Application of DNA self-assembly “from origami to computer”

2019/07/13 Akira Hirose

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Contents

1-1. What is DNA Nanotechnology? (1)

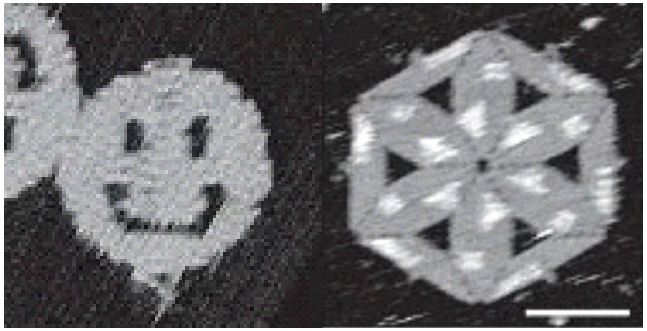
DNA:

- Carrier of genetic information
  in the form of sequence of 4 bases (ATGC)
- DNA →(transcription)→ RNA →(translation)→ proteins

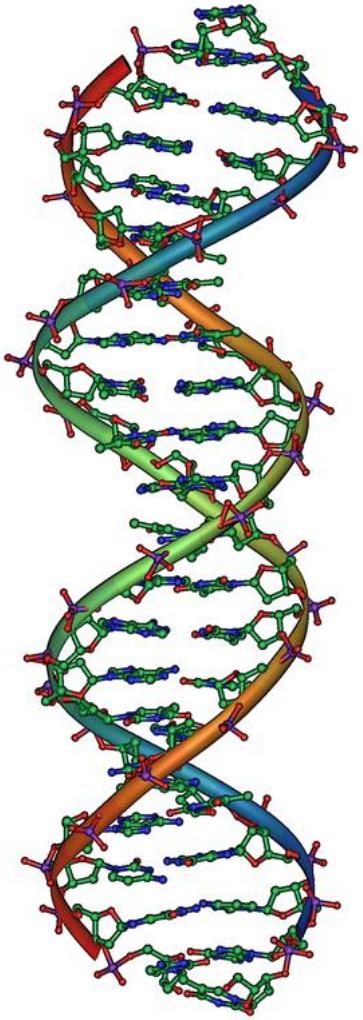
In DNA nanotechnology,

- DNA: non-biological functional molecule
- Various systems or structures are designed in nano scale
  using DNA as engineering materials.

DNA compututer*

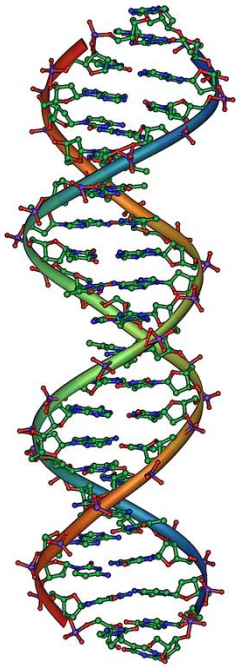


DNA nanostructure



* Please also refer to
  151114_LS_Keisuke_MASUDA_Information_containing_molecules

1-2. What is DNA Nanotechnology? (2)



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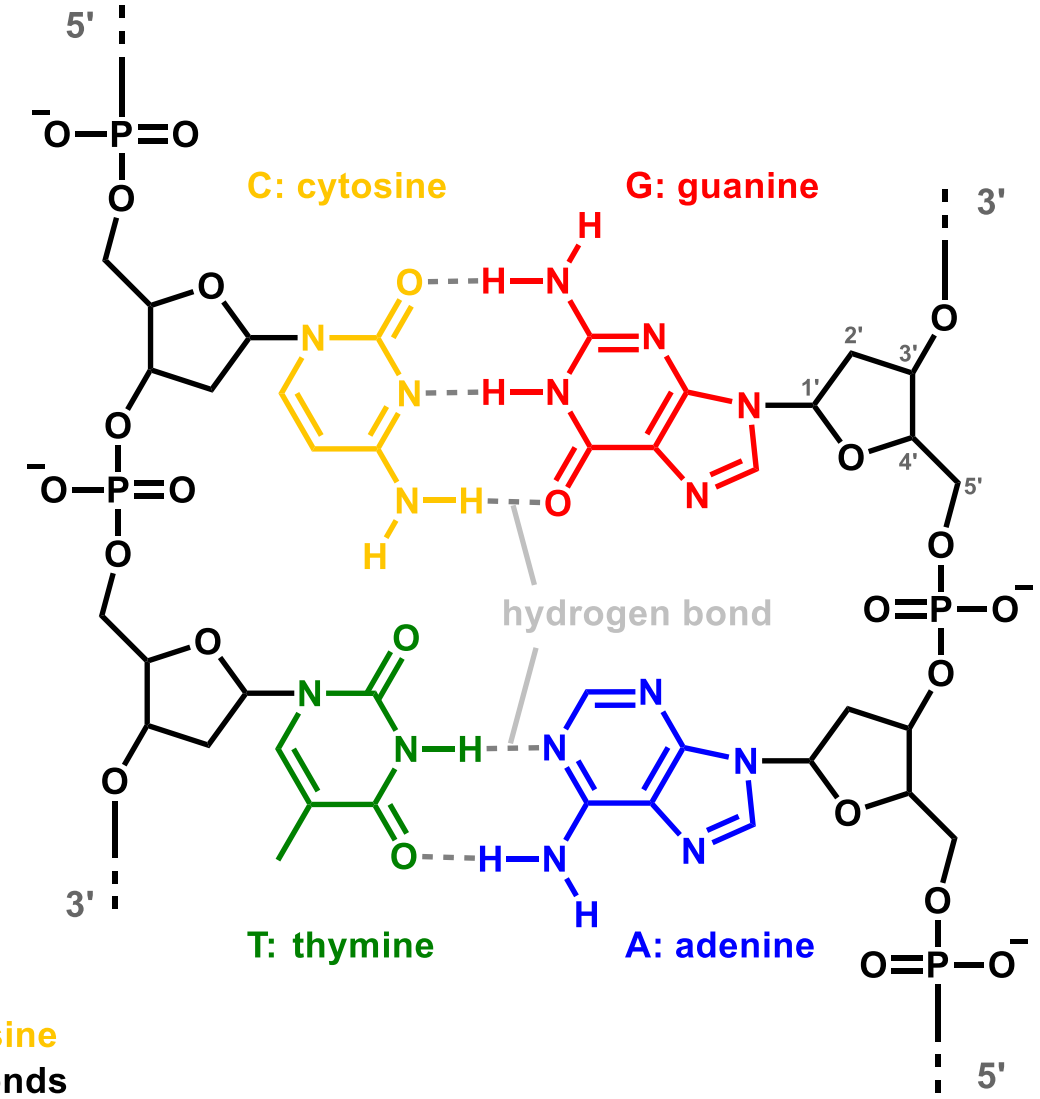
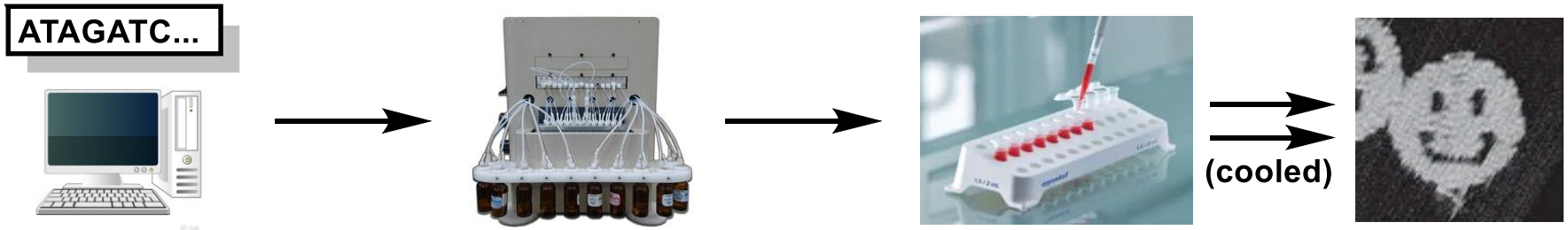


- 2 antiparallel chains form "double helix" structure.

- Adenine and Thymine / Guanine and Cytosine are bounded to each other by hydrogen bonds complementarily.
  → This base paring rule constrains the behavior of DNA

1-3. What is DNA Nanotechnology? (3)

General experimental procedure



1. DNA designing
2. DNA synthesis
3. DNA sequencing (reaction in buffer)

Why is DNA suitable for material?

1. Programmable design information

- DNAs include design information (base sequence) in themselves.
  = Information is equivalent to products.

2. Unnecessity of processing devices (other than DNA synthesizer)

- Nowadays DNA is easily available, provided by DNA synthesis service.

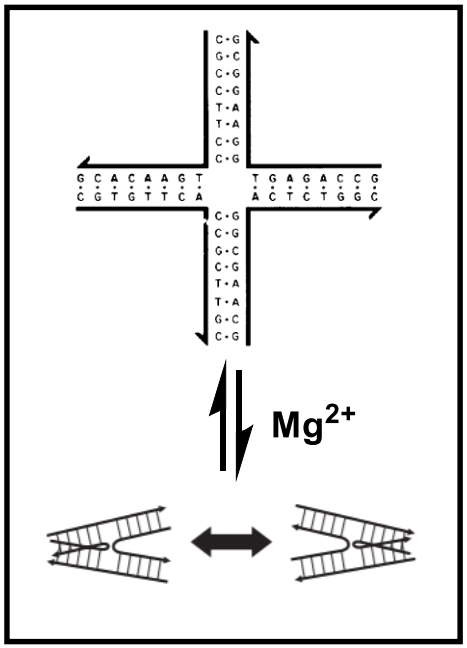
3. High processing accuracy

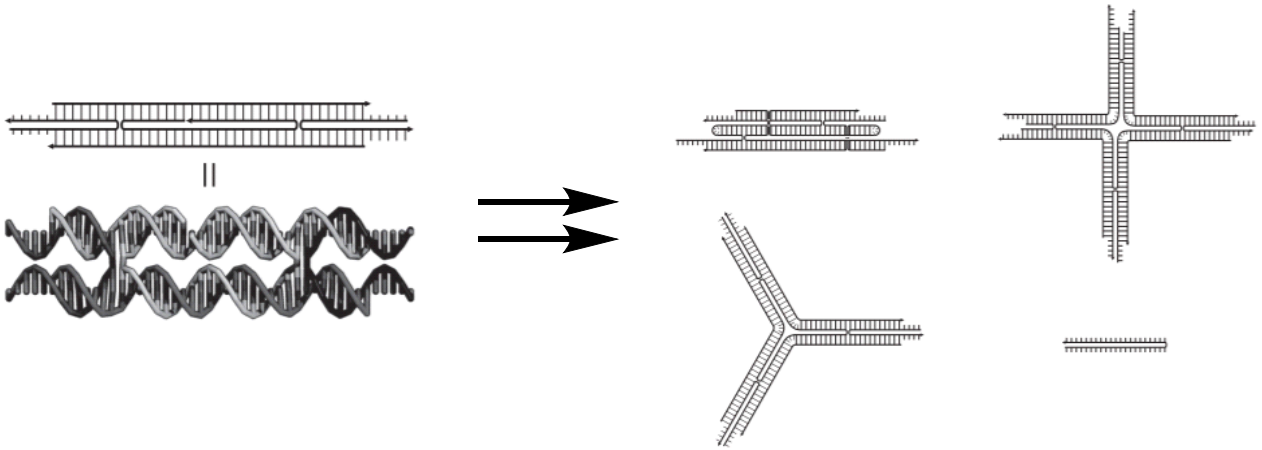
- DNA chains hybridize or self-assemble according to design information.
- Functional expression requires specific sequence design to avoid undesired hybridization.
  - technical development of calculation

