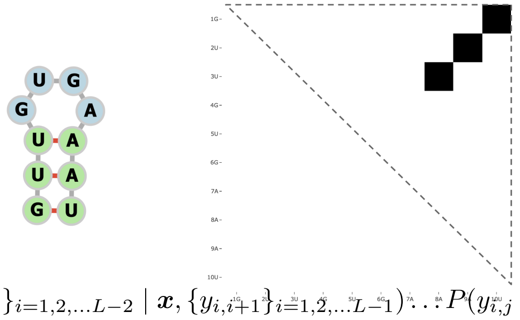

Figure 1: TODO

$$P(\boldsymbol{Y} \mid \boldsymbol{x}) = P(\{y_{i,i+1}\}_{i=1,2,...L-1} \mid \boldsymbol{x}) P(\{y_{i,i+2}\}_{i=1,2,...L-2} \mid \boldsymbol{x}, \{y_{i,i+1}\}_{i=1,2,...L-1}) \dots P(y_{i,j} \mid \boldsymbol{x}, \{y_{todo}\})$$

The generative process implied by such formulation is illustrated in Fig 2. We generated one off diagonal slice at a time, conditioned on the input sequence, starting from the one adjacent to the diagonal line, as shown in yellow on the plot. When generating the second slice (in green), we condition on the input sequence and the generated values in the first slice (in yellow). When generating the third one (in blue), we condition on the input sequence and both the yellow and green slices. This process continues until we fill the upper triangular matrix, where the last entry (in red TODO color plot) is generated conditioned on the input and the entire upper triangular matrix except for itself.

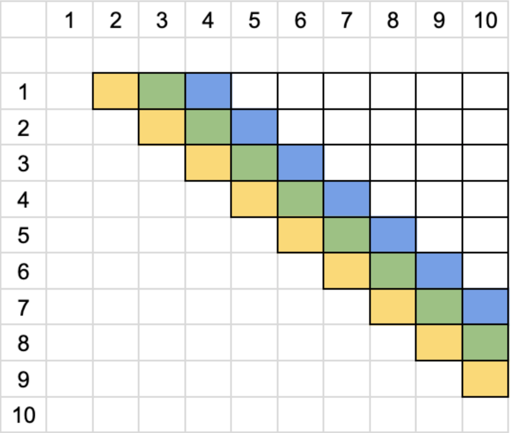

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Figure 2: TODO

### 3.2 Model

We propose an architecture that encourages learning basic rules of base pairing and local structures, to construct the global structure, without having hard-coded parameters, such that the entire model can be trained end-to-end using only sequences and structures. The architecture consists of the following components:

- two sets of 1D convolution layers on the 1-hot-encoded sequence
- Activations of each 1d conv layer (from both sets) are used to form a 2D feature map, where the (i, j)-th entry is the dot-product (can be replaced by a fully connected NN) between the activation of first set at position i, and the activation of second set at position j.
- Multiple 2D feature maps (formed via multiple layers of 1D conv) are concatenated, followed by a couple of 2D conv layers.

- Activation of the last 2D conv layer is concatenated with target from the previous "timestamp" $y^{t-1}$ (todo define notation), and the output is generated by an upper triangular convolution, which masks "future" timestamps and ensure the output is generated in auto-regressive fashion. (TODO more details on masked conv)
- Finally, there is a fully connected layer along the feature (3rd) dimension, with sigmoid activation to produce an output between 0 and 1, for each position in the upper triangular matrix.

At training time, different timestamps can be trained in parallel. At test time, we need to initialize the output at time $t = -1$, typically with a matrix of all zeros, then sample one slice at a time, until the full upper triangular matrix is filled. For a sequence of length $L - 1$, we need to run $L - 1$ steps sequentially. Note that multiple outputs can be sampled in parallel at test time.

TODO plot for NN architecture

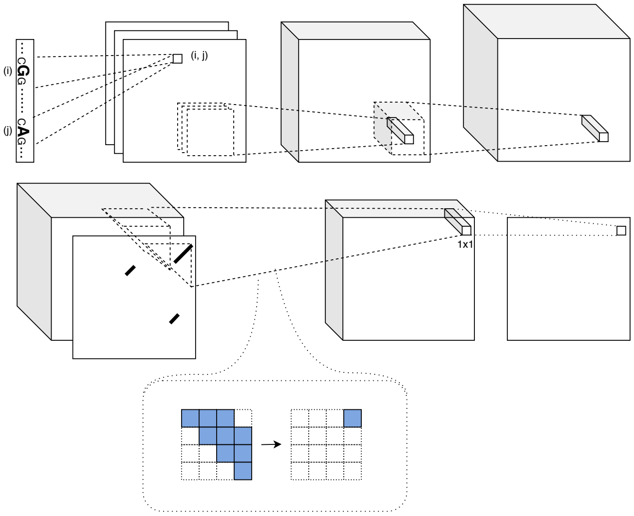

Figure 3: TODO

### 3.3 Training

We trained the model using a synthetic dataset, constructed by sampling $50000$ random sequences with length between $10$ and $100$. For each sequence, we ran RNAfold (TODO ref) with the default parameters and record the minimum free energy structure.

