# Supplementary Information file: Design and Characterization of RNA Nanotubes

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# Contents

# 1 Sequences

RNA sequences were identified starting from tile variants published by Rothemund *et al.*[4] RNA was transcribed in house from linear DNA templates. PAGE purified DNA sequences were ordered from IDT DNA (Coralville, IA, USA). Templates include 3 base "sealing" domains (5' end of non-template strand) to prevent breathing at the 5' end of the promoter site. All genes include the T7 promoter sequence. The +1 to +6 promoter region (transcription start site) was modified relative to the generally recommended sequence (GGGAGA),[2] to avoid altering or constraining the sequence content of RNA tiles.

## 1.1 DNA templates

**22-6 tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCG TAG AGC ACC ACT GAC AAG GTA -3' / 5'- TAC CTT GTC AGT GGT GCT CTA CGC AGA CTA TAG TGA GTC GTA TTA GAA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4** 5'- TTC TAA TAC GAC TCA CTA TAG CAG ACA GTT TCG UGG TCA TCC TAC CTT -3' / 5'- AAG GTA GGA TGA CCA CGA AAC TGT CTG CTA TAG TGA GTC GTA TTA GAA -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'
**23-7 tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-2b** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCC GTA GAG CAC CAC TGA CCA AGG TA -3' / 5'- TAC CTT GGT CAG TGG TGC TCT ACG GCA GAC TAT AGT GAG TCG TAT TAG AA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-2b** 5'- TTC TAA TAC GAC TCA CTA TAG GCA GAC AGT TTC GUG GTC ATC CTA CCT TG -3' / 5'- CAA GGT AGG ATG ACC ACG AAA CTG TCT GCC TAT AGT GAG TCG TAT TAG AA -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'
**24-8 tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-4b** TTC TAA TAC GAC TCA CTA TAG GTC TGC CGT AGA GCA CCA CTG ACA CAA GGT A -3' / 5'- TAC CTT GTG TCA GTG GTG CTC TAC GGC AGA CCT ATA GTG AGT CGT ATT AGA A -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC

GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-4b** 5'- TTC TAA TAC GAC TCA CTA TAG GCA GAC CAG TTT CGT GGT CAT CCU ACC TTG T -3' / 5'- ACA AGG TAG GAT GAC CAC GAA ACT GGT CTG CCT ATA GTG AGT CGT ATT AGA A -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'

**25-9 tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-6b** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCA TCG TAG AGC ACC ACT GAC AGG AAG GTA -3' / 5'- TAC CTT CCT GTC AGT GGT GCT CTA CGA TGC AGA CTA TAG TGA GTC GTA TTA GAA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-6b** 5'- TTC TAA TAC GAC TCA CTA TAG ATG CAG ACA GTT TCG UGG TCA TCC TAC CTT CCT -3' / 5'- AGG AAG GTA GGA TGA CCA CGA AAC TGT CTG CAT CTA TAG TGA GTC GTA TTA GAA -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'

**26-10 tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-8b** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCT ATC GTA GAG CAC CAC TGA CAA GGA AGG TA -3' / 5'- TAC CTT CCT TGT CAG TGG TGC TCT ACG ATA GCA GAC TAT AGT GAG TCG TAT TAG AA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-8b** 5'- TTC TAA TAC GAC TCA CTA TAG ATA GCA GAC AGT TTC GTG GTC ATC CTA CCT TCC TT -3' / 5'- AAG GAA GGT AGG ATG ACC ACG AAA CTG TCT GCT ATC TAT AGT GAG TCG TAT TAG AA -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'

**23-7 toeholded tile**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-2b** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCC GTA GAG CAC CAC TGA CCA AGG TA -3' / 5'- TAC CTT GGT CAG TGG TGC TCT ACG GCA GAC TAT AGT GAG TCG TAT TAG AA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'

**S4-2b-TH** 5'- TTC TAA TAC GAC TCA CTA TAG AAT AGG CAG ACA GTT TCG TGG TCA TCC TAC CTT G -3' / 5'- CAA GGT AGG ATG ACC ACG AAA CTG TCT GCC TAT TCT ATA GTG AGT CGT ATT AGA A -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'
**24-5 tile**
**S1-5bSE** 5'- TTC TAA TAC GAC TCA CTA TAT GTG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC TGG T -3" / 5'- ACC AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACA CAT ATA GTG AGT CGT ATT AGA A -3"
**S2-4b-5bSE** 5'- TTC TAA TAC GAC TCA CTA TAG GTC CGC CGT AGA GCA CCA CTG ACA CAA GGT G -3' / 5'- CAC CTT GTG TCA GTG GTG CTC TAC GGC GGA CCT ATA GTG AGT CGT ATT AGA A -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-4b-5bSE** 5'- TTC TAA TAC GAC TCA CTA TAG GAC CAC CAG TTT CGT GGT CAT CCT ACC ACC T -3' / 5'- AGG TGG TAG GAT GAC CAC GAA ACT GGT GGT CCT ATA GTG AGT CGT ATT AGA A -3'
**S5-5bSE** 5'- TTC TAA TAC GAC TCA CTA TAG TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA CGG C -3' / 5'- GCC GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ACT ATA GTG AGT CGT ATT AGA A -3'
**23-7 two tile system**
**S1** 5'- TTC TAA TAC GAC TCA CTA TAG TCA GTG GAC AGC CGT TCT GGC AGC GTT GGA CGA AAC T -3' / 5'- AGT TTC GTC CAA CGC TGC CAG AAC GGC TGT CCA CTG ACT ATA GTG AGT CGT ATT AGA A -3'
**S2-2b-T1** 5'- TTC TAA TAC GAC TCA CTA TAG AAC GAC GTA GAG CAC CAC TGA CCA AGG TA -3' / 5'- TAC CTT GGT CAG TGG TGC TCT ACG TCG TTC TAT AGT GAG TCG TAT TAG AA -3'
**S2-2b-T2** 5'- TTC TAA TAC GAC TCA CTA TAG TCT GCC GTA GAG CAC CAC TGA CTG AAT GA -3' / 5'- TCA TTC AGT CAG TGG TGC TCT ACG GCA GAC TAT AGT GAG TCG TAT TAG AA -3'
**S3** 5'- TTC TAA TAC GAC TCA CTA TAG CCA GAA CGG CTG TGG GCT AAA CAG TAA CCG AAG CAC CAA CGC T -3' / 5'- AGC GTT GGT GCT TCG GTT ACT GTT TAG CCC ACA GCC GTT CTG GCT ATA GTG AGT CGT ATT AGA A -3'
**S4-2b-T1** 5'- TTC TAA TAC GAC TCA CTA TAG GCA GAC AGT TTC GTG GTC ATC CTC ATT CA -3' / 5'- TGA ATG AGG ATG ACC ACG AAA CTG TCT GCC TAT AGT GAG TCG TAT TAG AA -3'
**S4-2b-T2** 5'- TTC TAA TAC GAC TCA CTA TAG TCG TTC AGT TTC GTG GTC ATC CTA CCT TG -3' / 5'- CAA GGT AGG ATG ACC ACG AAA CTG AAC GAC TAT AGT GAG TCG TAT TAG AA -3'
**S5** 5'- TTC TAA TAC GAC TCA CTA TAG GAT GAC CTG CTT CGG TTA CTG TTT AGC CCT GCT CTA C -3' / 5'- GTA GAG CAG GGC TAA ACA GTA ACC GAA GCA GGT CAT CCT ATA GTG AGT CGT ATT AGA A -3'

