Combinatorial Optimization in Bioinfo

Folding RNA *in silico*

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AMIBio Team
CNRS & École Polytechnique

Outline

Foreword . . .

. . . or how to make a million bucks by giving change parsimoniously!!

Problem: You have access to unlimited amount of **1**, **20** and **50** cents coins. A client prefers to travel light, i.e. to minimize the #coins.
How to give N cents back in change without losing a customer?

Strategy #1: Start with *heaviest* coins, and then complete/fill-up with coins of *decreasing* value.

$21 = ??$

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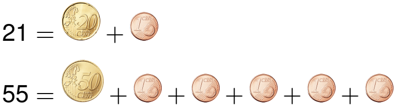

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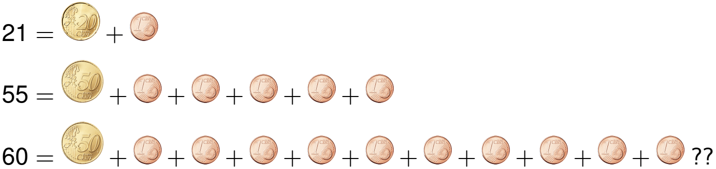

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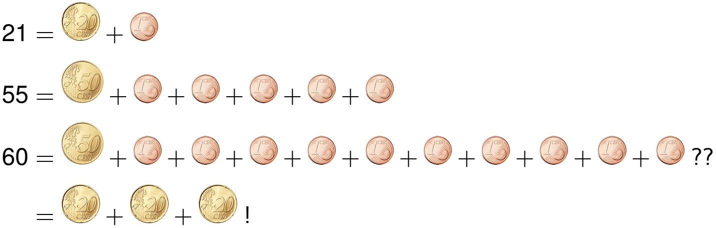

Problem *a priori (?!)* non-solvable using such a *greedy* approach, as a (simpler) problem is already NP-complete (thus Efficient solution $\Rightarrow$ 1M$).

Foreword

Strategy #2: Brute force enumeration $\rightarrow$ $\#\text{Coins}^N$ (Ouch!)

Strategy #3: The following recurrence gives the minimal number of coins:

$Min\#Coins(N) = \text{Min}$

-  $\rightarrow 1 + Min\#Coins(N-1)$
-  $\rightarrow 1 + Min\#Coins(N-20)$
-  $\rightarrow 1 + Min\#Coins(N-50)$

With some memory (N intermediate computations), the minimum number of coins can be obtained after $N \times \#\text{Coins}$ operations. An actual set of coins can be reconstructing by tracing back the choices performed at each stage, leading to the minimum.

Remark: We still haven't won the million, as N has exponential value compared to the length of its encoding, so the algorithm does not qualify as *efficient* (i.e. polynomial).

Still, this approach is much more efficient than a brute-force enumeration:

$\Rightarrow$ Dynamic programming.

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$\Rightarrow$ Dynamic programming.

Dynamic programming: General principle

Dynamic programming = General optimization technique.
Prerequisite: Optimal solution for problem P can be derived from solutions to sub-problems of P.

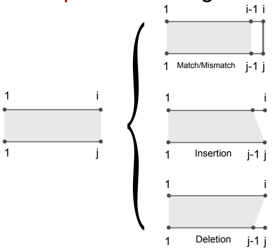
Bioinformatics :

Discrete solution space (alignments, structures...)

\+ Additively-inherited objective function (cost, log-odd score, energy...)

$\Rightarrow$ Efficient dynamic programming scheme

Example: Local Alignment(Smith/Waterman)



$$W(i,0) = 0$$
$$W(0,j) = 0$$
$$W(i,j) = \max \begin{cases} W(i-1,j-1) + m_{i,j} \\ W(i-1,j) + p_i \\ W(i,j-1) + p_d \end{cases}$$

Algorithmic details

Dynamic programming scheme defines a space of (sub)problems and a recurrence that relates the score of a problem to that of smaller problems.

Given a scheme, two steps :

- Matrix filling: Computation and tabulation of best scores (Computed from smaller problems to larger ones).
- Traceback: Reconstruct best solution from contributing subproblems.

Complexity of algorithm depends on:

- Cardinality of sub-problem space
- Number of alternatives considers at each step (#Terms in recurrence)

Smith&Waterman example:

- i: $1 \rightarrow n+1 \Rightarrow \Theta(n)$
- j: $1 \rightarrow m+1 \Rightarrow \Theta(m)$
- 3 operations at each step

$\Rightarrow \Theta(m.n)$ time/memory

$$
\begin{aligned}
W(i,0) &= 0\\
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W(i,j) &= \max \begin{cases} W(i-1,j-1)+m_{i,j} \\ W(i-1,j)+p_i \\ W(i,j-1)+p_d \end{cases}
\end{aligned}
$$

Properties of DP schemes

Necessary properties:

- Correctness: $\forall$ sub-problem, the computed value must indeed maximize the objective function .

Proofs usually inductive, and quite technical, but very systematic.

Desirable properties of DP schemes:

- Completeness of space of solutions generated by decomposition.
  Algorithmic tricks, by *cutting branches*, may violate this property.
- Unambiguity: Each solution is generated at most once.

$\Rightarrow$ Under these properties, one can enumerate solution space.

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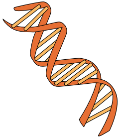
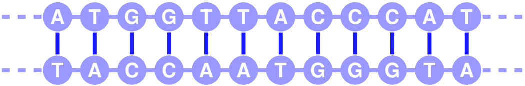
$\{A, C, G, T\}^\star$





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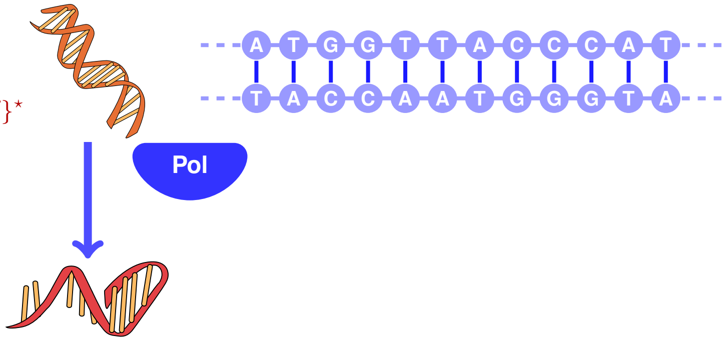

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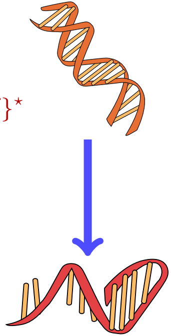

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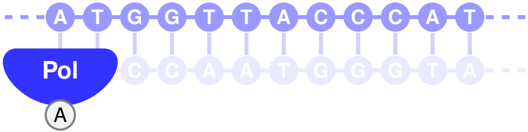

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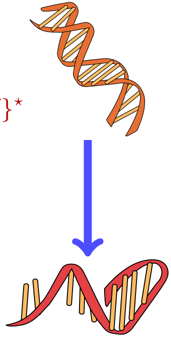

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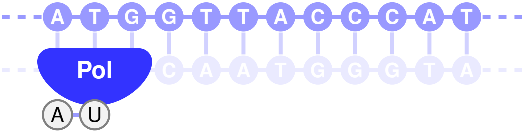

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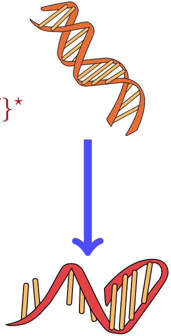

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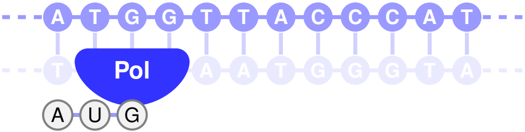

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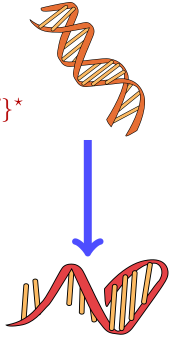

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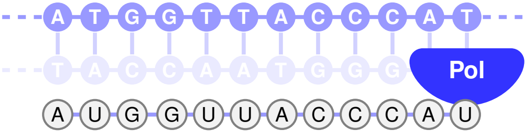

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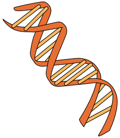

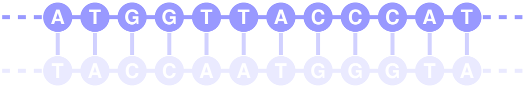

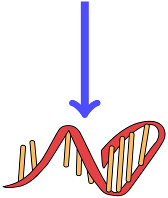

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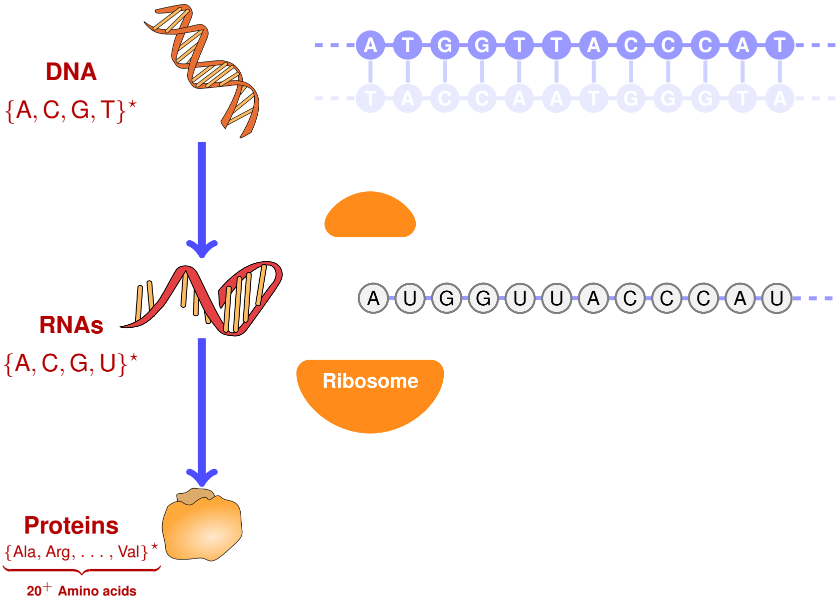

