# Supporting Information

## DNA Nanostructures that Self-Heal in Serum

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## SI 1: Nanotube tile design and conjugation of PEG to DNA

M13mp18 scaffold strand was purchased from Bayou Biolab. All other DNA strands used in this study were synthesized by Integrated DNA Technologies, Inc. (IDT). The DNA nanotube tile and adapter strands were desalted while Cy3, Atto647 and Atto488 fluorophore-labeled strands, biotin-labeled, and amino-modified strands were HPLC purified. Concentrations for DNA strands were determined either by measuring absorbance at 260 nm wavelength or by using IDT's stated yields to determine solution concentrations.

### 1.1 DNA tile design and tile strand sequences

The tile design and sequence in this study are adapted from Rothemund *et al* [1]. To make nanotubes that were stable at 37°C, we extended the sticky ends of the DNA tiles from 5 base pairs to 6 by shortening the double-stranded tile region so as to maintain a proper distance between crossover points. We began the study using nanotubes formed from DNA tiles as shown in Figure S1.

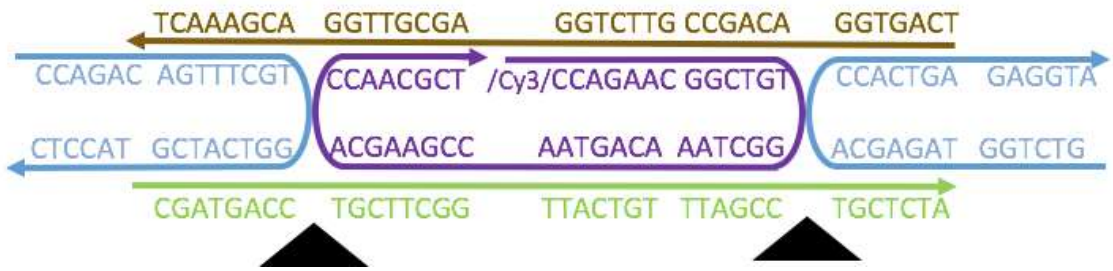


**Figure S1:** Schematic showing the architecture of the DNA tiles without PEG conjugation used in this study. Black triangles indicate crossover points. Cy3 fluorophores allow for nanotube visualization on the fluorescence microscope.

*DNA tile sequences:*

SEs_1: TCAGTGGACAGCCGTTCTGGAGCGTTGGACGAAACT

SEs_2: CCAGACAGTTTCGTGGTCATCGTACCTC

SEs_3-5'Cy3: /CY3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCACCAACGCT

SEs_4: GTCTGGTAGAGCACCACTGAGAGGTA

SEs_5: CGATGACCTGCTTCGGTTACTGTTTAGCCTGCTCTA

/Cy3/ denotes a Cy3 fluorophore covalently attached to the 5' end of DNA.

### 1.2 Conjugation of PEG chains to DNA tiles

After adding serum-supplemented medium to the seeded nanotubes anchored to glass cover slips, we observed that DNA nanotubes formed from the tiles in Figure S1 adhered to the glass and to one another, making it difficult to observe degradation and also suggesting that the function of these structures would be limited by unintended interactions (Figure S2). To reduce these effects, we developed a modified tile that presented a polyethylene glycol (PEG) chain. To make these tiles, we modified the central DNA tile strand (SEs_3-5'Cy3 in Figure S1) to present a 5-base thymine spacer with a 3'-end primary amine at its end (Figure S3). The amine-modified DNA strand, 'SEs_3-5'Cy3_3'amine', was purchased from IDT in PAGE purified form. To attach a PEG polymer to this strand, we reacted the amine on 3' end of the central DNA tile strand with N-hydroxylsuccinimide (NHS) functionalized polyethylene glycol (PEG) valeric acid with molecular weight of 20,000 Da (NANOCS, PG1-SVA-20K) as described in the main text Methods.

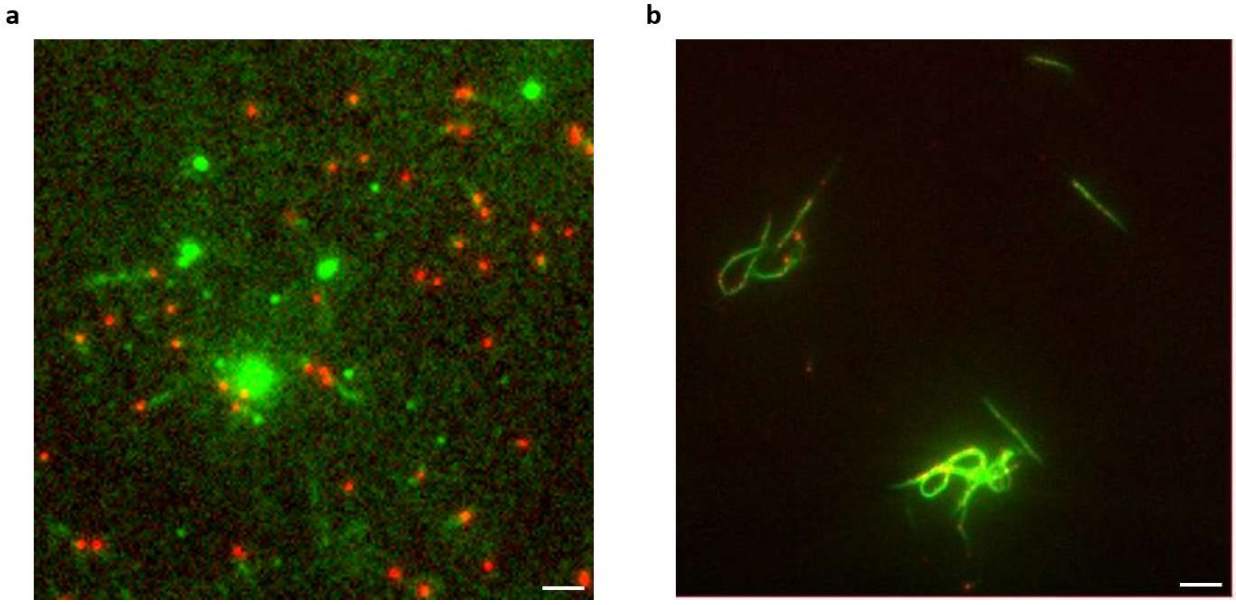


**Figure S2**. (a), Multicolor fluorescence images showing seeded nanotubes anchored to a glass dish after incubation in 10% FBS supplemented DMEM at 37°C for 12 hours. The anchored nanotubes adhered to glass surface and to each other. DNA structures and tiles that appeared to be degraded also adhered to surface, creating background noise in the image. DNA nanotubes are labeled with Cy3 dye (green) and seeds with Atto647 dye (red). (b), Seeded nanotubes without PEG coating incubation in 10% FBS supplemented DMEM at 37°C for 90 minutes in a 200 µl PCR tube then 6 µl of nanotube solution was plated onto a glass slide for imaging. Undesired clusters of nanotubes are observed, suggesting that unmodified nanotubes incubated in test tubes also aggregated. DNA nanotubes are labeled with Cy3 dye (green) and seeds with ATTO647 dye (red). Scale bars, 5 µm.

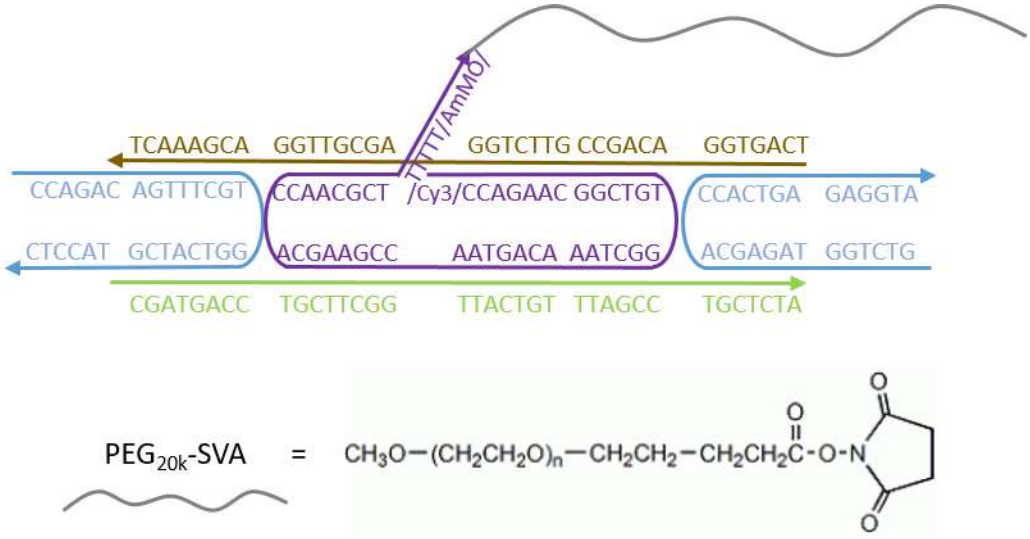


**Figure S3: Primary amine-modified SEs tiles are conjugated to succinimidyl valeric acid PEG with a molecular weight of 20 kDa ($PEG_{20k}$-SVA).**

SEs_3-5'Cy3_3'amine: /Cy3/CCAGAACGGCTGTGGCTAAACAGTAACCGAAGCACCAACGCTTTTTT/AmMO/

/AmMO/ denotes an amino group covalently attached to the 3' end of the DNA.

