SUPPORTING INFORMATION

Reconfiguring DNA Nanotube Architectures *via* Selective Regulation of Terminating Structures

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1.1 RE and SE nanotube monomer designs and sequences

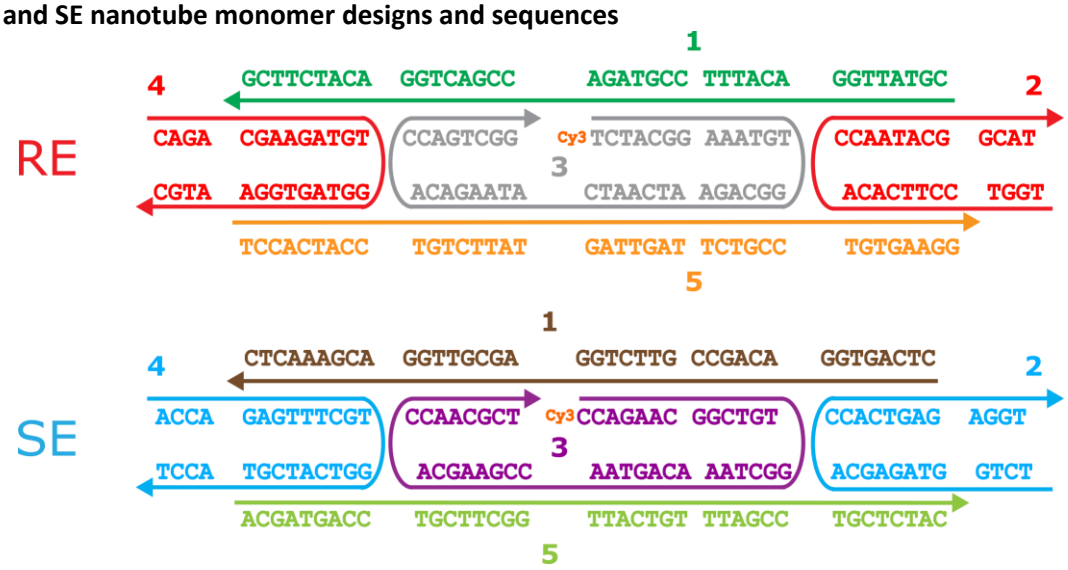


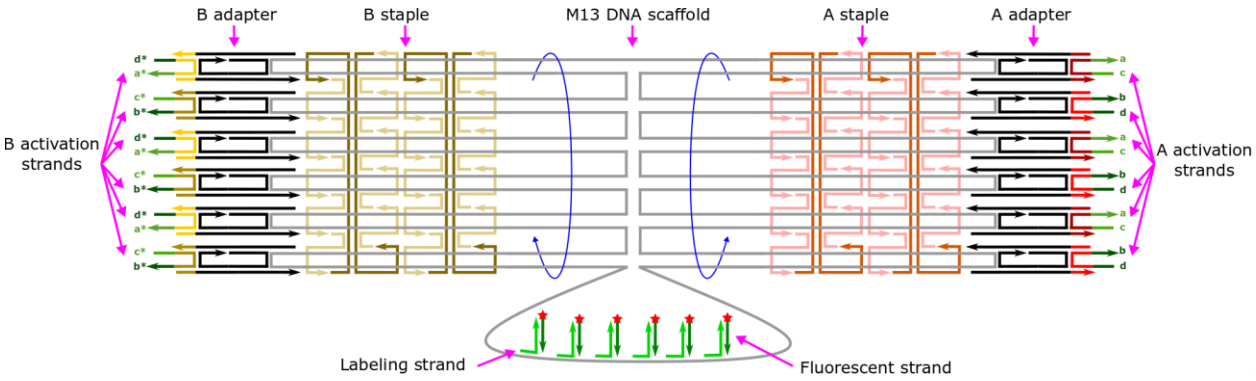
Table S1: Monomer strand sequences. All strands were ordered from Integrated DNA Technologies, Inc (IDT). Strands 1, 2, 4, and 5 were ordered PAGE purified and strand 3 was ordered HPLC purified. Modifications marked with / / are labels as defined by IDT.

Strand name	Sequence
RE – 1	CGTATTGGACATTTCCGTAGACCGACTGGACATCTTCG
RE – 2	TGGTCCTTCACACCAATACGGCAT
RE – 3	/5Cy3/TCTACGGAAATGTGGCAGAATCAATCATAAGACACCAGTCGG
RE – 4	CAGACGAAGATGTGGTAGTGGAATGC
RE – 5	TCCACTACCTGTCTTATGATTGATTCTGCCTGTGAAGG
SE – 1	CTCAGTGGACAGCCGTTCTGGAGCGTTGGACGAAACTC
SE – 2	TCTGGTAGAGCACCACTGAGAGGT
SE – 3	/5Cy3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCACCAACGCT
SE – 4	ACCAGAGTTTCGTGGTCATCGTACCT
SE – 5	ACGATGACCTGCTTCGGTTACTGTTTAGCCTGCTCTAC

1.2 Design of DNA origami termini

The design of the DNA origami termini was adopted from previous studies.[1,2] Each terminus is composed of a scaffold strand (M13mp18 DNA (7,240 bases) purchased from New England Biolabs), 24 staple strands, 18 adapter strands, and 2 activation strands (strands on the adapters that present the monomer sticky end sequences). Staples that link the top and bottom of the rows of the terminus in the diagram below are shown in color a darker shade than the other staples for clarity. The staple strand sequences are the same as those used in a previously work[1] and include hairpin domains (not depicted in the diagram below) that face outward after cyclization, and were added to control the direction of cyclization so that the direction of curvature would match that of the nanotubes.[1] The A and B termini are folded from different domains of the M13 DNA scaffold as shown below. To prepare A termini, the A staples and A adapter strands were mixed with M13 scaffold strand and annealed, while to prepare the B termini, B staples and B adapter stands were mixed with the M13 scaffold strand and annealed. Excess staples and adapters were removed after annealing via filtration, preventing cross-assembly (Methods).

The origami termini were fluorescently labeled as described previously.[3] Briefly, the portion of M13 DNA that was not used for folding either of the termini was used as binding sites for one hundred unique DNA strands (labeling strands). Each labeling strand is complementary to a unique 25-base region of the unfolded M13 at its 5' end and has the a common 15-base sequence at its 3' end. The 15-base sequence at the 3' end of each labeling strand served as a binding site for a DNA strand that was modified with a fluorescent tag at its 5' end (either atto488 or atto647 in this study). The labeling strands and fluorescent strand sequences are the same for both A and B termini.



1.3 Sequences of A termini

A termini

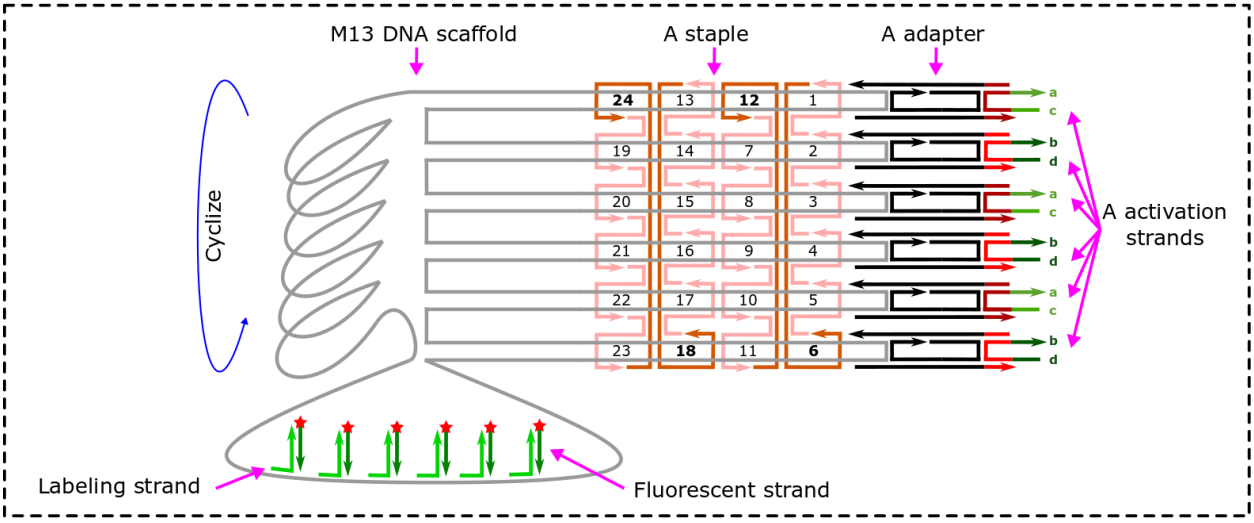


Table S2: Sequences of A termini staples. These strands were used to assemble all of the A termini. All staple strands were ordered unpurified from IDT. Numbers beside strand names correspond to the numbers identifying individual staples in the diagram above. As in previous nanotube seed designs, hairpins (highlighted in red) were incorporated into the staples to induce a directional preference for cyclization.[1]

Strand Name	Sequence
(1) T5R2E_HP	5′ ACAAACAACTGCCTATCACGACGCTTTTGCGTCGTGTTTTCGGAACCTGAGACT 3′
(2) T5R4E_HP	5′ TCGGCATTCCGCCGCCGTCGCTGCTTTTGCAGCGACTTAGCATTGATGATATTC 3′
(3) T5R6E_HP	5′ ATTGAGGGAATCAGTACGGAGCACTTTTGTGCTCCGTTGCGACAGACGTTTTCA 3′
(4) T5R8E_HP	5′ GAAGGAAAAATAGAAAGCCTAGCGTTTTCGCTAGGCTTATTCATATTTCAACCG 3′
(5) T5R10E_HP	5′ CTTTACAGTATCTTACCGCTCGTGTTTTCACGAGCGTTCGAAGCCCAGTTACCA 3′
(6) T5R12E_CYC_HP	5′ CCTCAAGATCCCAATCCGTGGAGCTTTTGCTCCACGTTCAAATAAGATAGCAGC 3′
(7) T5R2F_HP	5′ AATGCCCCATAAATCCGCTCGGACTTTTGTCCGAGCTTTCATTAAAAGAACCAC 3′
(8) T5R4F_HP	5′ CACCAGAGTTCGGTCAGCCGAGCGTTTTCGCTCGGCTTTAGCCCCCTCGATAGC 3′
(9) T5R6F_HP	5′ AGCACCGTAGGGAAGGTCGGAGGCTTTTGCCTCCGATTTAAATATTTTATTTTG 3′
(10) T5R8F_HP	5′ TCACAATCCCGAGGAACTGGTGGCTTTTGCCACCAGTTACGCAATAATGAAATA 3′
(11) T5R10F_HP	5′ GCAATAGCAGAGAATACCGCAGGCTTTTGCCTGCGGTTACATAAAAACAGCCAT 3′
(12) T5R12F_CYC_HP	5′ ATTATTTAGAAGGATTGCCATCGCTTTTGCGATGGCTTAGGATTAGAAACAGTT 3′
(13) T3R2E_HP	5′ GGAAAGCGGTAACAGTGTGGCAGCTTTTGCTGCCACTTGCCCGTATCGGGGTTT 3′
(14) T3R4E_HP	5′ GTTTGCCACCTCAGAGACCAGGCGTTTTCGCCTGGTTTCCGCCACCGCCAGAAT 3′
(15) T3R6E_HP	5′ TTATTCATGTCACCAAGCTCGCTGTTTTCAGCGAGCTTTGAAACCATTATTAGC 3′
(16) T3R8E_HP	5′ ATACCCAAACACCACGCCTACCGCTTTTGCGGTAGGTTGAATAAGTGACGGAAA 3′
(17) T3R10E_HP	5′ GCGCATTAATAAGAGCCTGGACGCTTTTGCGTCCAGTTAAGAAACAATAACGGA 3′
(18) T3R12E_CYC_HP	5′ TGCTCAGTGCCAGTTAGGTGGTCGTTTTCGACCACCTTCAAAATAAACAGGGAA 3′
(19) T3R2F_HP	5′ TGCCTTGACAGTCTCTGTCGGTGCTTTTGCACCGACTTGAATTTACCCCTCAGA 3′
(20) T3R4F_HP	5′ GCCACCACTCTTTTCACGGTCGGCTTTTGCCGACCGTTTAATCAAATAGCAAGG 3′
(21) T3R6F_HP	5′ CCGGAAACTAAAGGTGGACCTGGCTTTTGCCAGGTCTTAATTATCATAAAAGAA 3′
(22) T3R8F_HP	5′ ACGCAAAGAAGAACTGTCGGCTCGTTTTCGAGCCGATTGCATGATTTGAGTTAA 3′
(23) T3R10F_HP	5′ GCCCAATAGACGGGAGCACAGGCGTTTTCGCCTGTGTTAATTAACTTTCCAGAG 3′
(24) T3R12F_CYC_HP	5′ CCTAATTTACCAGGCGTCGGAGCGTTTTCGCTCCGATTGATAAGTGGGGGTCAG 3′

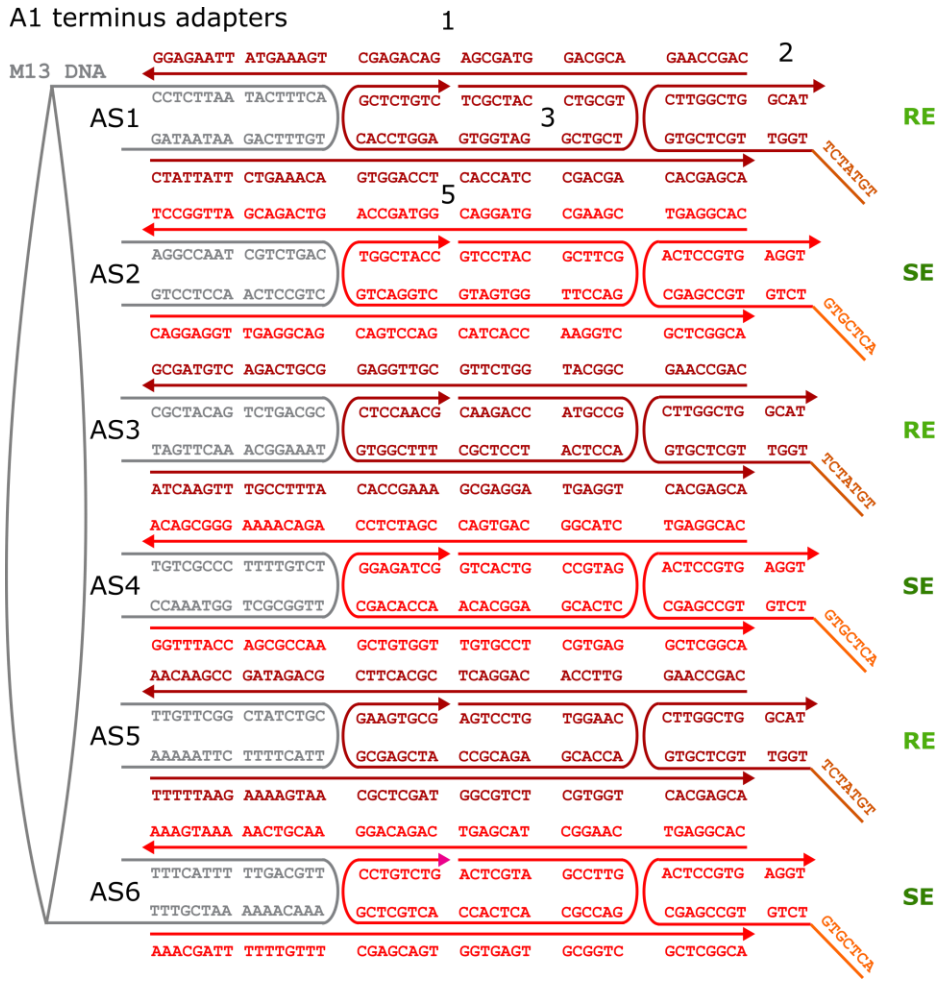


Table S3: A1 DNA origami terminus adapter strands. All strands were ordered unpurified from IDT.

