RNA folding and Matrix Field Theory

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Collaboration with

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Outline

- Review of basic properties of RNA
- Secondary structures
- Matrix field theory for RNA
- Large N expansion
- Recursion relations
- Exact enumeration of RNA structures
- Topological classification of RNA
- Monte Carlo approach

Review of basic properties of RNA

- RNA is a biopolymer
  - RNA (length ~ 70 – 2000)
  - DNA (length ~ 10^6 – 10^9)
  - Proteins (length ~ 10^2)
  - Polysaccharides (length ~ 10^3)

Composition of Cell (in weight)

- Water 70%
- Proteins 15%
- DNA 1%
- RNA 6%
- Polysaccharides 3%
- Lipids 2%
- Mineral ions 3%
- Etc…

Central dogma of Biology

DNA (information storage)

↓ transcription

RNA (information transmission)

↓ translation

Proteins (biological function)

Several forms of RNA

- Messenger : mRNA (L ~ 1000)
- Transfer: tRNA (L ~ 70)
- Ribosomal: rRNA (L ~ 3000)
- Micro: μRNA (L ~ 25)
- Huge amounts of non-coding RNA in "junk" DNA

Why does the 3d structure of RNA matter?

Important discovery in the 80s: RNA can have enzymatic activity

Important discovery since 2000: μRNA play crucial role in cell regulation

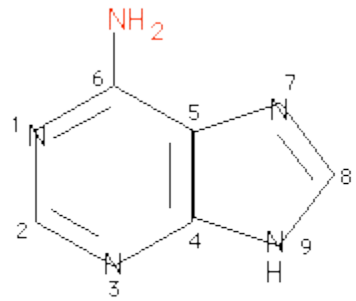
Function strongly related to shape

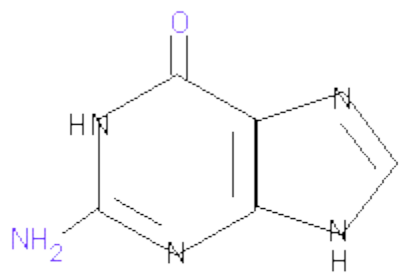
⟹ Must know 3d structure of RNA

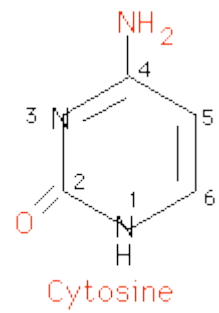
Chemistry of RNA

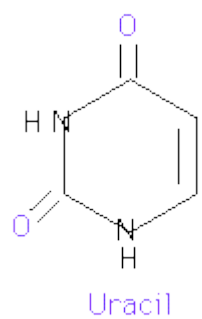
- RNA is a heteropolymer
- Four bases:
  - Adenine (A)
  - Guanine (G)
  - Cytosine (C)
  - Uracil (U)

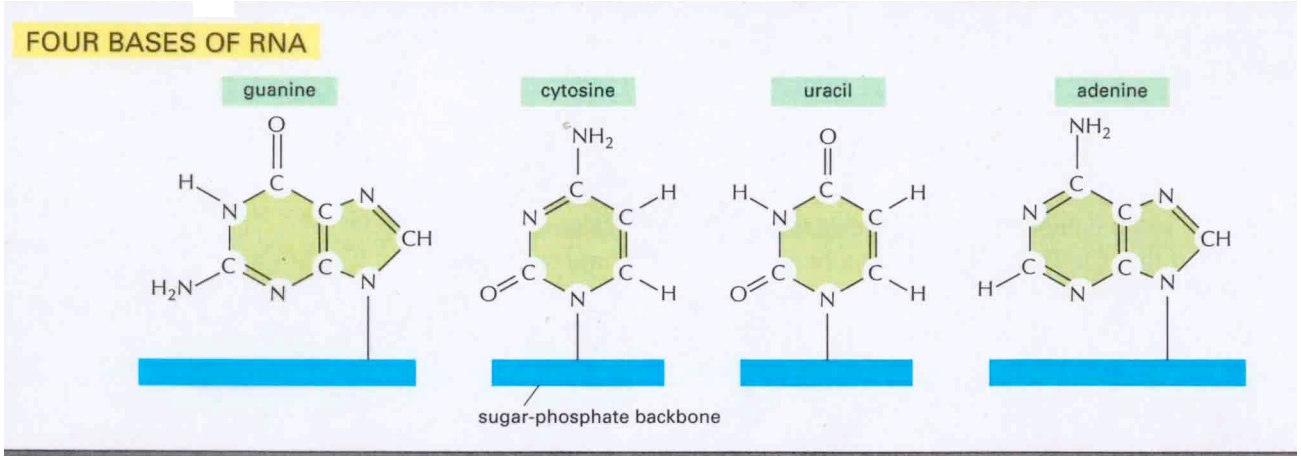
  The sugar phosphate backbone polymerizes into a single stranded charged (–) polymer

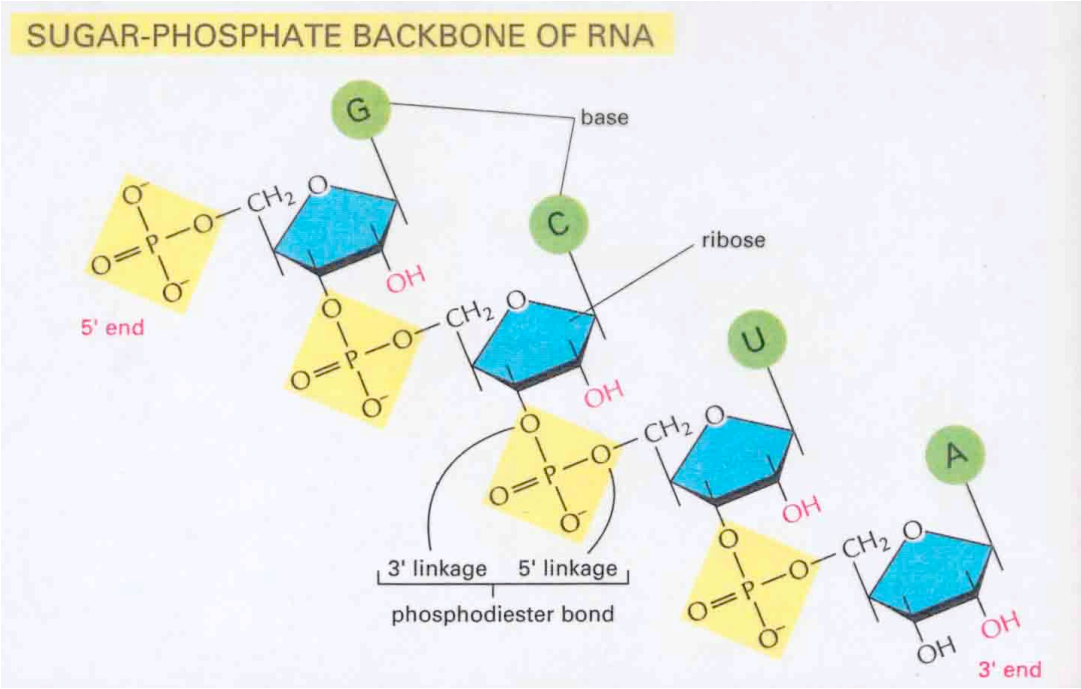









FOUR BASES OF RNA
guanine
cytosine
uracil
adenine
sugar-phosphate backbone


SUGAR-PHOSPHATE BACKBONE OF RNA
G
C
U
A
base
ribose
5' end
3' end
3' linkage
5' linkage
phosphodiester bond