→ Various fields on science have been supplying research projects

1-4. DNA Nanostructure (1) – Junction –

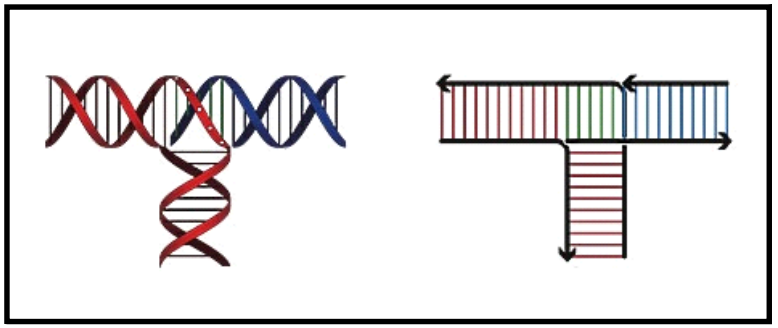
Crossover junction by Seeman[1)]

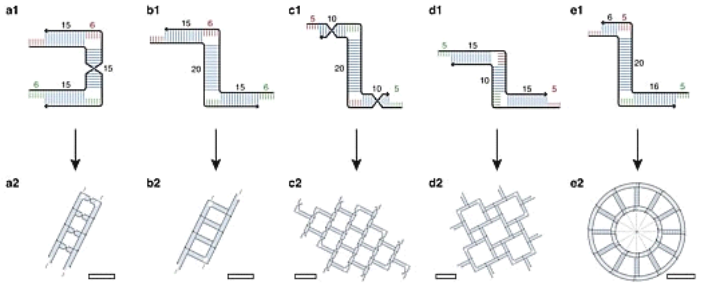




Various DNA tiles

T-junction by Hamada and Murata[2)]

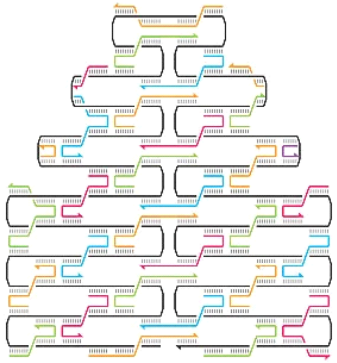




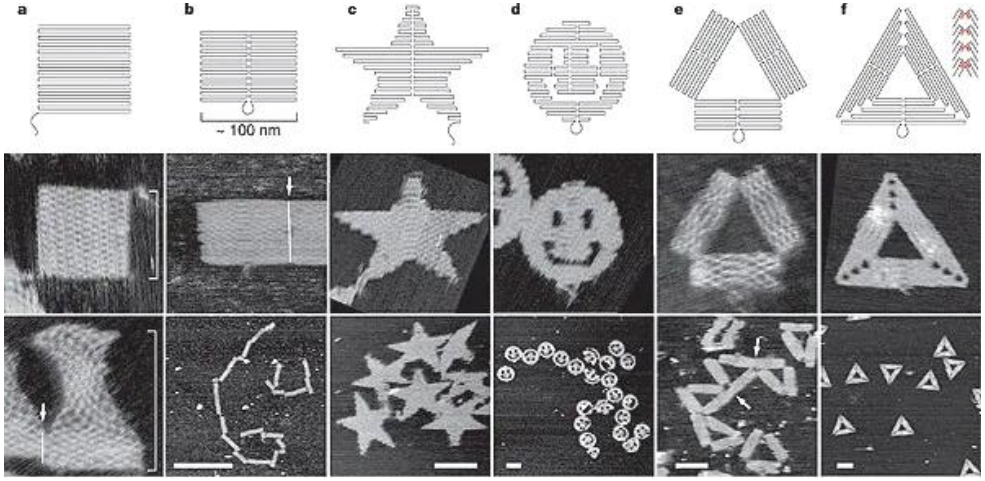
1) (a) Seeman, N. C. *J. Theor. Biol.* **1982**, *99*, 237. (b) Fu, T.-J.; Seeman, N. C. *Biochemistry* **1993**, *32*, 3211.
2) Hamada, S.; Murata, S. *Angew. Chem. Int. Ed.* **2009**, *48*, 6820.

1-5. DNA Nanostructure (2) – DNA Origami –

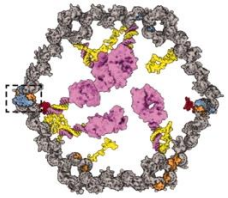
DNA origami by Rothemund [1)]



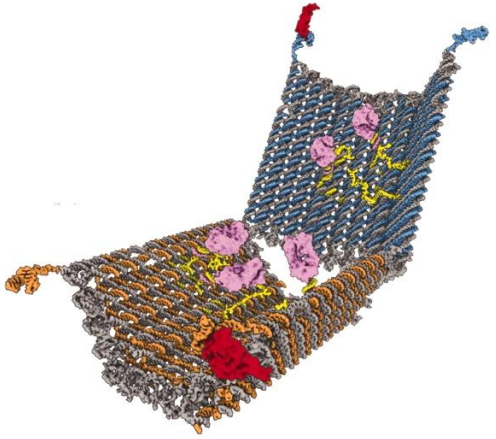
long single-stranded chain
+ many short staple-strands



Example of application:



unlock DNA aptamer
by target molecules



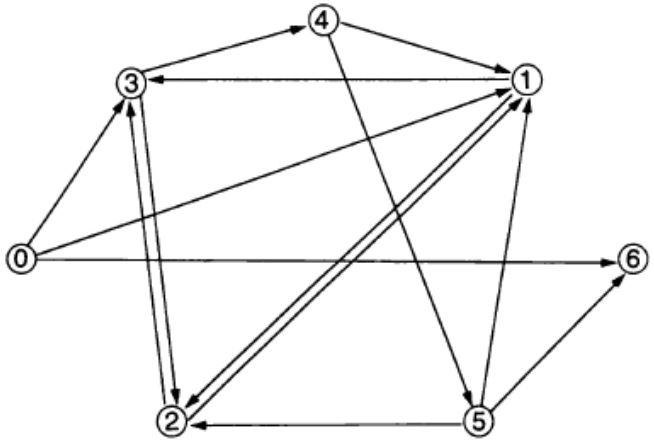
Douglas invented a drag-delivering nanorobot using DNA origami as a body material. [2)]

1) Rothemund, P. W. K. *Nature* **2006**, *440*, 297.
2) Douglas, S. M.; Bachelet, I.; Church, C. M. *Science* **2012**, *335*, 831.

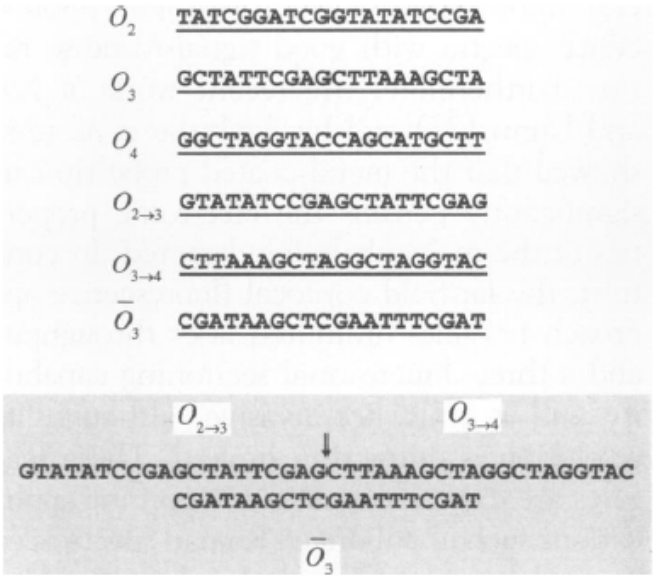
Contents

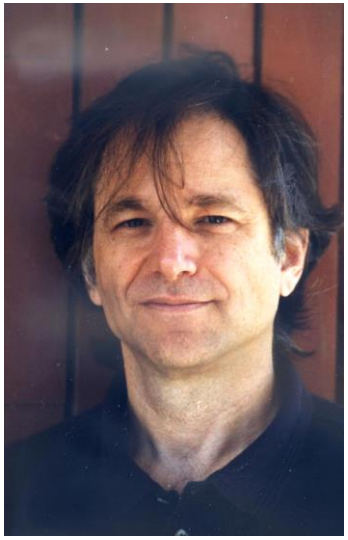
2-1. Dawn of DNA-based Computing

The first study on data processing by DNA



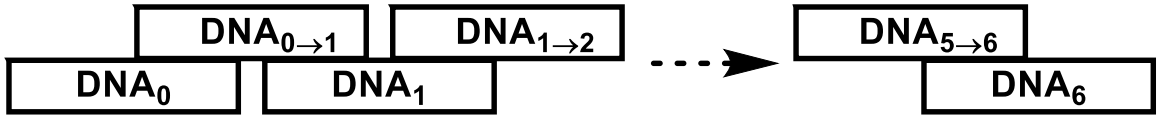
Q. Does this directed graph have Hamiltonian path?