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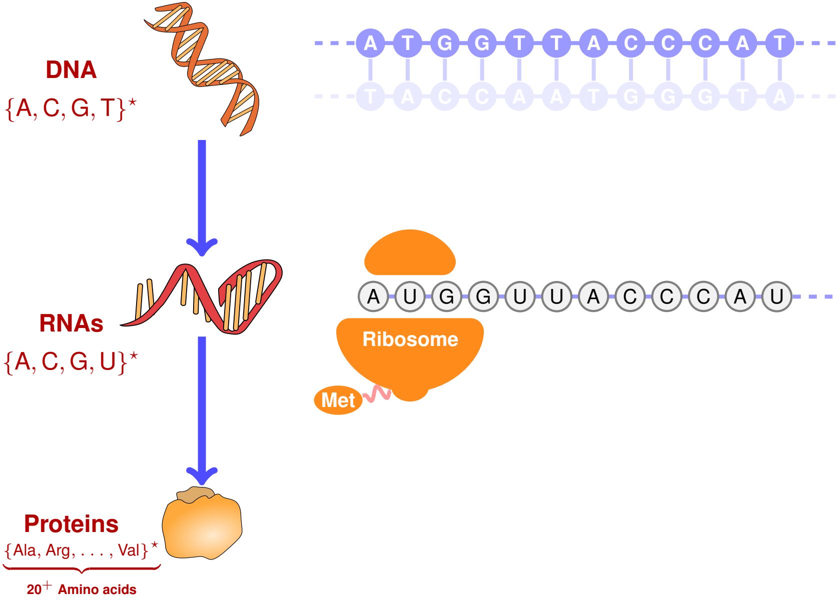

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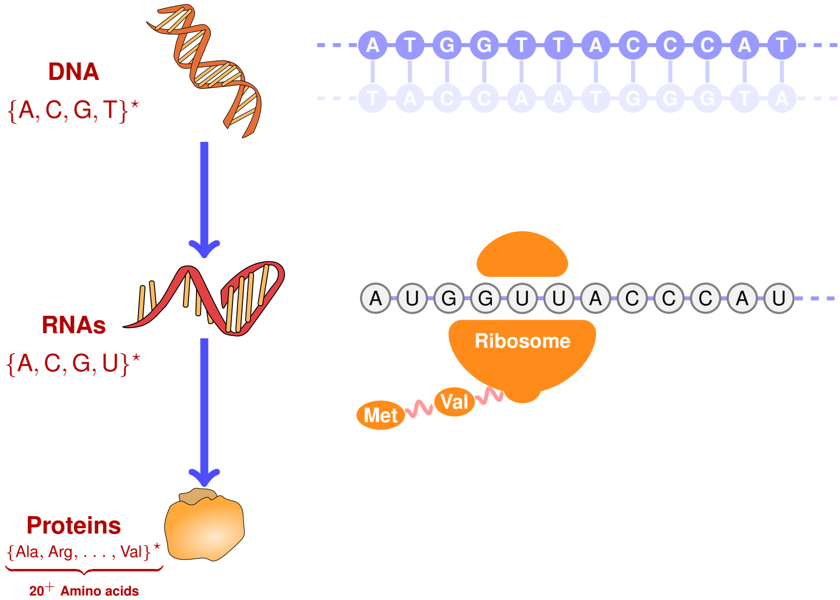

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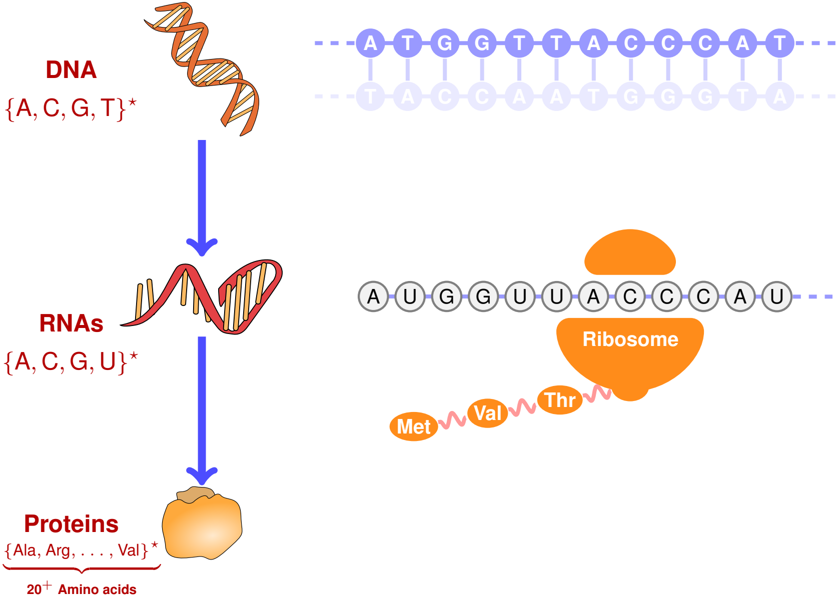

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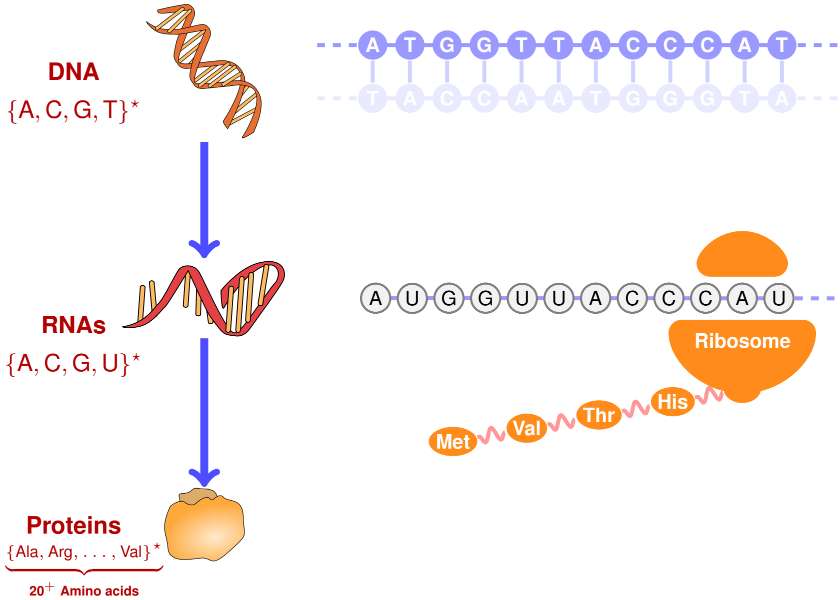

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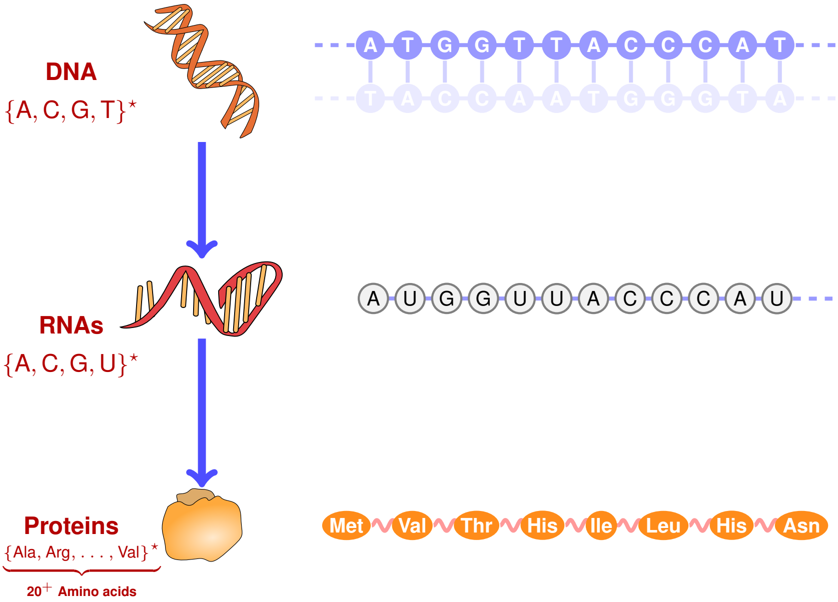

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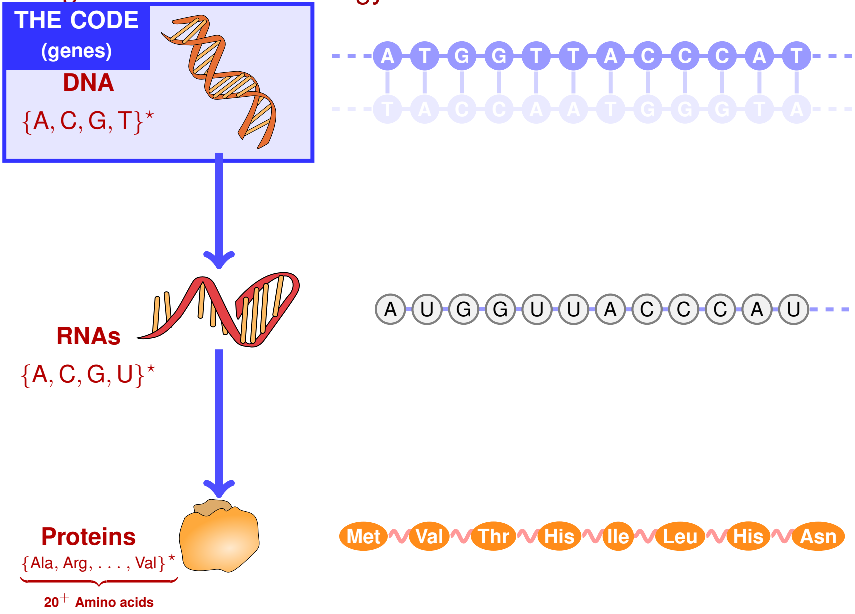