Energy scales

- Crick–Watson: conjugate pairs

  C – G

  A – U

Pairing due to Hydrogen bonds between bases ⇨ RNA folding

Stacking of aromatic groups

Electrostatics (Mg^{++} ions) controls 3d structure

Energy scales

C — G : 3kCal/mole = 5 kT

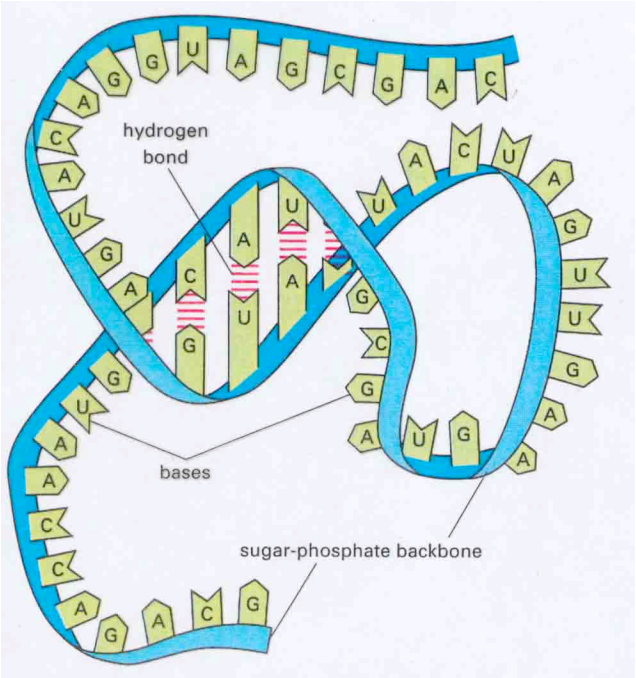
A — U : 2kCal/mole = 3.3 kT

G — U : 1kCal/mole =1.6 kT

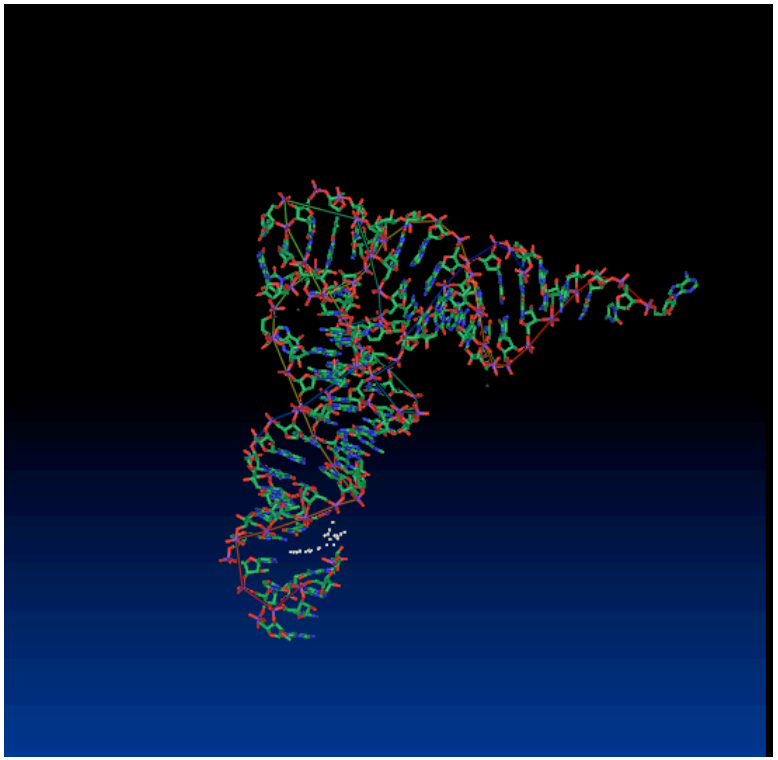
300 K = 0.6 kCal/mole = 1/40 eV

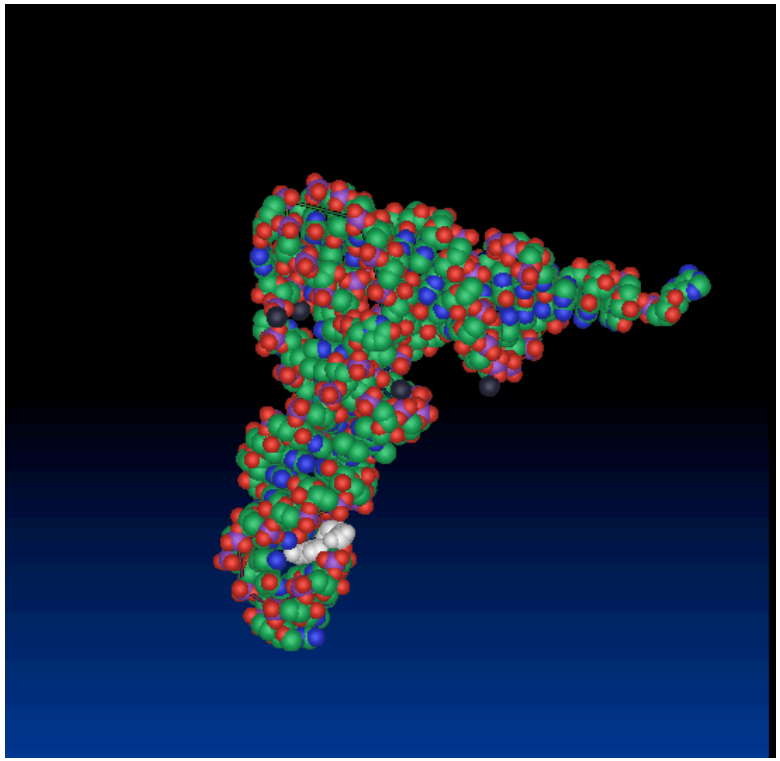
Base pairing

- Induces helical strands (like in DNA)
- Induces secondary structure of RNA



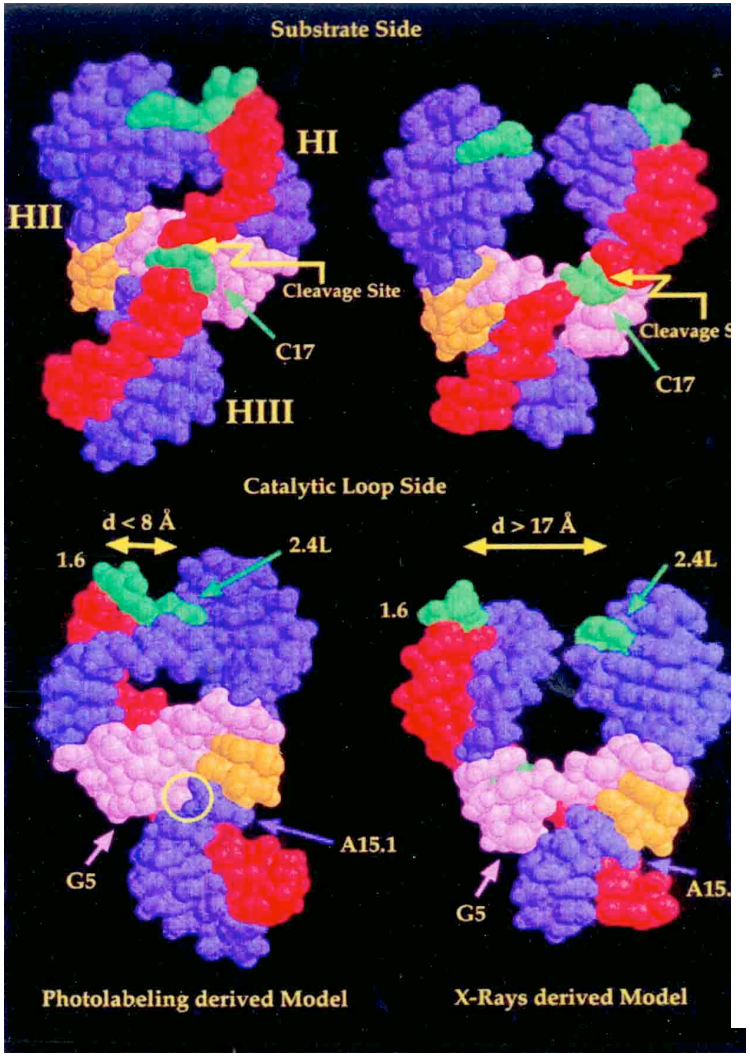
Pictures of RNA



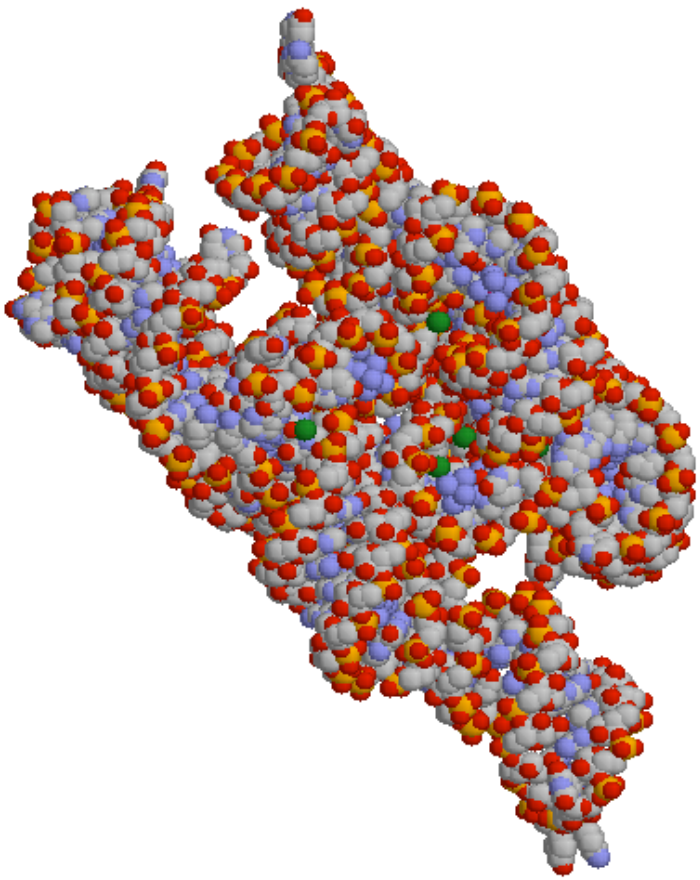


Transfer RNA

Hammerhead Ribozyme

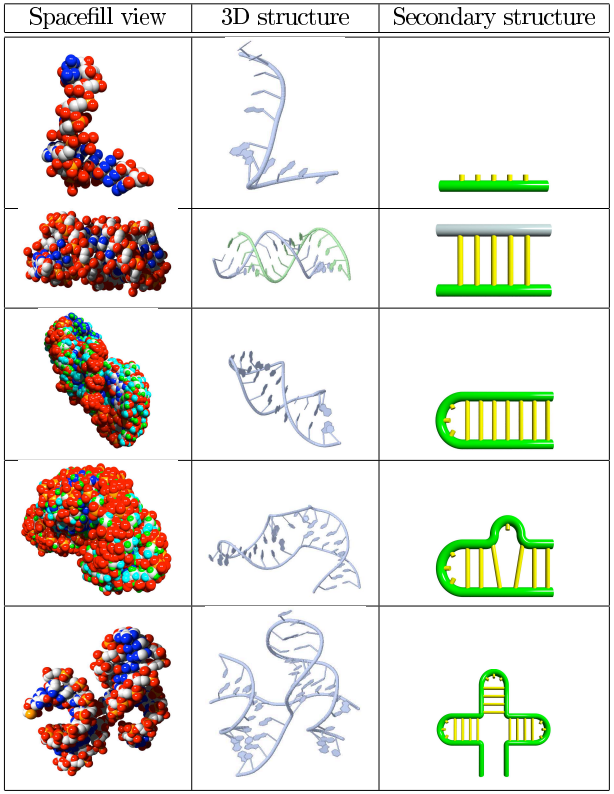


Ribosomal RNA



Secondary structures

- In RNA, there are helical stems with loops
- and bulges

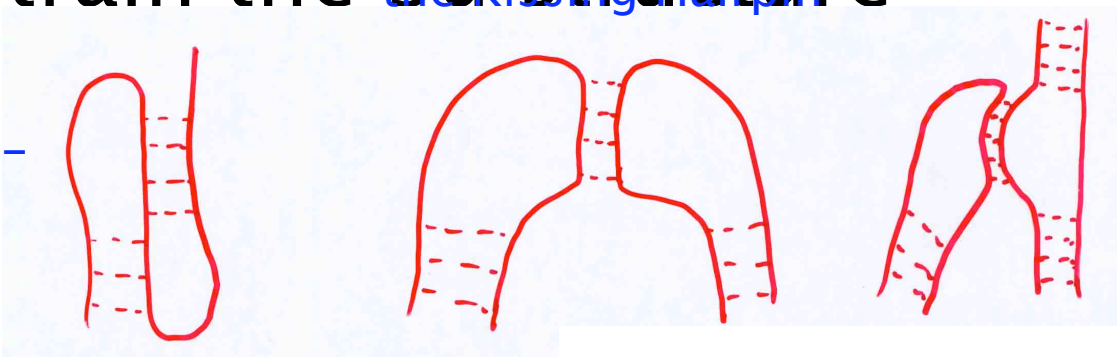


Pseudo-knots in RNA

- In addition to secondary structure, there are "pseudo-knots" which constrain the 3d structure

the Kissing Hairpin

The H pseudo-knot



Loop-bulge

- 3d folding controlled by concentration of Mg^{++} ions.

