RNA secondary structure prediction

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Lecture based on Mark Craven's class at University of Wisconsin

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Intelligent Data Analysis
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http://cw.felk.cvut.cz/wiki/courses/b4m36bin/start

Overview

- Key concepts
  - RNA secondary structure,
  - secondary structure features: stems, loops, bulges,
  - pseudoknots,
  - Nussinov algorithm,
  - Adapting Nussinov to take free energy into account.

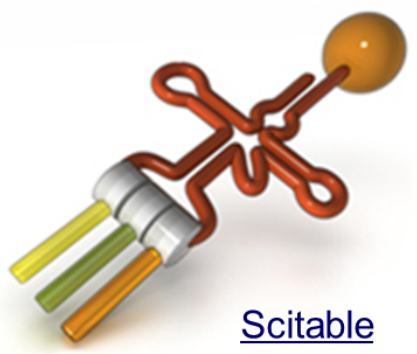
Why RNA is interesting

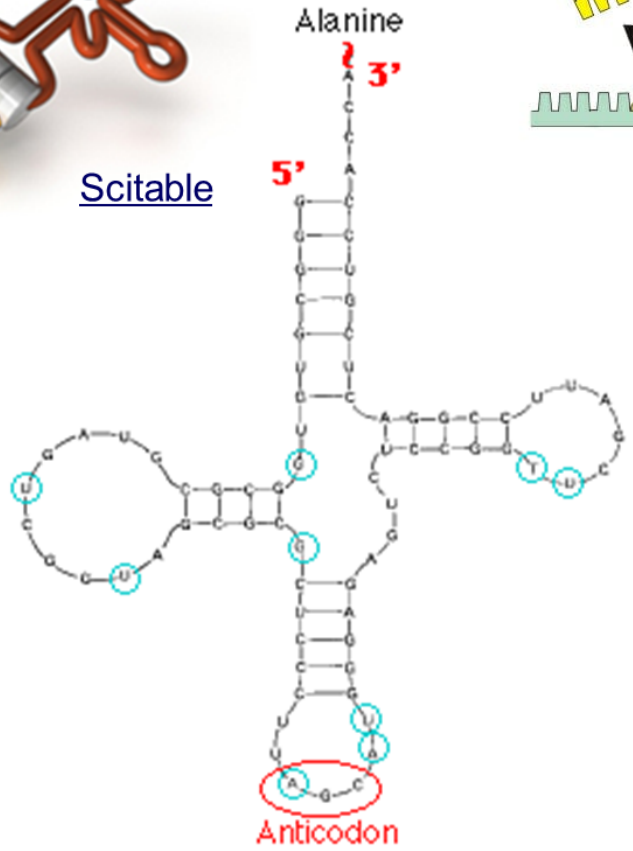
- Messenger RNA (mRNA) is not the only important class of RNA
  - ribosomal RNA (rRNA)
    - ribosomes are complexes that incorporate several RNA subunits in addition to numerous protein units,
  - transfer RNA (tRNA)
    - transport amino acids to the ribosome during translation,
  - the spliceosome, which performs intron splicing
    - a complex with several RNA units,
  - microRNAs and other ncRNAs that play regulatory roles,
  - many viruses (e.g. HIV) have RNA genomes,
  - guide RNA
    - sequence complementarity determines whether to cleave DNA,
  - folding of an mRNA can be involved in regulating the gene's expression.

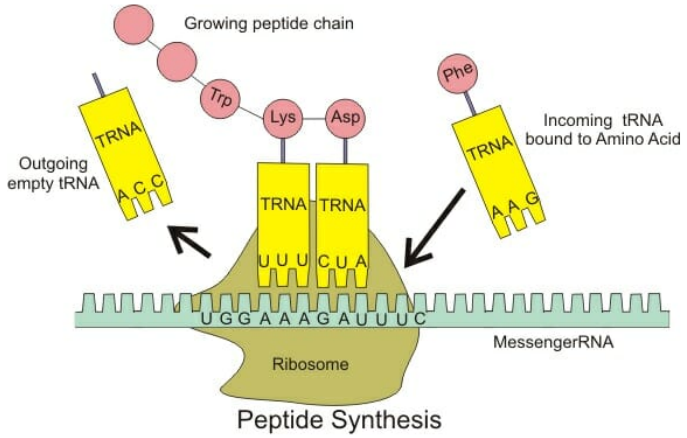
RNA secondary structure

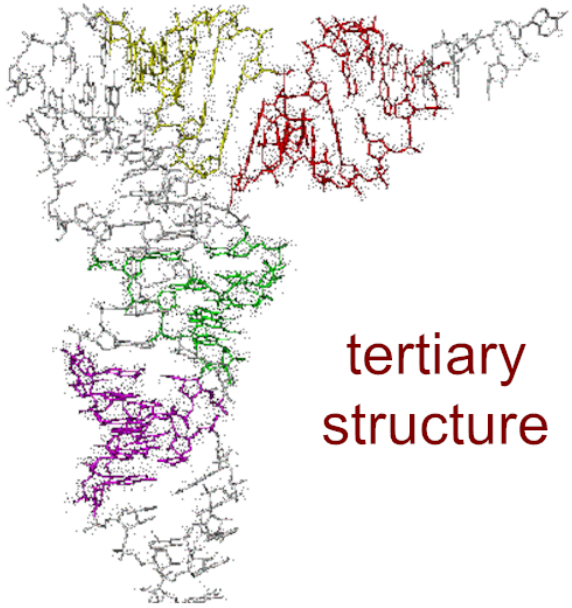
- RNA is typically single stranded,
- folding, in large part is determined by base-pairing,
- **A-U** and **C-G** are the canonical base pairs
  - **C-G** pairs form 3 hydrogen bonds, while **A-U** form only two,
  - other bases will sometimes pair, especially **G-U**,
- base-paired structure is referred to as the secondary structure of RNA,
- related RNAs often have homologous secondary structure
  - without significant sequence similarity.

