RNA Secondary Structure Prediction

BMI/CS 776
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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

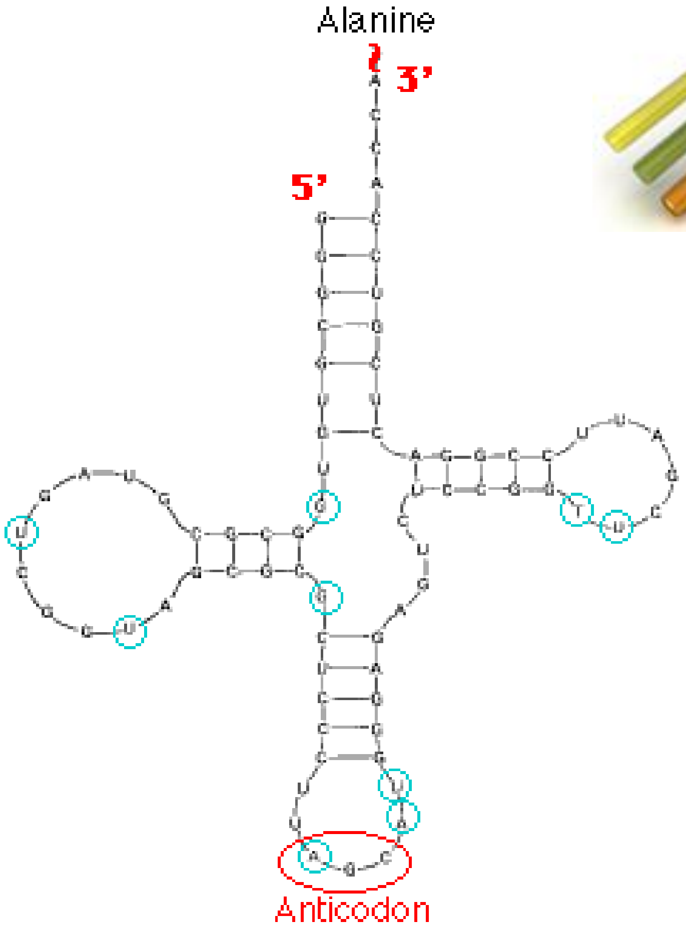
- Messenger RNA (mRNA) isn’t the only important class of RNA
  - ribosomal RNA (rRNA)
    - ribosomes are complexes that incorporate several RNA subunits in addition to numerous protein units
  - transfer RNA (tRNA)
    - transport amino acids to the ribosome during translation
  - the spliceosome, which performs intron splicing, is a complex with several RNA units
  - microRNAs and others that play regulatory roles
  - many viruses (e.g. HIV) have RNA genomes
  - guide RNA
    - sequence complementary determines whether to cleave DNA

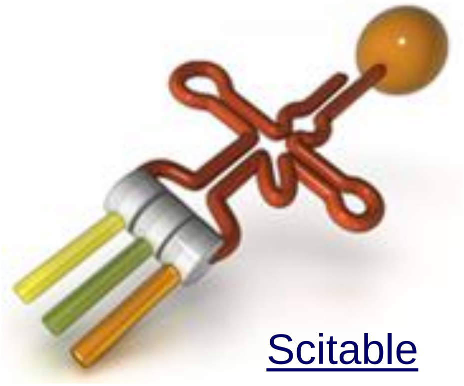
- Folding of an mRNA can be involved in regulating the gene’s expression

RNA Secondary Structure

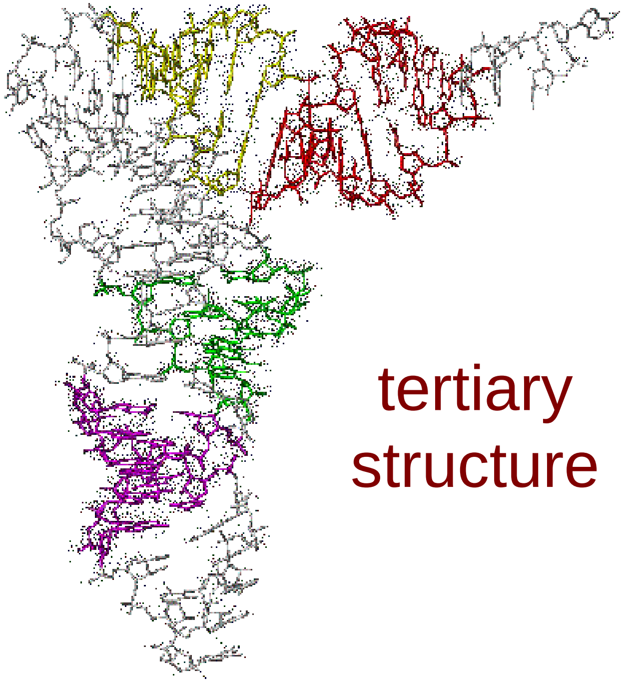
- RNA is typically single stranded
- Folding, in large part is determined by base-pairing
  - `A–U` and `C–G` are the canonical base pairs
  - other bases will sometimes pair, especially `G–U`
- Base-paired structure is referred to as the *secondary structure* of RNA
- Related RNAs often have homologous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

