CSE 527
Lecture 21, 12/8/03

RNA Secondary Structure Prediction

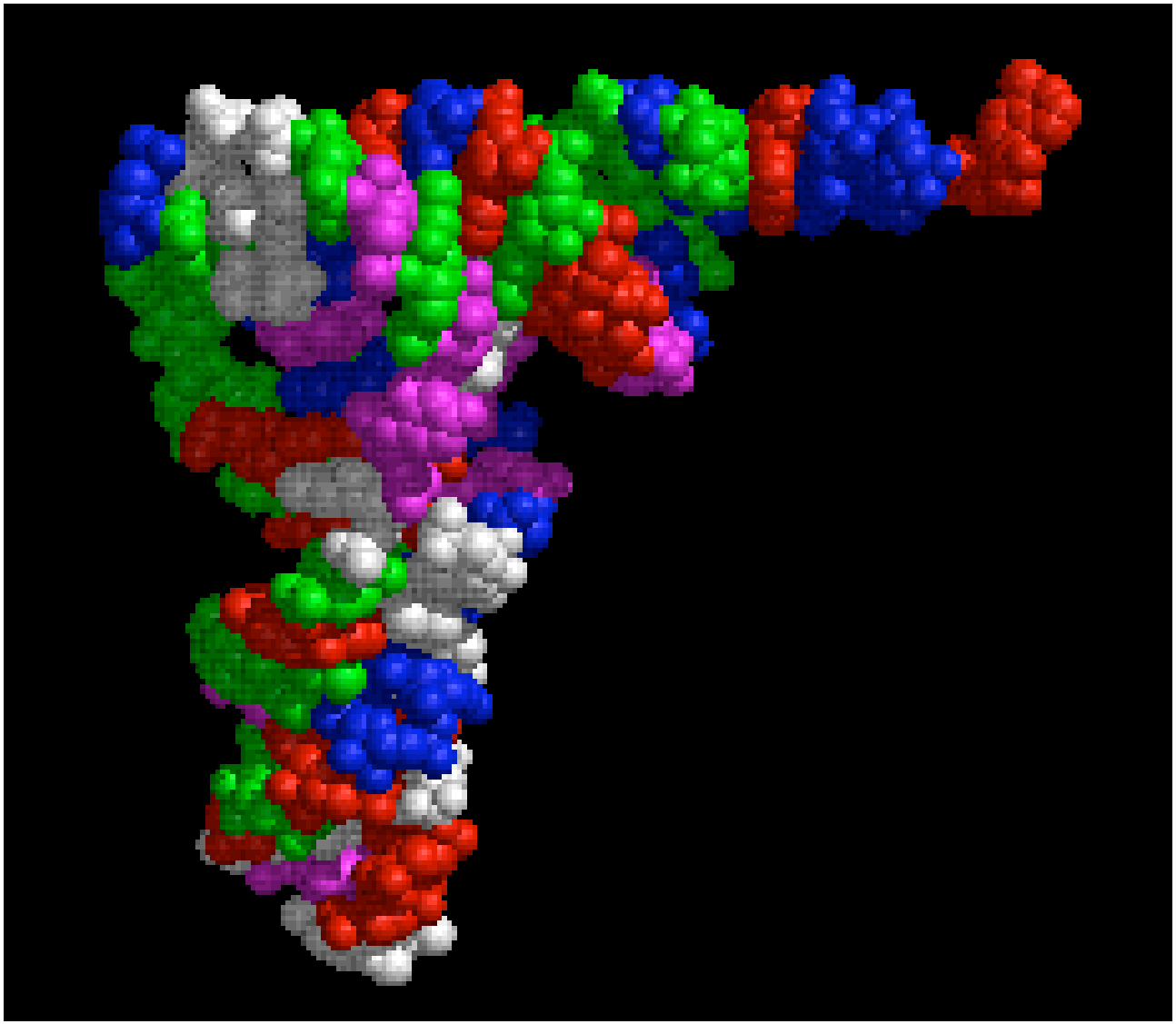
Outline

- 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

A tRNA 3d Structure



tRNA - Alt. Representations

