

M2 BIM – STRUCT – Lecture 1

Folding RNA *in silico*

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CNRS LIX, École Polytechnique

Outline

Introduction

Dynamic programming 101

Why RNA?

RNA folding

RNA Structure(s)

Some representations of RNA structure

Some flavours of folding prediction

Thermodynamics vs Kinetics

Dynamic programming: Reminder

Free-energy minimization

Nussinov-style RNA folding

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 =??

55

60

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$$21 = \text{50c} + \text{1c}$$

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$$60 = \text{50c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} + \text{1c} ??$$
$$= \text{20c} + \text{20c} + \text{20c} !$$

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 #Coins^N (Ouch!)

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

$$\text{Min\#Coins}(N) = \text{Min} \left\{ \begin{array}{l} \text{1€} \rightarrow 1 + \text{Min\#Coins}(N - 1) \\ \text{2€} \rightarrow 1 + \text{Min\#Coins}(N - 20) \\ \text{5€} \rightarrow 1 + \text{Min\#Coins}(N - 50) \end{array} \right.$$

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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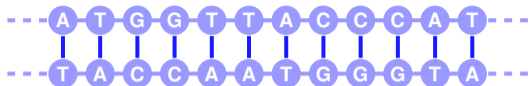
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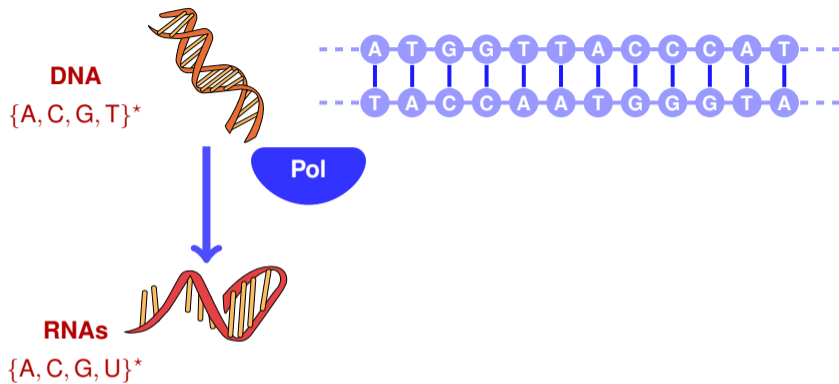
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Fundamental *dogma* of molecular biology