### 1.3 Design of inactivated tiles and a corresponding activation strand

To ensure that the tiles that were added to the serum supplemented DMEM had not themselves assembled into nanotubes before the start of the experiment, we modified the tiles to create tiles that were annealed in an inactive form, adapted from design of Zhang et al. [3]. These inactive tiles could then be activated, *i.e.* reach a conformation that allowed assembly into nanotubes, by a strand-displacement reaction with an activation strand (Figure S4). The inactive tiles are designed such that one of the sticky ends is double-stranded, preventing the tiles from forming a lattice by sticky end joining. The activation strand, 'SEs_activation', upon addition to the solution, displaces the 'SEs_inactive_strand5_right' strand and exposes a single-stranded sticky end where a double-strand end was previously. The resulting products have four exposed sticky ends, allowing assembly of DNA nanotubes.

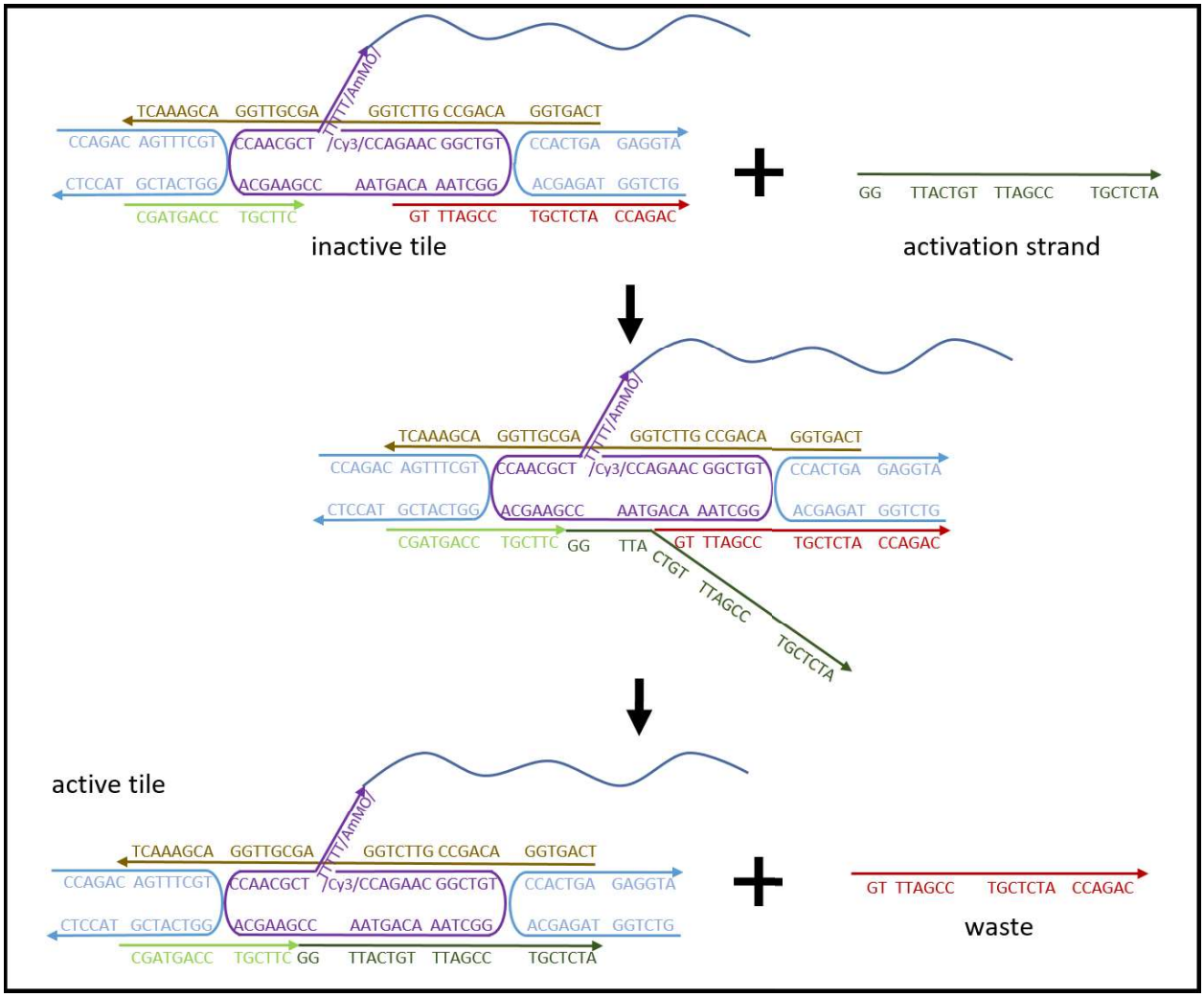


**Figure S4:** Schematic of the reaction in which the activation strand reacts with an inactive tile by displacing the strand that covers one of the sticky ends. The resulting reaction "activates" the tile.

*Inactive tile strands and activation strand sequences:*

Sequences of SEs_1, SEs_2, and SEs_4 tile strands are listed in S1.1.

Sequence of central tile strand is given in S1.2.

SEs_inactive_strand5_left: CGATGACCTGCTTC

SEs_inactive_strand5_right: GTTACTGTTTAGCCTGCTCTACCAGAC

SEs_activation: GGTTACTGTTTAGCCTGCTCTA

## SI 2. Seed design and sequences

The sequences of staple strands used to fold DNA nanotube seeds in this work are the same as those in Mohammed *et al* [2]. The following seed adapter strands, added along with staple strands at annealing, were modified to present the sticky end sequences of the tiles used this study:

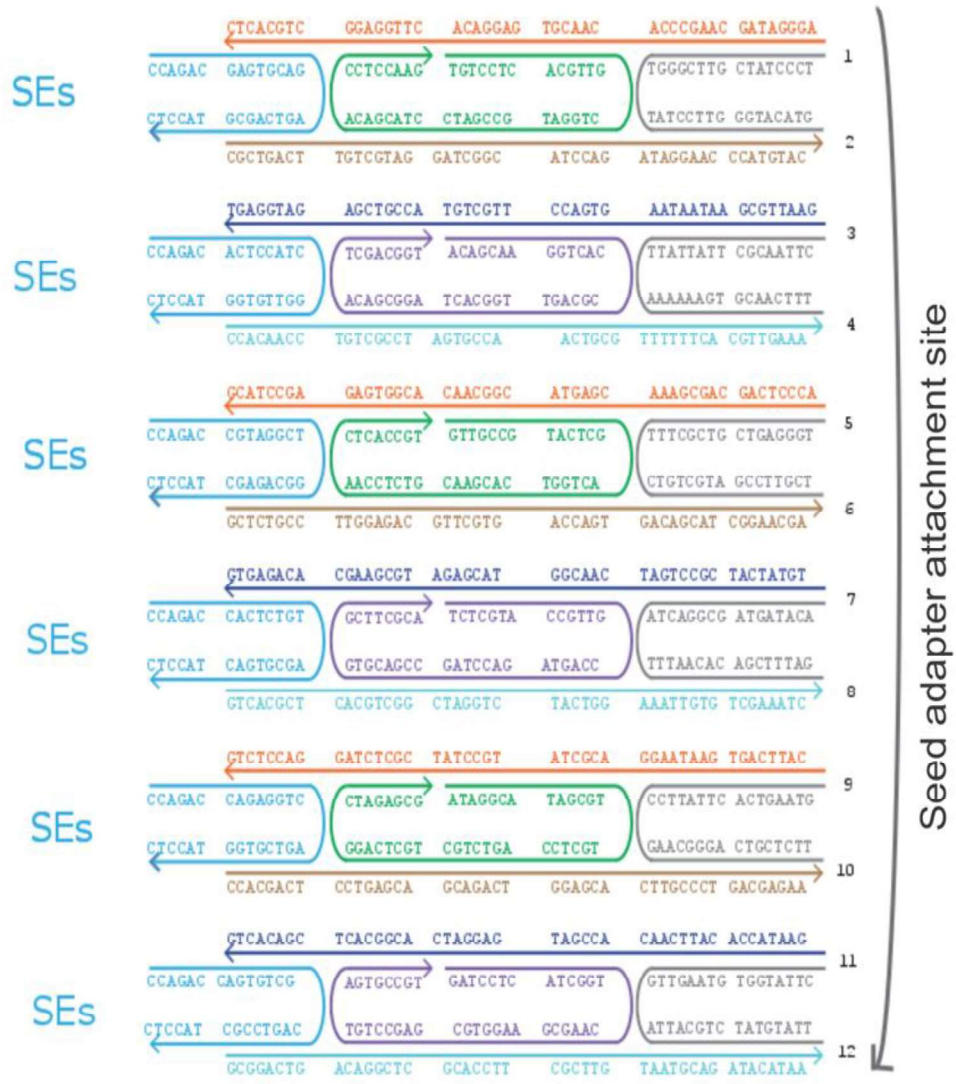


**Figure S5:** Schematic of the assembled adapter tiles for the seeds. The gray lines and their corresponding sequences are components of the M13mp18 scaffold.

