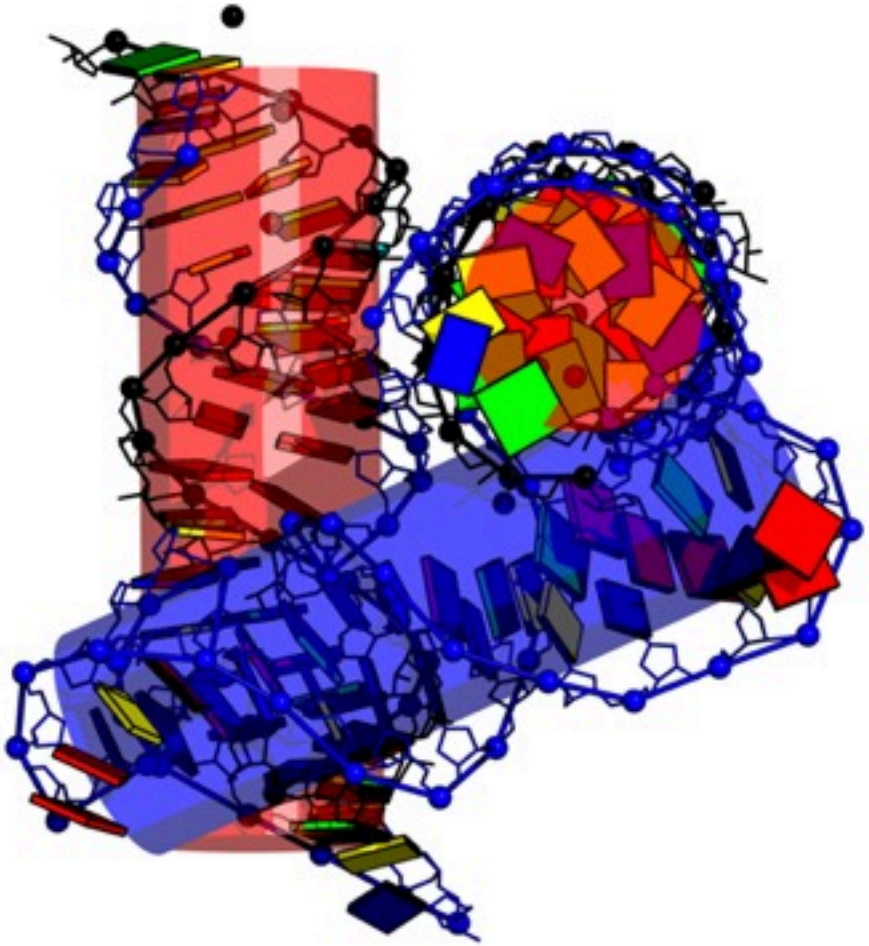
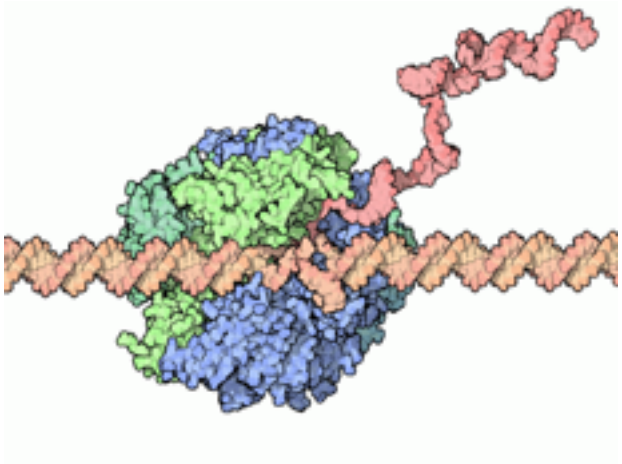


-old homepage-

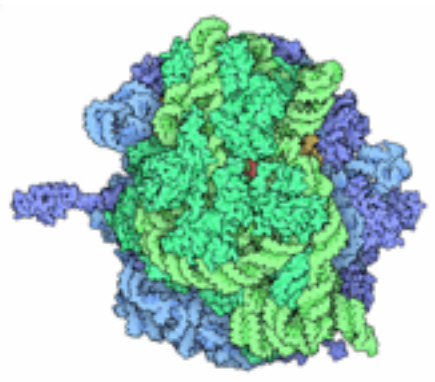
Post Doctoral Researcher
Department of Biosciences & Nutrition
Karolinska Institutet
Phone number: (+46)-08-524-81079 (lab)
(+46)-073-678-5334 (cell)
e-mail: mauricio dot esguerra at ki dot se

http://mesguerra.net

Biology's Central Dogma and Molecular Machines



TRANSCRIPTION
RNA Pol II



TRANSLATION
Ribosome, aka rRNA

DNA ⇒ RNA ⇒ mRNA ⇒ Protein

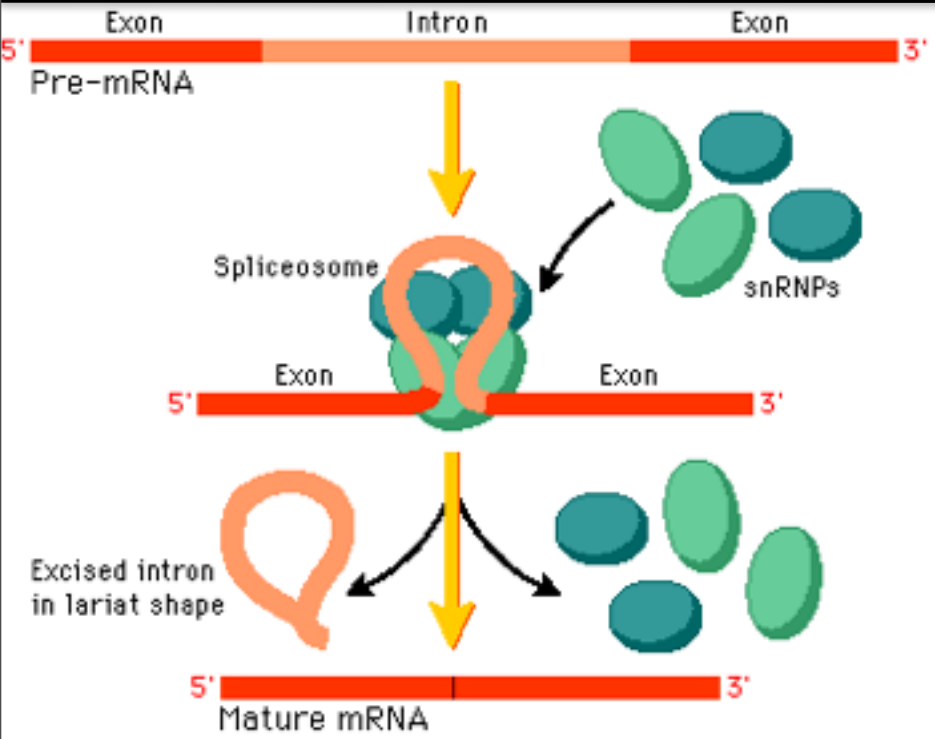
antigene RNA (agRNA)

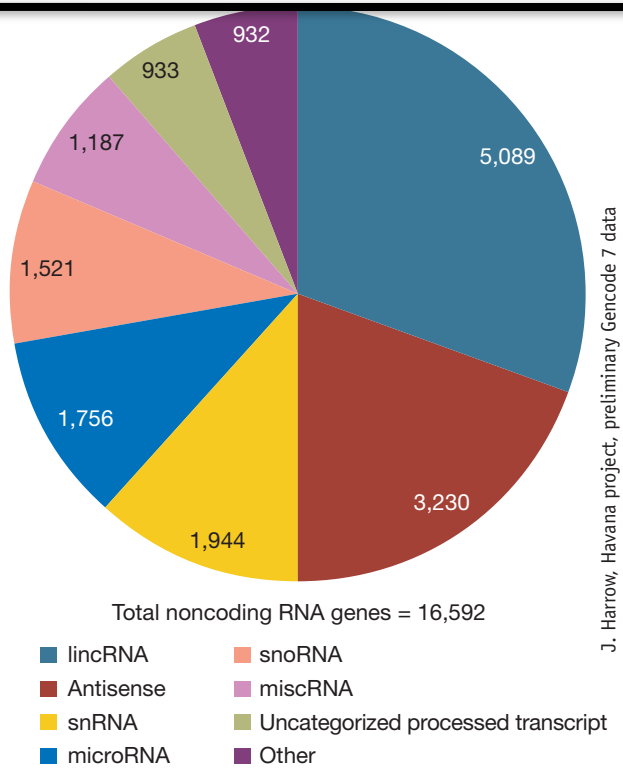
ncRNA (miRNA, siRNA, ...)

