Constrained RNA Folding - New Results

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Outline

Motivation

- RNA switch
  one RNA sequence $\Rightarrow$ different structures $\Rightarrow$ different functions
- Aptamer
  Aim: Finding a RNA sequence, which can fold into a special structure

QUESTION:

On what conditions is it possible to find a single RNA sequence which is compatible to given structures?

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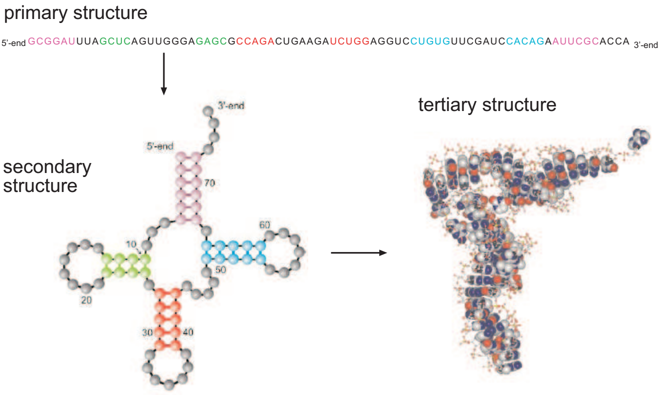
RNA Structure



RNA Structure

Definition

A length n **RNA sequence** or **primary structure** is considered as a word s in the space $C_n = \{A, C, G, U\}^n$, i.e.

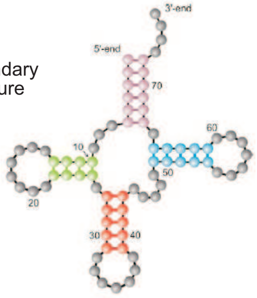
$$s = s_1 \dots s_n \in C_n = \{A, C, G, U\}^n.$$

RNA Structure

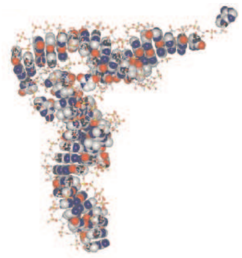
primary structure

5'-end GCGGAUUUAGCUCAGUUGGGAGAGCGCCAGACUGAAGAUCUGGAGGUCCUGUGUUCGAUCCACAGAAUUCGCACCA 3'-end

secondary structure

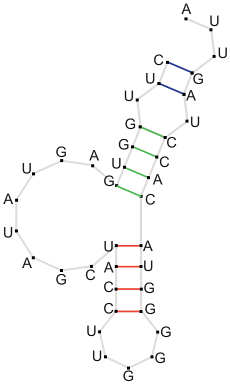


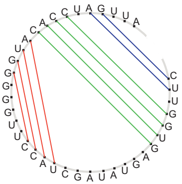
tertiary structure

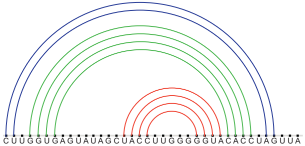


RNA Structure

A **shape** S of size n is a graph $S = (V_n, E)$ with set $V_n = \{v_1, \ldots, v_n\}$ of vertices and set E of independent edges such that for any two edges $\{v_i, v_j\}, \{v_k, v_l\} \in E$, where $i < j$ and $k < l$, it is not the case that $i < k < j < l$.

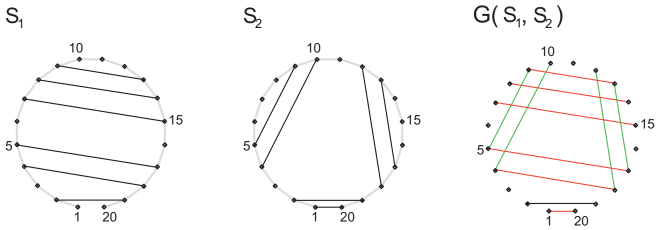






RNA Structure

For given shapes $S_1, \ldots, S_k$ of the same size n, we define the **graph of shapes** $G(S_1, \ldots, S_k)$ as $G(V_n, \cup_{i=1}^{k} E(S_i))$



Realization

On what conditions is it possible to find a single string, which is realizing any given shapes $S_1, \ldots, S_k$ of the same size?

