(NP-hard?) RNA inverse folding can be solved in linear time for structures without isolated stacks or base pairs

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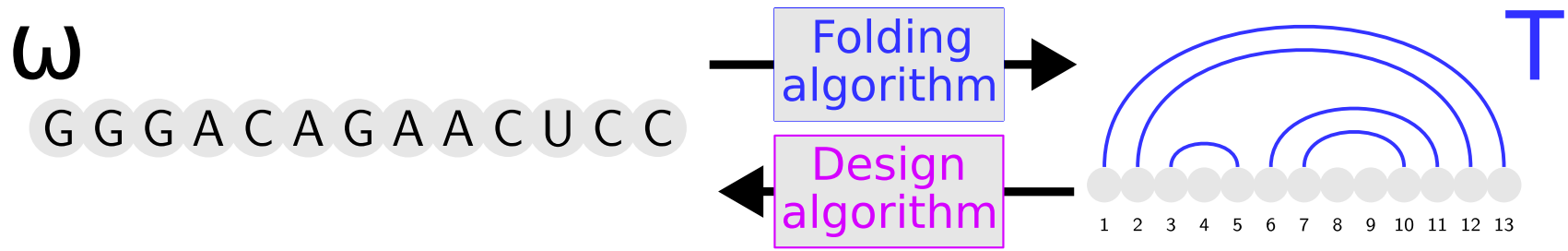
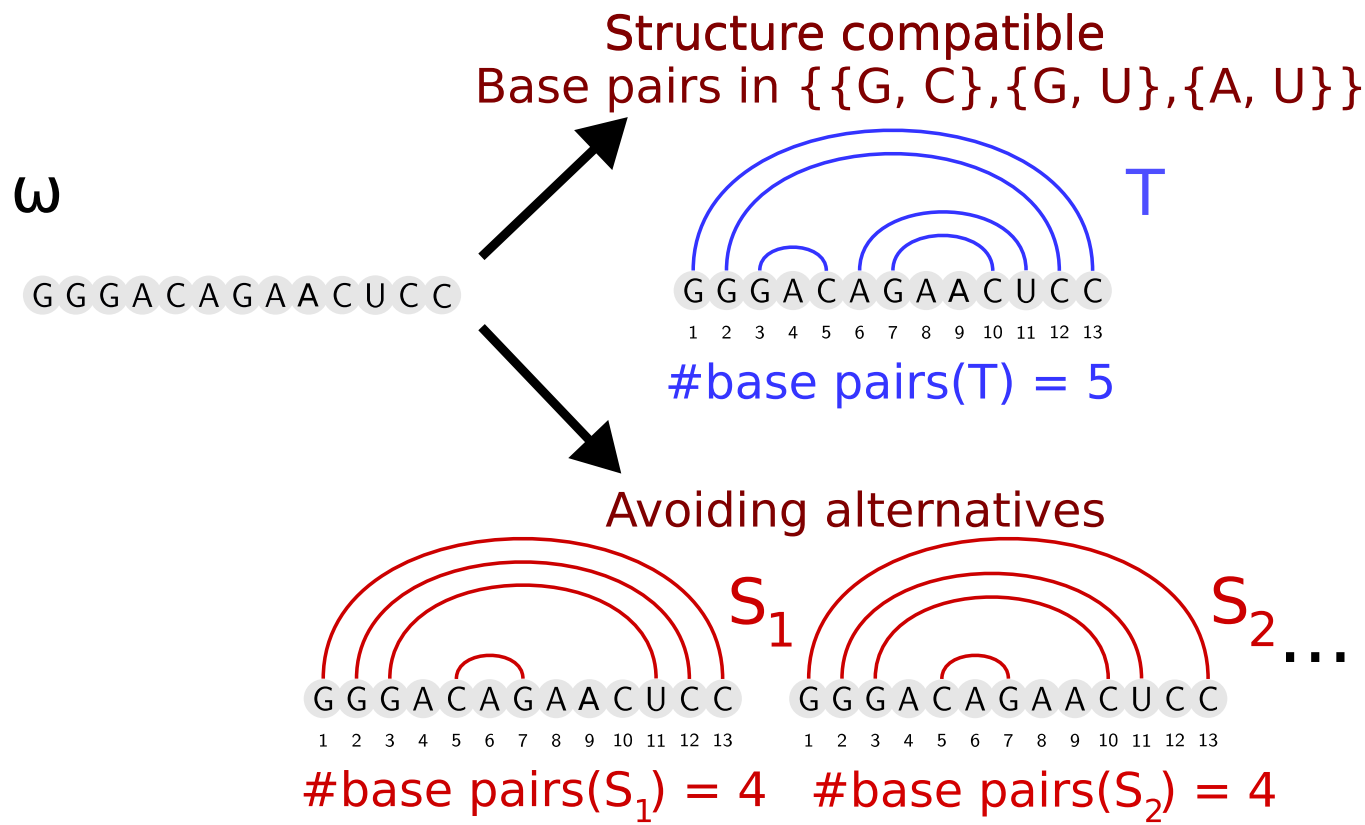
RNA 2D folding vs RNA structural design



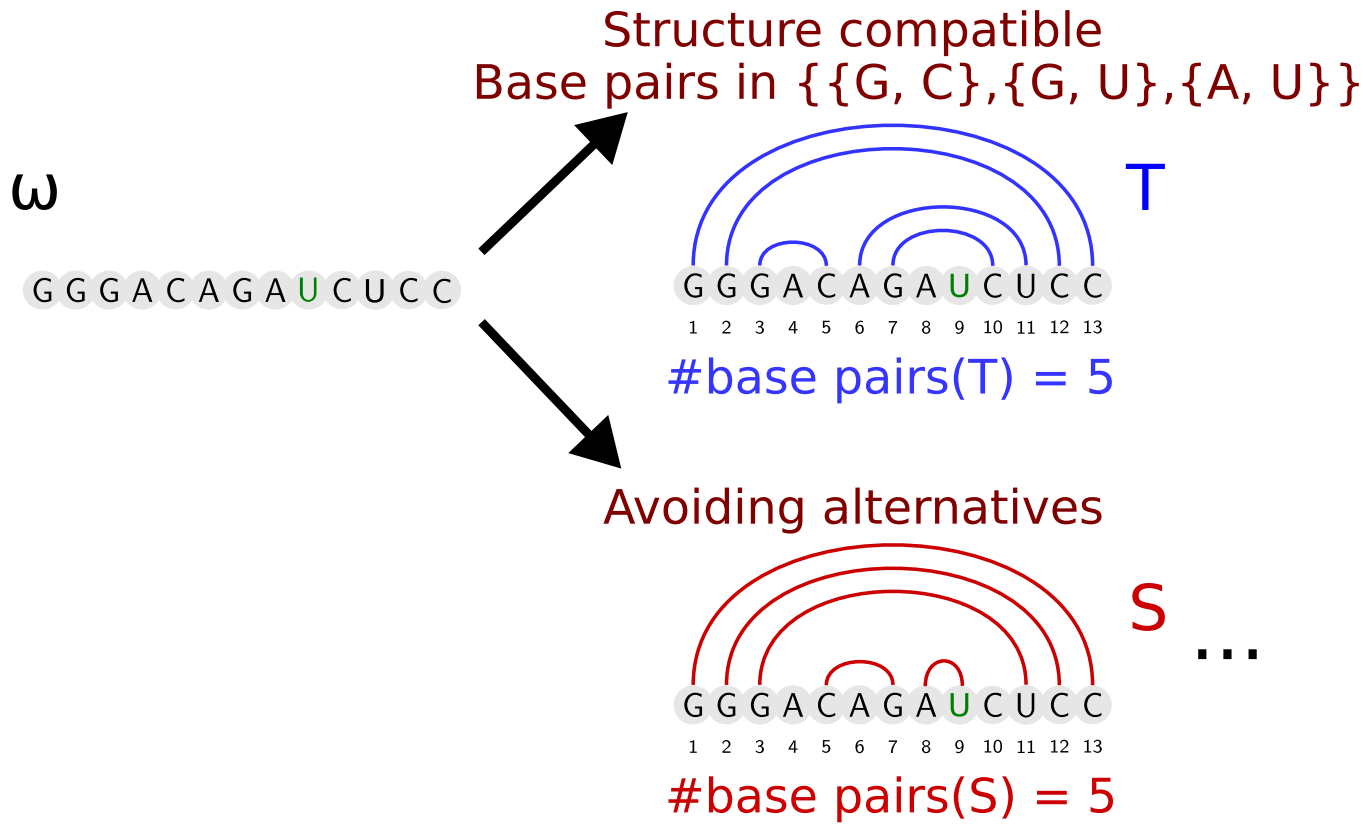
Inverse folding (IF): Formal definition



Goal: Find ω such that T is unique+optimal+valid fold for ω

$$\forall S \neq T, S \text{ comp. with } \omega, \#\text{BasePairs}(S) < \#\text{BasePairs}(T)$$

