M2 BIM – STRUCT - Lecture 2

Boltzmann equilibrium and beyond

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Outline

Physics-based structure prediction
- Turner energy model
- MFold/Unafold

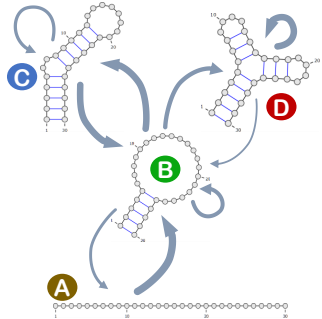
Boltzmann ensemble
- Nussinov: Minimisation $\Rightarrow$ Counting
- Computing the partition function
- Statistical sampling

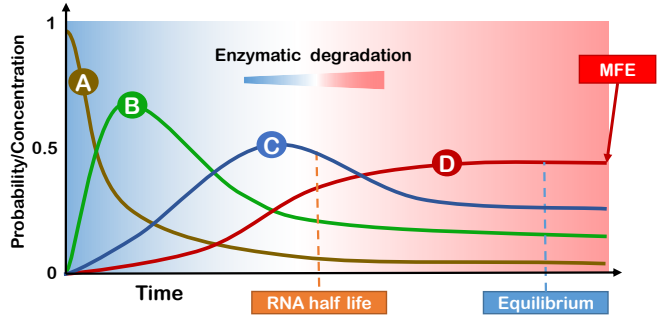
Performances
- Overall picture
- Family-level evaluation
- Evaluation issues

The specific case and issues of ML
- Deep learning: Beauty and the beast
- ML performances as advertised by authors
- Surprising limitations
- Takeaways

Extended Alorithmics/DP techniques
- Suboptimal structures
- Pseudoknots

Paradigms in RNA structural bioinformatics





A – Kinetic Landscape

Continuous-time Markov chain

B – Evolution of concentrations

Given free-energy $E : \{A, C, G, U\}^\star \times \mathcal{S} \to \mathbb{R}$, at the Boltzmann equilibrium:

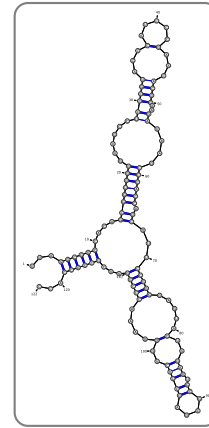
$$\mathbb{P}(S \mid w) \propto e^{-E(w,S)/RT}$$

- Minimum Free-Energy (MFE): Relevant structure = Most stable/probable
- Partition function: Equilibrium properties of Boltzmann ensemble
- Kinetics: Finite-time evolution of concentrations/probabilities

Turner energy model

Based on unambiguous decomposition of 2^{ary} structure into loops:

- Internal loops
- Bulges
- Terminal loops
- Multi loops
- Stackings



Free-energy Δ G of a loop depend on bases, assymmetry, dangles ...

Experimentally determined
+ Interpolated for larger loops.

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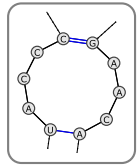

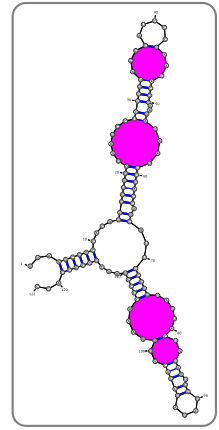

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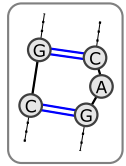

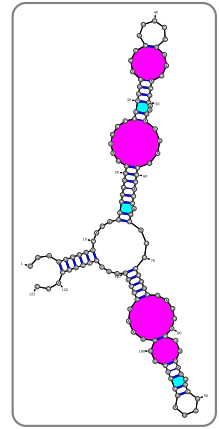

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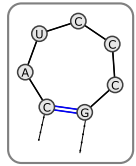

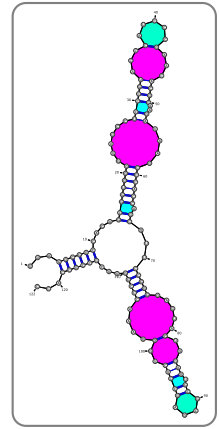

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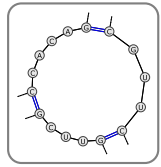

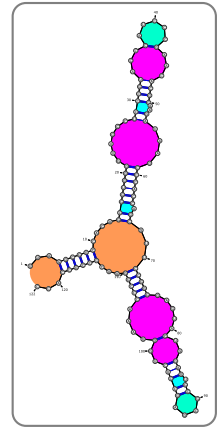

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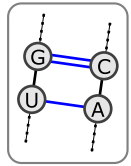

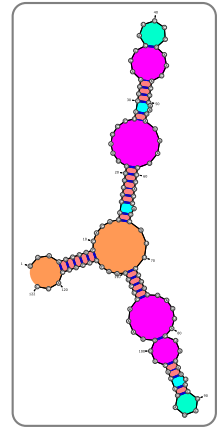

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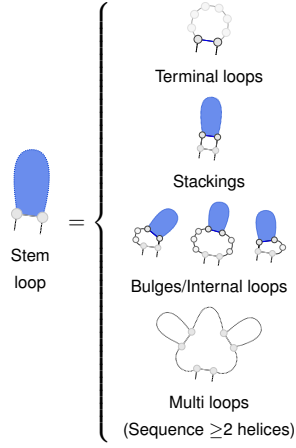
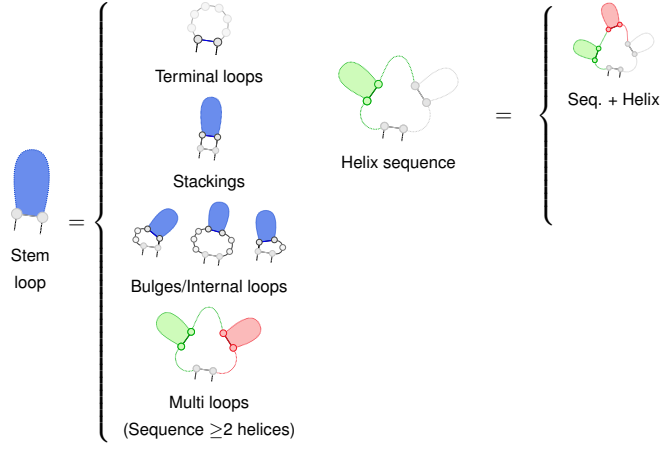
MFE DP equations



MFE DP equations



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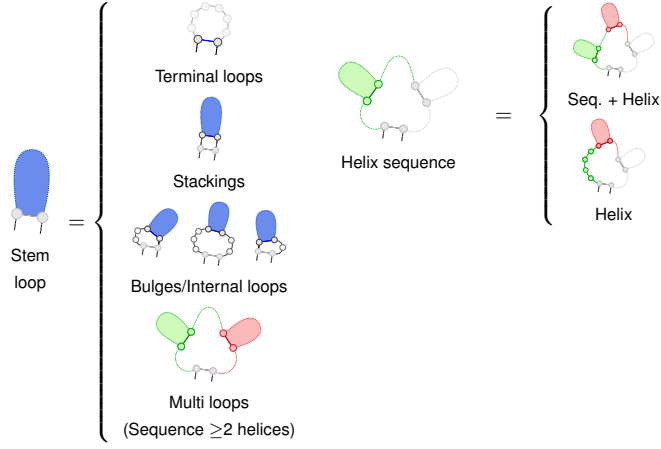

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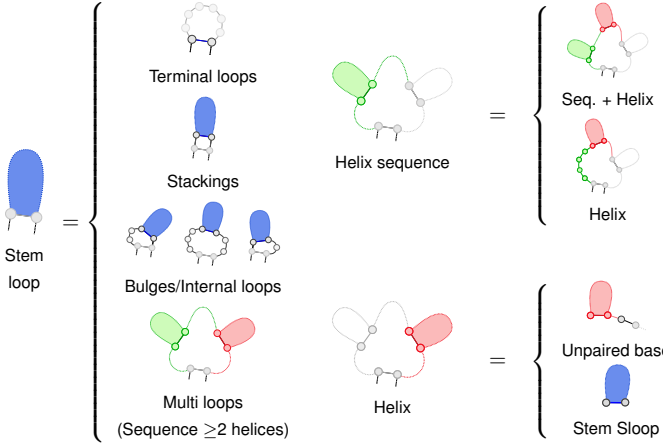
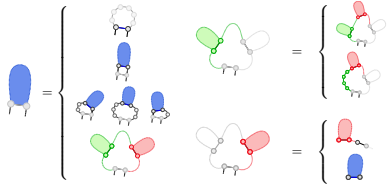


MFold Unafold

- $E_H(i,j)$: Energy of terminal loop *enclosed by* (i,j) pair
- $E_{BI}(i,j)$: Energy of bulge or internal loop *enclosed by* (i,j) pair
- $E_S(i,j)$: Energy of stacking $(i,j)/(i+1,j-1)$
- Penalty for multi loop (a), and occurrences of unpaired base (b) and helix (c) in multi loops.



