Free Energy Minimization

Idea:

- Overcome the main drawback of Nussinov's algorithm: non-realism of base pair maximization!
- Define an energy model for RNA that can be parameterized by experimentally measured energies
- Devise an algorithm that minimizes the free energy of RNA according to this model
- Algorithm (by Zuker) will be similar to Nussinov's algorithm

Gibbs Free Energy

Definition (Gibbs Free Energy)

The *Gibbs Free Energy G* of a system (e.g. dilution of RNAs) is

$$G = H - TS$$

where H is the enthalpy (potential to perform work), T the absolute temperature and S the entropy (measure of disorder).

Remarks:

- For RNA, we will compute the free energy of (a certain amount $N_A \approx 6 \cdot 10^{23}$ of molecules, a "mol") of a certain structure P. More precisely, we compute the *change of free energy* ΔE due to folding into P from $P_{\text{unfolded}} = \{\}$.
- The (change of) Gibbs free energy corresponding to P can be computed by summing free energy contributions from single "structural elements".
- Those contributions (for loops, stacks, ...) can be measured experimentally (Turner). They consist of enthalpic and entropic terms. Due to the latter, they depend on temperature.

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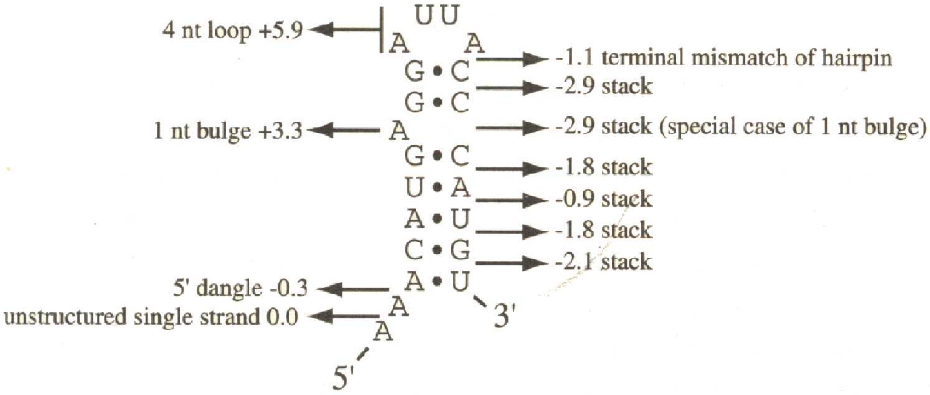
Free Energy — Example



overall $\Delta G = -4.6$ kcal/mol

Free Energy Model of RNA — Definitions

Definition (Secondary structure elements/Loops)

Let S RNA sequence of length n, P RNA structure of S. Call $1 \leq i \leq n$ *unpaired in* P, iff there is no j, s.t. $(i,j) \in P$ or $(j,i) \in P$.

- $(i,j) \in P$ *closes a* **hairpin loop** iff all $k : i < k < j$ unpaired in P
- $(i,j) \in P$ *closes a* **stacking loop** iff $(i+1, j-1) \in P$
- $(i,j) \in P$ *and* $(i',j') \in P$ *form an* **internal loop** (i,j,i',j') iff
    - $i < i' < j' < j$
    - (i,j) does not close a stacking loop
    - all $i+1, \ldots, i'-1$ and $j'+1, \ldots, j-1$ unpaired in P

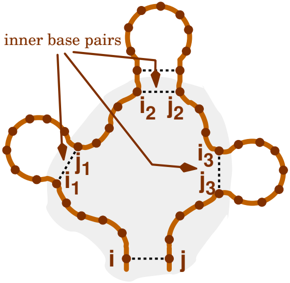
Free Energy Model of RNA — Definitions, ctd.

- An internal loop (i, j, i', j') is called **left** *(***right***)* **bulge**, iff $j = j' + 1$ ($i' = i + 1$), respectively.
- A ***k*-multiloop** consists of k base pairs $(i_1, j_1) \dots (i_k, j_k) \in P$ and a closing base pair $(i, j) \in P$ with the property that
    - $i < i_1 < j_1 < i_2 < j_2 < \dots < i_k < j_k < j$
    - $i + 1 \dots i_1 - 1; j_1 + 1 \dots i_2 - 1; \quad \dots \quad ; j_{k-1} + 1 \dots i_k - 1; j_k + 1 \dots j - 1$ unpaired in P

  $(i_1, j_1) \dots (i_k, j_k)$ close the **inner base pairs** *of the multiloop*.