*Adapter strands sequences*

AD1SEs6bp_1-: AGGGATAGCAAGCCCACAACGTGAGGACACTTGGAGGCTGCACTC

AD1_2SEs6bp_3-: TGTCCTCACGTTGCTGGATGCCGATCCTACGACACCTCCAAG

AD1_2SEs6bp_5-: CGCTGACTTGTCGTAGGATCGGCATCCAGATAGGAACCCATGTAC

AD2SEs6bp_2-: CCAGAC GAGTGCAGAGTCAGCGTACCTC

AD3SEs6bp_1-: GAATTGCGAATAATAAGTGACCTTGCTGTACCGTCGAGATGGAGT

AD3_4SEs6bp_3-: ACAGCAAGGTCACCGCAGTTGGCACTAGGCGACATCGACGGT

AD4SEs6bp_5-: CCACAACCTGTCGCCTAGTGCCAACTGCGTTTTTTCACGTTGAAA

AD3_4SEs6bp_2-: CCAGAC ACTCCATCGGTTGTGGTACCTC

AD5SEs6bp_1-: ACCCTCAGCAGCGAAACGAGTACGGCAACACGGTGAGAGCCTACG

AD5_6SEs6bp_3-: GTTGCCGTACTCGACTGGTCACGAACGTCTCCAACTCACCGT

AD6SEs6bp_5-: GCTCTGCCTTGGAGACGTTCGTGACCAGTGACAGCATCGGAACGA

AD5_6SEs6bp_2-: CCAGAC CGTAGGCTGGCAGAGCTACCTC

AD7SEs6bp_1-: TGTATCATCGCCTGATCAACGGTACGAGATGCGAAGCACAGAGTG

AD7_8SEs6bp_3-: TCTCGTACCGTTGCCAGTAGACCTAGCCGACGTGGCTTCGCA

AD8SEs6bp_5-: GTCACGCTCACGTCGGCTAGGTCTACTGGAAATTGTGTCGAAATC

AD7_8SEs6bp_2-: CCAGAC CACTCTGTAGCGTGACTACCTC

AD9SEs6bp_1-: CATTCAGTGAATAAGGACGCTATGCCTATCGCTCTAGGACCTCTG

AD9_10SEs6bp_3-: ATAGGCATAGCGTTGCTCCAGTCTGCTGCTCAGGCTAGAGCG

AD10SEs6bp_5-: CCACGACTCCTGAGCAGCAGACTGGAGCACTTGCCCTGACGAGAA

AD9_10SEs6bp_2-: CCAGAC CAGAGGTCAGTCGTGGTACCTC

AD11SEs6bp_1-: GAATACCACATTCAACACCGATGAGGATCACGGCACTCGACACTG

AD11_12SEs6bp_3-: GATCCTCATCGGTCAAGCGAAGGTGCGAGCCTGTAGTGCCGT

AD12SEs6bp_5-: GCGGACTGACAGGCTCGCACCTTCGCTTGTAATGCAGATACATAA

AD11_12SEs6bp_2-: CCAGAC CAGTGTCGCAGTCCGC TACCTC

Nanotube seeds were labeled with Atto647 fluorophore dyes for fluorescence imaging. The labeling system consists of 100 attachment strands, each of which contains 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 a labeling strand that has Atto647 fluorophore dye on the 5' end, 'labeling_strand_ATTO647N'. The sequences of the attachment strands are the same as those listed in Mohammed *et al*. [2]

labeling_strand_ATTO647N:  /5ATTO647NN/AAGCGTAGTCGGATCTC

## SI 3. Preparing seeded nanotubes

### 3.1 Preparation of nanotube seed annealing solution

To assemble seeds, 50 µl annealing mixture was prepared containing M13mp18 scaffold, staple strands, adapter strands, fluorescent attachment strands and biotin attachment strands in 1x TAE $Mg^{2+}$ buffer in the quantities shown below.

*Recipe for preparing nanotube seed annealing solution*:

| | Desired final concentration (nM or fold) | Stock concentration (nM or fold) | Volume added (µl) |
|---|---|---|---|
| $H_2O$ | | | 27.6 |
| TAE $Mg^{2+}$ buffer | 1 | 10 | 5 |
| Seed staple mix (concentrations are per strand) | 200 | 1389 | 7.2 |
| Seed adapter strand mix (concentrations are per strand) | 100 | 1000 | 5 |
| M13 scaffold | 5 | 100 | 2.5 |
| Fluorescent attachment strand mix (concentrations are per strand) | 25 | 1000 | 1.25 |
| Biotin attachment strand mix (concentrations are per strand) | 300 | 10000 | 1.5 |
| Total | | | 50 |

All of the following DNA strand mixes are prepared by dissolving DNA strands in MilliQ water:

*Seed staple mix:* Mixture containing all 72 seed strands at equal concentrations. 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 adapter strand mix:* solution containing all 24 seed adapter strands (as shown in Figure S5) in equal concentrations of 1 µM per strand.

*Fluorescent attachment strand mix:* solution containing 100 attachment strands in equal concentrations of 1 µM per strand for the fluorescence labeling of seeds. Sequences of fluorescent attachment strands are as given in Mohammed *et al*. [2]

*Biotin attachment strands mix:* mixture containing all 6 biotin attachment linker strands (Sequences as described in Section S4.3).

**3.2 Seed annealing protocol**

Seeds were annealed by running the following thermal schedule on the prepared annealing mixture with a thermocycler (Eppendorf Mastercycler):

1. 5 mins at 90°C
2. 90°C to 45°C at 1°C/min
3. 45°C for 60 mins
4. 45°C to 37°C at 1°C/10mins
5. 37°C hold until sample retrieval

**3.3 Seed purification and fluorescent labeling**

Seeds were separated from excess staple, adapter, biotin attachment, and fluorescent attachment strands by the following purification process:

After annealing, 50 µl of seed solution and 350 µl TAE $Mg^{2+}$ buffer were added to a 100kDa Amicon µltra-0.5mL centrifugal filter (UFC510096) and centrifuged at 3000 RCF for 4 min in a fixed-angle centrifuge. The sample was washed two more times by adding 350 µl TAE $Mg^{2+}$ buffer into the remaining solution and repeating centrifugation. Purified seed solution was collected by spinning the inverted filter in a new tube.

To fluorescently label the seeds, 12 µl of 1 µM Atto647 labeling strand was added to approximately 40 µl purified seeds collected from the filter unit. The mixture was incubated at room temperature for at least 15 minutes.

The concentration of purified seeds was measured by adopting the method developed by Agrawal *et al*[4]*.* After purification, an imaging solution was prepared by mixing 0.3 µL seed solution with 19.7 µL tile mix solution (containing 0.05 mg/ml BSA). 6 µl mixture was then transferred to a glass slide to be imaged under a fluorescence microscope with 60x objective. We continued to dilute the purified seeds until

100-200 seeds per field of view (87 µm x 87 µm) were observed, indicating an approximate seed concentration of 6pM in the imaged solution.

### 3.4 Annealing inactive tiles

DNA tiles were annealed separately from seeds before they were mixed with purified seeds. 88 µl of solution containing 398 nM of each of the inactive SEs tile strands (as listed in S1.3) in TAE $Mg^{2+}$ buffer was annealed using the same annealing protocol as seed annealing (S3.2).

### 3.5 Self-assembly of seeded nanotubes

6 µl purified seeds, diluted to 0.8 nM concentration, prepared as in S3.1-3 were mixed with 88 µl of the annealed inactive tile solution prepared as in S3.4 and incubated at 37°C. 4 µl of activation strand to a final concentration of 400 nM was then added to activate annealed tiles so that the tile concentration was 350 nM after the addition of purified seeds and the activation strands. The mixture was incubated at 37°C for at least 24 hours to allow the seeded nanotube to grow to their maximum lengths.