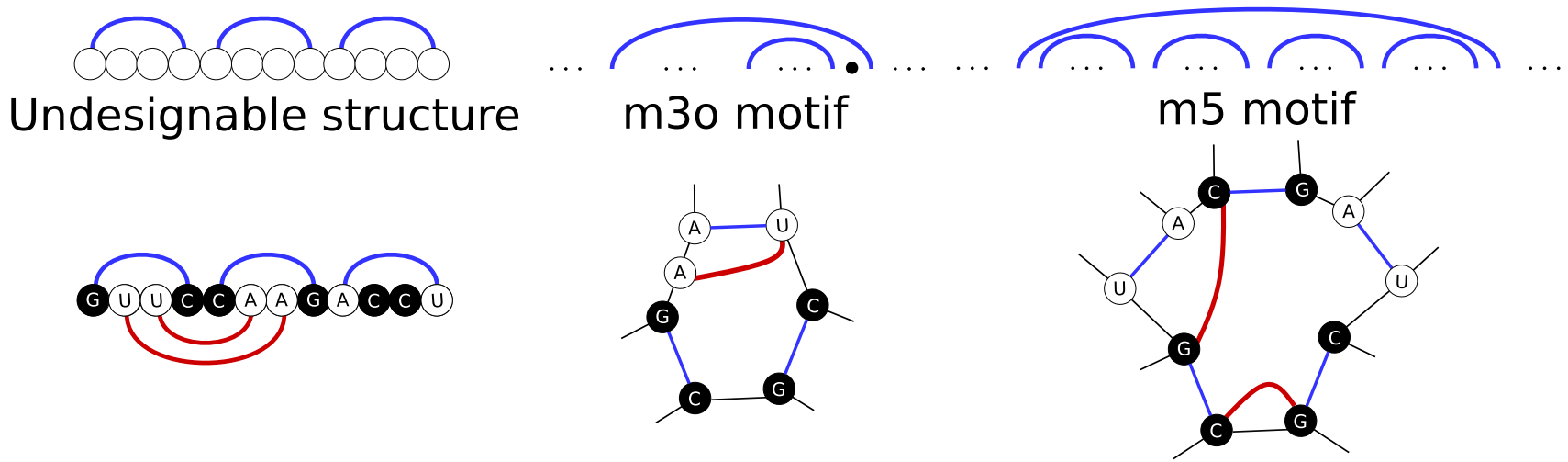
Inverse folding (IF): Formal definition



Goal: Find ω such that T is unique+optimal+valid fold for ω

$$\forall S \neq T, S \text{ comp. with } \omega, \#\text{BasePairs}(S) < \#\text{BasePairs}(T)$$

Minimal undesignable structures [Halès et al, 2017]

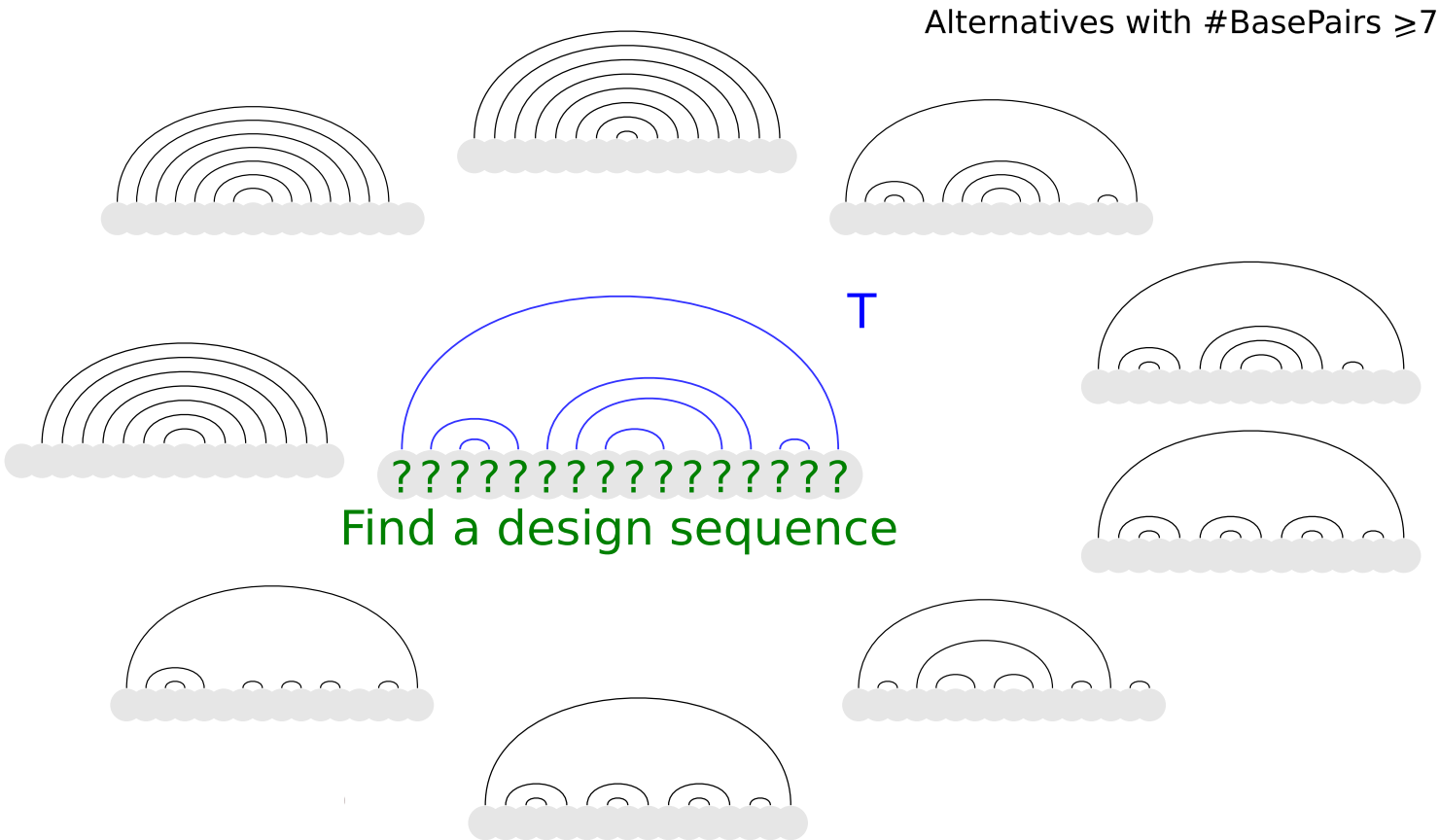


- Any target featuring occurrence of m3o or m5 is not designable

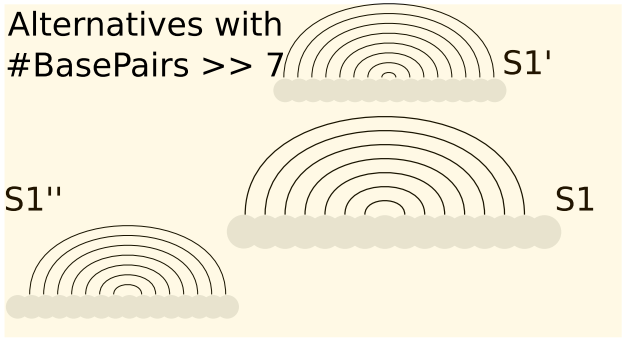
- Inverse folding (+ minor constraints) is NP-HARD [Bonnet et al, 2018]
  → Infinite (+ exp. growth) list of min undesignable motifs
  (unless P=NP)