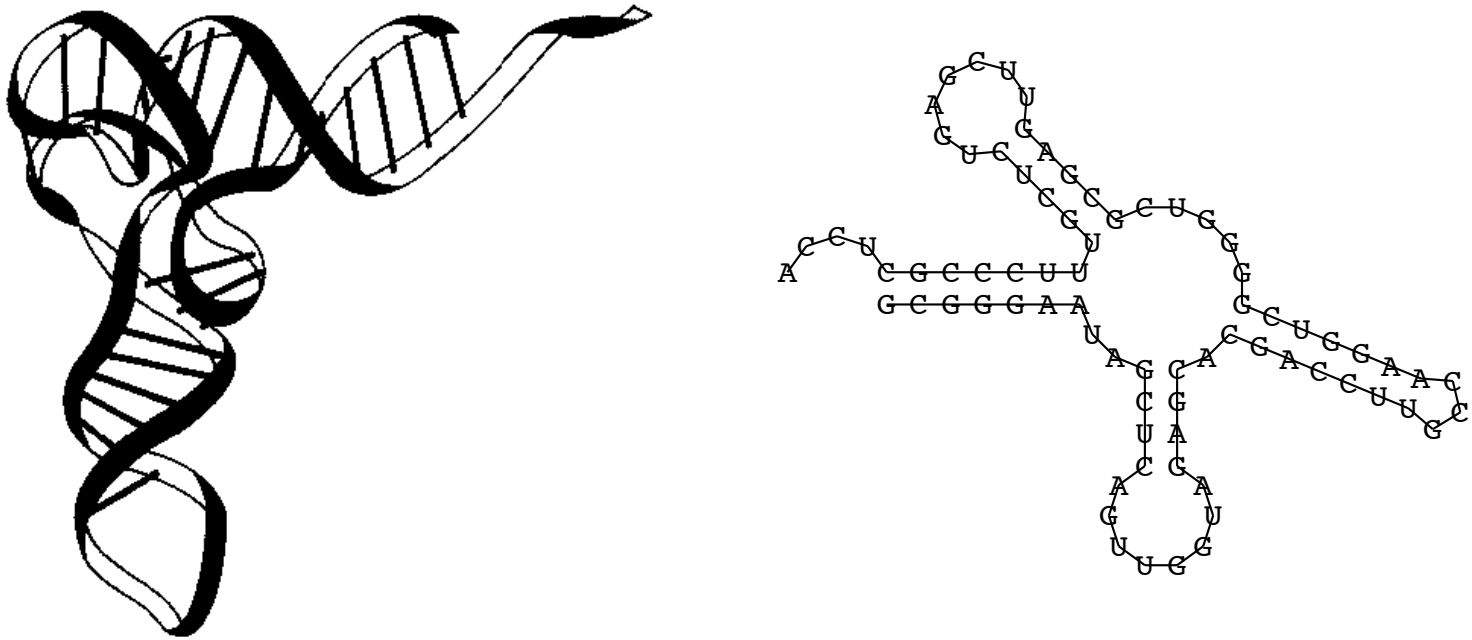


Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

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

A "Mountain" diagram

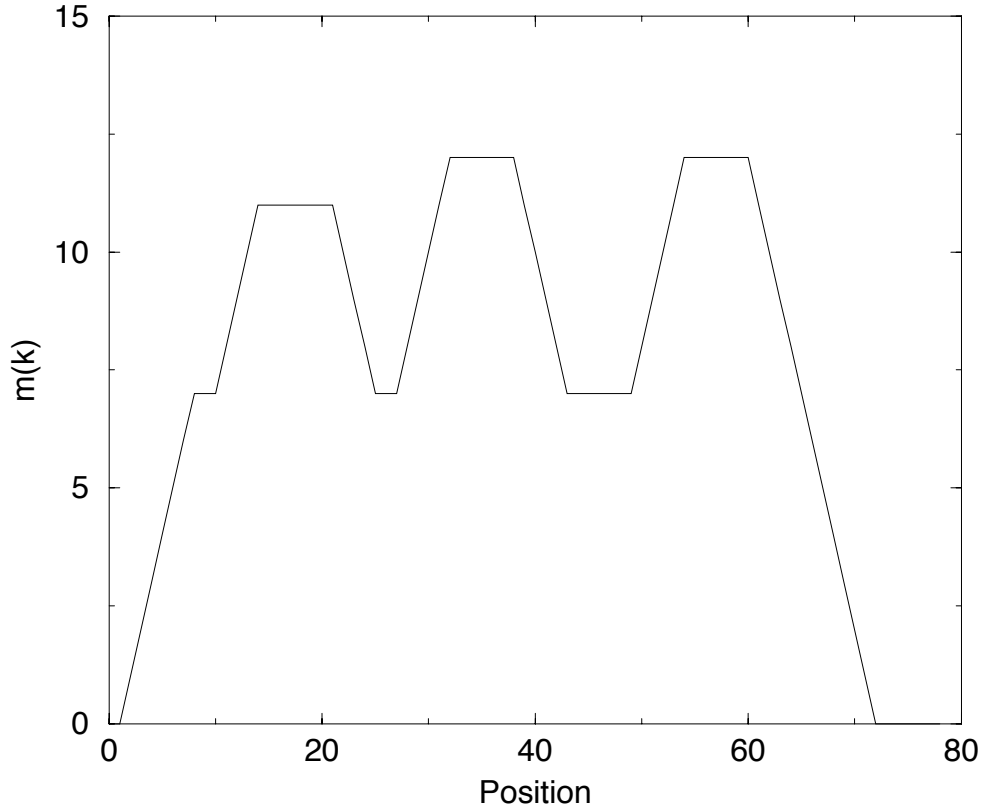
