Combinatorial Optimization in Bioinformatics

RNA Structure Prediction—Part II

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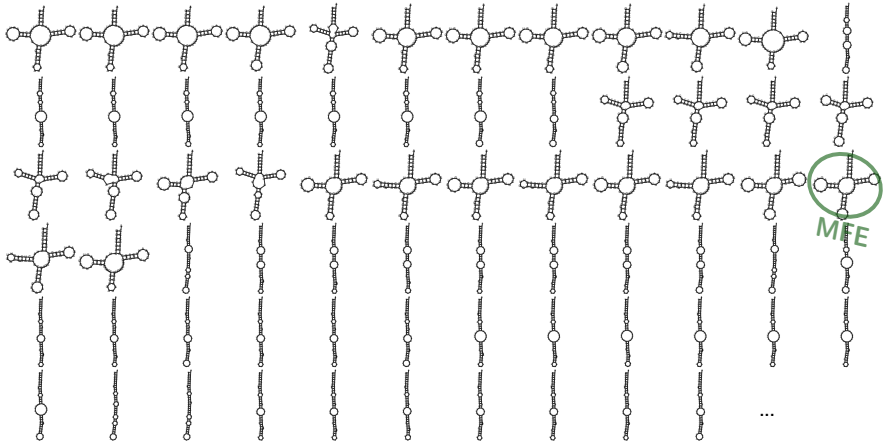
Beyond predicting single RNA structures

- so far: optimization over all (non-crossing) RNA structures
  - from simple to more complex objective functions
  - (further) bioinformatics example of optimization by DP: "optimal substructure", recursive decompsosition
  - first look beyond: relevance of ambiguity, counting
- today: beyond finding the optimal solution—more power of DP
  - **statistical mechanics**: energies correspond to probabilities
  - here: directly **probabilities of structures** in thermodynamic equilibrium, but technique is generally useful to assign probabilities to combinatoric objects.
  - calculate partition functions by DP—**sum over all structures** (in place of optimize)
  - inside + outside algorithm—**probability of features**
  - structure prediction from **multiple sequences**, **comparative analysis**

RNA structure ensembles, Probabilities

GGGCUAUUAGCUCAGUUGGUUAGAGCGCACCCCUGAUAAGGGUGAGGUCGCUGAUUCGAAUUCAGCAUAGCCCA



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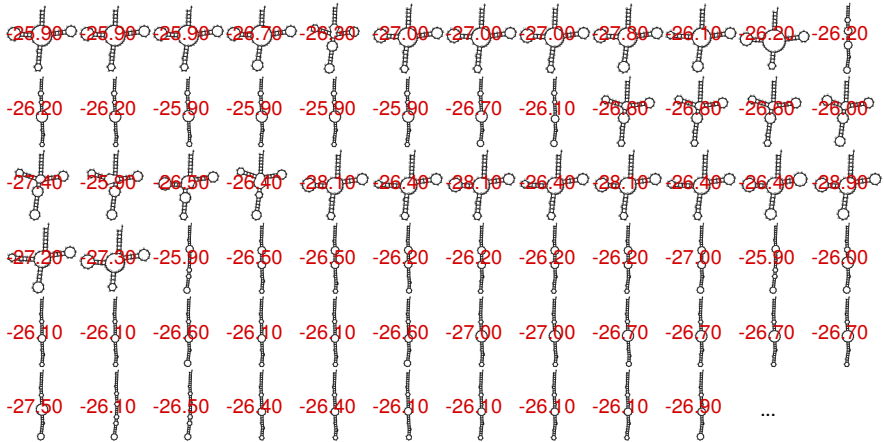
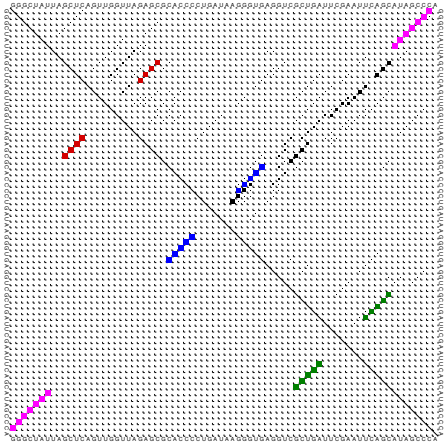
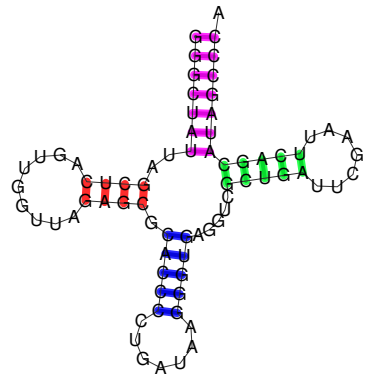


Energies → Structure Probabilities

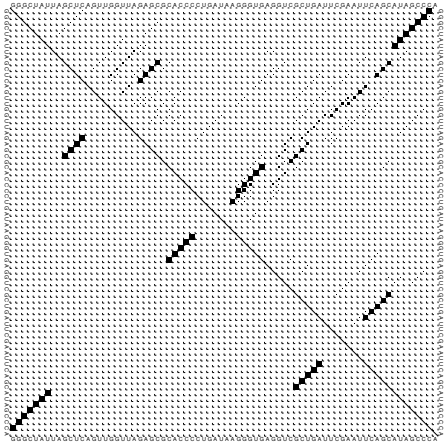
Structure Probabilities → Base Pair Probabilities