DP recurrence

$$
\begin{aligned}
\mathcal{M}'_{i,j} &= \min \begin{cases} E_H(i,j) \\ E_S(i,j) + \mathcal{M}'_{i+1,j-1} \\ \mathrm{Min}_{i',j'}(E_{BI}(i,i',j',j) + \mathcal{M}'_{i',j'}) \\ a + c + \mathrm{Min}_k(\mathcal{M}_{i+1,k-1} + {\mathcal{M}^1}_{k,j-1}) \end{cases} \\
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Backtracking

Backtracking to reconstruct MFE structure:

$$
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Complexity:

For each min, $\mathcal{O}(n)$ potential contributors
$\Rightarrow$ Worst-case complexity in $\mathcal{O}(n^2)$ for naive backtrack.
Keep best contributor for each Min $\Rightarrow$ Backtracking in $\mathcal{O}(n)$

$\Rightarrow$ `UnaFold` [MZ08]/`RNAFold` [HFS+94] compute the MFE for the Turner model in overall[1] time/space complexities in $\mathcal{O}(n^3)/\mathcal{O}(n^2)$

[1] Using a trick/restriction for internal loops...

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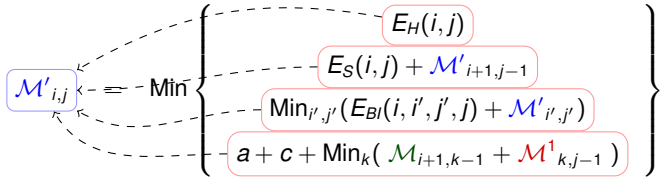

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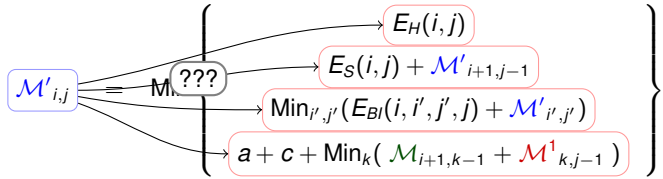

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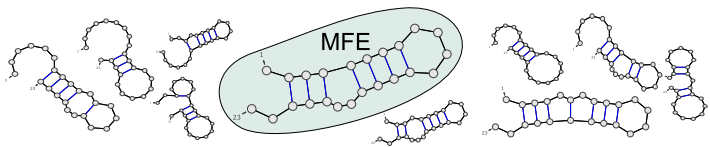
The canonical Boltzmann Ensemble

RNA *breathes* $\Rightarrow$ There is no more than a single conformation.

New paradigm

The conformations of an RNA coexist in the Boltzmann distribution.



Consequence: The MFE probability can be arbitrarily small.
$\Rightarrow$ To understand how RNA acts, one must account for the set of alternative structures.

In particular, structurally close structures may *ally*, and become the most realistic candidate in the search for a functional conformation.

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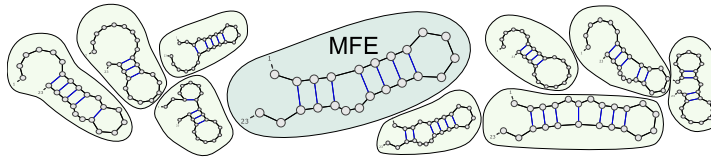

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Boltzmann Distribution: Definition

For each structure S compatible with an RNA ω, the Boltzmann distribution associates a Boltzmann factor $\mathcal{B}_{S,\omega} = e^{\frac{-E_{S,\omega}}{RT}}$, where:

- $E_{S,\omega}$ is the free-energy S (kCal.mol^{-1})
- T is the temperature (K)
- R is the perfect gaz constant (1.986.10^{-3} kCal.K^{-1}.mol^{-1})

To obtain a distribution, one simply renormalizes by the partition function

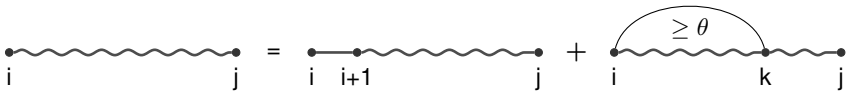
$$\mathcal{Z}_\omega = \sum_{S \in \mathcal{S}_\omega} e^{\frac{-E_{S,\omega}}{RT}}$$

where $\mathcal{S}_\omega$ is the set of conformations that are compatibles with ω.

The Boltzmann probability of a structure S is simply given by

$$P_{S,\omega} = \frac{e^{\frac{-E_{S,\omega}}{RT}}}{\mathcal{Z}_\omega}.$$

Nussinov/Jacobson DP scheme

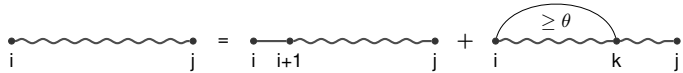


$$
\begin{aligned}
N_{i,t} &= 0, \quad \forall t \in [i, i+\theta] \\
N_{i,j} &= \min \begin{cases} N_{i+1,j} & i \text{ unpaired} \\ \min\limits_{k=i+\theta+1}^{j} \Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j} & i \text{ paired with } k \end{cases}
\end{aligned}
$$

Ambiguity? Consider i: Either unpaired, or paired to k.
Sets of structures generated in these two cases are clearly disjoint.
(also holds for various values of k) $\Rightarrow$ Unambiguous decomposition

Completeness?True, since scheme explores every possible outcome for i.
+ Induction on interval length $\Rightarrow$ Complete decomposition

Nussinov/Jacobson DP scheme



Recurrence for minimal free-energy of a fold :

$$
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N_{i,j} &= \min \begin{cases} N_{i+1,j} & (i \text{ unpaired}) \\ \min_{k=i+\theta+1}^{j} E_{i,k} + N_{i+1,k-1} + N_{k+1,j} & (i \text{ comp. with } k) \end{cases}
\end{aligned}
$$

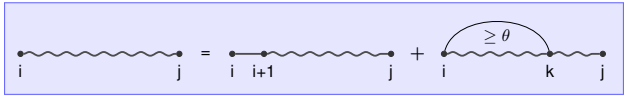
Recurrence for counting compatible structures :

$$
\begin{aligned}
C_{i,t} &= 1, \quad \forall t \in [i, i+\theta] \\
C_{i,j} &= \sum \begin{cases} C_{i+1,j} & (i \text{ unpaired}) \\ \sum_{k=i+\theta+1}^{j} 1 \times C_{i+1,k-1} \times C_{k+1,j} & (i \text{ comp. with } k) \end{cases}
\end{aligned}
$$

Decomposition matters, and the rest (MFE, count…) follows!

Partition function

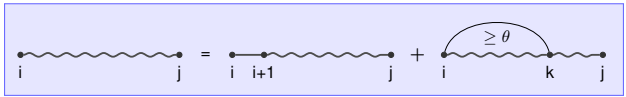
Partition function = Weighted count over compatible structures



$$\begin{aligned} \mathcal{Z}_{i,t} &= 1, \quad \forall t \in [i, i+\theta] \\ \mathcal{Z}_{i,j} &= \sum \left\{ \sum_{k=i+\theta+1}^{j} 1 \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} \right. \end{aligned}$$

Partition function

Partition function = Weighted count over compatible structures

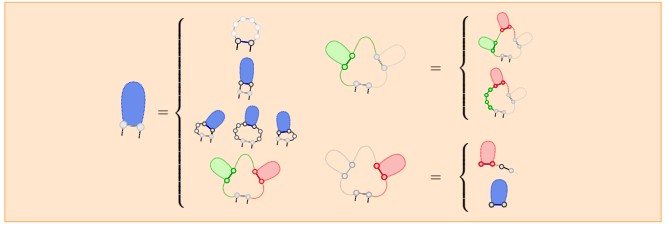


$$\mathcal{Z}_{i,t} = 1, \quad \forall t \in [i, i+\theta]$$

$$\mathcal{Z}_{i,j} = \sum \left\{ \sum_{k=i+\theta+1}^{j} e^{\frac{-E_{bp}(i,k)}{RT}} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} \right. \mathcal{Z}_{i+1,j}$$

Partition function

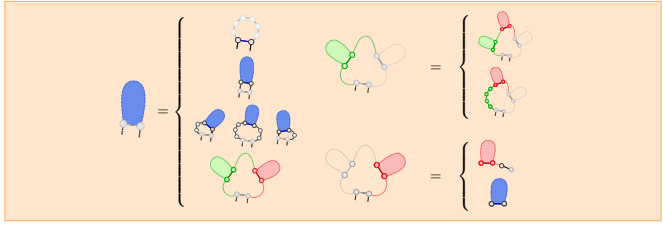
Partition function = Weighted count over compatible structures



$$
\begin{aligned}
\mathcal{M}'_{i,j} &= \text{Min}\begin{cases} E_H(i,j) \\ E_S(i,j) + \mathcal{M}'_{i+1,j-1} \\ \text{Min}(E_{BI}(i,i',j',j) + \mathcal{M}'_{i',j'}) \\ a + c + \text{Min}\left(\mathcal{M}_{i+1,k-1} + \mathcal{M}^1{}_{k,j-1}\right) \end{cases} \\
\mathcal{M}_{i,j} &= \text{Min}\left\{\text{Min}\left(\mathcal{M}_{i,k-1}, b(k-1)\right) + \mathcal{M}^1{}_{k,j}\right\} \\
\mathcal{M}^1{}_{i,j} &= \text{Min}\left\{b + \mathcal{M}^1{}_{i,j-1}, c + \mathcal{M}'_{i,j}\right\}
\end{aligned}
$$

