Statistical sampling and rational design of RNA

Yann Ponty

Analytical Genomics
A. Carbone's lab
INSERM U511/Paris 6
Paris, France

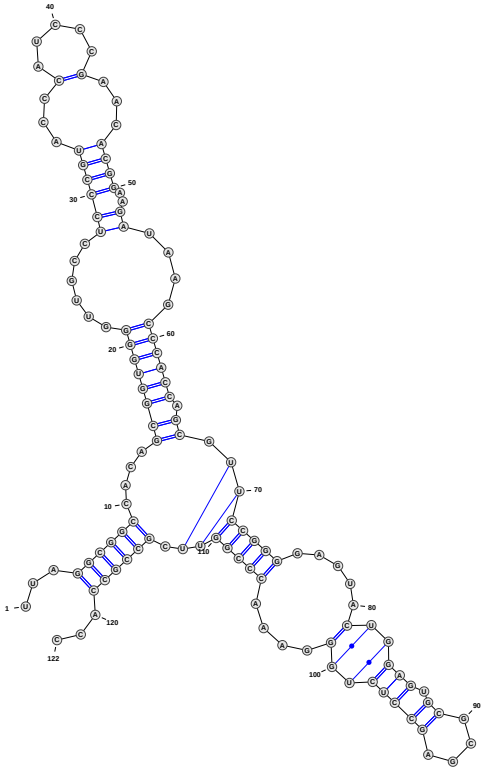
October 8, 2008

RNA structure

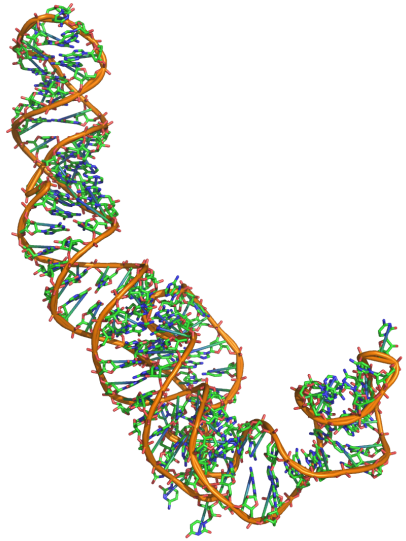
Three[1] levels of detail:

UUAGGCGGCCACAGC
GGUGGGGUUGCCUCC
CGUACCCAUCCCGAA
CACGGAAGAUAAGCC
CACCAGCGUUCCGGG
GAGUACUGGAGUGCG
CGAGCCUCUGGGAAA
CCCGGUUCGCCGCCA
CC

Primary structure



Secondary structure



Tertiary structure

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

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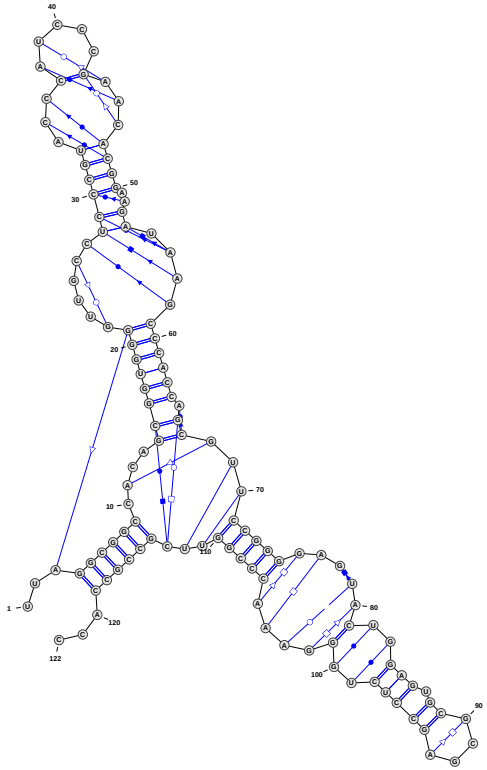
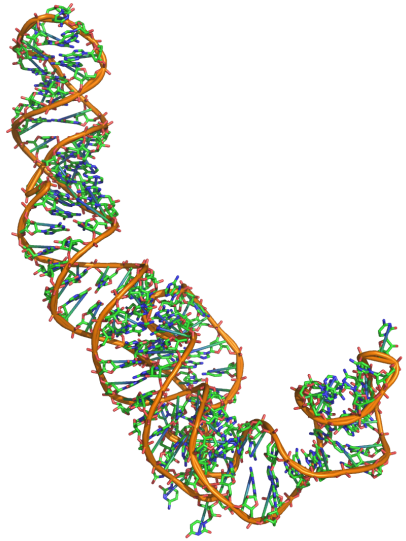


Secondary^{+} structure



Tertiary structure

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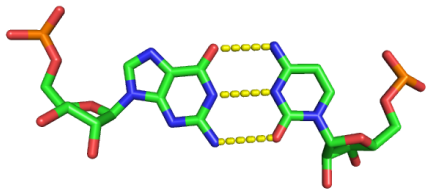
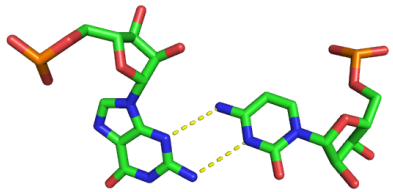
[1] Well, almost ...

- Non-canonical base-pairings:
  Base-pairs other than {(A-U), (C-G), (G-U)}
  Or interacting in a non WC/WC-Cis way [LW01].

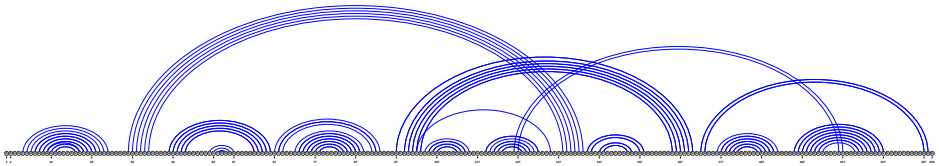


Canonical CG (WC/WC-Cis)



Non-canonical CG (Sugar/WC-Trans)

- Pseudoknots:



Pseudoknotted structure of a Group I Ribobozyme (PDBID: 1Y0Q:A)

Finding the best pseudoknotted structure:
$\Rightarrow$ NP-Complete [LP00] ...
... but polynomial for restricted classes [CDR+04].

State of the art

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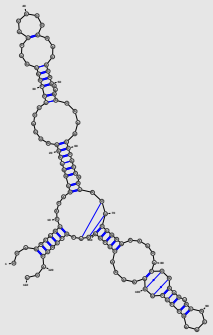
Sequence

Size Sens
<700 70-73%
[MSZT99, DCCG04]
16s,23s ∼50%
MCC∼ 0.5 [GG04]
Thermodynamics

Sens Spe MCC
∼75% ∼75% 0.8
Comparative [GG04]



2^{ary} structure

Reminder: $MCC = \frac{t^+ t^- - f^+ f^-}{\sqrt{(t^+ + f^+)(t^+ + f^-)(t^- + f^+)(t^- + f^-)}}$

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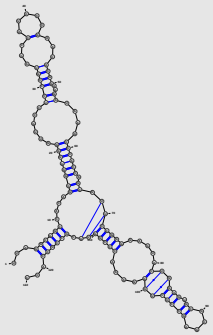

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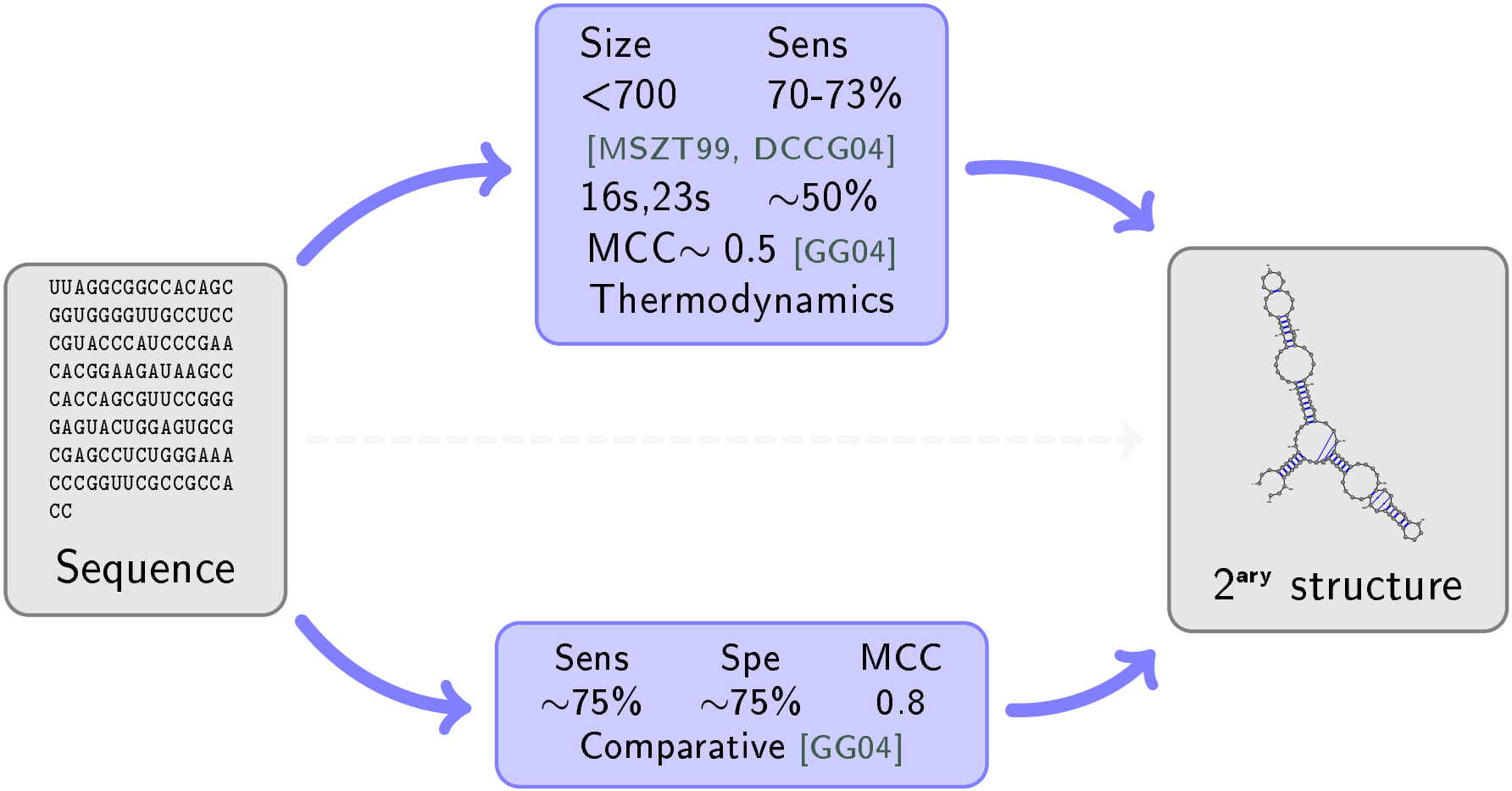

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The future: Toward 3D automated prediction

Goal: Go straight from sequence to 3D models!!!

- Comparative modeling + Molecular Dynamics: `RNA2D3D` [SYKB07]
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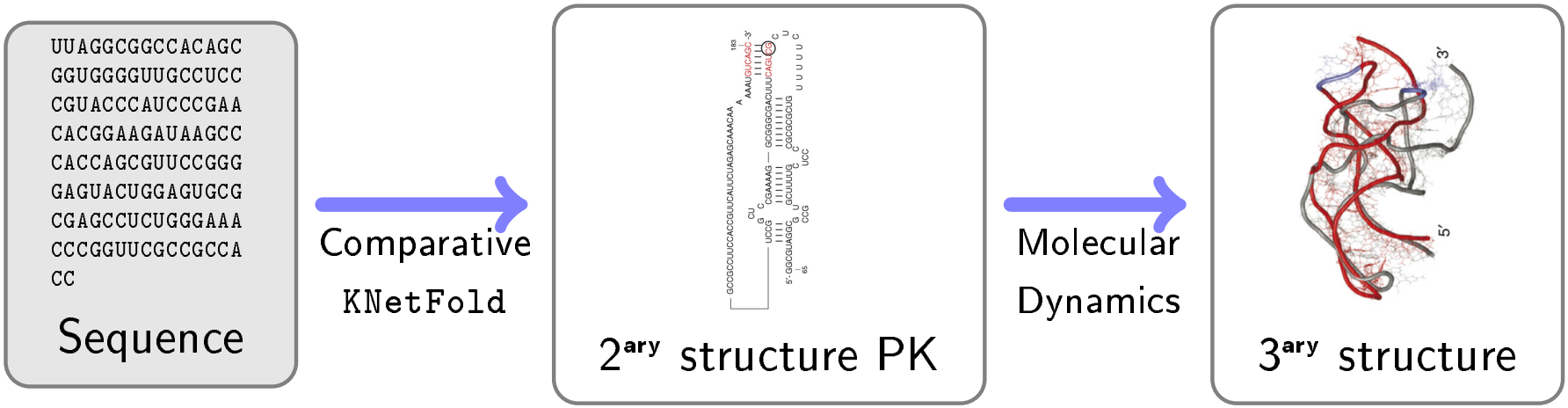

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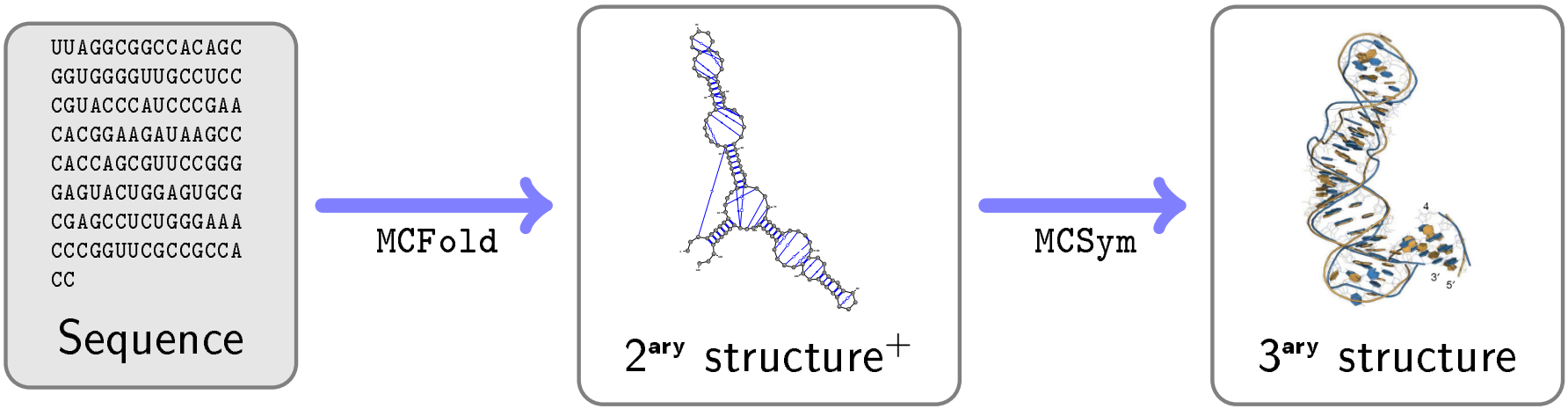
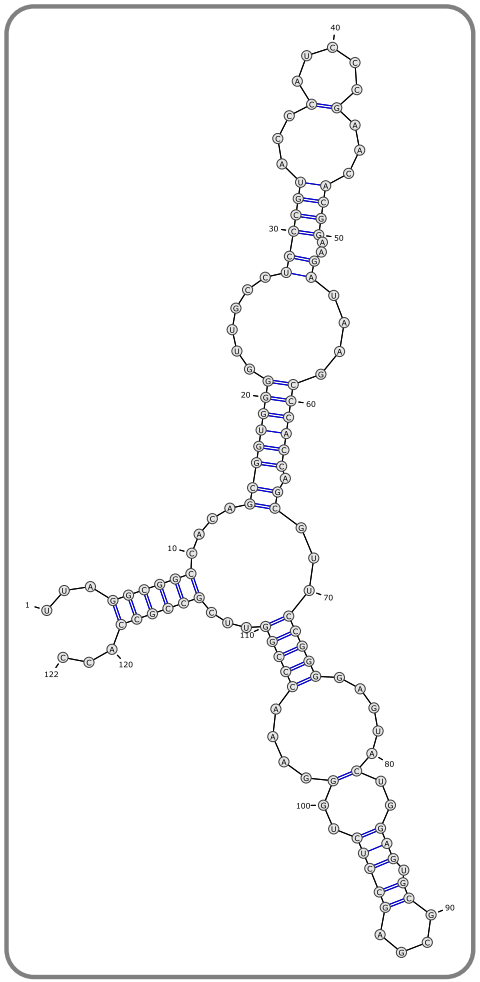


Turner model

Relies on an unambiguous decomposition of 2^{ary} into a set of loops:

- Interior Loops
- Bulges
- Terminal Loops, aka Hairpin Loops
- Multiple Loops
- Stacking pairs



Experimentally-determined values and interpolations for individual contributions of loops.

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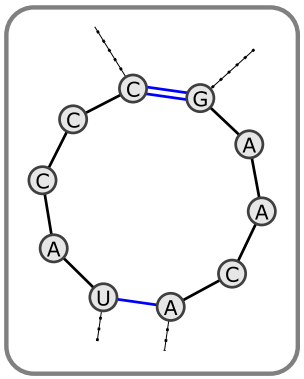

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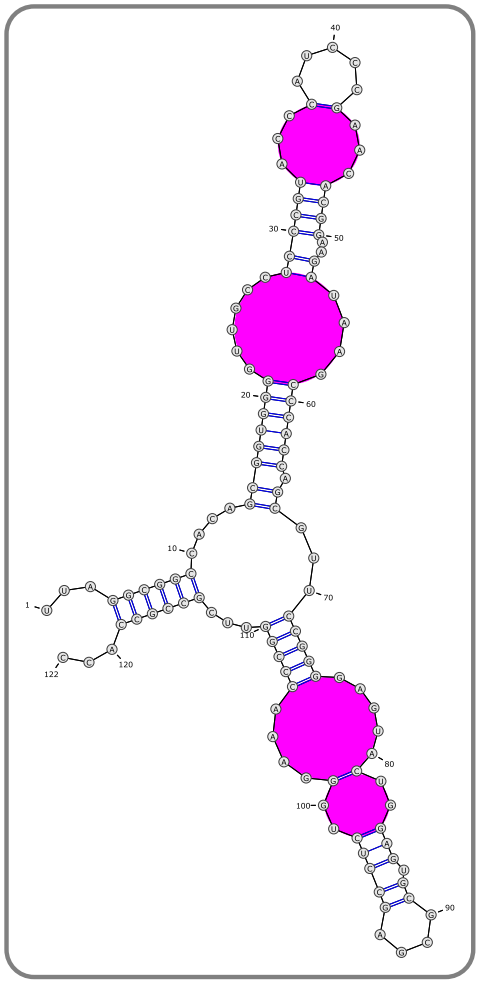

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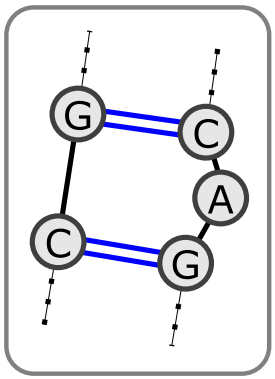

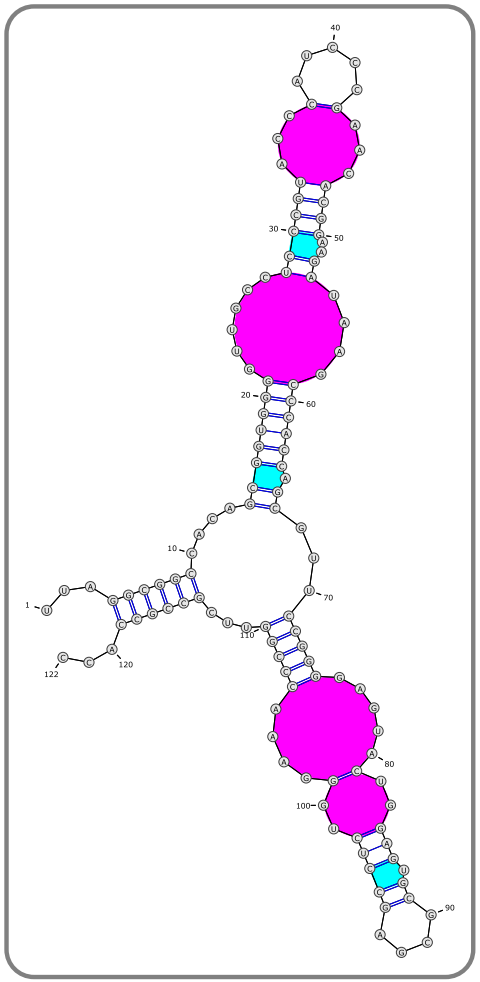

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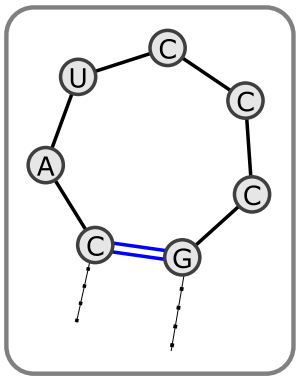

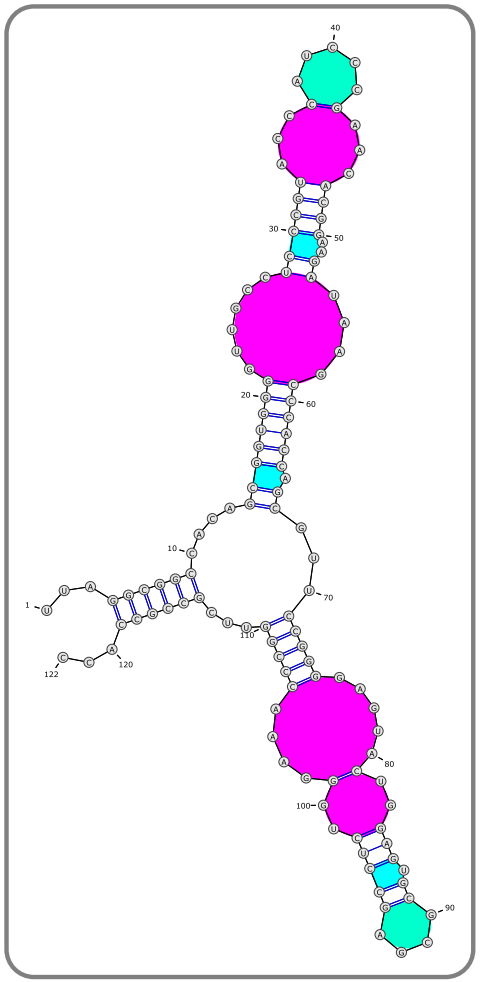

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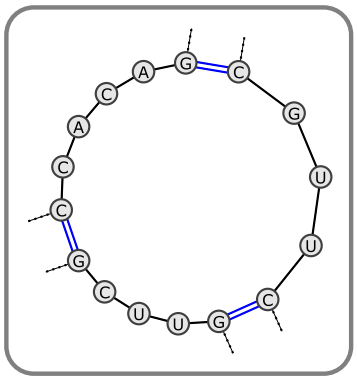

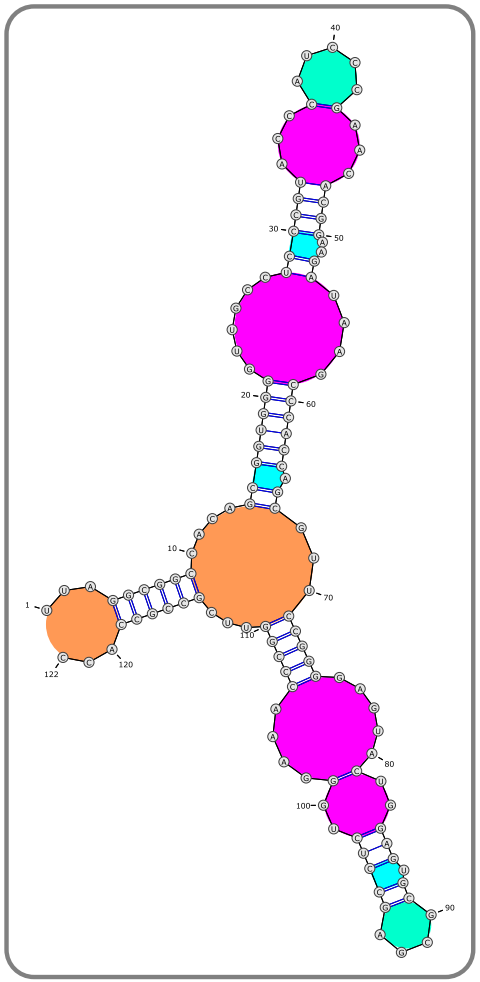

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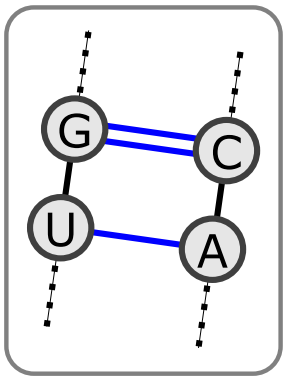

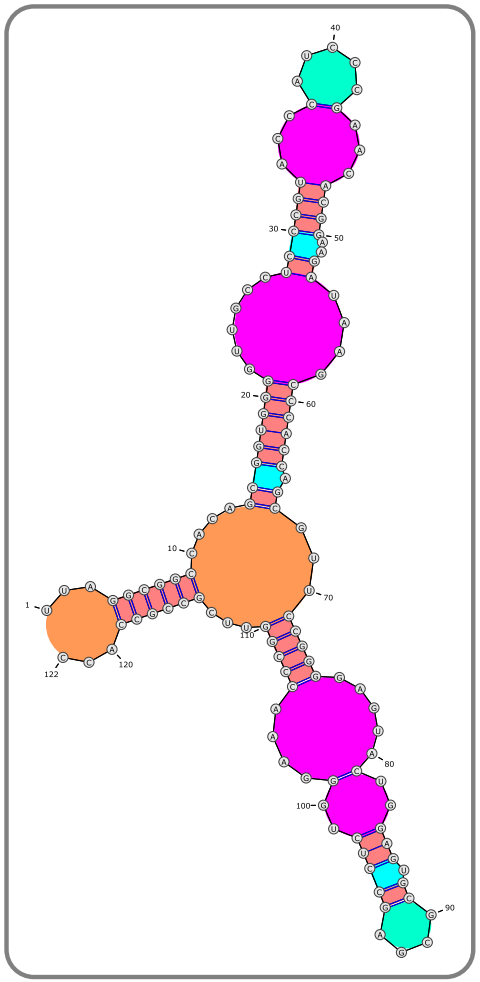

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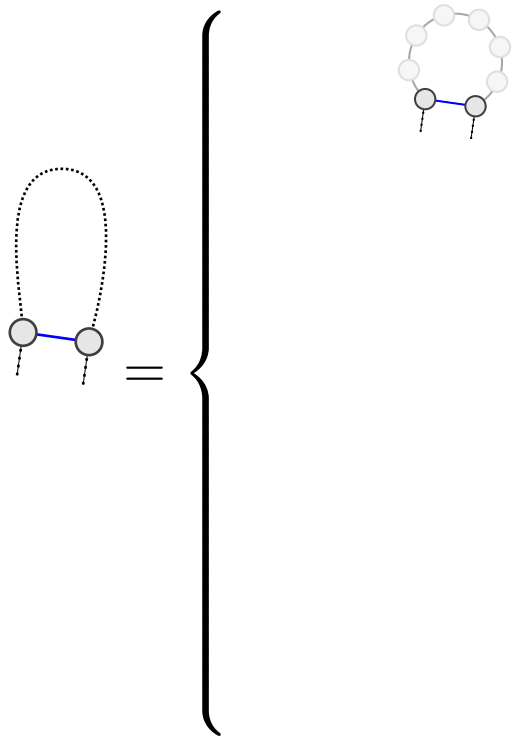
MFE folding

Theorem (MFE hypothesis)

RNA folds into its minimum free-energy conformation

But $\sim 1.8^n$ secondary structures compatible with S of size n [ZS84].
$\Rightarrow$ Dynamic programming, i.e. enumeration



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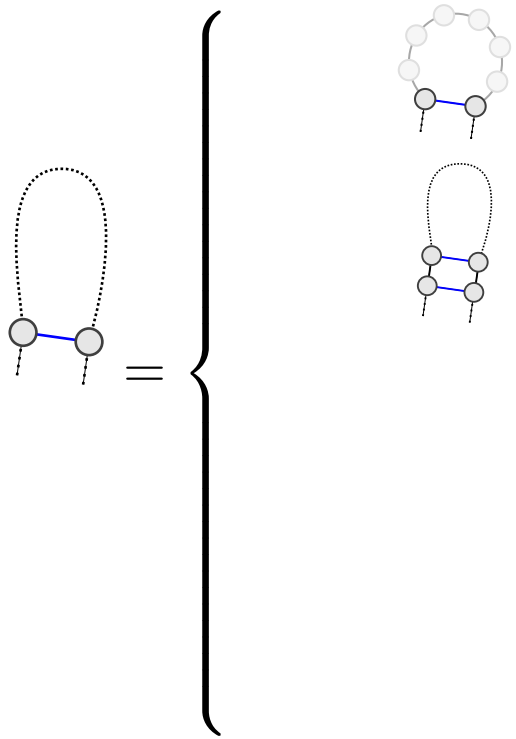

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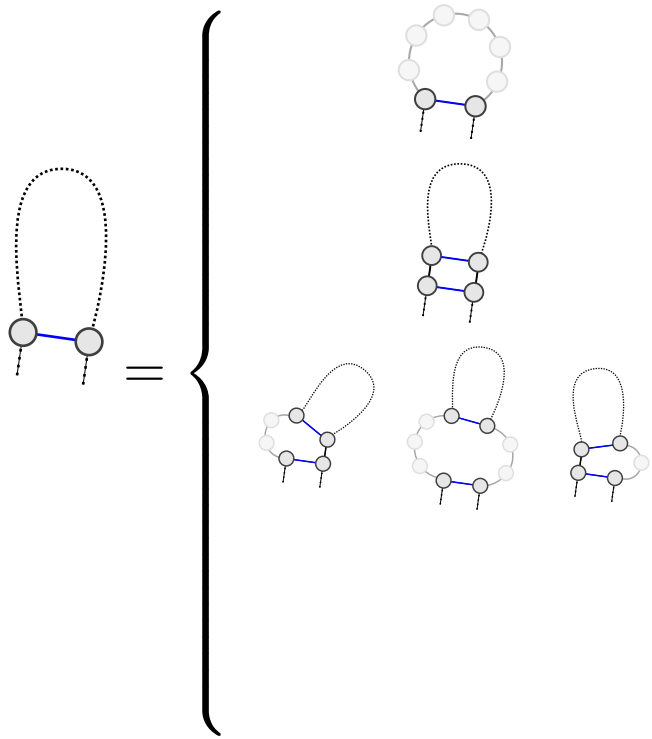

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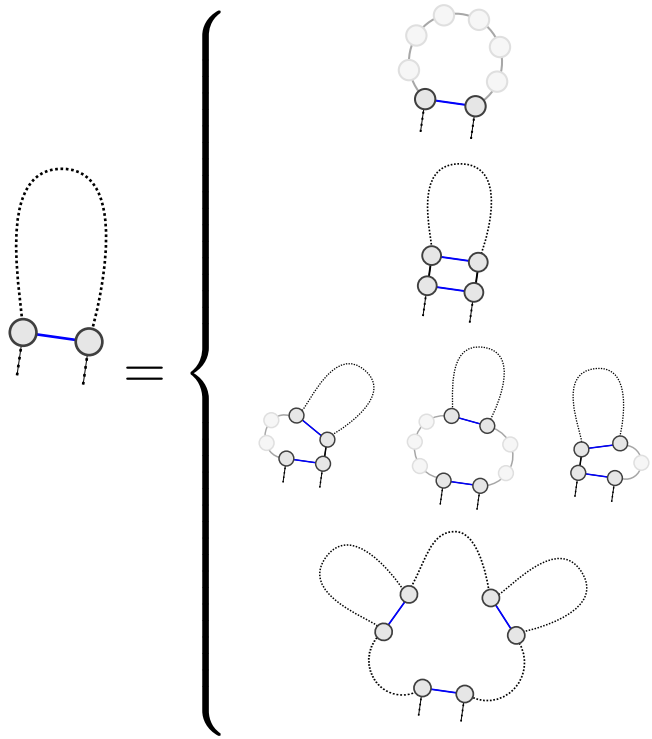

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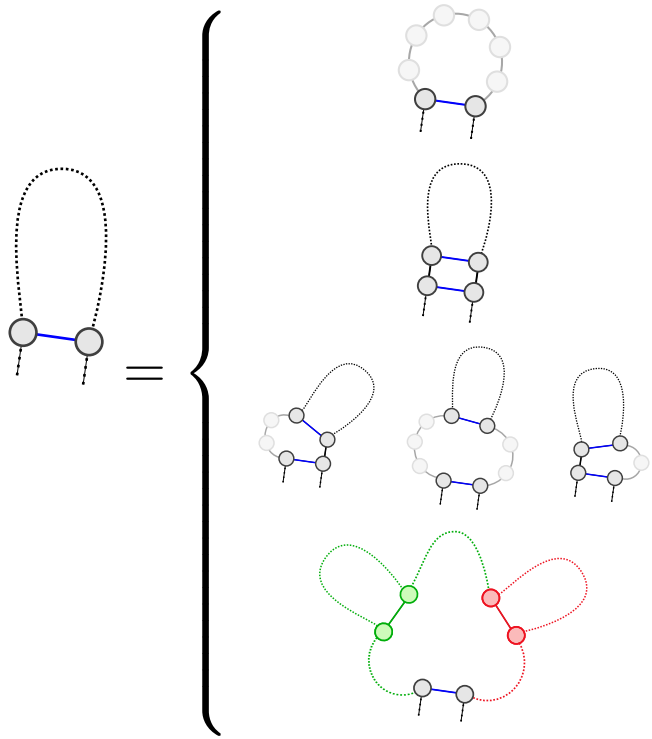

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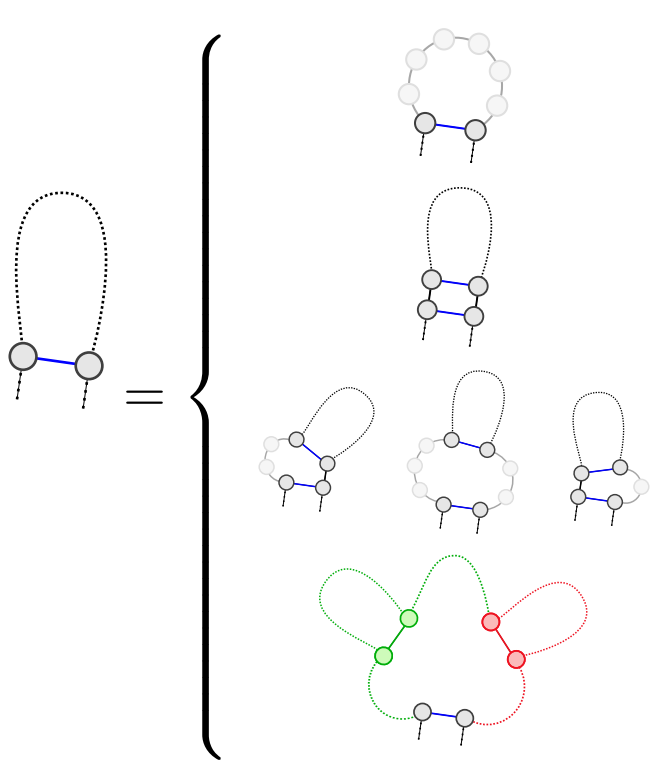

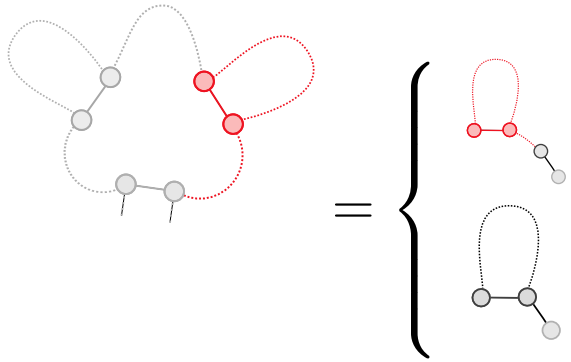

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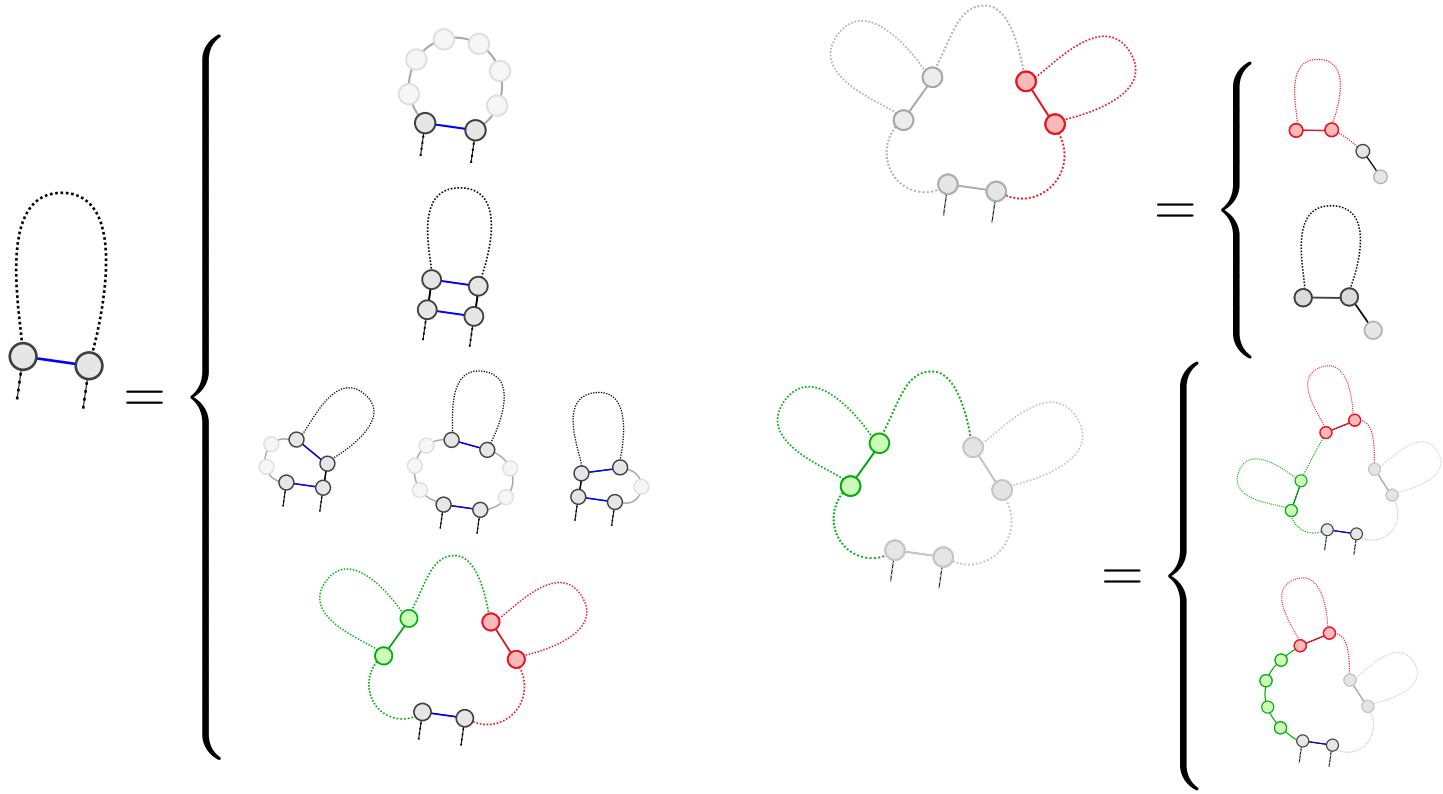
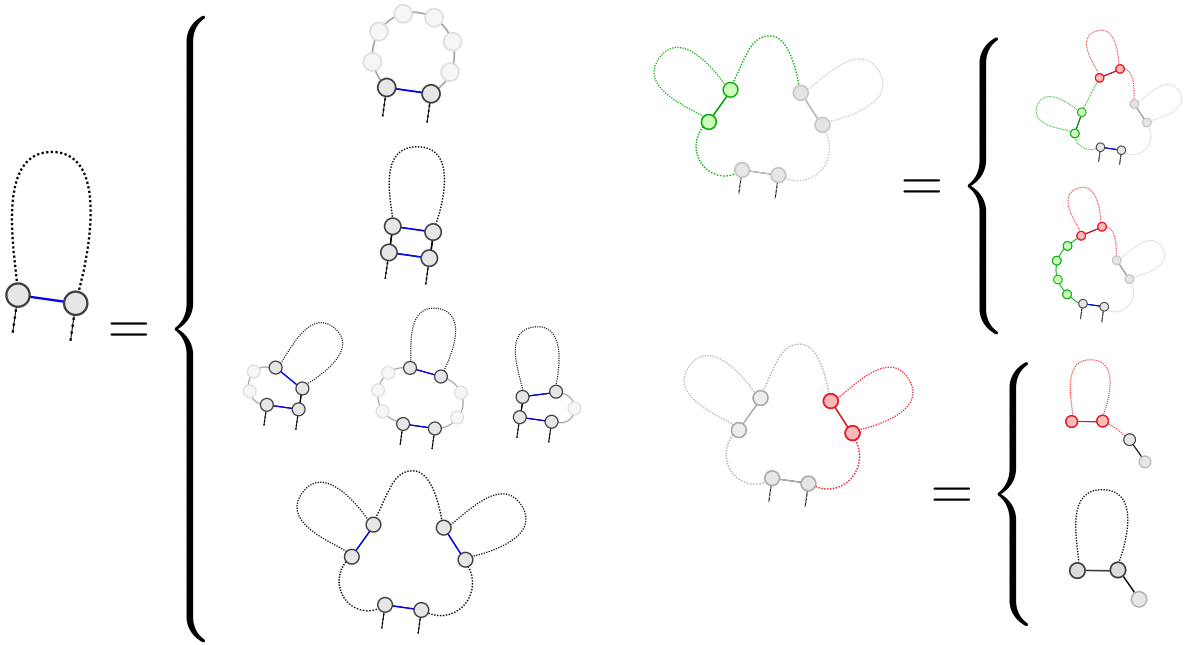


Validation

Proof of unambiguity $\Rightarrow$ Enumerative combinatorics
Waterman counted Sec. Str. [Wat78] and found the gen. fun. to be

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



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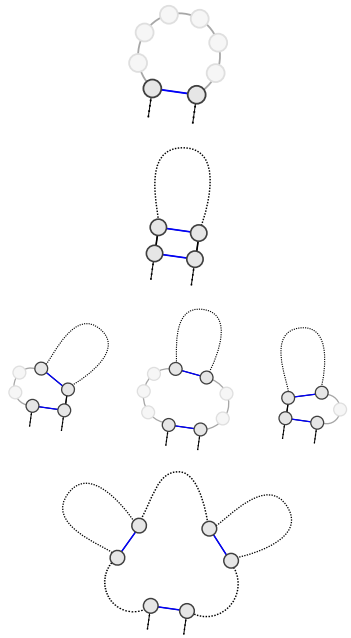

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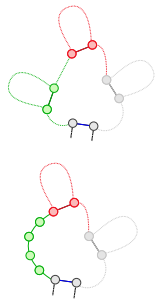

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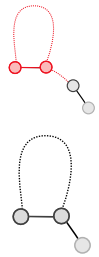

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$$\mathcal{S}(z) = 1 + z\mathcal{S}(z)$$

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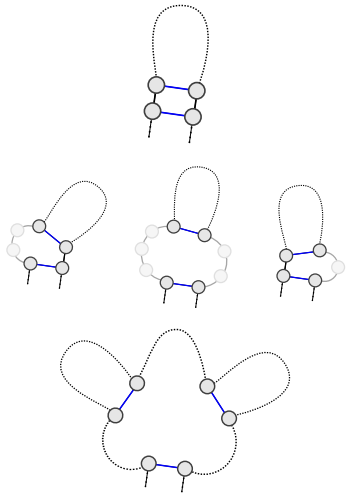

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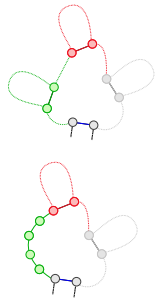

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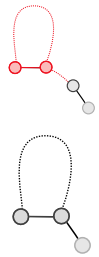

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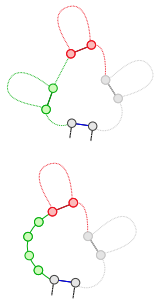

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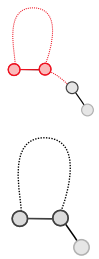

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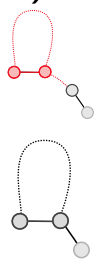

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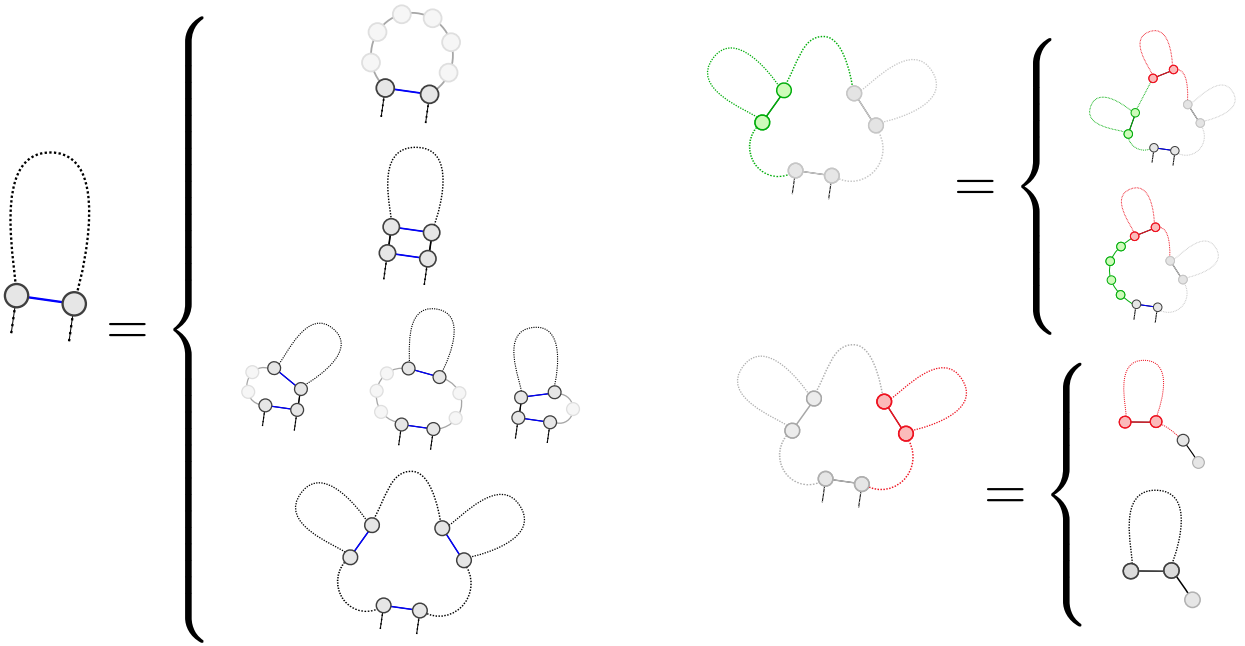
$$\begin{aligned} \Rightarrow \mathcal{A}(z) &= \frac{1 - z - z^2 - \sqrt{1 - 2z - z^2 - 2z^3 + z^4}}{2z^2} \\ &= \mathcal{W}(z) - 1 \end{aligned}$$

Woops, we forgot the empty structure for size 0 RNAs!

MFE folding

- $E_H(i,j)$: Energy of hairpin loop with closing pair (i,j)
- $E_{BI}(i,j)$: Energy of bulge or internal loop with closing pair (i,j)
- $E_S(i,j)$: Energy of stacking pairs $(i,j)/(i+1,j-1)$
- a,c,b: Penalties for multiloop, hairpins and unpaired bases in multiloop.



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While reconstructing the m.f.e. conformation:

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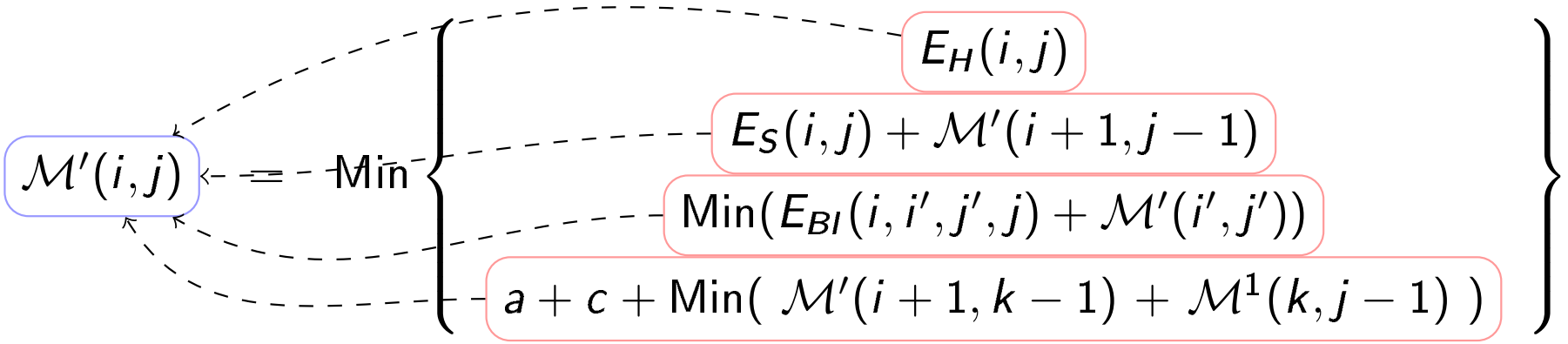

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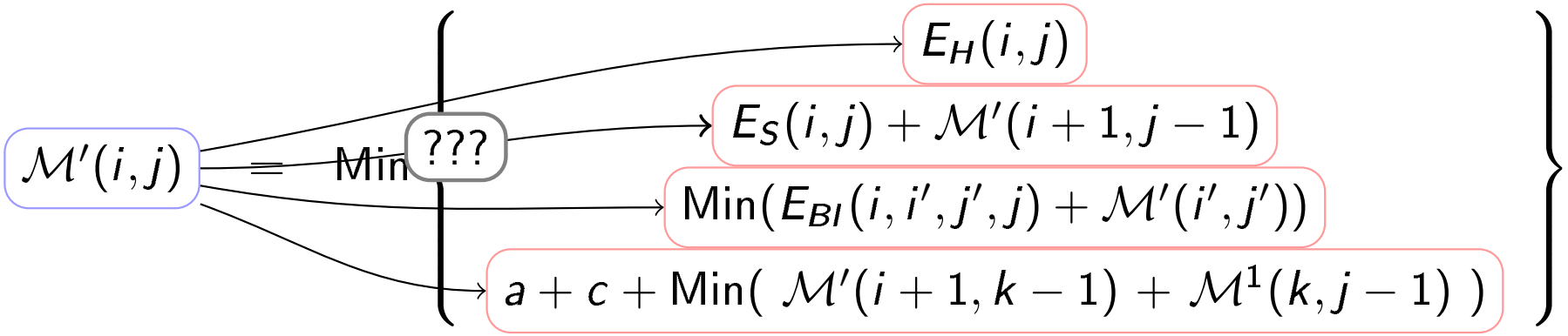

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Prob.: Approximation of energy function (Pseudoknots, NC base-pairs), so the real (native) structure could be underestimated and ignored.

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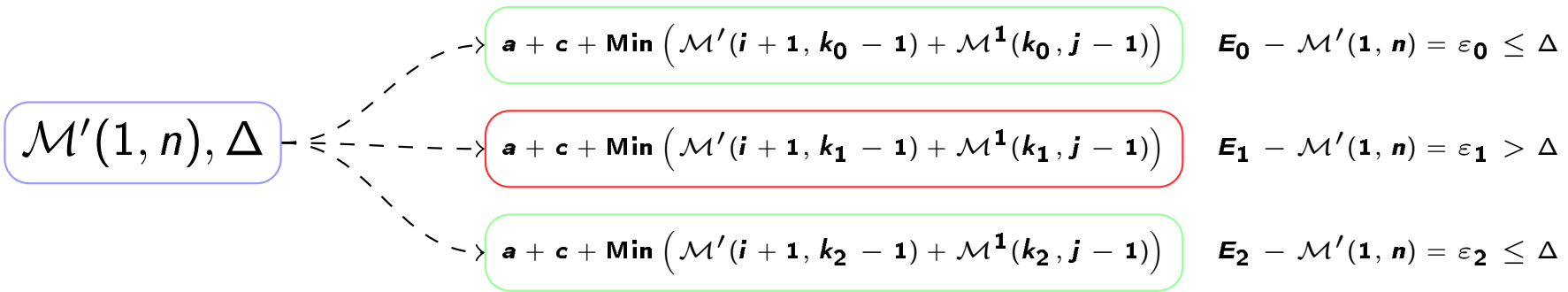

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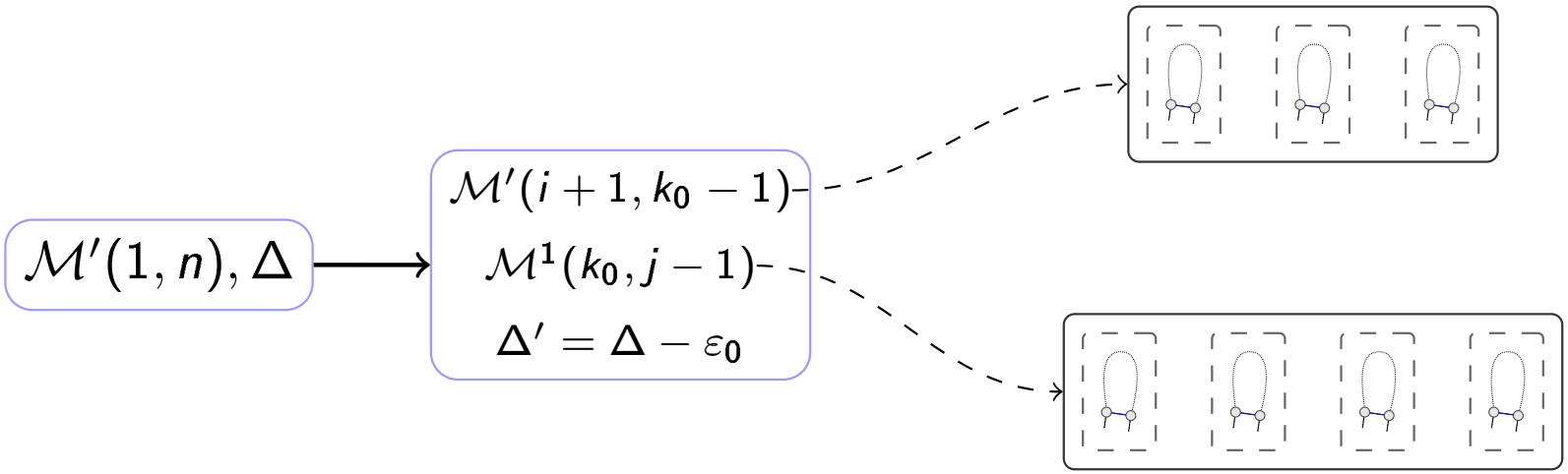

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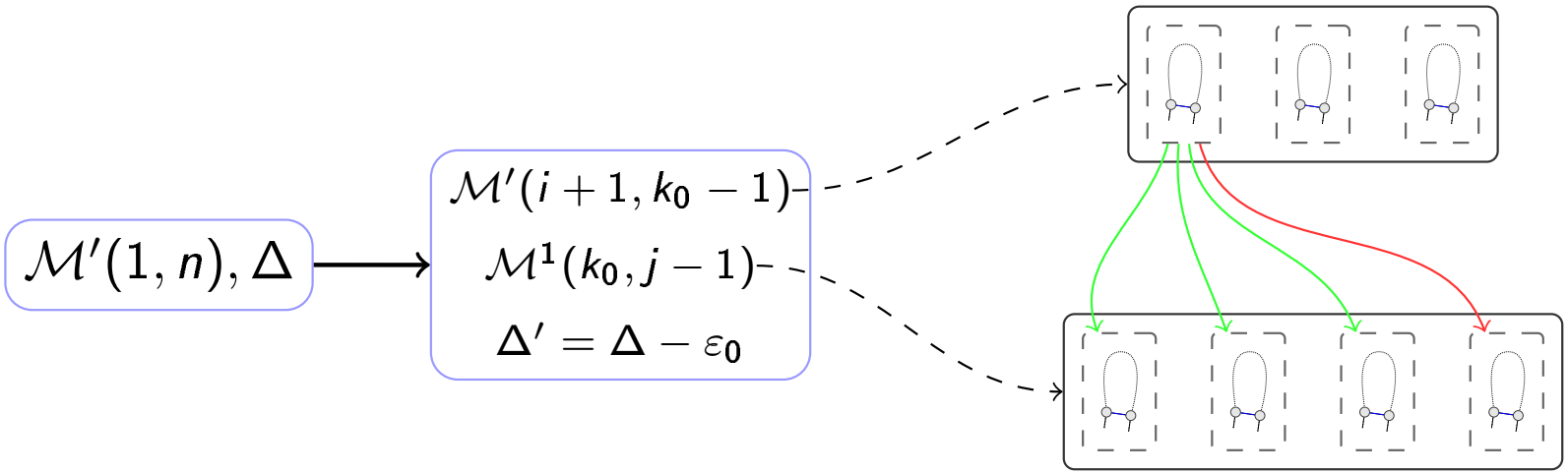

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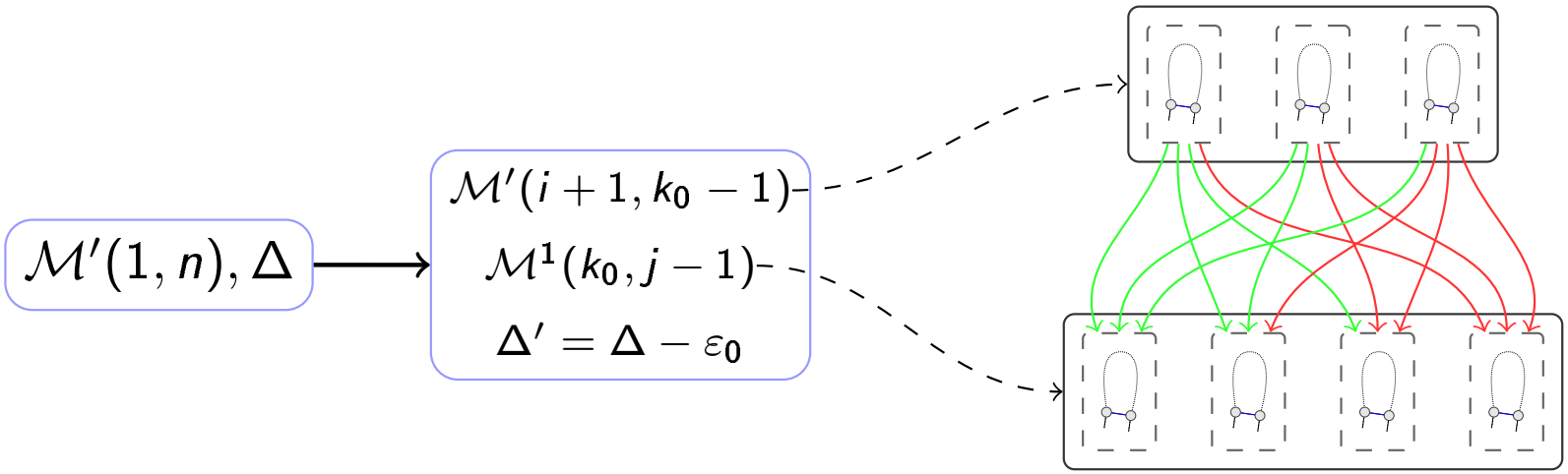

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Partition function/Boltzmann probability

- Let ω be an RNA sequence,
- $\mathcal{S}_\omega$ be the set of sequences compatible with ω,

$$\textbf{Partition function} \quad \mathcal{Z}_\omega = \sum_{S \in \mathcal{S}_\omega} e^{\frac{-E_{S,\omega}}{RT}}$$

where T is temperature in Kelvin and R is the universal gas constant.

$$\textbf{Boltzmann probability} \quad P_{S,\omega} = \frac{e^{\frac{-E_{S,\omega}}{RT}}}{\mathcal{Z}_\omega}$$

$P_{S,\omega}$ is the probability of observing ω in conformation S.

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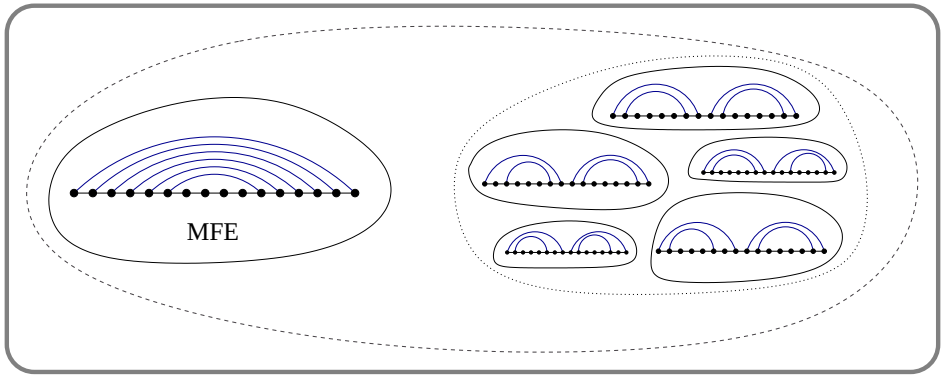
Now, we can restrict the sums to compute unpaired/paired base probabilities, base-pair prob., hairpin loops prob. ...

Statistical sampling of RNA

Apology for statistical sampling

The m.f.e. (Highest Boltzmann probability) $\mathcal{M}$ can be isolated and less probable than a set $\mathcal{B}$ of structurally similar suboptimals.
In this setting, native structure closer to $\mathcal{B}$ than to $\mathcal{M}$ [DCL05].



Strategy:

- Sample structures with Boltzmann probability
- Cluster structures
- Build and return a consensus structure from the best cluster

$\Rightarrow$ Relative improvements for specificity (+17.6%) and sensitivity (+21.74%, except for group II Introns)

Statistical sampling through stochastic traceback

Algorithm `SFold` [DL03]:

1. Generate a random number in $[0, \mathcal{Z}'(i,j))$
2. Subtract to r individual contributions to $\mathcal{Z}'(i,j)$, until $r < 0$
3. Recurse over substructures

$$\mathcal{Z}'(i,j) = \sum \left\{ \begin{array}{cc} e^{\frac{-E_H(i,j)}{RT}} + e^{\frac{-E_S(i,j)}{RT}} \mathcal{Z}'(i+1,j-1) & \text{A} \\ \sum \left(e^{\frac{-E_{BI}(i,i',j',j)}{RT}} \mathcal{Z}'(i',j') \right) & \text{B} \\ e^{\frac{-(a+c)}{RT}} \sum \left(\mathcal{Z}'(i+1,k-1) \mathcal{Z}^1(k,j-1) \right) & \text{C} \end{array} \right\}$$

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r

$A_1 | A_2 | B_i | B_{i+1} | \ldots | B_{j-1} | B_j | C_i | C_{i+1} | \ldots | C_{j-1} | C_j$

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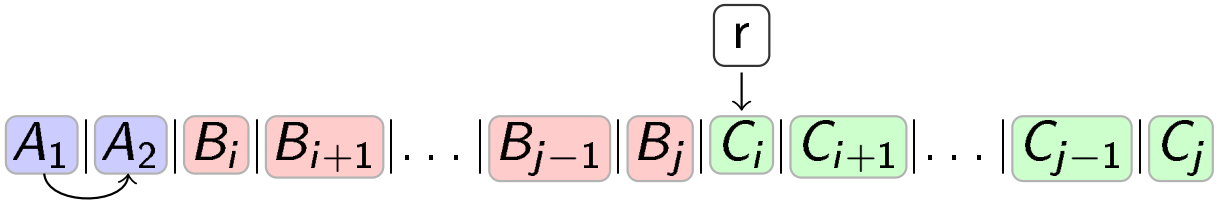

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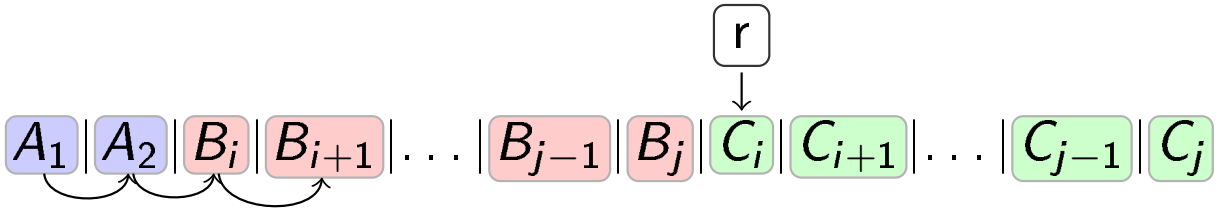

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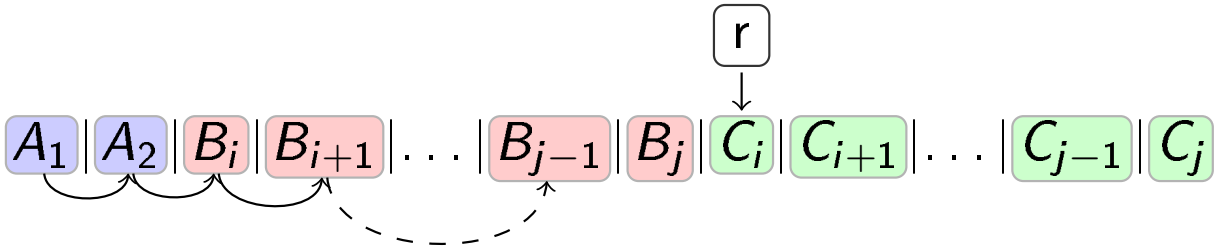

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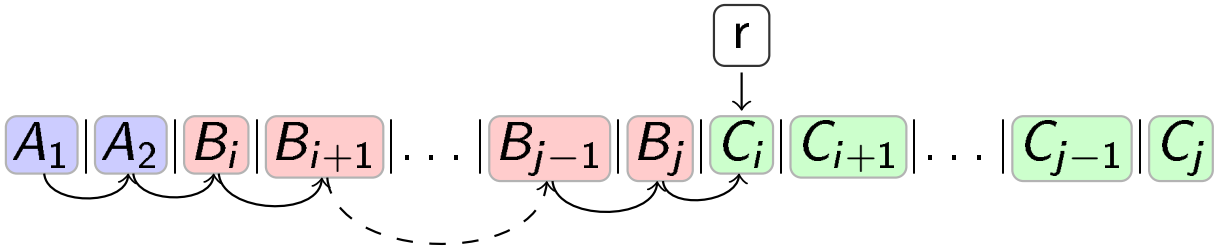

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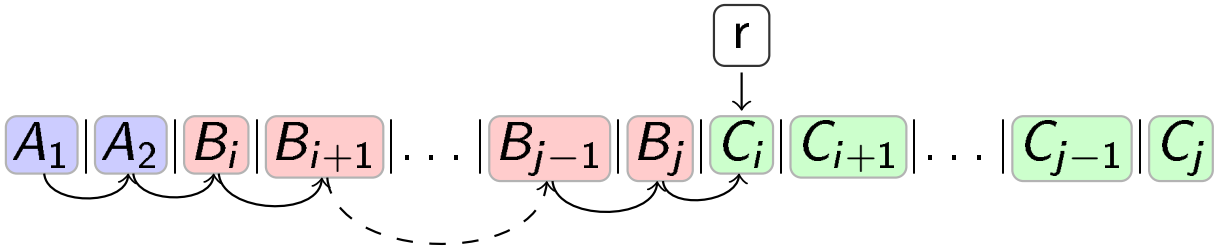

After $\Theta(n)$ operations, recurse over size $n-1$ interval
$\Rightarrow$ Worst-case time complexity for k samples in $\mathcal{O}(n^2 k)$

Remark: This is a weighted instance of the so-called recursive random generation of decomposable objects.

Efficient statistical sampling

How to improve statistical sampling?

- Improve time complexity:
  Average-case time complexity in $\Theta(kn\sqrt{n})$ [Pon08]
  ($\Theta(n^2)$ arises from recursing on $n - \mathcal{O}(1)$ after $\Theta(n)$ ops)
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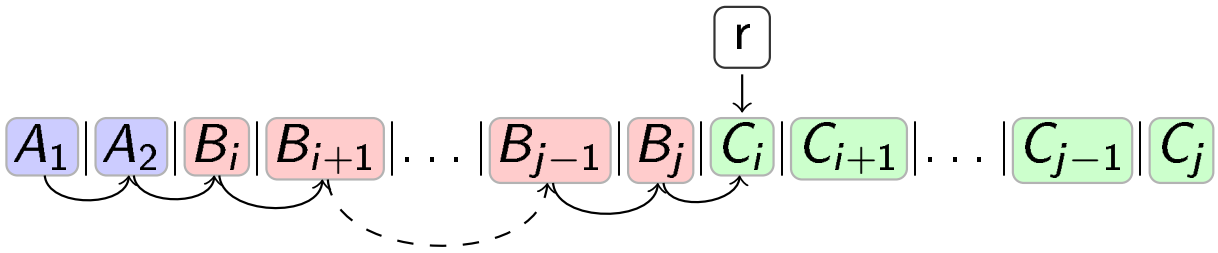
  - Boustrophedon [FZV94]
    Investigate uneven decompositions first, then even ones !
- Non-redundant generation



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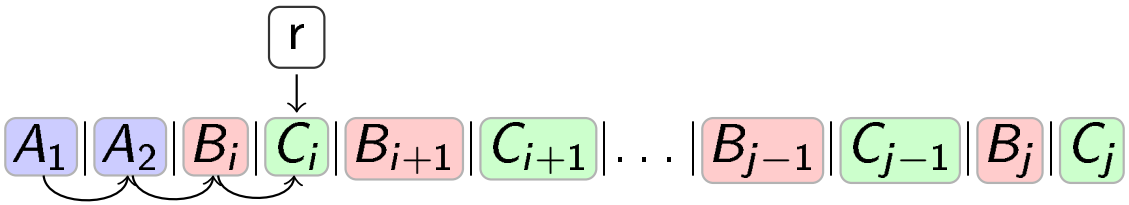

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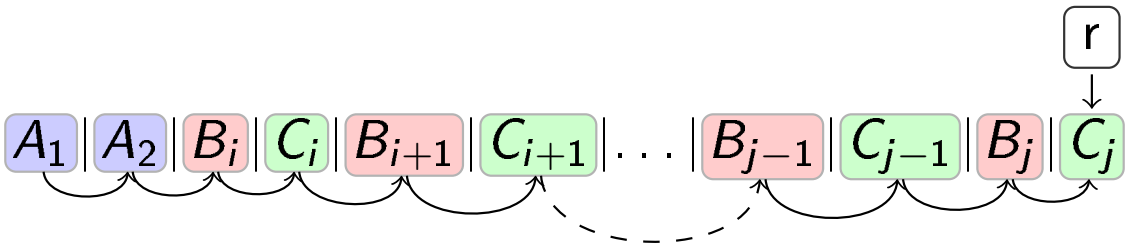

$\Rightarrow$ Some terms from B and C are reached in $\mathcal{O}(1)$
But still $\Theta(n^2)$, since $\mathcal{Z}'(i,j) \rightarrow (\mathcal{Z}'(i+1,k-1), \mathcal{Z}^1(k,j-1))$

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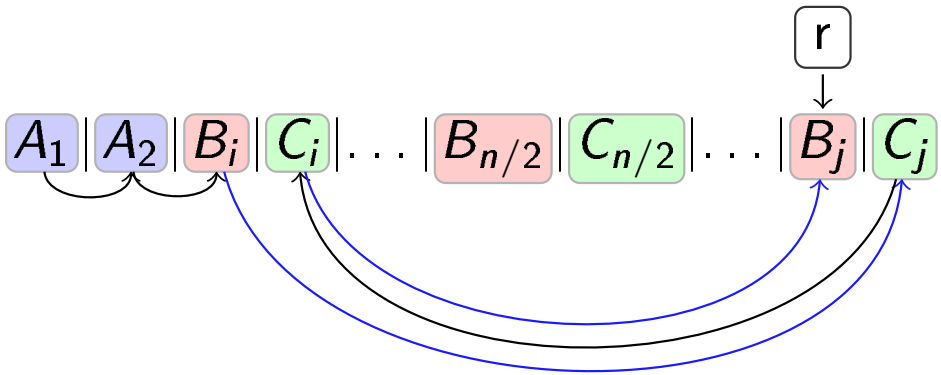

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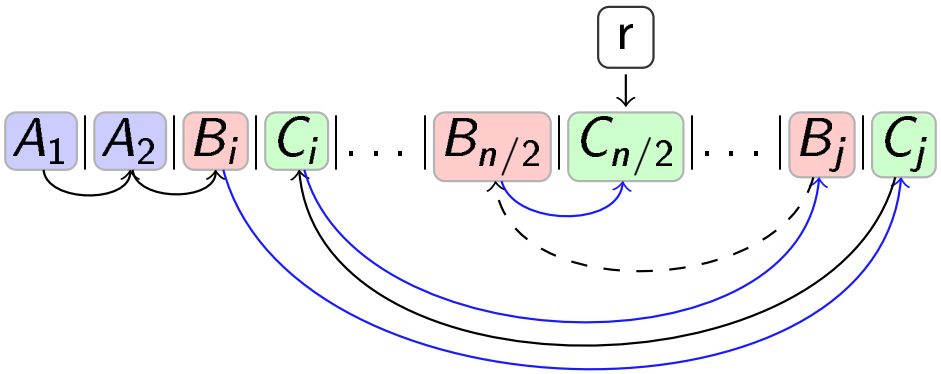

Worst-case: Divide exactly at each step [GK81] $\Rightarrow \Theta(n \log(n))$

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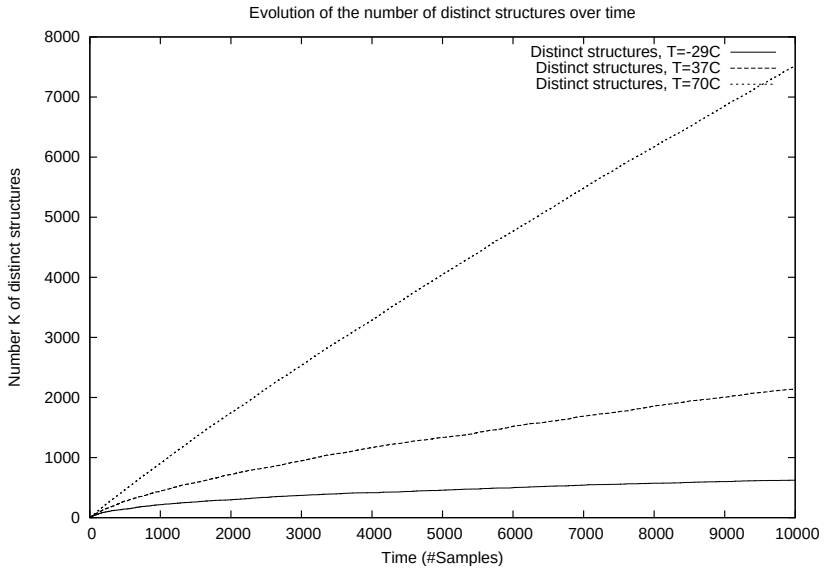

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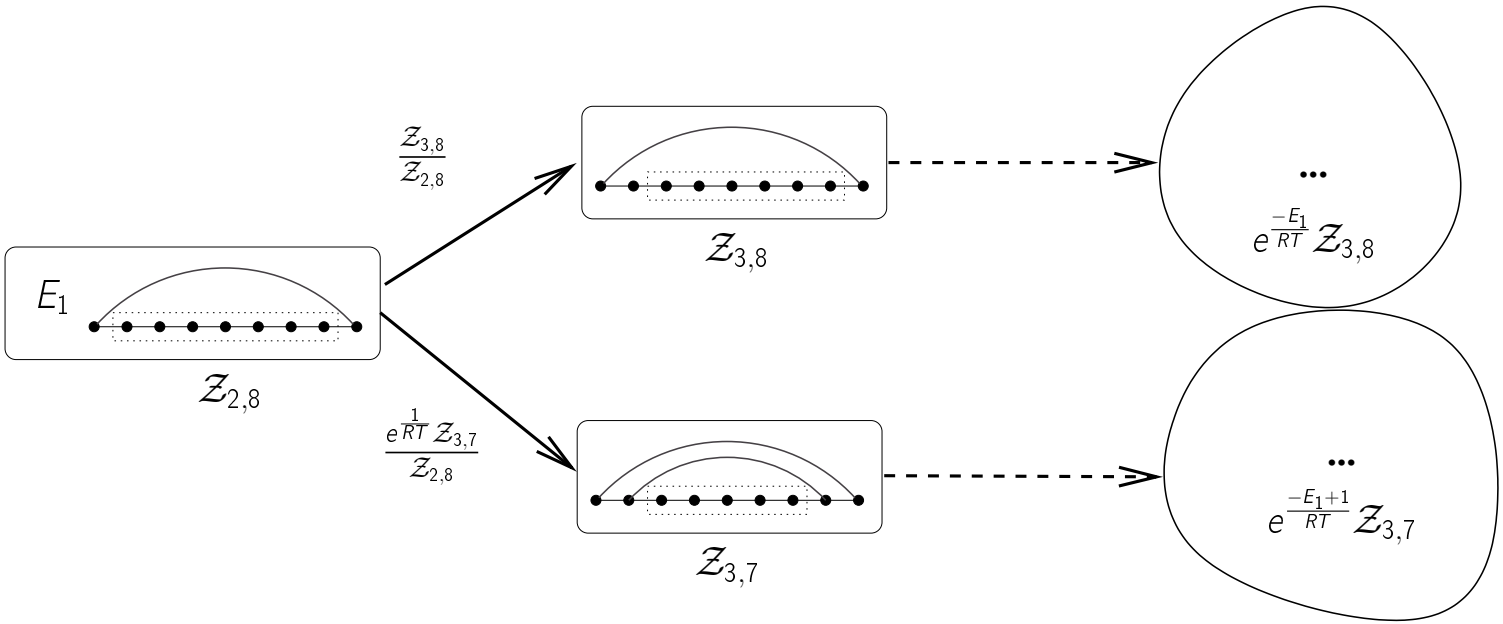

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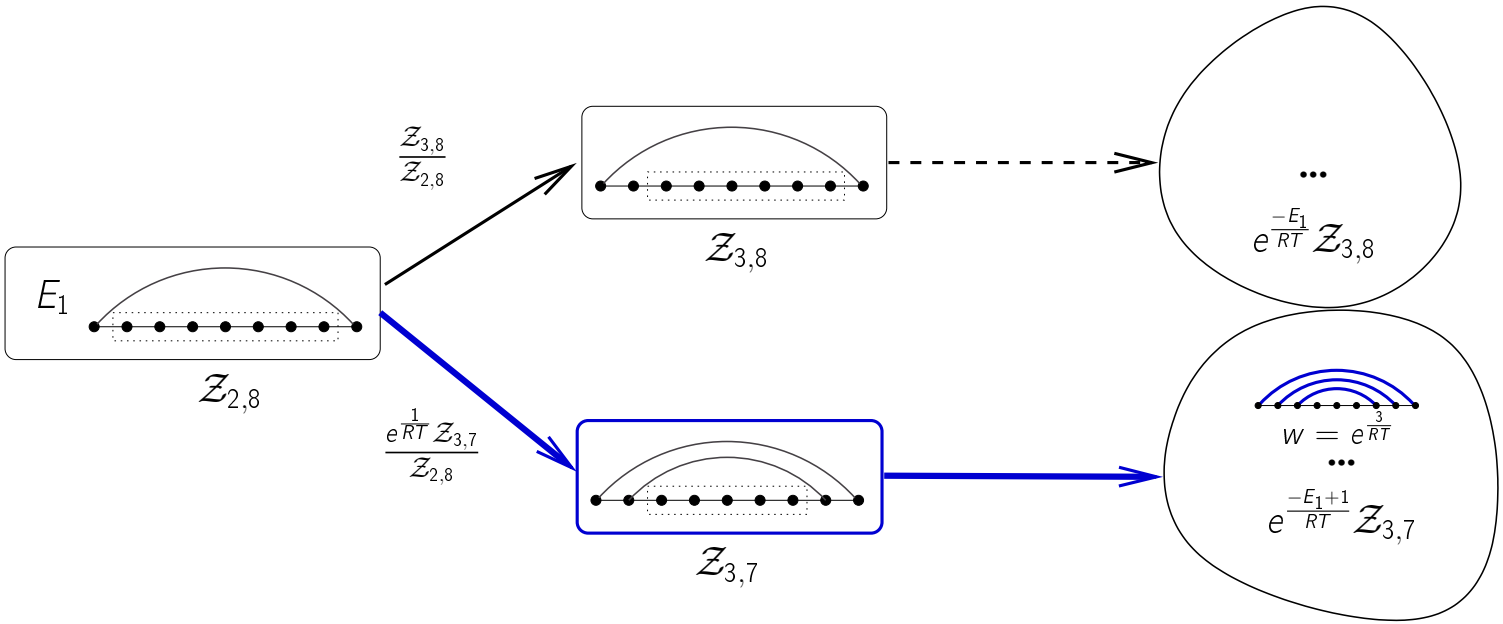

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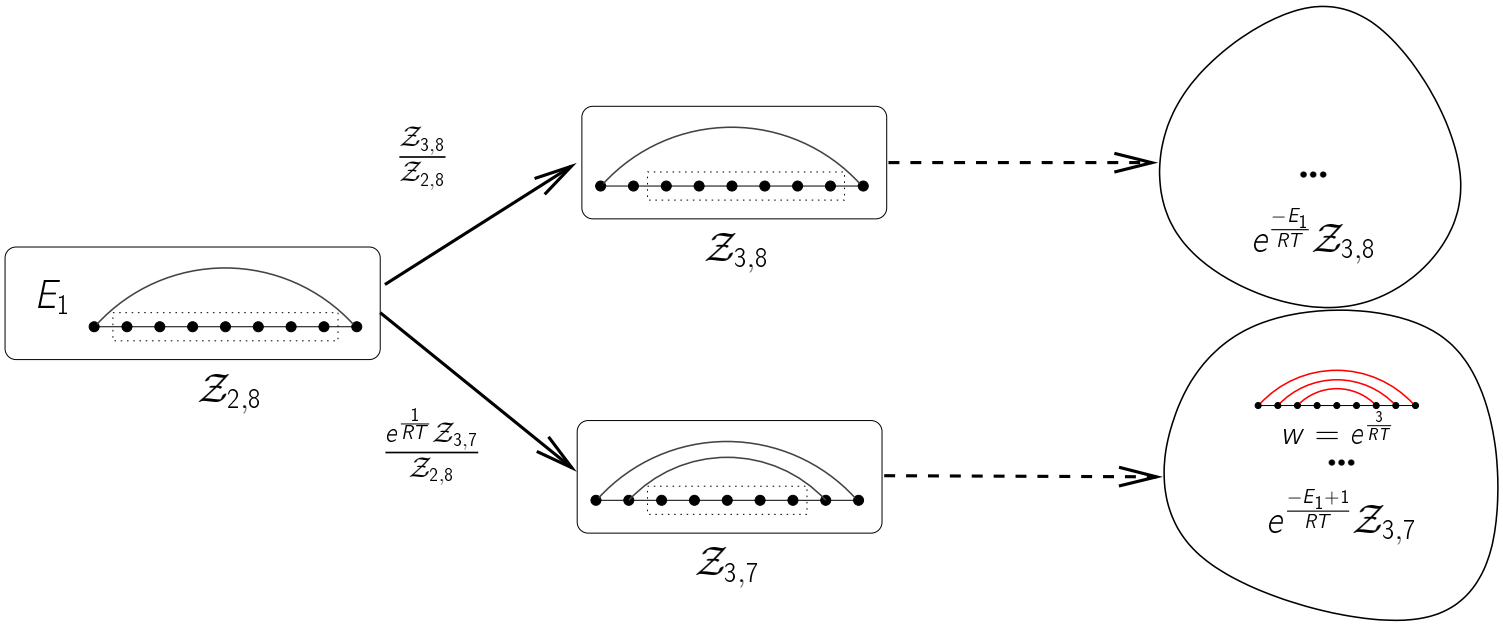

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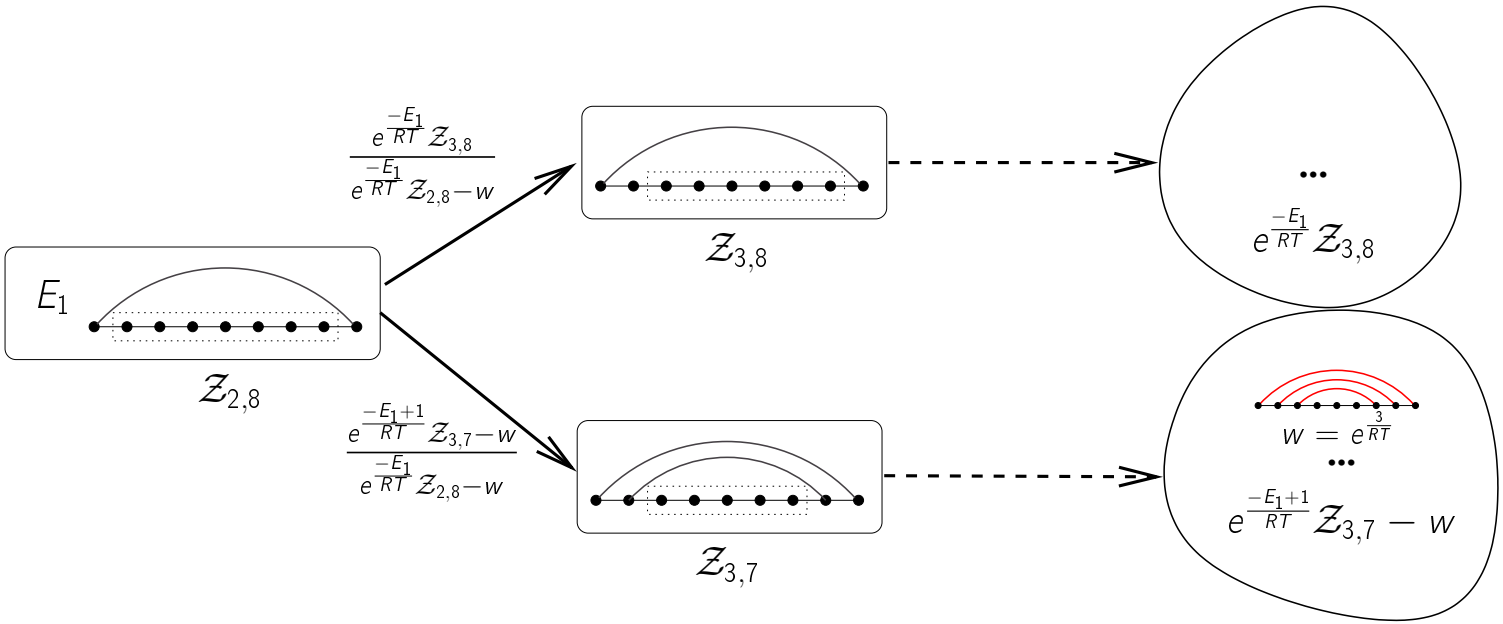

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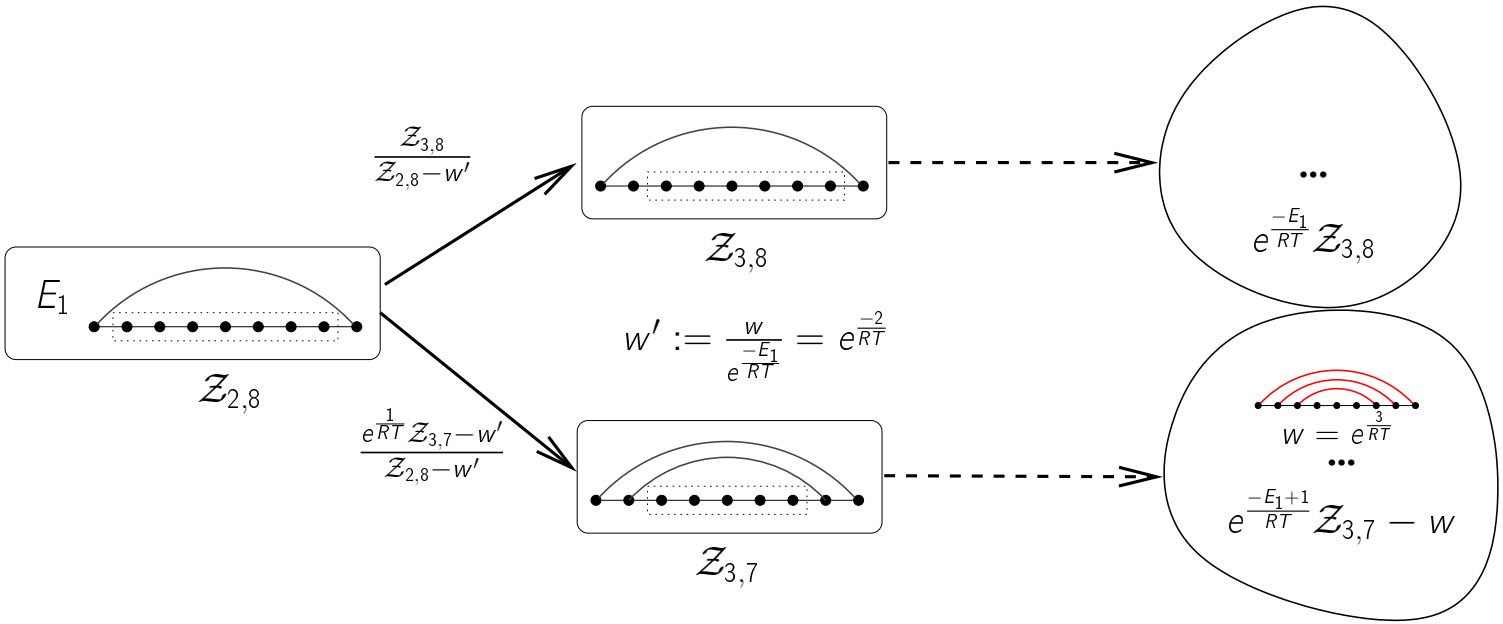

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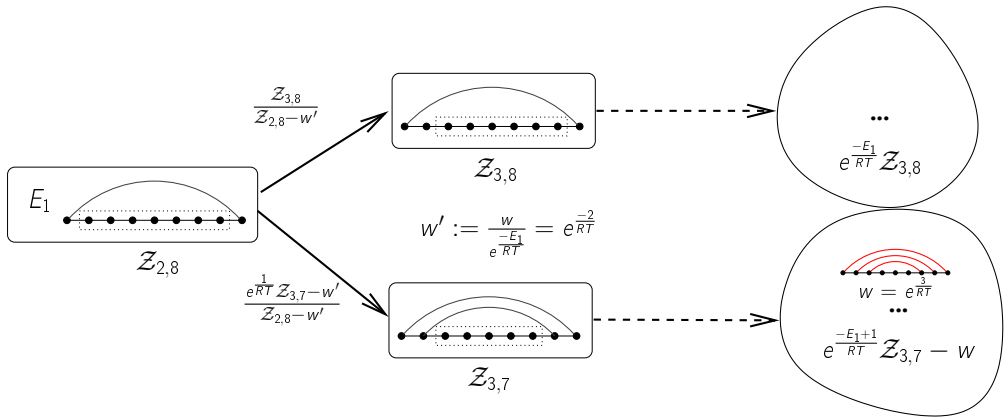
  - Build prefix tree for parse traces, storing in each node the contributions $K = \sum_{S \in \mathcal{R}} e^{\frac{-E_S}{RT}}$ of already sampled structures $\mathcal{R}$
  - During traceback, modify contributions of terms using K [Pon08]



(Partial?) Conclusion

In structural biology, the following conditions:

- Additivity of energy function
- Sweetly enumerable conformational space (No $-$ or min)

allowed for an exhaustive (polynomial) exploration through:

- Generate suboptimal foldings (`RNASubopt`)
- Compute partition function (`McCaskill`)
- Partition conformation landscape (`RNAMutants`, `RNABor`, `RNAShapes`)
- Perform statistical sampling in the Boltzmann ensemble (`SFold`)
- Simulate simple hybridization (`hybrid`)

Additional motivations for enriching the conformational space:

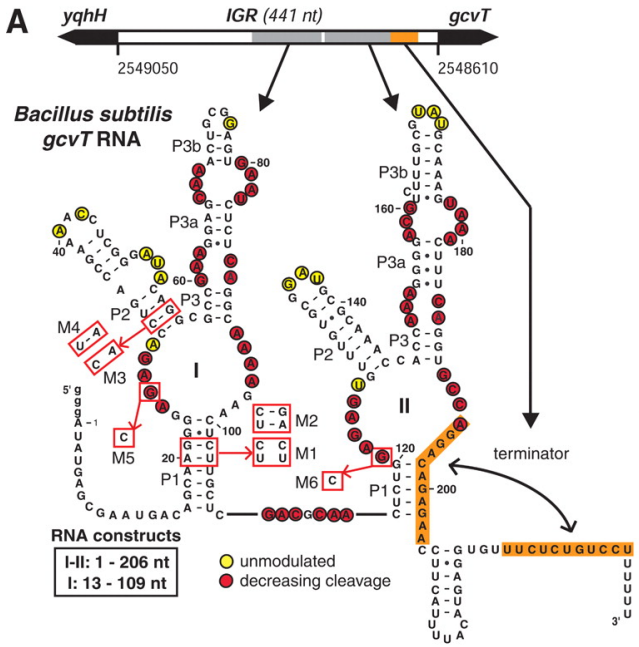
$\Rightarrow$ Better predictions (PK, non-canonical)

$\Rightarrow$ Predict interactions

Toward RNA design

Use our understanding of folding mechanisms to design

- Small interfering RNA engineering
- RNA switches, bistable RNAs
- Self-assembly and nanostructures



Science-Mandal *et al*-2004

Toward RNA design

RNA inverse folding problem

Given a target structure S, and a predictive algorithm P find an RNA sequence ω such $P(\omega) = S$.

Existing approaches:

- Local search approaches [AFH+04, AHHC07, BB06]
- No or few constraints
- Connectivity of the sequence space?

Open question B: Complexity of RNA design

What is the theoretical complexity of RNA-design?

Seems there is a hole in inverse-optimization theory...

Open question B': Stable RNA design

Given a target structure S, find an RNA sequence ω that both satisfies the RNA design problem, and has energy $E_{S,\omega} > E^{(2)}_{S,\omega} + \Delta$.

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Proof of average-case complexity

Theorem

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

Proof.

Homodimer model: All pair of positions can form a base-pair.
Boltzmann distribution: Based on Nussinov model.

Then the generating function $C(z) = \sum_{S \in \mathcal{S}} e^{\frac{bp(S)}{RT}} c(\omega) z^{|S|}$ holding the (unnormalized) average cost of a sampling scenario can be expressed in term of the **partition function** generating function $P_f(z) = \sum_{S \in \mathcal{S}} e^{\frac{bp(S)}{RT}} z^{|S|}$

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

□

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Moreover, $P_f(z)$ is solution of a system of algebraic equations induced by Waterman's context-free grammar for RNA secondary structures.

$$\begin{cases} P_f(z) &= z^2 e^{\frac{1}{RT}} P_f^{\geq\theta}(z) P_f(z) + z P_f(z) + 1 \\ P_f^{\geq\theta}(z) &= z^2 e^{\frac{1}{RT}} P_f^{\geq\theta}(z) P_f(z) + z P_f(z) + z^\theta. \end{cases}$$

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Extracting $A_n := [z^n]C(z)$ and $B_n := [z^n]P_f(z)$ using singularity analysis yields an average-case complexity $A_n/B_n \in \Theta(n\sqrt{n})$. □