Strand Name	Sequence
A1_AS1_RE_1	5′ CAGCCAAGACGCAGGTAGCGAGACAGAGCTGAAAGTATTAAGAGG 3′
A1_AS1_RE_3	5′ TCGCTACCTGCGTTCGTCGGATGGTGAGGTCCACGCTCTGTC 3′
A1_AS1_RE_5	5′ CTATTATTCTGAAACAGTGGACCTCACCATCCGACGACACGAGCA 3′
A1_AS2_SE_1	5′ CACGGAGTCGAAGCGTAGGACGGTAGCCAGTCAGACGATTGGCCT 3′
A1_AS2_SE_3	5′ GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC 3′
A1_AS2_SE_5	5′ CAGGAGGTTGAGGCAGCAGTCCAGCATCACCAAGGTCGCTCGGCA 3′
A1_AS3_RE_1	5′ CAGCCAAGCGGCATGGTCTTGCGTTGGAGGCGTCAGACTGTAGCG 3′
A1_AS3_RE_3	5′ CAAGACCATGCCGACCTCATCCTCGCTTTCGGTGCTCCAACG 3′
A1_AS3_RE_5	5′ ATCAAGTTTGCCTTTACACCGAAAGCGAGGATGAGGTCACGAGCA 3′
A1_AS4_SE_1	5′ CACGGAGTCTACGGCAGTGACCGATCTCCAGACAAAAGGGCGACA 3′
A1_AS4_SE_3	5′ GTCACTGCCGTAGCTCACGAGGCACAACCACAGCGGAGATCG 3′
A1_AS4_SE_5	5′ GGTTTACCAGCGCCAAGCTGTGGTTGTGCCTCGTGAGGCTCGGCA 3′
A1_AS5_RE_1	5′ CAGCCAAGGTTCCACAGGACTCGCACTTCGCAGATAGCCGAACAA 3′
A1_AS5_RE_3	5′ AGTCCTGTGGAACACCACGAGACGCCATCGAGCGGAAGTGCG 3′
A1_AS5_RE_5	5′ TTTTTAAGAAAAGTAACGCTCGATGGCGTCTCGTGGTCACGAGCA 3′
A1_AS6_SE_1	5′ CACGGAGTCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA 3′
A1_AS6_SE_3	5′ ACTCGTAGCCTTGGACCGCACTCACCACTGCTCGCCTGTCTG 3′
A1_AS6_SE_5	5′ AAACGATTTTTTGTTTCGAGCAGTGGTGAGTGCGGTCGCTCGGCA 3′
Activation strands	
A1_AS135_RE_2	5′ TGTATCTTGGTTGCTCGTGCTTGGCTGGCAT 3′
A1_AS246_SE_2	5′ ACTCGTGTCTGTGCCGAGCACTCCGTGAGGT 3′
Inactivation strands	
A1_AS135_RE_inact	5′ ATGCCAGCCAAGCACGAGCAACCAAGATACA 3′
A1_AS246_SE_inact	5′ ACCTCACGGAGTGCTCGGCACAGACACGAGT 3′

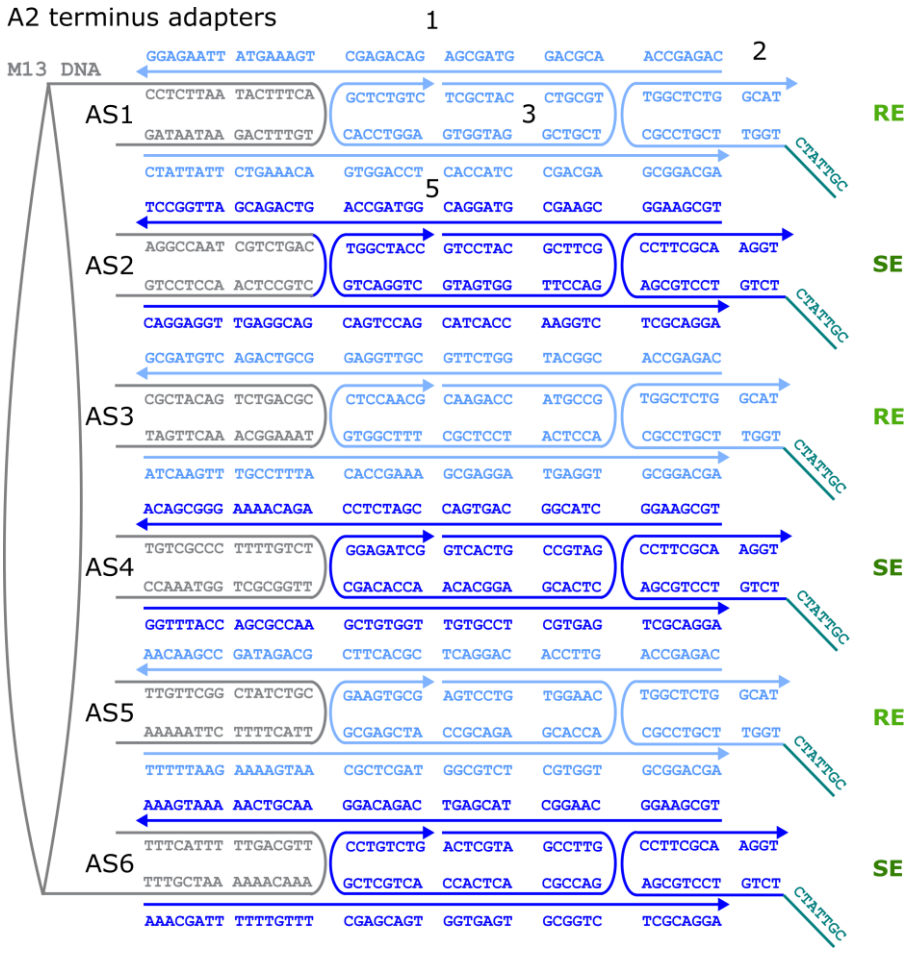


Table S4: A2 DNA origami terminus adapter strands. All of the _3 strands have the same sequences as the corresponding strands of the A1 terminus. All strands were ordered unpurified from IDT.

Strand Name	Sequence
A2_AS1_RE_1	5′ CAGAGCCAACGCAGGTAGCGAGACAGAGCTGAAAGTATTAAGAGG 3′
A2_AS1_RE_3	5′ TCGCTACCTGCGTTCGTCGGATGGTGAGGTCCACGCTCTGTC 3′
A2_AS1_RE_5	5′ CTATTATTCTGAAACAGTGGACCTCACCATCCGACGAGCGGACGA 3′
A2_AS2_SE_1	5′ TGCGAAGGCGAAGCGTAGGACGGTAGCCAGTCAGACGATTGGCCT 3′
A2_AS2_SE_3	5′ GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC 3′
A2_AS2_SE_5	5′ CAGGAGGTTGAGGCAGCAGTCCAGCATCACCAAGGTCTCGCAGGA 3′
A2_AS3_RE_1	5′ CAGAGCCACGGCATGGTCTTGCGTTGGAGGCGTCAGACTGTAGCG 3′
A2_AS3_RE_3	5′ CAAGACCATGCCGACCTCATCCTCGCTTTCGGTGCTCCAACG 3′
A2_AS3_RE_5	5′ ATCAAGTTTGCCTTTACACCGAAAGCGAGGATGAGGTGCGGACGA 3′
A2_AS4_SE_1	5′ TGCGAAGGCTACGGCAGTGACCGATCTCCAGACAAAAGGGCGACA 3′
A2_AS4_SE_3	5′ GTCACTGCCGTAGCTCACGAGGCACAACCACAGCGGAGATCG 3′
A2_AS4_SE_5	5′ GGTTTACCAGCGCCAAGCTGTGGTTGTGCCTCGTGAGTCGCAGGA 3′
A2_AS5_RE_1	5′ CAGAGCCAGTTCCACAGGACTCGCACTTCGCAGATAGCCGAACAA 3′
A2_AS5_RE_3	5′ AGTCCTGTGGAACACCACGAGACGCCATCGAGCGGAAGTGCG 3′
A2_AS5_RE_5	5′ TTTTTAAGAAAAGTAACGCTCGATGGCGTCTCGTGGTGCGGACGA 3′
A2_AS6_SE_1	5′ TGCGAAGGCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA 3′
A2_AS6_SE_3	5′ ACTCGTAGCCTTGGACCGCACTCACCACTGCTCGCCTGTCTG 3′
A2_AS6_SE_5	5′ AAACGATTTTTTGTTTCGAGCAGTGGTGAGTGCGGTCTCGCAGGA 3′
Activation strands	
A2_AS135_RE_2	5′ CGTTATCTGGTTCGTCCGCTGGCTCTGGCAT 3′
A2_AS246_SE_2	5′ CGTTATCTCTGTCCTGCGACCTTCGCAAGGT 3′
Inactivation strands	
A2_AS135_RE_inact	5′ ATGCCAGAGCCAGCGGACGAACCAGATAACG 3′
A2_AS246_SE_inact	5′ ACCTTGCGAAGGTCGCAGGACAGAGATAACG 3′

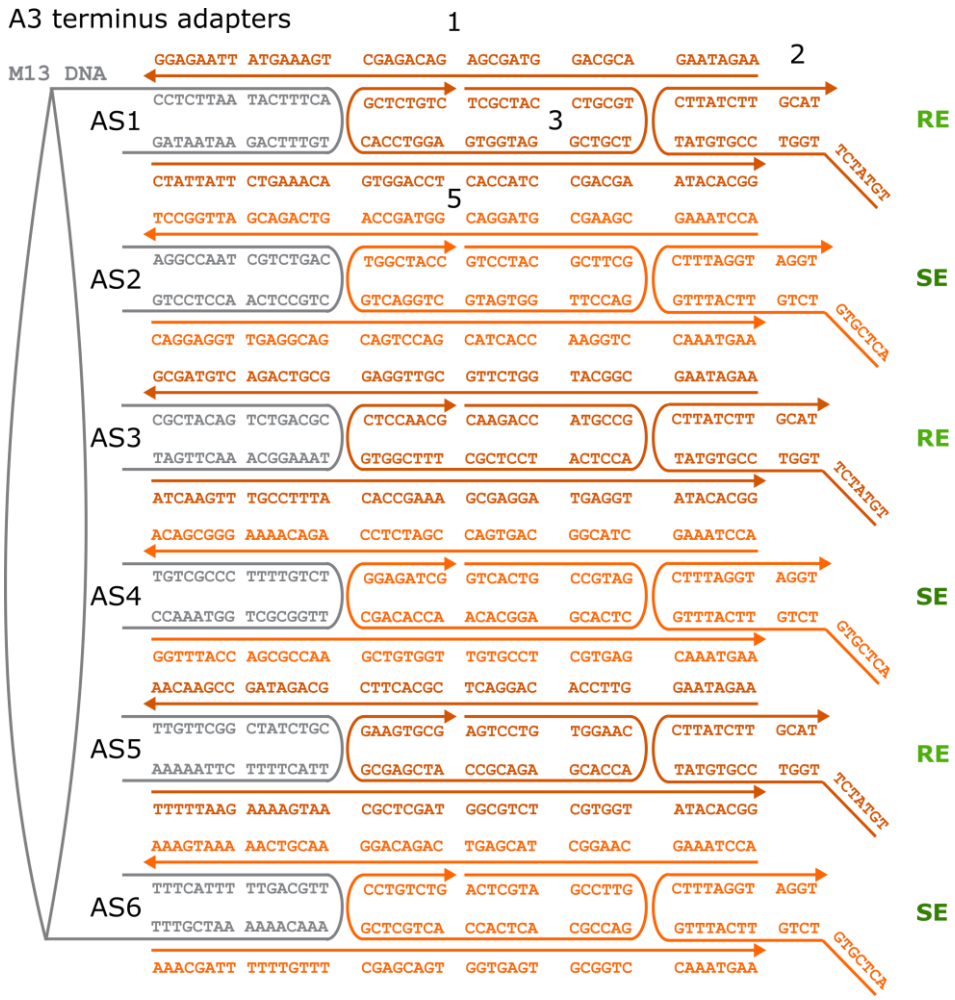


Table S5: A3 DNA origami terminus adapter strands. The activation strands for this terminus were designed to have no predicted secondary structure (Supporting Section 5). All of the _3 strands have the same sequences as the corresponding strands of the A1 and A2 termini. Two sets of activation strands were ordered, one set that were unpurified and another set that were PAGE purified from IDT. All other strands were ordered unpurified from IDT.

Strand Name	Sequence
A3_AS1_RE_1	5′ AAGATAAGACGCAGGTAGCGAGACAGAGCTGAAAGTATTAAGAGG 3′
A3_AS1_RE_3	5′ TCGCTACCTGCGTTCGTCGGATGGTGAGGTCCACGCTCTGTC 3′
A3_AS1_RE_5	5′ CTATTATTCTGAAACAGTGGACCTCACCATCCGACGAATACACGG 3′
A3_AS2_SE_1	5′ ACCTAAAGCGAAGCGTAGGACGGTAGCCAGTCAGACGATTGGCCT 3′
A3_AS2_SE_3	5′ GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC 3′
A3_AS2_SE_5	5′ CAGGAGGTTGAGGCAGCAGTCCAGCATCACCAAGGTCCAAATGAA 3′
A3_AS3_RE_1	5′ AAGATAAGCGGCATGGTCTTGCGTTGGAGGCGTCAGACTGTAGCG 3′
A3_AS3_RE_3	5′ CAAGACCATGCCGACCTCATCCTCGCTTTCGGTGCTCCAACG 3′
A3_AS3_RE_5	5′ ATCAAGTTTGCCTTTACACCGAAAGCGAGGATGAGGTATACACGG 3′
A3_AS4_SE_1	5′ ACCTAAAGCTACGGCAGTGACCGATCTCCAGACAAAAGGGCGACA 3′
A3_AS4_SE_3	5′ GTCACTGCCGTAGCTCACGAGGCACAACCACAGCGGAGATCG 3′
A3_AS4_SE_5	5′ GGTTTACCAGCGCCAAGCTGTGGTTGTGCCTCGTGAGCAAATGAA 3′
A3_AS5_RE_1	5′ AAGATAAGGTTCCACAGGACTCGCACTTCGCAGATAGCCGAACAA 3′
A3_AS5_RE_3	5′ AGTCCTGTGGAACACCACGAGACGCCATCGAGCGGAAGTGCG 3′
A3_AS5_RE_5	5′ TTTTTAAGAAAAGTAACGCTCGATGGCGTCTCGTGGTATACACGG 3′
A3_AS6_SE_1	5′ ACCTAAAGCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA 3′
A3_AS6_SE_3	5′ ACTCGTAGCCTTGGACCGCACTCACCACTGCTCGCCTGTCTG 3′
A3_AS6_SE_5	5′ AAACGATTTTTTGTTTCGAGCAGTGGTGAGTGCGGTCCAAATGAA 3′
Activation strands	
A3_AS135_RE_2	5′ TGTATCTTGGTCCGTGTATCTTATCTTGCAT 3′
A3_AS246_SE_2	5′ ACTCGTGTCTGTTCATTTGCTTTAGGTAGGT 3′

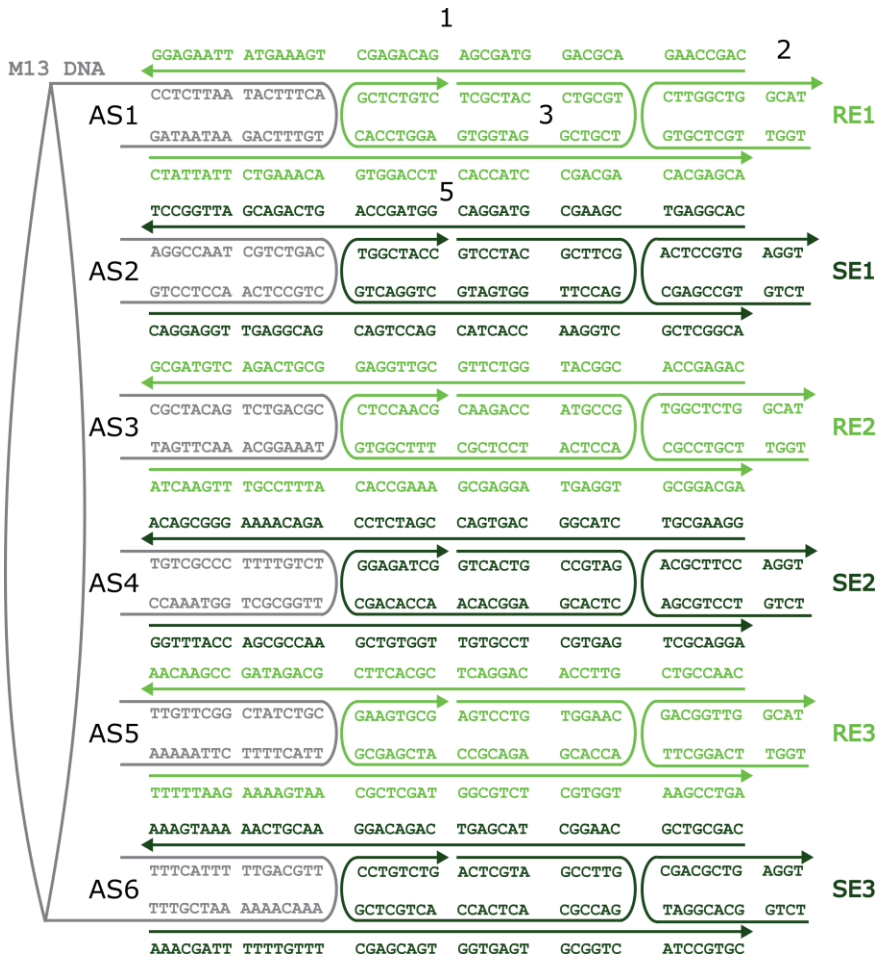


Table S6: A0 DNA origami terminus adapter strands. All strands were ordered unpurified from IDT.

