Self-Assembling DNA Nanotubes to Connect Molecular Landmarks

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1. DNA Nanotube and Seed Design with Sequences

All DNA strands used in this study were synthesized by Integrated DNA Technologies, Inc. (IDT) except the M13mp18 scaffold strand, which was purchased from Bayou Biolabs. The DNA nanotube tile and adapter strands were PAGE purified while Cy3, ATTO488 and ATTO647N fluorophore strands and biotin-modified strands were HPLC purified. Concentrations for DNA tile and adapter strands were determined by measuring absorbance at 260 nm; for other strands we relied on IDT to determine solution concentrations.

1.1. DNA nanotube design

The tile design and sequences in this study are adopted from Rothemund et al. Nanotubes contained two types of DNA tiles, termed REd(iagonal) and SEd(iagonal) arranged in a diagonal lattice (Fig. 1c).

In this study, 2-tile DNA nanotubes were used instead of 1-tile DNA nanotubes as we have found that nucleation yields of the 2-tile nanotubes used here are higher than for two types of single tile nanotubes we have nucleated with seeds (data not shown). It may also be the case that having 2 tile types restricts the orientation of nanotubes that are possible before joining, possibly decreasing the probability of introducing defects during joining.

Supplementary Fig. S1: Schematic showing the architecture of the REd and SEd tiles. Black triangles indicate crossover points. Cy3 fluorophores present on all the tiles allow for their visualization on the fluorescence microscope.
**REd and SEd nanotube tile sequences:**

- **RE-1:** CGTATTGGACATTTCCGTAGACCAGACCTGAGACATCTTC
- **RE-2EE01:** CTGGTCCTTCACCCACCGCATT
- **RE-3Cy3:** \(/Cy3/TCTACGGAAATGTGGCAGAATCAATCATAAGACACCAGTCGG\)
- **RE-4:** CAGACGAAGATGTGGTAGTGGAATGC
- **RE-5:** CCACTACCTGTCTTTATGATTGATTTCTGCCTGTGTAAGG

- **SE-1:** CTCAGTGGACAGCCGTTCTGGAGCGTGGACGAAACT
- **SE-2DIAG:** GTCTGGTAGAGCACCATGGAGAGGTA
- **SE-3Cy3:** \(/Cy3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCAACACGCT\)
- **SE-4DIAG:** ACCAGAGTTTCGTGGTCATCGTACCT
- **SE-5:** CGATGACCTGCTTCGGTTACTGTTTAGCTGCTCTAC

\(/Cy3/\) denotes Cy3 fluorophore covalently attached to the 5’ end of DNA.
1.2. Design of nanotube seeds

The DNA nanotube seeds used in this work are DNA origami nanostructures. Each nanotube seed consists of a long scaffold DNA strand (M13mp18, Bayou Biolabs) folded by 72 staple strands into a cylindrical shape. The end of the structure is modified with adapter tile strands to resemble a nanotube in cross-section. The adapter strands determine which nanotube end the seed resembles and presents the corresponding sticky ends. The two types of seeds (A and B) have different sets of adapter tiles, allowing each to nucleate a nanotube from one of two opposite nanotube ends.

The design for the central staples of the seed was adopted from Mohammed et al. Nanotube seed A has adapter strands that bind to sequences on one side of the seed while nanotube seed B has adapter strands that bind to the other side of the seed as shown in Supplementary Fig. S2. Mohammed et al. contains a complete list of staples sequences and origami design parameters.
Supplementary Fig. S2: Detailed staple map of origami seeds showing individual staples, locations of hairpins and adapter strand binding sites.
Adapter strand design:

The sequences for the adapter strands for nanotube seed A (Supplementary Fig. S3) are the same as in Mohammed et al.\(^4\) Nanotube seed B adapter strands (Supplementary Fig. S4) were designed to serve as a template for REdSEd nanotube growth starting from the opposite end as from nanotube seed A (Supplementary Fig. S2). Growth of nanotubes occurs via reversible DNA sticky end hybridization and can occur from both ends at similar rates\(^5\).

Supplementary Fig. S3: Structure of the assembled adapter tiles for Seed A. The gray lines and corresponding sequences are parts of the M13mp18 scaffold.
Supplementary Fig. S4: Structure of the assembled adapter tiles for seed B. The gray lines and corresponding sequences are parts of the M13mp18 scaffold.

Seed A adapter tile sequences

AD1REd_1: CAGCCAGAAGACGCAGCAGGAGACGGAGCTGAAAGTATTAAGAGG
AD1_2REd_3: TCGCTACCTGCGTTCGTCGGATGGTGAGGTCCACGCTCTGTC
AD1_2REd_5: CTATTATTCTGAAACAGTGGACCTCACCATCCGACGACACGAGCA
AD2REd_2: CTGGTTGCTCGTGCTTGGCTGGCATT
AD3SEd_1: CACGGAGTCGAAGCGTAGGACGGTAGCCAGTCAGACGATTGGCCT
AD3_4SEd_3: GTCCTACGCTTCGGACCTTGGTGATGCTGGACTGTGGCTACC
AD4SEd_5: CAGGAGGTTGAGGCAGCAGTCCAGCATCACCAAGGTCGCTCG GCA
AD3_4SEd_2: GTCTGTGCCGAGCACTCCGTGAGGTA
AD5REd_1: CAGAGCCACGGCATGGTGATCTGGCTGTCGACCATGAGGAGC

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Seed B adapter tile sequences

seedB_AD1REd_1: AGGGGATGCAAAGCCCACAACGTGAGGACACTTTGGAGGCTGCACTC
seedB_AD1_2REd_3: TGTGCCATCGCTCGGATCGGCTACGACACCTCCAAG
seedB_AD2REd_5: CAGCTGACTTGTCGTAGGATCGGCATCCAGATAGGAACCCATGTAC
seedB_AD1_2REd_4: CAGACGAGTGCAGAGTCAGCGAATGC
seedB_AD3SEd_1: GAATTGCGAATAATAAGTGACCTTGCTGTACCGTCGAGATGGAGT
seedB_AD3_4SEd_3: ACAGCAAGGTCACCGCAGTTGGCACTAGGCGACATCGACGGT
seedB_AD4SEd_5: CCACAACCTGTCGCCTAGTGCCAACTGCGTTTTTTCACGTTGAAA
seedB_AD3_4SEd_4: ACCAGACTCCATCGGTTGTTGCTACCT
seedB_AD5REd_1: ACCCTCAGCAGCGAAACGAGTACGGCAACACGGTGAGAGCCTACG
seedB_AD5_6REd_3: GTTGCCGTACTCGACTGGTCACGAACGTCTCCAACTCACCGT
seedB_AD6REd_5: GCTCTGCCTTGGAGACGTTCGTGACCAGTGACAGCATCGGAACGA
seedB_AD5_6REd_4: CAGACCGTAGGCTGGCAGAGCAATGC
seedB_AD7SEd_1: TGTATCATCGCCTGATCAACGGTACGAGATGCGAAGCACAGAGTG
seedB_AD7_8SEd_3: TCTCGTACCGTTGCCAGTAGACCTAGCCGACGTGGCTTCGCA
seedB_AD8SEd_5: GTCACGCTCACGTCGGCTAGGTCTACTGGAAATTGTGTCGAAATC
seedB_AD7_8SEd_4: ACCAGCACTCTGTAGCGTGACTACCT
seedB_AD9REd_1: CATTCAGTGAATAAGGACGCTATGCCTATCGCTCTAGGACCTCTG
seedB_AD9_10REd_3: ATAGGCATAGCGTTGCTCCAGTCTGCTGCTCAGGCTAGAGCG
seedB_AD10REd_5: CCACGACTCCTCGTACCGTGCAAGCGACTTGCGCTTCGCTGAGAAATC
seedB_AD9_10REd_4: CAGACCGATAGGCTGGCAGAGCAATGC
seedB_AD11SEd_1: GAATACCACATTCAACACCGATGAGGACACTTTGGAGGCTGCACTC
seedB_AD11_12SEd_3: GATCCTCATCGGTCAAGCGAAGGTGCGAGCCTGTAGTGCCGT
seedB_AD12SEd_5: GCGGACTGACAGGCTCGCACCTTCGCTTGTAATGCAGATACATAA
seedB_AD11_12SEd_4: ACCAGACTCTGTAGCGTGACTACCT
seedB_AD13SEd_1: CAGCGTCGCAAGGCTACGAGTCAGACAGGAACGTCAAAAATGAAA
seedB_AD13_12SEd_3: ACTCGTACGCTCTGAGACGTTCGTGACCAGTGACAGCATCGGAACGA
seedB_AD14SEd_5: AAACGATTTTTTTGGGTAGCGTTGAGGTGAGTCGGCTATCCGCTG
seedB_AD13_12SEd_2: GTCTGGCACGGATCGACGCGACATCGGTA
1.3. Fluorescent labelling of nanotube seeds

In experiments involving both A and B seeds, the A seed was labelled with ATTO647N and the B seed was labelled ATTO488 fluorophore dye. The labelling system for each consists of 100 attachment strands containing a subsequence that binds to the section of the M13mp18 scaffold that is not folded by staples. The reminder of the attachment strand binds to either an ATTO 647N or ATTO 488 labelling strand that has an ATTO647N or ATTO488 fluorophore dye respectively on the 5' end (Supplementary Fig. S5a). Supplementary Fig. S5b and Supplementary Fig. S5c show fluorescence images of labelled A seeds and B seeds respectively.

**Supplementary Fig. S5:** a, Schematic showing labelling of seed A with ATTO647N fluorophore dyes b, Fluorescence microscopy images showing (from left to right) A seeds (in ATTO 647N filter), nanotubes (in Cy3 filter) and composite. Scale bar 5 µm. c, Fluorescence microscopy images showing (from left to right) B seeds (in ATTO 488 filter), nanotubes (in Cy3 filter) and composite. Here and elsewhere images were prepared for publication by flat fielding of images to remove any uneven illumination and merging the respective individual filter images to make a composite multicolor image. Scale bar 5 µm.
Labeling strand sequences

Labeling_strand_ATTO488 /5ATTO488N/AAGCGTAGTCGGATCTC
Labeling_strand_ATTO647N /5ATTO647NN/AAGCGTAGTCGGATCTC

Attachment strand sequences

Unused_m13mp18_01 AAATTCTTACCATGATATAAAGCCAACTTTTGAGATCCGACTACGC
Unused_m13mp18_02 GCCGGCTTTAGATCATATGGCTATATTGGATATCCGACTACGC
Unused_m13mp18_03 ACAACCGGACTAATATTACATAGAATATTGAGATCCGACTACGC
Unused_m13mp18_04 TTTAATGTCTTTGAAATACCGGGCCTTTTTTGGAGATCCGACTACGC
Unused_m13mp18_05 TTGATTAAATTCTCTTTCTGTGCTATTATTGAGATCCGACTACGC
Unused_m13mp18_06 TTTAATTCAAAATCATAGGTCTGAGTTTTGAGATCCGACTACGC
Unused_m13mp18_07 ATCTTCTTAAACTCCCGGCTTTTTGAGATCCGACTACGC
Unused_m13mp18_08 ATACATCTCTCCTTCTGCTGATTGACTTTTGAGATCCGACTACGC
Unused_m13mp18_09 CTACGCTATTAATATTTTCCCTTAGTTTTGAGATCCGACTACGC
Unused_m13mp18_10 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_11 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_12 ATGTAATACATTCTTCCTCTATTTTTGAGATCCGACTACGC
Unused_m13mp18_13 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_14 ATGTAATACATTCTTCCTCTATTTTTGAGATCCGACTACGC
Unused_m13mp18_15 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_16 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_17 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_18 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_19 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_20 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_21 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_22 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_23 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_24 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_25 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_26 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_27 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_28 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_29 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_30 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_31 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_32 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_33 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
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Unused_m13mp18_45 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
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Unused_m13mp18_47 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_48 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
Unused_m13mp18_49 AGTGTAAACCTTTTGCTGAAAAATTTTTGAGATCCGACTACGC
2. Methods and Protocols

2.1. Glass surface treatment and attachment of seeds to glass

The goal of glass treatment was to attach DNA nanotube seeds to the glass via a specific biotin-streptavidin linker chemistry while minimizing nonspecific DNA nanotube and DNA seed interactions with the glass. To maximize the availability of the binding site on the DNA seed for tiles, we attached DNA nanotube seeds to the surface via the ends.

The glass was passivated by creating a biotin-labelled PEG silane monolayer by adopting a previously developed protocol onto which neutravidin or streptavidin was added (Supplementary Fig. S6), as described in detail below. DNA nanotube seeds were then attached to neutravidin molecules via biotinylated DNA strands attached to the seeds (Supplementary Figs. S6, S7).

**Supplementary Fig. S6:** Graphic showing the major steps involved in glass surface treatment and DNA nanotube seed attachment.

*Protocol for neutravidin presentation on glass surface:*

Glass-bottom dishes (D29-20-1-N, In Vitro Scientific) were cleaned by sonication in 10% NaOH for 20 minutes. Dishes were then washed with water to remove residual NaOH followed by a methanol wash. 500 µl of 1% biotin PEG silane MW 3400 (Biotin-PEG-SIL-3400-500mg, Layson Bio) solution (in solvent containing 95% methanol, 4% acetic acid, and 1% water) was prepared and added to dishes. Dishes were then stored overnight in the fume hood with a Parafilm seal to prevent evaporation. The next day, dishes were first rinsed with methanol, then with water to remove residual methanol. The glass bottom surfaces of the dishes were blow dried by pressurized nitrogen. Dishes were baked in oven at 90°C for 1 hour to cure and crosslink the biotin PEG silane. 500 µl of 1% BSA blocking solution (in TNT buffer) was added to dishes and incubated for 1.5 hours. Dishes were then washed with TNT buffer to remove excess blocker. 500 µl of 1
mg/mL Neutravidin (31000, Thermo Fisher Scientific) or 0.2 mg/mL Streptavidin (21122, Thermo Fisher Scientific) in TNT buffer was added to dishes and incubated for 30 minutes. Dishes were washed with TNT buffer followed by 3 washes with TAE Mg buffer. 500 µl of TAE Mg buffer was added to dishes after the washing steps. Nanotube seeds that have been biotin labelled (by annealing the seeds with the relevant biotin attachment linker strands for the relevant seed and the universal biotin attachment strand as listed below) were added to dishes and incubated for 5 minutes to produce the seed density on 29 mm glass bottom dishes shown in this work.

**TNT buffer:** 10 mM Tris-HCl, 0.1 M NaCl, 0.05% Tween-20, pH 7.5

**TAE Mg buffer:** 40 mM Tris-Acetate, 1 mM EDTA, 12.5 mM Mg$^{2+}$

**Note:** Silane powders must be equilibrated to room temperature before the containers are opened (stored in -20°C) and MUST have excess water/air removed from above powders before resealing (store 15 minutes under light nitrogen flow then recap in nitrogen).

**Supplementary Fig. S7:** Schematic illustrations of the passivated glass surface and the attachment of DNA nanotube seeds to the surface via biotin-neutravidin chemistry. Neutravidin molecules were deposited on the treated glass surface. 6 biotin attachment linker strands (green strands) each were bound to the M13mp18 scaffold and to a universal biotin attachment strand (black strand with pink disc). The universal biotin strands bound to neutravidin molecules present on the glass surface.

**Biotin attachment linker strand sequences for Nanotube Seed A**

- **Biotin_leftside_01** AGGGATAGCAAGCCCATTTTCACATCGTCACTCCT
- **Biotin_leftside_02** GAATTGCGAATAATAATTTTCACATCGTCACTCCT
- **Biotin_leftside_03** ACCCTCAGCAGCGAAATTTTCACATCGTCACTCCT
Biotin_leftside_04 TGTATCATCGCCTGATTTTTCACATCGTCACTCCT
Biotin_leftside_05 CATTCAGTGAATAAGGTTTTCACATCGTCACTCCT
Biotin_leftside_06 GAATACCACATTCAACTTTTCACATCGTCACTCCT

Biotin attachment linker strand sequences for Nanotube Seed B

Biotin_rightside_01 CTATTATTCTGAAACATTTTCACATCGTCACTCCT
Biotin_rightside_02 CAGGAGGTGGAGGCGCATTTCACATCGTCACTCCT
Biotin_rightside_03 ATCAAGTTTGCCTTTATTTTCACATCGTCACTCCT
Biotin_rightside_04 GTTTTAACCAGCGCCAATTTTCACATCGTCACTCCT
Biotin_rightside_05 TTTLTTAGAAAAAGTAATTTTCACATCGTCACTCCT
Biotin_rightside_06 AAACGATTTTTTGTGTTTTTCACATCGTCACTCCT

Universal biotin attachment strand sequence

Universal_biotin_attachment_strand /5BiosG/AGGAGTGACGATGTG

To confirm that seed attachment to the glass is mediated via biotin-neutravidin interaction, we prepared two dishes, one to which neutravidin was added to the glass surface and one that was prepared identically except that no neutravidin was added. We added equal amounts of seed solution to both the dishes, incubated for 10 minutes and washed with TAE Mg buffer to remove any non-specific or unstable attachments.