## 1.2 RNA sequences

**22-6 tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'

**S2** 5'- GUCUGCGUAGAGCACCACUGACAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4** 5'- GCAGACAGUUUCGUGGUCAUCCUACCUU -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**23-7 tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-2b** 5'- GUCUGCC GUAGAGCACCACUGAC CAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-2b** 5'- GGCAGAC AGUUUCGUGGUCAUCC UACCUUG -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**24-8 tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-4b** 5'- GGUCUGCC GUAGAGCACCACUGAC ACAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-4b** 5'- GGCAGACC AGUUUCGUGGUCAUCC UACCUUGU -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**25-9 tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-6b** 5'- GUCUGCAUC GUAGAGCACCACUG ACAGGAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-6b** 5'- GAUGCAGAC AGUUUCGUGGUCAUCC UACCUUCCU -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**26-10 tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-8b** 5'- GUCUGCUAUC GUAGAGCACCACUGAC AAGGAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-8b** 5'- GAUAGCAGAC AGUUUCGUGGUCAUCC UACCUUCCUU -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**23-7 toeholded tile**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-2b** 5'- GUCUGCC GUAGAGCACCACUGAC CAAGGUA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-2b-TH** 5'- GAAUA GGCAGAC AGUUUCGUGGUCAUCC UACCUUG -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

**24-5 tile**
**S1-5bSE** 5'- UGUGUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACUGGU -3'
**S2-4b-5bSE** 5'- GGUCCGCCGUAGAGCA CCACUGACACAAGGUG -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-4b-5bSE** 5'- GGACCACCAGUUUCGU GGUCAUCCUACCACCU -3'
**S5-5bSE** 5'- GUAGGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUACGGC -3'

**23-7 two tile system**
**S1** 5'- GUCAGUGG ACAGCCGUUCUGGCAGCGUUGG ACGAAACU -3'
**S2-2b-T1** 5'- GAACGAC GUAGAGCACCACUGAC CAAGGUA -3'
**S2-2b-T2** 5'- GUCUGCC GUAGAGCACCACUGAC UGAAUGA -3'
**S3** 5'- GCCAGAACGGCUGU GGGCUAAACAGUAACCGAAGCA CCAACGCU -3'
**S4-2b-T1** 5'- GGCAGAC AGUUUCGUGGUCAUCC UCAUUCA -3'
**S4-2b-T2** 5'- GUCGUUC AGUUUCGUGGUCAUCC UACCUUG -3'
**S5** 5'- GGAUGACC UGCUUCGGUUACUGUUUAGCCC UGCUCUAC -3'