Remarks

- *k*-**multiloop**



- Usually hairpin loops have minimal loop size of $m = 3$
  $\Rightarrow$ for all $(i, j) \in P$: $i < j - 3$.
- each secondary structure element is defined uniquely by its closing basepair
- for any basepair (i, j) we denote the corresponding secondary structure element with $Sec(i, j)$.

Energy of Secondary Structure Elements

Definition (Energy contribution of loops)

Energy contributions of the various structure elements:

- **hairpin loop** (i,j): $\mathrm{eH}(i,j)$
- **stacking** (i,j): $\mathrm{eS}(i,j)$
- **internal loop** (i,j,i,j'): $\mathrm{eL}(i,j,i',j')$
- **multiloop**: $\mathrm{eM}(i,j,i_1,j_1,\ldots,i_k,j_k)$

Remark

General multi loop contribution will be too expensive in prediction: exponential explosion!
$\Rightarrow$ Use a simplified contribution scheme.

Definition (Simplified energy contribution of multiloops)

- **multiloop** $\mathrm{eM}(i,j,k,k') = a + bk + ck'$

  $a, b, c =$ weights, $a =$ energy contribution for closing of loop
  $k =$ number of inner base pairs
  $k' =$ number of unpaired bases within loop

Loop Energy and Free Energy of an RNA

Definition (Free Energy of an RNA)

Given an RNA structure P of an RNA sequence S.

loop free energy: $E_{ij}^P :=$ energy contribution of $Sec(i,j)$

total free energy: $E(P) := \sum_{(i,j)\in P} E_{ij}^P$

Remark

more precisely we could write $E_S(P)$, since energy of P also depends on S

$\rightarrow$ we assume S is fix

Problem of Free Energy Minimization

Definition (RNA Structure Prediction by Energy Minimization)

- IN: RNA sequence S
- OUT: non-crossing RNA structure P of S, such that

$$E(P) = \min_{P' \text{ non-crossing RNA structure of } S} E(P')$$

Zuker's Algorithm for RNA Energy Minimization

Remarks

- Plan: the Zuker-Algorithm will be specified by defining matrix entries and giving recursion equations. Analogously to Nussinov, those recursions can be evaluated effictiently by DP. The optimal structure is obtained by Traceback.
- Do we need a *completely* new algorithm?

Definition (W-matrix)

For an RNA sequence S, define the Zuker-matrix W as a matrix of entries W_{ij} for $1 \leq i \leq j \leq n$ by

$$W_{ij} := \min\{E(P) \mid P \text{ non-crossing RNA } ij\text{-substructure of } S\}.$$

Remark

$E(P)$ can be used to evaluate a ij-substructure P, since P is still an RNA structure. Tacitely, we assume that sequence outside of base pairs does not contribute to the energy.

Zuker Recursion, Take 1

Initialisation: (for $j - i \leq m$)

$$W_{ij} = 0$$

Recursion: (for $i < j - m$)

$$W_{ij} = \min \begin{cases} W_{ij-1} & \text{— } j \textit{ unpaired} \\ \min_{i \leq k < j-m} W_{ik-1} + W_{k+1j-1} + E(???) & \text{— } j \textit{ paired} \end{cases}$$

Zuker Recursion: W-Recursion and V-matrix

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Definition (V-matrix)

For an RNA sequence S, define the Zuker-matrix V as a matrix of entries V_{ij} for $1 \leq i \leq j \leq n$ by

$$V_{ij} := \min \left\{ E(P) \;\middle|\; \begin{array}{l} P \text{ non-crossing RNA } ij\text{-substructure of } S, \\ \text{where } (i,j) \in P \end{array} \right\}.$$

"minimal energy of any closed ij-substructure of S"

V-Recursion, Take 1

Initialization: (for $j - i \leq m$)

$$V_{ij} = \infty$$

Recursion: (for $i < j - m$)

$$V_{ij} =$$

$$\min \begin{cases} \mathrm{eH}(i,j) & \text{— } \textit{hairpin loop} \\ V_{i+1,j-1} + \mathrm{eS}(i,j) & \text{— } \textit{stacking loop} \\ \min_{i<i'<j'<j} V_{i',j'} + \mathrm{eL}(i,j,i',j') & \text{— } \textit{interior loop/bulge} \\ \min_{k,i<i_1<j_1<\dots<i_k<j_k<j} \mathrm{eM}(i,j,i_1,j_1,\dots,j_k,j_k) & \text{— } \textit{multi-loop} \\ \qquad + \sum_{1\leq k'\leq k} V_{i_{k'}j_{k'}} & \end{cases}$$

