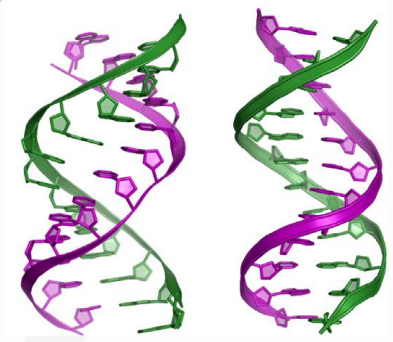
RNA Co-transcriptionality

Shinnosuke Seki
@ FLAT seminar Nov. 20, 2024

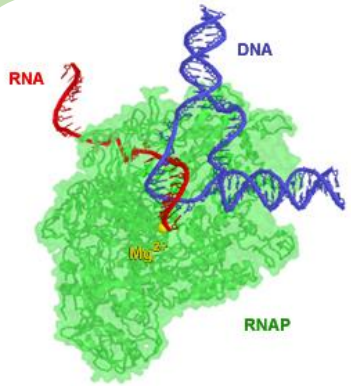
ORITATAMI LAB
@ENS Lyon and UEC Tokyo


Building blocks for life.
LEGO



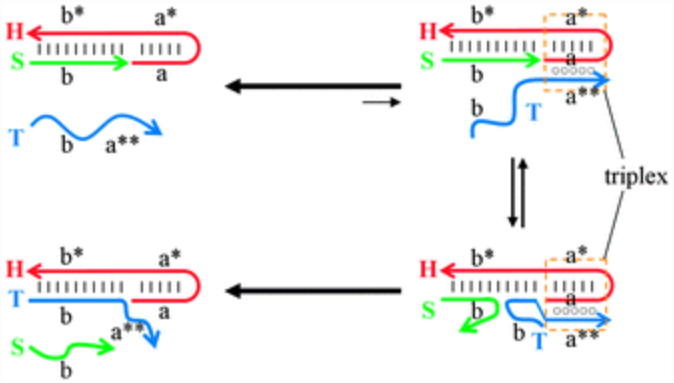
Substrate

+

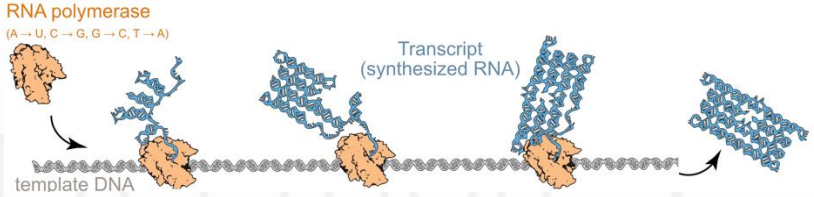


RNA polymerase

Natural Computers (enzymes) if needed



(DNA triplex) toehold-mediated strand displacement

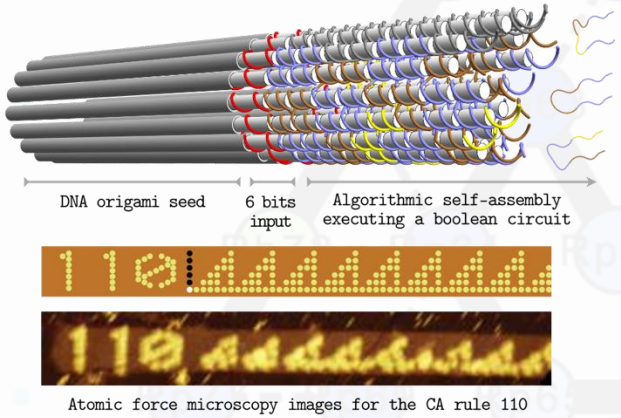


RNA co-transcriptional folding

⋮

Programming Platforms

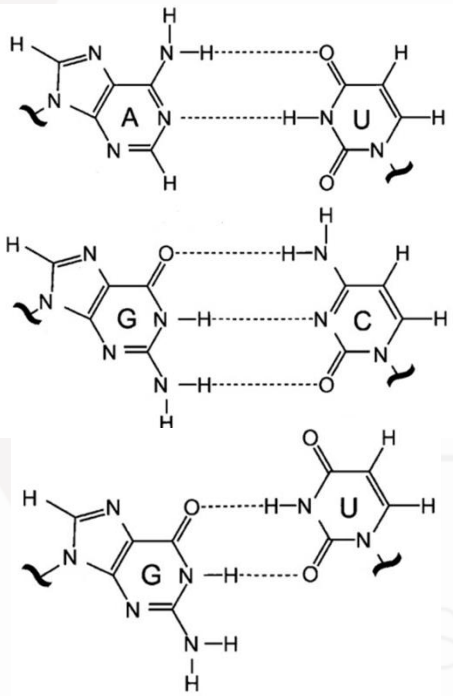
=



DNA-based rule-110 cellular automata (Woods et al. 2019)

(Re-)programmable Molecular Computers

Substrate



DNA/RNA are a chain of 4 kinds of bases A, G, C, T/U.

They may *hybridize* with each other primarily by 3 types of base pairs (shown left) between purines (A, G) and pyrimidines (C, T/U).

-- AcGucAu ->

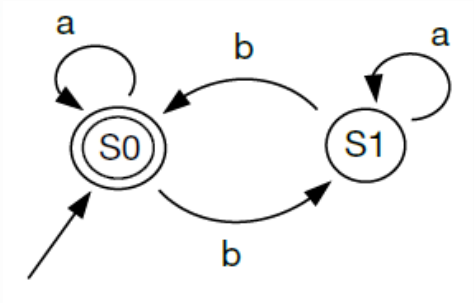
<- uGcGGcA --

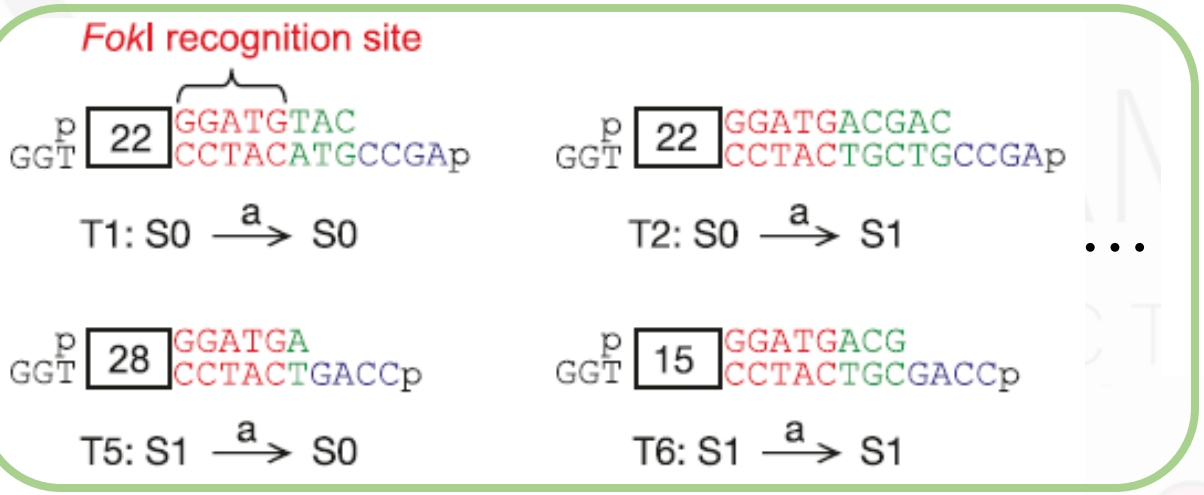
⇔

-- AcGucAu ->

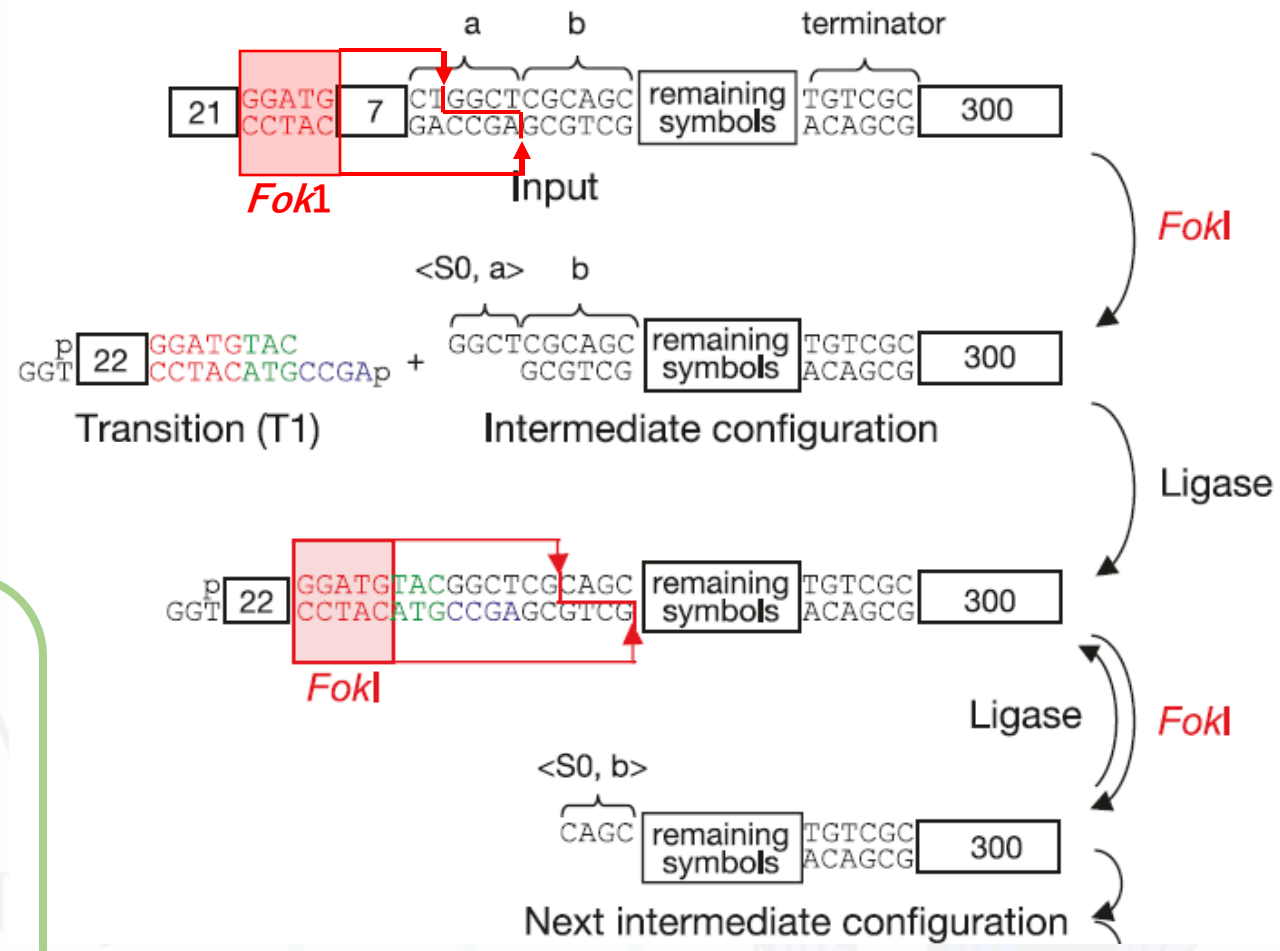
<- uGcGGcA --

*Fok*1-driven FA





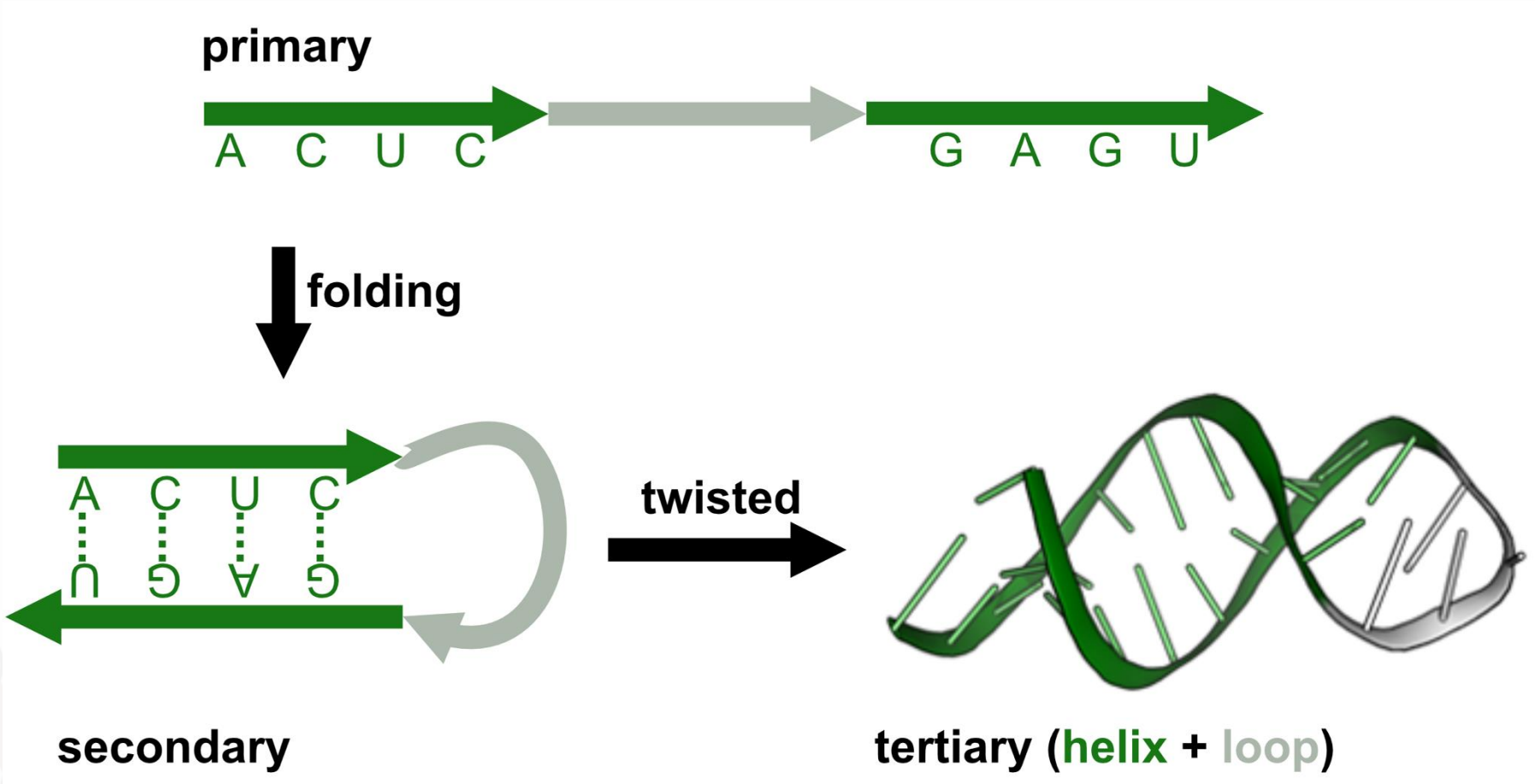
Transition molecules



Benenson et al. *Nature* 414, pp.430-434, 2001

Helix

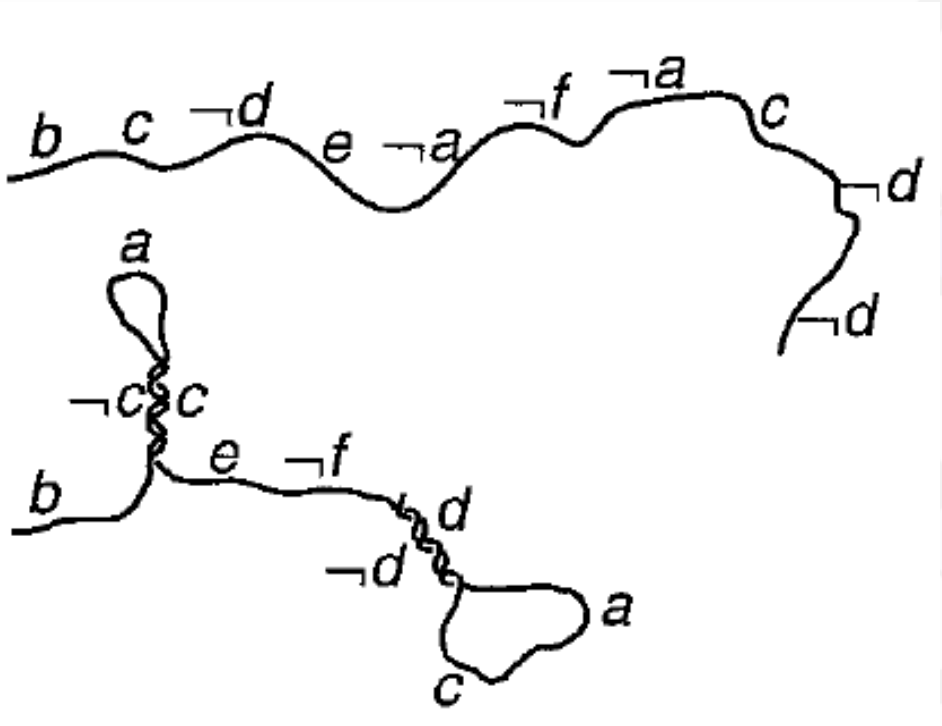
Atoms of single-stranded RNA structures



Enzyme-driven single-stranded computation

A molecular SAT solver.

