STRUCTURAL ALIGNMENTS OF PSEUDO-KNOTTED RNA-MOLECULES IN POLYNOMIAL TIME

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Abstract. An RNA molecule is structured on several layers. The primary and most obvious structure is its sequence of bases, i.e. a word over the alphabet \{A, C, G, U\}. The higher structure is a set of one-to-one base-pairings resulting in a two-dimensional folding of the one-dimensional sequence. One speaks of a secondary structure if these pairings do not cross and of a tertiary structure otherwise.

Since the folding of the molecule is important for its function, the search for related RNA molecules should not only be restricted to the primary structure. It seems sensible to incorporate the higher structures in the search. Based on this assumption and certain edit-operations a distance between two arbitrary structures can be defined. It is known that the general calculation of this measure is NP-complete [ZWM02]. But for some special cases polynomial algorithms are known. Using a new formal description of secondary and tertiary structures, we extend the class of structures for which the distance can be calculated in polynomial time. In addition the presented algorithm may be used to approximate the edit-distance between two arbitrary structures with a constant ratio.

1. Introduction

Ribonucleic acid (RNA) is structured on three levels. The primary and most obvious structure is the underlying sequence of bases. The higher layers of structure are given by its folding, i.e. its pattern of base pairings. As long as the structure is nested, one speaks of a secondary structure. If crossed pairs or pseudoknots exist, the molecule is of tertiary structure\(^1\). Since the folding and the embedding into the three-dimensional space are important for the functional properties of an RNA molecule, it is of some interest to compare different molecules based on the secondary and tertiary structure and not only on the primary structure.

Restricting to the primary structure, the comparison of two or more RNA strands is efficiently solvable by the same techniques used for the alignment of DNA sequences [Gus97]. For a given set of (weighted) edit operations, the edit-distance, i.e. the minimal number of operations needed for the transformation of one sequence into the other, is calculated. This results in an alignment of the two structures.

In the literature this approach is transferred to higher structures of RNA molecules in various ways. Some of them rely on the tree representation of secondary structures and measure the tree-edit-distance (eg. [SZ90, Zha96a, Zha96b]), some

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\(^1\)There exist two definitions of tertiary structure, one as given above, and an alternative definition as the spatial arrangement of bases.
Covered structures | time | space | reference
--- | --- | --- | ---
(plain, plain) | $O(n^2)$ | $O(n^2)$ | eg. [SK83]
(nested, any) | $O(n^4)$ | $O(n^2)$ | [ZWM02]
(decomposable, any) | $O(n^{12})$ | $O(n^8)$ | this paper
(any, any) | NP-complete | | [ZWM02]

**Figure 1.** Complexities of alignment algorithms

 involve *stochastic context-free grammars* [SBH94a, SBH94b]. Additional methods can be found in [BMR95] and [LRV98]. But most of them, mostly due to formal restrictions, cover only nested sequences.

As K. Zhang et al. do in [ZWM02] and [JLMZ02], we treat unpaired bases and base-pairings as atomic units. There exist several variations of this approach. First of all, one has to choose a set of basic edit-operations. Secondly, one has to chose the scores or weights for these operations, which may possibly depend on the underlying bases. Since we view unpaired bases and pairings as unbreakable units, it is only allowed to replace an unpaired base/pairing by another one, to remove it or to introduce a new one. It is not allowed to remove only one partner of a pairing, to break a pairing without removing the bases etc.

These edit-operations are quite restrictive, compared to the more flexible set used in [JLMZ02]. But nonetheless, the general problem of finding a minimal alignment (sequence of edit-operations) is NP-complete, as long as the edit-operations do rely on the higher structures, i.e. the pairings (this is known for several choices of edit-operations (ZWM02, JLMZ02). So far polynomial algorithms only are known for alignments of a secondary structure with an arbitrary one\(^2\).

We are going to present a new description of higher structures of RNA, using a formal system closely related to graph grammars. In the first section the formalism is defined and a set of generators, i.e. certain small structures which are used to construct larger, so-called *decomposable* structures, is presented. As we will see, this description includes nested structures and a wide variety of pseudoknots, but not all. Following that, we are going to describe our edit-operations and the resulting type of alignment of two tertiary structures. Connecting this with our formalism leads to the essential observation that the alignments of decomposable structures are of a special type, called *semi-decomposable*, meaning that a core structure of the alignment, consisting of all matched/mismatched pairings and bases, still is decomposable. This results in the framework of an algorithm calculating the *exact* score of a minimal alignment of a so-called *decomposable* structure with an arbitrary one. This algorithm runs in a time polynomial in the number of bases of the aligned sequences and polynomial space. The degrees of the polynomials depend on the choice of generators. In fact it is possible to extend the set of decomposable structures easily by the introduction of additional generators. This increases the degree of the polynomials, but the required time and space remain polynomial, even though the general problem is NP-complete.

Finally we will give some results about the approximation ratio of the algorithm for arbitrary pairs of RNA molecules, depending on the chosen scores.

\(^2\)The algorithm of Zhang can be adapted to cover certain restricted H-like pseudoknots instead of nested structures. But the details are not given in the cited paper.
Unfortunately the runtime and especially the space requirements prohibit an actual implementation of this algorithm. But nonetheless, they may be the foundation of a family of more efficient algorithms. Ideas, how this basic algorithm can be improved, are given in the last section of this paper.

2. Higher RNA Structures

Usually an RNA molecule is represented by a sequence of bases, i.e. a word \( s \) over the alphabet \( \Sigma_{\text{RNA}} = \{A, C, G, U\} \) together with a set of pairings, i.e. a set of pairs \( (i, j) \) with \( 1 \leq i < j \leq |s| \). Graphically these are represented by a structure (multi-)graph with vertex set \( \{1, \ldots, |s|\} \). The \( i \)-th base corresponds to the vertex \( i \), and two subsequent vertices/bases are connected by a backbone (-edge). In addition each pairing \( (i, j) \) is represented by an edge between \( i \) and \( j \).

