RNA Secondary Structure Prediction

BMI/CS 776

www.biostat.wisc.edu/bmi776/

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Goals for Lecture

Key concepts

- RNA secondary structure
- secondary structure features: stems, loops, bulges
- Pseudoknots
- Nussinov algorithm
- adapting Nussinov to take free energy into account

Why RNA is Interesting

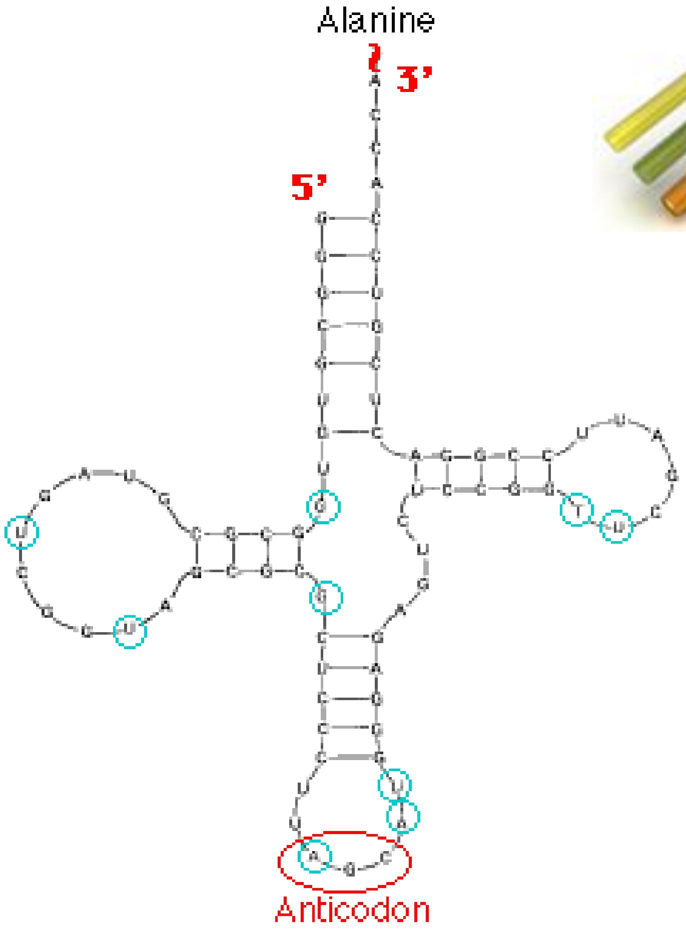
- In addition to messenger RNA (mRNA), there are other RNA molecules that play key roles in biology
  - ribosomal RNA (rRNA)
    - ribosomes are complexes that incorporate several RNA subunits in addition to numerous protein units
  - transfer RNA (tRNA)
    - transport amino acids to the ribosome during translation
  - the spliceosome, which performs intron splicing, is a complex with several RNA units
  - microRNAs and others that play regulatory roles
  - the genomes for many viruses (e.g. HIV) are encoded in RNA
  - etc.

- Folding of an mRNA can be involved in regulating the gene’s expression

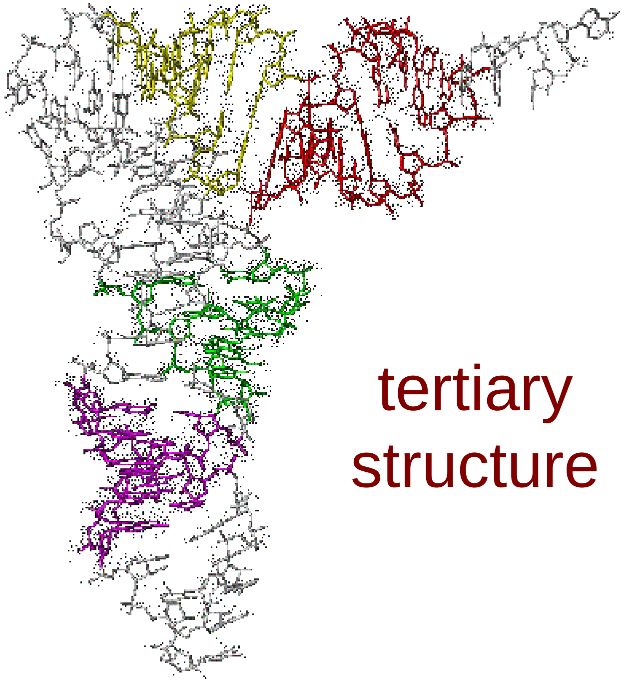
RNA Secondary Structure

- RNA is typically single stranded
- Folding, in large part is determined by base-pairing
  - **`A–U`** and **`C–G`** are the canonical base pairs
  - other bases will sometimes pair, especially **`G–U`**
- Base-paired structure is referred to as the *secondary structure* of RNA
- Related RNAs often have homologous secondary structure without significant sequence similarity