tRNA Secondary Structure



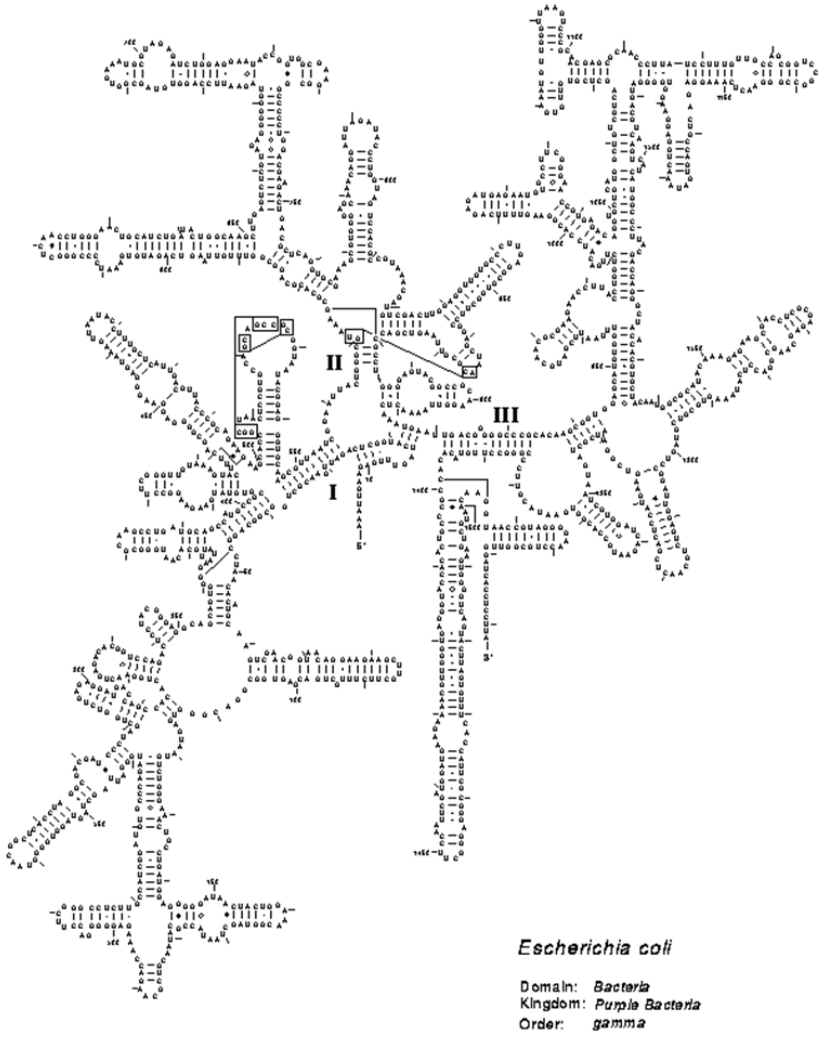






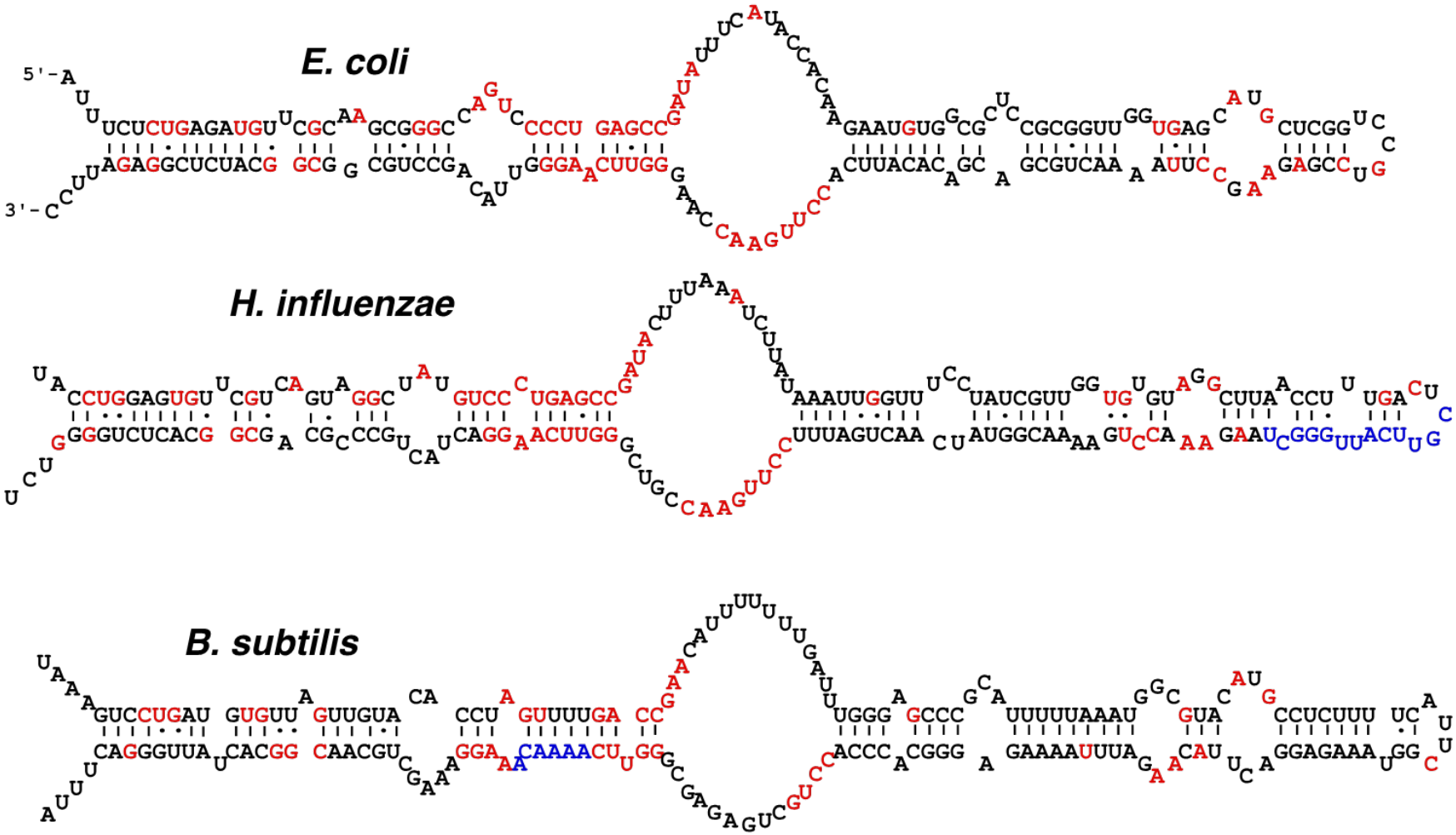
Marc Craven, BMI/CS 576, www.biostat.wisc.edu/bmi576.