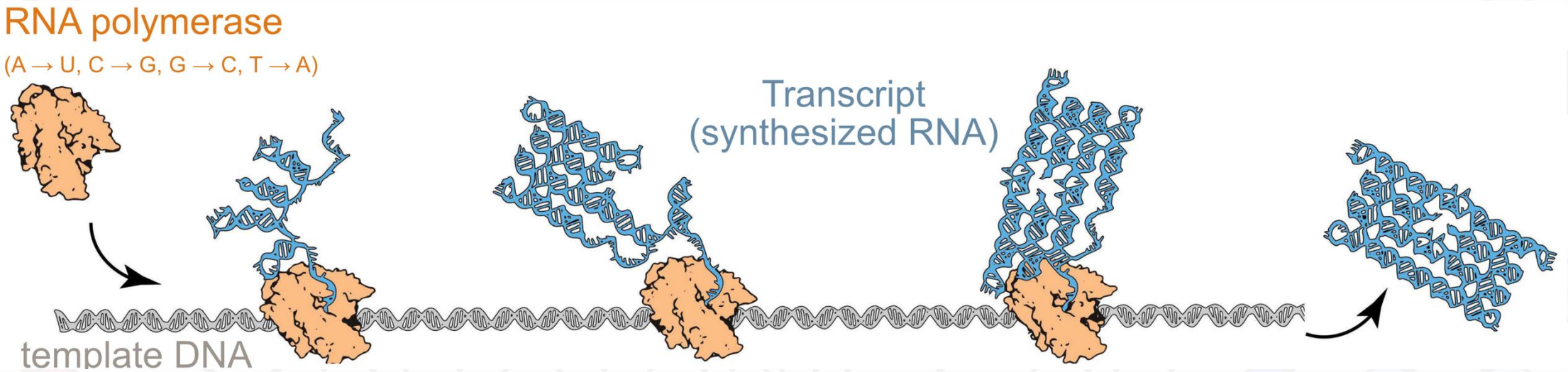
1. Literals are assigned with DNA sequences in such a way that
   - x and ¬y hybridize with each other iff x = y
   - x and y never hybridize, or neither do ¬x and ¬y
2. A SAT instance is programmed as a pool made of DNA sequences obtained by choosing one literal from each clause and catenating the corresponding DNA sequences.
3. Such a DNA sequence forms a hairpin iff both x and ¬x of a variable are involved.
4. A restriction enzyme cuts a hairpin.
5. It suffices to check if some DNA sequence has "survived."

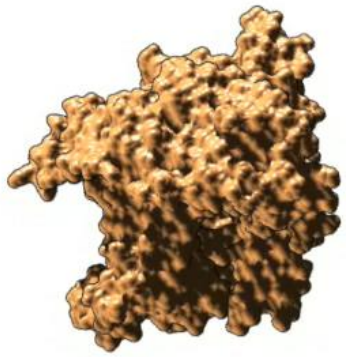


Sakamoto et al. *Science* 288, pp.1223-1226, 2000

RNA co-transcriptionality

Programmable platform for in vitro/vivo computations


RNA polymerase
(A → U, C → G, G → C, T → A)
Transcript
(synthesized RNA)
template DNA



Geary, Rothemund, and Andersen, *Science* **345** (6198): 799-804, 2014

RNA polymerase

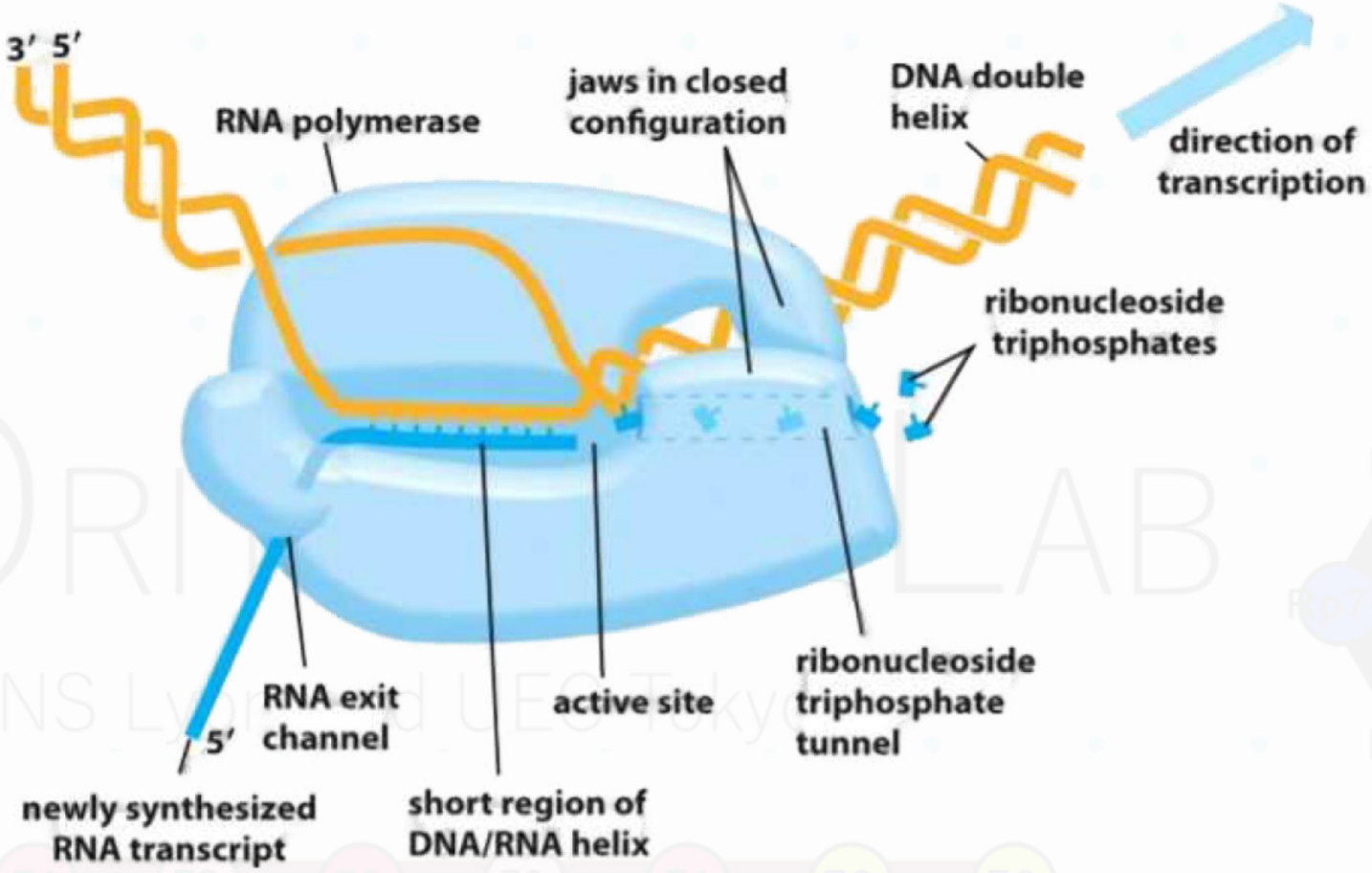
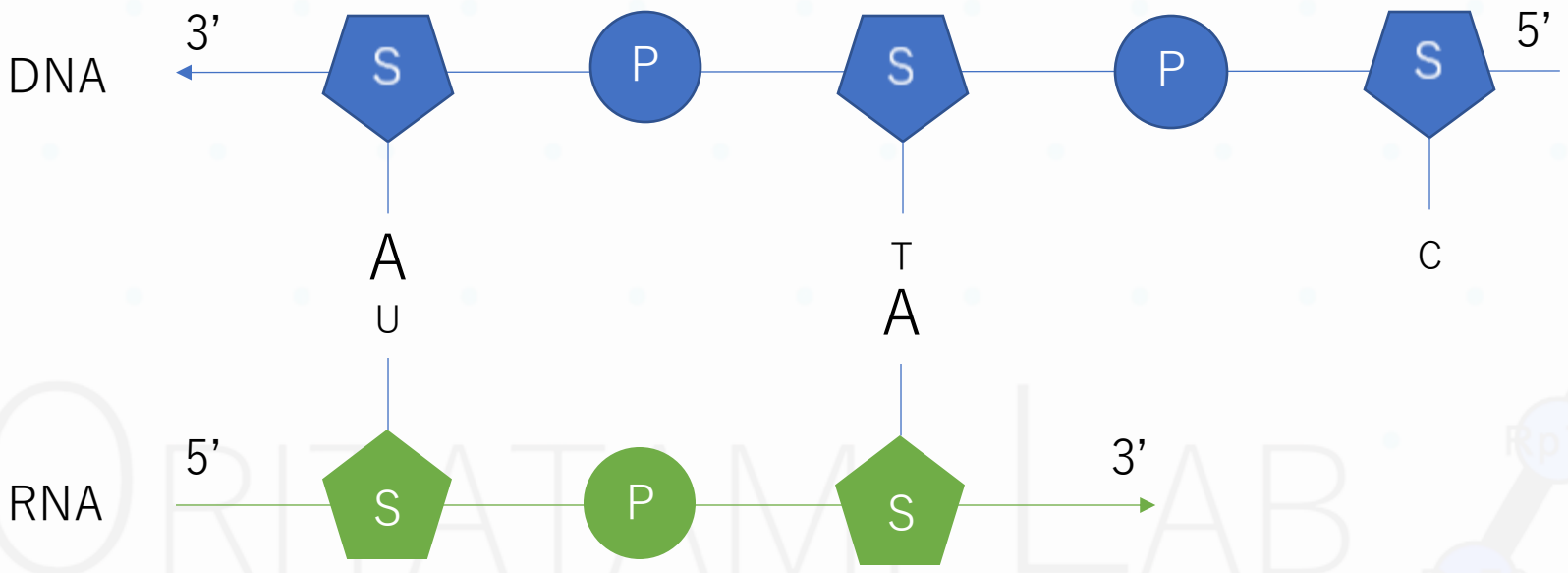
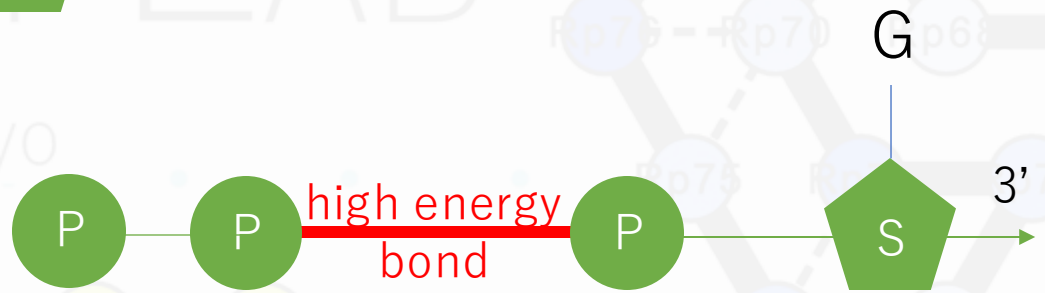


Figure 7-7 *Essential Cell Biology* (©Garland Science 2010)

RNA polymerase

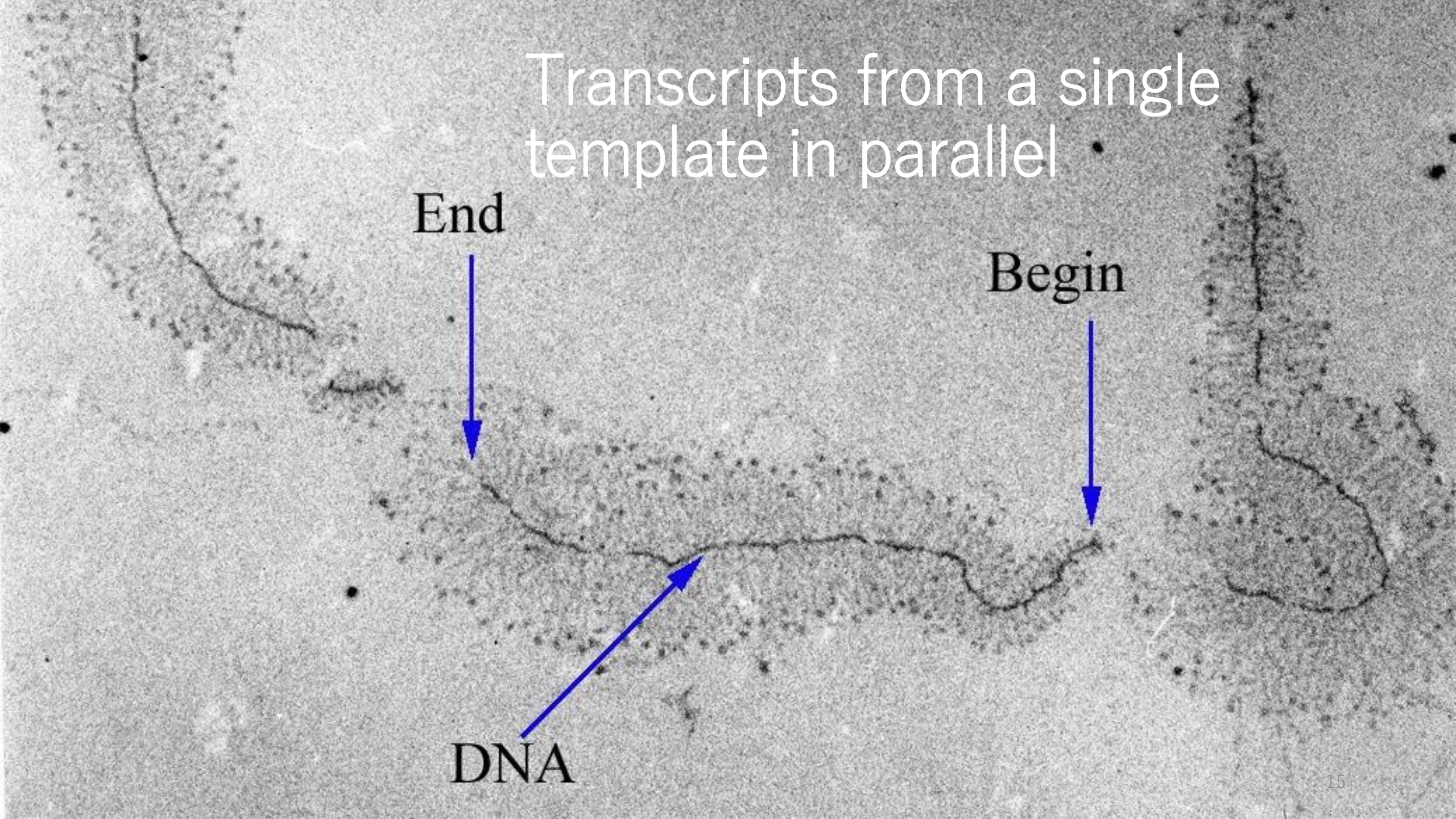


A → U, C → G, G → C, T → A



The idea of this diagram is from Feynman Lectures on Computation, 1996

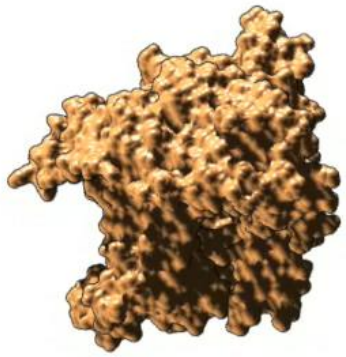
Transcripts from a single template in parallel



RNA origami

architecture for hard-coding a structure into CF

```
int main() {
    while (1) {
        std:cout << “Hello RNA World!!”;
    }
    return 0;
}
```