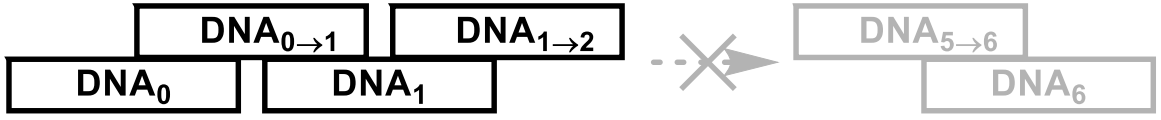
Leonard Adleman: inventor of RSA

- If yes,



All DNAs would be bound straight.

- If no,



All DNAs would not be bound straight.

2-2. Development of DNA-based Computing

Adleman's DNA computer,

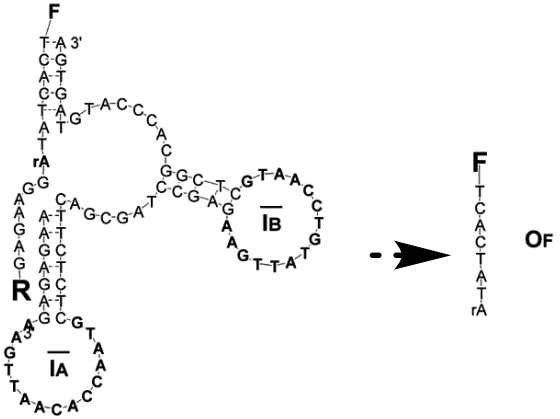
- would need the more DNAs, the more information was processed.
- could not process multi-step calculation.

Autonomous DNA computer for multi-step calculation

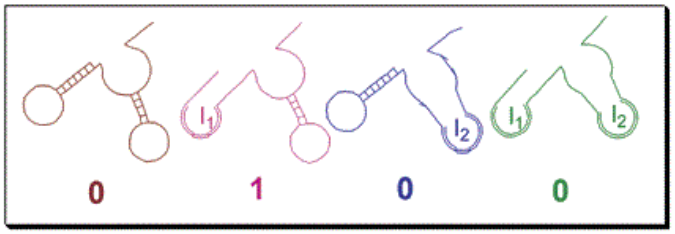
- Finite automaton using Class IIs restriction enzyme [1)]

- "Whiplash PCR": a chain reaction using DNA polymerase [2)]

- Logic gate using E6 DNAzyme [3)]
  e.g. ANDNOT ($A \wedge \neg B$) gate



I_A	I_B	O_F	I_{520}
0	0	0	<30
1	0	1	>50
0	1	0	<30
1	1	0	<30



NOT input DNA *A* → E6 DNAzyme is inactive.
input DNA *B* → E6 DNAzyme become inactive form.

1) Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. *Nature* **2001**, *414*, 430.
2) Sakamoto, K.; Kiga, D.; Komiya, K.; Gouzu, H.; Yokoyama, S.; Ikeda, S.; Sugiyama, H.; Hagiya, M. *Biosynthesis* **1999**, *52*, 81.
3) Stojanovic, M. N.; Mitchell, E. T.; Stefanovic, D. *J. Am. Chem. Soc*. **2002**, *124*, 3555.

2-3. Prof. Erik Winfree

career:

1991	B.S. @University of Chicago
1991-92	working with Stephen Wolfram and Matthew Cook
1992-94	Graduate student @Caltech (Prof. Al Barr)
1998	Ph.D in Computation and Neural Systems program @Caltech (Prof. John Hopfield)
1998-99	P. D. study on molecular biology @Princeton University (Prof. LewisThomas)
2000-	Assistant professo @Caltech
2006-	Associate professor @Caltech
2010-	Professor @Caltech

Research Projects:

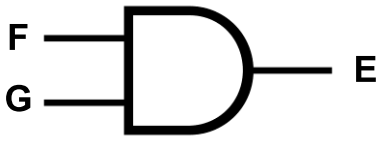
- DNA computation: Enzyme-free DNA logic gates (→ section 2)

- DNA nanotechnology: Algorithmic self-assembly using DNA tiles (→ section 3)

- Molecular Programming:
  Self-replicating molecular systems, evolution, and the origin of life

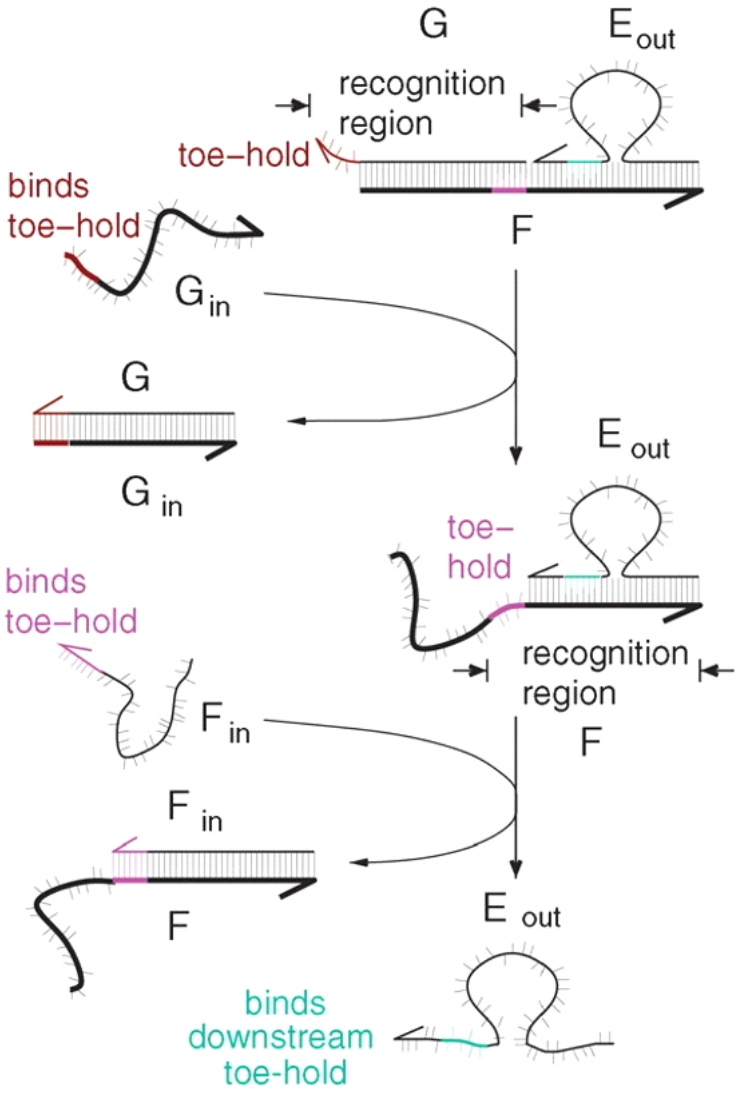
2-4. Enzyme-Free DNA Logic Circuits (1)

AND gate



F	G	E
0	0	0
0	1	0
1	0	0
1	1	1

	REACTANTS	PRODUCTS
1	EFG, NO INPUTS	SAME AS REACT.
2	EFG + F_{in}	SAME AS REACT.
3	EFG + G_{in}	EF + GG_{in}
4	EFG + F_{in} + G_{in}	E + FF_{in} + GG_{in}



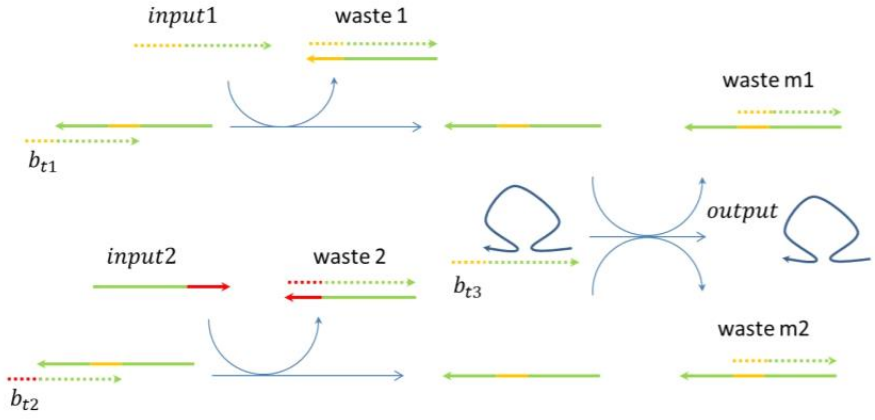
Seelig, G.; Soloveichik, D.; Zhang, D. Y.; Winfree, E. *Science* **2006**, *314*, 1585.

2-5. Enzyme-Free DNA Logic Circuits (2)

OR gate

input 1
input 2
output

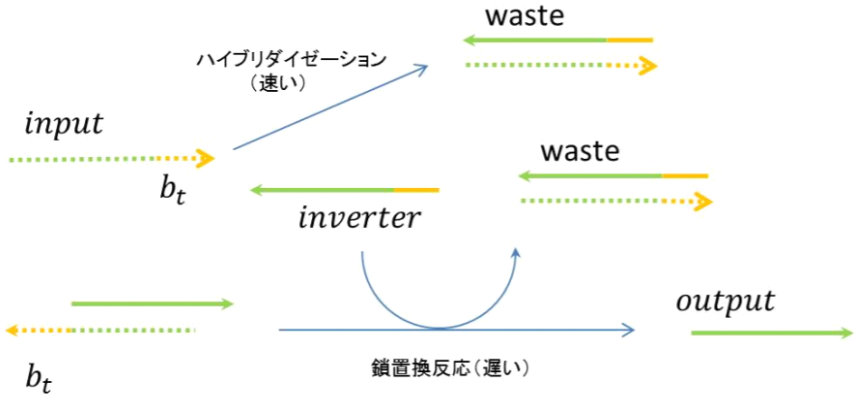
i1	i2	O
0	0	0
0	1	1
1	0	1
1	1	1



NOT gate

input
output

i	O
0	1
1	0



Seelig, G.; Soloveichik, D.; Zhang, D. Y.; Winfree, E. *Science* **2006**, *314*, 1585.

Contents

1. Introduction
   - What is DNA nanotechnology?
   - DNA origami

2. Previous Studies on DNA Computers and Winfree's Enzyme-Free Nucleic Acid Logic Circuits

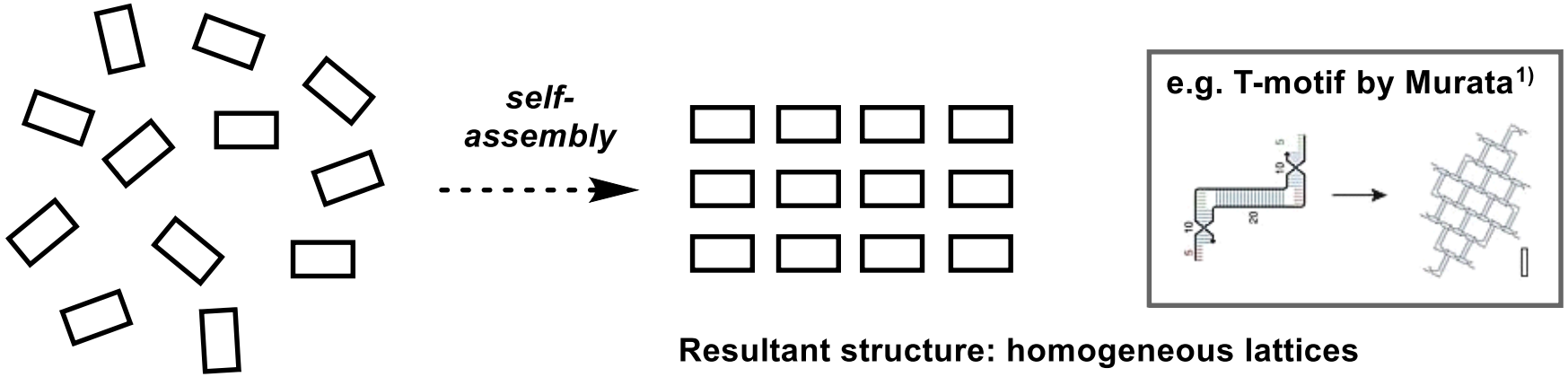
   Seelig, G.; Soloveichik, D.; Zhang, D. Y.; Winfree, E. *Science* **2006**, *314*, 1585.

3. **Diverse and robust molecular algorithms using reprogrammable DNA self-assembly (main topic)**

   Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E. *Nature* **2019**, *567*, 366.

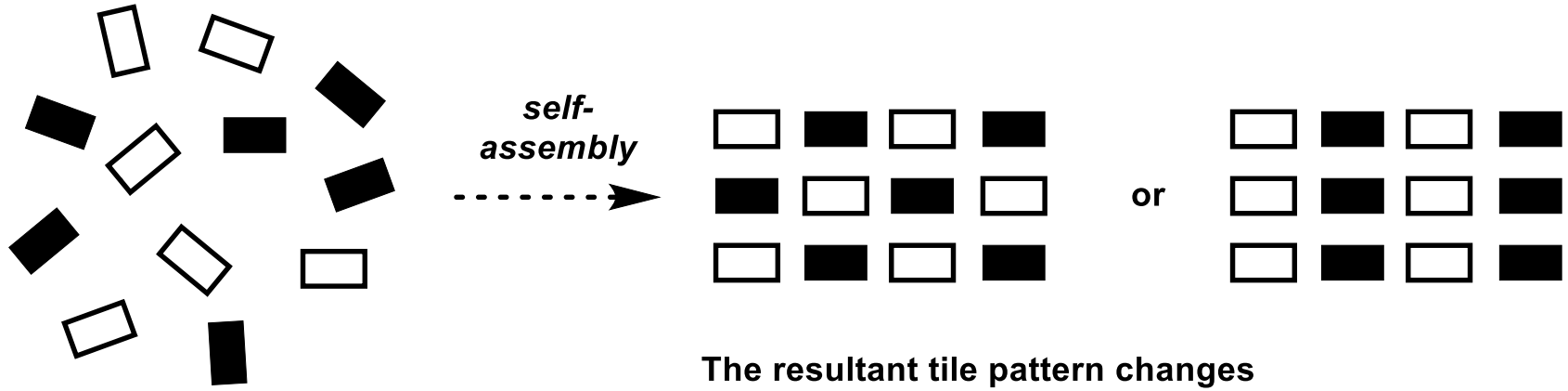
3-1. “Algorithmic” Self-Assembly

Self-assembly of one type of motif



Resultant structure: homogeneous lattices
→ A tile is bound to a tile of the same structure

Algorithmic self-assembly of motifs

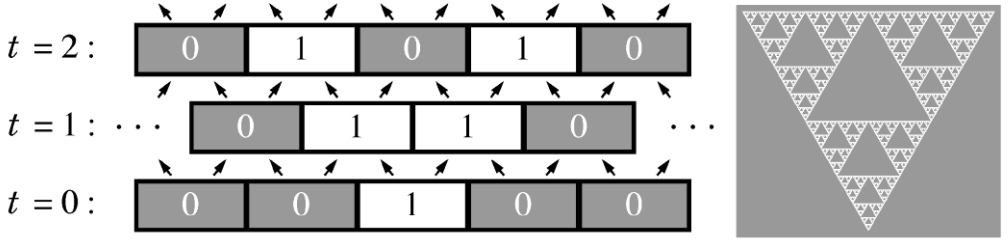


The resultant tile pattern changes
depending on the assembling rule

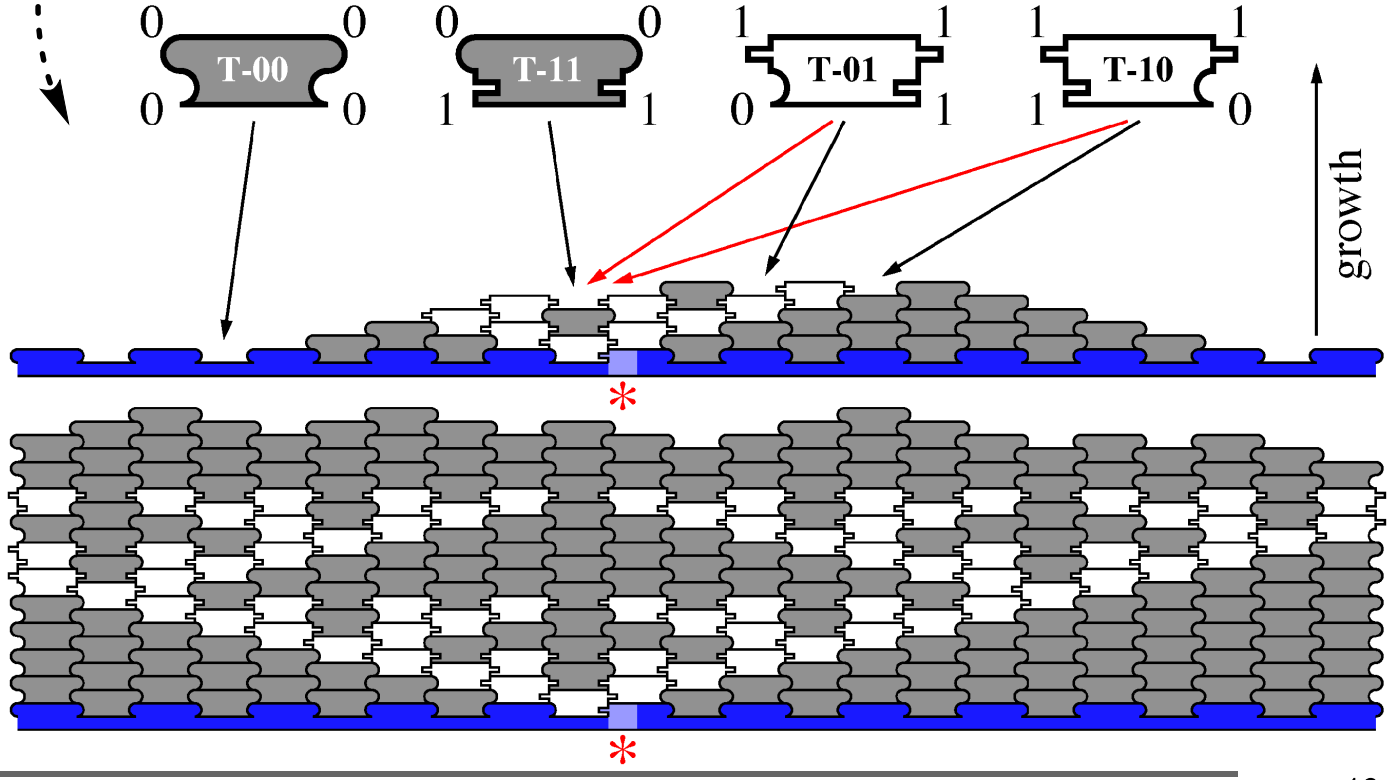
1) Hamada, S.; Murata, S. *Angew. Chem. Int. Ed.* **2009**, *48*, 6820.

3-2. Previous Work: DNA Sierpinski Triangles (1)

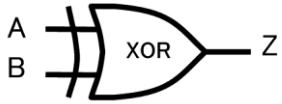
Sierpinski Triangles:
fractale which contains infinitely repeated equilateral triangle



implemented by DNA tiles



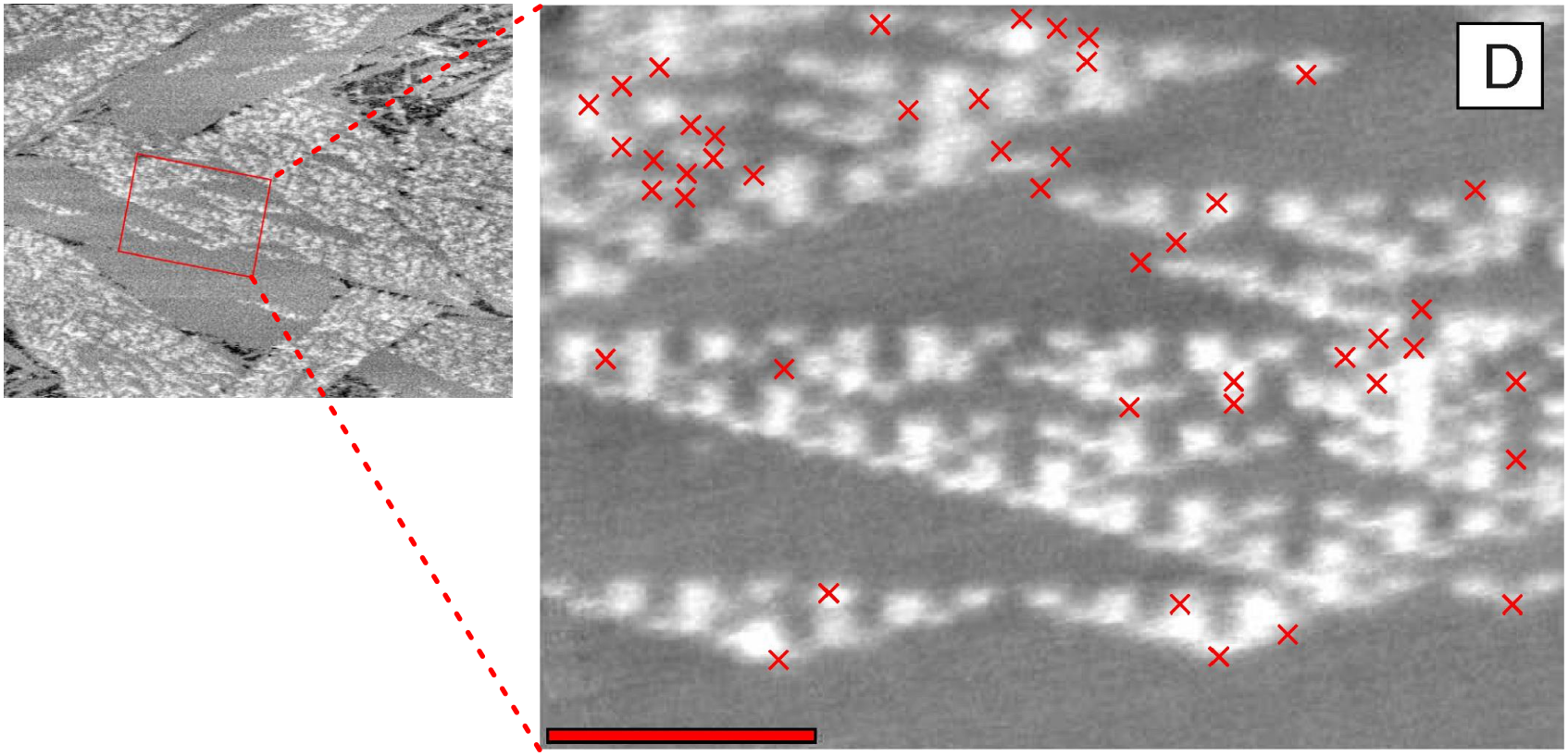
*** 4 tiles function as XOR gate.**



A	B	Z
0	0	0
0	1	1
1	0	1
1	1	0

Rothemund, P. W. K.; Papadakis, N.; Winfree, E. *PLos Biol.* **2004**, *2*, 2041.

3-3. Previous Work: DNA Sierpinski Triangles (2)



Desired pattern was successfully obtained.

Error rates: 1%~10%
- **controlling temperature and concentration**
- **tile design**
- **a floppy and slowly straightening nucleating structure**

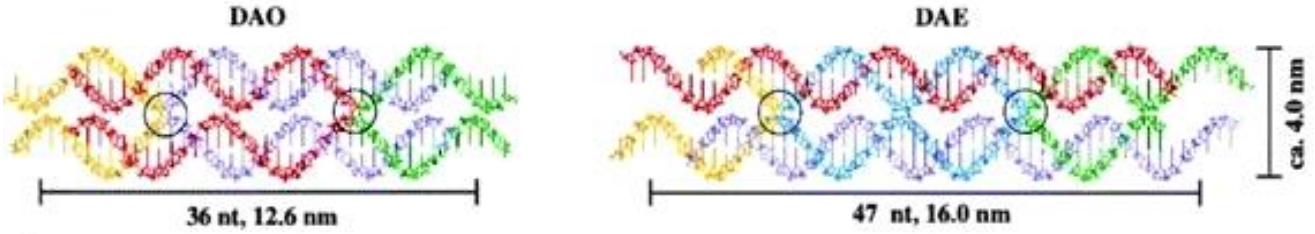
Rothemund, P. W. K.; Papadakis, N.; Winfree, E. *PLos Biol.* **2004**, *2*, 2041.

3-4. "Reprogrammable" and Single-Stranded Tile

Sierpinski Triangles consist DX tiles

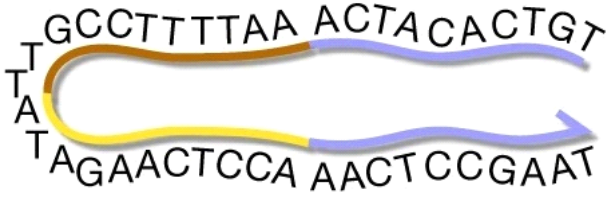
- DAO tile and DAE tile
- consisting four strands of DNA

→ difficult for tile systems to scale up (necessary for reprogrammable tile system)



Single-Stranded tile (SST) for scaling up

- instead of DX or tripple-crossover tiles



→ **However, there are substantial challenges in terms of SST using.**

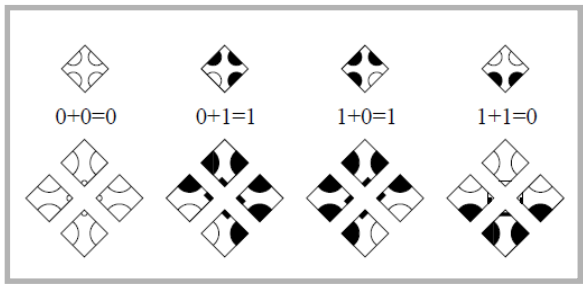
= SST is much more floppy than DX or TX tiles and would be effected by undesired interactions.

- Tile-attachment errors
- Disordered spurious nucleation
- binding to itself

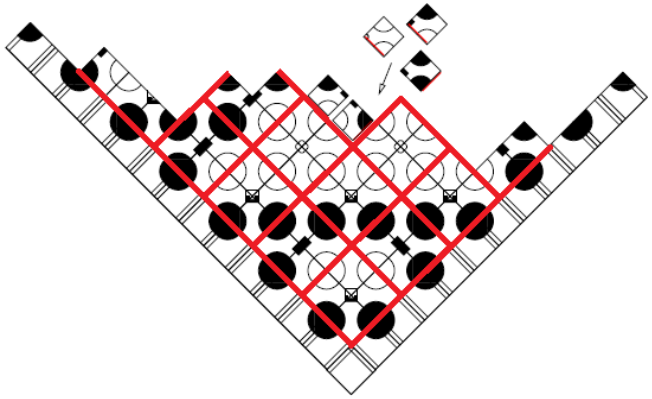
Winfree, E.; Liu, F.; Wenzler, L. A.; Seeman, N. C. *Nature* **1998**, *394*, 539.

3-5. Approaches toward Reducing Errors

Proofreading tile [1)]

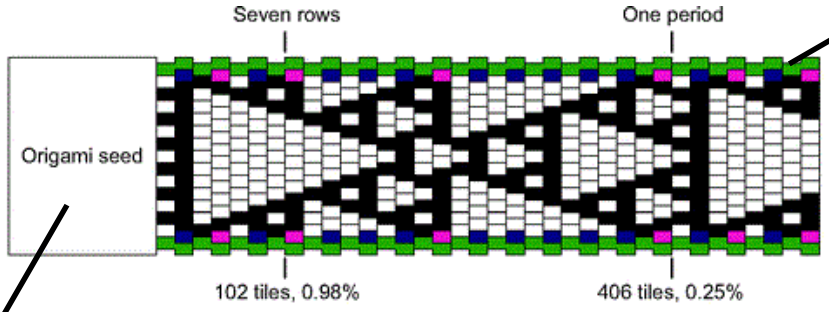


DNA tile devided into N×N
(e.g. 2×2)

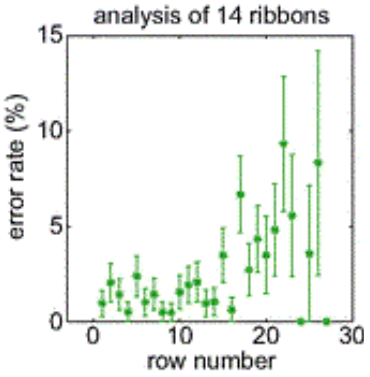


One tile error requires
two times of continuous
tile mismaches
→ Tile binding would be
more specifically.

Fixed-width ribbon using DNA origami seed [2)]



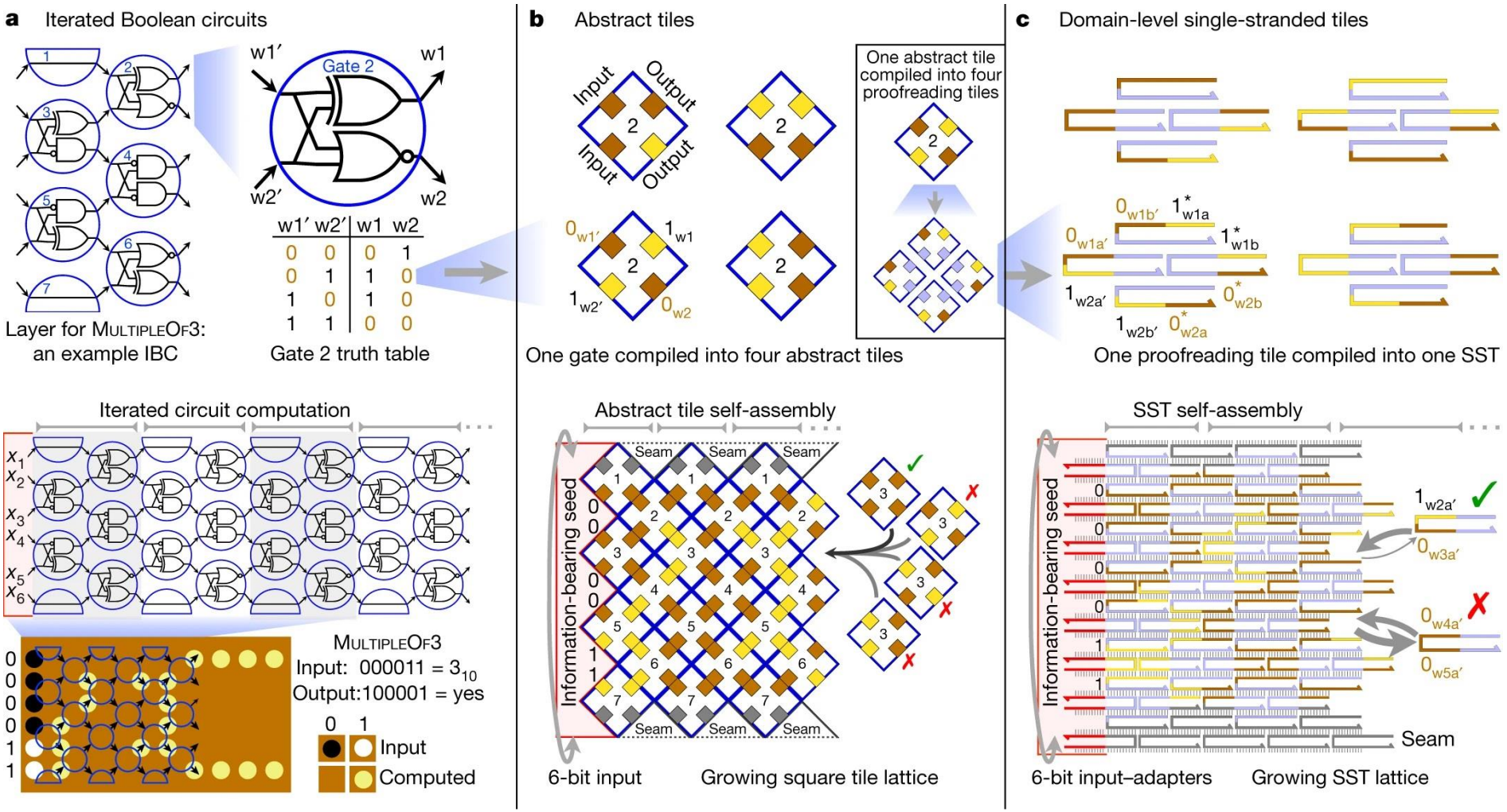
Boundary tiles keep the width of the ribbon.
→ enabling quantitative evaluation of error rates



Seed structure was made by DNA origami.
→ more rigid than the single-stranded scaffold strands
→ exellent control over arbitrary nucleation bit patterns

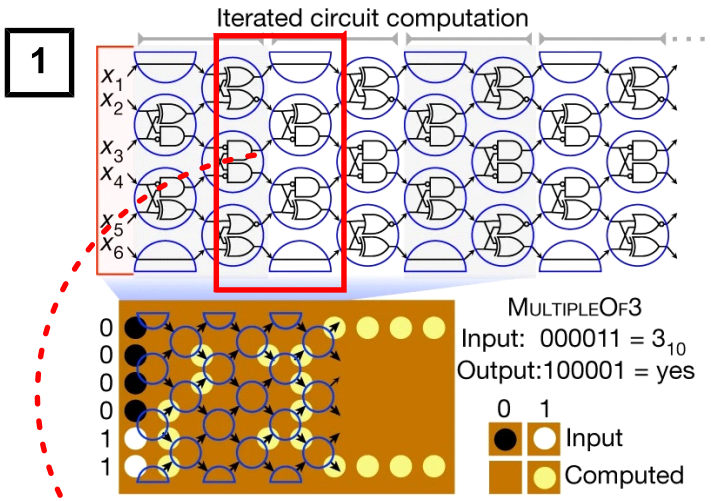
1) Winfree, E.; Bekbolatov, R. *DNA Computing.* **2003**, 126.
2) Fujibayashi, K.; Hariadi, R.; Park, S. H.; Winfree, E.; Murata, S. *Nano Lett.* **2007**, *8*, 1791.

3-6. Conception of Algorithm Design

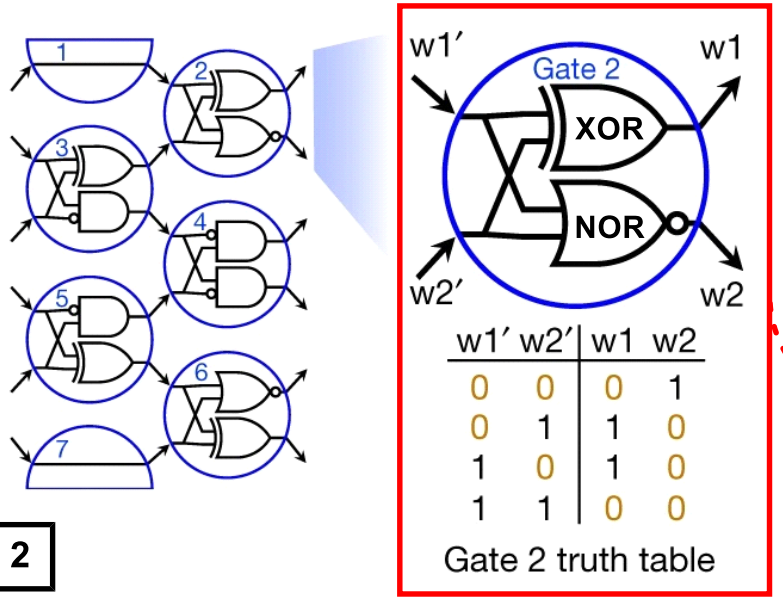


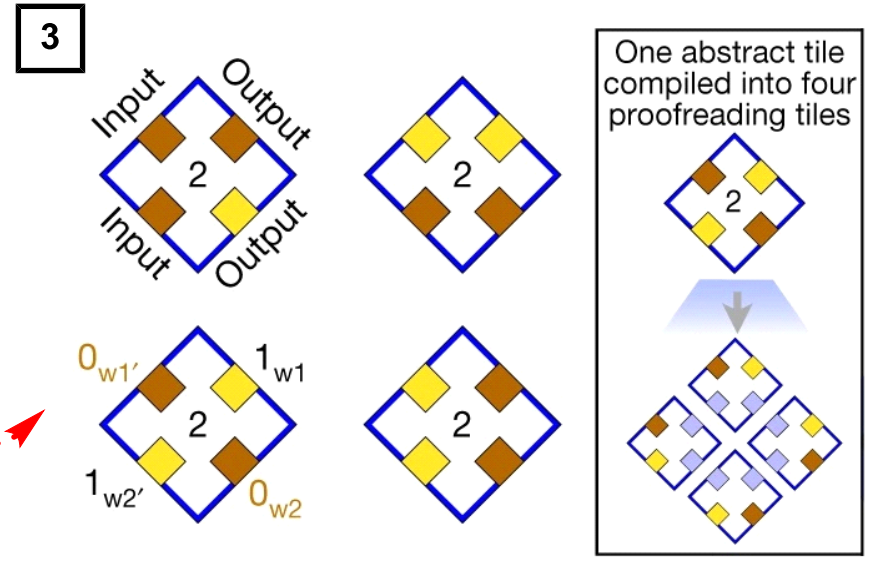
3-7. Design of Circuits and Logic Gates

(e.g. MULTIPLEOF3 circuit)



1. **6-bit 1-layer Iterated Boolean Circuit was chosen for molecular implementation.**
2. **One layer contains five logic gates.**
3. **Each gate was compiled into four abstract tiles. (further devided into 2×2 proofreading tiles)**

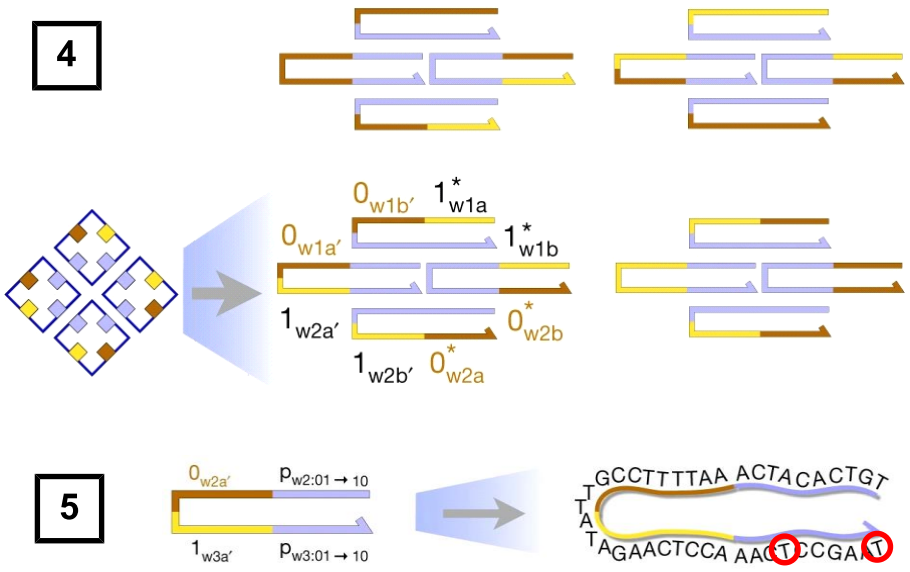




→ **Gate 2 compiled into four tiles**

Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E. *Nature* **2019**, *567*, 366.

3-8. Design of Each Single-Stranded Tile



4. One proofreading tile was compiled into one single-stranded tile (SST).

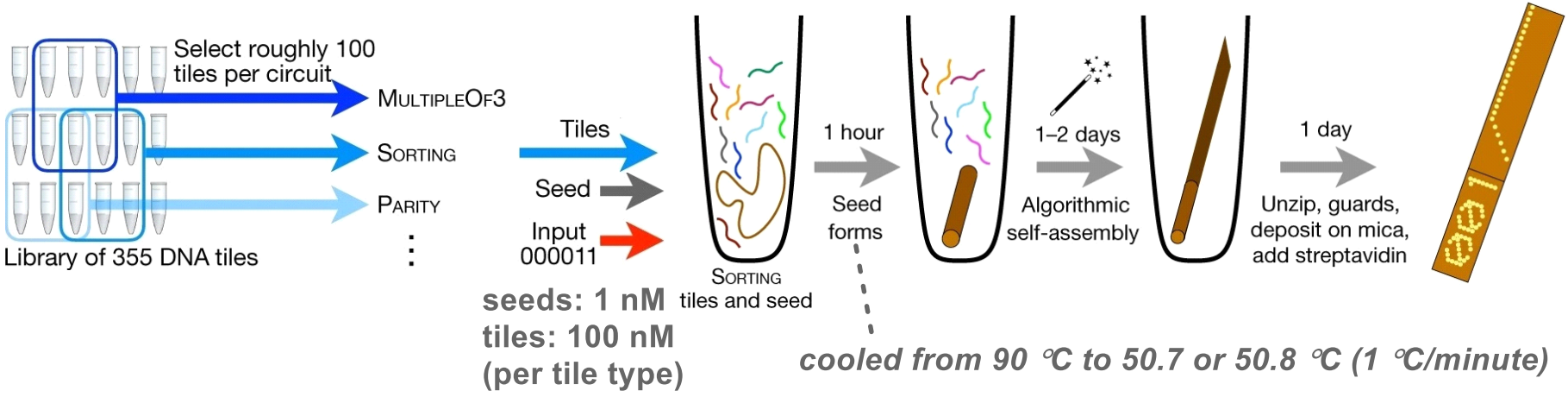
5. SSTs were divided into four distinct binding domains, and 42(10+11+11+10)-base DNA sequances were designed.
   * Thymine bases placed on proofreading blocks were biotin-modified for analysis.

6. Designed SSTs would self-assemble.

Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E. *Nature* **2019**, *567*, 366.

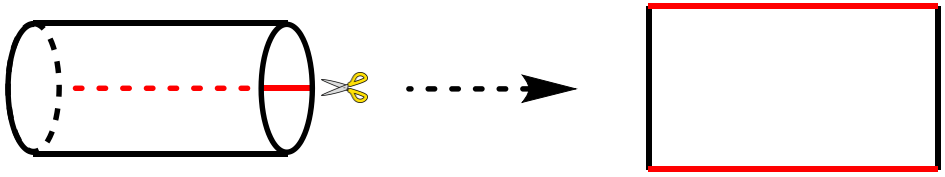
3-9. Experimental Protocol

Experimental protocol (e.g. SORTING circuit)



- Unzip = adding unzipping strands

→ **removing the seam strands and opening up nanotubes**



- Guards = adding guard strands

→ **shutting down the assembly by inactivating any remaining tiles in solution**

- Streptavidin binds to biotin which modifies "1" bits in tiles.

*** Input adaptor strands did have biotin modifications.**

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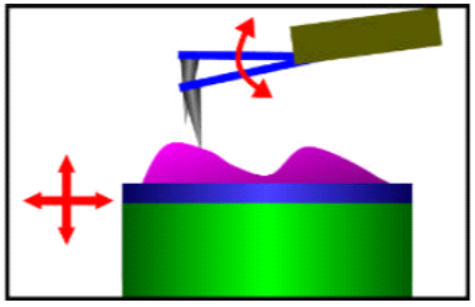
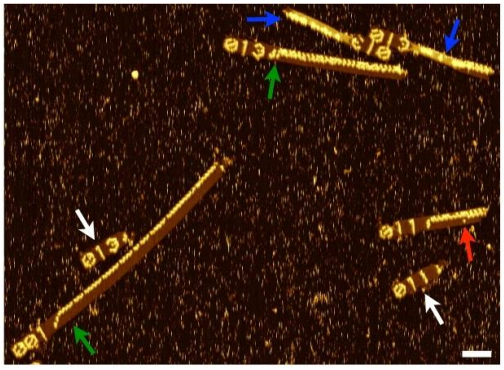
3-10. Final Analysis by Atomic Force Microscope

The Atomic Force Microscope (AFM)



AFM measures atomic force by detecting the motion of the cantilever
- In observation of DNA nanostructure,
  "tapping mode" is most frequently adopted.

Analysis by AFM

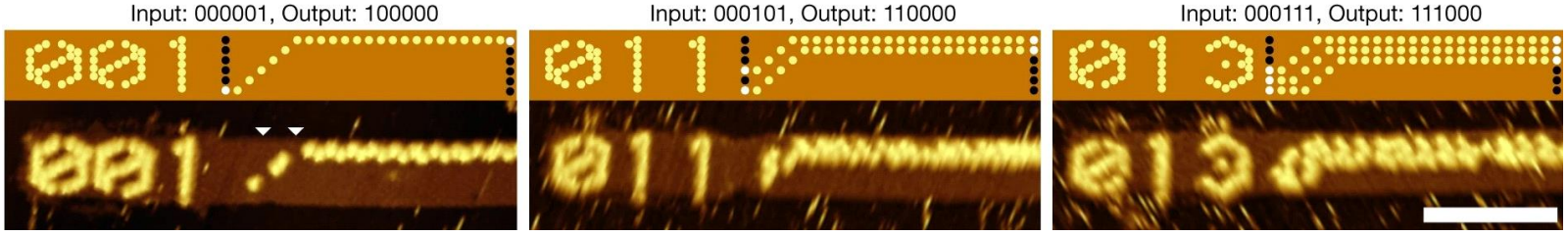


green arrows: nanotubes with correct growth

white arrows: failed to grow nanotube (63.3% of 1,299 analyzed)

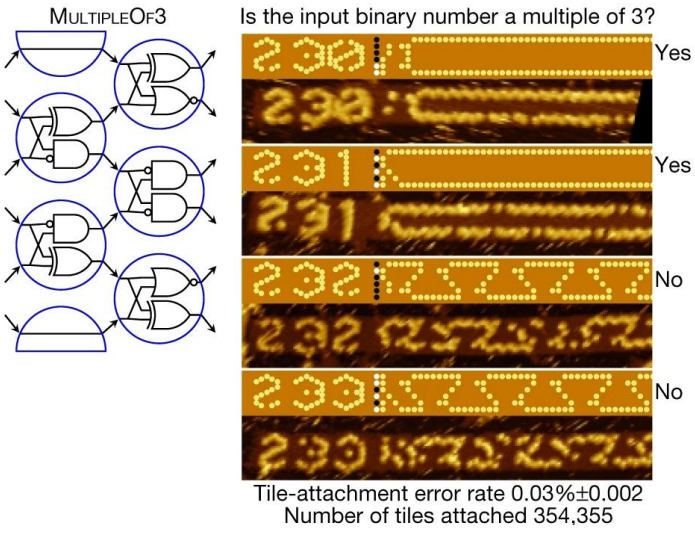
blue arrows: failed to unzip and could not be read

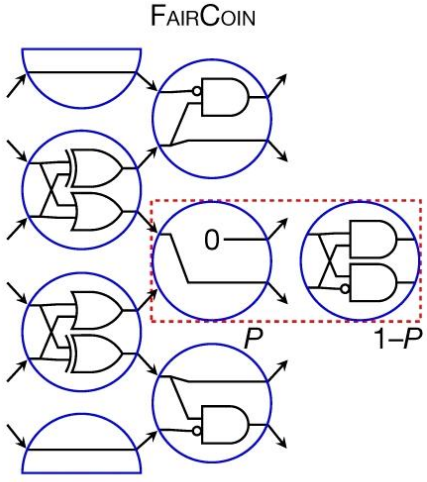
red arrows: logical errors
→ Tile-attachment error rate was estimated to be 0.03%
(counted 77 errors/estimated 269,028 attachments)

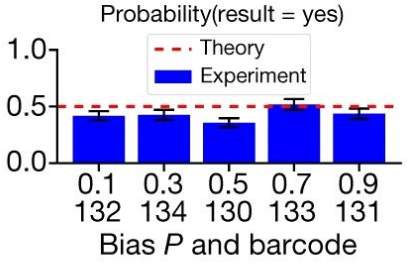


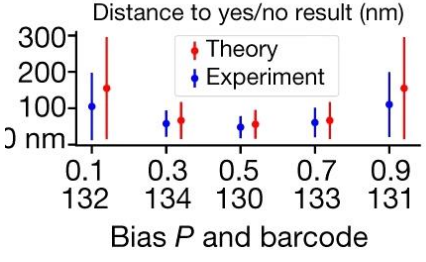
Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E. *Nature* **2019**, *567*, 366.

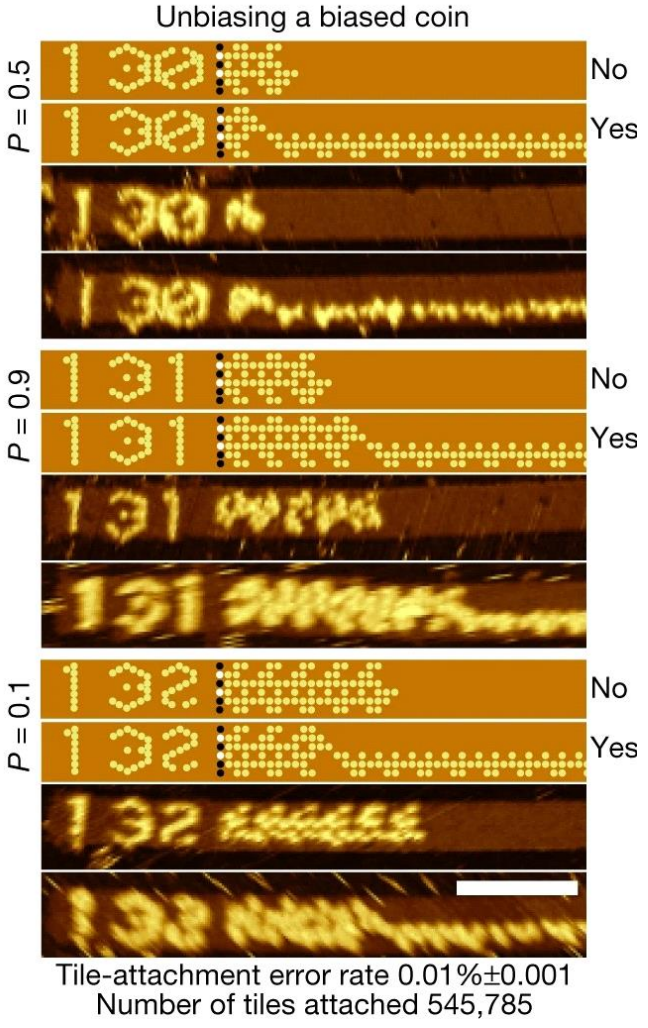
3-11. Randomized Algorithm Using Self-Assembly











In addition to deterministic computation (e.g. MULTIPLEOF3), randomized computation* can be implemented (e.g. FAIRCOIN).**

