Combinatorial approaches to RNA folding
Part II: Energy minimization via dynamic programming

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Overview

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In this lecture, we will study the energy minimization approach.

Dynamic programming

Dynamic programming (DP) is a method for solving complex problems by solving simplier subproblems and combining their solutions to obtain the overall solution.

The CG pairing uses 3 hydrogen bonds, whereas AU and UG each use 2.

The wobble pair UG is unstable, having roughly half the strength of an AU bond.

Given an RNA sequence $\mathbf{b} = b_1 b_2 \cdots b_n$, define the energy function of a pair:

$$e(i,j) = \begin{cases} 3 & \{b_i, b_j\} = \{\mathrm{C}, \mathrm{G}\} \text{ and } i \leq j-4 \\ 2 & \{b_i, b_j\} = \{\mathrm{A}, \mathrm{U}\} \text{ and } i \leq j-4 \\ 1 & \{b_i, b_j\} = \{\mathrm{G}, \mathrm{U}\} \text{ and } i \leq j-4 \\ 0 & \text{otherwise} \end{cases}$$

Assume energy is additive:

$$E(\mathbf{b}, S) = \sum_{\text{bp's } (i,j)} e(i,j)\,.$$

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Goal

Maximize the energy score $E(\mathbf{b}, S)$ over all possible secondary structures S into which $\mathbf{b}$ can fold.

Warm-up Exercise

Consider the RNA sequence $\mathbf{b} = \mathrm{G\,G\,G\,A\,C\,C\,U\,U\,C\,C}$. Find all possible ways that it can fold into a secondary structure S, without leaving any "allowed" unpaired bases. Draw the arc diagram and a "realistic sketch" of the folded RNA strand. Compute the energy score $E(\mathbf{b}, S)$ of each.

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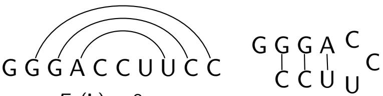
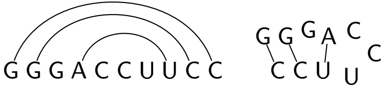


$E_S(\mathbf{b}) = 8$



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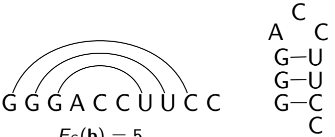
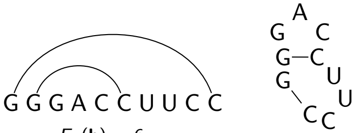
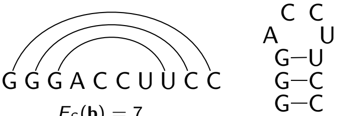


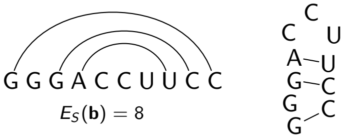
$E_S(\mathbf{b}) = 5$



$E_S(\mathbf{b}) = 6$



$E_S(\mathbf{b}) = 7$



$E_S(\mathbf{b}) = 8$

Dynamic programming

Dynamic Programming (DP) process

1. Use the optimal energy score of subsequences of **b** to determine the optimal (maximum) energy score of **b**.
2. Traceback to reconstruct the actual secondary structure that realizes this maximum.

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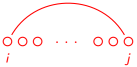
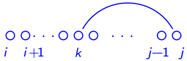
We compute $E(i,j)$ recursively. If $i < j-4$, then we return $E(i,j)=0$. Otherwise, there are four ways to recurse on the sequence $\mathbf{b}_{i,j}$:

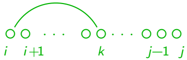
1. *(i,j) forms a basepair*: Recurse on the sequence $\mathbf{b}_{i+1,j-1}$.



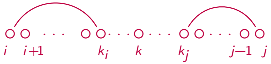
2. *i is unpaired but (k,j) is a basepair*: Recurse on the sequence $\mathbf{b}_{i+1,j}$.



3. *(i,k) is a basepair but j is unpaired*: Recurse on the sequence $\mathbf{b}_{i,j-1}$.