For each minibatch, we zero-pad the sequence array and structure matrix to the maximum length in the minibatch. When computing the loss and gradient, entries in structure matrix that were padded are being masks, in addition to the lower triangular entries (since we're only predicting the upper triangular matrix).

Note that although we present a single output structure for each input sequence at training time, the model is capable of generating a distribution of structures at test time.

TODO hyperparameters

### 3.4 Sampling structures

At test time, we can sample structures conditioned on the input sequence. As described in Section ?, we initialize the output structure with a matrix of all zeros, then sample one slice at a time until the upper triangular matrix is filled with sampled values. At each step, we sample a binary label for each position in the current slice based on the bernoulli probability predicted by the model. To ensure the sampled structure is valid, when sampling the label for location (i, j), if i-th or j-th position is already paired with another position (from samples in the previous timestamps), then we set $y_{i,j}$ to 0 without sampling from the model output.

## 4 Analysis

### 4.1 Generate distribution of structures

As an example, for a 323-base long sequence (TODO link to DB), we sampled 20 structure from the model output, as shown in Fig 4. On the left we show the binary upper triangular matrix sampled by the model (TODO it's too tiny to see anything), on the right we show the corresponding secondary structure rendered as a graph (only showing 4 due to space limit).

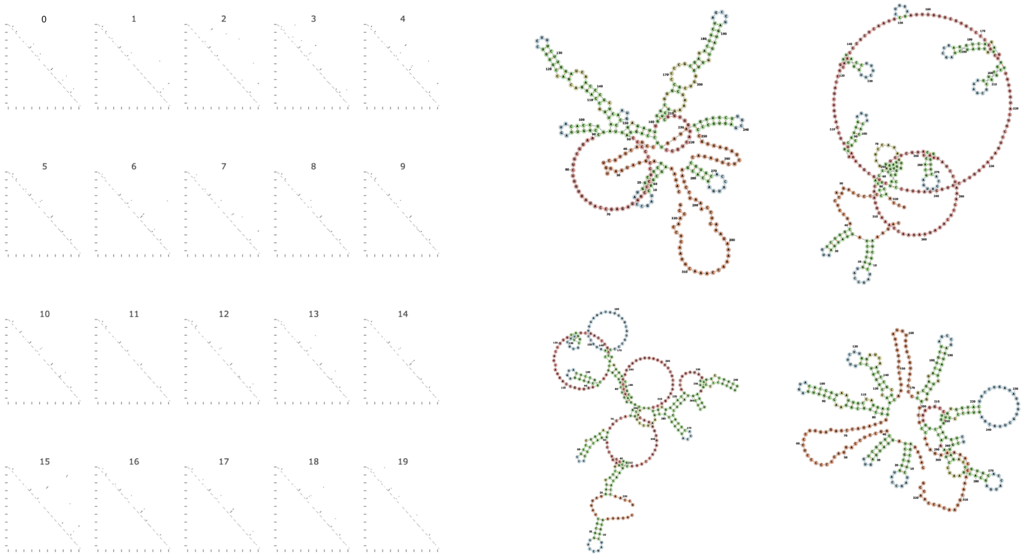

Figure 5: TODO

### 4.2 Run time comparison

### 4.3 Performance comparison

cross validation test set (real sequences)

### 4.4 Interesting cases

### 4.5 Differentiable model

## 5 Conclusion

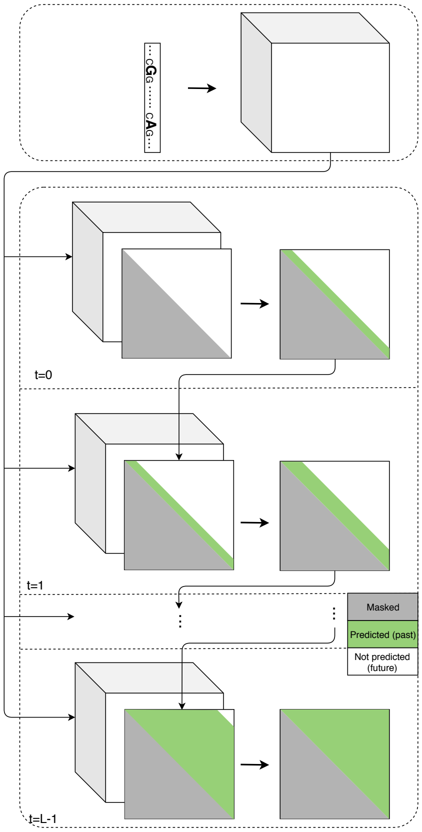

Figure 4: TODO