Grand Plan

RNA very basic structure very quick

3D structure

Secondary structure / predictions

The RNA world

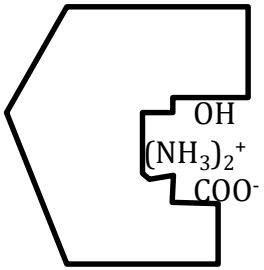
Roles of molecules

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

Think about binding…

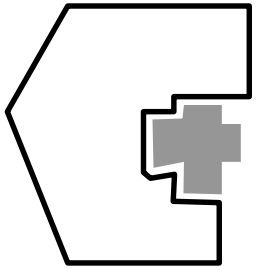
Specificity and binding

How do proteins work ?



Some site decorated with special groups

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



Chemical choice ?

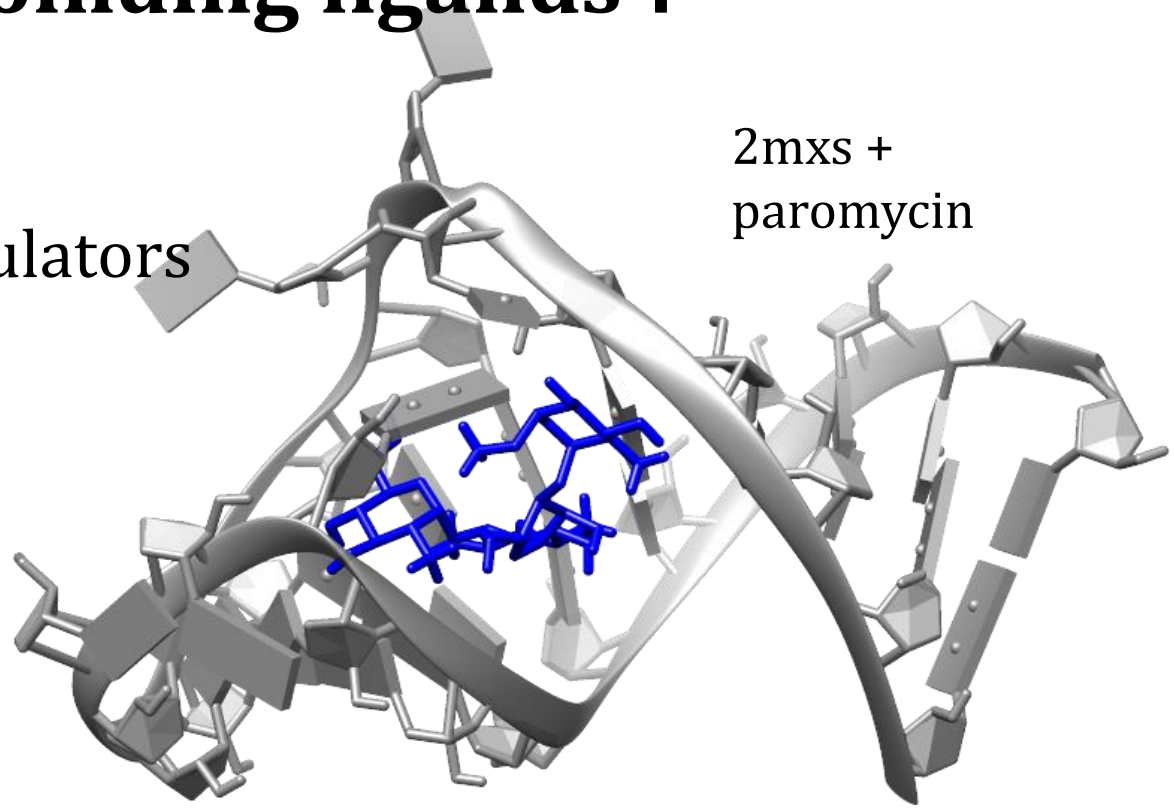
- 20 kinds of amino acid
- half a dozen really different types

Do you see this with nucleotides ? ..

RNA binding ligands ?

Examples

- riboswitches / regulators
- catalysts



Two consequences

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

DNA binding ligands ?

Very specific binding to proteins

- promoters / repressors
- DNA cleavage enzymes
- who is responsible for specificity ? (DNA or protein) ?

DNA ligand binding ? catalysis ?

- in laboratory ? – a bit
- in nature ? not really

Structure

DNA

- mostly thought of as double helix

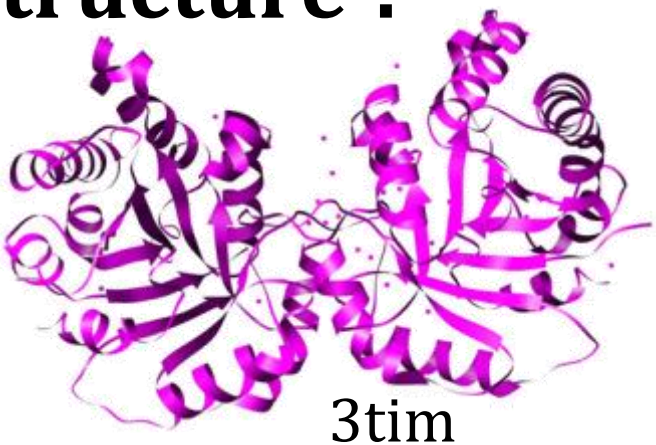
Protein (simple dogma)

- from a specific sequence to a well defined structure
- less often – floppy, unstructured

RNA

- does an RNA sequence fold up to a well defined structure ?
  - all possible RNA's ?
  - biological RNA's ?
  - some RNA's ?

How do we talk about structure ?



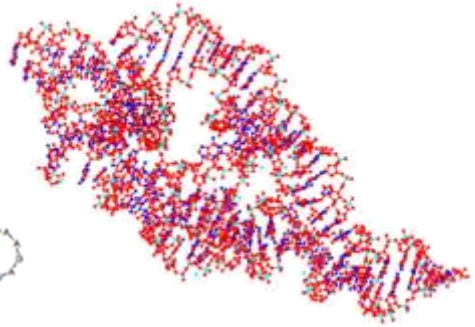
3tim

Protein

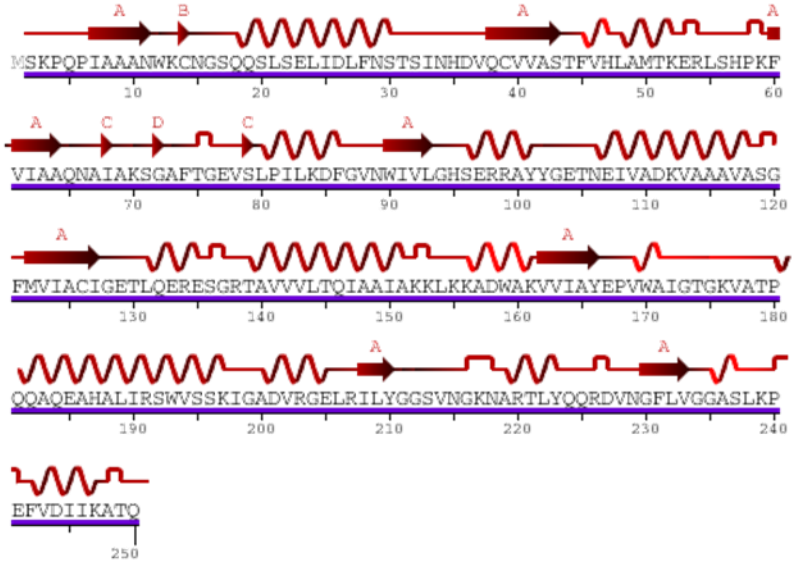
- usually 3D
- rarely secondary structure

RNA

- usually secondary structure
- rarely 3D



1u9s



Structural Data

Proteins

- 1.3×10^5 or about 3×10^4 interesting ones

RNA

- 3.5×10^3 structures with some RNA
- 1226 with pure RNA - many small and boring
- 430 pure RNA ≥ 40 residues (lots of redundancy)

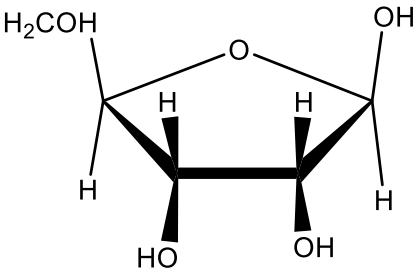
Why so few RNA structures ?

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

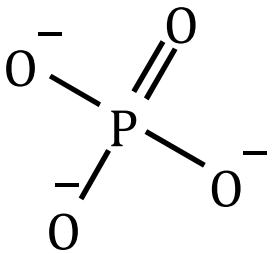
RNA structure

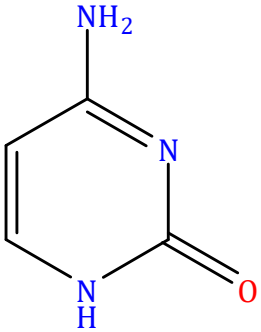
3 components

- ribose (sugar)
- phosphate (PO_4)
- base (nucleotide)

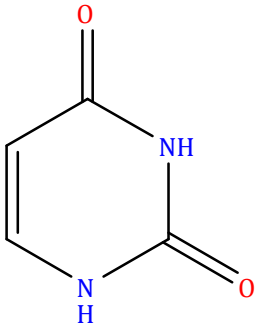


ribose

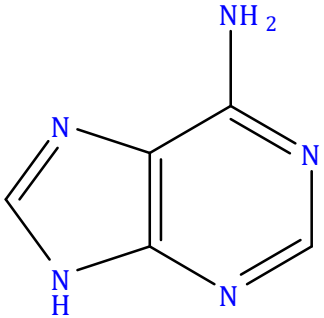




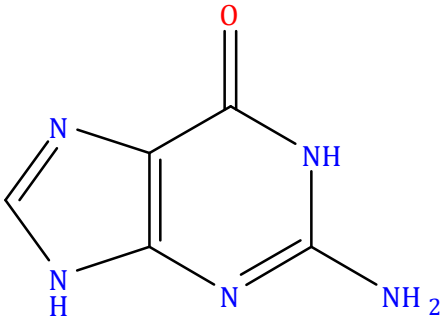
cytosine



uracil



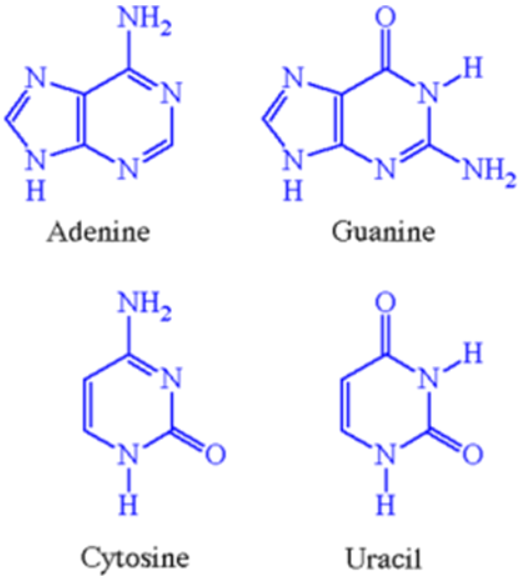
adenine



guanine

RNA Bases

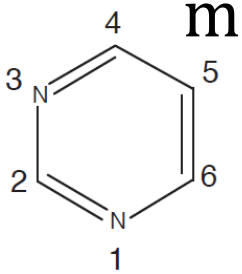
purines



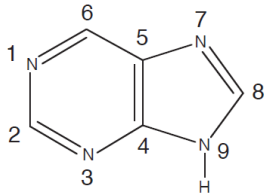
Are they like protein residues ?

- not classified by chemistry
- do they have interactions ?
  - yes (polar, H-bonds, van der Waals)

mother shapes



pyrimidine

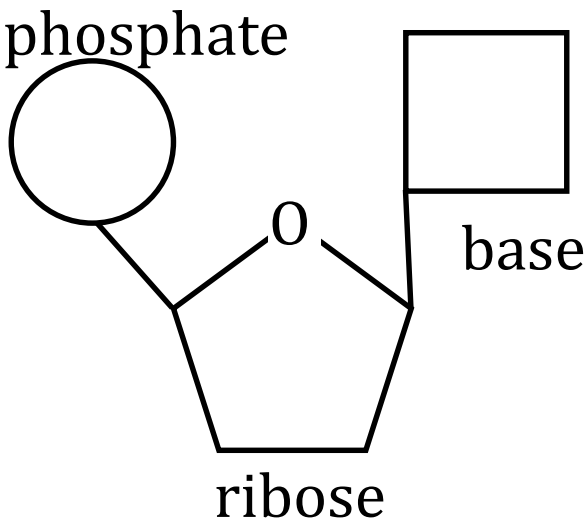


purine

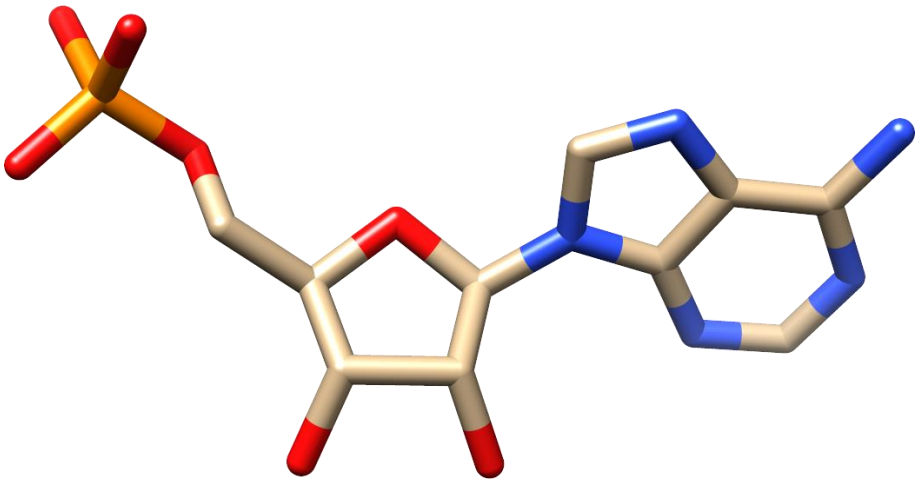
pyrimidines

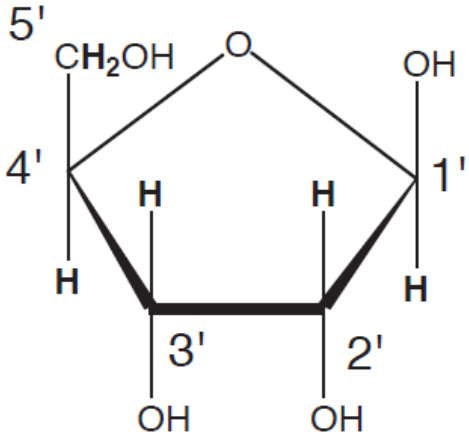
- no exam questions on numbering (from me)
- putting pieces together…

RNA structure



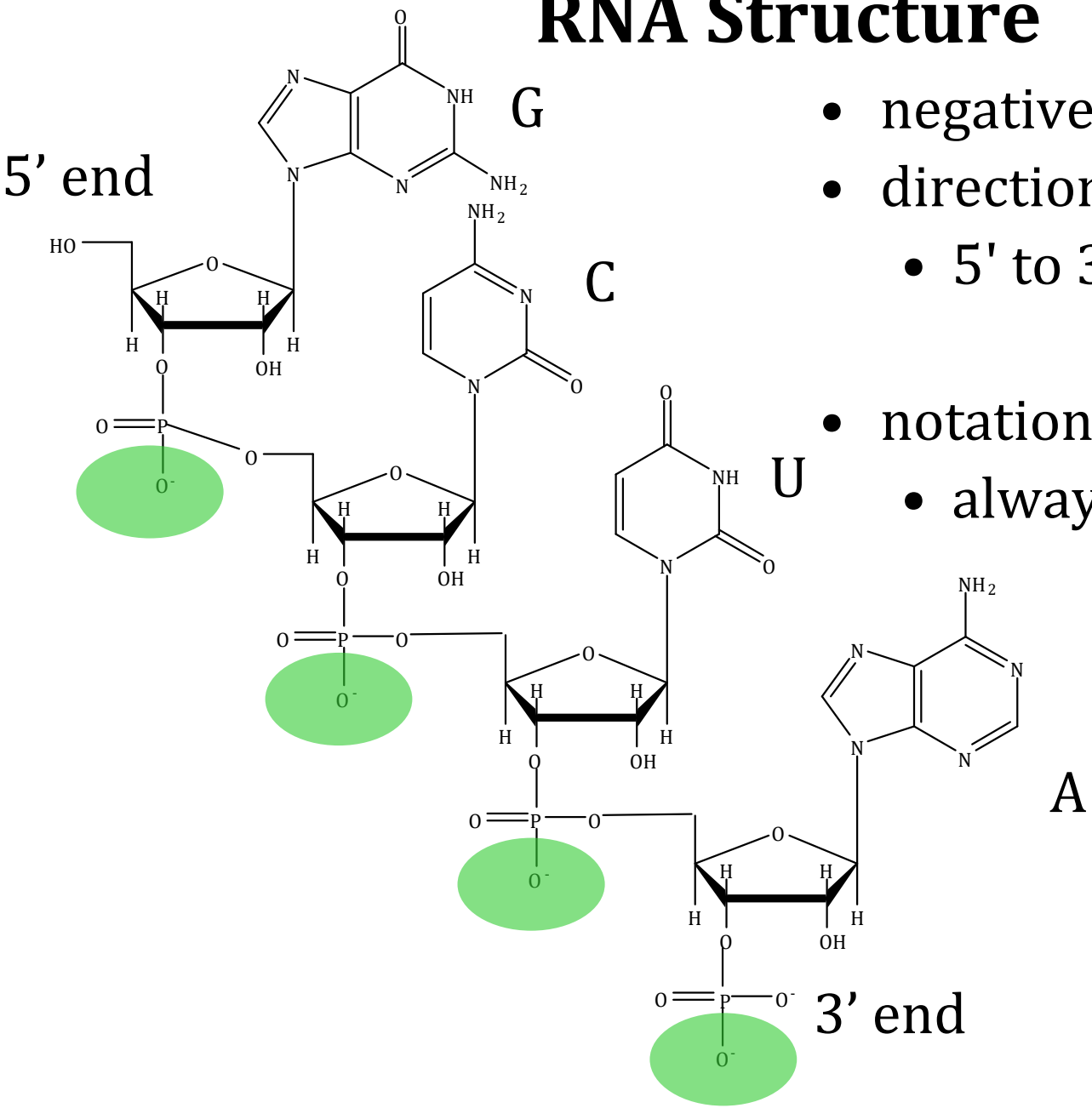
adenosine 5'-monophosphate





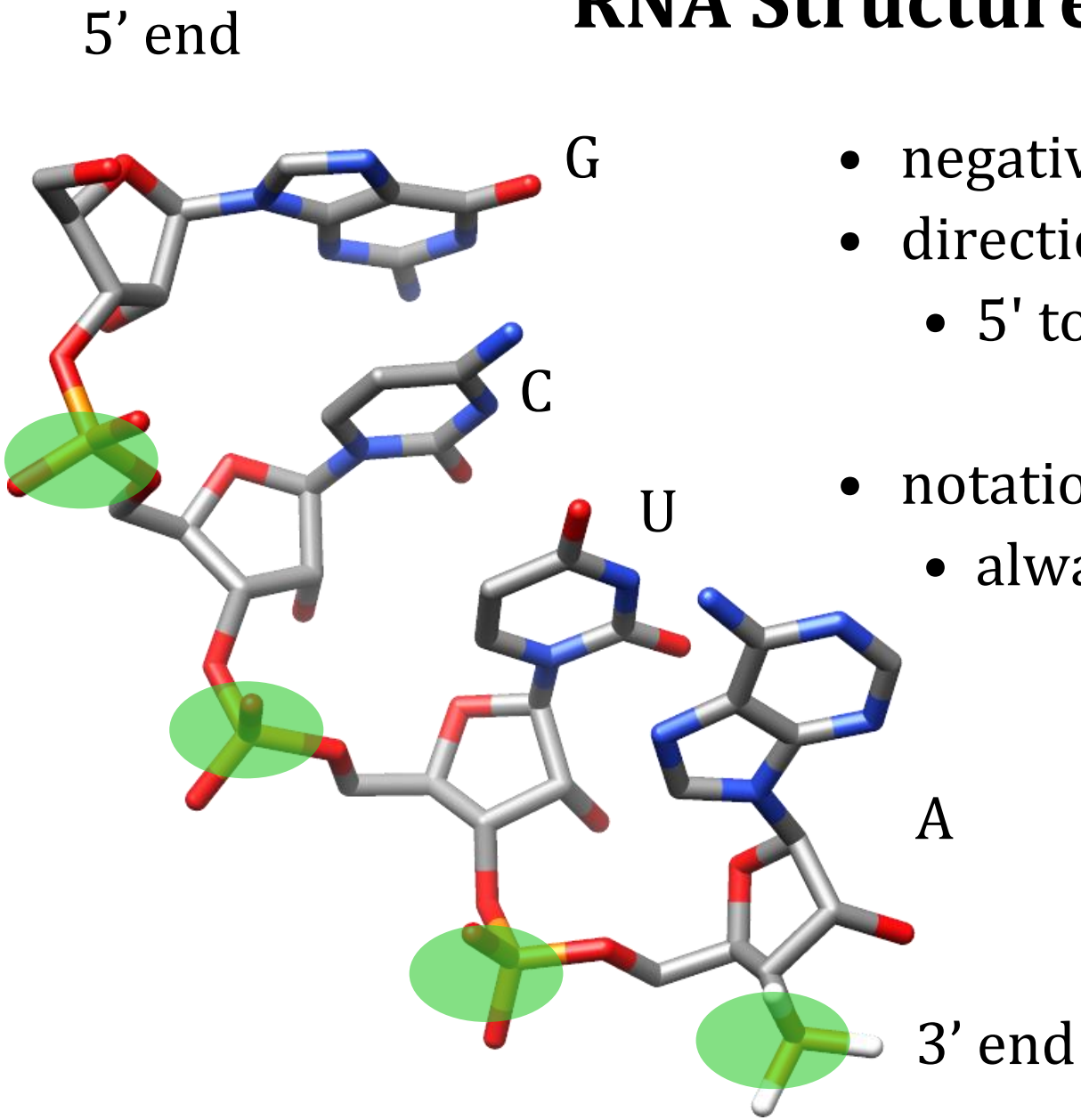
numbering on sugar ring is important

RNA Structure



- negative charges
- directional
  - 5' to 3'
- notation
  - always 5' to 3'

