7.91 / 20.490 / 6.874 / HST.506

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C. Burge Lecture #10

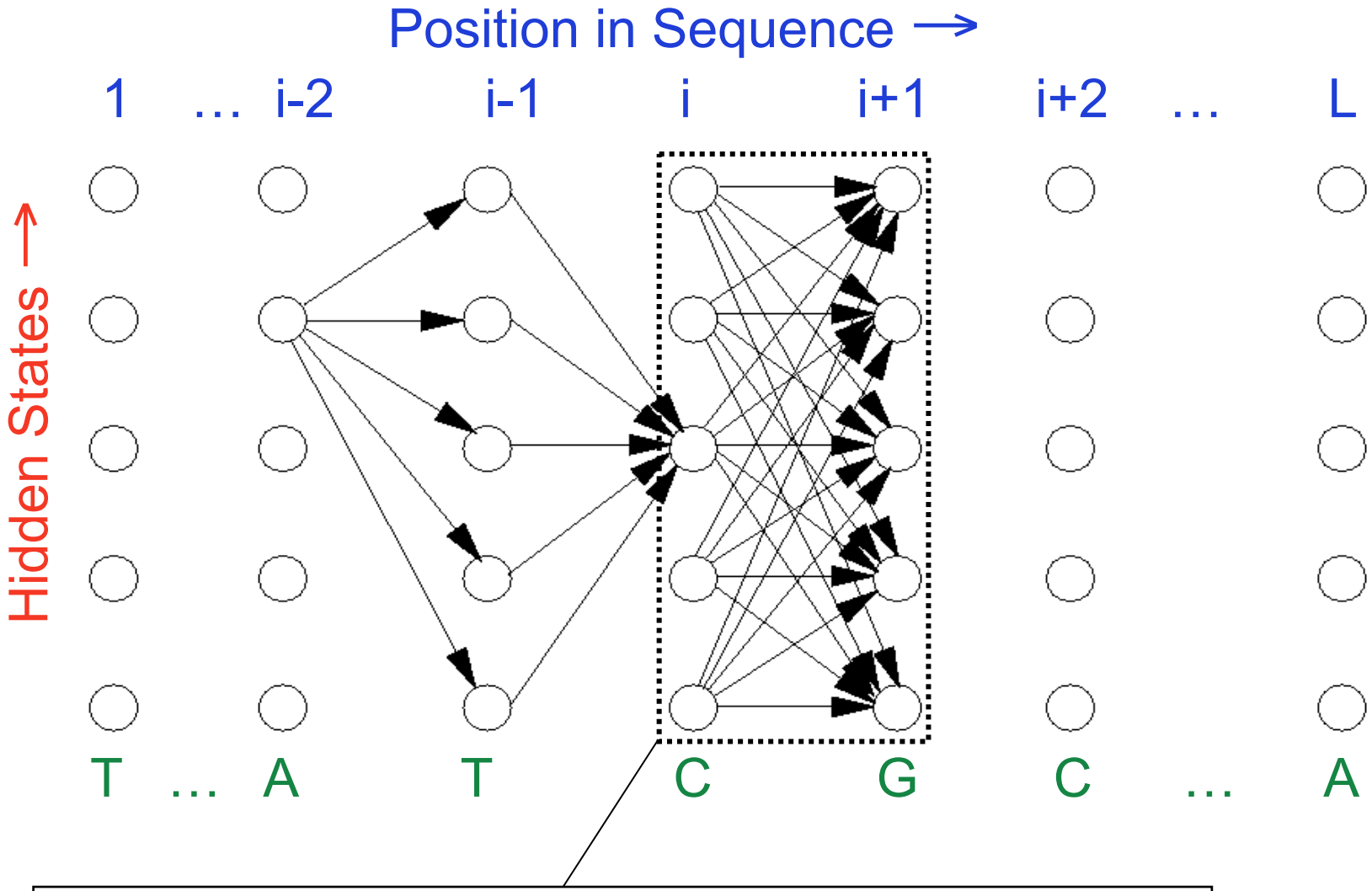
March 13, 2014

RNA Secondary Structure - Biological Functions & Prediction

Hidden Markov Models of Genomic & Protein Features

- Hidden Markov Model terminology
- Viterbi algorithm
- Examples
  - CpG Island HMM
  - TMHMM (transmembrane helices)

“Trellis” Diagram for Viterbi Algorithm



CpG Island HMM

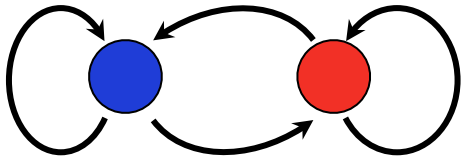
Rabiner notation

"Initiation probabilities" π_j

$P_g = 0.99$, $P_i = 0.01$

$P_{gg} = 0.99999$ $P_{ig} = 0.001$

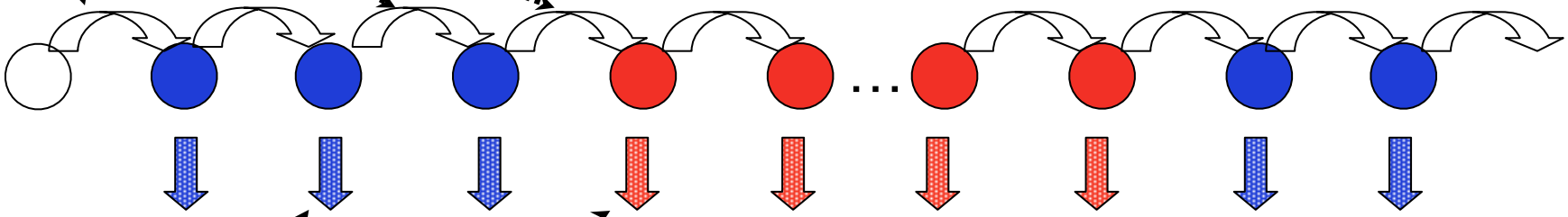
Genome



$P_{ii} = 0.999$

"Transition probabilities" a_{ij}

$P_{gi} = 0.00001$ Island



"Emission Probabilities" $b_j(k)$

	C	G	A	T
CpG Island:	0.3	0.3	0.2	0.2
Genome:	0.2	0.2	0.3	0.3

More Viterbi Examples

What is the optimal parse of the sequence for the CpG island HMM defined previously?

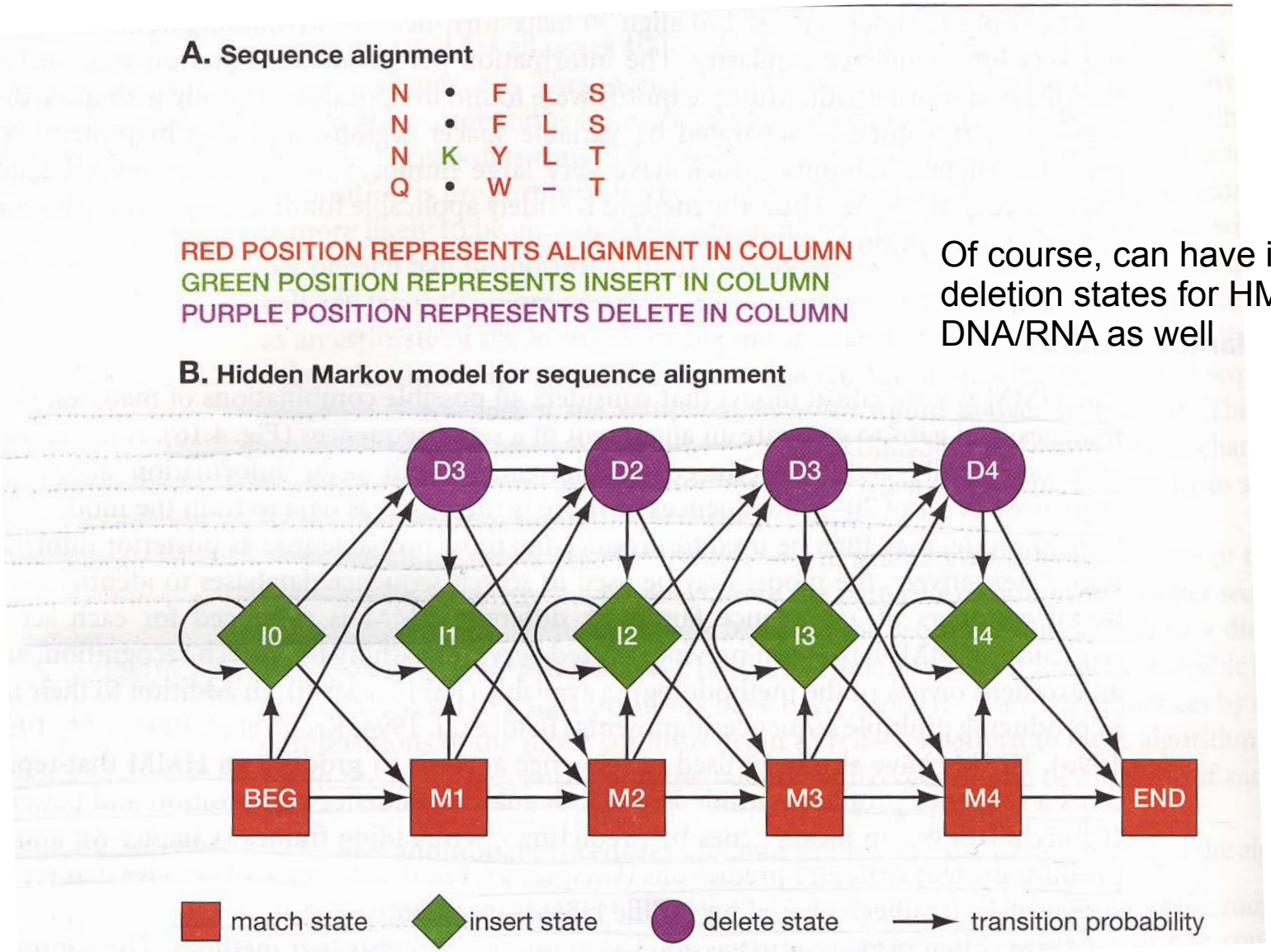
- $(ACGT)_{10000}$
- $A_{1000}C_{80}T_{1000}C_{20}A_{1000}G_{60}T_{1000}$

<u>Powers of 1.5:</u>

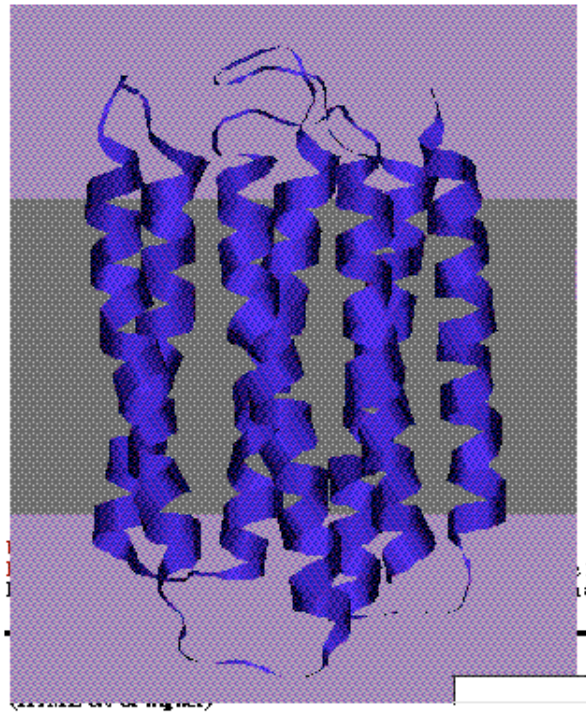
N =	20	40	60	80
$(1.5)^N$ =	$3x10^3$	$1x10^7$	$3x10^{10}$	$1x10^{14}$

Real World HMMs

“Profile HMM” with insertions/deletions



Of course, can have insertion/ deletion states for HMM models of DNA/RNA as well



TMHMM (v. 2.0)

Prediction of transmembrane helices in proteins

Help/Information (updated Sept 13, 2001)

One of the World Wide Web Prediction Servers
from the Center for Biological Sequence Analysis

limit each submission to at most 4000 proteins.
each large submission.