Small subunit ribosomal RNA



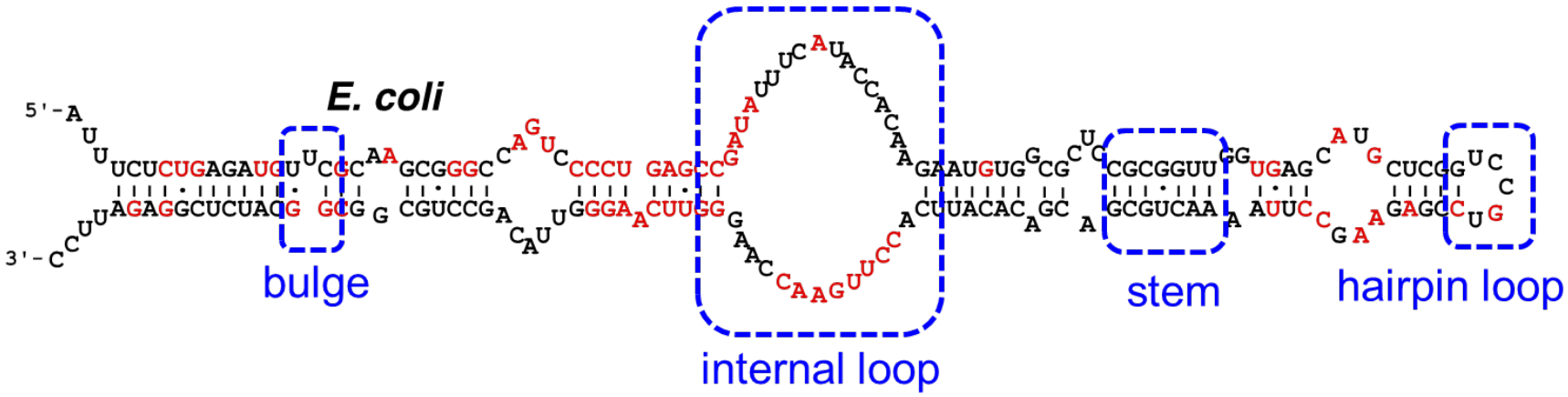
O'Connor, Nucleic acids research, 1997.

6S RNA secondary structure



Cavanagh, Annual review of microbiology, 2014.