## SI 4. Anchoring seeds to and detaching seeds from passivated glass

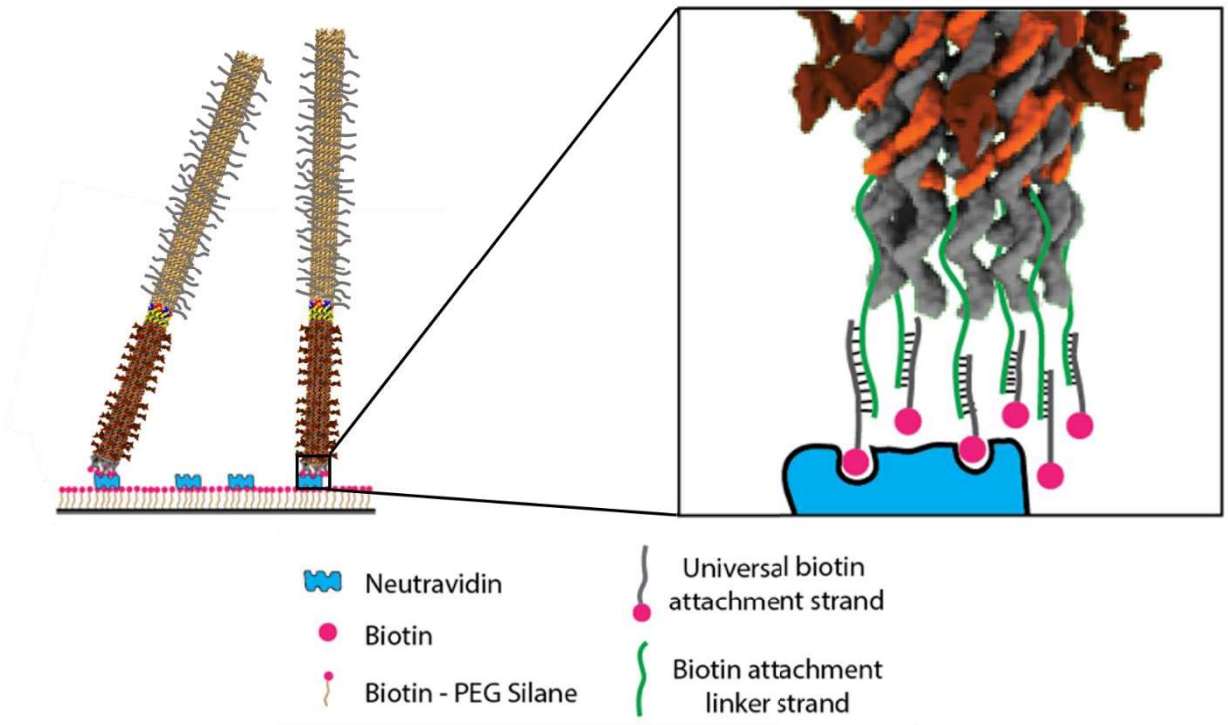


**Figure S6**. Schematic illustrations of the passivated glass surface and the anchoring of DNA nanotube seeds to the surface via biotin-NeutrAvidin chemistry. NeutrAvidin molecules were deposited on the treated glass surface. 6 biotin attachment linker strands (green strands) each were bound to the M13mp18 scaffold and to a universal biotin attachment strand (gray strand with biotin, pink). The universal biotin strands bound to NeutrAvidin molecules present on the glass surface.

*Biotin attachment linker strands sequences for the nanotube seed:*

Biotin_rightside_01: CTATTATTCTGAAACATTTTCACATCGTCACTCCT

Biotin_rightside_02: CAGGAGGTTGAGGCAGTTTTCACATCGTCACTCCT

Biotin_rightside_03: ATCAAGTTTGCCTTTATTTTCACATCGTCACTCCT

Biotin_rightside_04: GGTTTACCAGCGCCAATTTTCACATCGTCACTCCT

Biotin_rightside_05: TTTTTAAGAAAAGTAATTTTCACATCGTCACTCCT

Biotin_rightside_06: AAACGATTTTTTGTTTTTTTCACATCGTCACTCCT

Universal biotin attachment strand: /5BiosG/AGGAGTGACGATGTG

/5BiosG/ denotes a biotin protein covalently conjugated to 5' end of a DNA strand.

### 4.1 Protocol for NeutrAvidin presentation on the glass surface

The surface of glass-bottom dishes with a 50 µm labeled grid (ibidi µ-Dish 35 mm, high Grid-50 Glass Bottom) were modified with NeutrAvidin protein using the same protocol as described in Mohammed *et al*. [2]

### 4.2 Design of detachable nanotube anchor

The extended biotin attachment strand was designed by adding a 5-bp toehold domain to the universal biotin strand. In experiments where seeds were detached from the surface, seeds were anchored by hybridizing the extended biotin attachment strand on the surface rather than the universal biotin attachment strand. A biotin displacement strand, the full complement of the biotin attachment strand (excluding a spacer) was used to detach the reversibly anchored seeded nanotubes on passivated glass by binding to the extended biotin attachment strand and displacing the biotin attachment linker strands on seeds.

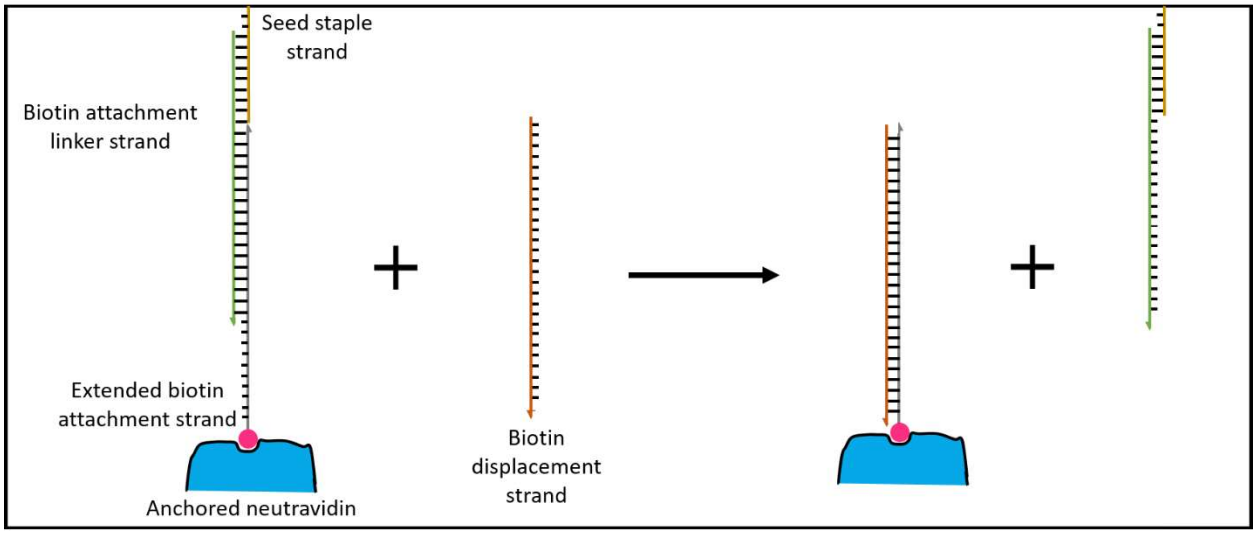


**Figure S7**. Schematic illustration showing a biotin attachment linker strand on a seed and how it can be detached from an extended biotin attachment strand anchored on a passivated glass via a strand displacement reaction involving a biotin displacement strand. The biotin displacement strand (orange strand), hybridizes to a toehold on the extended biotin attachment strand (gray strand with a biotin, pink), and displaces the biotin attachment linker strand (green strand) on the seed. As a result, the seed detaches from the glass surface.

Extended biotin attachment strand: /5BiosG/TTTT GTGAG AGG AGT GAC GAT GTG

Biotin displacement strand: CACATCGTCACTCCTCTCAC

## SI 5. Fluorescence images and length distribution of PEG-coated DNA nanotubes

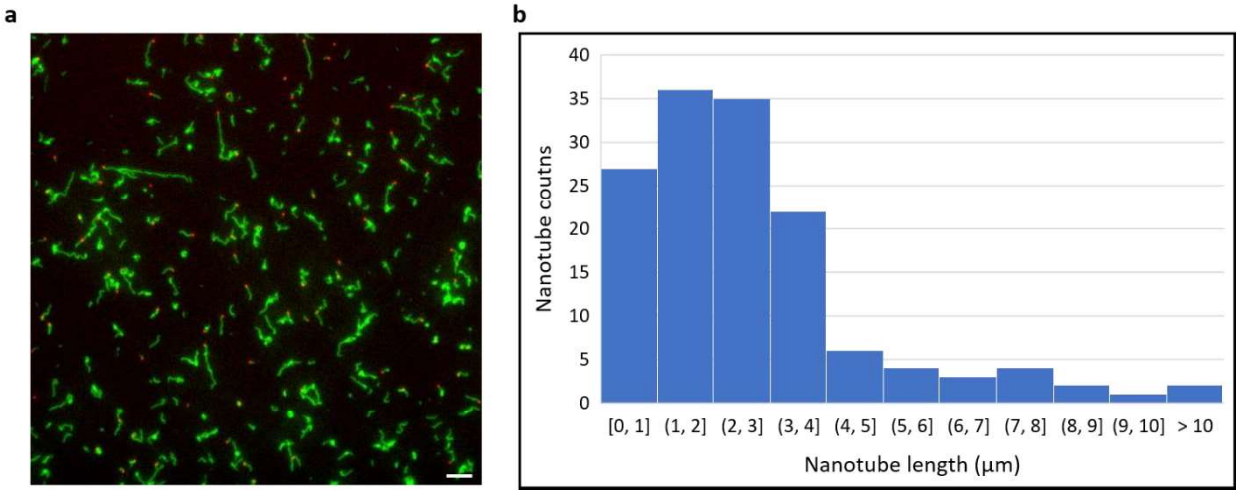


**Figure S8.** (a) Wide-field fluorescence image of PEG-coated seeded nanotubes grown as described in SI 3 and then plated on a glass slide. DNA nanotubes are labeled with Cy3 dye (green) and seeds with Atto647 dye (red). Scale bar, 5 µm. (b) Lengths of nanotubes in two fields on view were measured and the results are shown in a histogram graph of length distribution of seeded nanotubes with a PEG coating. N = 142.