## 1.3 RNA tile schematics

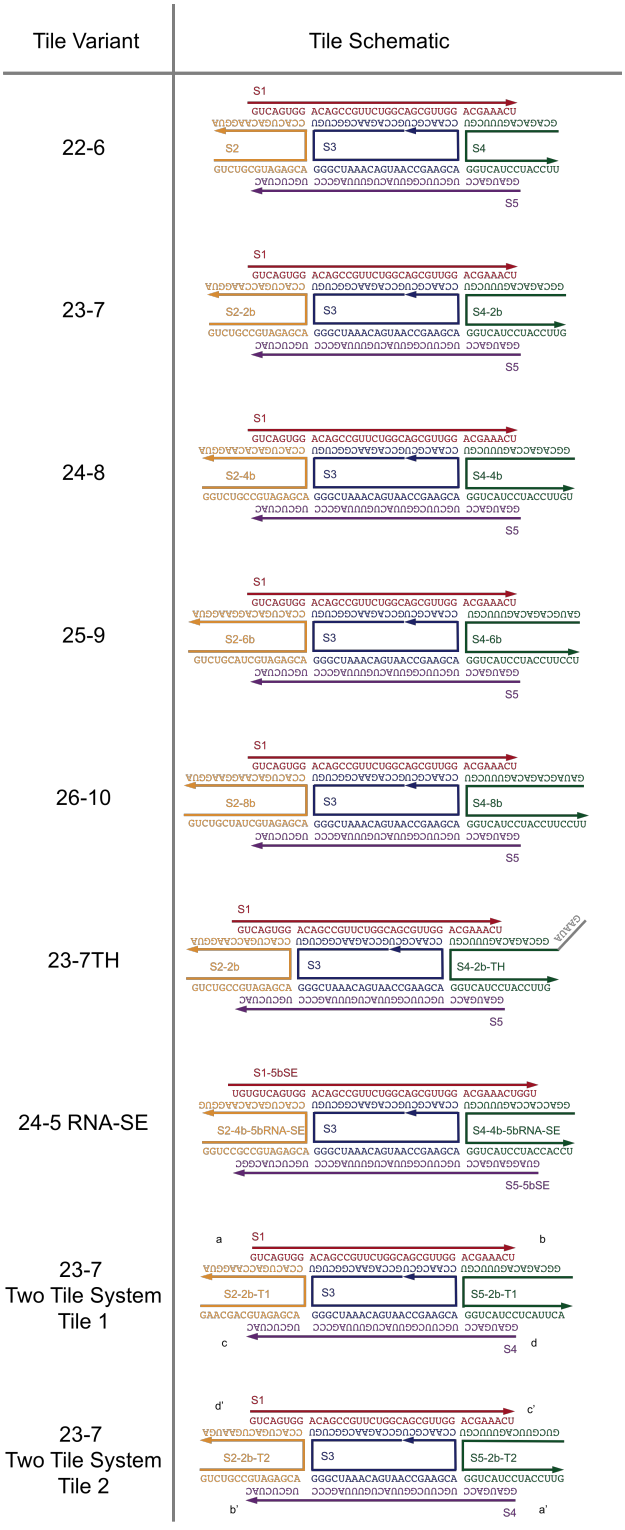


Figure S1: **RNA tile sequences for all tile variants**

# 2 Methods

## 2.1 Atomic Force Microscopy (AFM)

AFM images were obtained in tapping mode under buffer using a Digital Instruments Multimode AFM with a Nanoscope®III controller. We used either a Bruker SNL-10 silicon tip on a nitride lever with a spring constant of ≈ 0.24 N/m and a drive frequency of ≈9-10 kHz or an Olympus BL-AC40TS-C2 with a spring constant of ≈ 0.09 N/m and a drive frequency of ≈27-28 kHz. AFM buffer consisted of the same buffer used for annealing unless otherwise noted.

## 2.2 Fluorescence microscopy

Images were obtained with a NIKON TI-E inverted fluorescence microscope. A Cy3 filter was used to image tiles with Cy3 labeled S3 strand and a Cy5 filter was used to image tiles with Atto-647N labeled S3 strand. 60x oil immersion objective was used to collect all images, with a standard exposure time of 600 ms.

### 2.2.1 Fluorescence microscopy data processing

Fluorescence microscopy images were processed using ImageJ plugin Skeletonize to collect nanotube length distributions. Branching or looping nanotubes were eliminated from the length dataset using a MATLAB script. Pixels were converted to $\mu$m using conversion factor 1 pixel = 0.11 $\mu$m. Due to camera resolution limitations, nanotubes shorter than 0.33 $\mu$m were also eliminated.

Nanotube length distributions measured in fluorescence microscopy experiments are shown as violin plots in Fig. S2. These plots were prepared using `distributionPlot`, a MATLAB File Exchange script; in each violin plot, length data are plotted as a histogram normalized individually to have a maximum width of 0.8.

## 2.3 Denaturing polyacrylamide gel electrophoresis

Pre-mix was prepared by adding 42 g of urea to 25 mL of nanopure water (for a final volume of 100 mL), the mixture was then heated until the urea completely dissolved. This mixture was allowed to cool to room temperature, then a 40% (v/v) 19:1 acrylamide/bis-acrylamide solution was added in the appropriate volume for the desired percentage. The pre-mix was added in appropriate ratios with TBE and nanopure water, ammonium persulfate (APS), and tetramethylethylenediamine (TEMED) to start polymerization. Gels were cast in 10 x 10 cm, 1 mm thick disposable mini gel cassettes (Thermo Scientific, #NC2010) and allowed to polymerize for at least 2 hours before electrophoresis. Gels were run at room temperature at 100 V in 1X TBE unless otherwise noted. After electrophoresis the gels were stained in SYBR®Gold Nucleic Acid Gel Stain or ethidium bromide and then imaged using the Bio-Rad ChemiDoc MP system.

## 2.4 Non-denaturing polyacrylamide gel electrophoresis

40% solution of 19:1 acrylamide/bis-acrylamide, TAE, $MgCl_2$, APS, and TEMED were added together at appropriate concentrations for the desired polyacrylamide percentage, then cast in 10 x 10 cm, 1 mm thick disposable mini gel cassettes (Thermo Scientific, #NC2010) and allowed to polymerize for at least 2 hours before electrophoresis. Gels were run at 4°C at 150 V in 1X TBE buffer. After electrophoresis gels were stained in SYBR®Gold Nucleic Acid Gel Stain or ethidium bromide and then imaged using the Bio-Rad ChemiDoc MP system.

### 2.5 RNA extraction

Using the AmpliScribe-T7-Flash Transcription Kit the following components were mixed at room temperature: RNase-free water, 1-1.5 $\mu$g gene, AmpliScribe T7-Flash 1X Reaction Buffer (Epicentre, #ASF3507), 9 mM NTPs, 40 U/$\mu$L RiboGuard RNase Inhibitor (Epicentre, #RG90925), and 1 unit of AmpliScribe T7-Flash Enzyme Solution (Epicentre,#ASF3507). This mix was incubated at 37°C for 4 hours. After incubation, 20 $\mu$L of loading dye was added to the 20 $\mu$L transcription solution, and 8 $\mu$L of the transcription/dye mix was added to each of the middle 5 lanes of the gel. The gel was run at 100 V at room temperature in 1X TBE.

After electrophoresis the gel was stained in 80 mL 1X TBE and 1 $\mu$L SYBR®Gold Nucleic Acid Gel Stain (Thermo Scientific, # S-11494) for 20-30 minutes. The gel was then placed on a TLC silica gel 60 W F254S aluminum sheet (EMD Millipore, #1055590001) covered in plastic wrap. The gel was illuminated with UV light and the desired RNA band was cut out and chopped into equal amounts and placed into 0.5 mL DNA Lobind tubes (Eppendorf, #022431005), then 200 $\mu$L of 0.3 M sodium acetate at pH 5.3 was added to the Lobind tubes. The samples were then incubated at 42°C for 20 hours. After incubation, the sodium acetate was removed and placed into 1.7 mL RNase/DNase free tubes. The old Lobind tubes were rinsed with 100 $\mu$l of 0.3 M sodium acetate pH 5.3, which was added to the new samples. Then 1 mL of freezer cold 100% ETOH and 1 $\mu$L of glycogen were added into each tube and the sample was incubated at -20°C for 20 hours.

Next, the samples were spun at 13,500 rpm at 4°C for 15 minutes. The white precipitate pellet (RNA) at the bottom of the tube was located and the supernatant was carefully pipetted out of the tubes avoiding removal of the pellet. Then, 500 $\mu$l of 70% freezer cold ETOH was added to the tubes and spun at 13,500 rpm at 4°C for 5 minutes. The supernatant was carefully decanted again with a pipette. This washing procedure was repeated a third time. After the last wash, as much supernatant was removed as possible, then the samples were opened and placed in the vacuum concentrator and allowed to spin at room temperature for 15 minutes. The samples were then re-suspended in 10-15 $\mu$L of Ambion nuclease free water (Ambion, #AM9932).