After washing, almost no seeds remained in the dish without neutravidin (Supplementary Fig. S8), indicating seed attachment is neutravidin-mediated.

Supplementary Fig. S8: Fluorescence microscope images of nanotube seeds (type A) attached to two dishes, one with neutravidin on the treated glass surface (top row) and the other prepared with the same protocol without the neutravidin addition (bottom row). “Pre-wash” refers to images of dishes 10 minutes after seeds solution addition while “post-wash” refers to images of the same dishes after a TAE Mg buffer washing step is performed to remove any non-specific attachments.
To test the functionality of seeds attached to glass surface, we added DNA nanotube tiles to glass dishes and observed nanotube growth from seeds over 50 hours. Fluorescence images show successful attachment of seeds to the glass surface and nanotubes growing from them when incubated with nanotube tiles (Supplementary Fig. S9), implying seeds can nucleate nanotubes when they are attached to the treated glass surfaces used in the experiments in this paper.

**Supplementary Fig. S9:** Fluorescence images of DNA nanotubes growing from Seed A (top row) and Seed B (bottom row) that were attached to glass surface. Scale bars 10 µm.
2.2. Attachment of nanotube seeds to polystyrene beads

To attach seeds to carboxyl polystyrene beads, we first coated the bead surface with M13toAM DNA strand via carboxy-amine chemistry. An amine-labelled linker DNA strand (M13toAM) binds to a sequence region on the M13mp18 scaffold that is not part of the seed structure and is modified to have an amine group at its 5’ end. Nanotube seeds are added to M13toAM coated beads to attach seeds to beads (Supplementary Fig. S10). A detailed protocol follows. Seeds are labelled with ATTO488 dye using the fluorescent labelling scheme shown in Supplementary Fig. S5.

**Supplementary Fig. S10:** Schematic illustrating attachment of nanotube seeds to polystyrene beads.

*Protocol for attaching M13toAM strand to 3 µm carboxyl labeled polystyrene beads:*

20 µl of 5% (w/v) beads (CP-30-10, Spherotech) and 200 µl 0.1 M MES buffer (2-(N-morpholino)ethanesulfonic acid) were mixed and vortexed for 15 min. Beads were then centrifuged (1000 g for 3 min), decanted and resuspended in 200 µl 0.1 M MES buffer. In a separate Eppendorf tube, 150 µl of freshly prepared 10 mg/ml EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) in 0.1 M MES buffer and 20 µl of 50 µM M13toAM DNA were mixed and vortexed for 10 min. This M13toAM DNA solution was then added to the previously prepared beads (in MES buffer) and vortexed overnight in an Eppendorf tube covered with dark aluminum foil. The following day, beads with DNA solution were centrifuged (1000 g for 10 min), decanted and resuspended in 200 µl TE 0.1% SDS buffer. This TE 0.1% SDS buffer washing step was repeated twice. Beads were then incubated at 65°C for 15 min to remove any unstable M13toAM DNA bindings. Next, beads were washed twice by centrifugation, decantation and resuspension.
in TE 0.1% SDS buffer. M13toAM labelled beads were transferred to TAE Mg buffer by centrifugation, decantation and resuspension in 100 µl TAE Mg buffer to obtain a solution with 1% beads (assuming no loss).

**TE 0.1% SDS buffer:** 10x TE buffer with 0.1% SDS
* Recipe for -------------- 10 ml
* DI Water --------------- 8 ml
* Tris (100 mM)-------- 121 mg
* Na2EDTA (10 mM)---- 37 mg
* 10% SDS ------------- 1000 µl
* Adjust pH to 8.5 using HCl. After mixing everything increase the total volume to 10 ml by adding the required amount of DI water.

**M13toAM strand sequence**

/5AmMC6/TTTTTTATGACAAGACATCGATGGCTTAGAGCTTAATTGCTGAATATAATGCTGTAGCT

**Protocol for attaching nanotube seeds to M13toAM DNA coated beads:**

Recipe: B seeds with seed labeling mixture (ATTO488)

<table>
<thead>
<tr>
<th></th>
<th>Final desired concentration (nM)</th>
<th>Stock (nM)</th>
<th>Amount to add (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
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<td></td>
<td>82.4</td>
</tr>
<tr>
<td>Seed staples mix</td>
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<td>1.2</td>
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<tr>
<td>Seed B adapter strand mix</td>
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<td>1000</td>
<td>0.4</td>
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<tr>
<td>M13mp18 scaffold strand</td>
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<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>Seed labeling attachment strands mix</td>
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<td>100</td>
<td>0.6</td>
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<tr>
<td>Labeling strand ATTO488</td>
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<td>1000</td>
<td>5</td>
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<tr>
<td>10x TAE Mg buffer</td>
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<tr>
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</tbody>
</table>

*Seed staple mix:* Mixture containing all 72 seed strands in equal volume. Mixing individual seed staple strands (each stock at 100 µM) in equal volume makes the final concentration of staples in the mixture to be 1.389 µM (100 µM/72).

*Seed B adapter strand mix:* Mixture containing all seed B adapter strands (see Supplementary Fig. S4) at a concentration of 1 µM except the strands with sticky ends [seedB_AD2Red_4, seedB_AD3_4SEd_4, seedB_AD5_6Red_4, seedB_AD7_8SEd_4, seedB_AD9_10Red_4, seedB_AD11_12SEd_4], which are at 2 µM.

*Seed labelling attachment strands mix:* Mixture containing all 100 labelling attachment strands (see Supplementary Section 1.3) at a concentration of 100 nM.
To attach Seed B to M13toAM coated beads, we annealed 4 pM of B seeds with the seed labelling mixture (ATTO488) from 90°C to 20°C at 1°C/min. M13toAM coated beads were added to the solution at when the mixture reached 70°C in the following proportion:

76 µl TAE Mg buffer, 4 µl beads (1% w/v), 20 µl 4 pM seeds

After annealing, the mixture was centrifuged (100 g for 10 min), decanted and resuspended in 50 µl TAE Mg buffer to remove any unattached seeds from solution. This TAE Mg buffer wash step was repeated twice.

The above protocol should produce a solution of type B seeds with ATTO488 labelling attached to 3 µm beads in TAE Mg buffer. Each bead had 1-10 seeds attached to it.
2.3. Self-assembling nanotube connections between A and B seeds that are attached to a glass surface

Glass bottom dishes (D29-20-1-N, In Vitro Scientific) for attachment to seeds were prepared a day in advance. For glass dish preparation protocol, refer to Supplementary Section 3.1.

Nanotube Seeds

Seeds were mixed with a solution of tiles shortly before attachment because we found that optimal yields of growth of nanotubes was achieved when the seeds were incubated with nanotube tiles prior to growth.

Recipe: Type A nanotube seeds with seed labels (ATTO647) and biotin attachment strands

<table>
<thead>
<tr>
<th></th>
<th>Final desired concentration (nM)</th>
<th>Stock (nM)</th>
<th>Amount to add (µl)</th>
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<tbody>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>71.48</td>
</tr>
<tr>
<td>Seed staple mix</td>
<td>16</td>
<td>1389</td>
<td>1.2</td>
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<tr>
<td>Seed A adapter strand mix</td>
<td>4</td>
<td>1000</td>
<td>0.4</td>
</tr>
<tr>
<td>M13mp18 scaffold strand</td>
<td>0.04</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>Seed labelling attachment strands mix</td>
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<td>0.6</td>
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<tr>
<td>Labelling strand ATTO647N</td>
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<tr>
<td>Biotin attachment linker strand mix (A)</td>
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<tr>
<td>Biotin attachment strand</td>
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<td>0.8</td>
</tr>
<tr>
<td>REd SEd tiles (Cy3 labelled)</td>
<td>40</td>
<td>400</td>
<td>10</td>
</tr>
<tr>
<td>10x TAE Mg buffer</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
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<td>100</td>
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</table>

Seed staple mix: Mixture containing all 72 seed strands in equal volume. Mixing individual seed staple strands (each stock at 100 µM) in equal volume makes the final concentration of staples in the mixture to be 1.389 µM (100 µM/72).

Seed A adapter strand mix: Mixture containing all seed A adapter strands (see Supplementary Fig. S3) at a concentration of 1 µM except the strands with sticky ends [AD2REd_2, AD3_4SEd_2, AD5_6REd_2, AD7_8SEd_2, AD9_10REd_2, AD11_12SEd_2] which are at 2 µM.

Seed labelling attachment strands mix: Mixture containing all 100 labelling attachment strands (see Supplementary Section 1.3) at a concentration of 100 nM.
**Biotin attachment linker strand mix (A):** Mixture containing all six-biotin attachment linker strands for seed A (Biotin_leftside_01 to Biotin_leftside_06) at 100 nM (See Supplementary Section 3.1).

**REd SEd tiles (Cy3 labelled):** Mixture containing tile strands RE-1, RE-3Cy3, RE-5, SE-1, SE-3Cy3 and SE-5 at 400 nM, and the short strands RE-2EE01, RE-4, SE-2DIAG and SE-4DIAG at 800 nM (see Supplementary Section 1.1).

Recipe: Type B nanotube seeds with seed labels (ATTO488) and biotin attachment strands

<table>
<thead>
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<td>4</td>
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<td>0.4</td>
</tr>
<tr>
<td>M13mp18 scaffold strand</td>
<td>0.04</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>Seed labelling attachment strands mix</td>
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<td>0.6</td>
</tr>
<tr>
<td>Labelling strand ATTO488</td>
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<td>5</td>
</tr>
<tr>
<td>Biotin attachment linker strand mix (B)</td>
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<td>0.12</td>
</tr>
<tr>
<td>Biotin attachment strand</td>
<td>0.8</td>
<td>100</td>
<td>0.8</td>
</tr>
<tr>
<td>REd SEd tiles (Cy3 labelled)</td>
<td>40</td>
<td>400</td>
<td>10</td>
</tr>
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<tr>
<td><strong>Total</strong></td>
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**Seed B adapter strand mix:** Mixture containing all seed B adapter strands (see Supplementary Fig. S4) at a concentration of 1 µM except the strands with sticky ends [seedB_AD2REd_4, seedB_AD3_4SEd_4, seedB_AD5_6REd_4, seedB_AD7_8SEd_4, seedB_AD9_10REd_4, seedB_AD11_12SEd_4], which are at 2 µM.

**Biotin attachment linker strand mix (B):** Mixture containing all six-biotin attachment linker strands for seed A (Biotin_rightside_01 to Biotin_rightside_06) at 100 nM (See Supplementary Section 3.1).

We prepared the above solutions and annealed each in a thermocycler (Eppendorf Mastercycler) using the following annealing schedule:

- 5 mins at 90°C
- 90°C to 45°C at 1°C/min
- 45°C for 60 mins
- 45°C to 32°C at 1°C/10mins
- 32°C for 2 hours
Both the solutions were transferred to treated glass dishes that were maintained at 32°C in an enclosed microscope chamber. 10 µl each of 0.04 nM seeded nanotubes produced the seed density on 29 mm glass bottom dishes shown in this work.

We incubated the solutions for about 10 minutes in the dish then washed the dishes twice with about 500 µl of TAE Mg buffer to remove unbound seeds.

**Nanotube tiles**

Adapter strands are reversibly bound to seeds. To prevent the binding sites of seed structures from becoming unusable for tiles due to adapter strand unbinding, the tile mixture used for nanotube growth included additional adapter strands.

**Recipe: DNA nanotube tiles**

<table>
<thead>
<tr>
<th></th>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

We prepared the above solution and annealed it in a thermocycler (Eppendorf Mastercycler) using the following annealing protocol:

- 5 mins at 90°C
- 90°C to 45°C at 1°C/min
- 45°C for 60 mins
- 45°C to 32°C at 1°C/10mins

We transferred the annealed nanotube solution (500 µl) to glass bottom dishes containing A and B seeds already attached to the glass surface and maintained at 32°C in a microscope chamber.

Dishes were then sealed with Parafilm to prevent solution evaporation. Time-lapse acquisition was carried out at 32°C over several hours on a fluorescence microscope using custom acquisition software.

For monitoring connection yields after different assembly times, we imaged nanotubes in TAE buffer without excess tiles via buffer exchange, as image acquisition of DNA nanotubes in presence of excess DNA tiles was less ideal due to the higher background fluorescence. Hence, at every acquisition time, we first did a buffer exchange to remove
excess tiles, captured ~100 images at random locations, and then replenished the dishes with freshly annealed DNA nanotube tiles to continue the assembly reaction.
2.4. Detection of isolated seed pairs and connection yield calculations

Connection yields were determined using a set of 3-color fluorescence micrographs (ATTO488, ATTO647N and Cy3) that were captured at random locations within the dish using pre-programmed image capture software. To efficiently determine nanotube connection yield, we developed an automated image analysis program that located isolated seed A/B pairs within the captured images and established whether a nanotube connection was present between them. This allowed us to process a high number of images for accurate yield determination.

Supplementary Fig. S11: A sample composite image after flat fielding showing the detected isolated A/B seed pairs (enclosed in yellow ellipses). White dotted square represents where the image was cropped to remove border artifacts due to flat fielding. Purple line indicates area of image considered for detecting isolated seed pairs.

Steps involved in image analysis:

To remove low frequency background variations due to such effects as uneven illumination, flat fielding algorithm (see below for matlab code) was applied to images from all 3 filters (Cy3, ATTO488 and ATTO647N). Because flat fielding cannot correctly process the images at regions near the edges, images were cropped (white dashed line in Supplementary Fig. S11) by 5% from each side to remove such border artifacts before automated location of isolated pairs. To ensure that we could visualize the area around potential isolated seed pairs, we only considered pairs that were more than 10
µm from the cropped border, i.e. within the purple dashed box in Supplementary Fig. S11.

Flat fielding matlab code:

```matlab
function [Image_flat] = flatfielding(Image)

    h = fspecial('gaussian',50,50);
    B = imfilter(Image,h);
    % substract background
    Image2 = Image-B;
    % local contrast enhancement
    A = adapthisteq(Image2,'clipLimit',0.02, 'NBins', 400, 'Distribution',
                    'exponential', 'NumTiles', [100 100]);
    % despeckle
    Image_flat = medfilt2(A, [2 2]);
```

We found isolated pairs by locating pairs of A and B seeds which obeyed the constraints described in Supplementary Fig. S12. To precisely localize the seeds (and therefore measure the distance between them), ATTO647N and ATTO488 images were converted to binary images based on an intensity threshold. The Matlab shrink operation was then used to shrink objects that were determined to be A and B seed coordinates respectively to single points; inter-seed distance was computed as the distance between the two points. For seed pairs separated by more than 6 µm that were close to 10 µm window, we also manually checked that an area around these pairs of at least 1.5 times the distance between them was fully captured and that there were no seeds in this area. In order to focus our study on the how the kinetics of the assembly process affect interconnect yield, we excluded the relatively small fraction of nanotube seeds from which no nanotubes grew from our analysis.

**Supplementary Fig. S12:** Isolated seed pairs are pairs of A and B seeds are defined as those where the A seed is closer to its partner than to all other B seeds by at least a factor of 1.5 and the B seed is closer to its partner than to all other A seeds by at least a factor of 1.5.
To determine whether a nanotube connection existed between an isolated pair of seeds, the images of the nanotubes growing from the seeds (from the Cy3 filter) were first converted to binary images using an intensity threshold filter. Next, Matlab’s connected component labeling algorithm (http://www.mathworks.com/help/images/ref/bwconncomp.html) was applied to the binary images to identify all unconnected nanotubes. Our algorithm declared that a nanotube connection existed between an A-B seed pair if both the A and B seeds lay on the same nanotube. To ensure that automated analysis did not skew our yield findings, we further manually checked all images for incorrect nanotube connection determination.

Inter-seed distance was calculated as the Euclidian distance between two isolated seed pairs A/B. The contour lengths of nanotube connectors was determined using the ImageJ plugin Jfilament 2D (http://athena.physics.lehigh.edu/jfilament/).
2.5. Time-lapse tracking of individual connection formation

Microscope software that allows for autonomous time-lapse capture of multiple fluorescence channels at multiple dish locations over several hours was used for tracking the formation of individual nanotube connections (Fig. 2b and Supplementary Fig. S50). 3 µm carboxy-coated beads were deposited on the glass surface to serve as fiduciary markers to aid an autofocus algorithm in tracking the glass surface over the duration of imaging. At each time point during the time-lapse capture, an ATTO488 (Seed B) filter image, an ATTO647 (Seed A) filter image and 3 Cy3 (nanotubes) filter images were captured at a particular location. Time-lapse acquisition was done with 30 minute intervals for 20 hours after the addition of DNA tiles to the dishes.