Remarks

- V-recursion for *general* multi-loop energy
- complexity: multi-loop case exponential
- now: optimize using simplified multi-loop energy

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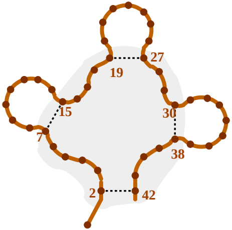
Simplified Multi-loop Energy — Example

- In general: multi-loop energy depends on everything: inner base pairs $(i_1, j_1) \dots (i_k, j_k)$, closing base pair (i, j), and sequence.
- Simplification: dependency only on number of inner base pairs k and number of unpaired bases k'.
- Example:



general: eM$(2, 42, 7, 15, 19, 27, 30, 38)$
simplified: eM$(2, 42, k, k') = a + bk + ck'$, where
$k = 3$: inner base pairs within loop
$k' = 12$: unpaired bases within multi-loop

- We will use: New multi-loop energy is additive

Efficient V-Recursion and WM-matrix

Initialization: (for $j - i \leq m$) $V_{ij} = \infty$ "as before"
Recursion: (for $i < j - m$)

$$V_{ij} = \min \begin{cases} \mathrm{eH}(i,j) & \text{— \textit{hairpin loop}} \\ V_{i+1,j-1} + \mathrm{eS}(i,j) & \text{— \textit{stacking loop}} \\ \min_{i<i'<j'<j} V_{i',j'} + \mathrm{eL}(i,j,i',j') & \text{— \textit{interior loop/bulge}} \\ \min_{i<k<j} WM_{i+1k} + WM_{k+1j-1} + a & \text{— \textit{multi-loop}} \end{cases}$$

Definition (WM-matrix)

For an RNA sequence S, the Zuker-matrix WM has entries WM_{ij} for $1 \leq i \leq j \leq n$:

$$WM_{ij} := \min \left\{ E^m_{ij}(P) \;\middle|\; \begin{array}{l} P \text{ non-crossing RNA } ij\text{-substructure of } S, \\ P \text{ not empty} \end{array} \right\},$$

where E^m_{ij} evaluates P as part of a multi-loop (i.e. including energy contributions b,c due to inner base pairs, unpaired bases).

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where E^m_{ij} evaluates P as part of a multi-loop (i.e. including energy contributions b,c due to inner base pairs, unpaired bases).

Remarks to Definition of WM-matrix

we defined:

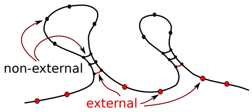
"$WM_{ij} := \min\{E^m_{ij}(P) \mid P$ RNA ij-substructure of S, P not empty$\}$, where E^m_{ij} evaluates P as part of a multi-loop"

Remarks

- "P not empty" ensures that the multi-loop case in the V-recursion cannot recurse to non-multiloops
- "$E^m_{ij}(P)$ evaluates P as part of a multi-loop" means that E^m_{ij} adds to $E(P)$ contributions c for unpaired bases (here we need i and j) and contributions b for inner base pairs of this part of a complete multi-loop. Define

$$E^m_{ij}(P) := E(P) + kb + k'c,$$

where k is the number of *external* base pairs and k' the number of *external* unpaired bases in P.



WM-Recursion

Initialization: (for $j - i \leq m$)

$$WM_{ij} = \infty \quad (ij\text{-substructure } P \text{ non-empty!})$$

Recursion: (for $i < j - m$)

$$WM_{ij} = \min \begin{cases} WM_{ij-1} + c & \text{— } \textit{j unpaired} \\ WM_{i+1j} + c & \text{— } \textit{i unpaired} \\ V_{ij} + b & \text{— } \textit{closed} \\ \min_{i<k<j} WM_{ik} + WM_{k+1j} & \text{— } \textit{non-closed} \end{cases}$$

Remark

decomposition complete — cases not distinct (which is ok for minimization!)