## 3 Stability of RNA nanotubes

We monitored the length distribution of annealed 23-7 and 24-8 RNA nanotubes for 30 hours. The violin plots in Fig. S2 show that the mean length of the nanotubes remains roughly constant; the overall distribution also appears to fluctuate only marginally over time.

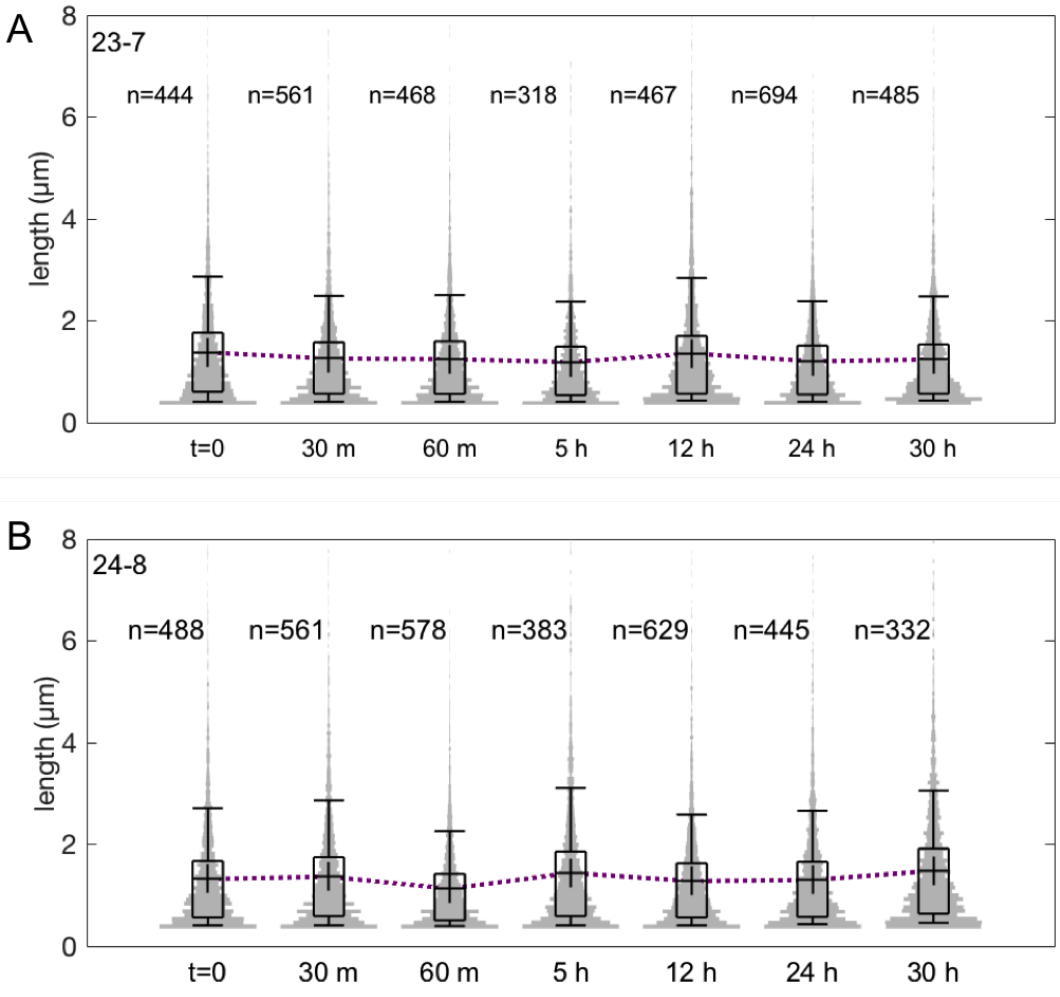


Figure S2: **The distribution of nanotube length over time does not fluctuate significantly** Violin plots of nanotube length for 23-7 and 24-8 tile variants. Data processing is described in Section 2.2.1.

## 4 Joining of RNA nanotubes is not observed

To test whether joining of RNA nanotubes occurs over time, we annealed two samples of the 24-8 tile variant. One of the samples was labeled with 10% Cy3 located on the 5' end of S3 and the other sample was labeled with 10% Atto-647N located on the 5' end of S3. Annealed Cy-3 and Atto-labeled nanotubes were then mixed and incubated at room temperature for 30 hours. In contrast with observations made on DNA nanotubes, the images collected indicate that nanotubes of distinct colors do not join, suggesting that joining is a rare event (Fig. S3).