## SI 6: Additional AFM images of PEG coated seeded nanotubes

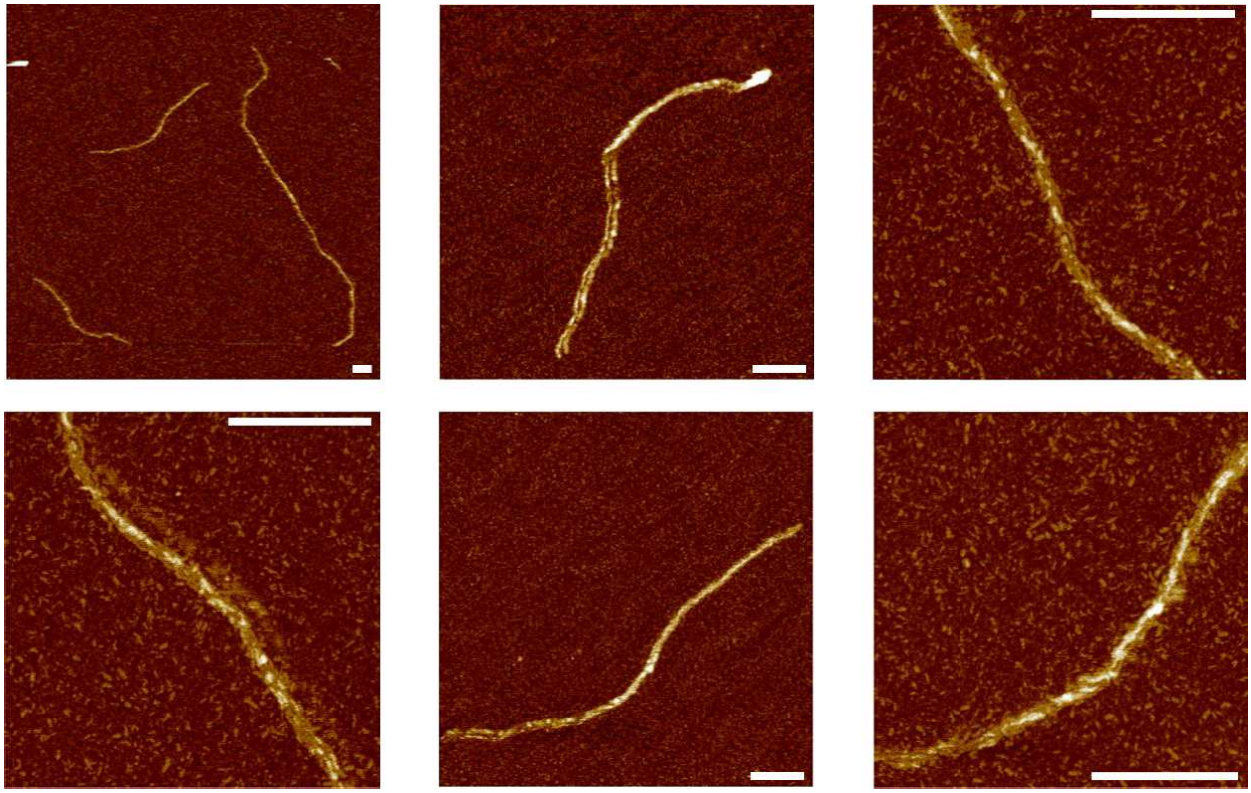

**Figure S9**. Sample AFM images of PEG coated DNA nanotubes. Scale bars, 200 nm.

## SI 7. Fluorescence images of nanotubes after different periods of serum-supplemented medium incubation

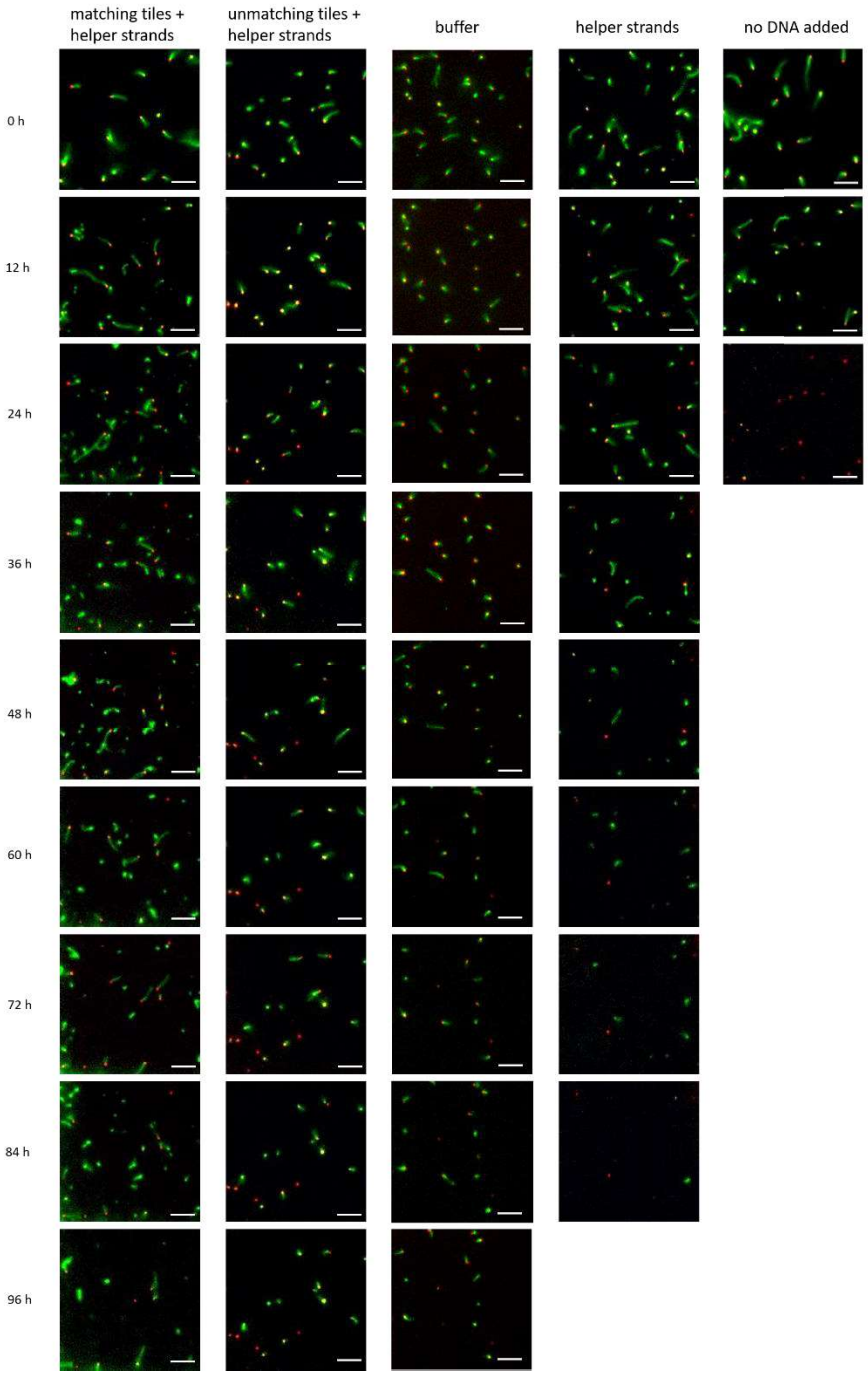


**Figure S10**. Additional fluorescence microscopy images showing the breakdown of end-anchored PEG-coated seeded nanotubes after different incubation times in four different conditions. DNA nanotubes are labeled with Cy3 dye (green), seeds with Atto647 dye (red). Scale bars, 5 μm.