RNA Structure



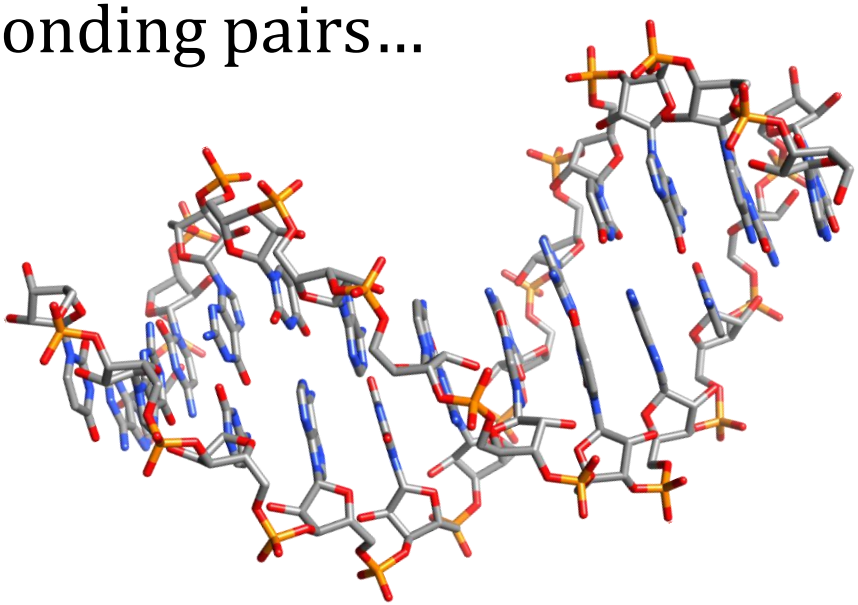
- negative charges
- directional
  - 5' to 3'
- notation
  - always 5' to 3'

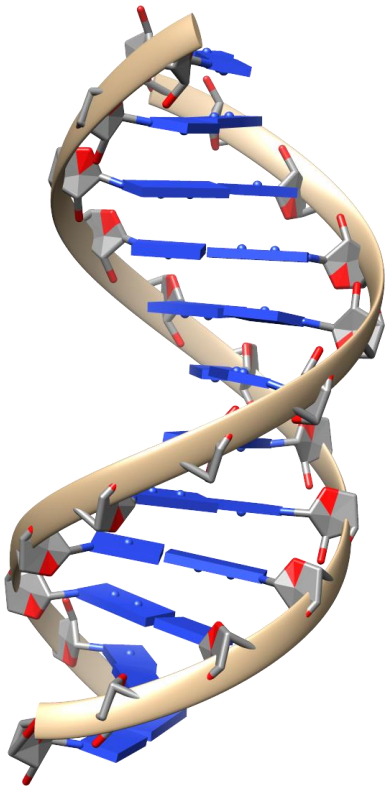
H bonding

What holds the pairs of a helix together ? H-bonds

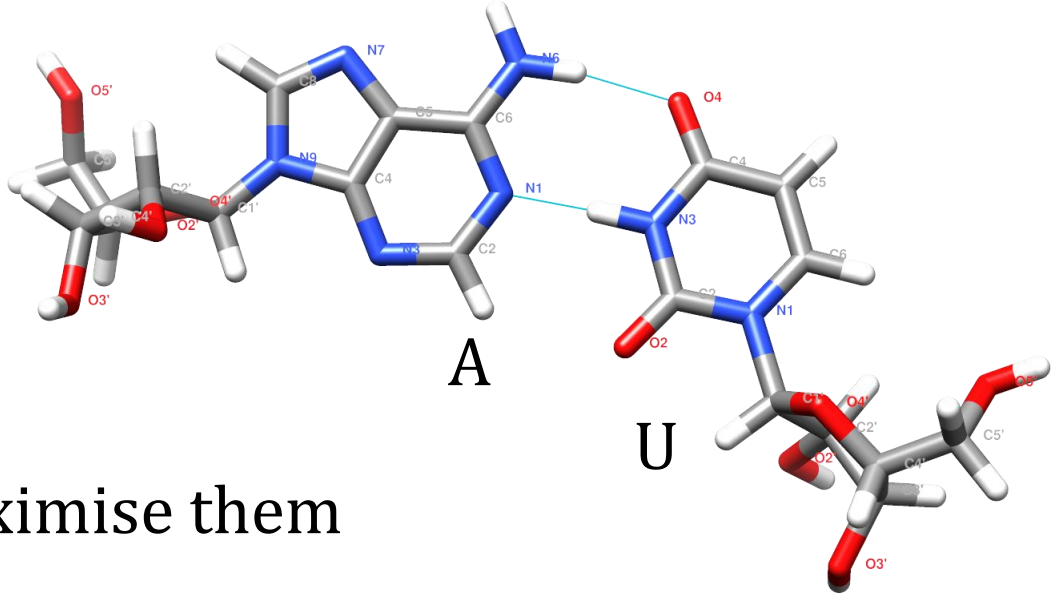
- applies to RNA
- rules from proteins
  - H-bond donors are NH, OH
  - acceptors – anything with partial –’ve

Historic H-bonding pairs…



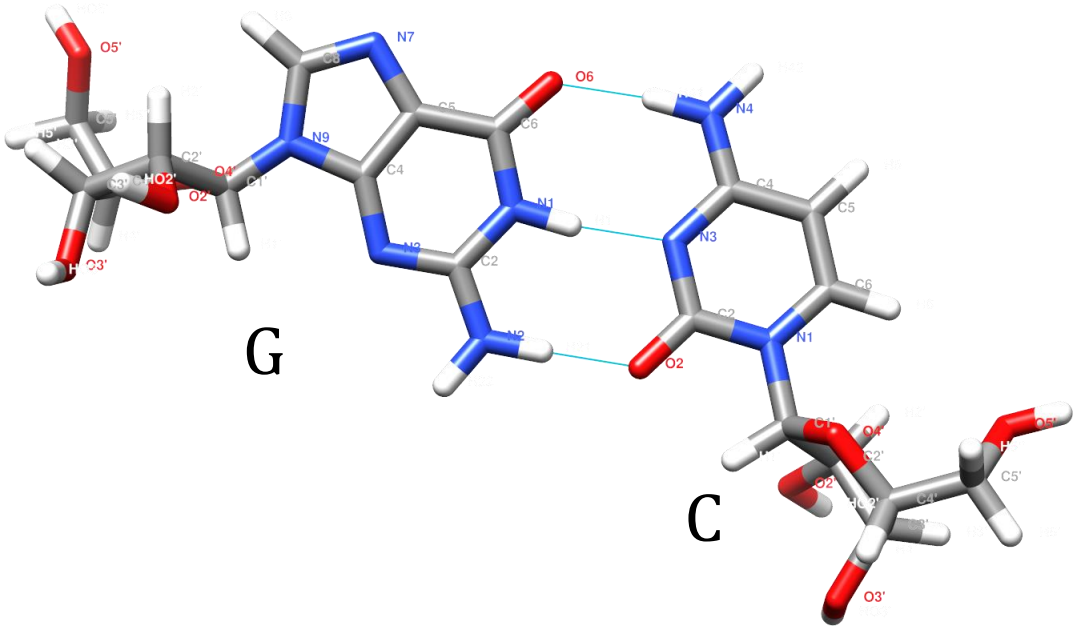


Historic H-bonding pairs



Count H bonds

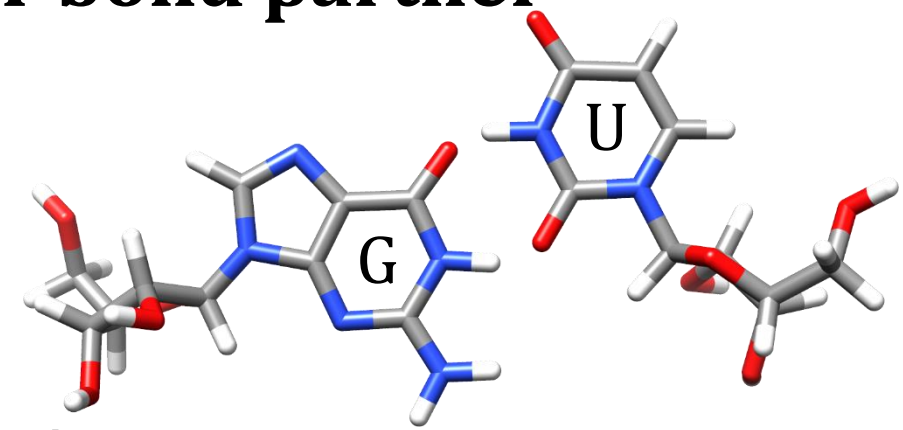
Structures like to maximise them



Historic viewpoint

- RNA has 4 bases + GC, AU base pairs
- H-bond pairs look flat
  - not true

Other common H-bond partner



Contrast with DNA (GC and AT)

- almost no mismatches in DNA

RNA (GC, AU) much more interesting

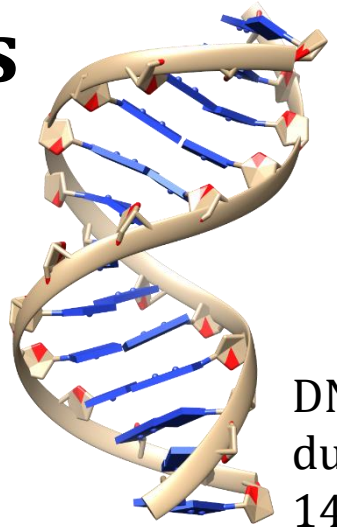
- third base pair GU (rather common)
- lots of weaker pairs possible

Possible RNA structures

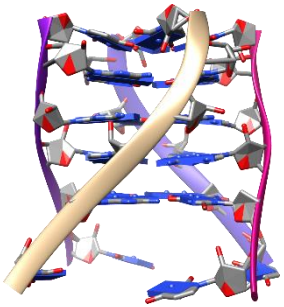
DNA ? nearly always similar helix

RNA

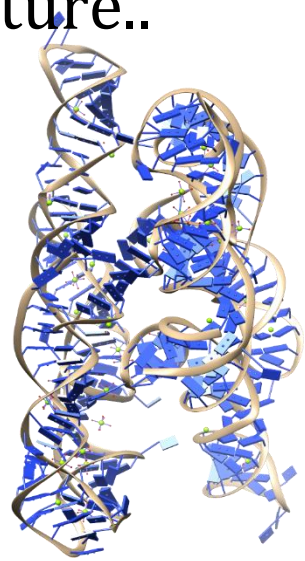
- lots of varieties known
- nomenclature..



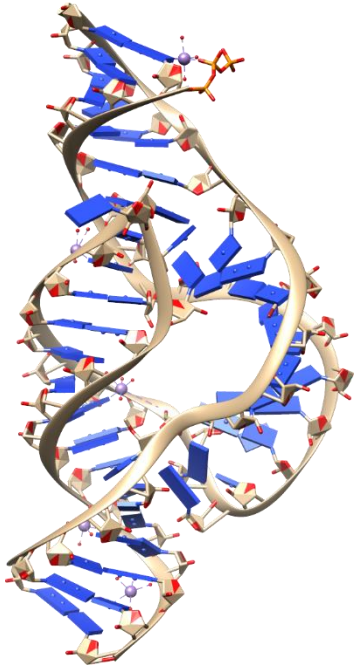
DNA duplex 140D



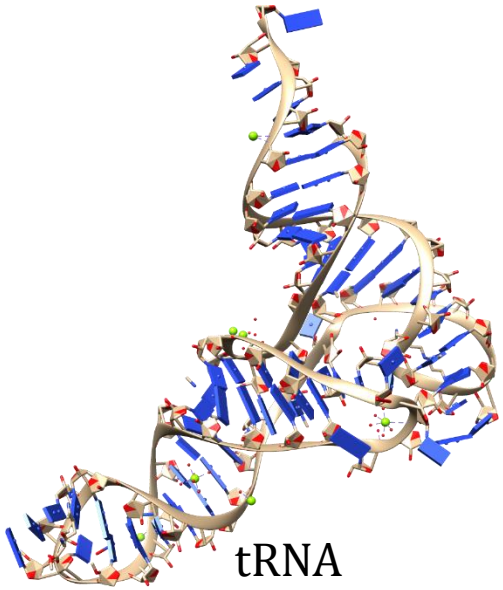
tetraplex 1mdg



group I intron 1hr2



hammerhead 2oeu



tRNA 1evv

What can we see in RNA structures ?

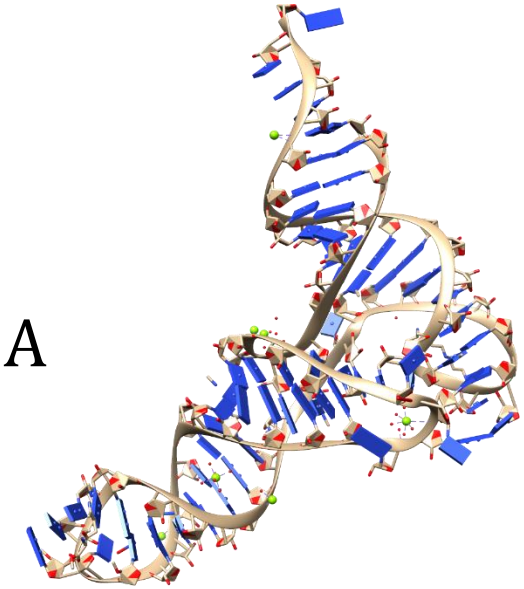
Not just canonical base pairs

H-bonds from bases

- to non-canonical sites in other bases
- to sugars

Even something small, common like tRNA

- lots of interesting interactions to maintain L-shape



Are there some common motifs ?

motifs / patterns

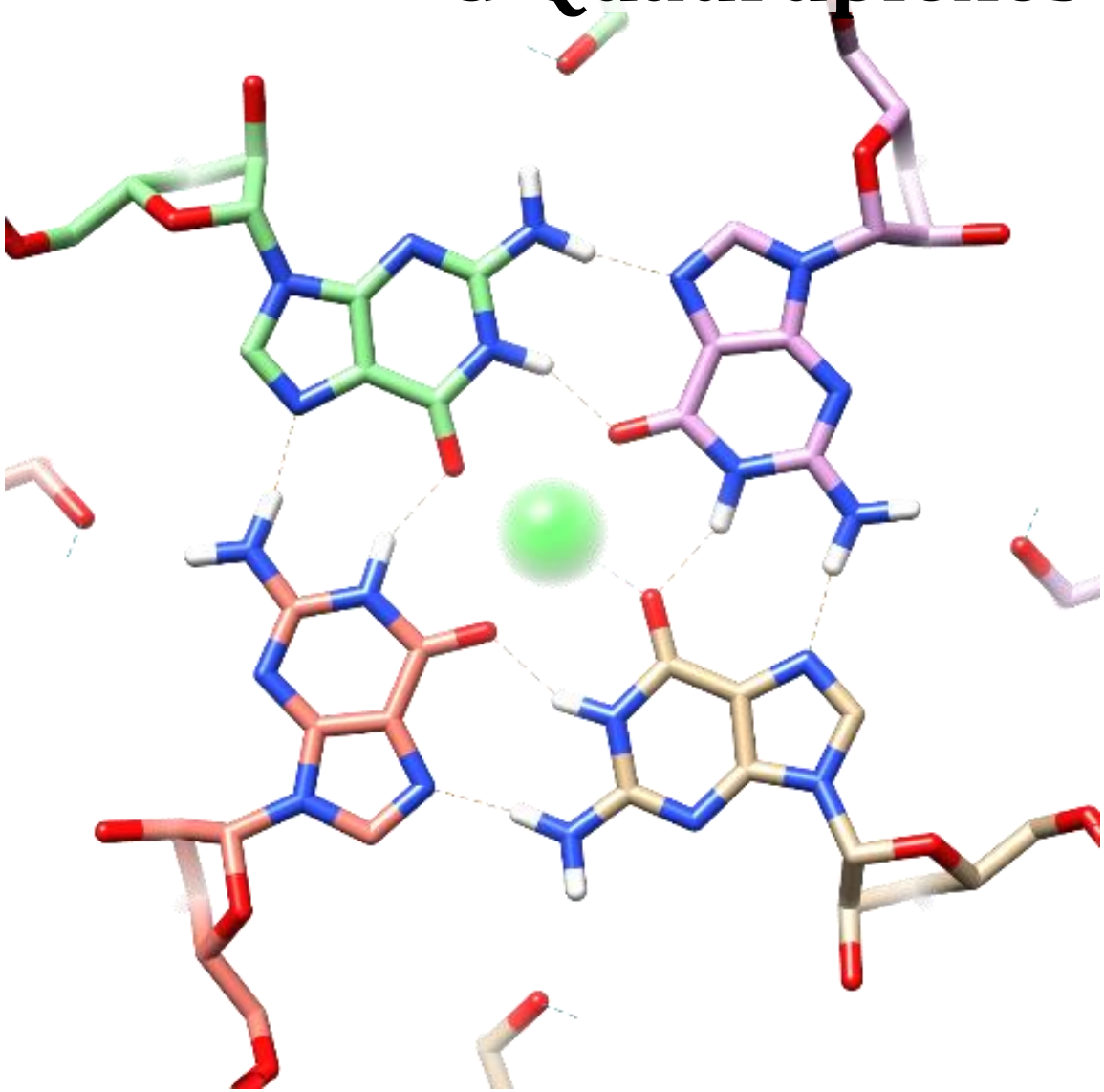
What do we do with proteins ?

- look for motifs we know α-helices, β-strands, turns
- they are held together by H-bonds, stable, common

What should we do with nucleotides ? The same

- a double helix is common, held together by H-bonds
- RNA tries to form stable, H-bonded structures
- important common motif – the quadruplex

G-Quadruplexes

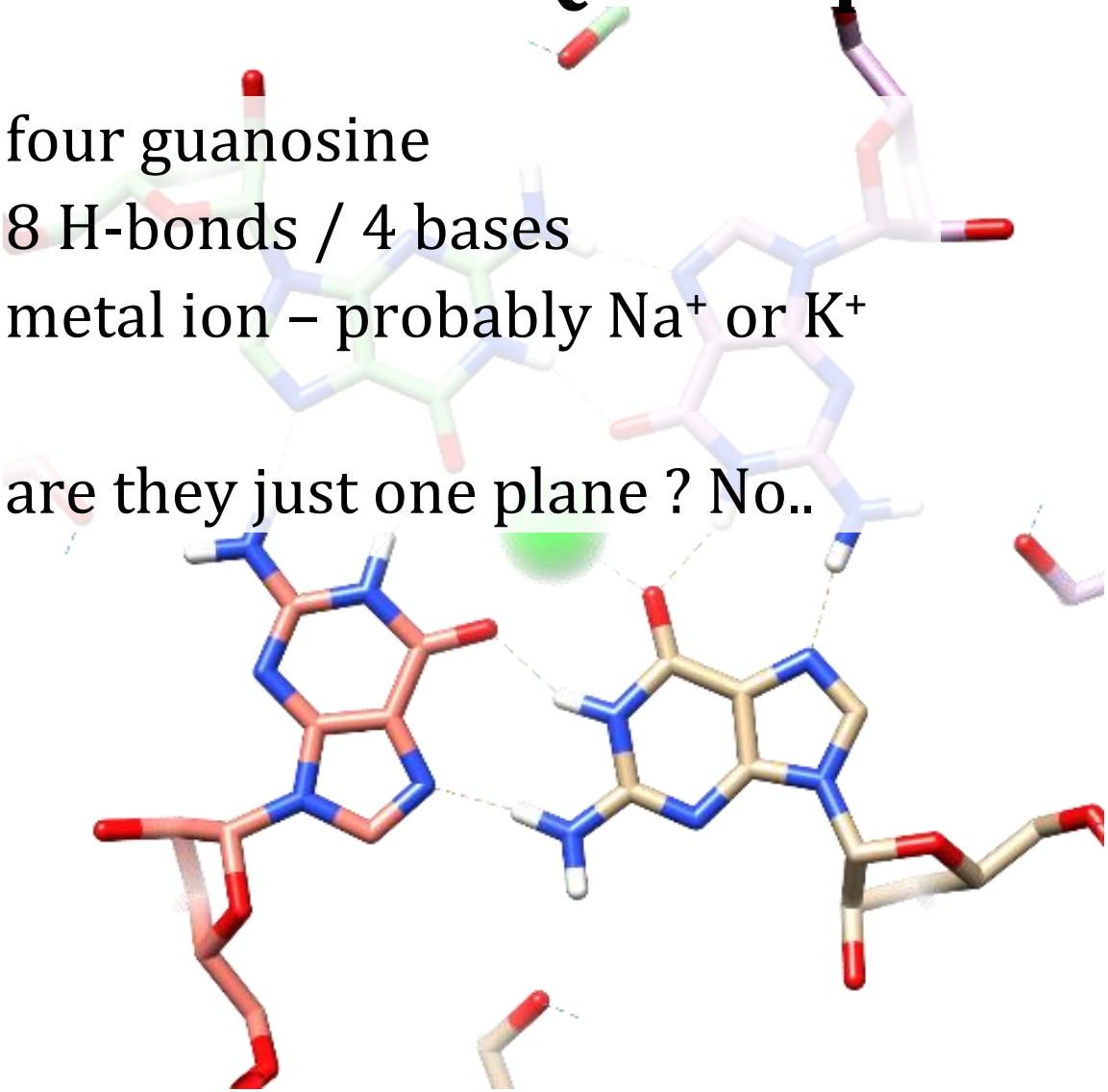


4rkv

G-Quadruplexes

- four guanosine
- 8 H-bonds / 4 bases
- metal ion – probably Na^+ or K^+

- are they just one plane ? No..