Replication

Genotype → **Gene Expression (Activation, Silencing)** → Phenotype

RNA is Spliced by the Spliceosome After Transcription.





Long noncoding RNAs are just one of many noncoding transcripts being annotated. lincRNA, long intergenic noncoding RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; and miscRNA, miscellaneous RNA.

http://genome.ucsc.edu/ENCODE/

http://www.gencodegenes.org/

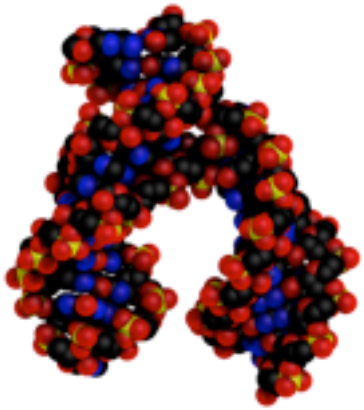
~3200 Million base-pairs (Humans)
< 2% makes mature mRNA (protein)
~50% transcribed ncRNA.

Monya Baker, *Nature Methods*, **8**, 379-383, 2011

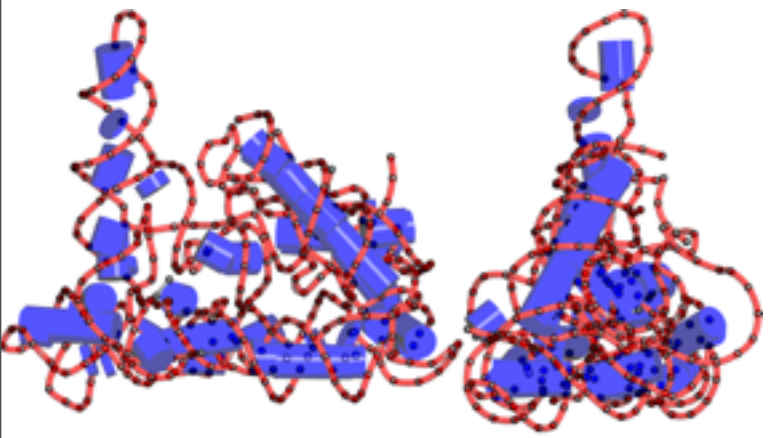
Non-coding RNA's

- RNApol I rRNA not 5S (~ 50% cell RNA)
- RNApol II precursors of mRNA and most snRNA and microRNA
- RNApol III 5S rRNA, tRNA and other small RNAs
- piwiRNA
- siRNA
- http://rfam.sanger.ac.uk/family/browse/with_structure#A
-