## SI 8. Additional fluorescence confocal images of repaired nanotubes

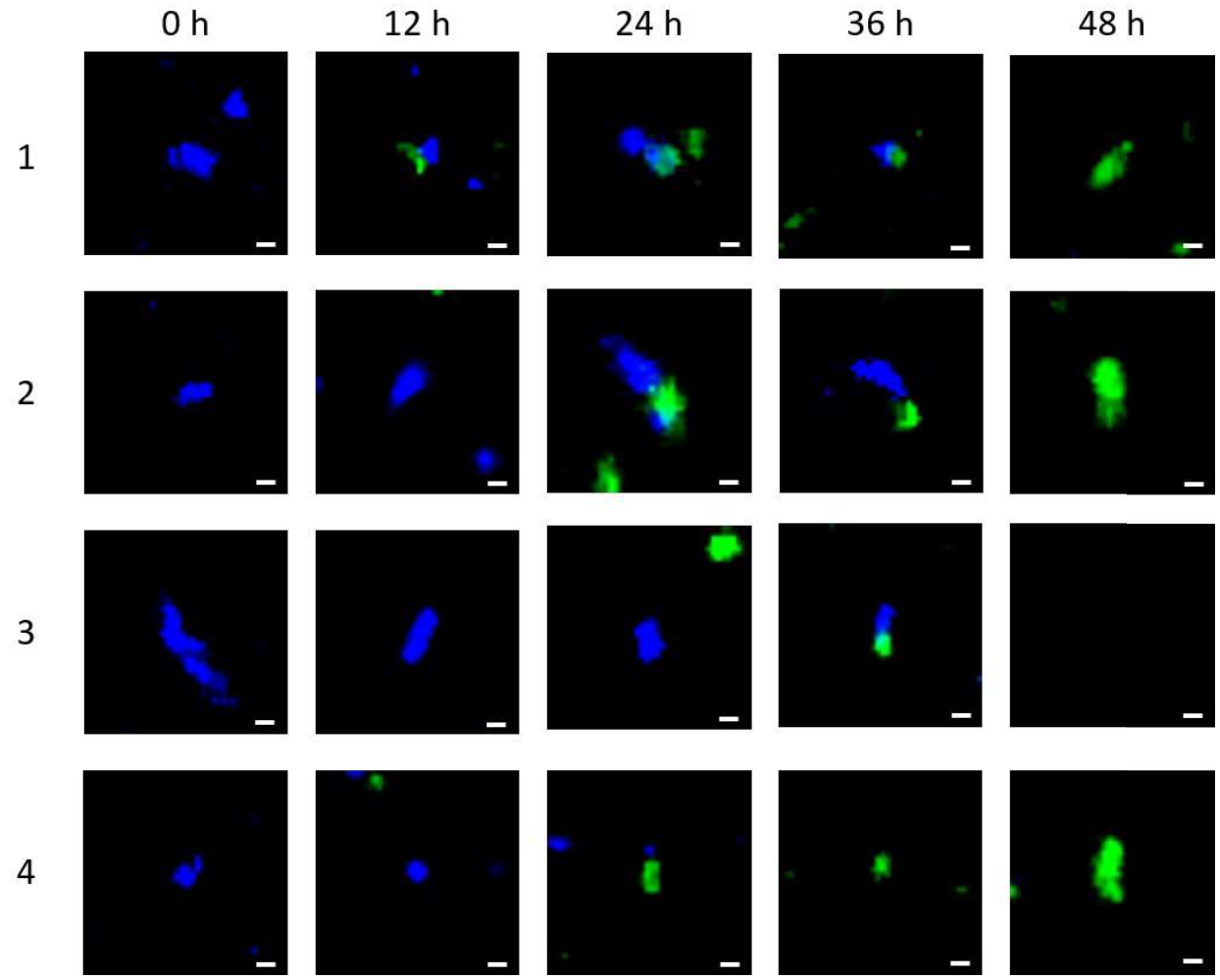


**Figure S11**. Additional multicolor time-lapse fluorescence microscopy images showing free tiles (green, Cy3) joined and incorporated into original anchored nanotubes (blue, Atto488) incubated in serum-supplemented medium at 37°C. Scale bars, 2 μm.

## SI 9. Additional simulations of nanotube degradation and repair

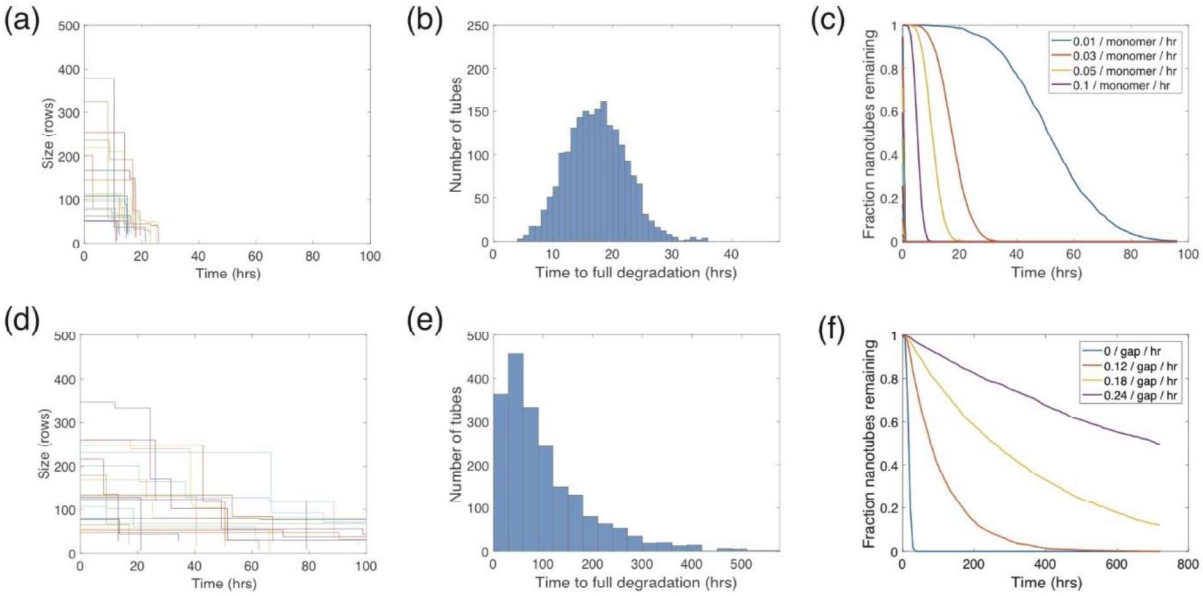


**Figure S12**: Simulated degradation and repair of nanotubes in which the initial distribution of lengths of nanotubes was chosen from the set of all nonzero initial lengths of nanotubes measured in the degradation experiments performed in this work. (a-c), Repeat of the simulations in Figure 4a-c using the length initial distribution of nanotubes from experiments. (d-e), Repeat of the same simulations as shown in Figure 4(d-f) using the initial distribution of nanotubes drawn from experimental measurements. The results show the same qualitative effects of degradation (a tendency for nanotubes to degrade within a clustered range of times) and repair (the creation of a long tail in the distribution of nanotube lifetimes that can dramatically extend the times over which nanotubes persist).

## SI 10. Incorporation of tiles into nanotubes in TAE $Mg^{2+}$ buffer

In order to show that tiles in solution can incorporate within DNA nanotubes, we first studied how DNA tiles incorporate into DNA nanotubes in a standard buffer where degradation is not expected to occur. To distinguish tiles that were incorporated after the nanotubes were annealed from the tiles that became incorporated after assembly, the tiles present in the original anneal and the tiles added subsequently were labeled with different fluorescent dyes. To test whether tiles would still incorporate despite the differences in tile interactions that might be caused by differences in fluorescent labeling, three different fluorescence schemes for labeling the nanotubes and free tiles in solution were tested. We compared growth and incorporation of Cy3-labeled nanotubes and Atto647-labeled free tiles, Atto647-labeled nanotubes and Cy3-labeled free tiles, and Cy3-labeled nanotubes and Atto488 labeled free tiles. In each fluorescently labeled tile, the fluorophore was present at the same location as in Cy3 labeled tiles (Fig. S3).

This comparison was done using seeded nanotubes without PEG coating. Seeds were annealed and purified following the steps described in S3.1-3. 18 µl inactive tiles (Fig. S3), were annealed at 167 nM concentration in TAE $Mg^{2+}$ buffer using the annealing protocol in S3.2. Then, 1.6 µl purified seeds and 0.4 µl of activation strand to a final concentration of 200 nM were mixed with 18 µl of the annealed inactive tile solution. The tile concentration becomes 150 nM after addition of purified seeds and activation strand. The mixture was incubated at 37°C for at least 24 hours to all seeded nanotube to grow to their maximum lengths.

After preparing seeded nanotubes, we annealed 19.4 µl free tiles in their inactive form (Fig. S4) at 204 nM concentration following the same annealing protocol as described in S3.2. Then, 0.6 µl activation strand to a final concentration of 300 nM was added to the annealed tiles, which were kept in 37°C incubation. Right after adding the activation strand, 5 µl prepared seeded nanotubes were mixed with 15 µl activated free tiles so that free tile concentration became 150 nM after addition. The mixed solution was kept incubated at 37°C for at least 12 hours before imaging.

Incorporation of tiles into nanotubes was observed by plating 0.6 µl of each sample onto a bare glass slide and imaging using epi-fluorescence microscope (Olympus IX71). As shown in Figure S13, for each color scheme experiment, the free tiles both joined at the growing ends of the existing nanotubes and incorporated within the existing nanotubes. The tiles added to the already assembled nanotubes also themselves assembled into new, unseeded nanotubes. The amount of tile incorporations differs among different color schemes, possibly because different fluorescent labeling molecules affected the kinetics of nanotube assembly, joining or monomer incorporation. We observed more tile incorporation into Cy3-labeled nanotubes by Cy5-labeled free tiles and into Cy5-labeled nanotubes by Cy3-labeled free tiles than into Cy3-labeled nanotubes by Atto488-labeled free tiles. Although the results suggested Cy3-labeled tiles and Cy5-labeled tiles were more compatible, we used Atto488 labeling and Cy3 labeling for the nanotubes and free tiles in the experiment that observed tile incorporation during degradation (Fig.

3), because this color combination allowed us to use a spinning disk confocal microscope that could capture the Atto488 and Cy3 channels simultaneously and because PEG-coated Cy3-labeled free tiles exhibited the least non-specific interaction with the surface of the three types of free tiles when incubated in serum-supplemented medium.

