

Fast prediction of RNA-RNA Interaction

Raheleh Salari¹, Rolf Backofen², and S. Cenk Sahinalp¹

¹ School of Computing Science, Simon Fraser University, Burnaby, Canada

² Institute of Computer Science, Albert-Ludwigs-Universität, Freiburg, Germany

Abstract. Regulatory antisense RNAs are a class of ncRNAs that regulate gene expression by prohibiting the translation of an mRNA by establishing stable interactions with a target sequence. There is great demand for efficient computational methods to predict the specific interaction between an ncRNA and its target mRNA(s). There are a number of algorithms in the literature which can predict a variety of such interactions - unfortunately at a very high computational cost. Although some existing target prediction approaches are much faster, they are specialized for interactions with a single binding site.

In this paper we present a novel algorithm to accurately predict the minimum free energy structure of RNA-RNA interaction under the most general type of interactions studied in the literature. Moreover, we introduce a fast heuristic method to predict the specific (multiple) binding sites of two interacting RNAs. We verify the performance of our algorithms for joint structure and binding site prediction on a set of known interacting RNA pairs. Experimental results show our algorithms are highly accurate and outperform all competitive approaches.

1 Introduction

Regulatory non-coding RNAs (ncRNAs) play an important role in gene regulation. Studies on both prokaryotic and eukaryotic cells show that such ncRNAs usually bind to their target mRNA to regulate the translation of corresponding genes. Many regulatory RNAs such as microRNAs and small interfering RNAs (miRNAs/siRNAs) are very short and have full sequence complementarity to the targets. However some of the regulatory antisense RNAs are relatively long and are not fully complementary to their target sequences. They exhibit their regulatory functions by establishing stable joint structures with target mRNA initiated by one or more loop-loop interactions.

In this paper we present an efficient method for RNA-RNA interaction prediction (RIP) problem with multiple binding domains. Alkan et al. [1] proved that RIP, in its general form, is an NP-complete problem and provided algorithms for predicting specific types of interactions and two relatively simple energy models - under which RIP is polynomial time solvable. We focus on the same type of interactions, which to the best of our knowledge, are the most general type of interactions considered in the literature; however the energy model we use is the joint structure energy model recently presented by Chitsaz et al. [5] which is more general than the one used by Alkan et al.

In what follows below, we first describe a combinatorial algorithm to compute the minimum free energy joint structure formed by two interacting RNAs. This algorithm has a running time of $O(n^6)$ and uses $O(n^4)$ space - which makes it impractical for long

RNA molecules. Then we present a fast heuristic algorithm to predict the joint structure formed by interacting RNA pairs. This method provides a significant speedup over our combinatorial method, which it achieves by exploiting the observation that the independent secondary structure of an RNA molecule is mostly preserved even after it forms a joint structure with another RNA. In fact there is strong evidence [7, 11] suggesting that the probability of an ncRNA binding to an mRNA target is proportional to the probability of the binding site having an unpaired conformation. The above observation has been used by different methods for target prediction in the literature (see below for an overview). However, most of these methods focus on predicting interactions involving only a single binding site, and are not able to predict interactions involving multiple binding sites. In contrast, our heuristic approach can predict interactions involving multiple binding sites by: (1) identifying the collection of accessible regions for both input RNA sequences, (2) using a matching algorithm, computing a set of "non-conflicting" interactions between the accessible regions which have the highest overall probability of occurrence.

Note that an accessible region is a subsequence in an RNA sequence which, with "high" probability, remain unpaired in its secondary structure. Our method considers the possibility of interactions being formed between one such accessible region from an RNA sequence with more than one such region from the other RNA sequence. Thus, in step (1), it extends the algorithm by Mückstein et al. for computing the probability of a specific region being unpaired [12] to compute the joint probability of two (or more) regions remaining unpaired. Because an accessible region from an RNA typically interacts with no more than two accessible regions from the other RNA, we focus on calculating the probability of at most two regions remaining unpaired: within a given an RNA sequence of length n , our method can calculate the probability of any pair of regions of length $\leq w$ each, in $O(n^4 \cdot w)$ time and $O(n^2)$ space. In step (2), on two input RNA sequences of length n and m ($n \leq m$), our method computes the most probable nonconflicting matching of accessible regions in $O(n^2 \cdot w^4 + n^3/w^3)$ time and $O(w^4 + n^2/w^2)$ space.