We require an additional type of RNA-structures, introducing a gap between two bases, as already used by Rivas and Eddy in [RE99]. These will be called gapped or 1-structures (An example is given in Fig. 2). In addition we are going to separate the structure from the sequence of bases, providing us with a simplified notation.

For \( n \in \mathbb{N} \) and \( n \geq 1 \) let \( \mathbf{n} \) denote the finite set \( \{1, \ldots, n\} \subset \mathbb{N} \) and \( \emptyset \) the empty set.

**Definition 2.1 (0- and 1-Structures).** A 0-structure \( \sigma = (n, P) \) (or structure of type 0) consists of a natural number \( n \) and a set \( P \subset \mathbf{n} \times \mathbf{n} \) of pairs \( (i, j) \) with \( i < j \), such that for \( (i, j), (i', j') \in P \) the intersection \( \{i, j\} \cap \{i', j'\} \) is either empty or \( \{i, j\} = \{i', j'\} \).

A 1-Structure \( \sigma = (n, P, k) \) (or structure of type 1) consists of a 0-structure \( (n, P) \) and a natural number \( 0 \leq k \leq n \).

The elements of \( \mathbf{n} \) are the bases and the pairs \( (i, j) \in P \) the (base-)pairings. A base \( i \in \mathbf{n} \) of \( \sigma \) is paired if there exist a pairing \( (i, j) \) or \( (j, i) \), and unpaired otherwise. Furthermore each base is paired with at most one other base. The pairings and unpaired bases are also called structural elements. In a 1-structure \( \sigma = (n, P, k) \) the sequence of bases is split after base \( k \) into two intervals \([i, k]\) and \([k + 1, n]\) called legs.

We explicitly allow the legs to be empty (by setting \( k = 0 \) and \( k = n \)). This allows us to view 0-structures as 1-structures with one empty leg. Therefore we may use these cases to relate to 0- and 1-structures without mentioning them explicitly.

![RNA structure with 8 bases and pairings 
{(1, 6), (2, 5), (3, 8), (4, 7)}](image)

The same structure with a gap between bases 4 and 5.

**Figure 2. Examples of RNA structures**
Since all pairings \((i, j)\) satisfy \(i < j\), we do not need to differentiate between \((i, j)\) and \((j, i)\). Furthermore, if a pairing \((i, j)\) is given we assume \(i < j\), unless stated otherwise. Furthermore we simply write \((i, j) \in \sigma = (n, P)\) for pairings in \(P\) and \(i \in \sigma\) for unpaired bases.

There exist four trivial structures containing at most one structural element. The identities simply consist of a free base or a single pairing. The empty structures are structures with no bases. In the graphical representation (see Fig. 3) we use an empty circle “◦” for empty legs.

Two pairings \((i, j)\) and \((i', j')\) with \(i < i'\) are called independent if \(i < j < i' < j'\), nested if \(i < i' < j' < j\) and crossed if \(i < i' < j < j'\). A 0- or 1-structure \(\sigma\) is called nested if any two pairings are either independent or nested (see Fig. 4). A structure containing crossed pairings is called pseudoknot or pseudoknotted.

The natural numbers induce a total order on the structural elements. More precisely we define

**Definition 2.2 (The Order of Structural Elements).** Let \(\sigma\) be a structure. The relation \(<_\sigma\) on the set of structural elements is given by:

- \(i <_\sigma j\) if and only if \(i < j\).
- \(i <_\sigma (i', j')\) if and only if \(i < i'\).
- \((i', j') <_\sigma i\) if and only if \(i' < i\).
- \((i, j) <_\sigma (i', j')\) if and only if \(i < i'\).

### 2.1. Structures and Sequences

Up to this point we only described the structure of RNA-molecules, but not the sequence of bases. In fact we put some effort in the separation of the secondary structures from the actual sequence of nucleotides.

**Definition 2.3 (Folded \(\Sigma\)-Sequences).** Let \(\Sigma\) be an alphabet, i.e. a finite non-empty set. A \(\Sigma\)-sequence of type 0 is a word in \(\Sigma^*\) and a \(\Sigma\)-sequence of type 1 is a pair \(s = (s_1, s_2)\) of words in \(\Sigma^*\).

A folded \(\Sigma\)-sequence is a pair \((\sigma, s)\) consisting of a structure \(\sigma\) and a \(\Sigma\)-sequence \(s\) of the same type as \(\sigma = (n, P, k)\), such that

- \(|s| = n\) if \(\sigma\) is a 0-structure and
- \(|s_1| = k\) and \(|s_2| = n - k\) if it is a 1-structure.

For RNA molecules the standard alphabet is \(\Sigma_{RNA} = \{A, C, G, U\}\). But nonetheless it is useful to use this general definition, because in the context of alignments bases may be deleted or inserted, resulting in “empty” bases, which correspond to the fifth letter “◦”.
2.2. The Compositions. We will especially consider structures constructed from smaller ones by two basic operations, called \textit{compositions}. The first replaces an unpaired base of an arbitrary structure by a whole 0-structure. The second composition does exactly the same with a pairing and a 1-structure. More formally we use the following definition.

\textbf{Definition 2.4 (The Compositions).}

(1) Let $\sigma = (n, P, k)$ be a structure and $i$ one of its unpaired bases. For each 0-structure $\tau = (m, Q)$ the structure $\sigma \circ_i \tau$ is defined by

$$\sigma \circ_i \tau := (n + m - 1, P \circ_i Q, k')$$

with

$$k' = \begin{cases} k & \text{if } k < i \\ k + m - 1 & \text{if } k \geq i \end{cases}$$

and $P \circ_i Q$ contains the following pairings

- $(j'_1, j'_2)$ for all $(j_1, j_2) \in P$ with $j'_x = \begin{cases} j_x & \text{if } j_x < i \\ j_x + m - 1 & \text{if } i < j_x \end{cases}$
- $(j_1 + i, j_2 + i)$ for all $(j_1, j_2) \in Q$.