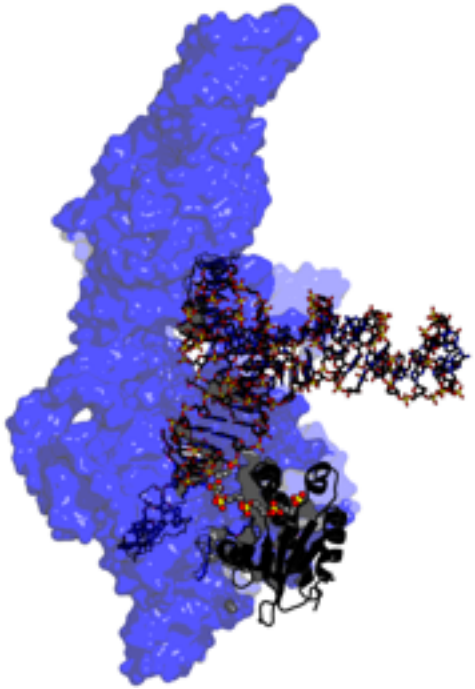
The RNA Zoo



Hammerhead Ribozyme
1hmh.pdb



GroupII Intron
3bwp.pdb



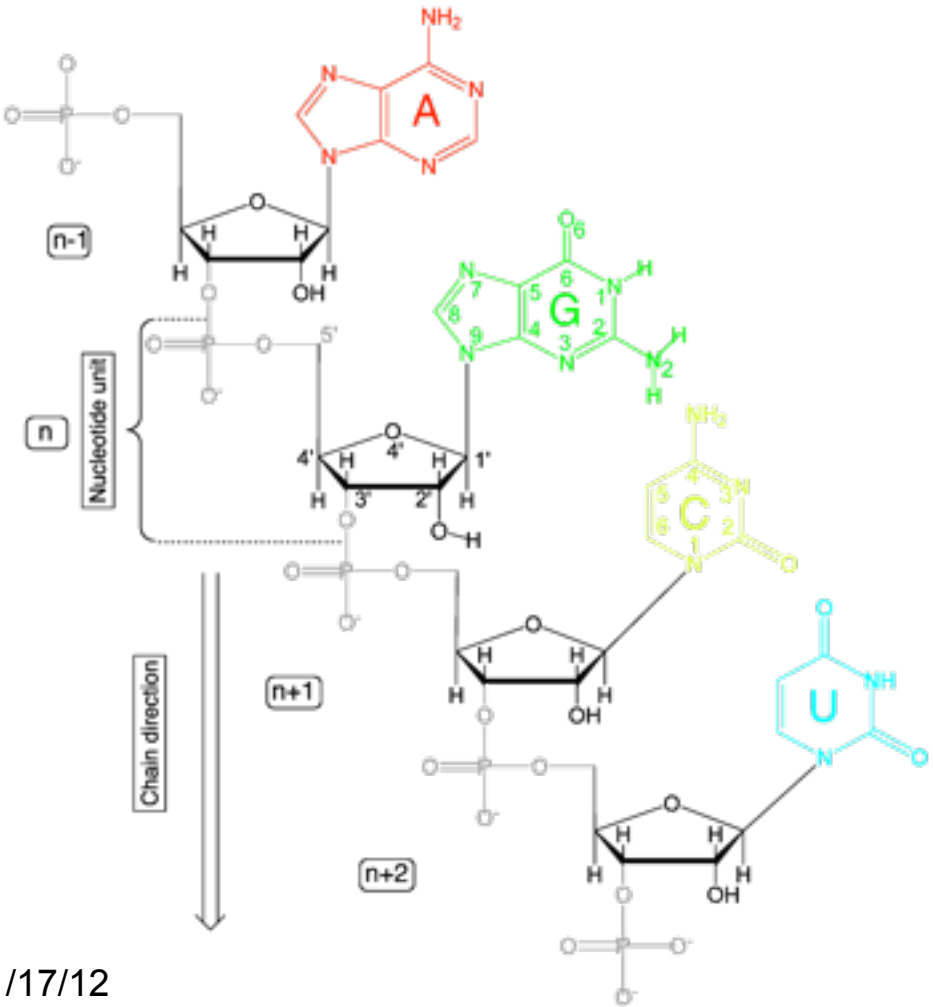
Rnase P

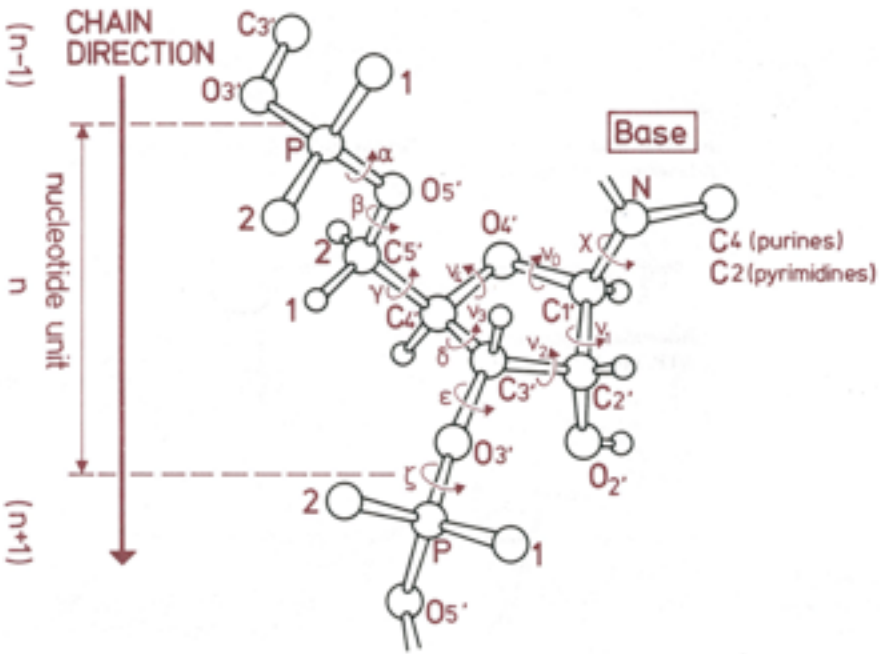
Structure Determines Function

RNA Structure Can Be Understood at Levels.

Primary Structure (Sequence)

```
GCGGAUU UA gCUC AGuuGGGA GAGC gCCAGAc UgAAg APcUGGA GgUC cUGUG uPCGaUC CACAG AAUUCGC ACCA
1234567 89 0123 45678901 2345 6789012 34567 8901234 5678 90123 4567890 12345 6789012 3456
```

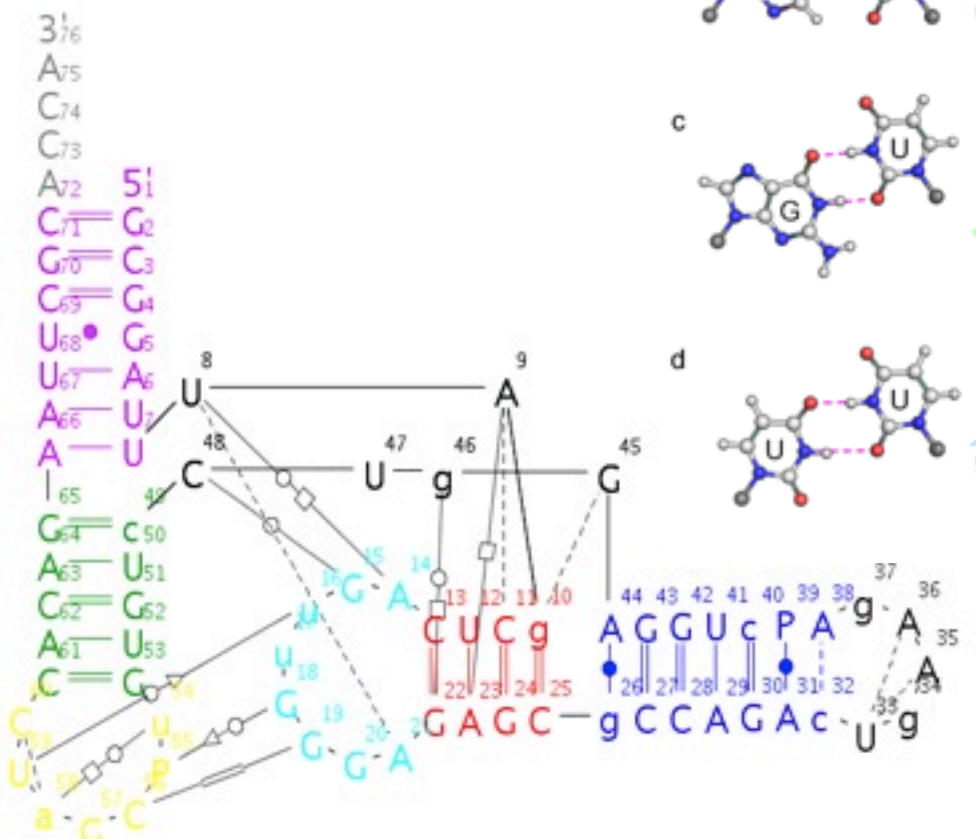


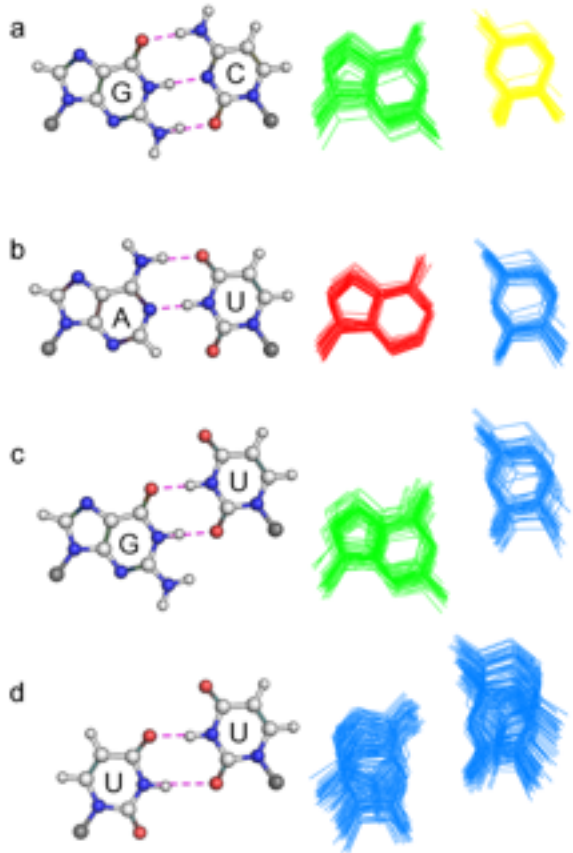


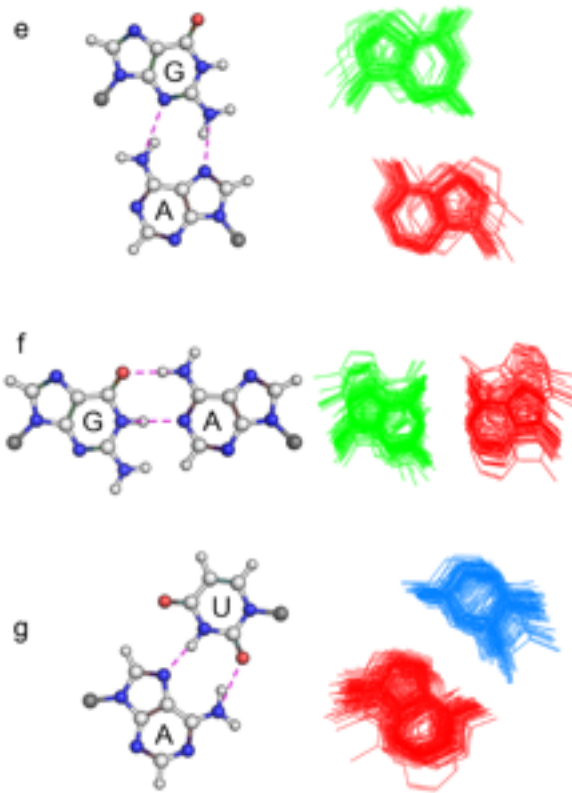
Backbone covalently linked through phosphodiester groups with 5' to 3' sense.