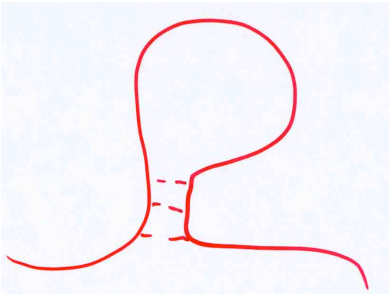
In fact base pairing is not good enough: need also stacking energies.

However:

- saturation of Crick-Watson pairing
- pseudo-knot free energy << free energy of secondary structure

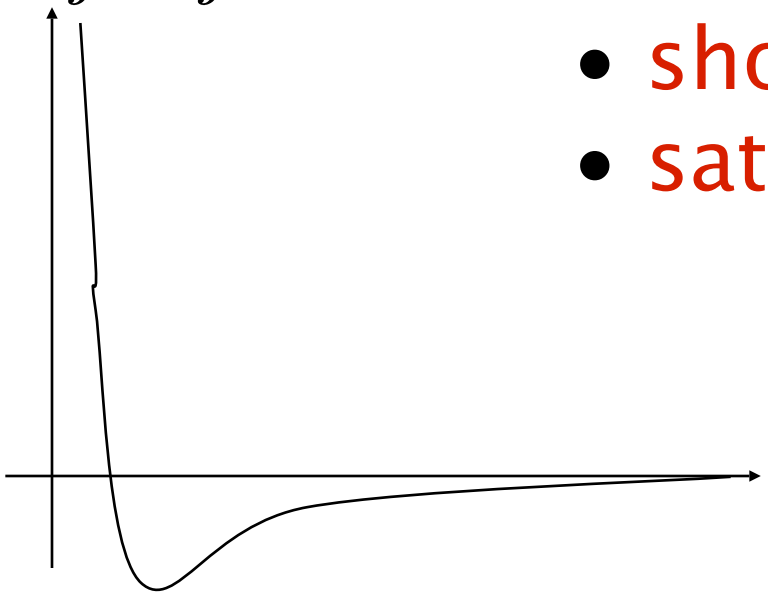
⇒ RNA folding much easier than protein folding

Partition function



$v_{ij}(\vec{r}_{ij})$: interaction of base i and j

- short range
- saturating



Partition function

$$Z = \int \prod_{i=1}^{L} d^3 r_i \prod_{i=1}^{L-1} \underbrace{f(\vec{r}_{i+1}, \vec{r}_i)}_{\text{Chain connectivity}} Q(\{\vec{r}_i\})$$

Interactions

$$\Rightarrow \begin{cases} \delta(|\vec{r}_{i+1} - \vec{r}_i| - a) \\ e^{-\frac{3}{2a^2}(\vec{r}_{i+1} - \vec{r}_i)^2} \end{cases}$$

$$Q = e^{-\frac{\beta}{2}\sum_{i \neq j} v_{ij}(\vec{r}_{ij})} + \text{solvent} + \text{electrostatics}$$

Further simplifications:

- Saturation of interactions
- Watson–Crick pairing

Define $V_{ij} = e^{-\beta\varepsilon_{ij}}\theta(|i-j|-4)$

Base pair energy

Chain rigidity

- Approximation

$$Z = \sum_{\substack{\text{sterically}\\ \text{allowed}\\ \text{configurations}}} Q_0$$

where

$$Q_0 = 1 + \sum_{i<j} V_{ij} + \sum_{i<j<k<l} (V_{ij}V_{kl} + V_{ik}V_{jl} + V_{il}V_{jk})$$

$$+ \ldots + \sum_{i<j<k<l<\ldots<p<q} V_{ij}V_{kl}\ldots V_{pq}$$

- sum is mainly combinatorial
- any index appears once and only once (saturation)

- In using this partition function, we have not taken into account the entropy of loops.
- For a loop of size l, the entropy is

$$S = l \log \mu - c \log l$$

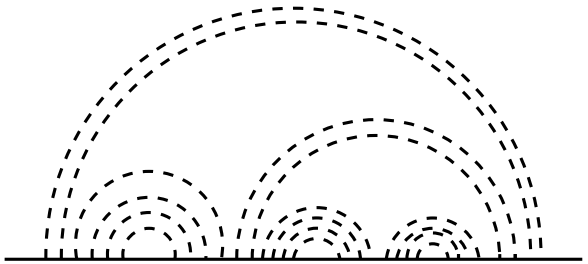
- In fact the $\log \mu$ goes into the free energies of pairing, so that

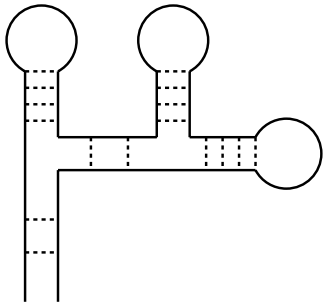
$$S = -c \log l$$

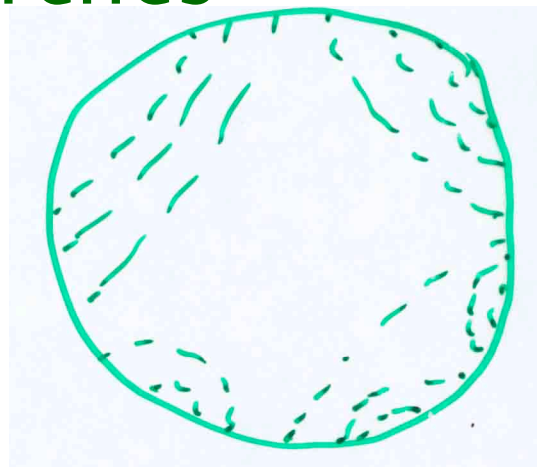
- with $c = 3/2$ (Gaussian chain)
- $c = 1.75$ (Self Avoiding Walk)

Secondary structures

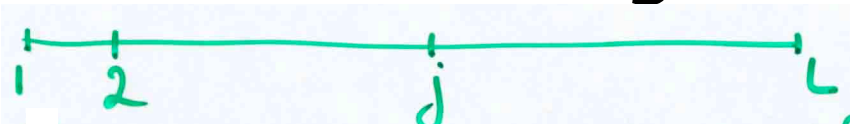
- We work on Q_0
- Secondary structures = Arches





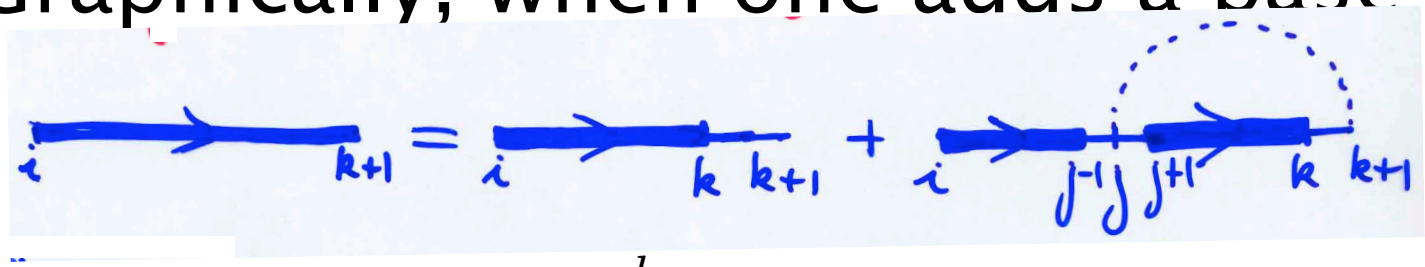


- Define $Z(i,j)$ as the
- partition function of segment (i,j)



Recursion relation

- Graphically, when one adds a base



$$Z(i,k+1) = Z(i,k) + \sum_{j=1}^{k} V_{j,k+1} Z(i,j-1) Z(j+1,k)$$

- with

$$V(i,j) = e^{-\beta\varepsilon(i,j)}\theta(|i-j|-4)$$

- by iterating this recursion, one can generate all possible secondary structures, with correct Boltzmann weights.
- This is the best tool for predicting secondary structures in RNA : more than 85% of base pairings correctly predicted
- Algorithm scales as N^3
- One can include Entropies and Stacking Energies
- http://www.tbi.univie.ac.at/~ivo/RNA

