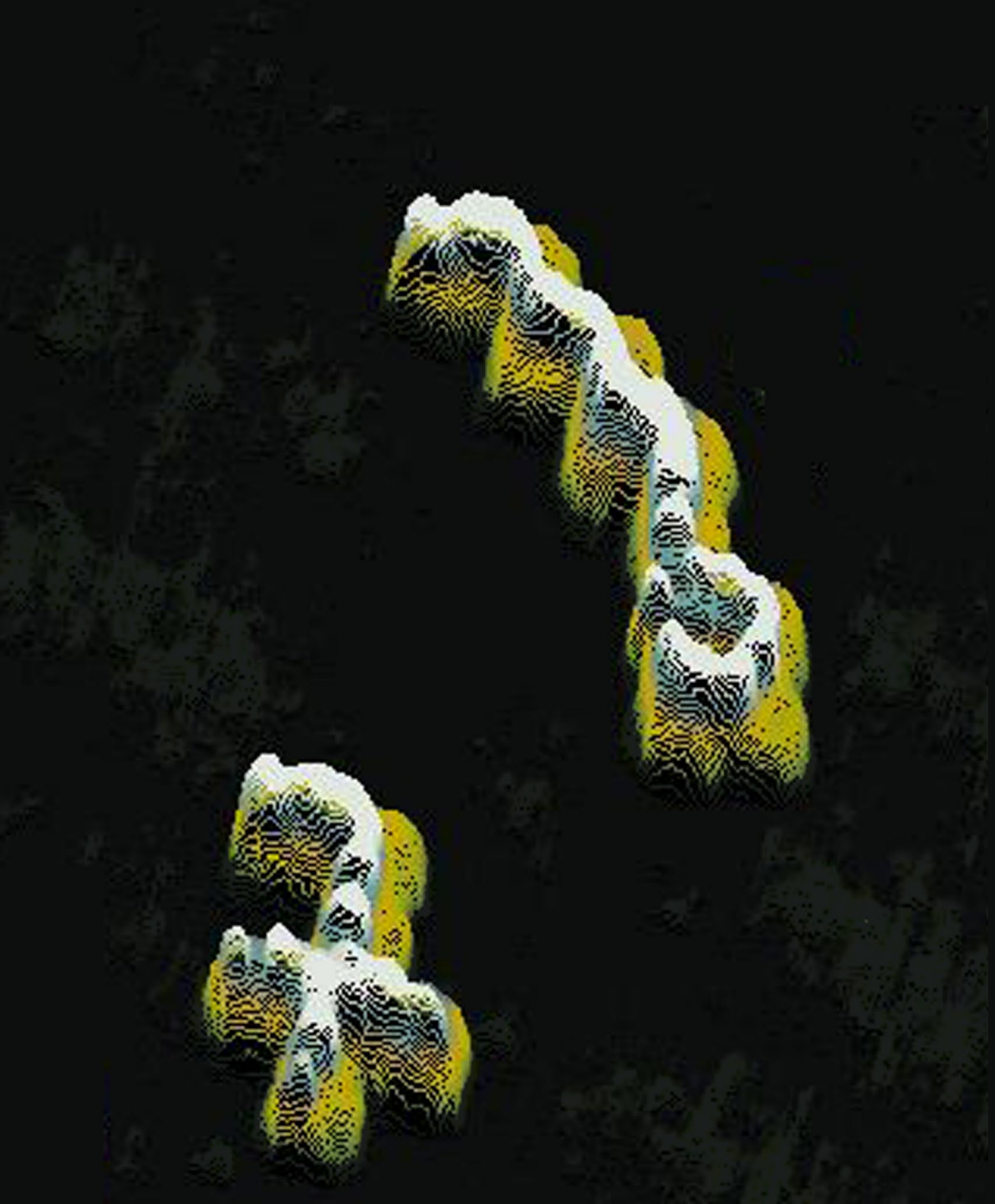


Modular reconfiguration of DNA origami assemblies using tile displacement

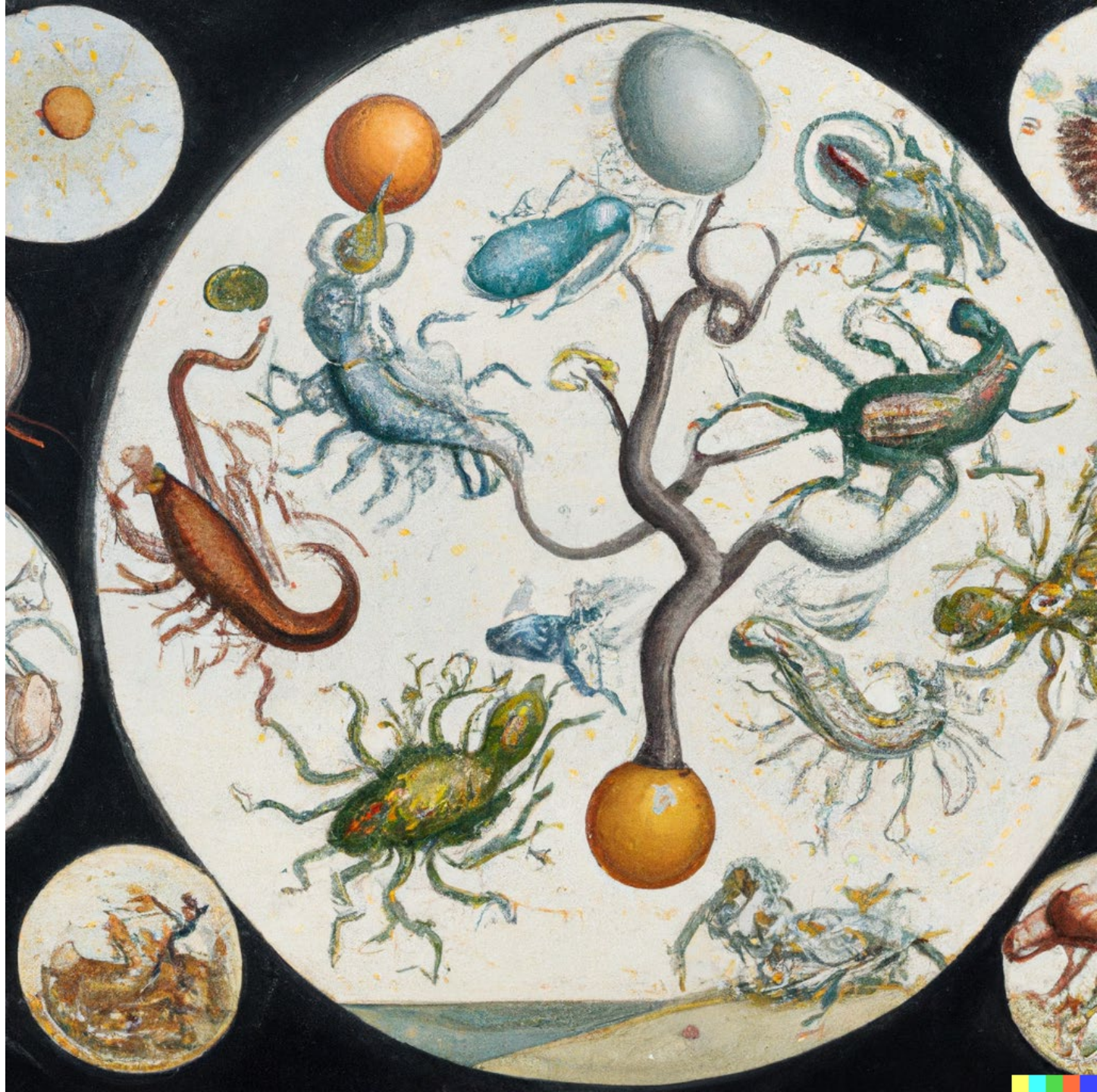
Namita Sarraf, Kellen Rodriguez, and Lulu Qian

Bioengineering
Computer Science
Computation & Neural Systems

Caltech

DNA29, September 14, 2023

The power of natural evolution lies in the adaptability of biological organisms but is constrained by the time scale of genetics and reproduction.



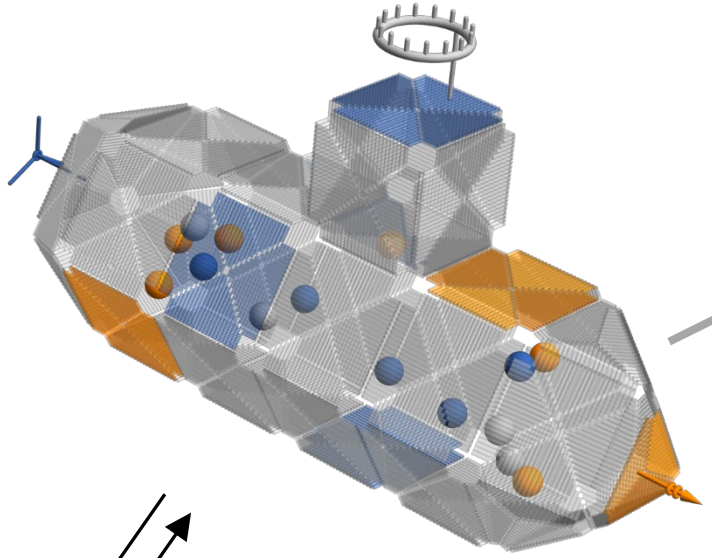
Engineering artificial molecular machines should not only include adaptability as a core feature but also apply it within a larger design space and at a faster time scale.

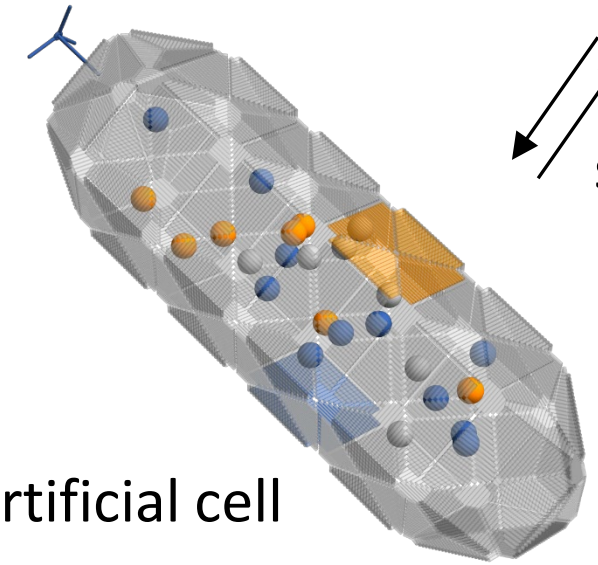
Modular electromechanical robots

CEBOT (Fukuda et al. 1988)
Crystalline (Rus et al. 2001)
MTRAN (Murata et al. 2002)
PolyBot (Yim et al. 2002)
Programmable Parts (Klavins et al. 2005)
Molecubes (Zykov et al. 2005)
SuperBot (Shen et al. 2006)
Miche (Rus et al. 2006)
SMORES (Davey et al. 2012)
M-Block (Romanishin et al. 2015)
ModQuad (Saldana et al. 2018)
⋮

Cubelets

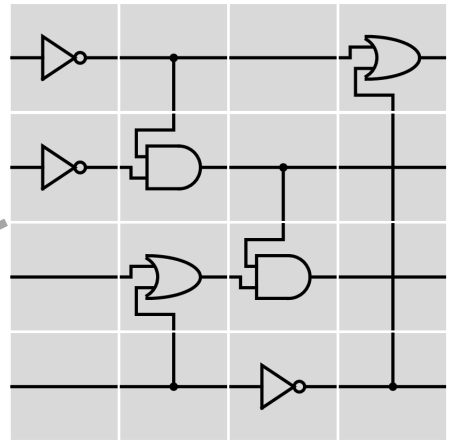
Future cell-scale molecular robots

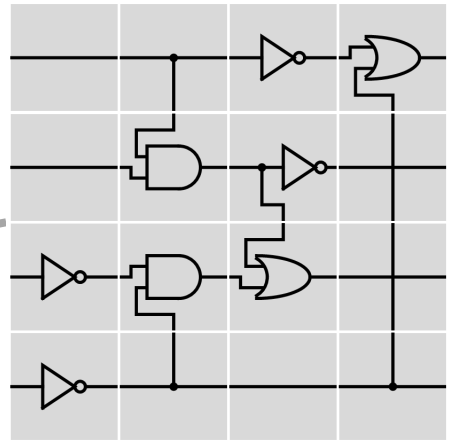


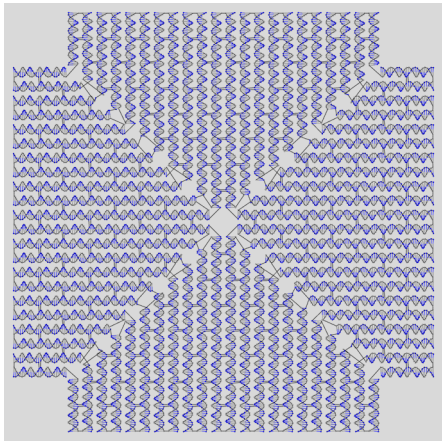


self-reprogramming

artificial cell

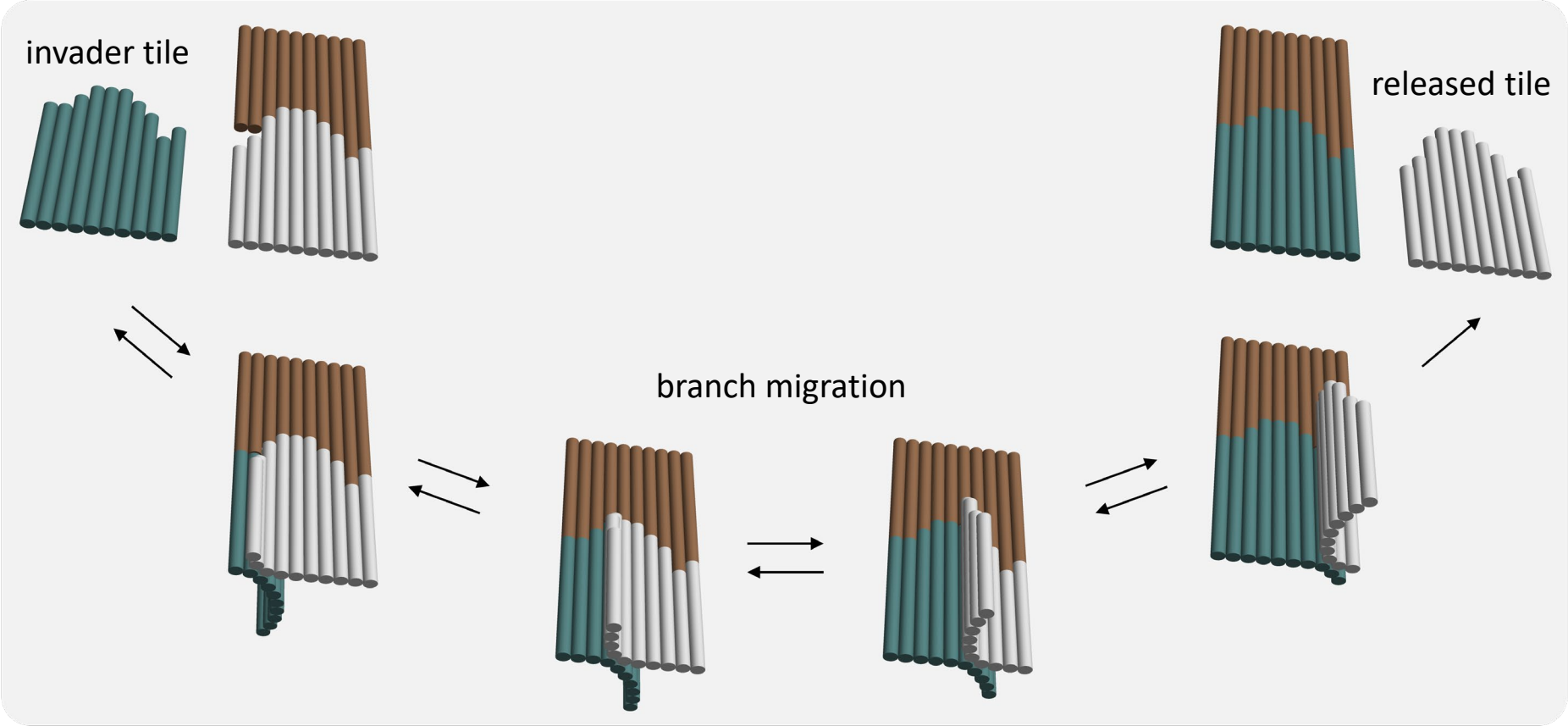




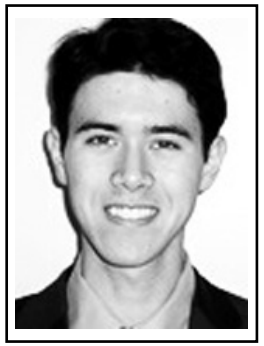


circuits attached to DNA origami tiles

Concept of DNA tile displacement

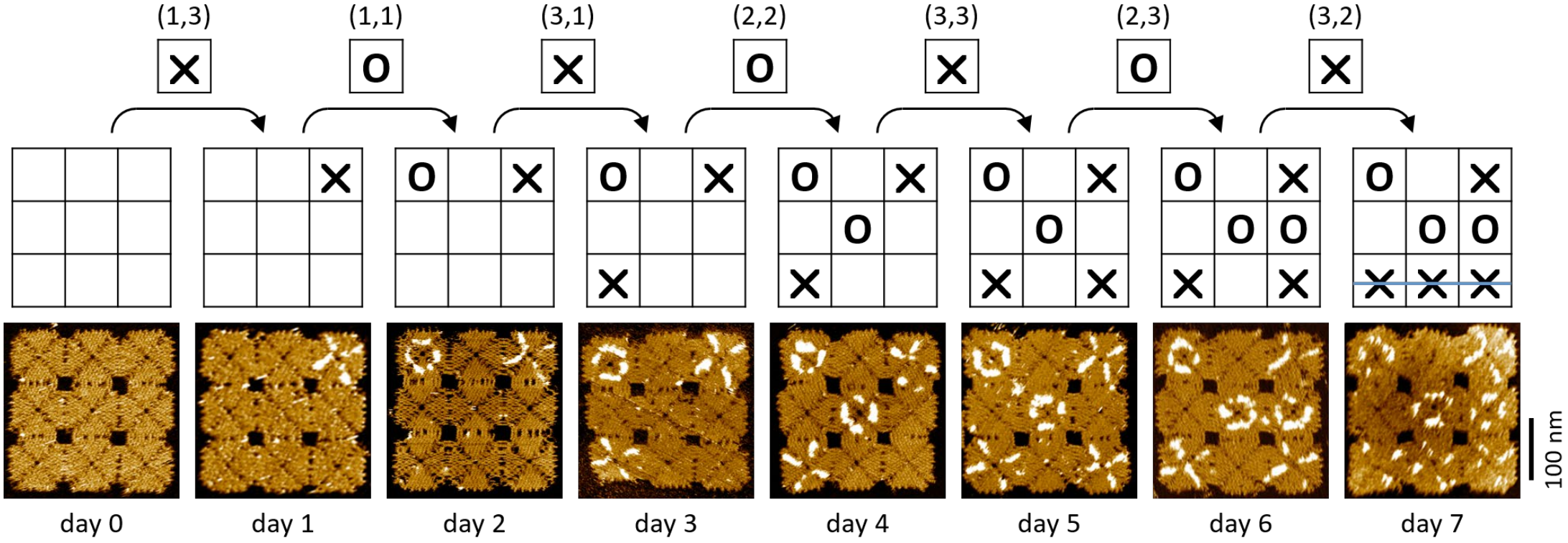


A tic-tac-toe game



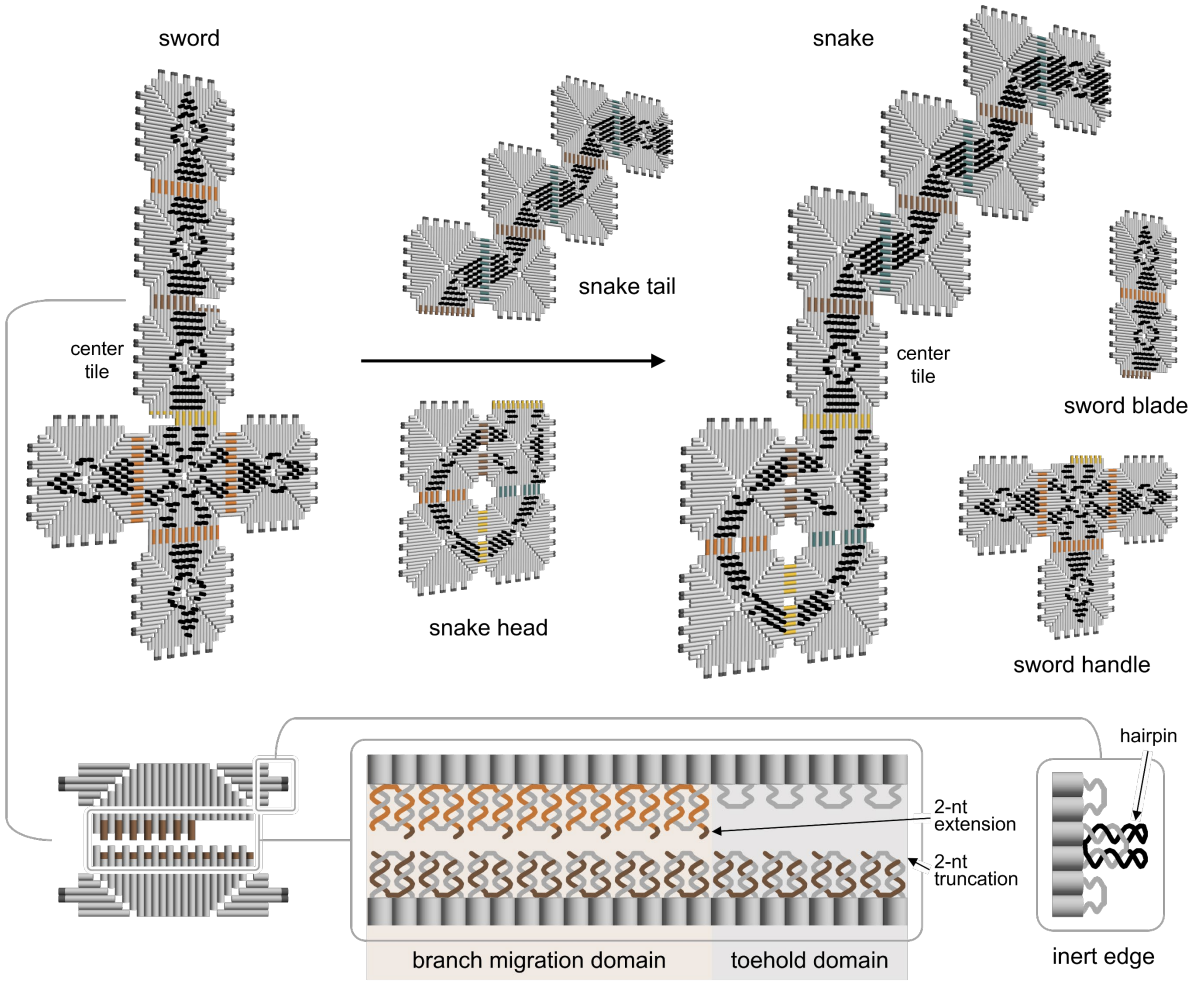
Philip Petersen

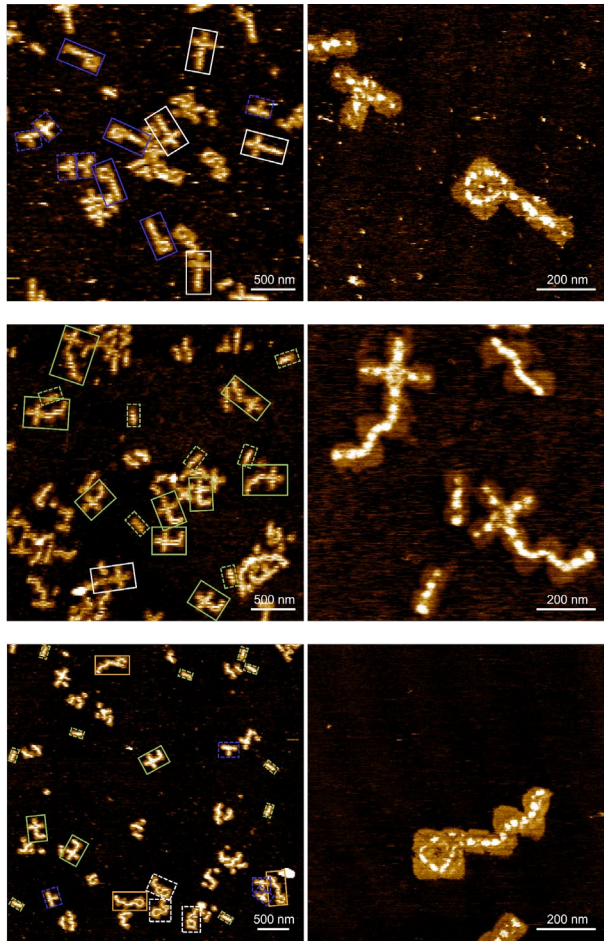
Greg Tikhomirov



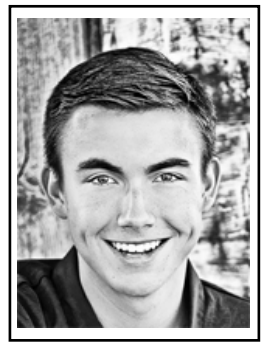
Petersen, Tikhomirov, and Qian, *Nature Communications* (2018)

Modular reconfiguration of DNA origami assemblies





Namita Sarraf

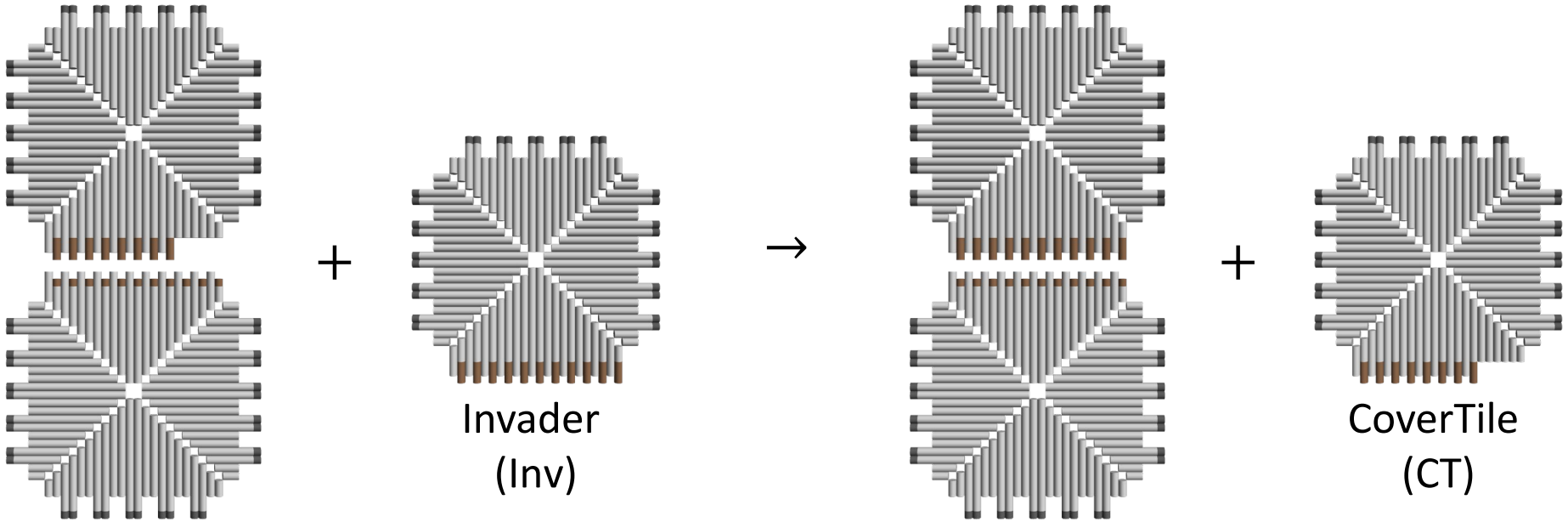


Kellen Rodriguez

Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

A simple tile displacement reaction

CT:BT + Inv → Inv:BT + CT



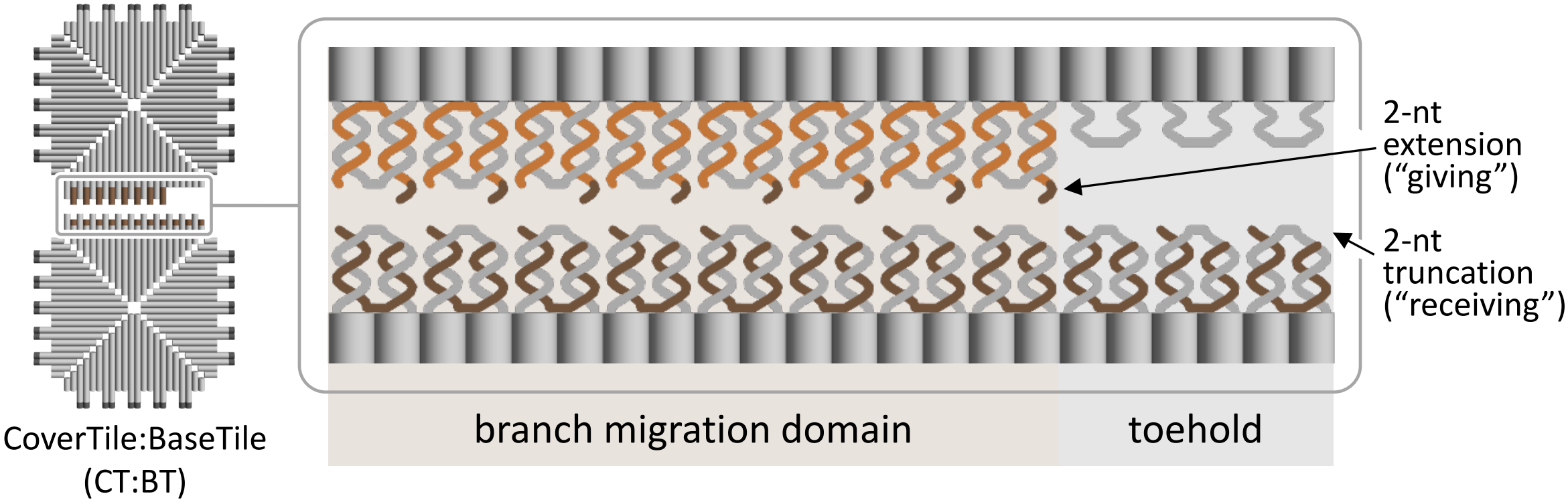
CoverTile:BaseTile
(CT:BT)

Invader
(Inv)

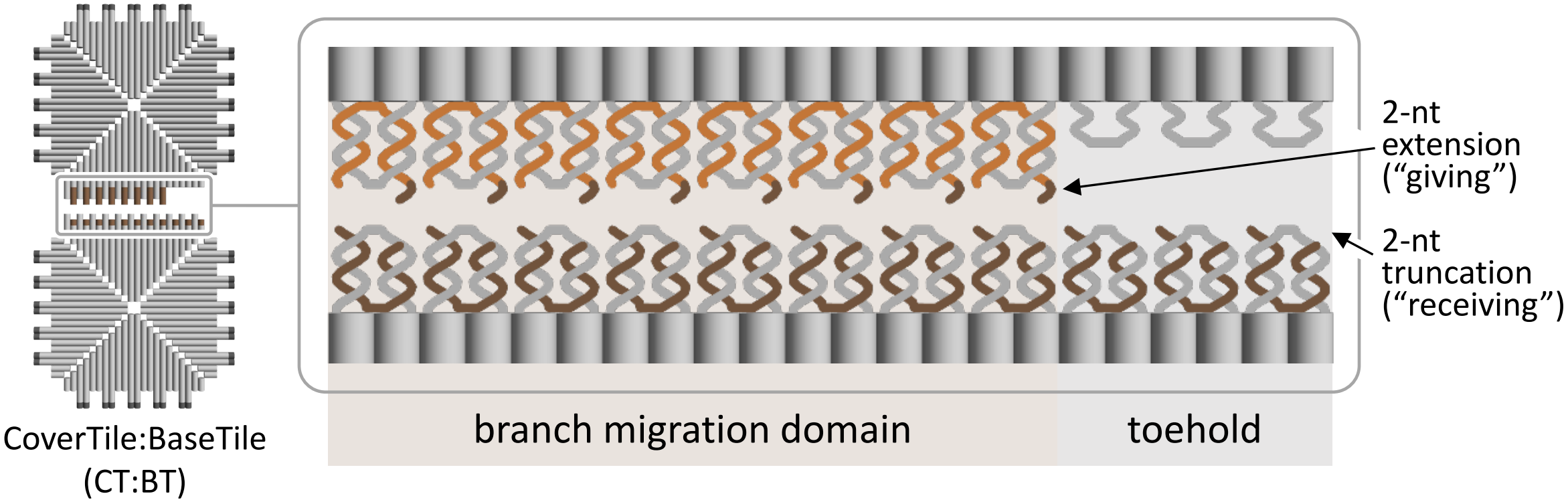
Invader:BaseTile
(Inv:BT)

CoverTile
(CT)

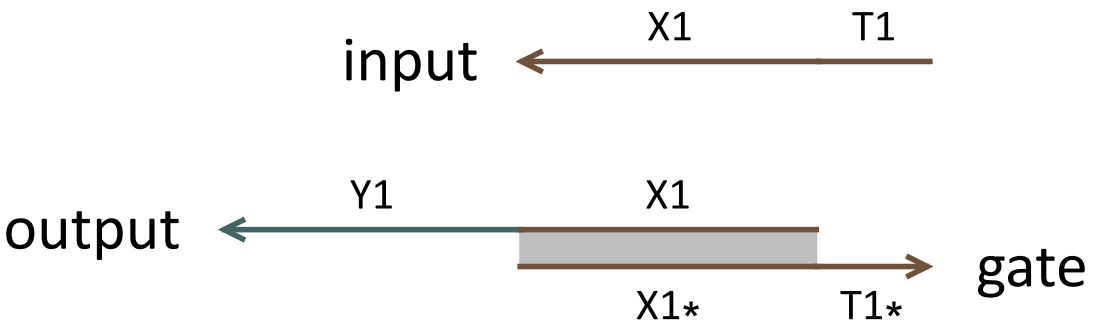
A simple tile displacement reaction



How many unique tile displacement reactions can be created using a square DNA origami tile that has a given width and a given scaffold sequence?