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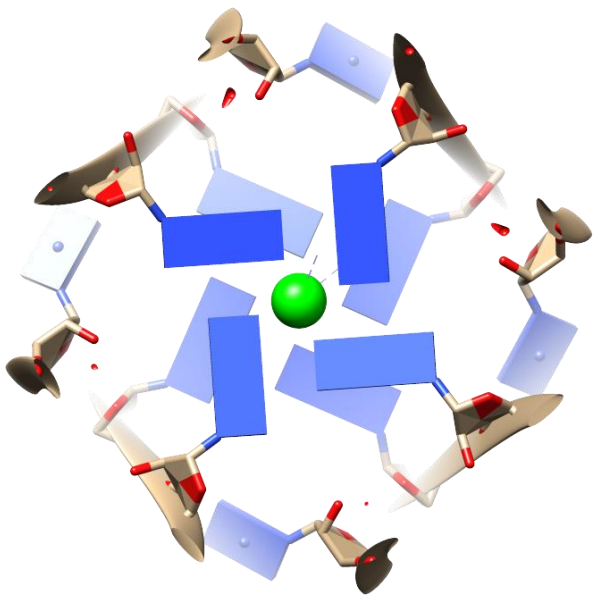
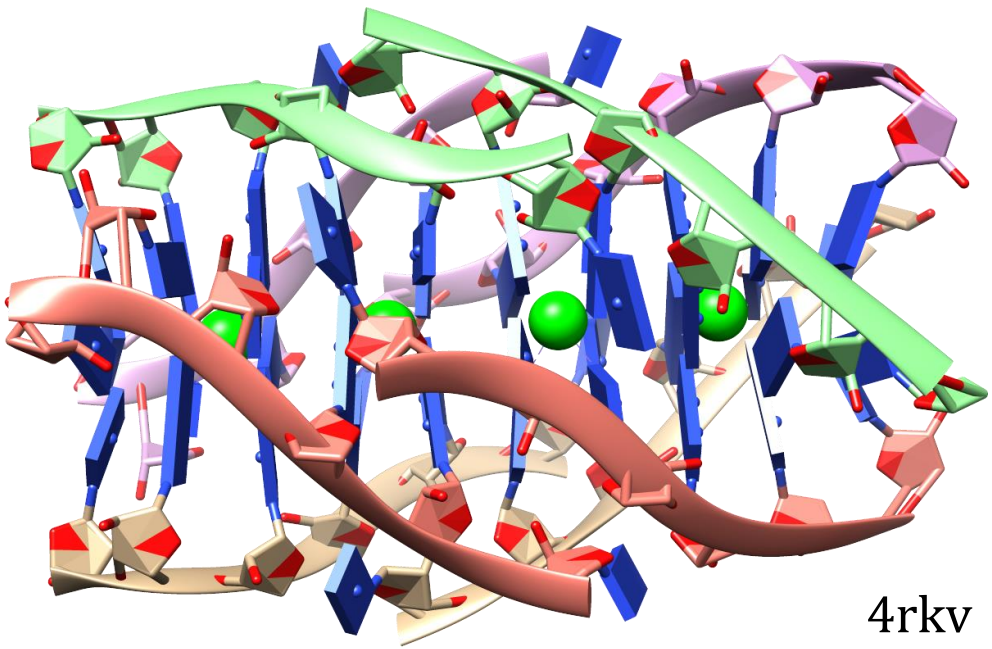




G-Quadruplexes

At the sequence level..

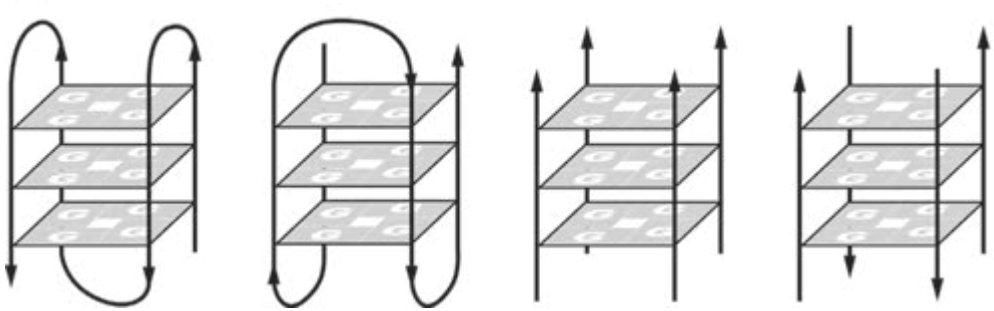
GGG (X) $_m$**GGG** (X) $_n$**GGG** (X) $_p$**GGG**

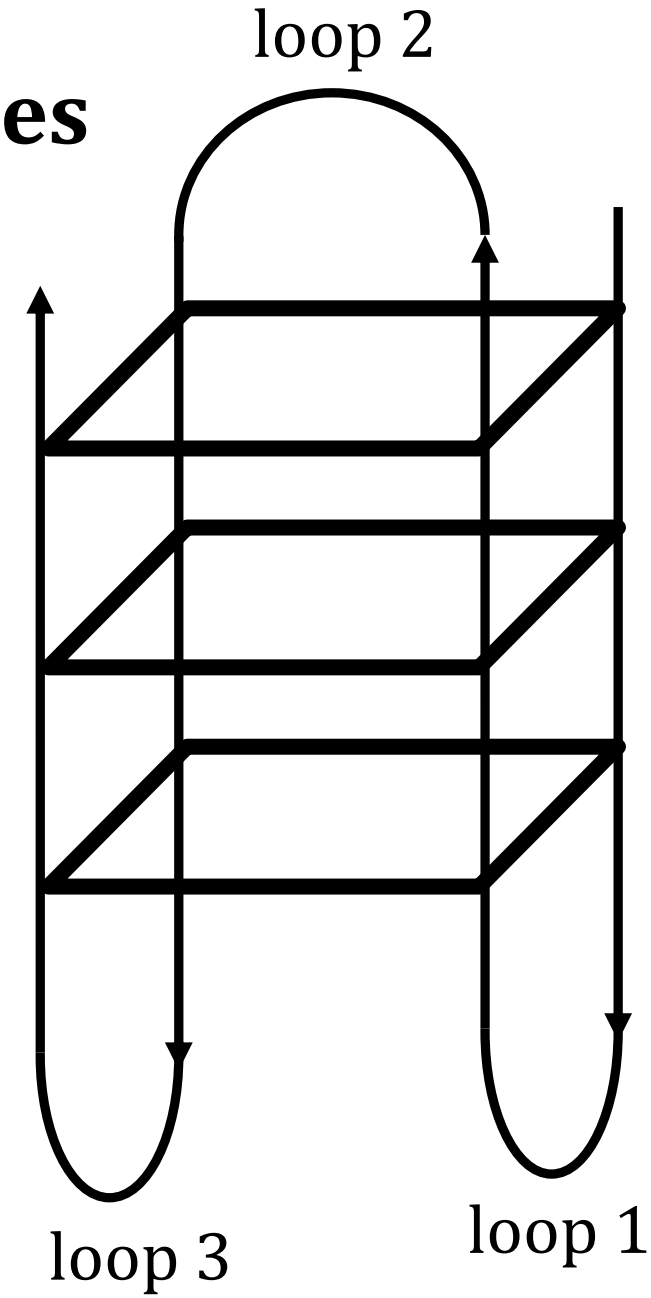
How long are m, n, p ? loop 1, 2, 3 ?

- everything is possible
- maybe 1 – 7 are common

Topologies

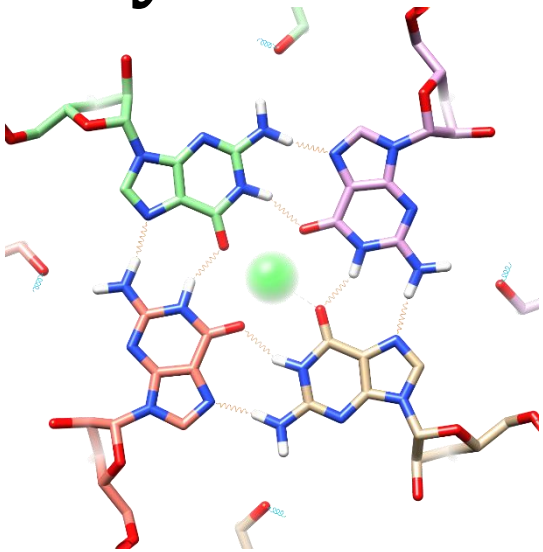
- parallel, anti-parallel





Rhodes, D. Lipps, H.J. Nucleic Acids Res, 43, 8627 (2015)

G-Quadruplexes - stability



In double-stranded structures

- 2 bases, 2 or 3 H-bonds
  (4 bases 4 to 6 H-bonds)

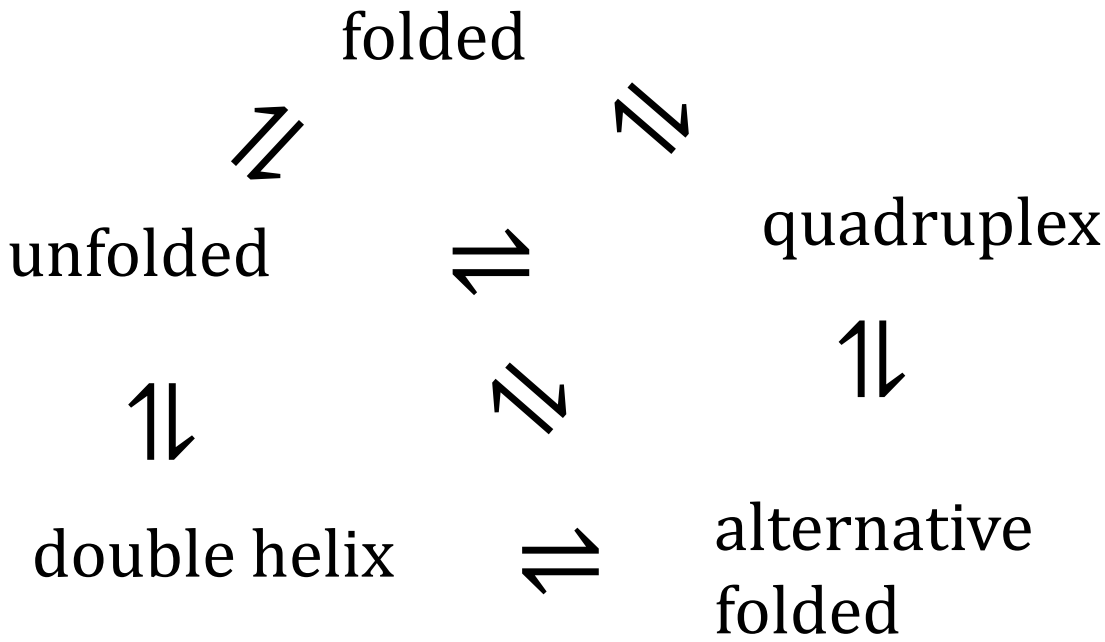
Quadruplexes

- 4 bases, 8 H-bonds
- similar strength to double-stranded

- stacking of guanosines

- implication ?

How important ?



Consider A $\overset{\Delta G}{\rightleftharpoons}$ B equilibrium

- for some sequences, ΔG will favour a quadruplex population

G-Quadruplexes – how common ?

search for **GGGX**$_{1\text{-}7}$**GGGX**$_{1\text{-}7}$**GGGX**$_{1\text{-}7}$**GGG** at DNA level

- 10^5 examples
- conservation of these motifs
- not evenly distributed (DNA examples)

Structure / Biology

in vitro or *in vivo* ? Are they real ?

- lots of *in vitro* examples – crystallography, NMR
- best evidence ?
  - conservation
    implies evolutionary pressure /function

An alternative structure

- changes which groups are accessible
- must affect accessibility / susceptibility to enzymes / regulators

More from Dr Czech

RNA coordinates / nomenclature

As for proteins: PDB format

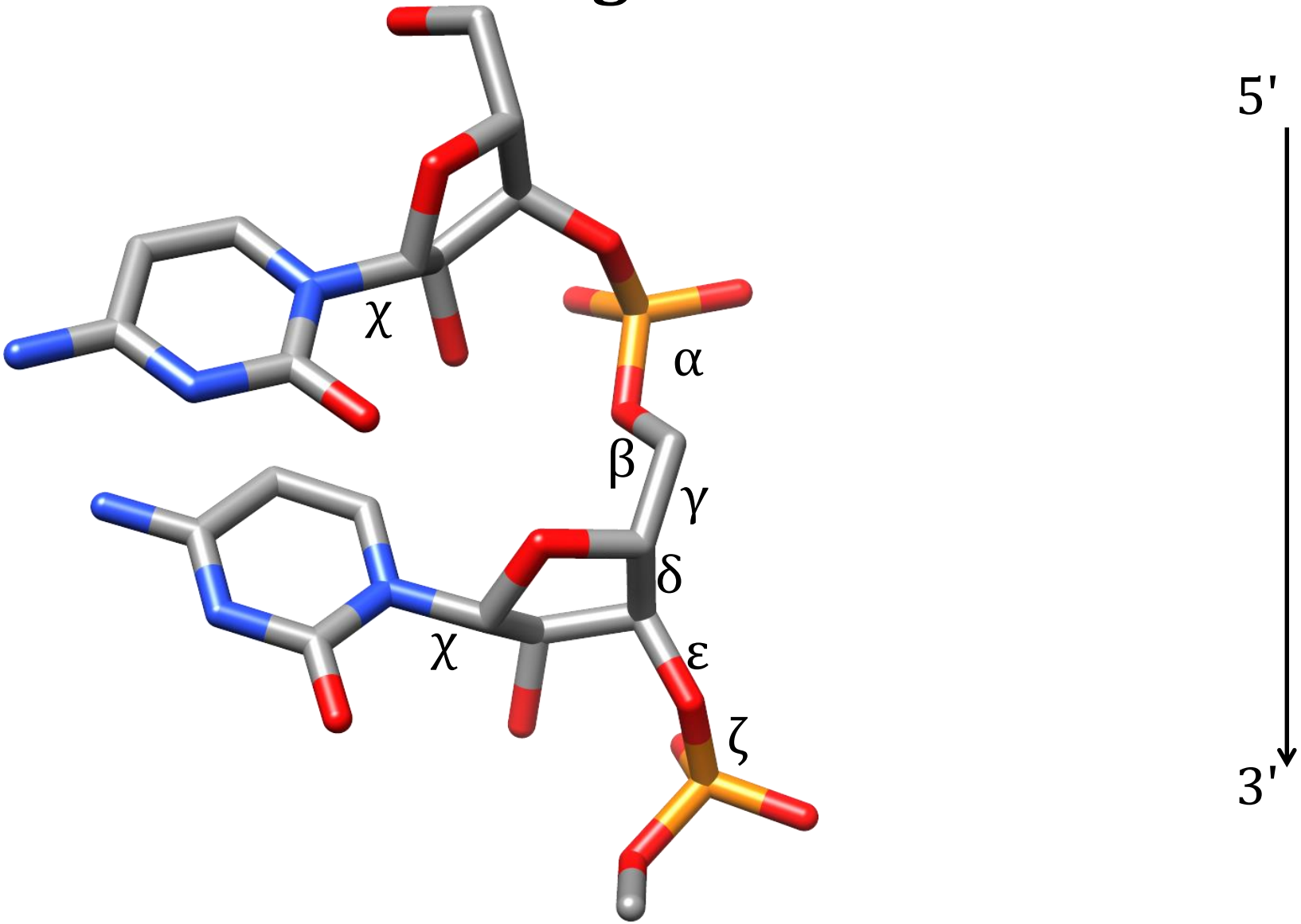
```
ATOM      1  O5*   G A 103      58.355  47.332  91.116  1.00175.32
ATOM      2  C5*   G A 103      57.373  48.210  90.636  1.00175.32
ATOM      3  C4*   G A 103      56.962  47.802  89.224  1.00175.19
ATOM      4  O4*   G A 103      58.148  47.463  88.474  1.00175.34
ATOM      5  C3*   G A 103      56.096  46.543  89.152  1.00175.03
```

As for proteins

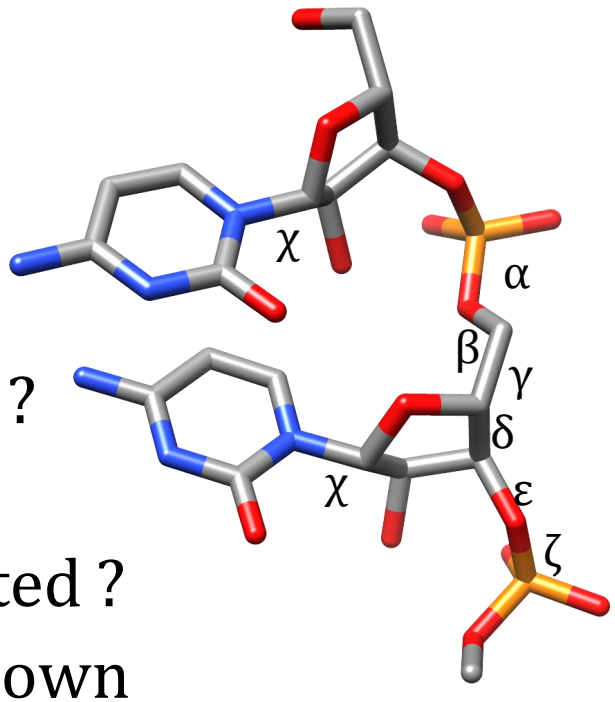
- dihedral angles are useful

Unlike proteins (φ,ψ) there are 6 (α, β, γ...)

dihedral angle nomenclature



dihedral angle nomenclature



6 backbone angles

- α, β, γ, δ, ε, ζ
- χ for base
- too many for me – how to simplify ?

what if two angles are highly correlated ?

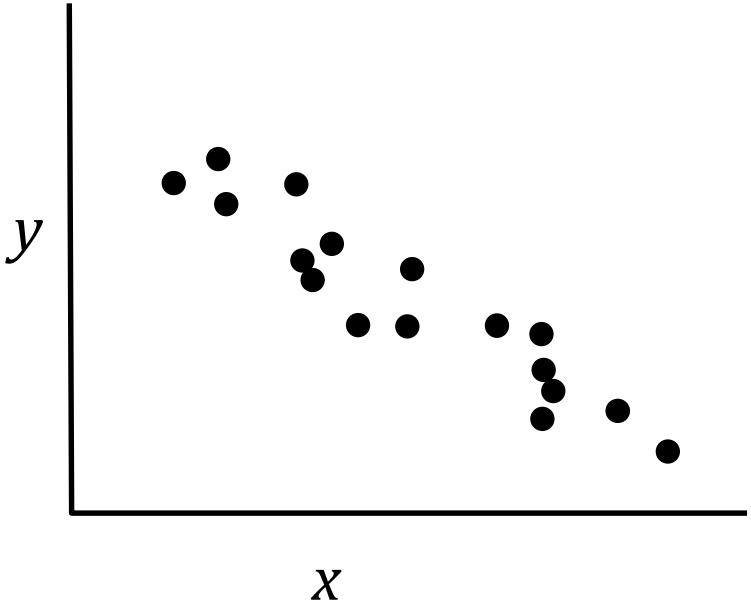
- if we know *x*, then *y* is probably known

ideas for classification…

Describing conformations

The question

- How many variables do I need to describe my data ?