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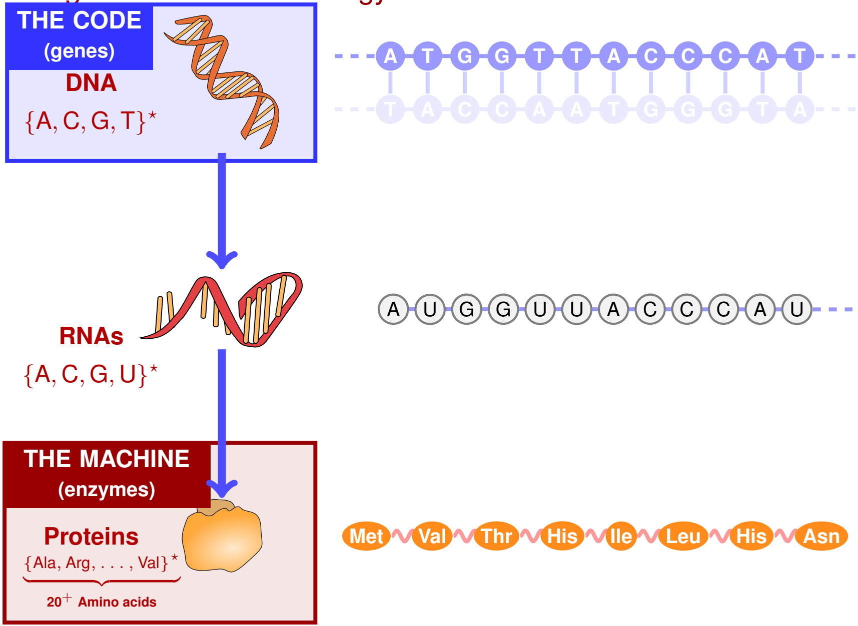

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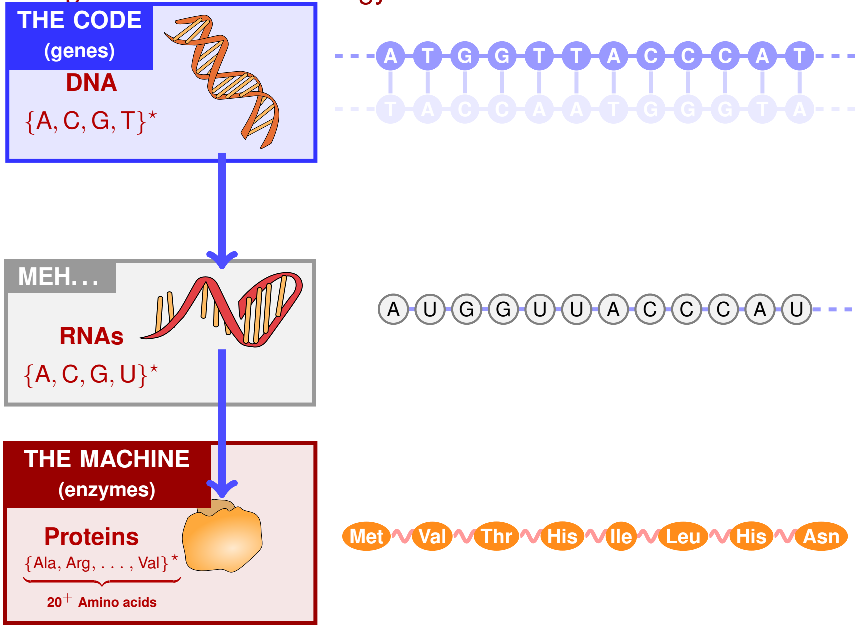

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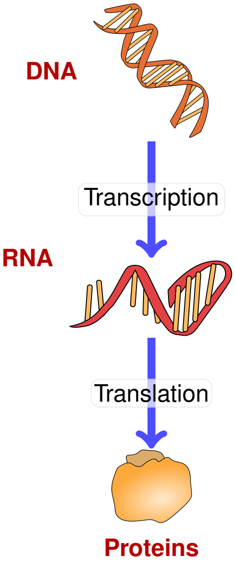

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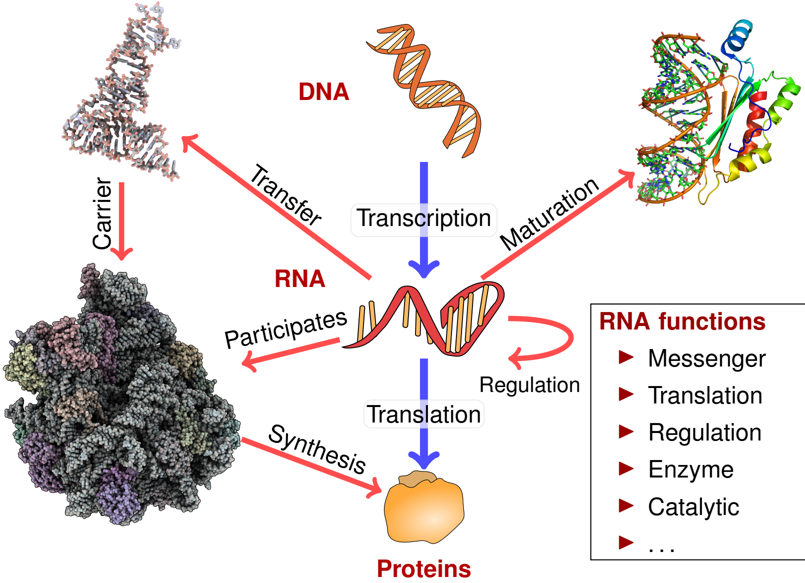

RNA functions

- Messenger
- Translation
- Regulation
- Enzyme
- Catalytic
- ...

RiboNucleic Acids (RNAs) in Human biology/health: Friends **and** Foes!

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Encodes proteins
mRNA Vaccines
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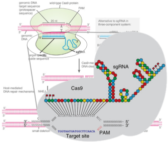

Hendel et al, 2015; Agrotis & Ketteler, 2015

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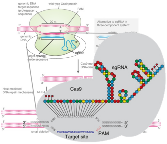

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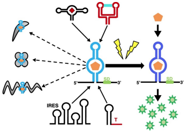

Berens & Suess 2015

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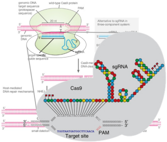

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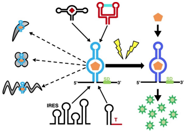

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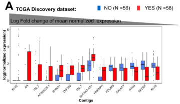

[NGuyen *et al*, 2021]

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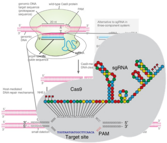

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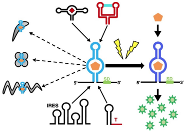

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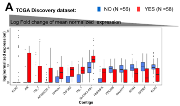
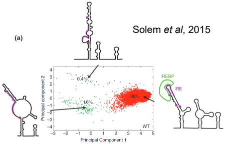


[NGuyen *et al*, 2021]

Solem *et al*, 2015



Non-coding mutations
lncRNAs, miRNAs, structure-associated (RiboSnitches)
β-thalassemia, duchenne muscular dystrophy,
Cystic fibrosis, Rett syndrome...

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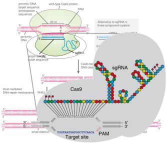
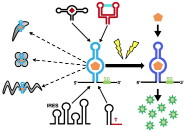


Hendel et al, 2015; Agrotis & Ketteler, 2015



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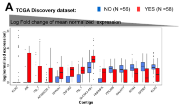

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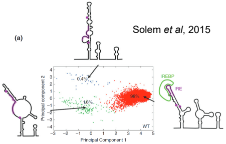

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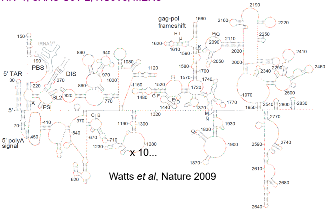
Genomic material for Human pathogens
HIV-1, SARS-CoV 2, HCoVs, MERS



Watts *et al*, Nature 2009

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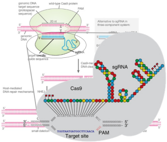

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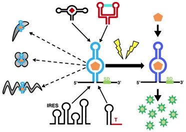

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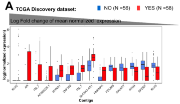
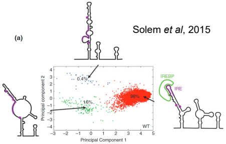


[NGuyen *et al*, 2021]

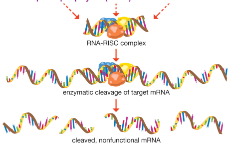


Solem *et al*, 2015

Non-coding mutations
lncRNAs, miRNAs, structure-associated (RiboSnitches)
β-thalassemia, duchenne muscular dystrophy,
Cystic fibrosis, Rett syndrome...

