Nucleotides

Mostly RNA

- complement RNA course
- more DNA in sequence context
- RNA does more biochemistry
  - RNAzymes, regulators

Assumed

- you remember
  - ACGT in DNA
  - ACGU in RNA
- always write from 5’ to 3’

Roles of molecules

	RNA	DNA	proteins
genetic information	X	X	
structure	usually single stranded	duplex	lots
regulation/interactions	X	X	X
ligand binding / catalysis	X		X

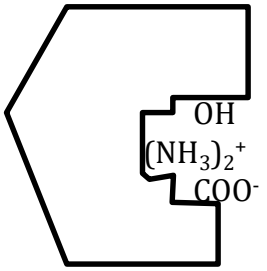
If RNA does all this interesting chemistry

- it has interesting structure

How do proteins work ?

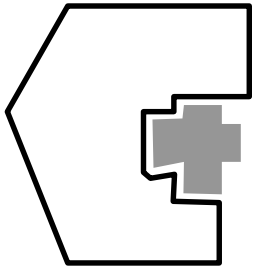
Some site decorated with special groups

+ / -, neutral, polar / non-polar, big / small



Chemical choice ?

- 20 kinds of amino acid
- half a dozen really different types

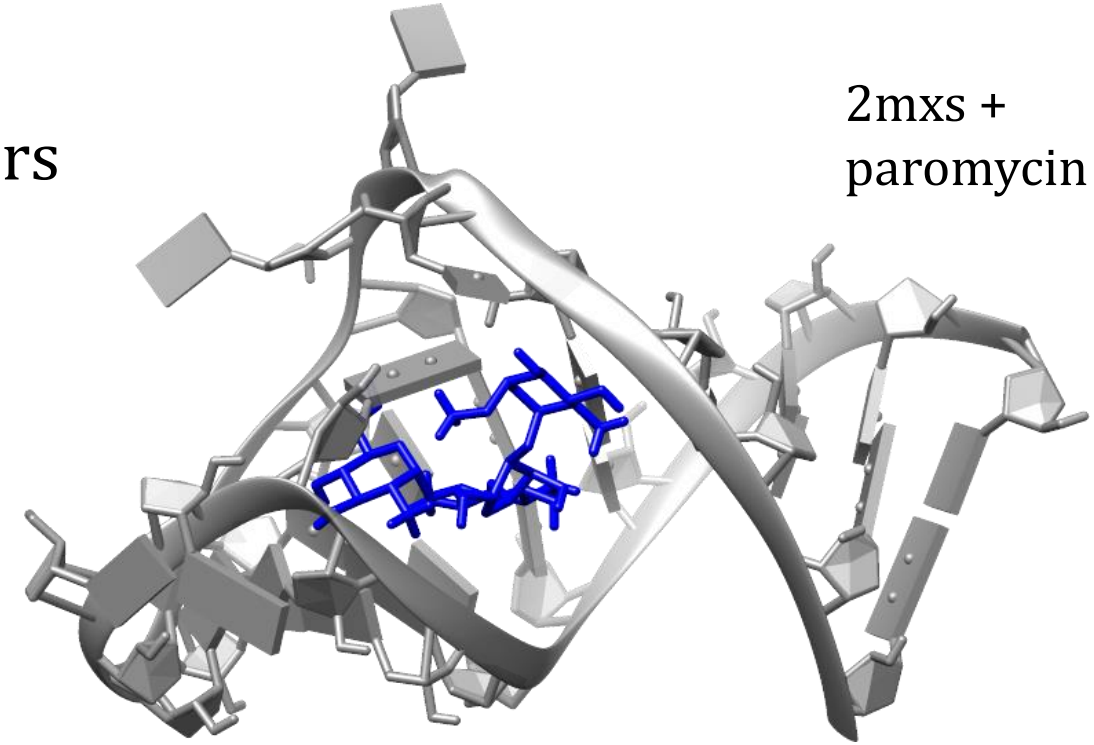


Do you see this with nucleotides ?

RNA binding ligands ?

Examples

- riboswitches / regulators
- catalysts



2mxs + paromycin

Two consequences

1. RNA must fold to certain shape
2. Exposed chemical groups give specificity / strength

Do not see this much with DNA

Structures / type of molecule

Protein

- specific structure depends on sequence
- sometimes floppy – not structured

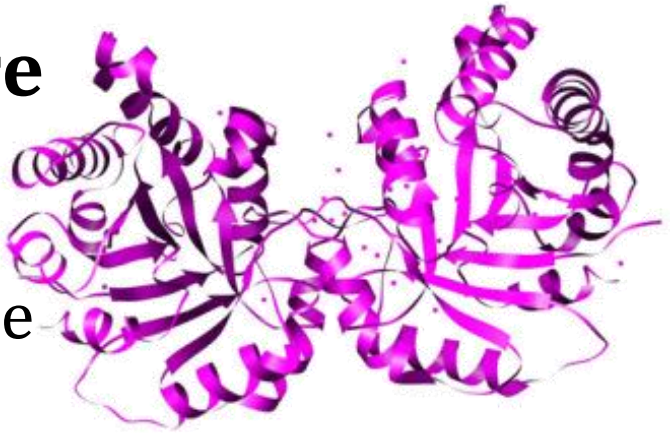
DNA

- double helix

RNA

- do they fold to nice, well-defined shape ?
  - all RNA ?
  - all biologically-interesting RNA ?
  - some ?

Views of structure

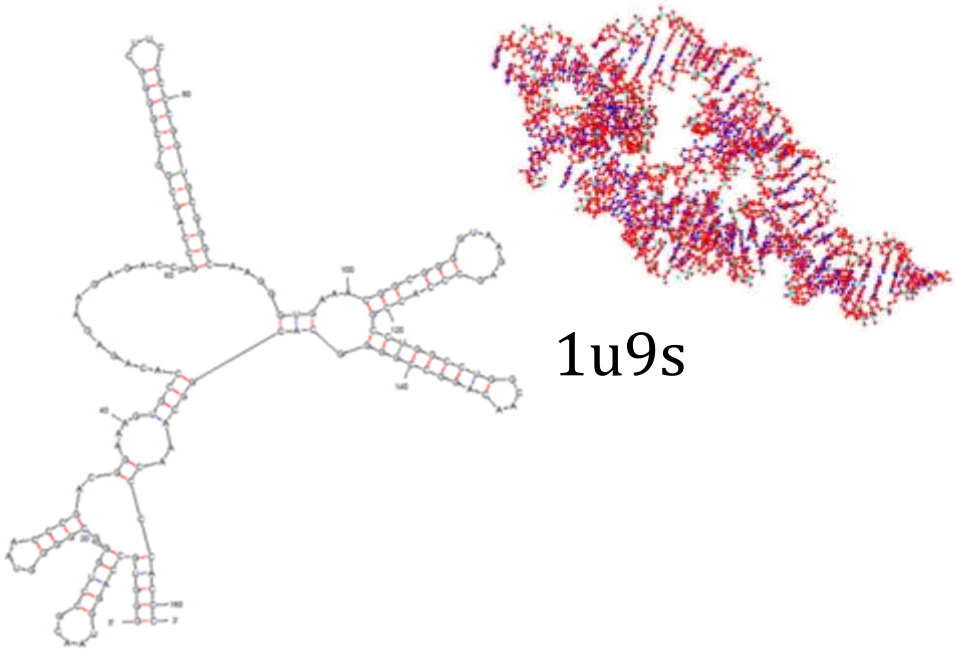


Proteins

- usually 3D – rarely secondary structure

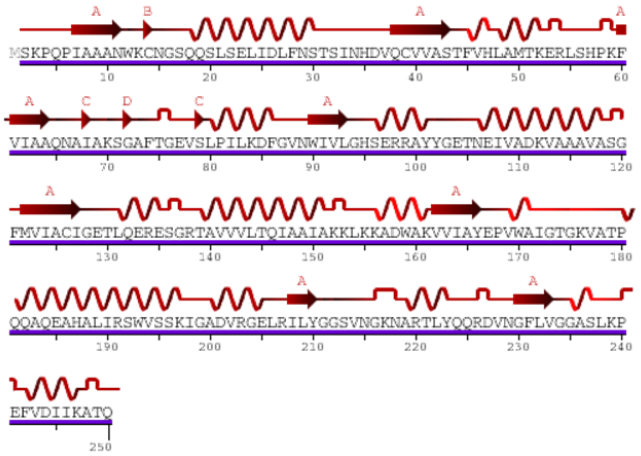
RNA

- usually 2D – less 3D information



1u9s

3tim



RNA – how much information ?

Proteins

- 1.4×10^5 or about 3×10^4 interesting ones

RNA

- 4.2×10^3 structures with some RNA
- 1443 with pure RNA - many small and boring
- 485 pure RNA ≥ 40 bases (really less - lots of redundancy)

Why so few RNA structures ?

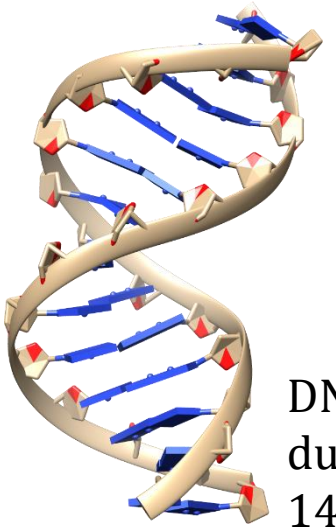
- RNA hard to handle (RNases)
- crystallography
- NMR
  - assignments very difficult (only 4 kinds of base)

Features of RNA

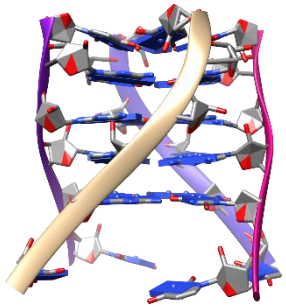
What dominates literature ?

- base pairing

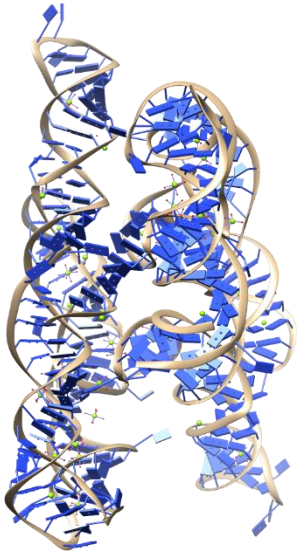
Need more interactions to explain all these shapes



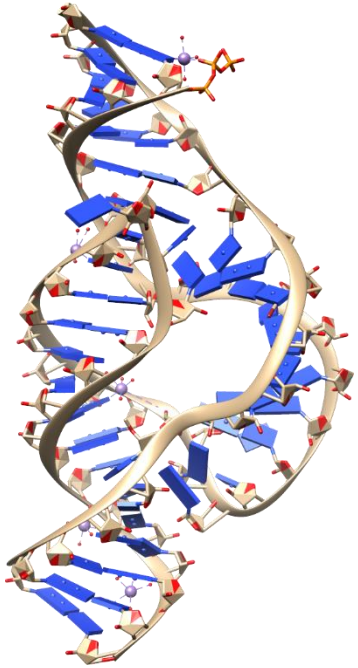
DNA
duplex
140D



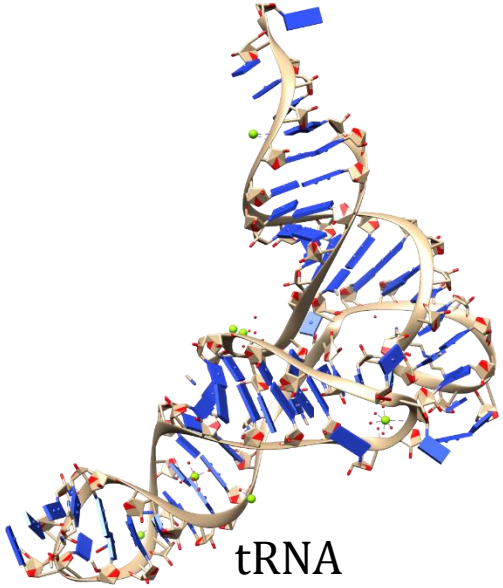
tetraplex
1mdg



group I intron
1hr2



hammerhead
2oeu



tRNA
1evv

Important for RNA structures

Energies ?

- As in previous lectures
- bonds, bond angles, torsion angles
- non-bonded (electrostatics, van der Waals)

Details coming ..

- H-bonds
- charges
- stacking

Is my description consistent ?

- H-bonds/charges/stacking vs electrostatics/van der Waals

non-bonded terms / convenience

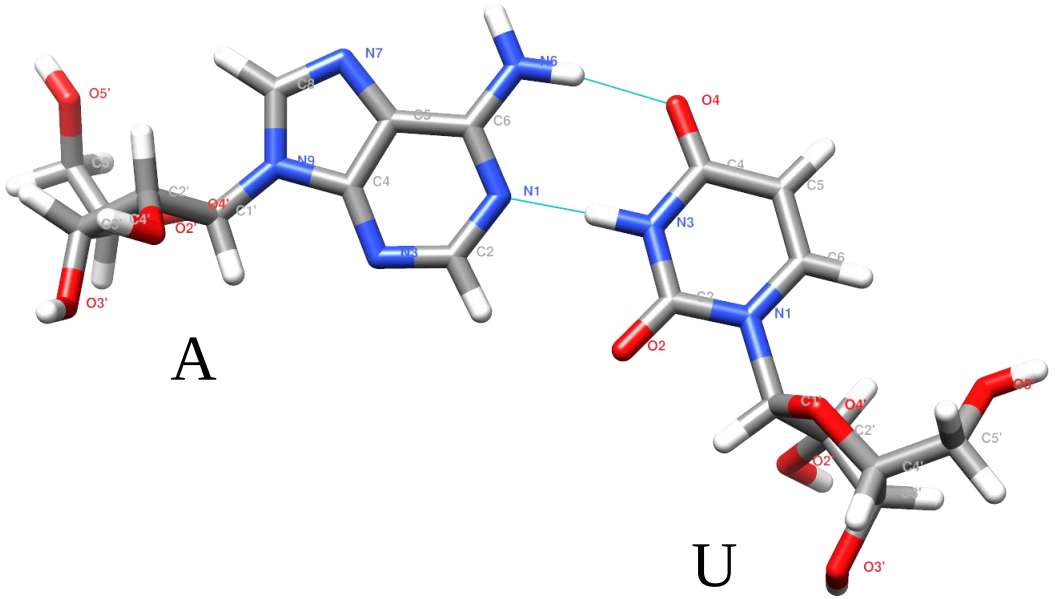
Physics not changed

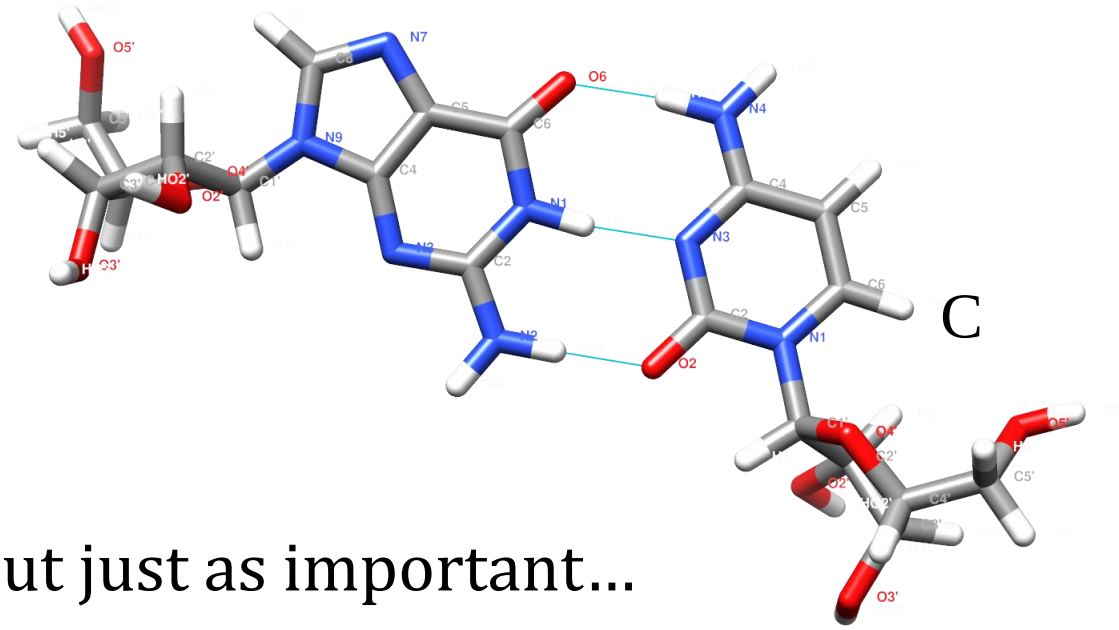
- convenient to talk in terms of H-bonds, charges and stacking

interaction	physics	relevance
H-bonds	van der Waals electrostatics	base-pairing + bit more
charges	electrostatics	backbone
stacking	van der Waals	bases

