Supplementary Information

Terminating DNA Tile Assembly with Nanostructured Caps

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Supplementary Note S1: RS Nanotube tile sequences

We modified the design of previously studied DAE-E nanotube tiles [1] by reducing the length of sticky ends from 5 base pairs to 4 in order to enable seeds to control whether nanotubes nucleate at room temperature (i.e. 20 °C) when tiles are present at concentrations around 40-50 nM. An additional base pair was added to the double stranded region of the tiles to maintain the proper distance between crossovers.

**R tile sequences:**

<table>
<thead>
<tr>
<th>RE-4bp-1:</th>
<th>CGTATTGGACATTTCCGTAGACCGACTGGACATCTTCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE-4bp-2EE01:</td>
<td>TGGTCCCTTCACACCAATACGGGAT</td>
</tr>
<tr>
<td>RE-4bp-3Cy3:</td>
<td>/Cy3/TCTACGGAAATGTGGCAGAATCAATCATAAGACACCAGTCGG</td>
</tr>
<tr>
<td>RE-4bp-4:</td>
<td>CAGACGAAGATGTGGTAGTGGGAATGC</td>
</tr>
<tr>
<td>RE-4bp-5:</td>
<td>TCCACTACCTGTCTTATGATTGATTCTGCTGTGAAGG</td>
</tr>
</tbody>
</table>

**S tile sequences:**

<table>
<thead>
<tr>
<th>SE-4bp-1:</th>
<th>CTCAGTGGAACAGCCGTTCTGGAGCGTTGGACGAAACTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE-4bp-2DIAG:</td>
<td>TCTGGTAGACCCACTGAGAGGT</td>
</tr>
<tr>
<td>SE-4bp-3Cy3:</td>
<td>/Cy3/CCAGAACCGCTGGCTAAACAGTAACCGAAGCACAAGCT</td>
</tr>
<tr>
<td>SE-4bp-4DIAG:</td>
<td>ACCAGATTTCGGTTAGTACGTACCT</td>
</tr>
<tr>
<td>SE-4bp-5:</td>
<td>ACGATGACCTGCTGTTACTGTCCCTGCTCAC</td>
</tr>
</tbody>
</table>

Here /Cy3/ denotes a covalently attached Cy3 fluorophore.
Supplementary Note S2: UV Nanotube tile sequences

In order to grow two different types of nanotubes in a single pot reaction, we used UV RT tiles. UV RT tiles were modified from those developed previously [1] by shortening their sticky ends so to enable them to grow from seeds at room temperatures at concentrations similar to those used for RT tiles.

Supplementary Figure S1: Schematic showing the architecture of the U and V tiles. Cy3 represents a covalently attached /Cy3/ fluorophore.

U tile sequences:

UE1_4bp-1: CGT TAA GGA CGA CGC AAT TCT CAC ATC GGA CGA GTA GG
UE2DIAG_4bp-1: TCT CTG GTT TCA CCT TAA CGC AAG
UE3_Cy3_4bp-1: /Cy3/AGA ATT GGC TCG TGG TTA GGT ATC GCT ACC GAT GTG
UE3_4bp-1: AGA ATT GGC TCG TGG TTA GGT ATC GCT ACC GAT GTG
UE4DIAG_4bp-1: TCA GCC TAC TCG TGG ATC TAT GCT TG
UE5_4bp-1: CAT AGA TCC TGA TAG CGA GAC CTA GCA ACC TGA AAC CA

V tile sequences:

VE1_4bp-1: CCA TTC GGA CGT TTG CGG TAA AGA TTA GGA CAT TGA AG
VE2DIAG_4bp-1: CTG ATC CGA GCA CCG AAT GGC GTA
VE3_Cy3_4bp-1: /Cy3/TCA CGG CAA ACG TGG CGA GTG ATC GAC TAC ACC TAA TCT
VE3_4bp-1: TTA CGG CAA ACG TGG CGA GTG ATC GAC TAC ACC TAA TCT
VE4DIAG_4bp-1: GAG ACT TCA ATG TGG CGT TCA CTA CG
VE5_4bp-1: GTG ACG GCC TGT AGT CGT ATC ACA CTC GCC TGC TCG GA
Supplementary Note S3: RT A seed design and sequences

RT A seeds were designed by modifying the nanotube nucleation structures from Mohammed et al [2] to present sticky ends corresponding to the tiles in Fig. 1a / Supp. Note S1 and by reducing the number of staples used to fold the assembled structure.

RT A seed architecture

Supplementary Figure S2: RT A seed architecture. Crossover diagram (top) and a strand map (bottom) showing the names of the staple strands at different positions (sequences for each strand are given below) in the RT A seed. The scaffold regions shown as unbound on the right end side of the diagram were used as binding sites for adapter strands, while the remainder of the scaffold structure (including the regions shown as unbound on the left side of the crossover diagram) remained unfolded (See Supp. Note S18).
RT A seed staple sequences:

T3R2F_HP: TGCCTTGACAGTCTCTGTGGTGTTTGGCACCGACTTTGAATTACCCTCAGA
T3R4F_HP: GCCACCACTCTTTTACGGTGTTTTGGCGACCCTTTAACTAAATAGCAAGG
T3R6F_HP: CCGGAAAATCAGTGACCTGCTTTTTGCACCCAGCTTTAATTCTACAAAGAA
T3R8F_HP: ACGCAAAAGAAGAAGACTTGCAGCTTTTTGAGCCGATTGTGACATTTGGATTTA
T3R10F_HP: GCCCAATAGACGGGAGACAAGGCTTTTGAGCTTTTAAATTACTCTTCAGAG
T3R12F_CYC_HP: CCTAATTTACCAGCGCTCGAGGTATTGTTGAGTGGG

T3R2E_HP: GGAAAGCGGTAAACAGTGGGAGCTTTTTGCACCCCTGCCGATTCGGG
T3R4E_HP: GCTTGCCAATCTACACGACCAGGGTTTGCGTTTTCCGCACCCACAGAAAT
T3R6E_HP: TTATGTCATGACTCCTCTGCTTTTGACGAGCTTTGAAACCATATTAGC
T3R8E_HP: ATACCCCAAACACACGGCCATTCTGTTTGGTAGATTGAAATAGTCGAGGA
T3R10E_HP: GCCATTTAATAAGAGCCCTGGAGCTTTTGGGCTCTAAGAAACATAGCT
T3R12E_CYC_HP: TGCTCATGGCCAGTTGCTCTGCTTTTGGAGCGTTTAAACATAAAAGCAGG

T5R2F_HP: AATGCCCTATAATCCTCGCTGGAGCTTTTGCACCCCTGGGACCTTTACATTAAAGAAACAC
T5R4F_HP: CACCAAGTGTCGTCGACGGGAGAAGGGTTTGCGTTTTCCGCACCCAGTTAGC
T5R6F_HP: AGCACTGGAGGGAATGTCAGGCTTTTGCCCTCCCATTTAAAATATTATTTG
T5R8F_HP: CCAAACTCCTCGAGGAATGTCGAGCTTTTGCCAACCTACGTCAGAAAATAATGAA
T5R10F_HP: GCACATACGAGAATAACGCAACGGCTTTTGACGCTTTTACATAAAAAACACCAT
T5R12F_CYC_HP: ATTATTTAAGAGATTGCCATCGCTGCTTGGAGAACATTAGAAACGTT

T5R2E_HP: ACAAACAACTGCTGTACGTGCAGGCTTTTGGCTCTGGTTTCCGAGACTGAGAC
T5R4E_HP: TCGCATTCCGCCGAGCTGCTCTTGCTAGCCAGCTTACAGCATTGATATTTC
T5R6E_HP: ATTGAGGGAATACGATTACCGGACTTTTGCAGTCTCCTCCGCTAGAGAAGCCCT
T5R8E_HP: GAAGGAAAAATAGAAGACCTAGGAGCTTTTGCGCATAGTTTACATATTTCAACCG
T5R10E_HP: CTATGCTATCTTTACGCTCTGTTTTACGAGGCTGCTGAGGACCCAGTTACCA
T5R12E_CYC_HP: CCTCAAGATCCCCATCCTCGGAGCTTTTGCCTCCAGTTAAATAGATACGC

Hairpins (highlighted in red) were incorporated into staples to induce a preference in the direction of cyclization.
RT A seed adapters:

Supplementary Figure S3: **RT A seed adapters.** Structure of assembled adapters for RT A seed. The gray lines and associated sequences are portions of the M13mp18 scaffold.
RT A seed adapter strand sequences:

A-4bp-1REd_1: CAGCCAGACGCAGTGAGCGGACAGAGCTGAAGATATTAAAGAGG
A-4bp-1_2REd_3: TCGCTACCTGCTGCTGAGCTGAGGATGCGCTCGTCT
A-4bp-1_2REd_5: CTTAATCTCTGAACAGTGAGCTACCATCCACGACGACACGAGCA
A-4bp-2REd_2: TGGTGCTGCTGCTGCTGCAGCTGCCAT
A-4bp-3SEd_1: CACGGATGCACTCCGTAGCCAGTACCGACACATGCTGGCCT
A-4bp-3_4SEd_3: GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC
A-4bp-3_5SEd_5: CTATTATTCTGAAACAGTGGACCTCACCATCCACGACGACACGAGCA
A-4bp-4SEd_2: TGGTTGCTGCTGCTGCTCCAGCTGCCAT
A-4bp-5REd_1: CAGAGCCACGCGATGGTCTTGCGTTGGAGGCGTCAGACTTGAGCG
A-4bp-5_6REd_3: CAAGACCATGCCGACCATCCTCGCTTTCGGTGCTCCAACG
A-4bp-6REd_5: ATCAAGTTTGCCTTTACACCGAAAGCGAGGATGAGGTGCGGACGA
A-4bp-6_6REd_2: TGGTTGCTGCTGCTGCTCCAGCTGCCAT
A-4bp-7SEd_1: CACGGAGTCTACGCCAGCGATCCTCCAGACAAAAAGCGAC
A-4bp-7_8SEd_3: GTCAGGCGCCAGGAGACCTCGTGAGGGACTGCTGAGCG
A-4bp-8SEd_5: GTTTTACCGCGAAGCGTGTGGTGGCTCCTGCCAGCTGAGGTGGAGG
A-4bp-7_6REd_2: TGGTTGCTGCTGCTGCTCCAGCTGCCAT
A-4bp-9REd_1: CAACCGTCGTTCCACAGGACTACGCTGCTCCAGATAGCGCAACAA
A-4bp-9_10REd_3: AGTCTCTGAGGACACCCAGAGACCGACCATCGAGCGAAAGTGCG
A-4bp-10SRd_5: TTTTAAAGAAAGCTAACGCTGATGGCGTCTCGTGGTAAGCTGA
A-4bp-9_10SRd_2: TGGTTGCTGCTGCTGCTCCAGCTGCCAT
A-4bp-11SEd_1: CACGGAGTCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA
A-4bp-11_12SEd_3: ACTCGTAGCCTTGGACCGCAGCTACCATCGACGCGAGGGCT
A-4bp-12SEd_5: AAACGATTATTTTTGTTTCGACGAGTGAGTGTCGCGGTCGCGG
A-4bp-11_12SEd_2: TGGTTGCTGCTGCTGCTCCAGCTGCCAT

In the dish experiments, RT A seeds were attached to Biotin attachment linker strands and Biotin attachment strand, their sequences are:

**Biotin attachment linker strand sequences for RT A Seeds**

Biotin_positive_01 AGGGATAGCAAGCCCATTTTCACATCGTCACTCCT
Biotin_positive_02 GAATTGCGAATAATAATTTTCACATCGTCACTCCT
Biotin_positive_03 TGTATCATCGGACACGCACTCGTCGCTCGTCGCTCG
Biotin_positive_04 TTTTAAAGAAAGCTAACGCTGATGGCGTCTCGTGGTAAGCTGA
Biotin_positive_05 ACTCGTAGCCTTGGACCGCAGCTACCATCGACGCGAGGGCT
Biotin_positive_06 AAACGATTATTTTTGTTTCGACGAGTGAGTGTCGCGGTCGCGG

**Biotin attachment strand sequence**

Biotin_attachment_strand /5BiosG/AGGAGTGACGATGTG
Supplementary Note S4: RT C seed design and sequences

RT C seeds use the same set of staples as the RT A seeds but a different set of adapters to provide complementary binding sites to UV tiles.

RT C seed adapters:

Supplementary Figure S4: RT C seed adapters. Structure of assembled adapters for RT C seed. The gray lines and associated sequences are portions of the M13mp18 scaffold.
RT C seed adapter strand sequences:

AD1UEd_1:  GAC ACG GAA GCG GAT GTG GAA GCA CTA GCT GAA AGT ATT AAG AGG
AD1_2UEd_3:  TTC CAC ATC CAC TCT GTC AGT CAC CTC GCA TCA GGC TAG TGC
AD2UEd_5:  CTA TTA TTC TGA AAC ACT GTG GCG AGG TGA CTG GCA GCA GAT GGT
AD1_2UEd_2:  TCT CAC CAT CTG TCC GTG TCC TCA
AD3VEd_1:  GTG CGA AGT GCT TGT CGT CAC AGG CAT CTG TCA GAC GAT TGG CCT
AD3_4VEd_3:  GTG ACA AGA AGA CAC TGC ACT CAG CCT TCG GAG ATG CCT
AD4VEd_5:  CAG GAG GTT GAG GCA GCC GAA GGC TGA GTG CAG TGT CCT GGT AGG
AD3_4VEd_2:  CTG ACC TAC CAG CTT CGC ACC ACC
AD5UEd_1:  GAT GAG CAT CTA GGT GGC TTC ACC TAC TGG CGT CAG ACT GTA GCG
AD5_6UEd_3:  GAA GCC ACG TCG AGA CCA GTC CTA CCA CAG CTC GCA GTA GGT
AD6UEd_5:  ATC AAG TTT GCC TTT ACG AGC TGT GGT AGG ACT GGT CGC TAT GGC
AD5_6UEd_2:  TCT CGG CAT AGC TTC TCA AAG
AD7VEd_1:  TCG CAC TGC TAC TCC AGC ATG GCA ACG GTA GAC AAA AGG GCC ACA
AD7_8VEd_3:  CAT GCT GGA GTA GAC AGG TGA GGA TGT CCA GAC CAC CGT TGC
AD8VEd_5:  GGT TTA CCA GCG CCA AGG TCT GGA CAT CTT CAC CTG TAG GCA CGA
AD7_8VEd_2:  CTG ATC GTG CCT CAG TGC GAC GTA
AD9UEd_1:  GTG CTC GAC TGA TCG CCT TGG CAT TCG CTA CCA
AD9_10UEd_3:  ATG CCA AGG CGA TGT CGA GGT TGT CTT GCT CGG TAG CGA
AD10UEd_5:  AAA CGA TTT TTT GTT TGA GCA AGA GCC CAA CCT CGA CTC CTA GCC
AD9_10UEd_2:  TCT CTA GGT CGT TGC AGC ACC AGG
AD11VEd_1:  GTC GAC CGT TGA TCA CTA GCA TGA TCG CAT TCG CTA CCA AGC TCA AAA CTG TCA CTA
AD11_12VEd_3:  ATG CCA AGG CGA TGT CTA GGT GGT TGT CTT GCT CGG TAG CGA
AD12VEd_5:  AAA CGA TTT TTT GTT TGA GCA AGA GCC CAA CCT CGA CTC CTA GCC
AD11_12VEd_2:  CTG AGG CTA GGA CAG GTC ACC GTA
Supplementary Note S5: Design and sequences of the RT B seed (rigid cap)

The RT B seed, which serves a nucleation site for RS nanotube tiles from their B interface, was designed by following the same design principles used in designed RT A seed.

Supplementary Figure S5: RT B seed architecture. Crossover diagram (top) and a strand map (bottom) showing the names of the staple strands at different positions (sequences below) in the RT B seed. The scaffold regions shown as unbound on the left side of the diagram were used as binding sites for adapter strands.
RT B Seed Staple sequences:

T_5R2F_HP: TGGAGTTTCAAGGAACGTCACCCGTTTTCGGTGGACTTAACCTAAGATCTCCA
T_5R4F_HP: AAAAAAGGCCTTTTGCAGTCTGCCCTTTTCCCACTTTGGATCGTTGGATGCA
T_5R6F_HP: ACGGCTCAAGTAACACTGCAGCTTTTTGTGCGAGTTCCGAGATTCGACCT
T_5R8F_HP: GCTCCATGACGTAACCAAGGACGGCTTTCGATCCTTAAGCTGCTACACAG
T_5R10F_HP: ACGAGTAGATCAGGTCAGCCAGTGGTTTAGTTAGATTTAGCCCAAAA
T_5R12F_CYC_HP: GGATACCACCACCCTGAGCCCTTTTCCGCTCACTTTTACATTTGCA
T_5R12E_CYC_HP: GGCTTCAAATTTGGGAGGAGCTTTTTGCTTCGTCCGCTAAGAGCATG
T_5R10E_HP: AAGATTTCTATTGAACGCCGAGATTTCTGTGCAAGATTCATACAG
T_5R8E_HP: CGGAAACCAAGAGCTTGTCTGCGTTTTCGACAGACTTGTAGGAGGAGTT
T_5R6E_HP: CGGAGTCGTTTTCGGAGTCTTACCGCTTTTCTGCAACCGGAGTT
T_5R4E_HP: GGAATAGGCTACACGGAACCCGTTTTCGGTTTAGAATAATCTG
T_5R2E_HP: TGAGTTTCAAGGAACGTCACCCGTTTTCGGTGGACTTAACCTAAGATCTCCA

Hairpins (highlighted in red) were incorporated to induce a preference in the direction of cyclization.
RT B seed adapters design:

Supplementary Figure S6: **RT B seed adapter architecture.** Structure of assembled adapters for RT B seeds. The gray lines and associated sequences are portions of the M13mp18 scaffold.