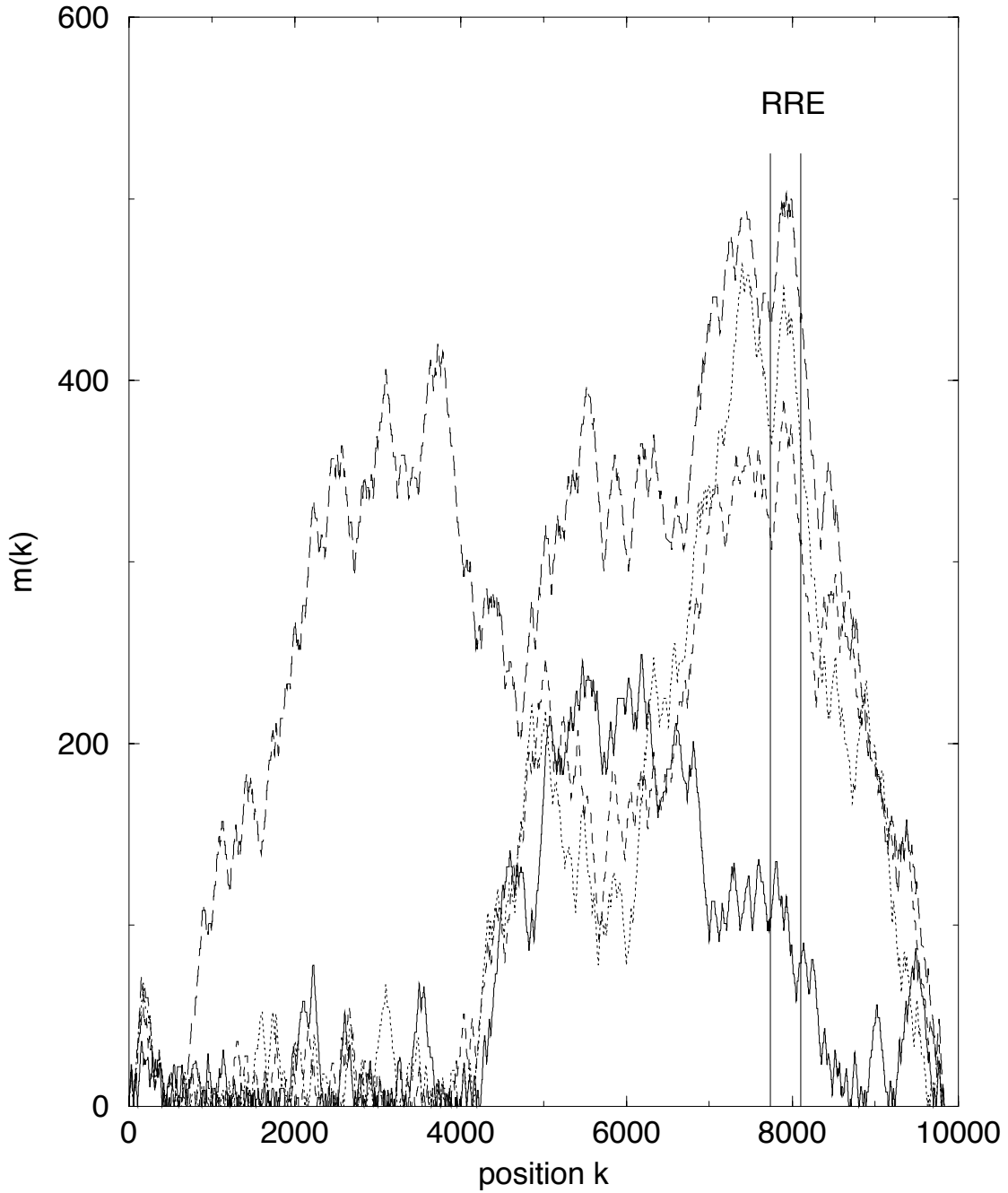


Figure 3: Mountain representation of the tRNA secondary structure shown in Figure 1. The three plateaus correspond to the three hairpin loops of the clover leave structure.