The operation $- \circ_i -$ is called composition along the (unpaired) base $i$.

(2) Let $\sigma = (n, P, k)$ be a structure and $(i, j)$ one of its pairings. For each 1-structure $\tau = (m, Q, l)$ the structure $\sigma \circ_{(i,j)} \tau$ is defined by

$$\sigma \circ_{(i,j)} \tau := (n + m - 2, P \circ_{(i,j)} Q, k')$$

with

$$k' = \begin{cases} k & \text{if } k < i \\ k + l - 1 & \text{if } i \leq k < j \\ k + m - 2 & \text{if } j \leq k \end{cases}$$

and $P \circ_{(i,j)} Q$ contains the following pairings

- $(j'_1, j'_2)$ for all $(j_1, j_2) \in P \setminus \{(i, j)\}$ with $j'_x = \begin{cases} j_x & \text{if } j_x < i \\ j_x + l - 1 & \text{if } i < j_x < j \\ j_x + m - 2 & \text{if } j \leq j_x \end{cases}$
- $(j'_1, j'_2)$ for all $(j_1, j_2) \in Q$ with $j'_x = \begin{cases} j_x + i - 1 & \text{if } j_x < l \\ j_x + j - 1 & \text{if } l \leq j_x \end{cases}$

The operation $- \circ_{(i,j)} -$ is called composition along the pairing $(i, j)$.

By definition the compositions preserve the type of the first argument, i.e. if it is a 0/1-structure, then the resulting structure again has type 0/1.
Obviously composition with an identity $id_k$ has no effect and composition with the empty structures $empty_k$ deletes the structural element along which it is composed.

Since the composition along an unpaired base or a pairing does not affect the remaining structural elements, we may compose simultaneously along all of them. This is denoted by $\sigma \circ (\tau_1, \ldots, \tau_l)$, where $\tau_i$ is composed with $\sigma$ along the $i$-th structural element, ordered by $<_\sigma$.

As already mentioned, the compositions provide means to build larger structures from smaller ones. To get an efficient description, one needs to use a set of basic structures, called generators, and to restrict to decomposable structures, i.e., those which are compositions of generators, reducing the search space.

**Definition 2.5 (Decomposable structure).** Let $\Gamma$ be a finite set of 0- and 1-structures. Its elements are called generators. A structure $\sigma$ is $\Gamma$-decomposable if

1. it is an identity ($id_0$ and $id_1$),
2. or there exists a generator $\tau \in \Gamma$ and $\Gamma$-decomposable structures $\sigma_i$ for $1 \leq i \leq l$, such that $\sigma = \tau \circ (\sigma_1, \ldots, \sigma_l)$.

For biological purposes we suggest the generators given in Fig. 6. In [Bau04] experiments confirmed that all pseudoknots in PseudoBase are decomposable with respect to these generators. This is a strong indication that indecomposable structures are biologically less relevant.

The generator $\text{disconn}$ allows the construction of disconnected 1-structures, consisting of two 0-structures.
Observe, that all nested structures can be described as compositions of *concat*, *loop*, *lconcat*, *rconcat* and *nest*. Nonetheless, not all structures can be build from the generators in $\mathcal{G}$ (e.g. the structure shown in Fig. 7).

### 3. Alignments

One way to measure the similarity between two folded sequences is the calculation of the edit distance, i.e. the minimal number (or score) of allowed (usually reversible) operations needed to construct one sequence from the other. In the unstructured case this distance is precisely the score of an *alignment* of the two sequences ([Gus97]). This also holds for folded sequences, as long as one restricts to certain edit operations, which are:

1. *base-replacement* or *base-mismatch*, replacing the character at one unpaired base with a different one
2. *base-deletions*, removing an unpaired base
3. *base-insertions*, adding an unpaired base
4. *pair-replacement*, replacing the characters at the ends of a pairing and changing at least one of them
5. *pair-deletion*, removing both ends of a pairing
6. *pair-insertion*, adding a pairing

For the representation of inserted and deleted characters we use *blanks* $\circ$. For an arbitrary alphabet $\Sigma$ let $\Sigma'$ be the disjoint union of $\Sigma$ with the blank $\{\circ\}$ (Graphically we represent blanks by empty circles, while non-blanks (i.e. characters) are represented by discs).

For an arbitrary $\Sigma$-sequence $s$ the $\Sigma$-sequence $\pi(s)$ is obtained by removing all occurrences of the blank from $s$. In the same way the folded $\Sigma$-sequence $\pi(\sigma, s)$ may be constructed from a folded $\Sigma'$-sequence $(\sigma, s)$, i.e. all bases of $\sigma$ associated with a blank are removed. If at least one base of a pairing is associated to a blank, the whole pairing is deleted.

A (structural) alignment is the description of two folded sequences obtained from each other by a sequence of basic edit operations, without remembering them in detail. But in fact a possible edit sequence between both structures may easily be constructed from an alignment, and vice versa.

![Figure 7. A non-decomposable structure](image_url)

![Figure 8. The graphical representation of the edit operations](image_url)
Definition 3.1 (Alignment). Let \((\sigma_k, s_k) = ((n_k, P_k), s_k), k = 1, 2,\) be two folded sequences of same type. A (structural) alignment \((\sigma, t_1, t_2)\) between \((\sigma_1, s_1)\) and \((\sigma_2, s_2)\) is a structure \(\sigma\) of same type, together with two \(\bar{\Sigma}\)-sequences \(t_1, t_2\), such that

- \((\sigma_k, s_k) = \pi(\sigma, t_k)\) for \(k = 1, 2\) and
- \((s_k[i], s_k[j]) \in \Sigma \times \Sigma \cup \{(\circ, \circ)\}\) for \((i, j) \in \sigma\) and \(k = 1, 2\).