OR by pasting sequence(s) in FASTA format:

Output format: Extensive, with graphics
Extensive, no graphics
One line per protein

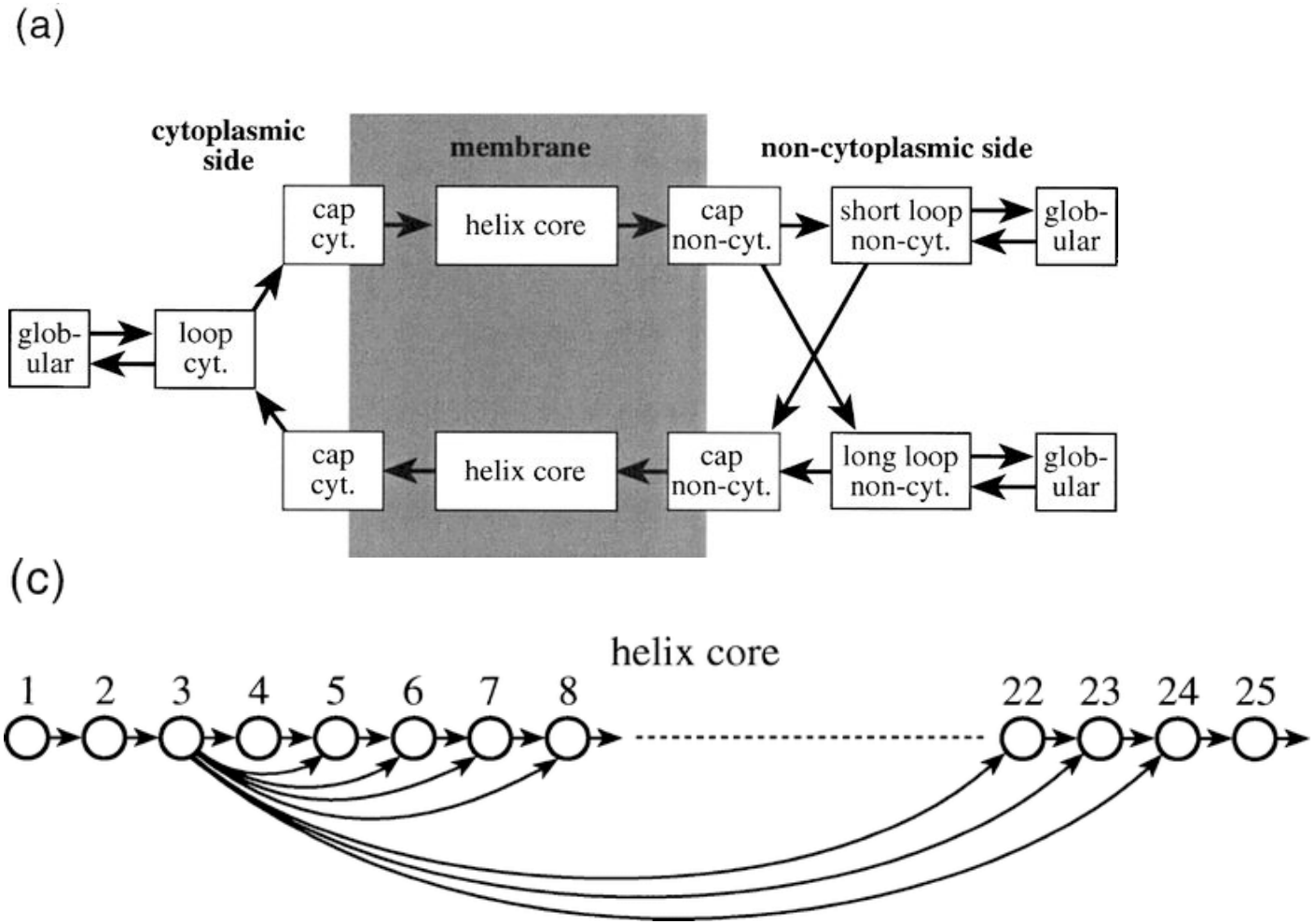
Other options: Use old model (version 1)