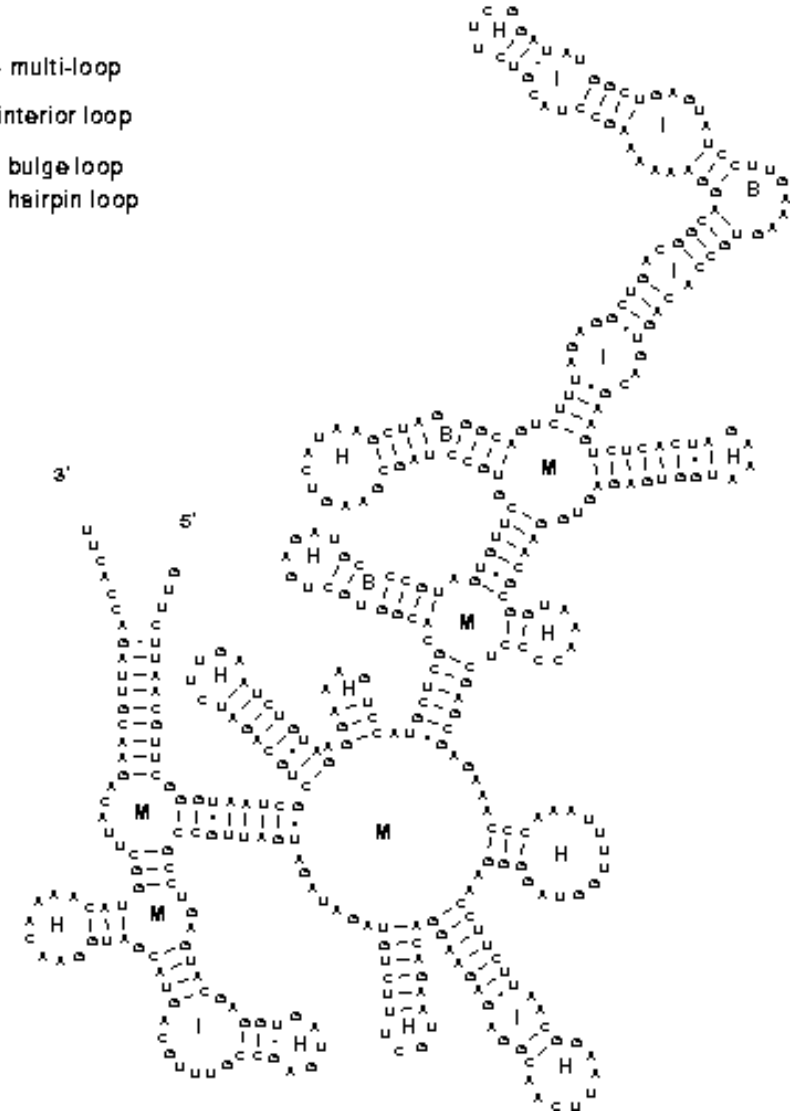
Bacillus subtilis RNase P RNA

M - multi-loop

I - interior loop

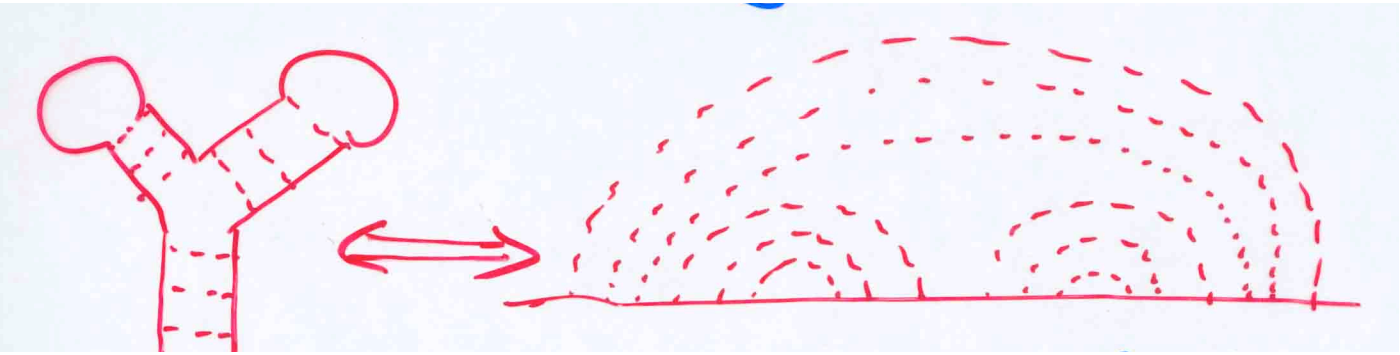
B - bulge loop

H - hairpin loop



- Recursion equation looks like Hartree equations (tree diagrams)
- No Pseudo-Knots
- Is it possible to find a field theory such that secondary structures are the Hartree graphs?
- Then, Pseudo-Knots would appear as the corrections to Hartree approximation.

Matrix Field Theory



$$Q_0 = 1 + \sum_{i<j} V_{ij} + \sum_{i<j<k<l} (V_{ij}V_{kl} + V_{ik}V_{jl} + V_{il}V_{jk})$$

$$+ \ldots + \sum_{i<j<k<l<\ldots<p<q} V_{ij}V_{kl}\ldots V_{pq}$$

Wick Theorem

- Simple representation: consider an RNA sequence of length L

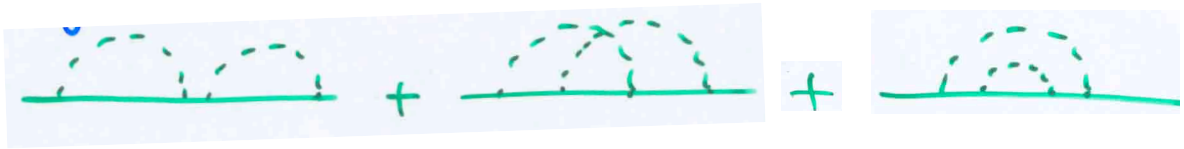
$$Q_0 = \frac{1}{\mathcal{N}} \int \prod_{i=1}^{L} d\phi_i e^{-\frac{1}{2}\sum_{i,j}\phi_i V_{ij}^{-1}\phi_j} \prod_{i=1}^{L}(1+\phi_i)$$

- due to Wick theorem

$$V_{ij} = \frac{1}{\mathcal{N}} \int \prod_{i=1}^{L} d\phi_i e^{-\frac{1}{2}\sum_{i,j}\phi_i V_{ij}^{-1}\phi_j} \phi_i\phi_j$$

Wick Theorem

$$V_{ij}V_{kl} + V_{ik}V_{jl} + V_{il}V_{jk} = \frac{1}{\mathcal{N}} \int \prod_{i=1}^{L} d\phi_i e^{-\frac{1}{2}\sum_{i,j}\phi_i V_{ij}^{-1}\phi_j} \phi_i\phi_j\phi_k\phi_l$$



- However, this form gives same weight to all pairings. No penalty for Pseudo-Knots.
- Experimentally, few pseudo-knots.

- We look for a parameter N such that

$$N \to +\infty \equiv$$ Secondary structures

- Corrections in $\frac{1}{N} \equiv$ Pseudo-Knots

- Pseudo-knots are tunable by $[Mg^{++}]$ concentration. $\frac{1}{N}$ plays the role of $[Mg^{++}]$

- TOPOLOGY=MATRIX FIELD THEORY

Matrix Field Theory: a Short Tutorial

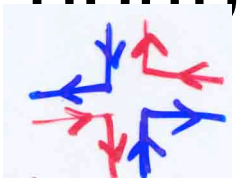
- Vector field theories: $O(n)$ models count number of connected component of a graph. n is the fugacity of a loop.
- Matrix field theories: "count" ϕ^4 topology.
- Consider the generalization of the scalar $\phi_{ab}(x)$, $N \times N$ x field theory (t'Hooft, 1973)
- Consider the fields : a

Matrix Field Theory

- A matrix ϕ^4 field theory is defined by

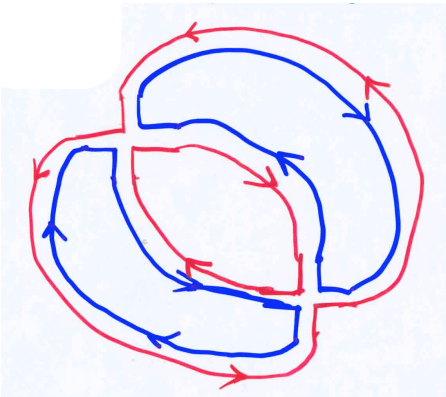
$$Z = \int \mathcal{D}\phi_{ab}(x) e^{-\frac{N}{2}\int dx Tr\phi(x)(-\nabla^2+m^2)\phi(x) - \frac{gN}{4!}\int dx Tr\phi^4(x)}$$

- represent $\phi_{ab}(x)$ by a double 

- Vertex: $NTr\phi^4_{ab}(x)$ ⇒  N
  factor
- Propagator: $\frac{1}{N}G(x-y)$ ⇒  $\frac{1}{N}$

Feynmann Graphs

- V: vertices
- I: internal propagators → N^{V-I+L}
- L: loops



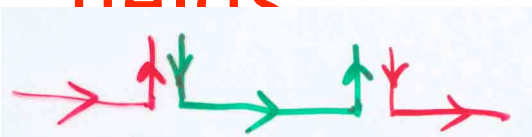
- V=2
- I=4
- L=4
- Euler characteristic: $\chi = V - I + L$

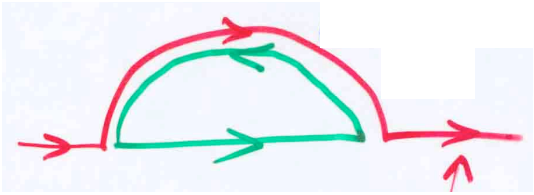
Euler characteristic and the Genus

- Consider a graph with Euler characteristics χ
- Theorem: this graph can be drawn without crossings on a surface of genus g given by $g = \frac{2-\chi-c}{2}$ where c is the
- number of boundaries of the graph
- The genus g is the number of handles of the embedding surface