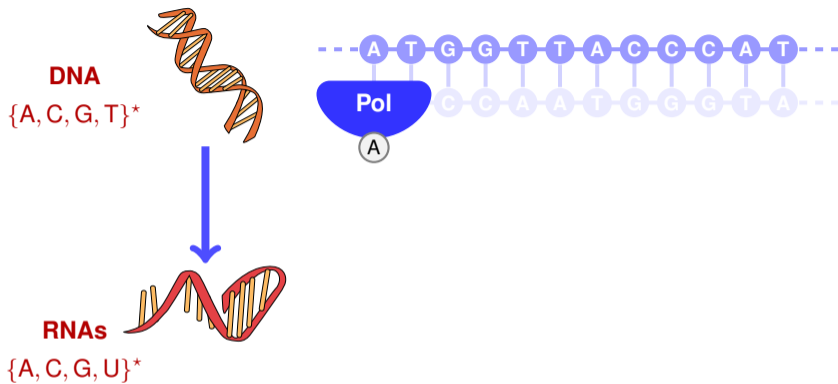
DNA
{A, C, G, T}^{*}



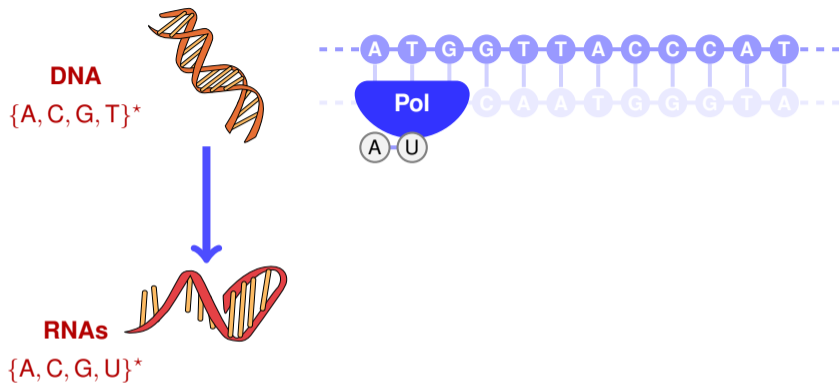
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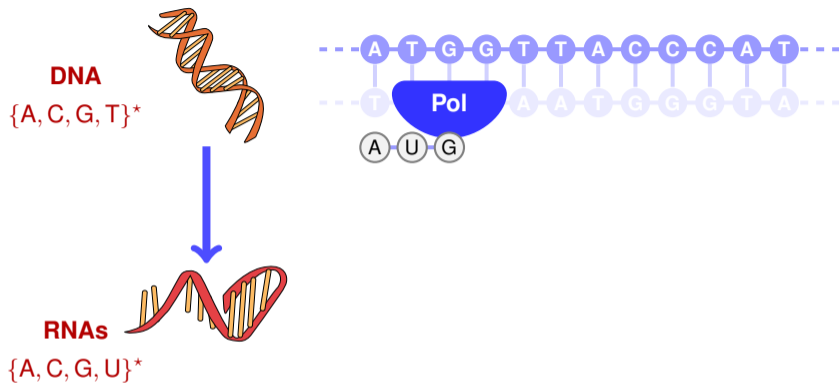
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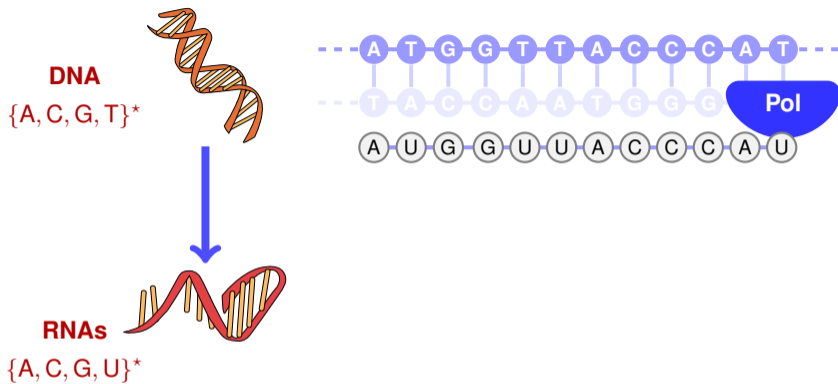
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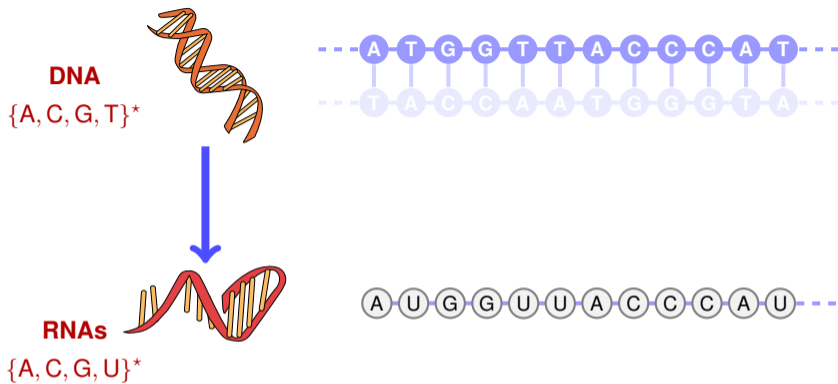
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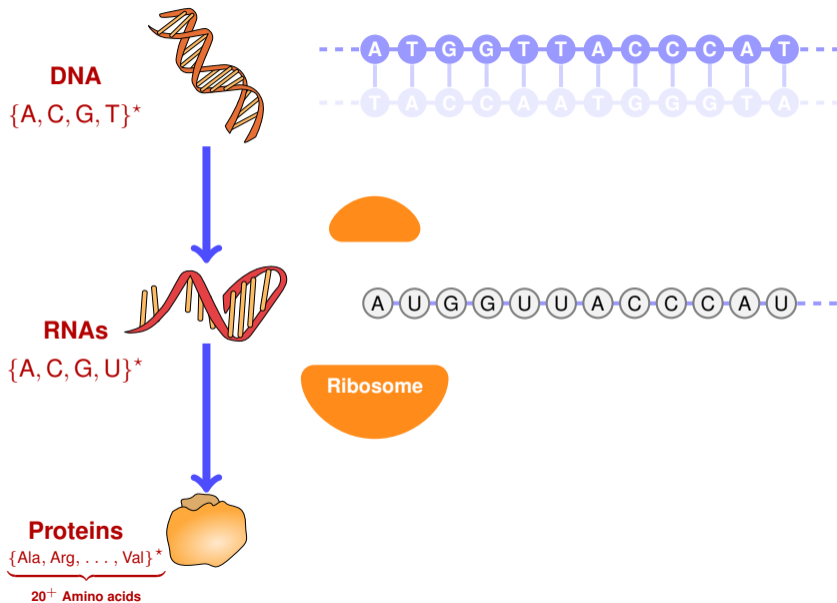
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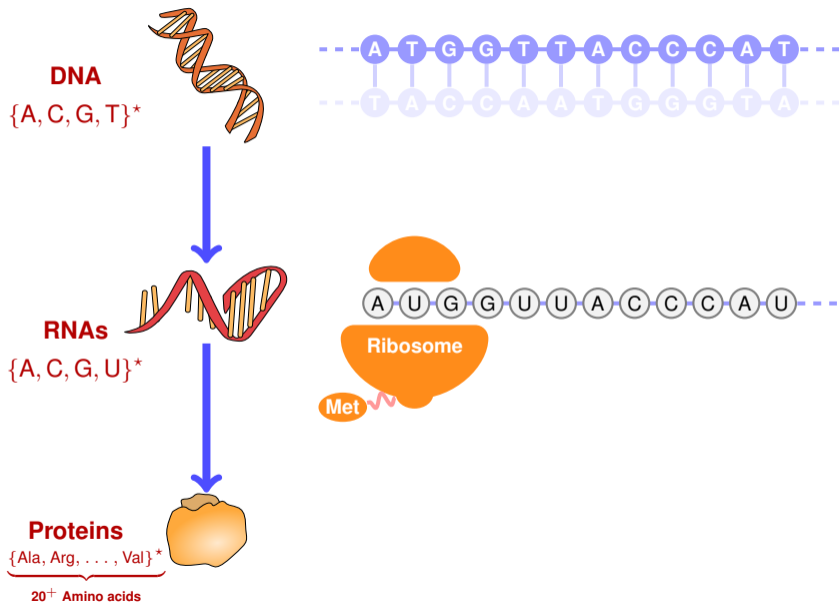
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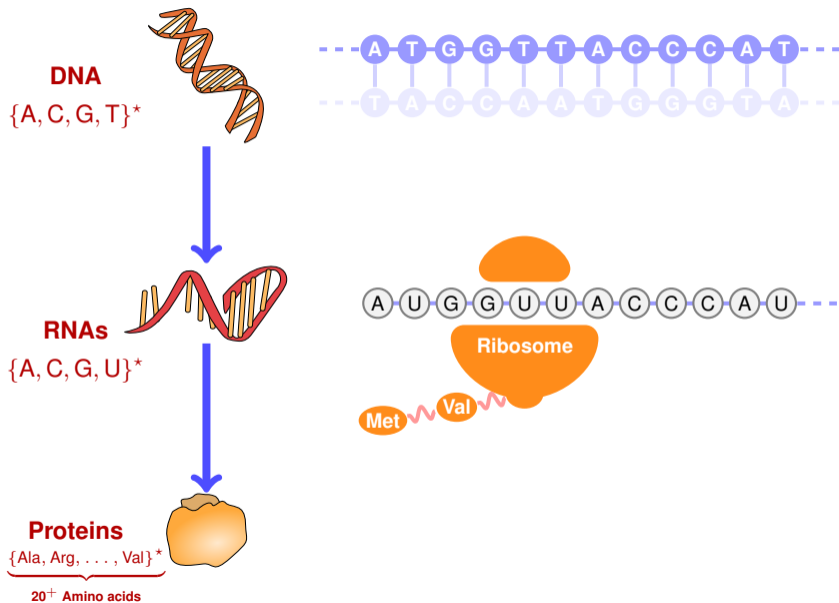
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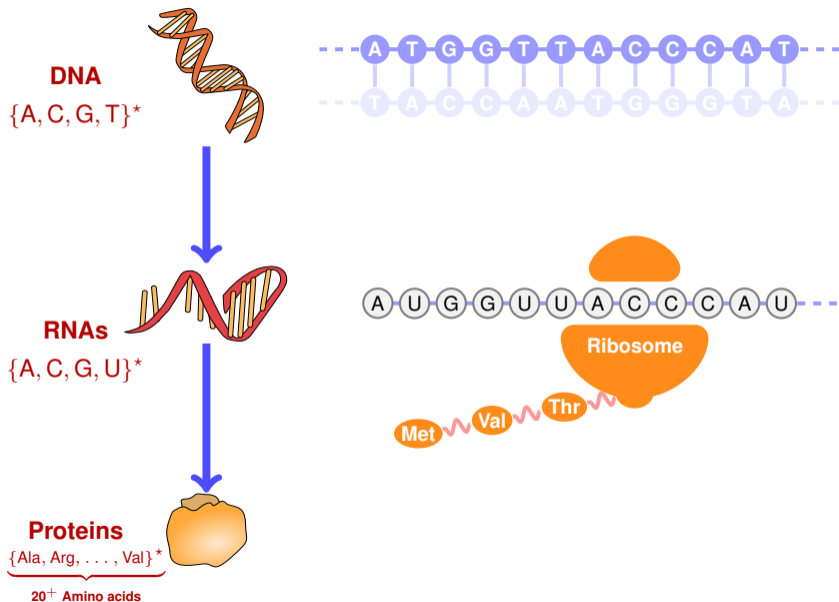
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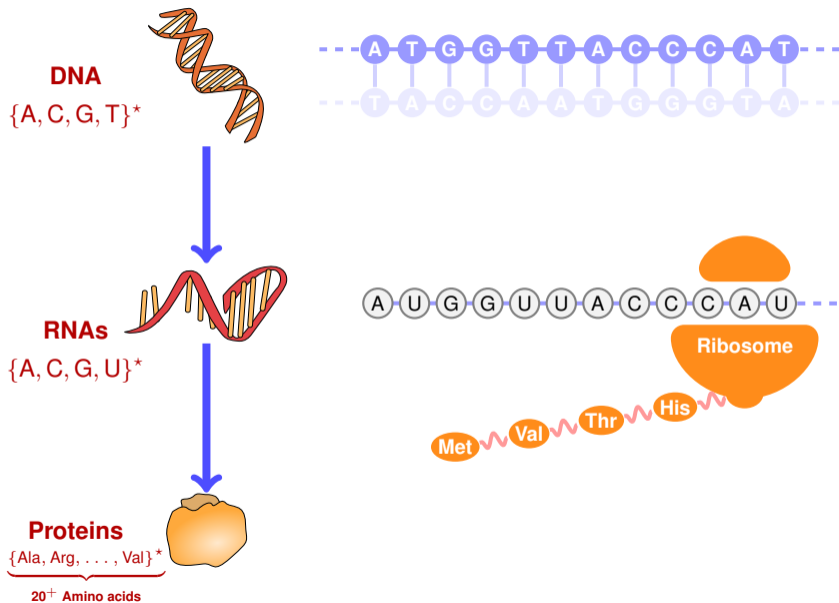
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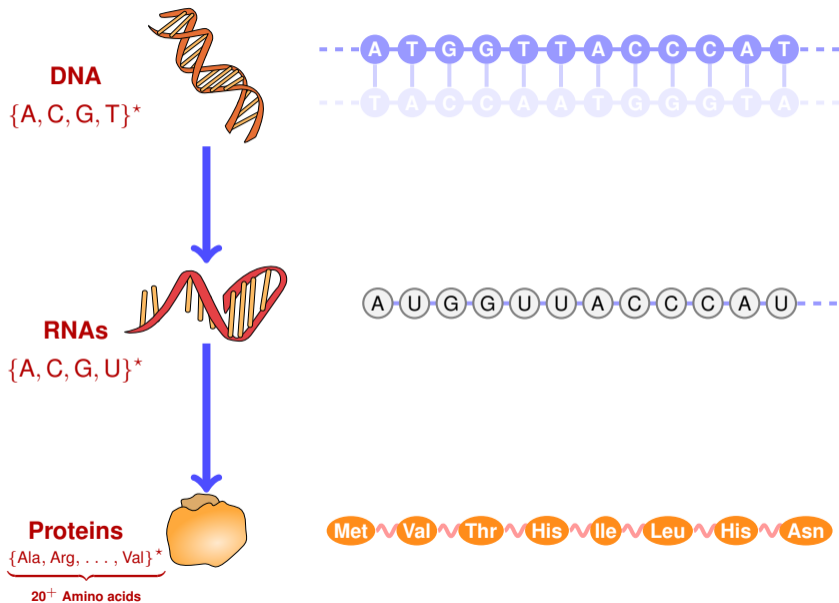
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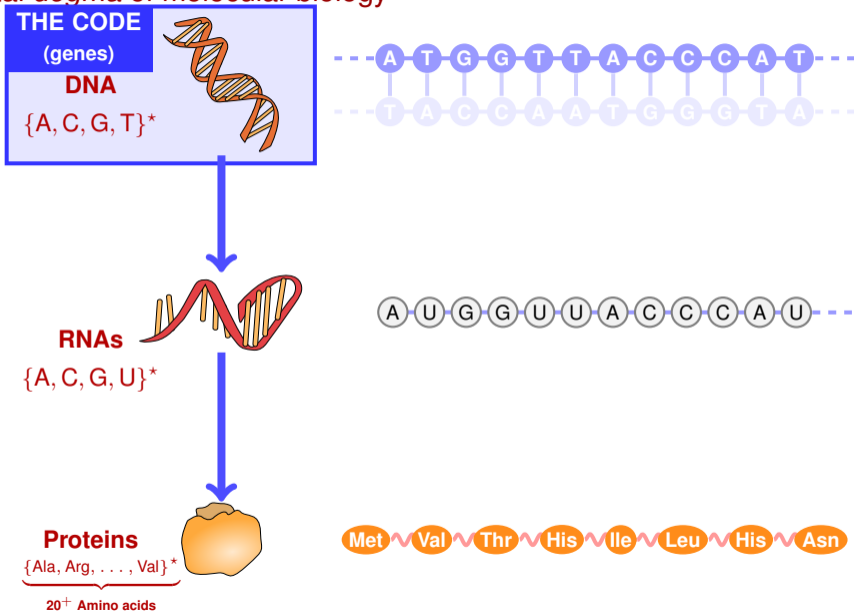
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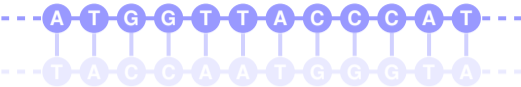