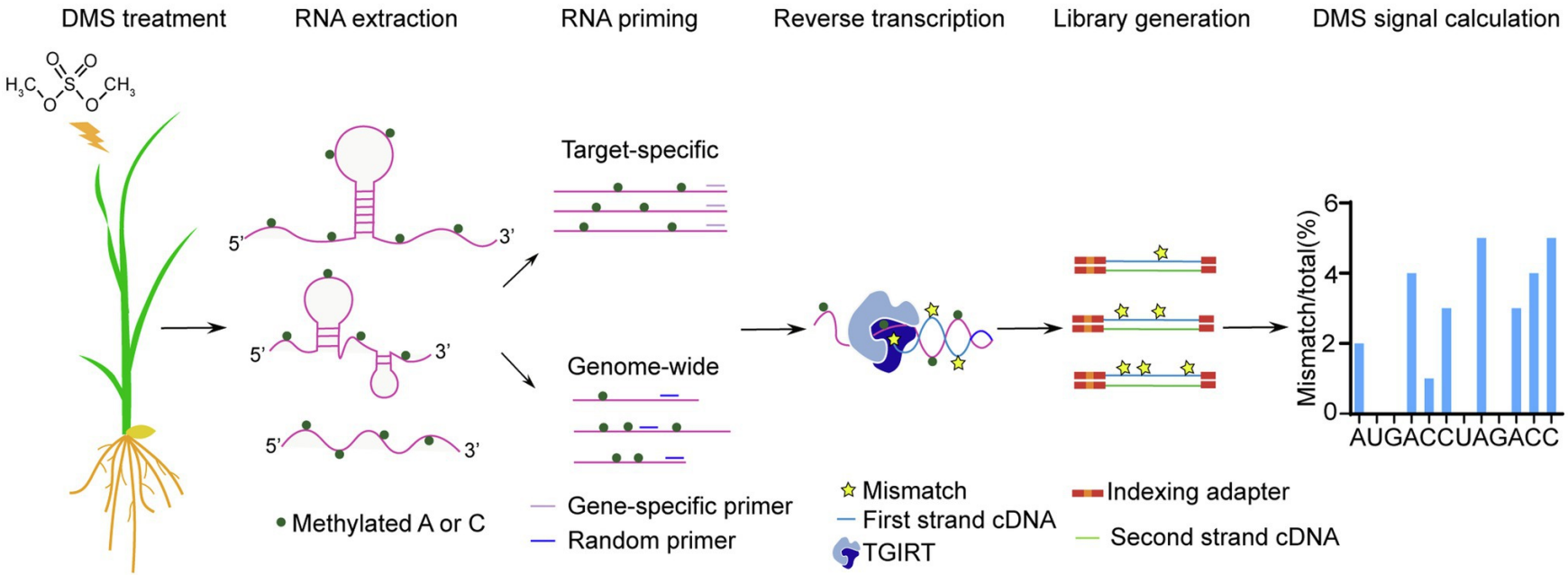
Secondary structure features



Marc Craven, BMI/CS 576, www.biostat.wisc.edu/bmi576.

Probing RNA secondary structure in vivo

- Dimethyl sulfate (DMS) mutational profiling
  - DMS methylates exposed **A** and **C** bases = those that do not pair,
  - modified RNA is then reversely transcribed using DNA polymerase,
  - methylated bases typically result in termination of reverse transcription.

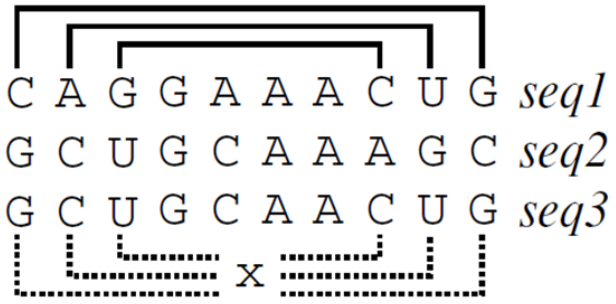


Jin et al.:Probing in vivo RNA Structure With Optimized DMS-MaPseq in Rice, Front. Plant Sci.2022.

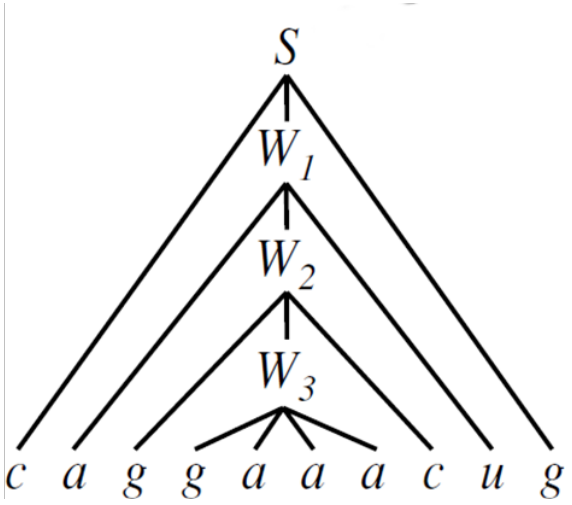
Secondary structure as CFG

- Context-free grammar (CFG) is a suitable formalism for representing palindrome languages.

seq1	*seq2*	*seq3*
A A	C A	C A
G A	G A	G A
G • C	U • A	U × C
A • U	C • G	C × U
C • G	G • C	G × G



$$
\begin{aligned}
S &\rightarrow aW_1u \mid cW_1g \mid gW_1c \mid uW_1a \\
W_1 &\rightarrow aW_2u \mid cW_2g \mid gW_2c \mid uW_2a \\
W_2 &\rightarrow aW_3u \mid cW_3g \mid gW_3c \mid uW_3a \\
W_3 &\rightarrow gaaa \mid gcaa.
\end{aligned}
$$



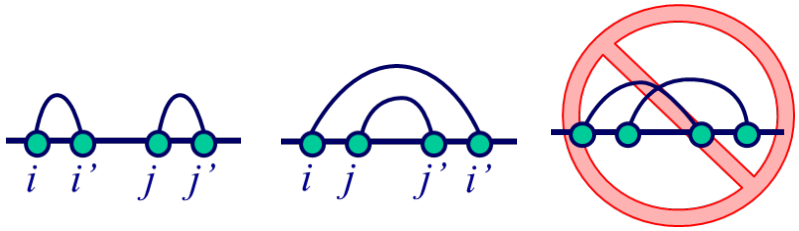
Durbin, Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids.