More intuitively, an alignment consists of a structure \(\sigma\), such that the structures \(\sigma_1\) and \(\sigma_2\) can be obtained by removing bases and pairings. The bases and pairings which have to be removed are indicated by the position of the blanks in the sequences. The second condition ensures that either both bases in a pairing are associated to blanks, or none. Two examples of alignments are shown in Fig. 9.

Instead of counting the minimal number of edit operations needed for the transformation of one sequence into another, we assign a non-negative score \(S(\sigma, t_1, t_2)\) to an alignment and try to minimize it. The score is the sum of scores of the structural elements of \(\sigma\), depending on both, the corresponding bases of the aligned structures, and the underlying structural elements. For each unpaired base of the alignment we use scores

\[
S(x, y) \quad S(x, \circ) \quad S(\circ, y)
\]

where \(x\) and \(y\) are arbitrary characters. For pairings we have scores of the following forms.

\[
S(x_1, x_2, y_1, y_2) \quad S(x_1, x_2, \circ, \circ, y_1, y_2) \quad S(\circ, \circ, y_1, y_2)
\]

The score of an alignment \((\sigma, t_1, t_2)\) is defined by

\[
S(\sigma, t_1, t_2) := \sum_{i \in \sigma} S(t_1[i], t_2[i]) + \sum_{(i, j) \in \sigma} S(t_1[i], t_1[j], t_2[i], t_2[j]).
\]

In addition to the scores being non-negative, we require

\[
S(\circ, \circ) = S(x, x) = 0 = S(x, y, x, y) = S(\circ, \circ, \circ, \circ).
\]

The score of a minimum alignment of two folded sequences is

\[
S((\sigma_1, s_1), (\sigma_2, s_2)) := \min \{S(\sigma, t_1, t_2) \mid \pi(\sigma, t_k) = (\sigma_k, s_k), k = 1, 2\}.
\]

3.1. Semi-decomposable Alignments. As shown in [ZWM02] the general problem of finding the score of a minimum alignment is NP-complete. But we are going to describe an algorithm calculating the exact minimum alignment of a decomposable folded sequence and an arbitrary one, using the fact that an arbitrary alignment of these is semi-decomposable, as defined below.

![Figure 9](image-url) Two structural alignments of two folded sequences
In the following $\Gamma$ is a finite set of generators including those in Fig. 9. The notions of semi- and decomposability will always refer to this set.

Let $P$ be a set of pairings in $\sigma$, then $\sigma \setminus P$ is the structure obtained by removing all pairings (including their bases) in $P$. If $(\sigma, s)$ is a folded sequence we define $(\sigma, s) \setminus P := (\sigma \setminus P, s \setminus P)$, where $s \setminus P$ is constructed from $s$ by removing all letters associated to the bases of pairings in $P$.

As we will see an alignment of a decomposable folded sequence with an arbitrary one is semi-decomposable in the following sense. This observation and Lemma 3.3 give us a possibility to calculate the minimal alignment by decomposition.

**Definition 3.2 (Semi-decomposable).** An alignment $(\sigma, t_1, t_2)$ of two folded sequences $(\sigma_1, s_1)$ and $(\sigma_2, s_2)$ is called semi-decomposable if there exists a set $P$ of pairings in $\sigma$, such that

1. $(t_k[i], t_k[j]) = (\sigma, \sigma)$ for one $k \in \{1, 2\}$ and each $(i, j) \in P$,
2. and the structure $\sigma \setminus P$ is decomposable.

For an arbitrary folded 0-sequence $(\sigma, s)$ of length $n$ and $1 \leq i < j \leq n$ let $\sigma[i, j]$ be the structure obtained from $\sigma$ by removing all unpaired bases outside the interval $[i, j]$ and all pairings with at least one end outside of it. Furthermore we define $(\sigma, s)[i, j] := (\sigma[i, j], \bar{s})$, where $\bar{s}$ is obtained from $s$ by deletion of the letters assigned to the deleted bases. Similar the 1-structure $(\sigma, s)[i_1, j_1; i_2, j_2]$ is defined for $i_1 \leq j_1 < i_2 \leq j_2$.

Let $\tau$ be an arbitrary structure with $m$ bases and $\sigma$ one of same type with $n$ bases. A $\tau$-splitting of $\sigma$ is a partition of the interval $[1, n]$ into $m$ subintervals $I^1, \ldots, I^m$ with $I^l = [i^l, j^l]$, which respect the gaps if $\tau$ and $\sigma$ have type 1, i.e.

- $i^l = 1, j^l = 1 + i^{l+1}$ for $1 \leq k < m$ and $j^m = n$,
- $j^l = k$ if $\sigma$ if the gap in $\tau$ is between $l$ and $l + 1$ and the gap in $\sigma$ between $k$ and $k + 1$.

A pairing $(i, j)$ of $\sigma$ is called incompatible with the splitting, if $i \in I^{i'}$ and $j \in I^{j'}$ with $i' \neq j'$ and $(i', j')$ is not a pairing in $\tau$, i.e. the two bases of the pairing lie in two different intervals of the splitting, which aren’t paired in $\tau$. A $\tau$-splitting of $\sigma$ is called proper if the induced splitting of $\tau \setminus P$ contains no empty interval, where $P$ is the set of incompatible pairings. In other words a $\tau$-splitting is proper if and only if each interval contains at least one unpaired base or one end of a compatible pairing.

The notion of proper splittings allows an equivalent description of semi-decomposable structures.