Partition function

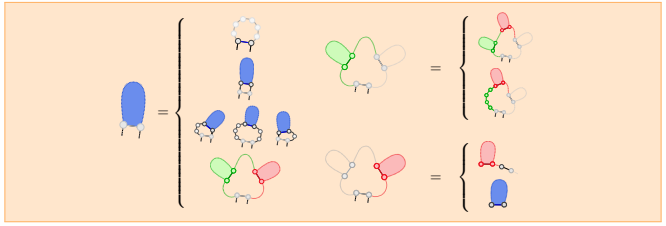
Partition function = Weighted count over compatible structures



$$
\begin{aligned}
\mathcal{M}'_{i,j} &= \text{Min}\left\{
\begin{array}{c}
e^{\frac{-E_H(i,j)}{RT}} \\
e^{\frac{-E_S(i,j)}{RT}} + \mathcal{M}'_{i+1,j-1} \\
\text{Min}\left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} + \mathcal{M}'_{i',j'}\right) \\
e^{\frac{-(a+c)}{RT}} + \text{Min}\left(\mathcal{M}_{i+1,k-1} + \mathcal{M}^1{}_{k,j-1}\right)
\end{array}
\right. \\
\mathcal{M}_{i,j} &= \text{Min}\left\{\text{Min}\left(\mathcal{M}_{i,k-1}, e^{\frac{-b(k-1)}{RT}}\right) + \mathcal{M}^1{}_{k,j}\right\} \\
\mathcal{M}^1{}_{i,j} &= \text{Min}\left\{e^{\frac{-b}{RT}} + \mathcal{M}^1{}_{i,j-1}, e^{\frac{-c}{RT}} + \mathcal{M}'_{i,j}\right\}
\end{aligned}
$$

Partition function

Partition function = Weighted count over compatible structures



$$
\begin{aligned}
\mathcal{M}'_{i,j} &= \mathrm{Min}\begin{cases} e^{\frac{-E_H(i,j)}{RT}} \\ e^{\frac{-E_S(i,j)}{RT}}\mathcal{M}'_{i+1,j-1} \\ \mathrm{Min}\left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}}\mathcal{M}'_{i',j'}\right) \\ e^{\frac{-(a+c)}{RT}}\mathrm{Min}\left(\mathcal{M}_{i+1,k-1}\mathcal{M}^1{}_{k,j-1}\right) \end{cases} \\
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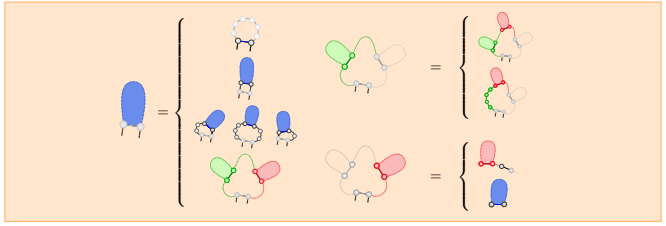
\mathcal{M}^1{}_{i,j} &= \mathrm{Min}\left\{e^{\frac{-b}{RT}}\mathcal{M}^1{}_{i,j-1}, e^{\frac{-c}{RT}}\mathcal{M}'_{i,j}\right\}
\end{aligned}
$$

Partition function

Partition function = Weighted count over compatible structures



$$
\begin{aligned}
\mathcal{Z}'(i,j) &= \sum \begin{cases} e^{\frac{-E_{BI}(i,j)}{RT}} \\ e^{\frac{-E_S(i,j)}{RT}} \mathcal{Z}'(i+1,j-1) \\ +\sum \left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} \mathcal{Z}'(i',j') \right) \\ +e^{\frac{-(a+c)}{RT}} \sum \left(\mathcal{Z}(i+1,k-1) \mathcal{Z}^1(k,j-1) \right) \end{cases} \\
\mathcal{Z}(i,j) &= \sum \left(\mathcal{Z}(i,k-1) + e^{\frac{-b(k-1)}{RT}} \right) \mathcal{Z}^1(k,j) \\
\mathcal{Z}^1(i,j) &= e^{\frac{-b}{RT}} \mathcal{Z}^1(i,j-1) + e^{\frac{-c}{RT}} \mathcal{Z}'(i,j)
\end{aligned}
$$

Partition function

Partition function = Weighted count over compatible structures

$$\begin{aligned} \mathcal{Z}_{i,t} &= 1, \quad \forall t \in [i, i+\theta] \\ \mathcal{Z}_{i,j} &= \sum \left\{ \sum_{k=i+\theta+1}^{j} e^{\frac{-E_{\text{bp}}(i,k)}{RT}} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} \right. \end{aligned}$$

Validity of a partition function computation:

- Completeness/Unambiguity of decomposition scheme
- Correctness of Boltzmann factor

  Weight induced by backtrack = Product of derivations weights

  $e^{-E/RT} \rightarrow$ Weight products $\Leftrightarrow$ Summing energy terms

$$\begin{aligned} e^{-E_{\text{bp}}(i,k)/RT} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} &= \cdot \sum_{x} e^{-E(x)/RT} \cdot \sum_{y} e^{-E(y)/RT} \\ &= \sum_{x,y} e^{-a/RT} \cdot e^{-E(x)/RT} \cdot e^{-E(y)/RT} \\ &= \sum_{x,y} e^{-(E_{\text{bp}}(i,k)+E(x)+E(y))/RT} \end{aligned}$$

Partition function

Partition function = Weighted count over compatible structures

$$\begin{aligned} \mathcal{Z}_{i,t} &= 1, \quad \forall t \in [i, i+\theta] \\ \mathcal{Z}_{i,j} &= \sum \left\{ \sum_{k=i+\theta+1}^{j} e^{\frac{-E_{\text{bp}}(i,k)}{RT}} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} \right. \end{aligned}$$

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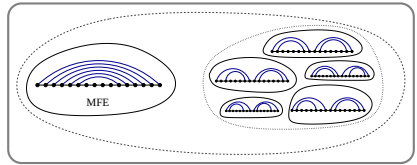
Statistical sampling of RNA 2$^{\text{ary}}$ structures

MFE ($\Leftrightarrow$ Max probability) may be heavily dominated by a set $\mathcal{B}$ of structurally similar suboptimal structures.
$\Rightarrow$ Functional conformation probably closer to $\mathcal{B}$ than to MFE.



Proof-of-concept: [DCL05]

- Sample structures within Boltzmann probability
- Cluster structures
- Build and return consensus structure of the heaviest cluster

$\Rightarrow$ Relative improvement for specificity (+17.6%) and sensitivity (+21.74%, except group II introns)

Problem

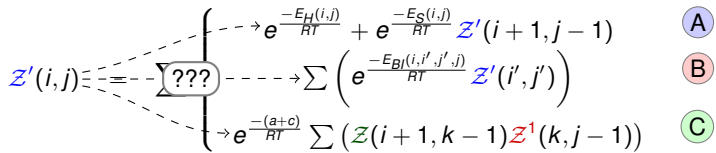
How to sample from the Boltzmann ensemble?

Stochastic backtrack (adapted from `SFold`)

Goal [DL03]: From sequence ω, draw S with prob. $e^{-E_S/RT}/\mathcal{Z}$

Principle: Choose derivation with prob. prop. to its contribution to part. fun.
Precomputation: Compute part. fun. versions of matrices ($\mathcal{Z}$, $\mathcal{Z}'$, $\mathcal{Z}^1$).
Stochastic backtrack:

1. Draw uniform random number $r \in [0, \mathcal{Z}'(i,j))$
2. Subtract from r the contributions of $\mathcal{Z}'(i,j)$ until $r < 0$
3. Recurse over associated regions/matrices



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$$\mathcal{Z}'(i,j) = \sum \begin{cases} e^{\frac{-E_H(i,j)}{RT}} + e^{\frac{-E_S(i,j)}{RT}} \mathcal{Z}'(i+1,j-1) & \text{A} \\ \sum \left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} \mathcal{Z}'(i',j') \right) & \text{B} \\ e^{\frac{-(a+c)}{RT}} \sum \left(\mathcal{Z}(i+1,k-1) \mathcal{Z}^1(k,j-1) \right) & \text{C} \end{cases}$$

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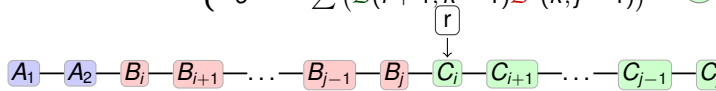

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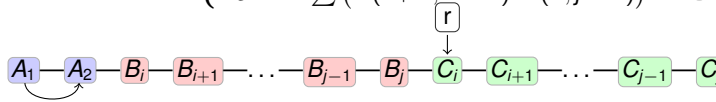

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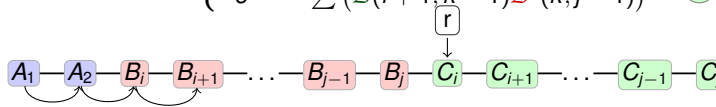

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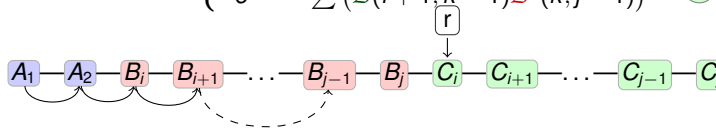

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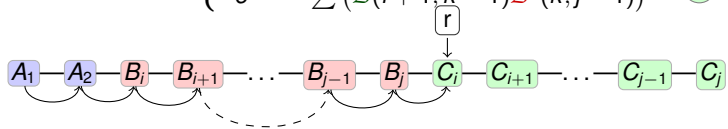

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Correctness: Each $S \in \mathcal{S}_\omega$ uniquely generated (DP scheme unambiguity)
Therefore the probability of generated S is

$$p_S = \frac{\mathcal{B}(E_1)}{\mathcal{B}(\mathcal{S}_w)} \cdot \frac{\mathcal{B}(E_2)}{\mathcal{B}(E_1)} \cdot \frac{\mathcal{B}(E_3)}{\mathcal{B}(E_2)} \cdots \frac{\mathcal{B}(\{S\})}{\mathcal{B}(E_m)}$$