Post processing of the images involved flat fielding to remove any uneven background illumination, merging the 3 grey scale filter images (ATTO488, ATTO647N and Cy3) as a single composite 3-color image and aligning the composite image stacks using seed coordinates as a reference to correct for any x-y stage drift during imaging.
2.6. Self-assembling nanotube connections between B seeds on beads and A seeds attached to glass surface

Glass bottom dishes (D29-20-1-N, In Vitro Scientific) for attachment to seeds were prepared a day in advance. For glass dish preparation protocol, refer Supplementary Section 3.1.

Nanotube Seeds

See Supplementary Section 3.3 for protocols for synthesizing Type A Seeded Nanotubes with Seed labeling (ATTO488) and Biotin attachment strands.

Annealed seed A solution was transferred to treated glass dishes within a 32°C in microscope chamber. We found that 10 µl of 0.04 nM seeded nanotubes gave us the best seed density on 29 mm glass bottom dishes.

We incubated the solution for about 10 minutes in the dish then washed the dishes twice with about 500 µl of TAE Mg buffer to remove unbound seeds.

Nanotube tiles

Adapter strands are reversibly bound to seeds. To prevent the binding sites of seed structures from becoming unusable for tiles due to adapter strand unbinding, the tile mixture used for nanotube growth included additional adapter strands.

Recipe: DNA nanotube tiles

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<tr>
<td>REd SEd tiles (Cy3 labelled)</td>
<td>40</td>
<td>400</td>
<td>50</td>
</tr>
<tr>
<td>Seed A adapter strand mix</td>
<td>0.4</td>
<td>1000</td>
<td>0.2</td>
</tr>
<tr>
<td>Seed B adapter strand mix</td>
<td>0.4</td>
<td>1000</td>
<td>0.2</td>
</tr>
<tr>
<td>10x TAE Mg buffer</td>
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<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

We prepared the above solution and annealed it in a thermocycler using the following annealing protocol:

- 5 mins at 90°C
- 90°C to 45°C at 1°C/min
- 45°C for 60 mins
- 45°C to 32°C at 1°C/10mins
We transferred the annealed nanotube solution (500 µl) to glass bottom dishes containing A seeds already attached to the glass surface and maintained at 32°C in a microscope chamber. We then added 10 µl of 1% 3 µm polystyrene beads decorated with B seeds (ATTO488 labelling) to the dishes. See Supplementary Section 3.2 for the protocol for attaching seeds to beads.

Dishes were sealed with Parafilm to prevent solution evaporation and then incubated at 32°C for 20 hours after which they were imaged.
2.7. Self-assembling nanotube connections between A seeds and B seeds on two respective glass surfaces separated by 3 to 5 microns

Two types of glass coverslips, small -18 mm x 18 mm (470019-002, VWR) and large -22 mm x 40 mm (470019-010, VWR) for attachment to seeds were prepared a day in advance through passivation, following the protocol in Supplementary Section 3.1.

Nanotube Seeds

Type A seeded nanotubes with ATTO647N labels and Type B seeded nanotubes with ATTO488 labels, both with biotin attachment strands, were synthesized following the protocols in Supplementary Section 3.3.

The annealed seed A solution was transferred to treated small glass coverslips while the annealed seed B solution was transferred to treated large glass coverslips within a 32°C glove box chamber. We found that 100 µl of 0.04 nM seeds resulted in a seed density on the coverslips suitable for reliable connection and visualization of the results.

We incubated the solutions for about 10 minutes on the coverslips then submerged the coverslips in TAE Mg++ buffer solution to remove any unbound seeds.

Nanotube tiles

Recipe

<table>
<thead>
<tr>
<th></th>
<th>Final desired concentration (nM)</th>
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<td>50</td>
</tr>
<tr>
<td>Seed A adapter strand mix</td>
<td>0.4</td>
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<td>0.2</td>
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<tr>
<td>Seed B adapter strand mix</td>
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<tr>
<td>10x TAE Mg buffer</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>500</td>
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</table>

Additional adapter strands for the seeds were included to enable reattachment of strands that fall off seeds during the assembly process.

We prepared the above solution and annealed it in a thermocycler (Eppendorf Mastercycler) using the following annealing protocol:

- 5 mins at 90°C
- 90°C to 45°C at 1°C/min
- 45°C for 60 mins
• 45°C to 32°C at 1°C/10mins

All the following steps were performed in a glove box chamber maintained at 32°C. We transferred the annealed tile solution (50 µl) to the large coverslips to which B seeds were already attached. Next, we placed two small 4 µm thick aluminium foil spacers (Geistnote) between the two glass coverslips. Finally, we placed the small coverslips to which A seeds were already attached onto the large coverslips and spacers (Supplementary Fig. S13). Excess nanotube solution between the two coverslips was removed by blotting with Kimwipes. The coverslips were sealed together into a closed fluid chamber using epoxy cured at 32°C for 10 mins. The closed fluid chamber was kept at 32°C for 15 hours to allow connections to form before imaging.

Z-stack image acquisition was carried out at 32°C on a confocal fluorescence microscope.

Supplementary Fig. S13: Schematic illustrating the steps to assemble a closed fluid chamber with different types of seeds attached on top and bottom glass coverslips for the formation of point-to-point nanotube connections in 3 dimensions.
3. Additional Data and Analysis

3.1. Lengths of connected and unconnected nanotubes after different point-to-point assembly times

**Supplementary Fig. S14:** Lengths of connected and unconnected nanotubes for point-to-point assembly experiments carried out over 10 hours, 20 hours, 30 hours, 40 hours and 70 hours.
70 hours. Left column: Scatter plots relating nanotube connector length and the distance between seeds for successful nanotube connections. The dashed blue lines indicate the shortest possible connector length for a given connection distance. Inset text indicates the number of connected nanotube pairs measured. Right column: Scatter plots relating the sum of nanotube connector lengths and distance between seeds for pairs of seeds where a connection did not form. The dashed blue lines indicate the shortest possible connector length for a given connection distance. Inset texts indicate the percentage of the total measured unconnected nanotube pairs that were long enough (above the dashed line) and not long enough (below the dashed line) or their ends to be able to reach one another.
3.2. Determination of whether nanotube rotational diffusion is restricted, \textit{i.e.} whether nanotubes prefer certain angular orientations over others.

\textbf{Supplementary Fig. S15:} Example rotationally unrestricted and restricted nanotubes growing from seeds attached to the glass surface. Left: polar histogram plots showing relative times spent in different orientations. Right: randomly selected images of nanotubes over 23 hours of observation. Scale bars 3 \(\mu\)m.

Qualitative observation of fluorescence microscopy movies of rotational diffusion of nanotubes suggested that nanotube rotational diffusion was biased, with some nanotubes spending long periods of time in a subset of angular orientations without exploring the remaining potential orientations (Supplementary Fig. S15). This bias limited the diffusive exploration of nanotubes, and likely lowered connection yields by, for example, preventing nanotubes from being able to form straight-line connections in some cases. To understand the extent of this effect and thus how it might have affected the yields of nanotube connections, we systematically analysed the rotational diffusion of randomly chosen nanotubes during growth by capturing 3 images every 40 minutes for 23 hours. We characterized their rotational dynamics as either restricted (indicating some angles were preferred over others) or unrestricted (indicating that we could not confidently declare that some angles were preferred over others).

To determine whether the bias in orientation observed during growth was statistically significant, we first identified seed locations (\textit{i.e.} coordinates) for each image in the time series we collected as described in Supplementary Section 3.5. We used these seed coordinates as a reference to correct for any x-y stage drift during the time the series was collected. For each of the captured frames, we also determined the angle of the nanotube by converting the nanotube image into a binary image using an appropriate intensity threshold on a Cy3 gray scale image and then fitting the section of the nanotube closest to the seed to a line.

This data was used to produce a polar histogram of the relative time an individual seeded nanotube spent in different angular configurations during the growth period for each of the 70 seeded nanotubes characterized (Supplementary Fig. S16). An ideal freely rotating nanotube would explore all available angles (360\(^\circ\)) and spend the same amount of time...
on average in each angular configuration. These plots show that the majority of the nanotubes only explored 25-50% of the available angles over 23 hours.

**Rotational restriction analysis**

We used the data shown in Supplementary Fig. S16 to determine statistically whether individual nanotubes appeared to exhibit bias in their exploration of possible angular configurations. To do so, we first observed that nanotube angles could be expressed as x and y coordinates along a unit circle. In an unbiased angular diffusion process the average x and y coordinates would each converge to 0 given enough angle samples. Our approach to determining bias was to compute the actual average x and y coordinates for a set of angles for a given nanotube as well as their confidence intervals. We called a nanotube rotationally restricted if its mean x or y coordinate was nonzero with greater than 95% confidence.

For a given nanotube, the average x and y coordinates were given by

\[
\hat{x} = \frac{1}{N} \sum_{j=1}^{N} \sin (\theta_j)
\]

\[
\hat{y} = \frac{1}{N} \sum_{j=1}^{N} \cos (\theta_j)
\]

The sample standard deviations of x and y are given by

\[
s_x = \sqrt{\frac{1}{N-1} \sum_{j=1}^{N} (x_j - \hat{x})^2}
\]

\[
s_y = \sqrt{\frac{1}{N-1} \sum_{j=1}^{N} (y_j - \hat{y})^2}
\]

The 95% confidence interval for \(\hat{x}_i\) and \(\hat{y}_i\) are given by

\[
x \in \left[ \hat{x} - \frac{\Phi^{-1}(0.95) s_x}{\sqrt{2N}}, \hat{x} + \frac{\Phi^{-1}(0.95) s_x}{\sqrt{2N}} \right]
\]

\[
y \in \left[ \hat{y} - \frac{\Phi^{-1}(0.95) s_y}{\sqrt{2N}}, \hat{y} + \frac{\Phi^{-1}(0.95) s_y}{\sqrt{2N}} \right]
\]

where \(\Phi^{-1}\) is the inverse cumulative normal distribution function.

If 0 does not fall into the 95% confidence intervals for both the x and y coordinates for a given nanotube, that nanotube was deemed rotationally restricted.

We quantified the amount of restriction to a nanotube’s free diffusion using the restriction score (S):
\[ S = \frac{\hat{x}^2 + \hat{y}^2}{2} \]

In principle, information about the distribution of angular orientations of nanotubes could also be used to establish with confidence that the angular distribution of some nanotubes had no bias (or restriction) by using a technique such as equivalence testing to establish bounds on \( S \). However, the number of independent samples of nanotube angular position required to show this with confidence (several hundred to one thousand using our methods) was larger than the number of samples we collected. Here a nanotube labelled as rotationally unrestricted is therefore one for which we could not be 95\% confident the motion was restricted.
Supplementary Fig. S16: Polar histogram plots showing the time distribution of angular configurations of 70 individual seeded nanotubes. S is the rotational restriction score (see Supplementary Section 4.2) and R = 1 indicates nanotubes whose rotational diffusion could be classified as restricted with 95% confidence. R = 0 indicates nanotubes that could not be shown with confidence to have restricted rotational diffusion. Plots are sorted in increasing order of rotational restriction score, i.e. from least to most restricted.
3.3. Estimating the DNA nanotube growth rate in conditions similar to those used in point-to-point assembly experiments

Successful interconnection requires a balance of nanotube growth and diffusive exploration of space by a nanotube end. To better understand how these two processes couple in the process we designed, we measured the growth rate of nanotubes, which determines how quickly the contour length of nanotubes changes over time.

To measure nanotube growth rate, we collected fluorescence microscopy movies of anchored seeded nanotubes over time. Specifically, type-A seeded nanotubes were attached to passivated glass bottom dishes (Supp. Section 2.1) similar to the ones used to demonstrate point-to-point assembly in dishes (Supp. Section 2.3). Seeded nanotubes were deposited to produce densities similar to those used in point-to-point experiments: 10 µl of 0.04 nM seeded nanotubes on 29 mm glass bottom dishes. Sets of one image filtered for ATTO647N fluorescence and three images filtered to capture Cy3 fluorescence were captured every 40 minutes for 24 hours.

Accurate measurement of nanotube contour length is important for estimating nanotube growth rate. However, there are several inherent challenges in accurately measuring the contour lengths of diffusing nanotubes as opposed to nanotubes that are completely adhered to glass surface. Specifically, a) moving nanotubes can go out of the imaging focal plane and, b) we can only assess the 2D projection of DNA nanotube length as we imaged the diffusing nanotubes through the bottom of the glass dish using epi-fluorescence. We tried to minimize the effects caused by the above-mentioned challenges on measuring a nanotube contour length by taking 3 images of the same nanotube around a particular time point and taking the longest measured nanotube length from those images at a particular time point. This estimation of length appears to be a fairly accurate method for measuring nanotube length, at least for the set of nanotube lengths considered here.

Plotting the measured nanotube length with time of 35 individual nanotubes showed nanotube length increased a more or less at a constant rate over 24 hours (Supplementary Fig. S17). Linear regression of the nanotube length data with time gave a nanotube growth of $0.11 \pm 0.01 \, \mu\text{m/hr.}$

This rate is consistent with a measured forward rate constant$^5$ of tile hybridization $6 \times 10^5 \, \text{M}^{-1}\text{s}^{-1}$ and of the energy$^4$ of Double-Antiparallel-Even-Even tile attachment $-10.9 \, \text{kcal mol}^{-1}$ at 32 °C. While the forward rate constant we used in the paper was measured for a slightly different type of DNA tile$^5$, similar forward rates in the range of $10^5 \, \text{M}^{-1}\text{s}^{-1} – 10^6 \, \text{M}^{-1}\text{s}^{-1}$ have been measured for other tiles in a variety of contexts$^8$ as well as for DNA oligonucleotides$^9$ and DNA origami nanostructures$^{10}$. We thus felt that such an approximation was reasonable. We estimated the off rate by using this on rate and the $\Delta G^\circ$ of tile-tile interactions of the exact nanotubes used in this study$^4$.

While it was not possible to explicitly quantify the amount of spontaneous nucleation of nanotubes because such nanotubes were not attached to seeds and would not necessarily
have been visible at the surface, these growth measurements suggest that spontaneous nucleation was not a major factor in the point-to-point assembly process. If there were many such nanotubes in solution, we would expect them to occasionally attach to seeded nanotubes via end-to-end joining. However, in our observation of 35 individual seeded nanotubes over 24 hours we observed only 3 points consistent with a joining event (indicated by arrows in Supp. Fig. S17).

Supplementary Fig. S17: Increase in nanotube length over time, Changes in lengths of 35 individual seeded nanotubes (each shown in a different colour) that were tracked over 24 hours using fluorescence micrographs. 2D projection of nanotube length was used as an estimate for nanotube length. Arrows indicate 3 instances when a free-floating nanotube in solution attached to the growing end of the nanotubes being tracked.
3.4. Estimating the rotational diffusion constant ($D_r$) of seed-anchored nanotubes

Successful interconnection requires a balance of nanotube growth and diffusive exploration of space by a nanotube end. To better understand how these two processes couple in the process we designed, we measured the rotational diffusion rate of nanotubes, which determines how quickly their angular position with respect to the seed changes over time.

To measure the rotational diffusion constant, we collected fluorescence microscopy movies of anchored nanotubes of different lengths and used this information to characterize how the nanotube changed its angle at the point of the seed anchor. Specifically, seeded nanotubes were grown over 20 hours at 32°C from A seeds (we assume the measured rate would be the same had B seeds been used) that were attached to a glass surface in dishes (see Supplementary Section 3.4). Movies (100 frames at 0.1 sec exposure taken via continuous capture) were recorded for several randomly selected locations within the dish after 5, 10 and 20 hours. Movies at different times were captured to determine rotational diffusion constants across a broad range of nanotube lengths to account for change in the rotational diffusion constant with length due to hydrodynamic drag. Our method takes into account only the rotational motion about the axis perpendicular to the glass plane passing through the seed and not the change in the angle between the nanotube and glass plane due to the challenge of capturing motion in the $z$ direction with enough time resolution to measure nanotube diffusion rates.

We used an automated algorithm to identify the coordinates of individual seeds (see Supplementary Section 3.5). The angle of a nanotube was determined semi-automatically for each of the 100 frames, since occasionally nanotubes would be close to perpendicular to the glass surface making it difficult to perform automated angle determination; these cases required user input. We then characterized the diffusive motion of the nanotube end over time relating a standard formula relating mean square angular displacement (MSAD) of nanotubes and $Z_r$:

$$\langle (\theta(t + \Delta t) - \theta(t))^2 \rangle = 2Z_r \Delta t$$  \hspace{1cm} (1)

where $\theta$ is nanotube angle with respect to a reference, $Z_r$ is an effective diffusion constant characterizing the rate of free nanotube end motion when the other nanotube end is tethered, $t$ is time and $\Delta t$ is time interval between angle measurements. For a particular nanotube, we determined the MSAD at various integral ($n$) multiples of $\Delta t$ ($\Delta t = 0.1$ seconds is the time interval between consecutive frames of the movie) by calculating the square of the difference in nanotube angle between images with time interval $n\Delta t$ between them as follows:

$$\langle (\theta(t + n\Delta t) - \theta(t))^2 \rangle = 2Z_r n\Delta t$$  \hspace{1cm} (2)

The plot of MSAD for a nanotube as a function of time interval ($n\Delta t$) should produce a line with slope $2Z_r$ (Supplementary Fig. S18). We determined $Z_r$ for individual nanotubes by fitting this plot to a line, determining the slope and dividing this slope by 2.13
Supplementary Fig. S18: Calculating $Z_r$ for individual nanotubes of varying lengths.
Mean squared angle displacement of 75 individual nanotubes versus time. Slope of the individual lines is equal to $2Z_r$.