Is this really two-dimensional data ?

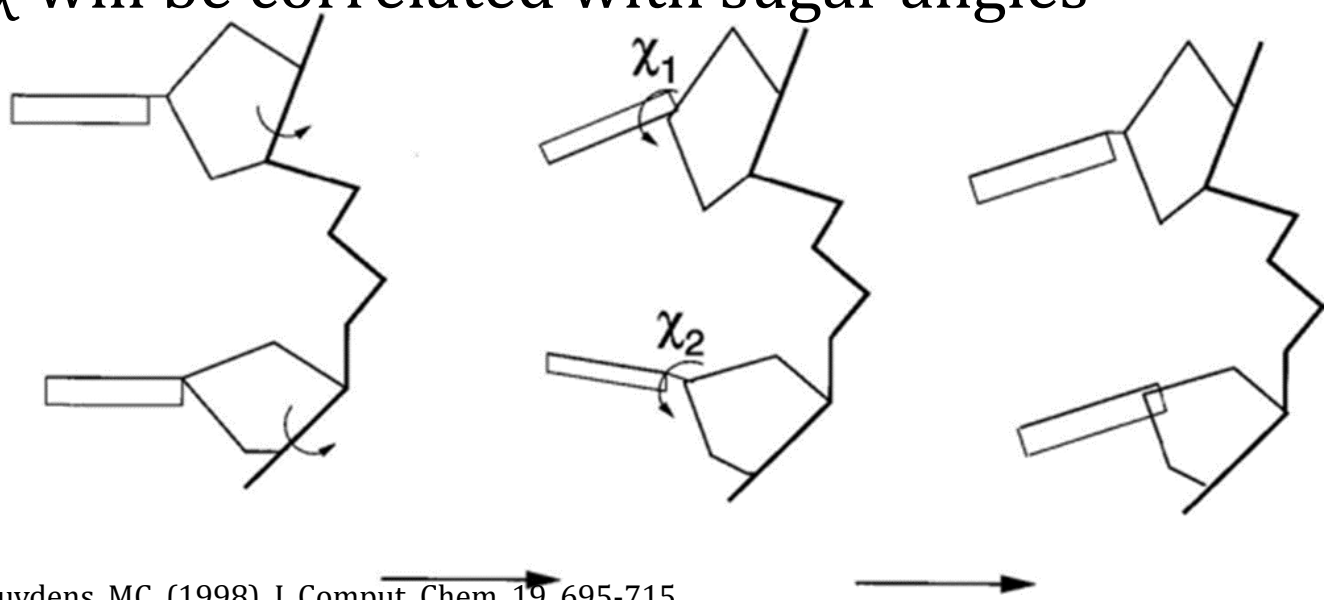
Describing RNA conformation

Example approach – look for correlations

- principle component analysis (quick detour if necessary)

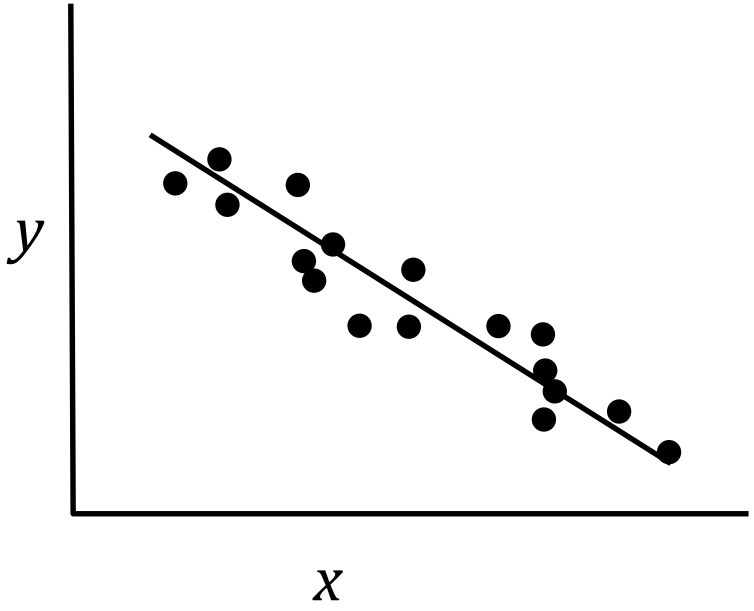
What if sugars move in two residues ?

- energetically, would like to maintain base pairing…
- sugar angles move, χ will compensate
  - χ will be correlated with sugar angles



Beckers, MLM & Buydens, MC, (1998), J. Comput. Chem. 19, 695-715.

PCA reminder



I have two dimensional data

- could well be described by a first (component) and
- maybe second component

n-dimensional data

- how much of variance is described by 1st, 2nd, … components

Consequence – describing conformation

I have many angles ($\alpha, \beta, \gamma, \ldots$)

- the number of interesting variables is much smaller

- people have used reduced sets of variables to describe conformations
- Claim..
  - RNA geometry is well described by 3 angles

- Is this useful ? how can it help you

How would you use reduced variables ?

- Collect data for all angles
- Use principle component analysis to see what is important

- classify and look for properties with the three most important variables

An alternative

- do not think in terms of classic angles

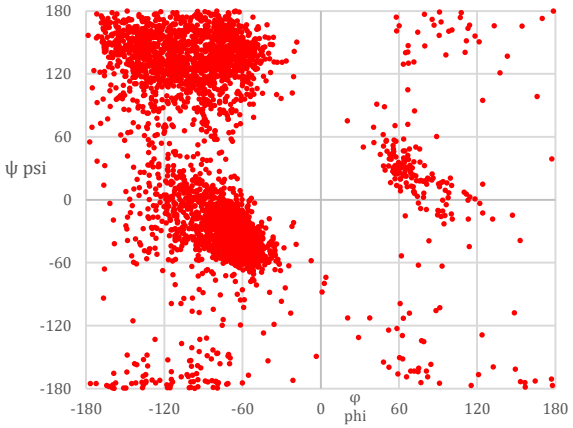
Describing RNA conformation

Alternative…

- do not work in terms of real dihedral angles
- invent reference points
- example study…
  - Duarte, CM & Pyle, AM, (1998) 284, 1465-1478

remember ramachandran plots in proteins

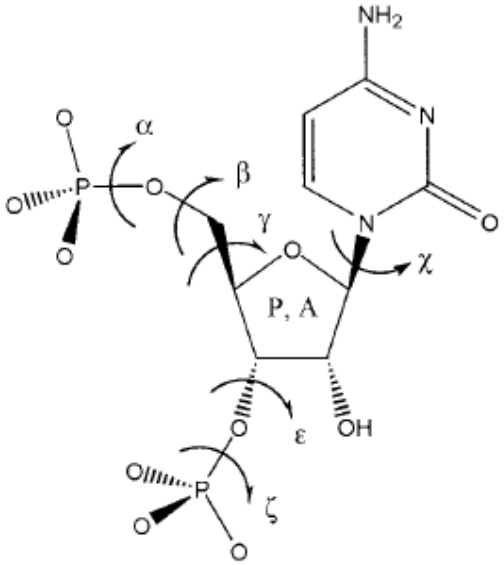
- can one do something similar in RNA ?



Reduced RNA conformation

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}

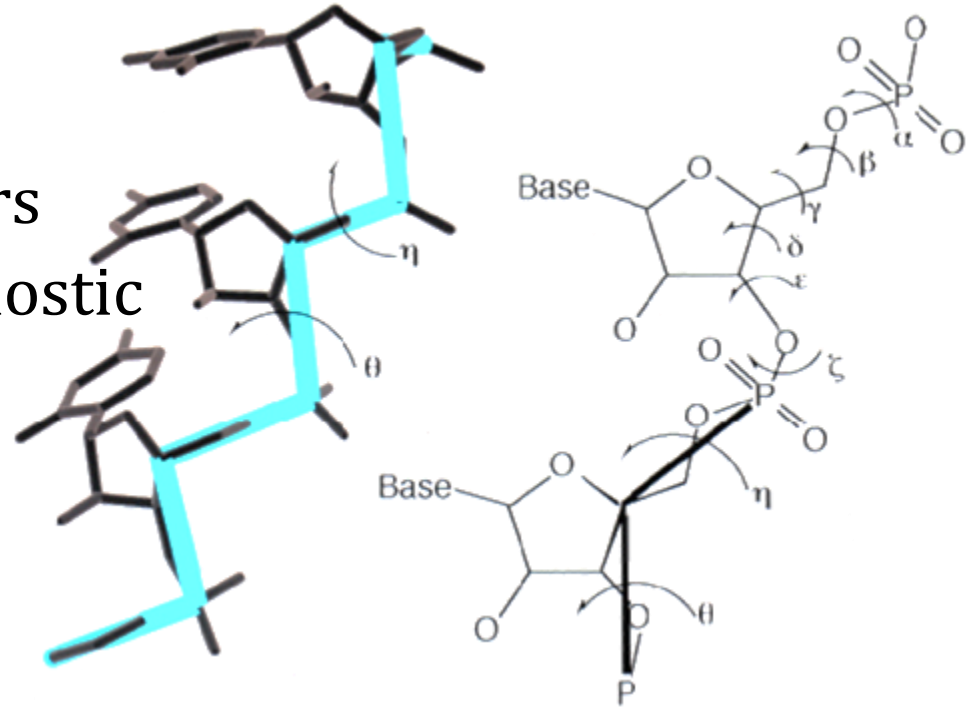
θ

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

Reduced RNA conformation

Plan of authors

- take 52 structures
  - (≈700 nucleotides)
  - collect η, θ
    - see if there are clusters
    - see if angles are diagnostic

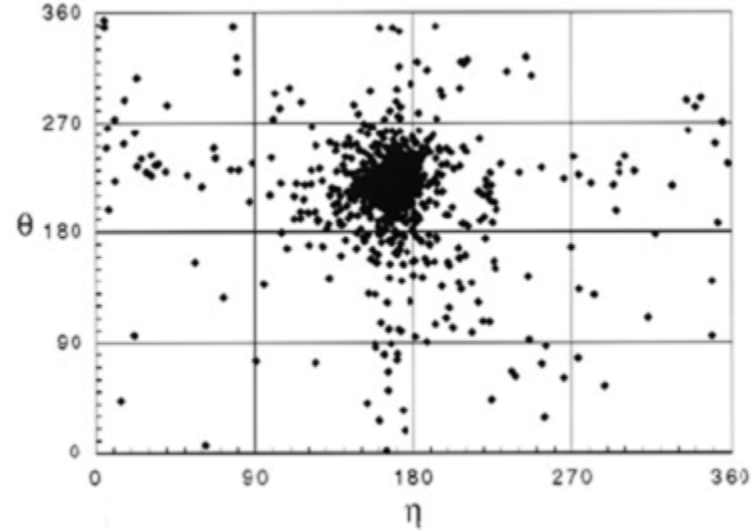


Duarte, CM & Pyle, AM, (1998) 284, 1465-1478

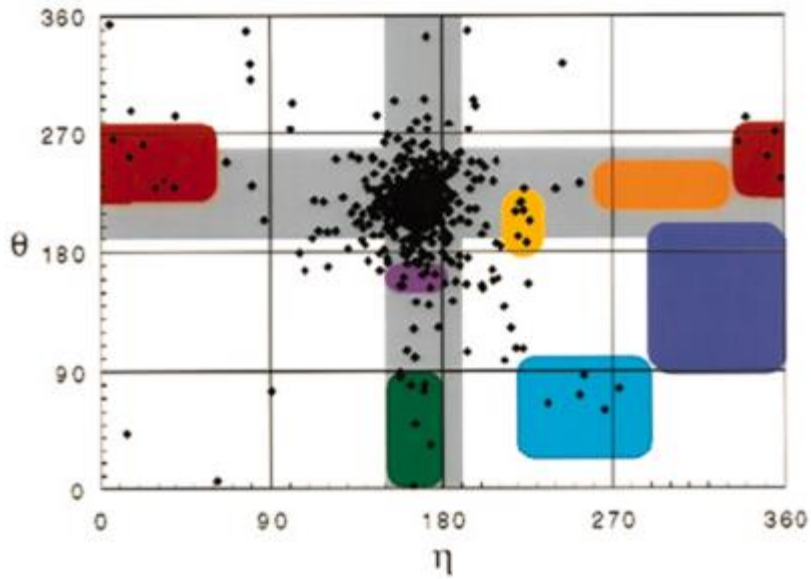
Reduced RNA conformation

Do you see clusters ?

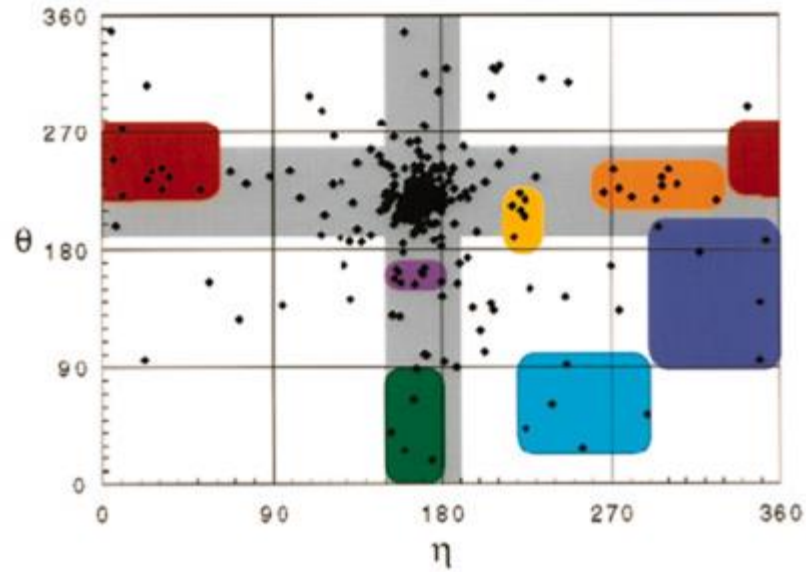
- main set of points …
- boring RNA helix



- a big claim



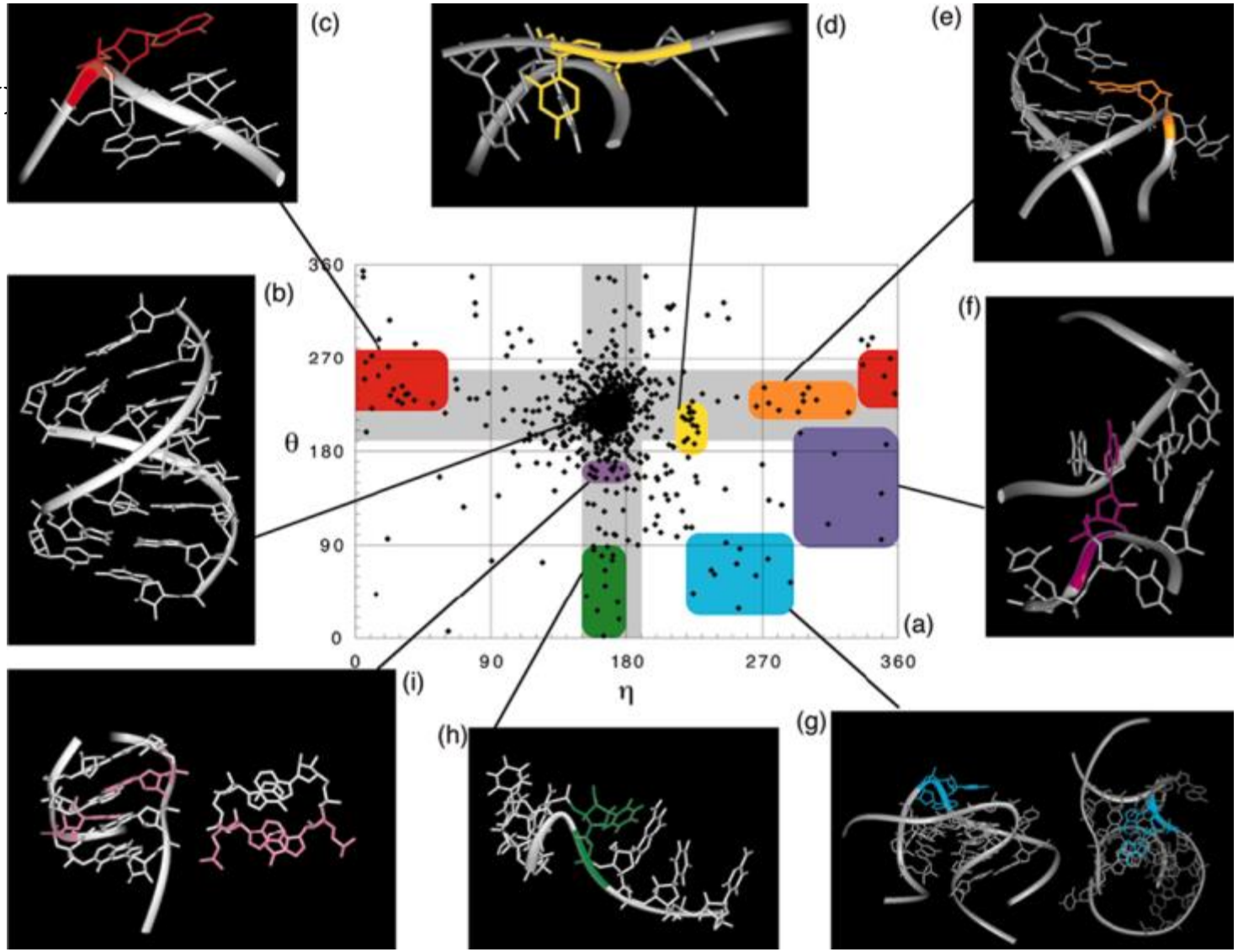
no tertiary interactions



yes tertiary interactions

Duarte, CM & Pyle, AM, (1998) 284, 1465-1478

Reduced RNA conformation



Duarte, CM & Pyle, AM, (1998) 284, 1465-1478

Reduced RNA conformation

We are interested in a critical look at ideas

How to read this…

- if you measure a pair of η, θ pseudo-angles
  - could you guess if something is wrong in structure ?
  - could you use this to categorise the conformation ?
- are there better ways to categorise structure ?