Submit Clear

Correctly predicts ~97% of transmembrane helices according to authors

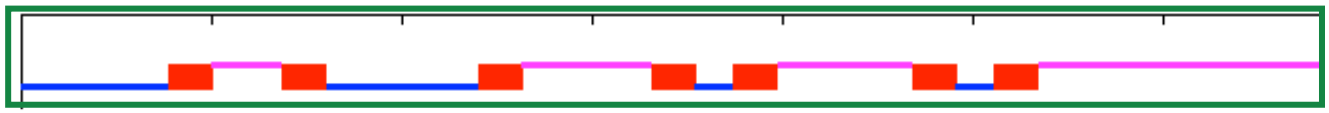
A. Krogh et al. *J. Mol. Biol.* 2001

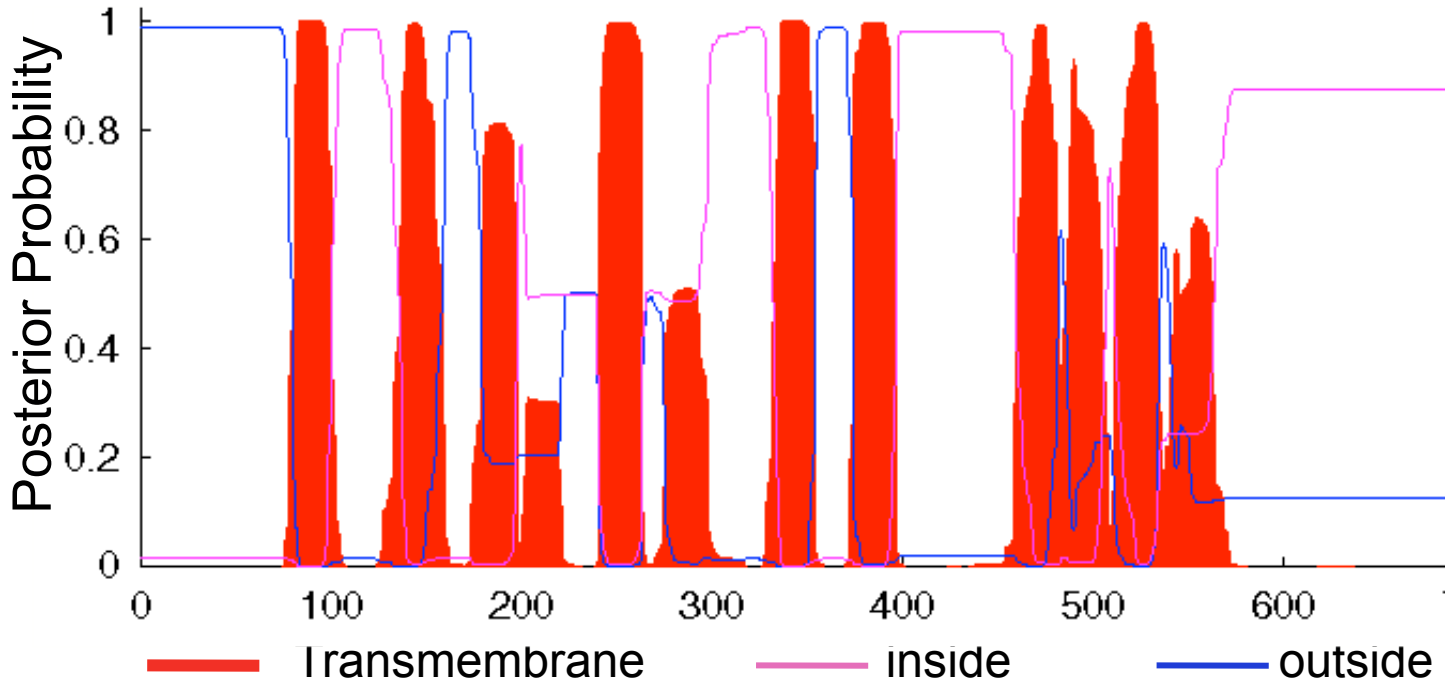
Architecture of TMHMM



TMHMM Output for Mouse Chloride Channel CLC6

Optimal Parse





RNA Secondary Structure

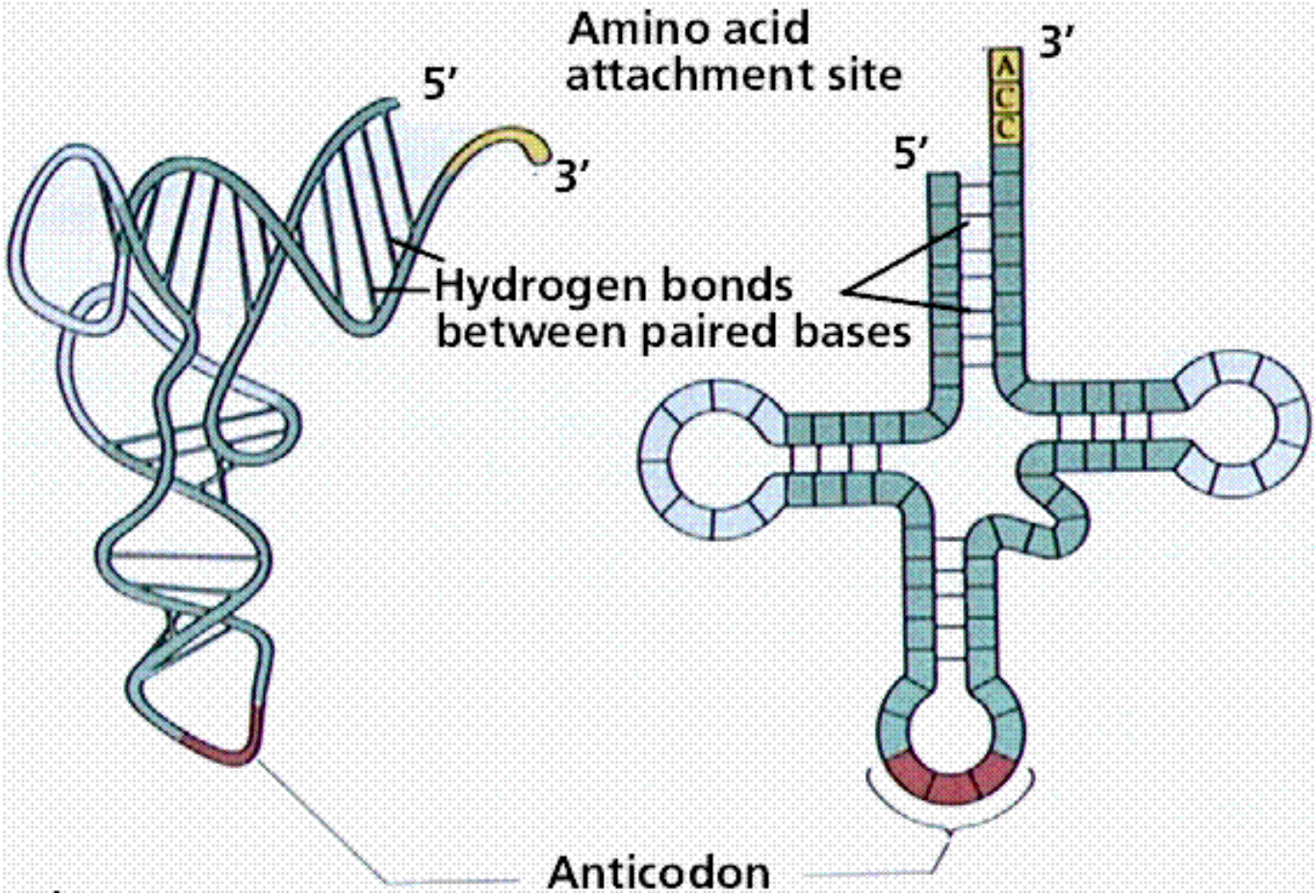
- Biological examples of RNA structure
- Predicting 2° structure by covariation
- Predicting 2° structure by energy minimization

Readings

NBT Primer on RNA folding, Z&B Ch. 11.9

RNA Secondary and Tertiary Structure

Example: tRNA

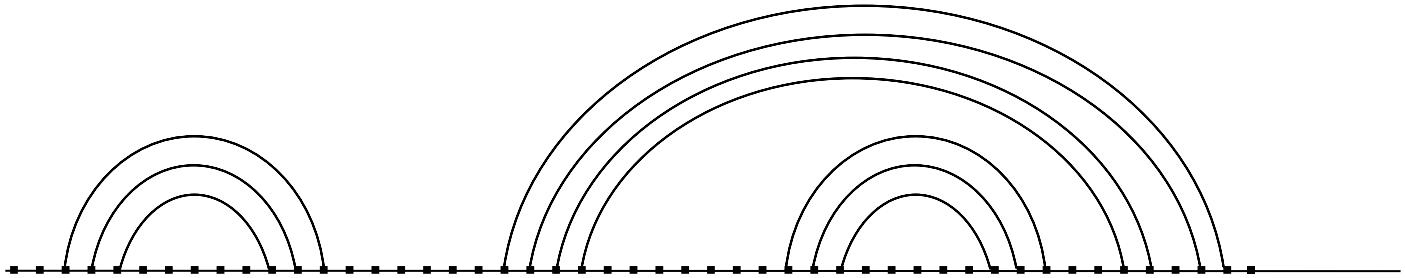


RNA Secondary Structure Notation

Parentheses notation

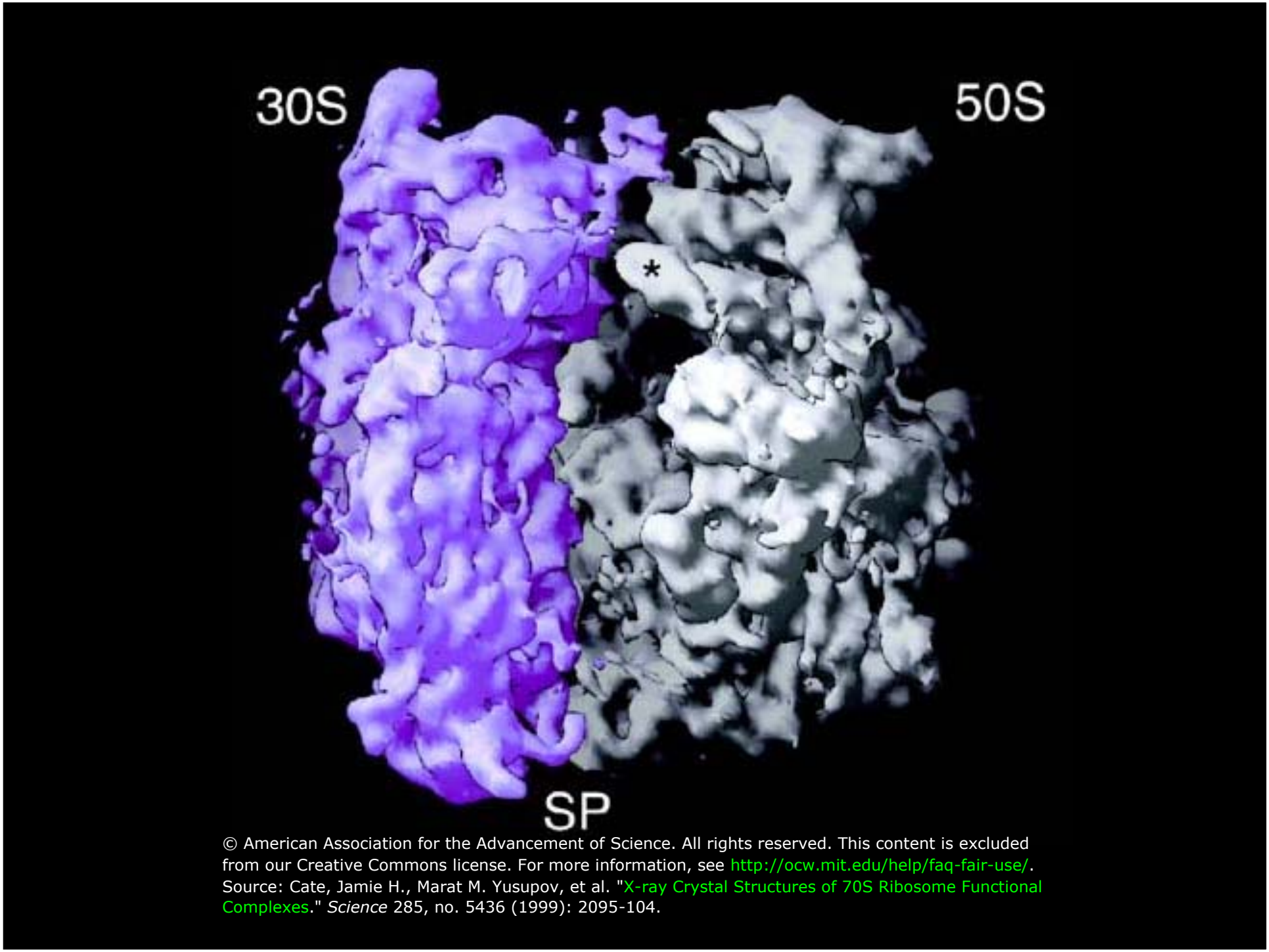
..(((…..)))……((((……................)).))…

Arc (‘rainbow’) notation



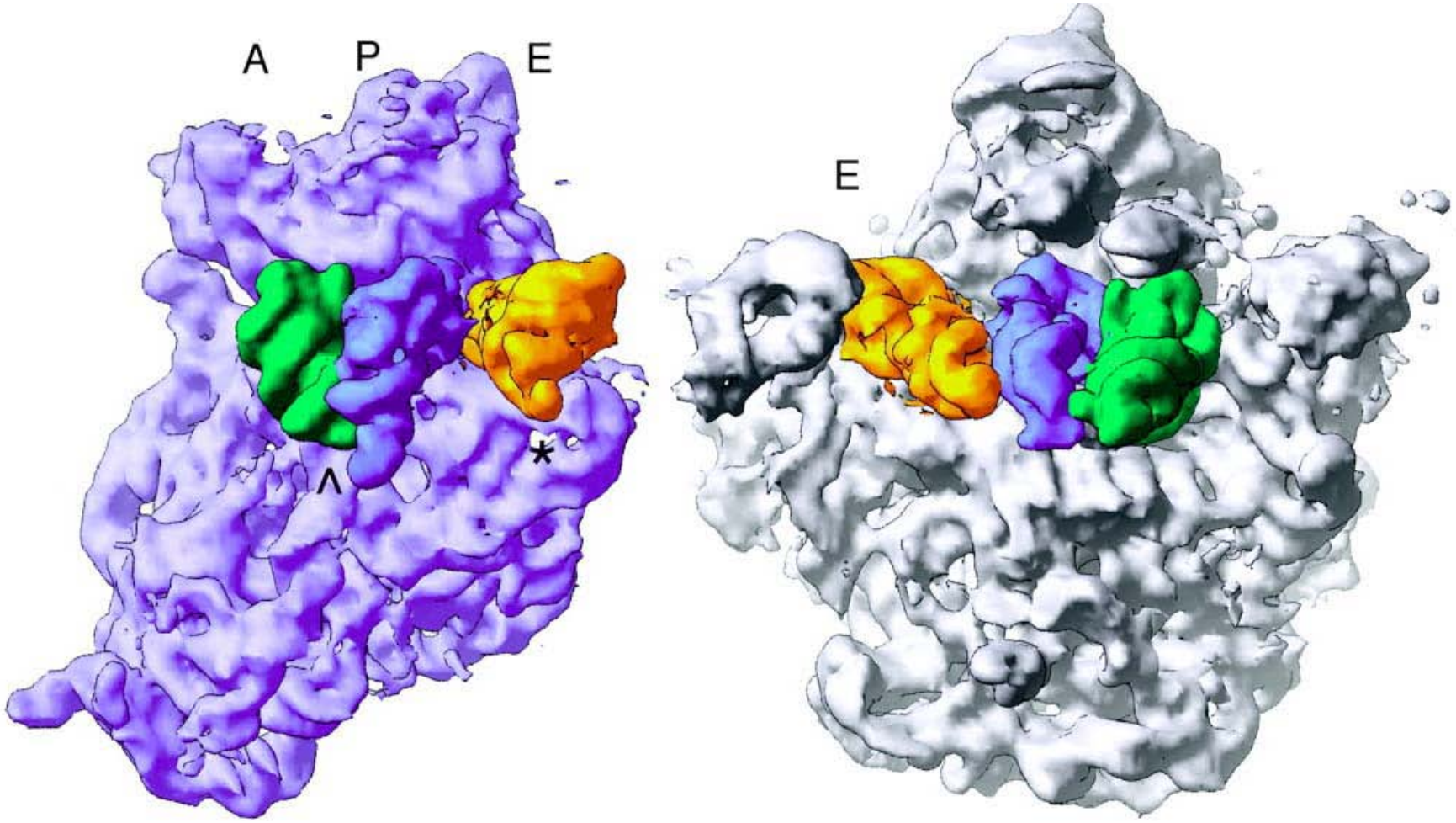
What do these structures look like?