Double line graphs

- In our problem, if we use matrix fields





$\phi_{ab}(x)$: NxN matrix

Propagator: 1/N

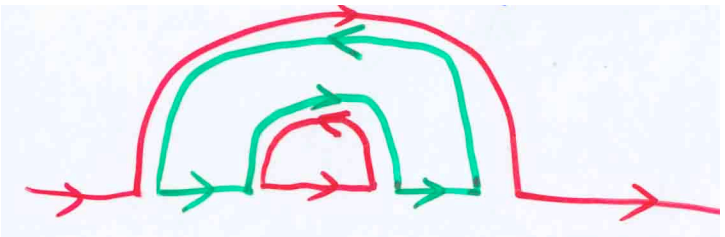
Loop: N

- If we use same rule:

$$N \times \frac{1}{N} = 1$$

- Above graph:

- Other graph

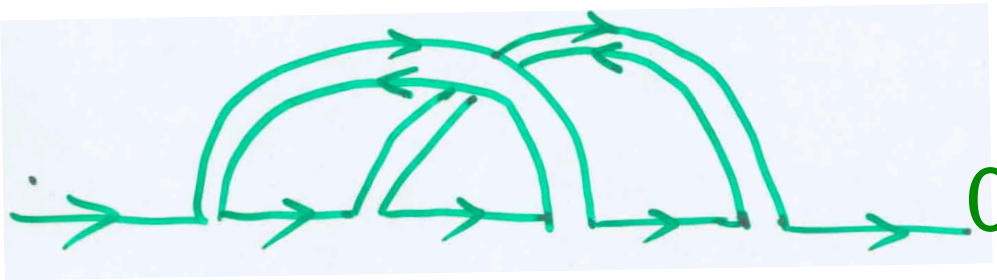


2 internal lines: $1/N^2$

2 Loops: N^2

Order 1

- Arches are of order 1



2 internal lines: $1/N^2$

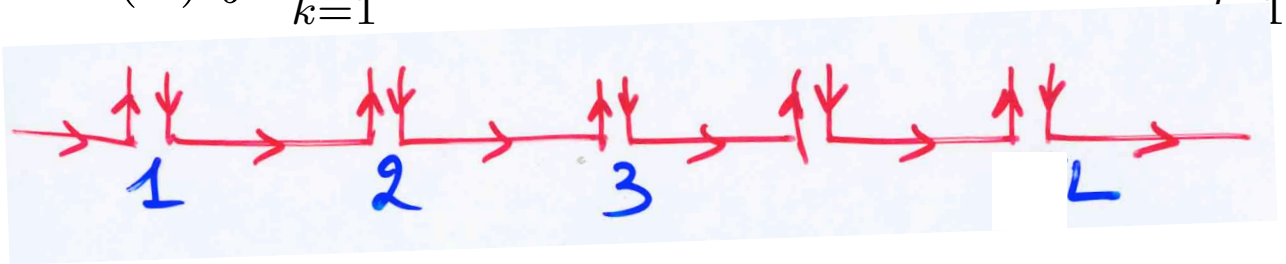
0 Loops: 1

- Pseudo-knots are of higher order in

Matrix field representation of RNA folding

- We thus generalize the Wick theorem

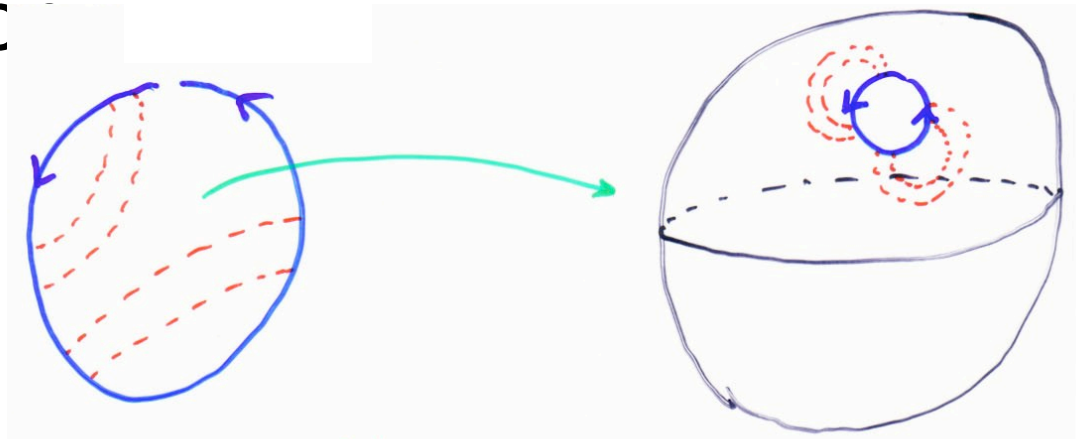
$$Z(1,L) = \frac{1}{A(L)} \int \prod_{k=1}^{L} d\varphi_k e^{-\frac{N}{2}\sum_{ij}(V^{-1})_{ij}\,\mathrm{tr}(\varphi_i\varphi_j)} \frac{1}{N}\,\mathrm{tr}\prod_{l=1}^{L}(1+\varphi_l)$$



- By looking at a few diagrams, it seems to do what we want: Hartree diagrams are of order 1, pseudo-knots are of higher order.

Topological classification of RNA folds

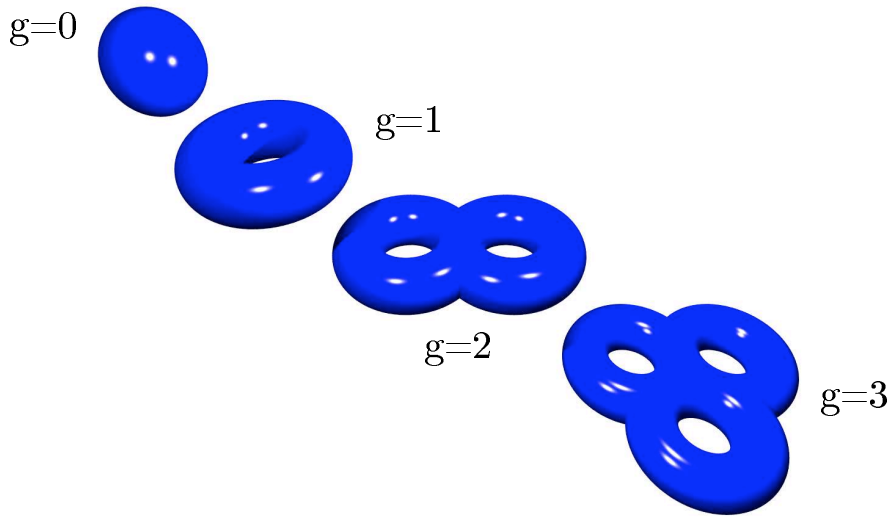
- An RNA fold can be characterized by its topolc



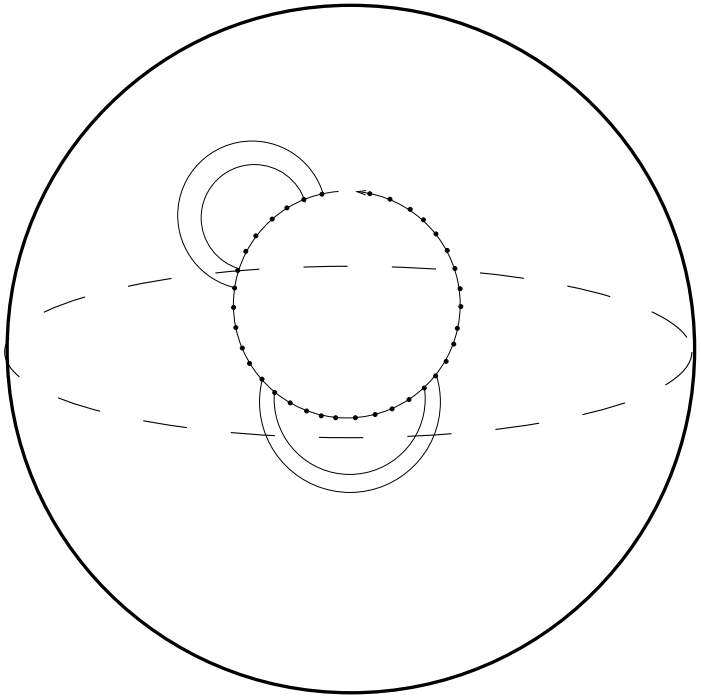
- Number of handles of embedding surface

$$g = \frac{P - L}{2}$$

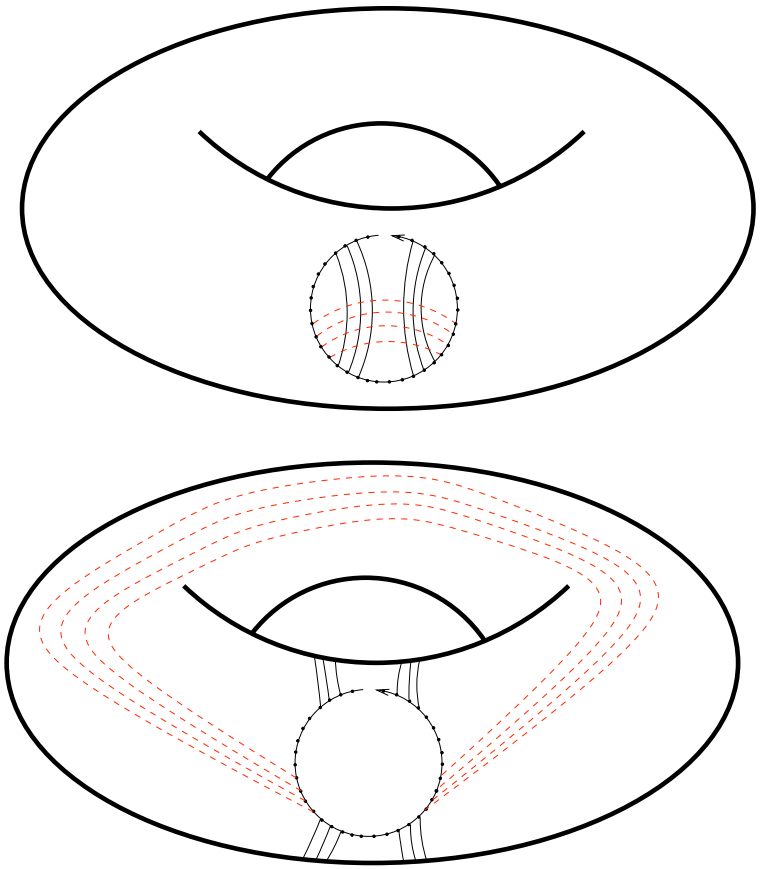
Topological expansion of closed oriented surfaces