Strand Name	Sequence
A0_AS1_RE_1	5′ CAGCCAAGACGCAGGTAGCGAGACAGAGCTGAAAGTATTAAGAGG 3′
A0_AS1_RE_3	5′ TCGCTACCTGCGTTCGTCGGATGGTGAGGTCCACGCTCTGTC 3′
A0_AS1_RE_5	5′ CTATTATTCTGAAACAGTGGACCTCACCATCCGACGACACGAGCA 3′
A0_AS2_SE_1	5′ CACGGAGTCGAAGCGTAGGACGGTAGCCAGTCAGACGATTGGCCT 3′
A0_AS2_SE_3	5′ GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC 3′
A0_AS2_SE_5	5′ CAGGAGGTTGAGGCAGCAGTCCAGCATCACCAAGGTCGCTCGGCA 3′
A0_AS3_RE_1	5′ CAGAGCCACGGCATGGTCTTGCGTTGGAGGCGTCAGACTGTAGCG 3′
A0_AS3_RE_3	5′ CAAGACCATGCCGACCTCATCCTCGCTTTCGGTGCTCCAACG 3′
A0_AS3_RE_5	5′ ATCAAGTTTGCCTTTACACCGAAAGCGAGGATGAGGTGCGGACGA 3′
A0_AS4_SE_1	5′ GGAAGCGTCTACGGCAGTGACCGATCTCCAGACAAAAGGGCGACA 3′
A0_AS4_SE_3	5′ GTCACTGCCGTAGCTCACGAGGCACAACCACAGCGGAGATCG 3′
A0_AS4_SE_5	5′ GGTTTACCAGCGCCAAGCTGTGGTTGTGCCTCGTGAGTCGCAGGA 3′
A0_AS5_RE_1	5′ CAACCGTCGTTCCACAGGACTCGCACTTCGCAGATAGCCGAACAA 3′
A0_AS5_RE_3	5′ AGTCCTGTGGAACACCACGAGACGCCATCGAGCGGAAGTGCG 3′
A0_AS5_RE_5	5′ TTTTTAAGAAAAGTAACGCTCGATGGCGTCTCGTGGTAAGCCTGA 3′
A0_AS6_SE_1	5′ CAGCGTCGCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA 3′
A0_AS6_SE_3	5′ ACTCGTAGCCTTGGACCGCACTCACCACTGCTCGCCTGTCTG 3′
A0_AS6_SE_5	5′ AAACGATTTTTTGTTTCGAGCAGTGGTGAGTGCGGTCATCCGTGC 3′
Activation strands	
A0_AS1_RE1_2	5′ TGGTTGCTCGTGCTTGGCTGGCAT 3′
A0_AS2_SE1_2	5′ TCTGTGCCGAGCACTCCGTGAGGT 3′
A0_AS3_RE2_2	5′ TGGTTCGTCCGCTGGCTCTGGCAT 3′
A0_AS4_SE2_2	5′ TCTGTCCTGCGAACGCTTCCAGGT 3′
A0_AS5_RE3_2	5′ TGGTTCAGGCTTGACGGTTGGCAT 3′
A0_AS6_SE3_2	5′ TCTGGCACGGATCGACGCTGAGGT 3′

1.4 Sequences of B termini

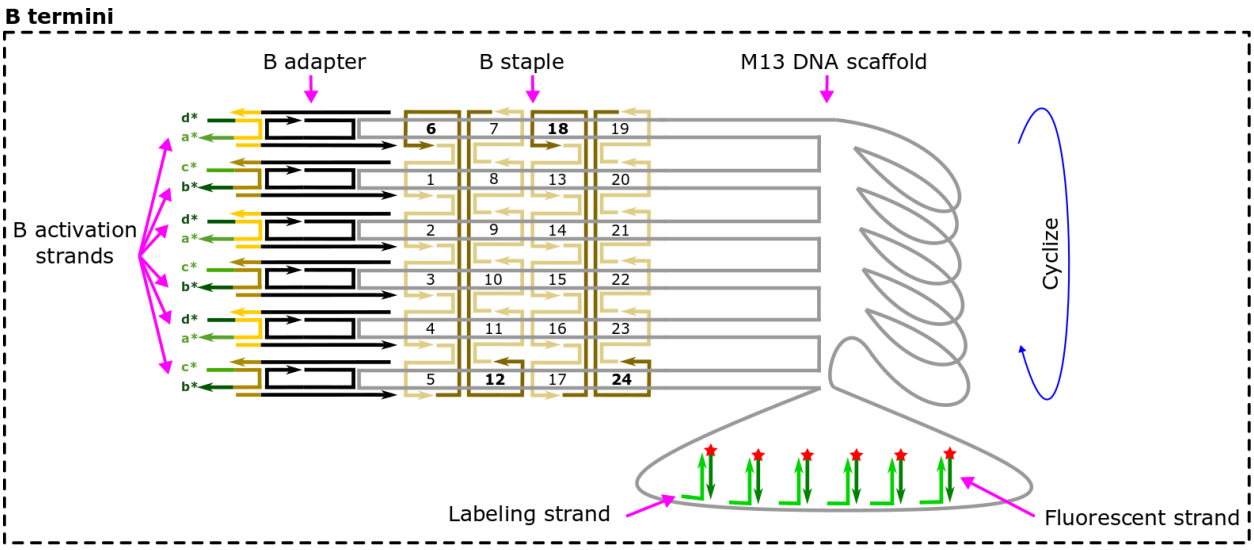


Table S7: Sequences of the B termini staples. These strands were used to assemble all the B termini. All staple strands were ordered unpurified from IDT. Numbers beside the strand names correspond to the numbered staples in the diagram above. As previously described, hairpins (highlighted in red) were incorporated into the staples to induce a directional preference for cyclization.[1]

Strand Name	Sequence
(1) T_5R2F_HP	5′ TGAGTTTCAAAGGAACGTCCACCGTTTTCGGTGGACTTAACTAAAGATCTCCAA 3′
(2) T_5R4F_HP	5′ AAAAAAGGCTTTTGCGGTGGTCCGTTTTCGGACCACTTGGATCGTCGGGTAGCA 3′
(3) T_5R6F_HP	5′ ACGGCTACAAGTACAACTCGGCACTTTTGTGCCGAGTTCGGAGATTCGCGACCT 3′
(4) T_5R8F_HP	5′ GCTCCATGACGTAACACGGATCGCTTTTGCGATCCGTTAAGCTGCTACACCAGA 3′
(5) T_5R10F_HP	5′ ACGAGTAGATCAGTTGCACCGCTGTTTTCAGCGGTGTTAGATTTAGCGCCAAAA 3′
(6) T_5R12F_CYC_HP	5′ GGAATTACCACCACCCGTGAGGCGTTTTCGCCTCACTTTCATTTTCCGTAACAC 3′
(7) T_5R2E_HP	5′ GAGAATAGGTCACCAGCGGAACCGTTTTCGGTTCCGTTTACAAACTCCGCCACC 3′
(8) T_5R4E_HP	5′ AAAGGCCGCTCCAAAACCGTGGCGTTTTCGCCACGGTTGGAGCCTTAGCGGAGT 3′
(9) T_5R6E_HP	5′ GCGAAACAAGAGGCTTGTGCTGCGTTTTCGCAGCACTTTGAGGACTAGGGAGTT 3′
(10) T_5R8E_HP	5′ CCAAATCATTACTTAGACGCTGGCTTTTGCCAGCGTTTCCGGAACGTACCAAGC 3′
(11) T_5R10E_HP	5′ AAAGATTCTAAATTGGCGACGGACTTTTGTCCGTCGTTGCTTGAGATTCATTAC 3′
(12) T_5R12E_CYC_HP	5′ CTCAGAGCGAGGCATAGGCTCCGCTTTTGCGGAGCCTTGTAAGAGCACAGGTAG 3′
(13) T_3R2F_HP	5′ TGTAGCATAACTTTCAGGCATCCGTTTTCGGATGCCTTACAGTTTCTAATTGTA 3′
(14) T_3R4F_HP	5′ TCGGTTTAGGTCGCTGGCTGACGCTTTTGCGTCAGCTTAGGCTTGCAAAGACTT 3′
(15) T_3R6F_HP	5′ TTTCATGATGACCCCCACCAGCCGTTTTCGGCTGGTTTAGCGATTAAGGCGCAG 3′
(16) T_3R8F_HP	5′ ACGGTCAATGACAAGACGGAGGCGTTTTCGCCTCCGTTACCGGATATGGTTTAA 3′
(17) T_3R10F_HP	5′ TTTCAACTACGGAACACTCGCTGCTTTTGCAGCGAGTTACATTATTAACACTAT 3′
(18) T_3R12F_CYC_HP	5′ CATAACCCACCGCCACCTGGCTCGTTTTCGAGCCAGTTCCTCAGAAACAACGCC 3′
(19) T_3R2E_HP	5′ TGCTAAACTCCACAGAGCCAGTGCTTTTGCACTGGCTTCAGCCCTCTACCGCCA 3′
(20) T_3R4E_HP	5′ ATATATTCTCAGCTTGCCGTCCGCTTTTGCGGACGGTTCTTTCGAGTGGGATTT 3′
(21) T_3R6E_HP	5′ CTCATCTTGGAAGTTTCGGATGGCTTTTGCCATCCGTTCCATTAAACATAACCG 3′
(22) T_3R8E_HP	5′ AGTAATCTTCATAAGGTCTGGTCGTTTTCGACCAGATTGAACCGAACTAAAACA 3′
(23) T_3R10E_HP	5′ ACGAACTATTAATCATGGCACCTGTTTTCAGGTGCCTTTGTGAATTTCATCAAG 3′
(24) T_3R12E_CYC_HP	5′ CCCTCAGATCGTTTACCGCTTGCGTTTTCGCAAGCGTTCAGACGACTTAATAAA 3′

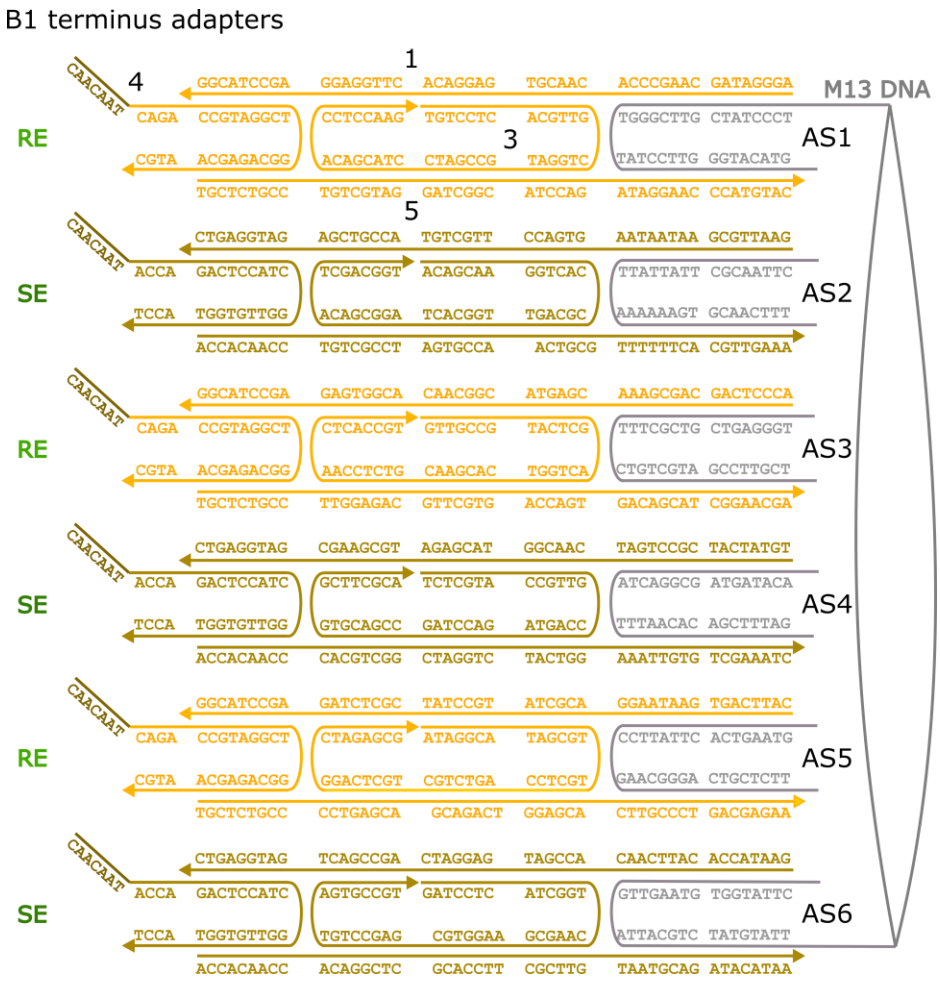


Table S8: B1 DNA origami terminus adapter strands. All strands were ordered unpurified from IDT.