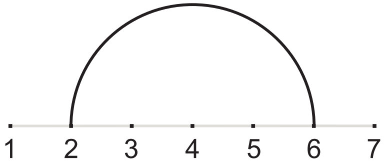
Definition

A string $s = s_1 \ldots s_n \in \{A, C, G, U\}^n$ **realizes** a shape S of size $n \Leftrightarrow \forall \{v_i, v_j\} \in E(S)$ holds:

$$s_i s_j \in \mathscr{B} = \{AU, UA, CG, GC, GU, UG\}.$$

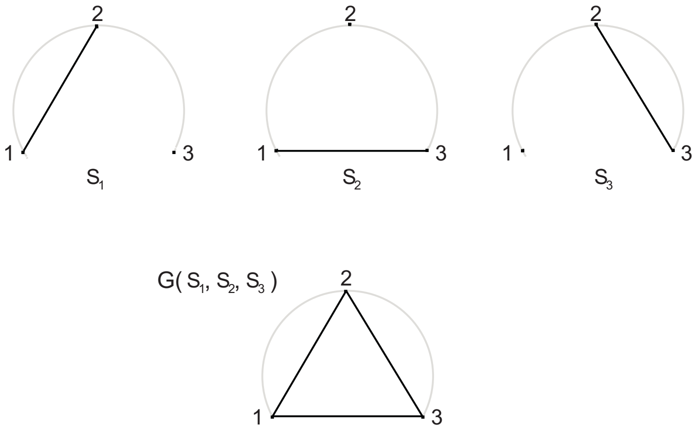
- $\mathscr{B}$ denotes the pairing rules.

Realization



$s_2 s_6 \in \{AU, UA, CG, GC, GU, UG\}$

Realization



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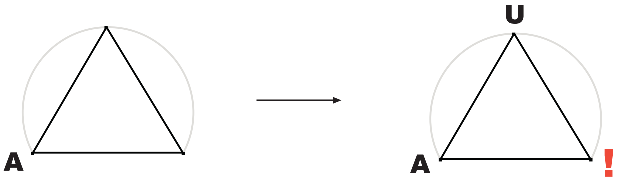
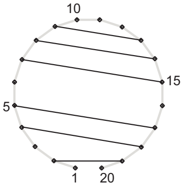


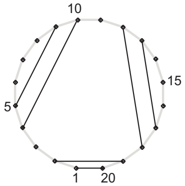
$$\mathscr{B} = \{AU, UA, CG, GC, GU, UG\}$$

Realization

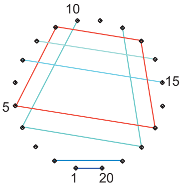
S_1



S_2



$G(S_1, S_2)$



Question:

On what conditions is it possible to find a single string, which is realizing any given shapes $S_1, \ldots, S_k$ of the same size?
If there is a single string, which is realizing all edges in the shapes $S_1, \ldots, S_k$, then this string realizes all the edges in the graph $G(S_1, \ldots, S_k)$.
Accordingly we can ask: What properties have to be fulfilled in the graph $G(S_1, \ldots, S_k)$, that there exists a single string, which is realizing all these shapes $S_1, \ldots, S_k$?

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Intersection Theorem

- $R(S)$ denotes the set of all sequences that are compatible with shape S.

1. **Intersection Theorem** [Reidys et al. 1995]
   For any two secondary structures S_1 and S_2 of same size holds: $R(S_1) \cap R(S_2) \neq \emptyset$
2. **Generalized Intersection Theorem** [Flamm et al. 2001]
   $\bigcap_{i=1}^{k} R(S_i) \neq \emptyset \Leftrightarrow$
   - $G(S_1, \ldots, S_k)$ is bipartite
   - there are no odd cycles in $G(S_1, \ldots, S_k)$
   - the shapes $S_1, \ldots, S_k$ are realizable by a single binary string, i.e. $\exists s \in \{0,1\}^n \Leftrightarrow \forall \{v_i, v_j\} \in E(S_l)$ holds: $s_i \neq s_j$

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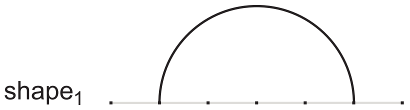
Why Extended Shapes?