Summary

- RNA structure as per Watson-Crick, old text books
- How are RNA structures different to DNA ?
- What are the biological roles ?
- Where do motifs like quadruplexes / base pairs come from ? Energies
- Is there evidence that they are important
- Can we neatly summarise RNA structures ?
  - see what information (angles) are necessary
  - define alternative angles

- Next..
  - predicting secondary structure

RNA structure, predictions

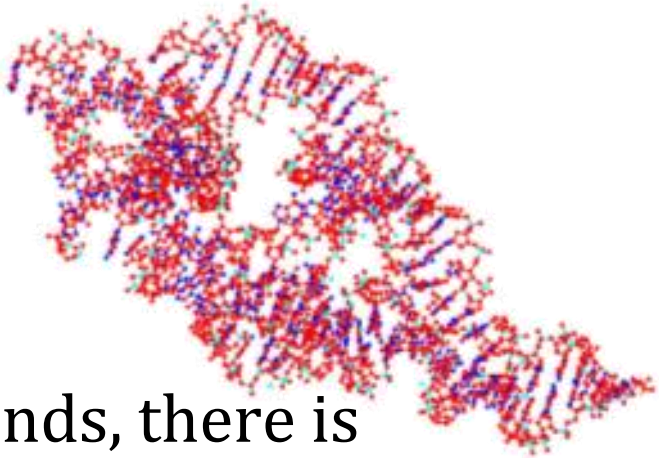
Themes

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

Structure – protein vs RNA

Middle of proteins

- hydrophobic core - soup of insoluble side chains



Middle of RNA

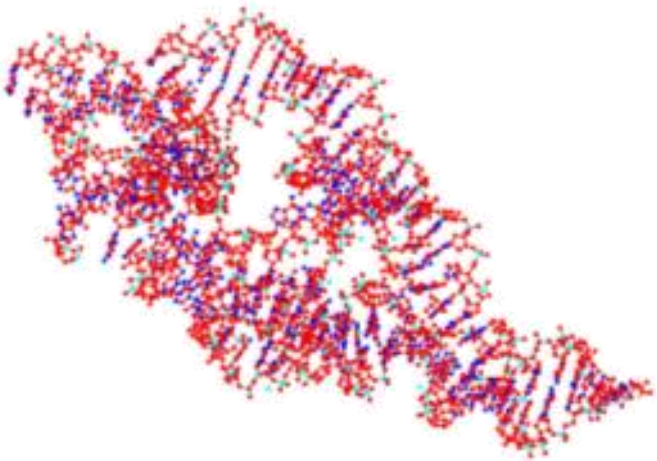
- base-pairing / H-bonds
- much more soluble
  - if something wants to forms H-bonds, there is competition from water

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

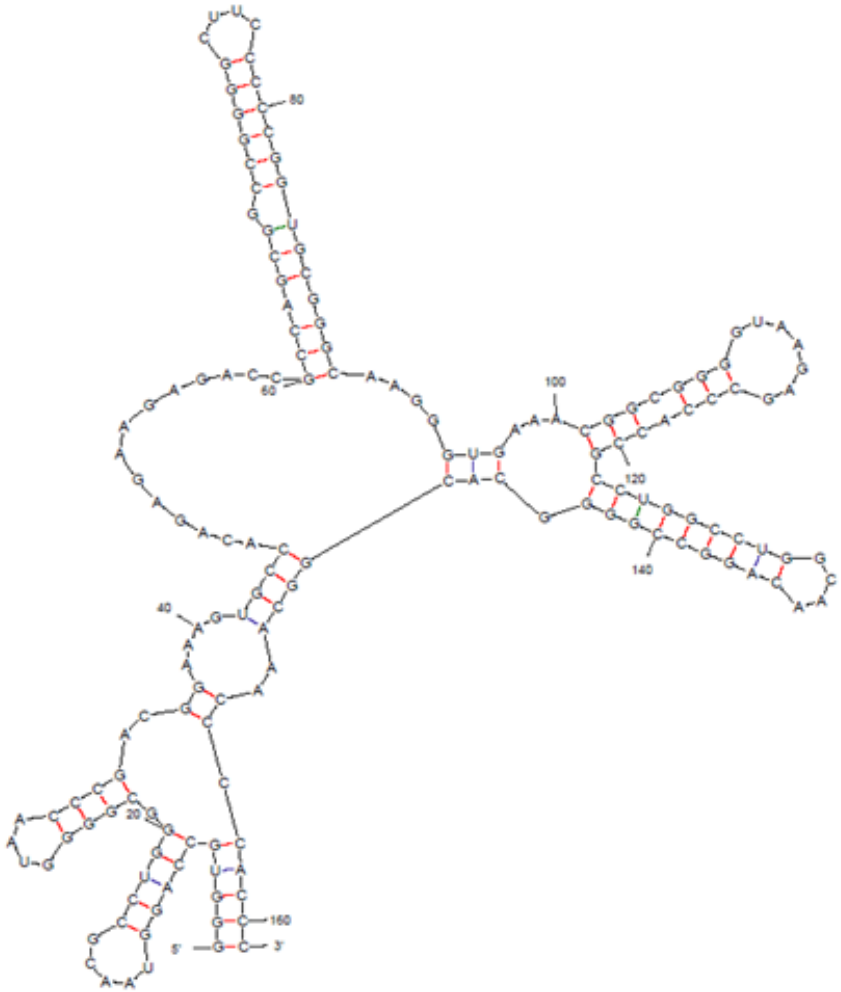


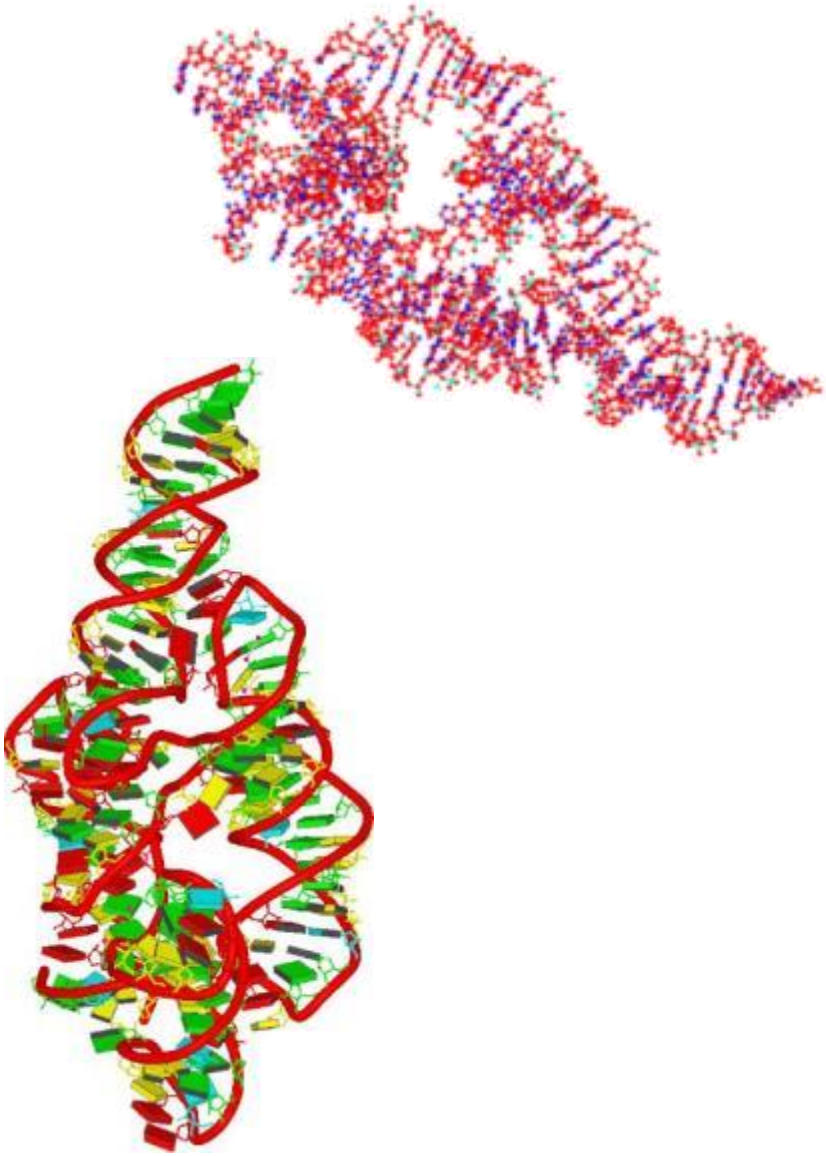
What do we do ?

- mostly ignore it

How realistic is 2D ? How relevant ?

3D versus 2D



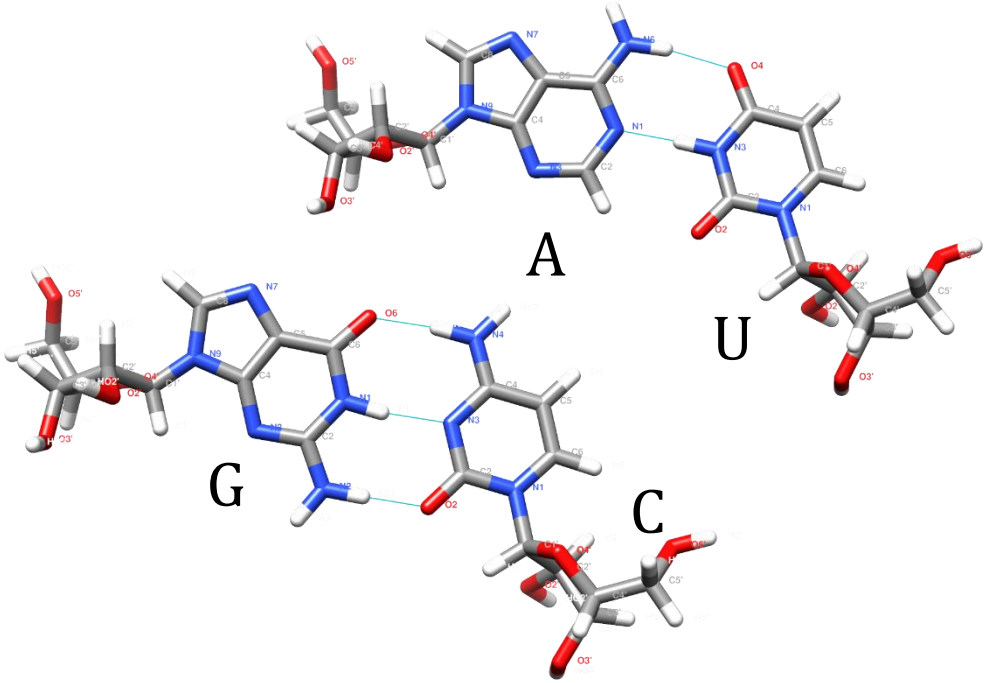


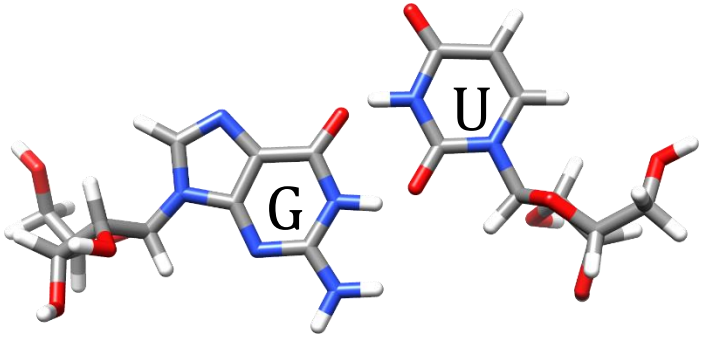
PDB acquisition code 1u9s

2D why of interest ?

1. computationally tractable (fügsam / machbar)

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

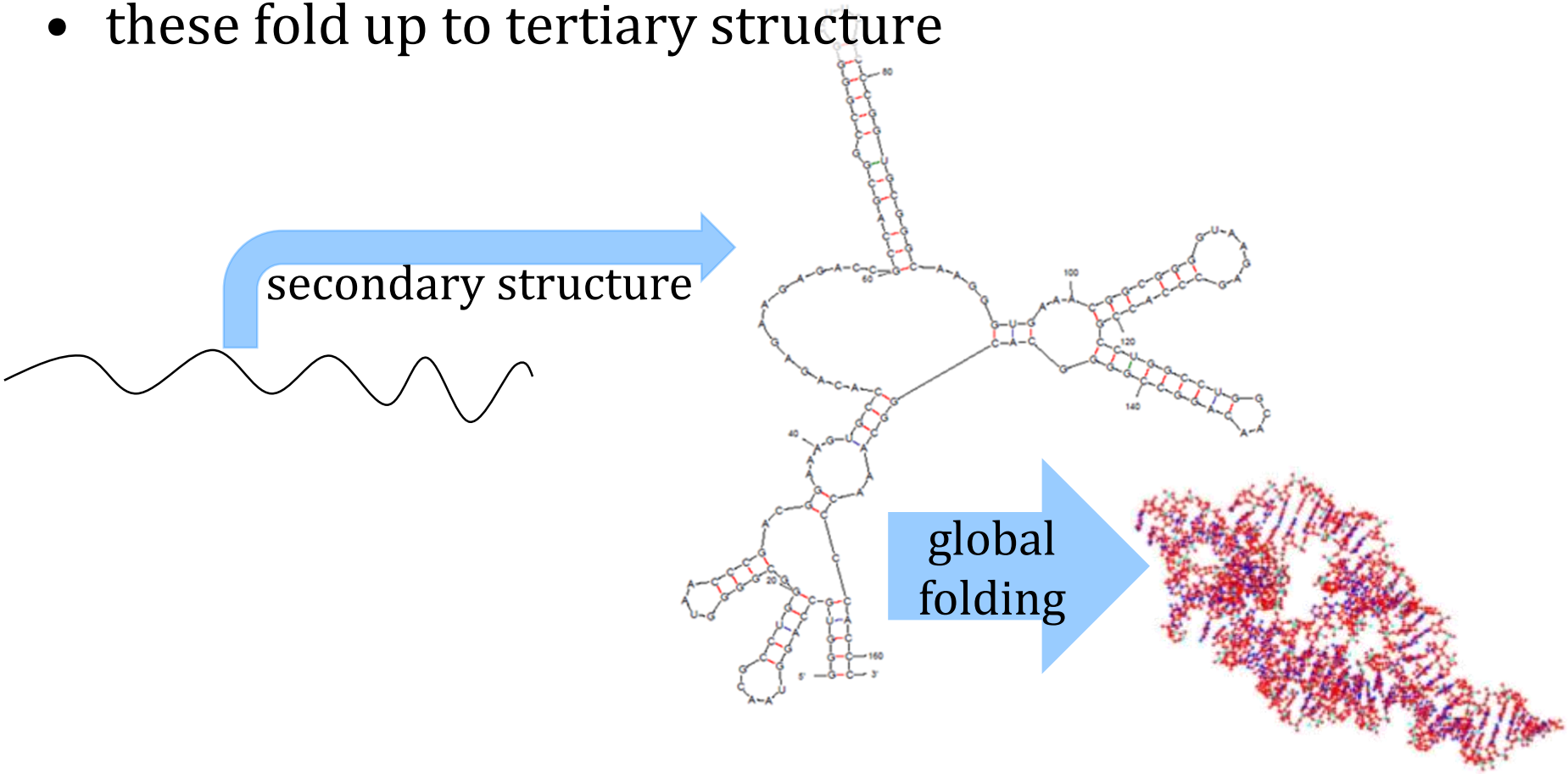




2D why of interest ?

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 ?

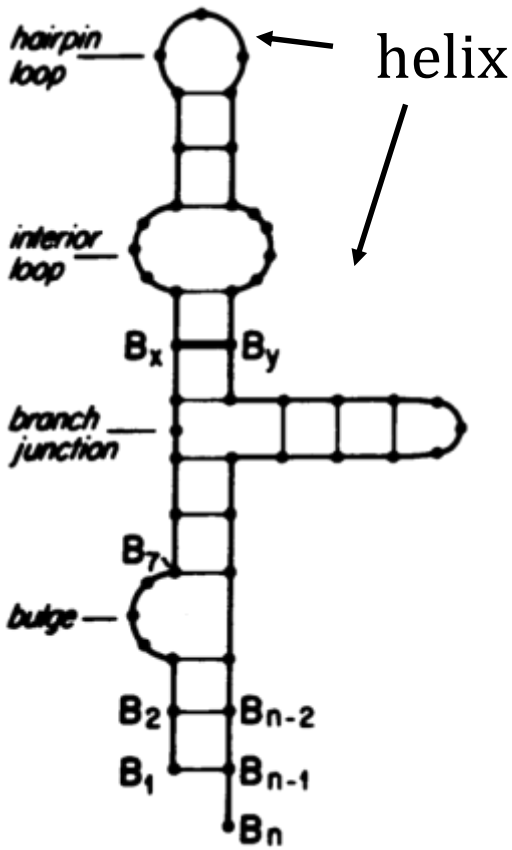
- RNA double strand helices are believed to be stable