RiboNucleic Acids (RNAs)

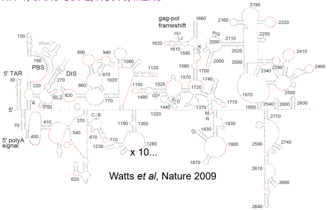
Regulation of gene expression
RNAi therapies (FDA approved)
Primary hyperoxaluria type 1 (PH1),
Hereditary transthyretin amyloidosis (ATTRv),
Acute hepatic porphyria (AHP)



Encyclopaedia Brittannica, Inc 2013

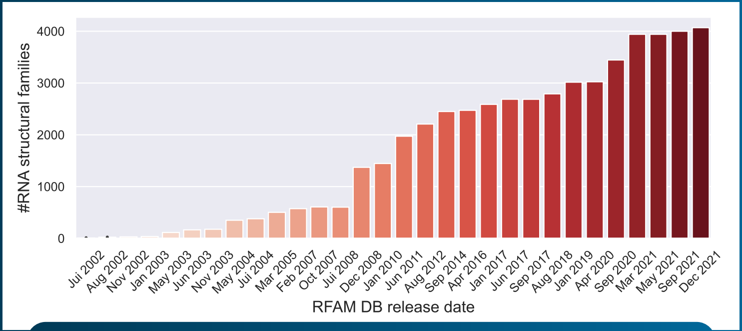
Encodes proteins
mRNA Vaccines
COVID-19, Malaria (Zika, CMV, Cancers?)

Genomic material for Human pathogens
HIV-1, SARS-CoV 2, HCoVs, MERS



Watts *et al*, Nature 2009

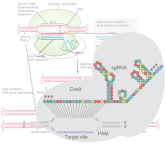
RiboNucleic Acids (RNAs) in Human biology/health: Friends **and** Foes!



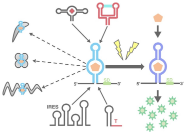
RNA functional diversity is (largely) enabled by deep structural diversity

RiboNucleic Acids (RNAs) in Human biology/health: Friends **and** Foes!

Targeting system for DNA Editing
CRISPR therapies
Sickle-cell anemia, β-thalassamia, Leber congenital amaurosis (LCA), cancers...



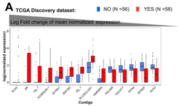
Hendel et al, 2015; Agrotis & Ketteler, 2015



Berens & Suess 2015

Sensor of metabolites
Riboswitches

Quantitative expression
Transcriptomic signatures
Cancer diagnosis/prognosis/relapse...



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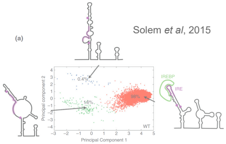

Solem *et al*, 2015

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lncRNAs, miRNAs, structure-associated (RiboSnitches)
β-thalassemia, duchenne muscular dystrophy,
Cystic fibrosis, Rett syndrome...

Rational design

RiboNucleic Acids (RNAs)

(2D) Structure Modeling

Regulation of gene expression
RNAi therapies (FDA approved)
Primary hyperoxaluria type 1 (PH1),
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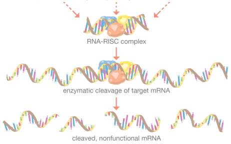

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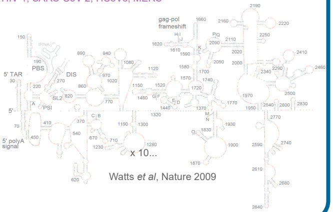
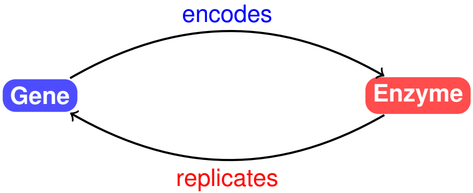


Watts *et al*, Nature 2009

RNA world: Resolving the *chicken vs egg* paradox at the origin of life. . .



A gene big enough to specify an enzyme would be too big to replicate accurately without the aid of an enzyme of the very kind that it is trying to specify. So the system *apparently cannot get started*.

[. . .] This is the RNA World. To see how plausible it is, we need to look at why proteins are good at being enzymes but bad at being replicators; at why DNA is good at replicating but bad at being an enzyme; and finally why *RNA might just be good enough at both roles to break out of the Catch-22.*

R. Dawkins. *The Ancestor's Tale: A Pilgrimage to the Dawn of Evolution*

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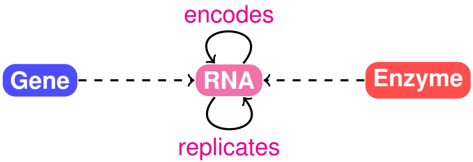

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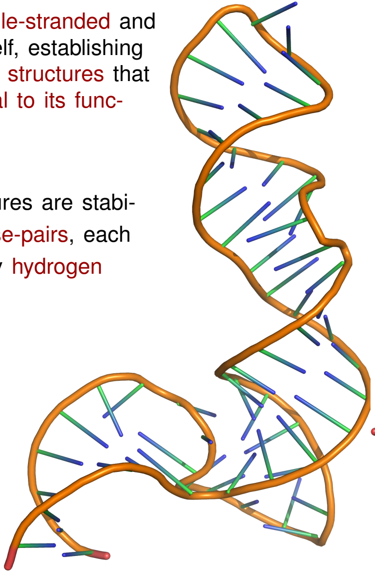
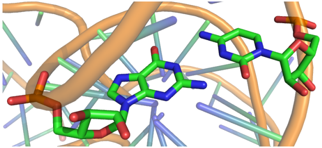
R. Dawkins. *The Ancestor's Tale: A Pilgrimage to the Dawn of Evolution*

RNA folding

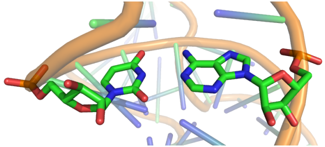
RNA is single-stranded and folds on itself, establishing complex 3D structures that are essential to its function(s).

RNA structures are stabilized by base-pairs, each mediated by hydrogen bonds.



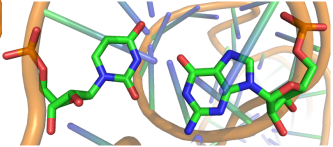


G/C



U/A

Watson/Crick base-pairs



U/G

Wobble base-pair

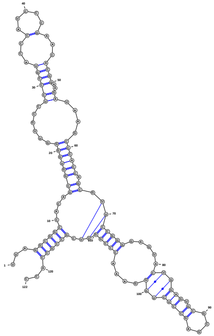
Canonical base-pairs

RNA Structure(s)

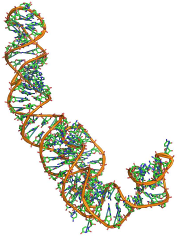
Three[1] levels of representation:

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC
```

Primary structure



Secondary structure



Tertiary structure

Source: 5s rRNA (PDB 1K73:B)

[1] Well, mostly...

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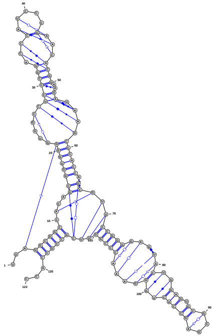
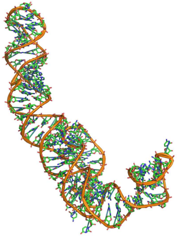
Three[1] levels of representation:

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC
```

Primary structure



Secondary⁺ structure



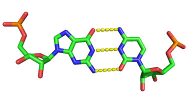
Tertiary structure

Source: 5s rRNA (PDB 1K73:B)

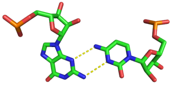
[1] Well, mostly...

Ignored by secondary structure

- **Non-canonical base-pairs**
  Any base-pair other than {(A-U), (C-G), (G-U)}
  Or interacting on non-standard edge ($\neq$ WC/WC-Cis) [LW01].

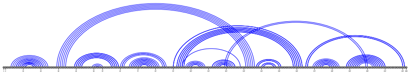


Canonique CG pair(WC/WC-Cis)



Non-canonique CG pair (Sugar/WC-Trans)

- **Pseudoknots (PKs)**



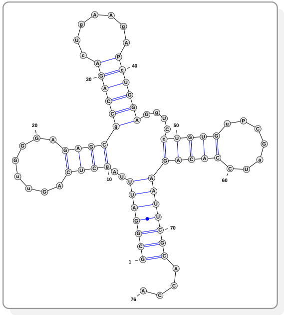
Pseudoknoted structure of group I ribozyme (PDBID: 1Y0Q:A)

Considering PKs may lead to better predictions, but:

- Some PK conformations are simply unfeasible;
- Folding *in silico* with general pseudoknots is NP-complete [LP00];

Still, folding on restricted classes of conformations seems promising [CDR+04].

Various representations for a versatile biomolecule



Outer-planar graphs

Hamiltonian-path, $\Delta(G) \leq 3$, 2-connected*

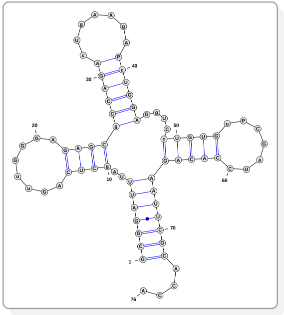
Supporting intuitions

Different representations

Common combinatorial structure

*Additional steric constraints

Various representations for a versatile biomolecule



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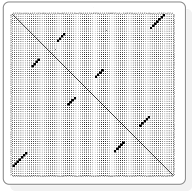

Dot plots

Adjacency matrices*

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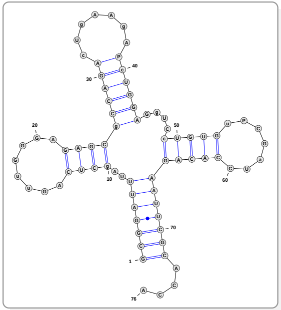

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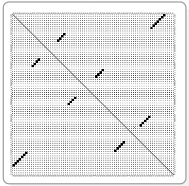

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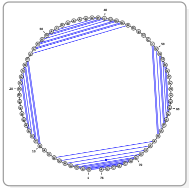

Non-crossing arc diagrams*

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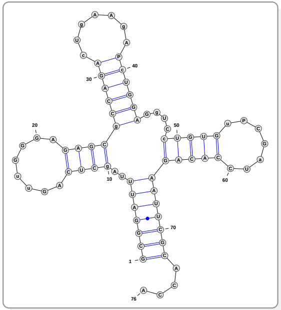

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Hamiltonian-path, $\Delta(G) \leq 3$, 2-connected*