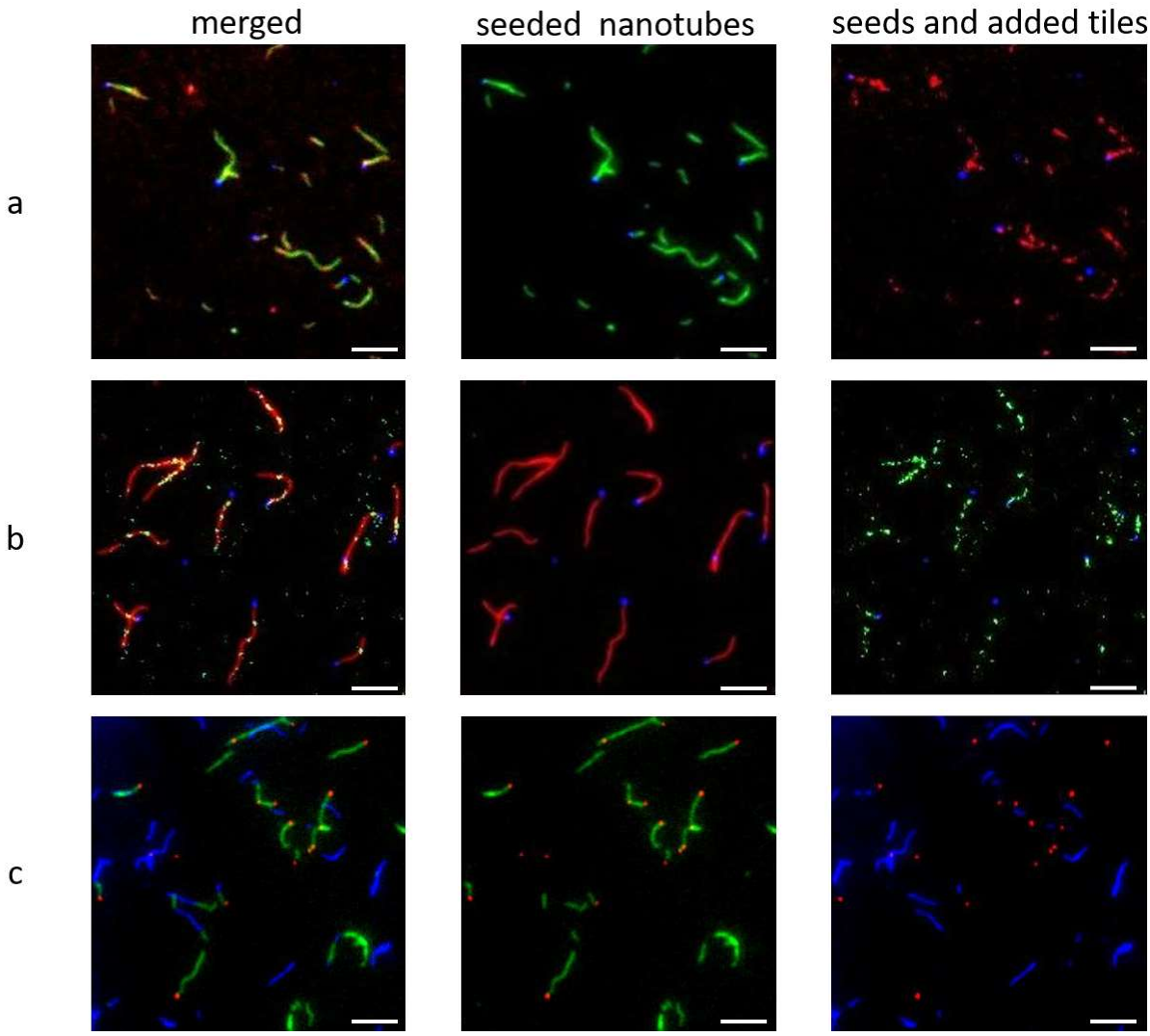


**Figure S13**. Fluorescence images of nanotubes grown in solution to which free tiles with different fluorescence labels are added. (a) Atto647-labeled (red) tiles incorporated into Cy3-labeled (green) nanotubes nucleated from Atto488-labeled (blue) seeds, (b) Cy3-labeled tiles incorporated into Atto647-labeled nanotubes nucleated from Atto488-labeled seeds, and (c) Atto488 labeled tiles incorporated into Cy3-labeled nanotubes nucleated from Atto647-labeled seeds. Scale bars 5 μm.

## SI 11. Inactive tiles do not form nanotubes

To verify that inactive tiles do not form nanotubes, 20 µl of Cy3-labeled inactive tiles at 300 nM concentration were annealed following the protocol described in S3.2, and incubated at 37°C for 30 hours before imaging with fluorescence microscope. To then verify that these tiles would form nanotubes after activation, 1.2 µl of activation strand at 10 µM concentration was added to 18.8 µl tile solution to reach a final concentration of 600 nM activation strand in solution. The mixture was incubated at 37°C for nanotube growth and imaged with fluorescence microscope after 22 hours.

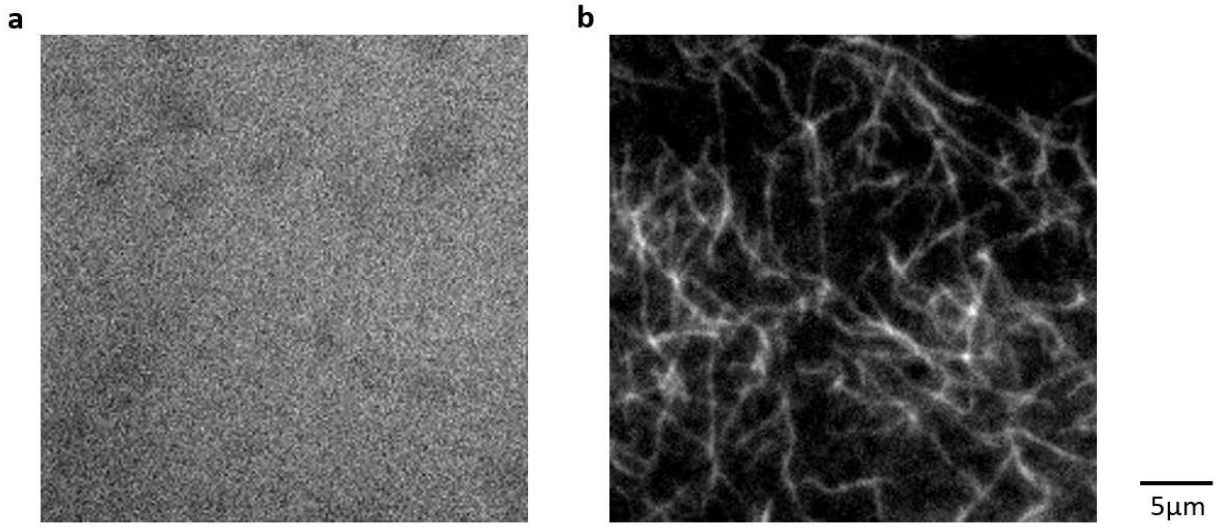


**Figure S14**. Monochrome fluorescence images of a) inactive tiles alone after incubation at 37°C for 30 hours and b) the same tiles formed nanotubes after addition of activation strand and subsequent 22-hour incubation at 37°C. No nanotubes formed from inactive tiles but many nanotubes formed after the tiles were activated.

## SI 12: Nonmatching tiles

U tiles, which have the same structure and size but different sequences than SEs tiles [4], were added to nanotubes in serum-supplemented medium as part of control experiments to test the effect of a given concentration of DNA on the rate of nanotube degradation. To ensure a consistent comparison between the effects of U and SEs tiles in solution on degradation rates, U tiles were, like SEs tiles, conjugated with PEG polymer molecules. Because U tiles have completely different sticky end sequences, they are not expected to interact with SEs tiles or DNA nanotubes consisting of SEs tiles. U tiles also do not have sticky end sequences that would allow them to interact with each other or form nanotubes alone after annealing.

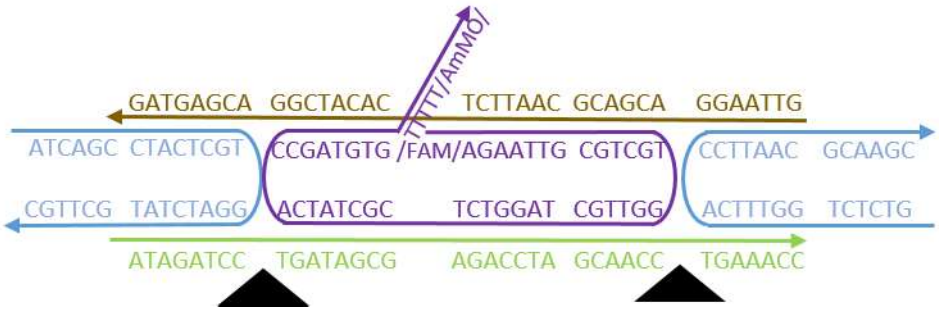


**Figure S15. Schematic showing the architecture of U tiles.**

*U tile sequences:*

U_1: GTTAAGGACGACGCAATTCTCACATCGGACGAGTAG

U_2: ATCAGCCTACTCGTGGATCTATGCTTGC

U_3-5'Cy3-3amine: /56FAM/AGAATTGCGTCGTGGTTGCTAGGTCTCGCTATCACCGA- TGTG TTTTT/AmMO/

U_4: GTCTCTGGTTTCACCTTAACGCAAGC

U_5: ATAGATCCTGATAGCGAGACCTAGCAACCTGAAACC

/FAM/ denotes FAM fluorophore covalently attached to the 5' end of DNA.

## SI 13: Helper strand analogs without sequence interactions with seeds or nanotube tiles

To ensure that the same amount of DNA was added to the serum-supplemented medium in the control group ('nonmatching tiles and helper strands') as in the experimental group ('matching tiles and helper strands') when the rate of nanotube degradation was measured, we designed DNA that matched the structure and total length of the helper strands in the experimental group but lacked sequences that allowed for binding interactions between the helper strands and seeded nanotubes. The helper strands used in the experiments include the activation and adapter strands. The activation strand added in the control group was the same strand used to activate inactive SEs tiles (S1.3). No interaction between this strand and the nanotubes was expected because the SEs tiles in the nanotubes anchored to the surface are already in the active form. The adapter strands used in place of the helper strands consisted of two domains. The first domain was complementary to adapter binding domain on seeds, and the second domain had randomly generated sequences so that added tiles would not interact with seeds. The adapter strands used in the other control group, where only helper strands were added to DNA nanotubes in serum-supplemented medium, were the same as the strands used in the experimental group and are listed in Section SI2.