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$$p_S = \frac{1}{\mathcal{B}(\mathcal{S}_w)} \cdot \frac{1}{1} \cdot \frac{1}{1} \cdots \frac{\mathcal{B}(\{S\})}{1}$$

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Therefore the probability of generated S is

$$p_S = \frac{\mathcal{B}(\{S\})}{\mathcal{B}(\mathcal{S}_w)} = \frac{e^{-E_S/RT}}{\mathcal{Z}} = P_{S,\omega}$$

Complexity

Goal [DL03]: From sequence ω, draw S with prob. $e^{-E_S/RT}/\mathcal{Z}$

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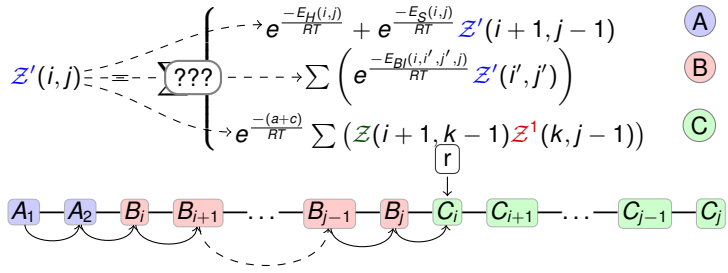

Average-case complexity in $\Theta(k \times n\sqrt{n})$ (homopolymer model) [Pon08].
Boustrophedon search $\Rightarrow \mathcal{O}(k \times n \log n)$ worst-case [Pon08].

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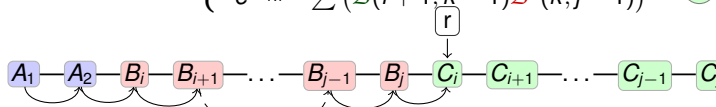
$$\mathcal{Z}'(i,j) = \sum \begin{cases} e^{\frac{-E_H(i,j)}{RT}} + e^{\frac{-E_S(i,j)}{RT}} \mathcal{Z}'(i+1,j-1) & \text{A} \\ \sum \left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} \mathcal{Z}'(i',j') \right) & \text{B} \\ e^{\frac{-(a+c)}{RT}} \sum \left(\mathcal{Z}(i+1,k-1) \mathcal{Z}^1(k,j-1) \right) & \text{C} \end{cases}$$



After $\Theta(n)$ operations, recurse over region of length $n-1$

$\Rightarrow$ Worst-case complexity in $\mathcal{O}(k \times n^2)$ for k samples

Average-case complexity in $\Theta(k \times n\sqrt{n})$ (homopolymer model) [Pon08].

Boustrophedon search $\Rightarrow \mathcal{O}(k \times n \log n)$ worst-case [Pon08].

Outline

- Physics-based structure prediction
  - Turner energy model
  - MFold/Unafold
- Boltzmann ensemble
  - Nussinov: Minimisation $\Rightarrow$ Counting
  - Computing the partition function
  - Statistical sampling
- Performances
  - Overall picture
  - Family-level evaluation
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Historical paradigms towards 2D prediction

Definition (Ab initio folding)

Starting from sequence, find conformation that minimizes free-energy.

Advantages:

- Mechanical nature allows the (in)validation of models
- Reasonable complexity $\mathcal{O}(n^3)/\mathcal{O}(n^2)$ time/space
- *Exhaustive* nature

Limitations:

- Hard to include PKs
- Highly dependent on energy model
- No cooperativity
- Limited performances

Definition (Comparative approach)

Starting from homologous sequences, postulate common structure and find best possible tradeoff between folding & alignment.

Avantages :

- Better performances
- (Limited) cooperativity
- Self-improving

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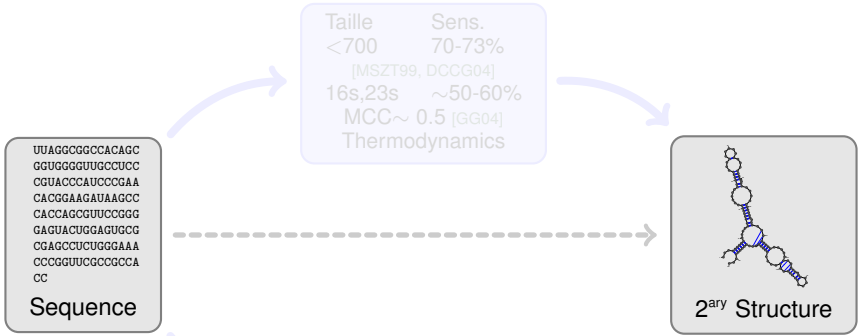
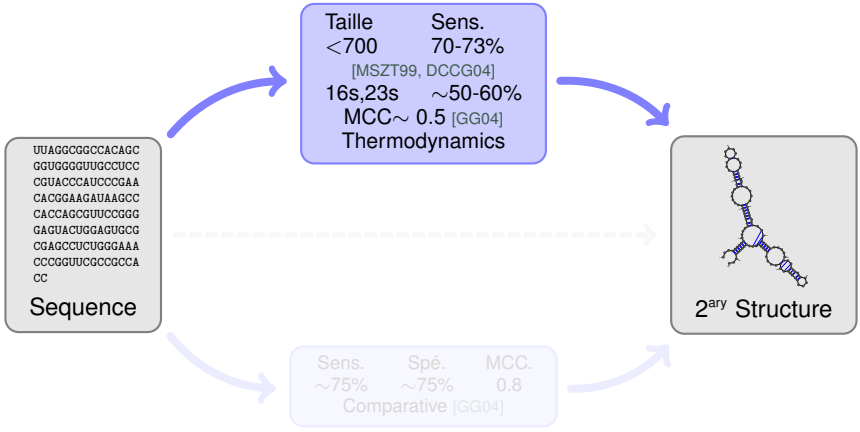
Typical performances



Reminder: $MCC = \frac{t^+t^- - f^+f^-}{\sqrt{(t^+ + f^+)(t^+ + f^-)(t^- + f^+)(t^- + f^-)}}$

Typical performances



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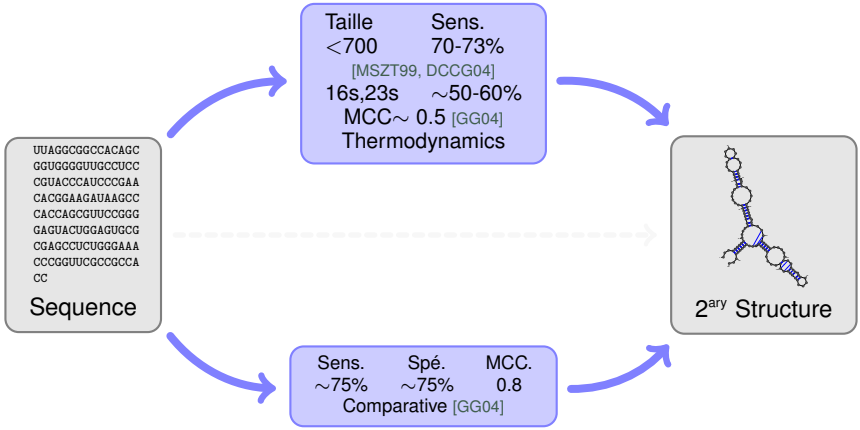

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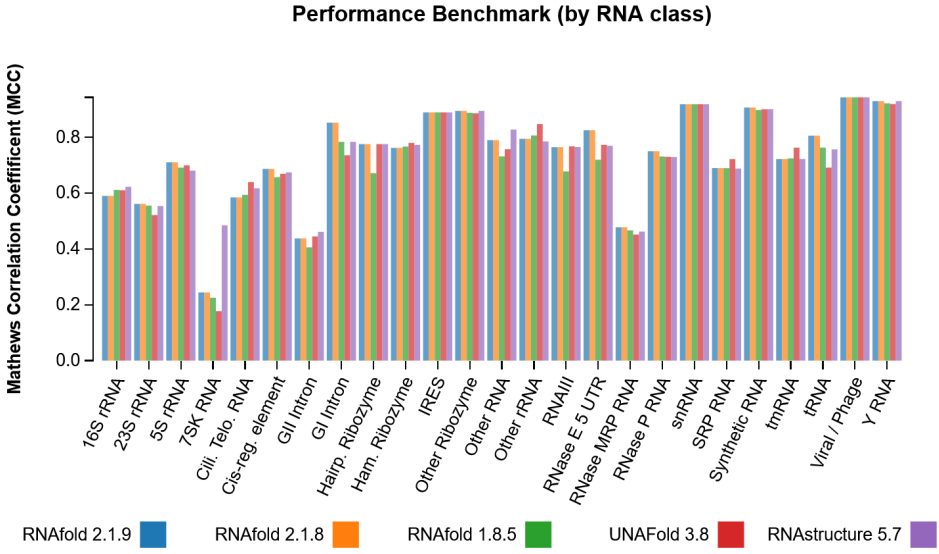
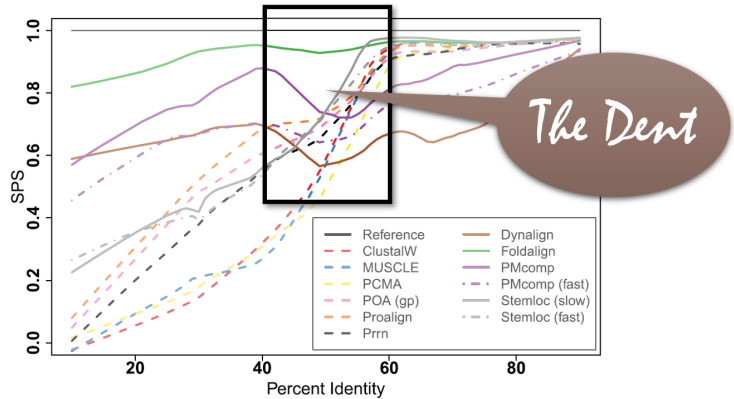
Detailed performances of 2D folding algorithms



Biased benchmarks: precedent in comparative folding/alignment

Bralibase: Benchmark for comp. RNA folding [Gardner,Wilm & Washietl, NAR 2005]



[Löwes *et al*, Briefings in Bioinfo 2016]

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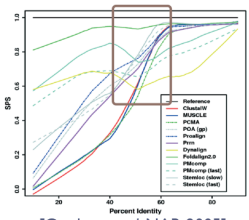

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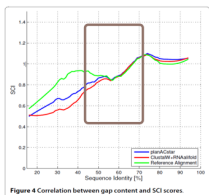
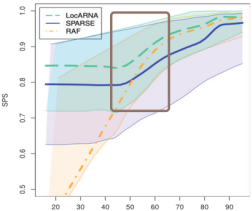
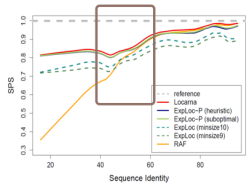


Figure 4 Correlation between gap content and SCI scores.