**RT B seed adapter strand sequences:**

B-4bp-1REd_1: AGGGTAGCAAGCCACAAACGTCAGGACACTTTGGGAGGTGCTGACACTCG
B-4bp-1_2REd_3: TGTCCTCAGTCGTTGGATGGGATGCGATCTTACGACGACACTTTGGGAGGTGCTGACACTCG
B-4bp-2REd_5: TCGCTGACTTTGCTGAGGATGGGATGCGATCTTACGACGACACTTTGGGAGGTGCTGACACTCG
B-4bp-1_2REd_4: CAGACGAGTGCTGACGAGTGCAGAGTCAGCGAATGC
B-4bp-3SEd_1: GAATTGCGAATAATAAGTGACCTTGCTGTACCGTCGAGATGGAGTC
B-4bp-3_4SEd_3: ACAGCAAGGTCACCGCAGTTGGCACTAGGCGACATCGACGGT
B-4bp-4SEd_5: ACCACAACCTGTCGCTAGTGCCAACTGCGTTTTTTCACGTTGAAA
B-4bp-3_4SEd_4: ACCAGACTCCATCGGTTTGTGGTACCT

B-4bp-5REd_1: ACCCTCAGCAGCGAAACGAGTGACGGAACAGGTAGCCTACGG
B-4bp-5_6REd_3: GTTGGCGGTACTCGACTGGTCAGCAACGTTCAACTCACTACGG
B-4bp-6REd_5: TGCTCTGCCTTGGAGAGCGGAGGTTCTTGAGACCAGTGACGACATCGGAACGA
B-4bp-5_6REd_4: CAGACCGTAGGCTGGCAGGAGCAATGCG

B-4bp-7SEd_1: TGTATCATTGCACTGATCAACCGTACGAGATGCGAAGACAGAGTGCG
B-4bp-7_8SEd_3: TCTCGTACCGGCTGACGCTAGCGACGGGCTGGCTTGAGGACTACCT
B-4bp-8SEd_5: AGTCAGCTACGCTCGGCTAGGTCTAGCTACTGAAATTTGTGGTGAAATC
B-4bp-7_8SEd_4: ACCAGCAGCTCTGCTACGTCGTACTACCT

B-4bp-9REd_1: CATTCAAGCTGATACAGGTACTGCTATGCTTACCTAGCCTCTG
B-4bp-9_10REd_3: ATAGGCATAAGCTGCTGCTATGCTGAAGTCTAGGACGCG
B-4bp-10REd_5: TCCAGCATCCTGGAGCGAGTGAGACTGGACAATTGCCCTGACGAGA
B-4bp-9_10REd_4: CAGACGAGGTGCATCGTGGAAATGC

B-4bp-11SEd_1: GAATACCACATTCAACAGGTAGAGATCAGGCACTCGACACTGC
B-4bp-11_12SEd_3: GATCTCCTCAGCTGAACGGAAGTGCGACGCTGACTGAGCG
B-4bp-12SEd_5: AGCGGACTGACAGGCCCTCGACTCGCTGATGGAATGCAGATACATAA
B-4bp-11_12SEd_4: ACCAGCAGTGTCAGTGCTCAGCTACCT
Supplementary Note S6: Labeling seeds and caps with fluorophores

To label seeds with fluorescent dyes for visualization via fluorescence microscopy, we included 100 attachment strands as part of each assembled seed or cap. One domain of each adapter strand bound to a specific respective section of the M13mp18 scaffold that was not folded by staples for any of the seeds or caps. The other domain of each attachment strand was bound to a labeling strand with an Atto fluorophore dye on one of its ends. The labeling scheme was changed by varying which labeling strand was included during seed or cap annealing. Unless otherwise noted, RT A and C seeds were labeled with Atto 647N dye and RT B seeds or caps were labeled with Atto 488 dye. D caps were labeled with a dye mixture containing equimolar concentrations of Atto 647N and Atto 488 dye strands (see Supp. Note S8).

**Dye labeling strand sequences**

| Labeling_strand_ATTO647N_seed   | /5ATTO647NN/AACCGTAGTCGAATCTC |
| Labeling_strand_ATTO488_cap    | /5ATTO488N/AACCGTAGTCGAATCTC |

**Dye attachment strand sequences**

| Unused_m13mp18_01 | AAATTCTTACCAGTATATAAGCCAAACTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_02 | GCCTGTATAGTGATATGCTGATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_03 | ACACCGGAATATCATATAGCTGATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_04 | GATAAAATAAGCGTTAATAAGAAATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_05 | TTTAATGTTTTGAAATACCGACGTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_06 | TTAGTTATATATATCTTCTTCTGACCTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_07 | ACGCGAGAAAACCTTTTCAAATATATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_08 | GATGCAAAATCTCACGAAAGACAAATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_09 | TGGGTATATATACTATATGTAATATTTTTGAGATCCGACTACGC |
| Unused_m13mp18_10 | ACTACCTTTTTAATCTCCGCTTGTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_11 | AATTTATCAAAATCTAGGCTTAGTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_12 | TTAAGACGCTGAGAAGAGATCTAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_13 | TCTCTGAAAAACATAGCGTAGCTTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_14 | TCGCTAAATCAATAATTTCTCTAGTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_15 | AGTTGAATACCTTCTCCTGAAATTTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_16 | GAAAACAGTACATAAAATCAATATAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_17 | ATTTTACCTTTGAAATTTTTTTTTTTTTTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_18 | AGAAAAAACAAATATACCTATTTTTTTTTTTTTTTTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_19 | CAAAAGGAGATGAGAAACAAACACATTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_20 | GCACGCTAAATTTTACATATTTATCTGTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_21 | AATACCATAGTAAATACGAGTAAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_22 | CAATACCGGTAGTGAGAAGAGTCTAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_23 | TAAACGCTACCCTTTTACATCGGAGATTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_24 | CAGGTAAAAAGCTAGTAGTAAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_25 | CAGAATAAAATTTGCGTGAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_26 | CCAATTCTAATAATTTGCAGTTAAATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_27 | TCTGTAAATAATGAGAAGGGTTAAGATACCTTTTTTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_28 | TATAATCCGATTTTGGATTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_29 | GATTTACGAGATTGAGGAAATCTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_30 | AGAGACGGAATATACATATATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_31 | CATTCTTTCCGACACAAATTTTTTTTGTGAGATCCGACTACGC |
| Unused_m13mp18_32 | TAATTTTAATAATGTTTAGAATACATTTTTTTTATTTTTTTGAGATCCGACTACGC |
| Unused_m13mp18_33 | GTATTTAATCTTTTTCGAAAGACTTTTTTTTTTTTTTATTTTTTTTTGAGATCCGACTACGC |
Unused_m13mp18_90  CTCTTCGCTATTACCCAGCCTGCGTTTTGAGATCCGACTACGC
Unused_m13mp18_91  CTGTTGGAAGGGCGATCGGTGCGGTTTTGAGATCCGACTACGC
Unused_m13mp18_92  GCGCCATTGCGCCATTCCAGCTCGCTTTTGAGATCCGACTACGC
Unused_m13mp18_93  CGCTTCCTGTGCCGCCAAACCAGGCAATTGAGATCCGACTACGC
Unused_m13mp18_94  ATCGCACTCCAGCCAGCTTTCCGGCTTTTGAGATCCGACTACGC
Unused_m13mp18_95  GACGACGACAGTATCGGCCTCAGGATTTTGAGATCCGACTACGC
Unused_m13mp18_96  GTAACCGTGACTCTGCCAGTTTGAGTTTTGAGATCCGACTACGC
Unused_m13mp18_97  GGTACCGTTGCTGATCGGCGCATTTTGAGATCCGACTACGC
Unused_m13mp18_98  AAACGGCGGATAGCGGCTAATTGGGATTTTGAGATCCGACTACGC
Unused_m13mp18_99  ACAACCCGTCGGATTCTCCGTGGGATTTTGAGATCCGACTACGC
Unused_m13mp18_100 TTCATCAACATTAATGAGCGAGTTTTGAGATCCGACTACGC
Supplementary Figure S7: Map of staples positions of 8 different caps. Map of staples positions of 8 different DNA origami structures that were used as caps to terminate the RT A seeded nanotubes’ growth. Graphical images showing how each of these adapter sets bind on the scaffold are given in Supp. Fig. S18. Structure 1 is the rigid cap (RT B seed) that has all the staple strands while structure 8 is just the M13mp18 scaffold with no staple strands or adapters. Structure 7 is referred to as a flexible cap as it has only adapters but no staple strands. Structures 2 and 6 have only B1, B2 and B3 SEd adapters while the rest of structures have all 6 adapters.
Supplementary Note S7: Adapters for flexible D caps

Flexible D caps have the same DNA origami structure as the flexible B caps do (see Supp. Fig. S7) but use a different set of adapters so that flexible D caps sticky ends bind the sticky ends of UV tiles.

