Variations on RNA folding: Locally stable structures and RNA hybridization

Defense Talk

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Outline

1. Introduction
   RNA structure
   RNA structure prediction

2. Bimolecular Secondary structures of RNA molecules
   Introduction
   Implementation
   Application

3. Local partition function
   RNAplfold
   Results
   Accessibility
   Results
RNA biology

Biological functions of RNA

- tRNA, mRNA, rRNA
- maturation: RNAse P, snoRNAs
- guide RNAs for editing
- spliceosomal RNAs
- functional motifs in mRNA
- Signal recognition particle
- miRNA, siRNA, piRNA
- T-Box RNA
- vault RNAs
- telomerase RNAs
- RNAse MRP

Ribosome
Levels of RNA structure description
Primary, secondary and tertiary structure
RNA secondary structure

Definition

List of base pairs $i, j$ such that:
- only GC, AU or GU base pair
- only one base-pair per base
- minimum distance 3
- base pairs do not cross
RNA secondary structure

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RNA secondary structure
Visualization
RNA secondary structure
Energy - Loop decomposition
RNA secondary structure
Energy - Loop decomposition

Hairpin-, Interior- and Multiloops
RNA secondary structure
Energy - Loop decomposition

Every Loop type is assigned an energy
- experimentally derived
- Energies are relative to open chain
- Energies are additive, independent

Hairpin-, Interior- and Multiloops
RNA secondary structure
Mfe structure prediction

- Structure of minimal energy for given sequence
- Loop energies are independent, additive
- Base pairs divide structure into inner and outer part
- Dynamic Programming
Dissect a problem into small, easy to solve sub-problems, solutions of sub-problems are tabulated

- Start with small sub-sequence
- Add bases one by one
- each base can either be unpaired or paired
Basic RNA Folding

\[ F_{ij} = \min \{ F_{i+1,j}, \min_k C_{i,k} + F_{k+1,j} \} \]
Basic RNA Folding

$$F_{ij} = \min \{ F_{i+1,j}, \min_k C_{i,k} + F_{k+1,j} \}$$
Basic RNA Folding

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F_{ij} = \min \{ F_{i+1,j}, \min_k C_{i,k} + F_{k+1,j} \}
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Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]

\[ C_{i,j} = \text{hairpin} \]

\[ M_{i,j} = \text{interior} \]

\[ M^l_{i,j} = \]

\[ C_{i,j} = \min\{H(i,j), \min_{k,l} I(ij, kl)C_{k,l}, \min_u M(ij)M_{i+1,u}M^l_{u+1,j-1} \} \]
Basic RNA Folding

\[ F_{ij} = F_{i+1,j} \]

\[ C_{ij} = F_{i,k} F_{k+1,j} \]

\[ F_{ij} = \text{hairpin}_{ij} \]

\[ M_{ij} = \text{interior}_{i,j} \]

\[ C_{ij} = M_{i,j} \]

\[ M_{il} = M_{il} \]

\[ C_{i,j} = \min \{ \mathcal{H}(i,j), \min_{k,l} \mathcal{I}(ij,kl) \mathcal{C}_{k,l}, \min_{u} \mathcal{M}(ij) M_{i+1,u} M_{u+1,j-1} \} \]
Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]

\[ F_{i,j} = C_{i,k}F_{k,j}F_{i,k+1} \]

\[ C_{i,j} = \text{hairpin} \]

\[ C_{i,j} = \text{interior} \]

\[ C_{i,j} = M_{i+1,j} \]

\[ C_{i,j} = M_{j-1,j} \]

\[ M^l_{i,j} = M^l_{i,j} \]

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Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]

\[ C_{i,k,k+1,j} = \text{hairpin} \]

\[ M_{i,j} = \text{interior} \]

\[ M^l_{i,j} = \text{M}^l_{i,j-1} \]

\[ M_{i,j} = \min\{\min_u (u - i + 1)a + C_{u+1,j}, \min_u M_{i,u} + C_{u+1,j}, M_{i,j-1}a\} \]
Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]

\[ C_{i,j} = \text{hairpin} \]

\[ M_{i,j} = \text{interior} \]

\[ M^l_{i,j} = \text{hairpin} \]

\[ M_{i,j} = \min\{\min_u (u - i + 1) \alpha + C_{u+1,j}, \min_u M_{i,u} + C_{u+1,j}, M_{i,j-1} + \alpha\} \]
Basic RNA Folding

\[ F_{ij} = F_{i+1,j} \]

\[ C_{ij} = \text{hairpin} \]

\[ M_{ii} = M_{i,j+1} \]

\[ M_{ij} = \min \{ \min_u (u - i + 1)a + C_{u+1,j}, \min_u M_{i,u} + C_{u+1,j}, M_{i,j-1} + a \} \]
Basic RNA Folding

\[ F_{i \rightarrow j} = F_{i+1 \rightarrow j} + C_{i \rightarrow j} \]

\[ C_{i \rightarrow j} = \text{hairpin}_{i \rightarrow j} + \text{interior}_{i \rightarrow j} \]

\[ M_{i \rightarrow j} = M_{i \rightarrow u+1} \]

\[ M^1_{i \rightarrow j} = M^1_{i \rightarrow j-1} + C_{i \rightarrow j} \]

\[ M^1(i, j) = \min \{ M^1(i, j-1), a, C(i, j) \} \]
Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]  
\[ C_{i,j} = \text{hairpin} \]  
\[ M_{i,j} = \text{interior} \]  
\[ M^l_{i,j} = M^l_{i,j-1} \]

\[ M^1(i, j) = \min M^1(i, j - 1) a, C(i, j) \]
RNA secondary structure
Partition function