Four key problems

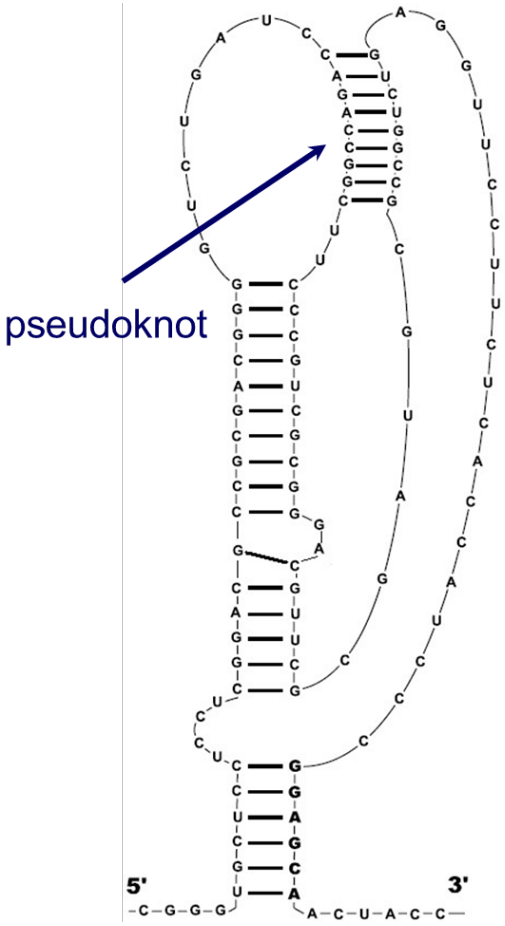
- Predicting RNA secondary structure (**Focus for today**)
  - Given: RNA sequence,
  - Do: predict secondary structure that sequence will fold into,
- Searching for instances of a given structure
  - Given: an RNA sequence or its secondary structure,
  - Do: find sequences that will fold into a similar 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 and pseudoknots

- We will 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),
  - or $i < j < j' < i'$ or $j < i < i' < j'$ (nested),
- cannot have $i < j < i' < j'$ or $j < i < j' < i'$
  - these crossings are called **pseudoknots**,
  - dynamic programming breaks down with them,
  - fortunately, they are not very frequent.



not nested nested crossing



Seliverstov et al. BMC Microbiology, 2005.

Predicting RNA secondary structure

- Given:
  - an RNA sequence,
  - the constraint = pseudoknots not allowed,
- Do:
  - find a secondary structure for the RNA,
  - it maximizes the number of base pairing positions,
- Nussinov algorithm
  - key ideas
    - do this using dynamic programming,
    - start with small subsequences,
    - progressively work to larger ones.

DP in the Nussinov algorithm

- Let

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

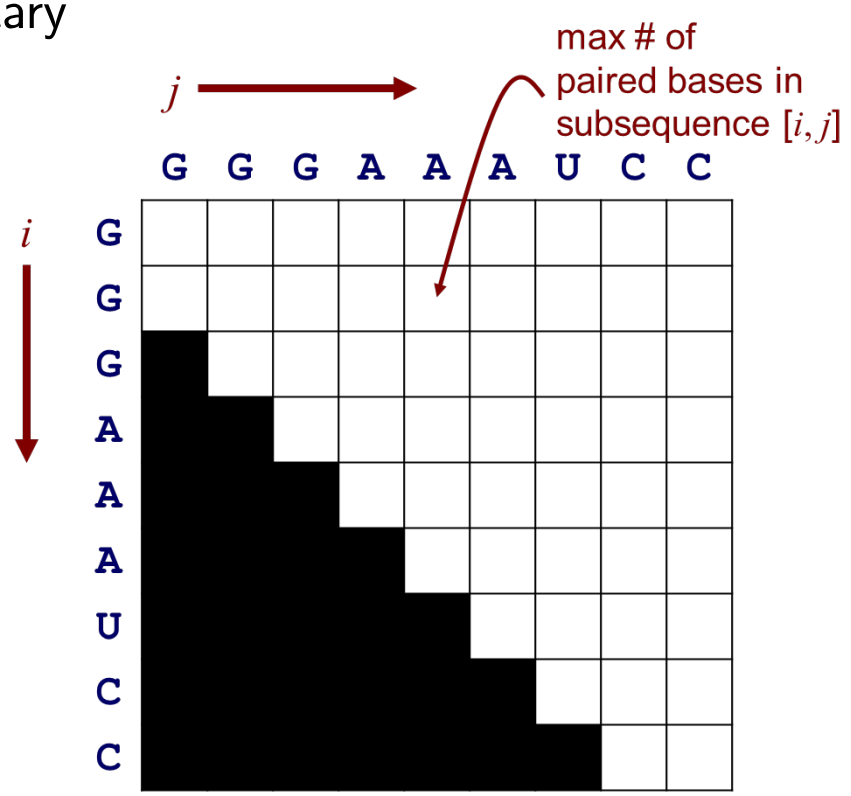
- initialization

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

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

- recursion

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



Durbin, Biological Sequence Analysis:
Probabilistic Models of Proteins and Nucleic Acids.

