

# Kinetic Trans-Assembly of DNA Nanostructures

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## 1 DNA strand sequences

The sequences of all the complexes used in the experiments are shown below, first as a sequence map (denoted with functional domain names, *e.g.* s1 and Tm1, and strand names in bold) followed by a table listing the individual strand names, sequences, and strand lengths.

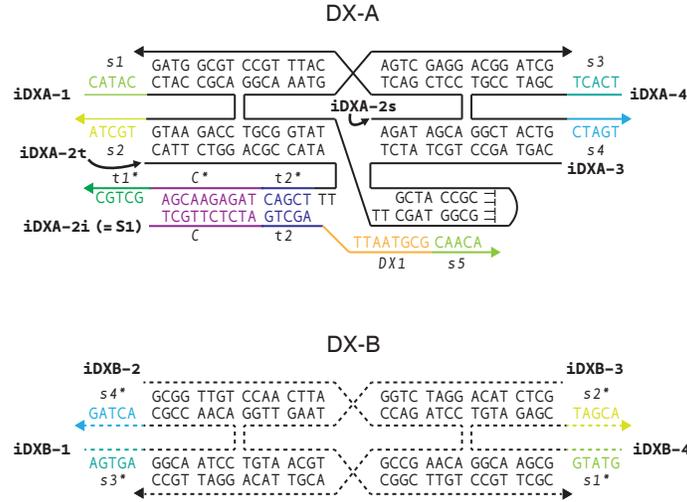


Figure S1: Sequence map of the DX-A and DX-B tiles.

Strand name	Sequence (5' → 3')	Length
iDXA-1	CATACTACCGCACCAGAATGTGCTA	26mers
iDXA-2s	AGATAGCACCTCGACTCATTGCGCTGCGGTAG	32mers
iDXA-2t	CATTCTGGACGCCATATTCGACTAGAGAACGAGCTGC	38mers
iDXA-2i (= S1)	TCGTTCTCTAGTCGATTAATGCGCAACA	28mers
iDXA-3hp	CAGTAGCCTGCTATCTGCTACCGCTTTTGGCGTAGCTTTATGGCGTGGCAAATGAGTCGAGGACGGATCG	70mers
iDXA-4	TCAGCTCTCC TGCC TAGC	26mers
iDXB-1	AGTGAGGCAATCCACAACCGCACTAG	26mers
iDXB-2	GCGGTTGTCCAACCTACCAGATCCACAAGCCGACGTTACAGGATTGCC	48mers
iDXB-3	GCTCTACAGGATCTGGTAAGTTGGTGTAAACGTCGGCTTGTCCGTTCCGC	48mers
iDXB-4	GTATGGCGAACGGGTAGAGCTAGCA	26mers

Table S1: Sequence details of the DX-A and DX-B tiles.

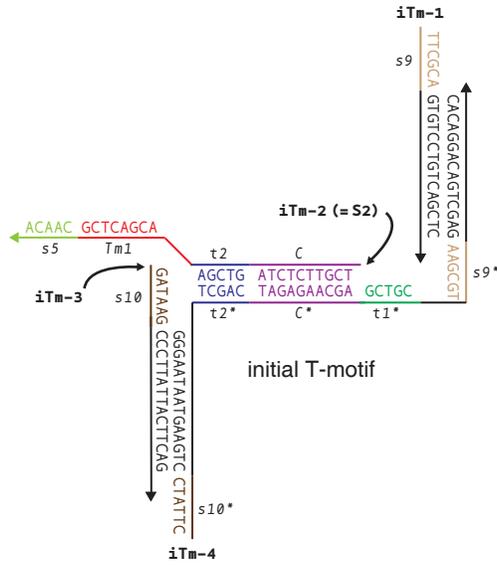


Figure S2: Sequence map of the initial T-motif.

Strand name	Sequence (5' → 3')	Length
iTm-1	TTCGCA GTCTCTCAGCTC	21mers
iTm-2 (= S2)	TCGTTCTCTAGTCGAACGACTCGCAACA	28mers
iTm-3	GATAAGCCCTTACTTTCAG	21mers
iTm-4	CTTATCCTGAAGTAATAAGGGTCGACTAGAGAACGAGCTGCTGCGAAGAGCTGACAGGACAC	62mers

Table S2: Sequence details of the initial T-motif.