- Stacking energies in same range as thermal energy
- Probability of structure $s \propto e^{-\frac{E_s}{RT}}$
- $Q = \sum_s e^{-\frac{E_s}{RT}}$
- Probability of structure $s = e^{-\frac{E_s}{RT}} / Q$
- Any probability of a structural feature can be computed
- E.g. probability to get certain base pairs
- Use mfe decomposition, min $\rightarrow \sum$, $+ \rightarrow *$
RNA secondary structure

Partition function

\[
Q(i, j) = Q(i + 1, j) + \sum_{i < k \leq j} Q^B(i, k)Q(k + 1, j)
\]

\[
Q^B(i, j) = H(i, j) + \sum_{i < k < l < j} I(ij; kl)Q^B(k, l) + \sum_{k} M(i + 1, k)M^1(k + 1, j - 1)
\]

\[
M(i, j) = M(i + 1, j) + \sum_{i < k < j} Q^B(k, j) + \sum_{i < k < j} M(i, k - 1)Q^B(k, j)
\]

\[
M^1(i, j) = M^1(i, j - 1) + Q^B(i, j)
\]
Outline

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   RNA structure
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2. Bimolecular Secondary structures of RNA molecules
   Introduction
   Implementation
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3. Local partition function
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RNA RNA interaction as highly selective targeting
Examples:
  • miRNA/siRNA – mRNA interaction
  • snoRNA/rRNA interaction
  • Hfq dependent regulation in prokaryotes, e.g. RyhB, OxyS
  • Many ncRNAs of unknown function
Bimolecular secondary structures of RNA Molecules

Recursions

Computation of joint partition function:

- Concatenate Sequences
- Keep track of concatenation point \((c_p)\)
- Treat loops containing \(c_p\) as exterior loops
- Add duplex initiation penalty
- Penalty added in post-processing step
Bimolecular secondary structures of RNA Molecules

Recursions

Computation of joint partition function:

- Concatenate Sequences
- Keep track of concatenation point ($c_p$)
- Treat loops containing $c_p$ as exterior loops
- Add duplex initiation penalty
- Penalty added in post-processing step
Bimolecular secondary structures of RNA Molecules

Recursions

Basic RNA Folding

\[ F_{i,j} = F_{i+1,j} \]

\[ C_{i,j} = \text{hairpin} \]

\[ M_{i,j} = \text{interior} \]

\[ M^l_{i,j} = \text{loop} \]
Bimolecular secondary structures of RNA Molecules

Recursions

RNA Cofolding

\[ F_{ij} = F_{i+1j} \]

\[ C_{ij} = F_{ij} \]

\[ M_{ij} = C_{ij} \]

\[ M^l_{ij} = M^l_{ij} \]
Two RNA molecules $A, B$ with concentration $[A]_0$ and $[B]_0$, resp. give rise to 5 species:

\[
\begin{align*}
A + B & \xrightleftharpoons{K_{AB}} AB \\
A + A & \xrightleftharpoons{K_{AA}} AA \\
B + B & \xrightleftharpoons{K_{BB}} BB
\end{align*}
\]

Equilibrium constants computed out of partition functions:

\[
\begin{align*}
K_{AB} &= \frac{Q_{AB}}{Q_A Q_B} \\
K_{AA} &= \frac{Q_{AA}}{Q_A Q_A} \\
K_{BB} &= \frac{Q_{BB}}{Q_B Q_B}
\end{align*}
\]
Bimolecular secondary structures of RNA Molecules
Concentration Dependency

- Use Mass conservation and equilibrium constants
- Generate two quadratic equations in two variables
- Use Newton’s iteration method to solve system

\[
0 = f([A], [B]) := [A] + K_{AB}[A][B] + 2K_{AA}[A][A] - [A]_0
\]
\[
0 = g([A], [B]) := [B] + K_{AB}[A][B] + 2K_{BB}[B][B] - [B]_0
\]
Case study: role of GU base pairs and miR efficacy

Difference dot plots
Case study: Role of GU base pairs and miR efficacy

Doench and Sharp

- Investigated effects of mutations on miRNA efficacy
- Introducing GU base pairs reduced efficacy
- Loss of miRNA function not due to difference in binding energy
- Explained effect as due to GU base pairs unfavorable for siRNA function

Case study: Role of GU base pairs and miR efficacy
Doench and Sharp
Case study: Role of GU base pairs and miR efficacy

Doench and Sharp
Case study: Role of GU base pairs and miR efficacy
Doench and Sharp

![Graph showing the relationship between miRNA 5' region ΔG and fold repression. The graph includes sequences such as AGCUAGCA, AGCUGACA, AGUAAACA, and AGUUGGCA at their respective positions.]
Case study: Role of GU base pairs and miR efficacy
Doench and Sharp

[Graph showing the relationship between miRNA 5' region ΔG and fold repression, with specific sequences for mRNA and siRNA highlighted.]
Case study: Role of GU base pairs and miR efficacy

Brennecke et. al.