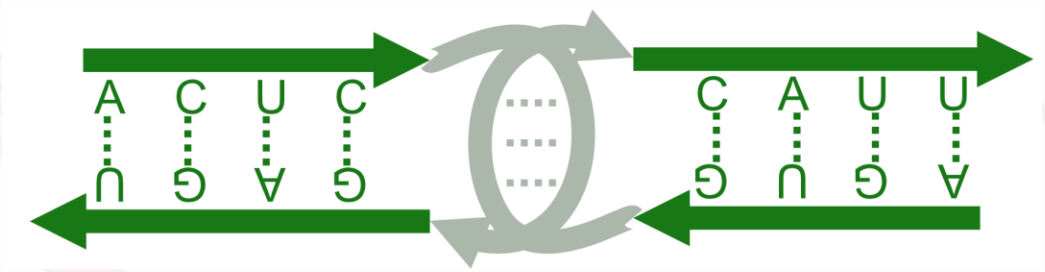
Geary, Rothemund, and Andersen, *Science* **345** (6198): 799-804, 2014

Helix

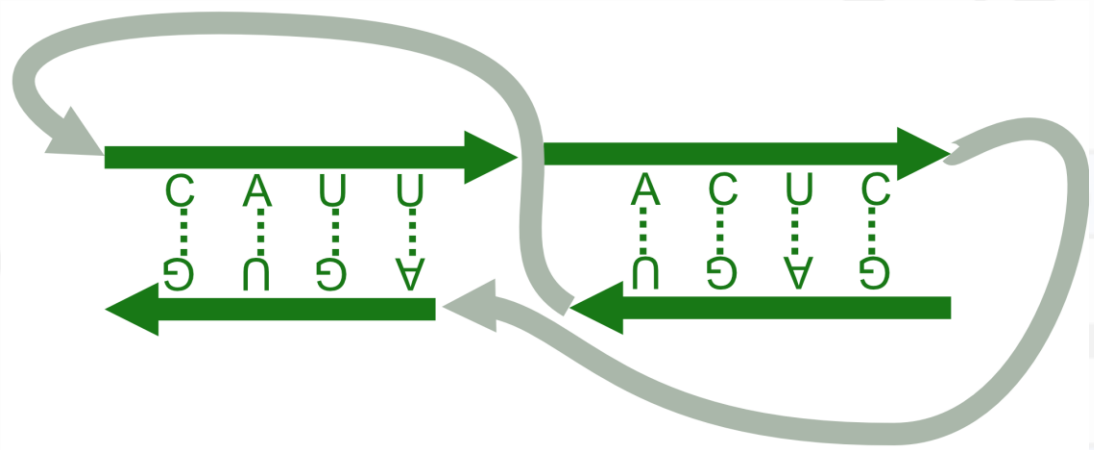
co-axial stacking

Helices are stabilized **co-axially** via base-stacking at their interface.

Two common motifs involving co-axial stacking are:



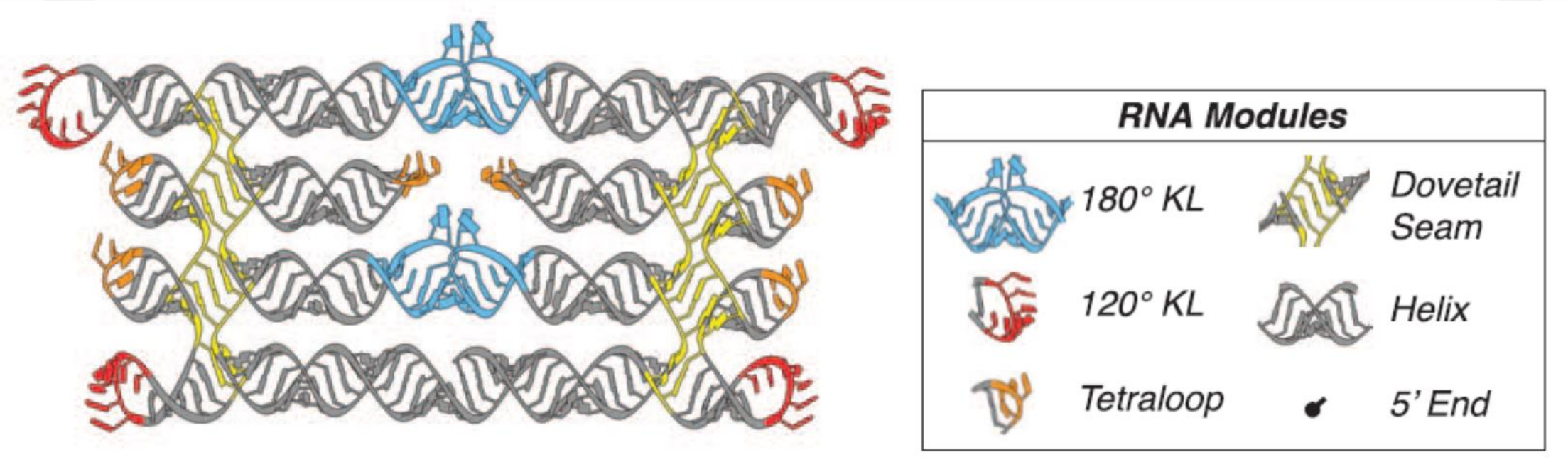
Kissing loop



Pseudoknot

RNA origami

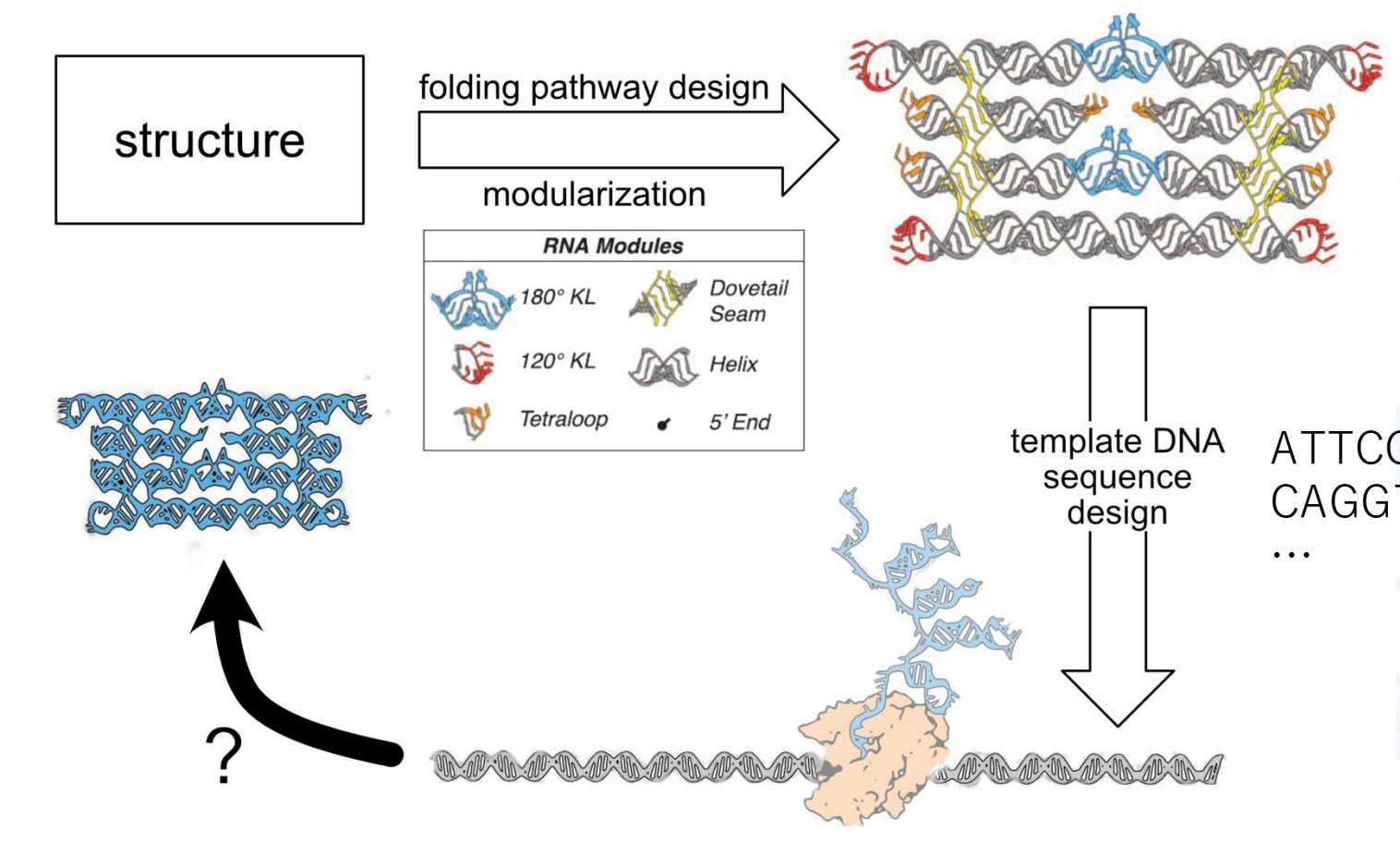
modular design of an RNA tile



Geary, Rothemund, and Andersen, *Science* **345** (6198): 799-804, 2014

RNA origami

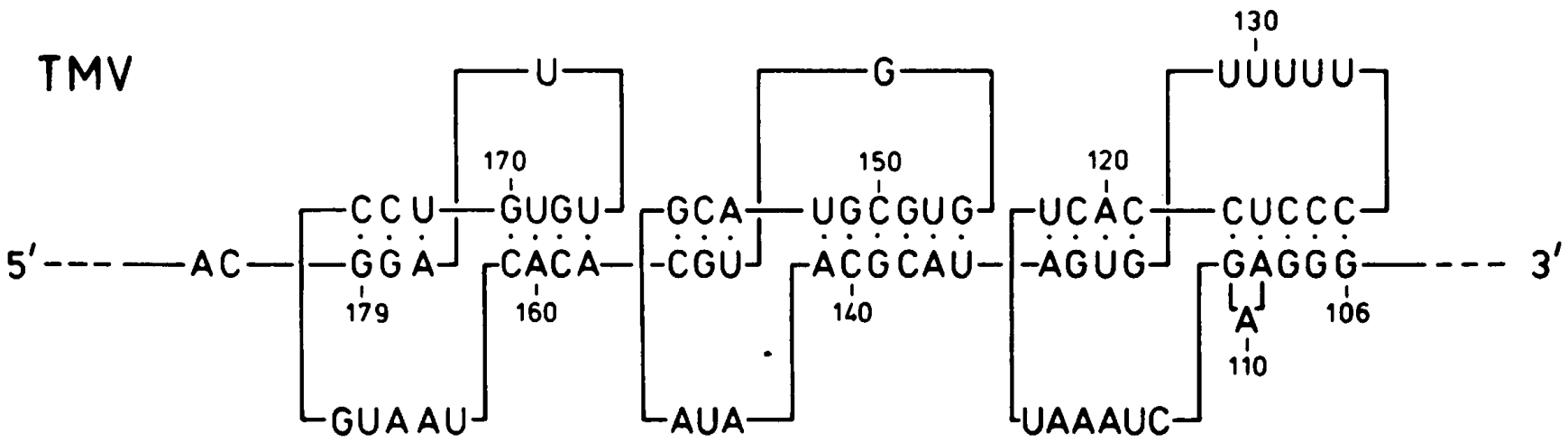
hard-coded CF



Geary, Rothemund, and Andersen, *Science* **345** (6198): 799-804, 2014

Helix

co-axially stacked into a viral backbone

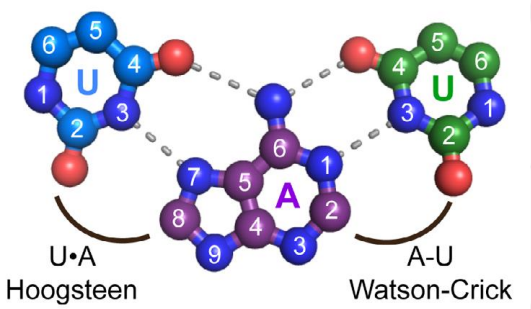


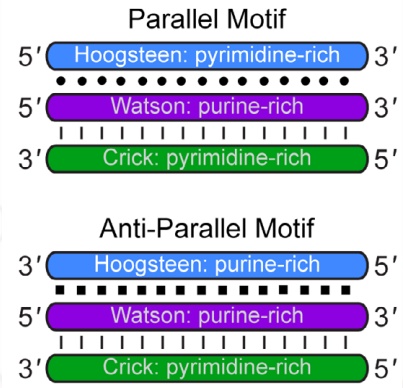
Helix

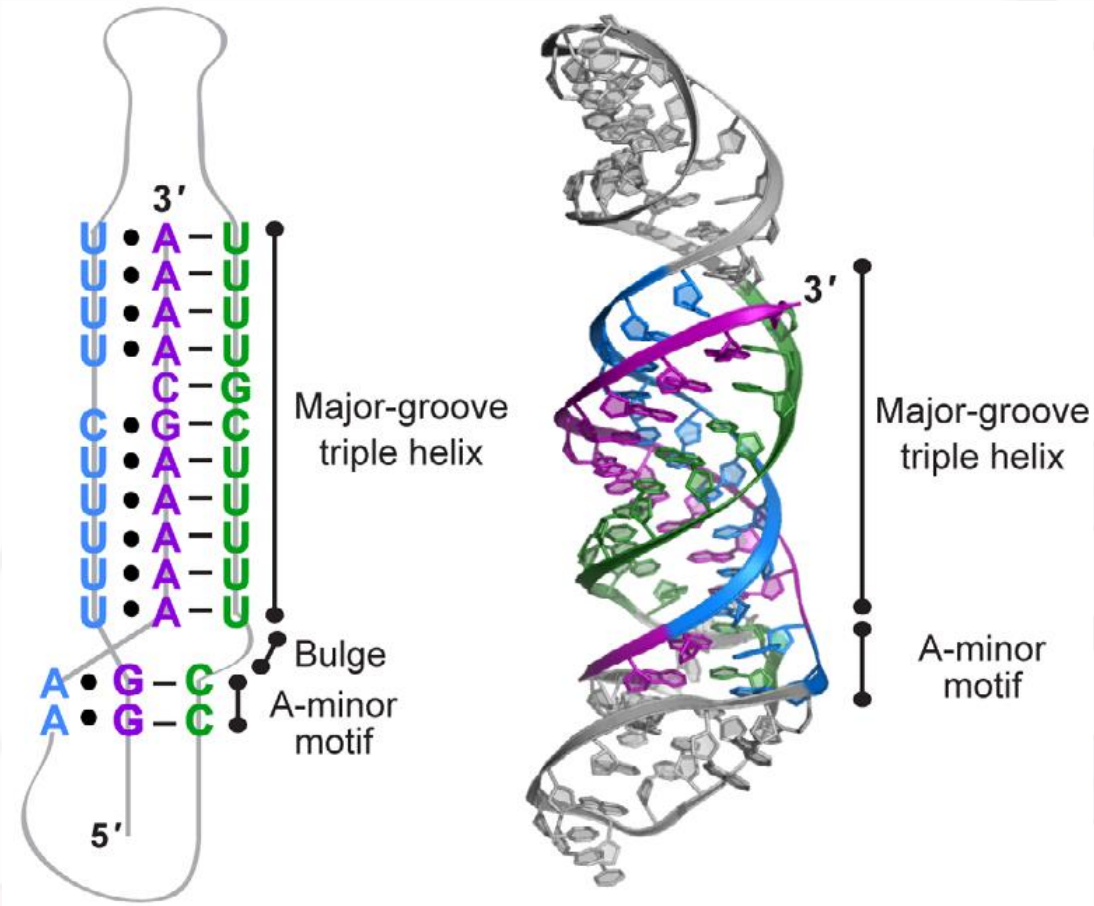
RNA triple helix

RNA sequences are capable of keeping their 3'-end away from their 5'-end, thus,

- folding into non-tree structures, transcending the bound of CFL;
- avoiding to be degraded by ribonuclease (RNase).



