Combinatorial insight into RNA structure

Analysis and Design of *ab-initio* RNA algorithms

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April 24, 2011

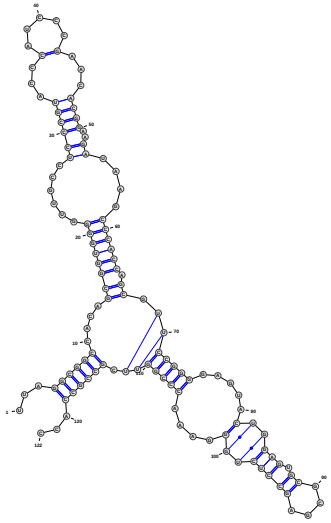
Outline

Part I

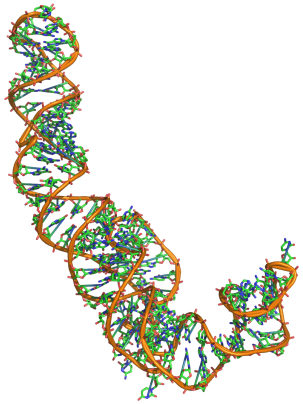
Foreword/Generating functions 101

```
UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC
```

Primary structure



Secondary structure



Tertiary structure

Source: 5s rRNA (PDBID: 1K73:B)

Definition

Secondary structures of RNA =
Maximal non-crossing subset of canonical base-pairs.

RNA structure

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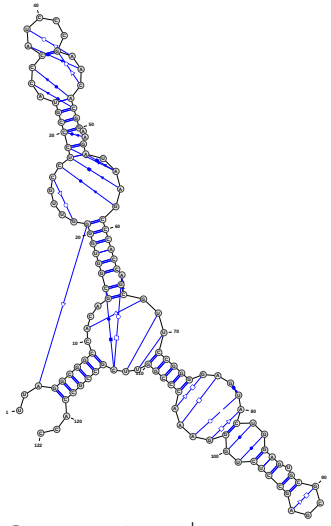

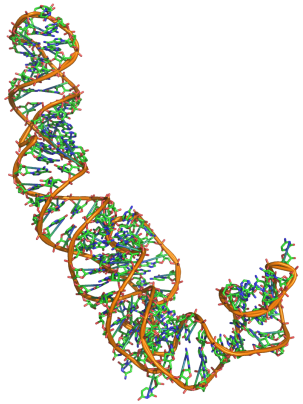

Primary structure | Secondary$^+$ structure | Tertiary structure

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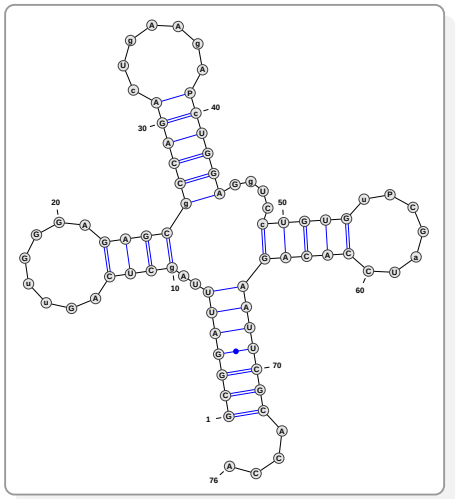
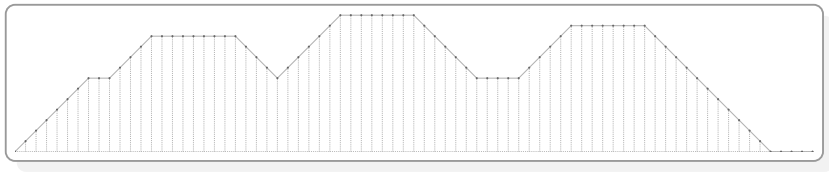
Various representations for a versatile molecule

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

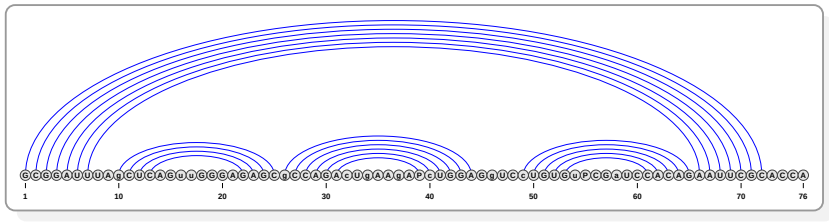
Well-parenthesized expression



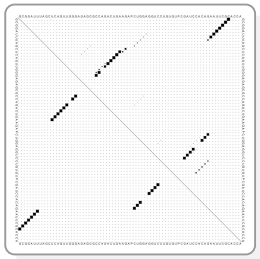
Outer planar graph



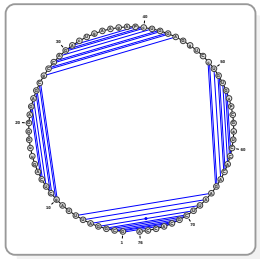
Mountain view



Non intersecting arcs



Dot plot



Feynman's diagram

Different objects
yet
Common combinatorial structure

Minimum Free-Energy (MFE)

Functional folding of an RNA = Minimum free-energy structure.

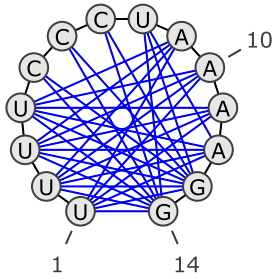
Nussinov/Jacobson (NJ) energy model: Simplest *nearest-neighbor* model.

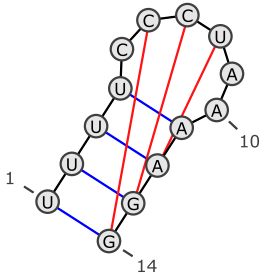
$$\text{Free-energy} = -\#\text{Base-pairs}$$

Folding in NJ $\Leftrightarrow$ Maximizing number of base-pairs.
($\Leftrightarrow$ Max independent set in circle graphs $\Leftrightarrow$ weighted CYK . . .)

Example:

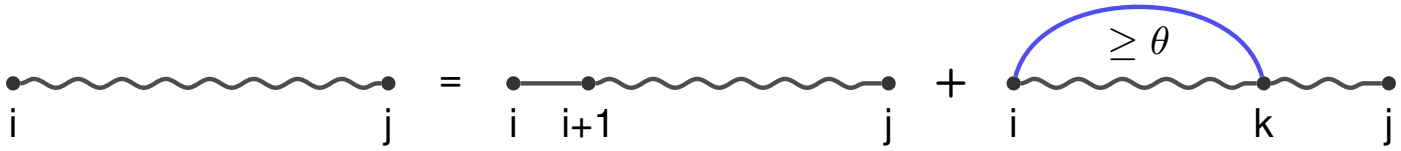
UUUUCCCUAAAAGG





Alt. : Assign weight to each base-pairs based on number of hydrogen bonds
$\Delta G(G{\equiv}C) = -3$ $\Delta G(A{=}U) = -2$ $\Delta G(G{-}U) = -1$

Minimization in Nussinov/Jacobson



Recurrence on the Minimal Free-Energy $N_{i,j}$ of any subinterval of an RNA :

$$
\begin{array}{rcl}
N_{i,t} & = & 0, \quad \forall t \in [i, i+\theta] \\
N_{i,j} & = & \min \begin{cases} N_{i+1,j} & (i \text{ unpaired}) \\ \min_{k=i+\theta+1}^{j} E_{i,j} + N_{i+1,k-1} + N_{k+1,j} & (i \text{ comp. with } k) \end{cases}
\end{array}
$$

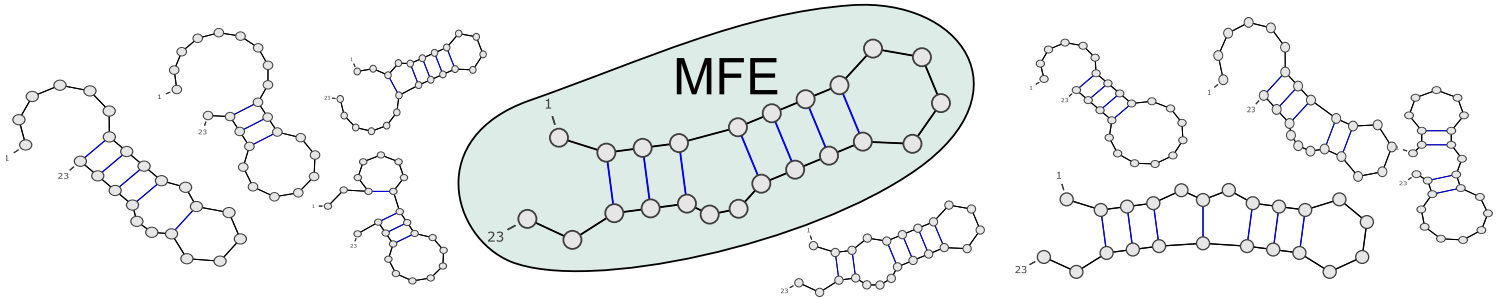
After computing N, the choices elected by min allow to rebuild (backtrack over) the MFE structure.

$\Rightarrow$ Dynamic programming for folding [NJ80].

Remark: Overly simplistic, but more complex energy models will typically already predict 73% of experimentally-determined base-pairs...

2004– : Boltzmann-ensemble paradigm

RNA *breathes* ⇒ There is not necessarily one functional conformation!



Boltzmann-ensemble paradigm

All possible secondary structures for an RNA coexist in a probability distribution, the Boltzmann distribution.

Bonus: MFE structure probability can be significantly remote, pointing toward unstructured RNAs.
Conversely, base-pairs having probability $> .99$ are valid 95% of the time.

- ⇒ Similar structures can bundle up.
- ⇒ Functional structures must be sought in sub-optimal structures.

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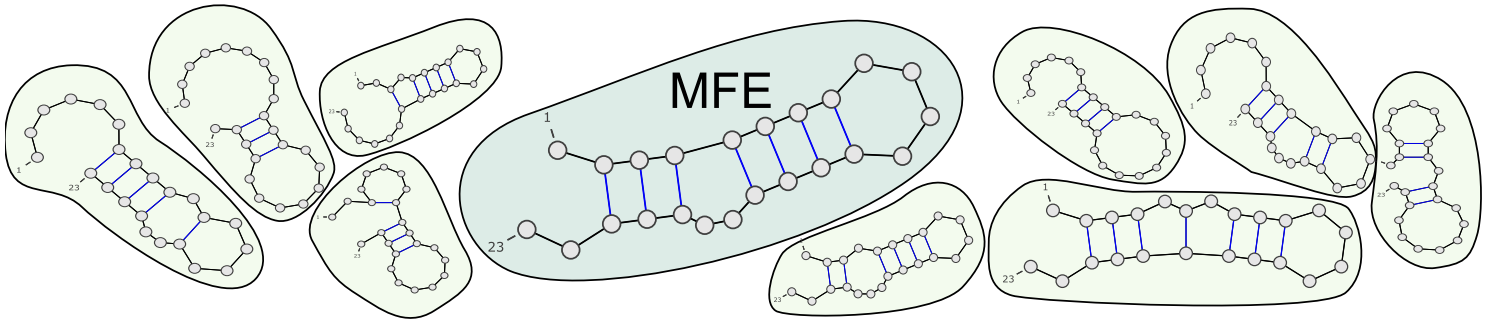

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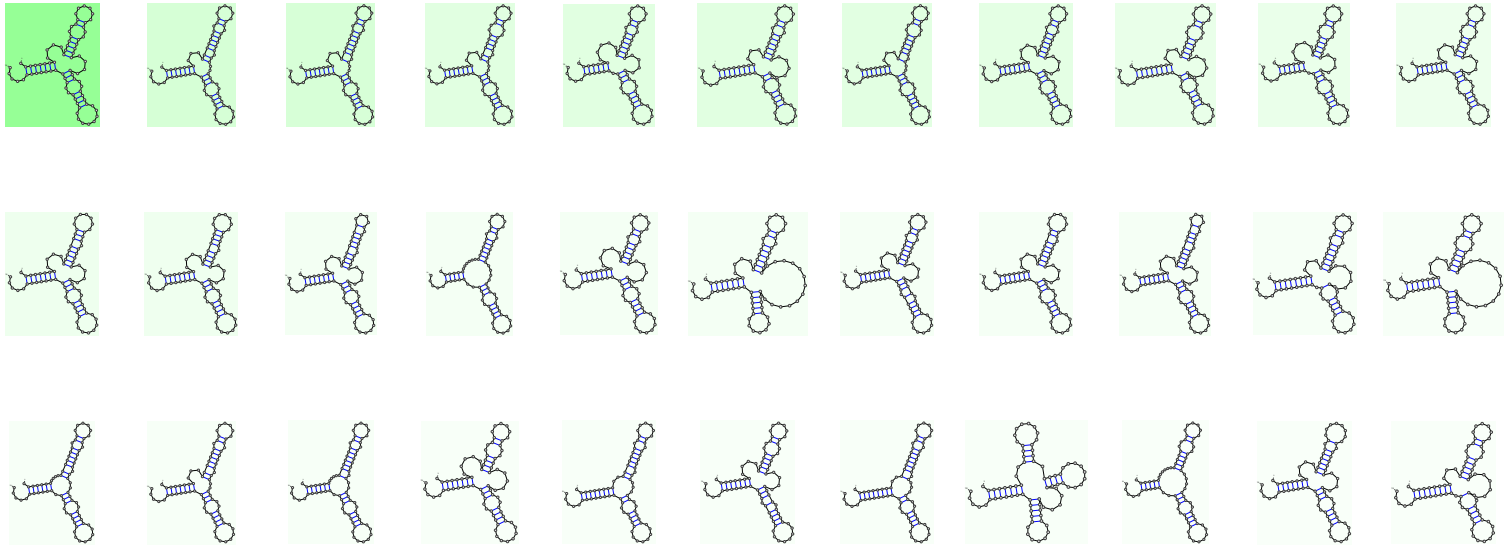
Why use combinatorics?

Boltzmann ensemble is a (weighted) combinatorial class.



Studying it as such *cleans out the details* and helps:

- Access asymptotic properties of secondary structures
- Investigate worst and average-case complexities
- Design better algorithms and methods for RNA

Generating functions

Let $|\,.\,|$ be a size function over objects (Sequences, trees, ...).
Combinatorial classes are (infinite) sets $\mathcal{C}$ of objects whose restrictions $\mathcal{C}_n$ to objects of size n are of finite cardinality.

Definition (Generating functions)

Let $\mathcal{C}$ be a combinatorial class and $c_n = |\mathcal{C}_n|$ the number of objects of size n in $\mathcal{C}$, then the generating function of $\mathcal{C}$ is $C(z)$ s. t.

$$C(z) = \sum_{s \in C} z^{|s|} = \sum_{n \geq 0} c_n z^n$$

Closed forms for $C(z)$ are often easy to find ...

DNA example: $\mathcal{D} := \{a, c, g, t\}^* \Rightarrow d_n = 4^n$
and $C(z) = 1 + 4z + 16z^2 + 64z^3 + \ldots = \sum_{n \geq 0} 4^n z^n = \frac{1}{1-4z}$

...and very often much simpler than for c_n!!!

DSV/symbolic method

From a class specification, one can directly establish the gen. fun.

Historically on languages, from Schützenberger's observation that

Gen. fun. are commutative images of languages

Grammar	Generating function	Coefficients
$C \to \varepsilon$	$C(z) = z^0 = 1$	$c_n = \mathbb{1}_{\{0\}}(n)$
$C \to t$	$C(z) = z^1 = z$	$c_n = \mathbb{1}_{\{1\}}(n)$
$C \to A \mid B$	$C(z) = A(z) + B(z)$	$c_n = a_n + b_n$
$C \to A.B$	$C(z) = A(z) \cdot B(z)$	$c_n = \sum_{k=0}^{n} a_k b_{n-k}$

Remark: One needs to ensure that unions are disjoint and concatenations unambiguous.

DNA example : $\{a, c, g, t\}^* \Leftrightarrow D \to a.D \mid c.D \mid g.D \mid t.D \mid \varepsilon$

$$\Rightarrow D(z) = z \cdot D(z) + z \cdot D(z) + z \cdot D(z) + z \cdot D(z) + 1$$

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DNA example : $\{a, c, g, t\}^* \Leftrightarrow D \to a.D \mid c.D \mid g.D \mid t.D \mid \varepsilon$

$$\Rightarrow D(z) = 4z \cdot D(z) + 1$$

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DNA example : $\{a, c, g, t\}^* \Leftrightarrow D \to a.D \mid c.D \mid g.D \mid t.D \mid \varepsilon$

$$\Rightarrow D(z) = \frac{1}{1 - 4z}$$

Disclaimer

What follows, although true in this context, is embarassingly simplistic.
A rigorous presentation can (and must) be found in Flaj./Sedg. 08.