Supplementary Figure S8: Structure of the assembled adapters for D caps. The gray lines and associated sequences are portions of the M13mp18 scaffold.
RT D seed adapter strand sequences:

AD1UEd_1: AGG GAT AGC AAG CCC ATA GAG CGT CAC AGC TTC GTC CAT CGC ACT C
AD1_2UEd_3: CTG TGA CGC TCT AGC TCT CAT GCT GGA CAG GCT AGG ACG AAG
AD2UEd_5: TGG TAC TGG TAG CCT GTC CAG CAT GAG AGC ATA GGA ACC CAT GTA C
AD1_2UEd_4: TCA GGA GTG CGA TCC AGT ACC ACT TG
AD3UEd_1: GAA TTG CGA ATA ATA AGA CAC TGC CAT CTG CGG TCT GTC CAA GGC G
AD3_4VEd_3: AGA TGG CAG TGT CCG AGA GTG ATG CTG ATC GGT GCA GAC CGC
AD4UEd_5: CCA GCC AAC CAC CGA TCA GCA TCA TCG TTT TTT CAC GTT GAA A
AD3_4VEd_4: GAG ACG CCT TGG AGT TGG CTG GTA CG
AD5UEd_1: ACC CTC AGC AGC GAA ACC AGT CTC ACC TAC CAC T
AD5_6UEd_3: TAG GTG AGA CTG GAG GTA CGA GTC CTA CGA TGT CGC TAG TGG
AD6UEd_5: TGC TTC ACG GAC ATC GTA GGA CTC GTA CCT GAC AGC ATC GGA ACG A
AD5_6UEd_4: TCA GTG TGG ATG ACG TGA AGC ACT TG
AD7UEd_1: TGT ATC ATC GCC TGA TGG AGA CGG CTT CAG CAC GAG TGC AGT GGC A
AD7_8VEd_3: TGA AGC CGT CTC CGC TCG ATT GCC GAT ACC GAC AAC TCG TGC
AD8UEd_5: CCT CAG TCC TGT CGG ATG ACG TGA AGC ACT TG
AD7_8VEd_4: GAG ATG CCA CTG CGG ACT GAG GTA CG
AD9UEd_1: CAT TCA GTG AAT AAG GTG GCT AGA CGC AGA CGA CAG CTC ACG CCA A
AD9_10UEd_3: CTG CGT CTA GCC AGT CCA CAG GTT GTG CCA TAC CGC TGT CGT
AD10UEd_5: CTA CTC GCA GGT ATG GCA CAA CCT GTG GAC CTT GCC CTG ACG AGA A
AD9_10UEd_4: TCA GTT GGC GTG ATG CGA GTA GCT TG
AD11VEd_1: GAA TAC CAC ATT CAA CGT GAG TCG AGG ATA GCC AAT CCG CAA GGC A
AD11_12VEd_3: ATC CTC GAC TCA CCG AAG CGT GAC TTC CAC CAA CGA TTG GCT
AD12VEd_5: GGT ACG GCT GTT GGT GGA AGT CAC GCT TCG TAA TGC AGA TAC ATA A
AD11_12VEd_4: GAG ATG CCT TGC GAG CCG TAC CTA CG
Supplementary Note S8: Stock preparations

Seed and cap staple mixes: Staple mixtures for all seeds and caps contained each of the staples at 4.17 μM suspended in water, except in experiments with glass-bottomed dishes, where the staple mixture contained the staples from [2] at 1388.89 nM each suspended in water.

Adapter strand mixes: Each adapter strand mix contained the respective REd and SEd adapter strands (Supp. Notes S3 and S5 respectively) that do not present sticky ends at 1 μM. The remaining adapters strands that have sticky ends were added at 2 μM (Supp. Notes S3 and S5). A similar protocol was followed to prepare the UEd and VEd adapter strand mix (Supp. Notes S4 and S7).

Fluorescent labeling attachment strands mix: The attachment strand mix contained 1 μM of each of the 100 labeling attachment strands (Supp. Note S6).

Fluorescent labeling strands mix: 100 μM stocks of Labeling_strand_ATTO647N_seed and Labeling_strand_ATTO488_cap were used to label respectively RT A or C seeds and RT B seeds or caps. The labeling strand mix for cap D contained 50 μM of each of the labeling strand (Labeling_strand_ATTO647N_seed, Labeling_strand_ATTO488_cap) suspended in water.

RT RS nanotube tiles (Cy3 labeled): The RS tile mixture contained each strand that does not present a sticky end at 600 nM and strands with sticky ends [RE-4bp-2EE01, RE-4bp-4, SE-4bp-2DIAG, SE-4bp-4DIAG] at 1200 nM.

RT UV nanotube tiles (25% Cy3 labeled): The UV tile mixture contained each 1 and 5 strands [UE1_4bp-1, UE5_4bp-1, VE1_4bp-1, VE5_4bp-1] at 400 nM and strands 2 and 4 with sticky ends [UE2DIAG-4bp-1, UE4DIAG-4bp-1, VE2DIAG-4bp-1, VE4DIAG-4bp-1] at 800 nM. To distinguish UV tiles from RS tiles, UV tiles were prepared with 100 nM each of Cy3 labeled UE3-Cy3_4bp-1 and VE3-Cy3_4bp-1 strands 300 nM each of UE3_4bp-1 and VE3_4bp-1 strands without a Cy3 label.

Biotin attachment linker strands mix: The linker strand mixture for A seeds contained each of the linker strands [Biotin_positive_01 - Biotin_positive_06] listed in Supp. Note S3 at 100 nM suspended in water.
Supplementary Note S9: Protocol for DNA origami preparation and purification

1. We made 50 μL mixtures (Supp. Table S1) containing M13mp18 scaffold, staple strands, adapter strands, fluorescent strand and attachment strands in 1 × TAE Mg²⁺ buffer (40 mM Tris-Acetate, 1 mM EDTA and 12.5 mM magnesium acetate). BSA biotin was included to reduce DNA absorption to PCR tubes [3].

Supplementary Table S1: Seed/Cap assembly mixture.

<table>
<thead>
<tr>
<th>Seed/Cap Assembly Mixture</th>
<th>Final desired concentration (nM or fold)</th>
<th>Stock concentration (nM or fold)</th>
<th>To add (ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>-</td>
<td>-</td>
<td>25.25</td>
</tr>
<tr>
<td>BSA Biotin solution</td>
<td>0.05 mg/ml</td>
<td>1 mg/ml</td>
<td>25.25</td>
</tr>
<tr>
<td>Staple strands mix</td>
<td>500 nM</td>
<td>4166.67 nM</td>
<td>6</td>
</tr>
<tr>
<td>Adapter strands mix</td>
<td>100 nM</td>
<td>1000 nM</td>
<td>5</td>
</tr>
<tr>
<td>M13mp18 scaffold</td>
<td>5 nM</td>
<td>100 nM</td>
<td>2.5</td>
</tr>
<tr>
<td>Fluorescent labeling attachment strands mix</td>
<td>25 nM</td>
<td>1000 nM</td>
<td>1.25</td>
</tr>
<tr>
<td>Fluorescent labeling strands mix</td>
<td>5000 nM</td>
<td>100000 nM</td>
<td>2.5</td>
</tr>
<tr>
<td>10 × TAE Mg++</td>
<td>1×</td>
<td>10×</td>
<td>5</td>
</tr>
<tr>
<td>Total volume</td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

The set of staple strands, adapter strands and dye strands included for RT A and C seeds and B and D caps were chosen as described in Supp. Notes S3-S8.

2. The seed assembly mixture was annealed using the following protocol:

Supplementary Table S2: Annealing protocol.

<table>
<thead>
<tr>
<th>Temperature range</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 °C</td>
<td>Hold for 5 min</td>
</tr>
<tr>
<td>90-45 °C</td>
<td>0.1 /6 sec</td>
</tr>
<tr>
<td>45 °C</td>
<td>Hold for 60 min</td>
</tr>
<tr>
<td>45-20 °C</td>
<td>0.01 /6 sec</td>
</tr>
<tr>
<td>20 °C</td>
<td>Hold until sample retrieval</td>
</tr>
</tbody>
</table>

3. Seeds were separated from excess adapter, staple, attachment and labeling strands by following the protocol described in the Methods section of the main paper; the concentration of seeds in the resulting solution was determined according to Supp. Note S10.
Supplementary Note S10: Protocol for determining the concentration of the purified seeds

Because of some expected loss of seeds or caps during purification, we measure the seed concentration in stock solutions after purification using the following procedure:

1. 6 µL of stock solution was pipetted onto an 18 mm × 18 mm cover slip which was then inverted onto a microscope slide. 4 micrographs of seeds at randomly selected locations on the slide were collected following the procedures outlined in the Methods section. The average number of seeds per 87 µm × 87 µm field of view as determined through manual counting.

2. We convert the number of seeds (or caps) per field of view into a concentration using a conversion formula based on the expected number of seeds observed at different concentrations. For 6 µL of solution deposited on an 18 mm × 18 mm cover slip, random deposition of seeds should result in an average number of seeds per image (N) per 87 µm × 87 µm field of view of

\[
N = [X] \times 10^{12} \times 6.022 \times 10^{23} \times 6 \times 10^{-6} \times (0.087/18)^{1/2}
\]

\[
N = 42 [X] \text{ (per field of view)}
\]

assuming that [X] is the concentration of the seeds (or caps) in pM. The final factor of 0.5 reflects the assumption that half the seeds adsorb to the cover slip, where seeds are imaged and half adsorb to the glass slide, where they are not imaged.

We tested this calibration procedure using unpurified seed stocks of known concentrations of 1, 3, 6, 12, 24 pM that were prepared according to Supp. Note S9. The measured and actual seed concentrations agreed closely (Supp. Fig. S9).