Related Work. Early attempts to compute the joint structure of interacting RNAs started by concatenating the two interacting RNA sequences and treated them as a single sequence `PairFold` [2] and `RNAcofold` [3]. As these methods typically use secondary structure prediction methods that do not allow pseudoknots, they fail to predict joint structures formed by non-trivial interactions between a pair of RNAs. Another set of methods ignore internal base-pairing in both RNAs, and compute the minimum free energy secondary structure for their hybridization (`RNAhybrid` [15], `UNAFold` [6, 9], and `RNA duplex` from Vienna package [3]). These approaches work only for simple cases involving typically very short strands. A further set of studies aim to compute the minimum free energy joint structure between two interacting RNAs. For example Pervouchine [14] devised a dynamic programming algorithm to maximize the number of base pairs among interacting strands. A follow up work by Kato et al. [8] proposed a grammar based approach to RNA-RNA interaction prediction. More generally Alkan et al. [1] studied the joint secondary structure prediction problem under three different models: 1) base pair counting, 2) stacked pair energy model, and 3) loop energy model. The resulting algorithms compute the optimum structure among all possible joint sec-

ondary structures that do not contain pseudoknots, crossing interactions, and *zigzags* (please see [1] for the exact definition). In fact the last set of algorithms above are the only methods that have the capability to predict joint secondary structures with multiple loop-loop interactions. However, these algorithms all require significant computational resources ($O(n^6)$ time and $O(n^4)$ spaces) and thus are impractical for sequences of even modest length. A final group of methods are based on the observation that interaction is a multi step process that involves: 1) unfolding of the two RNA structures to expose the bases needed for hybridization, 2) the hybridization at the binding site, and 3) restructuring of the complex to a new minimum free energy conformation. The main aim of these methods is to identify the potential binding sites which are going to be unfolded in order to form interactions. One such method presented by Alkan et al. [1], extends existing loop regions in independent structures to find potential binding sites. RNAup [13] presents an extension of the standard partition function approach to compute the probabilities that a sequence interval remains unpaired. IntaRNA [4] considers not only accessibility of a binding sites but also the existence of a seed to predict potential binding sites. All of these methods achieve reasonably high accuracy in predicting interactions involving single binding sites; however, their accuracy levels are not very high when dealing with interactions involving multiple binding sites.

2 Methods

We address the RNA-RNA Interaction Problem (RIP) based on the energy model of Chitsaz et al. [5] over the interactions considered by Alkan et al. [1]. Our algorithm computes the minimum free energy secondary structure among all possible joint secondary structures that do not contain pseudoknots, crossing interactions, and zigzags.

2.1 RNA-RNA Structure Prediction

Recently Chitsaz et al. [5] present an energy model for joint structure of two nucleic acids over the type of interactions introduced by Alkan et al. [1]. Based on the presented energy model they propose an algorithm that consider all possible cases of joint structures to compute the partition function. The specified algorithm with some minor changes can be used to compute the minimum free energy joint structure of two interacting nucleic acids. Following we shortly describe the algorithm.

We are given two RNA sequences \mathbf{R} and \mathbf{S} of lengths n and m . We refer to the i^{th} nucleotide in \mathbf{R} and \mathbf{S} by i_R and i_S respectively. The subsequence from the i^{th} nucleotide to the j^{th} nucleotide in one strand is denoted by $[i, j]$. We denote a base pair between the nucleotides i and j by $i \cdot j$. $MFE(i, j)$ denotes the minimum free energy structure of $[i, j]$, and $MFE(i_R, j_R, i_S, j_S)$ denotes the minimum free energy joint structure of $[i_R, j_R]$ and $[i_S, j_S]$.

Fig. 1 shows the recursion diagram of MFE for the joint structure of $[i_R, j_R]$ and $[i_S, j_S]$. In this figure a horizontal line indicates the phosphate backbone, a dashed curved line encloses a subsequence and denotes its two terminal bases which may be paired or unpaired. A solid vertical line indicates an interaction base pair, a dashed vertical line denotes two terminal bases which may be base paired or unpaired, and a

dotted vertical line denotes two terminal bases which are assumed to be unpaired. Grey regions indicate a reference to the substructure of single sequences.

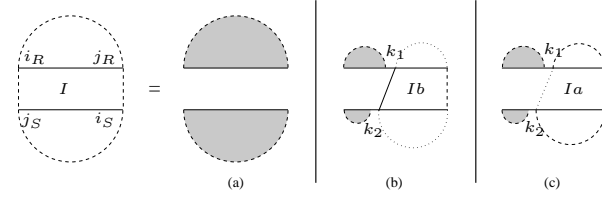


Fig. 1. Recursion for MFE joint structure of subsequences $[i_R, j_R]$ and $[i_S, j_S]$. Case a constitutes no interaction. In case b , the leftmost interaction bond is not closed by any base pair. In case c , the leftmost interaction bond is covered by base pair in at least one subsequence.

The joint structure of two subsequences derived from one of the following cases. The first case is when there is no interaction between the two subsequences. If there are some interaction bonds, the structure has two cases: either the leftmost bond is closed by base pair in at least one of the subsequences or not. If the joint structure starts with a bond which is not closed by any base pair we denote the case by Ib , otherwise the structure starts with a bond which is closed by base pair in at least one subsequence and the case is denoted by Ia . Therefore, $MFE(i_R, j_R, i_S, j_S)$ is calculated by the following dynamic programming:

$$MFE(i_R, j_R, i_S, j_S) = \min \left\{ \begin{array}{l} MFE(i_R, j_R) + MFE(i_S, j_S) \quad (a), \\ \min_{\substack{i_R \leq k_1 < j_R \\ i_S < k_2 \leq j_S}} \left\{ \begin{array}{l} MFE(i_R, k_1 - 1) + \\ MFE(k_2 + 1, j_S) + \\ MFE^{Ib}(k_1, j_R, i_S, k_2) \end{array} \right\} \quad (b), \\ \min_{\substack{i_R \leq k_1 < j_R \\ i_S < k_2 \leq j_S}} \left\{ \begin{array}{l} MFE(i_R, k_1 - 1) + \\ MFE(k_2 + 1, j_S) + \\ MFE^{Ia}(k_1, j_R, i_S, k_2) \end{array} \right\} \quad (c), \end{array} \right\} \quad (1)$$

in which $MFE^{Ib}(k_1, j_R, i_S, k_2)$ is the minimum free energy for the joint structure of $[k_1, j_R]$ and $[i_S, k_2]$ assuming $k_1 \cdot k_2$ is an interaction bond, and $MFE^{Ia}(k_1, j_R, i_S, k_2)$ is the minimum free energy for the joint structure of $[k_1, j_R]$ and $[i_S, k_2]$ assuming the leftmost interaction bond is covered by a base pair in at least one subsequence. The corresponding dynamic programming for computing the MFE^{Ib} and MFE^{Ia} can be derived from the cases explained in [5] in a similar fashion.

Similar to the partition function algorithm, the minimum free energy joint structure prediction algorithm has $O(n^6)$ running time and $O(n^4)$ space requirements. However the algorithm performs highly accurate (see section 3.2), but it requires substantial computational resources. This could be prohibitive for predicting the joint secondary structure of sufficiently long RNA molecules. Therefore, in the next section we present a fast heuristic algorithm to predict RNA-RNA interaction.

2.2 RNA-RNA Biding Sites Prediction

Our heuristic algorithm for RNA-RNA interaction prediction problem is based on the idea that the external interactions mostly occur between pairs of unpaired regions of single structures. We aim to predict interactions of multiple binding sites as long as they have no crossing. The heuristic algorithm contains the following steps:

- Predict the highly accessible regions in each strands. These regions include the loop regions in native structure of RNA strand. In order to predict accessible regions we chose all the regions which remain unpaired with high probability.
- Predict the optimal non-conflicting interactions between the accessible regions. For every pair of accessible regions of two interacting RNAs a cost of interaction is calculated. Then a matching algorithm runs to find the minimum cost non-conflicting subset of interactions.

Accessible Regions: For a single RNA sequence an accessible region is a subsequence that remains unpaired in equilibrium with high probability. The probability of an unpaired region can be calculated based on the algorithm presented in [12]. Here, we are interested in multiple unpaired regions. For this purpose one should compute the joint probabilities for any subset of possible intervals. Since the computation of all joint probabilities needs substantial time and space, in this paper we only consider the joint probability of two unpaired subsequences.

Denoting the set of secondary structures in which the sequence interval $[k, l]$ remains unpaired by $S^{u[k,l]}$, the corresponding partition function is

$$Q^{u[k,l]}(T) = \sum_{s \in S^{u[k,l]}} e^{-G_s/RT}, \quad (2)$$

where R is the universal gas constant and T is the temperature. In order to compute the $Q^{u[k,l]}$, the standard recursion for the partition function folding algorithm [10] can be extended as:

$$\begin{array}{c}
 \begin{array}{c} Q_{i,j}^{u[k,l]} \\ \text{---} \text{---} \text{---} \\ i \quad k \quad l \quad j \end{array} = \begin{array}{c} 1 \\ \text{---} \text{---} \text{---} \\ i \quad k \quad l \quad j \end{array} \left| \begin{array}{c} 1 \times Q_{i,j}^b \times Q_{k_2+1,j}^{u[k,l]} \\ \text{---} \text{---} \text{---} \\ i \quad k_1-1 \quad k_1 \quad k_2 \quad k_2+1 \quad k \quad l \quad j \end{array} \\
 \\
 \begin{array}{c} 1 \times Q_{k_1,k_2}^{b,u[k,l]} \times Q_{k_2+1,j} \\ \text{---} \text{---} \text{---} \\ i \quad k_1-1 \quad k_1 \quad k \quad l \quad k_2 \quad k_2+1 \quad j \end{array} \left| \begin{array}{c} 1 \times Q_{i,j}^b \times Q_{k_2+1,j} \\ \text{---} \text{---} \text{---} \\ ik \quad k \quad l \quad k_1-1 \quad k_1 \quad k_2 \quad k_2+1 \quad j \end{array}
 \end{array}$$

where $i \leq k \leq l \leq j$ and $k_1 \cdot k_2$ is the leftmost base pair. Partition functions $Q_{i,j}^{b,u[k,l]}$ (where $i \cdot j$) and $Q_{i,j}^{m,u[k,l]}$ (where $[i, j]$ is inside a multiloop and constitutes at least one base pair) while the interval $[k, l]$ remains unpaired are derived from the standard algorithm in a similar way. Furthermore, probability of a base pair $p \cdot q$ while $[k, l]$

remains unpaired, $\mathbb{P}(p \cdot q | u[k, l])$, can be calculated by applying the McCaskill algorithm [10] for computing the base pair probability on $Q^{u[k, l]}$. It is easy to see that the desired partition function $Q^{u[k, l]}$ and base pair probability $\mathbb{P}(p \cdot q | u[k, l])$ are computed in same time and space complexity as the standard algorithm by McCaskill ($O(n^3)$ and $O(n^2)$ respectively).