What is the difference between these two structures?



Source: Cate, Jamie H., Marat M. Yusupov, et al. "X-ray Crystal Structures of 70S Ribosome Functional Complexes." *Science* 285, no. 5436 (1999): 2095-104.

Ribosome at 7 Å with tRNAs

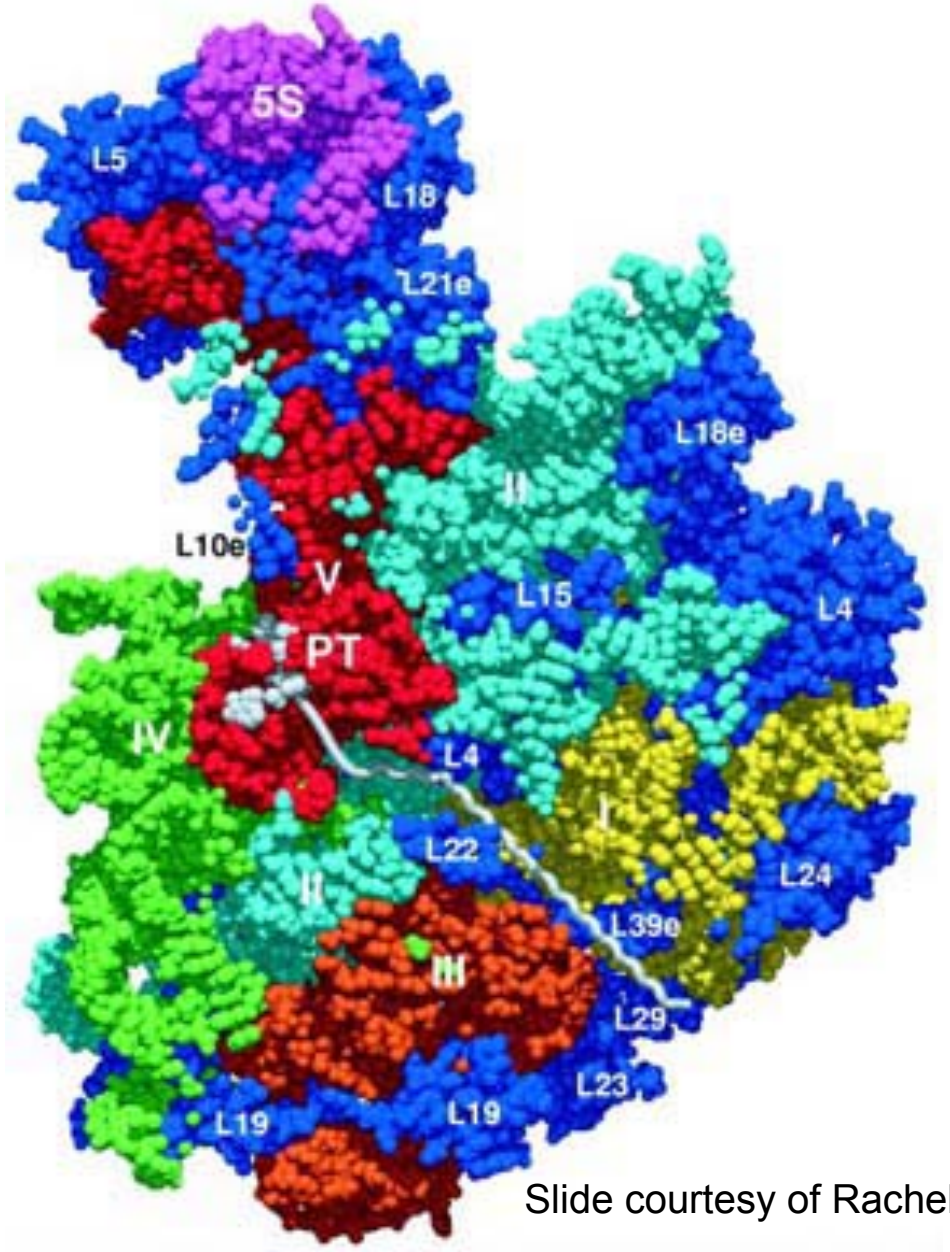


Source: Cate, Jamie H., Marat M. Yusupov, et al. "X-ray Crystal Structures of 70S Ribosome Functional Complexes." *Science* 285, no. 5436 (1999): 2095-104.

Slide courtesy of Rachel Green

Can build useful structures out of RNA

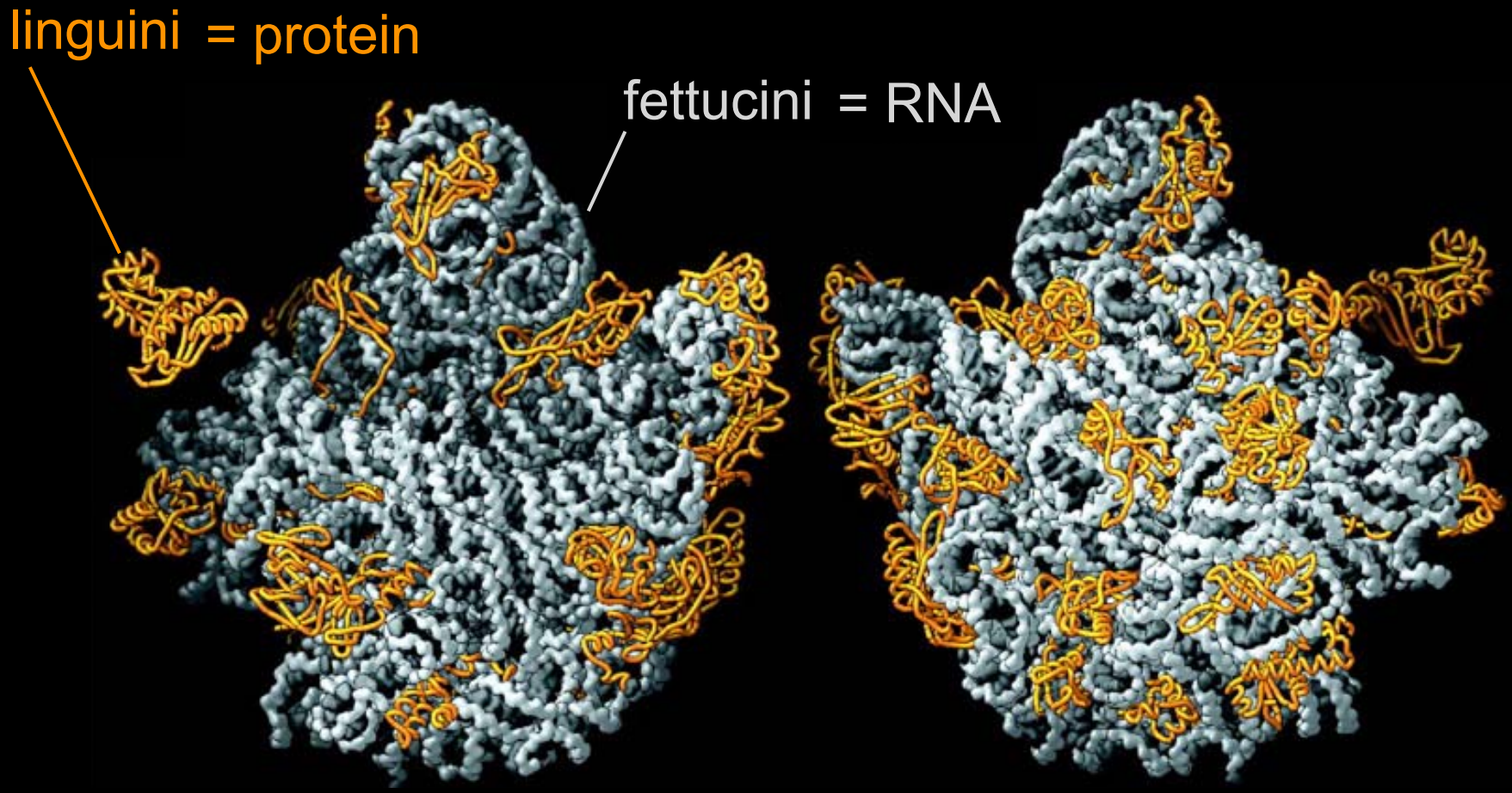
The exit channel for the growing polypeptide



Slide courtesy of Rachel Green

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.