The rotational diffusion constant $D_r$ of a rigid polymer describes the change in angle of a nanotube rotating about its centre. We used this formula to characterize the rotational motion of tethered DNA nanotubes. We approximated a DNA nanotube as a rod shaped particle with aspect ratio ($p = L/d$), where $L$ is nanotube length and $d$ is nanotube diameter, we expect a relationship between $p$ between $D_r$ and nanotube length of the form:

$$D_r = \frac{3k_B T (\ln(p) - \gamma)}{\pi \eta_s L^3}$$

In the above expression, $k_B$ is Boltzmann constant, $T$ is absolute temperature, $\eta_s$ is dynamic viscosity and $\gamma$ is a correction factor for rod shaped particle for more precise hydrodynamic calculation.

The above relationship describes how $D_r$, the angular change of a polymer about its central axis, changes with nanotube length. To determine the predicted value of $D_r$ as a function of nanotube length using our measurements of $Z_r$, we developed a simple conversion tool to produce a predicted $D_r$ value for each measured $Z_r$ value. The resulting predicted $D_r$ values and corresponding nanotube lengths were then used to fit $\gamma$ in Equation 3.

We derived this expression beginning from the observation that a diffusion constant $D$ is the ratio between $k_B T$ and a drag term. For the rotational diffusion of a rigid polymer, $D_r$ for a rotating rod is the ratio between $k_B T$ and the rotational friction constant ($\zeta$). To convert our values of $Z_r$ into a rotational diffusion constant $D_r$, we consider two rods, Rod...
A of length L rotating about its one end and Rod B of length 2L rotating about its center (Supplementary Fig. S19a). Rod B can be envisioned as two Rod As of length L joined end to end, such that each rod is rotating about its end. By symmetry, the rods that make up Rod A have the same rotational friction constant. The rotational frictional coefficient of Rod B can therefore be assumed to be twice that of Rod A, hence $Z_r$ for Rod A of length L is twice $D_r$ for a Rod B of length 2L.

Supplementary Fig. S19: Calculating $D_r$ of nanotubes as a function of nanotube length. a, Analytical relationship between $Z_r$ of a rod A of length L rotating about its one end and $D_r$ of a rod B of length 2L rotating about its centre. b, Scatter plot of the $D_r$ and nanotube length measurements corresponding to the measurements of $Z_r$ of individual nanotubes. The blue curve indicates the least squares fit of the equation 3 relating $D_r$ of a rod shaped particle to its aspect ratio $\rho$. Green dashed lines are confidence intervals representing two standard deviations.

On fitting Eq. 3 to our experimental data (Supplementary Fig. S19b), we get the following relationship for $D_r$ as a function of L:

$$D_r = \frac{3k_B T (\ln(\rho)+3.5)}{\pi \eta_s L^3} \quad (4)$$

where, $L$ is nanotube length and $d = 0.015 \mu m$. 

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3.5. Estimating the translational diffusion constant ($D_T$) of seed-anchored nanotubes

We used measured rates of nanotube growth and diffusion to set the timescale of our coarse-grained dynamic model of point-to-point assembly. To measure the length-dependent translational diffusion constant of DNA nanotubes ($D_T$) seeded DNA nanotubes were confined between PEG passivated coverslips and glass sides separated by ~2 microns. For glass surface passivation, we followed the same surface treatment protocol as dishes where we demonstrated nanotube interconnect assembly (Supplementary Section 3.1), except here we used mPEG-silane (MPEG-SIL-5000-1g, Layson Bio) instead of Biotin-PEG-silane, as no attachment of seeds to glass surface was required. We recorded 500 continuous frames each integrated over 0.1 seconds. We subsequently manually determined the centre of the nanotube contour of 13 randomly selected nanotubes of different lengths.

To calculate the $D_T$ for each nanotube, these centres of nanotube contours were then used to determine the translational motion of the nanotubes using a standard formula relating mean square displacement (MSD) of nanotubes and $D_T$:

$$< (U(t + \Delta t) - U(t))^2 > = 4D_T\Delta t$$

(5)

where $U(t)$ is the position vector of a nanotube at time $t$ with respect to a reference, $D_T$ is a 2-dimensional translational diffusion constant characterizing the rate of movement of nanotubes in 2D confinement, $t$ is time and $\Delta t$ is time interval between angle measurements. We calculated the MSD similar to our calculations for determining mean square angular displacement (MSAD) of nanotubes by integrating over different time intervals.

Specifically, for a particular nanotube, we determined the MSD at various integral ($n$) multiples of $\Delta t$ ($\Delta t = 0.1$ seconds is the time interval between consecutive frames of the movie) by calculating the square of the difference in nanotube coordinates between images with time interval $n\Delta t$ between them as follows:

$$< (U(t + n\Delta t) - U(t))^2 > = 4D_Tn\Delta t$$

(6)

The plot of MSD for a nanotube as a function of time interval ($n\Delta t$) should produce a line with slope $4D_T$ (Supplementary Fig. S20a). We determined $D_T$ for individual nanotubes by fitting this plot to a line, determining the slope and dividing this slope by 4.
**Supplementary Fig. S20: Calculating $D_T$ of nanotubes as a function of nanotube length.** a, Mean squared displacement of 13 individual nanotubes versus time. Slope of the individual lines is equal to $4D_T$. b, Scatter plot of $D_T$ vs. nanotube length measurements. The blue curve indicates the least squares fit of the equation relating $D_T$ of a rod shaped particle to its aspect ratio $p$. Green dashed lines are confidence intervals representing two standard deviations.

We approximated a DNA nanotube as a rod shaped particle with aspect ratio ($p = L/d$), where $L$ is nanotube length and $d$ is nanotube diameter. We thus expect a relationship between $p$, $D_T$ and nanotube length of the form:

$$D_T = \frac{k_B T (\ln (p) - \gamma)}{3 \pi \eta_s L}$$

(7)

In the above expression, $k_B$ is Boltzmann constant, $T$ is absolute temperature, $\eta_s$ is dynamic viscosity and $\gamma$ is a correction factor for a rod shaped particle for more precise hydrodynamic calculation.

On fitting Eq. 7 to our experimental data (Supplementary Fig. S20b), we get the following relationship for $D_T$ as a function of $L$:

$$D_T = \frac{k_B T (\ln (p) - 0.15)}{3 \pi \eta_s L}$$

(8)

where, $L$ is nanotube length and $d = 0.015 \mu m$. 
3.6. Measuring the persistence length of DNA nanotubes

Accurate simulation of DNA nanotube dynamics required knowing their persistence length in order to calibrate the energy penalties for polymer bending. Further, to maximize the yield of point-to-point assembly using landmarks separated on micron length scales, these simulations suggested that the connecting polymers should have micron-scale persistence lengths (Supp. Figs S36-38). We measured the persistence length of DNA nanotubes (the length over which correlations in the orientation of a thermally fluctuating polymer contour decay exponentially) as described below\textsuperscript{14}.

Seeded DNA nanotubes were confined between passivated coverslips and glass sides separated by ~2 microns similar to our experiments for measuring translational diffusion constant of DNA nanotubes (See Supplementary Section 3.4). We recorded 100 continuous frames each integrated over 0.2 seconds. Some sample images of nanotubes in 2-dimensional confinement are shown in Supplementary Fig. S21.

Supplementary Fig. S21: Selected fluorescence microscope images showing a DNA nanotube confined between two glasses separated by 2 microns.

Persistence length\textsuperscript{14} (P) in a two-dimensional system is defined by:

\begin{equation}
< \hat{t}(x) \cdot \hat{t}(x + \Delta x) > = e^{-\Delta x / 2P}
\end{equation}

where, \(< \hat{t}(x) \cdot \hat{t}(x + \Delta x) >\) is defined as the tangent correlation, representing the mean dot product between two unit tangent vectors separated by a distance \(\Delta x\) along the contour. Nanotubes contours were traced using an automated algorithm\textsuperscript{14} that assigns coordinates spaced by 4 pixels (0.68 \(\mu\)m) along the contour. For each nanotube, we plotted the correlations between the angles of the tangent vectors to these contours as a function of distance between the tangent points. We considered distances 2 \(\mu\)m < \(\Delta x\) < 7.5 \(\mu\)m for equation fitting to excluded tangent correlations with less than 100 tangent pairs in the data set, following previous measurements of persistence lengths of other DNA nanostructures\textsuperscript{14}. 
Supplementary Fig. S22: Calculating the persistence length of DNA nanotubes. Plot (black) relating the mean correlation of tangent vector angles and distance between points along a nanotube’s contour. The red curve indicates the least squares fit of the data to Equation 8.

Fitting the data to Equation 9 yielded a DNA tile nanotube persistence length of $8.7 \pm 0.5 \, \mu m$. 
3.7. Estimating the end-to-end joining rates of DNA nanotubes in free solution

To calibrate the nanotube end-to-end joining criteria in our physical model, we needed an estimate of nanotube joining rate. Because it was difficult to measure joining in confinement, we instead measured joining in free solution and calibrated our model so that free polymers in simulation joined at the measured rate. While it is expected that the rate of joining of nanotubes should be length dependent because length changes translational diffusion (following the scaling law of \( \ln \frac{L}{L_0} \))\(^{15}\), for the range of nanotubes considered (approximately 1-5 \( \mu \)m), we measured only a single joining rate.

**Supplementary Fig. S23**: Schematic showing the composition of the initial individual seeded nanotube solutions before mixing, including the labelling schemes for the different components, and the types of species expected at the end of joining reaction. By tabulating the number of each type of species, we were able to determine (roughly) the concentrations of unjoined and joined nanotubes at different time points. These concentrations were used to fit a (length-independent) rate of joining that was then used to fit the joining energy in our model.
Our approach to estimate nanotube joining rate was to mix pre-grown type A seeded nanotubes and type B seeded nanotubes in equimolar concentrations in free solution and measure the fraction of nanotubes in joined state as a function of time by counting the number of species of different types from reaction aliquots deposited onto slides (Supplementary Fig. S23).

We grew 50 pM of seeded nanotubes over 15 hours at 32°C from purified A seeds. Purified A seeds were made by annealing a solution containing scaffold, staples, adapter strands and fluorescent labelling strands. We then applied the annealed seed solution to Amicon Ultra 100 kDa filters, centrifuged at 1000 g for 20 mins and resuspended with 1x TAE Mg\textsuperscript{2+} buffer. We repeated this 3 times to get seeds without excess staples, adapter and fluorescent labelling strands. We determined that purifying seeds to be important as excess adapters in solution affected the rate of nanotube joining. Seed A was labelled in ATTO647N and nanotubes grown from seed A were labelled using ATTO488 dye. We refer to this solution of seeded nanotubes as solution 1. Similarly, we made solution 2 where seeded nanotubes were grown over 15 hours at 32°C from purified B seeds. Here, Seed B was labelled in ATTO647N and nanotubes grown from seed B were labelled in Cy3 dye. Both these solution were then mixed and incubated at 32°C to produce solution 3. This solution has equimolar concentrations of both types of seeded nanotubes. We captured several images of solution 3 in Cy3, ATTO647N and ATTO488 channels after 0 hours, 10 hours and 20 hours post mixing.

At the end of the joining reaction, we expected solution 3 to have the following different species -- type A seeded nanotubes, type B seeded nanotubes, A-B joined nanotubes, A joined nanotubes (type A seeded nanotube joined to a B seed), B joined nanotubes (type B seeded nanotube joined to a A seed) and empty seeds (see Supplementary Fig. S23). We considered A-B joined nanotubes, A joined nanotubes and B joined nanotubes as successfully joined nanotubes and other structures as species that had not participated in a joining reaction.

To estimate nanotube end-to-end joining rate, we modelled the change in nanotube ends in our experiment with a simple reaction:

\[
A + B \rightarrow J
\]  
(10)

where \( A \) are free (unjoined) nanotubes of type A, \( B \) are free nanotubes of type B (see Supp. Fig. S23) and \( J \) are joined nanotubes. \( k_J \) is the second order forward reaction rate constant for the joining reaction. Since \([A] = [B]\) at the start of the joining process by design and the two species are depleted equally through joining reactions, we model the process using a single concentration that is equal to the concentration of nanotubes of either type. We call this quantity \( E \). Thus, the rate law for the above reaction is given by:

\[
\frac{d[E]}{dt} = -k_J[E]^2
\]  
(11)
where, $E_0$ is the initial concentration of free nanotube ends of either type A or B (25 pM for our experiments) and $f$ is the fraction of nanotubes of type A or B that are in the joined state.

Integrating Equation 11 to solve for $E$ as a function of time and substituting the expression for $E$ in Equation 12 into the solution, we get

$$f = \frac{k_j E_0 t}{1 + k_j E_0 t}$$

(13)

where, $t$ is the time during which the joining reaction is allowed to proceed.

To find the most likely joining rate $k_j$, we plotted the fraction of nanotubes of type A that were in the joined state as a function of time (0, 10 and 20 hours) and fit the $k_j$ value that best fit the points to Equation 13, as shown in Supplementary Fig. S24. This fit produced a most likely value of joining of $(k_j) = 2.9\pm1.7 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$.

**Supplementary Fig. S24: Fitting the length-independent rate of nanotube end-to-end joining in free solution.** The blue curve indicates the least squares fit of the equation 13 relating fraction of nanotube ends in joined state ($f$) to time. Green dashed lines are confidence intervals representing two standard deviations.
3.8. Selective Melting of Unconnected Nanotubes

A simple thought argument suggests that it should be possible to melt unconnected nanotubes after point-to-point assembly while leaving connected nanotubes intact by either slightly heating the solution or by exchanging the buffer with a solution that contains no free tiles. Without free tiles, unconnected nanotubes should melt because the free tile concentration is close to 0, and the critical concentration of tiles attaching to nanotube ends by two sticky end bonds (e.g. at nanotube free ends) is about 15 nM (such a critical concentration implies that growth should occur when 36 nM of tiles are present, as during the assembly of nanotube connections). The detachment of tiles from the nanotube end exposes new tiles that are now attached to the nanotube by two sticky end bonds. Processive detachment of multiple tile layers would melt the entire nanotube. Likewise, heating the solution sufficiently would increase the critical concentration of tiles and thus also cause nanotubes to melt at their ends.

In contrast, connected nanotubes with no defects have no site where a tile is attached only by two sticky end bonds. As a result, scission of a connected nanotube or the introduction of other defects is required before melting can occur. However, it would be expected that such an inability to melt at different temperatures would require that nanotubes have few if any defects.

To test whether it was possible to selectively melt unconnected nanotubes while leaving unconnected nanotubes intact, we assembled nanotube interconnects between randomly deposited A and B seeds following the protocol described in Supplementary Section 3.3. After 20 hours of incubation, we exchanged the tile solution with reaction buffer containing no tiles while maintaining the temperature at 32°C. Time-lapse movies of DNA nanotubes were recorded over 15 hours.

After the 10 hours incubation period, we found that all (n=28) of the unconnected nanotubes tracked completely melted away, while majority (~95%, n=65 analysed) of the nanotube interconnections appeared to remain intact (Supplementary Fig. S25). Tracking nanotube lengths of unconnected nanotubes showed that nanotube length decreased monotonically at a constant rate of 0.30 ± 0.08 µm/hour for majority of the nanotubes (Fig 4e in the main paper).

These results suggested that simple separation via buffer exchange that would remove free tiles from solution very effectively removed undesired, unconnected nanotubes. Such a protocol would be useful in cases where it was of interest to connect a nanotube at one landmark A to one of many landmark B structures; after connection the nanotubes that grew from the landmark B structures that did not connect could be easily removed. Further, such a separation process could make it possible to iterate over multiple cycles to form a connection: the nanotubes growing from both landmarks in a pair that did not connect could be melted away; tiles could then be re-added to try to form a connection a second time.
Supplementary Fig. S25: Selected time-lapse images showing preferential melting of unconnected nanotubes. Yellow ellipses label nanotube interconnects; other nanotubes shown are connected to only one seed. Over the course of the experiment, nanotubes connected to only one seed melt, while nanotubes that connect two seeds stay intact. Scale bars are 10µm.

