CSE 527
Lecture 17, 11/24/04

RNA Secondary Structure Prediction

Outline

- What is it
- How is it Represented
- Why is it important
- Examples
- Approaches

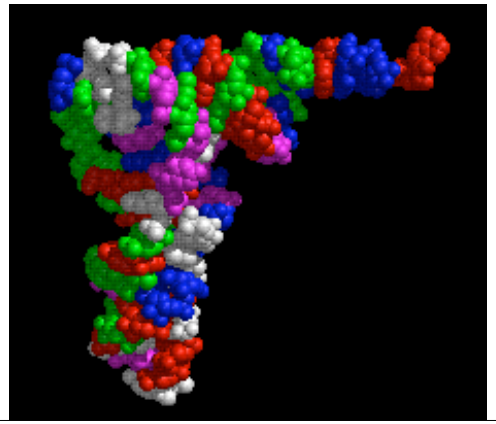
RNA Structure

- Primary Structure: Sequence
- Secondary Structure: Pairing
- Tertiary Structure: 3D shape

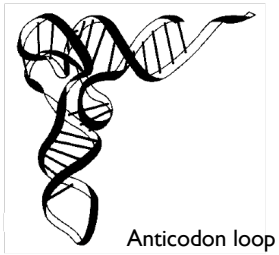
RNA Pairing

- Watson-Crick Pairing
  - C - G ~ 3 kcal/mole
  - A - U ~ 2 kcal/mole
- "Wobble Pair" G - U ~ 1 kcal/mole
- Non-canonical Pairs (esp. if modified)

A tRNA 3d Structure



tRNA - Alt. Representations



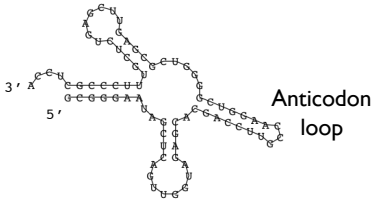
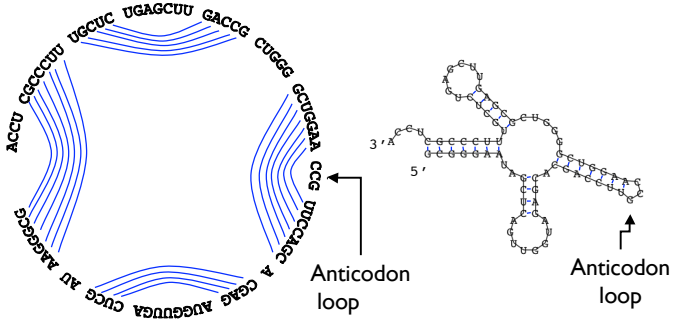


Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

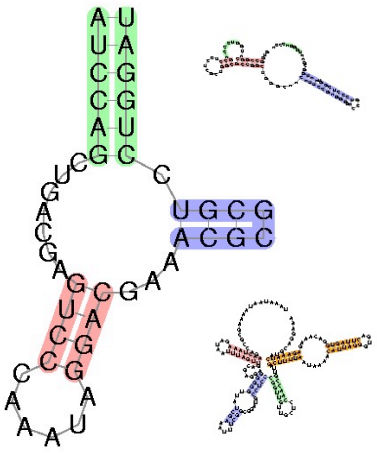
b) The secondary structure extracts the most important information about the structure, namely the pattern of base pairings.

tRNA - Alt. Representations



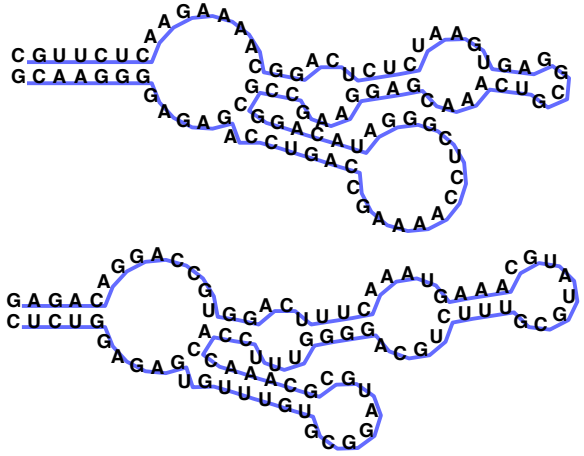
Why?

