Recitation 3-19

CB Lecture #10

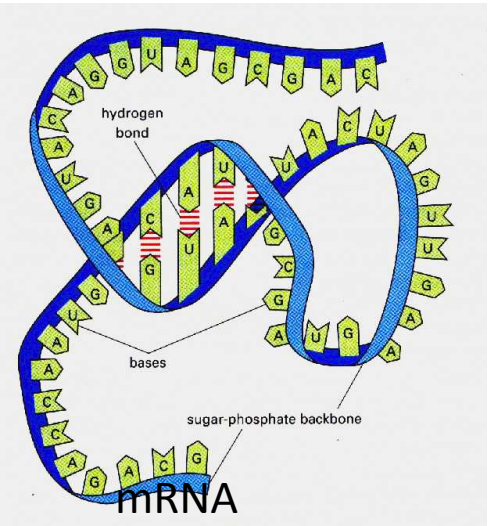
RNA Secondary Structure

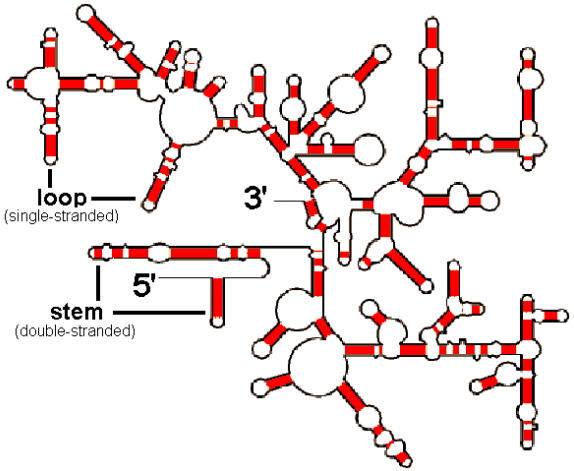
Announcements

- Exam 1 grades and answer key will be posted Friday afternoon
  - We will try to make exams available for pickup Friday afternoon (probably from 3:30-4pm and 5-5:30pm, before and after the Friday section)
- Pset #3 has been released, due April 3rd
  - much longer programming problem than Pset #2
  - Because of spring break, only one set of formal office hours before due date, but please email us with your questions
- Updated aims with research strategy will be due Friday April 4th

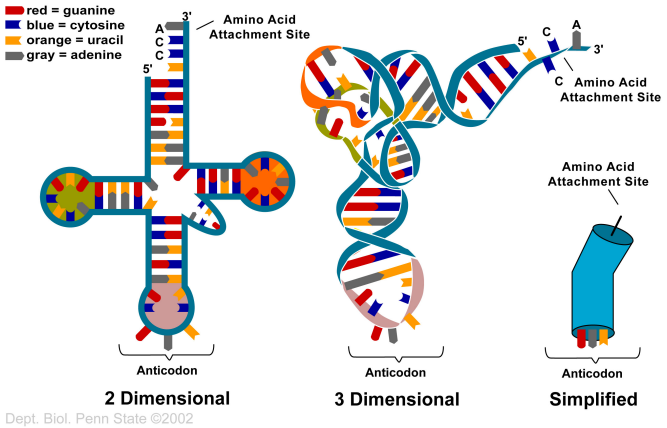
RNA Secondary Structure

- Just as protein can form secondary structure (α-helix and β-sheet), so too can single-stranded RNA by folding back on itself to form double-stranded regions





Source: http://www.mun.ca/biology/scarr/rRNA_folding.html



http://www.uic.edu/classes/phys/phys461/phys450/ANJUM04/RNA_sstrand.jpg

https://www.mun.ca/biology/scarr/rRNA_folding.html

https://wikispaces.psu.edu/download/attachments/54886630/figure_17_12.jpg

…and virtually every other RNA!

RNA Secondary Structure

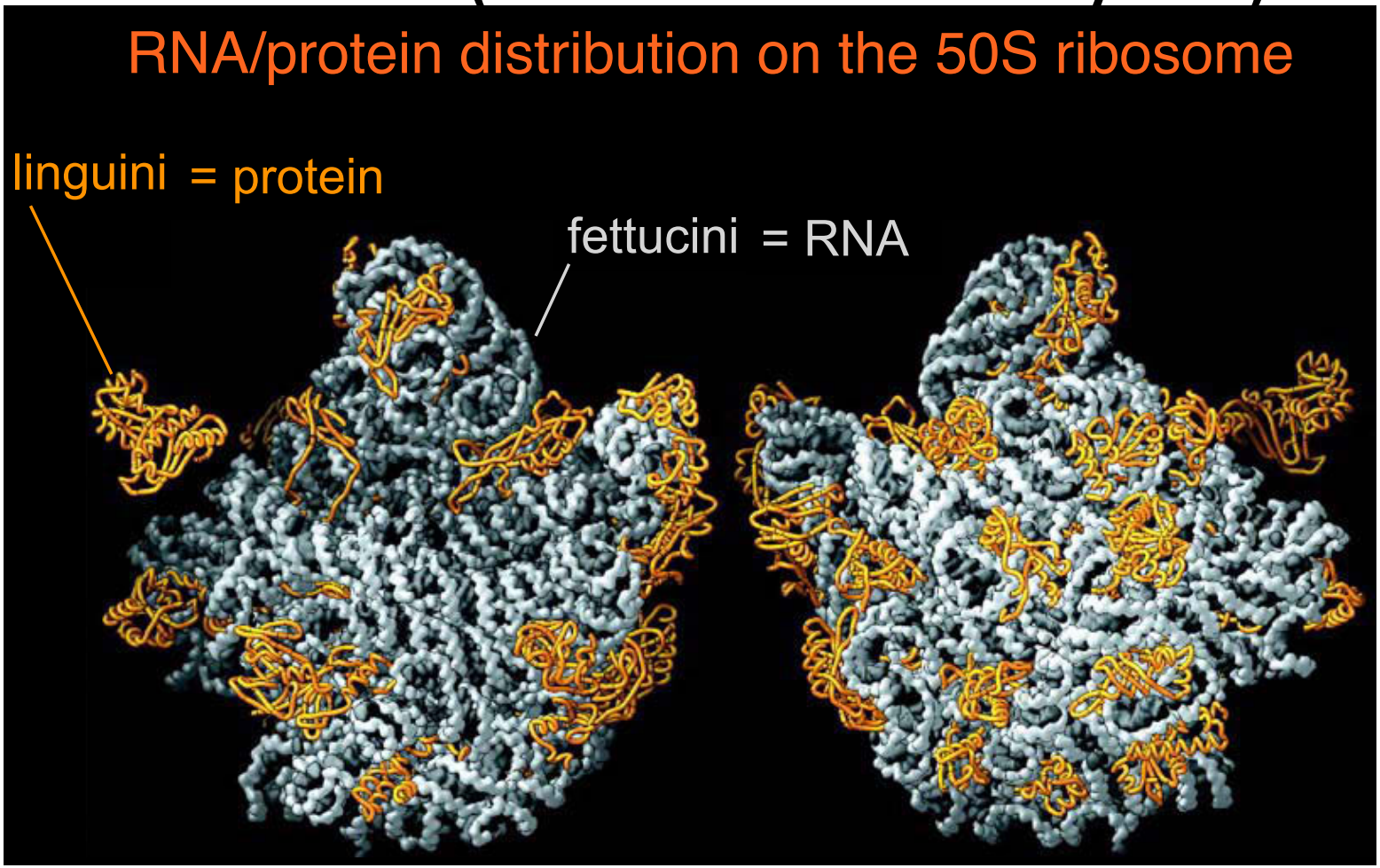
- RNA's secondary structure is often intimately tied with its function
  - rRNA and tRNA always adopt the same structure; function depends on it
  - mRNA may adopt different structures in different conditions – due to cell types, temperature, ion concentration, etc.
- mRNA's processing may depend on what structure is (or is not) present
  - Can inhibit or strengthen ability of RNA binding protein to bind mRNA and affect alternative splicing
  - Can inhibit the ribosome's ability to translate through the mRNA due to sequestration of ribosome binding site or hitting structured road block

Schematic diagram of an *E. coli* cell removed due to copyright restrictions. See the image here.

Riboswitches are metabolite-sensing RNAs, typically located in the non-coding portions of messenger RNAs, that control the synthesis of metabolite-related proteins

http://www-abell.ch.cam.ac.uk/images/riboswitchfig1.jpg

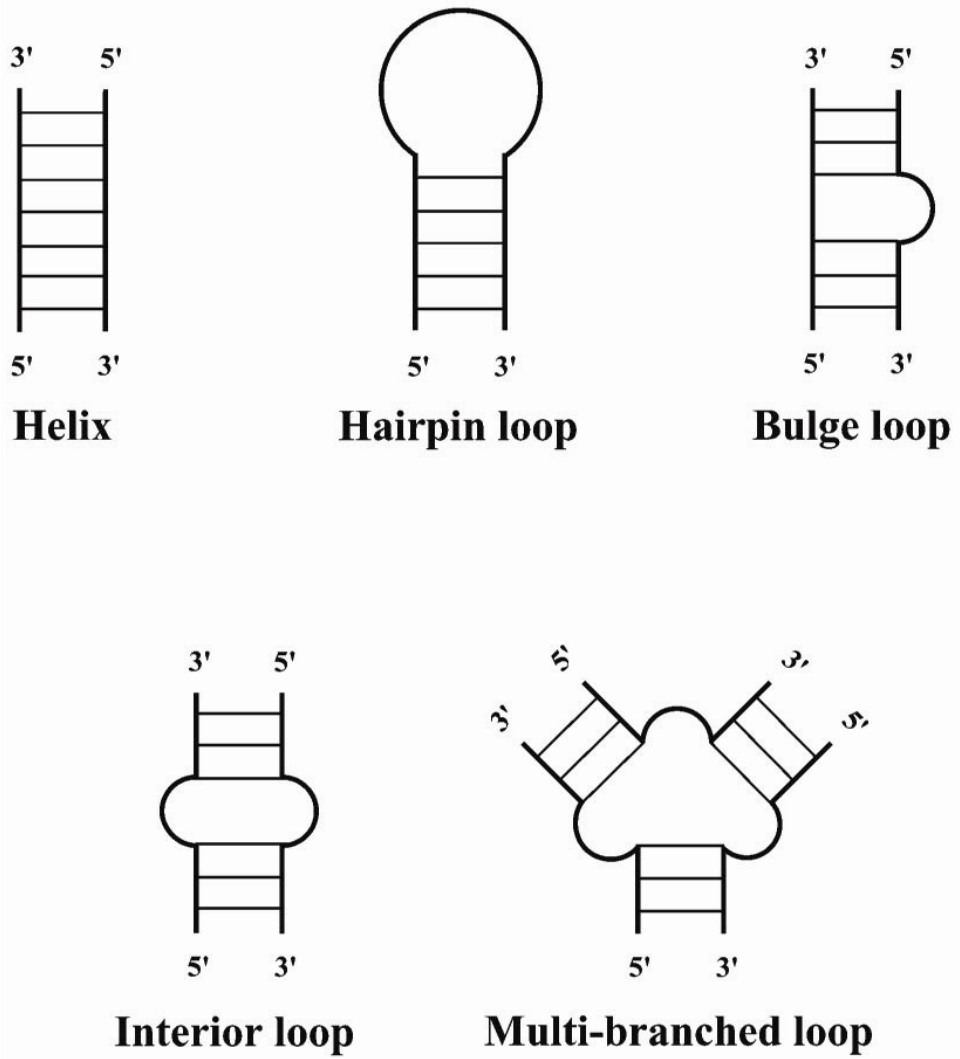
RNA is at the catalytic site of the ribosome (which is a ribozyme)