We further wanted to understand whether connected nanotubes did not melt quickly where unconnected nanotubes melt readily because the connected nanotubes must first disconnect before melting, and further, whether there was an energy barrier to disconnection. We performed three melting experiments at 32°C, 35°C and 37°C. In the case of the 35°C and 37°C experiments, the solution was heated by setting the external microscope temperature controller to the relevant temperature and waiting 1 hour for the nanotubes, dish and objective to equilibrate to the new temperature. After such equilibration, the buffer containing 36 nM of tiles was exchanged for a buffer without any tiles. We monitored the solutions over 10-18 hrs using snapshots taken every 20 minutes. In each case, a programmable motorized stage allowed us to follow about 50-75 isolated pairs of landmarks connected by nanotubes and about 20-60 nanotubes that were not connected at the start of the experiment.
As expected, we observed that unconnected nanotubes melted from their ends, with lengths shortening at increasing rates at increasing temperatures and with such decreases beginning immediately for all nanotubes that were tracked. All nanotubes melted through a process where nanotubes decreased gradually in length, supporting the idea that nanotubes melt row by row via the gradually detachment of monomers at the nanotube ends (Supp. Fig. 26).

Supplementary Fig. S26: Changes in length of nanotubes observed during melting processes at three different temperatures. The length of each nanotube decreases gradually, with the rate of decrease being determined by the temperature. Length data was processed using the inbuilt smooth function in Matlab to remove noise that arises due to the difficulty in measurement of nanotube contour length as nanotubes diffuse in and out of optical focus.

While all unconnected nanotubes began to decrease in length immediately after the start of the melting experiment, almost all connected nanotubes remained at a constant size for a significant period after a buffer exchange to remove free tiles and (in the case of experiments performed at 35ºC and 37ºC with an increase in temperature). In all cases, a decrease in connected nanotube length as part of the melting process was preceded by a scission event which exposed 1-2 nanotube ends. Scission events appeared at different times during the experimental time span with some nanotubes remaining connected at the end of the experiment. In the great majority of cases at all temperatures (~75%), scission occurred such that a nanotube became disconnected from a seed. The disconnection of nanotubes at the seed-nanotube interface would be expected to be more likely than scission of nanotubes in a region without lattice defects because the seed and the nanotube have slightly different DNA crossover patterns. Distortion is expected at the interface and has been observed in related structures previously16. The fact that such sites represent the most frequent type of scission suggests that most nanotubes have few if any sites with multiple lattice defects.

To understand how scission occurs, we further characterized when scission events at the nanotube-seed interface occurred. If scission is characterized by a single event that does not occur immediately because of an energy barrier to the initiation of scission, it would be expected that scission events would be observed at times that were exponentially distributed, with the rate of scission being the time constant $\tau$ of the distribution. The energy barrier to scission would be proportional to the log of this rate17.
To determine whether scission times were exponentially distributed, we initially plotted the times at which scission events at the seed-tube interface were observed. Qualitatively, these times appeared consistent with an exponential distribution (Supp. Fig. S27). However, more information was needed to determine an effective scission rate because some nanotubes did not break over the course of the experiment and others broke in the middle. To estimate the rate of seed-nanotube scission at each of the temperatures studied, we used a maximum likelihood approach that integrated information about all of the connected nanotubes tracked in a given experiment. While more data would be required for precise measurement of the scission energy, a plot of the log of the measured rates of scission as a function of inverse temperature (i.e. an Arrhenius plot) is linear for the temperatures measured (Supp. Fig. S28). In a linear Arrhenius plot, the slope of the line is the activation energy (scaled by -1/R, where R is the universal gas constant).

**Supplementary Fig. S27:** Histogram of times at which scission at the nanotube-seed interface was observed during experiments at 32°C, 35°C and 37°C. Only two scission events were observed at 32°C. Fits to the resulting time distributions are shown that either a) include only the observed scission events in a fit (neglecting the possibility that scission could occur after the experiment’s duration) in red and b) a fit to the distribution that includes all nanotubes tracked in green. The latter fit was used to determine the scission rate.
Supplementary Fig. S28: Arrhenius plot relating the rate of scission of nanotubes at the seed-nanotube interface to inverse temperature. The slope in such a plot is $-\frac{E}{R}$, where $E$ is the activation energy and $R$ is the universal gas constant.
4. Coarse-grained simulations of nanotube point-to-point assembly

To understand how different aspects of a point-to-point assembly process control its outcome, we develop a coarse-grained model in which we study point-to-point assembly. Within the model, each DNA nanotube attached to a landmark is represented as a chain of beads (shown in Supplementary Fig. S29a) such that the interactions between the beads are designed so that the dynamics of the chain reproduces worm-like chain (WLC) behaviour (Supplementary Fig. S29b). Growth of a nanotubes is modelled as occasional addition of beads to the chain. As described below, joining is allowed in the model when the chain ends come sufficiently close and their orientations are such that forming a single chain would not induce excessive bending energy in the fused chain (Supplementary Fig. S29c).

To make simulation computationally feasible, the model is highly coarse grained: mean distance between neighbouring beads in the chain represents ten rows of monomers, or 0.143 µm of polymer contour length. The energetics of bending are set by the persistence length of 8.7 µm that we measured for DNA nanotubes assembled under the conditions used in this study (Supplementary Section 3.6). The timescale of growth is set by experimental measurements, and the time scale of polymer motion is fit to either the measured rotational or translation diffusion constant, and the joining criteria is fit to the rates of nanotube joining in bulk.

![Supplementary Fig. S29](image)

Supplementary Fig. S29: (a) A schematic representation of anchored nanotubes as chains of connected beads. Growth is modeled as the stochastic kinetic addition of beads with rate constant $k_{growth}$. (b) Two neighbouring beads. Beads can rotate and the connections between them can stretch. The interaction potentials of beads depend on the distance between the beads, the length $r_{li+1}$ and their orientations, defined by the angles $\theta$ and $\psi$. (c) A schematic representation of how joining may occur in the model. Joining is allowed when the ends of the two nanotube chains are sufficiently close (see text) and the interaction potential of the beads involved in the joining process, $E_{join}$, is below the threshold $E_{cut}$.

We use these simulations first to determine whether a simple model of nanotube polymer motion, growth and joining can explain the observed measurements of point-to-point assembly yield and the lengths of polymer connections. While the coarse-grained nature of the model we use and the variation in experimental measurements mean that the model can make a variety of predictions, the model’s predictions of point-to-point assembly
outcomes (in terms of connection yields and lengths) are consistent with what is observed in experiments for some choices of model parameters. Having established this correspondence, we use the model to better understand how nanotube persistence length and the nature of diffusion may affect how point-to-point assembly proceeds.

4.1. The wormlike chain (WLC) model

4.1.1. Interaction potentials

A WLC is a standard model for describing semiflexible polymers such as DNA nanotubes. We model DNA nanotube dynamics as WLC dynamics with a chain of beads using the discrete model for WLC simulations introduced by Brackley et al. Dynamics of nanotube motion is then carried out as a molecular dynamics simulation.

The interaction potential $V_{\text{model}}$ determines the energy of a particular configuration of chains. Details of the simulation mechanisms and how the timescale of dynamics was set are described in the next section. The interaction potential of the beads in the model is

$$V_{\text{model}} = \sum_{i,j \notin <i,j>} V_{\text{exc}}(r_{ij}) + \sum_i \left[ V_{\text{FENE}}(r_{i+1}) + V_{\text{angle}}(u_i, u_{i+1}) + V_{\text{align}}(u_i, r_{i+1}) \right]$$

The specific energy terms are described in detail below. Briefly, the first sum accounts for excluded volume and is thus taken over all pairs of beads that are not adjacent. The second sum includes the energetics associated with bending and stretching of the chain and is the sum of energies between adjacent beads $i$ and $i+1$.

The excluded volume potential $V_{\text{exc}}$ ensures that no two portions of the chain are too close and that an excluded volume is maintained around the chain. It is defined as:

$$V_{\text{exc}}(r_{ij}) = \begin{cases} V_{\text{LJ}}(r, \sigma) & \text{if } r < r^* \\ V_{\text{smooth}}(r, b, r^c) & \text{if } r^* < r < r^c \\ 0 & \text{otherwise} \end{cases}$$

Where $V_{\text{LJ}}$ is a Lennard-Jones potential and $V_{\text{smooth}}$ is introduced so that $V_{\text{exc}}(r)$ is differentiable but remains short-range to make the simulation computationally tractable. These potentials are defined as follows:

$$V_{\text{LJ}} = 4 \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6$$

$$V_{\text{smooth}}(r, b, r^c) = b (r^c - r)^2$$

The potential $V_{\text{FENE}}$ governs stretching and ensures that each pair of beads is separated by an average distance $r_0$:
The energies of bending $V_{\text{angle}}$ and $V_{\text{align}}$ of the chain are defined as

$$V_{\text{angle}}(u_i, u_{i+1}) = \kappa_b (1 - \cos \theta_i)$$

and

$$V_{\text{align}}(u_i, r_{i+1}) = \kappa_a (1 - \cos \psi_i)$$

where $u_i$ and $u_{i+1}$ are the orientation vectors of beads $i$ and $i+1$ respectively and the angles $\theta_i$ and $\psi_i$ are defined as

$$\cos \theta_i = u_i \cdot u_{i+1}$$

and

$$\cos \psi_i = u_i \cdot r_{i+1} / \|r_{i+1}\|$$

Here, $r_{i+1}$ is the vector connecting the centres of mass of beads $i$ and $i+1$. These vectors and angles are schematically illustrated in Supplementary Fig. S29b.

To model the dynamics of the nanotubes such that one nanotube end is anchored to a surface, we introduce potential $V_{\text{anchor}}$ defined as

$$V_{\text{anchor}} = \sum_f V_{\text{spring}}(r_f) + \sum_i V_{\text{plane}}(z_i)$$

where the first sum is over all chains in the simulation and the second sum runs over all beads in the simulation. The spring potential between the center of mass of the first bead of the chain and the landmark is defined as follows:

$$V_{\text{spring}}(r_f) = k_{\text{spring}} (r_f - r_s)^2$$

where $r_f$ is the position of the centre of mass of the first bead of the chain and $r_s$ is the position of the landmark.

The repulsion potential $V_{\text{plane}}$ is included to model the situation where nanotubes are anchored on a glass surface (located at $z = 0$) through which the chain cannot diffuse, we include a repulsion potential that acts on all the beads of the chain, where $z_i$ is the $z$-coordinate the position of $i$-th bead:

$$V_{\text{plane}}(z_i) = \begin{cases} k_{\text{plane}} z_i^2 & \text{if } z_i < 0 \\ 0 & \text{if } z_i \geq 0 \end{cases}$$
In simulations of untethered chains (such as for parameterization of the bulk joining rates) the \( V_{spring} \) and \( V_{plane} \) terms are omitted from the potential, and the interaction potential in the simulation is

\[
V_{simulation} = V_{model}
\]  

as given by Eq. 14. For simulations of a chain (or chains) anchored to a surface (such as simulations of rotational diffusion or joining of two chains), the simulation potential is

\[
V_{simulation} = V_{model} + V_{anchor}
\]  

The values of the parameters of simulation potentials are listed in Supp. Table S1 in simulation units, where the energy unit \( k_B T = 1 \) and one length unit corresponds to 0.12 \( \mu \)m. The unit length for the simulation was chosen such that the mean distance between neighbouring beads sampled in the simulation corresponds to 0.143 \( \mu \)m, the length of 10 rows of DNA tiles within a nanotube. We chose \( V_{FENE} \) parameters from among the wide range of suitable parameters that reproduce the desired WLC behaviour. As we do not subject the chains to any tension in our simulations, the results of the simulations are not sensitive to particular choice of parameters for \( V_{FENE} \). The excluded volume interaction radius and the values that define the extent of the potential were chosen so that no chain intersection was observed. The parameter \( \kappa_b \) was set so that bending of the chain in the simulation would correspond to the measured persistence length of the nanotubes, 8.7 \( \mu \)m. The value of \( \kappa_a \) was chosen to be larger than \( \kappa_b \). As long as \( \kappa_a \) is on the same order or larger than \( \kappa_b \), the WLC behavior with the desired persistence length set by \( \kappa_b \) is reproduced. The value of \( k_{plane} \) is set so that no diffusion of beads across the location of the glass surface were observed in simulations. In order to minimize the effects of confining the coarse-grained chains to a set of configurations based on a specific anchor locations, we chose the value of \( k_{spring} \) that allows the end of the chain to move slightly, generally distances not larger than the size of individual beads. In tests, however, setting \( k_{spring} \) to a much larger value (100) did not significantly change the outcome of simulations.

<table>
<thead>
<tr>
<th>Backbone spring: ( V_{FENE} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \varepsilon = 76.8 )  ( \Delta = 1.6 )  ( \delta r^0 = 1.0 )</td>
</tr>
<tr>
<td>Excluded volume: ( V_{exc} )</td>
</tr>
<tr>
<td>( \sigma = 1.0 )  ( r^* = 0.9 )  ( b = 724.312 )  ( r^c = 0.995718 )</td>
</tr>
<tr>
<td>Bending: ( V_{angle} \wedge V_{align} )</td>
</tr>
<tr>
<td>( \kappa_b = 60.8 )  ( \kappa_a = 1000 )</td>
</tr>
<tr>
<td>Surface potentials: ( V_{spring} \wedge V_{plane} )</td>
</tr>
<tr>
<td>( k_{spring} = 1 )  ( k_{plane} = 1 )</td>
</tr>
</tbody>
</table>

**Supplementary Table S1:** Values for the parameters used in simulation, shown in simulation units.
4.1.2 Simulation

The model was implemented in the oxDNA simulation package (dna.physics.ox.ac.uk); the source code is available upon request. All the simulations were carried out using molecular dynamics (MD) simulations with an Andersen-like thermostat; details of the implementation of the thermostat can be found in Russo et al\(^{19}\). The thermostat couples the simulated system to a heat bath (with \(k_B T = 1\) in simulation energy units) so that the velocities and angular momenta of beads in the simulation follow the Maxwell-Boltzmann distribution. Motion was simulated using a molecular dynamics simulation that numerically integrates equations of motion using a Velocity Verlet algorithm for a given number of integration steps (103 in our case) with a time-step of \(\Delta t = 0.005\) simulation units. After this set of integration steps, the momenta of a randomly chosen fraction \(p\) of particles are resampled as random vectors drawn from a Gaussian distribution at corresponding temperature. The fraction \(p\) was chosen such that the translational diffusion coefficient of a single particle \(D_p\) in the simulation is 2.5 in simulation units. These values were chosen so that the simulation was numerically stable and such that the mean energy sampled in the MD simulation of diffusion of a chain did not deviate from the mean measured using Monte Carlo simulation of the same process. The chosen time-step \(\Delta t\) is significantly smaller than the timescale at which the chain moves in the simulation. Choosing a smaller \(\Delta t\) would therefore not affect predictions of the model. It would, however, increase simulation times.
4.2. Choosing model parameters to recapitulate observed kinetics of nanotube diffusion and joining

Supplementary Section 4.1 described the parameters of the model and how they were chosen to match observations of nanotube structure and stiffness. To develop a simulation in which the dynamics of polymer motions, growth and joining occur at the proper rates, it was necessary to find the proper relative time scales for these processes. Because our goal was to develop a simulation consistent with the experimental process of point-to-point assembly, we set these time scales by creating a correspondence between the timescale of the simulation and the timescale within experiments in such a way that the rates of experimental processes (growth, joining and rotational and translational diffusion) were similar in experiments and simulation. This correspondence also made it possible to compare simulations whose total time would be expected to be comparable to experiments lasting particular times.

As a caveat, however, it is notoriously difficult to properly set the timescale of chemical simulations to match the timescale of the corresponding experimental system. This difficulty is further exacerbated in coarse-grained simulations: the scaling between different processes that enable simulations and experiment to correspond may not be identical, so that there is no single timescale that properly represents all the motions of the experimental system. For example, in our simulation chains do not increase in size over the time it takes to add 5-10 tile rows, so that diffusion rates do not change during this period in the simulation. In reality, however, the addition of any tile changes the diffusion rate of the resulting structure. As a result, the correspondence should not be viewed as absolute, but instead as an approximate guideline.

To set reasonable timescales for the different processes in our simulation, diffusion, growth and joining, we chose simulation parameters that produced dynamics with a close overall fit to experimental measurements.

4.2.1. Timescales for nanotube diffusion

We set the timescale for polymer motion so that the rate of diffusion in the simulations matched the rate observed in experiments. We initially elected to build simulations based on a correspondence between rotational diffusion rates in simulation and experiment (rather than translational diffusion rates) because anchored nanotubes should not exhibit significant translational motion, and instead search for the corresponding end primarily through rotational motion.