How many unique strand displacement reactions can be created using a gate strand that has a given length and sequence?



n unique reactions:
$X_i \rightarrow Y_i$ where $1 \leq i \leq n$

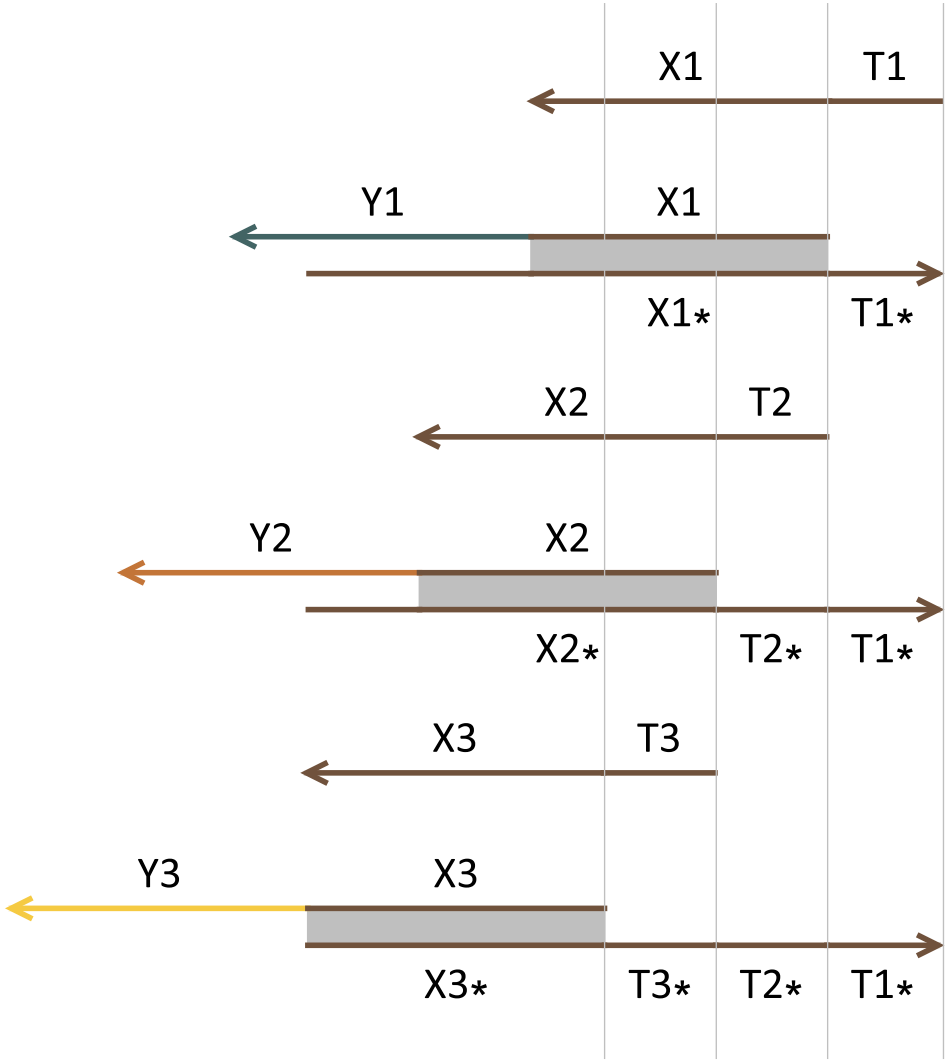
toehold = 6 nucleotides
branch migration = 16 nucleotides

How many unique strand displacement reactions can be created using a gate strand that has a given length and sequence?

n unique reactions:
$X_i \rightarrow Y_i$ where $1 \leq i \leq n$

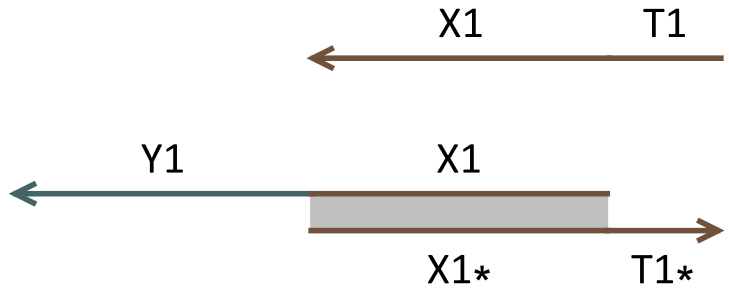
toehold = 6 nucleotides
branch migration = 16 nucleotides

34-nucleotide gate $\Longrightarrow$ 3 reactions



How many unique strand displacement reactions can be created using a gate strand that has a given length and sequence?

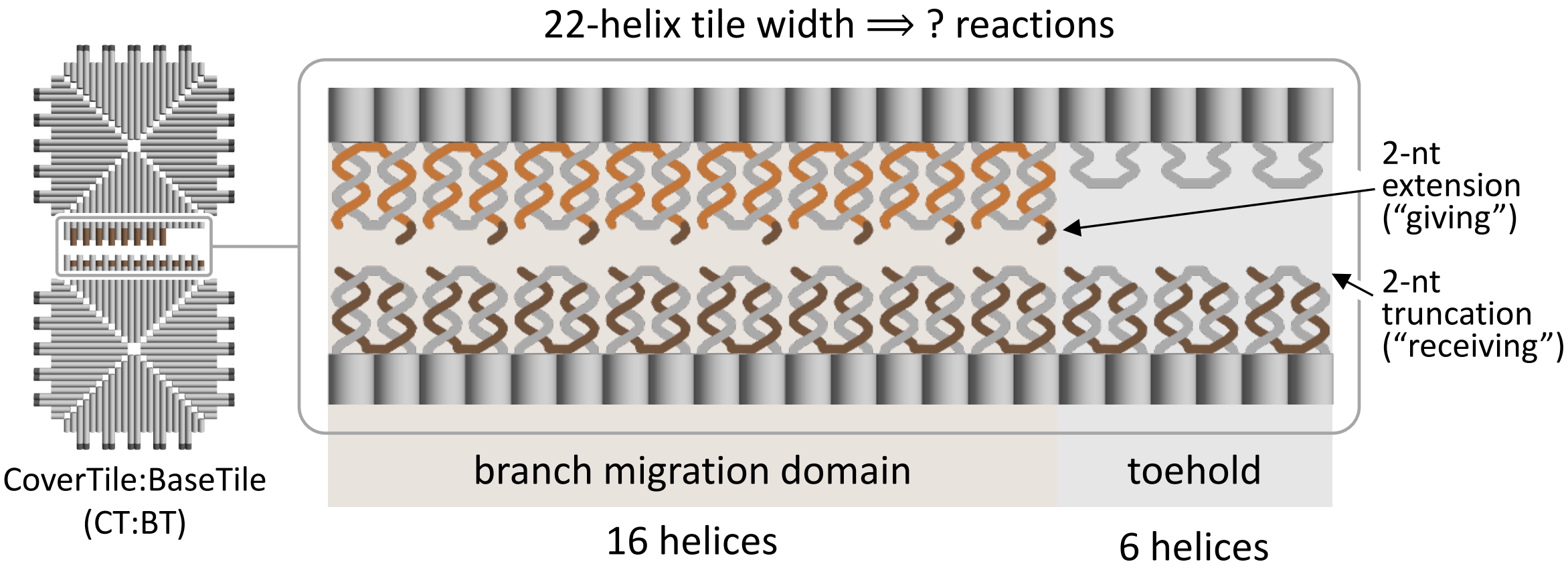
22-nucleotide gate $\Longrightarrow$ 1 reaction



n unique reactions:
$X_i \rightarrow Y_i$ where $1 \leq i \leq n$

toehold = 6 nucleotides
branch migration = 16 nucleotides

How many unique tile displacement reactions can be created using a square DNA origami tile that has a given width and a given scaffold sequence?



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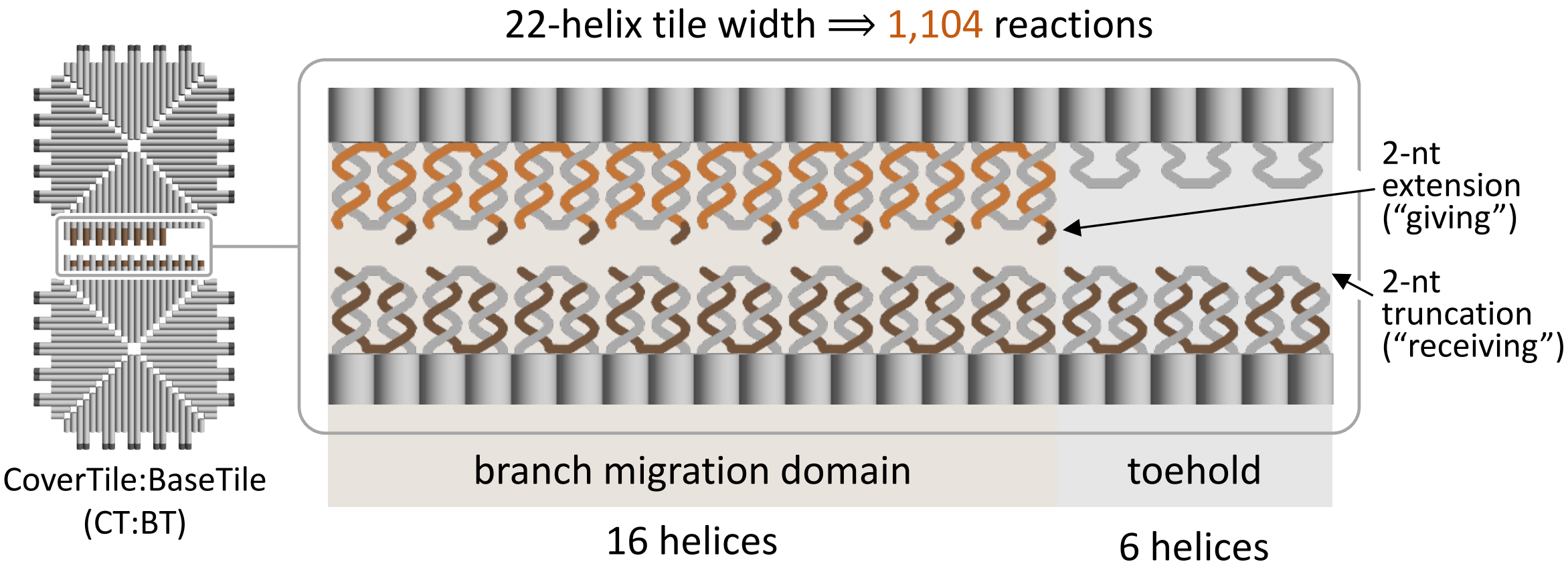

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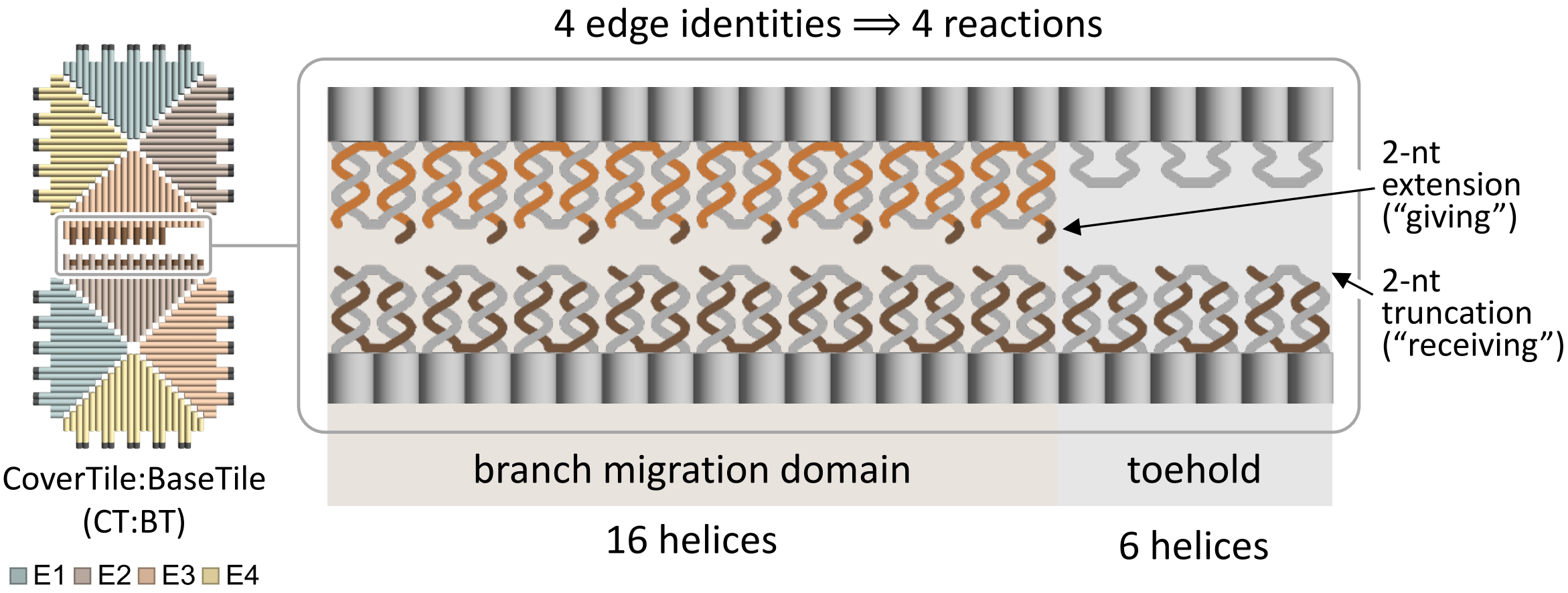

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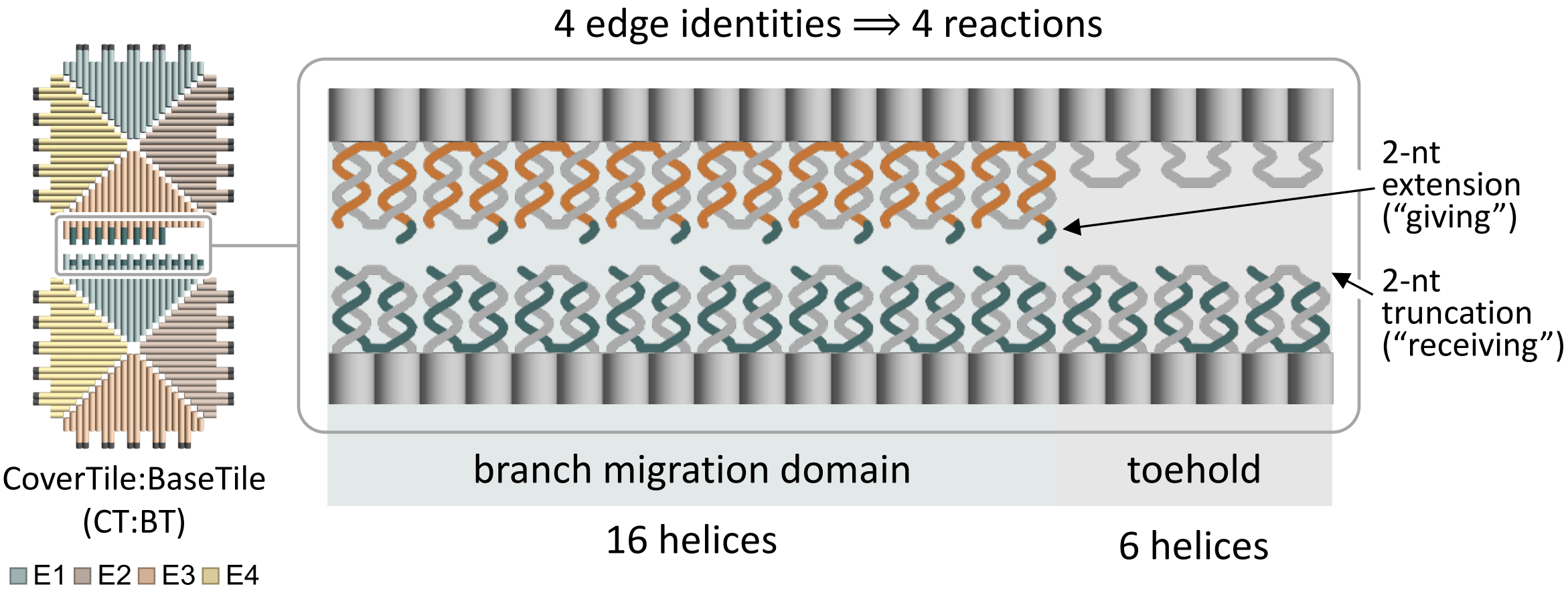
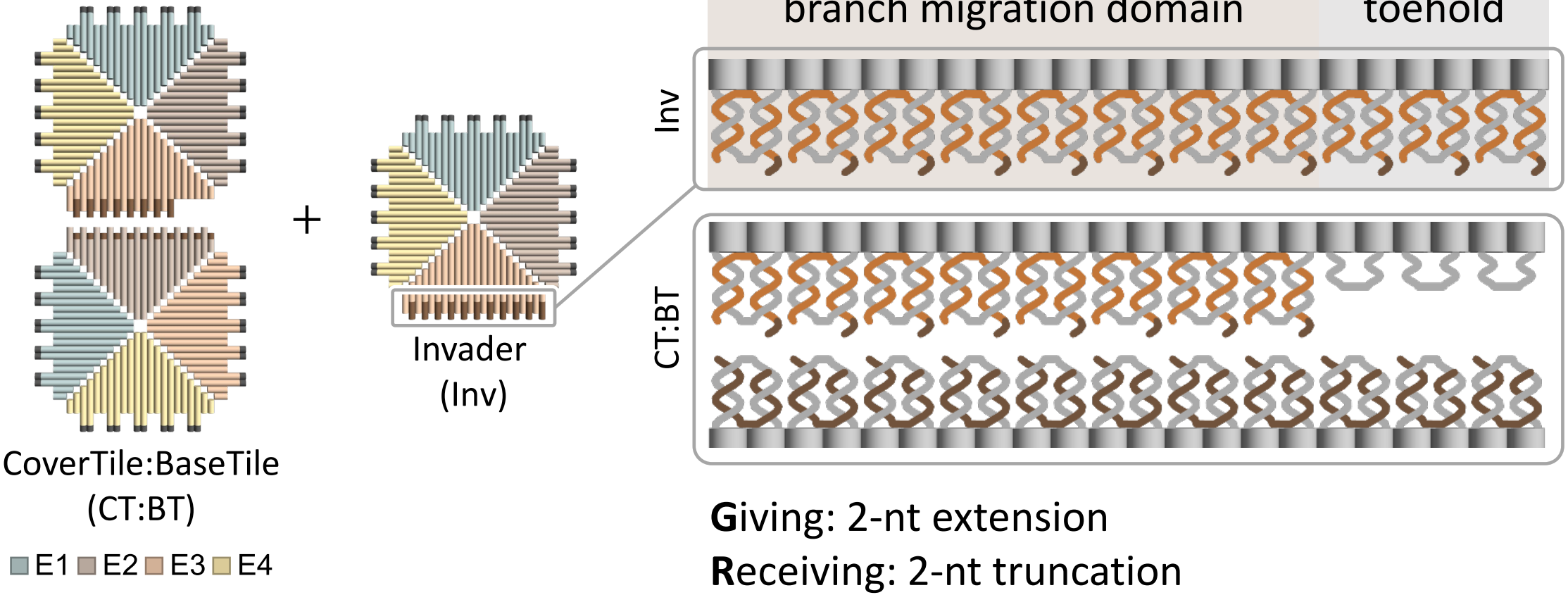


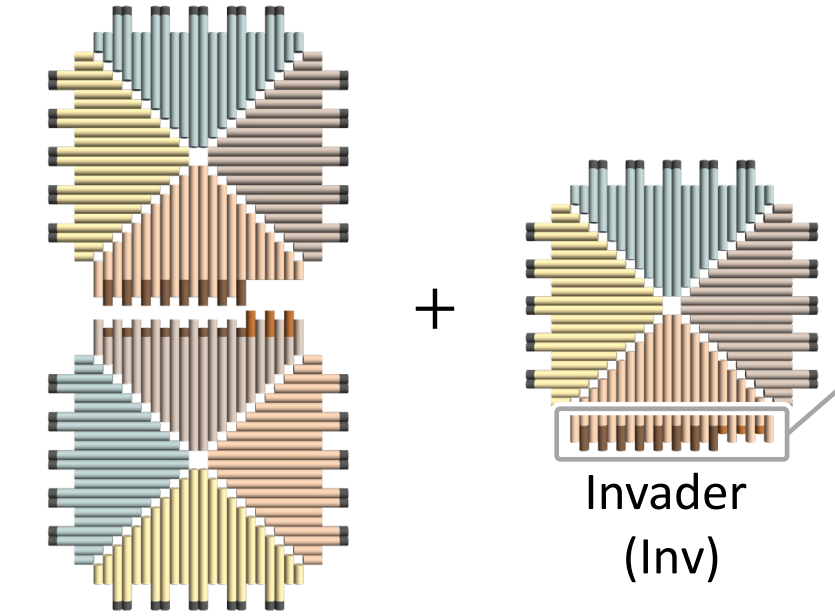
Same type of toehold and branch migration domains



Giving: 2-nt extension

Receiving: 2-nt truncation

Mixed types of toehold and branch migration domains

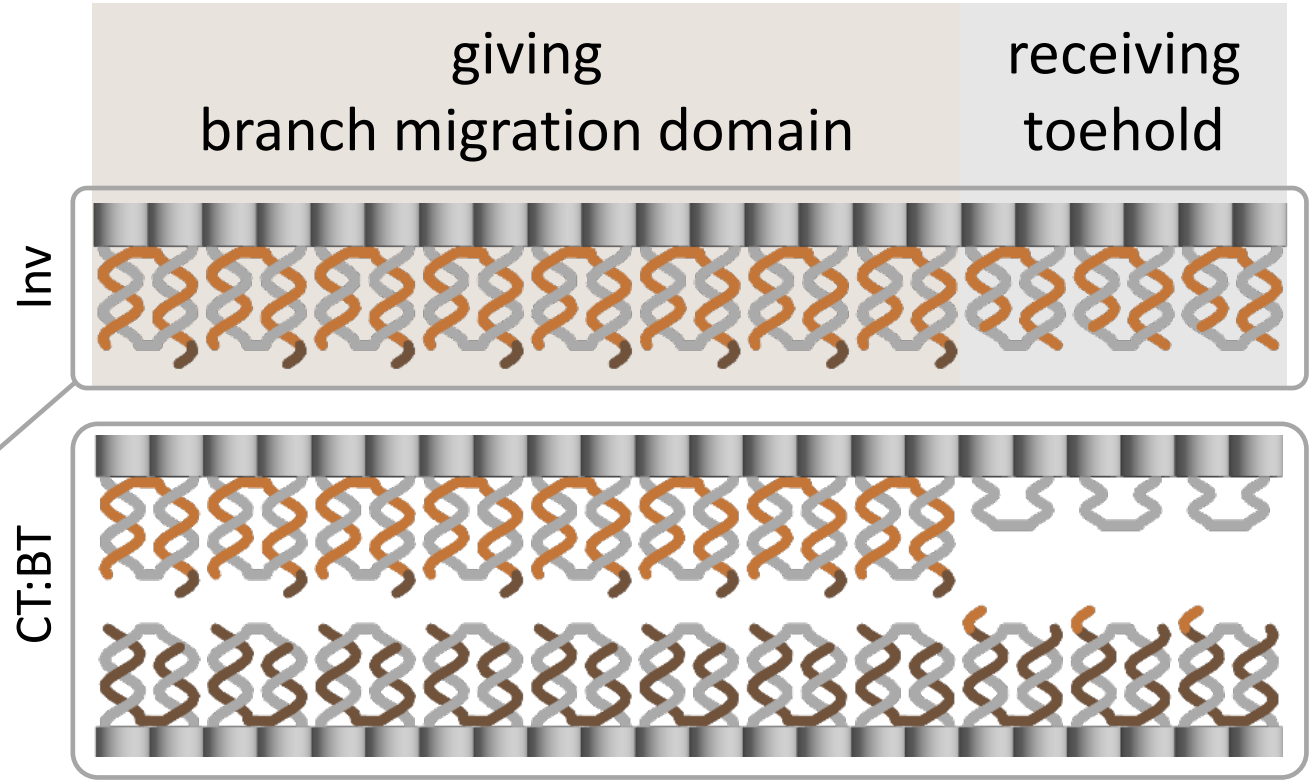


CoverTile:BaseTile
(CT:BT)

E1 E2 E3 E4

+

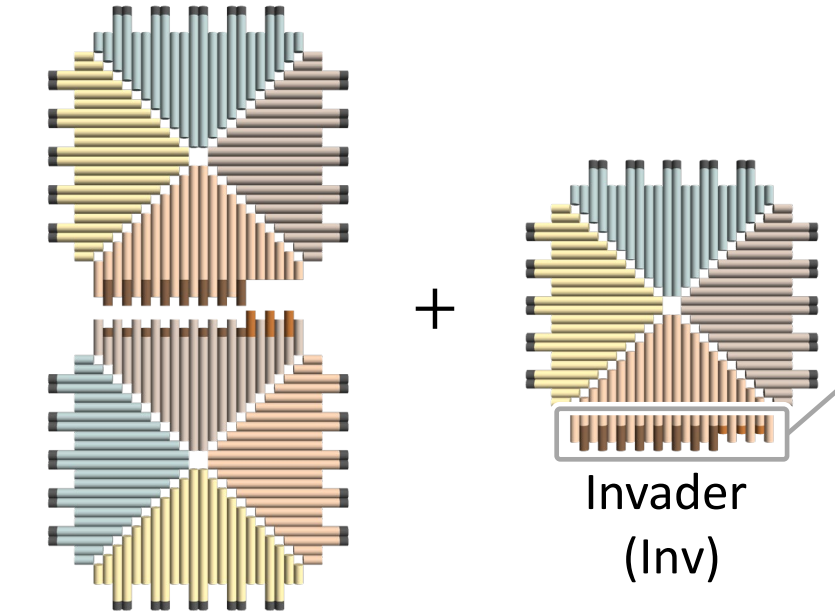
Invader
(Inv)



Giving: 2-nt extension
Receiving: 2-nt truncation

Mixed types of toehold and branch migration domains

4 toehold identities × 4 branch migration identities ⟹ 16 reactions

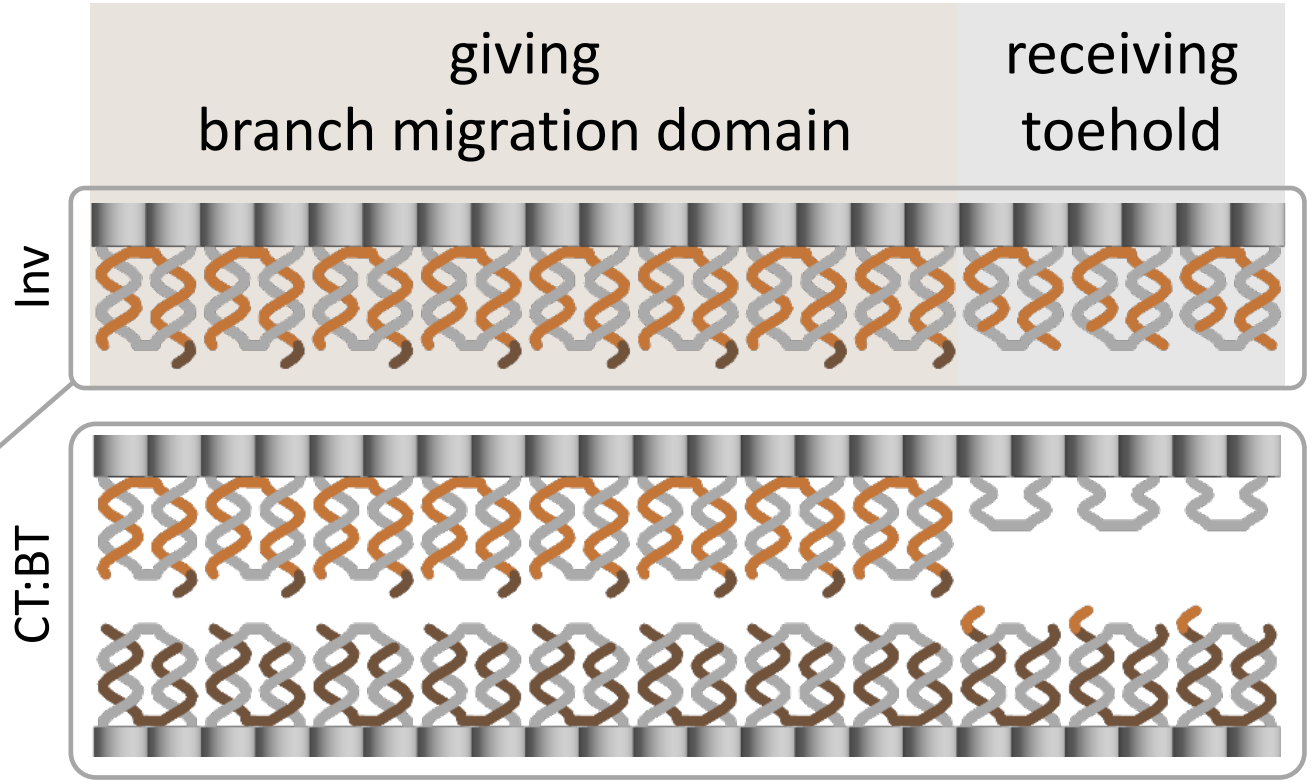


CoverTile:BaseTile
(CT:BT)

E1 E2 E3 E4

+

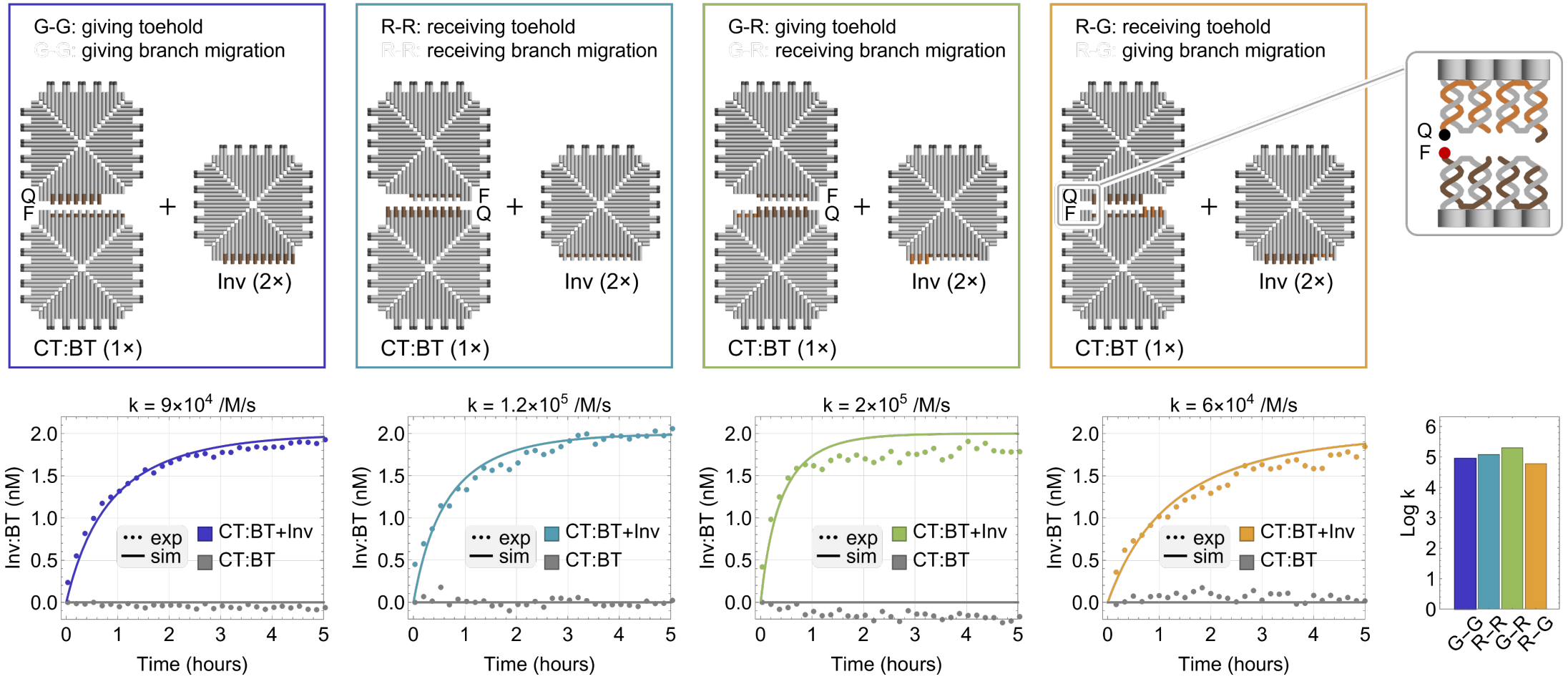
Invader
(Inv)



Giving: 2-nt extension

Receiving: 2-nt truncation

All four giving and receiving combinations of toehold and branch migration domains exhibited similar kinetics



Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

How many unique tile displacement reactions can be created using a square DNA origami tile that has a given width and a given scaffold sequence?