Nussinov algorithm traceback

- Determine one non-crossing RNA structure with maximal score.

```
push(1, L) onto stack
repeat until stack is empty
  pop(i, j)
  if i ≥ j continue
  else if γ(i + 1, j) = γ(i, j) push(i + 1, j)
  else if γ(i, j − 1) = γ(i, j) push(i, j − 1)
  else if γ(i + 1, j − 1) + δ(i, j) = γ(i, j)
    record i, j base pair
    push(i + 1, j − 1)
  else for k = i + 1 to j − 1:
    if γ(i, k) + γ(k + 1, j) = γ(i, j)
      push(k + 1, j)
      push(i, k)
      break
```

Predict RNA secondary structure by energy minimization

- Maximizing the number of base pairs oversimplifies prediction of folding,
- however, we can generalize the key recurrence relation by minimizing free energy instead.

$$E(i,j) = min \begin{cases} E(i+1,j) \\ E(i,j-1) \\ min_{i<k<j}[E(i,k) + E(k+1,j)] \\ P(i,j) \quad \leftarrow \quad \text{case that } i \text{ and } j \text{ are base paired} \end{cases}$$

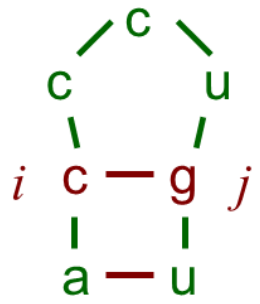
Predict RNA secondary structure by energy minimization

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

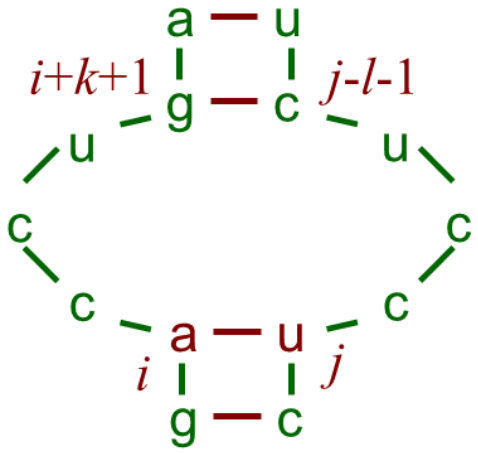
$$P(i,j) = min \begin{cases} \alpha(i,j) + \text{LoopEnergy}(j-i-1) \\ \alpha(i,j) + \text{StackingEnergy}(i,j,i+1,j-1) + P(i+1,j-1) \\ min_{k\geq 1}[\alpha(i,j) + \text{BulgeEnergy}(k) + P(i+k+1,j-1)] \\ min_{k\geq 1}[\alpha(i,j) + \text{BulgeEnergy}(k) + P(i+1,j-k-1)] \\ min_{k,l\geq 1}[\alpha(i,j)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

Predict RNA secondary structure by energy minimization

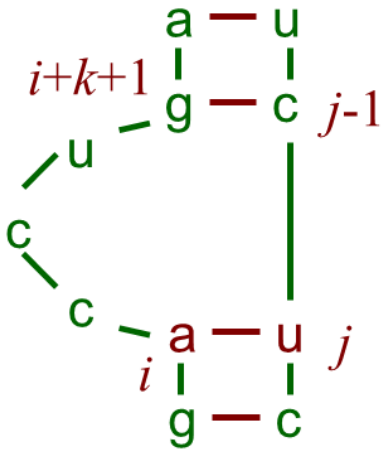
$\alpha(i,j) + \text{LoopEnergy}(j-i-1)$



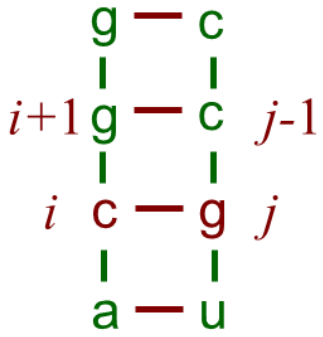
$min_{k,l \geq 1}[\alpha(i,j) + \text{LoopEnergy}(k+l) + P(i+k+1, j-l-1)]$