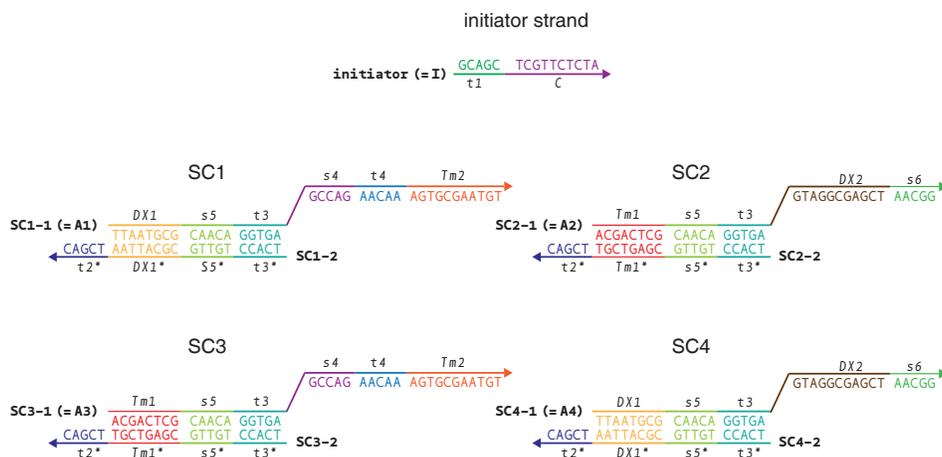


Figure S3: Sequence map of the initiator strand and the SC1-4 subcircuits.

Strand name	Sequence (5' → 3')	Length
initiator (= I)	GCAGCTCGTTCTCTA	15mers
SC1-1 (= A1)	TTAATGCGCAACAGGTGAGCCAGAACAAGTGC GAATGT	39mers
SC1-2	TCACCTGTTGCGCATTAAATCGAC	23mers
SC2-1 (= A2)	ACGACTCGCAACAGGTGAGTAGGCGAGCTAACGG	34mers
SC2-2	TCACCTGTTGCGAGTCGTTTCGAC	23mers
SC3-1 (= A3)	ACGACTCGCAACAGGTGAGCCAGAACAAGTGC GAATGT	39mers
SC3-2	TCACCTGTTGCGAGTCGTTTCGAC	23mers
SC4-1 (= A4)	TTAATGCGCAACAGGTGAGTAGGCGAGCTAACGG	34mers
SC4-2	TCACCTGTTGCGCATTAAATCGAC	23mers

Table S3: Sequence details of the initiator strand and SC1-4 subcircuits.

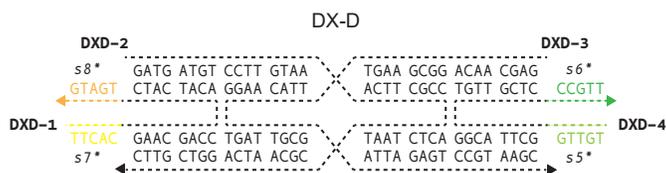
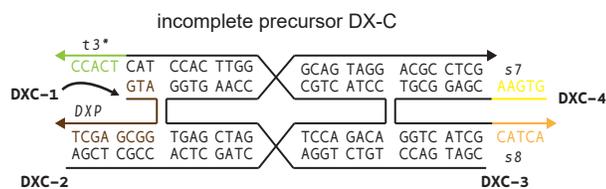


Figure S4: Sequence map of the incomplete precursor DX-C and DX-D tiles.

Strand name	Sequence (5' → 3')	Length
DXP	GTAGGCGAGCT	11mers
DXC-2	AGCTCGCCACTCGATCTCCAGACACCTACTGCGGTTACCTACTCACC	48mers
DXC-3	CGATGACCTGTCTGGAGATCGAGTGGTGAACCGCAGTAGGACGCCTCG	48mers
DXC-4	GTGAACGAGGCGTGGTCATCGCATCA	26mers
DXD-1	TTCACGAACGACCACATCATCTGATG	26mers
DXD-2	GATGATGTCCTTGAAACTTCGCCACTCTAATCGCAATCAGGTCGTT	48mers
DXD-3	GAGCAACAGGCGAAGTTACAAGGTGATTGCGATTAGAGTCCGTAAGC	48mers
DXD-4	TGTTGGCTTACGGTGTGCTCCCGTT	26mers

Table S4: Sequence details of incomplete precursor DX-C and DX-D tiles.