- RNA's fold, and function
- Nature uses what works



Importance

- Ribozymes (RNA Enzymes)
- Retroviruses
- Effects on transcription, translation, splicing...
- Functional RNAs: rRNA, tRNA, snRNA, snoRNA, micro RNA, RNAi, riboswitches, regulatory elements in 3' & 5' UTRs, ...



RNA Pairing

- Watson-Crick Pairing
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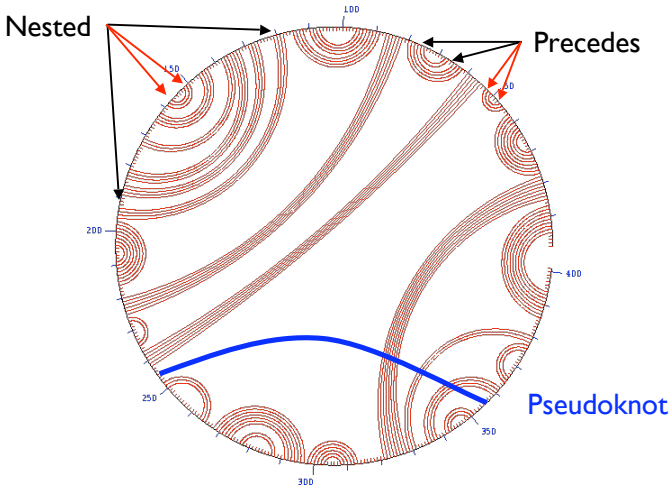
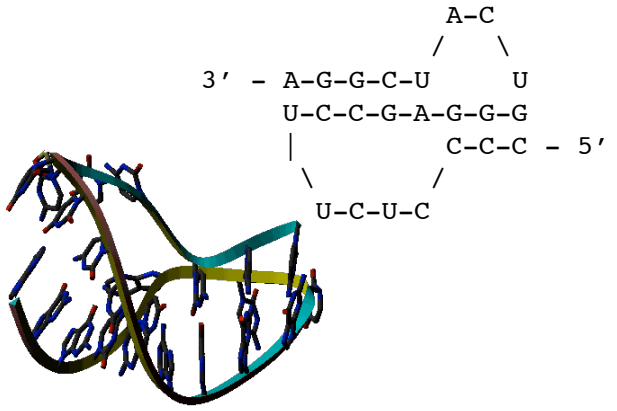
Definitions

- Sequence $^{5'}\, r_1\, r_2\, r_3 \ldots r_n\, ^{3'}$ in {A, C, G, T}
- A Secondary Structure is a set of pairs i•j s.t.
  1. $i < j-4$
  2. if i•j & i'•j' are two pairs with $i \le i'$, then
     - A. i = i' & j = j', or
     - B. j < i', or
     - C. i < i' < j' < j

  } First pair precedes 2nd, or is nested within it. No "pseudoknots."



A Pseudoknot



Approaches to Structure Prediction

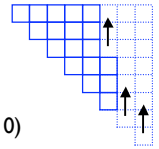
- Maximum Pairing
  + works on single sequences
  + simple
  - too inaccurate
- Minimum Energy
  + works on single sequences
  - ignores pseudoknots
  - only finds "optimal" fold
- Partition Function
  + finds all folds
  - ignores pseudoknots

Approaches, II

- Comparative sequence analysis
  + handles all pairings (incl. pseudoknots)
  - requires several (many?) aligned, appropriately diverged sequences
- Stochastic Context-free Grammars
  Roughly combines min energy & comparative, but no pseudoknots
- Physical experiments (x-ray crystalography, NMR)

Nussinov: Max Pairing

- B(i,j) = # pairs in optimal pairing of r_i ... r_j
- B(i,j) = 0 for all i, j with i ≥ j-4; otherwise
- B(i,j) = max of:
  1. B(i+1,j)
  2. B(i,j-1)
  3. B(i+1,j-1) +(if r_i pairs with r_j then 1 else 0)
  4. max { B(i,k)+B(k+1,j) | i < k < j }

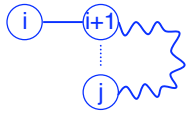


Time: $O(n^3)$

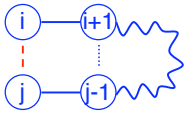
"optimal pairing of r_i ... r_j"