To determine a time scale in which the (length-dependent) rate of rotational diffusion in simulation matched rotational diffusion rate of nanotubes in experiments, we first measured the rotational diffusion rate of polymers in the simulation in simulation time units. We measured the mean square angular displacement (MSAD) of a simulated polymer anchored to a surface (using the simulation potential from Eq. 26) at one end using the same expression used to measure MSAD in experiments:
$MSAD = \left( (\theta(t_0 + \Delta t) - \theta(t_0))^2 \right) = 2Z_r\Delta t \quad (27)$

where $\theta(t_0 + \Delta t) - \theta(t_0)$ is the angle between the projections of the end-to-end vectors of the simulated chain onto the $z = 0$ plane at times $t_0 + \Delta t$ and $t_0$ respectively. We measured the MSAD of end-anchored simulated polymers for different lengths $L$ between different time intervals and used the method described in Supplementary Section 3.4 to fit a best value $Z_r^{\text{sim}}(L)$ for each polymer. We then converted each measured $Z_r^{\text{sim}}(L)$ value to the corresponding rotational diffusion coefficient $D_r^{\text{sim}}(2L)$ using the method shown in Supplementary Fig. S19a.

To obtain a time correspondence between simulation and experimental time from these simulated diffusion constants, we fit factor $\alpha$ so that $\alpha D_r^{\text{sim}}(L)$ best agreed with our experimental fit for the length-dependent rotational diffusion constants $D_r(L)$ (Eq. 4) over the range of lengths we examined. With such a scaling, one simulation time unit corresponds to $1/\alpha$ seconds. For our best fit, $1 tsim = 0.004$ s. The rotational diffusion coefficients measured for individual nanotubes in experiments, the fit to $D_r(L)$ based on these measurements and the values $\alpha D_r^{\text{sim}}(L)$ for the best fit $\alpha$ are plotted together in Supp. Fig. S30.

With this correspondence it takes about 1 s of computer time on a single 2.4 GHz processor to simulate 1 s of the diffusion of a single 3.6 $\mu$m long nanotube, so that simulating a single 70 hour point-to-point assembly process on such a computer takes several days. Because the simulation time increases as fourth power as the grain resolution of the chain modeled is decreased linearly, simulating such a point-to-point assembly process using a standard computer processor was not tractable for more fine-grained simulation.

Supplementary Fig. S30: Rotational diffusion coefficients ($D_r$) inferred from experimental measurements (red pluses) and in simulation (green crosses) given a correspondence between simulation time and real time where $1 tsim = 0.004$ s. The rotational diffusion constant as a function of polymer length inferred from experimental data, Eq. 4 is shown in blue.
We also performed an analogous process to use experimentally measured translational diffusion coefficients to establish a correspondence between experiments and simulations. While exploration of nanotube ends during point-to-point assembly occurs primarily through rotation, in principle an ideal simulation would have the same timescale relationships, so ensuring that this correspondence is close to the one established for rotational diffusion is an important check on the validity of the experimental measurements and the design of the simulation.

To establish a timescale where the translational diffusion rates in experiment and simulation would be the same, we measured the mean squared displacement (MSD) (as defined in Eq. 5, but with prefactor 6 instead of 4, as the MSD was measured in three dimensions) for the centres of mass of nanotubes in simulation ranging in length from 1 to 6 µm where the chains were freely diffusing in the bulk (with simulation potential from Eq. 25). We used these values to determine translational diffusion coefficients for each of the nanotubes following the methods in Supp. Section 3.4. We then found a value \( \alpha \) such that \( \alpha D_T^{\text{sim}}(L) \) best agreed with our experimental fit for the length-dependent rotational diffusion constants \( D_T(L) \) (Eq. 8). The time correspondence between simulations and experiment established using this procedure is \( t_{\text{sim}} = 0.002 \) s. The translational diffusion coefficients measured for individual nanotubes in experiments, the fit to \( D_T(L) \) based on these measurements and the values \( \alpha D_T^{\text{sim}}(L) \) for the best fit \( \alpha \) are plotted together in Supp. Fig. S31.

Thus, choosing a timescale based on measured (length-dependent) rates of rotational and translation diffusion thus results in slightly different correspondences between the timescales of the simulation and experiments. Some difference is expected because of the limitations of coarse-grained simulation. We consider the effect of choice of each of these timescales for comparison between simulation and experiment in Supplementary Section 4.3.
Supplementary Fig. S31: Translational diffusion coefficients ($D_T$) inferred from experimental measurements (red pluses) and in simulation (green crosses) given a correspondence between simulation time and real time where $1 \ t_{sim} = 0.004 \ s$. The translational diffusion constant as a function of polymer length inferred from experimental data, Eq. 8, is shown in blue.

4.2.2. Modelling nanotube growth

We model nanotube growth as the occasional addition of nanotube beads using Gillespie’s algorithm to calculate the next time for bead addition. The observed growth rate of the nanotubes measured using a change in the 2-dimensional project of nanotube length is $0.11 \ \mu m / hr$. To model the growth of the nanotube itself, we used a growth rate of $k_{growth} = 0.14 \ \mu m / hr$, which corresponds approximately to a rate of addition of one bead per hour. This rate is translated to a rate in simulation using the correspondence of simulation time and experimental time for polymer diffusion for the relevant simulation.

4.2.3. Chain joining

The final task in building our simulations was to develop a way to model the process of nanotube joining in such a way that the results were consistent with rates of nanotube joining that have been observed in bulk. This task was particularly challenging given that whether two nanotubes can join is likely dependent on the configuration and position of the nanotube ends, but our physical model of nanotube configuration consists of components with a size greater than 100 nm in chain length. Thus, the true endpoint of the chain is not tracked with precision in the simulation. Further, nanotube growth in simulation occurs in much larger steps (10 monomer rows) than in practice (single
monomers or monomer rows), so that after the addition of a bead, the position of the chain’s endpoint changes dramatically. Our goal in developing a simulation criterion was therefore not to closely model physical reality through the mechanism of simulation but instead to develop a process that recapitulated as best as was feasible the rate of joining observed in experimental systems.

Supplementary Fig. S32. Average joining rates observed in simulation between 1.6 μm long nanotubes in bulk for different values of $E_{\text{cut}}$ (green). The red line shows a linear fit to this data. The blue dashed lines show the upper and lower limits of joining values consistent with the standard deviations of the measured joining rate averaged across nanotubes of different lengths (i.e. those within 2 standard deviations).

Given the high degree of coarse-graining of chain length, both the location of last bead in the chain and the location of the second to last bead in the chain could both be considered as reasonable estimates for the true endpoint of the chain. To account for this fact, we developed a joining criterion where two chains could become joined if the position and the orientations of either the last or second to last bead of each chain were such that the energy costs of connecting the beads was below a given threshold. Specifically, to ask whether the chains could become joined between bead $i$ of chain A and bead $j$ of chain B, we evaluate the potential function:

$$V_{\text{join}}(r_{ij}, u_i, u_j, r_{ji}) = V_{\text{angle}}(u_j, u_i) + V_{\text{align}}(u_i, r_{ij}) + V_{\text{align}}(u_j, r_{ji}) + V_{\text{FENE}}(r_{ij})$$

(28)

where $r_{ij}$ and $r_{ji}$ are the vectors that connect the centres of mass of beads $i$ and $j$ and $i$ respectively, $u_i$ is the orientation vector for bead $i$ and $u_j$ is the orientation vector for bead $j$. If chain A has $N_a$ beads and chain B has $N_b$ beads, at each time step this potential function is applied to
1. the $Na^{ih}$ bead of chain A and the $Nb^{ih}$ bead of chain B,
2. the $Na^{1st}$ bead of chain A and the $Nb^{ih}$ bead of chain B,
3. the $Na^{ih}$ bead of chain A and the $Nb^{1st}$ bead of chain B,
4. the $Na^{1st}$ bead of chain A and the $Nb^{1st}$ bead of chain B.

If the smallest of the four resulting potentials is below a threshold energy value $E_{cut}$ then the chains are considered joined. A schematic of this joining criterion (for a single pair of beads) is shown in Supplementary Fig. S29c.

Given this mechanism of joining, we needed to choose a value of $E_{cut}$ that allowed joining to occur at a rate consistent with the rate observed in experiments. To do so, we first measured the rate of joining observed in simulation between two 1.6 µm long nanotubes in a simulation box with periodic boundary conditions of volume 14 µm³ for different values of $E_{cut}$ using the time correspondence between experiment and simulation obtained using the experimentally measured translational diffusion rate ($1 \ t_{sim} = 0.002$ s) and compared these rates with the average joining rate between nanotubes of a variety of lengths measured in experiment as described in Supp. Section 3.7. Supplementary Fig. S32 shows the simulated rates of joining for different values of $E_{cut}$ and how they correspond with the rate of joining measured in experiments. Roughly the rate of $E_{cut}$ values that are consistent with experimental values are those between 30 $k_BT$ and 80 $k_BT$. We chose the edges of this range, i.e. 30 $k_BT$ and 80 $k_BT$, as values of $E_{cut}$ to investigate further in simulation.

In developing this method for simulation of joining, we also considered different methods for determining whether and how joining should occur. Notably, we considered the possibility of using a stochastic criterion for joining, in which the specific orientation of chain termini is ignored, but such that whenever the ends of the two chains are closer than a particular distance the chains would become joined with probability $p_{join}$. This model reflects the limitations of a coarse-grained model to effectively the particular orientation of the chains at the joining tips. We found, however, that such a stochastic approach was not able to effectively recapitulate the rates of joining observed in experiments.
4.3. Simulations of point-to-point assembly and comparison with experiment

We ran our simulations using a complete set of parameters (or ranges of parameters) that were deemed in isolation consistent with measurements of individual rates, lengths, and other values from experiment and measured the yields of the formation of point-to-point connection and the range of connection lengths formed as a function of interlandmark distance. To collect this information, we chose a set of interlandmark distances between 1.7 \( \mu \)m and 12 \( \mu \)m, on order the range of interlandmark distances observed within experiments, and ran multiple simulations of point-to-point assembly for each distance.

Within each simulation, we consider the motion and growth of two chains, where the first bead of each respective chain is tethered using the \( V_{\text{spring}} \) potential to points on the simulated surface such that their separation across the plane is equal to the interlandmark distance being considered. Because connection is not possible until the chain length is close to or greater than interlandmark distances, simulation of chain motion begins when the total chain length nears the interlandmark distance. Yields are determined as the fraction of simulations for a particular distance where connections formed after the prescribed simulated amount of growth time, with error bars representing one standard deviation as determined using bootstrapping.

To determine whether any of our parameters produced yield and connection length results that were consistent with our experiments, we ran simulations with a single value of \( k_{\text{growth}} \) and parameter values set by the measured nanotube persistence length and three different sets possible conversion factors between the timescales of simulation and experiment and energy thresholds below which joining can occur.

We initially considered the case of lower rates of joining and faster growth rates, i.e. \( E_{\text{cut}} = 30 k_B T \) and \( 1 t^{\text{sim}} = 0.004 \) s (Supp. Fig. S33). These simulations showed yields of point-to-point significantly lower than those observed in experiment. Raising the rate of joining by raising the energy threshold below which joining could occur to \( E_{\text{cut}} = 80 k_B T \) significantly increased yields, although the results were still lower than those observed in experiments (Supp. Fig. S34). Further changing the time scale conversion to \( 1 t^{\text{sim}} = 0.002 \) s to allow more time for diffusive exploration further improved yields (Supp. Fig. S35). The resulting yields and connection lengths, using parameters that were each consistent with diffusion rates, joining rates and growth rates measured separately in experiments were consistent with point-to-point assembly yields and lengths of connections observed in experiments (Fig. 2c and Supp. Fig. S14).

It is likely that even better agreement between experimental data and simulation results could be obtained by further fine-tuning the value of \( E_{\text{cut}} \) and the time correspondence. However, given the uncertainty in the experimental measurements, the limitations of the simulations, and the computationally intensive nature of the simulation process, we did not pursue further optimization of simulation parameters governing joining rates or time scale.
Supplementary Fig. S33: Results of simulated point-to-point assembly using joining criterion $E_{cut} = 30k_B T$ and simulation and experiment time correspondence $t_{sim} = 0.004$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Ten simulated point-to-point assembly processes were run for each interlandmark distance considered.

Supplementary Fig. S34: Results of simulated point-to-point assembly using joining criterion $E_{cut} = 80k_B T$ and simulation and experiment time correspondence $t_{sim} = 0.004$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Twenty simulated point-to-point assembly processes were run for each interlandmark distance considered.
4.4. Exploring the effect of persistence length on point-to-point assembly yields and the lengths of connections using simulations

Having developed a simulation whose dynamics and parameters were consistent with the point-to-point assembly process performed experimentally, we used this simulation to study how point-to-point assembly would be affected by changes to the persistence length of the polymers forming the connections. One limitation of such a study is that a change in polymer persistence length would be likely not only to affect the degree of bending of the polymers participating in the process, but also the rates of diffusion of the polymers; in our study we ignored such changes in the diffusion rates because it was not clear how to predict these changes in a reasonable way.

Supplementary Fig. S35: Results of simulated point-to-point assembly using joining criterion $E_{\text{cut}} = 80k_BT$ and simulation and experiment time correspondence $1 \ t_{\text{sim}} = 0.002$ s. (Left) Yields. This data is also shown in Fig. 3e in the main text. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Twenty simulated point-to-point assembly processes were run for each interlandmark distance considered.
Supplementary Fig. S36: Results of simulated point-to-point assembly for persistence length 0.87 µm using joining criterion $E_{cut} = 80k_B T$ and simulation and experiment time correspondence $t_{sim} = 0.002$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Twenty simulated point-to-point assembly processes were run for each interlandmark distance considered.

We used the parameter values $E_{cut} = 80k_B T$ and simulation and experiment time correspondence $t_{sim} = 0.002$ s, which we found in the previous section to correspond best to our experimental results of the parameter values considered, and in several sets of simulations changed the $\kappa_h$ values that govern chain bending energies so that these values corresponded to persistence lengths that were both more flexible (0.87 µm) and more rigid (87 µm and 1 mm) than the DNA nanotubes studied here, whose persistence length was measured to be 8.7 µm. As a reference point, microtubules have a persistence length on order 1 mm. Given the degree of coarse-graining in our simulation, it was not possible to simulate point-to-point assembly of polymers significantly more flexible than 0.87 µm; the effects of choosing polymers that are flexible on the scale of the interlandmark distance is explored qualitatively in Supp. Section 5.

The results of simulations of point-to-point assembly for these persistence lengths are shown in Supplementary Fig.s S36-38. Snapshots of typically connected nanotubes for each persistence length are shown in Supplementary Fig. S39. For these simulations, we only considered interlandmark distances between 3.6 µm and 10 µm.
Supplementary Fig. S37: Results of simulated point-to-point assembly for persistence length 87 µm using joining criterion $E_{cut} = 80k_BT$ and simulation and experiment time correspondence $t_{sim} = 0.002$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Twenty simulated point-to-point assembly processes were run for each interlandmark distance considered.

The simulations illustrate how both decreasing and increasing persistence length while maintaining the interlandmark distances can decrease the effectiveness of point-to-point assembly. As shown in Supplementary Fig. S36, at a persistence length of 0.87 µm (smaller than the persistence length of the DNA tile nanotubes considered in this work), connections take longer to form, so that at a given time yields are lower, in particular at larger interlandmark distances. Further, the connections that form are longer on average and more varied in their lengths than when stiffer polymers are considered. The stiffest polymers considered, with a persistence length of 1mm (Supplementary Fig. S38), form connections at significantly lower rates than polymers of other stiffnesses. This lowered yield is due to the fact that the chains must be very well aligned for joining in order for chain bending energies not to exceed the bending energy threshold for joining. Such alignment is possible only within a short time window, when the sum of the growing chain lengths is almost exactly same as the interlandmark distance. When connections do form using such stiff polymers, however, they are very direct.
Supplementary Fig. S38: Results of simulated point-to-point assembly for persistence length 1 mm using joining criterion $E_{cut} = 80k_BT$ and simulation and experiment time correspondence $t_{sim} = 0.002$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. Twenty simulated point-to-point assembly processes were run for each interlandmark distance considered.

These simulations also indicate that a tight range of persistence lengths are not required for point-to-point assembly to be successful. Supplementary Fig. S37 shows the results of a simulated point-to-point assembly process where the chains have a persistence length 10 times larger than that of the DNA nanotubes used in experiments. Yields of point-to-point assembly in this case were similar to those of much more flexible polymers. Notably, the lengths of connections that form are much closer than the interlandmark distances across which these connections form than those observed in experiment. These results may suggest that using polymers with persistence lengths larger than, but still on the order of interlandmark distances can result in the reliable formation of straight, efficient linkages.