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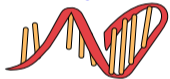
Fundamental *dogma* of molecular biology

THE CODE
(genes)

DNA
{A, C, G, T}^{*}




RNAs
{A, C, G, U}^{*}



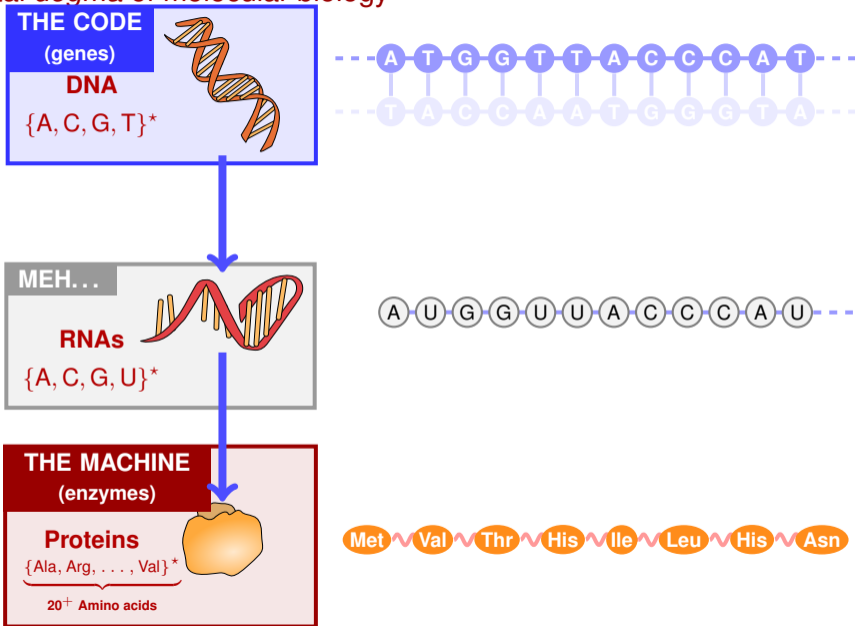
THE MACHINE
(enzymes)

Proteins
{Ala, Arg, . . . , Val}^{*}

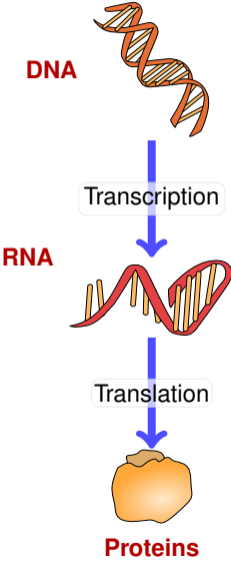
20⁺ Amino acids



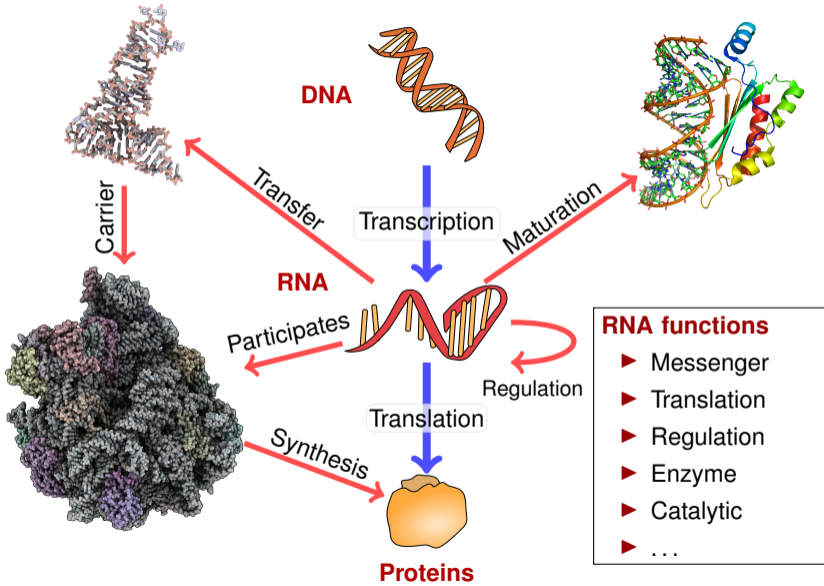
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RiboNucleic Acids (RNAs) in Human biology/health: Friends **and** Foes!

RiboNucleic Acids (RNAs)



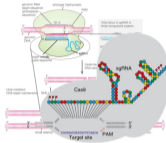
Encodes proteins
mRNA Vaccines
COVID-19, Malaria (Zika, CMV, Cancers?)

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Targeting system for DNA Editing

CRISPR therapies

Sickle-cell anemia, β -thalassamia, Leber congenital amaurosis (LCA), cancers...



Hendel et al, 2015; Agrotis & Ketteler, 2015

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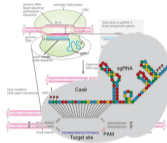
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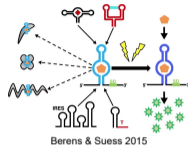
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Sensor of metabolites

Riboswitches

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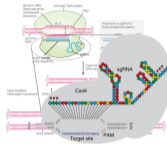
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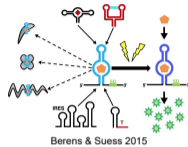
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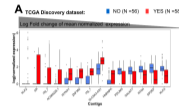
Berens & Suess 2015

Sensor of metabolites
Riboswitches

Quantitative expression

Transcriptomic signatures

Cancer diagnosis/prognosis/relapse...