Supplementary Figure S9: The number of seeds observed in fluorescence micrographs as a function of concentration agree with the number expected according to Equation 1.
Supplementary Note S11: Protocol for growing seeded nanotubes

1. Solutions of seeded nanotubes were prepared by first mixing 15.23 µL of purified water, 1 µL of 1 mg/ml BSA Biotin, 1.5 µL of RT RS nanotube tile solution (Supp. Note S8) and 1.97 µL 10 × TAE Mg²⁺ buffer to a final volume of 19.7 µL per tile solution.

2. Purified seeds were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. The concentration of the seed solution was adjusted by adding 1 × TAE Mg²⁺ so that the final seed concentration would be the reported concentration after adding 0.3 µL of the solution to the 19.7 µL of tile solution.

3. The tile solutions from step 1 were annealed from 90 °C to 20 °C using the protocol in Supp. Table S2. When the solution reached 20 °C, 0.3 µL of seed solution (to a final concentration of 2 pM) was added to 19.7 µL of the tile solution so that the tile concentration was 45 nM after addition of seeds, after which the mixtures were incubated for the time interval(s) described in the main text. Control experiments without seeds followed the above instructions except that 0.3 µL of 1 × TAE Mg²⁺ buffer was added in place of the seed solution.

4. To reduce imaging background due to single tile adsorption to glass, 0.3 µL of 1 µM of D01 (sequence below) was added before imaging. This strand adsorbed more effectively than poly-T sequences but did not interact with the tiles or adapter strands.

D01 strand: ATCAGAGAGTCAGAGGCGAGGTCGGTTTTCGACCGTGTTGTAATTGAACCAGTCA
Supplementary Note S12: Protocol for growing RT A seeded, B capped nanotubes

1. Solutions of seeded nanotubes were prepared by first mixing 14.96 µL of purified water, 1 µL of 1 mg/ml BSA Biotin, 1.5 µL of RT RS nanotube tile solution (Supp. Note S8) and 1.94 µL 10 × TAE Mg\(^{2+}\) buffer to a final volume of 19.4 µL per tile solution.

2. Purified A seeds were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. The concentration of the seed solution was adjusted by adding 1 × TAE Mg\(^{2+}\) buffer so that the final seed concentration would be the reported concentration after adding 0.3 µL of the solution to the tile solution after the addition of seeds and caps.

3. Purified B caps were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. The concentration of the cap solution was adjusted by adding 1 × TAE Mg\(^{2+}\) buffer so that the final seed concentration would be the reported concentration after adding 0.3 µL of the solution to the tile solution after the addition of seeds and caps.

4. The tile solutions from step 1 were annealed from 90 °C to 20 °C using the protocol in Supp. Table S2. When the solution reached 20 °C, 0.3 µL of seed solution (to a final concentration of 2 pM) was added to 19.4 µL tile solution, after which the mixture was incubated. After either 4 or 8 hours, 0.3 µL of cap solution (to a final concentration of 10 pM) was added to the mixture so that the tile concentration was 45 nM after addition of seeds and caps. Control experiments without caps followed the above instructions except that 0.3 µL of 1 × TAE Mg\(^{2+}\) buffer was added in place of the cap solution. For AFM experiments, 0.3 µL of seed solution (to a final concentration of 10 pM) was added to 19.4 µL tile solution and incubated for 2 hours and then 0.3 µL of cap solution (to a final concentration of 20 pM) was added to the mixture so that the tile concentration was 45 nM after addition of seeds and caps.

5. Slides were prepared as described in Supp. Note S11.
Supplementary Note S13: Protocol for dish glass surface treatment so that RT A seeds with linker strands can bind to the surface

The goal of this protocol is to enable seeds to bind on a monolayer formed on a glass surface via a specific biotin-neutravidin linker chemistry. Fixing the seeds to the surface allows individual nanotubes to be tracked during growth and capping processes. Briefly, a glass-bottomed dish with a biotin-PEG-silane monolayer and RT A seeds presented biotin are attached through biotin streptavidin chemistry. The protocol largely follows the methods in [4].

1. Glass-bottom dishes were cleaned by sonication in 10% NaOH for 40 minutes.

2. Glass-bottom dishes were then washed with water followed by methanol wash to remove residual NaOH.

3. 10 mg of biotin PEG silane MW 3400 was dissolved in 1000 µL of a solution containing 95% methanol, 4% acetic acid, and 1% water. This solution is then transferred onto glass-bottom dishes.

4. Dishes were sealed with Parafilm in an enclosed space overnight to prevent evaporation.

5. Dishes were washed with methanol followed by water to remove residual methanol.

6. The Parafilm was then removed from dishes and dishes were washed 3 times with methanol then 2 to 3 times with water. Water was then blown off the glass surface using nitrogen gas.

7. Dishes were then placed in oven at 90 °C for 1 hour.

8. 1000 µl of 1% BSA solution in TNT buffer (10 mM Tris-HCl, 0.05% Tween-20, 0.1 M NaCl, pH 7.5) was added to dishes and incubated for 1.5 hours.

9. Dishes were then washed with TNT buffer to remove excess 1% BSA solution.

10. 1000 µl of .2 mg/mL Neutravidin (31000, Thermo Fisher Scientific) in TNT buffer or 0.2 mg/mL Streptavidin (21122, Thermo Fisher Scientific) was added to dishes and incubated for approximately two hours.

11. Dishes were washed 3 times with TNT buffer followed by 3 washes with 1 × TAE Mg²⁺ buffer. 1000 µl of 1 × TAE Mg²⁺ buffer was left in each dish until use.
Supplementary Note S14: Protocol for tracking the growth of specific RT A Seeded nanotubes

1. To measure the seeded nanotubes growth rate, we prepared dishes (µ-Dish 35mm, high Grid-50 Glass Bottom, Ibidi) using the dish preparation protocol (see Supp. Note S13) a day in advance.

2. To ensure that the facet on the seed is not too close to the surface, seeds were folded using the staples from [2] with the adapters in Supp. Fig. S3. No difference in nucleation rates has been observed between seeds using this set of staples and the seeds with the staples used here [6]. To assemble the seeds with biotin attachment and linker strands, we made a 50 µL mixture (Supp. Table S3) containing M13mp18 scaffold, staple strands, adapter strands, dye strand and dye attachment strands, biotin attachment and attachment linker strands in 1 × TAE Mg²⁺ buffer. The seed assembly mixture was annealed using the protocol mentioned in Supp. Table S2.

Supplementary Table S3: Assembly mixture of RT A seeds for dish experiments.

<table>
<thead>
<tr>
<th>RT A Seed with linker strand Assembly Mixture</th>
<th>Final desired concentration (nM or fold)</th>
<th>Stock (nM or fold)</th>
<th>To add (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>2.95</td>
</tr>
<tr>
<td>RT A Seed staples mix for long seed</td>
<td>500</td>
<td>1388.89</td>
<td>18</td>
</tr>
<tr>
<td>RT A Seed adapter strand mix</td>
<td>100</td>
<td>1,000</td>
<td>5</td>
</tr>
<tr>
<td>M13mp18 scaffold strand</td>
<td>5</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>Fluorescent labeling attachment strands mix</td>
<td>25</td>
<td>1000</td>
<td>1.25</td>
</tr>
<tr>
<td>Fluorescent labeling strands mix</td>
<td>5000</td>
<td>100000</td>
<td>2.5</td>
</tr>
<tr>
<td>Biotin attachment strand mix</td>
<td>90</td>
<td>850</td>
<td>5.3</td>
</tr>
<tr>
<td>Biotin attachment linker strands mix</td>
<td>15</td>
<td>100</td>
<td>7.5</td>
</tr>
<tr>
<td>10 × TAE Mg²⁺ buffer</td>
<td>1</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

3. Purified seeds were prepared and their concentrations were measured as described in Supp. Note S10. The concentration of the seed solution was adjusted through the addition of 1 × TAE Mg²⁺ to achieve a seed density of about 100 seeds per field of view after the addition of 10 µL of the purified RT A seeds mixture into 1000 µL of the 1 × TAE Mg²⁺ solution contained in the dish. We allowed 15 minutes for seeds to attach on the dish glass surface after which time the glass surface was washed three times with 1 × TAE Mg²⁺ to remove unattached seeds.

4. After the seeds were attached to the glass surface, 1000 µL of 1 × TAE Mg²⁺ buffer was placed into the dish, and four different locations were identified using the markers embedded on the glass surface of the dish such as landmarks around which to track individual nanotubes. These locations were selected randomly. Once the locations were set, 1000 µL of 1 × TAE Mg²⁺ buffer
was replaced with 1000 µL of 75 nM annealed RT RS tiles mixture that has 1 × TAE Mg²⁺. Then the dish was sealed with Parafilm to prevent solution evaporation. In the dish experiments, we used a higher RS tile concentration (75 nM) compared to the experiments that were performed in the Eppendorf tubes (45 nM). This may be because of the absence of BSA in dish experiments. BSA prevents seeds and probably thus tiles from adhering to the walls of Eppendorf tubes [3]; more surface adsorption of tiles may have occurred in dishes.