tRNA Secondary Structure



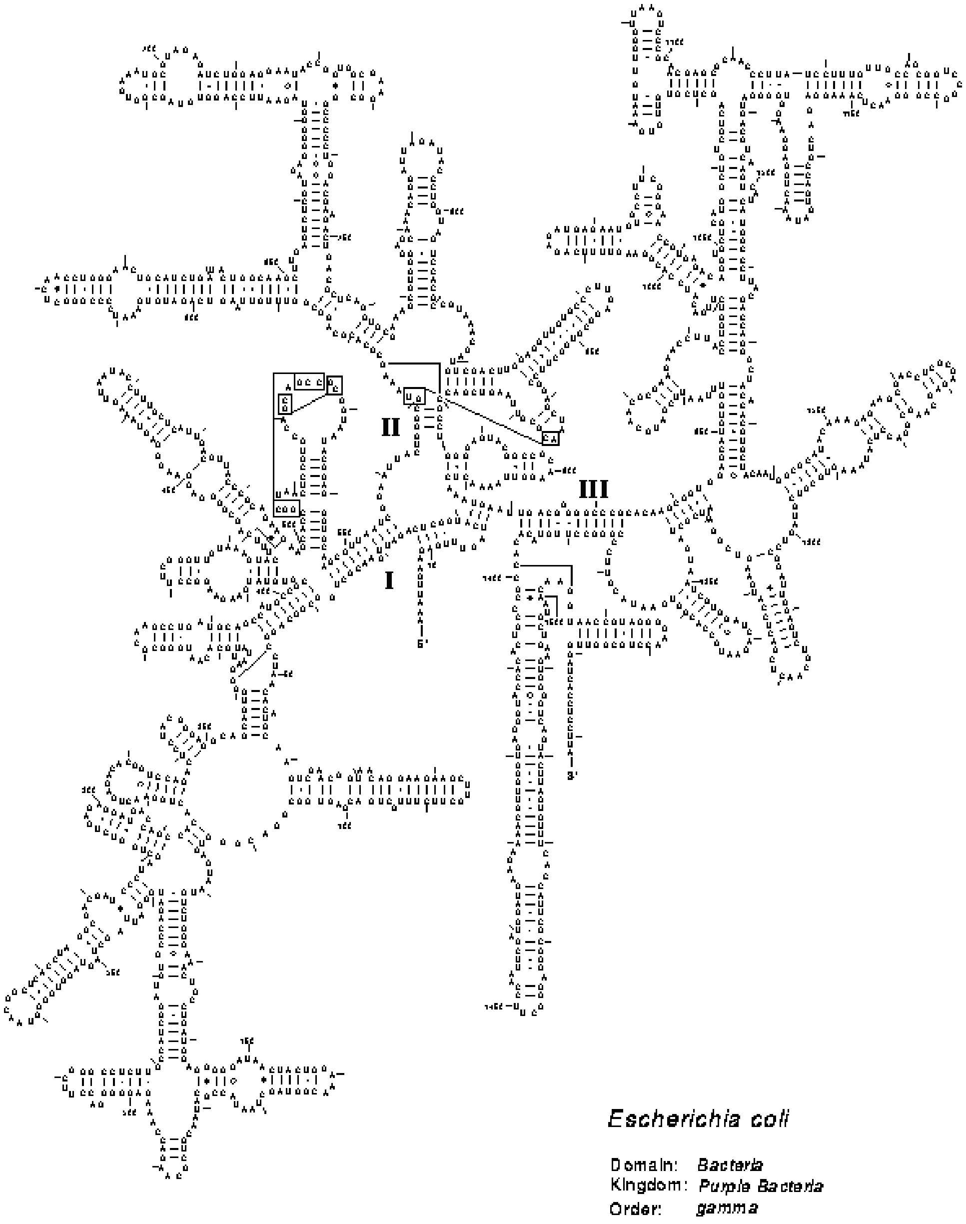


Scitable

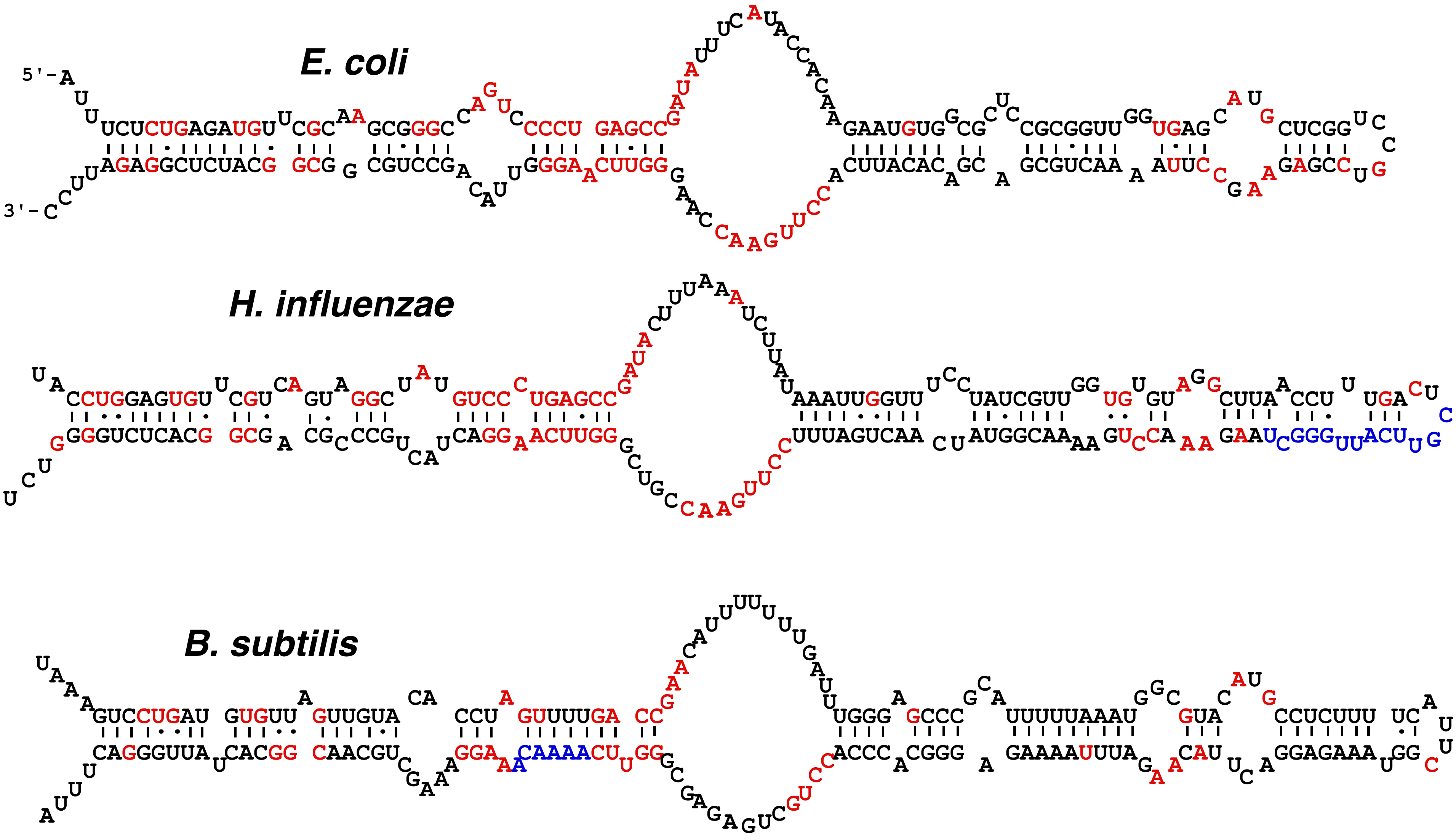


tertiary structure

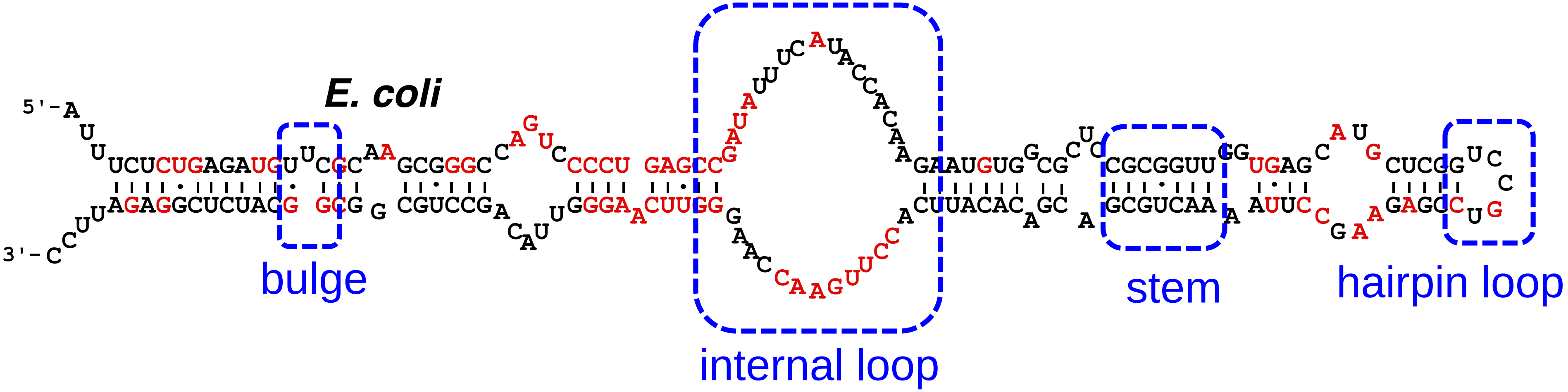
Small Subunit Ribosomal RNA



6S RNA Secondary Structure



Secondary Structure Features



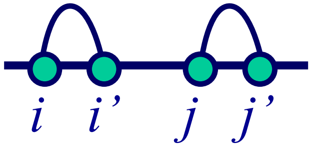
Four Key Problems

Focus for today

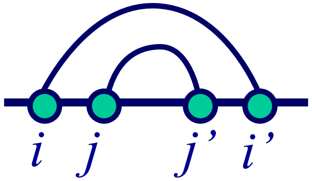
- Predicting RNA secondary structure
  Given: RNA sequence
  Do: predict secondary structure that sequence will fold into
- Searching for instances of a given structure
  Given: an RNA sequence or its secondary structure
  Do: find sequences that will fold into a similar structure
- Modeling a family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

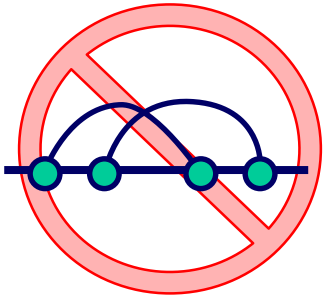


$i < i' < j < j'$ or $j < j' < i < i'$ (not nested)