[NGuyen et al, 2021]

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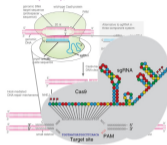
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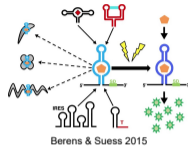
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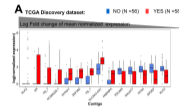
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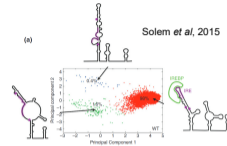
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[NGuyen et al, 2021]



Non-coding mutations

IncrRNAs, miRNAs, structure-associated (RiboSnitches)

β -thalassemia, duchenne muscular dystrophy, Cystic fibrosis, Rett syndrome...

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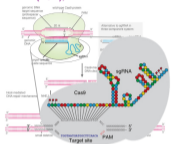
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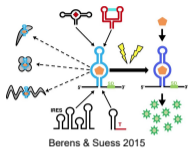
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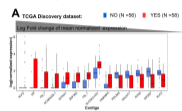
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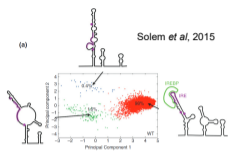
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[NGuyen et al, 2021]



Solem et al, 2015

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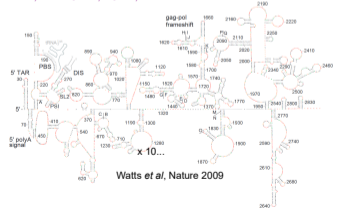
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Genomic material for Human pathogens

HIV-1, SARS-CoV 2, HCoVs, MERS

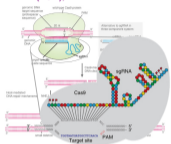


Watts et al, Nature 2009

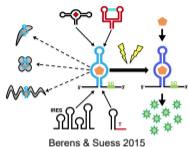
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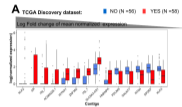
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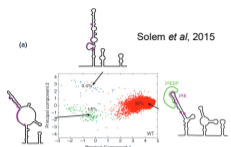
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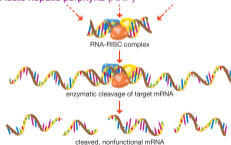
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RiboNucleic Acids (RNAs)



Regulation of gene expression

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



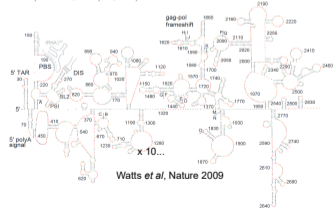
Encyclopaedia Britannica, Inc 2013



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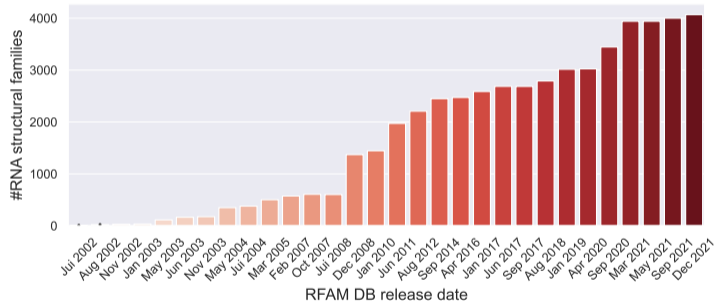
Sickle-cell anemia, β -thalassaemia, Leber congenital amaurosis (LCA), cancer

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Solem et al. 2015



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

Encyclopaedia Britannica, Inc. 2013

mRNA Vaccines

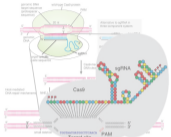
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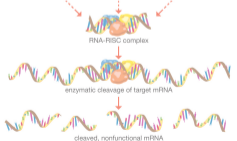


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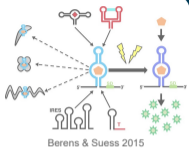
Rational design

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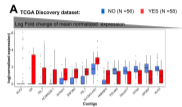


Berens & Suess 2015

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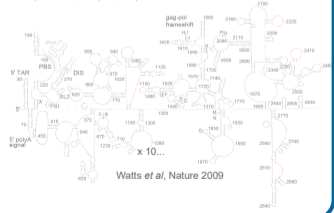
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(2D) Structure Modeling

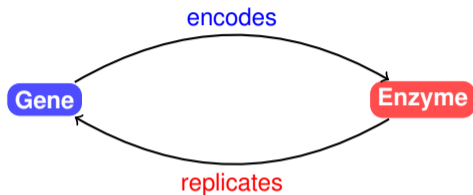
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Watts et al, Nature 2009

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

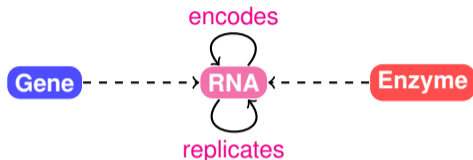


A **gene** big enough to specify **an enzyme** would be too big to replicate accurately without the aid of **an enzyme** of the very kind that it is trying to specify. So the system *apparently cannot get started*.

[...] 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*

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*.

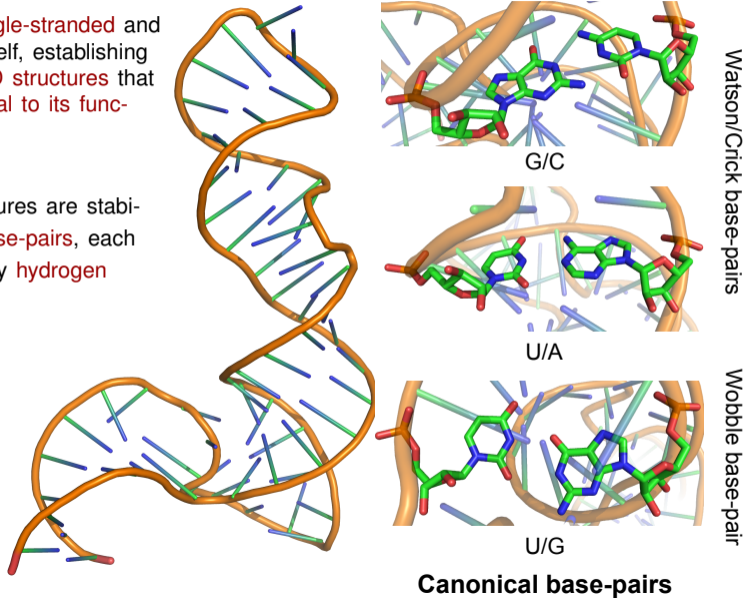
[...] This is the **RNA World**. To see how plausible it is, we need to look at why proteins are good at being enzymes but bad at being replicators; at why DNA is good at replicating but bad at being an enzyme; and finally why **RNA might just be good enough at both roles to break out of the Catch-22**.

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

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**.

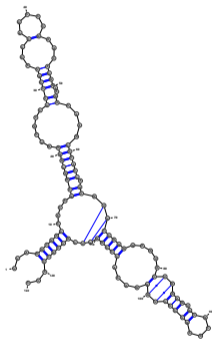


RNA Structure(s)

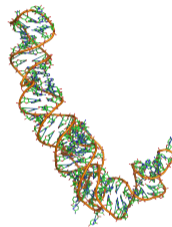
Three¹ levels of representation:

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCGAA
CACGGAAGAUAGCC
CACCAGCGUUCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGAAA
CCCGGUUCGCCCA
CC
```

Primary structure



Secondary structure



Tertiary structure

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

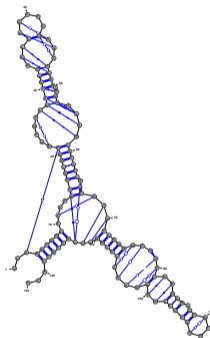
¹Well, mostly...

RNA Structure(s)

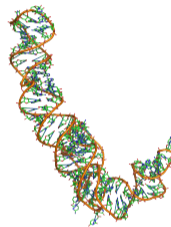
Three¹ levels of representation:

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCGAA
CACGGAAGAUAAAGCC
CACCAGCGUUCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGAAA
CCCGGUUCGCCGCA
CC
```

Primary structure



Secondary⁺ structure



Tertiary structure

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

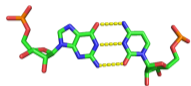
¹Well, mostly...