Secondary Structure

1. Sequence
2. Network Hydrogen Bonded Base-Pairs (Canonical and Non-Canonical)

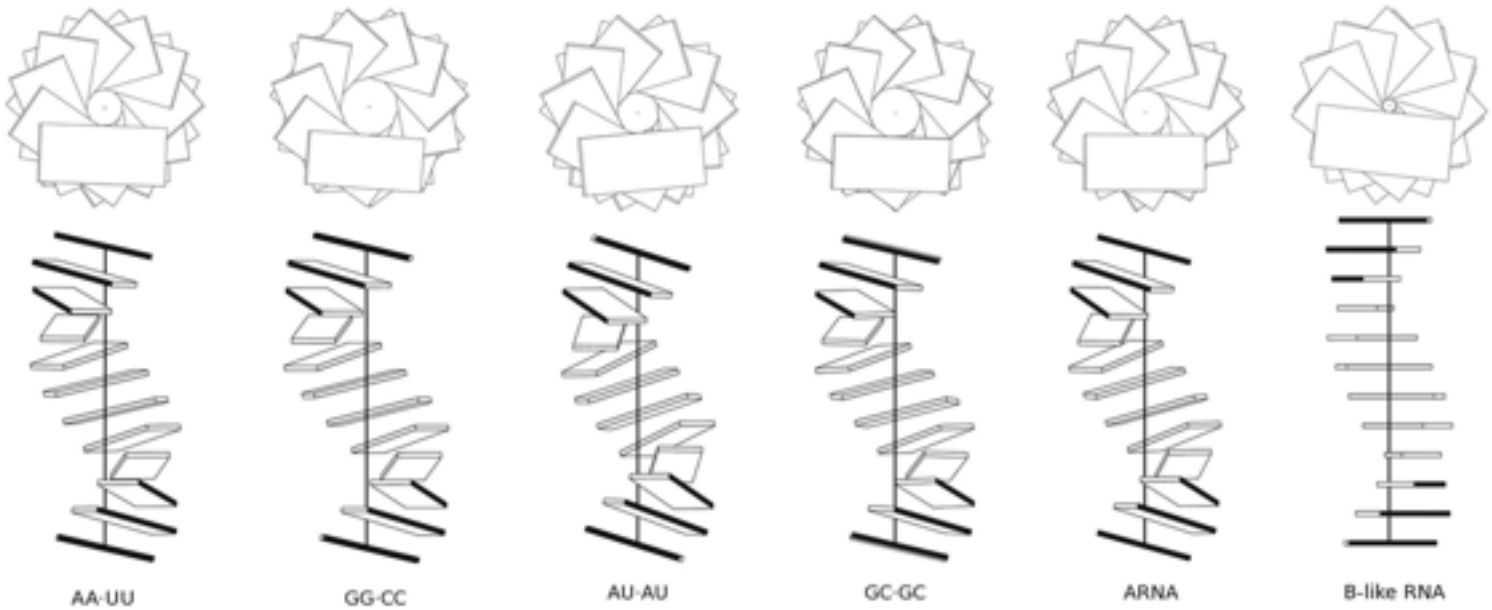






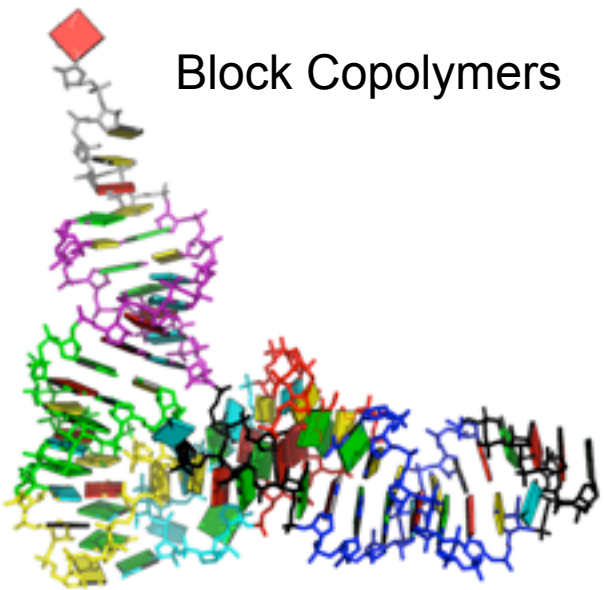
Secondary Structure “Motifs”

3D Structure (Subtle Conformational Variation of Helical Regions)



Homopolymers

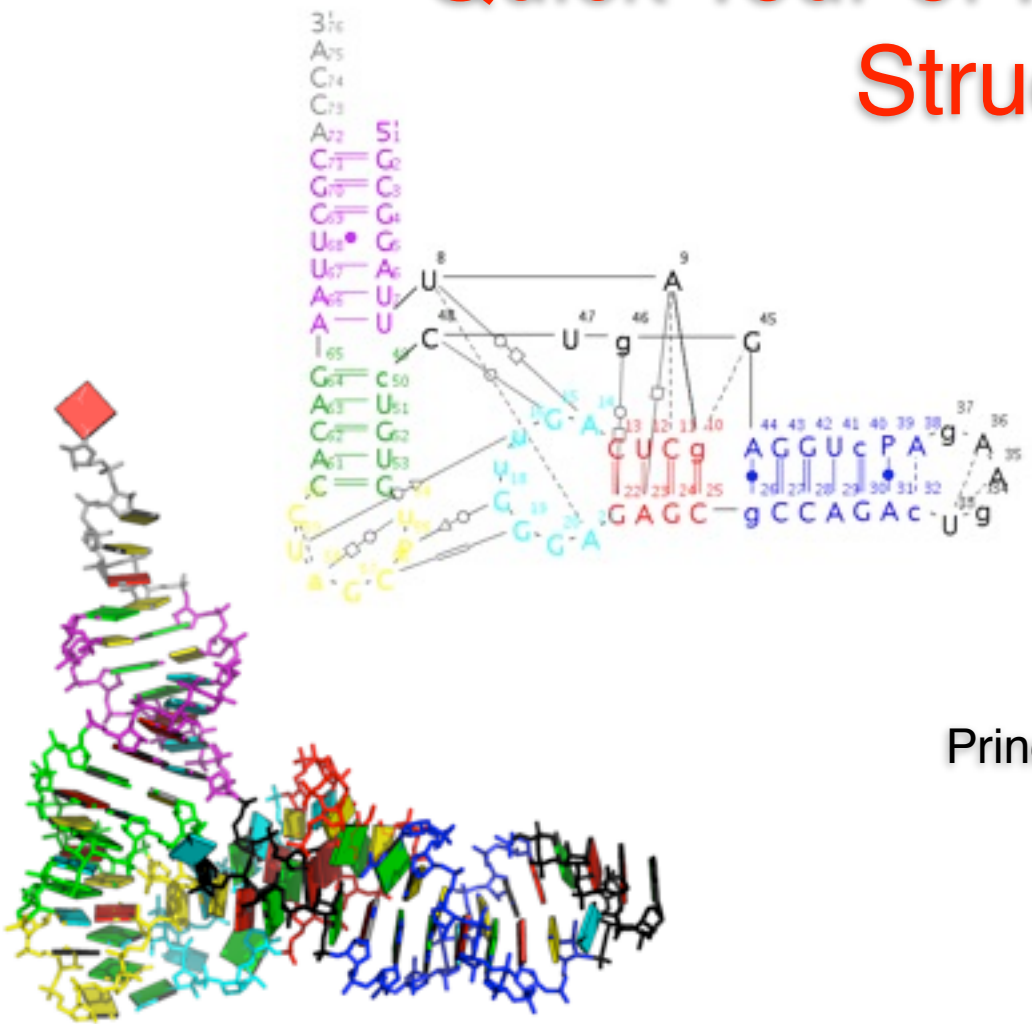
Block Copolymers



1. Sequence
2. Network Hydrogen Bonded Base-Pairs (Canonical and Non-Canonical)
3. Network of so-called “Tertiary Interactions” (Secondary-Motif interactions), e.g. ion-mediated, base-backbone, inter-helical base-pairs.

GCGGAUU UA gCUC AGuuGGGA GAGC gCCAGAc UgAAg APcUGGA GgUC cUGUG uPCGaUC CACAG AAUUCGC ACCA
1234567 89 0123 45678901 2345 6789012 34567 8901234 5678 90123 4567890 12345 6789012 3456

Quick Tour of RNA Secondary Structure Prediction



Mauricio Esguerra Neira

Lennart Nilsson Group

http://mesguerra.net

Principles of Nucleic Acid Structure

KI Doctoral Course 2430

Torsdag, Februari Två, 2012

13:00 - 16:00

Two Kinda ThreeTypes of RNA Secondary Structure Prediction

- RNA Sequence Covariation (Gutell)
- RNA Free Energy Minimization (Tinoco-Uhlenbeck)
- RNA Base-Pair Maximization (Nussinov)

Sequence Covariation

- Main Idea is:
- Gutell quite successful in getting rRNA.

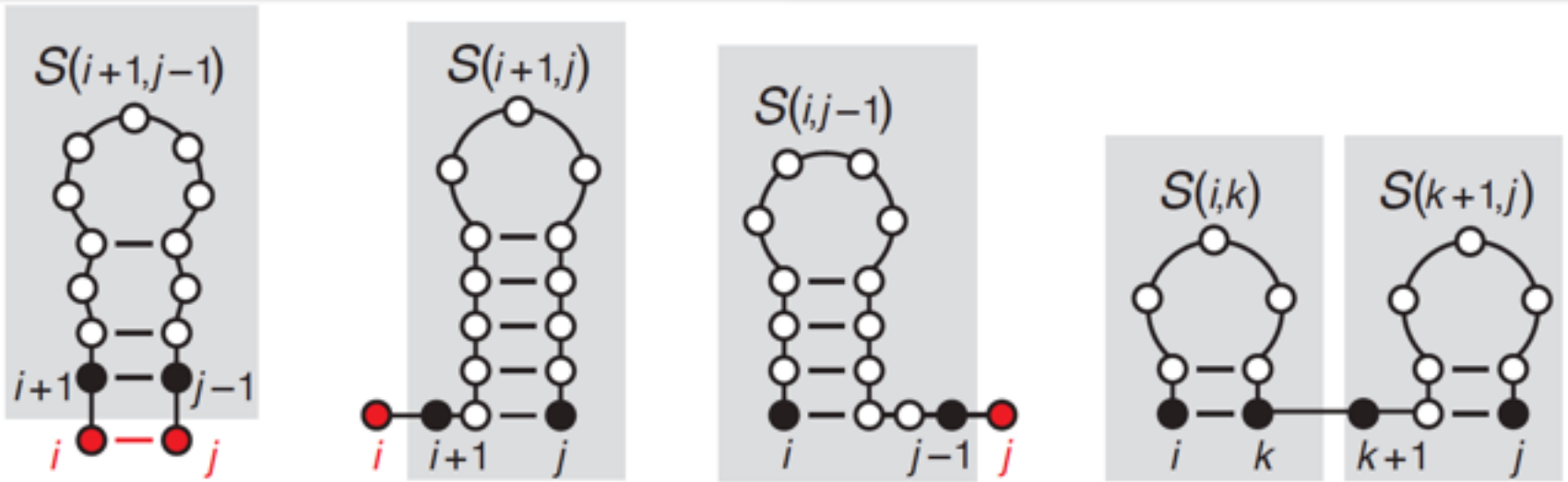
Delta G Minimization

- Tinoco-Uhlenbeck Postulate

  “Base-pair free energies are additive”

- Dynamic Programming Nussinov Algorithm

Nussinov Algorithm (Maximum Number of Base-Pairs)



1. *i,j* pair 2. *i* unpaired 3. *j* unpaired 4. Bifurcation

$$D(i,j) = \max\begin{Bmatrix} max_{i<k<j} D(i,k) + D(k+1,j) \\ D(i+1,j-1) + \ w(i,j) \\ D(i+1,j) \\ D(i,j-1) \end{Bmatrix}$$

Nussinov et al. *SIAM J. Appl. Math.* **35**, 68-82 (1978)

Initialization

$$D(i,j) = \max\begin{Bmatrix} D(i,i) = 0 & \forall i = 1..L \\ D(i,i-1) = 0 & \forall i = 2..L \end{Bmatrix}$$

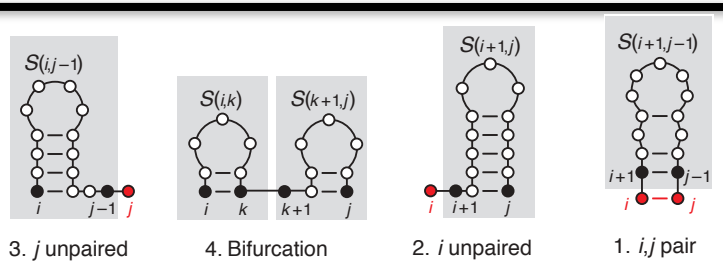
Recursion

$$D(i,j) = \max\begin{Bmatrix} D(i,k) + D(k+1,j) & where\ i \le k < j \\ D(i+1,j-1) + \ w(i,j) & \end{Bmatrix}$$

Nussinov and Jacobson *PNAS* **77**, 6309-6313 (1980)

The Algorithm in Action (4 MNBP)

$$D(i,j) = \max \begin{cases} D(i, j-1) \\ D(i+1, j-1) + w(i,j) \\ D(i+1, j) \\ \max_{i<k<j} D(i,k) + D(k+1, j) \end{cases}$$



S. Eddy, *Nature Biotech.* **22**, 1457-1458 (2004)

D(1,1) = max
- i<k<j NOT
- D(2,0) + w(1,1) = 0
- D(2,1) = 0
- D(1,0) = 0

D(2,5) = max
- D(2,4) = 1
- D(3,4) + w(2,5) = 0
- D(3,5) = 1
- k={3,4}
  max{
  D(2,3)+D(4,5) = 2
  D(2,4)+D(5,5) = 1
  } = 2

	j	1	2	3	4	5	6	7	8	9	10
i		A	C	G	G	C	A	A	C	G	U
1	A	0	0	1	1	2	2	2	2	3	4
2	C	0	0	1	1	2	2	2	2	3	4
3	G		0	0	0	1	1	1	2	2	3
4	G			0	0	1	1	1	1	2	3
5	C				0	0	0	0	0	1	2
6	A					0	0	0	0	1	2
7	A						0	0	0	1	2
8	C							0	0	1	1
9	G								0	0	0
10	U									0	0

Backtracking to Structure (Counterclockwise)

$$D(i,j) = \begin{cases} D(i,j-1) \\ D(i+1,j) \\ D(i+1,j-1) + w(i,j) \\ for\ _{i<k<j}\ do\ D(i,k) + D(k+1,j) \end{cases}$$

D(1,10) =

D(1,9) = 3 ≠ 4 ✖

D(2,10) = 4 = 4 ✓

D(2,9) + w(1,10) = 4 = 4 ✓

k={2,3,4,5,6,7,8,9}
D(1,2)+D(3,10) = 3
D(1,3)+D(4,10) = 4 ✓
D(1,4)+D(5,10) = 3
D(1,5)+D(6,10) = 4 ✓
D(1,6)+D(7,10) = 4 ✓
D(1,7)+D(8,10) = 3
D(1,8)+D(9,10) = 2
D(1,9)+D(10,10) = 3

	j	1	2	3	4	5	6	7	8	9	10
i		A	C	G	G	C	A	A	C	G	U
1	A	0	0	1	1	2	2	2	2	3	4
2	C	0	0	1	1	2	2	2	2	3	4
3	G		0	0	0	1	1	1	2	2	3
4	G			0	0	1	1	1	1	2	3
5	C				0	0	0	0	0	1	2
6	A					0	0	0	0	1	2
7	A						0	0	0	1	2
8	C							0	0	1	1
9	G								0	0	0
10	U									0	0

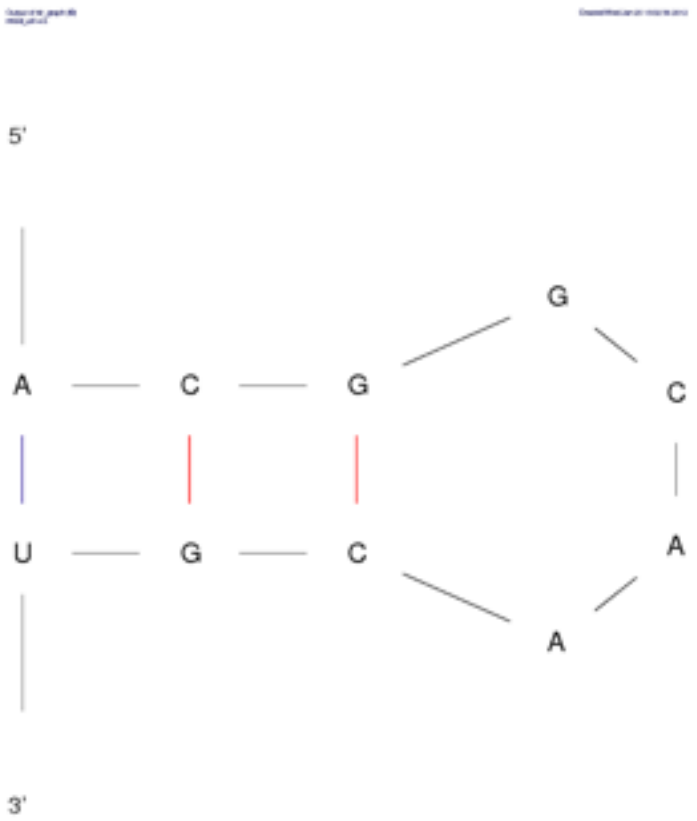
One Possible Structure With 4 Base-Pairs

ACGGCAACGU
((((()..))))
but |j-1| > 1
(((....)))

	j	1	2	3	4	5	6	7	8	9	10
i		A	C	G	G	C	A	A	C	G	U
1	A	0	0	1	1	2	2	2	2	3	4
2	C	0	0	1	1	2	2	2	2	3	4
3	G		0	0	0	1	1	1	2	2	3
4	G			0	0	1	1	1	1	2	3
5	C				0	0	0	0	0	1	2
6	A					0	0	0	0	1	2
7	A						0	0	0	1	2
8	C							0	0	1	1
9	G								0	0	0
10	U									0	0

One Possible Structure With 4 Base-Pairs

ACGGCAACGU
((((()..)))
but |j-1| > 1
(((....)))



	j	1	2	3	4	5	6	7	8	9	10
i		A	C	G	G	C	A	A	C	G	U
1	A	0	0	1	1	2	2	2	2	3	4
2	C	0	0	1	1	2	2	2	2	3	4
3	G		0	0	0	1	1	1	2	2	3
4	G			0	0	1	1	1	1	2	3
5	C				0	0	0	0	0	1	2
6	A					0	0	0	0	1	2
7	A						0	0	0	1	2
8	C							0	0	1	1
9	G								0	0	0
10	U									0	0

Online Java Tool Using the Nussinov Algorithm

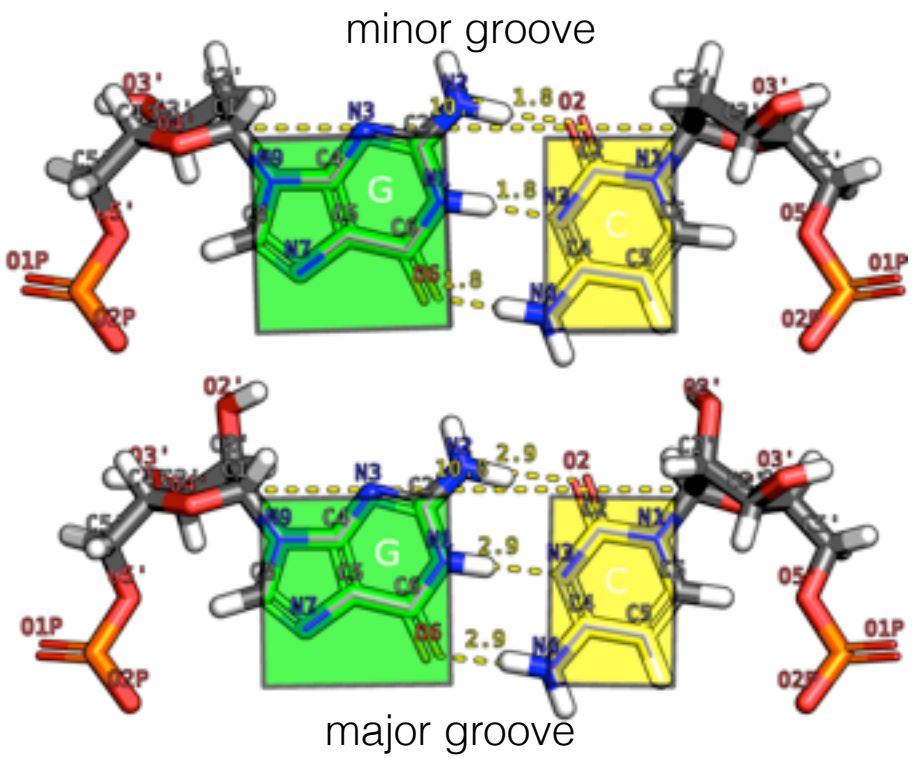
Nussinov algorithm - Ultrastudio.org

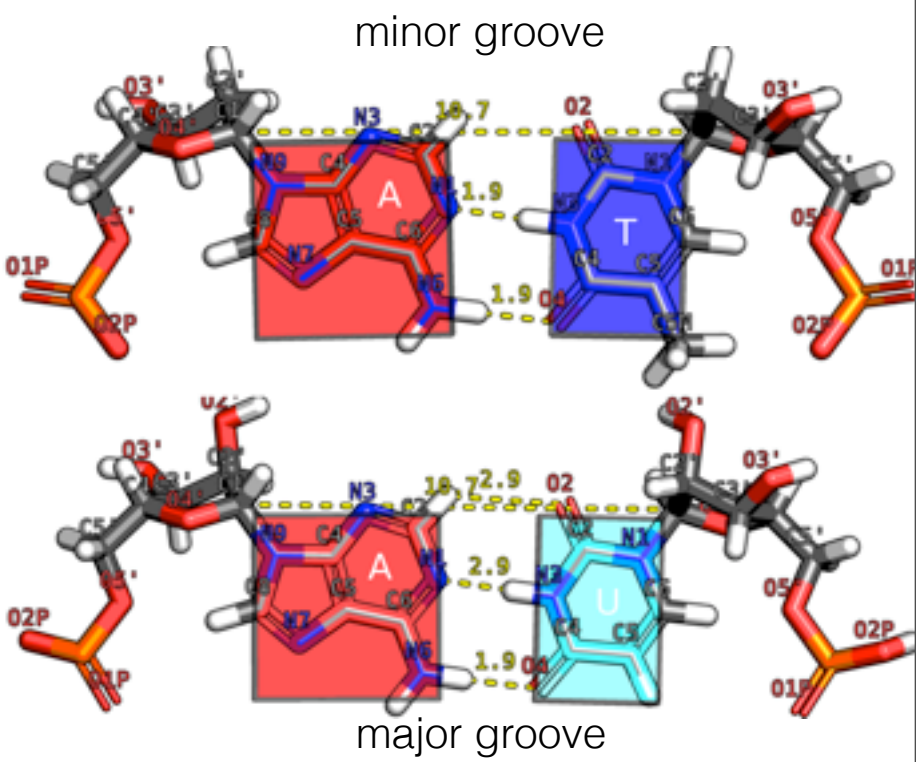
Visualization of Secondary Structure

- Standard
- Circular
- Dot Plot

RNA 3D STRUCTURE

NDB/PBD Color Convention

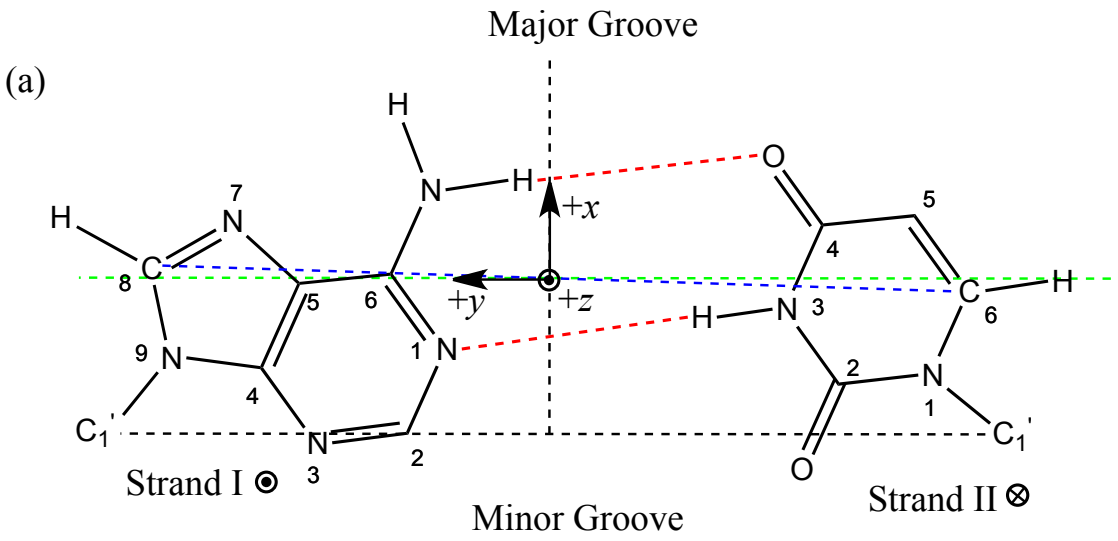




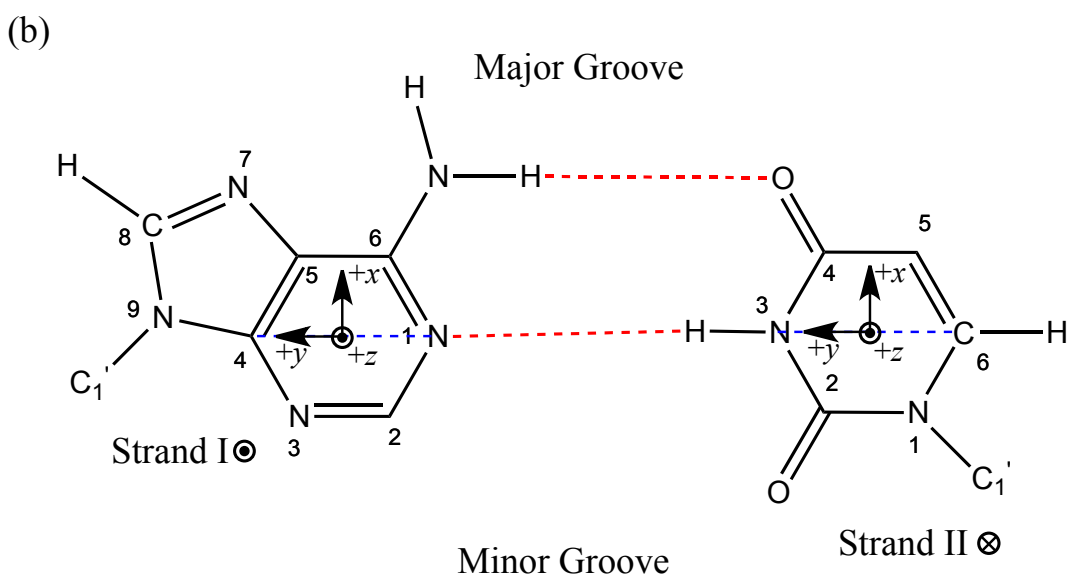
Purines aka **R** = Guanine (green) and Adenine (red)
Pyrimidines aka **Y** = Citosine (yellow), Thymine (blue) and Uracil (cyan)

Standard Reference Frame

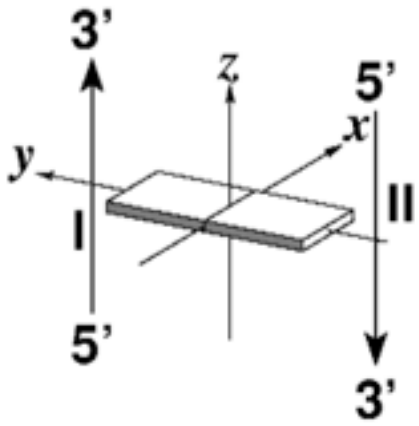
new
3DNA, Curves+
1999-2001



old
SCHNAaP
1997

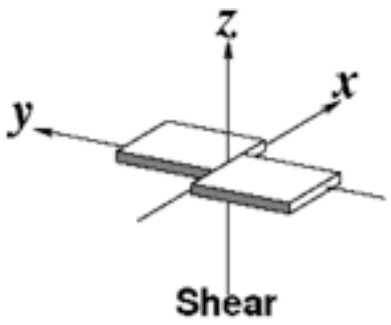


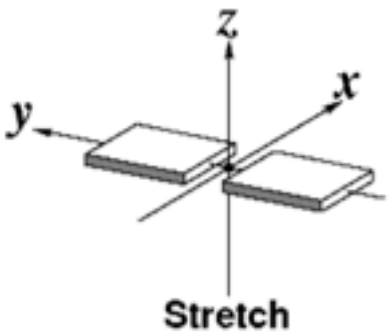
Calladine-Drew Rigid Block Models (Base-Pairs)