5. Images were captured at four time points- 0, 6, 12 and 24 hours. At 6 hours and all the follow-up time points, 1000 µL of the tile mixture, which was added at the earlier time points, was removed from the dish and stored in the Eppendorf tube at 20 ºC. This mixture was then transferred back into the dish after the image acquisition. After removing the tile mixture from the dish, three washing steps were performed with 1000 µL of 1 × TAE Mg²⁺ to minimize fluorescence noise. The embedded markers in the dishes were used to locate the specified dish locations. 10 images of Cy3 filter and 10 images of Atto 647N filter were collected in immediate series for all 4 locations using a time-lapse acquisition software. After the image acquisition at 6 hours and all the follow-up time points, we transferred back 1000 µL of the tile mixture in the dish to allow nanotubes to continue to grow. After transfer, dish was then sealed with Parafilm to prevent solution evaporation.

6. Nanotube lengths were estimated via ImageJ JFilament (http://athena.physics.lehigh.edu/jfilament/) [5] as the length of the 2D projection of the DNA nanotubes. 10 images of the same nanotube at each time point (6, 12 and 24 hours) were collected and the longest length determined for each time point was considered to best representation of the length of the nanotube at that time.
Supplementary Note S15: Protocol for measuring the rate of caps binding to RT A seeded nanotubes

1. To measure the rate of caps binding to RT A seeded nanotubes, we prepared glass dish surfaces (see Supp. Note S13) a day in advance and purified RT A seed mixture following the methods for these steps given in Supp. Note S14. We then followed the protocol for growing RT A seeded nanotubes mentioned in Supp. Note S11 using the following RS tile mixture.

Supplementary Table S4: RS tile solution used for growing nanotubes in glass dishes.

<table>
<thead>
<tr>
<th></th>
<th>Final Desired Concentration (nM or fold)</th>
<th>Stock (nM or fold)</th>
<th>To add (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td></td>
<td></td>
<td>15.23</td>
</tr>
<tr>
<td>RT A Seeds</td>
<td>0.003</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>RS tiles</td>
<td>75</td>
<td>600</td>
<td>2.5</td>
</tr>
<tr>
<td>10× TAE Mg²⁺</td>
<td>1</td>
<td>10</td>
<td>1.97</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

2. After 6 hours of growth, 6 µL of the seeded nanotube mixture was transferred onto the dish in the presence of 1000 µL of 1×TAE Mg²⁺ buffer. We allowed 15 mins for the seeded nanotubes to attach to the dish glass surface by the biotin labels on the seed. After 15 minutes, the glass surface was washed three times with 1× TAE Mg²⁺ to remove unattached seeds. Examples of seed density produced by this attachment process are shown after 0 hours in Supp. Fig. S26.

3. After washing 1000 µL of 1× TAE Mg²⁺ solution was placed onto the dish for image acquisition. Four specific locations were chosen using the markers embedded on the glass surface of the dish as regions for tracking nanotubes over time.

4. After choosing the locations for tracking, the buffer was replaced by 985 µL of annealed RS tile mixture and 15 µL purified flexible B caps, made using Supp. Notes S9 and S10. The solutions were made so that the final concentration of tiles was 75 nM and the final concentration of the B was 24 pM. The dish was then sealed with Parafilm and kept at room temperature on a microscope stage. The presence of free tiles in solution kept the nanotubes from melting over the course of the experiment.

5. Fluorescence images of the four previous determined locations were collected every ten minutes for 4 hours and after that every 30 minutes for next 2 hours using time-lapse acquisition software. One image of the RT A seeds (Atto 647N), three images of B caps (Atto 488), and three images of the nanotubes (Cy3) were collected at each location at each time point. The high background fluorescence levels in the Cy3 channel caused by the presence of the free tiles meant that nanotube images were not used in analysis.
6. During the image acquisition process, caps entered and left the image; occasional appearance of the caps near a seed could appear to be a capping event. We thus noted that a capping event occurred when a cap appeared near a seed over multiple consecutive time points.
**Supplementary Note S16: Protocol for growing RT A and C seeded nanotubes in a single pot reaction**

1. Solutions of RT A and C seeded nanotubes were prepared by first mixing 13.73 µL of purified water, 1 µL of 1 mg/ml BSA Biotin, 1.5 µL of each RT RS and UV nanotube tiles (Supp. Note S8) and 1.97 µL 10 × TAE Mg\(^{2+}\) buffer to a final volume of 19.7 µL per tile solution.

2. Purified seeds were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. Purified A and C seeds were mixed together and the combined seed solution was adjusted through the addition of 1 × TAE Mg\(^{2+}\) so that the final concentration would be the 2 pM (each) after adding 0.3 µL of the solution containing A and C seeds to the 19.7 µL of tile solution.

3. The tile solutions from step 1 were annealed from 90 °C to 20 °C using the protocol in Supplementary Table S2. When the solution reached 20 °C, 0.3 µL of seed solution (to a final concentration of 2 pM) was added to 19.7 µL of the tile solution so that the tile concentration would be 45 nM of RS tiles and 30 nM of UV tiles after addition of seeds. After mixing, the mixtures were incubated for the time interval(s) described in the main text. Control experiments without seeds followed the above instructions except that 0.3 µL of 1 × TAE Mg\(^{2+}\) was added in place of the seed solution.

4. Slides were prepared as described in Supp. Note S11.
**Supplementary Note S17: Protocol for growing RT A seeded, B capped and RT C seeded, D capped nanotubes in a single pot reaction**

1. Solutions of seeded nanotubes were prepared by combining 13.46 µL of purified water, 1 µL of 1 mg/ml BSA Biotin, 1.5 µL of RT nanotube RS tile solution and 1.5 µL of RT nanotube UV tile solution (Supp. Note S8) and 1.94 µL 10× TAE Mg²⁺ buffer to a final volume of 19.4 µL per tile solution.

2. Purified A and C seeds were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. Purified A and C seeds at the same concentrations were mixed together in equal volume and the combined seed solution was adjusted by adding 1× TAE Mg²⁺ so that the final concentration of each seed would be as specified in the main text when 0.3 µL of the combined A and C seeds solution is added to the tile solution.

3. Purified flexible B and D caps were prepared and their concentrations were measured as described in Supp. Notes S9 and S10. Purified flexible B and D caps at the same concentrations so they are mixed together with equal volume and the combined seed solution was adjusted by adding of 1× TAE Mg²⁺ so that the final concentration of each seed would be as specified in the main text when 0.3 µL of the combined B and D cap solution is added to the tile solution.

4. The tile solutions from step 1 were annealed from 90 °C to 20 °C using the protocol in Supp. Table S2. When the solution reached 20 °C, 0.3 µL of the combined A and C seed solution (to a final concentration of 2 pM) was added to 19.4 µL tile solution, after which the mixture was incubated. After either 4 or 8 hours, 0.3 µL of the combined B and D cap solution (to a final concentration of 10 pM) was added to the mixture so that the tile concentrations were set at 45 nM of RS tiles and 30 nM of UV tiles after addition of seeds and caps. Control experiments without caps followed the above instructions except that 0.3 µL of 1× TAE Mg²⁺ was added in place of the cap solution. In this experiment with only one type of caps being added, 0.3 µL of the corresponding caps (B or D caps) was added as reported above.

5. Slides were prepared as described in Supp. Note S11.
Supplementary Figure S10: **RT A seeds control the nucleation of RS nanotubes under ambient conditions.** *(a-b)* Nanotubes grow readily from RS tiles at room temperature when 2 pM RT A seeds were present. *(c-d)* RS tile nanotubes grew only rarely in the absence of RT A seeds. Micrographs taken after tiles and seeds for a-b were incubated for 25 hours at 20 °C. Scale bars are 4 µm. Four 85 × 85 µm field of view images for each of the two conditions was used to determine that the number of nanotubes in the presence and absence of RT A seeds.

The density of nanotubes on slides was 9200 ± 1020 mm⁻² for samples grown in the presence of seeds and 1600 ± 200 mm⁻² for samples grown in the absence of seeds. The mean lengths of nanotubes grown were 3.4 ± 0.14 µm in the presence of seeds and 3 ± 0.23 µm in the absence of seeds. When nanotubes grew in the presence of a higher concentration of seeds (4 pM), the density of nanotubes on slides was 18900 ± 5278 mm⁻² and the mean length of nanotubes was 2.4 ± 0.03 µm. That is, at a higher seed concentration, we observed more but shorter nanotubes [6].
Supplementary Figure S11: **RT B seeds control the nucleation of RS nanotubes under ambient conditions.** Example fluorescence micrographs of (a-b) nanotube growth from RT B seeds and (c-d) without seeds over 25 hours at 20 °C. Scale bars are 4 μm. A set of 4 85 × 85 μm field of view images for each of the two conditions was used to determine that the number of nanotubes in the presence and absence of RT B seeds. The density of nanotubes on slides was 8700 ± 900 mm² for samples grown in the absence of seeds. The density of nanotubes grown in the presence of seeds was 1500 ± 300 mm². The mean length of nanotubes was 3.4 ± 0.12 μm for samples grown in the absence of seeds. The mean length of nanotubes grown in the presence of seeds was 2.3 ± 0.3 μm.
Supplementary Figure S12: The densities of seeded and unseeded nanotubes in the presence of RT A seeds at different RS tile concentrations. The images were taken after 25 hours of growth. At 45 nM tile concentration, we observed that over 87% nanotubes were seeded while unseeded nanotubes were only 13% and the nucleation yield of RT A seeds was 76% ± 3.38%.
Supplementary Figure S13: Example fluorescence micrographs showing RT A seeded nanotubes to which no caps were added (left) and to which RT B seeds were added after 4 and 8 hours (middle and right). Tiles were labeled with Cy3 (green), RT A seeds were labeled with Atto 647N (red) and RT B seeds were labeled with Atto 488 (blue). RT B seeds could both bind to the growing ends of nanotubes and nucleate new nanotubes. Scale bars are 4 μm.
Supplementary Figure S14: Average length (left) and density of nanotubes (right) with RT A seeds before and after rigid caps were added.
Supplementary Figure S15: **Growth of different types of RS nanotubes in absence and presence of rigid caps.** Mean lengths, total lengths and mean number of tubes per unit area found in aliquots on microscope slides of different types where (a) only seeds were added at the start of the growth process, (b) rigid caps were added after 4 hours and (c) rigid caps were added after 8 hours.
Supplementary Figure S16: The length distributions of seeded nanotubes at different time points when caps were added after 8 hours of growth.