Importance

- Ribozymes (RNA Enzymes)
- Retroviruses
- Effects on transcription, translation, splicing...
- Functional RNAs: rRNA, tRNA, snRNA, snoRNA, micro RNA, RNAi, ...

Secondary Structure of Several HIV strains



"5-finger" motif in RRE region of many HIV strains

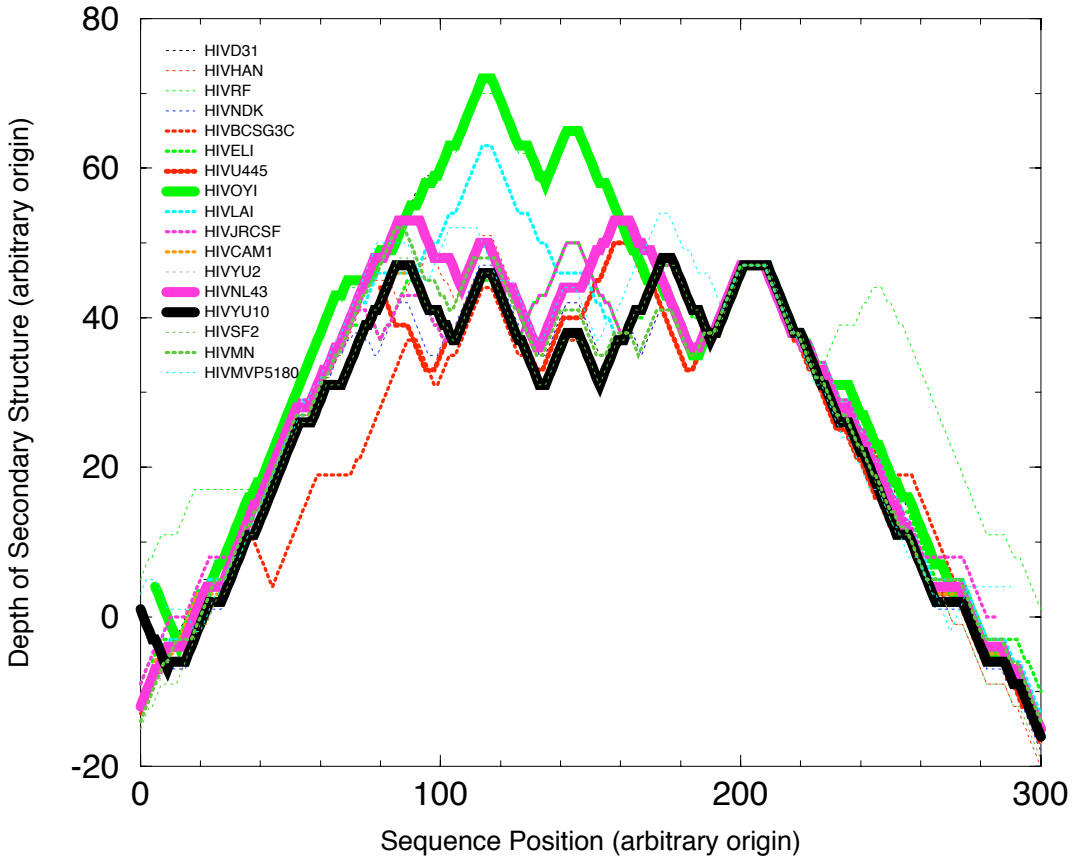


Figure 11: Alignment of the RRE regions of 17 sequences based solely on the minimum free energy secondary structure. The mountain representation reveals the five-fingered motif, the Roman numerals correspond to the numbering of the hairpins in (Dayton *et al.*, 1989). 5 out of 22 sequences showed a different pattern here. We find three different folding patterns each highlighted by one example. The first one (thick black line) corresponds to the consensus five-fingered motif that is presented in (Konings, 1992). The second one (light gray) is present among other in HIVLAI. It shown in (Huynen *et al.*, 1995) that this structure has high structural versatility; one of its alternatives

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)

Definitions

- Sequence $^{5'}$ r1 r2 r3 ... rn $^{3'}$ in {A, C, G, T}
- A Secondary Structure is a set of pairs i•j s.t.