**Lemma 3.3.** An alignment $(\sigma, t_1, t_2)$ of $(\sigma_1, s_1)$ and $(\sigma_2, s_2)$ is semi-decomposable, if and only if either $\sigma$ is an identity, a generator, or if

1. there exists a generator $\tau$ with $m$ bases and $i$ structural elements,
2. proper $\tau$-splittings $I^1_k, \ldots, I^m_k$ of $\sigma_k$ for $k = 1, 2$,
3. for each structural element $\chi$ of $\tau$ exists a semi-decomposable alignment $(\sigma^\chi, t_1^\chi, t_2^\chi)$ of $(\sigma_1, s_1)[I^1_1]$ and $(\sigma_2, s_2)[I^2_1]$ if $\chi = i \in \tau$, or of $(\sigma_1, s_1)[I^1_1; I^1_2]$ and $(\sigma_2, s_2)[I^2_1; I^2_2]$ if $\chi = (i, j) \in \tau$,
4. and $\sigma_k \setminus P_k = \tau \circ (\pi(\sigma^1, t_k), \ldots, \pi(\sigma^i, t_k))$ for $k = 1, 2$, where $P_k$ is the set of incompatible pairings of $\sigma_k$.

**Proof.** If $\sigma$ is an identity or a generator the lemma obviously holds.
Suppose that the alignment \((\sigma, t_1, t_2)\) is semi-decomposable. If \(\sigma\) is only an unpaired base, then it obviously is a decomposition and \(P' = \emptyset\). The same holds if \(\sigma\) has only a single pairing. Now assume that \(P\) is the set of pairings defined in the definition of semi-decomposability, i.e., \(\sigma\backslash P = \tau \circ (\sigma^1, \ldots, \sigma^m)\) for a generator \(\tau\) and decomposable structures \(\sigma^1, \ldots, \sigma^m\). Then one can choose a \(\tau\)-splitting of \(\sigma\), such that the induced splitting of \(\tau\backslash P\) is the one given by the decomposition (simply add the bases of the deleted pairings to appropriate intervals). Since the generators do not allow the deletion of bases or pairings, this splitting is proper and some of the pairings in \(P\) are compatible with it and some aren’t. Let \(P' \subseteq P\) be the subset of the latter ones. The compatible pairings can be added to the \(\sigma^i\) resulting in structures \(\hat{\sigma}^1, \ldots, \hat{\sigma}^m\). By induction these induce semi-decomposable subalignments, because they contain less structural elements than \(\sigma\) and removal of the added pairings results in decomposable structures. Furthermore we have \(\sigma\backslash P' = \tau \circ (\hat{\sigma}^1, \ldots, \hat{\sigma}^m)\).

Now assume that there exists a generator \(\tau\) and a proper \(\tau\)-splitting as stated in the lemma. Since the subalignments are semi-decomposable and contain less structural elements than \(\sigma\), there exists a set \(P_\chi\) of pairings in \(\sigma^\chi\) for each structural element \(\chi \in \tau\), such that \(\sigma^\chi\backslash P_\chi\) is decomposable and each pairing in \(P_\chi\) is assigned to blanks in at least one sequence. If \(P\) is the set of pairings incompatible to the splitting, this leads to \(\sigma\backslash P \cup \bigcup_{\chi \in \tau} P_\chi = \tau \circ (\sigma^1\backslash P_1, \ldots, \sigma^r\backslash P_r)\), proving the semi-decomposability of \((\sigma, t_1, t_2)\).

**Corollary 3.4.** Any alignment \((\sigma, t_1, t_2)\) of a decomposable folded sequence \((\sigma_1, s_1)\) and an arbitrary folded sequence \((\sigma_2, s_2)\) is semi-decomposable.

**Proof.** Choose \(P'\) as the set of pairings \((i, j)\) in \(\sigma\) matched by blanks, i.e., \((t_2[i], t_2[j]) = (\circ, \circ)\). Then \(\sigma\backslash P'\) is exactly \(\sigma_1\) with additional unpaired bases. Since unpaired bases may be added at any position by composition with generators, \(\sigma\backslash P'\) is decomposable.

As a consequence of Corollary 3.4 it is sufficient to find the minimum semi-decomposable alignment if at least one of the sequences is decomposable. An arbitrary semi-decomposable alignment can be constructed in the following way.

1. Choose a generator \(\tau\).
2. Choose two proper \(\tau\)-splittings of the structures.
3. Find all incompatible pairings.
4. Align the subsequences (without incompatible pairings) induced by the splittings.

Then the score of the alignment is the sum of the scores of the subalignments and the scores of incompatible pairings matched against blanks. This approach leads to the algorithm described in detail in the following section.

3.2. **The Algorithm.** The minimum alignment of two folded sequences is calculated using dynamic programming. We use two arrays, indexed by intervals. For two folded 0-sequences \((\sigma_1, s_1)\) and \((\sigma_2, s_2)\) the value \(S_0[I_1; I_2]\) is the score of a minimal alignment of the 0-structures \((\sigma_1, s_1)[I_1]\) and \((\sigma_2, s_2)[I_2]\). Similar \(S_1[I_1, J_1; I_2, J_2]\) is the score of a minimal alignment of the two 1-sequences \((\sigma_1, s_1)[I_1; J_1]\) and \((\sigma_2, s_2)[I_2; J_2]\).
and \((\sigma_2, s_2)[I_2; J_2]\). The values \(R_k[I; J]\) are the sums of all weights of pairings \((i, j)\) in \((\sigma_k, s_k)\) such that one end is in \(I\) and the other in \(J\), i.e.

\[
R_k[I; J] := \sum_{(i, j) \in \sigma_k, i \in I, j \in J} S\left(\frac{s_k[i]}{\circ}, \frac{s_k[j]}{\circ}\right).
\]

\(W(\sigma, s)\) is the score of the sequence \((\sigma, s)\) (aligned with blanks), i.e.

\[
W(\sigma, s) := \sum_{i \in \sigma} S\left(\frac{s[i]}{\circ}\right) + \sum_{(i, j) \in \sigma} S\left(\frac{s[i]}{\circ}, \frac{s[j]}{\circ}\right).
\]

For shorter notation we write:

\[
W_k[I] := W((\sigma_k, s_k)[I]) \text{ and } W_k[I; J] := W((\sigma_k, s_k)[I; J])
\]

The entries of the arrays \(S_0\) and \(S_1\) can be calculated recursively using scores for shorter intervals. The recursion stops if all intervals consist of only one base, i.e. \(I_k = [i_k, i_k]\) and \(J_k = [j_k, j_k]\). Then we have:

(1) \[
S_0[I_1; I_2] = \begin{cases} 
\min \left( S\left(\frac{s_1[i_1]}{\circ}, \frac{s_1[j_1]}{\circ}\right), W_1[I_1] + W_2[I_2] \right) & \text{if } i_1 \text{ and } i_2 \text{ are unpaired} \\
W_1[I_1] + W_2[I_2] & \text{otherwise}
\end{cases}
\]

(2) \[
S_1[I_1, J_1; I_2, J_2] = \begin{cases} 
\min \left\{ S\left(\frac{s_1[i_1]}{\circ}, \frac{s_1[j_1]}{\circ}, \frac{s_2[i_2]}{\circ}, \frac{s_2[j_2]}{\circ}\right), W_1[I_1; J_1] + W_2[I_2; J_2] \right\} & \text{if } (i_1, j_1) \text{ and } (i_2, j_2) \text{ are pairings} \\
W_1[I_1; J_1] + W_2[I_2; J_2] + W_1[I_2; J_2] & \text{only } (i_1, j_1) \text{ is a pairing} \\
W_2[I_1; J_2] + W_1[I_1] + W_1[I_1] & \text{only } (i_2, j_2) \text{ is a pairing} \\
W_1[I_1] + W_1[I_1] + W_2[I_2] + W_2[I_2] & \text{otherwise}
\end{cases}
\]

In general, we have to check every generator \(\tau\) of type 0 and every pair \(I_k = I_k^1 \ldots I_k^m, k = 1, 2\), of proper \(\tau\)-splittings of \(\sigma_k[I_k]\). The score of this decomposition is

(3) \[
X_0(\tau, I_k^1, \ldots, I_k^m) := \sum_{i \in \tau} S_0[I_1^i; I_2^i] + \sum_{(i, j) \in \tau} S_1[I_1^i, I_1^j; I_2^i, I_2^j] + \sum_{(i, j) \notin \tau} \left( R_1[I_1^i; I_1^j] + R_2[I_2^i; I_2^j] \right).
\]

The first two sums are the scores contributed by subalignments induced by the unpaired bases and pairings in \(\tau\). The third sum is the score of incompatible pairings.

Since the splittings are proper, the intervals \(I_k^i\) aren’t empty and therefore are shorter than \(I_k\). Hence \(X_0(\tau, I_k^1, \ldots, I_k^m)\) can be calculated from the scores of subalignments of shorter intervals.
The score $S_0[I_1; I_2]$ is the minimum over all generators of type 0 and all proper splittings. For $\text{concat}$ and $\text{loop}$ this leads to

$$S_0[I_1; I_2] = \min \left\{ \begin{array}{ll}
S_0[I_1^1; I_2^1] + S_0[I_1^2; I_2^2] & (\tau = \text{concat}) \\
+ R_1[I_1^1; I_2^1] + R_2[I_1^2; I_2^2] & \\
S_1[I_1^1, I_1^2; I_2^1, I_2^2] & (\tau = \text{loop})
\end{array} \right\}$$

where $I_k = I_k^1 I_k^2$, $k = 1, 2$, are decompositions of the intervals.

Similar, $S_1$ can be calculated as the minimum score over all decompositions using a generator $\tau$ of type 1. In general, one obtains the following score for $S_1[I_1, J_1; I_2, J_2]$ if $\tau$ has non-empty legs with $m$ and $l$ bases, $m, l \leq 1$:

$$X_1(\tau, I_k^1, \ldots, I_k^{m+l}) := \sum_{i \in \tau} S_0[I_i^1; I_i^2] + \sum_{(i,j) \notin \tau} S_1[I_i^1, I_i^2; I_j^1, I_j^2] + \sum_{(i,j) \in \tau} \left( R_1[I_i^1; I_i^2] + R_2[I_j^1; I_j^2] \right).$$

where $I_k = I_k^1 \ldots I_k^m$, $J_k = J_k^{m+1} \ldots J_k^{m+l}$ for $k = 1, 2$.

Again the score $S_1[I_1, J_1; I_2, J_2]$ is the minimum over all sums for all generators of type 1 and all appropriate splittings. For some of the generators given in Fig. 6 this results in the formulas given in Tab. 1.

\begin{algorithm}
\textbf{Algorithm 1}: Calculation of the score of a minimal structural alignment

\begin{algorithmic}
\State \textbf{Input}: Two folded sequences $(\sigma_k, s_k)$, $k = 1, 2$ of type 0, one of them has to be decomposable
\State \textbf{Output}: The score of a minimum structural alignment between both
\State \textbf{begin}
\State \hspace{1em} $n_1 \leftarrow$ number of bases in $\sigma_1$
\State \hspace{1em} $n_2 \leftarrow$ number of bases in $\sigma_2$
\State \hspace{1em} Mark all entries $S_0[I_1; I_2]$ and $S_1[I_1, J_1; I_2, J_2]$ as undefined;
\State \hspace{1em} \textbf{return} $S_0((\sigma_1, s_1), (\sigma_2, s_2), (1, n_1), (1, n_2))$;
\State \textbf{end}
\end{algorithmic}

\begin{function}
\textbf{Function} $S_0(S_1, S_2, I_1, I_2)$

\begin{function}
\State \textbf{Input}: Two folded 0-sequences $S_k = (\sigma_k, s_k)$ and two intervals $I_k$, $k = 1, 2$
\State \textbf{Output}: The score of a minimum structural alignment of $(\sigma_1, s_1)[I_1]$ and $(\sigma_2, s_2)[I_2]$
\State \textbf{begin}
\State \hspace{1em} if $S_0[I_1; I_2]$ is defined then \textbf{return} $S_0[I_1; I_2]$;
\State \hspace{1em} forall generators $\tau$ of type 0 do
\State \hspace{2em} forall proper $\tau$-splittings $I_k = I_k^1 \ldots I_k^m$, $m = |\tau|$ do
\State \hspace{3em} $x \leftarrow X_0(\tau, I_k^1, \ldots, I_k^m)$ as given by Eqn. 7 or 8;
\State \hspace{3em} if $x < S_0[I_1; I_2]$ then $S_0[I_1; I_2] \leftarrow x$;
\State \hspace{1em} end
\State \hspace{1em} end
\State \hspace{1em} \textbf{return} $S_0[I_1; I_2]$;
\State \textbf{end}
\end{function}
\end{function}
\[
S_1[I_1, J_1; I_2, J_2] = \begin{cases}
S_0[I_1; I_2] + S_0[J_1; J_2] + R_1[I_1; J_1] + R_2[I_2; J_2] & (\tau = \text{disconn}) \\
S_0[I_1^1; I_2^1] + S_1[I_2^2, J_1; I_2^2, J_2] + R_1[I_1^1; I_2^2] + R_1[I_1^1; J_1] & \text{for } I_1 = I_1^1 I_2^2 \text{ and } I_2 = I_1^1 I_2^2, I_1^1 \neq I_k \\
+ R_2[I_2^2; J_2^2] + R_2[I_2^2; J_2] & (\tau = \text{concat}) \\
S_1[I_1^1; I_2^1; I_2^2; J_1^1] + S_1[I_2^2, J_1^1; I_2^2, J_2] + R_1[I_1^1; I_2^2] + R_1[I_1^1; J_1^1] & \text{for } I_1 = I_1^1 I_2^2 I_1^3 \text{ and } I_2 = I_1^3 I_2^2 I_1^2, I_2^2 \neq I_k \\
+ R_2[I_2^2; J_2] + R_1[I_2^2; J_2] & (\tau = \text{hwarp}) \\
\end{cases}
\]

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Function & $S_1(S_1, S_2, I_1, J_1, I_2, J_2)$ \hline
Input: & Two folded 0-sequences $S_k = (\sigma_k, s_k)$ and four intervals $I_k, J_k$, \\
& $k = 1, 2$ \hline
Output: & The score of a minimum structural alignment of $(\sigma_1, s_1)[I_1, J_1]$ and \\
& $(\sigma_2, s_2)[I_2, J_2]$ \hline
begin & if $S_0[I_1, J_1; I_2, J_2]$ is defined then return $S_0[I_1, J_1; I_2, J_2]$; \\
& forall generators $\tau$ of type 1 do \\
& forall proper $\tau$-splittings $I_k = I_k^1 \ldots I_k^m, J_k = J_k^1 \ldots J_k^{m+1} \text{ do}$ \\
& \hspace{1cm} $x \leftarrow X_1(\tau, I_k^1, \ldots, I_k^{m+1}) \text{ as given by Eqn. 2) or 3) ;}$ \\
& \hspace{1cm} if $x < S_1[I_1, J_1; I_2, J_2]$ then $S_1[I_1, J_1; I_2, J_2] \leftarrow x$; \hline
& end \hline
& return $S_1[I_1, J_1; I_2, J_2]$; \hline
\end{tabular}
\end{table}
Theorem 3.5. Let $\Gamma$ be a set of generators, i.e. a finite set of 0- and 1-structures, including those in Fig. 4 and $m$ the maximal number of bases in any leg of any generator $\tau \in \Gamma$. The score of a minimum alignment of a $\Gamma$-decomposable folded sequence with $n_1$ bases, and an arbitrary one with $n_2$ bases, can be calculated in $O(n_1^{m+4}n_2^{m+4})$ time and $O(n_1^4n_2^4)$ space.

Proof. There are $O(n_k^2)$ weights $W((\tau_k, s_k)[I])$, which can be calculated in $O(n_k)$ each, leading to $O(n_1^3 + n_2^3)$ time and $O(n_1^2 + n_2^2)$ space. Similar the $R_k[I; J]$ need $O(n_1^4 + n_2^5)$ space and $O(n_1^5 + n_2^5)$ total time.

The arrays $S_0$ and $S_1$ have $O(n_1^2n_2^2)$ and $O(n_1^4n_2^4)$ entries. We assume that the calculation of $X_0(\tau, \ldots)$ and $X_1(\tau, \ldots)$ using equations (3) and (4) require constant time (this is the case if the equations for each generator $\tau$ are directly programmed and not dynamically evaluated). Furthermore already known scores are stored such that each score has to be calculated at most once.

Since the $R_k[I; J]$ and the weights $W$ are already known, the time required for each splitting of the intervals is constant, and the check whether a splitting is proper takes $O(n_1 + n_2)$ time. Hence it is sufficient to count the settings for each entry. For $S_0$ each interval is split into at most $m$ parts, resulting in $O(n_1^{m-1}n_2^{m-1})$ cases and $O(n_1^{m+2}n_2^{m+2})$ time. For $S_1$ each interval is split into at most $m$ subintervals, leading to $O(n_1^{m-1}n_2^{m-1})$ cases and $O(n_1^{m+4}n_2^{m+4})$ time for $S_1$. □

Corollary 3.6. For the set $\Gamma$ of generators given in Fig. 4 the score of a minimum alignment of a decomposable folded sequence with $n_1$ bases, and an arbitrary one with $n_2$ bases, can be calculated in $O(n_1^7n_2^2)$ time and $O(n_1^4n_2^4)$ space.