Supplementary Figure S17: Capping and nucleation yields of the rigid caps as a function of cap concentration. (a) Capping yields are high across a range of concentrations of rigid caps (RT B seeds). (b) As cap concentration increased, the fraction of nanotubes in the population that were nucleated by caps increased significantly. In this experiment, the concentration of RT A seeds was 2 pM and images were captured after 25 hours of growth.
Supplementary Figure S18: **How caps act as RS nanotube nucleation sites in the presence of RT A seeds.**

To characterize how often caps act as nucleation sites, we used 2 pM of RT A seeds to grow nanotubes and then added 10 pM of each different cap in different aliquots after 8 hours of growth. These mixes were imaged after 25 hours of growth. Column 2 presents the percentage of caps that were added that nucleated new nanotubes, analogous to column 2 in Figure 3. Because RT A seeds nucleate nanotubes and deplete the concentration of tiles, a smaller percentage of caps of all types nucleate nanotubes in the presence of RT A seeds than when RT A seeds were not present, as in Fig. 3. Column 3 gives the percentage of nanotubes at the end of the reaction that appear to have been nucleated by capping structures, i.e. they have a cap at one end and no seed at the other. This percentage captures how many structures were “side effects” of the capping process. Ideally no new assemblies would arise because of the addition of caps so this percentage should be as close to zero as possible.

<table>
<thead>
<tr>
<th>Cap Type</th>
<th>% of caps that nucleated nanotubes</th>
<th>% of all nanotubes that are nucleated by caps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Rigid Cap)</td>
<td>12.8 ± 0.7</td>
<td>38.6 ± 3.4</td>
</tr>
<tr>
<td>2</td>
<td>1.0 ± 0.3</td>
<td>6.2 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>3.8 ± 0.04</td>
<td>15 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>1.2 ± 0.32</td>
<td>13.2 ± 3.4</td>
</tr>
<tr>
<td>5</td>
<td>1.9 ± 0.3</td>
<td>9.86 ± 1.7</td>
</tr>
<tr>
<td>6</td>
<td>0.4 ± 0.2</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>7 (Flexible Cap)</td>
<td>1.2 ± 0.3</td>
<td>5.4 ± 1.4</td>
</tr>
<tr>
<td>8 (Scaffold Only)</td>
<td>0 (n=156)</td>
<td>0</td>
</tr>
</tbody>
</table>
Supplementary Note S18: M13mp18 scaffold regions

The unused sequence of M13mp18 scaffold has several complimentary sites that can bind to sticky ends at the B interface of RS tiles. These sites are highlighted in green. The sections of M13mp18 scaffold that are highlighted in red are bound by staples for the short seed or cap and the sections that are highlighted in yellow are binding sites for attachment strands.
Supplementary Figure S19: Example fluorescence micrographs showing RT A seeded RS nanotubes to which no caps were added (left, repeated from Supp. Fig. S13 to provide a basis for comparison) and to which flexible caps were added after 4 and 8 hours (middle and right). Tiles were labeled with Cy3 (green), RT A seeds were labeled with Atto 647N (red) and flexible caps were labeled with Atto 488 (blue). Scale bars are 4 μm.
Supplementary Figure S20: **Flexible caps stop the growth of seeded nanotubes.** The number of seeded nanotubes (left) and average length (right) before and after flexible caps were added. The data for nanotubes with seeds but no caps is also shown in Supp. Fig. S14 and is replotted here for comparison.
Supplementary Figure S21: **Percentages of different types of nanotubes that assemble in reactions involving rigid and flexible caps.** (a) In a reaction involving only tiles and 2 pM seeds, most nanotubes were seeded. (b-c) After addition of 10 pM rigid caps followed by (b) 4 hours and (c) 8 hours, most seeded nanotubes become capped and excess caps nucleate new nanotubes, which form the majority of structures when caps were in excess. (d-e) After addition of 10 pM flexible caps followed by (b) 4 hours and (c) 8 hours, most seeded nanotubes become capped. Excess flexible caps nucleated relatively few nanotubes as compared with excess rigid caps.
Supplementary Figure S22: Growth of different types of RS nanotubes in absence and presence of flexible caps. Mean lengths and density of tubes of different types where (a) only seeds were added at the start of the growth process, (b) flexible caps were added after 4 hours and (c) flexible caps were added after 8 hours.
Supplementary Figure S23: Length distributions of seeded nanotubes when flexible caps were added after 8 hours of growth.
Supplementary Figure S24: Time-lapse multicolor fluorescence micrographs showing the growth of specific RT A seeded nanotubes over 24 hours. Individual nanotubes were grown while seeds were anchored to glass-bottomed dishes as described in Supp. Note S14. Scale bars are 2 µm.
Supplementary Figure S25: **Change in the length of individual RT A seeded nanotubes over time.** 50 individual nanotubes were tracked and their lengths are reported here in dashed-dot lines. The mean of the trajectories is shown in solid black line. Lengths were measured as described in Supp. Note S14. The observed growth rate, computed using the average trajectory is $0.165 \pm 0.0052$ μm/hr. The error reported for the growth rate was determined using the standard error of the mean of the growth rate for each time point.
Supplementary Figure S26: **Multicolor fluorescence micrographs of the same sets of nanotubes anchored to glass bottom dishes as the capping process progresses.** RT A seeds were labeled with Atto 647N (red) and flexible B caps were labeled with Atto 488 (blue). Scale bars are 10 µm. Caps both bound to seeds and nonspecifically attached to the surface.
Supplementary Figure S27: **Multicolor fluorescence micrographs showing two specific seeded nanotubes before and after the caps attached to their ends.** The nanotubes were anchored to a glass bottom dish by biotin labels on their seeds (see Supp. Note S15). The top and bottom sets of images show the same pair of nanotubes before (top) and after (bottom) caps were added. The top set of images were taken before tiles were added so there is significantly less background noise. RT A seeds were labeled with Atto 647N (red), RS nanotube were labeled with Cy3 (green) and the flexible B cap were labeled with Atto 488 (blue). RS nanotubes grew from RT A seeds for 8 hours and then flexible B caps were added and after 2 hours we found these nanotubes capped (see Supp. Note S15). Scale bars are 2 µm.
Supplementary Note S19: Measuring the rate of capping

To estimate the rate at which flexible B caps bind to RT A seeded nanotube free ends, we considered the capping reaction as a reaction of the form

\[ A + B \xrightarrow{k_j} AB \]  

(2)

Here, \( A \) is a seeded RT nanotube, \( B \) is a free flexible B cap and \( AB \) is a nanotube with an RT A seed and B caps. \( k_j \) is the reaction rate for the capping reaction. We assumed that this capping reaction is irreversible, as we did not observed caps falling off in our study of the capping of 300 nanotubes. Using mass action kinetics, we can model the kinetics of this reaction as

\[ \frac{d[AB]}{dt} = -k_j[A][B] \]  

(3)

where \( t \) is the reaction time and [ ] represents the molar concentration of the species. We define \( X \) as the fraction of RT seeded capped nanotubes out of all seeded nanotubes (\([AB]/[A]_{t=0}\)). If we assume that \([B]_{t=0} \gg [A]_{t=0}\), and \([AB]_{t=0}=0\) we can solve equation (1) in terms of \( X \):

\[ X = 1 - e^{-k_j t[B]_{t=0}} \]  

(4)

To determine the most likely value of \( k_j \), we used the least squares fitting of the measured data (Fig. 5). In experiments, we used 2 pM of RT A seeds to grow RS nanotubes, therefore the upper bound of \([A]_{t=0} = 2 \) pM while \([B]_{t=0} = 24 \) pM (see Supp. Note S15).
Supplementary Figure S28: **Example fluorescence micrographs showing different types of RS and UV nanotubes that assemble in reactions involving RT A and C seeds and flexible B and D caps.**

RS tile nanotubes (Cy3 bright green) grew from RT A seeds labeled with Atto 647N (red) and UV tile nanotubes (Cy3 dim green at 25% incorporation) grew from RT C seeds labeled with Atto 647N (red). Flexible cap B was labeled with Atto 488 (blue) and flexible cap D was labeled with 50% Atto 647N and 50% Atto 488 dyes (purple). Images were taken after 25 hours of growth. (a) Both RT A and C seeded nanotubes grew long when no caps were added. Here, RT C seeded UV nanotubes are highlighted using white markers. (b) Only RT A seeded nanotubes stop growing when B caps were added after 8 hours. (c) Only RT C seeded nanotubes stop growing in the presence of D caps. (d) Both RT A and C seeded nanotubes stop growing in presence of B and D caps. In cases (b-d), caps were added after 8 hours of growth.
Supplementary Figure S29: The mean lengths of different types of RS and UV nanotubes that assemble in reactions involving RT A, C seeds and flexible B and D caps. (a) The mean lengths of RT A and C seeded nanotubes when no caps are added. (b) The mean lengths of RT A or C seeded nanotubes when flexible B caps were added at 8 hours. (c) The mean lengths of RT A or C seeded nanotubes when flexible D caps were added at 8 hours. (d) The mean lengths of RT A and C seeded nanotubes when flexible B and D caps were added at 8 hours.
Supplementary Note S20: Estimate of the Energetics of Entropic Inhibition of Nucleation by Caps

To understand why flexible caps can cap nanotubes but do not tend to nucleate them, we estimated the rates of nucleation and capping of rigid and flexible caps using thermodynamic and kinetic parameters measured in this work and drawn from the literature. We considered the case where a cap binds to a flat facet of a growing nanotube such that all of the sticky ends bind on the adapters hybridize to sticky ends on the nanotube (Supp. Fig. S30).