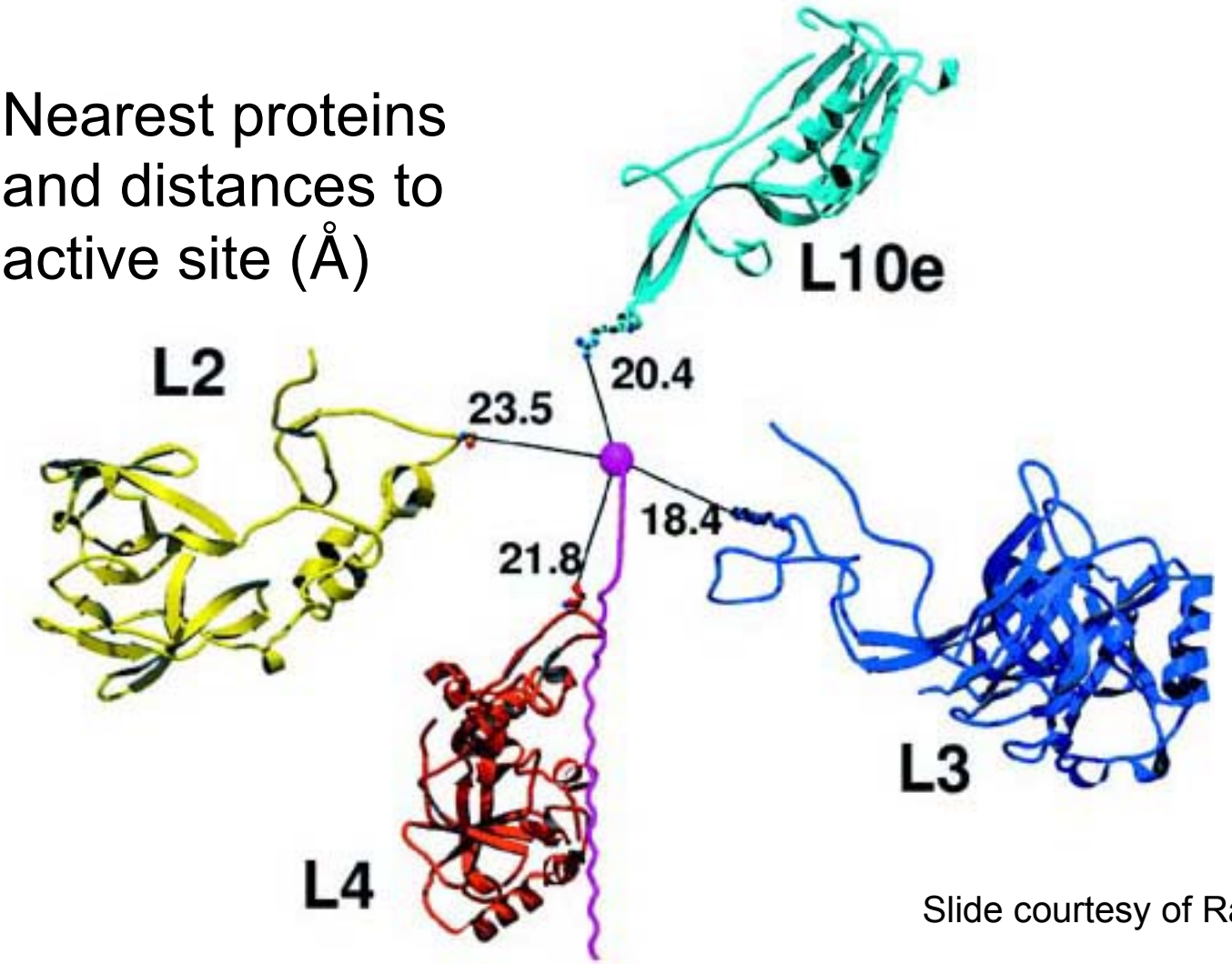
RNA/protein distribution on the 50S ribosome



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.

The ribosome is a ribozyme

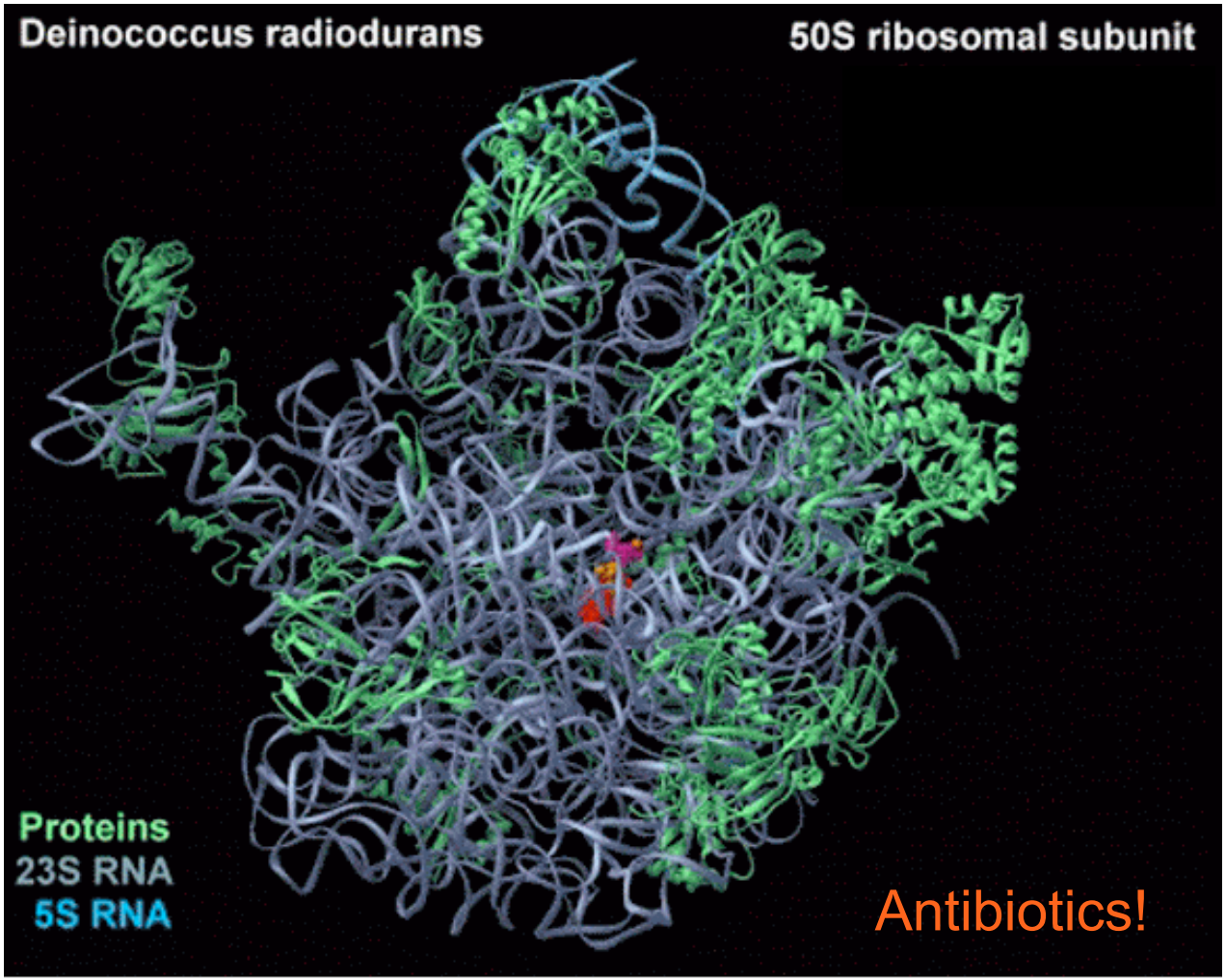
Nearest proteins and distances to active site (Å)



Slide courtesy of Rachel Green

Source: Nissen, Poul, Jeffrey Hansen, et al. "The Structural Basis of Ribosome Activity in Peptide Bond Synthesis." *Science* 289, no. 5481 (2000): 920-30.

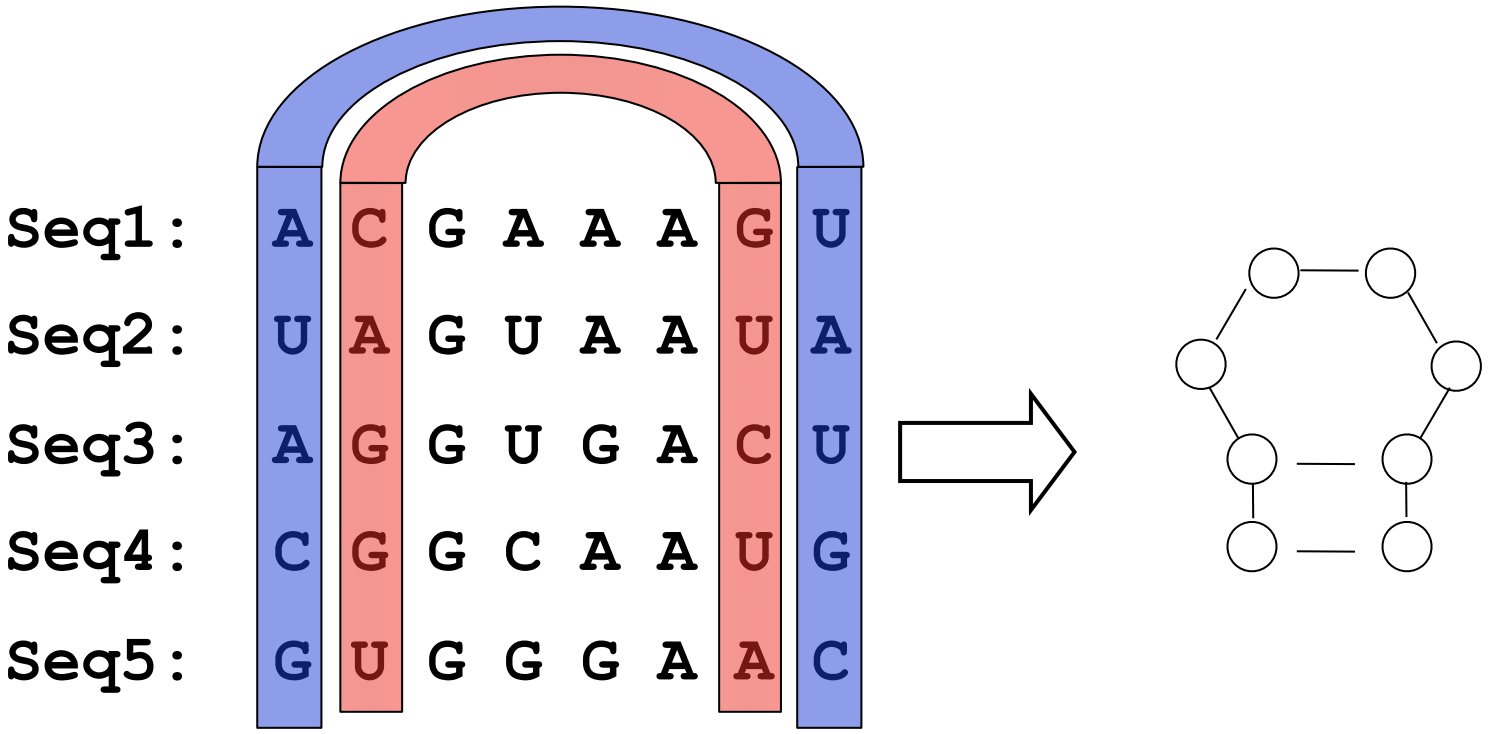
What are the practical applications of knowing the ribosome structure?



ncRNAs:
Challenges for Computational Biology

- Prediction of ncRNA structure
- Identification of ncRNA genes
- Prediction of ncRNA functions

RNA 2° structure by covariation / compensatory changes



Mutual information statistic for pair of columns in a multiple alignment

$$M_{ij} = \sum_{x,y} f_{x,y}^{(i,j)} \log_2 \frac{f_{x,y}^{(i,j)}}{f_x^{(i)} f_y^{(j)}}$$

$f_{x,y}^{(i,j)}$ = fraction of seqs w/ nt. *x* in col. *i*, nt. *y* in col. *j*

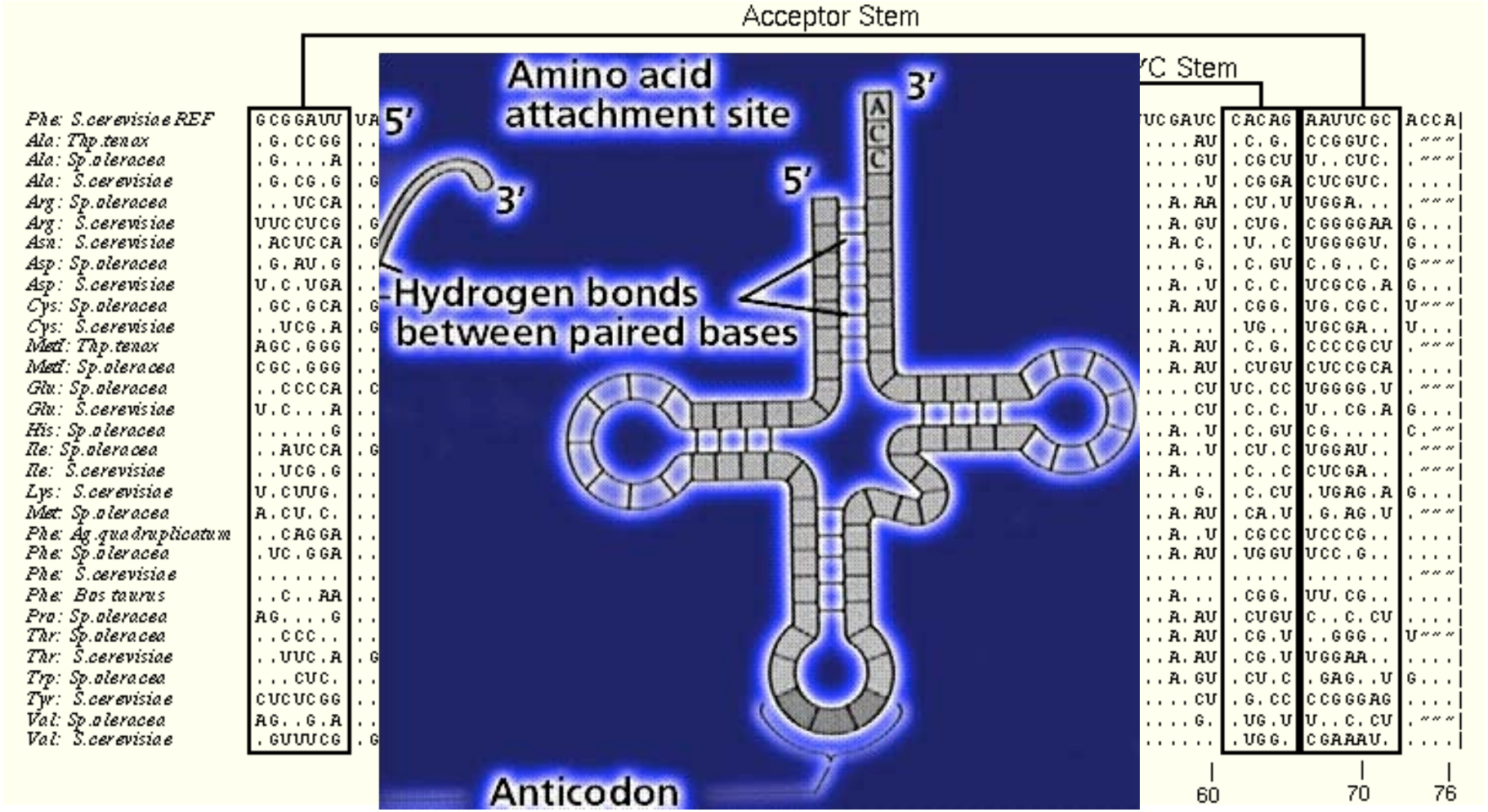
$f_x^{(i)}$ = fraction of seqs w/ nt. *x* in col. *i*

sum over *x*, *y* = A, C, G, U

M_{ij} is maximal (2 bits) if *x* and *y* individually appear at random (A,C,G,U equally likely), but perfectly covary (e.g., always complementary)

Could use other measure of dependence (e.g., chi-square statistic)

Inferring 2° structure from covariation



What is needed for accurate inference of RNA secondary structure by covariation?

- Secondary structure more highly conserved than primary sequence
- Sufficient divergence between homologs for many variations to have occurred, but not so much that can't be aligned
- Sufficient number of homologs sequenced

Classes of Non-coding RNAs

- tRNAs
- rRNAs
- UTRs
- snRNAs
- snoRNAs
- prok. terminators

…

- RNaseP
- SRP RNA
- tmRNA
- miRNAs
- lncRNAs
- riboswitches

…

Energy Minimization Approach

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

There are typically many possible folded states

- assumption that minimum energy state(s) will be occupied

$$\Delta G = \Delta H - T\Delta S$$

Enthalpy favors folding

Entropy favors unfolding

What environmental variables affect RNA folding?

How Do Energy Minimization Algorithms Work?

Consider Simple Model: Base Pair Maximization

Scoring System:

+1 for base pair (C:G, A:U)

0 for anything else

Maximizing score equivalent to minimizing folding free energy for a model which assigns same enthalpy to all allowed base pairs
(and ignores details such as base stacking, loops, entropy)

Nussinov algorithm: recursive maximization of base pairing

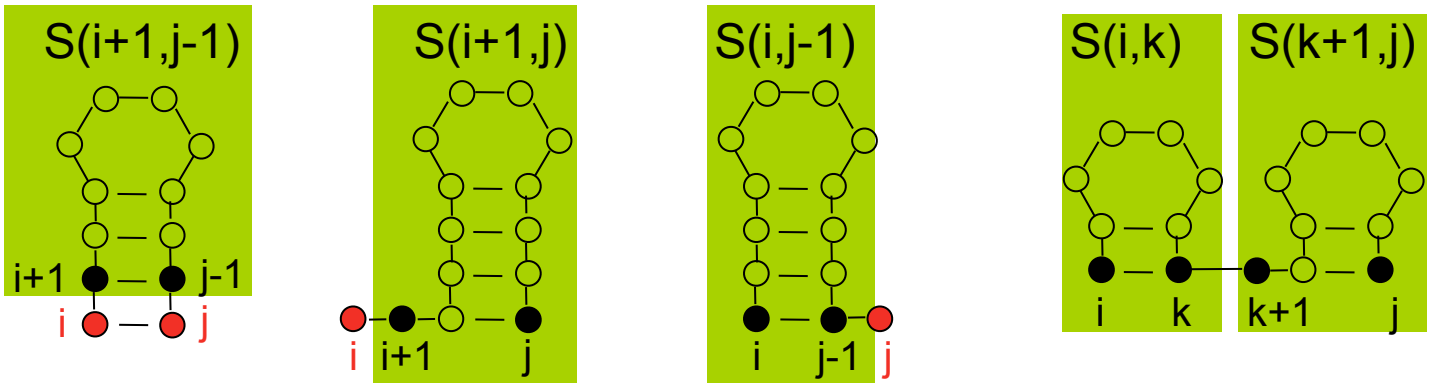
Recursive Maximization of Base Pairing

Given an RNA sequence of length N

Define S(i,j) to be the score of the best structure for the subsequence (i, j)

Notice that S(i,j) can be defined recursively in terms of optimal scores of smaller subsequences of the interval (i,j)

There are four possible ways that the score of the optimal structure on (i,j) can relate to scores of optimal structures of nested subsequences:



1. i,j pair 2. i unpaired 3. j unpaired 4. bifurcation

Source: Eddy, Sean R. "How do RNA Folding Algorithms Work?" *Nature Biotechnology* 22, no. 11 (2004): 1457-8.

Eddy, Nature Biotech. 2004

Base Pair Maximization Algorithm

S(i,j) = score of the optimal structure for the subsequence (i, j)

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

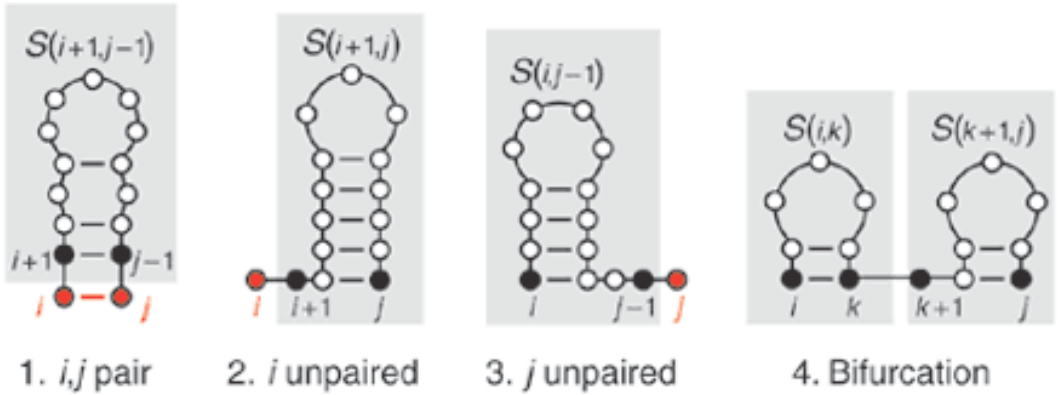
1) Initialize an N x N matrix S with S(i,i) = S(i,i-1) = 0

2) Fill in S(i,j) matrix recursively from the diagonal up and to the right (keep track of which choice was made at each step)

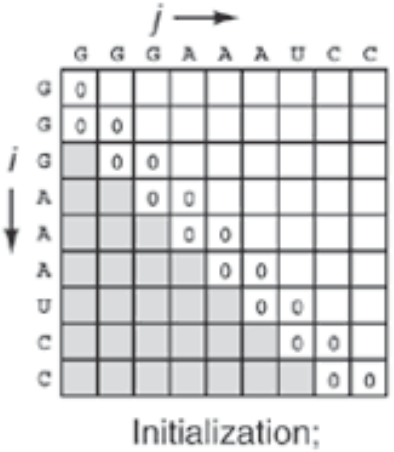
3) Trace back from S(1,N) (upper right corner of matrix) to diagonal to determine optimal structure

Dynamic Programming for Base Pair Maximization

Recursive definition of the best score for a sub-sequence *i,j* looks at four possibilities:



Dynamic programming algorithm for all sub-sequences *i,j*, from smallest to largest:



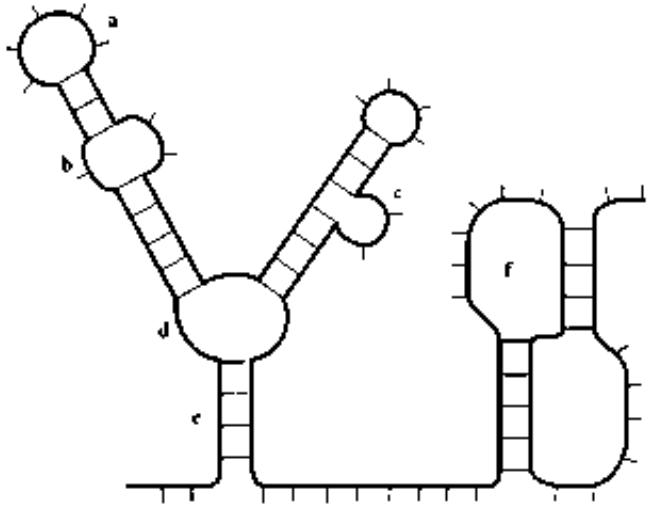
Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Eddy, Sean R. "How do RNA Folding Algorithms Work?" *Nature Biotechnology* 22, no. 11 (2004): 1457-8.