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

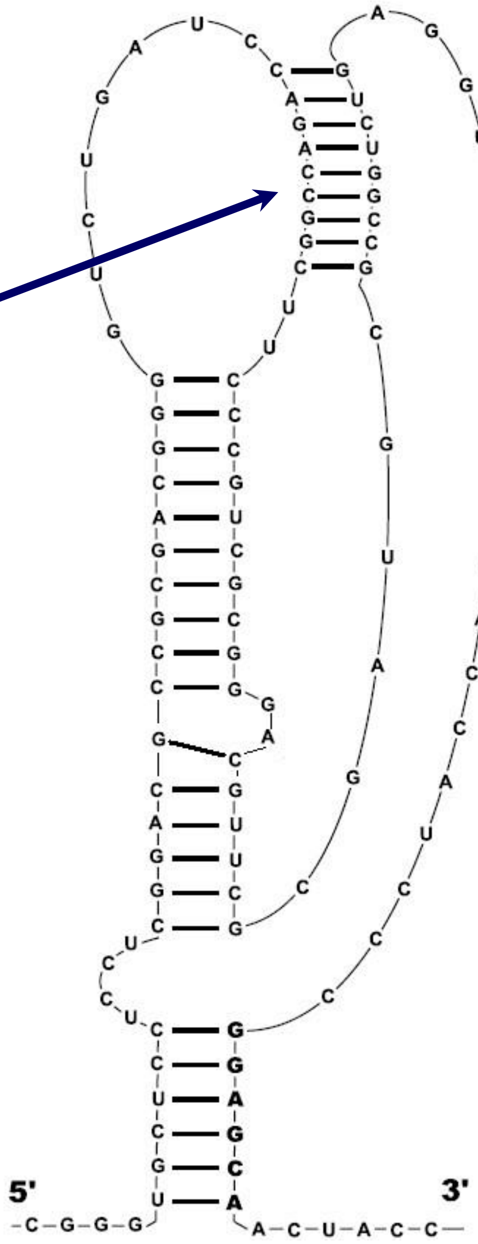
- Can't have $i < j < i' < j'$ or $j < i < j' < i'$