Ignored by secondary structure

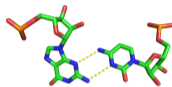
► Non-canonical base-pairs

Any base-pair **other than** {(A-U), (C-G), (G-U)}

Or interacting on non-standard edge (\neq WC/WC-Cis) [LW01].

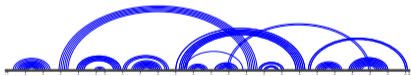


Canonique CG pair(WC/WC-Cis)



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

► Pseudoknots (PKs)



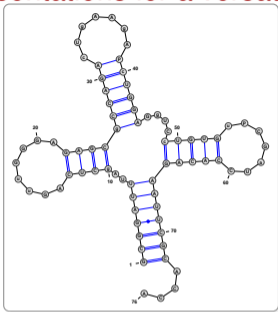
Pseudoknotted structure of group I ribozyme (PDBID: 1Y0Q:A)

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*

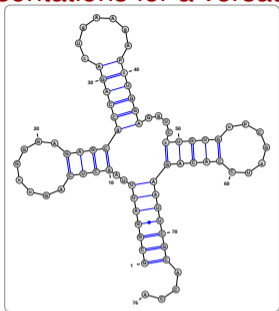
Supporting intuitions

Different representations

Common combinatorial structure

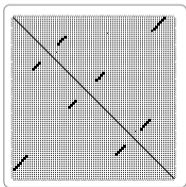
* Additional steric constraints

Various representations for a versatile biomolecule



Outer-planar graphs

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



Dot plots

Adjacency matrices*

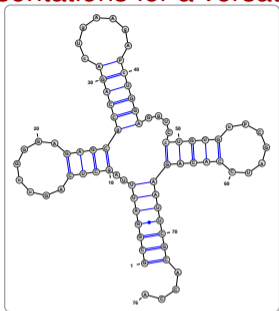
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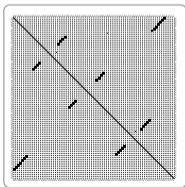
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Various representations for a versatile biomolecule

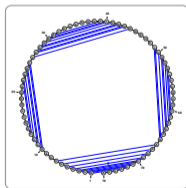


Outer-planar graphs

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



Dot plots
Adjacency matrices*



Non-crossing arc diagrams*

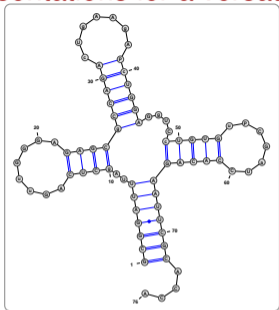
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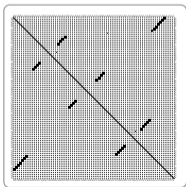


(((((((...(((.....))))))((((.....))))))....((((.....))))))....

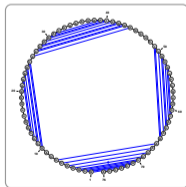
Motzkin words*

Outer-planar graphs

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



Dot plots
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Non-crossing arc diagrams*

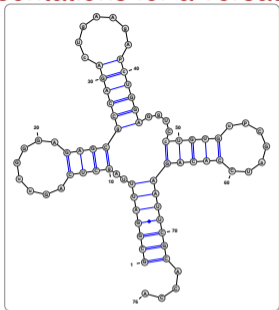
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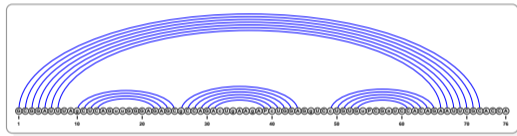


Outer-planar graphs

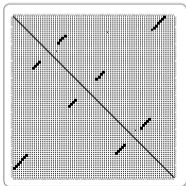
Hamiltonian-path, $\Delta(G) \leq 3$, 2-connected*

((((((...(((.....))))))(((.....)))))).... (((.....)))))))))....

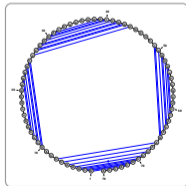
Motzkin words*



Non-crossing arc-annotated sequences*



Dot plots
Adjacency matrices*



Non-crossing arc diagrams*

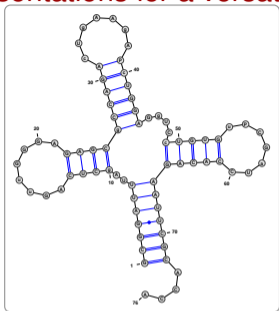
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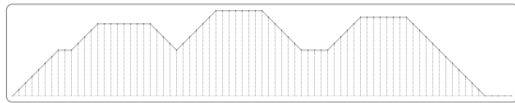


Outer-planar graphs

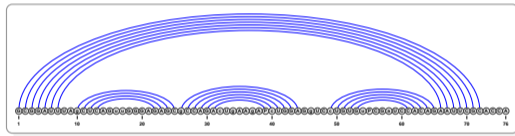
Hamiltonian-path, $\Delta(G) \leq 3$, 2-connected*

((((((...(((.....))))))(((.....))))))...(((.....)))))))))....

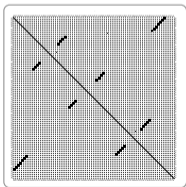
Motzkin words*



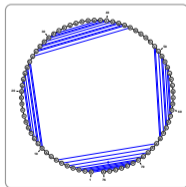
Positive 1D meanders* over $S = \{+1, -1, 0\}$



Non-crossing arc-annotated sequences*



Dot plots
Adjacency matrices*



Non-crossing arc diagrams*

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Outline

Introduction

Dynamic programming 101

Why RNA?

RNA folding

RNA Structure(s)

Some representations of RNA structure

Some flavours of folding prediction

Thermodynamics vs Kinetics

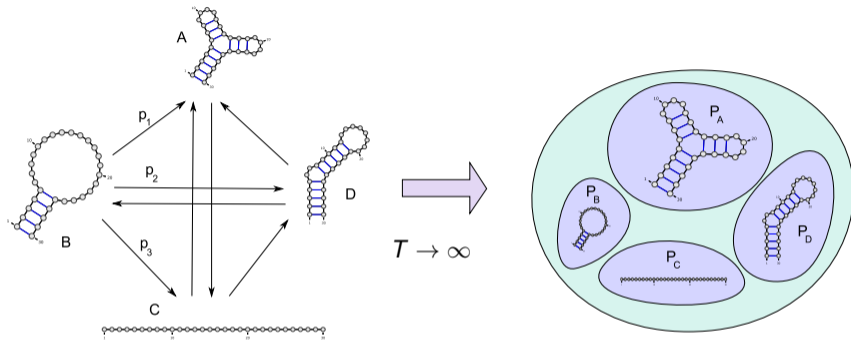
Dynamic programming: Reminder

Free-energy minimization

Nussinov-style RNA folding

Thermodynamics *aparté*

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



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

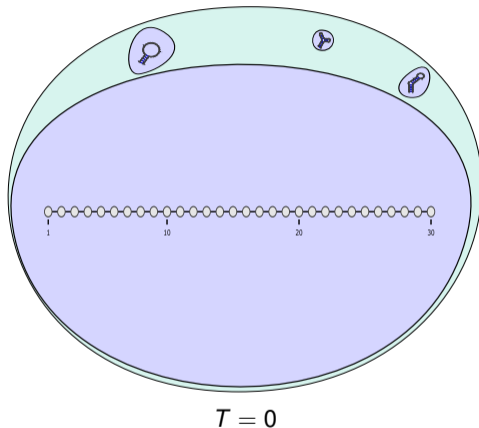
Corollary: Initial conformation does not matter.