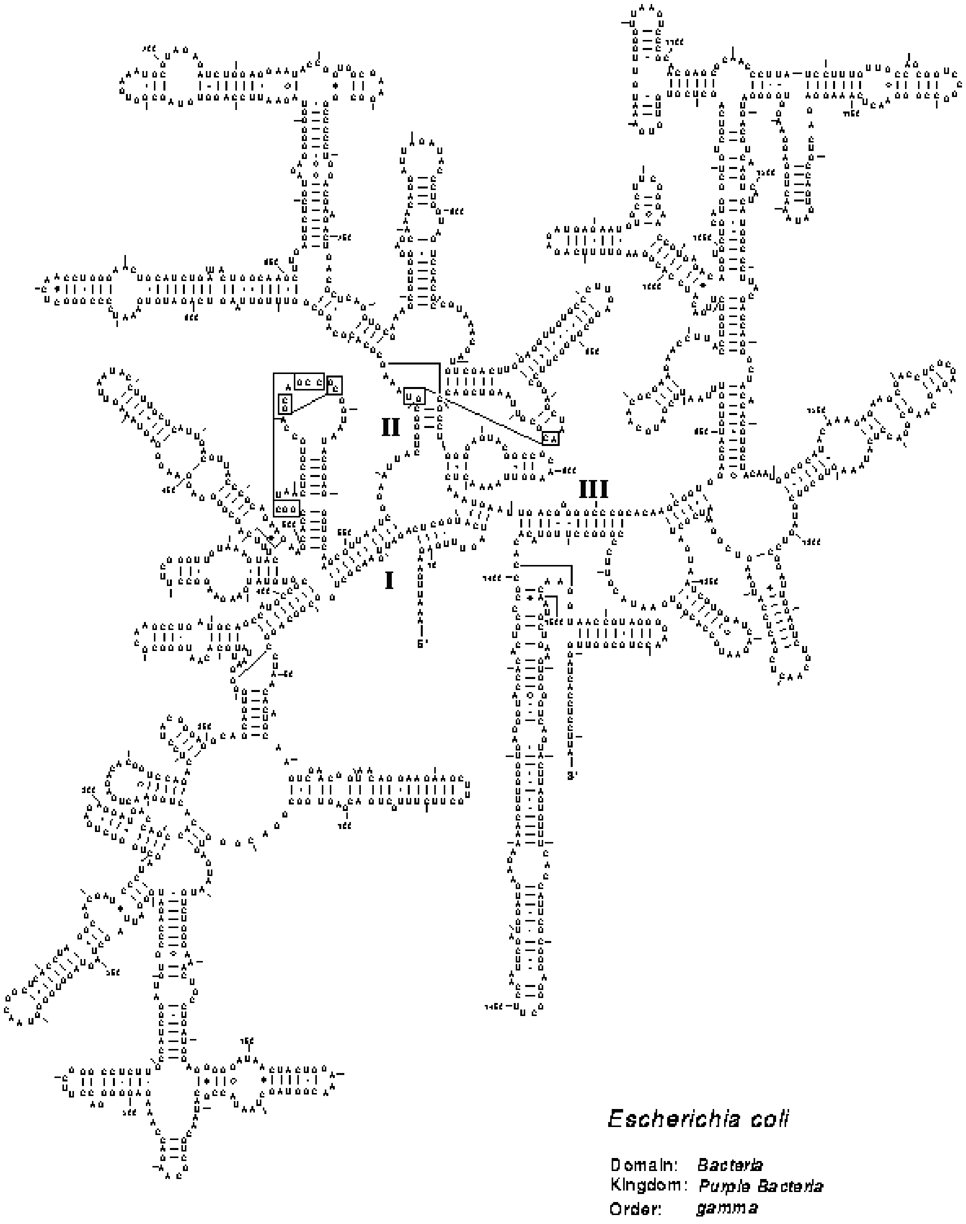
tRNA Secondary Structure




Scitable


tertiary structure

Small Subunit Ribosomal RNA

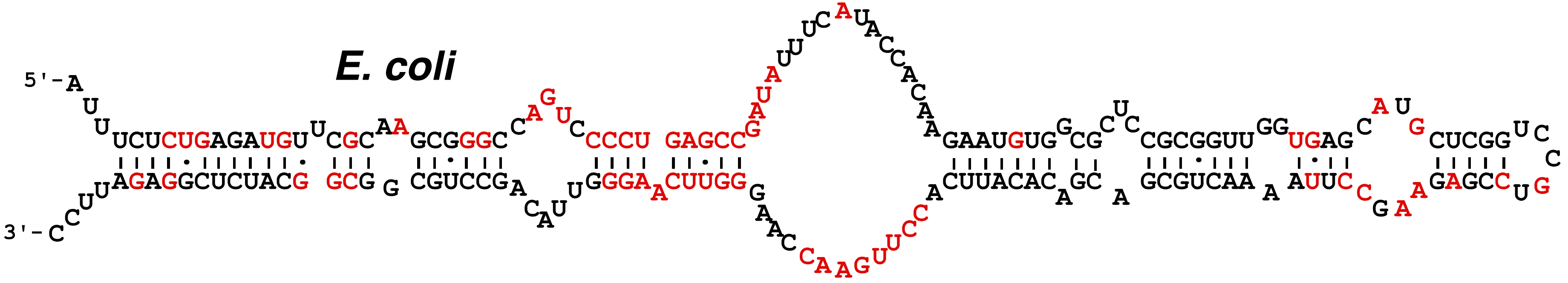


Escherichia coli

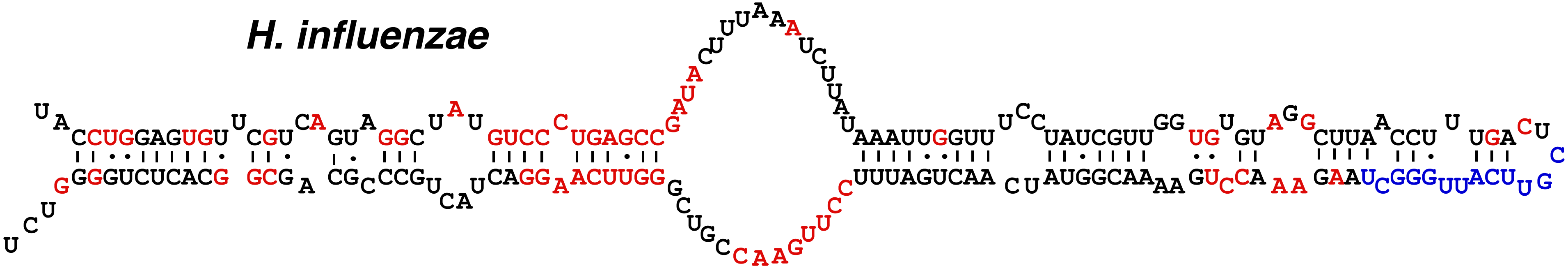
Domain: *Bacteria*
Kingdom: *Purple Bacteria*
Order: *gamma*