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



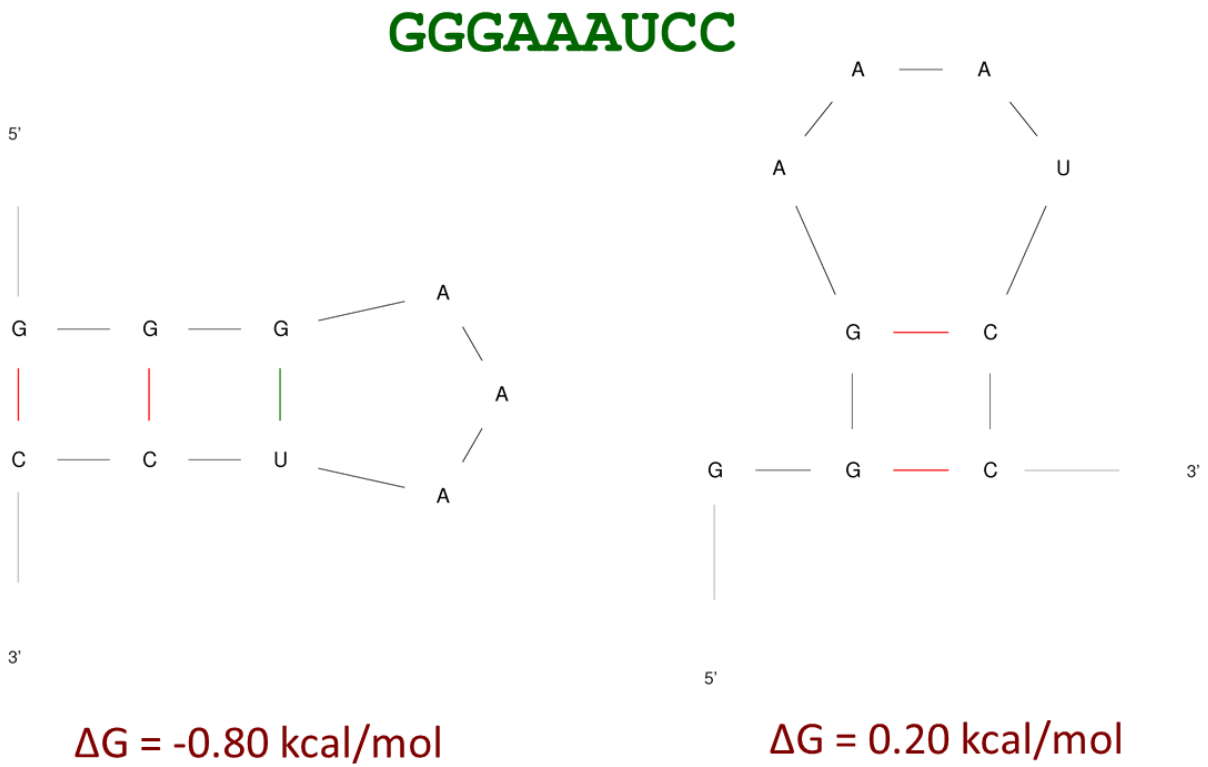
$\alpha(i,j) + \text{StackingEnergy}(i,j,i+1,j-1) + P(i+1,j-1)$



Marc Craven, BMI/CS 576, www.biostat.wisc.edu/bmi576.

Mfold example

- Mfold solutions with energy up to 5% from the best
  - different from Nussinov results (2 Watson-Crick base pairs only here).