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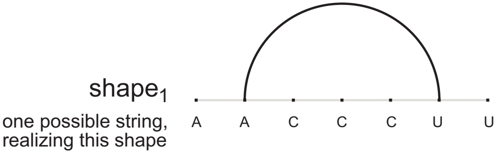

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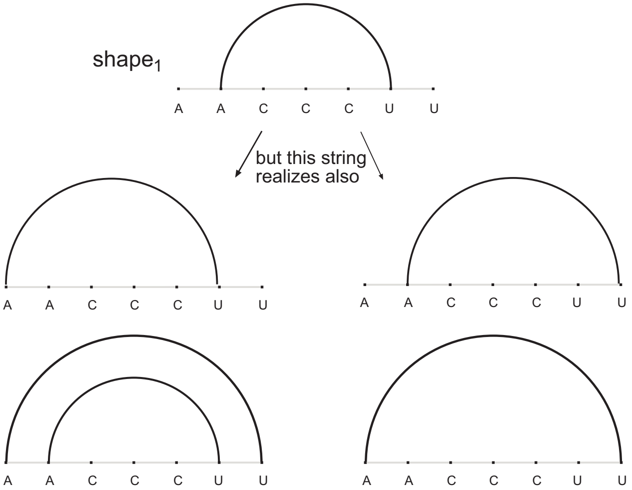

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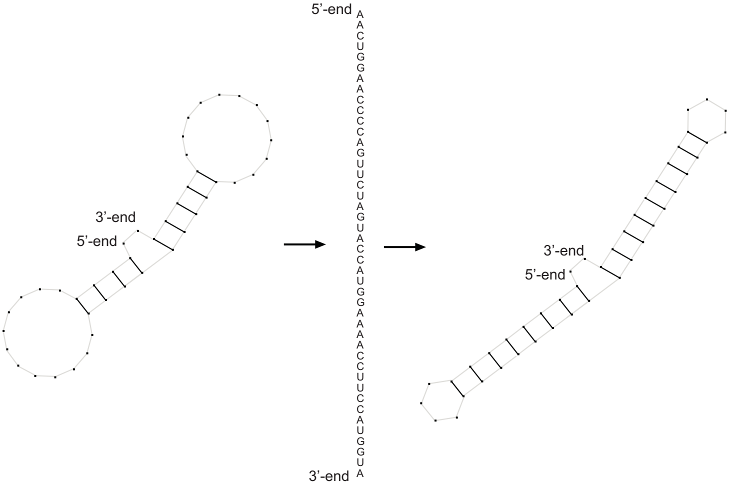
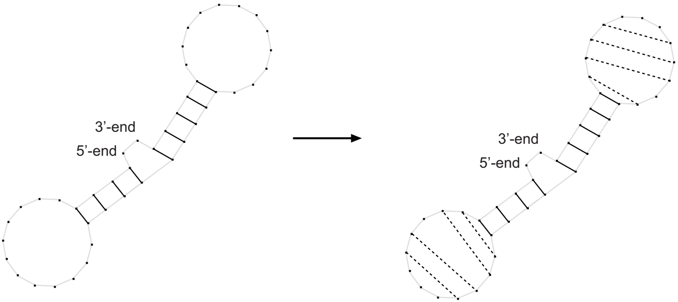


Why Extended Shapes?

How can we find sequences, which are realizing given shapes, but also conserving the structure even better than the current sequences, in such a way that unpaired positions are considered?

Pseudo Edges



In this case:

- insert pseudo edges
- ask for additional conditions to a string, which is realising a shape with pseudo edges

Extended shapes

Definition

Let $S = G(V_n, E)$ be a shape of size n. The set $V'(S) = \{v_i \in V_n \mid \nexists v_j \in V_n,\ s.t.\{v_i, v_j\} \in E\}$ is the set of all vertices that are not paired.

- Now arbitrary vertices $v_i, v_j \in V'(S)$ will get connected by new edges $\{v_i, v_j\}$.
- These new edges are called **pseudo edges**.
- But connecting new vertices with pseudo edges is restricted to: **The characteristics of a shape have to be conserved.**

For a better understanding we are calling the 'old' edges: **regular edges**.

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Extended shapes

Definition

Let $\hat{E}(S)$ be the set of all inserted *pseudo edges* in S. We call the graph $\hat{S} = (V_n, E(S), \hat{E}(S)) := (V_n, \tilde{E} := E(S) \cup \hat{E}(S), \gamma)$, that emerges from the shape S, **shape extension** of S or also **extended shape**.

Extended shapes

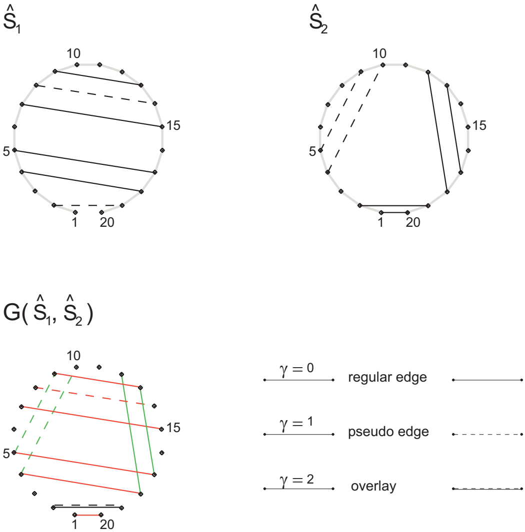
Definition

Let $\hat{S}_1 = (V_n, E(S_1), \hat{E}(S_1))$, ..., $\hat{S}_k = (V_n, E(S_k), \hat{E}(S_k))$ be extended shapes.

The **graph of extended shapes** $G(\hat{S}_1, \ldots, \hat{S}_k)$ is defined by:

$$(V, E, \hat{E}) := (V_n, \tilde{E} := \cup_{i=1}^{k} (E(S_i) \cup \hat{E}(S_i)), \gamma)$$

Extended shapes



Realizing Extended Shapes

For the sake of convenience we define the term *realizing an extended shape* at first for a binary alphabet.

Definition

A binary string $s = s_1 \ldots s_n \in \{0,1\}^n$ realizes an *extended shape* $\hat{S}$ of size $n \iff$

1. for all regular edges $\{v_i, v_j\} \in E(S)$ holds: $s_i \neq s_j$, i.e. $s_i s_j \in \mathscr{B} := \{01, 10\}$.
2. for all pseudo edges $\{v_i, v_j\} \in \hat{E}(S)$ holds: $s_i = s_j$, i.e. $s_i s_j \in \mathscr{B}_{PSE} := \{00, 11\}$.

Realizing Extended Shapes

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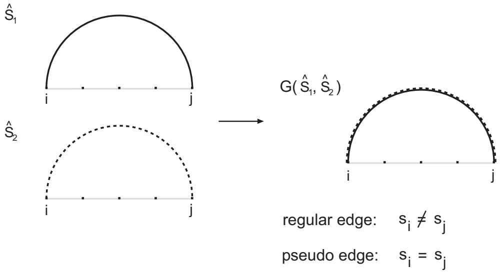


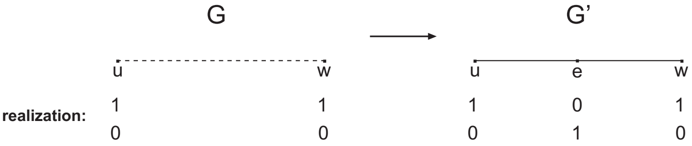
- Intersection Theorem isn't valid for extended shapes.

The same question, another answer

What properties have to be fulfilled in the graph of extended shapes $G(\hat{S}_1, \ldots, \hat{S}_k)$, s.t. there exists a single binary string, which is realizing all these extended shapes $\hat{S}_1, \ldots, \hat{S}_k$

New Results

Let $G(\hat{S}_1, \hat{S}_2)$ be the graph of two extended shapes. Furthermore let G' be the graph, that emerges from $G(\hat{S}_1, \hat{S}_2)$ by replacing all pseudo edges $e = \{u, w\}$ with two new regular edges $\{u, e\}, \{e, w\}$ with a new vertex e not contained in V_n, i.e. deg(e)=2.



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Lemma

Two arbitrary extended shapes $\hat{S}_1, \hat{S}_2$ of size n can be realized by a single binary string $\Longleftrightarrow$ The graph G' is bipartite.

New Results

Theorem

Any extended shapes $\hat{S}_1, \ldots, \hat{S}_k$ of size n can be realized by a single binary string $\Leftrightarrow$

1. $E(S_l) \cap \hat{E}(S_m) = \emptyset$ *mit* $l \neq m \in \{1, \ldots, k\}$, *i.e. there are no overlays and*
2. *the graph* $G(\hat{S}_1, \ldots, \hat{S}_k)$ *does not contain cycles with an odd number of regular edges.*

New Results

Similar conditions to the RNA alphabet:

- for all regular edges $\{v_i, v_j\} \in E(S)$ holds: $s_i s_j \in \{AU, UA, CG, GC, GU, UG\}$
- for all pseudo edges $\{v_i, v_j\} \in \hat{E}(S)$ holds: $s_i = s_j$, i.e. $s_i s_j \in \{AA, UU, GG, CC\}$

New Results

Theorem

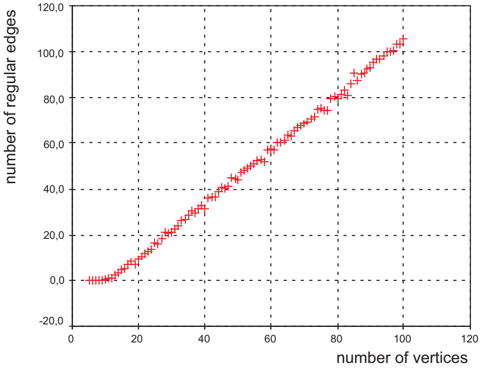
Any extended shapes $\hat{S}_1, \ldots, \hat{S}_k$ of size n can be realized by a single string $s \in \{A, C, G, U\}^n$ with the conditions above $\Leftrightarrow$ These extended shapes $\hat{S}_1, \ldots, \hat{S}_k$ of size n can be realized by a single binary string

New Results

- If we have found sequences, that realize extended shapes,
- then we have found sequences, that realize the corresponding shapes,
- BUT also conserve the structure even better, in the case that unpaired positions are considered.

Empirical Results

$G(S_1, S_2, S_3, S_4)$



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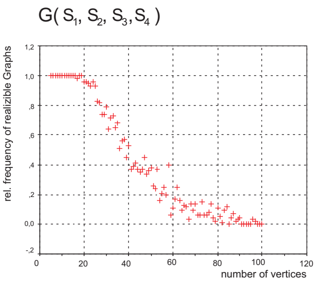
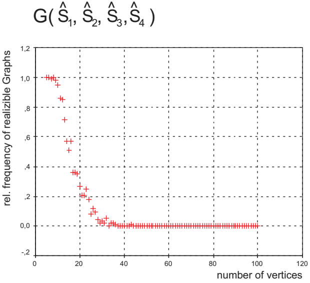
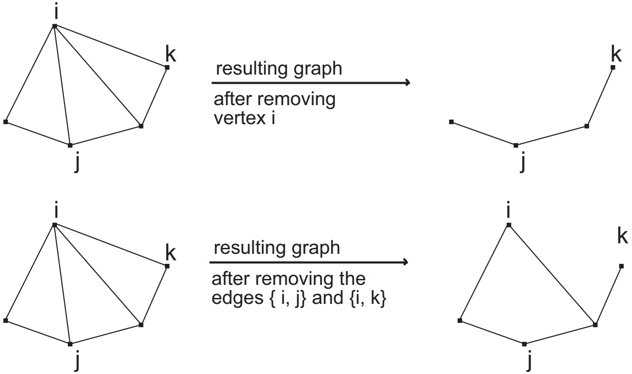




Problems


i
k
j
resulting graph
after removing vertex i
k
j
i
k
j
resulting graph
after removing the edges { i, j} and {i, k}
i
k
j

Let $S_1, \ldots, S_k$ and $\hat{S}_1, \ldots, \hat{S}_k$ be any shapes or extended shapes respectively of the same size n.

Problem

Compute the minimum number of

1. VERTICES, *that must be removed from the graph $G(S_1, \ldots, S_k)$, s.t. $S_1, \ldots, S_k$ are realizable by a single string.*
2. EDGES, *that must be removed from the graph $G(S_1, \ldots, S_k)$, s.t. $S_1, \ldots, S_k$ are realizable by a single string.*
3. VERTICES, *that must be removed from the graph $G(\hat{S}_1, \ldots, \hat{S}_k)$, s.t. $\hat{S}_1, \ldots, \hat{S}_k$ are realizable by a single string.*
4. REGULAR AND PSEUDO EDGES, *that have to be removed from $G(\hat{S}_1, \ldots, \hat{S}_k)$, s.t. $\hat{S}_1, \ldots, \hat{S}_k$ are realizable by a single string.*

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Conclusion

1. we started with "normal" shapes
2. aim: finding sequences, that realizes shapes
3. we extended the shapes by inserting **pseudo edges**
4. aim: finding sequences, that realizes **extended shapes**.
5. There are NP-complete problems, arising from the term *realizing shapes and extended shapes*.

Thank you for your attention.