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

- Determine most stable (Minimum Free-Energy) structure at equilibrium;
- Compute average properties of Boltzmann ensemble;

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure²

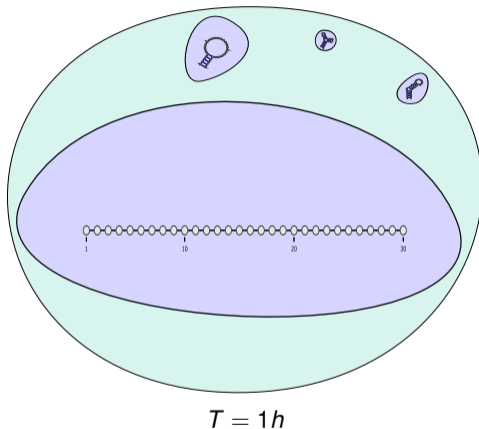


But most mRNAs are degraded before 7h (Org.: Souris [SSN⁺09]).

²Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure²

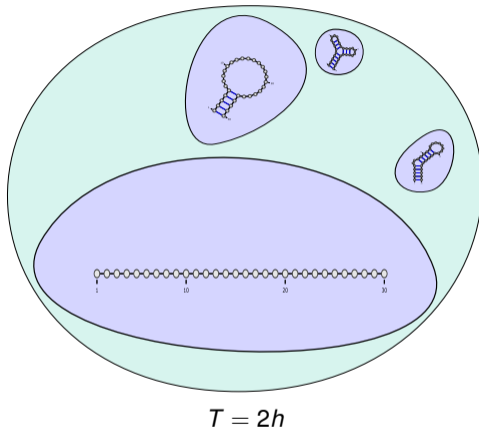


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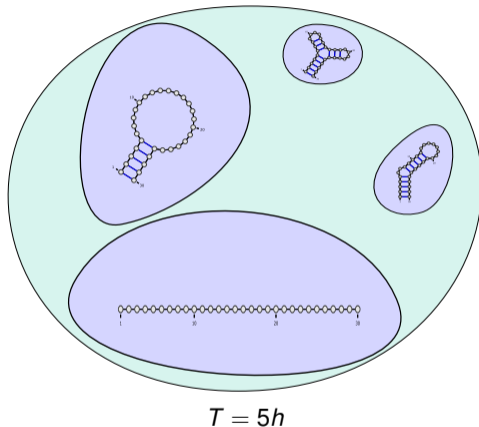


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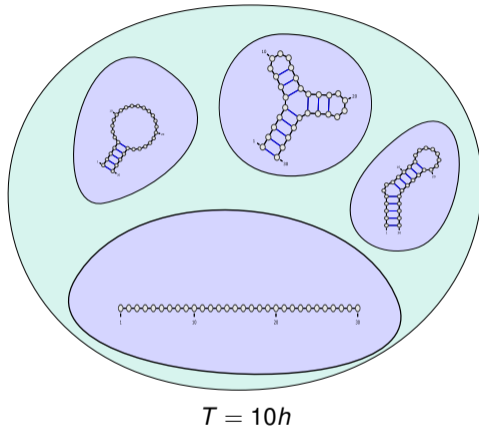


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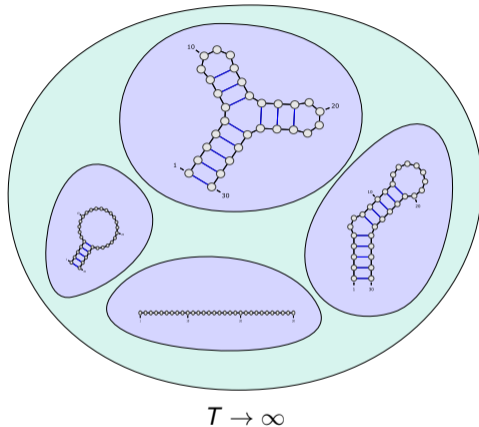


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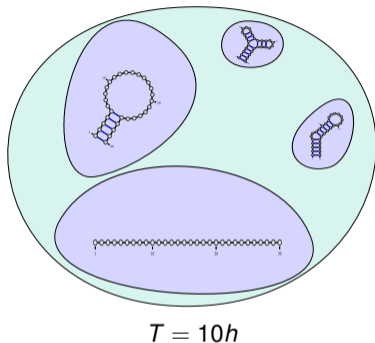


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²Except for co-transcriptional folding...

Away from equilibrium

Transcription: RNA synthesized, supposedly without structure²



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

- Determine most stable (Minimum Free-Energy) structure at equilibrium;
- Compute average properties of Boltzmann ensemble;
- Determine most likely structure at finite time T .

(c.f. H. Isambert through simulation, NP-complete deterministically [MTSC09])

²Except for co-transcriptional folding...

Dynamic programming: General principle

Dynamic programming = General optimization technique.

Prerequisite: Optimal solution for problem P can be derived from solutions to strict sub-problems.

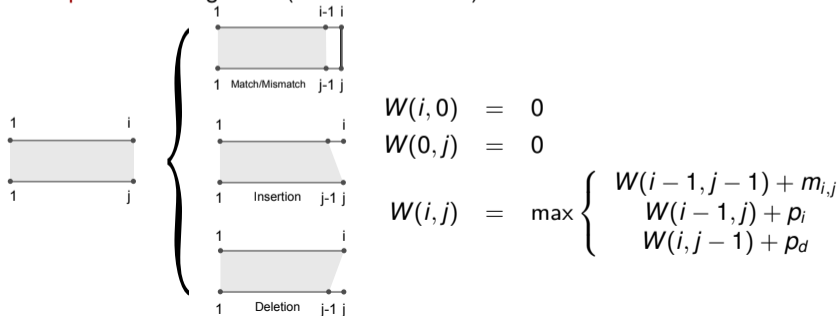
Bioinformatics :

Discrete solution space (alignments, structures...)

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

⇒ Efficient dynamic programming scheme

Example: Local Alignment(Smith/Waterman)



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

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0								
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACACTA

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	→ 2							
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

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$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1						
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2					
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1				
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Diagram illustrating the dynamic programming table for local alignment. The table shows the alignment of AGCACACA (rows) and ACACACTA (columns). The values in the table represent the maximum score for the alignment up to that point. Red arrows indicate the path of the optimal alignment: A (row 1) aligns with A (col 2), C (row 2) aligns with C (col 3), A (row 3) aligns with A (col 4), and C (row 4) aligns with C (col 5). A grey arrow points from the cell (3,4) to (3,5), indicating a deletion of 'A' in the second sequence.

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0								
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Diagram illustrating the dynamic programming table for local alignment of AGCACACA and ACACACTA. The table shows the maximum score $W(i, j)$ for each subproblem. Red arrows indicate the path of the optimal alignment: (0,0) to (1,1), (1,1) to (2,2), (2,2) to (3,3), (3,3) to (4,4), (4,4) to (5,5), (5,5) to (6,6), (6,6) to (7,7), (7,7) to (8,8), (8,8) to (9,9).

Complete example

Example: Local alignment of AGCACACA and ACACA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0								
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0	0	3	2	3	2	3	2	1
A	0								
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACAETA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0	0	3	2	3	2	3	2	1
A	0	2	2	5	4	5	4	3	4
C	0								
A	0								
C	0								
A	0								

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A	
	0	0	0	0	0	0	0	0	0	
A	0	0	2	1	2	1	2	1	0	2
G	0	0	1	1	1	1	1	1	0	1
C	0	0	0	3	2	3	2	3	2	1
A	0	0	2	2	5	4	5	4	3	4
C	0	0	1	4	4	7	6	7	6	5
A	0	0	2	3	6	6	9	8	7	8
C	0	0	1	4	5	8	8	11	10	9
A	0	0	2	3	6	7	10	10	10	12

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

		A	C	A	C	A	C	T	A
	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0	0	3	2	3	2	3	2	1
A	0	2	2	5	4	5	4	3	4
C	0	1	4	4	7	6	7	6	5
A	0	2	3	6	6	9	8	7	8
C	0	1	4	5	8	8	11	10	9
A	0	2	3	6	7	10	10	10	12

Complete example

Example: Local alignment of AGCACACA and ACACACTA

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Best alignment

		A	C	A	C	A	C	T	A
0	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0	0	3	2	3	2	3	2	1
A	0	2	2	5	4	5	4	3	4
C	0	1	4	4	7	6	7	6	5
A	0	2	3	6	6	9	8	7	8
C	0	1	4	5	8	8	11	10	9
A	0	2	3	6	7	10	10	10	12