Zuker-Algorithm: Summary

- 3 matrices:
  W — minimal energy of general substructure $i \dots j$
  V — minimal energy of closed substructure $i \dots j$
  WM — minimal energy of true part of a multi-loop $i \dots j$
- recursions equations

$$W_{ij} = \min \begin{cases} W_{ij-1} \\ \min_{i \le k < j-m} W_{ik-1} + V_{kj} \end{cases}$$

$$V_{ij} = \min \begin{cases} \mathrm{eH}(i,j),\ V_{i+1,j-1} + \mathrm{eS}(i,j) \\ \min_{i<i'<j'<j} V_{i',j'} + \mathrm{eL}(i,j,i',j') \\ \min_{i<k<j} WM_{i+1k} + WM_{k+1j-1} + a \end{cases}$$

$$WM_{ij} = \min \begin{cases} WM_{ij-1} + c,\ WM_{i+1j} + c,\ V_{ij} + b \\ \min_{i<k<j} WM_{ik} + WM_{k+1j} \end{cases}$$

immediate complexity: $O(n^4)$ time, $O(n^2)$ space

Complexity Revisited

$O(n^2)$ matrix entries
Multi-loop branching: “only” $O(n)$
Interior loop: $O(n^2)$ **limiting!**
Trick: reduce complexity of limiting case.
simplest: bound maximal interior loop size (e.g. 30)

Theorem. (Zuker)
Given an RNA sequence S, Zuker's algorithm predicts the non-crossing, minimal energy structure P of S in $O(n^3)$ **time and** $O(n^2)$ **space.**

Remarks

- Minimal free energy in W_{1n}
- We assume traceback is done analogously to Nussinov-Traceback. Same reduced complexity. Only extension: trace through three matrices, i.e. keep track of matrix.

Implementations

- Michael Zuker's `Mfold` / `Unafold`
- Ivo Hofacker's Vienna RNA Package: `RNAfold`
- David Mathew's `RNAstructure`
- Example:

```
ivo@tbi: $ RNAfold

Input string (upper or lower case); @ to quit
....,....1....,....2....,....3....,....4....,....5....,....6....,....7....,
GGGGGUAUAGCUCAGGGGUAGAGCAUUUGACUGCAGAUCAAGAGGUCCCUGGUUCAAAUCCAGGUGCCCCCU
length = 72
GGGGGUAUAGCUCAGGGGUAGAGCAUUUGACUGCAGAUCAAGAGGUCCCUGGUUCAAAUCCAGGUGCCCCCU
(((((((.((((.......)))).(((((((..((((....))))..))).)))))).....))))))).
minimum free energy = -26.70 kcal/mol
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additionally: produces file rna.ps

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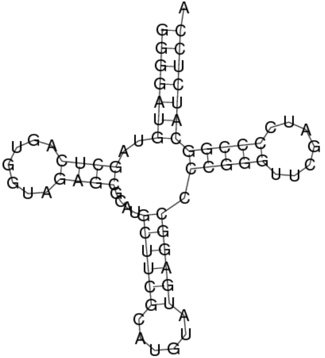
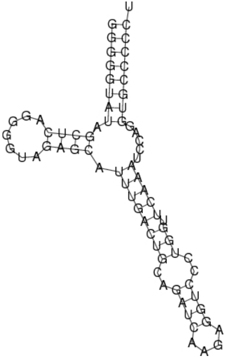
Example: tRNAs

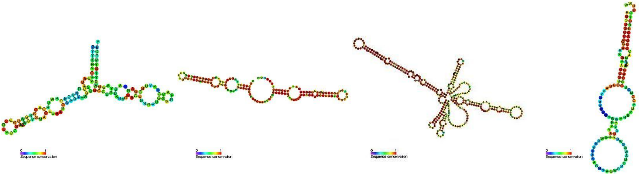
- Mouse tRNA-ALA:



- Mouse tRNA-CYS:



- A biologist finds new RNA (i.e. usually only RNA sequence!)
  - get (first idea of) structure by using RNAfold
  - see whether similarities to known structures exist. Can we guess the RNA family by characteristic shape?



5S rRNA H/ACA snoRNA 7SK Y RNA

recommended: browse Rfam, e.g.
`http://rfam.sanger.ac.uk/family/browse/top20`

- Biologist has several RNAs. Are they similar by structure?
- We have a sequence: could it be structural RNA?