Mückstein et al. [12] introduce an algorithm to compute the probability of unpaired region $\mathbb{P}(u[i, j])$ for a given sequence interval $[i, j]$. Here, we extend the specified algorithm to compute $\mathbb{P}(u[i, j] | u[k, l])$ which is the probability of unpaired region $[i, j]$ while $[k, l]$ remains unpaired. Clearly if some part of $[i, j]$ is within the interval $[k, l]$, the corresponding probability for that part is equal to one. Hence, for computing the probability only the parts of $[i, j]$ which are exterior to $[k, l]$ should be considered. Here, without loss of generality we assume $k \leq l < i \leq j$.

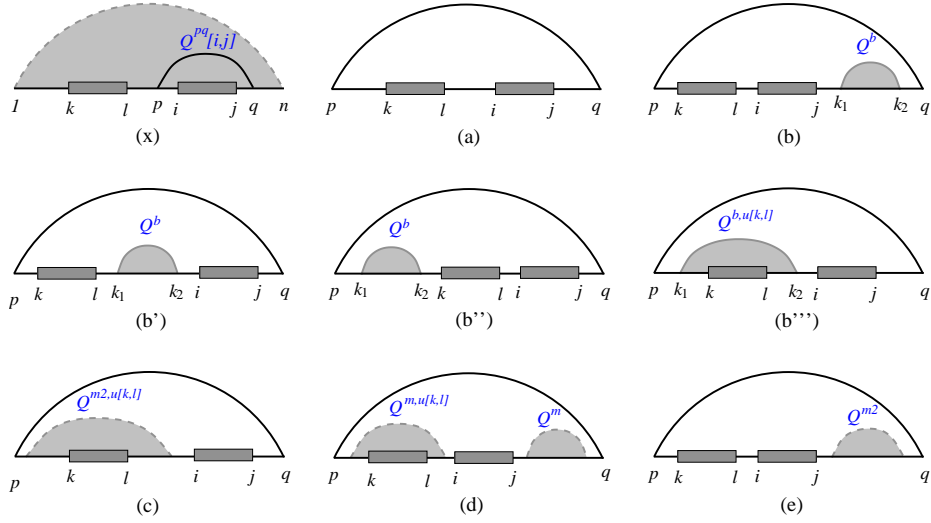


Fig. 2. Cases of unpaired interval $[i, j]$ within a loop enclosed by $p \cdot q$ while $[k, l]$ remains unpaired.

For unpaired interval $[i, j]$ there are two general cases: either it is not closed by any base pair, or it is part of a loop. Fig. 2 summarizes the cases of unpaired interval $[i, j]$ as a part of the loop enclosed by base pair $p \cdot q$ while interval $[k, l]$ remains unpaired. In case x interval $[p, q]$ does not contain interval $[k, l]$, and in the other cases $(a - e)$ interval $[k, l]$ lies in interval $[p, q]$. Probability $\mathbb{P}(u[i, j] | u[k, l])$ can be calculated as follows:

$$\begin{aligned}
 \mathbb{P}(u[i, j] | u[k, l]) &= \frac{Q_{1, i-1}^{u[k, l]} \times 1 \times Q_{j+1, n}}{Q^{u[k, l]}} \\
 &+ \sum_{l < p < i \leq j < q} \mathbb{P}(p \cdot q | u[k, l]) \times \frac{Q_{i, j}^{pq}}{Q_{p, q}^b} \quad (x) \quad (3) \\
 &+ \sum_{p < k \leq l < i \leq j < q} \mathbb{P}(p \cdot q | u[k, l]) \times \frac{Q^{pq, u[k, l]}[i, j]}{Q_{p, q}^{b, u[k, l]}} \quad (a - e)
 \end{aligned}$$

$Q^{pq}[i, j]$ which is introduced by Mückstein et al., counts all structures on $[p, q]$ that $[i, j]$ is part of the loop closed by base pair $p \cdot q$. The quantity $Q^{pq, u[k, l]}[i, j]$ is a variant of $Q^{pq}[i, j]$ while $[k, l]$ lies in $[p, q]$. Recursion of $Q^{pq, u[k, l]}[i, j]$ on cases (a–e) displayed in Fig. 2, is based on different types of loop and position of $[k, l]$. Therefore, we have