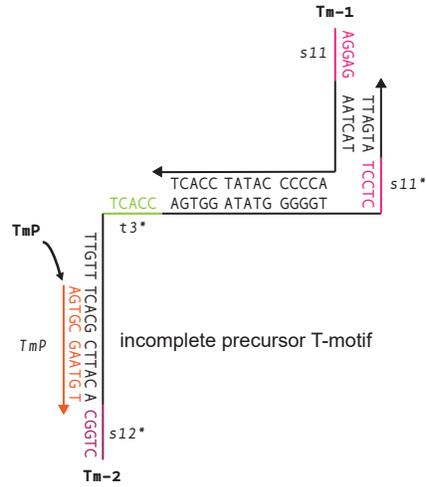


Figure S5: Sequence map of the incomplete precursor T-motif.

Strand name	Sequence (5' → 3')	Length
TmP	AGTGCGAATGT	11mers
Tm-1	AGGAGAATCATACCCCATATCCACT	26mers
Tm-2	CTGGCACATTCGCACATTTGTTTCACCAGTGATATGGGGTCTCCTATGATT	52mers

Table S5: Sequence details of the incomplete precursor T-motif.

## 2 Sample preparation for kinetic trans-assembly

A total of four different schemes (schemes I-IV) were prepared. For each scheme, stoichiometric amounts of the initial DNA structure, two DNA sub-circuits (SC1 and SC2 or SC3 and SC4), incomplete precursor T-motifs, incomplete precursor DX tiles, and DX-D tiles were pipetted into a single microtube. To start the reaction cascade,  $\times 10$  the amount of the initiator strand (compared to the other components) was pipetted into the microtube. The final concentration of the initiator strand was 300 nM and the final concentration of the rest of the components was 30 nM. The microtubes were kept at 30 C for 4 hours to facilitate the strand displacement and self-assembly reactions.

scheme I components	[nM]
DX-I	30
SC1	30
SC2	30
precursor T-motif	30
precursor DX-C	30
DX-D	30
initiator strand	300

scheme II components	[nM]
DX-I	30
SC3	30
SC4	30
precursor T-motif	30
precursor DX-C	30
DX-D	30
initiator strand	300

scheme III components	[nM]
ladder	30
SC1	30
SC2	30
precursor T-motif	30
precursor DX-C	30
DX-D	30
initiator strand	300

<u>scheme IV components</u>	<u>[nM]</u>
ladder	30
SC3	30
SC4	30
precursor T-motif	30
precursor DX-C	30
DX-D	30
initiator strand	300

### 3 Additional AFM data

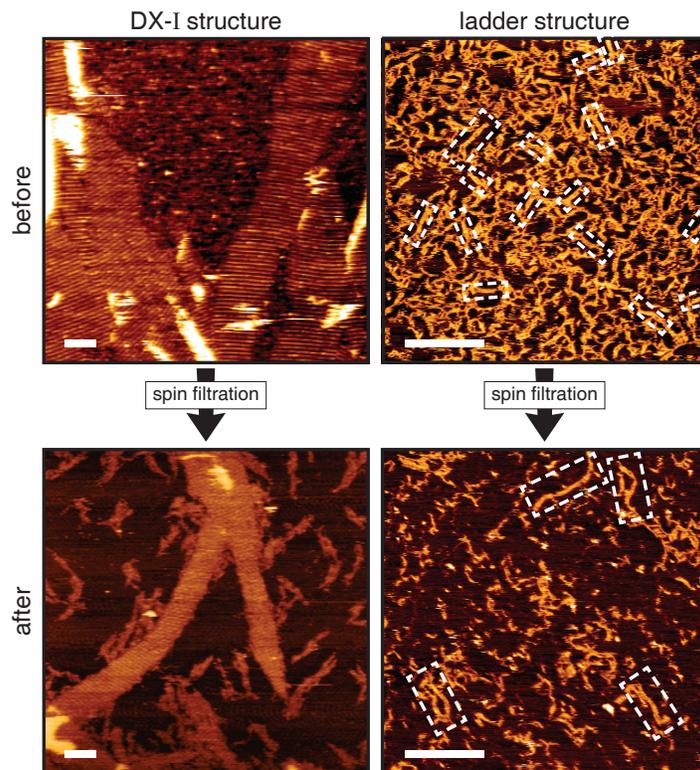


Figure S6: AFM data of the initial DNA structures used in this work (top row) before and (bottom row) after spin filtration. Spin filtration is done to remove any unattached single-stranded signal strands from the system to prevent leak reactions. After filtering, these initial structures were added to the system along with the other purified components (*e.g.* subcircuits and precursor tiles) in stoichiometric amounts. Once all the components have been placed into a microtube, the initiator strand is inserted to initiate the reaction cascade. Some of the ladder structures are shown inside dashed white boxes (white scale bars : 200 nm).