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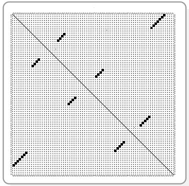
(((((((..((((........))))((((((.......))))))....(((((.......))))))))))))....
```

Motzkin words*



Dot plots

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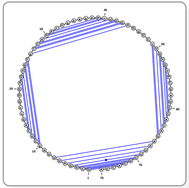

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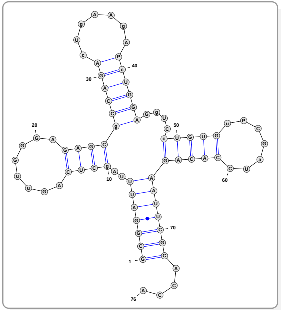

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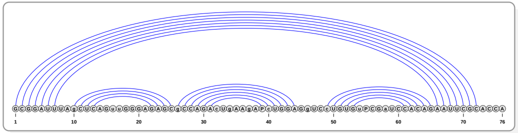
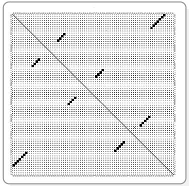


Non-crossing arc-annotated sequences*



Dot plots

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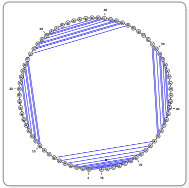

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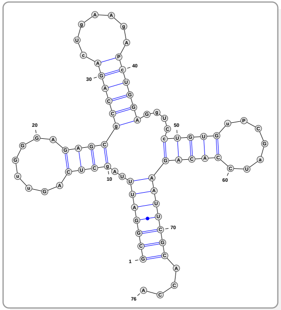

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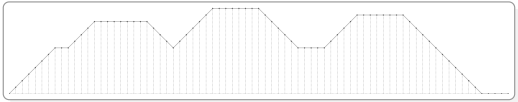
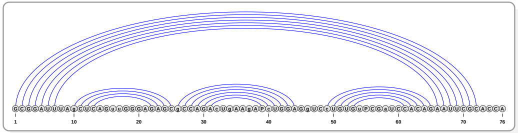
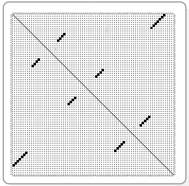


Positive 1D meanders* over $\mathcal{S} = \{+1, -1, 0\}$

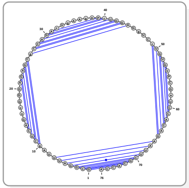


Non-crossing arc-annotated sequences*



Dot plots

Adjacency matrices*



Non-crossing arc diagrams*

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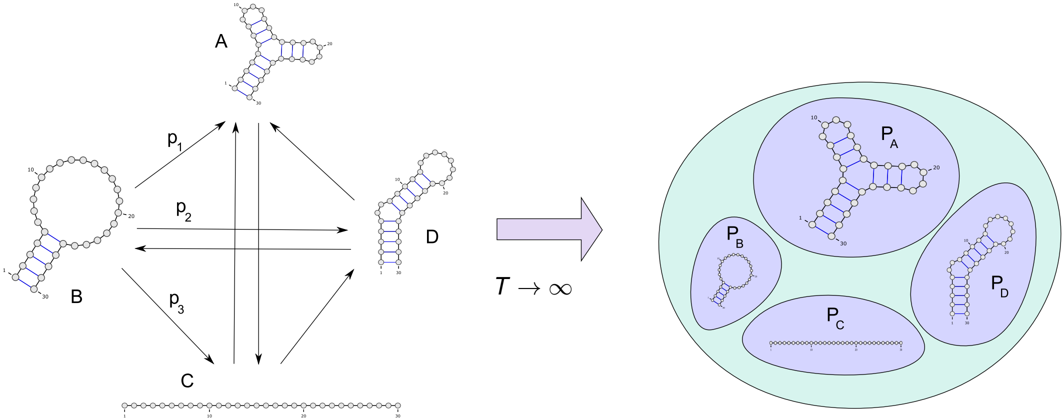
Thermodynamics *aparté*

At the nanoscopic scale, RNA structure *fluctuates* (≈ Markov process).



Convergence towards a stationary distribution at the Boltzmann equilibrium, where the probability of a conformation only depends on its free-energy.

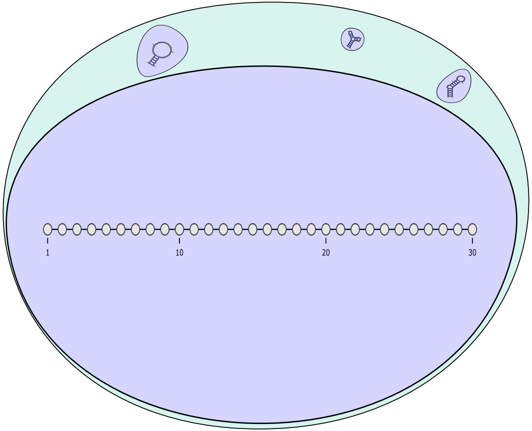
Corollary: Initial conformation does not matter.

Questions: For a given conformation space and free-energy model:

A. Determine most stable (Minimum Free-Energy) structure at equilibrium;

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



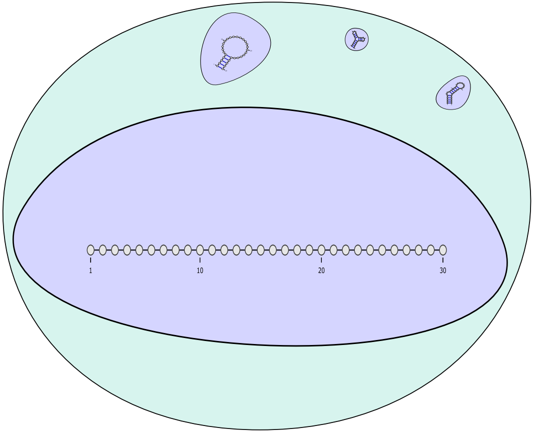
$T = 0$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2] Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



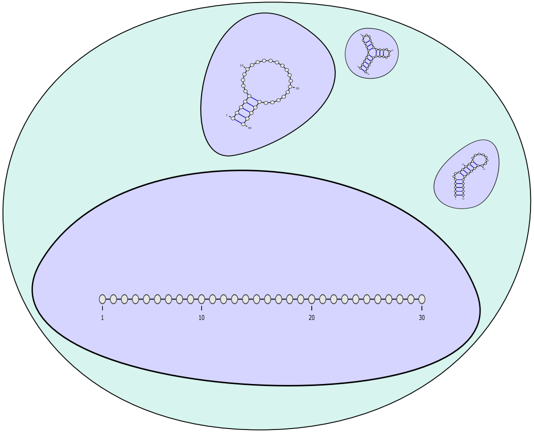
$T = 1h$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2]Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



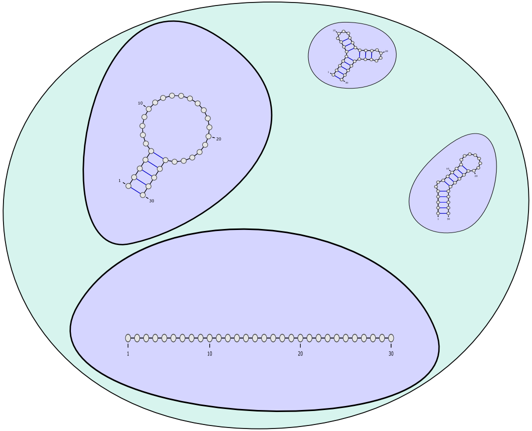
$T = 2h$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2] Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



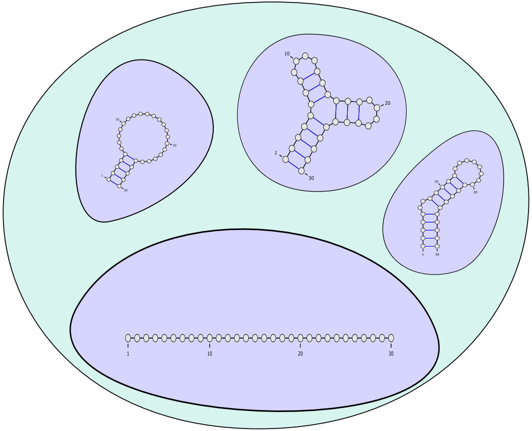
$T = 5h$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2]Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



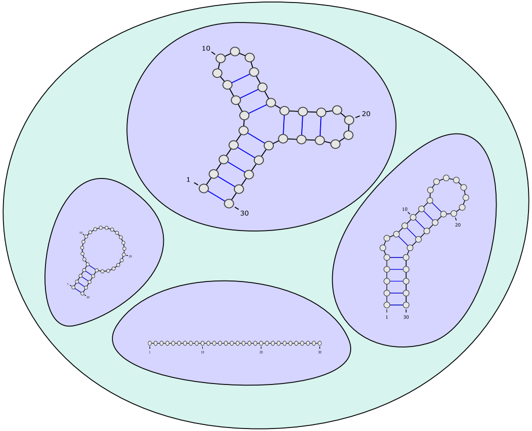
$T = 10h$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2] Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



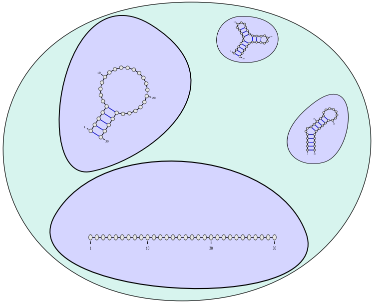
$T \to \infty$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

[2] Except for co-transcriptional folding. . .

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure[2]



$T = 10h$

But most mRNAs are degrade before 7h (Org.: Souris [SSN+09]).

A. Determine most stable (Minimum Free-Energy) structure at equilibrium;
B. Compute average properties of Boltzmann ensemble;
C. Determine most likely structure at finite time T.
(c.f. H. Isambert through simulation, NP-complete deterministically [MTSC09])

[2] Except for co-transcriptional folding...

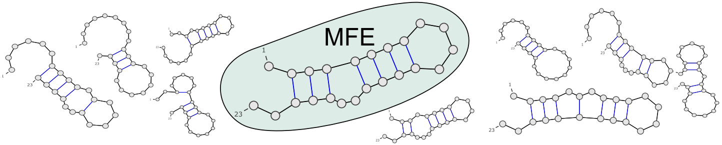
Outline

Folding by minimizing free-energy

Problem A: Determine Minimum Free-Energy structure (MFE).

Ab initio folding prediction =

Predict RNA structure from its sequence ω only.



- **Conformations:** Set S_ω of secondary structures compatible (w.r.t. base-pairing constraints) with primary structure ω .
- **Free-Energy:** Function $E_{\omega,S}$ (KCal.mol^{-1}), additive on motifs occurring in any sequence/conformation couple (ω, S).
- **Native structure:** Functional conformation of the biomolecule.

  Remarks:
  - Not necessarily unique (Kinetics, or bi-stable structures);
  - In presence of PKs $\rightarrow$ Ambiguous: Which is the native conformation?

Nussinov/Jacobson model

Nussinov/Jacobson energy model (NJ)

Base-pair maximization (with a twist):

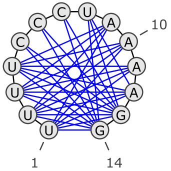
- Additive model on independently contributing base-pairs;
- Canonical base-pairs only: Watson/Crick (A/U,C/G) and Wobble (G/U)

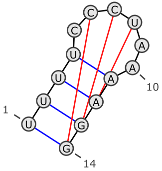
$$\Rightarrow E_{\omega,S} = -\#Paires(S)$$

Folding in NJ model $\Leftrightarrow$ Base-pair (weight) maximization

Example:

UUUUCCCUAAAAGG





Variant: Weight each pair with −#Hydrogen bonds

ΔG(G≡C) = -3 ΔG(A=U) = -2 ΔG(G−U) = -1

Nussinov/Jacobson model

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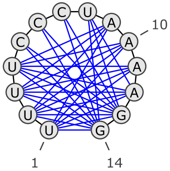

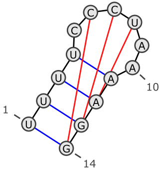

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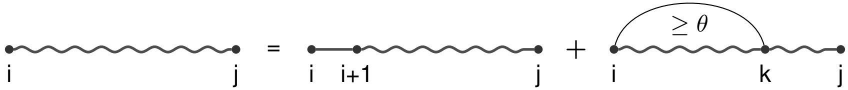
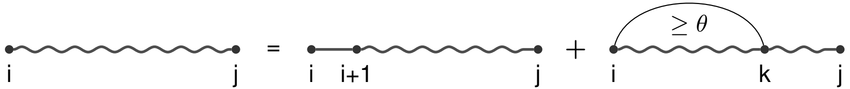
Nussinov/Jacobson DP scheme



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

Nussinov/Jacobson DP scheme



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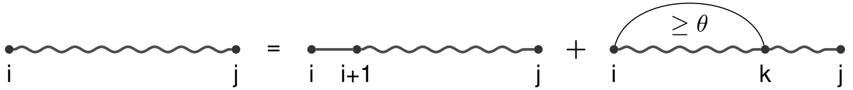
Correctness. Goal = Show that MFE over interval $[i, j]$ is indeed found in $N_{i,j}$ after completing the computation. Proceed by induction:

- Assume that property holds for any $[i', j']$ such that $j' - i' < n$.
- Consider $[i, j], j - i = n$. Let $\text{MFE}_{i,j} :=$ Base-pairs of best struct. on $[i, j]$.
  Then first position i in $\text{MFE}_{i,j} =$ is either:
  - Unpaired: $\text{MFE}_{i,j} = \text{MFE}_{i+1,j}$ $\rightarrow$ free-energy $= N_{i+1,j}$
  - Paired to k: $\text{MFE}_{i,j} = \{(i, k)\} \cup \text{MFE}_{i+1,k-1} \cup \text{MFE}_{k+1,j}$.
    (Indeed, any BP between $[i+1, k-1]$ and $[k+1, j]$ would cross (i, k))
    $\rightarrow$ free-energy $= \Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j}$

Nussinov/Jacobson DP scheme



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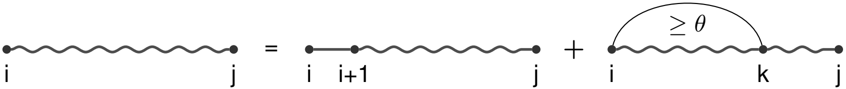
Nussinov/Jacobson DP scheme



$$
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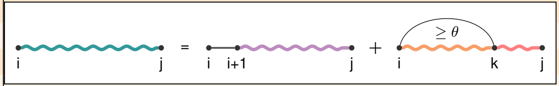
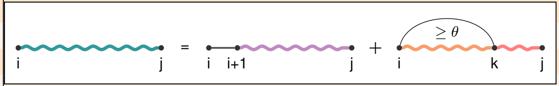
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



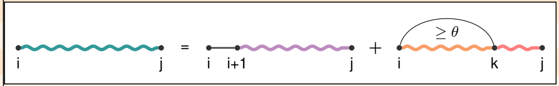
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



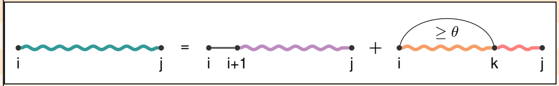
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
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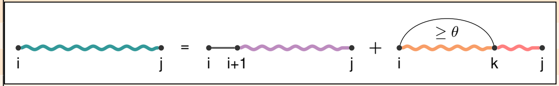
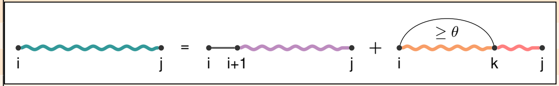
G																	0	0
A																		0



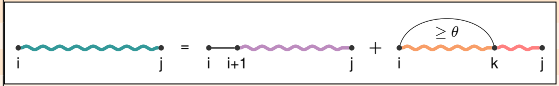