$$\begin{aligned}
Q^{pq, u[k, l]}[i, j] &= e^{-G_{p, q}^{\text{hairpin}}/RT} & (a) \\
&+ \sum_{\substack{j < k_1 < k_2 < q \\ l < k_1 < k_2 < i | p < k_1 < k_2 < k}} e^{-G_{i, k_1, k_2, j}^{\text{interior}}/RT} Q_{k_1, k_2}^b & (b, b', b'') \\
&+ \sum_{i < k_1 < k \leq l < k_2 < i} e^{-G_{i, k_1, k_2, j}^{\text{interior}}/RT} Q_{k_1, k_2}^{b, u[k, l]} & (b''') \\
&+ Q_{p+1, i-1}^{m2, u[k, l]} e^{-(a+b+c(q-i))/RT} & (c) \\
&+ Q_{p+1, i-1}^{m, u[k, l]} Q_{j+1, q-1}^m e^{-(a+b+c(j-i-1))/RT} & (d) \\
&+ Q_{j+1, q-1}^{m2} e^{-(a+b+c(j-p))/RT} & (e)
\end{aligned} \tag{4}$$

where Q^{m2} is the partition function of a subsequence inside a multiloop that constitutes at least two base pairs. Q^{m2} which is introduced in Mückstein et al. algorithm can be extended to calculate $Q^{m2, u[k, l]}$. Therefore, the joint probability of two unpaired regions is obtained using

$$\mathbb{P}(u[i, j], u[k, l]) = \mathbb{P}(u[i, j] | u[k, l]) \times \mathbb{P}(u[k, l]). \tag{5}$$

Mückstein et al. algorithm requires $O(n^3)$ running time and $O(n^2)$ space complexity to compute the probability of unpaired region $\mathbb{P}(u[i, j])$ for every possible interval $[i, j]$ assuming the interval length is limited to size w . Using the the extended algorithm, given sequence interval $[k, l]$ computing $\mathbb{P}(u[i, j], u[k, l])$ for every possible interval $[i, j]$ requires the same time and space complexity. Note that for each interval $[k, l]$, $Q^{u[k, l]}$ should be computed separately. Since there are $O(n \cdot w)$ different intervals for a limited interval length w , with $O(n^4 \cdot w)$ running time and $O(n^2)$ space complexity we are able to compute the joint probabilities for all pairs of unpaired regions. The same idea can be used to compute the joint probability of multiple unpaired regions. However, considering each extra interval increases the running time by a factor of $O(n \cdot w)$.

Interaction Matching Algorithm: We are given two lists of non-overlapping accessible regions $T_{\mathbf{R}} = \{r_1, r_2, \dots, r_{n'}\}$ and $T_{\mathbf{S}} = \{s_1, s_2, \dots, s_{m'}\}$ sorted according to their orders in interacting sequences \mathbf{R} and \mathbf{S} . We aim to calculate the optimal set of interaction bonds between the accessible regions under the following constraints: (1) Each accessible region can interact with at most two accessible regions from the other sequence. (2) There is no crossing interaction.

Let Q_{r_i, s_j} be the partition function of all possible joint structures of two interacting sequence r_i and s_j , which can be calculated by piRNA [5]. Define $Q_{r_i, s_j}^I = Q_{r_i, s_j} - Q_{r_i} Q_{s_j}$ as the partition function for the set of joint structures that contain some interactions. We denote the interaction between two accessible regions r_i and s_j

by $r_i \circ s_j$ which is considered if and only if $\mathbb{P}(r_i \circ s_j) = \frac{Q_{r_i, s_j}^I}{Q_{r_i, s_j}} > 1/2$. The cost of interaction between two accessible regions r_i and s_j , $C(r_i, s_j)$, is the sum of the following terms:

- $E_u(r_i)$ and $E_u(s_j)$: the energy difference between the complete ensemble and the ensemble in which the interacting subsequences are left unpaired for both accessible regions. We have $E_u(r_i) = (-RT)(\ln(Q_{\mathbf{R}}^{u[r_i]}) - \ln(Q_{\mathbf{R}})) = (-RT) \ln(\mathbb{P}(u[r_i]))$. Similar equation can be used to calculate $E_u(s_j)$.
- $E_I(r_i, s_j)$: the ensemble energy of interacting joint structure for the two accessible regions where $E_I(r_i, s_j) = (-RT) \ln(\mathbb{P}(r_i \circ s_j))$.

Cost of interaction between an accessible region r_i and two other accessible regions s_k and s_j is defined as $C(r_i, s_k s_j) = E_u(r_i) + E_u(s_k, s_j) + E_I(r_i, s_k s_j)$, where $s_k s_j$ is the concatenation of two subsequences, and $E_u(s_k, s_j) = (-RT) \ln(\mathbb{P}(u[s_k], u[s_j]))$. Similarly the cost of interaction between two accessible regions from \mathbf{R} and one accessible region from \mathbf{S} is defined.

As an option, one can use minimum free energy (*MFE*) instead of ensemble energy (E_I) to define the cost of interaction. Accessible regions r_i and s_j are considered to be able to interact if and only if $MFE(r_i, s_j) < MFE(r_i) + MFE(s_j)$, i.e. there are some interaction bonds in the minimum free energy joint structure. Therefore, we have $C(r_i, s_j) = E_u(r_i) + E_u(s_j) + MFE(r_i, s_j)$. The cost of interaction of an accessible region r_i with two other accessible regions s_k and s_j is defined as $C(r_i, s_k s_j) = E_u(r_i) + E_u(s_k, s_j) + MFE(r_i, s_k s_j)$.

With $H(i, j)$, we denote the minimum cost non-conflicting set of interactions between the accessible regions $\{r_1, \dots, r_i\}$ and $\{s_j, \dots, s_{m'}\}$. The following dynamic programming computes $H(i, j)$:

$$H(i, j) = \min \left\{ \begin{array}{ll} H(i-1, j+1) + C(r_i, s_j) & (i) \\ \min_{j < k \leq m'} \{H(i-1, k+1) + C(r_i, s_k s_j)\} & (ii) \\ \min_{1 \leq k < i} \{H(k-1, j+1) + C(r_k r_i, s_j)\} & (iii) \\ H(i-1, j) & (iv) \\ H(i, j+1) & (v) \\ \infty & (vi) \end{array} \right\} \quad (6)$$

where $1 \leq i \leq n'$ and $1 \leq j \leq m'$. The algorithm starts by calculating $H(1, m')$ and explores all $H(i, j)$ by increasing i and decreasing j until $i = n'$ and $j = 1$. The DP algorithm has $O(n'^2 \cdot m' + n' \cdot m'^2)$ time and $O(n' \cdot m')$ space requirements. Also we need $O(n' \cdot m' \cdot w^6)$ time and $O(w^4)$ space to compute the cost of interaction for every pair of accessible regions. Assuming $n' \geq m'$ and $n' \leq n/w$, we can conclude that this step of the algorithm requires $O(n^2 \cdot w^4 + n^3/w^3)$ time and $O(w^4 + n^2/w^2)$ space.

CopA-CopT is a well known antisense RNA-target complex observed in E.coli [16]. The joint structure of CopA-CopT contains two disjoint binding sites. Fig. 3 shows the identified accessible regions in CopA and CopT. Two regions connected by an edge are able to interact. Fig. 4 shows the known and predicted interaction bonds between CopA and CopT. Note that internal bonds of both RNAs are not displayed in this figure.

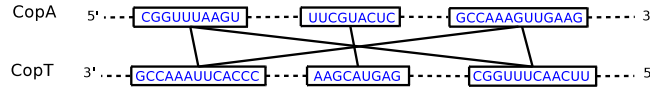


Fig. 3. Interaction between accessible regions of CopA-CopT: a simple example for interaction matching algorithm.

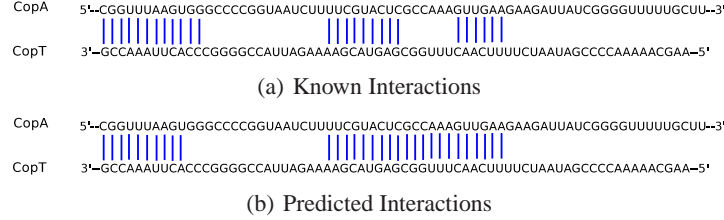


Fig. 4. Interaction between CopA and CopT. (a) Natural interactions. (b) Predicted interactions.

3 Results

3.1 Dataset

In our experiments we used a dataset of 23 known RNA-RNA interactions which includes two recently used test sets. Table 2 contains the list of these RNA pairs. The first 18 sRNA-target pairs are compiled and used as test set by `IntaRNA` [4]. Next 5 pairs of RNAs which are known to have loop-loop interactions have been used by Kato et al. [8] to evaluate the proposed grammatical parsing approach for RNA-RNA joint structure prediction.

3.2 Structure Prediction

In our first experiment, we assessed the performance of our prediction algorithm for minimum free energy joint structure. For this purpose we used the 5 RNA-RNA complexes from Kato et al. [8] test set. We compared our results with two state-of-the-art methods for joint structure prediction: (1) the grammatical approach by Kato et al. [8] (denoted by EBM as energy-based model), and (2) the DP methods for two models presented by Alkan et al. [1] (denoted by SPM as stacked-pair model and LM as loop model).

In order to estimate the accuracy of prediction, we measured the sensitivity and PPV defined as follows:

$$sensitivity = \frac{\text{number of correctly predicted base pairs}}{\text{number of true base pairs}}, \quad (7)$$

$$PPV = \frac{\text{number of correctly predicted base pairs}}{\text{number of predicted base pairs}}. \quad (8)$$

As another measure of accuracy we calculated F-measure which considers both sensitivity and PPV. F-measure is the harmonic mean of sensitivity and PPV, and its formula

is as follows:

$$F = \frac{2 \times \text{sensitivity} \times \text{PPV}}{\text{sensitivity} + \text{PPV}}. \quad (9)$$

Table 1. Prediction accuracy of competitive RNA-RNA joint structure prediction methods. Dataset is compiled by Kato et al. [8].