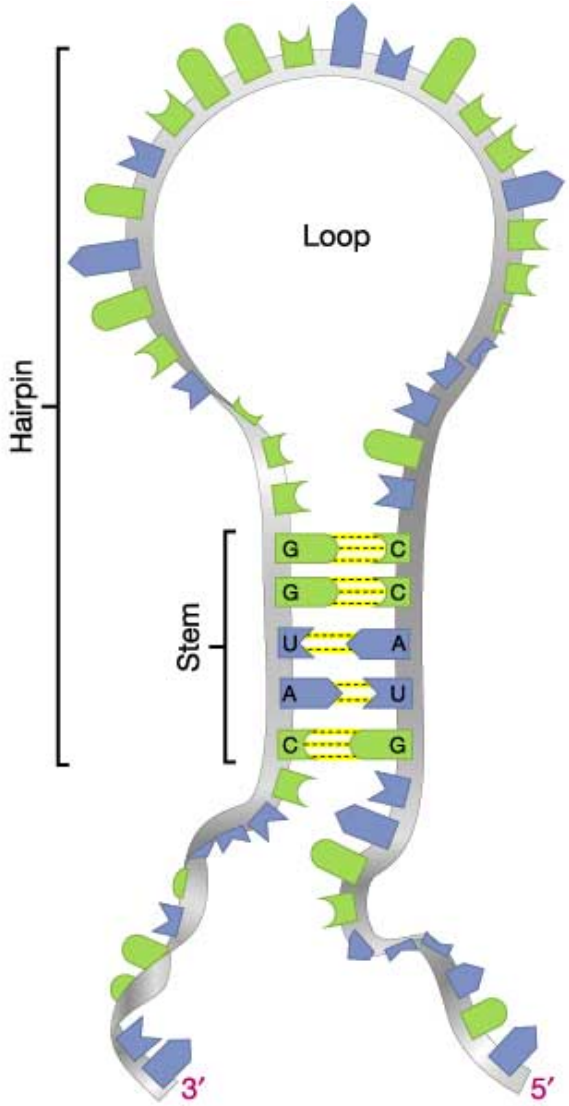
Source: Ban, Nenad, Poul Nissen, et al. "The Complete Atomic Structure of the Large Ribosomal Subunit at 2.4 Å Resolution." *Science* 289, no. 5481 (2000): 905-20.

-Ribozymes – RNAs capable of catalyzing biochemical reactions - provide support for "RNA world" hypothesis – that life evolved from a world with RNAs but no DNA or protein

Terminology



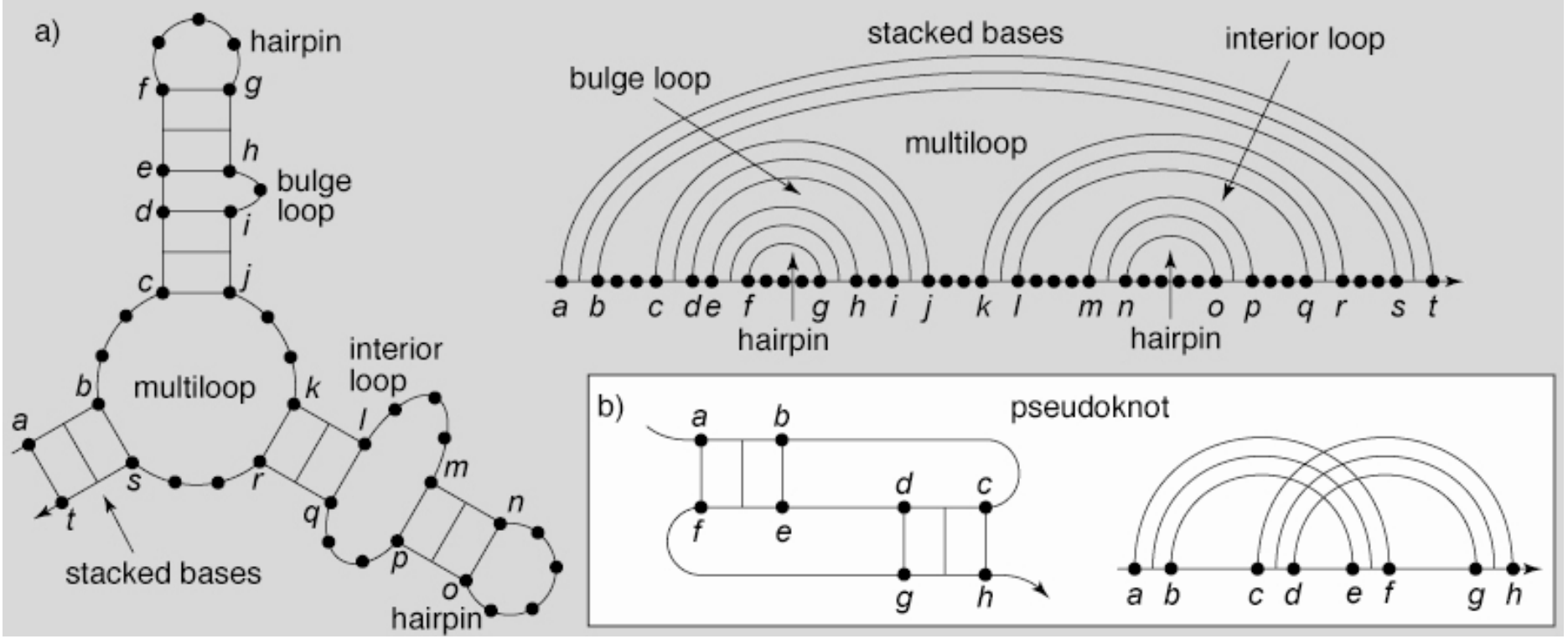
Source: Ding, Ye, and Charles E. Lawrence. "A Statistical Sampling Algorithm for RNA Secondary Structure Prediction." *Nucleic Acids Research* 31, no. 24 (2003): 7280-301.



http://www.ims.nus.edu.sg/Programs/biomolecular07/files/clote_tut2b.pdf

http://biology.kenyon.edu/courses/biol114/KH_lecture_images/transcription/FG03_15b.JPG

Arc Notation



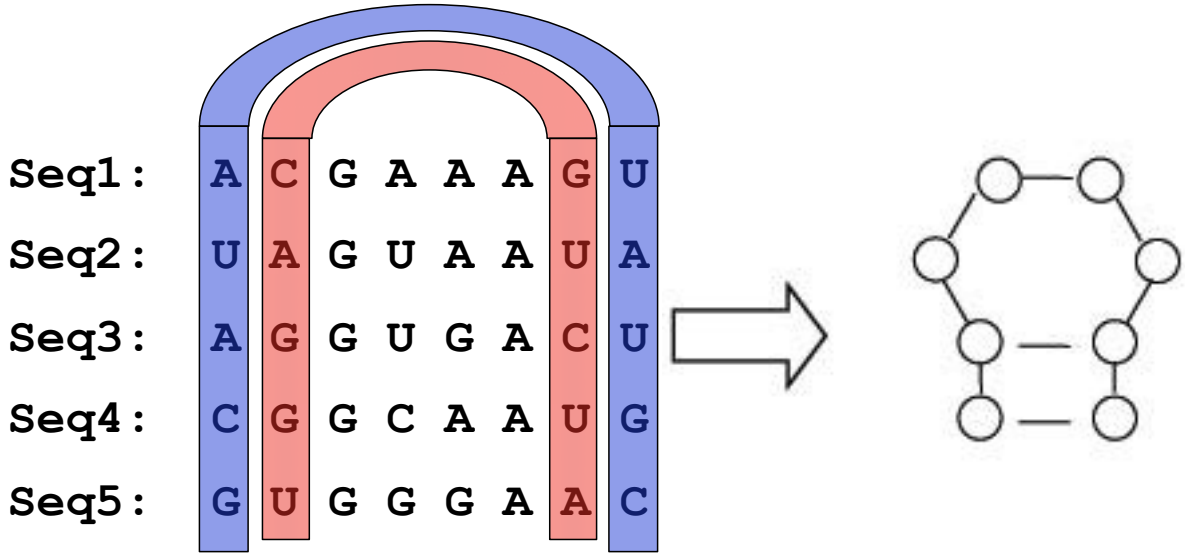
http://www.ims.nus.edu.sg/Programs/biomolecular07/files/clote_tut2b.pdf

non-coding RNAs (ncRNAs)

- Any RNA molecule that doesn't code for protein (any non-mRNA molecule)
  - tRNAs, rRNAs, miRNAs, snRNAs, snoRNAs, ribozymes (RNase P), lnRNAs, riboswitches
- Due to the central role of structure in facilitating RNA's function, we'd like the determine structure
  - 2 different approaches for secondary structure
    1. Covariation and compensatory changes through evolution
    2. Energy minimization

Covariation and compensatory changes

- Idea: If structure is contributing to function but actual sequence is not, we should see structure conserved but not necessarily sequence
  - So evolution allows mutations as long as secondary structure is maintained



Covariation and compensatory changes

- Need sufficient divergence so that a decent number of mutations and compensatory mutations have occurred, but not so much that sequences can't be aligned

- Need a large number of homologs sequenced to have power to detect compensatory mutations

Mutual Information (MI)

- The most common way of quantifying sequence covariation for the purpose of RNA secondary structure determination
- A measure of two variables' mutual dependence
  - Measures the information that *X* and *Y* share: it measures how much knowing one of these variables reduces uncertainty about the other
  - If *X* and *Y* are independent, then knowing *X* does not give any information about *Y* and vice versa, so their MI = 0
  - At the other extreme, if *X* is a deterministic function of *Y* and *Y* is a deterministic function of *X,* then all information conveyed by *X* is shared with *Y*: knowing *X* determines the value of *Y* and vice versa
    - As a result, in this case the mutual information is the same as the uncertainty contained in *Y* (or *X*) alone, namely the entropy of *Y* (= entropy of *X*)
  - Mutual information between aligned columns of nucleotides that are base-paired should be high
    - Knowing one of the nucleotides tells you everything about the other (if A, other is U; if C, other is G, etc.)