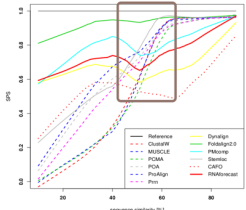
[Bremges *et al*, BMC Bioinfo, 2010]



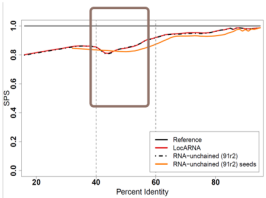
[Will *et al*, Bioinformatics 2015]



[Schmiedl *et al*, RECOMB 2012]



[Höchsmann *et al*, Unpublished]



[Bourgeade *et al*, J Comp Biol, 2015]

[Löwes *et al*, Briefings in Bioinfo 2016]

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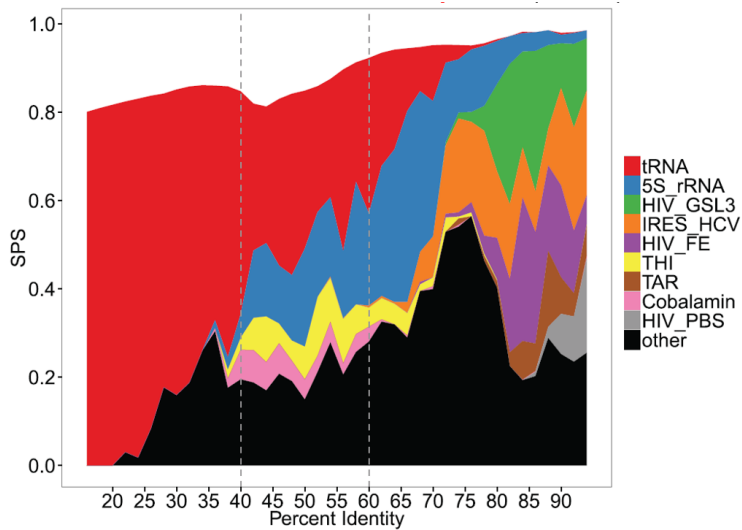

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The elephant in the room – **2010s version**

The elephant in the room – **2020s version**

A personal take on predictive Bioinformatics

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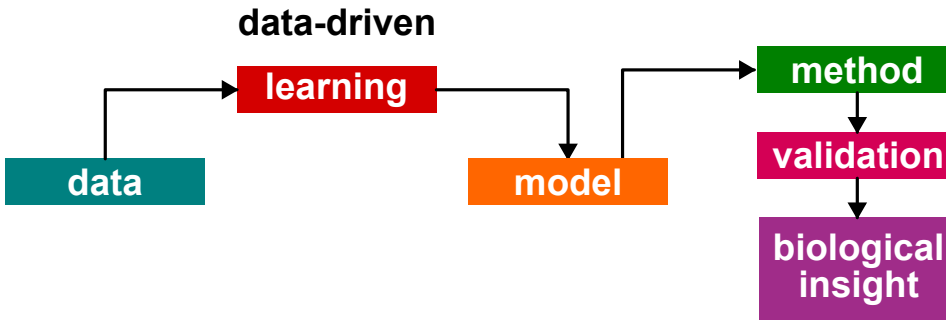

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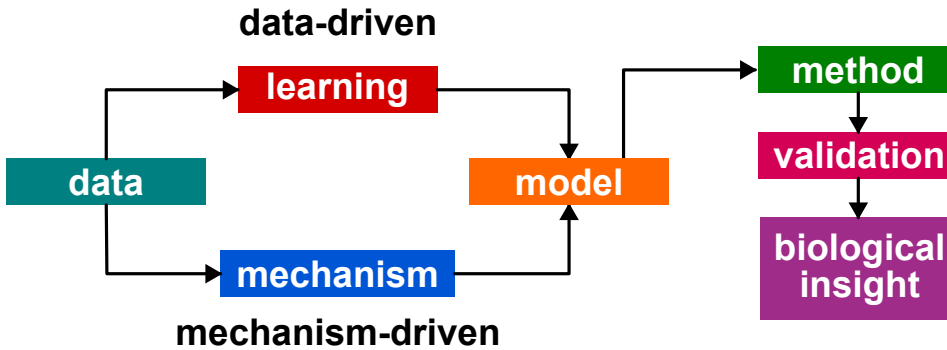


Method dev. as a modeling discipline:

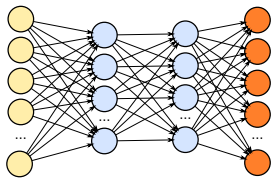
Mechanism-driven model + Exact/deterministic algorithms

$\rightarrow$ Performance as (in)validation of model

Machine Learning (ML): The beauty...

Machine Learning as a tool for scientific discovery

- Great promises
- Self-improving methods
- Generates/prioritizes hypotheses
- Available workforce (ubiquitous in curriculums)
- Highly promoted/funded by research institutions and glamorous journals...



Machine Learning (ML): The beauty... and the beast

Multiple (potential) pitfalls for ML in Bio*:

- Tricky evaluation (data leakage) $\rightarrow$ Extrapolation/generalization???
- Reproducibility issues (code/datasets availability, stability, retraining)
- Fishing expeditions/storytelling, selective reporting
- Educational deadend?
- Future(?) ecological disaster? Random blue checkmarks AI zealots on Twitter (grumble...)

(a) Husky classified as wolf (b) Explanation

[Ribeiro et al, KDD'16]

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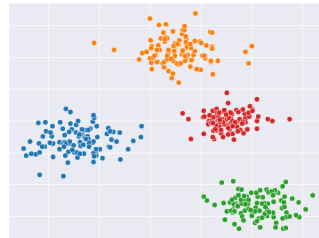

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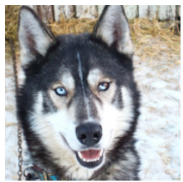

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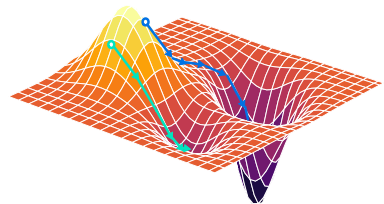
Available upon request

***aka* iff I'm in a good mood,**

PhD/postdoc still in lab, HDDs haven't burned,

pharma hasn't expressed interest in data...



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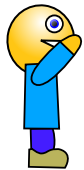
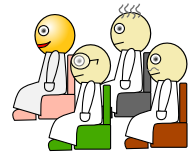


Fifth law of thermodynamics (continued)

```
                    ...
-0.31622776601683794 0.31622776601683794
-0.3157663248679193  0.3160839282916222
0.006806069733149146 0.17777128902976705
0.4472135954999579   1.433348584081719
-1.5736761136523203  1.433348584081719
-0.0002340648727882  0.4522609460629265
                    ...
                              24235/1020400
```



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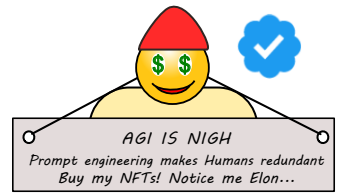


A crowded ML field for RNA 2D prediction

Method	Output	PKs?	Architecture	Availability
CONTRAfold	Pairwise contacts	No	CLLM	Code+weights+webserver
EternaFold	Pairwise contacts	No	CLLM	Code+weights+webserver
DMfold	DBN	Yes	bi-LSTM	Code only
RNA-state-inf	Binary paired/unpaired	N/A	bi-LSTM	Code only
SPOT-RNA2	Pairwise contacts	Yes	CNN	Code+weights+webserver
CROSS	Binary paired/unpaired	N/A	CNN-like	Webserver
RPRes	Binary paired/unpaired	N/A	bi-LSTM+CNN	Code only
2dRNA	Pairwise contacts	Yes	bi-LSTM+CNN	Webserver
2dRNA-LD	Pairwise contacts	Yes	bi-LSTM+CNN	Webserver
SPOT-RNA	Pairwise contacts	Yes	CNN+bi-LSTM	Code+weights+webserver
MXfold2	Pseudo-dG	No	CNN+bi-LSTM	Code+weights+webserver
CNNFold	Pairwise contacts	Yes	CNN(NxN input)	Code+weights
UFold	Pairwise contacts	Yes	CNN(NxN input)	Code+weights+webserver
CDPfold	DBN	No	CNN(N×Ninput)	Code
E2Efold	Pairwise contacts	Yes	Transformer+CNN	Code+weights
ATTfold	Pairwise contacts	Yes	Transformer+CNN	No