3.3. The Approximation of arbitrary alignments. Obviously the calculations can be made for two arbitrary folded sequences, resulting in a minimum semi-decomposable alignment. As we have seen, this leads to a globally minimum alignment, if at least one of them is decomposable. But what can be said about the calculated score if neither structure is decomposable.

Let $S_{\text{decomp}}(1, n_1; (1, n_2))$ be the score calculated by our algorithm. Then we have the following result regarding the quality of the approximation.

Theorem 3.7. If there exists a constant $c \geq 1$, such that

$$cS\left(\begin{array}{c} x, y \\ x', y' \end{array}\right) \geq S\left(\begin{array}{c} x, y \\ \emptyset, \emptyset \end{array}\right) + S\left(\begin{array}{c} \emptyset, \emptyset \\ x', y' \end{array}\right)$$

for all $x, y, x', y' \in \Sigma$ with $x \neq x'$ or $y \neq y'$, then

$$S_{\text{decomp}}((\sigma_1, s_1), (\sigma_2, s_2)) \leq cS((\sigma_1, s_1), (\sigma_2, s_2))$$

for all folded sequences $(\sigma_k, s_k), k = 1, 2$.

Proof. Let $(\sigma, t_1, t_2)$ be a minimum alignment. We are going to construct a semi-decomposable alignment, replacing all mismatched pairings by an insertion and a deletion. This procedure allows us to control the increase of the score and leads to the stated inequality, and it ensures that the constructed alignment remains semi-decomposable, since its decomposable core is constructed from the initial alignment by the deletion of pairings.
Let $M = \{(i,j) \in \sigma \mid t_1[i] \neq t_2[i] \text{ or } t_1[j] \neq t_2[j] \text{ and } t_k[i], t_k[j] \neq \circ \}$ be the set of mismatched pairings. Then we have:

$$S((\sigma_1, s_1), (\sigma_2, t_2)) = S(\sigma, t_1, t_2)$$

$$= \sum_{i \in \sigma} S\left(\frac{t_1[i]}{t_2[i]}\right) + \sum_{(i,j) \in \sigma} S\left(\frac{t_1[i], t_1[j]}{t_2[i], t_2[j]}\right)$$

$$= A + \sum_{(i,j) \notin M} S\left(\frac{t_1[i], t_1[j]}{t_2[i], t_2[j]}\right) + \sum_{(i,j) \in M} S\left(\frac{t_1[i], t_1[j]}{t_2[i], t_2[j]}\right)$$

$$\geq A + B + \sum_{(i,j) \in M} \frac{1}{c} \left(S\left(\frac{t_1[i], t_1[j]}{\circ, \circ}\right) + S\left(\frac{\circ, \circ}{t_2[i], t_2[j]}\right)\right)$$

$$\geq \frac{1}{c} (A + B)$$

$$+ \frac{1}{c} \sum_{(i,j) \in M} \left(S\left(\frac{t_1[i], t_1[j]}{\circ, \circ}\right) + S\left(\frac{\circ, \circ}{t_2[i], t_2[j]}\right)\right)$$

$$\geq \frac{1}{c} S_{\text{decomp}}((\sigma_1, s_1), (\sigma_2, s_2))$$

If the scores satisfy

$$S\left(\frac{x, y}{x', y'}\right) \leq S\left(\frac{x, y}{\circ, \circ}\right) + S\left(\frac{\circ, \circ}{x', y'}\right)$$

for at least one combination of $x, y, x', y' \in \Sigma$ with $x \neq x'$ or $y \neq y'$, then one may simply choose:

$$c := \max \left\{ \frac{S\left(\frac{x, y}{\circ, \circ}\right) + S\left(\frac{\circ, \circ}{x', y'}\right)}{S\left(\frac{x, y}{x', y'}\right)} \mid x \neq x' \text{ or } y \neq y' \right\} \geq 1.$$

In particular, one obtains:

**Corollary 3.8.** $S((\sigma_1, s_1), (\sigma_2, s_2)) = S_{\text{decomp}}((\sigma_1, s_1), (\sigma_2, s_2))$ for arbitrary folded sequences $(\sigma_k, s_k), k = 1, 2$, if

$$S\left(\frac{x, y}{x', y'}\right) = S\left(\frac{x, y}{\circ, \circ}\right) + S\left(\frac{\circ, \circ}{x', y'}\right)$$

for $x \neq x'$ or $y \neq y'$.

4. Conclusion

We presented a new formal description of secondary RNA structures incorporating a wide variety of pseudoknots, i.e. tertiary structures. Based on this description it is possible to calculate the exact score of a minimal structural alignment of a decomposable and an arbitrary structure. For other cases the algorithm provides an approximation of guaranteed quality, depending on the chosen weights for the underlying edit operations.
For the set of generators given in Fig. 6, the algorithm requires $O(n^{14})$ time and $O(n^8)$ space, where $n$ is the number of bases in the longer sequence. Hence, further improvements are necessary. For example, the structures may be reduced to their underlying stems, i.e., sequences of nested pairs, decreasing the number of nodes. On the other hand, it might be possible to restrict to special decompositions of the intervals, which would reduce the required space. This would at least lead to an approximative algorithm producing exact results in many cases. To which extent and at which quality remains to be analyzed.

On the other hand, the set of structures, for which the algorithm produces exact results, can be extended by the addition of generators. This causes an increasing runtime. But as long as only a finite number of generators is used, the algorithm stays polynomial, even though the general problem is NP-complete. Hence the addition of generators leads to a sequence of polynomial solvable problems (possibly) “converging” to an NP-complete problem. Hence it may be interesting to examine the gap between decomposable and non-decomposable structures.

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