6S RNA Secondary Structure

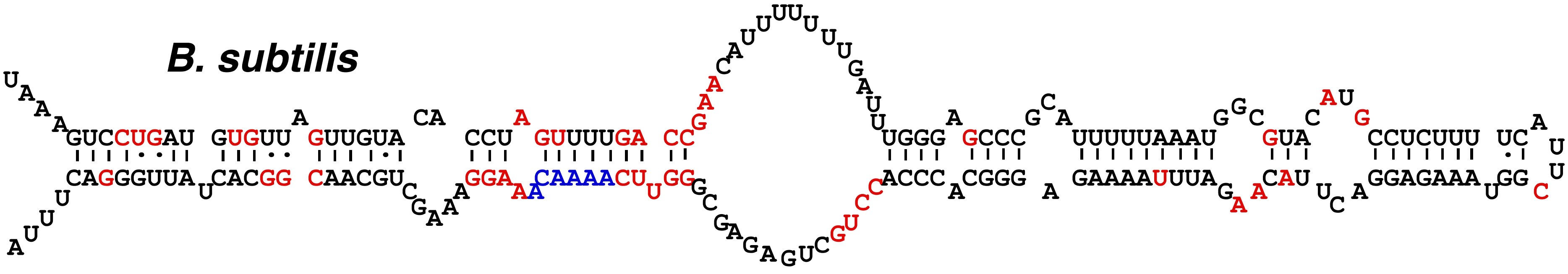
E. coli



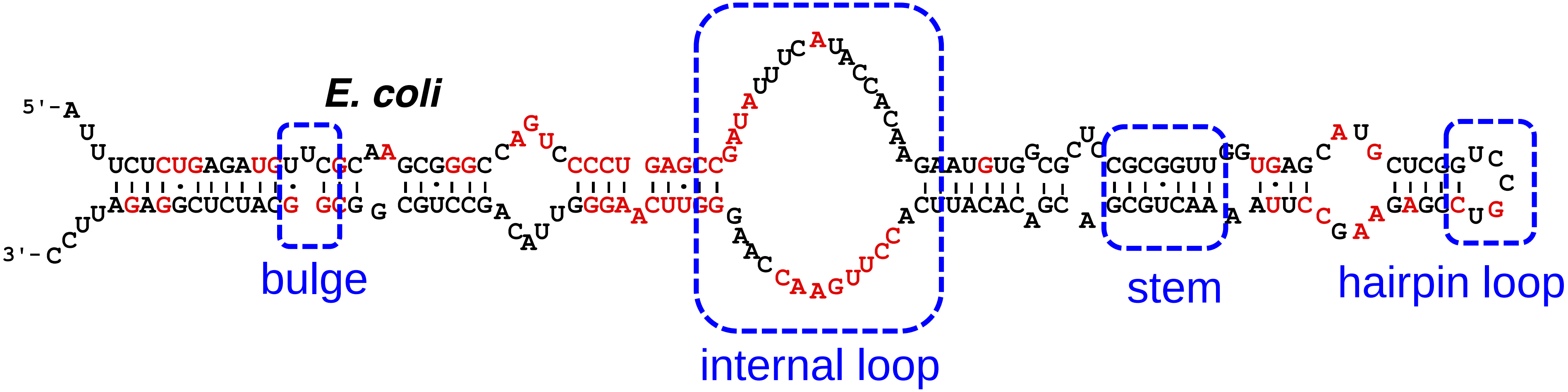
H. influenzae



B. subtilis



Secondary Structure Features



Four Key Problems

Focus for today

- Predicting RNA secondary structure
  Given: RNA sequence
  Do: predict secondary structure that sequence will fold into

- Searching for instances of a given structure
  Given: an RNA sequence or its secondary structure
  Do: find sequences that will fold into a similar structure

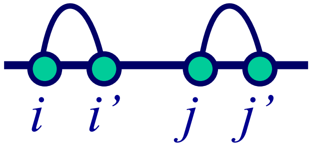
- Modeling a family of RNAs
  Given: a set of RNA sequences with similar secondary structure
  Do: construct a model that captures the secondary structure regularities of the set

- Identifying novel RNA genes
  Given: a pair of homologous DNA sequences
  Do: identify subsequences that appear to have highly conserved RNA secondary structure (putative RNA genes)

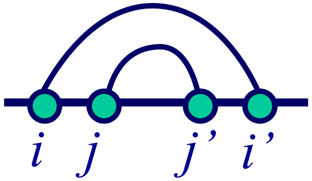
RNA Folding Assumption

- Algorithms we'll consider assume that base pairings do not cross

- For base-paired positions i, i' and j, j', with $i < i'$ and $j < j'$, we must have either

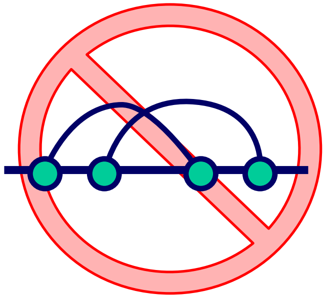


$i < i' < j < j'$ or $j < j' < i < i'$ (not nested)



$i < j < j' < i'$ or $j < i < i' < j'$ (nested)

- Can't have $i < j < i' < j'$ or $j < i < j' < i'$



Pseudoknots

- These crossings are called *pseudoknots*
- Dynamic programming breaks down if pseudoknots are allowed
- Fortunately, they are not very frequent

pseudoknot

Figure from Seliverstov et al. *BMC Microbiology*, 2005

Simplest RNA Secondary Structure Task

Given:

- An RNA sequence
- The constraint that pseudoknots are not allowed

Do:

- Find a secondary structure for the RNA that maximizes the number of base pairing positions

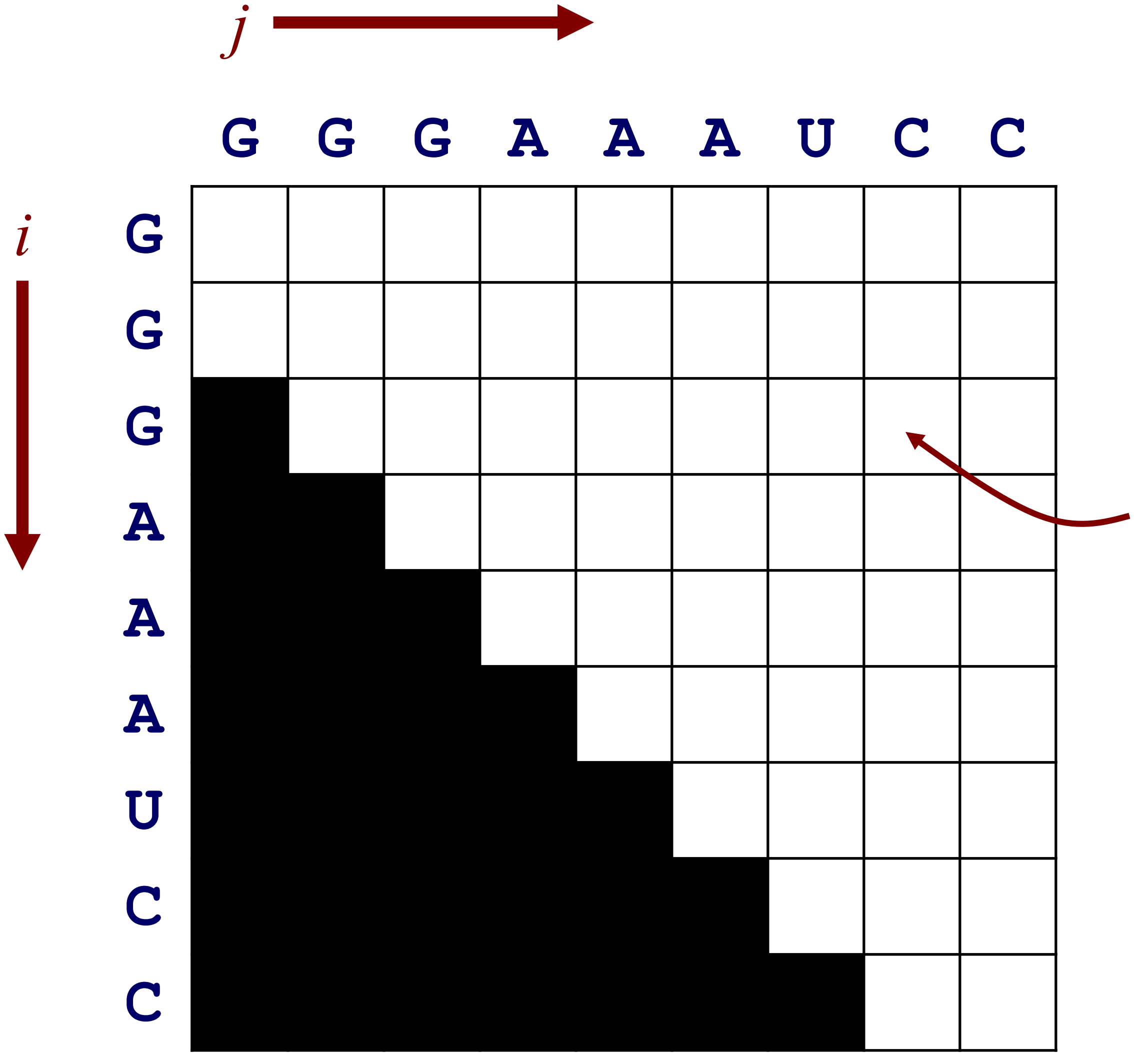
Predicting RNA Secondary Structure: the Nussinov Algorithm

[Nussinov et al., *SIAM Journal of Applied Mathematics* 1978]

Key idea:

- Do this using dynamic programming
  - start with small subsequences
  - progressively work to larger ones