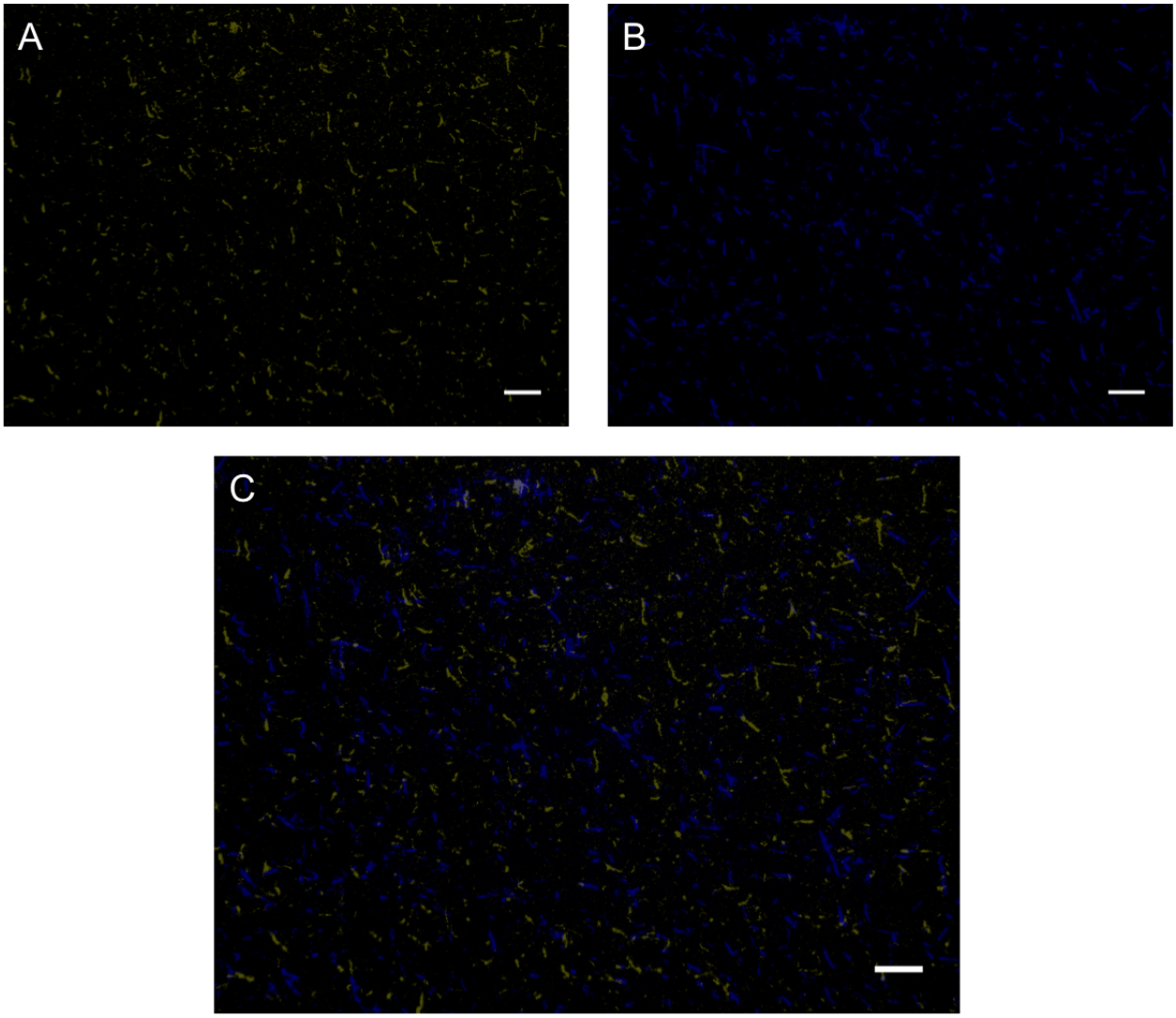


Figure S3: **Joining of RNA nanotubes is a rare event.** Nanotubes of different colors do not appear to join. A. 24-8 tubes labeled with Cy3. B. 24-8 tubes labeled with Atto-647N. C. Overlap of A and B. Scale bar 10 $\mu$m.

## 5 Nanotube nucleation requires high tile concentration

To identify the minimum concentration of tiles required to observe formation of assemblies, we annealed the 23-7 and 24-8 tile variants at different tile concentrations. After annealing, samples were imaged using both fluorescence microscopy and AFM. We annealed tiles at 1000 nM, 500 nM, 250 nM, and 100 nM; fluorescence microscopy images are shown in Fig. S4. AFM images of variant 23-7 annealed at different concentrations are shown in Fig. S5. Nanotube formation was observed at tile concentrations between 1000 nM and 250 nM. No nanotubes were observed at 100 nM. As the annealed tile concentration decreases we also notice a qualitative decrease in the lengths of the tubes that form. In previous work, we observed the formation of a different type of RNA nanotubes at 50 nM tile concentration. [7] Features such as sequence content and inter-tile crossover distance may play a role in determining minimum tile concentration required for nucleation.

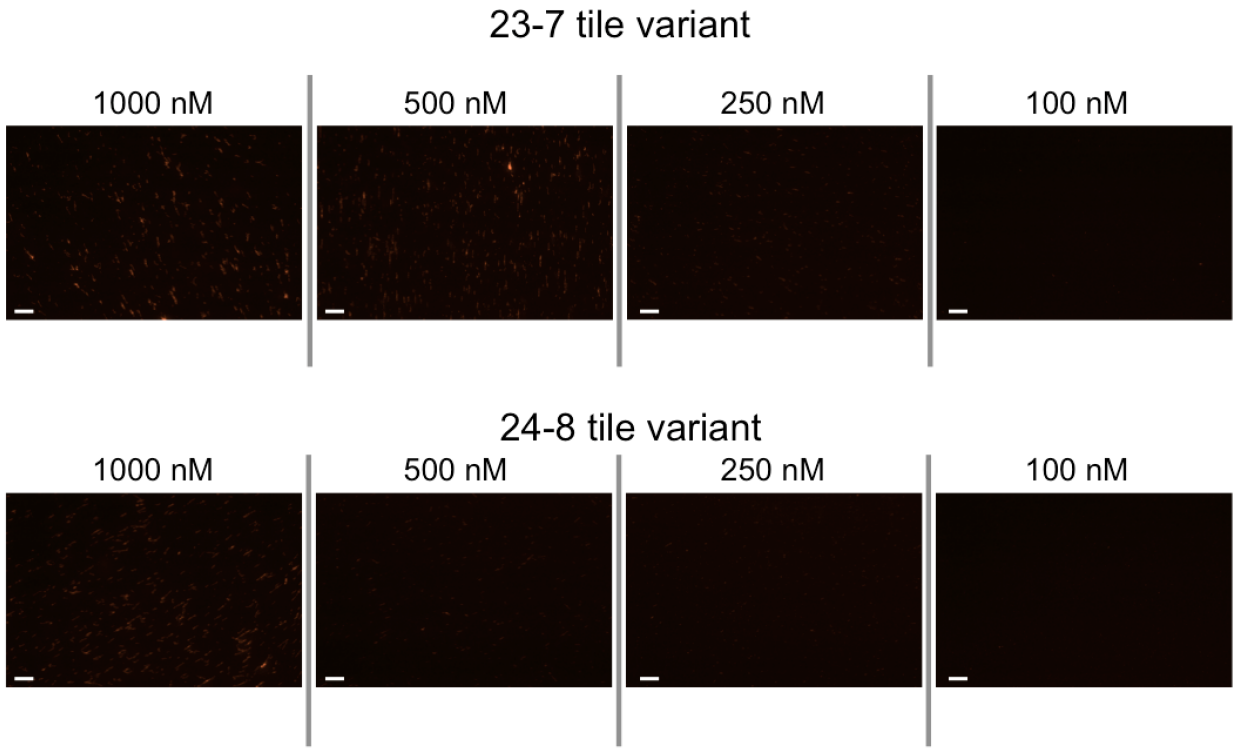


Figure S4: **Fluorescence microscopy images of nanotubes annealed at different tile concentration.** The scale bar is 10 $\mu$m.