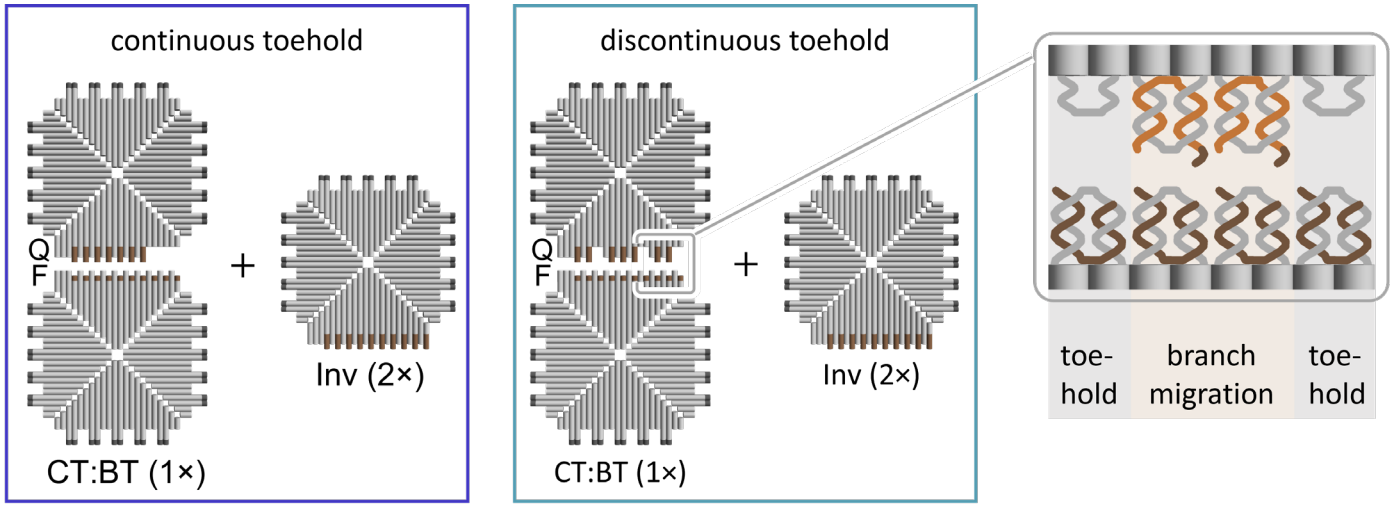
N_t: Number of toehold identities
N_b: Number of branch migration identities for a given toehold

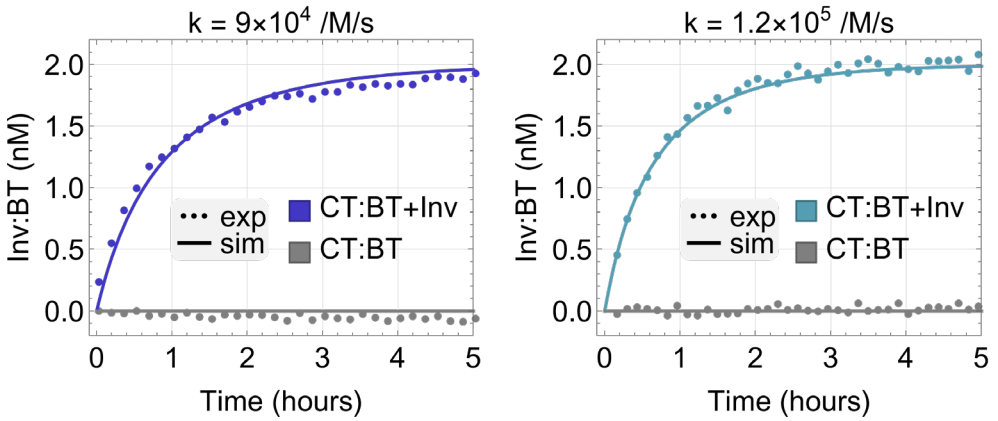
Number of distinct reactions: $N = N_t \times N_b$

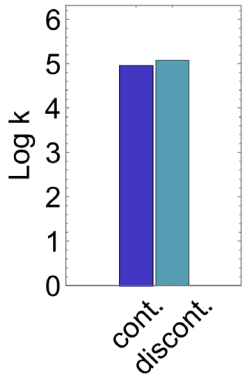
	N_t	N_b	N
G-G	4	1	4
R-R	4	1	4
G-R	4	4	16
R-G	4	4	16
Total			**40**

Giving toehold – **R**eceiving branch migration
Receiving toehold – **G**iving branch migration

Continuous and discontinuous toeholds exhibited similar kinetics

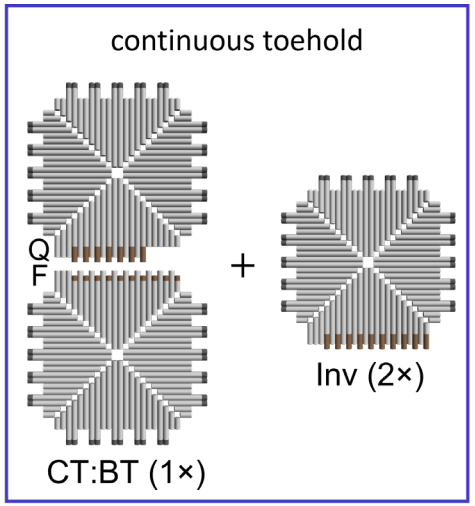


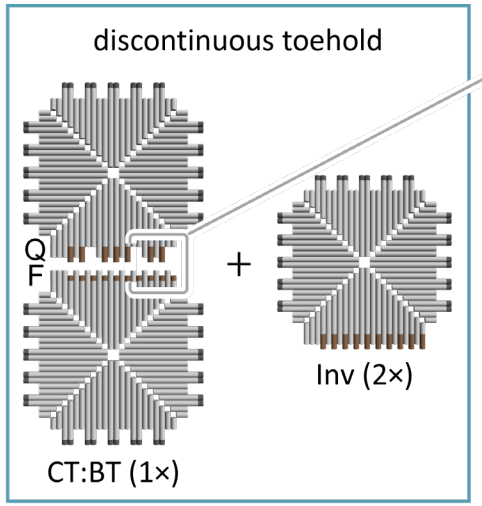


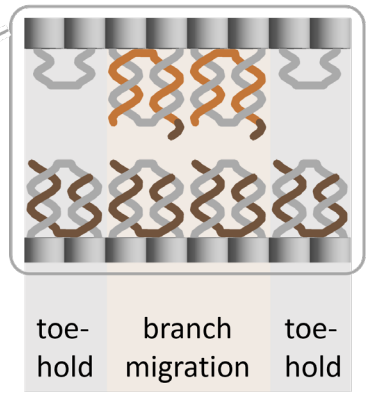


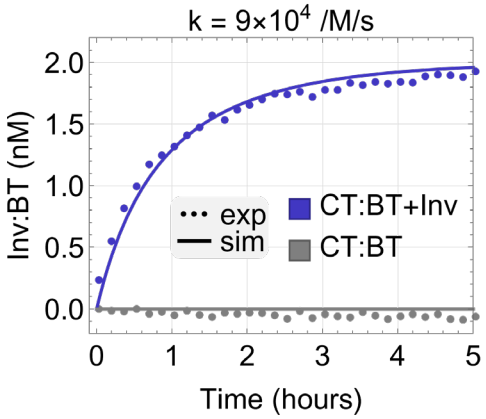
Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

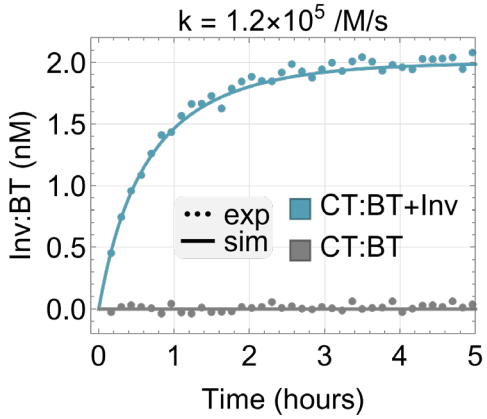
Continuous and discontinuous toeholds exhibited similar kinetics

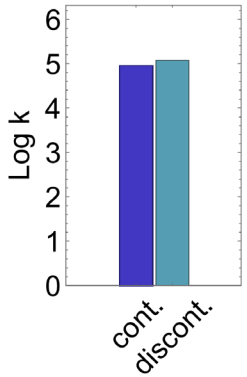




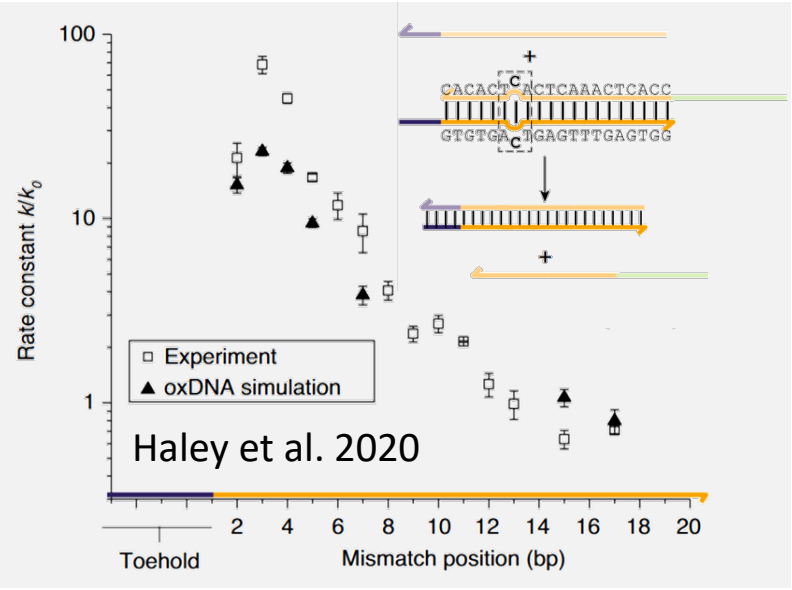






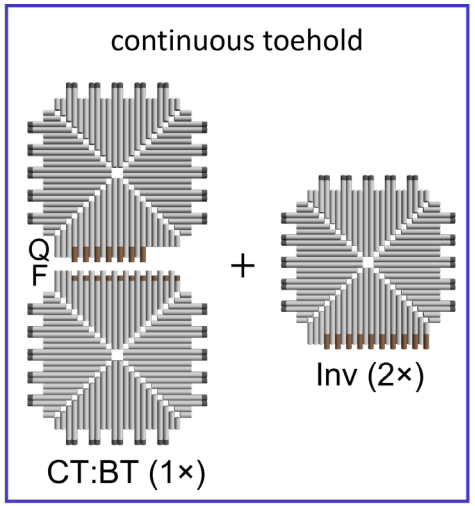


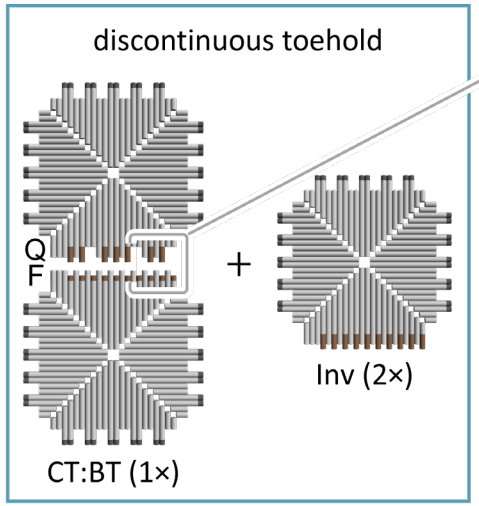
A discontinuous toehold is conceptually similar to mismatch elimination in strand displacement, but in comparison it has much faster kinetics. Why?

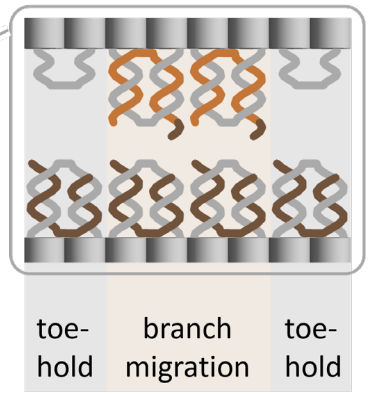


Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Continuous and discontinuous toeholds exhibited similar kinetics







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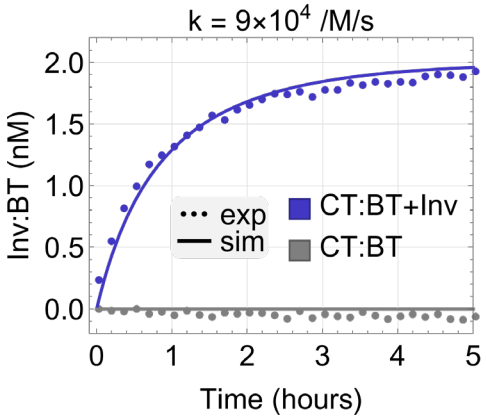
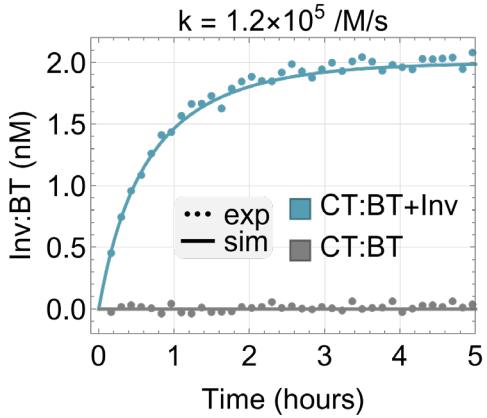
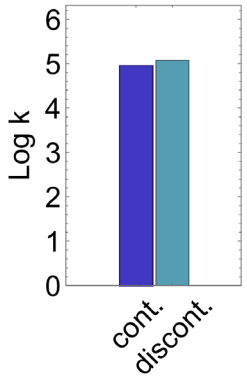




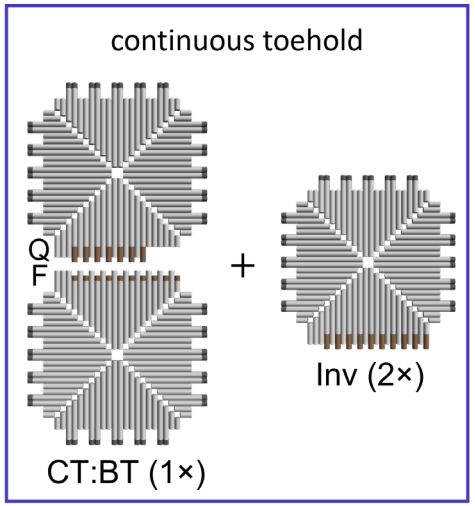


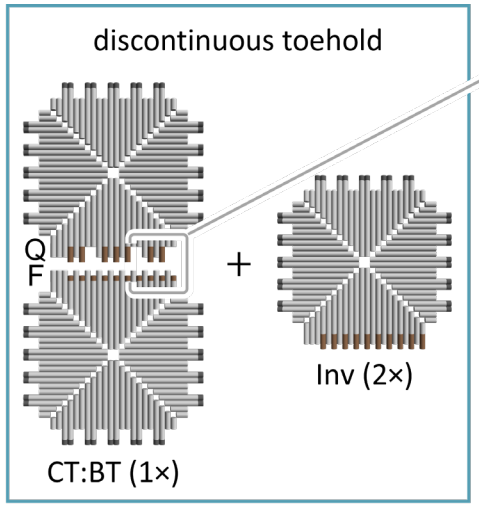
Explanations:

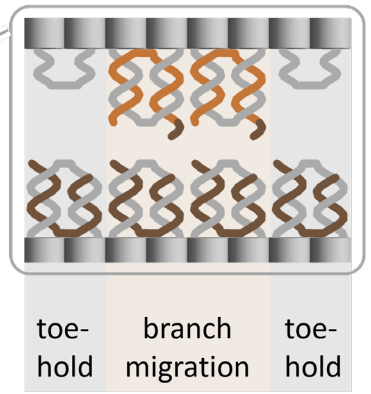
- less entropic cost

Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Continuous and discontinuous toeholds exhibited similar kinetics







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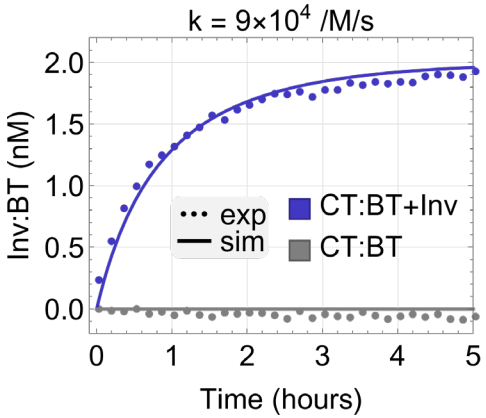
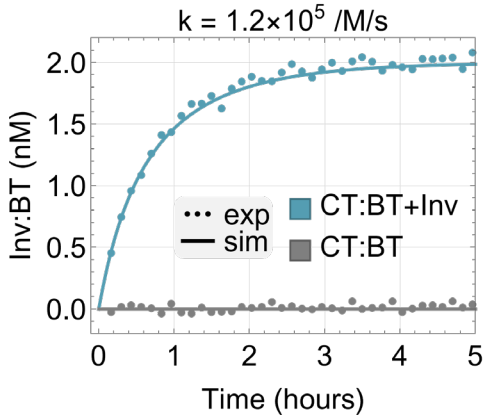
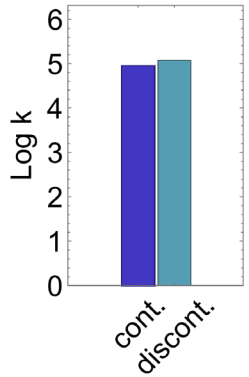






Explanations:

- less entropic cost
- larger space

Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

How many unique tile displacement reactions can be created using a square DNA origami tile that has a given width and a given scaffold sequence?

N_t: Number of toehold identities
N_b: Number of branch migration identities for a given toehold
N_p: Number of toehold locations

Number of distinct reactions: $N = N_t \times N_b \times N_p$

	N_t	N_b	N_p	N
G-G	4	1	1	4
R-R	4	1	1	4
G-R	4	4	17	272
R-G	4	4	17	272
Total				**552**

Toeholds consist of 3 out of 11 edge staples, no two share more than one staple in common.

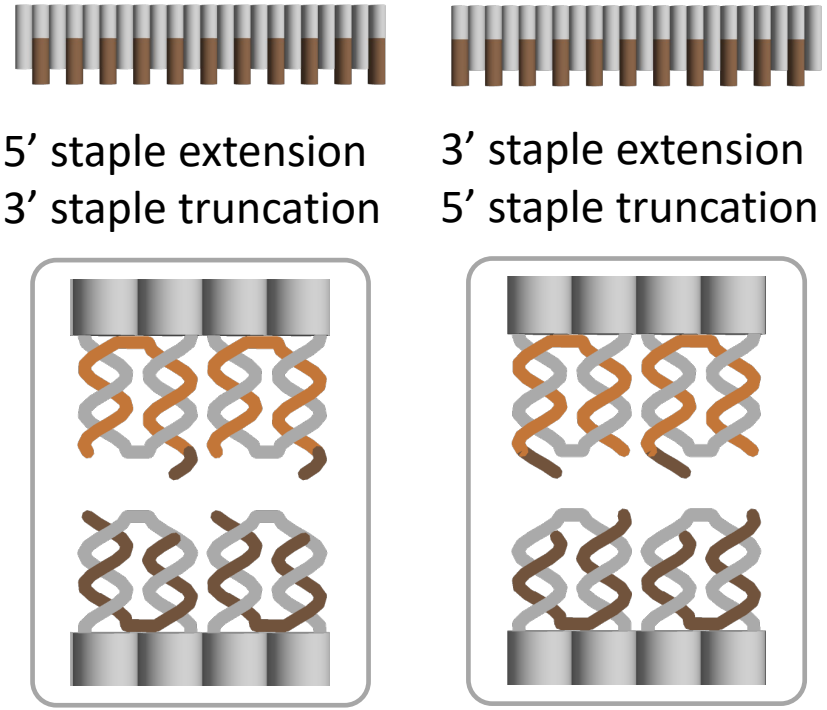
$N_p = 17$

How many unique tile displacement reactions can be created using a square DNA origami tile that has a given width and a given scaffold sequence?

N_t: Number of toehold identities
N_b: Number of branch migration identities for a given toehold
N_p: Number of toehold locations
N_e: Number of staple extension locations

Number of distinct reactions: $N = N_t \times N_b \times N_p \times N_e$

	N_t	N_b	N_p	N_e	N
G-G	4	1	1	2	8
R-R	4	1	1	2	8
G-R	4	4	17	2	544
R-G	4	4	17	2	544
Total					**1104**



$N_e = 2$

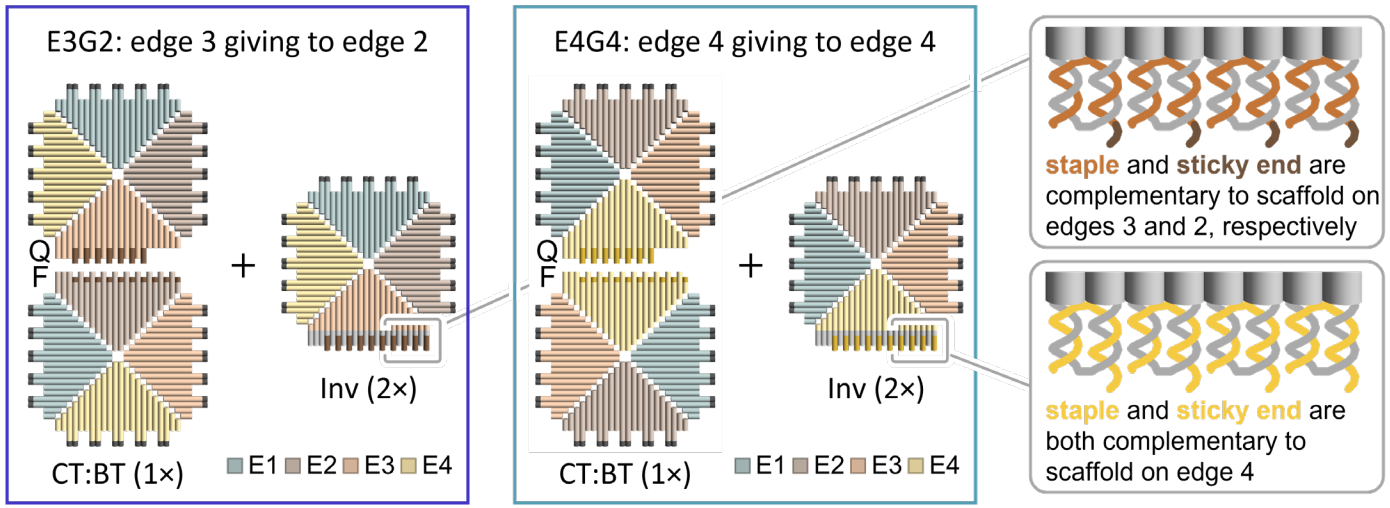
How robust are tile displacement reactions to spurious interactions such as cross-talk and occlusion?

N_t: Number of toehold identities
N_b: Number of branch migration identities for a given toehold
N_p: Number of toehold locations
N_e: Number of staple extension locations

Number of distinct reactions: $N = N_t \times N_b \times N_p \times N_e$

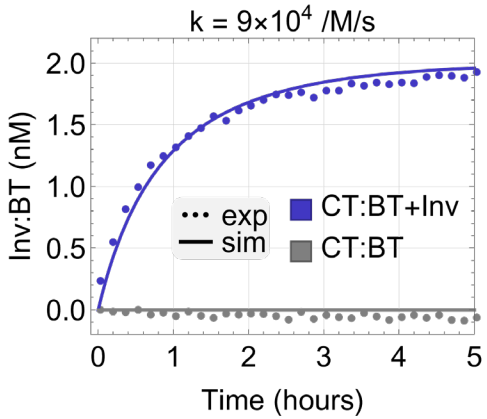
	N_t	N_b	N_p	N_e	N
G-G	4	1	1	2	8
R-R	4	1	1	2	8
G-R	4	4	17	2	544
R-G	4	4	17	2	544
Total					**1104**

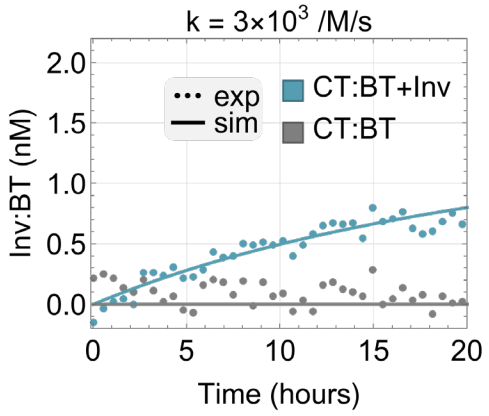
Self-occluding edges exhibited slower kinetics

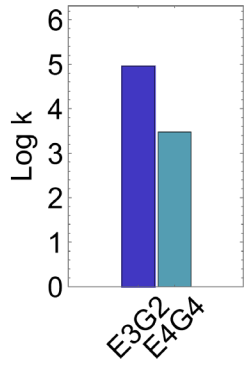


$x \neq y$

$x = y$







$ExGy$ indicates edge x giving to edge y, where $x, y \in \{1, 2, 3, 4\}$

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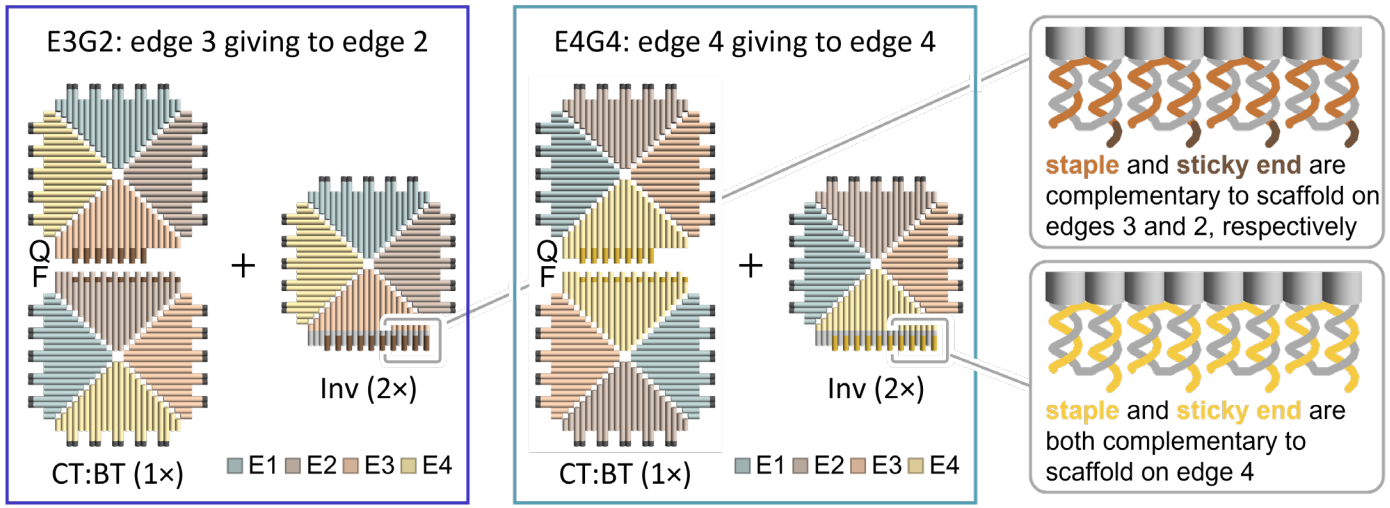
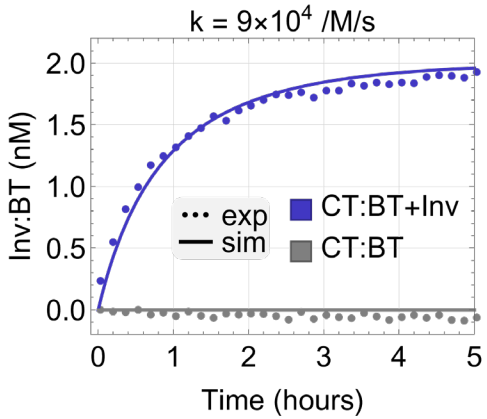
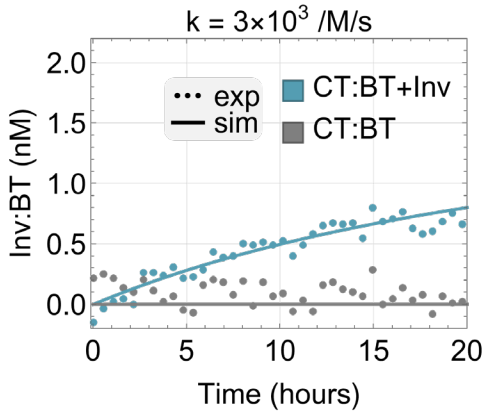
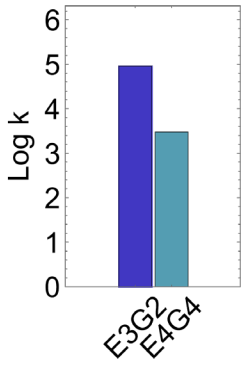
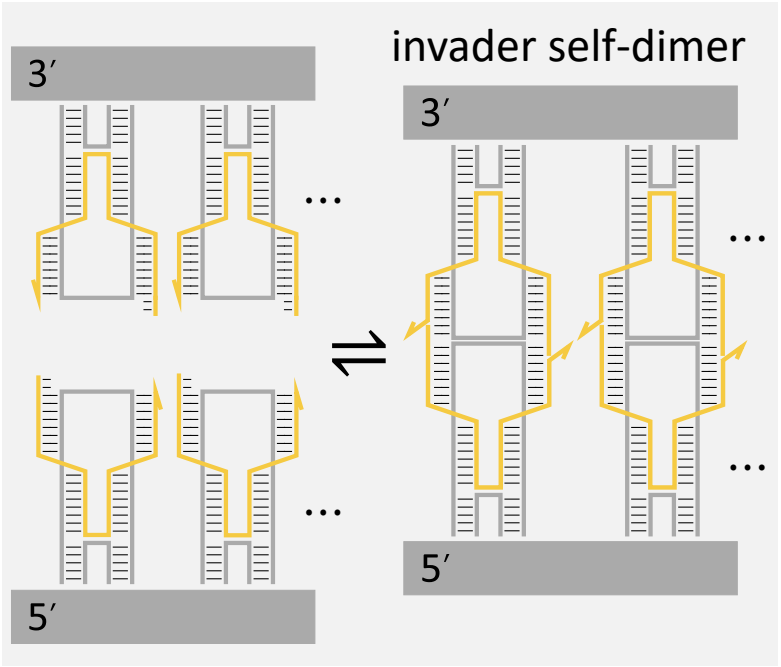
Self-occluding edges exhibited slower kinetics











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How robust are tile displacement reactions to spurious interactions such as cross-talk and occlusion?