Sequences of adapters with random sequence tile binding domain:

AD1UEd_1_rand: GACACGGAAGCGGATGTGGAAGCACTAGCTCGCGAAAGCACGTAG

AD1_2UEd_3_rand: TTCCACATCCGCTCTGGCAGTCACCTCGCATCAGGCTAGTGC

AD2UEd_5_rand: ATTGAATTTCAGCCCGCTGATGCGAGGTGACTGCCAGCAGATGGT

AD1_2UEd_2_rand: TCTCTGACCATCTGTCCGTGTCGCAAGC

AD5UEd_1_rand: GATGAGCATCGACGTGGCTTCACCTACTGCGTGAGATGCACGCAC

AD5_6UEd_3_rand: GAAGCCACGTCGAGACCAGTCCTACCACAGCTCGCAGTAGGT

AD6UEd_5_rand: TAAGTTTCGCCAAGAACGAGCTGTGGTAGGACTGGTCGCTATGCC

AD5_6UEd_2_rand: TCTCTGGGCATAGCTGCTCATCGCAAGC

AD9UEd_1_rand: GTGCTCGACTGACACCGAATCCGTGGATGGCGTCGTGATGCAGGC

AD9_10UEd_3_rand: GATTCGGTGTCAGAGCTGGTTGGACTCATGCCGTCATCCACG

AD10UEd_5_rand: GCTCAACACCCAATGTACGGCATGAGTCCAACCAGCTACGACCTA

AD9_10UEd_2_rand: TCTCTGTAGGTCGTTCGAGCACGCAAGC

## SI 14: Nonmatching tiles do not incorporate into nanotubes

To verify that U tiles, used in the control group for the amount of DNA in the experiment, do not incorporate into SEs tile nanotube, we captured images of the samples with fluorescence microscope at each time point to characterize how FAM-labeled U tiles became incorporated (or not) into anchored SEs nanotubes in serum-supplemented medium. At each time point (t = 12h, 24h, 36h, …), after washing the dish with TAE $Mg^{2+}$ buffer, one image in FAM channel was captured along with seeds and DNA nanotubes. As in other time-lapse experiments, fresh U tiles, helper strands, and serum-supplemented DMEM were replenished to the dish after each imaging. At no time point were U tiles observed in nanotubes.

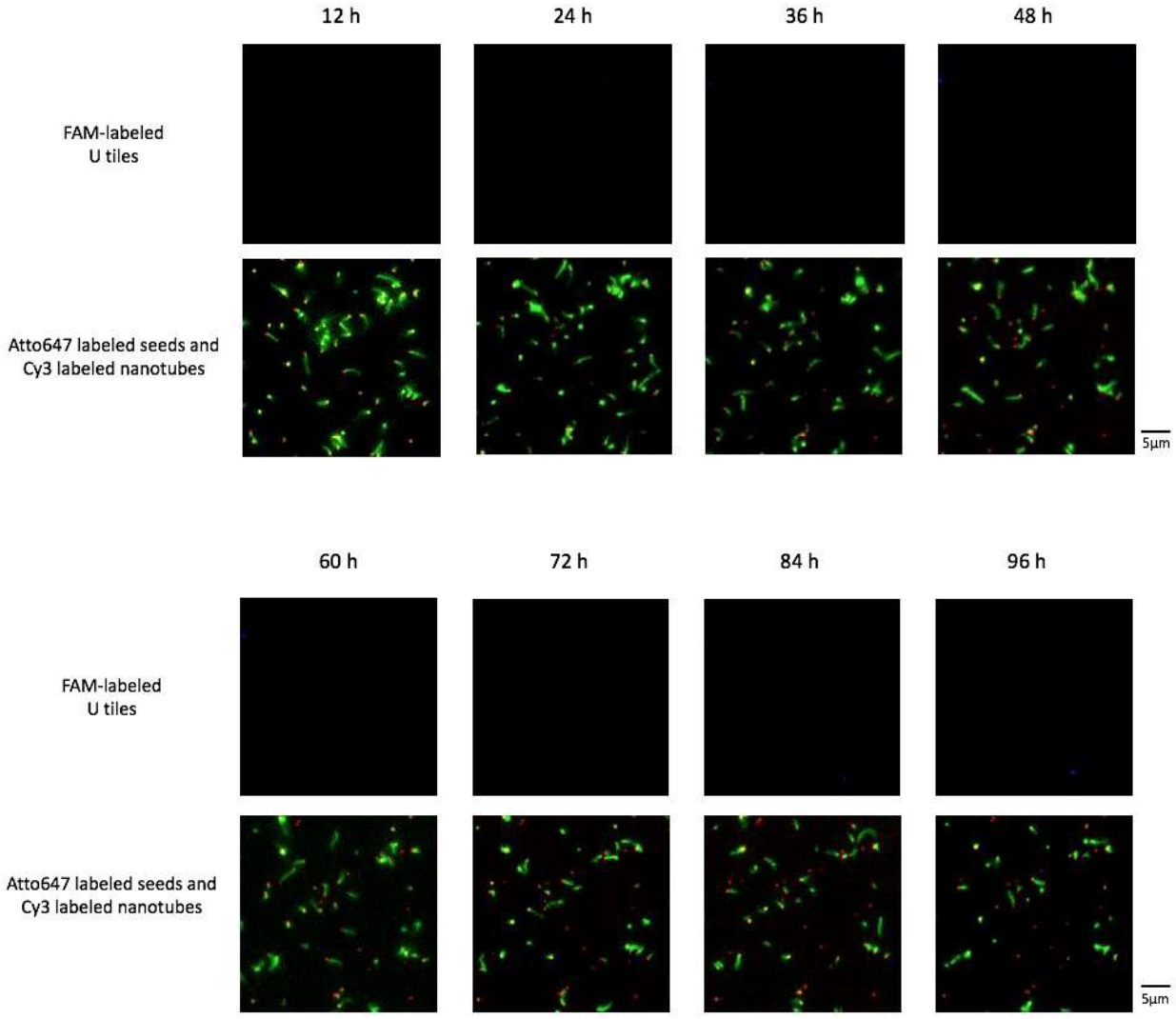


**Figure S16**. Fluorescence images of U tiles and multicolor images of seeded nanotubes on dish glass surface, captured after buffer washing at different time points. U tiles are labeled with FAM (blue),

nanotubes with Cy3 (green), and seeds with Atto647 (red). The images showed that all the U tiles added into the solution were washed away during buffer washing step, indicating that no U tiles incorporated into nanotubes after 12-hour incubations in serum at 37$^{o}$C.

## SI 15: Additional fluorescence images of anchored DNA nanotube degradation in serum

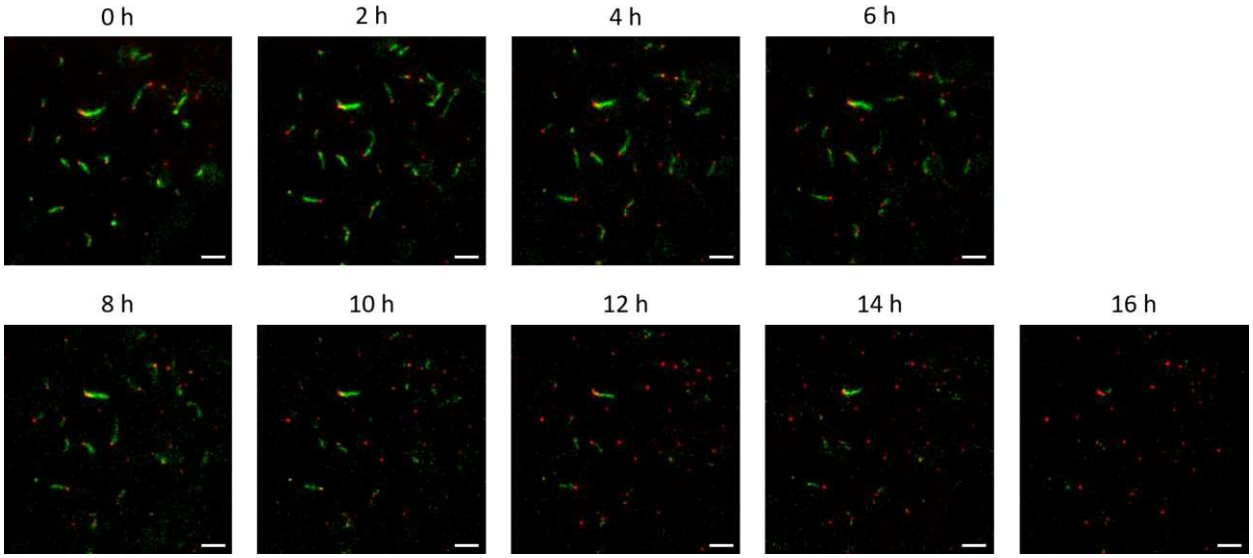


**Figure S17**. Time-lapse fluorescence images showing degradation of seeded DNA nanotubes anchored on a glass coverslip incubated in serum-supplemented medium at 37°C. Nanotubes are labeled with Cy3 (green) and seeds with Atto647 (red). DNA nanotube disassembled rapidly from t = 8 hours to t = 16 hours. Scale bars, 5 μm.

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