RNA-RNA interaction pairs	Sensitivity				PPV				F-measure			
	inRNAs	EBM	SPM	LM	inRNAs	EBM	SPM	LM	inRNAs	EBM	SPM	LM
CopA-CopT	1.000	0.909	0.955	0.864	0.846	0.800	0.778	0.760	0.917	0.851	0.857	0.809
DIS-DIS	1.000	0.786	0.786	0.786	1.000	0.786	0.786	0.786	1.000	0.786	0.786	0.786
IncRNA ₅₄ -RepZ	0.875	0.917	0.875	0.875	0.792	0.830	0.778	0.778	0.831	0.871	0.824	0.824
R1inv-R2inv	0.900	0.900	1.000	1.000	0.900	0.947	1.000	1.000	0.900	0.923	1.000	1.000
Tar-Tar*	1.000	1.000	1.000	1.000	0.875	0.933	0.875	0.875	0.933	0.965	0.933	0.933
Average	0.955	0.902	0.923	0.905	0.883	0.859	0.843	0.840	0.916	0.879	0.880	0.870

Table 1 shows comparison between the accuracy of our method and other competitors. We referred to our method by inRNAs as an algorithm for prediction the interactions between RNAs. As it can be seen, our method based on the three accuracy measures outperformed the competitors. For Tar-Tar* and R1inv-R2inv pairs that both RNAs are relatively short (~ 20 nt), all methods were accurate enough. However, for DIS-DIS which is not still long (35nt), only our method was able to predict the interaction while the other approaches returned no interaction. CopA-CopT and IncRNA₅₄-RepZ are a bit longer (~ 60 nt); CopA-CopT has two disjoint binding sites and IncRNA₅₄-RepZ has a continuous binding site. Our method outperformed the others in predicting the joint structure of CopA-CopT, while IncRNA₅₄-RepZ was predicted more accurately by EBM. We did not compare the running time between these methods due to the fact that each one uses different platform and hardware. Our method on one Sun Fire processor X4600 2.6 GHz with 64 GB RAM has been running ~ 4000 (sec) to predict the joint structures of CopA-CopT and IncRNA₅₄-RepZ.

3.3 Binding Sites Prediction

In another experiment, we focused on testing the performance of our heuristic algorithm for interaction prediction. For assessing the predictive power of our algorithm, we compared our algorithm with IntaRNA [4] and RNAup [13]. Based on the experimental results presented by IntaRNA, both IntaRNA and RNAup which incorporate accessibility of target regions, performed better than the other competitive programs.

The results of these two programs for the first 18 RNA pairs are as presented in [4]. For the next 5 RNA pairs, we run IntaRNA with its default settings and RNAup with the same setting that has been used by the experiment in [4]³. In order to estimate the accuracy of programs, we measured the sensitivity, PPV and F-measure such that only interacting base pairs are considered.

Table 2 shows the results of our programs as well as IntaRNA and RNAup. In this dataset OxyS-fhlA and CopA-CopT are the only ones that have two disjoint binding

³ RNAup has been run using parameter -b which considers the probability of unpaired regions in both RNAs and the maximal length of interaction to 80.

Table 2. Prediction accuracy of competitive RNA-RNA interaction prediction methods. Dataset is compiled by Busch et al. [4] and Kato et al. [8].

RNA-RNA interaction pairs	Sensitivity			PPV			F-measure		
	inRNAs	IntaRNA	RNAup	inRNAs	IntaRNA	RNAup	inRNAs	IntaRNA	RNAup
DsrA-RpoS	0.808	0.808	0.808	0.778	0.778	0.778	0.793	0.793	0.793
GcvB-argT	0.950	0.950	0.900	0.864	0.950	0.947	0.905	0.950	0.923
GcvB-dppA	1.000	1.000	1.000	0.850	0.586	0.459	0.919	0.739	0.629
GcvB-gltI	0.750	0.000	0.000	0.500	0.000	0.000	0.600	0.000	0.000
GcvB-livJ	0.634	0.955	0.955	0.824	0.955	0.955	0.717	0.955	0.955
GcvB-livK	0.540	0.542	0.542	0.570	0.565	0.565	0.555	0.553	0.553
GcvB-oppA	1.000	1.000	1.000	0.733	0.957	0.957	0.846	0.978	0.978
GcvB-STM4351	0.760	0.760	0.880	1.000	0.905	0.957	0.864	0.826	0.917
IstR-tisAB	0.722	0.879	0.667	1.000	0.960	1.000	0.839	0.918	0.800
MicA-ompA	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MicA-lamB	1.000	1.000	0.826	1.000	0.821	0.704	1.000	0.902	0.760
MicC-ompC	1.000	1.000	0.727	1.000	0.537	0.410	1.000	0.699	0.524
MicF-ompF	0.960	0.960	0.800	0.960	0.960	0.952	0.960	0.960	0.869
OxyS-flhA	0.813	0.500	0.375	1.000	1.000	1.000	0.897	0.667	0.545
RyhB-sdhD	0.618	0.588	0.794	0.955	1.000	0.794	0.750	0.741	0.794
RyhB-sodB	1.000	1.000	1.000	1.000	0.818	0.900	1.000	0.900	0.947
SgrS-ptsG	0.566	0.739	0.739	0.765	1.000	1.000	0.651	0.850	0.850
Spot42-galK	0.432	0.409	0.523	0.760	0.643	0.523	0.551	0.500	0.523
CopA-CopT	0.889	1.000	0.556	0.828	0.391	0.652	0.857	0.562	0.600
DIS-DIS	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IncRNA _{5,4} -RepZ	1.000	0.738	0.750	0.889	0.850	0.857	0.941	0.790	0.800
R1inv-R2inv	1.000	1.000	1.000	0.778	1.000	0.778	0.875	1.000	0.875
Tar-Tar*	1.000	1.000	1.000	0.833	0.833	0.833	0.909	0.909	0.909
Average	0.845	0.819	0.776	0.865	0.805	0.784	0.845	0.791	0.763