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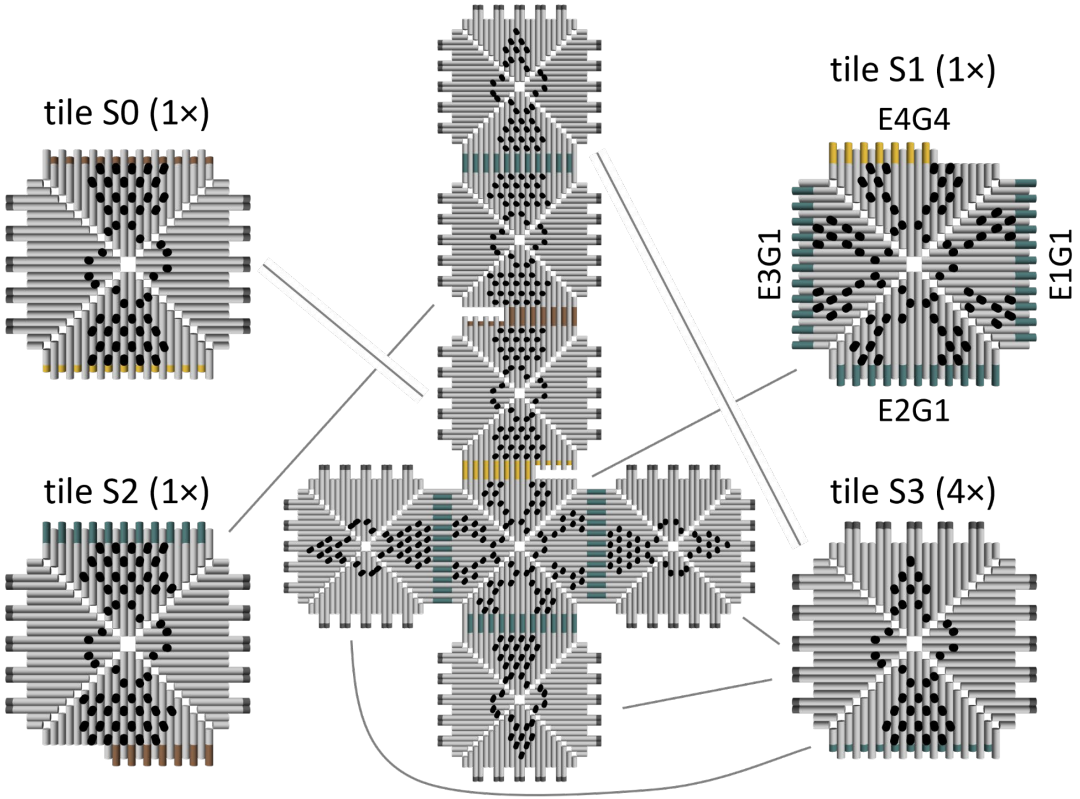
N_p: Number of toehold locations
N_e: Number of staple extension locations

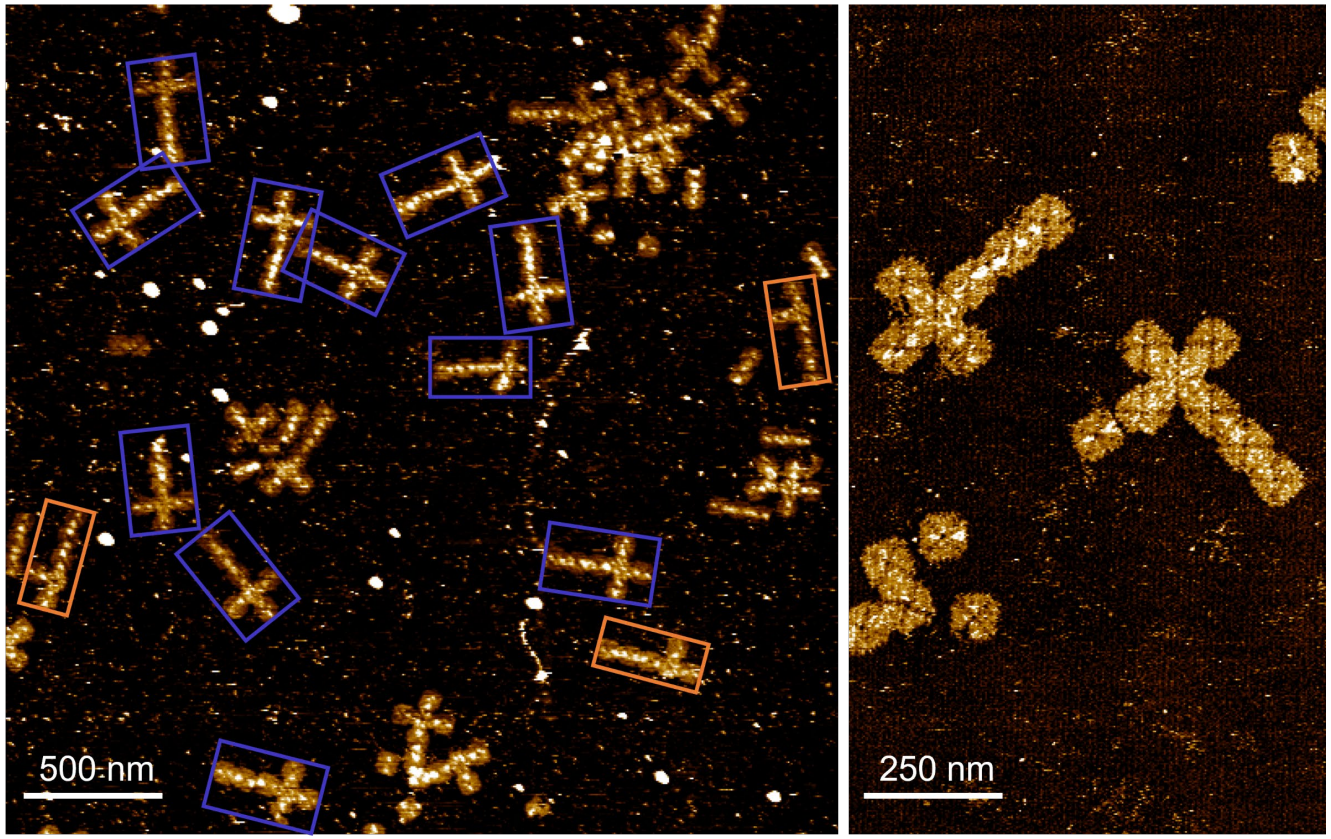
Number of distinct reactions: $\boldsymbol{N = N_t \times N_b \times N_p \times N_e}$

	N_t	N_b	N_p	N_e	N
G-G	4	1	1	2	8
R-R	4	1	1	2	8
G-R	4	3	17	2	408
R-G	4	3	17	2	408
Total					**832**

To maintain a controlled tile displacement rate, self-occluding edges should be avoided.

Does self-occluding edges affect the yield of tile assembly?





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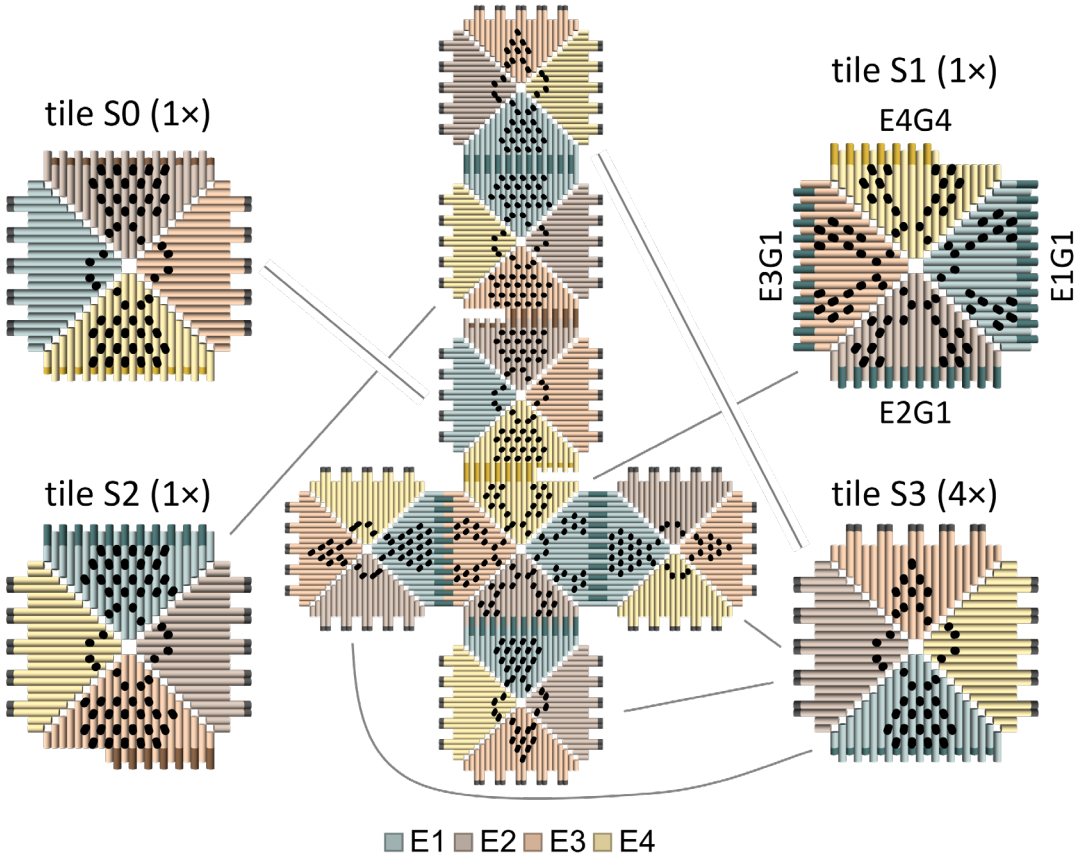
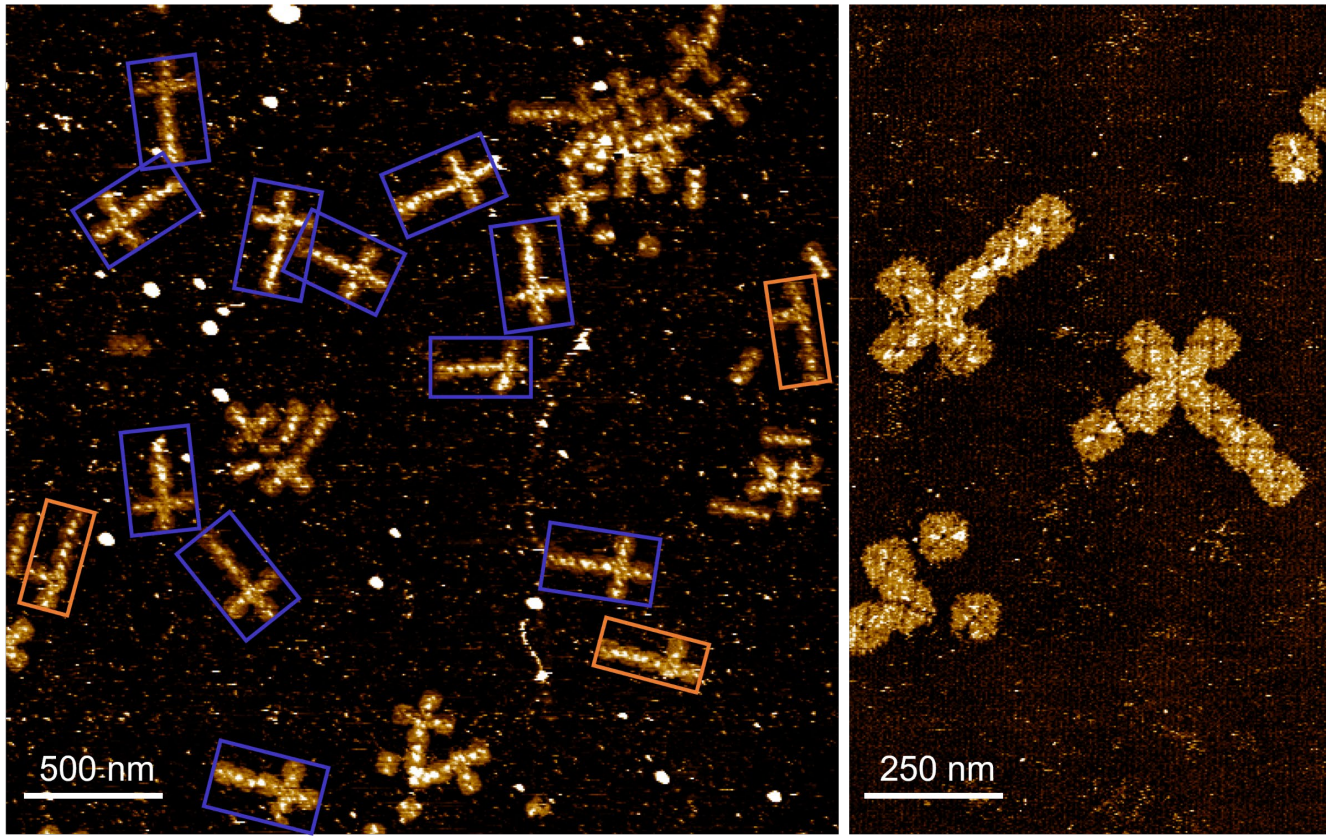
Does self-occluding edges affect the yield of tile assembly?





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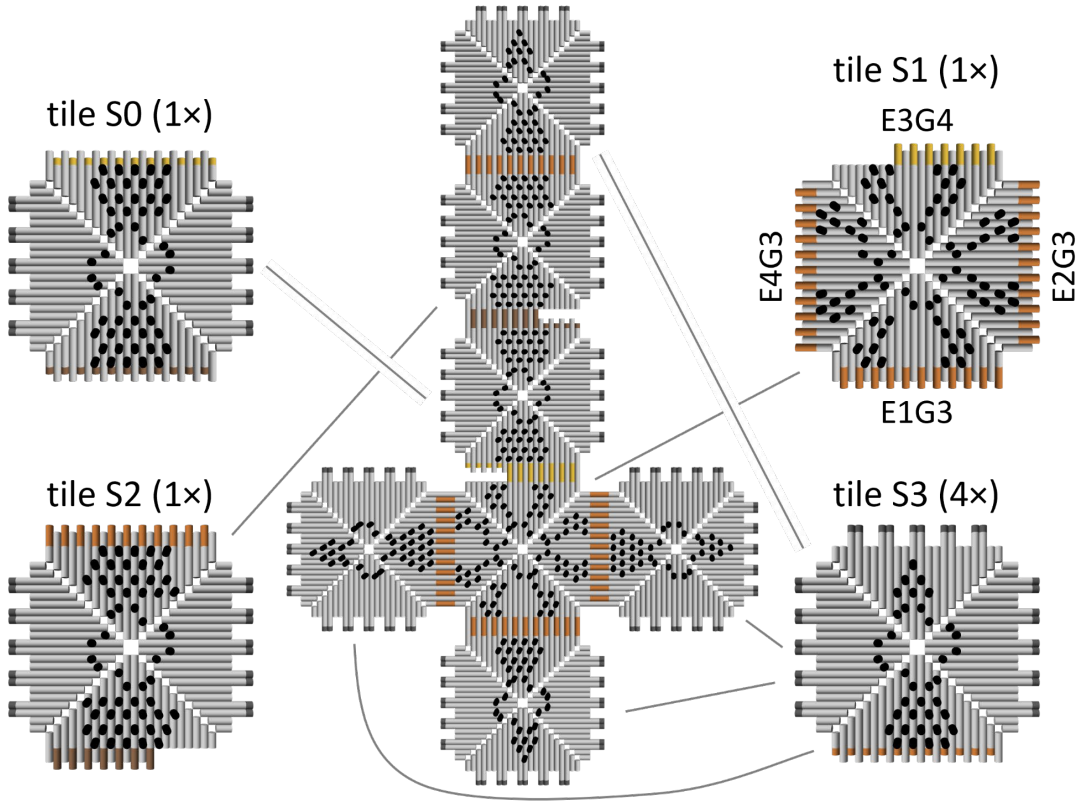
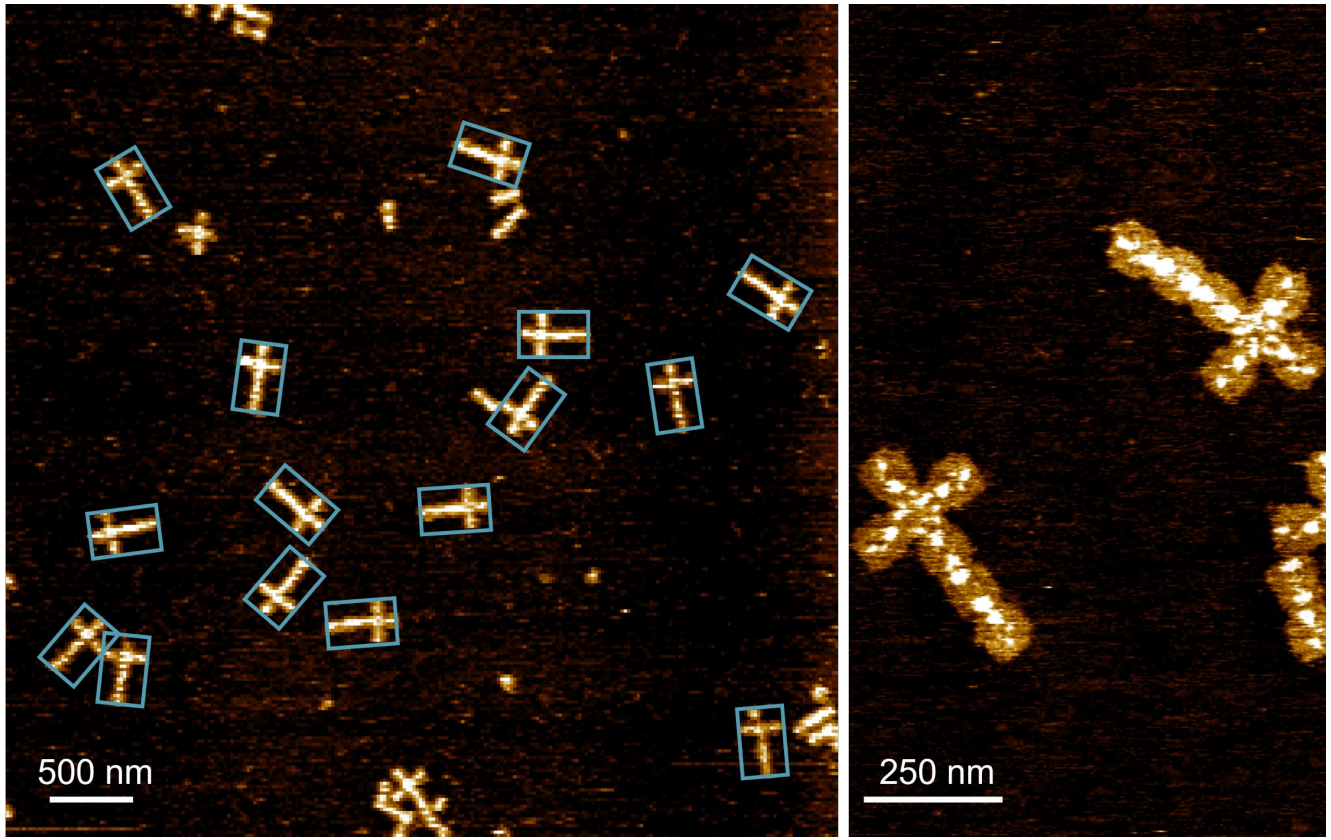
Does self-occluding edges affect the yield of tile assembly?





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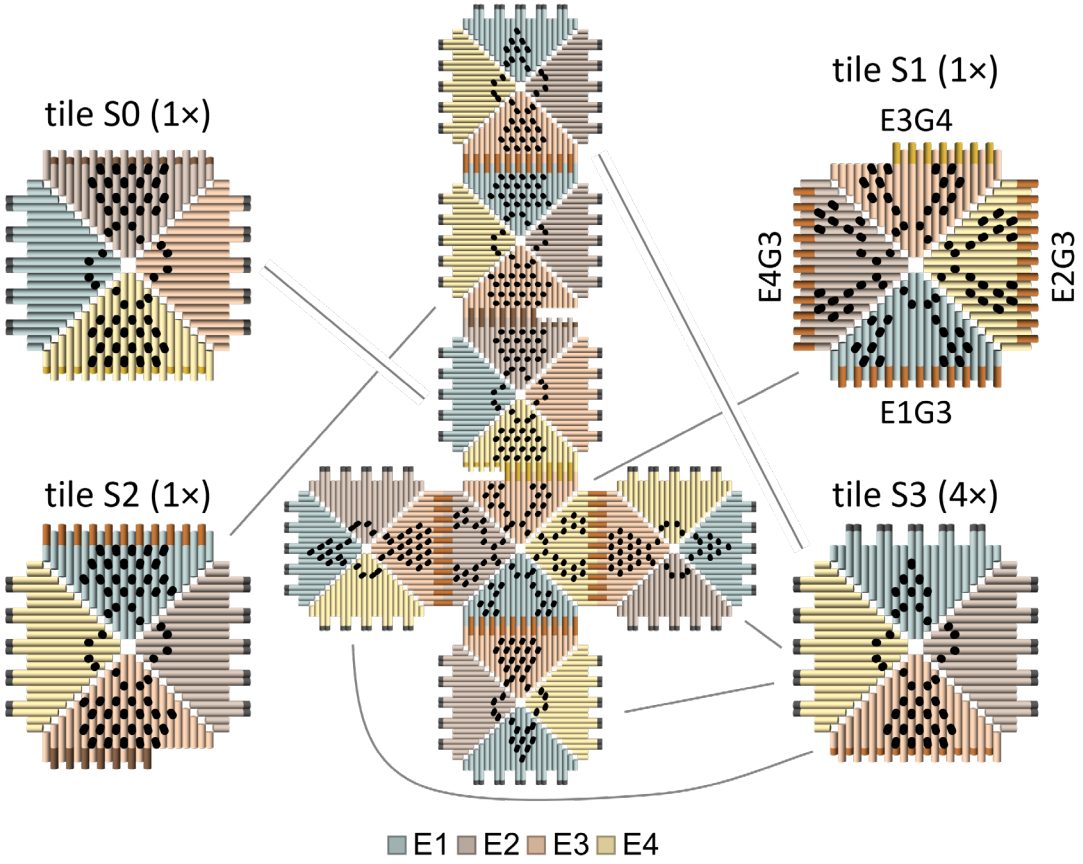
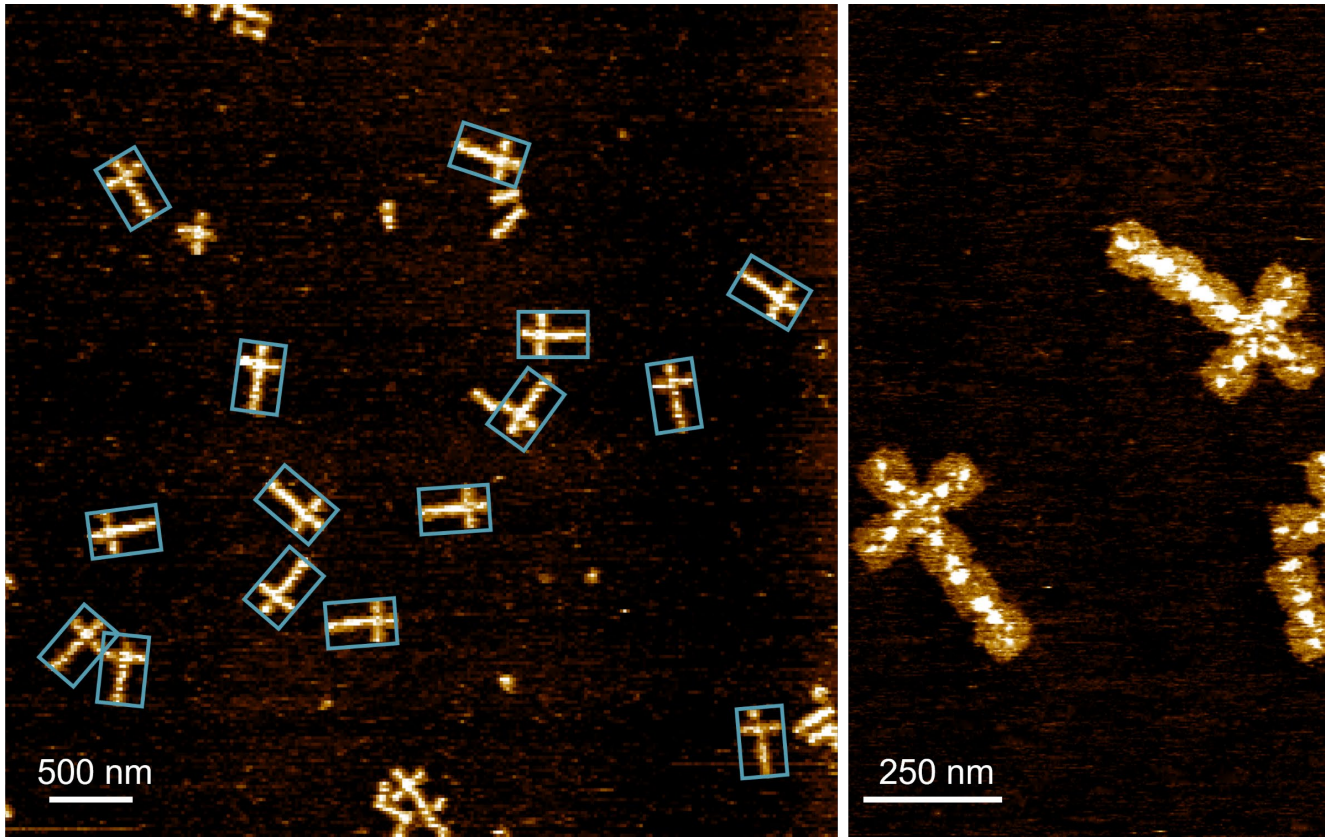
Does self-occluding edges affect the yield of tile assembly?





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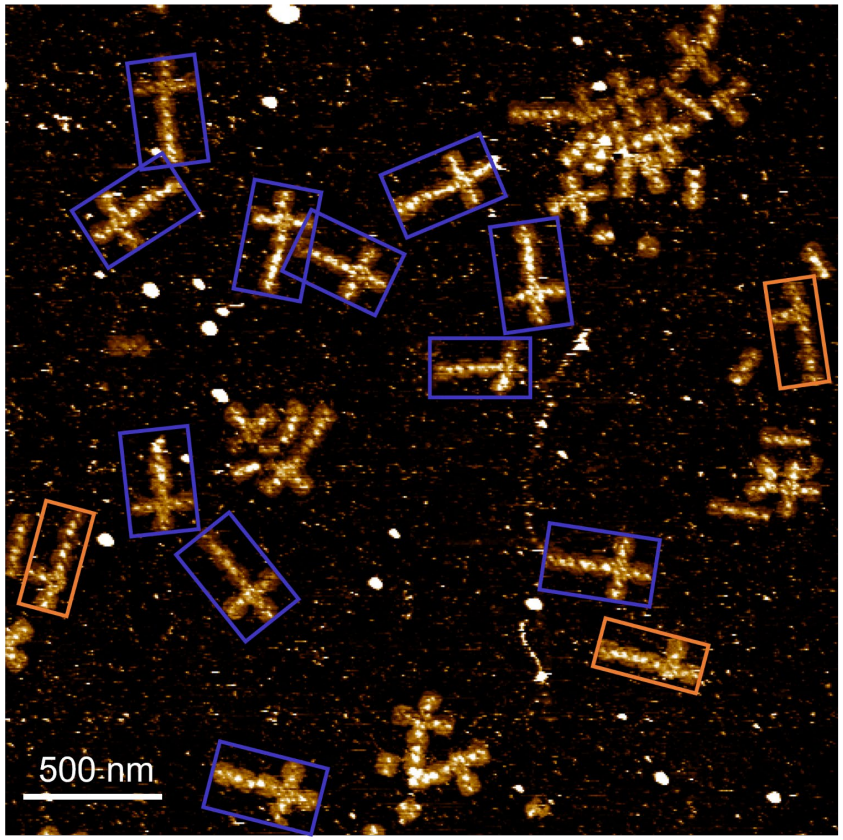
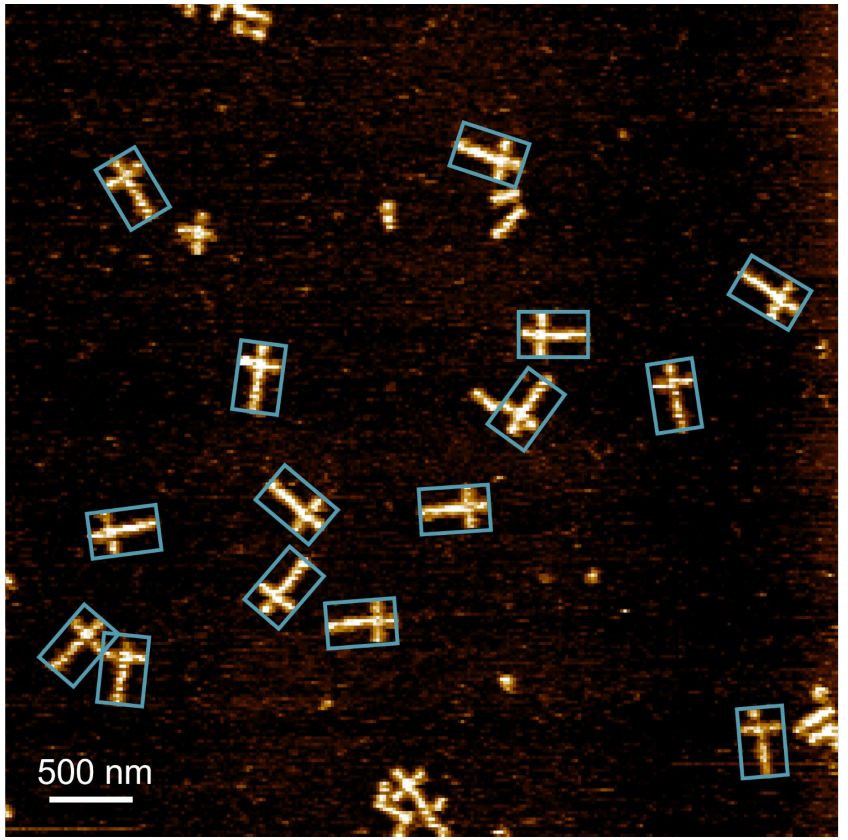
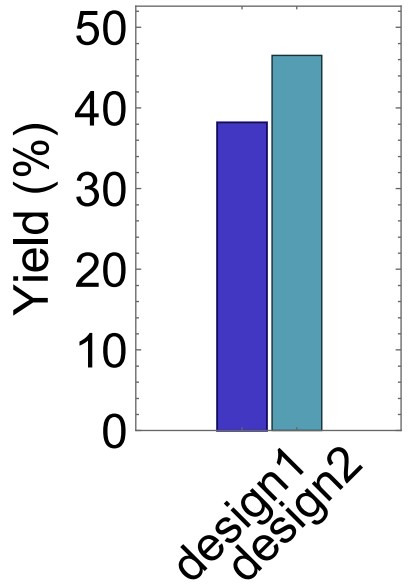
Does self-occluding edges affect the yield of tile assembly? Yes







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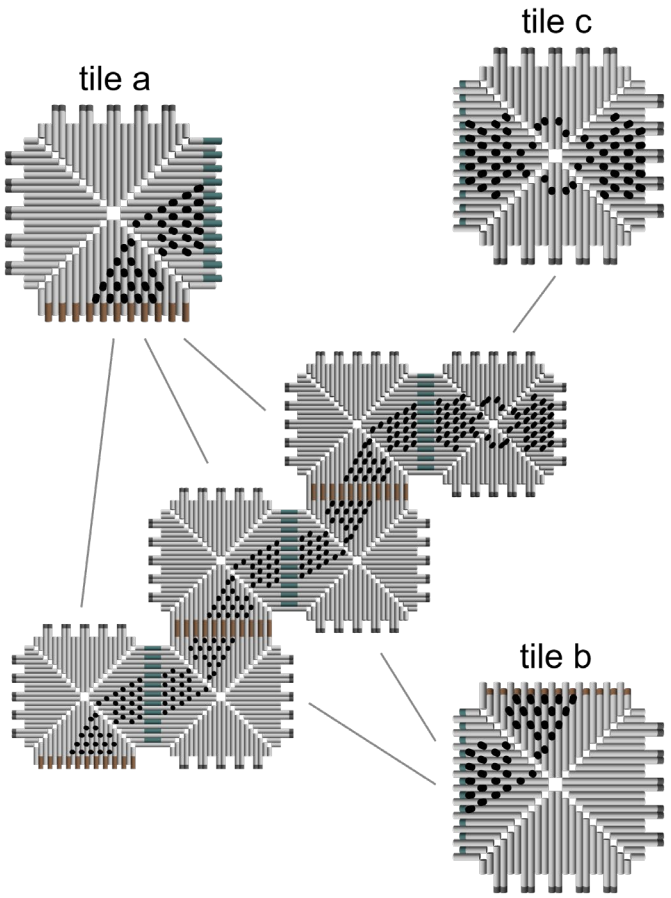
Invaders with variable sizes



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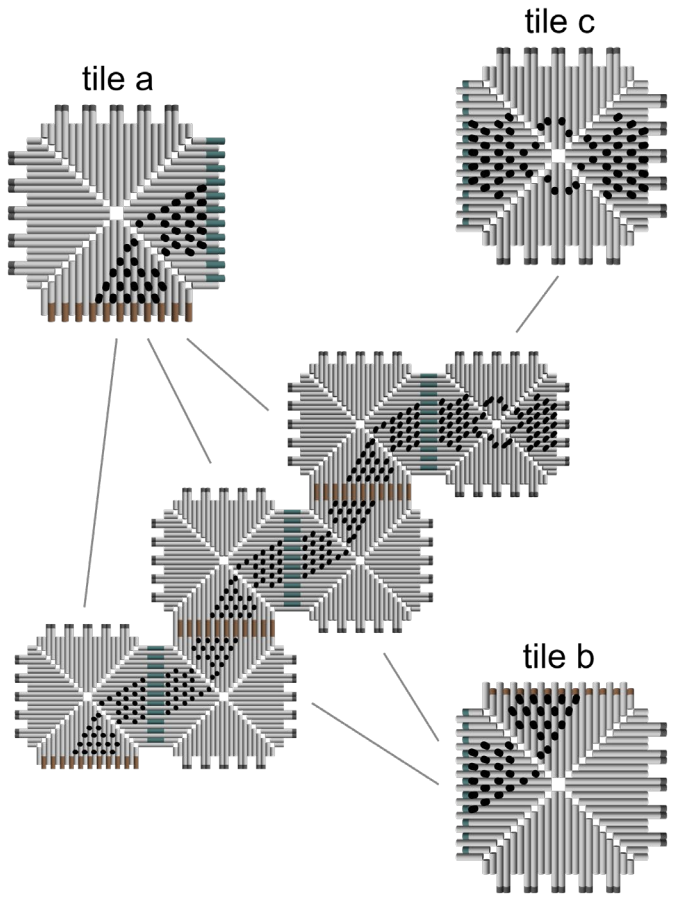
Invaders with variable sizes



monomers $= \{a, b, c\}$
rules $= \{\{a, b\}, \{a, c\}, \{b, a\}\}$
rates $= \{k, k, k\}$, where $k = 4.5 \times 10^5$
maxlength $= 20$
reactions $=$ polymerCRN[monomers, rules, rates, maxlength]

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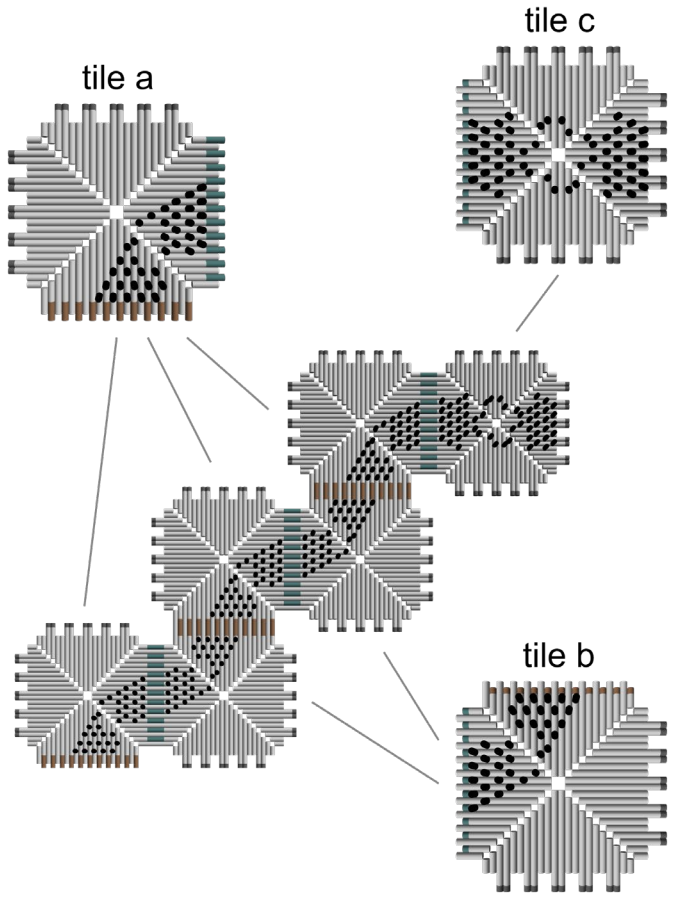
Invaders with variable sizes



monomers $= \{a, b, c\}$
rules $= \{\{a, b\}, \{a, c\}, \{b, a\}\}$
rates $= \{k, k, k\}$, where $k = 4.5 \times 10^5$
maxlength $= 20$
reactions $=$ polymerCRN[monomers, rules, rates, maxlength]

Example reactions enumerated with maxlength = 3:

$a + b \xrightarrow{k} ab$ $\quad b + a \xrightarrow{k} ba$ $\quad ba + b \xrightarrow{k} bab$

$a + c \xrightarrow{k} ac$ $\quad b + ab \xrightarrow{k} bab$ $\quad ba + c \xrightarrow{k} bac$

$ab + a \xrightarrow{k} aba$ $\quad b + ac \xrightarrow{k} bac$ $\quad a + ba \xrightarrow{k} aba$

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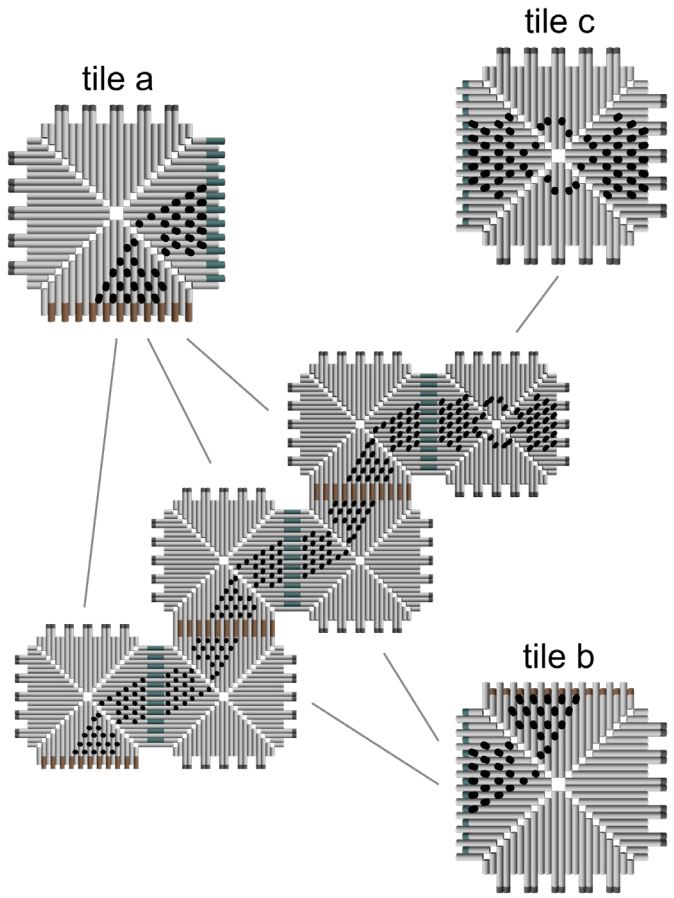
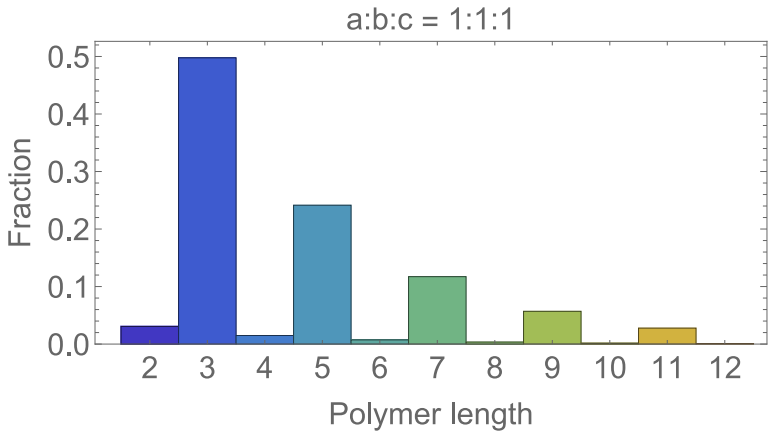
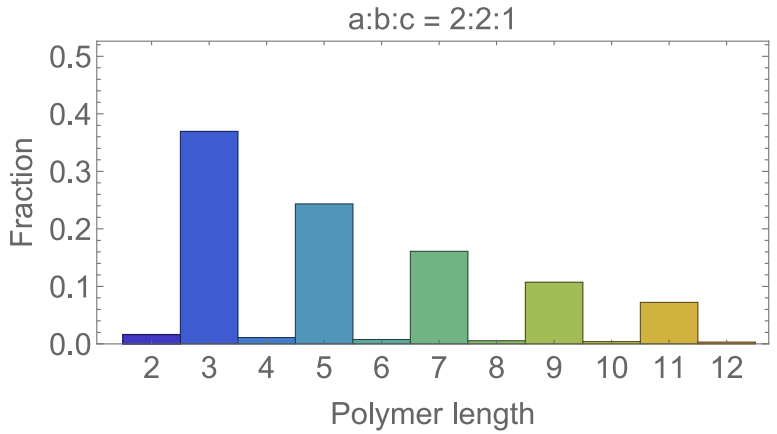
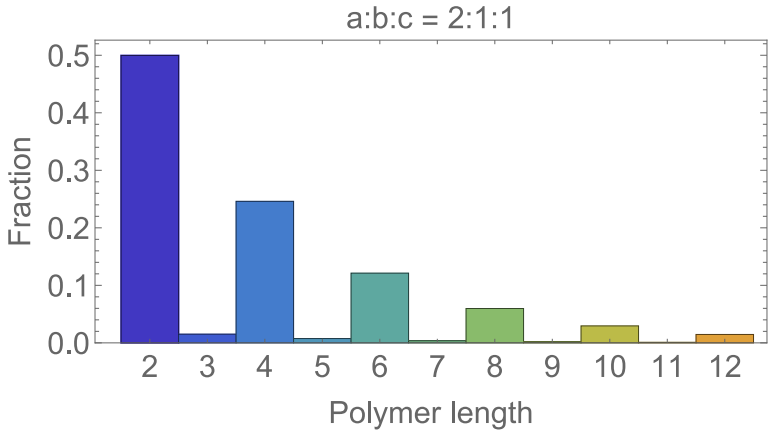
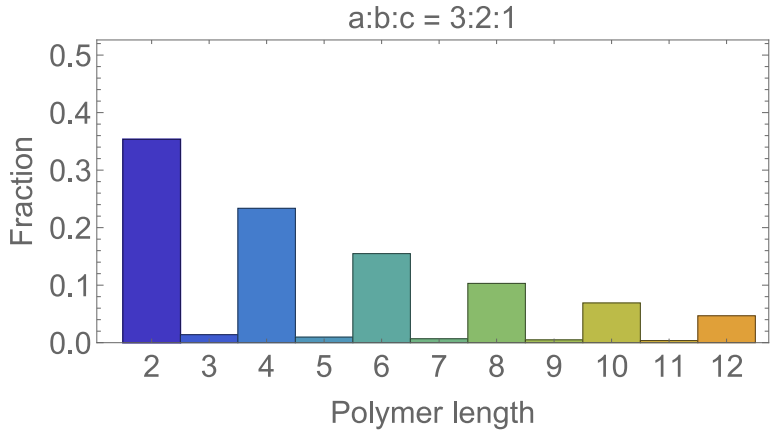
Invaders with variable sizes











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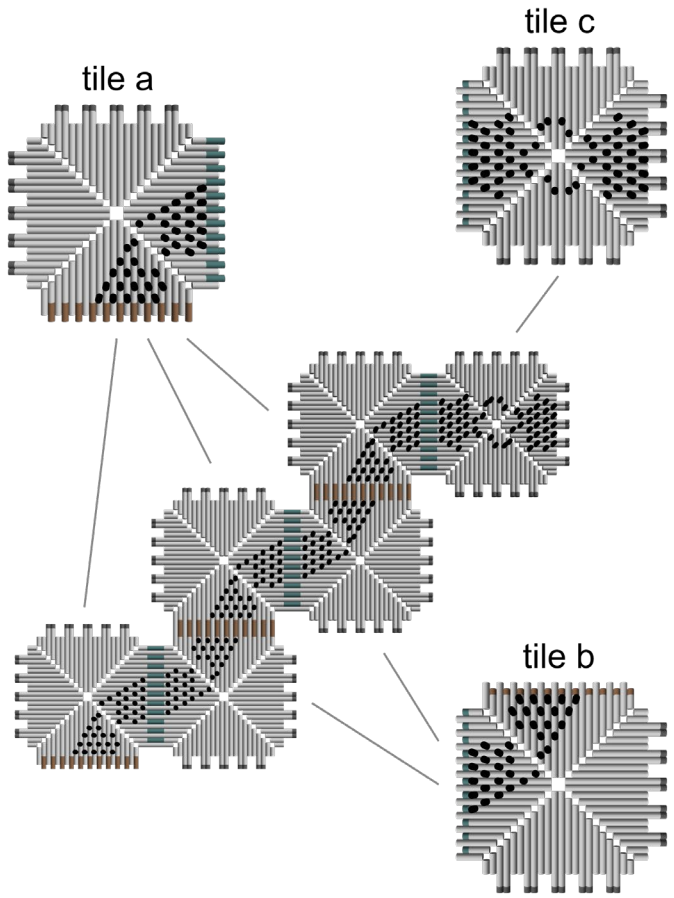
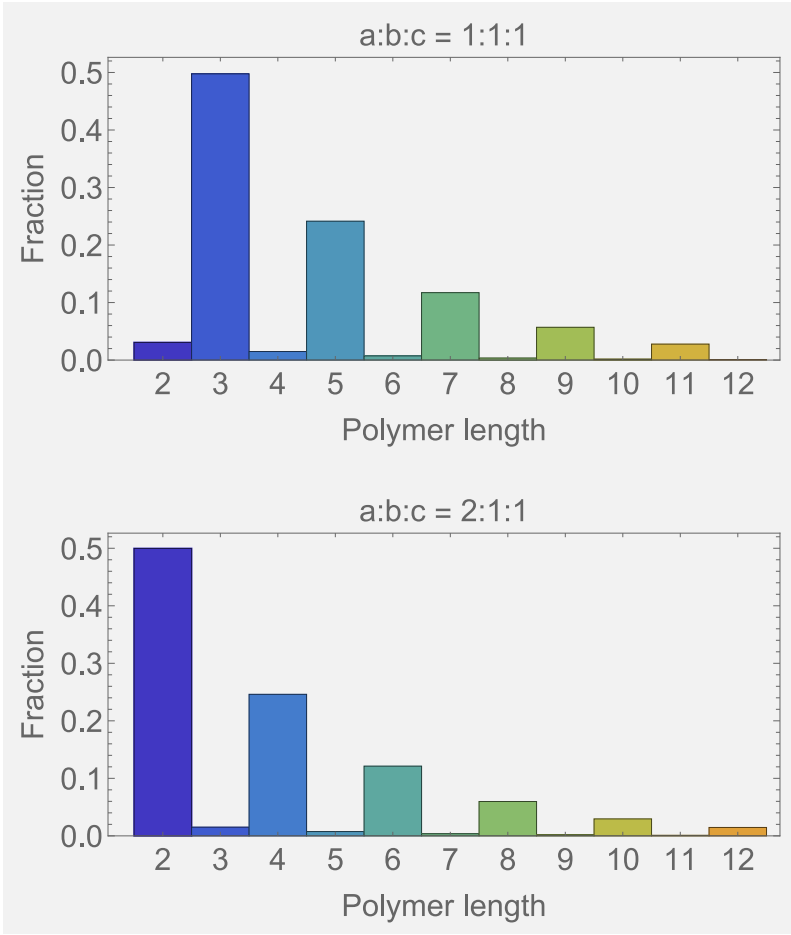
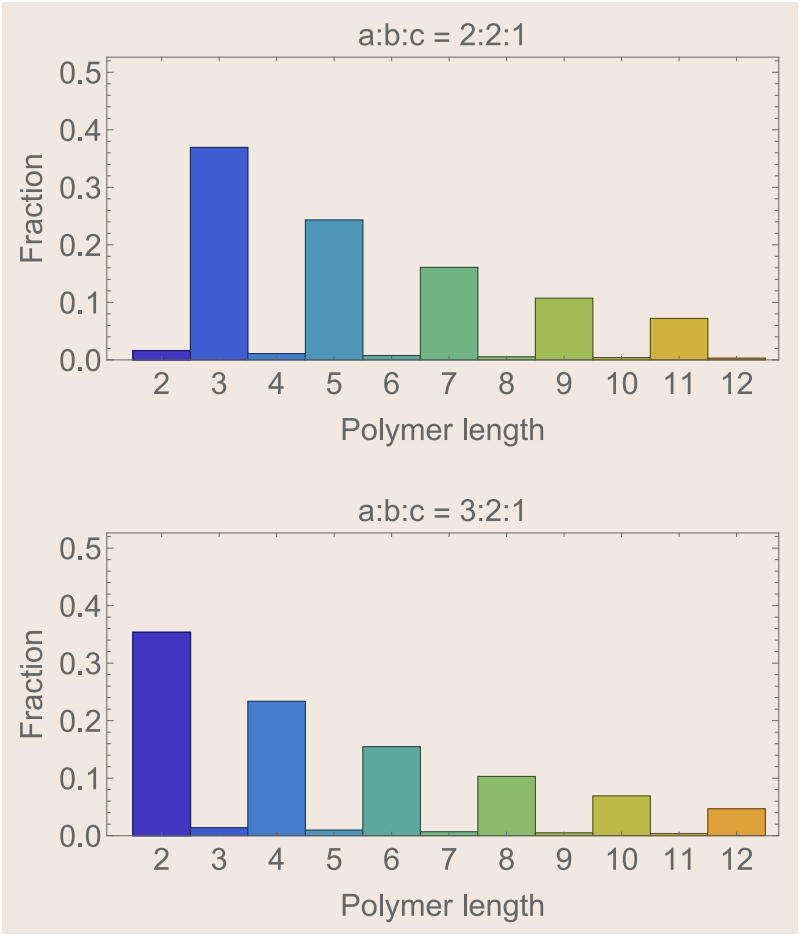
Invaders with variable sizes

lower tile ratio c: $(a + b)$ results in longer polymers







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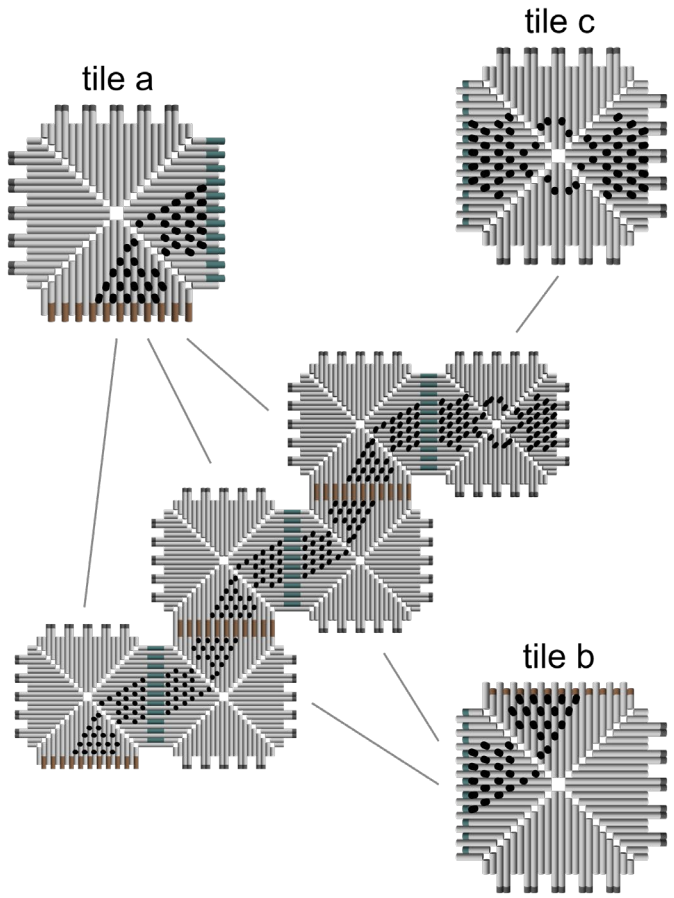
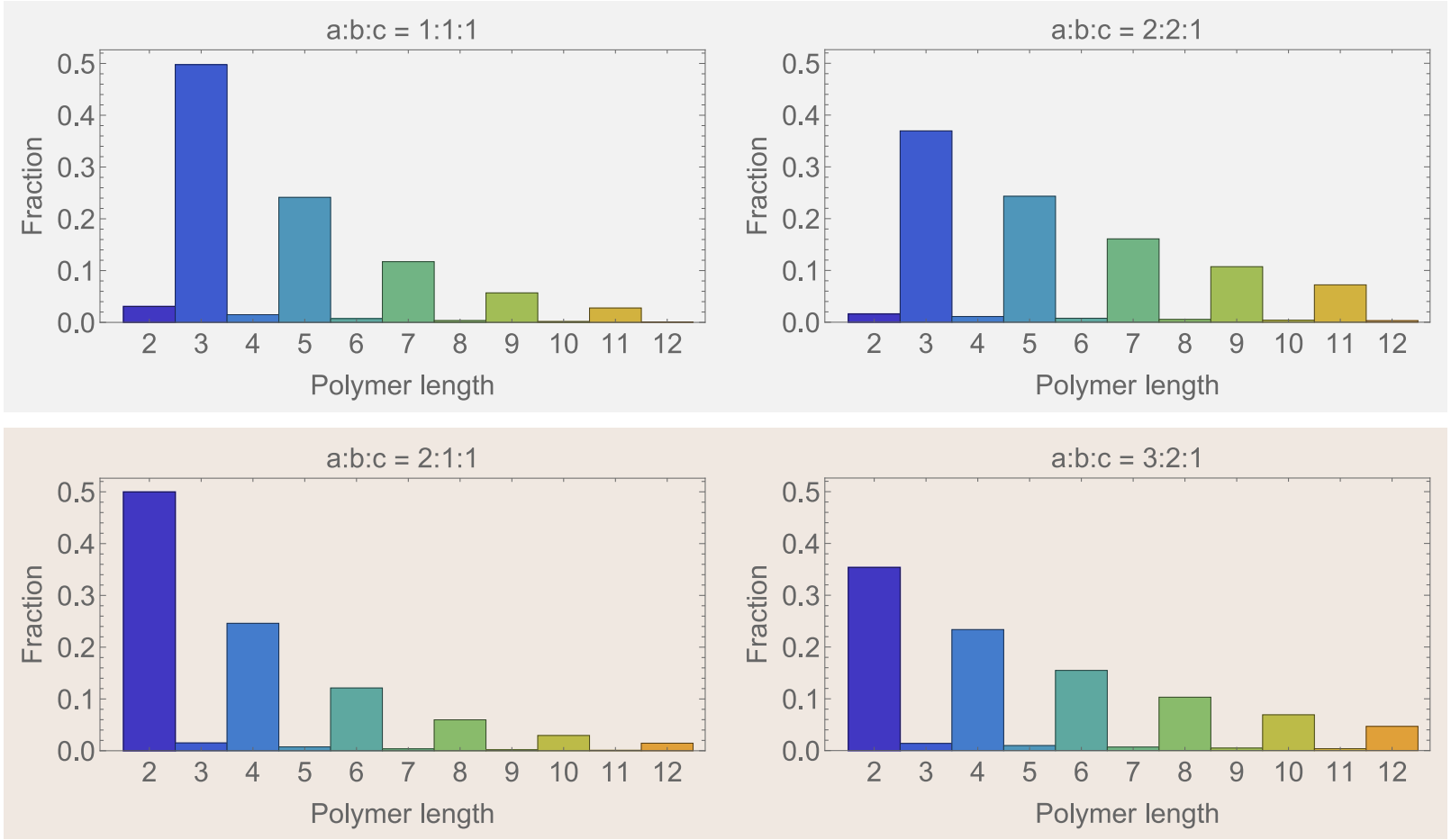
Invaders with variable sizes

tile ratio a: b determines whether even or odd-length polymers are favored





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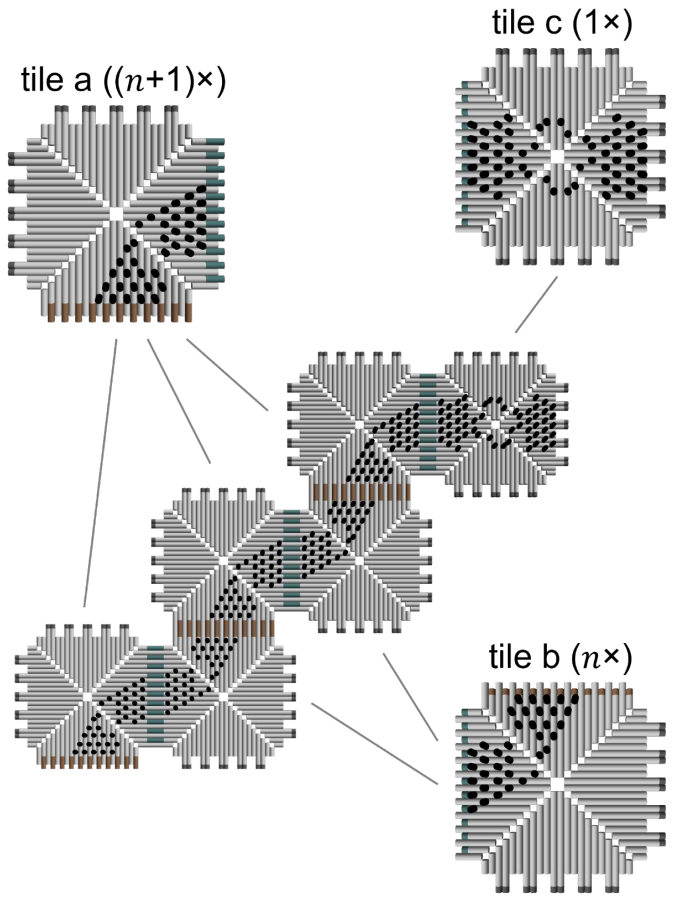
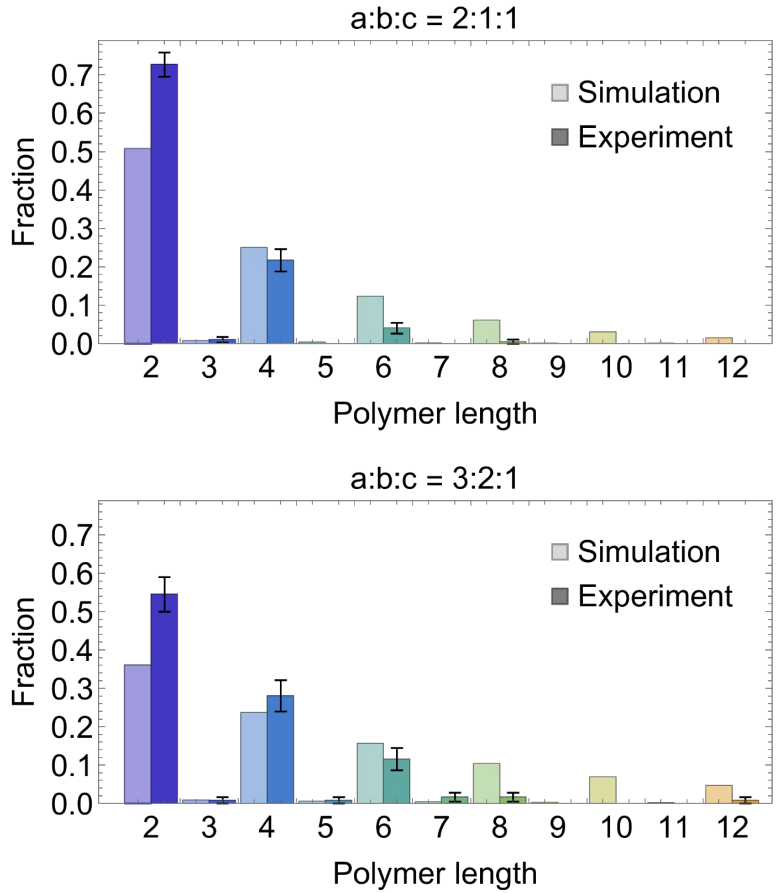
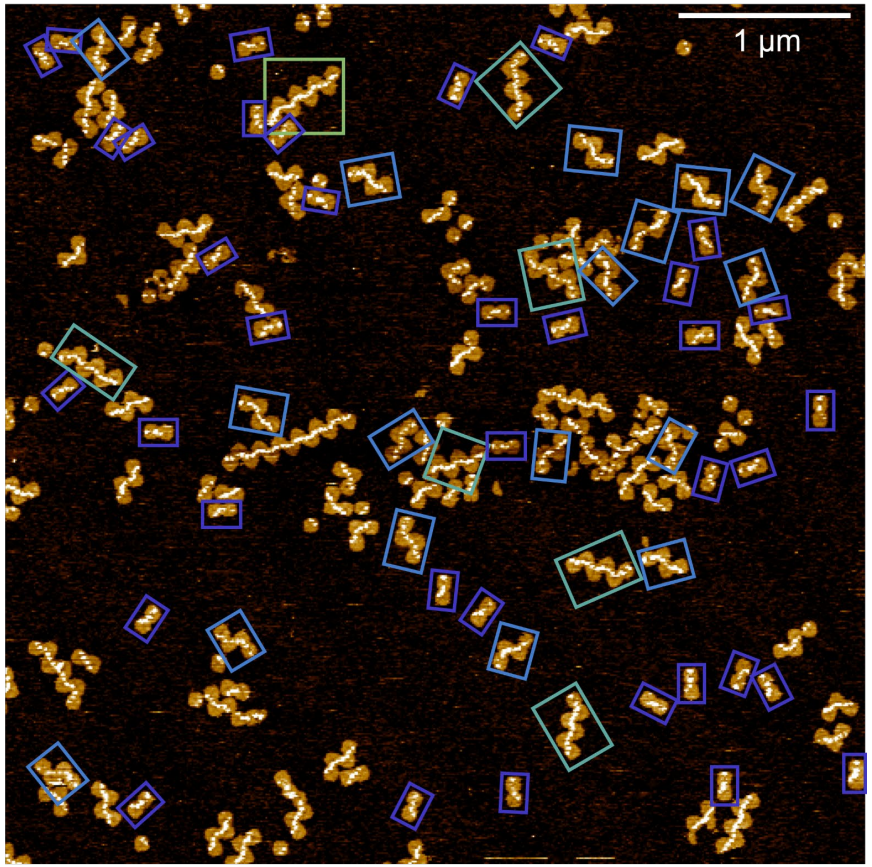
Invaders with variable sizes







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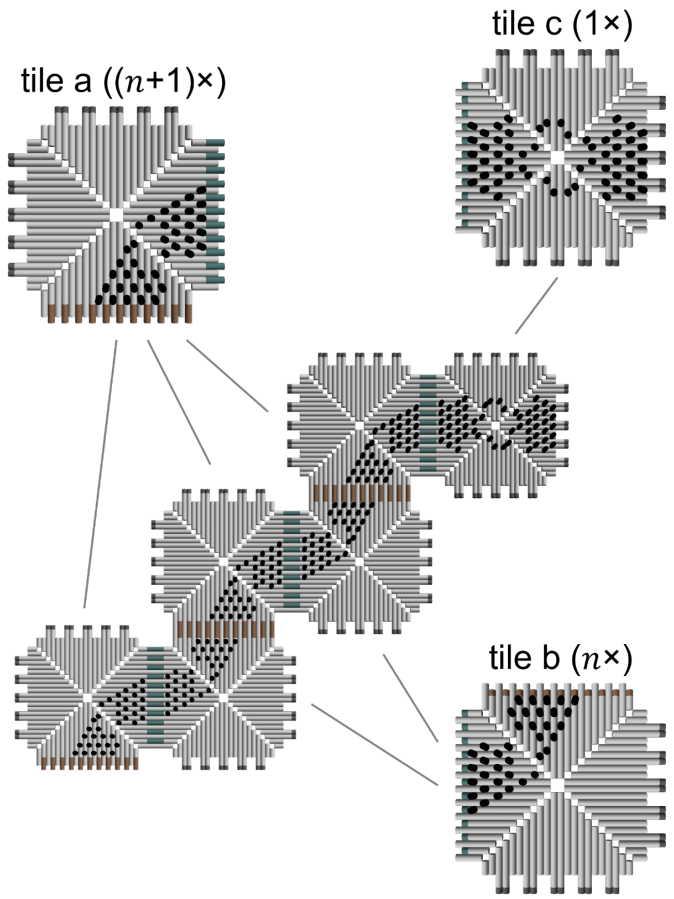
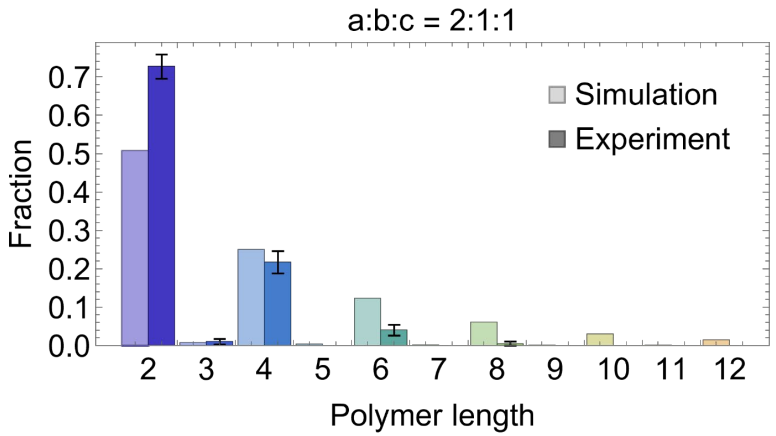
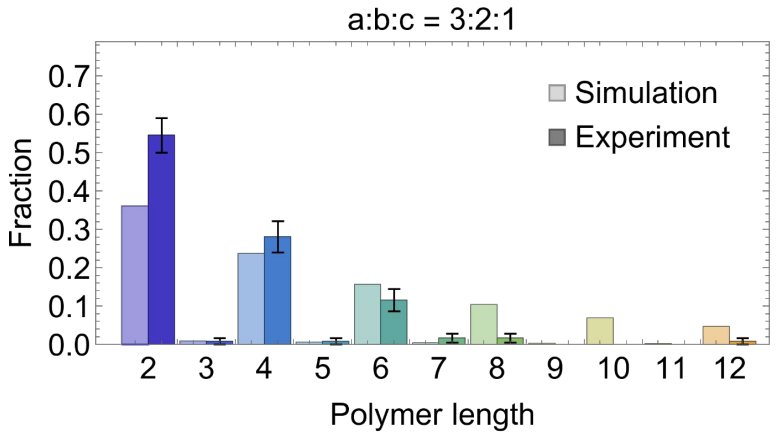
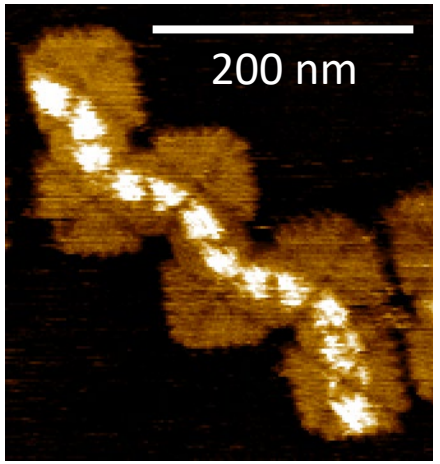
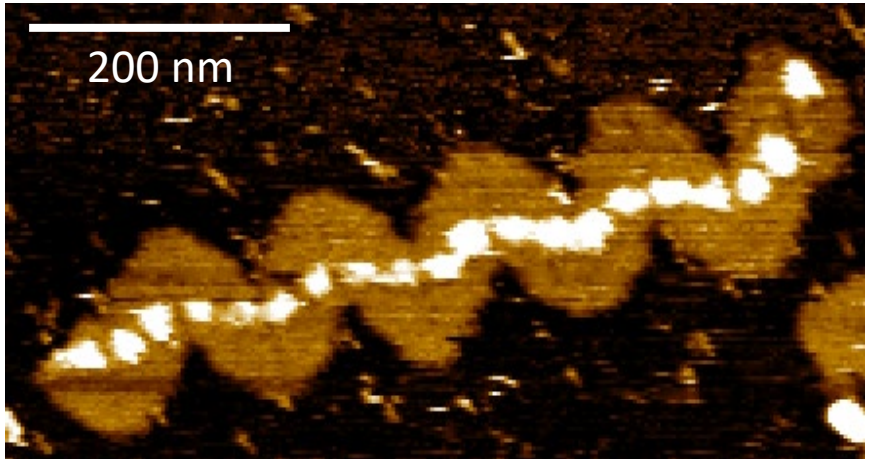
Invaders with variable sizes