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



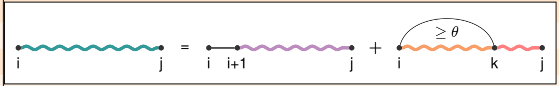
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



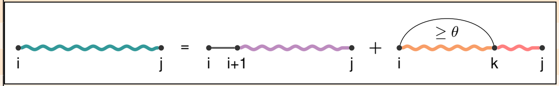
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
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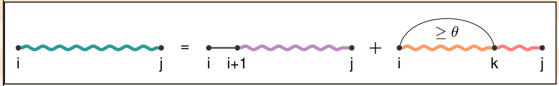

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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A															0	0	0	0
C																0	0	0
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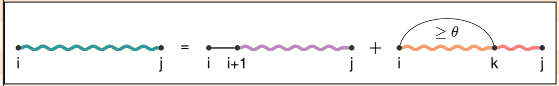

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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A															0	0	0	0
C																0	0	0
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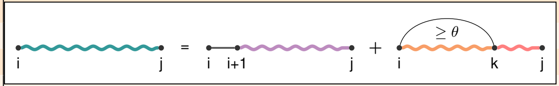

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
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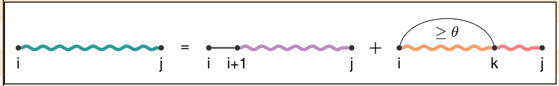

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	.	.	.	.	.	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
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U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
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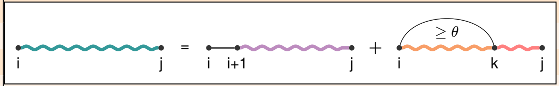

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	.	.	.	.	.	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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A															0	0	0	0
C																0	0	0
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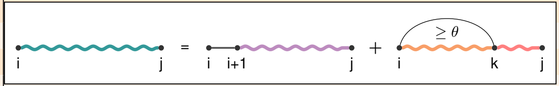
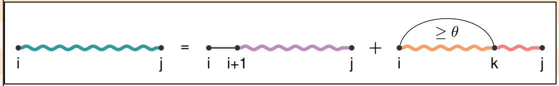


Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	.	.	.	.	.	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
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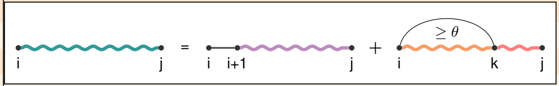

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
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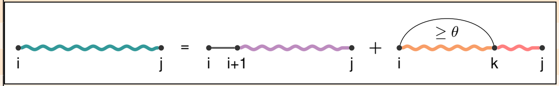

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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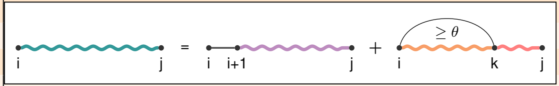
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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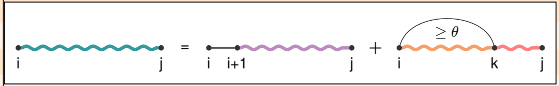

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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A															0	0	0	0
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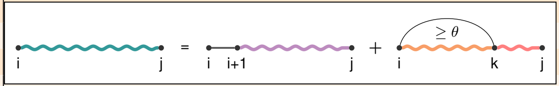

Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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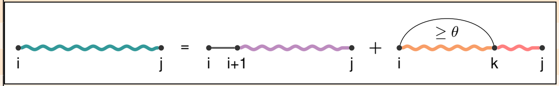
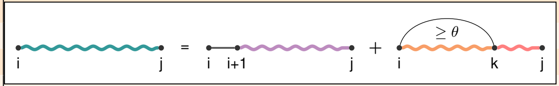


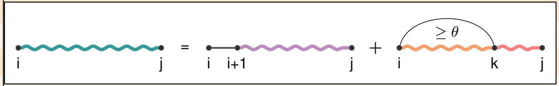
Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	.	.	.	.	.	.	.	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	(	.	.	.	.	.	)	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



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	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	(	.	.	.	.	.	)	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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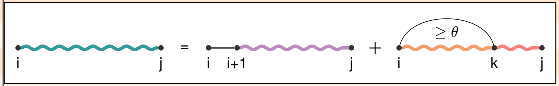
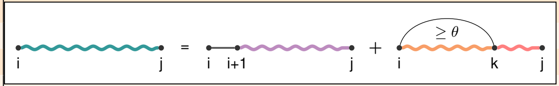


Nussinov/Jacobson

	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	(	.	.	.	.	.	)	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
G		0	0	0	0	0	3	4	4	6	6	6	6	7	9	11	11	11
G			0	0	0	0	3	3	3	5	5	5	5	6	8	10	10	10
A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
U					0	0	0	0	0	0	2	2	4	5	7	7	8	10
A						0	0	0	0	0	2	2	2	5	5	5	8	8
C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



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	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	(	(	.	.	.	)	)	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
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A				0	0	0	0	2	2	2	2	4	4	5	7	7	8	10
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C							0	0	0	0	0	0	2	5	5	5	8	8
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
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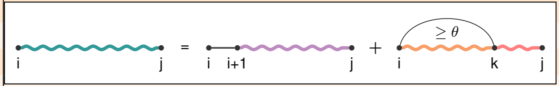

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	C	G	G	A	U	A	C	U	U	C	U	U	A	G	A	C	G	A
	(	(	(	.	.	.	)	.	(	(	.	.	.	)	)	)	)	.
C	0	0	0	0	0	0	3	4	4	6	6	6	6	9	9	11	14	14
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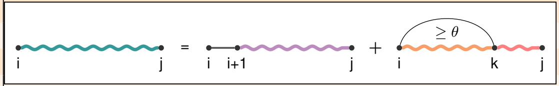
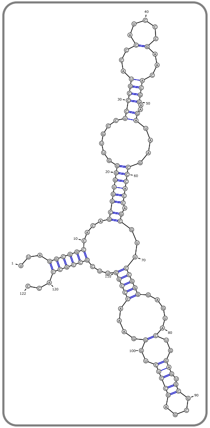
U								0	0	0	0	0	2	3	5	5	6	7
U									0	0	0	0	2	3	5	5	5	7
C										0	0	0	0	3	3	3	5	5
U											0	0	0	0	2	2	2	3
U												0	0	0	0	0	1	2
A													0	0	0	0	0	0
G														0	0	0	0	0
A															0	0	0	0
C																0	0	0
G																	0	0
A																		0



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.

Improved results by taking stacking into account.

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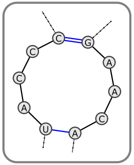

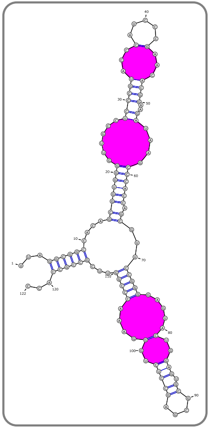

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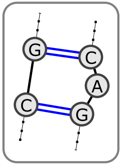

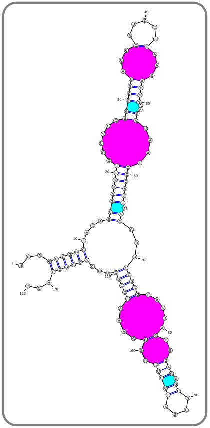

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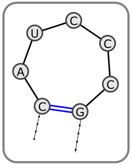

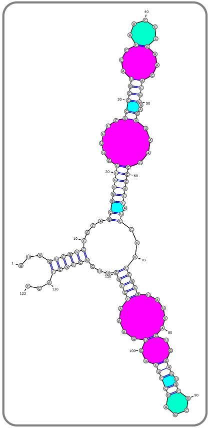

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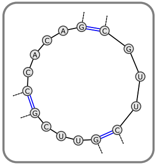

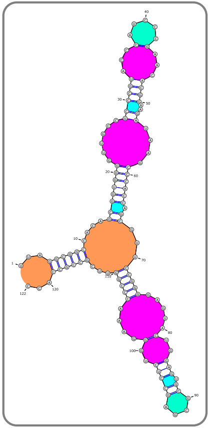

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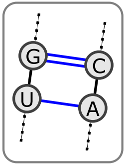

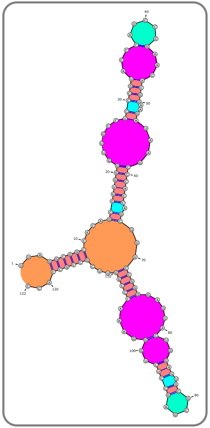

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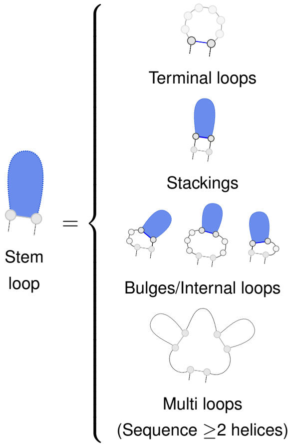
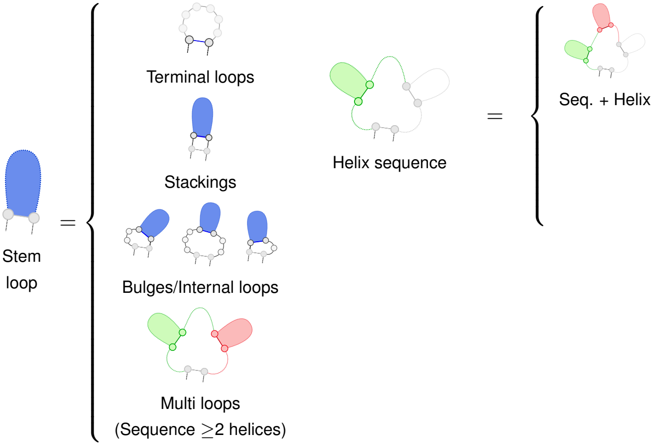
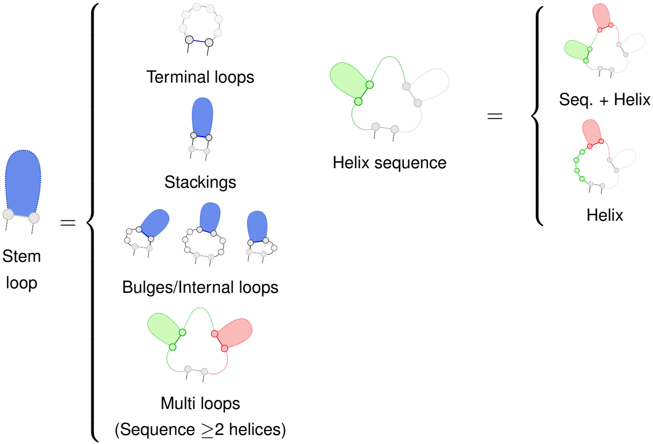
MFE DP equations



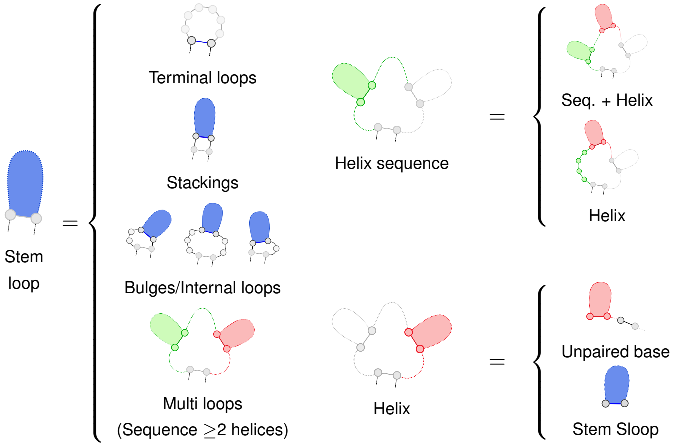
MFE DP equations



MFE DP equations

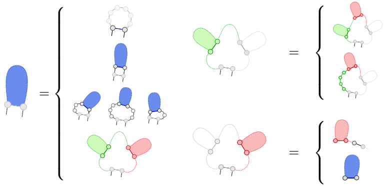


MFE DP equations



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

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

$$\mathcal{M}_{i,j} = \text{Min}_k \left\{ \min\left(\mathcal{M}_{i,k-1}, b(k-1)\right) + \mathcal{M}^1_{k,j} \right\}$$

$$\mathcal{M}^1_{i,j} = \text{Min}_k \left\{ b + \mathcal{M}^1_{i,j-1}, c + \mathcal{M}'_{i,j} \right\}$$

Backtracking

Backtracking to reconstruct MFE structure:

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

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Complexity:

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

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

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

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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[3] time/space complexities in $\mathcal{O}(n^3)/\mathcal{O}(n^2)$

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Backtracking

Backtracking to reconstruct MFE structure:

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Two main approaches

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

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

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- Self-improving

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Sequence

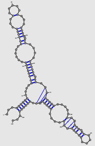
Taille	Sens.
<700	70-73%

[MSZT99, DCCG04]

16s,23s	$\sim$50%

MCC$\sim$ 0.5 [GG04]

Thermodynamics



2^{ary} Structure

Sens.	Spé.	MCC.
$\sim$75%	$\sim$75%	0.8

Comparative [GG04]

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

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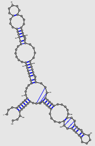

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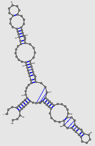

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Towards a 3D ab-initio prediction

Goal: From sequence to all-atom/coarse grain 3D models!!!

- Comparative models + Molecular dynamics: `RNA2D3D` [SYKB07]
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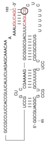
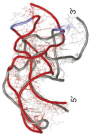
Séquence

→ Comparative `KNetFold` →



2^{ary} Structure + Pseudoknots

→ Molecular dynamics →



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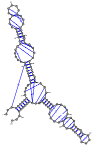
→ `MCFold` →



2^{ary+} Structure

→ `MCSym` →

3^{ary} Structure

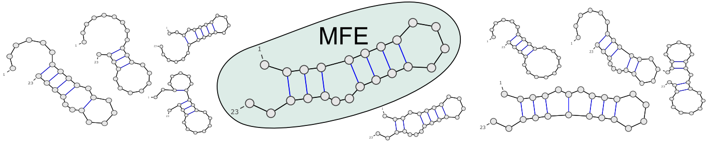
Outline

The canonical Boltzmann Ensemble

RNA *breathes* ⇒ 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.
⇒ 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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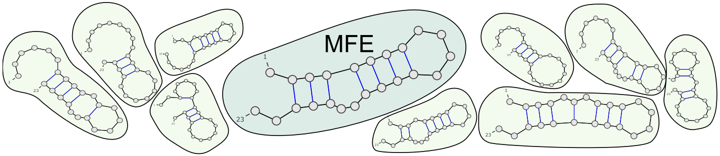

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In particular, structurally close structures may *ally*, and become the most realistic candidate in the search for a functional conformation.

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

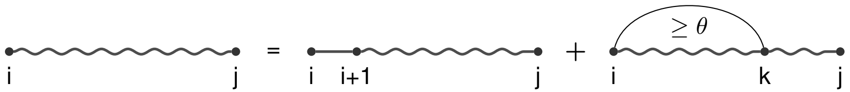
$$\mathcal{Z}_\omega = \sum_{S \in \mathcal{S}_\omega} e^{\frac{-E_{S,\omega}}{RT}}$$

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

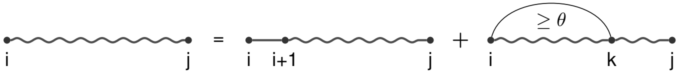


$$
\begin{aligned}
N_{i,t} &= 0, \quad \forall t \in [i, i+\theta] \\
N_{i,j} &= \min \begin{cases} N_{i+1,j} & i \text{ unpaired} \\ \min\limits_{k=i+\theta+1}^{j} \Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j} & i \text{ paired with } k \end{cases}
\end{aligned}
$$

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 :

$$
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\end{aligned}
$$

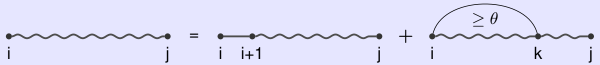
Recurrence for counting compatible structures :

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

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

Partition function

Partition function = Weighted count over compatible structures



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

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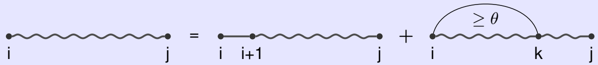

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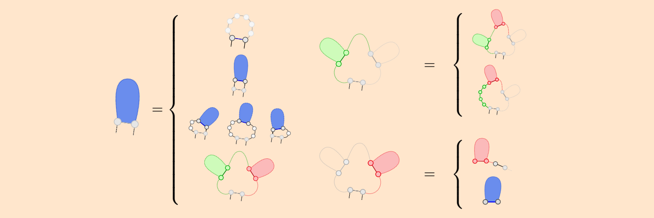

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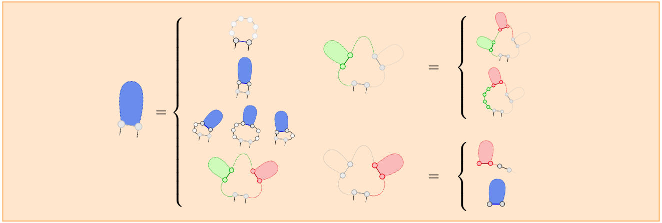

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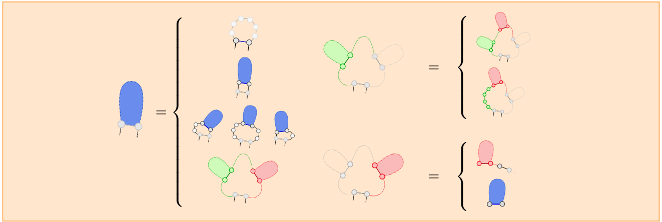

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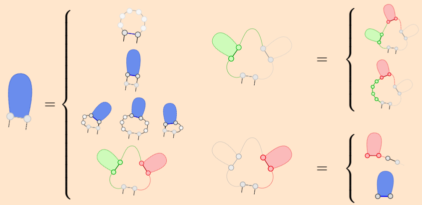

$$\mathcal{Z}'(i,j) = \sum \begin{cases} e^{\frac{-E_H(i,j)}{RT}} \\ e^{\frac{-E_S(i,j)}{RT}} \mathcal{Z}'(i+1,j-1) \\ +\sum \left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} \mathcal{Z}'(i',j') \right) \\ +e^{\frac{-(a)}{RT}} \sum \left(\mathcal{Z}(i+1,k-1)\mathcal{Z}^1(k,j-1) \right) \end{cases}$$

$$\mathcal{Z}(i,j) = \sum \left(\mathcal{Z}(i,k-1) + e^{\frac{-b(k-1)}{RT}} \right) \mathcal{Z}^1(k,j)$$

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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

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

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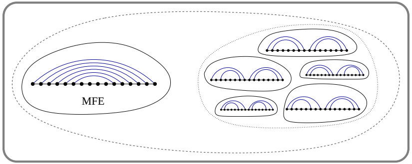
Statistical sampling of RNA 2^{ary} structures

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

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

Stochastic backtrack (adapted from `SFold`)

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

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r ↓

$A_1 | A_2 | B_i | B_{i+1} | \ldots | B_{j-1} | B_j | C_i | C_{i+1} | \ldots | C_{j-1} | C_j$

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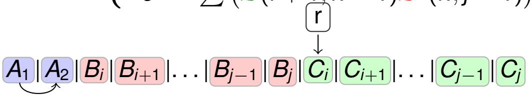

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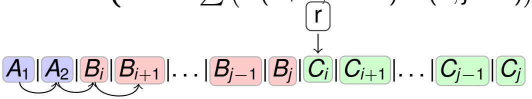

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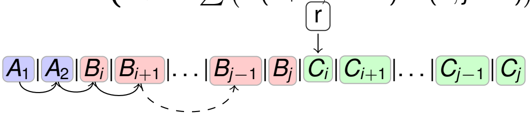

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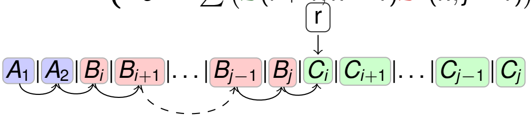

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Correctness: Each $S \in \mathcal{S}_\omega$ uniquely generated (DP scheme unambiguity)
Therefore the probability of generated S is

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

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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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Correctness: Each $S \in \mathcal{S}_\omega$ uniquely generated (DP scheme unambiguity)
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

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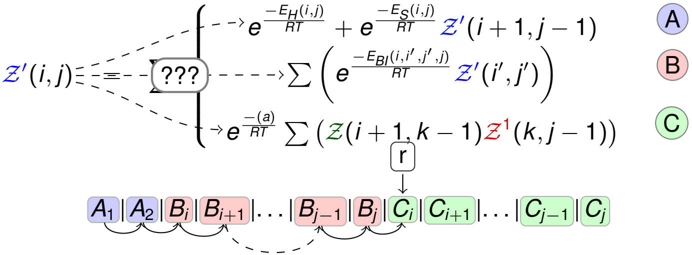

Average-case complexity in $\Theta(k \times n\sqrt{n})$ (homopolymer model) [Pon08].
Boustrophedon search $\Rightarrow \mathcal{O}(k \times n \log n)$ worst-case [Pon08].

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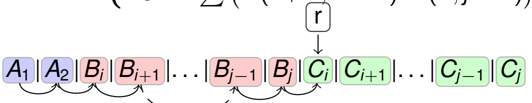

After $\Theta(n)$ operations, recurse over region of length $n-1$
$\Rightarrow$ Worst-case complexity in $\mathcal{O}(k \times n^2)$ for k samples

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].

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