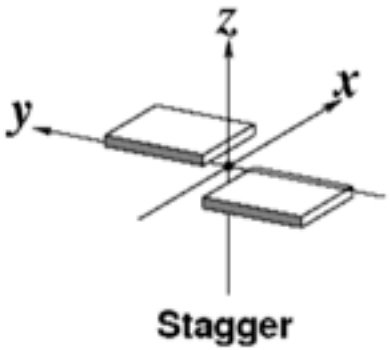


translation

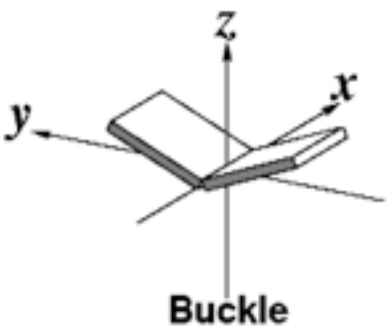
Base-Pair Parameters

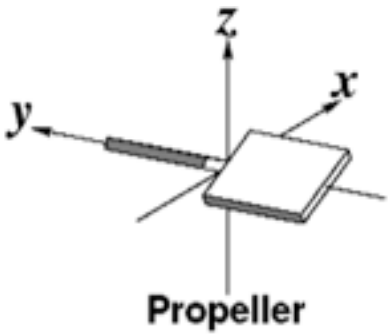


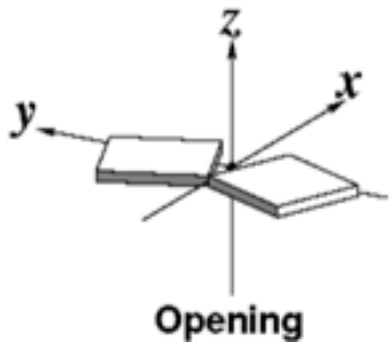




rotation

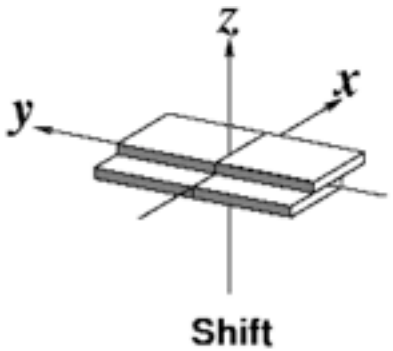




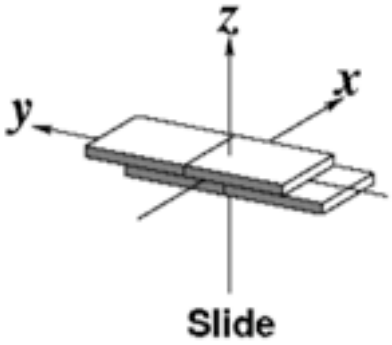


Rigid Block Model Base-Pair Steps

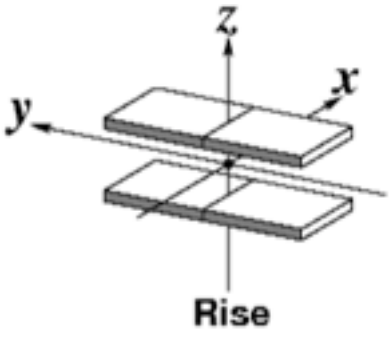
Base-Pair-Step Parameters



Shift

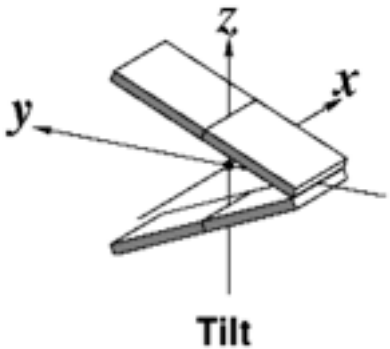


Slide

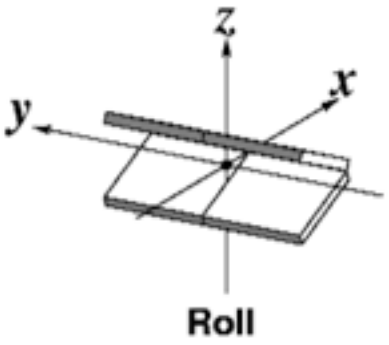


Rise

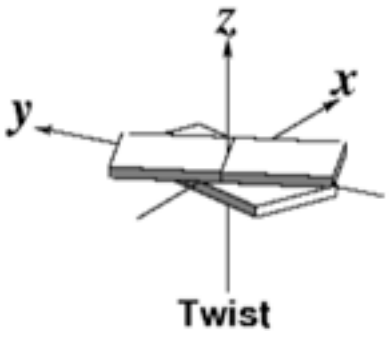
translation



Tilt



Roll



Twist

rotation

Software to Compute Rigid-Body Parameters

NEW

- 3DNA (Xiang-Jun Lu @ Columbia, Bussemaker lab.)
  - http://w3dna.rutgers.edu
  - http://rutchem.rutgers.edu/~xiangjun/3DNA
- 3dnaV2, UNIX preferred (linux, OS-X, freebsd, cygwin "yikes!")
- Curves+ (Richard Lavery @ Universite d' Lyon)

OLD

- FREEHELIX, RNA, SCHNAaP, SCHNArP, compDNA, NUPARM

What's a base-pair in 3DNA? (In misc_3dna.pra)

- The distance between the origins of the two bases (as defined by their standard reference frames) must be less than certain limit (15.0 Å by default) - otherwise, they would be too far away to be called a pair.
- The vertical separation (i.e., stagger) between the two bases must be less than certain limit (2.5 Å by default) - otherwise, they would be stacking instead of pairing.
- The angle between the two base z-axes (i.e., their normal vectors) is less than a cut-off (65.0° by default).
- There is at least one pair of nitrogen/oxygen base atoms that are within a H-bonding cut off distance (4.0 Å by default).

- Make same as previous slide but with cartoons.

Base-Pair Databases

- http://bps.rutgers.edu for RNA.
- http://rnasteps.rutgers.edu for RNA Steps.
- http://3dnascapes.rutgers.edu for DNA.

Classic Papers on Secondary Structure

- Classics
  - Tinoco - Uhlenbeck Nature 1971
- Books
  - Tinoco's Book
  - Bioinformatics Book Chapter by Mathews
- Online
- Recent Trends
- People

RNA Secondary Structure Prediction Groups

- Doug Turner University of Rochester (Turner Rules)
- David Mathews University of Rochester (Dynalign)
- Michael Zuker Rensselaer Polytechnic Institute (mfold)
- Ivo Hoffacker Wien (vienna)
- Peter Stadler Leipzig (vienna)
- Francois Major Montreal (mc-sym)
- Tamar Schlick NYU (graph-grammars)
- Ruth Nussinov Tel Aviv (1978 dynamic algorithm)
- Nacho Tinoco (1971 free-energy minimization)

Overview

PART I (Secondary Structure)

- Introduction
  - Motivation.
  - Levels of RNA Structure?
  - Secondary Structure Prediction
  - Representation
- Software
  - Prediction (mfold, vienna, dynalign)
  - Rendering (Annotation s2s, others.)
- Everyones Favorite FIKA!

Overview

PART II (Rigid Block Model for Nucleic Acids)

- NDB/PDB Color Conventions.
- 1991 Tsukuba - Standard Reference Frame.
- Calladine-Drew Rigid Block Model.
- Software (3DNA(ansi-C), Curves+(fortran))

Overview

PART III (Practical Workshop-Tutorial)

- Practical Examples.
  - Using paper and pencil alone do Nussinov algorithm to some sequence. This gives you some structure, not necessarily minimal energy I guess.
  - Use computer to go to mfold, or vienna webserver to do prediction for same sequence. (maybe mfold is better because it gives more cartoons)
  - Make A-DNA, B-DNA, Z-DNA. Chromosomal DNA.
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