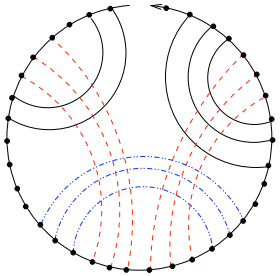
Genus 0: the Sphere

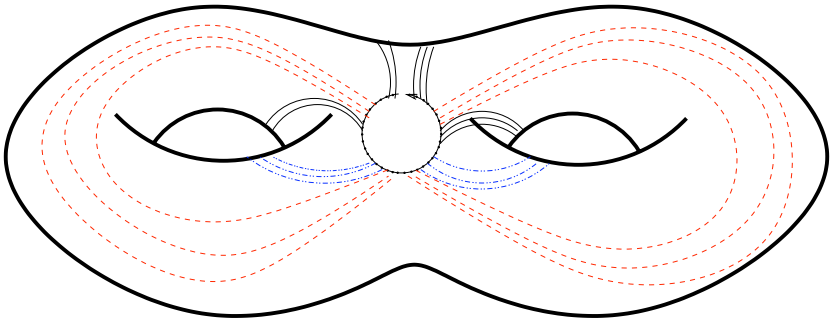


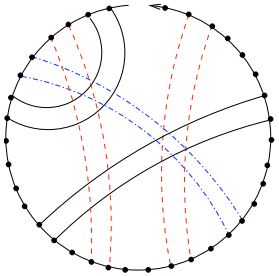
Genus 1: the Torus

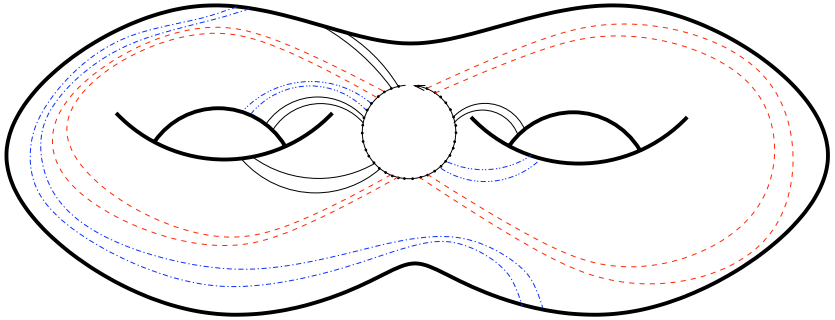
- 

Genus 2: the Bi-torus

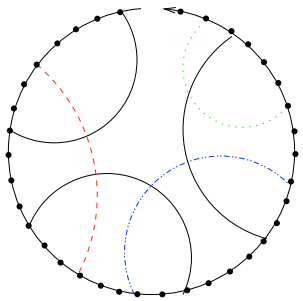


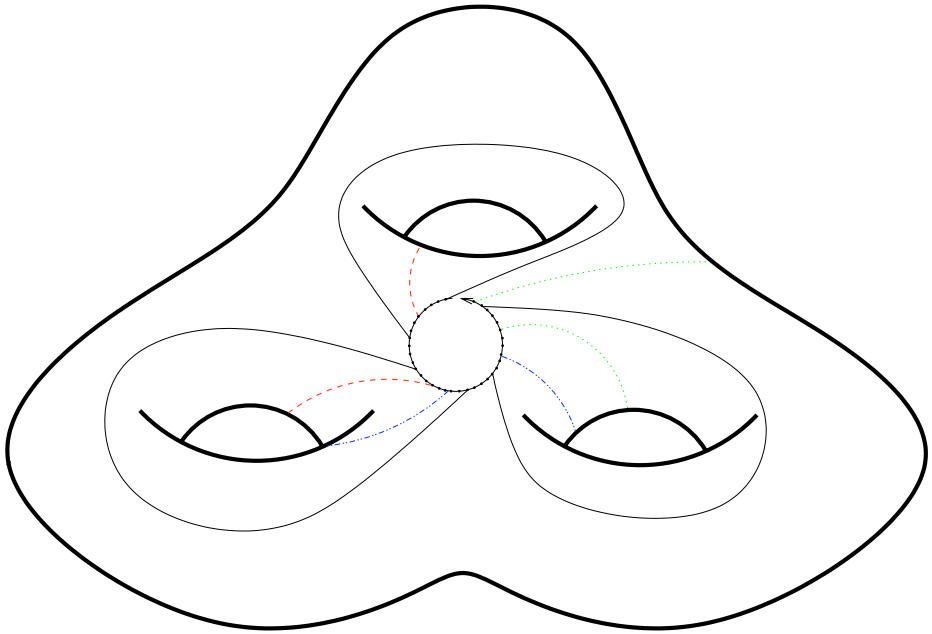






Genus 3





Large N expansion

- After some algebraic manipulations, one has the exact expression:

$$Z(1,L) = \frac{1}{C}\int dA e^{-\frac{N}{2}\mathrm{tr}A^2 + N\mathrm{tr}\log M(A)} M^{-1}(A)_{L+1,1}$$

- where $A_{ll'}$ is a $L \times L$ matrix and

$$M_{ij} = \delta_{ij} - \delta_{i,j+1} + i(V_{i-1,j})^{\frac{1}{2}} A_{i-1,j}$$

- The N dependence is explicit

one can perform a loop expansion (saddle-point)

The loop expansion

- Saddle-point equation

$$\frac{\partial S}{\partial A_{ll'}} = 0 \Longleftrightarrow$$ Hartree recursion equations

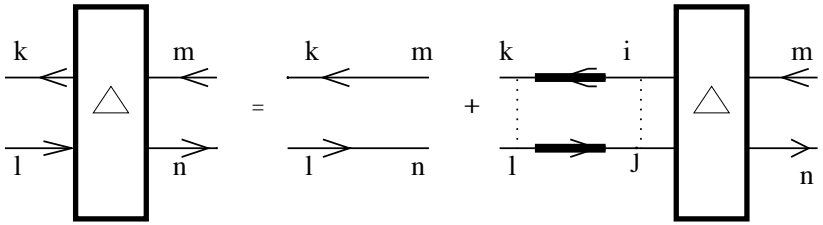
- Expansion in 1/N

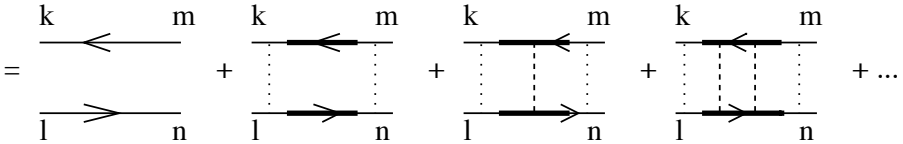
$$A_{ll'} = A^{(0)}_{ll'} + \frac{x_{ll'}}{\sqrt{N}}$$

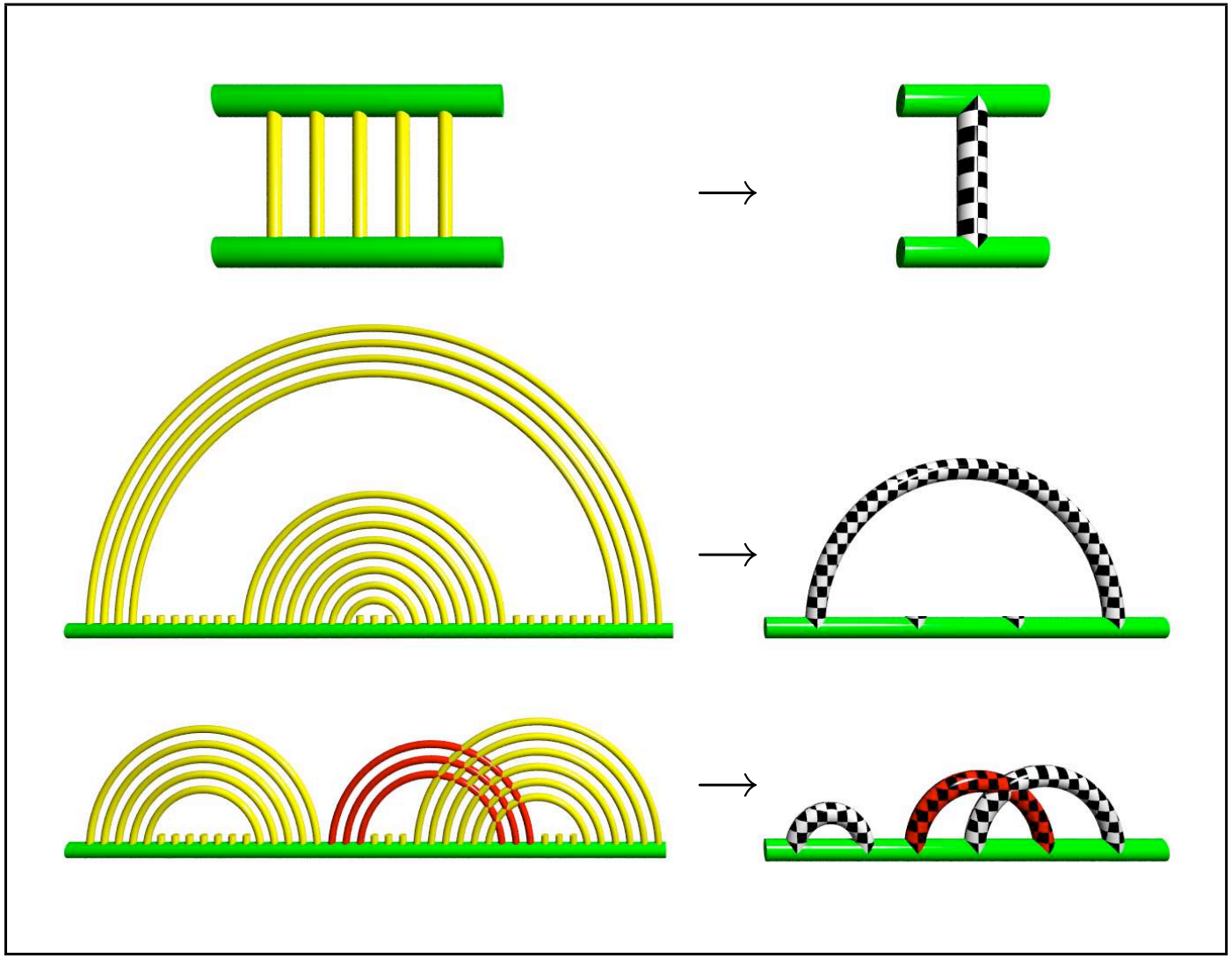
- Propagators of $x_{ll'}$ satisfy a Bethe-Salpeter equation