Base-pairing / H-bonds

Historic



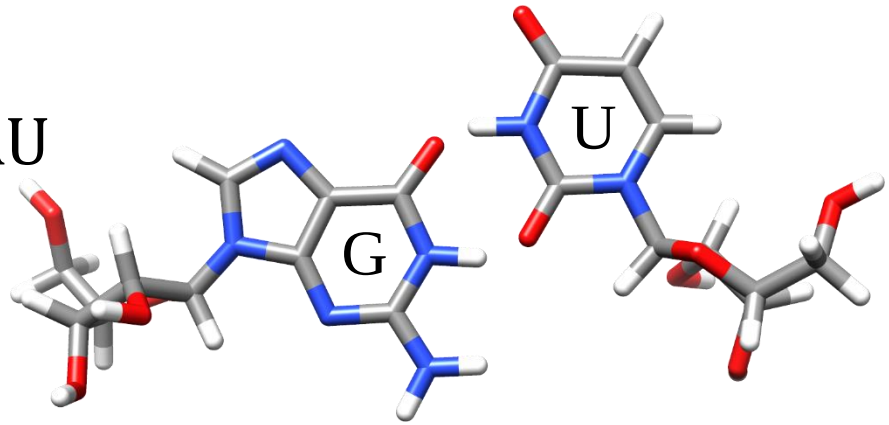


but just as important…

H-bonds wobble pairs

GU

- strength very comparable to AU



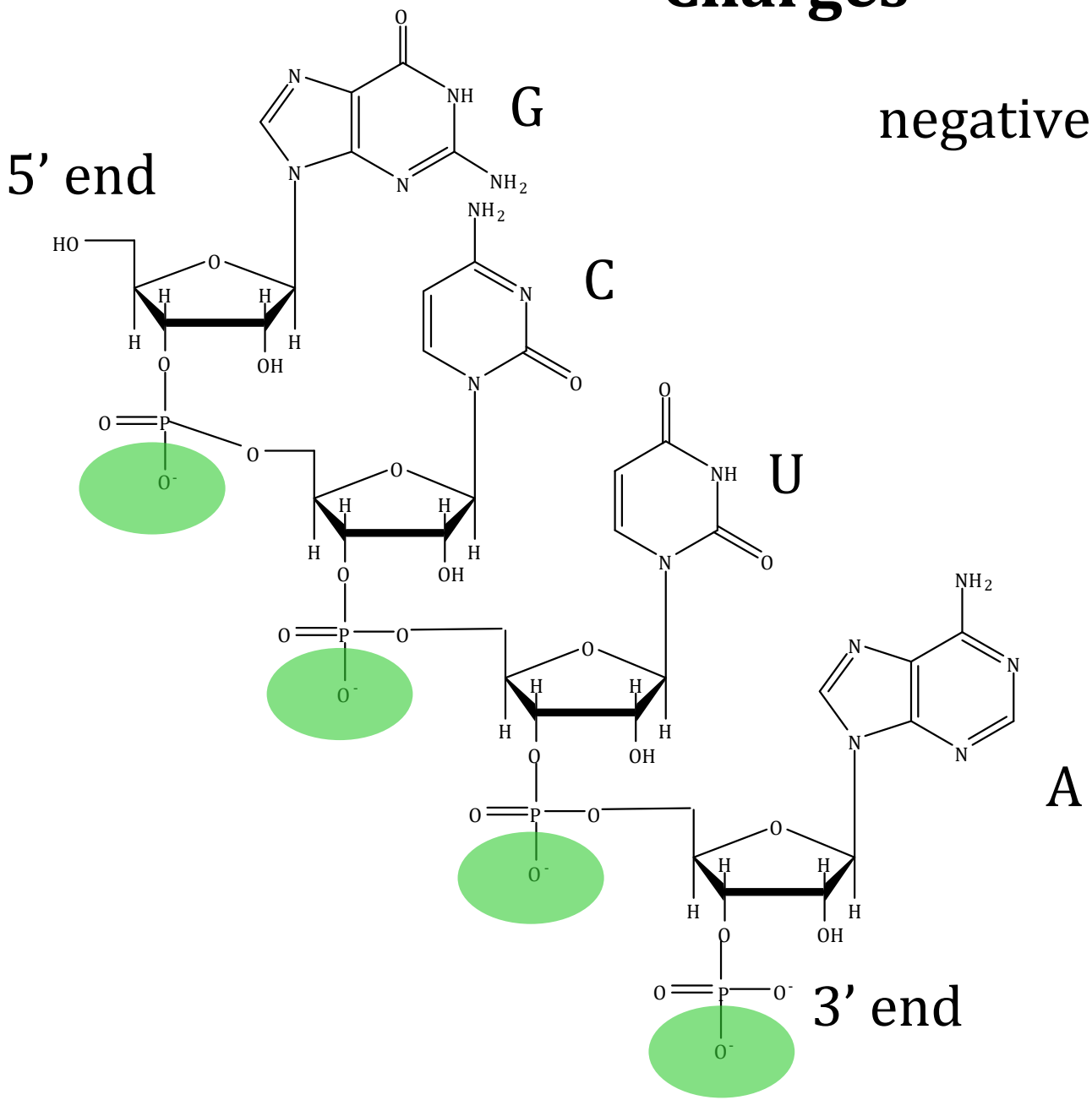
Compare with DNA

- mismatches – very rare

More generally

- count the H-bond donors and acceptors
- many H-bond possibilities
  - not limited to bases

Charges

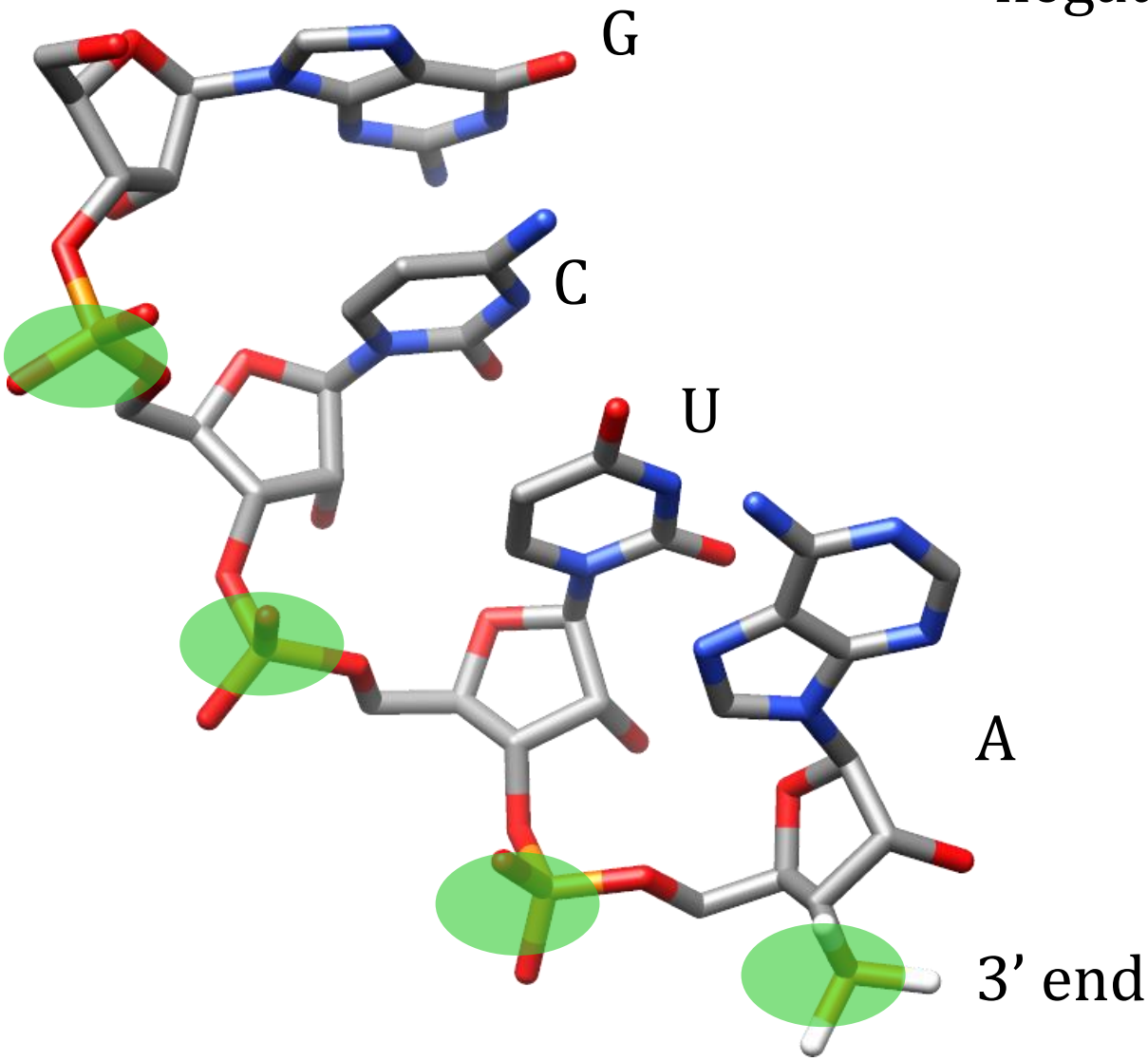


negative charges

Charges

5’ end

negative charges



Charges

Contrast with proteins

- mostly neutral, some charged residues

RNA and DNA

- full negative charge every base (at backbone)

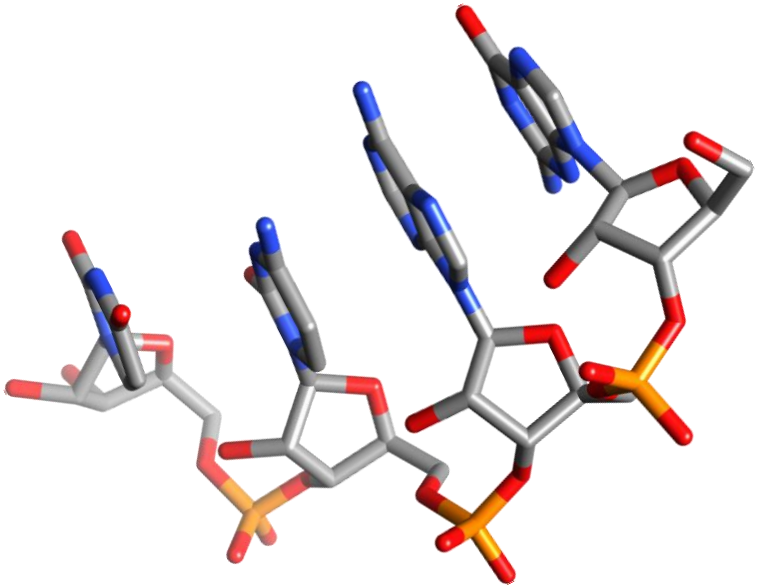
Consequences

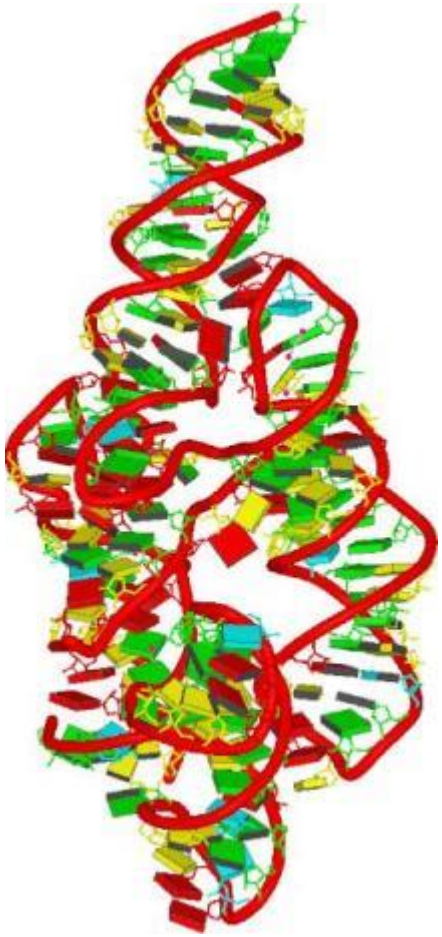
- strong interaction with
  - solvent
  - +ve ions
- shape of backbone
  - move PO_4^- away from each other

Stacking

Bases are large aromatic systems

Very strong preference to form stacks





Representation / storing 3D structures

Proteins – conventions and simplifications

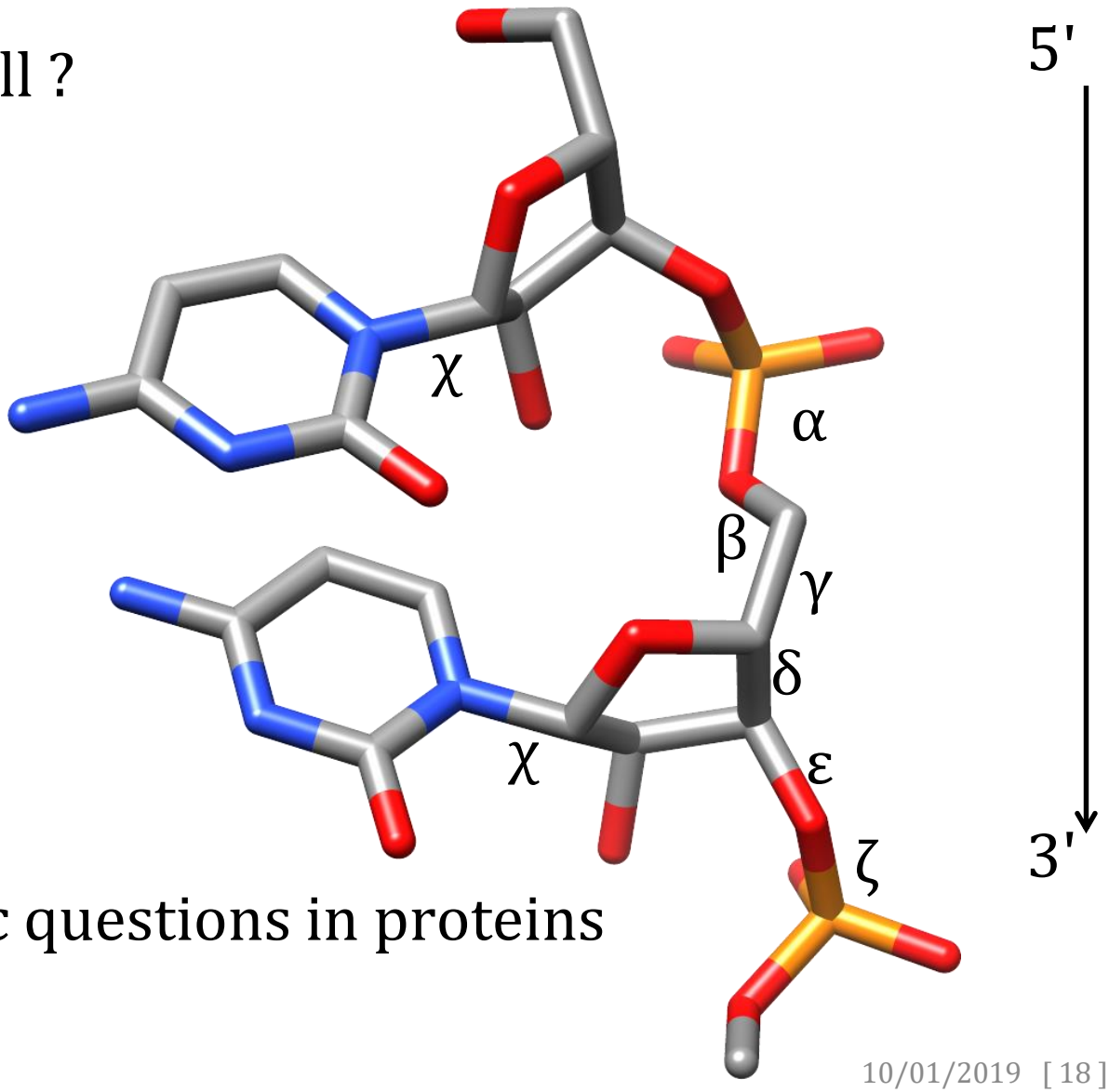
- diagrams – ribbon plots
- break into secondary structure and loops
- represent as a set of C^{α} atoms
- Ramachandran $/\phi,\psi$ plots

RNA - similar ideas ?

RNA – no Ramachandran plot

Many angles

- do we need them all ?



Two issues

- restricted freedom
  consider δ
- correlations
  - partly like steric questions in proteins

Use less than 6 angles

We do not need 6 independent descriptors (angles)

- want to simplify
  - for communication
  - calculations / storage

Easy – but no agreed scheme

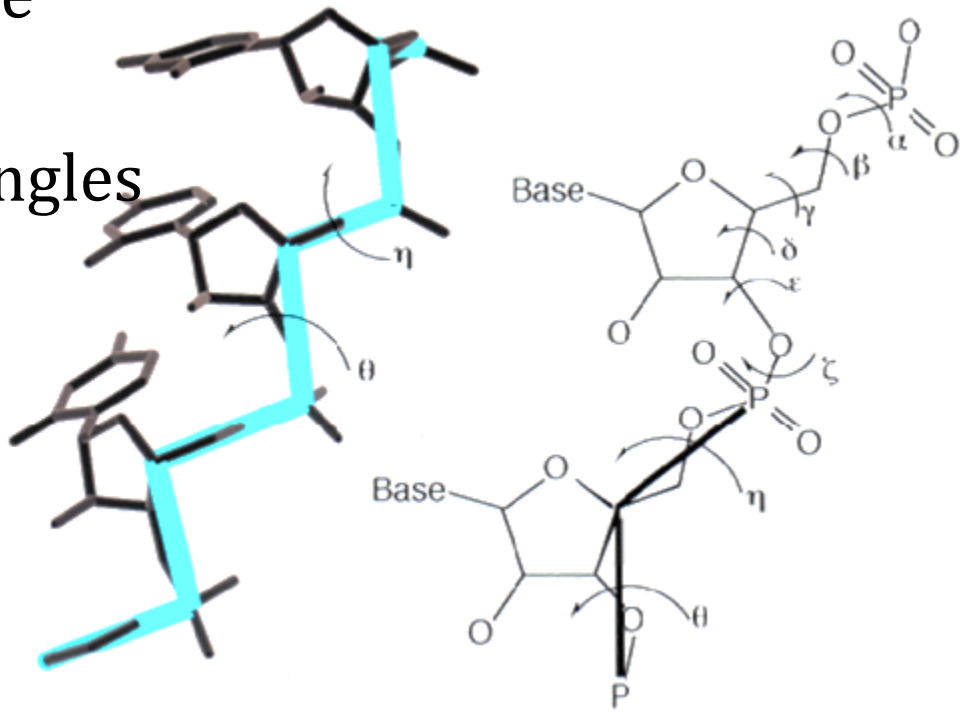
- a proposal

Torsion angles

Use atoms that are not bonded to each other

Basic idea

- pick 4 atoms that are not sequential
- define a simplified backbone
  - P-C_4-P-C_4-P-C_4-…
- leads to "pseudo-torsion" angles



η

$C4_{n-1}$-P_n-$C4_n$-P_{n+1}

θ

P_n-$C4_n$-P_{n+1}-$C4_{n+1}$

Duarte, CM & Pyle, AM, (1998) J. Mol. Biol 284, 1465-1478

End of structure introductions

- Nucleotide history dominated by base-pairing
- single-stranded RNA folds into shapes like an enzyme / receptor
- Energies - we use simplifications
- Must be more than just base-pairing
- Representations - not as nice as for proteins

Remember everything for next topic

- predicting secondary structure

RNA structure, predictions

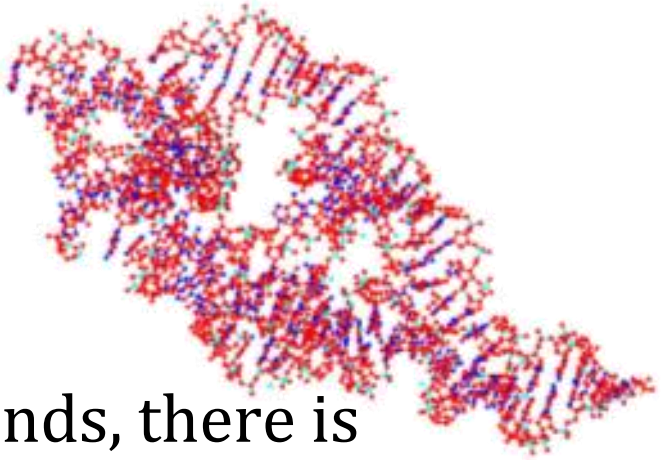
Themes

- 2D, 3D
- structure predictions
- energies
- kinetics

Structure – protein vs RNA

Middle of proteins

- hydrophobic core - soup of insoluble side chains



Middle of RNA

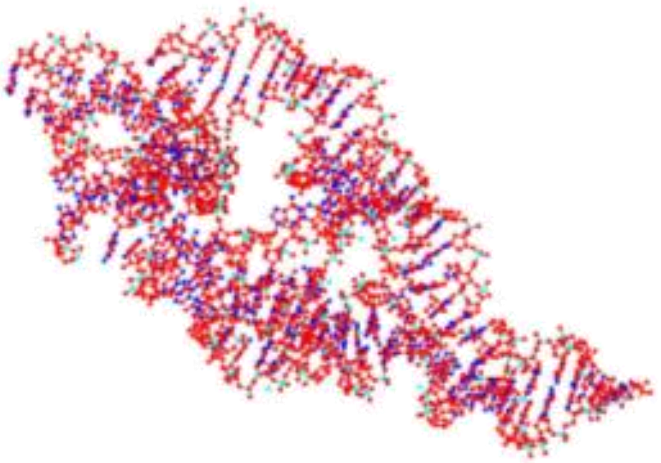
- base-pairing / H-bonds
- much more soluble
  - if something wants to forms H-bonds, there is competition from water

Protein structure lectures are not helpful today

RNA – how important is 3D structure ?

Binding of ligands (riboswitches, ribozymes)

- totally dependent on 3D shape - where functional groups are in space

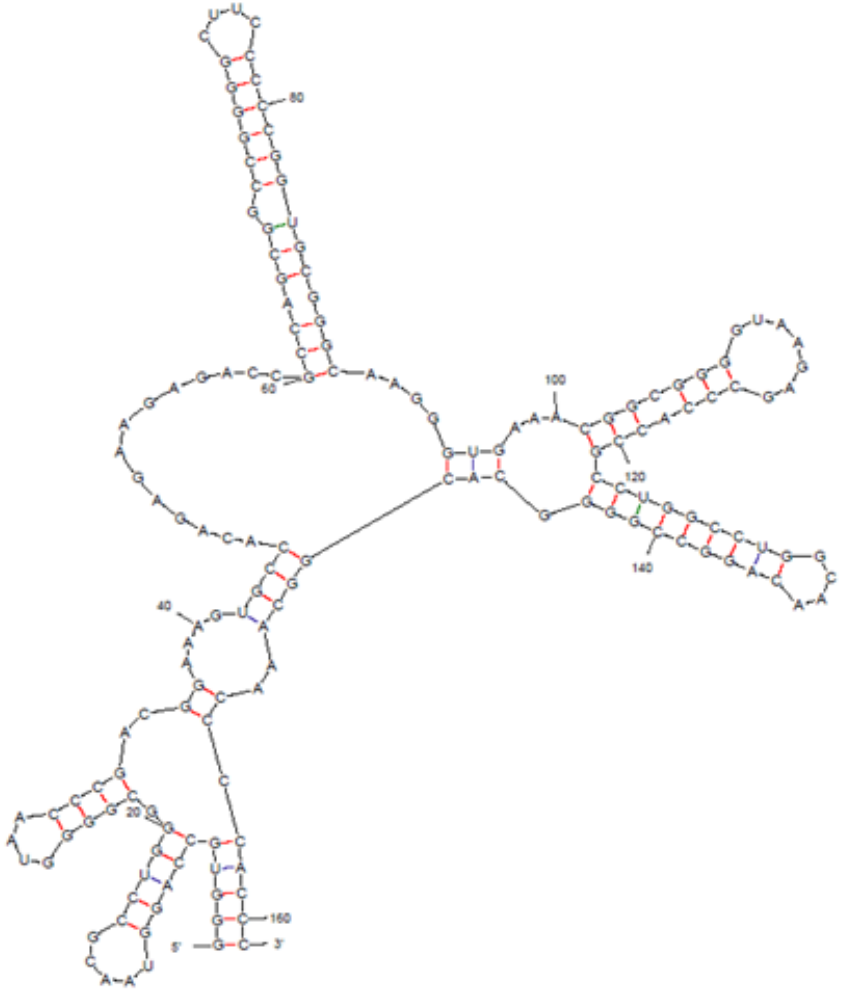


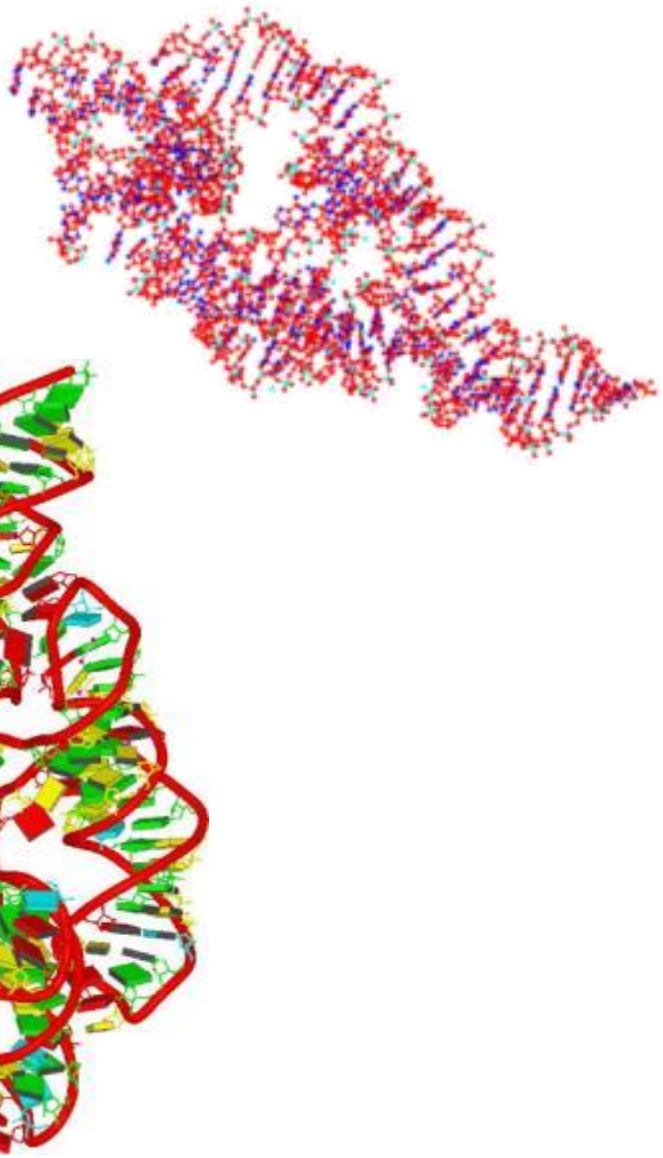
What do we do ?

- mostly ignore it

How realistic is 2D ? How relevant ?

3D versus 2D



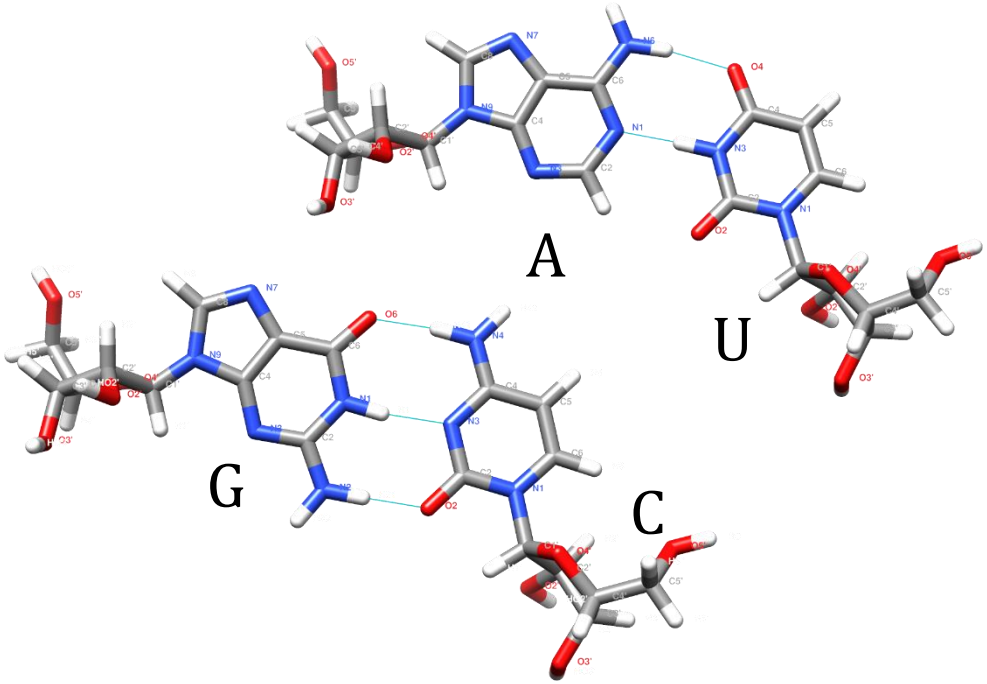


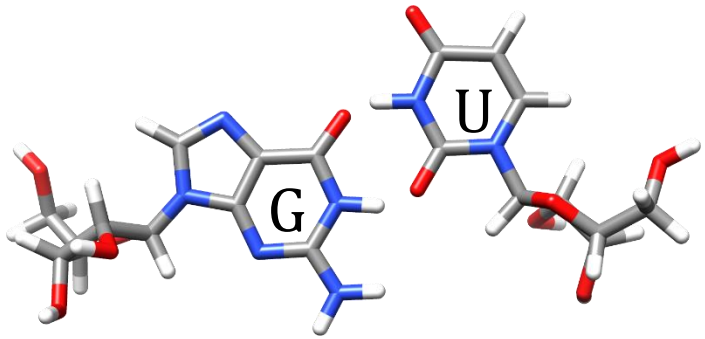
PDB acquisition code 1u9s

2D why of interest ?

1. (a) computationally tractable (fügsam / machbar)
   (b) can be checked by experiment (SHAPE)

2. historic – belief that nucleotides are
   dominated by base pairs + helices (classic and wobble)

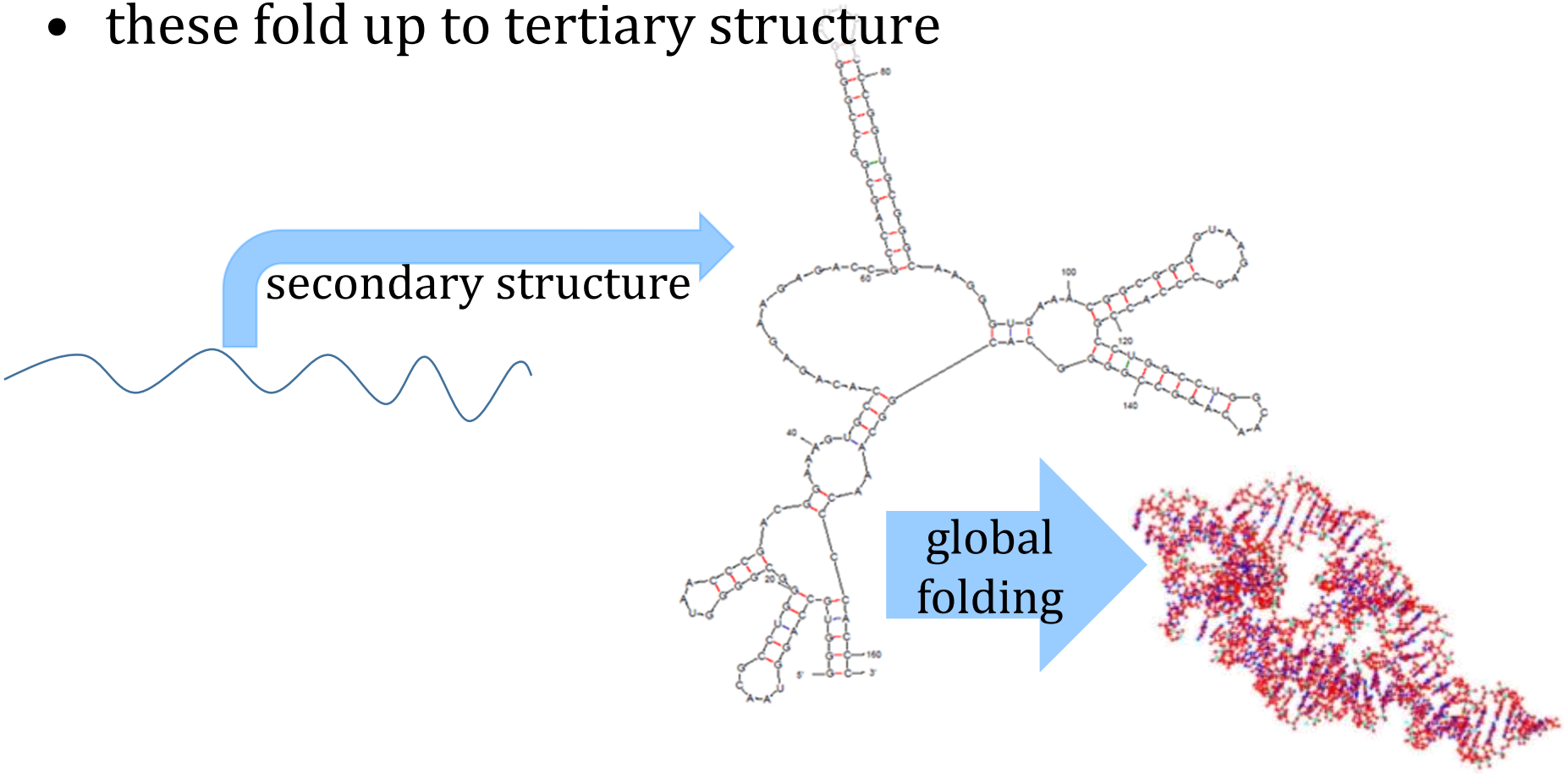




2D why of interest ?

3. Claim - RNA folds hierarchically

- secondary structure forms from bases near in sequence
- these fold up to tertiary structure



2D why of interest ?

3. Claim - RNA folds hierarchically

Contrary evidence in protein world

- isolated α-helices and β-strands are not stable in solution

Plausible in RNA world ?

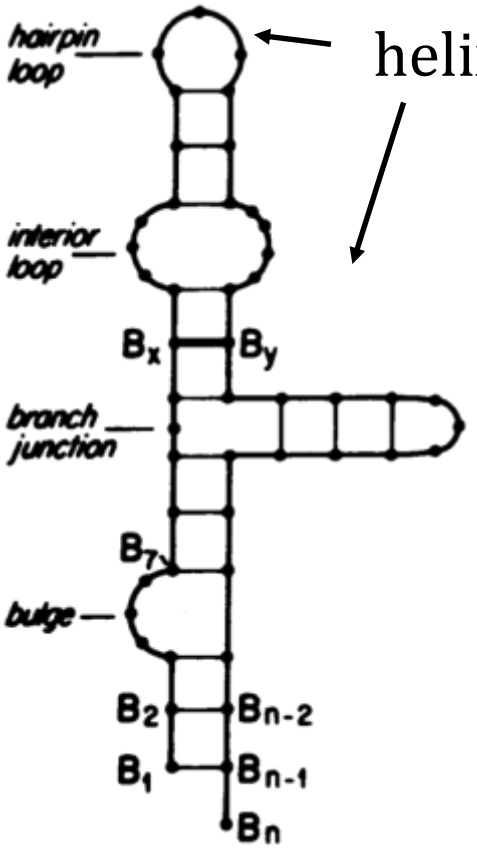
- RNA double strand helices are believed to be stable

Useful ? if true

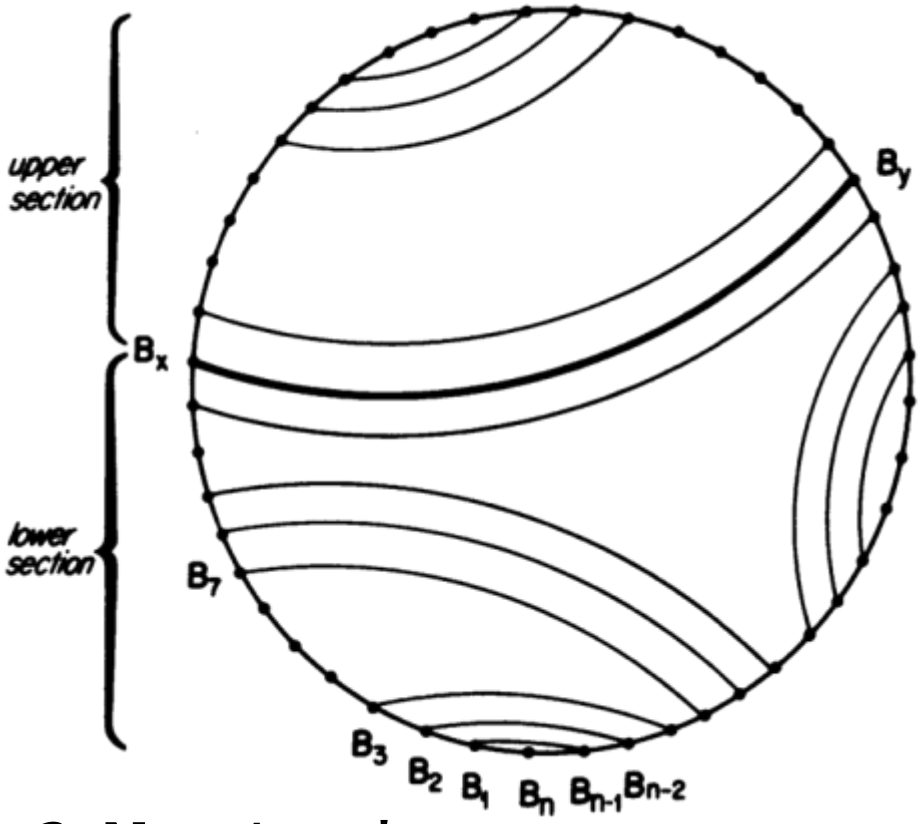
- 2D (H-bond pattern) prediction is the first step to full structure prediction

Four representations of flat RNA

1. conventional



+ on next slide

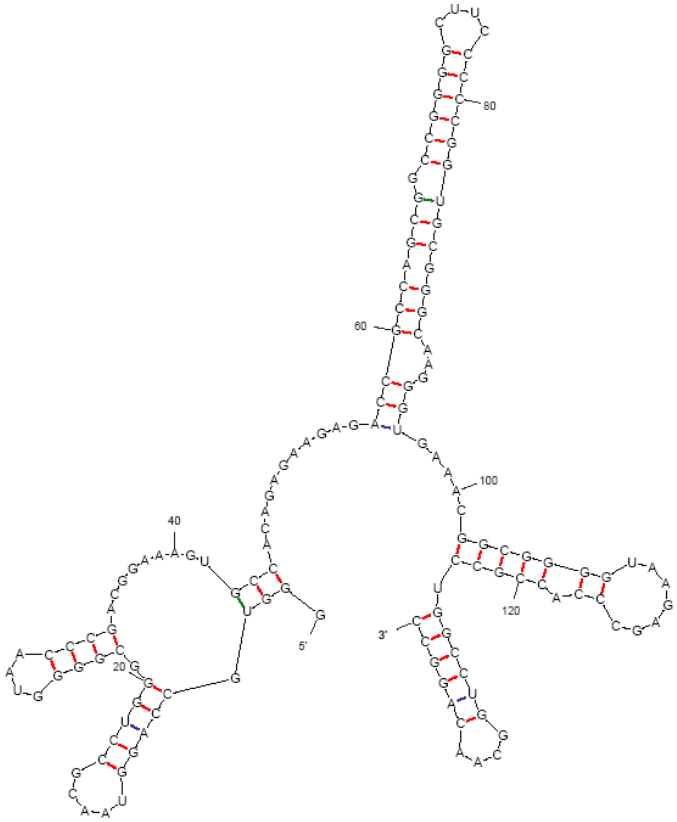


2. Nussinov's

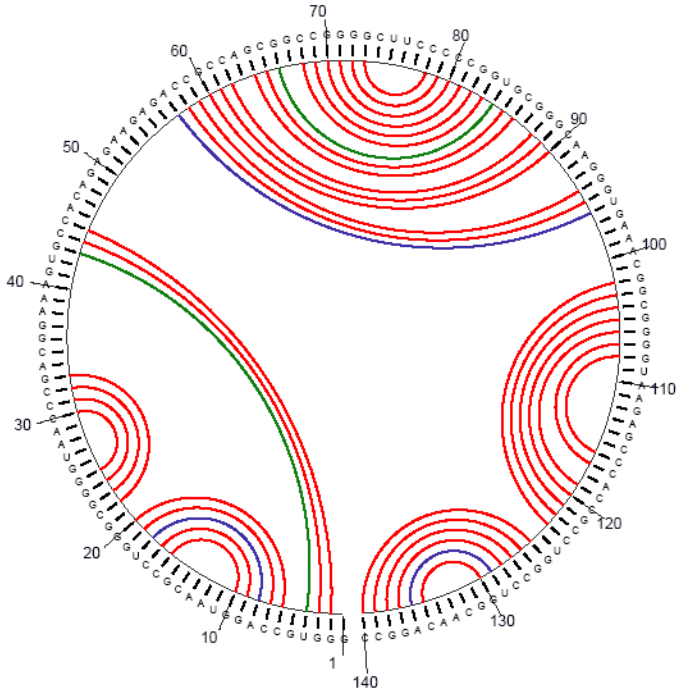
- write down bases on circle
- arcs (lines) may not cross

from Nussinov, R., Jacobson, A.B. Proc. Nati. Acad. Sci. USA, 77, 6309-6313(1980)

Four representations of flat RNA



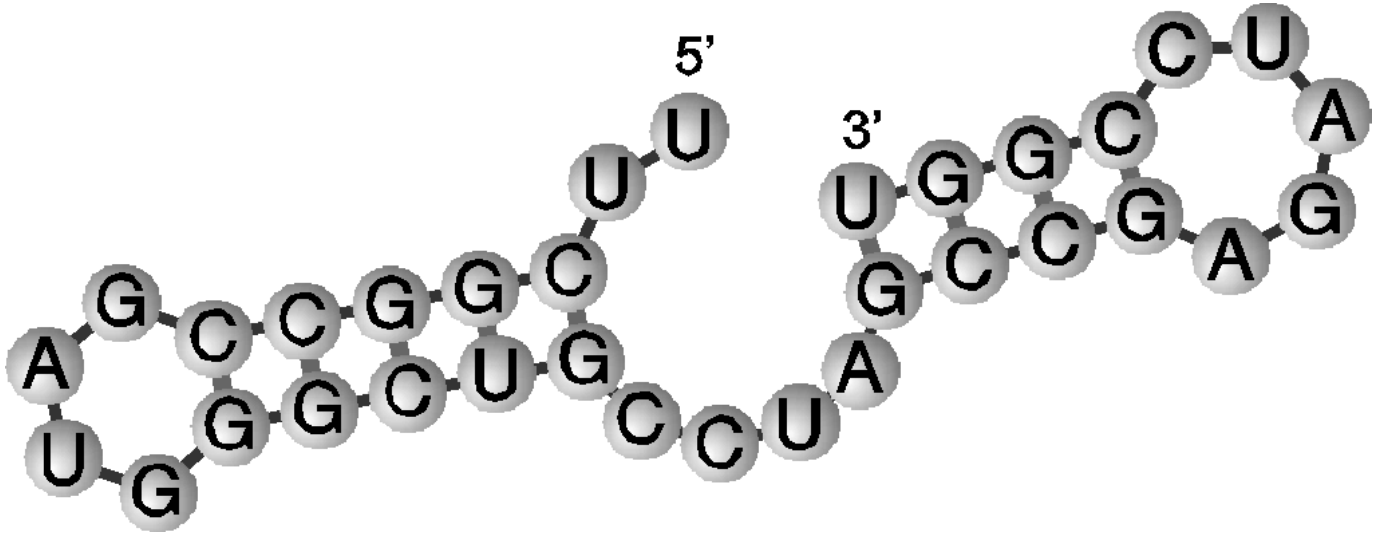
1. conventional representation



2. Nussinov's circle

Same features on both plots

Parentheses



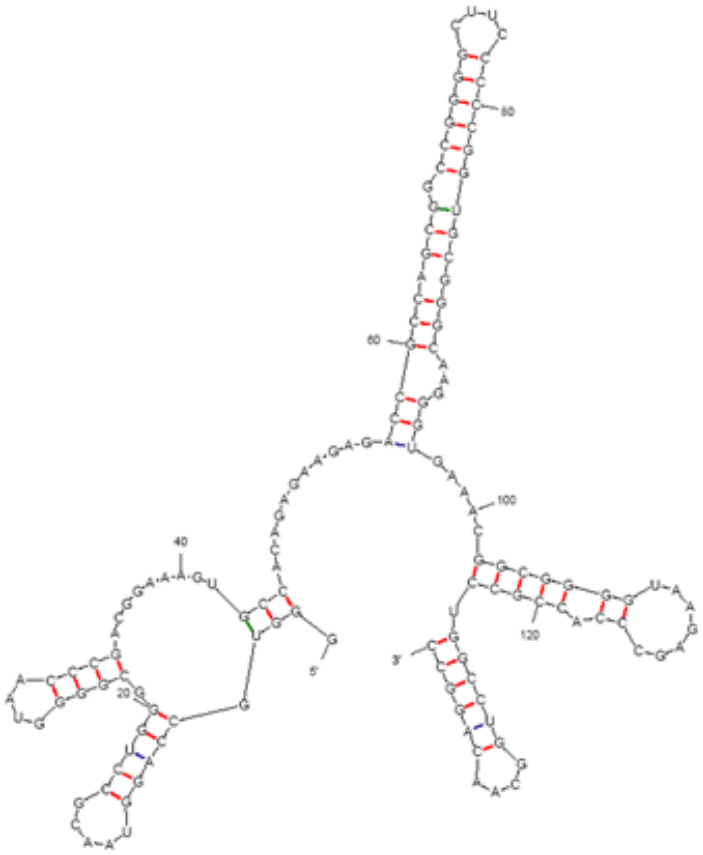
3. parentheses – most concise

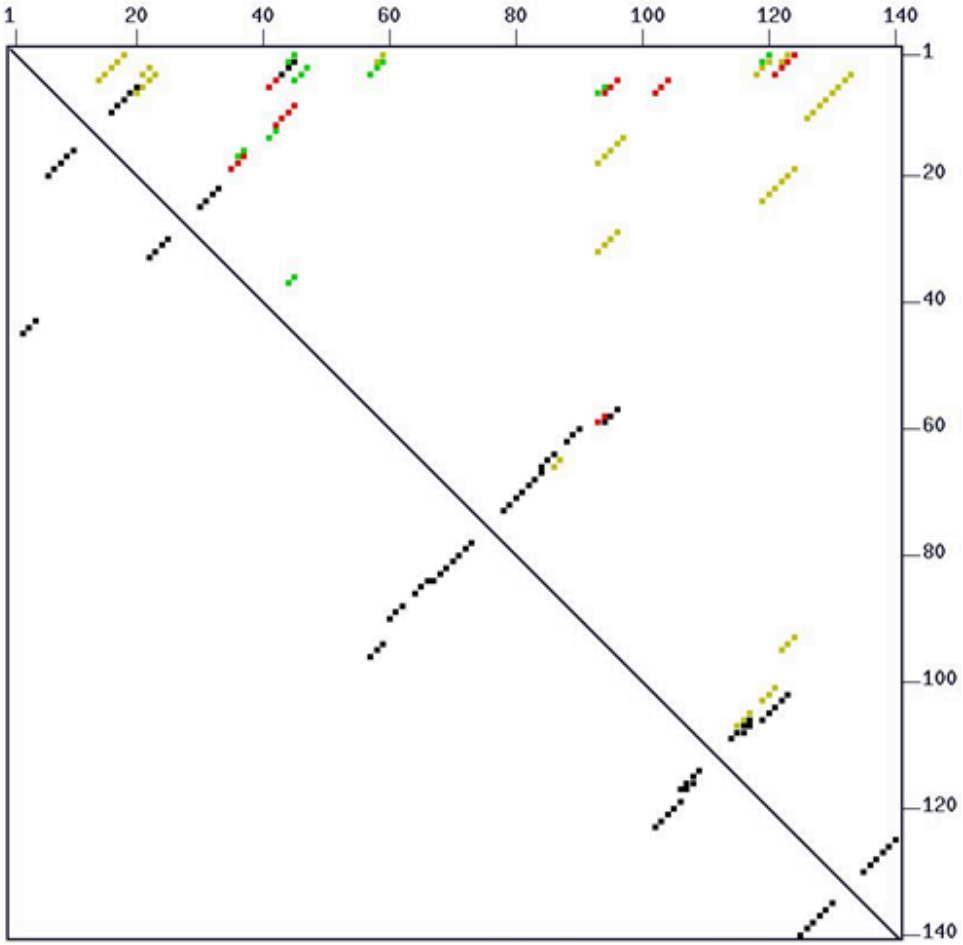
. . (((((. . . .))))) . . . . ((((. . . . .))))

- can be directly translated to picture
- easily parsed by machine (not people)

from Schuster, P., Rep. Prog. Phys. 69 (2006) 1419–1477

Dot plots





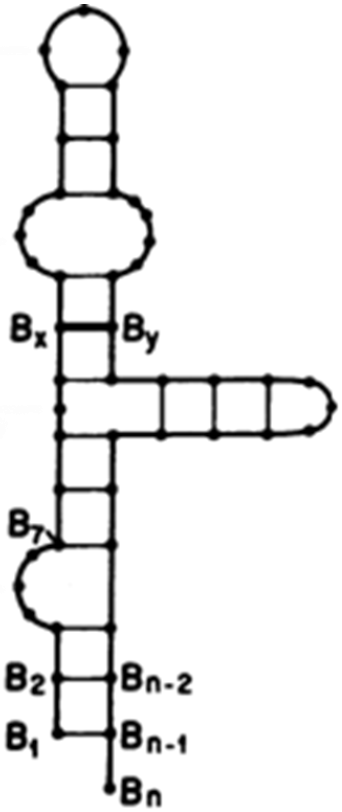
4. Dot plots

Same features in both plots

- look for long helix 57-97, bulges in long helix
- probabilities (upper right) – remember for later

made with mfold server

nomenclature / features

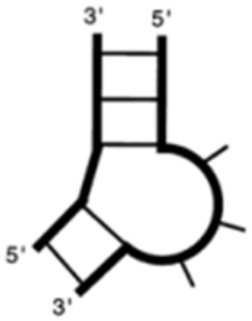


single strand

A-form double helix

Double helix with 5'-dangling end

single nucleotide bulge



three nucleotide bulge

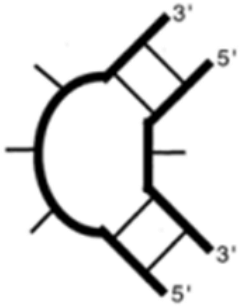
hairpin loop

For explanations later

- hairpin loop
- bulge (unpaired bases)

mismatch pair
or, symmetric internal loop of 2 nucleotides

symmetric internal loop



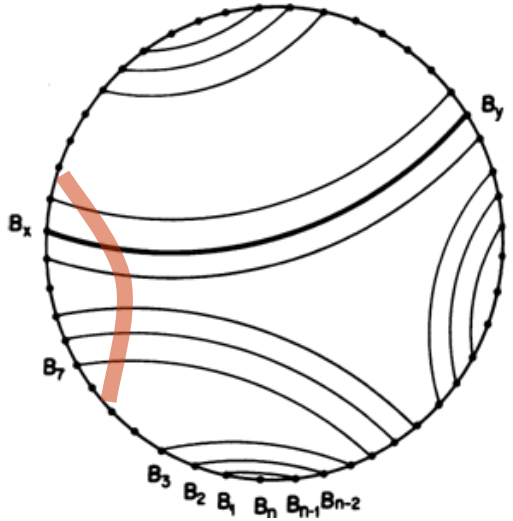
asymmetric internal loop

Nussinov, R., Jacobson, A.B. Proc. Nati. Acad. Sci. USA, 77, 6309-6313(1980)
Burkard, M.E., Turner, D.H., Tinoco Jr., I., in The RNA World, 2nd Edn, eds Gesteland, RF, Atkins, JF Cold Spring Harbor Laboratory Press (1999)

2D – properties and limitations

Declare crossing base pairs illegal

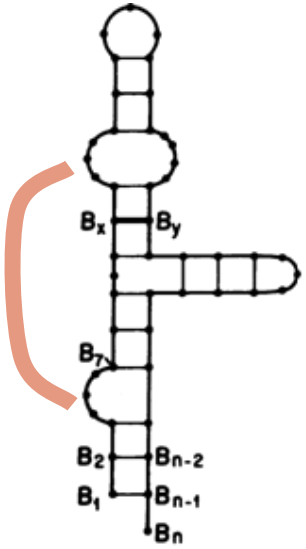
- think of parentheses
- discussed later



What do energies depend on ? (for now)

- just the identity of the partners
- 2 or 3 types of interaction
  - GC, AU, GU

What is the best structure for a sequence ?



Predicting secondary structure

How many structures are possible for n bases ?

$$cn^{3/2}d^n$$

for some constants c and d

- exponential growth (d^n)

Problem can be solved

- restriction on allowed structures
- clever order of possibilities

Best 2D structure (secondary)

First scoring scheme :

- each base pair scores 1 (more complicated later)

Problem

- some set of base pairs exists – maximises score

Our approach

- what happens if we consider all hairpins ?
- what happens if we allow hairpins to split in two pieces ?

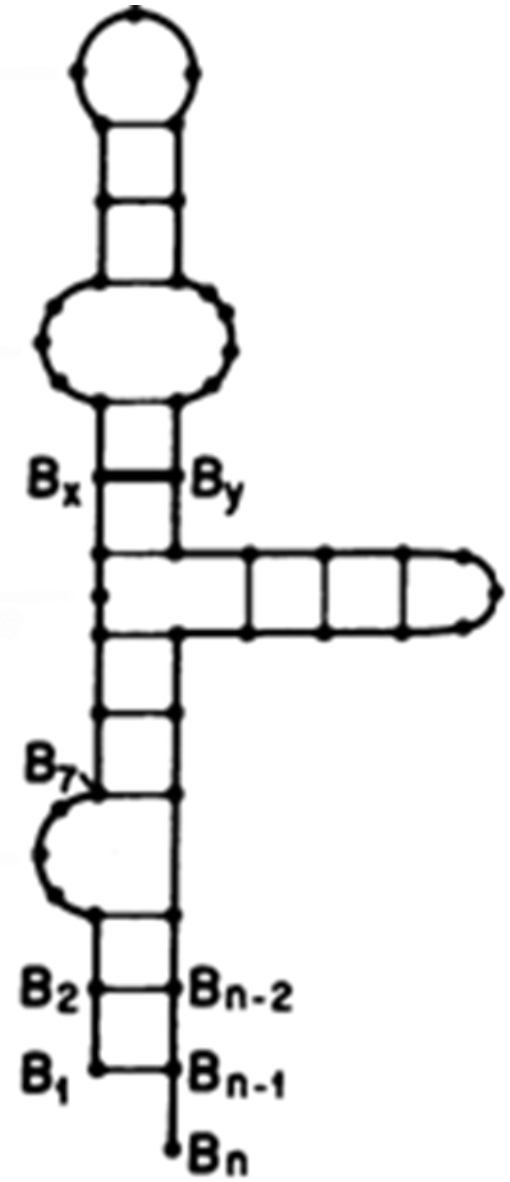
Philosophy

Structure is

- best set of hairpins (loops)
  - with bulges
    - loops within loops

Start by looking at scores one could have

- try extending each hairpin



hairpins / loops

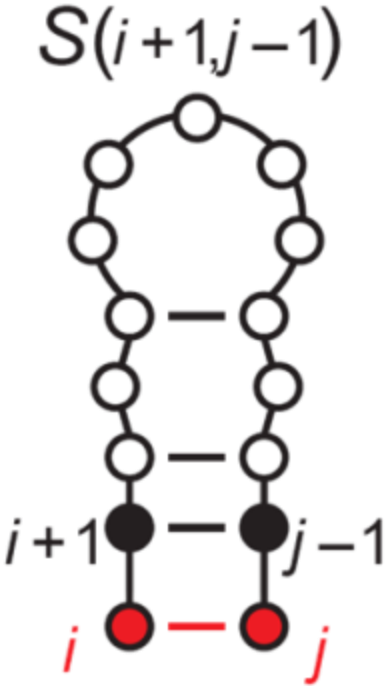
Start by looking for best possible hairpin

If we know the structure of the inner loop

- we can work out the next

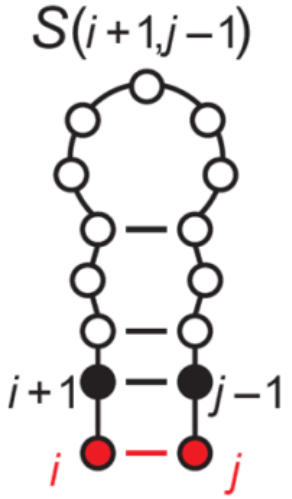
If we know the black parts

- we can decide what to do with the red ***i*** and ***j***



picture from Eddy, S.R. Nature Biotech 22, 909-911 (2004)

hairpins / loops

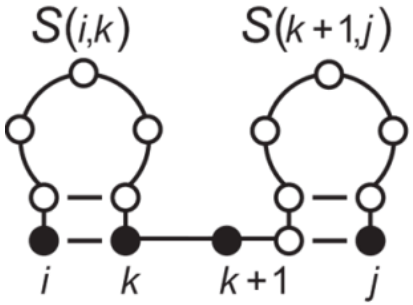


Important idea

- if I know the optimal inner loop
  try to extend it
- try to insert gaps - see if score is improved

Next important point

- walk along sequence 1..n see if score is better with two loops



Guarantees optimal solution, but…

picture from Eddy, S.R. Nature Biotech 22, 909-911 (2004)

Pseudoknots

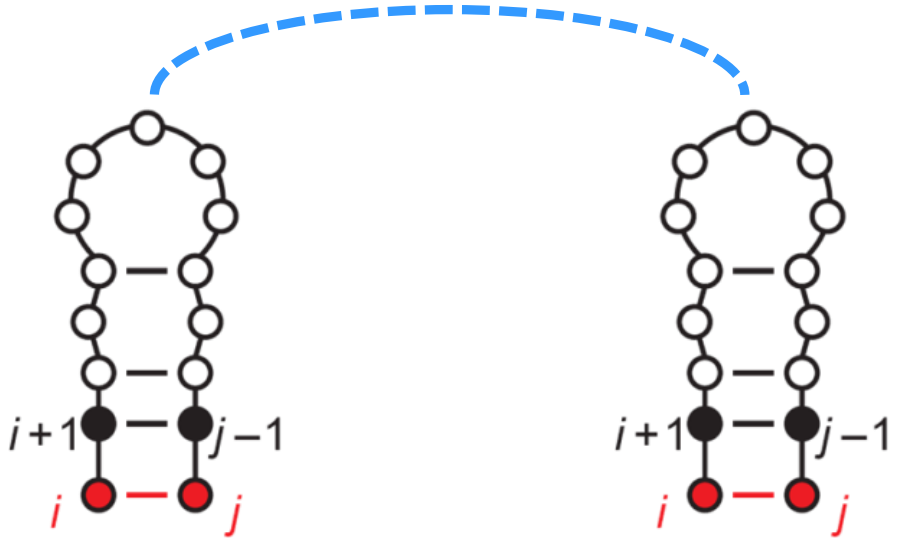
Have we considered .. ?

No !

Name – pseudoknot

Do we worry ?

- Stellingen – no
- here ? Probably.



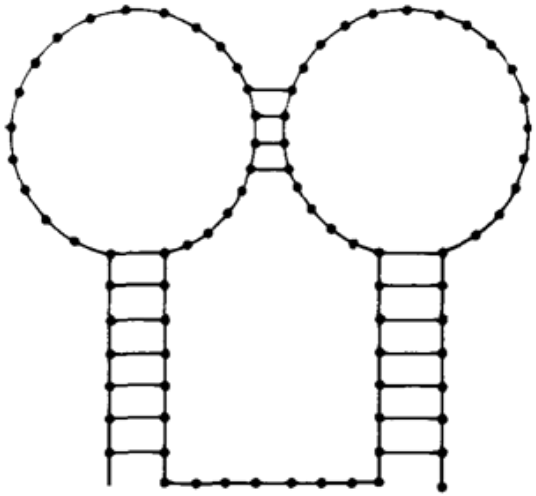
Pseudoknots

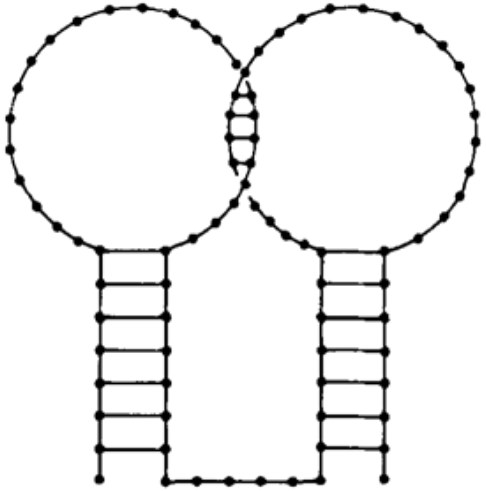
Pseudo-knot – not a knot

- why the name ?

Topologically like a knot

Would you expect them to occur ?





picture from Zuker & Sankoff, Bull. Math. Biol. 4, 591-621 (1984),
RNA secondary structures and their prediction

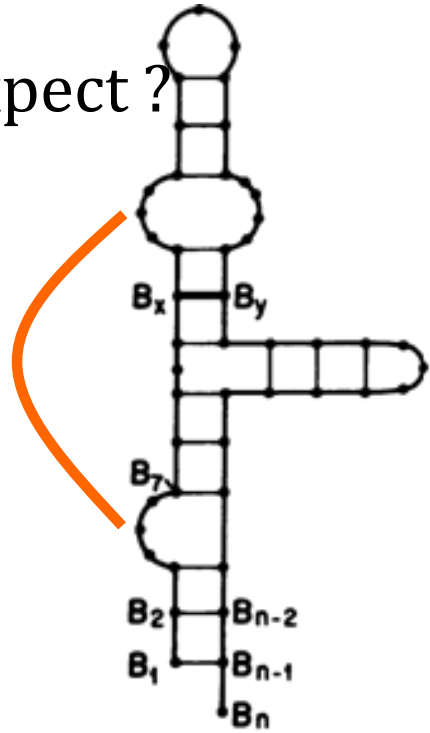
Pseudoknots

Given some unpaired bases, what would you expect ?

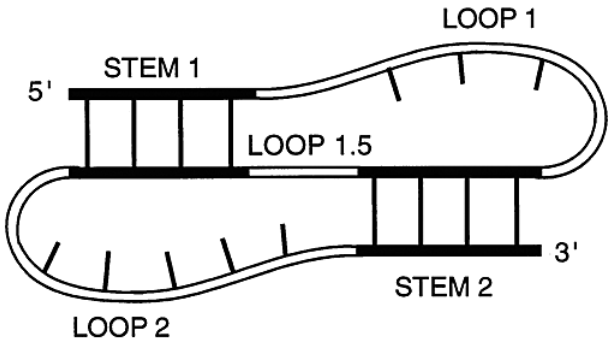
- solvate ?
- form more H-bonds ?
- pack bases against each other ?

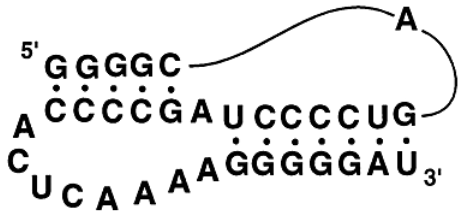
Cannot (practically) be predicted

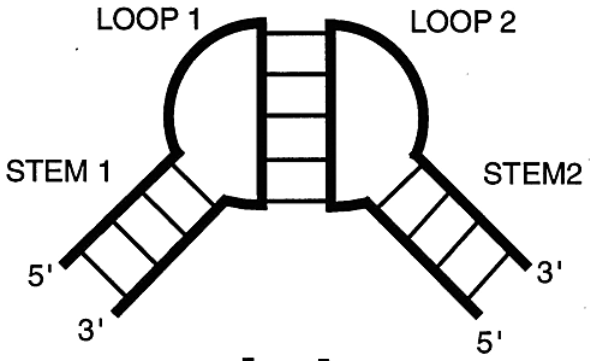
- order of steps in base-pairing methods

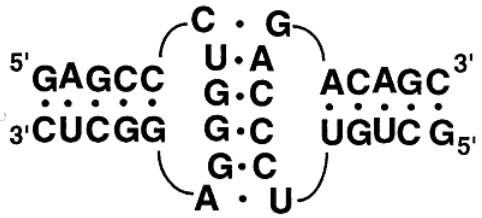


pseudoknots

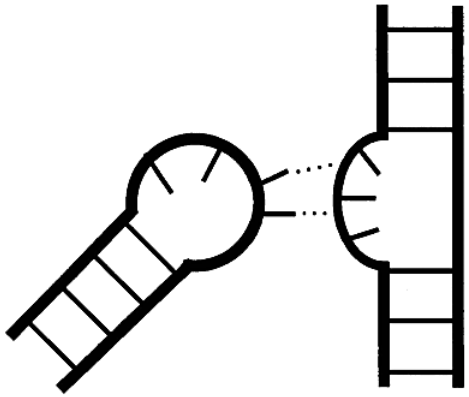


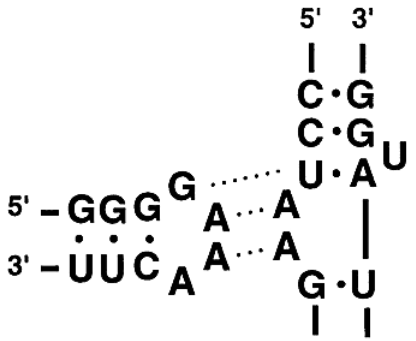






kissing hairpins





hairpin loop - bulge

from Burkard, M.E., Turner, D.H., Tinoco Jr., I., in The RNA World, 2nd Edn, eds Gesteland, RF, Atkins, JF Cold Spring Harbor Laboratory Press (1998)

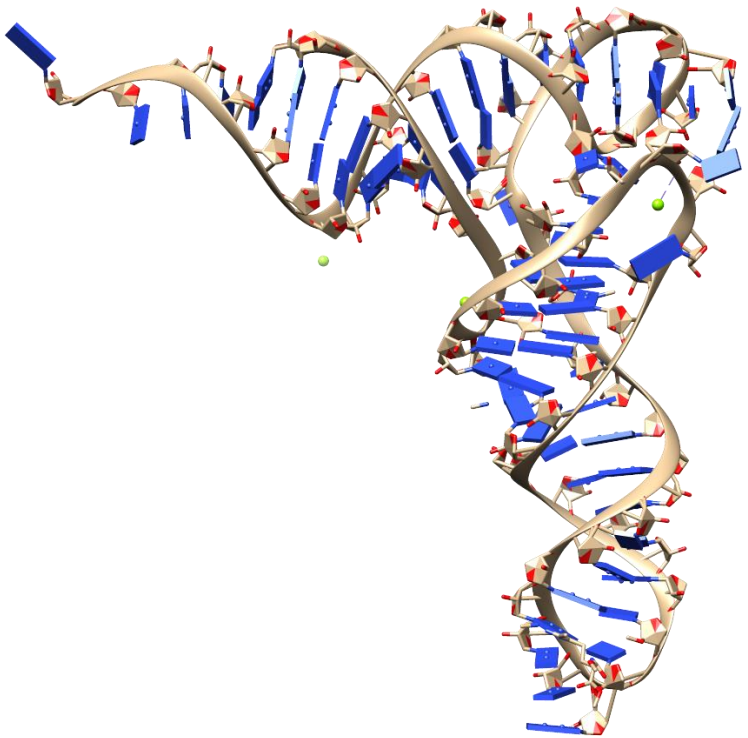
pseudoknots

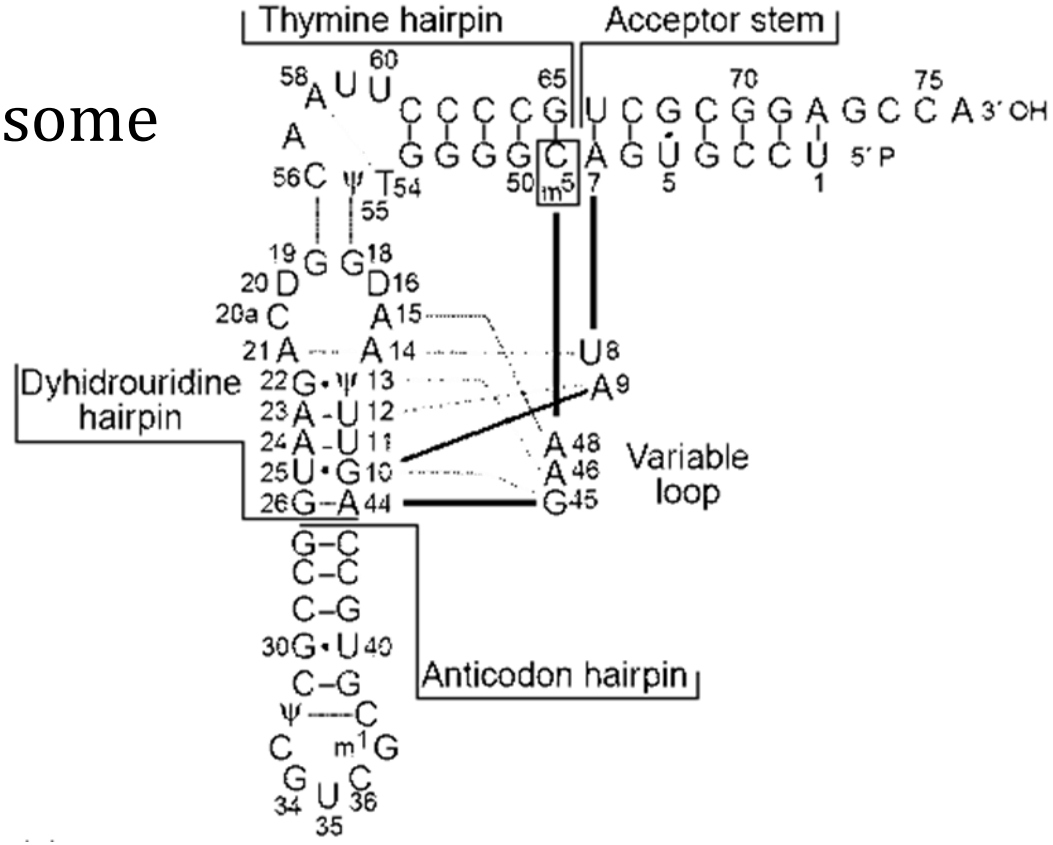
Frequency of pseudoknots ?

- a few % of all H-bonds / base pairs

Significant ?

- most structures will have some
- classic RNA example





pseudoknot summary

Fast algorithms cannot find pseudoknots

- in order to go fast, the algorithms work in a special order
- some base pairs come in "wrong" order
- most web servers, fast programs ignore the problem

A real limitation in the methods

How expensive are the methods ?

cost of predicting structure..

The methods are not perfect.. How expensive are they ?

for each i (growing loops)

test each j

try each k (splitting loops)

gives $n \times n \times n = O(n^3)$

Scoring schemes – H bonds

First step – from base pairs to H-bonds

We know

- GC 3 H-bonds
- AU 2 H-bonds
- GU 2 H-bonds

Compare a structure with

- 3 × GC versus 4 × AU
- 9 H-bonds versus 8 H-bonds

Scoring schemes – unpaired bases

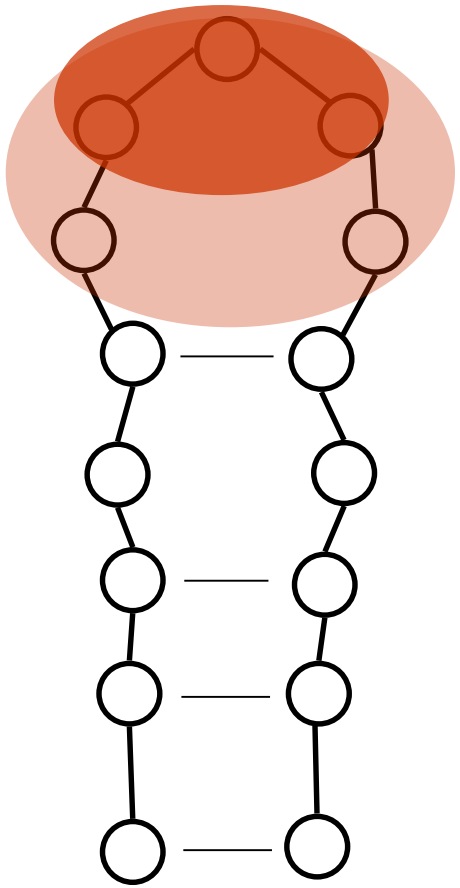
Second improvement

Consider unpaired bases

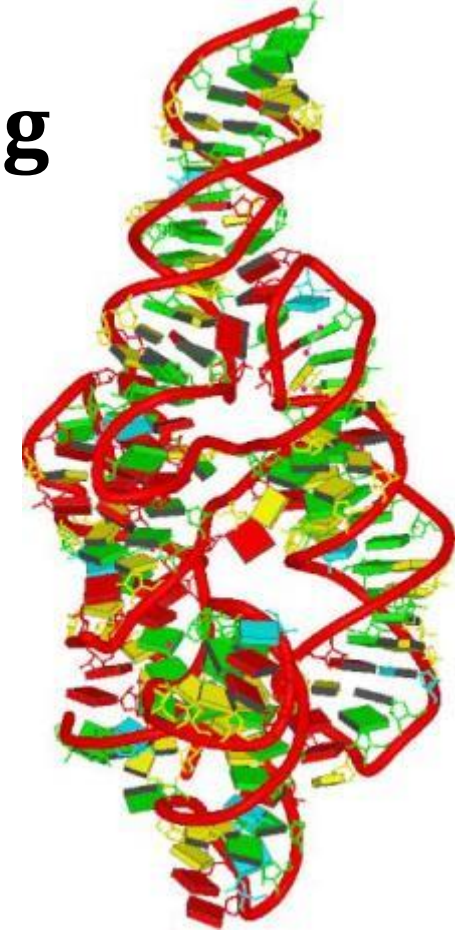
- counted for zero before
- compare loop of 3 / 5 / ..

Do these bases

- interact with each other ? solvent ?
- energy is definitely ≠ 0



Scoring schemes - stacking



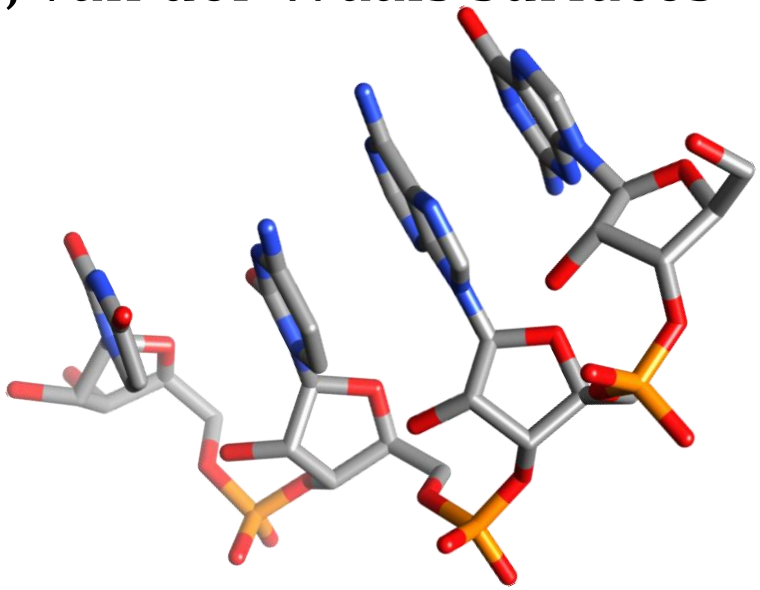
Third improvement

Bad assumption: each basepair is independent

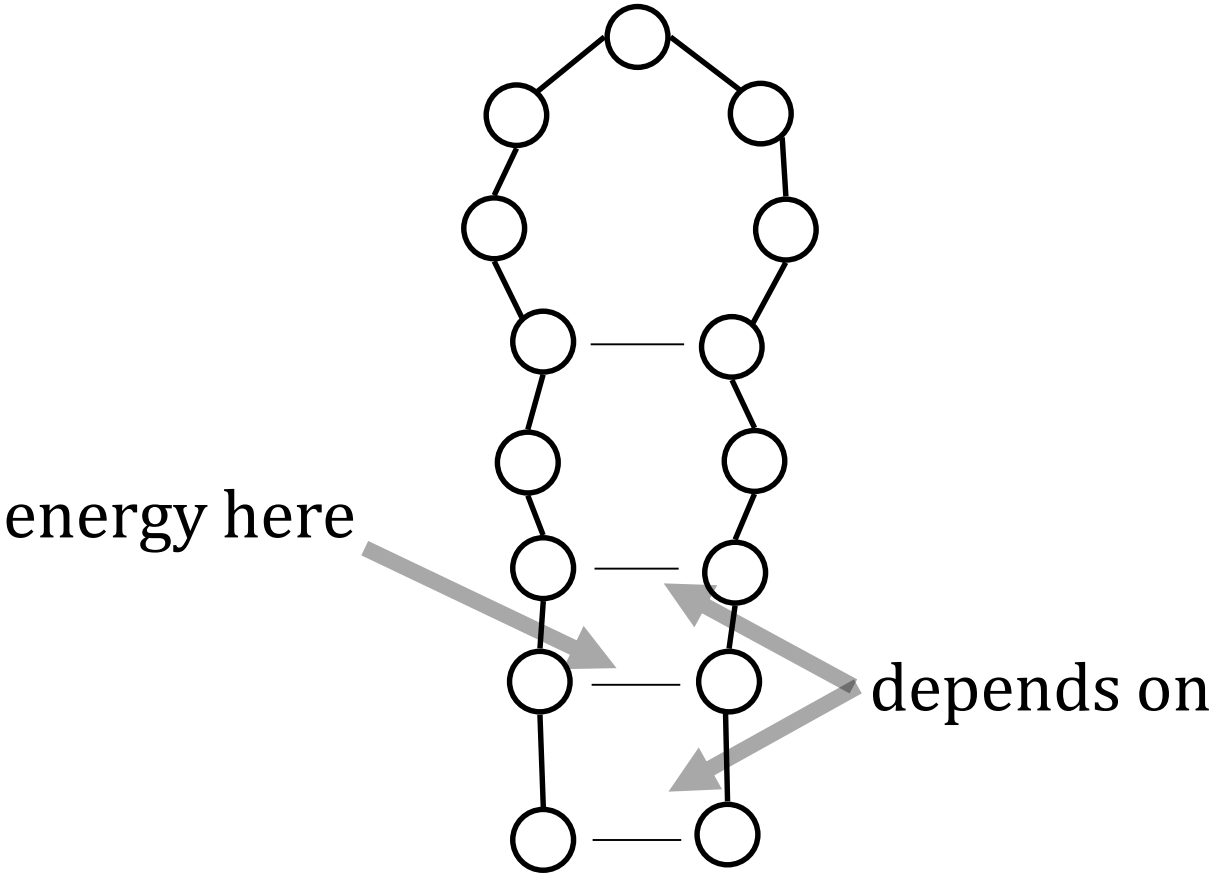
- $S(i,j)$ = base-pair + $S(i+1, j-1)$

Consider all the interacting planes

- partial charges, van der Waals surfaces



Scoring schemes - stacking



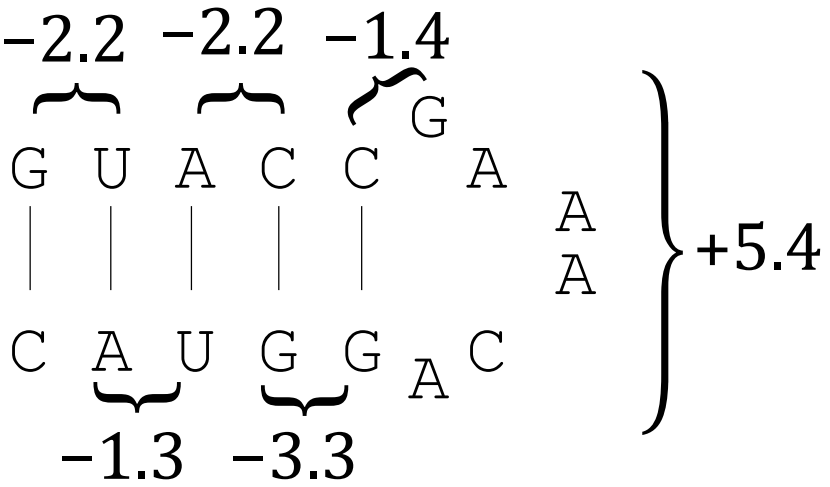
Goal

- incorporate most important effects
- do not add too many parameters … nearest neighbour model

Nearest neighbour model

Previously we added

GC + UA + AU + …



Now

(GU/CA) + (UA/AU) +…

= (−2.2) + (−1.3) + …

Terminal loop costs 5.4 kcal mol^{-1}

scoring summary

Approximation to free energies - $\Delta G_{folding}$

n base pairs	very primitive
n H-bonds	
loop sizes	
base-stacking	nearest neighbour model
tertiary interactions	ignored

Reliability

How accurate ?

- maybe 5 – 10 % errors in energies

How good are predictions ?

- maybe 50 – 75 % of predicted base pairs are correct

Why so bad ?

Reliability – alternative structures

Think of an "A"

- wants to pair with a U
- there are many many U's

Think of any base

- many possible good partners

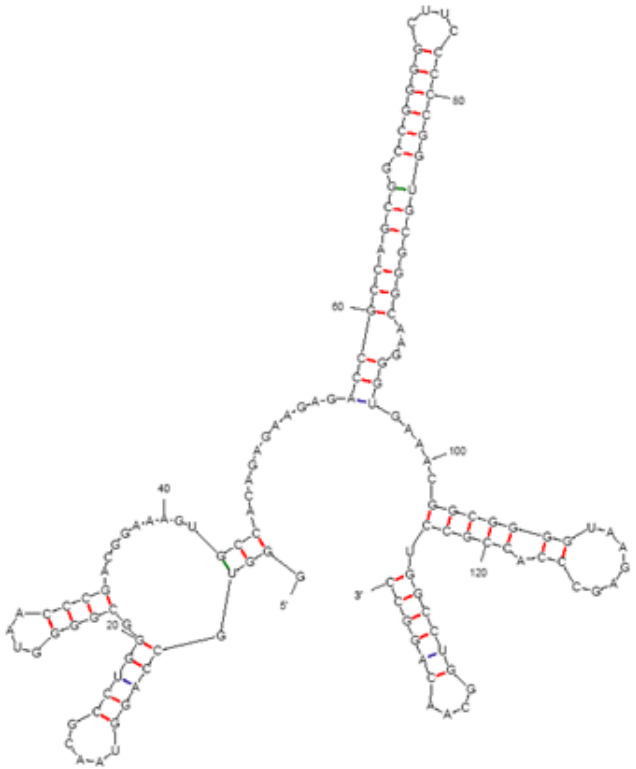
Consider whole sequence

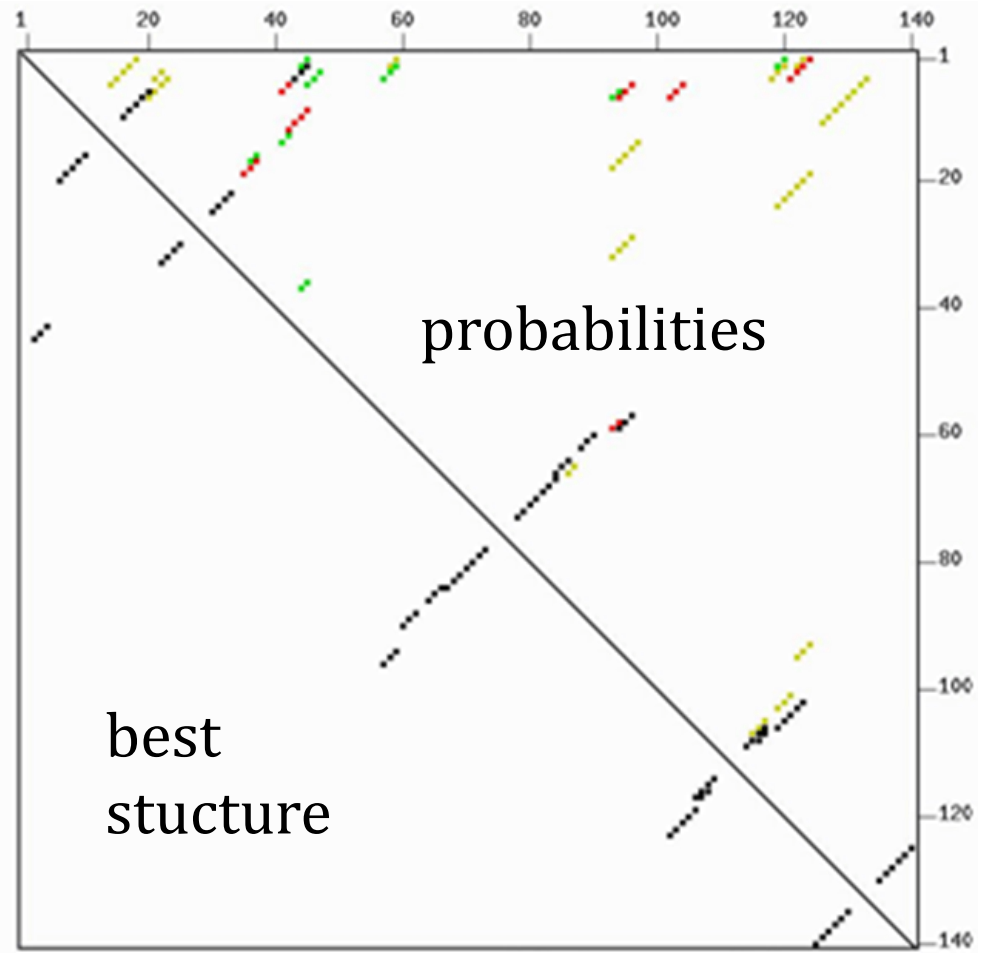
- there may be many structures which are almost as good (slightly sub-optimal)

Treat in terms of probabilities

Probabilities

- lower left – best structure
- upper right – probabilities of base-pairs



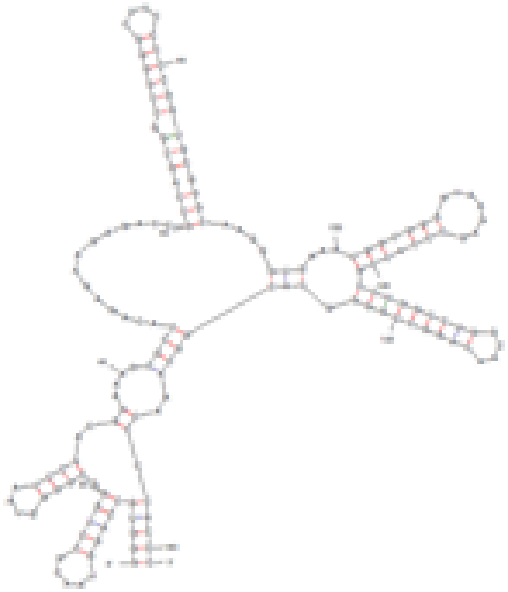


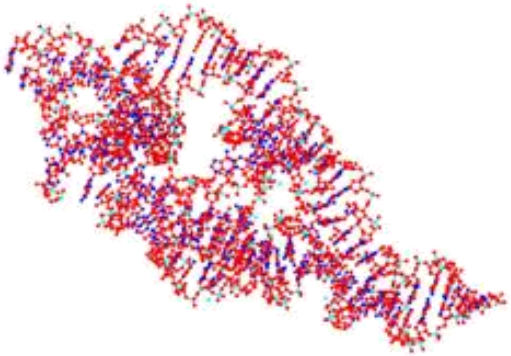
Reliability - Tertiary interactions

- miscellaneous H-bonds
- non-specific van der Waals

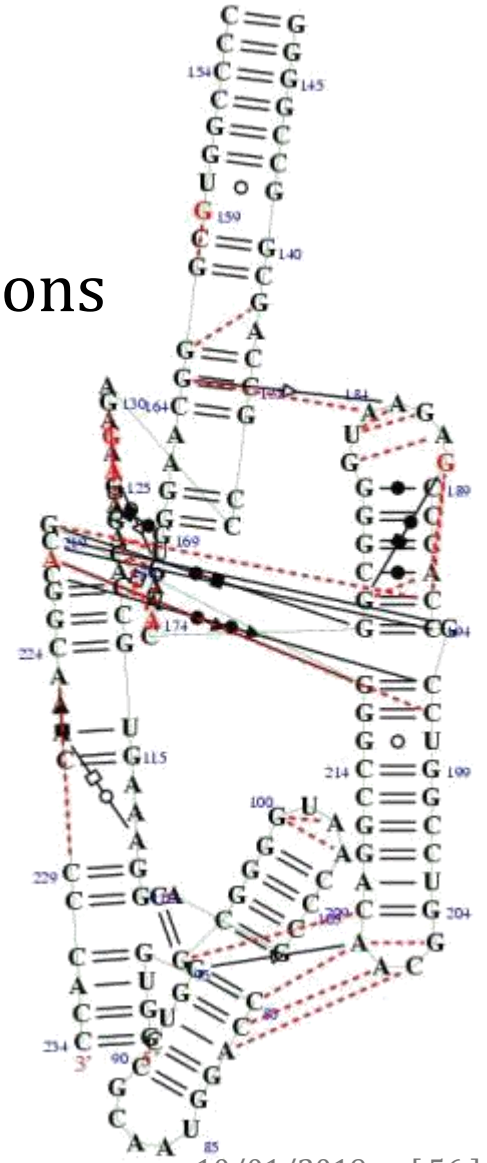
Most larger RNA's have many tertiary interactions

- relatively compact



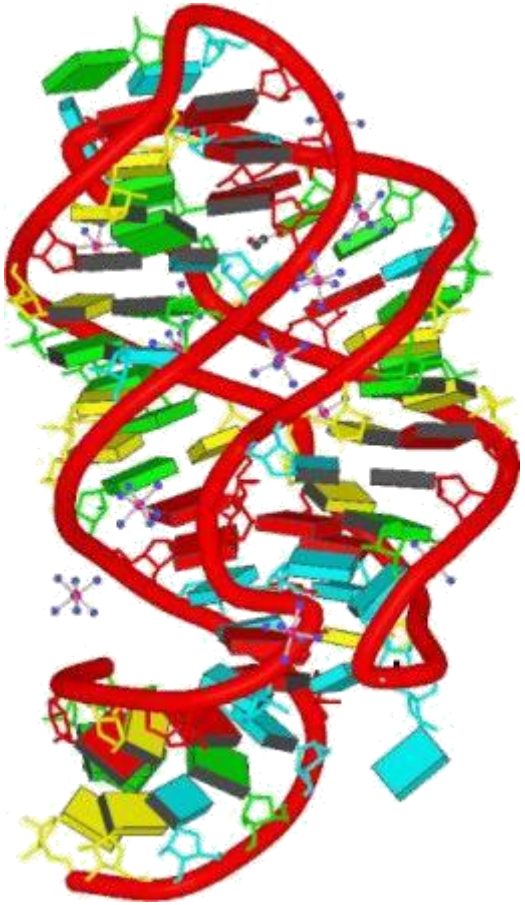


tertiary interactions
from crystal

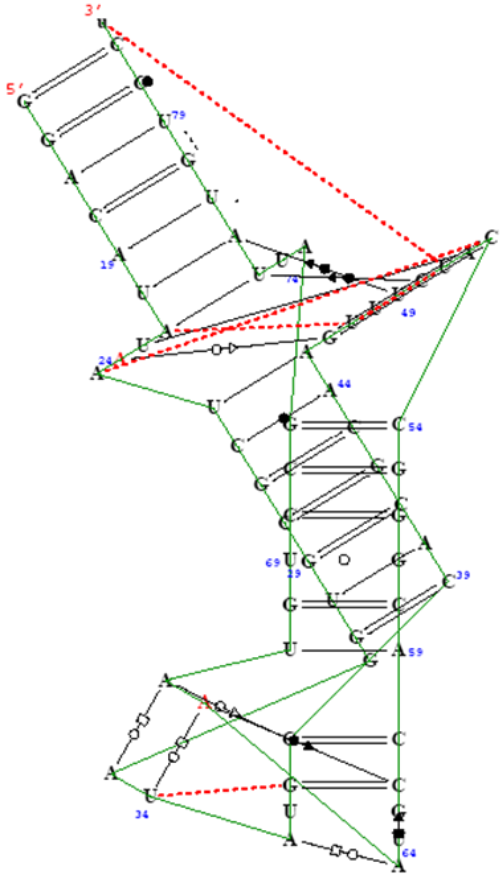


2D vs 3D

2g9c purine riboswitch

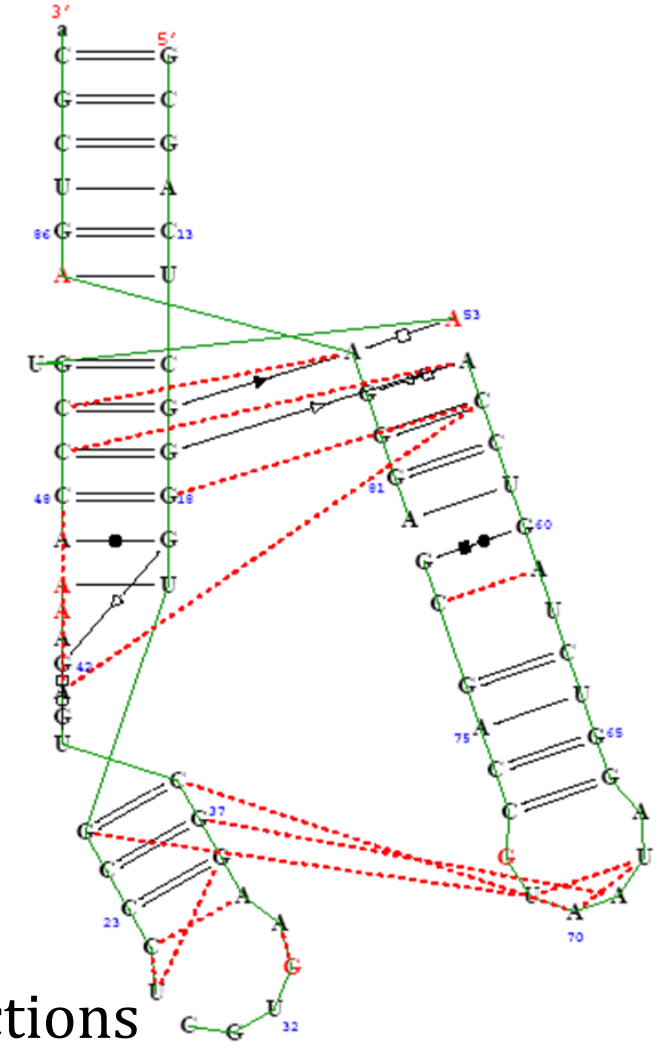


tertiary interactions from crystal



2D vs 3D

2hoj



tertiary interactions
from crystal

Reliability - summary

1. alternative structures with similar energies

- if the second best guess is the correct one
  - you will not see it

2. tertiary interactions are not accounted for

State-of-the-art predictors

Related sequences from other species fold the same way

Procedure

- collect closely related RNA sequences from data bank
- try to fold all simultaneously

Why is this good ?

- imagine our mistakes are random
- repeating the calculation averages over random errors

Imagine you could predict the best secondary structure perfectly. Is the problem solved ? …

Kinetics

Imagine you can predict 2D structures

- are you happy ?

Two possible scenarios

- kinetic trapping
- slow formation

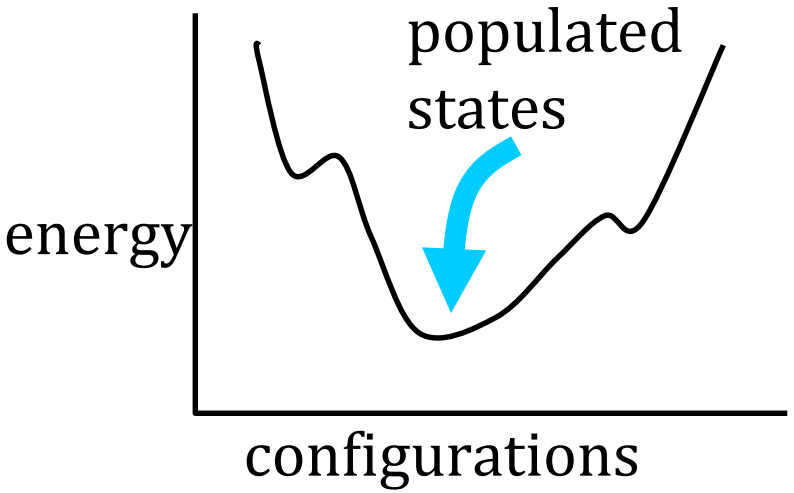
Kinetic trapping

Term from protein world

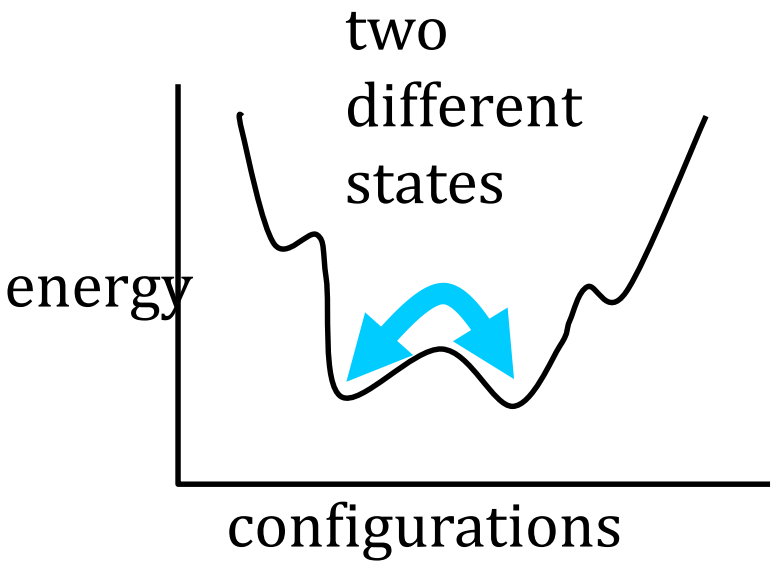
Wherever the molecule is

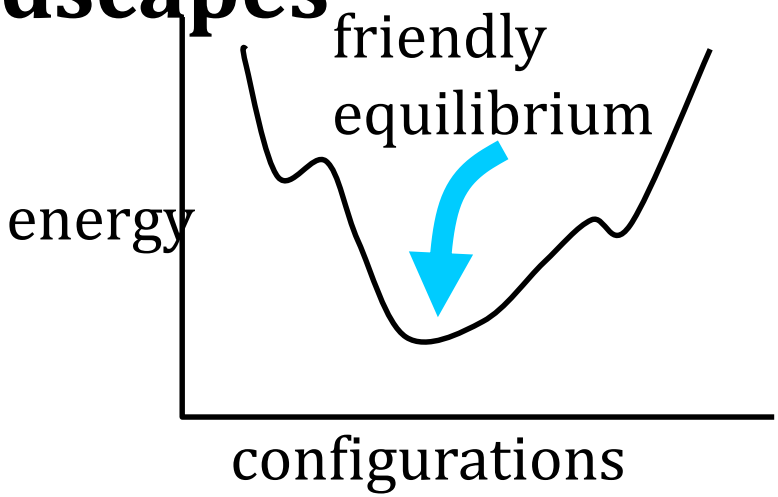
- it will probably go to energetic minimum

- less friendly landscape

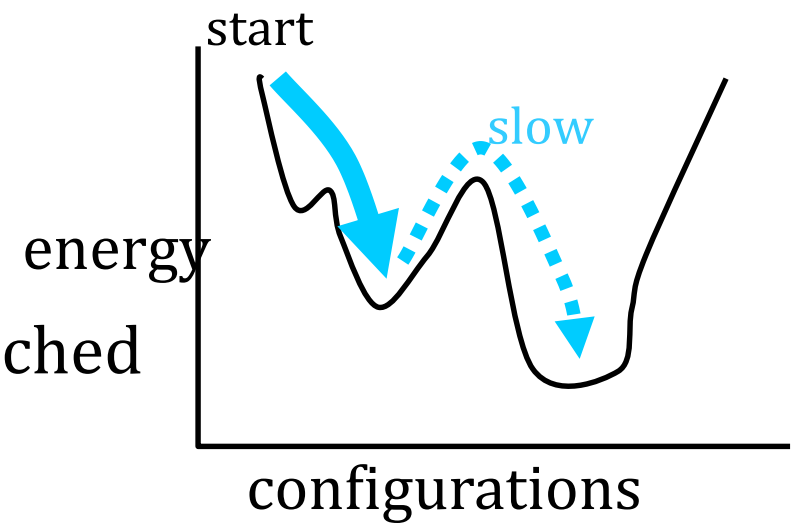


Energy landscapes





If barrier is too high, best conformation may never be reached



How real is the problem ?

Consider base of type G

- there are many C's he could pair with
- only one is correct

There are many local minima on the energy landscape

Landscapes / kinetics

Can one predict these problems ?

- not with methods so far

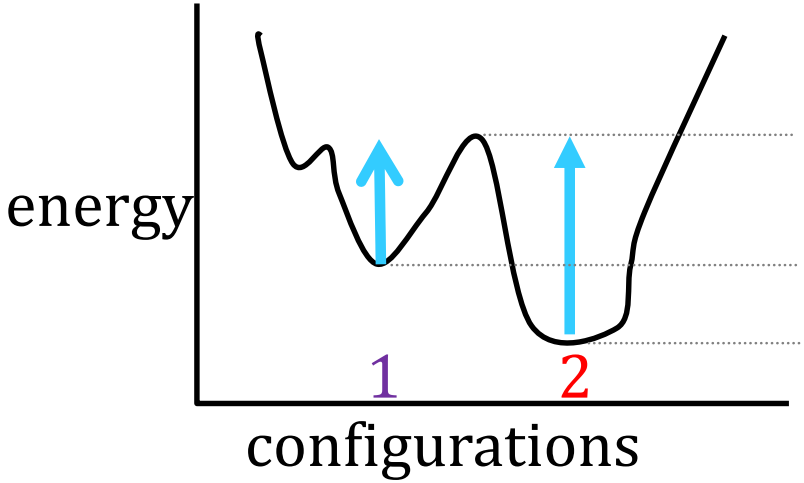
Try with simulation methods

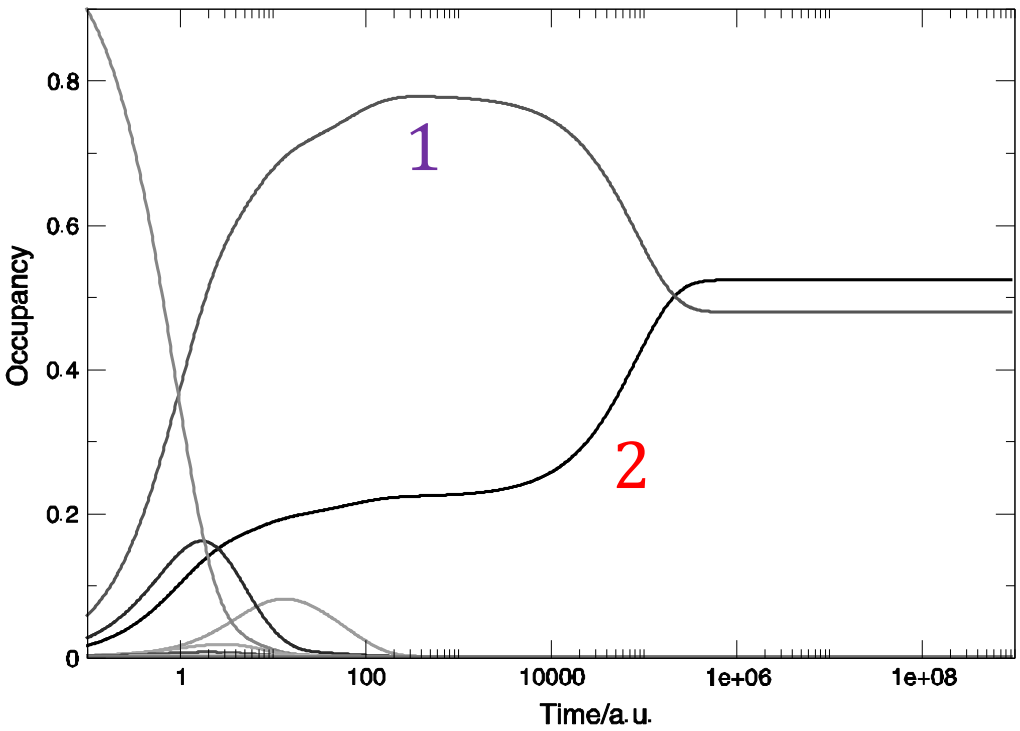
- Monte Carlo / time-based methods

- start with unfolded molecule
- use classic methods to get a set of low energy predictions
- simulate folding steps
  - measure amount of each good conformation with time..

Example calculation

- conformation 1 forms rapidly
- conformation 2 slowly forms
  - conformation 1 disappears



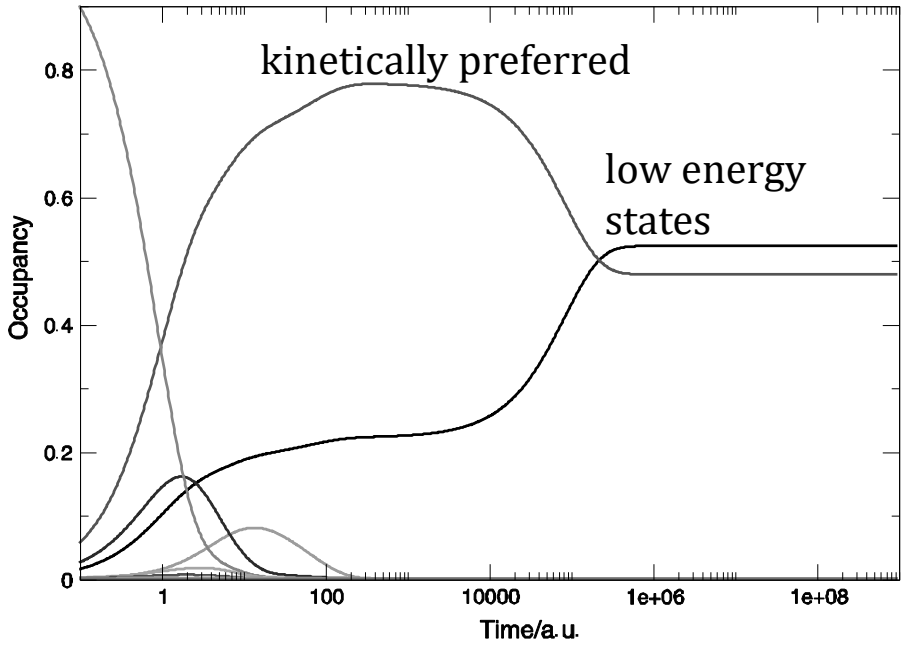


Implications

What if RNA is degraded ?

Molecule disappears before it finds best conformation

"kinetically preferred" conformations may be more relevant than best energy



summary

Tertiary structure very important (binding of ligands)

2D (secondary structure calculations)

- fast
- limits structures one can predict (no pseudoknots)
- predictions are not reliable
- used everywhere in literature (coming seminars)

You may lose anyway (kinetics)