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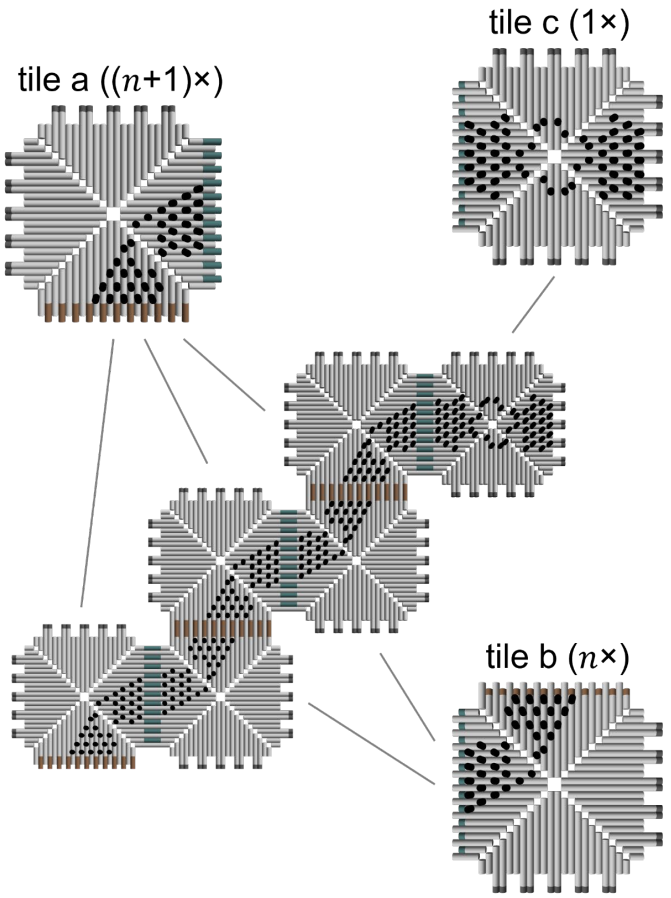
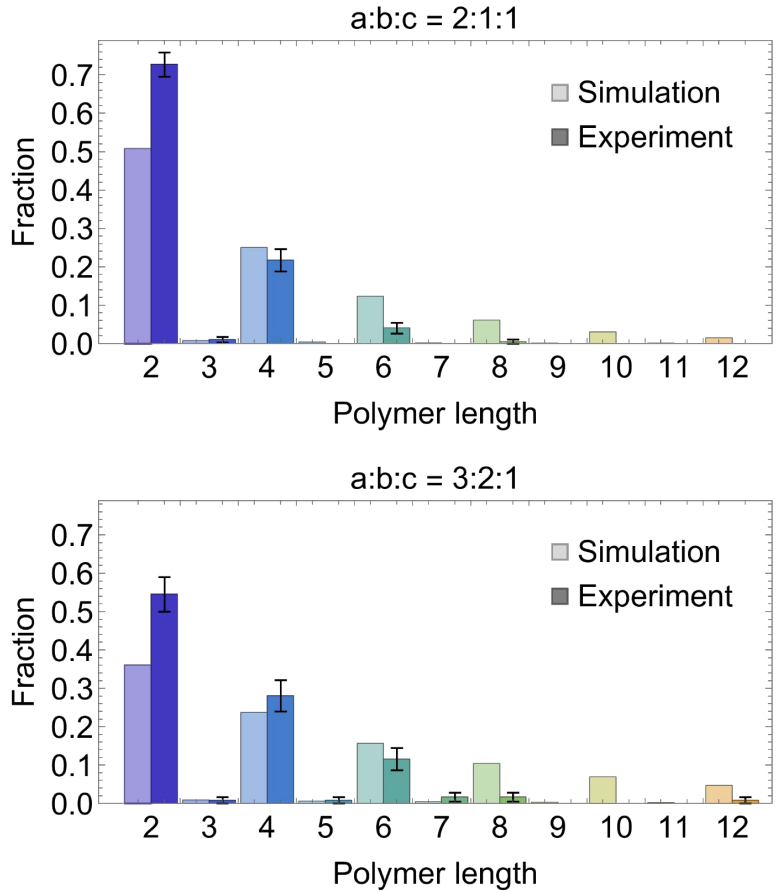
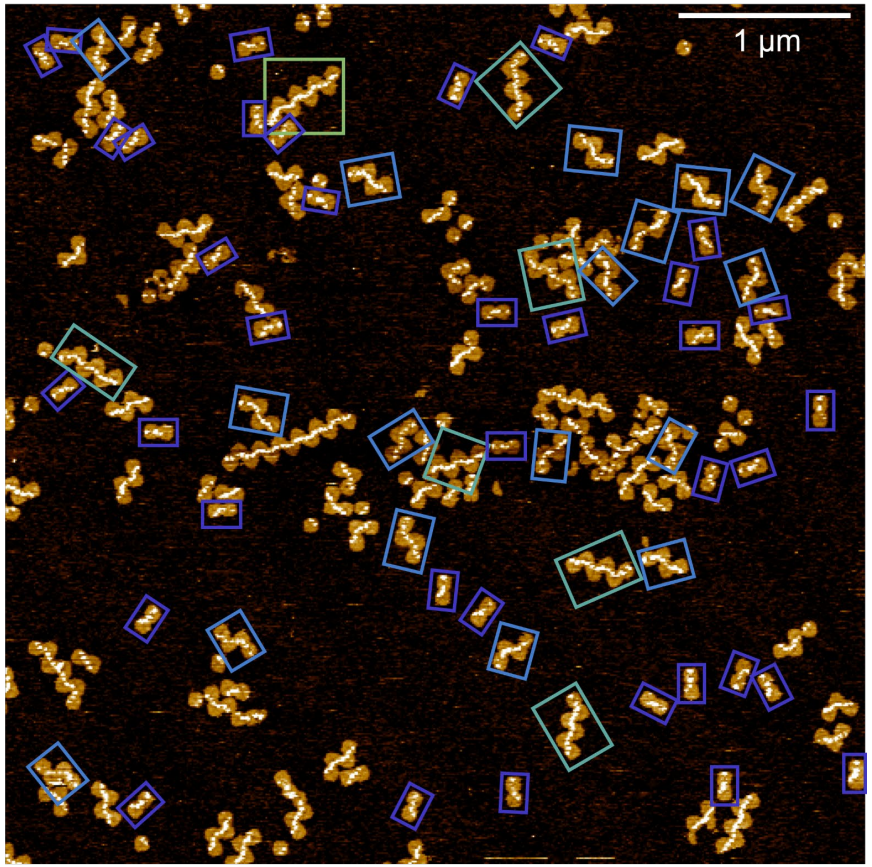
Invaders with variable sizes

Mystery 1: roughly 50% of the structures contained just tiles a and b, contradictory to the simulation prediction that the concentrations of ab-only polymers should be approximately zero.







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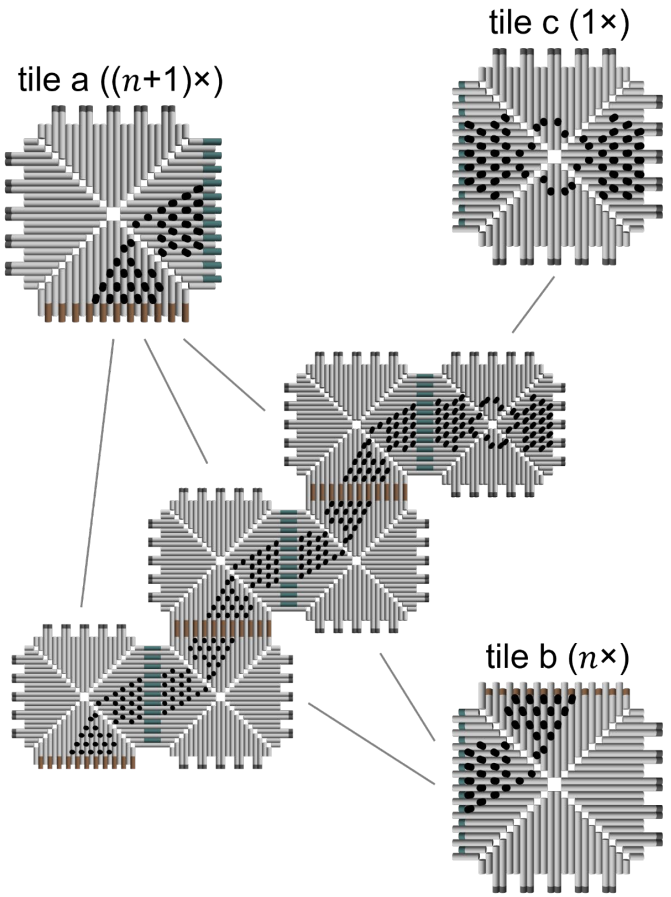
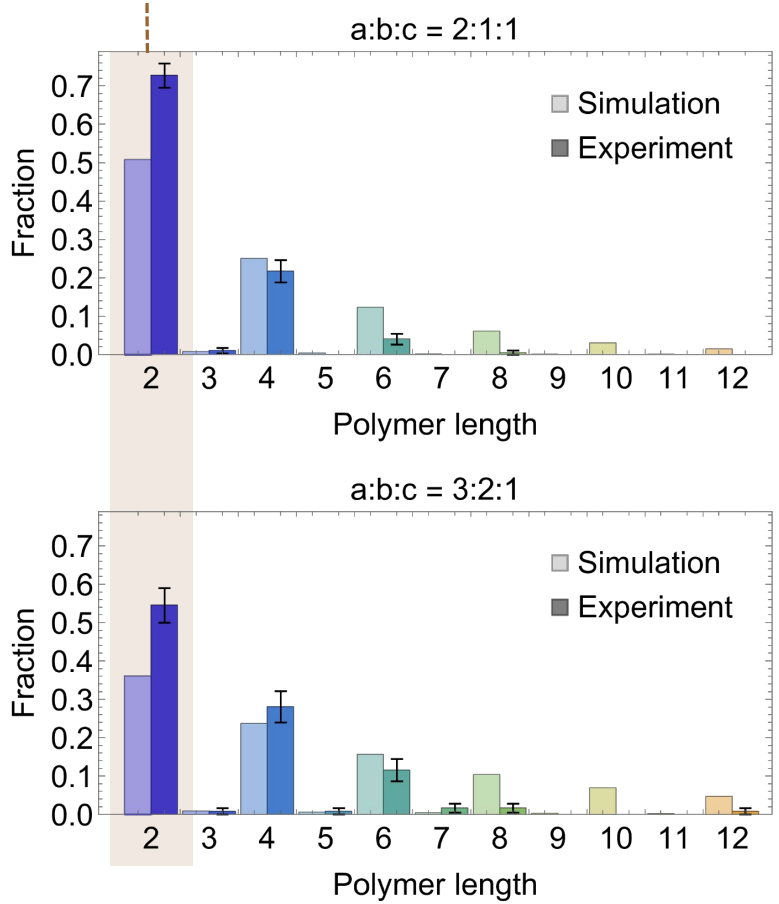
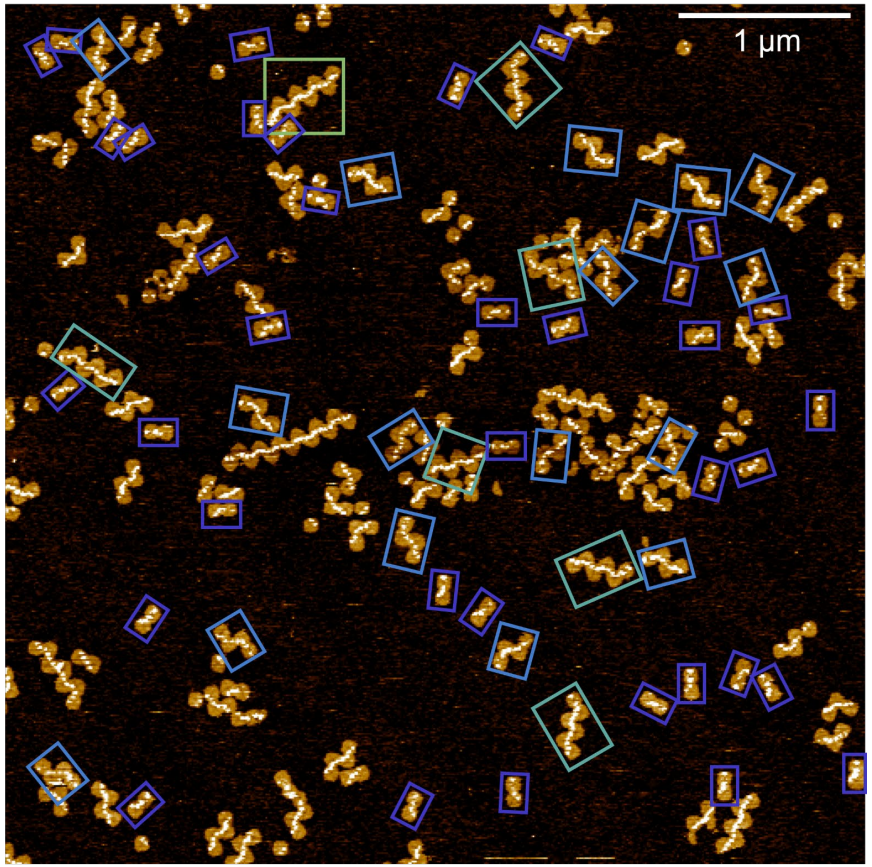
Invaders with variable sizes





Mystery 2: more ac dimers (length = 2) were observed in experiments than what simulations predicted.



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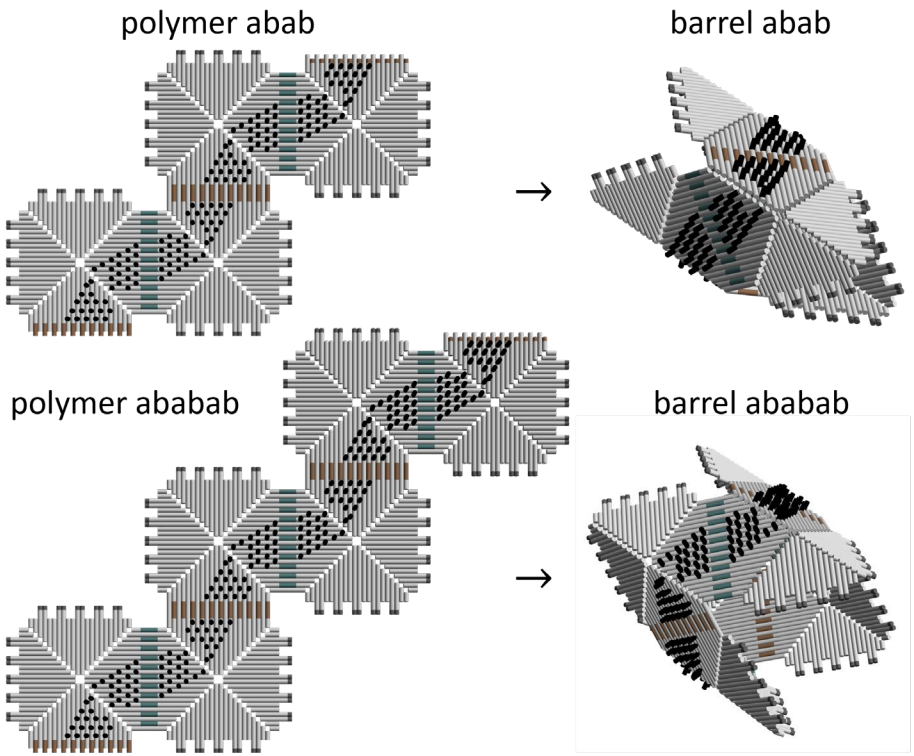
Hypothesis: barrel formation



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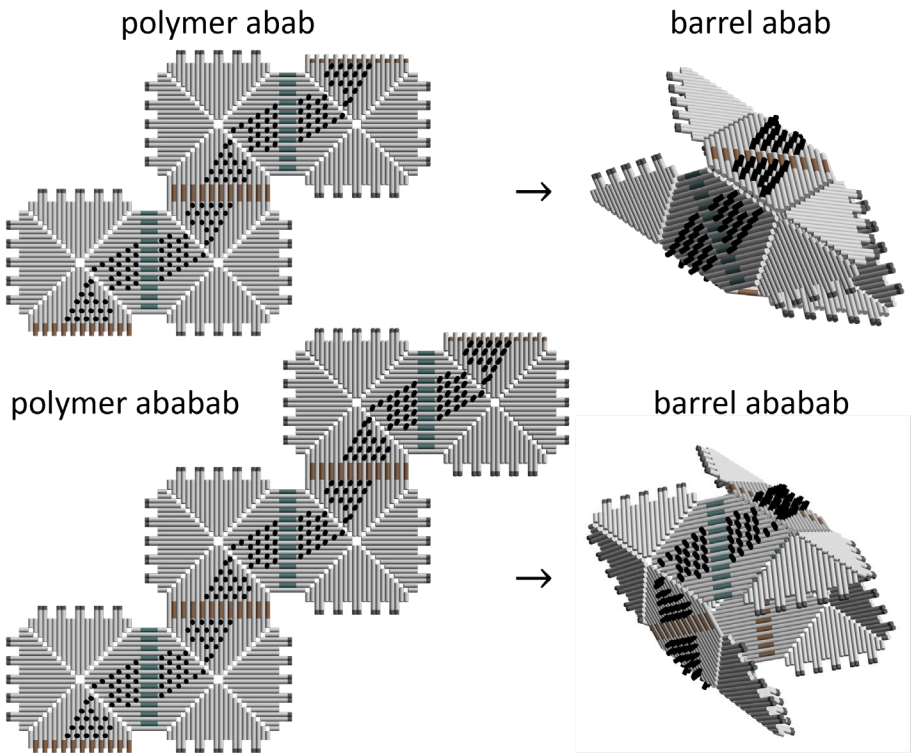
Hypothesis: barrel formation



Mystery 1: roughly 50% of the structures contained just tiles a and b, contradictory to the simulation prediction that the concentrations of ab-only polymers should be approximately zero. ✓

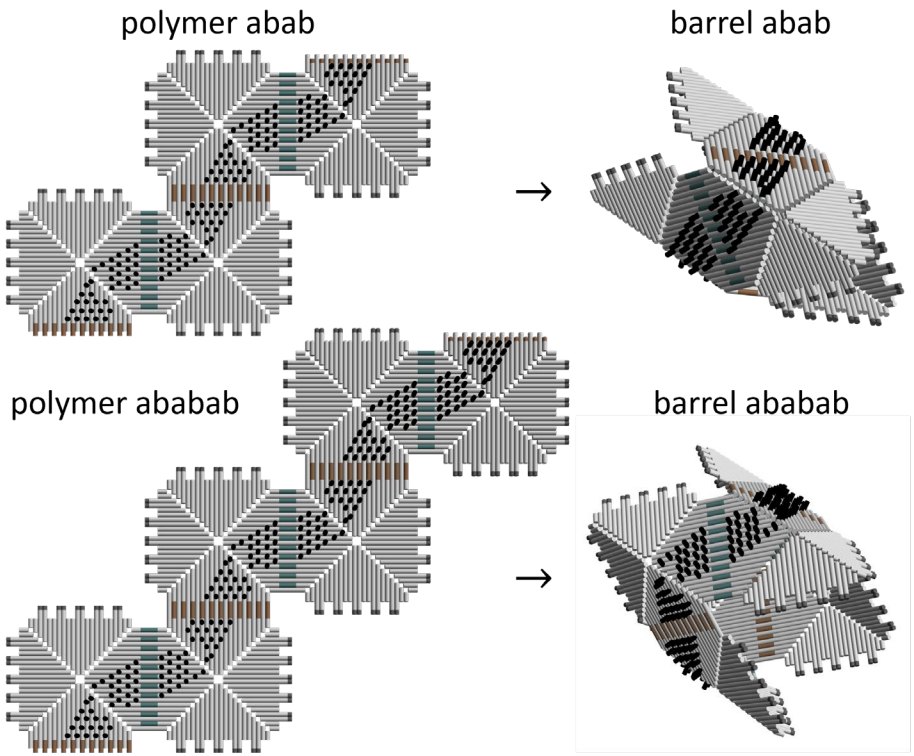
Mystery 2: more ac dimers (length = 2) were observed in experiments than what simulations predicted. ✓

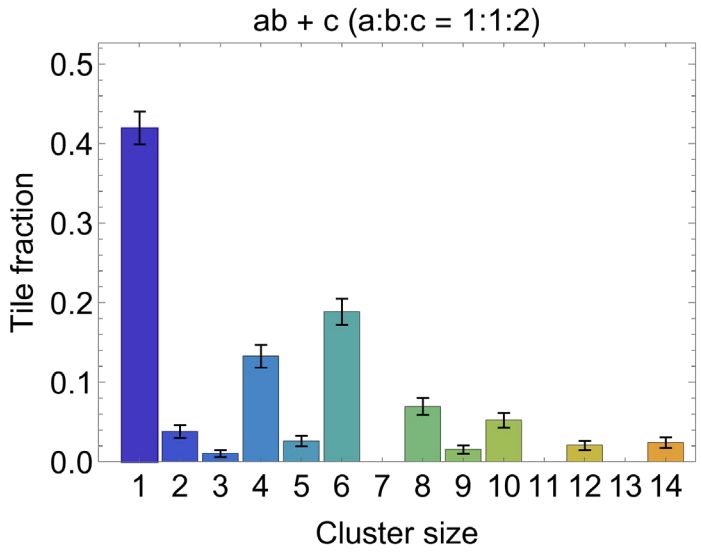
Reactions like $abab + ac \rightarrow ababac$ cannot take place if polymers like $abab$ have formed barrel structures.

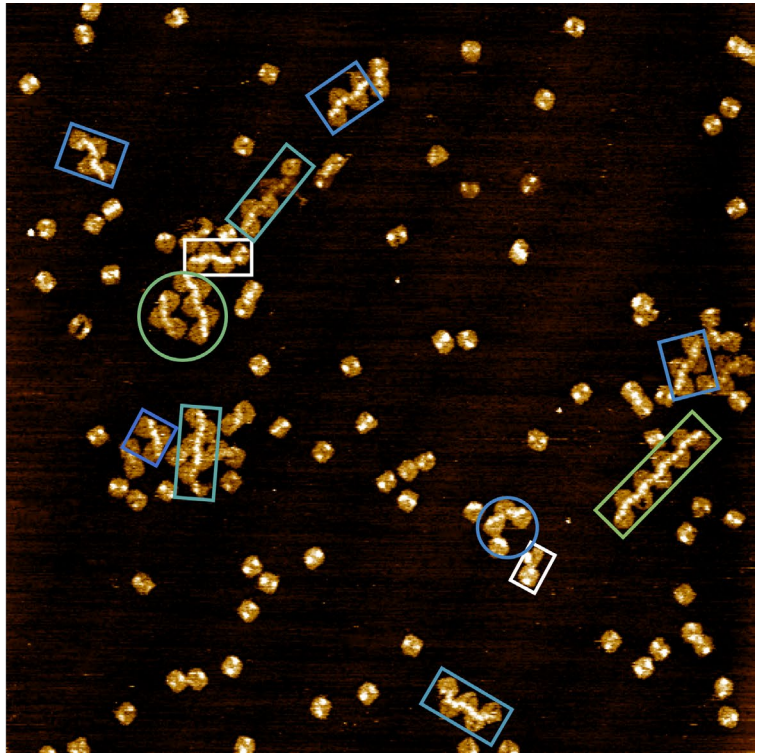
Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Hypothesis: barrel formation

annealed tiles a and b at 1:1 ratio and then added tile c to the mixture

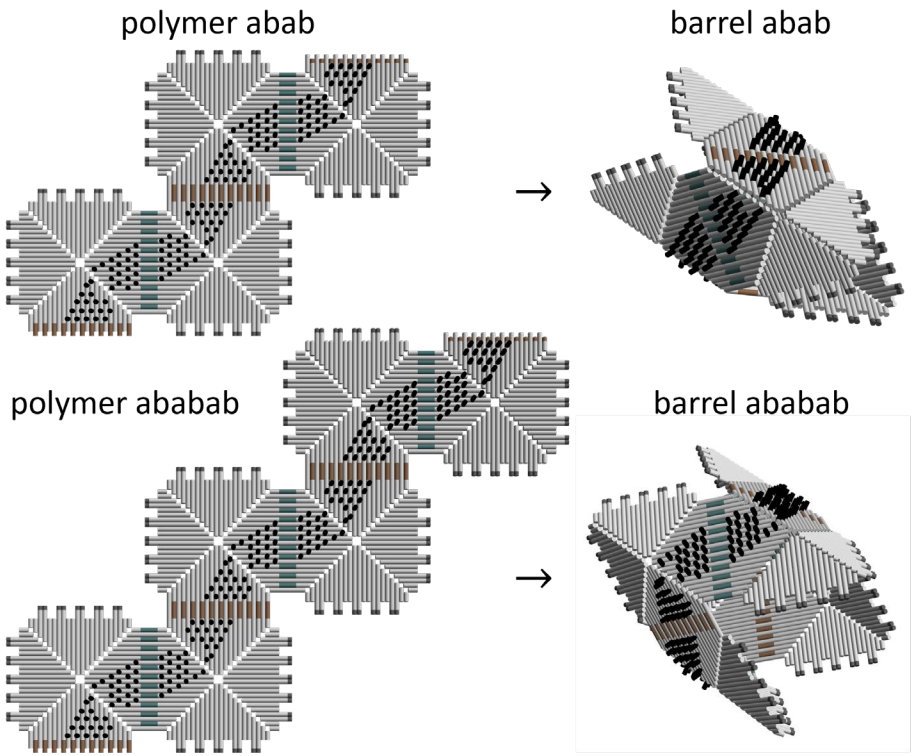


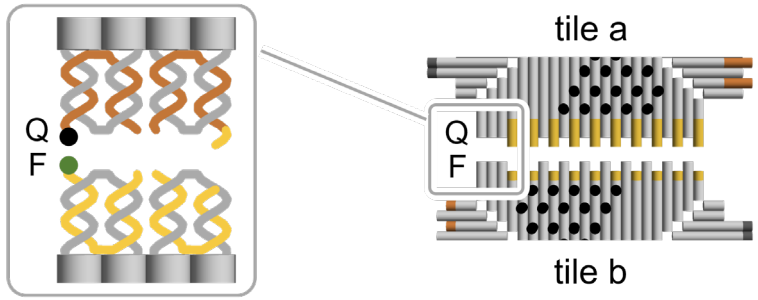


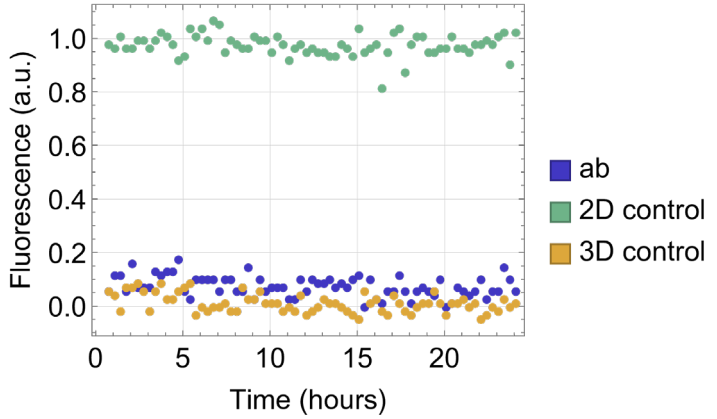


Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Hypothesis: barrel formation ✓

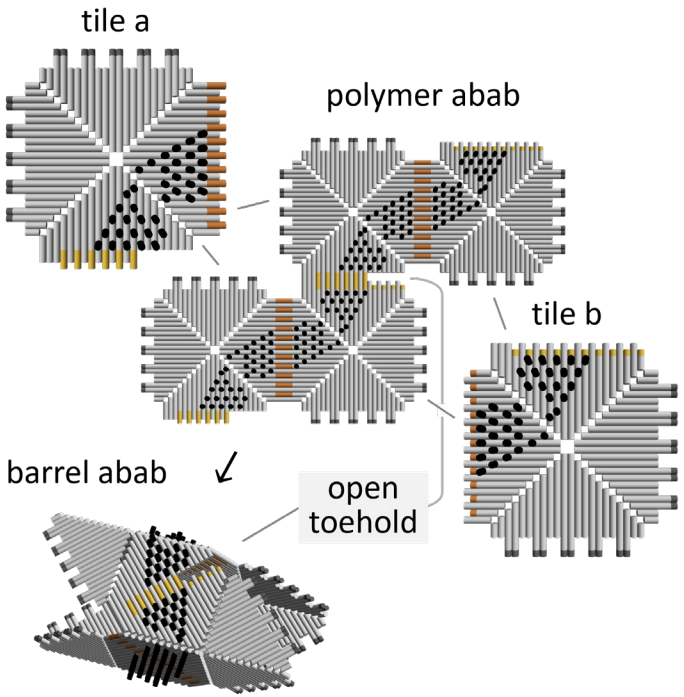






Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Reconfiguration of barrels to flat structures



Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

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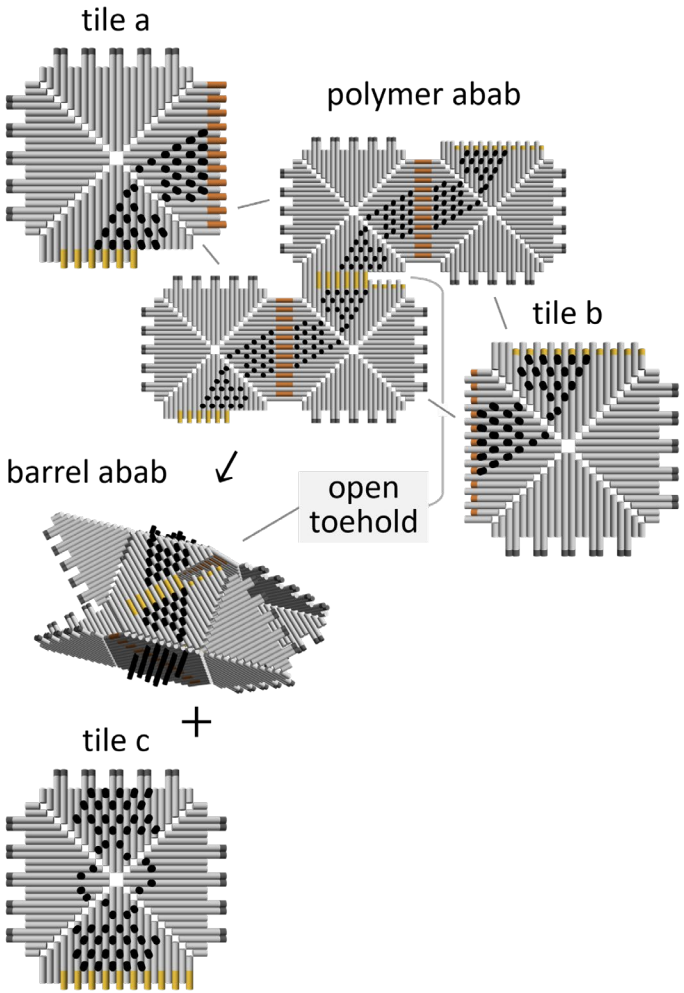