Mutual Information (MI)

MI between two columns *i* and *j*:

$$M_{ij} = \sum_{x=A,C,G,U} \sum_{y=A,C,G,U} f_{x,y}^{(i,j)} \log_2 \left(\frac{f_{x,y}^{(i,j)}}{f_x^{(i)} f_y^{(j)}} \right)$$

$f_{x,y}^{(i,j)}$: fraction of sequences with x in column *i* AND y in column *j*

$f_x^{(i)}$: fraction of sequences with x in column *i*

-Relative entropy of the joint distribution relative to the individual distributions of the nucleotides in columns *i* and *j*

-MI is maximal (2 bits) if x and y appear at random (all 4 nts equally likely) but perfectly covary (e.g. always complementary)

Mutual Information (MI)

MI is maximal (2 bits) if x and y appear at random (all 4 nts equally likely) but perfectly covary (e.g. always complementary)

$$M_{ij} = \sum_{x=A,C,G,U} \sum_{y=A,C,G,U} f_{x,y}^{(i,j)} \log_2 \left(\frac{f_{x,y}^{(i,j)}}{f_x^{(i)} f_y^{(j)}} \right)$$

What is $f_{x,y}^{(i,j)}$? Because *x* and *y* perfectly covary,

$f_{x,y}^{(i,j)} = \frac{1}{4}$ for the 25% of covarying events (e.g. $(x,y) = (\mathrm{A},\mathrm{U})$)

$f_{x,y}^{(i,j)} = 0$ for the 75% of non-existent events (e.g. $(x,y) = (\mathrm{A},\mathrm{A})$)

What is $f_x^{(i)} f_y^{(j)}$? $\frac{1}{4} * \frac{1}{4} = \frac{1}{16}$

Mutual Information (MI)

MI is maximal (2 bits) if x and y appear at random (all 4 nts equally likely) but perfectly covary (e.g. always complementary)

$$M_{ij} = \sum_{x=A,C,G,U} \sum_{y=A,C,G,U} f_{x,y}^{(i,j)} \log_2 \left(\frac{f_{x,y}^{(i,j)}}{f_x^{(i)} f_y^{(j)}} \right)$$

$$= \sum_{(x,y)=(A,U),(C,G),(G,C),(U,A)} f_{x,y}^{(i,j)} \log_2 \left(\frac{f_{x,y}^{(i,j)}}{f_x^{(i)} f_y^{(j)}} \right)$$

$$= \sum_{(x,y)=(A,U),(C,G),(G,C),(U,A)} \frac{1}{4} \log_2 \left(\frac{1/4}{1/16} \right)$$

$$= \sum_{(x,y)=(A,U),(C,G),(G,C),(U,A)} \frac{1}{4} * 2$$

$$= 2 \text{ bits}$$

2nd approach: Energy minimization

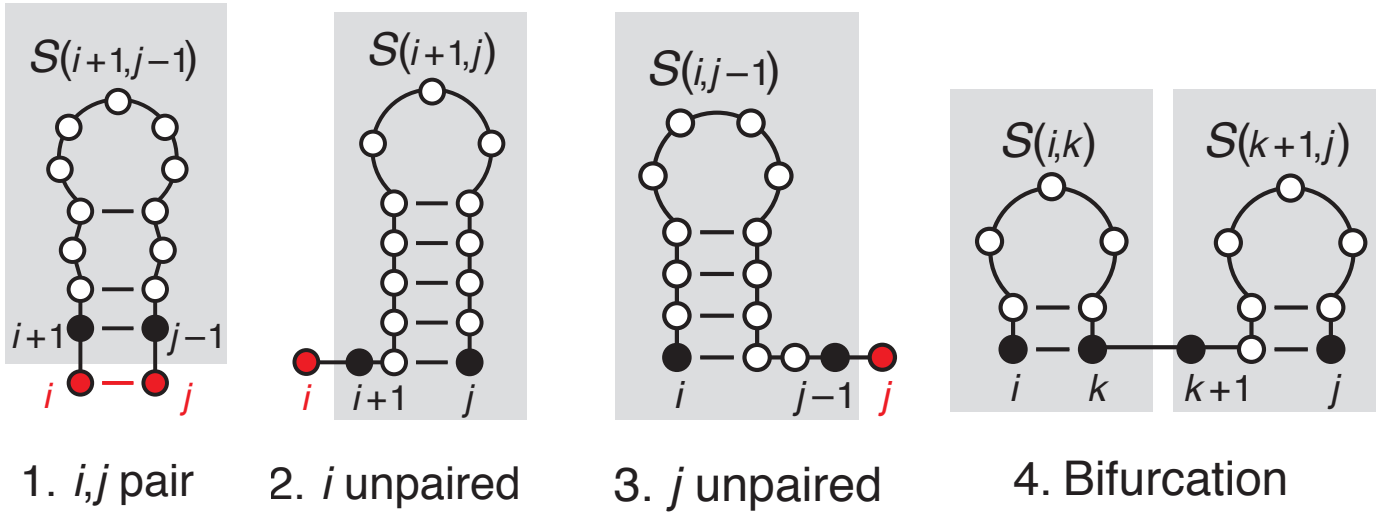
$$\Delta G_{folding} = G_{unfolded} - G_{folded}$$

- Assume that RNA will fold to its lowest energy state
- Simplest model: all base pairs contribute equally to lowering structure's energy
  - Base Pair Maximization (ignores energy contributions of base stacking, loops, entropy, etc.): +1 for paired bases, 0 for unpaired
    - Use the Nussinov algorithm of recursive maximization of base pairing

See the Eddy Nature Biotechnology Primer 2004

Nussinov algorithm

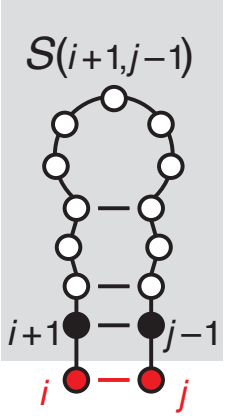
- Look at one contiguous sub-sequence from position *i* to position *j* in our complete sequence of length *N*, and calculate the score of the best structure for just that sub-sequence
- This optimal score (call it $S(i,j)$) can be defined recursively in terms of optimal scores of smaller sub-sequences
- Four possible ways that a structure of nested base pairs on *i*...*j* can be constructed
  1. *i*, *j* are a base pair, added on to a structure for *i*+1 ... *j*–1
  2. *i* is unpaired, added on to a structure for *i*+1 ... *j*
  3. *j* is unpaired, added on to a structure for *i* ... *j*–1
  4. *i*, *j* are paired, but not to each other; the structure for *i*...*j* adds together sub-structures for two sub-sequences, *i* ... *k* and *k*+1 ... *j* (a bifurcation)



Nussinov algorithm

1. *i*,*j* are a base pair, added on to a structure for *i*+1 ... *j*–1

- The score we add for the base pair *i*,*j* is independent of any details of the optimal structure on *i* + 1...*j* – 1
- Similarly, the optimal structure on *i* + 1...*j* – 1 and its score *S*(*i* + 1, *j* – 1) are unaffected by whether *i*, *j* are base paired or not (or anything else that happens in the rest of the sequence)
- Therefore, *S*(*i*, *j*) is just *S*(*i* + 1, *j* – 1) plus one, if *i*, *j* can base pair.



1. *i*,*j* pair

$$S(i,j) = S(i+1,j-1) + 1 \quad [\text{if } i,j \text{ base pair}]$$

Nussinov algorithm

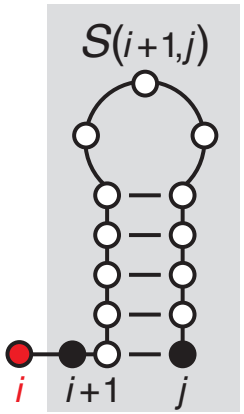
2. *i* is unpaired, added on to a structure for *i+1 … j*

- In case 2, the optimal score $S(i+1,j)$ is independent of the addition of an unpaired base *i*, so $S(i+1, j) + 0$ is the score of the optimal structure on *i,j* conditional on *i* being unpaired

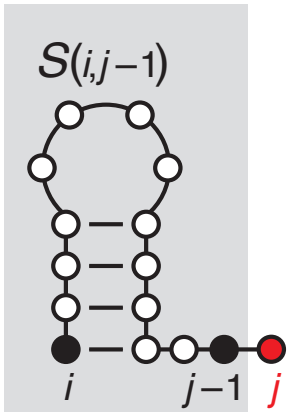
3. *j* is unpaired, added on to a structure for *i … j*–1

- Case 3 is the same thing, but conditional on *j* being unpaired

$$S(i,j) = S(i+1,j)$$



2. *i* unpaired



3. *j* unpaired

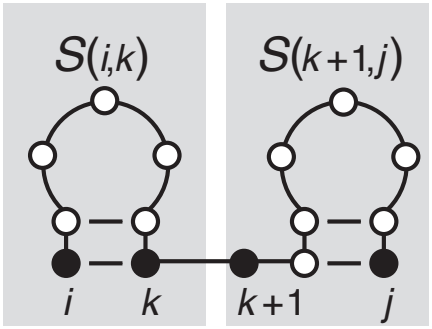
$$S(i,j) = S(i,j-1)$$

Nussinov algorithm

4. *i*,*j* are paired, but not to each other; the structure for *i*...*j* adds together sub-structures for two sub-sequences, *i* ... *k* and *k*+1 ... *j* (a bifurcation)