Complete example

Example: Local alignment of AGCACACA and ACACACTA

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

Best alignment

A
A

		A	C	A	C	A	C	T	A
0	0	0	0	0	0	0	0	0	0
A	0	2	1	2	1	2	1	0	2
G	0	1	1	1	1	1	1	0	1
C	0	0	3	2	3	2	3	2	1
A	0	2	2	5	4	5	4	3	4
C	0	1	4	4	7	6	7	6	5
A	0	2	3	6	6	9	8	7	8
C	0	1	4	5	8	8	11	10	9
A	0	2	3	6	7	10	10	10	12

Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

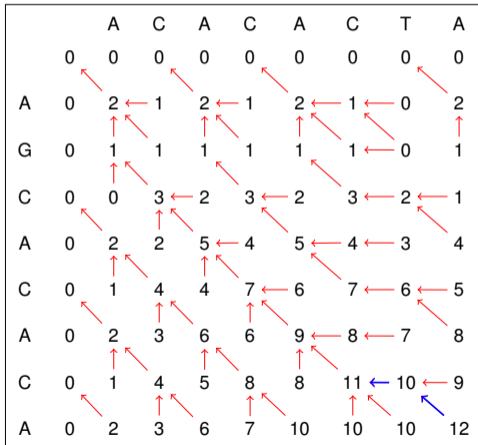
$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Best alignment

- A
T A



Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

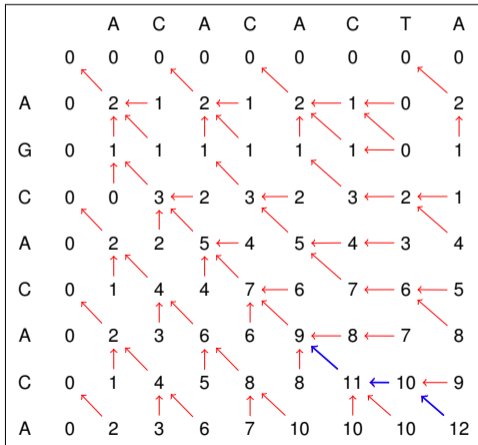
$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Best alignment

C - A
C T A



Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

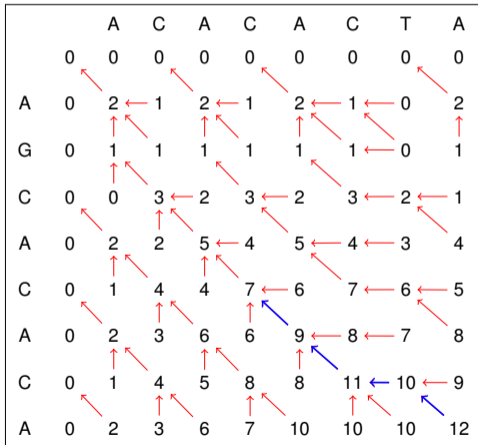
$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Best alignment

A C - A
A C T A



Complete example

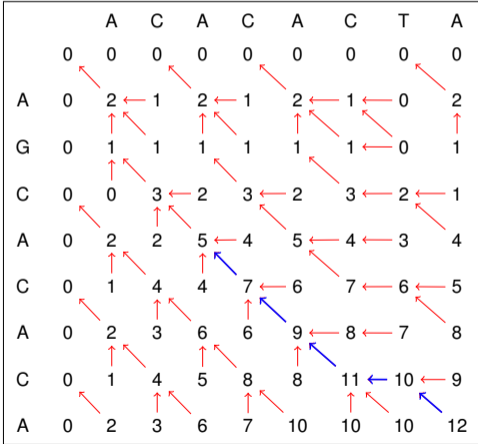
Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

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

Best alignment

C A C - A
C A C T A



Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

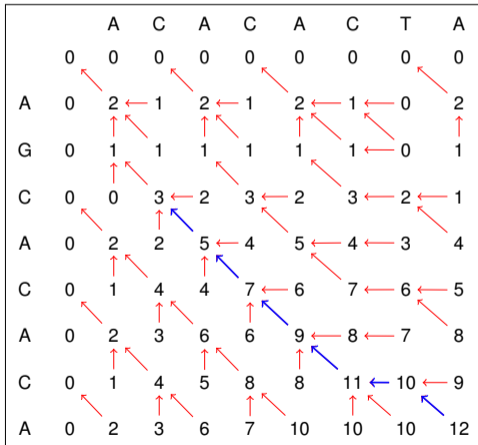
$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Best alignment

A C A C - A
 A C A C T A



Complete example

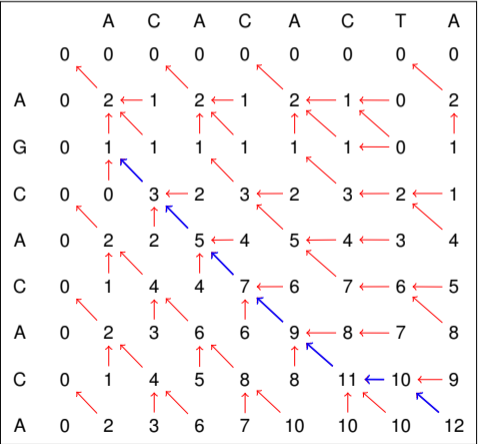
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Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

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

Best alignment

C A C A C - A
C A C A C T A



Complete example

Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

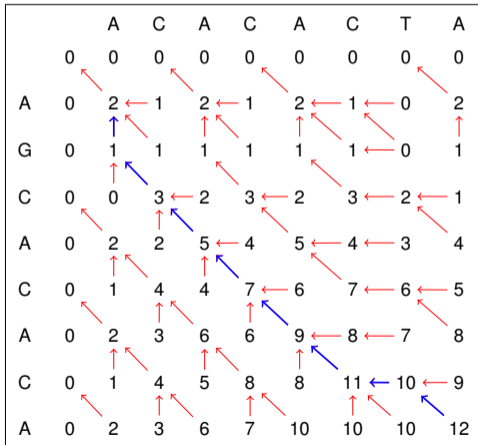
$$W(i, 0) = 0$$

$$W(0, j) = 0$$

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

Best alignment

G C A C A C - A
 - C A C A C T A



Complete example

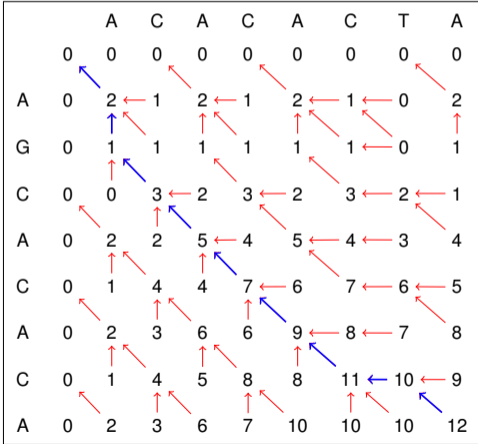
Example: Local alignment of AGCACACA and ACACACTA

Costs: Match $m_{i,j} = +2$, Insertion/Déletion $p_i = p_j = -1$

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

Best alignment

A G C A C A C - A
A - C A C A C T A



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.

Outline

Introduction

Dynamic programming 101

Why RNA?

RNA folding

RNA Structure(s)

Some representations of RNA structure

Some flavours of folding prediction

Thermodynamics vs Kinetics

Dynamic programming: Reminder

Free-energy minimization

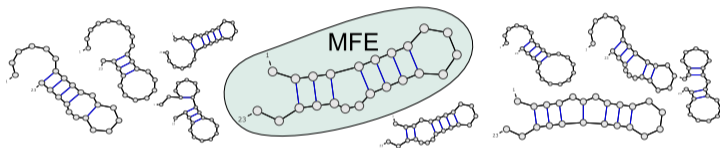
Nussinov-style RNA folding

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 → Ambiguous: Which is the native conformation?