Strand Name	Sequence
B1_AS1_RE_1	5′ AGGGATAGCAAGCCCACAACGTGAGGACACTTGGAGGAGCCTACGG 3′
B1_AS1_RE_3	5′ TGTCCTCACGTTGCTGGATGCCGATCCTACGACACCTCCAAG 3′
B1_AS1_RE_5	5′ TGCTCTGCCTGTCGTAGGATCGGCATCCAGATAGGAACCCATGTAC 3′
B1_AS2_SE_1	5′ GAATTGCGAATAATAAGTGACCTTGCTGTACCGTCGAGATGGAGTC 3′
B1_AS2_SE_3	5′ ACAGCAAGGTCACCGCAGTTGGCACTAGGCGACATCGACGGT 3′
B1_AS2_SE_5	5′ ACCACAACCTGTCGCCTAGTGCCAACTGCGTTTTTTCACGTTGAAA 3′
B1_AS3_RE_1	5′ ACCCTCAGCAGCGAAACGAGTACGGCAACACGGTGAGAGCCTACGG 3′
B1_AS3_RE_3	5′ GTTGCCGTACTCGACTGGTCACGAACGTCTCCAACTCACCGT 3′
B1_AS3_RE_5	5′ TGCTCTGCCTTGGAGACGTTCGTGACCAGTGACAGCATCGGAACGA 3′
B1_AS4_SE_1	5′ TGTATCATCGCCTGATCAACGGTACGAGATGCGAAGCGATGGAGTC 3′
B1_AS4_SE_3	5′ TCTCGTACCGTTGCCAGTAGACCTAGCCGACGTGGCTTCGCA 3′
B1_AS4_SE_5	5′ ACCACAACCCACGTCGGCTAGGTCTACTGGAAATTGTGTCGAAATC 3′
B1_AS5_RE_1	5′ CATTCAGTGAATAAGGACGCTATGCCTATCGCTCTAGAGCCTACGG 3′
B1_AS5_RE_3	5′ ATAGGCATAGCGTTGCTCCAGTCTGCTGCTCAGGCTAGAGCG 3′
B1_AS5_RE_5	5′ TGCTCTGCCCCTGAGCAGCAGACTGGAGCACTTGCCCTGACGAGAA 3′
B1_AS6_SE_1	5′ GAATACCACATTCAACACCGATGAGGATCACGGCACTGATGGAGTC 3′
B1_AS6_SE_3	5′ GATCCTCATCGGTCAAGCGAAGGTGCGAGCCTGTAGTGCCGT 3′
B1_AS6_SE_5	5′ ACCACAACCACAGGCTCGCACCTTCGCTTGTAATGCAGATACATAA 3′
Activation strands	
B1_AS135_RE_4	5′ CAACAATCAGACCGTAGGCTGGCAGAGCAATGC 3′
B1_AS246_SE_4	5′ CAACAATACCAGACTCCATCGGTTGTGGTACCT 3′
Inactivation strands	
B1_AS135_RE_inact	5′ GCATTGCTCTGCCAGCCTACGGTCTGATTGTTG 3′
B1_AS246_SE_inact	5′ AGGTACCACAACCGATGGAGTCTGGTATTGTTG 3′

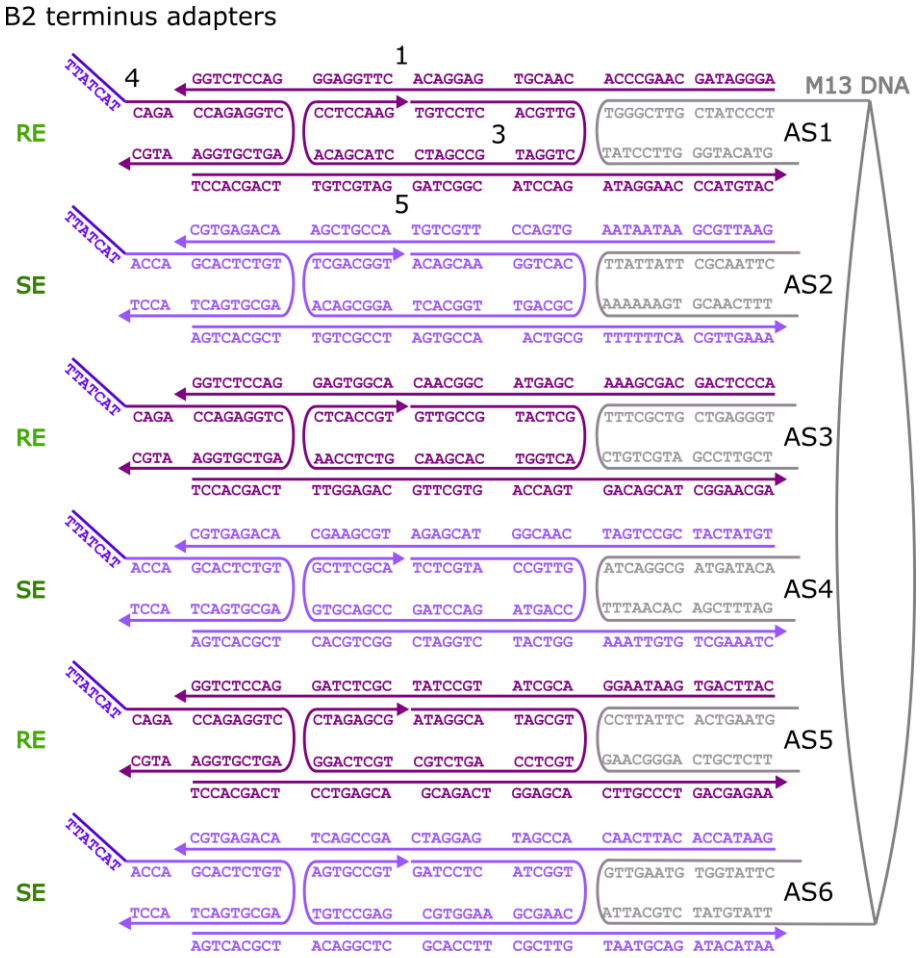


Table S9: B2 DNA origami terminus adapter strands. All of the _3 strands have the same sequences as the corresponding strands of the B1 termini. All strands were ordered unpurified from IDT.

Strand Name	Sequence
B2_AS1_RE_1	5′ AGGGATAGCAAGCCCACAACGTGAGGACACTTGGAGGGACCTCTGG 3′
B2_AS1_RE_3	5′ TGTCCTCACGTTGCTGGATGCCGATCCTACGACACCTCCAAG 3′
B2_AS1_RE_5	5′ TCCACGACTTGTCGTAGGATCGGCATCCAGATAGGAACCCATGTAC 3′
B2_AS2_SE_1	5′ GAATTGCGAATAATAAGTGACCTTGCTGTACCGTCGAACAGAGTGC 3′
B2_AS2_SE_3	5′ ACAGCAAGGTCACCGCAGTTGGCACTAGGCGACATCGACGGT 3′
B2_AS2_SE_5	5′ AGTCACGCTTGTCGCCTAGTGCCAACTGCGTTTTTTCACGTTGAAA 3′
B2_AS3_RE_1	5′ ACCCTCAGCAGCGAAACGAGTACGGCAACACGGTGAGGACCTCTGG 3′
B2_AS3_RE_3	5′ GTTGCCGTACTCGACTGGTCACGAACGTCTCCAACTCACCGT 3′
B2_AS3_RE_5	5′ TCCACGACTTTGGAGACGTTCGTGACCAGTGACAGCATCGGAACGA 3′
B2_AS4_SE_1	5′ TGTATCATCGCCTGATCAACGGTACGAGATGCGAAGCACAGAGTGC 3′
B2_AS4_SE_3	5′ TCTCGTACCGTTGCCAGTAGACCTAGCCGACGTGGCTTCGCA 3′
B2_AS4_SE_5	5′ AGTCACGCTCACGTCGGCTAGGTCTACTGGAAATTGTGTCGAAATC 3′
B2_AS5_RE_1	5′ CATTCAGTGAATAAGGACGCTATGCCTATCGCTCTAGGACCTCTGG 3′
B2_AS5_RE_3	5′ ATAGGCATAGCGTTGCTCCAGTCTGCTGCTCAGGCTAGAGCG 3′
B2_AS5_RE_5	5′ TCCACGACTCCTGAGCAGCAGACTGGAGCACTTGCCCTGACGAGAA 3′
B2_AS6_SE_1	5′ GAATACCACATTCAACACCGATGAGGATCACGGCACTACAGAGTGC 3′
B2_AS6_SE_3	5′ GATCCTCATCGGTCAAGCGAAGGTGCGAGCCTGTAGTGCCGT 3′
B2_AS6_SE_5	5′ AGTCACGCTACAGGCTCGCACCTTCGCTTGTAATGCAGATACATAA 3′
Activation strands	
B2_AS135_RE_4	5′ TTATCATCAGACCAGAGGTCAGTCGTGGAATGC 3′
B2_AS246_SE_4	5′ TTATCATACCAGCACTCTGTAGCGTGACTACCT 3′
Inactivation strands	
B2_AS135_RE_inact	5′ GCATTCCACGACTGACCTCTGGTCTGATGATAA 3′
B2_AS246_SE_inact	5′ AGGTAGTCACGCTACAGAGTGCTGGTATGATAA 3′

1.5 DNA origami terminus labeling strands

Table S10: DNA origami terminus strands for fluorescent labeling. All labeling strands were ordered unpurified from IDT. Fluorescent strands were ordered HPLC purified from IDT

Strand Name	Sequence
Fluorescent strands	
atto647 strand	/5ATTO647NN/AAGCGTAGTCGGATCTC 3′
atto488 strand	/5ATTO488N/AAGCGTAGTCGGATCTC 3′
Labeling strands	
Unused_m13mp18_01	5′ AAATTCTTACCAGTATAAAGCCAACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_02	5′ GCCTGTTTAGTATCATATGCGTTATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_03	5′ ACACCGGAATCATAATTACTAGAAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_04	5′ GATAAATAAGGCGTTAAATAAGAATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_05	5′ TTTAATGGTTTGAAATACCGACCGTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_06	5′ TTAGTTAATTTCATCTTCTGACCTATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_07	5′ ACGCGAGAAAACTTTTTCAAATATATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_08	5′ GATGCAAATCCAATCGCAAGACAAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_09	5′ TGGGTTATATAACTATATGTAAATGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_10	5′ ACTACCTTTTTAACCTCCGGCTTAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_11	5′ AATTTATCAAAATCATAGGTCTGAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_12	5′ TTAAGACGCTGAGAAGAGTCAATAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_13	5′ TCCTTGAAAACATAGCGATAGCTTATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_14	5′ TCGCTATTAATTAATTTTCCCTTAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_15	5′ AGTGAATAACCTTGCTTCTGTAAATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_16	5′ GAAACAGTACATAAATCAATATATGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_17	5′ ATTTCATTTGAATTACCTTTTTTAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_18	5′ AGAAAACAAAATTAATTACATTTAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_19	5′ CAAAAGAAGATGATGAAACAAACATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_20	5′ GCGAATTATTCATTTCAATTACCTGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_21	5′ AATACCAAGTTACAAAATCGCGCAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_22	5′ CAATAACGGATTCGCCTGATTGCTTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_23	5′ TAACAGTACCTTTTACATCGGGAGATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_24	5′ CAGGTTTAACGTCAGATGAATATACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_25	5′ CAGAAATAAAGAAATTGCGTAGATTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_26	5′ CCATATCAAAATTATTTGCACGTAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_27	5′ TCTGAATAATGGAAGGGTTAGAACCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_28	5′ TATAATCCTGATTGTTTGGATTATATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_29	5′ GATTATCAGATGATGGCAATTCATCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_30	5′ AAGGAGCGGAATTATCATCATATTCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_31	5′ CATTTTGCGGAACAAAGAAACCACCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_32	5′ TAATTTTAAAAGTTTGAGTAACATTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_33	5′ GTATTAAATCCTTTGCCCGAACGTTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_34	5′ TAGACTTTACAAACAATTCGACAACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_35	5′ ATAATACATTTGAGGATTTAGAAGTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_36	5′ CAACTAATAGATTAGAGCCGTCAATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_37	5′ TATCTAAAATATCTTTAGGAGCACTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_38	5′ ACTGATAGCCCTAAAACATCGCCATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_39	5′ GAATGGCTATTAGTCTTTAATGCGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_40	5′ AGAATACGTGGCACAGACAATATTTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_41	5′ ATAGAACCCTTCTGACCTGAAAGCGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_42	5′ ATAAAAGGGACATTCTGGCCAACAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_43	5′ GCAGATTCACCAGTCACACGACCAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_44	5′ ATCGTCTGAAATGGATTATTTACATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_45	5′ ATGGAAATACCTACATTTTGACGCTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_46	5′ CCAGCCATTGCAACAGGAAAAACGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_47	5′ CTGGTAATATCCAGAACAATATTACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_48	5′ GTAGAAGAACTCAAACTATCGGCCTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_49	5′ TGATTAGTAATAACATCACTTGCCTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_50	5′ AAATTAACCGTTGTAGCAATACTTCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_51	5′ CCGAGTAAAAGAGTCTGTCCATCACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_52	5′ GAAGTGTTTTTATAATCAGTGAGGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_53	5′ GACAGGAACGGTACGCCAGAATCCTTTTTGAGATCCGACTACGC 3′

Unused_m13mp18_54	5′ AACAGGAGGCCGATTAAAGGGATTTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_55	5′ TCCTCGTTAGAATCAGAGCGGGAGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_56	5′ GCTTTGACGAGCACGTATAACGTGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_57	5′ CGCCGCTACAGGGCGCGTACTATGGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_58	5′ TAACCACCACACCCGCCGCGCTTAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_59	5′ TGGCAAGTGTAGCGGTCACGCTGCGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_60	5′ AAGCGAAAGGAGCGGGCGCTAGGGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_61	5′ CGAACGTGGCGAGAAAGGAAGGGAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_62	5′ GATTTAGAGCTTGACGGGGAAAGCCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_63	5′ TAAATCGGAACCCTAAAGGGAGCCCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_64	5′ TTTTGGGGTCGAGGTGCCGTAAAGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_65	5′ TACGTGAACCATCACCCAAATCAAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_66	5′ AAACCGTCTATCAGGGCGATGGCCCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_67	5′ ACGTGGACTCCAACGTCAAAGGGCGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_68	5′ TTTGGAACAAGAGTCCACTATTAAATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_69	5′ CCGAGATAGGGTTGAGTGTTGTTCCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_70	5′ AAATCCCTTATAAATCAAAAGAATATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_71	5′ TGTTTGATGGTGGTTCCGAAATCGGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_72	5′ CTGGTTTGCCCCAGCAGGCGAAAATTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_73	5′ TGAGAGAGTTGCAGCAAGCGGTCCATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_74	5′ AGCTGATTGCCCTTCACCGCCTGGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_75	5′ TTTCTTTTCACCAGTGAGACGGGCATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_76	5′ GTTTGCGTATTGGGCGCCAGGGTGGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_77	5′ GAATCGGCCAACGCGCGGGGAGAGGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_78	5′ GAAACCTGTCGTGCCAGCTGCATTATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_79	5′ TGCGCTCACTGCCCGCTTTCCAGTCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_80	5′ GAGTGAGCTAACTCACATTAATTGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_81	5′ TAAAGTGTAAAGCCTGGGGTGCCTATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_82	5′ TTCCACACAACATACGAGCCGGAAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_83	5′ CTGTGTGAAATTGTTATCCGCTCACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_84	5′ ATTCGTAATCATGGTCATAGCTGTTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_85	5′ TAGAGGATCCCCGGGTACCGAGCTCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_86	5′ CAAGCTTGCATGCCTGCAGGTCGACTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_87	5′ ACGACGTTGTAAAACGACGGCCAGTTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_88	5′ TTGGGTAACGCCAGGGTTTTCCCAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_89	5′ AGGGGGATGTGCTGCAAGGCGATTATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_90	5′ CTCTTCGCTATTACGCCAGCTGGCGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_91	5′ CTGTTGGGAAGGGCGATCGGTGCGGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_92	5′ GCGCCATTCGCCATTCAGGCTGCGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_93	5′ CGCTTCTGGTGCCGGAAACCAGGCATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_94	5′ ATCGCACTCCAGCCAGCTTTCCGGCTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_95	5′ GACGACGACAGTATCGGCCTCAGGATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_96	5′ GTAACCGTGCATCTGCCAGTTTGAGTTTTGAGATCCGACTACGC 3′
Unused_m13mp18_97	5′ GGTCACGTTGGTGTAGATGGGCGCATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_98	5′ AAACGGCGGATTGACCGTAATGGGATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_99	5′ ACAACCCGTCGGATTCTCCGTGGGATTTTGAGATCCGACTACGC 3′
Unused_m13mp18_100	5′ TTCATCAACATTAAATGTGAGCGAGTTTTGAGATCCGACTACGC 3′

1.6 Design of Y-shaped termini for step-wise assembly of hierarchical nanostructures

The Y-shaped DNA origami termini used here are identical to those presented in[4] except for the sticky end adapter strands, which present 4-base sticky ends instead of the original 5-base sticky ends in order to match the monomer system used in this paper. The staples, labelling strands, and labelling attachment strands all match those presented in Jorgenson *et al*.[4] The sticky end adapter strands are presented below in Table S11.

Table S11: Ay sticky end adapter strands. All strands were ordered unpurified from IDT.