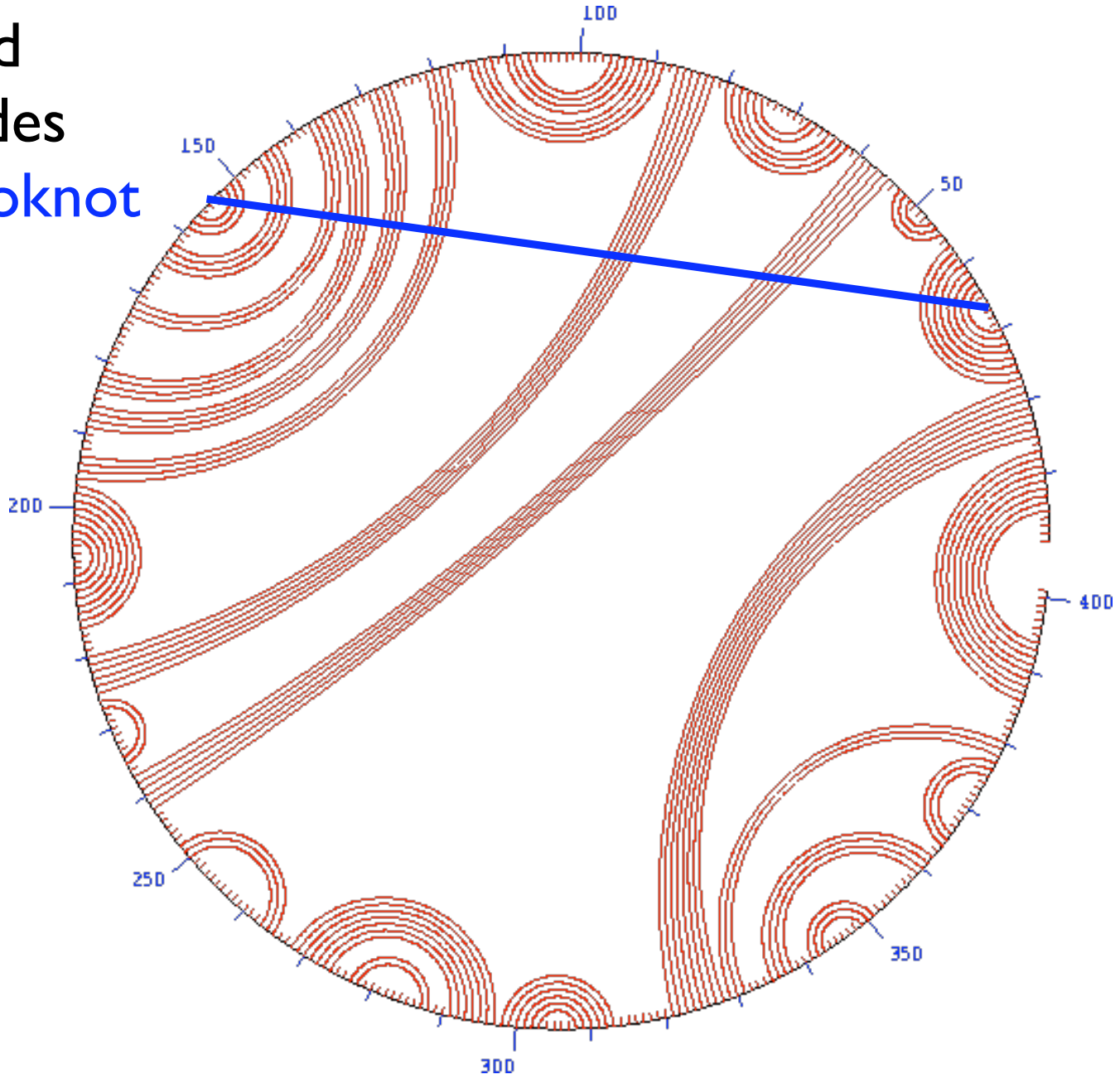
1. $i < j-4$
2. if i•j & i'•j' are two pairs with $i \leq i'$, then
   - A. $i = i'$ & $j = j'$, or
   - B. $j < i'$, or
   - C. $i < i' < j' < j$

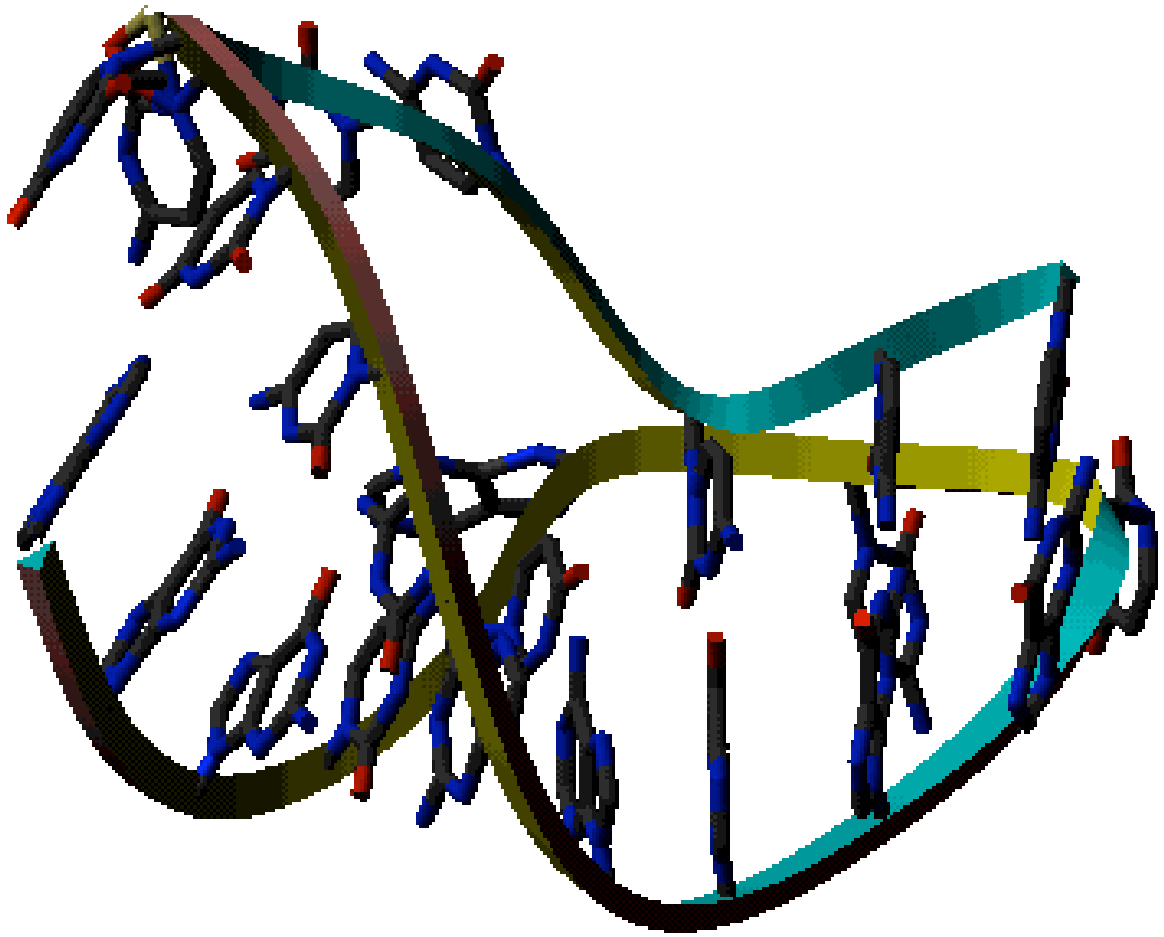
} First pair precedes 2nd, or is nested within it. No "pseudoknots."

Nested

Precedes

Pseudoknot





The corresponding secondary structure is:

```
                A–C
3' – A–G–G–C–U/   U
     U–C–C–G–A–G–G–G
      U         C–C–C – 5'
       C––U––C/
```

A Pseudoknot

Approaches to Structure Prediction

- Maximum Pairing
  + 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
- 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 ri ... rj
- 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 ri pairs with rj then 1 else 0)
  4. max { B(i,k)+B(k+1,j) | i < k < j }