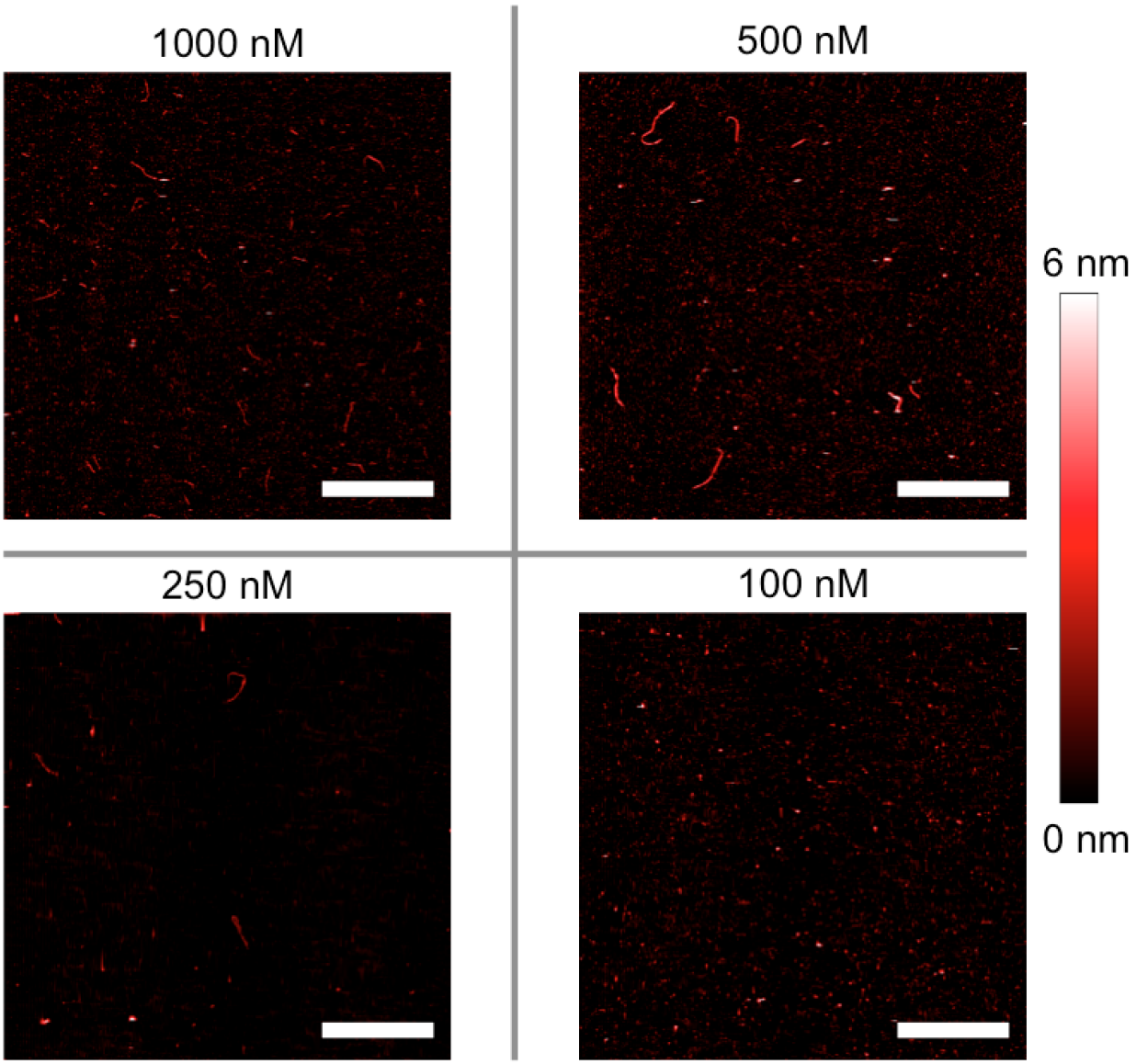


Figure S5: **AFM images of 23-7 annealed nanotubes at different tile concentrations.** The scale bar is 5$\mu$m

## 6 Assembly of a two-tile system does not occur at room temperature

We designed a two-tile system by modifying the 23-7 tile variant that yields tubular assemblies. We altered exclusively the sticky end sequences so that tiles assemble in a perpendicular pattern, following a similar two-tile DNA nanotube design in Rothemund *et al.*[4] Tiles were annealed separately (see Methods) and subsequently mixed to test whether nucleation and polymerization into nanotubes is possible at constant temperature. Unlike the two-tile DNA nanotubes, these RNA tiles do not appear to nucleate spontaneously and do not form nanotubes (Fig. S6).