DP in the Nussinov Algorithm



max # of
paired bases in
subsequence $[i, j]$

Figure 10.8 from textbook

DP in the Nussinov Algorithm

- Let $$\delta(i, j) = \begin{cases} 1 & \text{if } x_i \text{ and } x_j \text{ are complementary} \\ 0 & \text{otherwise} \end{cases}$$

- Initialization:

$$\gamma(i, i-1) = 0 \qquad \text{for } i = 2 \text{ to } L$$

$$\gamma(i, i) = 0 \qquad \text{for } i = 1 \text{ to } L$$

- Recursion

$$\gamma(i, j) = \max \begin{cases} \gamma(i+1, j) \\ \gamma(i, j-1) \\ \gamma(i+1, j-1) + \delta(i, j) \\ \max\limits_{i<k<j} \left[\gamma(i, k) + \gamma(k+1, j)\right] \end{cases}$$

max # of
paired bases in
subsequence $[i, j]$

Nussinov Algorithm Traceback

```
push $(1,L)$ onto stack
repeat until stack is empty
    pop $(i,j)$
    if $i \geq j$ continue
    else if $\gamma(i+1, j) = \gamma(i, j)$ push $(i+1, j)$
    else if $\gamma(i, j-1) = \gamma(i, j)$ push $(i, j-1)$
    else if $\gamma(i+1, j-1) + \delta(i, j) = \gamma(i, j)$
        record $i, j$ base pair
        push $(i+1, j-1)$
    else for $k = i+1$ to $j$-1 : if $\gamma(i,k) + \gamma(k+1, j) = \gamma(i, j)$
        push $(k+1, j)$
        push $(i,k)$
        break
```

Predicting RNA Secondary Structure by Energy Minimization

- It’s naïve to predict folding just by maximizing the number of base pairs
- However, we can generalize the key recurrence relation so that we’re <u>minimizing</u> free energy instead

$$E(i,j) = \min \begin{cases} E(i+1,j) \\ E(i,j\text{-}1) \\ \min\limits_{\mathrm{i<k<j}} \left[E(i,k) + E(k+1,j) \right] \\ P(i,j) \longleftarrow \end{cases}$$

case that i and j are base paired

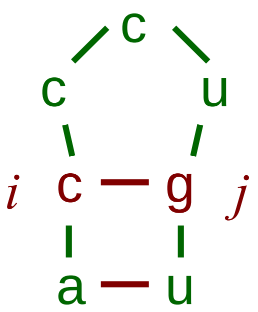
Predicting RNA Secondary Structure by Energy Minimization

- A sophisticated program, such as Mfold [Zuker et al.], can take into account free energy of the “local environment” of $[i, j]$

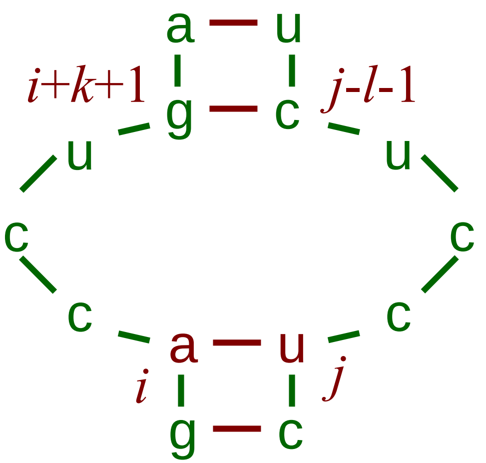
$$P(i,j) = \min \begin{cases} \alpha(i,j) + \mathrm{LoopEnergy}(j-i-1) \\ \alpha(i,j) + \mathrm{StackingEnergy}(i,j,i+1,j-1) + P(i+1,j-1) \\ \min\limits_{k\geq 1}\left[\alpha(i,j) + \mathrm{BulgeEnergy}(k) + P(i+k+1,j-1)\right] \\ \min\limits_{k\geq 1}\left[\alpha(i,j) + \mathrm{BulgeEnergy}(k) + P(i+1,j-k-1)\right] \\ \min\limits_{k,l\geq 1}\left[\alpha(i,j) + \mathrm{LoopEnergy}(k+l) + P(i+k+1,j-l-1)\right] \\ \min\limits_{j>k>i}\left[\alpha(i,j) + E(i+1,k) + E(k+1,j-1)\right] \end{cases}$$