Bethe-Salpeter equation

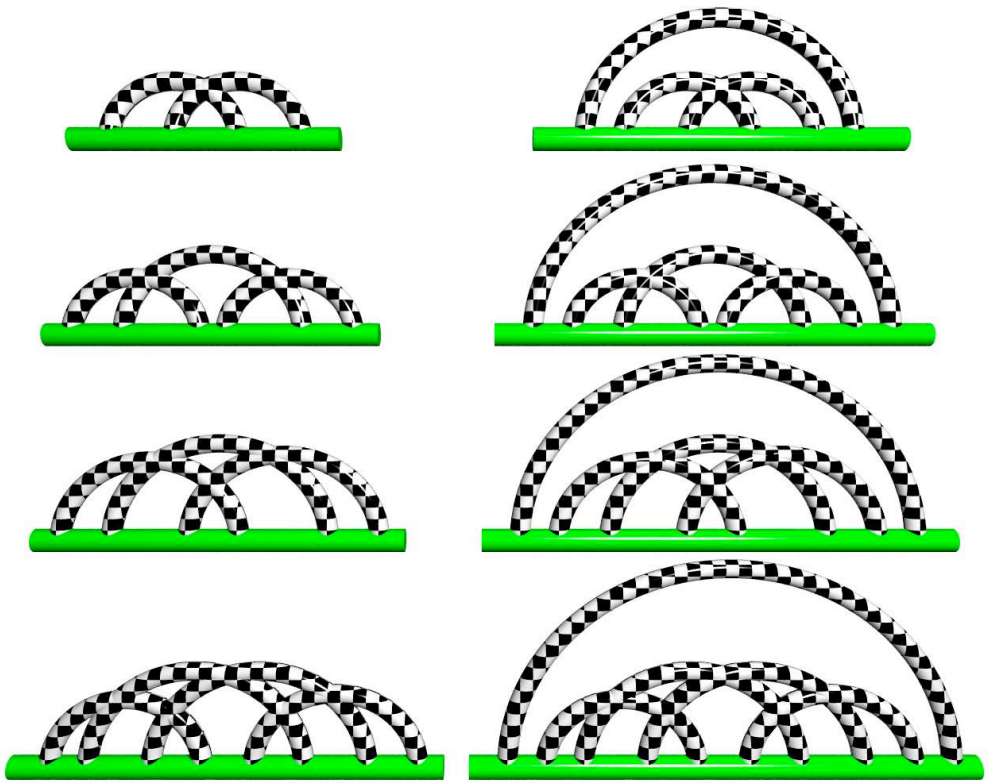
- No order 1/N correction







Eight Pseudo-knots of genus 1



ABAB: H PK

ABACBC: KHP

ABCABC

ABCADBCD

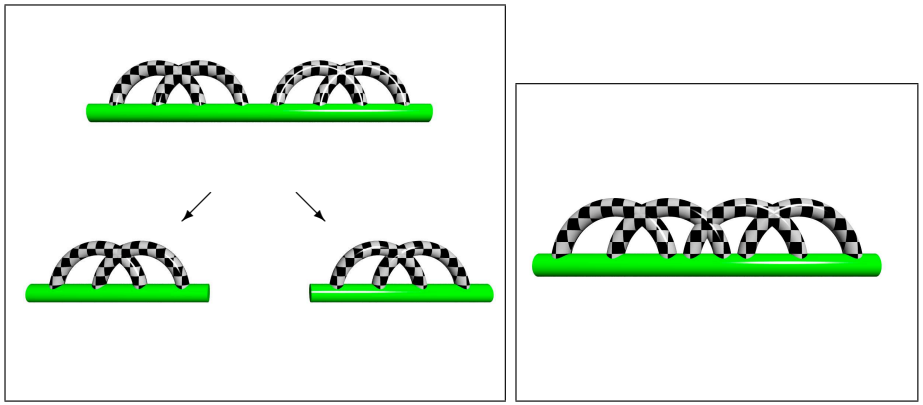
Recursion relations

- It is possible to obtain exact recursion relations for genus 1
- There is an exact relation

$$Z(1,L+1) = Z(1,L) + \sum_{k=1}^{L} V_{L+1,k} < \frac{1}{N} Tr \prod_{i=1}^{k-1} (1+\phi_i) \times \frac{1}{N} Tr \prod_{j=k+1}^{L} (1+\phi_j) >$$

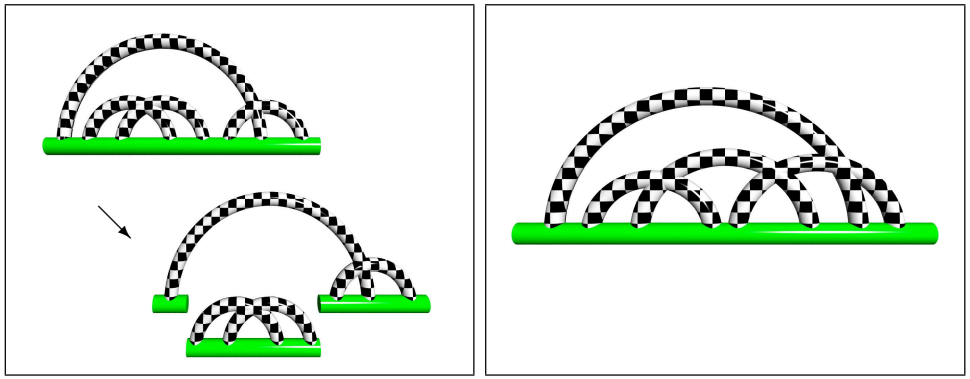
- which can be expanded in powers of $\frac{1}{N}$
- Algorithm scales as L^6 → too long!

Irreducibility and Nesting



Irreducible PK

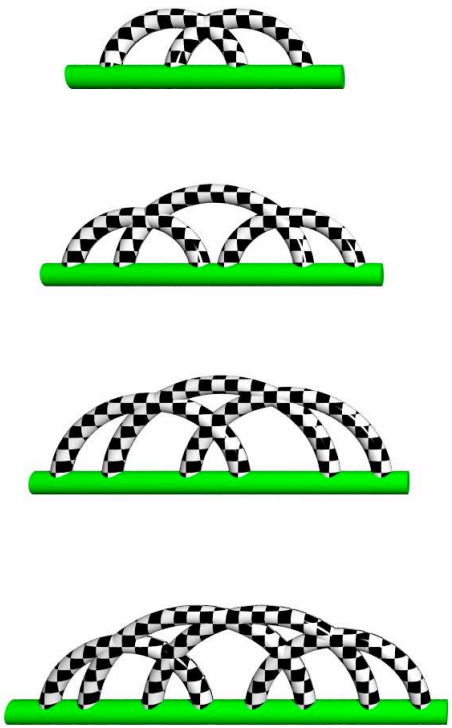
Genus is additive



Non nested PK

Primitive Pseudo-Knots

Irreducible and non-nested



Only 4 primitive PK of genus 1

Statistical study

- Look in database and calculate genii of pseudo-knots
- PseudoBase: around 245 pseudo-knots; all are of genus 1, except 1 of genus 2
- 237 H PK of the type ABAB
- 6 KHP of the type ABACBC
- 1 PK of the type ABCABC
- 1 PK of type ABCDCADB with genus 2

- Protein Data Bank (PDB): 850 RNA Structures
- 650 RNA have genus 0 (short fragments)
- Number of bases ranges from 22 (H PK with genus 1) to 2999 (with genus 15)
- Maximum total genus is 18. Maximum genus of primitive PK is 8.
- Transfer RNA (L=78) are KHP of genus 1

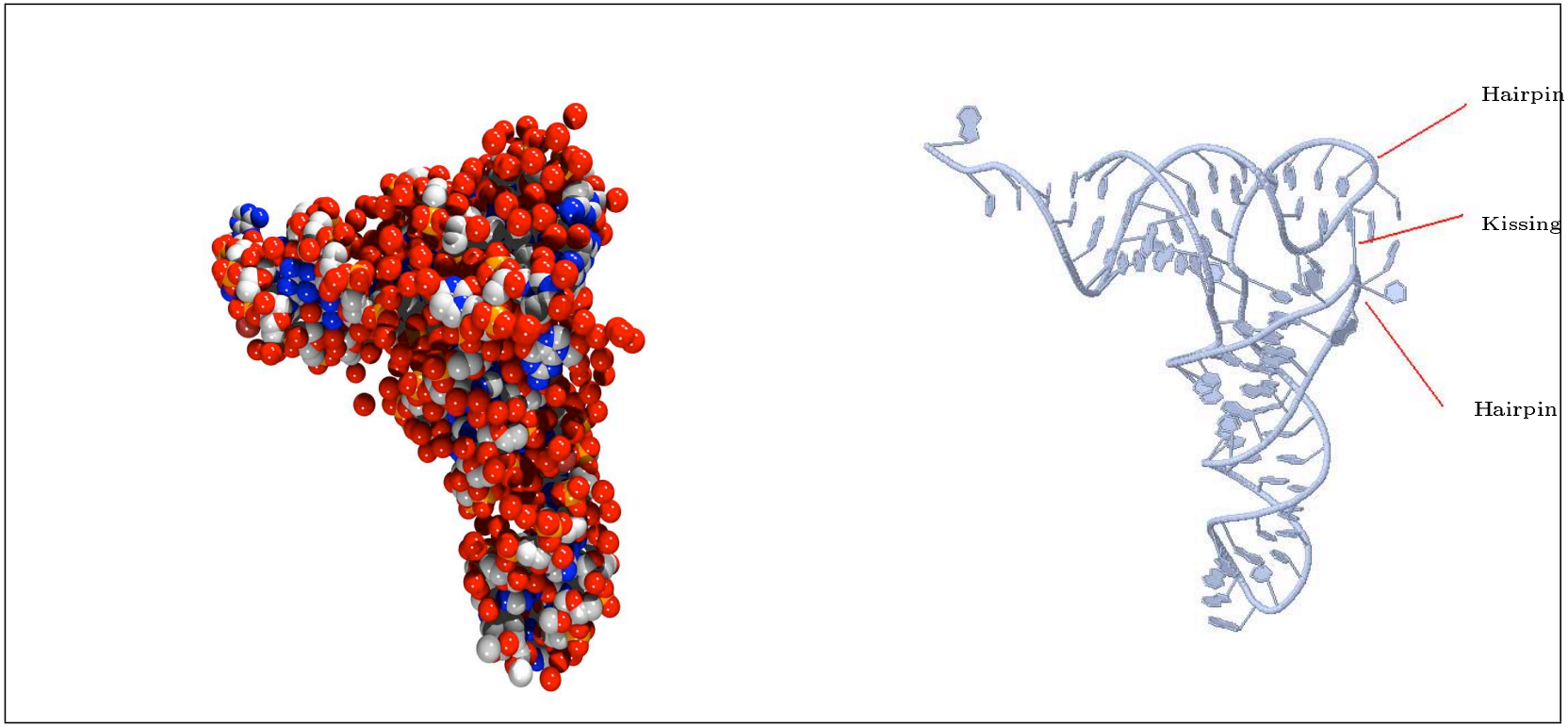


Figure 10: A typical tRNA (PDB ID 1evv [34]. It has the genus 1 of a kissing hairpin pseudoknot.

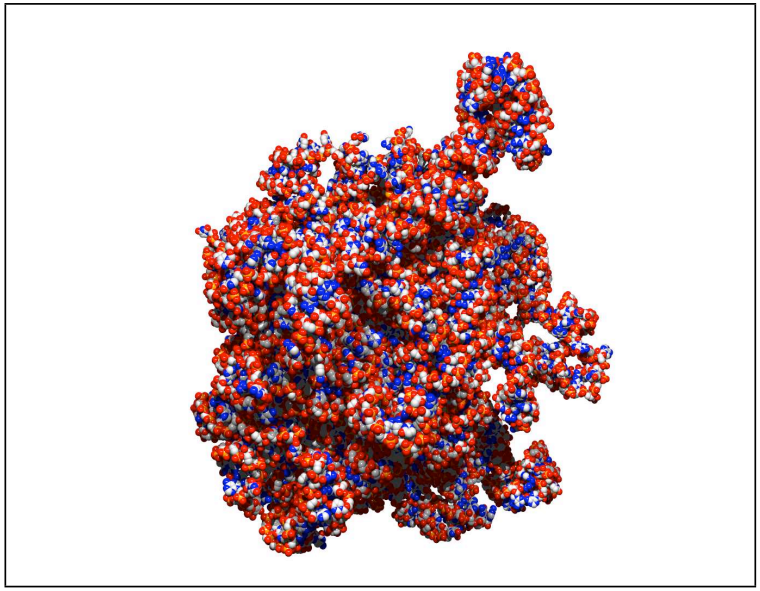


Figure 11: The B chain of 1vou.pdb is an RNA of genus 7 and of length 2825 bases.

- This PK of genus 7 is made of 3 HPK, 3 KHP nested in a large KHP
- Are these genii big?

Exact enumeration of RNA structures.

- Model: RNA in which any base can pair with any other base. All pairing energies are identical

$$V_{ij} = v$$

- Partition function of the model can be written as

$$Z_N(L) = \frac{1}{A} \int d\phi \; e^{-\frac{N}{2v} \mathrm{Tr} \phi^2} \frac{1}{N} \mathrm{Tr} \; (1+\phi)^L$$

- with only one NxN matrix ϕ

- This integral can be calculated exactly using random matrix theory (orthogonal polynomials).

$$Z_N(L) = \sum_{g=0}^{\infty} \frac{a_L(g)}{N^{2g}}$$

- and the asymptotic behaviors are given by

$$a_L(g) \approx_{L\to\infty} K_g (1+2v)^L L^{3g-3/2}$$

$$K_g = \frac{1}{3^{4g-3/2} 2^{2g+1} g! \sqrt{\pi}}$$

- The total number of diagrams with any genus is given by

$$\mathcal{N} \approx_{L\to\infty} L^{L/2} \frac{e^{-L/2+\sqrt{L}-1/4}}{\sqrt{2}}$$

- the average genus is given by

$$< g >_L \approx 0.25L$$

- for real RNA, the largest genus we found is 18 for ribosomes (size around 3000 bp). The genus should be around 750.
- What about Steric Constraints?

Enumeration of self-avoiding RNA structures.

- Self-avoiding polymer on a cubic lattice
- Saturating attraction between nearest-neighbor monomers.
- Monte Carlo growth method allows to calculate accurately free energies.

  Length of chains up to 1200

$$\langle g \rangle \approx 0.13L$$

- Still much bigger than for real RNA:

Monte Carlo method

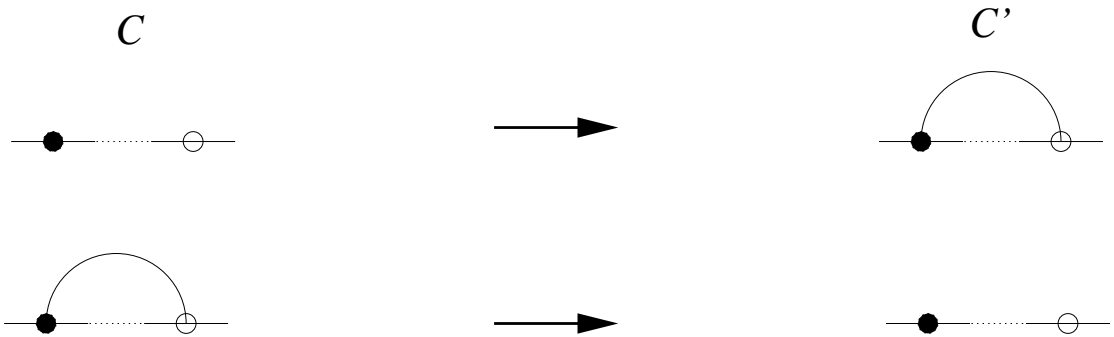
- Idea: forget matrix fields, keep genus
- Work in pairing space (contact map)

$$Z = \sum_{\text{possible pairings}} e^{-\beta E(\text{pairing})}$$

- Introduce a chemical potential for the topology: $e^{-\mu} = \frac{1}{N^2}$

$$Z = \sum_{\text{possible pairings}} e^{-\beta E(\text{pairing}) - \mu g(\text{pairing})}$$

Possible moves

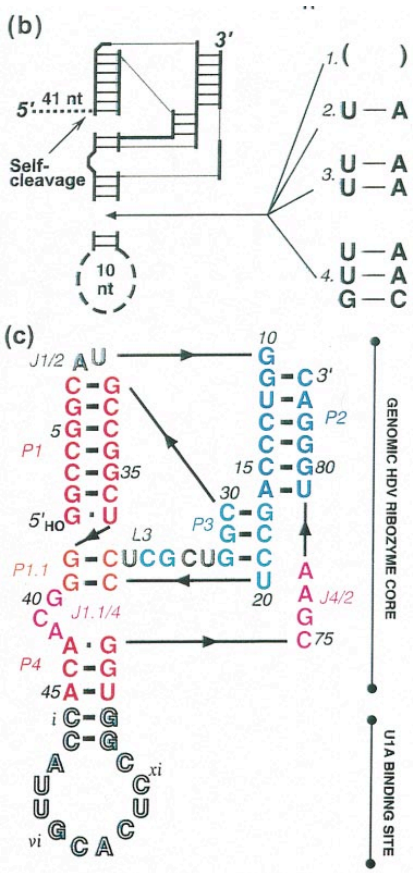


- Accept or reject move with probability

$$p = e^{-\beta \Delta E - \mu \Delta g}$$

- It is possible to
  - take into account the entropy
  - make it very fast
  - take into account steric constraint
- We are able to find the correct pseudo-knots in RNA up to size 200
  - transfer RNAs
  - Hepatitis delta virus ribozyme

The structure of the HDV ribozyme



Conclusion

- Matrix field theory introduces a natural classification of RNA folds according to their topological genus.
- One can write exact recursion equations for genus 0, 1, ...
- Most promising is the Monte Carlo calculation with chemical potential for the genus.