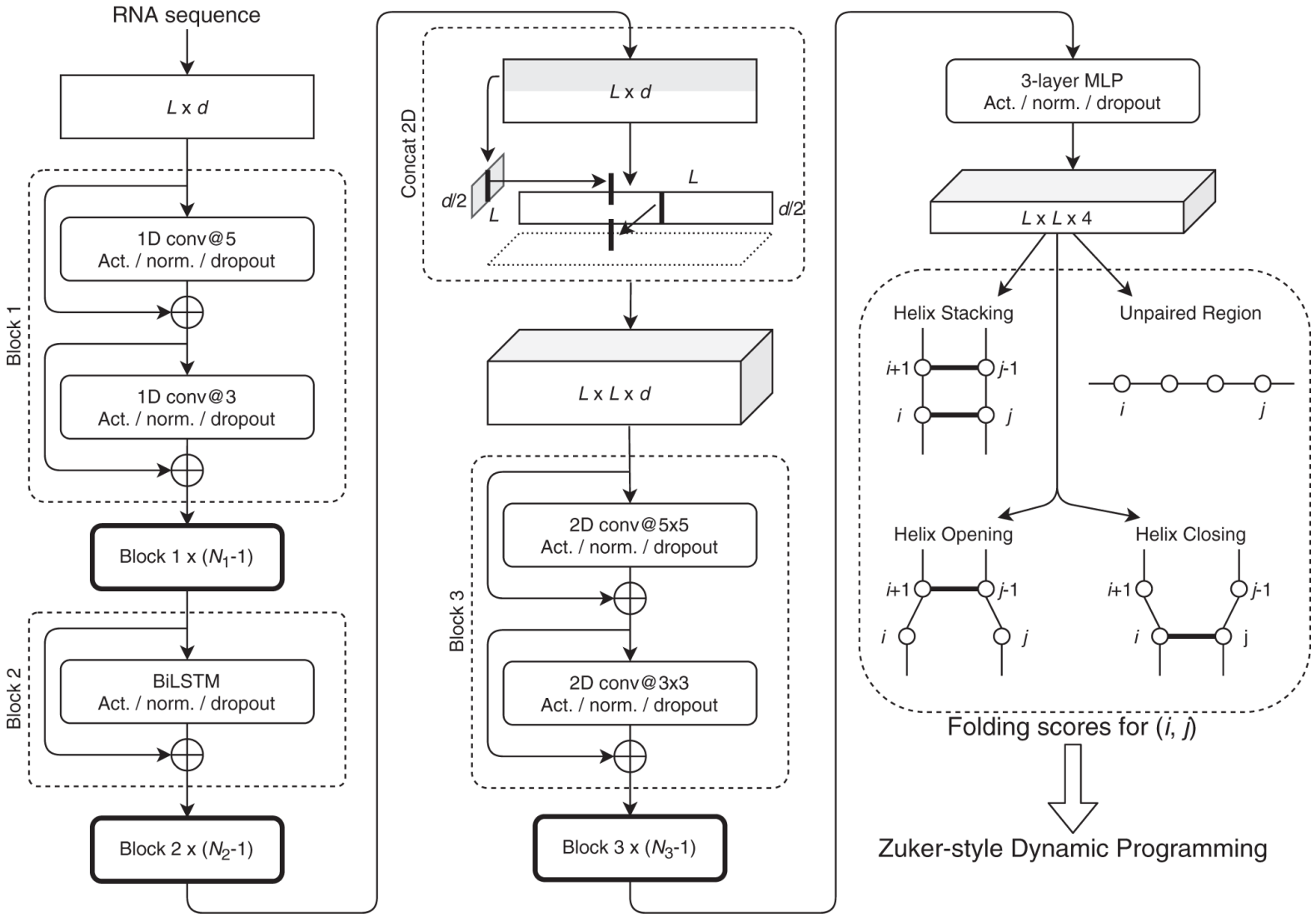


http://unafold.rna.albany.edu/

MXfold2 – a recent deep learning extension to Mfold

- Deep neural network computes four types of folding scores
  - unpaired, stacking, opening and closing score,
  - the scores obtained for each pair of nucleotides,
- then, MXfold2 predicts an optimal secondary structure
  - it maximizes the sum of the scores of the nearest-neighbor loops,
  - using Zuker-style dynamic programming as shown in Mfold,
- the deep neural network is trained using the max-margin framework
  - also known as structured support vector machine (SSVM),
  - it minimizes the structured hinge loss function with thermodynamic regularization,
- it prevents the folding score of the secondary structure from differing significantly from the free energy of the thermodynamic parameters.

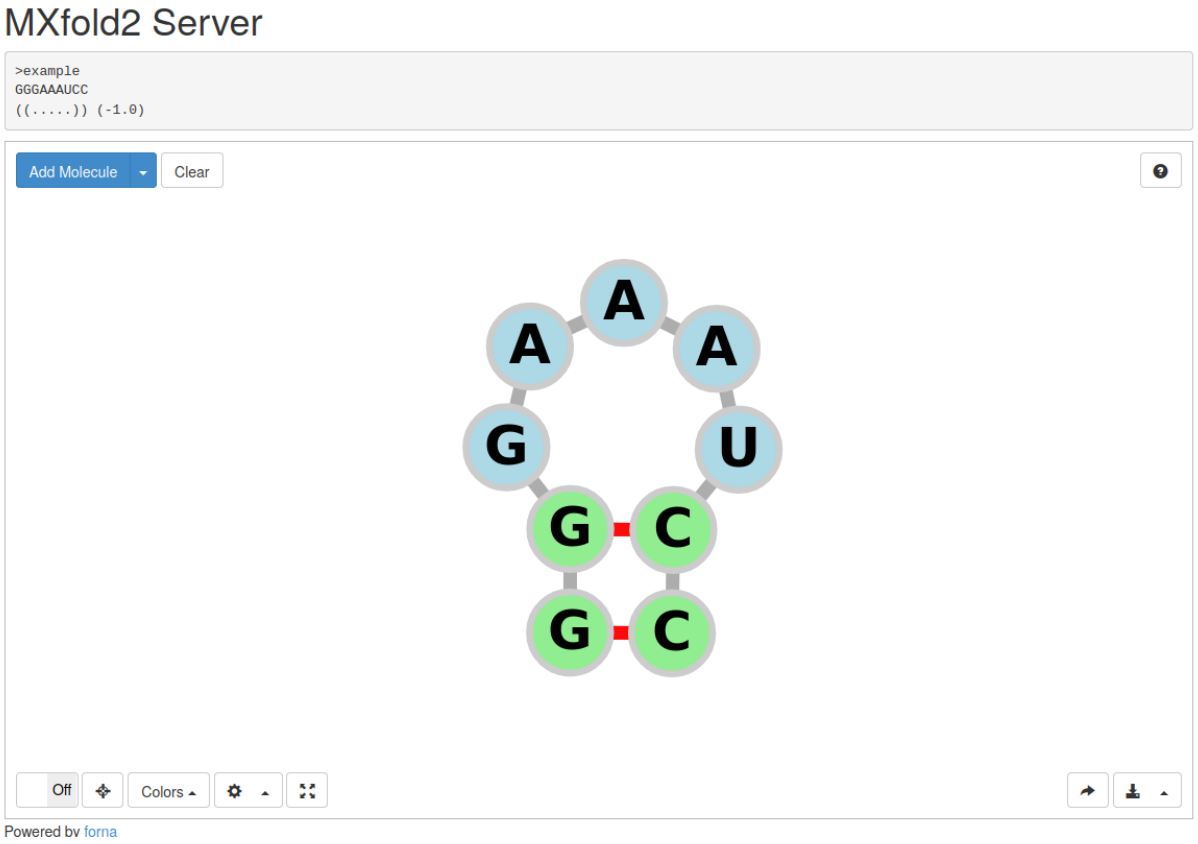
MXfold2 – a recent deep learning extension to Mfold



Sate et al: RNA secondary structure prediction using deep learning with thermodynamic integration, Nature Communications, 2021.

MXfold2 example

- MXfold2 server publicly available,
- yet another outcome for our small running example,



http://ws.sato-lab.org/mxfold2/predict

Summary

- RNA has numerous roles in translation, splicing, DNA replication, regulation,
- RNA structure understanding is important
  - substitutions possible, function preserved as long as they preserve structure,
- Secondary structure can be predicted
  - comparative sequence analysis
    - molecules with similar function will form similar structures,
    - it searches for positions that co-vary,
  - free energy minimization
    - take a sequence, search for energetically stable complementary regions,
    - in a simplified form discussed in this lecture,
    - many foldings lie close to the predicted global energy minimum,
  - current folding programs get on average 60-75% base pairs correct,
  - in general an intractable task,
- experimental methods such as dimethyl sulfate (DMS) probing exist.