sites, and our methods outperformed IntaRNA and RNAup by up to 30% improvement in F-measure. Both RNAup and IntaRNA could not predict any correct bond for GcvB-gltI, since they missed the binding site. However, IntaRNA could get 80% accuracy by considering the suboptimal prediction which is close to the accuracy that we have achieved. In overall, the results demonstrate that our method predicted RNA-RNA interactions more accurately in compare to competitive methods.

4 Conclusion

In this work, we introduced a fast algorithm for RNA-RNA interaction prediction. Our heuristic algorithm for RNA-RNA interaction prediction problem incorporates the accessibility of multiple unpaired regions, and a matching algorithm to compute the optimal set of interactions between the target regions. The algorithm requires $O(n^4 \cdot w)$ running time and $O(n^2)$ space complexity. The main advantage of our method is its ability to predict multiple binding sites which has been predictable only by expensive algorithms [1, 8] so far. On a set of several known RNA-RNA complexes, our proposed algorithm showed a reliable accuracy. Especially, for complexes with multiple binding sites our approach was able to outperform the competitive methods.

It would be interesting to design a method to efficiently compute the joint probability of multiple unpaired regions. Furthermore, the improvement of IntaRNA which got some benefit by considering seed features in comparison to RNAup, encourages us to take into account the existence of seed in the follow up work.

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References

1. C. Alkan, E. Karakoc, J.H. Nadeau, S.C. Sahinalp, and K. Zhang. RNA-RNA interaction prediction and antisense RNA target search. *Journal of Computational Biology*, 13(2):267–282, 2006.
2. M. Andronescu, Z.C. Zhang, and A. Condon. Secondary structure prediction of interacting RNA molecules. *J. Mol. Biol.*, 345:987–1001, 2005.
3. S.H. Bernhart, H. Tafer, U. Mückstein, C. Flamm, P.F. Stadler, and I.L. Hofacker. Partition function and base pairing probabilities of RNA heterodimers. *Algorithms Mol Biol*, 1:3, 2006.
4. A. Busch, A.S. Richter, and R. Backofen. IntaRNA: Efficient prediction of bacterial sRNA targets incorporating target site accessibility and seed regions. *Bioinformatics*, page btn544, 2008.
5. H. Chitsaz, R. Salari, S.C. Sahinalp, and R. Backofen. A partition function algorithm for two interacting nucleic acid strands. *Bioinformatics*, 25(12), 2009.
6. R.A. Dimitrov and M. Zuker. Prediction of hybridization and melting for double-stranded nucleic acids. *Biophysical Journal*, 87:215–226, 2004.
7. J. Hackermüller, N.C. Meisner, M. Auer, M. Jaritz, and P.F. Stadler. The effect of RNA secondary structures on RNA-ligand binding and the modifier RNA mechanism: a quantitative model. *Gene*, 345:3–12, 2005.
8. Y. Kato, Akutsu, T, and H. Seki. A grammatical approach to rna-rna interaction prediction. *Pattern Recogn.*, 42(4):531–538, 2009.
9. N.R. Markham and M. Zuker. UNAFold: software for nucleic acid folding and hybridization. *Methods Mol. Biol.*, 453:3–31, 2008.
10. J.S. McCaskill. The equilibrium partition function and base pair binding probabilities for RNA secondary structure. *Biopolymers*, 29:1105–1119, 1990.
11. N.C. Meisner, J. Hackermüller, V. Uhl, A. Aszdi, M. Jaritz, and M. Auer. mRNA openers and closers: modulating AU-rich element-controlled mRNA stability by a molecular switch in mRNA secondary structure. *Chembiochem*, 5:1432–1447, 2004.
12. U. Mückstein, H. Tafer, J. Hackermüller, S.H. Bernhart, M. Hernandez-Rosales, J. Vogel, P.F. Stadler, and I.L. Hofacker. Translational control by RNA-RNA interaction: Improved computation of RNA-RNA binding thermodynamics. *Bioinformatics Research and Development*, 13:114–127, 2008.
13. U. Mückstein, H. Tafer, J. Hackermüller, S.H. Bernhart, P.F. Stadler, and I.L. Hofacker. Thermodynamics of RNA-RNA binding. *Bioinformatics*, 22:1177–1182, 2006.
14. D.D. Pervouchine. IRIS: intermolecular RNA interaction search. *Genome Inform*, 15:92–101, 2004.
15. M. Rehmsmeier, P. Steffen, M. Hochsmann, and R. Giegerich. Fast and effective prediction of microRNA/target duplexes. *RNA*, 10:1507–1517, 2004.
16. E.G. Wagner and K. Flrdh. Antisense RNAs everywhere? *Trends Genet.*, 18:223–226, 2002.