We further study the effect of persistence length on point-to-point assembly yields and connection lengths using an alternative approach in Supplementary Section 5.
Supplementary Fig. S39: Sample configurations of chains at the point of connection formed in simulated point-to-point assembly processes. The landmarks separated by 3.6 μm. Configurations are shown for chain persistence lengths of (a) 0.87 μm (b) 8.7 μm (c) 87 μm and (d) 1 mm.
4.5. The effect of restrictions on the rotational diffusion of nanotubes on point-to-point assembly

Experimental characterization of nanotube rotation about the landmark indicated that a great majority of nanotubes do not rotate freely about their seed anchor, as was assumed in process design and in simulation (Supplementary Section 3.2). We further observed that different nanotubes diffused over different ranges of angles. From an intuitive point of view, the inability of nanotubes to explore all rotational angles could prevent nanotubes from being able to form configurations that would allow connection in many cases. For example, if a pair of nanotubes could only diffuse across angles in which their anchored ends were oriented directly away from one another, connection would be much more difficult (Supp. Fig. S40). Yet despite these potential limitations we observed that connections formed reliably between most pairs of landmarks given enough time to do so. We used simulations of point-to-point assembly to explore more rigorously how restricting the range of angles that nanotubes can be positioned at their anchor effects the process of point-to-point assembly.

Restrictions on nanotube rotational diffusion were characterized in experiments by characterizing the set of angles with respect to its landmark visited by a nanotube over a long time period. To simulate the effects of restriction, we limited the angles that could be visited to the set of angles visited by a randomly chosen nanotube that was observed in experiments. While in experiments it was straightforward to only characterize the range of angles visited with respect to the imaging plane, in simulation we assumed that restriction occurred with respect to this angle and with respect to the angle of incidence perpendicular to the plane in our simulations. We made this assumption because a) we assume that restrictions on the orientations over which diffusion can occur are present across both angles, even if the measurement of this restriction is not straightforward and b) our approach to simulating restricted diffusion was to introduce an energy potential to prevent rotation of the nanotubes across some angles. Introducing such a potential only in the direction of the projection of the chains onto the plane made the simulation numerically unstable.

**Supplementary Fig. S40:** Schematic illustration of how restrictions on the angle of rotation could make nanotube attachment more difficult and/or cause connecting nanotubes to be more meandering that would otherwise be expected. Grey area denotes the angular region through which the nanotubes can diffuse freely.
Specifically, to simulate the observed restricted orientation, for each chain in a simulation we randomly selected one of the ranges of angles across which nanotube diffusion was observed. We fix the initial orientation of the nanotube at a randomly chosen angle, defined as the fixed orientation vector $d$. We use the randomly chosen set of observations to determine an angle $\theta$ defined as half the range of angular orientations where the chosen nanotube was observed to diffuse. We constrain the position of the endpoint of the polymer, i.e. the first bead using a potential that acts on the first bead of the chain that is attached to the surface

$$V_{\text{restriction}} = \begin{cases} 0 & \text{if } 1 - d \cdot u_1 < w \\ k_r (1 - d \cdot u_1 - w)^2 & \text{otherwise} \end{cases} \quad (29)$$

such that $w = \cos \theta$.

This energy is added to the potential function given in Eq. 26. To ensure that diffusion is restricted rather than reduced, we made this energy large by setting $k_r = 10^3 k_B T$.

We ran a set of simulations using the same parameter values as in Supplementary Fig. S35, but adding the potential $V_{\text{restriction}}$ and considering only interlandmark distances between 3.6 \( \mu m \) and 10 \( \mu m \). 25 simulations were run for each interlandmark distance. In order to ensure a fair comparison of how yield changes with interlandmark distance, we chose 25 sets of orientation vectors and angles as described by the procedure above, and this same set was used to restrict orientation for each of the 25 samples at each distance.

The results are shown in Supplementary Fig. S41. Surprisingly, we found that introducing restricted rotational diffusion of nanotubes had relatively little effect on connection yields, although the yields at shorter interlandmark distances were lower than in simulations that did not incorporate restricted diffusion. These lower yields match the yields at short interlandmark distances observed in experiments, although other factors in experiments (for example defective seeds or the joining of nanotubes from solution to make connections longer quickly) might also account for these yields. The range of connection lengths observed in simulations that incorporated restricted rotational diffusion were also more similar to the range of lengths observed in experiments.

One might be tempted to assume that the results of these simulations were due to particularly lucky choices of initial orientations and rotational ranges. These results are, however, also compatible with the method of analysis of point-to-point assembly where rotational diffusion is restriction presented in Supplementary Section 5.3. Intuitively, while restriction of rotational diffusion can also make connection more difficult by orienting nanotubes away from one another, it can also enhance the probability of connection by restricting the space where nanotubes explore but not changing the range of locations where nanotube ends may meet. More generally, the effects of rotational diffusion are complex, with the same set of restrictions making connection at some nanotube lengths more difficult while making it easier to connect when the changes are different lengths. One important benefit of our simulations, therefore, while not exact
predictors of yield or the topology of connections, is to elucidate such unexpected phenomena.

**Supplementary Fig. S41:** Results of simulated point-to-point assembly that incorporate restrictions on the angles of nanotube rotation (see text) and using joining criterion $E_{cut} = 80k_BT$ and simulation and experiment time correspondence $t_{sim} = 0.002$ s. (Left) Yields. (Right) The lengths of chains that joined as a function of interlandmark distance after 70 hours of simulated assembly time. 25 simulated point-to-point assembly processes were run for each interlandmark distance considered.
5. Steady-state probabilities of polymer end intersection

To further gain intuition about how the stiffness (i.e. persistence length) of the growing polymers affects connection yields and the lengths of connections that tend to form during point-to-point assembly, we developed an analytical method for comparing the relative probabilities of connections and yields forming at different lengths.

The goal of this analysis is to qualitatively compare the probability of a connection forming under some situations relative to others. To do so, we develop a method for computing the chance that a set of polymers of a particular length separated by a particular distance will connect, assuming that the polymer motion (bending and diffusion) is at a steady state. That is, we assume that the probability that a polymer’s end is in a particular location at a particular time may be drawn from the equilibrium distribution of polymer configurations. We use this approach to calculate the probability that the two polymers’ ends are close enough to form a connection. We then compare these probabilities for polymers of different lengths to get an idea of the relative probabilities of polymers connecting at different lengths and distances.

This simple analysis is helpful for considering whether longer or shorter connections are likely to form and is computationally tractable enough to perform for many different polymer stiffnesses and interlandmark distances. However, it has several important biases that mean that its results should not be interpreted as quantitative predictions of yields or connection lengths. First, the probability of connections forming at a given length assumes nothing about what the position the polymers are in when they first grow to that length and it ignores the possibility that the polymers may have connected before reaching the length that is being considered. This analysis thus overestimates the relative probability of forming long connections over short ones. Second, this analysis does not consider the amount of time that polymers have to form a connection when they are at a particular length but instead computes the instantaneous probability of the two polymers being in a configuration where a connection could form. In our experiments, polymers grow at a constant rate, so roughly the same time is spent at each length, but diffusion rates slow as polymer length increases. Since we compare probabilities assuming the same number of configurations are sampled at each length, whereas in practice a smaller number of configurations are sampled at longer lengths than at shorter ones, we are again systematically overestimating the probability of forming longer connections. Despite these biases, the analysis is useful for comparing how distributions will change qualitatively as parameters such as the persistence length are varied while remaining simple enough to reason about. Because the trends in yields in connection length and connection yield observed in point-to-point assembly and predicted by simulations can be counterintuitive, we found this tool, which explained the potential reasons behind these trends, of value.
5.1. Relative probabilities of two rigid polymers forming point-to-point connections as a function of polymer length

We begin our analysis by considering a point-to-point assembly process involving two perfectly rigid polymers (such that the polymers have infinite persistence length, \( l_p = \infty \)). Let the separation between the landmarks be distance \( d \), such that the seed for polymer 1 is at \( (0,0,0) \) and the seed for polymer 2 is at \( (d,0,0) \). For simplicity, we assume that both polymers are the same length, as allowing them to be of different lengths changes the quantitative but not qualitative conclusions, and that polymers can explore all 3D space \( i.e. \) there are no nearby surfaces or objects that prevent their free diffusion.

Given that these polymers are perfectly rigid, if the ends of the polymers in our model are to meet, they must do so at \( x = \frac{d}{2} \) (Supp. Fig. S42a). To see this, consider the polymers as lines in space. In this case, the end of a perfectly rigid polymer of length \( L \) anchored at point \( (0,0,0) \) explores the surface of a sphere with radius \( L \) as it diffuses. The set of points where polymer ends may meet and join when the two polymers are of length \( L \) is therefore the circle with centre \( \left( \frac{d}{2},0,0 \right) \) with radius \( r = \sqrt{L^2 - \left( \frac{d}{2} \right)^2} \). In practice, however, the polymers have a finite diameter. We take this into account by modeling each polymer as a cylinder with diameter \( d_{tube} \) where \( d_{tube} \ll d \).

At steady state, the centre of the end of a perfectly rigid polymer of length \( L \) and no obstacles to diffusion has an equal chance of being at any position on the surface of a sphere with radius \( L \) centred at its anchored end. The polymers’ ends can meet where the surfaces of the two spheres overlap, which given that the polymers are of equal length, we approximate as an annulus in the y-z plane at \( x = \frac{d}{2} \). We estimate the area of this annulus, \( A_{annulus} \), to be the projection of a cylinder’s end, \( i.e. \), a circle with diameter \( d_{tube} \), onto the y-z plane across all coordinates where the two polymers can meet. The outer radius of this annulus is \( r + \frac{d_{tube}}{2} \cos(90 - \phi) \) and the inner radius is \( r - \frac{d_{tube}}{2} \cos(90 - \phi) \) where \( \phi \) is the polar angle in degrees \( i.e. \) angle between the positive z-axis and the polymer, such that \( \phi = \cos^{-1} \left( \frac{d}{2L} \right) \). Simplifying, \( A_{annulus} \) is mathematically defined as:

\[
A_{annulus} = 2\pi L d \cos (90 - \phi).
\]  

We compute the probability that at steady state the ends of polymers 1 and 2 of length \( L \) are in a configuration where they could join by multiplying the area of the annulus where the ends can intersect, \( A_{annulus} \), by the projected area of one polymer end on the annulus at a fixed coordinate such that the two polymer ends can intersect, \( A_{tube-intersection} \), and dividing this product by the product of the spherical surface areas that each end sweeps out. \( P(\text{intersect}) \), is therefore defined as:
\[ P(\text{intersect}) = \frac{A_{\text{annulus}} A_{\text{tube-intersection}}}{(4\pi)^2 L^4}, \]  

where \( A_{\text{tube-intersection}} \) is mathematically defined:

\[ A_{\text{tube-intersection}} = \frac{\pi d_{\text{tube}}^2 \cos(90 - \phi)}{4}. \]

In practice, rigid polymers are less likely to join if there is a small angle between them than if they meet head on because a sharp angle would require the ends to bend to join, which for a rigid polymer would be energetically unfavourable. In the case of nanotubes, meeting at such an angle would also limit the number of sticky ends contacting one another, thereby decreasing the energy of interaction. To consider the relative probabilities of polymers of different lengths joining and forming connections given that polymers meeting at short angles are unlikely to connect, we include a joining probability term that is a function of the angle at which the polymer ends meet in the probability. In this qualitative analysis, we consider different joining probability functions: one that does not vary, another that varies linearly and a third that varies exponentially with the angles between the polymer ends (Supp. Fig. S42b). These joining probabilities are defined mathematically:

\[ \alpha_{\text{none}} = 1, \]

\[ \alpha_{\text{linear}} = \gamma/180, \]

\[ \alpha_{\text{exp}} = \exp\left(\frac{\gamma-180}{30}\right), \]

where \( \gamma \) is the angle in degrees between the two polymers at their point of meeting (see inset in Supp. Fig. S42b).

To include this probability of joining, we multiply the probability that the ends of the two polymers are in a configuration where they can meet by a joining probability term \( \alpha \). The resulting probabilities are plotted in Supplementary Fig. S42c. When the total contour length \( 2L \) is close to the interlandmark distance \( d \), connection is unlikely because the area where intersection can occur is very small. This area increases as the total contour length increases. At large separation distances (when \( L \gg d \)), the area explored by each polymer end grows quadratically with \( L \), whereas the area of the annulus grows linearly with \( L \), so the probability decreases roughly as \( 1/L \). Thus, as \( L \) grows to infinity, the probability of the polymer ends meeting in a particular configuration goes back down to zero from some maximum. Including a linear or exponential joining probability decreases the probability of intersection with increasing length more quickly than when the probability of joining is assumed to be uniformly 1 because longer polymers must join at increasingly sharp angles.
Supplementary Fig. S42: Lengths of connections formed on average by perfectly rigid polymers. (A) Illustration showing the circle where the endpoints of two vectors (abstract models for perfectly rigid polymers), each of length $L$ and separated by distance $d$ can intersect. (B) To understand how a preference for polymer joining when polymers meet head on vs. at a sharp angle affects the relative probabilities of nanotubes joining at different lengths, we consider different probabilities of connection as a function of the angle at which the polymers meet, which are plotted here (see Eqs. 33-35). (C) The probability functions of two polymer ends meeting and forming a connection as a function of polymer length. The blue curve shows the probabilities when joining can always occur regardless of the angles between the polymers, and the red and black curves show the computed probability of connection when the probability of joining given that the ends meet is a linear (red) or exponential function of this angle.
5.2. Relative probabilities of two semiflexible flexible polymers forming point-to-point connections as a function of polymer length

While the end of a perfectly rigid anchored polymer explores the surface of a sphere when it diffuses, visiting each point that can be explored with equal probability, the end of a polymer that can bend can explore all regions of space within the sphere of radius equal to the contour length of the polymer (assuming again the polymer can explore all 3D space). The end of such a non-rigid polymer also visits different points within this sphere with different frequencies because some bending configurations are more favourable than others. To determine the relative probabilities that connections of different lengths are formed by polymers that can bend, we extend the geometric analysis developed in Supp. Section 5.1, in which we calculate the probability that two polymer ends are close enough to join, to consider the possibility that polymers can bend as well as rotate.

We begin this process by first reviewing the probabilities that semiflexible and flexible ends have different distances between their two ends (i.e. end-to-end distances). These probabilities determine how often the ends of the polymers are in the different regions of the sphere of space that can be explored by each.

5.2.1. The probability distribution of the end-to-end distance of a flexible polymer

To determine the probability distribution of the end-to-end distance of a flexible polymer with contour length $L$, we use the freely-joined chain (FJC) model\textsuperscript{20} in which we consider the polymer to consist of $N$ monomers of length $l$ such that $L = Nl$ and $L \gg l$. The probability density function of the end-to-end distance, $R$, of the polymer is then given by:

$$P(R) = \left(\frac{3}{2\pi N l^2}\right)^{3/2} e^{-\frac{3R^2}{2Nl^2}}.$$  (36)

Here, the probability distribution over $R$ for a particular contour length $L$ depends on the value of $l$. Smaller values of $l$ mean that there are more joints within the chain and thus that it is more flexible. To help the reader gain an intuitive feel for how this expression translates into actual end-to-end distance values, the distribution of end-to-end distances for the case where $l/L=0.001$ is plotted in Supplementary Fig. S43. To give the reader some perspective on values of interest for $l/L$, if one were to model ssDNA as a FJC, an approximate value for $l/L$ would 0.7 nm, or the distance between phosphate groups on the backbone\textsuperscript{21}. In this case, $l/L=0.001$ would correspond to a chain length of 700 nm.

5.2.2. The probability distribution of the end-to-end distance of a semiflexible polymer

The end-to-end distance, $R$, for semiflexible polymers (whose contour length is on order the persistence length) considered as a worm-like chain\textsuperscript{11} has been approximated by a simple expression \textsuperscript{22}:
\[ P(R) = \frac{4\pi N \left( \frac{R}{L} \right)^2}{\left( 1 - \left( \frac{R}{L} \right)^2 \right)^2} \exp \left( - \frac{9L}{8l_p \left( 1 - \left( \frac{R}{L} \right)^2 \right)} \right) \]  

(37)

where \( N \) is a normalization constant, \( L \) is the contour length and \( l_p \) is the persistence length.

This expression may be applied to polymers with a wide range of contour lengths and persistence lengths and agrees well with separate, more complex solutions and simulations of the probability distribution of the end-to-end distance.

The results of Eq. 37 for a range of \( l_p/L \) along with the results of Eq. 36 for flexible case where \( l/L = 0.001 \) are plotted in Supplementary Fig. S43.

**Supplementary Fig. S43: Probability densities of end-to-end distances for semiflexible polymers given by Eq. 37 and an example flexible polymer using Eq. 36.**

Increasing the persistence length relative to the contour length of a polymer tends to increase its average or typical end-to-end distance. The expected value of each end-to-end distance distribution is depicted by a dot in the corresponding colour underneath the distribution. The average end-to-end distance is not the same as the most frequent end-to-end distance in some cases because the distributions, while peaked, are highly asymmetric.

5.2.3. Algorithm for calculating the relative probabilities of connection of semiflexible or flexible polymers as a function of polymer length
Here we extend the approach developed in Supp. Section 5.1 for estimating the relative probability of two polymers of length $L$ forming a connection by calculating the probability that their ends are close enough to join. For simplicity, we assume that the two polymers are of the same length and that two polymer ends close enough to join can join regardless of the angle formed between them.