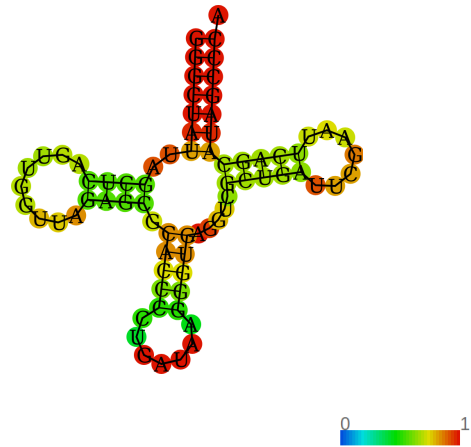
Dotplots and Reliabilities





Dotplots and Reliabilities





Thermodynamic Equilibrium / Statistical Mechanics

We assume that the probability of each possible structure P (in thermodynamic equilibrium) is proportional to its Boltzmann weight ($\implies$ **Boltzmann distribution**).

$$Pr(P) \propto exp(-\frac{E(P)}{RT})$$

(R = gas constant; T = temperature in Kelvin).

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Reason to assume **Boltzmann distribution**:

- Formally justified as distribution with maximal entropy in a closed physical system (with constant average energy).
- Boltzmann distributions models the distribution of free, dynamically folding structures after infinite time (and without additional forces).

Normalization: Partition function

$$Pr(P) \propto exp(-\frac{E(P)}{RT})$$

Normalize Boltzmann weights by Z:

$$Pr(P) = exp(-\frac{E(P)}{RT})/Z, \text{ such that}$$

$$\sum_{P} Pr(P) = 1$$

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Z is called partition function.

Efficient computation?

- weighted sum has similarities to counting (DP!)
- weights depend on free energy (Zuker-like DP?!)

Recall: Counting of Structures

$$N_{i,j} = \max \begin{cases} N_{i+1,j} \\ \max\limits_{\substack{i<k\leq j \\ S[i],S[j] compl.}} 1 + N_{i+1,k-1} + N_{k+1,j} \end{cases}$$

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$$\Downarrow$$

$$C_{i,j} = C_{i+1,j} + \sum_{\substack{i<k\leq j \\ S[i],S[j]\,compl.}} 1 \cdot C_{i+1,k-1} \cdot C_{k+1,j}$$

Exchange of operators: $\max \to \sum/+$, $\sum/+ \to \prod/\cdot$

From counting to partition function

$$C_{i,j} = C_{i+1,j} + \sum_{\substack{i<k\leq j \\ S[i],S[j] compl.}} 1 \cdot C_{i+1,k-1} \cdot C_{k+1,j}$$

$$\Longrightarrow Z_{i,j} = Z_{i+1,j} + \sum_{\substack{i<k\leq j \\ S[i],S[j] compl.}} \exp\left(\frac{-E_{bp}(i,k)}{RT}\right) \cdot Z_{i+1,k-1} \cdot Z_{k+1,j}$$

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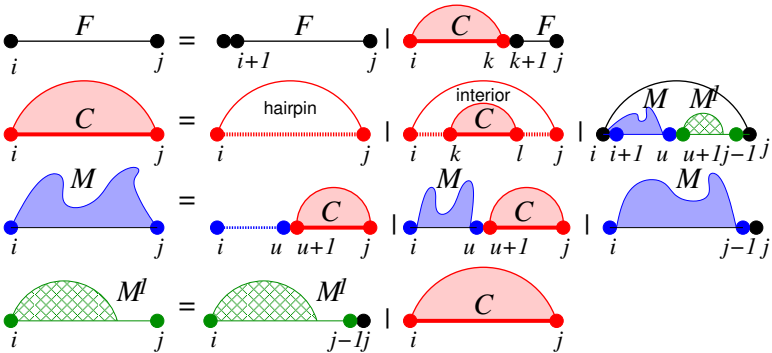
Validity?

$$\exp(\frac{-E_{bp}(i,k)}{RT}) \cdot Z_{i+1,k-1} \cdot Z_{k+1,j} = \exp(\frac{-E_{bp}(i,k)}{RT}) \cdot \sum_x \exp(\frac{-E(x)}{RT}) \cdot \sum_y \exp(\frac{-E(y)}{RT})$$

$$= \sum_{x,y} \exp(\frac{-E_{bp}(i,k)}{RT}) \cdot \exp(\frac{-E(x)}{RT}) \cdot \exp(\frac{-E(y)}{RT})$$

$$= \sum_{x,y} \exp(\frac{-(E_{bp}(i,k) + E(x) + E(y)}{RT}$$

Free energy minimization ('Zuker' algorithm)



Initialization: $F_{ii} = 0$; $C_{ii} = M_{ii} = M^1_{ii} = \infty$

Recursions: $F_{ij} = \min \left\{ F_{i+1,j}, \min\limits_{i<k\leq j} C_{ik} + F_{k+1,j} \right\}$

$$C_{ij} = \min \left\{ H(i,j), \min_{i<k<l<j} C_{kl} + I(i,j;k,l), \min_{i<u<j} M_{i+1,u} + M^1_{u+1,j-1} + a \right\}$$

$$M_{ij} = \min \left\{ \min_{i<u<j} (u-i+1)c + C_{u+i,j} + b, \min_{i<u<j} M_{i,u} + C_{u+1,j} + b, M_{i,j-1} + c \right\}$$

$$M^1_{ij} = \min \left\{ M_{i,j-1} + c, C_{ij} + b \right\}$$

Efficient computation of partition functions

Efficient computation: partition function algorithm (McCaskill, 1990) **can be inferred from the MFE algorithm** by *replacing minimum operations with sums and additions with multiplications*.

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$$
\begin{aligned}
Z_{i,j} &= Z_{i+1,j} + \sum_{i<k\leq j} Z^C_{i,k} Z_{k+1,j} \\
Z^C_{i,j} &= e^{-\beta H(i,j)} + \sum_{i<k<l<j} Z^C_{k,l} e^{-\beta I(i,j,k,l)} + \sum_{i<u<j} Z^M_{i+1,u} Z^{M1}_{u+1,j-1} e^{-\beta a} \\
Z^M_{i,j} &= \sum_{i<u<j} e^{-\beta(u-i+1)c} Z^M_{u+1,j} + \sum_{i<u<j} Z^M_{i,u} Z^C_{u+1,j} e^{-\beta b} + Z^M_{i,j-1} e^{-\beta c} \\
Z^{M1}_{i,j} &= Z^{M1}_{i,j-1} e^{-\beta c} + Z^C_{i,j} e^{-\beta b}
\end{aligned}
$$

(Un)-Ambiguity

Partition function by operator exchange “trick” would not work on ambigous recursions. Why?

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prerequisite, violated by ambiguous decomposition

$$\text{traceback} : \text{structure} = 1 : 1$$

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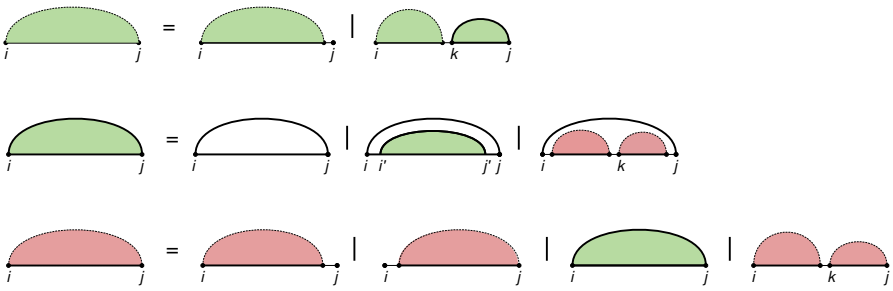
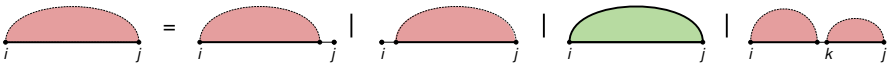
$$\text{traceback} : \text{structure} = 1 : 1$$

Challenge of ambiguity in (original) Zuker-recursions: split of multiloops

Decomposition of Structure Space by (original) Zuker



Ambiguity Example



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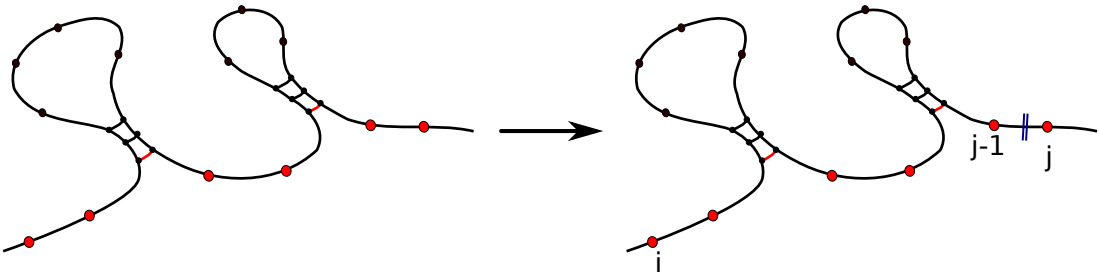

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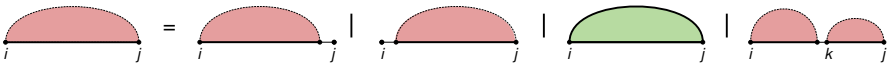

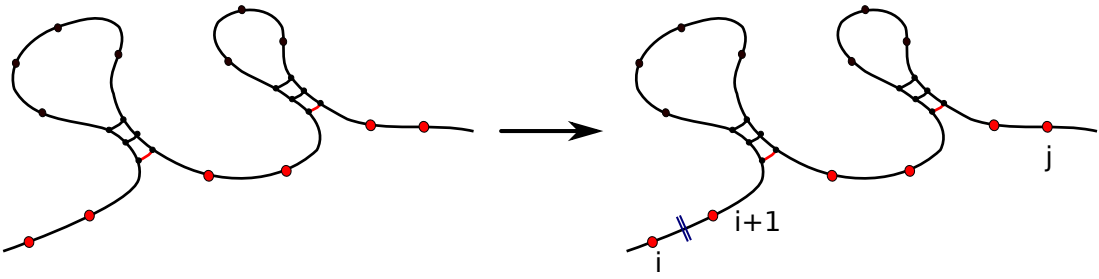

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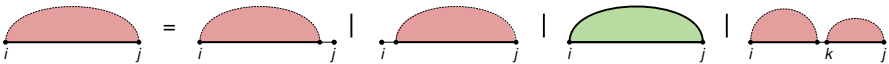

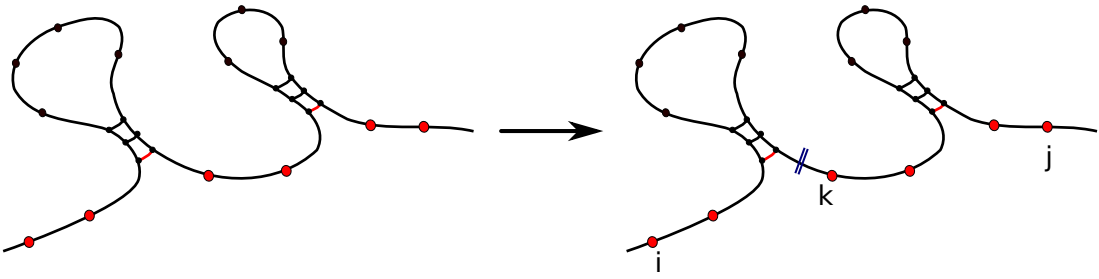

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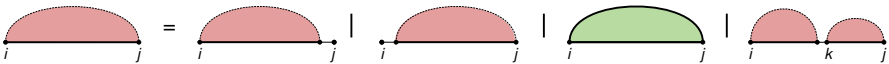

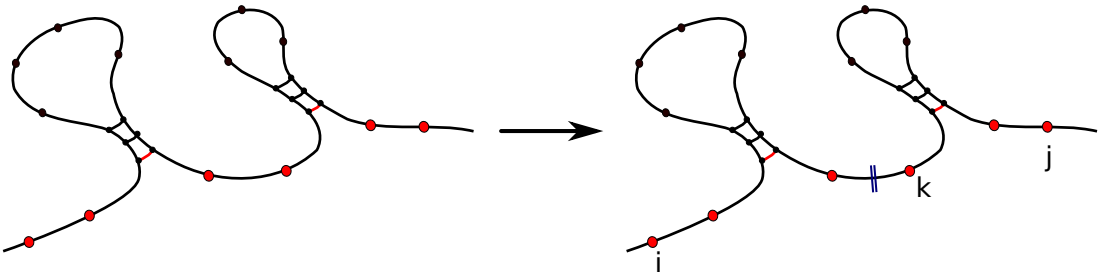

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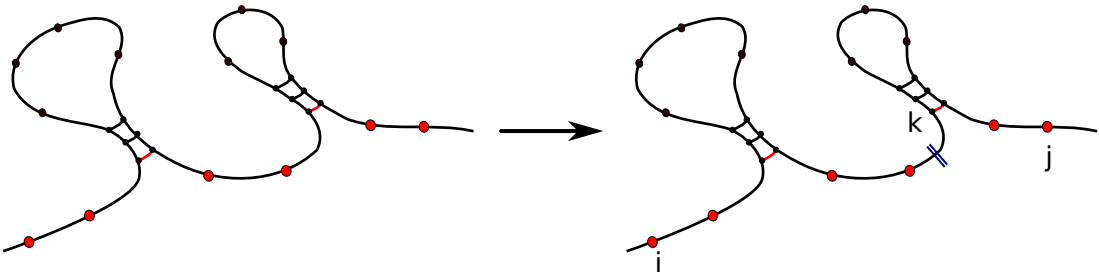

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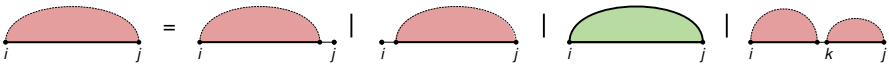
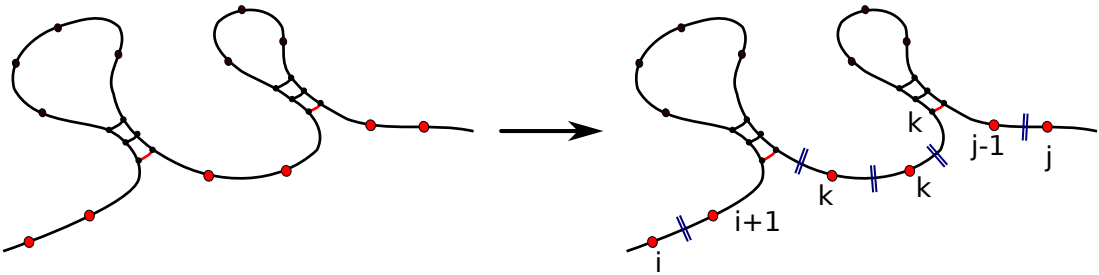
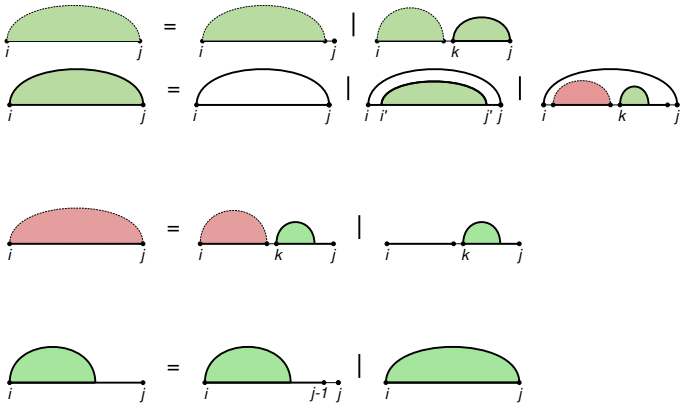




Discarding Ambiguity



Disambiguation due to “fourth” matrix.

Structure and base pair probabilities

The **probability of a specific structure** is given by:

$$Pr(P) = \frac{exp(-\frac{E(P)}{RT})}{Z}$$

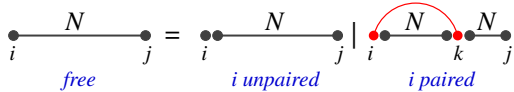
The **probability of a base pair** (i, j) is the fraction of all structures having this base pair and the number of all possible structures.

$$p_{ij} = \sum_{P \ni (i,j)} Pr(P)$$

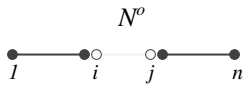
This can be computed efficiently from the product of the inside and *outside* partition functions of a base pair:

$$p_{ij} = \frac{Z_{\texttt{inside}(i,j)} \cdot Z_{\texttt{outside}(i,j)}}{Z}$$

Outside algorithms

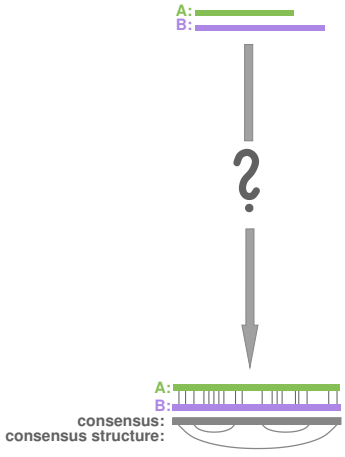


$$N_{i,j} = \max \begin{cases} N_{i-1,j} \\ \max_{i \le k < j} 1 + N_{i+1,k-1} + 1 + N_{k+1,j} : S_i \text{ and } S_k \text{ compl.} \end{cases}$$



$$N^o_{i,j} = \max \begin{cases} N^o_{i-1,j} & i-1 \text{ unpaired} \\ \max\limits_{S_k,\ S_{i-1} \text{ compl.}}^{1 \le k < i} N^o_{k,j} + 1 + N_{k+1,i-2} & i-1 \text{ paired left} \\ \max\limits_{S_{i-1},\ S_k \text{ compl.}}^{j \le k < n} N^o_{i-1,k} + 1 + N_{j,k-1} & i-1 \text{ paired right} \end{cases}$$

Comparative RNA Analysis



adopted from:
[Gardner & Giegerich BMC 2004]

Typical Scenario

Given: set of related RNA sequences

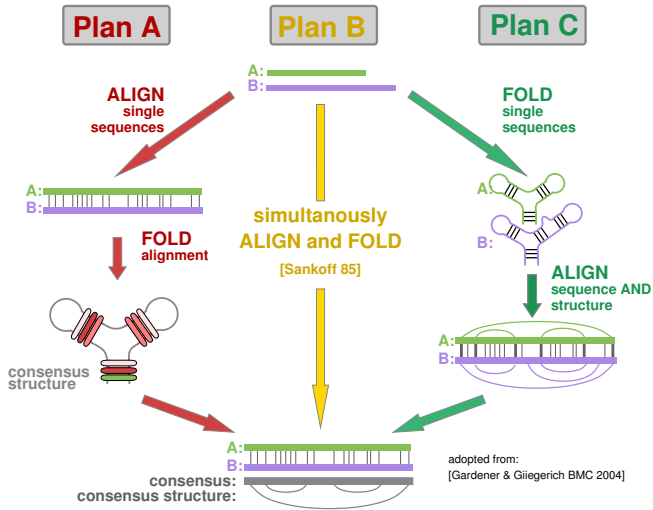
```
>AF008220
GGAGGAUUAGCUCAGCUGGGAGAGCAUCUGCCUUACAAGCAGAGGGUCGGCGGUUCGAGCCCGUCAUCCUCCA
>M68929
GCGGAUAUAACUUAGGGGUUAAAGUUGCAGAUUGUGGCUCUGAAAACACGGGUUCGAAUCCCGUUAUUCGCC
>X02172
GCCUUUAUAGCUUAGUGGUAAAGCGAUAAACUGAAGAUUUAUUUACAUGUAGUUCGAUUCUCAUUAAGGGCA
>Z11880
GCCUUCCUAGCUCAGUGGUAGAGCGCACGGCUUUUAACCGUGUGGUCGUGGGUUCGAUCCCCACGGAAGGCG
>D10744
GGAAAAUUGAUCAUCGGCAAGAUAAGUUAUUUACUAAAUAAUAGGAUUUAAUAACCUGGUGAGUUCGAAUCUCACAUUUUCCG
```

Wanted:

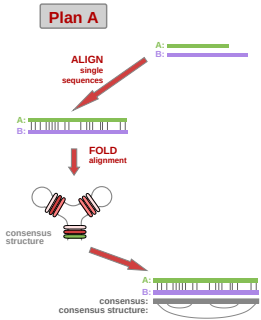
```
AF008220  GGAGGAUU-AGCUCAGCUGGGAGAGCAUCUGCCUUACAAGC---------AGAGGGUCGGCGGUUCGAGCCCGUCAUCCUCCA
M68929    GCGGAUAU-AACUUAGGGGUUAAAGUUGCAGAUUGUGGCUC---------UGAAAA-CACGGGUUCGAAUCCCGUUAUUCGCC
X02172    GCCUUUAU-AGCUUAG-UGGUAAAGCGAUAAACUGAAGAUU---------UAUUUACAUGUAGUUCGAUUCUCAUUAAGGGCA
Z11880    GCCUUCCU-AGCUCAG-UGGUAGAGCGCACGGCUUUUAACC---------GUGUGGUCGUGGGUUCGAUCCCCACGGAAGGCG
D10744    GGAAAAUUGAUCAUCGGCAAGAUAAGUUAUUUACUAAAUAAUAGGAUUUAAUAACCUGGUGAGUUCGAAUCUCACAUUUUCCG

consens   (((((((...((((........))))((((((.......)).........))))....(((((.......)))))))))))).
```

- Typically, only sequences are known, not their structures
- Evolutionary conserved for many RNAs: sequence and structure

RNA Alignment Approaches



Comparative RNA Analysis: Plan A

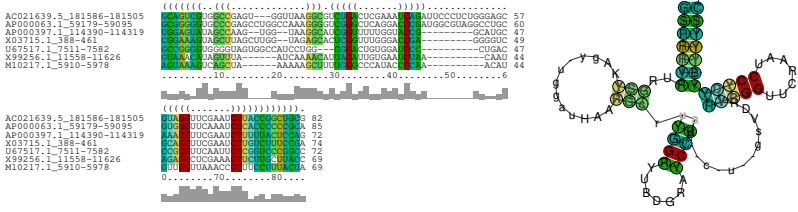


Remarks

- ALIGN: multiple sequence alignment
  (recall Needleman-Wunsch, progressive alignment)
- FOLD: generalize prediction for single sequences

Predict (optimal) consensus structure of an RNA alignment

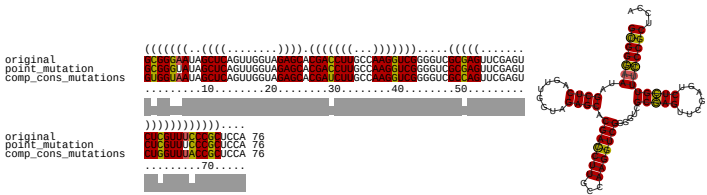
```
CLUSTAL W --- LocARNA 2.0.0RC7


AC021639.5_181586-181505 GCAGUCGUGGCCGAGU---GGUUAAGGCGUCUGACUCGAAAUCAGAUUCCCUCUGGGAGCGUAGGUUCGAAUCCUACCGGCUGCG
AP000063.1_59179-59095   GCGGGGGUGCCCGAGCCUGGCCAAAGGGGUCGGGCUCAGGACCCGAUGGCGUAGGCCUGCGUGGGUUCAAAUCCCACCCCCCGCA
AP000397.1_114390-114319 UGGAGUAUAGCCAAG--UGG--UAAGGCAUCGGUUUUUGGUACCG---------GCAUGCAAAGGUUCGAAUCCUUUUACUCCAG
X03715.1_388-461         CGGAAAGUAGCUUAGCUUGG--UAGAGCACUCCGGUUUUGGGACCGA---------GGGGUCGCAGGUUCGAAUCCUGUCUUUCCGA
U67517.1_7511-7582       GCCGGGGUGGGGUAGUGGCCAUCCUGG---GGGACUGUGGAUCCC----------CUGACCCGGGUUCAAUUCCCGGUCCCGGCC
X99256.1_11558-11626     GUAAACAUAGUUUA------AUCAAAACAUUAGAUUGUGAAUCUAA----------CAAUAGAGGCUCGAAACCUCUUGCUUACC
M10217.1_5910-5978       AGUAAAGUCAGCUA------AAAAAGCUUUUGGGCCCAUACCCCAA----------ACAUGUUGGUUAAACCCCUUCCUUUACUA
```



RNAalifold

IN: RNA alignment

OUT: **Consensus structure of aligned RNAs**

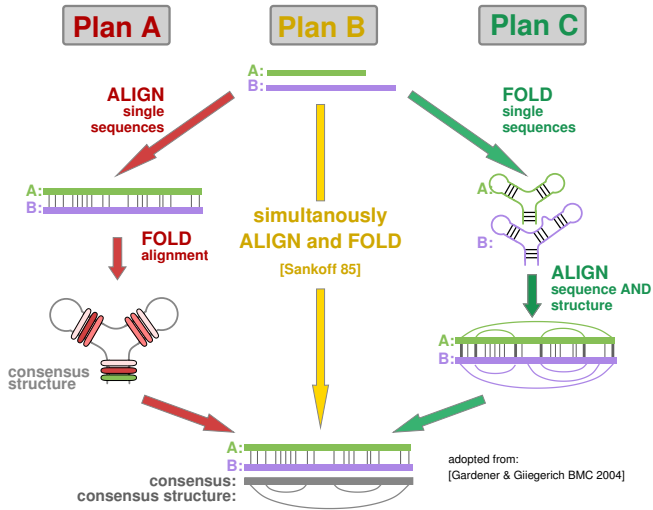


Optimizes **free energy** + **conservation score**

conservation score = compensatory and consistent mutations

- alignment as sequence of alignment columns. Folding of this sequence is analogous to folding of an RNA sequence, 'base pairs' are formed between alignment columns
- Thus, same decomposition as Zuker; but modified scoring: sum of loop energies over all sequences & add conservation score

RNA Alignment Approaches

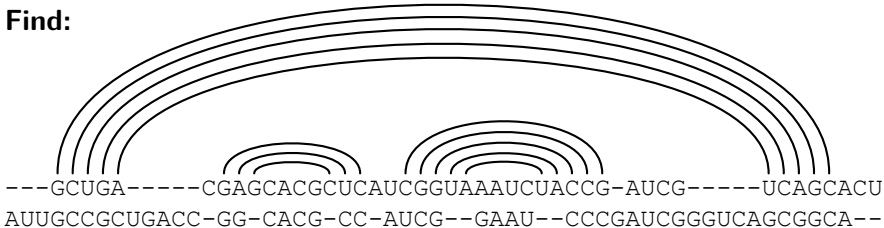


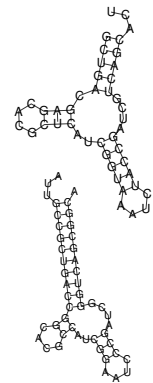
Simultaneous Alignment and Folding [Sankoff]

Given: A = GCUGACGAGCACGCUCAUCGGUAAAUCUACCGAUCGUCAGCACU

& B = AUUGCCGCUGACCGGCACGCCAUCGGAAUCCCGAUCGGGUCAGCGGCA

Find:





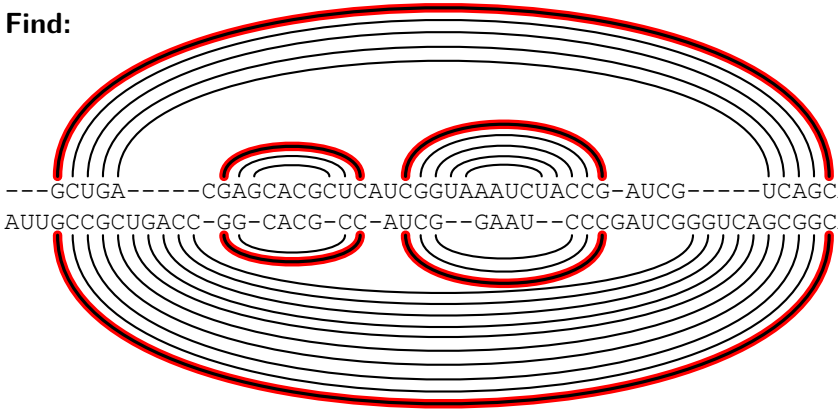
edit distance + energy A + energy B → opt

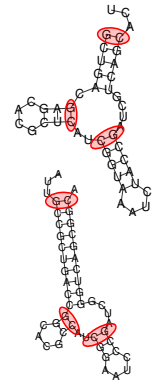
Simultaneous Alignment and Folding [Sankoff]

Given: A = GCUGACGAGCACGCUCAUCGGUAAAUCUACCGAUCGUCAGCACU

& B = AUUGCCGCUGACCGGCACGCCAUCGGAAUCCCGAUCGGGUCAGCGGCA

Find:





edit distance + energy A + energy B → opt

Sankoff Problem Definition

- Idea: Sankoff = Zuker Folding $\times$ Needleman/Wunsch Alignment

- IN: two sequences a and b
- find two **equivalent** structures P_a and P_b
  and **compatible** alignment A of a and b
  such that $Energy(a, P_a) + Energy(b, P_b) + EditDistance(A)$ minimal

- where: *Energy* yields (loop-based) Turner free energy,
  EditDistance is edit distance (base mismatch x, indel y)

- what means *compatible*
  A must align all base pairs $(i_f, i_g) \in P_a$ and $(j_f, j_g) \in P_b$ that enclose branches
  (red base pairs of previous slide)

Constraints

Summarizing, we need to find the optimal structures + alignment with the following constraints:

constraints on the predicted structures:

- must be equivalent (same shape)

constraints on the alignment:

- multiloops must be aligned to their equivalent partner
- hairpin loops must be aligned to their equivalent partner
- each 2-loop (includes stacking and bulge) must be aligned to exactly one other 2-loop or must be aligned to gaps entirely.

Edit distance of sub-sequences

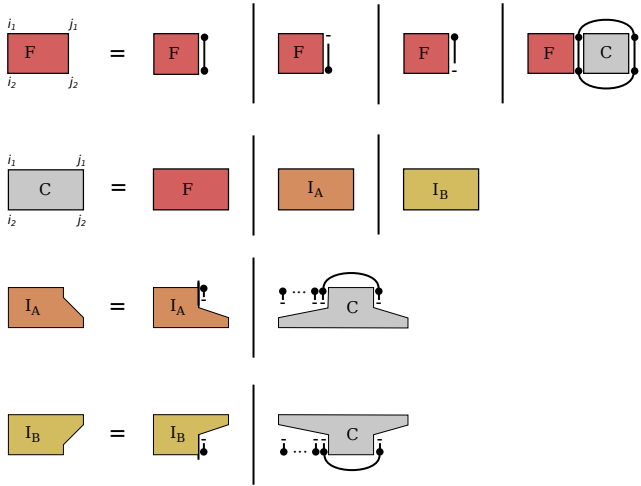
- distance based score
  - x = base mismatch
  - y = base deletion/insertion
- $D(i_1, j_1; i_2, j_2)$ minimum sequence alignment cost between sequences $a_{i_1} \dots a_{j_1}$ and $b_{i_2} \dots b_{j_2}$.
- Recursion: $D(i_1, j_1; i_2, j_2) = \min \begin{cases} D(i_1, j_1 - 1; i_2, j_2 - 1) + \begin{cases} x & \text{if } a_{j_1} \neq b_{j_2} \\ 0 & \text{otherwise} \end{cases} \\ D(i_1, j_1 - 1; i_2, j_2) + y \\ D(i_1, j_1; i_2, j_2 - 1) + y \end{cases}$
- Initialization: $D(i_1, i_1; i_2, i_2) = \begin{cases} x & \text{if } a_{i_1} \neq b_{i_2} \\ 0 & \text{else} \end{cases}$

Simplified Sankoff (without full energy model)

We define the following functions (which will be evaluated recursively):

- $F(i_1, j_1, i_2, j_2)$ best alignment between subsequences $a_{i_1} \dots a_{j_1}$ and $b_{i_2} \dots b_{j_2}$.
- $C(i_1, j_1, i_2, j_2)$ best "closed"alignment between the same subsequences, where we predict the base pairs (i_1, j_1) and (i_2, j_2).
- $I_A(i_1, j_1, i_2, j_2)$ alignment of the same subsequences, which deletes j_1
- $I_B(i_1, j_1, i_2, j_2)$ symmetrically (...which inserts j_2)

Simplified Sankoff (without full energy model)



Sankoff's extreme complexity

space complexity $O(n^4)$

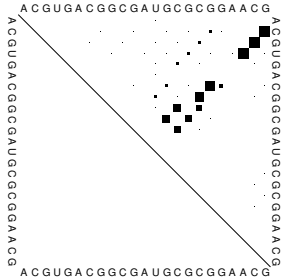
- constant number of matrices
- each of them has $O(n^4)$ entries

time complexity $O(n^6)$

- each entry of matrix D requires constant time
- each entry of F,C, and G requires $O(n^2)$ time (minimize over all h_1, h_2)
- hence: $n^4 \cdot n^2 = n^6$

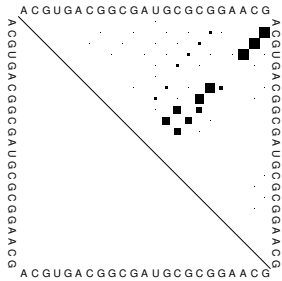
Ensemble-based sparsification (LocARNA)

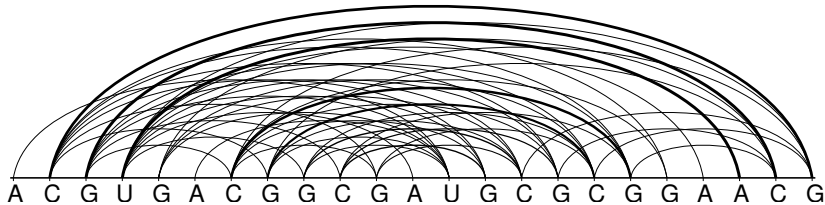
- **Sparsify structure ensemble**



Ensemble-based sparsification (LocARNA)

- **Sparsify structure ensemble**

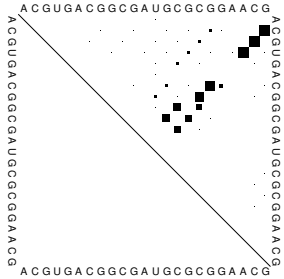


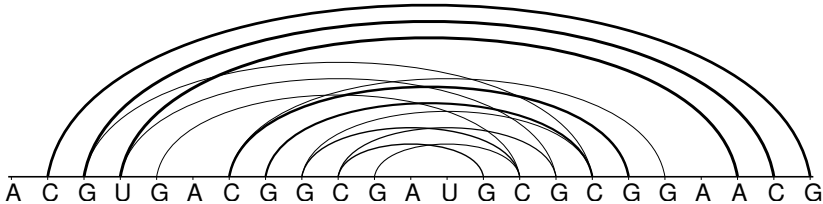


all base pairs

Ensemble-based sparsification (LocARNA)

- **Sparsify structure ensemble**

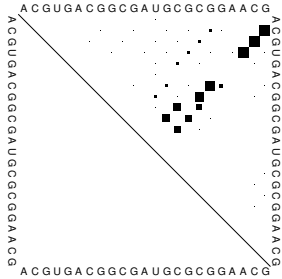


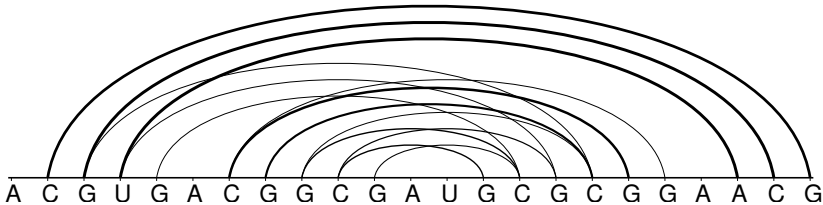


only probable base pairs

Ensemble-based sparsification (LocARNA)

- **Sparsify structure ensemble**





only probable base pairs

- **immediate time improvement** by $O(n^2)$
- **space improvement** by $O(n^2)$ requires rearrangement of recursion evaluation

Rfam / Infernal

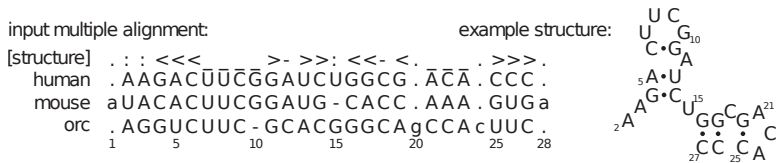
- Infernal: scan genomic data for RNA family members

  Inference of RNA alignments
- important tool for Rfam

  Rfam 10.1 (June 2011, 1973 families)

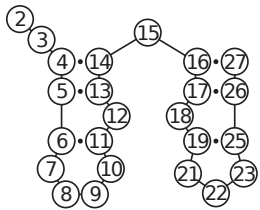
  `http://rfam.sanger.ac.uk/`
- in Rfam: 'hand-curated' seed alignments $\Rightarrow$ full alignments
- use Stochastic Context Free Grammars to model RNA families
- model of a family: *Consensus Model (CM)*



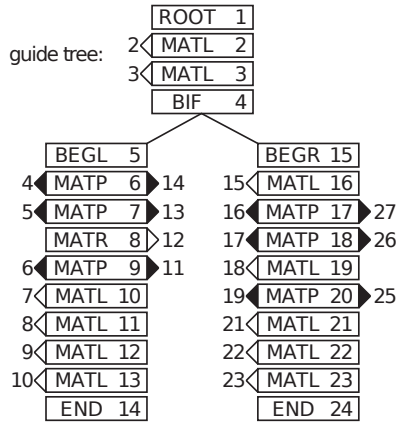
Infernal

Construct grammatical description

consensus structure:



guide tree:



Infernal

- Construct CM from guide tree
- Expand nodes of guide tree:
  Add match, insertion, and deletion states
- learn transition and output probabilities from alignment
- CM comparable to profile HMM for protein families (Pfam)