Time: $O(n^3)$

Pair-based Energy Minimization

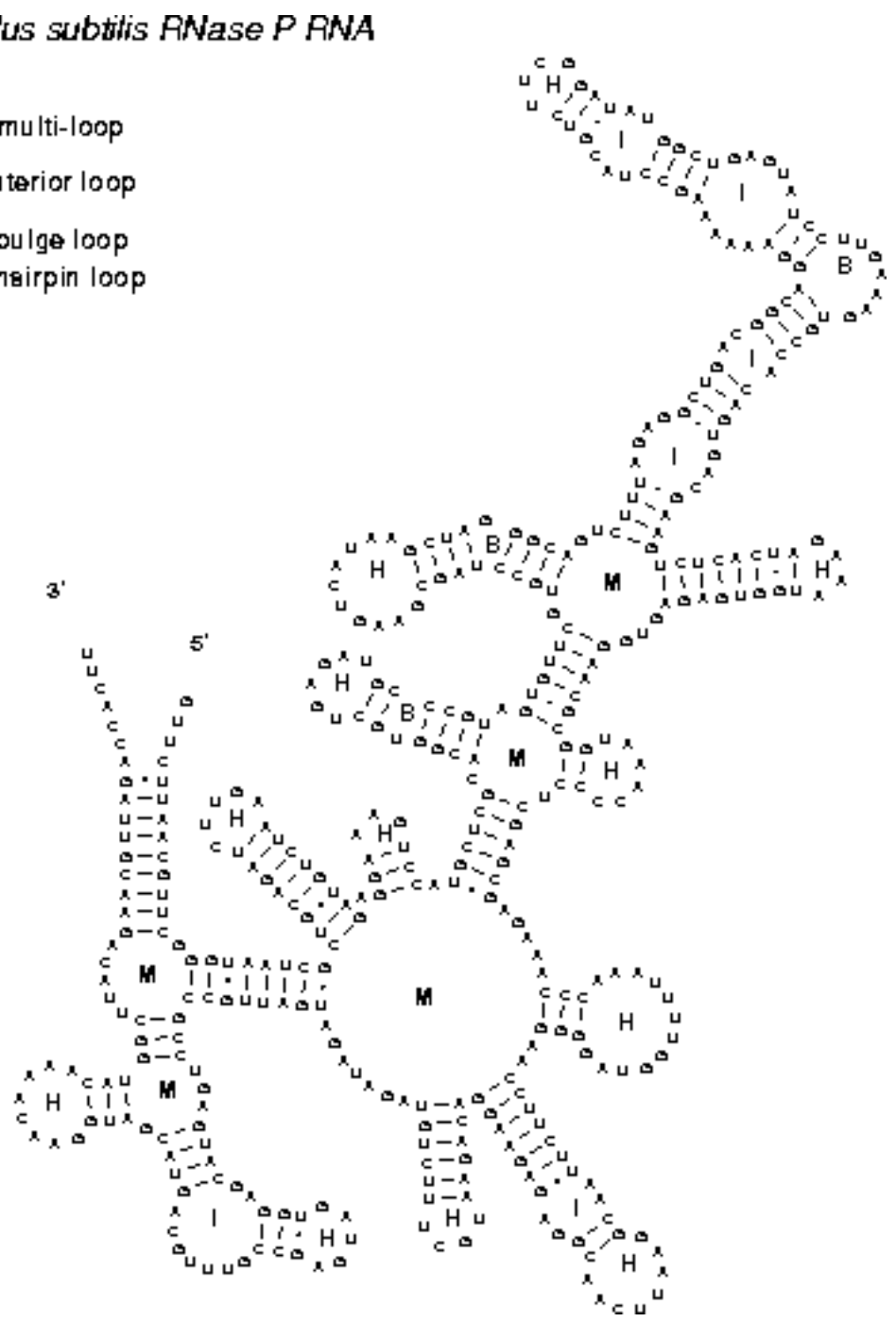
- E(i,j) = energy of pairs in optimal pairing of ri ... rj
- E(i,j) = ∞ for all i, j with i $\geq$ j-4; otherwise
- E(i,j) = min of:
  1. E(i+1,j)
  2. E(i,j-1)
  3. E(i+1,j-1) + e(ri,rj)
  4. min { E(i,k)+E(k+1,j) | i < k < j }

Time: $O(n^3)$

Loop-based Energy Minimization

- Detailed experiments show that it's more accurate to model based on loops, rather than just pairs
- Loop types
  - Stack
  - Hairpin loop
  - Bulge
  - Interior loop