Nussinov/Jacobson model

Nussinov/Jacobson energy model (NJ)

Base-pair maximization (with a twist):

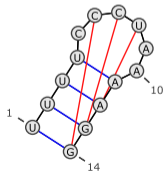
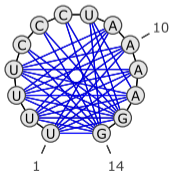
- ▶ Additive model on **independently contributing** base-pairs;
- ▶ **Canonical base-pairs** only: Watson/Crick (A/U,C/G) and Wobble (G/U)

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

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

Example:

UUUUCCCUAAAAGG



Variant: Weight each pair with $-\#Hydrogen\ bonds$

$$\Delta G(G \equiv C) = -3$$

$$\Delta G(A = U) = -2$$

$$\Delta G(G - U) = -1$$

Nussinov/Jacobson model

Nussinov/Jacobson energy model (NJ)

Base-pair maximization (with a twist):

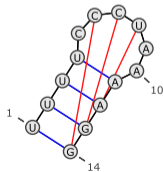
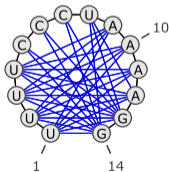
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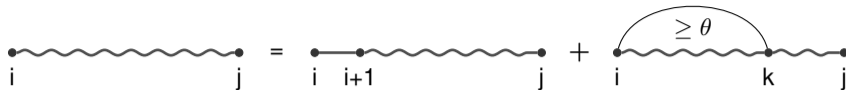
Variant: Weight each pair with $-\#Hydrogen\ bonds$

$$\Delta G(G \equiv C) = -3$$

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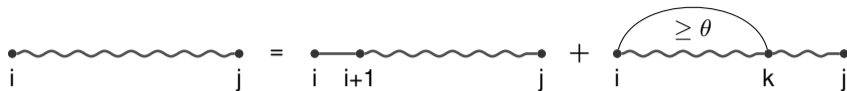
Nussinov/Jacobson DP scheme



$$N_{i,t} = 0, \quad \forall t \in [i, i + \theta]$$

$$N_{i,j} = \min \begin{cases} N_{i+1,j} & i \text{ unpaired} \\ \min_{k=i+\theta+1}^j \Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j} & i \text{ paired with } k \end{cases}$$

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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:** $\text{MFE}_{i,j} = \text{MFE}_{i+1,j}$

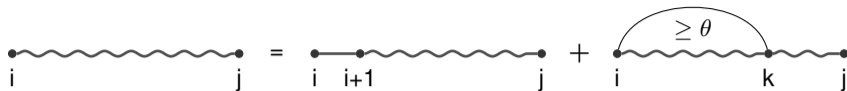
→ free-energy = $N_{i+1,j}$

▶ **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))

→ free-energy = $\Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j}$

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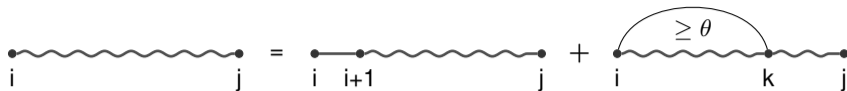
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(Indeed, any BP between $[i + 1, k - 1]$ and $[k + 1, j]$ would **cross** (i, k))

→ free-energy = $\Delta G_{i,k} + N_{i+1,k-1} + N_{k+1,j}$

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	2	2	2	2	4	4	4	5	7	7	8	10
U					0	0	0	0	0	2	2	4	4	5	7	7	8	10
A						0	0	0	0	2	2	2	5	5	5	8	8	8
C							0	0	0	0	0	2	5	5	5	8	8	8
U								0	0	0	0	2	3	5	5	6	7	7
U									0	0	0	2	3	5	5	5	7	7
C										0	0	0	3	3	3	5	5	5
U											0	0	0	2	2	2	3	3
U												0	0	0	0	1	2	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
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	2	2	4	5	7	7	8	10	10
A						0	0	0	0	2	2	2	5	5	5	8	8	8
C							0	0	0	0	0	2	5	5	5	8	8	8
U								0	0	0	0	2	3	5	5	6	7	7
U									0	0	0	0	2	3	5	5	7	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



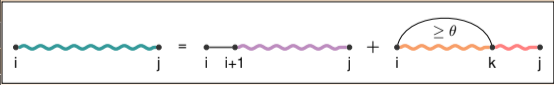
Nussinov/Jacobson

	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	2	2	4	5	7	7	8	10	10
A						0	0	0	0	2	2	2	5	5	5	8	8	8
C							0	0	0	0	0	2	5	5	5	8	8	8
U								0	0	0	0	2	3	5	5	6	7	7
U									0	0	0	2	3	5	5	5	7	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	1	2	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	2	2	2	2	4	4	5	7	7	8	8	10
U					0	0	0	0	0	2	2	4	5	7	7	8	8	10
A						0	0	0	0	2	2	2	5	5	5	8	8	8
C							0	0	0	0	0	2	5	5	5	8	8	8
U								0	0	0	0	2	3	5	5	6	7	7
U									0	0	0	2	3	5	5	5	7	7
C										0	0	0	3	3	3	5	5	5
U											0	0	0	2	2	2	3	3
U												0	0	0	0	1	2	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	
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	



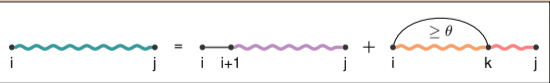
Nussinov/Jacobson

	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	2	2	2	2	4	4	5	7	7	8	10	10	
U					0	0	0	0	0	2	2	4	5	7	7	8	10	10	
A						0	0	0	0	2	2	2	5	5	5	8	8	8	
C							0	0	0	0	0	2	5	5	5	8	8	8	
U								0	0	0	0	2	3	5	5	6	7	7	
U									0	0	0	2	3	5	5	5	7	7	
C										0	0	0	3	3	3	5	5	5	
U											0	0	0	2	2	2	3	3	
U												0	0	0	0	1	2	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									0	0	0	2	3	5	5	5	7		
C										0	0	0	3	3	3	5	5		
U											0	0	0	2	2	2	3		
U												0	0	0	0	1	2		
A													0	0	0	0	0		
G														0	0	0	0		
A															0	0	0		
C																0	0		
G																	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									0	0	0	2	3	5	5	5	7		
C										0	0	0	3	3	3	5	5		
U											0	0	0	2	2	2	3		
U												0	0	0	0	1	2		
A													0	0	0	0	0		
G														0	0	0	0		
A															0	0	0		
C																0	0		
G																	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									0	0	0	2	3	5	5	5	7		
C										0	0	0	3	3	3	5	5		
U											0	0	0	0	2	2	3		
U												0	0	0	0	1	2		
A													0	0	0	0	0		
G														0	0	0	0		
A															0	0	0		
C																0	0		
G																	0		
A																		0	



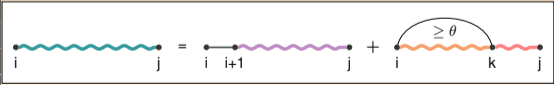
Nussinov/Jacobson

	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	



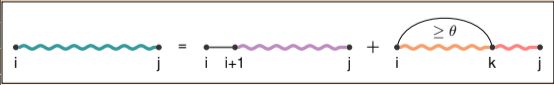
Nussinov/Jacobson

	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									0	0	0	2	3	5	5	5	7		
C										0	0	0	0	3	3	3	5	5	
U											0	0	0	2	2	2	3		
U												0	0	0	0	1	2		
A													0	0	0	0	0		
G														0	0	0	0		
A															0	0	0		
C																0	0		
G																	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	2	2	2	2	4	4	5	7	7	8	10	10	
U					0	0	0	0	0	2	2	4	5	7	7	8	10	10	
A						0	0	0	0	2	2	2	5	5	5	8	8	8	
C							0	0	0	0	0	2	5	5	5	8	8	8	
U								0	0	0	0	2	3	5	5	6	7	7	
U									0	0	0	2	3	5	5	5	7	7	
C										0	0	0	0	3	3	3	5	5	
U											0	0	0	2	2	2	3	3	
U												0	0	0	0	1	2	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									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	1	2		
A													0	0	0	0	0		
G														0	0	0	0		
A															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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									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	



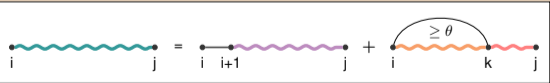
Nussinov/Jacobson

	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	2	2	4	5	7	7	8	10		
A						0	0	0	0	2	2	2	5	5	5	8	8		
C							0	0	0	0	0	2	5	5	5	8	8		
U								0	0	0	0	2	3	5	5	6	7		
U									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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