Brennecke et. al.
Difference dot plot

<table>
<thead>
<tr>
<th></th>
<th>0 GU</th>
<th>1 GU</th>
<th>2 GU</th>
<th>3 GU</th>
</tr>
</thead>
<tbody>
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<td>A</td>
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<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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Bimolecular secondary structures of RNA Molecules

Conclusion

- Sophisticated thermodynamical analysis in agreement with experimental data
- Can not explain reduced efficacy of all mutated binding sites
- No need to treat GU base pairs differently
- mRNA structure and therefore target site accessibility is important
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Local partition function

Motivation

- RNA folding is $O(n^3)$
- Huge amount of data
- Faster analysis tools
- Boundaries of transcripts often unknown
- Substructures of large Molecules (IRES, SECIS, ...)
- Prediction of long range base pairs bad anyway
Local partition function

Motivation

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Local partition function
sliding window approach

- Compute $Q$ and pair probabilities for a stretch of length $L$, starting at $u$
- Increment $u$, compute partition function, continue, until end of molecule
- We compute all possible windows (i.e. increment 1)
- Reusing entries computed earlier, complexity is $O(nL^2)$ instead of $O(nL^3)$
What is the pair probability?
Local Pair probability is the mean probability over all possible windows a pair can be in:

\[ \pi^L(i, j) = \frac{1}{1 - (j - i) + 1} \sum_{u=j-L}^{i} p^{u,L}(i, j) \]

Computing all \( p^{u,L}(i, j) \) is \( \mathcal{O}(nL^3) \), but we can derive a recursion for the averages directly:
Local partition function
Pair probabilities

- \( i, j \) not enclosed by a base pair
- Within interior loop
- Within multi loop

\[
\pi^L(i, j) = \sum_{u=j-L}^{i} \frac{Q(u, i-1)Q^B(i, j)Q(j+1, u+L)}{Q^{u,L}(u, u+L)} \\
+ \sum_{u\leq k<i; j<l\leq u+L} \pi^L(k, l) \frac{I(kl, ij)}{Q^B(k, l)} \\
+ \sum_{u\leq k<i; j<l\leq u+L} \pi^L(k, l) \frac{M(k+1, i-1) + M(j+1, l-1)}{Q^B(k, l)} \\
+ \sum_{u\leq k<i; j<l\leq u+L} \pi^L(k, l) \frac{M(k+1, i-1)M(j+1, l-1)}{Q^B(k, l)}
\]
green: annotated in RFam (human), red: unannotated in homologue (dog)
Predicting Accessibility

- Compute probability of a stretch of bases to be unpaired
- Equivalent to compute energy necessary to open a binding site
- Target site accessibility
- Local Version of RNAup
Predicting Accessibility

Hairpin:

Interior loop:

Multi loop:
Accessibility
Performance in separating non-working from working siRNAs

40 30 0

8.384e−06 7.162e−12 7.04e−13 3.285e−13 9.764e−11

4 4 8 8 12 12 16 16 19 19
I want to thank
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Consensus structures

- Often conservation on structural but not on sequence level
- Mutations can retain base pairs (consistent, compensatory)
- Predict structure of an alignment of RNA molecules
- Compute mean energy, add conservation score
- RNAalifold
Modifications of RNAalifold

- Result can be biased
  - Include sequence weighting
  - Gaps are scored like bases
  - Use energy evaluation dependent on sequences
Modifications of RNAalifold

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Modifications of RNAalifold

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Implementation

\[ Q(i, j) = \sum_{s \in \text{Sequences}} Q(i, j, s) \]

Energy evaluation:
Implementation

\[ Q(i, j) = \sum_{s \in \text{Sequences}} w(s) Q(i, j, s) \]

Energy evaluation:
Implementation

\[ Q(i, j) = \sum_{s \in \text{Sequences}} Q(i, j, s) \]

Energy evaluation:

```plaintext
sequence_2: AGCGUUCUUGCGC--GUGUUUUUCGCUUGCU 30
sequence_3: AGCGUUCUUGCGC--GU--UUUUGCCGCUUGCU 28
sequence_1: AGCGUUCUUGCGAUCG--GU--UUUUGCCGCUUGCU 32

old: ((((((....((((....))))....)))))).... -5.95
new: (((((....((((((..((....))))))..)))))) -5.83
```
Implementation

\[ Q(i, j) = \sum_{s \in \text{Sequences}} Q(i, j, s) \]

Energy evaluation:
- Use length in sequence, not in alignment
- Use next position in sequence, not in alignment (for gaps)
- Translational arrays, arrays with 3’, 5’ neighbors
blue, yellow: both wrong, red: both right, green: new right
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