*** Outputs of randomized gates change depending on the relative concentrations of tile types.**

**** P = 0.1~0.9. so FAIRCOIN is biased coin to be exact.**

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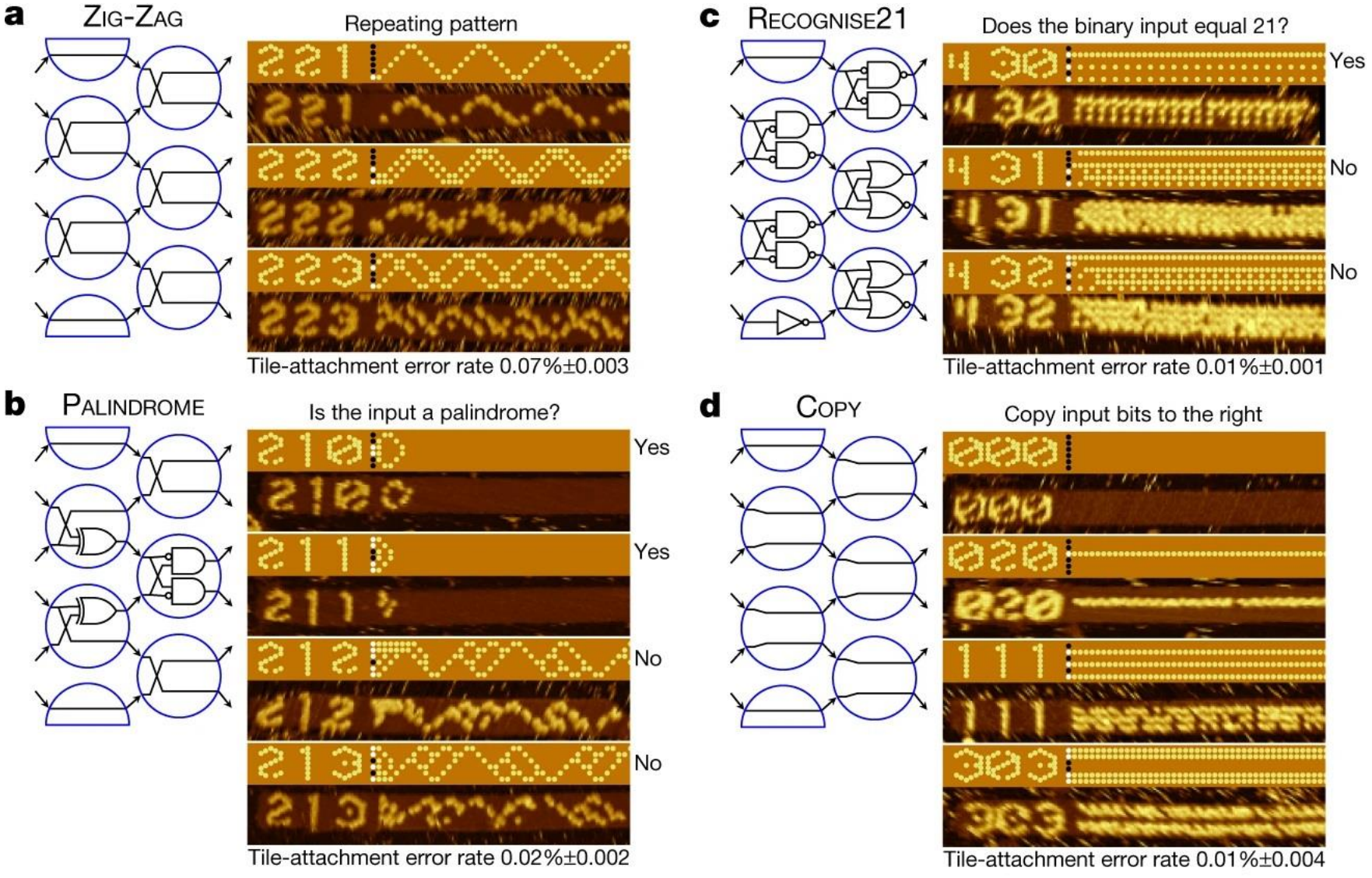
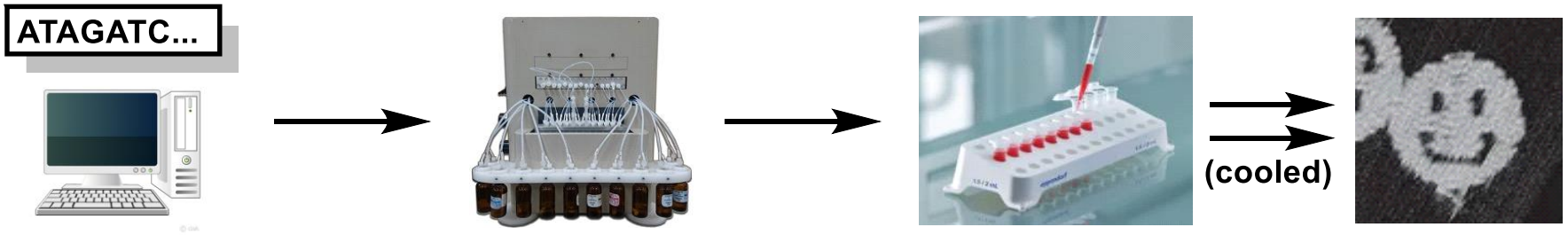
3-12. Realized Algorithms by 6-bit IBC Tile Set



Woods, D.; Doty, D.; Myhrvold, C.; Hui, J.; Zhou, F.; Yin, P.; Winfree, E. *Nature* **2019**, *567*, 366.

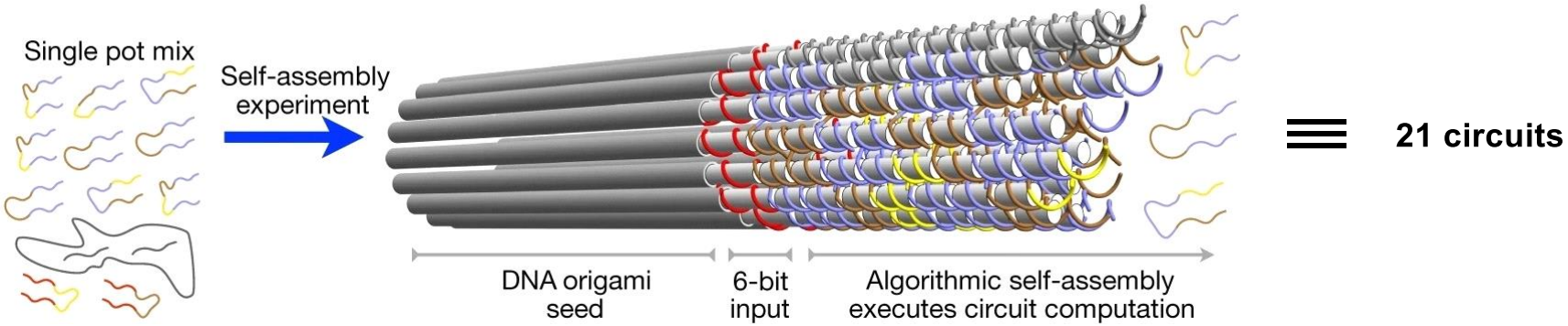
Summary

- DNA can be freely degined and
  construct various nanosystems/nanostructures by autonomous reaction



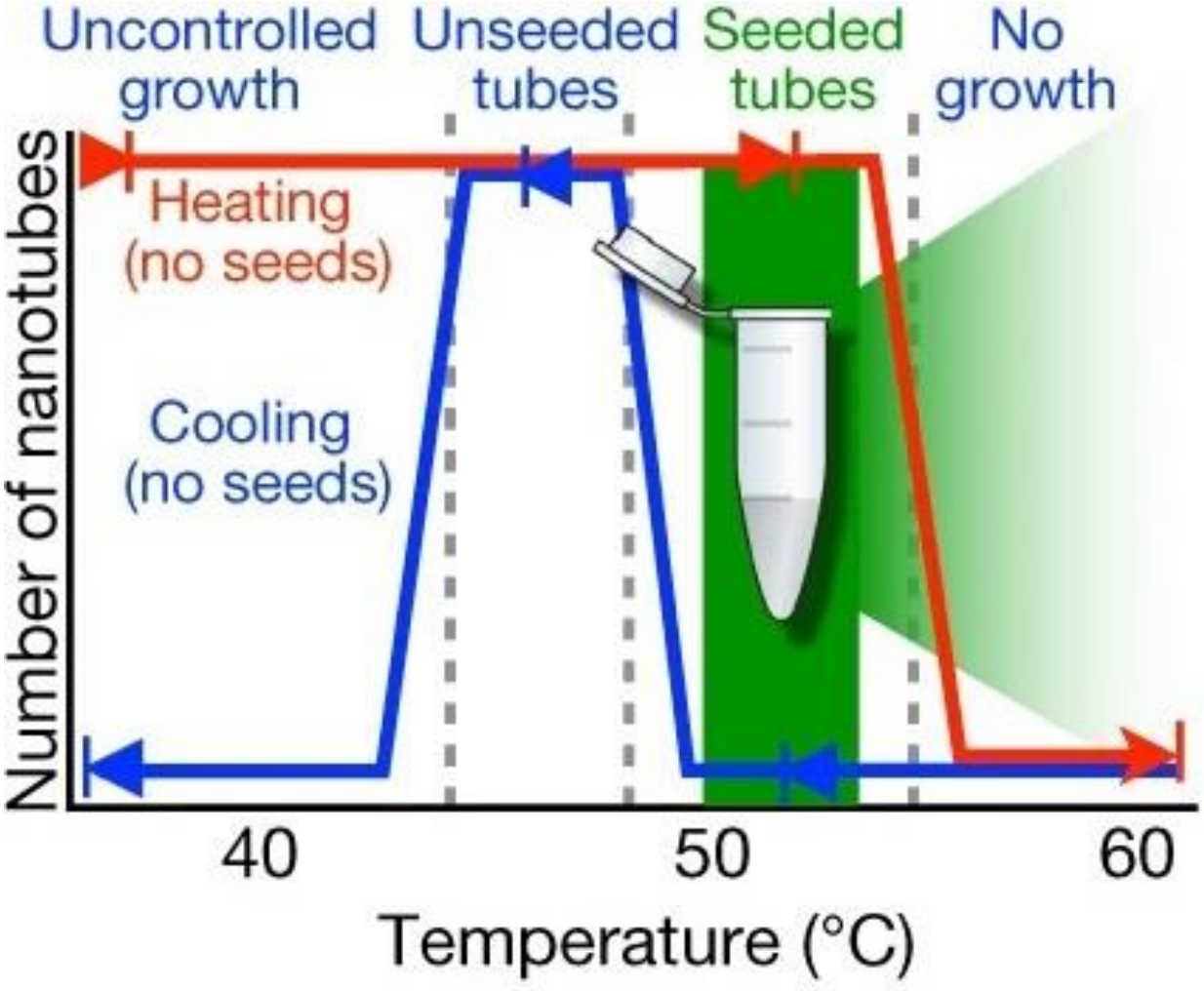
1. DNA designing
2. DNA synthesis
3. DNA sequencing
   (reaction in buffer)

- Prof. Winfree accomplished to create a reprogrammable self-assembly system
  containing 355 types of DNA tiles and implement 21 circuits.



Appendix

Determination of Reaction Temperature



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