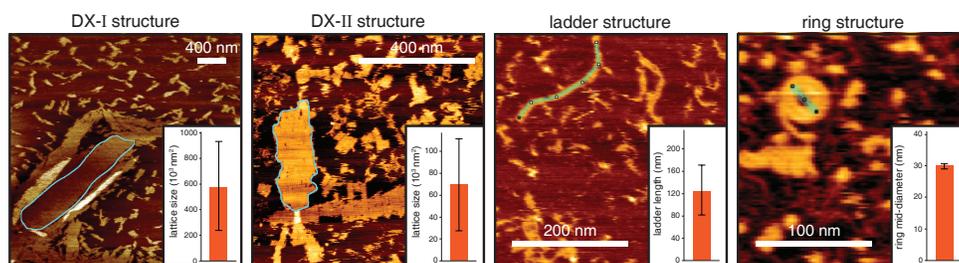


Figure S7: Size analysis of DX-I, DX-II, ladder, and ring structures from AFM images using ImageJ. The averages were calculated from 8 representative structures and the error bars indicate the standard deviation.

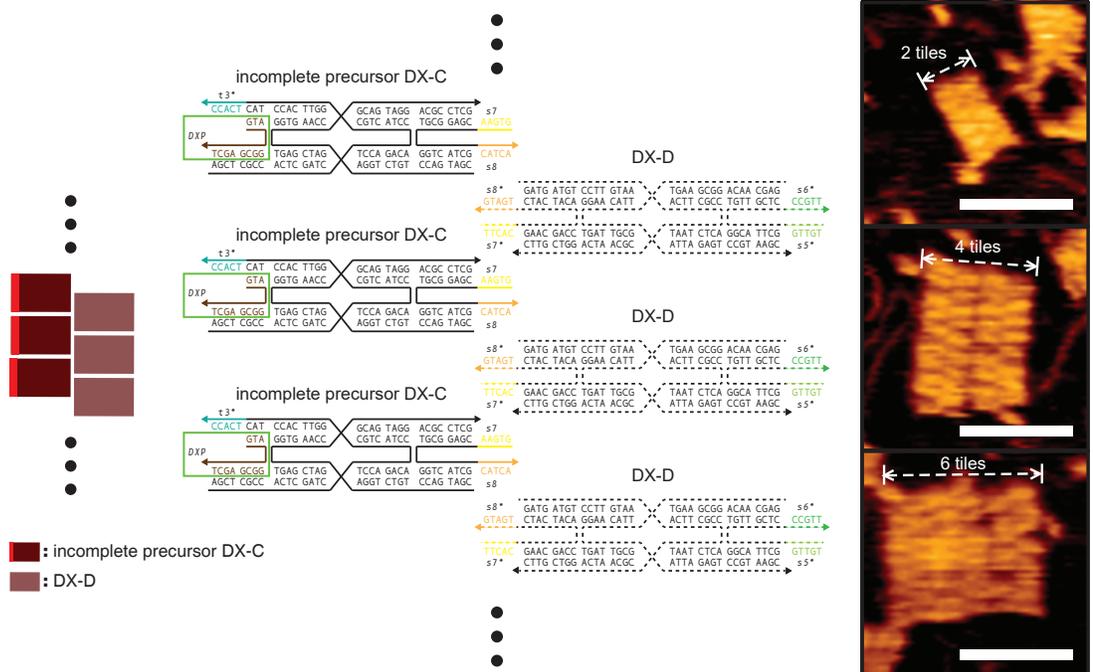


Figure S8: Formation of small lattice fragments from incomplete precursor DX-C and DX-D tiles. Since incomplete precursor DX-C tiles only possess 2 out of the normal 4 sticky-ends (the green boxes indicate where the missing s5 and s6 sticky-ends should be), growth cannot occur along the helical axes but can, to some degree, occur perpendicularly to the helical axes through the s7, s8, and their respective complementary sticky-ends to form 2-tile wide columnar units (shown in the cartoon, sequence map, and top AFM image). Units of these 2-tile wide columnar structures can be brought together by base-stacking and/or geometric stacking to form 4-tile wide (center AFM) or 6-tile wide (bottom AFM) lattice fragments (white scale bars : 50 nm).