Strand Name	Sequence
Arm 1	
Ay_AS1_RE_2	5′ TGGTCGATCCACGCTTGGCTGCAT 3′
Ay_AS3_RE_2	5′ TGGTTGAGCGTTAGTACCGAGCAT 3′
Ay_AS5_RE_2	5′ TGGTCACCGCTGCGTCTCCAGCAT 3′
Ay_AS2_SE_2	5′ TCTGGACACTGCACCTTCAGAGGT 3′
Ay_AS4_SE_2	5′ TCTGGCACGAGTACAAGTCGAGGT 3′
Ay_AS6_SE_2	5′ TCTGGTCTGCCGAGTGTCCGAGGT 3′
Arm 2	
Ay_AS1_RE_2	5′ TGGTCGCTCACGTCCTCGCTGCAT 3′
Ay_AS3_RE_2	5′ TGGTGTCAGGAACCTGAGTGGCAT 3′
Ay_AS5_RE_2	5′ TGGTCTCCGCATTGTGGACTGCAT 3′
Ay_AS2_SE_2	5′ TCTGTGTTCCGTGCCGAGTCAGGT 3′
Ay_AS4_SE_2	5′ TCTGGCGAGCAATCGGATACAGGT 3′
Ay_AS6_SE_2	5′ TCTGCGAGACACGATTGGCGAGGT 3′
Arm 3	
Ay_AS1_RE_2	5′ TGGTTCACTCCATGCCATTAGCAT 3′
Ay_AS3_RE_2	5′ TGGTCATGGCAACTGGTTCAGCAT 3′
Ay_AS5_RE_2	5′ TGGTCGATAGGAGCCTCATGGCAT 3′
Ay_AS2_SE_2	5′ TCTGTGGTTAGCACTTCCAGAGGT 3′
Ay_AS4_SE_2	5′ TCTGTCAAGCGATAGCCTCAAGGT 3′
Ay_AS6_SE_2	5′ TCTGAGTGCGACAGCGAGCCAGGT 3′

2. Experimental details

2.1 Concentrations and times of addition of activation and inactivation strands

The concentrations of the activation and inactivation strands that were added to nanotubes and the times they were added are tabulated below for all the experiments. The time of action reflects the amount of time that had passed since the start of the experiment. Each experiment started with the addition of the purified termini to the annealed monomer mixtures. Imaging refers to the removal of a sample of the reaction mixture from the Eppendorf tube to a slide, which was then imaged *via* fluorescence microscopy (Methods). For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Main text results:

Table S12: Details of the experiments that produced the results shown in Figure 2 of the main text and Supporting Figure S2. Final concentration refers to the final concentration of the strands that were added in the nanotube solution. A1a and A1i refer to the RE/SE activation and inactivation strands, respectively, for the A1 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Figure 2a and Supporting Figure S2a		
Action	**Final concentration**	**Time of action**
Imaging / A1a added	50 nM each	6 hours
Imaging	NA	21 hours
Figure 2b and Supporting Figure S2b		
Action	**Final concentration**	**Time of action**
Imaging / A1i added	100 nM each	22 hours
Imaging	NA	26 hours
Figure 2c,d		
Action	**Final concentration**	**Time of action**
Imaging / A1a added	50 nM each	9 hours
Imaging / A1i added	100 nM each	21.5 hours
Imaging / A1a added	200 nM each	25 hours
Imaging	NA	45 hours

Table S13: Details of the experiments that produced the results shown in Figure 4. Final concentration refers to the final concentration of the strands that were added in the nanotube solution. A1a and A1i refer to the RE/SE activation and inactivation strands, respectively, for the A1 terminus. B1a and B1i refer to the RE/SE activation and inactivation strands, respectively, for the B1 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Figure 4a,b		
Action	**Final concentration**	**Time of action**
Imaging / A1a added	50 nM each	2 hours
Imaging / B1a added	50 nM each	7 hours
Imaging / A1i added	100 nM each	20 hours
Imaging / B1i added	100 nM each	24 hours
Imaging	NA	43 hours

Table S14: Details of the experiments that produced the results shown in Figure 5. Final concentration refers to the final concentration of the strands that were added in the nanotube solution. A1a and A1i refer to the RE/SE activation and inactivation strands, respectively, for the A1 terminus. B2a and B2i refer to the RE/SE activation and inactivation strands, respectively, for the B2 terminus. A2a and A2i refer to the RE/SE activation and inactivation strands, respectively, for the A2 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Figure 5		
Action	**Final concentration**	**Time of action**
Imaging / A1a added	50 nM each	2.5 hours
Imaging / B2a added	50 nM each	21.5 hours
Imaging / A1i added	100 nM each	27.5 hours
Imaging / A2a added	50 nM each	31 hours
Imaging	NA	54.5 hours

Table S15: Details of the experiments that produced the results shown in Figure 6 in the main text. Final concentration refers to the final concentration of the strands that were added in the reaction mixture. A1i refers to the A1 inactivation strand.

Figure 6		
Action	**Final concentration**	**Time of action**
Imaging Y structures	N/A	21 hours
Imaging / B2 added	5 pM	20.5 hours
Imaging A1-B1 structures	N/A	33.5 hours
Adding A1i	100 nM	34.5 hours
Combine A1-B1 + Y structures	12:1 excess A1-B1 structures	34.5 hours
Imaging	NA	82.5 hours

Supporting Information results:

Table S16: Details of the experiments that produced the results shown in Supporting Figure S3. Final concentration refers to the final concentration of the strands that were added in the reaction mixture. A1a and A1i refer to the RE/SE activation and inactivation strands, respectively, for the A1 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Supporting Figure S3a,b		
Action	**Final concentration**	**Time of action**
Imaging / A1i added	100 nM each	6.5 hours
Imaging / A1a added	200 nM each	10.5 hours
Imaging	NA	23 hours

Table S17: Details of the experiments that produced the results shown in Supporting Figure S5. Final concentration refers to the final concentration of the strands that were added in the reaction mixture. B1a and B1i refer to the RE/SE activation and inactivation strands, respectively, for the B1 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Supporting Figure S5a,b		
Action	**Final concentration**	**Time of action**
Imaging / B1i added	100 nM each	23 hours
Imaging / B1a added	200 nM each	27 hours
Imaging	NA	45 hours
Supporting Figure S5c,d		
Action	**Final concentration**	**Time of action**
Imaging / B1a added	50 nM each	9.5 hours
Imaging / B1i added	100 nM each	24 hours
Imaging / B1a added	200 nM each	29 hours
Imaging	NA	48 hours

Table S18: Details of the experiments that produced the results shown in Supporting Figure S6. Final concentration refers to the final concentration of the strands that were added in the nanotube solution. A2a and A2i refer to the RE/SE activation and inactivation strands, respectively, for the A2 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Supporting Figure S6a,b		
Action	**Final concentration**	**Time of action**
Imaging / A2i added	120 nM each	16 hours
Imaging / A2a added	200 nM each	20 hours
Imaging	NA	40 hours
Supporting Figure S6c,d		
Action	**Final concentration**	**Time of action**
Imaging / A2a added	50 nM each	7 hours
Imaging / A2i added	100 nM each	23.5 hours
Imaging / A2a added	200 nM each	29.5 hours
Imaging	NA	45 hours

Table S19: Details of the experiments that produced the results shown in Supporting Figure S7. Final concentration refers to the final concentration of the strands that were added in nanotube solution. B2a and B2i refer to the RE/SE activation and inactivation strands, respectively, for the B2 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Supporting Figure S7a,b		
Action	**Final concentration**	**Time of action**
Imaging / B2i added	120 nM each	16 hours
Imaging / B2a added	200 nM each	20 hours
Imaging	NA	40 hours
Supporting Figure S7c,d		
Action	**Final concentration**	**Time of action**
Imaging / B2a added	50 nM each	7 hours
Imaging / B2i added	100 nM each	23.5 hours
Imaging / B2a added	200 nM each	29.5 hours
Imaging	NA	45 hours

Table S20: Details of the experiments that produced the results shown in Supporting Figure S8. Final concentration refers to the final concentration of the strands that were added in the nanotube solution. A1a and A1i refer to the RE/SE activation and inactivation strands, respectively, for the A1 terminus. B1a and B1i refer to the RE/SE activation and inactivation strands, respectively, for the B1 terminus. For steps with both imaging and strand additions, the imaging was conducted first and then the strands were added.

Supporting Figure S8a,b		
Action	**Final concentration**	**Time of action**
Imaging / A1a added	50 nM each	2 hours
Imaging / B1a added	50 nM each	11.5 hours
Imaging / A1i added	100 nM each	23.5 hours
Imaging / A1a added	200 nM each	27.5 hours
Imaging	NA	49.5 hours

2.2 Termini concentrations and fluorescence labeling schemes

The concentrations of the termini structures in each experiment were measured after their assembly and purification using a previously established heuristic[2] that relates the number of termini per field of view of a fluorescence micrograph of a sample in a terminus concentration. The mean number of termini per field of view were used to determine the termini concentrations for a given sample at a specific timepoint. Since the solution used for imaging at each timepoint for a given sample was placed in its own tube, the termini concentrations measured for the solutions corresponding to all timepoints of a particular sample (*i.e.* a set of timepoints) were then averaged to obtain the concentrations tabulated in Supporting Table S22.

Table S21: Labeling schemes for termini in different experiments. For termini labeled with both atto488 and atto647, 500 nM of each fluorophore modified DNA strand were added before annealing.

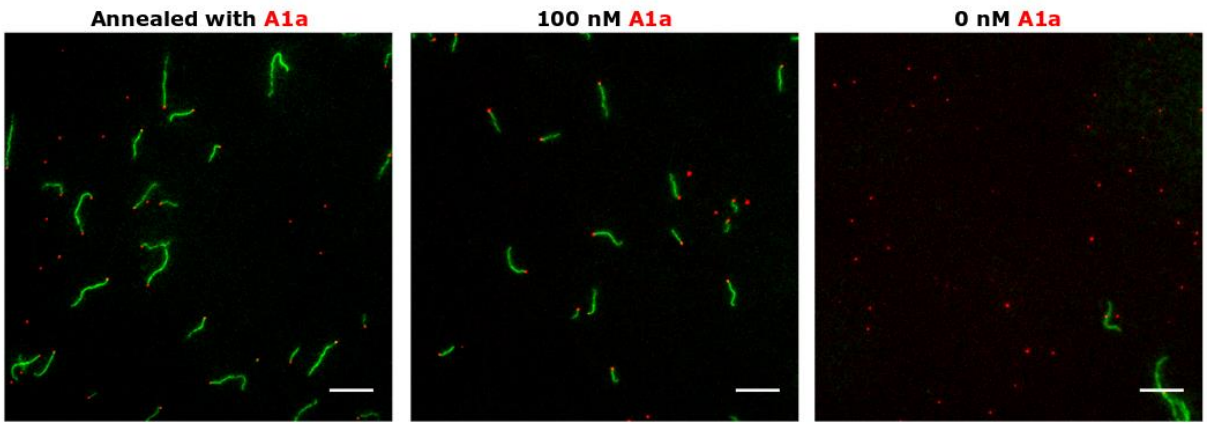
	Labeling dye				
Main text	**A1 term.**	**B1 term.**	**A2 term.**	**B2 term.**	**Ay term.**
Figure 2	atto488	N/A	N/A	N/A	N/A
Figure 4	atto488	atto647	N/A	N/A	N/A
Figure 5	atto647	N/A	atto488	atto488 + atto647	atto488
Figure 6	atto488 + atto647	atto647	N/A	N/A	N/A
Figure 7	atto647	N/A	N/A	N/A	N/A
Supporting Information					
Supporting Figure S1	atto488	N/A	N/A	N/A	N/A
Supporting Figure S2	atto488	N/A	N/A	N/A	N/A
Supporting Figure S3	atto488	N/A	N/A	N/A	N/A
Supporting Figure S4	atto488	N/A	N/A	N/A	N/A
Supporting Figure S5	N/A	atto488	N/A	N/A	N/A
Supporting Figure S6	N/A	N/A	atto488	N/A	N/A
Supporting Figure S7	N/A	N/A	N/A	atto488	N/A
Supporting Figure S8	atto488	atto647	N/A	N/A	N/A

Table S22: Concentrations of termini in experiments. A final concentration of 5 pM was desired; however, slightly different concentrations were obtained with each purification and from subsequent pipetting of the termini into each sample.

	Terminus concentrations			
Main text	**A1 terminus**	**B1 terminus**	**A2 terminus**	**B2 terminus**
Figure 2a	A1: 3.86 pM	N/A	N/A	N/A
Figure 2b	A1: 5.22 pM	N/A	N/A	N/A
Figure 2c,d	11.54 pM	N/A	N/A	N/A
Figure 4a,b	5.14 pM	3.99 pM	N/A	N/A
Figure 5	7.69 pM	N/A	10.42 pM	8.51 pM
Supporting Information				
Supporting Figure S2a	A1o: 3.20 pM	N/A	N/A	N/A
Supporting Figure S2b	A1o: 4.38 pM	N/A	N/A	N/A
Supporting Figure S3a,b	17.84 pM	N/A	N/A	N/A
Supporting Figure S5a,b	N/A	7.67 pM	N/A	N/A
Supporting Figure S5,d	N/A	2.40 pM	N/A	N/A
Supporting Figure S6a,b	N/A	N/A	9.65 pM	N/A
Supporting Figure S6c,d	N/A	N/A	4.99 pM	N/A
Supporting Figure S7a,b	N/A	N/A	N/A	15.57 pM
Supporting Figure S7c,d	N/A	N/A	N/A	7.54 pM
Supporting Figure S8a,b	9.80 pM	10.28 pM	N/A	N/A

3. Additional A1 termini activation experiments

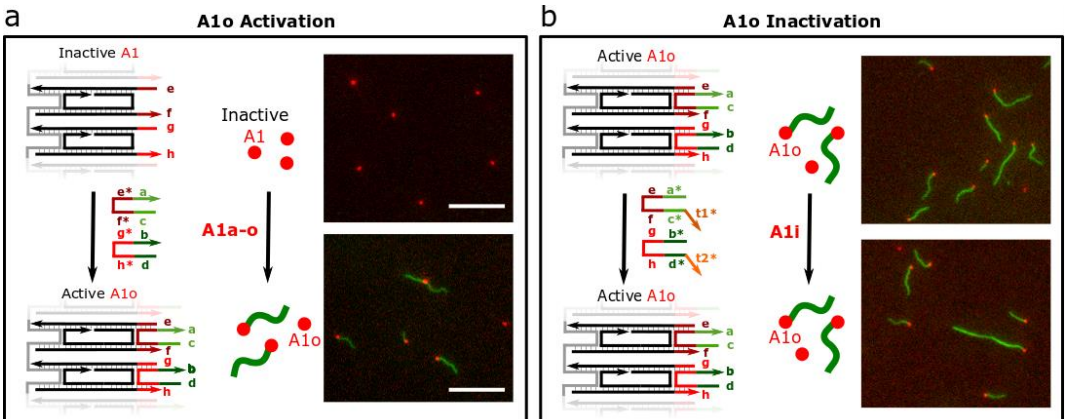
a



b

Fraction	24 hr	24 hr	24 hr
Nanotubes with termini	0.76 ± 0.04	0.83 ± 0.05	0.00 ± 0.00
Termini with nanotubes	0.63 ± 0.04	0.49 ± 0.05	0.00 ± 0.00

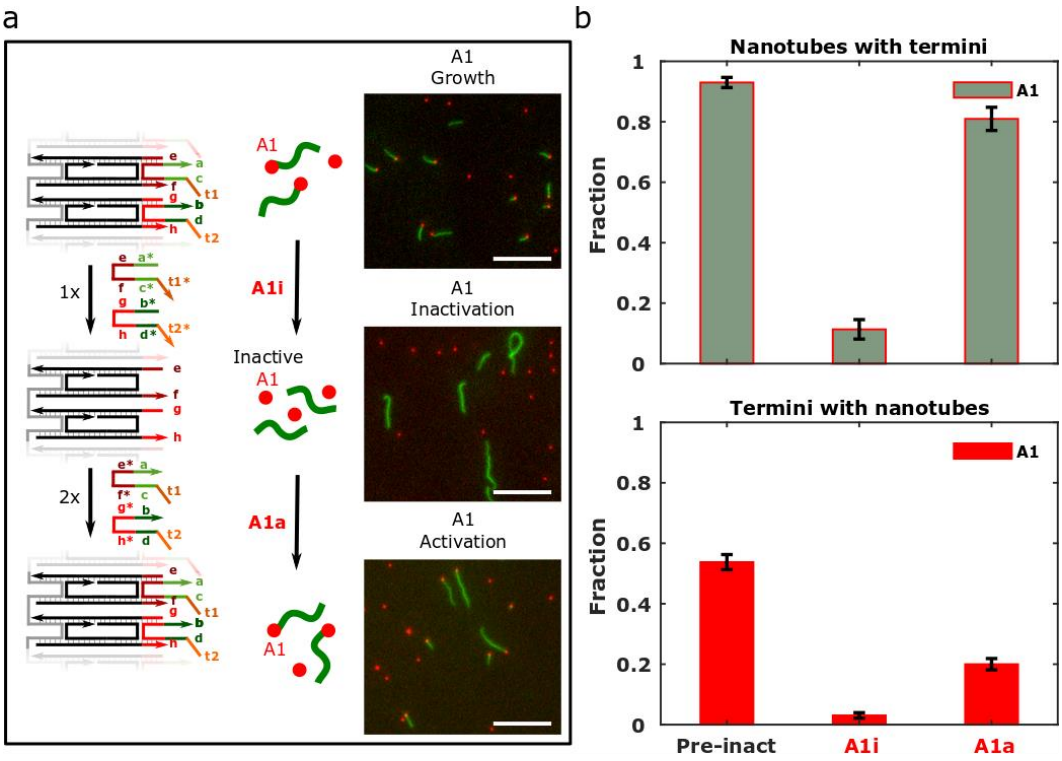
Supporting Figure S1: A1 terminus activation can be triggered after 24 hours and there is low unseeded nanotube growth with inactive termini. **a**) Representative fluorescence micrographs after activation of the A1 terminus with different activation strand concentrations. **b**) Fractions of nanotubes with termini and termini with nanotubes after activation of the A1 terminus quantified from the experiment in (a). Errors represent 95% confidence intervals of proportions. The activation strands (A1a) were added to the final concentrations above the micrographs as soon as the purified termini were added to the monomer mixes. The "Annealed" sample served as a positive control where the A1 terminus was annealed with the activation strands present. The samples were imaged after incubating 24 hours with the activation strands. A1 termini were at 3 pM for all samples. Experiments were otherwise conducted as described in the Methods of the main text.