Predicting RNA Secondary Structure by Energy Minimization

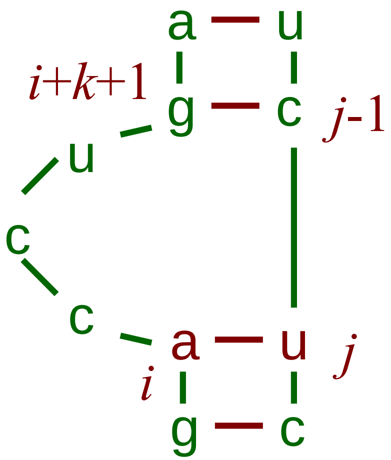
$$\alpha(i, j) + \text{LoopEnergy}(j - i - 1)$$



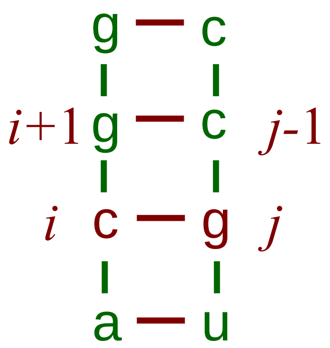
$$\min_{k,l \geq 1}\left[\alpha(i, j) + \text{LoopEnergy}(k + l) + P(i + k + 1, j - l - 1)\right]$$



$$\min_{k \geq 1}\left[\alpha(i, j) + \text{BulgeEnergy}(k) + P(i + k + 1, j - 1)\right]$$

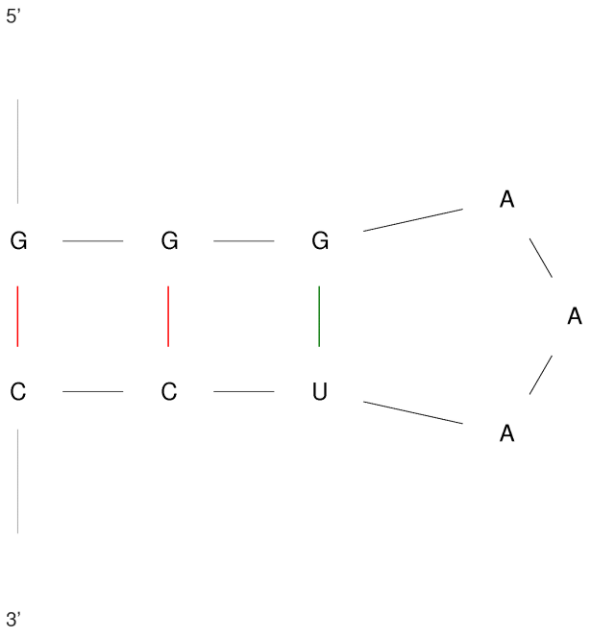


$$\alpha(i, j) + \text{StackingEnergy}(i, j, i + 1, j - 1) + P(i + 1, j - 1)$$

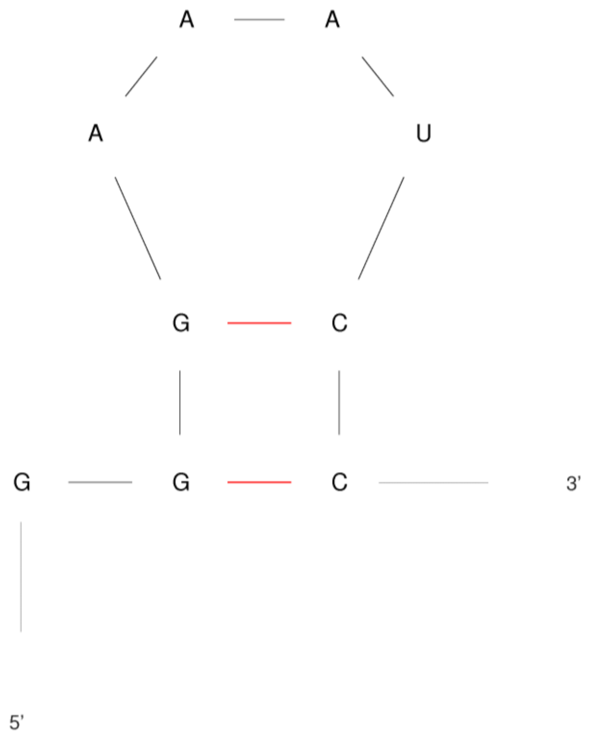


Mfold example

GGGAAAUCC



ΔG = -0.80 kcal/mol



ΔG = 0.20 kcal/mol

http://unafold.rna.albany.edu/