Several (overlapping, but exhaustive) possibilities

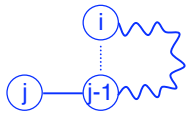
1. r_i is unpaired; look at best way to pair r_{i+1} ... r_j



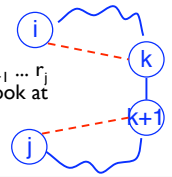
3. they pair with each other, so 1 + best r_{i+1} ... r_{j-1}



2. r_j is unpaired; look at best way to pair r_i ... r_{j-1}



4. They pair, but *not* to each other; i pairs with k for some i < k < j; so look at best r_i ... r_k + best r_{k+1} ... r_j (don't need to look at other k; why?)



Pair-based Energy Minimization

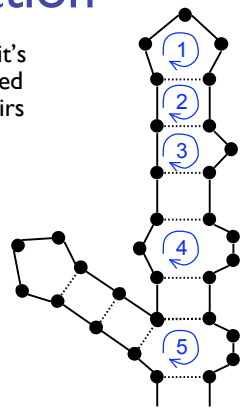
- E(i,j) = energy of pairs in optimal pairing of r_i ... r_j
- E(i,j) = ∞ for all i, j with i ≥ j-4; otherwise
- E(i,j) = min of:
  - E(i+1,j)
  - E(i,j-1)
  - E(i+1,j-1) + e(r_i, r_j)
  - min { E(i,k)+E(k+1,j) | i < k < j }

energy of one pair

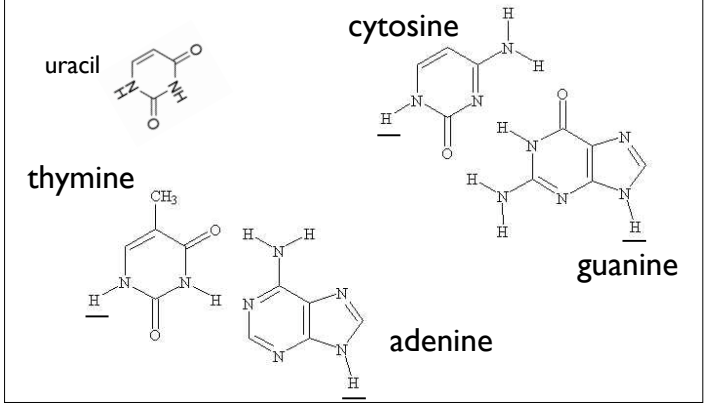
Time: $O(n^3)$

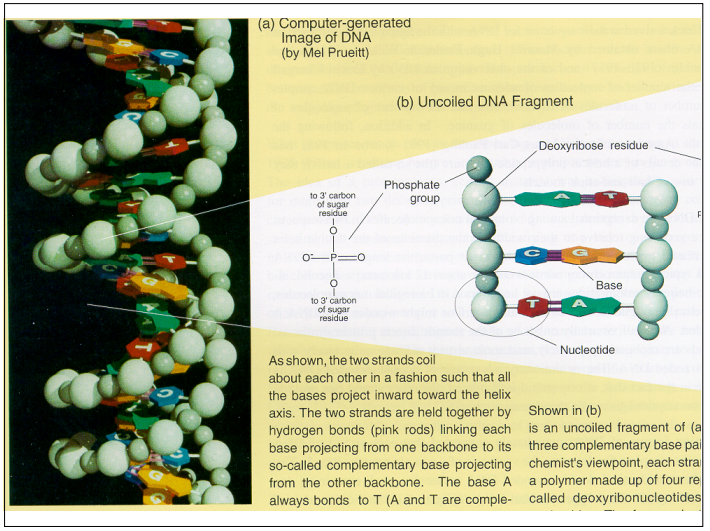
Loop-based Energy Minimization

- Detailed experiments show it's more accurate to model based on loops, rather than just pairs
- Loop types
  1. Hairpin loop
  2. Stack
  3. Bulge
  4. Interior loop
  5. Multiloop