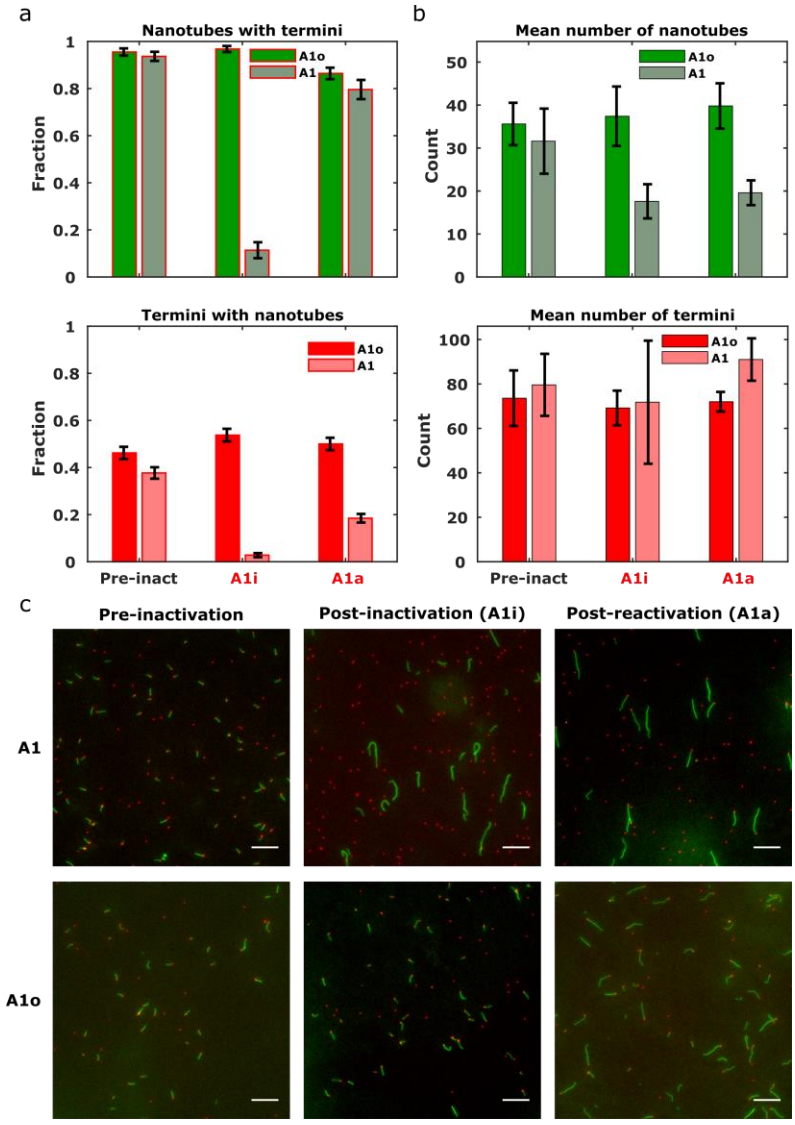
Fraction	Pre-act	A1a-o
Nanotubes with A1 termini	0.0 ± 0.0	0.82 ± 0.07
A1 termini with nanotubes	0.0 ± 0.0	0.44 ± 0.06

Fraction	Pre-inact	A1i
Nanotubes with A1 termini	0.80 ± 0.04	0.72 ± 0.05
A1 termini with nanotubes	0.55 ± 0.04	0.46 ± 0.04

Supporting Figure S2: A1 terminus activation and inactivation using activation strands without 7-base 5' toehold domains. Schematic of terminus activation (a) or terminus inactivation (b) with representative fluorescence micrographs of results. The fractions of nanotubes with termini and termini with nanotubes are tabulated below. Pre-act refers to the incubation period before the activation strands (A1a) were added. Pre-inact refers to the incubation period before the inactivation strands (A1i) were added. See Supporting Table S12 for further experimental details. Errors represent 95% confidence intervals of proportions. Scale bars: 10 μm.



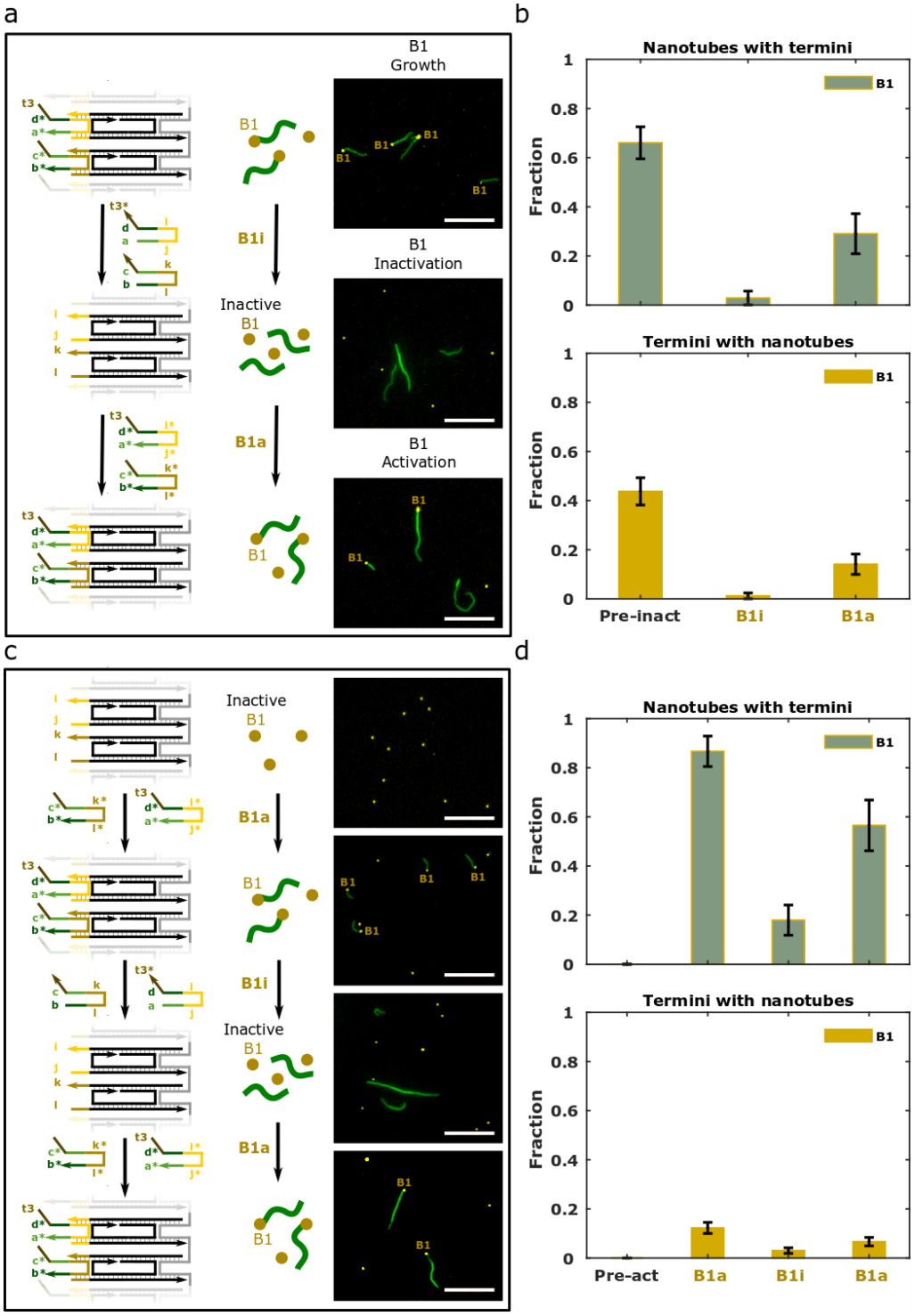
Supporting Figure S3: A1 terminus inactivation and reactivation. **a)** Schematic of an experiment in which termini were annealed in their active state. After nanotubes were grown from these active termini, the termini were inactivated then reactivated. Representative fluorescence micrographs at each stage of the process are shown. **b)** Fractions of nanotubes with termini and termini with nanotubes for the experiment in (**b**). See Supporting Table S16 for further experimental details. Error bars represent 95% confidence intervals of proportions. Scale bars: 10 μm.



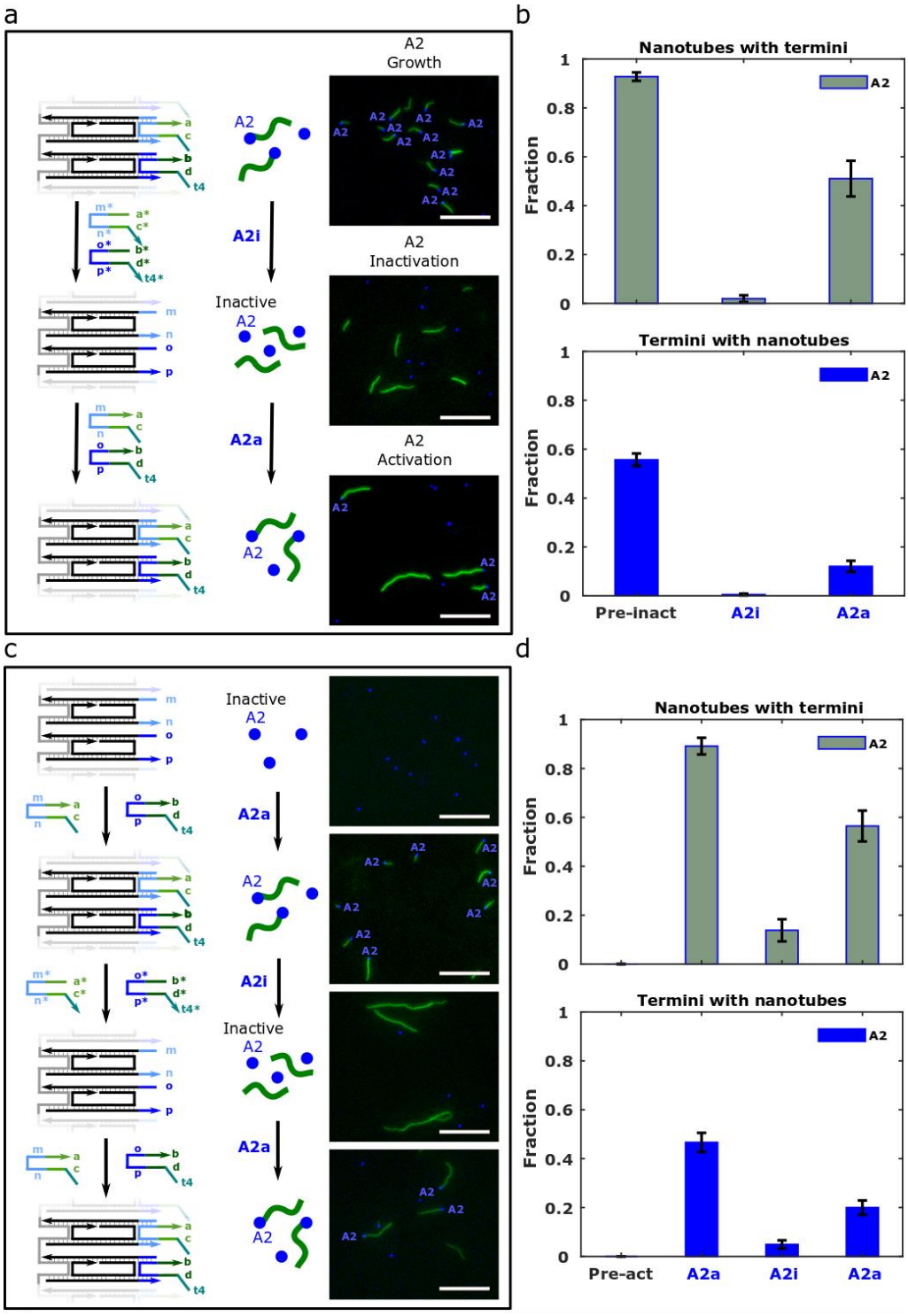
Supporting Figure S4: Nanotube joining after terminus inactivation decreases the fraction of termini with nanotubes after reactivation. **a)** Fractions of nanotubes with termini and termini with nanotubes during sequential terminus inactivation and reactivation of termini with adapter sticky end strands with (A1) and without (A1o) the single-stranded inactivation toehold domains. Error bars represent 95% confidence intervals. **b)** Mean number of nanotubes and termini per field of view from the images collected for this experiment. Error bars represent standard deviation across the images. During the incubation period after terminus inactivation, the mean number of nanotubes per field of view in the sample (A1) where the termini are removed from the nanotubes during the inactivation step decreases by about a factor of two. In contrast, the mean number of nanotubes does not change in the A1o sample (termini missing the 7-base 5' toehold domain on the activation strands) where the termini are not removed from the nanotubes by inactivation strands. This suggests that the number of nanotubes can decrease in the sample when both ends are exposed, presumably because end-to-end joining[5] can happen in this case. The decrease in the number of nanotubes in the A1 sample post-inactivation means that fewer nanotubes are available to attach to the termini after re-activation. Given that the number of termini is the same or larger than the number of nanotubes, the decrease in the number of nanotubes available lowers the fraction of termini attached to nanotubes post-reactivation than pre-inactivation. **c)** Fluorescence micrographs of samples of the reaction solution after the different stages of the experiment. Far fewer DNA nanotubes were observed in the images of the A1 sample post-inactivation than in pre-inactivation images. The nanotubes in the A1 sample post-inactivation (where the termini were removed from the nanotubes) were also visibly longer than the nanotubes in the A1o sample (where termini were not removed from the nanotubes), consistent with the end-to-end joining of nanotubes in the A1 sample during the period when the termini were inactive and both nanotube ends were free. A1o termini were present at 10.23 pM and A1 termini were as described for the experiment in Figure 2 of the main text. The A1 terminus data (top three images) is also presented in Figure 3 of the main text. Scale bars: 10 µm.