Loop Examples



Zuker: Loop-based Energy, I

- W(i,j) = energy of optimal pairing of ri ... rj
- 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

$$V(i,j) = \min(eh(i,j), es(i,j)+V(i+1,j-1), VBI(i,j), VM(i,j))$$

$$VM(i,j) = \min \{ W(i,k)+W(k+1,j) \mid i < k < j \})$$

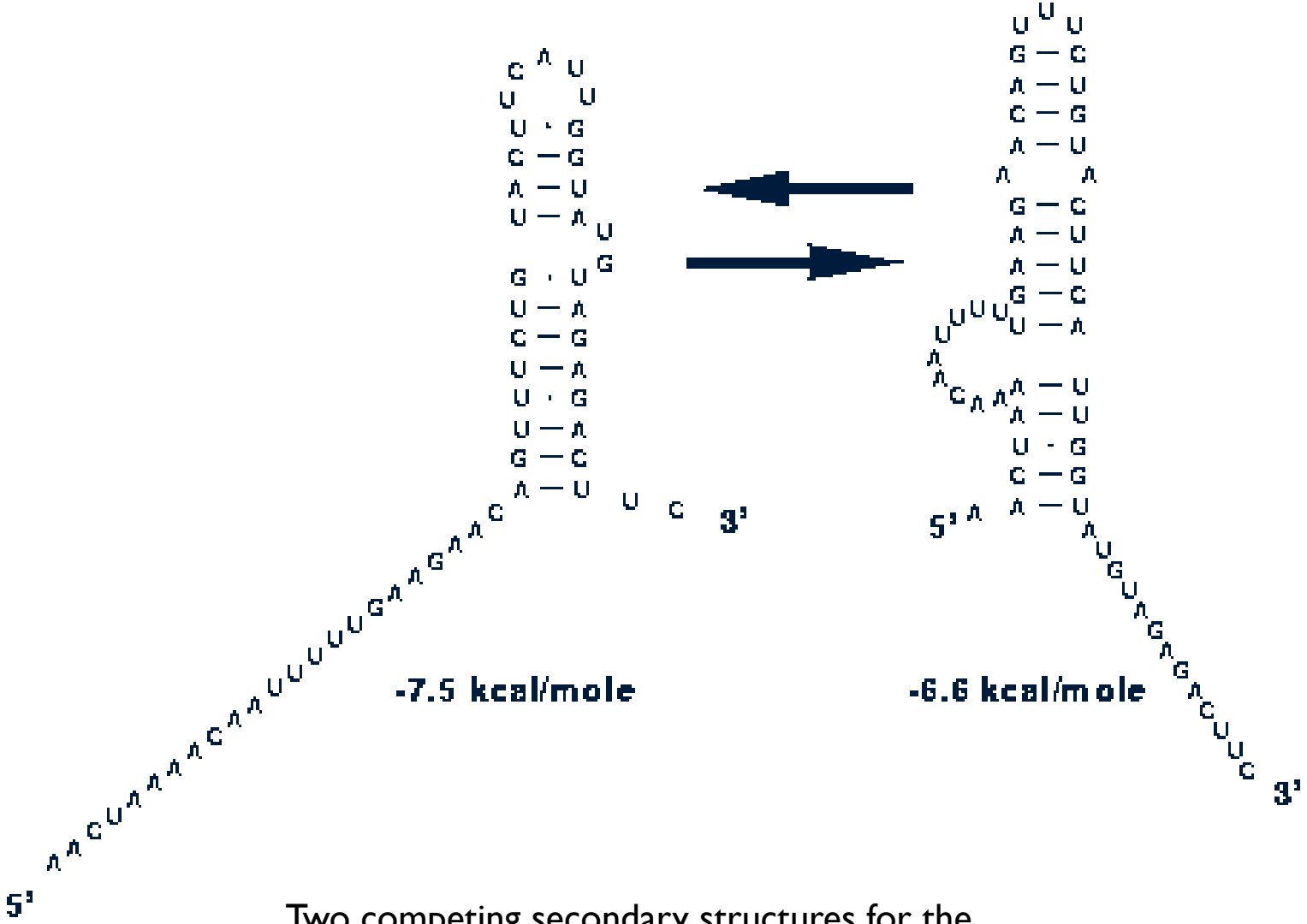
$$VBI(i,j) = \min \{ ebi(i,j,i',j') + V(i', j') \mid i < i' < j' < j \ \& \ i'-i+j-j' > 2 \}$$

Time: $O(n^4)$

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

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.



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

Example of suboptimal folding

Black dots:
pairs in opt fold

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