Eddy, Nature Biotech. 2004

Base Pair Maximization Algorithm Issues

- What is computational complexity of algorithm?
  (for sequence of length N)

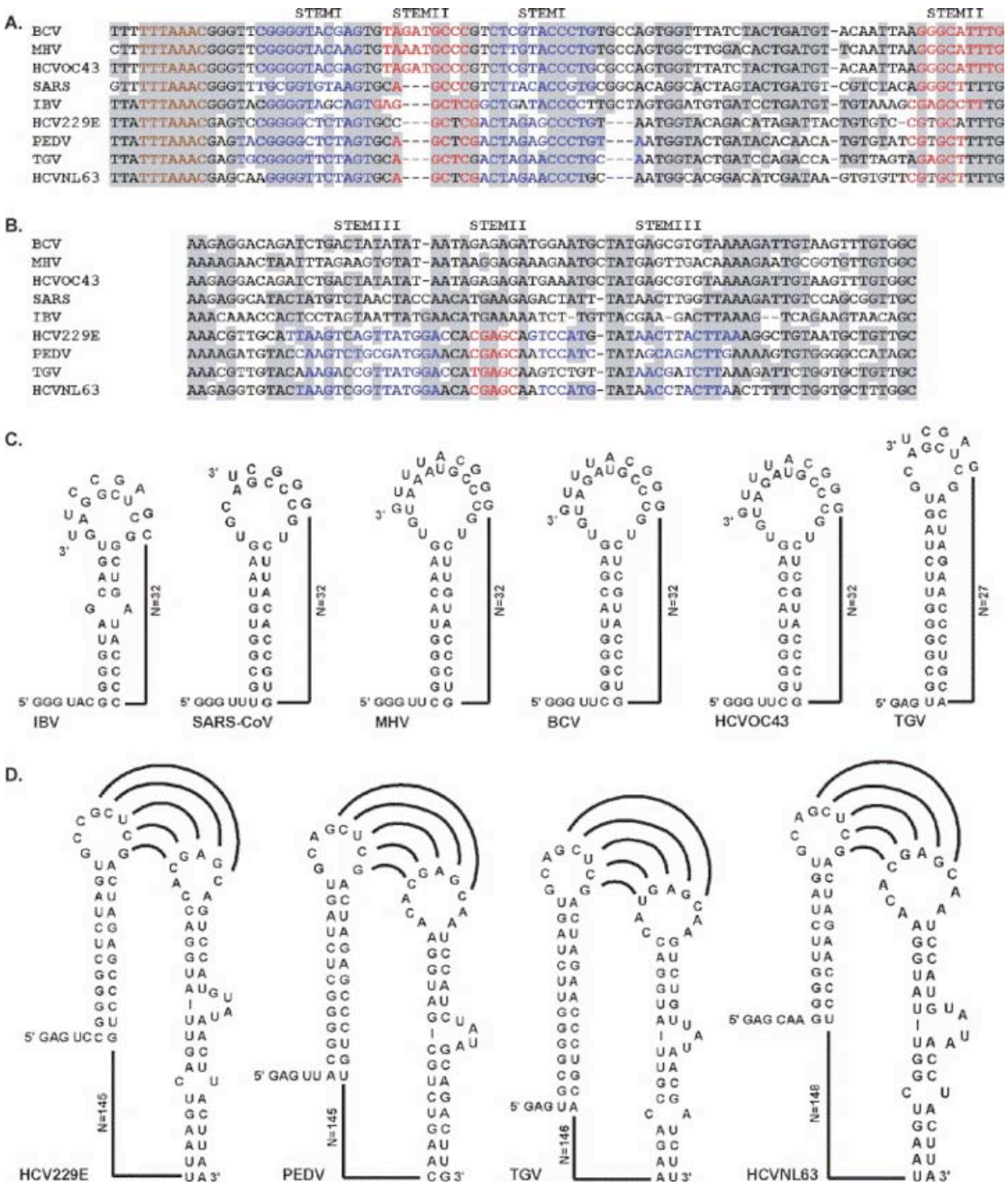
Answer: Memory - $O(N^2)$ Time - $O(N^3)$



- Can it handle pseudoknots?

Answer: No. Pseudoknots invalidate recursion for S(i,j)

Viral Pseudoknots and "Kissing loops"



Baranov et al. Virology 2005

RNA Energetics I

```
3′…CCAUUCAUAG…5′
   | | | | | |
5′…CGUGAGU…3′
```

Free energy contributions to helix formation come from:

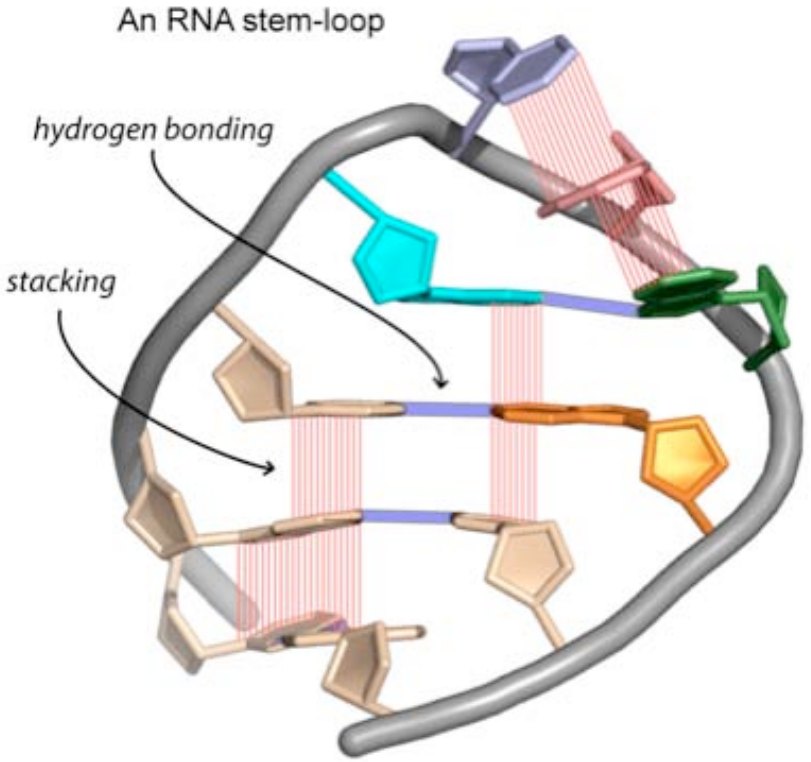
- base **pairing**:

```
G       A       G
‖   >   ‖   >   ‖
C       U       U
```

- base **stacking**:

```
G p A
‖ ⟺ |
C p U
```

Base stacking contributes more to free energy than base pairing



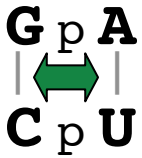
Source: Mohan, Srividya, Chiaolong Hsiao, et al. "RNA Tetraloop Folding Reveals Tension Between Backbone Restraints and Molecular Interactions." *Journal of the American Chemical Society* 132, no. 36 (2010): 12679-89.

RNA Energetics I

Free energy contributions from:

- base pairing: G:C > A:U > G:U

- base stacking:



are combined in
Doug Turner's Energy Rules:

Matrix for each X,Y stacking on each possibly base pair or free end

```
          5' --> 3'
             UX
             AY
          3' <-- 5'

                  X
Y      A       C       G       U
A      .       .       .    -1.30
C      .       .    -2.40      .
G      .    -2.10      .    -1.00
U  -0.90       .    -1.30      .
```

RNA Energetics II

Other Contributions to Folding Free Energy

- Hairpin loop destabilizing energies
  - a function of loop length
- Interior and bulge loop destabilizing energies
  - a function of loop length
- Terminal mismatch and base pair energies

RNA Energetics III

Folding by Energy Minimization

A more complex dynamic programming algorithm is used - similar in spirit to the Nussinov base pair maximization algorithm

Gives:

- minimum energy fold
- suboptimal folds (e.g., five lowest ΔG folds)
- probabilities of particular base pairs
- full partition function

Accuracy: ~70% of base pairs correct

Links & References

The Mfold web server:

http://mfold.rna.albany.edu/?q=mfold/rna-folding-form

The Vienna RNAfold package (free for download)

http://www.tbi.univie.ac.at/~ivo/RNA/

RNA folding references:

M. Zuker, et al. In *RNA Biochemistry and Biotechnology* (1999)

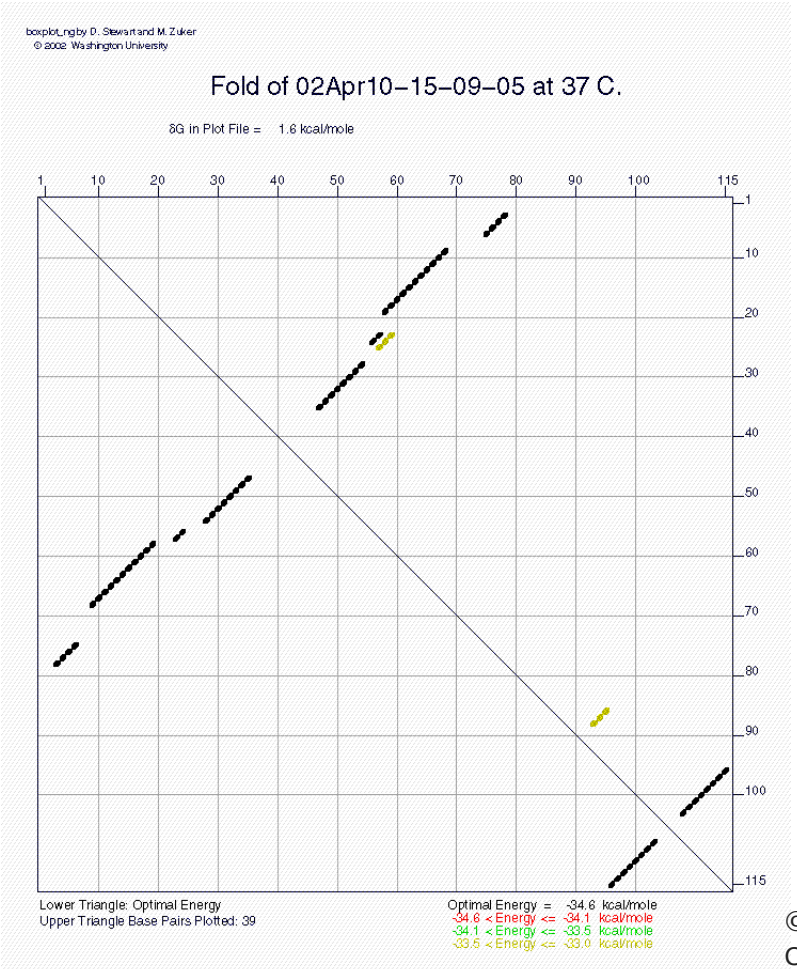
D.H. Mathews et al. *J. Mol. Biol.* **288**, 911-940 (1999)

Vienna package by Ivo Hofacker

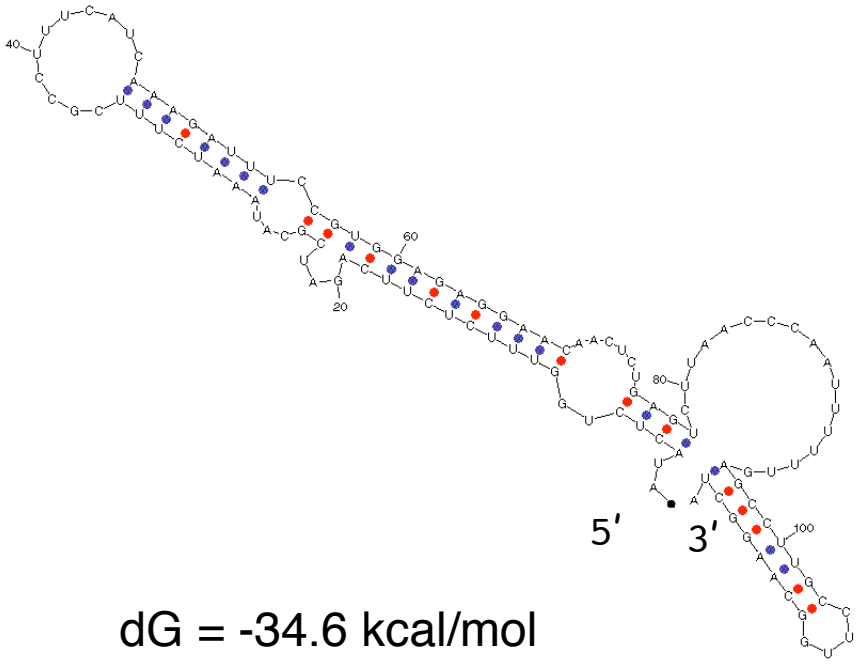
RNA Secondary Structure Prediction by Energy Minimization Summary

- Assumes folding energy decomposable into independent contributions of small units of structure
- Algorithms are guaranteed to find minimal free energy structure defined by the model
- In practice, algorithms predict ~70% of bp correct
- Errors result from
  - imprecision of the model/parameters
  - differences between *in vitro* and *in vivo* conditions
  - *in vivo* structure may not always have minimum free energy

Sample Mfold Output (Human U5 snRNA)



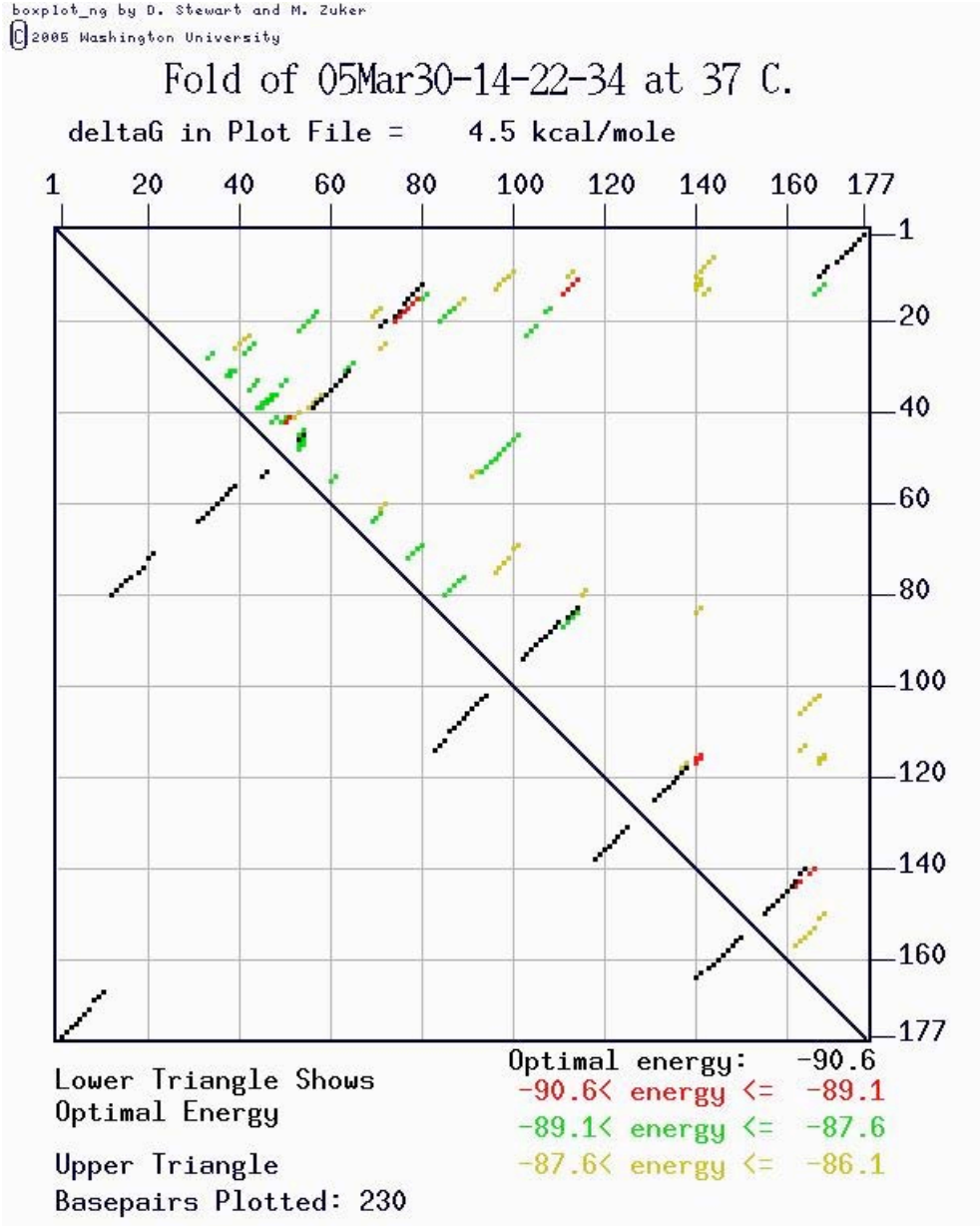
Energy dot plot



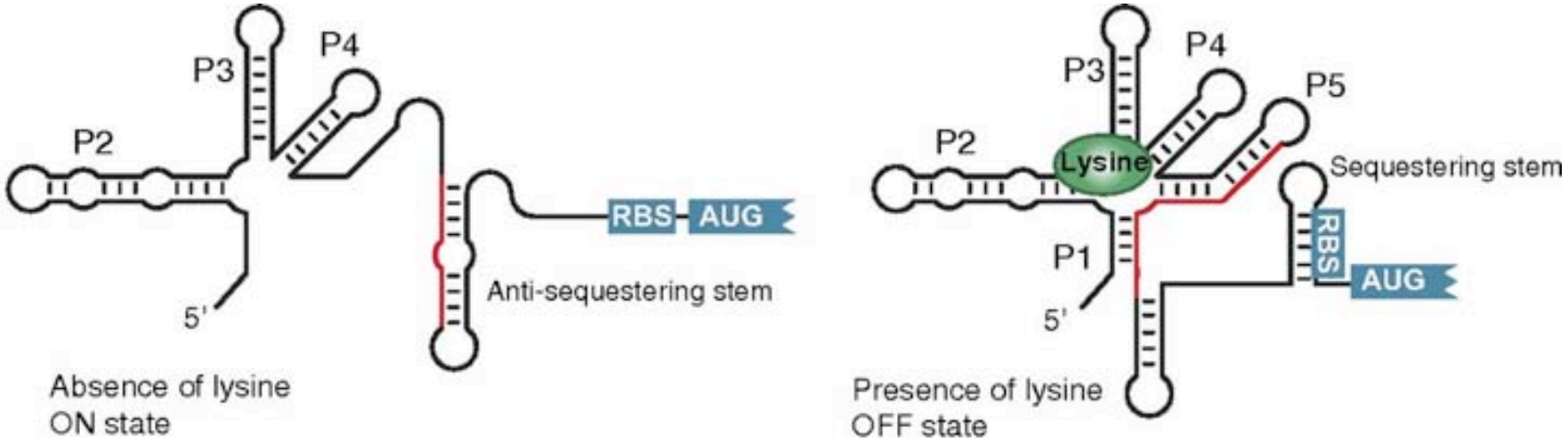
dG = -34.6 kcal/mol

Minimum free energy structure

Energy dot plot for a lysine riboswitch



Function of the lysine riboswitch



Lysine interacts with the junctional core of the riboswitch and is specifically recognized through shape-complementarity within the elongated binding pocket and through several direct and K+-mediated hydrogen bonds to its charged ends.

Controls expression of enzymes involved in biosynthesis and transport of lysine

Serganov et al. Nature 2008. Caron et al PNAS 2012

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