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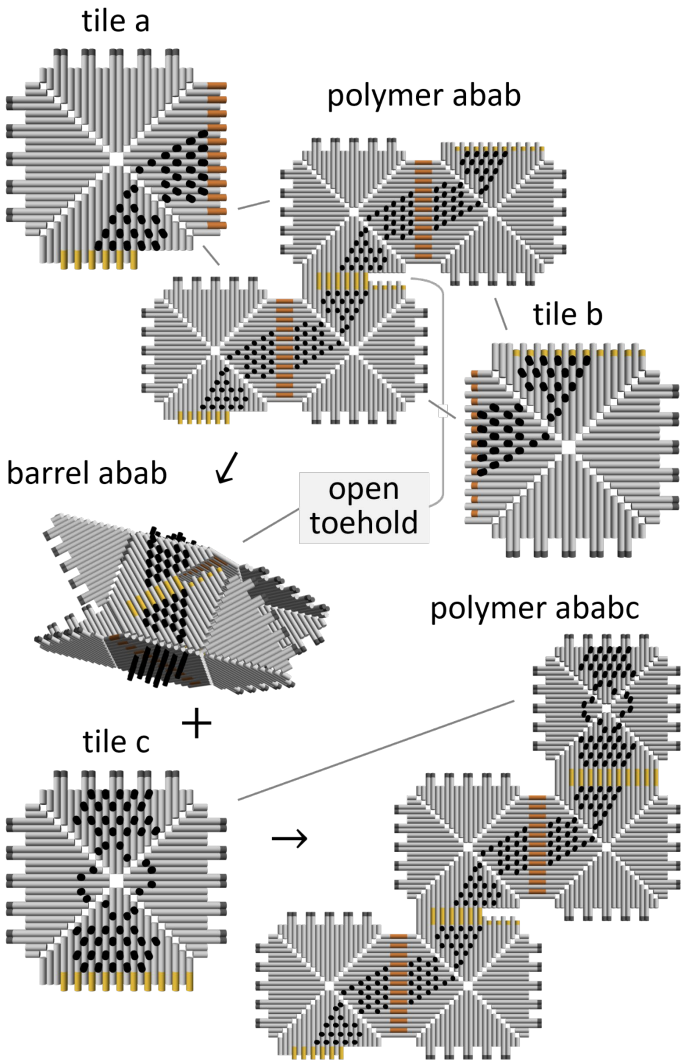
Reconfiguration of barrels to flat structures



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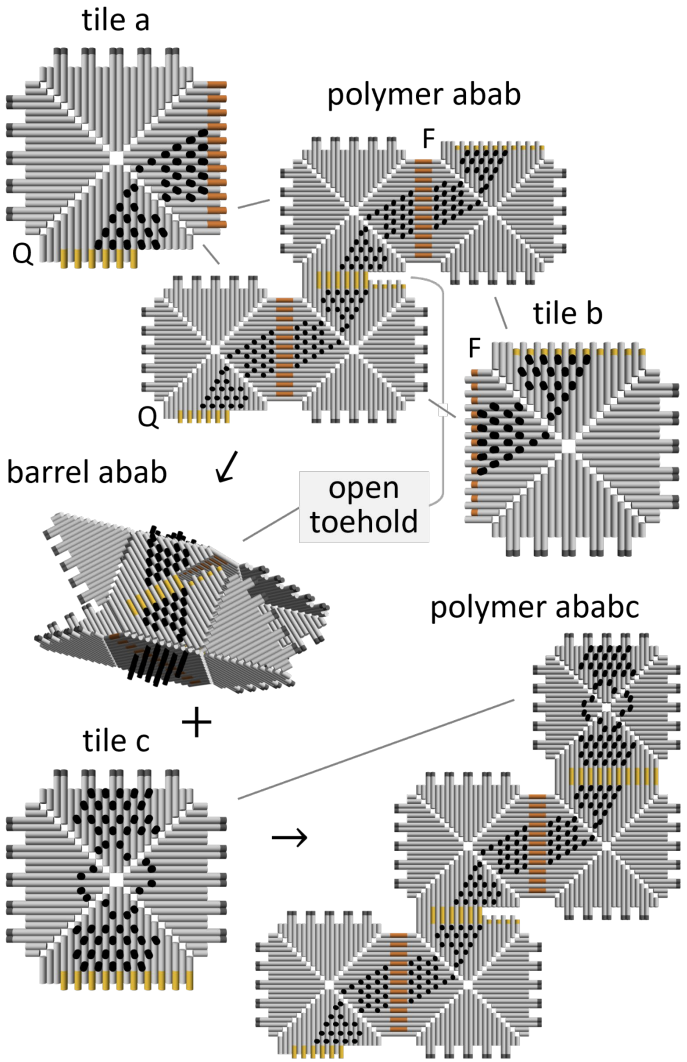
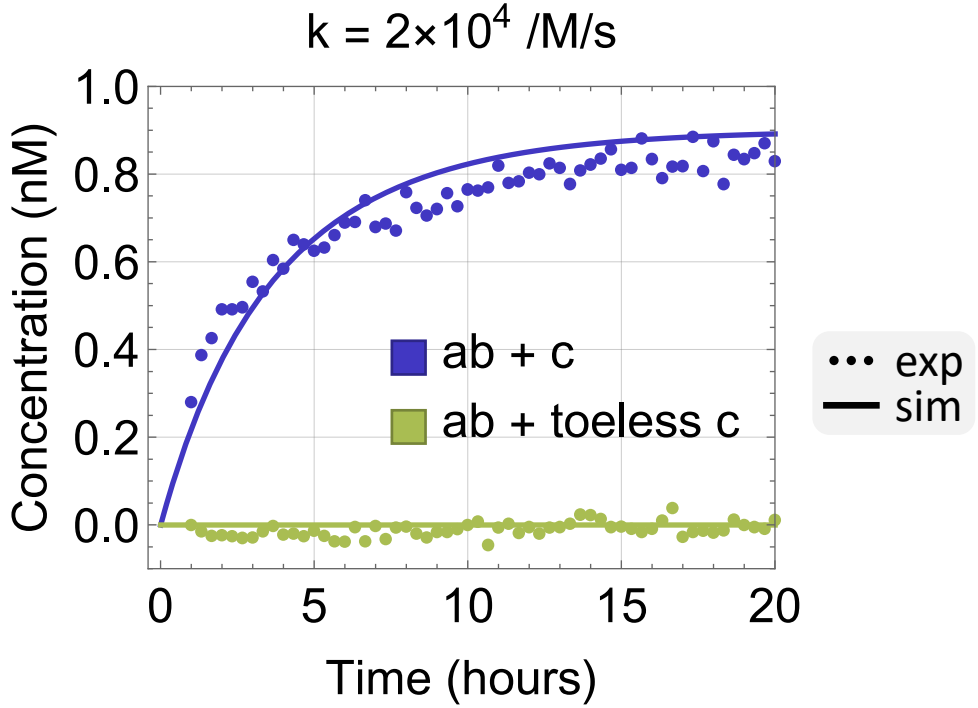
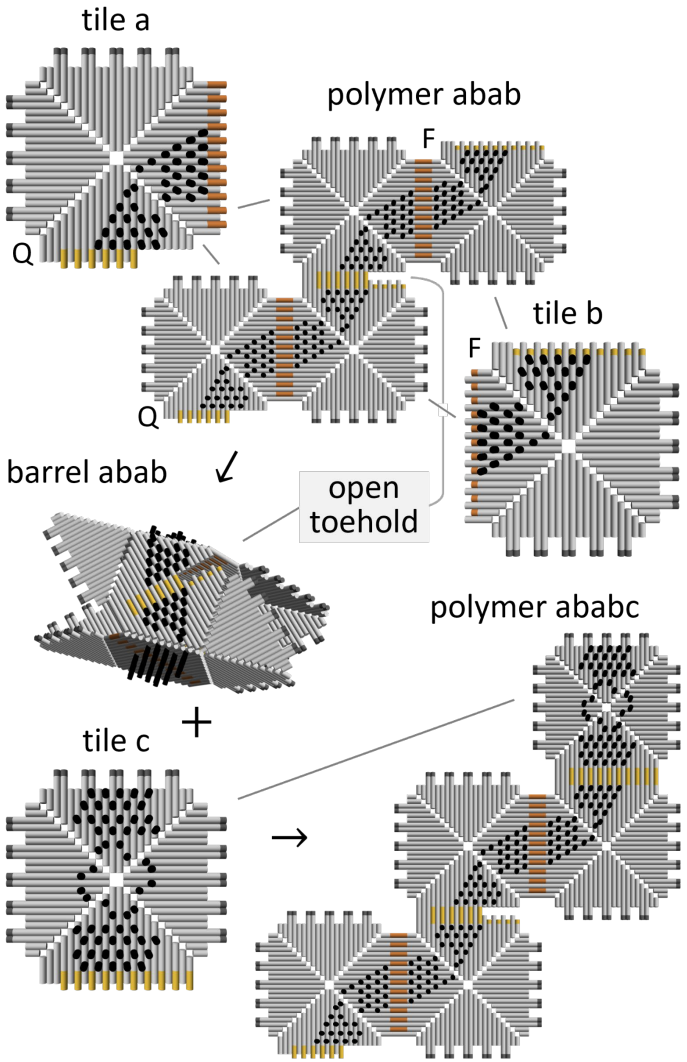
Reconfiguration of barrels to flat structures

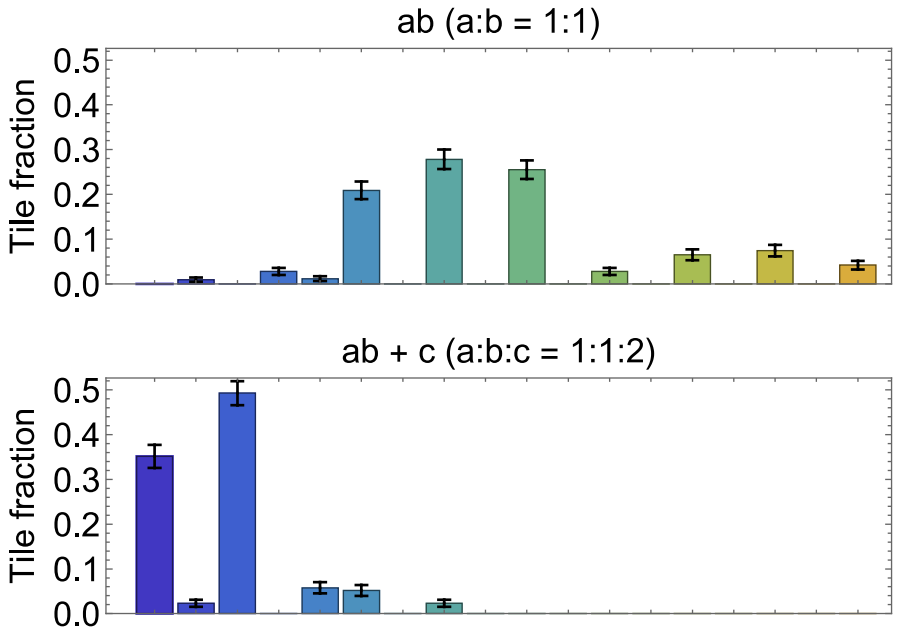




Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

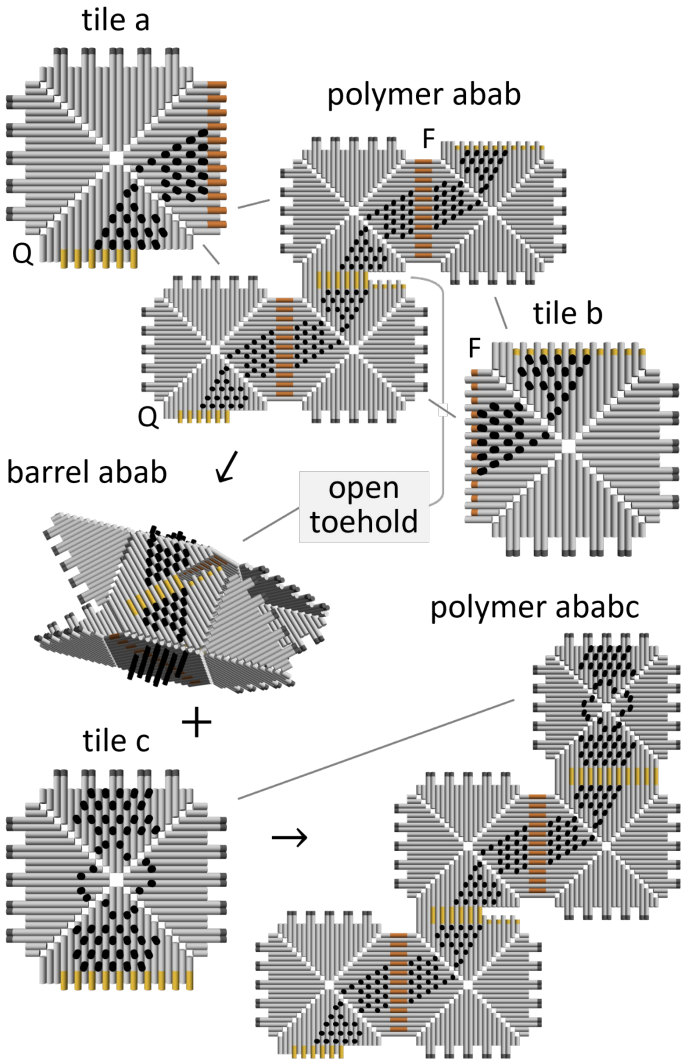
Reconfiguration of barrels to flat structures

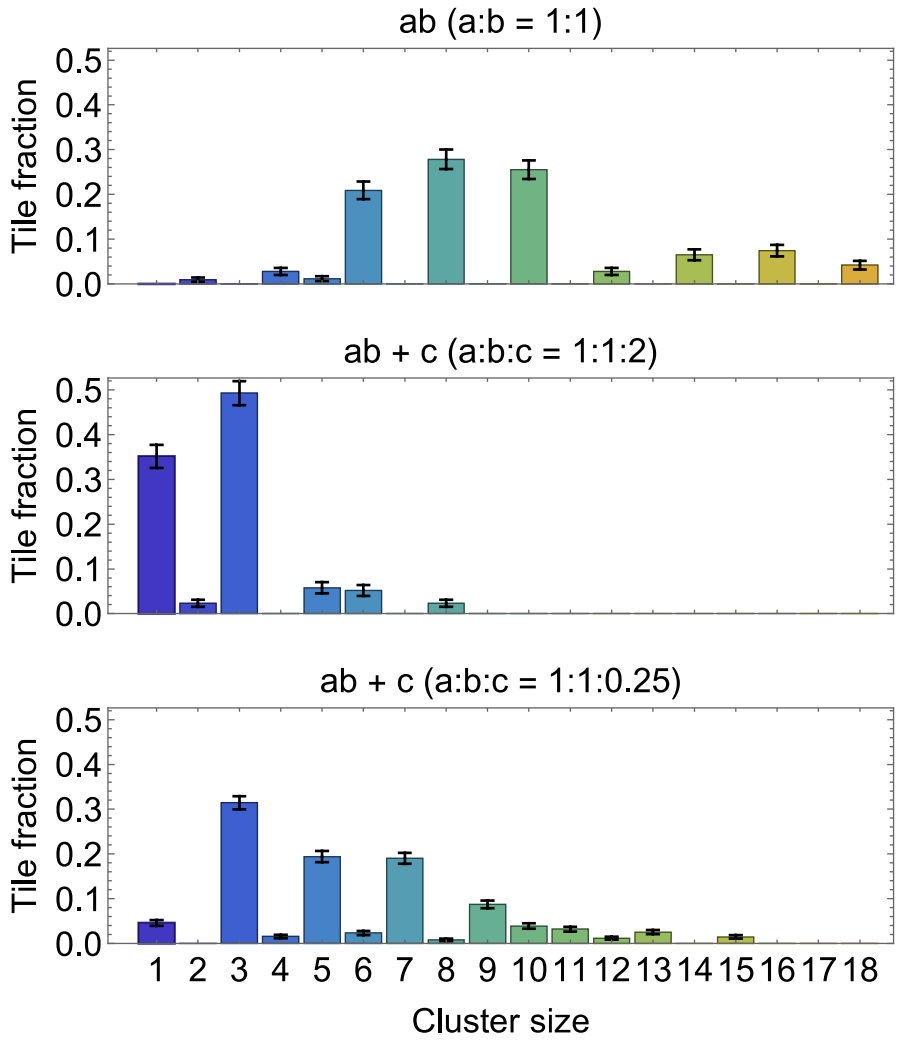




Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

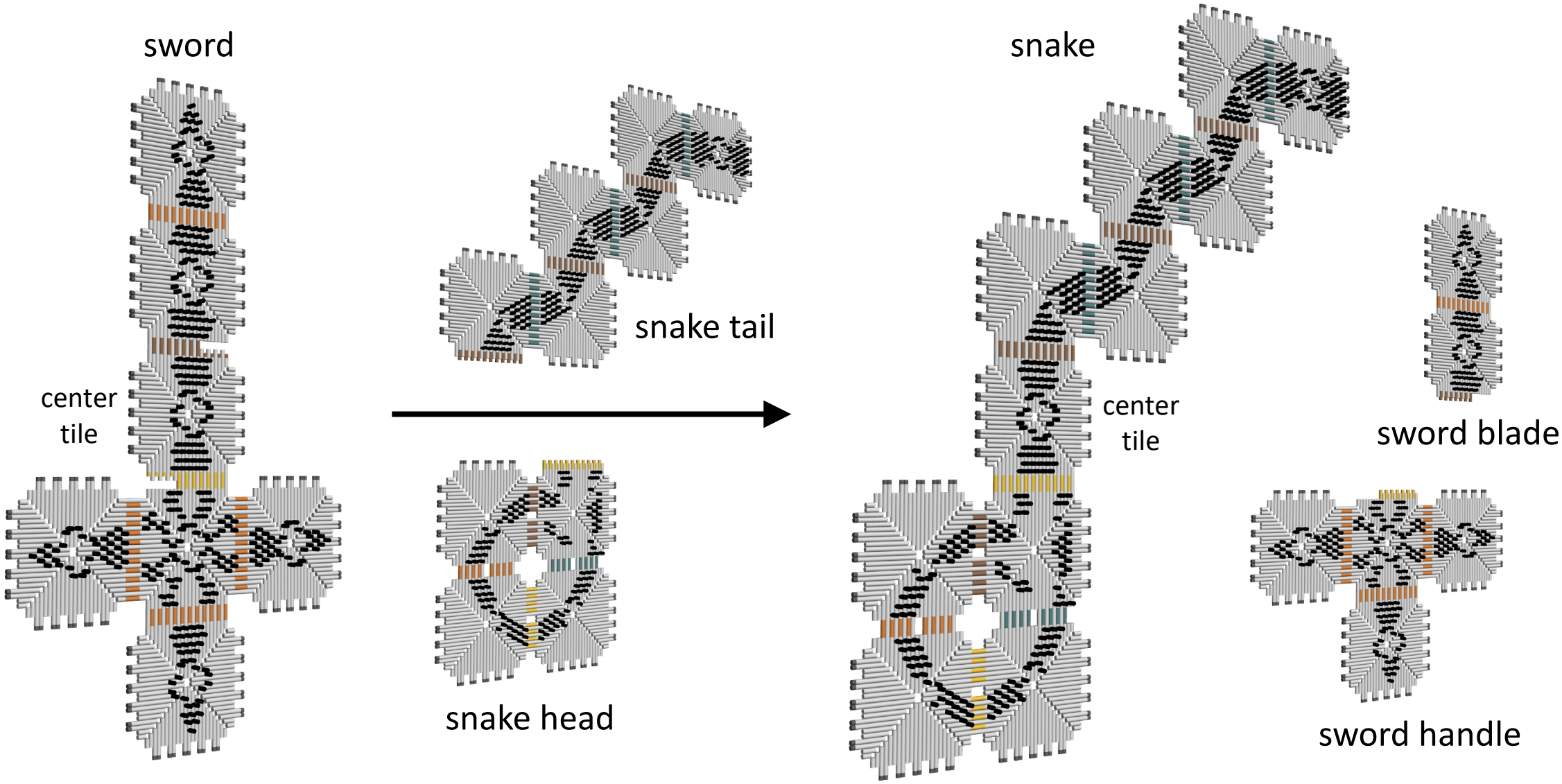
Reconfiguration of barrels to flat structures



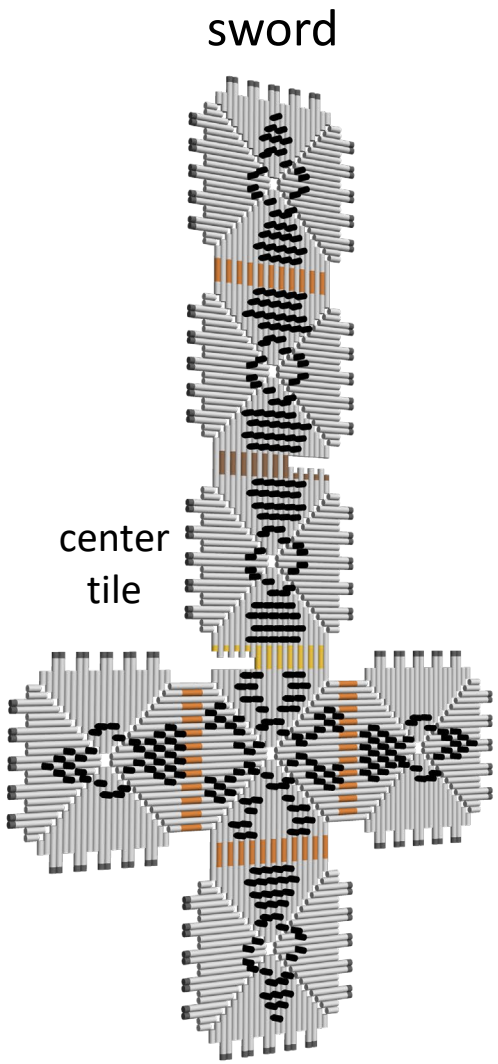


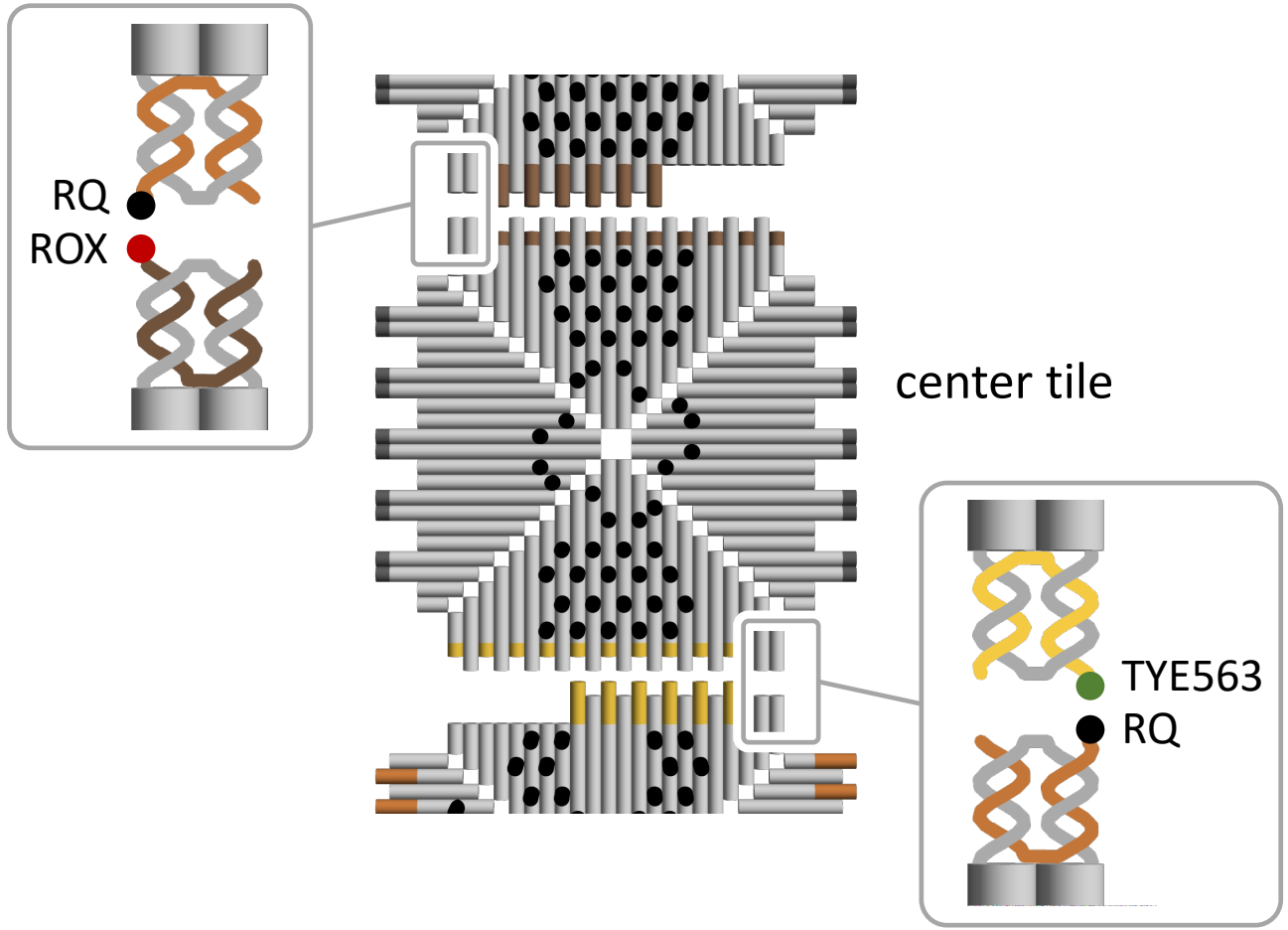
Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Simultaneous tile displacement