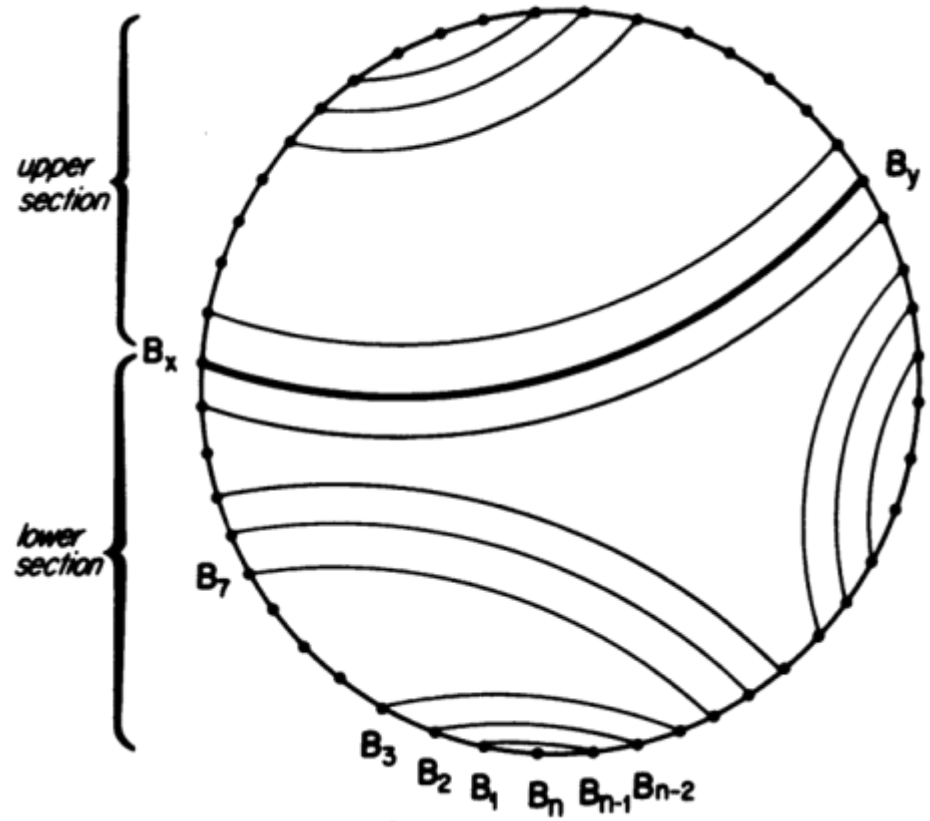
Useful ? if true

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

Four representations of flat RNA

1. conventional





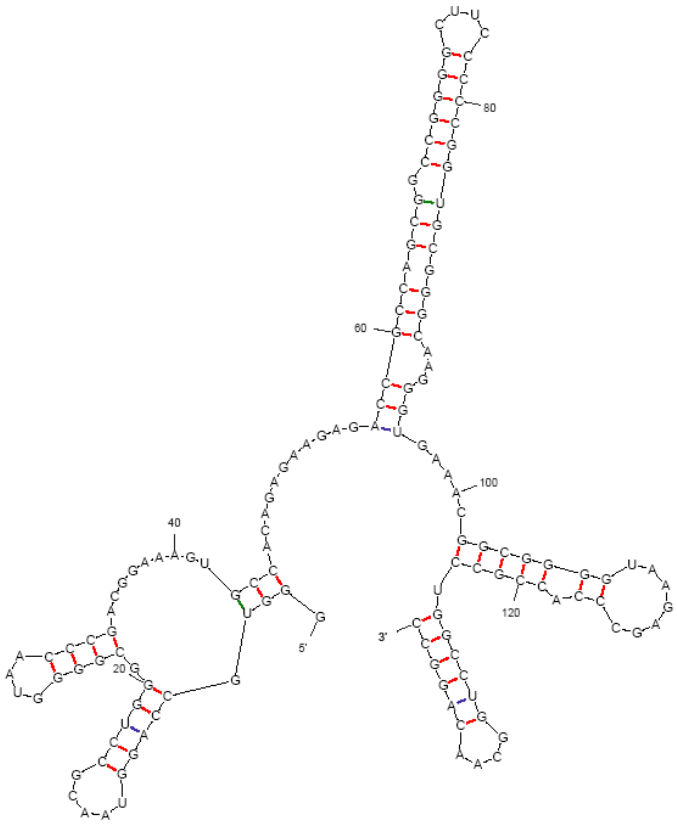
2. Nussinov's

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

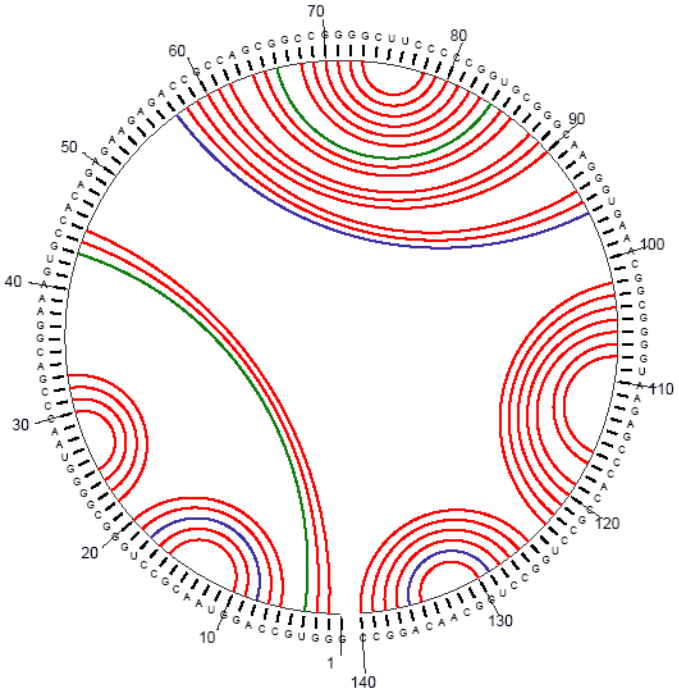
+ on next slide

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

Four representations of flat RNA



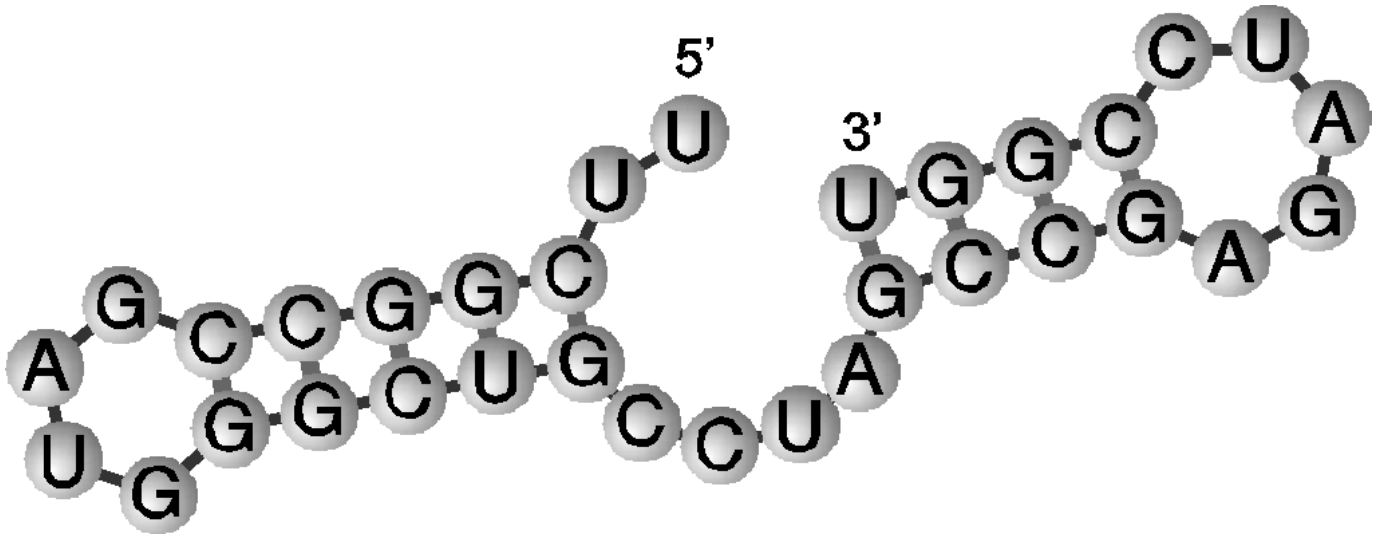
1. conventional representation



2. Nussinov's circle

Same features on both plots

Parentheses



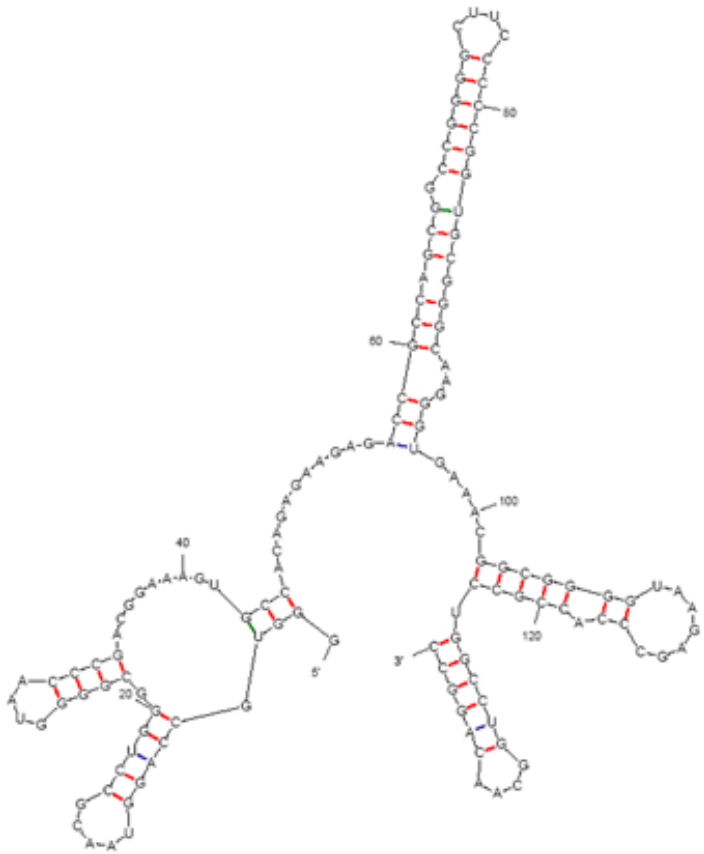
3. parentheses – most concise

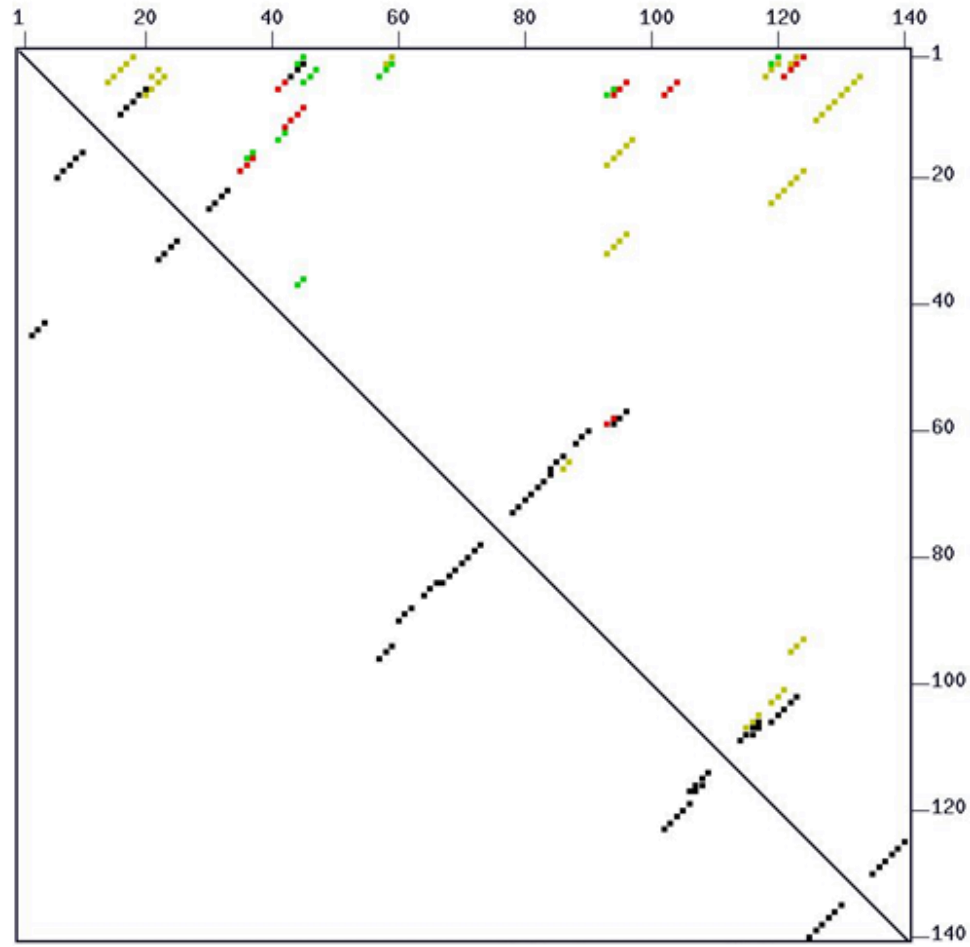
```
..((((....))))....((((.....))))
```

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

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

Dot plots





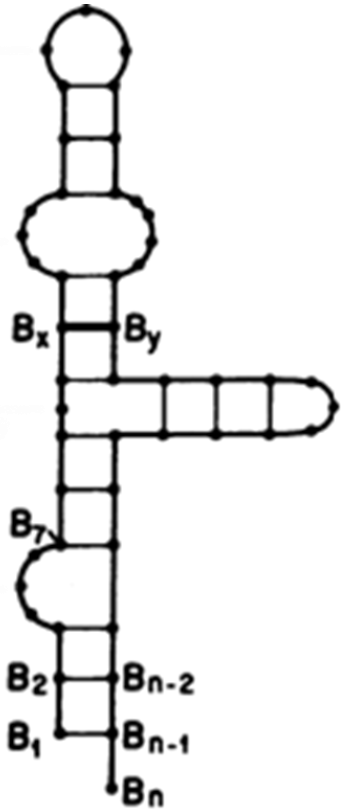
4. Dot plots

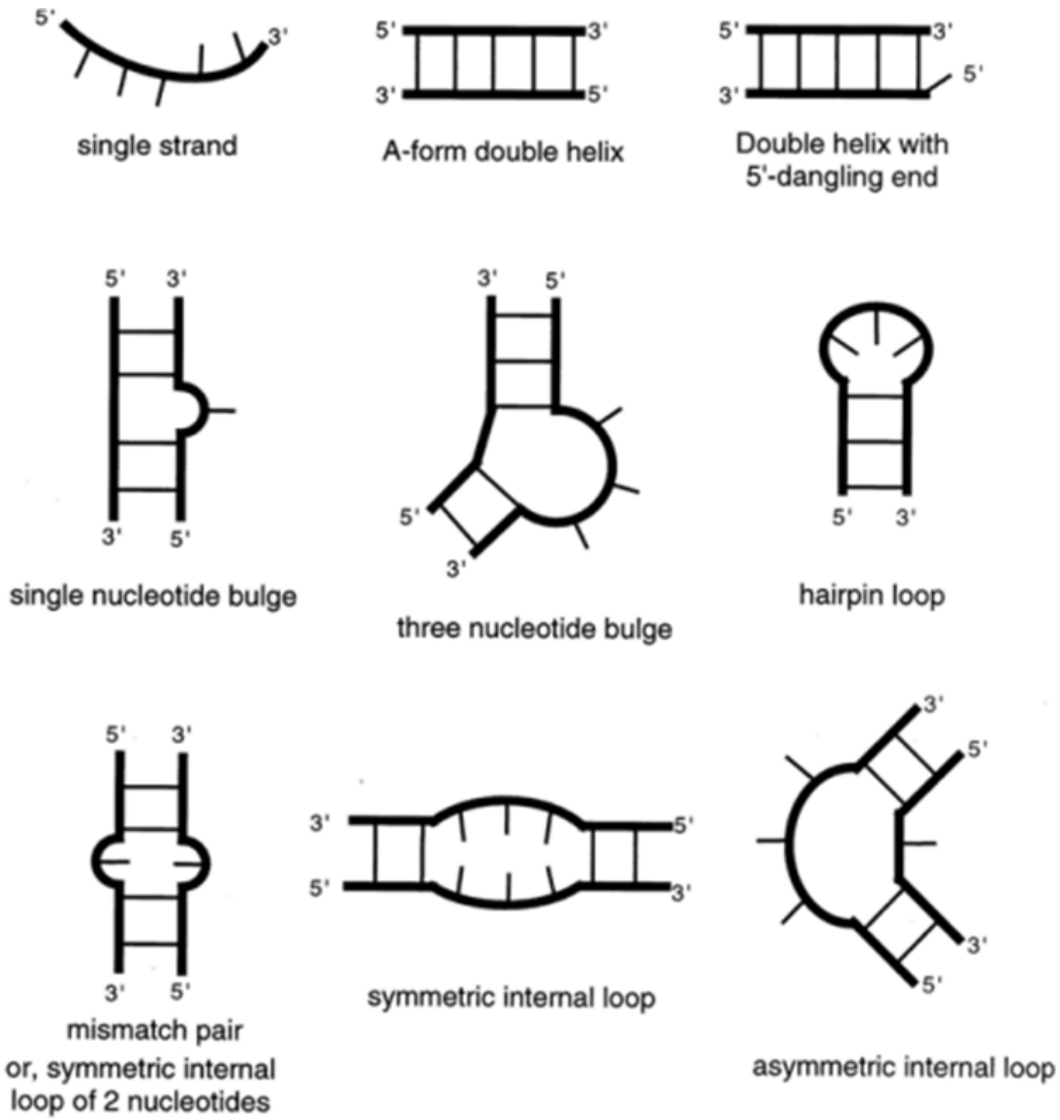
Same features in both plots

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

made with mfold server

nomenclature / features



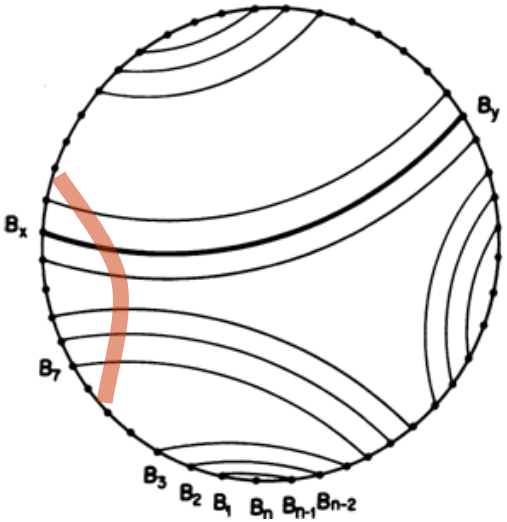


For explanations later

- hairpin loop
- bulge (unpaired bases)

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

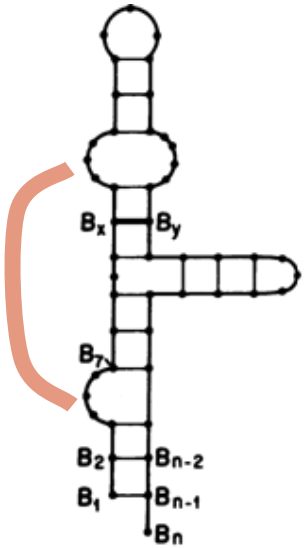


Declare crossing base pairs illegal

- 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 ?

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

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 ?

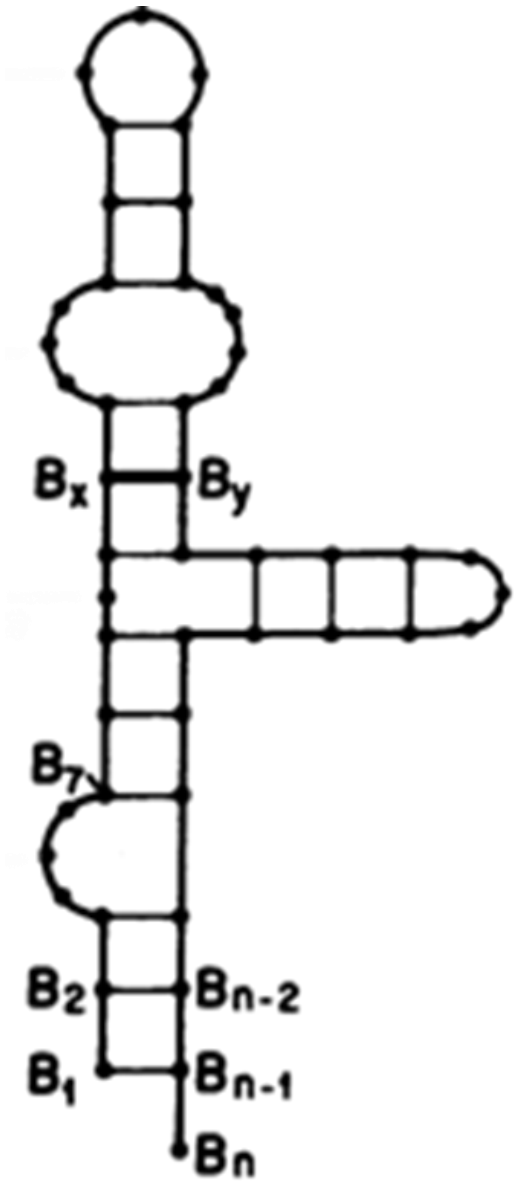
Philosophy

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

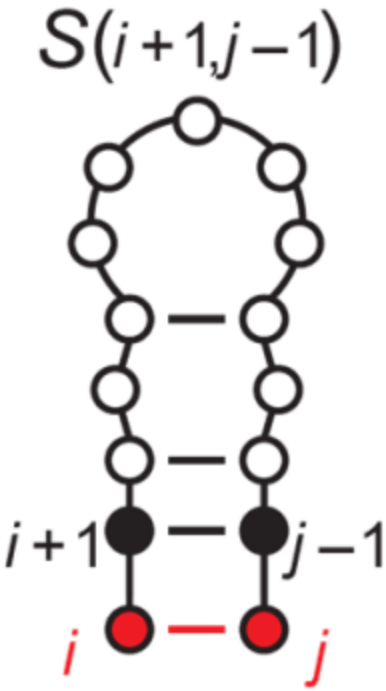
Start by looking for best possible hairpin

If we know the structure of the inner loop

- we can work out the next

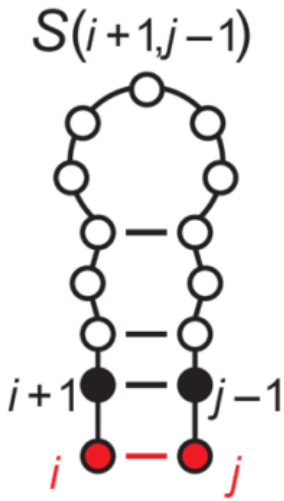
If we know the black parts

- 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

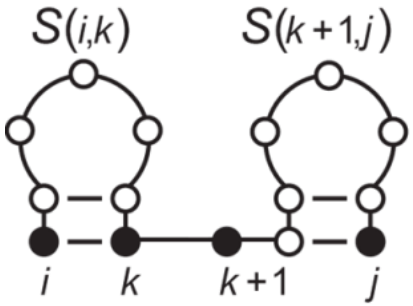


Important idea

- 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

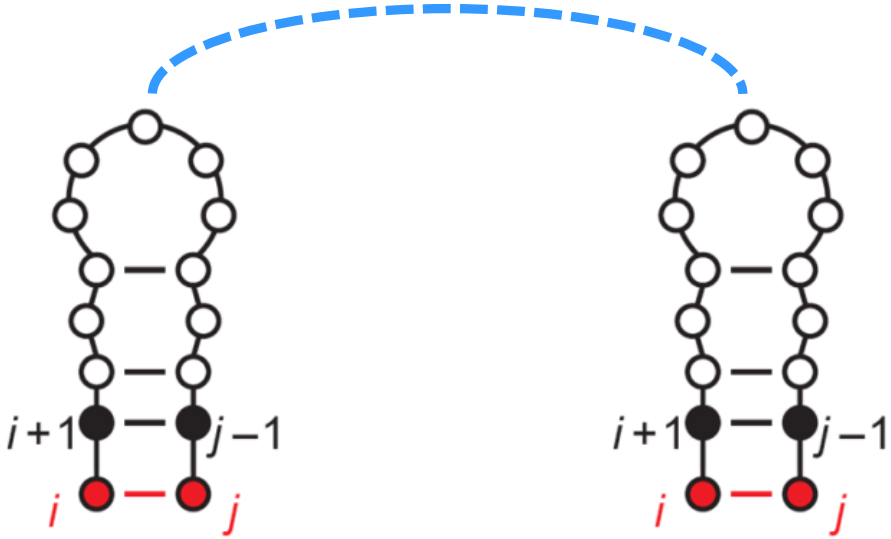
Have we considered .. ?

No !

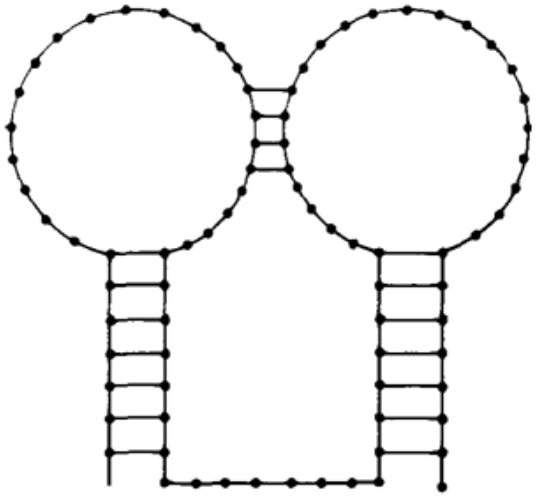
Name – pseudoknot

Do we worry ?

- Stellingen – no
- here ? Probably.



Pseudoknots

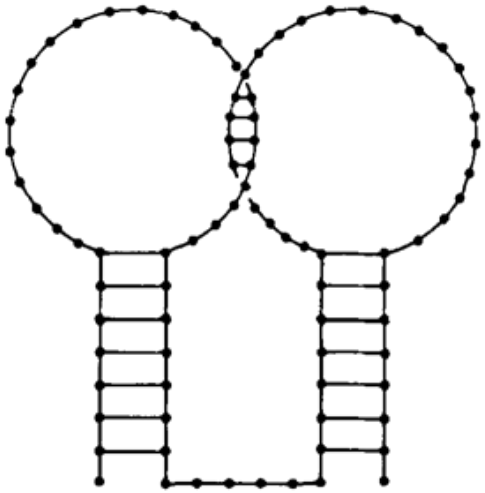


Pseudo-knot – not a knot

- 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

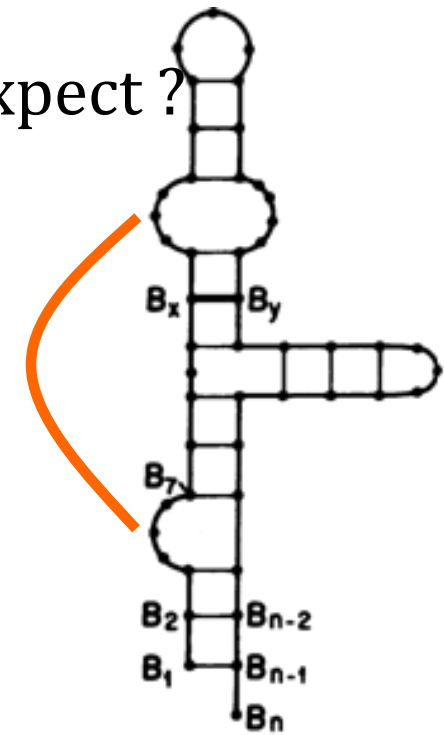
Pseudoknots

Given some unpaired bases, what would you expect ?