4. Activation and inactivation of B1, A2, and B2 termini

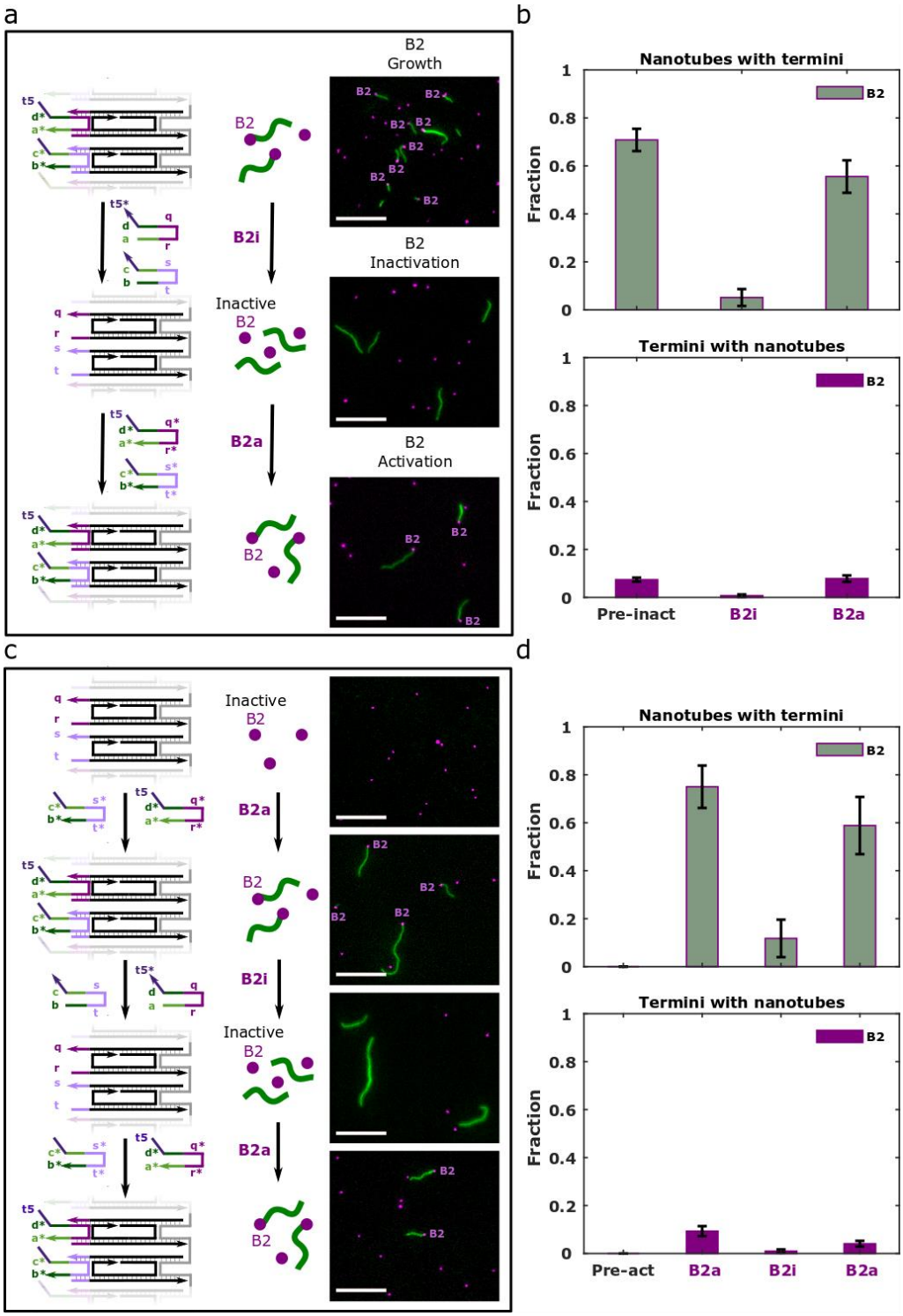
Note: in all the experiments in this section, the fraction of termini having attached nanotubes after the second sequential activation step is lower than the fraction of termini having attached nanotubes after the first activation step. The difference in these fractions may be caused by nanotube joining, which would lower the number of nanotubes after the termini were inactivated (Supporting Figure S4).



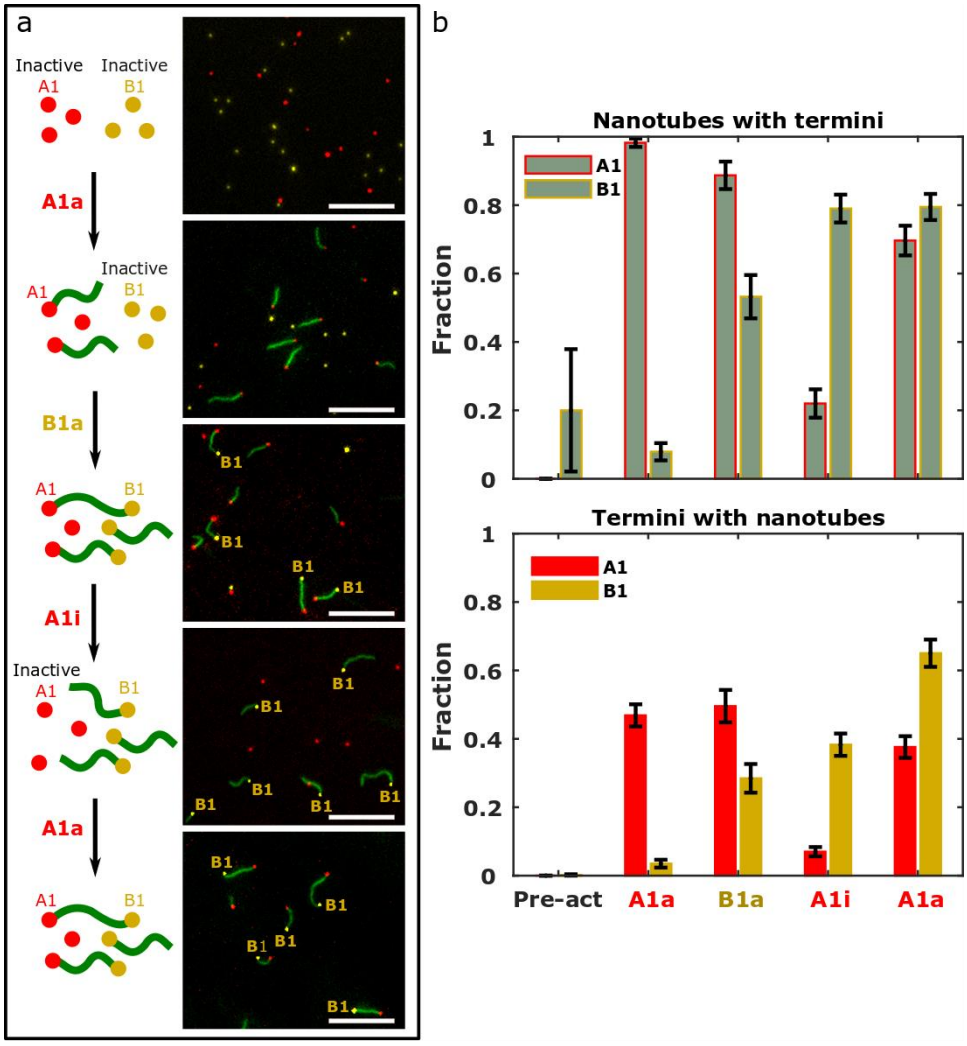
Supporting Figure S5: B1 terminus activation and inactivation. **a)** B1 terminus inactivation and reactivation performed in sequence with representative fluorescence micrographs. Initially active termini were used to grow nanotubes and subsequently inactivated by the addition of inactivation strands (B1i), then reactivated by activation strands (B1a). **b)** Fractions of nanotubes with termini and termini with nanotubes from (**a**). Pre-inact: the incubation period before addition of inactivation strands. **c)** Successive B1 terminus activation, inactivation, and reactivation with representative fluorescence micrographs from each step. **d)** Fractions of nanotubes with termini and termini with nanotubes from (**c**). Pre-act: the incubation period before addition of activation strands. B1 termini attached to nanotubes are labeled for clarity. See Supporting Table S17 for further experimental details. Error bars represent 95% confidence intervals of proportions. Scale bars: 10 μm.



Supporting Figure S6: A2 terminus activation and inactivation. **a)** A2 terminus inactivation and reactivation performed in sequence with representative fluorescence micrographs. Initially active termini were used to grow nanotubes and subsequently inactivated by the addition of inactivation strands (A2i) then reactivated by activation strands (A2a). **b)** Fractions of nanotubes with termini and termini with nanotubes from (**a**). Pre-inact: the incubation period before addition of inactivation strands. **c)** Successive A2 terminus activation, inactivation, and reactivation with representative fluorescence micrographs from each step. **d)** Fractions of nanotubes with termini and termini with nanotubes from (**c**). Pre-act: the incubation period before addition of activation strands. A2 termini attached to nanotubes are labeled for clarity. See Supporting Table S18 for further experimental details. Error bars represent 95% confidence intervals of proportions. Scale bars: 10 μm.



Supporting Figure S7: B2 terminus activation and inactivation. **a)** B2 terminus inactivation and reactivation performed in sequence with representative fluorescence micrographs. Initially active termini were used to grow nanotubes and subsequently inactivated by the addition of inactivation strands (B2i) then reactivated by activation strands (B2a). **b)** Fractions of nanotubes with termini and termini with nanotubes from (**a**). Pre-inact: the incubation period before addition of inactivation strands. **c)** Successive B2 terminus activation, inactivation, and reactivation with representative fluorescence micrographs from each step. **d)** Fractions of nanotubes with termini and termini with nanotubes from (**c**). Pre-act: the incubation period before addition of activation strands. B2 termini attached to nanotubes are labeled for clarity. See Supporting Table S19 for further experimental details. Error bars represent 95% confidence intervals of proportions. Scale bars: 10 μm.

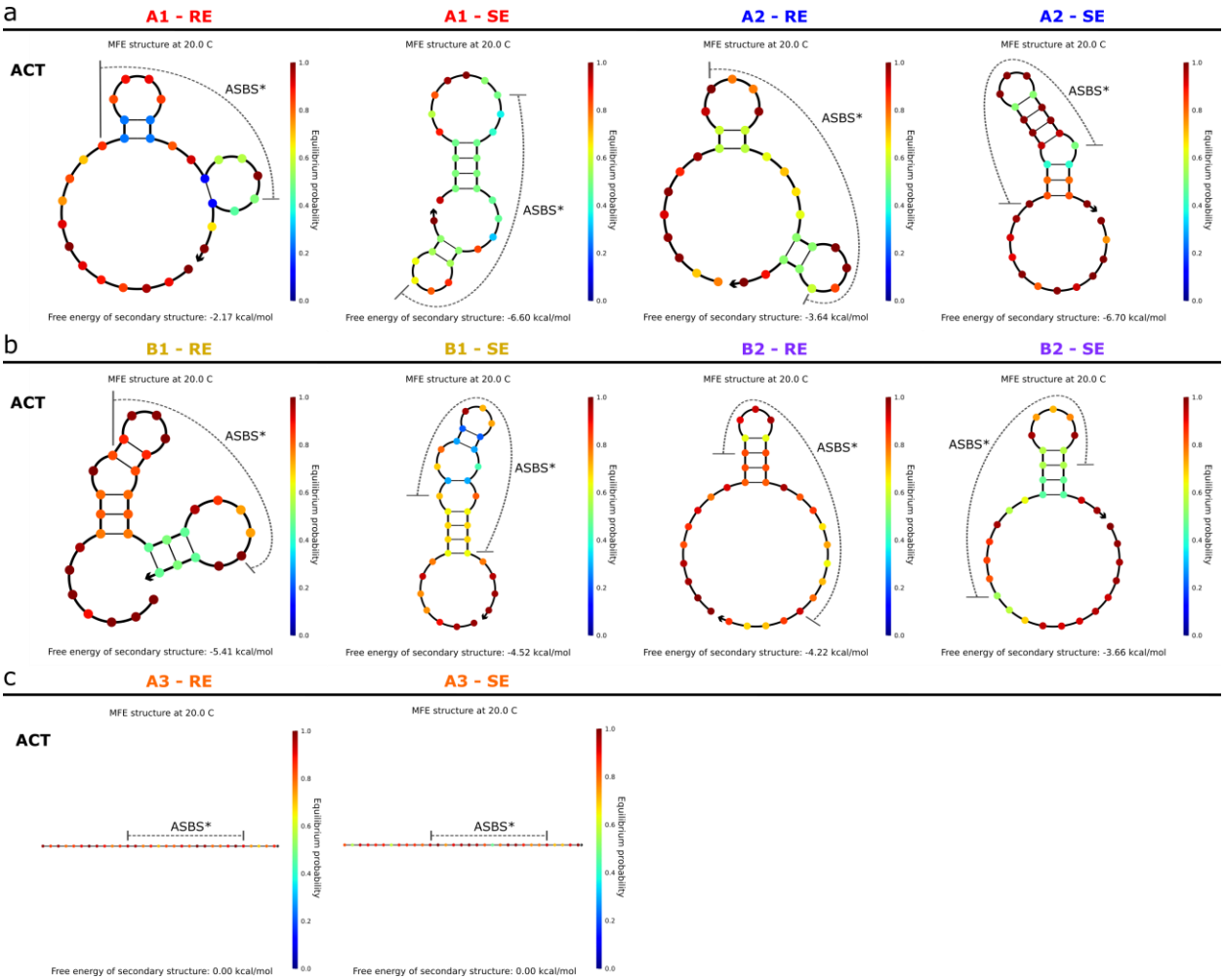


Supporting Figure S8: Successive assembly, disassembly, and reassembly of an A1-B1 terminated nanotube architecture. **a)** Schematic of the experiment with representative fluorescence micrographs after each step. **b)** Fractions of nanotubes with termini and termini with nanotubes for the experiment in (**a**). The yields of doubly terminated A1-B1 architectures (Supporting Section 6.3) are tabulated in Supporting Table S23. See Supporting Table S20 for further experimental details. Error bars represent 95% confidence intervals. Scale bars: 10 μm.

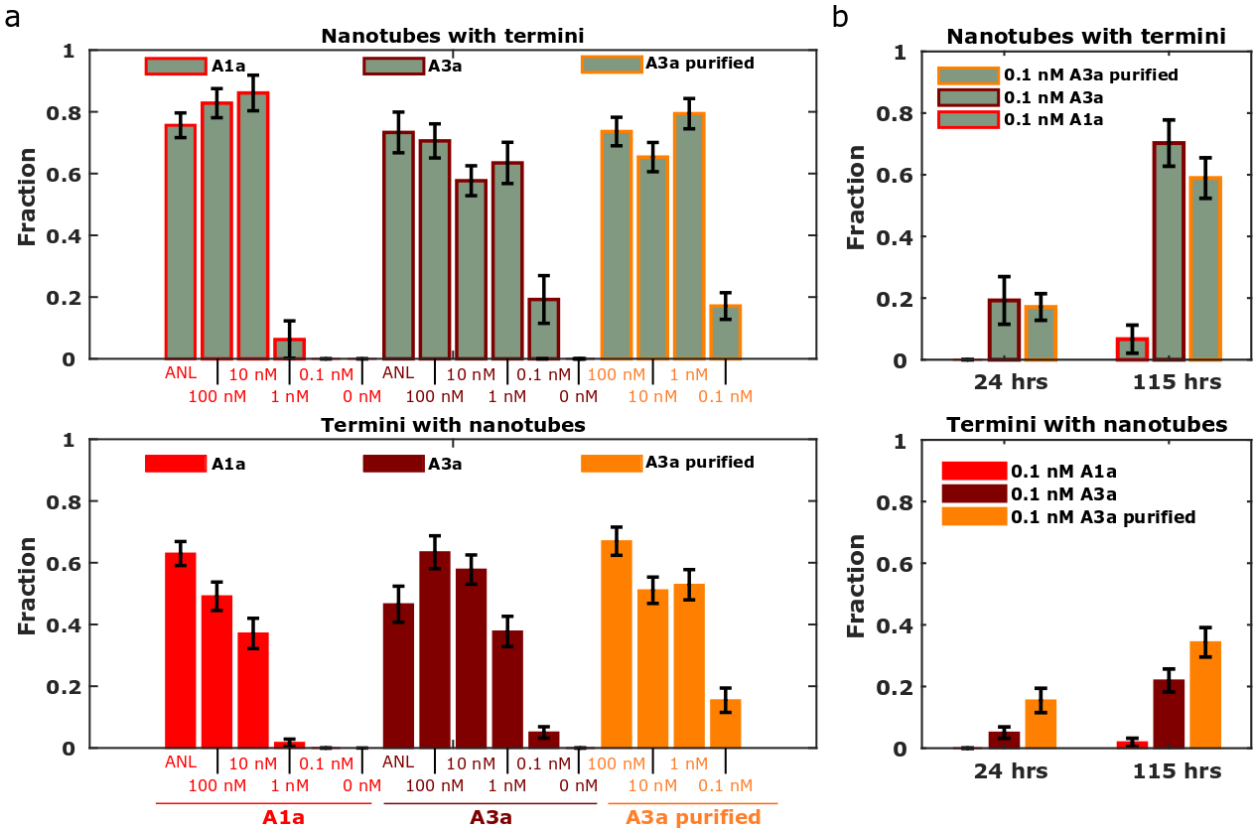
5. Activation strands concentration titrations with strands designed without secondary structure

Supporting Figure S9a,b show the results of NUPACK[6] analysis of secondary structure for the activation strands of the A1, A2, B1, and B2 termini at 20 °C. This analysis reveals that most of these activation strands have significant secondary structure in the regions where they are supposed to bind to their target termini, which could explain why the concentration of activation strands required for significant activation was much higher than expected (Figure 6 of the main text). Supporting Figure S9c shows the activation strands for the A3 terminus, which were designed with NUPACK to have no secondary structure at 20 °C.