A singularity is a point $z = \rho$ where $C(z)$ is no longer analytic.
Asymptotics of coeff c_n are driven by the singularities of $C(z)$.

1st principle

Location of the dominant (smallest) singularity ρ dictates the exponential growth $\Rightarrow \frac{c_n}{\rho^{-n}} = o(\alpha^n),\ \forall \alpha > 1$.

DNA example: $D(z) = 1/(1-4z) \Rightarrow \rho = 1/4 \Rightarrow d_n \sim 4^n P(n)$.

Basic scale

2nd principle

Nature of ρ dictates subexponential part $P(n)$ s.t. $c_n \sim \rho^{-n} P(n)$.

Basic scale: If one can rewrite $C(z)$ as

$$C(z) = f(z) + g(z)(1 - z/\rho)^{\alpha}$$

where f and g are analytic $\forall |z| < |\rho|$ and non-null at ρ, then

$$c_n \equiv [z^n]C(z) \sim \frac{g(\rho)\rho^{-n}}{\Gamma(-\alpha)n^{\alpha+1}}$$

Example: $D(z) = \frac{1}{1-4z} \Rightarrow c_n \sim 4^n$
$(\rho = 1/4, \alpha = -1, f(z) = 0,$ and $g(z) = 1)$

General methodology

Asymptotic estimates are obtained using a 4 steps meta-algorithm:

1. Find the right model
2. Translate into grammar
3. Translate into system and solve g. f.
4. Singularity analysis yields asymptotics

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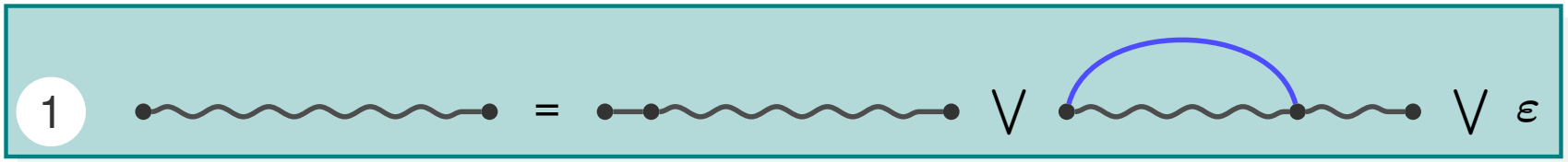
Appetizer: Motzkin words

Let us count dot-bracket notations (Motzkin words)



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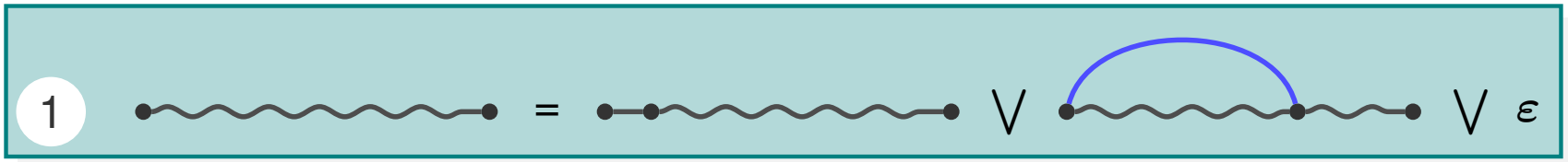

2 $M \rightarrow \bullet M \mid (M)M \mid \varepsilon$

Let us count dot-bracket notations (Motzkin words)

1 

2 $M \rightarrow \bullet\, M \mid (\, M \,)\, M \mid \varepsilon$

3 $M(z) = z \cdot M(z) + z \cdot M(z) \cdot z \cdot M(z) + 1$

$= \frac{1-z\pm\sqrt{1-2z-3z^2}}{2z^2}$

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3 $$M(z) = z \cdot M(z) + z \cdot M(z) \cdot z \cdot M(z) + 1$$

$$= \begin{cases} \frac{1-z+\sqrt{1-2z-3z^2}}{2z^2} = \frac{1}{z^2} - \frac{1}{z} - 1 - z - 2z^2 + \mathcal{O}(z^3) \\ \frac{1-z-\sqrt{1-2z-3z^2}}{2z^2} = 1 + z + 2z^2 + 4z^3 + 9z^4 + \mathcal{O}(z^5) \end{cases}$$

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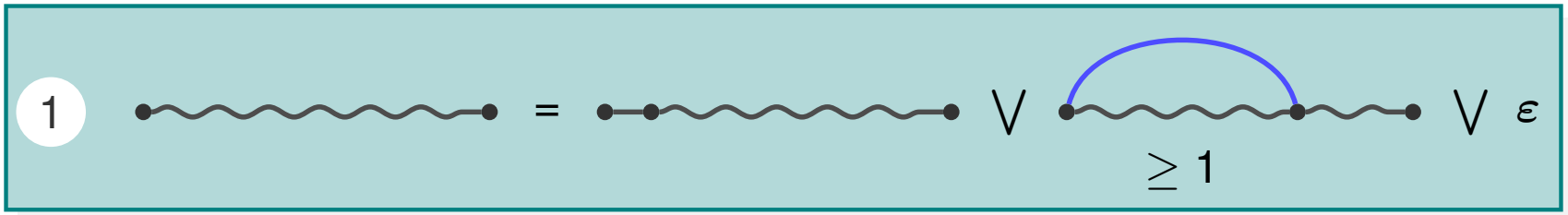
2 $M \quad \rightarrow \quad \bullet\, M \quad | \quad (\, M \,)\, M \quad | \quad \varepsilon$

3 $$M(z) = z \cdot M(z) + z \cdot M(z) \cdot z \cdot M(z) + 1$$
$$= \frac{1-z-\sqrt{1-2z-3z^2}}{2z^2}$$

4 $\rho = 1/3$, $M(z) = \frac{1-z}{2z^2} - g(z) \cdot \sqrt{1-z/\rho}$, and $g(z) := \frac{\sqrt{1+z}}{2z^2}$
$\Rightarrow s_n \equiv [z^n]M(z) \sim \frac{g(\rho)\rho^{-n}}{\Gamma(-\alpha)n^{\alpha+1}} = \frac{3\sqrt{3}}{2\sqrt{\pi}} \cdot \frac{3^n}{n\sqrt{n}}(1 + \mathcal{O}(1/n))$

Consider RNA secondary structures (Waterman 78)



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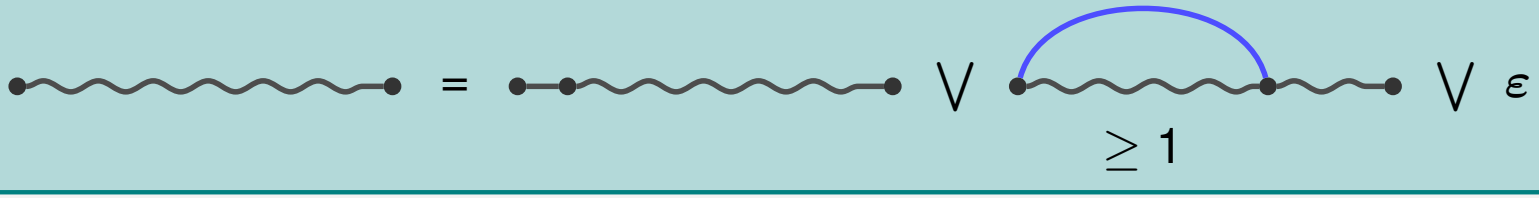
1 

2 $S \rightarrow \bullet\, S \mid (\, S_{>0}\,)\, S \mid \varepsilon$

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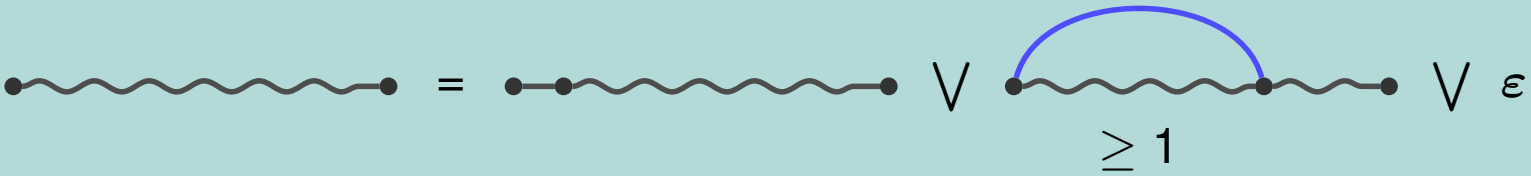

2

$$S \rightarrow \bullet\, S \mid (\,\mathbf{T}\,)\, S \mid \varepsilon$$

$$\mathbf{T} \rightarrow \bullet\, S \mid (\,\mathbf{T}\,)\, S$$

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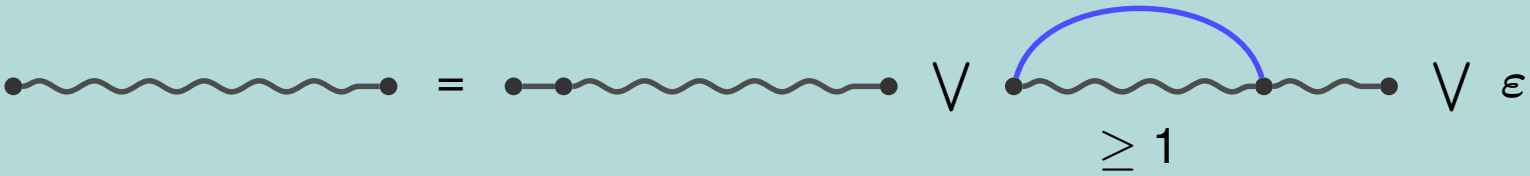
1 

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$$S(z) = \frac{1-z+z^2-\sqrt{1-2z-z^2-2z^3+z^4}}{2z^2}$$

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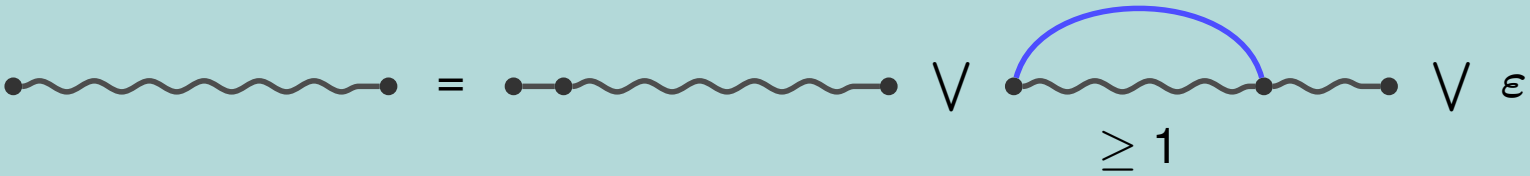
1 

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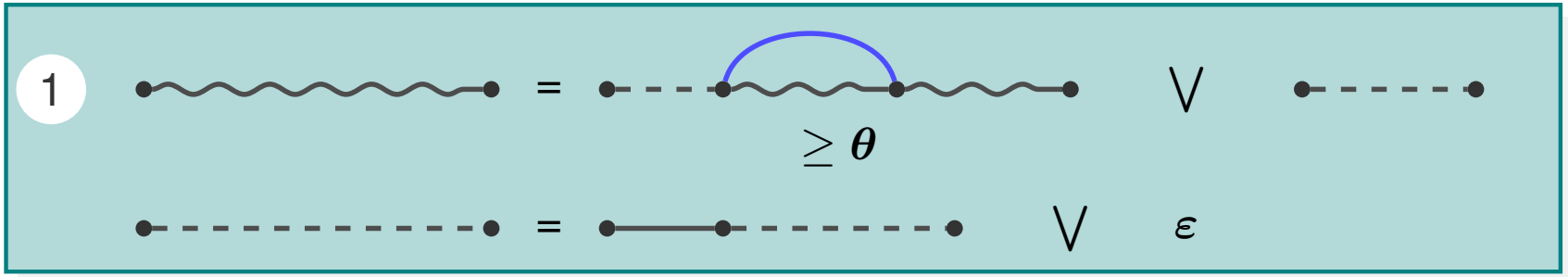
$$S(z) = \frac{1-z+z^2-\sqrt{1-2z-z^2-2z^3+z^4}}{2z^2}$$

4

$$\rho = \frac{3-\sqrt{5}}{2} = 1-\phi$$

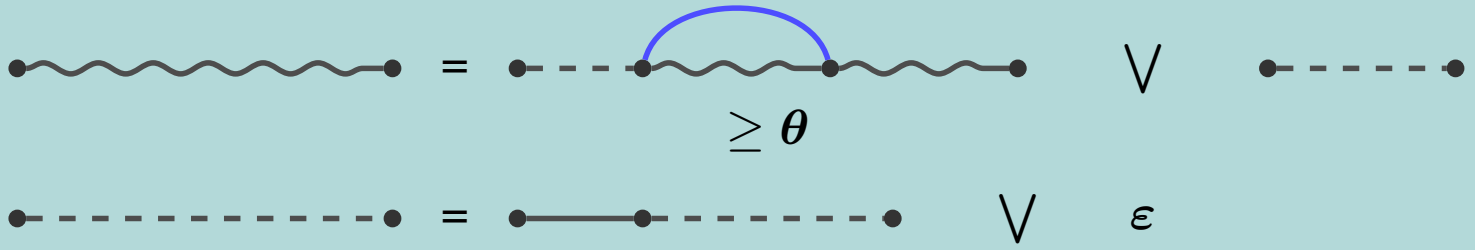
$$[z^n]S(z) = \sqrt{\frac{15+7\sqrt{5}}{8\pi}} \cdot \frac{\left(\frac{3+\sqrt{5}}{2}\right)^n}{n\sqrt{n}}(1+\mathcal{O}(1/n)) \sim 1.1 \cdot \frac{2.6^n}{n\sqrt{n}}$$

Let us generalize the θ constraint



Let us generalize the θ constraint

1



2 $S \rightarrow U\,(\,S_{\geq\theta}\,)\,S \mid U \qquad U \rightarrow \bullet\,U \mid \varepsilon$

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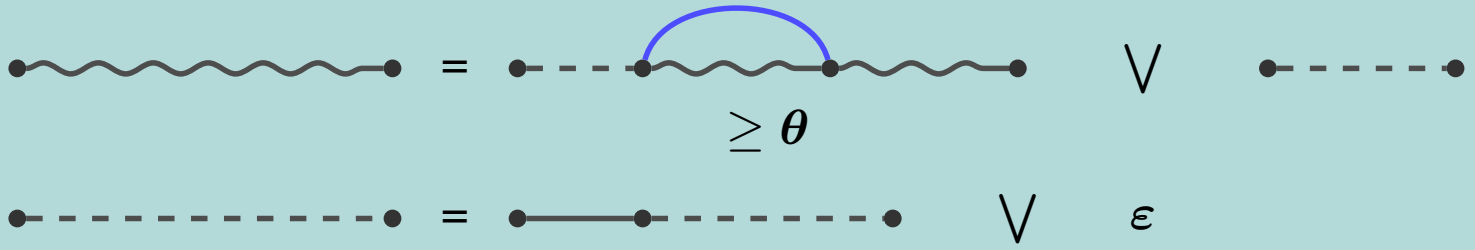

2

$$S \rightarrow U\,(\,\mathbf{T}\,)\,S \mid U \qquad U \rightarrow \bullet\, U \mid \varepsilon$$

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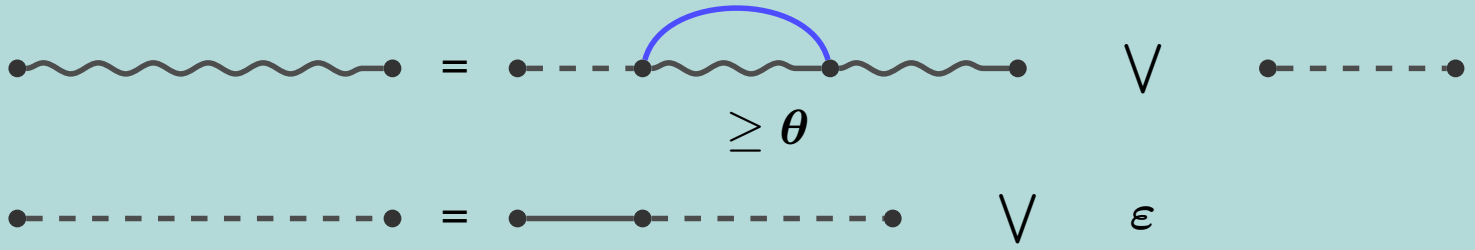

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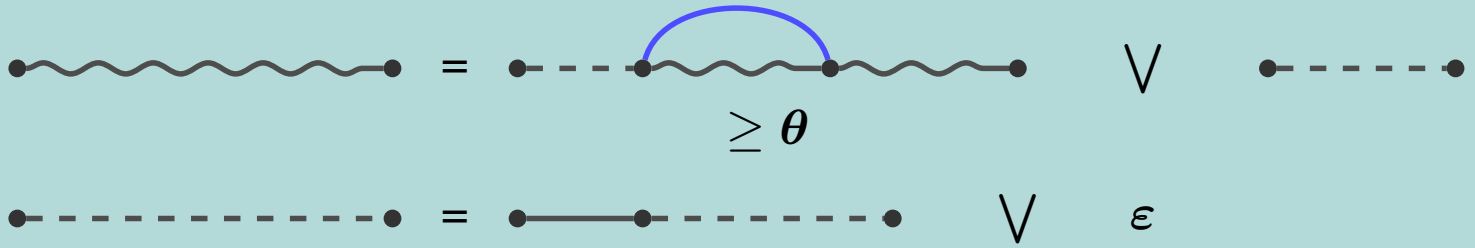

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4

$$s_n \sim K.\frac{\beta^n}{n\sqrt{n}}(1 + \mathcal{O}(1/n))$$

θ	0	1	3	10
β	3.	2.62	2.29	2.02

Technical summary

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Applying automatic theorems gives precise asymptotic equivalents.
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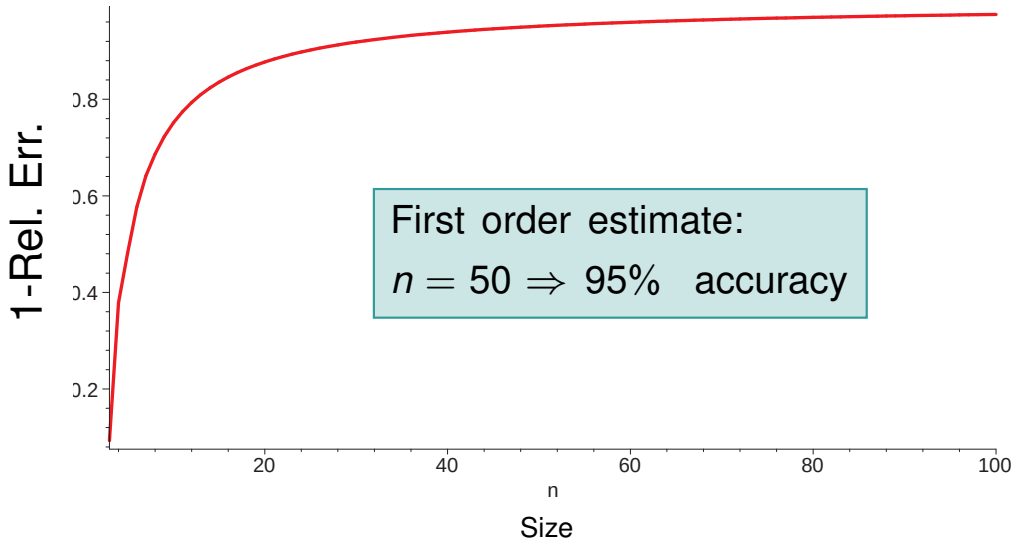

Secondary structures

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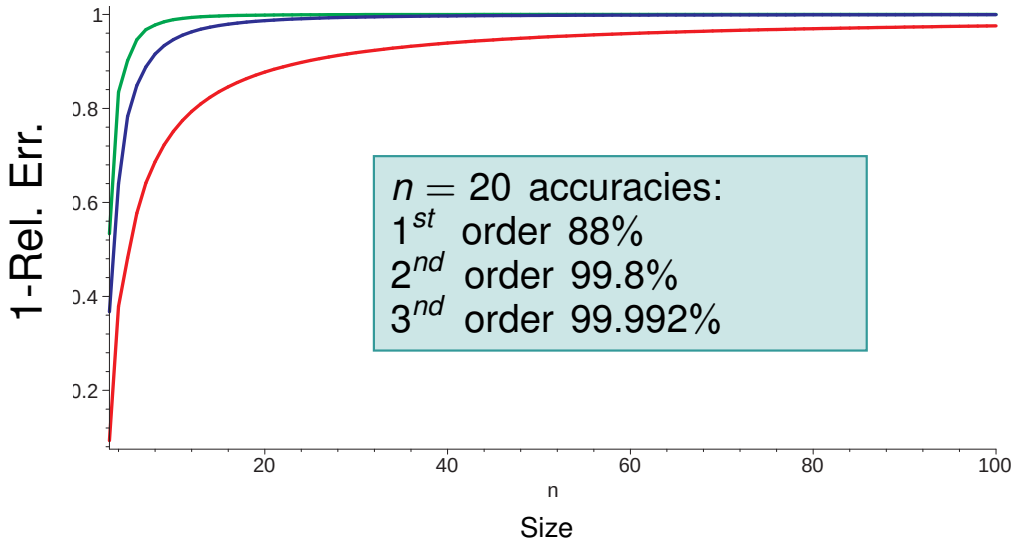

Secondary structures

Technical summary

Message #1

Finding the right decomposition (DP) is a combinatorial task.

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Applying automatic theorems gives precise asymptotic equivalents.
+ Asymptotic regime is reached for small lengths.

Message #3

There is a large exponential number of structures of size n:
Homopolymer model: $\Omega(2^n)$ Stickiness model: $\mathcal{O}(1.8^n/n^{3/2})$

Part II

Enumeration of RNA shapes

Definition (RNA shapes [Giegerich *et al*])

Coarse-grain representation hierarchy for RNA sec. struct.

Based on the *underlying backbone* structure.

Example

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Sec. str. ((((.((((..((((......))))))))((((......)))).))))
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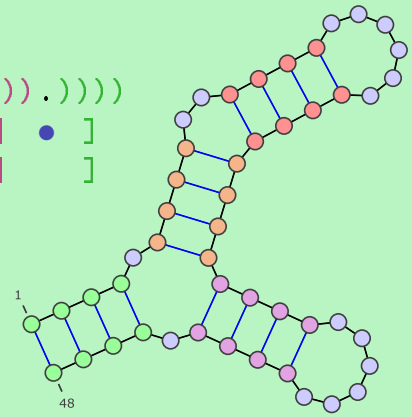
π-shape   [   [     -       - ] [       ]   ]
```



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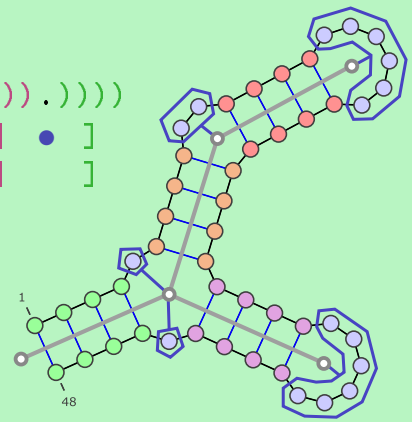

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```
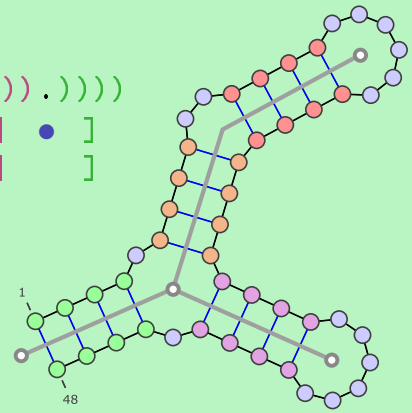
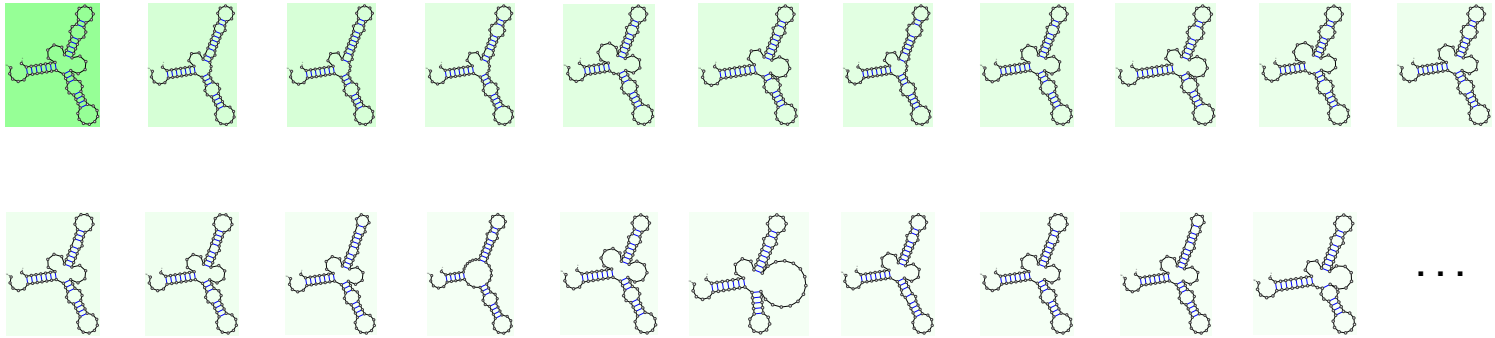

Remove unpaired regions
Contract nested helices



RNA shapes allow a hierarchical search in the Boltzmann ensemble



10000 samples ⇒ 1727 Secondary structures...

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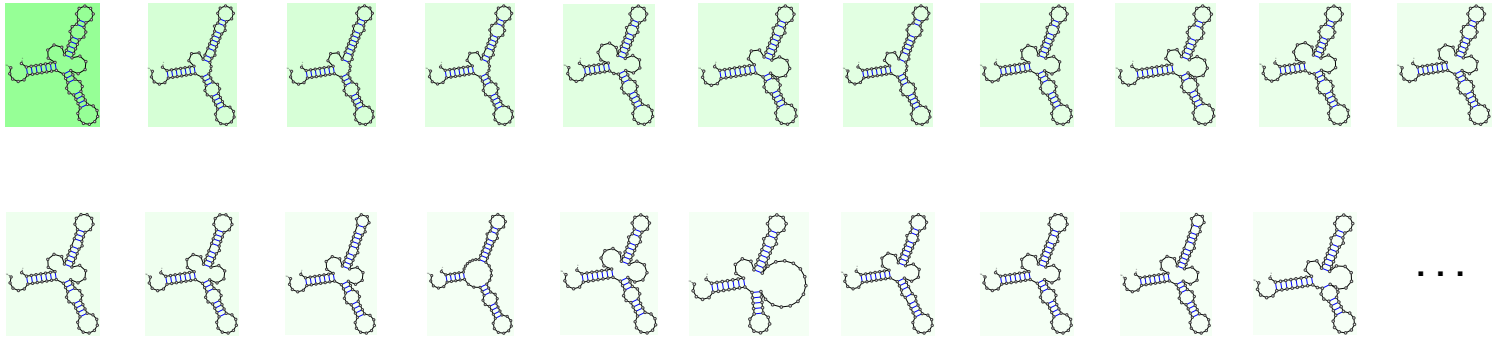

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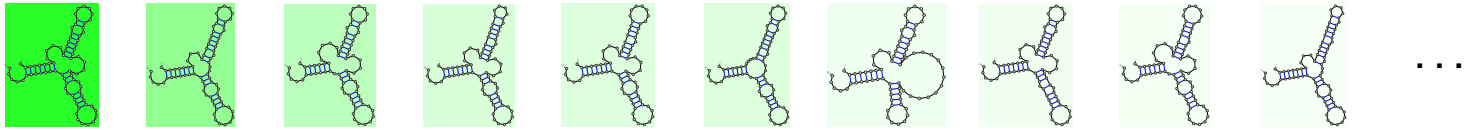

...406 π'-shapes...

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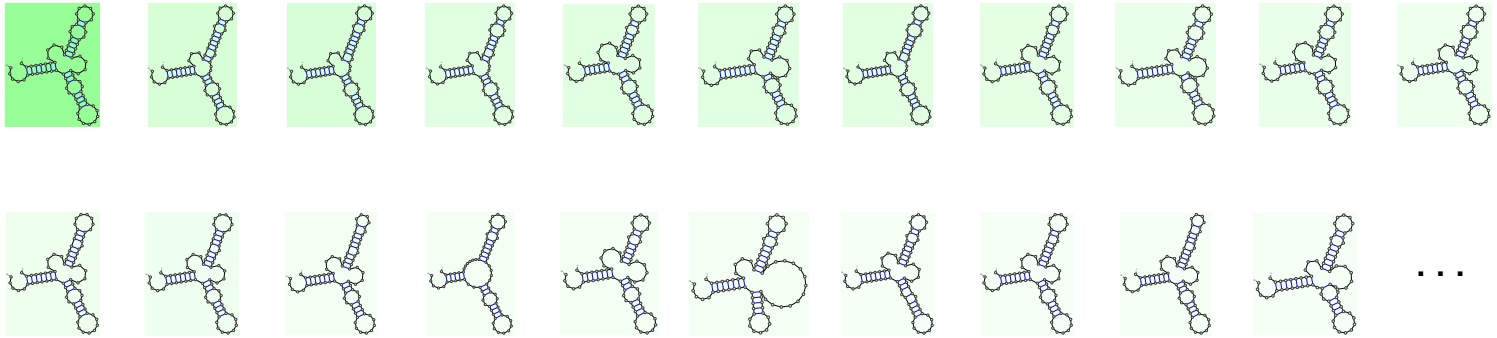

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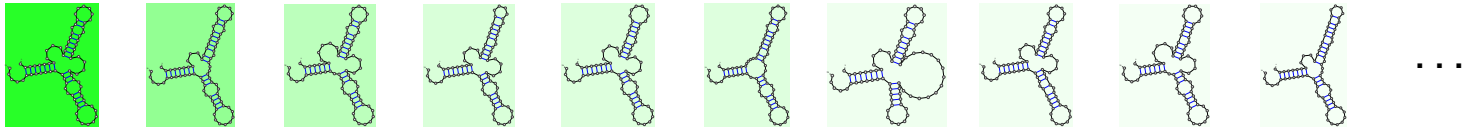

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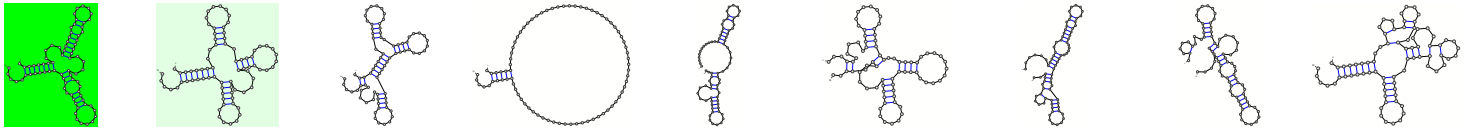
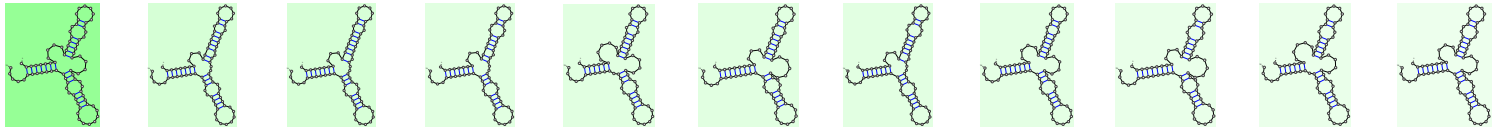
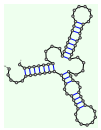


...but only 9 π-shapes!

RNA shapes allow a hierarchical search in the Boltzmann ensemble



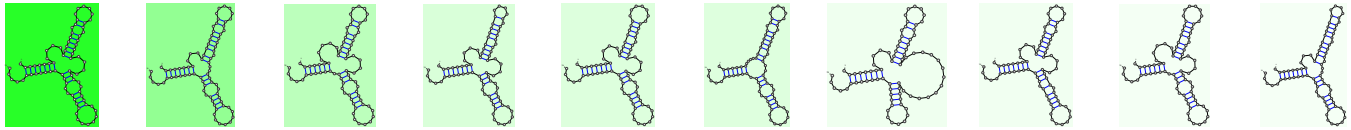


Can we reasonably perform an exhaustive search of all possible shapes compatible with input structure?

How many shapes must we investigate?

10000 samples ⇒ 1727 Secondary structures...

...



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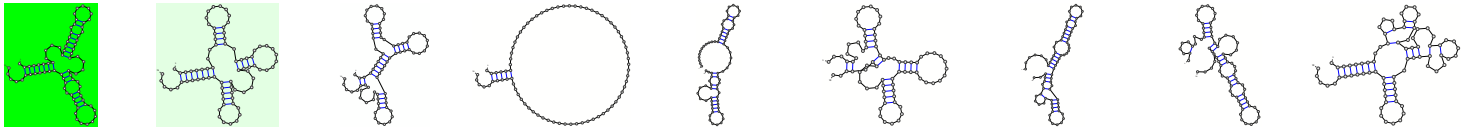
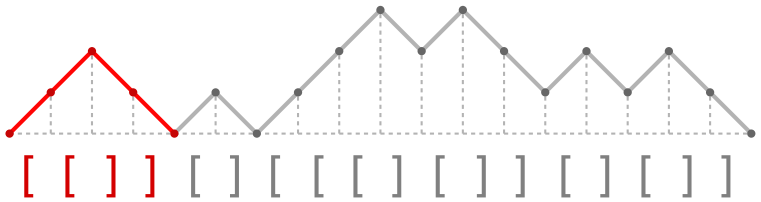


...but only 9 π-shapes!

Objective: Count π-shapes with $2n$ parentheses.



1 π-shapes are bracket words avoiding the `[[...]]` motif.

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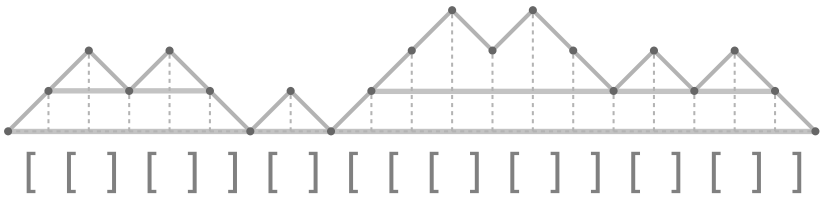

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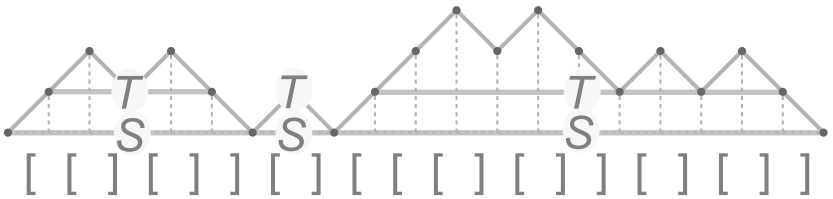

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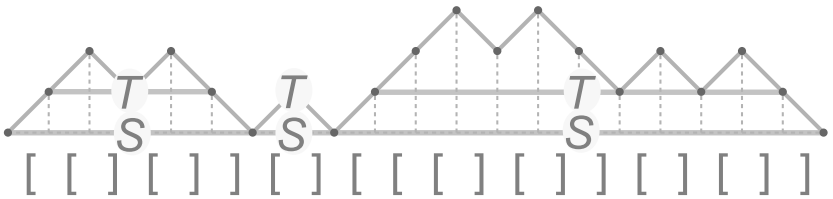

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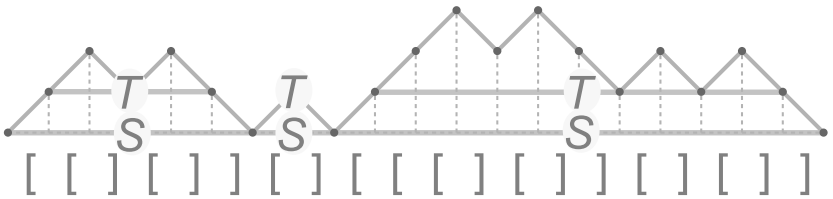

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Remark: Doesn't this look familiar?

These are Motzkin words, i.e. our first example.

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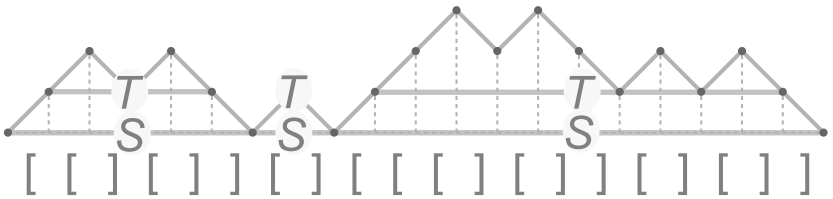

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Limitations

Number of π-shapes of size n

$\neq$

Number of π-shapes compatible with RNA of size n

Reasons:

1. Shapes of size $\leq n$ should be considered
2. Forming a hairpin loop [ ] *takes* at least $\theta + 2$ bases

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4 For n even: $s_{2n} \sim \frac{3\sqrt{3}}{4\sqrt{\pi}} \cdot \frac{3^n}{n\sqrt{\pi}}(1 + \mathcal{O}(1/n)) \approx 0.48 \cdot \frac{3^n}{n\sqrt{n}}$

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$$R \rightarrow \square\,\mathrm{S} \mid \varepsilon$$

3

$$R(z) = \frac{1 - z^2 - \sqrt{1 - 2z^2 - 3z^4}}{2z^2(\mathbf{1} - \mathbf{z})}$$

4

$$r_{2n} \sim \frac{3\sqrt{3}}{4\sqrt{\pi}} \cdot \frac{3^n}{n\sqrt{n}}(1 + \mathcal{O}(1/n)) \Rightarrow r_n \approx 2.07 \cdot \frac{1.73^n}{n\sqrt{n}}$$

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$$R(z) = \frac{1 - z^{\theta+2} - \sqrt{1 - 2z^{\theta+2} - 4z^{\theta+4} + z^{2\theta+4}}}{2z^2(1-z)}$$

4

$$\theta = 3 \Rightarrow r_n \approx 2.44\frac{1.32^n}{n\sqrt{n}}$$

Objective: Count π'-shapes compatible with RNA of length n.

1. π'-shapes = bracket words avoiding motifs `[[...]]` and $\bullet\bullet$

2. $R \to \square\, R \mid S \qquad S \to U\,[\,T\,]\,S \mid U \qquad U \to \diamond \mid \varepsilon$

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3. $\theta = 3,\ R(z) = \frac{1+2z^2+2z^3+z^4-z^5-z^6-\sqrt{1-4z^3-2z^4-2z^5+2z^6-7z^8-z^{10}+2z^{11}+z^{12}}}{2z^2(1-z^2)}$

4. $r_n \sim 1.27\,\frac{1.81^n}{n\sqrt{n}}$

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4. $$r_n \sim 1.27\frac{1.81^n}{n\sqrt{n}}$$

Summary

Model	Asymptotic number
Sec. str. on n – Combinatorial	$1.1 \cdot \frac{\mathbf{2.6}^n}{n\sqrt{n}}$
Sec. str. on n – Empirical	$0.04 \cdot \frac{\mathbf{1.4}^n}{n\sqrt{n}}$
π-shapes of size n	$1.38 \cdot \frac{\mathbf{1.73}^n}{n\sqrt{n}}$
π-shapes compatible with sec. str. on n	$2.44 \cdot \frac{\mathbf{1.32}^n}{n\sqrt{n}}$
π-shapes – Empirical	$0.21 \cdot \frac{\mathbf{1.1}^n}{n\sqrt{n}}$
π'-shapes of size n	$0.99 \cdot \frac{\mathbf{2.41}^n}{n\sqrt{n}}$
π'-shapes compatible with sec. str. on n	$1.28 \cdot \frac{\mathbf{1.81}^n}{n\sqrt{n}}$

Message #4

The number of π-shapes (coarsest) is upper-bounded by 1.3^n.
$\Rightarrow$ Shape-based indexing is feasible through exhaustive search.

Part III

Realistic models for random RNA structures

Motivation

Random generation can be used to:

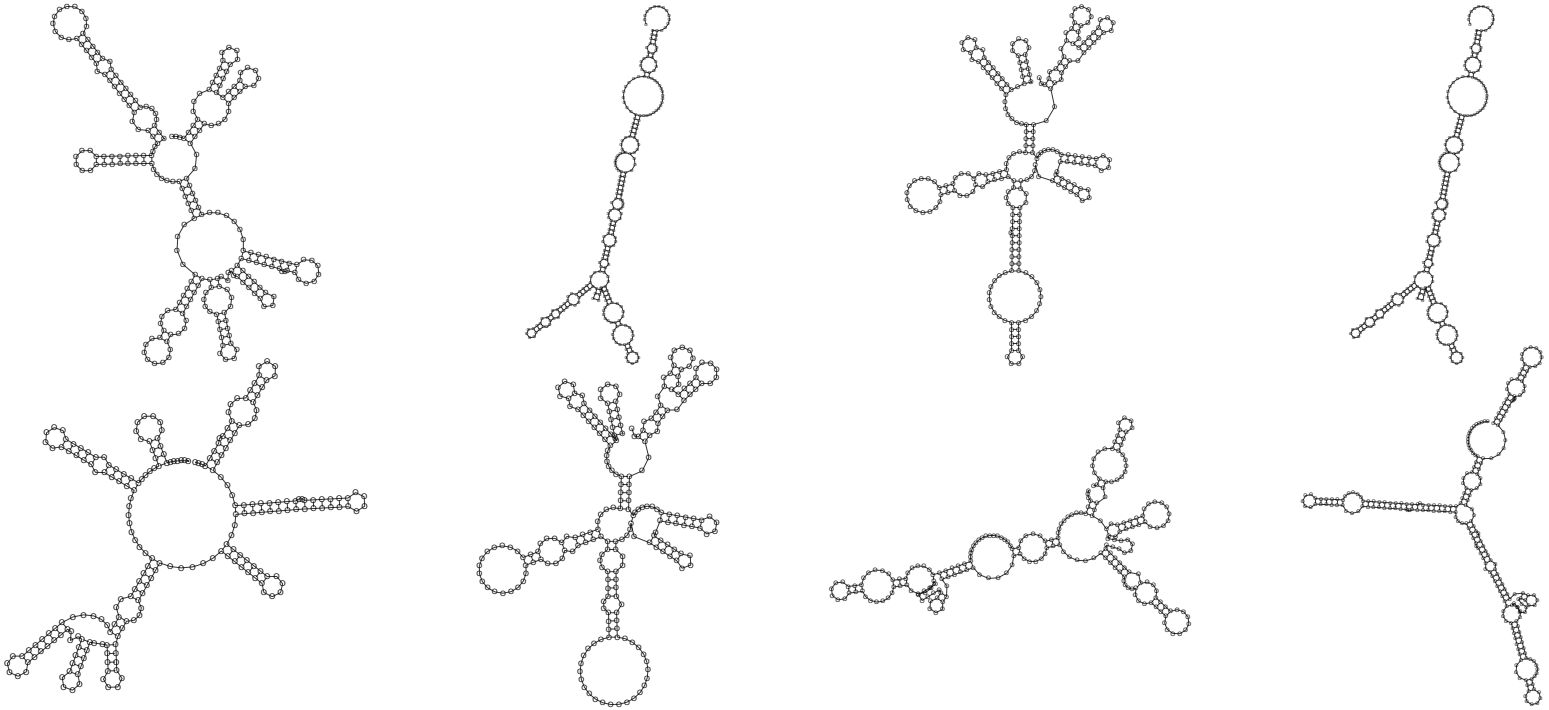
- Assess the significance of observed phenomena
- Estimate the practical complexity of algorithms

Worst-case complexity can be *misleading*:

- Statistical sampling: $\Theta(n^2) \to \mathcal{O}(n\sqrt{n})$
- Tree alignment: $\Theta(n^4) \to \mathcal{O}(n^2)$

Average case analysis: Hidden uniformity hypothesis
Yet all secondary structures are not equally likely observed as native structures!

RNA secondary structures (Mathews DB)



Grammar approach for the random generation

RNA secondary structures = ()-free Motzkin words [Wat78]

+ Uniform random generation (Boltzmann, recursive . . .)

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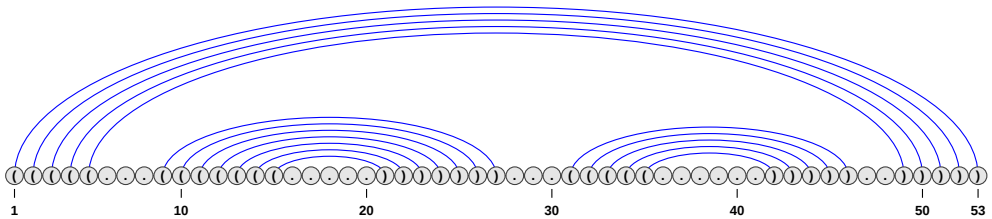

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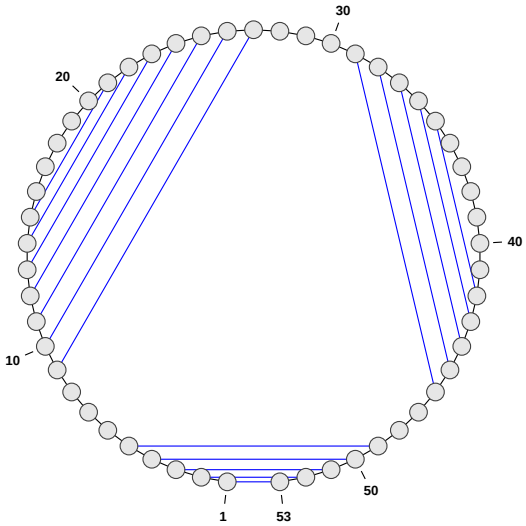

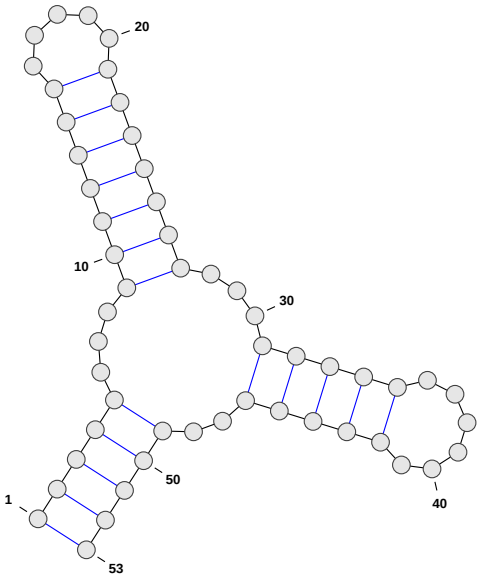

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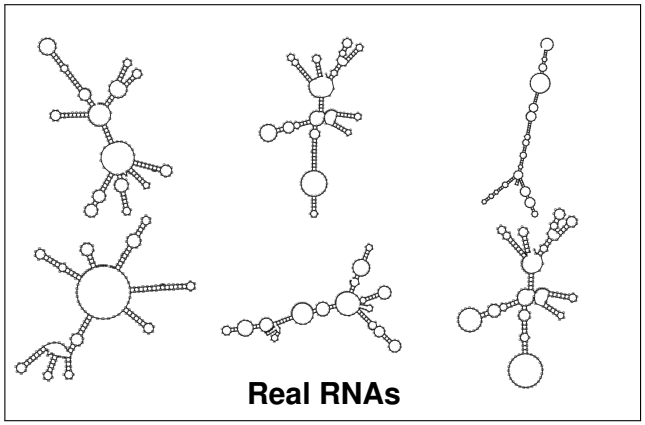
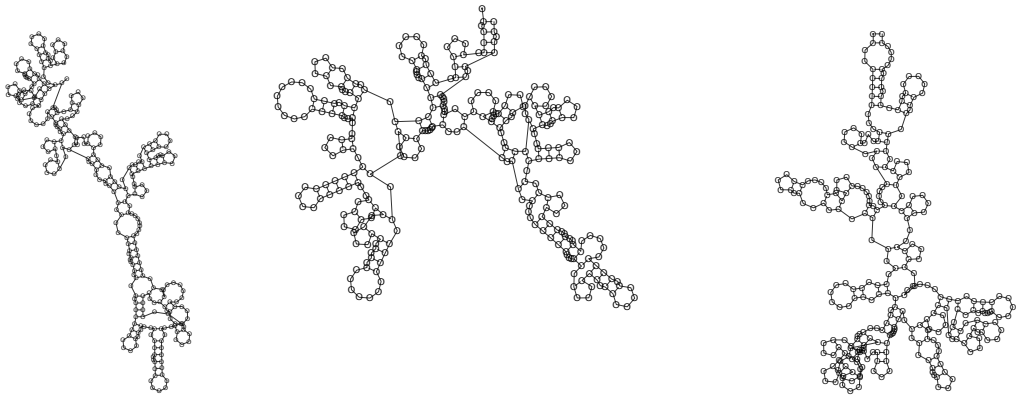
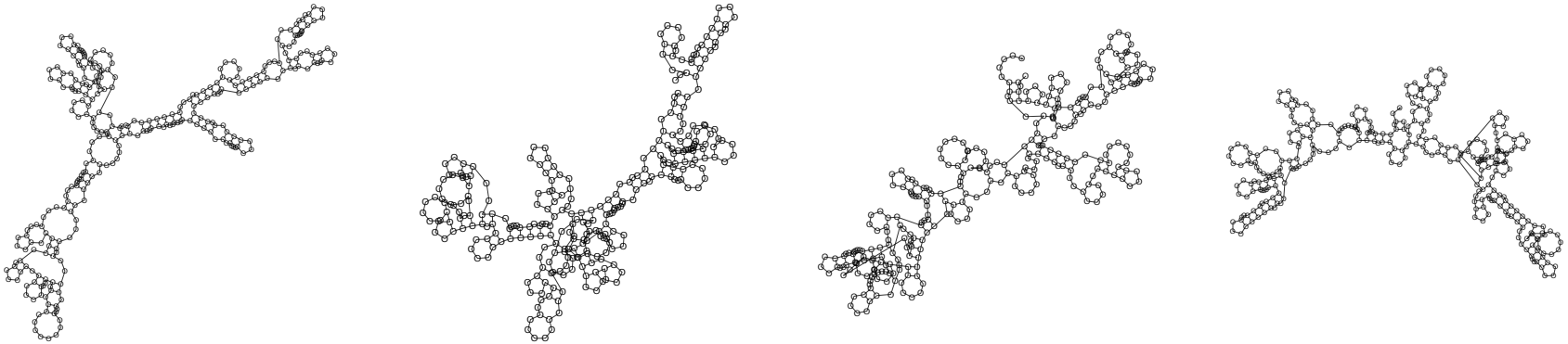




Random uniform RNAs



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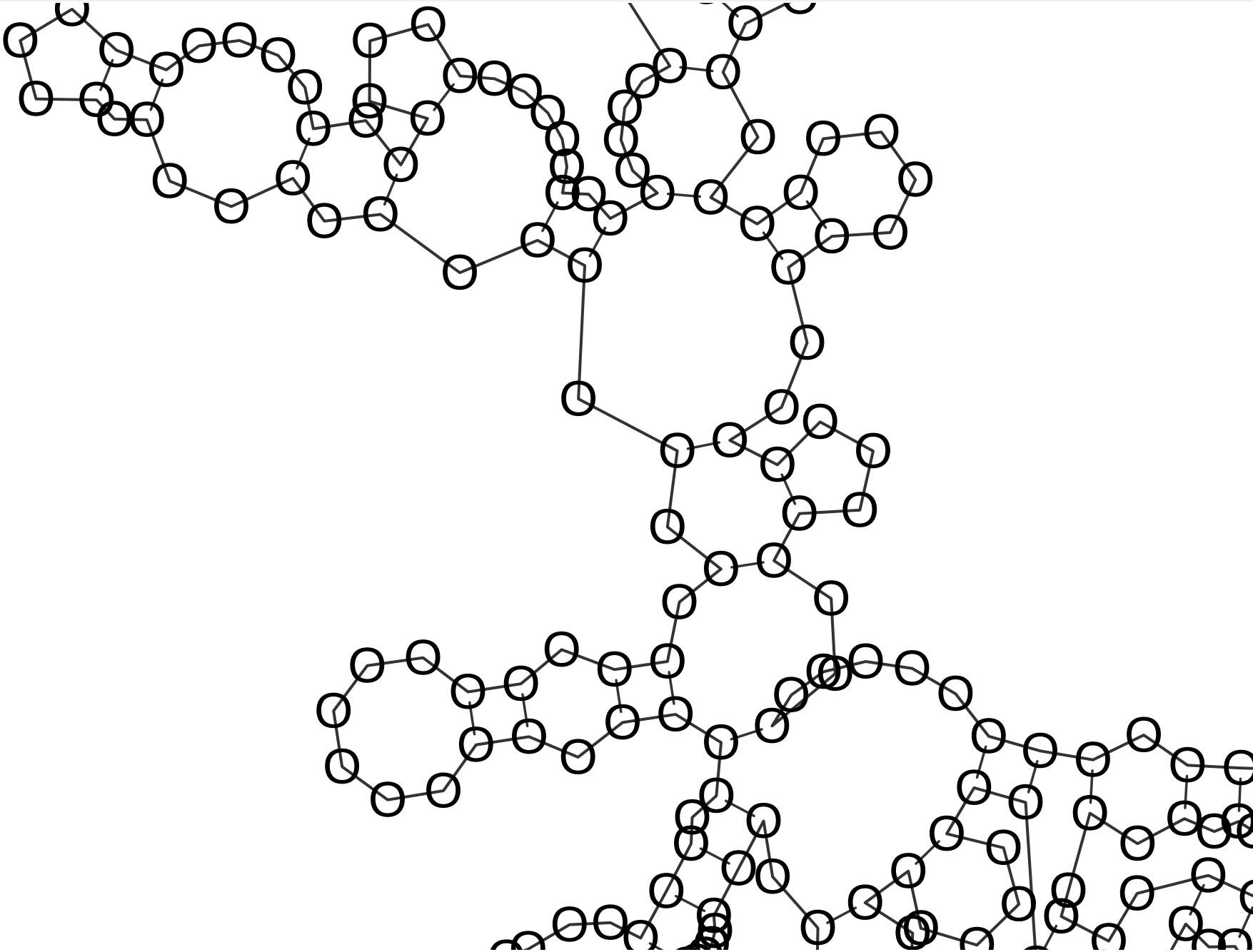

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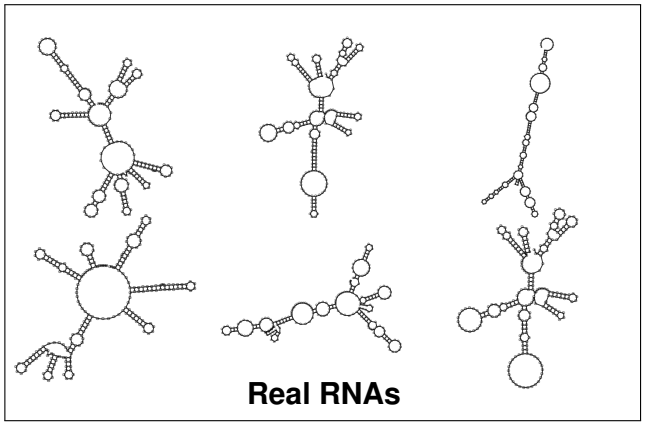
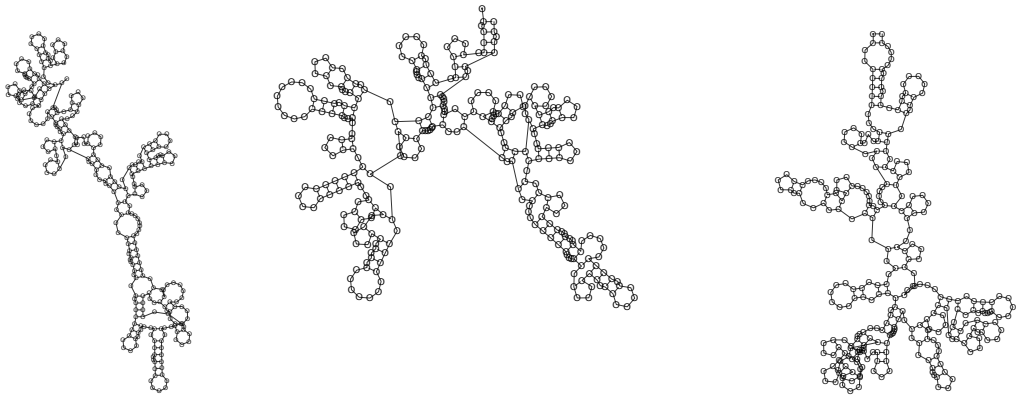
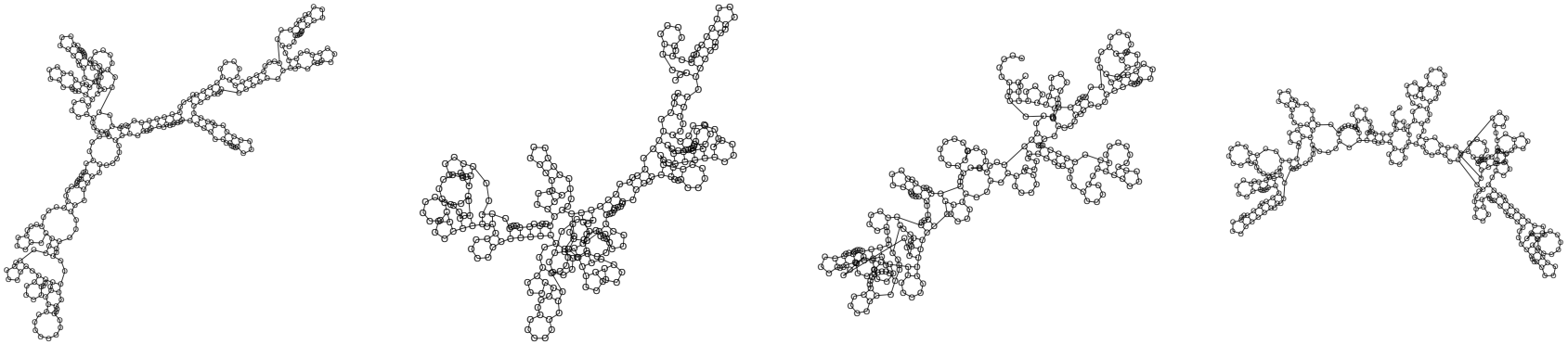




Random uniform RNAs



Grammar approach for the random generation

RNA secondary structures = ()-free Motzkin words [Wat78]

+ Uniform random generation (Boltzmann, recursive . . .)

+ Sequence/structure annotation (h: Base-pair, H: Helix, . . .)

Example:

```
( ( ( ( ( • • • ( ( ( ( ( ( ( • • • • • ) ) ) ) ) ) ) • • • ( ( ( ( ( • • • • • • ) ) ) ) ) • • ) ) ) ) )
H h h h h M m m H h h h h h h T t t t t h h h h h h h m m m H h h h h T t t t t t h h h h h m m h h h h h
```

Annotate native structures from Mathews database
$\Rightarrow$ Proportions of occurrences of each terminal

Grammar can be changed to generate already annotated structures
$\Rightarrow$ Evaluate expected proportions in the uniform model

Available techniques:

- Empirical
- Recursive/Dynamic programming
- Flajolet–Symbolic, . . .

Grammar approach for the random generation

RNA secondary structures = ()-free Motzkin words [Wat78]

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Feature	B	b	I	i	M	m	T	t	H	h
$\mathcal{M}_0$ (%)	7.2	5.6	2.8	7.3	3.7	7.6	5.2	14.5	**18.6**	**27.5**
Exper.	1.5	2.3	1.9	11.2	1.1	9.0	2.6	16.6	**4.8**	**48.9**

Proportions of symbols associated with structural features:
Uniform model $\mathcal{M}_0$ vs Observed ($n = 300$)

As suspected, avg helix length $(\mathrm{H} + \mathrm{h})/(2 * \mathrm{H})$ is way off!
($\mathcal{M}_0$: 1.23, Exp: 5.59)

Limits of the uniform model

Message #1

RNA secondary structures $\Rightarrow$ Uniform model = Bad!

Null-hypothesis too easily refuted/Overrated statistical significance
Bonzai structures might yield biased estimates.

Message #2

Need (at least) to account for constraints on min/avg sizes of features.

- Helices
- Sequences of unpaired bases
- Degree of branching loops

Constraints captured by grammars in two ways:

- Min: Tweak grammar
- Avg: Modify distribution $\Rightarrow$ Weighted probability distribution

Definition (Context-free grammar)

Context-free grammar = 4-tuple $(\Sigma, \mathcal{N}, \mathcal{P}, \mathcal{S})$:

- Σ: Alphabet.
- $\mathcal{N}$: Non-terminal symbols.
- $\mathcal{P}$: Set of production rules $N \to X \in \mathcal{N} \times \{\Sigma \cup \mathcal{N}\}^*$.
- $\mathcal{S}$: Axiom, or initial non-terminal.

Definition (Weighted context-free grammar [DRT00])

A **weighted** context-free grammar is a **5**-tuple $\mathcal{G}_\pi = (\Sigma, \mathcal{N}, \mathcal{P}, \mathcal{S}, \boldsymbol{\pi})$:

- Σ, $\mathcal{N}$, $\mathcal{P}$, $\mathcal{S}$: Same as previously.
- π: **Weight function** $\pi : \Sigma \to \mathbb{R}$.

Then $\mathcal{G}_\pi$ implicitly defines a **weighted probability distribution** $\mathcal{W}$:

$$\forall \omega \in \mathcal{L}(\mathcal{G}_\pi),\ \mathbb{P}(\omega) = \frac{\pi(\omega)}{\pi\left(\mathcal{L}(\mathcal{G}_\pi)\right)} = \frac{\prod_{i=1}^{|\omega|} \pi(\omega_i)}{\pi\left(\mathcal{L}(\mathcal{G}_\pi)\right)}.$$

Generation: Generating k words of size n takes $\mathcal{O}(n^2 + n\log(n).k)^*$ or $\mathcal{O}(n\log(n).k)^*$ using symbolic preprocessing (linear recurrences).

*Arithmetic operations $\Rightarrow$ $\Theta(n)$ overhead OR tricky interval arithmetics [DZ99]

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Summary

1 – Uniform models are bad!

2 – Let's use weighted distribution!

Which one?

Weights should be computed such that:

- Sampled structure follow Boltzmann distribution
  Not very good for modeling native structure
- Expectation is maximized
  Not very easy (WCFG$\neq$SCFG)
  Overfitting vs Inconsistance
- Aiming at observed average properties
  Average random objects should mimic features of the training set

How to compute weights targeting a given observation?

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How to compute weights targeting a given observation?

Computing suitable weights

$f^{\pi}_{n,i}$: Proportion of Z_i in words of size n drawn from a π-distribution
$f^{\pi}_{*,i}$: Asymptotic proportion of Z_i in π ($f^{\pi}_{*,i} = \lim_{n\to\infty} f^{\pi}_{n,i}$)

Problem

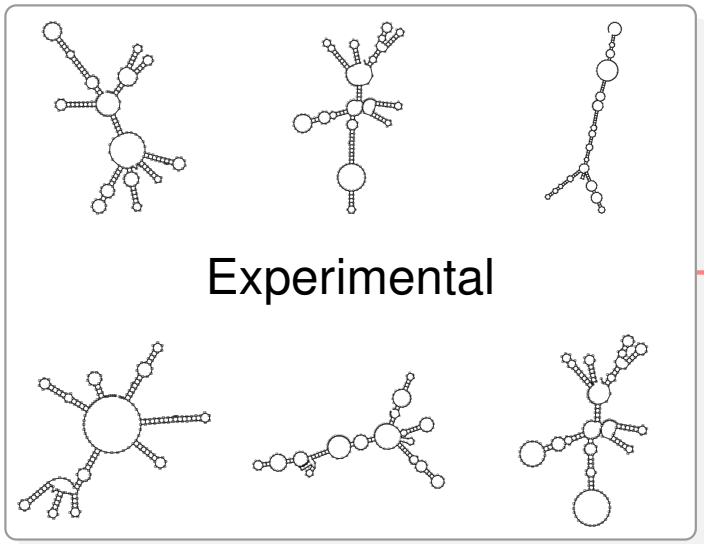
Input: Grammar $\mathcal{G}$, target frequencies (μ_i), length n
Goal: Find π_0 such that $\forall i \in [1, |\Sigma|],\ f^{\pi_0}_{n,i} = \mu_i$

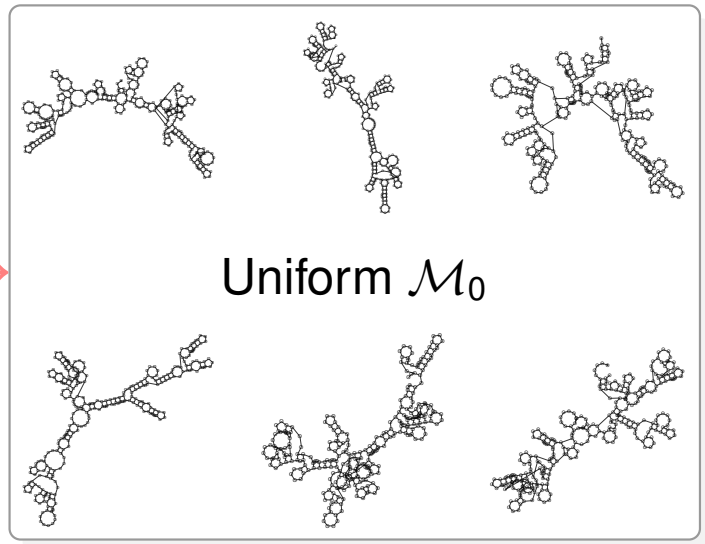
- Analytic (asymptotic) Rational
- Analytic (asymptotic) Algebraic
- Automatic heuristic (Fixed size)
  Computation of $f^{\pi}_{n,i}$ easier than that of weight vector π_0
  + *Partial monotonicity*: $(\pi(Z_i) \nearrow) \to (f^{\pi}_{n,i} \nearrow)$
  $\Rightarrow$ Rephrase as an continuous optimization problem

$$\text{Objective function: } g(\pi) = \sqrt{\sum_i \left(f^{\pi}_{n,i} - \mu_i\right)^2}$$

We use the CONDOR optimizer software package [Ber05].

Application





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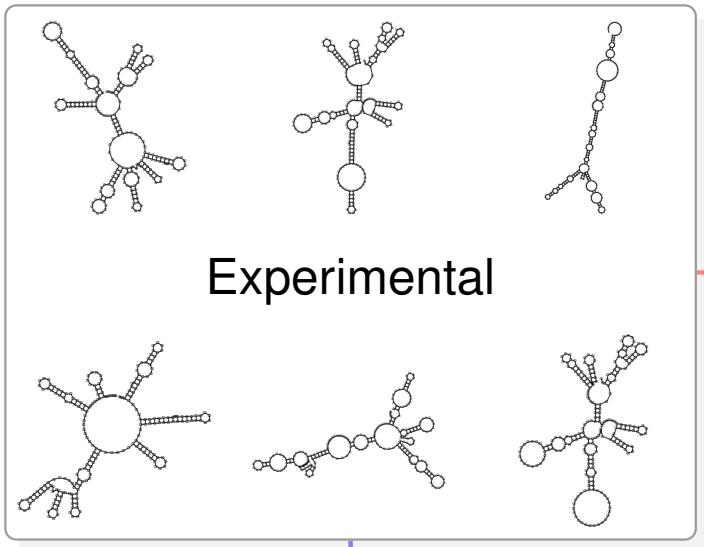

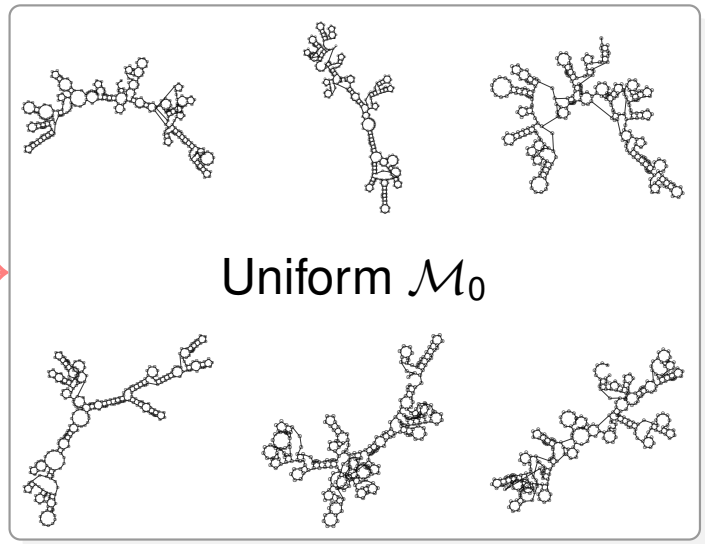

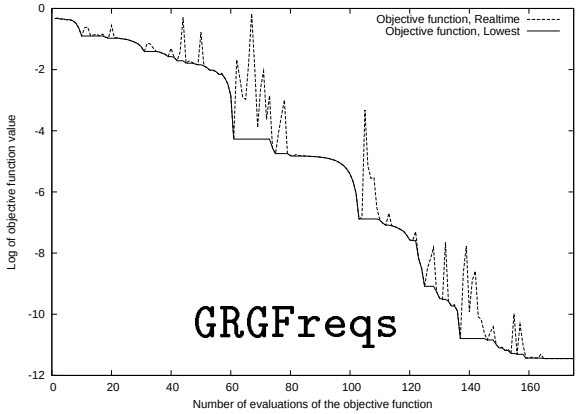

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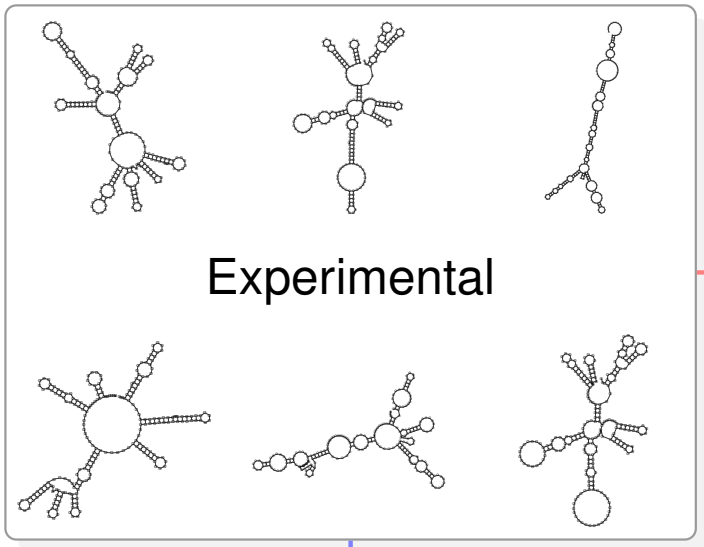

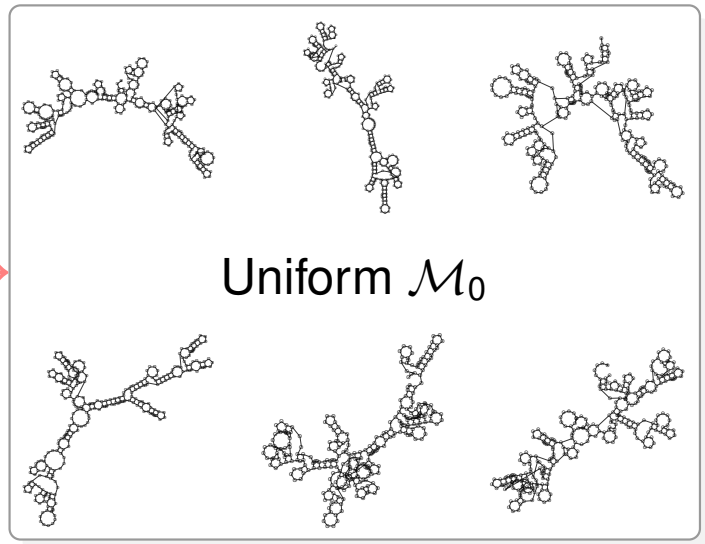

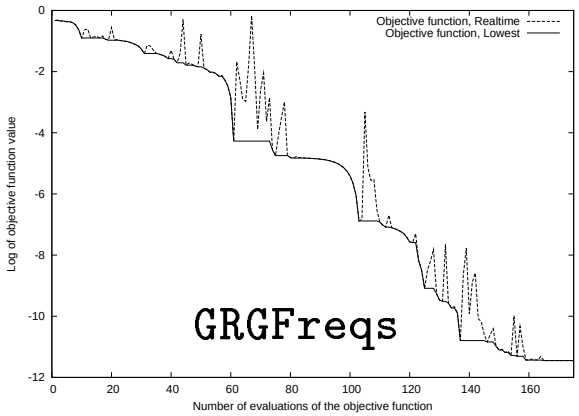

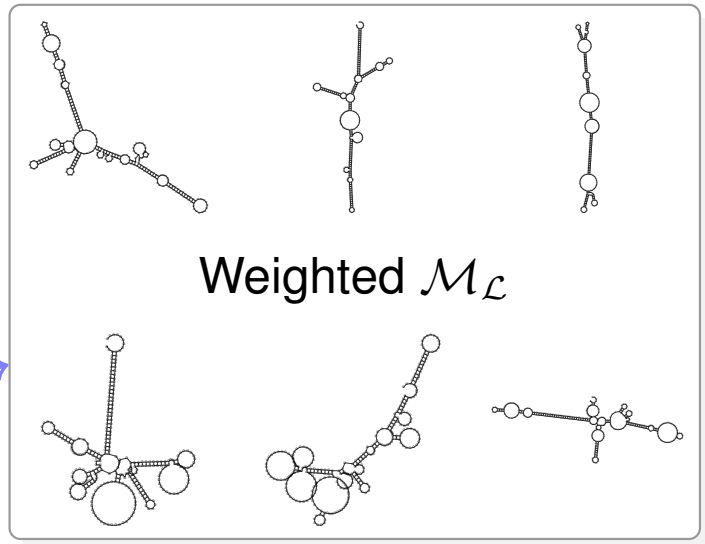

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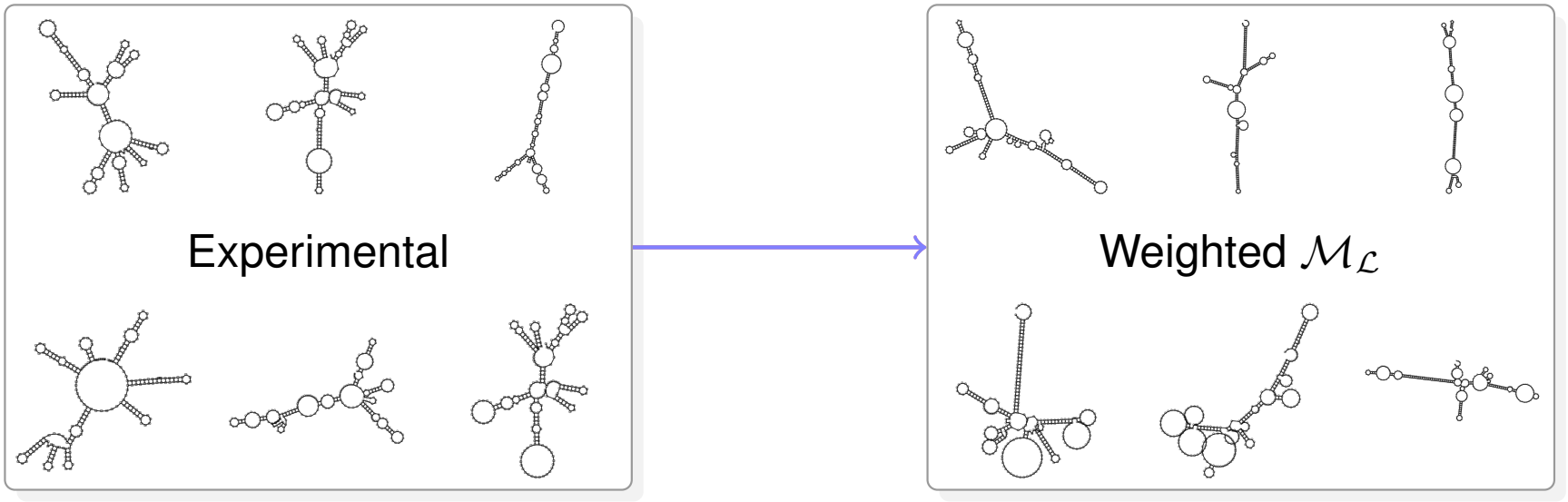
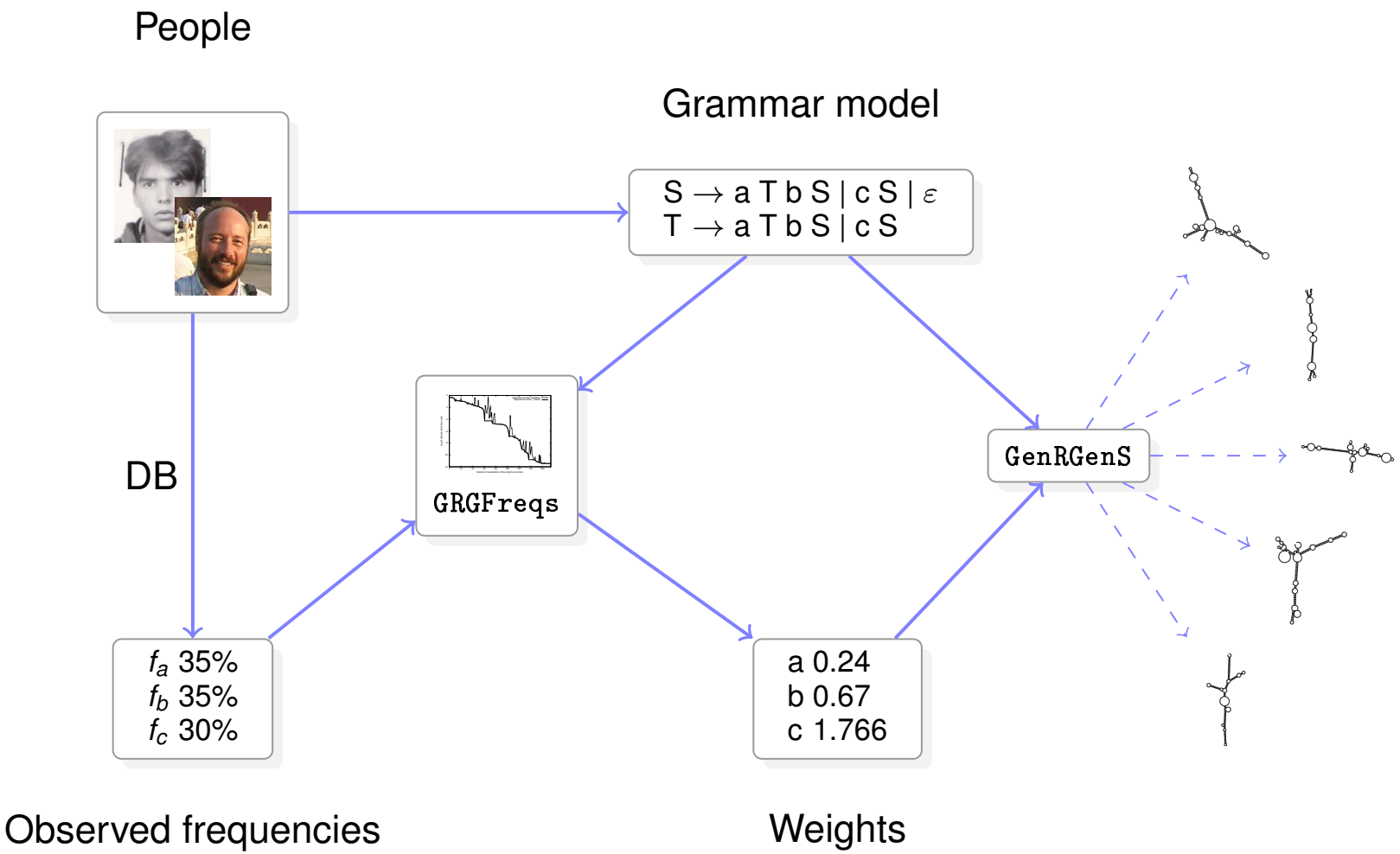


Feature	B	b	I	i	M	m	T	t	H	h
Weight	1.15	1.15	1.15	1.15	2.75	1	1.15	1.15	.00308	1.25
$\mathcal{M}_0$ (%)	7.2	5.6	2.8	7.3	**3.7**	**7.6**	5.2	14.5	**18.6**	**27.5**
$\mathcal{M}_{\mathcal{L}}$ (%)	0.6	2.9	1.5	16.1	**1.1**	**9.0**	1.8	13.3	**4.8**	**48.9**
Exp.	1.5	2.3	1.9	11.2	**1.1**	**9.0**	2.6	16.6	**4.8**	**48.9**

Conclusion/Perspectives



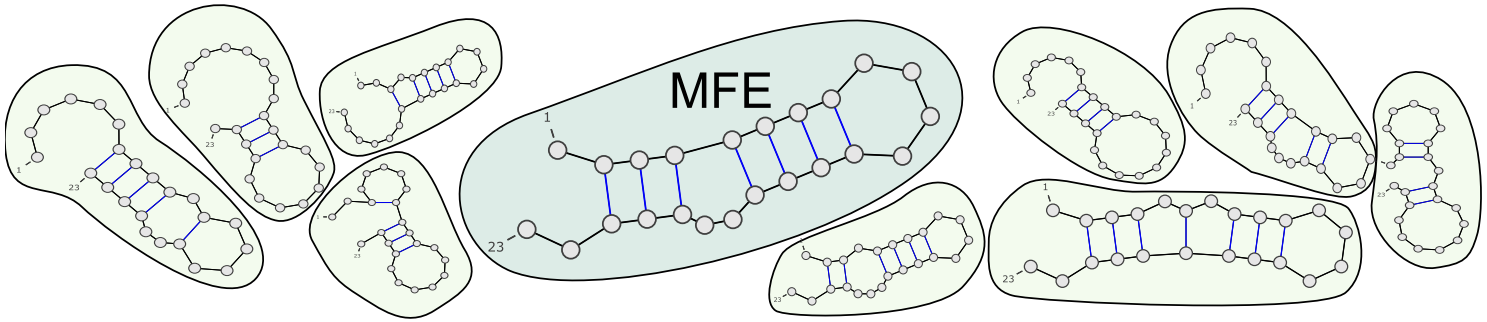
Now we can generate random structures fulfilling any desired properties.

Part IV

Improved statistical sampling

2004– : Boltzmann-ensemble paradigm

RNA *breathes* ⇒ There is not necessarily one functional conformation!



Boltzmann-ensemble paradigm

All possible secondary structures for an RNA coexist in a probability distribution, the Boltzmann distribution.

Consequently: MFE structure probability can be significantly remote (*unstructured RNAs?*).
Conversely, base-pairs having probability $> .99$ are valid 95% of the time.

- ⇒ Similar structures can bundle up.
- ⇒ Functional structures must be sought in sub-optimal structures.

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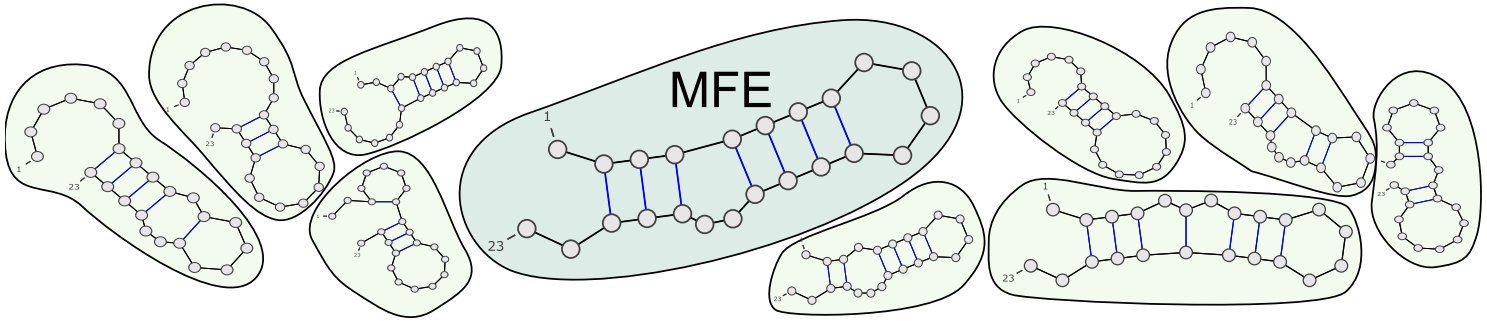


Boltzmann-ensemble paradigm

All possible secondary structures for an RNA coexist in a probability distribution, the Boltzmann distribution.

Experiment: [DCL05]

- Sample structures from Boltzmann probability
- Cluster based on structural distance
- Build consensus structure based on most-probable cluster

⇒ Relative improvement for specificity (+17.6%) and sensitivity (+21.74%, except Group II Introns)

Boltzmann distribution: Definitions

A Boltzmann distribution assigns a weight to each structure S for an RNA ω, called the Boltzmann factor $e^{\frac{-E_{S,\omega}}{RT}}$ where:

- $E_{S,\omega}$ is the free-energy of S (kCal.mol^{-1})
- T is the temperature (K)
- R is the Boltzmann *constant* (1.986.10^{-3} kCal.K^{-1}.mol^{-1})

Distribution is renormalized on $\mathcal{S}_\omega$ by the partition function

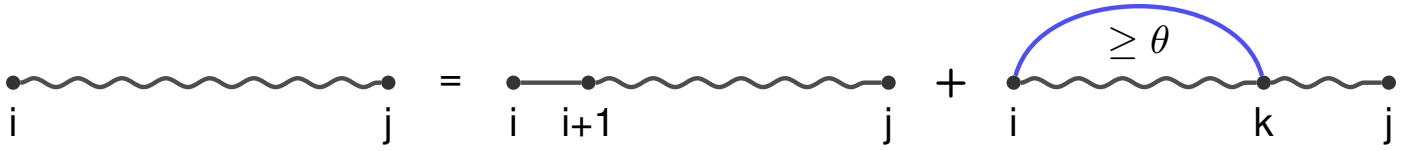
$$\mathcal{Z}_\omega = \sum_{S \in \mathcal{S}_\omega} e^{\frac{-E_{S,\omega}}{RT}}.$$

where $\mathcal{S}_\omega$ is the set of conformations compatible with ω.

The Boltzmann probability of a structure S is therefore

$$P_{S,\omega} = \frac{e^{\frac{-E_{S,\omega}}{RT}}}{\mathcal{Z}_\omega}.$$

Partition function



Recurrence on the minimal free-energy of a folding:

$$
\begin{aligned}
N_{i,t} &= 0, \quad \forall t \in [i, i+\theta] \\
N_{i,j} &= \min \begin{cases} N_{i+1,j} & (i \text{ unpaired}) \\ \min_{k=i+\theta+1}^{j} E_{i,j} + N_{i+1,k-1} + N_{k+1,j} & (i \text{ comp. with } k) \end{cases}
\end{aligned}
$$

+ Unambiguity

$\Rightarrow$ Recurrence on partition function :

$$
\begin{aligned}
\mathcal{Z}_{i,t} &= 1, \quad \forall t \in [i, i+\theta] \\
\mathcal{Z}_{i,j} &= \sum \begin{cases} \mathcal{Z}_{i+1,j} & (i \text{ unpaired}) \\ \sum_{k=i+\theta+1}^{j} e^{\frac{1}{RT}} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} & (i \text{ comp. with } k) \end{cases}
\end{aligned}
$$

+ Some slightly non-trivial computations. . .

= Base-pair probabilities in the Boltzmann ensemble [McC90].

Summands of $\mathcal{Z}$ $\Leftrightarrow$ Cumulated Boltzmann weight of all accessible structures.

$\Rightarrow$ Stochastic Backtrack recursively generates compatible structures with respect to the Boltzmann distribution [DL03].

$$\mathcal{Z}_{i,t} = 1, \quad \forall t \in [i, i+\theta]$$

$$\mathcal{Z}_{i,j} = \sum \begin{cases} \mathcal{Z}_{i+1,j} & \text{A} \\ \sum_{k=i+\theta+1}^{j} e^{\frac{1}{RT}} \times \mathcal{Z}_{i+1,k-1} \times \mathcal{Z}_{k+1,j} & \text{B}_k \end{cases}$$

$$A \,|\, B_{i+\theta+1} \,|\, B_{i+\theta+2} \,|\, B_{i+\theta+3} \,|\, \ldots \,|\, B_{j-2} \,|\, B_{j-1} \,|\, B_j$$

$\Rightarrow$ Worst-case complexity in $\mathcal{O}(n^2 k)$ for k samples.
Could we do better than that to sample more at the same computational cost?

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$\mathcal{Z}_{i+1,j}/\mathcal{Z}_{i,j}$ → $\mathcal{Z}_{i+1,j}$

$\frac{e^{\frac{1}{RT}} \mathcal{Z}_{i+1,k-1} \mathcal{Z}_{k+1,j}}{\mathcal{Z}_{i,j}}$ → r

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r

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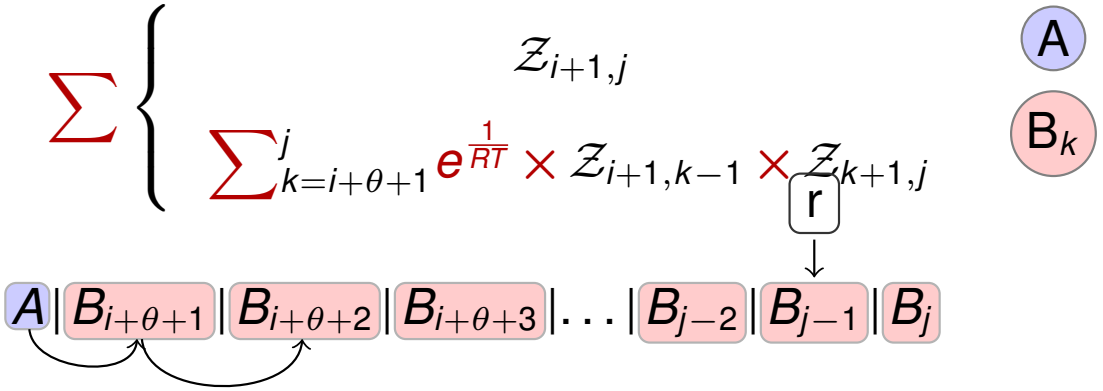

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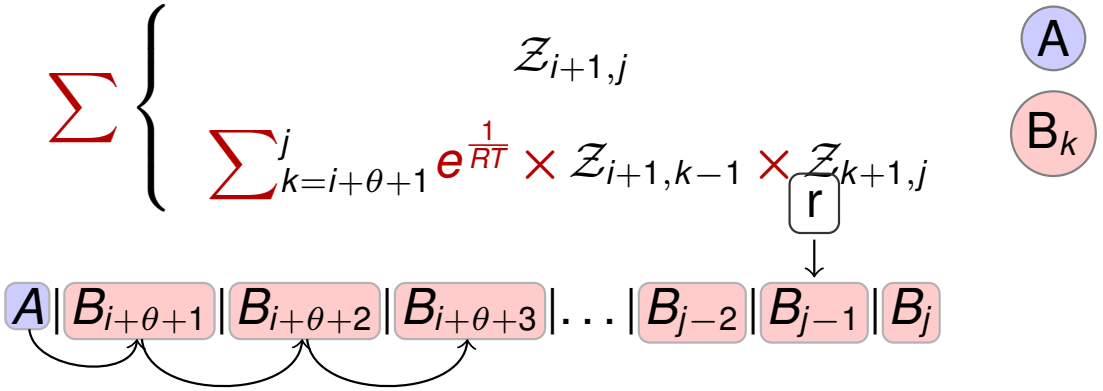

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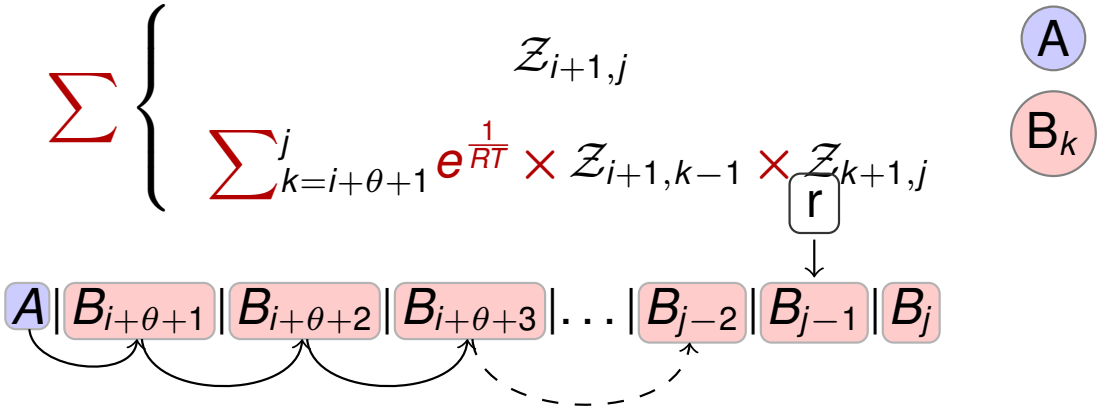

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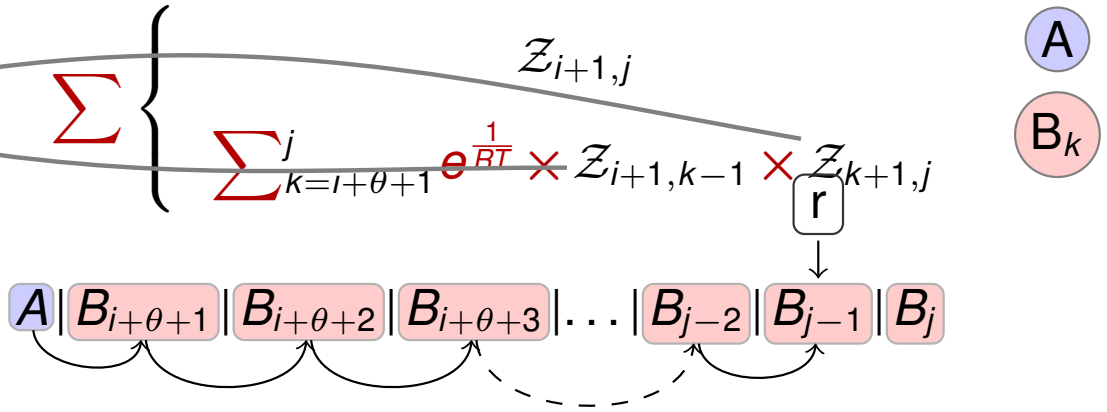

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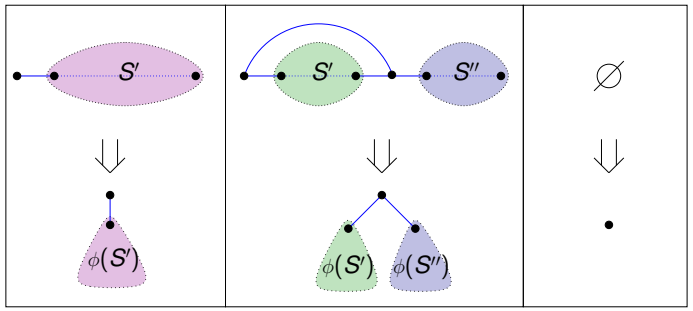
Average-case analysis

Homopolymer model: Any base-pair allowed.
Boltzmann distribution: Based on a Nussinov/Jacobson energy model.

Theorem

Let n be the RNA length et k the #samples.
The average-case complexity of statistical sampling is in $\Theta(n^3 + kn\sqrt{n})$.



Remark 1: Cost $c(S)$ of generating a structure S is dominated by the cumulated size of the leftward children $t \in \mathcal{T}_g(S)$ in the associated unary/binary tree:

$$c(S) = n - \#bp(S)/2 + \sum_{t \in \mathcal{T}_g(S)} |t| - \theta$$

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Theorem

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The average-case complexity of statistical sampling is in $\Theta(n^3 + kn\sqrt{n})$.

Let us focus on the expectation of the *generation cost* random variable X . We have

$$\mathbb{E}(X \mid n) = \sum_{|S|=n} c(S) \cdot \frac{e^{\frac{bp(S)}{RT}}}{\mathcal{Z}_n} = \frac{\sum_{|S|=n} c(S) \cdot e^{\frac{bp(S)}{RT}}}{\sum_{|S|=n} e^{\frac{bp(S)}{RT}}}.$$

Let us consider separately the two generating functions $C(z)$ and $P_f(z)$ such that

$$C(z) = \sum_{S \in \mathcal{S}} e^{\frac{bp(S)}{RT}} c(S) z^{|S|} \quad \text{et} \quad P_f(z) = \sum_{S \in \mathcal{S}} e^{\frac{bp(S)}{RT}} z^{|S|}.$$

Let us remark that $\mathbb{E}(X \mid n) = [z^n]C(z)/[z^n]P_f(z)$.

Average-case analysis

Homopolymer model: Any base-pair allowed.
Boltzmann distribution: Based on a Nussinov/Jacobson energy model.

Theorem

Let n be the RNA length et k the #samples.
The average-case complexity of statistical sampling is in $\Theta(n^3 + kn\sqrt{n})$.

$C(z)$ and $P_f(z)$ are the positive solutions of the system

$$\begin{aligned}
C(z) &= z\left(P_f(z) + C(z)\right) + z^2 e^{\frac{1}{RT}}(1-\theta)P_f^{\geq\theta}(z)P_f(z) \\
&+ z^3 e^{\frac{1}{RT}} \frac{\partial P_f^{\geq\theta}(z)}{\partial z} P_f(z) + z^2 e^{\frac{1}{RT}} C^{\geq\theta}(z)P_f(z) \\
&+ z^2 e^{\frac{1}{RT}} P_f^{\geq\theta}(z)C(z) \\
P_f(z) &= z^2 e^{\frac{1}{RT}} P_f^{\geq\theta}(z)P_f(z) + zP_f(z) + 1
\end{aligned}$$

where $S^{\geq\theta}(z)$ is the truncated gen. fun. $S(z)$, deprived of its terms of degrees $< \theta$.

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Let n be the RNA length et k the #samples.
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$\Rightarrow$ Big generating functions (Thanks to Maple !)...

+ Singularity analysis:

$$[z^n]P_f(z) \sim \frac{\kappa}{\rho^n n\sqrt{n}}(1 + \mathcal{O}(1/n)) \qquad [z^n]C(z) \sim \frac{\kappa'}{\rho^n}(1 + \mathcal{O}(1/\sqrt{n}))$$

We obtain an asymptotic equivalent for $\mathbb{E}(X \mid n)$:

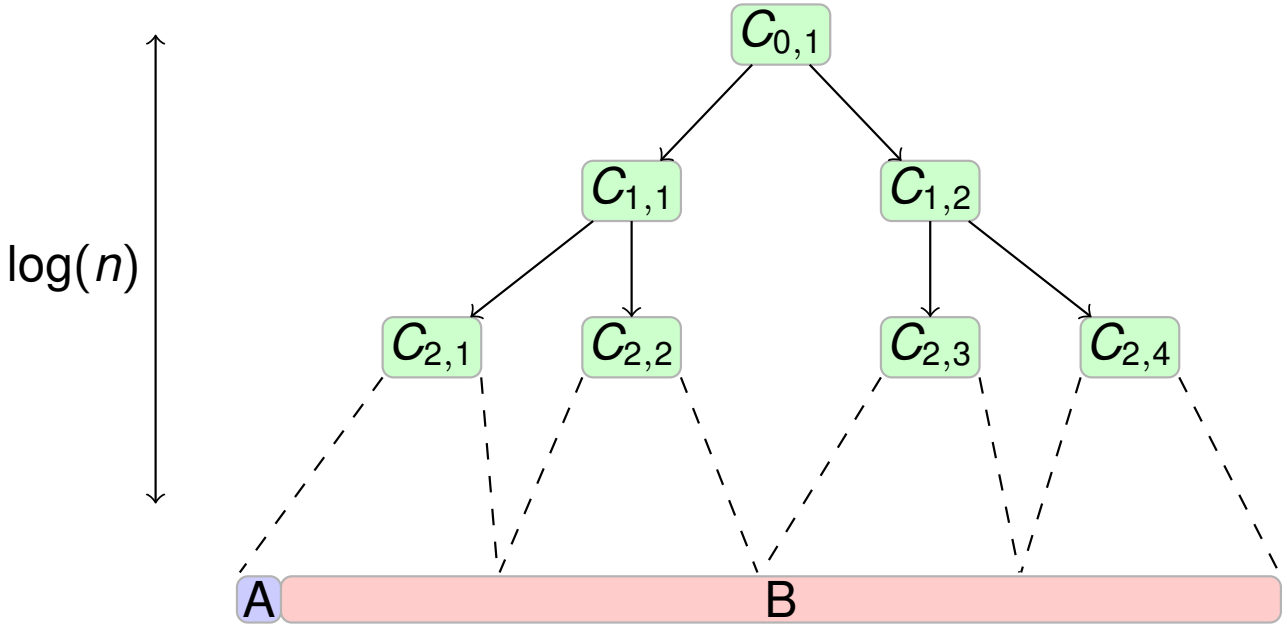
$$\mathbb{E}(X \mid n) = \frac{[z^n]C(z)}{[z^n]P_f(z)} \sim \frac{\kappa'}{\kappa} n\sqrt{n}(1 + \mathcal{O}(1/\sqrt{n})).$$

$\square$

Remark: Bivariate version of the 4 steps program...

Rationale: Most of the time is spent on testing unsuitable decompositions!

- Hierarchical organization of contributions



$\Rightarrow$ Generation in $\mathcal{O}(n^3 + kn\log(n))$, memory in $\Theta(n^3)$.

- Boustrophédon search

Unbalanced decompositions

$\Rightarrow$ Low *immediate* cost, recursion on intervals of lengths $\mathcal{O}(c)/\mathcal{O}(j\text{-}i\text{-}c)$.

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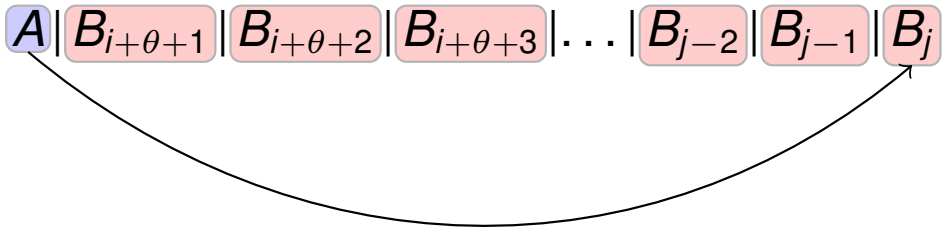

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Décomposition égale
$\Rightarrow$ *Immediate* cost is high ($\mathcal{O}(j\text{-}i\text{-}c)$), divide and conquer.

Worst-case complexity solution of

$$f(n) = \max_{k\in[1,n-1]}(f(k) + f(n-k) + 2\min(k, n-k)) \in \mathcal{O}(n\log(n)) \text{ [FZV94]}.$$

$\Rightarrow$ Generation in $\mathcal{O}(n^3 + kn\log(n))$ without any precomputation.

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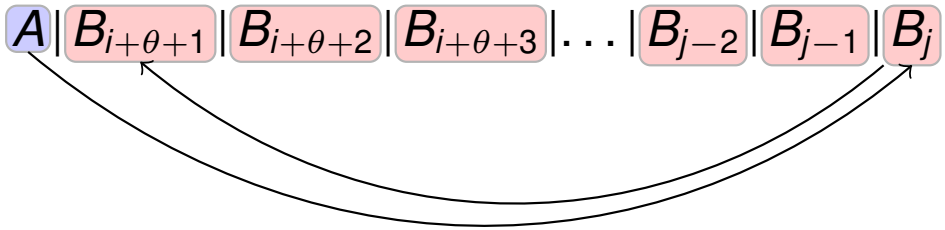

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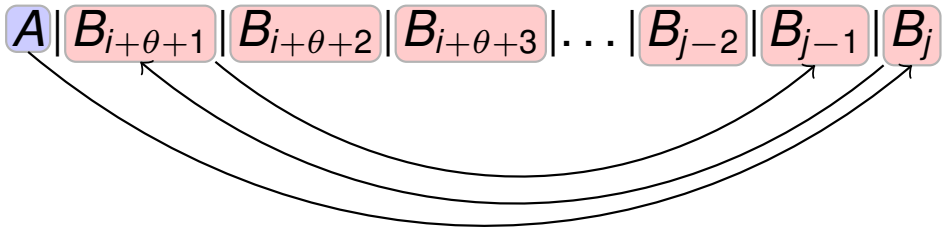

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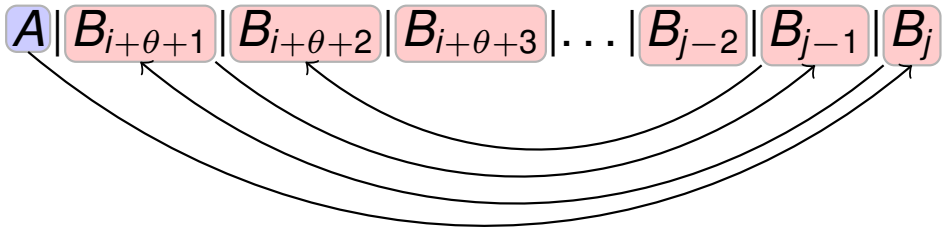

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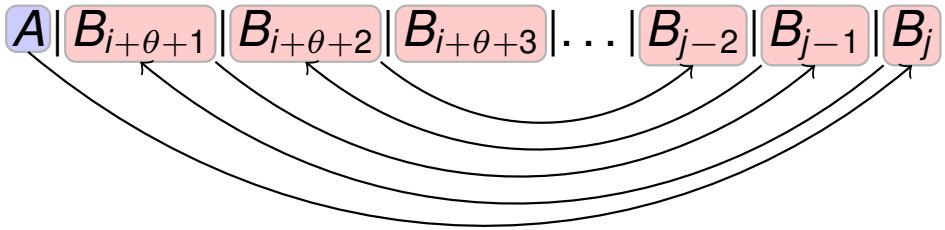

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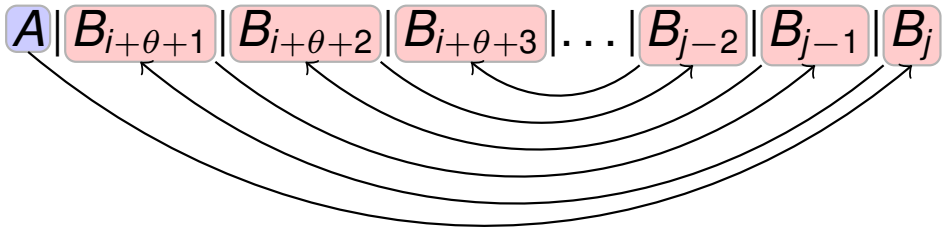

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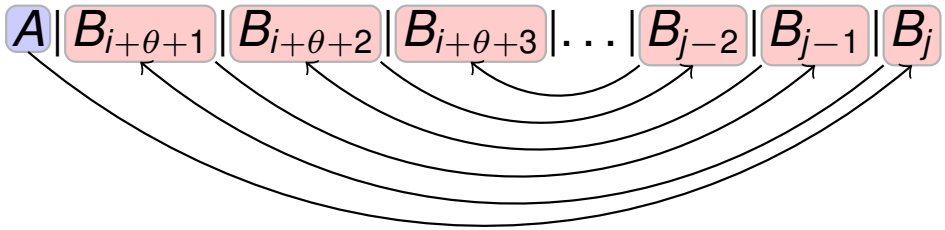

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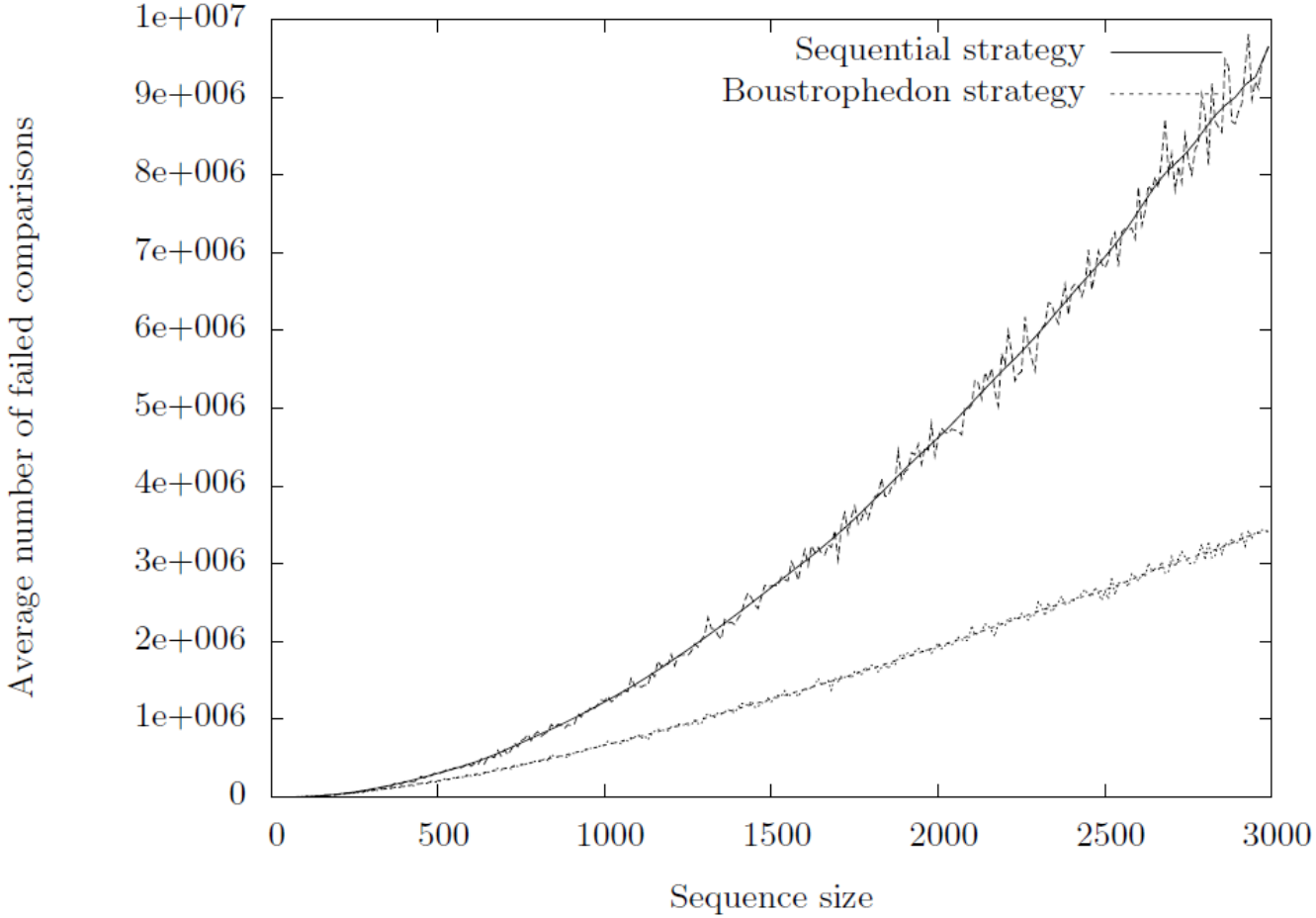
Results

- Implementation in `Unafold` (successor of `MFold`) using full Turner model.
- Random sequences: Markov order 1 model based on a 5s rRNA.

Implementation in `Unafold` (successor of `MFold`).



Part V

Conclusion

Summary

Using enumerative (models) and analytic (asymptotics) techniques, one can:

- Accurately enumerate RNA Shapes [JCB08].

- Improved random models for structures [Bioinf.06, TCS10].
- Analyze and optimize statistical sampling algorithms [JMB08].

- Analyze Boltzmann samples sets of structures [GASCOM10].

- Traverse the full mutational landscape [Recomb11].

- Explore enriched conformational spaces (PKs, MC-Fold) [...].

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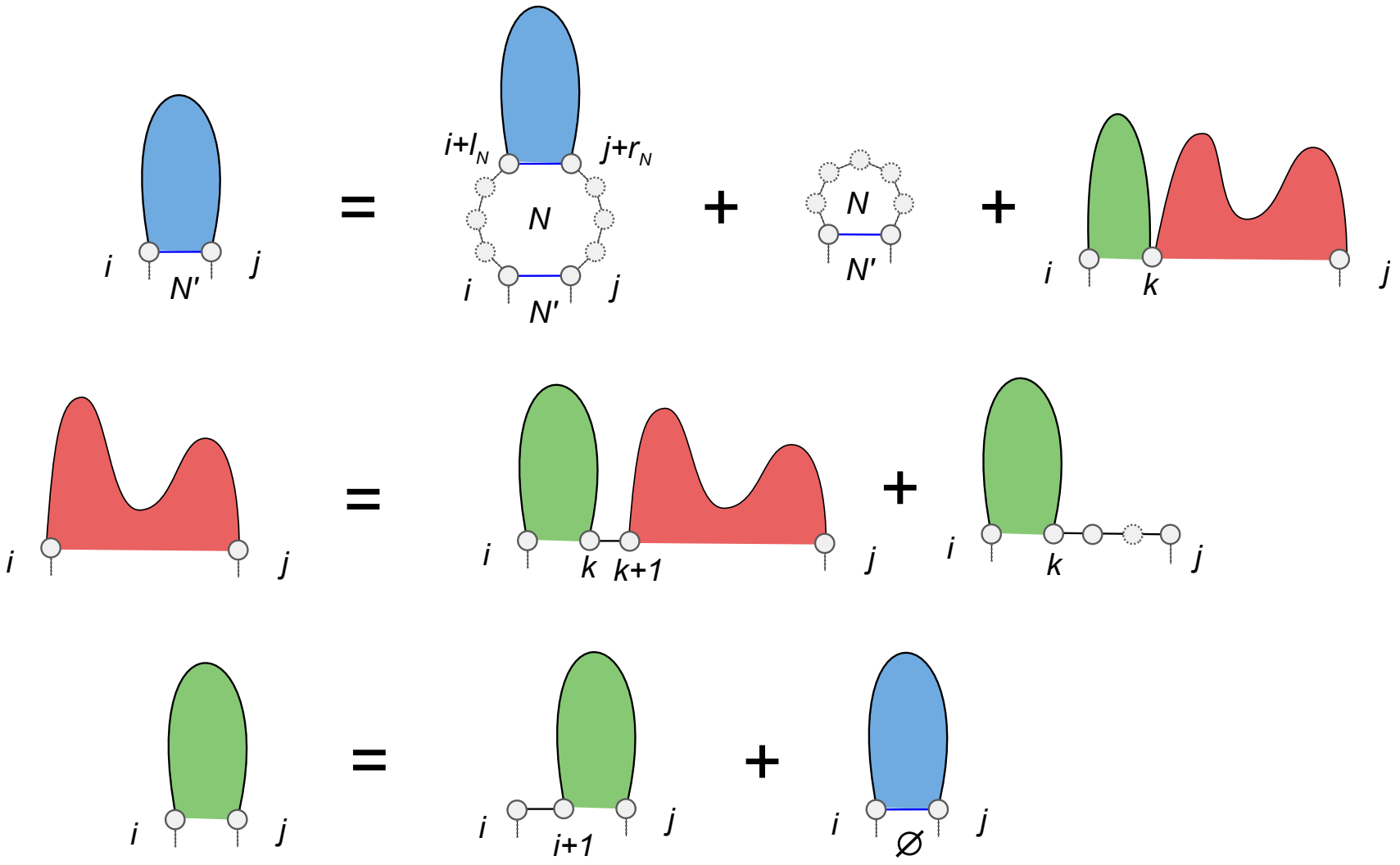
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