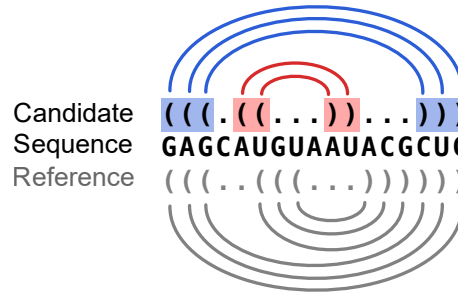
[Wu *et al*, Briefings in Bioinfo 2023]

Performances of 2D structure prediction

RNAStrand benchmark

[Adronescu *et al*, BMC Bioinf 2008]

Method	F_1
RNAfold 1.8.5	0.737
UNAfold 3.8	0.725
RNAstructure 5.7	0.744

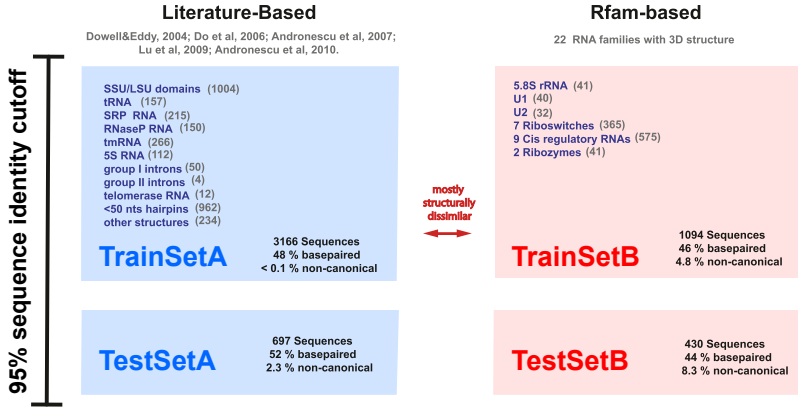


Sens = 3/6 = 0.5 PPV = 3/5 = 0.6

$F_1 = 0.545...$

$$F_1\text{-score} = \frac{2 \times \text{PPV} \times \text{Sens}}{\text{PPV} + \text{Sens}}$$

The TORNADO dataset



[Rivas *et al*, RNA 2012]

TrainSetA vs TestSetA: 95% sim. cutoff → Learn *k*-mer to template association

(May happen even for extreme cutoffs)

TrainSetA vs TestSetB: Rewards learning structurally generalizable models

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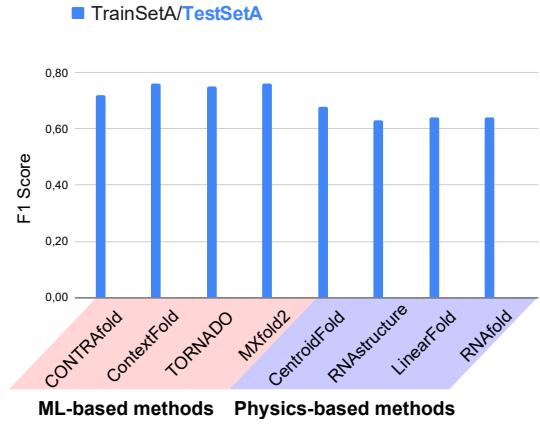

[Sato *et al*, Nature Comm 2021]

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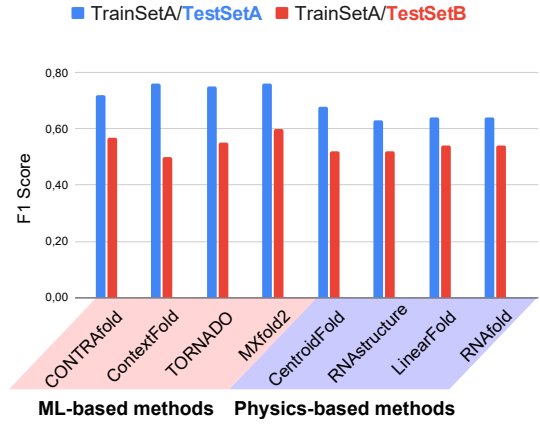

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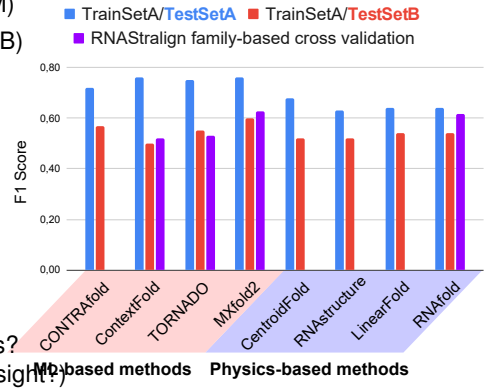
$$F_1\text{-score} = \frac{2 \times \text{PPV} \times \text{Sens}}{\text{PPV} + \text{Sens}}$$

The (nc)RNA datasphere

- 34M sequences, inc 22M presumably structured (RNACentral)
- 4000+ functional ncRNA families (RFAM)
- 250-300 non-redundant 3D models (PDB)



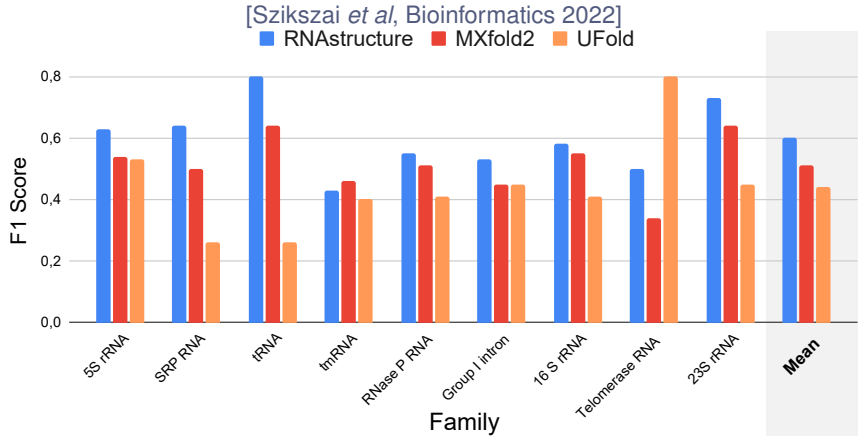
Existing methods trained on datasets:

- highly-redundant sequence-wise
- low-diversity structure-wise

Do ML methods generalize to new structures?
(Do ML perfs translate into *new* biological insight?)

[Sato *et al*, Nature Comm 2021]

Generalization to new families/structures remains problematic

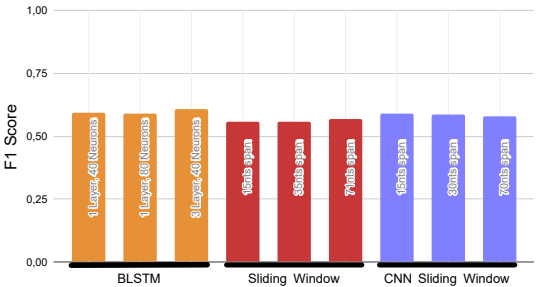


Family-fold cross-validation on ArchiveII dataset [Sloma & Mathews, RNA 2016]
3974 RNAs of length 77-438 (large rRNAs split into smaller domains)

What if you had access to (unbounded) additional data?

Idea: Assess NN models' capacity to emulate RNAfold on random sequences

[Flamm *et al*, Frontiers in Bioinfo 2022]



Perfs *plateau* at 80k seq/structs (70nts)

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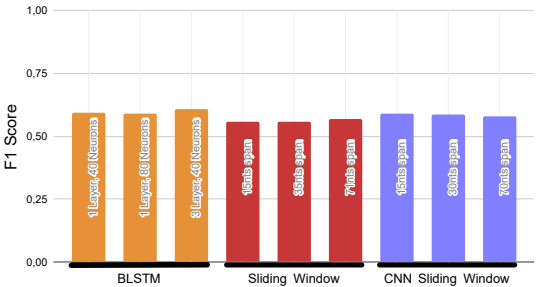

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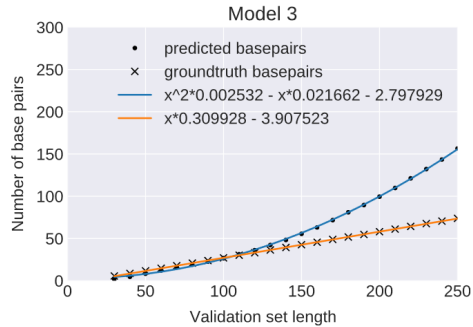
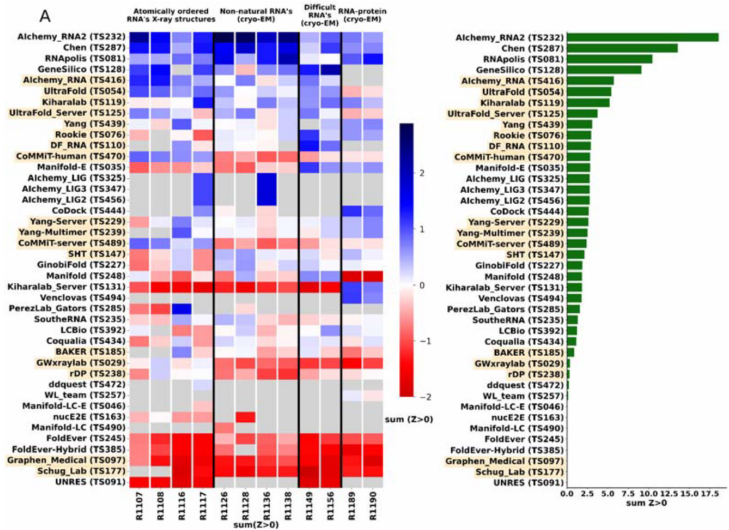


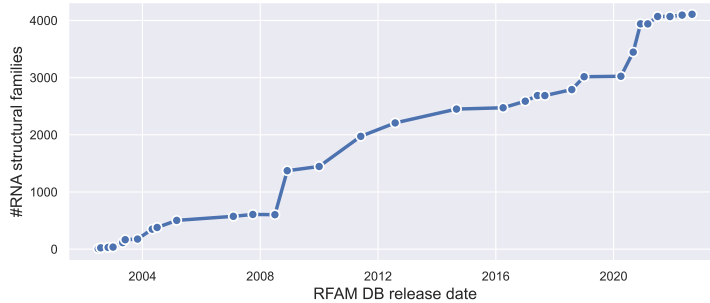
Popular CNN predicts $\Theta(n^2)$ BPs!

RNA 3D structure: No AlphaFold moment at CASP15



[Das *et al*, under review]

Conclusions and musings



- Still a need for improved RNA prediction (possibly ML-based)
- Purely combinatorial methods still $\pm$ state-of-the-art for new families...
- Hybrid approaches *à la* MxFold2: Best of both worlds?
- Assessing intrinsic limits of architectures: RNAFold as surrogate model

Conclusions and musings

So what's special about RNA?

- Modular but combinatorial structure
- New folds being routinely discovered (+ can be designed)
- Relatively scarce 3D data
- Opportunity: Tons of probing data (ML)
- Potential of LLMs/transformers (incoming)
- Pseudoknots-ready algorithms

Conclusions and musings

RNA/Bioinfo community needs to enforce stricter standards for ML publications:

- Enforce datasets and source code availability
  [Szikszai *et al*, Bioinfo'22] found 4/8 recent DL methods non-functional
- Realistic retraining mandatory
  Precondition for self-improvement, benchmarking of novel methods
- Consider mechanistic and ML methods as largely incomparable
- Better datasets/benchmarks needed, but perhaps not sufficient
- Sequence-based leakage should be systematically investigated

Outline

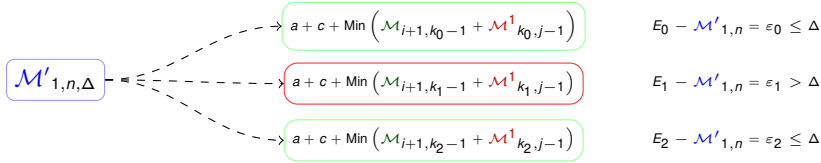
- Physics-based structure prediction
  - Turner energy model
  - MFold/Unafold
- Boltzmann ensemble
  - Nussinov: Minimisation $\Rightarrow$ Counting
  - Computing the partition function
  - Statistical sampling
- Performances
  - Overall picture
  - Family-level evaluation
  - Evaluation issues
- The specific case and issues of ML
  - Deep learning: Beauty and the beast
  - ML performances as advertised by authors
  - Surprising limitations
  - Takeaways
- Extended Alorithmics/DP techniques
  - Suboptimal structures
  - Pseudoknots

Suboptimal structures

Prob.: Simplified energy model (no pseudoknots, only canonical BPs)
$\Rightarrow$ Native structure (functional) could be overthrown.

$\Rightarrow$ Investigate suboptimal structures (`RNASubopt` [WFHS99]),
i.e. build all structures within Δ KCal.mol^{-1} of MFE:

- Compute minimum free-energy matrices
- Backtrack on any contribution within Δ of MFE;
- Update Δ such that future backtracks create ≥ 1 struct.
- Recursively generate subopts and combine (brute-force ou Sort)



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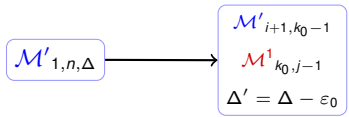

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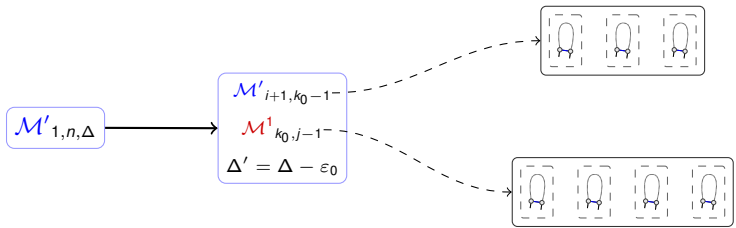

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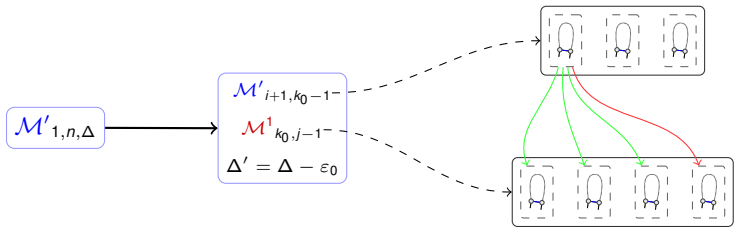

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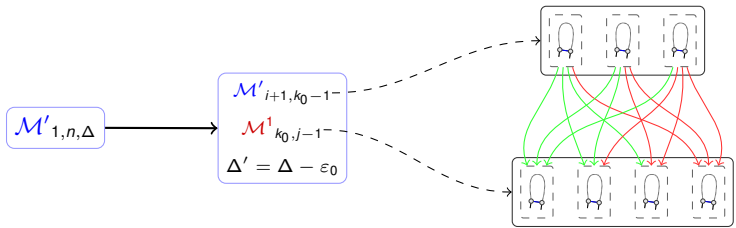

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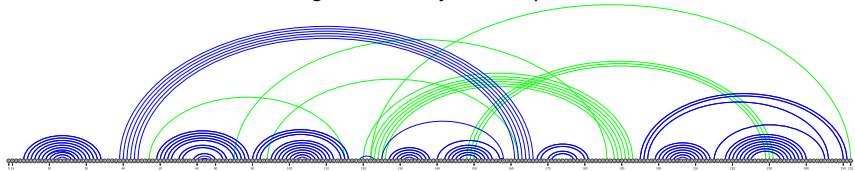
$$\Rightarrow \text{Time complexity (Sort)} : \mathcal{O}(n^3 + n \cdot k \log(k))$$

(k grows exponentially fast with Δ!)

Predicting pseudoknotted structures

Pseudoknots are essential to the folding and activity of multiple RNA families.



Groupe I Ribozyme

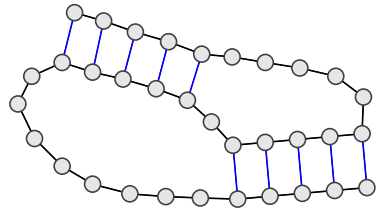
Their disregard within current folding algorithms stems both from **algorithmic** and **energetic** intricacies.

(Pseudoknots = Crossings $\Rightarrow$ foldings delimited by base-pair can no longer be assumed to be independent)

Type	Complexity	Reference
Secondary structures	$\mathcal{O}(n^3)$	[MSZT99]
L&P	$\mathcal{O}(n^5)$	[LP00]
D&P	$\mathcal{O}(n^5)$	[DP03]
A&U	$\mathcal{O}(n^5)$	[Aku00]
R&E	$\mathcal{O}(n^6)$	[RE99]
Unconstrained	NP-complete	[LP00]

Akutsu/Uemura Algorithm

Goal: Capture a category of simple, yet recurrent, pseudoknots.



Idea: When such a PK motif is rotated, one can deduce the MFE of a triplet (i, j, k) from the MFE of triplets directly below it.

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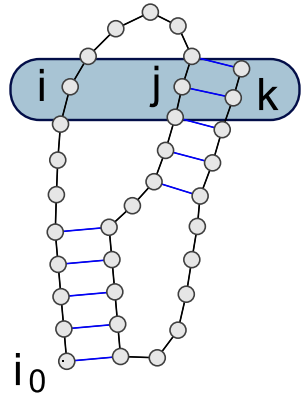

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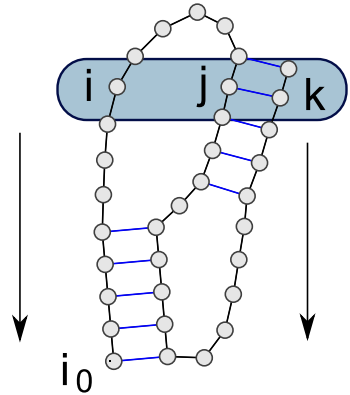

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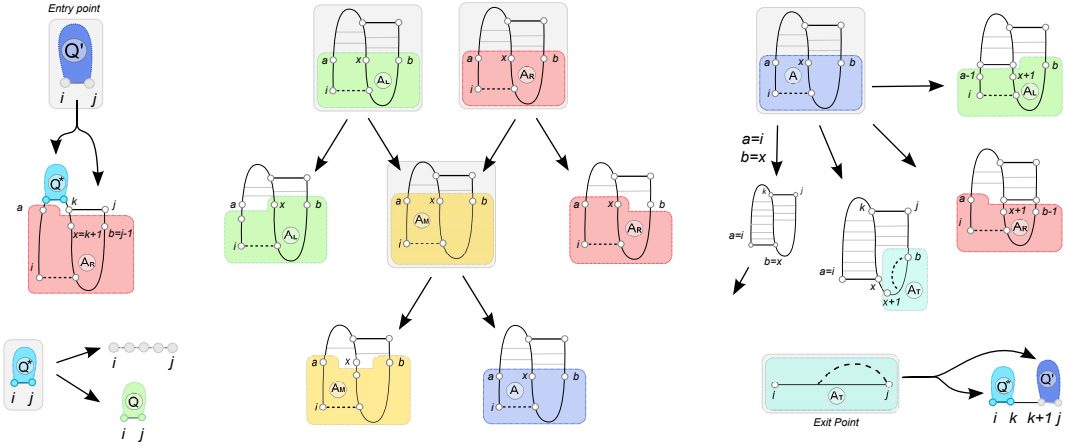
Akutsu/Uemura: Dynamic programming



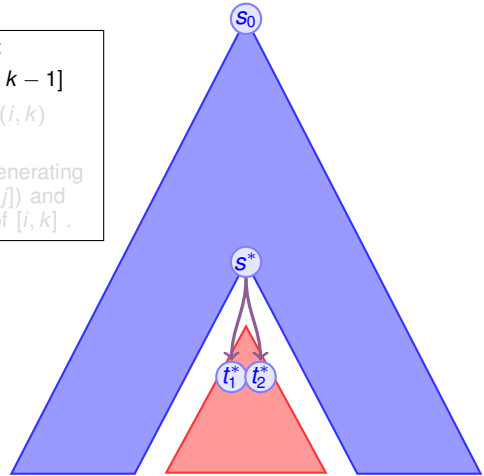
Application/Problem	Weight fun.	Time/Space	Ref.
Energy minimization	π_{bp}	$\mathcal{O}(n^4)/\mathcal{O}(n^4)$	[Aku00]
Partition function	$e^{\frac{-\pi_{bp}}{RT}}$	$\mathcal{O}(n^4)/\mathcal{O}(n^4)$	$\Theta(n^6)$[CC09]
BP probabilities	$e^{\frac{-\pi_{bp}}{RT}}$	$\mathcal{O}(n^4)/\mathcal{O}(n^4)$	–
Sampling (k-struct.)	$e^{\frac{-\pi_{bp}}{RT}}$	$\mathcal{O}(n^4 + kn\log n)/\mathcal{O}(n^4)$	–

Exercice: Write DP equation for MFE computation, counting and partition function.

Inside/outside algorithm

Structure including base pair (i, k):

- Inside: Structures over $[i+1, k-1]$
- Outside: Contexts of interval (i, k)
  - $\forall$ interval $[i, j], i < j \leq k$
  - Complete structure by generating brother intervals ($[k+1, j]$) and recurse over the father of $[i, k]$.



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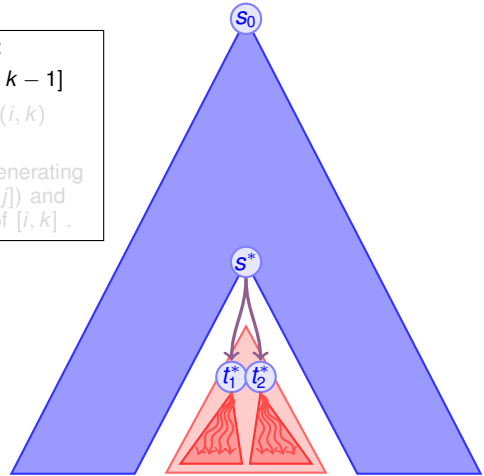

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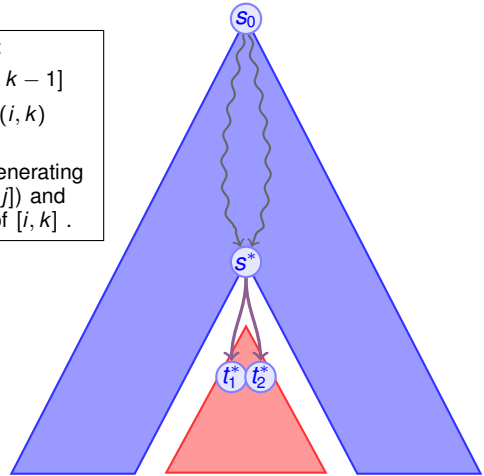

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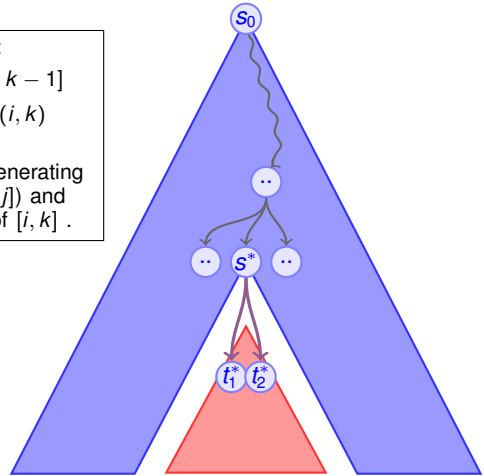

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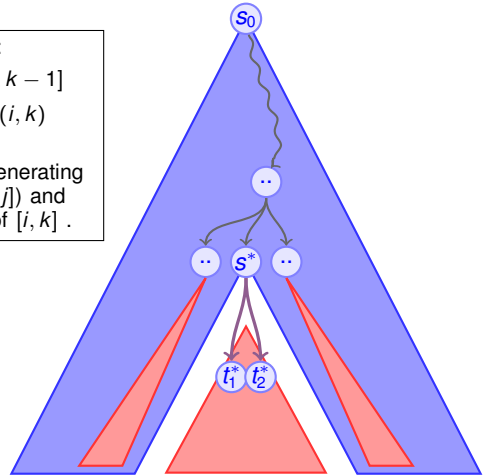

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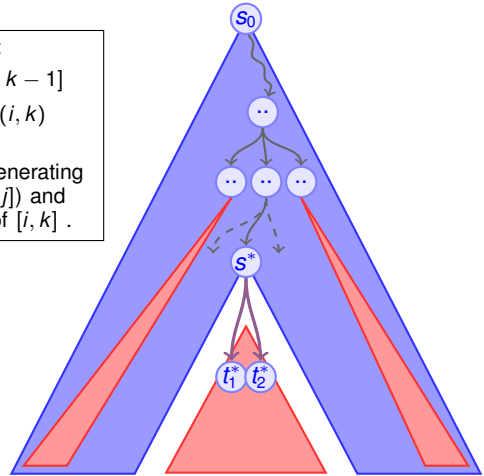

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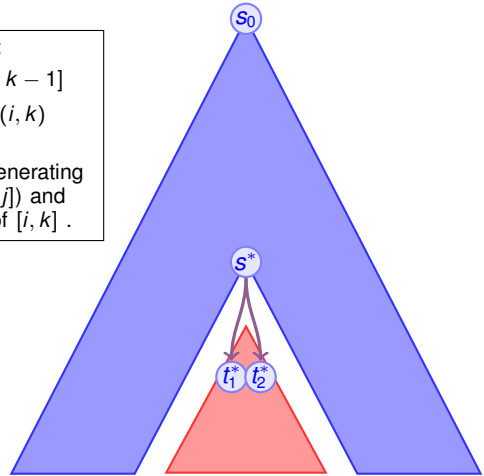


Whenever some further technical conditions are satisfied, this decomposition is complete and unambiguous, and implies a *simple recurrence* for computing the base pair probability matrix in $\Theta(n^3)$.

Alternatively: Duplicate sequence

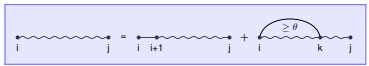
What is a good dynamic programming scheme?

Correction of a (Ensemble) dynamic programming scheme:

Objective function correctly computed/inherited at local level

\+ All the conformations can be obtained

$\Rightarrow$ Correct algorithm (Induction)



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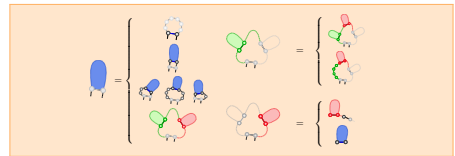

Enumerating search space helps but does not constitute a proof.

Need to show equivalence of DP schemes, *e.g.* use one to simulate the other and vice versa.

(Generating functions may help)

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$$C_{i,t} = 1, \quad \forall t \in [i, i+\theta]$$
$$C_{i,j} = \sum \left\{ \sum_{k=i+\theta+1}^{j} 1 \times C_{i+1,k-1} \times C_{k+1,j} \right.^{C_{i+1,j}}$$

Homopolymer (All pairs allowed) + $\theta = 1$
$\Rightarrow C_{1,n} = \mathbf{1}, 1, 1, 2, 4, 8, 17, 32, 82, 185, 423, \ldots$

$\Leftrightarrow$?

$$C'_{i,j} = \sum \left\{ \begin{array}{l} 1 \\ C'_{i+1,j-1} \\ \sum_{i',j'} C'_{i',j'} \\ \sum_k C_{i+1,k-1} \times C^1{}_{k,j-1} \end{array} \right.$$
$$C_{i,j} = \sum_k \left((C_{i,k-1} + 1) \times C^1{}_{k,j} \right)$$
$$C^1{}_{i,j} = C^1{}_{i,j-1} + C'_{i,j}$$

Homopolymer + $\theta = 1$
$\Rightarrow C'_{1,n} = \mathbf{0}, 1, 1, 2, 4, 8, 17, 32, 82, 185, 423, \ldots$

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