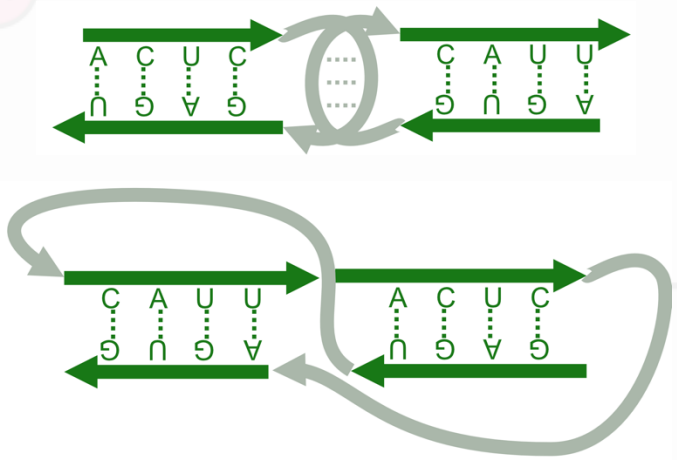




J. A. Brown. *WIREs RNA*. 2020;11:e1598

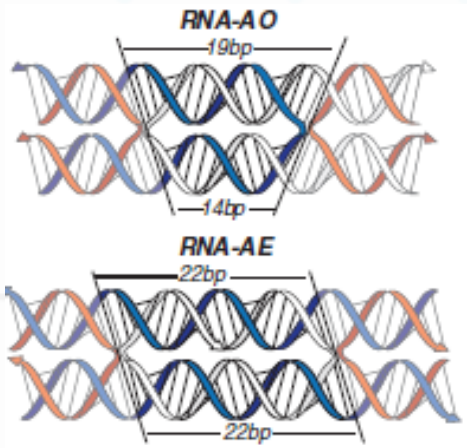
RNA Origami to Oritatami

Co-axial stackings



+

Cross-over motifs

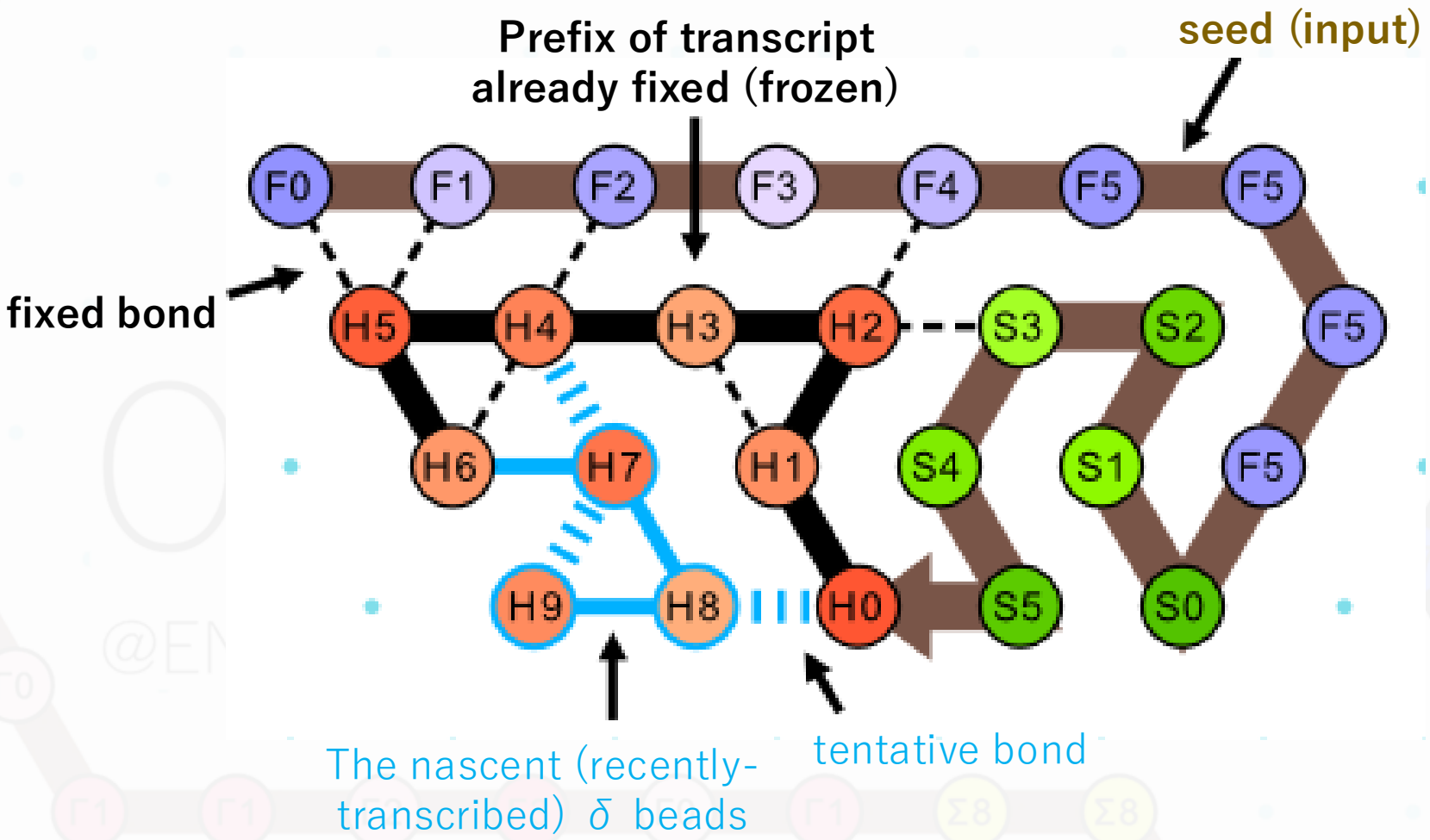


=

Quasi-2D engineering of co-transcriptional folding

Oritatami

model of CF-driven computing

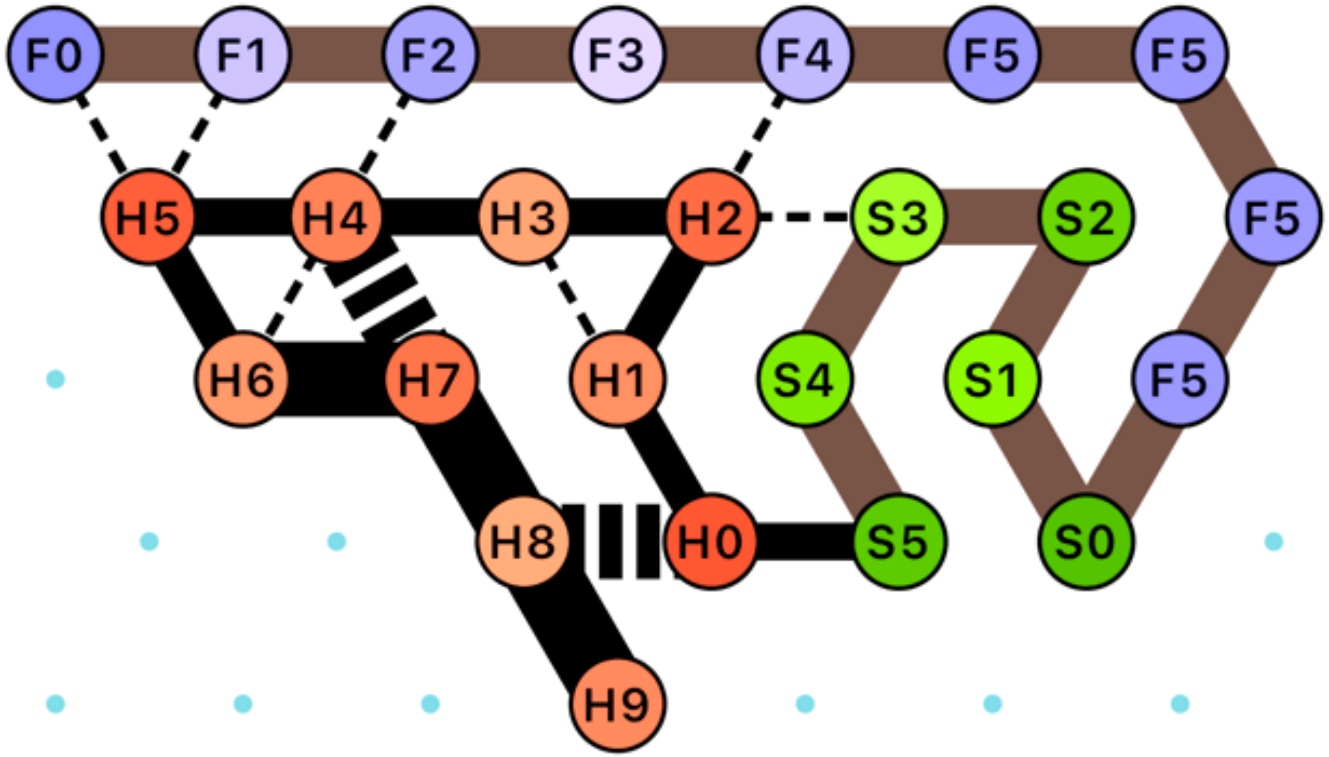


Geary et al. **MFCS2016**: 43:1-43:14

Oritatami

model of CF-driven computing

The nascent fragment tries to fold with as many bonds as possible.

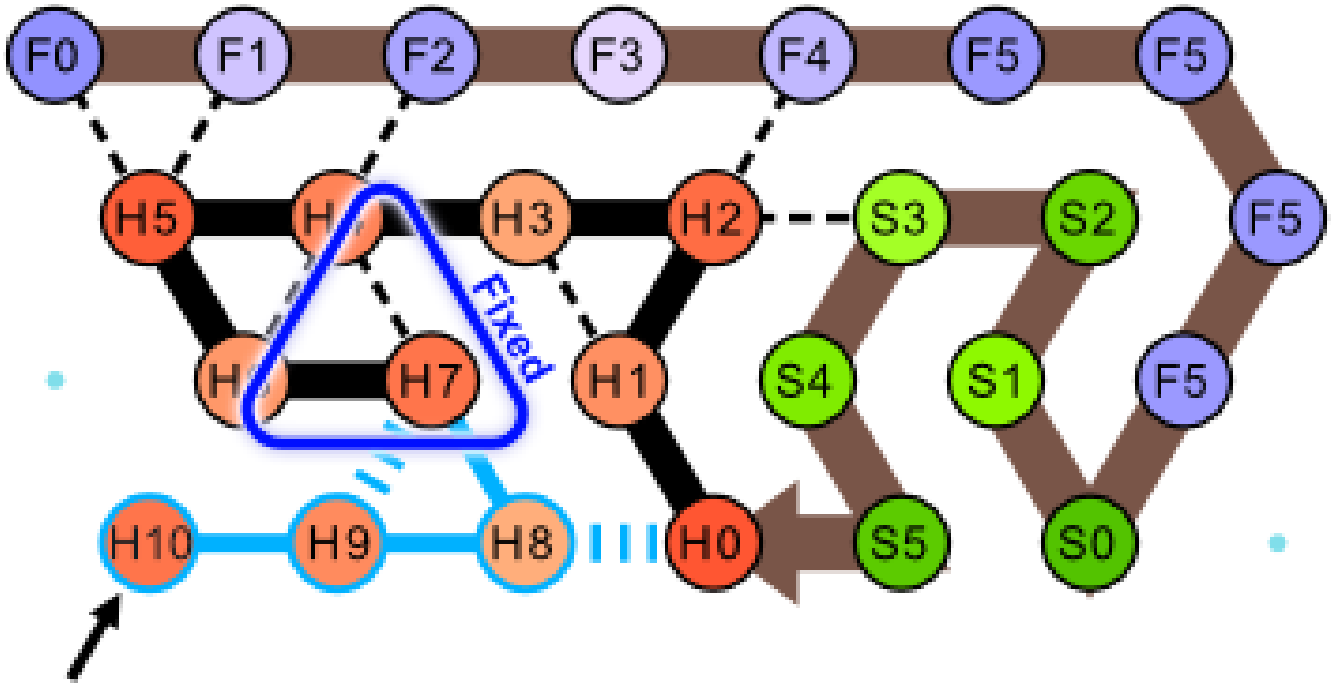


Geary et al. **MFCS2016**: 43:1-43:14

Oritatami

model of CF-driven computing

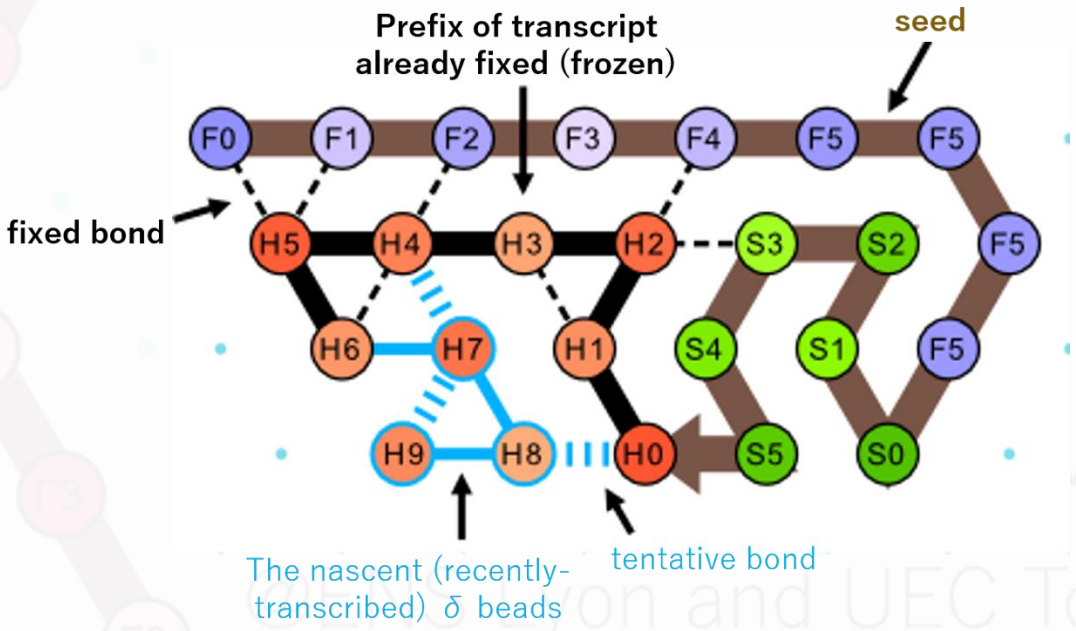
If there are more than one way of fixing H7 point-wise or bond-wise, then **nondeterminism**.



Transcribed! (according to the **preprogrammed** transcript)

Oritatami

model of CF-driven computing



An oritatami system consists of

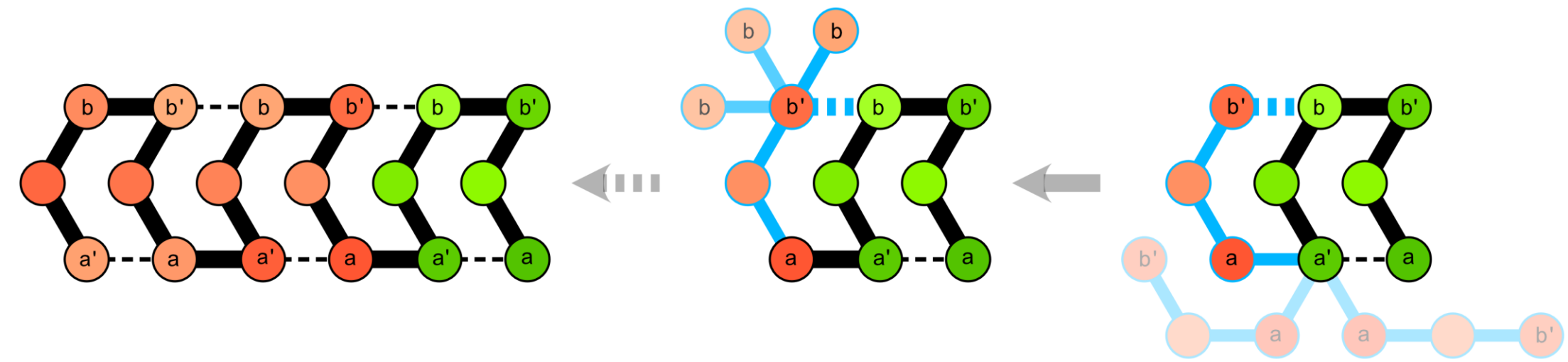
- A finite set Σ of types of abstract molecule (*bead*).
- $w \in \Sigma^*$ (*transcript*)
- $R \subseteq \Sigma \times \Sigma$ (*affinity/ binding rule*)
- δ (*delay*)
- α (*arity*), max # of bonds per bead, formed on the first-come-first-served basis

States by definition!? No! Implement them if needed.

Oritatami

Glider, a self-standing motif

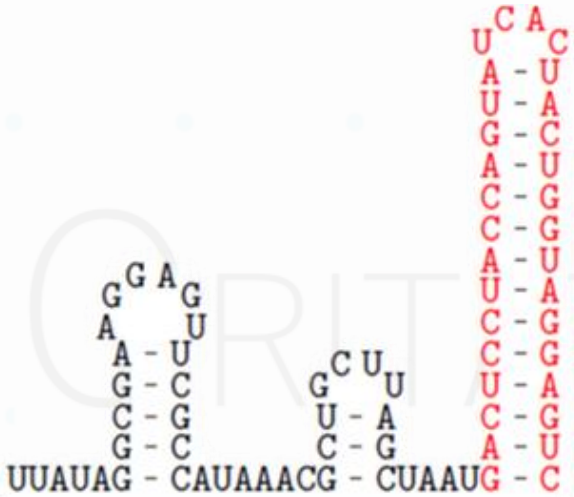
With $\delta = 3$, arbitrary arity $\alpha \geqq 1$, $R = \{(a, a'), (b, b')\}$, the periodic transcript a-•-b'-b-•-a'-a-•-⋯ folds into the self-standing *glider* motif.



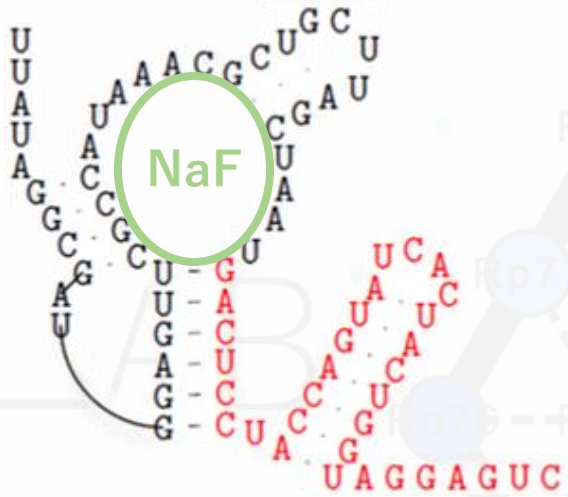
Oritatami

context-sensitive folding

A transcript can fold differently, depending on what are around (environment).



Terminator stem folded
(0mM NaF)



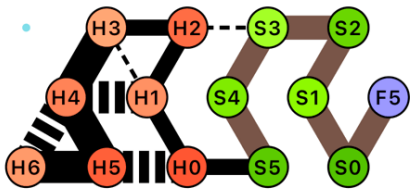
Terminator stem formation
failed (10mM NaF)

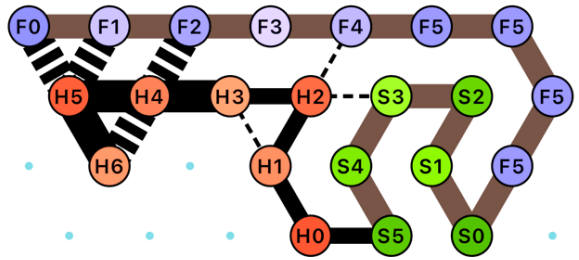
Watters et al. *Nat. Struct. Mol. Biol.* 23(12), pp.1124-1131, 2016

Oritatami

context-sensitive folding

A transcript can fold differently, depending on what are around (environment).





Oritatami

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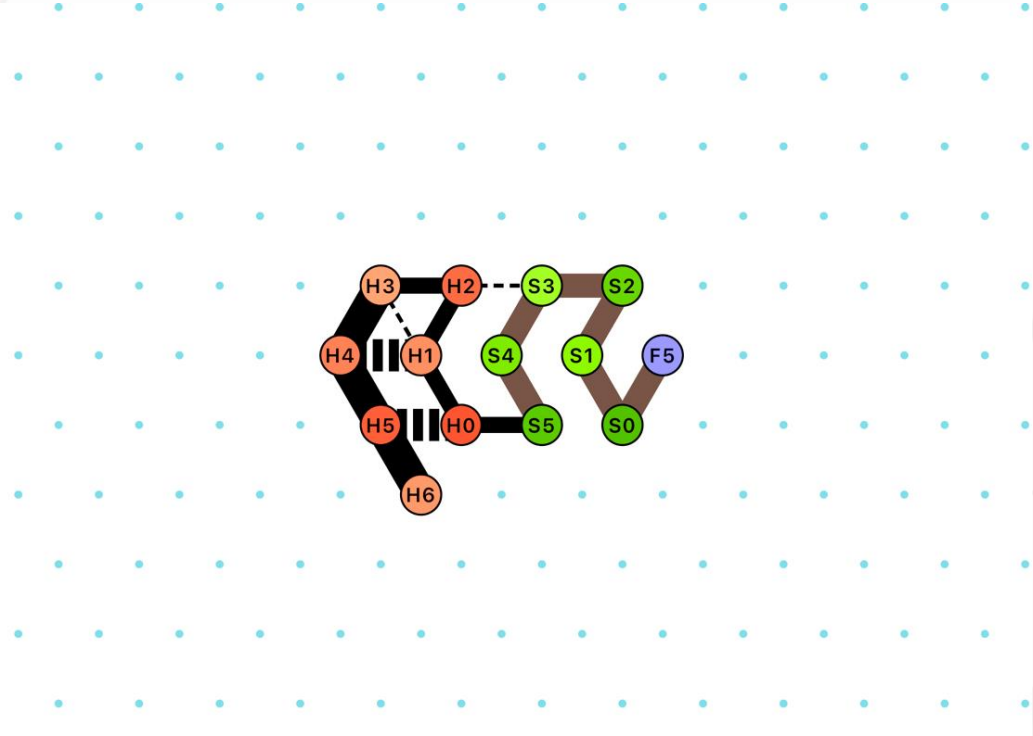
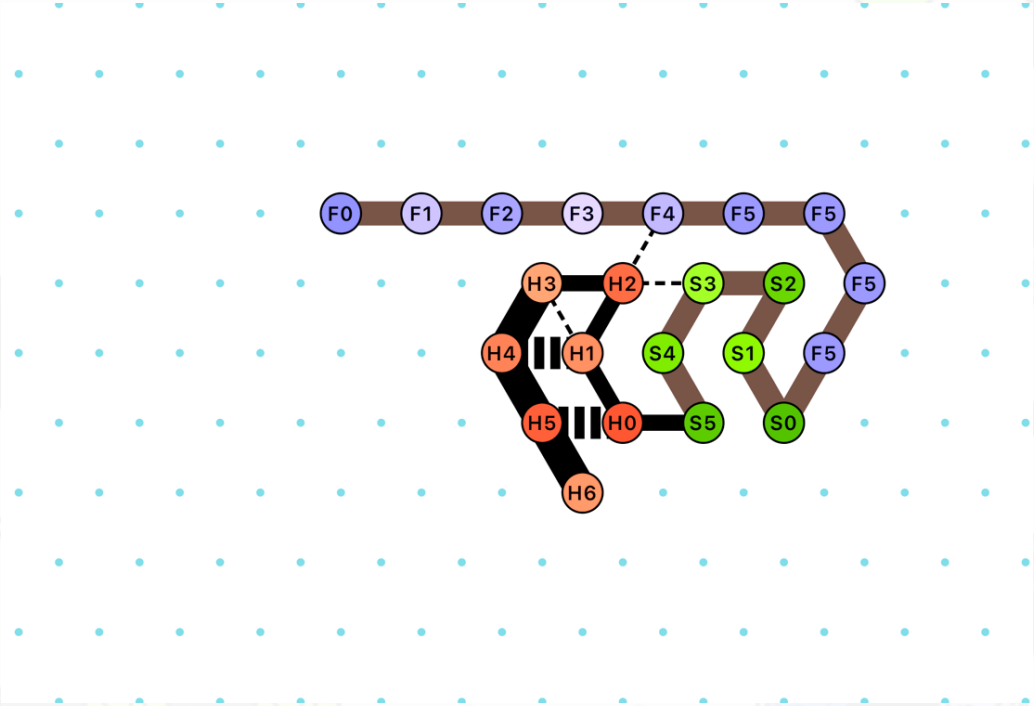
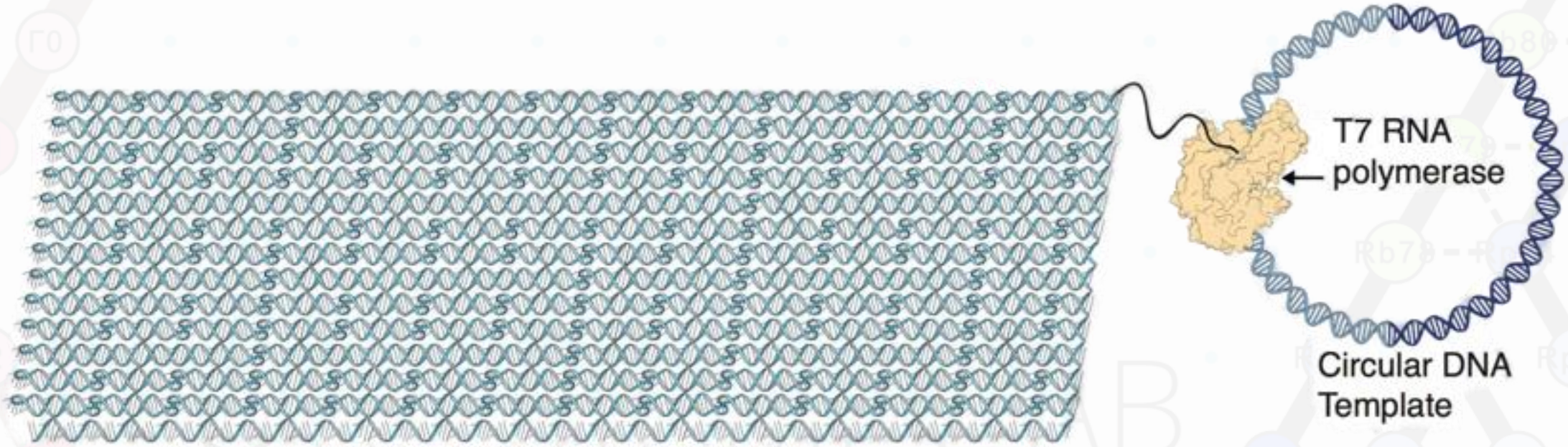




Homo-polymeric CF-driven computing



Complex structures are often made of simple identical (homo-) units (polymers) in nature.

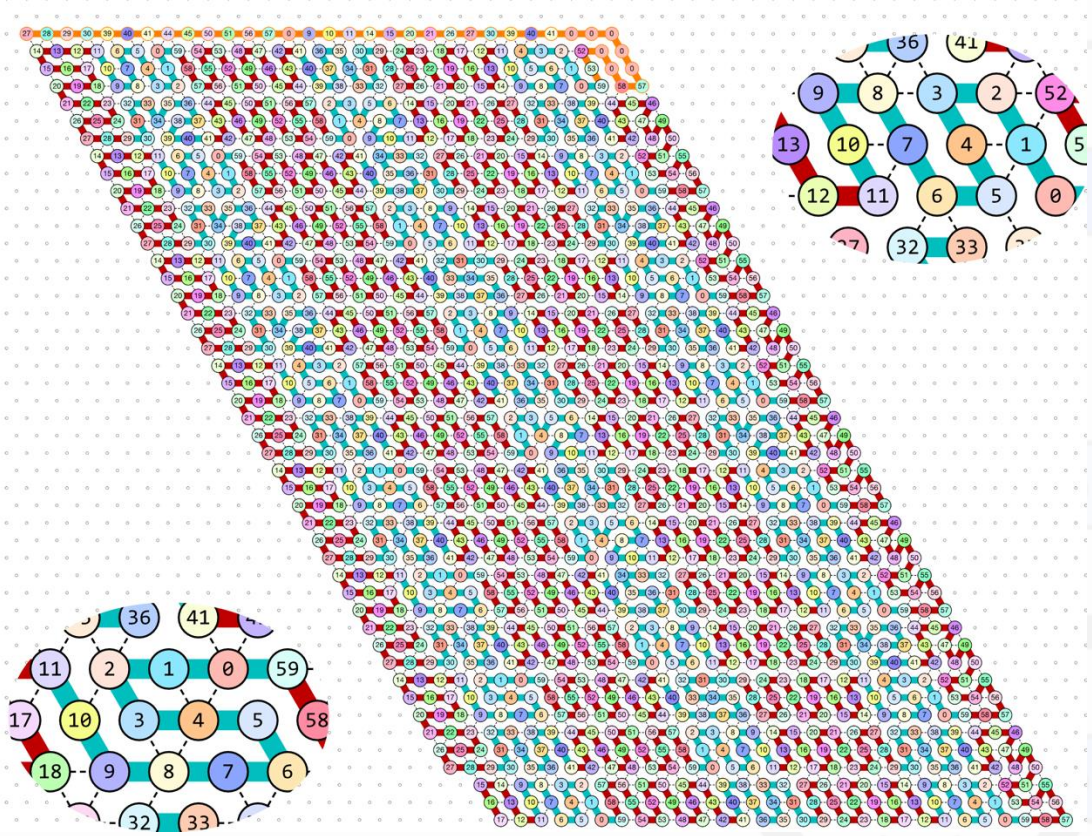
A periodic RNA transcript (homo-polymer) can be transcribed from a circular DNA.

Geary & Andersen, **DNA2014**: 1-19

Homo-polymeric CF-driven computing

Zigzag binary counter

- The first oritatami implementation
- Fixed bit-width (3 in the right figure), but later endowed with capability to widen by 1 bit at every overflow
- A transcript is of period 60 as **0-1-⋯-11-12-⋯-29-30-⋯-41-42-⋯-59-0-1-⋯**.
- Increment by 1 per zigzag.
- The factors **0-⋯-11** and **30-⋯-41** serve as a half-adder by folding into one of 4 possible conformations, depending on what are around.

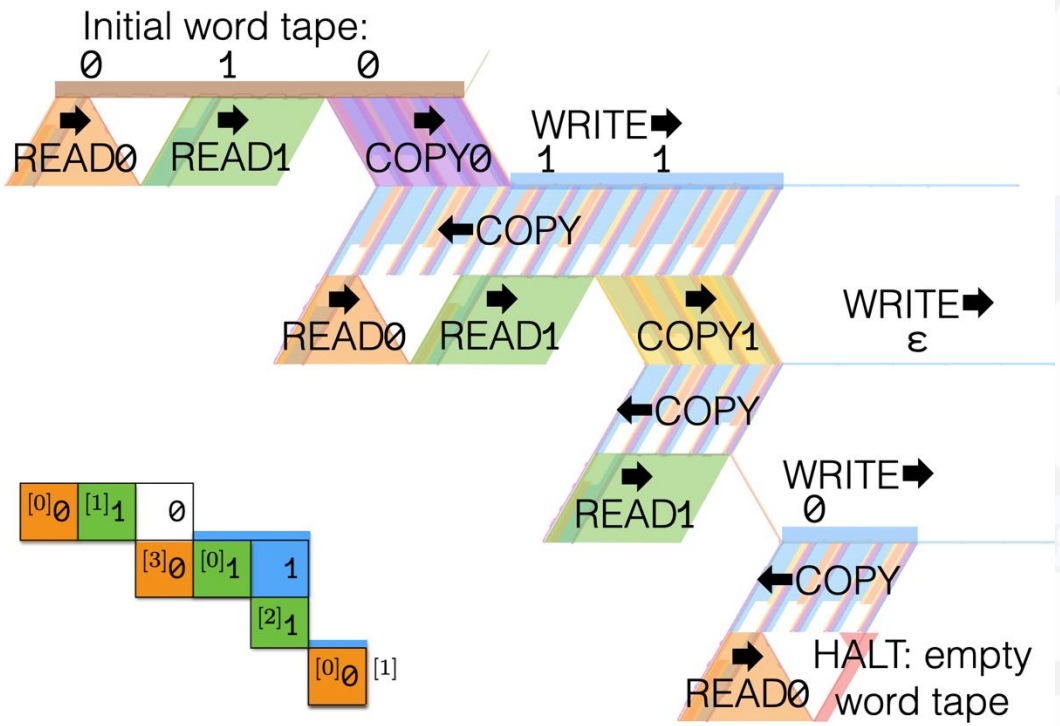


Geary et al. **MFCS 2016**. 43:1-43:14

Homo-polymeric CF-driven computing

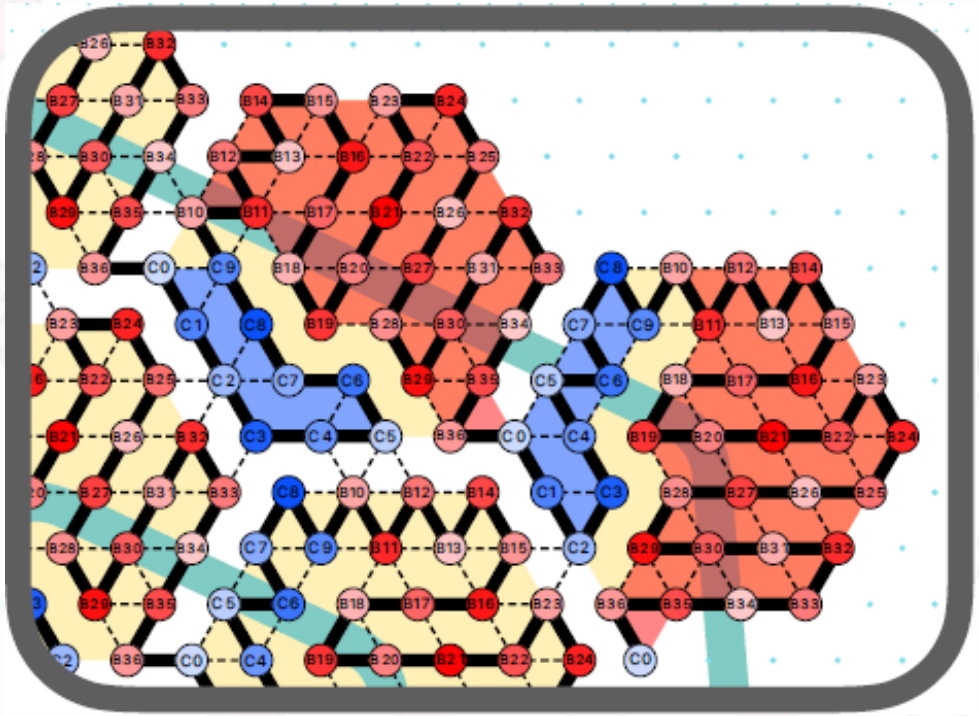
Turing-universality

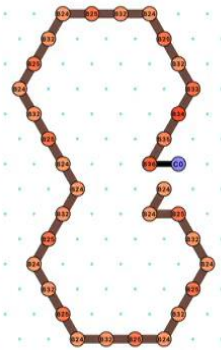
- Cyclic tag system (cts) is a binary rewriting system made of a cyclic list of $u_0, u_1, \cdots, u_{k-1} \in \{0, 1\}^*$ and a pointer $0 \leqq p < k$. It rewrites a word $w = a_1 a_2 \cdots a_n$ as:
  1. temp $= a_2 \cdots a_n$ (a_1 is deleted from w)
  2. If $a_1 = 1$, temp = temp $\cdot\, u_p$
  3. $w =$ temp
  4. p++ (mod k)



Homo-polymeric CF-driven computing

Tessellation by a transcript of period 37 at delay 3





Turedo

(Tur[-ing] + [Ter-]edo [navilis])

Programming language for CF

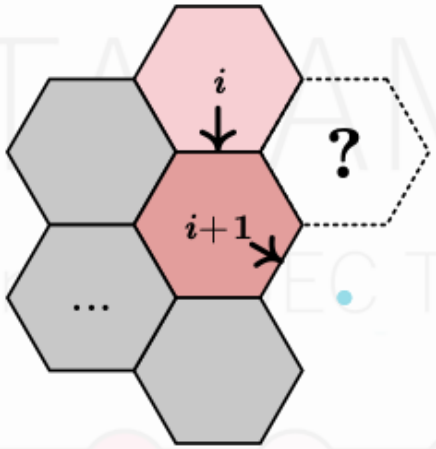
Turedo

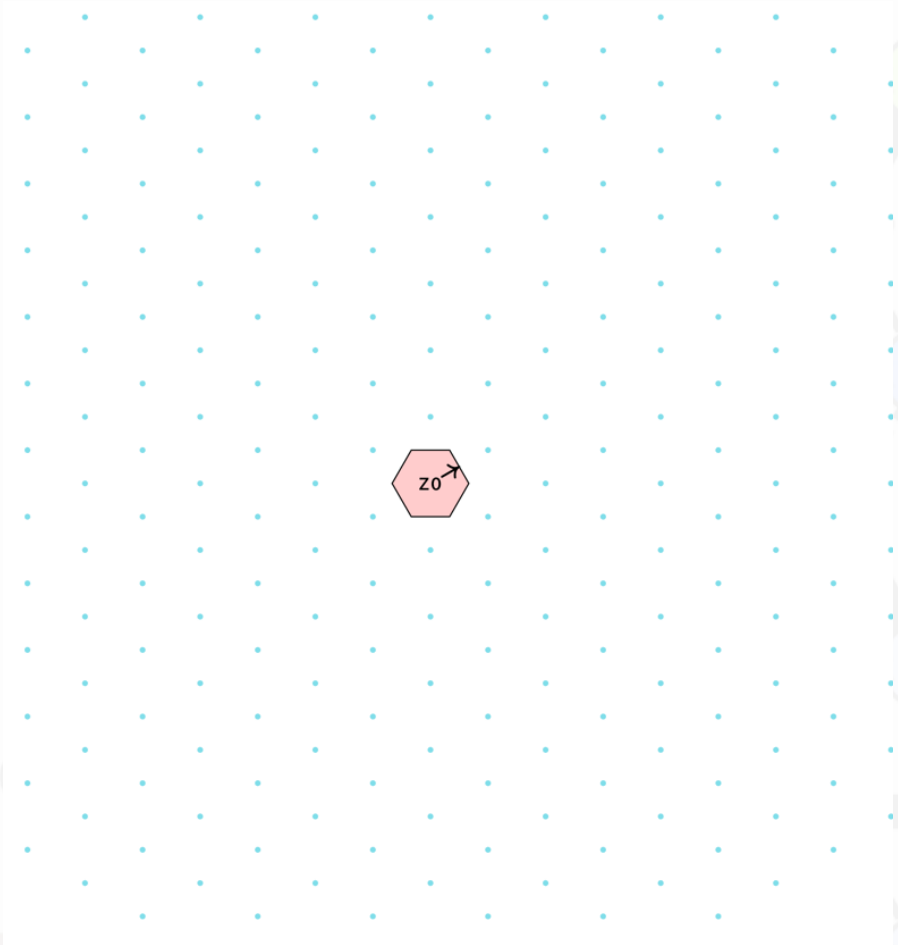
2D Turing machine on the hex grid that is *self-avoiding*, that is,

- Once visited, a cell won't be visited again.
- According to the configuration within the radius-r from its head, it colors the current cell and decides which neighbor to visit next, where r is a system parameter.

Example of radius-1 Turedo

(mod-4 clockwise walker)





Turedo

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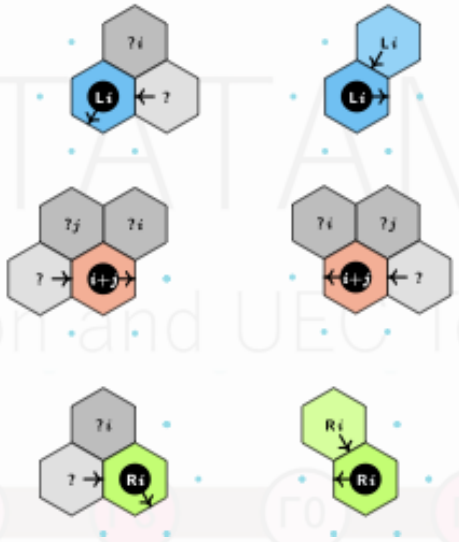
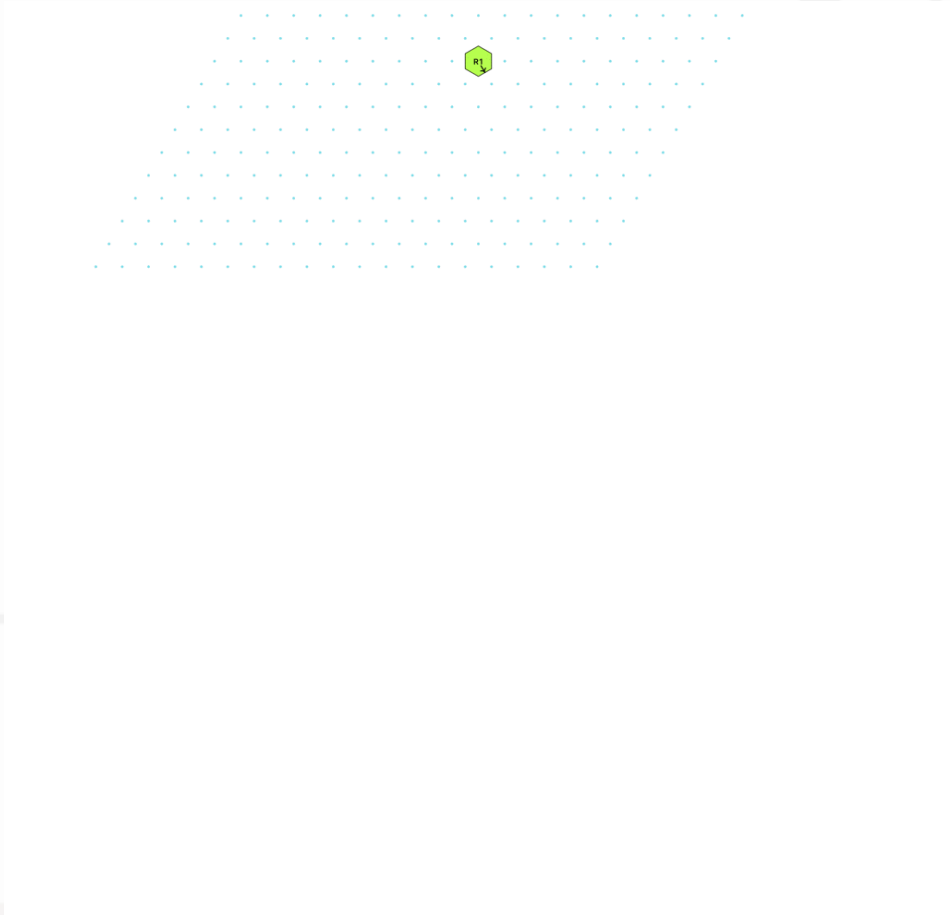
Example of radius-1 Turedo

(Sierpinski triangle)



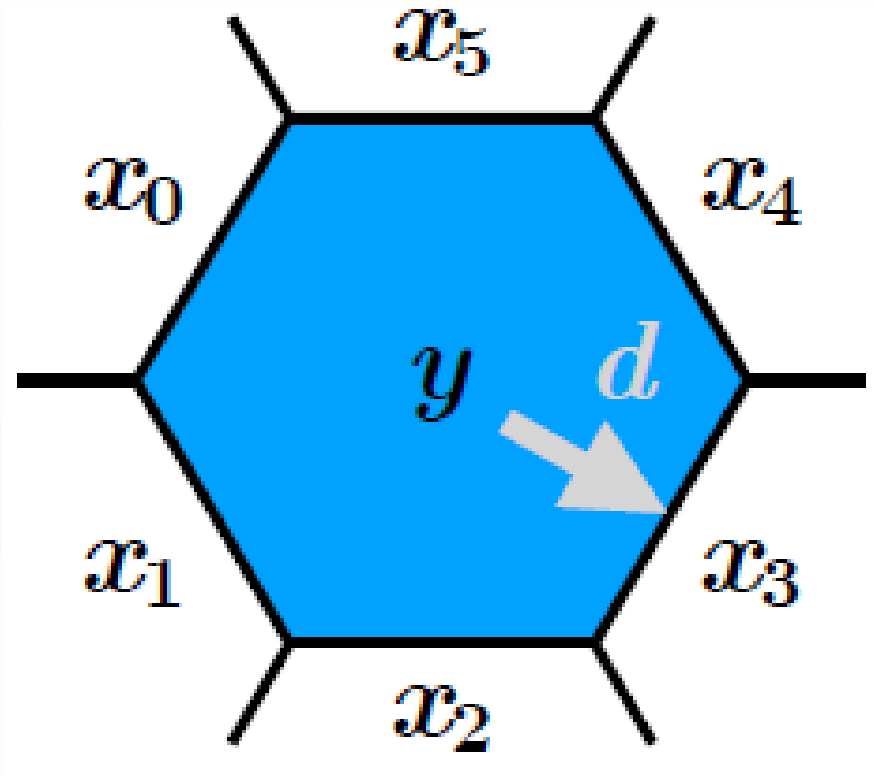


Pchelina et al. **STACS2022**: 51:1-51:23

Turedo

- A radius-1 stateless Turedo $T = (A, \delta)$ is a pair of a tape alphabet A including the blank $\perp$ and a transition function $\delta\colon A^6 \to A \times \{\mathrm{BR}, \mathrm{FR}, \mathrm{S}, \mathrm{FL}, \mathrm{BL}\}$.

- Suppose T has come from north, where x_5 is written. If $\delta(q, x_0, x_1, x_2, x_3, x_4, x_5) = (a, d)$, then it
  1. writes $a \in A$ at the current cell, and
  2. moves to the neighbor cell in the direction d, <u>as long as the cell is still blank</u>; otherwise, it halts.



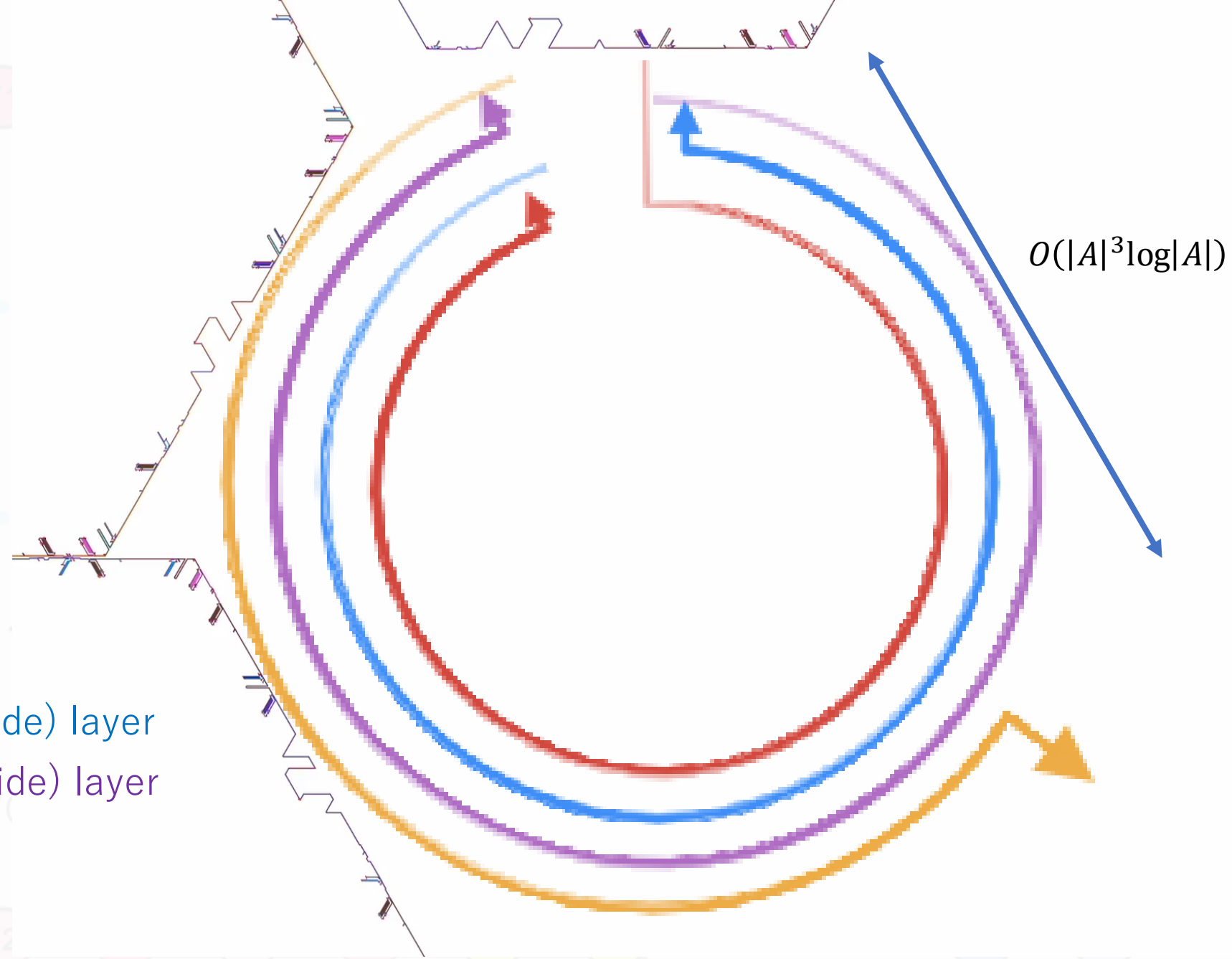
Turedo

Turedo-to-Oritatami Compiler Theorem.

A radius-1 stateless Turedo $T = (A, \delta)$ can be programmed into a transcript w of period $O(|A|^6 \log|A|)$ with which the deterministic delay-3 oritatami system $(\Sigma, w, R, 3, 6)$ simulates T intrinsically, where

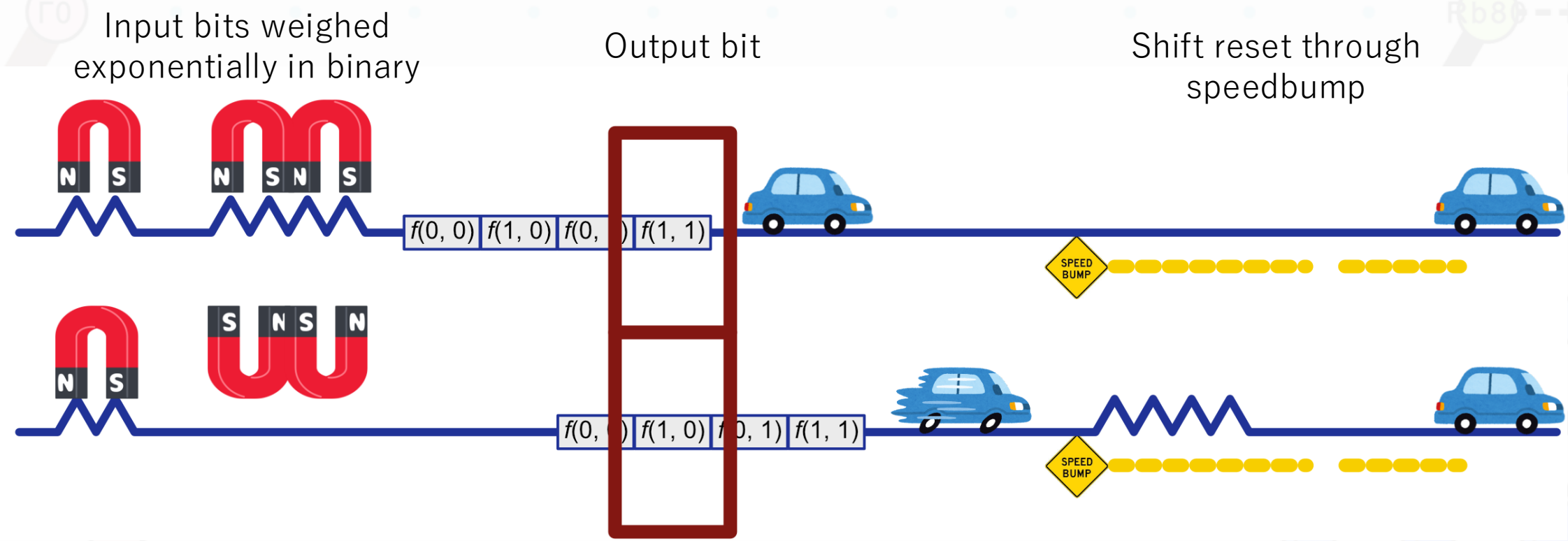
- Σ is universal, that is, independent of T, and consists of 1753 bead types.
- R is also universal.
- Each period of w folds into a **macrocell** of side length $O(|A|^3 \log|A|)$.

Macrocell



1. Scaffold layer
2. Read ($\log|A|$ bits/side) layer
3. Write ($\log|A|$ bits/side) layer
4. Exit layer

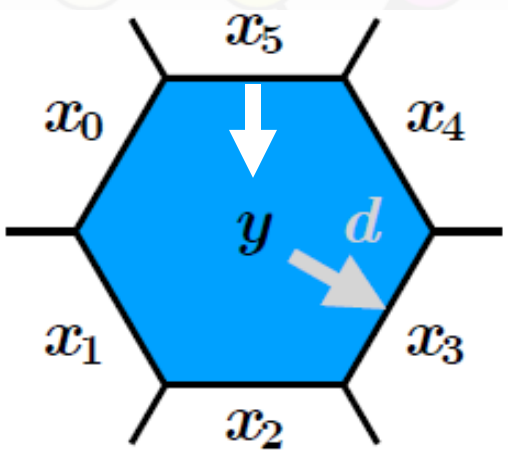
Shift-driven computing



$6 \log|A|$ bits → Max offset $O(|A|^6)$ → Length $O(|A|^6)$

Pchelina et al., **LATIN2020**, pp.425-436.

Period of transcript



A Turedo $T = (A, \delta)$ is encoded in the period of a transcript as:

Scaffold → **Read** → **Write** → **Speedbump** → **Exit** → Scaffold → ⋯

Scaffold hardcodes the macrocell's skeleton

Read $\boldsymbol{x} = (\boldsymbol{x_0}, \boldsymbol{x_1}, \boldsymbol{x_2}, \boldsymbol{x_3}, \boldsymbol{x_4}, \boldsymbol{x_5})$ with $x_s = (b_{s,0}, b_{s,1}, \dots, b_{s,\log|A|-1})$

- Weigh-sums the bits in $x_0, x_1, \dots\ x_5$ in this order as:

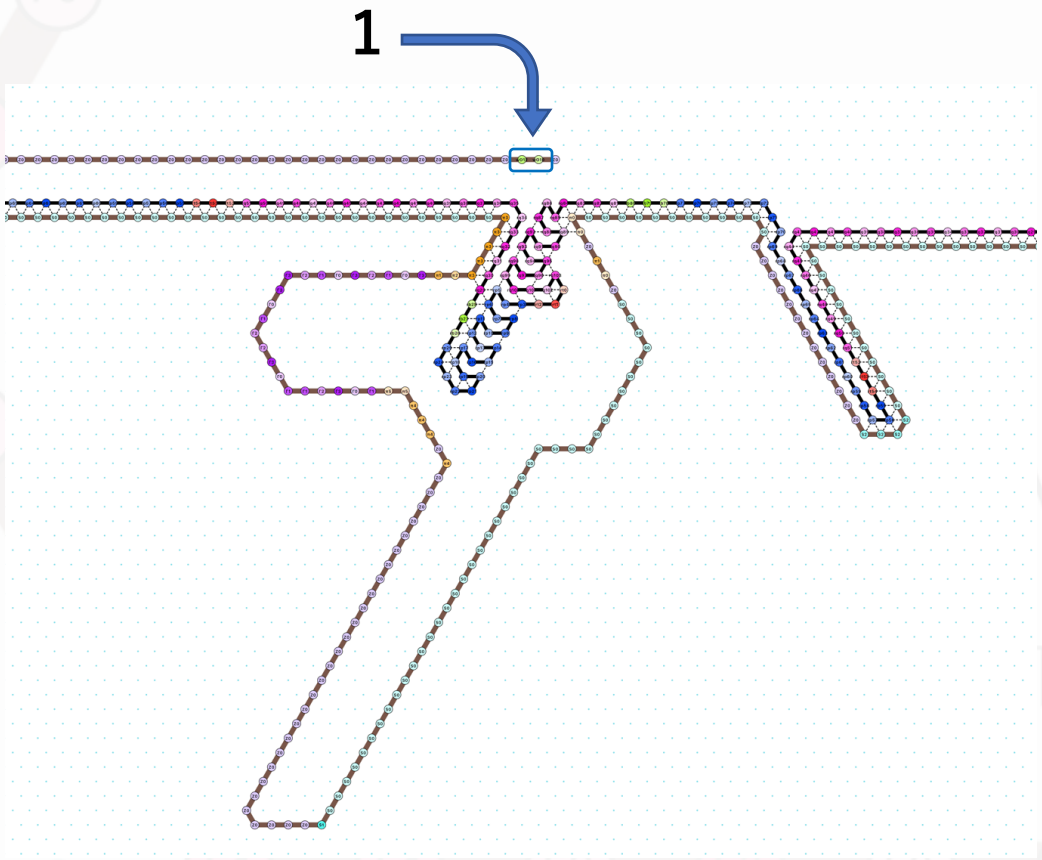
$$\Delta(x) = \sum_{s=0}^{5} \sum_{i=0}^{\log|A|-1} b_{s,i} 2^{s\log|A|+i}$$

- Pushes the remaining transcript forward by this offset.

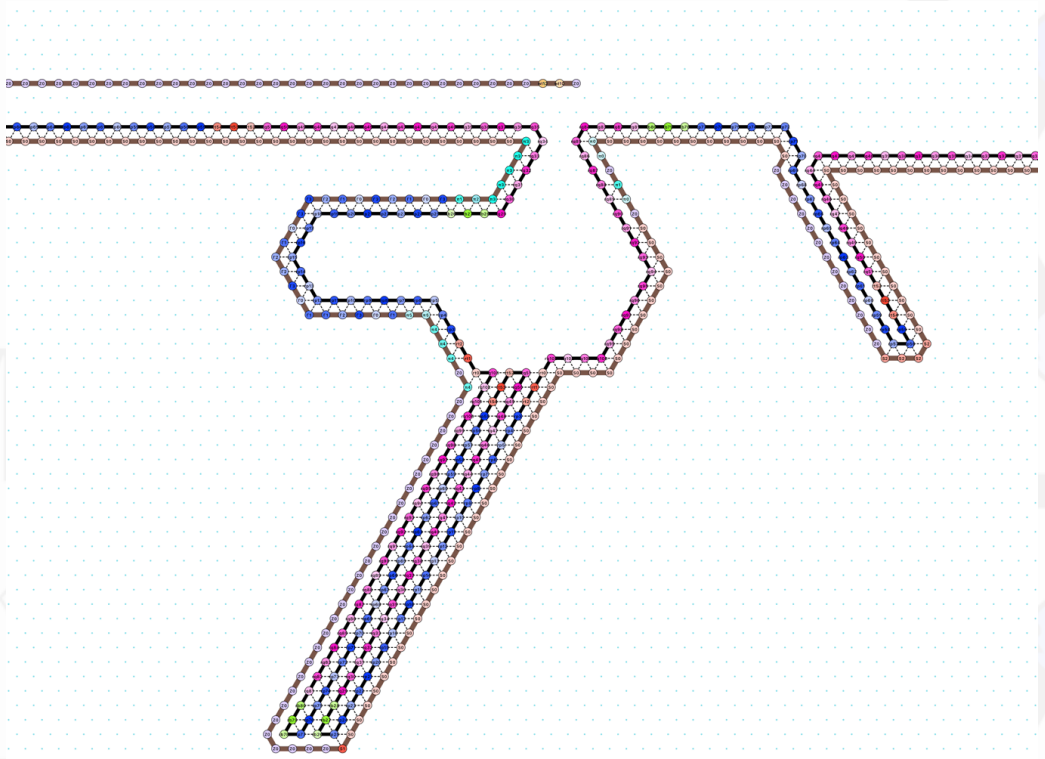
NOTE. these bits must have been written in a **uniform** format. It is uncomputable whether a cell will be visited; let alone from which direction it will be entered.

Read ↺: Reading pockets

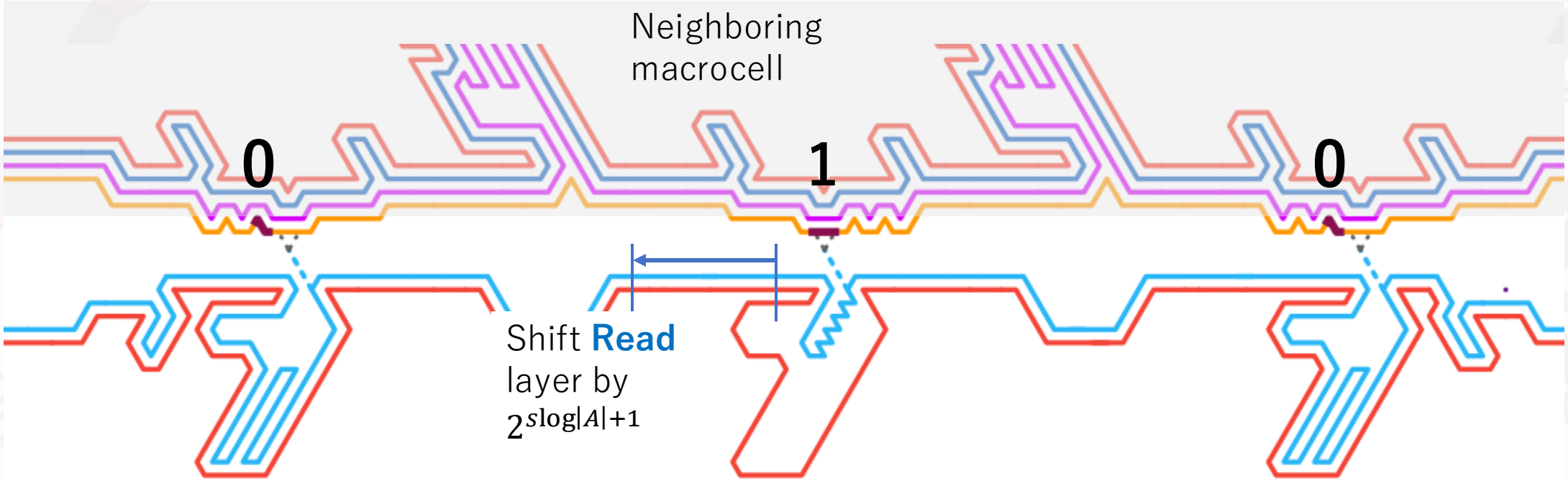
OK, readers, it's you who weighs bits!!



Otherwise



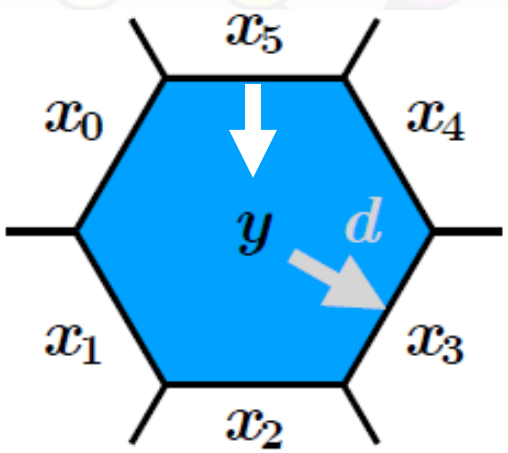
Read ↺: Reading pockets



Capacities (Pocket size) : $2^{s\log|A|+2}$ > $2^{s\log|A|+1}$ > $2^{s\log|A|+0}$

If the j-th bit is 1, then $\Delta(x) \mathrel{+}= 2^{s\log|A|+j}$

Period of transcript



A Turedo $T = (A, \delta)$ is encoded in the period of a transcript as:

Scaffold → **Read** → **Write** → **Speedbump** → **Exit** → Scaffold → ⋯

Write a and d (letter & direction)

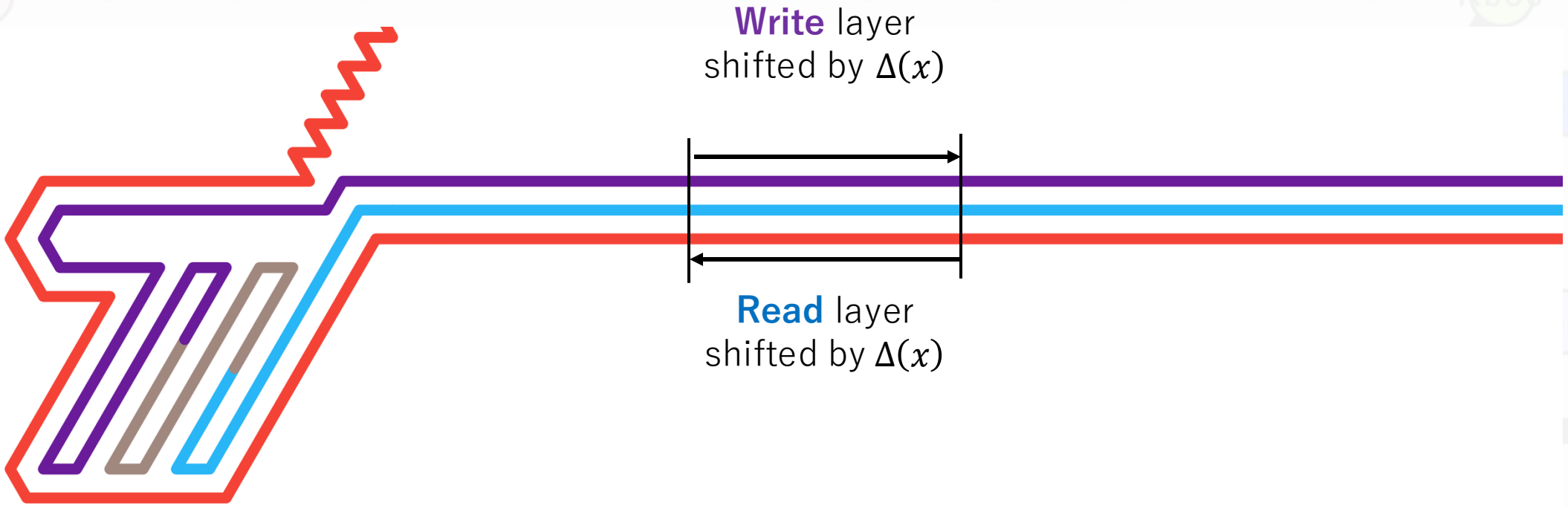
- All the transition tables for each bit and for exit-direction are encoded: $|A|^6$ entries per table.
- This layer is shifted by $\Delta(x)$ so that only the referred entry by x of each table is exposed at a position readable later while the others are "hidden."
- Outputs must be in a uniform format along all the sides.

Speedbump absorbs $\Delta(x)$

Exit at the side $d(x)$ specified by **Write**

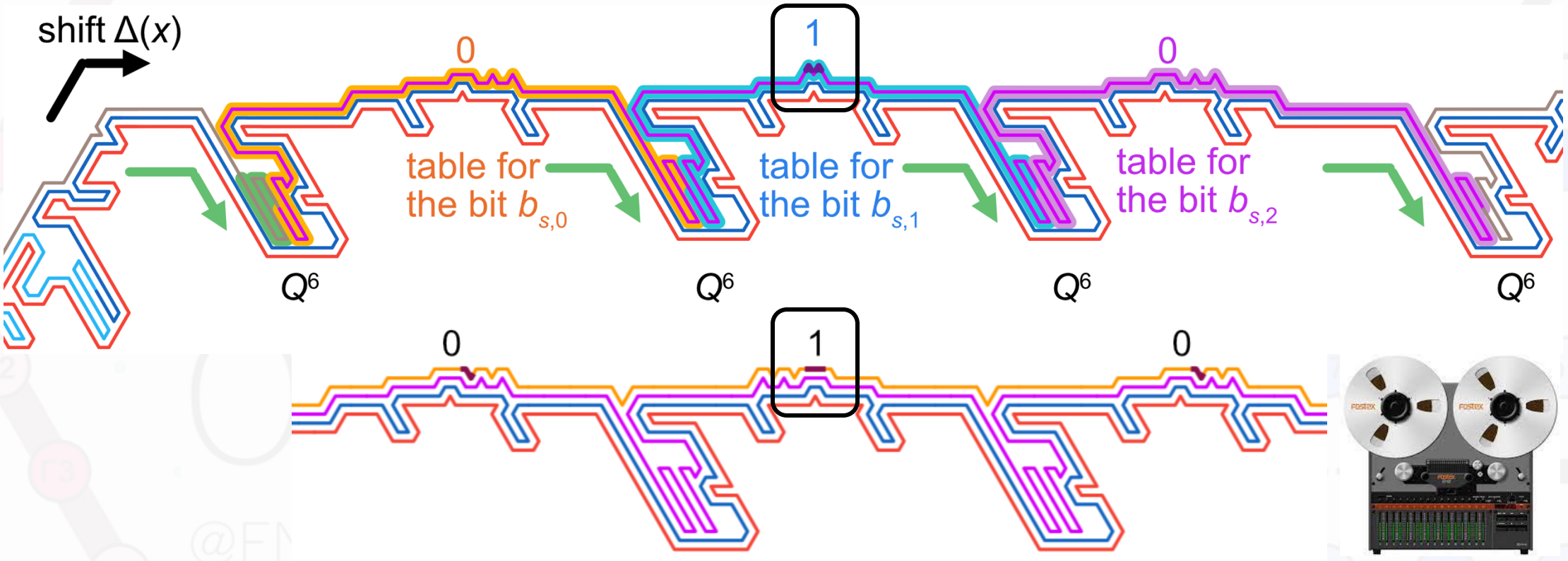
Read ↺ to Write ↻: U-turn pocket

The shift is transferred from **Read** to **Write**.



Capacity: $|A|^6$

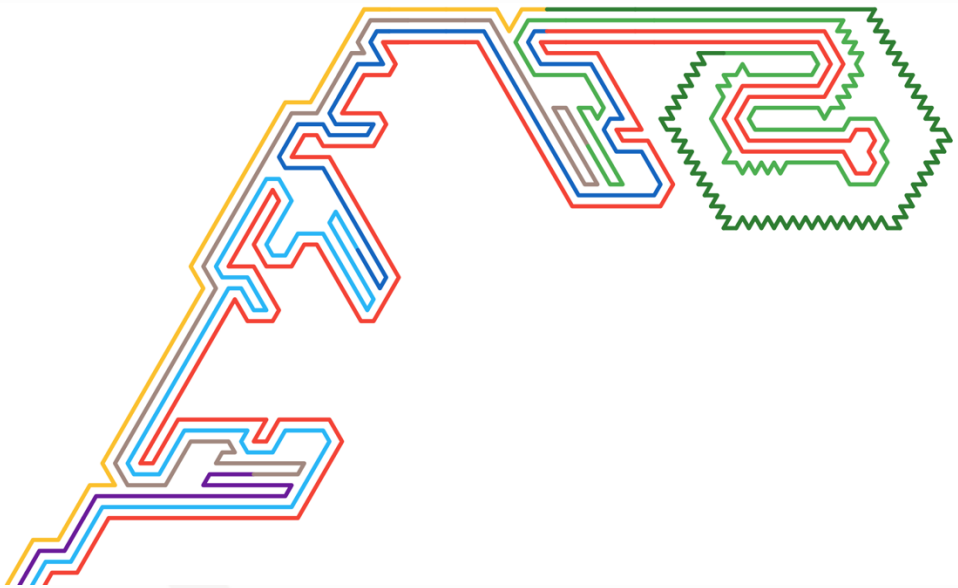
Write ↻: tables shifted by $\Delta(x)$

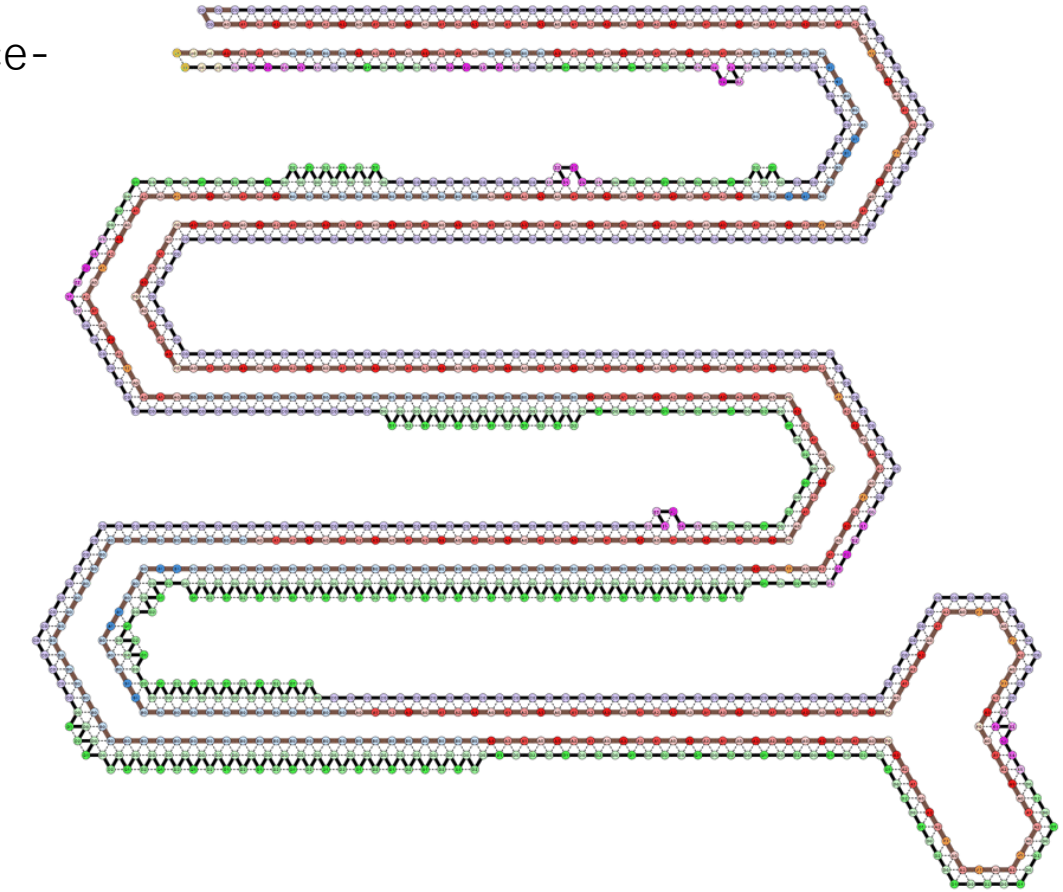


NOTE: The system knows in the stage of programming tables for each x, whether each bit will be covered by **Exit** according to the exit direction $d(x)$.

“Foldable” Speedbump: absorbing $\Delta(x)$

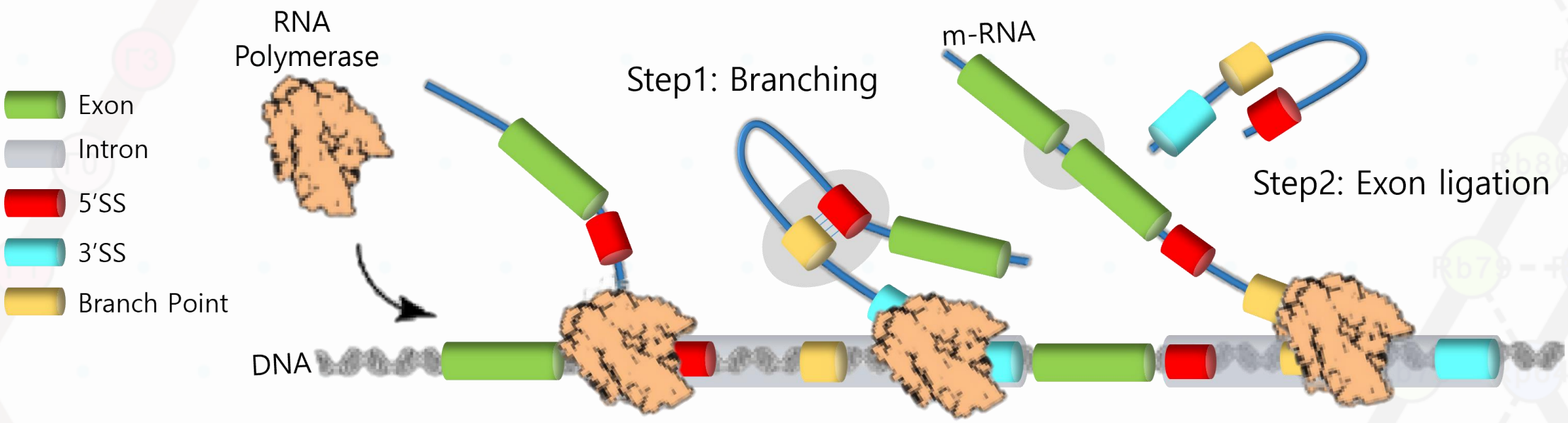
Based on the straight speedbump [PchelinaSSU20] but quadratically more space-efficient.





RNA Spinner

In vitro/vivo auto-synthesis of RNA components by NFA



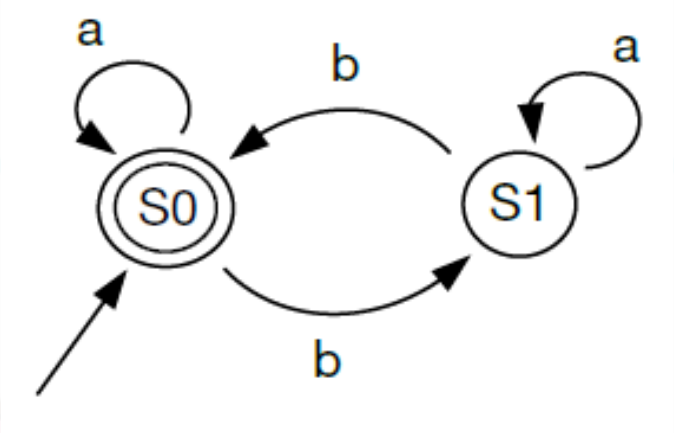
Co-transcriptional splicing

$uxs\ell\theta(s)yv \to uv$ if

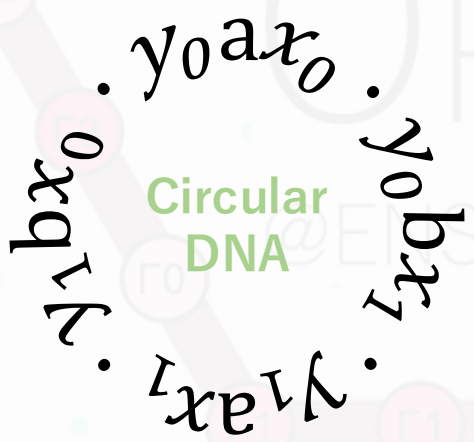
1. (x, y) is an enzymatically-recognizable context and
2. $s\ell\theta(s)$ is a *stable* hairpin, where θ is an antimorphic involution.

RNA spinner

An NFA for a *superset* of R.



encoding



Co-transcriptional splicing

Any molecular system consists of *finitely many* (*kinds*) of DNA and RNA sequences. Let R be the *finite* set of RNA sequences it involves.

Recall that RNAs are naturally degraded.

$$y_0 a\overrightarrow{x_0} \cdot y_0 b x_1 \cdot y_1 a x_1 \cdot y_1 b x_0 - \overleftarrow{y_0} a x_0 \cdot y_0 b x_1 \cdots$$

$$\rightarrow \quad y_0 a a\overrightarrow{x_0} \cdot \overleftarrow{y_0} b x_1 \cdot y_1 a x_1 \cdots$$

$$\rightarrow \quad y_0 a a b x_1 \cdot y_1 a x_1 \cdots$$

Problems to be solved for RNA Spinner

Problem 1.

Given R and a finite set D of domains (via which sequences in R interact with each other or with other molecules), construct an NFA A with as *few transitions* as possible s.t.

$$R \subseteq L(A) \subseteq R \cup \overline{\Sigma^* D \Sigma^*}$$

By setting $D = \Sigma$, this problem is reduced to the NP-hard problem of finding a transition-minimal NFA for finite languages [GruberH07]. Such a ubiquitous domain however turns any system into a chaotic soup.

Problems to be solved for RNA Spinner

A hairpin gets less stable with a longer loop and a shorter stem.

Contribution by stem is linear, while it remains open how a loop is penalized.

Problem 2

Propose a proper energy model for RNA hairpin stability, and study hairpin-related operations by considering only stable hairpins in the model.

Table 6. Free-energy increments for loops

Loop size	Internal loop*†	Bulge loop*‡	Hairpin loop*§
1	—	+3.3	—
2	+0.8	+5.2	—
3	+1.3	+6.0	+7.4
4	+1.7	+6.7	+5.9
5	+2.1	+7.4	+4.4
6	+2.5	+8.2	+4.3
7	+2.6	+9.1	+4.1
8	+2.8	+10.0	+4.1
9	+3.1	+10.5	+4.2
10	+3.6	+11.0	+4.3
12	+4.4	+11.8	+4.9
14	+5.1	+12.5	+5.6
16	+5.6	+13.0	+6.1
18	+6.2	+13.6	+6.7
20	+6.6	+14.0	+7.1
25	+7.6	+15.0	+8.1
30	+8.4	+15.8	+8.9

Freier et al., *PNAS* 83, pp.9373-9377, 1986

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References

- [FazekasIKMST24] S. Fazekas, N. Iwano, Y. Kihara, R. Matsuoka, S. Seki, and H. Takeuchi. *Theoretical Computer Science* 999: 114550, 2024
- [FazekasKMST22] S. Fazekas, H. Kim, R. Matsuoka, S. Seki, and H. Takeuchi. **ISAAC2022**, LIPIcs 248, 37:1-37:15
- [GearyMSS16] C. Geary, P-E. Meunier, N. Schabanel, and S. Seki. **MFCS2016**, LIPIcs 58, 43:1-43:14
- [GearyMSS18] C. Geary, P-E. Meunier, N. Schabanel, and S. Seki. **ISAAC2018**, LIPIcs 123, 23:1-23:13
- [GruberH07] H. Gruber and M. Holzer, **LATA2007**, 261-272, 2007
- [MaruyamaS21] K. Maruyama and S. Seki. *Natural Computing*, 20(2), pp.329-340, 2021
- [PchelinaSST22] D. Pchelina, N. Schabanel, S. Seki, and G. Theyssier. **STACS2022**, LIPIcs 219, pp.51:1-51:23
- [PchelinaSSU20] D. Pchelina, N. Schabanel, S. Seki, and Y. Ubukata. **LATIN2020**, LNCS 12118, pp.425-436