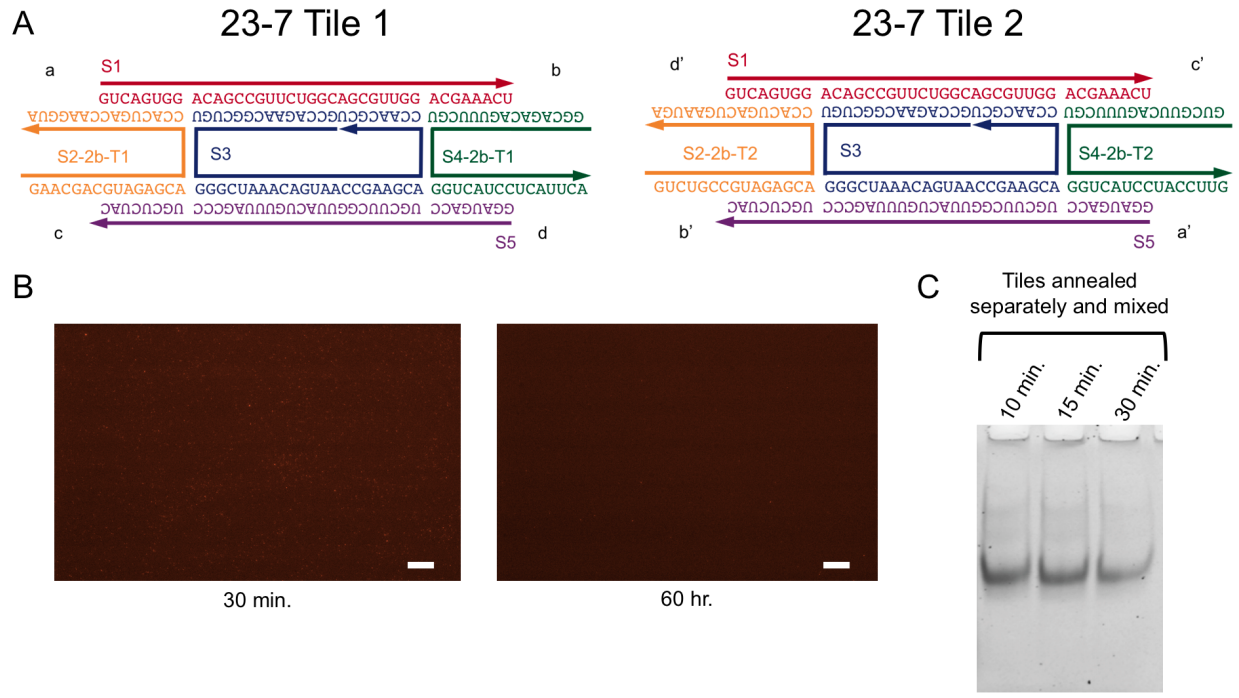


Figure S6: **23-7 two tile system.** A. Schematic of two tile system B. fluorescence microscopy images of separately annealed tiles after mixing them together and incubating for 30 minutes (right) and 60 hours (left). C. Native PAGE of the 23-7 two tile system and separately annealed and mixed for 10, 15, and 30 minutes. Scale bar is 10 $\mu$m.

## 7 Formation of nanotubes is influenced by sticky end length

To investigate the influence of the length of the sticky ends on the yield of assembled structures, we modified the 24-8 tile variant to shorten the sticky end length from 8 bases to 5 bases. We increased the length of the S1 and S5 strands by 6 bases each. We visualized the assemblies using AFM (Fig. S7). For the 24-5 tile variant we observe no formation of tubular structures, but rather filamentous assemblies.

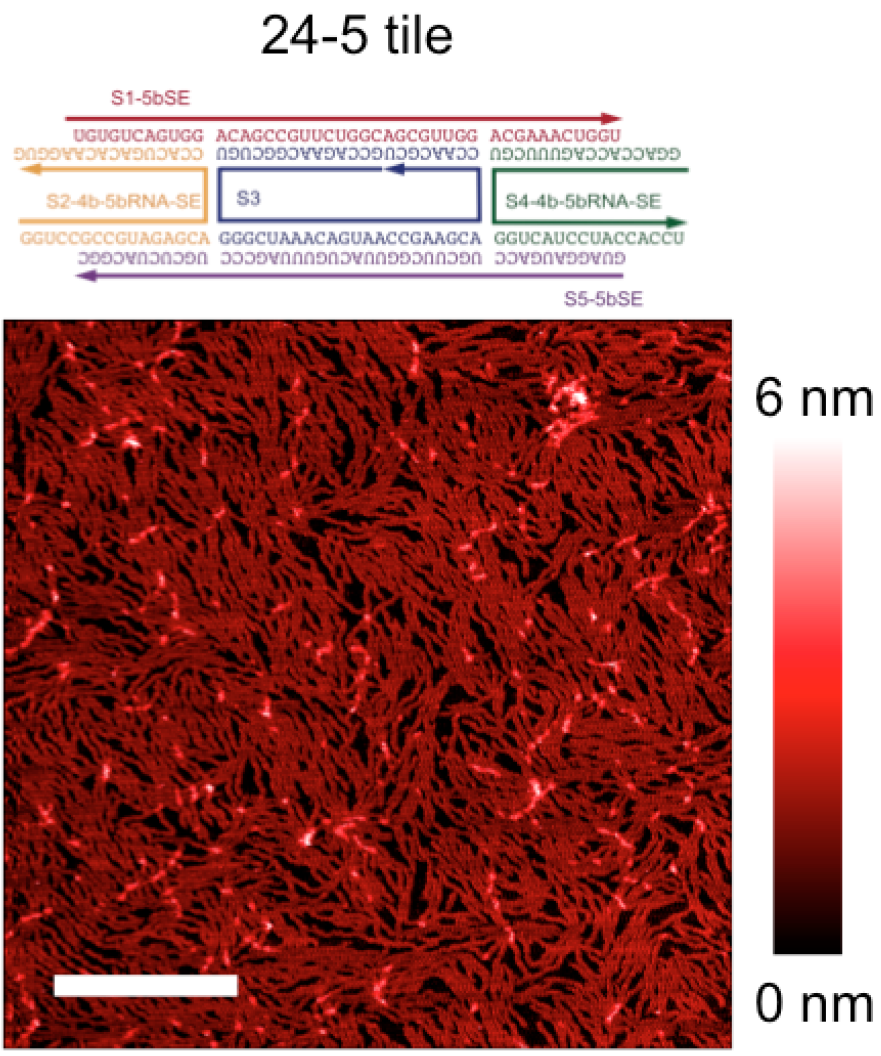


Figure S7: **Example AFM image of annealed 24-5 tile variant.** While the 24-8 tile variant yields nanotubes, this variant with shorter sticky ends does not result in nanotube assembly. Scale bar is 250 nm.