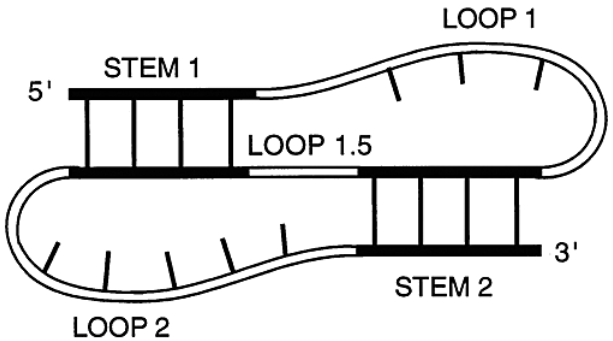
- solvate ?
- form more H-bonds ?
- pack bases against each other ?

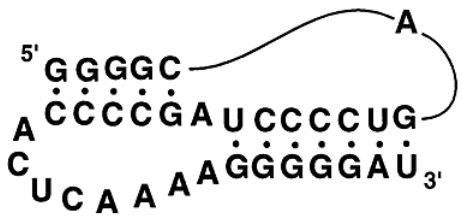
Cannot (practically) be predicted

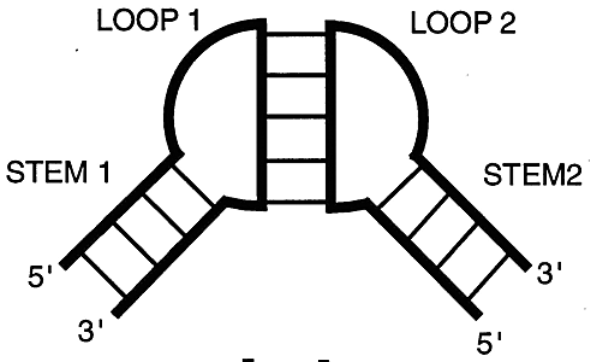
- order of steps in base-pairing methods

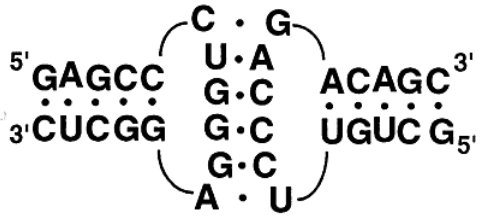


pseudoknots

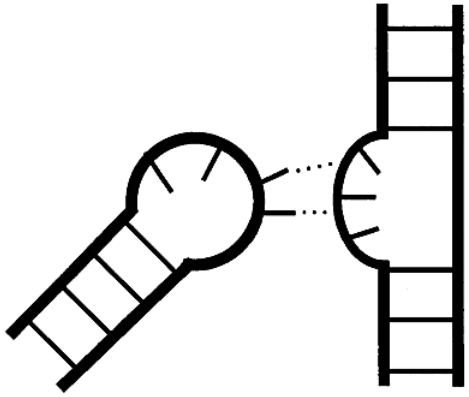


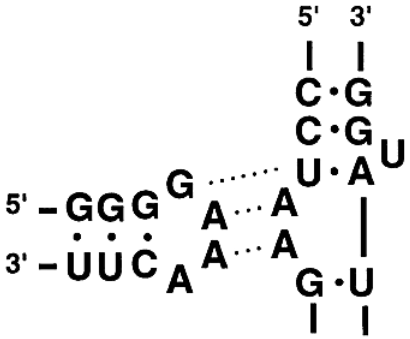






kissing hairpins





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)

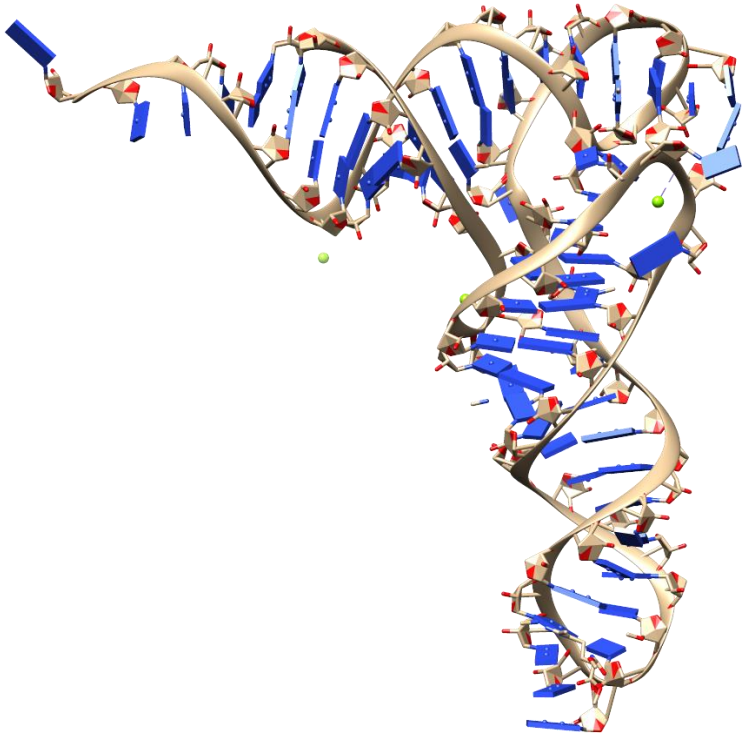
pseudoknots

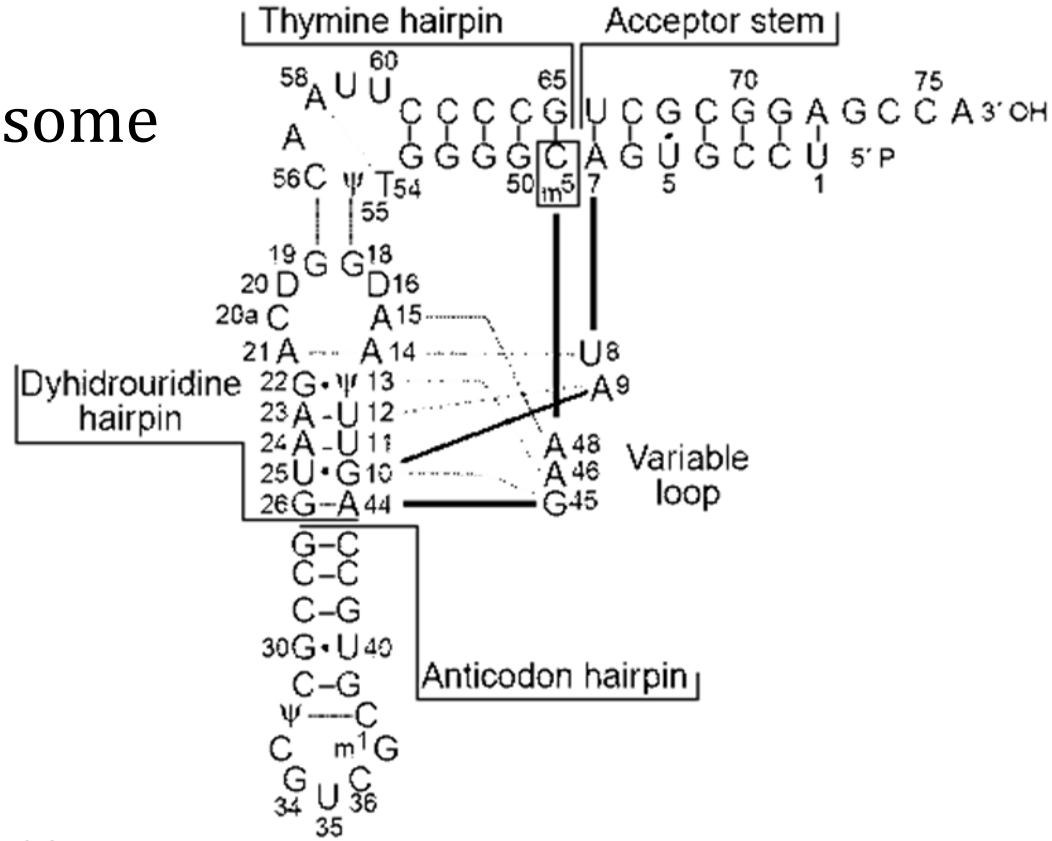
Frequency of pseudoknots ?

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

Significant ?

- most structures will have some
- classic RNA example





Westhof, E., Auffinger, P. in Encyclopedia of Analytical Chemistry R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester, 2000

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

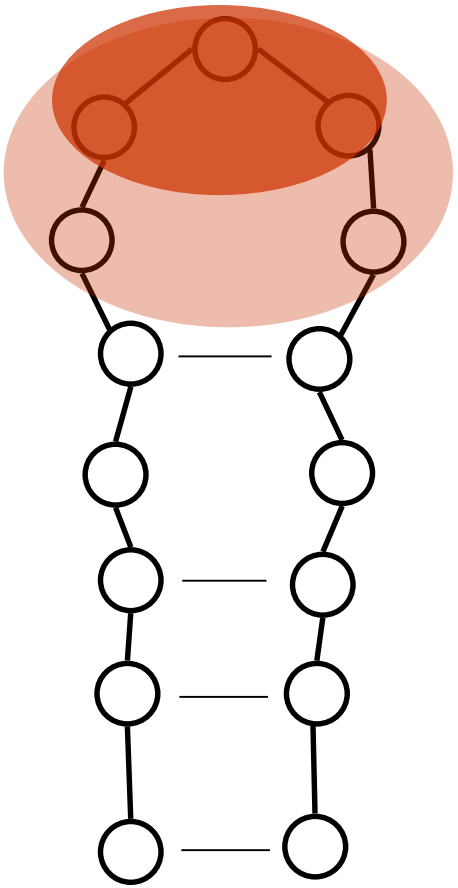
Second improvement

Consider unpaired bases

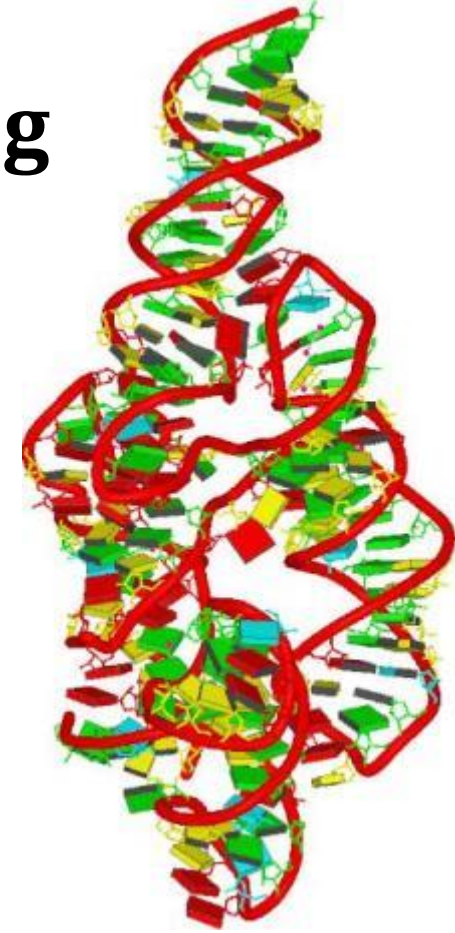
- counted for zero before
- compare loop of 3 / 5 / ..

Do these bases

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



Scoring schemes - stacking



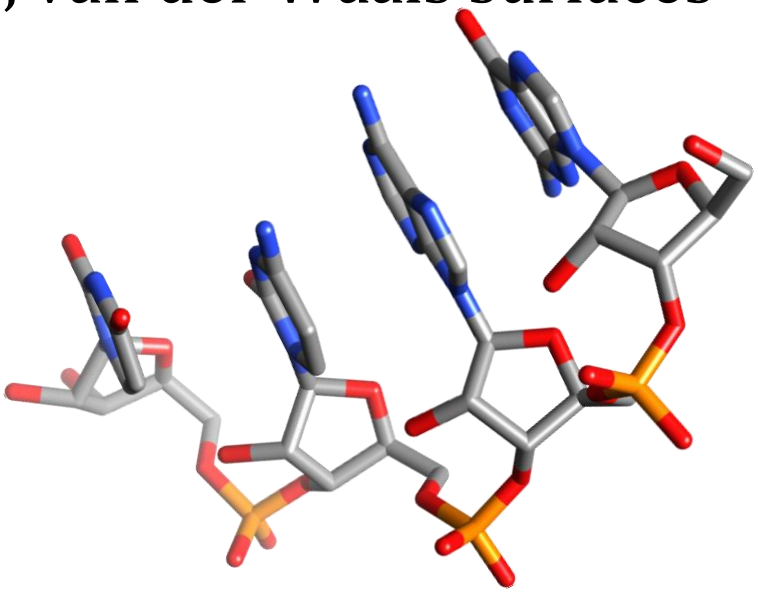
Third improvement

Bad assumption: each basepair is independent

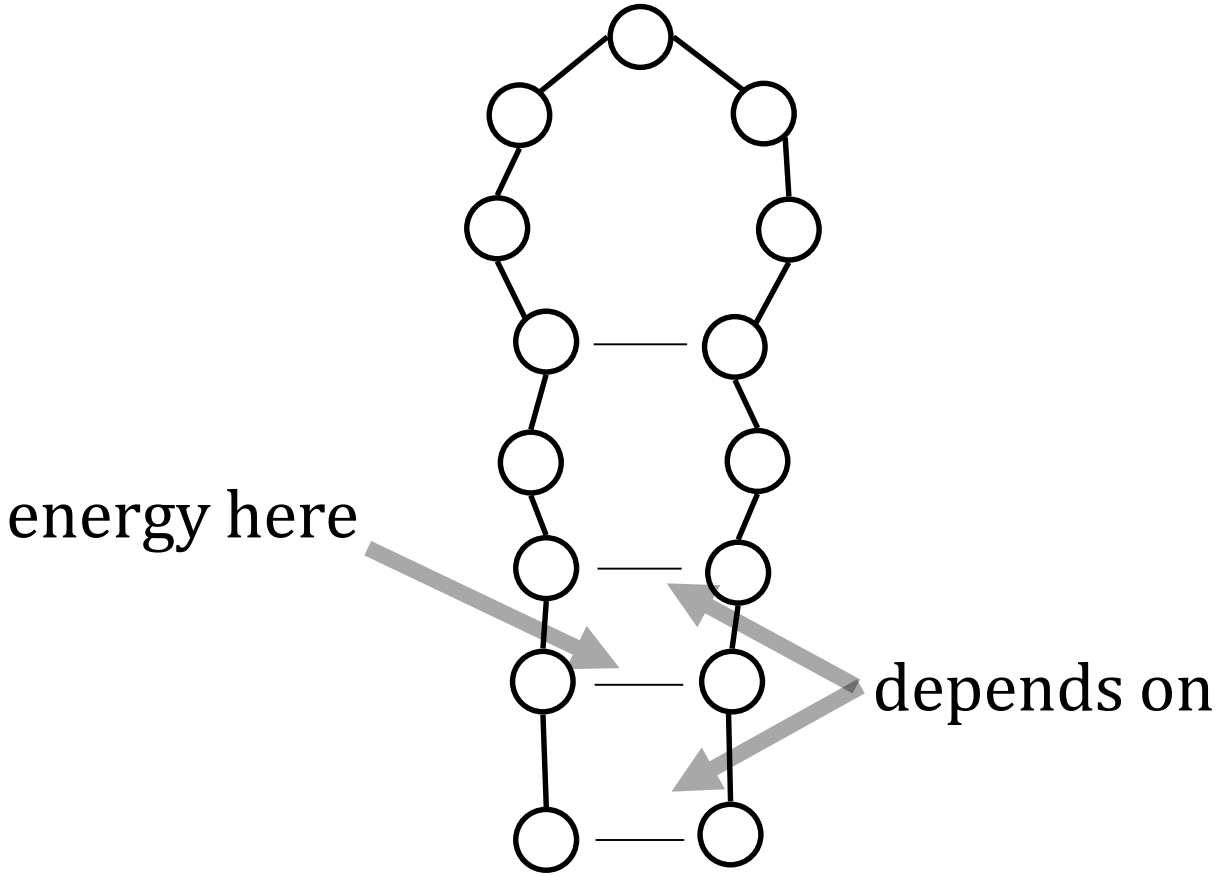
- $S(i,j)$ = base-pair + $S(i+1, j-1)$

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

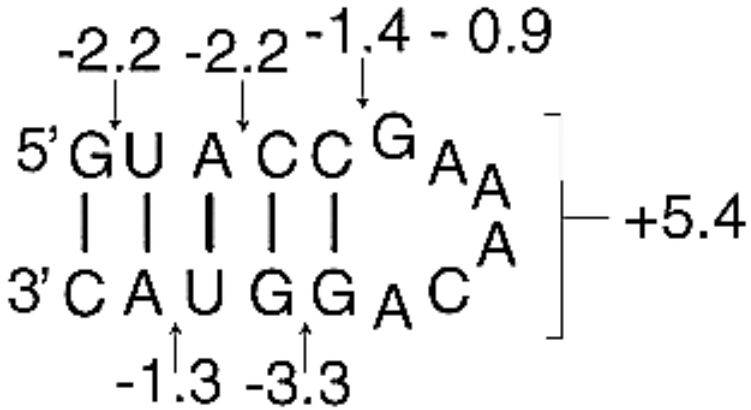
Nearest neighbour model

Previously we added

- GC + UA + AU + …

Now

- (GU/CA) + (UA/AU) +..



- terminal loop costs 5.4 kcal mol^{-1}

Mathews, DH, Schroeder, SJ, Turner, DH, Zuker, M in The RNA World 3rd ed, eds Gesteland, RF, Cech, RT, Atkins, JF, CSHL Press (2006)

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

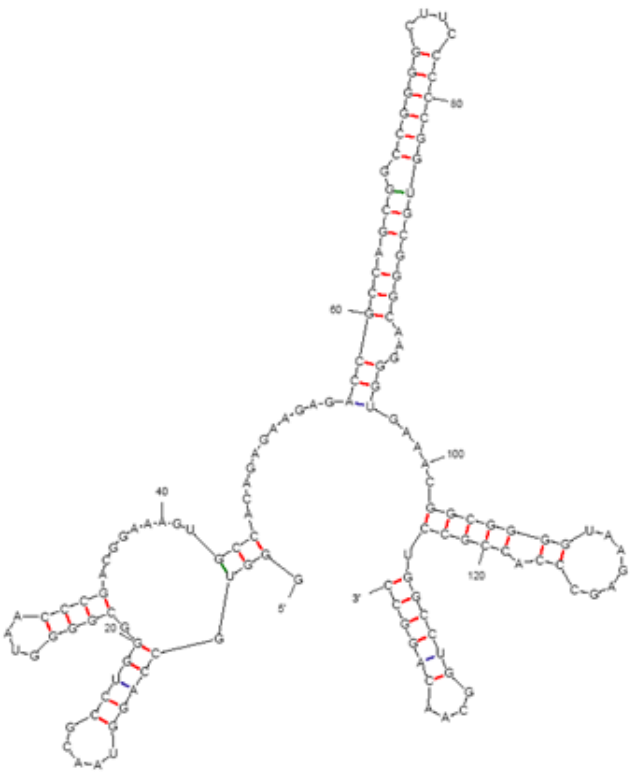
Consider whole sequence

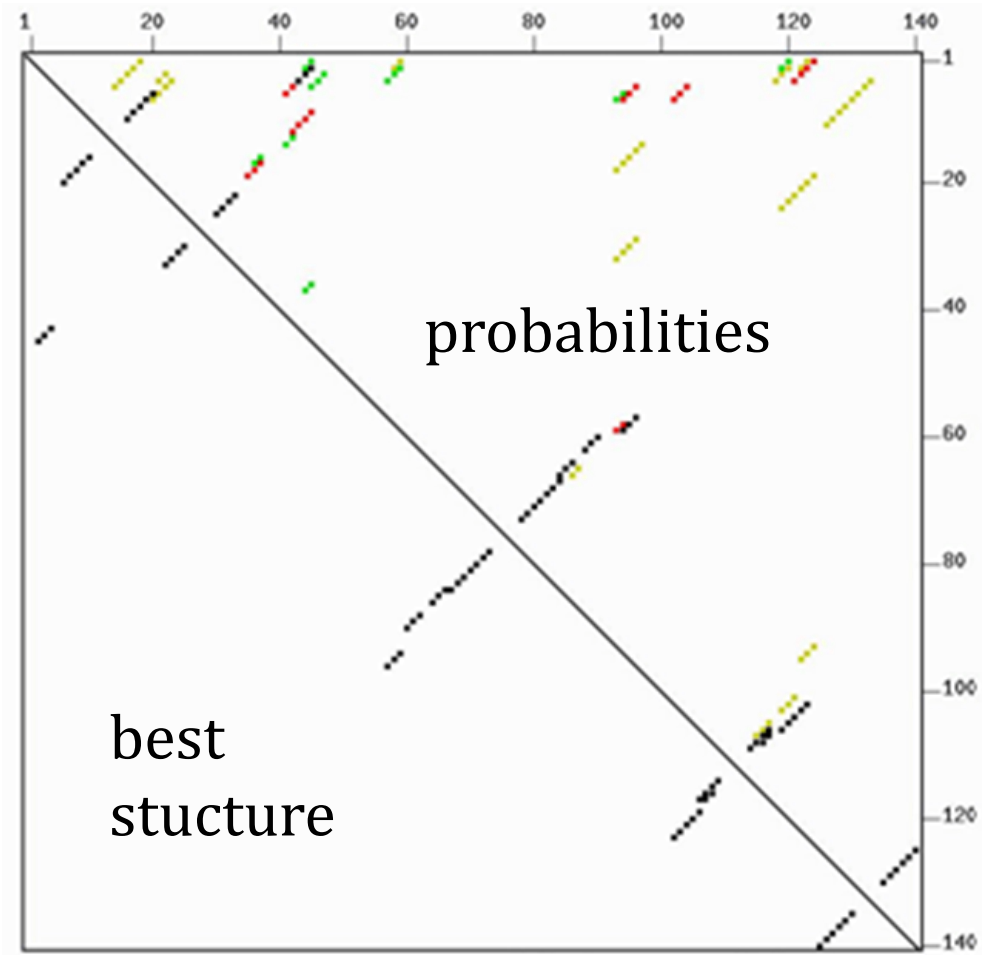
- there may be many structures which are almost as good (slightly sub-optimal)

Treat in terms of probabilities

Probabilities

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



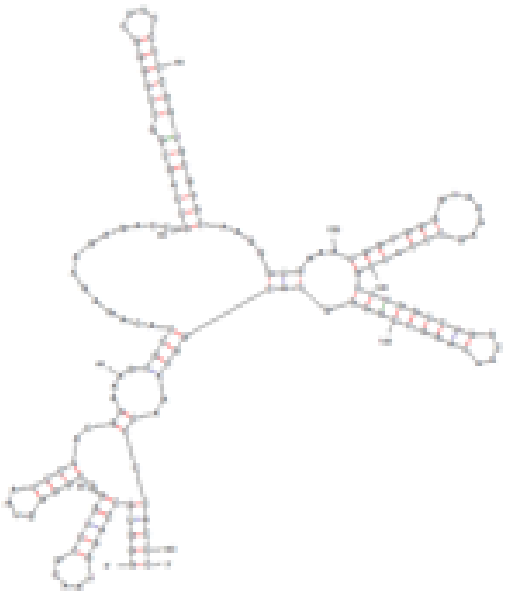


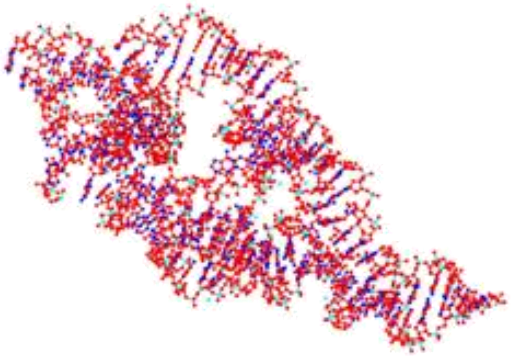
Reliability - Tertiary interactions

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

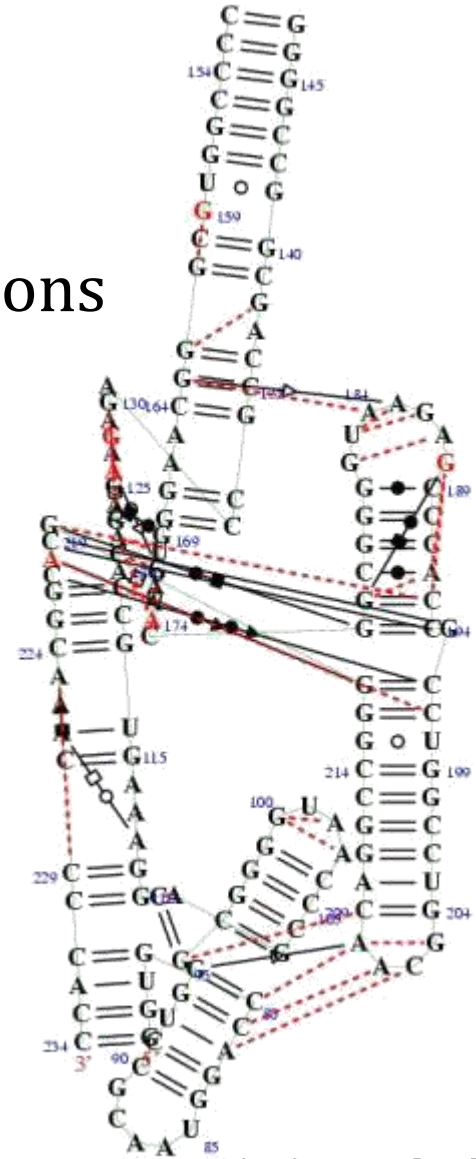
Most larger RNA's have many tertiary interactions

- relatively compact



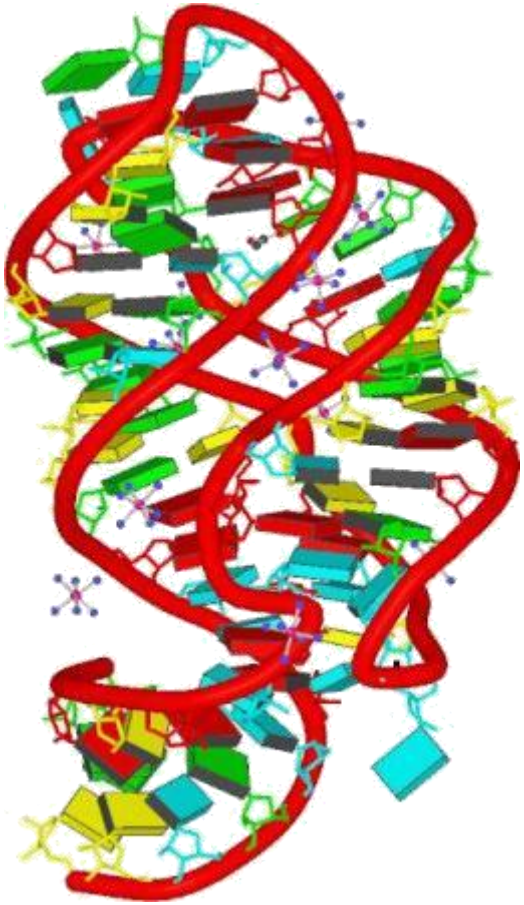


tertiary interactions
from crystal

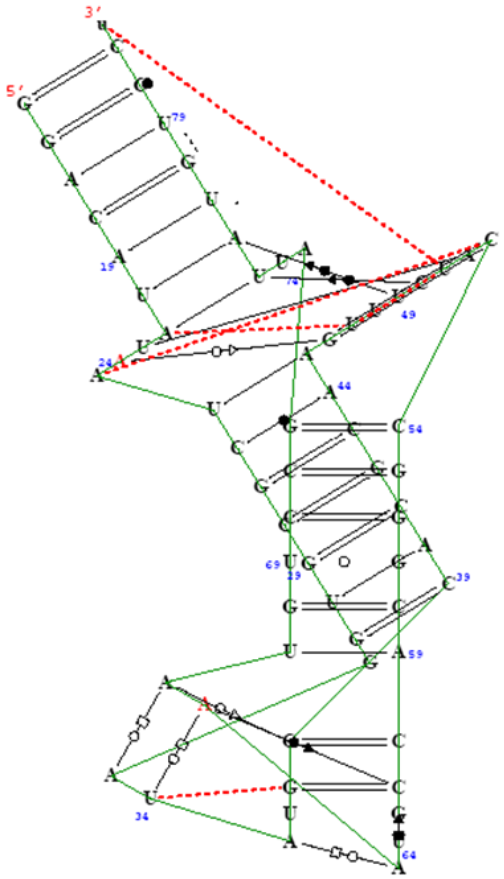


2D vs 3D

2g9c purine riboswitch

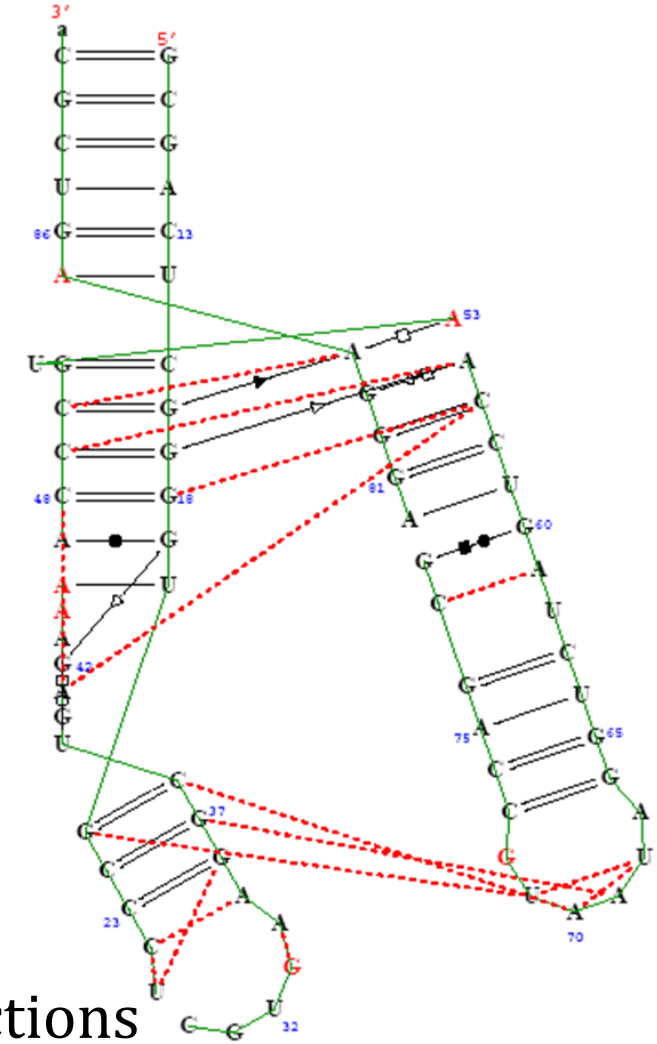


tertiary interactions from crystal



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

Kinetics..

Imagine you can predict 2D structures

- are you happy ?

Two possible scenarios

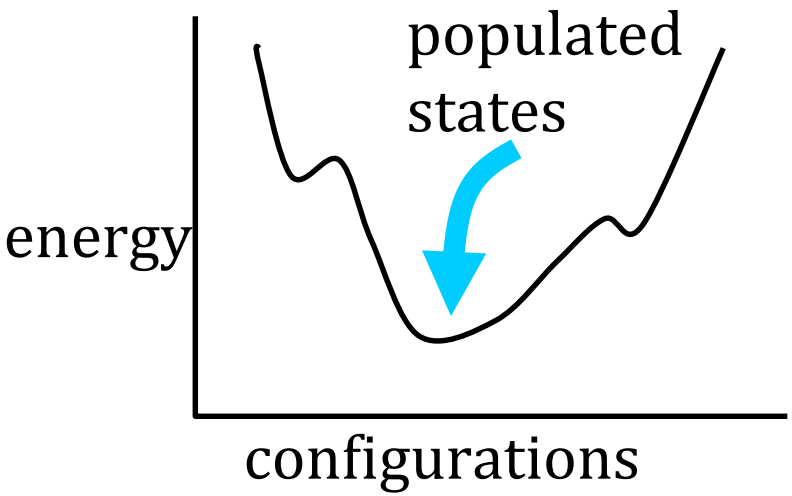
- kinetic trapping
- slow formation

Kinetic trapping

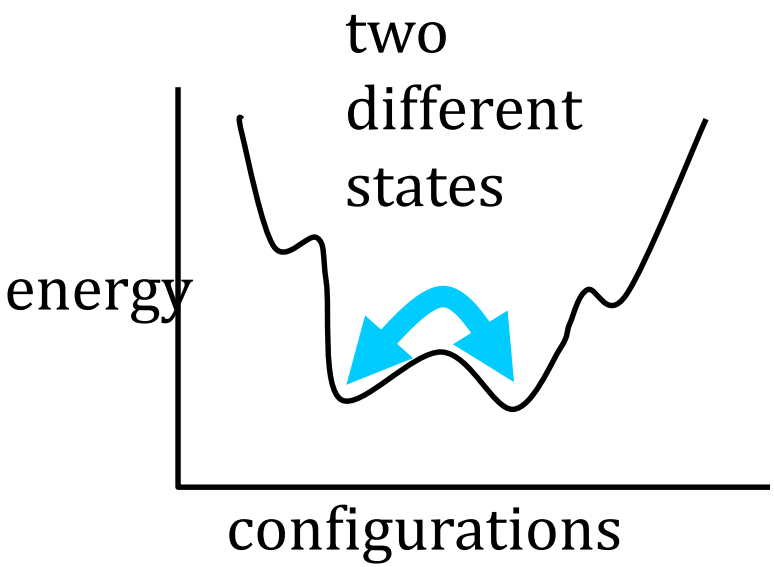
Term from protein world

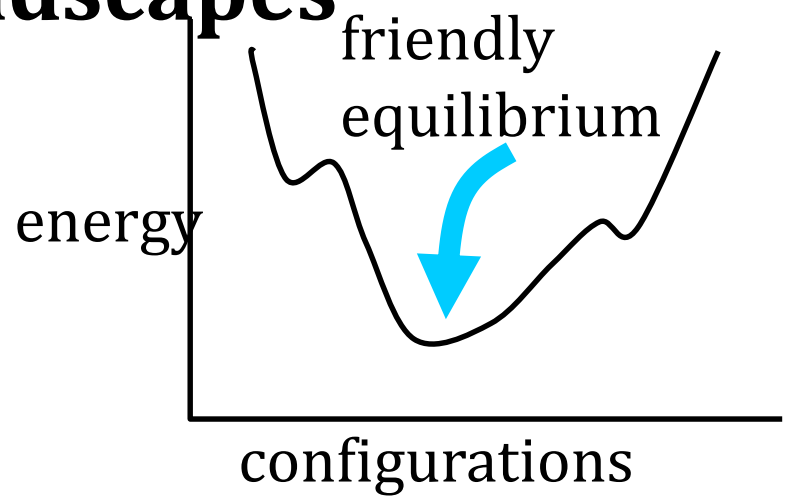
Wherever the molecule is

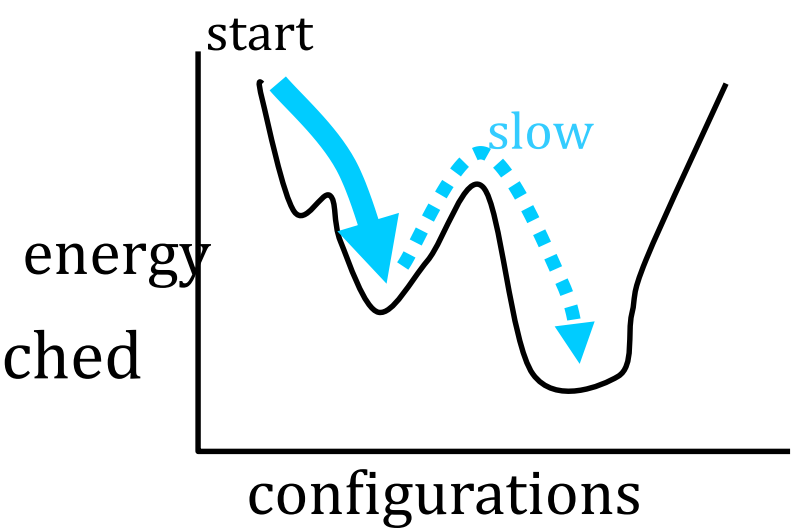
- it will probably go to energetic minimum
- less friendly landscape



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 lots of false (local) minima on the energy landscape

Landscapes / kinetics

Can one predict these problems ?

- not with methods so far

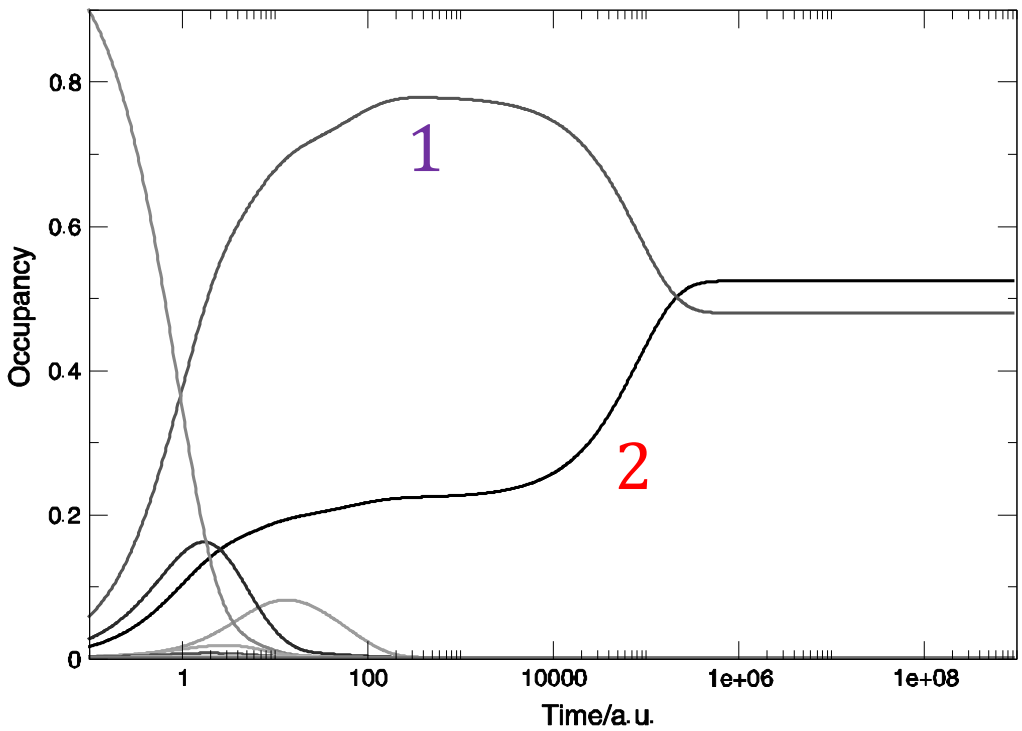
Try with simulation methods

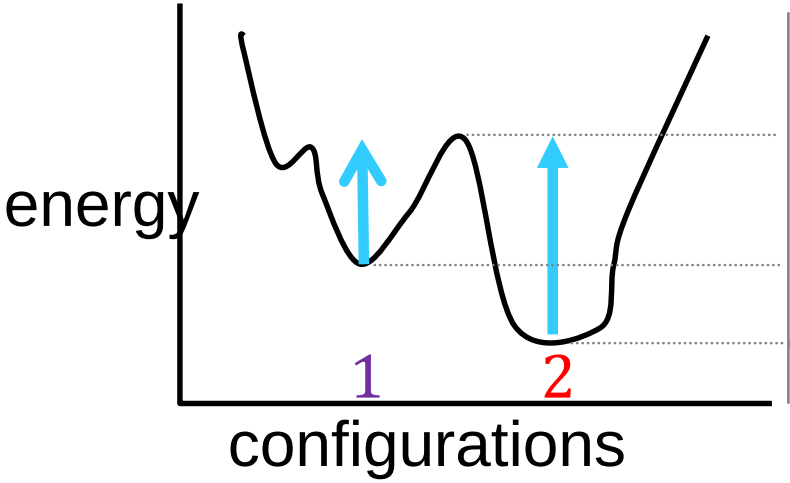
- Monte Carlo / time-based methods

- 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





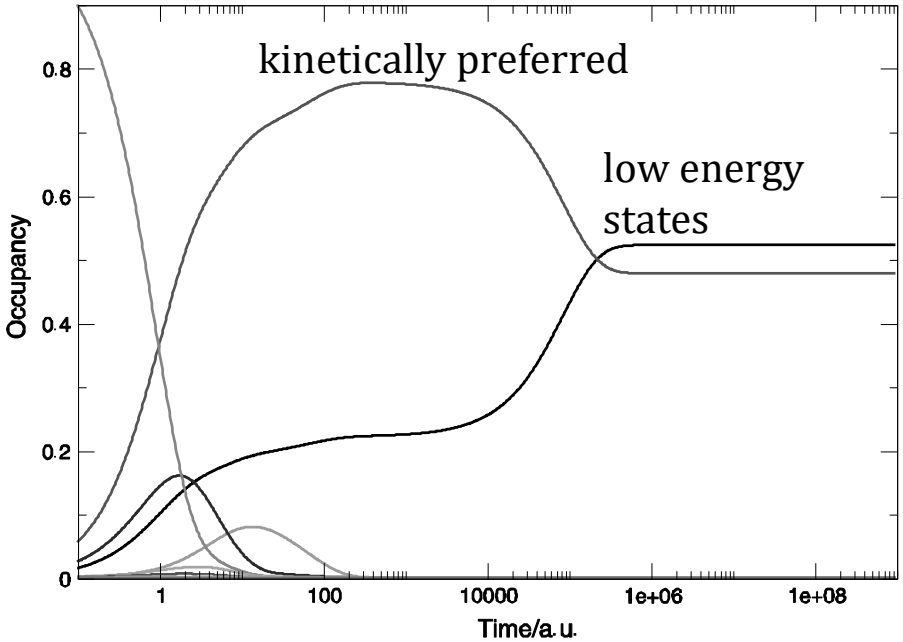
Flamm, C & Hofacker, I.L., Monatsh Chem 139, 447-457 (2008) Beyond energy minimization ...

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)