4. *(i,k_i) and (k_j,j) are paired for some $k_i < k_j$.* Recurse on the two subsequences $\mathbf{b}_{i,k}$ and $\mathbf{b}_{k+1,j}$, for some $k_i \leq k < k_j$. *We need to consider all possible values of $k = i+4,\ldots,j-4$.*



Dynamic programming

Taking the maximum energy score over each of four ways to recurse on the subsequence $\mathbf{b}_{i,j}$ yields a recurrence for the the maximum score $E(i,j)$:

$$E(i,j) = \max \begin{cases} E(i+1,j-1) + e(i,j) \\ E(i+1,j) \\ E(i,j-1) \\ \max\limits_{i<k<j} E(i,k) + E(k+1,j). \end{cases}$$

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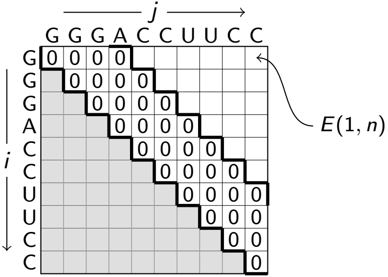
The values of $E(i,j)$ can be arranged in a table. *The optimal energy score $E(\mathbf{b}, S)$ is simply $E(1,n)$.*



	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0						
G		0	0	0	0					
G			0	0	0	0				
A				0	0	0	0			
C					0	0	0	0		
C						0	0	0	0	
U							0	0	0	0
U								0	0	0
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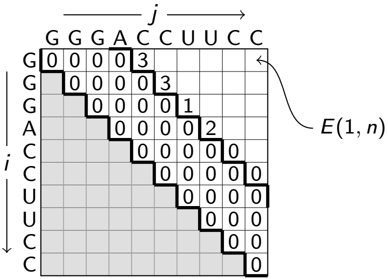

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3					
G		0	0	0	0	3				
G			0	0	0	0	1			
A				0	0	0	0	2		
C					0	0	0	0	0	
C						0	0	0	0	0
U							0	0	0	0
U								0	0	0
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Exercise

Fill out the remaining table for the sequence $\mathbf{b} = \mathrm{G\,G\,G\,A\,C\,C\,U\,U\,C\,C}$.

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3					
G		0	0	0	0	3				
G			0	0	0	0	1			
A				0	0	0	0	2		
C					0	0	0	0	0	
C						0	0	0	0	0
U							0	0	0	0
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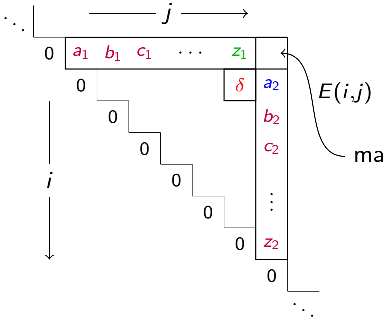

$$\max\begin{cases} E(i+1,j-1)+e(i,j)=\delta+e(i,j) \\ E(i+1,j)=a_2 \\ E(i,j-1)=z_1 \\ \max\limits_{i<k<j}\{E(i,k)+E(k+1,j)\} \\ \qquad =\max\{a_1+a_2,\,b_1+b_2,\ldots,z_1+z_2\} \end{cases}$$

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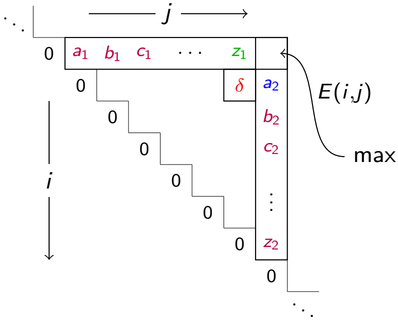

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	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4			
G		0	0	0	0	3	3	3		
G			0	0	0	0	1	2	5	
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
U							0	0	0	0
U								0	0	0
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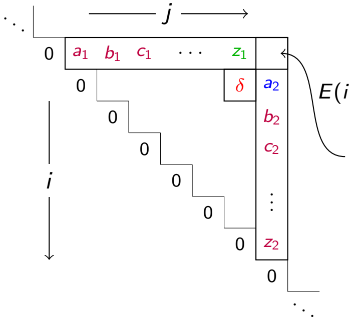

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G		0	0	0	0	3	3	3	5	
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
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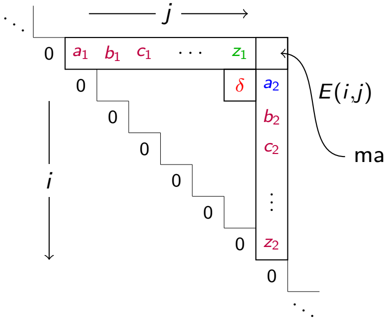

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G	0	0	0	0	3	3	4	4	6	
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
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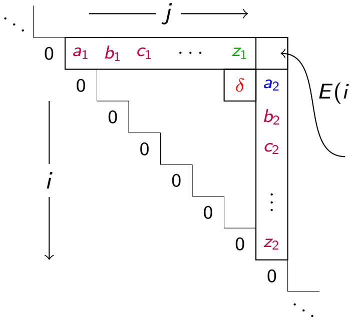

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G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
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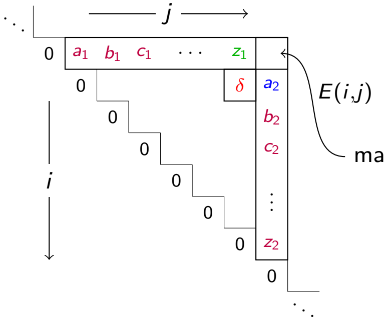

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Dynamic programming: traceback step

Goal

Now that we know the optimal energy score, we need to determine which secondary structure realizes that score.

Exercise

"Trace back" the following table to find a minimal free energy secondary structure for $\mathbf{b} = \text{G G G A C C U U C C}$.

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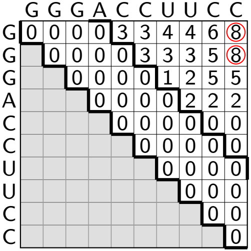

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
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U								0	0	0
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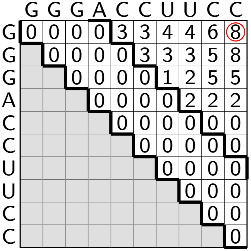

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
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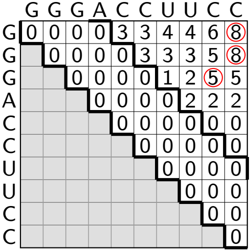
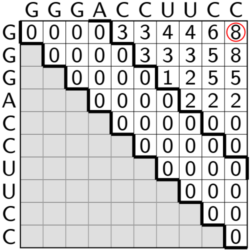


	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	⑧
G		0	0	0	0	3	3	3	5	⑧
G			0	0	0	0	1	2	⑤	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
U							0	0	0	0
U								0	0	0
C									0	0
C										0



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

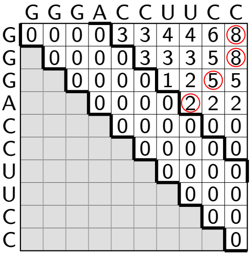
Dynamic programming: traceback step

Goal

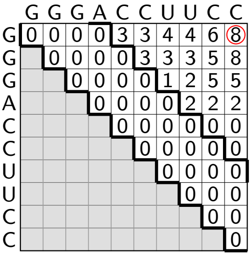
Now that we know the optimal energy score, we need to determine which secondary structure realizes that score.

Exercise

"Trace back" the following table to find a minimal free energy secondary structure for $\mathbf{b} = \mathrm{G\,G\,G\,A\,C\,C\,U\,U\,C\,C}$.



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



	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	⑧
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
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C						0	0	0	0	0
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Now that we know the optimal energy score, we need to determine which secondary structure realizes that score.

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"Trace back" the following table to find a minimal free energy secondary structure for $\mathbf{b} = GGGACCUUCC$.

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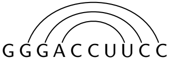

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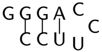

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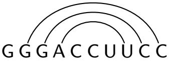

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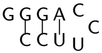

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G			0	0	0	0	1	2	5	5
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C						0	0	0	0	0
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	G	G	G	A	C	C	U	U	C	C
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G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
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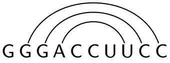

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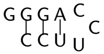

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A				0	0	0	0	2	2	2
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U							0	0	0	0
U								0	0	0
C									0	0
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G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
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U								0	0	0
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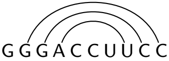

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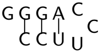

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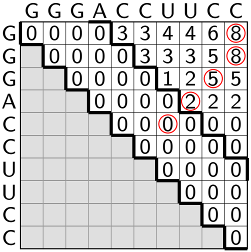

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
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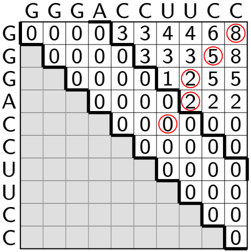

	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
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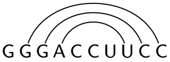

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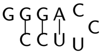

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	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
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U							0	0	0	0
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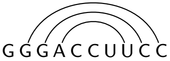

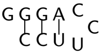

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	G	G	G	A	C	C	U	U	C	C
G	0	0	0	0	3	3	4	4	6	8
G		0	0	0	0	3	3	3	5	8
G			0	0	0	0	1	2	5	5
A				0	0	0	0	2	2	2
C					0	0	0	0	0	0
C						0	0	0	0	0
U							0	0	0	0
U								0	0	0
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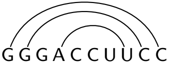

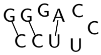

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Model validity and weaknesses

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Did we *really* find the most thermodynamically stable folds of the RNA sequence $\mathbf{b} = \mathrm{G\,G\,G\,A\,C\,C\,U\,U\,C\,C}$?

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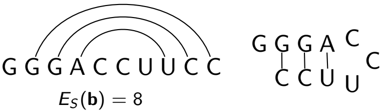

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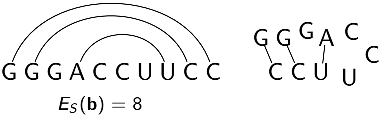

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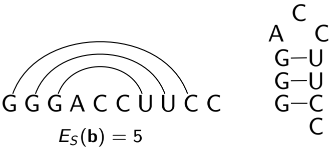
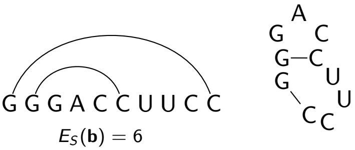
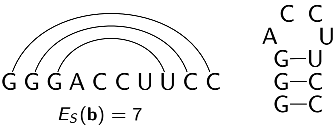


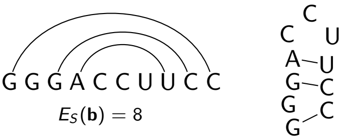
$E_S(\mathbf{b}) = 5$



$E_S(\mathbf{b}) = 6$



$E_S(\mathbf{b}) = 7$



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(And technically, our energy scores should have been negative, with our goal being to *minimize* free energy.)

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Note that this takes into account the negative energy contributions by the base pairs.

We assume that loop energy contributions are additive, and thus the free energy is:

$$E_S(\mathbf{b}) = \sum_{\text{all loops } \mathbf{L}} e(\mathbf{L}) = e(\mathbf{L}_0) + \left(\sum_{(i,j)} e(L((i,j))) \right).$$

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There are many of these "special cases," which lead to energy penalities or bonuses.

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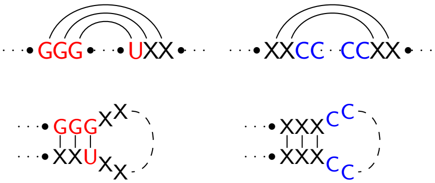
Two examples of these "special cases" are GGG-loops and poly-C hairpin loops:



Incorporating loop structure

The energy contribution of a loop depends on a number of parameters:

- its type
- its size
- its "closing base pairs"
- many other technicalities and special cases

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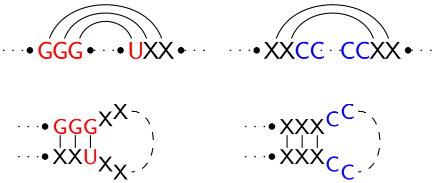

These special cases result in the model having hundreds of parameters, most of which are experimentally determined.

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In the theory of polymers, the free energy of a hairpin loop **L** of size l_s is approximately

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UNIFold stores these parameters in text files, which are called upon when the algorithm runs.

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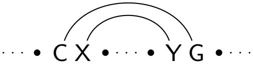

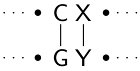

X \ Y	A	C	G	U
A	∞	∞	∞	-2.1
C	∞	∞	-3.3	∞
G	∞	-2.4	∞	-1.4
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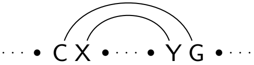

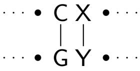

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The table above is for pairs stacked above a CG bond. Since there are 6 possibilities for what (X, Y) can be stacked under, UNAFold has 6 tables like the one above.

UNAFold

UNAFold "Unified Nucleic Acid Folding" is a comprehensive software package for nucleic acid folding and hybridization prediction.

It was developed by Michael Zuker's research group at RPI and SUNY Albany. It is freely available from the following website:

`http://mfold.rna.albany.edu/`

Also available there is extensive documentation and literature about the software and the algorithms.

It essentially runs a complicated dynamic programming algorithm, albeit with a hundreds of special cases and parameters.