Pseudoknots

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

- Modern software does support them
  - Akiyama et al. 2018



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

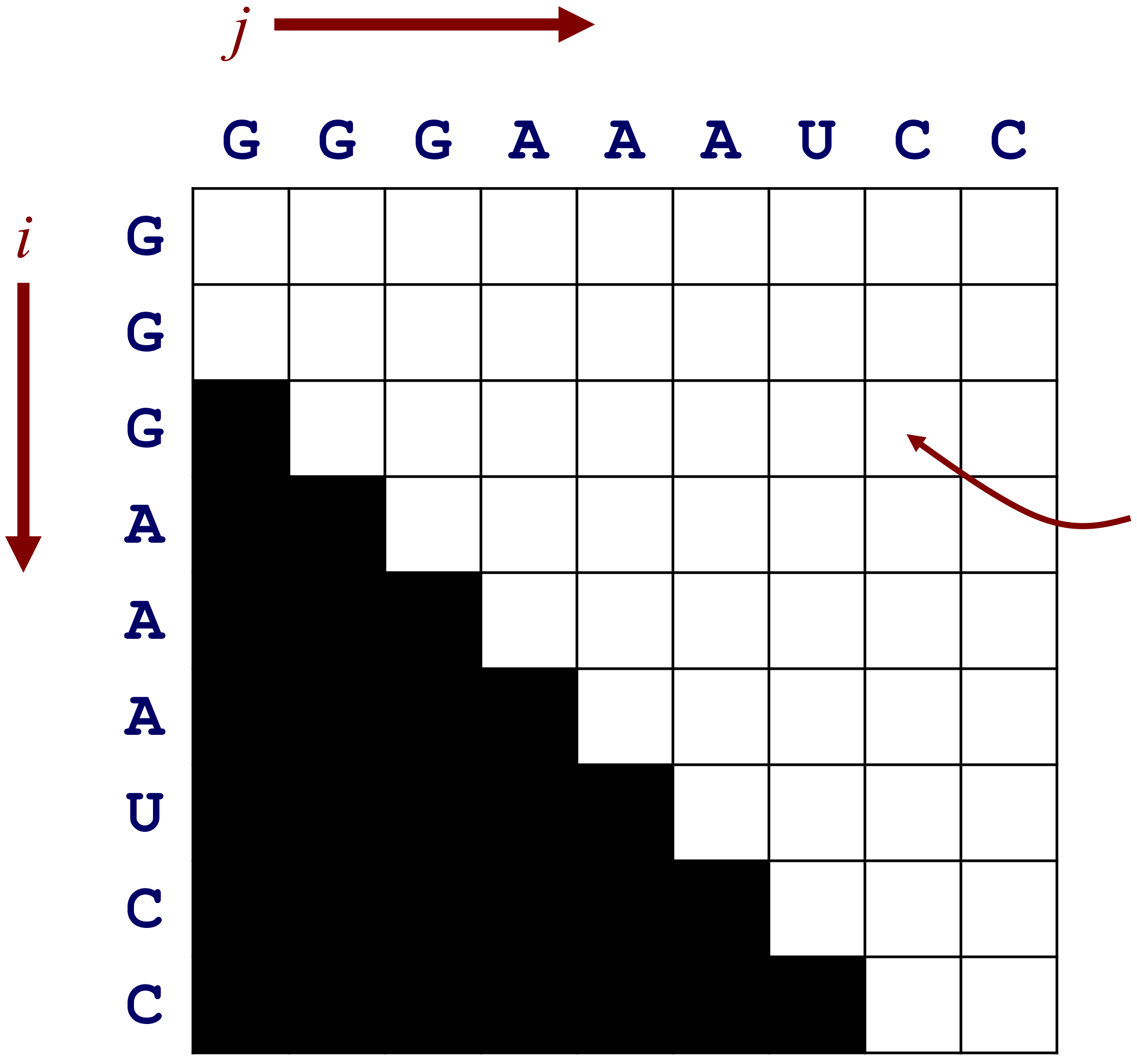
Predicting RNA Secondary Structure: the Nussinov Algorithm

[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 minimizing free energy instead

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

case that i and j are base paired

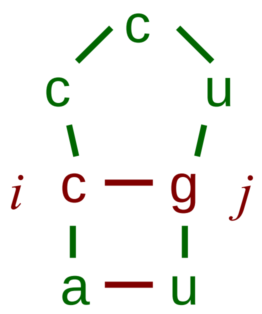
Predicting RNA Secondary Structure by Energy Minimization

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

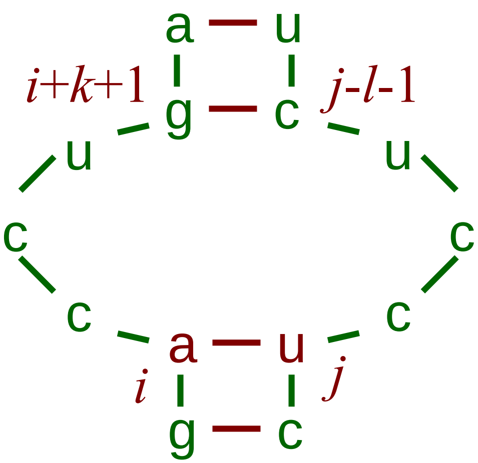
$$P(i,j) = \min \begin{cases} \alpha(i,j) + \text{LoopEnergy}(j-i-1) \\ \alpha(i,j) + \text{StackingEnergy}(i,j,i+1,j-1) + P(i+1,j-1) \\ \min\limits_{k \geq 1}\left[\alpha(i,j) + \text{BulgeEnergy}(k) + P(i+k+1,j-1)\right] \\ \min\limits_{k \geq 1}\left[\alpha(i,j) + \text{BulgeEnergy}(k) + P(i+1,j-k-1)\right]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