For a given set of polymers with contour length $L$, persistence length $l_p$, and whose endpoints are separated by distance $d$, we calculate the relative probability that the polymer ends can connect by assuming that connections will form if they are sufficiently close.

For computational convenience here we define sufficient closeness here by discretizing the total volume the polymers can explore $V$ into small voxels that are cubes with dimensions $\delta^3$ where $V \gg \delta^3$ and assuming polymers can join if their two ends are in the same voxel. We chose the voxel value to be small enough so that doubling the volume of the voxel did not significantly change the results of the calculation. We calculate the probability that both polymer ends intersect to form a connection at a particular time for a particular connection distance and polymer length by calculating the probability that both polymer ends are in each voxel $v$ and then summing these probabilities for all voxels.

To find the probability that the end of a given polymer is within voxel $v$, we first determine the minimum and maximum end-to-end distance, $R_{max}$ and $R_{min}$, which would enable the polymer to occupy $v$ by calculating the Euclidean distance between the 8 corners of $v$ and the anchor point of the polymer. Next, to determine the probability of the given polymer having an end-to-end distance that allows it to occupy voxel $v$, we integrate the end-to-end distribution function (as described by Eqns. 37 and 36 for the semi-flexible and flexible case, respectively) between the minimum and maximum end-to-end distance: $p_{\text{Length}} = \int_{R_{min}}^{R_{max}} P(R) dR$. We then compute the entire volume $dV$ the given polymer can explore at such end-to-end distances, which has the shape of a spherical shell, using the formula $dV = 4/3 \pi (R_{max}^3 - R_{min}^3)$. Given the end-to-end distance of a polymer enables the end of the polymer to occupy voxel $v$, the probability that the end of the polymer can be found in voxel $v$ is approximately the probability that the polymer is between the given limits multiplied by the ratio of the volume of voxel $v$ to the total volume the end of the polymer can explore at such end-to-end distances $dV$: $p_v = p_{\text{Length}} \frac{\delta^3}{dV}$. It is important to note that we are introducing a small error into the calculations here by mixing coordinate systems when computing volumes: cubic voxels map to Cartesian coordinates whereas spherical shells map to spherical coordinates. While this error should be minimized by decreasing the volume of the voxel i.e. as the volume of the voxel shrinks to become infinitely small so too does this error, a linear decrease in $\delta$ corresponds to a cubic increase in the number of voxels and thus computation, so there is a tradeoff between minimizing error and computational time. Because in our case doubling the volume of each voxel did not significantly change any of our results, this error should be very small.
Next, to find the probability that the ends of both polymers are in voxel \( v \), we multiply the probabilities that each polymer end is in the voxel: \( P_v = p_{v,1}p_{v,2} \), where \( p_{v,1} \) and \( p_{v,2} \) correspond to the probabilities of polymer 1 and polymer 2, respectively. This quantity is then summed across all voxels in \( V \) order to determine the probability that the two polymer ends will intersect at a given contour length \( L \), assuming a fixed persistence length: \( P_{\text{intersect}}(L) = \sum_v P_v \). We call this quantity the probability of intersection and show this value of Supplementary Fig. S44a for semiflexible and rigid polymers and S44b for flexible polymers.

Supplementary Fig. S44: Probabilities of two polymers connecting as a function of contour length (normalized by interlandmark distance) and persistence length. (A) The probability of connection (see text) of two anchored semi-flexible polymers (using Eq. 37 to determine the probabilities of end-to-end distance) as a function of the total contour length of the connected polymers normalized by the point-to-point distance, \( d \) for a range of persistence lengths \( l_p \). Both polymers are assumed to have the same contour length. (B) The probability of connection (see text) of two flexible, anchored polymers intersecting as a function of the normalized total contour length for flexible polymers with different monomer lengths \( l \) normalized by \( d \). Here we use Eq. 36 to describe the probability function of the end-to-end distance of the flexible polymer. Inset plot depicts zoomed-in view with log scale y-axis to show extremely low, but non-zero, probability curves. Expected value is depicted as a dot in the corresponding colour underneath the distribution(s).

This qualitative analysis suggests that shorter persistence lengths tend to favour the formation of connections by longer polymer lengths (subject to the caveat that the analysis is biased for all systems for favour longer connections over shorter ones). They show that using a more flexible polymer would require accepting the formation of longer, possibly more tangled connections and waiting longer for such connectors to grow.
5.3. Relative probabilities of two rigid polymers forming point-to-point connections as a function of polymer length when rotationally restricted

One significant experimental observation we made when characterizing the point-to-point assembly process was that some nanotubes appeared to diffuse only over a subset of possible angular orientations (see Supp. Section 3.2 for more detail), i.e. these nanotubes were rotationally restricted. Here we use the analysis methods developed in this section to understand how rotational restriction might affect the probabilities of connection. This analysis found that, as would be expected, rotational restriction can prevent some pairs of nanotubes from connecting. However, as described below, rotational restriction could also enhance the probability of two polymers connecting in other cases, for example, if two restricted regions highly overlap.

Here we qualitatively characterize these two potential effects by extending the framework for computing the probability of point-to-point connection by rigid polymers in supplementary section 5.1 to include rotational restriction in the $\theta$ dimension (x-y plane in Supp. Fig. S42a).

As a simple model of rotational restriction, we constructed pairs of polymers with different angular ranges that could be explored and determined how these ranges affected the probabilities of connection at different lengths. We constrained the angle $\theta$ of each rigid polymer to a set of continuous angles through which the polymer could freely diffuse (examples are shown as the pink and green shaded areas in Supp. Fig. S45a). We chose the range for each polymer randomly from the set of ranges observed randomly in experiments (Supplementary Fig. S16). As $\theta$ was the only dimension in which we could experimentally measure restricted rotational diffusion, we restricted rotation in this dimension only. The smallest range of $\theta$ for a polymer was 90 degrees (1/50 polymers had this range) and the largest 360 degrees (4/50), with a mean range of 234 degrees. The assigned angular range of a given polymer was centred about $\theta_0$, where $\theta_0$, is drawn from a uniform random distribution between 0-360 degrees (see Supp. Fig S45b,c).

To calculate the likelihood of two rotationally restricted rigid polymers of length $L$ connecting, we modified our calculations in Supp. Section 5.1 to include a term representing the fraction of the annulus on which the two rods can form a connection, $f_a$, which as described in Supp. Section 5.1, is determined by the range of angles the two polymer can explore and the length of the polymers. For an unrestricted pair, $f_a = 1$ for all polymer lengths. In contrast, for a pair of polymers each with tight angular ranges facing away from the other, such that it is impossible for polymers to connect at any length, $f_a = 0$ for all polymer lengths.

Not only do we have to modify the annular area across which the polymers can meet, but we also have to modify the areas that each polymer sweeps out when they are not in an orientation in which connection is possible. The surface area that the end of an anchored, rigid polymer sweeps out is directly proportional to fraction of angles, $f_\theta$, in the $\theta$ dimension the polymer can explore, so we multiply the spherical area by $f_\theta$. Finally,
incorporating the joining penalty introduced in Supp. Section 5.1, we obtain the expression:

\[ P_{\text{intersect}} = \frac{f_a A_{\text{annulus}} A_{\text{tube-intersection}}}{(4\pi)^2 L^4 f_{\theta,1} f_{\theta,2}} \alpha, \]  

(38)

where \( f_{\theta,1} \) and \( f_{\theta,2} \) indicate the fraction of angles that the first and second polymer in the pair can explore, respectively.

To understand influence of rotational restriction in forming a connection, we calculated a simple score, \( s \), defined as the ratio of probabilities of intersection for the potentially rotationally restricted case to the unrestricted case (in the unrestricted case \( s = 1 \)) at a given \( L/d \). We found that rotational restriction could both reduce and enhance the probability of connection depending on the polymer length and the angles the polymers were allowed to explore. For example, at polymer lengths 1.2\( L/d \) and 1.8\( L/d \), respectively, 8/25 and 10/25 rotationally restricted pairs had a higher probability of connecting than the unrestricted case and 9/25 and 7/25 pairs had zero probability of connecting (see Supp. Fig. S45b,c). Almost all (24/25 pairs) could connect if the length of each polymer was at least 2.5\( d \). More generally, rotational restriction can cause a range of nonlinear effects in the probability of connection, suggesting that there is no simple explanation for its effect on the connection process (See Supp. Fig. S45d-g).
Supplementary Fig. S45: Rotational restriction of rigid polymers can both increase and decrease the probability of connections forming. Schematic diagrams showing (A) an example pair of rigid polymers that are rotationally unrestricted and (B-C) 25 example pairs of rigid polymers that are rotationally restricted in the $\theta$ dimension. The pink region denotes the range of angles $\theta$ through which one polymer can diffuse and the green region denotes angles through which another polymer can diffuse. The bounds shown here are (A-B) $L/d = 1.2$ and (C) $L/d = 1.8$. Dark dots indicate seed positions. Rigid polymers are shown in example orientations by either dark red or dark green lines,
in all schematics with a dimensionless length of $L/d = 0.5$, in order more easily visualize the relative size of the shaded regions. (B-C) The range of exploration permitted to each polymer was randomly selected from the set of experimentally measured ranges (Supplementary Fig. S16). Values above the pairs indicate the score, $s$, of the pair, defined as the probability of intersection relative to the unrestricted case (in the unrestricted case $s = 1$) for that $L/d$. Pairs with colored square borders correspond to the bold lines of the same colors in plots (D-G). Estimated probability of intersection as a function of total contour length normalized by point-to-point distance for all 25 pairs of rigid polymers where (D) connections may always form regardless of the angle between polymers and where the probability of joining decreases (E) linearly and (F) exponentially with polymer angle. Diagrams in (D) depict the schematic restriction diagrams for various pairs at different values of total contour length / d. (G) The likelihood of intersection relative to the unrestricted case (or the score, $s$) as a function of total contour length relative to the likelihood of forming a connection without any rotational restriction as a function of point-to-point distance.
6. Additional Microscopy Images

6.1. Selected images of successful nanotube connections after 30 hours of point-to-point assembly.

**Supplementary Fig. S46**: Additional multi-colour fluorescence microscopy images of isolated A and B seeds showing successful nanotube after 30 hours of point-to-point assembly. Scale bars are 5µm.
6.2. Selected images of isolated seed pairs with nanotubes that were still unconnected after 30 hours of point-to-point assembly.

Supplementary Fig. S47: Additional multi-colour fluorescence microscopy images of isolated A and B seeds with nanotubes that were still unconnected after 30 hours of point-to-point assembly. Scale bars are 5µm.
6.3. Selected images of nanotube connections collected after the 70 hours point-to-point assembly experiment

Supplementary Fig. S48: Additional multi-colour fluorescence microscopy images of isolated A and B seeds between which nanotube connections formed after 70 hours of point-to-point assembly. Scale bars are 5μm.
6.4. Selected images of isolated seed pairs with nanotubes that were still unconnected after 70 hours of point-to-point assembly.

To understand why nanotube connections failed to form between some pairs of seeds even after extended nanotube assembly times, we sorted the images of unconnected nanotubes collected after the 70 hour point-to-point assembly experiment into 4 categories representing different potential causes. Categories include insufficient nanotube length, nanotube interaction with the glass surface that would prevent free diffusion, restriction of the rotational diffusion of nanotubes that did not involve nanotubes sticking to the surface and other, which includes pairs of nanotubes that did not connect for any apparent reason. Selected examples from each category are represented as image series from a movie over 2 seconds in Supplementary Fig. S49.

11% of the unsuccessful connections had nanotubes with insufficient lengths making it physically impossible for a connection to form. Unconnected nanotubes in which at least one of the nanotubes appears partially stuck to the glass surface comprised 22% of this group. 39% of cases contained “potentially rotationally restricted” nanotubes displaying persistent orientational bias over time scales far larger than those for rotational diffusion. Here, nanotubes did not appear to interact with the glass, suggesting that the limitations on rotation stem from interactions between the seeds and the glass. For this categorization analysis, we classified a nanotube to be potentially rotationally restricted if its mean square angular displacement (MSAD) was less than half the MSAD of a nanotube of similar length calculated using measured nanotube Dr (Supplementary Fig. S19). We could not use the statistical tests for rotational restriction described in Supplementary Section 4.2 due to the lack of the number of independent samples (we only had 20 frames at 0.1 sec exposure as compared to 100 frames at 0.1 sec exposure). Finally, the remaining 28% of unsuccessful connections had no clear reason for lack of a connection. Our models suggest that a reason for failed connections not accounted for by the other effects is that the respective ends of the two nanotubes had simply failed to come into contact over the course of the experiment. Connection is impossible before the sum of the contour lengths reaches the length of the distance between the seed landmarks, and then the probability of connection at time points after that is a function of nanotube length, stiffness and diffusion rate. Notably, we do not expect all nanotubes to connect – once the individual nanotubes grow much longer than the inter-landmark distance, formation of connections can become challenging as the meeting of ends may require that nanotubes bend into unfavourable configurations.
Length limited - Individual nanotubes not long enough to form a connection (n = 2, 11%)

At least one nanotube stuck on glass surface preventing free rotational diffusion (n = 4, 22%)

At least one nanotube possibly rotationally restricted at the seed (n = 7, 39%)

Other cause for failure (n = 5, 28%)

**Supplementary Fig. S49:** Selected examples of failed connections after 70 hours of point-to-point assembly. Each row of images shows images of a single pair of seeds spread over 2 seconds to show a short duration of nanotube motion. Images are categorized into groups based on potential factors that could have contributed to the failure to form connections as described in Supplementary Note 5.4. Scale bars are 5µm.
6.5. Additional time-lapse images of the process of nanotube connection formation

Supplementary Fig. S50: Additional multicolour time-lapse fluorescence microscopy images showing steps in a successful nanotube connection process. The last example shows the case where a nanotube from solution attaches to a seed (indicated by yellow arrows) during the growth process, resulting in a large sudden increase in nanotube length at that seed. See also Supplementary Movie S1. Scale bars are 5µm.
6.6. Selected additional images of DNA nanotube connections formed across 3 dimensions

Supplementary Fig. S51: Additional three-dimensional reconstructed confocal fluorescence microscopy images showing nanotubes interconnections between A seeds on the top glass surface (red, ATTO647N) and B seeds on the bottom glass surface (blue, ATTO488).
6.7. Selected additional images of three dimensional organization of DNA nanotubes between seeds attached to 3 µm beads and seeds on a glass surface.

**Supplementary Fig. S52:** Additional multi-colour fluorescence microscopy images showing nanotubes connected between seeds on the glass surface (red, ATTO647N) and seeds on 3 µm beads (blue, ATTO488). Scale bars are 5µm.
7. Supplementary Movie captions

Supplementary Movie S1: Time-lapse movie of an isolated seed A - B pair showing nanotube connection via simultaneous growth and diffusion of nanotube ends. Seed A, seed B and nanotubes are labelled in ATTO647N (red), ATTO488 (blue) and Cy3 (green) respectively. Movie is captured over 6.5 hours and is sped up 5,000-fold. Scale bar 5 µm.

Supplementary Movie S2: Three-dimensional reconstructed movie showing one complete 360º rotation perspective view of multiple DNA nanotube connections (green, Cy3 dye) spanning A seeds (red, ATTO647n dye) on one glass surface and B seeds (blue, ATTO488 dye) on another glass surface such that the two parallel glass surfaces are separated by 3.5 µm. The images are captured using confocal microscopy. The two gray surfaces were rendered into the movie post-capture to serve as visual markers for the location of the two glass surfaces on which seeds were attached.

Supplementary Movie S3: Real-time movie showing nanotubes connecting B seeds (blue) on a 3 µm bead and A seeds (red) on a glass surface after a point-to-point assembly reaction. Scale bar 5µm.

Supplementary Movie S4: Preferential melting of unconnected nanotubes. Just before this movie, free tiles were removed from the dish via buffer exchange while maintaining the temperature of the dish at 32ºC. Seed A, seed B and nanotubes are labelled in ATTO647N (red), ATTO488 (blue) and Cy3 (green) respectively. 3 frames were captured every 20 minutes over 10 hours so that the movie is sped up (on average) 6,000-fold. Scale bar 5µm.

Supplementary Movie S5: Time-lapse movie showing nanotube connection formation in coarse-grained molecular dynamics simulations of point-to-point assembly. The two nanotubes are anchored to a surface and the anchor points are separated by 6 µm. The movie corresponds to 6.5 simulated hours of assembly and is sped up 5000-fold, i.e. a timescale identical to Supplementary Movie S1. The x y z arrows (red, green, blue) show the coordinate system within the simulation. The simulation stops after the chains join. Multiple copies of the frame showing the connected state are inserted into the movie to make it easier to see it within the animation.
8. Supplementary References