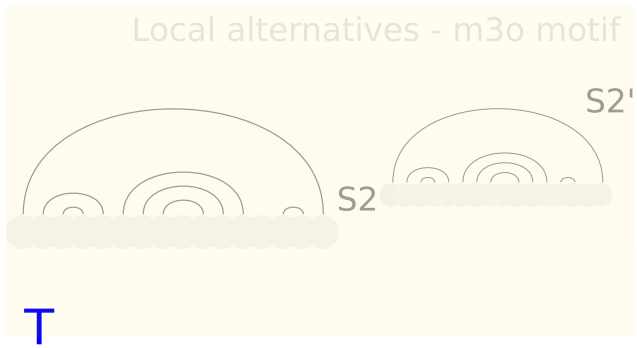
- Decision version of Inverse Folding not reducible to pattern matching

Finding a criteria to solve Inverse Folding

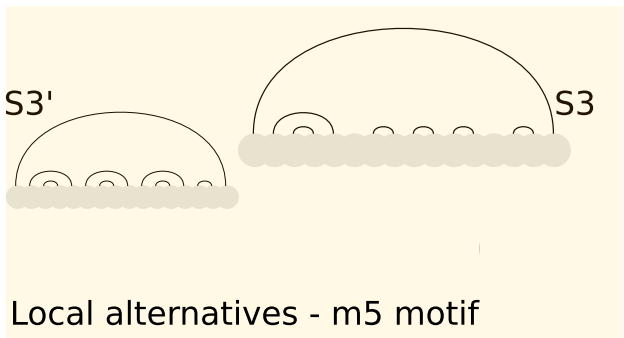


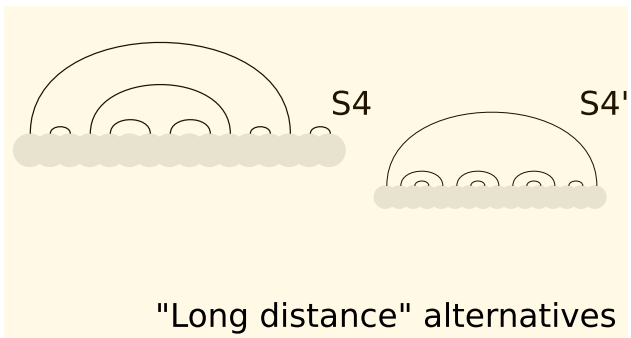
Finding a criteria to solve Inverse Folding





A compatible sequence is not enough

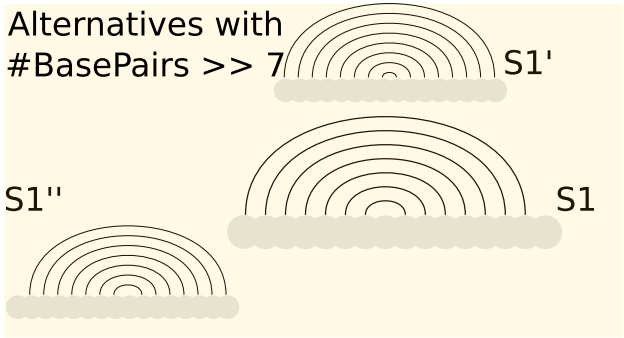


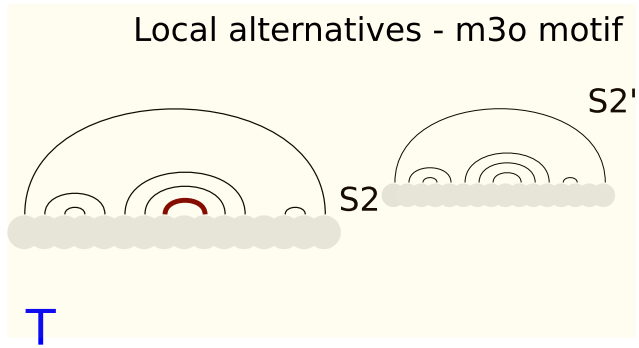


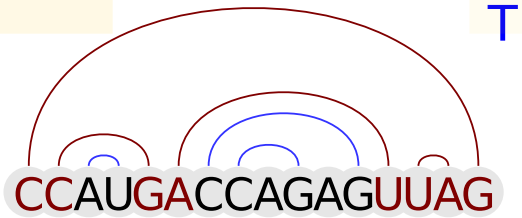
Complexity: 

- Compatible is necessary

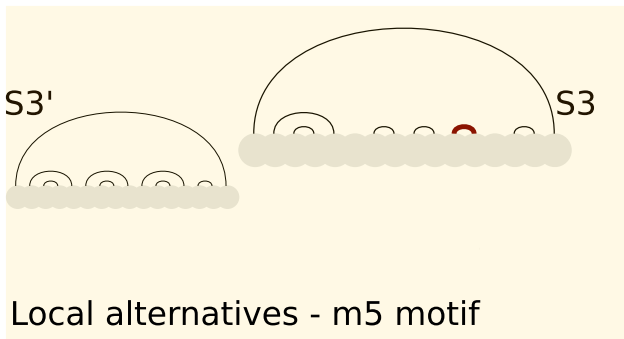
Finding a criteria to solve Inverse Folding

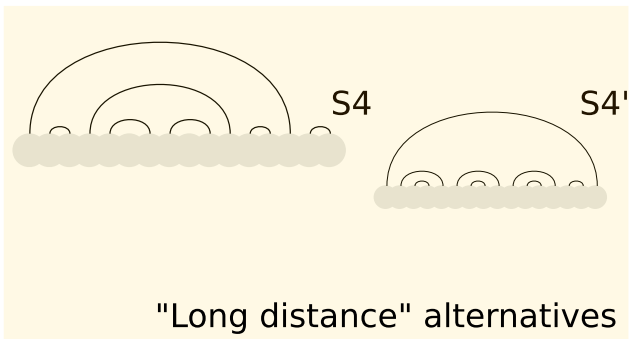






A proper sequence is not enough

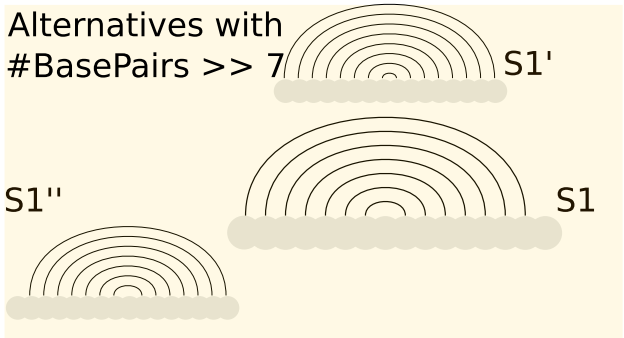


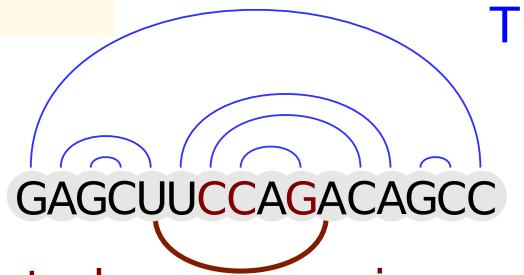


Complexity: 

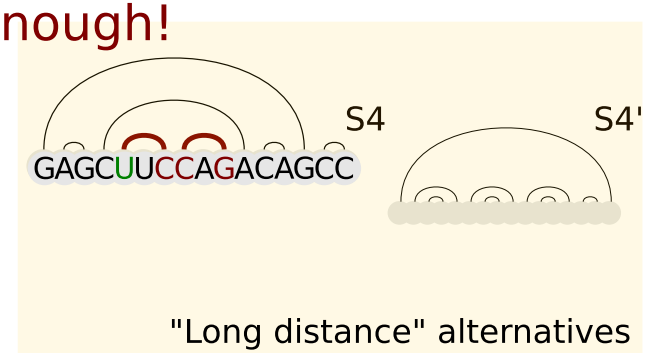
- Compatible + proper is necessary

Finding a criteria to solve Inverse Folding





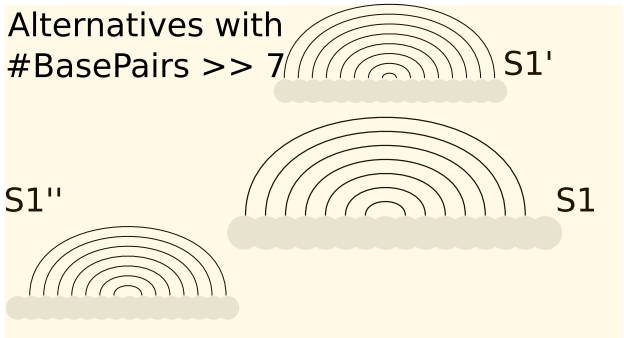
A separated sequence is enough!

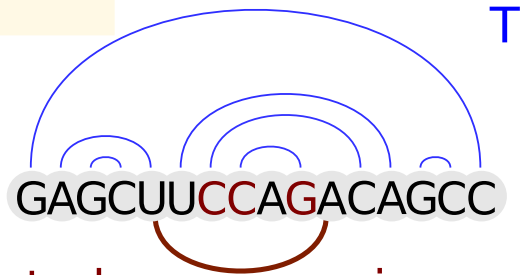


Complexity:

- Compatible + proper + separated not necessary, but sufficient
- Separated: All A-Us delimit $\#G - \#C \neq 0$

Finding a criteria to solve Inverse Folding





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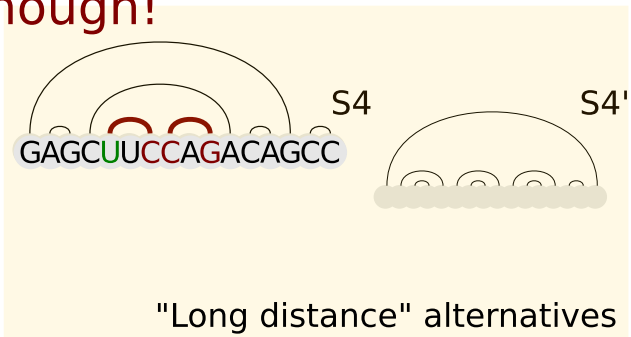
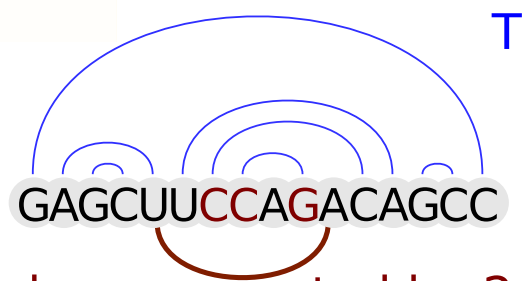


Complexity: NP-HARD

- Compatible + proper + separated not necessary, but sufficient
- Separated: All A-Us delimit $\#G - \#C \neq 0$

Finding a criteria to solve Inverse Folding



One can replace separated by 2-separated

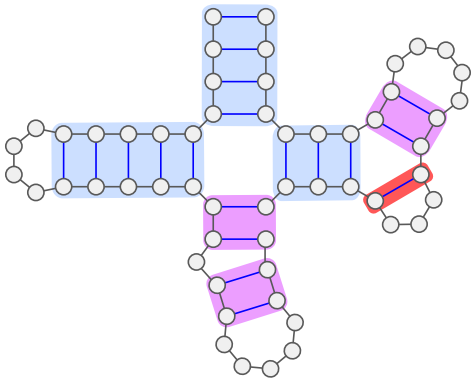
Complexity: 

- Separated $\leftrightarrow$ peer-to-peer, 2-Separated $\leftrightarrow$ local

Inverse Folding efficiently solved for non-bonsai structures

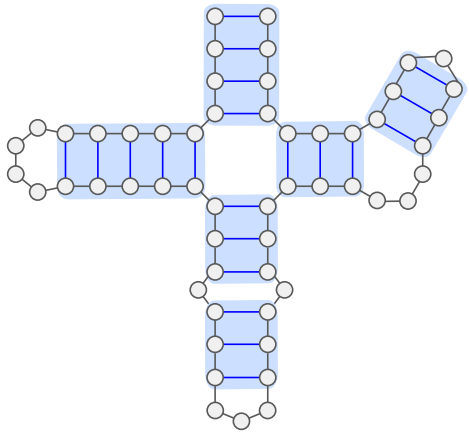
h_{min} = Minimum #base pairs in an helix

Structure with h_{min}= 1



Presumably NP-complete to design...

Structure with h_{min}= 3



Designed in linear time!

Helices of size 3+

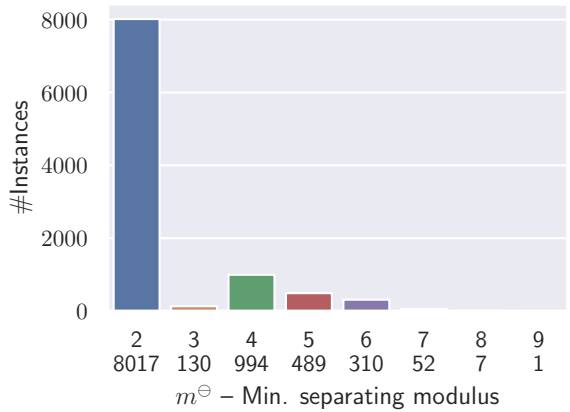
Isolated stacks (e.g. helices of size 2)

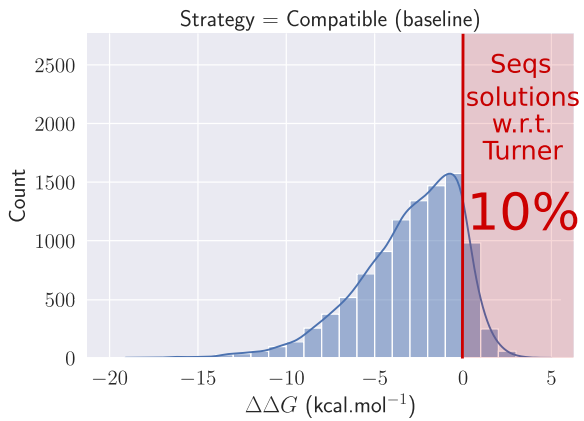
Isolated base pairs (e.g. helices of size 1)

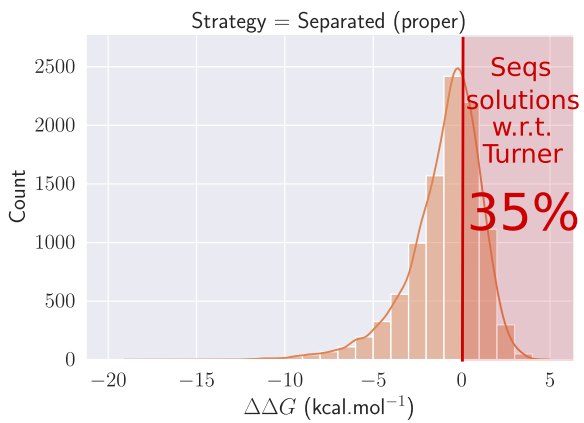
Result: [Boury et al, WABI 2024]

- Core: Structures with $h_{min} = 3 \rightarrow$ 2-separable
- In general: m-separated design is $\Theta(n.m.2^m)$ time and $\Theta(m.n)$ space (e.g. Fixed-Parameter Tractable in m)
- Corollary: Structures with $h_{min} = 3$ solved in $O(n)$!
- Bonus: Uniform sampling of sequences

Experimental results at a glance

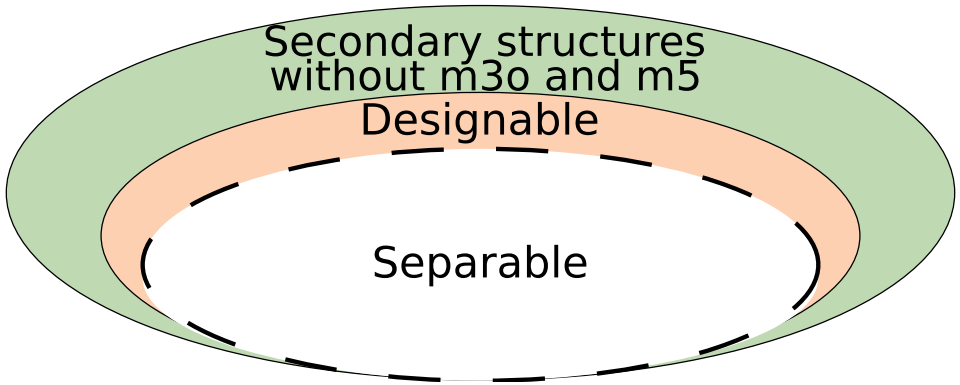






- Structures with $h_{min} \leqslant 2 \rightarrow$ Mainly designable in practice (around 100 nucleotides), but exponential decay of numbers of solutions
- Sampled solutions from 2-separable structures are more promising according to the Turner energy model than random compatible sequences

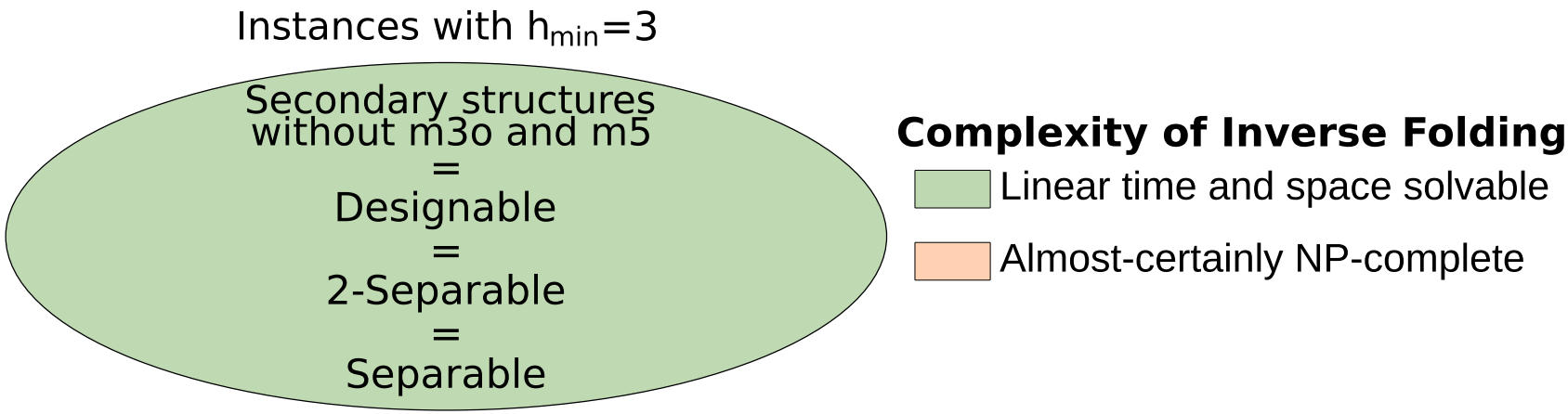
Conclusion



Complexity of Inverse Folding

- Linear time and space solvable
- Almost-certainly NP-complete

Conclusion



Future work:

- Extensions to more realistic energy models
- Solid foundation towards concrete design methodologies even with smaller helices
- Hard to make the problem "difficult" in base pairs model

Thanks to...

Yann Ponty

Laurent Bulteau

And to the other members of the AMIBio team:

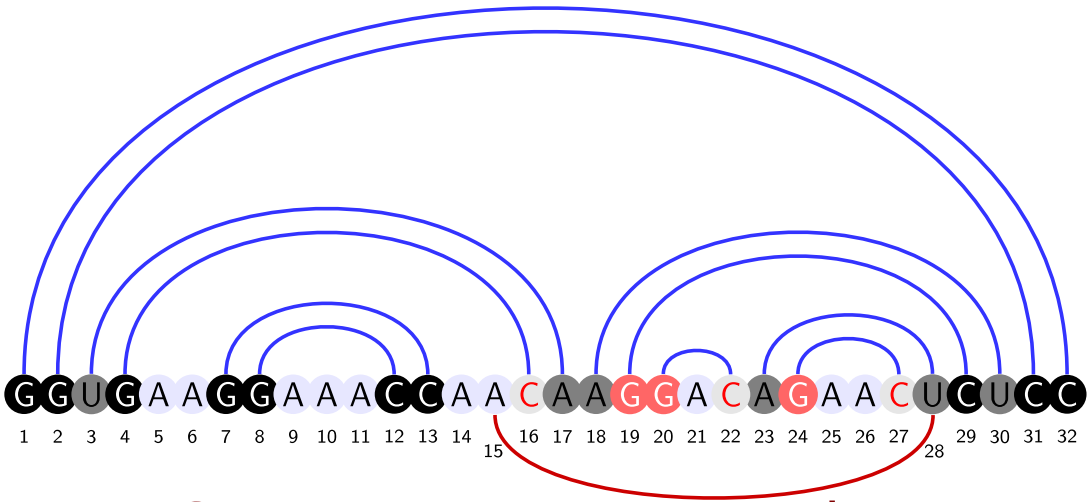
Sarah Berkemer
Jean-Marc Steyaert
Sebastian Will
Alan Azede
Nan Pan
Taher Yacoub

Link to the paper

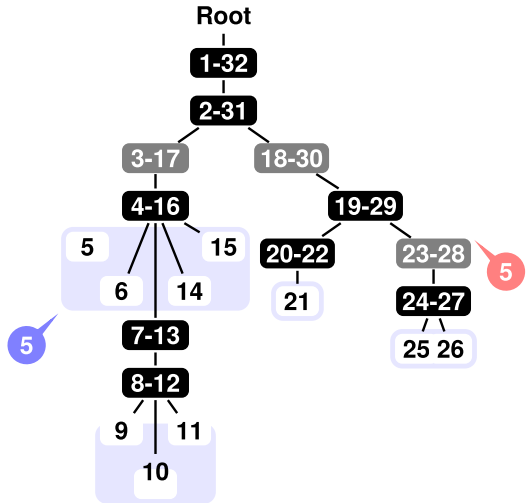
You!

ENS DE LYON

Tree formalism



Structure representation



Tree representation

Definition (Levels): Given a tree coloring, the level $L : V(T) \to \mathbb{Z}$ of a node v is $L(v) := |p|_{\bullet} - |p|_{\circ}$ where p denotes the color vector associated with the node sequence from parent(v) to Root.

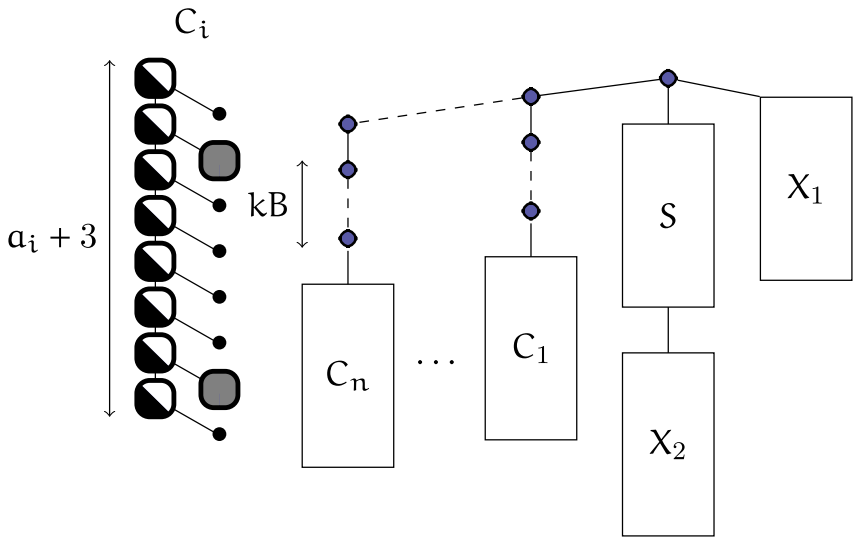
Decide separability is NP-complete

Problem 1 (Interval Packing):

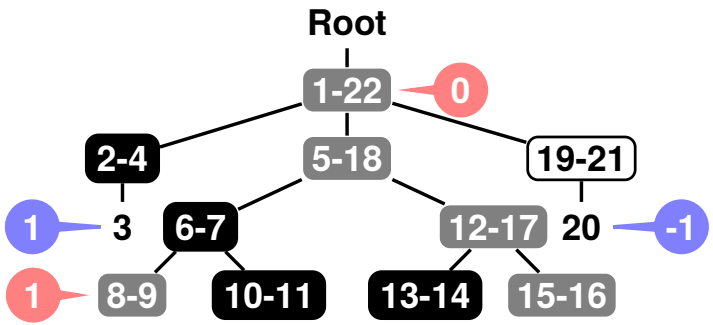
Input: set of distinct integers $A = \{a_1, \cdots, a_n\}$, integers k and B

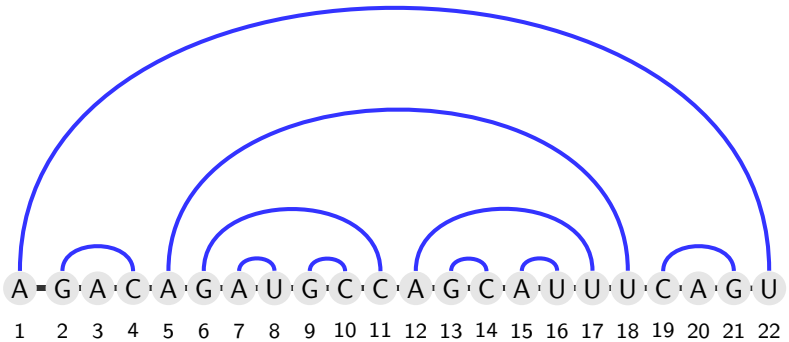
Output: function x from A to intervals of $[\![0, kB-1]\!]$ such that:

- $x(a_i)$ is an interval of size a_i
- $x(a_i)$ and $x(a_j)$ are disjoint for $i \neq j$
- $x(a_i)$ does not contain both $jB-1$ and jB for any i, j.



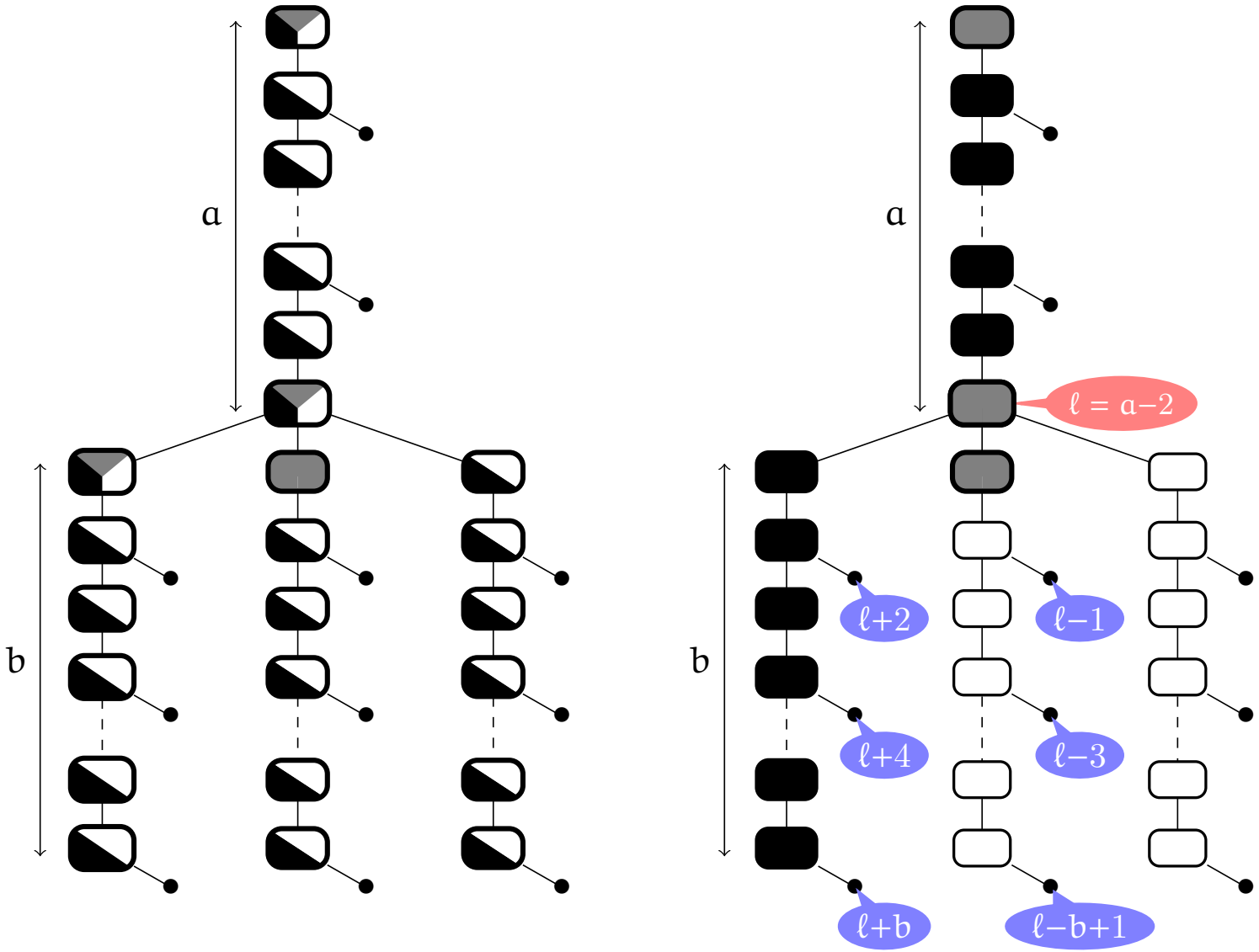
Which instances are non-separable but designable?





- Harder to find with helices of size 2 or more. (currently more than 1000 nucleotides long)

Core widget of the designable non-separable instances with helices of size 2



Modulo-separability

Definition ((Modulo) m-separability): Let m be an integer. A coloring *Color* is m-separated (or separated with modulus m) for a target secondary structure T, if an only if

$$\{Lv(v) \bmod m \mid Color(v) = \bullet\} \cap \{Lv(v) \bmod m \mid v \text{ is a leaf}\} = \varnothing$$

- Modulo separability coincides with separability with $m \geqslant \frac{n}{2}$

Problem 2 (Modulo Separability):
Input: A tree T (with no $m_{3\bullet}$ or m_5 motif), a modulus $m \in \mathbb{N}$

Output: A coloring of T that is m-separated, or $\perp$ if no such coloring exists.

Dynamic programming scheme for modulo separability

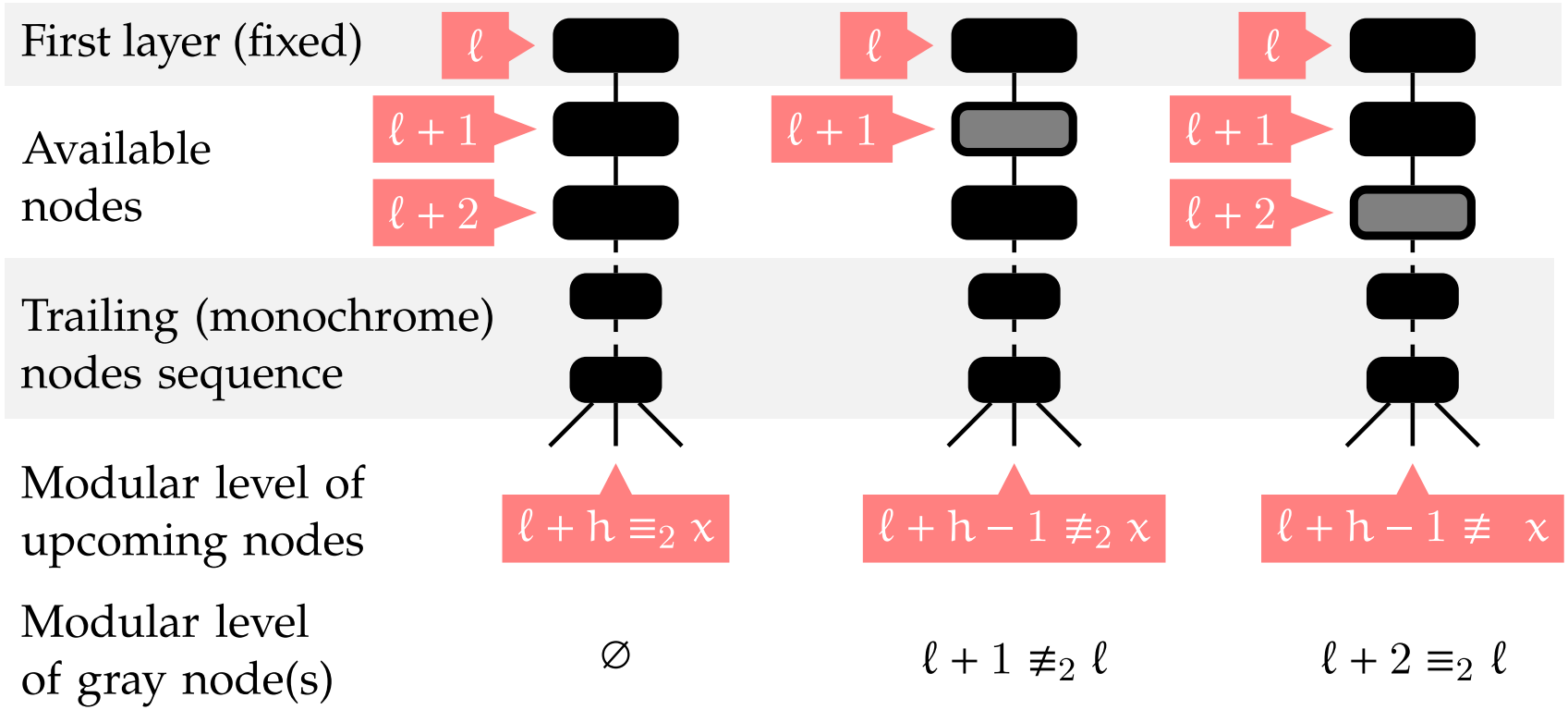
$$\mathrm{d}^{\xi_L}_{v\to c,l} = \begin{cases} \text{False} & \text{if } \ell \in \xi_L \wedge c = \bullet \\ & \text{or } \ell' \notin \xi_L, \text{ and} \\ & \exists \text{ leaf in children}(v) \\ \text{True} & \text{if children}(v) = \emptyset \\ \bigvee_{\substack{c' \text{ "valid" coloring of} \\ \text{children}(v) \\ \text{given } v\to c}} \bigwedge_{v' \in \text{children}(v)} \mathrm{d}^{\xi_L}_{v'\to c'(v'),\ell'} & \text{otherwise.} \end{cases}$$

with $\ell' := \ell + \delta(c) \bmod m$

- $\mathrm{d}^{\xi_L}_{v\to c,\ell}$: existence of a valid assignment for a subtree of T rooted at internal node v, with v occurring at level ℓ, and being assigned a prior color c.
- ξ_L: Leaves levels (thus $[\![0, m[\![\setminus \xi_L$ are $\bullet$ levels.)
- δ: level increment induced by a color c

Instances with helices of size 3 or more are all separable

$$\mathrm{x} := \ell + \mathrm{h} \bmod 2$$



Theorem: Secondary structures with helices of size 3 or more are 2-separable (thus designable) in linear time

Inverse folding: Complexity Zoo

- NP-hard, 2008, Schnall-Levin et al $\cdots$
- Linear, 2017, Halès et al $\cdots$

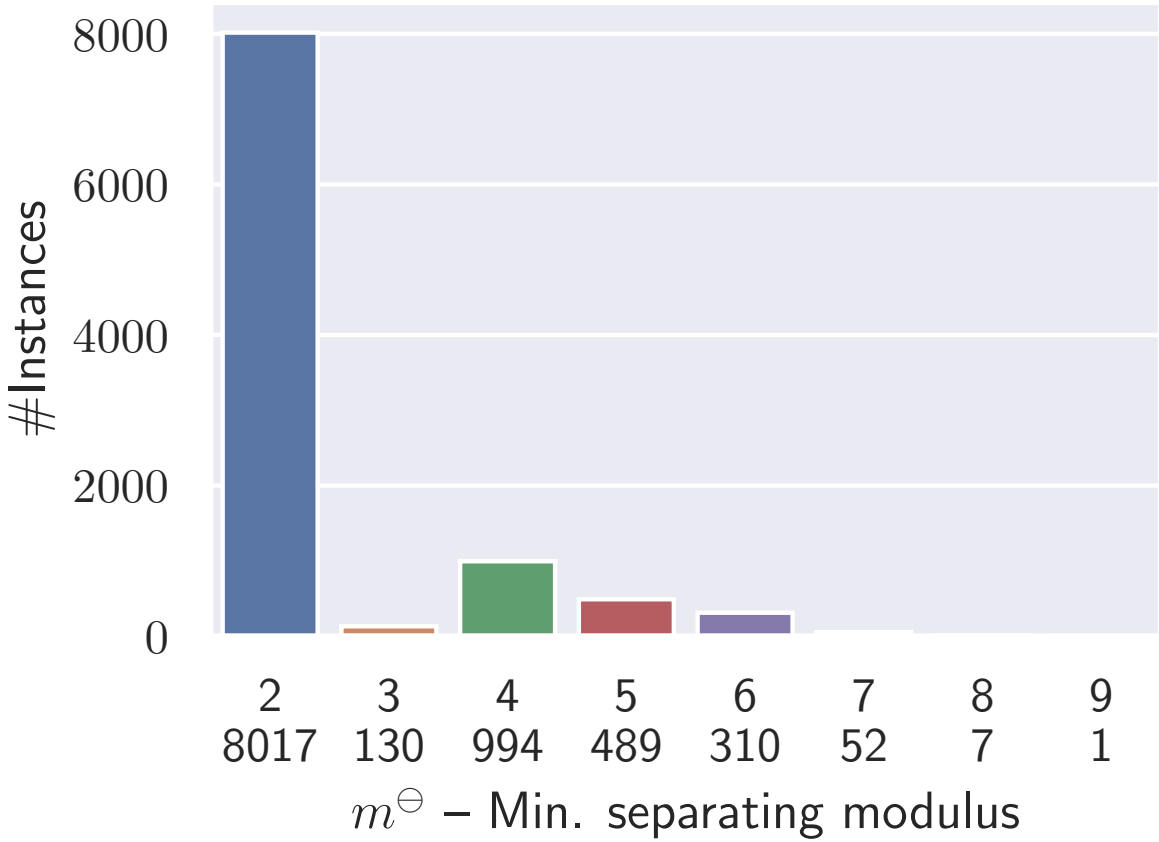
  But only on a subset called "separable instances".
- NP-hard, 2018, Bonnet et al $\cdots$

  But only an extension with constrained base pairs.
- Our contribution:

  Linear by avoiding isolated base pairs and stacks, 2024, Boury et al.

Beyond helices of size 3: instances with helices of size 2

There is no certainty that these instances are Modulo *m*-separable!

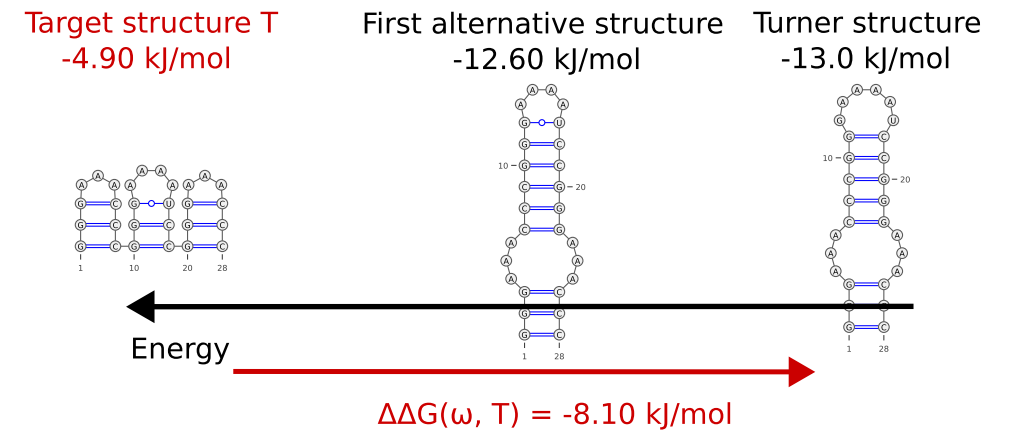


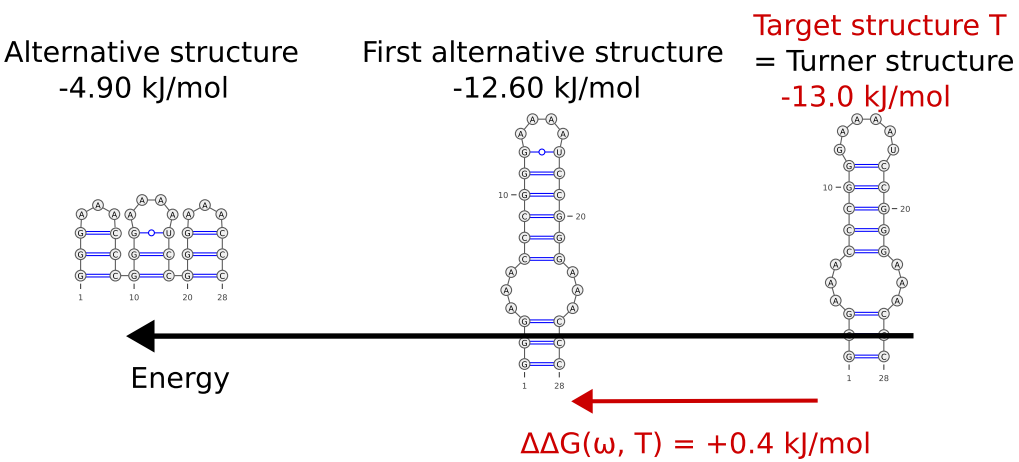
- Surprisingly enough, all instances containing helices of size 2 were found Modulo *m*-separable thus designable.

Turner energy of designed sequence with helices of size 3

$$\Delta\Delta G(\omega, T) := \Delta G(\omega, \alpha(w, T)) - \Delta G(\omega, T)$$

$$\alpha(\omega, T) := \min\{\Delta G(\omega, T') \mid |T' \triangle T| \geq 3\}$$





Turner energy of designed sequence with helices of size 3

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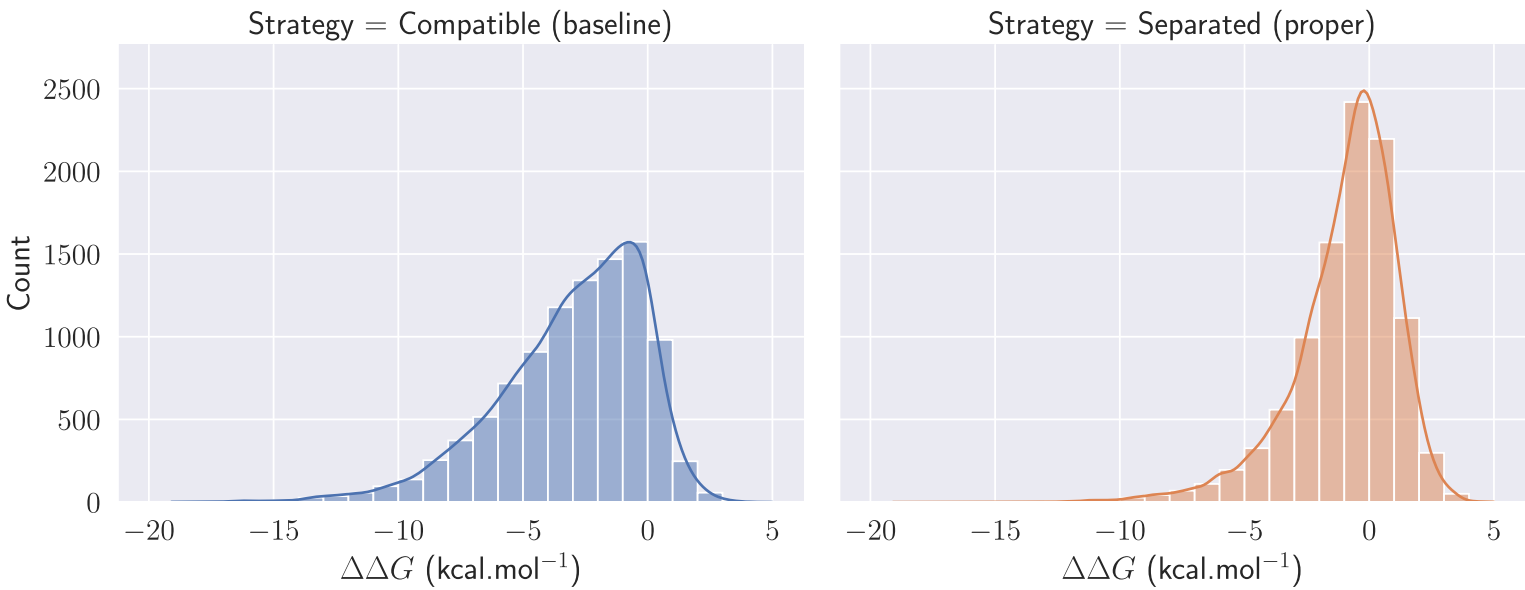

- Even if guarantied only in a base pairs model, our sequences represent better competitor in Turner energy model than simply compatible sequences