Predicting RNA Secondary Structure by Energy Minimization

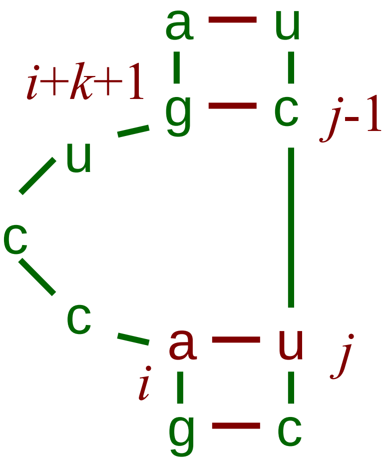
$$\alpha(i,j) + \text{LoopEnergy}(j-i-1)$$



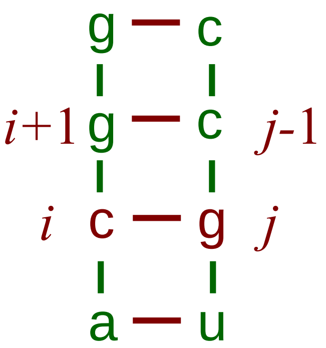
$$\min_{k,l\geq 1}\left[\alpha(i,j) + \text{LoopEnergy}(k+l) + P(i+k+1,\, j-l-1)\right]$$



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

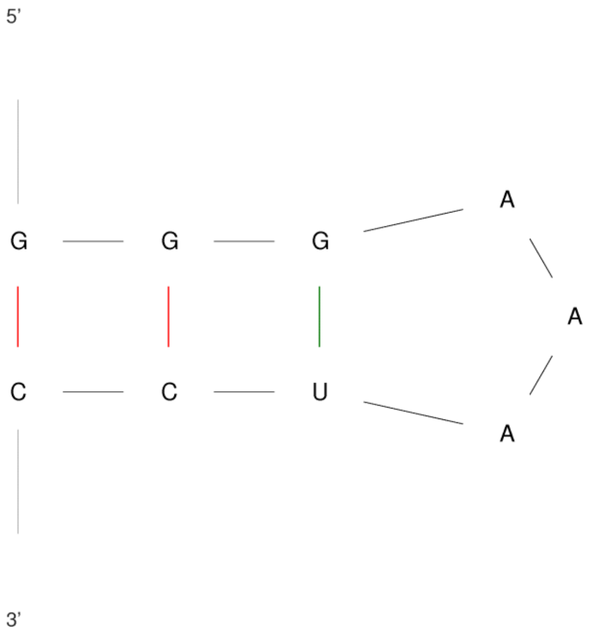


$$\alpha(i,j) + \text{StackingEnergy}(i,j,i+1,j-1) + P(i+1,\, j-1)$$

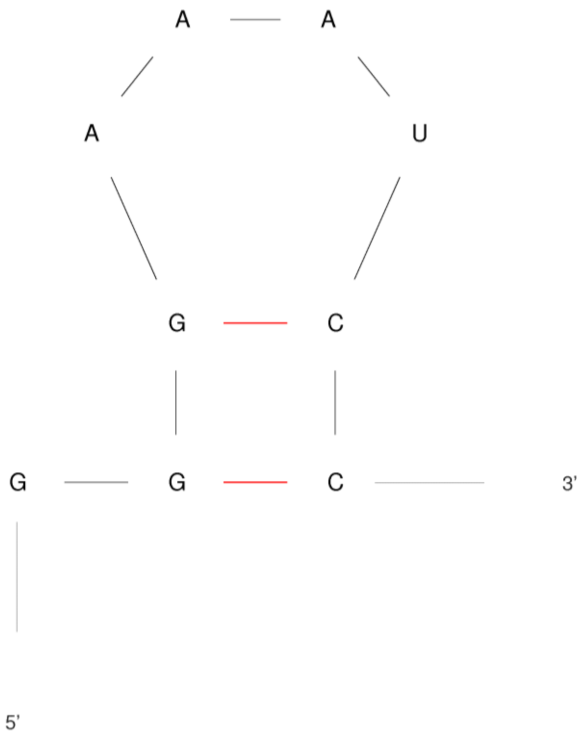


Mfold example

GGGAAAUCC



ΔG = -0.80 kcal/mol



ΔG = 0.20 kcal/mol

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