Supporting Figure S10 shows the activation yields of A1 and A3 termini with different concentrations of their activation strands. For the A3 termini, both unpurified and PAGE purified strands were tested. The PAGE purified A3 activation strands that have no predicted secondary structure result in the highest activation yields, producing >35% of termini with nanotubes after long incubation times.



Supporting Figure S9: Predicted secondary structures of activation strands for A1 and A2 termini (**a**), B1 and B2 termini (**b**), and for an A3 terminus that was specifically designed so that its activation strands would not have secondary structure (**c**). Secondary structures were predicted using NUPACK[6] with the default settings at 20 °C. Color coding of the bases represents the probability that a given base will be in the depicted conformation at equilibrium. The gray dashed lines labeled ASBS* are the domains of activation strands that hybridize to the activator strand binding sites (ASBS) on the termini. For the A1, A2, B1, and B2 termini, the ASBS* domains all have some predicted structure which could lower their binding affinity for their target termini.



Supporting Figure S10: Activation strands designed to have no secondary structure can activate termini at much lower concentrations than activation strands with significant amounts of predicted secondary structure. **a**, Fractions of nanotubes with termini and termini with nanotubes after activation of the A1 terminus (A1a) or A3 terminus using unpurified (A3a) or PAGE purified activation strands (A3a purified) from IDT. Error bars represent 95% confidence intervals of proportions. The activation strands were added to the final concentrations listed on the x-axes of the plots as soon as the purified termini were added to the monomer mixes. The "ANL" samples served as positive controls: these termini were annealed with their activation strands present. The samples were imaged after incubating them for 24 hours with the activation strands. The fraction of termini with nanotubes is also presented in Figure 6 of the main text. **b**, Fractions of nanotubes with termini and termini of the samples activated 0.1 nM of activation strands after 24 and 115 hours. The fraction of A3 termini that are activated increases significantly with longer incubation time. Error bars represent 95% confidence intervals of proportions. Termini were at 3 pM across the samples. Experiments were otherwise conducted as described in the Methods of the main text. All termini fluorescently labeled with atto488. Adapter and activation strand sequences are listed in Supporting Section 1.3.

6. Image analysis

The fluorescence micrographs of nanotubes and/or termini were processed using custom MATLAB scripts for quantitative analysis. Three to five images were typically processed and analyzed for a specific sample at a given timepoint. Below is the workflow for the image analysis process.

Detecting objects

A fluorescence micrograph of DNA nanotubes and a corresponding fluorescence micrograph of DNA origami termini were imported simultaneously for analysis. Canny edge detection[7] was used to detect the edges of objects in both the DNA nanotube image and the DNA origami termini image and produce binary images of the object edges. The detected objects were then filled in with pixels using MATLAB's *bwmorph( )* function. No further processing was done to the DNA origami termini image.

For the DNA nanotube image, morphological operations were applied with MATLAB's *bwmorph( )* function to skeletonize all the detected objects to be 1 pixel in width. This processed image was used for the quantification of the fraction of termini with nanotubes (referred to as qFTwN below- Supporting Figure S11).

6.1 Analysis of samples with termini only labeled with a single fluorophore

Quantification of fraction of termini with nanotubes

To quantify the fraction of termini with nanotubes, the locations of the endpoints of each object in the qFTwN image were determined. A radius (typically 2 to 4 pixels) around each of these endpoint locations was searched in the processed DNA origami termini image and if a terminus was found in the search radius, this terminus was counted as having a nanotube attached to it (Supporting Figure S11, middle). The fraction of termini with nanotubes was then calculated as the total number of identified termini that had a nanotube attached to them divided by the total number of termini in all the images processed for a given set of conditions (experiment and timepoint). The total number of termini in each image was determined by counting all the individual objects in the processed DNA origami termini image. Error bars for the fraction of termini with nanotubes represent the 95% confidence intervals of proportions for large samples (Eq. 1).

$$(1) \quad CI = \pm 1.96\sqrt{p(1-p)/n}$$

Quantification of fraction of nanotubes with termini

To quantify the fraction of nanotubes with termini, the objects in the qFTwN nanotube image that extended past the boundary of the image were removed as it is not possible to determine what types of termini (if any) are present at both the ends of a nanotube that extends past the image boundary. We will term this processed nanotube image with the nanotubes that extended past the boundaries removed qFNwT below. To determine the fraction of nanotubes with termini, the locations of the endpoints of each nanostructure in the qFNwT image were determined. A radius (typically 2 to 4 pixels) around each of these endpoint locations was searched in the processed DNA origami terminus image and if a terminus was found in the search radius, a nanotube with a terminus was counted (Supporting Figure S11, right). The fraction of nanotubes was then calculated as the total number of nanotube

endpoints that had a specific terminus attached to them over the total number of nanotubes across all the images processed for a given sample at a specific timepoint. Since some nanotubes cross over in the images and result in branched objects with more than two endpoints in the processed images, the total number of nanotubes in an image was calculated as:

$$(2)\quad ceil\left(\frac{\#\ of\ endpoints}{2}\right)$$

Where ceil(x) rounds to the lowest integer greater than or equal to x. So, an object with two endpoints would be counted as a single nanotube, an object with three or four endpoints would be counted as two nanotubes, an object with five or six endpoints would be counted as three nanotubes, etc. Error bars for the fraction of termini with nanotubes represent the 95% confidence intervals of proportions (Eq. 1).

6.2 Analysis of samples with termini labeled with two fluorophores

For samples with termini that were labeled with two different fluorophores (Figure 6 of the main text), adjustments to the analysis in Supporting Section 6.1 were made to account for termini labeled with two fluorophores. First, termini labeled with two fluorophores were identified by identifying image locations where the pixels of an object in the processed atto488 binary micrograph overlapped more than 50% of the pixels of an object in the processed atto647 binary micrograph. Second, the total number of termini labeled with only a single fluorophore in each image were then quantified by counting all the individual objects in the processed termini binary micrograph of a single fluorophore and subtracting from that quantity the total number of identified termini labeled with two fluorophores. Once the number and location of all the different termini we located the fraction of nanotubes with termini and termini with nanotubes could be calculated as described in Supporting Section 6.1. Supporting Figure S11b shows representative processed images from this analysis.

6.3 Quantification of yields of doubly terminated nanotubes

In experiments where both an A terminus and a B terminus were activated, the yield of nanotubes attached to both an A and B terminus was calculated. We termed such nanotubes with termini on both ends, doubly terminated nanotubes. The yield of forming doubly terminated nanotubes was determined by analyzing the qFNwT processed nanotube images. The yield was then calculated as the total number of nanotubes attached to both the termini of interest divided by the total number of nanotubes in all the images processed for a given sample at a specific timepoint (Eq. 3).

$$(3)\quad \frac{\#\ of\ nanotubes\ attached\ to\ T_1\ and\ T_2}{total\ \#\ of\ nanotubes}$$

where T_1 is the terminus that was activated first and T_2 is the terminus that was activated second in the experiments.

Yields were also calculated by dividing by the number of nanotubes attached to either T_1 or T_2 (Eqs. 4 and 5). For example, for the results in Figure 5b the yield with respect to A1 nanotubes is the number of nanotubes to both and A1 and B1 terminus divided by all the nanotubes attached to an A1 terminus.

$$(4)\quad \frac{\#\ of\ nanotubes\ attached\ to\ T_1\ and\ T_2}{\#\ of\ nanotubes\ attached\ to\ T_1}$$

(5) $$\frac{\#\ of\ nanotubes\ attached\ to\ T_1\ and\ T_2}{\#\ of\ nanotubes\ attached\ to\ T_2}$$

Table S22-S24 show the yields from all relevant experiments.

Table S22: Yields of doubly terminated A1-B1 nanotube architectures from the experiments presented in Figure 4 of the main text. The yields were calculated as the total number of nanotubes in an A1-B1 architecture divided by either all nanotubes, the nanotubes attached to A1 termini, or the nanotubes attached to B1 termini (Eqs. 3 – 5, respectively). Errors represent 95% confidence intervals of proportions. N represents the number of nanotubes counted for each calculation (used as the denominator in either Eq. 3, 4, or 5). Note that the number of nanotubes attached to A1 termini after A1i addition is low, which results in a relatively high A1-B1 yield when considering only A1 nanotubes compared to A1-B1 yields considering all nanotubes or only B1 terminated nanotubes.

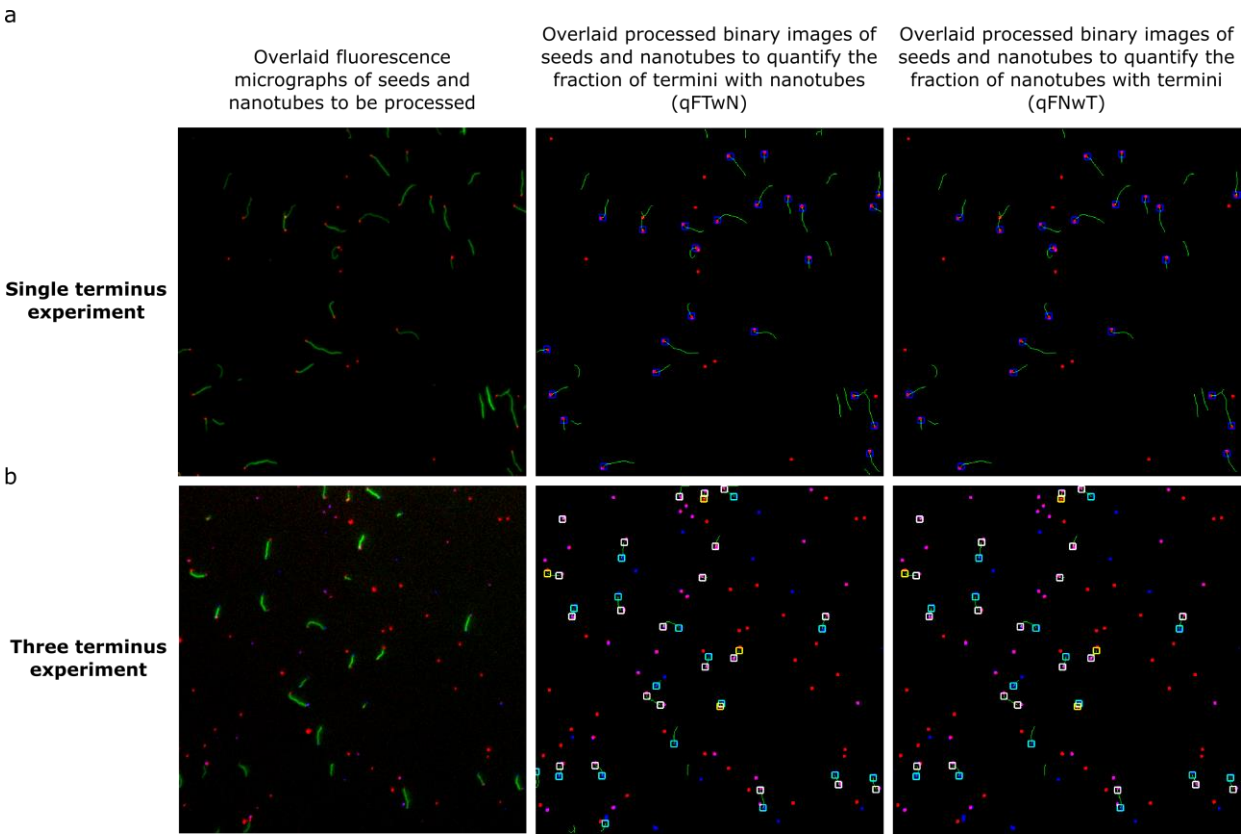
	A1 – B1 yields (%)	**A1 – B1 yields** (%)	**A1 – B1 yields** (%)
Eq.3:	All: 45.7 ± 4.4 (N = 127)	All: 7.4 ± 2.9 (N = 81)	All: 0.0 (N = 59)
Eq.4:	A1: 50.0 ± 4.6 (N = 116)	A1: 50.0 ± 14.4 (N = 12)	A1: 0.0 (N = 6)
Eq.5:	B1: 67.4 ± 5.0 (N = 86)	B1: 12.8 ± 4.9 (N = 47)	B1: 0.0 (N = 3)
	After **B1a** addition	After **A1i** addition	After **B1i** addition

Table S23: Yields of doubly terminated A1-B1 nanotube architectures from the experiments presented in Supporting Figure S8. The yields were calculated as the total number of nanotubes in an A1-B1 architecture divided by either all nanotubes, the nanotubes attached to A1 termini, or the nanotubes attached to B1 termini (Eqs. 3 – 5, respectively). Errors represent 95% confidence intervals of proportions. N represents the number of nanotubes counted for each calculation (used as the denominator in either Eq. 3, 4, or 5). Note that the number of nanotubes attached to A1 termini after A1i addition is low, which results in a relatively high A1-B1 yield when considering only A1 nanotubes compared to A1-B1 yields considering all nanotubes or only B1 terminated nanotubes.

	A1 – B1 yields (%)	**A1 – B1 yields** (%)	**A1 – B1 yields** (%)
Eq.3:	All: 41.9 ± 6.3 (N = 62)	All: 15.0 ± 3.6 (N = 100)	All: 46.4 ± 4.7 (N = 112)
Eq.4:	A1: 47.3 ± 6.7 (N = 55)	A1: 68.2 ± 9.9 (N = 22)	B1: 58.4 ± 5.2 (N = 78)
Eq.5:	B1: 78.0 ± 7.1 (N = 33)	B1: 19.0 ± 4.4 (N = 79)	A1: 66.7 ± 5.3 (N = 89)
	After **B1a** addition	After **A1i** addition	After **A1a** addition

Table S24: Yields of doubly terminated A1-B2 and B2-A2 nanotube architectures from the experiments presented in Figure 5 of the main text. The yields for A1-B2 structures were calculated as the total number of nanotubes in an A1-B2 architecture divided by either all nanotubes, the nanotubes attached to A1 termini, or the nanotubes attached to B2 termini (Eqs. 3 – 5, respectively). The yields for B2-A2 structures were calculated as the total number of nanotubes in an B2-A2 architecture divided by either all nanotubes, the nanotubes attached to B2 termini, or the nanotubes attached to A2 termini. Errors represent 95% confidence intervals of proportions. N represents the number of nanotubes counted for each calculation (used as the denominator in either Eq. 3, 4, or 5). Note that the number of nanotubes attached to A1 termini after A1i addition is low, which results in a relatively high A1-B2 yield when considering only A1 nanotubes compared to A1-B2 yields considering all nanotubes or only B2 terminated nanotubes.

	A1 – B2 yields (%)	**A1 – B2 yields** (%)	**B2 – A2 yields** (%)
Eq.3:	All: 50.9 ± 4.7 (N = 114)	All: 16.8 ± 3.6 (N = 107)	All: 42.5 ± 4.7 (N = 113)
Eq.4:	A1: 58.6 ± 5.0 (N = 99)	A1: 64.3 ± 9.1 (N = 28)	A1: NA
Eq.5:	B2: 85.3 ± 4.3 (N = 68)	B2: 20.7 ± 4.3 (N = 87)	B2: 51.6 ± 5.2 (N = 93)
Eq.5:	A2: N/A	A2: N/A	A2: 75.0 ± 5.4 (N = 64)
	After **B2a** addition	After **A1i** addition	After **A2a** addition



Supporting Figure S11: Representative images from the MATLAB image analysis algorithm. **a**,**b**, Left: The overlaid fluorescence micrographs of the image to be processed. Nanotubes are green. Middle: The overlaid binary image output that was analyzed by MATLAB to quantify the fraction of termini with nanotubes. Right: The overlaid binary image output that was analyzed by MATLAB to quantify the fraction of nanotubes with termini. Note that nanotubes at the edge of the image have been removed. In the single termini experiment, the blue squares indicate the A1 termini that the algorithm identified as being attached to a nanotube. In the three termini experiment, the boxes indicate termini that the algorithm identified as being attached to a nanotube; yellow box: A1 terminus, white box: B2 terminus, cyan box: A2 terminus.

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