- We deal with putting two independent sub-structures together, the optimal score *S*(*i*,*k*) is independent of anything going on in *k*+1 ... *j*, and vice versa
- Must consider all possible *k*'s between *i* and *j*

$$S(i,j) = \max_{i<k<j} S(i,k) + S(k+1,j)$$

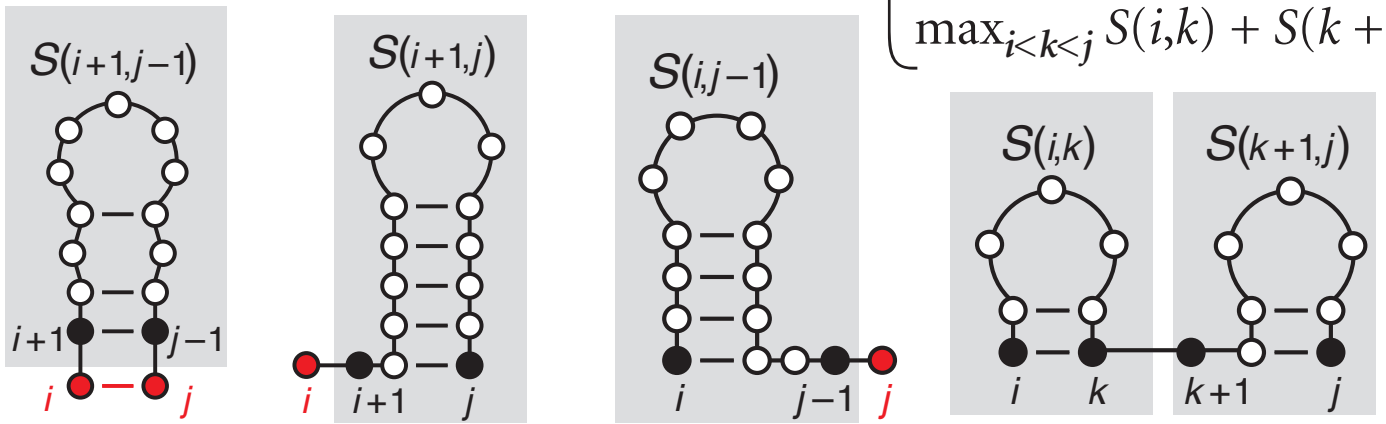


4. Bifurcation

Nussinov algorithm

- Since these are the only four possible cases, the optimal score $S(i, j)$ is just the maximum of the four possibilities
- We've thus defined the optimal score $S(i,j)$ recursively as a function only of optimal scores of smaller sub-sequences, so we only need to remember these scores, not the combinatorial explosion of possible structures

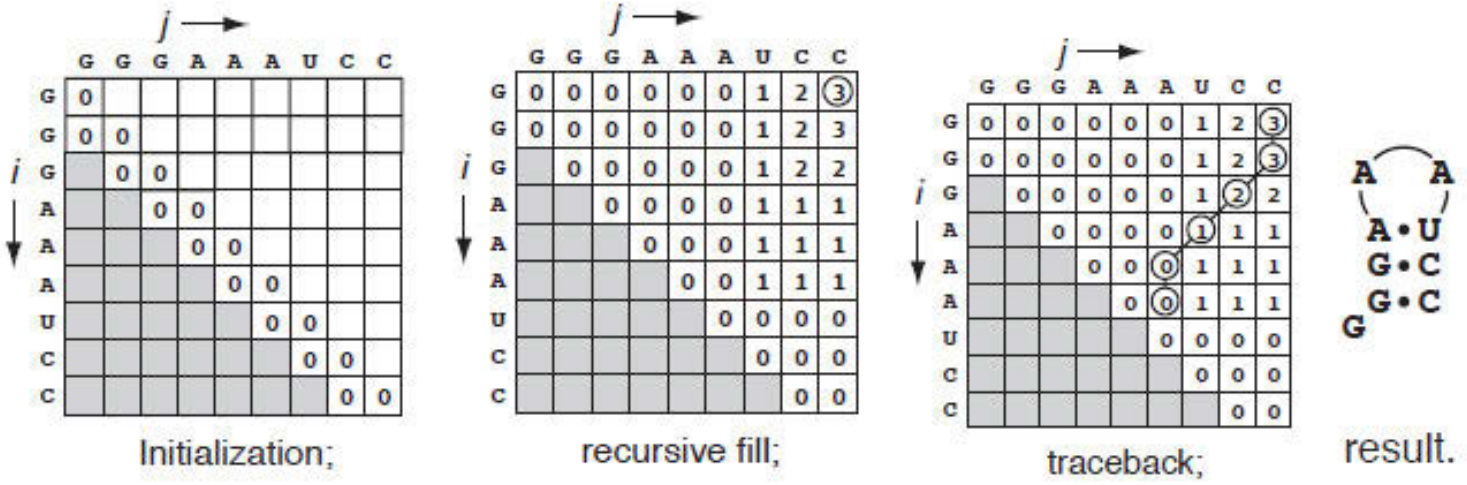
$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$



1. *i,j* pair 2. *i* unpaired 3. *j* unpaired 4. Bifurcation

Nussinov algorithm

- To run this recursion efficiently, we need to make sure that whenever we try to compute an $S(i,j)$, we already have calculated the scores for smaller sub-sequences.
  - This sets up a dynamic programming algorithm.
- We tabulate the scores $S(i, j)$ in a triangular matrix. We initialize on the diagonal; subsequences of length 0 or 1 have no base pairs, so $S(i,i) = S(i, i-1) = 0$ (by convention, the $i, i-1$ cells represent zero length sequences; the recursion must never access an empty matrix cell).
- Work outwards on larger and larger sub-sequences, until we reach the upper right corner.
  - This corner is $S(1, N)$, the score of the optimal structure for the complete sequence from i=1 to j=N.
  - From that point, recover the optimal structure by tracing back the optimal path that got us into the upper corner, one step in the structure at a time.



Nussinov algorithm

- Storing the $S(i, j)$ matrix requires memory proportional to N^2, similar to what sequence alignment algorithms need

- However, the innermost loop of having to find optimal potential bifurcation points k means that the folding algorithm requires time proportional to N^3, a factor of N more time-intensive than sequence alignment
  - RNA folding calculations often require a large amount of computer power

Nussinov Algorithm Example

We want to fold the following RNA sequence:

AAGUUCG

(1) Write the sequence along the top and left side of the matrix

(2) Initialize the diagonal of the matrix and one-below to zero

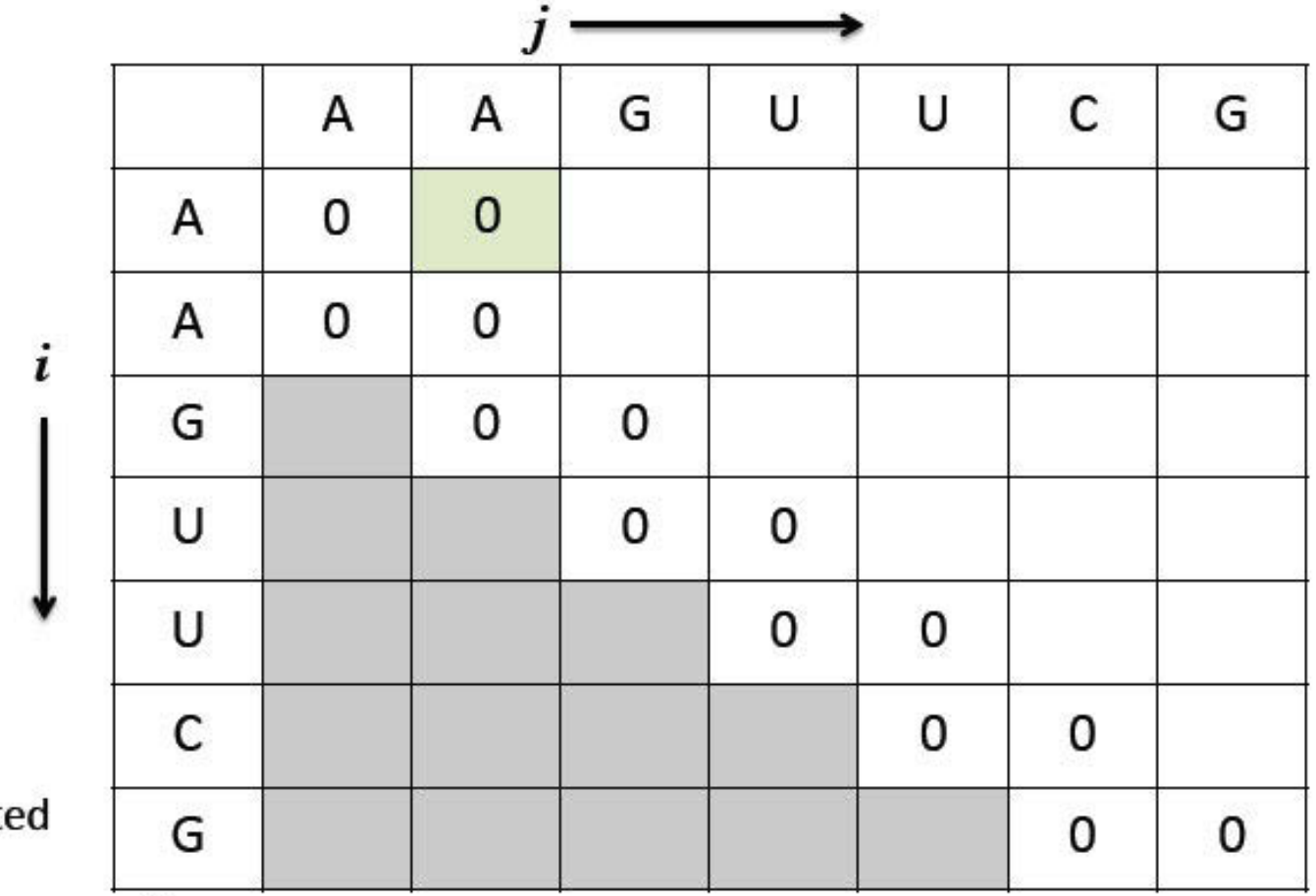
(3) Fill in *i, j*th entries according to

$$S(i,j) = \max \begin{cases} S(i+1,j-1) + 1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

Nussinov Algorithm - initialization

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

Nussinov Algorithm



Fill in highlighted square:

(i = 1,j = 2)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) & \\ S(i,j-1) & \\ \max_{i<k<j} S(i,k) + S(k+1,j) & \end{cases}$$

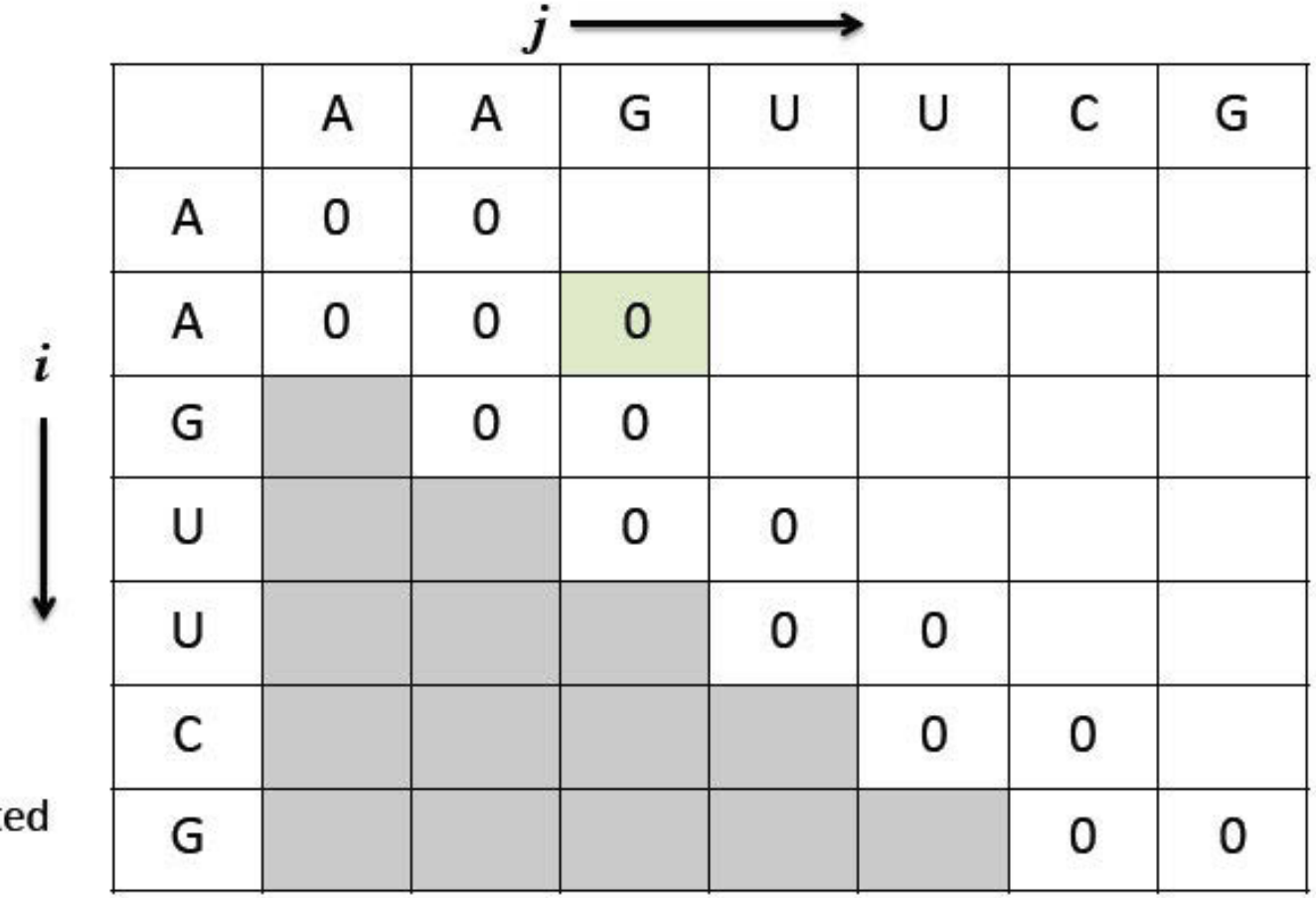
A-A don't base pair

$= 0$

$= 0$

Since i = 1, j = 2, no k such that $i < k < j$

Nussinov Algorithm



Fill in highlighted square:

(i = 2,j = 3)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & \text{[if } i,j \text{ base pair]} \\ S(i+1,j) & \\ S(i,j-1) & \\ \max_{i<k<j} S(i,k) + S(k+1,j) & \end{cases}$$

A-G don't base pair

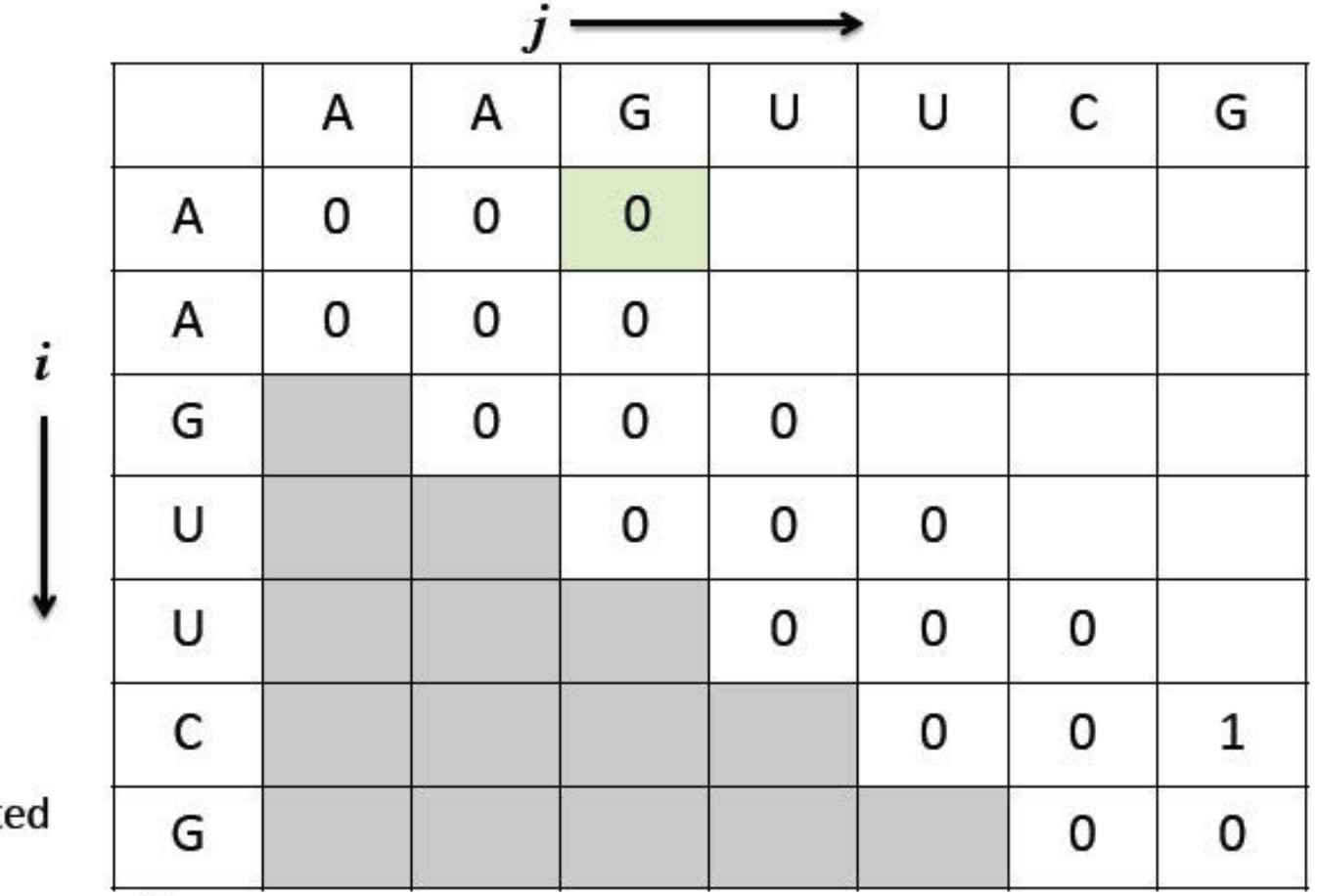
$= 0$

$= 0$

Since i = 2, j = 3, no k such that $i < k < j$

Fill in the rest of this diagonal!

Nussinov Algorithm



Fill in highlighted square:

(i = 1,j = 3)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) & \\ S(i,j-1) & \\ \max_{i<k<j} S(i,k) + S(k+1,j) & \end{cases}$$

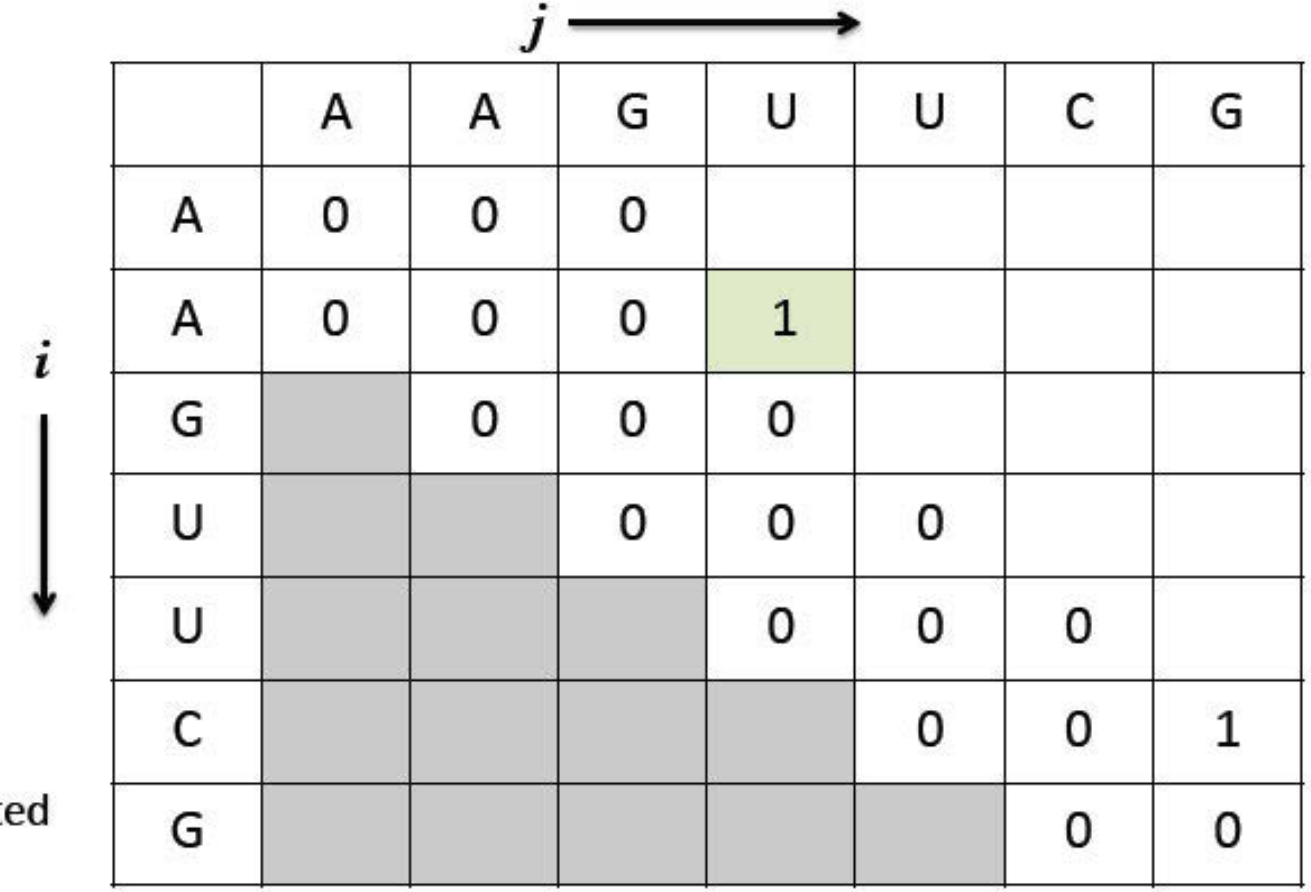
A-G don't base pair

$= 0$

$= 0$

$k = 2$: $S(1,2) + S(3,3) = 0+0$

Nussinov Algorithm



Fill in highlighted square:

(i = 2,j = 4)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

A-U **do** base pair: 0+1

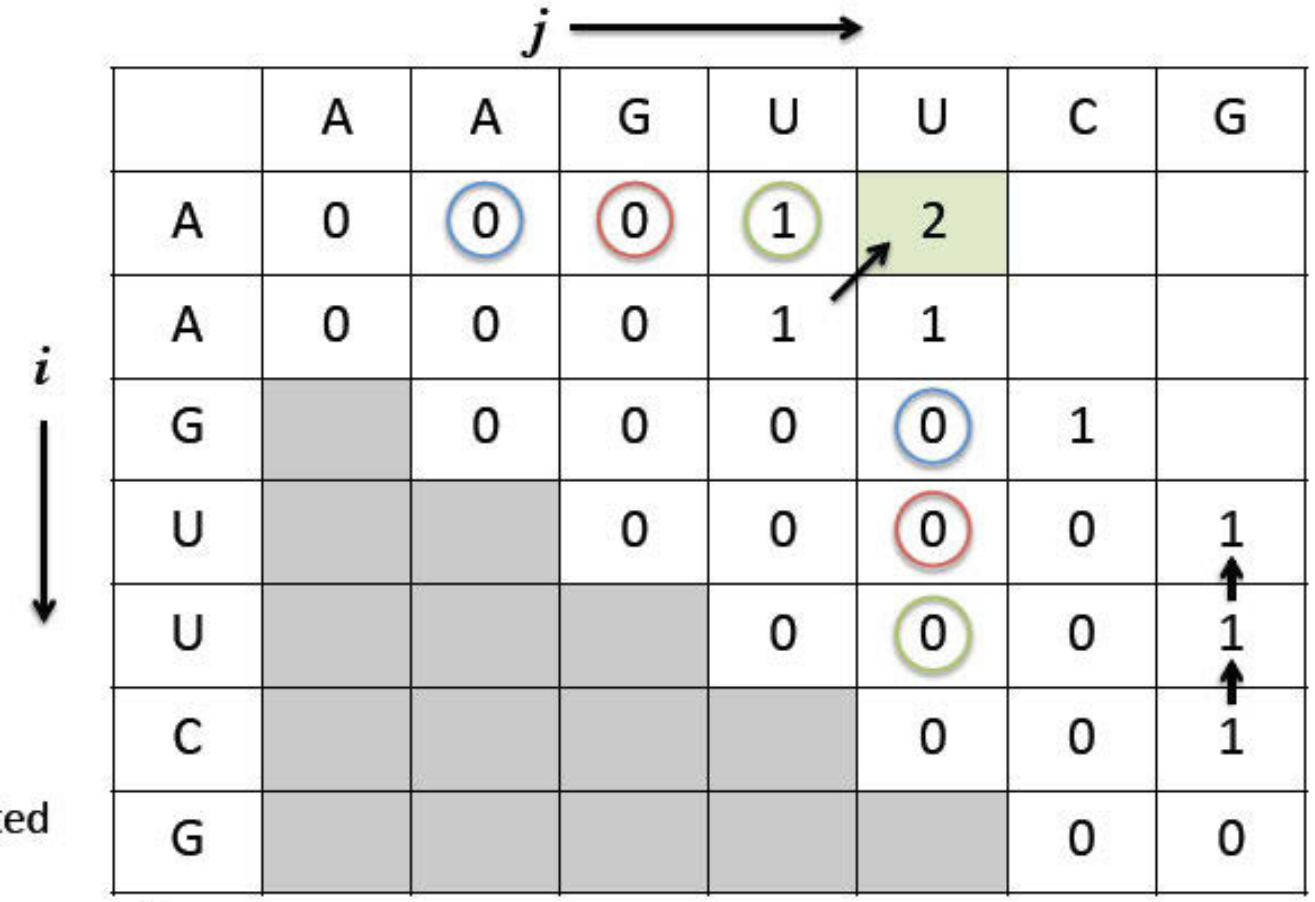
= 0

= 0

k = 3: S(2,3) + S(4,4) = 0+0

Fill in the rest of this diagonal and the one above it

Nussinov Algorithm



Only need to draw arrows between nonzero entries

Fill in highlighted square:

(i = 1,j = 5)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & \text{[if } i,j \text{ base pair]} \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

A-U **do** base pair: 1+1 = 2

= 1

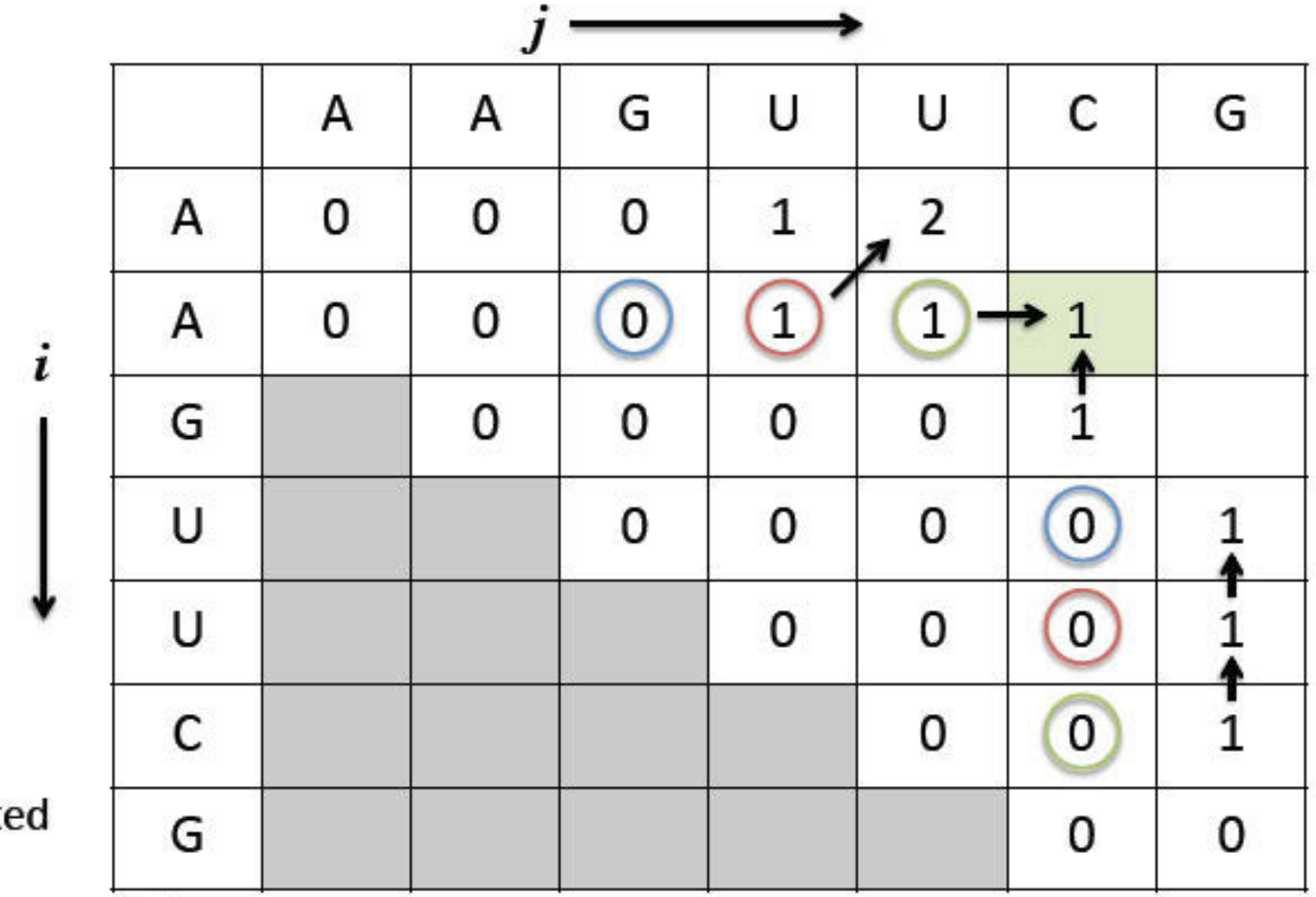
= 1

k = 2: S(1,2) + S(3,5) = 0+0

k = 3: S(1,3) + S(4,5) = 0+0

k = 4: S(1,4) + S(5,5) = 1+0 = 1

Nussinov Algorithm



Fill in highlighted square:

(i = 2, j = 6)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & \text{[if } i,j \text{ base pair]} \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

A-C don't base pair

$= 1$

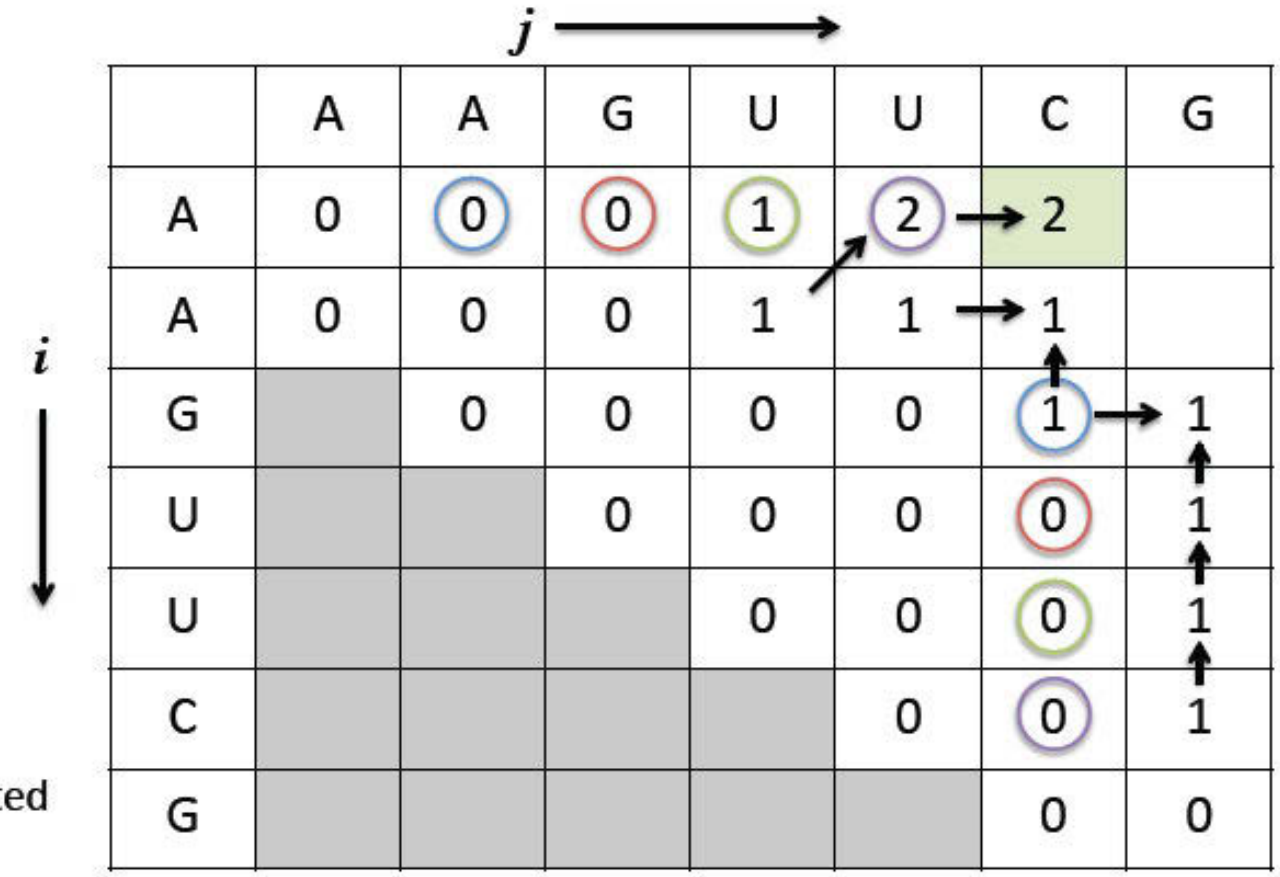
$= 1$

k = 3: S(2,3) + S(4,6) = 0+0

k = 4: S(2,4) + S(5,6) = 1+0 = 1

k = 5: S(2,5) + S(6,6) = 1+0 = 1

Nussinov Algorithm



Fill in highlighted square:

(i = 1,j = 6)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

A-C don't base pair

= 1

= 2

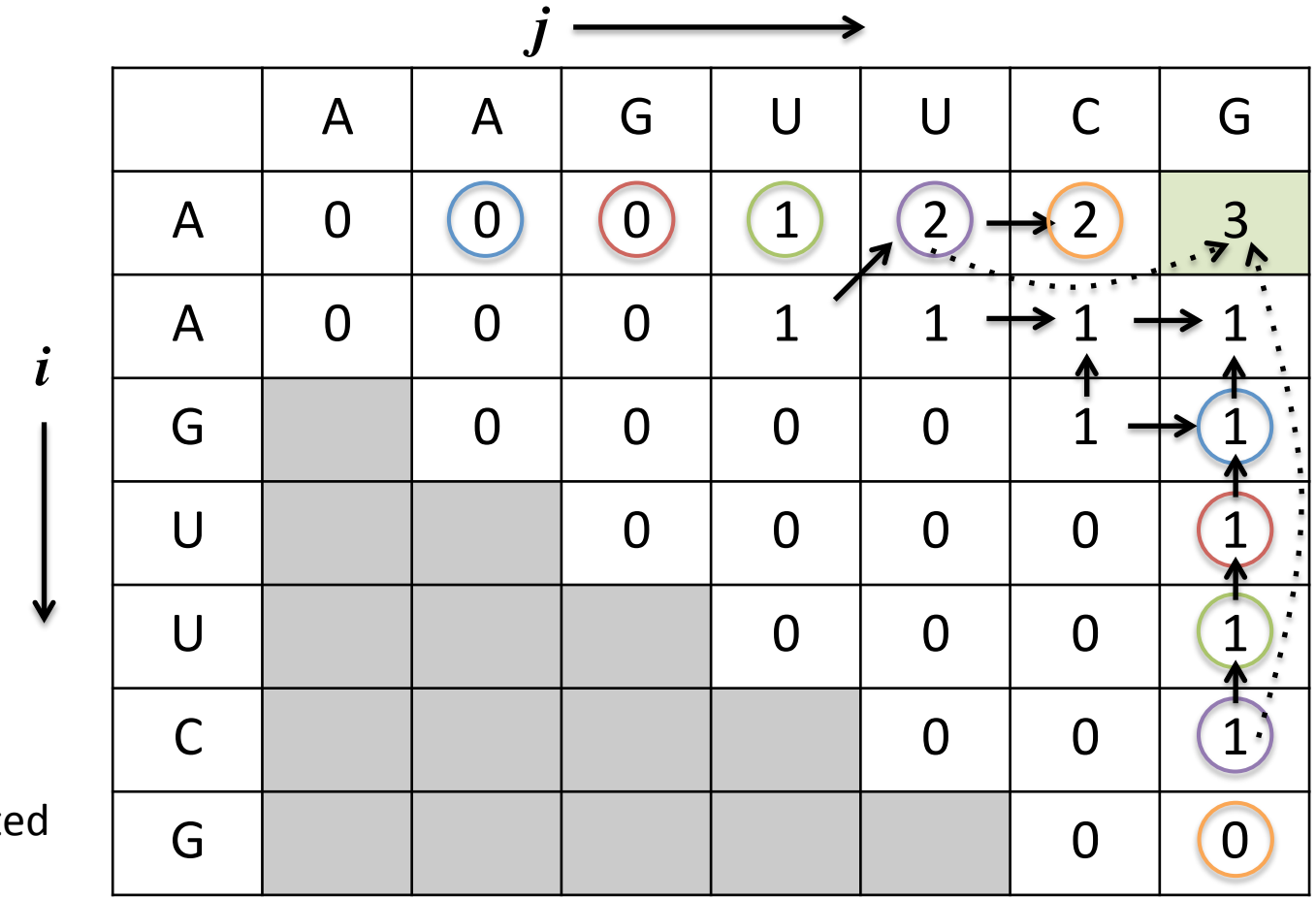
k = 2: S(1,2) + S(3,6) = 0+1 = 1

k = 3: S(1,3) + S(4,6) = 0+0 = 0

k = 4: S(1,4) + S(5,6) = 1+0 = 1

k = 5: S(1,5) + S(6,6) = 2+0 = 2

Nussinov Algorithm



Fill in highlighted square:

(i = 1,j = 7)

$$S(i,j) = \max \begin{cases} S(i+1,j-1)+1 & [\text{if } i,j \text{ base pair}] \\ S(i+1,j) \\ S(i,j-1) \\ \max_{i<k<j} S(i,k) + S(k+1,j) \end{cases}$$

A-G don't base pair

= 1

= 2

k = 2: S(1,2) + S(3,7) = 0+1 = 1

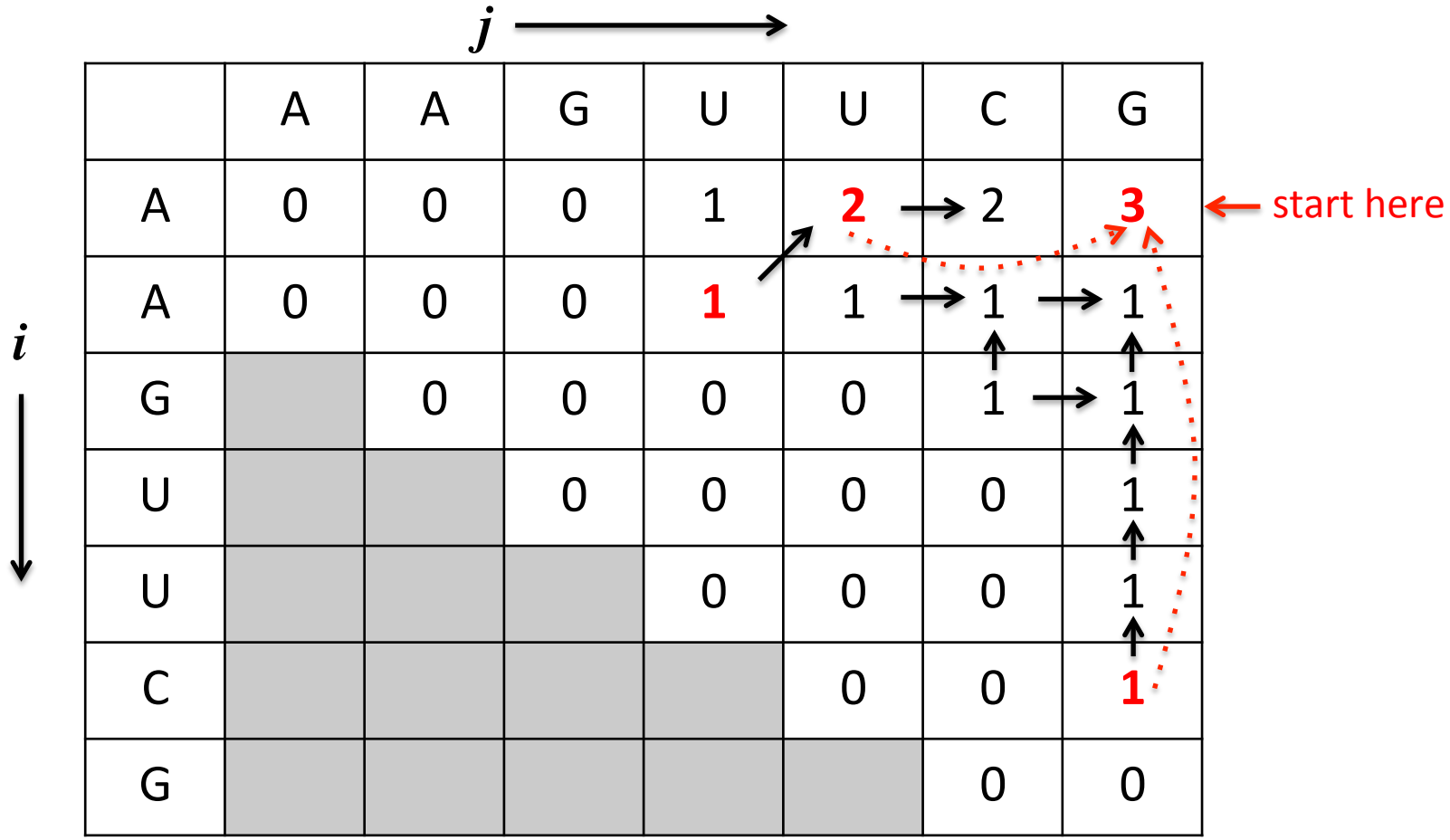
k = 3: S(1,3) + S(4,7) = 0+1 = 1

k = 4: S(1,4) + S(5,7) = 1+1 = 2

k = 5: S(1,5) + S(6,7) = 2+1 = 3

k = 6: S(1,6) + S(7,7) = 2+0 = 2

Nussinov Algorithm -traceback



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

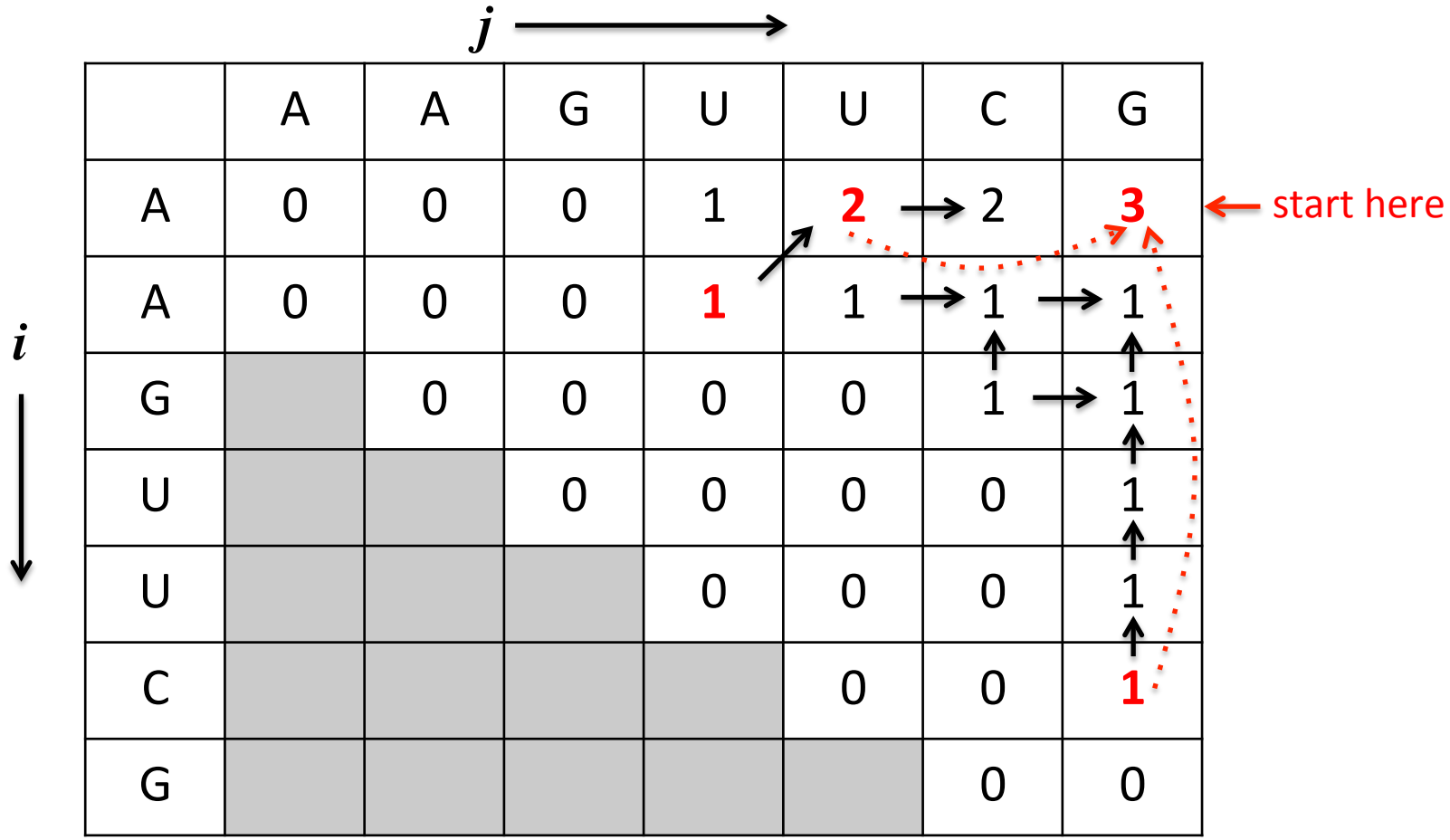
Can you draw this folded RNA?

k = 5: S(1,5) + S(6,7) = 2+1 = 3

Optimal sub-structure from 1-5 (with 2 matches)

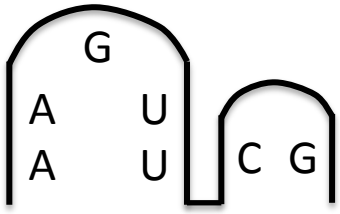
Optimal sub-structure from 6-7 (with 1 match)

Nussinov Algorithm -traceback



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

Can you draw this folded RNA?

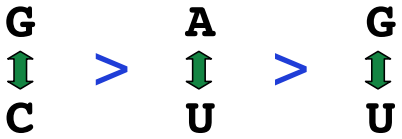


- note that in reality, stems can't form if the loop is less than 3bp due to restrictions on backbone angles

Improvements on Nussinov algorithm

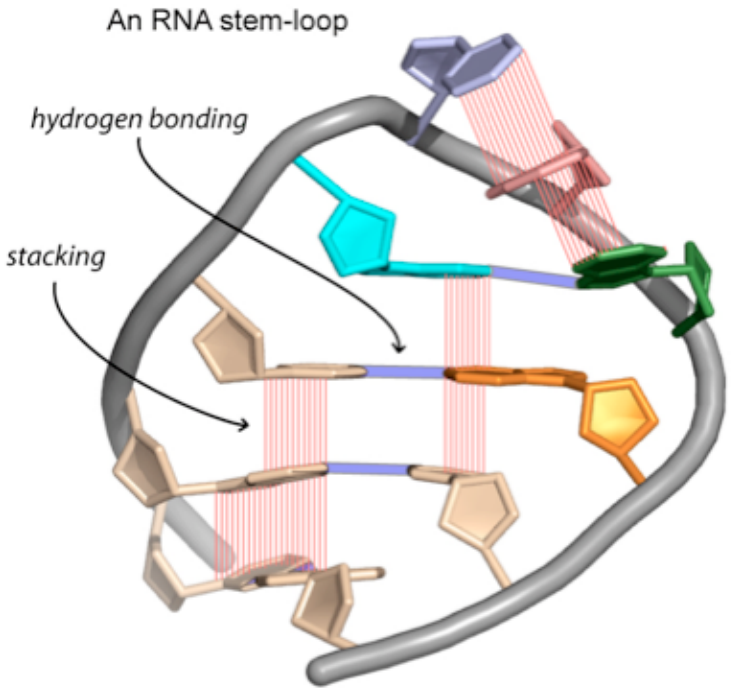
- Nussinov is the "core" of most RNA folding programs, but they all have bells & whistles
  - Take into account that loop must be 3 or more nucleotides
  - Not all base pairs are equal in reality (we treated them all at +1 in Nussinov)
  - Base stacking interactions

• base **pairing**:



• base **stacking**:

Base stacking contributes more to free energy than base pairing



Improvements on Nussinov algorithm

- Nussinov is the "core" of most RNA folding programs, but they all have bells & whistles
  - Take into account that loop must be 3 or more nucleotides
  - Not all base pairs are equal in reality (we treated them all at +1 in Nussinov)
  - Base stacking interactions
  - Penalizes interior bulges
  - Extra terms at terminal ends of RNA exposed to solvent
  - -Nussinov algorithm cannot detect pseudoknots, since these do not satisfy the recursive assumption that each structure can be split into smaller self-contained sub-structures - more advanced algorithms
  - With all these additions, mfold gets ~70% of bases correctly folded; pretty good on average but would likely want to do *in vivo* structure profiling of your RNA if you really want to know its structure

Happy Spring Break!

7.91J / 20.490J / 20.390J / 7.36J / 6.802J / 6.874J / HST.506J Foundations of Computational and Systems Biology
Spring 2014