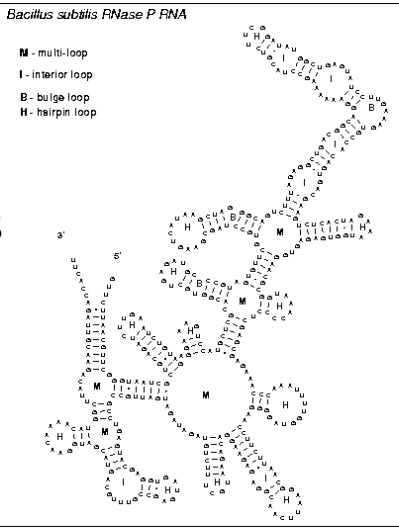


Base Pairs and Stacking





Loop Examples



Zuker: Loop-based Energy, I

- W(i,j) = energy of optimal pairing of r_i ... r_j
- V(i,j) = as above, but forcing pair i•j
- W(i,j) = V(i,j) = ∞ for all i, j with i ≥ j-4
- W(i,j) = min(W(i+1,j), W(i,j-1), V(i+1,j-1), min { E(i,k)+E(k+1,j) | i < k < j })

Zuker: Loop-based Energy, II

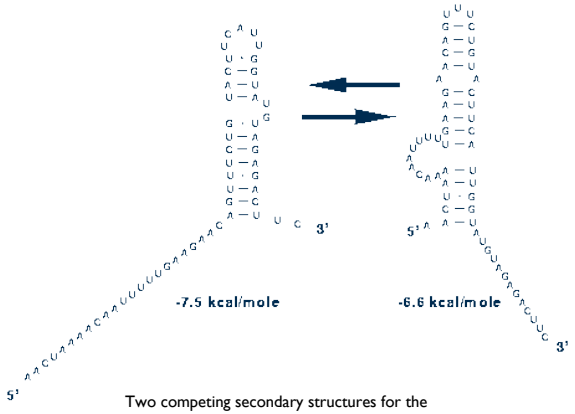
hairpin stack bulge/ interior multi- loop

- V(i,j) =min(eh(i,j), es(i,j)+V(i+1,j-1), VBI(i,j), VM(i,j))
- VM(i,j) = min { W(i,k)+W(k+1,j) | i < k < j })
- VBI(i,j) = min { ebi(i,j,i',j') + V(i', j') | i < i' < j' < j & i'-i+j-j' > 2 }

bulge/ interior

Time: $O(n^4)$

$O(n^3)$ possible if ebi(.) is "nice"



Two competing secondary structures for the Leptomonas collosoma spliced leader mRNA.

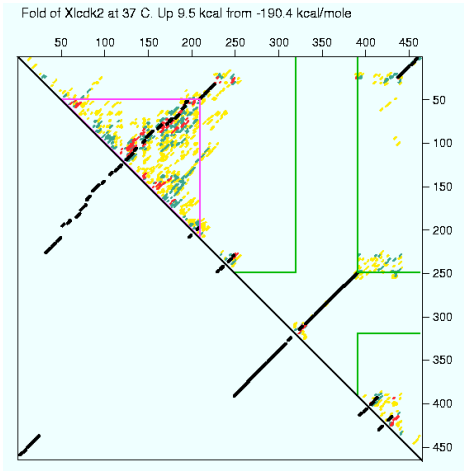
Suboptimal Energy

- There are always alternate folds with near-optimal energies. Thermodynamics predicts that populations of identical molecules will exist in different folds; individual molecules even flicker among different folds
- Zuker's algorithm can be modified to find suboptimal folds
- McCaskill gives a more elaborate dynamic programming algorithm calculating the "partition function," which defines the probability distribution over all these states.

Example of suboptimal folding

Black dots: pairs in opt fold

Colored dots: pairs in folds 2-5% worse than optimal fold



A “Mountain” diagram

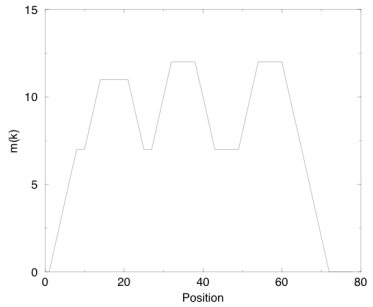


Figure 3: Mountain representation of the tRNA secondary structure shown in Figure 1. The three plateaus correspond to the three hairpin loops of the clover leave structure.