## 8 Large area scan AFM images

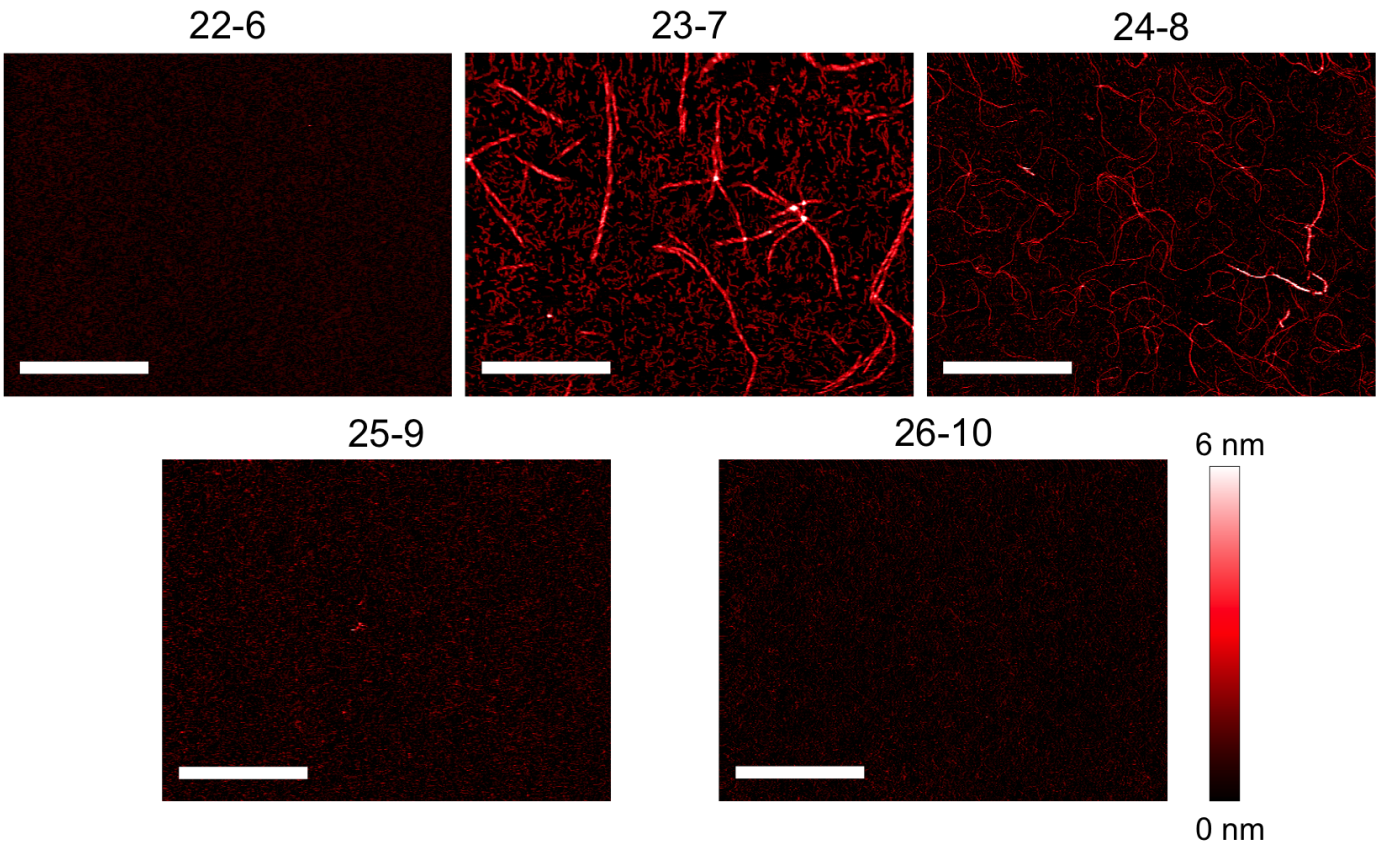


Figure S8: **Large scan area AFM images of all tile variants.** Scale bar: 2 $\mu$m

## 9 The presence of a fluorophore on S3 affects nanotube formation

We observed that the presence of a fluorescent label on strand S3 affects the number and the mean length of nanotubes in fluorescence microscopy images; this influence depends on what fraction of strand S3 includes a fluorophore (Fig. S9). When the labeled S3 strand is 25% (of total S3 concentration), the yield of nanotubes decreases significantly. Thus, we decided to perform fluorescence experiments incorporating 10% of fluorophore-labeled strand S3 because it presented the brightest fluorescence and a high yield of long nanotubes. We hypothesize that, similarly to what occurs in DNA labeled strands, when Cy3 is attached to a single stranded RNA the dye becomes more rigid due to stacking interactions. This stacking causes steric hindrance, which in turn displays a high energy barrier for the rotation of the bond of Cy3, thus increasing fluorescence, however, this increase in steric hindrance may also affect the assembly potential of structures.[5]

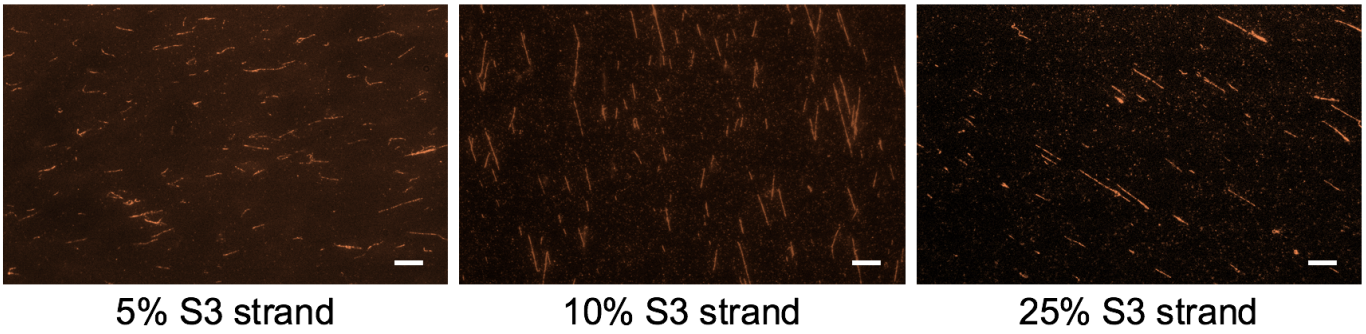


Figure S9: **Fluorescence microscopy images of 23-7 tile with varying percentages of S3 strand labeled with Cy3.** Scale bar: 10 $\mu$m

## 10 Computer modeling of RNA tile assemblies

Computer models of individual RNA tiles were prepared as described in.[1] RNA helices are aligned in a three-dimensional RNA modeling environment, UCSF Chimera,[3] to systematically build up a model of the RNA tile with ideal A-form RNA geometry. A detailed step-by-step tutorial of this RNA modeling approach is also described in.[6] Multiple models of the RNA tile are then loaded into the modeling environment and aligned according to the direction of their connecting ends to depict how additional multimer units would coaxially-stack and link to the structure. In cases where joining units would cause major steric overlap we leave the connector ends unpaired.

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