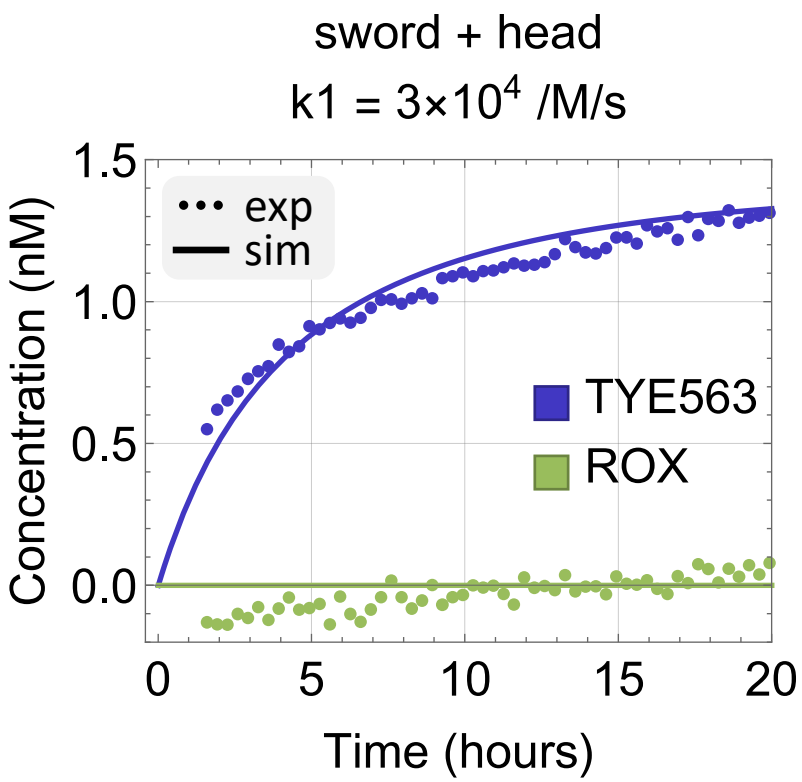


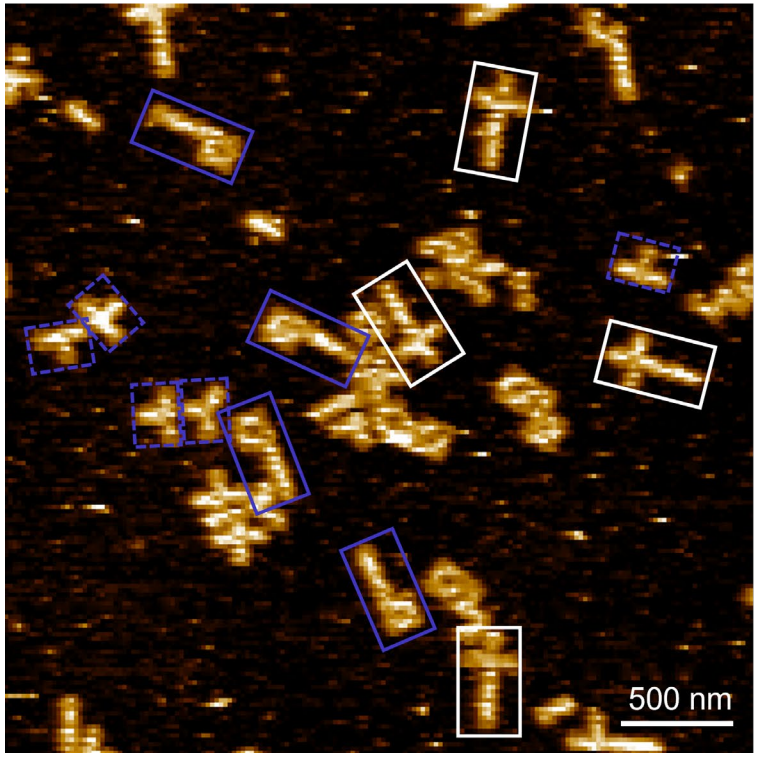
Simultaneous tile displacement

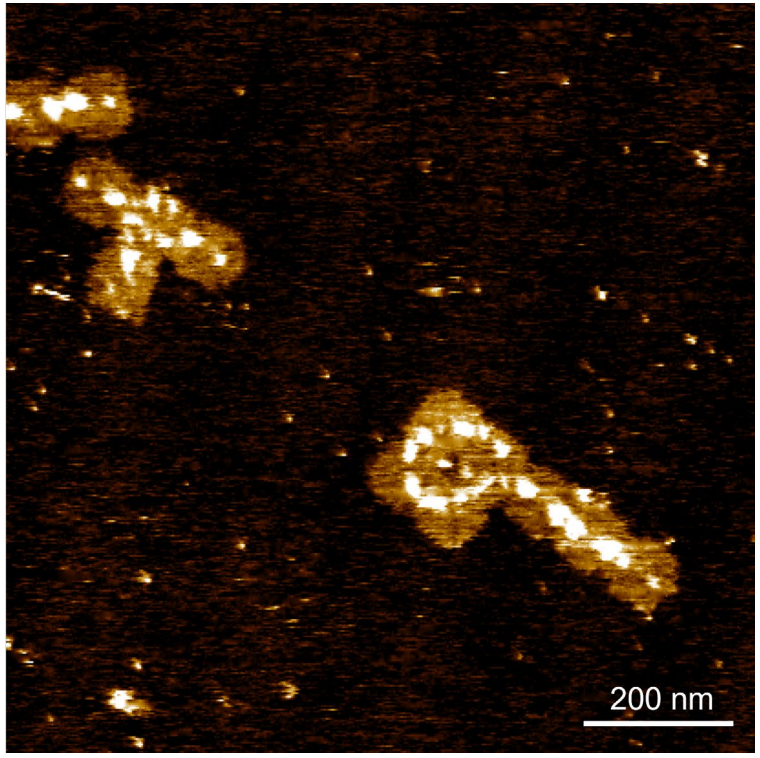




Simultaneous tile displacement

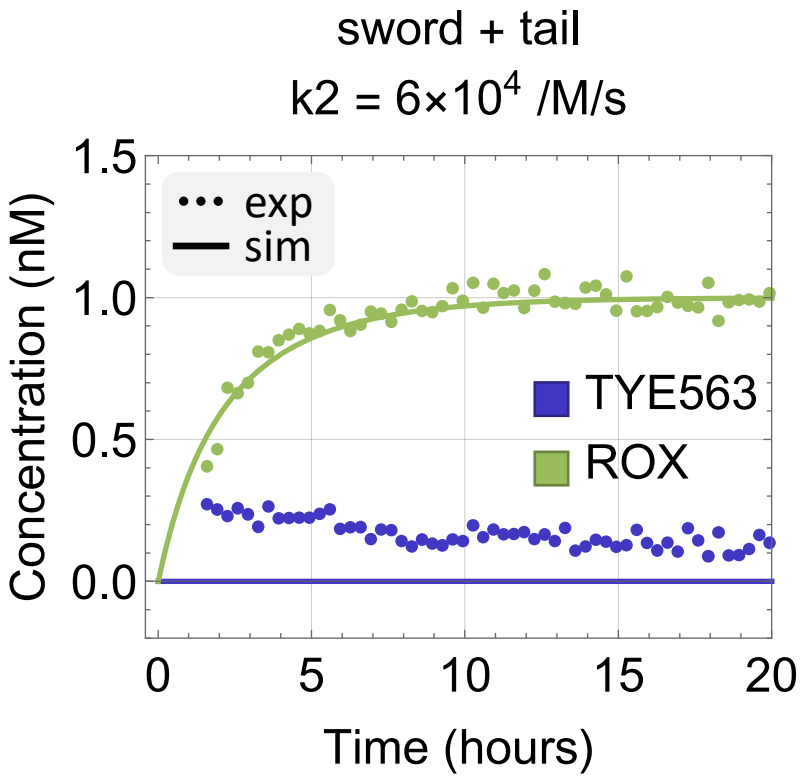


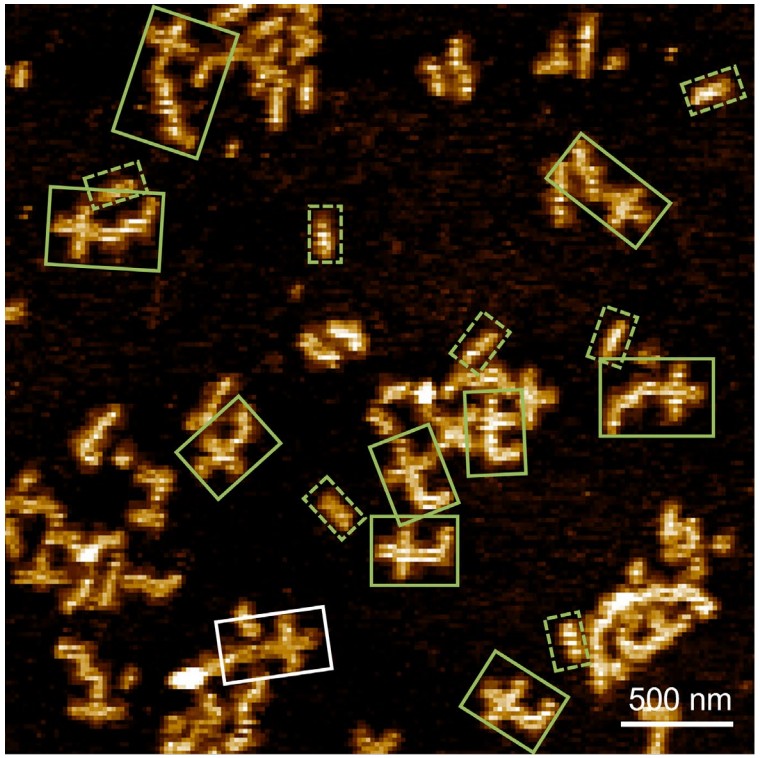


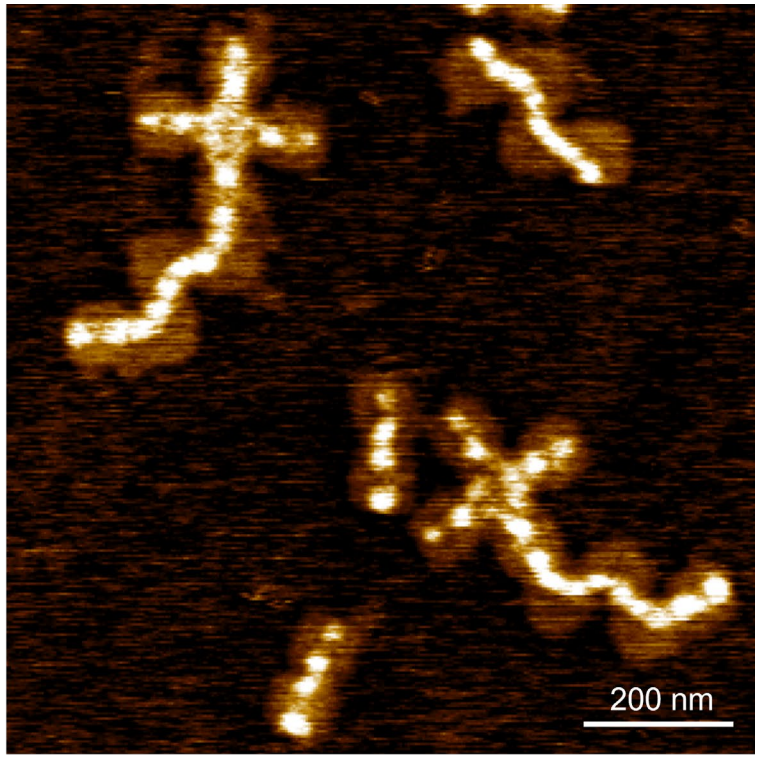


Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

Simultaneous tile displacement







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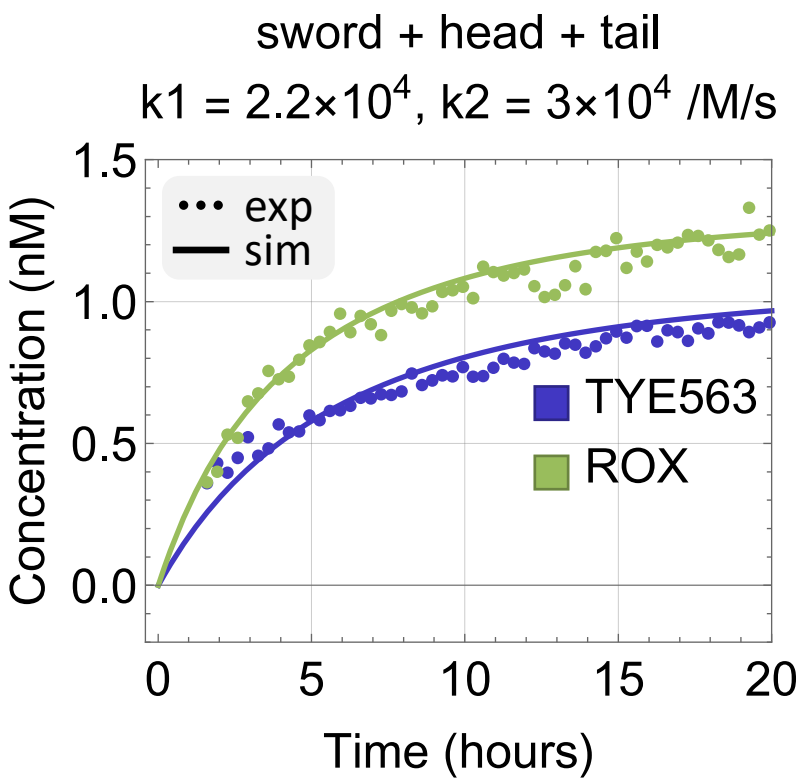
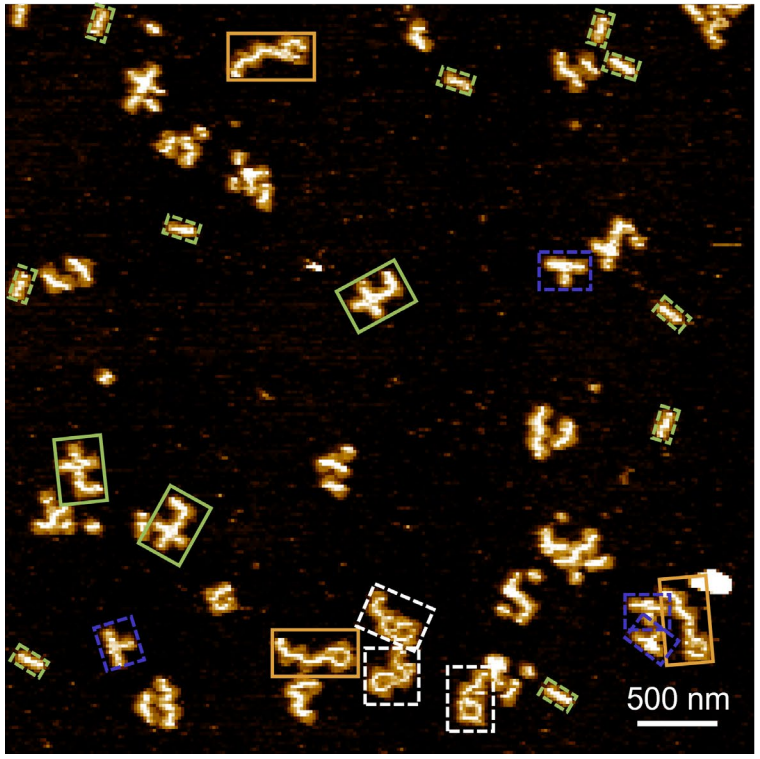
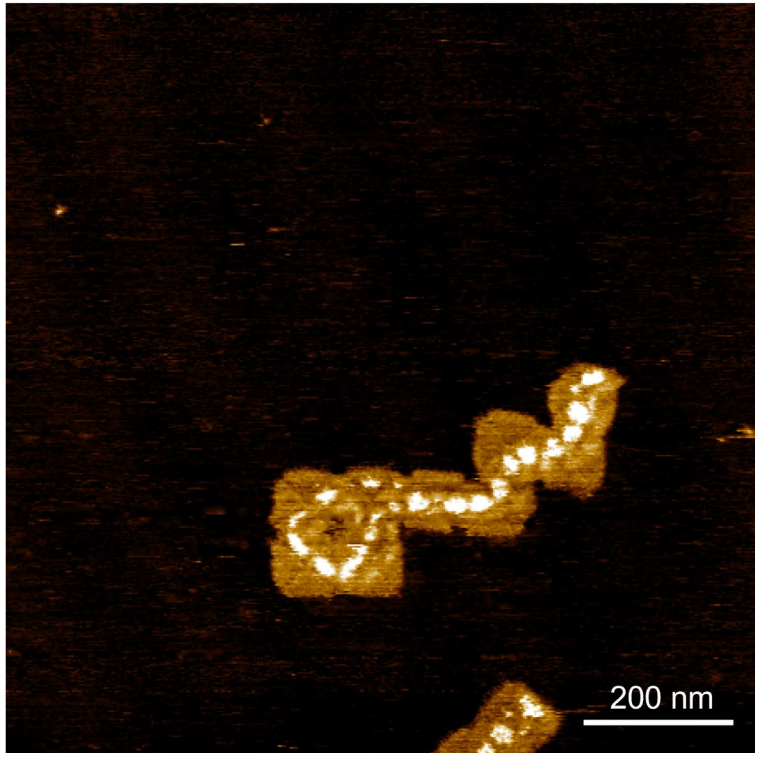
Simultaneous tile displacement

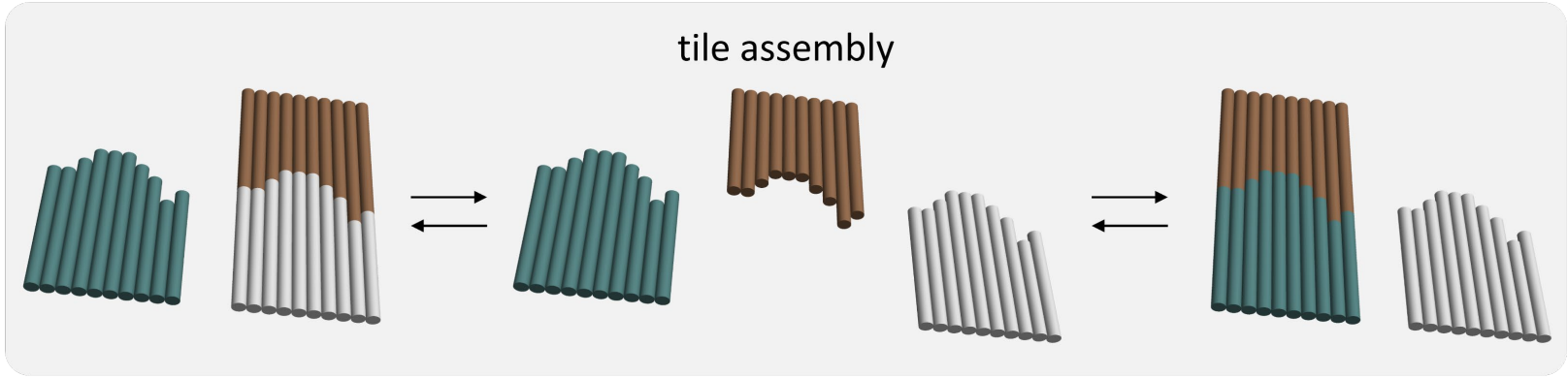






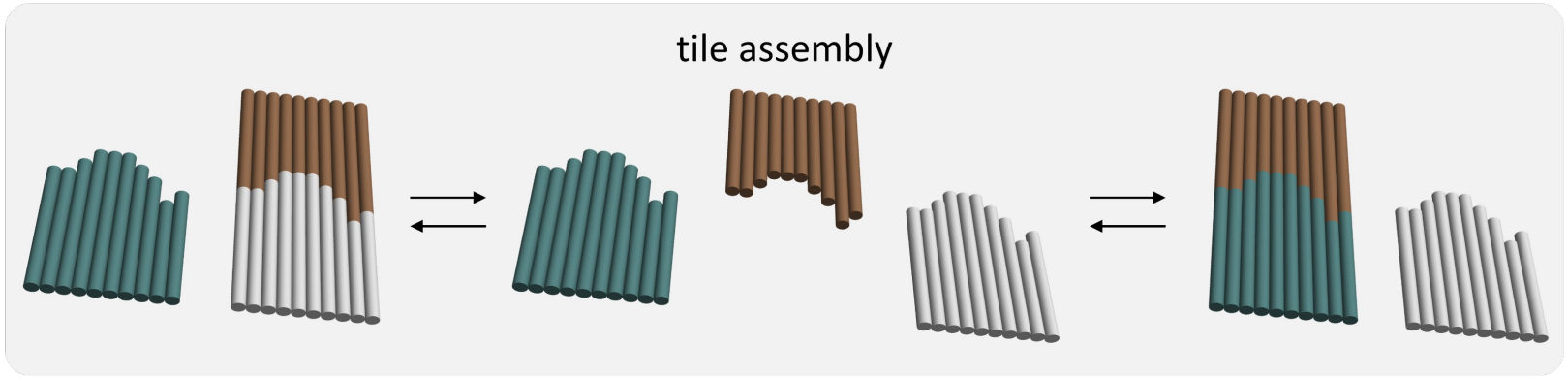
Sarraf, Rodriguez, and Qian, *Science Robotics* (2023)

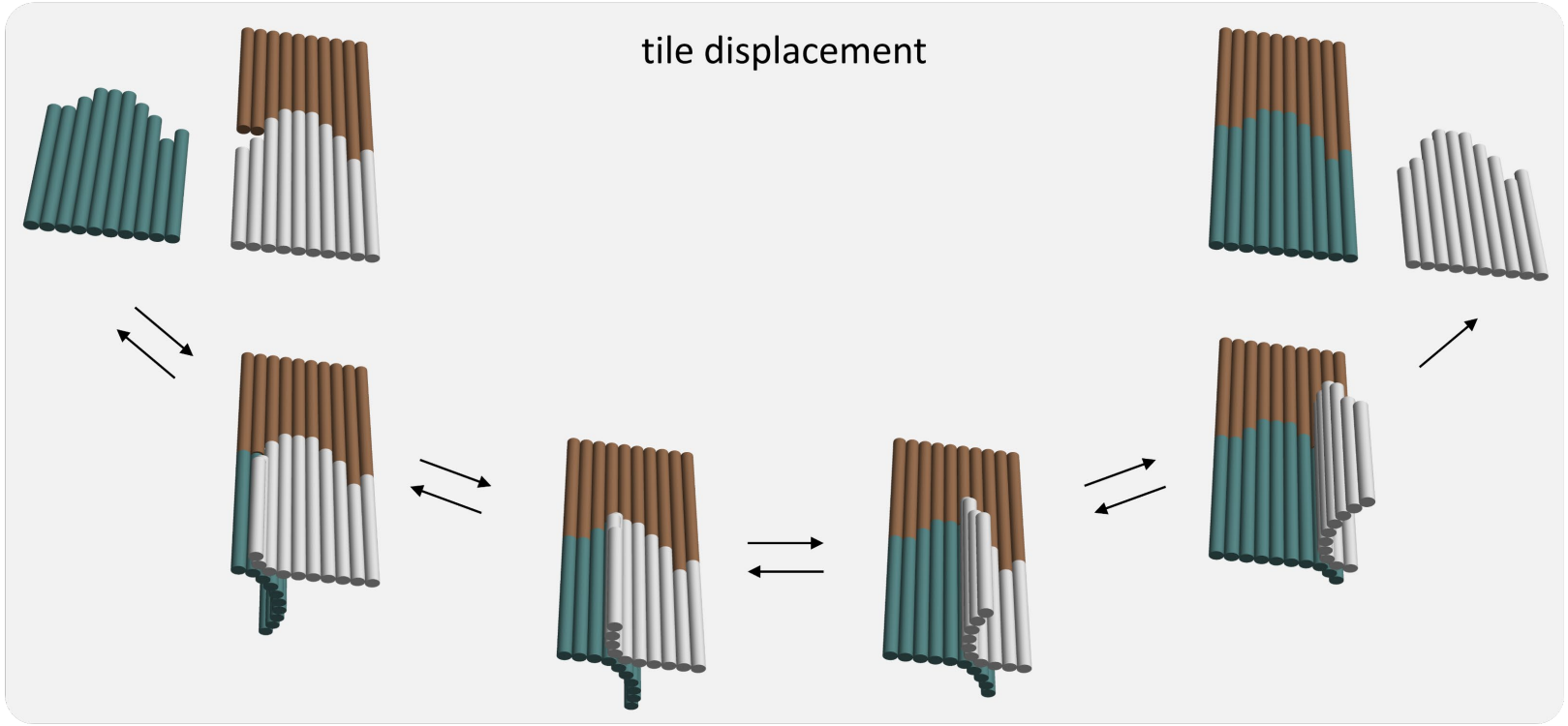
Binding between DNA tiles has limited specificity, and thus tile assembly systems must operate near melting temperature.

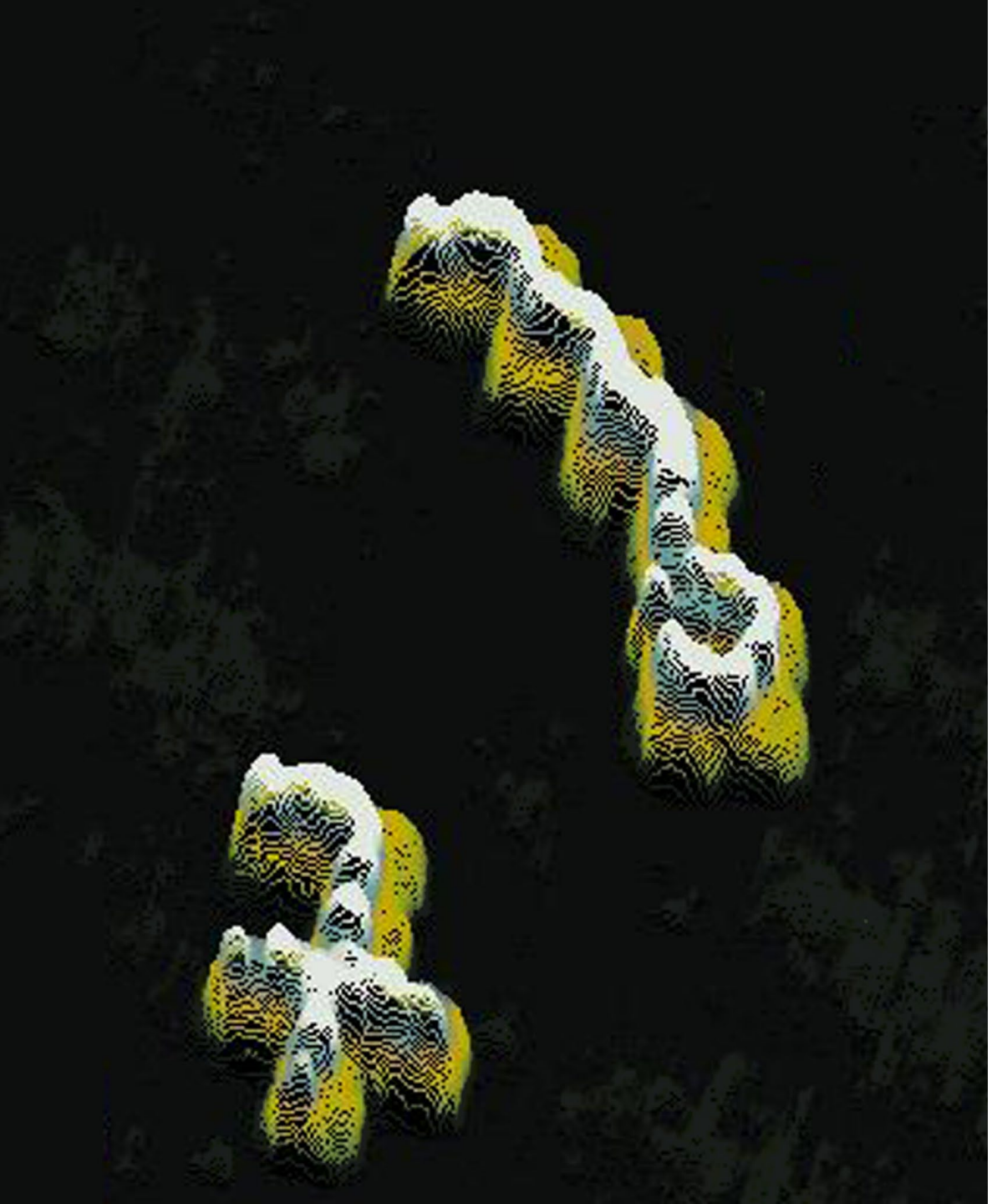


Binding between DNA tiles has limited specificity, and thus tile assembly systems must operate near melting temperature.

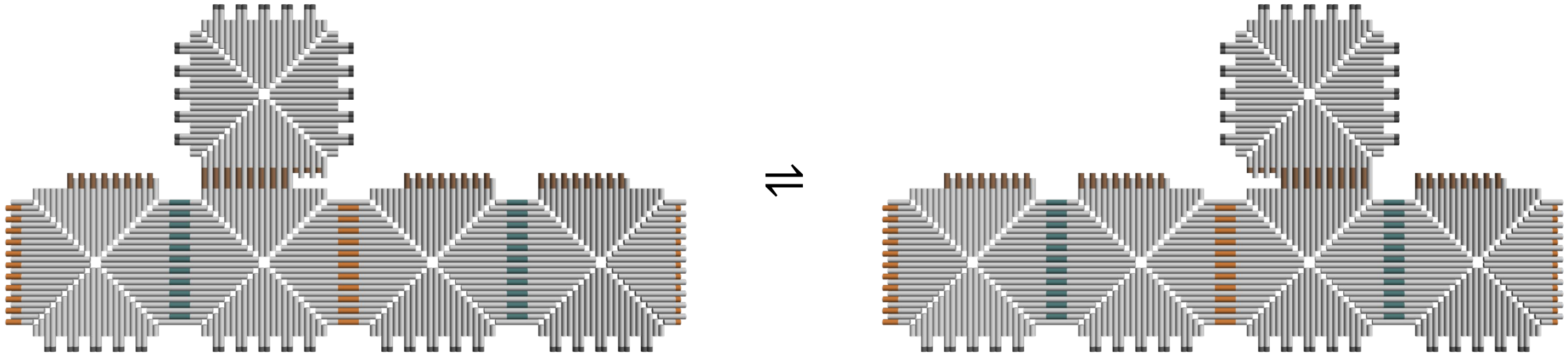
Tile displacement systems operate at room temperature with little crosstalk between distinct reactions, serving as a mechanism for modular reconfiguration robust to temperature and tile concentration.

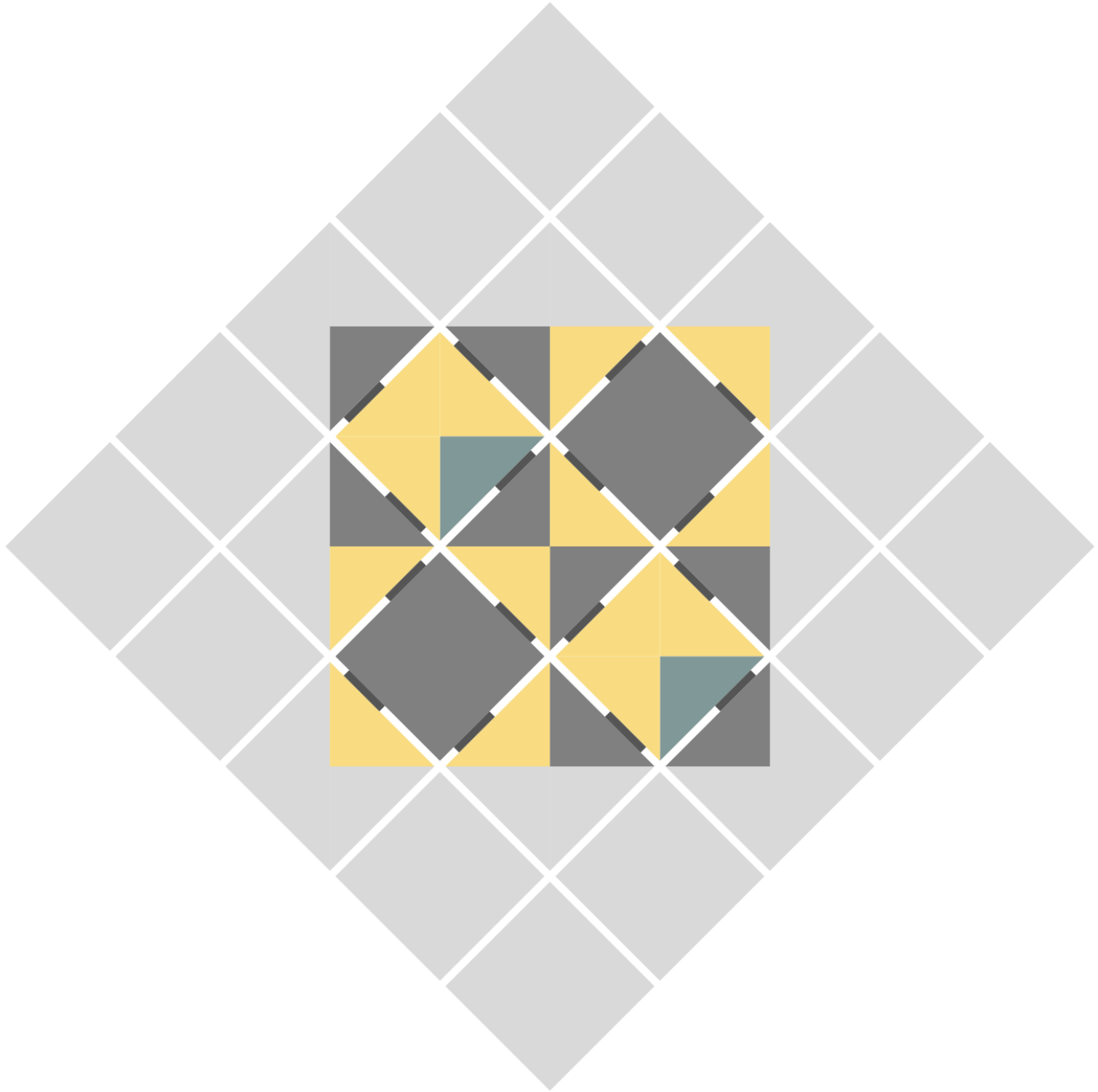






Future work: localized tile displacement





Tile displacement cellular automata

Erik Winfree and Lulu Qian

Bioengineering
Computer Science
Computation & Neural Systems

Caltech

DNA29, September 14, 2023

The Qian lab @ Caltech

Lulu Qian

Greg Tikhomirov

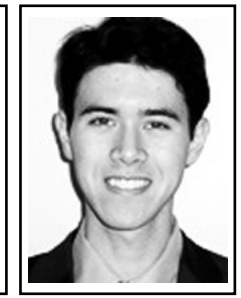
Wei Li

Tianqi Song

Dominic Scalise

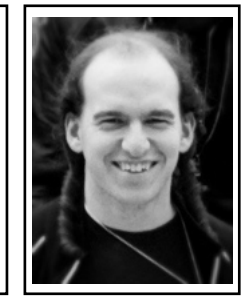
Boya Wang

Anu Thubagere



Philip Petersen

Kevin Cherry



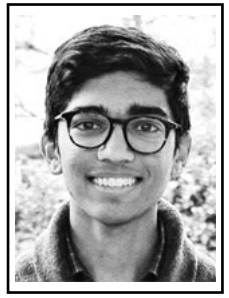
Hope Johnson

Namita Sarraf

Sam Davidson

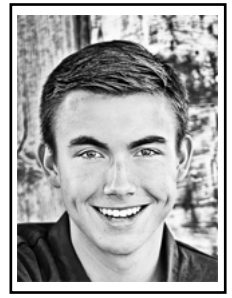
Olivia Zou

Matthew Plazola



Gokul Gowri

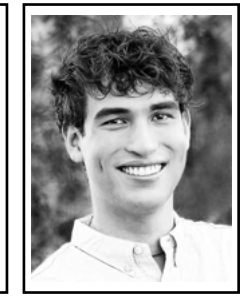
Dallas Taylor



Kellen Rodriguez

Allison Glynn

Spencer Winter



Martin Holmes

SCHMIDT FUTURES

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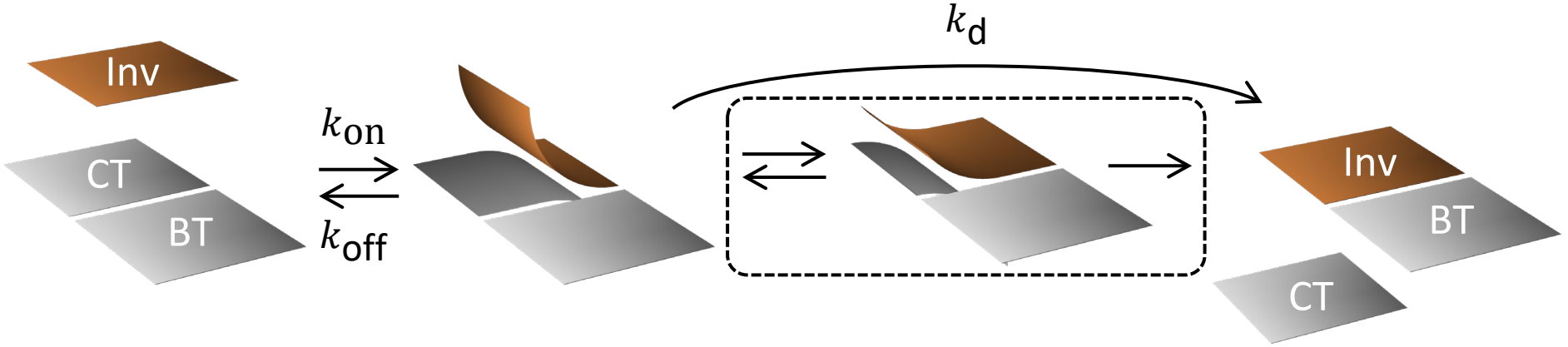
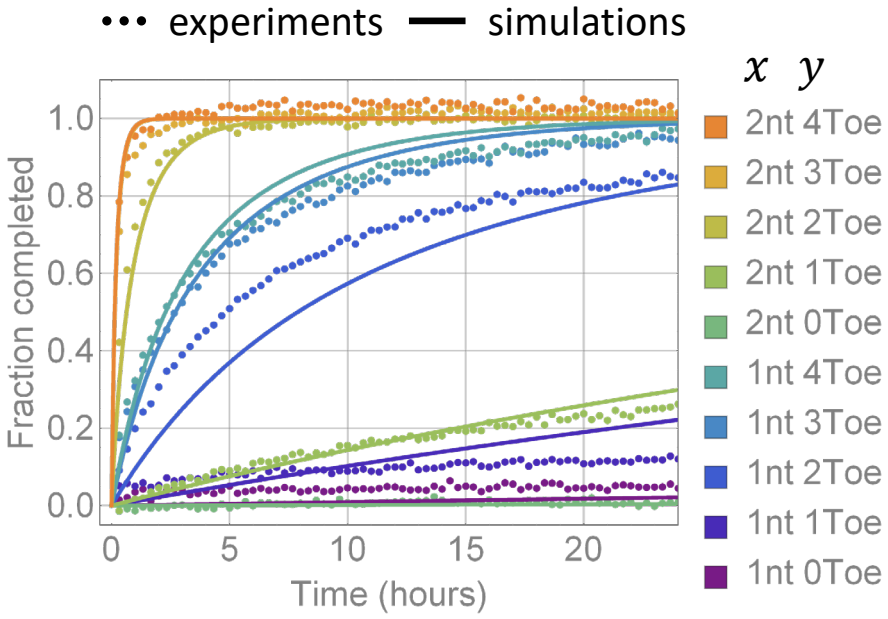
Kinetics of DNA tile displacement





	Tile displacement		Strand displacement
binding rate (k_{on})	$x = 1$	2.5×10^4/M/s	2×10^6/M/s
	$x = 2$	4.5×10^5/M/s	
dissociation rate (k_{off})	$x = 1$	$10^{1-1.1y}$/s	10^{6-L}/s
	$x = 2$	10^{3-2y}/s	
displacement rate (k_d)	0.025/s		1/s

Petersen, Tikhomirov, and Qian, *Nature Communications* (2018)