![Model reaction between a nanotube facet and the flexible cap.](image)

Supplementary Figure S30: **Model reaction between a nanotube facet and the flexible cap.**

The binding process between each tile on the nanotube facet and the cap creates two hybridized sticky ends, an energetically favorable process. This energy gain is offset, however, by an entropic cost, because the loop of single-stranded DNA between two adapter tiles on the cap must take on a specific configuration for both sticky ends to hybridize. Supplementary Figure S31 shows one example of how such closure could occur during the binding of a single tile. A similar process could also allow the cap to bind to a single tile at a nanotube facet. Other types of loop closure would occur if a tile bound the end on non-adjacent adapter tiles, which would have similar or greater entropic cost.

![Schematic of loop closure of the flexible cap required for a tile to bind to the flexible cap by two sticky ends.](image)

Supplementary Figure S31: **Schematic of loop closure of the flexible cap required for a tile to bind to the flexible cap by two sticky ends.**

To estimate the energy gained through the binding of a tile on the nanotube facet to a flexible cap, we computed the net free energy change as the difference in the free energy gained through the hybridization of the sticky ends and the entropy (and thus free energy) loss that occurs because of the loop’s closure.

We estimated the free energy gained due to the attachment of one sticky end on a nanotube end to one end on the cap using the nearest neighbor model. We consider here just the sticky ends on R and S tiles and their corresponding binding sites on B caps. The sticky end sequences on these tiles are shown in Supp. Fig. S32.
We followed methods used to calculate sticky energies developed in [7] and calculated hybridization energies using NuPack [8] at 20 °C, 0.1 M NaCl and 12.5 mM Mg^{2+}. This approach includes the flanking bases in the calculation of sticky end free energies. At 20 °C, this model predicts a free energy change of -6.8 kcal/mol for three of the sticky ends and -5.9 kcal/mol for the remaining one.

To calculate the entropy loss incurred by orienting the sticky ends from two adjacent adapters to both bind to a single tile, we use a method for calculating the entropy cost of forming single-stranded DNA loops developed in [9]. This approach estimates this energy as

\[ \Delta S_{loop} \approx -R(3\nu - \sigma_4) \ln l \]  

where \( l \) is the number of base pairs in the loop, \( \nu \) is the Flory exponent, \( \sigma_4 \) is a topological exponent associated with the vertex with four outgoing legs and \( R \) is the universal gas constant [9]. In practice the ends of the loop are 2-3 nm apart and electrostatics due the adapter complexes probably play a role, but such an approach should provide a reasonable and simple estimate. Therefore, in this calculation \( l=192 \) bps, the length of the single stranded region from the one end of the adapter strand on the M13 scaffold to other end. Other constants values are used as within [9]: \( \nu=0.588, \sigma_4=-0.48 \) and \( R= 1.98 \text{ cal mol}^{-1}\text{K}^{-1} \). Using this approach, the predicted entropic cost of a loop formation is \( \Delta S^\circ_{loop}=-23.36 \text{ cal mol}^{-1}\text{K}^{-1} \), or \( \Delta G^\circ_{loop} = -6.84 \text{ kcal/mol at 20°C} \).

The net free energy for the attachment of a single tile by both sticky ends, whether within a tube or free, is thus the sum of the two sticky end energies minus the free energy loss due to the loop entropy cost. For the two pairs of energies, these net free energy changes are -6.2 kcal/mol or -7.1 kcal/mol for the two tile types. We can similarly calculate the net free energy change of binding all the tiles on a nanotube facet to all the adapter tiles on a flexible cap. A diagram of the binding reaction is shown in Supp. Fig. S33. This free energy change is the difference between the free energy gained by the formation of 12 sticky ends bonds (3 of each of the types shown) and the free energy lost due to 5 loop closures, or -46.7 kcal/mol. Thus, capping should be an irreversible process, even when considering the entropic loss due to the required loop closures to form the bond.

Supplementary Figure S32: Sticky end pairs on the RS tiles shown with several flanking bases.
To understand why caps do not nucleate nanotubes nearly as well as rigid caps, we next estimated the relative rates of nucleation by rigid and flexible caps using a very simple model of nucleation that should hopefully provide a conservative estimate of nucleation rates of both structures.

Specifically, a lower bound for the nucleation rate might be the rate at which two adjacent tiles attach to a nucleation site and a single tile then attaches to these tiles, forming a 3-tile complex with a seed or cap. We compared nucleation rates for the two structures by estimating the rates of formation of such a complex on a seed or flexible cap.

Estimating these rates requires estimating or measuring the on and off rates of tiles at the seed interface, the flexible cap interface and the on and off rates of a tile from two tiles attached to each of these interfaces. We assumed that for all these cases, the forward rate of tile attachment was the same and was $5.99 \times 10^5$ kcal/mol, which was measured between 30-37°C in Hariadi et al. for DAO-O 6 base pair tiles [10]. Because this rate was used for all types of tile binding, using a different value would not qualitatively affect our estimates of the relative rates of nucleation on the two interface types.

For simplicity, we assumed that the on and off rates of tiles from the two tiles attached to either a seed or cap were the same as the on or off rate of a tile from a fully formed nanotube. In our measurements of nanotube growth (Supp. Fig. S25), we found that the growth rate of nanotubes at 75 nM free tiles of $0.165 \pm 0.005$ μm/hr. We used stochastic kinetics simulations of nanotube growth developed in [2] to find the off rate of tiles that would produce this net growth rate. Free energies of -9.50 to -9.55 kcal/mol produced growth rates of 0.13 to 0.23 μm/hr in simulation.

To estimate the off rates of tiles from the seed interface, we began with the observation from [2] that tile binding to a seed incurred a free energy penalty, perhaps because of effects such as lattice mismatch between the DAE-E lattice and the origami structure. The result was that tile binding to seeds was mildly, but not strongly disfavored. We assuming the energy penalty for nucleation measured in [2] was the same in our study. We used the estimated binding energy of tiles to caps calculated above as the energy change when tiles bound to flexible caps. Using these two energies we estimated the net off rates for tiles from seeds and flexible caps.

To compare the net rates at which three tile complexes would form, we assumed 45 nM of tiles, which were used in both of our experiments and created a simple stochastic kinetic simulation where three-tile
complexes could form on seeds or caps through all possible combinations of single tile binding and unbinding to a pair of attachment sites. These simulations predicted that three-tile complexes would form on seeds under these conditions at a rate of $3 \times 10^{-5} \text{s}^{-1}$ to $9 \times 10^{-5} \text{s}^{-1}$ or 1 per about 3-9 hours, which is consistent with rates observed. In contrast, these simulations predicted that the same complexes would form on flexible caps at $5 \times 10^{-7} \text{s}^{-1}$ or 1 per 23 days, assuming the energies of attachment of the two tile types calculated above. If the true critical nucleus size is larger, we would expect this simple analysis to underestimate the difference between the nucleation rates on the two structures.
Supplementary Movie S1: Time-lapse movie of nanotube growth. Time-lapse movie showing the growth of an RT A seeded RS nanotube. The nanotube is anchored to a glass surface at the seed by a biotin-streptavidin linkage (see Supp. Note S14). The RT A seeds were labeled with Atto 647N (red) and the RS nanotube tiles were labeled with Cy3. The movie consists of 40 images, where each 10 images were recorded after 0, 6, 12 and 24 hours of growth. Each set of 10 images was taken in immediate series. Each image’s exposure time was 200 milliseconds. Scale bar is 2 µm.

Supplementary Movie S2: Time-lapse movie of nanotube capping. Time-lapse movie of a capping process where RS nanotubes grew from an RT A seed and flexible B caps were added to solution after 8 hours of growth (see Supp. Note S15). The RT A seeds were labeled with Atto 647N (red), the RS nanotube tiles were labeled with Cy3 (green) and the flexible B cap was labeled with Atto 488 (blue). The movie consists of 12 images, where each image was recorded at 10 min intervals for 2 hours once the caps were added. Each image’s exposure time was 200 milliseconds. Scale bar is 2 µm.

References:


